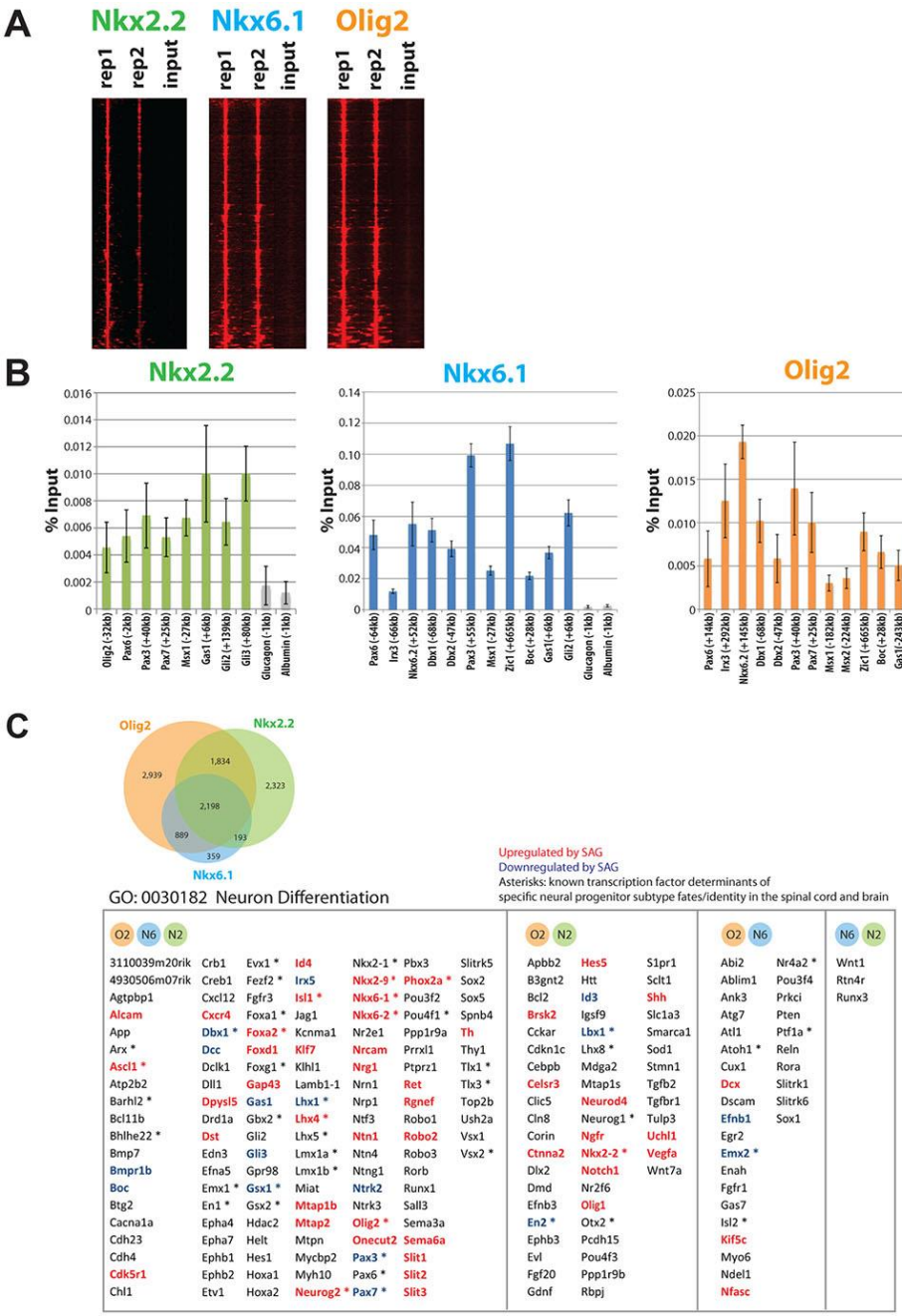
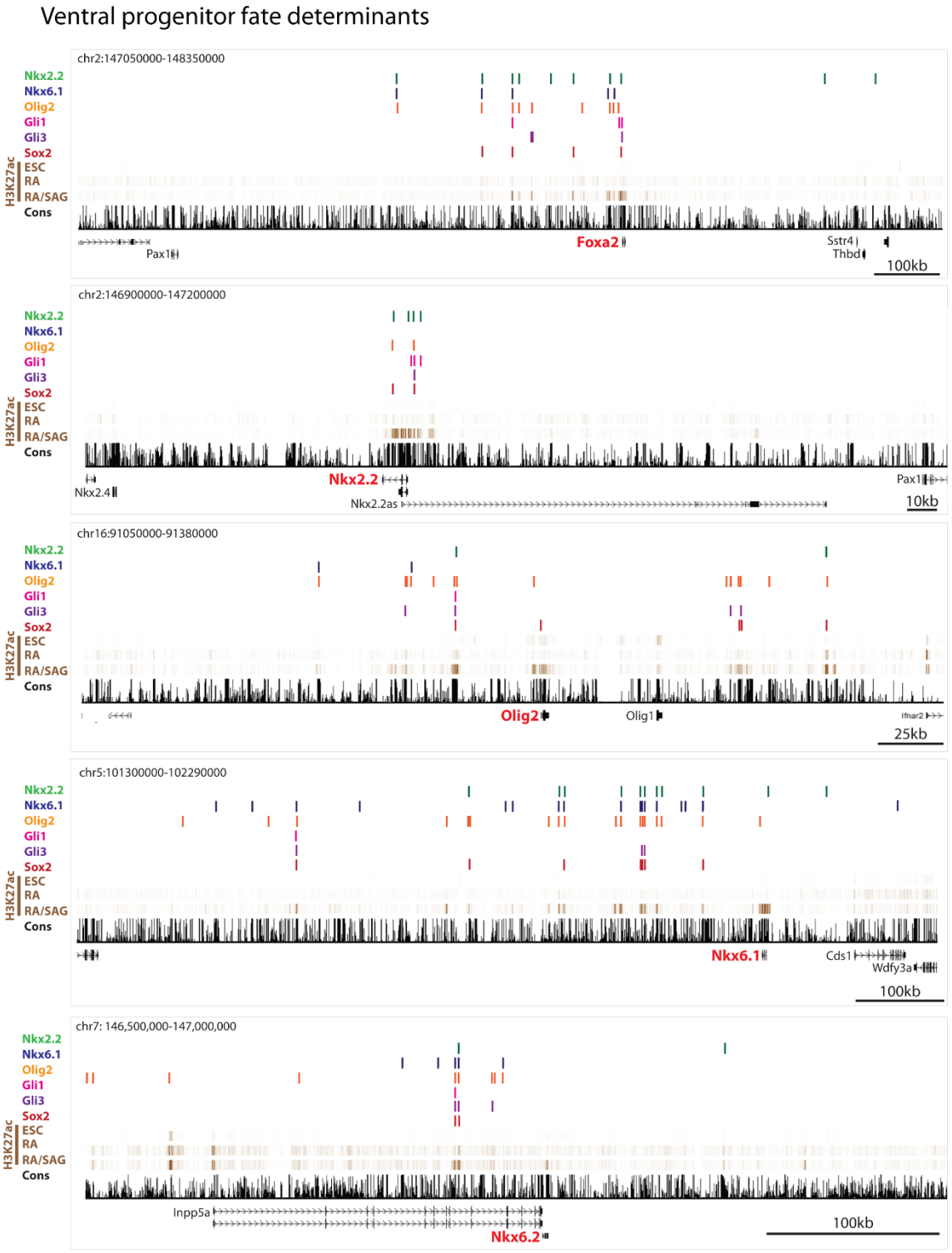


