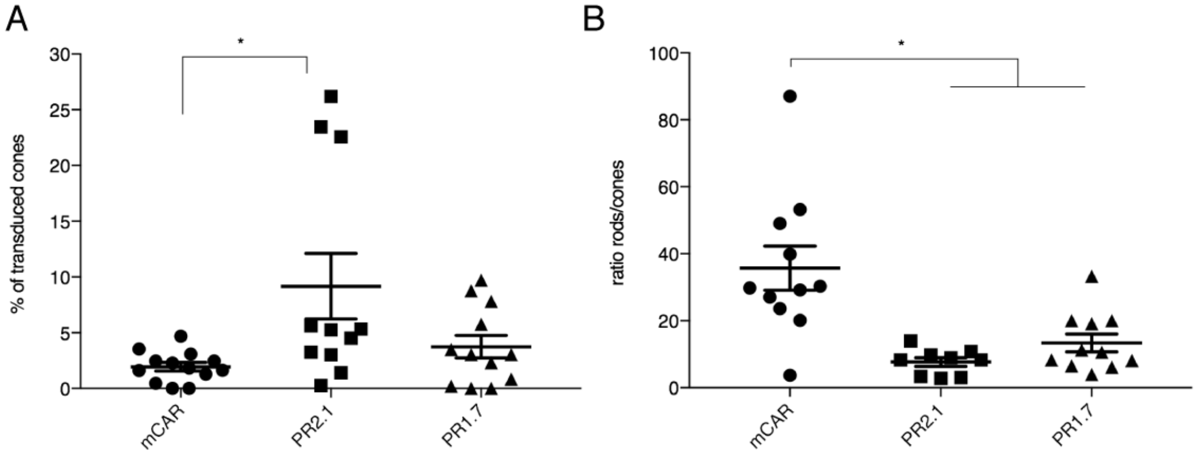
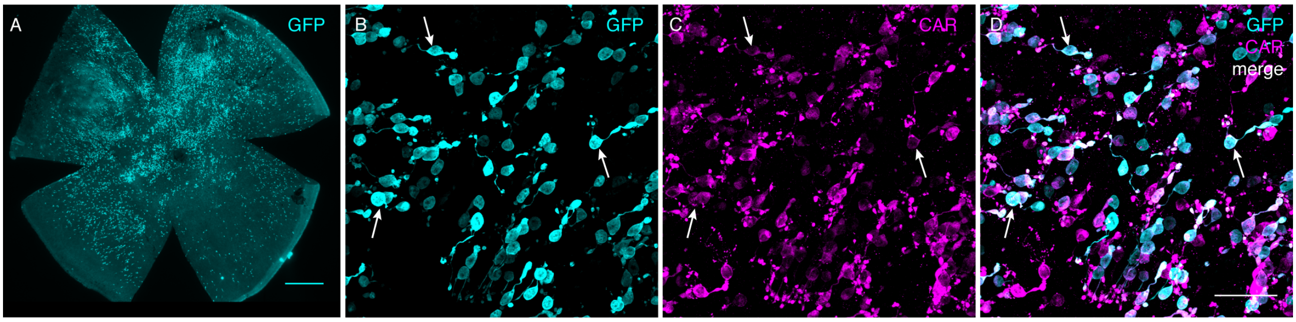
Supplemental data

