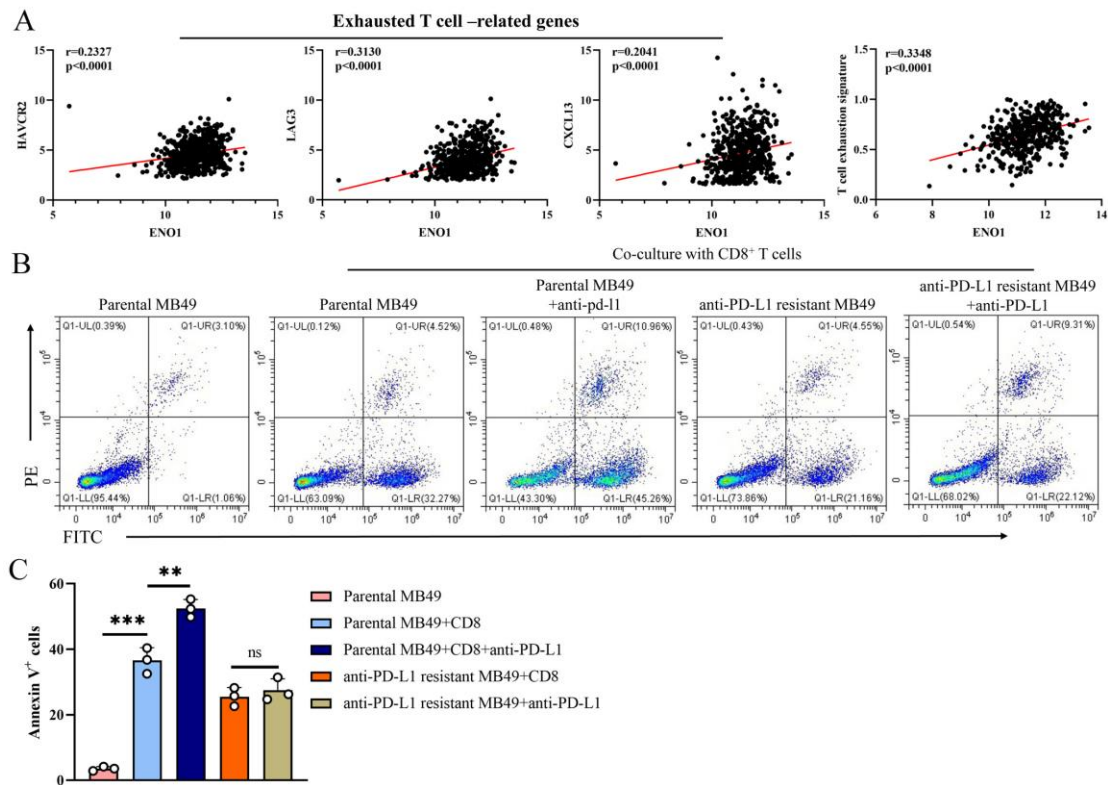
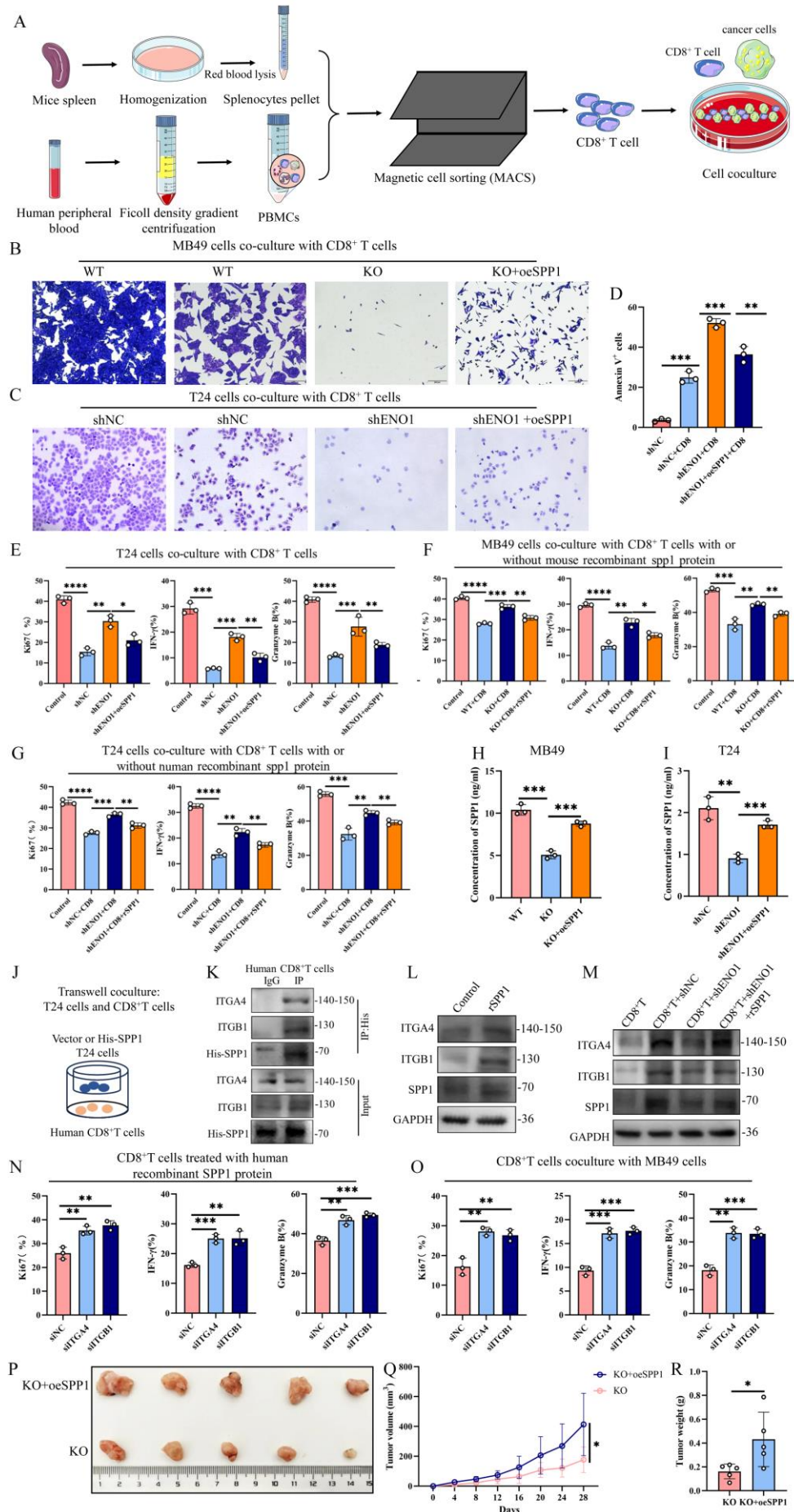