Figure S1. Characterization of Nkx2.2, Nkx6.1, and Olig2 binding and their target genes

(A) Heatmap analysis of two ChIP-seq biological replicates. (B) ChIP-qPCR analysis on E10.5 embryonic trunk preparation. Error bars are standard error based on 2 biological replicates. (C) Genes co-targeted by Nkx2.2, Nkx6.1, and Olig2 that fall into Neuron Differentiation GO term are shown. SAG: agonist for Shh pathway. Genes upregulated and downregulated by SAG are associated with ventral and dorsal progenitor identities, respectively.



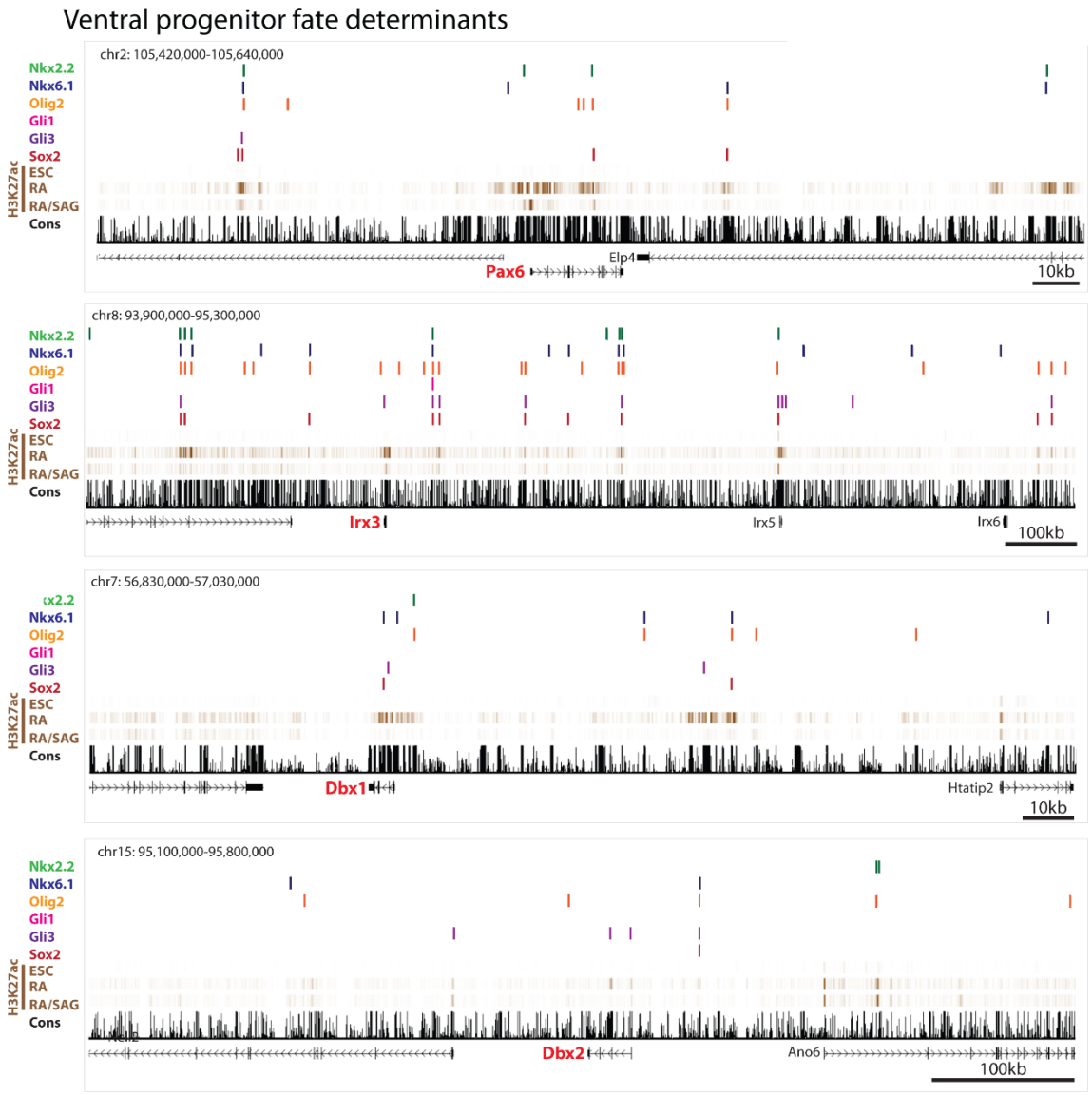


Figure S2. Transcription factor binding and H3K27ac status at ventral neural fate determinants

Indicated transcription factor binding is shown as ticks and H3K27ac signal and phastcon conservation score as heatmap at ventral neural fate determinant loci. RA: neural progenitor culture treated with RA for 72hrs. RA/SAG: neural progenitor culture treated with RA and SAG for 72hrs.

