Expanded View Figures

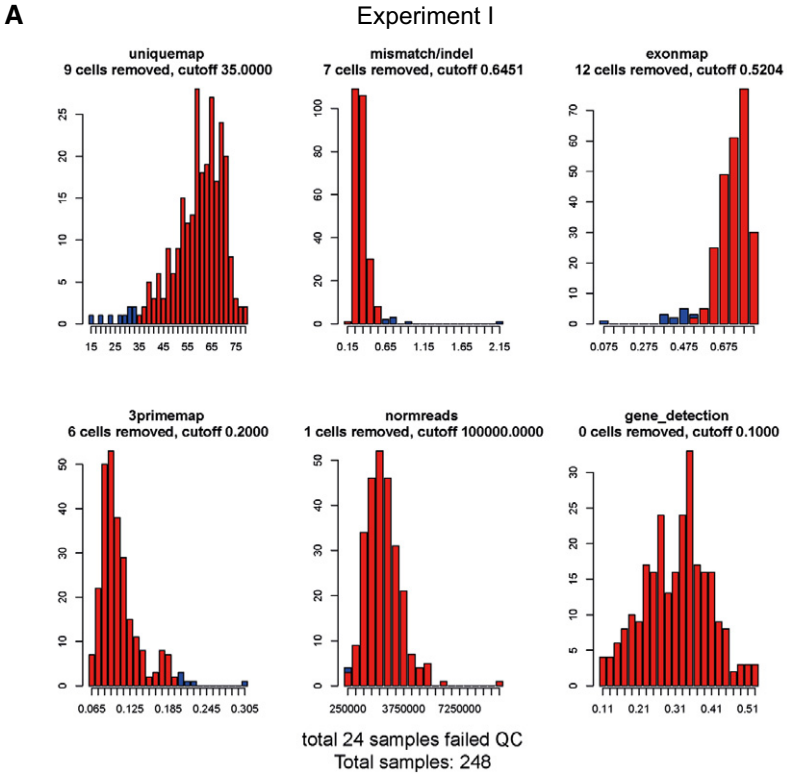

Figure EV1. Quality control (qc) for single-cell RNA-seq.

A, B Histograms showing percent uniquely mapping reads, fraction mismatches, fraction exon-mapping reads, fraction of reads mapping to a region at the 10% most 3 prime end of each transcript, fraction of reads mapping to mRNA (for normalization), and fraction of gene detection (> 1 RPKM) from two independent experiments (in A and B). Blue bars represent samples that did not pass the filtering criteria, and red bars represent wells passing the criteria. Filtering cutoffs are indicated above each histogram (n = 392).

