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Supporting Information for

A microfluidic platform enables comprehensive gene expression profiling of mouse retinal stem cells

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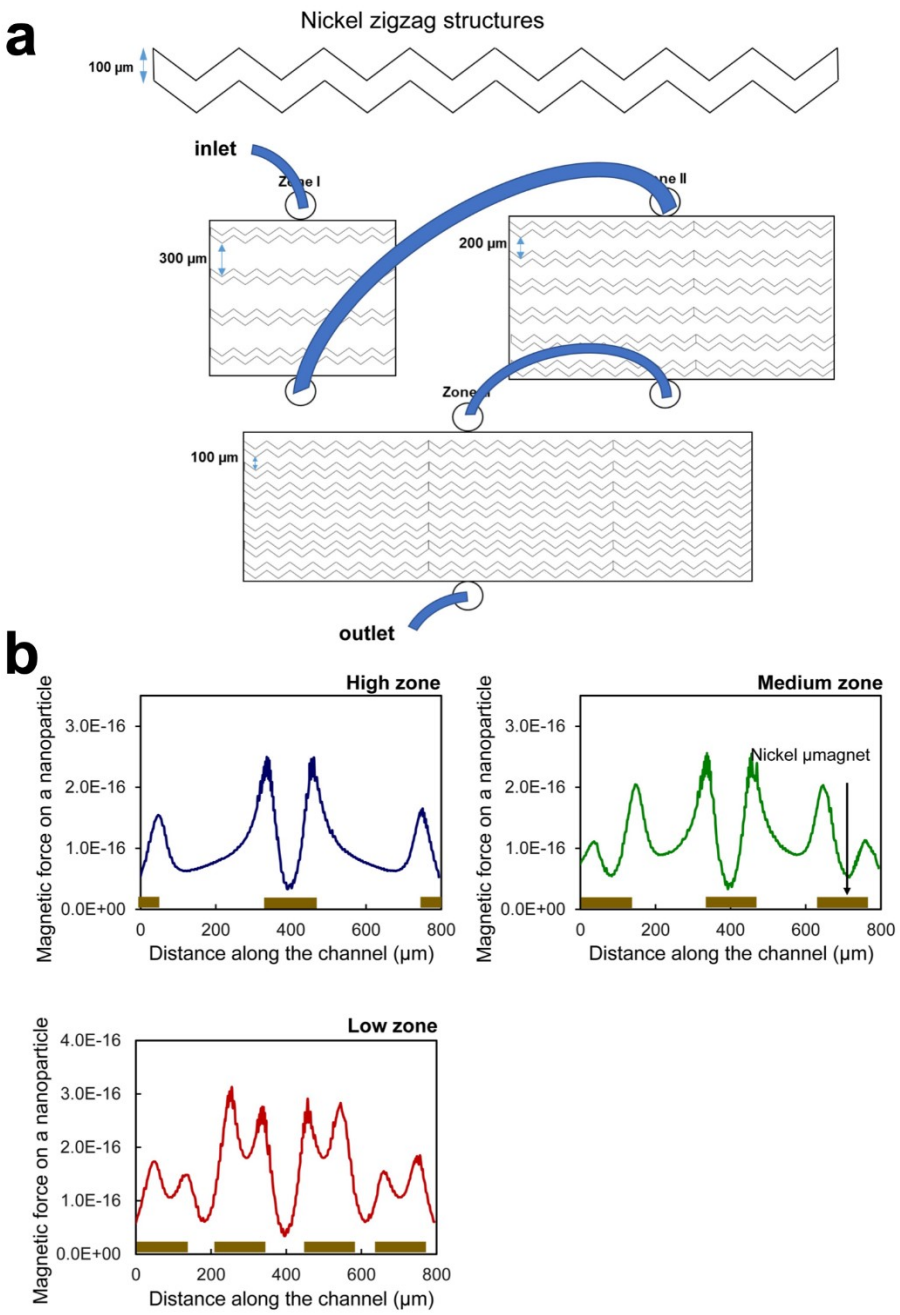

Figure S1. A representation of the microfluidic devise used for cell capture and release and the magnetic force involved. (a) Schematic representing the microfluidic device used for retinal stem cells capture and release. The device consists of three capture zones connected via tubing. Nickel micromagnets are incorporated inside the capture zones to enhance the external magnetic field. (b) Simulation of the magnetic force acting on a single magnetic nanoparticle inside each capture zone as a function of the distance along the channel at a height of 10 µm. The brown boxes show the position of nickel micro-magnets. The distance between the nickel structures is 300 µm, 200 µm and 100 µm, in high, medium, and low capture zones, respectively.

