SUPPLEMENTAL INFORMATION

Fig S1. Genes with reduced expression in the SCN after light stimulation. *Related to Figure 1.*

(A) Heatmap of the light suppressed genes.

(B-E) Representative light suppressed genes that were significantly changed after light stimulation at 30 min-, 1-, 3-, or 6- hours of

(F-H) Pathway analysis of light-reduced genes at different timepoints.

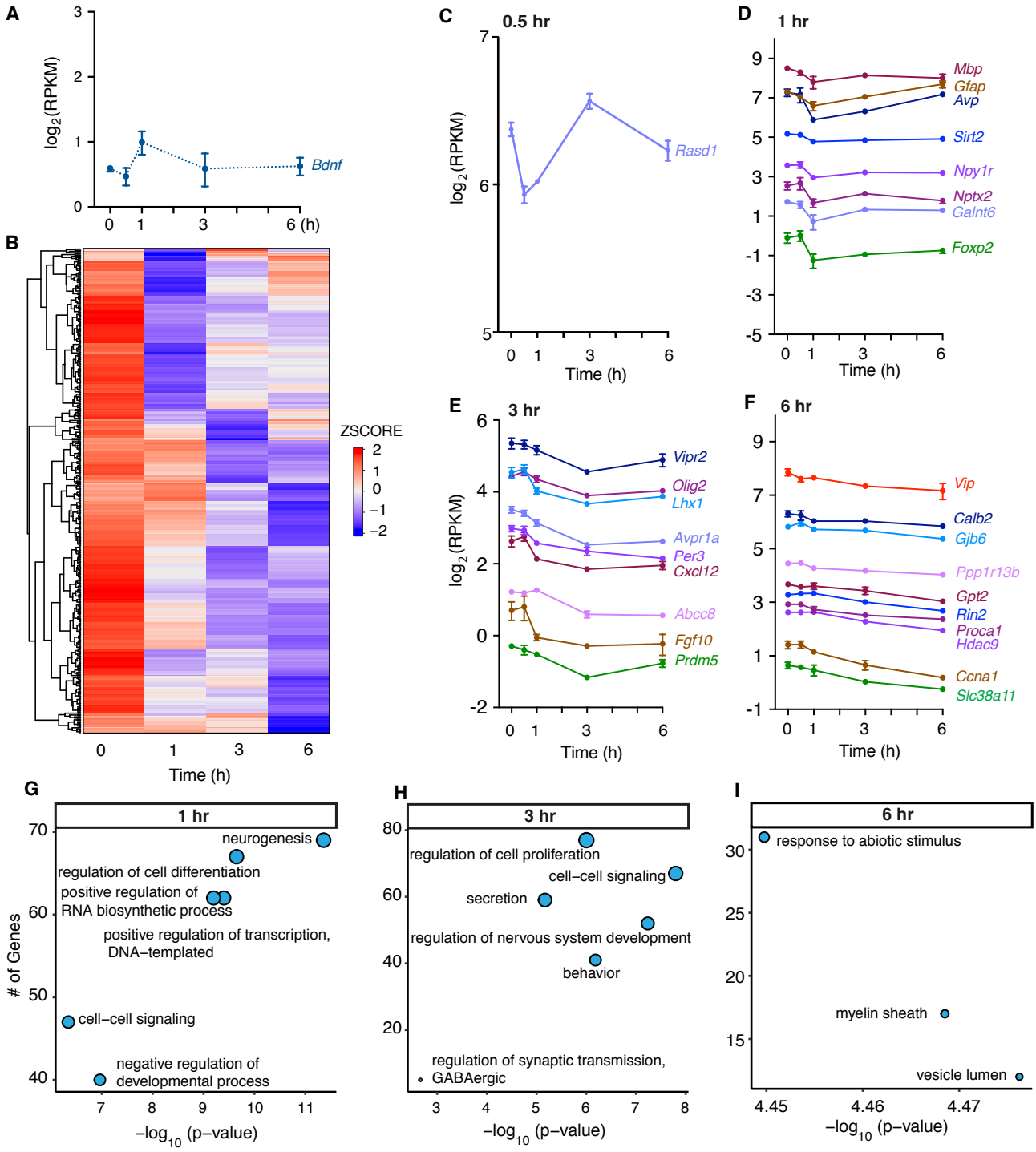

A
log2(RPKM)
Bdnf
0 1 3 6 (h)
B
ZSCORE
2
1
0
−1
−2
0 1 3 6
Time (h)
C
0.5 hr
log2(RPKM)
Rasd1
Time (h)
D
1 hr
Mbp
Gfap
Avp
Sirt2
Npy1r
Nptx2
Galnt6
Foxp2
Time (h)
E
3 hr
log2(RPKM)
Vipr2
Olig2
Lhx1
Avpr1a
Per3
Cxcl12
Abcc8
Fgf10
Prdm5
Time (h)
F
6 hr
Vip
Calb2
Gjb6
Ppp1r13b
Gpt2
Rin2
Proca1
Hdac9
Ccna1
Slc38a11
Time (h)
G
1 hr
# of Genes
neurogenesis
regulation of cell differentiation
positive regulation of RNA biosynthetic process