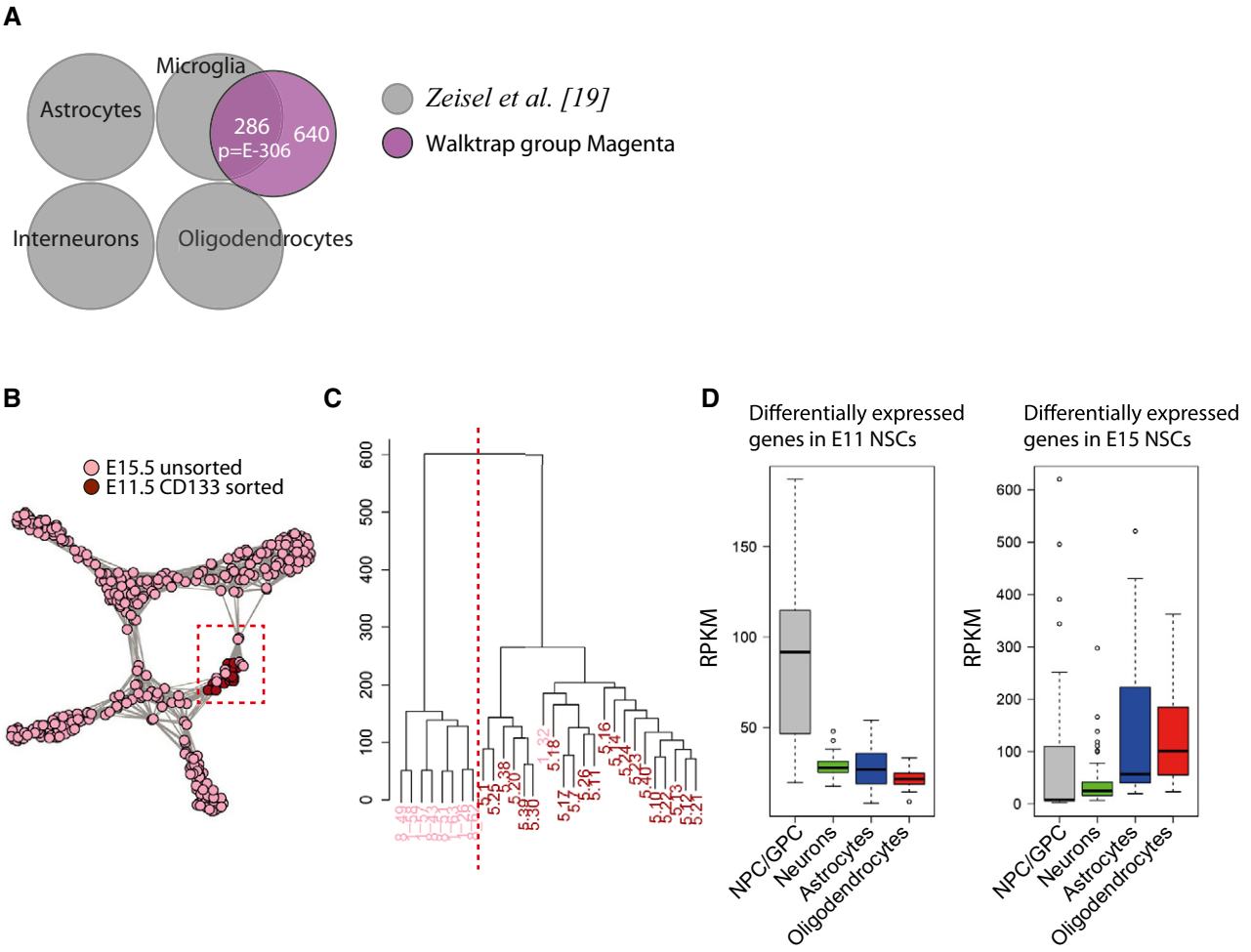

Figure EV2. Cells from E11.5 and E15.5 cluster together.

A Differentially expressed genes from Walktrap cell cluster colored by magenta significantly overlap with genes expressed in microglial cells from adult mouse brain [19]. *P*-values (phyper, R) are calculated from the total number of genes for mm10 (23,389).

B *t*-SNE graph of cells from scRNA-seq that were colored according to their developmental stage. Cells from E11.5 and E15.5 spinal cords were similar enough to cluster together (a Walktrap cell cluster defined as NPC/GPC, marked by red box).

C Histogram graph of cells from the NPC/GPC Walktrap cell cluster. Cells were divided into two major clusters through hclust software (R), which were highly overlapping to the developmental stages of the cells (pink E11.5; red E15.5).

D Genes that were significantly higher expressed in E11.5 progenitor cells compared to E15.5 progenitor cells were genes expressed in NPC/GPC. Genes that had increased expression in E15.5 cells were instead genes expressed in the NPC/GPC, astrocytes, and oligodendrocytes. In the boxplots, all genes expressed at RPKM > 1 are shown and include therefore genes expressed in more than one cell type. Boxplot whiskers show 1.5 IQR of highest and lowest quartile, outliers are included (dots). n = 194 for NPC/GPC, 1309 for neurons, 434 for astrocytes, and 301 for oligodendrocytes.