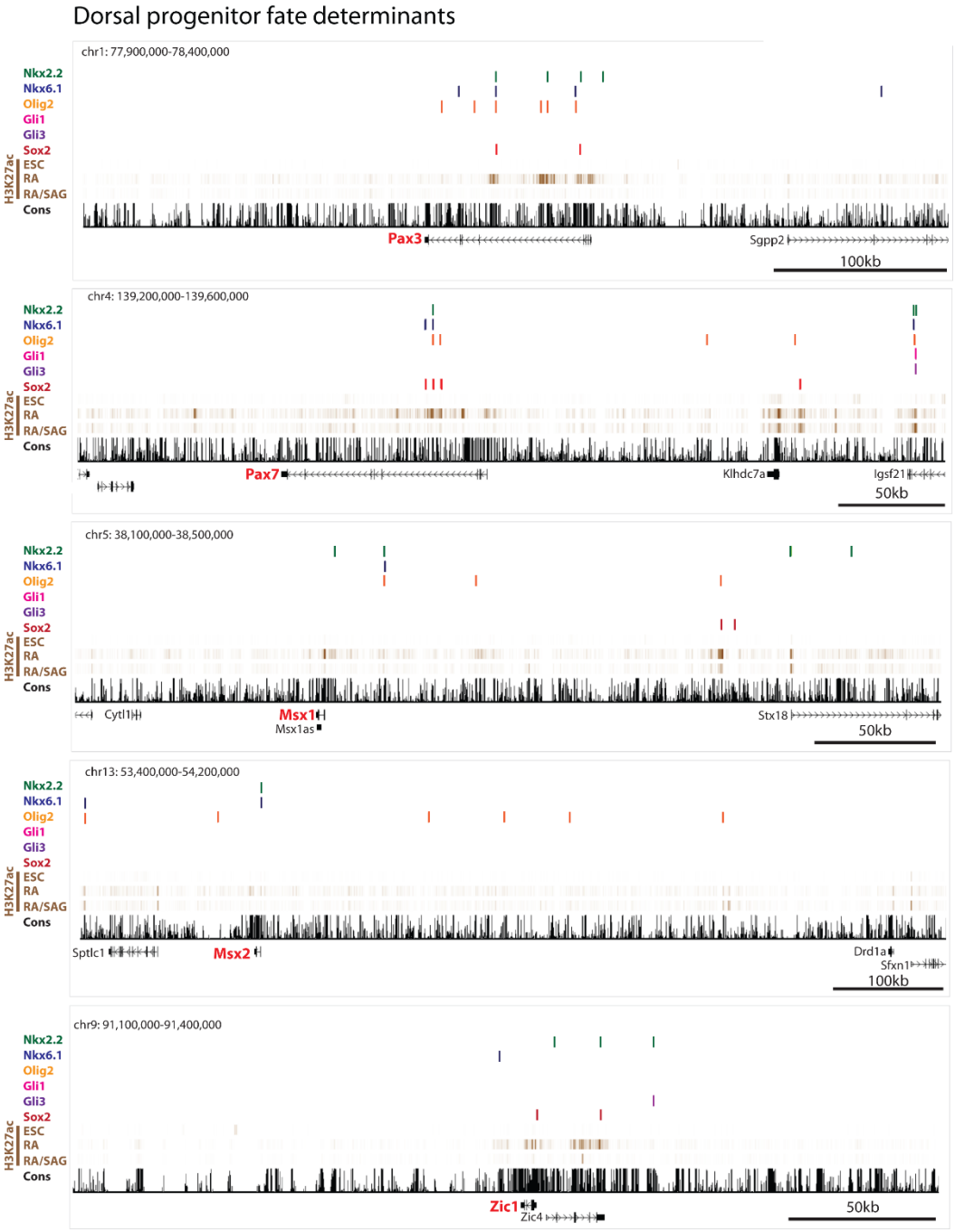
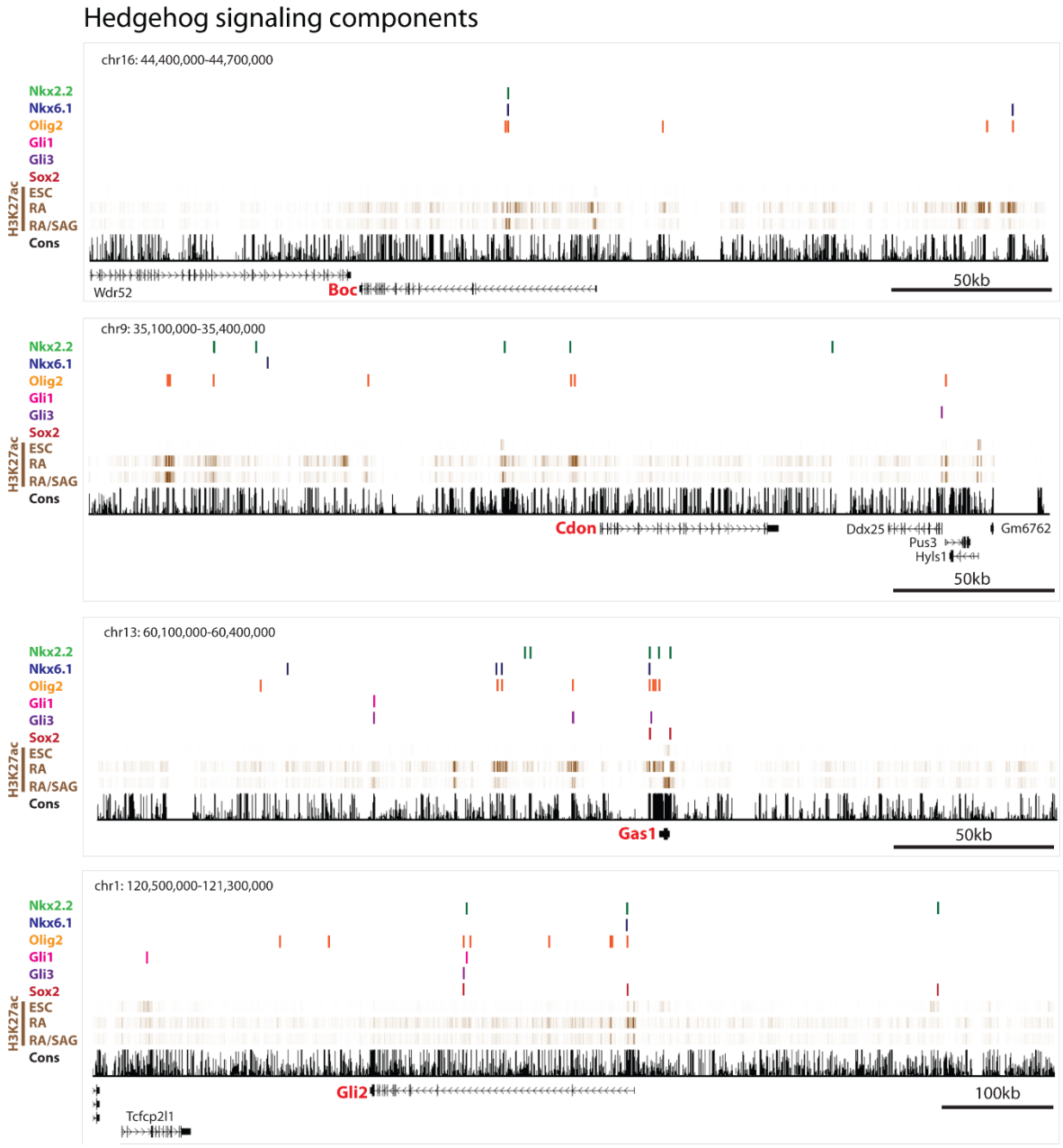


Figure S3. Transcription factor binding and H3K27ac status at dorsal neural fate determinants

Indicated transcription factor binding is shown as ticks and H3K27ac signal and phastcon conservation score as heatmap at dorsal neural fate determinant loci. RA: neural progenitor culture treated with RA for 72hrs. RA/SAG: neural progenitor culture treated with RA and SAG for 72hrs.



