

Supplementary Information

PAX8 and MECOM are interaction partners driving ovarian cancer

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Supplementary Figure 1. Identification of PAX8 interacting partners

A) Schematic representation of BioID-HA-T2A-mCherry cassette integrated at the C-terminus of the PAX8 locus by CRISPR. B) Western blot analysis of bulk cell population transfected with BioID-cassette repair template before and after mCherry FACS sorting. C) Western blot analysis of single cell clones isolated from mCherry sorted IGROV-1-PAX8-BioID-T2A-mCherry. D) Gene Ontology Biological Process enrichment analysis of proteins identified from Bio-ID MS experiment in Figure 1A. BH p-val refers to an adjusted p-value of the enrichment corrected for multiple testing according to Benjamini-Hochberg. E) List of proteins identified by BioID-MS involved in Chromatin/Transcriptional Complexes, Histones or DNA Damage. P-value refers to two-sided t-test of enrichment corrected for multiple testing. F) Ratio of expression between PRDM3 and EVI1 from TCGA dataset. Data are presented as boxplot (representing median and first and third quartiles, whiskers extend to 95th percentile), omitting outliers. Number of data points for each lineage is represented on the x-axis. Source data for western blots are provided as a Source Data file.

Supplementary Figure 2. Biochemical/Biophysical confirmation of PAX8-PRDM3 interaction

A) Western blot analyses from IVTT reactions to express LgBit tagged PAX8 truncation mutants and full length PRDM3. The picture display one representative image out of three independent experiments. B) Western blot analyses from IVTT reactions to express LgBit tagged PAX8 domains. Asterisks denote unspecific bands. The picture display one representative image out of three independent experiments. C) Representative gels from purification of the indicated recombinant proteins utilized for *in-vitro* assays. Data have been reproduced independently of recombinant protein batches produced. (D) Methyl region of 1D proton spectra recorded for different PAX8 (9–135) and PRDM3 (2–345) samples. The two top traces are the reference spectra of each protein. The third trace, PAX+PRDM3 (sum) was obtained as the mathematical sum of the two reference spectra at the top. The bottom trace, PAX+PRDM3 (experimental) was measured with an equimolar mixture of PAX8 (9–135) and PRDM3 (2–345). All spectra were recorded, processed and plotted with identical parameters. The peak labeled DSS corresponds to the internal standard DSS-d₆ used in all samples. The peaks marked with asterisks are buffer impurities. E) SDS-PAGE of recombinant PAX8 (2-328) mixed with PRDM3 (75-434) untreated or treated with DSSO. The picture display one representative image out of three independent experiments. F) MALDI-MS analysis of mixture of PAX8 and PRDM3 recombinant proteins. Note that PRDM3 (75-434) tends to dimerize even in absence of crosslinking agent. G) Peptides involved in intermolecular crosslinks from peptide mapping of CX-MS. Source data for western blots and interaction assay are provided as a Source Data file.

