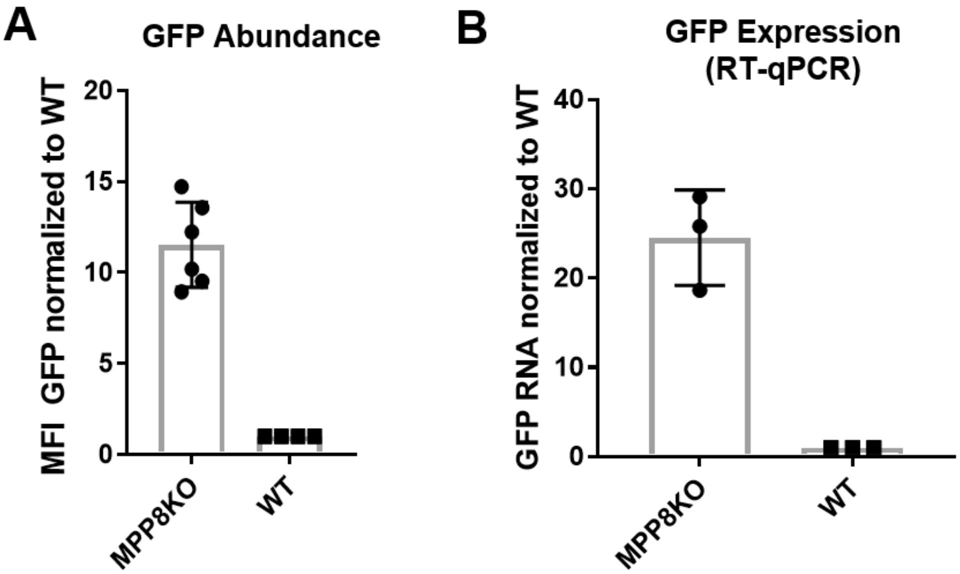
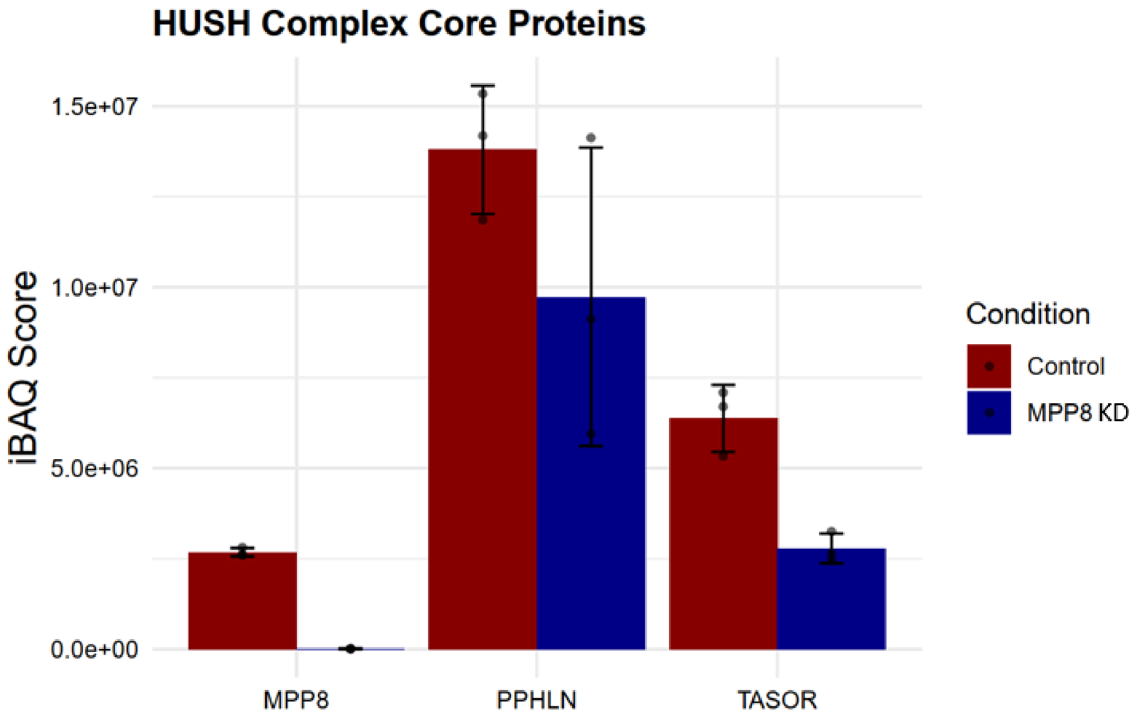