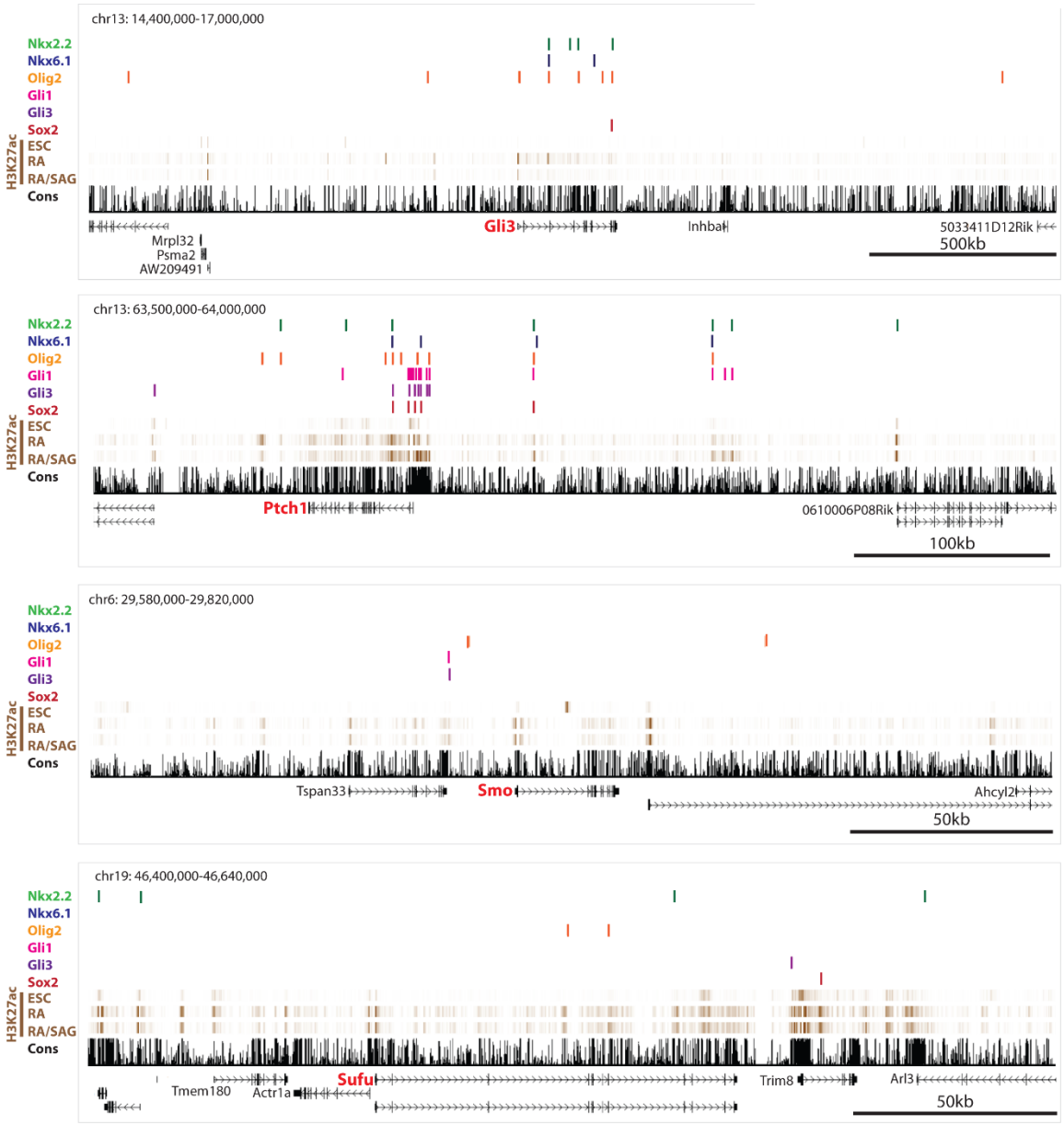


Figure S4. Transcription factor binding and H3K27ac status at Hedgehog pathway components

Indicated transcription factor binding is shown as ticks and H3K27ac signal and phastcon conservation score as heatmap at Hedgehog pathway component loci. RA: neural progenitor culture treated with RA for 72hrs. RA/SAG: neural progenitor culture treated with RA and SAG for 72hrs.

