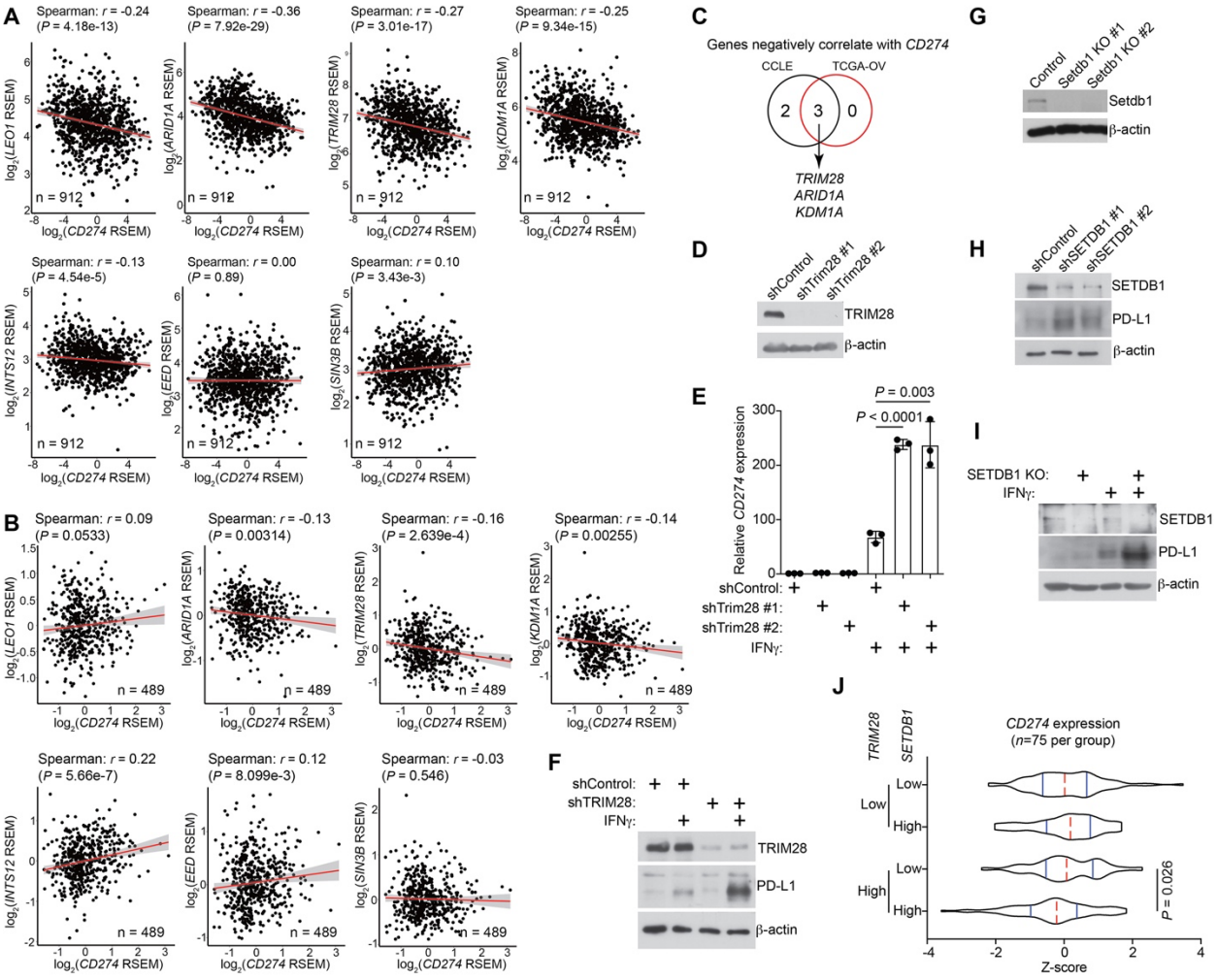
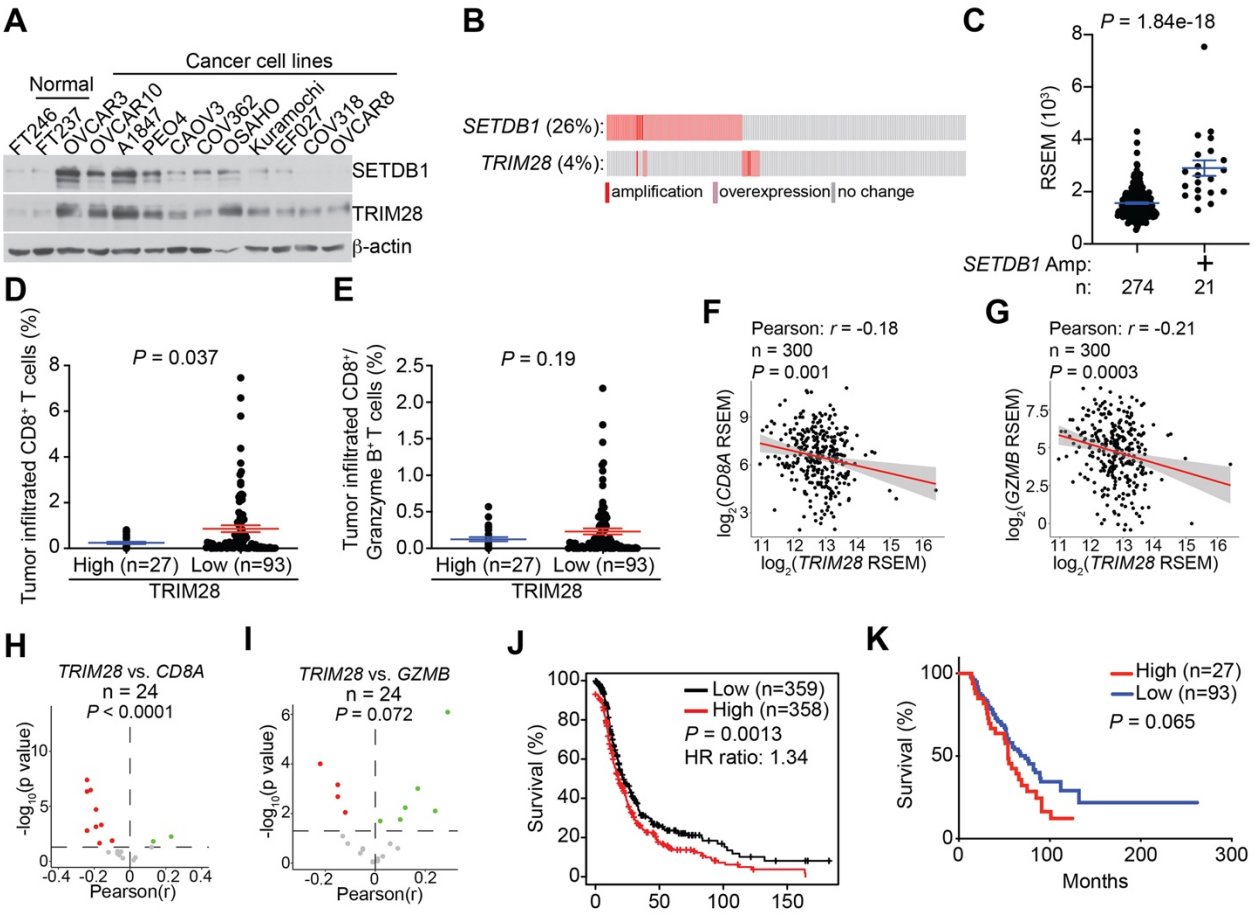
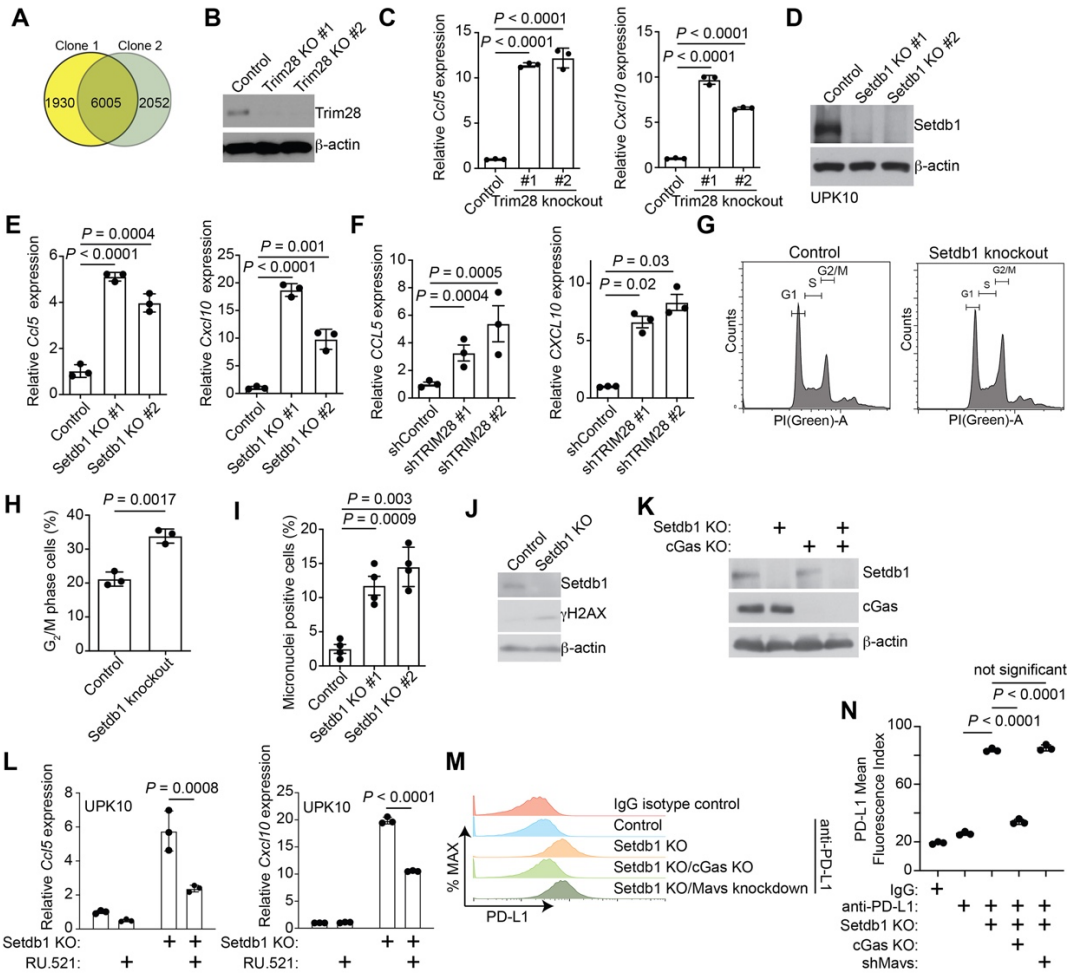


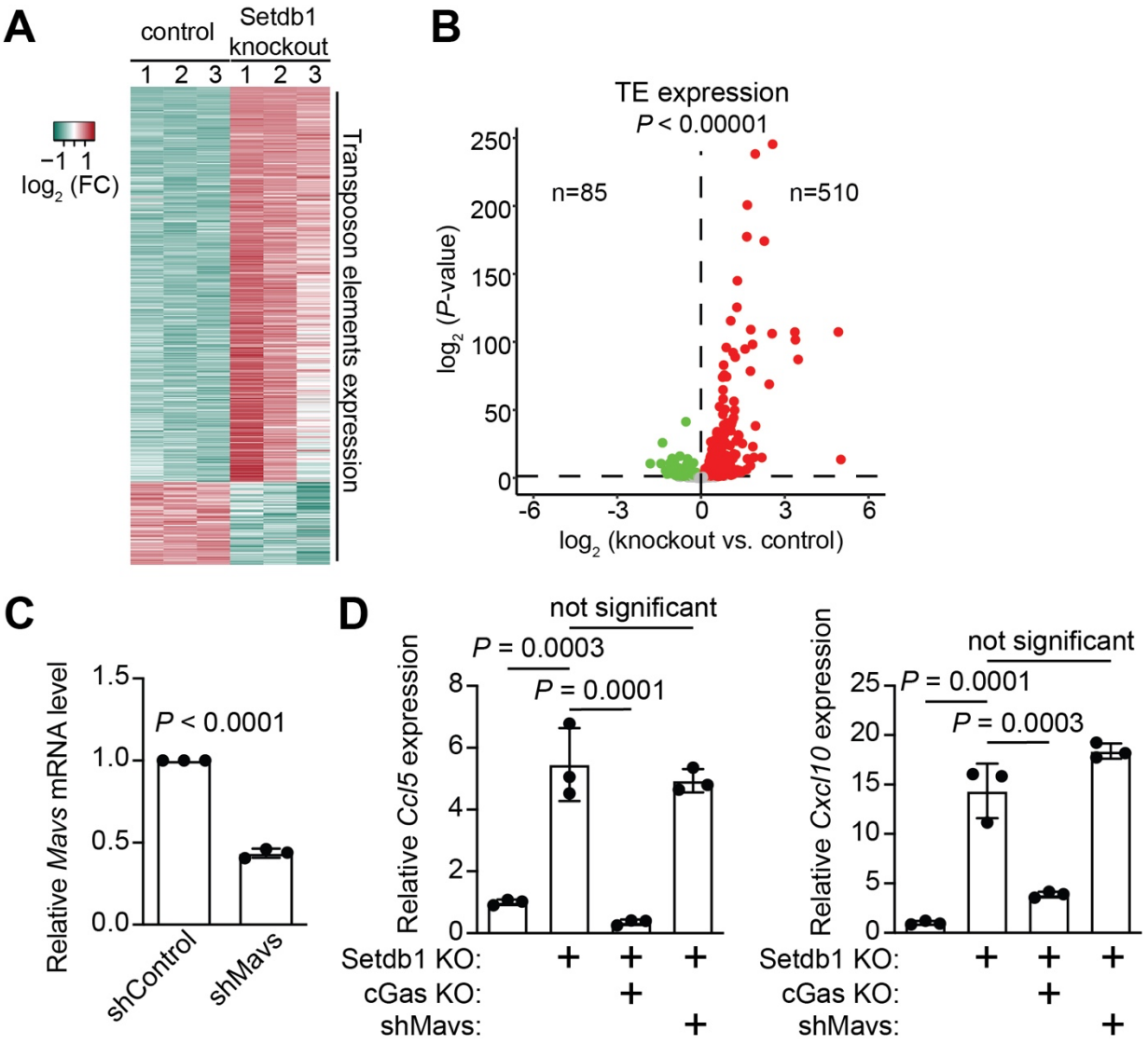
Supplementary Figure 1: Trim28 is a negative regulator of PD-L1 expression. A-C, Correlations between expression of the indicated top hits from the screen with *CD274* expression in Broad Institute Cancer Cell Line Encyclopedia (CCLE) dataset (**A**) or TCGA ovarian cancer dataset (**B**). And