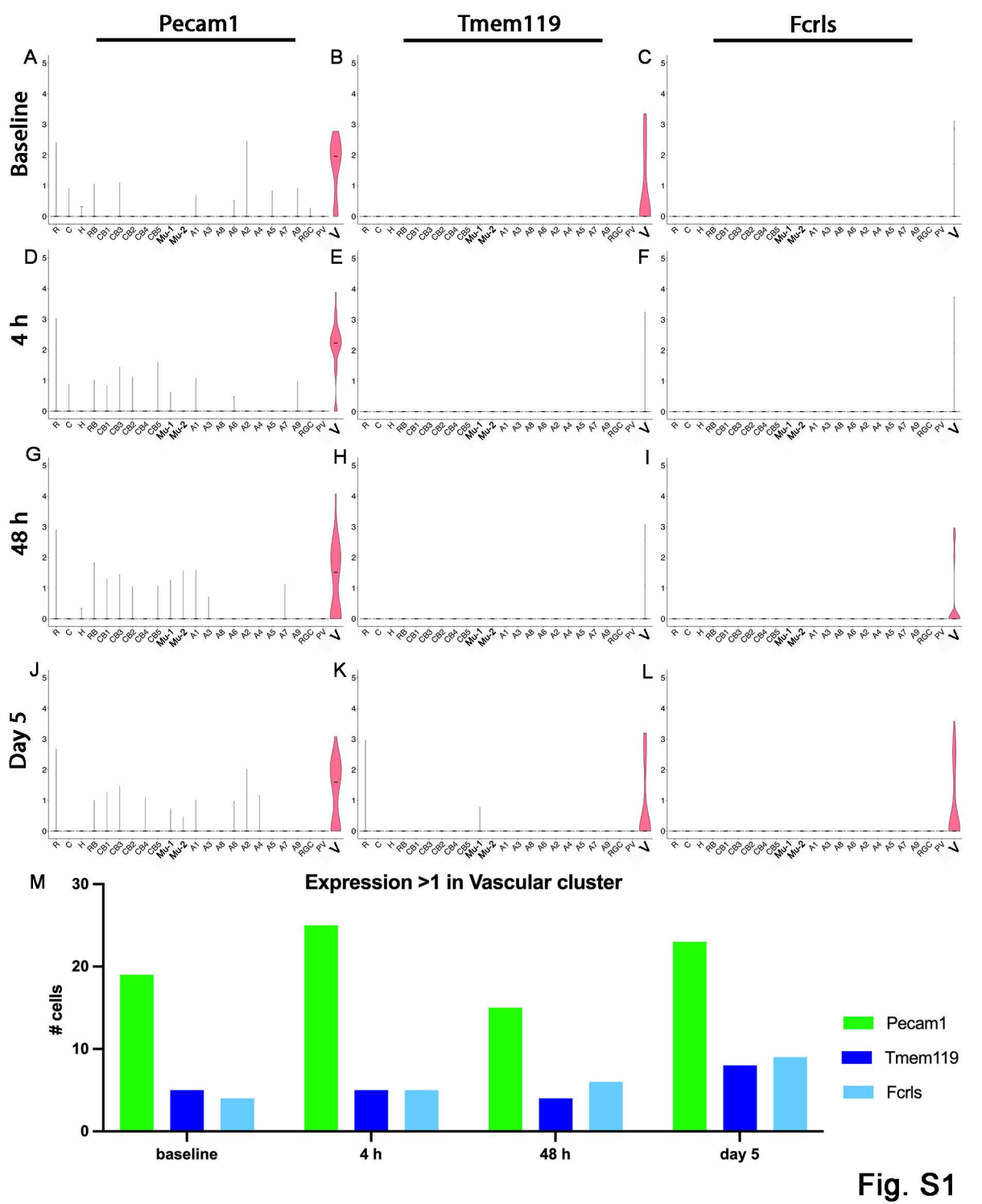
Pecam1
Tmem119
Fcrls
A
B
C
D
E
F
G
H
I
J
K
L
Baseline
4 h
48 h
Day 5
M
Expression >1 in Vascular cluster
cells
baseline
4 h
48 h
day 5
Pecam1
Tmem119
Fcrls

