Figure S1. ENO1 knockdown attenuates lactate production, migration and viability of prostate cancer cells. PC-3 cells were transfected with ENO1-targeting siRNA (si-ENO1 #1 or #2) or control siRNA (scramble sequence) for 72 h, and the efficiency of ENO1 depletion was confirmed by (A) western blotting. GAPDH served as the loading control. (B) ENO1-knockdown PC-3 cells were cultured for an additional 48 h, and then the supernatant was collected for determination of lactate levels. (C) Transwell migration assay, (D) cell viability assay and (E) measurement of cell surface ENO1 were performed using ENO1-knockdown PC-3 cells. All results are presented as the mean ± SD of three independent experiments. P-values were calculated with one-way ANOVA (with Tukey's post hoc test). ENO1, enolase-1; siRNA, small interfering RNA.

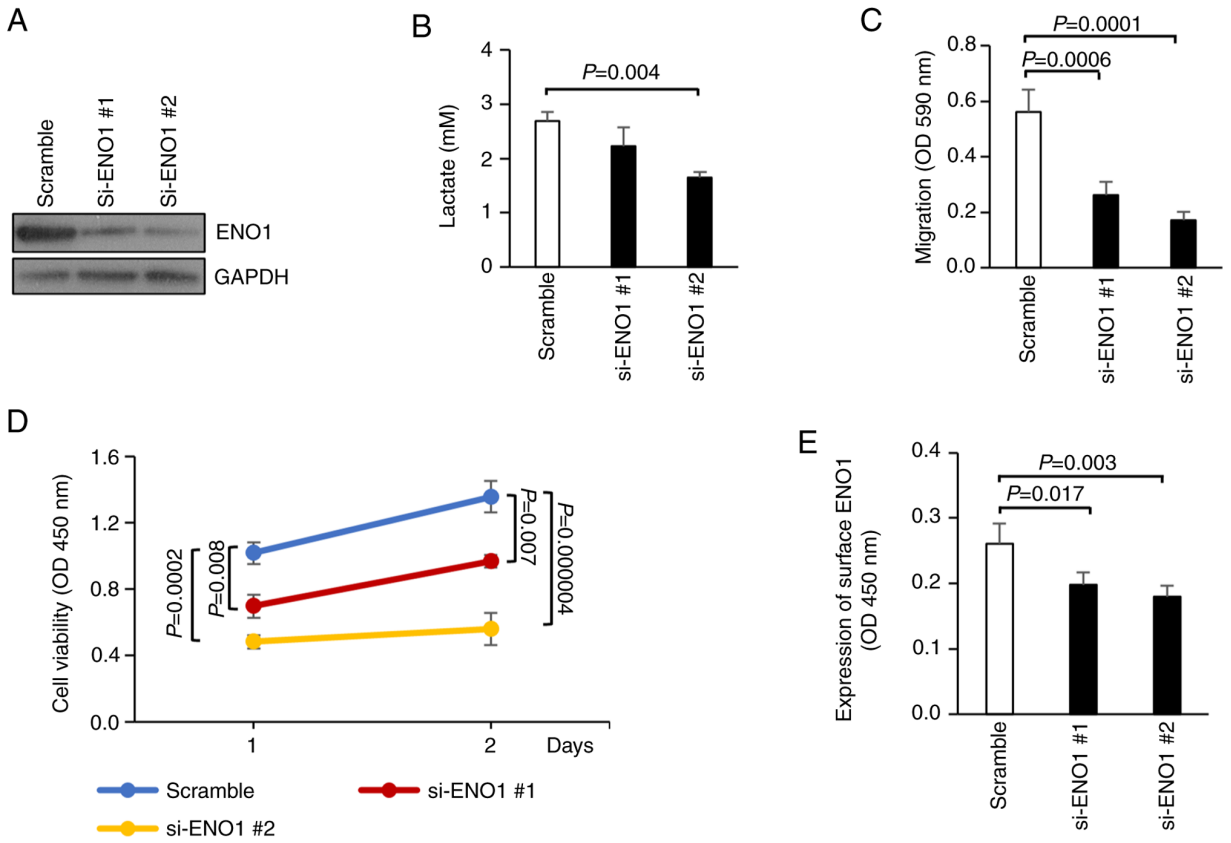

Figure S2. Extracellular ENO1-specific regulation of glycolysis and pro-cancer activity of prostate cancer cells. PC-3 cells were treated with the indicated concentrations of ENO1-WT and allowed to migrate for additional 18 h in a Transwell assay. The studies were conducted in the presence or absence of 100 μg/ml ENO1 mAb. The cell-conditioned media and cell pellets from the Transwell inserts were collected for the measurement of (A) lactate concentration and (B) intracellular LDH activity. (C) Cell migration of PC-3 cells was measured by Transwell assay. (D) Cell migration of PC-3 cells was measured after treatment with the indicated concentrations of ENO1-WT, ENO1-S40A and ENO1-D245R using a Transwell assay. (E) PC-3 cells were pre-treated with 20 ng/ml TNF-α for 4 h and allowed to migrate in the presence or absence of the indicated concentration of ENO1 mAb for an additional 18 h in a Transwell assay. hIgG1 was used as a control for the non-specific antibody binding effect. (F) The lactate concentration of the cell-conditioned media from the Transwell inserts was measured. All results are presented as the mean ± SD of three independent experiments. P-values were calculated with one-way ANOVA (with Tukey's post hoc test). ENO1, enolase-1; ENO1-WT, wild-type ENO1; hIgG1, human IgG1; LDH, lactate dehydrogenase; mAb, monoclonal antibody.

