
Figures and figure supplements

A β -catenin-driven switch in TCF/LEF transcription factor binding to DNA target sites promotes commitment of mammalian nephron progenitor cells

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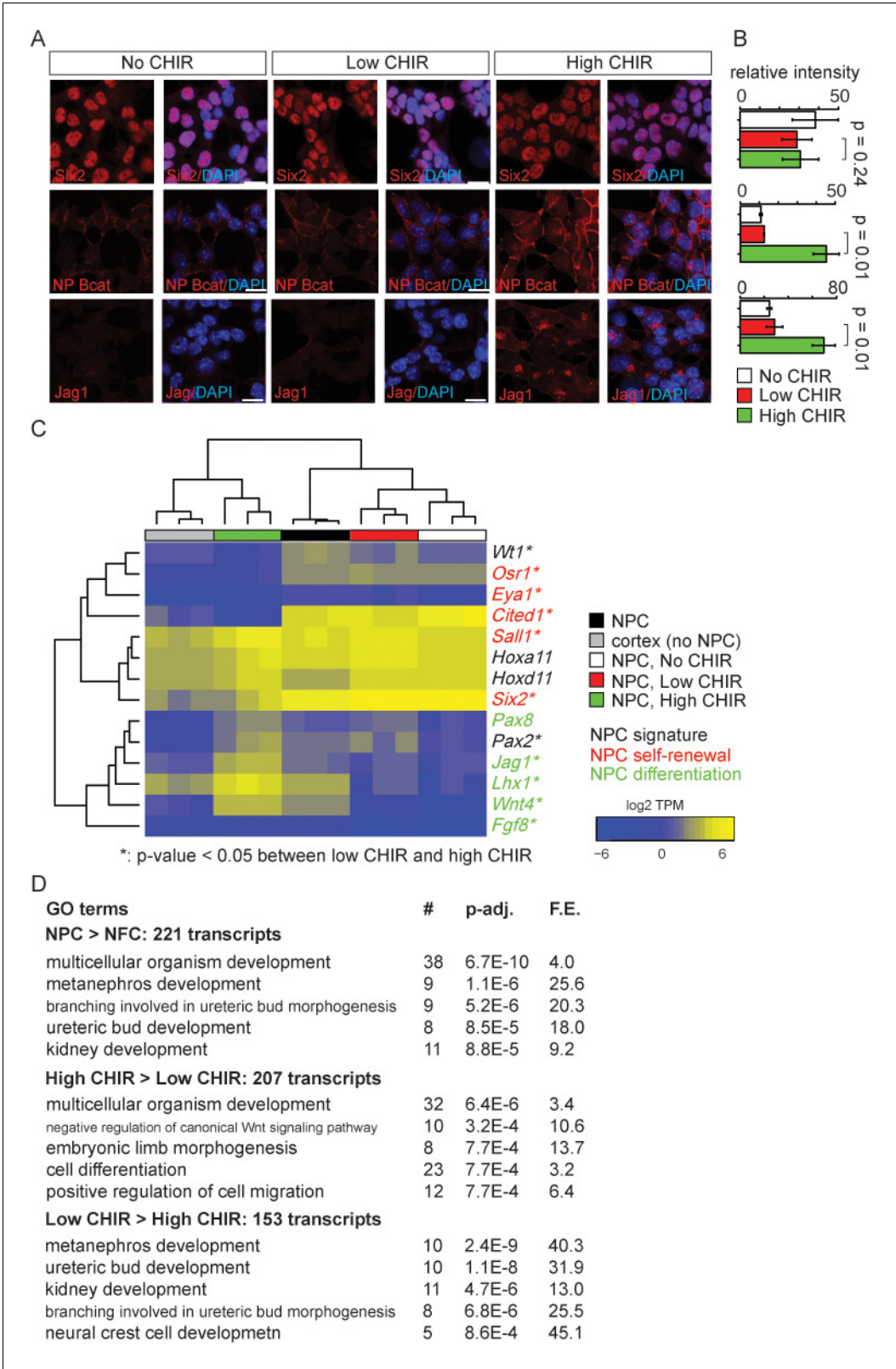


Figure 1. Nephron progenitor expansion medium (NPEM) supplemented with differential levels of CHIR99021 (CHIR) models nephron progenitor cell maintenance or differentiation in a plate. (A) Immunofluorescence (IF) staining showing expression level of Six2, non-phospho (NP) β -catenin and Jag1 in nephron progenitor cell (NPC) cultured in NPEM supplemented with various CHIR dosages. (B) Relative intensity

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of IF signals from individual cells in experiment associated with A. (C) Heatmap/hierarchical cluster of expression levels of NPC signature, self-renewal, and differentiation marker genes. (D) Top five enriched Gene Ontology (GO) terms of indicated differentially expressed gene lists, analyzed by DAVID. Link to high-definition figure: <https://www.dropbox.com/s/76chyrs07m0toen/Fig%201.pdf?dl=0>.

