Functional Genomic Screening Identifies FOXP1 as a Tumor-Intrinsic Mediator of Immune Evasion in Pancreatic Cancer

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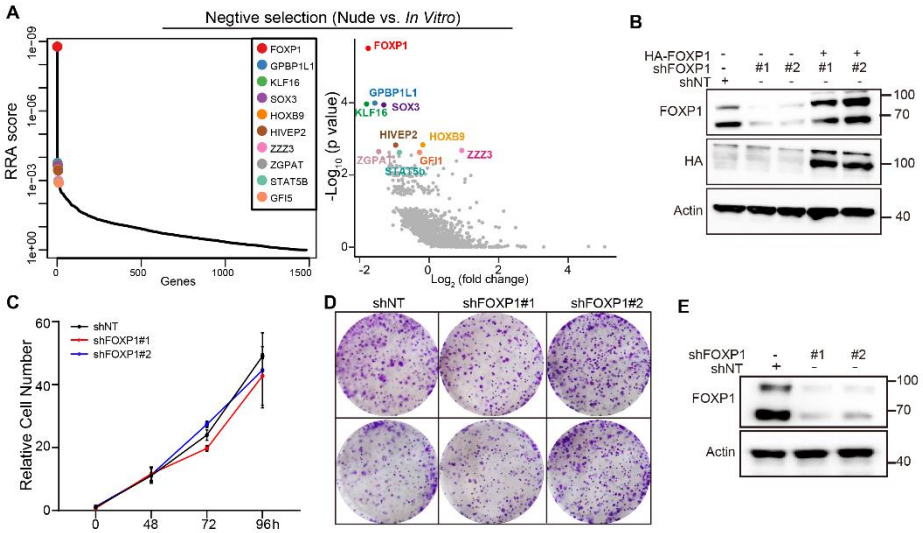


Figure S1. ***In vivo*** **CRISPR/Cas9 screen identifies FOXP1 as a mediator of immune evasion in PDAC.** Related to Figure 1. **A** Robust rank aggregation (RRA) analysis showing the top depleted genes in immunodeficient mice relative to the in vitro cell population. **B** Validation of FOXP1 knockdown and rescue efficiency in KPC cells by western blot analysis. **C** Cell viability of FOXP1-knockdown KPC cells assessed by CCK8 assay. **D** Representative images of colony formation in shNT and FOXP1-knockdown KPC cells. **E** Validation of FOXP1 knockdown efficiency in PANC02 cells by western blot analysis. Data are presented as Mean ± SD.

