Supplemental Tables and Figures

Generation of a Retina Reporter hiPSC Line to Label Progenitor, Ganglion, and Photoreceptor Cell Types

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Supplemental Table S1: Primers used for genotyping in figures 1 and 2

Location	**Forward (5'-3')**	**Reverse (5'-3')**	**Size**
Outside *VSX2* 5'HA to Cerulean	CCAAGTGGAGGAAGCGGGAGAAGT (FW1)	CGGCGGCGGTCACGAAC (RV1)	2053bp
Puro to outside *VSX2* 3'HA	GCGTTGGCTACCCGTGAT (FW2)	GCCCCAGCTCCTTATTCC (RV2)	1870bp
Outside *BRN3b* 5'HA to eGFP	TATTCGGCGGGCTGGATGAGAGTC (FW3)	GCCGTCGCCGATGGGGGTGTT (RV3)	1673bp
Blas to outside *BRN3b* 3'HA	TCGACTAGAGCTTGCGGAACC (FW4)	AACCAGGCCATATACAGAACTCAA (RV4)	1528bp
Outside *RCVRN* 5'HA to mCherry	AGCTTTGTTGAGCACCGACT(FW5)	GTTCTCCTCCACGTCTCCAG (RV5)	1167bp
Neo to outside *RCVRN* 3'HA	TCGCCTTCTTGACGAGTTCT (FW6)	TGGATCTGGTCCTCTCCATC (RV6)	1493bp
Outisde *VSX2* 5'HA to outside *VSX2* 3'HA	CCAAGTGGAGGAAGCGGGAGAAGT (FW1)	GCCCCAGCTCCTTATTCC (RV2)	2627bp
Outisde *BRN3b* 5'HA to outside *BRN3b* 3'HA	TATTCGGCGGGCTGGATGAGAGTC (FW3)	AACCAGGCCATATACAGAACTCAA (RV4)	2438bp
Outisde RCVRN 5'HA to outside *RCVRN* 3'HA	AGCTTTGTTGAGCACCGACT(FW5)	TGGATCTGGTCCTCTCCATC (RV6)	2221bp

Supplemental Table S2: Primers used for CRISPR off-target screening

Off-Target Screening VSX2-sgRNA				
Name	**Gene**	**Sequence**	**PAM**	**Off-target Score***
VSX2, Chr14		GTCAAGGCGCGCTCAGATGC	CGG	100
Chr19 non-gene sequence		GTCAAGGCGTACTCAGATGC	GAG	2.668116758
Chr19 non-gene sequence		GTGAAGAAGTGCTCAGATGC	CAG	0.916843223
Sytabulin, Chr8	ENSG00000147642	GTGAAGACACCCTCAGATGC	TGG	0.349165048
VEGF-A, Chr6	ENSG00000112715	GTCAAGGCGTGCTCCGATGG	GGG	0.317986706
KIF16B, Chr20	ENSG00000089177	GTCGAAGCGGGCTCCGATGC	AGG	0.252128661
STAT2, Chr12	ENSG00000170581	GTCAATGGGAGCTCTGATGC	AGG	0.234357477

Off-Target Screening BRN3b-sgRNA				
Name	**Gene**	**Sequence**	**PAM**	**Off-target Score***
BRN3b (POU4F2), Chr4	ENSG00000151615	AAGAGTCTTCTAAATGCCGG	CGG	100
RP11-1100L3.7, Chr12	ENSG00000257663	AGCAGTCTTCCAGATGCCGG	CAG	0.371654759
RP11-45M11.7, Ch6	ENSG00000275846	AAGCTCCTTCTAAATGCCAG	TAG	0.351773802
TC2N, Chr14	ENSG00000165929	TAAAGTCTTCTAAATGCCAA	TAG	0.331943062
FAM83F, Chr22	ENSG00000133477	AAGAGAATTGGAAATGCCGG	CAG	0.302192873
RP11-484K9.4, Chr3	ENSG00000272844	AAGACTCTTTGAAATGCCTG	CGG	0.288247111
RP11-321M21.1 , Chr18	ENSG00000266774	AATAGTCCTCCAAATGCTGG	CAG	0.202171083

Off-Target Screening RCVRN-sgRNA				
Name	**Gene**	**Sequence**	**PAM**	**Off-target Score***
Recoverin, Chr17	ENSG00000109047	AGGGAGGACAGCTGAACAGT	TGG	100
Chr4 non-gene sequence		AGGGAGGCCAGCTGAAGAGT	GGG	3.099576271
Chr2 non-gene sequence		GGAGAGGGCAGCTGAACAGT	TAG	2.726928675
Chr14 non-gene sequence		AGAGAGATCAGCTGAACAGT	GGG	1.740860136
Chr17 non-gene sequence		AGAAAGGACAGCTGAACTGT	AGG	0.741790707
Chr17 non-gene sequence		AGTGAGGATAGCTGGACAGT	AGG	0.541032634

* Off-target scores provided by Benchling.