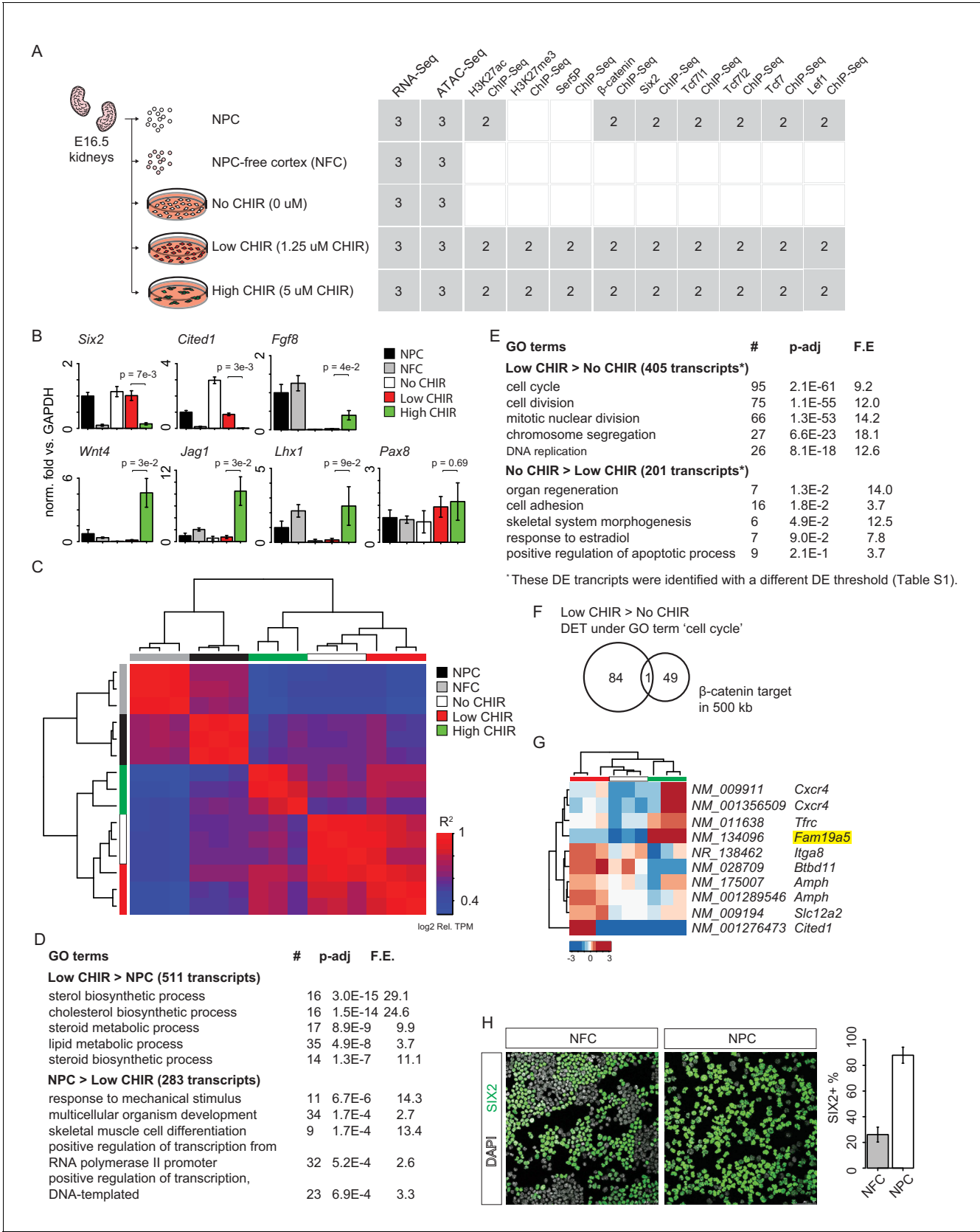


Figure 1—figure supplement 1. Supplementary RNA-Seq data analysis. (A) Overview of experiment design and data available (gray). (B) Bar plots show RT-qPCR measurement of relative expression of the indicated genes, as verification of results in **Figure 1C**. (C) Hierarchical cluster of R-square

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values between transcriptome-wide TPM of the indicated pair of replicate RNA-Seq data sets. (D) Top five enriched Gene Ontology (GO) terms of genes differentially expressed between low CHIR99021 (CHIR) condition and uncultured nephron progenitor cell (NPC). (E) Top five enriched GO terms of genes differentially expressed between low CHIR and no CHIR conditions. (F) Venn diagram shows overlap of β -catenin target genes with all genes associated to the GO term 'cell cycle' that are highly expressed in low CHIR versus no CHIR condition. (G) Heatmap and hierarchical cluster showing log2 relative TPM (TPM divided by mean across samples) to reflect change of gene expression (at isoform level) of **Karner et al., 2011** class II genes (differentially expressed low CHIR > no CHIR or high CHIR > low CHIR) in our data set. The highlighted gene is supported by genetic evidence of regulation by β -catenin in the current data. (H) Immunofluorescence (IF) staining of SIX2 in unpurified NPC-free cortex (NFC) cells and NPC freshly purified from NFC; bar plots showing percentage of SIX2+ cells in the preps.

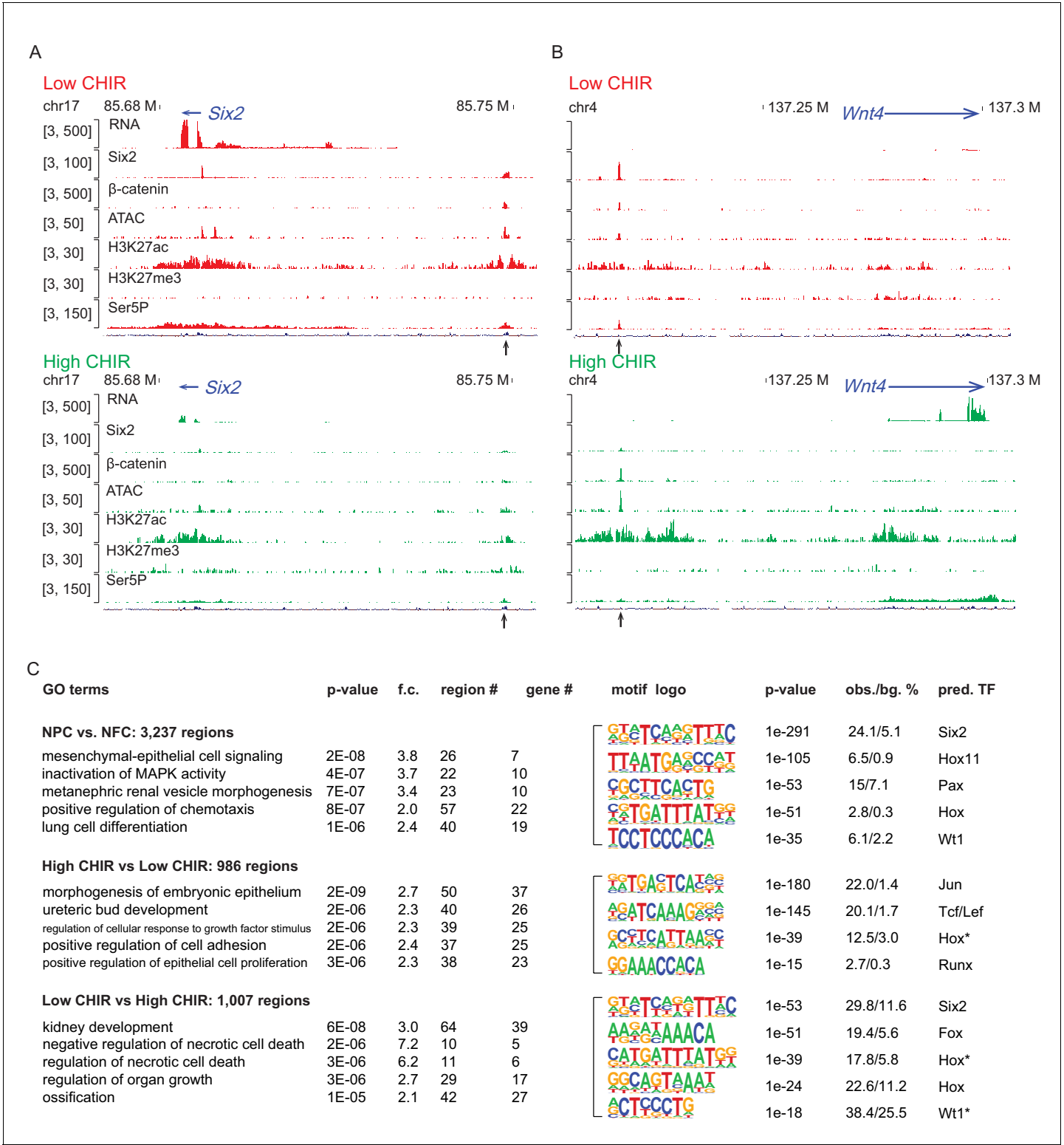


Figure 2. High dosage of CHIR99021 (CHIR) triggered change of nephron progenitor cell (NPC) epigenome. (A, B) Genome browser view of RNA-Seq, ATAC-Seq, as well as *Six2*, H3K27ac, H3K27me3, and Ser5P ChIP-Seq data near *Six2* (A) and *Wnt4* (B) in low CHIR (left) and high CHIR (right) conditions. Black arrow indicates *Six2*DE and *Wnt4*DE, respectively. (C) Display of differentially accessible regions (DARs) generated by the indicated comparisons. (Left) Heatmaps showing log2 normalized read counts of top 500 most significant DARs; (middle) top five most significant Gene Ontology (GO) terms associated with the DARs; (right) top five most enriched motifs discovered de novo in the DARs; * indicates less well-conserved motifs for the factor. NFC: NPC-free cortex.