Fig. S1

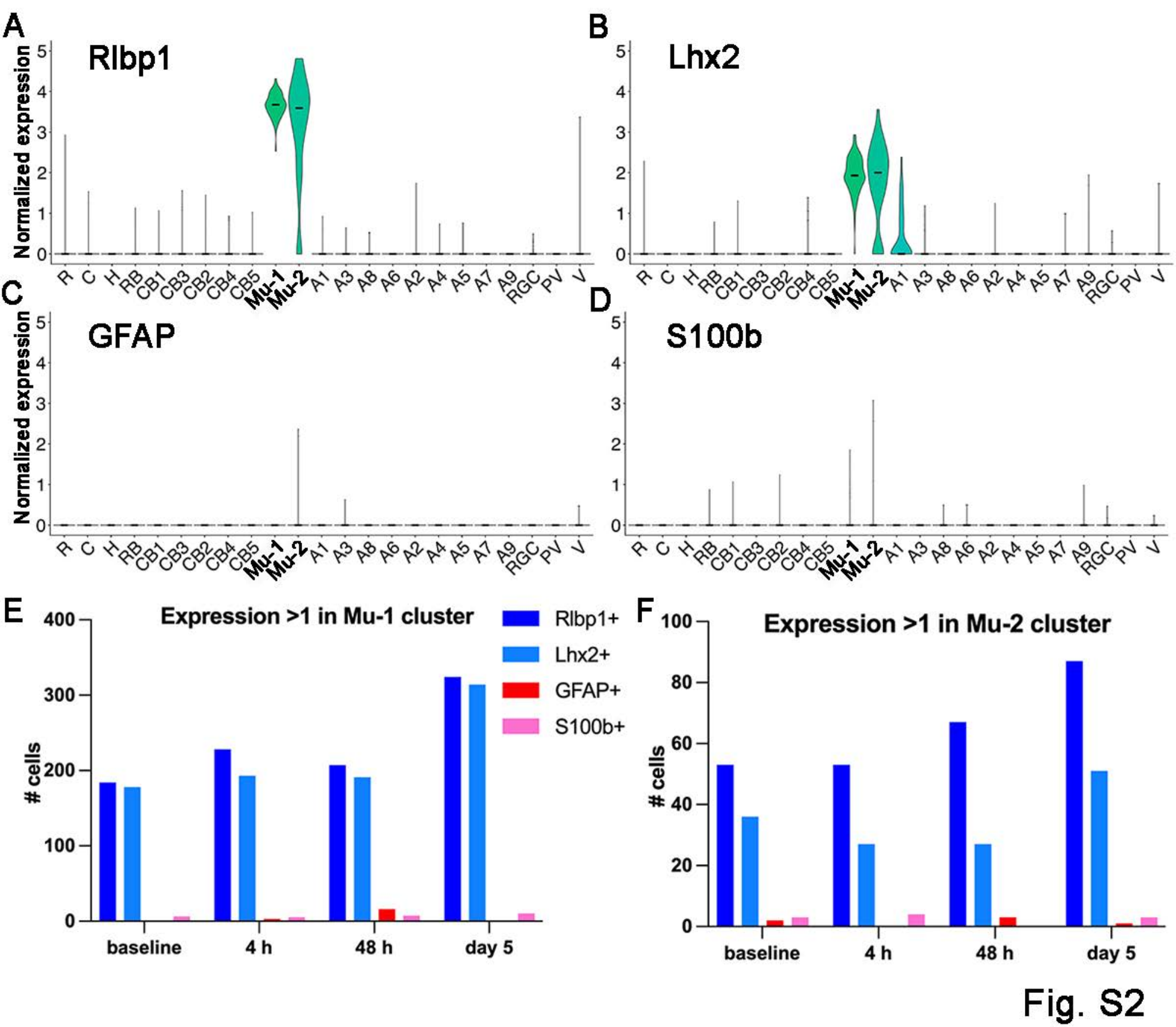
A
Rlbp1
Normalized expression
B
Lhx2
C
GFAP
D
S100b
R C H RB CB1 CB3 CB2 CB4 CB5 Mu-1 Mu-2 A1 A3 A8 A6 A2 A4 A5 A7 A9 RGC PV V
E
Expression >1 in Mu-1 cluster
Rlbp1+
Lhx2+
GFAP+
S100b+
cells
baseline
4 h
48 h
day 5
F
Expression >1 in Mu-2 cluster

Fig. S2

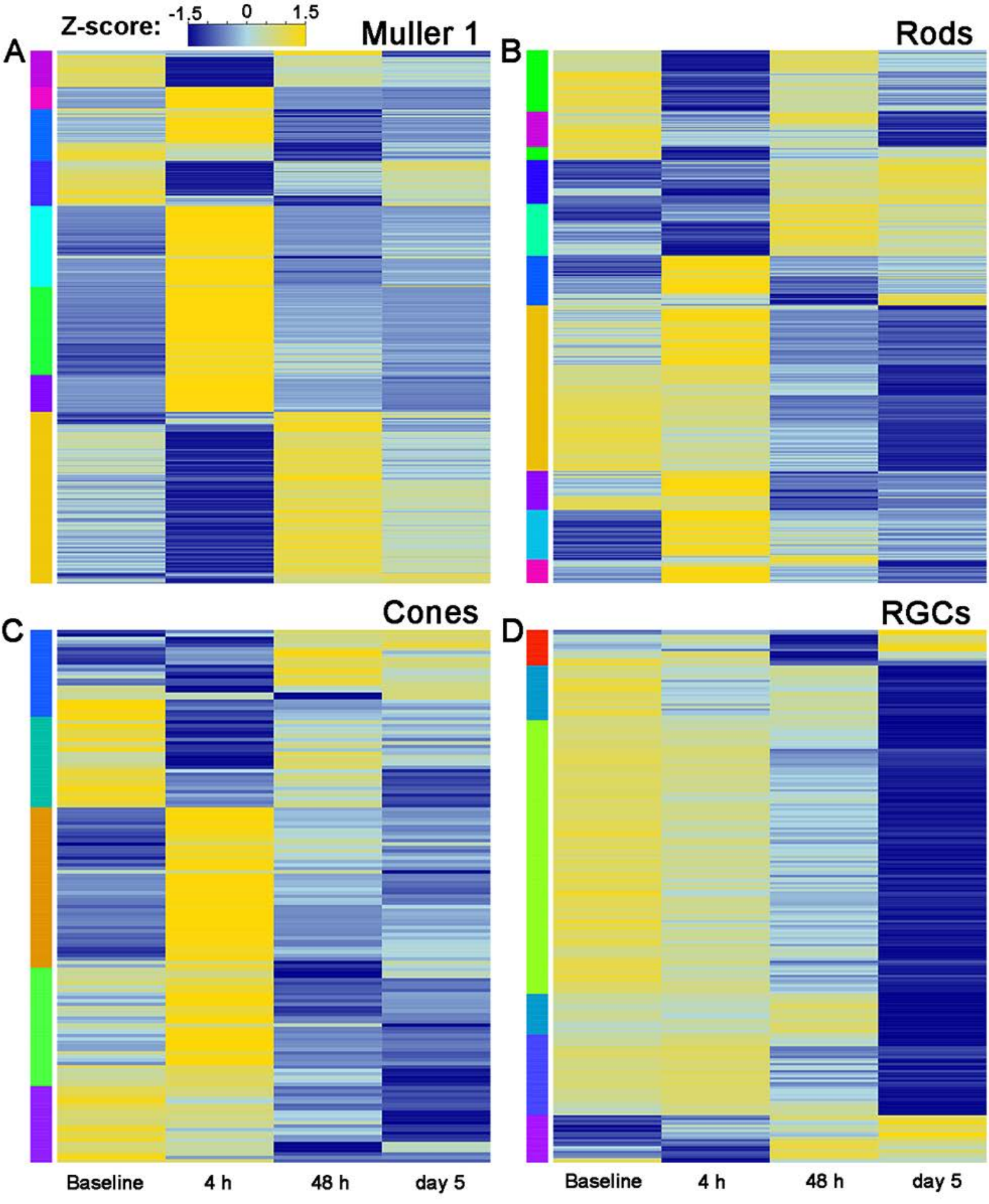
Z-score:
-1.5
0
1.5
A
Muller 1
B
Rods
C
Cones
D
RGCs
Baseline
4 h
48 h
day 5
Baseline
4 h
48 h
day 5