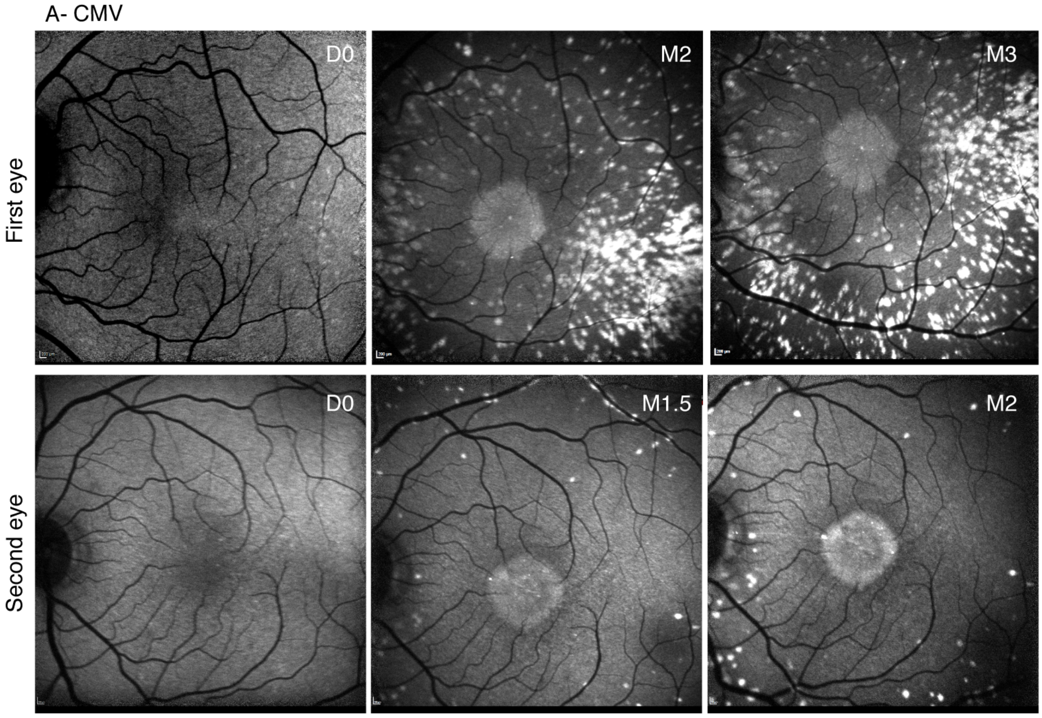
Supplemental Figure and Figure Legends



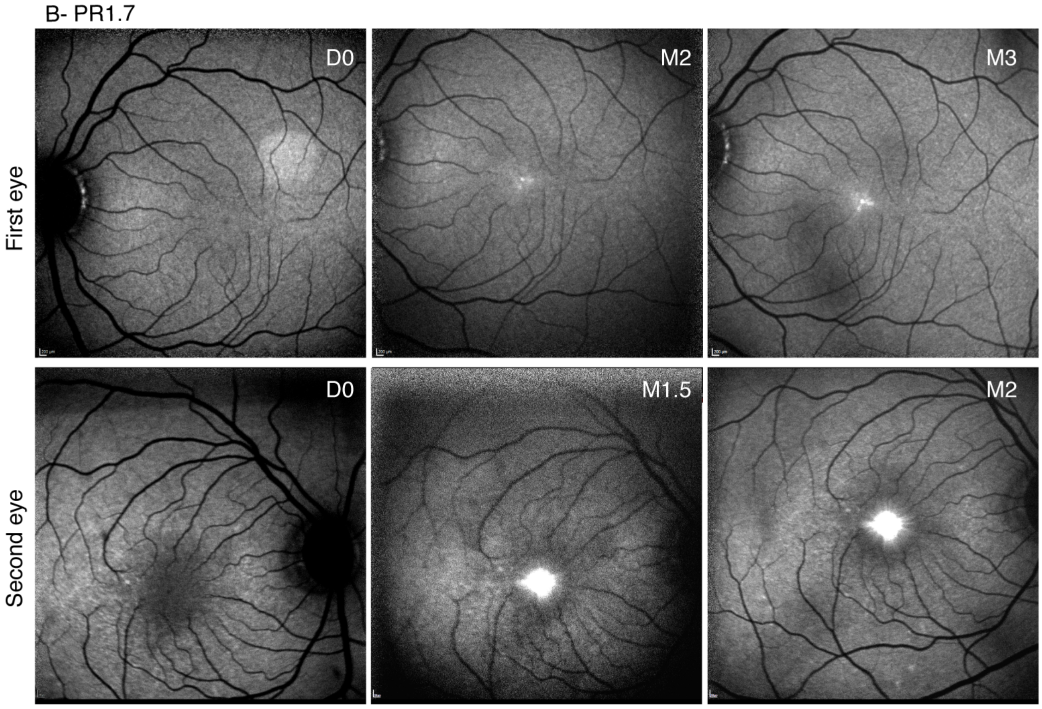
Supplemental Figure 1: **Quantification of cone transduction efficiency after intravitreal administration of AAV2-7m8-GFP in mice using mCAR, PR1.7 or PR2.1 promoters (n=4 eyes per condition). (A)** Quantification of the percentage of transduced cones based on flatmount images and colocalization of GFP and cone arrestin stainings. **(B)** Evaluation of the specificity of the promoters in the photoreceptor layer expressed as the ratio of number of transduced rods over the number of transduced cones. Data represent mean ± SEM and were analyzed with ANOVA multiple comparison test. AAV: adeno-associated virus; mCAR: mouse cone arrestin promoter; PR1.7 and PR2.1: promoters of 1.7 and 2.1 kilobases in length, respectively, based on the human red opsin gene enhancer and promoter sequences.



