Table S1. 4Cseq-Dlx5/6UCE Bait counts on mouse chr6:

Evf2$^{+/+}$*: Evf2* (+) regulated

Evf2$^{TS/TS}$*: Evf2* (-) regulated

Conserved: *Evf2* (I) independent

Sox2 regulated: *Sox2*$^{fl/fl}$ (cre negative, Sox2 (+) regulated), *Sox2*$^{fl/fl}$*:Dlx5/6cre* (Sox2 (-) regulated

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Fig_S1

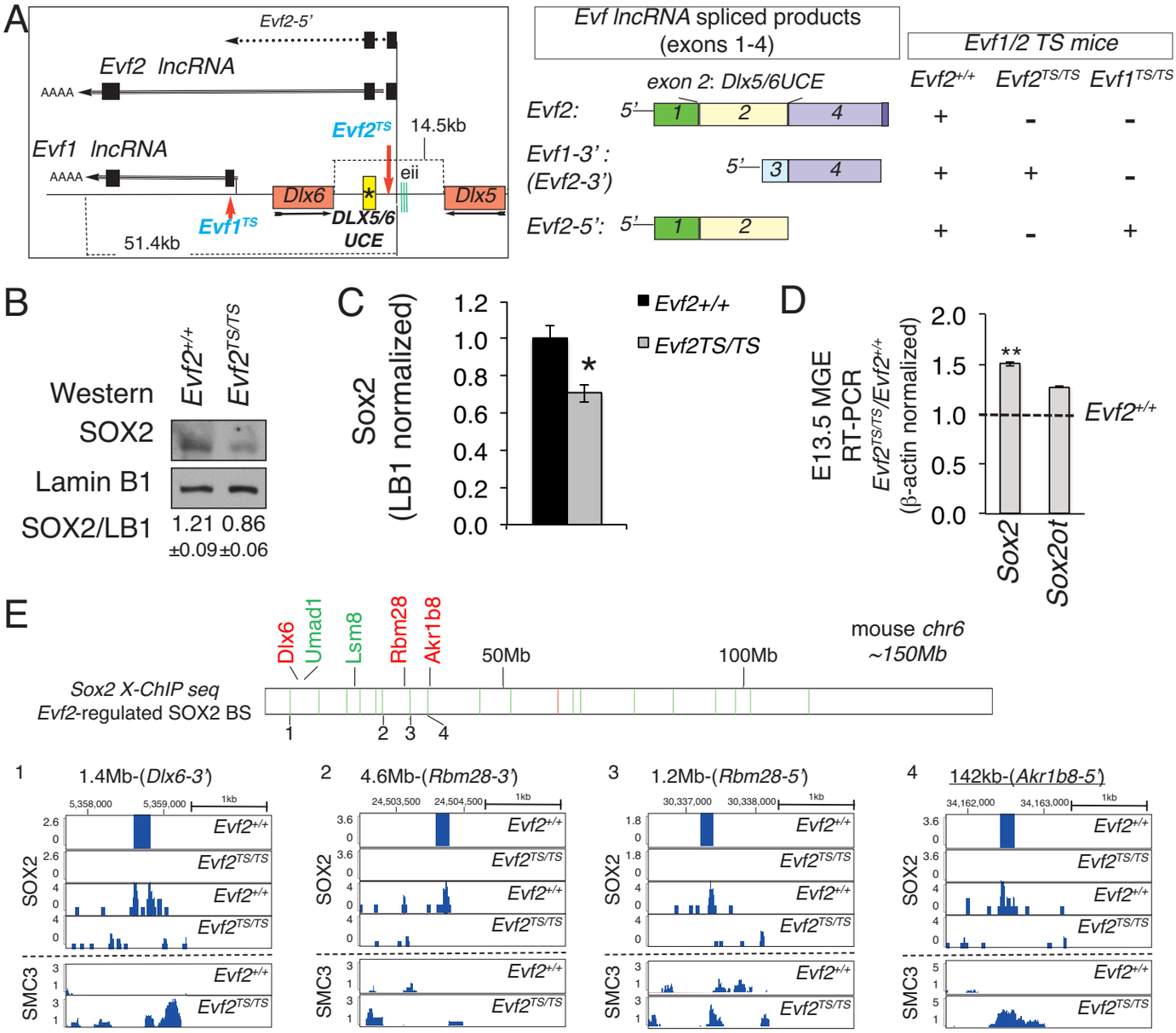


Figure S1. *Evf2* regulation of *Evf2* Sox2 RNP. A. (Left) On mouse chr6, the sites of *Evf1/2* transcription stop insertions (TS, blue) are shown with respect to repressed genes Dlx5 and Dlx6 (red boxes), and polyadenylated and spliced *Evf1, Evf2, and Evf2-5'* transcripts (exons 1-4 in black). *Dlx5/6UCE* sequences are in yellow with star. (Right) Evf contains 4 exons; depending on placement of the TS insertion, different combination of Evf spliced forms are produced. **B.C.**. Western analysis of Sox2 protein levels in *Evf2* expressing (*Evf2*$^{+/+}$) and *Evf2* lacking (*Evf2*$^{TS/TS}$) E13.5GE extracts, (normalized to Lamin B1) shows ~25% decrease in Sox2 protein levels in the absence of *Evf2*. **D.** Taqman qRT-PCR of Sox2 and Sox2ot (a ~118kb overlapping transcript) (RNA levels in *Evf2*$^{+/+}$ and *Evf2*$^{TS/TS}$ E13.5GE shows ~50% increase in Sox2 RNA, but no change in Sox2ot RNA (normalized to β-actin). A-C. n=3/genotype, Student's *t* test. **E.** *Evf2* regulation of *Evf2* RNPs Sox2 and Smc3. Sox2 ChIPseq profiles across mouse chr6 from crosslinked chromatin isolated from *Evf2* expressing (*Evf2*$^{+/+}$) and *Evf2* lacking (*Evf2*$^{TS/TS}$) E13.5GE. *Evf2* positively regulated Sox2 binding sites (BS) (green bars), and negatively regulated (red bars) are shown. *Evf2* repressed gene targets are labeled in red (Dlx, Rbm28, Akr1b8), activated gene targets in green (Umad1, Lsm8). Peaks from sites labelled 1-4 overlap with Evf2 positively regulated Sox2 binding (differential MACS2 (peak calling algorithm used for ChIPseq, Zhang et al. 2008) binding site shown in top two rows) and Smc3 binding peaks. Site 1: Sox2 (+) Smc3 (-), Site 2: Sox2 (+) Smc3 (no peak), Site 3: Sox2 (+) Smc3 binding (but not Evf2 regulated), Site 4:Sox2 (+) Smc3 (-), n=2/genotype, Sites 1-4 top two rows (differential MACS2), rows 3-6 (MACS2. FDR<0.05).