Supplemental Table S3: sg RNA sequences for targeting

Specific guide RNA	**5' - 3'**
VSX2 .sgRNA	GTCAAGGCGCGCTCAGATGC
BRN3b .sgRNA	AAGAGTCTTCTAAATGCCGG
RCVRN .sgRNA	AGGGAGGACAGCTGAACAGT

Supplemental Table S4: Primers used for Gibson Assembly to make the HDR template

Gene	Forward (5'-3')	Reverse (5'-3')
VSX2.5'HA	ATTGGGTACCGGGCCTCCTGTGAGAACAGTGTG	CCGCTTCCGTCGACCAAAGCCATGTCCTCCAGC
VSX2.3'HA	ATACGAAGTTATTAGGTGTAGGTCAAGGCGCGCTCA	CTCCACCGCGGTGGCGCCAGATTGGGTTGTTCAAGG
RCVRN.5'HA	CTATAGGGCGAATTGGGTACTGCCTTCCCCGCCAGGTC	GTCGACCTCGAGGGGGGGCCTGGCGTTCTTCATCTTTTCCTTCACTTTTTG
RCVRN.3'HA	ATACGAAGTTATTAGGTGTGAACACACATGCACACA	CTCCACCGCGGTGGCCAAAAGCTTATTCATCGGG
BRN3b.5'HA	GGCGAATTGGAGCTCCACCGCGGTGGCCGCCGAGGCTCTGGCAGC	ATACAGCACAGCATAGGTCCAGGGTTCTCCTCCACG
BRN3b.3'HA	CCACTAGTTCTAGAAATAGAAGACTCTTGGCCTCTCC	TTGATATCGAATTCCTGCAGCCCGGGGTGCATCGGTCATGCTTCC

Supplemental Table S5: Primers flanking the sgRNA cut site used to generate PCR fragments for sequencing

Set of primers used to screen for off-target cutting efficiency of **VSX2.sgRNA** (*) symbol at the end indicates that this primer is good to use as probe for sequencing			
Gene	Forward (5'-3')	Reverse (5'-3')	Size
Sytabulin	GCACCGCATGGCTTCTCACC (*)	GGCCCCATCAAAATAAAACCATC	1.2kb
VEGFA	TGTGGCGGCCTCCCTTCATCTG (*)	CCCGCTCGCTCGCTCGCTCAC	887bp
Kinesis	GCCTGGCACCCTTGACATT	AGCAGGCAGAGCATCCCATCC (*)	913bp
Stat2	TTGAGGGGCTGGAGAAAGATAAGT (*)	TGGGGAGCAGAGACAAATAGAGAA	906bp
Chr19	CACTGCCCACTACCCACTACTAAG (*)	CGGGAGCAATATGGGAAATGGTC	941bp

Set of primers used to screen for off-target of cutting efficiency **RCVRN.sgRNA** (*) symbol at the end indicates that this primer is good to use as probe for sequencing			
Gene	Forward (5'-3')	Reverse (5'-3')	Size
chr4	TGTTCCCGGCCATTTGTA (*)	ATCTTGCCAGCATCCATTATCT	844bp
chr2	AAGCCCACTGGAAAGGTATGAACT (*)	AATGGGAAGGGGACTGAACAAA	833bp
chr14	AGTTTACGGGAGGGAGGTCAGC (*)	TGGCAGGGAGAAACAGTAGAA	596bp
chr17	GGGTGGCGGCAGCTTGATAAA (*)	CCCCGAGGATAGCACTGTTGG	497bp
chr17	GAGCCCCCGGAAGCACAAATACAG (*)	GGCAGGCGTCTCCGTTCTCACAC	648bp

Set of primers used to screen for off-target cutting efficiency of **BRN3b.sgRNA** (*) symbol at the end indicates that this primer is good to use as probe for sequencing			
Gene	Forward (5'-3')	Reverse (5'-3')	Size
1100L3.7	CTTCCCGGCACCAAATCACTCTAC (*)	GCCCCTCCCCTGCTTATCTGG	1.0kb
45M11.7	ACCCCTTTTATTCGTGCTCTATTG (*)	AGTCCCGCGTCCTGCTCTC	1.0kb
FAM83F	TGGCCTTTTGCTTTTTCACACC	CACCCCCGGCGTCCTTTACCTG (*)	854bp
RP11-484K9	CCGTAGGGGGCGAGGAACC (*)	GTGAAGGCGGAAATACAAACAGTC	691bp
RP11-321M21	GGGGCAAGCTTCTCCACTATTATC	GTTCCATCCTGCGGCTCTTC (*)	931bp

Supplemental Table S6: Primers for RT-qPCR

Gene	Forward (5'-3')	Reverse (5'-3')
Oct4	TGTACTCCTCGGTCCCTTTC	TCCAGGTTTTCTTTCCCTAGC
NANOG	CAGTCTGGACACTGGCTGAA	CTCGCTGATTAGGCTCCAAC
PAX6	CGGAGTGAATCAGCTCGGTG	CCGCTTATACTGGGCTATTTTGC
SIX3	CCGGAAGAGTTGTCCATGTT	CGACTCGTGTTTGTTGATGG
VSX2	TCATGGCGGAGTATGGGCT	TCCAGCGACTTTTTGTGCATC
BRN3a	GGGCAAGAGCCATCCTTTCAA	CTGTTCATCGTGTGGTACGTG
BRN3b	CTCGCTCGAAGCCTACTTTG	GACGCGCACCACGTTTTTC
RCVRN	CCAGAGCATCTACGCCAAGTT	CCGTCGAGGTTGGAATCGAAG
MITF	GACATGCGCTGGAACAAGGGAACC	CCGGGGGACACTGAGGAAAGGAG
BEST-1	AACTGAGCCTACCACACAACA	CGGATTCGACCTCCAAGCC