<u>**Supplemental Figure 2:**</u> **Cone transduction after intravitreal administration of AAV2-7m8-GFP in rd10 mice using PR1.7 promoter. (n=4 eyes) (A)** GFP expression in a 3-months old rd10 retinal whole-mount two months after injection. The retina is mounted with photoreceptor layer facing upwards. Scale bar is 500 µm. (B-D) Zoom into the whole-mount retina shown in D. Scale bar is 40 µm. **(B)** Cone cell bodies remaining after degeneration expressing GFP (cyan). **(C)** Cone arrestin immunostaining is shown in magenta. (D) Colocalization of GFP and cone arrestin stainings. AAV: adeno-associated virus; PR1.7: Promoter 1.7kilobases in length, based on the human red opsin gene enhancer and promoter sequences; rd10: retinal degeneration 10 mouse model for retinitis pigmentosa.

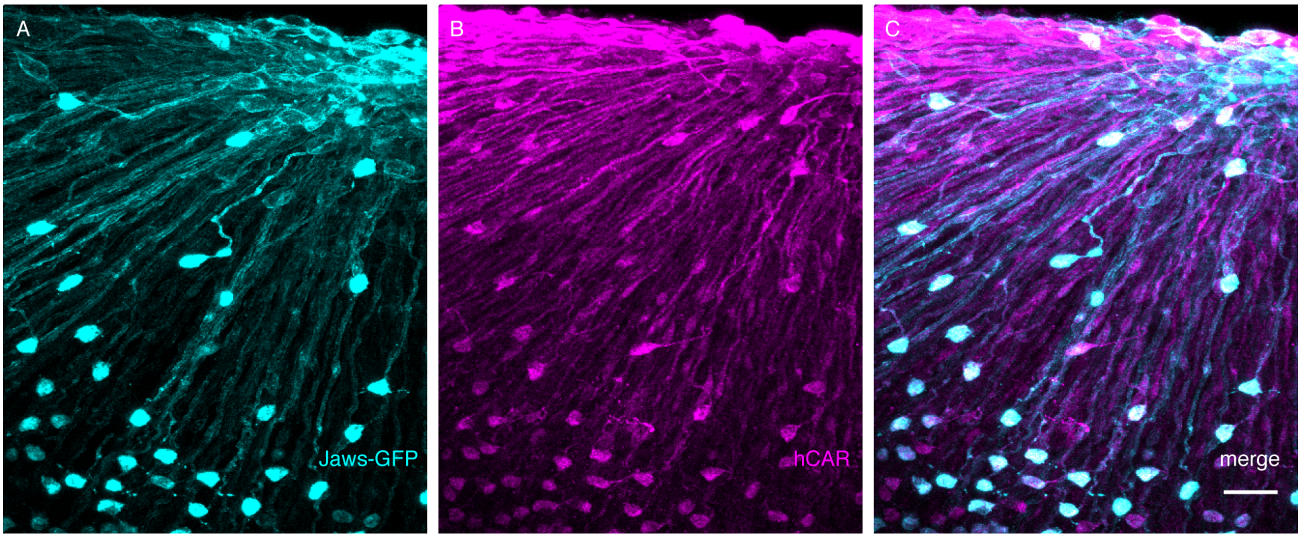


A- CMV

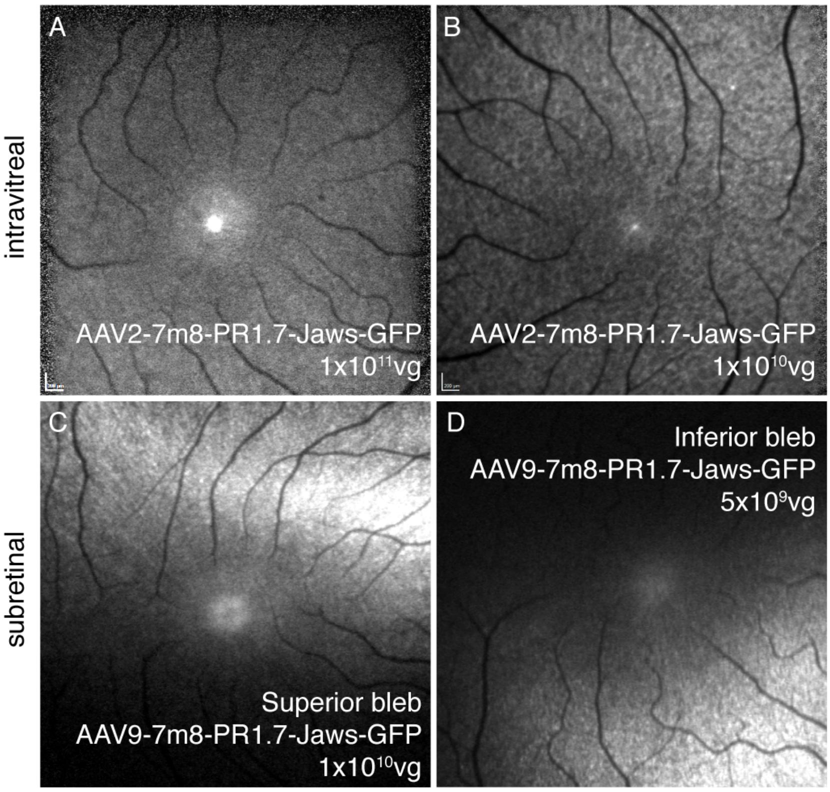


B- PR1.7

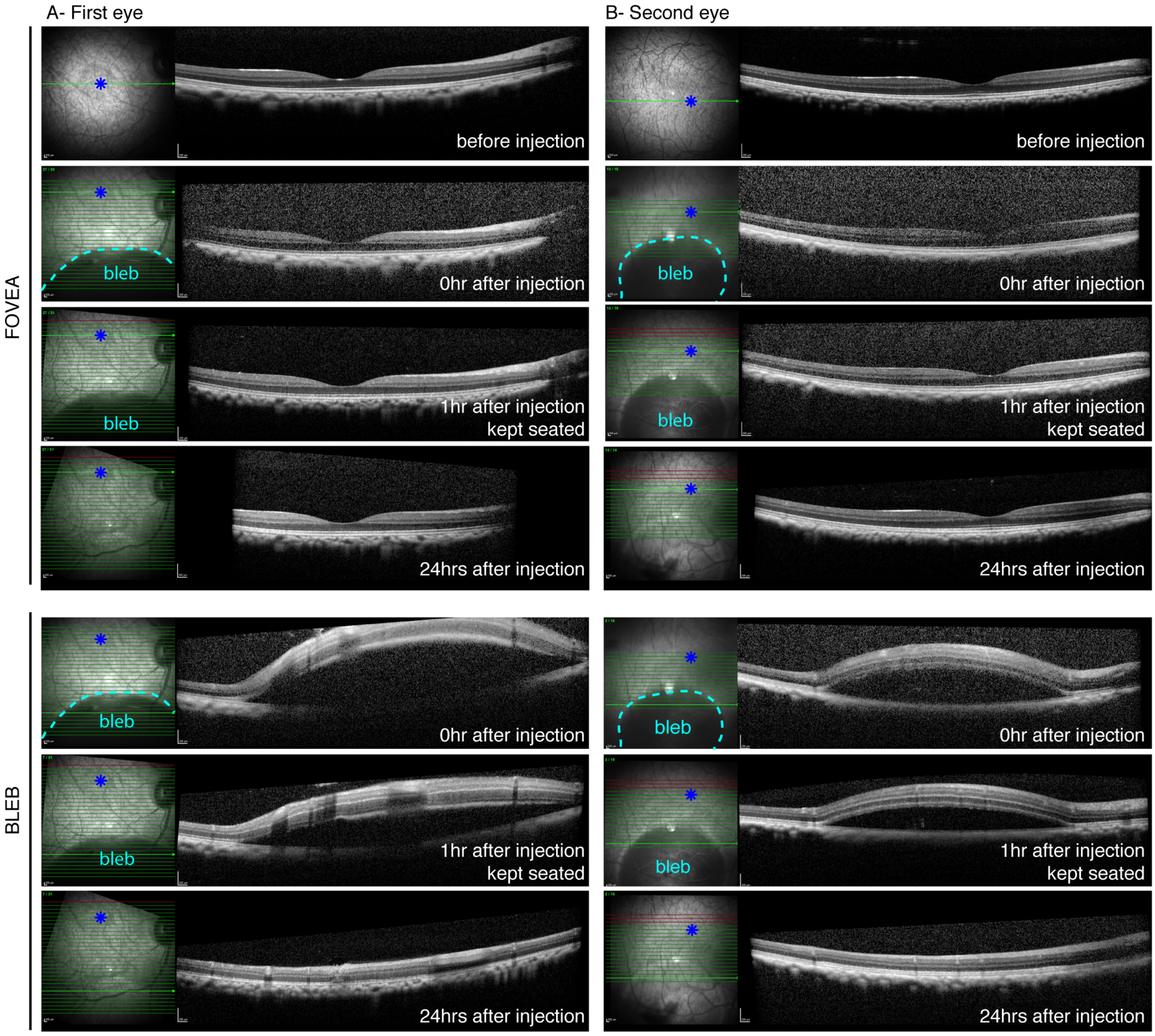
<u>Supplemental Figure 3:</u> **GFP expression follow-up after intravitreal administration of AAV2-7m8 under the control of CMV and PR1.7 promoters (n=2 per condition). (A)** Eye fundus images of CMV treated eyes. **(B)** Eye fundus images of PR1.7 treated eyes. D0: Day of injection, pre-dose; M1.5, 2, 3: Month 1.5, 2, 3 after injection. AAV: adeno-associated virus; CMV: cytomegalovirus promoter; PR1.7: Promoter 1.7kilobases in length, based on the human red opsin gene enhancer and promoter sequences.