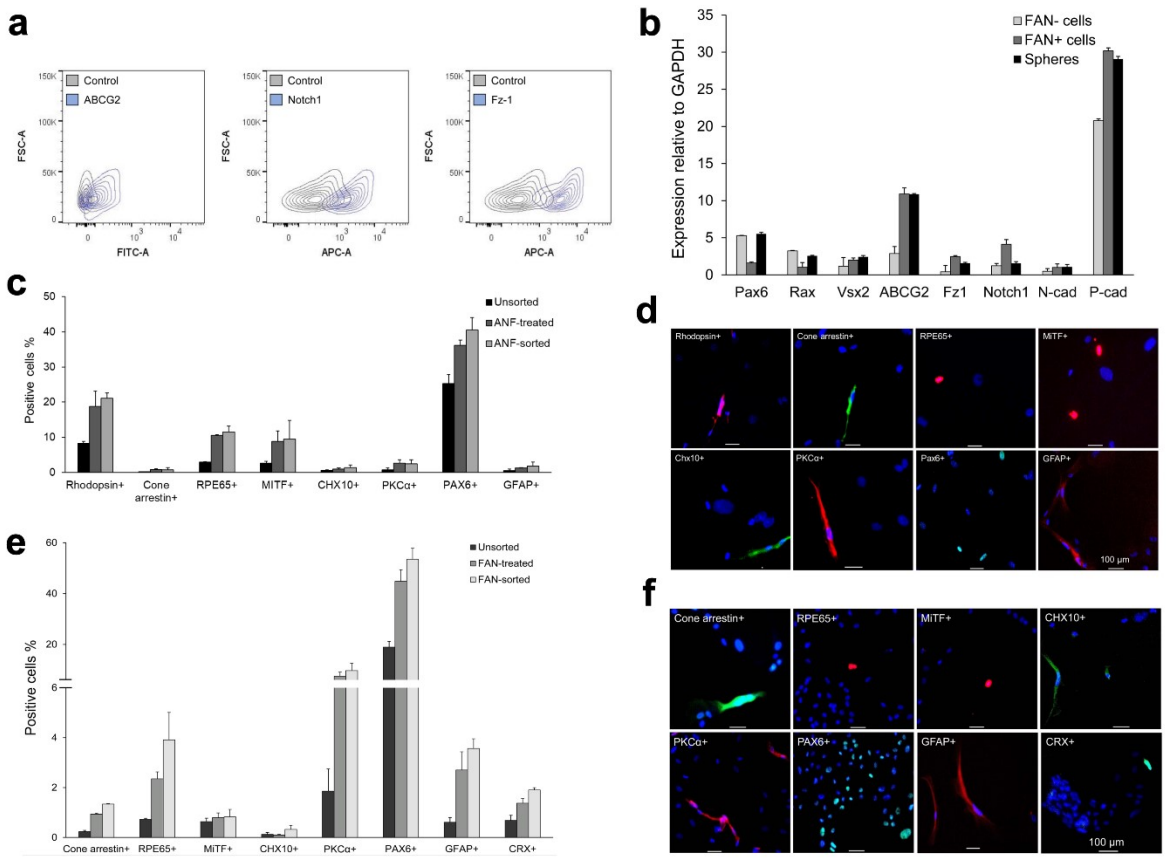

Figure S2. Characterization of ANF$^+$ ciliary epithelial (CE) cells populations. (a) Flow cytometric analysis of CE cells after immunostaining for ABCG2, Notch1 and Frizzled1 (Fz1). Control experiments were carried out using the isotypes. (b) RT-qPCR analysis of various retinal genes. These genes include *Abcg2*, *Notch1*, and *Fzd1* in sorted FAN$^+$, FAN$^-$ cells and formed spheres. Expression is calculated using ΔΔCT/RQ/FC. (c) Differentiation of mouse RSC spheres. Spheres isolated from unsorted/unlabeled and the enriched ABCG2$^+$/Notch1$^+$/Fz1$^+$ populations were cultured for 28 days under pan retinal conditions (1%FBS+FGF2 on laminin). Data are reported relative to total number of cells (DAPI$^+$). (d) The cells were immunostained for markers specific for rod photoreceptors: rhodopsin$^+$ cells (red), cone photoreceptors: cone arrestin$^+$ cells (green), mature retinal pigmented epithelia: RPE65$^+$ cells (red), immature retinal pigmented epithelia: MiTF$^+$ (red), bipolar and progenitor cells: Chx10$^+$ cells (green), bipolar cells: PKCα$^+$ cells (green), amacrine and progenitors: Pax6$^+$ cells (green), Müller glia: GFAP$^+$ cells (green). Nuclei were stained with DAPI (blue). (e) Differentiation of human RSC spheres. Spheres isolated from unsorted/unlabeled and the enriched ABCG2$^+$/Notch1$^+$/Fz1$^+$ population were cultured for 28 days under pan retinal conditions. The cells were cultured for 40 days under pan retinal conditions (1%FBS+FGF2 on laminin). Data are reported relative to total number of cells (DAPI$^+$). (f) Representative images of cone arrestin$^+$ cells (green), RPE65$^+$ cells (red), MiTF$^+$ cells (red), CHX10$^+$ cells (green), PKCα$^+$ cells (red), PAX6$^+$ cells (green), GFAP$^+$ cells (red), and CRX$^+$ cells (green). Nuclei were stained with DAPI (blue). Secondary antibodies were incubated directly with differentiated cells without primary antibodies as negative controls.

a

	Cluster '0'	Cluster '1'	Cluster '2'	Cluster '3'	Cluster '4'
1	Selenow	Nov	Ankrd13b	Prl2c5	Pvr