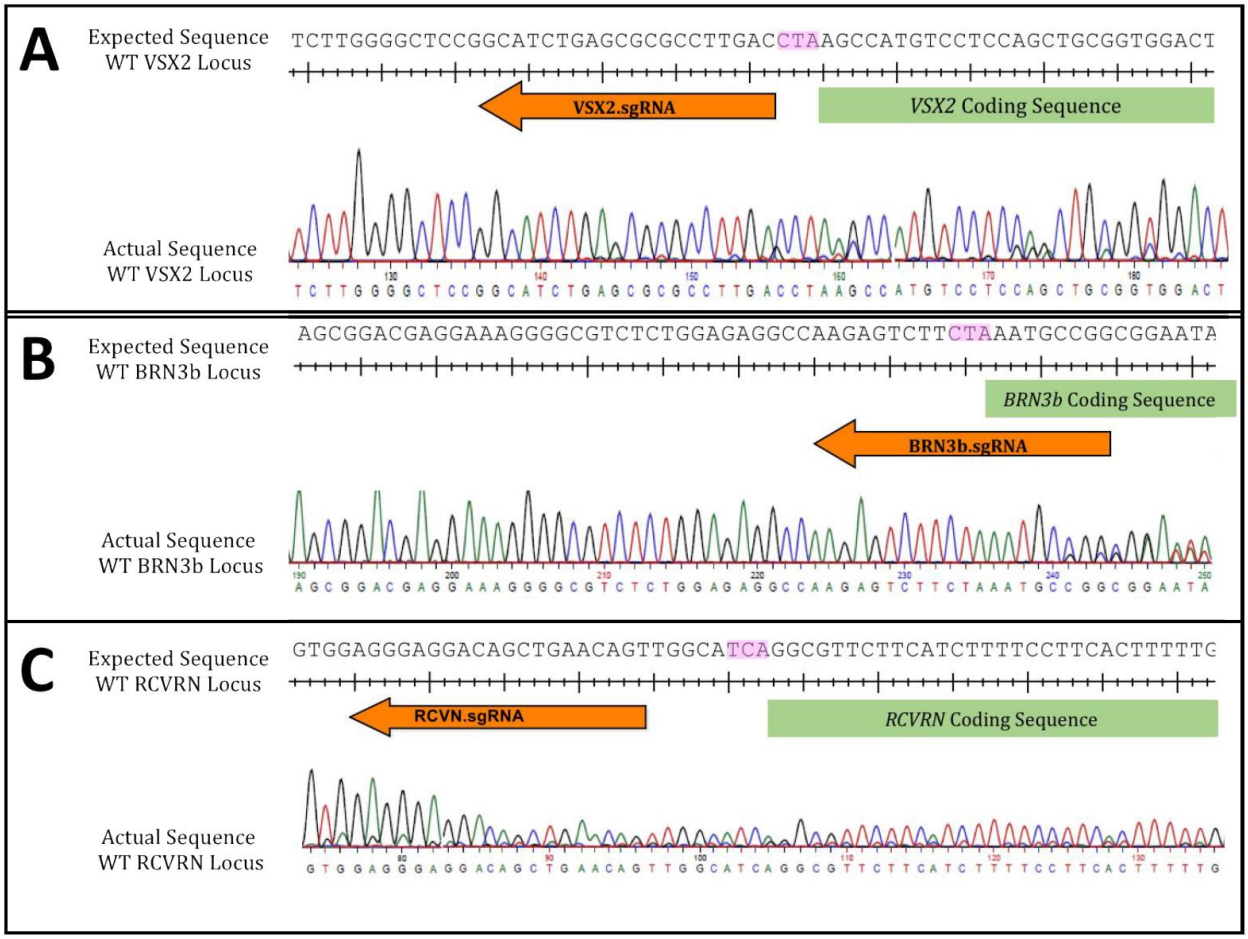
Supplemental Table S7: Antibodies used for immunohistochemistry

Antibodies	Supplier	Species	Type	Dilution	Reference
VSX2	Millipore	Sheep	Polyclonal	1:500	ab9016
CFP	Abcam	Rabbit	Polyclonal	1:100	ab6556
BRN3	Santa Cruz	Goat	Polyclonal	1:1000	sc-6026X
MCM2	Abcam	Rabbit	Polyclonal	1:1000	ab4461
Prox-1	Millipore	Rabbit	Polyclonal	1:2000	ab5475
Cralbp	Abcam	Mouse	Monoclonal	1:500	ab15051
Ap2-alpha	DSHB	Mouse	Monoclonal	1:35	3B5a
Recoverin	Millipore	Rabbit	Polyclonal	1:500	ab5585
Oct4	Abcam	Rabbit	Polyclonal	1:500	ab19857
Sox2	Santa Cruz	Goat	Polyclonal	1:500	Sc-17319
Pax6	Santa Cruz	Mouse	Polyclonal	1:100	Sc-32766
Six3	Santa Cruz	Mouse	Polyclonal	1:100	Sc-365519
Rx	Santa Cruz	Mouse	Polyclonal	1:150	Sc-271889