the overlap between these two analyses reveals the indicated three hits whose expression negatively correlates with *CD274* expression (**C**). **D,** Confirmation of Trim28 knockdown in ID8 cells by the indicated shRNAs determined by immunoblot. β-actin was used as a loading control. **E,** Expression of CD274 in control and the indicated shTrim28 ID8 cells treated with or without 30 ng/ml IFNγ for 24 h determined RT-qPCR. n = 3 biologically independent experiments. **F**, Expression of TRIM28, PD-L1 and a loading control β-actin in OVCAR3 human ovarian cancer cell lines expressing shControl or shTRIM28 treated with or without 30 ng/ml IFNγ for 24 h determined by immunoblot. **G,** Validation of Setdb1 knockout in ID8 cells determined by immunoblot. β-actin was used as a loading control. **H,** Expression of SETDB1, PD-L1 and a loading control β-actin in OVCAR3 human ovarian cancer cell lines expressing shControl or the indicated shTRIM28. **I**, Expression of SETDB1, PD-L1 and a loading control β-actin in control and SETDB1 knockout OVCAR3 human ovarian cancer cell lines treated with or without 30 ng/ml IFNγ for 24 h determined by immunoblot. **J**, *CD274* expression in the indicated groups based on TCGA ovarian cancer dataset. Data represent mean ± SEM. *P* values were calculated using a two-tailed student *t* test in S1E and by Spearman analysis in S1A-B.