Fig_S2

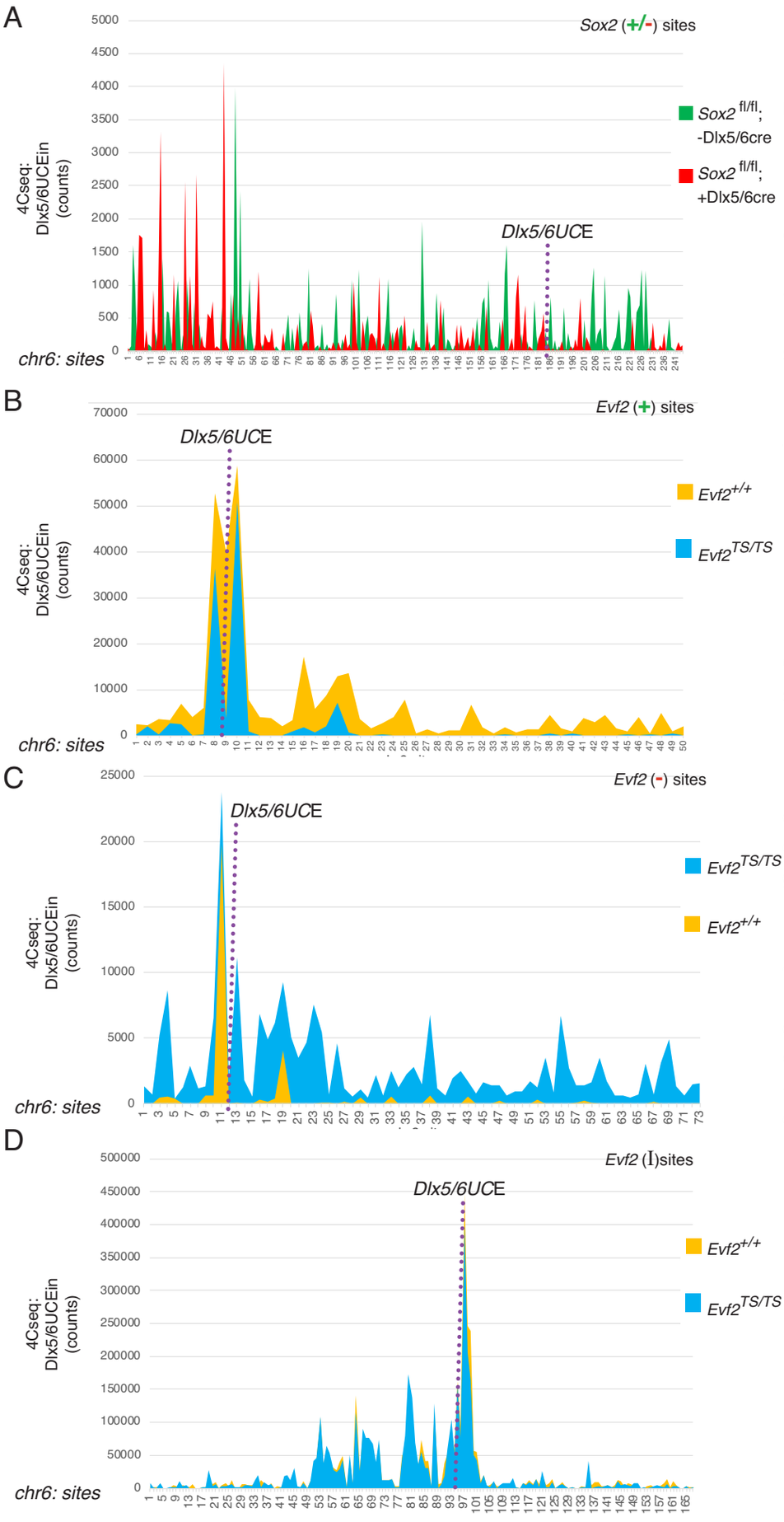


Figure S2. 4Cseq counts identify Sox2 and *Evf2* regulated *Dlx5/6UCEins* across mouse *chr6* in E13.5GEs **A**. *Sox2 (+)* positively regulated *Dlx5/6UCE*ins from Sox2fl/fl;-Dlx5/6cre (presence of Sox2), and Sox2 (-) negatively regulated Dlx5/6UCEins, Sox2fl/fl; +Dlx5/6cre (absence of Sox2). n=4/each genotype 4Cseq intersection of two computational methods (FourCseq and DEseq). Scaled counts for FDR <0.01 are plotted.

B. *Evf2 (+)* positively-regulated *Dlx5/6UCEin* sites (orange) $Evf2^{+/+}$ (presence of *Evf2*), and Evf2 (-) negatively regulated Dlx5/6UCEins (blue) $Evf2^{TS/TS}$ (absence of *Evf2*).

C. *Evf2* (-) negatively regulated sites (blue) $Evf2^{+/+}$ (presence of *Evf2*), and $Evf2^{TS/TS}$ (absence of *Evf2*).

D. Dlx5/6UCEins independent of Evf2 (Evf2 (I) sites). In B-D data is summarized from Cajigas et al 2018.

note:

-*Y-axis:* count numbers from 4Cseq using Dlx5/6UCE as bait

-*X-axis:* numbers correspond to chr6 location sites listed for each genotype (Excel).