Fig. S3

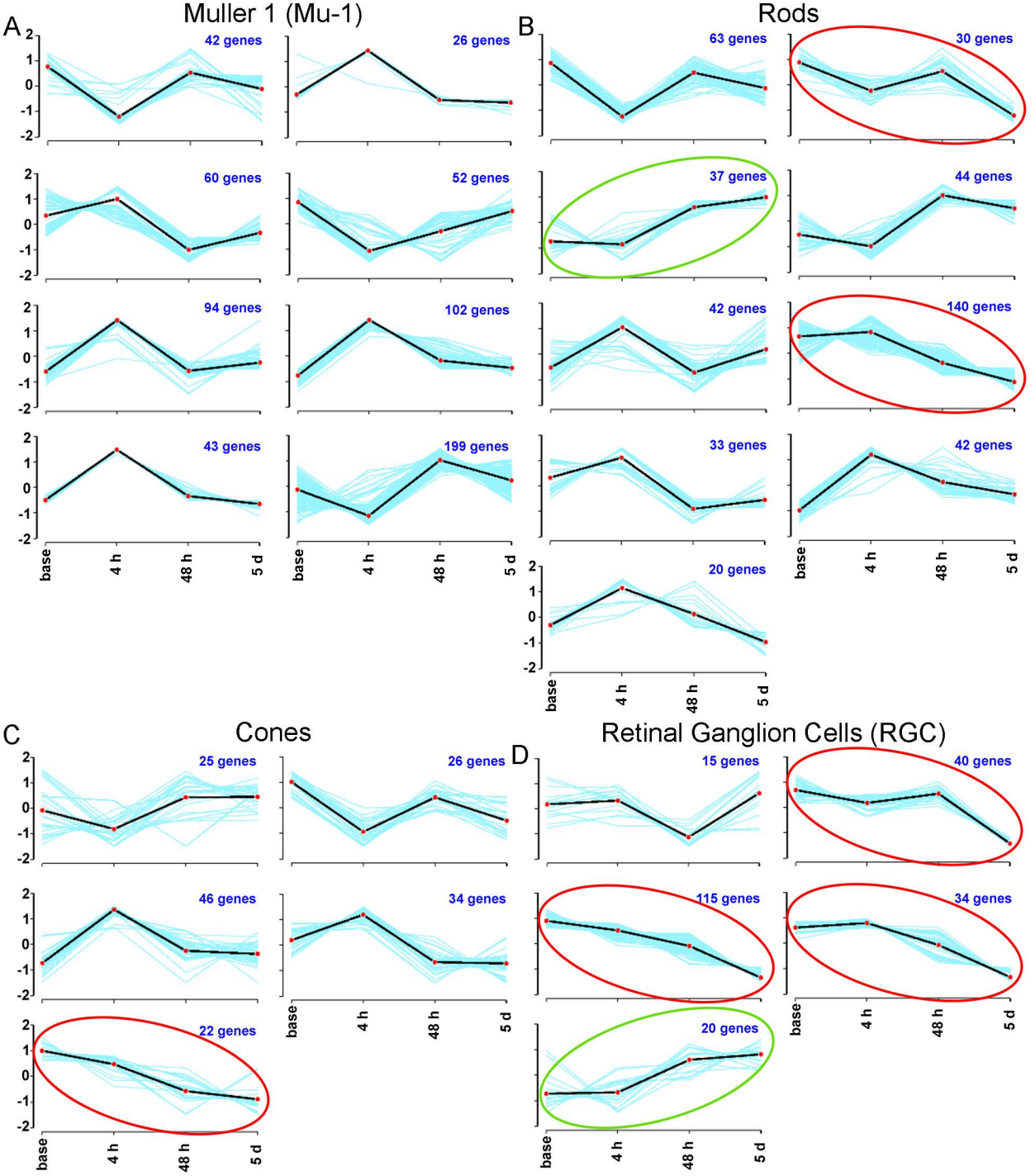
A
Muller 1 (Mu-1)
42 genes
26 genes
60 genes
52 genes
94 genes
102 genes
43 genes
199 genes
B
Rods
63 genes
30 genes
37 genes
44 genes
42 genes
140 genes
33 genes
42 genes
20 genes
C
Cones
25 genes
26 genes
46 genes
34 genes
22 genes
D
Retinal Ganglion Cells (RGC)
15 genes
40 genes
115 genes
34 genes
20 genes
base
4 h
48 h
5 d

