

## Additional file 2

**Figure S1.** Comparing the gene expression levels of *Sox2* and *hnRNPK* in mESCs. The relative transcript levels of endogenous *Sox2* and *hnRNPK* in control (shscramble), *Sox2*-KD (shSox2-1), and *hnRNPK*-KD (shRNPK-1) E14 ESCs were analyzed by qRT-PCR. Barplots represent the mean  $\pm$  SD (n=3). ANOVA was used to assess statistical significance (\*, \*\*, and \*\*\* indicate P-values of <0.05, <0.01, and <0.001, respectively).

**Figure S2.** Relationship between differential isoform usage and differential gene expression. The X-axis represents the log<sub>2</sub> fold change (log<sub>2</sub>FC) in gene expression between KD conditions (shhnRNPK, shSOX2) and the control (shscramble). The Y-axis represents the relative isoform fraction expression (dIF) in the same comparison. IF is quantified as the fraction of the parent gene originating from a specific isoform. Significant isoform usage events, defined by |dIF| > 0.1 and q-value < 0.05, are highlighted in red.

**Figure S3.** Correlation analysis of alternative splicing events affected by SOX2 and hnRNPK in the same direction. **A.**  $\Delta$ PSI correlation revealed a strong correlation (R = 0.89, P-value < 2.2e-16) between alternative splicing (AS) events after the KD of *hnRNPK* and those following the KD of *Sox2*. The  $\Delta$ PSI of *Eif4a2* and *Ash2l* AS events are indicated in red or green. Statistical significance was performed by Pearson correlation test. **B.** The top ten genes with the highest number of changed AS occurrence. The AS events used in **(A)** and **(B)** are from Figure 4A.

**Figure S4.** QRT-PCR assessment of the relative transcript levels of selected pluripotency markers in *hnRNPK*-KD (shhRNPK-2) and *Sox2*-KD (shSox2-2) E14 ESCs after 48 or 72 hours of Dox-induced expression of *Ash2l-b* and *Eif4a2<sup>PTC</sup>*. Uninduced: before Dox induction. Data are represented as mean  $\pm$  SD (n=3). ANOVA was used to assess significance (\*\*\*\* and \*\* indicate *P*-values of  $< 0.0001$  and  $< 0.01$ , respectively; ns indicates not significant).

**Figure S5.** Characterization of nucleotide composition around 5' splice sites and 3' splice sites. Nucleotide frequencies around the 5' splice sites and 3' splice sites of exons in alternative splicing effected by *Sox2* (**A**), *hnRNPK* (**B**) or *Cdk9* (**C**).

