Supplementary Material

Nanostructured recombinant protein particles raise specific antibodies against the NNV coat protein in sole

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Supplementary Figures and Tables

FIGURE S1

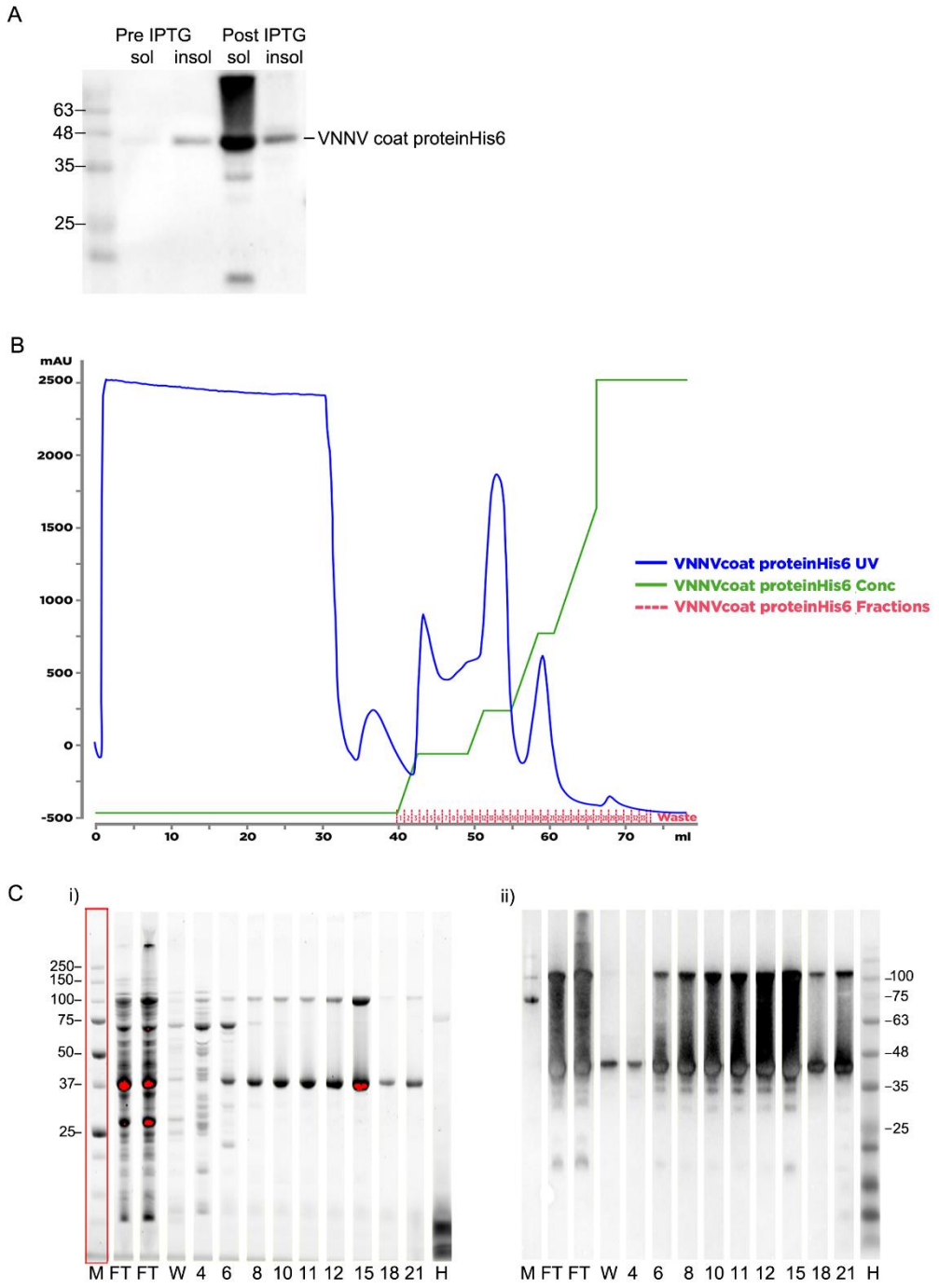

FIGURE S1: Soluble NNV coat protein production. Using the VNNV-C^{NP} clone, cultures were induced at 37 ºC with 1 mM IPTG & then grown slowly overnight at 16 ºC.