-*Dlx5/6UCE* bait location indicated by purple dotted lines

Fig_S3

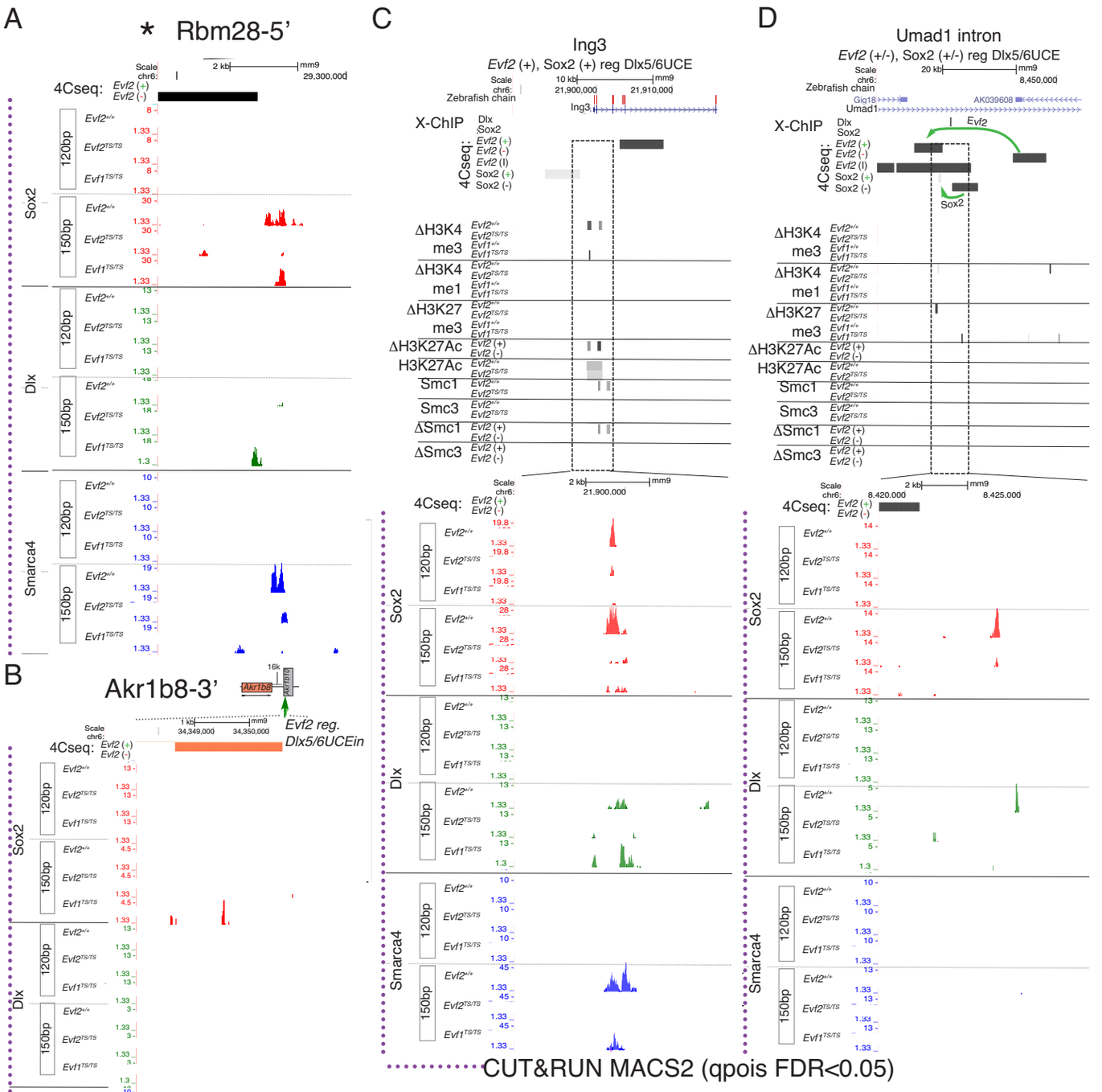
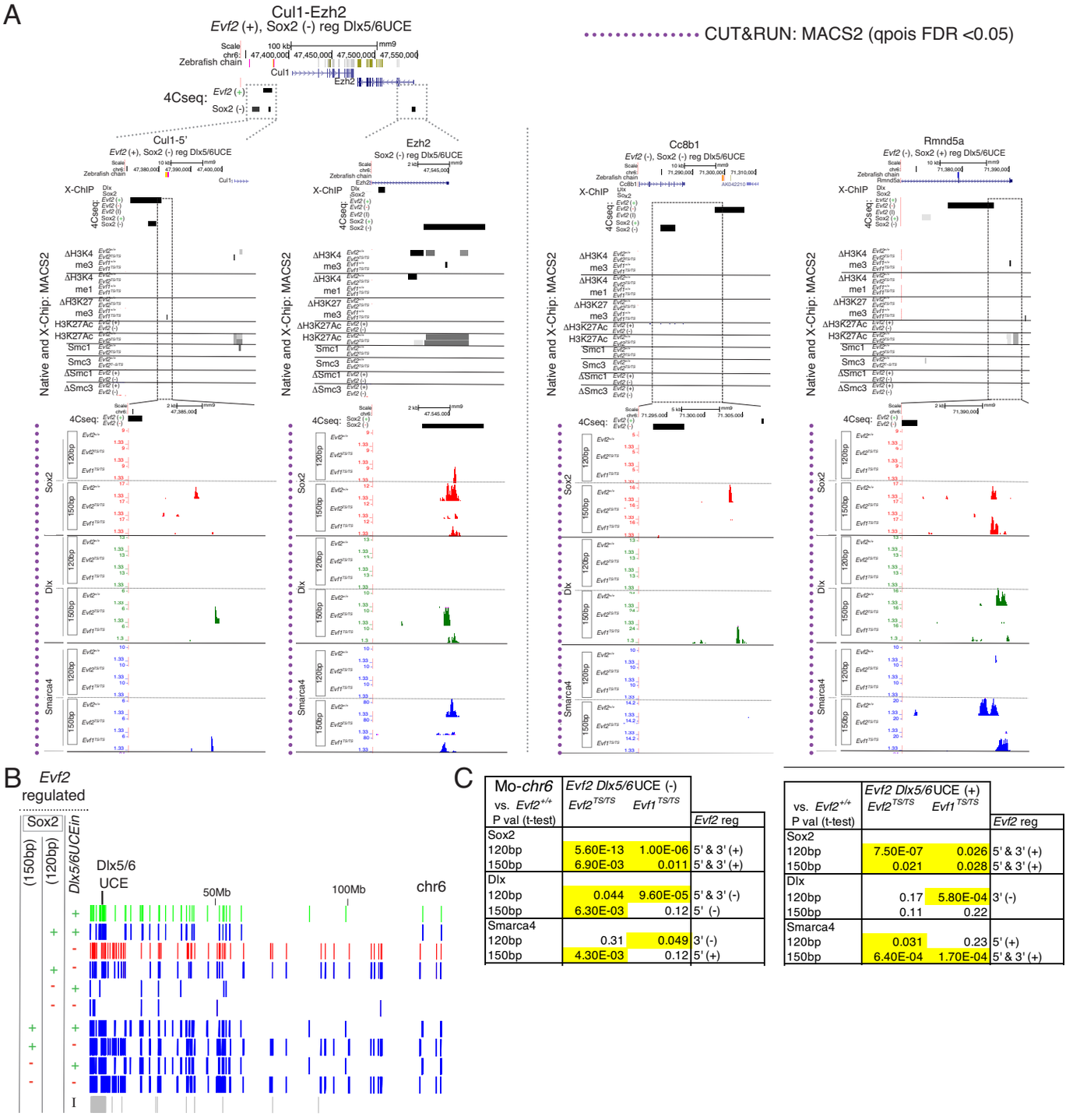