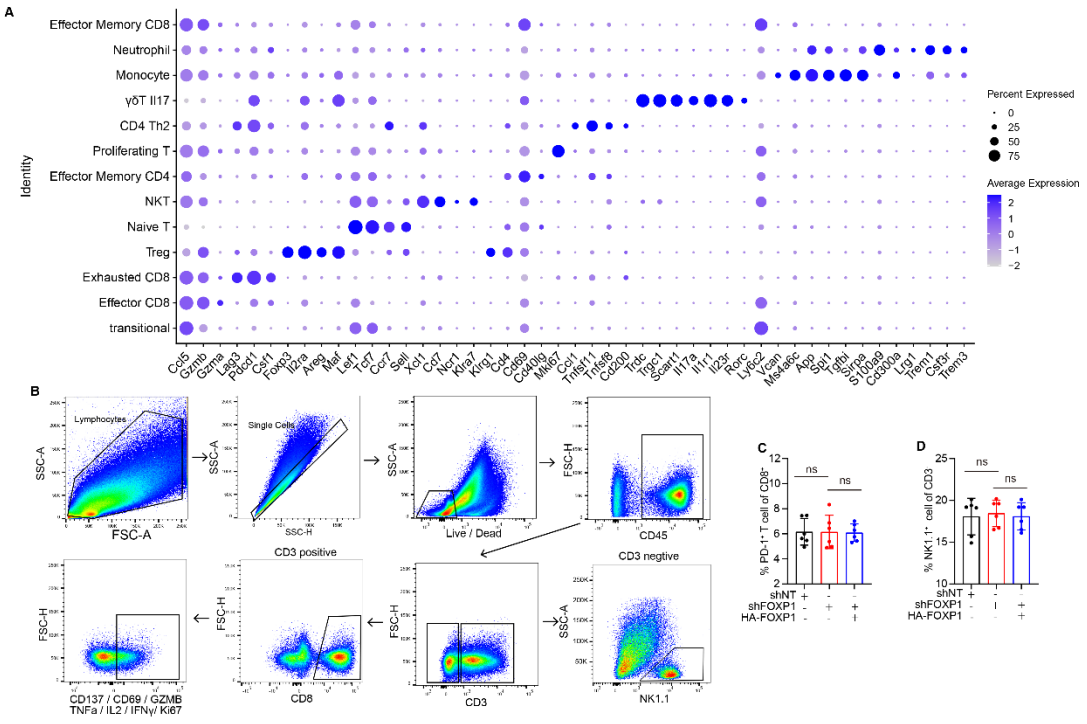


Figure S2 FOXP1 Knockdown alters the Immune Landscape of PDAC. Related to Figure 2. **A** Dotplot showing the expression of representative marker genes across individual cell clusters. **B** Flow cytometry gating strategy for the analysis of tumor-infiltrating lymphocyte subsets. **C** Representative of flow cytometry plots and quantification of PD-1^{+} CD8^{+} T cells isolated from end-stage subcutaneous shNT and shFOXP1 tumors. **D** Representative of flow cytometry plots and quantification of tumor-infiltrating NK cells isolated from end-stage subcutaneous shNT and shFOXP1 tumors. Data are presented as Mean ± SD, n = 6 per group, two-tailed *t* test, * $p < 0.05$.

