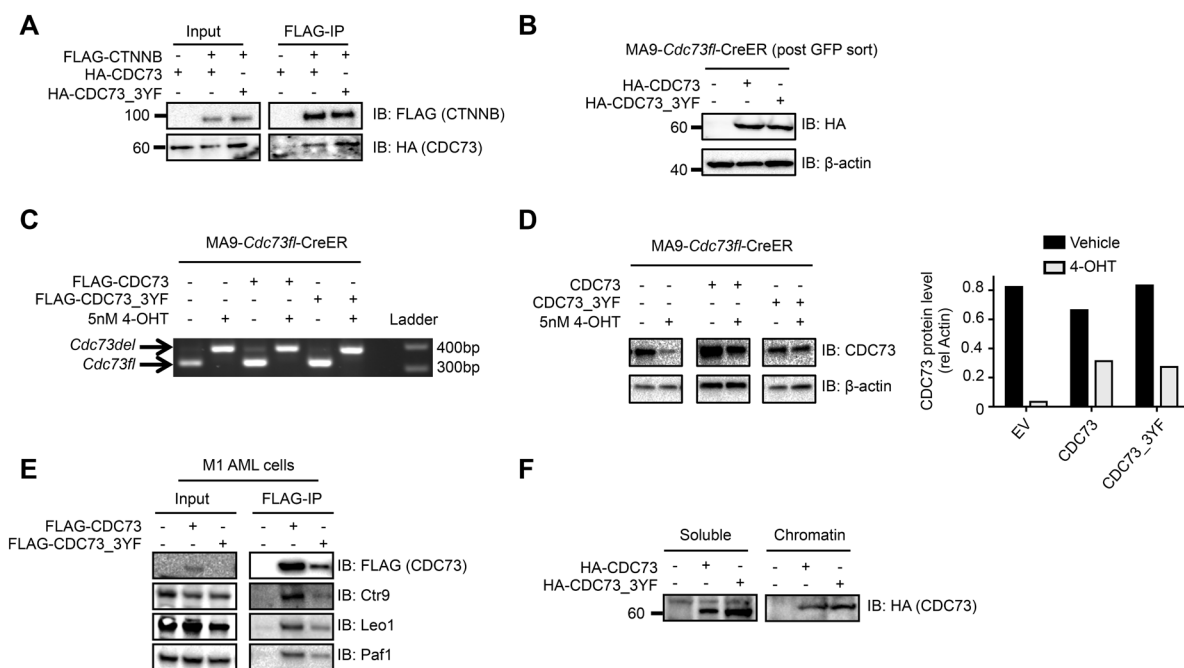
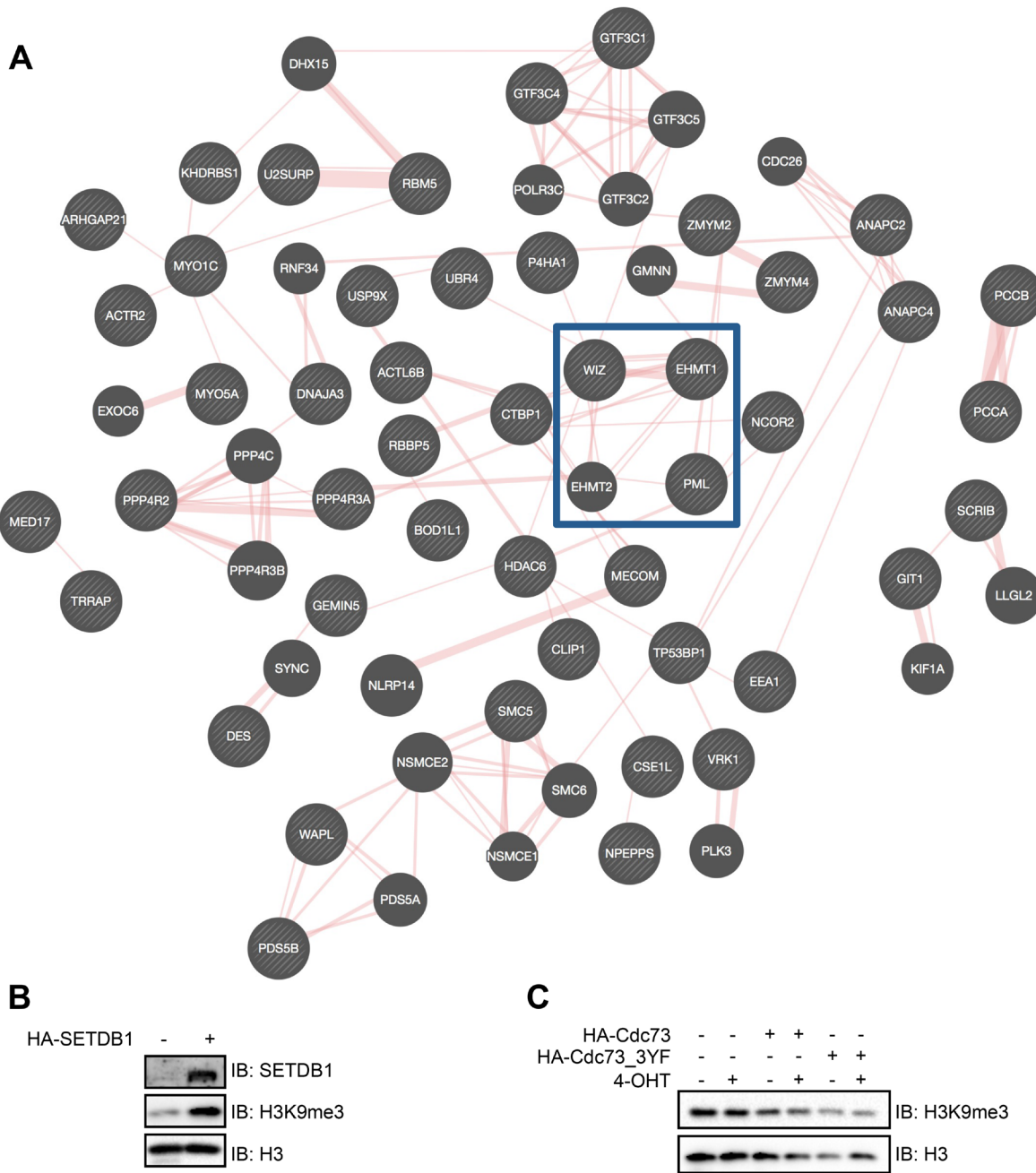


