

**DECODING LUPUS NEPHRITIS: SPATIAL OMICS
INSIGHTS INTO INFLAMMATORY PATHWAYS
AND CELLULAR DYNAMICS IN TREATMENT RESPONSE**

Sebastián Sandoval Moreno

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DOCTORAL THESIS

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Barcelona, 2026

"What is essential is invisible to the eye."

– Antoine de Saint-Exupéry

Acknowledgements

This thesis represents the culmination of many years of training and learning, a journey during which I have been fortunate to count on the guidance and support of exceptional people.

I would like to express my deepest gratitude to Dr. Josefina Cortés, my thesis director, for instilling in me a profound passion and curiosity for autoimmune diseases, and particularly for systemic lupus erythematosus. Her guidance, trust, and teachings throughout these years have been a cornerstone of my development as a physician and researcher.

I am equally indebted to Dr. Cristina Solé, my thesis co-director, who generously and patiently taught me laboratory techniques and the essential skills to grow as a researcher. Her unwavering support has been decisive at every stage of this work.

My sincere thanks also go to the Lupus Unit team at VHIR for their continuous support and collaboration, as well as to the Rheumatology Department at Hospital Vall d'Hebron, where I received much of my training as a rheumatologist. I am also deeply grateful to my current colleagues at the Rheumatology Department of Hospital de Granollers, whose warm support and understanding greatly facilitated the completion of this work.

To our dear patients, without whom this work would not have been possible. Thank you for your invaluable contribution to science, for your strength and determination, and above all for your willingness to help us investigate and move towards a future where autoimmune diseases can be better controlled.

To my friends, who made this journey lighter and brighter, even through the most challenging times.

And above all, I dedicate this thesis to my beloved family. Though far away, they have always been present, their unconditional love and constant support being the true driving force behind every step of this path.

Abbreviations

ABCs - Age-Associated B Cells
ABIN1 - A20 Binding and Inhibitor of NF-kappaB 1
ACR - American College of Rheumatology
AI - Activity Index
AMP - Accelerating Medicines Partnership
Anti-C1q - Anti-Complement Component 1q Antibodies
Anti-dsDNA - Anti-double-stranded DNA Antibodies
AP-1 - Activator Protein-1
APCs - Antigen-Presenting Cells
APOL1 - Apolipoprotein L1
APRIL - A Proliferation-Inducing Ligand
AZA - Azathioprine
BAFF - B-Cell Activating Factor
cLN - Childhood-Onset Lupus Nephritis
C1Q - Complement Component 1q
C1R - Complement Component 1R
C1S - Complement Component 1S
C2 - Complement Component 2
C3 - Complement Component 3
C4 - Complement Component 4
CAMs - Cell Adhesion Molecules
CCL-20 - C-C Motif Chemokine Ligand 20
CCR2 - C-C Motif Chemokine Receptor 2
CD14 - Cluster of Differentiation 14
CD163 - Cluster of Differentiation 163
CD163+ - Cluster of Differentiation 163 Positive
CD27 - Cluster of Differentiation 27
CD56 - Cluster of Differentiation 56
CG - Cytosine-Guanine
CKD - Chronic Kidney Disease
CI - Chronicity Index
CpG-DNA - Cytosine-phosphate-Guanine DNA
CsA - Cyclosporine A
CYC - Cyclophosphamide
CXCL-12 - C-X-C Motif Chemokine Ligand 12
CXCL2 - C-X-C Motif Chemokine Ligand 2
DCs - Dendritic Cells
DLK1-DIO3 - Delta-Like 1 Homolog - Dioxygenase Cluster 3
DNA - Deoxyribonucleic Acid
DNTs - Double-Negative T Cells

DNASE1L3 - Deoxyribonuclease 1 Like 3
 DNMTs - DNA Methyltransferases
 DSP - Digital Spatial Profiling
 EGF - Epidermal Growth Factor
 eGFR - Estimated Glomerular Filtration Rate

 ESRD - End-Stage Renal Disease
 EULAR - European League Against Rheumatism
 EXT1/2 - Exostosin 1/Exostosin 2
 FCAR - Fc Alpha Receptor
 Fc - Fragment Crystallizable Region
 FcGR - Fc Gamma Receptor
 FcGR1 - Fc Gamma Receptor 1 (CD64)
 FcGR2A - Fc Gamma Receptor 2A (CD32a)
 FcGR3A - Fc Gamma Receptor 3A (CD16a)
 FcGRs - Fc Gamma Receptors
 FD - Factor D
 FAS - Fas Cell Surface Death Receptor
 FN1 - Fibronectin 1
 GC - Glucocorticoids
 GECs - Glomerular Endothelial Cells
 GFR - Glomerular Filtration Rate
 GN - Glomerulonephritis
 GSEA - Gene Set Enrichment Analysis
 GWAS - Genome-Wide Association Study
 HAS2 - Hyaluronan Synthase 2
 HCQ - Hydroxychloroquine
 HDAC3 - Histone Deacetylase 3
 HDL - High-Density Lipoprotein
 HIV - Human Immunodeficiency Virus
 HLA - Human Leukocyte Antigen
 HLA-DP - Human Leukocyte Antigen-DP
 HLA-DQ - Human Leukocyte Antigen-DQ
 HLA-DR - Human Leukocyte Antigen-DR
 HMGB1 - High-Mobility Group Box 1
 HNP - Human Neutrophil Peptides
 ICAM1 - Intercellular Adhesion Molecule 1
 ICs - Immune Complexes
 IF - Immunofluorescence
 IFN- α - Interferon Alpha
 IgG1 - Immunoglobulin G Subclass 1
 IgG2 - Immunoglobulin G Subclass 2
 IgG3 - Immunoglobulin G Subclass 3
 IL-1 alpha - Interleukin-1 Alpha

IL-1 beta - Interleukin-1 Beta
 IL12 - Interleukin 12
 IL15-RA - Interleukin-15 Receptor Alpha
 IL1RAP - Interleukin 1 Receptor Accessory Protein
 IL6 - Interleukin 6
 IL8 - Interleukin 8
 INF - Interferon
 IRF5 - Interferon Regulatory Factor 5
 ISGs - Interferon-Stimulated Genes
 ISN/RPS - International Society of Nephrology/Renal Pathology Society
 JAK/STAT - Janus Kinase/Signal Transducer and Activator of Transcription Pathway
 JAM-A - Junctional Adhesion Molecule A
 KDIGO - Kidney Disease: Improving Global Outcomes
 KEGG - Kyoto Encyclopedia of Genes and Genomes
 Ki67+ - Marker of Cellular Proliferation (Ki-67 Positive)
 LN - Lupus Nephritis
 MCP-1 - Monocyte Chemoattractant Protein-1
 MAPK - Mitogen-Activated Protein Kinase
 MCs - Mesangial Cells
 MHC - Major Histocompatibility Complex
 miRNAs - MicroRNAs
 ML - Machine Learning
 MMF - Mycophenolate Mofetil
 mRNA - Messenger Ribonucleic Acid
 mTOR - Mechanistic Target of Rapamycin
 NCAM1 - Neural Cell Adhesion Molecule 1
 NETosis - Neutrophil Extracellular Trap Formation
 NETs - Neutrophil Extracellular Traps
 NEBT - NanoString Elements Barcoding Technology
 NGAL - Neutrophil Gelatinase-Associated Lipocalin
 NGS - Next-Generation Sequencing
 NK cells - Natural Killer Cells
 NO2 - Nitrogen Dioxide
 NRP-1 - Neuropilin-1
 OR - Odds Ratio
 PCR - Protein C Reactive
 PDGF - Platelet-Derived Growth Factor
 PDGF-BB - Platelet-Derived Growth Factor-BB
 PDGFRA - Platelet-Derived Growth Factor Receptor Alpha
 PM2.5 - Particulate Matter ≤ 2.5 Micrometers
 RNA - Ribonucleic Acid
 ROIs - Regions of Interest
 RTX - Rituximab
 SARS-CoV-2 - Severe Acute Respiratory Syndrome Coronavirus 2

scRNA-seq - Single-Cell RNA Sequencing
SLE - Systemic Lupus Erythematosus
SLEDAI-2K - Systemic Lupus Erythematosus Disease Activity Index 2000
SLICC - Systemic Lupus International Collaborating Clinics
SMD - Small Nuclear Ribonucleoprotein D
SNP - Single Nucleotide Polymorphism
SSR - Spanish Society of Rheumatology
ST - Spatial Transcriptomics
STAT - Signal Transducer and Activator of Transcription
STAT4 - Signal Transducer and Activator of Transcription 4
STING - Stimulator of Interferon Genes
TAC - Tacrolimus
TGF - Transforming Growth Factor
TGFB-1 - Transforming Growth Factor Beta 1

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Abstract

Background

Lupus nephritis (LN) is a severe complication of systemic lupus erythematosus (SLE), affecting up to 60% of patients. Despite advances in therapy, 10–30% of LN patients progress to end-stage renal disease (ESRD) within a decade. The complex and heterogeneous pathogenesis of LN underscores the need for improved disease classification and a deeper understanding of its underlying mechanisms. Emerging omics technologies hold promise for enhanced patient stratification. Transcriptomic analysis of serial kidney biopsies has revealed dynamic gene expression changes associated with clinical treatment responses, offering insights into potential targeted therapies. Spatial transcriptomics further enhances this approach by integrating tissue morphology with cell–cell interactions and region-specific gene expression, enabling comprehensive molecular insights while preserving histological context.

Methods

We conducted spatial transcriptomic and proteomic analyses on formalin-fixed, paraffin-embedded kidney biopsies from 20 patients with proliferative lupus nephritis (LN), collected at both flare and post-treatment stages (n=8). Patients were stratified as responders or non-responders using the American College of Rheumatology (ACR) response criteria for proliferative and membranous LN in SLE clinical trials. Six regions of interest (ROIs) per biopsy, comprising a total of 12 areas of interest (AOIs), were selected from histologically active glomerular and tubular compartments. AOIs were defined by immunofluorescence markers: CD45⁺ (immune cells), CD31⁻ (mesangial cells), CD31⁺ (endothelial cells), and PanCK⁺ (tubular epithelial cells).

Results

Of the 20 patients treated with mycophenolate mofetil (MMF), 11 patients (55%) achieved complete renal response. Eight patients underwent repeat biopsy after one year of therapy. Baseline demographics were similar between groups, although non-responders had significantly lower estimated glomerular filtration rate (eGFR) at baseline. Trends toward higher proteinuria, SLEDAI-2K scores, and anti-dsDNA titers were also observed in non-responders.

Following treatment, significant reductions were observed in SLEDAI-2K scores, proteinuria, anti-dsDNA antibody levels, and the histological activity index. The chronicity index modestly increased in follow-up biopsies, particularly in non-responders. Immune cells in renal tissue were predominantly localized in tubular regions, with plasmacytoid dendritic cells, natural killer cells, and macrophages as the most abundant subsets during flare. A trend toward macrophage enrichment was observed in non-responders (p=0.057). Over time, non-responders demonstrated persistent upregulation of immune activation genes and pathways involving integrins, plexin-B, and T-cell receptor signaling, whereas responders showed upregulation of

transcriptional recovery programs, including RNA polymerase and splicing factor pathways.

In mesangial compartment, repeat biopsies in non-responders exhibited strong activation of inflammatory pathways such as NF- κ B, AP-1(-like), and interferon signaling, which synergically amplify cytokine-driven responses (e.g., TNF- α and IL-1 β) with interferon signaling further amplifying NF- κ B activation. In tubular regions, T-cell receptor signaling remained upregulated in non-reponders.

Spatial proteomics confirmed the importance of immune and interferon-driven pathways by revealing significant differences between responders and non-responders. In CD45⁺ regions, non-responders exhibited overexpression of CD14, IL-1 α , CD27, TIM-3, STAT1, IL-15RA, and TMS1, while STING protein was abundantly enriched in endothelial (CD31⁺) regions.

Conclusions

Our study highlights the critical role of cellular interactions between immune cells, mesangial cells, and tubular cells in sustaining renal inflammation in LN. Persistent activation of T-cell activation, interferon, and NF- κ B pathways characterized non-responders across serial biopsies, underscoring mechanisms of treatment resistance. Spatial multi-omics thus provides a powerful framework for understanding refractory LN and identifying potential biomarkers and therapeutic targets.

RESUMEN

La nefritis lúpica (NL) sigue siendo una de las complicaciones más graves del LES y su evolución depende de procesos inflamatorios locales que no siempre se reflejan en los parámetros clínicos habituales. En este trabajo aplicamos transcriptómica y proteómica espaciales sobre biopsias renales pareadas (brote y post-tratamiento) para mapear, con contexto histológico, la interacción entre células inmunes y células residentes. La mitad de los pacientes alcanzó respuesta completa clínica; sin embargo, las segundas biopsias revelaron que un subgrupo mantenía actividad tisular, evidenciando la brecha entre remisión clínica y remisión histológica.

Los no respondedores conservaron firmas inflamatorias persistentes en varios compartimentos: activación del interferón tipo I, NF- κ B, señalización del receptor de células T e integrinas; en glomérulo, las células glomerulares mostraron activación de TNF-R y TLR3/4; en el túbulo, se mantuvieron AP-1/JAK-STAT/MAPK y el diálogo inflamatorio con linfocitos. Predominaron macrófagos en las células inmunes en el intersticio, con tendencia a mayor abundancia en no respondedores y sobreexpresión de genes de respuesta temprana (FOS, JUNB, EGR1), lo que sugiere programas intrarrenales que sostienen el daño pese a la inmunosupresión. En contraste, los respondedores transitaban hacia rutas de reparación y homeostasis (remodelado de matriz, reparación del ARN, vías metabólicas y reguladoras como PPAR/Plexinas/PTP).

Complementariamente, la proteómica espacial y el análisis urinario identificaron CD14 y TIM-3 como marcadores asociados a no respuesta, mientras que CD163 apareció en menor medida, apuntando a un predominio de macrófagos proinflamatorios en la enfermedad refractaria. En conjunto, estos hallazgos sitúan al compartimento tubulointersticial como nodo crítico de activación macrofágica e interferón; apoyan el valor de la biopsia de control para estratificar riesgo más allá de la clínica, y proponen un panel urinario combinatorio (CD14, TIM-3, CD163 y quimio/citoquinas) para monitorizar de forma no invasiva la actividad intrarrenal. La NL refractaria, más que depender de una inflamación sistémica uniforme, parece emerger de programas inflamatorios persistentes y compartimento-específicos dentro del riñón, lo que respalda estrategias terapéuticas dirigidas al microambiente inmune renal.

INTRODUCTION

1.1 Lupus nephritis

Lupus Nephritis (LN) is one of the most severe complications of Systemic Lupus erythematosus (SLE), affecting up to 60% of patients over the course of their disease. This condition is characterized by immune-mediated damage to the kidneys, which can lead to chronic kidney disease (CKD) or end-stage renal disease (ESRD) if left untreated. Despite advances in diagnosis and treatment, LN remains a leading cause of morbidity and mortality in SLE patients (1). This section explores the epidemiology, pathogenesis, and clinical implications of LN, highlighting recent advances and persisting challenges in its management.

1.1.1 Epidemiology

SLE affects all ages, sexes, and ethnic groups but predominantly women of reproductive age, with a female-to-male ratio of about 9:1. Globally, its incidence is estimated at 5.1 cases per 100,000 person-years and prevalence at 43.7 per 100,000, affecting roughly 3.4 million people, although data remain unavailable for nearly 80% of countries (2). The EPISER2016 study by the Spanish Society of Rheumatology reported an SLE prevalence of 210 per 100,000 adults (95% CI: 110–400) (3).

LN is one of the most severe and frequent SLE manifestations, driving much of its morbidity and mortality. It typically develops within the first five years of SLE diagnosis and may present as the initial disease manifestation (1). The female-to-male ratio in LN is approximately 5:1 (4–6). Its cumulative incidence is higher among Asian (55%), African (51%), and Hispanic (43%) populations than among Caucasians (14%), and it tends to be more aggressive in children but less common in older adults (7,8).

Despite therapeutic advances, 10–30% of LN patients progress to ESRD within 10 years of diagnosis (4). Individuals of African and Hispanic ancestry show greater disease activity, relapse rates, and mortality, whereas in Caucasians, male sex and high baseline creatinine are independent predictors of CKD progression (4,9,10). In high-income countries, LN-related ESRD incidence fell markedly from the 1970s to the 1990s and has since stabilized (11). Nonetheless, socioeconomic disadvantage remains a strong, independent risk factor for poor renal outcomes, irrespective of ethnicity (12).

1.1.2 Pathogenesis

The pathogenesis of LN is complex and multifactorial, involving genetic, epigenetic, hormonal, environmental, and ethnic factors. Genetic susceptibility interacts with environmental triggers to disrupt immune tolerance to nuclear autoantigens, leading to production of antinuclear antibodies. Nuclear material released from apoptotic or netting neutrophils activates innate and adaptive immunity through Toll-like receptor (TLR) signaling and a type I interferon (IFN- α)–driven response. Dendritic cells, T helper cells, B cells, and plasma cells together sustain dysregulated polyclonal autoimmunity. Within the kidney, LN is driven by immune complex deposition, complement activation,

and Fcγ receptor–mediated pathways. In addition, tertiary lymphoid structures may form in renal tissue, containing B cells and plasma cells that produce autoantibodies locally and amplify inflammation (13,14).

In the following subsections, we summarize the main genetic and epigenetic contributors to LN, key environmental factors, and fundamental immunological mechanisms involving infiltrating immune cells and resident renal cells.

1.1.2.1 Genetics and epigenetics

1.1.2.1.1 Genetics

SLE develops in genetically predisposed individuals exposed to environmental triggers. GWAS and candidate gene studies have identified more than 100 susceptibility loci, most involved in innate and adaptive immunity. Several of these variants also increase the risk or severity of LN, influencing pathways such as immune complex clearance, nucleic acid sensing, complement activation, and type I IFN signaling (15,16). Below are key loci most relevant to LN.

1.1.2.1.1.1 The major histocompatibility complex (MHC)

The strongest genetic association with SLE and LN lies in the MHC region on chromosome 6, which contains the human leukocyte antigen (HLA) genes. Class II alleles, especially HLA-DR2/DR3 and DR15, are well-established SLE risk factors and have been linked to increased risk of LN, whereas other alleles such as HLA-DR4 and DR11 may be protective (16–18). These alleles likely promote autoreactive T-cell activation through enhanced presentation of self-antigens, consistent with links between HLA-DR3, SmD-reactive T cells, and anti-Ro antibodies (18,19).

1.1.2.1.1.2 Interferon regulatory factor 5 (IRF 5)

IRF5, located on chromosome 7, encodes a transcription factor that promotes inflammatory and type I IFN–related gene expression downstream of TLR activation. The association between *IRF5* and SLE was first described by Sigurdsson et al. in 2005 (20), and a functional risk haplotype has since been linked to increased serum IFN-α activity and higher *IRF5* expression (20–27). This *IRF5* risk haplotype is also associated with LN, particularly proliferative forms and severe renal dysfunction (28,29). By amplifying type I IFN responses in the presence of autoantibodies, *IRF5* variants likely contribute both to systemic autoimmunity and to intrarenal inflammatory amplification.

1.1.2.1.1.3 Fc gamma receptors (FCGRs)

Fcγ receptors include one high-affinity activating receptor (FcγRI) and several low-affinity receptors (FcγRIIA, FcγRIIIA) plus the inhibitory FcγRIIB. They differ in IgG subclass binding and are mainly expressed on myeloid cells (30). Polymorphisms in **FCGR2A** and **FCGR3A** affect IgG subclass binding and immune complex handling (30–38).

The FCGR2A rs1801274 variant (H131R) reduces IgG2 binding, impairing immune complex clearance and increasing LN risk (39–41).

FCGR3A F158V: while the low-affinity F allele is linked to LN susceptibility, the high-affinity V/V genotype has been associated with faster progression to ESRD (42). Collectively, these variants influence LN onset (poor clearance) and progression (enhanced inflammation).

1.1.2.1.1.4 TNFAIP3-interacting protein 1 (TNIP 1)

TNIP1 encodes ABIN-1, a negative regulator of NF-κB. Animal models show that loss of ABIN-1 function induces lupus-like nephritis (45). Human TNIP1 variants are associated with SLE and LN across multiple populations (28,45–49). Reduced ABIN-1 expression in risk haplotypes supports a mechanism of exaggerated NF-κB activation and renal inflammation (50).

1.1.2.1.1.5 Signal transducer and activator of transcription 4 (STAT4)

STAT4 polymorphisms, particularly rs7574865, are major SLE risk alleles and are strongly linked to LN and anti-dsDNA antibodies (28,51,52). Risk carriers show heightened sensitivity to type I IFNs and enhanced Th1/Th17 differentiation, promoting proinflammatory T-cell responses in the kidney (53,54).

1.1.2.1.1.6 Tumor necrosis factor superfamily member 4 (TNFSF4)

TNFSF4, on chromosome 1, encodes OX40 ligand (OX40L/CD252), a costimulatory molecule expressed on antigen-presenting cells that supports T cell activation and survival. Variants such as rs2205960 and rs10489265 are associated with LN in European and Asian cohorts (55,56). Serum TNFSF4 levels and expression of its receptor on CD4⁺ T cells are higher in SLE patients with nephritis and correlate with disease activity (56,57). These findings implicate the OX40–OX40L axis in intrarenal T cell–mediated inflammation and LN pathogenesis.

HLA, IRF5, FCGR, STAT4, and TNFSF4 thus define a core set of SLE susceptibility genes, some variants of which are also enriched in LN. However, none is specific for LN, and GWAS focusing directly on nephritis have identified additional loci more selectively associated with renal involvement, including APOL1, PDGFRA, and HAS2.

1.1.2.1.1.7 Apolipoprotein L1 (APOL 1)

APOL1, on chromosome 22, encodes a component of high-density lipoprotein (HDL) involved in innate immunity and trypanolysis (58–60).

The G1 and G2 risk variants, common in individuals of African ancestry, confer protection against *Trypanosoma brucei rhodesiense* but markedly increase the risk of several kidney diseases, including focal segmental glomerulosclerosis (FSGS), HIV-associated nephropathy, and ESRD (59–63). In African American SLE patients, APOL1 G1/G2 variants are strongly associated with progression to ESRD in LN (64), whereas no similar association has been observed in European Americans or in milder forms of LN (65). APOL1 is induced by TLR3 agonists and interferons and is involved in autophagy (66). Although not a general SLE susceptibility gene, APOL1 acts as a modifier of nephritis risk and progression in African-ancestry populations, contributing to the burden and severity of LN in these groups.

1.1.2.1.1.8 Platelet-derived growth factor receptor alpha (PDGFRA)

PDGFRA variants (e.g., rs1364989) have been associated with LN (69). PDGFR signaling promotes mesangial proliferation and fibrosis (67,68). In lupus models, PDGFR inhibition reduces nephritis (70,71), highlighting its role in disease progression.

1.1.2.1.1.9 Hyaluronan synthase 2 (HAS2)

HAS2 synthesizes hyaluronan, a key extracellular matrix component in fibrosis. Mesangial and tubular cells upregulate HAS2 in response to IL-1 β and PDGF, and HA accumulation is increased in active LN (73–77). The HAS2 variant rs7834765 is associated with LN risk and likely contributes by promoting matrix expansion and fibrosis (69).

1.1.2.1.1.10 Complement genes

Deficiencies in early classical pathway components (C1q, C1r, C1s, C2, C4) are among the strongest monogenic risk factors for SLE and lupus-like syndromes. C1q deficiency, although rare, is associated with SLE in ~90% of cases, while deficiencies in C1r, C1s, and C4 show penetrance of 65–80%. Complete C2 deficiency is more frequent but confers lower SLE penetrance (~25%) (78).

Complement genes are also expressed by resident renal cells. Transcriptomic analyses of serial LN biopsies have shown marked upregulation of alternative pathway genes during flares, particularly C3 and **FD**, which increased by ~80% and 122%, respectively, in patients who failed to respond to standard induction therapy. In contrast, complement gene expression tended to normalize in patients achieving complete clinical remission (79). These findings underscore the involvement of the alternative complement pathway in LN and support its consideration as a therapeutic target.

1.1.2.1.2 Epigenetics

Epigenetics refers to heritable changes in gene expression that occur without alterations in the DNA sequence. Evidence indicates that epigenetic mechanisms play a crucial role in the development and progression of LN (80). The main epigenetic regulators are DNA methylation, histone modifications, and non-coding RNAs (miRNAs and lncRNAs).

DNA methylation at CpG sites usually leads to chromatin condensation and gene silencing, whereas hypomethylation favours transcription. Aberrant methylation in CD4⁺ T cells from SLE patients was first described more than two decades ago (81). Genome-wide studies have since shown distinct methylation signatures in SLE, including hypomethylation of type I IFN-regulated genes, which are central to disease pathogenesis (82–85). Coit et al. reported that these changes are even more pronounced in SLE patients with LN compared with those without nephritis (86). At the renal level, demethylation of GALNT18 in neutrophils has been associated with LN in longitudinal studies, and inhibition of the histone methyltransferase EZH2 ameliorated nephritis in murine models by modifying H3 methylation patterns (87,88)

Non-coding RNAs add a further layer of epigenetic control. miRNAs and lncRNAs can both be regulated by DNA methylation and histone marks and, in turn, modulate epigenetic enzymes. A subset of so-called “epi-miRNAs” targets epigenetic regulators such as DNA methyltransferases, creating feedback loops that influence inflammation, proliferation, and fibrosis in renal cells and urinary exosomes (89–92). For example, HDAC3-dependent repression of miR-30d has been linked to podocyte injury, while EZH2 overexpression may enhance T-cell migration through methylation of junctional adhesion molecule A (93–99). Overall, miRNAs and other epigenetic mechanisms are emerging as key modulators of LN, but the underlying regulatory networks remain incompletely understood and warrant further investigation.

A summary of the association and role of genetics in the development of LN is illustrated in Figure 1

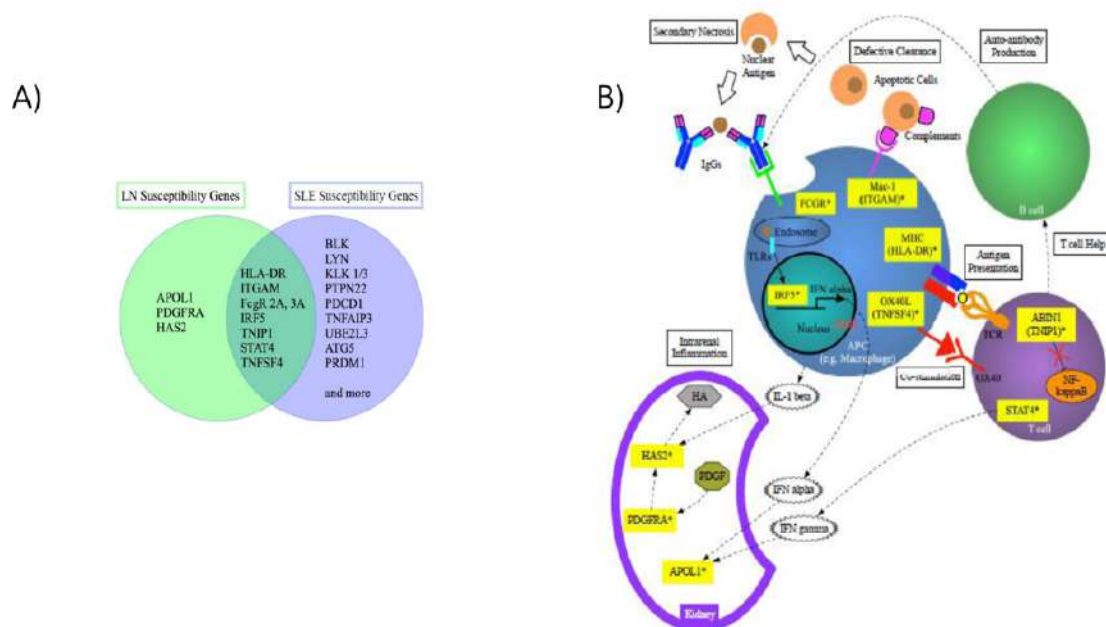


Figure 1. Genetic involvement in the immune system's role in LN pathogenesis: a) Venn diagram of SLE and LN susceptibility genes. b) Mutations in complement receptors (Mac-1) and FcγRs impair apoptotic cell clearance, causing secondary necrosis and release of nuclear antigens that activate TLRs. Altered *IRF5* function increases IFN-α production, while co-

stimulatory molecules such as HLA and OX40L enhance T-cell activation and *STAT4*-mediated IFN-gamma production. *TNIP1* variants lead to ABIN1 dysfunction and dysregulated NF-kappaB signaling. Activated T cells support B-cell autoantibody production, and IFNs upregulate *APOL1* expression in the kidney. Pro-inflammatory IL-1 beta from activated APCs (e.g., macrophages) and *PDGF-PDGFR* signaling promote hyaluronan synthesis via *HAS2*. *Adapted from Iwamoto et al.* LN, lupus nephritis; SLE, systemic lupus erythematosus ABIN1, A20 binding and inhibitor of NF-kappaB 1; APC, antigen presenting cell; *APOL1*, apolipoprotein L-1; *FCGR*, Fc gamma receptor; HA, hyaluronan; *HAS2*, hyaluronan synthase 2; HLA, human leukocyte antigen; *IFN*, interferon; IL, interleukin; *IRF5*, interferon regulatory factor 5; *ITGAM*, integrin alpha M; *OX40L*, *OX40* ligand; *PDGFR*, platelet-derived growth factor receptor alpha; *STAT4*, signal transducers and activators of transcription 4; TLRs, Toll-like receptors; *TNFSF4*, tumor necrosis factor super family 4.

1.1.2.2 Environmental factors

Ultraviolet B (UVB) radiation is a well-recognized environmental trigger of SLE and LN. UVB not only exacerbates cutaneous disease but also promotes neutrophil migration to the kidneys in an IL-17A-dependent manner, supporting the concept of a “kidney–skin axis” (100). Air pollution has also emerged as a potential contributor to LN. In a multicentre Chinese study of 2,672 patients, exposure to PM_{2.5} and NO₂ significantly increased LN risk within one month, with interquartile range increases in PM_{2.5} and NO₂ corresponding to odds ratios of 1.16 and 1.19, respectively (101).

Viral infections may trigger or unmask LN, with recent cases linking de novo LN to SARS-CoV-2 infection (102). Increasing attention has also been given to dysbiosis and microbial antigens. Bacterial components and metabolites may mimic autoantigens and influence immune activation in LN (103). Experimental models show that a “leaky gut” allows pathogenic microbes to translocate into the bloodstream, form immune complexes (ICs), and deposit in the kidney (104). Finally, dietary factors represent another modifiable environmental influence. Vitamin D, vitamin E, and omega-3 fatty acids have been associated with improvements in inflammatory markers and endothelial function in SLE (105). Curcumin may exert anti-inflammatory, antioxidant, and antiproteinuric effects in LN, though further studies are needed (104,106).

1.1.2.3 Vitamin D deficiency

LN patients often show lower serum vitamin D levels compared with SLE patients without renal involvement. Reduced expression of vitamin D receptors in kidney tissue correlates inversely with histological activity, and decreased expression of the vitamin D-synthesizing enzyme 1 α -hydroxylase in PBMCs is also more pronounced in LN and negatively associated with SLEDAI scores (107,108). This deficiency can result from photosensitivity-related sun avoidance, medication effects (e.g., glucocorticoids, hydroxychloroquine), or the presence of anti-vitamin D antibodies. Low vitamin D levels have been linked to low complement C3/C4, higher disease activity, and impaired immunoregulation (109).

Vitamin D also modulates the renin–angiotensin–aldosterone system, inhibits renal inflammation, maintains nephrin and podocin expression, and protects podocytes from autoantibody-induced autophagy (110). Collectively, vitamin D likely influences both immune dysregulation in SLE and renal injury in LN, with potential nephroprotective effects.

1.1.2.4 Immunological mechanisms

SLE and LN are characterized by the dysregulation of numerous immune system components. This section focuses on two critical aspects of intrarenal immunological mechanisms: the role of pathogenic autoantibodies and the interaction between infiltrating immune cells and resident kidney cells.

1.1.2.4.1 Role of autoantibodies

Autoantibodies and immunocomplexes (ICs) are central mediators of renal injury in LN. Damage occurs via deposition of circulating ICs, binding of autoantibodies to planted antigens, or in situ IC formation within glomeruli. Extracellular DNA released from dying cells acts as a potent immunological trigger (111). Autoantibodies against nuclear antigens form ICs that deposit in glomeruli and the interstitium, activate complement, and recruit inflammatory cells (112).

Anti-dsDNA and anti-C1q antibodies are the most extensively studied. Anti-dsDNA contributes to glomerular deposition and activation of nucleic acid sensors, triggering cytokine production (1). DNA- and RNA-containing ICs resemble viral particles and stimulate TLRs on B cells and dendritic cells, amplifying autoimmunity (113). IgG2 appears to be a key isotype in LN, especially for glomerular anti-dsDNA, suggesting that IgG2 should be prioritized in association studies (115). Other autoantibodies, including anti-C1q, anti-nucleosome, anti- α -actinin, and anticardiolipin, contribute to LN pathogenesis (112). Moroni et al. showed that anti-C1q IgG correlates strongly with LN activity, particularly in proliferative classes, although anti-C1q lacks specificity and is also found in other autoimmune conditions (116,117).

More recently identified autoantibodies such as anti-ENO1, anti-histone H2 IgG2, and anti-SOD2 (“second-wave antibodies”) may more accurately reflect renal activity and respond to immunosuppression (112,118). Autoantibody glycosylation also affects cytotoxicity, with sialylation reducing and fucosylation enhancing pathogenicity (119,120).

Distinct autoantibody profiles may associate with specific classes of LN. Anti-Annexin A2 and anti-Moesin antibodies have been linked to proliferative LN (122,123), whereas anti-NCAM1 antibodies have been identified in a subset of membranous LN (124). EXT1/EXT2-positive subepithelial deposits have been described in patients with PLA2R-negative membranous nephropathy and may include some LN cases (125,126).

1.1.2.4.2 Role of complement

Complement abnormalities have long been linked to LN. Low CH50 and decreased C3/C4 levels occur in ~75% of patients with focal nephritis and 90% with diffuse nephritis, and

the classic “full house” pattern (IgG, IgA, IgM with C1q, C4, C3, and C5b-9) is characteristic of LN (127).

Complement has dual roles: hereditary deficiencies of classical pathway components (e.g., C1q, C4) confer strong susceptibility to SLE, yet IC-mediated complement activation drives renal inflammation and tissue injury (128). Although classical pathway activation was initially considered dominant, alternative and lectin pathways also contribute to LN (129). Decreased plasma C3 and C4 reflect ongoing IC formation, with C3 reduction particularly associated with renal flares.

Complement split products such as C3dg and C4d correlate with disease activity, whereas renal C4d deposition is linked to worse renal outcomes (130,131). Complement also promotes thrombosis, especially in SLE patients with antiphospholipid antibodies, in whom complement deposition on platelets (C1q, C3d, C4d) is associated with venous thrombosis (132).

The mechanisms involved in the pathogenesis of LN are illustrated in Figure 2.

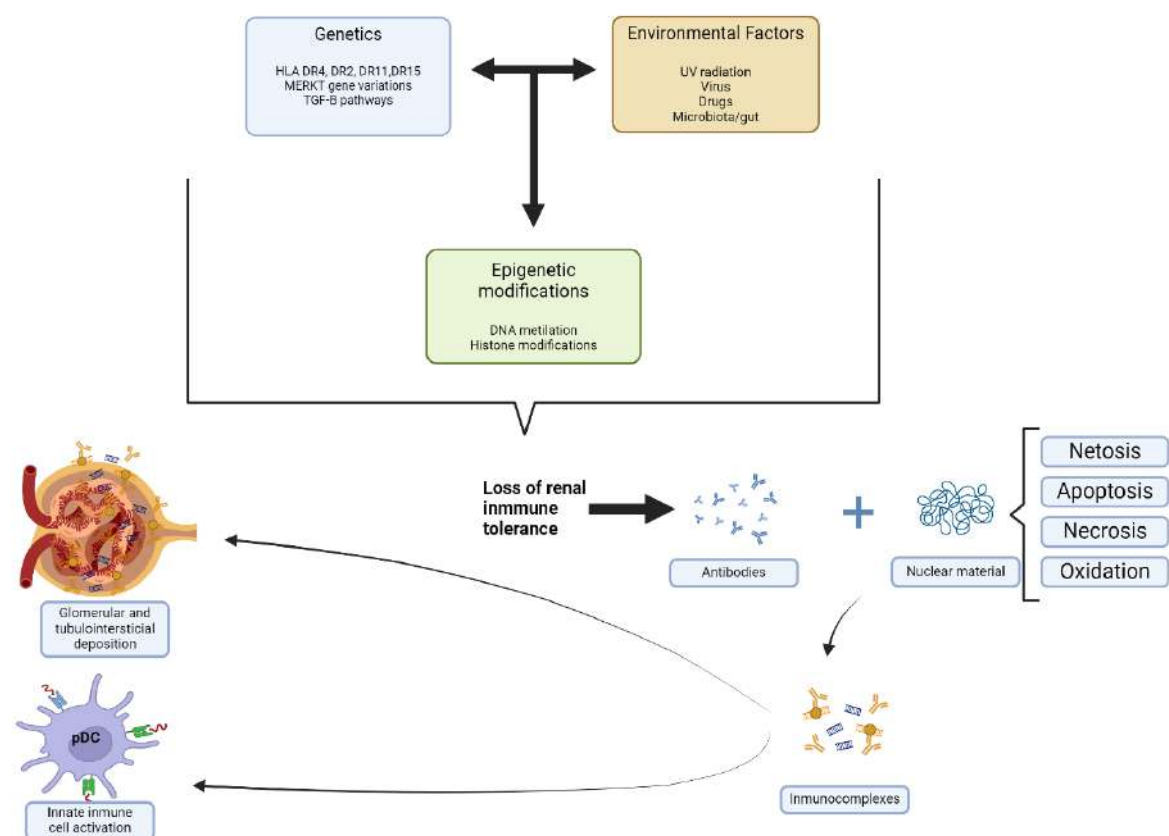


Figure 2. Pathogenesis of LN: Relationship between genetic and environmental factors driving epigenetic changes, loss of immune tolerance, and autoantibody formation. Autoantibodies combine with nuclear material generated by NETosis, oxidation, necrosis, and apoptosis to form immune complexes. These accumulate in the glomerulus and activate innate immunity, including dendritic cell–mediated cytokine production. (Created with BioRender.com). MERTK,

Mer-tyrosine kinase; TGF- β , Transforming Growth Factor- β ; UV, ultraviolet; DNA, deoxyribonucleic acid; pDC, plasmacytoid dendritic cell.

1.1.2.4.3 Role of infiltrating immune cells

Advances in high-dimensional and spatial technologies have greatly refined our understanding of the intrarenal immune landscape in LN. Infiltrating immune cells are key drivers of inflammation, tissue injury, and fibrosis, acting through complex interactions with resident kidney cells. The main contributing populations include neutrophils, monocytes/macrophages, lymphocytes, dendritic cells, and NK cells.

Neutrophils:

For many years, LN research focused mainly on adaptive immunity (DCs, T cells, B cells), but recent studies have revealed a strong neutrophil signature closely linked to SLE and LN activity (133). Neutrophils contribute to autoimmunity through NETosis, the release of neutrophil extracellular traps (NETs) composed of DNA, chromatin, and immunogenic proteins such as LL-37 and HNP (135). SLE patients show enhanced granulocytic activation and increased NETs, particularly in LN, where NET levels correlate with anti-dsDNA titers and complement consumption (C3, C4) (14). NETs connect neutrophils to IFN- α signaling by exposing autoantigens, promoting autoantibody production, activating complement, and stimulating IFN- α release by DCs (40).

In LN kidneys, persistent NETs containing IL-17A and tissue factor have been found even in the absence of intact neutrophils, suggesting a sustained contribution to thrombo-inflammation and fibrosis. Impaired NET clearance—due to reduced DNase I activity or C1q deficiency—leads to NET accumulation, increased antigen exposure, anti-NET autoantibody formation, and a self-perpetuating cycle of inflammation (41,42). NET remnants and the neutrophil-to-lymphocyte ratio have been proposed as biomarkers of LN activity and prognosis (43).

However, the nephritogenic role of NETs is not entirely linear. In lupus-prone mice, NOX2 deficiency reduces NET formation but worsens nephritis, whereas NOX2 activation increases NETosis yet improves disease, indicating that NET biology in LN may be context dependent (136).

Monocytes/Macrophages:

Monocytes and macrophages are key effectors in LN. Circulating monocytes are recruited into inflamed renal tissue through endothelial activation (e.g., TLR signaling) and chemokine pathways such as CCR2 and TNFR2, then differentiate into macrophages within glomerular and tubulointerstitial compartments (137,138). The kidney also contains a network of tissue-resident macrophages that contribute to local immune responses.

Macrophage infiltration correlates strongly with disease severity and progression to ESRD. Transcriptomic studies show robust type I IFN and inflammatory activation in renal macrophages, which shift from patrolling cells to proinflammatory macrophages

and antigen-presenting cells. These cells secrete complement components, exhibit impaired efferocytosis, and thereby exacerbate tissue injury (139–141).

Macrophages span an M1–M2 activation spectrum (142–144): M1 macrophages are proinflammatory, while M2 subsets participate in regulation, repair, or fibrosis. In LN, M1 polarization—driven partly by the S1P/S1PR1 axis—amplifies renal inflammation and fibrotic remodeling (145). CD163⁺ M2c-like macrophages are the dominant subset in many LN biopsies, whereas M2a macrophages have been associated with disease progression (146,147). Consistently, urinary CD163, a marker of M2c-like cells, is elevated in active LN and correlates with activity, prognosis, fibrosis, and progression to kidney failure (148–150).

Monocyte recruitment is further promoted by MCP-1 (CCL2), a key mediator and promising biomarker of early renal inflammation and declining renal function (151). Experimental models show that macrophage depletion or NF-κB inhibition in myeloid cells reduces renal macrophage infiltration, inflammatory cytokines, and overall kidney injury, underscoring their pathogenic role (152,153).

Lymphocytes:

T and B lymphocytes accumulate selectively in LN kidneys and participate in local immune responses and chronic inflammation. Infiltrating CD4⁺ and CD8⁺ T cells contribute to tissue damage and progression to kidney failure (154,155). CD8⁺ T cells are particularly abundant in periglomerular infiltrates, and their density correlates with higher activity indices and serum creatinine, predicting poor outcomes in class III/IV LN (156).

Among CD4⁺ cells, T follicular helper (TFH) cells are especially pathogenic. An increased TFH:Treg ratio is observed in active and proliferative LN (157). TFH cells are frequently detected in biopsy specimens and associate with marked tubulointerstitial inflammation, higher creatinine, and lower eGFR (158). Their infiltration is linked to PD-L1 and IL-4 expression, which favors TFH2 differentiation and promotes B-cell activation (159).

Double-negative T cells (DNTs), lacking both CD4 and CD8, are expanded in active SLE and produce high levels of IL-17. The Th17–IL-17 axis fosters kidney inflammation by inducing cytokine production, epithelial-to-mesenchymal transition, and leukocyte recruitment (160,161). In contrast, Sm-specific Tregs show protective effects in experimental LN by suppressing pro-inflammatory responses and delaying disease progression (162).

B cells in LN display mitochondrial dysfunction and abnormal oxidative phosphorylation, enhancing antibody production and sustaining autoimmunity (163,164). Multiplex confocal microscopy has shown large periglomerular aggregates of B cells and TFH cells in human LN kidneys (165). A distinct subset of age-associated B cells (ABCs) accumulates in SLE, correlates with anti-chromatin antibodies and disease activity (166–169), and scRNA-seq has revealed local ABC-like activation within LN kidneys (170).

Plasma cell infiltration in the renal medulla has been associated with greater disease severity, although it does not directly correlate with tubulointerstitial IC deposition, suggesting additional antibody-independent mechanisms (171). Formation of tertiary lymphoid structures (TLS) in the interstitium, driven in part by BAFF, provides local niches for antigen presentation, B-cell maturation, and antibody production, thereby amplifying tubulointerstitial inflammation and clinical severity (172).

Dendritic cells (DCs):

Dendritic cells (DCs), particularly plasmacytoid DCs (pDCs), play a central role in LN by producing type I interferons (IFN-I) in response to nucleic acid-containing immune complexes. Peripheral DC profiles correlate with disease severity: severe LN is associated with reduced pDCs, whereas milder disease shows increased myeloid DCs with heightened costimulatory activity. Through endosomal TLRs, pDCs sense immune complexes and release IFN- α and TNF- α , amplifying inflammation (173).

A novel subset, CD163⁺ DCs, is enriched in LN kidneys. These cells localize in periglomerular regions near CD3⁺ T cells, promoting inflammation by enhancing T-cell activation and skewing responses toward Th1 and Th17 phenotypes (174). CD163⁺ DCs express high levels of Fc γ receptors, facilitating immune complex uptake and antigen presentation, and secrete cytokines such as IL-12 and IL-6, sustaining intrarenal inflammation. Injured tubular epithelial cells (TECs) likely recruit these DCs, highlighting the interplay between resident and infiltrating cells.

CD163⁺ DCs also contribute to tertiary lymphoid structure (TLS) formation—sites of localized immune activation and antibody production. Their presence correlates with disease activity and histologic damage, underscoring their potential as a therapeutic target in LN (174).

Natural killers (NK) cells:

In SLE, especially in LN, circulating NK cells are reduced and functionally impaired, with diminished cytotoxicity (175). An increased proportion of Ki67⁺ (proliferating) NK cells has been associated with disease severity and active nephritis (176). In kidney tissue, NK cells and NK-like T cells (CD56⁺) are mainly found in the interstitium and peri-glomerular regions, and only rarely within glomeruli (177).

