

**Supplemental files for:**  
**Partitioning of gene expression among zebrafish photoreceptor subtypes**

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**Supplemental Table S1**

Revised gene names and accession numbers

gene name	original name*	Gene ID (NCBI or Ensembl)
<i>pde6hb</i>	<i>zgc:73359</i>	393810 (NCBI gene ID)
<i>guca1e.2</i>	<i>si:ch211-103a14.5</i>	ENSDARG00000100747
<i>cngb1b</i>	<i>si:dkey-44k1.5</i>	ENSDARG00000042107
<i>fibcd1a</i>	<i>CU639468.1-si:dkeyp-51b7.3</i>	ENSDARG00000074640
<i>mir726</i>	<i>CT573282.1</i>	ENSDARG00000082495
<i>hsf4-like</i>	<i>si:dkey-18a10.3</i>	ENSDARG00000090814
<i>lbh-like</i>	<i>si:ch1073-303d10.1</i>	ENSDARG00000103006

\* “original name” is the gene name in the assembled transcript annotation that was used in our scRNA-seq analysis (See Methods).

## Supplemental Table S2

Zebrafish lines used for quantitative PCR analysis

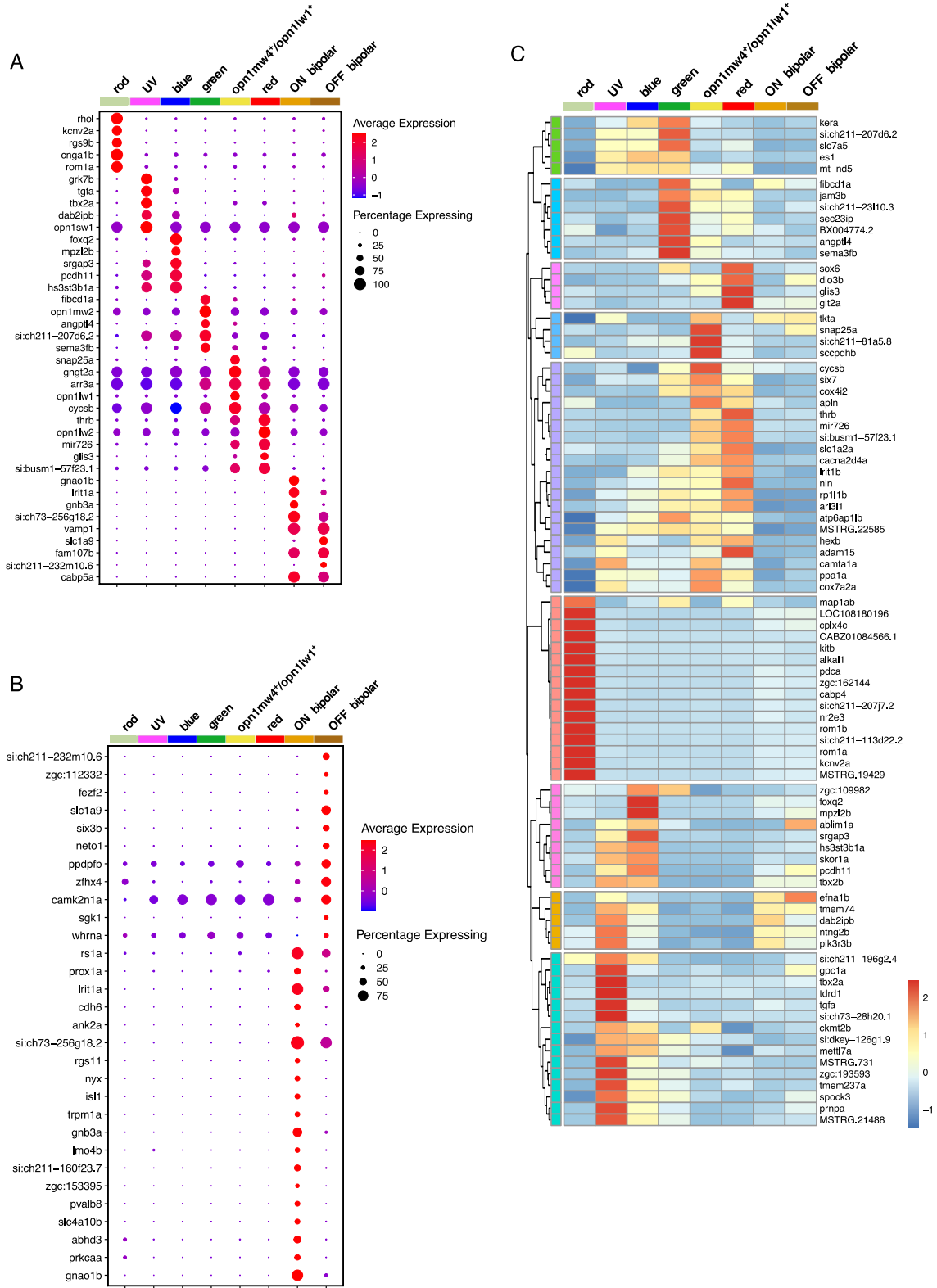
Genotype	Age (months)	Reference
<i>Tg(rho:EGFP)ja2Tg</i>	7-8	(1)
<i>Tg(-5.5opn1sw1:EGFP)kj9Tg</i>	10-11	(2)
<i>Tg(-3.5opn1sw2:EGFP)kj11Tg</i>	9	(3)
<i>Tg(opn1mw2:EGFP)kj4Tg</i>	9	(4)
<i>Tg(thrb:Tomato)q22Tg</i>	18	(5)
<i>TgBAC(vsx1:GFP)nns5Tg</i>	10	(6)

