

**Targeted Chromatin Conformation (T2C) analysis identifies novel distal neural enhancers of *ZEB2* in pluripotent stem cell differentiation.**

**Supplementary Figure 1. Schematic overview of published enhancers and chromatin architecture changes and overview of T2C Region of Interest (ROI) in this study.**

A) Known identified *ZEB2* intragenic enhancers as described in Bar-Yaacov et al (31) and of predicted looping between SNPs located in *TEX41* (a non-coding RNA) and *ZEB2* (34). B) T2C ROI considered for this study with protein-encoding genes (black boxes) and non-coding RNAs (orange boxes). < or > indicates the direction of transcription.

**Supplementary Figure 2. Normalized steady-state RNA values of *ZEB2* and selected marker genes at the considered time points of neural differentiation.**

The same genes considered for differential expression in Figure 1 (see main text) are assessed here for their temporal dynamics.

**Supplementary Figure 3. Distribution and frequency of fragments generated by *ApoI* in the T2C region of interest and T2C quality controls.**

A) Density plot of fragment distribution according to the size of the obtained fragments. B) fragment counts versus the size of the individual fragments generated by *ApoI*. C) Number of *cis* and *trans* targeted read-pairs in the proximity matrix. D) Proportion of targeted interaction over 1 kb apart, as compared with their score in read pairs. E) Cumulative number of read-pairs versus the distance of binned (per 50 kb) targeted interactions.

**Supplementary Figure 4. *ApoI* fragments-based high resolution T2C maps of *ZEB2* gene chromatin dynamics during differentiation.**

T2C maps can be reconstructed at *ApoI* fragments high resolution for local regions of ~100-150kb as in the case of *ZEB2* (136kb) confirming the existence of several intragenic loopings.

**Supplementary Figure 5. mRNA expression of annotated genes and long non-coding RNAs located in the considered T2C region of interest (ROI).**

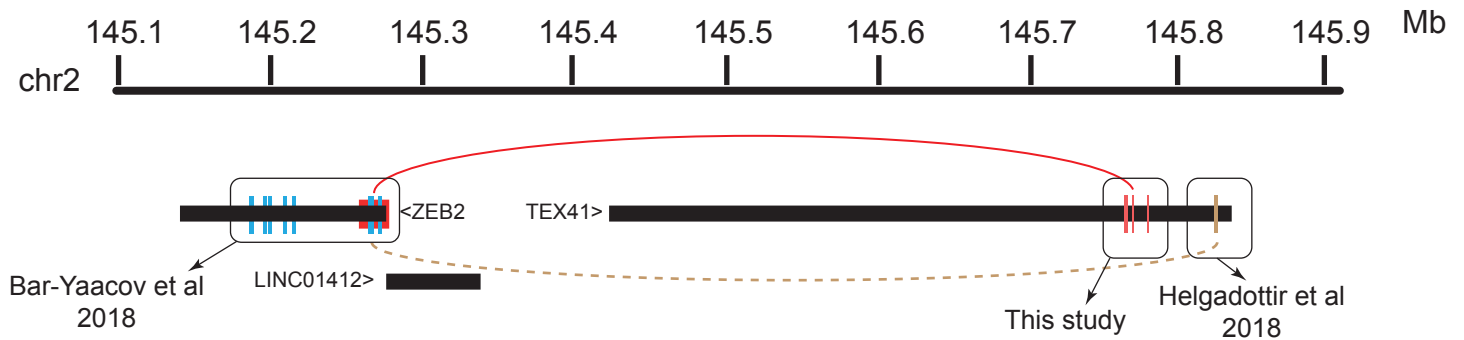
A) Normalized values and log2 Fold Change heatmap of genes. B) non-coding RNAs located in the T2C ROI.

**Supplementary Figure 6. mRNA expression of transcription factors for which a consensus motif has been identified in the newly identified enhancers.**