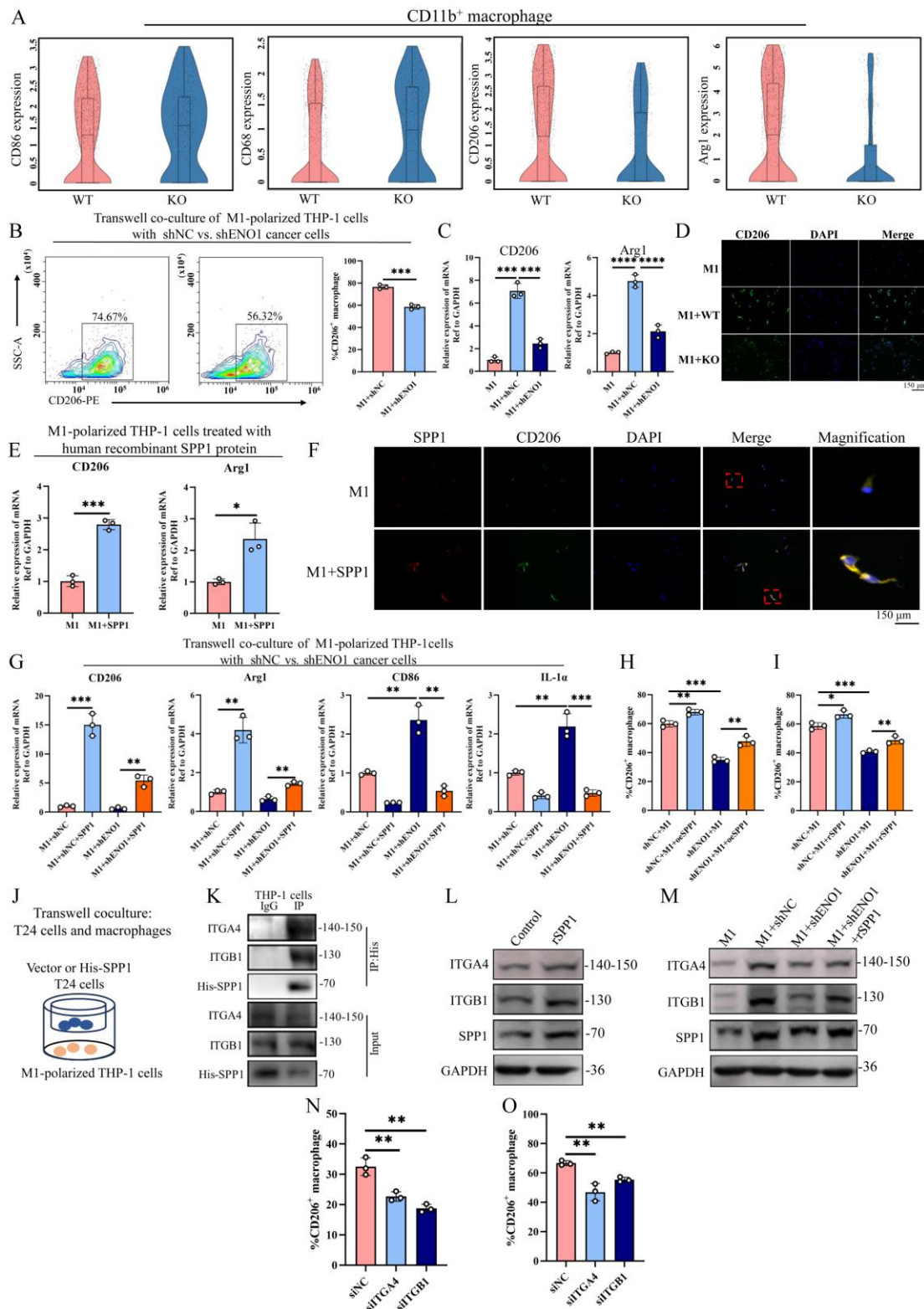
Additional file1. (A) Correlation analysis between ENO1 expression with exhausted T cells related markers (HAVCR2, LAG3, CXCL3) and T cell exhaustion signature in the TCGA bladder urothelial carcinoma (TCGA-BLCA) dataset. Two-tailed Spearman correlation is reported. **(B, C)** Flow cytometric analysis of CD8⁺ T cell-mediated killing of parental MB49 cells and anti-PD-L1-resistant MB49 cells in the presence or absence of anti-PD-L1 antibody. n = 3 biologically independent samples per group. One-way ANOVA with Dunnett's multiple comparisons test. Data are presented as mean values $\pm$ SD.

