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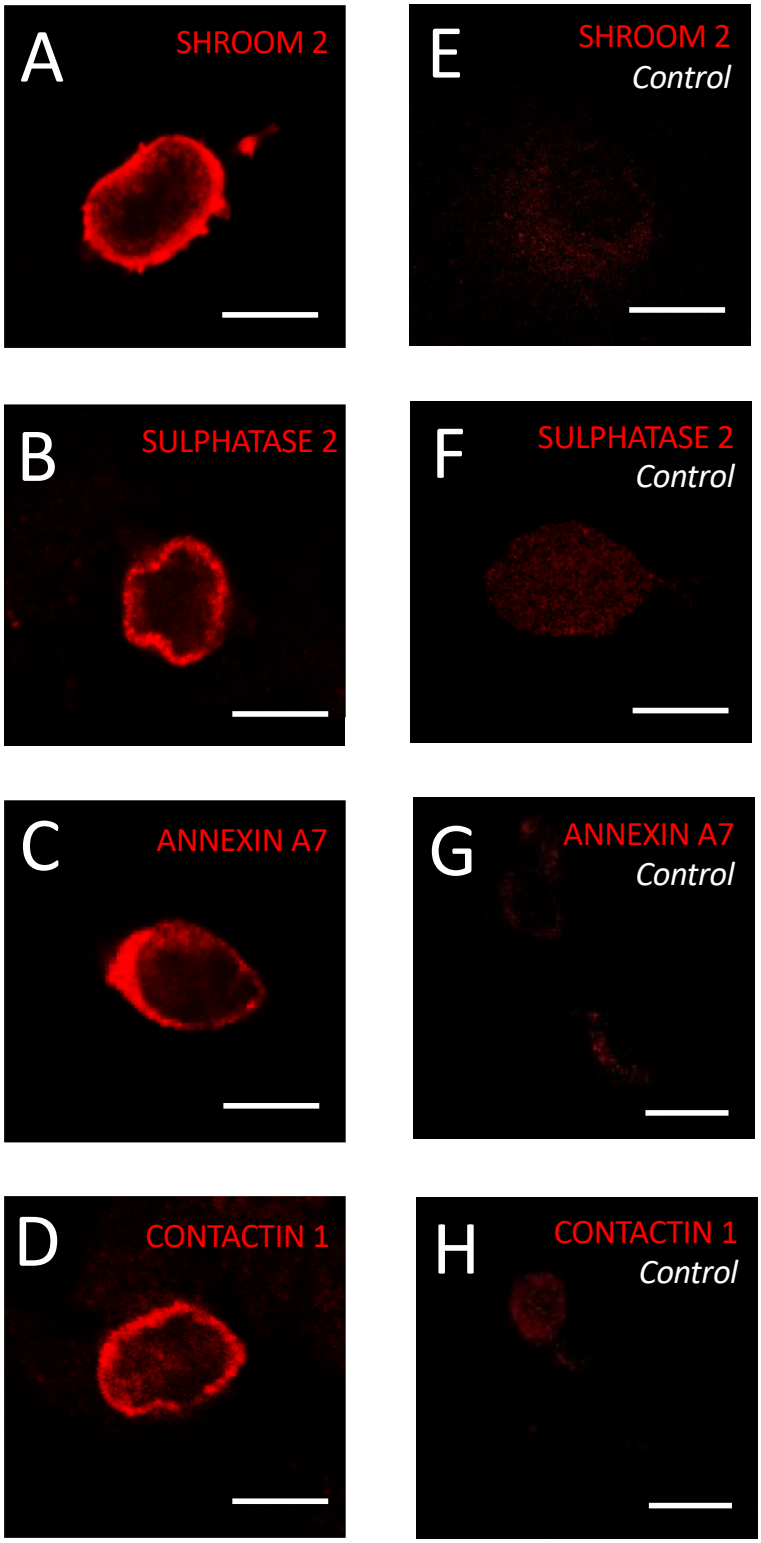
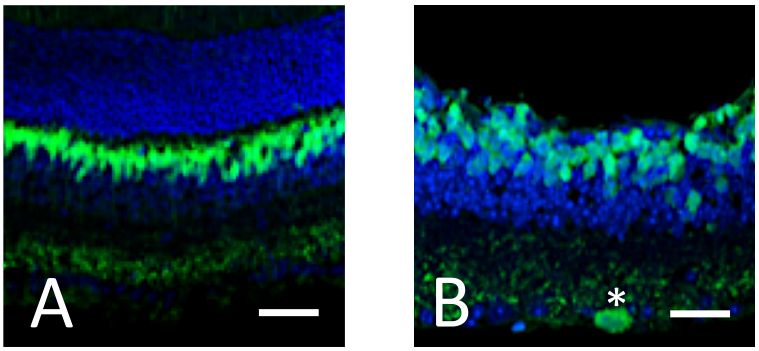
“Retinal bipolar cell gene expression during retinal degeneration: implications for optogenetic visual restoration”