Fig. S4

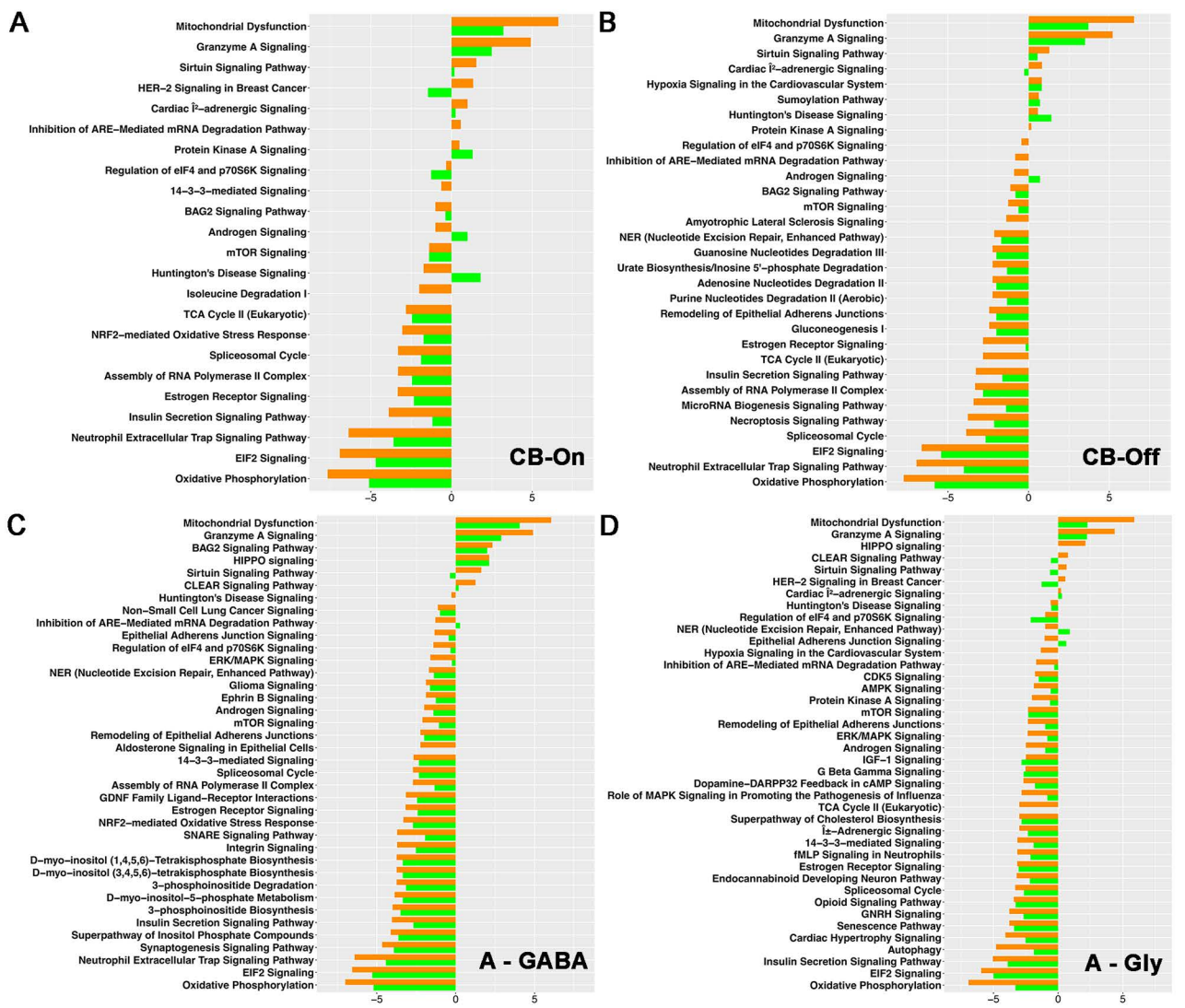
A
Mitochondrial Dysfunction
Granzyme A Signaling
Sirtuin Signaling Pathway
HER–2 Signaling in Breast Cancer
Cardiac Î²–adrenergic Signaling
Inhibition of ARE–Mediated mRNA Degradation Pathway
Protein Kinase A Signaling
Regulation of eIF4 and p70S6K Signaling
14–3–3–mediated Signaling
BAG2 Signaling Pathway
Androgen Signaling
mTOR Signaling
Huntington's Disease Signaling
Isoleucine Degradation I
TCA Cycle II (Eukaryotic)
NRF2–mediated Oxidative Stress Response
Spliceosomal Cycle
Assembly of RNA Polymerase II Complex
Estrogen Receptor Signaling
Insulin Secretion Signaling Pathway
Neutrophil Extracellular Trap Signaling Pathway
EIF2 Signaling
Oxidative Phosphorylation
−5
0
5
CB-On
B
Mitochondrial Dysfunction
Granzyme A Signaling
Sirtuin Signaling Pathway
Cardiac Î²–adrenergic Signaling
Hypoxia Signaling in the Cardiovascular System
Sumoylation Pathway
Huntington's Disease Signaling
Protein Kinase A Signaling
Regulation of eIF4 and p70S6K Signaling
Inhibition of ARE–Mediated mRNA Degradation Pathway
Androgen Signaling
BAG2 Signaling Pathway
mTOR Signaling
Amyotrophic Lateral Sclerosis Signaling
NER (Nucleotide Excision Repair, Enhanced Pathway)
Guanosine Nucleotides Degradation III
Urate Biosynthesis/Inosine 5'–phosphate Degradation
Adenosine Nucleotides Degradation II
Purine Nucleotides Degradation II (Aerobic)
Remodeling of Epithelial Adherens Junctions
Gluconeogenesis I
Estrogen Receptor Signaling
TCA Cycle II (Eukaryotic)
Insulin Secretion Signaling Pathway
Assembly of RNA Polymerase II Complex
MicroRNA Biogenesis Signaling Pathway
Necroptosis Signaling Pathway
Spliceosomal Cycle
EIF2 Signaling
Neutrophil Extracellular Trap Signaling Pathway
Oxidative Phosphorylation
−5
0
5
CB-Off
C
Mitochondrial Dysfunction
Granzyme A Signaling
BAG2 Signaling Pathway
HIPPO signaling
Sirtuin Signaling Pathway
CLEAR Signaling Pathway
Huntington's Disease Signaling
Non–Small Cell Lung Cancer Signaling
Inhibition of ARE–Mediated mRNA Degradation Pathway
Epithelial Adherens Junction Signaling
Regulation of eIF4 and p70S6K Signaling
ERK/MAPK Signaling
NER (Nucleotide Excision Repair, Enhanced Pathway)
Glioma Signaling
Ephrin B Signaling
Androgen Signaling
mTOR Signaling
Remodeling of Epithelial Adherens Junctions
Aldosterone Signaling in Epithelial Cells
14–3–3–mediated Signaling
Spliceosomal Cycle
Assembly of RNA Polymerase II Complex
GDNF Family Ligand–Receptor Interactions
Estrogen Receptor Signaling
NRF2–mediated Oxidative Stress Response
SNARE Signaling Pathway
Integrin Signaling
D–myo–inositol (1,4,5,6)–Tetrakisphosphate Biosynthesis
D–myo–inositol (3,4,5,6)–tetrakisphosphate Biosynthesis
3–phosphoinositide Degradation
D–myo–inositol–5–phosphate Metabolism
3–phosphoinositide Biosynthesis
Insulin Secretion Signaling Pathway
Superpathway of Inositol Phosphate Compounds
Synaptogenesis Signaling Pathway
Neutrophil Extracellular Trap Signaling Pathway
EIF2 Signaling
Oxidative Phosphorylation
−5
0
5
A - GABA
D
Mitochondrial Dysfunction
Granzyme A Signaling
HIPPO signaling
CLEAR Signaling Pathway
Sirtuin Signaling Pathway
HER–2 Signaling in Breast Cancer
Cardiac Î²–adrenergic Signaling
Huntington's Disease Signaling
Regulation of eIF4 and p70S6K Signaling
NER (Nucleotide Excision Repair, Enhanced Pathway)
Epithelial Adherens Junction Signaling
Hypoxia Signaling in the Cardiovascular System
Inhibition of ARE–Mediated mRNA Degradation Pathway
CDK5 Signaling
AMPK Signaling
Protein Kinase A Signaling
mTOR Signaling
Remodeling of Epithelial Adherens Junctions
ERK/MAPK Signaling
Androgen Signaling
IGF–1 Signaling
G Beta Gamma Signaling
Dopamine–DARPP32 Feedback in cAMP Signaling
Role of MAPK Signaling in Promoting the Pathogenesis of Influenza
TCA Cycle II (Eukaryotic)
Superpathway of Cholesterol Biosynthesis
Î±–Adrenergic Signaling
14–3–3–mediated Signaling
fMLP Signaling in Neutrophils
Estrogen Receptor Signaling
Endocannabinoid Developing Neuron Pathway
Spliceosomal Cycle
Opioid Signaling Pathway
GNRH Signaling
Senescence Pathway
Cardiac Hypertrophy Signaling
Autophagy
Insulin Secretion Signaling Pathway
EIF2 Signaling
Oxidative Phosphorylation
−5
0
5
A - Gly

