ADDITIONAL FILE 1

Additional Materials and Methods

Ex vivo fatty acid synthase activity assay

FASN activity assay was done 12 hours after the last intraperitoneal (i.p.) injection. Tumor tissues were minced and homogenized in ice-cold lysis buffer (300 μL: 1 mM EDTA, 150 mM NaCl, 100 μg/mL PMSF, 50 mM Tris-HCl, pH 7.5) using the TissueRuptor. Then, tissues were sonicated during 30 minutes at 4ºC (PSelecta ultrasons) and centrifuged for 15 minutes at 4ºC to obtain supernatants particle-free. A supernatant sample was taken to measure protein content by the Lowry-based BioRad assay (BioRad). FASN activity assay was done as we previously described [8-9, 13]. Briefly, one-hundred and twenty micrograms of substrate were pre-incubated during 15 minutes at 37ºC in 0.2 M of potassium phosphate buffer pH 7.0, for temperature equilibration. The sample was then added to the reaction mixture: 200 mM potassium phosphate buffer pH 7.0, 1 mM EDTA, 1 mM dithiothreitol, 30 μM acetyl-CoA and 0.24 mM NADPH in 0.3 mL reaction volume were monitored at 340 nm for 3 min to measure background NADPH oxidation (Lambda Bio 20, Perkin Elmer, EUA; using UV Kinlab 2.80.02 software). After the addition of 50 μM of malonyl-CoA, the reaction was assayed for an additional 10 min to determine FASN-dependent oxidation of NADPH. Rates were corrected for the background rate of NADPH oxidation in the presence of acetil-CoA. FASN activity was expressed in nmol NADPH oxidized x min^{-1} x mg $protein^{-1}$.

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