The physiological role of NK cells in healthy kidney and their exact function in LN remain incompletely defined. Kidney-infiltrating NK cells have been described in lupus-prone mice, and scRNA-seq of human LN kidneys has identified at least two NK populations, including a cytotoxically active subset and a more tissue-adapted renal subset. Their potential contribution to cytotoxic injury, cytokine production, and cross-talk with myeloid and T cells is an area of ongoing investigation.

1.1.2.4.4. Role of kidney resident cells

Kidney-resident cells are now recognized as active contributors to LN pathogenesis rather than passive targets. Tubular epithelial cells (TECs), podocytes, mesangial cells

(MCs), and glomerular endothelial cells (GECs) respond to immune complexes and inflammatory cytokines, amplifying renal inflammation, fibrosis, and tissue remodeling (Figure 3).

Tubular epithelial cells (TECs):

Tubular epithelial cells (TECs) play an active role in the tubulointerstitial pathology of LN. They produce cytokines, chemokines, and fibrogenic molecules and engage in bidirectional interactions with infiltrating immune cells. Proximal TECs can express antigen-presentation machinery, enabling them to activate and proliferate CD4⁺ T cells. TECs also contribute to the formation of tertiary lymphoid structures (TLS), sites of localized immune activation in LN. TEC dysfunction disrupts the tubular barrier, facilitating immune cell infiltration and amplifying tissue injury (178).

TECs further sustain inflammation by secreting chemokines such as CXCL12 and CCL20, which recruit lymphocytes and other leukocytes into the kidney. They express costimulatory molecules including B7-H4, enhancing T-cell activation and promoting persistent intrarenal inflammation. Binding of anti-dsDNA antibodies to TECs triggers the release of inflammatory mediators, including type I interferons (IFN-I) and BAFF, thereby exacerbating immune activation and supporting TLS development (179).

Compared with the wealth of data on infiltrating immune cells, our understanding of resident cell contributions—such as those of TECs, remains limited and is based largely on isolated studies that require broader validation. Nonetheless, accumulating evidence suggests that TEC-derived inflammatory and profibrotic signals are likely key amplifiers of kidney injury in LN.

Podocytes:

Podocytes, specialized epithelial cells in the glomerulus, are crucial for maintaining the integrity of the glomerular filtration barrier. In LN, podocytes are directly damaged by circulating autoantibodies, such as those targeting dsDNA, which exacerbate glomerular inflammation and proteinuria. In addition to their structural role, injured podocytes activate the innate immune system by expressing TLRs and releasing pro-inflammatory cytokines. These changes lead to further activation of the immune response and enhance kidney damage (180,181).

Podocytes also participate in crescent formation, a hallmark of severe LN, by interacting with parietal epithelial cells in the Bowman's capsule. This interaction contributes to the expansion of the glomerular crescent and the development of fibrosis in the kidney (180).

Mesangial cells (MCs):

Mesangial cells (MCs), located within the glomerulus, play a central role in LN pathogenesis by responding early to immune complex (IC) deposition. Upon stimulation by lupus-relevant signals, MCs proliferate and produce a broad range of proinflammatory and profibrotic mediators, promoting leukocyte recruitment,

activation, and extracellular matrix remodeling. Of myeloid origin, MCs also exhibit antigen-presenting functions, modulating CD4⁺ T-cell activation and contributing to local immune amplification. They express TLRs and can produce type I interferons, further intensifying intraglomerular inflammation (183).

MCs contribute to glomerular fibrosis through activation of key signaling pathways, including JAK/STAT, PI3K/AKT, and MAPK, which drive mesangial expansion and extracellular matrix deposition (182). In addition, MCs interact with infiltrating immune cells—particularly macrophages—via the CXCL12/dipeptidyl peptidase-4 axis, promoting MC proliferation and exacerbating glomerular inflammation (182).

It is important to note that much of our current understanding of MC biology in LN is derived from in vitro studies and murine models, highlighting the need for more comprehensive human tissue studies.

Glomerular endothelial cells (GECs):

Glomerular endothelial cells (GECs) line the glomerular capillaries and are essential for maintaining the filtration barrier. In LN, subendothelial immune complex deposition activates GECs, leading to endothelial dysfunction and impaired barrier integrity, which contributes to proteinuria. Activated GECs produce inflammatory mediators, including type I interferons (IFN-I) via TLR3 stimulation, thereby amplifying intraglomerular inflammation (183). Immune complex-mediated activation of GECs also disrupts the coagulation–fibrinolysis balance, promoting thrombosis and endothelial apoptosis, further exacerbating glomerular injury. In addition, GECs help recruit immune cells into the kidney and modulate local immune responses. They also communicate with podocytes—for example, via mTOR signaling—shaping glomerular inflammation and structural injury (184).

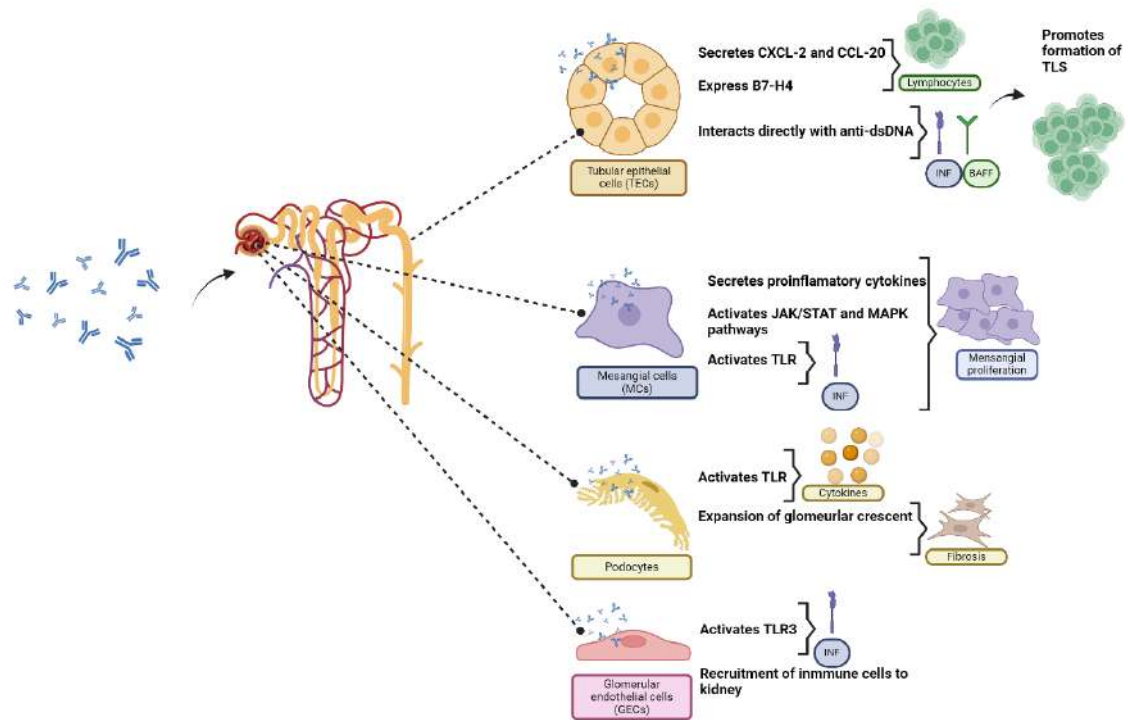


Figure 3. Role of kidney resident cells. Autoantibodies can directly target renal cells, which actively perpetuate inflammation and tissue injury in LN. Tubular cells recruit lymphocytes and contribute to tertiary lymphoid structure (TLS) formation. Mesangial cell activation triggers pro-inflammatory cytokine production and proliferation, while podocyte injury promotes cytokine release and fibrosis. Endothelial cells further amplify inflammation by producing cytokines and recruiting immune cells. (Created with BioRender.com). TLS, tertiary lymphoid structures; anti-dsDNA, anti-double-stranded DNA antibodies; TLR, Toll-like receptor; IFN, interferon; BAFF, B-cell-activating factor.

1.1.3 Diagnosis

1.1.3.1 Clinical presentation

LN can present with a broad clinical spectrum, ranging from silent renal involvement to rapidly progressive disease leading to kidney failure. Many patients remain asymptomatic, with normal urinalysis and kidney function, whereas others develop overt renal impairment, such as nephrotic syndrome (>3.5 g/day proteinuria), acute nephritic syndrome, or rapidly progressive renal failure. Typical urinary abnormalities include haematuria, leukocyturia, cellular casts, and mild proteinuria; the presence of acanthocytes and red blood cell casts is strongly suggestive of glomerular inflammation and active LN. Severe cases may exhibit nephrotic-range proteinuria or acute kidney injury with elevated serum creatinine and reduced GFR (185). The spectrum and prevalence of these manifestations are summarized in **Table 1**.

According to the 1982 ACR classification criteria, renal involvement is defined by persistent proteinuria (>0.5 g/day or $\geq 3+$ on dipstick) or the presence of cellular casts (red blood cell, haemoglobin, granular, tubular, or mixed) (186). The 2012 SLICC criteria

similarly define renal disease as a protein–creatinine ratio or 24-hour protein excretion ≥ 0.5 g/day, or red blood cell casts in the urine sediment (187).

Table 1: Clinical manifestations in patients with LN (188)

Manifestation	Prevalence (%)
Proteinuria	100
Nephrotic syndrome	45–65
Granular casts	30
Hematic casts	10–30
Microscopic haematuria	33–80
Macroscopic haematuria	1–5
Impaired renal function	40–80
Rapidly progressive impairment	15–30
Hypertension	15–50
Hyperkalaemia	15
Tubular abnormalities	60–80

The value of renal biopsy in diagnosing LN is underscored in the 2019 EULAR/ACR classification criteria, in which positive ANA together with biopsy-proven proliferative LN (class III or IV) is sufficient for SLE classification (189).

In recent decades, LN has tended to present with milder clinical features, likely due to earlier diagnosis and more frequent monitoring. Consequently, nephritic syndrome and rapidly progressive forms are now less common. Improved surveillance—particularly regular assessment of renal function and proteinuria—allows earlier detection of LN, including silent cases, and helps prevent progression to irreversible kidney damage (190).

1.1.3.2 Disease biomarkers in clinical practice

1.1.3.2.1 Serum biomarkers

Serum biomarkers are crucial for diagnosing and monitoring LN, providing insights into disease activity, prognosis, and treatment response.

a. Traditional markers

-Glomerular filtration rate (GFR): GFR is a central indicator of kidney function in LN. Declining GFR reflects injury from inflammation, glomerulosclerosis, or tubulointerstitial damage. Serial monitoring helps assess treatment response and identify patients at risk of CKD or ESRD, making GFR indispensable for risk stratification and long-term follow-up (191).

-Serum creatinine: Serum creatinine is routinely used to evaluate renal impairment but may fail to detect early or mild injury. Therefore, it is interpreted

alongside other markers—particularly proteinuria and the urine protein-to-creatinine ratio—to more accurately assess renal involvement in SLE (191).

b. Autoantibodies

Anti-dsDNA and anti-C1q antibodies are the most widely used autoantibody biomarkers for LN and are recommended in clinical guidelines.

Anti-dsDNA antibodies are the best-studied and correlate with renal disease activity, especially in proliferative LN. Rising titres may precede flares, but their sensitivity and specificity are limited, as not all patients with high levels develop nephritis (193).

Anti-C1q antibodies—directed against the first component of the classical complement pathway—show high specificity for LN and help distinguish it from other nephritides (192). They are useful for identifying active LN, and studies have shown that anti-C1q levels can predict renal flares months in advance. Combined measurement of anti-C1q and anti-dsDNA improves prediction of poor renal outcomes (193). Although not superior to anti-dsDNA, anti-C1q provides valuable complementary information.

Emerging biomarkers include anti- α -enolase (anti-ENO1) antibodies, which show good discrimination between LN and non-renal SLE (AUC 0.81), and anti-CRP antibodies, which correlate with LN activity and poor treatment response (16,194,195).

Despite ongoing research, only anti-dsDNA, anti-C1q, and antiphospholipid antibodies are routinely used in clinical practice. Antiphospholipid antibodies are particularly relevant when evaluating renal thrombotic microangiopathy and can inform assessment of LN severity (6,8,10,196).

c. Complement

The complement system is a key mediator of inflammation in LN. Complement activation contributes to immune complex formation and drives subsequent renal injury. Reduced serum **C3** and **C4** levels are classic indicators of active disease and increased immune complex deposition.

In LN, **low C3** correlates strongly with renal flares and is a valuable marker of activity, while **C4**, although less specific, also reflects ongoing complement activation. Increasing attention has been given to complement **split products**, such as **C3dg** and **C4d**, which may provide more precise information on intrarenal complement activity. Elevated **C4d** is associated with more severe renal involvement and worse prognosis. Notably, **C4d deposition** in peritubular capillaries serves as a key marker of kidney injury and predicts adverse outcomes in LN (130,131).

1.1.3.2.2 Urine biomarkers

Urinary biomarkers are increasingly recognized as valuable tools for diagnosing and monitoring LN. Their major advantage is non-invasiveness, allowing more frequent assessment than serum markers or renal biopsies.

Urinalysis remains a simple but informative screening test. Common abnormalities, microscopic hematuria (>5 RBCs/HPF), granular or cellular casts, and occasional macroscopic hematuria, often reflect active renal inflammation and may occur even without significant proteinuria. In patients with established LN and clinical renal involvement, proteinuria is universal, with nephrotic-range levels present in approximately 50% of cases (197).

Although 24-hour urine collection is the gold standard for quantifying proteinuria, it is impractical for routine use. The protein-to-creatinine ratio (PCR) is widely adopted due to its strong correlation with 24-hour measurements. Proteinuria >0.5 g/day is an indication for renal biopsy in SLE (198,199). Proteinuria also serves as the current gold-standard marker for monitoring LN, treatment response, and long-term prognosis. Post-treatment proteinuria levels are robust predictors of renal outcomes (200–202). However, proteinuria has important limitations: ESKD, nephrotic syndrome, and urinary tract infections can confound interpretation (203). Moreover, proteinuria may persist long after inflammation has resolved—normalizing in fewer than 50% of patients within two years (204). Persistent proteinuria often reflects chronic structural damage, not ongoing activity; in one study, 62% of patients with complete histologic remission still showed clinical proteinuria (205), underscoring the challenge of distinguishing activity from damage. Because of these limitations, there is strong interest in developing novel urinary biomarkers that more accurately reflect LN activity, severity, and treatment response.

1.1.3.2.3. Novel biomarkers:

1.1.3.2.3.1 Serum:

Cytokines:

Cytokines are key regulators of the immune response and play a central role in LN pathogenesis. Pro-inflammatory cytokines such as IFN- γ , IL-1 β , IL-6, IL-8, and IL-17A are upregulated during flares and drive immune activation and renal inflammation. Elevated IFN- γ and related cytokines are particularly associated with severe, proliferative LN (103,206). Among the most studied is BAFF, which promotes B-cell activation and autoantibody production. Serum BAFF levels correlate with LN activity, and BAFF inhibition with belimumab has shown clinical benefit in active LN (106). Soluble APRIL (sAPRIL) is also increased in active disease and may help predict treatment failure (1). Additional cytokines, including IL-10 and TRAF6, have been linked to LN activity and treatment response (119,207).

Despite their relevance in research, cytokine measurements are not yet used routinely in clinical practice for LN diagnosis or monitoring.

1.1.3.2.3.2 Urinary

Cytokines:

Urinary cytokines, such as MCP-1 (Monocyte Chemoattractant Protein-1), IL-17, and TGF- β 1, have shown strong potential as LN. uMCP-1 is elevated in active LN, correlates with disease activity, and can predict renal flares 2-4 months in advance. A meta-analysis reported 89% sensitivity and 63% specificity for distinguishing active from inactive LN (107,208). Urine IL-17 and TGF- β 1 are also emerging as potential biomarkers for LN. Increased levels have been associated with severe LN and may provide useful information about the disease's progression, with AUC values of 71.70% and 66.50%, respectively (209). These biomarkers may help to distinguish between severe and mild forms of LN, aiding in the assessment of disease severity and the tailoring of treatment strategies.

Cell adhesion molecules (CAMs):

Urinary CAMs, including VCAM-1, ALCAM, and ICAM-1, reflect leukocyte recruitment during inflammation and correlate with LN activity. These markers discriminate active from inactive LN and from non-renal SLE, with consistently high AUC values (151). uVCAM-1 is particularly associated with proliferative LN, which carries worse renal outcomes (110,131,208). Combining urinary biomarkers (e.g., uVCAM-1, uCystatin C, and uKIM-1) improves diagnostic performance (AUC 0.80) (206).

Other urinary proteins:

Urine biomarkers such as NGAL (neutrophil gelatinase-associated lipocalin), CD163, and epidermal growth factor (EGF) have shown potential in monitoring LN. NGAL levels in urine have been associated with treatment response, and elevated levels can predict renal flare, particularly in the early stages of the disease (76, 80). uCD163, a protein expressed on monocytes and macrophages, demonstrates an AUC of 0.998 in distinguishing active from inactive LN, indicating its potential as a highly reliable biomarker of disease activity (174).

Urinary epidermal growth factor (EGF) levels are reduced in patients with LN and correlate with poor renal outcomes, including proteinuria and elevated serum creatinine levels. These levels are useful for assessing the risk of progression to ESRD (209).

In animal models, urinary NRP-1 > 34.40 ng/mL predicted favourable renal response with 100% sensitivity and 88% specificity, highlighting its potential as a marker of histologic recovery (210).

MicroRNAs:

MicroRNAs (miRNAs) have been identified as potential biomarkers in LN, given their stability in body fluids and their role in regulating gene expression. Several miRNAs, such as miR-146a, miR-204, and miR-30c, have been found to be associated with LN and its specific pathways, including nucleic acid processes and inflammation. Urinary levels of

miR-146a are reduced in patients with active LN and correlate with disease activity, making it a potential marker for predicting renal flares and ESRD progression (211)

Other miRNAs, such as miR-29c, are also promising for their ability to predict chronicity in LN. In fact, exosomal miR-29c levels negatively correlate with the histological chronicity index and glomerular sclerosis, providing a potential non-invasive tool to assess the severity of kidney damage (200)

A recent meta-analysis highlighted four urinary miRNAs encapsulated within extracellular vesicles (EVs) that are linked to various pathways implicated in LN (212). Notably, baseline levels of miR-146a have been associated with the presence of albuminuria and the occurrence of renal flares (213), while miR-135b appears to serve as a biomarker for identifying individuals who respond to immunosuppressive (IS) therapy (186).

1.1.3.3 Genetics

Genetic risk scores combining HLA and non-HLA loci have been associated with the onset and severity of LN. High polygenic risk scores (PRS) for SLE correlate with earlier disease onset and more severe nephritis (214). Likewise, a weighted genetic risk score based on 112 SLE-associated SNPs and HLA haplotypes independently predicts LN development and anti-Sm antibody production (215), suggesting that SLE susceptibility loci may contribute more strongly to proliferative than to non-proliferative LN (216). Although promising, current models capture only part of the genetic contribution to LN. A more comprehensive gene-based risk score could help identify SLE patients at highest risk for nephritis and better predict long-term outcomes.

1.1.3.4 Renal biopsy

Renal biopsy remains the most reliable method for identifying the pathological changes underlying clinical manifestations such as proteinuria or urinary sediment abnormalities. It confirms the diagnosis of LN and determines the histological class. Biopsies are usually performed percutaneously once bleeding risks are addressed. Evaluation should include light microscopy, immunofluorescence, and electron microscopy. An adequate specimen typically contains at least ten glomeruli. The main indications for renal biopsy are summarized in Table 2.

Table 2: Indications for Kidney Biopsy in LN (1)

Indication	
Persistent proteinuria (≥ 0.5 g/day)	Particularly when associated with haematuria and/or cellular casts in the urinary sediment.
Persistent isolated haematuria or pyuria	After ruling out other potential causes, such as infections.
Unexplained chronic kidney disease (CKD)	Despite normal findings in the urinary sediment.

1.1.3.4.1. Histological Classification

The introduction of renal biopsy in the 1950s and immunofluorescence in the 1960s greatly advanced the understanding of LN pathology. The first World Health Organization (WHO) classification appeared in 1974 (217), focusing primarily on glomerular lesions. It was updated in 1982 by the International Study of Kidney Disease in Children, which introduced subdivisions of classes III and IV into active, chronic, or mixed forms (218), and again revised in 1995 (219).

In 2003, the International Society of Nephrology/Renal Pathology Society (ISN/RPS) proposed a new classification to address ambiguities in the WHO system and incorporate accumulated knowledge (220). The ISN/RPS classification, endorsed by ACR and EULAR/ERA-EDTA (220,221) remains the recommended system (Table 3) (222,223). It includes activity and chronicity indices (Table 4), along with assessment of tubulointerstitial and vascular pathology. Higher activity and chronicity scores correlate with poorer renal outcomes (15).

The current histological classes of LN are summarized in **Table 3**

Table 3: Lupus Nephritis Classification According to ISN/RPS (2003)

Class	Description
Class I: Minimal Mesangial Lupus Nephritis	Normal glomeruli under light microscopy, with minimal mesangial deposits observed through immunofluorescence (IF).
Class II: Mesangial Proliferative Lupus Nephritis	Mild mesangial hypercellularity and expansion in light microscopy, with mesangial deposits detected by IF. Subepithelial or subendothelial deposits may also be observed through IF or electron microscopy.
Class III: Focal Lupus Nephritis	Lesions affecting >50% of glomeruli, with endocapillary or extracapillary involvement and subendothelial deposits, with or without mesangial alterations. Subclassified into: Class III (A): Active lesions (focal proliferative lupus nephritis). Class III (A/C): Active and chronic lesions (focal proliferative and sclerosing lupus nephritis). Class III (C): Chronic inactive lesions with scarring (focal sclerosing lupus nephritis).
Class IV: Diffuse Lupus Nephritis	Lesions affecting ≥50% of glomeruli, with diffuse subendothelial deposits, with or without mesangial involvement. Lesions may be segmental (S) or global (G) and subdivided into: Class IV-S (A): Active segmental lesions (diffuse segmental proliferative lupus nephritis). Class IV-G (A): Active global lesions (diffuse global proliferative lupus nephritis). Class IV-S (A/C): Active and chronic segmental lesions (diffuse segmental proliferative and sclerosing lupus nephritis). Class IV-G (A/C): Active and chronic global lesions (diffuse global proliferative and sclerosing lupus nephritis).

	Class IV-S (C): Chronic segmental lesions (diffuse segmental sclerosing lupus nephritis). Class IV-G (C): Chronic global lesions (diffuse global sclerosing lupus nephritis). Includes "wire loop" deposits characteristic of this class.
Class V: Membranous Lupus Nephritis	Thickening of the glomerular basement membrane with global or segmental immune deposits on the subepithelial side of the membrane. May include mesangial expansion and coexist with Class III or IV lesions. Advanced sclerosis may be present. Early stages resemble idiopathic membranous nephropathy.
Class VI: Advanced Sclerosing Lupus Nephritis	Involvement of more than 90% of glomeruli, with no residual activity.

Table 4. Indices for Activity and Chronicity in Lupus Nephritis (223).

Index	Category	Abnormalities	Scoring
Activity Index	Glomerular	- Cellular proliferation	0 = Absent, 1 = Mild, 2 = Moderate, 3 = Severe
		- Fibrinoid necrosis or karyorrhexis	
		- Cellular crescents	
		- Hyaline thrombi or wire loops	
		- Leukocyte infiltration	
	Tubulointerstitial	- Mononuclear cell infiltration	
Chronicity Index	Glomerular	- Glomerular sclerosis	0 = Absent, 1 = Mild, 2 = Moderate, 3 = Severe
		- Fibrous crescents	
	Tubulointerstitial	- Interstitial fibrosis	
		- Tubular atrophy	

The term *lupus nephritis* refers to immune complex–mediated kidney injury, typically marked by deposits of IgG, IgA, IgM, C1q, C3, and C4 on immunostaining. Kidney biopsy is particularly valuable for detecting silent LN (179) and for assessing the degree of inflammation and irreversible tissue damage, which is essential for guiding therapy. Biopsy also helps exclude or identify other conditions that can mimic LN, such as IgA nephropathy, diabetic nephropathy, antiphospholipid antibody–associated nephropathy (APLN), hypertensive nephrosclerosis, and thin basement membrane disease, thereby improving diagnostic accuracy (178,180,224,225).

1.1.3.4.2 Second Biopsies

Repeat kidney biopsy has gained importance in LN management. The most accepted indication is persistent or recurrent renal activity despite adequate therapy. A second, more debated indication is biopsy during clinical remission to guide immunosuppression

tapering, particularly when serological abnormalities persist. Repeat biopsy can reveal shifts in LN class, residual activity, or chronic damage not evident clinically (226).

The optimal timing remains uncertain: biopsies at 6–8 months may be too early to reflect full tissue response (227,228), whereas those performed ≥ 2 years later may miss ongoing activity requiring intervention (229). Active lesions often decline with treatment but may persist for years (230). In a prospective study, histologic activity during clinical remission predicted relapse after treatment withdrawal, with an NIH Activity Index >2 associated with higher flare risk (231).

Despite these insights, repeat biopsies are not routine due to their invasive nature and potential complications.

1.1.4 Treatment

Renal response in LN should be monitored according to the 2019 EULAR/ERA-EDTA targets, which include a $\geq 25\%$ reduction in proteinuria at 3 months, $\geq 50\%$ at 6 months, and proteinuria <500 – 700 mg/day by 12 months, while maintaining GFR within 10% of baseline (232). Standard induction therapy consists of high-dose glucocorticoids (GCs) combined with either cyclophosphamide (CYC) or mycophenolate mofetil (MMF), followed by maintenance therapy with low-dose GCs plus MMF or azathioprine (AZA). Rituximab (RTX) is mainly used as rescue therapy (232).

Recent LN treatment updates have been issued by EULAR, KDIGO, and the ACR. EULAR's recommended treatment approach is summarized in Figure 4.

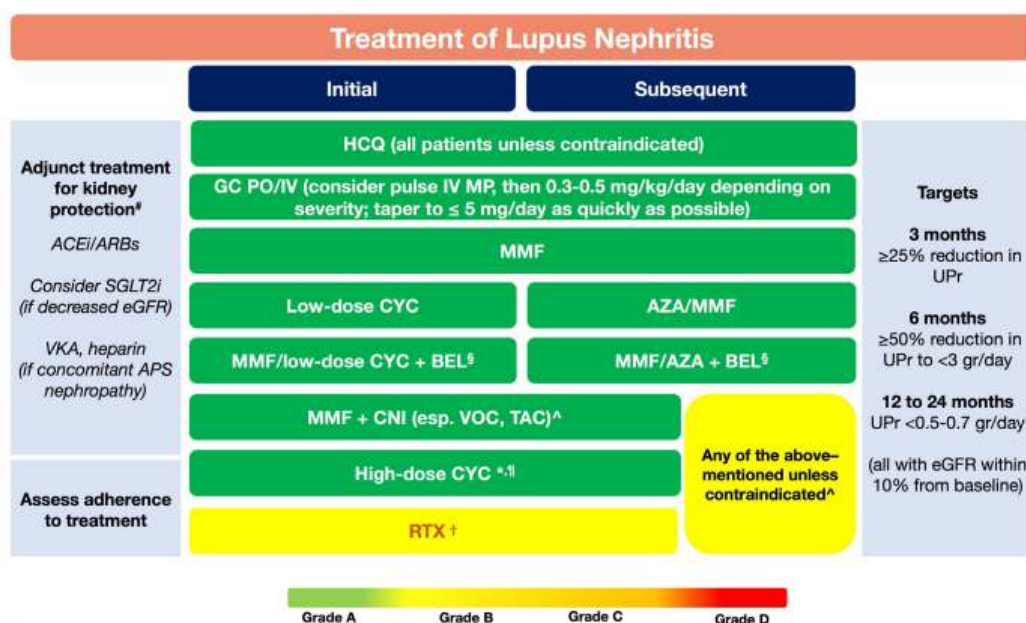


Figure 4. EULAR 2023 Guidelines for the Treatment of Lupus Nephritis. Adapted from Fanouriakis, A., et al. (122).

Conventional regimens remain effective with acceptable toxicity, but 30–40% of patients still develop renal or extra-renal flares, and long-term progression to ESRD (10–30%) has changed little over decades. This has driven the development of new therapies. Since 2020, the FDA has approved belimumab (BEL) and voclosporin (VOC) for induction therapy. When added to MMF/MPA and glucocorticoids, they form modern “triple therapy” regimens that expand on traditional dual therapy. Step-up (dual → triple) and step-down (triple → simplified) strategies are emerging and support a shift away from the classic induction–maintenance model toward more flexible, continuous combination therapy (233,234).

Belimumab, an anti-BAFF monoclonal antibody, was approved for LN in 2020 based on the BLISS-LN trial, which enrolled 448 patients with class III–V LN. BEL improved renal response rates, reduced steroid exposure, and lowered the risk of renal events or death (236,237). It may be preferred in severe LN with reduced kidney function or frequent flares, while VOC may be more effective when proteinuria is high (>3 g/day) (235,238). Voclosporin, a next-generation calcineurin inhibitor, offers improved pharmacokinetics and fewer metabolic side effects than cyclosporine or tacrolimus, with no intrinsic nephrotoxicity—though transient GFR reductions and hypertension may occur (239,240). In the AURORA 1 trial, VOC + MMF achieved complete renal response in 41% vs 23% with MMF alone and rapidly reduced proteinuria (<500 mg/day by 6 months). Steroids were tapered to 2.5 mg/day by week 16 (241). AURORA 2 showed stable renal function over 3 years without CNI-related pathology (239). VOC is reshaping LN therapy by broadening options beyond MMF- and steroid-based regimens.

Beyond triple therapy, KDIGO (242) and recent studies have proposed sequential regimens (switching between BEL and VOC) and four-drug induction (BEL + VOC + MMF/MPA + GCs), though these require further validation (243).

For maintenance, therapy should follow the induction regimen. MMF used during induction (alone or with BEL/VOC) should be continued for ≥3 years. After low-dose CYC induction, MMF or AZA is recommended, continuing BEL if used initially. Long-term immunosuppression of at least 3 years remains standard for proliferative LN (232).

1.1.5 Prognosis

Although the prognosis of LN has improved markedly over recent decades, it remains a leading cause of morbidity and mortality in SLE. Up to 70% of patients do not achieve complete renal remission within six months of standard induction therapy, and 10–30% progress to end-stage renal disease (ESRD) within ten years—outcomes that significantly increase cardiovascular risk, susceptibility to infections, and overall mortality (244). Prognosis is shaped by several factors, including histological class, the extent of chronic damage at diagnosis, treatment adherence, and comorbidities such as hypertension or diabetes. Persistent proteinuria, declining GFR, and recurrent renal flares are strong predictors of poor long-term outcomes. Preventing relapses, preserving renal function, and minimizing treatment toxicity—particularly from prolonged glucocorticoid

exposure—remain major clinical challenges, as GC-related adverse effects contribute substantially to infection rates and mortality (245–247).

Newer therapies such as belimumab and voclosporin show promise in improving remission rates and reducing glucocorticoid burden, although their long-term safety and effectiveness are still being evaluated (241,249). The marked heterogeneity of LN further underscores the need for improved risk stratification tools (250,251). Emerging genomic, transcriptomic, and urinary biomarkers, together with artificial intelligence–based predictive models, are expected to refine prognosis and support a more personalized therapeutic approach (252–256). Ultimately, optimizing long-term renal outcomes in LN will require early detection, individualized treatment strategies, careful management of comorbidities, and accurate identification of patients at highest risk for progression (189).

1.2 Multiomics studies in LN

Recent advances in multiomics technologies applied to human kidney tissue are providing critical mechanistic insights into the pathways driving cellular injury and kidney loss. These approaches enable the identification of cell-specific and region-specific molecules that regulate cell fate, activation state, and intercellular communication—information essential for understanding lupus-related kidney damage. The marked clinical heterogeneity of SLE complicates diagnosis and management. RNA sequencing and other omics platforms have revealed multiple disease-associated molecular signatures, but complex disorders like SLE arise from interactions among numerous genes, proteins, and metabolites. Thus, single biomarkers or isolated pathways rarely capture the full biological landscape.

Integrative multiomics analysis is therefore key to unraveling the molecular networks underlying SLE and LN. Combining transcriptomic, proteomic, and metabolomic data—supported by pathway enrichment tools such as KEGG—offers a more comprehensive view of disease mechanisms (257). In parallel, single-cell RNA sequencing (scRNA-seq) has revolutionized our ability to profile cellular composition, differentiation, and intercellular communication (258), providing high-resolution insights into organ-specific immune dysregulation in LN.

1.2.1 RNA sequencing technologies

a) Blood and PBMCs

Bulk RNA sequencing and scRNA-seq of blood and PBMCs in lupus nephritis have revealed disease-specific transcriptional signatures, notably the upregulation of type I interferon-stimulated genes (ISGs) across multiple immune cell types, including monocytes, T cells, B cells, and plasma cells (259). Cross-ethnic comparisons revealed notable differences: plasmablast, cell-cycle, and erythropoiesis modules were more enriched in African American patients, whereas neutrophil, myeloid, and inflammation

modules predominated in Hispanic and Caucasian patients. IFN-, plasmablast-, and B-cell-related signatures appeared even in mild disease, while neutrophil/myeloid/inflammatory signatures were particularly enriched in patients with renal involvement, supporting a model of stepwise disease progression.

scRNA-seq has enabled the identification of expanded cytotoxic CD8⁺ T cell populations and dysfunctional regulatory CD4⁺ T cell clusters in patients with active lupus nephritis.^{[6][16-17]} Differential gene expression in PBMCs, such as TNFRSF17, correlates with renal involvement and proteinuria, and may serve as a therapeutic target (261). Additionally, circulating long non-coding RNAs (lncRNAs) and tRNA-derived small RNAs (tsRNAs) have emerged as promising noninvasive biomarkers for disease activity and flare prediction.

b) Kidney tissue

Bulk transcriptomic analyses of LN kidney biopsies have identified key pathways associated with inflammation, tissue injury, and treatment response. Across kidney diseases—including LN—reduced expression of epidermal growth factor (EGF) strongly correlates with lower eGFR, highlighting impaired tubular repair as a shared mechanism of progression (262,263). In LN-specific cohorts, unsupervised clustering of bulk RNA-seq data has revealed upregulation of genes involved in antigen presentation, T- and B-cell activation, and type I interferon (IFN) signaling, with these signatures closely associated with impaired renal function and increased proteinuria (158,264). Tubulointerstitial IFN activation is particularly linked to proteinuria, while adaptive immune effector infiltration correlates with tubular damage and reduced GFR. Serial biopsy studies further show divergent molecular trajectories with treatment: higher NCAM1 expression associates with complete response, whereas elevated IL1RAP and FCAR characterize non-response, supporting the idea that persistent innate and complement-driven inflammation underlies refractory disease (79,265).

Single-cell RNA sequencing (scRNA-seq) has expanded these findings by mapping the cellular diversity and activation states present in LN kidneys. In a landmark study, scRNA-seq identified 21 immune cell subsets (170), including multiple macrophage clusters, diverse T- and NK-cell populations (tissue-resident and cytotoxic CD8⁺ T cells, NK cells, TFH-like and regulatory T cells), plasmacytoid and conventional dendritic cells, and several naïve, memory, and antibody-secreting B-cell subsets. A proliferating cluster of T, NK, and myeloid cells reflected active immune cycling within the inflamed tissue. Th17 cells have been detected within LN kidneys, whereas exhausted CD8⁺ T-cell phenotypes—typical of chronic viral infections—appear largely absent, suggesting a predominance of effector and tissue-resident cytotoxic programs (269,270).

Data from the Accelerating Medicines Partnership (AMP) further demonstrated broad upregulation of interferon-stimulated genes (ISGs) across nearly all renal cell types, including tubular, endothelial, podocyte, and mesangial cells (170,271,272). Kidney ISG signatures strongly correlated with ISG expression in blood, pointing to a systemic type I IFN-driven process with local amplification by plasmacytoid dendritic cells and kidney-resident myeloid cells in response to nucleic acid-containing immune complexes and

HMGB1. Collectively, bulk and single-cell transcriptomic studies depict LN kidneys as sites of dense, heterogeneous immune infiltration interacting with activated resident parenchymal cells, embedded within a sustained type I IFN–dominant molecular environment. These insights highlight the complex cellular interactions driving LN pathogenesis and underscore the potential of transcriptomic profiling to refine prognosis and inform targeted therapeutic strategies.

Together, they provide a detailed view of the immune cell networks contributing to renal inflammation and tissue damage in LN (Fig. 5).

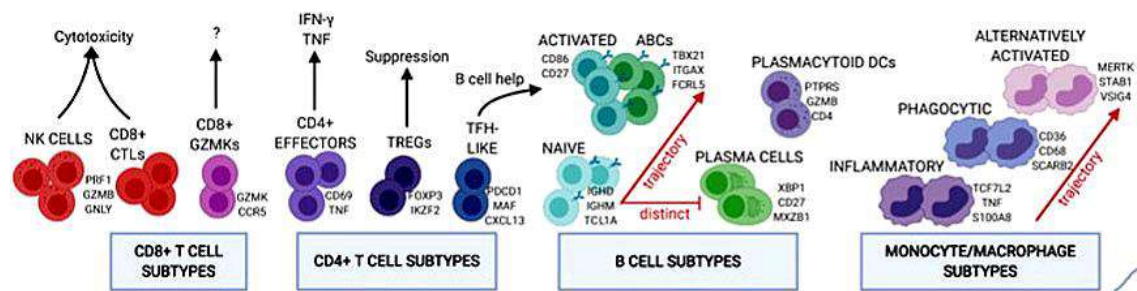


Figure 5. Immune cells subsets in LN. scRNA-seq of leukocytes in lupus LN kidney tissues has uncovered a diverse array of immune cell subtypes, each defined by distinct gene expression profiles. Adapted from Rao *et al.* (268) CCR5, C-C motif chemokine receptor 5; CXCL13, C-X-C motif chemokine ligand 13; FCRL5, Fc receptor like 5; FOXP3, forkhead box P3; GNLY, granulysin; GZMB, granzyme B; GZMK, granzyme K; IGHD, immunoglobulin heavy constant delta; IGHM, immunoglobulin heavy constant mu; IKZF2, IKAROS family zinc finger 2; ITGAX, integrin subunit alpha X; MAF, MAF bZIP transcription factor; MERTK, MER proto-oncogene, tyrosine kinase; PDCD1, programmed cell death 1; PRF1, perforin 1; S100A8, S100 calcium binding protein A8; SCARB2, scavenger receptor class B member 2; STAB1, stabilin 1; TCL1A, T cell leukemia/lymphoma 1A; PTPRS, protein tyrosine phosphatase receptor type S; TCF7L2, transcription factor 7 like 2; TBX21, T-box transcription factor 21; TNF, tumor necrosis factor; VSIG4, V-set and immunoglobulin domain containing 4.

c) Urine

Urine-based transcriptomics, including scRNA-seq and exosomal RNA profiling, have demonstrated that urinary immune cell gene expression closely mirrors intrarenal signatures, supporting urine as a surrogate for kidney biopsy. Urinary exosomal tsRNAs (e.g., tRF3-Ile-AAT-1, tiRNA5-Lys-CTT-1) and cytokines (e.g., CCL2, CXCL10) are elevated in active lupus nephritis and can discriminate LN from SLE without nephritis, offering a noninvasive approach for diagnosis and monitoring. Urine proteomics has identified IL-16 and other markers that reflect intrarenal disease activity and response to therapy. These findings underscore the potential of urine transcriptomics for dynamic, noninvasive assessment of LN (274).

1.2.2 Spatial transcriptomics

A key limitation of conventional RNA-seq is its inability to preserve the spatial organization of cells—an essential feature for understanding tissue physiology and disease (275,276). High-resolution spatial omics technologies have transformed this landscape by enabling molecular profiling directly within intact tissue architecture, integrating cellular localization with gene and protein expression (277–280). These methods overcome a long-standing challenge in biomedical research by allowing unbiased, simultaneous quantification of biomolecules while retaining their spatial context (281). Spatial omics now provides the ability to define both the cellular composition of tissues and the molecular mechanisms underlying tissue organization and pathological lesions.

Spatial transcriptomics (ST) has been instrumental in producing detailed cellular atlases for several organs, including the kidney (280), heart (283), lung (284), and brain (285). These techniques are broadly classified into two categories:

1. **Next-Generation Sequencing (NGS)-based approaches**, which encode positional information onto transcripts prior to sequencing (286,287).
2. **Imaging-based methods**, comprising in situ sequencing (288–290) or hybridization (291,292) techniques where transcripts are directly amplified or labeled within tissue sections.

In this project, we employed the GeoMx Digital Spatial Profiling (DSP) technology, a cutting-edge imaging-based method with several distinct features:

- **Hybridization and microscopy combination:** GeoMx DSP integrates methods from imaging-based technologies to provide precise spatial information (293,294).
- **Non-extraction-based RNA analysis (NEBT):** This ex-situ approach avoids RNA extraction, making it suitable for formalin-fixed, paraffin-embedded (FFPE) samples and tissues with degraded RNA. While NEBT offers high spatial resolution, it often targets a limited number of RNA transcripts (293,294).
- **Region of Interest (ROI) profiling:** Users can define tissue ROIs ranging from 20 to 300 cells, enabling transcriptomic profiling via photocleavable barcoded probes. These probes, after targeted hybridization, are selectively cleaved, extracted, and sequenced to map RNA within the predefined ROI (293,294).

Analysis of spatial transcriptomics data often requires integration with external datasets and prior knowledge. Approaches such as Multimodal Intersection Analysis (MIA) and Gene Set Enrichment Analysis (GSEA) characterize gene sets by comparing them with annotated pathways, enabling functional enrichment through resources like Gene Ontology, KEGG, and Hallmark databases (294–296).

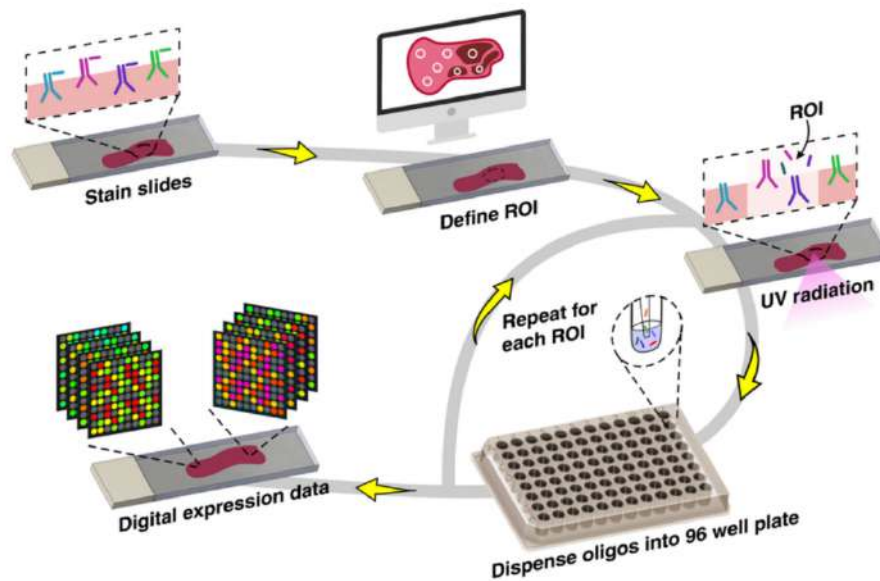


Figure 5. Workflow of the nanoString GeoMx digital spatial profiler (DSP). Schematic overview of the DSP workflow, including ROI selection on tissue sections, fluorescent labeling, UV-mediated release of indexed oligonucleotides, and digital quantification. This method enables multiplex molecular profiling while preserving tissue architecture. Adapted from NanoString Technologies (ResearchGate).

1.2.2.1 Applications to kidney research

The kidney has become a major focus of spatial transcriptomics (ST) research, with more than 30 studies applying ST technologies to investigate renal physiology and disease. This interest reflects the kidney's pronounced morphological heterogeneity—its distinct compartments (glomeruli, tubules, vessels, interstitium) make spatially resolved analysis essential for understanding renal pathology (282,297–303).

In glomerular disease models, Marshall et al. (301) used ST to identify structural alterations in the filtration barrier and blood flow regulatory system in diabetic kidney disease. Smith et al. (304) applied GeoMx DSP to examine spatial gene expression in collapsing glomerulopathy associated with HIV or SARS-CoV-2, while Clair et al. (305) used GeoMx to characterize distinct transcriptional signatures in Alport syndrome, focal segmental glomerulosclerosis, and membranous nephropathy.

1.2.2.2 Applications in LN

In lupus nephritis, Richoz et al. (306) used ST in a mouse model to demonstrate divergent roles of renal macrophage subsets: resident macrophages directed leukocyte recruitment, whereas monocyte-derived macrophages mediated immune complex uptake and antigen presentation. In LN, GeoMx DSP studies are not published. However, Danaher et al. recently used CosMx—an emerging platform integrating spatial profiling with single-cell resolution—to create the first spatial single-cell atlas of childhood-onset LN (cLN) (307). Eleven immune cell types were mapped, with macrophages constituting

the dominant population (73% in controls, 64% in cLN). Although total macrophage numbers were similar, cLN progression featured increased B-lineage cells (B cells, plasma cells) and plasmacytoid dendritic cells (pDCs). Myeloid cells localized to inflamed glomeruli, whereas B cells clustered in tubulointerstitial immune hotspots. Gene expression signatures varied by anatomical compartment, highlighting the spatial complexity of LN.

Spatial transcriptomics is transforming kidney research by linking molecular programs to specific anatomical niches, improving diagnostic precision, and revealing region-specific biomarkers often missed by bulk approaches.