### Supplemental Table S3

#### Primers used for quantitative PCR

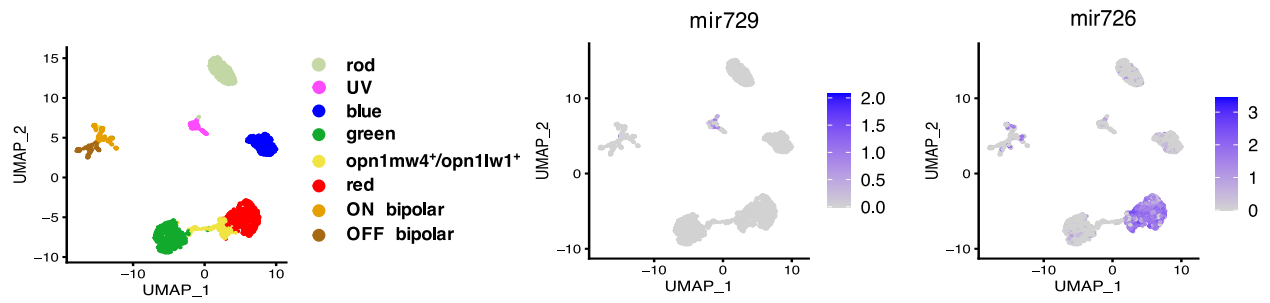
Target gene	Forward (5' to 3')	Reverse (5' to 3')
<i>rho</i>	ACTTCCGTTTCGGGGAGAAC	GAGTGCGGGTGTAGTAGTCG
<i>opn1sw1</i>	CGAGAGATATGTGGTCATCTG	TGTATCTGCTCCATCCAAAG
<i>opn1sw2</i>	GGAGGAATGGTGAGTTTGTG	GGTCTTGAAGGTAAAGTTCC
<i>opn1mw2</i>	GCGTGGGTAGATTAGTTGTG	GGCTTATGCTCAGATTTAGTGG
<i>opn1lw1</i>	TTATGTCTTCATGAACCGACAG	ATTTTCATCTTTTCCCCATGTC
<i>rpl13a</i>	TCCCAGCTGCTCTCAAGATT	ACTTCCAGCCAACCTCATGG
<i>angptl4</i>	GAGATGACACCCGAAGGAGG	GCCACCTTTGGACACAGAATG
<i>cabp5a</i>	GCCAAAATTGACCGAGAGCTGG	CAGGTTCCCCAGATCCTTACAG
<i>si:busm1-57f23.1</i>	GCTAGCACTCAGGTTACAAATAGG	CCATGGTGACCTCAAACCTGTC
<i>mpz12b</i>	TAAATCCGCCCGACATCCAG	CCAGAAATCCCGATTTCAGAG
<i>tbx2a</i>	ACTGGACCAGCAACAAGTCC	GTCTTGACTCTGTCCTCGC
<i>gnb3a</i>	ATGCATTGGGGGACAGACTC	TTGCATGCACCTTATTGGTGG
<i>gnb3b</i>	TGCTCACACAGGTTACTTGTC	AGTCTCCAAATCCCACAGTGC
<i>grk1a</i>	GAGACCTCAAGCCGGAGAAC	GCGCCATGAACCCTGGAG
<i>grk7a</i>	GAGCACAAGAACTTCGACGC	ATTAGTCCAGCCTCCAAGCG
<i>grk7b</i>	GGACCGCCTTGGCAGC	TGTCACCTGTGTCCTTAGCG
<i>rcvrn2</i>	CTGCCAGGCTATCTTTAAGCTG	TAAACTCGCCCTCAGCCAAT
<i>rcvrn3</i>	CTAAAGCTGGAGTGGGCCTT	TTTTCTCTGGCGTGCTCTCA
<i>gngt1</i>	CGTAAGCAGCAAAAATGCCG	GGATCTTCATCCGCTCCTCC
<i>gngt2a</i>	GTGACCTGTTGCCTCCATCG	TTTAGAGACAGGCTCTCTGGT
<i>gngt2b</i>	ACACACTTCTCTGACTGCCG	TTGCTGAAACCGCGGTACG
<i>gucal1a</i>	CTGGATCCAAAAGCCAATGCC	CGGCCACGTACTCCATAAAAG
<i>gucal1c</i>	TCACGTTTGACATGGACGGG	CCTGGATGGCCTTGAATATGGT
<i>gucal1e</i>	GCTCTTCGATATGGACGGCA	GACAACTCGCCGTCTCCATT
<i>gucal1e.2</i>	AGCTGCGCTGGTATTTCAAG	ACACTCCGTCTGCATTGAGG