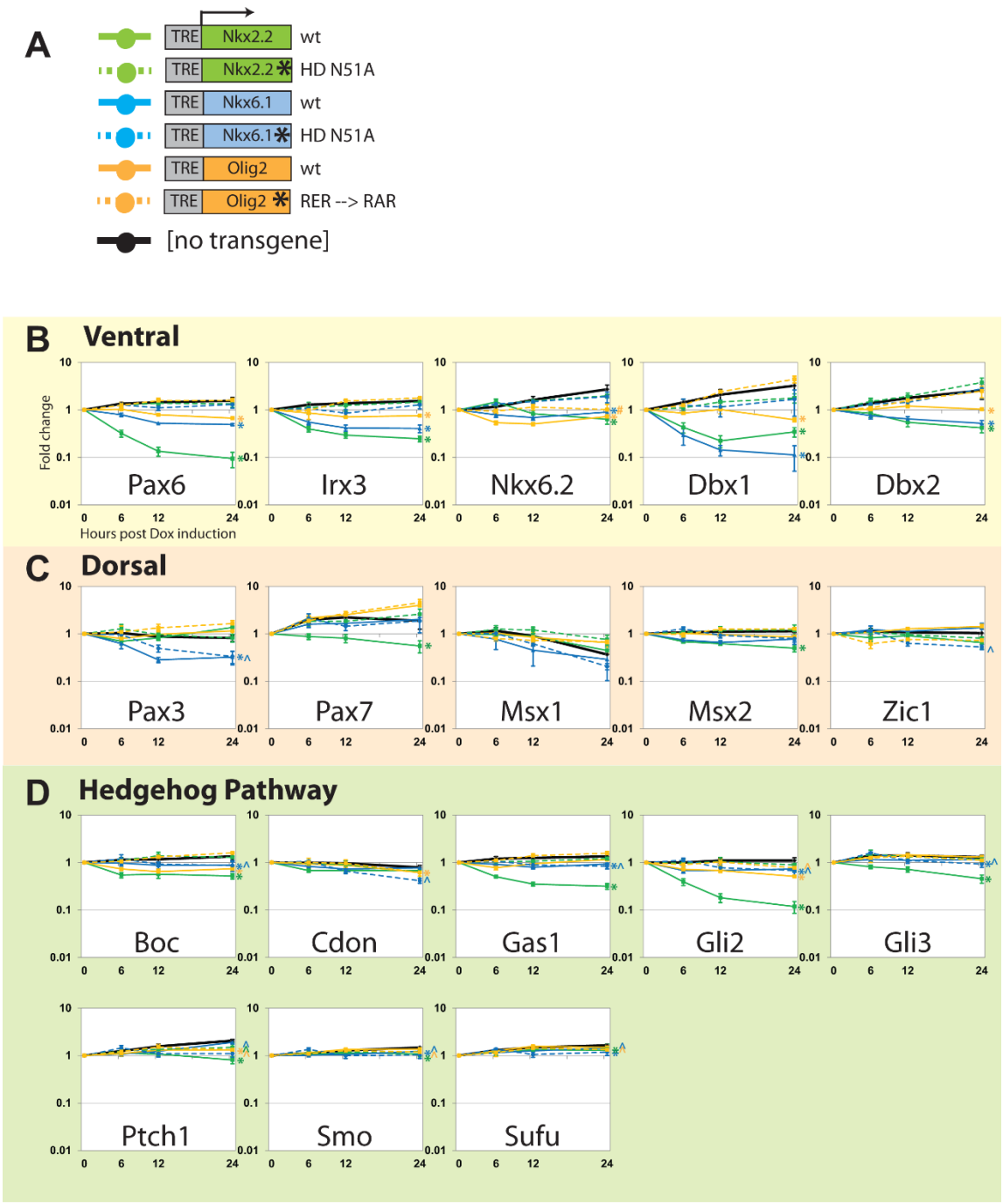


Figure S5. Overexpression assays with DNA binding deficient Nkx2.2, Nkx6.1, Olig2 mutants

(A) A schematic describing transgene structures. (B-D) Expression change plot. See panel A for color and stroke designations. X-axis: hours post Dox induction, y-axis: fold change from Dox induction (t=0). Error bars: standard error based on 3 biological replicates.