2	Tmsb10	Bbs12	Nradd	Olfr1406	Fam107b
3		Prim1	Btbd11	Fam19a1	Il15ra
4		Paqr4	Zfp518a	Atoh1	Ciart
5		Angptl2	Hus1	B3gnt5	Cftr
6		Cst6	Nov	Slc3811	Prr51
7		Nprl3	St6galnac4	Ttk	Uhrf1bp11
8		Bivm	Tmem179	Tmem91	Tnfrsf12a
9		Pgap2	Creld1	Vmn1r13	Zbtb10
10		Gchfr	Pecr	Dnah3	Gdf15

b

ScRNA Seq: Transcription Factors		
	Cluster '2'	Cluster '4'
1	NRF	ATF4
2	KLF13	SMAD4
3	CREB1	PLAU
4	Crx (Otx3)	HIVEP1
5	Elk1	NFKB1
6	HIF1A	MAX
7	MyoD1 (Myf3)	NR5A2
8	SERBF1	REPIN1
9	ZBTB7A	RELA
10	SRF	SND1
11	SIRT3	CLOCK
12	KLF1	NRF1
13	XrccA	SIN3A
14	AIRE	TP53 (P53)
15	POLE	EP300
16	EPAS1	TPAP2C
17	Notch1	NCOR1
18	CCNE1 (Cyclin	STAT5B
19		STAT6

c

Molecular signature of Non-RSC containing clusters				
Cluster 0: Potentially Trabecular Meshwork				
Col17a1	Tmsb10	Selenow	Krt5	Krt12
Apod	Tgfbi	Bbs12	Aldh3a1	
Cluster 1: Potentially Pigmented Ciliary Epithelium				
Tyr	Bbs12	Nov	Serpinf1	
Cluster 3: Potentially Corneal/Limbal cells				
Olfr1406	Piezo2	Hoxa9	Krt27	Vmnlr13
Slc38a11	Mki67	Krt5	Aldh3a1	Krt12
Pkp1				

Figure S3. Molecular characterization of the clusters. (a) Top 10 differentially expressed (DE) genes in each cluster. Wilcoxon rank-sum tests with false detection rate correction were used. Cluster '0' only has two DE marker genes. Colors correspond to tSNE plot cluster colors. (b) Enrichr analysis of RSCs-containing cluster 2 vs. cluster 4 showing the 19 most differentially expressed transcription factors based on gene expression. The yellow highlighted TFs are obtained from the Transfac & Jaspar position weight matrix (PWM) analysis and the blue highlighted TFs are obtained from protein-protein interaction (TF-PPI), the green highlighted TF was highly expressed in both the PWM and PPI analysis.[1] (c) Molecular signatures obtained from published papers and suggesting the identity of the 3 non-RSC clusters. None of the clusters fall perfectly into any category but do have higher expression of specific genes that are known to be enriched in trabecular meshwork, pigmented ciliary epithelium and the corneal/limbal region of the eye.[2-4]