Supplemental Table S8: RT-qPCR primers for FACS sorted cells

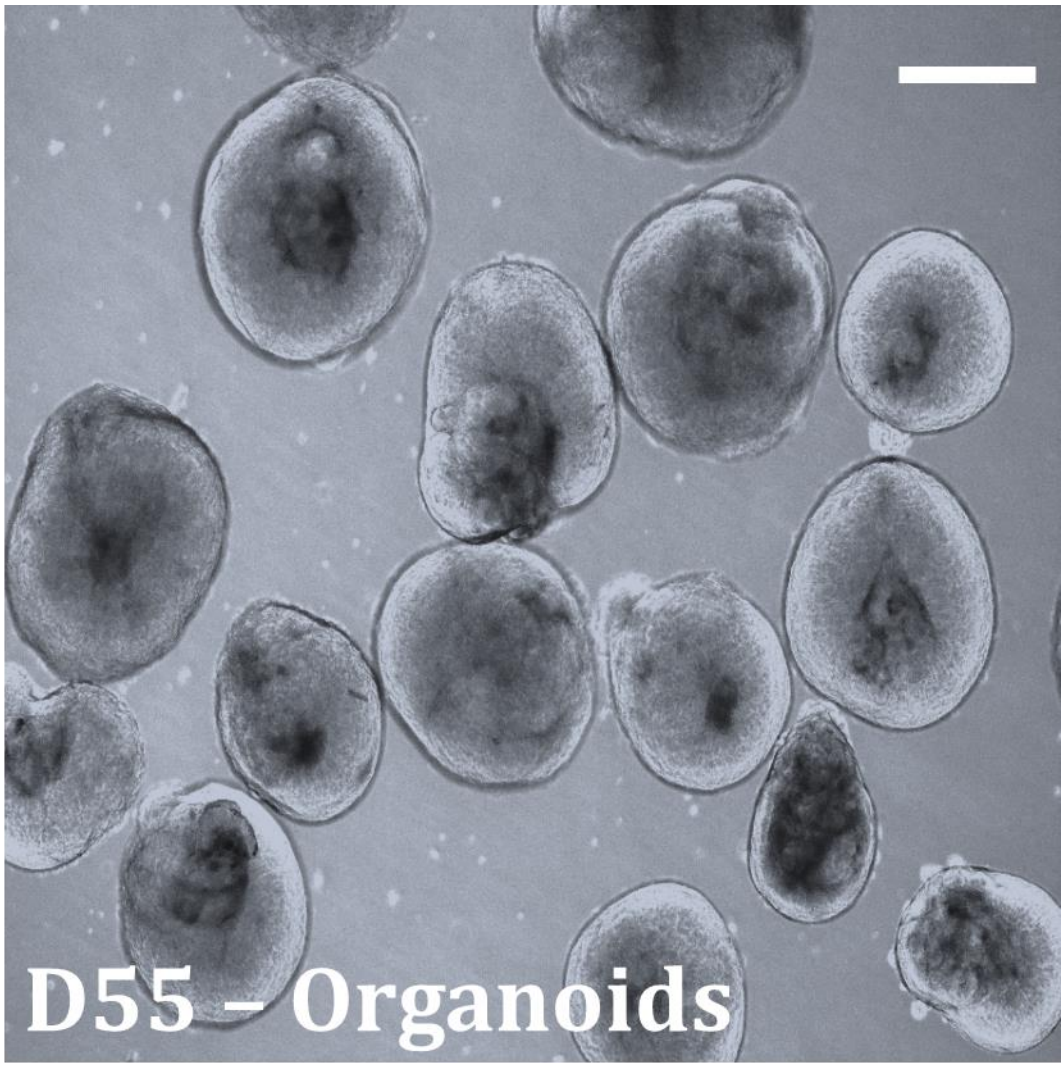
Gene	**Forward (5'- 3')**	**Reverse (5' - 3')**
Cerulean	AAGCTGACCCTGAAGTTCATCTGC	CTTGTAGTTGCCGTCGTCCTTGAA
VSX2	TCATGGCGGAGTATGGGCT	TCCAGCGACTTTTTGTGCATC
mCherry	GATAACATGGCCATCATCAAGGA	CGTGGCCGTTCACGGAG
RCVRN	CCAGAGCATCTACGCCAAGTT	CCGTCGAGGTTGGAATCGAAG
eGFP	GACCAAAAGATCATGGTGAGC	GAACTTCAGGGTCAGCTTGC
BRN3b	CTCGCTCGAAGCCTACTTTG	GACGCGCACCACGTTTTTC
GAPDH	CAATGACCCCTTCATTGACC	GACAAGCTTCCCGTTCTCAG

