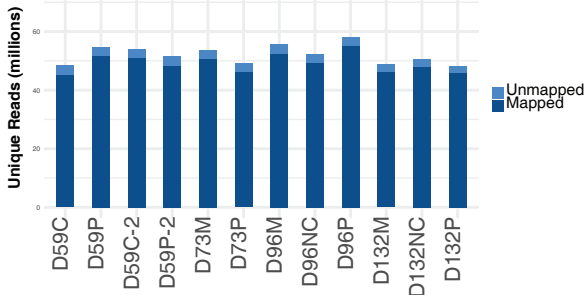
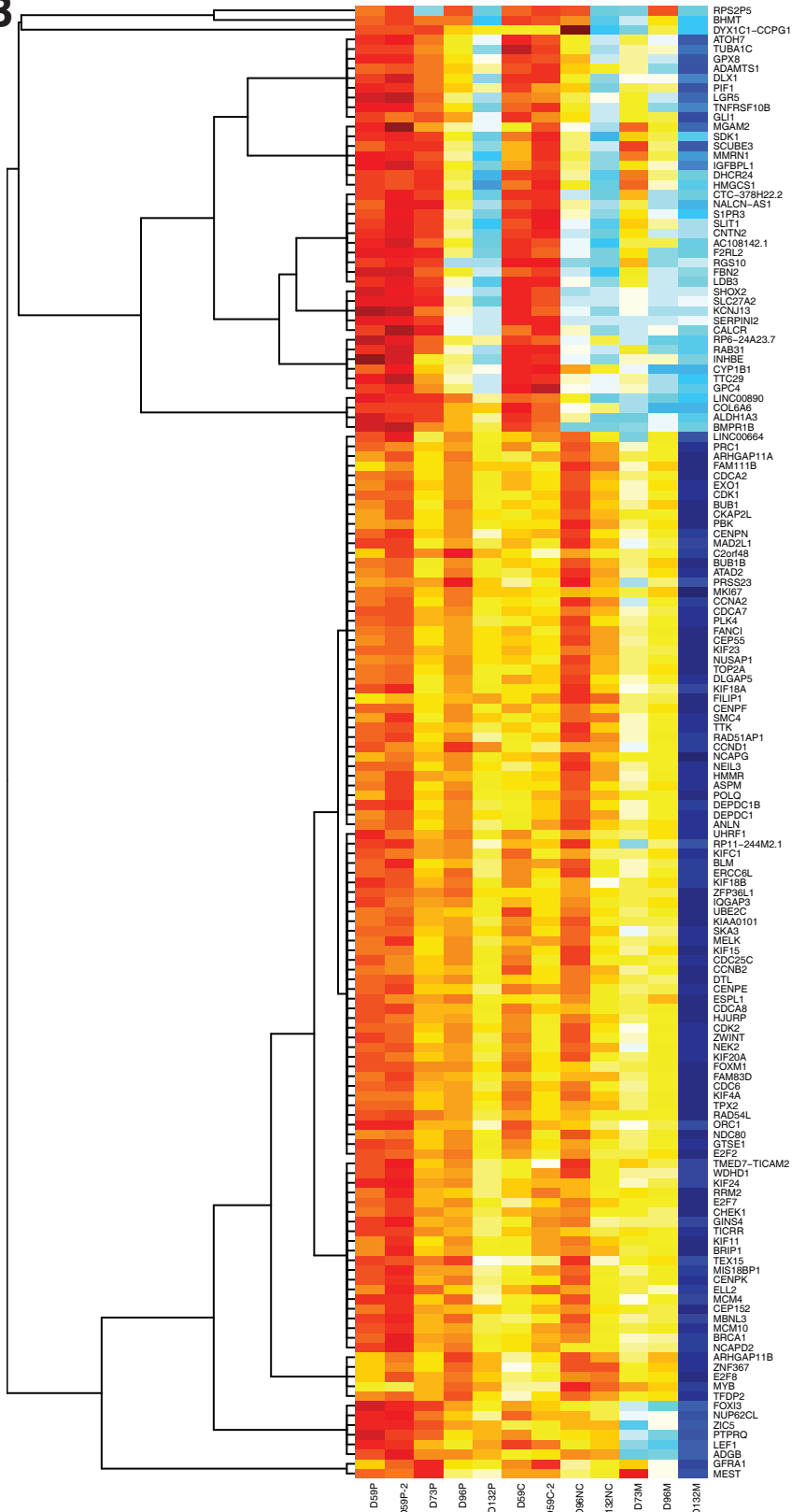
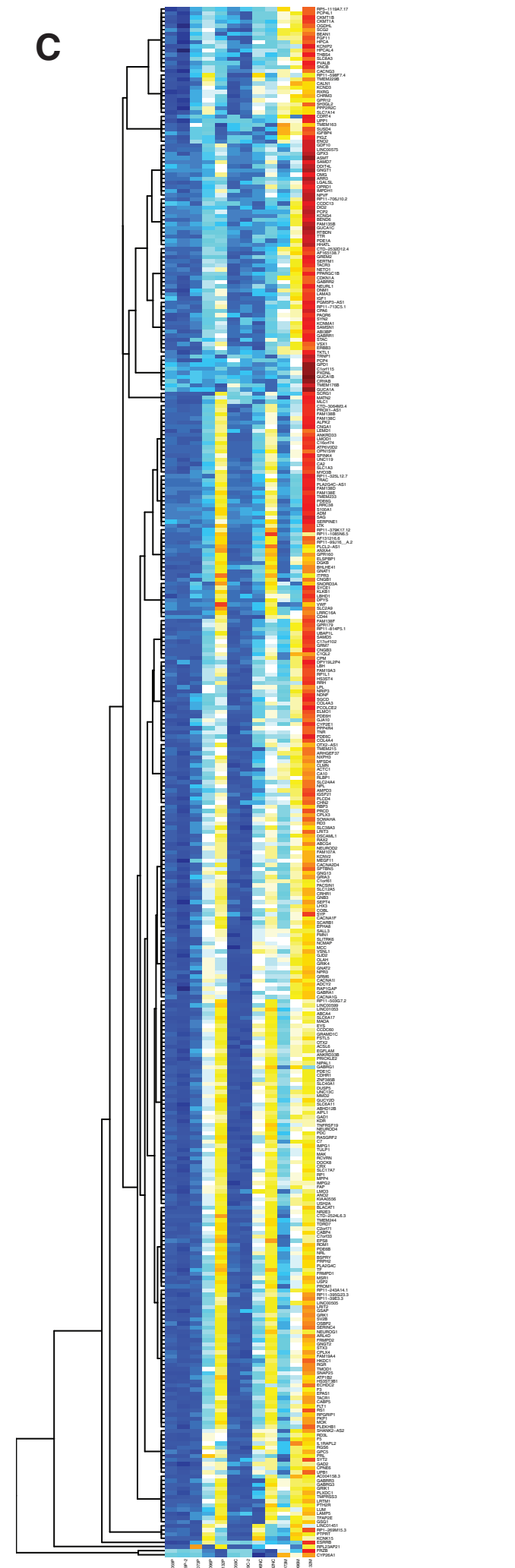
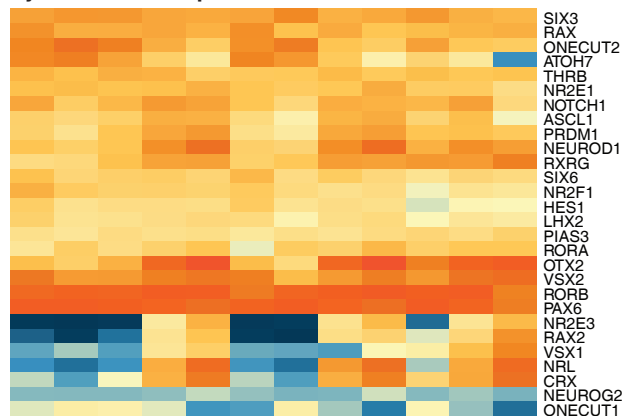