**Figure S1**

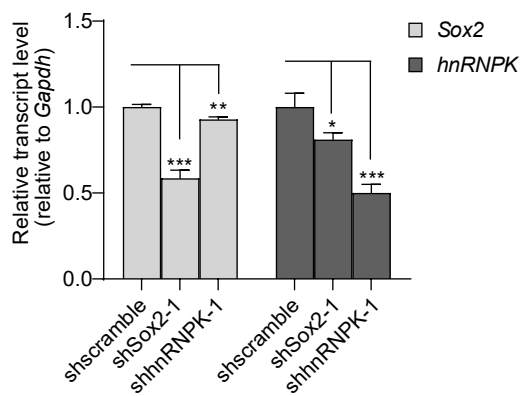


Figure S2

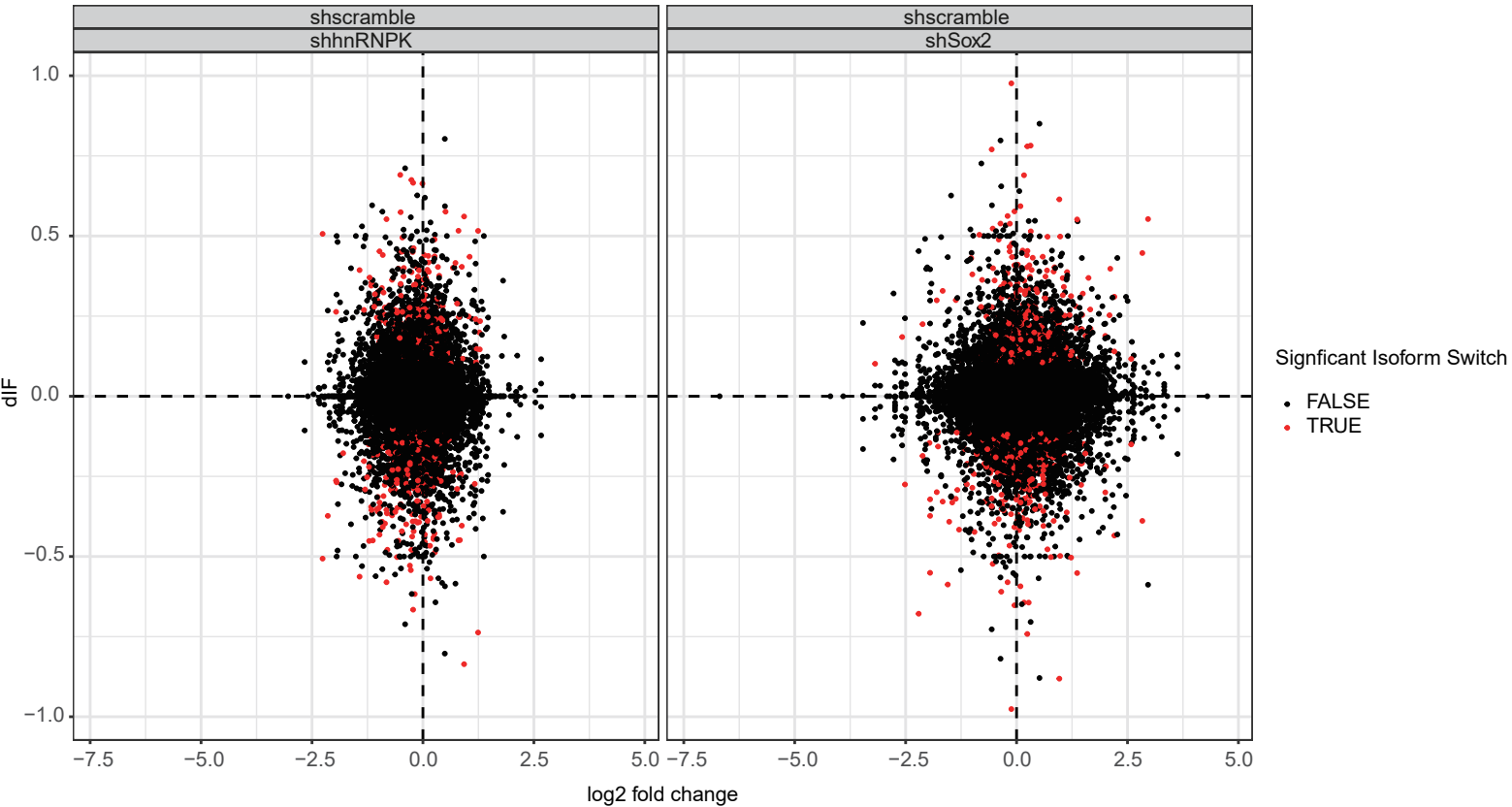