Supplemental Figure 1

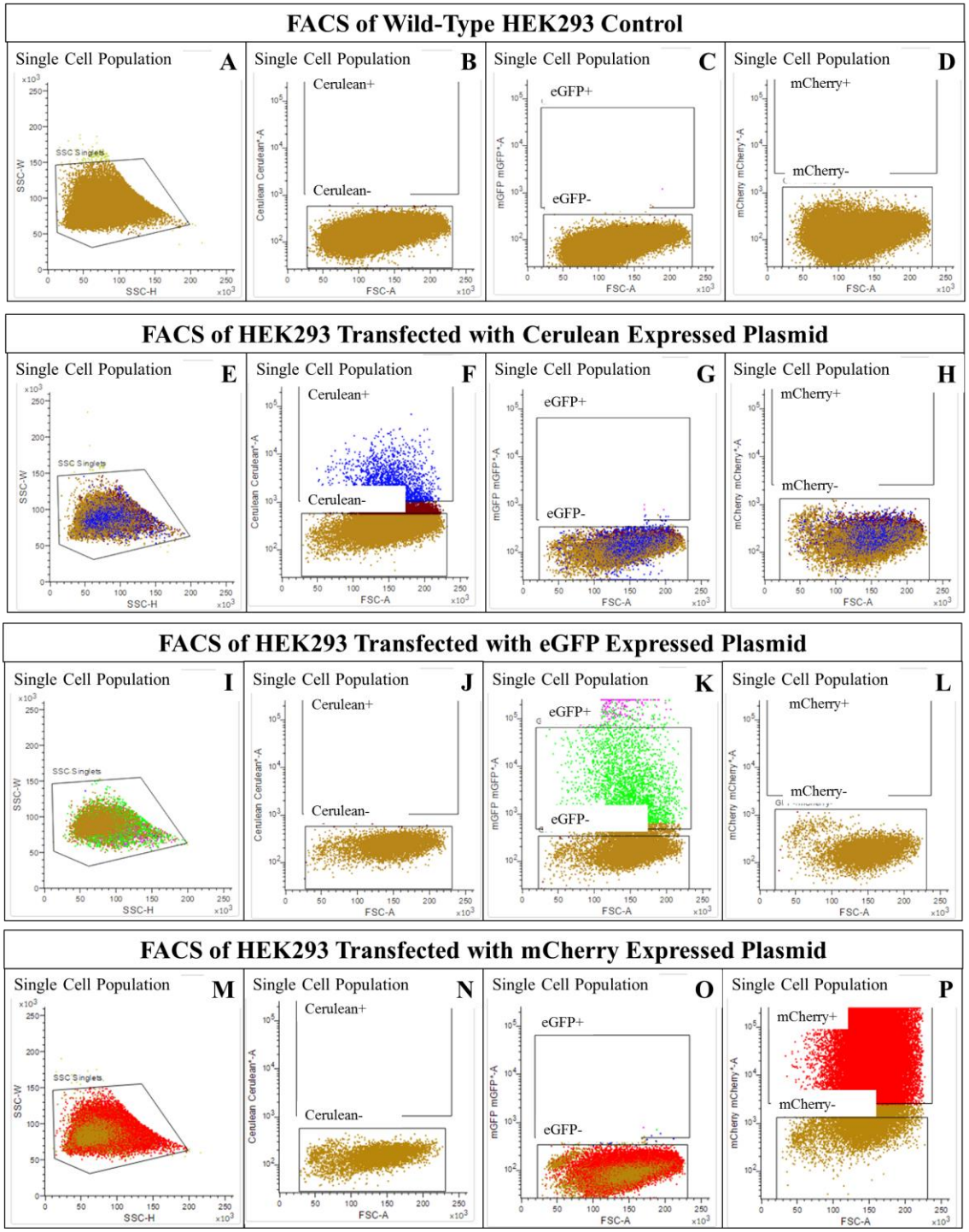


Supplemental Figure 2

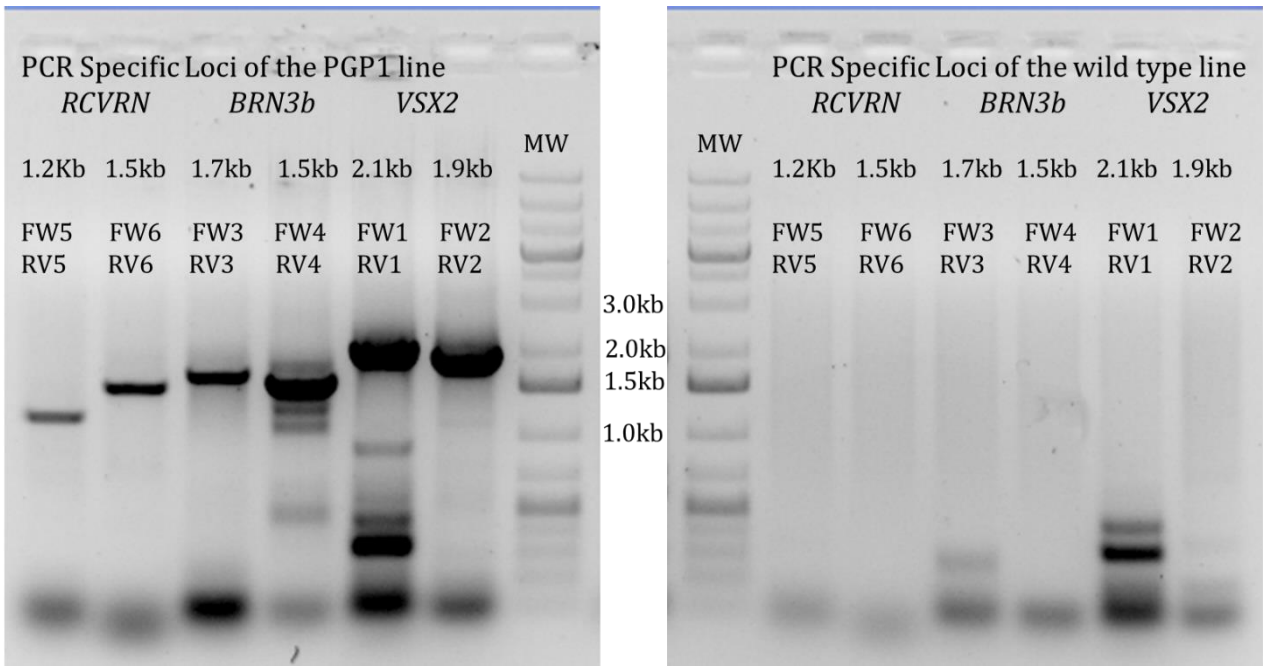
Supplemental Figure 3



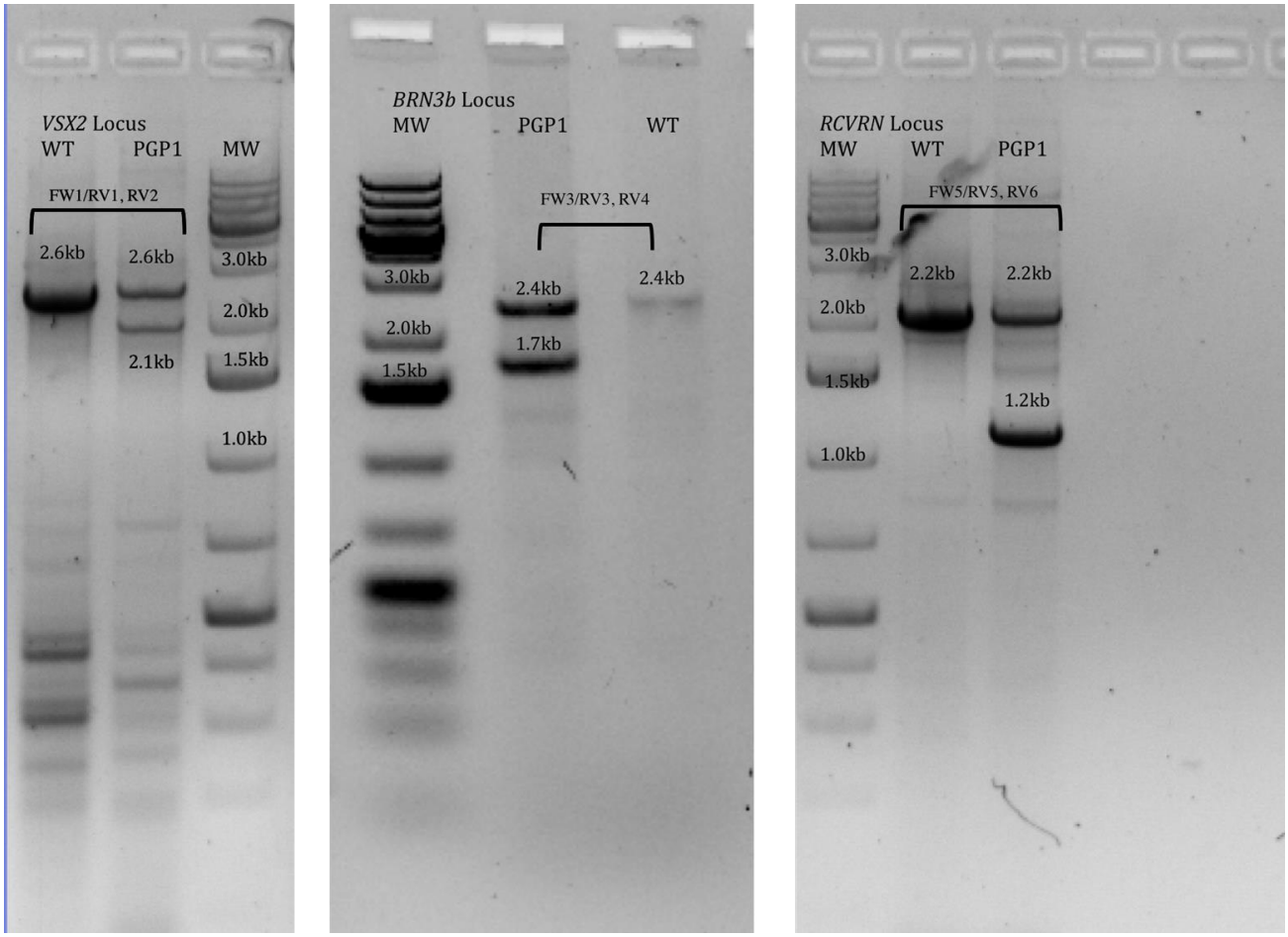
Supplemental Figure 4



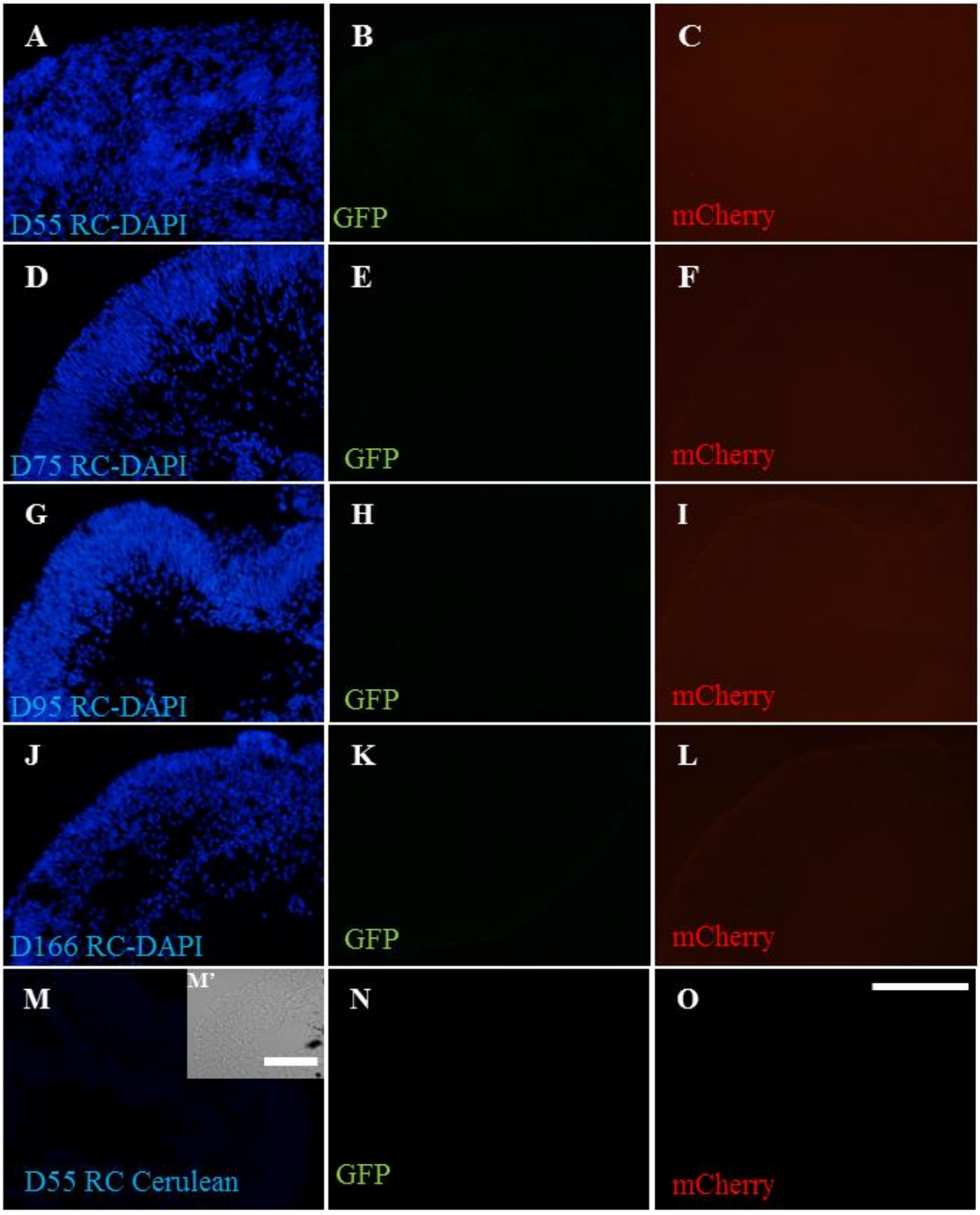