Figure S3. Relationships between *Evf2*-regulated *Dlx5/6UCEin*, RNP binding, and histone modifications at specific sites on mouse *chr6*. CUT&RUN 120bp and 150bp profiles of *Evf2*-regulated binding *Evf2* RNPs Sox2, Dlx, Smarca4, histone modifications (H3K27ac/me3, H3K4me3, H3K4me1), and Smc1a and Smc3 at select *Evf2*-regulated *Dlx5/6UCEin*s from *Evf2*$^{+/+}$, *Evf2*$^{TS/TS}$, and *Evf1*$^{TS/TS}$ E13.5GE. **A.** *Evf2* regulates Sox2 and Smarca4 binding near the *Evf2* negatively regulated *Rbm28-5'-Dlx5/6UCEin*. **B.** *Evf2* regulates Smarca4, but not Sox2 at the *Evf2* positively regulated *Dlx5/6UCEin* located 16kb 3' of *Evf2* repressed target gene Akr1b8. **C-D.** ChIPseq profiles of histone modifications (native chromatin:H3K27me3, H3K4me3, H3K4me1 cross linked: H3K27ac), Smc1a/Smc3 (crosslinked chromatin), Sox2, Dlx, Smarca4 (CUT&RUN) overlapping with Evf2 regulated Dlx5/6UCEins generated from 4Cseq analysis. ChIPseq MACS2 peak (FDR<0.05), n=2-4/genotype, histone modification ChIPseq and Smc1/3 ChiPseq, and 4Cseq FourCseq intersection with DEseq data was based on previously reported in Cajigas et al. 2018.