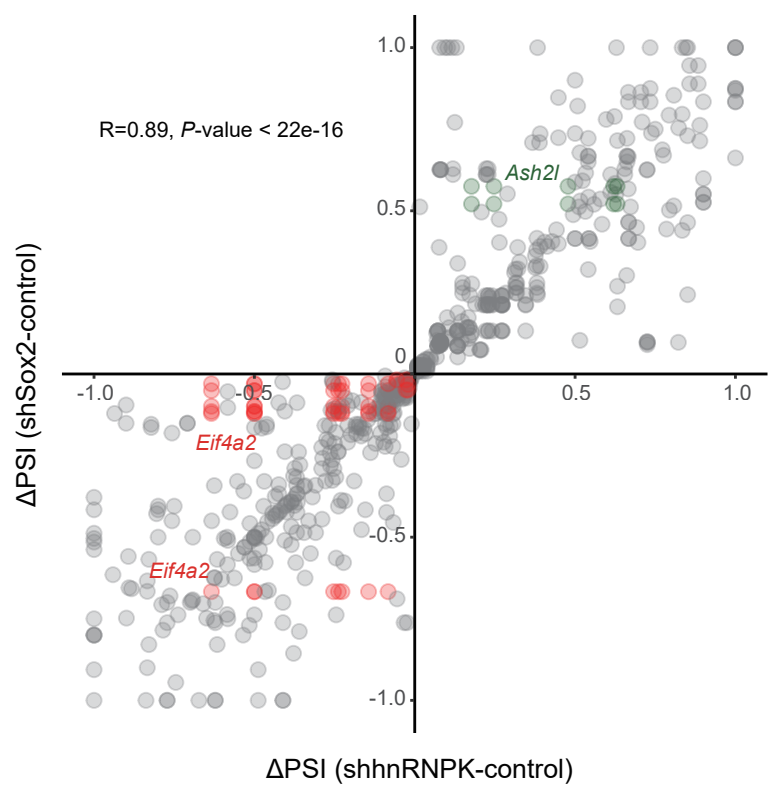
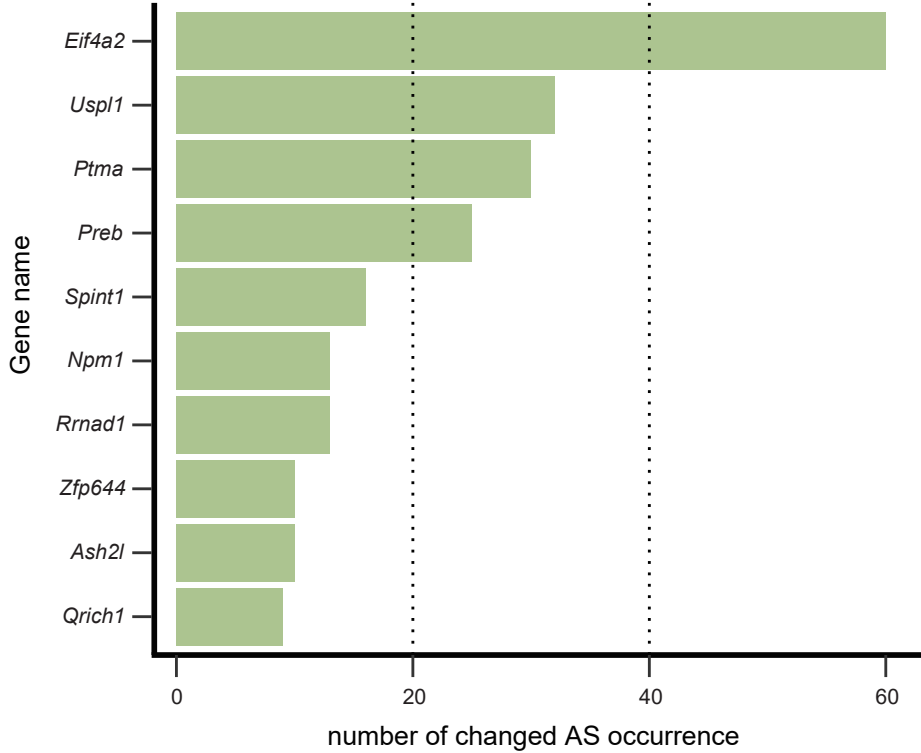