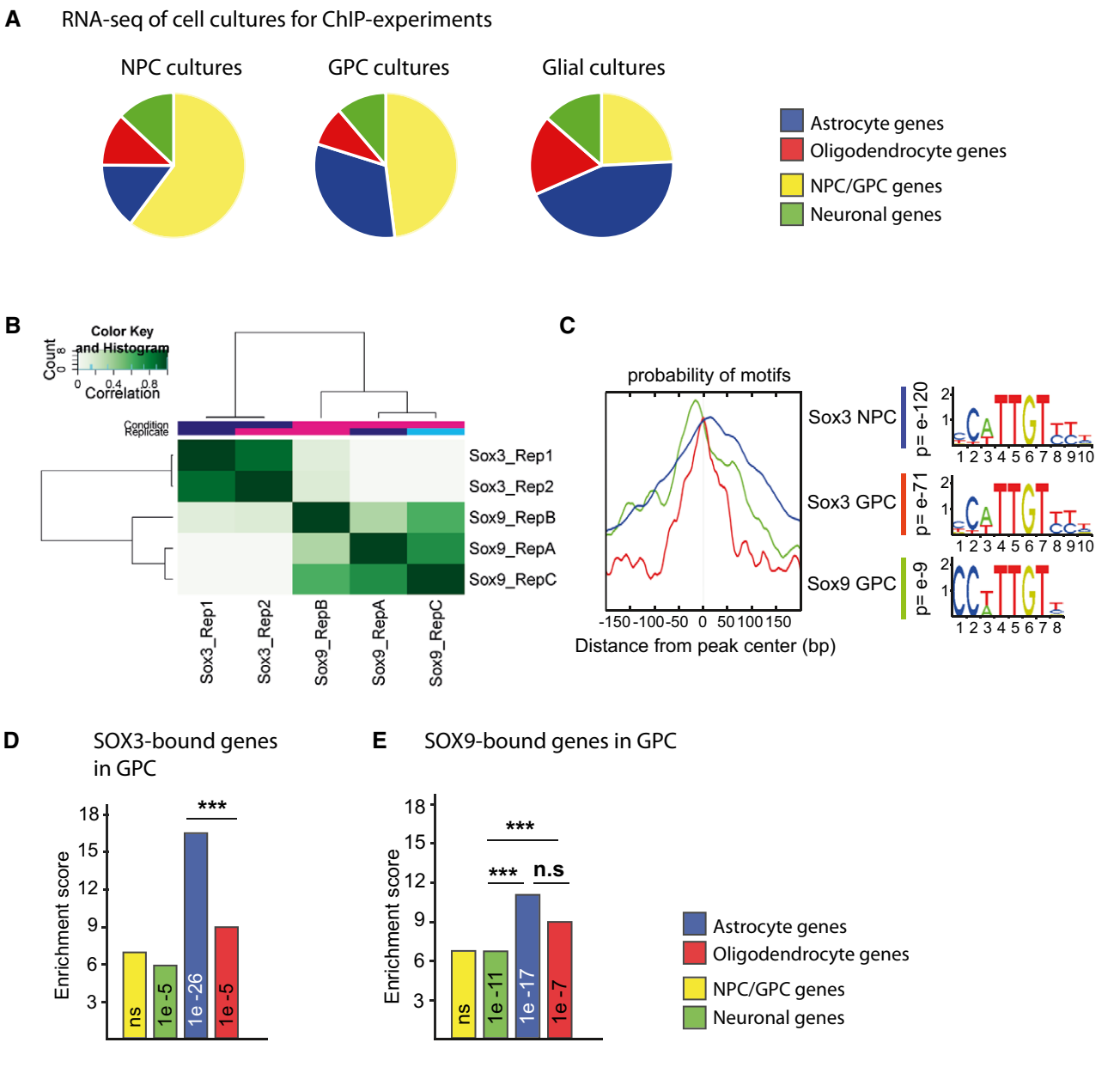

Figure EV3. Supportive data for SOX ChIP-seq experiments.