PAF1 complex interactions with SETDB1 mediate promoter H3K9 methylation and transcriptional repression of *Hoxa9* and *Meis1* in acute myeloid leukemia

SUPPLEMENTARY MATERIALS



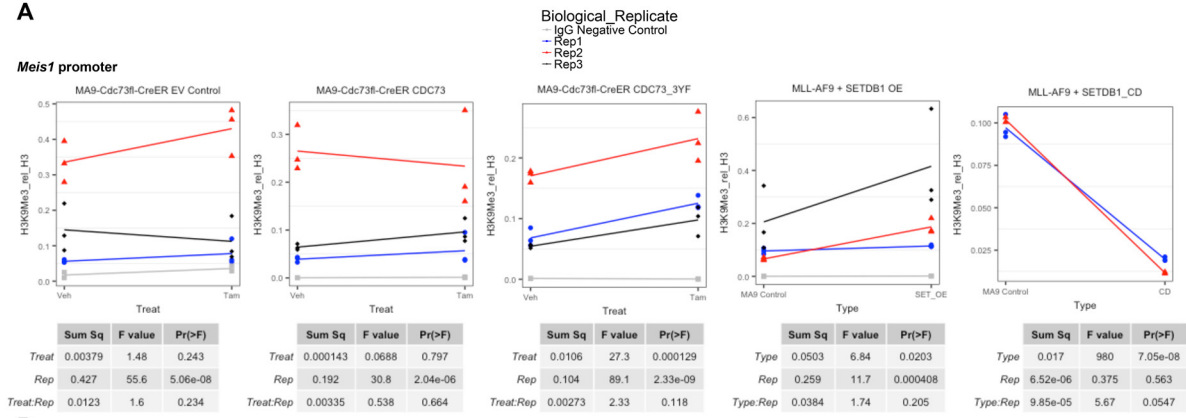
Supplementary Figure 1: (A) HEK293T cells were transiently co-transfected with either HA-CDC73 or HA-CDC73_3YF and FLAG-CTNNB. FLAG-IPs were performed and immunoblotted with the indicated antibodies. (B) Western blot of whole cell lysates from MLL-AF9 transformed *CDC73fl/fl*-CreER¹² CDC73 or CDC73_3YF re-expression cells and immunoblotted with the indicated antibodies demonstrating expression of HA-CDC73 and HA-CDC73_3YF. (C) Genotyping of MLL-AF9-*Cdc73fl/fl*-CreER¹² cells treated with 4-OHT or vehicle control to confirm genetic excision of the *Cdc73* allele. (D) Whole cell lysates from MLL-AF9-*Cdc73fl/fl*-CreER¹² cells treated with 4-OHT or vehicle control immunoblotted with anti-CDC73 antibody (left). Densitometry was performed on CDC73 protein bands and values were plotted relative to β -actin protein band signal (right). (E) M1 mouse AML cells that express stable retroviral FLAG-CDC73 or FLAG-CDC73_3YF are subjected to FLAG-IPs. PAF1c proteins are detected using western blotting with the indicated antibodies. (F) HEK293T cells were transiently transfected with CDC73 or CDC73_3YF. Whole cell lysates were collected and run as soluble fraction. Cells were lysed, the insoluble chromatin fraction was spun down and washed three times, then immunoblotted with HA antibody as the chromatin fraction.