A. Soluble (sol) & insoluble (insol) fractions of NNV coat proteinHis6 pre IPTG induction & 14 h post IPTG at 16 ºC. Each well on western blot shows protein produced in an equivalent number of cells**.**

B. Chromatogram purification of NNV coat proteinHis6 by FPLC (fast protein liquid chromatography). Soluble protein purified using 0.1 M $NiCl_2$ on 1 mL HiTrap Chelating HP affinity column, eluting with imidazole gradient. mAU = milli-Absorbance Units. Prominent peaks: fractions 4-6, 10-15 & 18-21.

C. FPLC fractions (B) tested on western blot for presence of NNV coat proteinHis6 (~38 K Daltons), using **i) total protein detection ii) Anti-His antibody**. M = stain free system total protein marker FT = flow through, W = waste, H = protein marker for western, detecting Histag.

FIGURE S2

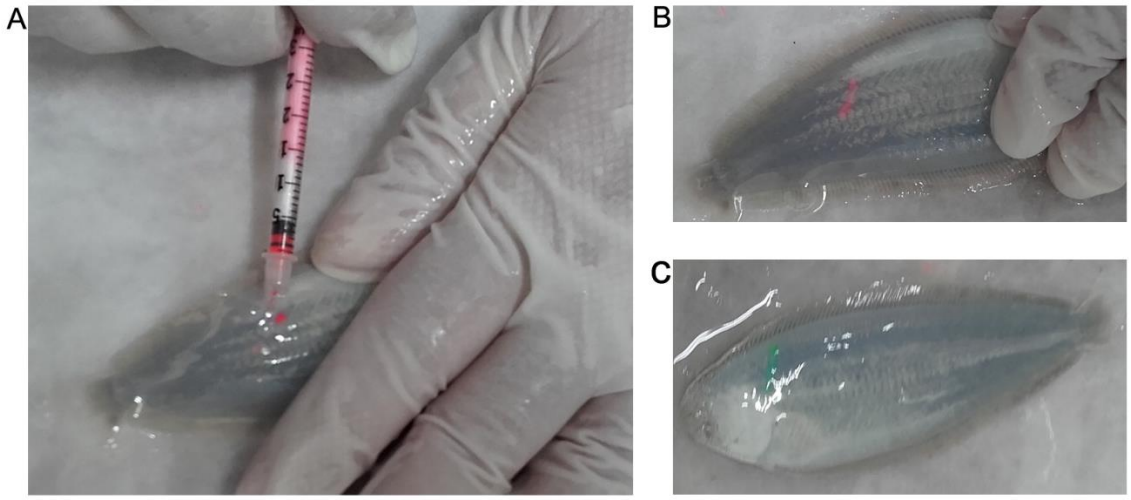

FIGURE S2: Colour coding of sole. A. Fish were colour tagged by injecting a thin line of dye (Visible Implant Elastomer) under the ventral skin. **B**. Red at the distal end for fish destined for oral intubation. **C**. Green at the proximal end for fish to be injected i.p.