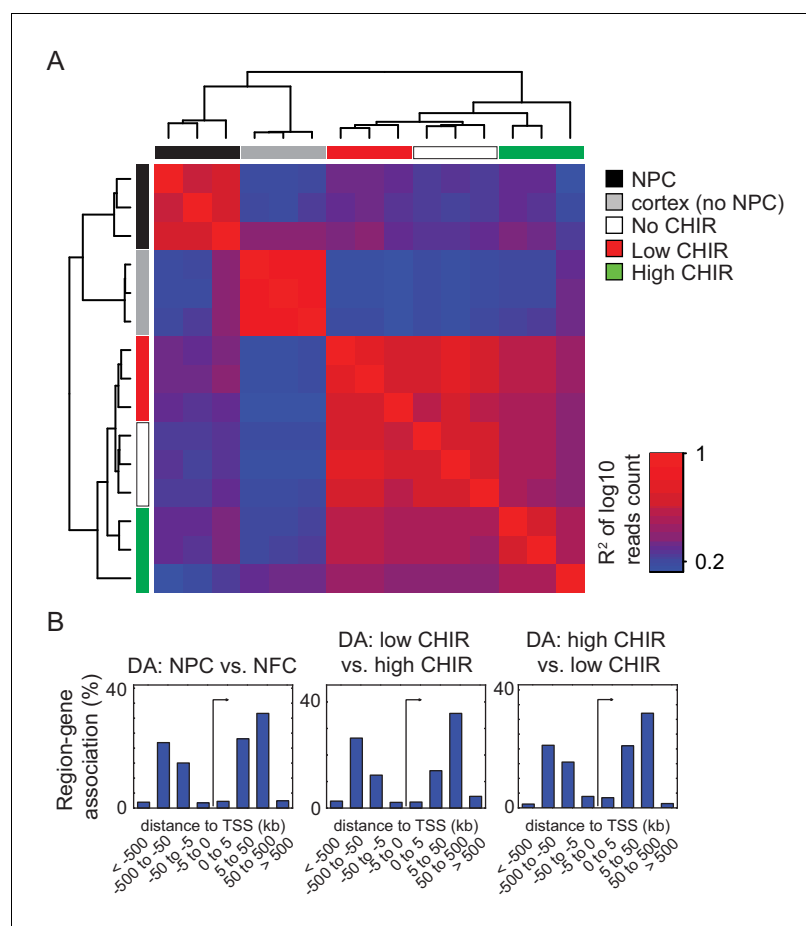


Figure 2—figure supplement 1. Supplementary ATAC-Seq data analysis. (A) Hierarchical cluster of R-square values between normalized ATAC-Seq reads within merged peaks from all samples. (B) Histograms of distances from differentially accessible (DA) chromatin regions to transcriptional start site (TSS) implicate a predominant enhancer feature. CHIR: CHIR99021; NPC: nephron progenitor cell; NFC: NPC-free cortex.

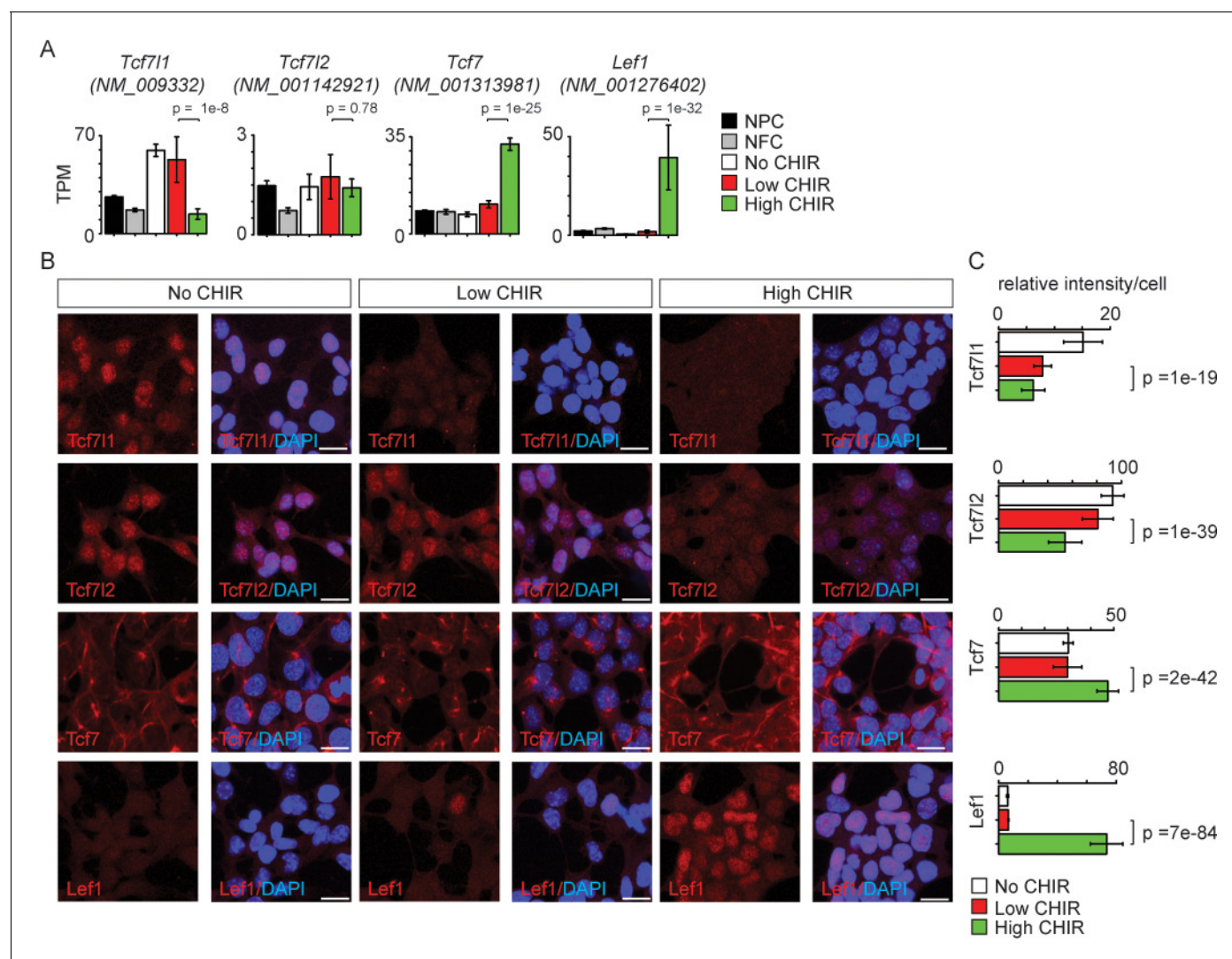


Figure 3. Differential expression of TCF/LEF family transcription factors in nephron progenitor cell (NPC) in response to distinct level of CHIR99021 (CHIR). **(A)** Bar plots showing RNA-Seq measured expression levels of TCF/LEF family factors in NPC cultured in nephron progenitor expansion medium (NPEM) culture supplemented with various concentrations of CHIR. **(B)** Immunofluorescence (IF) staining of TCF/LEF family factors in NPEM cultured with conditions indicated. **(C)** Relative intensity of IF signals from individual cells in experiment associated with B. Link to high-definition figure: <https://www.dropbox.com/s/pvhu1ffhoujt39p/Fig%203.pdf?dl=0>. NFC: NPC-free cortex.