# Supplemental figure



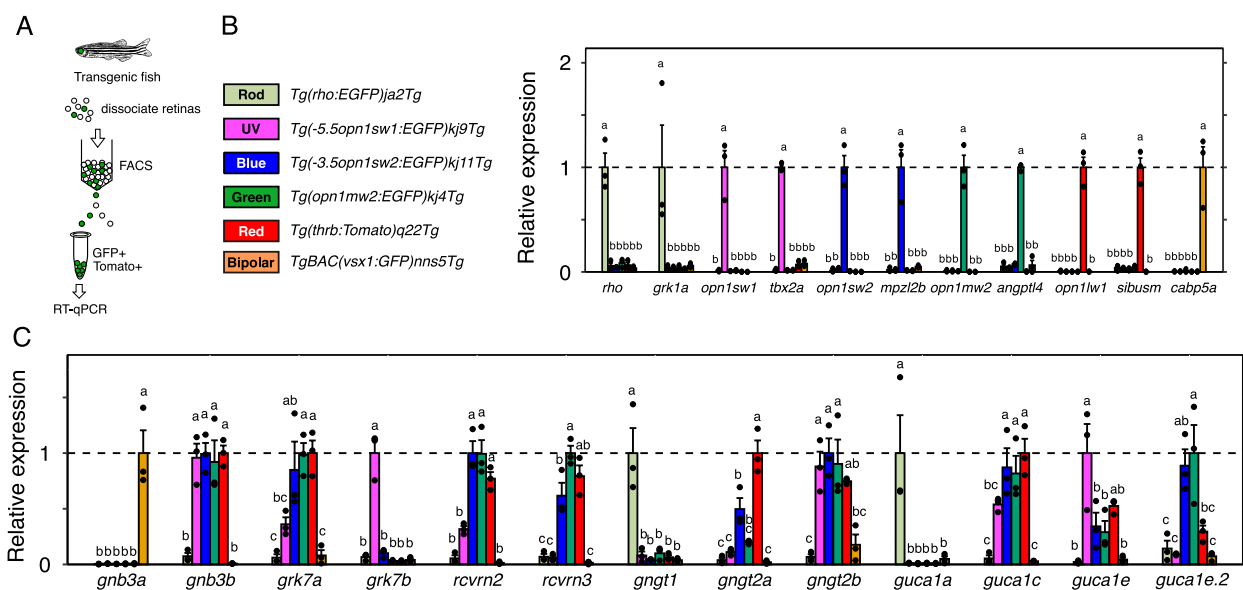
**Figure S1. Expression patterns of cluster-enriched marker genes and phototransduction genes**