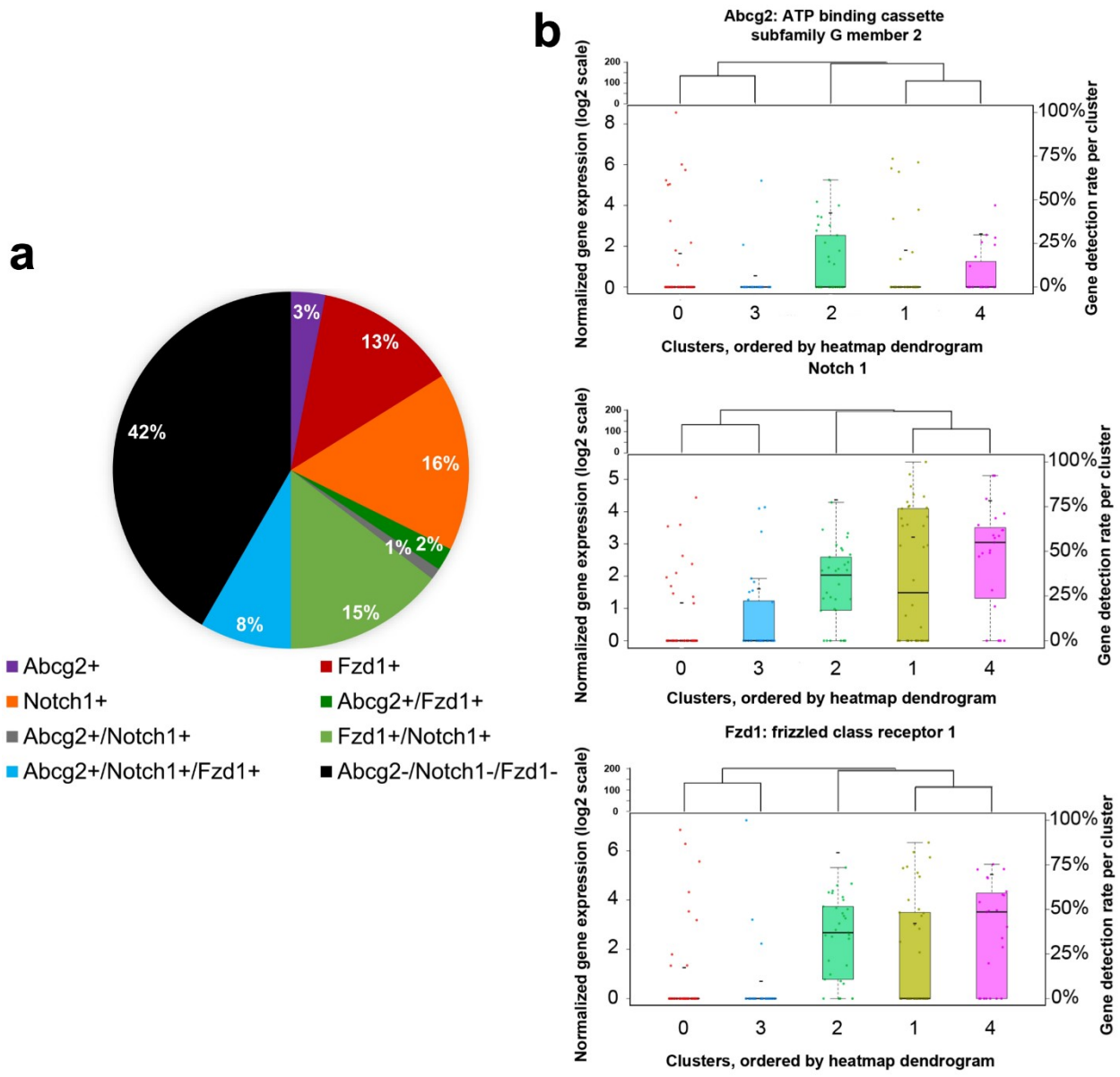

Figure S4. Expression analysis of *Abcg2*, *Notch1*, and *Fzd1* genes in the sequenced cells. (a) The genes were expressed in 58% of the cells. A subset of the sorted cells was plated for clonal sphere assay and 50% of the cells were able to form spheres. These data indicate that the RSCs express either one, two or a combination of all three markers. (b) Box plots showing interquartile range of *Abcg2*, *Notch1*, and *Fzd1* gene expression across clusters. Dots indicate normalized gene expression per cell, and the black dash indicates the proportion of cells in each cluster.