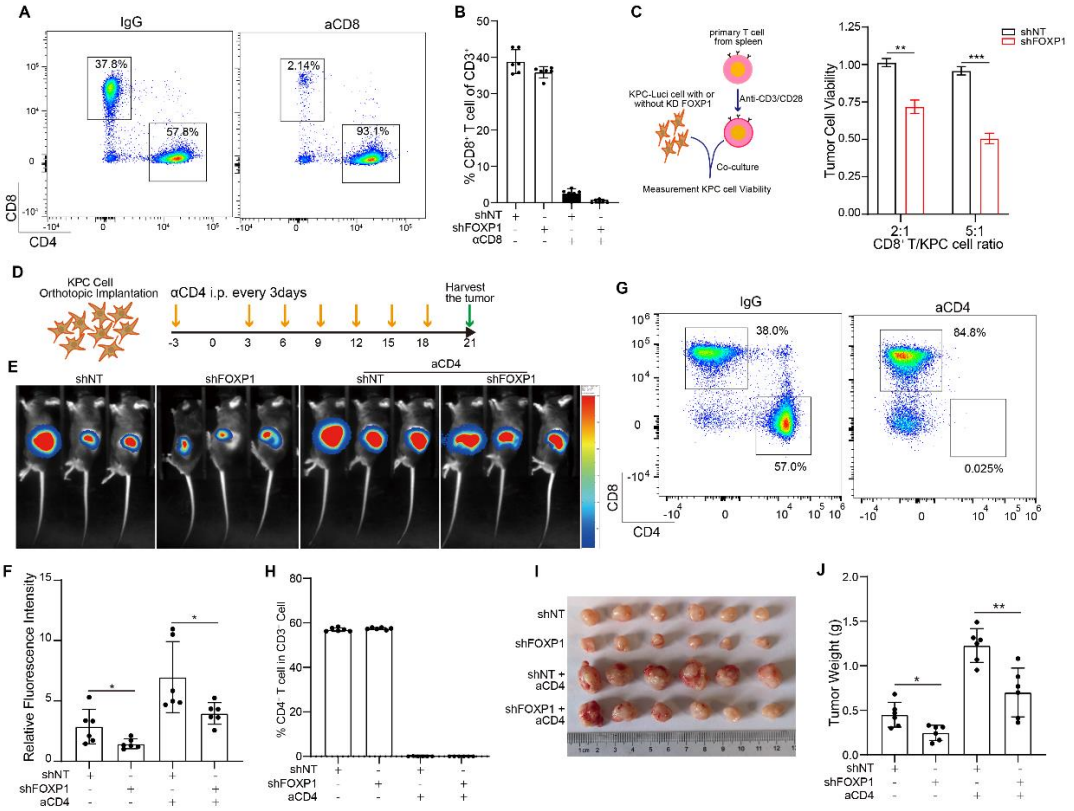


Figure S3 FOXP1-mediated immune evasion is dependent on CD8⁺ T Cells and the PD-1 pathway. Related to Figure 3. **A** Representative flow cytometry plots showing CD8⁺ T and CD4⁺ T cells in the spleens of mice treated with aCD8. **B** Quantification of splenic CD8⁺ T-cell depletion. **C** Cytotoxicity of activated CD8⁺ T cells against shNT and shFOXP1 KPC cells following 24 h of co-culture, measured by luciferase activity. **D** Schematic of the anti-CD4 (aCD4) antibody treatment regimen in mice orthotopically implanted with shNT or shFOXP1 KPC cells. **E** and **F** Representative of bioluminescence images (**E**) and quantification of tumor bioluminescence intensity (**F**) in mice treated with or without aCD4 antibody. **G** Representative flow cytometry plots showing splenic CD8⁺ and CD4⁺ T cells following aCD4 antibody treatment. **H** Quantification of splenic CD4⁺ T-cell depletion. **I** Representative images of orthotopic tumors from mice bearing shNT or shFOXP1 KPC cells with or without aCD4 treatment. **J** Quantification of tumor weights shown in **I**.

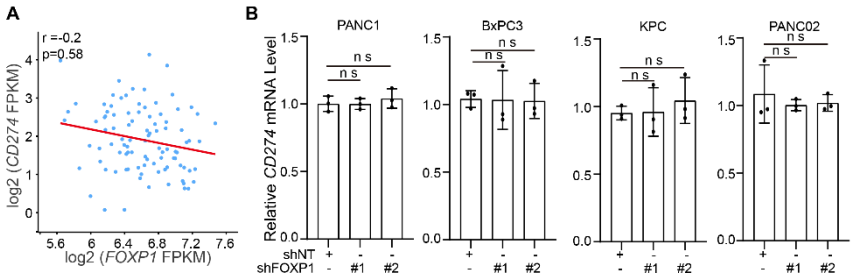


Figure S4 FOXP1 promotes PD-L1 protein expression by inhibiting lysosomal degradation. Related to Figure 4. **A** Correlation analysis between *CD274* and *FOXP1* mRNA expression in TCGA pancreatic cancer dataset. **B** RT-qPCR analysis of *CD274* mRNA levels in FOXP1 knockdown cells.