(A) Dot plot showing the expression patterns of the top five differentially enriched genes for each cluster. Genes were ranked by adjusted p-value and grouped by cell cluster. Dot size reflects the percentage of cells within the cluster expressing the gene, and dot color indicates average expression level within the cluster. Values are row-wise Z-scored gene-expression values. Note that our single-cell data shows evidence of cross-cluster contamination with ambient RNA from several genes, most notably *opn1sw1*, *arr3a*, and *gngt2a*. (B) Dot plot showing the expression patterns of the 30 most differentially enriched genes between ON and OFF bipolar cells. (C) Heatmap showing average expression levels of the top 15 differentially expressed genes. Phototransduction isoform genes were excluded in this analysis. Values are row-wise Z-scored gene-expression values. The marker genes were arranged by hierarchical clustering with the ‘complete’ agglomeration method using the average gene expression levels.



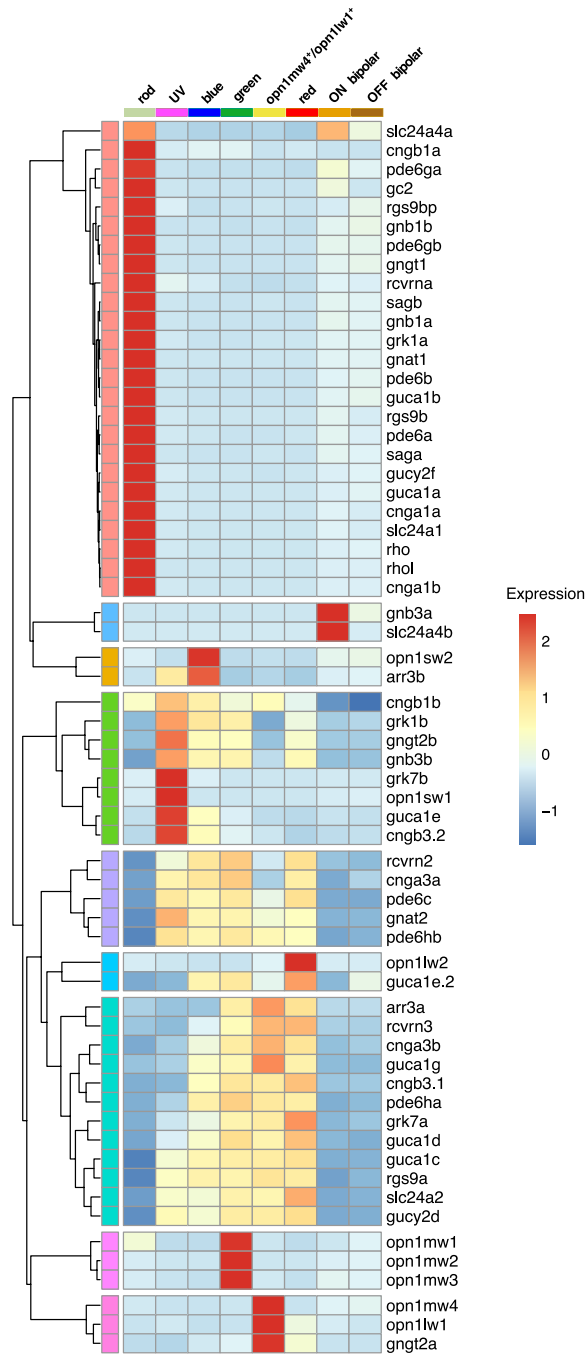
**Figure S2. Expression patterns of pre-microRNA genes**

Left: UMAP plot of cell clusters from Fig. 1C, reproduced here for reference. Right: Expression patterns of pre-microRNA genes.