Fig. S5

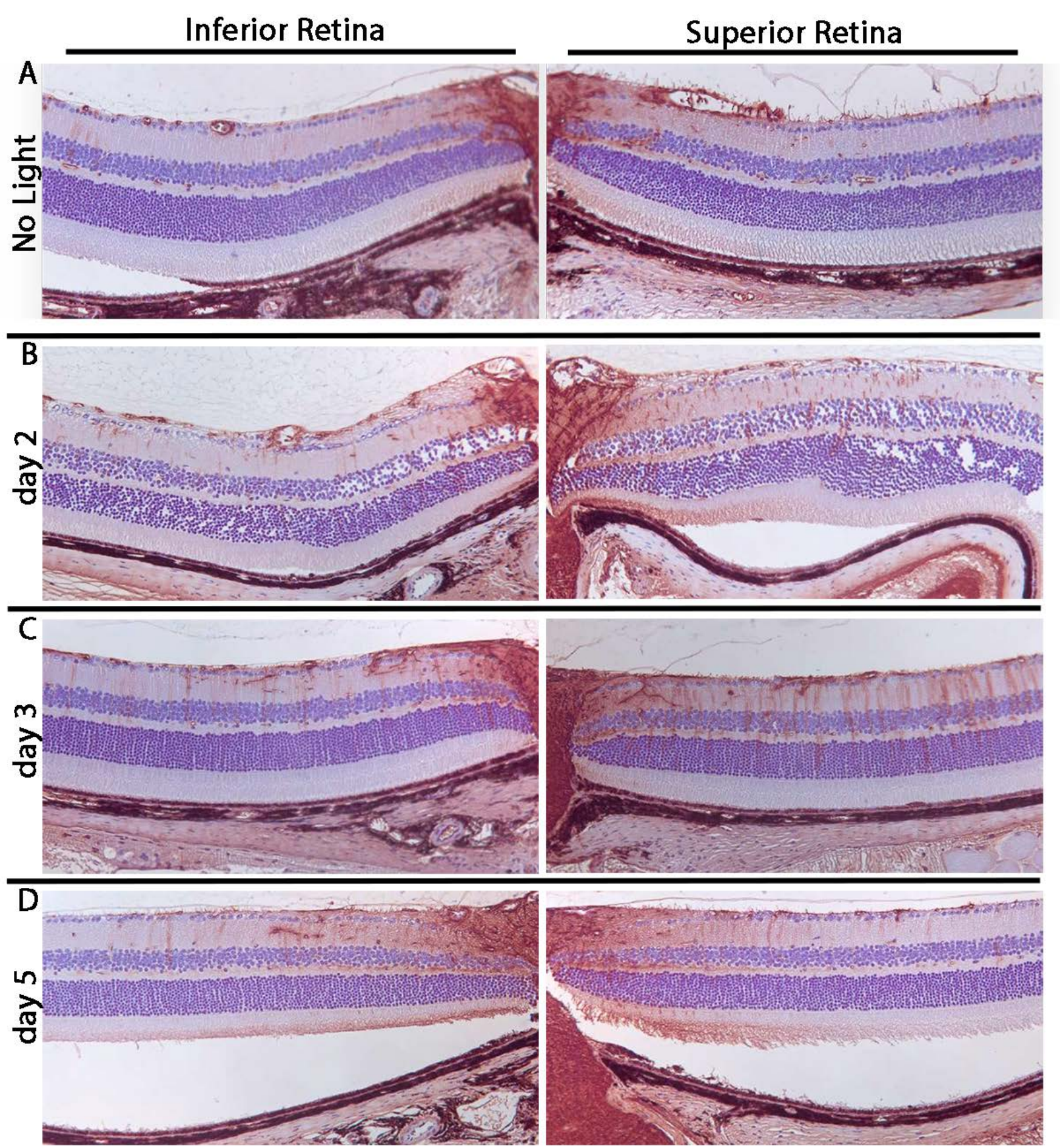
Inferior Retina
Superior Retina
A
No Light
B
day 2
C
day 3
D
day 5