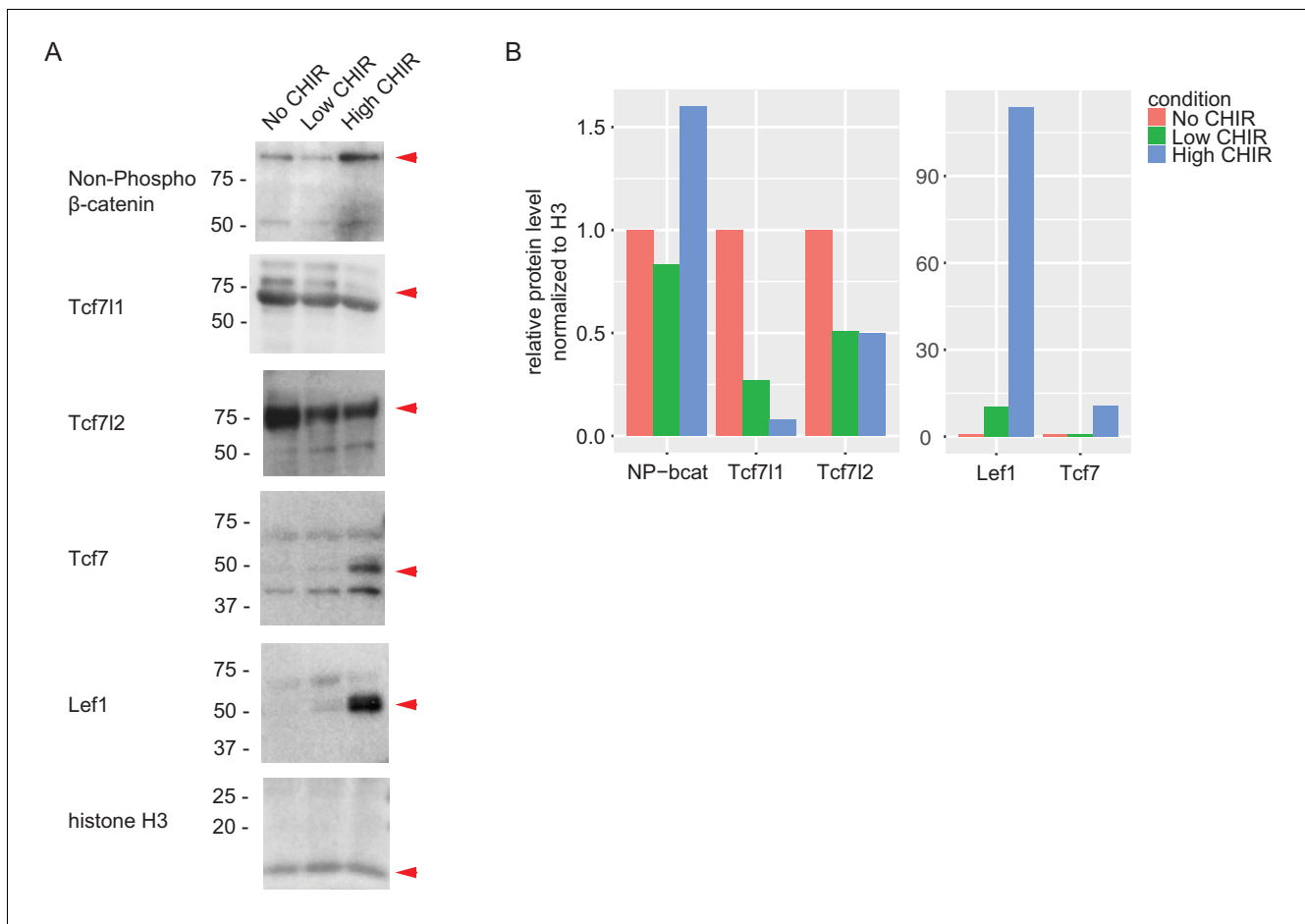


Figure 3—figure supplement 1. Expression of TCF/LEF factors measured by immunoblots. (A) Immunoblots of TCF/LEF family factors in nephron progenitor cell cultured in nephron progenitor expansion medium with the indicated treatment; red arrows indicate target band. (B) Quantification of target band intensity normalized to histone H3 intensity. CHIR: CHIR99021.