A Gene expression of RNA-seq of NPC and GPC cultures used for ChIP-seq experiments and their correlation to differentially expressed genes in NPC/GPC, neuronal, astrocytic, and oligodendrocyte cell clusters from scRNA-seq. NPC and GPC cultures correlate mostly to the NPC/GPC-specific genes from scRNA-seq. Also, correlation of gene expression from the GPC-derived glial culture is shown. In the graphs, all genes expressed at RPKM > 1 are shown and include therefore genes expressed in more than one cell type.

B Histogram showing the correlation between ChIP experiments for SOX3 (two replicates) and SOX9 (three replicates) in GPCs.

C Sox DNA motifs were centrally enriched in consensus peaks (from replicates shown in A) for SOX3 ChIPs in GPCs and SOX9 in GPCs. Peaks from SOX3 ChIP experiments in NPCs also contain centrally enriched Sox motifs [7].

D, E (D) Correlation between SOX3-bound genes in GPCs and genes specifically expressed in NPC/GPC, neurons, astrocytes, and oligodendrocytes. (E) Correlation between SOX9-bound genes in GPCs and genes specifically expressed in NPC/GPC, neurons, astrocytes, and oligodendrocytes. *P*-values (phyper, R) are calculated using the total number of protein-coding genes in mm10 assembly (23,389). Significance calculated by prop.test R, ***$P > 0.001$, based on the size of the gene groups (NPC/GPC = 194, neurons = 1309, astrocytes = 434, oligodendrocytes = 301).

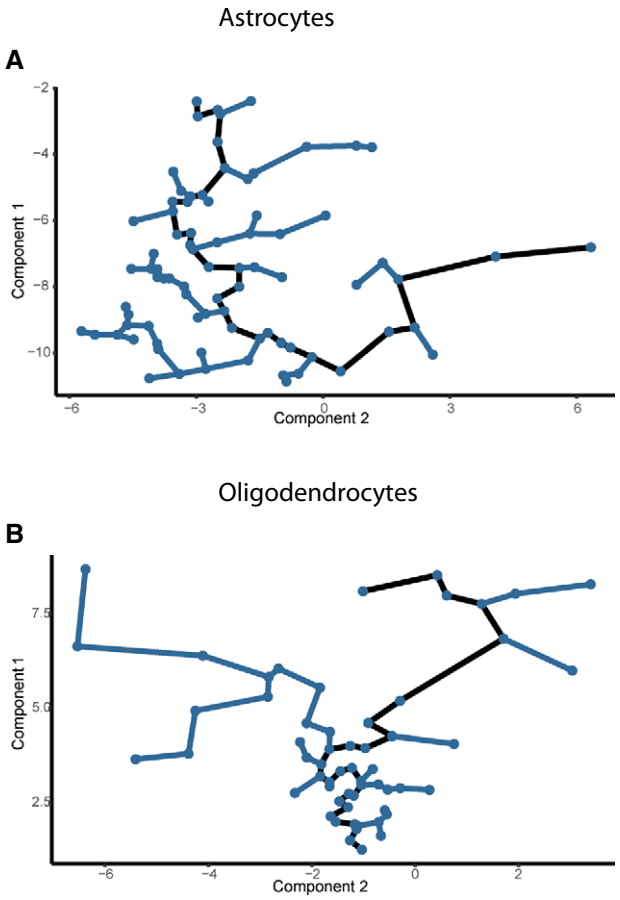

Figure EV4. Astrocytes and Oligodendrocytes arranged in a pseudotemporal order.

A, B Cell expression profiles (points) for astrocytes (A) and oligodendrocytes (B) in a two-dimensional independent component space. Black lines indicate the main backbones of Monocle's pseudotime ordering of the cells.