positive regulation of transcription, DNA−templated
cell−cell signaling
negative regulation of developmental process
−log10 (p−value)
H
3 hr
regulation of cell proliferation
cell−cell signaling
secretion
regulation of nervous system development
behavior
regulation of synaptic transmission, GABAergic
−log10 (p−value)
I
6 hr
response to abiotic stimulus
myelin sheath
vesicle lumen
−log10 (p−value)

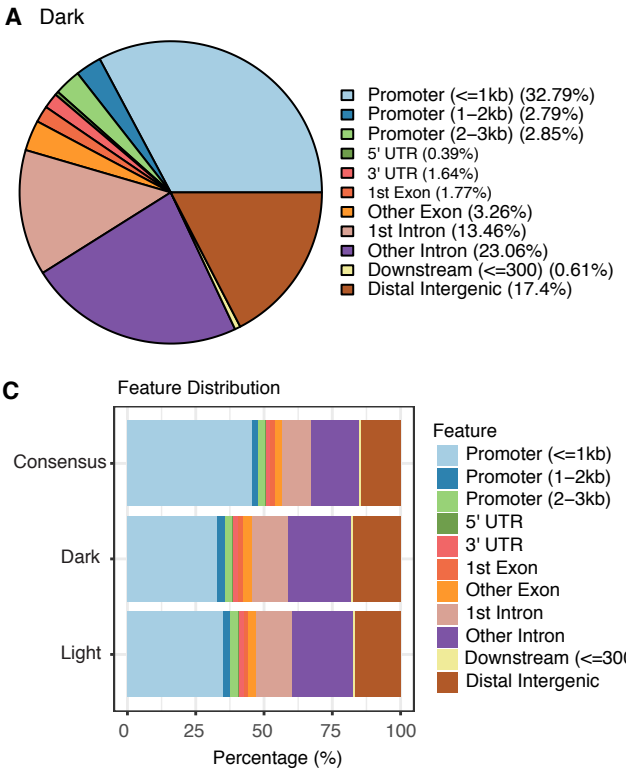
Fig S2. Genomic annotation of binding sites for H3K27ac. *Related to Figure 2.* Binding peaks were annotated in Chipseeker, promoter (≤ 1kb), promoter (1-2kb), promoter (2-3kb), 5'UTR, 3'UTR, 1st Exon, other Exon, 1st Intron, other Intron, downstream (≤300) and distal intergenic.

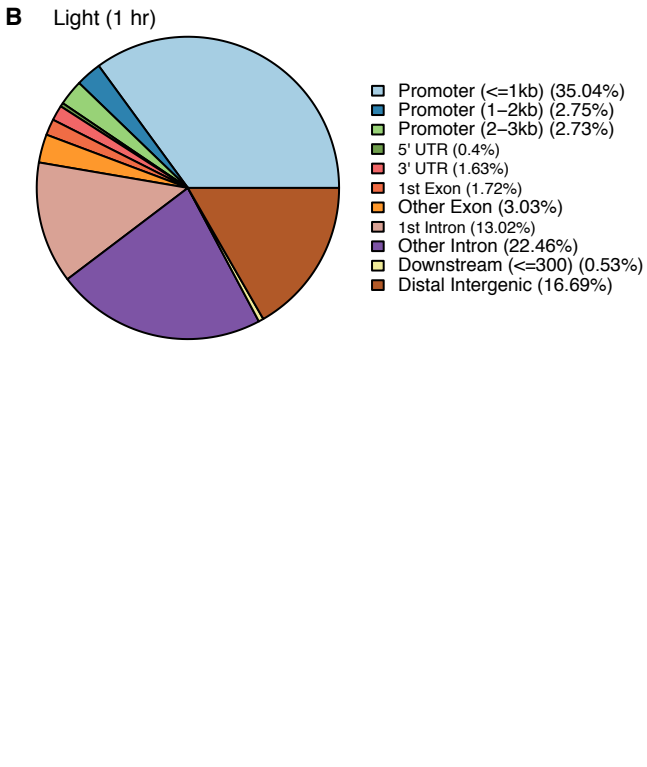
(A) Genomic annotation of the binding sites for H3K27ac from CT17 dark SCN.

