Table S1 sgRNA Oligonucleotide Sequences

Target	Sequence
Negative Control #1	GTTTCAACATCTAATTTCTCAGG
Negative Control #2	TTGCAGCCTTTATGAAGTTGTGG
PPHLN1	ATTTACATGAAATAGGTGAGCGG
MPP8	AGAAAAATTTGTCGAATCCCAGG
TASOR	GTTTCCTTATAAAACAGTGCTGG
TASOR2 #1	GACGACTCATAGCCATCTGTGGG
TASOR2 #2	TCTCGGATTTGGACGTAAGGCGG

Table S2 Antibodies

Antibody	Application	Vendor (Catalog Number)
LINE-1 ORF1p	Immunoblot	CST (D3W90)
TASOR	Immunoblot and ChIP	ATLAS (HPA006735)
MPP8	Immunoblot and ChIP	Proteintech (16796-1-AP)
PPHLN1	Immunoblot and ChIP	ATLAS (HPA038903)
Anti-FLAG/DYKDDDDK	Immunoblot	Invitrogen (PA1-984B)
GAPDH	Immunoblot	Millipore (MAB374)

Figure S1: TASOR2 Isoforms and Interactions

A) Gene Expression Profiling in Diverse Cell Lines: Gene expression data from the Genotype-Tissue Expression (GTEx) project illustrates the differential expression of TASOR2 isoforms across various cell lines. The heatmap depicts the expression levels of distinct TASOR2 isoforms, providing insight into the tissue-specific variations. Highlighted within the figure are key domains of TASOR2, aiding in the identification and characterization of functional regions within the protein.

B) Interactome Analysis Reveals Dynamic Complex Recruitment: Utilizing Immunoprecipitation-Mass Spectrometry/Mass Spectrometry (IP-MS/MS), the interaction landscape of TASOR2 is explored. The data reveals the recruitment of diverse protein complexes to TASOR2, particularly in association with the HuSH and HuSH2 complexes. This comprehensive analysis sheds light on the dynamic interactions that contribute to the functional versatility of TASOR2, uncovering potential regulatory mechanisms and pathways involved in its cellular roles.

C-D) Peptide Spectra Detected by IP LC-MS/MS: Sequence of TASOR2 isoform 5 (longest isoform) including the PARP domain, with starting methionine of isoform 1 (dominant isoform expressed) without the PARP domain underlined and bolded. All peptides detected in LC-MS/MS from both FLAG-PPHLN1 and FLAG-MPP8 IPs are highlighted. C and D are two technical replicates of IP LC-MS/MS.

Figure S2: TASORs Expression in Diverse Cell Lines

A) The expression profile of TASOR across a spectrum of human cell lines, as determined by the Human Protein Atlas. The heatmap illustrates the varying levels of TASOR in different cellular contexts, shedding light on its dynamic presence and potential functional significance.

B) Comprehensive analysis of TASOR2 expression in diverse cell lines, as documented by the Human Protein Atlas. The depicted data unveils the nuanced expression patterns of TASOR2 across various cellular environments, providing insights into its potential roles and regulatory mechanisms within distinct cellular contexts. The differential expression observed highlights the intricate nature of TASOR2 in cellular processes and hints at its potential implications in diverse biological pathways.

Figure S3: PPHLN1 ChIP-seq Analysis in HuSH KO Backgrounds

A) The heatmap and peak profiles illustrate the comprehensive PPHLN1 ChIP-seq results obtained from K-562 cells within the context of HuSH knockdown (KO). The ChIP-seq experiments were meticulously conducted in two distinct HuSH KO backgrounds, namely TASOR KO and TASOR2 KO, with each condition being replicated biologically (n=2). The peak calling analysis was performed using MACS2, resulting in the identification of distinct genomic regions associated with PPHLN1 binding. Specifically, two nonoverlapping sets of peaks were delineated, referred to as HuSH2 and HuSH sites, corresponding to TASOR KO and

TASOR2 KO backgrounds, respectively. This segmentation provides a nuanced view of PPHLN1's preferential binding patterns and highlights potential regulatory elements associated with the HuSH complex.