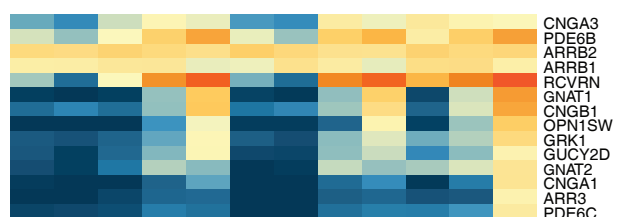
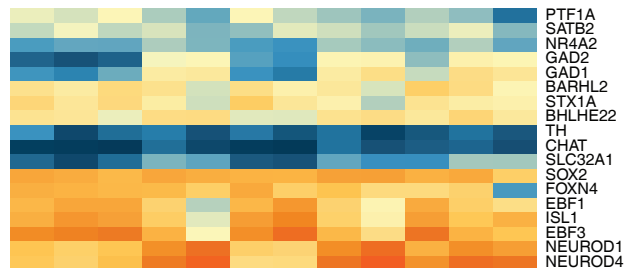
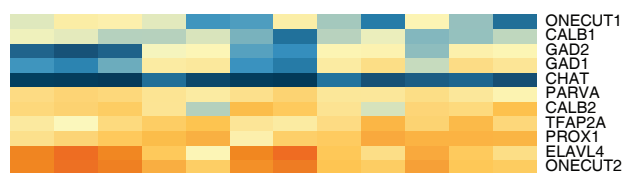