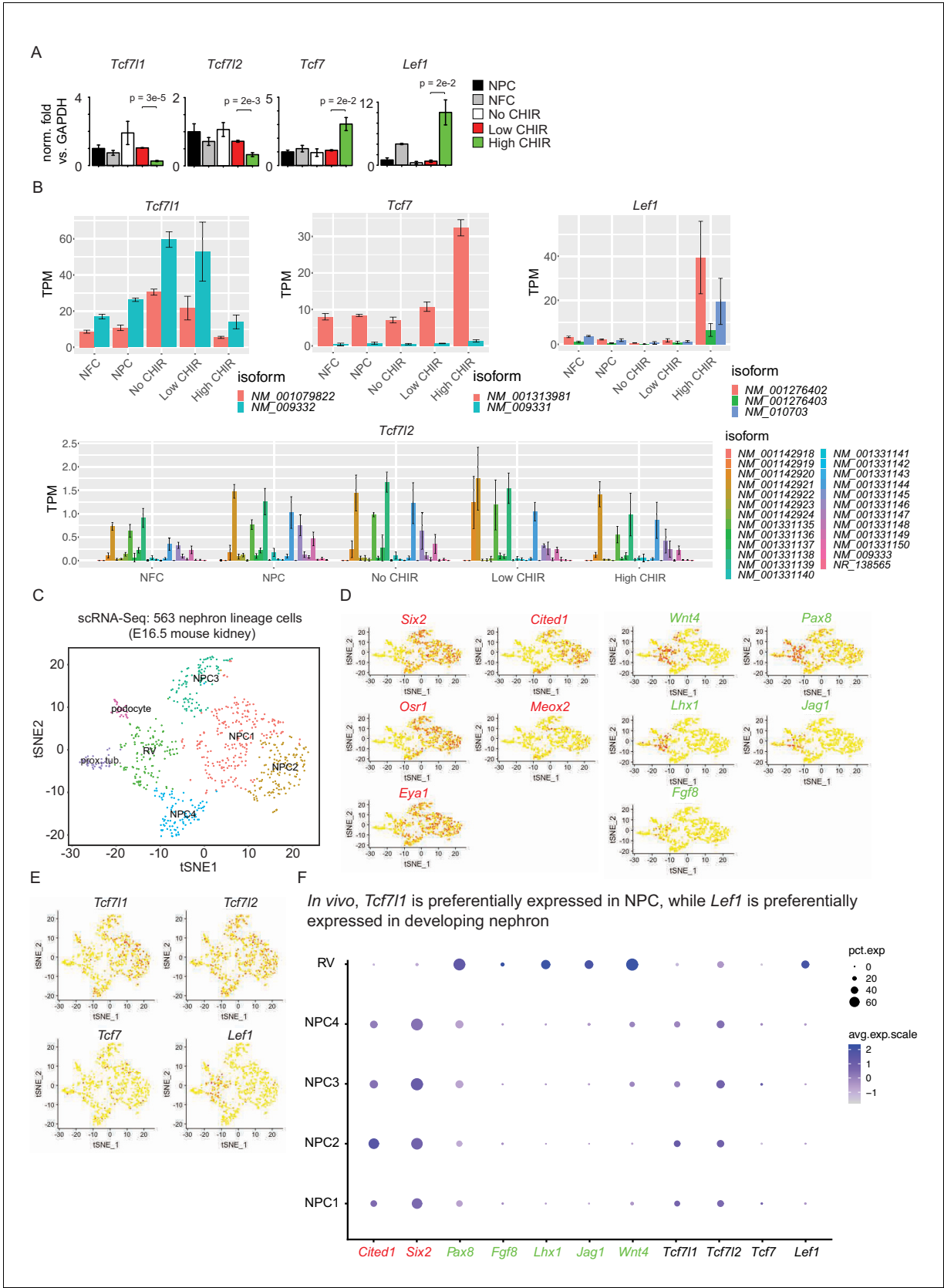


Figure 3—figure supplement 2. Supplementary evidence for differential expression of TCF/LEF factors. (A) Bar plots show RT-qPCR measurement of relative expression of TCF/LEF family factors, as verification for results in **Figure 3A**. (B) Bar plots show expression of individual transcripts of TCF/LEF **Figure 3—figure supplement 2 continued on next page**

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factors in our RNA-Seq data. (C) tSNE plot displaying unbiased cluster of nephron lineage cells profiled by single-cell RNA-Seq. (D) Feature plots displaying distribution of self-renewal (red) and differentiation (green) marker genes transcripts on the tSNE plot. (E) Feature plots showing distribution of TCF/LEF factors transcripts on the tSNE plot. (F) Dot plots showing accumulated expression level of marker genes as well as TCF/LEF factor in selected clusters of cells. NPC: nephron progenitor cell; NFC: NPC-free cortex; CHIR: CHIR99021.

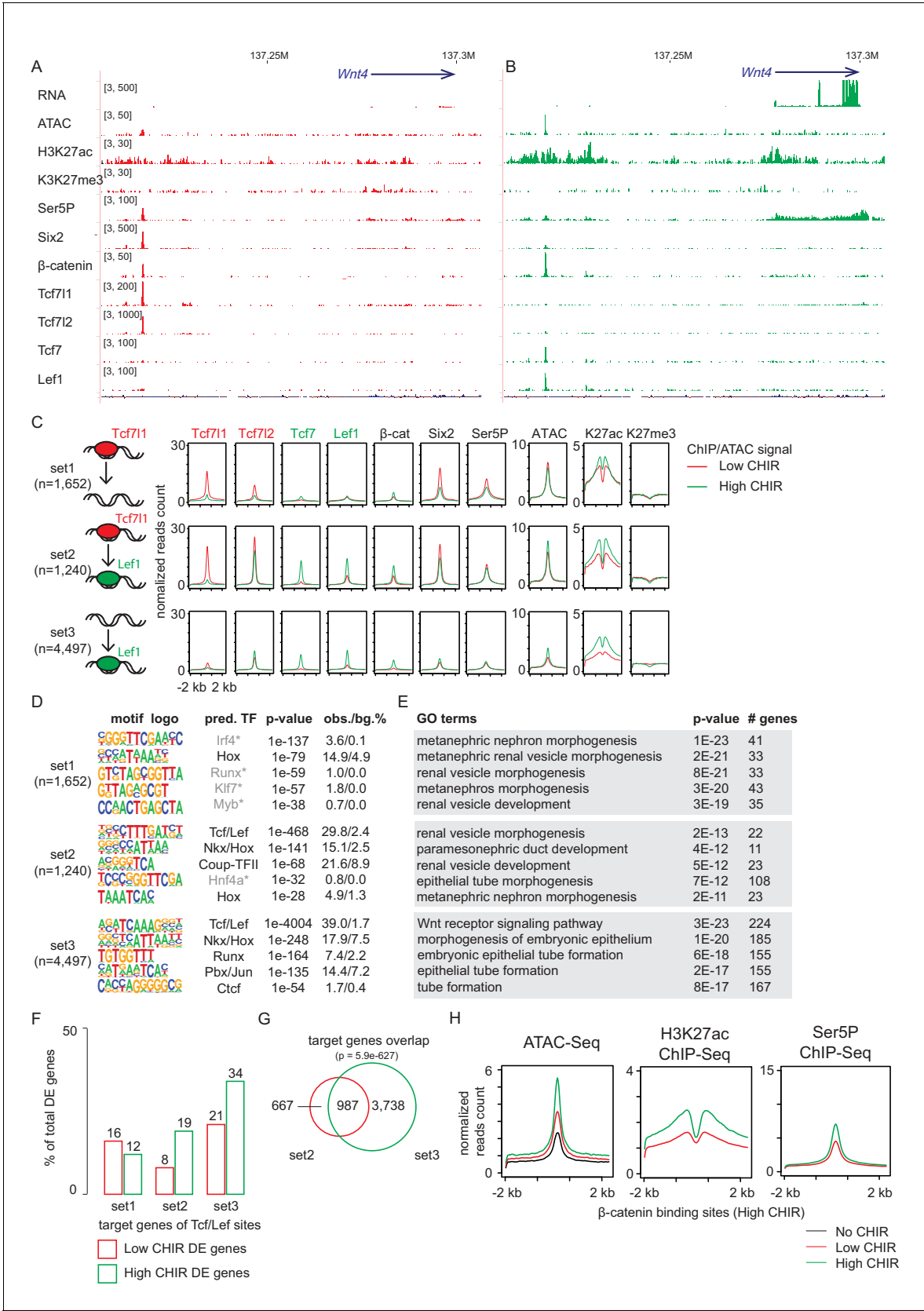


Figure 4. Increased CHIR99021 (CHIR) dosage induces a switch of TCF/LEF factors binding to the genome. (A, B) Genome browser view of *Wnt4* enhancer locus showing ChIP-Seq signal of TCF/LEF factors in low and high CHIR conditions. (C) Histograms showing binding intensity of TCF/LEF factors in low and high CHIR conditions. (D) Motif logos and predicted transcription factors for the three sets of genes. (E) GO terms for the three sets of genes. (F) Bar graph showing the percentage of total differentially expressed genes for the three sets of genes. (G) Venn diagram showing the overlap of target genes between set2 and set3. (H) Genomic tracks showing ATAC-Seq, H3K27ac ChIP-Seq, and Ser5P ChIP-Seq signal for β -catenin binding sites in high CHIR conditions.