Figure S4: Homer Analysis Reveals Distinct Localization Patterns of HuSH and HuSH2

A) Venn diagram illustrating the identification of MPP8 ChIP-seq peaks designated as HuSH and HuSH2 using MACS2 in biological replicates (n=2) of TASOR KO and TASOR2 KO, resulting in the definition of two nonoverlapping regions.

B) Bar chart presenting the observed over expected ratio of HuSH and HuSH2 peak localization at key genomic elements, as determined by Homer peak analysis.

C) Nested bar chart depicting all MPP8 peaks found at HuSH or HuSH2 sites, categorized by their closest genomic elements.

D) Pie chart illustrating the distribution of HuSH peaks across LINE elements of varying ages, providing insights into the preferential association of HuSH with specific LINE subtypes. This analysis contributes to a comprehensive understanding of the genomic landscape and regulatory roles of HuSH and HuSH2.

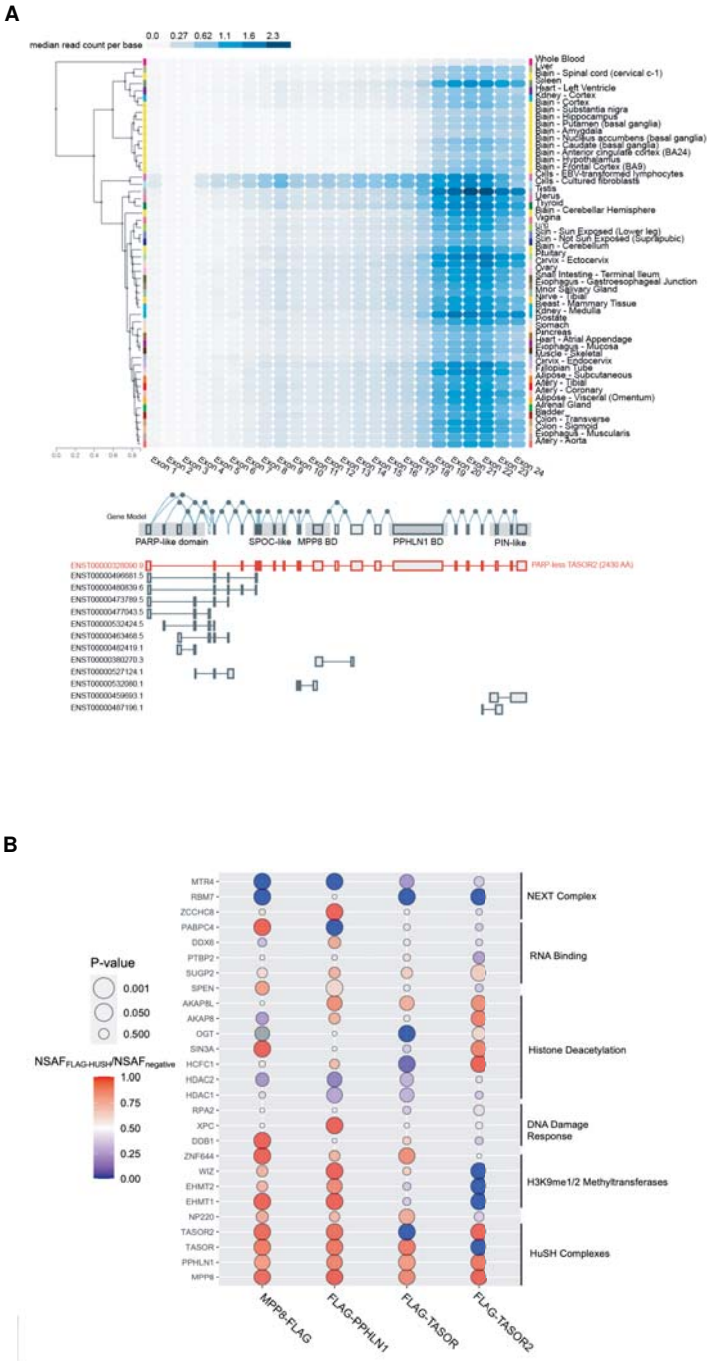
Figure S5: AlphaFold analysis of MPP8 interaction with TASOR/2.