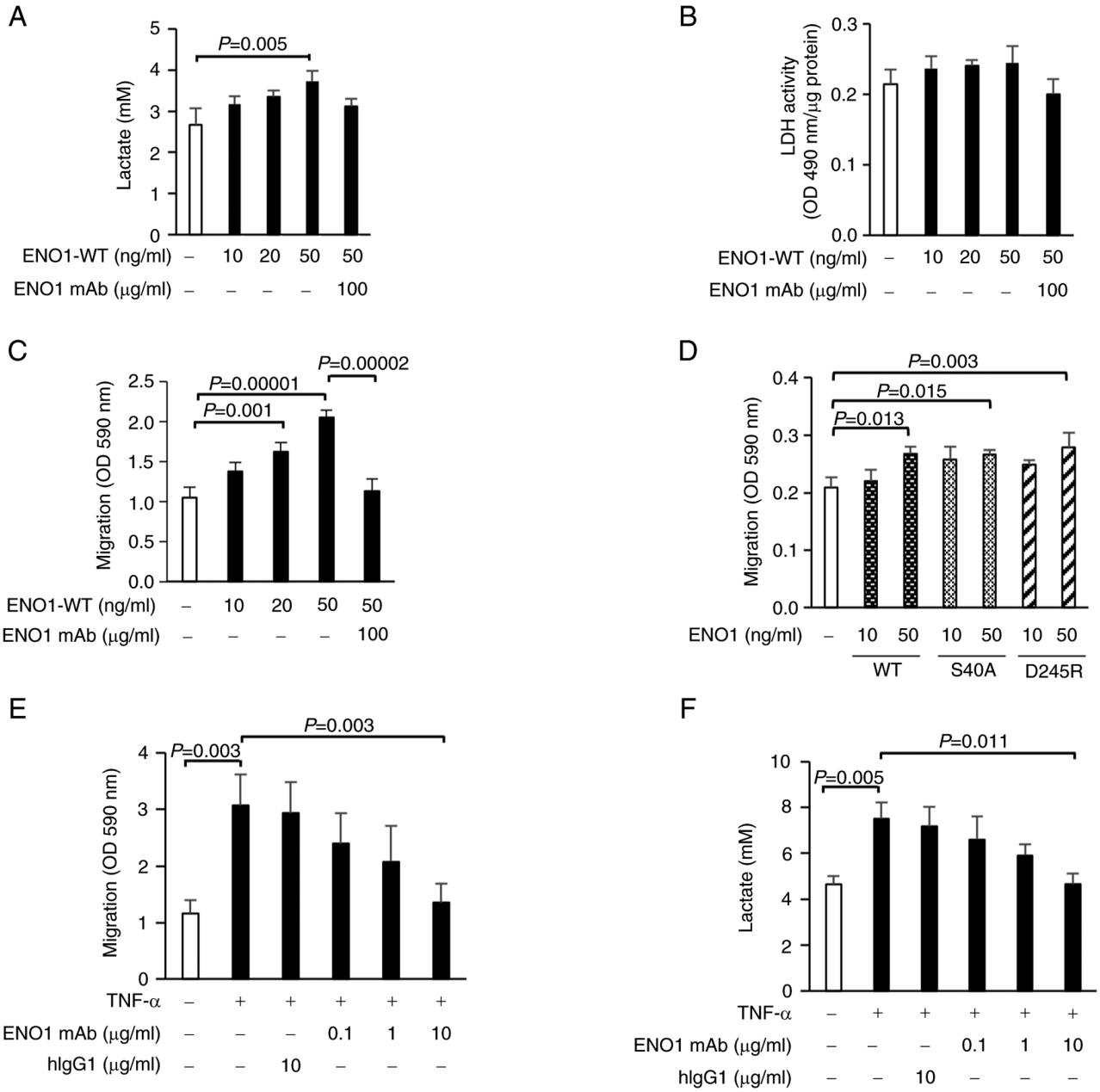

Figure S3. HIF-1α may not be regulated in a NF-κB-dependent manner upon ENO1 stimulation. RPMI-8226 cells were pre-treated with or without BAY 11-7085 (0.25 μM) for 1 h. The protein levels of HIF-1α and IκBα were measured 24 h after treatment with or without ENO1-WT (100 μg/ml). The amounts of studied proteins were first normalized with GAPDH, and then the Rel was calculated by comparing with the untreated cells, of which the value is set to 1.0. ENO1, enolase-1; ENO1-WT, wild-type ENO1; HIF-1α, hypoxia-inducible factor 1-α; NF-κB, nuclear factor-κB; Rel., relative ratio.

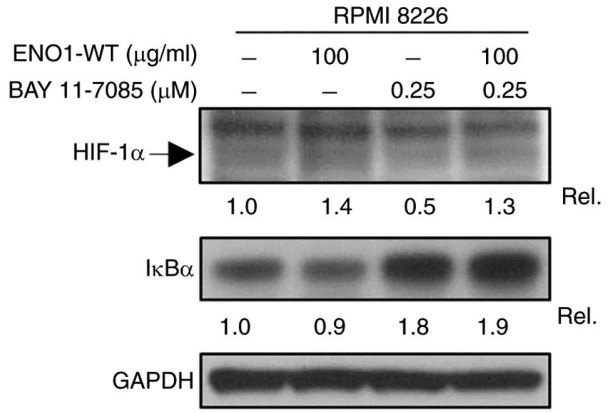

Figure S4. PHD2 may not be regulated upon ENO1 stimulation. RPMI 8226 cells were treated with or without ENO1-WT (100 μg/ml) for 24 h. The protein levels of PHD2 were analyzed by immunoblotting. The amounts of studied proteins were first normalized with GAPDH, and then the Rel value was calculated by comparing with that of untreated cells, which is set to 1.0. PHD2, HIF-prolyl hydroxylase 2; Rel., relative ratio.