FIGURE S3

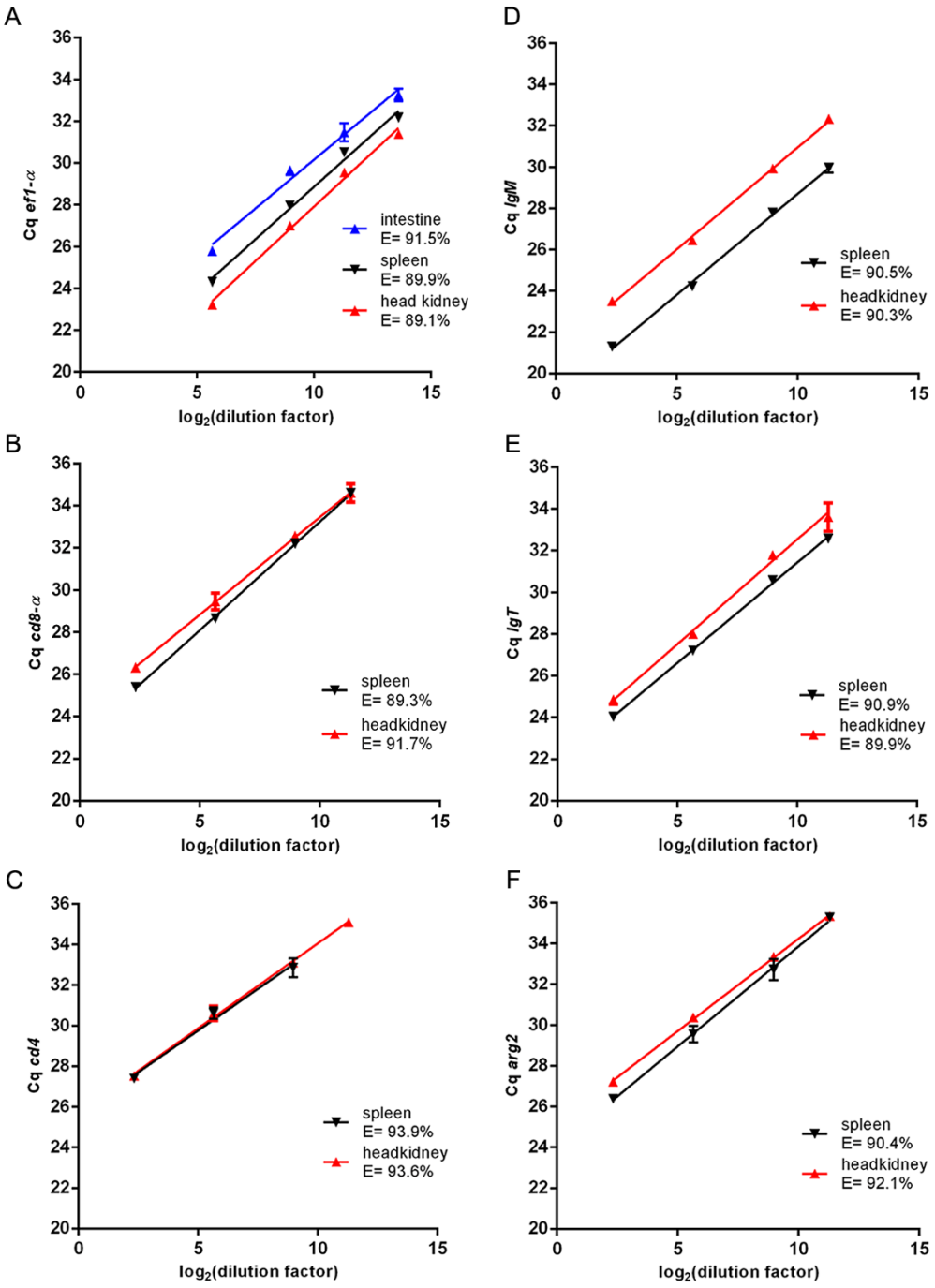

FIGURE S3: Efficiencies of *S. senegalensis* primers for qPCR.

Primer efficiencies determined via qPCR using serial dilutions in triplicate of control cDNA. Efficiencies ($E= 10^{-1/slope}$) calculated via linear regression for primers:**A.** *eef1-α* **B**. *cd8-α* **C**. *cd4* **D**. *igm* **E**. *igt* **F**. *arg2*