samples. **(E-F)** RT-qPCR and western blotting analyses the expression level of ENO1 in WT or ENO1-KO cancer cells. n = 3 biologically independent samples per group. Two-side unpaired Student's t-test. Data are presented as mean $\pm$ SD. **(G-K)** In vivo bioluminescence imaging of lung metastases. Intravenous injection of luciferase-labeled WT or ENO1-KO MB49 cells into C57BL/6J mice via the tail vein (n=6 per group), followed by measurement of experimental lung metastases growth on day 20 using an IVIS Spectrum In Vivo imaging system (G, I). Images and hematoxylin & eosin staining of lung metastases (H, J) and statistical analysis of lung metastatic nodules (K). Scale bars: 200 μ m. Two-side unpaired Student's t-test. Data are presented as mean $\pm$ SD. **(L-P)** In vivo bioluminescence imaging of lung metastases. Intravenous injection of luciferase-labeled WT or ENO1-KO MB49 cells into C57BL/6J mice via the tail vein (n=5 per group), followed by treatment with 8mg/kg ENOblock and measurement of experimental lung metastases growth on day 14 using an IVIS Spectrum In Vivo imaging system (L, N). Images and hematoxylin & eosin staining of lung metastases (M, O) and statistical analysis of lung metastatic nodules (P). Scale bars: 200 μ m. Two-side unpaired Student's t-test. Data are presented as mean $\pm$ SD. **(Q)** GSEA revealing immune-associated pathways correlated with ENO1 expression in the RNA-seq dataset. *p < 0.05, **p < 0.01, ***p < 0.001.