A

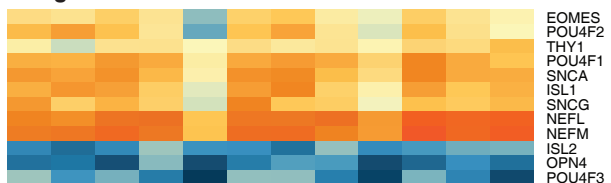
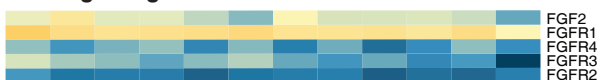
STAR 2-pass Alignment

**B****C**

A**Eye field Transcription Factors****Phototransduction****Amacrine****Horizontal**

D59P D59P-2 D73P D96P D132P D59C D59C-2 D96NC D132NC D73M D96M D132M

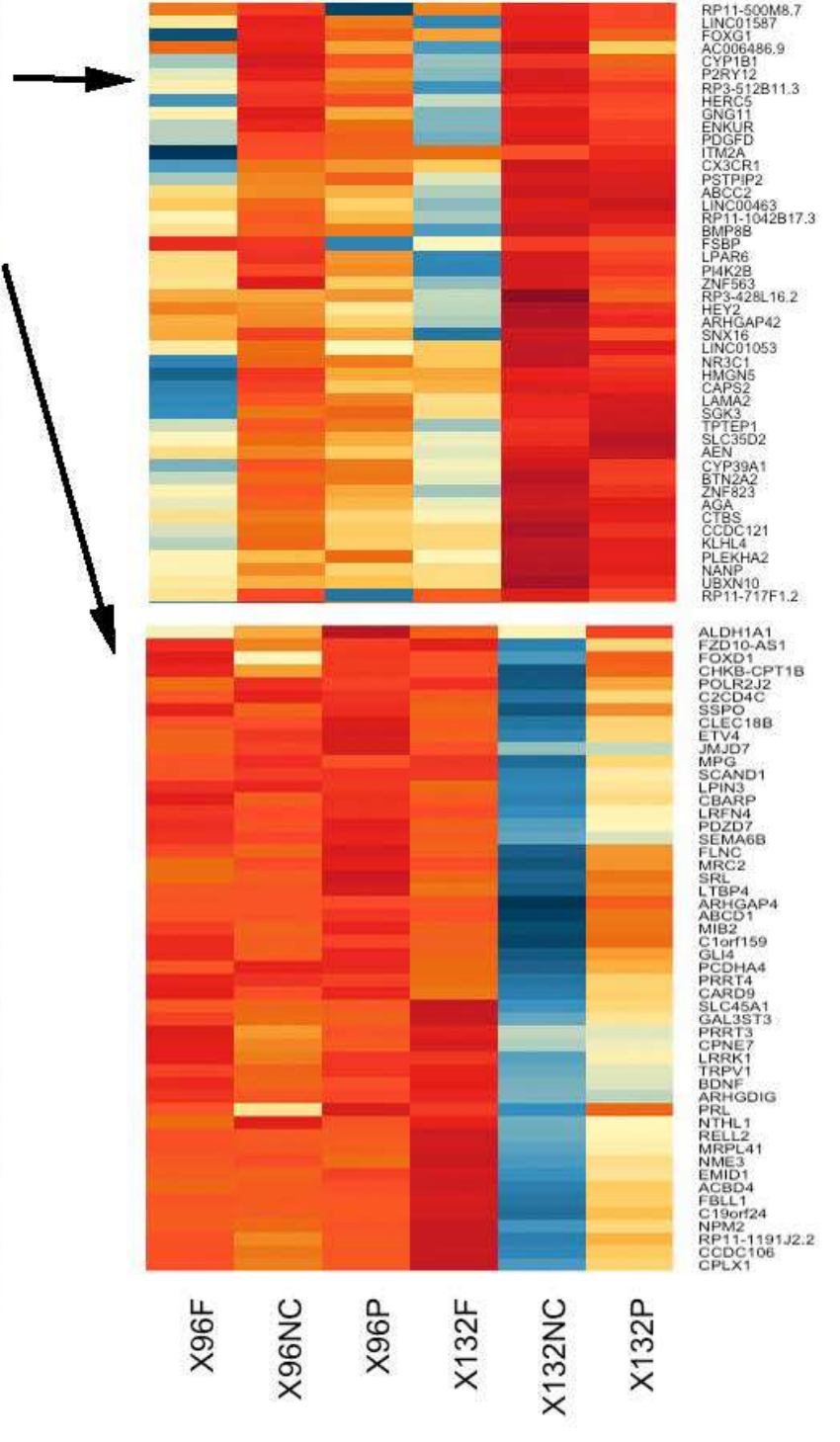
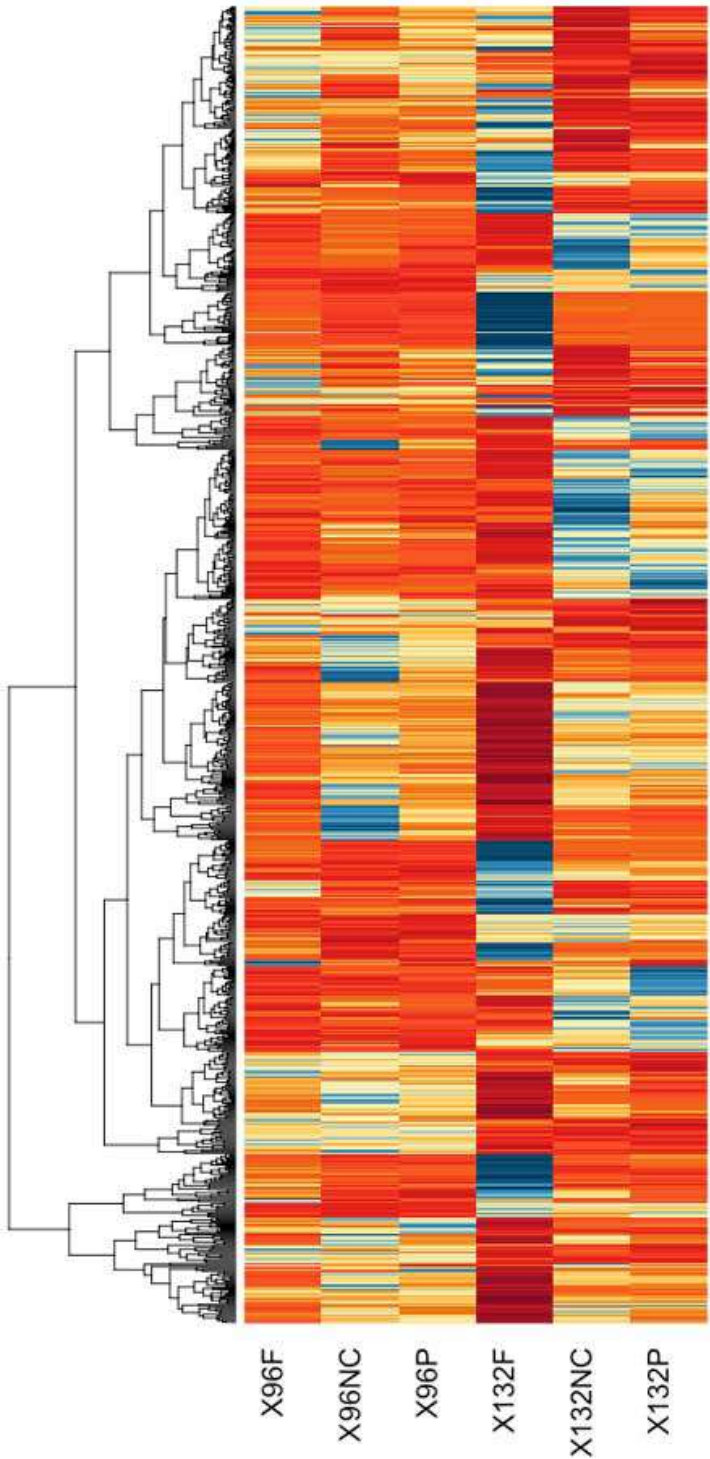
Periphery Central Macula