Supplementary Figures & Tables

February 2021


A
SHROOM 2
E
SHROOM 2
Control
B
SULPHATASE 2
F
SULPHATASE 2
Control
C
ANNEXIN A7
G
ANNEXIN A7
Control
D
CONTACTIN 1
H
CONTACTIN 1
Control

(Fig S1.)


A
B
*

(Fig S2.)

Figure S1. Validation of antibodies. ICC images of HEK293T cells transfected to express the protein corresponding to the labelled antibody. As can be seen, all four stain at a level far above that of a non-transfected control. Note staining pattern of all four antibodies is consistent with protein in or around the cell membrane.
A – D Appropriately transfected HEK293T cells stained with the respective antibody
E – H Untransfected HEK293T cells stained with the respective antibody
Scale bars - 10μm

Figure S2. L7.Cre.EYFP mice express EYFP in rod and cone type 2 & 6 bipolar cells as well as a small subset of bistratified retinal ganglion cells (circa 1,500 in retina, denoted by asterix). Blue = DAPI, Green = YFP Scale bars = 15μm

(A) On a *Pde6b*$^{wt/wt}$ background
(B) On a *Pde6b*$^{rd1/rd1}$ background

Probe/gene name	log_2 fold change	p value
Rod Specific		
Gnat1	-21.14	0.0002
Pdc	-1.83	0.0421
Pde6b	-3.47	0.0004
Nrl	-2.79	0.0380
Sag	-6.01	0.0009
Slc24a1	-5.27	0.0231
Pde6g	-3.02	0.0473
Reep6	-2.56	0.0380
Esrrb	-2.00	0.0153

Probe/gene name	log_2 fold change	p value
Rod contentious		
Gpnmb	-4.14	0.0035
Ldha	-4.61	0.0014
Car2	-2.94	0.0004
Adcy1	-2.97	0.0004

Table S1. (A) Genes with rod specific ontological annotation; (B) Genes with literature suggesting contentiousness due to low bipolar compared to photoreceptor protein expression. A positive log fold change represents upregulation in degenerate samples. *"p* value" represents the adjusted p-value following False Detection Rate (FDR) testing.