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factors and chromatin markers on the three sets of TCF/LEF binding sites assigned by overlap of low CHIR Tcf7l1 and high CHIR Lef1 binding sites. (D) Result from de novo motif discovery of the three sets of TCF/LEF binding sites described in A. (E) Top Gene Ontology (GO) terms associated with the corresponding sets of TCF/LEF binding sites shown in B. (F) Percentage of TCF/LEF target genes belonging to different sets in differential expressed genes specific to low CHIR or high CHIR conditions as described in **Figure 1**. (G) Venn diagram showing overlap of set 2 and 3 target genes assigned by GREAT (**McLean et al., 2010**). (H) Histograms showing quantification of reads from the indicated data sets in ± 2 kb of β -catenin binding sites in high CHIR condition.

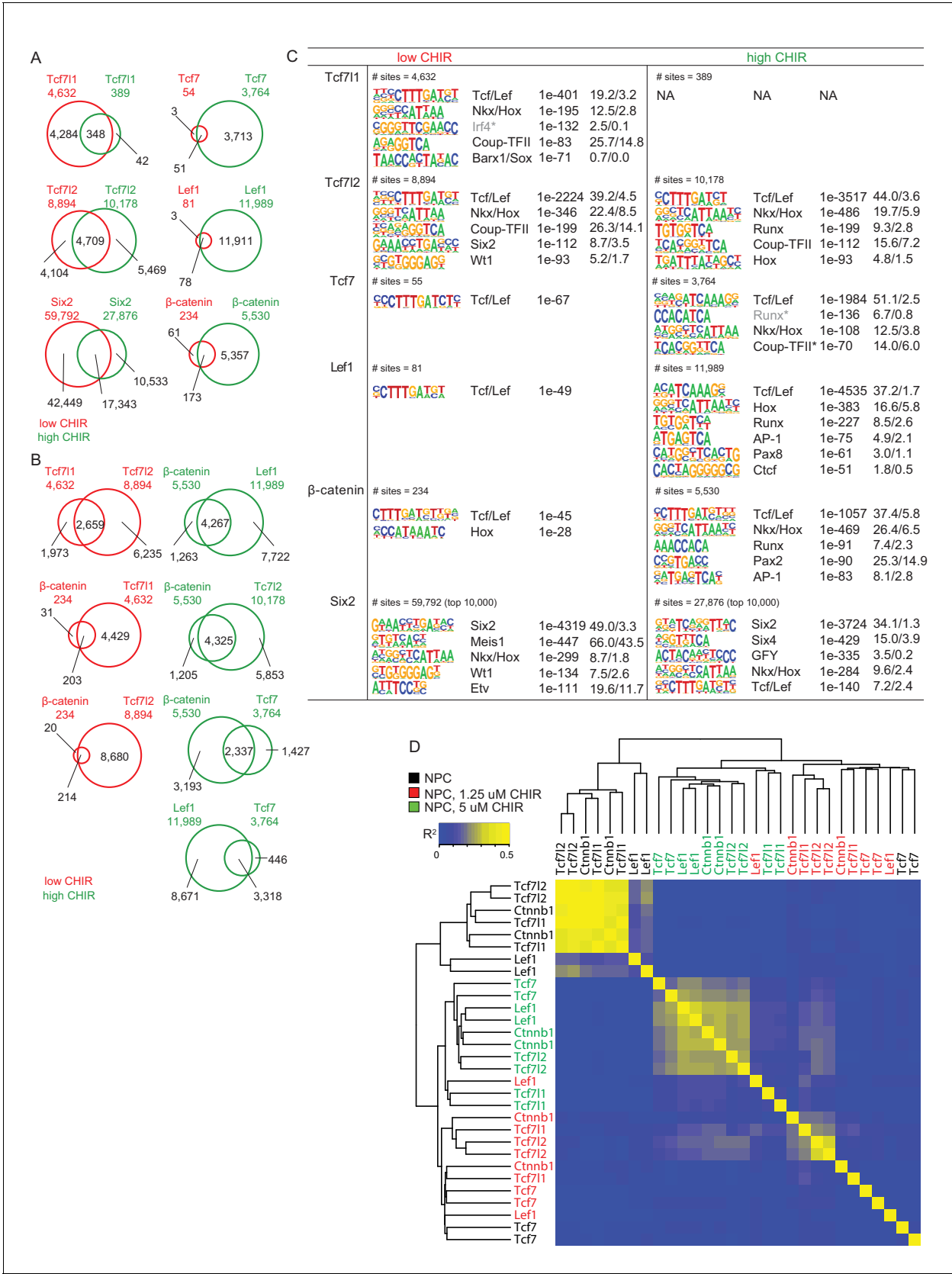


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de novo discovery (Homer) from binding sites of the data sets indicated. (D) Hierarchical clustering of normalized read counts of ChIP-Seq data sets on merged binding sites. NPC: nephron progenitor cell; CHIR: CHIR99021.