B**Cones****Rods****Ganglion****Muller glia****Synapse****FGF signaling**

D59P D59P-2 D73P D96P D132P D59C D59C-2 D96NC D132NC D73M D96M D132M

Periphery Central Macula





ATOH7
D89

Temporal

Fovea

Optic
nerve

Nasal

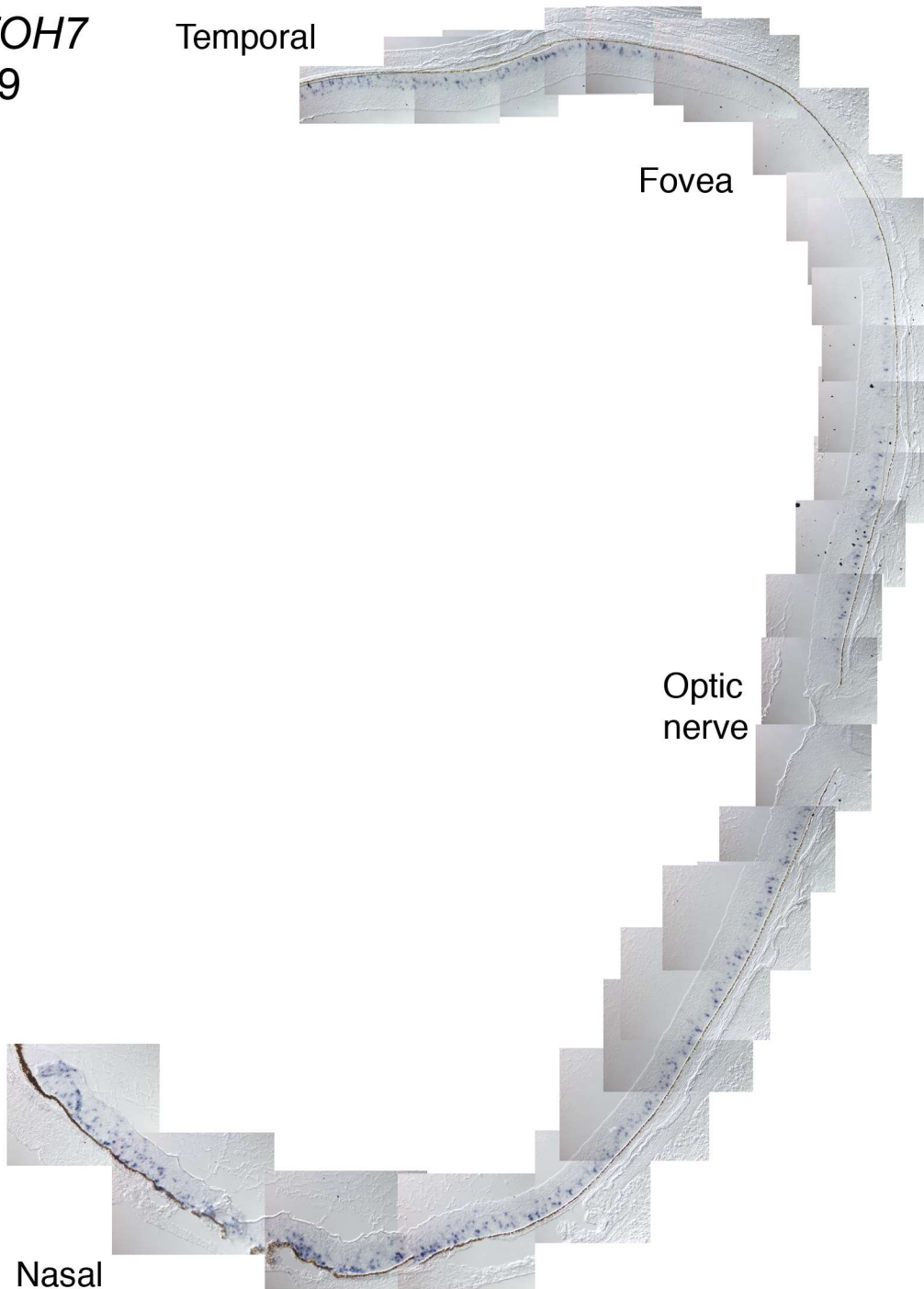


Table S1

Sample_ID	Sample_day	RIN
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rsem_star_trimmed_R42015-066pf_53d-F12-IR_Aligned.toTranscriptome.genes.results	D53	10
rsem_star_trimmed_R42015-057pf_57d-F3-IR_Aligned.toTranscriptome.genes.results	D57	7.7
rsem_star_trimmed_R42015-070pf_57d-F16-IR_Aligned.toTranscriptome.genes.results	D57-2	9.2
rsem_star_trimmed_R42015-060pf_67d-F6_Aligned.toTranscriptome.genes.results	D67	7.9
rsem_star_trimmed_R42015-065pf_67d-F11-IR_Aligned.toTranscriptome.genes.results	D67-2	8.9
rsem_star_trimmed_R42015-064pf_70d-F10-IR_Aligned.toTranscriptome.genes.results	D70	8.8
rsem_star_trimmed_R42015-058pf_80d-F4-IR_Aligned.toTranscriptome.genes.results	D80	7.8
rsem_star_trimmed_R42015-061pf_87d-F7_Aligned.toTranscriptome.genes.results	D87	9.7
rsem_star_trimmed_R42015-067pf_94d-F13-IR_Aligned.toTranscriptome.genes.results	D94	9
rsem_star_trimmed_R42015-068pf_94d-F14-IR_Aligned.toTranscriptome.genes.results	D94-2	8.7
rsem_star_trimmed_R42015-071pf_105d-F17-IR_Aligned.toTranscriptome.genes.results	D105	9.3
rsem_star_trimmed_R42015-062pf_107d-F8-IR_Aligned.toTranscriptome.genes.results	D107	9.3
rsem_star_trimmed_R42015-069pf_115d-F15-IR_Aligned.toTranscriptome.genes.results	D115	8.7