Fig_S4



Mo-*chr6* vs. *Evf2*$^{+/+}$ P val (t-test)	*Evf2 Dlx5/6*UCE (-) *Evf2*$^{TS/TS}$	*Evf1*$^{TS/TS}$	*Evf2* reg
Sox2			
120bp	5.60E-13	1.00E-06	5' & 3' (+)
150bp	6.90E-03	0.011	5' & 3' (+)
Dlx			
120bp	0.044	9.60E-05	5' & 3' (-)
150bp	6.30E-03	0.12	5' (-)
Smarca4			
120bp	0.31	0.049	3' (-)
150bp	4.30E-03	0.12	5' (+)

vs. *Evf2*$^{+/+}$ P val (t-test)	*Evf2 Dlx5/6*UCE (+) *Evf2*$^{TS/TS}$	*Evf1*$^{TS/TS}$	*Evf2* reg
Sox2			
120bp	7.50E-07	0.026	5' & 3' (+)
150bp	0.021	0.028	5' & 3' (+)
Dlx			
120bp	0.17	5.80E-04	3' (-)
150bp	0.11	0.22	
Smarca4			
120bp	0.031	0.23	5' (+)
150bp	6.40E-04	1.70E-04	5' & 3' (+)

Figure S4. *Evf2*-regulated *Dlx5/6UCEin*, RNP binding, and histone modifications on mouse *chr6*. CUT &RUN profiles of *Evf2*-regulated *Evf2* RNPs Sox2, Dlx, Smarca4, histone modifications (H3K27ac/me3, H3K4me3, H3K4me1) and *Evf2*-regulated *Dlx5/6UCEin*s from *Evf2*$^{+/+}$, *Evf2*$^{TS/TS}$, and *Evf1*$^{TS/TS}$ E13.5GE. **A.** Relationships between ChIPseq profiles of histone modifications (crosslinked and native chromatin), Smc1a/Smc3 (crosslinked chromatin), and Sox2, Dlx, Smarca4(CUT&RUN, native chromatin) overlapping with *Evf2* regulated *Dlx5/6UCEins* generated from 4Cseq analysis. **B.** *Evf2* regulated Sox2 binding sites overlapping with *Evf2* positively regulated (+) and negatively regulated (-) and independent *Dlx5/6UCEins* across *chr6*. **C**. Statistically significant differences (yellow highlights) in RNP binding (CUT&RUN ChIPseq) at *Evf2* positively and negatively regulated *Dlx5/6UCEins* (4Cseq) from *Evf2*$^{+/+}$, *Evf2*$^{TS/TS}$, and *Evf1*$^{TS/TS}$ E13.5GE.