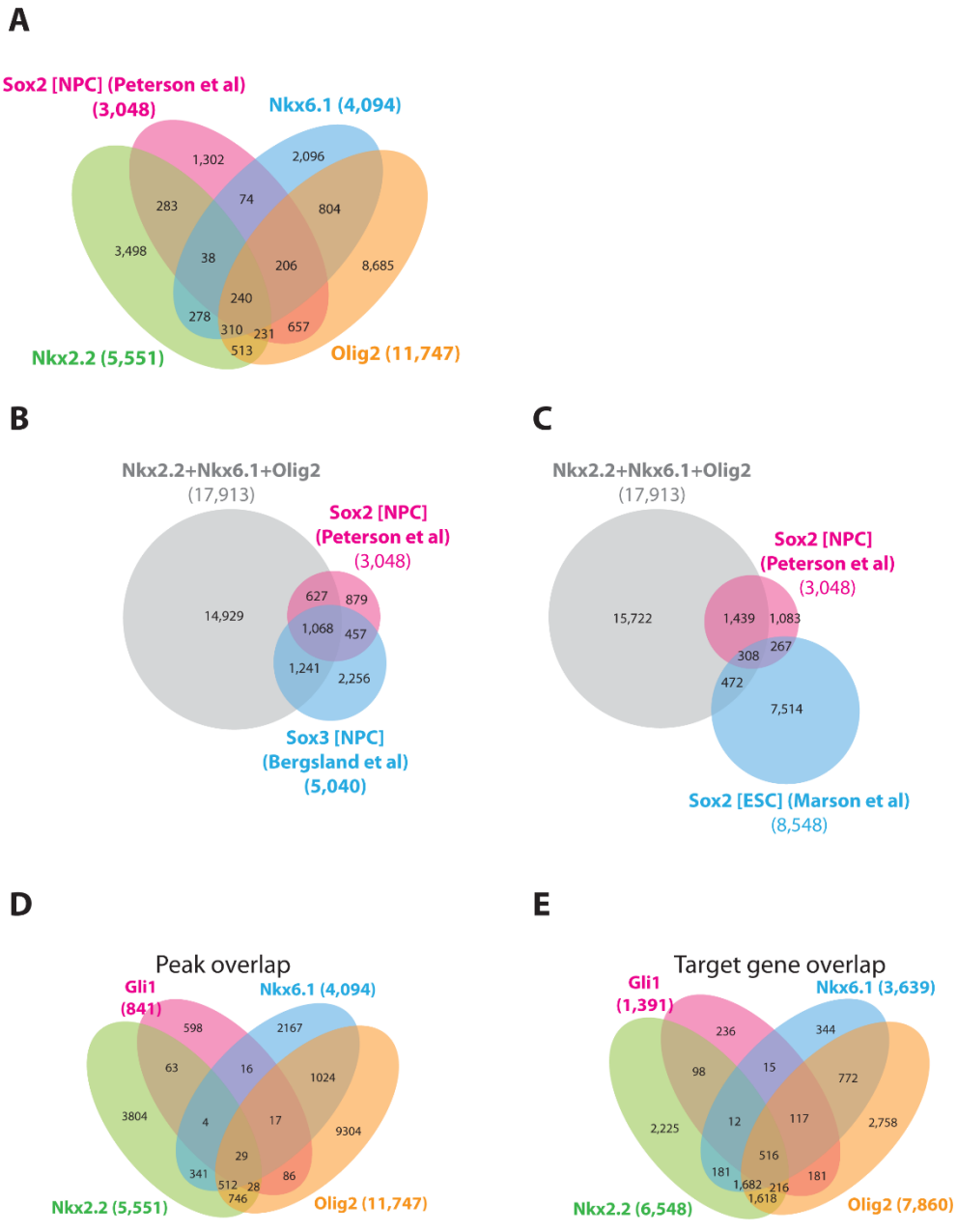


Figure S6. Sox2 and Gli1 binding region intersectional analysis

(A) 4-way intersectional analysis between Nkx2.2, Nkx6.1, Olig2, and Sox2. (B) Venn diagram intersection between the union of Nkx2.2, Nkx6.1, and Olig2 binding regions, Sox2, and Sox3. (C) Venn diagram intersection between the union of Nkx2.2, Nkx6.1, and Olig2 binding regions, Sox2 binding regions in neural progenitors, and Sox2 binding regions in ESCs. (D, E) Venn diagram for binding region overlap (D) and target gene overlap (E) between Nkx2.2, Nkx6.1, Olig2, and Gli1.