Symbol	Probe/gene name	log_2 fold Δ	p value
A - Probes upregulated in degeneration			
Igfn1	Immunoglobulin-like and fibronectin type III domain containing 1	4.10	0.0181
Cgn	Cingulin	3.52	0.0138
Msrb2	Methionine sulfoxide reductase B2	3.03	0.0344
Cabyr	Calcium binding tyrosine-(Y)-phosporylation regulated	2.25	0.0380
Efnb1	Ephrin-B1	2.15	0.0312
Ccdc171	Coiled-coil domain containing 171	1.99	0.0458
Phc1	Polyhomeotic homolog 1	1.93	0.0276
Sfxn1	Sideroflexin 1	1.92	0.0195
Fam19a3	Family with sequence similarity 19 (chemokine (C-C motif)-like) A3	1.87	0.0458
Shroom2	Shroom family member 2	1.81	0.0416
Sfrs1	Serine/arginine-rich splicing factor 1	1.72	0.0417
Sept4	Septin 4	1.54	0.0324
Sulf2	Sulfatase 2	1.43	0.0344
B - Probes downregulated in degeneration			
Nrm	Nurim (nuclear envelope membrane protein)	-1.40	0.0416
Gm362	Predicted gene 362	-1.46	0.0138
Pde9a	Phosphodiesterase 9A	-1.55	0.0416
Ndufb2	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 2	-1.55	0.0414
Gabrg2	Gamma-aminobutyric acid (GABA) A receptor, gamma 2	-1.56	0.0494
Anxa7	Annexin A7	-1.56	0.0244
Fam195a	Family with sequence similarity 195, member A	-1.61	0.0053
Atox1	Antioxidant 1 copper chaperone	-1.62	0.0414
Socs5	Suppressor of cytokine signaling 5	-1.64	0.0149
Csrnp2	Cysteine-serine-rich nuclear protein 2	-1.65	0.0276
Rnf11	Ring finger protein 11	-1.75	0.0244
Cntn1	Contactin 1	-1.79	0.0494
COXIII	Mitochondrial cytochrome oxidase II subunit	-1.83	0.0244
Ap3m2	Adapter-related protein complex 3, mu 2 subunit	-1.85	0.0231
Rnf11	Ring finger protein 11	-1.88	0.0060
Mif	Macrophage migration inhibitory factor	-1.91	0.0337
Pde1c	Phosphodiesterase 1C, calmodulin-depdendent 70kDa	-1.96	0.0053
Pcdha7	Protocadherin alpha 7	-1.98	0.0264
Extl3	Exostosin-like glycosyltransferase 3	-2.05	0.0291
Unc13a	Unc-13 homolog A	-2.11	0.0060
Gm5478	Predicted pseudogene 5478	-2.13	0.0103
Tmsb10	Thymosin beta 10	-2.15	0.0494
Pgk1	Phosphoglycerate kinase 1	-2.30	0.0414
Stmnd1	Stathmin domain containing 1	-2.42	0.0034
Mt2	Metallothionein 2	-2.50	0.0126
Yaf2	YY1 associated factor 2	-2.54	0.0473
Clip1	CAP-GLY domain containing linker protein 1	-2.75	0.0060
Slc7a3	Solute carrier family 7 (cationic amino acid transporter, Y+ system), 3	-2.87	0.0103
Abca1	ATP-binding cassette, sub-family A (ABC1), member 1	-3.10	0.0171
Them6	Thioesterase superfamily member 6	-3.24	0.0011
Hdac9	Histone deacetylase 9	-3.69	0.0029
Ptprr	Protein tyrosine phosphatase, receptor type, R	-3.72	0.0135
Naa11	N(alpha)-acetyltransferase 11, NatA catalytic subunit	-3.83	0.0004
Lynx1	Ly6/neurotoxin 1	-8.26	0.0135
Mt1	Metallothionein 1	-10.73	0.0015

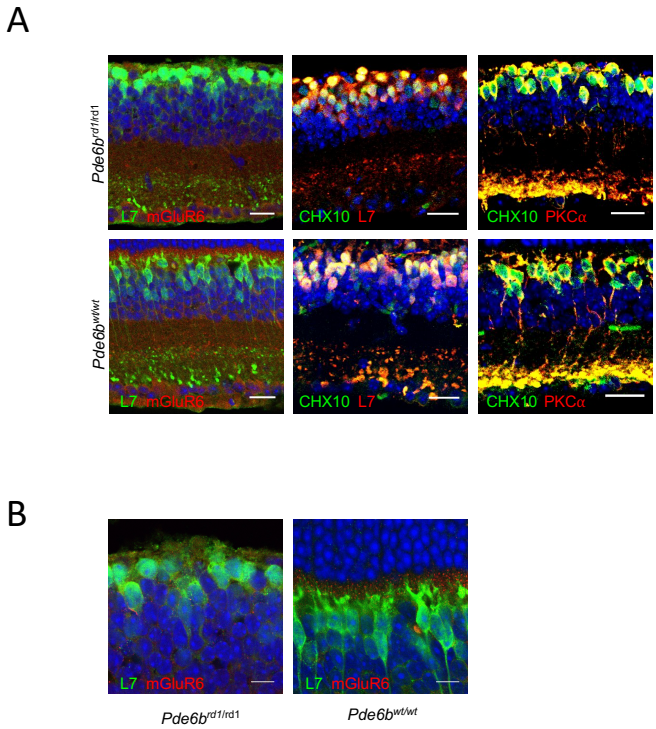
Table S2. Differentially expressed genes in a microarray study comparing FACS-isolated EYFP-positive cells from degenerate and non-degenerate L7-Cre EYFP mouse retinas. Statistically significant, differentially expressed, probes are listed according to log fold change. A positive log fold change represents upregulation in the degenerate samples. "*p* value" represents the adjusted p-value following False Detection Rate (FDR) testing.