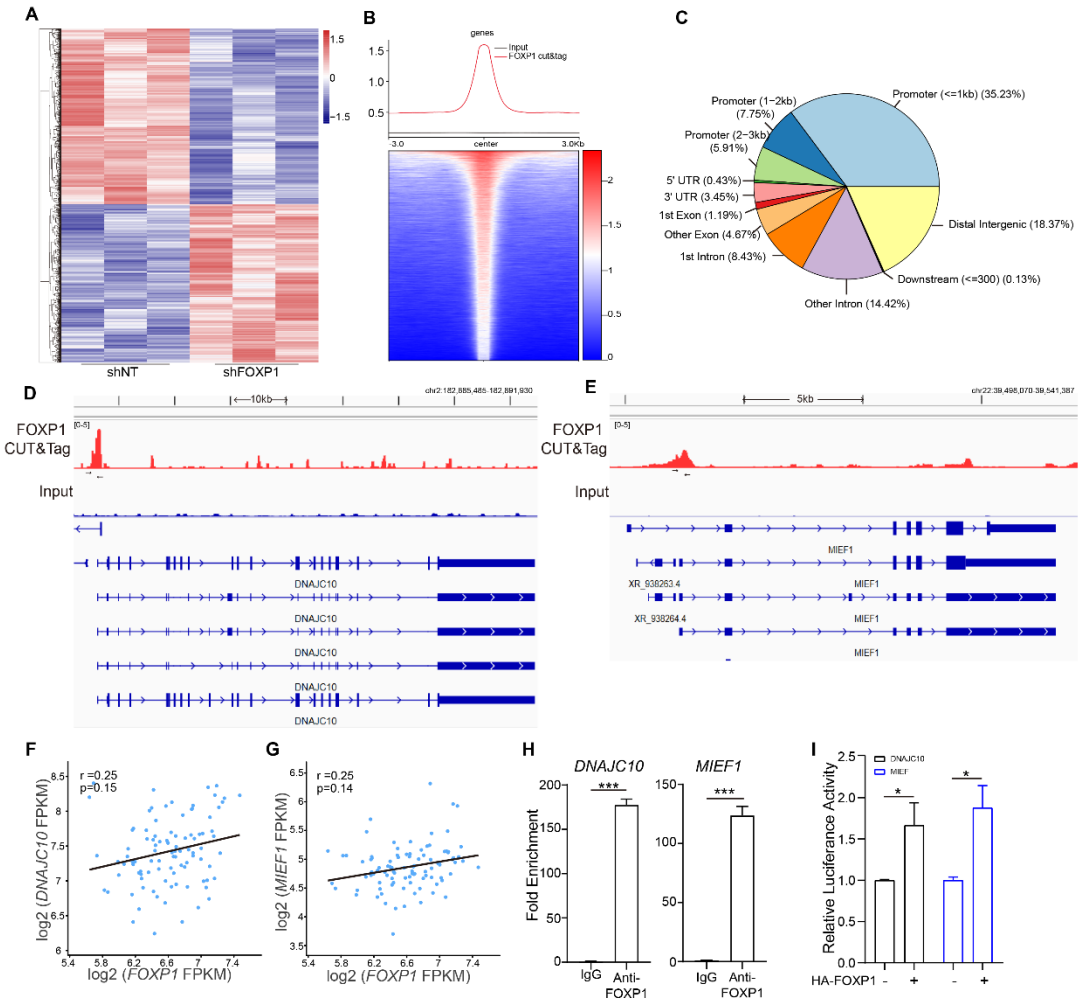


Figure S5 FOXP1 transcriptionally activates CMTM6 expression. Related to Figure 5. **A** Heatmap displaying the significantly altered genes caused by FOXP1 knockdown. **B** Heatmap of FOXP1 CUT&Tag signals in PANC1 cells. **C** The chromosomal distribution of FOXP1-related peaks. **D** FOXP1 occupancy at promoter of *DNAJC10* gene using CUT&Tag. **E** FOXP1 occupancy at and around promoter of *MIEF1* gene using CUT&Tag. Black arrows indicate the locations of ChIP-qPCR primers. **F** Representative analysis of the correlation between *DNAJC10* and *FOXP1* mRNA expression in TCGA pancreatic cancer dataset. **G** Representative analysis of the correlation between *MIEF* and *FOXP1* mRNA expression in TCGA pancreatic cancer dataset. **H** FOXP1 enrichment at the promoter of indicated genes were shown by ChIP-qPCR in PANC1 cells. **I** Representative analysis of the indicated genes promoter activity was activated by FOXP1. The indicated gene promoter was co-transfected with FOXP1 in HEK293T cells, the promoter activity was measured. Data was shown as Mean ± SD, two-tailed *t* test, * $p < 0.05$, *** $p < 0.001$.