rsem_star_trimmed_R42015-063pf_125d-F9-IR_Aligned.toTranscriptome.genes.results	D125	8.1
rsem_star_trimmed_R42015-055pf_132d-F1-IR_Aligned.toTranscriptome.genes.results	D132	8.3
rsem_star_trimmed_R42015-056pf_136d-F2-IR_Aligned.toTranscriptome.genes.results	D136	8.2
rsem_star_trimmed_R42017-023pf_59dH-27008Central_merge_Aligned.toTranscriptome.genes.results	D59C	7.2
rsem_star_trimmed_R42017-024pf_59dH-27008Periphery_merge_Aligned.toTranscriptome.genes.results	D59P	9
rsem_star_trimmed_R42017-025pf_59dH-27031Central_merge_Aligned.toTranscriptome.genes.results	D59C-2	9.3
rsem_star_trimmed_R42017-026pf_59dH-27031Periphery_merge_Aligned.toTranscriptome.genes.results	D59P-2	9.8
rsem_star_trimmed_R42017-027pf_73dFovea_merge_Aligned.toTranscriptome.genes.results	D73M	8.7
rsem_star_trimmed_R42017-028pf_73dPeriphery_merge_Aligned.toTranscriptome.genes.results	D73P	7.8
rsem_star_trimmed_R42017-029pf_96dFovea_merge_Aligned.toTranscriptome.genes.results	D96M	8.8
rsem_star_trimmed_R42017-030pf_96dNasalCentral_merge_Aligned.toTranscriptome.genes.results	D96NC	7.5
rsem_star_trimmed_R42017-031pf_96dPeriphery_merge_Aligned.toTranscriptome.genes.results	D96P	8.7
rsem_star_trimmed_R42017-032pf_132dFovea_merge_Aligned.toTranscriptome.genes.results	D132M	6.8
rsem_star_trimmed_R42017-033pf_132dNasalCentral_merge_Aligned.toTranscriptome.genes.results	D132NC	8.7
rsem_star_trimmed_R42017-034pf_132dPeriphery_merge_Aligned.toTranscriptome.genes.results	D132P	6.8