**Figure S3. RT-qPCR analysis of differentially expressed genes using cDNA from isolated photoreceptors subtypes and bipolar cells**

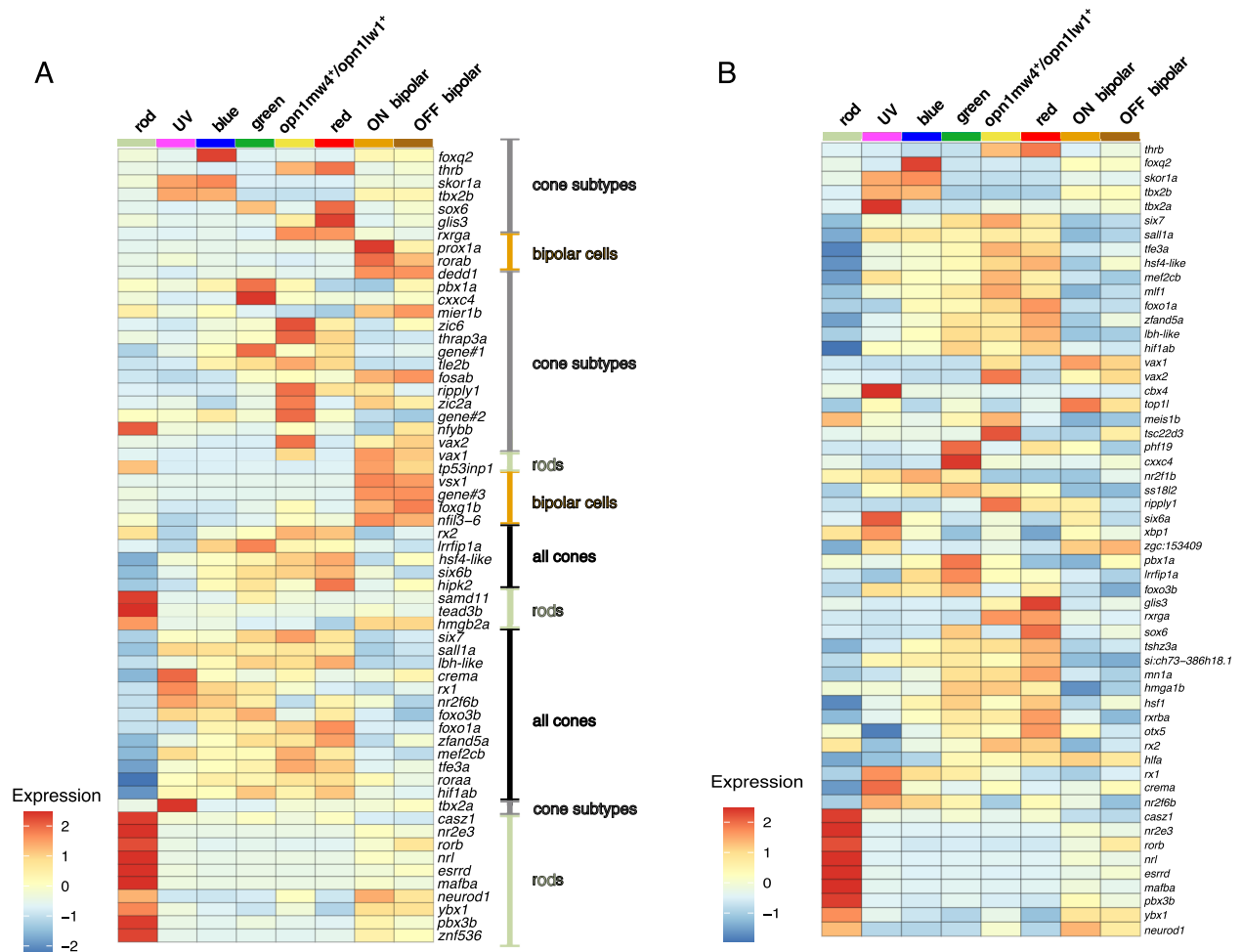
(A) Approach for isolating rods, cone subtypes, and bipolar cells from the indicated transgenic zebrafish lines. GFP- or tdTomato-positive cells were collected from adult fish of each line. (B-C) RT-qPCR of cluster-enriched marker genes (B) and phototransduction genes (C). Data are represented as mean  $\pm$  SEM ( $n = 3$ ), while individual values are shown as markers. The transcript levels were normalized to ribosomal protein L13a (*rpl13a*) transcript levels. qPCR results were evaluated by Tukey's honest statistical difference (HSD) test. Distinct letters indicate statistically significant differences ( $P < 0.05$ ). "sibusm" represents *si:busm1-57f23.1*.