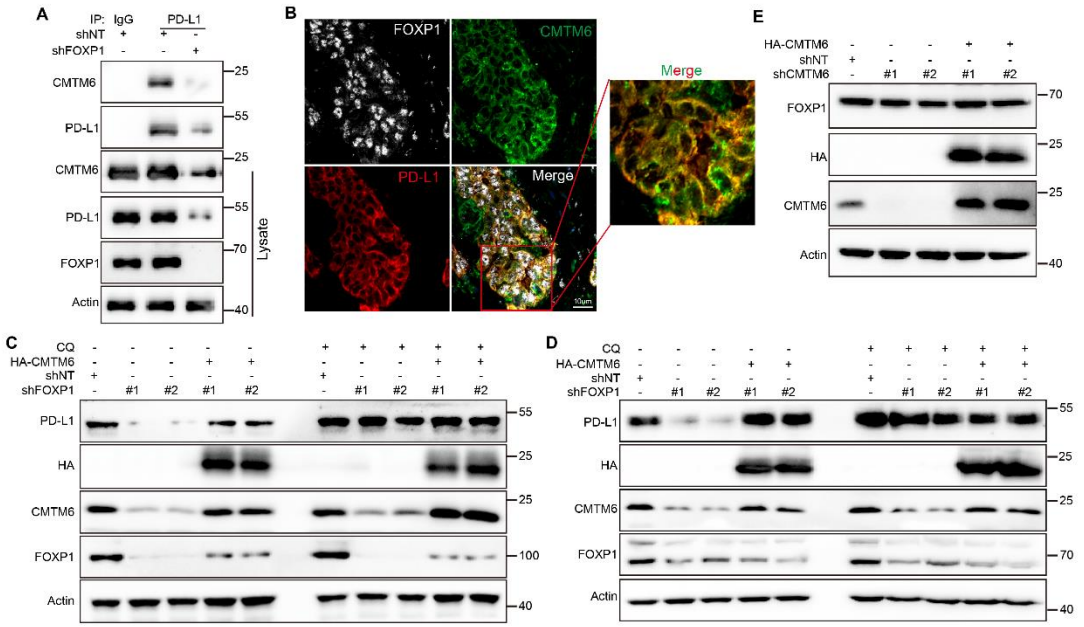


Figure S6 FOXP1 promotes tumor immune evasion through CMTM6. Related to Figure 6. **A** Representative analysis of endogenous co-immunoprecipitation of CMTM6 and PD-L1 in KPC cells with or without FOXP1 knockdown. **B** Representative images of multiplexed IHC staining of FOXP1, CMTM6 and PD-L1 in human PDAC tissue sections. **C** Western blot analysis of PD-L1 in PANC1 cells following FOXP1 knockdown and CMTM6 re-expression. Cells were treated with CQ (50 μM) for 3 h before harvest. **D** Western blot analysis of PD-L1 in KPC cells after FOXP1 knockdown and CMTM6 re-expression. Cells were treated with CQ (50 μM) for 24 h before harvest. **E** Western blot analysis of CMTM6 knockdown efficiency in KPC cells infected with shCMTM6 lentivirus.

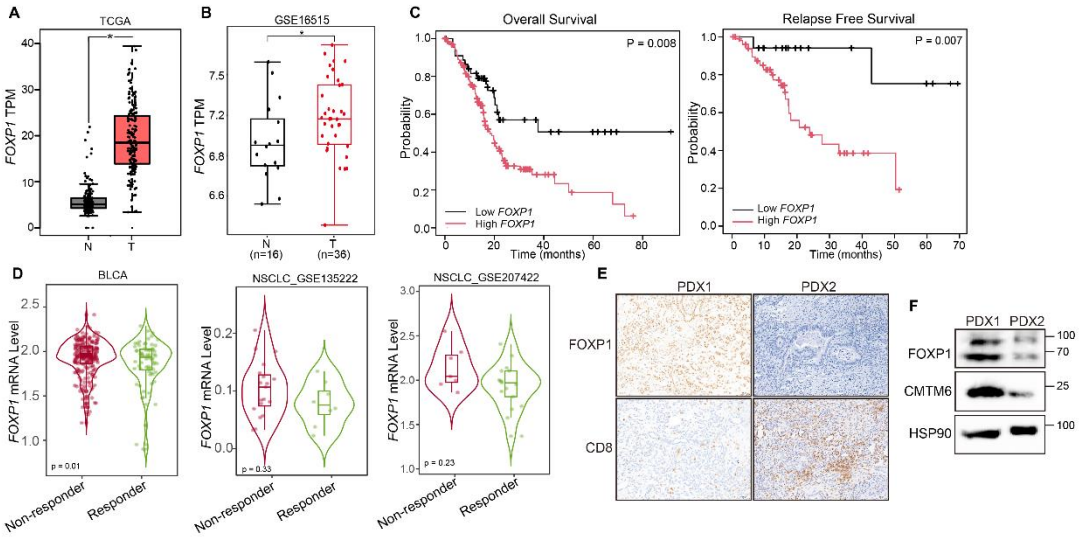


Figure S7. Conserved immune evasion function of FOXP1 in human PDAC. Related to Figure 7. **A-B** *FOXP1* mRNA expression levels in PDAC transcriptomic data from TCGA and GSE16515 datasets. **C** *FOXP1* mRNA expression levels were correlated with patients' overall survival and relapse free survival. **D** Representative analysis of *FOXP1* mRNA levels correlated with patients PFS in human BLCA and NSCLC ICB cohorts. **E** Representative image of immunohistochemical analyses of FOXP1 and CD8 level in the corresponding patient-derived tumors. **F** Western blot analysis of the original patient tumors used to establish PDX models. * $p < 0.05$.