<u>Supplemental Figure 4:</u> Cone transduction in the fovea after intravitreal administration of 1x10^{11} vg of AAV2-7m8-PR1.7-Jaws-GFP in one macaque eye (A) Jaws-GFP expression in foveal whole-mount two months after injection (cyan). **(B)** Human cone arrestin immunostaining is shown in magenta. **(C)** Colocalization of GFP and cone arrestin stainings. Scale bar is 20 µm. AAV: adeno-associated virus; hCAR: human cone arrestin; PR1.7: Promoter 1.7kilobases in length, based on the human red opsin gene enhancer and promoter sequences.



<u>Supplemental Figure 5:</u> Macaque eye fundus images for characterization of dose response. Jaws-GFP expression two months after intravitreal injection of 10^{11} particles (n=1 eye) **(A)** and 10^{10} particles **(B)** of AAV2-7m8-PR1.7-Jaws-GFP (n=1 eye). Jaws-GFP expression two weeks after subretinal injection of $1x10^{10}$ particles (n=1 eye, superior bleb) **(C)** and $5x10^{9}$ particles (n=2 eyes, inferior blebs) **(D)** of AAV9-7m8-PR1.7-Jaws-GFP**.** AAV: adeno-associated virus, vg: viral genome; PR1.7: Promoter 1.7kilobases in length, based on the human red opsin gene enhancer and promoter sequences.



<u>Supplemental Figure 6:</u> Distal inferior subretinal administration follow-up using in vivo eye fundus and optical coherence tomography (OCT) imaging. Images were acquired before and after peripheral injection of AAV9-7m8-PR1.7-Jaws-GFP, $5x10^9$ viral particles (n=2 eyes). Eye fundus infrared image is centered on the macula and OCT image was taken at the level of the fovea or at the level of the bleb shortly before and after the injections. Follow-up images were acquired one hour after injections while the animal was kept seated. Another image was acquired 24 hours after injections. Bleb: subretinally injected fluid; hrs: hours; AAV: adeno-associated virus, PR1.7: Promoter 1.7kilobases in length, based on the human red opsin gene enhancer and promoter sequences. Dark blue asterisk: fovea; bold green arrows: OCT image of the retina highlighted with the dark green arrow, is shown on the right part of each image; dashed cyan line: delimitation of the bleb.

Supplemental Tables

Supplemental Table 1: Transcription factors’ binding sites analysis for red opsin gene based promoters, PR2.1 and PR1.7.

