



Figure S4. NAPRT is a cytosolic and nuclear protein that regulates cancer cell metabolism and susceptibility to FK866. A, 2×10^6 scr-shRNA-, or NAPRT-sh2-expressing OVCAR-4, OVCAR-5, OVCAR-8 and Capan-1 cells/flask were seeded in 175 cm² flasks, allowed to grow up to 80% confluence and subsequently used for the isolation of mitochondria and cytosolic fractions. Thereafter, Ubiquinol-Cytochrome C Reductase Core Protein 1 (UQCRC1), phosphofructokinase (PFK), histone 3 (H3), NAPRT and β -actin levels were detected by immunoblotting. B, H, 3×10^6 OVCAR-5 cells expressing the scr-shRNA or NAPRT-sh2/flask were seeded in 75 cm² flasks, allowed to adhere overnight and then stimulated for 24 h w/ or w/o 100 nM FK866. Thereafter, cells were used for the generation of lysates in which P/O ratio, ATP, AMP, as well as hexokinase, phosphofructokinase, pyruvate kinase and lactate dehydrogenase activity were determined. I, 10^5 Capan-1 cells expressing the scr-shRNA, NAPRT-sh1 or NAPRT-sh2/well were plated in 6-well plates, allowed to adhere overnight and then stimulated for 24 h w/ or w/o 100 nM FK866. Thereafter, cells were used for cell cycle determination. A, One representative experiment out of three is presented. B-I, Data are presented as means of three separate experiments. ns: non-significant; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$. In C-H, the experimental values obtained with scr-sh+FK866, NAPRT-sh2 and NAPRT-sh2+FK866 all had $p < 0.05$ compared to those obtained with the scr-sh with the exception of the ATP and AMP values obtained with the scr-shRNA+FK866, which were non-significant.