In the present study, we apply GeoMx DSP to LN kidney tissue, profiling morphologically distinct regions of interest to uncover spatially resolved mechanisms of injury that remain obscured in conventional analyses.

2.HYPOTHESIS

We hypothesize that in patients with lupus nephritis (LN), the persistence of inflammatory and immune activation–related transcriptomic and proteomic signatures within distinct renal compartments—specifically CD45⁺ immune cells, PanCK⁺ tubular epithelial cells, CD31⁻ glomerular cells, and CD31⁺ endothelial cells—following treatment with mycophenolate mofetil (MMF) is associated with incomplete clinical response, ongoing histological activity despite apparent clinical remission, and therapeutic resistance.

Furthermore, we propose that characterizing immune pathway activation at the time of diagnostic kidney biopsy—and assessing how these pathways are modulated by standard-of-care therapy—will provide critical mechanistic insights into the limitations of current LN treatments. By performing longitudinal spatial transcriptomic and proteomic profiling of paired biopsies, we aim to identify both injury-related and resolution-associated pathways that may serve as therapeutic targets to improve long-term outcomes in LN.

3.OBJECTIVES

3.1 Main objective:

- To elucidate the molecular mechanisms underlying differential treatment responses in patients with lupus nephritis by employing spatial transcriptomic and proteomic analyses of distinct renal cell populations in kidney biopsies.

3.2 Secondary objectives:

1. Compare the transcriptomic and proteomic profiles of areas of interest (CD45⁺, PanCK⁺, CD31⁻, and CD31⁺) in initial renal biopsies between patients who achieve complete response and those who do not respond to treatment.
2. Identify gene and protein expression signatures, as well as signaling pathways that differ between response groups and determine which are associated with persistent inflammation and therapeutic resistance.
3. Evaluate changes in molecular profiles between the initial biopsy (Bx1) and the follow-up biopsy (Bx2) to determine the evolution of molecular signatures in relation to clinical and histological responses.
4. Correlate the molecular findings (transcriptomic/proteomic signatures) with clinical and laboratory parameters (such as SLEDAI, proteinuria, eGFR, and anti-dsDNA) to identify potential predictive biomarkers of treatment response in lupus nephritis.

4. MATERIALS AND METHODS

4.1 Patients

The SLE Unit at Vall d'Hebron Hospital, led by Dr. Josefina Cortés-Hernandez, is part of the Department of Rheumatology and serves as a nationally accredited center of excellence for systemic autoimmune diseases, recognized by the CSUR program. The unit manages a cohort of more than 900 patients and receives highly complex cases for specialized treatment and follow-up.

SLE patients undergoing clinically indicated renal biopsy were recruited from the Lupus Unit at Vall d'Hebrón Hospital between 2018 and 2023. The study was ethically approved by Vall d'Hebrón Hospital Ethics Committee and all participants provided written informed consent prior to inclusion (PR(AG)59/2022).

Inclusion and exclusion criteria are detailed below:

a. Inclusion criteria

- Age 18-59
- Fulfill the 2019 ACR/EULAR classification criteria for SLE (189)(see Figure 6).
- Biopsy-proven type III-IV proliferative glomerulonephritis with/without type V membranous
- Sufficient glomeruli (> 10 per section) in the biopsy
- Recent biopsy (≥2018) to preserve sample quality and reliability
- Signed informed consent

b. Exclusion criteria

- Evidence of thrombotic microangiopathy, vasculopathy, or podocytopathy in renal biopsies.
- Pathology slides unavailable, or with insufficient material for the project.
- Immunosuppressive treatment prior to biopsy
- Presence of other concomitant confounding pathologies (e.g. diabetes mellitus) or malignancies

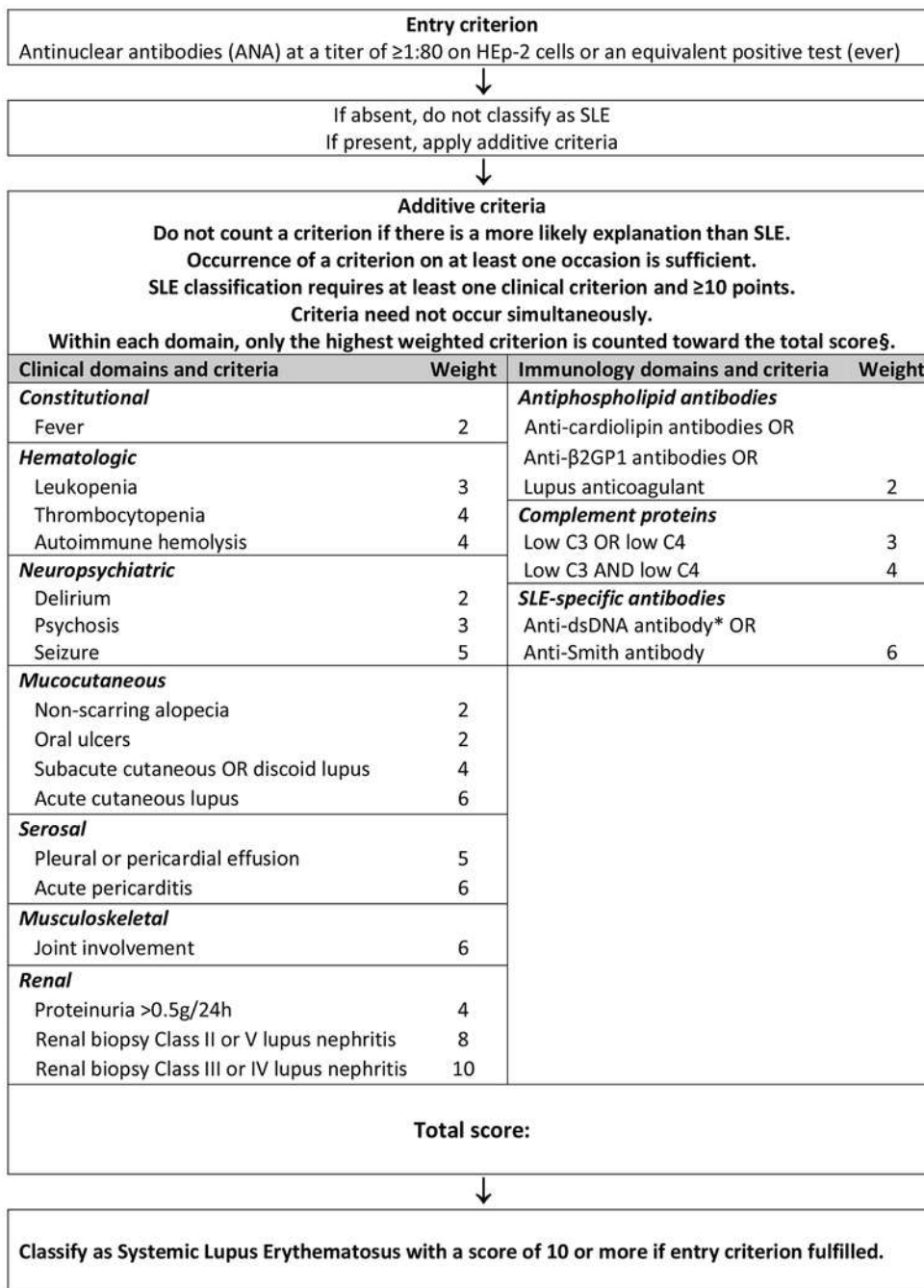


Figure 6. ACR/EULAR 2019 SLE criteria. Adapted from Aringer et al.(189)

All patients received intravenous methylprednisolone (500 mg for three days) followed by a tapering course of oral prednisone starting at 1 mg/kg/d, reaching 5 mg/day by week 8, and MMF as induction and maintenance therapy. All patients received ARBs (Angiotensin II Receptor Blockers). The same nephrologist and rheumatologist treated all patients.

Clinical responses were assessed according to the American College of Rheumatology (ACR) criteria and membranous response guidelines for proliferative and membranous lupus nephritis used in SLE clinical trials (308), using achieved levels of serum creatinine

concentration (SCr) and 24-hour urine protein following 12 months of treatment (Table 5). Overall, 11 patients achieved a complete response (CR), 3 patients achieved a partial response (PR) and 6 patients had non-response (NR) to standard induction therapy.

Table 5. Criteria for Assessing Treatment Response in Patients with Lupus Nephritis

Response Type	Proteinuria Criteria	SCr Criteria
Complete Response (CR)	Proteinuria < 0.5 g/d	Normalization of SCr
Partial Response (PR),	Reduction > 50% to < 3 g/d but > 0.5 g/d	Stable or improved SCr
No Response (NR)	Reduction < 50% or proteinuria > 3 g/d	Stable or improved SCr

Proteinuria was measured at the time of biopsy (Bx1) and 12 months post-treatment. Collected data included demographic, biochemical, hematological, and immunological parameters such as anti-dsDNA antibodies and complement levels. A second biopsy (Bx2) was performed in 8 patients (45%) within 12 months after Bx1 (median:12 months; range: 11-15 months). In patients who underwent serial biopsies, we performed a secondary classification of treatment response based on histological findings, categorizing them as either histological responders or non-responders. An AI of 0 at Biopsy 2 was considered to represent complete histologic remission (206)

4.2 Sample collection

4.2.1 Urine sample collection

Urine samples were collected from patients at two specific time points: prior to kidney biopsy and 12 months after the initiation of treatment. Immediately following collection, the samples were centrifuged at 3.900 rpm for 30 minutes at 4°C to remove cellular components and debris. The resulting supernatants were carefully aliquoted and stored at -80°C until further analysis.

4.2.2. FFPE tissues samples and immunohistochemistry

At the time of kidney biopsy, all patients provided informed consent permitting the use of residual needle-core biopsy specimens for research purposes, once their primary diagnostic evaluation had been completed. Renal tissue samples were embedded in formalin-fixed, paraffin-embedded (FFPE) blocks for future research applications or retrospective diagnostic assessment.

Conventional histological evaluation of renal biopsies was performed by two independent, experienced nephropathologists, each blinded to the other's assessments. The specimens were examined using both light microscopy and immunofluorescence techniques. Biopsies were classified according to the International Society of Nephrology/Renal Pathology Society (ISN/RPS) classification system and scored for activity index (AI) and chronicity index (CI). Following evaluation, the paraffin-embedded renal biopsy blocks were stored in the pathology department under stringent quality control conditions to ensure long-term preservation and integrity.

4.3 Spatial transcriptomic analysis

Each slide contained four tissue sections from archival formalin-fixed, paraffin-embedded (FFPE) kidney biopsy samples, arrayed on separate slides. The slides were prepared according to the manufacturer's guidelines (NanoString GeoMx DSP Manual Slide Preparation; MAN-10150-01). To minimize batch effects, three slides were prepared, each containing four biopsy specimens, equally distributed between responders and non-responders.

For FFPE blocks, 5 μm -thick tissue sections were mounted on Superfrost microscope slides (Fisher Scientific, Waltham, MA, USA) and baked at 60°C for 30 minutes to ensure that the tissue sections fit within the scanned area of the slide. The following day, the slides were stained with SYTO13 (NanoString, 121300303, FITC) for nuclear staining, CD45 (Novus, NBP2-3427, 2B11 + PD7/26) as an immune cell marker, CD31 (Abcam, ab215912, AF647) and PanCK (Novus, NBP2-33200, AF594) as cellular markers for characterization of kidney tissue morphology. The slides were then loaded into the GeoMx DSP instrument for scanning and selecting regions and areas of interest (ROIs and AOIs).

In situ hybridizations were performed with the GeoMx Human Whole Transcriptome Atlas Panel (WTA, 18,676 total targets) according to the manufacturer's instructions. Probes were added to each slide in a hybridization chamber, covered with a coverslip, and incubated at 37°C overnight. After incubation, the slides were washed to remove unbound probes and blocked in 200 μl of Buffer W, then incubated in a humidity chamber. Each slide was scanned using a 20X objective and default scan parameters. For each tissue section, six ROIs with 12 AOIs were selected (average area and nuclei count of 262.43 μm^2 and 187, respectively).

ROIs were meticulously selected based on evaluations conducted by the Vall d'Hebron renal pathology team, following a standardized and comprehensive assessment protocol. Only active glomerular and tubular regions, identified by specific histopathological criteria, were included in the analysis to focus on areas with ongoing inflammation and cellular activity. Regions exhibiting chronic damage features, including glomerulosclerosis, interstitial fibrosis, tubular atrophy, or fibrous crescents, were systematically excluded. Samples with a chronicity index (CI) equal to or greater than 2 were omitted to minimize confounding by irreversible tissue damage. This approach ensured that only zones of acute pathological activity were analyzed, optimizing the accuracy and relevance of subsequent spatial proteomics analyses.

In each ROI, areas of interest (AOIs) were selected based on immunofluorescence staining of CD45⁺ cells, CD31^{-/+} cells, or PanCK⁺ cells. After ROI placement, the GeoMx DSP instrument photocleaves the UV-cleavable barcoded linker of the bound RNA probes from each ROI, and the individual segmented areas are collected into separate wells in the DSP collection plate.

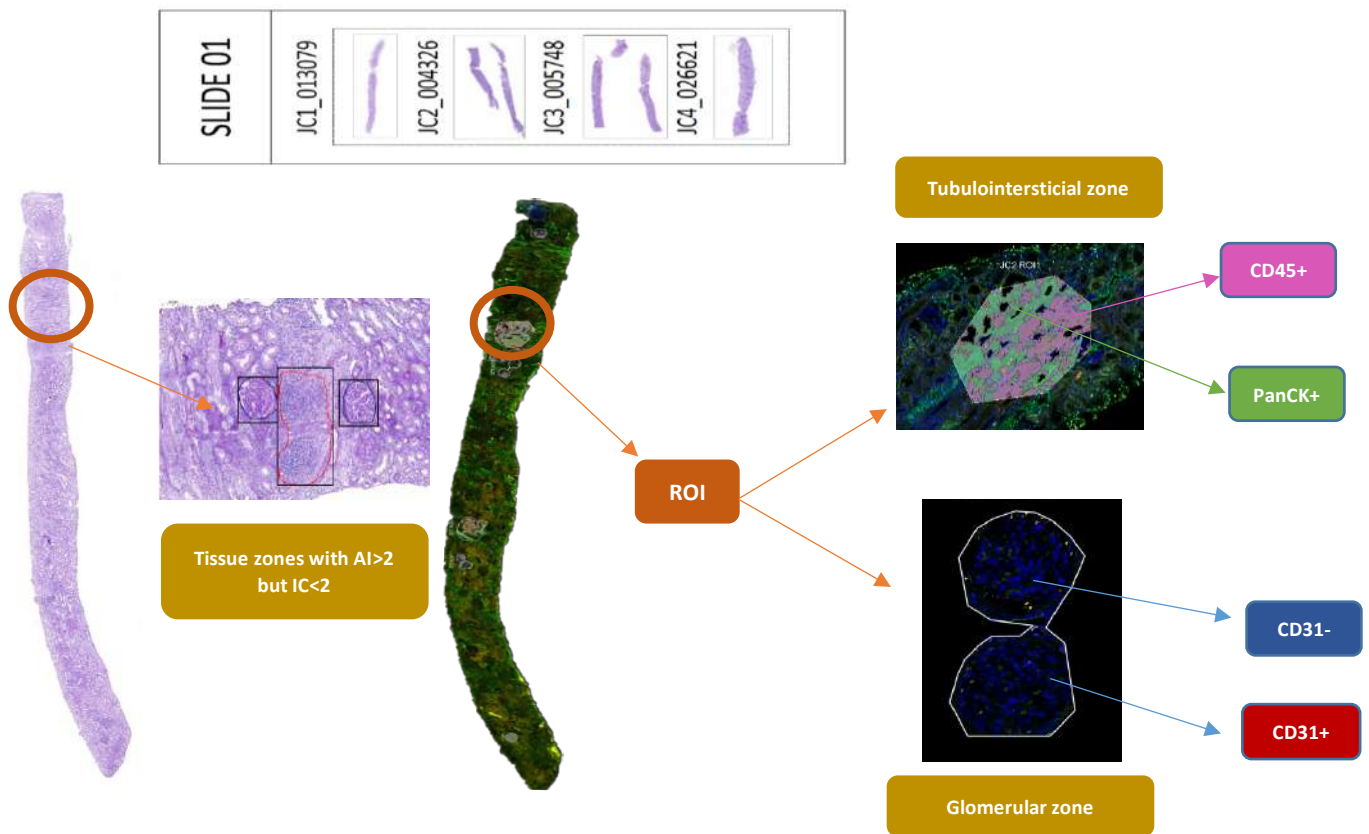


Figure 7. Tissue Characterization and ROI Selection in Kidney Biopsies Using GeoMx DSP. The diagram illustrates the workflow for analyzing kidney tissue sections from archival FFPE biopsies using the GeoMx Digital Spatial Profiler (DSP). Slides were stained with SYTO13 (blue) for nucleic acid visualization, CD45 (pink) as an immune cell marker, CD31⁻ (blue) and PanCK (green) to assess cellular and tissue morphology, and CD31⁺ (red) as an endothelial cell marker. Regions of interest (ROIs) were selected using the GeoMx DSP platform based on detailed histopathological evaluation by the renal pathology team at Vall d'Hebron.

4.3.1 Library preparation and generation of expression matrices

Total target counts per DSP collection plate for sequencing were calculated from the total samples areas (μm^2). For sequencing of whole transcriptome analysis (WTA) libraries, the target sequencing depth was 100 counts/ μm^2 . Sequencing libraries were generated by polymerase chain reaction (PCR) from the photo-released indexing oligos with ROI-specific Illumina adapter sequences and unique i5 and i7 sample indices were added. Each PCR reaction used 4 μl of indexing oligos, 4 μl of indexing primer mix and 2 μl of Nanostring 5X PCR Master Mix. Thermocycling conditions were 37°C for 30 min, 50°C for 10 min, 95°C for 3 min; 18 cycles of 95°C for 15 sec, 65°C for 1 min, 68°C for 30 sec; and 68°C 5 min. PCR reactions were pooled and purified twice using AMPure XP beads (Beckman Coulter, A63881) according to manufacturer's protocol. Pooled libraries were sequenced at 2x27 base pairs and with the dual-indexing workflow on an Illumina NovaSeq. Reads were trimmed, merged, and aligned to retrieve probe identity, and the unique molecular identifier of each read was used to remove PCR duplicates, converting reads to digital counts for each target within an individual ROI.

4.3.2 Analysis of spatial RNA sequencing data

Data analysis was provided by NanoString's Spatial Data Analysis Services Data first underwent a quality control assessment. Each ROI/ AOI was tested for raw sequencing reads, % aligned, % trimmed or % stitched sequencing reads. Segments with less than 1000 raw reads or below ~80% for one or more of these QC parameters were removed (11 were removed). Segments below ~50% sequencing saturation were not analyzed. To identify samples with high background noise (no-specific probe binding), we first calculated the limit of quantification (LOQ), which is 2 standard deviations above the geometric mean of the negative probes for each ROI/AOI. The percentage of genes detected above the LOQ value was then calculated and samples removed from the analysis if they fell below a 5% gene detection rate. This left 310 of the 321 ROI/AOI for downstream analysis. Gene counts were normalized using Q3 normalization. Uniform Manifold Approximation and Projection (UMAP) plots were generated with normalized expression data using the umap packages (v0.2.8.0; RRID:SCR_018217) under default settings. Coefficients of variation (CV) were calculated for each gene ($\text{CVg} = \text{SDg}/\text{meang}$). The genes with the highest 10% CVs were plotted as a heatmap using unsupervised hierarchical clustering based on Pearson distance. Differential gene expression between groups was performed using a Linear mixed effect model from the GeomxTools package (v3.1.1). Each model included a random intercept term for patient ID to account for the non-independence of multiple ROIs placed per tissue. Genes were considered significantly differentially expressed with p adjusted < 0.05 and $|\log_2 \text{fold change}| > 0.5$. They were visualized in volcano plots and violin plots using the ggplot2 package (v3.3.6; RRID:SCR_014601). We next performed cell type deconvolution with the spatialdecon Bioconductor/R package (309). Advances in mixed cell deconvolution enable quantification of cell types in spatial transcriptomic data (Danaher, P., Kim, Y., Nelson, B., Griswold, M., Yang, Z., Piazza, E., & Beechem, J. M. (2022). Advances in mixed cell deconvolution enable quantification of cell types in spatial transcriptomic data. *Nature communications*, 13(1), 385). We used the Human Cell Atlas kidney scRNAseq dataset [Stewart et al., (2019)] to estimate tissue-specific cell type abundances in our GeoMx

data. Pathway and gene set enrichment analysis (GSEA) was performed on each ROI using single sample GSEA from the Gene Set Variation Analysis (GSVA, doi: 10.18129/B9.bioc.GSVA, Bioconductor) package against the Kegg Brite database (<https://www.genome.jp/kegg/kegg1.html>). Data generated using the Nanostring GeoMx DSP platform are available from GEO under accession number GSE302584. Any other data generated in this study are available upon request from the corresponding author.

4.4 Spatial proteomics analysis

The spatial proteomics analysis was performed using the GeoMx IO Proteome Atlas (IPA) platform on renal biopsies from patients with lupus nephritis. This cutting-edge platform enables high-throughput profiling of over 570 proteins (Appendix, Table 1) across FFPE tissue sections. GeoMx IPA technology allows for the simultaneous quantification of protein expression in distinct tissue compartments, including glomerular and tubular regions, as well as immune cell infiltrates within the renal microenvironment.

The analysis was conducted on renal biopsies from eight patients who underwent serial biopsies before and after treatment with conventional therapy. Among these patients, five were classified as responders, and three as non-responders.

Tissue sections from archival FFPE kidney biopsy samples were mounted on independent slides. The slides were prepared following the manufacturer's guidelines (NanoString GeoMx DSP Manual Slide Preparation; MAN-10150-01). For FFPE blocks, 5 μ m-thick tissue sections were mounted on Superfrost microscope slides (Fisher Scientific) and baked at 60°C for 30 minutes to ensure proper adhesion within the scanned area. In each slide, four tissue samples from different analysis groups were included to minimize batch effects.

The following day, slides were stained with SYTO13 (NanoString, 121300303, FITC) for nucleic acid staining, CD45 (Novus, NBP2-3427, 2B11 + PD7/26) for global immune inflammation, and CD31 (Abcam, ab215912, AF647) and PanCK (Novus, NBP2-33200, AF594) as cellular markers for kidney tissue morphology characterization. Proteomic data generated using the Nanostring GeoMx DSP platform are available from GEO under accession number GSE301097.

4.4.1 Protein quantification

Using GeoMx's proprietary technology, an extensive protein panel was analyzed, encompassing over 570 proteins associated with critical biological pathways such as cell signaling, immune regulation, inflammation, and metabolism. The analysis focused on defining distinct protein expression patterns in specific tissue compartments, including glomerular and tubular regions as well as immune cell infiltrates within the renal microenvironment.

Each FFPE tissue section underwent high-resolution digital imaging to identify regions of interest (ROIs) with precision. ROIs were selected based on their morphological and pathological features to capture representative areas for spatial analysis. This approach ensured that key microenvironmental differences, such as immune infiltration or tissue damage, were accurately captured.

The selected ROIs were subjected to digital quantification of protein abundance using barcoded oligonucleotide tags conjugated to antibodies targeting specific proteins. These barcoded tags allowed for multiplexed protein detection within a single assay. Next-Generation Sequencing (NGS) was performed on the Illumina NextSeq platform, which decoded the barcoded tags. The sequencing output generated comprehensive and high-dimensional data on protein expression levels, enabling precise characterization of protein localization and abundance across tissue compartments.

This methodology offered several advantages, including high sensitivity, reproducibility, and the ability to profile hundreds of proteins simultaneously, even in limited tissue samples. By integrating spatial resolution with proteomic quantification, this approach provided insights into the functional and pathological mechanisms at play within the renal biopsies.

4.4.2 Data analysis of protein spatial distribution

The data processing pipeline began with aligning sequencing reads to a reference library of barcoded oligonucleotides, ensuring accurate identification of target proteins. Normalization steps were applied to account for potential batch effects and variability introduced during sample preparation or sequencing. The normalized data was then subjected to rigorous statistical analysis to quantify relative protein abundance across ROIs.

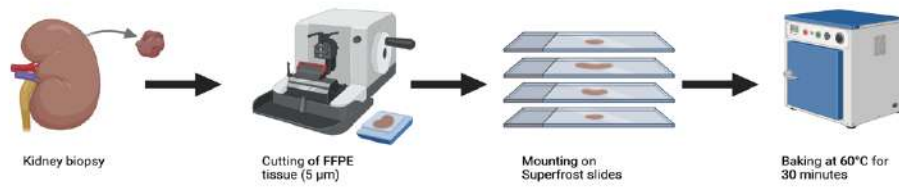
GeoMx software facilitated the comprehensive analysis of protein expression data, generating detailed pathway annotations and functional insights. The software enabled correlation of spatial protein distribution with clinical phenotypes, identifying biomarkers linked to immune-mediated pathology, inflammation, and tissue remodeling in LN.

Further analysis included comparisons between responders and non-responders to therapy, revealing differential protein expression patterns and highlighting pathways potentially associated with treatment response. Proteins involved in immune activation, complement cascade, and cellular stress pathways were closely examined for their role in disease progression and treatment outcomes.

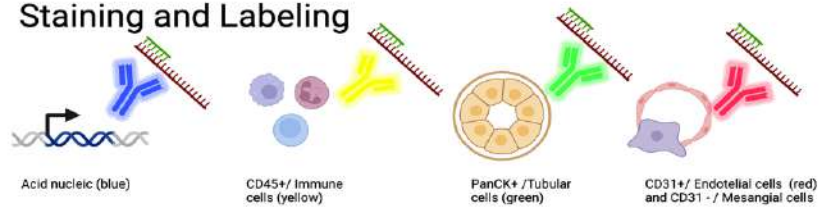
Data visualization tools, including heatmaps, volcano plots, and spatial maps of protein expression, were utilized to illustrate key findings. These visualizations helped identify hotspots of pathological activity and their association with histological features, enhancing the interpretation of the results.

The integration of spatial proteomics with high-throughput sequencing allowed for an unprecedented understanding of the molecular and spatial heterogeneity in lupus nephritis. This approach provided a robust platform for biomarker discovery and paved the way for potential therapeutic targets in this complex immune-mediated disease.

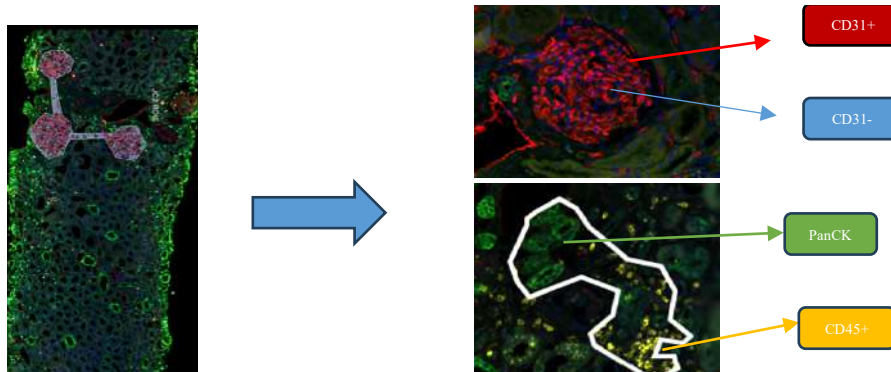
1 Samples preparation



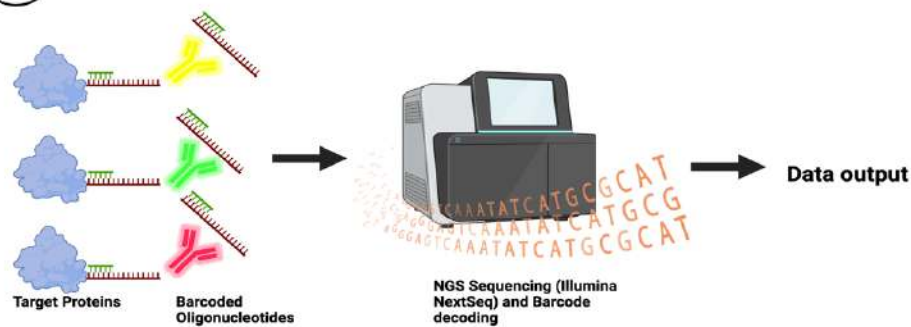
2 Staining and Labeling



3 ROI selection



4 Protein quantification



5 Data analysis and results

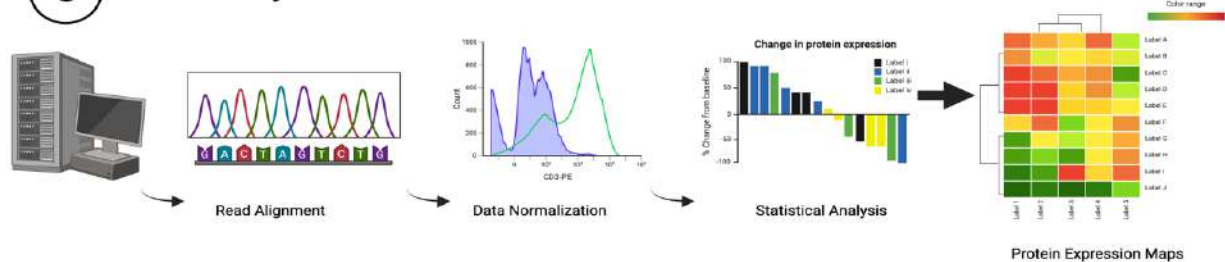


Figure 8. Workflow of the GeoMx Platform for Spatial Proteomics Analysis in Renal

Biopsies. Spatial proteomics analysis was performed using the GeoMx IO Proteome Atlas platform. 1,2,3. FFPE renal biopsies were prepared and stained with specific markers to identify regions of interest (ROIs). 4. Protein quantification was performed by detecting barcoded oligonucleotides, followed by NGS sequencing. 5. Data were processed and analyzed to generate protein expression maps across different tissue compartments.

4.5 Validation of selected proteins in urine samples

Validation of selected proteins in urine samples was performed using enzyme-linked immunosorbent assays (ELISAs) targeting proteins that were identified as upregulated in renal tissue proteomic analyses from non-responders to treatment. Urine samples were collected at two defined time points: prior to treatment initiation and following treatment completion (n = 20; 11 responders and 9 non-responders).

The proteins selected for validation included IL-1, IL-15, CD14, CD27, and STING. Quantification was carried out using the following commercial ELISA kits: Human IL-1 alpha ELISA Kit (BMS243-2, Thermo Scientific, Waltham, Massachusetts, USA); Human IL-15 ELISA Kit (BMS2106, Thermo Scientific, Waltham, Massachusetts, USA); Human CD14 ELISA Kit (EHCD14, Thermo Scientific, Waltham, Massachusetts, USA); Human CD27 Ligand/TNFSF7 ELISA Kit (EH38RB, Thermo Scientific, Waltham, Massachusetts, USA); STING ELISA Kit (ELH-TMEM173-1, Raybiotech, Peachtree Corners, Georgia, USA).

All assays were performed in triplicate and in accordance with the manufacturers' protocols, ensuring standardized sample processing and consistent quantification across the study.

4.6 Statistical analysis

The sample size was estimated based on the variability observed in comparable transcriptomic studies using techniques such as scRNA-seq and bulk RNA-seq. These studies indicate that a minimum of 7 patients per comparison subgroup are sufficient to detect statistically significant transcriptomic and proteomic differences. To compare responders and non-responders, and accounting for an estimated 40–50% non-response rate, a minimum of 15 patients was deemed necessary.

Urine samples from our cohort (n = 28) were used to validate urinary biomarkers identified from tissue proteomics. Assuming a 30 percentage point difference in clinical response rates between groups, this sample size provides 80% power to detect the difference at a significance level of 0.05.

Statistical analyses and graphical representations were performed using GraphPad Prism (RRID: SCR_002798), version 10.2.2 (GraphPad Software, San Diego, CA, USA). Data are presented as mean $\pm$ standard deviation (SD) or standard error of the mean (SEM), as specified in the figure legends.

Group comparisons were conducted using two-tailed, paired or unpaired Student's t-tests, or one-way ANOVA where appropriate. For post hoc multiple comparisons,

Tukey's test or the Benjamini-Hochberg method for false discovery rate correction was applied, depending on the dataset.

Receiver operating characteristic (ROC) curve analysis was used to evaluate the discriminatory power of individual biomarkers. For multi-biomarker assessment, CombiROC (<http://combiroc.eu>), an online platform, was used to perform multiplex ROC analysis and identify optimal biomarker combinations for improved diagnostic performance. Statistical significance is indicated as follows: $p \leq 0.05$: *, $p \leq 0.01$: **, and $p \leq 0.001$: ***.

4.7 Ethical considerations

This study received ethical approval from the Vall d'Hebrón Hospital Ethics Committee (Reference: PR(AG)59/2022). All participants provided written informed consent prior to enrolment, in full accordance with the principles set forth in the Declaration of Helsinki.

The PhD student ensured that all participants were clearly and thoroughly informed about the nature and objectives of the study. Detailed information was provided in a dedicated participant information sheet, specifically prepared to address the purpose, procedures, and ethical considerations of the research. Upon agreeing to participate, each individual signed an informed consent form, prepared in duplicate: one copy was given to the participant for their records, and the second was securely archived in the clinical documentation.

5. RESULTS

5.1. Study design

To characterize the molecular signatures of proliferative LN and their association with treatment response, we performed spatial transcriptomic profiling in a cohort of 20 patients with biopsy-proven LN at disease onset. All patients received standard induction and maintenance therapy, consisting of corticosteroids pulses and MMF, and were followed prospectively. After one year, patients were stratified based on clinical response: 11 achieved complete clinical remission (55%), while 9 were classified as non-responders (45%).

Spatial transcriptomic profiling was conducted at baseline across four key renal compartments: CD45⁺ immune infiltrates, PanCK⁺ tubular epithelial cells, CD31⁻ glomerular cells, and CD31⁺ endothelial cells. We then analyzed the resulting molecular profiles in relation to both clinical outcomes and histological findings.

A subset of 8 patients underwent a second kidney biopsy after one year of treatment, allowing for longitudinal assessment of transcriptomic and proteomic changes and the identification of molecular pathways associated with therapeutic response. Additionally, we performed a sub-analysis within the group of clinical responders to evaluate differences between those who achieved histological remission and those who did not, aiming to uncover mechanisms underlying persistent tissue-level inflammation despite apparent clinical improvement.

Key tissue proteins identified from these analyses were further validated in urine samples as potential non-invasive biomarkers.

A detailed summary of the study design and analytical workflow is provided in Figure 9.

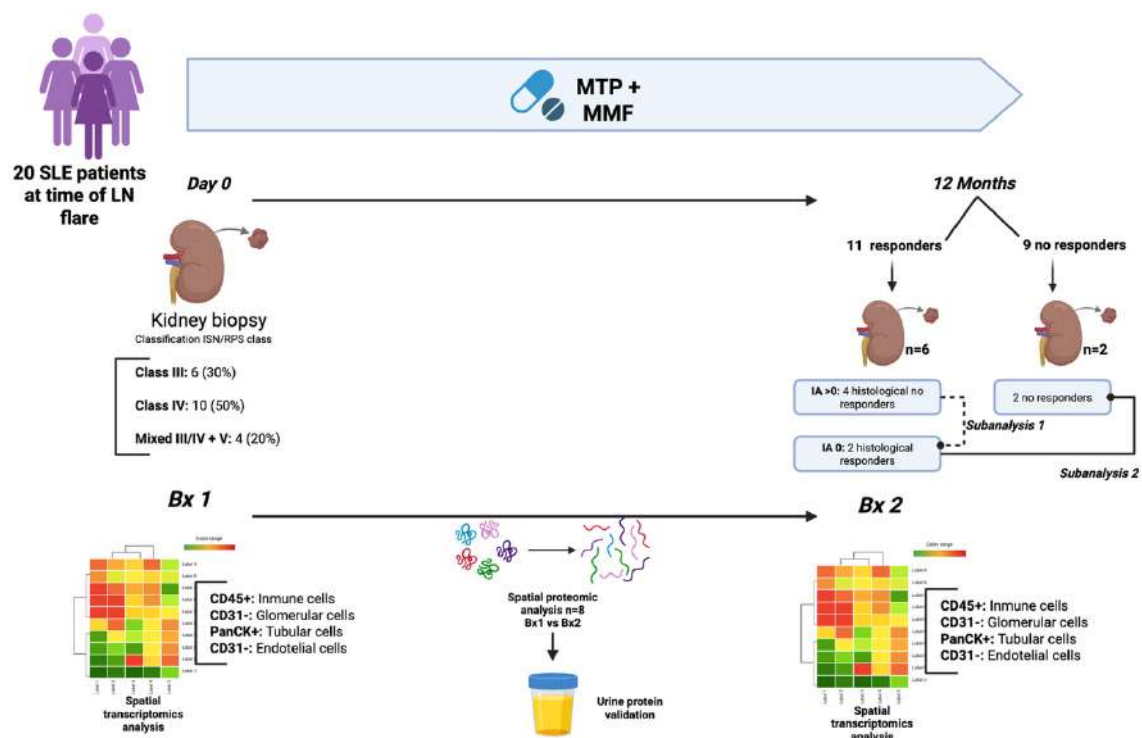


Figure 9. Study workflow. Twenty SLE patients with LN flare underwent kidney biopsy (Bx1) at baseline and received standard treatment with MTP and MMF. Spatial transcriptomic profiling was performed in four renal compartments. After 12 months, clinical response was assessed: 11 patients were responders and 9 non-responders. A second biopsy (Bx2) was obtained in 8 patients, enabling paired spatial proteomic analysis and urine protein validation. Among clinical responders with Bx2 (n=6), patients were stratified by histological response based on the activity index (IA): IA = 0 (histological responders) vs IA > 0 (non-responders). Two sub-analyses were conducted: **(1)** histological vs non-histological responders and **(2)** histological vs clinical non-responders.

5.2 Patient characteristics

The clinical and demographic characteristics of patients with LN, included in the molecular analysis, stratified by renal outcomes, are summarized in Table 1. This study enrolled 20 consecutive patients with biopsy-proven proliferative lupus nephritis. After 52 weeks of standard therapy, 11 patients (55%) achieved a complete renal response (CRR). Both groups, CRR and non-renal response displayed comparable baseline characteristics regarding age, gender distribution, ethnicity, and the prevalence of relapsing renal disease. However, at baseline, eGFR was significantly lower in NR than in CRR patients ($p < 0.05$). This latter group also tended to have higher 24-hour urine protein levels, global SLEDAI-2K scores, and anti-dsDNA titres, although these differences did not reach statistical significance.

Per protocol, repeated biopsies were performed post-treatment in six CRR patients and 2 non-responders. Histologic findings at baseline (Bx1) and post-treatment (Bx2) are summarized in Table 2. At Bx1, non-responders had a significantly higher chronicity index, predominantly driven by increased interstitial fibrosis ($p < 0.05$). Notably, all baseline biopsies showed less than 30% glomerulosclerosis and interstitial fibrosis (IF). At the post-treatment time point (Bx2), glomerulosclerosis and IF remained below 50% across all samples.

Table 6. Baseline demographic and clinical characteristics.

	Complete Renal Response (n = 11)	No Response (NR) (n = 9)	P Value
Age (years), mean (range)	37,4 (26-55)	35,3 (18-52)	0,641
Time from SLE diagnosis to LN flare (years), mean (range)	4,1 (0-14)	5,5 (0-16)	0,320
Female, n (%)	10 (90,9)	9 (100)	0,999
Race/ethnicity, n (%)			
Caucasian	10 (90,9)	7 (77,7)	0,566
Hispanics	3 (27,7)	1 (11,1)	0,591
SLEDAI-2K global score, mean (range)	15,2 (6-26)	18,2 (8-32)	0,204
Lupus nephritis			
De novo, n (%)	10 (90,9)	7 (77,7)	0,566
Relapsing flare, n (%)	1 (9,0)	2 (22,2)	0,566
Proliferative class, n (%)			
Class III	6 (54,5)	3 (33,3)	0,999
Class IV	5 (45,4)	6 (66,6)	0,999
Laboratory parameters at biopsy time, mean (SD)			
Proteinuria, g/24h	2,1 (1,5)	4,3 (3,7)	0,179
Serum creatinine, mg/ml	0,83 (0,2)	1,7 (1,3)	0,154
eGFR (mL/min/1.73 m ²)	80,0 (4,5)	59,1 (35)	0,064
Anti-dsDNA Abs, n (%)	8 (72,7)	7 (77,8)	0,411
Anti-dsDNA Abs, IU/mL	314 (287)	289 (244)	0,411
Low Serum C3, n (%)	8 (72,7)	6 (66,6)	0,243
Low Serum C4, n (%)	7 (63,6)	6 (66,6)	0,640
Histological variables of first kidney biopsy^a			
Total glomeruli biopsy (mean, [range])	28,5 (13-56)	20,8 (7-32)	0,159
Activity Index biopsy 1 (mean, [range])	7,4 (5-12)	9,3 (2-18)	0,119
Endocapillary proliferation	1,2 (2,5)	1,7 (2)	0,332
Subendothelial deposits	0,2 (1)	0,7 (1)	0,145
Neutrophils/karyorexis	0,8 (1)	1,6 (2)	0,105
Fibrinoid necrosis	0,9 (2)	1,5 (2)	0,367
Cellular/fibrocellular crescents	1,0 (2)	1,7 (2)	0,372

Interstitial inflammation	0,3 (1)	1 (1)	0,137
Chronicity Index biopsy 1(mean, [range])	1,5 (0-3)	2,6 (0-4)	0,177
Glomerulosclerosis	0,4 (1)	0,7 (1)	0,984
Fibrous crescents	0,09 (0)	0,2 (0)	0,491
Tubular atrophy	0,2 (1)	0,6 (1)	0,125
Interstitial fibrosis	0,1 (0)	0,8 (1)	0,137
Response status, n (%)			
Complete renal response	11 (100)	0 (0)	0,001
Partial renal response	0 (0)	6 (66,6)	0,002
No response	0 (0)	3 (33,3)	0,073
Time to remission (months), mean (SD)	8 (5,3)	n/a	n/a

Data are expressed in mean (standard deviation) and p value group by t-student test analysis. Abbreviations: SLE= Systemic lupus erythematosus; SLEDAI-2K= Systemic Lupus Erythematosus Disease Activity Index; eGFR= Estimated glomerular filtration rate; n/a= not applicable. Complete renal response= urinary protein-to-creatinine ratio (UPCR) of less than 0.5 mg/mg or 500 mg/day or less, glomerular filtration rate (GFR) greater than 90 mL/min/1.73 m² and a serum creatinine level that is normal or 125% less than baseline if abnormal. Partial renal response= 50% reduction in proteinuria compared to baseline, while still above 500 mg/day. No response= failure to meet the criteria for a complete or partial response (308). Reference ranges are as follows: anti-double-stranded DNA antibodies, <15 IU per millilitre; serum C3 (mg/dL), 85 to 110; serum C4 (mg/dL), 10 to 40.

^aData reported represent consensus values determined by nephropathologists who independently reviewed the cases and resolved discrepancies through discussion.

5.3. Histopathology and DSP RNA quality control

Representative kidney biopsy samples stained with periodic acid-Schiff (PAS) were analyzed to identify optimal regions of interest (ROIs) exhibiting significant activity and minimal chronicity for GeoMx Digital Spatial Profiling (DSP) Whole Transcriptome Assay (WTA). Immunofluorescence markers were employed to delineate specific areas of interest (AOIs) within each ROI. These AOIs were categorized as CD31⁺ (endothelial cells), CD31⁻ (mesangial cells), PanCK⁺ (tubular cells), and CD45⁺ (lymphocytes), representing glomerular and tubular structures (Figure 10a).

Each tissue section contained 12 geometric AOIs with a mean area of 262.43 μm^2 (IQR: 143–208 μm^2) and a median of 187 cells per AOI (IQR: 139–211 cells). Quality control (QC) identified and excluded 11 segments with gene detection rates below 5% (Figure 10b). Read counts for the remaining 50 segments were normalized using the quartile 3 (Q3) method. Genes detected in fewer than 10% of the segments were excluded, yielding a final dataset of 8,876 genes (Figure 10c). QC confirmed robust normalization by validating separation between the geometric mean of negative control probes and the Q3 of gene read counts across both segment and distribution levels (Figure 10d).

Expression profiling revealed tissue-specific gene enrichment. Glomerular ROIs demonstrated higher expression of glomerulus-specific genes (*PDGFRB*, *PODXL*, *NPHS2*, *NPHS1*) compared to tubular-specific genes, including *SLC34A1* (proximal tubule), *SLC12A3* (distal tubule), and *AQP2* (collecting duct) (Figure 10e). Conversely, tubular ROIs showed elevated expression of tubular-specific genes, with *AQP2* being most prominent (Figure 10e). These results confirm that GeoMx DSP effectively captured distinct transcriptomic profiles for glomerular and tubular compartments. Uniform Manifold Approximation and Projection (UMAP) analysis of overall gene expression identified three clusters (Figure 10f). Samples separated according to segmentation strategy, with PanCK⁺ and CD45⁺ segments forming distinct clusters, while CD31⁺ and CD31⁻ segments showed substantial overlap (Figure 10f).