Gene identification number	Gene title	Protein symbol	OPN1LW Promoter fragment			Probe set	Signal Intensity	
			Site number			Identification number	Robust Multi-array Average (RMA)	
			2061 bp	1724 bp	337 bp		Mean	Standard deviation
196	Aryl hydrocarbon receptor	AHR	1	1	0	202820_at	48.4	1.8
467	Activating transcription factor 3	ATF3	2	2	0	202672_s_at	51.6	14.4
1051	CCAAT/enhancer binding protein beta	CEBPB	55	41	14	212501_at	206.3	35.5
1406	Cone-rod homeobox	CRX	12	11	1	217510_at	1638.3	96.4
2002	ELK1. ETS transcription factor	ELK1	11	9	2	203617_x_at	86.8	2.6
2353	Fos proto-oncogene. AP-1 transcription factor subunit	FOS	9	8	1	209189_at	744.1	174.7
2969	General transcription factor Iii	GTF2I	54	44	10	210892_s_at	41.6	4.8
3091	Hypoxia inducible factor 1 alpha subunit	HIF1A	1	1	0	200989_at	810.1	29.1
3725	Jun proto-oncogene. AP-1 transcription factor subunit	JUN	27	23	4	201464_x_at	377.4	50.9
4150	MYC associated zinc finger protein	MAZ	3	3	0	212064_x_at	157.3	6.9
4205	Myocyte enhancer factor 2A	MEF2A	1	1	0	212535_at	203.8	3.5
4782	Nuclear factor I C	NFIC	36	38	8	226895_at	195.6	11.3
4800	Nuclear transcription factor Y subunit alpha	NFYA	4	3	1	228433_at	43.3	1.7
10062	Nuclear receptor subfamily 1 group H member 3	NR1H3	12	11	1	217370_x_at	174.6	17.2
7025	Nuclear receptor subfamily 2	NR2F1 /	1	0	1	209505_at	128.6	10.2

	group F member 1	COUP-TF1						
2908	Nuclear receptor subfamily 3 group C member 1	NR3C1	173	140	33	201865_x_at	164.7	8.6
5451	POU class 2 homeobox 1	POU2F1	3	2	1	227254_at	192.6	7.2
5465	Peroxisome proliferator activated receptor alpha	PPARA	6	5	1	223437_at	232.0	19.6
5914	Retinoic acid receptor alpha	RARA	3	3	0	216300_x_at	40.0	1.9
5915	Retinoic acid receptor beta	RARB	6	6	0	205080_at	64.4	3.0
3516	Recombination signal binding protein for immunoglobulin kappa J region	RBPJ	2	2	0	211974_x_at	435.6	29.7
5970	RELA proto-oncogene. NF-kB subunit	RELA	3	3	0	201783_s_at	53.3	3.7
6256	Retinoid X receptor alpha	RXRA	26	22	4	202449_s_at	146.0	6.8
6667	Sp1 transcription factor	SP1	2	1	1	224754_at	158.2	6.6
6772	Signal transducer and activator of transcription 1	STAT1	4	4	0	200887_s_at	160.0	13.7
6908	TATA-box binding protein	TBP	14	11	3	203135_at	76.7	2.7
6925	Transcription factor 4	TCF4	2	2	0	212386_at	526.0	30.8
6934	Transcription factor 7 like 2	TCF7L2	6	5	1	212761_at	140.6	8.4
8463	TEA domain transcription factor 2	TEAD2	8	5	3	243766_s_at	53.4	2.4
7020	Transcription factor AP-2 alpha	TFAP2A	28	23	5	204653_at	264.8	29.5
7068	Thyroid hormone receptor beta	THRB	10	9	1	229657_at	106.4	5.3
7392	Upstream transcription factor 2	USF2	5	5	0	202152_x_at	176.8	9.4
7528	YY1 transcription factor	YY1	18	15	3	201901_s_at	379.0	22.4

PR1.7 and PR2.1: promoters of 1.7 and 2.1 kilobases in length, respectively, based on the human red opsin gene enhancer and promoter sequences; bp: base pairs.

Supplemental Table 2: Transcription factors' binding sites analysis for mouse cone arrestin (mCAR) promoter.