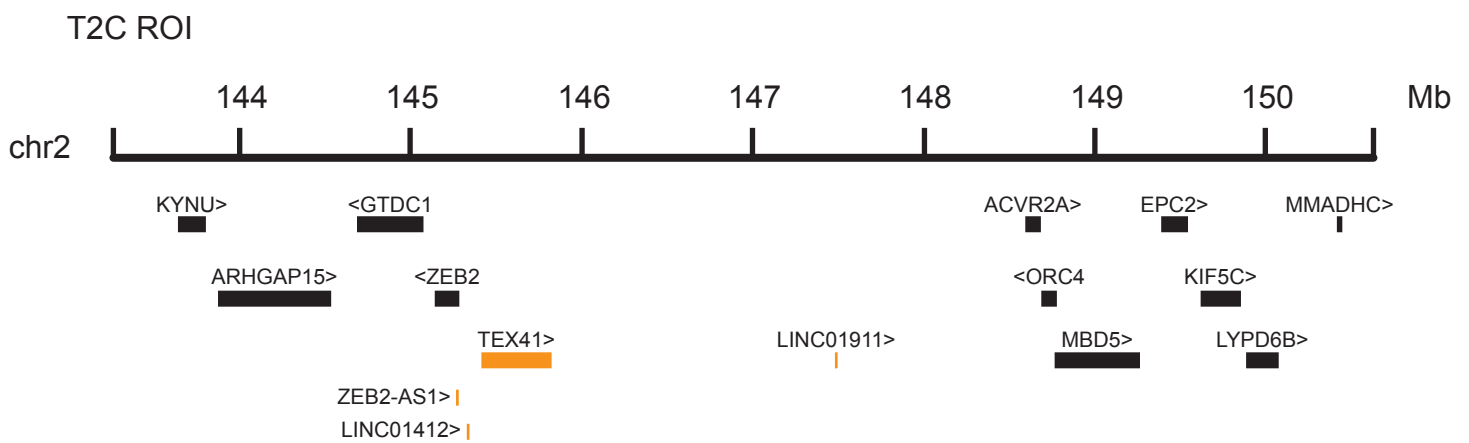
**Supplementary File 1. Coordinates of all *ApoI* fragments interactions for the considered T2C region of interest.**

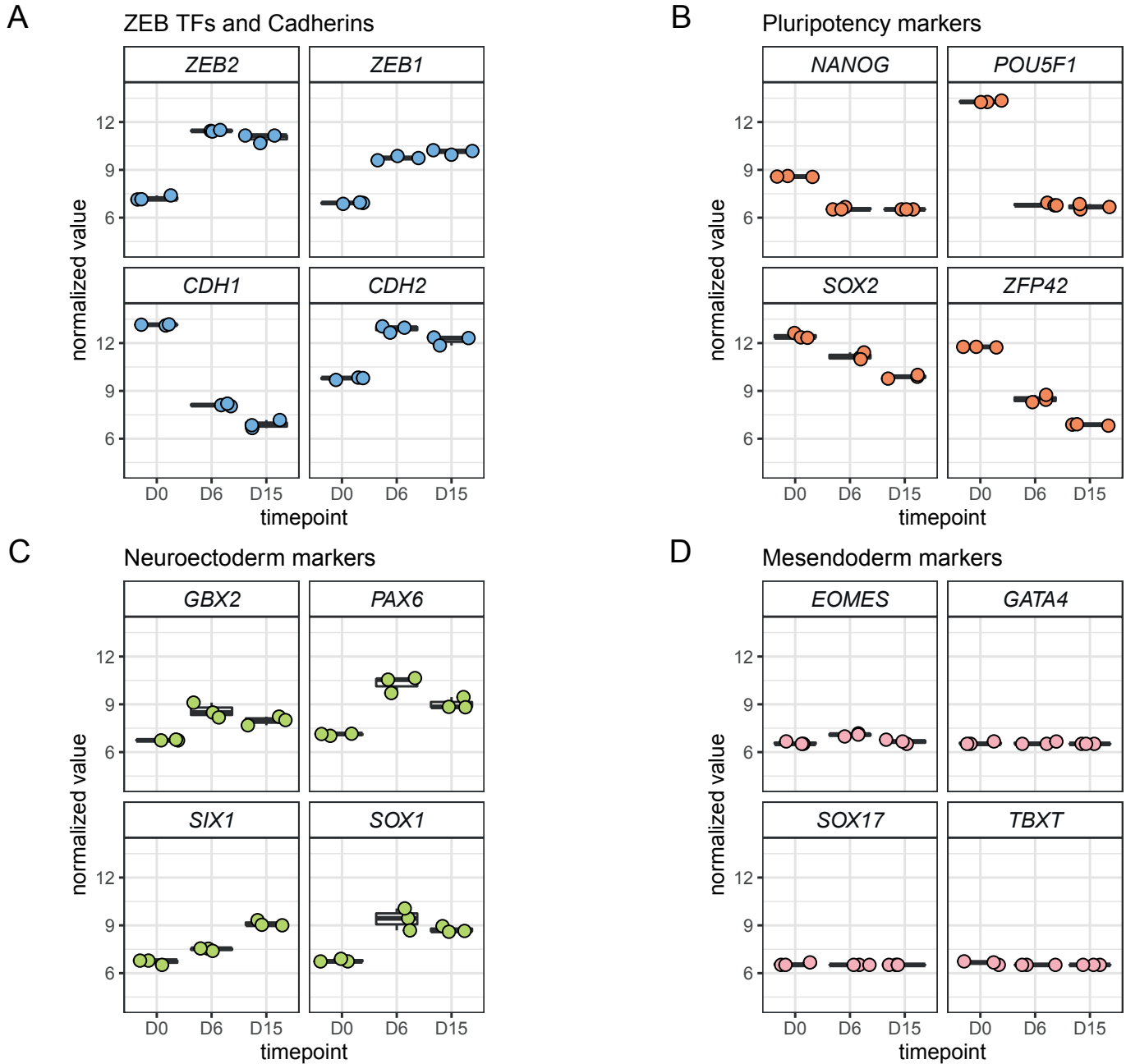
**Supplementary File 2. Coordinates of all proximity interactions as obtained from the 20-kb maps (in main text Figure 3) for the three TADs.**