A) Protein sequence alignment of TASOR and TASOR2 orthologs depicting the conserved MPP8 binding domain. Transgenes with amino acid substitutions at the highlighted residues are illustrated in Figure 6.

B, C) AlphaFold-predicted structure of the TASOR/2 SPOC domain interacting with the Ankyrin Repeats of MPP8.

D) Protein domain diagram illustrating the sequences in TASOR/2 associated with MPP8.

Figure S1



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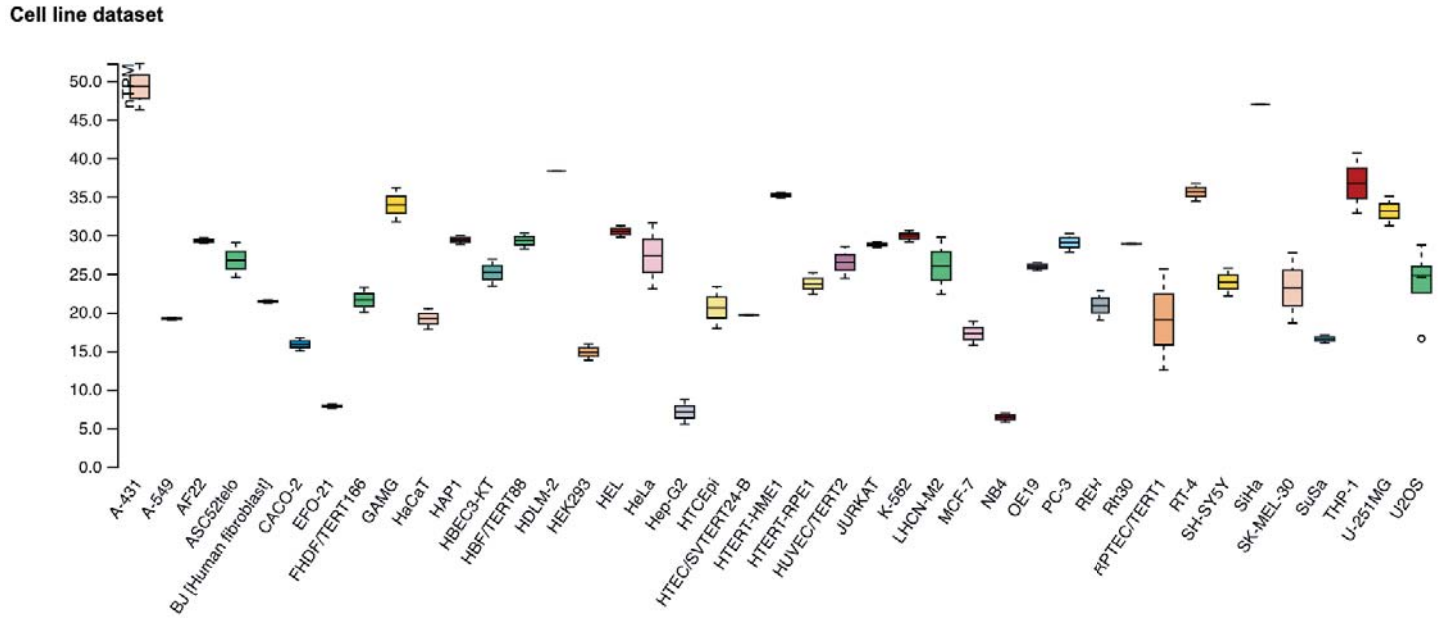