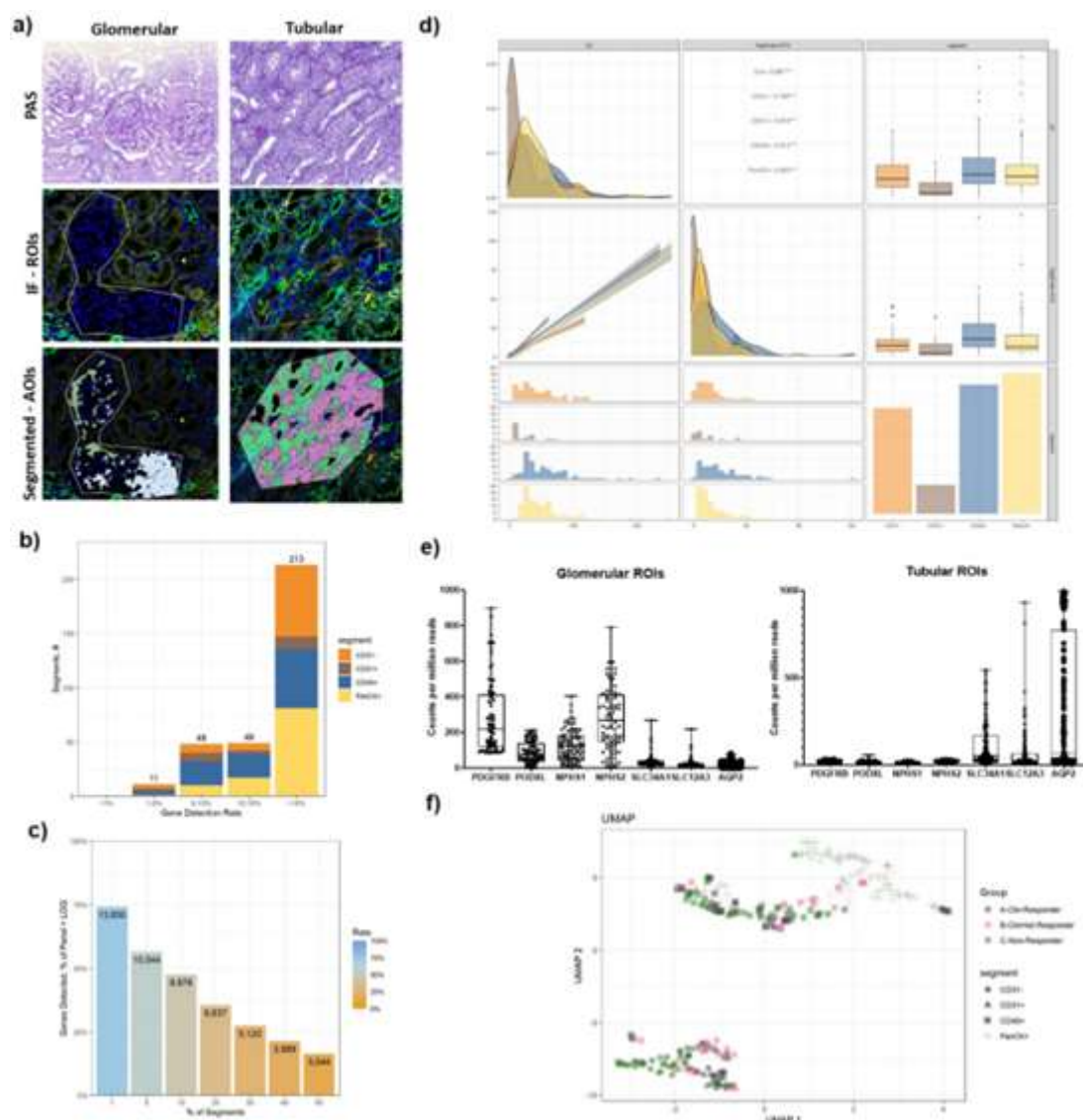


Figure 10. Spatial data analysis of kidney tissue from LN patients. A) Representative periodic acid-Schiff (PAS)-stained sections identifying glomerular and tubular regions, alongside immunofluorescence images used for region and area of interest (ROI and AOI) selection. Immunofluorescence markers include SYTO13 (blue), CD31 (red), PanCK (green), and CD45

(pink), guiding NanoString GeoMx DSP analysis. Scale bar: 500 μ m. **B)** Distribution of AOI segments by the percentage of genes detected above the limit of quantification (LOQ), based on negative control probes. Low-signal segments were excluded to ensure analytical accuracy. **C)** Gene detection summary showing 8,785 genes were above LOQ in at least 10% of segments. **D)** Scatter plot showing a strong correlation between the geometric mean of negative control probes and the 75th quantile (Q3) of gene expression, validating Q3 normalization. **E)** Tissue-specific gene expression revealed enrichment of glomerular-specific markers (*PODXL*, *NPHS2*, *NPHS1*) in glomerular ROIs and tubular markers (*AQP2*, *SLC34A1*, *SLC12A3*) in tubular ROIs. Data shown as box plots with individual values. **F)** UMAP plot illustrating transcriptomic clustering. CD45+ and PanCK+ AOIs formed distinct clusters (square and cross markers), while CD31+ and CD31- AOIs partially overlapped (triangle and dot markers). Subtle separation was observed between responder (grey and pink) and non-responder (green), suggesting transcriptomic differences related to treatment outcomes.

5.4. Transcriptomic insights into renal flare in responders and non-responders

5.4.1 CD45+ immune cell landscape

We characterized the immune cell infiltrate in LN during flare by analysing CD45+ areas of interest (AOIs) in kidney biopsies from responders and non-responders. The mean number of CD45+ cells per AOI did not differ significantly between groups (responders, mean: 183 cells and non-responders, mean: 152 cells). In both groups, the majority of CD45+ AOIs were in tubular regions, accounting for 96% and 94% of identified areas, respectively (Figure 11a).

Spatial deconvolution analysis, using published single-cell transcriptomic data (310) estimated the relative abundance of immune cell types within these AOIs. Plasmacytoid dendritic cells, natural killer cells, and macrophages were the most prevalent immune cell types identified in LN kidney biopsies, with normalized mean counts of 1.986, 1.554, and 1.314, respectively (Figure 11b). While overall immune composition did not differ significantly, between groups, macrophages showed a trend toward enrichment in non-responders ($p = 0.057$, Figure 11c).

We further examined immune cell distributions across different histological subtypes of LN. Pure proliferative classes (III and IV) displayed the highest immune cell infiltration, primarily composed of innate immune cells, including pDCs, tissue macrophages, monocytes, neutrophils, and NK cells. In contrast, adaptive immune cells—such as B cells and CD4+/CD8+ T lymphocytes—were less prominent. Mixed classes (III/V or IV/V) exhibited the lowest immune cell density, with pDCs and NK cells being the predominant populations identified (Figure 12a).

Differential gene expression analysis identified seven significantly dysregulated genes in CD45+ AOIs, ranked by significance: *FOS*, *EGR1*, *PITX1*, *JUNB*, *HYAL2*, *AFAP1L1*, and *KRTAP5-8*. Of these, *HYAL2* and *AFAP1L1*, associated with cellular structure and remodelling, were upregulated in responders (Figure 11d). In contrast, non-responders showed higher expression of inflammation- and cytokine-related genes (*FOS*, *EGR1*, and

JUNB), transcriptional regulators (*PITX1*) and keratin-related genes (*KRTAP5-8*) (Figure 11e). These transcriptional patterns were consistent across patients and AOs, indicating robust transcriptional differences (Figure 11f).

To explore their biological relevance, pathway enrichment analysis was performed using Gene Set Variation Analysis (GSVA) R package on gene sets from the KEGG Brite database [<https://www.genome.jp/kegg/kegg1.html>]. Among 752 tested pathways (5-500 genes each), 56 showed significant enrichment in CD45⁺ AOs. Nine gene sets achieved nominal significance ($p < 0.05$), and 47 achieved suggestive significance ($p < 0.2$). In non-responders, enriched pathways involved cytokine signalling, cell cycle regulation, and chromatin remodelling. These genes included SOCS box targets, Cyclin-dependent kinase regulators, Aurora kinase regulators, the SRCAP complex, intraflagellar transport (IFT) complex, and the SWR1 complex (Figure 11f). Conversely, responders demonstrated enrichment in pathways related to Histone/nucleic acid organization and Tyrosine kinase/phosphatase regulation, including Nucleotide excision repair, Tubulin pathways, Pericentriolar material (PCM), the MLL3/MLL4 complex, Cysteine endopeptidases, PPM1 phosphatases (PP2C), and Platelet-derived growth factor (PDGF) signalling pathways (Figure 11f).

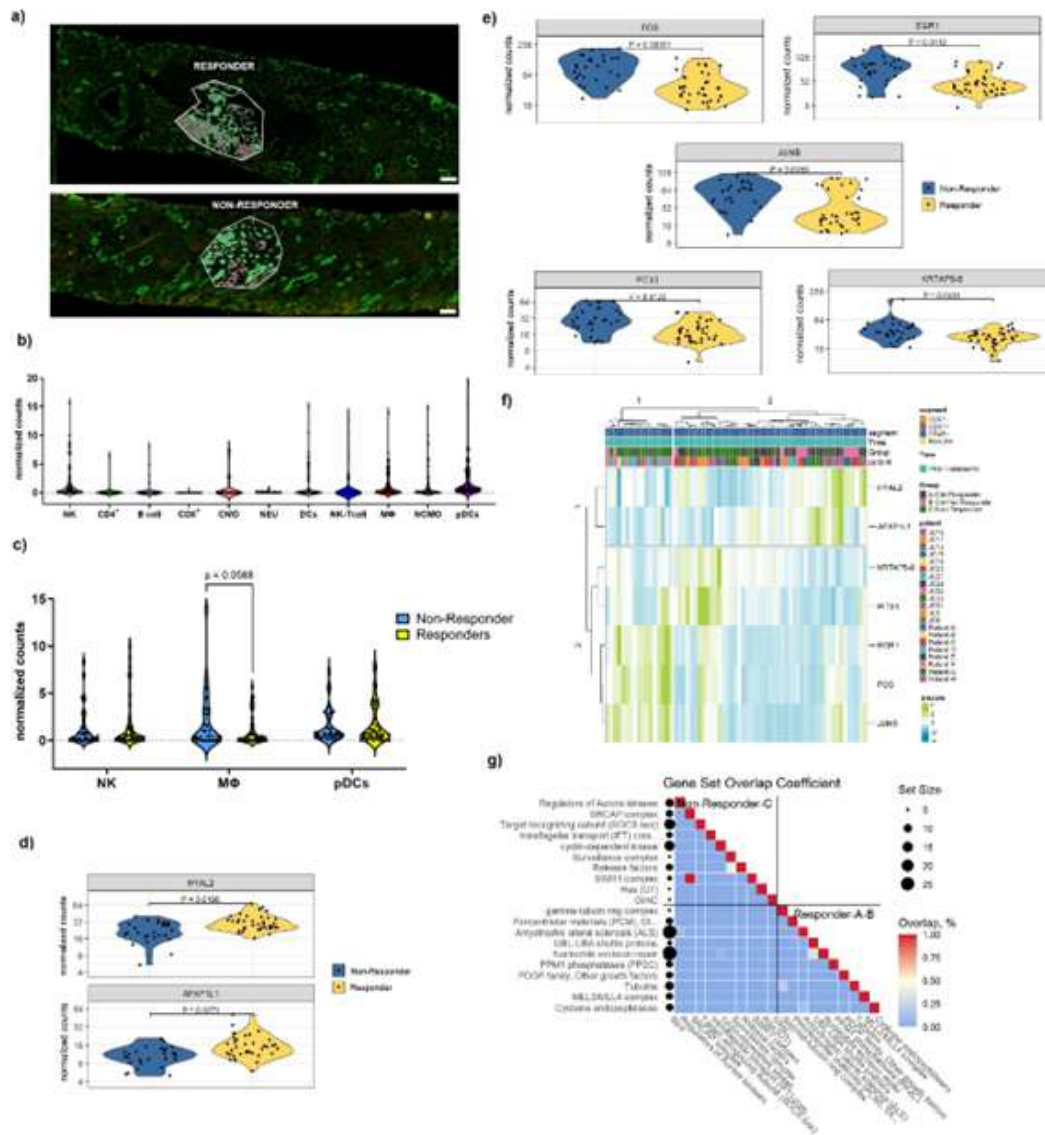


Figure 11. Distinct transcriptomic signatures in CD45+ immune cells of responder and non-responder LN patients. A) Representative immunofluorescence images of CD45+ immune cells in kidney biopsies from responders and non-responders. CD45 is shown in pink, and PanCK in green. Scale bar: 100 μ m. B) Violin plots illustrating the abundance of immune cell subtypes within CD45+ AOIs. Immune cell subtypes include natural killer cells (NK cells), classical monocytes (CMO), neutrophils (NEU), dendritic cells (DCs), macrophages (MΦ), non-classical monocytes (NCMO), and plasmacytoid dendritic cells (pDCs). Each dot represents data from an individual CD45+ AOI. C) Violin plots showing the proportion of NK cells, macrophages (MΦ), and pDCs within CD45+ AOIs, stratified by responder and non-responder groups. P-values were determined by two-way ANOVA with Benjamini-Hochberg adjustment for multiple comparisons. (D, E) Differential gene expression analysis identifies significant transcriptional differences between responders and non-responders. (D) Responder exhibited upregulation of *HYAL2* and *AFAP1L1*, while (E) non-responders showed elevated expression of *FOS*, *EGR1*, *JUNB*, *PITX1*, and *KRTAP5-8*. Violin plots show individual data points, with P-values from the Wilcoxon rank-sum test, adjusted using the Benjamini-Hochberg method. F) Heatmap of representative gene expression profiles (population averages) for CD45+ AOIs in responders and non-responders, with unsupervised hierarchical clustering based on Pearson distances of z-score-normalized data, revealing distinct patterns. g) Gene Set Variation Analysis (GSVA) of KEGG datasets shows

overlapping gene sets in CD45⁺ AOIs between responders and non-responders. The heatmap displays the relative overlap of gene sets, quantified by the Szymkiewicz-Simpson overlap coefficient. Higher overlap values (red) indicate shared biological processes. Bubble size reflects the relative size of each gene set, and color intensity represents overlap degree.

5.4.2 Distinct glomerular signatures in LN response

Using CD31⁻ areas of interest (AOIs) within glomerular regions, mesangial and podocyte cells were identified in an approximate 1.7:1 ratio, based on the expression of *PDGFRB*, *AGTR1* and *ITGA8* (mesangial cell markers) and *PODX1*, *NPHS1* and *NPHS2* (podocyte markers; Figure 1e). Endothelial cells, by contrast, were localized to CD31⁺ AOIs. Differential gene expression analysis identified 341 differentially expressed genes (DEGs) in CD31⁻ AOIs and 1,234 DEGs in CD31⁺ AOIs between responders and non-responders (p -value < 0.05, $|\log_2$ fold change| > 0.5; (see Tables A2 and A3, Appendix). Heatmap clustering of CD31⁺ and CD31⁻ AOIs showed treatment response as the primary driver of transcriptional differences across AOIs (Figure 13a).

In non-responders, CD31⁺ AOIs showed marked upregulation of genes associated with proliferation, differentiation, and cytokine signalling, including *DUS1L*, *EGR1*, *JUNB*, *ZFP36L1*, *TMED2*, *MYADM*, *RPS26*, *FN1*, *ARPC2*, and *S100A4*. STRING-based protein-protein interaction (PPI) analysis highlighted networks involving transcription factors *JUNB* and *EGR1*, and ribosomal proteins *RPS18* and *RPL8*, suggesting activation of translation and stress response pathways (Figure 13b). Conversely, CD31⁻ AOIs in non-responders showed downregulation of *CCT3*, *BAK1*, *DUSP6*, *RPS18*, *MYC*, *VASP*, *RPL8*, *ADAMTS1*, *S100A9*, and *ITGA5*.

Gene Set Variation Analysis (GSVA) identified 80 significant gene sets in CD31⁻ AOIs and 48 in CD31⁺ AOIs, with the top five represented in a volcano plot (Figure 13c). In CD31⁻ cells, pathway analysis identified upregulation of immune and inflammatory pathways, including *P53*, *AP-1*, *Toll-like receptor*, *TNF*, *NF-kappa B*, and *extracellular matrix remodelling* pathways (Figure 3d). CD31⁺ AOIs further showed enrichment of *P53*, *Toll-like receptor*, *NF-kappa B*, *Class I cytokine receptor*, *apoptosis*, *SOCS*, and *FoxO* pathways (Figure 13e).

In responders, no significant protein-protein interactions were observed among cluster genes. Nonetheless, GSVA revealed distinct pathway enrichment: CD31⁻ AOIs showed upregulation of Plexin A/B, mTOR, PPAR, and protein tyrosine phosphatase receptor (PTPs) signalling (Figure 13G), while CD31⁺ AOIs were enriched for DNA repair-related pathways, including RNA polymerase activity, claudin (CLDN) signalling, histone deacetylase activity, and PIKK family pathways (Figure 13G).

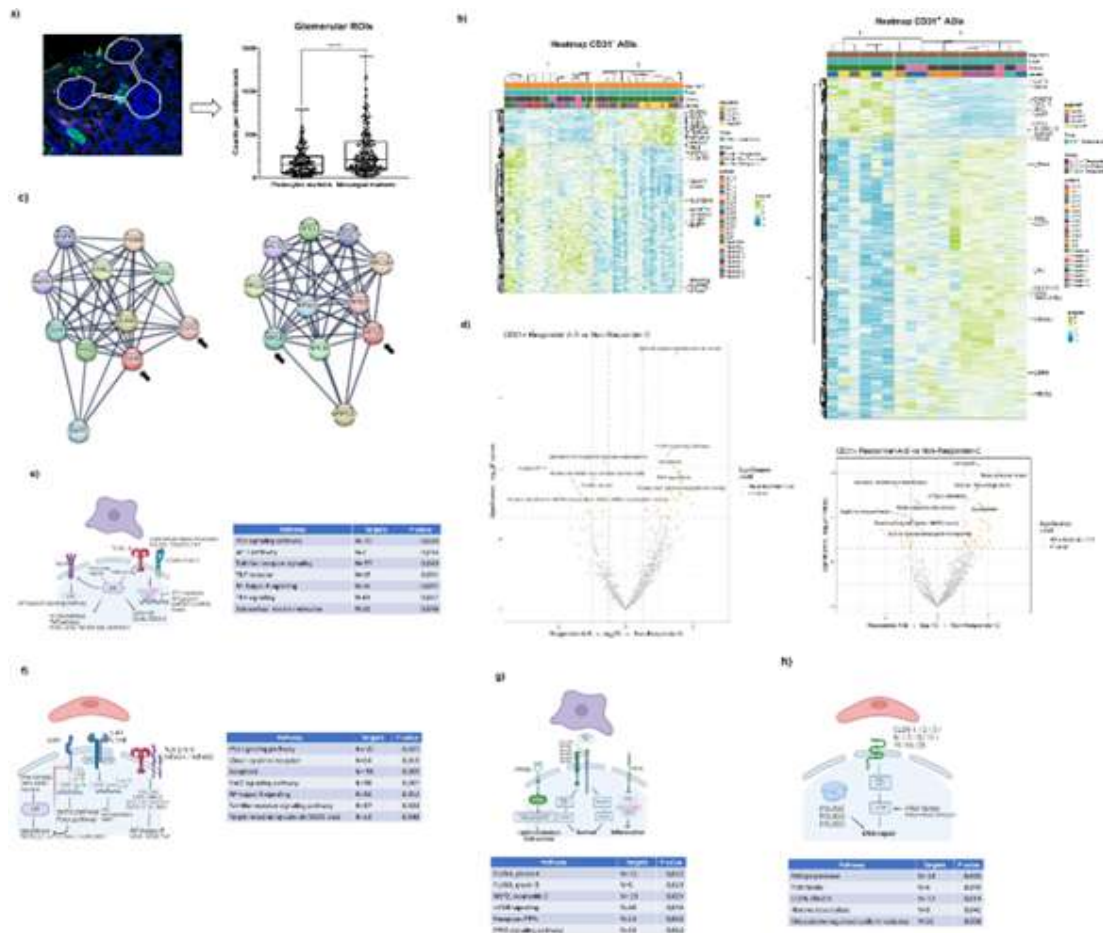


Figure 13. Distinct clustering and pathway differences in glomerular cells between responders and non-responders in lupus nephritis. **A)** CD31⁻ AOIs within glomerular ROIs were analyzed to estimate the mesangial-to-podocyte ratio using specific cellular markers. A significantly higher proportion of mesangial cells was observed (1.7:1 ratio; ***p < 0.0001, Student's t-test). **B)** Heatmap of CD31⁻ and CD31⁺ glomerular AOIs, showing marker gene expression stratified by treatment response. Unsupervised hierarchical clustering reveals distinct transcriptional profiles between responders and non-responders. **C)** Protein-protein interaction (PPI) network analysis of genes from CD31⁻ or CD31⁺ clusters in non-responders, generated using the STRING database. Only gene pairs with at least one significant interaction are shown, emphasizing functional connections between transcription factors, ribosomal proteins, and other key regulators. **D)** Volcano plots of differentially enriched pathways in responders versus non-responders for CD31⁻ (left) and CD31⁺ (right) AOIs. Significantly enriched pathways (FDR-adjusted p < 0.05) are shown in orange, non-significant in grey. Differential analysis was performed using the Limma package with empirical Bayes moderated t-statistics. The x-axis represents log₂(fold-change), and the y-axis represents -log₁₀ (P-value). **E-H)** Gene Set Variation Analysis (GSVA) based on KEGG Brite pathways. Pathways enriched in non-responders are shown for CD31⁻ (**E**) and CD31⁺ (**F**) AOIs, while pathways upregulated in responders are shown for CD31⁻ (**G**) and CD31⁺ (**H**) AOIs. Pathway significance was assessed using hypergeometric testing via ClusterProfiler.

5.4.3 PanCK⁺ tubular transcriptomic profiles by response

PanCK⁺ cells were selected from tubular ROIs to isolate tubule-specific transcriptomes and minimize glomerular contamination. Spatial deconvolution revealed a significantly higher abundance of proximal tubular cells in responders ($p=0.007$, Figure 15a), consistent with their role in glomerular filtrate reabsorption. In contrast, non-responders showed enrichment of peritubular capillary endothelial cells, essential for delivering blood and oxygen to surrounding tubular cells ($p=0.021$, Figure 14a).

Differential gene expression analysis identified 364 within tubular ROIs ($p<0.05$ and $|\log_2 \text{fold change}| > 0.5$, Figure 15b). Non-responders upregulated genes involved in stress response and signaling (e.g. *YWHAZ*, *RNF10*, *CXXC5*, *MCL1*, *ITM2C*, *LLGL2*, *DDX17*, *HOXD4*, *FOS*, and *PPP1R15A*), while responders exhibited higher expression of genes such as *GRHPR*, *PKLR*, *GAMLM*, *XYLB*, *FUOM*, *SULT1A2*, *FGD5*, *PNPO*, *MRPL44*, and *DNAJC22*. Although no direct protein-protein interactions were identified, responder-enriched genes (*PKLR*, *PNPO*, *GRHPR*, *XYLB*, and *SULT1A2*) were significantly associated with metabolic processes ($p = 0.0199$).

Pathway analysis identified 160 significantly enriched gene sets ($p < 0.05$). In responders, upregulated pathways were predominantly associated with the metabolism of amino acids, carbohydrates, fatty acids, and nucleotides (e.g., *Pantothenate and CoA biosynthesis*, *Glycine, Serine, and Threonine metabolism* (Figure 15c). In contrast, non-responders showed enrichment in pathways related to transcriptional regulation, stress responses, and inflammation, including *Target Recognizing Subunit SOCS Box*, *TREX Complex*, and *DNA Helicase* (Figure 15c). Additionally, non-responders exhibited significant upregulation of pathways associated with cytokine and growth factor signaling, inflammation, and apoptosis. These included *AP-1(-like) components*, *Jak-STAT*, *TNF*, and *MAPK signaling pathways* (Figure 15d).

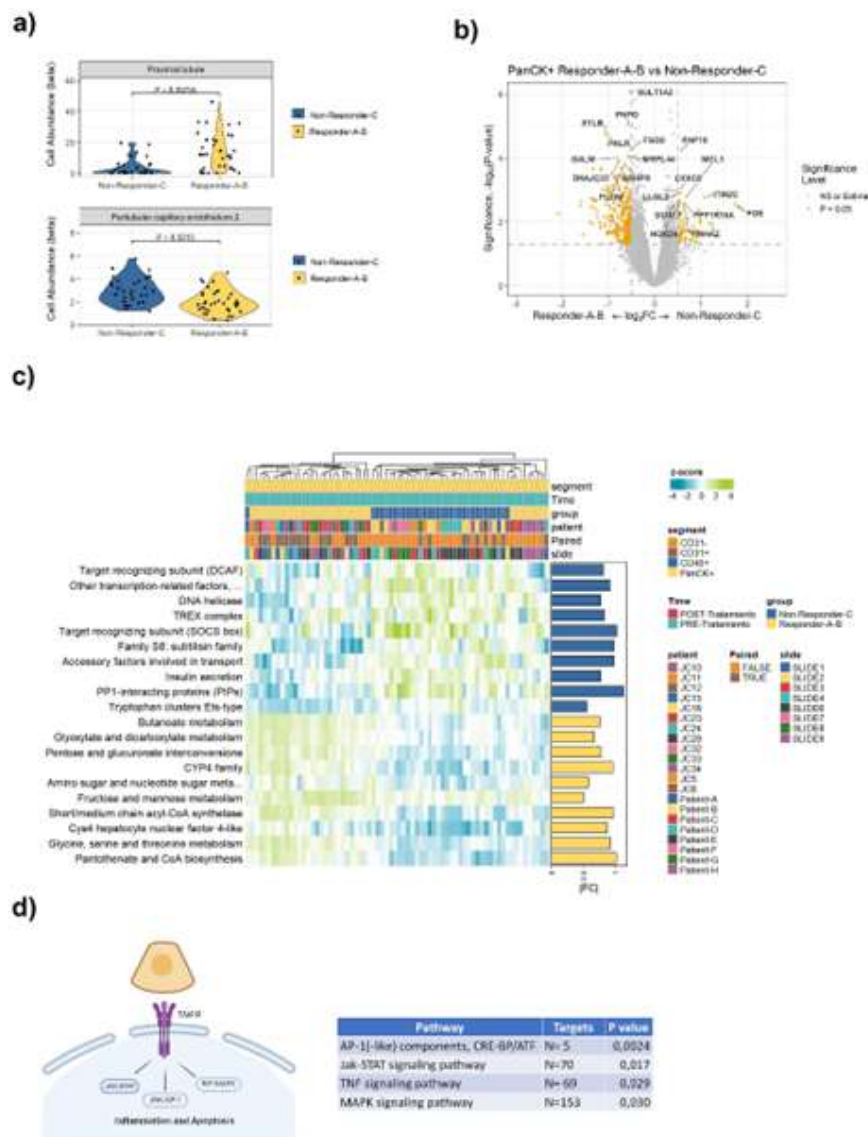


Figure 15. Transcriptomic profiling of PanCK+ tubular cells distinguishes responders from non-responders in LN. A) Representative images of PanCK+ AOs from kidney biopsy tubular regions of responders and non-responders. Scale bar = 100 μ m. **B)** Violin plots showing proportions of proximal tubular cells and peritubular capillary endothelial cells within PanCK+ AOs in responder and non-responder patients. P-values were calculated using two-way ANOVA with Benjamini-Hochberg correction for multiple testing. **C)** Volcano plot of differentially expressed genes (DEGs) in PanCK+ AOs. Significant genes (FDR-adjusted $p < 0.05$) are highlighted in orange, non-significant genes in grey. DEGs were identified using empirical Bayes moderated t-statistics with the R package *Limma*. The x-axis represents $\log_2$ (fold-change), and the y-axis represents $-\log_{10}$ (P-value). **D)** Heatmap of pathway enrichment based on Gene Set Variation Analysis (GSVA) using KEGG Brite pathways, illustrating distinct transcriptomic signatures in responders vs. non-responders. **E)** Immune-related pathways were specifically enriched in non-responders, as determined by hypergeometric testing with ClusterProfiler.

5.4.4. Correlation of ROI transcriptomes with LN activity

First, we performed the correlation between immune cell populations and clinical markers of disease activity. We found no significant associations between immune cell populations and clinical markers of lupus activity. Among all cell types analyzed, only natural killer (NK) cells showed a non-significant trend toward higher abundance in patients with proteinuria >500 mg/24h ($p = 0.06$). No correlations were observed between immune cell populations and anti-dsDNA positivity or complement levels (Fig. 12b).

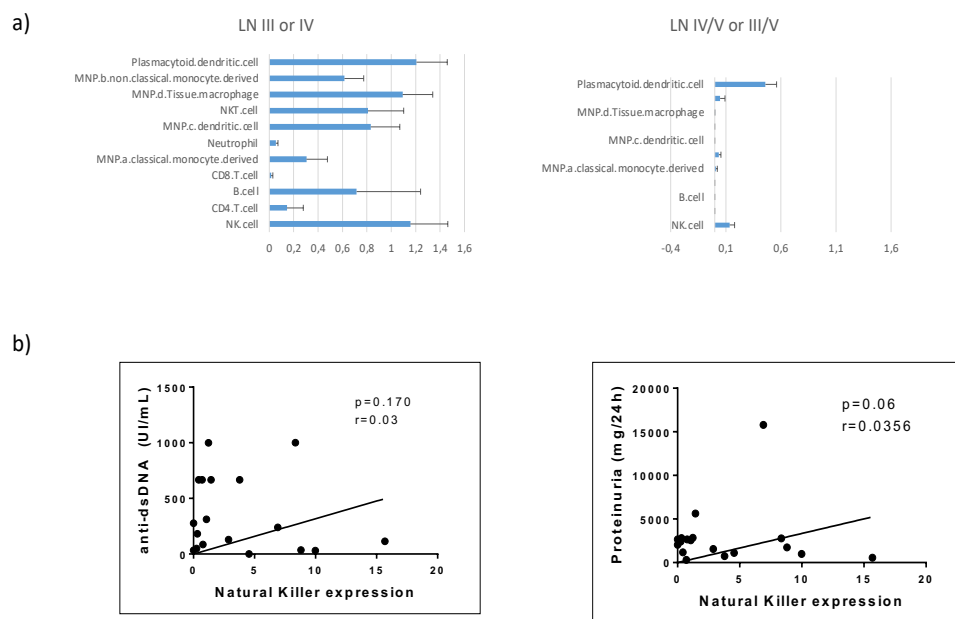


Figure 12. Immune Cell Differences and NK Cell–Clinical Correlations: a.Differences in immune cell types between pure proliferative forms and mixed forms of lupus nephritis. b. Pearson correlation between NK cell expression and anti-DNA antibody levels ($p = 0.1$, $r = 0.03$) as well as proteinuria ($p = 0.06$, $r = 0.03$).

Second, Top dysregulated genes across $CD45^+$, $CD31^-$, $CD31^+$, and $PanCK^+$ AOs distinguishing responders from non-responders were correlated with clinical and histological markers of LN activity (Figure A1 and A2, Appendix). No significant correlations were found between the seven differentially expressed genes in $CD45^+$ AOs (*FOS*, *EGR1*, *PITX1*, *JUNB*, *HYAL2*, *AFAP1L1*, and *KRTAP5-8*) and any clinical or histological metrics (Appendix Figure A1). In $CD31^-$ AOs, *S100A9* expression showed a strong positive correlation with proteinuria ($r = 0.638$, $p = 0.002$), suggesting its link to disease severity (Appendix Figure A2). In $CD31^+$ AOs, *JUNB* and *EGR1* were significantly correlated with the chronicity index ($r = 0.517$, $p = 0.019$ and $r = 0.653$, $p = 0.002$, respectively), indicating roles in long-term tissue remodelling or fibrosis (Appendix Figure A2). Similarly, in $PanCK^+$ AOs, *FOS* expression was positively correlated with the chronicity index ($r = 0.434$, $p = 0.049$), further supporting its involvement in disease

progression (Appendix Figure A2). No significant correlations were observed with anti-dsDNA antibodies or complement levels.

5.5. Treatment response signatures in post-biopsy transcriptomes

Of the 20 patients with active LN who were enrolled, eight underwent a second biopsy after one year of treatment, as per protocol (Table 2). To evaluate the impact of treatment on renal transcriptomic profiles, gene expression changes were analyzed between baseline (Bx1) and follow-up (Bx2) biopsies in responders (n=6) and non-responders (n=2). The cohort consisted entirely of female patients, predominantly Caucasian (87.5%), with 87% newly diagnosed with lupus nephritis and a mean age of 35 years. Treatment led to significant reductions in SLEDAI-2K scores, proteinuria, anti-dsDNA antibody levels, and the histological activity index scores, although a modest increase in chronicity index was observed. Only, 25% of responders (n=2) achieved an histological activity index (AI) score of 0. Follow-up intervals were comparable between groups

Table 7: Clinical and histological characteristics at Bx1 and Bx2

	Bx1 (n = 8)	Bx2 (n = 8)	P Value
<i>SLEDAI-2K global score, mean (range)</i>	15,6 (6-26)	3 (0-8)	<0.001
<i>Laboratory parameters, mean (IRQ)</i>			
Proteinuria, g/24h	2.2 (1.3)	0.95 (1.7)	0.121
Serum creatinine, mg/ml	0,81 (0,4)	1,04 (0,8)	0.479
eGFR (mL/min/1.73 m²)	91,3 (6,2)	84.25 (25)	0.452
Anti-dsDNA Abs, IU/mL	316,6 (270)	62,1 (77)	0.023
Low Serum C3, n (%)	6 (75)	2 (25)	0.132
<i>Histological variables</i>			
Total glomeruli biopsy (median [IRQ])	26 (12-56)	11,7 (10-50)	0.357
Activity Index (median [range])	8,9 (5-18)	3 (0-12)	0.119
Chronicity Index (median [range])	1,6 (0-3)	2,1 (0-4)	0.937
Histological class, n (%)			
Class I	0 (0)	2	0,467
Class II	0 (0)	2	0,467
Class III	2 (25)	0	0,467
Class IV	4 (50)	2	0,601
Class V	0	1	0,999
Class III o IV/V	2 (25)	1	0,999

In the Bx2 cohort, 33 ROIs were analyzed for CD45⁺/CD31⁻ cells and 38 for PanCK⁺ cells. Only 4 AOs contained sufficient CD31⁺ cells within the glomerulus for analysis. As a result, CD31⁺ cells were excluded from the subanalysis. Applying a dual threshold of $p < 0.05$ and $|\log_2 \text{ fold change}| > 0.5$, we identified 118, 377, and 191 significantly differentially expressed genes in CD45⁺, CD31⁻, and PanCK⁺ ROIs, respectively. Heatmap clustering revealed clear transcriptional divergence between responders and non-responders at Bx2 (Figure 16a), with previously observed DEGs normalizing and new ones emerging post-treatment.

5.5.1 Immune cell pathways in CD45⁺ follow-up samples

In CD45⁺ cells, 16 DEGs were identified in non-responders, compared to 101 in responders. Analysis of immune cell composition revealed a significant increase in neutrophils ($p = 0.01$) and tissue macrophages ($p = 0.03$) in non-responders. Additionally, there was a trend toward a higher abundance of dendritic cells in non-responders compared to responders ($p = 0.07$).

Non-responders upregulated immune activation and differentiation genes (e.g., *KDM4B*, *YWHAH*, and *DUSP1*) and pathways link to integrins, plexin-B, and T-cell receptor signaling (*ILF2*, *GPX1*, *CCN1*, *RHOA*). Responders, in contrast, showed increased expression of transcriptional recovery genes (*POLR2J2*, *LYRM9*, *SFXN5*) and enrichment in RNA polymerase and splicing factor pathways (Figure 16b).

5.5.2 CD31⁻ transcriptomic and pathway changes

In CD31⁻ AOs, non-responders upregulated immunoglobulin gamma genes (*IGHG1*, *IGHG2*, *IGHG3*, *IGHG4*), interferon signalling components (*IFNGR2*, *IFNAR2*), along with significant upregulation of NF- κ B-related genes, evidenced by increased expression of *IL1R1*, *CD14*, and *VCAM1* (Figure 16c), reflecting persistent immune activation. In contrast, responders exhibited increased expression of upregulation of tissue repair and remodeling genes such as *CDC42BPB*, *CDC42BPA*, *IGFBP5*, *EFNB2*, *EFNB1*, and *EFNA1*, associated with the DMPK and PDGF pathways (Figure 16c).

5.5.3 Transcriptomic changes in panCK⁺ tubular cells

In PanCK⁺ AOs, responders downregulated genes such as *WFDC2*, *DCDC2*, *HS6ST1*, *HLA-DRB1*, *RPLP0*, *RPL23*, *RPLP1*, and *RPS18*, related to ribosomal function, DNA repair, and transcription, indicating reduced cellular stress (Figure 16d). Conversely, non-responders upregulated T-cell receptor signaling genes (e.g. *PIK3AP1*, *MAPK4*, *CD74*, and *RHOA*), consistent with ongoing inflammation (Figure 16d).

5.6 Transcriptomic and pathway differences by histological response

We conducted a subanalysis of clinical complete responders, stratifying them by histological outcome into histological responders (AI = 0, n = 2) and non-histological responders (AI > 0, n = 4), which revealed distinct gene expression profiles.

All patients in this subanalysis were women, predominantly Caucasian, and of comparable age. Table 8 summarizes the clinical and histological characteristics of both biopsies, comparing histological responders and non-responders. Notably, all histological responders (100%) had purely proliferative LN, whereas 75% of non-histological responders had a proliferative class and 25% had a mixed class. At baseline, non-histological responders presented with higher levels of proteinuria compared to histological responders. In contrast, histological responders had a higher initial activity index as well as a greater chronicity index.

Table 8. Clinical and histological characteristics at Bx1 and Bx2 of histological no responders and histological responders

	Histological no Responder (n = 4)		Histological Responder (n = 2)		P Value
	<i>Bx1</i>	<i>Bx2</i>	<i>Bx1</i>	<i>Bx2</i>	
<i>SLEDAI-2K score, mean (range)</i>	11 (4-17)	1 (0-2)	11,5 (10-13)	2 (0-2)	0,997 (0,985)
Proliferative class, n (%)					
Class III	25	0	50	0	>0,999 (>0,999)
Class IV	50	0	50	0	>0,999 (>0,999)
Class III or IV/V	25	25	0	0	>0,999 (>0,999)
<i>Laboratory parameters at biopsy time, mean (SD)</i>					
Proteinuria, g/24h	2,9 (0,86)	0,11 (0,84)	0,65 (0,13)	0,12 (0,48)	0,0021 (>0,999)
Serum creatinine, mg/ml	0,7 (0,1)	0,7 (0)	0,7 (0,2)	0,6 (0)	>0,999 (0,4406)
eGFR (mL/min/1.73 m ²)	89,2 (1,1)	90 (0)	84 (8,4)	90 (0)	0,1612 (>0,999)
Anti-dsDNA Abs, IU/mL	253,5 (235,9)	76,7 (113,1)	390,8 (390,4)	60,6 (19,6)	0,7245 (0,9954)
Low Serum C3, n (%)	50	25	50	0	>0,999 (>0,999)

Low Serum C4, n (%)	0	0	50	0	0,333 (>0,999)
Activity Index biopsy (median [range])	4 (0-12)	2 (0-5)	9 (8-10)	0,5 (0-1)	0,496 (0,934)
Endocapillary proliferation	1,25 (0,8)	0,5 (0)	1,5 (0)	0 (0)	0,817 (0,471)
Subendothelial depositis	0,5 (0,5)	0 (0)	1 (0,7)	0 (0)	0,329 (>0,999)
Neutrophils/karyorexis	0,5 (0,5)	0,25 (0)	0,5 (0,7)	0 (0)	>0,999 (0,734)
Fibrinoid necrosis	1 (1,5)	0	2 (0)	0 (0)	0,428 (>0,999)
Cellular/fibrocellular crescents	0,5 (0,7)	0	3 (1,4)	0 (0)	0,004 (>0,999)
Interstitial inflammation	0,25 (0,3)	0	1 (0)	0,5 (0,7)	0,045 (0,186)
Chronicity Index biopsy 1(range)	1,2 (0-3)	1,7 (0-3)	2 (1-3)	2,5 (1-4)	0,845 (0,845)
Glomerulosclerosis	0,5 (0,5)	0,75 (0,5)	1 (0)	1 (1,4)	0,647 (0,893)
Fibrous crescents	0,25 (0,3)	0 (0)	0 (0)	0 (0)	0,2855 (>0,999)
Tubular atrophy	0,25 (0,3)	0,25 (0,5)	0,5 (0,7)	1 (0)	0,775 (0,156)
Interstitial fibrosis	0,25 (0,3)	0 (0)	0,5 (0,7)	0,5 (0,7)	0,736 (0,331)

Clinical and histological parameters of histological non-responders (n = 4) and histological responders (n = 2) at baseline (Bx1) and follow-up biopsy (Bx2). Values are presented as mean (SD), mean (range), or number (%), as appropriate. P values correspond to comparisons between histological non-responders and responders at Bx1; values in parentheses correspond to comparisons at Bx2. Categorical variables were compared using Fisher's exact test, and continuous variables using two-way ANOVA.

5.6.1 CD45⁺ immune cell transcriptomic changes

In CD45⁺ AOIs, 458 DEGs genes were identified. Non-histological responders showed upregulation of TNF receptor family genes (*cTNFRSF11B*, *C1QTNF1*, *TNF*, *C1QTNF7*) and NRP1 associated pathways (*SEMA3G*, *SERPING1*) (Figure 16e). Conversely, histological responders exhibited upregulation of PP1-interacting proteins (*IKZF5*, *PPP1R18*, *PPP1R17*, *PPP1CB*, *RPL8*, *RPL39*, *MCM7*) and FoxO signaling pathway genes (*MAP3K14*, *MAP1B*, *PIK3C2B*, *MAP2K6*, *SMAD9*, *FOXO4*, *FOXO6*, *FOXO1*), showing transcriptional regulation and cell survival processes (Figure 16e).

5.6.2 Glomerular cell transcriptional signatures in CD31⁻ Cells

In CD31⁻ AOs, 1,020 DEGs were mainly upregulated in non-histological responders, linked to mesangial cell activation (e.g. *LILRB2*, *DTX3*, *BSG*), lipid metabolism (e.g. *SPP1*, *SULT1A2*, *SLCO2A1*, *ABCG1*) and signal transduction pathways (e.g. *LAT*, *ARHGEF40*, *PRKCI*, *SORL1*). Conversely, 36 genes upregulated in histological responders were linked to RNA splicing/processing (*MZT2B*, *CIRBP*, *GTF2I*, *EMC10*), cell cycle/DNA regulation (*RSF1*, *RBBP7*, *HMG1*, *HMG20B*), and protein degradation (*PSMB4*, *CTSD*, *VPS4A*, *ATP6V1G1*) (Figure 16f).

5.6.3 Tubular cell transcriptional profiles in panCK⁺ cells

In PanCK⁺ AOs, 584 DEGs were identified, with most upregulated in histological responders, emphasizing tubular cell activation in kidney repair and associations with mitochondrial and metabolism pathways. Only 59 DE genes were upregulated in non-histological responders, with significant involvement in the FoxO signaling pathway (*MAP3K14*, *MAP1B*, *PIK3C2B*, *MAP2K6*) (Figure 16g).

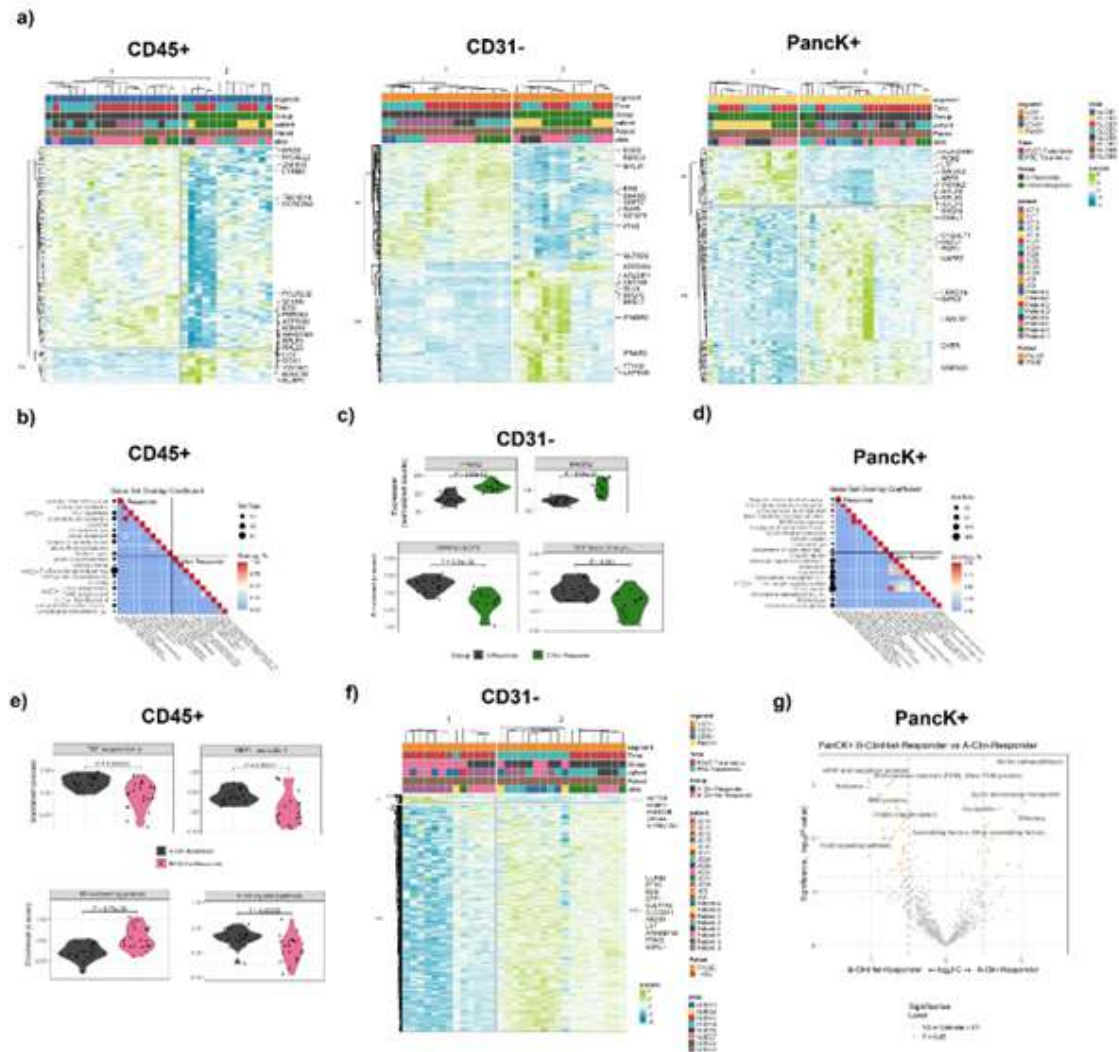


Figure 16. Transcriptomic Changes in LN Biopsies Between Initial (Bx1) and Follow-Up (Bx2) Biopsies. **A)** Heatmap of gene expression profiles from CD45⁺, CD31⁻, and PanCK⁺ AOs, showing distinct clustering between responders from non-responders. Colour intensity represents differential gene expression levels. **(B) and (D)** Gene set overlap analysis for CD45⁺ **(B)** and PanCK⁺ **(D)** AOs between Bx1 and Bx2. Heatmap colors indicate overlapping coefficients, with red denoting greater overlap. Bubble size represents the relative gene set size, highlighting convergence of biological pathways over time. **(C)** Violin plot showing significantly upregulated genes and pathways in responders and non-responders, with corresponding p-values. **(E)** Similarly, violin plots show differential gene expression between histological responders and non-responders in CD45⁺ AOs. **(F)** Heatmap of key differentially expressed genes in CD31⁻ AOs, comparing histological responders and non-responders, and illustrating pathway-specific differences. **(G)** Volcano plot comparing histological responders versus non-responders, highlighting significantly differentially expressed genes within PanCK⁺ AOs.