Fig_ S5

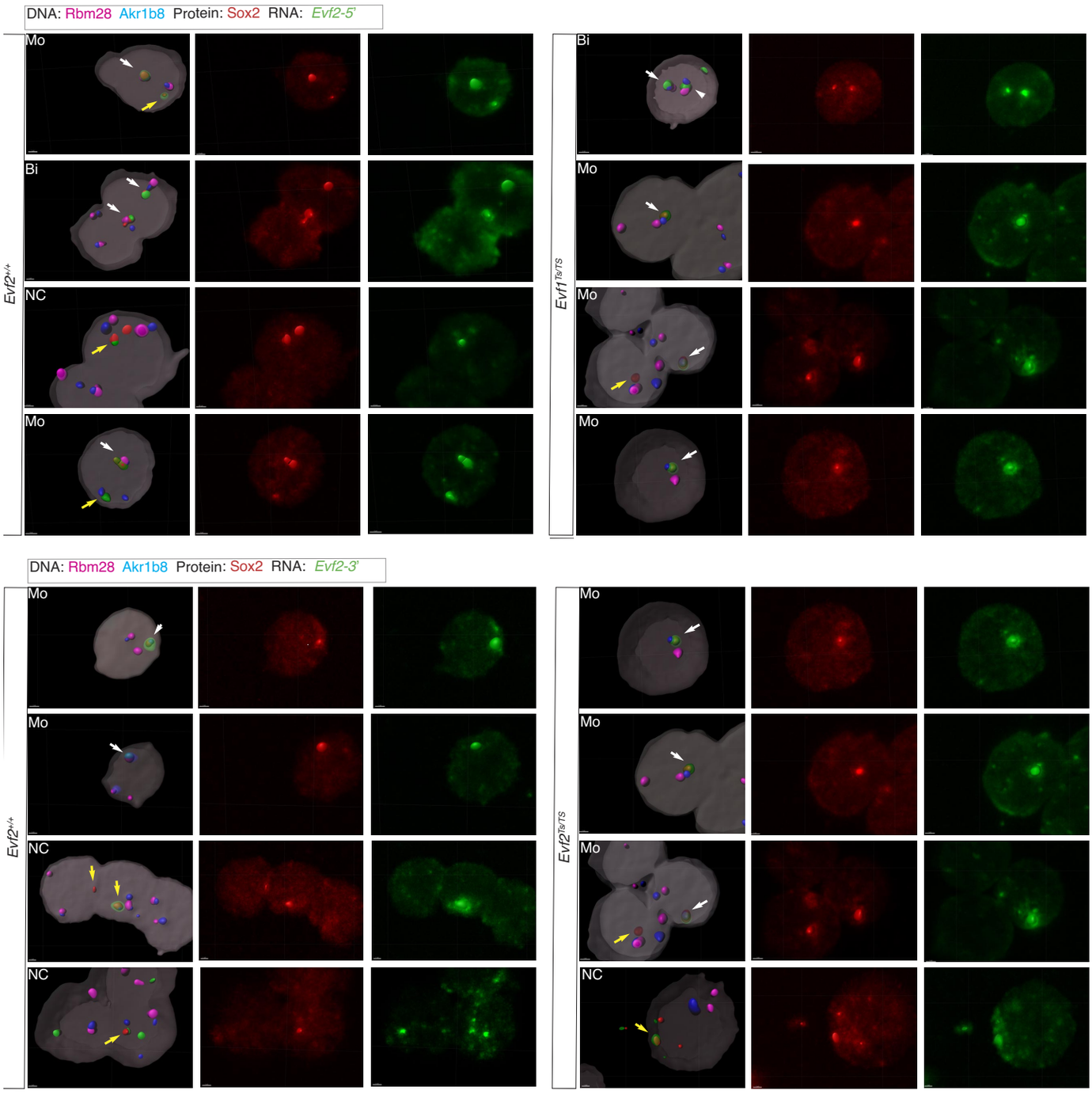


Figure S5. Additional examples of Evf2 RNA cloud and Sox2 protein pool association with re-pressed target genes Akr1b8 and Rmb28 in Evf2 mutants. Imaris 3D reconstructions based on confocal analysis in *Evf2*$^{+/+}$, *Evf2*$^{TS/TS}$, *Evf1*$^{TS/TS}$ E13.5 GE nuclei: FISH (fluorescent in situ hybridization of DNA probes) Rbm28 DNA (pink), Akr1b8 DNA (blue), Evf2-5' RNA (green), Evf2-3' RNA (green) and detection of Sox2 protein using anti-Sox2 antibody: Sox2 protein pools (red). Mo indicates monoallelic, Bi indicates biallelic, white arrows indicate colocalization of RNA or protein with a DNA target, yellow arrows indicate RNA or protein not colocalized (NC) with a DNA target.