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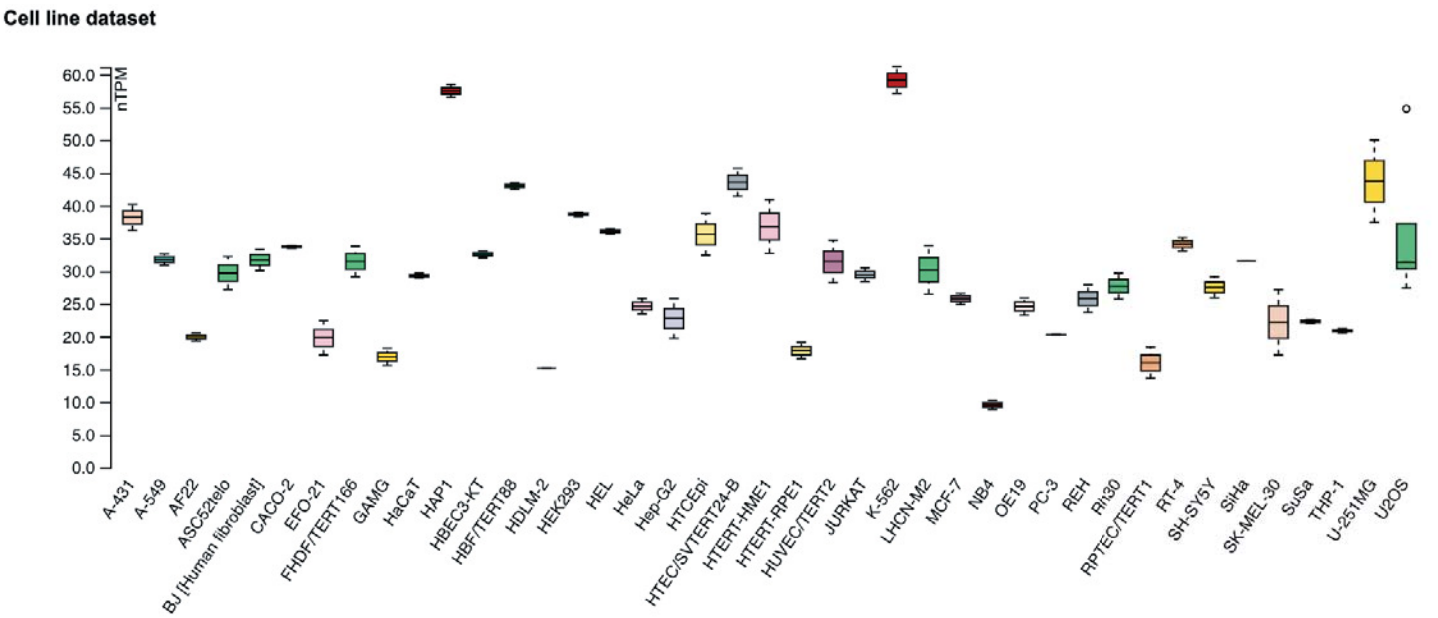