Fig. S6

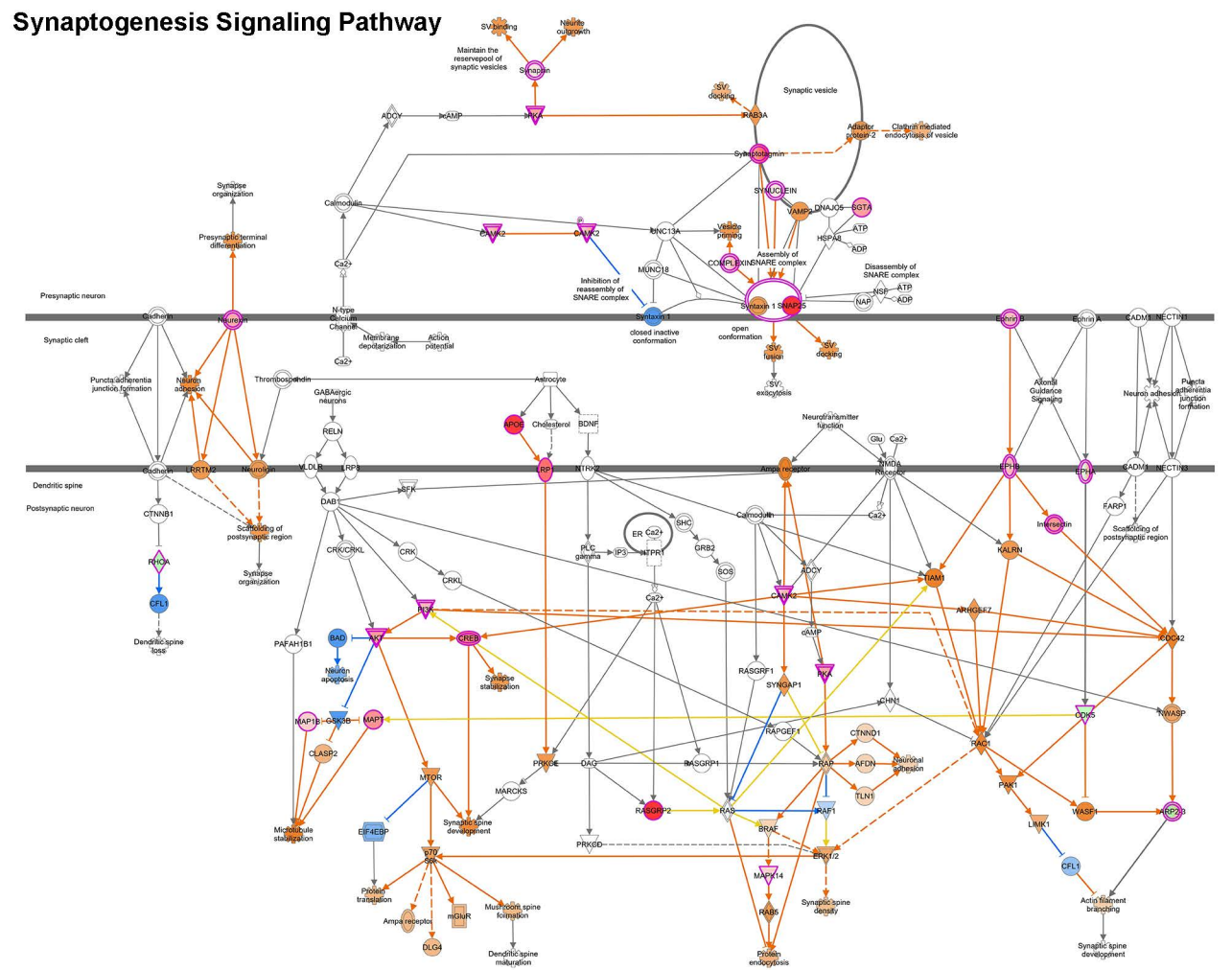
Synaptogenesis Signaling Pathway
SV binding
Neurite outgrowth
Maintain the reserve pool of synaptic vesicles
Synapsin
Synaptic vesicle
SV docking
ADCY
cAMP
PKA
RAB3A
Adaptor protein-2
Clathrin mediated endocytosis of vesicle
Synaptotagmin
SYNUCLEIN
VAMP2
DNAJC5
SGTA
ATP
HSPA8
ADP
Synapse organization
Calmodulin
Presynaptic terminal differentiation
CAMK2
CAMK2
UNC13A
Vesicle priming
COMPLEXINS
Assembly of SNARE complex
Disassembly of SNARE complex
NSF
ATP
ADP
NAP
Ca2+
MUNC18
Inhibition of reassembly of SNARE complex
Presynaptic neuron
Cadherin
Neurexin
N-type Calcium Channel
Membrane depolarization
Action potential
Ca2+
Syntaxin 1
SNAP25
closed inactive conformation
open conformation
SV fusion
SV docking
SV exocytosis
Synaptic cleft
Ephrin B
EphrinA
CADM1
NECTIN1
Puncta adherentia junction formation
Neuron adhesion
Thrombospondin
Astrocyte
GABAergic neurons
Axonal Guidance Signaling
Neuron adhesion
Puncta adherentia junction formation
RELN
APOE
Cholesterol
BDNF
Neurotransmitter release
Glu
Ca2+
Cadherin
LRRTM2
Neurexin
VLDLR
RP3
RP2
NTRK2
Ampa receptor
NMDA Receptor
EPHB
EPHA
CADM1
NECTIN3
Dendritic spine
Postsynaptic neuron
FYN
DAB1
CTNNB1
Scaffolding of postsynaptic region
FARP1
Intersectin
Scaffolding of postsynaptic region
RHOA
Synapse organization
CRK/CRKL
CRK
PLC gamma
ER
Ca2+
IP3
ITPR1
SHC
GRB2
SOS
Calmodulin
Ca2+
KALRN
TIAM1
CFL1
CRKL
ADCY
CAMK2
Ca2+
ARHGEF7
Dendritic spine loss
PI3K
cAMP
CDC42
PAFAH1B1
BAD
AKT
CREB
Neuron apoptosis
Synapse stabilization
RASGRF1
SYNGAP1
PKA
CHN1
MAP1B
GSK3B
MAP1
CLASP2
RAPGEF1
CDK5
WNASP
CTNND1
RAC1
PRKCE
DAG
RASGRP1
RAP
AFDN
Neuronal adhesion
MARCKS
TLN1
PAK1
RASGRP2
RAS
RAF1
WASF1
ARP2/3
Microtubule stabilization
EIF4EBP
MTOR
Synaptic spine development
BRAF
LIMK1
PRKCD
ERK1/2
p70 S6K
MAPK14
CFL1
Protein translation
Synaptic spine density
Actin filament branching
Ampa receptor
mGluR
DLG4
Mushroom spine formation
RAB5
Dendritic spine maturation
Protein endocytosis
Synaptic spine development