Table S4

NRL
NR2E3
VSX1
THRB
NEUROG2
PIAS3
RORA
CRX
HES1
NR2F1
NOTCH1
PRDM1
LHX2
SIX6
RAX
ONECUT1
ONECUT2
OTX2
RXRG
NEUROD1
ASCL1
ATOH7
SIX3
PAX6
RORB
VSX2
NR2E1
GAD2
GAD1
TFAP2A
PROX1
PARVA
ELVAL3
CALB2
CALB1
ELAVL4
SYP
DLG4
GABARAPL2
UNC119
GRINA
CLSTN3
CLSTN1
SYT1
GNAI2
SYT11
EGFLAM
ROBO2

Table S4

STX3
SLC17A7
EPHB2
CTBP2
SYT4
CHRNA4
AQP4
CAV1
CAV2
KCNJ10
SOX9-AS
CA2
RLBP1
SLC1A3
SOX2
GLUL
CABP5
GRM6
PRDM8
LHX4
BHLHE22
PRKCA
RCVRN
AIPL1
ROM1
GUCA1
GNGT2
PDE6H
OPN1SW
GNAT2
PDE6B
GNAT1
CNGB1
CNGA1
RHO
NEFM
POU4F2
EBF1
ISL1
SYT13
NRP1
IRX2
POU4F1
GFI1
POU4F3
WT1
SNCG
GAP43

Table S4

INA
PTF1A
LHX1
TH
SLC18A3
CHAT
SLC32A1
FOXN4
SLC6A9
STX1A
PDE6A
GUCA1A
GUCA1B

Table S5

NRL
NR2E3
VSX1
THRB
NEUROG2
PIAS3
RORA
CRX
HES1
NR2F1
NOTCH1
PRDM1
LHX2
SIX6
RAX
ONECUT1
ONECUT2
OTX2
RXRG
NEUROD1
ASCL1
ATOH7
SIX3
PAX6
RORB
VSX2
NR2E1
GAD2
GAD1
TFAP2A
PROX1
PARVA
CALB2
CALB1
ELAVL4
SYP
DLG4
GABARAPL2
UNC119
GRINA
CLSTN3
CLSTN1
SYT1
GNAI2
SYT11
EGFLAM
ROBO2
STX3

Table S5

SLC17A7
EPHB2
CTBP2
SYT4
CHRNA4
CAV1
CAV2
KCNJ10
CA2
RLBP1
SLC1A3
SOX2
GLUL
CABP5
GRM6
PRDM8
LHX4
BHLHE22
PRKCA
RCVRN
AIPL1
ROM1
GNGT2
PDE6H
OPN1SW
GNAT2
PDE6B
GNAT1
CNGB1
CNGA1
RHO
NEFM
POU4F2
EBF1
ISL1
SYT13
NRP1
IRX2
POU4F1
POU4F3
SNCG
GAP43
INA
PTF1A
LHX1
TH
CHAT
SLC32A1

Table S5

FOXN4
SLC6A9
STX1A
PDE6A
GUCA1A
GUCA1B
RAX2
NEUROD4
HES5
HEY2
DLX1
DLX2
EOMES
BARHL2
EBF2
EBF3
EBF4
SALL3
NR4A2
SATB2
NR2F2
RB1
IRX5
SOX8
SOX9
GNB1
GNB3
GNGT1
PDE6C
PDE6G
CNGA3
CNGB3
GUCA1C
GUCY2D
RGS9
GNB5
ARR3
GRK1
SLC24A1

Supplementary Figure Legends

Figure S1, related to Figure 1. **A.** Workflow for whole retina RNA-seq analysis. **B.** Principal components analysis (PCA) plot showing the four outliers removed from the study. **C.** The number of mapped reads per sample. **D–E.** AP clustering of genes that were differentially expressed between the two youngest (D52/53 and D53) and two oldest (D132, D136) samples. The clustering yielded 62 clusters (**C**). The tree was cut at the indicated red line in (**C**), which yielded 6 superclusters (SCs).