(B) Genomic annotation of the binding sites for H3K27ac from SCN after 1-hour light exposure.

(C) Comparison of H3K27ac sites distribution between dark and light exposed SCN.

(D-E) UCSC genome browser views of *Nr4a1* and *Per2*. NPAS4 ChIP-seq (Kim et al., 2010) is shown in dark blue. H3K4me3, H3K27ac ChIP-seq, and light pulse bulk RNA-seq are shown as indicated. The bed file of the promoter (-1kb - +100 bp) is shown in green while the bed file of H3K27ac sites is shown in red. The H3K27ac sites for *Fos* and *Nr4a1* are shaded blue. The promoter region is highlighted in shaded grey.


A
Dark
Promoter (<=1kb) (32.79%)
Promoter (1–2kb) (2.79%)
Promoter (2–3kb) (2.85%)
5' UTR (0.39%)
3' UTR (1.64%)
1st Exon (1.77%)
Other Exon (3.26%)
1st Intron (13.46%)
Other Intron (23.06%)
Downstream (<=300) (0.61%)
Distal Intergenic (17.4%)


B
Light (1 hr)
Promoter (<=1kb) (35.04%)
Promoter (1–2kb) (2.75%)
Promoter (2–3kb) (2.73%)
5' UTR (0.4%)
3' UTR (1.63%)
1st Exon (1.72%)
Other Exon (3.03%)
1st Intron (13.02%)
Other Intron (22.46%)
Downstream (<=300) (0.53%)
Distal Intergenic (16.69%)

C
Feature Distribution
Consensus
Dark
Light
0
25
50
75
100
Percentage (%)
Feature
Promoter (<=1kb)
Promoter (1–2kb)
Promoter (2–3kb)
5' UTR
3' UTR
1st Exon
Other Exon
1st Intron
Other Intron
Downstream (<=300)
Distal Intergenic

Fig S3. 10x sample sequencing metrics and quality control plots. *Related to Figure 3.*

X axis: a total of ten 10X Genomics single-nucleus libraries were involved in the study. Each library was generated from 7 pooled male SCN pairs.

(A) Number of UMIs detected in each library. Nuclei containing more than 10,000 UMI were filtered out. Dashed line: 10,000 UMI. Number of genes detected in each library. Cells containing more than 4,000 genes were filtered out. Dashed line: 4000 UMI.

(B) Quality control screening for mitochondria contamination and potential doublets. Left: Scatter plot showing the number of UMI (X-axis) vs Percentage of mitochondria (Y-axis). Right: Scatter plot showing the number of UMI (X-axis) vs Number of genes (Y-axis). Nuclei containing more than 10% mitochondrial transcripts and more than 10,000 UMI were filtered out.

(C) Distribution of UMIs and detected genes per cells in 45 clusters. An average of 1901 UMI counts and 1100 genes were obtained per cell across all clusters.

(D) Cell distribution for each cell type. Open bar, cells from light sample libraries; filled bar, cells from dark sample libraries.

(E) Total extracted reads per library, unique mapped reads per library, and assigned reads per library.