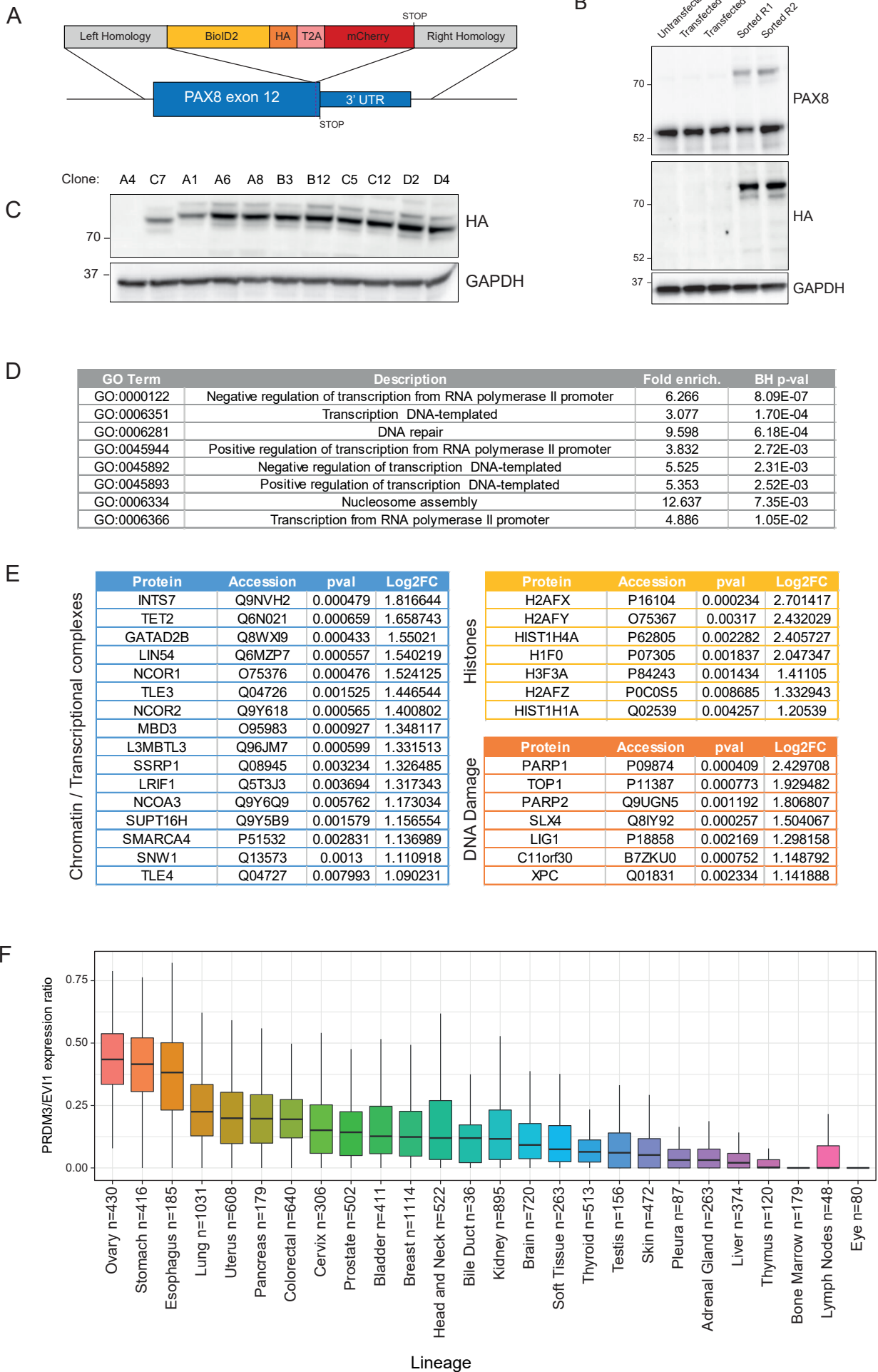
Supplementary Figure 3. PAX8/MECOM silencing for functional experiments

A) Heatmap of ChIP-signal for PAX8 and PRDM3 (two replicates) centered around the union of all PAX8 or PRDM3 peaks. B) ChIP-qPCR validation for PAX8 (in red) or PRDM3 (in blue) upon PAX8 knockdown or MECOM knockdown. CTGF -8.3Kb is used as negative control region. Data are presented as mean values \pm SD from 4 biological replicates. C) Western blot analysis of NIH:OVCAR3 cells bearing shRNAs against PAX8 or MECOM used in this study. VINCULIN is used as a loading control. (*) depicts shPAX8_1581 in a different vector utilized in previous studies⁹. The picture display one representative image out of three independent experiments. D) qPCR analyses validating the efficiency of the shPAX8 (in red) or shMECOM (in blue) constructs used for RNA-seq studies in 5 ovarian cancer cell lines. Data are presented as mean values \pm SD from 3 biological replicates. E) KEGG pathway enrichment analyses for the 58 genes included in the PAX8-MECOM gene module identified in this study. P-values refer to two-sided T-test. F) Tumor volume growth (left) and qPCR validation (right) for shPAX8 (in orange shades) or shMECOM (in blue shades) in-vivo experiments for RNA-seq. Tumor growth data are presented as individual mice ($n > 4$ per group), qPCR validation data are presented as mean values \pm SD from 4 independent mice. Source data for western blots and qPCRs are provided as a Source Data file.