5.7 Spatial proteomics of CD45⁺ and CD31⁺ cells

To identify potential tissue biomarkers, we performed spatial protein analysis on paired biopsies (Bx1 and Bx2) from eight patients. The analysis focused on glomerular and tubular regions of interest (ROIs), selecting the corresponding areas of interest (AOIs) (Figure 18a). Quality control of housekeeper proteins and background (isotypes) confirmed that 186 Areas of Interest (AOIs) and 570 proteins met validation criteria (Figure S). Most proteins demonstrated a signal-to-noise ratio above background, though a few showed relatively low signals (Figure S). UMAP plots revealed that sample segregation was primarily driven by AOI segmentation rather than clinical classification (Figure 18b).

Significant differences in protein expression were observed predominantly in CD45⁺ AOIs between responder and non-responder patients. In non-responders, CD14, IL1-alpha, CD27, TIM3, STAT1, phospho-STAT1, and IL15RA were significantly upregulated. These proteins are associated with immune activation, inflammation, and immune regulation, often linked to pathways driving chronic or persistent inflammatory conditions. Conversely, responder patients demonstrated significant upregulation of Histone H2B (acetyl K5) (Figure 18c). Histone acetylation is known to activate anti-inflammatory gene expression and promote the production of pro-resolving lipid mediators, facilitating the resolution of inflammation and preventing its chronic progression.

In CD31⁺ AOIs, 35 areas were identified as sufficient for comparison between clinical groups. In this subset, non-responder patients exhibited significant upregulation of STING and C-reactive protein (CRP) ($p = 0.03$ and $p = 0.04$, respectively, Figure 18d). These proteins are critical regulators of inflammation; STING activates immune responses to pathogens and cellular damage, while CRP enhances immune clearance of infections and tissue injury. However, chronic CRP elevation may contribute to persistent inflammation and tissue damage over time.

For CD31⁻ and PanCK⁺ AOIs, no significant protein differences were observed. Although not statistically significant, VCAM1 and Fibronectin showed a trend towards upregulation in these areas ($p = 0.078$ and $p = 0.098$, respectively, Figure 19). VCAM1 primarily regulates cell trafficking fibronectin facilitates cell migration and extracellular matrix (ECM) remodeling. These proteins may contribute to fibrosis and tissue damage in chronic inflammatory states.

Sub-analysis comparing histological and non-histological responders did not reveal any significantly differentially expressed proteins.

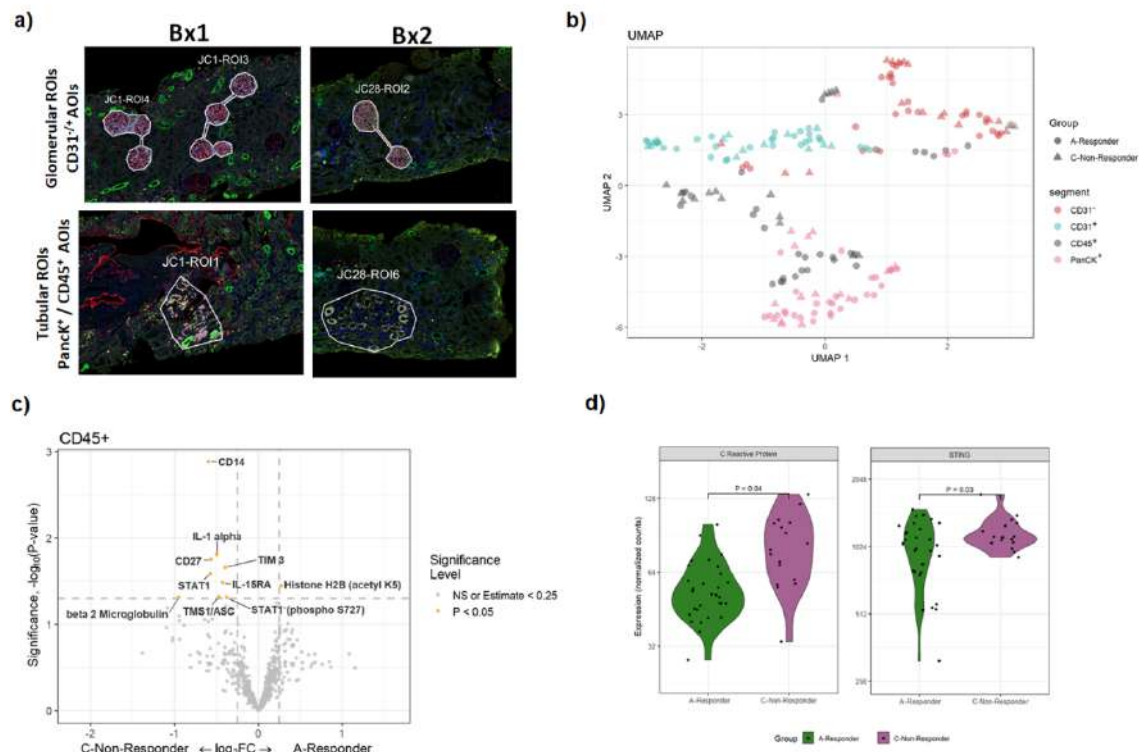


Figure 18. Spatial Protein Analysis of Paired Renal Biopsies (Bx1 and Bx2) in Responders and Non-Responders with Lupus Nephritis. **a)** Spatial protein analysis was performed on paired renal biopsies (Bx1 and Bx2) from eight lupus nephritis patients. Regions of interest (ROIs) were defined within the glomerular and tubular compartments, and corresponding areas of interest (AOIs) were selected for detailed molecular profiling. **b)** UMAP analysis of AOIs for CD31⁺ (red and blue), CD45⁺ (grey), and PanCK⁺ (pink) revealed distinct clustering based on AOI origin (glomerular or tubular) rather than clinical response. Responders are represented as squares, while non-responders are shown as triangles. **c)** A volcano plot highlights the most significantly altered proteins in CD45⁺ AOIs from paired renal biopsies of responders versus non-responders. Proteins significantly upregulated (p < 0.05) in non-responders are shown on the right, while those upregulated in responders are on the left. **d)** Among CD31⁺ AOIs, only c-reactive protein and STING demonstrated significant differences between responders and non-responders. Individual p-values for these comparisons are indicated. Statistical analysis was conducted using one-way ANOVA.

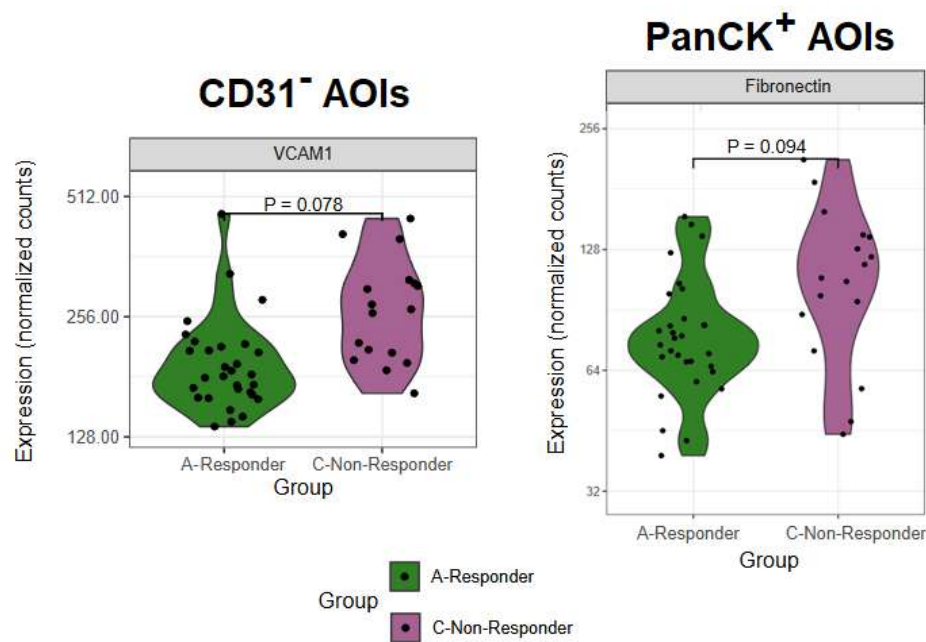


Figure 19. Violin plot for protein with a significant trend in CD31⁻ and PanCK⁺ AOIs. No significant p-values were observed between the responder and non-responder groups for proteins in CD31⁻ and PanCK⁺ AOIs. However, a trend was observed for VCAM1 in CD31⁻ AOIs and for Fibronectin in PanCK⁺ AOIs. Corresponding p-values are displayed in the violin plots.

5.8 Urinary analysis of biopsy-derived proteins

To evaluate the translational potential of spatially identified proteins as urinary biomarkers of LN response, we measured their levels in urine samples. All top differentially expressed proteins from CD45⁺ AOIs (CD14, CD27, IL-1 α , IL-15, and TIM-3) were detectable in urine, consistent with tissue expression. However, urinary levels of CD27, IL-1 α , and IL-15 did not significantly differ between responders and non-responders. In contrast, CD14 and TIM-3 levels were significantly higher in non-responders post-treatment (2157 vs. 1021 ng/mL and 4716 vs. 1206 ng/mL, $p = 0.007$ and 0.001, respectively; Figure 20 A–B), and significantly decreased in responders after treatment, suggesting an association with renal recovery ($p = 0.012$ and 0.028; Figure 20C). At flare, TIM-3 remained elevated in non-responders (6509 vs. 3765 ng/mL, $p = 0.022$; Figure 20D).

Receiver operating characteristic (ROC) analysis confirmed the discriminatory potential of urinary TIM-3 at both flare (AUC = 0.778, $p = 0.037$) and post-treatment (AUC = 0.92, $p = 0.001$; Figure 20E). In contrast, CD14 showed predictive value only post-treatment (AUC = 0.901, $p = 0.002$; Figure 20F). A combined ROC model using both markers yield an AUC of 0.938 ($p = 0.003$; Figure 20G), with no significant improvement over TIM-3 alone. Cut-off values of 1966 ng/mL (CD14) and 2534 ng/mL (TIM-3) achieved 100%

sensitivity and 75% specificity in identifying non-responders. In comparison, traditional biomarkers including proteinuria, low complement (C3 or C4), and anti-dsDNA, had lower discriminatory performance (AUC range: 0.510–0.767; Figure 21)., with only proteinuria reaching statistical significance post-treatment ($p = 0.044$, AUC = 0.767).

Finally, urinary STING, upregulated in CD31⁺ AOs of non-responders, was detected at low levels in urine and did not significantly differ between groups (Figure 22).

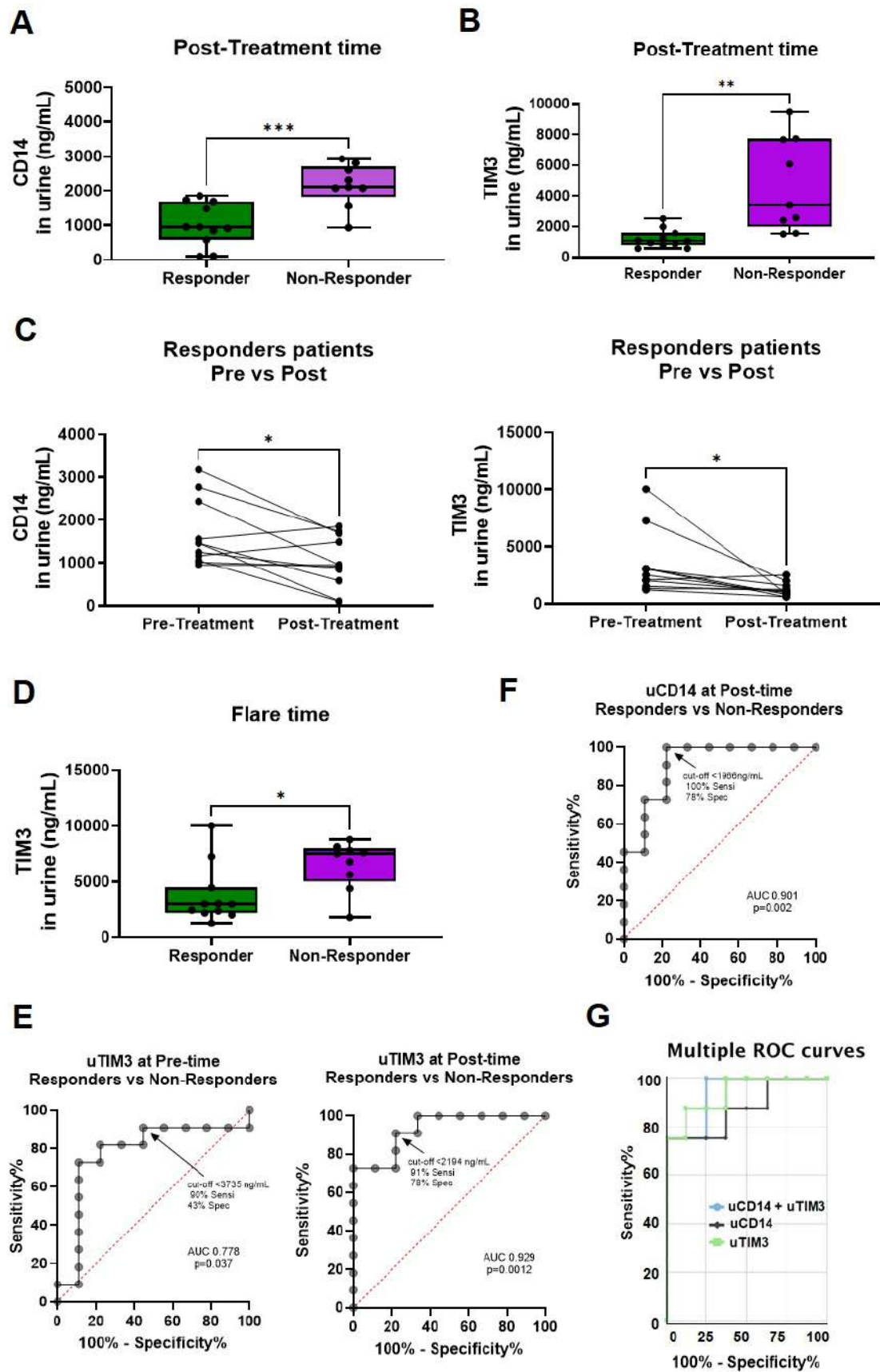


Figure 20. Urinary levels of CD14 and TIM-3 distinguish treatment response and highlight their biomarker potential. (A–B) Box plots showing significantly elevated post-

treatment urinary CD14 **(A)** and TIM-3 **(B)** levels in non-responders compared to responders ($p < 0.01$). **(C)** Paired analysis in responders demonstrates significant post-treatment decreases in urinary CD14 and TIM-3 levels ($p < 0.05$). **(D)** Urinary TIM-3 levels at flare were significantly higher in non-responders versus responders ($p < 0.05$). **(E)** ROC analysis of urinary TIM-3 shows moderate predictive performance at flare (AUC = 0.778, $p = 0.037$; sensitivity 90%, specificity 43%) and improved discrimination post-treatment (AUC = 0.929, $p = 0.0012$; sensitivity 91%, specificity 78%). **(F)** Post-treatment urinary CD14 demonstrates strong predictive value (AUC = 0.901, $p = 0.002$; sensitivity 100%, specificity 78%). **(G)** Combined ROC analysis of urinary CD14 and TIM-3 outperforms individual biomarkers.

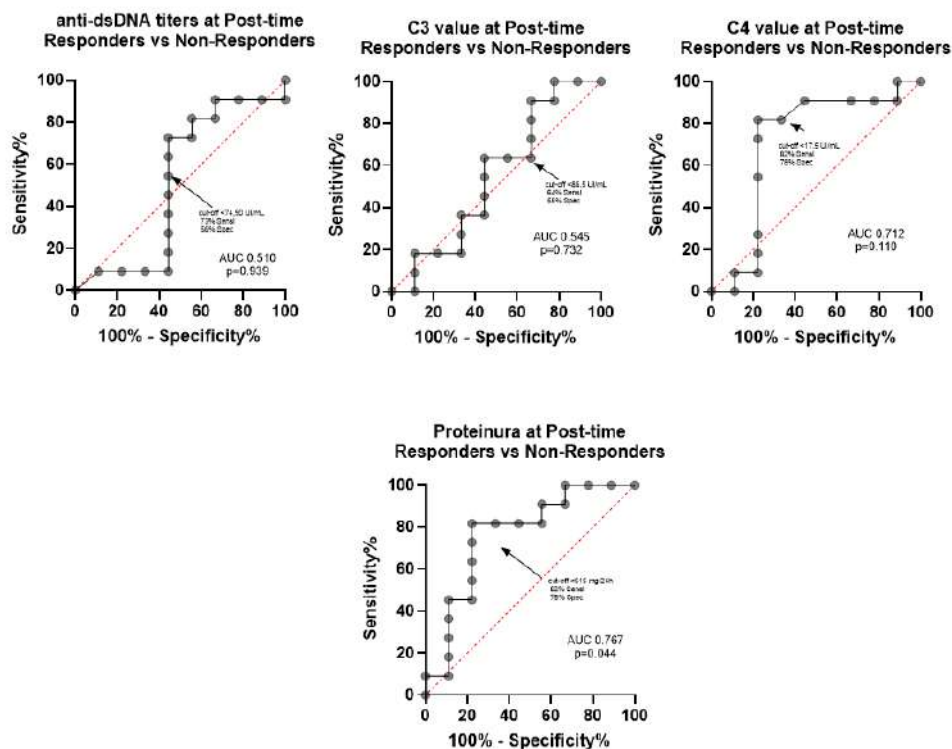


Figure 21. ROC analysis of traditional biomarkers, including proteinuria, low complement (C3 or C4), and anti-dsDNA. Only proteinuria demonstrated significant power to discriminate between responder and non-responder patients post-treatment. The AUC values and corresponding p-values are displayed in the graph.

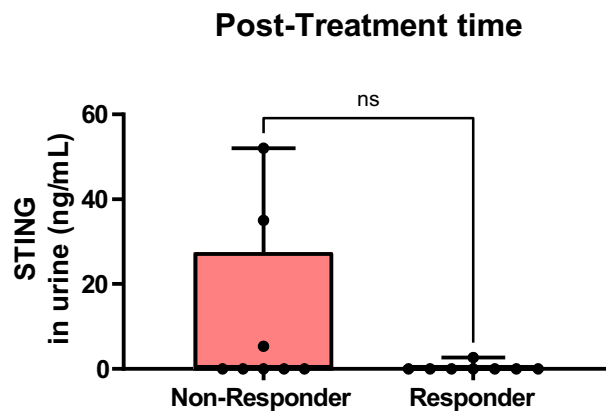


Figure 22. Detection of STING protein in urine. A low number of samples tested positive for STING protein in urine, and no significant differences were observed between the clinical groups studied (ns = not significant).

6.DISCUSSION

Our spatial transcriptomic analysis of LN provides new insights into how treatment response is shaped by local, compartment-specific interactions between immune and kidney-resident cells. Rather than being sustained by systemic immune activity alone, resistance to treatment emerges from persistent inflammatory programs within renal compartments. By analyzing paired baseline and follow-up biopsies, we found that non-responders retained strong inflammatory signatures across multiple compartments, while complete responders transitioned toward pathways associated with repair and homeostasis. The tubulointerstitial compartment emerged as a central hub of macrophage activation, epithelial stress responses, and persistent interferon signaling. Notably, a subset of clinically defined responders did not achieve histological remission, underscoring the gap between clinical parameters and tissue-level disease activity (149).

Spatial transcriptomics has begun to reshape our understanding of LN by revealing how inflammatory and repair programs are distributed within renal compartments. While studies in diabetic nephropathy, IgA nephropathy, and experimental lupus have demonstrated the value of spatial profiling (310–312), most prior LN work has relied on bulk or single-cell approaches, methods such as Visium HD and MERFISH provide cellular resolution but are constrained by targeted panels (313). In contrast, our use of the GeoMx Digital Spatial Profiler allowed unbiased, transcriptome-wide profiling across anatomically defined regions, enabling a more comprehensive view of immune–epithelial interactions (294). By focusing on actively inflamed rather than chronically scarred regions, we enriched areas of ongoing immune activity and gained deeper insight into the mechanisms driving disease progression.

In our study, transcriptomic analysis of CD45⁺ immune cells during active lupus nephritis revealed a predominance of immune transcripts localized to the tubulointerstitial compartment—a region increasingly recognized as central to progressive renal damage. Across biopsies, we observed enrichment of leukocyte-associated transcripts, including signatures of natural killer (NK) cells, plasmacytoid dendritic cells (pDCs), monocytes, and macrophages. While overall immune composition did not differ markedly between treatment groups, macrophages showed a trend toward enrichment in non-responders. Although we could not resolve specific macrophage subtypes, this pattern supports their role in sustaining inflammation and contributing to treatment resistance (139,314,315).

These findings extend prior transcriptomic studies in lupus nephritis. Bulk and single-cell analyses have consistently highlighted interferon-regulated transcripts, macrophage activation, and complement-related pathways as dominant features of systemic and renal disease activity. Peterson et al. (265) described a strong glomerular interferon and monocyte signature, while Parikh (256) showed that non-remitting patients maintained these pathways despite immunosuppressive therapy. Similarly, blood-based studies from Chiche (316) and Banchereau (259) reported persistent type I interferon activity in

severe SLE, reinforcing the concept that systemic interferon priming drives disease severity. Our results are consistent with these observations but add spatial resolution, revealing not only which genes are expressed, but also where they persist and within which cellular neighborhoods. This spatial perspective is particularly important in lupus nephritis, where glomerular and tubulointerstitial involvement carry distinct prognostic implications.

Macrophages emerged as key mediators of refractory disease in our cohort. These cells infiltrate both glomerular and tubulointerstitial compartments, where their abundance correlates with histologic severity and poor renal outcomes. Functionally, they display striking plasticity: pro-inflammatory M1-like subsets amplify tissue injury through cytokine production, oxidative stress, and leukocyte recruitment, while M2-like subsets promote repair through debris clearance and immunoregulation—but can also drive fibrosis when dysregulated (139). Persistent macrophage activation has been strongly linked to treatment resistance and progression to end-stage renal disease(149,315). Our data reinforce this central role, showing enrichment of macrophage-related transcripts in the tubulointerstitial compartment of non-responders. Although precise M1/M2 classification was not possible with our platform, the sustained activation observed underscores their contribution to chronic intrarenal inflammation and highlights them as promising therapeutic targets.

Gene expression profiling revealed distinct molecular signatures between responders and non-responders. Complete responders (CRs) upregulated genes involved in extracellular matrix remodeling and tissue repair, such as *HYAL2* and *AFAP1L1*, whereas non-responders (NRs) displayed marked enrichment of immediate-early response genes, including *FOS*, *PITX1*, *JUNB*, *KRTAP5-8*, and *EGR1*. These genes converge on AP-1 signaling, a pathway that amplifies inflammatory cascades and promotes tissue injury. *FOS* and *JUNB*, expressed in macrophages and T cells, enhance cytokine production, leukocyte recruitment, and tissue damage, while *EGR1*—a zinc-finger transcription factor induced by cytokines and growth factors—further contributes to macrophage activation and tubulointerstitial inflammation, linking it to disease progression (317–319). *PITX1*, a developmental homeobox transcription factor, was also detected in LN infiltrating cells, although its role in immune-mediated kidney injury remains unclear. Additionally, aberrant expression of epithelial-associated genes such as *KRTAP5-8* within immune infiltrates suggests possible transdifferentiation events contributing to fibrosis. Together, these signatures highlight unresolved inflammation in NRs and the activation of repair pathways in CRs.

Beyond the renal compartment, dysregulation of these early response genes has also been implicated in SLE and other glomerulopathies. Overexpression of *c-JUN* in PBMCs modulates the *FCGR2B* promoter and T-cell differentiation, with genetic polymorphisms linked to SLE susceptibility (320–322). *FOS* has been associated with DNA damage, telomere dysfunction, neutrophil activation, and podocyte injury, while its

downregulation correlates with disease severity in IgA nephropathy, suggesting a potential role as a biomarker of glomerular injury (323). Similarly, *EGR1* regulates B and T cell activity, induces pro-inflammatory cytokines (TNF- α , IL-6, IL-8, MCP-1), and upregulates adhesion molecules (CD44, ICAM-1) as well as epithelial-mesenchymal transition factors (VEGF, PDGF) (324). Persistent upregulation of these early response genes in NRs after therapy underscores a failure to suppress inflammation, further supported by continued activation of T-cell receptor and integrin pathways alongside impaired transcriptional regulation via RNA polymerase II.

These results align with prior bulk and single-cell transcriptomic studies but advance them by adding spatial context. By anchoring gene expression to defined renal compartments, our data reveal that macrophage-driven tubulointerstitial inflammation and sustained early response gene activation are hallmarks of non-response, whereas engagement of tissue repair programs distinguishes effective therapeutic responses.

Kidney-resident cells are not passive bystanders but active regulators of renal homeostasis and immune responses during disease (179). Within the glomerulus, mesangial cells, which constitute 30–40% of glomerular cells, are essential for maintaining structural integrity and modulating inflammation (325). They express toll-like receptors (TLRs) and respond to inflammatory stimuli such as lipopolysaccharides (LPS), immune complexes, and interleukin-1 (IL-1) (228). Once activated, MCs proliferate, secrete proinflammatory mediators (e.g., IL-6, CCL2), and promote extracellular matrix accumulation (326). These signals recruit and polarize macrophages toward a proinflammatory M1 phenotype, amplifying TNF- α production and sustaining glomerular injury in a self-perpetuating cycle (139,182).

In our study, NRs exhibited upregulation of TNF receptor (TNF-R) signaling, TLR3/4 pathways, and NF- κ B activation within glomerular regions, highlighting mechanisms of persistent immune activation, interferon-driven responses, and maladaptive tissue remodeling that may underlie treatment resistance and disease progression in LN. Mesangial cells express both TNF receptor 1 (TNFR1, p55) and TNF receptor 2 (TNFR2, p75), which, upon engagement by TNF- α , activate overlapping but distinct downstream cascades: TNFR1 drives NF- κ B and MAPK signaling (ERK, JNK, p38), induces chemokines (e.g., MCP-1, RANTES), and adhesion molecules (ICAM-1, VCAM-1), and in some contexts promotes apoptosis via caspase-8 whereas TNFR2 amplifies NF- κ B activity and supports cell survival. Elevated TNF- α in serum and renal tissue of LN patients, particularly within inflamed glomeruli, synergizes with type I interferons to enhance interferon-stimulated gene expression and sustain local immune activation. Clinically, TNF/TNFR activity correlates with histologic severity and poor therapy response, supported by biopsy studies demonstrating glomerular TNF- α deposition and experimental models confirming its pathogenic role in glomerulonephritis (327,328).

Mesangial cells also express multiple TLRs, including TLR3 and TLR4, which recognize pathogen- and damage-associated molecular patterns (PAMPs and DAMPs) (228). TLR3 recognises viral double-stranded RNA and endogenous apoptotic debris, while TLR4 responds to LPS and endogenous ligands such as HMGB1 and fibronectin fragments. Signaling through TRIF and MyD88 activates NF- κ B and IRF3/7, driving both type I interferon production and proinflammatory cytokine release (329). NF- κ B acts as a central hub, integrating TNF-R and TLR signals to amplify cytokine and chemokine expression, adhesion molecule upregulation, extracellular matrix deposition, mesangial expansion, and glomerulosclerosis. Persistent activation of these pathways promotes leukocyte recruitment and tissue injury, consistent with murine studies where blockade of TLR3, TLR4, or NF- κ B reduced cytokine production, leukocyte infiltration, and glomerular damage (330). Human biopsy data mirror these findings, with elevated TLR4, NF- κ B, and IRF3 expression correlating with proteinuria, histologic severity, and non-response to therapy. Collectively, these data underscore the central role of TNF-R, TLR3/4, and NF- κ B signaling in mesangial cell-driven inflammation and progression of lupus nephritis (113,331).

In contrast, CRs exhibited enrichment of pathways linked to lipid metabolism, immune regulation, and inflammation resolution, including PPAR, Plexin A/B, and protein tyrosine phosphatase (PTP) signaling—all known negative regulators of NF- κ B. This aligns with experimental models showing that PPAR activation attenuates renal inflammation by suppressing IL-17, STAT3, and TNF- α (182)., while promoting metabolic reprogramming toward anti-inflammatory macrophage phenotypes (332). Similarly, PTP and Plexin signaling have been shown to counteract NF- κ B-driven transcriptional programs and facilitate tissue repair. Together, these observations suggest that successful treatment responses in LN may depend not only on direct immunosuppression but also on the engagement of endogenous regulatory and metabolic pathways that restrain inflammation and promote renal recovery.

Second kidney biopsies in LN provide crucial insights that cannot be captured by clinical or serological monitoring alone. Standard measures such as proteinuria, serum creatinine, and autoantibody titers frequently underestimate the true degree of intrarenal inflammation. In our cohort, repeat biopsies revealed persistent immune activation in a subset of patients who had achieved apparent clinical remission, changes that would have gone undetected without histological reassessment. These findings echo those of Malvar *et al.*, who reported that 30–40% of patients in clinical remission still exhibited histological activity, which strongly predicted future flares and worse long-term renal outcomes (206,241). Together, these observations underscore the prognostic value of follow-up biopsies, which can detect subclinical inflammation, identify patients at risk of progression despite treatment, and refine therapeutic decision-making. By directly visualizing ongoing tissue injury, second biopsies highlight

the limitations of relying solely on clinical endpoints in LN and point to the need for more precise tools to monitor disease activity.

Transcriptomic analysis of repeated biopsies further revealed that, despite treatment, non-responders (NRs) maintained elevated expression of TNF and TLR pathway components in the glomerular compartment, reflecting persistent innate immune activation. Importantly, NRs also upregulated immunoglobulin-related transcripts and type I interferon (IFN-I) pathways post-treatment, suggesting the emergence of an evolving antibody- and IFN-driven intrarenal disease program.

Immunoglobulin transcripts have previously been detected in non-B cells within LN kidneys, including mesangial cells and proximal tubular epithelial cells, pointing to local in situ immunoglobulin production beyond classical B cell sources (333,334). This is highly relevant because locally produced antibodies can drive immune complex formation within glomeruli, activating complement, stimulating mesangial cells, and perpetuating a cycle of cytokine and matrix production, mesangial proliferation, and glomerulosclerosis. Importantly, this process may sustain renal inflammation independently of circulating autoantibodies, offering a potential explanation for persistent immune activation and treatment resistance in some patients.

In parallel, upregulation of IFN-I pathways in NRs highlights a second axis of maladaptive immune activation. Type I interferons, particularly IFN- α and IFN- β , are canonical mediators of SLE pathogenesis, traditionally produced by plasmacytoid dendritic cells in response to nucleic acid-containing immune complexes (335). However, recent evidence indicates that resident renal cells, including mesangial cells, podocytes, and tubular epithelial cells, can also generate IFN-I and sustain intrarenal interferon signaling. Activation of IFN-I pathways induces interferon-stimulated genes (ISGs) that enhance antigen presentation, cytokine and chemokine secretion, and immune cell recruitment, thereby creating a self-amplifying loop of glomerular and tubulointerstitial injury (336). Spatial and single-cell transcriptomic analyses confirm widespread IFN-I activity within LN kidneys, with ISG-expressing immune and parenchymal cells clustering at sites of tissue injury (337). Clinically, stronger renal IFN-I signatures correlate with proteinuria, histologic severity, and poor therapeutic response, positioning IFN-I as a key mediator that links systemic autoimmunity to local kidney damage (338).

In the tubulointerstitial compartment, RTECs play a central role in LN pathogenesis and progression to ESRD by producing inflammatory and fibrotic mediators and interacting with infiltrating immune cells (178). Reflecting patterns observed in the glomerulus, non-responders (NRs) exhibited persistent activation of cytokine signaling, enzymatic regulation, and stress-response pathways, including AP-1, JAK-STAT, TNF, and MAPK cascades. Activation of AP-1 and JAK-STAT signaling has been linked to tubular atrophy, epithelial-mesenchymal transition (EMT), and cellular senescence—processes that perpetuate chronic renal injury (339). While Yes-associated protein (YAP), a Hippo

pathway effector, was not differentially expressed in our dataset, prior studies show that YAP can directly promote AP-1 expression in tubular epithelial cells, suggesting a potential upstream role in sustaining this pathogenic pathway (340). These proinflammatory transcriptional programs remained elevated in NRs after treatment and were accompanied by persistent T-cell receptor signaling, pointing to ongoing immune–epithelial crosstalk as a driver of non-response.

In contrast, complete responders (CRs) maintained expression of genes involved in DNA repair, metabolic homeostasis, and epithelial recovery, consistent with resolution of inflammation and initiation of repair programs. This compartment-specific divergence underscores the spatial complexity of LN and highlights the importance of regional immune–epithelial interactions in shaping treatment outcomes.

T cells emerged as particularly important mediators of tubulointerstitial injury. Both CD4⁺ and CD8⁺ T cells infiltrated LN kidneys, often displaying activated, memory, or cytotoxic phenotypes, with evidence of clonal expansion consistent with antigen-driven responses. These infiltrates release proinflammatory cytokines and cytotoxic mediators that amplify local tissue injury and sustain immune activation(341). Notably, CD8⁺ T cells have been observed within renal tubules, and follow-up biopsies confirm that subsets of T cells persist in the tubulointerstitial compartment despite treatment (170,337). Their persistence correlates with tubular atrophy, fibrosis, and poorer outcomes, emphasizing that adaptive immune responses within the kidney—beyond circulating autoantibodies—are key drivers of progression (342). These findings underscore the therapeutic potential of targeting intrarenal T cell activity to disrupt chronic inflammation and promote renal recovery.

Although limited by sample size, deeper analysis within the CR group revealed substantial heterogeneity in histological outcomes. Strikingly, some clinically defined responders failed to show parallel histological improvement. These “non-histological responders” displayed ongoing immune activation, including upregulation of TNF receptor family genes and NRP1-associated signaling in CD45⁺ immune cells, persistent mesangial activation, and enrichment of lipid metabolism and signal transduction pathways in the CD31⁺ compartment. Sustained FoxO signaling in tubular regions further illustrated the compartment-specific complexity of LN and highlighted a disconnect between clinical remission and unresolved tissue inflammation (343).

Among non-responders, those failing to achieve both clinical and histological remission exhibited the most pronounced immune activation. In CD45⁺ immune cells, NRs upregulated TNF receptor family genes (*TNFRSF11B*, *TNFAIP8*), AP-1 signaling, interferon pathways (*IFNAR2*, *IFNGR2*), and histone modification genes (*H2AC11*, *H2AC8*), indicating persistent inflammatory and epigenetic dysregulation. In contrast, histological responders upregulated genes linked to mitochondrial function, chromatin remodeling, and metabolic adaptation, consistent with immune resolution and cellular

recovery. Similarly, in the glomerular CD31⁺ compartment, NRs showed enrichment of mesangial activation and extracellular matrix remodeling, while histological responders expressed genes associated with cytoskeletal organization and matrix stability, reflecting improved repair. In the tubular PanCK⁺ compartment, NRs maintained expression of inflammatory mediator pathways (*IFNAR2*, *PIK3AP1*), whereas histological responders upregulated oxidative stress response and repair programs (FoxO, FGF signaling), indicative of epithelial recovery.

Together, these findings demonstrate that persistent immune activation and impaired repair pathways distinguish non-responders from both complete and histological responders. They provide mechanistic insight into ongoing renal injury despite treatment and highlight the limitations of clinical remission as a surrogate for tissue recovery.

Because transcript levels do not always correspond to protein abundance or functional activity, we complemented our spatial transcriptomic analysis with targeted proteomic profiling to capture the actual effector pathways driving tissue injury and repair in LN. Proteomics directly quantifies the protein products, which can be more relevant to cellular function than mRNA, but current spatial proteomic platforms assay only a limited panel of targets. Within this constraint, only two proteins showed significant differential expression between complete responders and non-responders in our study: CD14 and TIM-3.

CD14 is a TLR4 co-receptor and canonical monocyte/macrophage marker that mediates innate inflammation (344). In LN, CD14⁺ monocyte infiltration correlates strongly with histologic activity and kidney injury. Its urinary form (uCD14) has been reported as a non-invasive biomarker of active proliferative LN, reflecting ongoing monocyte-driven inflammation (14,345). We found CD14 enriched in non-responders, reinforcing the view that pro-inflammatory, M1-like macrophages contribute to treatment resistance. TIM-3, an immune checkpoint receptor on chronically stimulated T cells (and some myeloid cells), normally helps limit excessive T cell activation; however, persistent TIM-3 expression has been associated with immunosuppression and progressive tissue injury (346,347). Notably, CD14⁺ monocytes can differentiate into TIM-3-expressing suppressor cells that inhibit T-cell responses, suggesting a self-reinforcing loop in non-responders where CD14-driven inflammation persists while TIM-3-mediated checkpoint activity blunts adaptive immunity.

The persistence of these proteins in non-responders underscores our transcriptomic finding that LN involves local intrarenal immune microenvironments sustaining dysregulated inflammation. Clinically, this is important because conventional measures (proteinuria, serum creatinine, serologies) often fail to capture early or compartmentalized renal inflammation, and serial biopsy is impractical. In contrast, urine biomarkers provide a non-invasive “liquid biopsy” of the kidney, collection is

painless, completely non-invasive and easily repeatable, enabling frequent monitoring of kidney-specific immune processes. Importantly, we detected both CD14 and TIM-3 in patient urine, supporting their roles as accessible surrogates of intrarenal immune dysregulation. These results align with prior studies showing that urinary CD14 levels correlate with LN activity and proteinuria, and that urinary TIM-3 rises in active disease with worse outcomes (348,349). They also extend earlier work on macrophage-related urine biomarkers. CD163, an M2-like macrophage marker, has been linked to proliferative LN and favourable response (149). In our cohort, urinary CD163 was present but at lower levels than CD14, suggesting that M1-like macrophage activation dominates in refractory disease; CD163 and other M2-associated markers may instead reflect reparative or resolving processes. TIM-3 adds a further dimension by capturing checkpoint-mediated immunoregulation within the inflamed kidney.

Together, these findings support developing a combinatorial urinary biomarker panel — including CD14, TIM-3, CD163, and relevant cytokines/chemokines (e.g. MCP-1/CCL2, CXCL10, BAFF, TWEAK) — to provide a more nuanced, mechanistic readout of intrarenal immune activity than any single marker alone. Such a panel, which is relatively easy and low-cost to implement via standard urinary assays, could improve patient stratification, enable real-time monitoring without repeat biopsies, and guide targeted therapy. Our tissue proteomics also detected CD163 in non-responders, albeit at lower abundance than CD14, confirming that CD14+ macrophages are the dominant drivers of treatment resistance in our cohort.

In summary, these results highlight how spatially resolved proteomics and urinary biomarkers together reveal persistent compartmentalized inflammation in LN, underscoring the need for precision strategies that directly target the kidney's immune microenvironment.

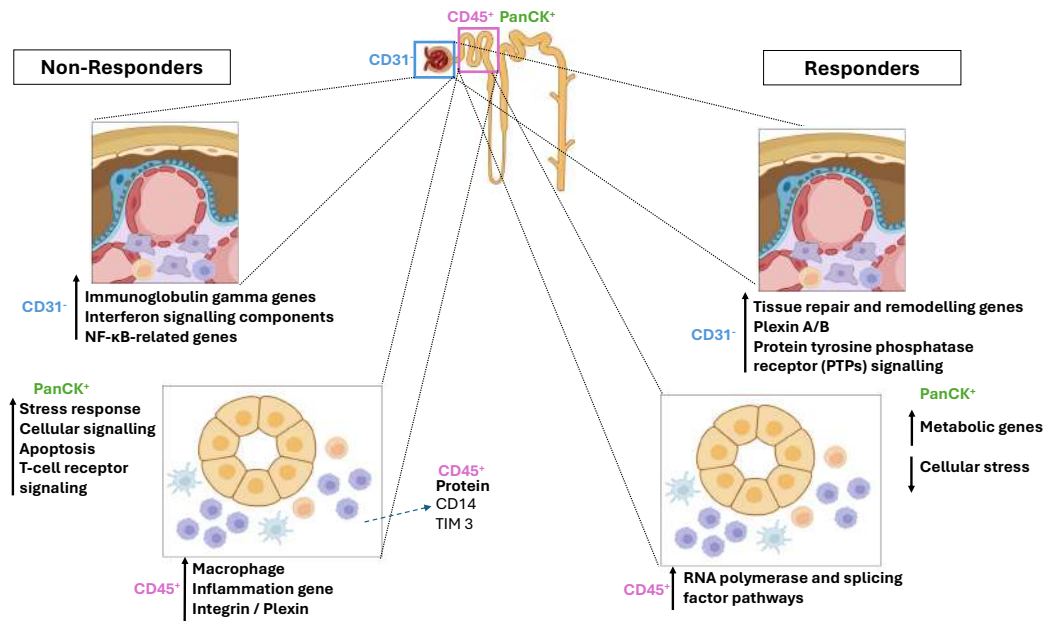


Figure 23. Integrative summary of compartment-specific transcriptional and proteomic signatures in lupus nephritis responders versus non-responders.

Schematic illustration of spatially resolved findings. In glomerular CD31⁻ regions, non-responders (NRs) exhibited persistent activation of immunoglobulin transcripts, interferon signaling, and NF-κB pathways, while responders (Rs) engaged tissue repair and remodeling programs including Plexin A/B and protein tyrosine phosphatase (PTP) signaling. In tubular PanCK⁺ regions, NRs displayed stress responses, apoptosis, and T-cell receptor signaling, in contrast to Rs, which upregulated metabolic and repair-associated genes. In immune CD45⁺ infiltrates, NRs were enriched for macrophage/inflammatory genes and integrin/plexin signaling, whereas Rs showed enrichment in RNA polymerase and splicing factor pathways. Proteomic analysis confirmed enrichment of CD14 and TIM-3 proteins in NRs, consistent with ongoing intrarenal immune dysregulation. Together, these data highlight that persistent, compartment-specific immune activation distinguishes non-responders, whereas responders engage repair and homeostatic programs. Data output from: spatial transcriptomic and proteomic profiling of lupus nephritis renal biopsies (GeoMx DSP).

7. CONCLUSIONS

1. Treatment response in lupus nephritis is driven by local, compartment-specific interactions between immune and kidney-resident cells, rather than by systemic immune activity alone.
2. Non-responders sustain persistent inflammatory programs—including IFN-I, NF- κ B, AP-1, TCR signaling, and integrins—most prominently within the tubulointerstitial compartment, which emerges as a central hub of macrophage activation, epithelial stress responses, and interferon signaling.
3. Responders shift toward repair and homeostatic pathways, characterized by extracellular matrix remodeling, transcriptional recovery, and regulatory/metabolic programs such as PPAR, Plexins, and PTP, with consistent signatures of resolution across both epithelial and mesangial compartments.
4. Macrophages emerge as key mediators of refractoriness: they are enriched in non-responders, and their transcriptional and proteomic footprints associate with structural damage, persistent histological activity, and poorer long-term prognosis.
5. Spatial proteomics and urine-based biomarkers reinforce these findings: a combinatorial urinary panel (CD14, TIM-3, CD163, MCP-1/CCL2, CXCL10, BAFF, TWEAK) provides a mechanistic and non-invasive readout of intrarenal immune activity, offering a promising tool for patient stratification and longitudinal monitoring without the need for repeat biopsies.

8. FUTURE DIRECTIONS

This study provides new insights into the intrarenal immune landscape of lupus nephritis, but several important questions remain. Validation of our findings in larger and more diverse patient cohorts, spanning different histological classes and ethnic backgrounds, is needed to confirm the generalizability of these molecular patterns and to capture the full heterogeneity of the disease.