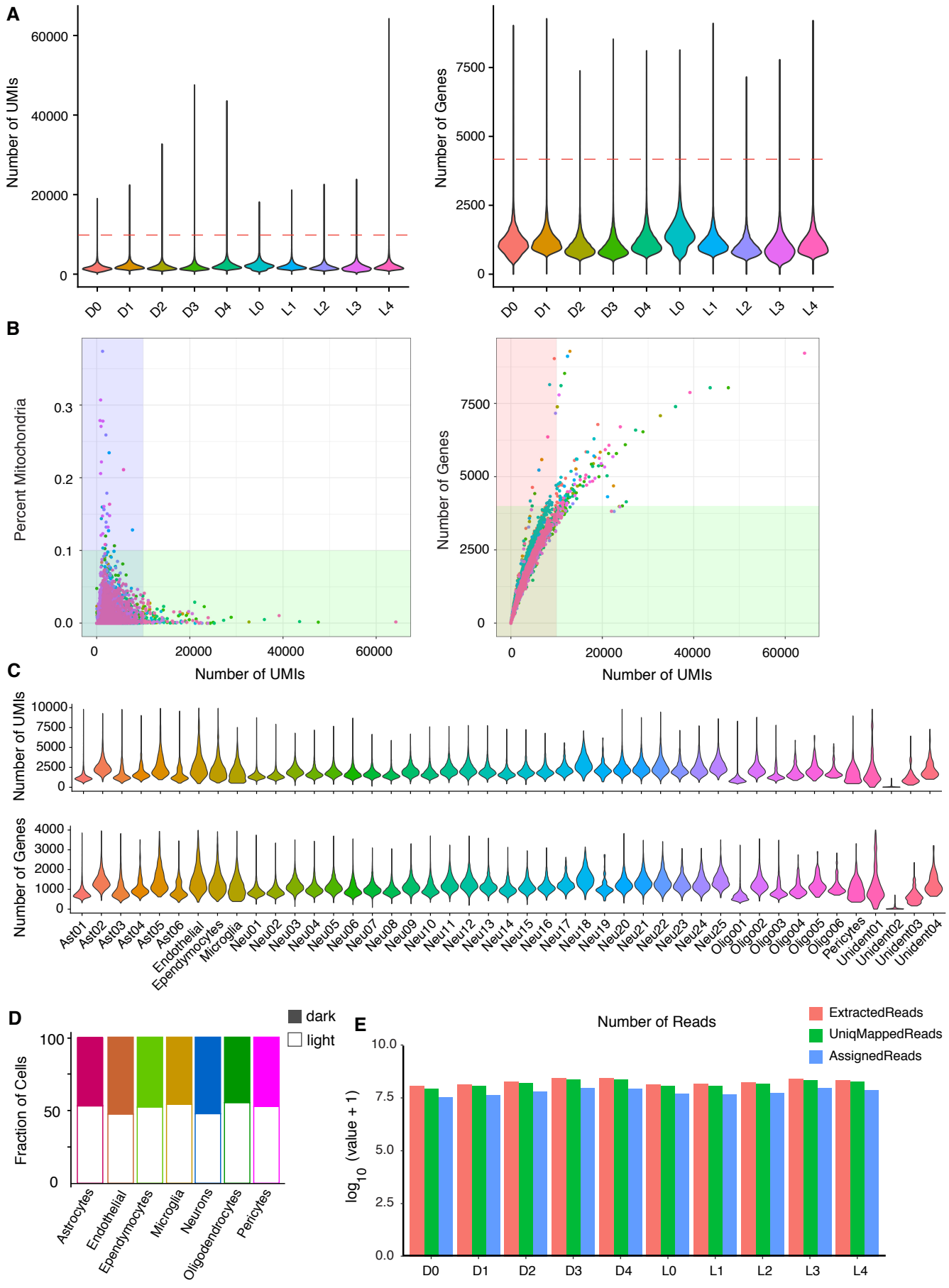
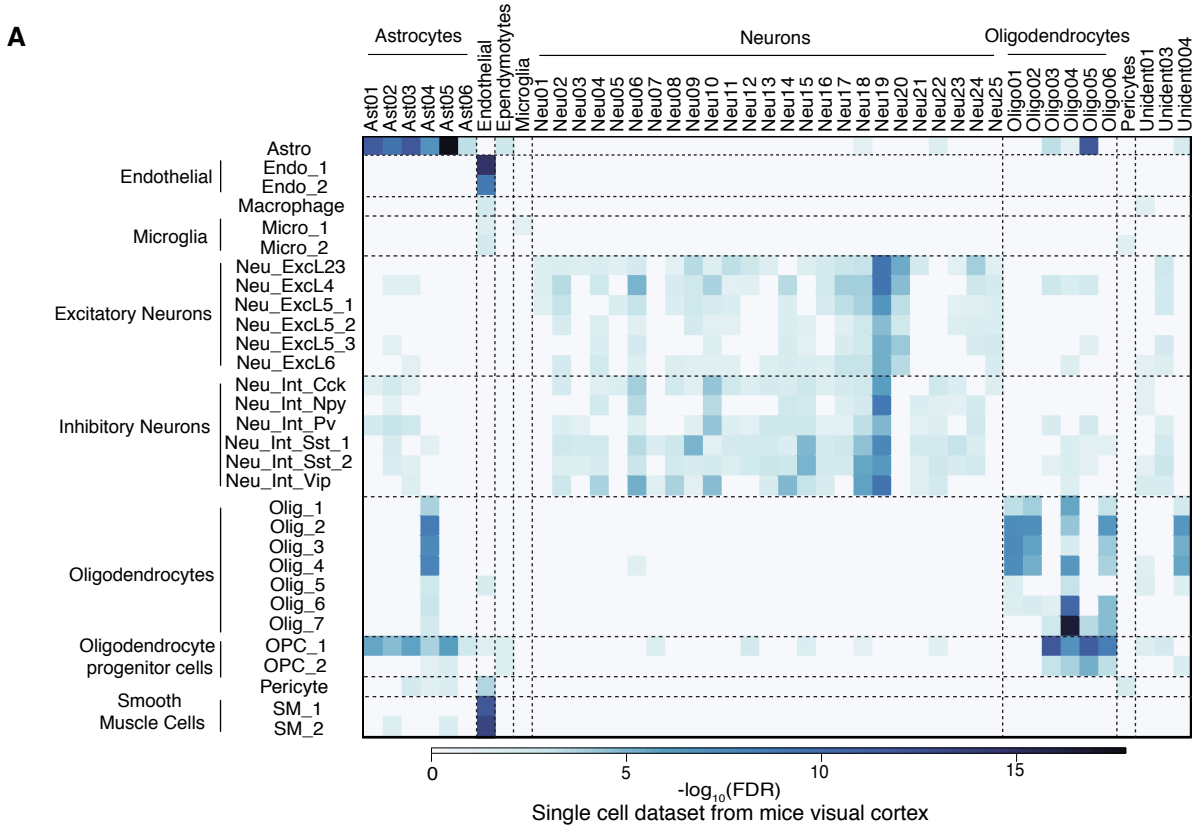