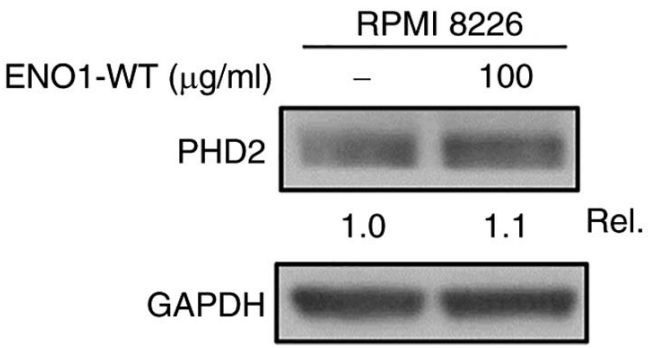

Figure S5. Overexpression of ENO1 is associated with glycolysis and pro-cancer activities. KMS-11 cells were treated with transfection reagent alone (mock) or stably transfected with oe-ENO1, followed by (A) analysis of HIF-1α, HK2, GLUT1, ENO1 (Myc/DDK-tagged) and ENO1 (endogenous) protein levels. The amounts of studied proteins were first normalized with GAPDH, and then the Rel. was calculated by comparing with the mock cells, of which the value was set to 1.0. The concentration of (B) lactate, (D) IL-6 and (E) VEGF in the culture medium and (F, lower panels) the migratory cells in the Transwell assay were determined 48 h after seeding. Representative images were shown at x10 magnification. (C) Cell viability assay was measured at 72 h after seeding. All results are presented as the mean ± SD of three independent experiments. P-values were calculated with a two-tailed paired Student's t-test. ENO1, enolase-1; oe-ENO1, ENO1-expressing vector; GLUT1, glucose transporter 1; HIF-1α, hypoxia-inducible factor 1-α; HK2, hexokinase 2; IL-6, interleukin 6; Rel, relative ratio; VEGF, vascular endothelial growth factor.