Fig. S7

Ephrin Receptor Signaling Pathway

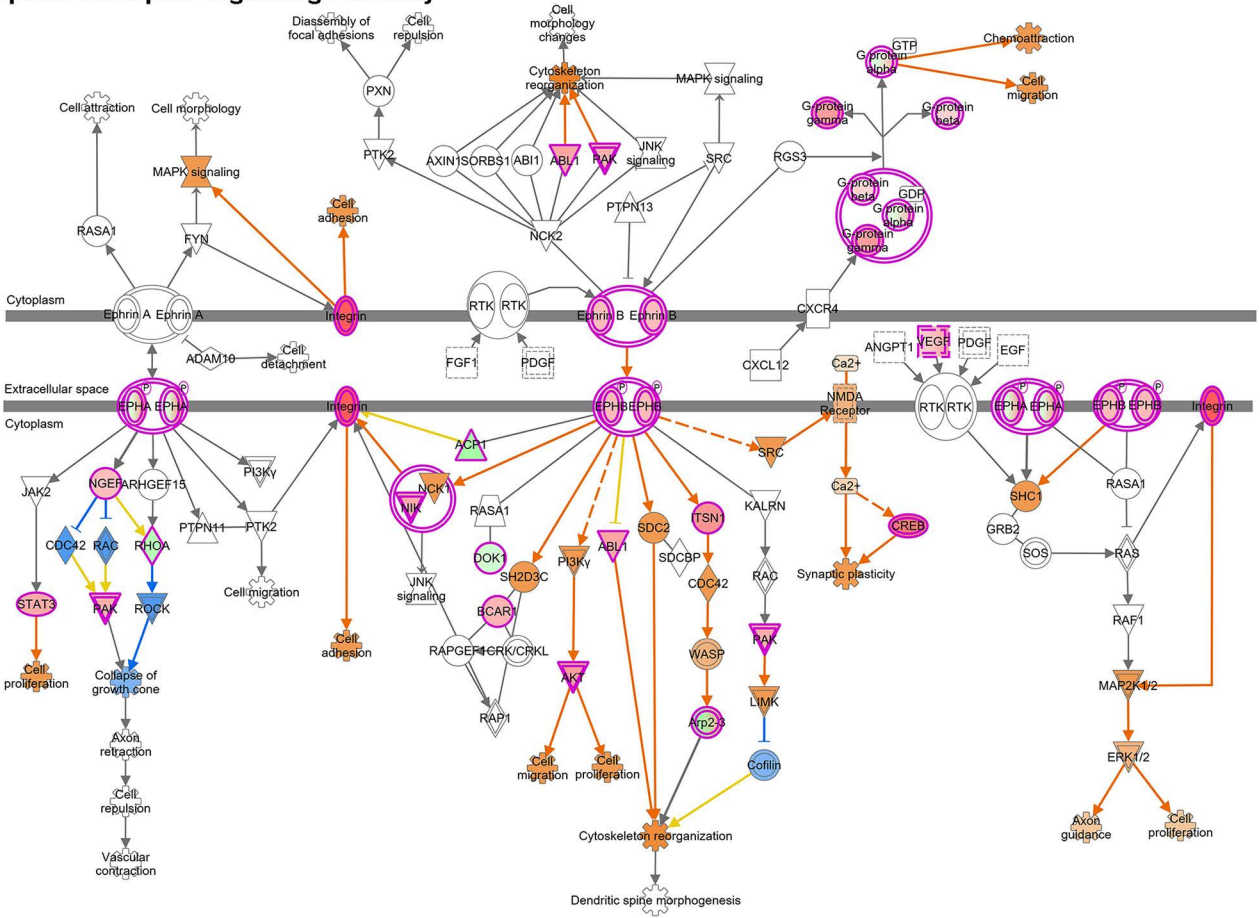

Fig. S8

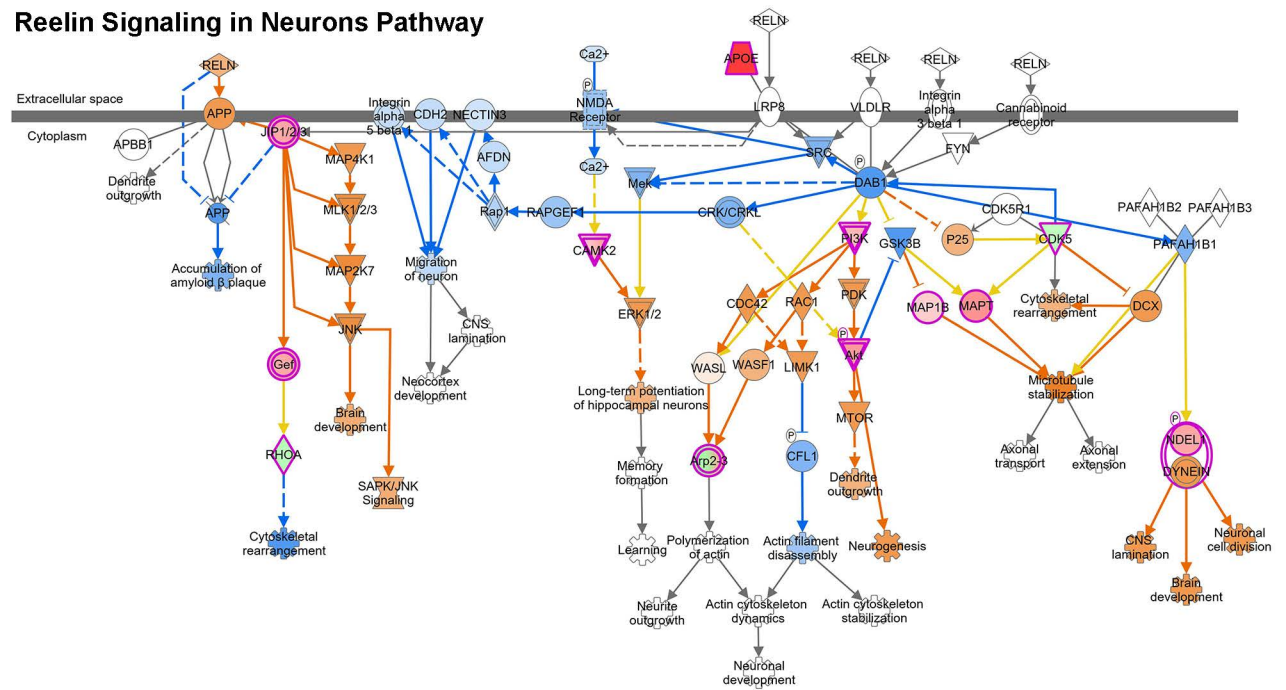
Reelin Signaling in Neurons Pathway
Extracellular space
Cytoplasm
RELN
APP
APBB1
Dendrite outgrowth
Accumulation of amyloid β plaque
JIP1/2/3
MAP4K1
MLK1/2/3
MAP2K7
JNK
Gef
RHOA
Cytoskeletal rearrangement
Brain development
SAPK/JNK Signaling
Integrin alpha 5 beta 1
CDH2
NECTIN3
AFDN
Migration of neuron
CNS lamination
Neocortex development
Rap1
RAPGEF1
Ca2+
NMDA Receptor
CAMK2
ERK1/2
Long-term potentiation of hippocampal neurons
Memory formation
Learning
APOE
LRP8
VLDLR
Integrin alpha 3 beta 1
Cannabinoid receptor
SRC
FYN
DAB1
Mek
CRK/CRKL
PI3K
PDK1
Akt
GSK3B
CDC42
RAC1
WASL
WASF1
LIMK1
Arp2/3
CFL1
Polymerization of actin
Actin filament disassembly
Neurite outgrowth
Actin cytoskeleton dynamics
Actin cytoskeleton stabilization
Neuronal development
MTOR
Dendrite outgrowth
Neurogenesis
CDK5R1
P25
CDK5
MAP1B
MAPT
Cytoskeletal rearrangement
Microtubule stabilization
Axonal transport
Axonal extension
PAFAH1B2
PAFAH1B3
PAFAH1B1
DCX
NDEL1
DYNEIN
CNS lamination
Neuronal cell division
Brain development