A
Number of UMIs
60000
40000
20000
0
D0 D1 D2 D3 D4 L0 L1 L2 L3 L4
Number of Genes
7500
5000
2500
0
B
Percent Mitochondria
0.3
0.2
0.1
0.0
0 20000 40000 60000
Number of UMIs
C
Number of UMIs
10000
7500
5000
2500
0
Number of Genes
4000
3000
2000
1000
0
Ast01 Ast02 Ast03 Ast04 Ast05 Ast06 Endothelial Ependymocytes Microglia Neu01 Neu02 Neu03 Neu04 Neu05 Neu06 Neu07 Neu08 Neu09 Neu10 Neu11 Neu12 Neu13 Neu14 Neu15 Neu16 Neu17 Neu18 Neu19 Neu20 Neu21 Neu22 Neu23 Neu24 Neu25 Olig001 Olig002 Olig003 Olig004 Olig005 Olig006 Pericytes Unident01 Unident02 Unident03 Unident04
D
Fraction of Cells
100
50
0
dark
light
Astrocytes Endothelial Ependymocytes Microglia Neurons Oligodendrocytes Pericytes
E
Number of Reads
ExtractedReads
UniqMappedReads
AssignedReads
log10 (value + 1)
10.0
7.5
5.0
2.5
0.0
D0 D1 D2 D3 D4 L0 L1 L2 L3 L4

Fig S4. Comparison with cell types described in analogous studies. *Related to Figure 4.*

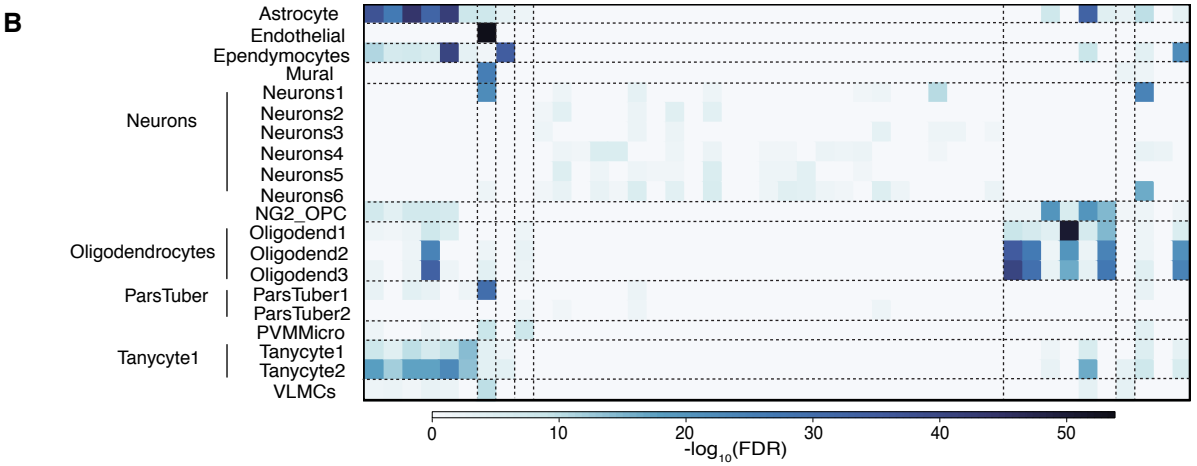
(A) Heatmap shows the correlation between cell types discovered in a dataset from mouse light-pulsed visual cortex (Hrvatin et al., 2018) and this study. Fisher's exact test and color reflects the adjusted *p* value.