**Figure S4. Heatmap showing hierarchically clustered expression patterns of phototransduction genes**

Related to Fig. 3C. Heatmap shows average expression levels of genes. Values are row-wise Z-scored gene-expression values. The genes were arranged by hierarchical clustering with the ‘complete’ agglomeration method using the average gene expression levels. All phototransduction genes except for *guca1e.2*, *guca1g*, *rcvrnb*, and *slc24a4b* were differentially expressed among photoreceptor and bipolar populations.

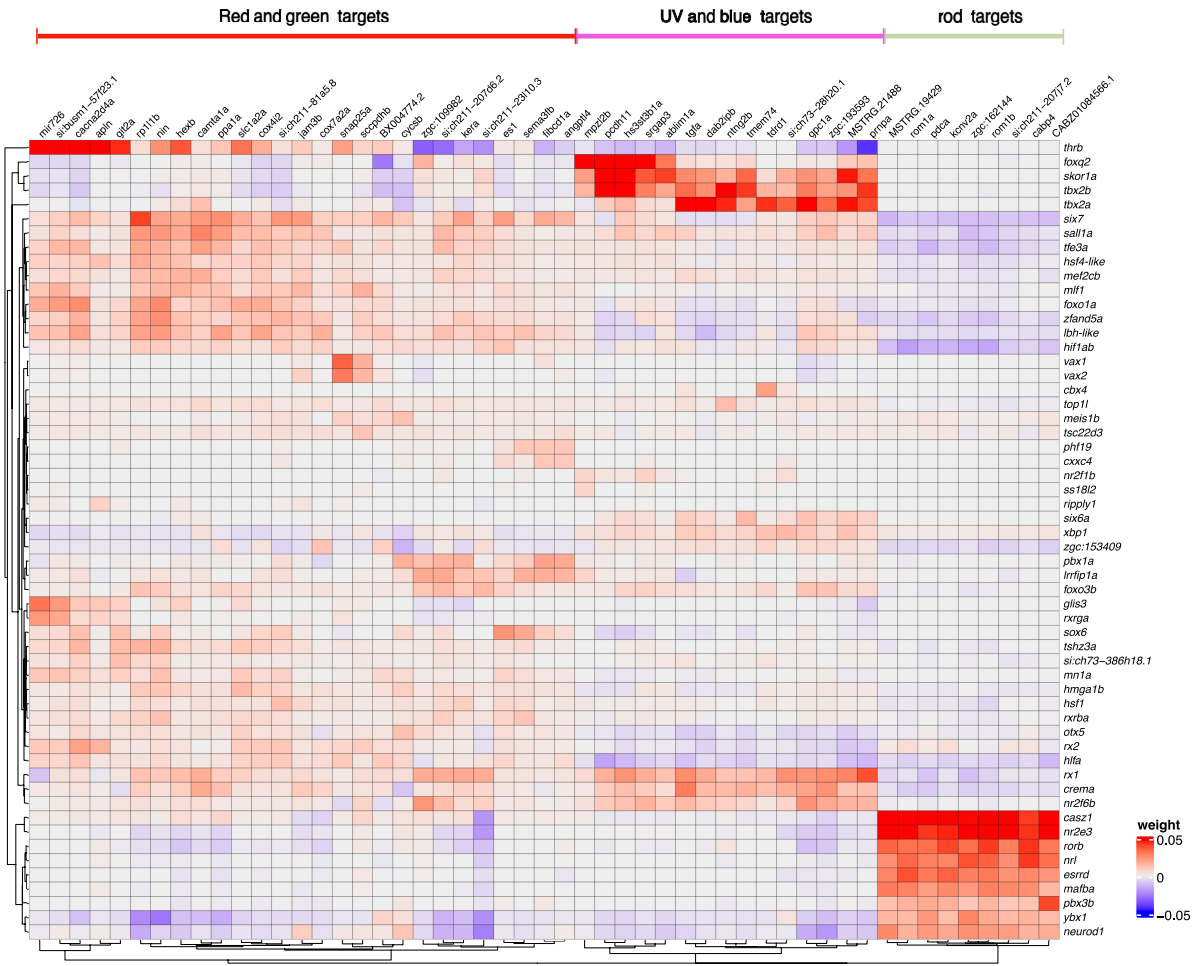




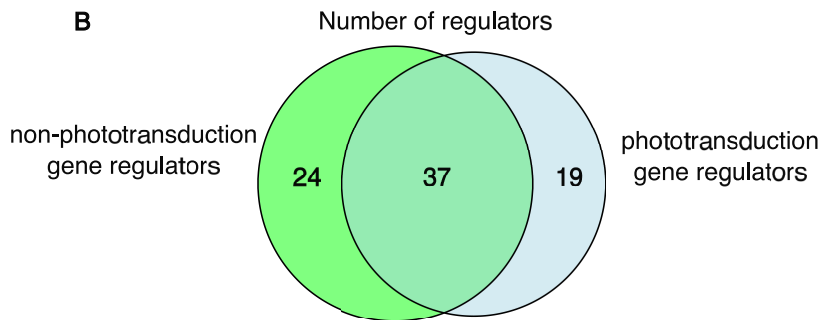
**Figure S5. Expression patterns of transcriptional regulators identified by SCENIC analysis**

(A) Heatmap showing the expression patterns of transcriptional regulators associated with phototransduction genes, presented in the same order as in Fig. 4. Gene#1: zgc:114046; Gene#2: zgc:110269; Gene#3: si:ch211-288g17.3. (B) Heatmap showing the expression patterns of transcriptional regulators associated with non-phototransduction genes, presented in the same order as in Fig. S6.

**A**



**B**



**Figure S6. Candidate transcriptional regulators responsible for expression of non-phototransduction genes**

Heatmap showing positive (red) and negative (blue) associations between transcriptional regulators (transcription factors and cofactors) and differentially expressed non-phototransduction genes (target genes) calculated by the GENIE3 algorithm in SCENIC. Rows and columns are arranged according to divisive hierarchical clustering (dividing clusters in a top-down manner). The (dis)similarity of observations was