Supplementary Figure 2: (A) Interaction network output by GeneMANIA for proteins that co-purified with CDC73_3YF in the AP-MS experiment. The interactions were filtered to include only those described as physical interactions. Circles containing diagonal lines indicate proteins included in the search node. The blue rectangle indicates the sub-network of proteins that was found to be associated with transcriptional repression. The proteins contained in this rectangle were used in the subsequent targeted GeneMANIA search. **(B)** Western blot of acid extracted histones from MLL-AF9 control cells or MLL-AF9 cells co-transduced with SETDB1 overexpression vector. Western blots were blotted with the indicated antibody. The top panel is a whole cell lysate demonstrating overexpression of SETDB1. **(C)** Western blots of acid extracted histones from MLL-AF9 transformed *CDC73^{fl/fl}-CreER^{T2}* CDC73 re-expression cells. Western blots were blotted with the indicated antibody.

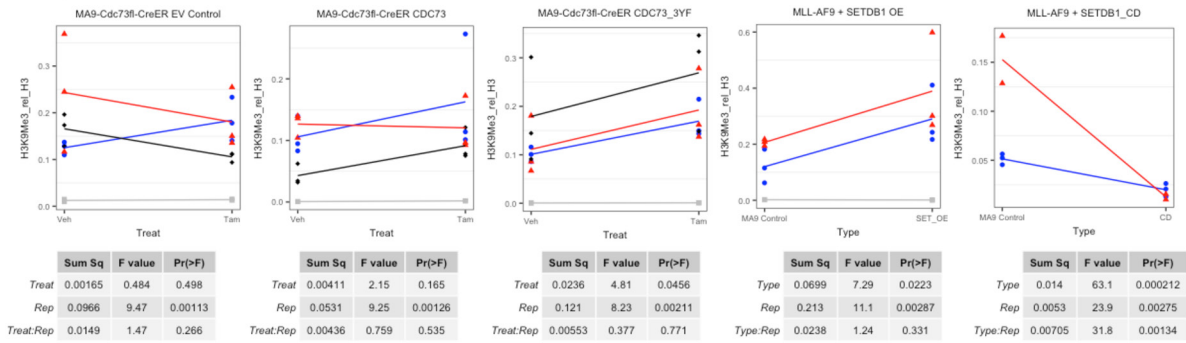
A

Meis1 promoter



B

Hoxa9 promoter



Supplementary Figure 3: Linear regression models were run to test the difference between treated groups or MLL-AF9 control vs MLL-AF9 + SETDB1 or MLL-AF9 + SETDB1_CD overexpression for ChIP-qPCR at the (A) *Meis1* or (B) *Hoxa9* promoter locus. Technical replicate values are represented as the same color, whereas biological replicates are indicated by different colors. ANOVA tables are shown below the interaction plots generated by the linear model. Type=Cell Line, Rep=Biological_Replicate, Treat:Rep=Interaction.

Supplementary Table 1: xlsx document containing all proteins inferred from AP-MS data as well as statistical analysis for confidence of potential interactions and the comparison of CDC73 and CDC73_3YF interactomes.

The bait/ prey proteins are annotated with a PROTID corresponding to the UniProt ID for the given bait/ prey protein, a Gene Symbol corresponding to the gene that codes for the identified protein, and a Gene Name that corresponds to the full name of that gene/ protein that was identified.

Sheets:

PSM_Scoring contains all spectral counting values for all replicates of CDC73, CDC73_3YF, and Control AP-MS experiments.

All_SAIN_T_probabilities contains all SAINT probabilities (SP) calculated for the AP-MS experiments using the CRAPome online tool.

Overlapping_proteins contains a list of proteins that are found in the interactome of both CDC73 and CDC73_3YF with a SAINT probability of ≥ 0.7 .

CDC73_3YF_enriched contains a list of proteins that are found in the interactome of CDC73_3YF with a SAINT probability of ≥ 0.7 and have a SAINT probability of ≤ 0.7 in the CDC73 AP-MS.

CDC73_enriched contains a list of proteins that are found in the interactome of CDC73 with a SAINT probability of ≥ 0.7 and have a SAINT probability of ≤ 0.7 in the CDC73_3YF AP-MS.

Key contains a brief definition for any abbreviations used in the other sheets.

See Supplementary File 1