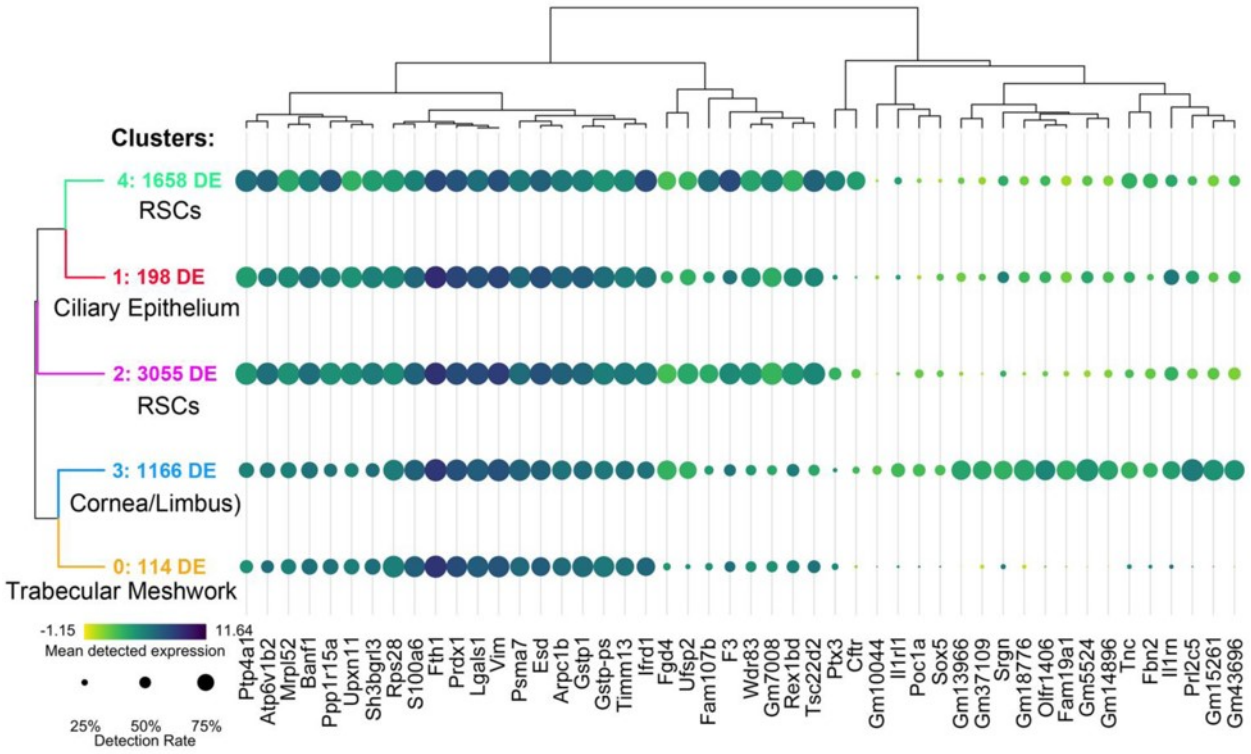

Figure S5. Modified heatmap generated by scClustvis software for differentially expressed (DE) genes *vs.* neighbour genes for all five clusters. This shows the top 10 genes that are most positively DE per cluster compared to their nearest neighbour. Wilcoxon rank-sum tests with false detection rate correction were used. The dot diameter indicates the number of cells within the cluster that are expressing that specific gene and the dot colors reflect the average normalized gene expression in the cells harboring the gene (i.e. the larger the dot, the more cells expressing the gene and the darker the dot, the higher the gene expression in those cells).