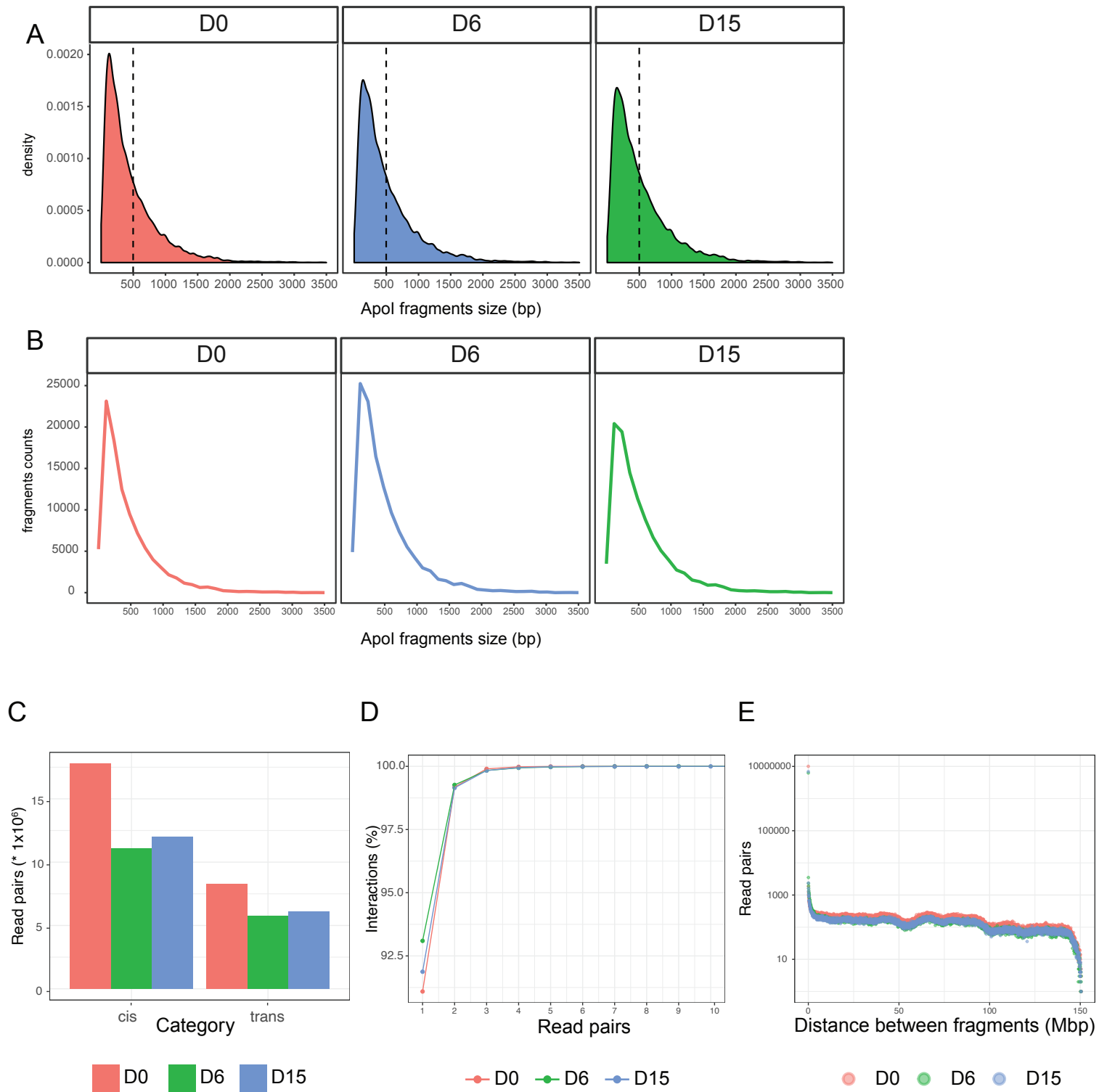
A

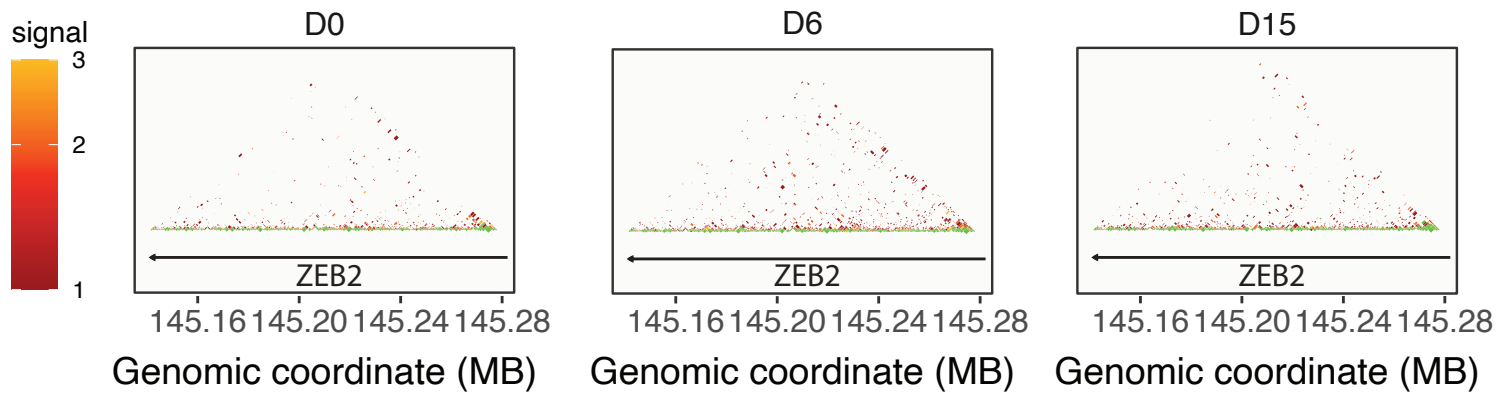


B



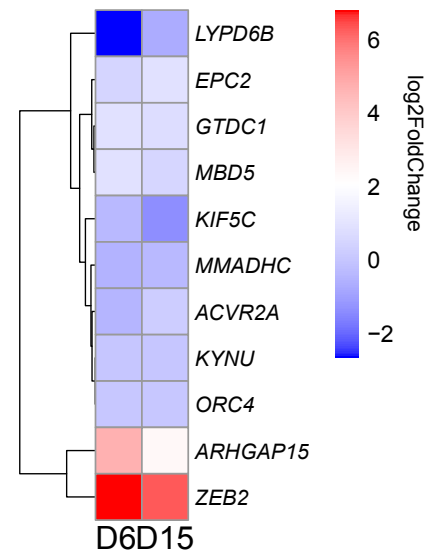