(B) Heatmap shows the correlation between cell types discovered in a single cell dataset from mouse arcuate hypothalamus and median eminence (Campbell et al., 2017) and this study. Fisher's exact test and color reflects the adjusted *p* value.

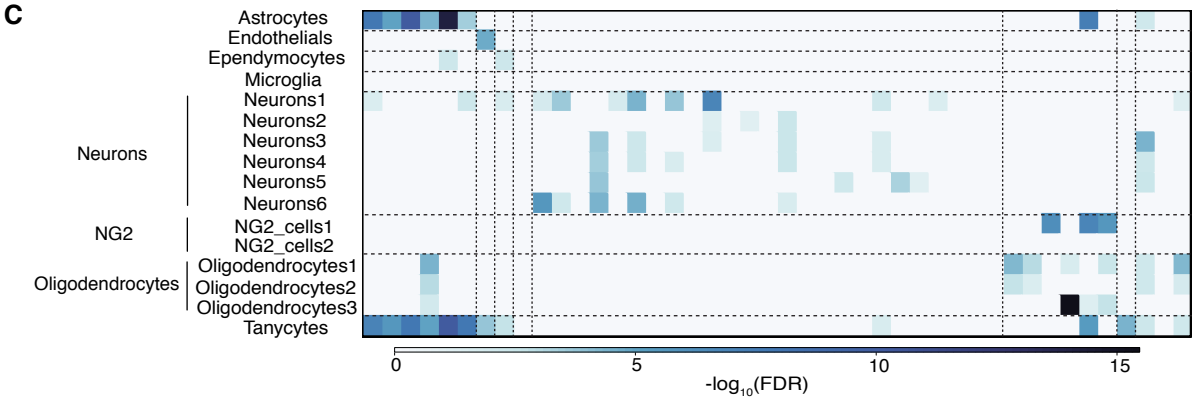
(C) Heatmap shows the correlation between cell types discovered in a dataset from mouse SCN (Wen et al., 2020) and this study. Fisher's exact test and color reflects the adjusted *p* value.



Single cell dataset from mice visual cortex
(Hrvatin *et. al.*, 2017)



Single cell dataset from mice hypothalamic arcuate-median eminence complex
(Campbell *et. al.*, 2017)



Single cell dataset from mice SCN
(Wen *et. al.*, 2020)

Fig S5. Genes used to identify SCN cells and FISH confirmation of IEG expression in SCN peptidergic neurons. *Related to Figure 5.*

(A) Three groups of marker genes were used to identify potential SCN cell clusters: canonical SCN marker genes, non-SCN marker genes, and unspecific marker genes. The clusters marked with Y are potential SCN clusters and they were used for reclustering. The clusters marked with X do not express canonical SCN markers and therefore were not used for the reclustering. Size of the dot reflects the percentage of cells that expressed genes of interest and the color intensity reflects the average expression.