calculated using Euclidean distances. Cell type expression patterns of the transcriptional regulators are presented in Fig. S5B. **(B)** Venn diagram showing the number of transcriptional regulators identified by SCENIC analysis using two different sets of target genes: phototransduction gene (Fig. 4) and non-phototransduction genes (Fig. S6A).

## Supplementary Data S1. List of genes differentially expressed among adult zebrafish photoreceptors and bipolar cells

“cluster” represents cell cluster, in which the gene is differentially expressed compared to the cells in all the other clusters. “p\_val” represents unadjusted p-val. “p\_val\_adj” represents adjusted p-value, based on bonferroni correction using all genes in the dataset. “avg\_log2FC” represents the log fold change of average expression. “pct.1” represents the percentage of cells in the cluster, in which the gene is expressed, while “pct.2” represents the average percentage of cells in all the other clusters, in which the gene is expressed. “seqnames”, “strand”, “start”, “end”, and “width” represent gene location in the genome. “original name” is the gene name in our scRNA-seq analysis using the updated transcript annotation (See Methods). “gene” and “Geneid” represents gene name and gene id, respectively, in the annotation file (v4.3.2.gtf) used as an initial guide for the transcript assembly (See Methods). The columns (rod, UV, blue, green, opn1mw4\_opn1lw1, red, ON bipolar, and OFF bipolar) represent normalized pseudo-bulk RNA sequencing reads (transcripts per million, TPM).

## References

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