Fig_ S6

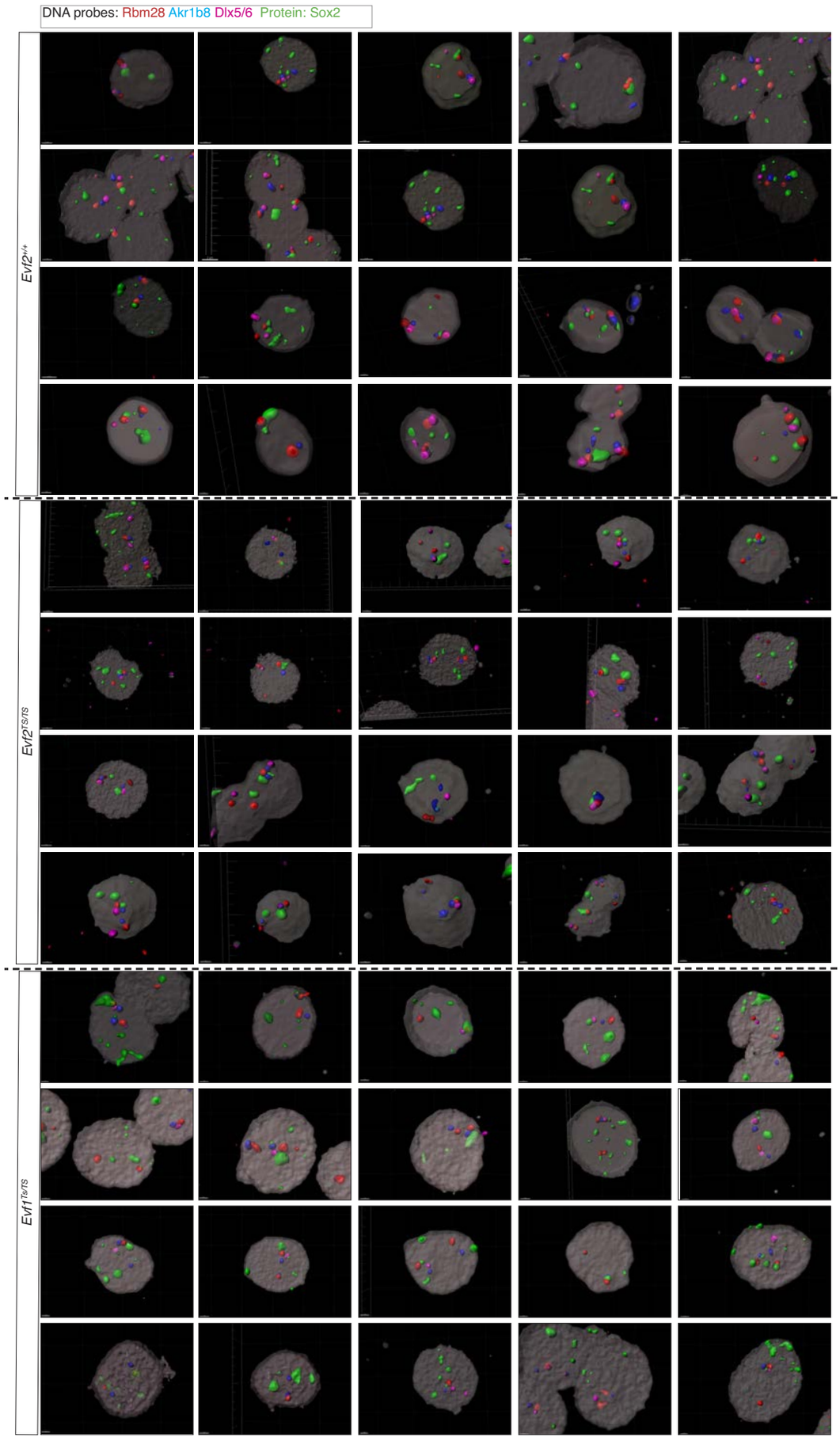


Figure S6. Additional examples of Sox2 protein pools associated with repressed gene targets Rbm28 and Akr1b8 and Dlx5/6 in Evf2 mutants. Imaris 3D reconstructions based on Z-stacks from confocal analysis in *Evf2*$^{+/+}$, *Evf2*$^{TS/TS}$, *Evf1*$^{TS/TS}$ E13.5 GE nuclei: FISH (fluorescent in situ hybridization of DNA probes) Rbm28 DNA (red), Akr1b8 DNA (blue), Dlx5/6 DNA (pink), and detection of Sox2 protein using anti-Sox2 antibody: Sox2 protein pools (green).