FIGURE S4

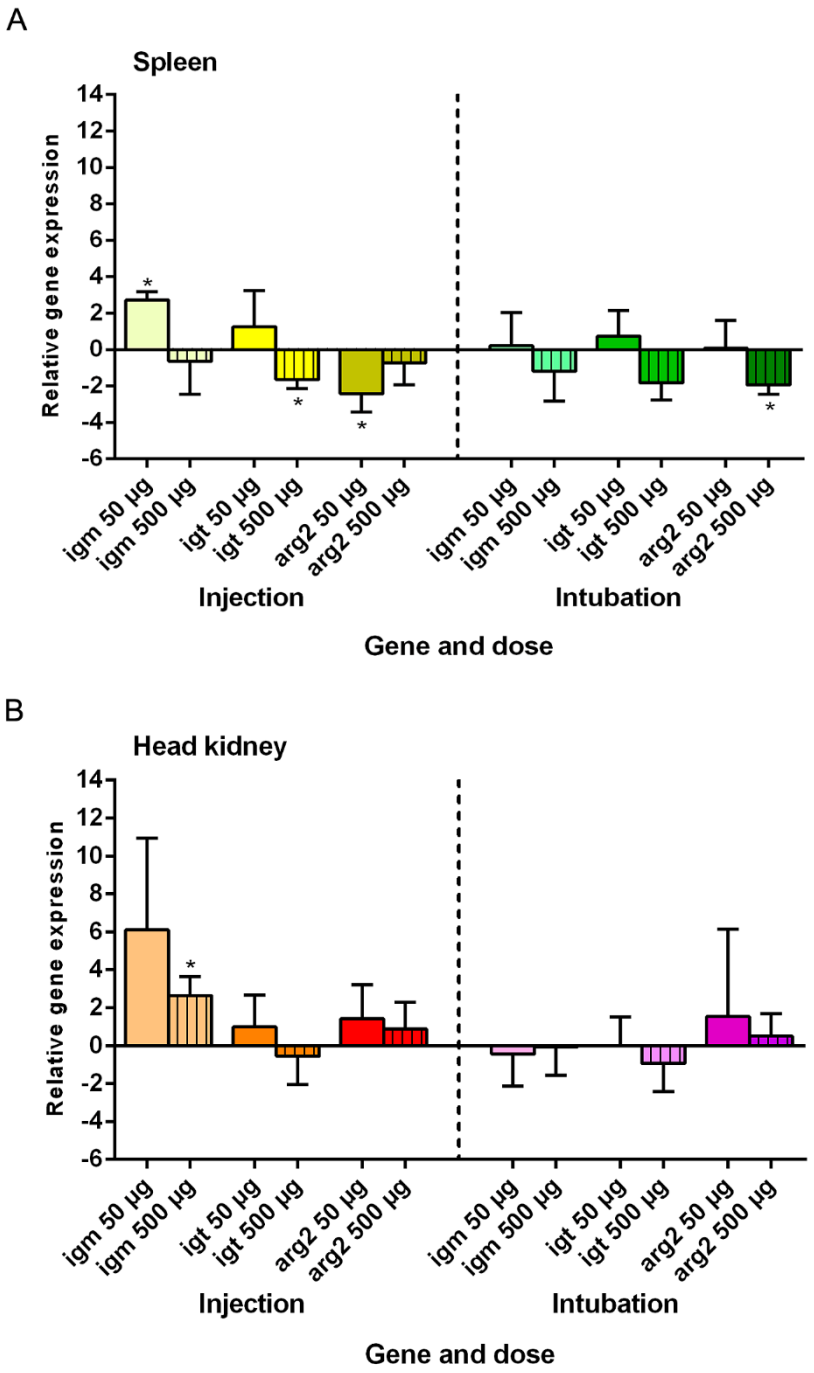

FIGURE S4: Gene expression post VNNV-C^{NP} administration via both routes and two doses in A. Spleen and B. Headkidney. Fish (~15 g) were administered 50 or 500 µg of VNNV-C^{NP} in PBS on days 0 and 14 (booster) by i.p. injection (left) or oral intubation (right). At 3 days post booster tissues were sampled from 4 fish/ treatment for RNA extraction. Gene expression determined by qPCR with *eef1-α* as reference gene and PBS control as the calibrator group using the Livak method. Data are mean ± SD (n = 4). Differences between each treatment mean & control were analysed by unpaired one-sided t-tests with Welch's correction for unequal variances. Significance levels *p < 0.05

TABLES

TABLE S1: *S. senegalensis* primers for qPCR

Gene	Sequence 5'-3'	Amplicon Size (bp)	Reference/ Accession #/ SoleaDB
eef1a	**F**: GATTGACCGTCGTTCTGGCAAGAAGC **R**: GGCAAAGCGACCAAGGGGAGCAT	142	Infante, 2008 [1] Genebank: AB326302
cd8a	**F:** GTCGCAGTTCTGCTCTCCGC **R:** TCGGTTGCAGTAGAGGACGG	97	solea_v4.1_unigene59609
cd4	**F:** AGCAGGGCAGAGAAGAAGACG **R:** GCAGCTGGCCGGGATGTAAG	142	solea_v4.1_unigene450963
igm	**F:** TGAAACATTGACACAGCCAGCC **R:** CGTGTGAGCTTCCAATCCACTC	149	solea_v4.1_unigene691100
igt	**F:** AGTGGTAAAGCGGCCTGGAG **R:** GCCTTTCCTTCAGCTTGTCTG	108	solea_v4.1_unigene625671
arg2	**F:** ACCGCGTCGTTAGCAGTTGA **R:** TGCTCTGTGTCGTCCTTCGCC	107	solea_v4.1_unigene32066

[1] C. Infante, M.P. Matsuoka, E. Asensio, J.P. Canavate, M. Reith, M. Manchado, Selection of housekeeping genes for gene expression studies in larvae from flatfish using real-time PCR, BMC Mol Biol 9 (2008) 28.