Additional file3. (A) Illustration shows the in vitro procedure of human and mouse CD8⁺ T cells isolation and cocultivation with cancer cells. (B) Proliferation assay was conducted to evaluate WT, ENO1-KO, or ENO1-KO+oeSPP1 cells in the presence of mouse CD8⁺ T cells. n = 3 biologically independent samples per group. (C, D) Proliferation and apoptosis assays were conducted to evaluate shNC, shENO1, or shENO1+oeSPP1 cells in the presence of human CD8⁺ T cells. n = 3 biologically independent samples per group. One-way ANOVA with Dunnett's multiple comparisons test. Data are presented as mean values $\pm$ SD. (E) Flow cytometry analysis showed Ki67, IFN- γ , GZMB expression of mouse CD8⁺ T cells that were cocultured with shNC, shENO1, shENO1+oeSPP1 cells. n = 3 biologically independent samples per group. One-way ANOVA with Dunnett's multiple comparisons test. Data are presented as mean values $\pm$ SD. (F, G) Flow cytometry analysis showed Ki67, IFN- γ , GZMB expression of mouse/human CD8⁺ T cells that were cocultured with MB19 or T24 cells treated with mouse/human recombinant SPP1 protein. n = 3 biologically independent samples per group. One-way ANOVA with Dunnett's multiple comparisons test. Data are presented as mean values $\pm$ SD. (H, I) The ELISA assay detected the concentration of SPP1 in the coculture system. n = 3 biologically independent samples per group. One-way ANOVA with Dunnett's multiple comparisons test. Data are presented as mean values $\pm$ SD. (J) A schematic representation of a transwell coculture assay involving human CD8⁺ T cells combined with His-SPP1 T24 cells. (K) Co-immunoprecipitation assays of CD8⁺ T cells were performed using anti-His antibody. n = 3 biologically independent samples per group. (L) Western blotting assay detected ITGA4 and ITGB1 protein expression in human CD8⁺ T cells with or without recombinant mouse SPP1 protein treatment. n = 3 biologically independent samples per group. (M) Western blotting assay detected ITGA4 and ITGB1 protein expression in human CD8⁺ T cells cocultured with WT or ENO1-KO cells, with or without recombinant mouse SPP1 protein. n = 3 biologically independent samples per group. (N) Flow cytometric analysis of Ki67, IFN- γ and GZMB expression in siITGA4 or siITGB1 CD8⁺ T cells treated with recombinant human SPP1 protein. n = 3 biologically independent samples per group. (O) Flow

cytometric analysis of Ki67, IFN- γ and GZMB expression in siTGA4 or siTGB1 CD8⁺ T cells cocultured with T24 cells. n = 3 biologically independent samples per group. Two-side unpaired Student's t-test. Data are presented as mean $\pm$ SD. **(P-R)** ENO1-KO or ENO1-KO+oeSPP1 cells were subcutaneously inoculated into C57BL/6J mice (n=5 per group). Tumor sizes (O), volumes (P), and weight (Q) were measured. Two-side unpaired Student's t-test. Data are presented as mean $\pm$ SD. *p < 0.05, **p < 0.01, ***p < 0.001.

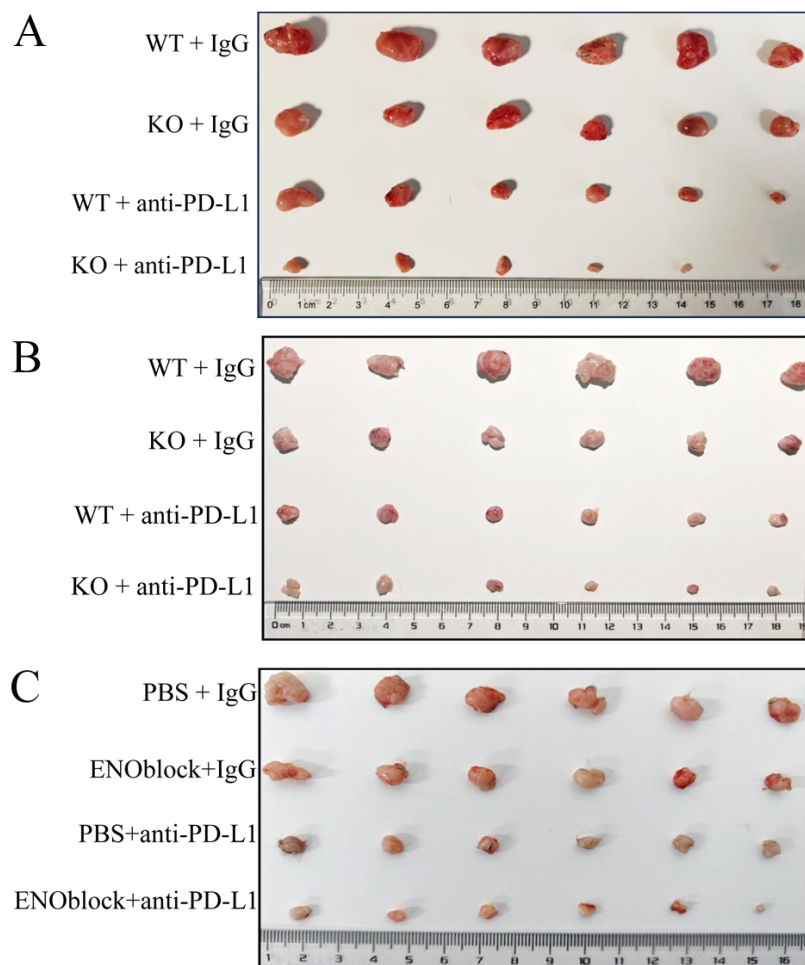


Additional file4. ENO1 promotes the polarization of M2 TAMs via SPP1 in TME.