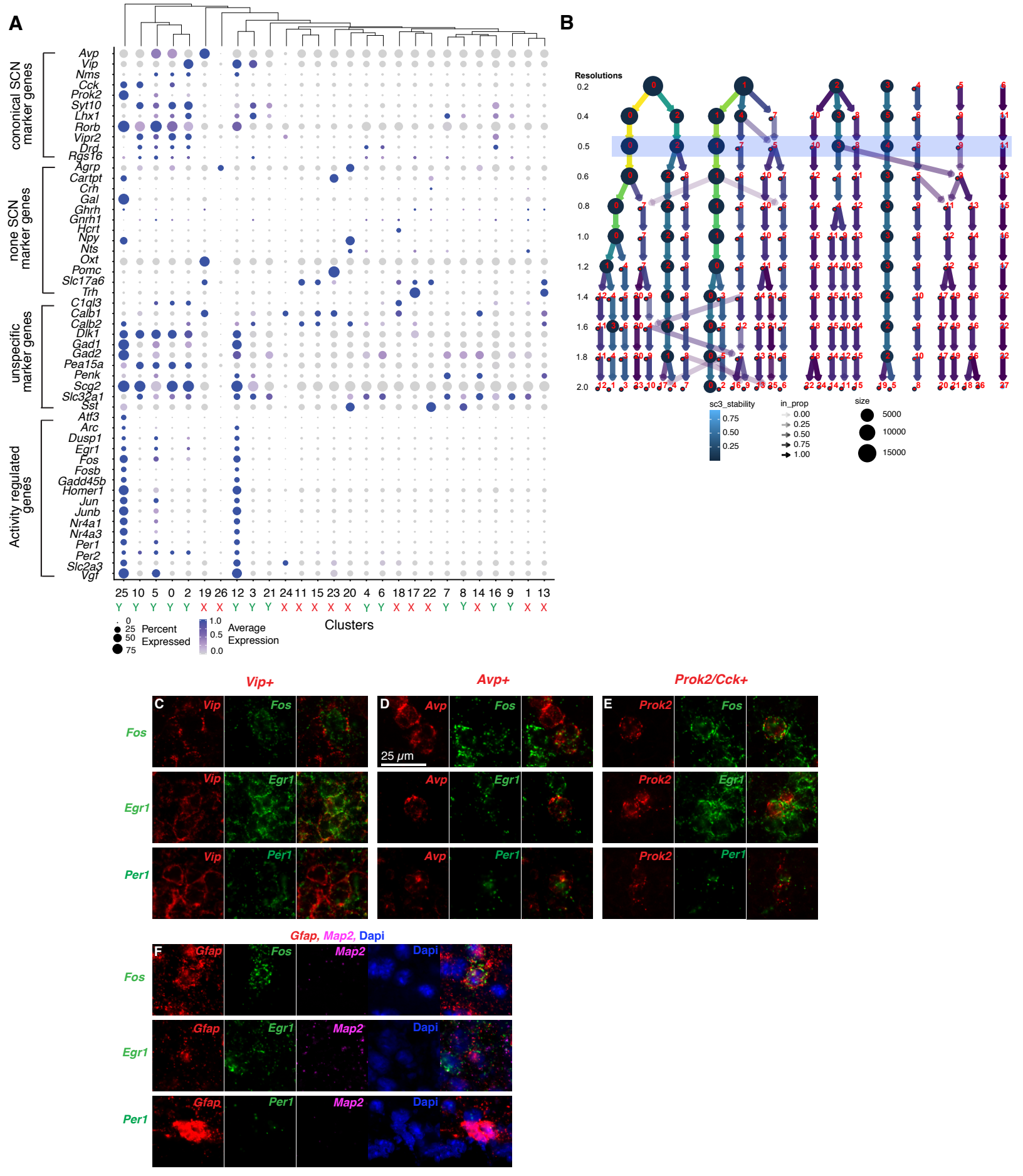
(B) Clustree analysis depicting the stability of the cluster across different resolutions. Nodes are colored by stability score. Size corresponds with the number of nuclei. Arrow width corresponds with the proportion of nuclei shared between clusters at different resolutions.

(C) Expression of *Fos, Egr1 and Per1* (green) in *Vip* (red) cells.

(D) Expression of *Fos, Egr1 and Per1* (green) in *Avp* (red) cells.

(E) Expression of *Fos, Egr1 and Per1* (green) in *Prok2* (red) cells.

(F) Expression of *Fos, Egr1 and Per1* (green) in *Gfap* (red) cells. Dapi (blue) was used to stain nuclei. *Map2* is a neuronal marker. SCNs were dissected after a 1-hour light pulse at CT17. Scale bar 25 μm.


A
cononical SCN marker genes
none SCN marker genes
unspecific marker genes
Activity regulated genes
Avp
Vip
Nms
Cck
Prok2
Syt10
Lhx1
Rorb
Vipr2
Drd
Rgs16
Agrp
Cartpt
Crh
Gal
Ghrh
Gnrh1
Hcrt
Npy
Nts
Oxt
Pomc
Slc17a6
Trh
C1ql3
Calb1
Calb2
Dlk1
Gad1
Gad2
Pea15a
Penk
Scg2
Slc32a1
Sst
Atf3
Arc
Dusp1
Egr1
Fos
Fosb
Gadd45b
Homer1
Jun
Junb
Nr4a1
Nr4a3
Per1
Per2
Slc2a3
Vgf
25 10 5 0 2 19 26 12 3 21 24 11 15 23 20 4 6 18 17 22 7 8 14 16 9 1 13
Y Y Y Y Y X X Y Y Y X X X X X Y Y X X X Y Y X Y Y X X
Clusters
Percent Expressed
Average Expression
B
Resolutions
sc3_stability
in_prop
size