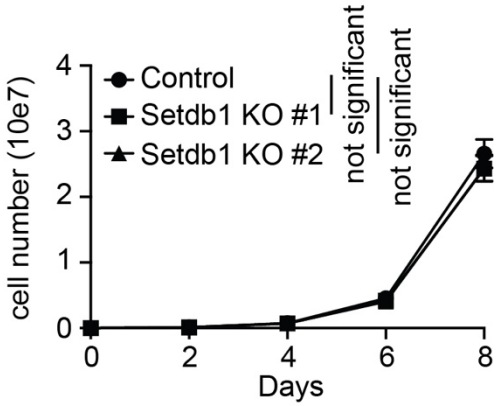
Supplementary Figure 2: TRIM28 expression negatively correlates with effector CD8⁺ T cell infiltration. A, Expression of SETDB1, TRIM28 and a loading control β-actin in the indicated normal fallopian tube epithelial cells and high-grade serous ovarian cancer cell lines determined by immunoblot. **B,** *SETDB1* amplification and overexpression profile in the TCGA HGSOC dataset. **C,** Expression of *SETDB1* in the TCGA HGSOC cases with or without *SETDB1* amplification. **D-E,** High SETDB1 expression correlates with a lower tumor infiltrated CD8⁺ T cells (D) and Granzyme B⁺/CD8⁺ T cells (E). **F-G,** Negative correlation between *TRIM28* and *CD8A* (F) or *GZMB* (F) based on RNA-seq analysis in the TCGA HGSOC dataset. **H-I,** Significant negative correlation between *TRIM28* and *CD8A* (H) *or GZMB* (I) expression based on RNA-seq analysis from 24 TCGA cancer types with at least 100 patients. **J,** Kaplan-Meier survival of SETDB1 high and low patients from a publicly microarray dataset (7). **K,** Overall survival of patients with high or low TRIM28 from the tumor microarray based on Kaplan-Meier analysis. *P* values were calculated using a two-tailed student *t* test except S2F-G by Pearson r analysis and S2J-K by log rank test.



Supplementary Figure 3: SETDB1-TRIM28 loss activates the cGAS-STING pathway. A, Overlap of differentially expressed genes (DEGs) in two Setdb1 knockout clones compared with control ID8 cells. **B,** Validation of Trim28 knockout in the indicated ID8 cells determined by immunoblot. β-actin was used as a loading control. **C,** Expression of *Ccl5* and *Cxcl10* in control and the indicated Trim28 knockout ID8 cells determined by RT-qPCR analysis. **D,** Expression of Setdb1 and a loading control β-actin in the indicated UPK10 cell lines determined by immunoblot. **E,** Expression of *Ccl5* and *Cxcl10* in control and the indicated Setdb1 knockout UPK10 cells determined by RT-qPCR analysis. **F,** Expression of *CCL5* and *CXCL10* in control and the indicated TRIM28 knockdown OVCAR3 cells determined by RT-qPCR analysis. **G-H,** Cell cycle profile of control and Setdb1 knockout ID8 cell lines determined by flow cytometry (G). And percentage of G_2/M cell cycle in the indicated cells was quantified (H). **I,** Percentage of micronuclei positive cells from the indicated control or Setdb1 knockout UPK10 cells was quantified. **J**, Expression of Setdb1, γH2AX and a loading control β-actin in the indicated ID8 cell lines determined by immunoblot. **K,** Expression of Setdb1, cGas and a loading control β-actin in the indicated ID8 cell lines determined by immunoblot. **L,** Expression of *Ccl5* and *Cxcl10* in control and the indicated Setdb1 knockout UPK10 cells treated with or without cGAS inhibitor RU.521 (2.5 μg/ml for 48 h) determined by RT-qPCR analysis. **M,** Expression of PD-L1 was determined by FACS analysis in control, Setdb1 knockout, Setdb1 knockout/cGas knockout and Setdb1/Mavs knockdown ID8 cells. An isotype matched IgG was used as a negative control. **N**, Quantification of M, the mean fluorescence index of PD-L1 was determined. Data represent mean ± SEM, n = 3 biologically independent experiments. *P* values were calculated using a two-tailed student *t* test.



Supplementary Figure 4: *Mavs* knockdown does not affect expression of *Ccl5* or *Cxcl10* induced by Setdb1 knockout. **A,** Heatmap of differentially expressed TEs (n = 490) with *P* value < 0.05 and FDR < 5%. **B,** Volcano plot illustrating the TEs upregulated (red) or downregulated (green) up Setdb1 knockout. **C,** Confirmation of *Mavs* knockdown by shRNA in ID8 cells using RT-qPCR analysis. **D,** Expression of *Ccl5* and *Cxcl10* in the indicated ID8 cells was determined by RT-qPCR analysis. Data represent mean ± SEM, n = 3 biologically independent experiments. *P* values were calculated using a two-tailed student *t* test except S4B by Fisher's Exact Test.



Supplementary Figure 5: Setdb1 knockout does not significantly affect the growth of ID8 cells. Cell growth curves for the indicated control or Setdb1 knockout ID8 cells. n = 3 biologically independent experiments. *P* values were calculated using a two-way ANOVA.