(A) The expression levels of M1-like TAM marker genes (CD86, CD68) and M2-like TAM marker genes (CD206, Arg1) in CD11b⁺ macrophage. (B) Flow cytometric analysis of CD206 expression in M1-polarized THP-1 cells cocultured with shNC or

shENO1 cells. n = 3 biologically independent samples per group. Two-side unpaired Student's t-test. Data are presented as mean $\pm$ SD. **(C)** The mRNA expression of M2 TAM-like marker gene (CD206, Arg1) in M1-polarized THP-1 cells cocultured with shNC or shENO1 cells. n = 3 biologically independent samples per group. Two-side unpaired Student's t-test. Data are presented as mean $\pm$ SD. **(D)** Immunofluorescence analysis of CD206 expression in M1-polarized BMDMs cocultured with WT or ENO1-KO cells. n = 3 biologically independent samples per group. **(E)** The mRNA expression of M2 TAM-like marker gene (CD206, Arg1) in M1-polarized THP-1 cells cocultured with or without human SPP1 recombinant protein. n = 3 biologically independent samples per group. Two-side unpaired Student's t-test. Data are presented as mean $\pm$ SD. **(F)** Immunofluorescence analysis for CD206 expression for M1-polarized BMDMs cocultured with or without mouse SPP1 recombinant protein. n = 3 biologically independent samples per group. **(G)** The mRNA expression of M2 TAM-like marker gene (CD206, Arg1) and M1-like marker gene (CD86, IL-1 α) in M1-polarized THP-1 cells cocultured with shNC or shENO1 cells, with or without SPP1 overexpression. n = 3 biologically independent samples per group. One-way ANOVA with Dunnett's multiple comparisons test. Data are presented as mean $\pm$ SD. **(H, I)** Flow cytometric analysis of CD206 expression in M1-polarized THP-1 cells cocultured with WT or ENO1-KO cells, with or without SPP1 overexpression or recombinant mouse SPP1 protein. n = 3 biologically independent samples per group. One-way ANOVA with Dunnett's multiple comparisons test. Data are presented as mean $\pm$ SD. **(J)** A schematic representation of a transwell coculture assay involving ex vivo programmed M1-polarized THP-1 cells combined with Vector or His-SPP1 T24 cells. **(K)** Co-immunoprecipitation assays of M1-polarized THP-1 cells were performed using anti-His antibody. n = 3 biologically independent samples per group. **(L)** Western blotting assay detected ITGA4 and ITGB1 protein expression in M1-polarized THP-1 with or without recombinant mouse SPP1 protein treatment. **(M)** Western blotting assay detected ITGA4 and ITGB1 protein expression in M1-polarized BMDMs cocultured with WT or ENO1-KO cells, with or without recombinant mouse SPP1 protein. n = 3 biologically independent samples per group. **(N)** Flow cytometric analysis of CD206

expression in siITGA4 or siITGB1 M1-polarized THP-1 cells treated with recombinant mouse SPP1 protein. n = 3 biologically independent samples per group. **(O)** Flow cytometric analysis of CD206 expression in siITGA4 or siITGB1 M1-polarized THP-1 cells cocultured with T24 cells. n = 3 biologically independent samples per group. Two-side unpaired Student's t-test. Data are presented as mean $\pm$ SD. **p < 0.01, ***p < 0.001, ****p < 0.0001.



Additional file5. (A) C57BL/6J mice (n=6 per group) were subcutaneously inoculated with WT or ENO1-KO cells. Mice received intraperitoneally treated with 200 μ g anti-PD-L1 or IgG on days 7, 9, 11, 13, and 15 after tumor inoculation. Tumor sizes were measured. **(B)** In vivo bioluminescence imaging of bladder orthotopic tumor model. Luciferase-labeled WT or ENO1 MB49 cells were subcutaneously inoculated into the bladder of C57BL/6J mice (n=6 per group). Mice were intraperitoneally treated with 200 μ g anti-PD-L1 or IgG on days 7, 9, 11, and 13 after tumor inoculation. Tumor sizes were measured. **(C)** MB49 cells were subcutaneously inoculated into C57BL/6J mice

and then treatment with ENOblock and anti-PD-L1 (n=6 per group). Tumor sizes were measured.