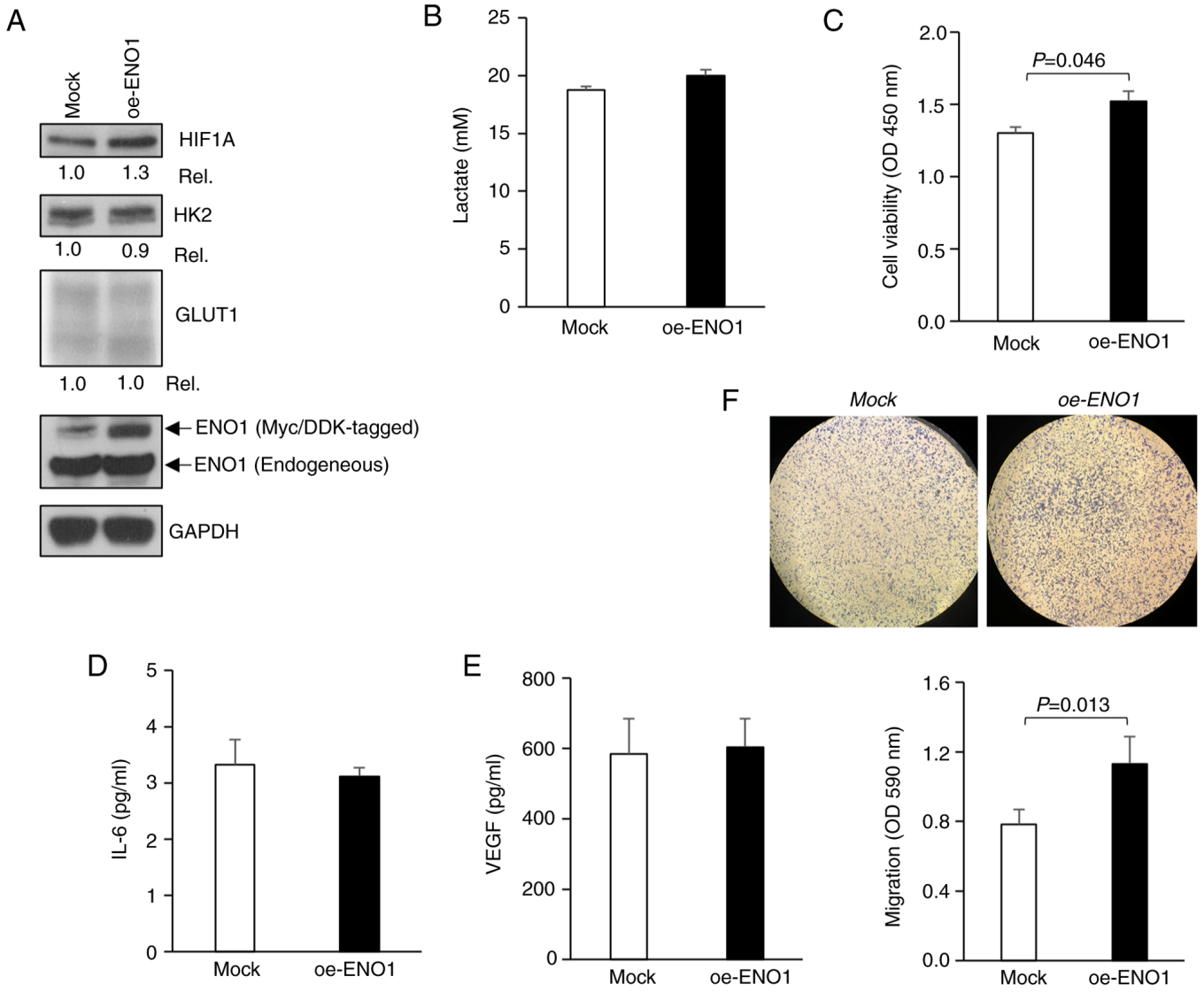

Figure S6. ENO1 mAb reduces cell viability of multiple myeloma cells. (A) RPMI-8226 or (B) U266 cells were treated with the indicated concentrations of ENO1 mAb or hIgG1. Cell viability was assayed 1, 2 or 3 days after treatment using the Cell Counting Kit-8. The OD 450 nm value was positively associated with the number of viable cells. The results are presented as a percentage of a reduction in viability of ENO1 mAb and hIgG1-treated cells comparing with the untreated cells. P-values were calculated with one-way ANOVA (with Tukey's post hoc test). ENO1, enolase-1; hIgG1, human IgG1; mAb, monoclonal antibody; Ut, untreated.