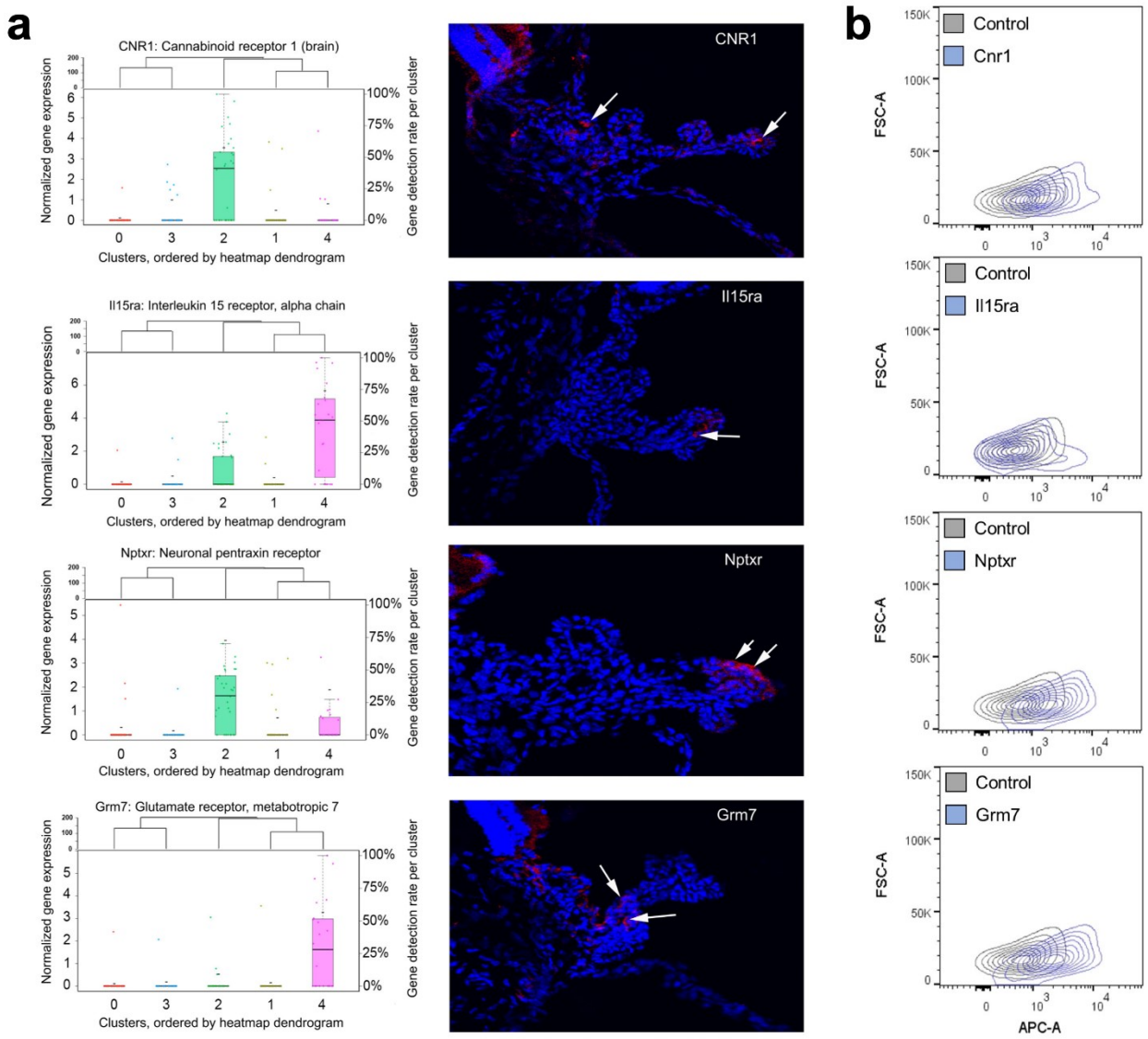

Figure S6. Validation of the cell-surface markers identified in clusters 2 and 4. (a) Box plots and images showing the expression of four novel cell-surface markers identified in clusters 2 and 4. *CNR1* is almost exclusively expressed in cluster 2 of RSCs. *Grm7* is almost exclusively expressed in cluster 4 of RSCs. *Il15ra* and *Nptxr* are almost exclusively expressed in clusters 2 and 4 of RSCs. Each box plot shows interquartile range of the gene expression across clusters, with dots indicating normalized gene expression per cell, and the black dash indicating the proportion of cells in each cluster. Fluorescence images of CE cells immunostained with antibodies specific for Cnr1, Il15ra, Nptxr, and Grm7. (b) Flow cytometric analysis of CE cells after immunostaining for Cnr1, Il15ra, Nptxr, and Grm7. Dissociated CE cells were incubated with primary antibodies specific to either Cnr1, Il15ra, Nptxr, and Grm7, followed by incubation with APC-streptavidin. Control experiments were carried out in which the cells were incubated first with biotin-labeled rabbit isotype control followed by incubation with APC-streptavidin.