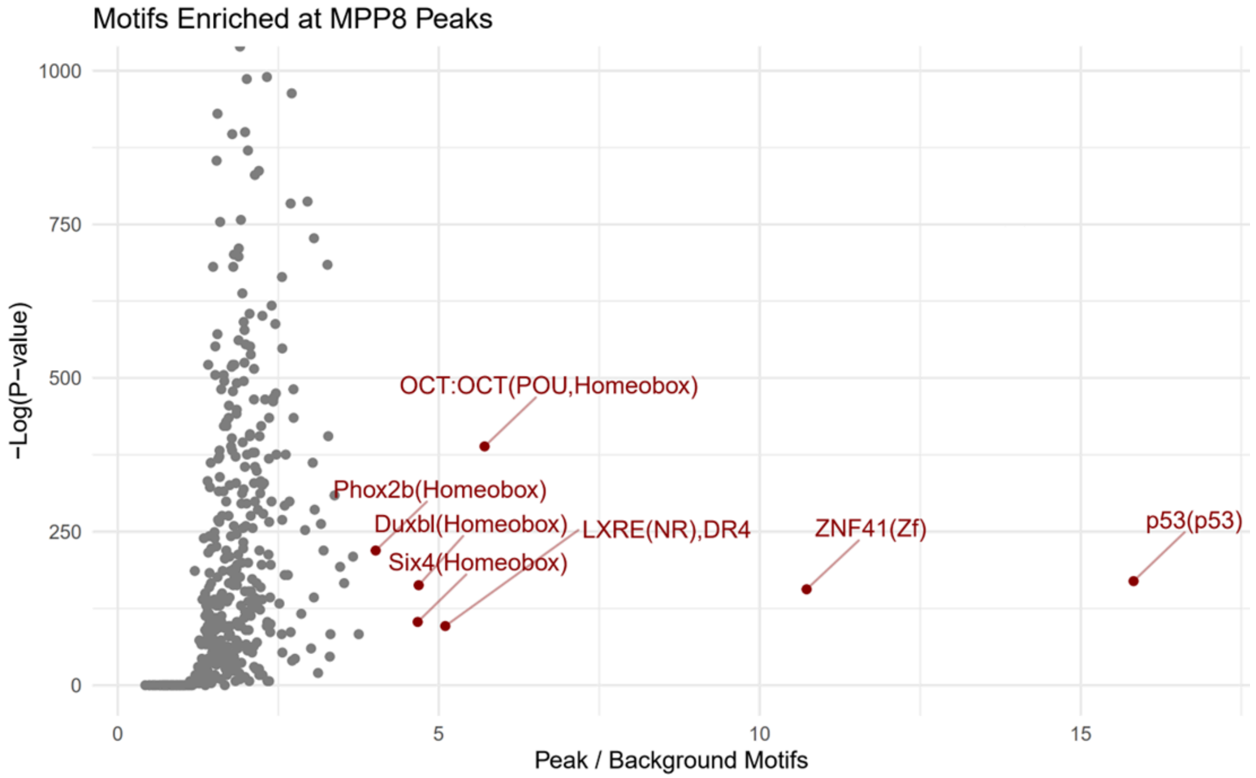