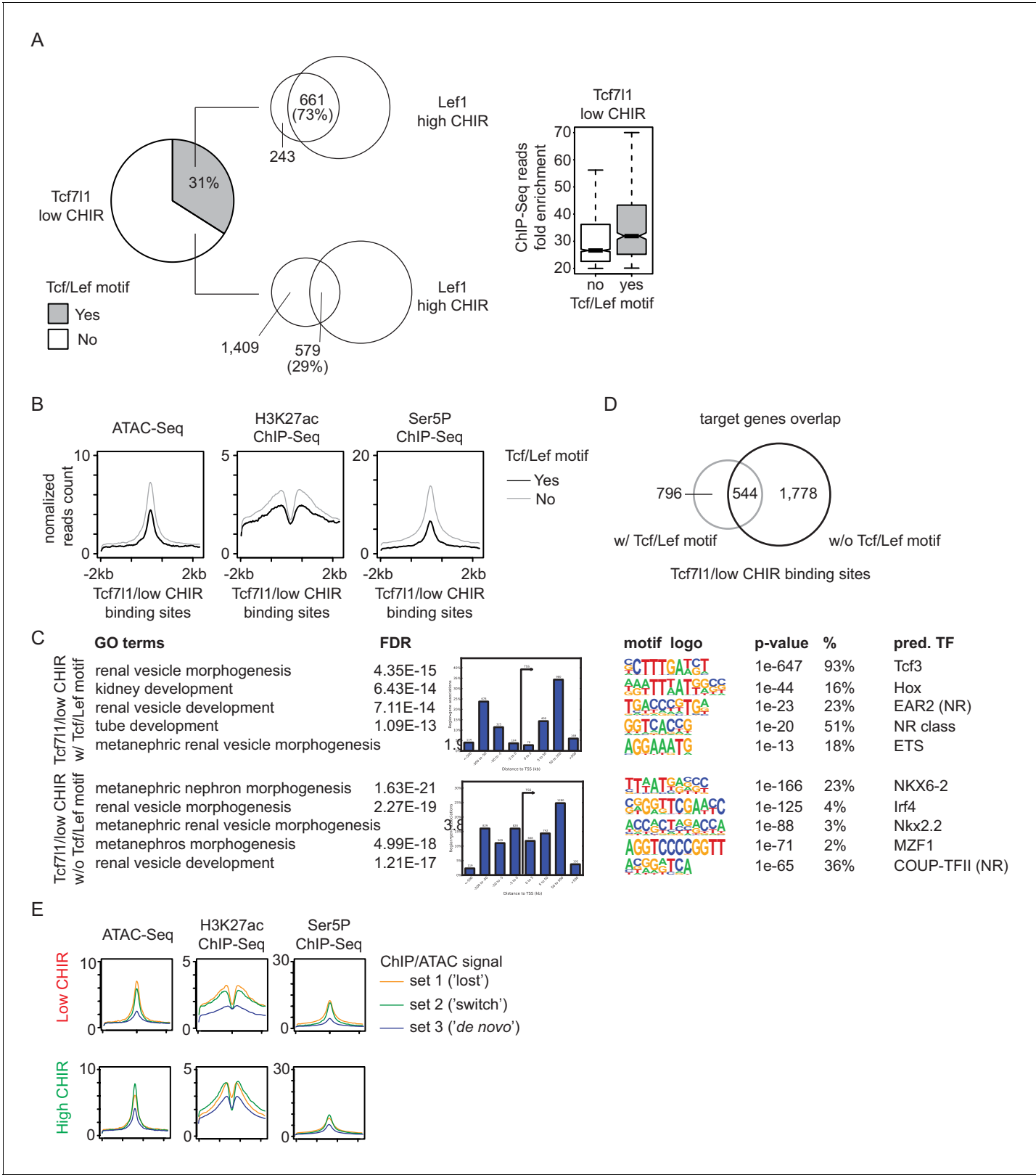


Figure 4—figure supplement 2. Analysis of Tcf711 binding in low CHIR99021 (CHIR). (A) Overlap of direct and indirect Tcf711 binding in low CHIR with Lef1 binding in high CHIR (left) and enrichment of ChIP-Seq reads on the two types of binding events. (B) Distribution of ATAC-Seq, H3K27ac ChIP-Seq, and Ser5P ChIP-Seq signals on Tcf711 binding sites in low CHIR with or without TCF/LEF motifs. (C) Gene Ontology (GO) analysis by GREAT on Tcf711 binding sites. (D) Venn diagram showing target genes overlap between Tcf711/low CHIR binding sites with and without Tcf/Lef motifs. (E) Genomic tracks for ATAC-Seq, H3K27ac ChIP-Seq, and Ser5P ChIP-Seq around Tcf711 binding sites for set 1 ('lost'), set 2 ('switch'), and set 3 ('de novo').

Figure 4—figure supplement 2 continued

binding sites in low CHIR with or without TCF/LEF motifs. (D) Overlap of predicted target genes between Tcf7l1 binding sites in low CHIR with or without TCF/LEF motifs. (E) Histograms showing intensities of ATAC-Seq or ChIP-Seq signals around set 1, 2, and 3 TCF/LEF binding sites in low CHIR and high CHIR conditions.

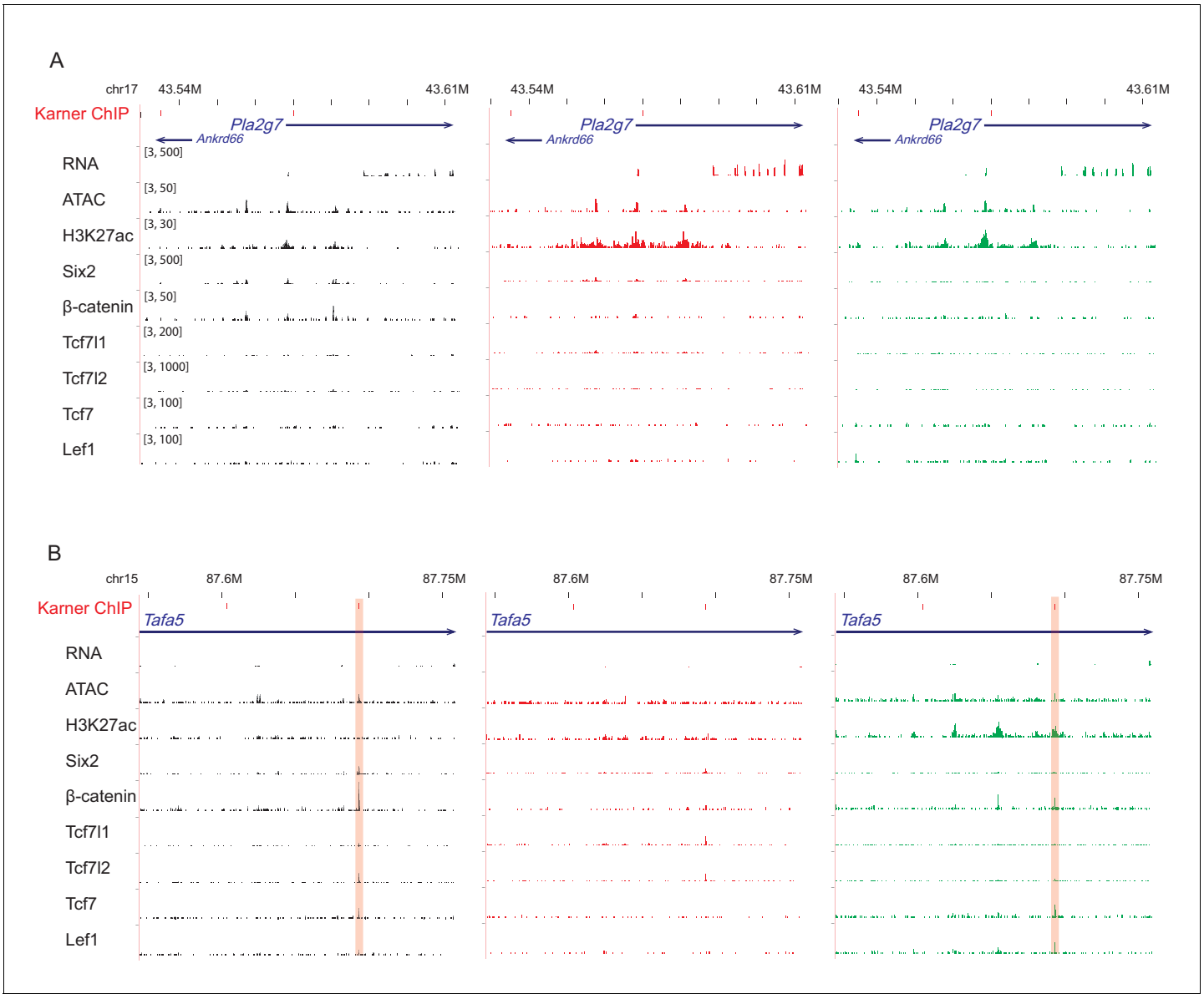


Figure 4—figure supplement 3. β -Catenin binding sites near (A) *Pla2g7* and (B) *Tafa5*, two β -catenin target genes reported in *Karner et al., 2011*. The ChIP-qPCR target sites were marked as red bins on the top track. The highlighted region is where our data is consistent with Karner's.