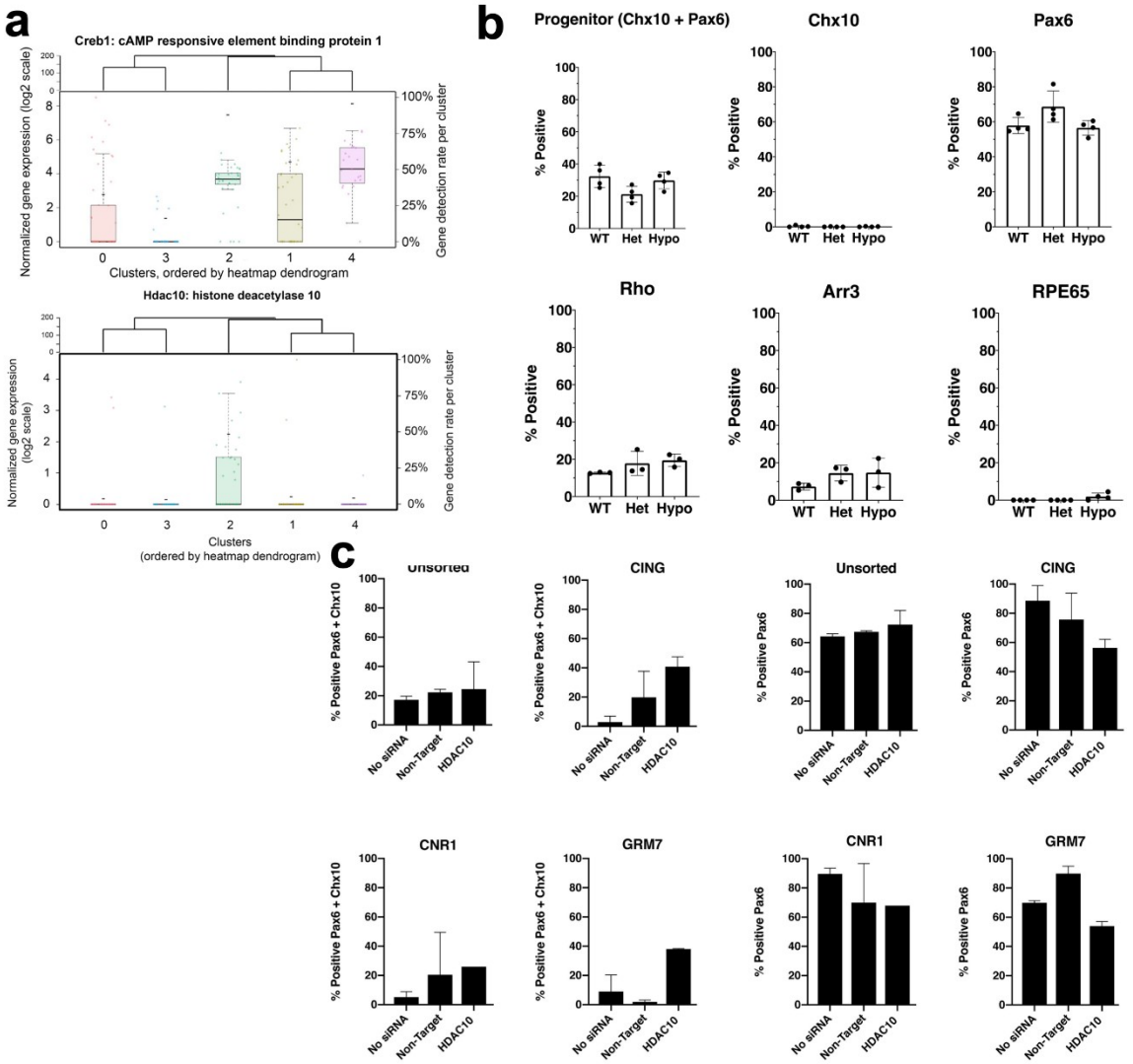

Figure S7. Validation of transcription factors identified in clusters 2 and 4. (a) Box plots showing the expression of two novel transcription factors identified in clusters 2 and 4. *Creb1* is expressed in both clusters 2 & 4 but has more downstream target genes expressed in cluster 2. *Hdac10* is almost exclusively expressed in cluster 2. (b) Creb1 mouse RSC sphere differentiation. Pan-retinal differentiation of RSC spheres derived from adult *Creb1* wild-type (WT), heterozygous hypomorphic (Het) and homozygous hypomorphic (Hypo) mice. There are no differences across the genotypes in their differentiation into progenitors/RSC ($Pax6^+/Chx10^+$), bipolar cells ($Chx10^+$), amacrine cells ($Pax6^+$), rod photoreceptors (Rho^+), cone photoreceptors ($Arr3^+$) or retinal pigmented epithelia ($RPE65^+$). (c) siRNA treated RSC sphere differentiation. Pan-retinal differentiation of RSC spheres derived from adult CD1 mice sorted by targeting Cnr1, Grm7 and CING ($Cnr1^+/Grm7^+/Il15ra^+/Nptxr^+$) and differentiated in pan-retinal medium in the presence of either SFM, control NT siRNA or *Hdac10* siRNA (Cluster 2 TF). There were no significant differences observed across the groups in the differentiation into progenitors/RSC ($Pax6^+/Chx10^+$) or amacrine cells ($Pax6^+$ alone). There were no $Chx10^+/Pax6^-$ cells observed, suggesting that there were no bipolar cells differentiating under any condition (not shown).