Supplemental Figure 5



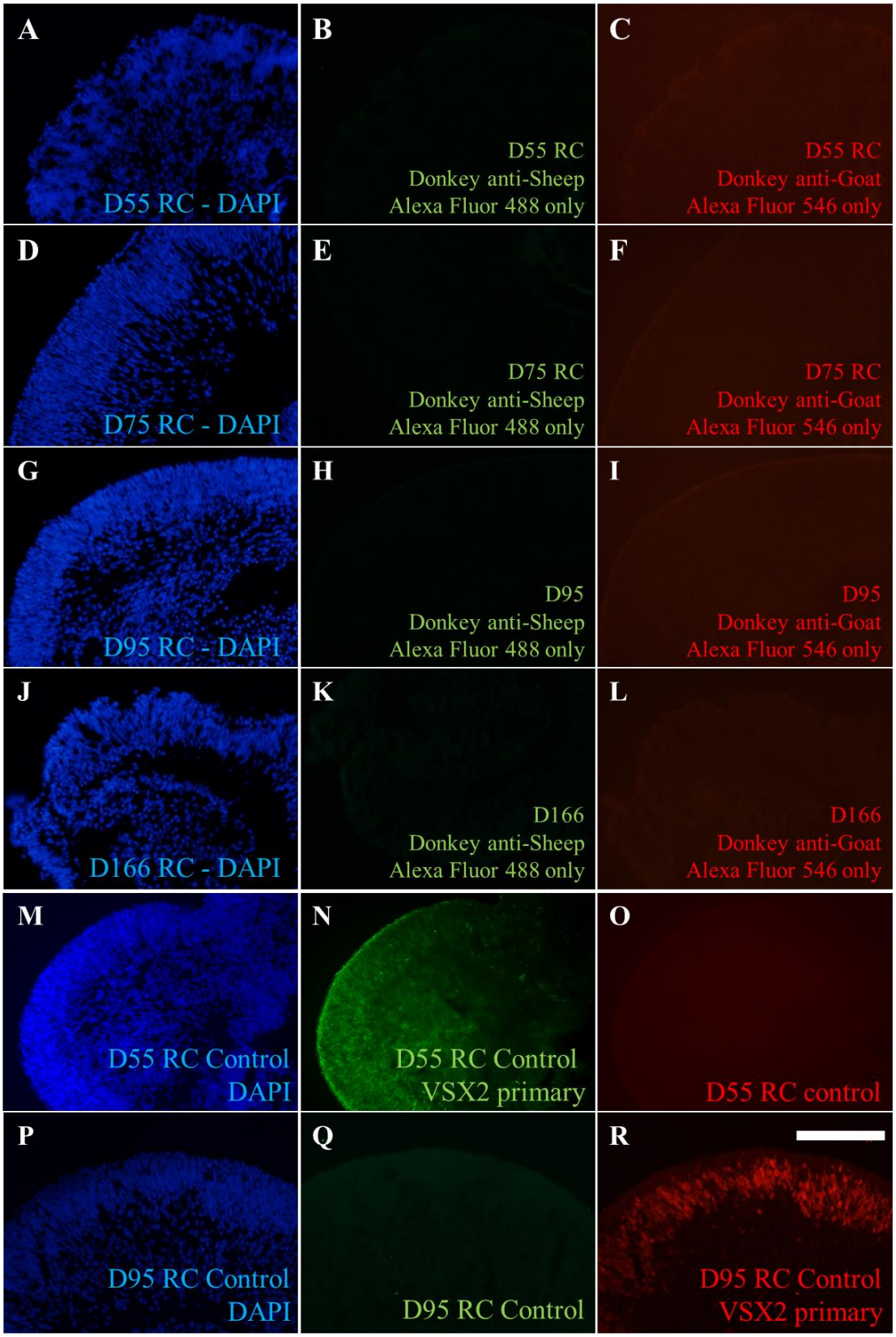
Supplemental Figure 6



Supplemental Figure 7



Supplemental Figure 8



SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1: Lack of Indel Mutations in the Non-Targeted Alleles of PGP1. Sequence analysis of the WT alleles of VSX2 (A), BRN3b (B) and RCVRN (C) failed to detect any Cas9-mediated indel mutations in PGP1. Orange arrows represent the sgRNA sequence, the endogenous stop codons are shaded in pink and coding sequences are represented by green rectangles.