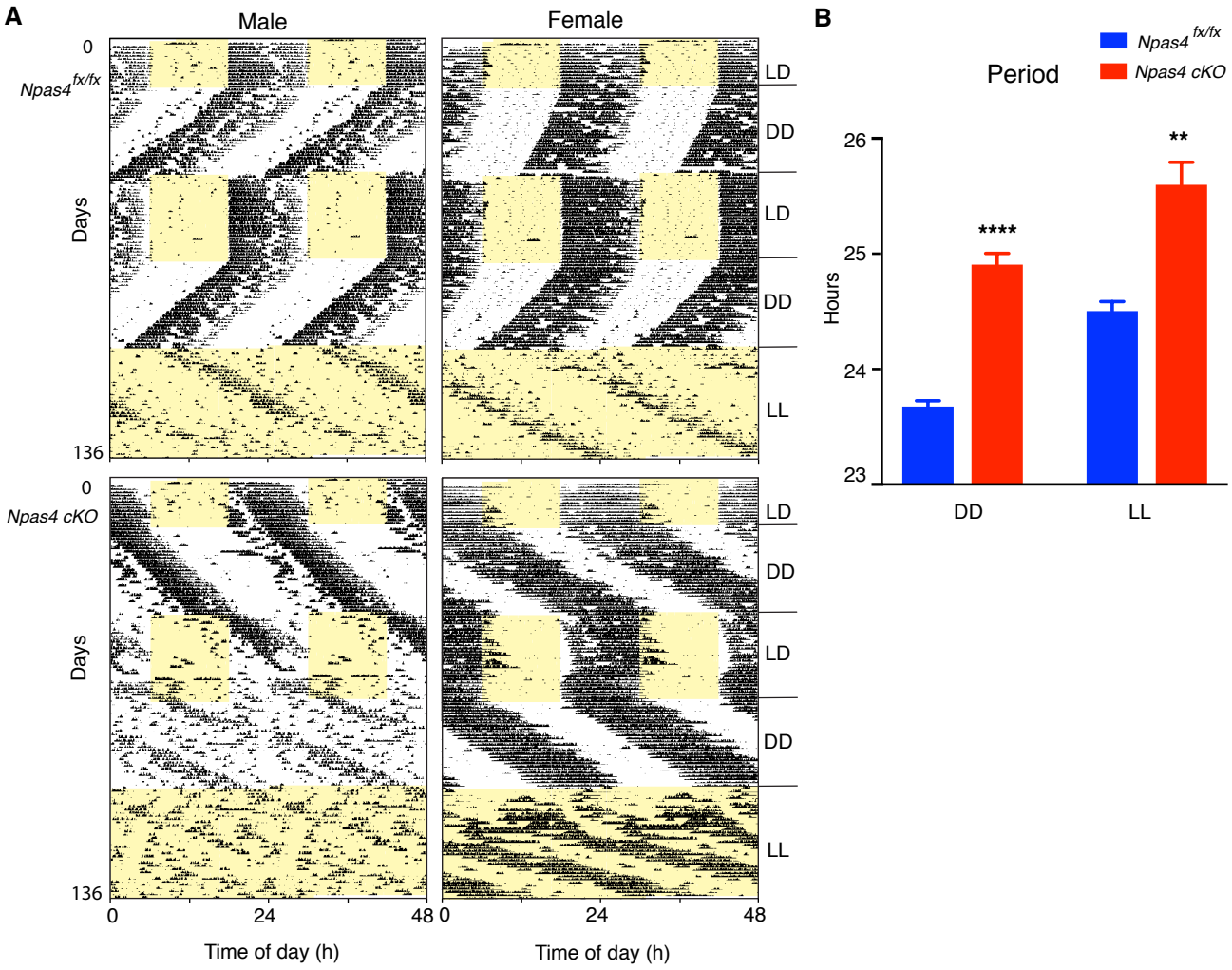
Vip+
Avp+
Prok2/Cck+
C
D
E
Vip
Avp
Prok2
Fos
Egr1
Per1
25 μm

Gfap, Map2, Dapi
F
Gfap
Fos
Egr1
Per1
Map2
Dapi

Fig S6. Phase of entrainment in *Npas4* mutants to 12-h light:12-h dark (LD 12:12). *Related to Figure 6.*

(A) Representative actogram of *Npas4*$^{fx/fx}$ and *Npas4* cKO per sex. Records are double-plotted, and each horizontal line represents 48 hr of activity.

(B) Average period in constant darkness and constant light (*Npas4*$^{fx/fx}$, n = 12, *Npas4* cKO, n = 13). Mean ± s.e.m. are shown. (**** $p < 0.0001$, ** $p < 0.01$, unpaired Student's *t* test)


A
Male
Female
0
Npas4 fx/fx
Days
136
LD
DD
LD
DD
LL
0
Npas4 cKO
Days
136
LD
DD
LD
DD
LL
0
24
48
Time of day (h)
0
24
48
Time of day (h)
B
Period
Npas4 fx/fx
Npas4 cKO
Hours
26
25
24
23
****
**
DD
LL