Probe	Log_2 fold Δ	p value
Receptor		
Grm6	1.054	0.8573
Gα subunits		
Gna11	1.04	0.7896
Gna11	-1.07	0.8574
Gna14	-1.08	0.7764
Gnai1	-1.09	0.9360
Gnai2	-1.04	0.8810
Gnai3	1.01	0.9863
Gnao1	-1.23	0.7900
Gnao1	1.02	0.9835
Gnaq	-1.40	0.0539
Gnaq	-1.03	0.8832
Gβγ subunits		
Gnb3	1.91	0.1685
Gnb4	-1.05	0.9335
Gnb5	-1.01	0.9670
Gng2	-1.10	0.8044
Gng3	1.34	0.8414
Gng5	1.04	0.8175
Gng7	1.64	0.6041
Gng7	1.07	0.7679
Gng10	-1.08	0.7839
Gng11	-1.05	0.8035
Gng13	-1.13	0.7109
Bipolar cell-specific		
Cabp5	1.48	0.5394
Pcp2	-1.12	0.8044
Prkca	-1.09	0.7514
Scgn	1.40	0.8044
Scgn	-1.02	0.9746
Vsx1	1.04	0.9102
Vsx2	1.21	0.8056
Vsx2	1.10	0.9021
Kinases		
Grk4	1.13	0.7241
Grk4	-1.07	0.7896
Grk5	1.09	0.7918
Grk5	1.04	0.8642

Probe	Log_2 fold Δ	p value
Intracellular effectors		
Adcy1	-2.97	0.0004
Adcy1	1.05	0.8044
Adcy2	1.77	0.5721
Adcy3	1.01	0.9480
Adcy8	1.06	0.8086
Adcy9	1.05	0.8035
Pde1b	-1.37	0.5764
Pde1b	-1.41	0.6178
Pde1c	-1.96	0.0053
Pde1c	-1.22	0.1861
Pde1c	-1.09	0.7629
Pde1c	1.19	0.7932
Pde1c	-1.10	0.8337
Pde1c	1.00	0.9986
Pde3a	-1.03	0.8542
Pde3a	1.01	0.9441
Pde3a	1.00	1.0000
Pde3b	-1.02	0.9259
Pde4a	-1.12	0.5721
Pde4b	-1.26	0.4073
Pde4b	-1.20	0.7671
Pde4b	1.27	0.8311
Pde4b	-1.02	0.9409
Pde4d	-2.05	0.5154
Pde4d	-1.00	0.9918
Pde7a	-1.06	0.7887
Pde7b	-1.16	0.6154
Pde8b	-1.02	0.8908
Pde9a	-1.55	0.0416
Plcb3	1.07	0.7536
Plcb4	1.24	0.8044
TRP channels		
Trpc6	-1.05	0.7749
Trpc7	-1.29	0.7887
Trpm1	1.19	0.7847
Trpm1	-1.13	0.8035
Trpm1	1.09	0.8220
Trpm1	1.19	0.8401
Arrestin		
Arrb2	-1.11	0.8673

Housekeeping genes					
Actb	1.45	0.6114	*Gapdh*	-1.51	0.1950
Actb	-1.31	0.7128	*Rplp0*	-1.15	0.8035
Actb	-1.03	0.9801	*Rplp0*	1.02	0.9900
Actb	-1.03	0.9802			

Table S3. In a microarray study comparing FACS-isolated EYFP-positive cells from degenerate and non-degenerate L7-Cre EYFP mouse retinas, genes related to intracellular communication, the native on-bipolar signal transduction cascade. A selection of "housekeeping" genes are included for comparison. A positive log fold change represents upregulation in degenerate samples. "p value" represents the adjusted p-value following False Detection Rate (FDR) testing. Note that multiple probes for some genes are reported in the above table, for clarity, all probes are labelled only with the associated gene name.


A
Pde6b rd1/rd1
Pde6b wt/wt
L7 mGluR6
CHX10 L7
CHX10 PKCα
B
L7 mGluR6
Pde6b rd1/rd1
Pde6b wt/wt
C
Colocalised staining at P90
ns
ns
ns
1.0
0.5
0.0
Colocalised Signal
Pde6b wt/wt L7 & mGluR6
Pde6b rd1/rd1 L7 & mGluR6
Pde6b wt/wt CHX10 L7
Pde6b rd1/rd1 CHX10 L7
Pde6b wt/wt CHX10 PKCa
Pde6b rd1/rd1 CHX10 PKCa
Group

(Fig S3.)