Figure S3

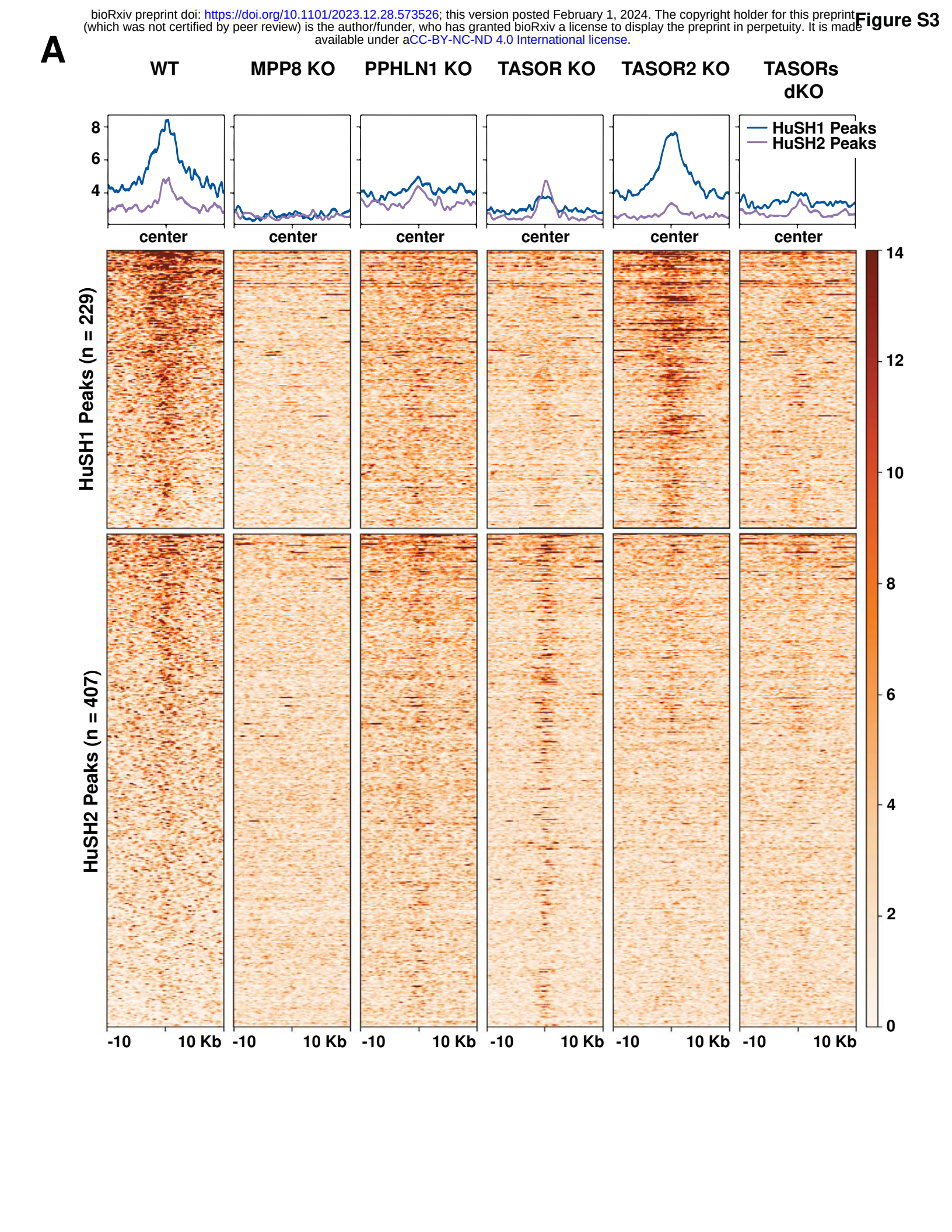

Figure S4

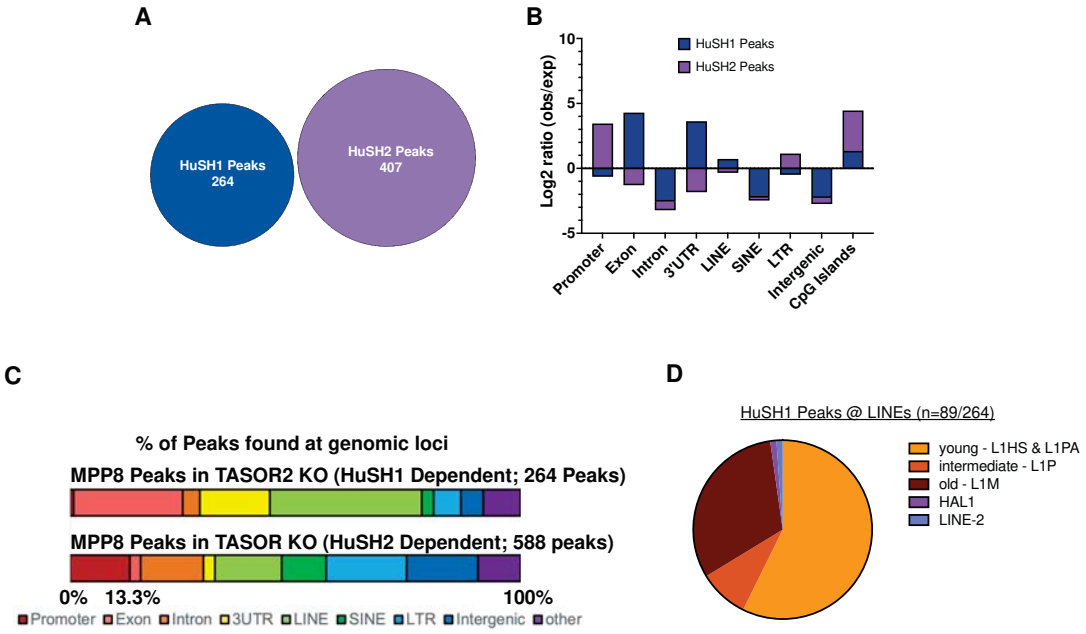

Figure S5