Figure S2, related to Figure 2. **A.** Single cell RNA-seq data from Pollen et al.,: 84 cells from D63 retina were clustered using *SOX2* (progenitor) and *POU4F2* (ganglion cell). Two predominant clusters were apparent corresponding to these two cell types. Right panel: a D59 peripheral retina immunostained with *SOX2* (magenta) and *POU4F2* (green). **B.** Hierarchical clustering of just the three main clusters generated from *SOX2*⁺ cells. Cells in red and blue contained other cell cycle genes characteristic of progenitor cells. Furthermore, the red cluster was identified as “early retinal progenitors (ERPs)” expressing *PRTG*, while the blue cluster was *PRTG*⁻ and labeled “late retinal progenitors (LRPs)”. **C.** The top 1000 differentially expressed genes in ERPs and LRPs were compared against the genes present in our superclusters (SCs) 1–6; ERP genes associated more with SCs 1 and 2, whereas more LRPs were represented in SCs 3 and 4. **D.** Genes in the three cortical progenitor populations—ventricular radial glia (vRG), intermediate progenitors (tRG), and outer radial glia (oRG)—were compared to retinal genes in SCs 1–6. Our analysis revealed that vRG genes were more likely to be in SCs 1 and 2. In contrast, more oRG genes were detected in SCs 2–4.

Figure S3, related to Figure 5. **Synapse formation in the fovea. A.** Three pre-synaptic markers, SYP, CTBP2 (ribbon), and SLC17A7 (glutamatergic) expression were observed first in the inner plexiform layer (IPL) as early as D67 and in both IPL and outer plexiform layer (OPL) from D96. The post-synaptic marker, DLG4, was observed from D67/73. All of the synaptic markers were visualized in green. **C.** Synapse-related genes were identified using GO term analysis, and the Counts Per Million (CPM) values of a subset of the genes were Log₂ transformed and plotted as a heatmap. GCL: Ganglion Cell Layer; INL: Inner Nuclear Layer; NBL: Neuroblastic layer; ONL: Outer Nuclear Layer.

Figure S4, related to Figure 1. **Comparison of fetal RNA-seq datasets from present and previously published Aldiri, *et al.* study. A.** Principal component analysis (PCA) of the gene level expression data from Aldiri, *et al.* Principal component (PC) 1 vs. PC2 in the upper panel and PC2 vs. PC3 in the lower panel demonstrate discordance of FW18 and FW10 from the remaining samples, indicating that these samples are outliers in the data. **B.** PCA of the gene level expression data of the Aldiri *et al.*, (cool hues with sample names starting with “F”) and the data from this report (hot hues with sample names starting with “D”). PC1 vs. PC2 in the upper panel indicates the largest variance to be associated with experimental factors attributed to the two investigations, and the second largest variance to be linked to the age of the samples. PC2 vs. PC3 in the lower panel further show FW18 to be an outlier from other samples. **C.** Heatmap comparing expression values from the two reports of genes from 3 epochs described in

Figure 1C. **D.** Heatmap comparing expression values from the two reports of photoreceptor genes highlighted in Figure 4C.

Figure S5, related to Figure 6A, B. **RNA-seq of macula, nasal central, and peripheral retina.** **A.** Alignment results after RNA-seq data processing. **B and C.** Genes that were differentially downregulated (**B**) or upregulated (**C**) (as identified by DESeq) between D59 central and D132 macula samples.

Figure S6, related to Figure 6C. **Heatmaps of genes known to be related to retinal cell types, synapses, and FGF signaling.**

Figure S7, related to Figure 6. **Hierarchical clustering of genes using K-median and cosine correlation.** Genes that are upregulated or downregulated early in the macula vs. nasal central and periphery are highlighted.

Figure S8, related to Figure 6E. ***In situ* hybridization for ATOH7 in a nasal-temporal section of D89 retina.** *ATOH7* is no longer expressed in the fovea at this stage, but *ATOH7*-expressing cells are present in peripheral and posterior temporal retina (asterisk) and the entire nasal retina. This is consistent with the hypothesis that foveal development is accelerated with respect to the rest of the retina. NBL: neuroblastic layer; GCL: ganglion cell layer; INL: inner nuclear layer, ONL: outer nuclear layer.

Table S1, related to Figure 1. List of samples and their RNA Integrity Number (RIN).

Table S2, related to Figure 1. List of 3072 differentially expressed genes between the youngest two (D52/54 and D53) and oldest two (D132/D136) retina. Also incorporated are the supercluster information and the correlation with mouse.

Table S3, related to Figure 6. List of genes that are differentially expressed between D59 central retina and D132 macula.

Table S4, related to Figure 7. List of 109 genes whose expression was used to compare the development stages between mouse and human retina. These genes are expressed in various cell types of the retina and share homology between mouse and human.

Table S5, related to Figure 8. List of 135 genes whose promoters were used for scanning transcription factor binding sites. The binding site data from this list of genes was used to construct the potential gene network.