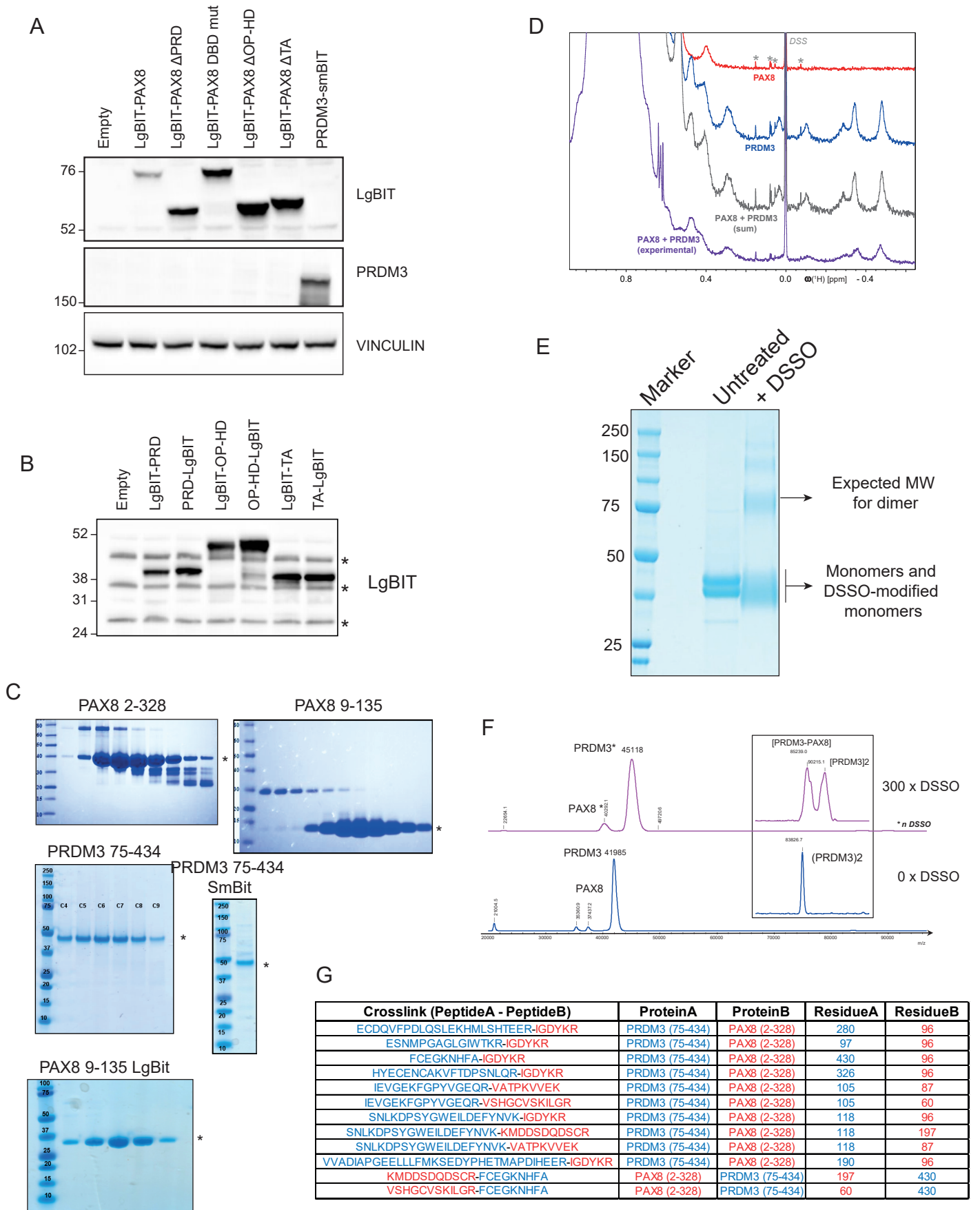
Supplementary Figure 4. PAX8 and MECOM regulate a specific gene expression module.

A) Barplot showing sensitivity to PAX8 or MECOM KO as per Combined RNAi screens reported in DepMap portal. Bars are color coded by MECOM expression. B) Relative proliferation effect as measured by quantification of Colony Formation Assay experiments in multiple ovarian cancer cell lines bearing shPAX8 or shMECOM constructs. C) Western blot analysis of tumors from Figure 4C, on samples derived 1 week after treatment start. D-E) Boxplot of normalized Z-score expression of 58 genes constituting the PAX8-PRDM3 gene module identified in this study in CCLE ovarian cancer cell lines (D) or Patient derived xenografts collection (E). Cell line / PTX models are ranked by median Signature score. PAX8 and MECOM z-score expression are plotted as heatmap. Pearson Correlation (r) between PAX8 or MECOM z-score and median Signature score is reported. *** = p-value < 0.001 . Source data for western blots and qPCRs are provided as a Source Data file.