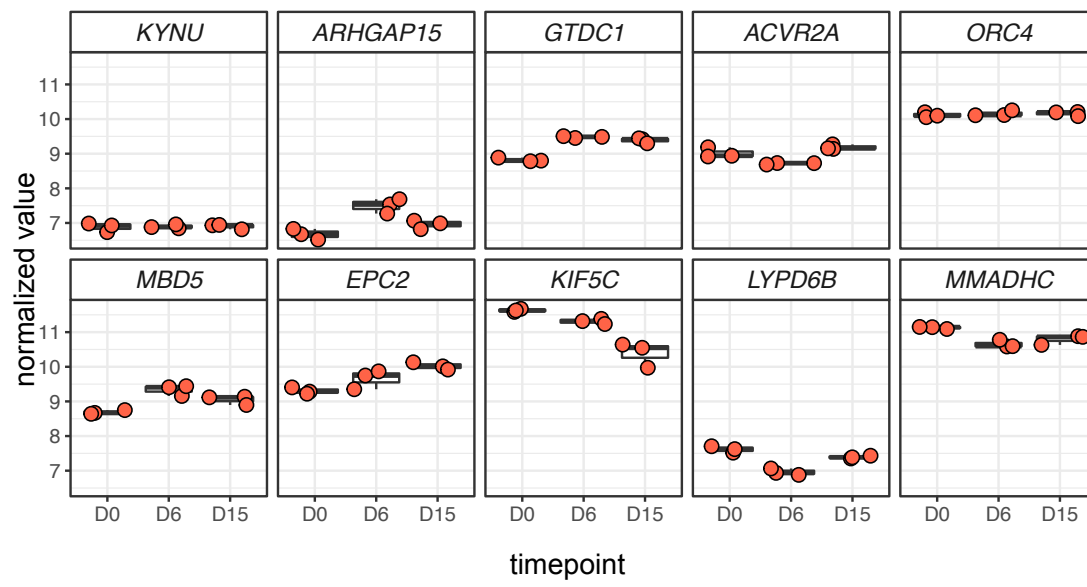






A

genes in T2C ROI chr2:143–151 Mb



B

long non coding RNAs