Figure S3.

A. Immunohistochemical staining of retina from *Pde6b*$^{rd1/rd1}$ and *Pde6b*$^{wt/wt}$ mice culled at P90 for proteins associated with the bipolar signalling cascade (mGluR6, PKCα & L7 – none of which demonstrated significant changes in gene expression between *Pde6b*$^{rd1/rd1}$ and *Pde6b*$^{wt/wt}$ - see table S3). In all panels, DAPI staining is in blue, other colours as indicated in panels scale bar = 20μm main panels.
B. Higher magnification views of mGluR6 staining in the outer plexiform layer . Note particularly the punctate pattern of staining of the bipolar cells dendrites in the wild type. Note L7 staining covers cell bodies and dendrites. Scale bars =10μm.
C. Colocalised pixels above threshold - a semiquantitative index of protein expression based on immunohistochemical staining. Normalised to highest value over all retinae stained for the same proteins. t-tests did not reveal a difference in mean values between *Pde6b*$^{rd1/rd1}$ and *Pde6b*$^{wt/wt}$ retinae stained for the same proteins ($p>0.05$). NS = not-significant.

Probe	Log_2 fold Δ	p value
Kainate type		
Grik1	1.57	0.6786
Grik1	1.07	0.7896
Grik1	1.13	0.7934
Grik1	1.09	0.8785
Grik1	-1.03	0.9705
Grik4	-1.01	0.9784
Vglut transporter		
Slc17a6	-1.05	0.8679
Slc17a6	-1.01	0.9865
Slc17a7	-1.05	0.8592
Slc17a8	-1.08	0.7415

Probe	Log_2 fold Δ	p value
AMPA type		
Gria1	-1.39	0.7587
Gria1	1.05	0.8440
Gria1	1.04	0.8681
Gria1	1.04	0.8708
Gria2	1.27	0.6526
Gria2	1.21	0.7415
Gria3	-1.04	0.8035
Gria3	-1.06	0.9259
Gria4	1.07	0.7587
NMDA type		
Grin2c	-1.02	0.9094

GABA and Glycine receptor subunits

GABA		
Gabra1	-1.05	0.8824
Gabra3	1.07	0.8194
Gabrb1	1.01	0.9632
Gabrb2	1.11	0.6142
Gabrb2	1.01	0.9870
Gabrb3	1.12	0.7500
Gabrb3	-1.11	0.8013
Gabrb3	-1.03	0.9382
Gabrg2	-1.56	0.0494
Gabrg2	-1.48	0.6302
Gabrg2	-1.36	0.6308
Gabrg2	1.06	0.7420
Gabrg2	-1.06	0.8721
Gabrg2	-1.10	0.9477
Gabrr1	-1.56	0.5706
Gabrr1	-1.04	0.8718
Gabrr2	-1.16	0.7896

Glycine		
Glra2	-1.06	0.8015
Glra4	1.09	0.7753
Glrb	1.07	0.7969

Table S4. In a microarray study comparing FACS-isolated EYFP-positive cells from degenerate and non-degenerate L7-Cre EYFP mouse retinas, genes relating to glutamate, glycine and gaba receptors;. A positive log fold change represents upregulation in degenerate samples. “P value” represents the adjusted p-value following False Detection Rate (FDR) testing. Note that multiple probes for some genes are reported in the above table, for clarity, all probes are labelled only with the associated gene name.

Protein of Interest	Antibody (source & product number)	Plasmid used in validation ICC
ANNEXINa7	Abcam ab197586	Source bioscience plasmid no. 3157926
CONTACTIN1	Abcam ab66265	Sino biologic plasmid no. MG50933
SHROOM2	Atlas hpa051646	GenScript plasmid no. OMu14281D
SULPHATASE2	Abcam ab113405	Addgene plasmid no. 13008
CHX10	Abcam ab16141	See Kim et al [REFERENCE Kim et al 2008]
PKCα	Abcam ab32376	See Ruether et al [REFERENCE Ruether et al 2010]

(Table S5)

Table S5. Antibodies and plasmids used in their validation

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