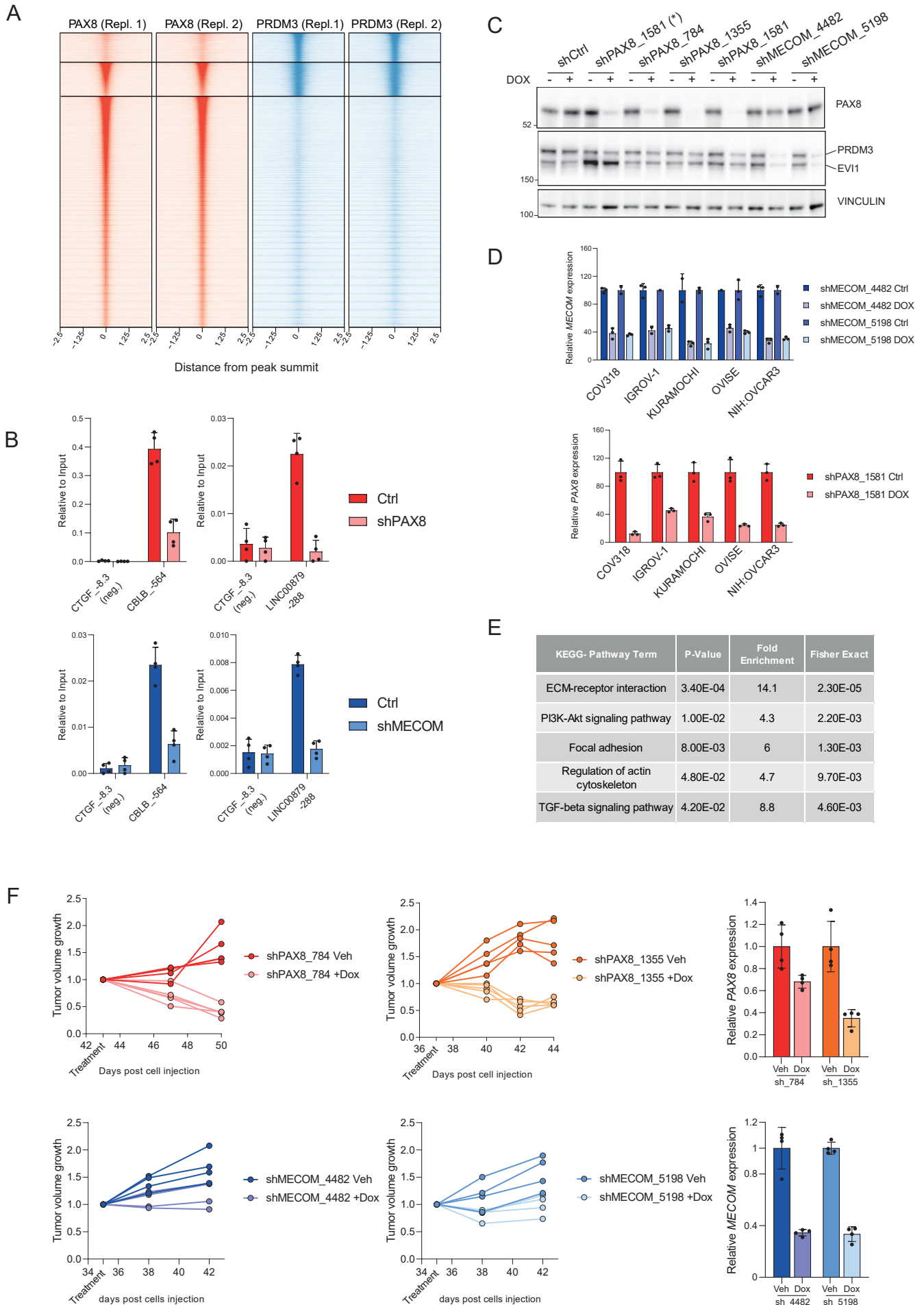
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4