Supplemental Figure 2: **Cerulean Positive Retina Progenitors Appear Before eGFP or mCherry Positive Cells During PGP1 Retinal Organoid Differentiation**. After 20 days of differentiation, retinal domains (A) first express Cerulean (blue) (B), but not eGFP (C) or mCherry (D). The composite of the bright field and VSX2/Cerulean (E). Magnification bar 20µm.

Supplemental Figure 3: Brightfield View of Three Dimension Retinal Organoids at D55 of differentiation. Free-floating organoids have variable size but all maintain a three dimensional shape with characteristic spherical structure with a distinct thick exterior and hollower interior. Scale bar 40µM

Supplemental Figure 4: Establishing FACS Gates Using Transiently Transfected HEK293 Cells. Wild-type HEK293 cells (A-D) or HEK293 cells transiently transfected with expression plasmids for Cerulean (E-H), eGFP (I-L), or mCherry (M-P) were dissociated into single cell populations (A, E, I, M) and Gates were established for each fluorescent protein based on parameters that would lead to capturing the appropriate fluorescent protein expressing cells

without capturing any wild-type cells. We confirmed that the captured cells in the Cerulean positive gate (F) the eGFP positive gate (K) and the mCherry positive gate (P) expressed the appropriate fluorescent protein when sorted and cultured.

Supplemental Figure 5: Original PCR Gels Supporting Figure 1. The original ethidium bromide stained PCR gels used to support figure 1. PCR reactions using genomic DNA as template with the primers indicated above each lane. The expected band sizes for each targeted allele are shown above each lane. The template DNA for the gel on the left came from the PGP1 clone while the template for the gel on the right was from a wild-type hiPSC clone. MW indicates a DNA size ladder run on each gel.

Supplemental Figure 6: Original PCR Gels Supporting Figure 2. The original ethidium bromide stained PCR gel that was cropped for clarity in figure 2. The PGP1 cell line and wild-type hiPSCs (WT) provided the genomic DNA template for a three primer PCR strategy to detect the wild-type and targeted alleles for the *VSX2, BRN3b* and *RCVRN* loci. The primers used for each reaction are indicated above the relevant lanes. MW indicates DNA size ladders run in duplicate on the gel.

Supplemental Figure 7: Confirmation that Fluorescent Protein Expression in PGP1-Derived Retinal Cup Organoids Does Not Survive Fixation and Frozen Sectioning. Sections of PGP1 hiPSC-derived retinal organoids were prepared after fixation in 4% paraformaldehyde, overnight incubation in 30% sucrose at 4ºC, and embedding in OCT compound. Organoid sections from D55 (A-C), D75 (D-F), D95 (G-I), and D166 (J-L) of differentiation were visualized following DAPI

staining on the blue DAPI filter (A, D, G, J), the green FITC filter for GFP (B, E, H, K) and the red Texas Red filter for mCherry (C, F, I, L). No green or red signals consistent with eGFP or mCherry were detected in organoids of any age. Using an LSM 800 confocal system, an unstained organoid section from D55 was visualized for cerulean expression (Ex.433nm, Em.475) (M), eGFP (Ex.493nm, Em. 517) (N), and mCherry (Ex.577, Em.603) (O). The insert (M') showed the D55 RC section in brightfield. Ex is excitation wavelength and Em is Emission wavelength. Magnification bar for (M') is 50 μm, and 100 μm for all other images.

Supplemental Figure 8: Additional Controls for PGP1-Derived Retinal Cup Organoids Prepared for Immunohistochemistry. To ensure the signals from immunofluorescent staining experiments are specific for the intended antigens, the retina cup organoids were stained for DAPI and secondary antibody only (A-L). The staining for DAPI, and the secondary antibodies Donkey anti Sheep Alexa Fluor 488, and Donkey anti Sheep Alexa Fluor 546 were done for sections from organoids at D55 (A-C), D75 (D-F), D95 (G-I), and D166 (J-L) of differentiation. To ensure that the DAPI signal did not represent the VSX2-Cerulean signal, D55 organoid sections were stained with DAPI, and a sheep anti-VSX2 primary antibody and secondary anti sheep Alexa Fluor 488 antibody (M-O). D95 organoid sections were stained with DAPI, and a sheep anti-VSX2 primary antibody and a secondary antisheep Alexa Fluor 546 antibody (P-R). Note that only a subset of the DAPI signals in (M) and (P) are VSX2 positive (N, R). Images in the first column (A, D, G, J, M, P) were photographed with a DAPI filter, while images in the middle (B, E, H, K, N, Q) were photographed with a FITC filter and the last column (C, F, I, L, O, R) were photographed with a Texas Red filter. Magnification Bar. 50μm, applies to all images.

Supplemental Movie 1: Three-dimensional image of a Day 55 retinal cup Organoid made from the PGP1 cell line. A portion of a retinal cup organoid made from the PGP1 cell line was imaged at 55 days of differentiation for the expression of Cerulean, eGFP and mCherry to visualize cells expressing *VSX2*, *BRN3b* and *RCVRN*, respectively. At this stage many Ceruelan-expressing cells are found in the organoid. At this stage of development, eGFP-expressing cells exhibit long extensions typical of ganglion cell axons and only a few mCherry-expressing cells appear at this stage. Images taken on an Olympus FV3000 confocal system.