Figure S3

A

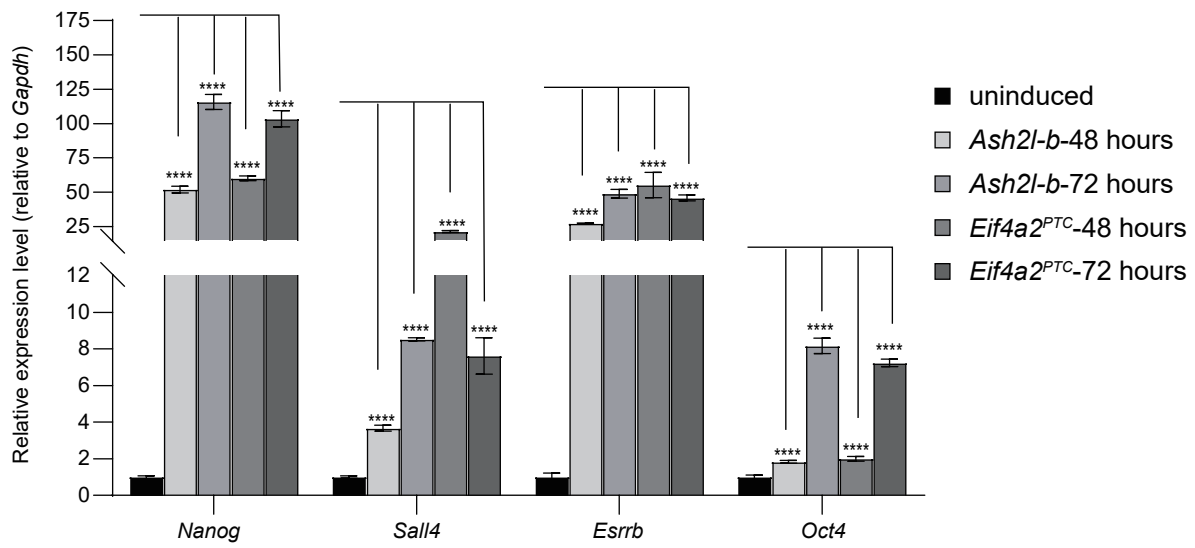


B

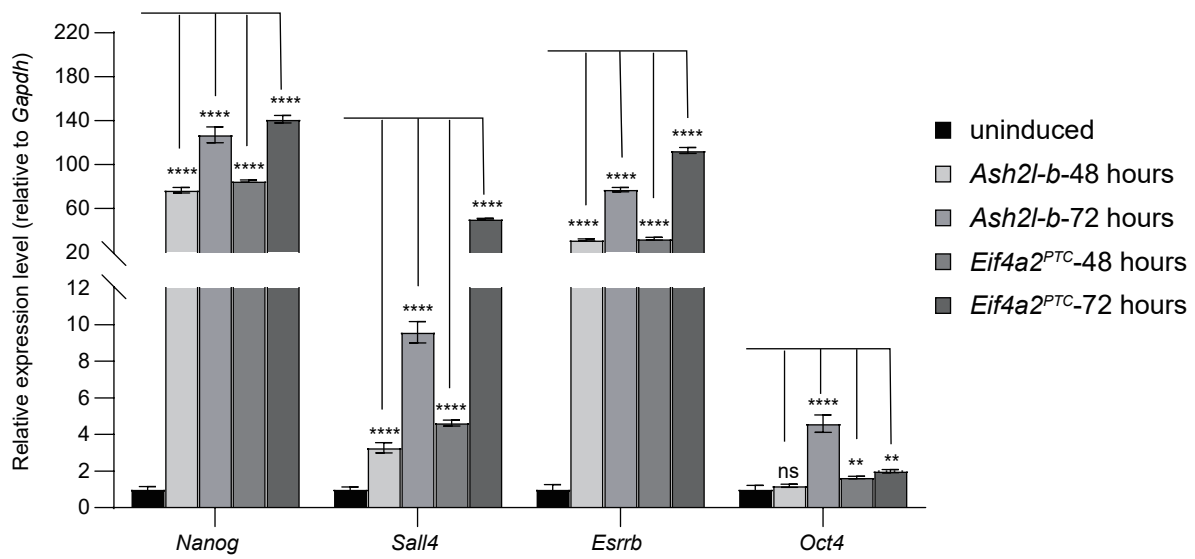


# Figure S4

**A**

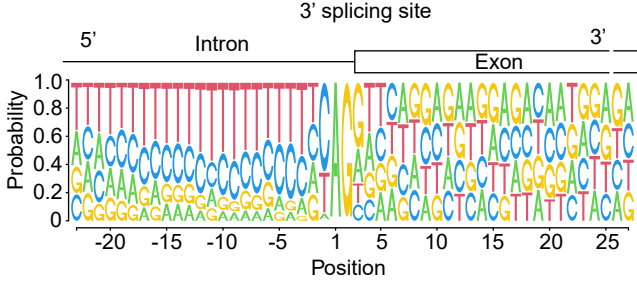
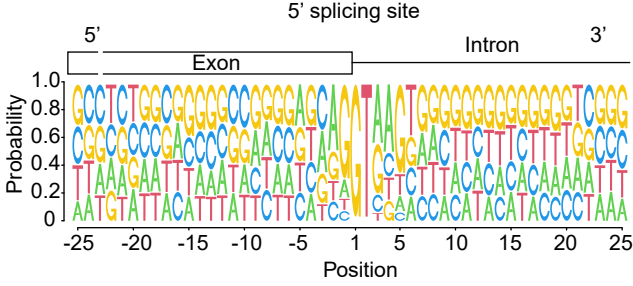


**B**

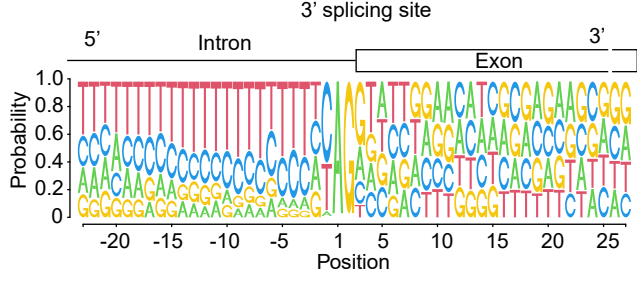
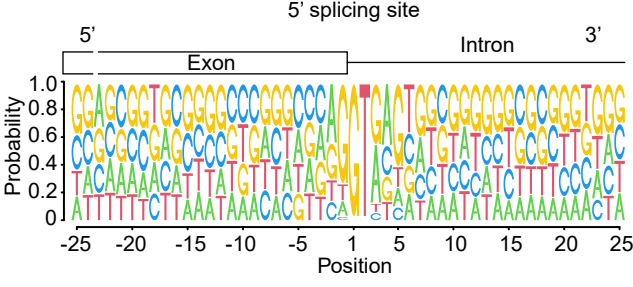


# Figure S5

## A. Sox2-KD



## B. hnRNPK-KD



## C. Cdk9-KD