Gene identification number	Gene title	Protein symbol	Mouse *Arr3* Promotor fragment		Probe set	Signal Intensity	
			Site number		Identification number	Robust Multi-array Average (RMA)	
			3207 bp (-3170/+37)	521 bp (-510/+11)		Mean	Standard deviation
8546	Adaptor-related protein complex 3. beta 1 subunit	AP3B1	5	0	203142_s_at	55.3	2.1
1386	Activating transcription factor 2	ATF2	1	0	212984_at	150.2	5.2
467	Activating transcription factor 3	ATF3	5	0	202672_s_at	51.6	14.4
79365	Basic helix-loop-helix family member e41	BHLHE41	5	0	221530_s_at	1083.9	64.2
1051	CCAAT/enhancer binding protein beta	CEBPB	221	37	212501_at	206.3	35.5
1406	Cone-rod homeobox	CRX	19	6	217510_at	1638.3	96.4
1998	E74 like ETS transcription factor 2	ELF2	2	0	203822_s_at	91.9	2.8
2002	ELK1. ETS transcription factor	ELK1	10	2	203617_x_at	86.8	2.6
2353	Fos proto-oncogene. AP-1 transcription factor subunit	FOS	146	25	209189_at	744.1	174.7
2969	General transcription factor Iii	GTF2I	3	0	210892_s_at	41.6	4.8
3280	Hes family bHLH transcription factor 1	HES1	26	0	203394_s_at	522	71.3
3725	Jun proto-oncogene. AP-1 transcription factor subunit	JUN	85	8	201464_x_at	377.4	50.9
3726	JunB proto-oncogene. AP-1 transcription factor subunit	JUNB	2	0	201473_at	369.1	77.6
3727	JunD proto-oncogene. AP-1 transcription factor subunit	JUND	20	2	203752_s_at	580.8	63.8

4150	MYC associated zinc finger protein	MAZ	14	3	212064_x_at	157.3	6.9
4205	Myocyte enhancer factor 2A	MEF2A	4	0	212535_at	203.8	3.5
4520	Metal regulatory transcription factor 1	MTF1	2	1	227150_at	112.8	4.9
4782	Nuclear factor I C	NFIC	110	28	226895_at	195.6	11.3
4784	Nuclear factor I X	NFIX	1	0	227400_at	101	8.1
4800	Nuclear transcription factor Y	NFYA	53	0	204108_at	44.4	1.8
4801		NFYB			218127_at	104.1	6.1
4802		NFYC			202215_s_at	101	4.6
10062	Nuclear receptor subfamily 1 group H member 3	NR1H3	26	2	217370_x_at	174.6	17.2
7025	Nuclear receptor subfamily 2 group F member 1	NR2F1 / COUP-TF1	2	1	209505_at	128.6	10.2
2908	Nuclear receptor subfamily 3 group C member 1	NR3C1	297	46	201865_x_at	164.7	8.6
5080	Paired box 6	PAX6	1	0	205646_s_at	1430.4	87.3
5451	POU class 2 homeobox 1	POU2F1	3	0	227254_at	192.6	7.2
5465	Peroxisome proliferator activated receptor alpha	PPARA	6	0	223437_at	232	19.6
5914	Retinoic acid receptor alpha	RARA	3	1	216300_x_at	40	1.9
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5970	RELA proto-oncogene. NF-kB subunit	RELA	16	2	201783_s_at	53.3	3.7
6256	Retinoid X receptor alpha	RXRA	41	6	202449_s_at	146	6.8
6670	Sp3 transcription factor	SP3	3	1	213168_at	155.7	7.5
6722	Serum response factor	SRF	3	1	202401_s_at	58.6	4.1
6772	Signal transducer and activator of transcription 1	STAT1	15	1	200887_s_at	160	13.7
6908	TATA-box binding protein	TBP	110	17	203135_at	76.7	2.7

6925	Transcription factor 4	TCF4	2	0	212386_at	526	30.8
6934	Transcription factor 7 like 2	TCF7L2	8	1	212761_at	140.6	8.4
8463	TEA domain transcription factor 2	TEAD2	1	0	243766_s_at	53.4	2.4
7020	Transcription factor AP-2 alpha	TFAP2A	61	7	204653_at	264.8	29.5
7024	Transcription factor CP2	TFCP2	5	0	209338_at	100.1	3.6
7030	Transcription factor binding to IGHM enhancer 3	TFE3	65	3	212457_at	226.9	7
7068	Thyroid hormone receptor beta	THRB	5	0	229657_at	106.4	5.3
7528	YY1 transcription factor	YY1	67	5	201901_s_at	379	22.4

bp: base pairs.