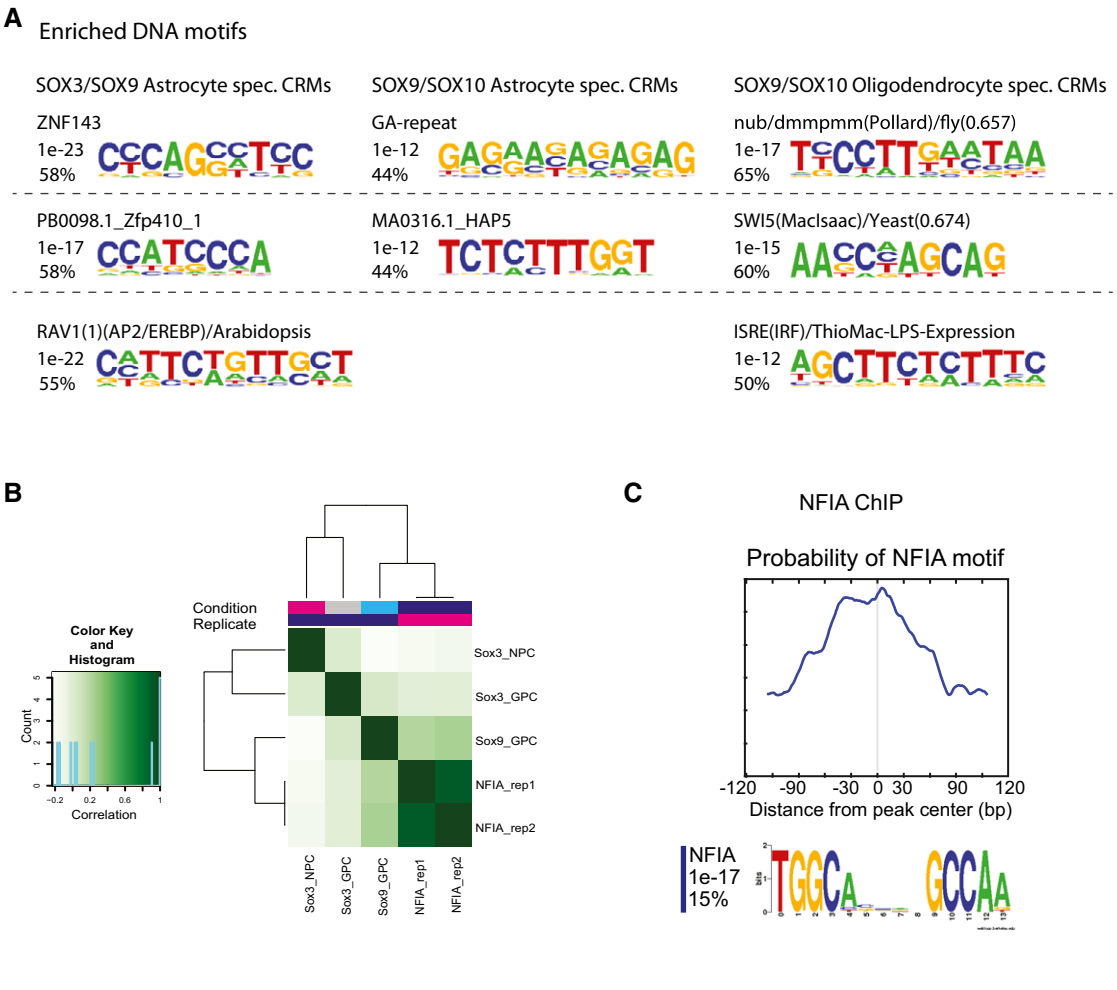

Figure EV5. NFIA ChIP-seq in GPCs.

A DNA motifs enriched in three groups of CRMs: SOX3/SOX9-bound CRMs associated with astrocyte genes, SOX9/SOX10-bound CRMs associated with astrocyte genes, and SOX9/SOX10 CRMs associated with oligodendrocyte genes. Figure shows the two to four most frequent motifs enriched for each group over the other two groups (the most frequent motifs from each group are shown in Fig 4E). Analysis was performed with HOMER software with default settings except for background settings (see Materials and Methods section).

B Histogram showing the correlation between NFIA ChIP experiments (two replicates) and SOX3 ChIP in NPC and GPC and SOX9 ChIP in GPC.

C Centrally enriched NFIA motifs were found in the NFIA consensus peak set.

D NFIA ChIP peaks overlap with 28% of Sox9-ChIP peaks and with 8% only of the Sox3-ChIP peaks in GPCs.