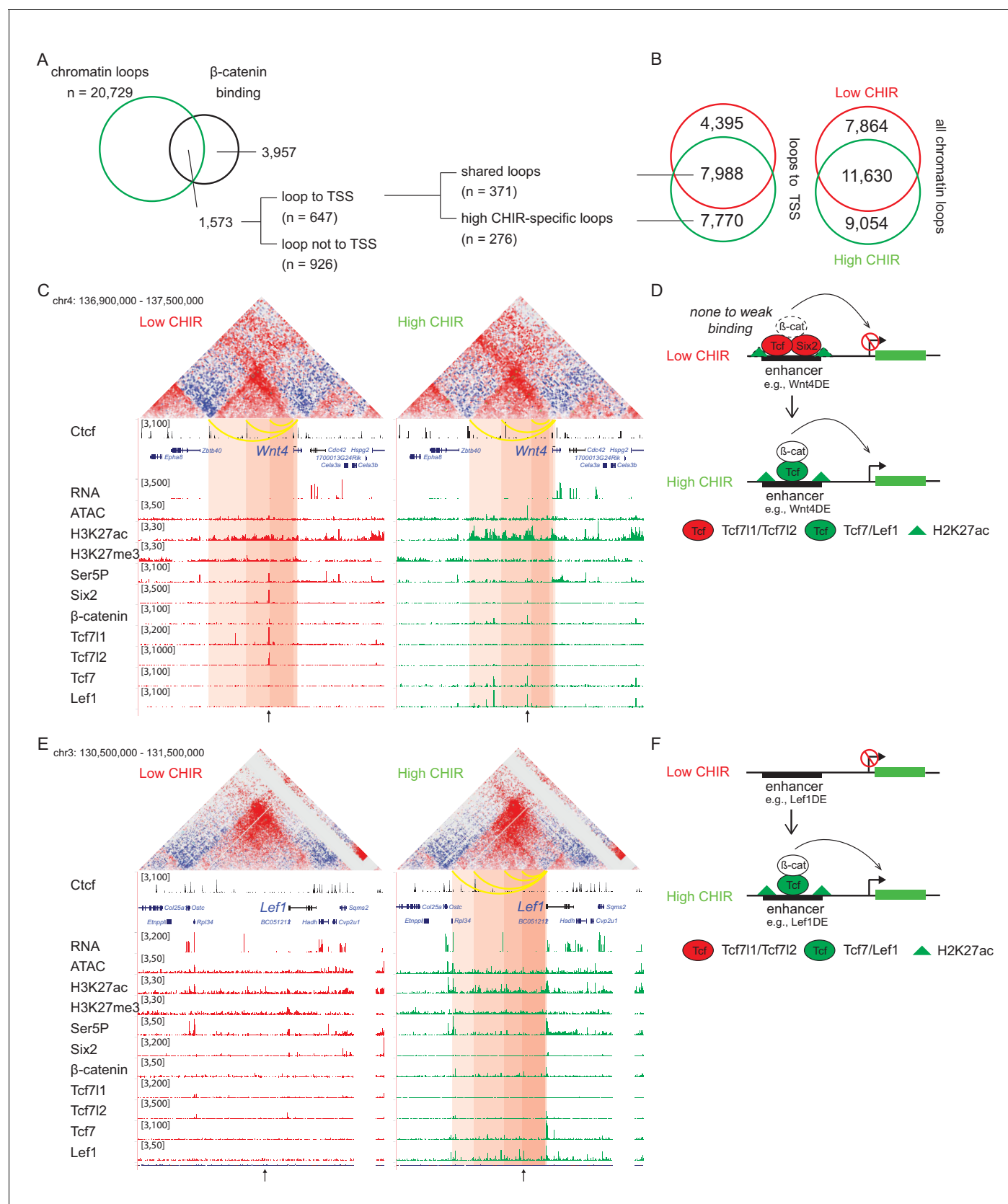


Figure 5. β -Catenin activates gene expression through both stable and de novo enhancer–promoter loops. (A) β -Catenin binding sites that overlap with an anchor of chromatin loop in high CHIR99021 (CHIR), the proportion that connects to a transcriptional start site (TSS) (gray in the pie chart) and Figure 5 continued on next page

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segregation between two types of loops defined in B. (B) Overlap of chromatin loops between low CHIR and high CHIR conditions. Examples (C, E) and schematics (D, F) of β -catenin utilizing low/high CHIR-shared enhancer–promoter loops to activate *Wnt4* (C, D) or high CHIR-specific loops to activate *Lef1* (E, F). Black arrow at the bottom indicates the β -catenin binding sites involved in the loops.

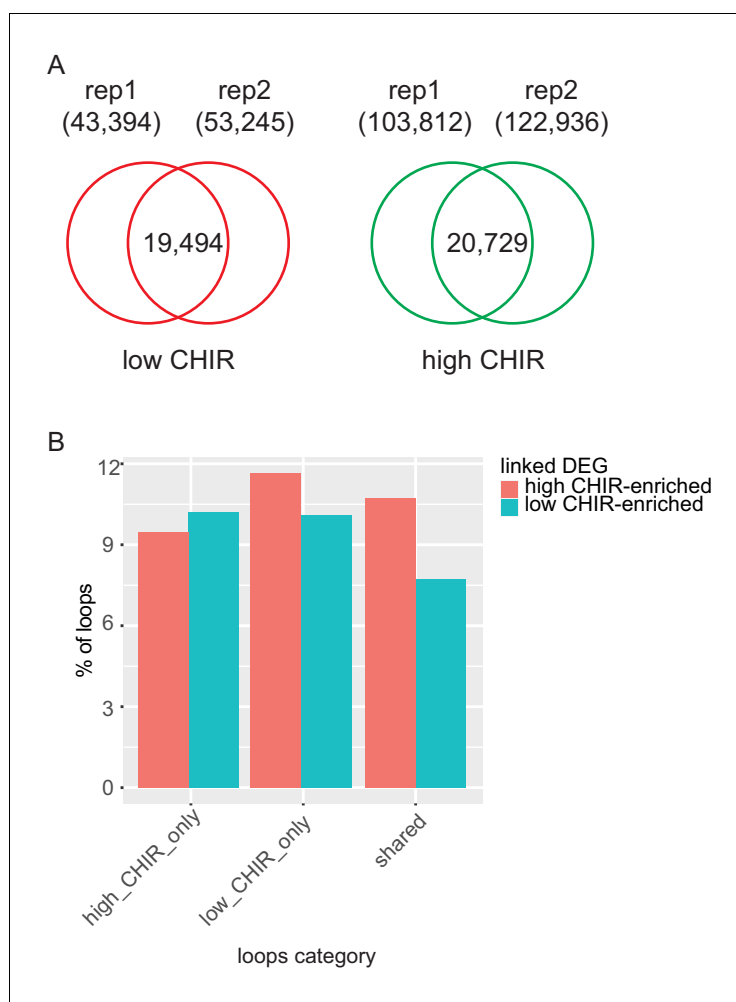


Figure 5—figure supplement 1. Supplementary Hi-C data analysis. (A) Reproducible loops between replicates. (B) Bar plots showing the percentage of loops in the corresponding category (high CHIR99021 [CHIR] only, low CHIR only or share) that are linked to differentially expressed genes (DEG) that are high in low CHIR or high CHIR.