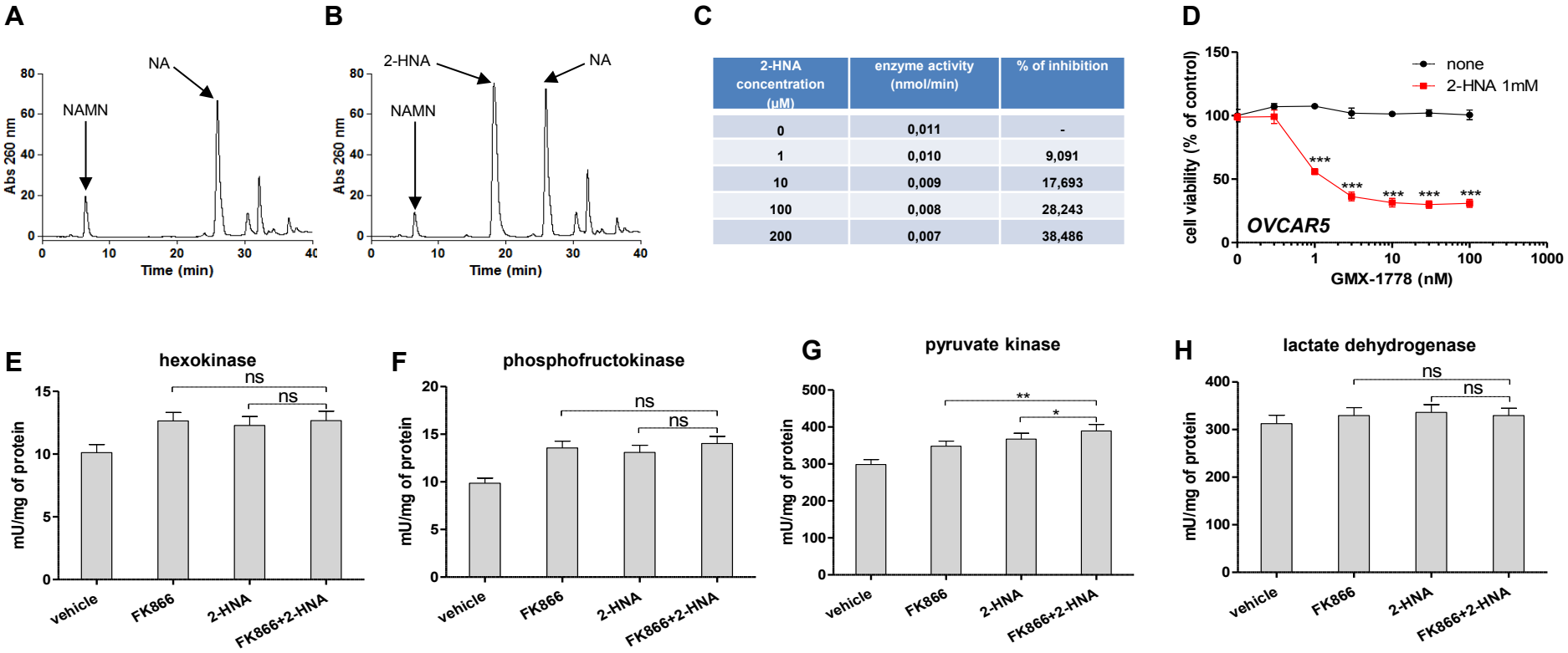

Figure S5. 2-HNA inhibits NAPRT enzymatic activity and increases glycolysis in OVCAR-5 cells. A-C. The enzymatic activity of recombinant NAPRT was assayed using different concentrations of NA and PRPP, in presence or absence of 2-HNA at the indicated concentrations. Formation of the NAPRT product, NA mononucleotide (NAMN) was analyzed by reverse-phase C18-HPLC. NAPRT activity inhibition by 2-HNA was calculated comparing the amount of NAMN in reactions containing 2-HNA against the reactions without it. D, 2 x 10^3 OVCAR-5 cells/well were plated in 96-well plates, allowed to adhere overnight and then incubated for 72 h w/ or w/o GMX-1778 at the indicated concentrations in the presence or absence of 1 mM 2-HNA. Subsequently, cell viability was detected with SRB. E-H, 3 x 10^6 OVCAR-5 cells/flask were plated in 75 cm^2 flasks, allowed to adhere overnight and then treated for 24 h w/ or w/o 100 nM FK866 in the presence or absence of 1 mM 2-HNA. Thereafter, cell lysates were generated and hexokinase, phosphofructokinase, pyruvate kinase and lactate dehydrogenase activities were determined. A-C, One representative experiment out of three is presented. D-G, Data are presented as means ± SD of three separate experiments. ns: non-significant; *: p<0.05; **: p<0.01; ***: p<0.001. In E-H, the experimental values for FK866, 2-HNA and FK866+2-HNA all had p<0.05 compared to those obtained with the vehicle.