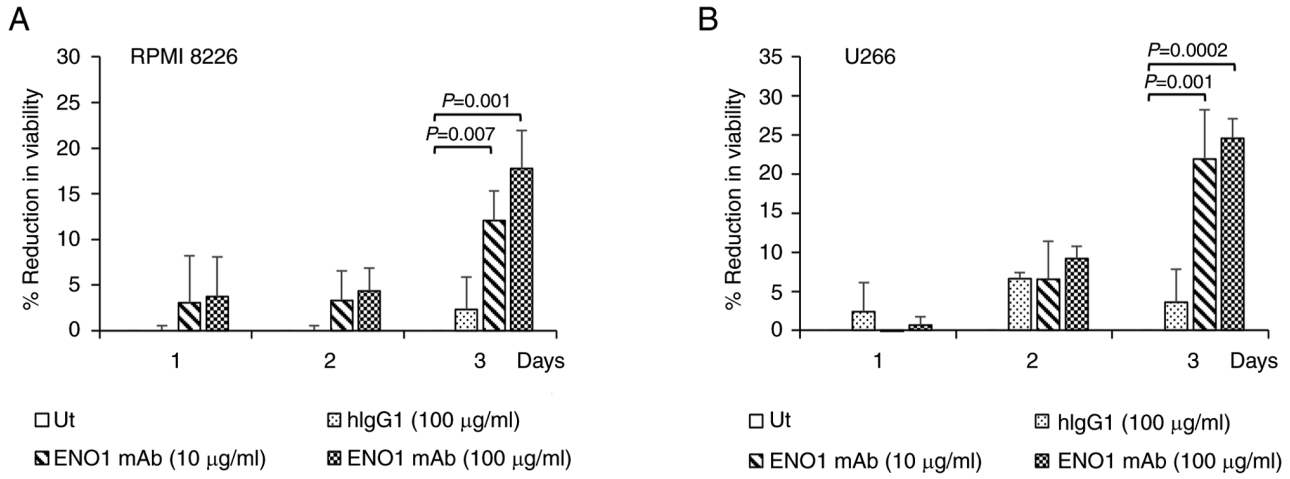

Figure S7. The MBP-1 protein levels and c-MYC mRNA levels are not regulated by ENO1 mAb. RPMI 8226 cells were treated with indicated concentrations of ENO1 mAb or hIgG1 for 48 h. (A) The MBP-1 protein levels were analyzed by immunoblotting. The amounts of studied proteins were first normalized with GAPDH, and then the Rel. was calculated by comparing with that of untreated cells, which was set to 1.0. (B) The *c-MYC* mRNA levels were quantified by using reverse transcription-quantitative PCR. All results are presented as the mean ± SD of three independent experiments. P-values were calculated with one-way ANOVA (with Tukey's post hoc test). ENO1, enolase-1; hIgG1, human IgG1; mAb, monoclonal antibody; MBP-1, c-myc promoter binding protein 1; Rel., relative ratio.

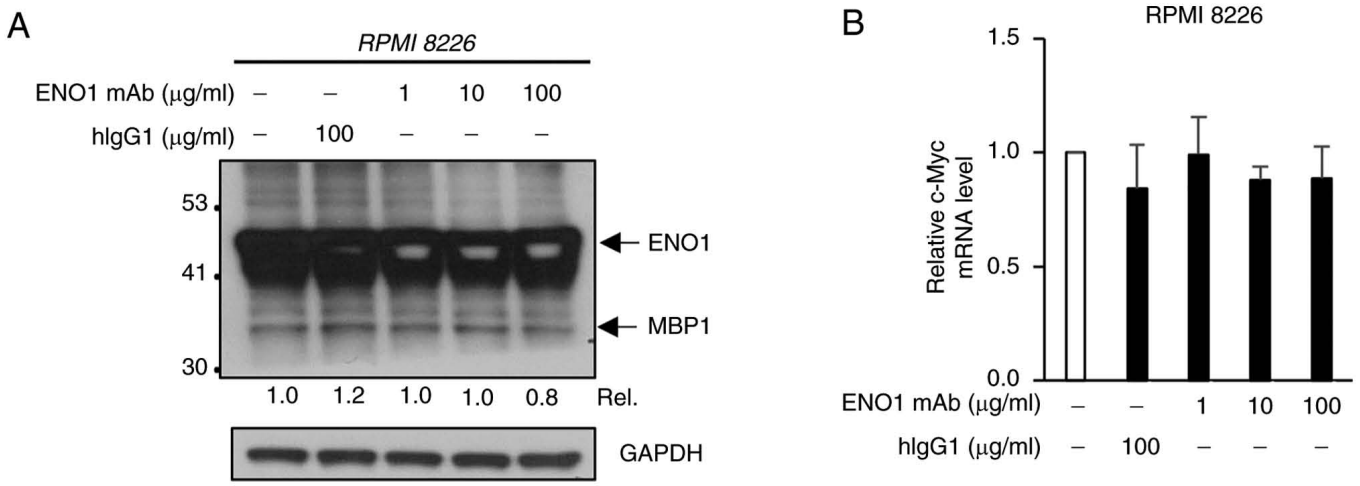

Figure S8. ENO1 mAb, but not hIgG1, reduces tumor growth and glycolysis in the RPMI-8226 subcutaneous xenograft model. Male nude mice were subcutaneously implanted with RPMI-8226 cells and randomized when tumor size reached >100 mm^3 (n=5). ENO1 mAb (30 mg/kg) or isotype control hIgG1 (30 mg/kg) was intraperitoneally injected twice a week at the indicated time points. (A) Each data point represents the mean volume ± SD from the ENO1 mAb-treated, hIgG1 treated or vehicle control groups. Mice were sacrificed on day 23 and (B) representative images of excised tumors are shown. (C) Tumor weight was measured and sera were collected for (D) the measurement of lactate concentration. (E) The mice body weight was measured at the indicated time points. P-values were calculated with one-way ANOVA (with Tukey's post hoc test). ENO1, enolase-1; hIgG1, human IgG1; mAb, monoclonal antibody.