Fig. S9

Supplemental Figure Legends

Supplemental Figure S1. Very few microglia cells were identified in mouse retina and were included within the vascular endothelial cluster. A-L) Violin plots show that there is little to no expression of microglia markers Tmem119 (B, E, H, K) and Fcrls (C, F, I, L) as compared to the vascular marker Pecam1 (A, D, G, J) at the 4 time points. M) The number of cells with normalized expression greater than 1 for the vascular marker Pecam1 (green bars), and microglia specific markers (Tmem119, dark blue and Fcrls, light blue) are shown.

Supplemental Figure S2. Very few astrocytes were identified in the mouse retina and were included within the Muller glia cluster. A-D) Sample violin plots from the baseline timepoint show that there is little to no expression of astrocyte markers GFAP (C) and S100b (D) as compared to Muller glia markers Rlbp1 (A) and Lhx2 (B). E-F) Quantification for all timepoints is shown for the M1 cluster (E) and M2 cluster (F); the number of cells with normalized expression greater than 1 for the Muller glia markers Rlbp1 (dark blue bars) and LhX2 (light blue bars) compared to the astrocyte markers GFAP (red bars) and S100b (pink bars) are included.

Supplemental Figure S3. Heat maps of average gene expression for selected cell types. The four cell types shown are (A) Muller-1 cells, (B) Rod cells, (C) Cone cells, and (D) RGCs. Genes with statistically significant expression changes (FDR < 0.05) between time points are shown at baseline, 4 h, 48 h, and day 5 post light injury.

Supplemental Figure S4. Different cell types vary in their temporal patterns of gene expression changes. Spaghetti plot representation of differentially expressed genes grouped by their temporal patterns of expression changes. A) Eight patterns were generated for Muller-1 cells with no clear trend of decrease or increase in the late time point in any cluster. B) Five patterns were generated for cone cells, with one cluster (red oval) showing a clear downward trend at the late time point. C) Nine patterns were generated for rod cells, with one cluster showing an upward trend (green oval) and two clusters showing a downward trend (red ovals). (D) Five patterns were generated for RGCs with one cluster being upregulated (green oval) and three clusters showing a downward trend (red ovals).

Supplemental Figure S5. IPA results at 48 h and day 5. Results of IPA for the top up- or down-regulated pathways at 48 h (green bars) and day 5 (orange bars) post light injury are shown as bar plots for selected cell types. Cell types shown are: A) ON-cone bipolar cells (CB-On), B) OFF-cone bipolar cells (CB-Off), C) GABAergic amacrine cells (A. GABA) and D) glycinergic amacrine cells (A. Gly). The top 1500 statistically differentially expressed genes ($p < 0.05$) between 48 h and baseline and between day 5 and baseline were used as input for IPA. Pathways are considered as significantly regulated if they meet a p-value <0.05 and $|Z\text{-score}| > 2.0$.

Supplemental Figure S6. GFAP Immunohistochemistry of freeze-substitution fixed, paraffin processed retinal sections from mice with and without light injury (FCD-LIRD). Representative images of GFAP-stained retinal sections showing the inferior (left column) and superior (right column) retina of eyes collected after no light injury (A), 2 days after light injury (B), 3 days after light injury (C) and 5 days after light injury (D). Light injury eyes were subjected to 45 Klux following the 4@3 FCD-LIRD protocol. GFAP positive staining (brown diaminobenzidine-chromogen) is seen as brown streaks following the Muller glia cellular distribution. Minimal staining is seen at baseline (A). Increased staining is seen at day 2 (B) and day (3) followed by a reduction at day 5 (D). Images were taken at 20x magnification on a Leica DM200 microscope.

Supplemental Figure S7. The *Synaptogenesis signaling pathway* is upregulated in Muller-1 cells at day 5 post light injury. A schematic representation of the synaptogenesis pathway in Muller-1 cells at day 5 post light injury compared to the baseline. The top 1500 statistically differentially expressed genes ($p < 0.05$) between these two time points were used as input for IPA. The diagram was generated by Ingenuity Pathway Analysis (QIAGEN).

Supplemental Figure S8. The *Ephrin receptor signaling pathway* is upregulated in Muller-1 cells at day 5 post light injury. A schematic representation of the Ephrin receptor pathway in Muller-1 cells at day 5 post light injury compared to the baseline. The top 1500 statistically differentially expressed genes ($p < 0.05$) between these two time points were used as input for IPA. The diagram was generated by Ingenuity Pathway Analysis (QIAGEN).

Supplemental Figure S9. The *Reelin signaling in neurons pathway* is upregulated in Muller-1 cells at day 5 post light injury. A schematic representation of the Reelin signaling in neurons pathway in Muller-1 cells at day 5 post light injury compared to the baseline. The top 1500 statistically differentially expressed genes ($p < 0.05$) between these two time points were used as input for IPA. The diagram was generated by Ingenuity Pathway Analysis (QIAGEN).