Integrating spatial transcriptomics with single-cell RNA sequencing, spatial proteomics, and epigenomics will be critical to achieve a multi-layered understanding of kidney inflammation. Such approaches could more precisely define effector cell populations and signaling networks that drive either tissue injury or repair.

Equally important is longitudinal sampling. Systematic follow-up biopsies and paired urine collections can provide unique insights into disease evolution, allowing early identification of molecular predictors of treatment response or flare and laying the foundation for biomarker-based patient stratification.

Our results highlight macrophage-related pathways and early-response gene signatures as central to treatment resistance. Functional studies aimed at validating these mechanisms may uncover novel therapeutic targets for patients unresponsive to conventional immunosuppression.

Finally, the detection of CD14 and TIM-3 in urine supports their potential as non-invasive markers of intrarenal immune activity. Prospective validation of these and related biomarkers in clinical trials could enable real-time monitoring of therapeutic efficacy, reducing reliance on repeated biopsies.

Ongoing work in our group is focused on functional validation of key pathways identified here, with the goal of translating these molecular insights into more precise, personalized treatment strategies for lupus nephritis.

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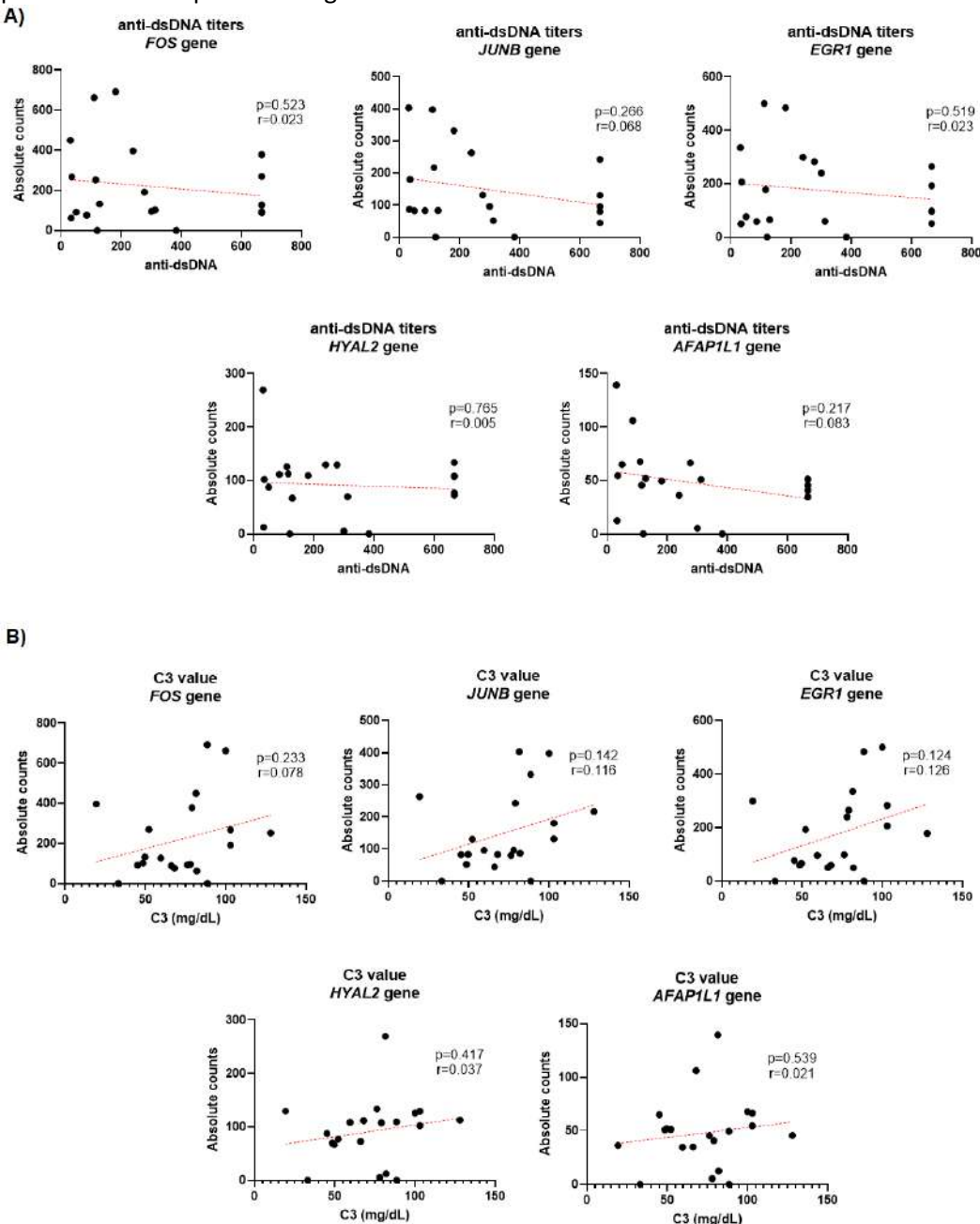
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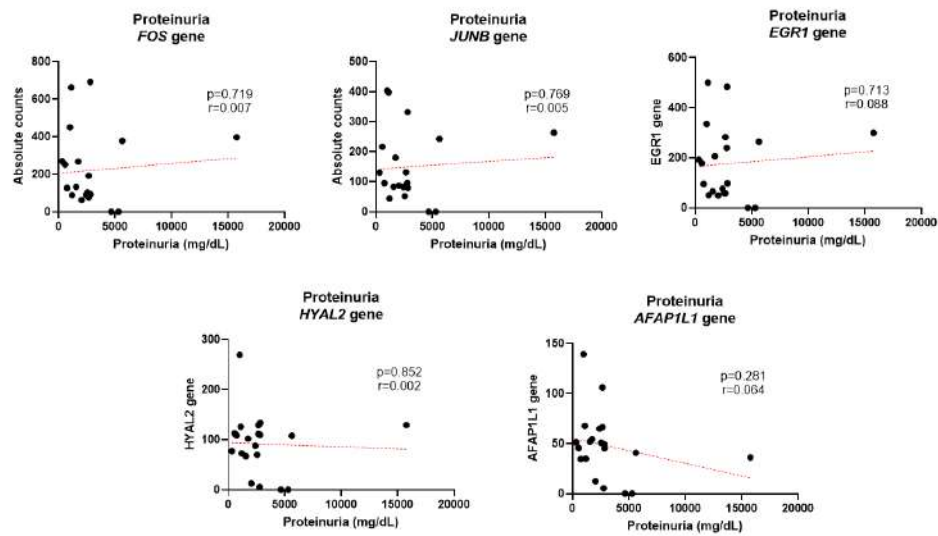
10.APPENDIX

10.1 Figures

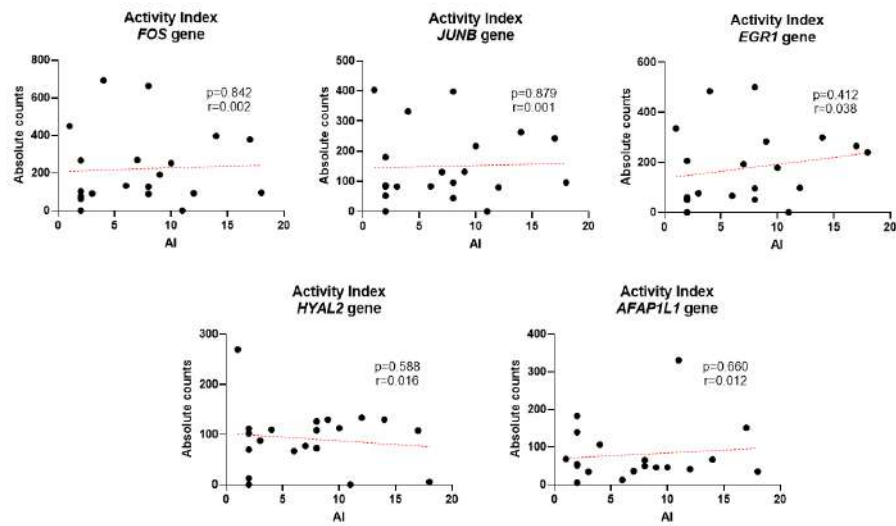
Appendix, Figure A1. Correlation of the top differentially expressed kidney transcripts at flare in CD45⁺ AOIs with clinical or histological parameters. Absolute gene counts obtained from spatial transcriptomic analysis of CD45⁺ AOIs were correlated with clinical parameters, including anti-dsDNA (A), C3 (B) and proteinuria values (C), as well as the activity (D) or chronicity index (E) (AI or CI). The corresponding p-values and correlation coefficient are provided in each panel. No significant correlations were found in CD45⁺ AOIs.



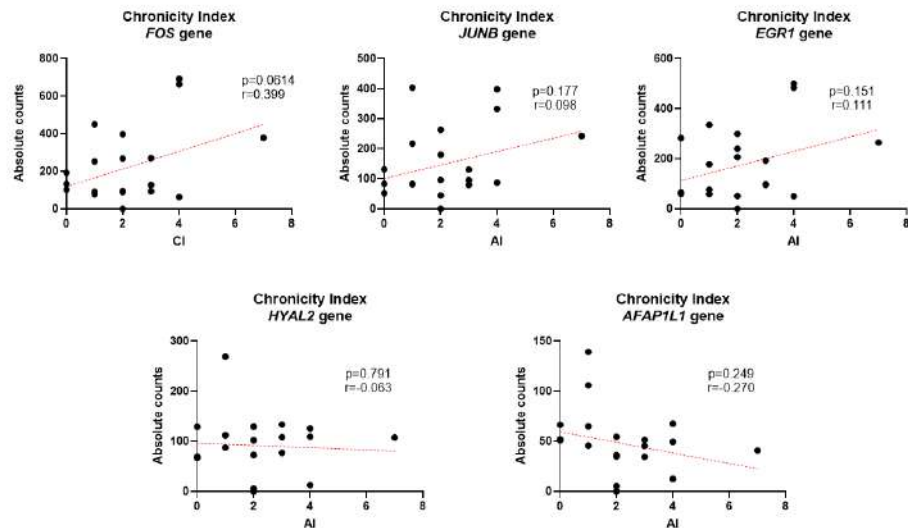
C)



D)

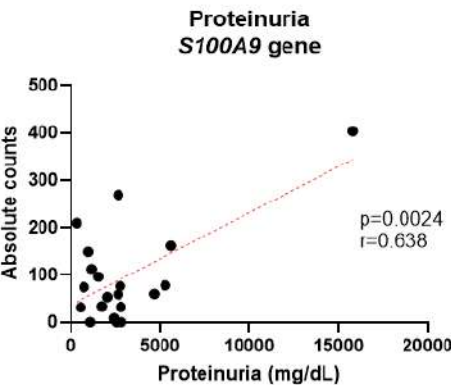


E)

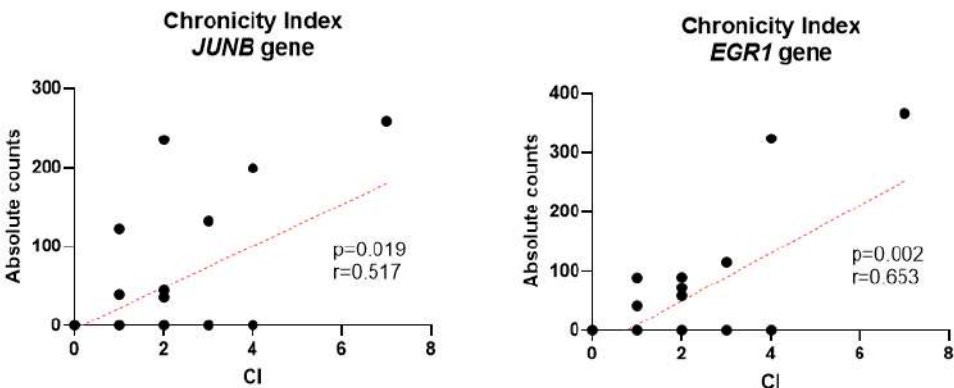


Appendix figure 2. Significant correlation of the top differentially expressed kidney transcripts at flare with clinical or histological parameters. Significant correlations are shown between absolute gene counts obtained in CD31⁻ (A), CD31⁺ (B) and PanCK⁺ (C) AOs with proteinuria and chronicity index (CI), respectively. The corresponding p-values and correlation coefficient are provided in each panel.

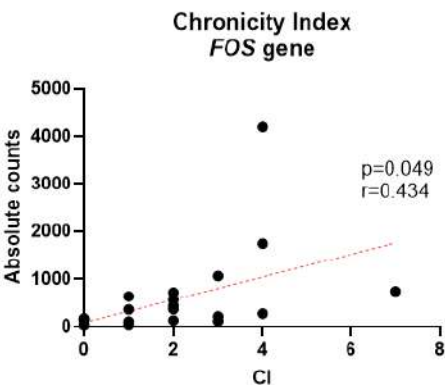
A) CD31⁻ AOs



B) CD31⁺ AOs



C) PanCK⁺ AOs



10.2 TABLES

Appendix, Table 1. Proteins Analyzed in the GeoMx IO Proteome Atlas for the Study of Renal Biopsies in Patients with Lupus Nephritis

PROTEIN NAME	GENE	Ab ID	AbCam-Predicted Reactive Species
15 Lipoxygenase 1	ALOX15	ab245201	House Mouse, Human, Norway Rat
15-PGDH	HPGD	ab243942	Human
2B4	CD244	ab275892	Human
5 Lipoxygenase/5-LO	ALOX5	ab227991	Dog, House Mouse, Human, Norway Rat
ACE2	ACE2	ab239924	House Mouse, Human, Norway Rat
Actin	ACTA1	ab219733	Chicken, House Mouse, Human, Norway Rat
active YAP1	YAP1	ab223126	House Mouse, Human
Adenosine Receptor A2a	ADORA2A	ab273879	House Mouse, Human, Norway Rat
AIF	TXNDC12,AIF M1	ab220215	House Mouse, Human, Norway Rat
AKR1B10	AKR1B10	ab232623	Human
AKT1 (phospho S473)	AKT1	ab215873	House Mouse, Human, Norway Rat
AKT1 (phospho T450)	AKT1	ab247614	House Mouse, Human, Norway Rat
AKT3 + AKT2 + AKT1	AKT2,AKT1,AKT3	ab219588	Cattle, House Mouse, Human, Norway Rat
ALDH1A1	ALDH1A1	ab206396	Human
Aldolase	ALDOA	ab273636	House Mouse, Human, Norway Rat
Aldolase B+Aldolase C	ALDOC,ALDOB	ab248483	House Mouse, Human, Norway Rat
ALK	ALK	ab271941	Human
alpha smooth muscle Actin	ACTA2	ab220795	House Mouse, Human, Norway Rat
AMPK alpha 1	PRKAA1	ab210714	House Mouse, Human, Norway Rat
Androgen Receptor	AR	ab271891	House Mouse, Human, Norway Rat
APC	APC	ab239828	House Mouse, Human, Norway Rat
APG5L/ATG5	ATG5	ab227084	Human
ARID1A	ARID1A	ab217154	House Mouse, Human, Norway Rat
ARID3A	ARID3A	ab249528	House Mouse, Human, Norway Rat
ASS1	ASS1	ab231684	Cattle, House Mouse, Human, Norway Rat
ATF2	ATF2	ab247240	Human
ATF3	ATF3	ab256534	House Mouse, Human
ATG12	ATG12	ab247876	House Mouse, Human, Norway Rat
ATG7	ATG7	ab227564	Human
ATM	ATM	ab216617	Human
ATM (phospho S1981)	ATM	ab208775	Human
ATR (phospho S428)	ATR	ab230831	House Mouse, Human, Norway Rat
Aurora A	AURKA	ab232013	Human
Axl	AXL	ab240396	Human
Bad	BAD	ab220116	Dog, House Mouse, Human, Norway Rat

Bak	BAK1	ab220790	Human
Bax	BAX	ab216985	Cattle, House Mouse, Human, Norway Rat
Bcl-2	BCL2	ab185002	Human
Bcl6	BCL6	ab243920	House Mouse, Human, Norway Rat
Bcl-XL	BCL2L1	ab199099	House Mouse, Human, Norway Rat, Pig
Beclin 1	BECN1	ab232461	House Mouse, Human, Norway Rat
beta 2 Microglobulin	B2M	ab237032	House Mouse, Human, Norway Rat
beta Actin	ACTB	ab264083	House Mouse, Human, Norway Rat
beta Arrestin 1	ARRB1	ab206776	Cattle, House Mouse, Human, Norway Rat
beta Catenin	CTNNB1	ab196204	
beta glucuronidase (GUSB)	GUSB	ab249385	Human
beta Tubulin	TUBB	ab226028	House Mouse, Human, Norway Rat, Vervet
BFL-1/GRS	BCL2A1	ab253247	Human
Bid	BID	ab247217	Human
Bim	BCL2L11	ab170589	House Mouse, Human, Norway Rat
BRAF	BRAF	ab189351	House Mouse, Human, Norway Rat
BRCA1	BRCA1	ab264518	Human
Brd4	BRD4	ab289893	House Mouse, Human, Norway Rat
BRG1	SMARCA4	ab222230	House Mouse, Human, Norway Rat
BTK	BTK	ab227812	House Mouse, Human, Norway Rat
C Reactive Protein	CRP	ab256546	Human
C4a	C4B,C4A	ab249610	Human
C5 / C5b	C5	ab275933	House Mouse, Human, Norway Rat
Carbonic Anhydrase 3/CA3	CA3	ab250486	House Mouse, Human, Norway Rat
Carbonic Anhydrase 9/CA9	CA9	ab270401	House Mouse, Human, Norway Rat
Caspase-10/CASP-10	CASP10	ab249980	Human
Caspase-3	CASP3	ab197202	House Mouse, Human
Caspase-3 p12	CASP3	ab238936	House Mouse, Human, Norway Rat
Catalase	CAT	ab227116	Human
Cathepsin G	CTSG	ab282121	Human
Cathepsin K	CTSK	ab223140	Human
Cathepsin S	CTSS	ab248706	Human
Caveolin-1	CAV1	ab240332	House Mouse, Human
CCL18	CCL18	ab300058	House Mouse, Human, Norway Rat
CCR3	CCR3	ab271832	House Mouse, Human, Macaques, Norway Rat
CCR5	CCR5	ab287963	Human
CCR6	CCR6	ab243852	Human
CCR7	CCR7	ab272938	House Mouse, Human, Norway Rat
CD103	ITGAE	ab254201	House Mouse, Human, Norway Rat
CD11a	ITGAL	ab227119	Crab-Eating Macaque, Human
CD11b	ITGAM	ab187537	Human
CD11c	ITGAX	ab216655	Human
CD127	IL7R	ab282011	Human
CD133	PROM1	ab284397	House Mouse, Human
CD134 / OX40L receptor	TNFRSF4	ab264470	Human

CD14	CD14	ab230903	Human
CD15	FUT4	ab240171	Human
CD16	FCGR3A	ab252908	Human
CD163	CD163	ab213612	House Mouse, Human, Norway Rat
CD177	CD177	ab256510	Human
CD18	ITGB2	ab307407	House Mouse, Human, Norway Rat
CD20	MS4A1	ab236434	Cattle, Dog, House Mouse, Human, Norway Rat
CD22	CD22	ab240404	Dog, Human
CD27	CD27	ab256583	Human
CD272/BTLA	BTLA	ab254387	Human
CD276	CD276	ab240407	House Mouse, Human, Norway Rat
CD28	CD28	ab243557	House Mouse, Human, Norway Rat
CD3 epsilon	CD3E	ab271850	Human
CD31	PECAM1	ab225883	House Mouse, Human, Norway Rat
CD33	CD33	ab281568	Human
CD34	CD34	ab198395	African Savanna Elephant, Dog, House Mouse, Human, Norway Rat, Sheep
CD36	CD36	ab221605	Domestic Guinea Pig, Human
CD38	CD38	ab236232	Human, Rhesus Monkey
CD39	ENTPD1	ab236038	House Mouse, Human
CD3D	CD3D	ab226073	Human
CD3G	CD3G	ab229282	House Mouse, Human
CD4	CD4	ab181724	Human
CD40	CD40	ab223546	Human
CD40 Ligand	CD40LG	ab303611	House Mouse, Human, Norway Rat
CD44	CD44	ab216647	House Mouse, Human, Norway Rat
CD44v6	CD44	ab255931	Human
CD45	PTPRC	ab214437	Human
CD45RA	PTPRC	ab256137	Human
CD47	CD47	ab284138	Human
CD63	CD63	ab215821	Human
CD64	FCGR1A	ab302902	House Mouse, Human, Norway Rat
CD66b	CEACAM8	ab300123	House Mouse, Human, Norway Rat
CD68	CD68	ab227458	Human
CD70	CD70	ab300084	House Mouse, Human, Norway Rat
CD73	NT5E	ab271895	Human
CD74	CD74	ab247655	Human
CD79a	CD79A	ab239891	Human
CD79b	CD79B	ab240083	House Mouse, Human, Norway Rat
CD8 alpha	CD8A	ab251596	Human, Norway Rat
CD83	CD83	ab275032	Human
CD86	CD86	ab269593	Human
CD90 / Thy1	THY1	ab181885	Human, Norway Rat
CD94	KLRD1	ab238166	Human
CD99	CD99	ab240990	Human
CDK1	CDK1	ab264084	African Clawed Frog, Chicken, House Mouse, Human, Norway Rat
CDK1 (phospho T161) + CDK2 / CDK3 (phospho T160)	CDK1,CDK3,CDK2	ab251327	House Mouse, Human, Norway Rat
CDK1 + Cdk2 + Cdk3 (phospho T14)	CDK1,CDK3,CDK2	ab219586	Cattle, House Mouse, Human, Norway Rat

CDK1 + CDK2 + CDK3 + CDK5 (phospho Y15)	CDK1,CDK3,CDK2,CDK5	ab248508	
Cdk2	CDK2	ab240937	House Mouse, Human, Norway Rat
Cdk4	CDK4	ab213216	Human
Cdk6	CDK6	ab222395	Human
CDKN2A/p14ARF	CDKN2A	ab271999	Human
CDKN2A/p16INK4a	CDKN2A	ab186932	Human
c-Fos	FOS	ab302668	House Mouse, Human, Norway Rat
Chk1	CHEK1	ab210964	Human
Chk2	CHEK2	ab227998	Human
cIAP2	BIRC3	ab239800	Human
c-Jun	JUN	ab247284	House Mouse, Human, Norway Rat
c-Jun (phospho S63)	JUN	ab227533	Cattle, House Mouse, Human, Norway Rat
c-Kit	KIT	ab216450	Human, White-Tufted-Ear Marmoset
Cleaved PARP1	PARP1	ab272005	House Mouse, Human, Norway Rat
c-Maf	MAF	ab219213	House Mouse, Human, Norway Rat
c-Myb (phospho S11)	MYB	ab217945	Human
c-Myc	MYC	ab168727	House Mouse, Human, Norway Rat
c-Myc (phospho S62)	MYC	ab232691	House Mouse, Human, Norway Rat
Collagen I	COL1A1,COL1A2	ab215969	Cattle, Human
Collagen IV	COL4A3,COL4A2,COL4A1,COL4A4,COL4A5	ab256353	House Mouse, Human, Norway Rat
COX2 / Cyclooxygenase 2	PTGS2	ab227528	House Mouse, Human, Norway Rat
CPT1A	CPT1A	ab234906	Human
CREB	CREB1	ab221216	Cattle, House Mouse, Human, Norway Rat
CREB (phospho S133)	CREB1	ab220798	Cattle, Chicken, House Mouse, Human, Norway Rat, Zebrafish
CREBBP	CREBBP	ab283283	House Mouse, Human, Norway Rat
CSF-1-R	CSF1R	ab240265	Human, Rat
CTLA4	CTLA4	ab251599	House Mouse, Human, Rhesus Monkey
Cullin 1/CUL-1	CUL1	ab202555	House Mouse, Human, Norway Rat
CXCL10/IP-10	CXCL10	ab306588	Human
CXCL5 + CXCL6	CXCL6,CXCL5	ab284855	Human
CXCR3	CXCR3	ab288446	House Mouse, Human, Norway Rat
CXCR4	CXCR4	ab271934	House Mouse, Human
CXCR5	CXCR5	ab272936	House Mouse, Human, Norway Rat
CXCR6	CXCR6	ab281934	Human
Cyclin A2	CCNA2	ab231164	House Mouse, Human, Norway Rat
Cyclin B1	CCNB1	ab156447	Human
Cyclin D1	CCND1	ab156448	House Mouse, Human, Norway Rat
Cyclin E1	CCNE1	ab245729	Human
Cyclin E2	CCNE2	ab239833	Human
Cyclophilin A	PPIA	ab248157	House Mouse, Human, Norway Rat
Cytokeratin 1	KRT1	ab250844	House Mouse, Human, Norway Rat
Cytokeratin 14	KRT14	ab236439	Cattle, House Mouse, Human, Norway Rat, Pig

Cytokeratin 17	KRT17	ab239986	House Mouse, Human, Norway Rat
Cytokeratin 19	KRT19	ab195872	House Mouse, Human
Cytokeratin 5	KRT5	ab236216	Cattle, Dog, House Mouse, Human, Norway Rat, Pig
Cytokeratin 8	KRT8	ab217173	House Mouse, Human, Norway Rat
Cytokeratin 8 (phospho S431)	KRT8	ab284783	Human
Dihydrofolate reductase (DHFR)	DHFR	ab248017	House Mouse, Human, Norway Rat
Dnmt1	DNMT1	ab191382	Human
DR5	TNFRSF10B	ab243847	Human
DUSP6	DUSP6	ab220811	House Mouse, Human, Norway Rat
E Cadherin	CDH1	ab256580	Human
EGFR	EGFR	ab227459	House Mouse, Human, Norway Rat
EGFR (phospho Y1068)	EGFR	ab182618	House Mouse, Human
EMR1/ADGRE1	ADGRE1	ab270798	Human
ENO1 + ENO2 + ENO3	ENO3, ENO1, ENO2	ab251571	House Mouse, Human
eNOS	NOS3	ab282129	House Mouse, Human
EpCAM	EPCAM	ab225894	Human
ErbB2 / HER2	ERBB2	ab194979	House Mouse, Human
ErbB2 / HER2 (phospho Y877)	ERBB2	ab238437	Human
ErbB3 / HER3	ERBB3	ab236220	Human
ErbB4 / HER4 (phospho Y1162)	ERBB4	ab247386	Human
ErbB4 / HER4+ErbB2 / HER2	ERBB4, ERBB2	ab263027	House Mouse, Human
ERG	ERG	ab214796	House Mouse, Human, Norway Rat
ERK1	MAPK3	ab214168	House Mouse, Human
ERK1 (phospho T202 + Y204) + ERK2 (phospho T185 + Y187)	MAPK1, MAPK3	ab242418	Human
ERK1 (phospho T202) + ERK2 (phospho T185)	MAPK1, MAPK3	ab232370	House Mouse, Human, Norway Rat
ERK2	MAPK1	ab269567	House Mouse, Human, Norway Rat
ETS1	ETS1	ab238645	House Mouse, Human, Norway Rat
FABP4	FABP4	ab219595	House Mouse, Human, Norway Rat
FADD	FADD	ab232045	Human
FAK	PTK2	ab271836	Cattle, House Mouse, Human, Norway Rat
FANCI	FANCI	ab273628	Human
Fas	FAS	ab178076	Human
Fatty Acid Synthase	FASN	ab221934	House Mouse, Human, Norway Rat
FBP2	FBP2	ab248391	House Mouse, Human, Norway Rat
FGF2	FGF2	ab246354	Human
FGFR2	FGFR2	ab289993	Human
Fibronectin	FN1	ab268023	House Mouse, Human, Norway Rat
Fos B	FOSB	ab240288	House Mouse, Human, Norway Rat
FOXO1A	FOXO1	ab240212	House Mouse, Human, Norway Rat
FOXO4/AFX	FOXO4	ab248170	Human

FOXP3	FOXP3	ab96048	Human
Galectin 3	LGALS3	ab251504	Human
gamma H2A.X (phospho S139)	H2AX	ab215967	House Mouse, Human, Norway Rat, Sheep
GATA3	GATA3	ab214804	House Mouse, Human
GGT1/GGT	GGT1	ab247856	Human
GITR	TNFRSF18	ab251600	Human
GLB1/Beta- galactosidase	GLB1	ab2305175	House Mouse, Human, Norway Rat
GLP-1	GCG	ab226036	Human
GLP-1R	GLP1R	ab274381	Human
Glucocorticoid Receptor (phospho S226)	NR3C1	ab256549	Human
Glucose 6 Phosphate Dehydrogenase	G6PD	ab231828	House Mouse, Human, Norway Rat
Glucose Transporter GLUT1	SLC2A1	ab252403	House Mouse, Human, Norway Rat
Glutaminase	GLS	ab214802	Human
Glutathione Peroxidase 4	GPX4	ab219592	House Mouse, Human, Norway Rat
GNLY/Granulysin	GNLY	ab241511	Human
Granzyme A	GZMA	ab251499	Human
Granzyme B	GZMB	ab226162	Human
Granzyme K	GZMK	ab282714	Human
GRP78 BiP	HSPA5	ab226042	House Mouse, Human, Norway Rat
GSK3 beta	GSK3B	ab183177	Human, Recombinant fragment
GSK3 beta (phospho S9)	GSK3B	ab219359	Human
HDAC1	HDAC1	ab213701	House Mouse, Human, Norway Rat
HDAC2	HDAC2	ab213700	House Mouse, Human, Norway Rat
HDAC3	HDAC3	ab247459	House Mouse, Human, Norway Rat
HDAC4 + 5 + 9	HDAC4, HDA C5, HDAC9	ab271355	House Mouse, Human, Norway Rat
HDAC6	HDAC6	ab210472	Human, Old World Monkeys <Cercopithecidae>, Vervet
HDAC9	HDAC9	ab239979	Human
Heme Oxygenase 1	HMOX1	ab219360	Domestic Guinea Pig, House Mouse, Human
Hexokinase 1	HK1	ab233837	House Mouse, Human, Norway Rat
Hexokinase II	HK2	ab228819	House Mouse, Human, Norway Rat
HIF-1 alpha	HIF1A	ab210073	Human
Histone H2A (acetyl K5)	H2AC8, H2AC 21, H2AC4, H2 AC20	ab247302	House Mouse, Human, Norway Rat, Roundworm <Caenorhabditis Elegans>
Histone H2A (phospho T120)	H2AC8, H2AC 21, H2AC4, H2 AC20	ab249963	Human
Histone H2A.X	H2AX	ab256544	Human
Histone H2B (acetyl K5)	H2BC21, H2B C12L	ab247300	House Mouse, Human, Norway Rat, Roundworm <Caenorhabditis Elegans>
Histone H3 (acetyl K14)	H3C4, H3C6, H3C11, H3C7, H3C2, H3C12,	ab203952	Human, Mouse, Rat

	H3C3,H3C10, H3C1,H3C8		
Histone H3 (acetyl K18)	H3C4,H3C6, H3C11,H3C7, H3C2,H3C12, H3C3,H3C10, H3C1,H3C8	ab239413	House Mouse, Human, Norway Rat
Histone H3 (acetyl K27)	H3C4,H3C6, H3C11,H3C7, H3C2,H3C12, H3C3,H3C10, H3C1,H3C8	ab302877	House Mouse, Human, Norway Rat
Histone H3 (acetyl K9)	H3C4,H3C6, H3C11,H3C7, H3C2,H3C12, H3C3,H3C10, H3C1,H3C8	ab203951	House Mouse, Human, Norway Rat
Histone H3 (di methyl K4)	H3C4,H3C6, H3C11,H3C7, H3C2,H3C12, H3C3,H3C10, H3C1,H3C8	ab173324	Cattle, Chicken, House Mouse, Human, Norway Rat, Old World Monkeys <Cercopithecidae>, Rice, Vervet
Histone H3 (di methyl K79)	H3C4,H3C6, H3C11,H3C7, H3C2,H3C12, H3C3,H3C10, H3C1,H3C8	ab249947	House Mouse, Human, Norway Rat
Histone H3 (mono methyl K4)	H3C4,H3C6, H3C11,H3C7, H3C2,H3C12, H3C3,H3C10, H3C1,H3C8	ab239402	House Mouse, Human, Norway Rat
Histone H3 (phospho S10)	H3C4,H3C6, H3C11,H3C7, H3C2,H3C12, H3C3,H3C10, H3C1,H3C8	ab238673	Human, Mouse, Drosophila melanogaster
Histone H3 (phospho S28)	H3C4,H3C6, H3C11,H3C7, H3C2,H3C12, H3C3,H3C10, H3C1,H3C8	ab215532	Domestic Guinea Pig, House Mouse, Human, Norway Rat
Histone H3 (tri methyl K27)	H3C4,H3C6, H3C11,H3C7, H3C2,H3C12, H3C3,H3C10, H3C1,H3C8	ab222481	House Mouse, Human, Norway Rat
Histone H3 (tri methyl K36)	H3C4,H3C6, H3C11,H3C7, H3C2,H3C12, H3C3,H3C10, H3C1,H3C8	ab282596	House Mouse, Human, Norway Rat
Histone H3 (tri methyl K9)	H3C4,H3C6, H3C11,H3C7, H3C2,H3C12,	ab232324	House Mouse, Human, Norway Rat

	H3C3,H3C10, H3C1,H3C8		
HLA A	HLA-A	ab216653	Human, Norway Rat
HLA B7	HLA-B	ab247757	Human
HLA E	HLA-E	ab300554	House Mouse, Human, Norway Rat
HLA F	HLA-F	ab248133	Human
HLA G	HLA-G	ab283278	Human
HLA-C	HLA-C	ab307362	Human
HLA-DPB1	HLA-DPB1	ab246351	Human
HLA-DR	HLA-DRA	ab209968	House Mouse, Human, Norway Rat
HMGB1	HMGB1	ab216986	House Mouse, Human, Norway Rat
HMGB2	HMGB2	ab239992	House Mouse, Human, Norway Rat
Hsp27	HSPB1	ab247352	Human
Hsp27 (phospho S78)	HSPB1	ab247262	Human
Hsp70	HSPA1B,HSP A1A	ab250639	Human
Hsp90	HSP90AA1,H SP90AB1	ab240366	Human, Mouse, Rat, Recombinant fragment
HuR / ELAVL1	ELAVL1	ab242410	House Mouse, Human, Norway Rat
ICAM1	ICAM1	ab271852	Human
ICOS	ICOS	ab236226	House Mouse, Human
IDH1	IDH1	ab242078	House Mouse, Human, Norway Rat
IDH2	IDH2	ab230796	House Mouse, Human, Norway Rat
IFIT1	IFIT1	ab305302	House Mouse, Human, Norway Rat
IFNGR1	IFNGR1	ab226151	Human
IGF1 Receptor	IGF1R	ab267345	Human
IGF2BP1/IMP1	IGF2BP1	ab229700	Human
Ikaros	IKZF1	ab300417	House Mouse, Human, Norway Rat
IKB alpha	NFKBIA	ab183134	House Mouse, Human, Norway Rat
IKK alpha	CHUK	ab171362	Human
IKK beta	IKBKB	ab171364	House Mouse, Human, Norway Rat
IKK gamma/NEMO	IKBKG	ab230832	House Mouse, Human, Norway Rat
IKKi/IKKe	IKBKE	ab247993	Human
IL-1 alpha	IL1A	ab300502	House Mouse, Human, Norway Rat
IL-1 beta	IL1B	ab283822	House Mouse, Human, Norway Rat
IL-12A	IL12A	ab214433	House Mouse, Human
IL-12RB1	IL12RB1	ab280892	House Mouse, Human, Norway Rat
IL-15RA	IL15RA	ab282288	Human
IL-18	IL18	ab243295	Human
IL-1RA	IL1RN	ab303491	House Mouse, Human, Norway Rat
IL-2 Receptor alpha	IL2RA	ab215378	Human
IL22 RA2/IL-22BP	IL22RA2	ab267789	Human
IL-22RA1	IL22RA1	ab283701	Human
IL-2RG	IL2RG	ab282585	House Mouse, Human, Norway Rat
IL-33	IL33	ab236036	Human, Norway Rat
IL3RA/CD123	IL3RA	ab280370	Human
IL4	IL4	ab300139	House Mouse, Human, Norway Rat
IL-5RA	IL5RA	ab251603	Human
IL-6R	IL6R	ab280573	Human
IMP3	IGF2BP3	ab250113	Human
Indoleamine 2_3- dioxygenase	IDO1	ab271990	Human
iNOS	NOS2	ab239990	House Mouse, Human, Norway Rat
Insulin	INS	ab282464	House Mouse, Human, Norway Rat

Insulin Receptor alpha	INSR	ab283717	House Mouse, Human, Norway Rat
Insulin Receptor beta	INSR	ab278104	House Mouse, Human, Norway Rat
Integrin alpha 5	ITGA5	ab239400	House Mouse, Human, Norway Rat
Integrin alpha 6	ITGA6	ab240252	House Mouse, Human, Norway Rat
Integrin beta 1	ITGB1	ab192456	Human
Interferon gamma	IFNG	ab231301	Human, Recombinant fragment
IRF3	IRF3	ab201809	House Mouse, Human
IRF4	IRF4	ab240071	Human
IRF5	IRF5	ab231163	House Mouse, Human, Norway Rat
ISG15	ISG15	ab285370	Human
JAK3	JAK3	ab232005	Human
JNK1 + JNK2 + JNK3 (phospho T183+T183+T221)	MAPK10,MAPK8,MAPK9	ab219584	House Mouse, Human, Norway Rat
JunD	JUND	ab250511	House Mouse, Human, Norway Rat
KDM3A / JHDM2A	KDM3A	ab272695	House Mouse, Human, Norway Rat
Ki67	MKI67	ab197547	House Mouse, Human, Norway Rat, White-Tufted-Ear Marmoset
KIR2DL1 + KIR2DL2	KIR2DL1	ab255857	Human
KIR2DL3	KIR2DL3	ab241538	Human
KIR3DL1 + KIR3DS1	KIR3DL1	ab256542	Human, Recombinant fragment
KMT6 / EZH2	EZH2	ab231165	House Mouse, Human, Norway Rat
KMT6/EZH2 (phospho T487)	EZH2	ab300568	House Mouse, Human, Norway Rat
Lactate Dehydrogenase	LDHB,LDHA	ab219591	House Mouse, Human, Norway Rat
LAG-3	LAG3	ab209740	Human
Lamin A + Lamin B1 + Lamin C	LMNA,LMNB1	ab226043	House Mouse, Human, Norway Rat, Thirteen-Lined Ground Squirrel
LAMP1	LAMP1	ab247693	Human
LAMP2A	LAMP2	ab240018	House Mouse, Human, Norway Rat
LC3B	MAP1LC3B	ab221794	House Mouse, Human, Norway Rat
Lck	LCK	ab229379	House Mouse, Human, Norway Rat
LDL Receptor	LDLR	ab271846	House Mouse, Human
LEF1	LEF1	ab215999	House Mouse, Human, Norway Rat
LILRB3	LILRB3	ab271297	Human
Liver Arginase	ARG1	ab211961	Human
LYVE1	LYVE1	ab282748	House Mouse, Human
Macrophage Inflammatory Protein 1 alpha / CCL3	CCL3L1,CCL3L3,CCL3	ab277944	Human, Mouse, Recombinant fragment
Macrophage Inflammatory Protein 3 alpha	CCL20	ab261730	Human
Mannose Receptor	MRC1	ab254471	Human
M-CSF	CSF1	ab234259	House Mouse, Human, Norway Rat
MDM2 (phospho S166)	MDM2	ab249560	House Mouse, Human, Norway Rat
MEK1	MAP2K1	ab239802	Cattle, Dog, House Mouse, Human, Norway Rat
MEK1 (phospho S298)	MAP2K1	ab214445	House Mouse, Human, Norway Rat

MEK2	MAP2K2	ab233731	House Mouse, Human
MEK4/MKK4 (phospho S80)	MAP2K4	ab284698	House Mouse, Human
MEKK3	MAP3K3	ab239826	House Mouse, Human
MelanA	MLANA	ab271839	Human
Melanoma gp100	PMEL	ab190913	Human
Met (c-Met)	MET	ab271998	Human, Recombinant fragment
Met (c-Met) (phospho Y1349)	MET	ab247359	Human
MICA + MICB	MICB,MICA	ab241513	Human, Recombinant fragment
MLH1	MLH1	ab214441	House Mouse, Human, Norway Rat
MMP1	MMP1	ab271845	Human
MMP14	MMP14	ab271840	Cattle, House Mouse, Human, Norway Rat
MMP3	MMP3	ab214794	Human, Mouse, Rat, Recombinant fragment
MMP7	MMP7	ab271977	Human
MMP8	MMP8	ab224265	House Mouse, Human, Norway Rat
MMP9	MMP9	ab204850	Human, Rat, Recombinant fragment
Monoamine Oxidase A/MAO-A	MAOA	ab240031	House Mouse, Human, Norway Rat
MRP8	S100A8	ab271863	House Mouse, Human
MS4A2	MS4A2	ab280570	Human
MSH2	MSH2	ab228334	Human
MSH6	MSH6	ab214454	House Mouse, Human, Norway Rat
mTOR	MTOR	ab218525	House Mouse, Human, Norway Rat
mTOR (phospho S2448)	MTOR	ab177734	House Mouse, Human, Pig
MUC1	MUC1	ab238994	Human
Mucin 5AC	MUC5AC	ab212636	House Mouse, Human, Norway Rat
Musashi 1 / Msi1	MSI1	ab221797	Chicken, Human, Mouse, Quail
MX1	MX1	ab284604	Human
MyD88	MYD88	ab199247	Human
Myeloperoxidase	MPO	ab226155	Human
N Cadherin	CDH2	ab271856	House Mouse, Human, Norway Rat
NAK/TBK1	TBK1	ab186470	House Mouse, Human, Norway Rat
Nanog	NANOG	ab218524	Human
NCAM1	NCAM1	ab248809	Human
NCR1	NCR1	ab254209	Human
Nectin 2	NECTIN2	ab239346	Human
Nectin-4	NECTIN4	ab251110	Human
Neutrophil Elastase	ELANE	ab243923	Human
NFAT2	NFATC1	ab264530	Human
NFkB p105 / p50	NFKB1	ab283716	Human
NF-kB p65	RELA	ab207297	House Mouse, Human
NKG2A + NKG2E + NKG2C	KLRC1,KLRC3 ,KLRC2	ab241456	Human
NKG2D	KLRK1,KLRC4 -KLRK1	ab302908	House Mouse, Human, Norway Rat
Notch1	NOTCH1	ab221603	Cattle, House Mouse, Human
NQO1	NQO1	ab264434	House Mouse, Human, Norway Rat
NRF1	NRF1	ab221792	House Mouse, Human, Norway Rat
NSD3	NSD3	ab300490	House Mouse, Human, Norway Rat
Occludin	OCLN	ab224526	Dog, House Mouse, Human, Norway Rat
Osteopontin	SPP1	ab236213	Human

p27 KIP 1	CDKN1B	ab206927	House Mouse, Human, Norway Rat
p27 KIP 1 (phospho S10)	CDKN1B	ab228877	House Mouse, Human, Norway Rat, Old World Monkeys <Cercopithecidae>
p38 (phospho T180)	MAPK14	ab236527	Human
p38 beta/MAPK11 + p38 alpha/MAPK14	MAPK11,MAPK14	ab227995	House Mouse, Human, Norway Rat
p40 - DeltaNp63	TP63	ab269956	House Mouse, Human, Norway Rat
p53	TP53	ab225531	House Mouse, Human, Norway Rat
p53 (acetyl K373)	TP53	ab239856	Human
p53 (phospho S392)	TP53	ab284810	Human
p53 (phospho S46)	TP53	ab239880	Human
p57 Kip2	CDKN1C	ab284805	Human
p63	TP63	ab214790	House Mouse, Human, Norway Rat
p73	TP73	ab251550	Human
PAK1	PAK1	ab302507	House Mouse, Human, Norway Rat
PAK1+PAK2+PAK3 (phospho S141 + S144 + S154)	PAK1,PAK2,PAK3	ab239830	House Mouse, Human, Norway Rat
pan Cytokeratin	KRT77,KRT76	ab264485	House Mouse, Human, Norway Rat
Pan Trk	NTRK1,NTRK3,NTRK2	ab188825	Human, Mouse, Rat, Recombinant fragment
PARP1	PARP1	ab221923	Human
PCNA	PCNA	ab264494	Human, Rat
PD1	PDCD1	ab201811	Human
PDGF B	PDGFB	ab252808	Human
PDGFR alpha	PDGFRA	ab234965	House Mouse, Human, Norway Rat
PDGFR alpha + PDGFR beta	PDGFRA,PDGFRB	ab215978	House Mouse, Human, Norway Rat
PD-L1	CD274	ab251611	Human, Recombinant fragment
PD-L2	PDCD1LG2	ab288308	Human
Periostin	POSTN	ab227049	House Mouse, Human, Norway Rat
PF4	PF4	ab248336	Human, Norway Rat
PI 3 Kinase catalytic subunit alpha/PIK3CA	PIK3CA	ab243914	Cattle, Chicken, House Mouse, Human, Norway Rat
PI 3 Kinase catalytic subunit gamma/PI3K-gamma	PIK3CG	ab302959	House Mouse, Human, Norway Rat
PI 3 Kinase p85 alpha	PIK3R1	ab236440	Cattle, House Mouse, Human, Norway Rat
PI 3 Kinase p85 beta	PIK3R2	ab231161	Human, Norway Rat
PKC beta 1	PRKCB	ab232518	House Mouse, Human, Norway Rat
PLCG 2	PLCG2	ab248535	Human
Podoplanin / gp36	PDPN	ab237033	Human
Poliovirus Receptor/PVR	PVR	ab267794	Human
PP2A alpha + beta	PPP2CA,PPP2CB	ab247238	House Mouse, Human, Norway Rat
PRAME	PRAME	ab232571	Human
PRAS40	AKT1S1	ab249037	House Mouse, Human