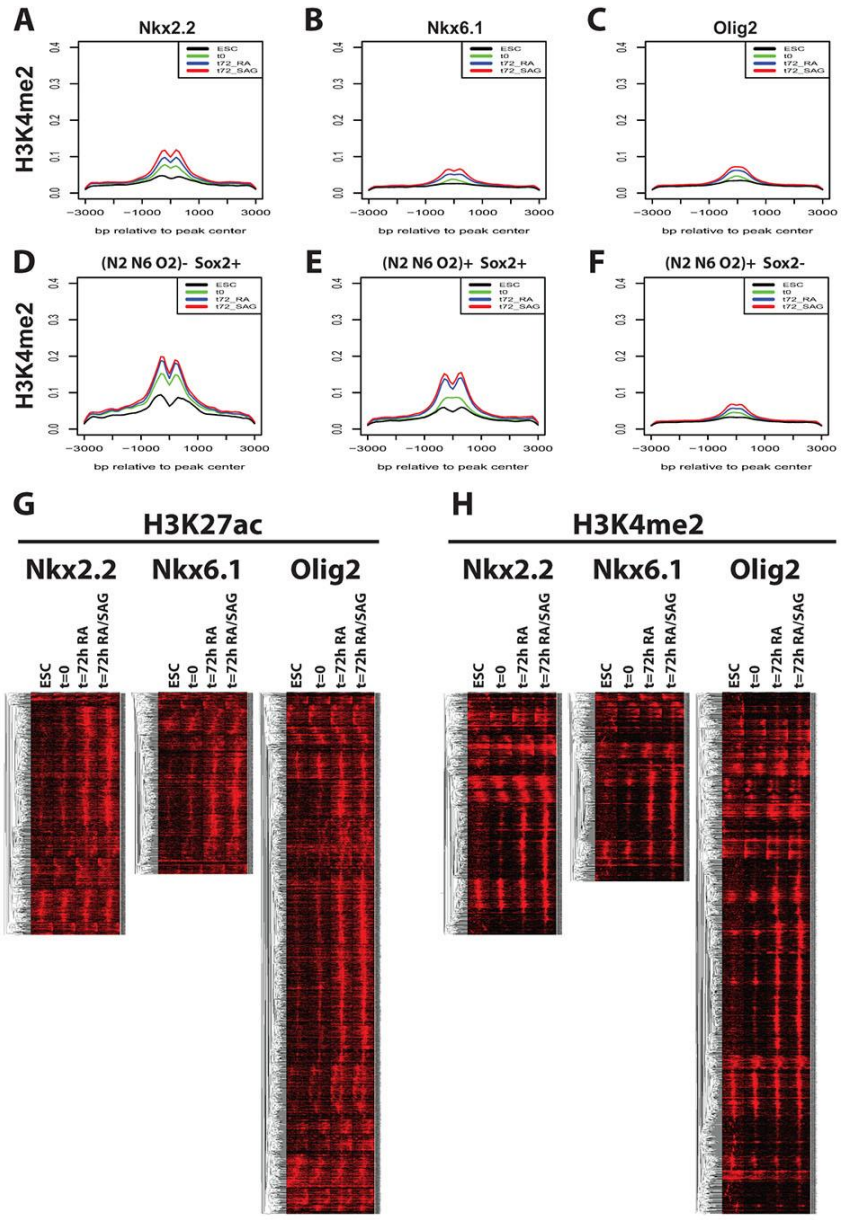


Figure S7. Analysis of active chromatin signatures at Nkx2.2, Nkx6.1, Olig2 and Sox2 binding regions

(A-F) Aggregate plot for H3K4me2 at Nkx2.2, Nkx6.1, Olig2, and Sox2 binding regions. (A-C) Individual binding regions. (D) Sox2 binding regions that do not overlap with Nkx2.2, Nkx6.1, or Olig2. (E) Sox2 binding regions that overlap with Nkx2.2, Nkx6.1, or Olig2. (F) Nkx2.2, Nkx6.1, or Olig2 binding region that do not overlap with Sox2 binding. (G, H) Heatmap clustering analysis of H3K4me2 and H3K27ac modifications at Nkx2.2, Nkx6.1, and Olig2 binding regions.

Table S1. Nkx2.2, Nkx6.1, and Olig2 binding regions

(Tab1-3) Primary peak call and annotation. Column A: peak rank according to peak score. Column B-D: binding peak coordinates. Column E-U: genes associated with the peak. Nearest gene: the gene whose transcriptional start site is the closest to the peak. Nearest left gene: the closest gene located left to the peak, Nearest right gene: the closest gene located right to the peak. 5x Nearest left/right genes: five closest genes to the left and to the right from the peak. Gene symbol: official gene name. refseq: Refseq ID. Relative2peak: relative location of the peak to the gene. TSS_upstream: the binding peak is upstream of the transcriptional start site of the gene. TES_downstream: the binding peak is downstream of the transcriptional end site of the gene. Dist2TSS: relative distance of the peak to the transcriptional start site of the gene in bp. (Tab4) Integrated peak list and annotation for Nkx2.2, Nkx6.1, Olig2, Sox2, and Gli1. See above for designations for column B-L. Column M-Q: binding peak count for indicated transcription factors. Column R-AK: transcription factor binding motif count in the binding region. See Fig. 3A-C for motif details. Column AL-AP: mouse pronuclear injection enhancer assay test results from http://enhancer.lbl.gov/.

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Table S2. Repression assay RNAseq data

(Tab1) rpkm values for all samples and all analyzed genes. (Tab2) Expression value ratio between indicated overexpression line and the parental line for genes repressed by 2-fold or greater in at least one overexpression group. This data was used for hierarchical clustering shown in Fig 3C. (Tab3) List of genes down-regulated in each transgene expression experiment. Down-regulation cutoff was 2-fold relative to parental cell line using replicate average. Genes associated with binding of respective factor(s) are indicated in red. (Tab4-9) DAVID GO term analysis on down-regulated genes. (Tab4) Genes down-regulated at least in one cell line. (Tab5-9) Genes down-regulated in each cell line from Tab3.

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Table S3. Primers used in this study

(Tab1) Primer sequences for the RT-qPCR assays in this study. Column A: target gene. Column B,C: left primer. Column D,E: right primer. (Tab2) Primers used in the embryonic ChIP-qPCR assay. Column A: target gene. Column B: distance from the assay region to the TSS of the target gene. Column C: transcription factor bound to the assay region. Column D, E: left primer. Column F, G: right primer.

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