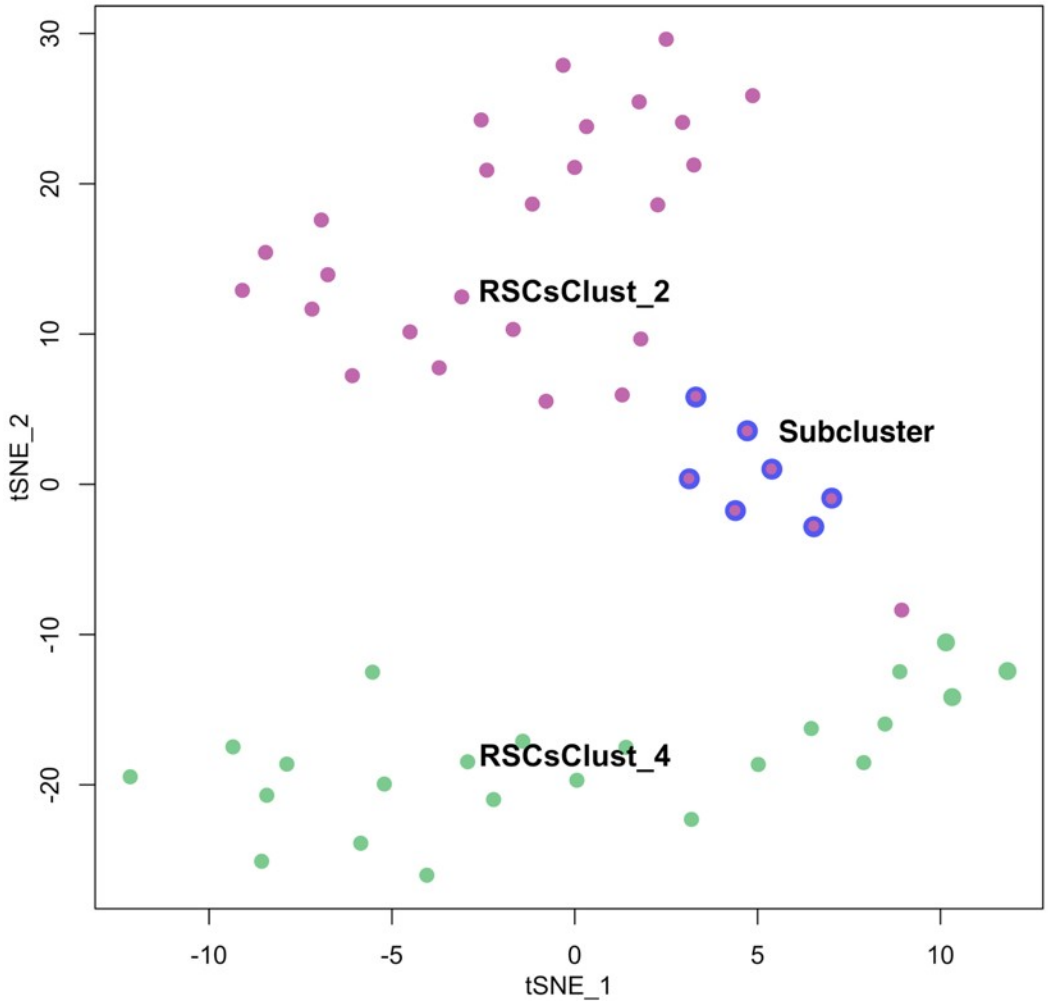

Figure S8. tSNE plot showing the reclustering analysis of RSCs-containing clusters 2 and 4. The purple dots represent the cells previously identified as cluster 2, the green dots represent the cells previously identified as cluster 4 and the pink dots with blue rings in the middle represent the newly identified cluster, which is identified based on genes with significant differential gene expression compared to either previously identified clusters.

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