PRDM1/Blimp1	PRDM1	ab307645	Human
Pro Caspase-8	CASP8	ab208774	House Mouse, Human, Norway Rat
Progesterone Receptor	PGR	ab206926	Human
Progesterone Receptor (phospho S190)	PGR	ab247409	Human
Prostate Specific Antigen	KLK3	ab271858	Human
PSMA	FOLH1	ab226141	Human
PTCH2	PTCH2	ab300037	House Mouse, Human, Norway Rat
PTEN	PTEN	ab243940	Dog, House Mouse, Human
PTEN (phospho T366)	PTEN	ab208104	House Mouse, Human, Norway Rat
PTGES2/Gbf1	PTGES2	ab300053	House Mouse, Human, Norway Rat
PU.1/Spi1	SPI1	ab302624	House Mouse, Human, Norway Rat
Rad51	RAD51	ab234634	Human
RAGE	AGER	ab228861	House Mouse, Human, Norway Rat
Raptor	RPTOR	ab239827	House Mouse, Human, Norway Rat
Ras	NRAS,KRAS, HRAS	ab275884	House Mouse, Human, Norway Rat
Rb	RB1	ab218526	House Mouse, Human, Vervet
Rb (phospho S608)	RB1	ab208693	House Mouse, Human, Norway Rat
Rb (phospho S807)	RB1	ab215530	House Mouse, Human, Norway Rat
Rb (phospho T252)	RB1	ab250779	Human
Rb (phospho T356)	RB1	ab247461	Human
Rb (phospho T373)	RB1	ab219158	Human
Rb (phospho T780)	RB1	ab240174	Human
Rel B	RELB	ab247280	Human
Ret	RET	ab214791	House Mouse, Human, Norway Rat
Retinoic Acid Receptor beta	RARB	ab247968	House Mouse, Human, Norway Rat
Rho	RHOC,RHOA, RHOB	ab247214	House Mouse, Human, Norway Rat
Rho A + B + C	RHOC,RHOA, RHOB	ab221161	House Mouse, Human, Norway Rat
ROCK1	ROCK1	ab230799	House Mouse, Human, Norway Rat
ROCK2 + ROCK1	ROCK2,ROCK 1	ab219587	Cattle, House Mouse, Human, Norway Rat
ROS1	ROS1	ab238986	Human
RPS6 (phospho S235 + S236)	RPS6	ab236224	House Mouse, Human
RPS6 (phospho S240 + S244)	RPS6	ab225578	House Mouse, Human, Norway Rat
RSK1 p90	RPS6KA1	ab239805	House Mouse, Human, Norway Rat, Pig
RSK1 p90 (phospho T359)	RPS6KA1	ab171376	House Mouse, Human, Norway Rat
RUNX1 / AML1 + RUNX3 + RUNX2	RUNX2,RUN X3,RUNX1	ab220117	House Mouse, Human, Norway Rat
S100 beta	S100B	ab215989	House Mouse, Human, Macaques, Norway Rat, Zebrafish
S100A12/CGRP	S100A12	ab273051	Human
S100A4	S100A4	ab216003	Human
S100A8 + S100A9	S100A9	ab288578	House Mouse, Human, Norway Rat
S100A9	S100A9	ab271864	Human

S100P	S100P	ab225543	Human
S6K1	RPS6KB1	ab203558	House Mouse, Human, Norway Rat
Scavenging Receptor SR-BI	SCARB1	ab272003	House Mouse, Human, Norway Rat
SCF	KITLG	ab247320	Human
SDHA	SDHA	ab240098	House Mouse, Human, Norway Rat
SFRP1	SFRP1	ab240023	Human
SFRP4	SFRP4	ab240126	House Mouse, Human, Norway Rat
SHP1	PTPN6	ab240949	House Mouse, Human, Norway Rat
SHP2	PTPN11	ab300580	House Mouse, Human, Norway Rat
SIGLEC5	SIGLEC5	ab307435	House Mouse, Human, Norway Rat
SIRT1	SIRT1	ab220807	Human
SIRT1 (phospho S47)	SIRT1	ab247432	Human
SIRT2	SIRT2	ab223534	House Mouse, Human, Norway Rat
SIRT3	SIRT3	ab223531	Human
SLC7A5/LAT1	SLC7A5	ab305252	House Mouse, Human, Norway Rat
Smac/Diablo	DIABLO	ab239797	House Mouse, Human, Norway Rat
SMAD1 + SMAD5 + SMAD9 (phospho S463 + S465 + S467)	SMAD5,SMA D9,SMAD1	ab168252	House Mouse, Human, Norway Rat
Smad2	SMAD2	ab157371	Human, Norway Rat
Smad2 (phospho S255)	SMAD2	ab219598	House Mouse, Human, Norway Rat
Smad2 (phospho S467)	SMAD2	ab280897	House Mouse, Human, Norway Rat
Smad3	SMAD3	ab157372	House Mouse, Human, Norway Rat
Smad3 (phospho S423 + S425)	SMAD3	ab172202	Fruit Fly <Drosophila Melanogaster>, House Mouse, Human
Smad4	SMAD4	ab215968	House Mouse, Human, Norway Rat
SMAD5	SMAD5	ab238952	House Mouse, Human, Norway Rat, Vervet
SOCS3	SOCS3	ab280889	House Mouse, Human, Norway Rat
SOD2/MnSOD	SOD2	ab227846	House Mouse, Human, Norway Rat
SOD2/MnSOD (acetyl K68)	SOD2	ab218529	House Mouse, Human, Norway Rat
Somatostatin Receptor 2	SSTR2	ab271907	House Mouse, Human, Norway Rat
Sonic Hedgehog	SHH	ab175180	Human
SOX9	SOX9	ab225541	Cattle, Dog, House Mouse, Human, Norway Rat, Pig, White-Tufted-Ear Marmoset
SQSTM1 / p62	SQSTM1	ab227992	Human
Src	SRC	ab222220	House Mouse, Human, Norway Rat
ST2	IL1RL1	ab291094	House Mouse, Human, Norway Rat
STAT1	STAT1	ab234904	Human
STAT1 (phospho S727)	STAT1	ab215820	House Mouse, Human, Norway Rat
STAT2	STAT2	ab239811	House Mouse, Human, Norway Rat
STAT3	STAT3	ab171359	House Mouse, Human, Norway Rat
STAT3 (phospho Y705)	STAT3	ab280202	Human
STAT4	STAT4	ab247366	Human

STAT5 (phospho Y694)	STAT5A,STAT 5B	ab219607	House Mouse, Human
STAT5a	STAT5A	ab213219	House Mouse, Human, Norway Rat
STAT5b	STAT5B	ab240211	House Mouse, Human, Norway Rat
STAT6	STAT6	ab215995	House Mouse, Human
STAT6 (phospho Y641)	STAT6	ab263950	House Mouse, Human, Norway Rat
STING	STING1	ab242019	Human
Sumo 1	SUMO1	ab248491	House Mouse, Human, Norway Rat
Survivin	BIRC5	ab192675	Human
Syk	SYK	ab190176	House Mouse, Human, Norway Rat
Syk (phospho Y323)	SYK	ab169865	Human
Syk (phospho Y352) +ZAP70 (phospho Y319)	SYK,ZAP70	ab300410	House Mouse, Human, Norway Rat
Syndecan-1	SDC1	ab226108	House Mouse, Human, Norway Rat
TAK1	MAP3K7	ab222394	House Mouse, Human, Norway Rat
TAP2	TAP2	ab307283	House Mouse, Human, Norway Rat
T-bet / Tbx21	TBX21	ab307194	House Mouse, Human, Norway Rat
TBR2 / Eomes	EOMES	ab261913	House Mouse, Human, Norway Rat
TEF1/TEAD-1	TEAD1	ab219647	House Mouse, Human, Norway Rat
TGF beta 1	TGFB1	ab229856	House Mouse, Human, Norway Rat
Thymidylate Synthase	TYMS	ab240947	Human
TIM 3	HAVCR2	ab242080	House Mouse, Human, Norway Rat
Tissue Factor	F3	ab254007	Human
TLR7	TLR7	ab210403	Human
TLR8	TLR8	ab260002	Human
TLR9	TLR9	ab250911	Human
TMPRSS2	TMPRSS2	ab242384	Human
TMS1/ASC	PYCARD	ab283712	Human
TNF alpha	TNF	ab307165	House Mouse, Human, Norway Rat
TNFAIP3	TNFAIP3	ab227987	House Mouse, Human
Topoisomerase I	TOP1	ab219735	House Mouse, Human, Norway Rat
Topoisomerase II alpha	TOP2A	ab181828	House Mouse, Human, Norway Rat
Topoisomerase II alpha (phospho S1106)	TOP2A	ab247394	Human
Topoisomerase II alpha (phospho T1343)	TOP2A	ab247333	Human
TRAF1	TRAF1	ab300076	House Mouse, Human, Norway Rat
TRAF2	TRAF2	ab249405	Human
TRAF6	TRAF6	ab218575	House Mouse, Human, Norway Rat, Zebrafish
Transferrin Receptor	TFRC	ab232376	House Mouse, Human
TROP2	TACSTD2	ab272014	House Mouse, Human
Tuberin	TSC2	ab247339	Human
Tuberin (phospho S1254)	TSC2	ab248503	Human
Tuberin (phospho T1462)	TSC2	ab247850	House Mouse, Human
UBE2C	UBE2C	ab269592	House Mouse, Human, Norway Rat

Ubiquitin	UBB	ab230145	House Mouse, Human, Norway Rat
uPA Receptor/U- PAR	PLAUR	ab251557	Human
Urokinase	PLAU	ab236060	House Mouse, Human, Norway Rat
VCAM1	VCAM1	ab271899	House Mouse, Human, Norway Rat
VEGF Receptor 1	FLT1	ab210608	House Mouse, Human
VEGF Receptor 2	KDR	ab243912	House Mouse, Human
VEGFA	VEGFA	ab185238	Human, Mouse
VEGFD	VEGFD	ab232447	House Mouse, Human, Norway Rat
Vimentin	VIM	ab193555	House Mouse, Human, Norway Rat, Rhesus Monkey, Vervet
VISTA	VSIR	ab238886	Human
Von Willebrand Factor	VWF	ab250094	Human
Wee1	WEE1	ab288729	House Mouse, Human
Wee1 (phospho S642)	WEE1	ab307516	House Mouse, Human, Norway Rat
Wilms Tumor Protein	WT1	ab216646	House Mouse, Human
Wnt3a	WNT3A	ab234099	House Mouse, Human, Norway Rat
YAP1	YAP1	ab172373	Human
YAP1 (phospho S127)	YAP1	ab172374	House Mouse, Human, Norway Rat
YKL-40/CHI3L1	CHI3L1	ab255864	Human
ZAP70	ZAP70	ab247258	Human

Appendix, Table A2. Differentially expressed genes in CD31⁺ AOs between responders and non-responders.

	Gene name	log2 fold change	P value	FDR
1	NUPR2	-0,774	0,000049	0,33
2	PFKFB2	-0,646	0,00021	0,33
3	AHDC1	-0,557	0,00028	0,33
4	SCAMP3	-0,811	0,00032	0,33
5	SLC12A3	-0,861	0,00036	0,33
6	DPP6	-0,752	0,00047	0,33
7	ZNRF3	-0,664	0,00051	0,33
8	BMP7	-0,535	0,00058	0,33
9	ADHFE1	-0,674	0,00058	0,33
10	RASEF	-0,569	0,00063	0,33
11	AJAP1	-0,539	0,00069	0,33
12	MYADM	0,669	0,00069	0,33
13	CYFIP2	-0,592	0,00071	0,33
14	LOX	-0,762	0,00071	0,33
15	PABIR1	-0,556	0,00072	0,33
16	ATG4D	-0,543	0,00078	0,33
17	KLF8	-0,531	0,00079	0,33
18	CYP3A5	-0,996	0,00087	0,35
19	NDUFS1	-0,58	0,00093	0,36
20	AGT	-0,696	0,0011	0,37
21	AQP7	-0,514	0,0011	0,37
22	UBR3	-0,805	0,0012	0,37
23	RNF186	-0,66	0,0013	0,37
24	SIAE	-0,564	0,0013	0,37
25	GPR155	-0,585	0,0014	0,37
26	NR1I3	-0,513	0,0015	0,37
27	KCNK5	-0,702	0,0015	0,37
28	GAMT	-0,806	0,0017	0,37
29	FLRT3	-0,601	0,0018	0,37
30	FABP3	-0,516	0,0018	0,37
31	SFXN5	-0,547	0,0018	0,37
32	ZFP36L1	0,707	0,0019	0,37
33	ZFP41	-0,567	0,0019	0,37
34	ARPC2	0,602	0,0019	0,37
35	EGR1	1,698	0,0021	0,39
36	NDNF	-1,285	0,0023	0,39
37	ZBTB18	-0,794	0,0023	0,39
38	DSG4	-0,798	0,0024	0,39
39	DUS1L	0,596	0,0025	0,39
40	ATXN3	-0,682	0,0026	0,39
41	ARMH4	-0,985	0,0026	0,39

42	LMX1B	-0,553	0,0026	0,39
43	TMED2	0,558	0,0028	0,40
44	PHYHIP	-0,811	0,0028	0,40
45	CLASP1	-0,607	0,0031	0,42
46	FN1	2,129	0,0033	0,42
47	EXOC8	-0,611	0,0034	0,42
48	ADORA1	-0,636	0,0035	0,42
49	TPD52	-0,547	0,0036	0,42
50	ITIH5	-0,586	0,0038	0,42
51	TANK	-0,856	0,0039	0,42
52	SPTLC3	-0,817	0,004	0,42
53	FMO1	-0,683	0,0041	0,42
54	BRPF3	-0,647	0,0042	0,42
55	RFK	-0,503	0,0045	0,42
56	NFASC	-0,79	0,0045	0,42
57	EHHADH	-0,503	0,0046	0,42
58	TLR3	-0,577	0,0047	0,42
59	S100A4	0,687	0,0048	0,42
60	PLG	-0,6	0,005	0,42
61	PTGR2	-0,72	0,005	0,42
62	MORN3	-0,564	0,0052	0,42
63	JUNB	1,277	0,0052	0,42
64	RPS26	0,849	0,0053	0,42
65	TSPAN7	-0,925	0,0053	0,42
66	MKLN1	-0,545	0,0055	0,42
67	TNNT2	-0,825	0,0055	0,42
68	PDPN	-0,692	0,0057	0,42
69	LTO1	-0,513	0,0058	0,42
70	ITGA5	0,568	0,0058	0,42
71	TMOD1	-0,626	0,0059	0,42
72	CD248	0,634	0,0059	0,42
73	RPL32	0,562	0,006	0,42
74	BRCC3	-0,527	0,0061	0,42
75	IFNAR2	0,516	0,0064	0,42
76	LRRC8A	-0,527	0,0066	0,42
77	SOX4	0,62	0,0066	0,42
78	CIT	-0,571	0,0067	0,42
79	CRHBP	-1,802	0,0067	0,42
80	FBXL8	-0,534	0,0068	0,42
81	MGAM	-0,538	0,0068	0,42
82	RAB17	-0,507	0,0068	0,42
83	RPL12	0,584	0,007	0,42
84	HMGCS2	-0,628	0,0071	0,42
85	TSHZ1	-0,58	0,0072	0,42
86	TNIP2	-0,552	0,0073	0,42

87	CCNDBP1	-0,591	0,0073	0,42
88	SERPINH1	0,555	0,0077	0,42
89	VTI1A	-0,512	0,0077	0,42
90	IQGAP1	0,525	0,0077	0,42
91	ITGA11	0,715	0,0078	0,42
92	PRX	-0,888	0,0078	0,42
93	PIK3C2G	-0,601	0,0078	0,42
94	ZFYVE9	-0,628	0,0079	0,42
95	OGDHL	-0,528	0,008	0,42
96	PROSER1	-0,589	0,008	0,42
97	AAMDC	-0,629	0,0081	0,42
98	GSTA2	-0,611	0,0086	0,42
99	SEMA3B	-0,882	0,0087	0,42
100	RPL23	0,636	0,0087	0,42
101	DUSP6	0,669	0,009	0,42
102	EXPH5	-0,671	0,009	0,42
103	CRIP1	-0,606	0,0091	0,42
104	ITGB4	-0,514	0,0092	0,42
105	COBL	-0,699	0,0092	0,42
106	PAPPA2	-0,574	0,0092	0,42
107	PC	-0,52	0,0093	0,42
108	ENPP4	-0,705	0,0093	0,42
109	MAB21L4	-0,567	0,0095	0,42
110	ATP6V0A4	-0,705	0,0095	0,42
111	HS6ST2	-0,546	0,0096	0,42
112	HBB	-1,164	0,0096	0,42
113	GRB10	-0,54	0,0097	0,42
114	THOP1	-0,712	0,0099	0,42
115	PRPF4	-0,531	0,01	0,42
116	MFSD4A	-0,708	0,01	0,42
117	CD276	0,764	0,01	0,42
118	KL	-0,647	0,01	0,42
119	HNH4A	-0,755	0,01	0,42
120	MCL1	0,671	0,011	0,42
121	C1orf21	-0,524	0,011	0,42
122	CMTM8	-0,541	0,011	0,42
123	MAP2K6	-0,732	0,011	0,42
124	MCCD1	-0,643	0,011	0,42
125	EHD3	-1,38	0,011	0,42
126	RWDD4	-0,631	0,011	0,42
127	GINM1	-0,509	0,011	0,42
128	BCL9	-0,77	0,011	0,42
129	CYP8B1	-0,576	0,011	0,42
130	UGT2A3	-0,589	0,011	0,42
131	UMOD	-0,934	0,011	0,42

132	CRIP1	0,779	0,011	0,42
133	MRGPRF	-0,712	0,011	0,42
134	DNAJC22	-0,574	0,012	0,42
135	PTPRQ	-0,707	0,012	0,42
136	TMEM178A	-0,553	0,012	0,42
137	YWHAH	0,68	0,012	0,42
138	ANKS1A	-0,661	0,012	0,42
139	INSYN1	-0,64	0,012	0,42
140	COL6A2	0,654	0,012	0,42
141	SLC25A24	-0,509	0,012	0,42
142	TUB	-0,827	0,012	0,42
143	PLCG2	-0,749	0,012	0,42
144	CLCNKB	-0,654	0,012	0,42
145	TLR4	-0,503	0,012	0,42
146	USP47	-0,511	0,013	0,42
147	AEBP1	0,959	0,013	0,42
148	TST	-0,573	0,013	0,42
149	CORO1C	0,685	0,013	0,42
150	CCDC126	-0,637	0,013	0,42
151	PAQR7	-0,531	0,013	0,42
152	GRHPR	-0,645	0,013	0,42
153	ABHD8	-0,559	0,013	0,42
154	CRB2	-0,663	0,014	0,42
155	COL1A1	1,145	0,014	0,42
156	RUNX1	0,773	0,014	0,42
157	SMTN	-0,555	0,014	0,42
158	KAZN	-0,607	0,014	0,42
159	CTH	-0,679	0,014	0,42
160	SOD2	0,694	0,014	0,42
161	TMEM139	-0,653	0,014	0,42
162	HNF4G	-0,759	0,014	0,42
163	GALNT3	-0,714	0,015	0,42
164	TMEM45B	-0,684	0,015	0,42
165	SMAD6	-0,705	0,015	0,42
166	RPL31	0,575	0,015	0,43
167	GTF2A2	-0,575	0,015	0,43
168	SEMA4G	-0,643	0,015	0,43
169	AVPI1	-0,52	0,016	0,43
170	UGT8	-0,618	0,016	0,43
171	FAM120C	-0,62	0,016	0,43
172	HDDC2	-0,547	0,016	0,43
173	RPS24	0,695	0,016	0,43
174	ST6GALNAC3	-0,583	0,016	0,43
175	PLPPR1	-0,647	0,016	0,43
176	IQGAP2	-0,741	0,016	0,43

177	TCEA3	-0,628	0,017	0,43
178	PICK1	-0,584	0,017	0,44
179	ZCRB1	-0,535	0,017	0,44
180	NPR3	-0,516	0,018	0,44
181	PVR	-0,606	0,018	0,44
182	UFSP2	-0,522	0,018	0,44
183	SLC16A1	-0,506	0,018	0,44
184	DUSP11	-0,626	0,018	0,44
185	HCAR1	-0,581	0,019	0,44
186	PABPC1L	-0,572	0,019	0,44
187	ZBTB10	-0,508	0,019	0,44
188	JUN	0,898	0,019	0,44
189	VIM	0,631	0,019	0,44
190	TPRKB	-0,618	0,019	0,44
191	TMSB10	0,673	0,02	0,44
192	MTMR3	-0,502	0,02	0,44
193	LRP11	-0,508	0,02	0,44
194	AGMAT	-0,518	0,02	0,44
195	GIPC2	-0,706	0,02	0,44
196	FXVD5	0,504	0,02	0,44
197	PXMP2	-0,633	0,02	0,44
198	RPS4X	0,64	0,02	0,44
199	NET1	-0,561	0,02	0,44
200	RAP2B	0,574	0,021	0,44
201	PI4KB	-0,503	0,021	0,44
202	ELMOD2	-0,54	0,021	0,44
203	FRMD1	-0,642	0,021	0,44
204	OGT	-0,536	0,021	0,44
205	ODR4	-0,585	0,021	0,44
206	NPHS1	-1,085	0,021	0,44
207	FOS	1,527	0,021	0,44
208	RPS27	0,54	0,022	0,44
209	LIMS3	0,523	0,022	0,44
210	ELP1	-0,503	0,022	0,44
211	FNBP1L	-0,5	0,022	0,44
212	SMPDL3B	-0,577	0,022	0,44
213	KLF15	-0,659	0,022	0,44
214	KIAA1549	-0,504	0,022	0,44
215	SERPINA5	-0,658	0,023	0,44
216	DACH1	-0,734	0,023	0,44
217	SEMA3G	-0,659	0,023	0,44
218	SYNGR3	-0,559	0,023	0,44
219	DAO	-0,505	0,023	0,44
220	KCNJ1	-0,658	0,023	0,44
221	TIMP1	1,079	0,023	0,44

222	PSPH	-0,516	0,023	0,44
223	GAS2L1	-0,554	0,024	0,44
224	ATP6V1G3	-0,533	0,024	0,44
225	ALB	-0,615	0,024	0,44
226	PTPN21	-0,612	0,024	0,44
227	RPL5	0,516	0,024	0,44
228	CLCN2	-0,565	0,024	0,44
229	PCOLCE2	-0,978	0,024	0,44
230	VCAM1	0,58	0,024	0,44
231	ARRDC4	-0,587	0,024	0,44
232	TACO1	-0,6	0,025	0,44
233	EMCN	-1,119	0,025	0,44
234	CDKN1C	-0,79	0,025	0,44
235	PLGRKT	-0,596	0,025	0,44
236	TGFBR3	-0,771	0,025	0,44
237	FRK	-0,539	0,025	0,45
238	NEK8	-0,61	0,026	0,45
239	CCR1	0,615	0,026	0,45
240	CCL2	1,084	0,026	0,45
241	NEBL	-0,535	0,026	0,45
242	CCL3	0,663	0,026	0,45
243	HBA2	-0,821	0,026	0,45
244	PEAR1	-0,576	0,026	0,45
245	BTNL9	-1,089	0,026	0,45
246	CDC23	-0,592	0,026	0,45
247	TNFRSF11B	0,574	0,026	0,45
248	CTS2	0,648	0,026	0,45
249	SIM1	-0,62	0,026	0,45
250	BCR	-0,527	0,027	0,45
251	BGN	0,623	0,027	0,45
252	TNFAIP3	0,541	0,027	0,45
253	CHRD1	-0,709	0,028	0,45
254	LDHA	0,507	0,028	0,45
255	SLC6A13	-0,501	0,028	0,45
256	DEFB1	-0,708	0,028	0,45
257	HSD11B2	-0,537	0,028	0,45
258	RORC	-0,592	0,029	0,45
259	COL1A2	1,115	0,029	0,45
260	MTO1	-0,501	0,029	0,45
261	TRIB1	0,583	0,029	0,45
262	CAPG	0,74	0,029	0,45
263	KCNH6	-0,657	0,03	0,45
264	RDH12	-0,544	0,03	0,45
265	PKHD1	-0,639	0,031	0,45
266	RAVER1	-0,511	0,031	0,46

267	CLEC14A	-0,609	0,032	0,46
268	CTSV	-0,687	0,032	0,46
269	FGF1	-0,804	0,032	0,46
270	FRMD3	-0,73	0,032	0,46
271	MRPS33	-0,568	0,032	0,46
272	RPS8	0,56	0,032	0,46
273	SUSD2	-0,621	0,032	0,46
274	SLC16A12	-0,556	0,032	0,46
275	CLIC5	-0,848	0,032	0,46
276	GLIPR1	0,589	0,033	0,46
277	ESRP1	-0,659	0,034	0,46
278	TNFRSF12A	0,791	0,034	0,46
279	MME	-0,905	0,034	0,46
280	RASGRP3	-0,683	0,034	0,46
281	S100A11	0,644	0,034	0,46
282	B3GNT8	-0,709	0,034	0,46
283	SMPDL3A	-0,555	0,035	0,46
284	PRODH	-0,571	0,035	0,46
285	TMEM192	-0,524	0,035	0,46
286	ABRAXAS2	-0,634	0,035	0,46
287	FTH1	0,634	0,036	0,46
288	LRRC2	-0,587	0,036	0,46
289	CSRP2	-0,553	0,037	0,46
290	TMEM150C	-0,584	0,037	0,46
291	COL5A1	0,672	0,037	0,46
292	C3orf52	-0,513	0,037	0,46
293	RELCH	-0,517	0,037	0,46
294	RPS3A	0,507	0,037	0,46
295	B3GALNT1	-0,528	0,037	0,46
296	MCEE	-0,584	0,038	0,46
297	ZFP36	1,026	0,038	0,46
298	HECW2	-0,755	0,038	0,46
299	ATP9A	-0,507	0,038	0,46
300	GSTA3	-0,677	0,038	0,46
301	IL13RA2	-0,918	0,039	0,47
302	ABCA5	-0,606	0,039	0,47
303	NOL8	-0,838	0,04	0,47
304	DHRS3	-0,615	0,04	0,47
305	PPP1R13B	-0,54	0,04	0,47
306	ITM2A	-0,544	0,041	0,47
307	CKMT2	-0,507	0,041	0,47
308	RPL28	0,532	0,041	0,47
309	CLEC1A	-0,66	0,041	0,47
310	MMRN2	-0,504	0,041	0,47
311	TMEM240	-0,501	0,041	0,47

312	SLC28A1	-0,656	0,042	0,47
313	PRSS23	0,6	0,042	0,47
314	TCF21	-0,665	0,042	0,47
315	LYNX1	-0,558	0,042	0,48
316	NDRG1	-0,634	0,043	0,48
317	FLT3LG	-0,516	0,043	0,48
318	RPL7A	0,534	0,043	0,48
319	AGXT	-0,517	0,043	0,48
320	FZD4	-0,525	0,044	0,48
321	STC1	-0,602	0,044	0,48
322	ANKRD28	-0,543	0,044	0,48
323	ZNF549	-0,742	0,044	0,48
324	CLCN5	-0,577	0,045	0,48
325	CD68	0,838	0,045	0,48
326	PRKAR2B	-0,553	0,045	0,48
327	VSIG8	-0,541	0,045	0,48
328	VEGFA	-0,88	0,046	0,48
329	ZDHHC6	-0,639	0,046	0,48
330	TYRO3	-0,611	0,046	0,48
331	APLNR	-0,888	0,046	0,48
332	IL17RC	-0,649	0,046	0,48
333	TEK	-0,922	0,047	0,48
334	CENPC	-0,553	0,047	0,48
335	AP4E1	-0,61	0,047	0,48
336	HIF3A	-0,924	0,048	0,48
337	CABLES1	-0,616	0,048	0,48
338	TFAP2A	-0,67	0,048	0,48
339	ROBO4	-0,637	0,05	0,48
340	ELF5	-0,539	0,05	0,48
341	HEBP2	-0,564	0,05	0,48

log2 fold change = change in gene expression between conditions, expressed on a log base 2 scale; FDR= false discovery rate (adjusted for multiple testing).

Appendix Table A3. Differentially expressed genes in CD31⁺ AOs between responders and non-responders.

	Gene name	log2 fold change	P value	FDR
1	NR3C2	-1,208	0,00000021	0,0019
2	GGT7	-1,703	0,0000017	0,0074
3	CDKAL1	-1,35	0,0000088	0,023
4	ITGA5	1,553	0,00001	0,023
5	ODR4	-1,722	0,000018	0,024
6	VASP	1,087	0,000018	0,024
7	TMEM178A	-1,429	0,000021	0,024
8	LTN1	-0,917	0,000024	0,024
9	RETREG1	-1,711	0,000024	0,024
10	OSM	-1,298	0,000031	0,024
11	RP9	-1,581	0,000031	0,024
12	LTA4H	-1,207	0,000032	0,024
13	CINP	-1,465	0,000041	0,028
14	TMEM8B	-1,394	0,00005	0,031
15	OGT	-1,436	0,000057	0,031
16	TFAP2A	-1,322	0,000058	0,031
17	BANP	-1,193	0,00006	0,031
18	TFE3	-1,337	0,000064	0,031
19	TMEM98	-0,844	0,000066	0,031
20	TRAPPC6A	-1,511	0,00011	0,046
21	GSTZ1	-1,429	0,00011	0,046
22	HIP1R	-1,349	0,00012	0,049
23	MYC	1,015	0,00014	0,053
24	DUSP6	1,188	0,00015	0,055
25	ATP8B1	-1,536	0,00016	0,057
26	SCO1	-1,5	0,00018	0,061
27	SLC4A11	-1,086	0,0002	0,064
28	JCHAIN	-1,662	0,00022	0,068
29	BAK1	0,864	0,00022	0,068
30	ADAMTS1	1,433	0,00024	0,068
31	PTH1R	-0,871	0,00025	0,068
32	CNST	-1,044	0,00028	0,075
33	ACVR1B	-1,131	0,00034	0,087
34	PRMT7	-1,004	0,00035	0,087
35	NGLY1	-0,721	0,00036	0,087
36	TSPAN8	-1,187	0,00041	0,096
37	CDKL1	-1,506	0,00041	0,096
38	DIS3L	-1,034	0,00045	0,10
39	KAT8	-1,09	0,00046	0,10
40	NUP58	-0,815	0,00048	0,10
41	SCOC	-1,439	0,00051	0,10

42	BLOC1S2	-0,846	0,00054	0,10
43	PRR35	-1,266	0,00055	0,10
44	TMEM63B	-1,019	0,00056	0,10
45	RPS18	0,634	0,00056	0,10
46	OBSL1	-1,458	0,00056	0,10
47	S100A9	2,579	0,00057	0,10
48	RPL8	0,502	0,00059	0,10
49	CCT3	0,663	0,0006	0,10
50	CD24	-1,563	0,00063	0,11
51	ABCC5	-1,164	0,00064	0,11
52	NARS2	-1,002	0,00065	0,11
53	UMOD	-2,917	0,00066	0,11
54	MAL	-1,337	0,00067	0,11
55	FAAP100	-0,88	0,00068	0,11
56	CTSF	-1,35	0,00071	0,11
57	C1orf21	-0,98	0,00072	0,11
58	PTK7	-1,116	0,00076	0,11
59	RNF150	-0,913	0,00081	0,12
60	WDR12	-0,929	0,00083	0,12
61	H4C12	0,93	0,00083	0,12
62	KLF5	-1,058	0,00084	0,12
63	CEP68	-0,947	0,00088	0,12
64	SZRD1	0,605	0,00089	0,12
65	ZFYVE26	-0,925	0,00092	0,12
66	ZC3H4	-1,284	0,00092	0,12
67	RHOBTB3	-0,984	0,00093	0,12
68	GGNBP2	-0,643	0,00094	0,12
69	CLCNKB	-1,591	0,00098	0,12
70	PLOD1	0,622	0,00099	0,12
71	TPD52	-1,015	0,001	0,12
72	SLC16A1	-0,895	0,0011	0,13
73	BEX2	-1,242	0,0011	0,13
74	KBTBD11	-0,893	0,0011	0,13
75	RPAP3	-0,83	0,0011	0,13
76	HSDL2	-1,25	0,0012	0,13
77	NDUFA6	-0,627	0,0012	0,13
78	EVI2B	0,891	0,0012	0,14
79	LEMD3	-0,86	0,0012	0,14
80	IFT122	-1,217	0,0013	0,14
81	SIGIRR	-1,006	0,0013	0,14
82	ZNF696	-1,14	0,0013	0,14
83	ZNF217	-0,608	0,0013	0,14
84	TESC	-1,195	0,0014	0,14
85	ABLIM2	-0,752	0,0014	0,14
86	OCM	-0,865	0,0014	0,14

87	PGM2	0,503	0,0014	0,14
88	SERGEF	-0,787	0,0014	0,14
89	SH2B1	-0,765	0,0015	0,14
90	SPRY1	0,965	0,0015	0,14
91	ZNF496	-0,977	0,0015	0,14
92	CLCN2	-1,414	0,0015	0,14
93	MAPK9	-1,192	0,0015	0,14
94	EIF4EBP3	-1,122	0,0015	0,14
95	POLR2J	-1,083	0,0016	0,14
96	RNASE4	-1,052	0,0016	0,14
97	FAM98C	-1,142	0,0016	0,14
98	NVL	-1,185	0,0017	0,15
99	IBTK	-1,079	0,0017	0,15
100	SFI1	-0,743	0,0017	0,15
101	DUSP3	0,959	0,0018	0,15
102	CLINT1	-0,737	0,0018	0,15
103	C12orf29	-1,018	0,0018	0,15
104	DCDC2	-0,933	0,0018	0,15
105	KDEL2	0,692	0,0019	0,15
106	MAN1A1	-0,91	0,0019	0,15
107	CRTC2	-0,53	0,0019	0,16
108	CLIP4	-1,561	0,002	0,16
109	PTCRA	-0,953	0,002	0,16
110	PARD3B	-1,033	0,002	0,16
111	TMEM240	-1,057	0,0021	0,16
112	TENT5C	-1,091	0,0021	0,16
113	METTL5	-0,888	0,0021	0,16
114	ERAP1	-0,585	0,0021	0,16
115	ZNF888	-0,813	0,0021	0,16
116	ADCK2	-1,083	0,0021	0,16
117	ZNF141	-0,688	0,0021	0,16
118	DMAP1	-0,949	0,0022	0,16
119	SLC26A7	-1,104	0,0022	0,16
120	TMED5	-0,948	0,0022	0,16
121	BBS2	-1,105	0,0022	0,16
122	RETSAT	-1,204	0,0023	0,16
123	HNF1B	-1,165	0,0023	0,16
124	PPM1K	-1,086	0,0023	0,16
125	DHX29	-0,927	0,0023	0,16
126	CLDN11	-1,227	0,0025	0,17
127	ZNF608	-1,222	0,0025	0,17
128	BMP7	-0,997	0,0025	0,17
129	CEMIP2	-0,965	0,0026	0,17
130	C7orf26	-0,791	0,0026	0,17
131	ZNF251	-1,014	0,0026	0,17

132	SLC46A1	-1,176	0,0027	0,17
133	ALG13	-0,9	0,0027	0,17
134	MTMR11	-1,245	0,0027	0,17
135	NPAS3	-1,066	0,0027	0,17
136	RNPEP	-0,537	0,0027	0,17
137	CDH6	-0,763	0,0027	0,17
138	LMCD1	0,733	0,0027	0,17
139	ANAPC2	-0,841	0,0027	0,17
140	GLB1	-0,85	0,0027	0,17
141	CSNK1G2	-0,999	0,0027	0,17
142	PBX2	0,569	0,0028	0,17
143	GSTM2	-1,101	0,0028	0,17
144	EPS8L1	-0,781	0,0028	0,17
145	RRM2B	-1,065	0,0028	0,17
146	SUPT16H	-0,989	0,0028	0,17
147	RPS9	0,582	0,0028	0,17
148	CTXN1	-0,536	0,0029	0,17
149	KANK1	-0,983	0,0029	0,17
150	CCDC8	-1,229	0,0029	0,17
151	SCNN1G	-1,044	0,0029	0,17
152	FAM3A	-0,557	0,0029	0,17
153	SLC38A1	-1,035	0,003	0,17
154	RNF146	-0,719	0,003	0,17
155	ZNF76	-0,788	0,003	0,17
156	LAPTM5	1,558	0,0031	0,17
157	IFI27L1	-1,373	0,0031	0,17
158	SSU72	0,58	0,0032	0,17
159	NUAK2	-1,003	0,0033	0,18
160	MIA3	-0,763	0,0033	0,18
161	KCNJ16	-1,333	0,0034	0,18
162	GOLGA3	0,684	0,0034	0,18
163	GTPBP4	-0,599	0,0035	0,19
164	PSAT1	-1,274	0,0035	0,19
165	MPDZ	-0,979	0,0036	0,19
166	COL4A3	-1,304	0,0036	0,19
167	ORAI2	-0,864	0,0036	0,19
168	TRIM6	-1,282	0,0037	0,19
169	SH2B3	1,15	0,0037	0,19
170	FXN	-0,836	0,0037	0,19
171	PRKD3	-0,825	0,0038	0,19
172	STRIP2	-1,192	0,0038	0,19
173	CTXN3	-1,023	0,0038	0,19
174	SLC25A36	-0,956	0,0039	0,19
175	GRAMD1C	-1,095	0,0039	0,19
176	TNF	-1,639	0,004	0,19

177	ARHGAP12	-1,128	0,004	0,19
178	CPD	-0,582	0,0041	0,19
179	ACSM5	-1,162	0,0041	0,19
180	SLC25A23	-0,969	0,0041	0,19
181	DDX3X	0,711	0,0041	0,19
182	PLRG1	-1,1	0,0041	0,19
183	TCF4	1,542	0,0041	0,19
184	PNPLA8	-1,254	0,0042	0,20
185	TYROBP	1,299	0,0042	0,20
186	MRPL46	-0,859	0,0042	0,20
187	PLEKHA6	-1,054	0,0042	0,20
188	ARPC1B	1,225	0,0043	0,20
189	UPK3A	-0,981	0,0043	0,20
190	GPC4	-1,122	0,0043	0,20
191	TNFSF12	0,658	0,0045	0,20
192	PPP2R3A	-0,821	0,0046	0,21
193	CIPC	-0,969	0,0046	0,21
194	ERV3-1	-0,792	0,0046	0,21
195	PFDN5	0,539	0,0046	0,21
196	ITIH5	-0,941	0,0046	0,21
197	USP33	-0,712	0,0047	0,21
198	HEMK1	-1,439	0,0047	0,21
199	USF3	-1,057	0,0048	0,21
200	STXBP3	-1,133	0,0048	0,21
201	EFHD2	0,765	0,0049	0,21
202	NIPSNAP1	-0,94	0,0049	0,21
203	CCNB1IP1	-1,039	0,0049	0,21
204	LITAF	0,806	0,005	0,21
205	GOLGA6L9	-0,982	0,005	0,21
206	IFITM10	-1,07	0,0051	0,21
207	DGKH	-0,719	0,0052	0,21
208	ZNF318	-1,022	0,0052	0,21
209	ANKRD46	-0,925	0,0052	0,21
210	CHMP6	-0,987	0,0052	0,21
211	MED25	-0,82	0,0053	0,21
212	SCAF4	-0,959	0,0053	0,21
213	PIP4P1	-1,099	0,0053	0,22
214	ERC1	-0,897	0,0054	0,22
215	RHOA	0,505	0,0055	0,22
216	COQ2	-0,87	0,0055	0,22
217	DEAF1	-1,023	0,0055	0,22
218	GPI	-1,045	0,0055	0,22
219	WDR27	-1,176	0,0056	0,22
220	L1CAM	-1,059	0,0056	0,22
221	CHRFAM7A	-0,861	0,0057	0,22

222	IDUA	-0,761	0,0057	0,22
223	PRRT2	-1,187	0,0057	0,22
224	FLRT3	-0,788	0,0058	0,22
225	RRAD	-0,917	0,0058	0,22
226	MMUT	-0,805	0,0058	0,22
227	IGSF3	-0,778	0,0058	0,22
228	H4C13	-0,61	0,0059	0,22
229	MRPL10	-1,068	0,0059	0,22
230	SYNRG	-0,986	0,006	0,22
231	LIG3	-0,903	0,006	0,22
232	ABITRAM	-0,999	0,0061	0,22
233	RAB29	-1,431	0,0062	0,23
234	H2AC11	-1,334	0,0062	0,23
235	VPS45	-0,819	0,0063	0,23
236	TCTN3	-1,107	0,0063	0,23
237	RASEF	-0,713	0,0063	0,23
238	TMEM52B	-1,592	0,0063	0,23
239	RPS7	0,55	0,0064	0,23
240	ARPC2	0,987	0,0064	0,23
241	NOTCH2	-1,053	0,0064	0,23
242	CLCN3	-0,699	0,0064	0,23
243	FAM83H	-1,331	0,0065	0,23
244	TEX30	-0,848	0,0066	0,23
245	FRAT2	-0,802	0,0066	0,23
246	MAGOHB	-0,756	0,0066	0,23
247	CAP1	0,782	0,0068	0,23
248	ANKLE1	-1,169	0,0068	0,23
249	WLS	-0,923	0,0068	0,23
250	ZNF708	-0,847	0,0069	0,23
251	SLC33A1	-0,847	0,0069	0,23
252	ORAI3	-0,932	0,0069	0,23
253	ATP13A3	0,824	0,0069	0,23
254	TARS2	-1,089	0,007	0,23
255	CLVS1	-1,181	0,007	0,23
256	PRADC1	-1,175	0,007	0,23
257	PHTF1	-1,075	0,007	0,23
258	TMEM245	-0,943	0,007	0,23
259	DEFB1	-2,531	0,0071	0,23
260	BLVRB	-0,981	0,0072	0,24
261	PIGM	-0,916	0,0072	0,24
262	CPSF6	-0,92	0,0073	0,24
263	BZW1	0,73	0,0074	0,24
264	PI4K2B	-0,688	0,0074	0,24
265	ZFP36	2,091	0,0074	0,24
266	HMG3	-0,88	0,0074	0,24

267	ABCA5	-1,058	0,0074	0,24
268	SUFU	-0,56	0,0075	0,24
269	MINDY4B	-1,3	0,0075	0,24
270	TSPOAP1	-1,021	0,0075	0,24
271	SGTB	-1,135	0,0075	0,24
272	KAZN	-0,982	0,0075	0,24
273	FN3K	-1,216	0,0076	0,24
274	PRPS1	-0,77	0,0076	0,24
275	GPALPP1	-0,744	0,0077	0,24
276	MORN3	-1,082	0,0077	0,24
277	FBXO8	-0,566	0,0077	0,24
278	CKS1B	0,555	0,0077	0,24
279	CLIC1	1,134	0,0078	0,24
280	PISD	-1,016	0,0078	0,24
281	RBM7	0,78	0,0078	0,24
282	BBLN	0,601	0,0078	0,24
283	UXS1	-0,947	0,0078	0,24
284	CTC1	-0,853	0,0079	0,24
285	THOC1	-0,695	0,0079	0,24
286	USP20	-1,027	0,008	0,24
287	DCAF6	-0,865	0,0081	0,24
288	NOC2L	-1,163	0,0082	0,24
289	ATP1A1	-0,726	0,0082	0,24
290	COG1	-0,983	0,0083	0,24
291	ZMYM2	-0,948	0,0083	0,24
292	WWP1	-0,74	0,0083	0,24
293	COA8	-0,959	0,0083	0,24
294	UBTD2	0,728	0,0084	0,24
295	BTBD3	-0,737	0,0084	0,24
296	MFN2	0,666	0,0084	0,24
297	SLC25A33	-0,917	0,0084	0,24
298	TLK1	-0,846	0,0085	0,24
299	TMEM205	-1,539	0,0085	0,24
300	PFN1	1,139	0,0085	0,24
301	ZNF226	-0,784	0,0086	0,24
302	YBX1	0,914	0,0087	0,24
303	SYPL2	-1,1	0,0087	0,24
304	SNAPC4	-1,048	0,0087	0,24
305	SMC5	-0,632	0,0088	0,24
306	CLASP1	-1,223	0,0088	0,24
307	FIBIN	-1,381	0,0088	0,24
308	LMAN2L	-0,981	0,0088	0,25
309	DUX5	-0,789	0,0089	0,25
310	FAXDC2	-0,828	0,0089	0,25
311	NIBAN2	0,885	0,0089	0,25