Supplementary Figure 1: Transcriptional repression of pscALP-PLXIN-gagGFP-WPRE by the HUSH complex. (A) GFP mean fluorescence intensity (MFI) of control (WT) or MPP8 knockout (MPP8KO) HEK293T cells transduced with pscALP-PLXIN-gagGFP-WPRE. (B) RT-qPCR enrichment of pscALP-PLXIN-gagGFP-WPRE RNA in HEK293T control (WT) or MPP8 knockout (MPP8KO) HEK293T cells transduced with pscALP-PLXIN-gagGFP-WPRE.

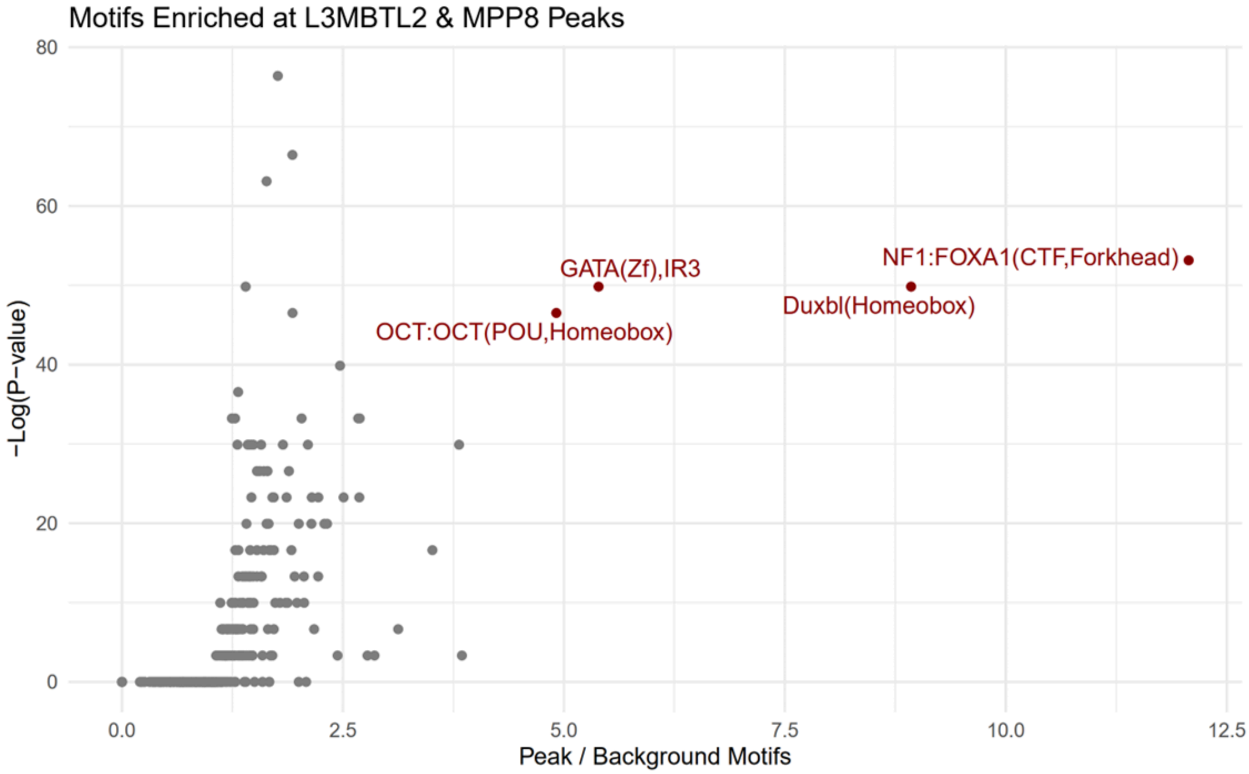


Supplementary Figure 2: HUSH core proteins detected by Provirus Proximity Proteomics. HEK293T cells were transduced with dCas9-APEX2 and expressed sgRNA targeting the lentiviral provirus (sgLTR). Control cells were transduced with an shRNA targeting luciferase. shMPP8 cells are transduced with an shRNA targeting MPP8 (core HUSH component). iBAQ values across 3 biological replicates and depicted for control and *MPP8* knockdown conditions. For data associated with this figure please see Extended Data Table 1.



Supplementary Figure 3: Motif enrichment at MPP8 peaks

De novo motif discovery applied to DNA sequences of top 10,000 MPP8 peaks (by CUT&Tag signal), subtracting “simple repeat” regions (RepeatMasker) likely to result from spurious mapping. 24.6% of peaks overlap with annotated transcription start sites. Genomic sequences from 10,000 randomly selected “promoter-like” cis regulatory elements (ENCODE) were used as background signal. Points represent individual motifs. X axis values represent the proportion of motif occurrences in the test (MPP8) regions over those found in the background regions. Y axis values are the –Log(p-value) calculated by Homer. Red points depict motifs $x > 4$ and $p < 0.001$. For data associated with this figure please see Extended Data Table 10 and SRA Bioproject PRJNA869850.



Supplementary Figure 4: Motif enrichment at L3MBTL2 and MPP8 peaks

De novo motif discovery applied to DNA sequences of intersecting regions from top 10,000 MPP8 peaks and top 10,000 L3MBTL2 peaks (by CUT&Tag signal), subtracting "simple repeat" regions (RepeatMasker) likely to result from spurious mapping. 800 overlapping peaks from both factors intersect. 56.75% of peaks overlap with annotated transcription start sites. Genomic sequences from 10,000 randomly selected "promoter-like" cis regulatory elements (ENCODE) were used as background signal. Points represent individual motifs. X axis values represent the proportion of motif occurrences in the test regions over those found in the background regions. Y axis values are the –Log(p-value) calculated by Homer. Red points depict motifs $x > 4$ and $p < 0.001$. For data associated with this figure please see Extended Data Table 11 and SRA Bioproject PRJNA869850.