312	SERPINA6	-1,193	0,009	0,25
313	CHMP5	-0,976	0,009	0,25
314	UQCC1	-0,682	0,009	0,25
315	GET1	-1,232	0,009	0,25
316	ALG12	-0,835	0,0091	0,25
317	ADM2	-0,935	0,0091	0,25
318	ZNF529	-0,918	0,0092	0,25
319	JUNB	2,006	0,0093	0,25
320	GALNT18	-1,061	0,0093	0,25
321	SELENOP	-0,638	0,0093	0,25
322	CDH3	-0,962	0,0094	0,25
323	SYK	-0,957	0,0094	0,25
324	EPG5	-0,724	0,0095	0,25
325	FAM172A	-0,699	0,0095	0,25
326	MFSD4A	-1,069	0,0096	0,25
327	STK4	0,774	0,0096	0,25
328	RPS26	0,906	0,0097	0,25
329	TSPAN3	-0,682	0,0097	0,25
330	DNM1	-0,892	0,0097	0,25
331	TUBA1C	1,573	0,0097	0,25
332	GPRC5A	-0,95	0,0098	0,25
333	RAB19	-0,977	0,0098	0,25
334	LLPH	0,503	0,0099	0,25
335	HS6ST3	-1,782	0,0099	0,25
336	REST	-0,882	0,0099	0,25
337	WDR75	-0,978	0,0099	0,25
338	CRYM	-1,444	0,01	0,25
339	POLR3K	-0,774	0,01	0,25
340	SETDB2	-0,537	0,01	0,25
341	TPP1	0,589	0,01	0,25
342	ACSL4	0,509	0,01	0,25
343	GPR78	-1,319	0,01	0,25
344	PEX11G	-0,862	0,01	0,25
345	SLC12A1	-1,934	0,01	0,25
346	MFAP3	-0,956	0,01	0,25
347	GATB	-0,705	0,01	0,25
348	LARP7	-1,001	0,01	0,25
349	NAGLU	-0,818	0,01	0,25
350	FRA10AC1	-0,781	0,01	0,25
351	BCAS2	-0,907	0,01	0,25
352	RAB40B	-1,406	0,01	0,25
353	RORC	-1,143	0,01	0,25
354	PPP1R9A	-0,928	0,01	0,25
355	ANXA3	-0,873	0,01	0,25
356	WDPCP	-1,138	0,01	0,25

357	ING5	-0,586	0,011	0,25
358	CCL3	0,929	0,011	0,25
359	ARID2	-0,995	0,011	0,25
360	RAB11FIP5	-0,752	0,011	0,25
361	ECSCR	1,312	0,011	0,26
362	SLC30A1	-1,115	0,011	0,26
363	MDN1	-1,165	0,011	0,26
364	AVPI1	-0,822	0,011	0,26
365	MRGPRF	-0,638	0,011	0,26
366	SHPRH	-0,87	0,011	0,26
367	OXER1	-1,429	0,011	0,26
368	WDR83OS	-0,767	0,011	0,26
369	GPHN	-0,991	0,011	0,26
370	ST13	-0,968	0,011	0,26
371	H2AC21	-1,387	0,011	0,26
372	ADHFE1	-0,939	0,011	0,26
373	PHF3	-1,1	0,011	0,26
374	RASD1	0,806	0,011	0,26
375	TMEM19	-0,964	0,011	0,26
376	POM121L12	-1,939	0,011	0,26
377	GPR108	-0,668	0,011	0,26
378	CCDC91	-0,831	0,011	0,26
379	CAMK2G	-0,834	0,011	0,26
380	AKR1E2	-0,891	0,012	0,26
381	EIF5A	0,64	0,012	0,26
382	TREH	-1,192	0,012	0,26
383	OGFOD3	-0,877	0,012	0,26
384	MVP	0,539	0,012	0,26
385	H3-4	-1,47	0,012	0,26
386	ATP6V1G3	-0,926	0,012	0,26
387	NFATC2IP	-1,343	0,012	0,26
388	ARPC5	0,592	0,012	0,26
389	PER3	-0,857	0,012	0,26
390	SMPD4	-0,85	0,012	0,26
391	TMEM116	-1,078	0,012	0,26
392	DDX18	-0,766	0,012	0,26
393	CA12	-1,035	0,012	0,26
394	TMED4	-0,57	0,012	0,26
395	PAK1	-0,502	0,012	0,26
396	CHAMP1	-1,24	0,012	0,26
397	PDE4C	-0,994	0,012	0,26
398	CCDC148	-1,233	0,012	0,26
399	CMTR1	-0,816	0,012	0,26
400	AMACR	-1,106	0,012	0,26
401	ATP10D	-0,788	0,012	0,26

402	MUS81	-1,462	0,012	0,26
403	CLDN19	-1,094	0,012	0,26
404	SLF2	-0,614	0,012	0,26
405	ZSWIM1	-0,953	0,012	0,26
406	CALB1	-0,869	0,012	0,26
407	FKBP1A	1,226	0,012	0,26
408	MAP4K4	0,975	0,012	0,26
409	KCNH3	-0,801	0,012	0,26
410	ZBTB7C	-1,195	0,012	0,26
411	FAM117B	-0,933	0,012	0,26
412	LIMCH1	-0,601	0,013	0,26
413	MYCBP2	-0,549	0,013	0,26
414	KDM5B	-0,977	0,013	0,26
415	HSPG2	1,32	0,013	0,26
416	ALB	-0,884	0,013	0,26
417	TCTN1	-1,361	0,013	0,26
418	DOCK5	-0,797	0,013	0,26
419	MORN2	-0,807	0,013	0,26
420	SLC3A2	0,746	0,013	0,26
421	FN1	2,417	0,013	0,26
422	LCP2	0,903	0,013	0,26
423	CCDC90B	-0,754	0,013	0,26
424	COA5	-0,739	0,013	0,26
425	SMIM11A	-0,94	0,013	0,26
426	TBCB	0,913	0,013	0,26
427	SCAMP3	-1,249	0,013	0,26
428	HINT2	-0,567	0,013	0,26
429	RNF24	-0,662	0,013	0,26
430	ELMO2	-0,794	0,013	0,26
431	PTPRC	1,013	0,013	0,26
432	SIPA1	1,226	0,013	0,26
433	CYBRD1	-0,945	0,013	0,26
434	MOGS	-0,724	0,013	0,26
435	PIAS3	-0,9	0,013	0,26
436	RAB17	-1,485	0,013	0,26
437	ZNF419	-0,71	0,013	0,26
438	ACTR3	1,012	0,013	0,26
439	SSBP4	0,896	0,013	0,26
440	DDIT3	-0,549	0,014	0,26
441	C1QB	1,091	0,014	0,26
442	ABCG1	1,122	0,014	0,26
443	ZNF821	-0,965	0,014	0,26
444	ECHDC2	-0,739	0,014	0,26
445	ATP6V0B	-0,858	0,014	0,26
446	UBE2D3	0,648	0,014	0,26

447	ITFG1	-0,579	0,014	0,26
448	CLN6	-1,328	0,014	0,26
449	GLT8D1	-0,721	0,014	0,26
450	BTBD6	-0,716	0,014	0,26
451	DNAJB11	-0,6	0,014	0,26
452	THBS3	-0,793	0,014	0,26
453	TNFRSF12A	1,009	0,014	0,26
454	CDH16	-1,192	0,014	0,26
455	GDA	-0,957	0,014	0,26
456	RPL14	0,765	0,014	0,26
457	LMNA	1,279	0,014	0,26
458	HOXB2	-0,674	0,014	0,27
459	CAAP1	-0,93	0,014	0,27
460	QSER1	-0,882	0,014	0,27
461	MICU1	-0,969	0,014	0,27
462	AKTIP	-0,952	0,014	0,27
463	TANK	-1,111	0,014	0,27
464	ACAT2	-0,76	0,014	0,27
465	RPL39L	-1,66	0,015	0,27
466	SLC2A8	-0,575	0,015	0,27
467	HBP1	-0,872	0,015	0,27
468	MPV17	-0,633	0,015	0,27
469	PLD1	-1,365	0,015	0,27
470	CSE1L	-0,83	0,015	0,27
471	STK38	-0,89	0,015	0,27
472	CDC14B	-0,892	0,015	0,27
473	PRKAR2B	-0,801	0,015	0,27
474	MAP3K11	0,659	0,015	0,27
475	BCL2	-0,737	0,015	0,27
476	TNNT2	-1,2	0,015	0,27
477	PNPT1	-1,071	0,015	0,27
478	TCAIM	-0,78	0,015	0,27
479	AP2A1	0,618	0,015	0,27
480	CEACAM18	-1,193	0,015	0,27
481	GLYATL1	-1,074	0,015	0,27
482	RIOX2	-1,399	0,015	0,27
483	NECAP2	0,687	0,015	0,27
484	MOB1B	-0,68	0,015	0,27
485	FAM171A1	-1,171	0,015	0,27
486	TMED2	0,726	0,015	0,27
487	CFL1	0,965	0,015	0,27
488	TNIP2	-0,656	0,015	0,27
489	CCDC198	-1,562	0,015	0,27
490	LNX1	-1,066	0,016	0,27
491	ZNF611	-0,828	0,016	0,27

492	OGFOD1	-1,059	0,016	0,27
493	NR2F1	-0,591	0,016	0,27
494	CSTF1	-0,667	0,016	0,27
495	LYG1	-1,212	0,016	0,27
496	CDK2AP1	0,959	0,016	0,27
497	SIK1	1,318	0,016	0,27
498	IFIT5	-0,851	0,016	0,27
499	UBXN6	0,706	0,016	0,27
500	C2CD2L	-0,899	0,016	0,27
501	UBE2D4	-0,684	0,016	0,27
502	TRIM47	0,707	0,016	0,27
503	KCTD2	-1,584	0,016	0,27
504	KCTD21	-0,875	0,016	0,28
505	SRGN	1,979	0,016	0,28
506	TIMM50	-1,024	0,016	0,28
507	CD99L2	-0,841	0,017	0,28
508	JKAMP	-0,83	0,017	0,28
509	TUBA3E	-1,063	0,017	0,28
510	WDR59	-0,739	0,017	0,28
511	HSPA12A	-0,642	0,017	0,28
512	TRHDE	-0,868	0,017	0,28
513	LUZP6	-0,591	0,017	0,28
514	REEP6	-1,05	0,017	0,28
515	NTN4	-0,911	0,017	0,28
516	CERKL	-1,274	0,017	0,28
517	GPX1	0,98	0,017	0,28
518	S100A16	0,879	0,017	0,28
519	STARD7	0,642	0,017	0,28
520	USH1C	-1,424	0,017	0,28
521	CTSS	1,278	0,017	0,28
522	ZNF687	-0,736	0,017	0,28
523	FZR1	-1,009	0,017	0,28
524	FCN1	0,731	0,017	0,28
525	FUCA1	-0,667	0,017	0,28
526	SLC12A3	-1,36	0,017	0,28
527	PLA2G6	-0,75	0,017	0,28
528	ECM2	-0,867	0,017	0,28
529	ARHGEF12	-0,856	0,017	0,28
530	SOD3	-0,626	0,017	0,28
531	FBLN5	-0,763	0,017	0,28
532	CRYZ	-0,984	0,017	0,28
533	SMPD1	-0,833	0,017	0,28
534	PGAP4	-0,993	0,017	0,28
535	ARMCX2	-0,741	0,017	0,28
536	SGSM2	-0,862	0,017	0,28

537	CYCS	0,538	0,017	0,28
538	MAPKAPK2	0,583	0,017	0,28
539	ANK3	-0,802	0,017	0,28
540	MYH11	-1,205	0,018	0,28
541	MED29	-0,807	0,018	0,28
542	MIEF2	-1,103	0,018	0,28
543	RANGRF	-0,899	0,018	0,28
544	IKZF5	-1,39	0,018	0,28
545	NATD1	-1,87	0,018	0,28
546	CC2D1B	-0,965	0,018	0,28
547	DNAJC22	-0,915	0,018	0,28
548	EPHA2	-0,712	0,018	0,28
549	POLD2	-0,795	0,018	0,28
550	IGHM	-1,263	0,018	0,28
551	KIAA0825	-0,969	0,018	0,28
552	PPFIBP2	-1,126	0,018	0,28
553	SLMAP	-0,911	0,018	0,28
554	PTP4A2	0,63	0,018	0,28
555	SMDT1	-1,426	0,018	0,28
556	SLC5A10	-1,637	0,018	0,28
557	CLDN25	-1,344	0,018	0,28
558	RAI14	-1,173	0,018	0,28
559	HCK	0,639	0,018	0,28
560	H4C3	-1,185	0,018	0,28
561	MAP3K13	-0,906	0,018	0,28
562	UGT8	-1,25	0,018	0,28
563	KNG1	-1,851	0,018	0,28
564	FGFRL1	-0,789	0,018	0,28
565	PFKFB2	-0,716	0,018	0,28
566	WBP1	-0,526	0,019	0,28
567	DYRK2	-0,937	0,019	0,28
568	RFTN1	0,695	0,019	0,28
569	PTPN9	-0,615	0,019	0,28
570	HGS	-0,508	0,019	0,28
571	SLC6A18	-0,971	0,019	0,28
572	ULK3	-0,955	0,019	0,28
573	PROM1	-0,772	0,019	0,28
574	PLK2	0,899	0,019	0,28
575	C11orf80	-1,258	0,019	0,28
576	GBP4	-1,007	0,019	0,28
577	C1QTNF7	-1,091	0,019	0,28
578	ZMAT5	-0,874	0,019	0,28
579	LHX1	-0,792	0,019	0,28
580	TRMT112	0,576	0,019	0,28
581	NPR3	-0,562	0,019	0,28

582	TNFAIP3	1,235	0,019	0,28
583	UNK	-1,008	0,019	0,28
584	DVL3	-0,586	0,019	0,28
585	EPS15	-1,15	0,019	0,28
586	ABCF2	-0,879	0,019	0,28
587	OVOL1	-0,83	0,019	0,28
588	CYTH1	0,943	0,019	0,28
589	VEGFB	-0,676	0,019	0,28
590	RPL12	0,512	0,02	0,28
591	NUP107	-0,784	0,02	0,28
592	EML2	-1,165	0,02	0,28
593	MRPL50	-0,993	0,02	0,28
594	SLC22A24	-1,022	0,02	0,28
595	BCL9L	0,558	0,02	0,28
596	CNIH4	-0,614	0,02	0,28
597	C18orf32	-0,521	0,02	0,28
598	COL4A1	1,809	0,02	0,28
599	TIMM10B	-0,907	0,02	0,28
600	CTNNBIP1	-0,639	0,02	0,28
601	MCM5	-0,768	0,02	0,28
602	ATP9A	-0,895	0,02	0,28
603	SS18L1	-0,789	0,02	0,28
604	KDM4C	-0,966	0,02	0,28
605	SPNS2	-0,849	0,02	0,28
606	LCLAT1	-1,242	0,02	0,28
607	TSPYL1	-0,969	0,02	0,28
608	CLNK	-1,337	0,02	0,28
609	CHI3L1	-1,375	0,02	0,28
610	TSPYL4	-0,78	0,02	0,28
611	HSD11B2	-1,245	0,02	0,28
612	ZDHHC7	-0,676	0,02	0,28
613	SLC25A13	-0,818	0,02	0,28
614	SF3B6	-0,904	0,021	0,28
615	UBR4	-0,729	0,021	0,28
616	NUDT16	-0,666	0,021	0,28
617	FGD4	-0,955	0,021	0,28
618	ARHGEF10	-0,884	0,021	0,28
619	ACVR1	-0,646	0,021	0,28
620	GALNT14	-1,004	0,021	0,28
621	MACROH2A1	0,747	0,021	0,29
622	NUDT22	-0,776	0,021	0,29
623	ESPN	-0,865	0,021	0,29
624	NDE1	-0,869	0,021	0,29
625	ERGIC2	-0,88	0,021	0,29
626	DUSP1	1,376	0,021	0,29

627	ATP5PF	-0,51	0,021	0,29
628	CCDC6	-0,743	0,021	0,29
629	TBCE	-0,825	0,021	0,29
630	LRRC27	-0,877	0,021	0,29
631	HSP90AB1	0,675	0,021	0,29
632	IFI30	1,737	0,022	0,29
633	TCEA3	-1,275	0,022	0,29
634	C17orf49	0,749	0,022	0,29
635	MYO18A	0,615	0,022	0,29
636	SRRD	-0,826	0,022	0,29
637	WFDC1	0,87	0,022	0,29
638	NCOA5	-0,989	0,022	0,29
639	OR51S1	-0,78	0,022	0,29
640	GLOD4	-0,503	0,022	0,29
641	PMS2	-0,772	0,022	0,29
642	SLC40A1	-0,621	0,022	0,29
643	MSN	0,81	0,022	0,29
644	TSEN15	-0,91	0,022	0,29
645	NOA1	-0,688	0,022	0,29
646	EIF4ENIF1	-1,281	0,022	0,29
647	TAGLN2	1,343	0,022	0,29
648	DYNC1I2	0,529	0,022	0,29
649	SNAP91	-1,353	0,022	0,29
650	TMEM45B	-1,339	0,022	0,29
651	NRK	-1,459	0,022	0,29
652	FAM110A	-0,787	0,022	0,29
653	CEP44	-1,39	0,022	0,29
654	PUM3	-0,737	0,022	0,29
655	SLC22A12	-1,159	0,022	0,29
656	RUSC1	-0,733	0,022	0,29
657	CLN3	-0,505	0,023	0,29
658	RDH12	-1,244	0,023	0,29
659	SLC30A2	-1,121	0,023	0,29
660	SYT3	-1,428	0,023	0,29
661	KRTAP10-10	-0,983	0,023	0,29
662	STX4	0,655	0,023	0,29
663	PILRB	-0,953	0,023	0,29
664	MPPED2	-1,179	0,023	0,29
665	OLA1	-0,739	0,023	0,29
666	C1orf116	-1,12	0,023	0,29
667	SCIN	-0,618	0,023	0,29
668	CYB5R4	-0,728	0,023	0,29
669	OXA1L	-0,654	0,023	0,29
670	RPS14	0,553	0,023	0,29
671	DOCK6	0,951	0,023	0,29

672	GPR176	-0,715	0,023	0,29
673	ACSS1	-0,564	0,023	0,29
674	RPS2	0,94	0,023	0,29
675	ARHGEF28	-0,787	0,023	0,29
676	BPNT1	-1,026	0,023	0,29
677	DSG4	-1,12	0,023	0,29
678	CIAPIN1	-0,752	0,023	0,29
679	SMAD9	-0,672	0,023	0,29
680	HMCES	-0,825	0,023	0,29
681	PI4KB	0,703	0,024	0,29
682	PCNX1	-0,757	0,024	0,30
683	PAIP2	-0,634	0,024	0,30
684	FUT6	-1,568	0,024	0,30
685	ASF1A	-0,947	0,024	0,30
686	ACTR2	0,622	0,024	0,30
687	COL4A2	1,443	0,024	0,30
688	PTPN7	-0,757	0,024	0,30
689	GNPDA1	-1,075	0,024	0,30
690	ASPRV1	-1,679	0,024	0,30
691	NAA60	0,504	0,024	0,30
692	ALG10	-0,948	0,024	0,30
693	DYSF	0,967	0,024	0,30
694	MTFR1L	-1,134	0,024	0,30
695	FRMD1	-1,504	0,024	0,30
696	SPPL2B	-0,562	0,024	0,30
697	FCGR2C	0,935	0,024	0,30
698	MRPS15	-0,896	0,024	0,30
699	GATAD1	-0,846	0,024	0,30
700	SLC5A9	-1,157	0,025	0,30
701	CA4	-1,114	0,025	0,30
702	SYNJ2BP	-0,822	0,025	0,30
703	SBF1	-0,738	0,025	0,30
704	ABHD6	-1,031	0,025	0,30
705	GPM6A	-1,341	0,025	0,30
706	HDHD3	-1,086	0,025	0,30
707	TNFRSF1A	0,713	0,025	0,30
708	CGN	-0,943	0,025	0,30
709	HUNK	-0,91	0,025	0,30
710	SPRY4	0,902	0,025	0,30
711	TCF7L2	-0,587	0,025	0,30
712	KCNJ1	-0,989	0,025	0,30
713	HNRNPF	0,833	0,025	0,30
714	HTR6	-1,193	0,025	0,30
715	NT5DC2	0,878	0,025	0,30
716	HERPUD1	-0,667	0,025	0,30

717	EHD4	-0,877	0,025	0,30
718	PRR16	-1,064	0,025	0,30
719	RAB3GAP1	-0,785	0,025	0,30
720	H4C9	0,608	0,025	0,30
721	TTC4	-0,84	0,025	0,30
722	BRAF	-0,628	0,025	0,30
723	TGFB1	0,744	0,026	0,30
724	ADH5	-0,915	0,026	0,30
725	MAP2K6	-0,901	0,026	0,30
726	KIT	-0,965	0,026	0,30
727	C20orf96	-1,103	0,026	0,30
728	CCR1	0,6	0,026	0,30
729	SPAG16	-1,102	0,026	0,30
730	CLRN3	-1,05	0,026	0,30
731	MCOLN3	-1,353	0,026	0,30
732	ARAP3	0,594	0,026	0,30
733	ABHD12	-0,988	0,026	0,30
734	GATA2	-0,962	0,026	0,30
735	TFCP2L1	-0,524	0,026	0,30
736	MTLN	-1,122	0,026	0,30
737	WAPL	-1,128	0,026	0,30
738	ATIC	-0,907	0,026	0,30
739	AP1G2	-0,954	0,026	0,30
740	RHOB	1,166	0,026	0,30
741	JAM3	0,541	0,026	0,30
742	HSDL1	-0,925	0,026	0,30
743	HSPA12B	-0,631	0,027	0,30
744	MSH6	-0,811	0,027	0,30
745	PHF13	-1,237	0,027	0,30
746	JCAD	0,689	0,027	0,30
747	ABRAXAS2	-0,746	0,027	0,30
748	ARID1B	-1,076	0,027	0,30
749	NUP42	-0,733	0,027	0,30
750	LRR61	-1,054	0,027	0,30
751	CLUAP1	-0,772	0,027	0,30
752	LCN12	-0,863	0,027	0,30
753	MYBBP1A	-1,218	0,027	0,30
754	CLMN	-0,791	0,027	0,30
755	SLC27A5	-1,197	0,027	0,30
756	CD68	1,066	0,027	0,30
757	LDHA	0,881	0,027	0,30
758	C9orf78	-0,643	0,027	0,30
759	OR4C46	-0,925	0,027	0,30
760	VIRMA	-0,875	0,027	0,30
761	SLK	-0,974	0,027	0,30

762	PDGFC	-0,818	0,027	0,30
763	OSER1	-0,651	0,028	0,30
764	COPE	-0,542	0,028	0,31
765	RAC3	-1,025	0,028	0,31
766	HEXA	-0,893	0,028	0,31
767	MAN2C1	-0,547	0,028	0,31
768	ARNT2	-0,831	0,028	0,31
769	RPL7A	0,722	0,028	0,31
770	RNF111	-0,641	0,028	0,31
771	CYP2W1	-1,331	0,028	0,31
772	OGDHL	-1,321	0,028	0,31
773	ZNF3	-0,984	0,028	0,31
774	DOCK1	-0,896	0,028	0,31
775	EIF4A1	0,888	0,028	0,31
776	STAU2	-0,819	0,028	0,31
777	DYNC2LI1	-0,812	0,028	0,31
778	TNFAIP1	0,774	0,028	0,31
779	IGF2BP2	-0,773	0,029	0,31
780	MINDY3	-0,521	0,029	0,31
781	ENPP4	-1,062	0,029	0,31
782	NDUFA5	-0,981	0,029	0,31
783	CCDC184	-1,401	0,029	0,31
784	ZBED1	-0,95	0,029	0,31
785	MAD2L1BP	-0,89	0,029	0,31
786	CRB2	-0,834	0,029	0,31
787	TMEM238	-0,772	0,029	0,31
788	SCNM1	-1,27	0,029	0,31
789	NDUFS2	-0,884	0,029	0,31
790	QRSL1	-1,484	0,029	0,31
791	DIS3	-0,773	0,029	0,31
792	TMEM47	0,792	0,029	0,31
793	FZD7	-1,105	0,029	0,31
794	DMTN	-0,74	0,029	0,31
795	SLC28A1	-1,11	0,029	0,31
796	TRAPPC6B	-0,706	0,029	0,31
797	MTMR3	-0,665	0,029	0,31
798	NCBP1	-0,696	0,029	0,31
799	RPL15	0,542	0,029	0,31
800	UBE2Q2	-0,621	0,029	0,31
801	CBX8	-0,854	0,029	0,31
802	VAT1	0,539	0,029	0,31
803	ZNF12	-0,813	0,03	0,31
804	RECQL5	-0,764	0,03	0,31
805	RBM19	-0,787	0,03	0,31
806	MRPL51	-0,701	0,03	0,31

807	SH3BGRL3	0,978	0,03	0,31
808	ZCRB1	-1,028	0,03	0,31
809	CD28	-1,361	0,03	0,31
810	MFHAS1	-0,784	0,03	0,31
811	PREX2	-0,605	0,03	0,31
812	MRPL16	-0,735	0,03	0,31
813	CRCP	-0,562	0,03	0,31
814	SIRT2	0,74	0,03	0,31
815	USP2	-1,17	0,03	0,31
816	NSUN5	-1,07	0,03	0,31
817	MBOAT2	-0,964	0,03	0,31
818	ATP6V1F	0,627	0,03	0,31
819	APBB3	-0,614	0,03	0,31
820	YTHDF2	-0,787	0,03	0,31
821	TENT5A	-1,576	0,03	0,31
822	EXD2	-0,782	0,03	0,31
823	PMFBP1	-1,203	0,03	0,31
824	PRAP1	-0,929	0,03	0,31
825	TMEM213	-0,897	0,03	0,31
826	MRPS31	-0,634	0,03	0,31
827	PABPC1	0,733	0,03	0,31
828	VAMP1	-1,105	0,031	0,31
829	OSMR	1,008	0,031	0,31
830	MSMO1	-0,953	0,031	0,31
831	THNSL2	-1,019	0,031	0,31
832	HOXD10	-0,893	0,031	0,31
833	TRMT44	-0,769	0,031	0,31
834	SMIM15	-0,671	0,031	0,31
835	EGF	-0,943	0,031	0,31
836	PPP1R3B	-0,547	0,031	0,31
837	GALK2	-0,944	0,031	0,31
838	FXD4	-1,237	0,031	0,31
839	EPCAM	-0,722	0,031	0,31
840	TSPO	0,586	0,031	0,31
841	TERF2IP	-0,804	0,031	0,31
842	MRPL28	-0,824	0,031	0,31
843	COX18	-0,784	0,031	0,31
844	YDJC	-1,285	0,031	0,31
845	CD9	1,17	0,031	0,31
846	CABP2	-1,525	0,031	0,31
847	ZGPAT	-0,762	0,031	0,31
848	THY1	0,565	0,031	0,31
849	MED13L	-1,273	0,031	0,31
850	MYH14	-0,994	0,031	0,31
851	DDI2	-0,906	0,031	0,31

852	BRAP	-0,805	0,032	0,31
853	TFDP2	-0,693	0,032	0,31
854	CTH	-1,075	0,032	0,31
855	SELENOS	-1,104	0,032	0,31
856	ATRN	-0,604	0,032	0,31
857	SCPEP1	-1,123	0,032	0,31
858	S100A11	1,435	0,032	0,31
859	RAB5IF	0,638	0,032	0,31
860	SMPDL3B	-1,344	0,032	0,31
861	PRUNE2	-0,739	0,032	0,31
862	TPCN2	-1,439	0,032	0,31
863	C1orf210	-1,433	0,032	0,31
864	CASTOR2	-1,239	0,032	0,31
865	HNF4G	-1,038	0,032	0,31
866	KIF13B	-0,947	0,032	0,31
867	CMTR2	-0,812	0,032	0,31
868	SHTN1	-0,818	0,032	0,31
869	SEPTIN9	0,953	0,033	0,31
870	MFSD6	-1,197	0,033	0,31
871	R3HCC1	-1,162	0,033	0,31
872	IARS1	-0,502	0,033	0,31
873	ITSN1	-0,822	0,033	0,31
874	SLC26A4	-0,621	0,033	0,31
875	IRF2BP1	-1,205	0,033	0,31
876	RHEX	-0,556	0,033	0,31
877	RAPGEF6	-0,953	0,033	0,31
878	TMEM106B	-0,747	0,033	0,31
879	NID2	0,598	0,033	0,31
880	LIPA	0,672	0,033	0,31
881	IYD	-0,889	0,033	0,31
882	PPA2	-0,816	0,033	0,31
883	SLC35A4	0,786	0,033	0,31
884	MARCKSL1	1,189	0,033	0,31
885	RPL30	0,535	0,033	0,31
886	TRAPPC4	-0,636	0,033	0,31
887	HS6ST2	-0,83	0,033	0,31
888	UXT	-0,71	0,033	0,31
889	ZNF263	-0,776	0,033	0,31
890	SGSM3	-0,581	0,033	0,31
891	HSD17B8	-0,6	0,034	0,31
892	H3-3A	0,691	0,034	0,31
893	NR4A1	1,77	0,034	0,31
894	PLEKHG5	0,865	0,034	0,31
895	ZYX	0,973	0,034	0,31
896	INSYN1	-0,833	0,034	0,31

897	NDN	-0,948	0,034	0,31
898	DUSP4	-1,231	0,034	0,31
899	VPS72	-0,712	0,034	0,31
900	FSCN1	1,178	0,034	0,31
901	C17orf97	-0,842	0,034	0,31
902	CBLB	-0,974	0,034	0,31
903	OXNAD1	-1,182	0,034	0,31
904	RPS24	0,611	0,034	0,31
905	AMY2B	-0,88	0,034	0,31
906	CTDP1	-0,989	0,034	0,31
907	KLK14	-1,251	0,034	0,31
908	TM2D3	-0,839	0,034	0,31
909	ZNF644	-0,763	0,034	0,31
910	GTF2F1	-1,34	0,034	0,31
911	ACP1	-0,976	0,034	0,31
912	CSNK1E	0,904	0,034	0,31
913	PTCH1	0,724	0,034	0,31
914	PCGF3	-0,765	0,034	0,31
915	ACOT2	-0,925	0,034	0,31
916	PRDM5	-1,049	0,034	0,31
917	PBRM1	-0,709	0,034	0,31
918	GASK1B	0,636	0,034	0,31
919	LRRC2	-0,848	0,035	0,31
920	CMTM3	0,986	0,035	0,31
921	TERF2	-0,667	0,035	0,31
922	TTC14	-0,636	0,035	0,31
923	SOX12	-1,059	0,035	0,31
924	TBL3	-0,67	0,035	0,31
925	DNAJC19	-0,785	0,035	0,31
926	IQGAP1	0,833	0,035	0,31
927	VAMP2	-0,53	0,035	0,31
928	CDH5	1,059	0,035	0,31
929	MOV10	-0,876	0,035	0,31
930	ACSS3	-1,245	0,035	0,31
931	PDP1	-0,786	0,035	0,31
932	UBE2M	-1,202	0,035	0,31
933	KIF21A	-1,426	0,035	0,31
934	TBX3	1,006	0,035	0,31
935	SPEF2	-0,796	0,035	0,31
936	HMGN1	0,53	0,035	0,31
937	UBXN7	-1,152	0,035	0,31
938	YWHAZ	0,956	0,035	0,31
939	ELANE	-1,375	0,035	0,31
940	H4-16	-0,862	0,035	0,31
941	ACADM	-1,32	0,035	0,31

942	TOM1L2	-0,698	0,035	0,31
943	EXOSC6	-0,795	0,035	0,31
944	ADGRA3	-0,92	0,036	0,31
945	ST3GAL3	-0,529	0,036	0,31
946	FRS2	-0,914	0,036	0,31
947	ADGRE5	1,066	0,036	0,31
948	VPS37C	-0,767	0,036	0,31
949	DDX49	-0,598	0,036	0,31
950	TMUB1	-0,73	0,036	0,31
951	KRT28	-0,556	0,036	0,31
952	DPP6	-0,991	0,036	0,31
953	MAGEA10	-1,135	0,036	0,31
954	COG5	-0,647	0,036	0,32
955	ARIH1	-0,701	0,036	0,32
956	TACC2	-1,166	0,036	0,32
957	LYZ	1,166	0,036	0,32
958	EZH1	-0,661	0,036	0,32
959	FZD1	-0,592	0,036	0,32
960	CFAP410	-0,799	0,036	0,32
961	NFS1	-0,602	0,036	0,32
962	SCYL3	-1,089	0,036	0,32
963	HOXA3	-0,856	0,036	0,32
964	C2orf74	-1,099	0,036	0,32
965	GRB7	-0,858	0,037	0,32
966	AGPAT2	-0,696	0,037	0,32
967	FOXJ3	-0,597	0,037	0,32
968	PPHLN1	-0,8	0,037	0,32
969	CD59	0,896	0,037	0,32
970	TMEM68	-0,581	0,037	0,32
971	F8A2	-1,088	0,037	0,32
972	MTMR14	-0,63	0,037	0,32
973	CBX1	0,574	0,037	0,32
974	NAA10	0,811	0,037	0,32
975	H2AX	-0,636	0,037	0,32
976	RPL21	0,68	0,037	0,32
977	ITGB4	-0,999	0,037	0,32
978	SLC50A1	0,509	0,037	0,32
979	FOSL2	1,537	0,037	0,32
980	TMEM234	-0,91	0,037	0,32
981	COBL	-1,236	0,037	0,32
982	NOPCHAP1	-0,888	0,037	0,32
983	ADH6	-1,086	0,037	0,32
984	RBMX	-1,068	0,037	0,32
985	FAM120C	-0,875	0,037	0,32
986	MGAM	-1,156	0,037	0,32

987	COMMD8	-0,587	0,037	0,32
988	CYB561A3	-0,959	0,037	0,32
989	DMRT2	-0,929	0,037	0,32
990	SRRM1	-0,721	0,038	0,32
991	FAM168A	-0,74	0,038	0,32
992	SMR3A	-0,663	0,038	0,32
993	AJUBA	-0,832	0,038	0,32
994	KPNA3	-0,633	0,038	0,32
995	INO80D	-0,806	0,038	0,32
996	NAMPT	1,583	0,038	0,32
997	SURF6	-0,699	0,038	0,32
998	MRPS17	-1,384	0,038	0,32
999	ESYT2	-0,734	0,038	0,32
1000	LRRK2	-0,795	0,038	0,32
1001	FHL2	0,519	0,038	0,32
1002	FAM162A	-0,758	0,038	0,32
1003	EIF1	0,804	0,038	0,32
1004	CSTF2	-0,956	0,038	0,32
1005	ZNF521	-1,273	0,038	0,32
1006	NPAS2	-1,059	0,038	0,32
1007	PEX3	-0,533	0,038	0,32
1008	DOCK2	-0,602	0,038	0,32
1009	POTEE	-1,113	0,038	0,32
1010	ERICH4	-1,388	0,038	0,32
1011	NAAA	-1,121	0,038	0,32
1012	SLFN11	0,601	0,039	0,32
1013	TMCO3	-0,916	0,039	0,32
1014	SLC17A5	-0,835	0,039	0,32
1015	PRDX6	-0,897	0,039	0,32
1016	URGCP	-0,642	0,039	0,32
1017	HNF4A	-1,267	0,039	0,32
1018	CMC2	-0,738	0,039	0,32
1019	AUP1	-0,537	0,039	0,32
1020	SCMH1	-0,669	0,039	0,32
1021	CFD	-1,018	0,039	0,32
1022	PVALB	-1,114	0,039	0,32
1023	PLVAP	1,355	0,039	0,32
1024	FOXN3	-0,883	0,039	0,32
1025	MAGEB6	-1,59	0,039	0,32
1026	MRPS6	-0,709	0,039	0,32
1027	RSPRY1	-1,139	0,039	0,32
1028	ME3	-0,809	0,039	0,32
1029	CD47	-0,755	0,039	0,32
1030	GK2	-0,867	0,039	0,32
1031	DDX42	-0,964	0,039	0,32

1032	SNX27	-1,056	0,039	0,32
1033	ADORA1	-0,665	0,039	0,32
1034	CASC3	-0,705	0,039	0,32
1035	ALDH1A1	-0,773	0,039	0,32
1036	PMP22	-0,69	0,04	0,32
1037	PLEKHG3	-0,609	0,04	0,32
1038	LRRC32	1,296	0,04	0,32
1039	FRAS1	-1,265	0,04	0,32
1040	CCNG1	-0,646	0,04	0,32
1041	LYST	-0,545	0,04	0,32
1042	POU5F1	-0,726	0,04	0,32
1043	EXOSC9	-0,597	0,04	0,32
1044	RPL28	0,631	0,04	0,32
1045	ZNF589	-0,955	0,04	0,32
1046	TMEM208	-0,904	0,04	0,32
1047	PHGDH	-1,179	0,04	0,32
1048	ANKRD28	-1,255	0,04	0,32
1049	WTIP	-0,832	0,04	0,32
1050	ITPR2	-0,752	0,04	0,32
1051	FAM149A	-1,028	0,04	0,32
1052	STIP1	0,61	0,04	0,32
1053	FOXRED2	-0,73	0,041	0,32
1054	PABPC1L	-0,795	0,041	0,32
1055	ARHGEF37	-0,908	0,041	0,32
1056	THAP5	-1,139	0,041	0,32
1057	ULK1	-1,012	0,041	0,32
1058	RAP2B	0,889	0,041	0,32
1059	EMX2	-0,787	0,041	0,32
1060	TRAFD1	-0,51	0,041	0,32
1061	BACE2	-0,655	0,041	0,32
1062	HDGFL2	-0,535	0,041	0,32
1063	PALM	-0,852	0,041	0,32
1064	SPRYD4	-1,429	0,041	0,32
1065	MT1E	-0,884	0,042	0,32
1066	ZW10	-0,618	0,042	0,32
1067	TFCP2	-1,147	0,042	0,32
1068	NNT	-0,58	0,042	0,32
1069	ATR	-0,892	0,042	0,32
1070	ZC3HC1	-0,838	0,042	0,32
1071	SERPINA5	-1,254	0,042	0,32
1072	EPDR1	-0,897	0,042	0,32
1073	TSN	-0,651	0,042	0,32
1074	ACD	-0,951	0,042	0,32
1075	KIAA0754	-0,772	0,042	0,32
1076	OBSCN	-0,715	0,042	0,32

1077	CIAO2A	-0,906	0,042	0,32
1078	CHPT1	-0,754	0,042	0,32
1079	WDR1	0,87	0,042	0,32
1080	BDH1	-0,56	0,042	0,32
1081	FPGS	-0,825	0,042	0,32
1082	GNE	-1,007	0,042	0,32
1083	PAF1	-0,689	0,042	0,32
1084	GOLM1	-1,02	0,042	0,32
1085	CDC42EP5	0,601	0,042	0,32
1086	SDHAF4	-0,63	0,042	0,32
1087	LURAP1	-0,973	0,043	0,32
1088	TRIOBP	0,541	0,043	0,32
1089	MRPS33	-0,916	0,043	0,32
1090	KL	-0,818	0,043	0,32
1091	PAFAH2	-1,165	0,043	0,32
1092	TPRKB	-0,738	0,043	0,32
1093	CTRB1	-0,819	0,043	0,32
1094	SESN3	-0,738	0,043	0,32
1095	MEA1	-0,509	0,043	0,32
1096	UCHL3	-1,046	0,043	0,32
1097	LIN52	-1,074	0,043	0,32
1098	NFE2	-0,793	0,043	0,32
1099	AATF	-0,815	0,043	0,32
1100	TAB2	-0,772	0,043	0,32
1101	ARPC3	0,637	0,043	0,32
1102	CDH2	-0,856	0,043	0,32
1103	NIFK	-0,717	0,043	0,32
1104	ZNF836	-0,872	0,043	0,32
1105	COQ8B	-1,129	0,043	0,32
1106	NDUFB11	-1,122	0,043	0,32
1107	PTPN13	-0,701	0,043	0,33
1108	LCP1	1,024	0,043	0,33
1109	LRRC41	-0,676	0,043	0,33
1110	SMTNL2	-1,143	0,044	0,33
1111	CERS5	-1,414	0,044	0,33
1112	TRIP4	-0,654	0,044	0,33
1113	MSH3	-1,072	0,044	0,33
1114	MAB21L4	-0,877	0,044	0,33
1115	EGFR	-0,935	0,044	0,33
1116	LYPD1	-1,331	0,044	0,33
1117	NDUFA8	-1,479	0,044	0,33
1118	PLAGL1	-0,759	0,044	0,33
1119	TMC4	-1,09	0,044	0,33
1120	GLYCTK	-0,736	0,044	0,33
1121	EFTUD2	-0,697	0,044	0,33

1122	TRPM3	-0,654	0,044	0,33
1123	CRLF3	-1,025	0,044	0,33
1124	ST3GAL6	-0,749	0,044	0,33
1125	MCEE	-1,186	0,044	0,33
1126	GON7	-0,529	0,044	0,33
1127	IMMP2L	-0,889	0,044	0,33
1128	KIR2DS1	0,966	0,045	0,33
1129	ACP3	-0,724	0,045	0,33
1130	BLOC1S6	-0,586	0,045	0,33
1131	MRPL22	-0,867	0,045	0,33
1132	SOWAHC	-1,342	0,045	0,33
1133	SIM2	-0,741	0,045	0,33
1134	PIK3CA	-0,822	0,045	0,33
1135	TNFRSF14	-0,84	0,045	0,33
1136	CHD1L	-0,816	0,045	0,33
1137	TAPBPL	-1,102	0,045	0,33
1138	SUDS3	-0,583	0,045	0,33
1139	TUBB2B	-0,855	0,045	0,33
1140	PRPF38B	-0,509	0,045	0,33
1141	DGLUCY	-0,528	0,045	0,33
1142	CCND1	1,05	0,045	0,33
1143	PRPSAP1	-1,189	0,045	0,33
1144	HOOK1	-0,858	0,045	0,33
1145	MTAP	-1,08	0,045	0,33
1146	TNFRSF10C	-0,819	0,045	0,33
1147	EZR	-0,686	0,046	0,33
1148	EEF1AKNMT	-1,048	0,046	0,33
1149	KIF12	-0,98	0,046	0,33
1150	FAM114A1	-0,77	0,046	0,33
1151	HM13	0,594	0,046	0,33
1152	SLC16A12	-1,537	0,046	0,33
1153	NFASC	-1,161	0,046	0,33
1154	ARMCX1	0,667	0,046	0,33
1155	MCCD1	-0,937	0,046	0,33
1156	TMTC2	-0,869	0,046	0,33
1157	DAB2IP	-0,764	0,046	0,33
1158	RTCA	-0,548	0,046	0,33
1159	DCAF10	-0,698	0,046	0,33
1160	RAP1B	0,634	0,046	0,33
1161	UBL7	-0,647	0,046	0,33
1162	CCZ1	-0,576	0,046	0,33
1163	RHBDD1	-0,786	0,046	0,33
1164	ARHGAP24	-0,86	0,047	0,33
1165	MRPL2	-0,797	0,047	0,33
1166	DMGDH	-1,298	0,047	0,33

1167	MRPS22	-0,882	0,047	0,33
1168	BCAP29	-0,621	0,047	0,33
1169	OSBPL1A	-0,672	0,047	0,33
1170	TNRC6B	-1,112	0,047	0,33
1171	HOXA5	-0,69	0,047	0,33
1172	PPIA	0,734	0,047	0,33
1173	TLR3	-0,686	0,047	0,33
1174	DMAC1	-1,187	0,047	0,33
1175	CMTM7	-0,539	0,047	0,33
1176	PURB	-0,501	0,047	0,33
1177	BSCL2	-0,797	0,047	0,33
1178	CHID1	-0,52	0,047	0,33
1179	LCORL	-0,752	0,047	0,33
1180	ATP6V1B1	-0,914	0,047	0,33
1181	FABP3	-1,252	0,047	0,33
1182	TMEM18	-1,043	0,048	0,33
1183	DUSP9	-0,865	0,048	0,33
1184	LSG1	-0,748	0,048	0,33
1185	ZNF274	-0,626	0,048	0,33
1186	OAZ1	0,786	0,048	0,33
1187	SINHCAF	-0,626	0,048	0,33
1188	CPT1B	-0,796	0,048	0,33
1189	PPP1R14B	0,512	0,048	0,33
1190	VAV3	-0,99	0,048	0,33
1191	RBPMS2	-0,515	0,048	0,33
1192	MOB3B	-0,809	0,048	0,33
1193	PHYHD1	-0,83	0,048	0,33
1194	DNAJB12	-0,638	0,048	0,33
1195	TEX264	-0,981	0,048	0,33
1196	SEMA4F	-0,993	0,048	0,33
1197	CENPB	0,626	0,048	0,33
1198	IPP	-1,091	0,048	0,33
1199	CASP2	-0,682	0,048	0,33
1200	NPY1R	-0,573	0,048	0,33
1201	RPS5	0,591	0,048	0,33
1202	CHIC1	-0,556	0,048	0,33
1203	LMF1	-0,691	0,048	0,33
1204	WDFY2	-0,577	0,049	0,33
1205	ERCC5	-0,68	0,049	0,33
1206	PRODH	-1,075	0,049	0,33
1207	OPHN1	-0,9	0,049	0,33
1208	STX17	-0,661	0,049	0,33
1209	IQCK	-0,63	0,049	0,33
1210	C12orf57	0,653	0,049	0,33
1211	HOXB6	-0,684	0,049	0,33

1212	PKHD1	-1,165	0,049	0,33
1213	TAB3	-0,905	0,049	0,33
1214	MVB12B	-0,981	0,049	0,33
1215	MAP2K3	0,501	0,049	0,33
1216	NR2C2	-0,624	0,049	0,33
1217	ZNF124	-0,75	0,049	0,33
1218	AGMAT	-1,595	0,049	0,33
1219	RPL27A	0,767	0,049	0,33
1220	AP1M1	-0,567	0,049	0,33
1221	LOXL1	-0,692	0,049	0,33
1222	APBB1	-0,72	0,049	0,33
1223	RPL29	0,612	0,049	0,33
1224	VPS4B	-0,834	0,049	0,33
1225	MAP1LC3B	0,577	0,049	0,33
1226	RHCG	-0,62	0,049	0,33
1227	LBH	0,579	0,05	0,33
1228	TIMM21	-0,815	0,05	0,33
1229	GIT1	-0,652	0,05	0,33
1230	IGIP	-0,654	0,05	0,33
1231	TBC1D19	-1,23	0,05	0,33
1232	CHMP4B	-0,838	0,05	0,33
1233	EIPR1	-0,849	0,05	0,33
1234	NIPAL3	-0,79	0,05	0,33