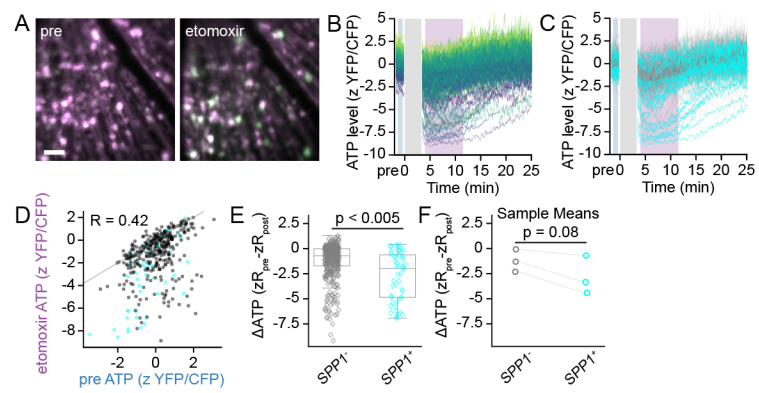


Figure	Animal	RGC Count	Experiment
1	2493 1R	127	Baseline
1	3139 2R	126	Baseline
1	3590 2R	77	Baseline
1	3657 0R	167	Baseline
1	7702 2R	41	Baseline
1	1410 3R	39	Repeat
1	1411 0L	54	Repeat
1	1411 0R	82	Repeat
1	1411 1R	37	Repeat
1	1411 2L	62	Repeat
1	1411 2R	65	Repeat
1	3091 3R	42	Repeat
1	2493 1R	160	Typing
1	3139 2R	178	Typing
1	3590 2R	101	Typing
1	3657 0R	220	Typing
1	7702 2R	113	Typing
2	2557 3R	92	Rotenone
2	2558 1L	90	Rotenone
2	2558 2L	89	Rotenone
2	3657 0L	161	Rotenone
2	974 0L	120	Rotenone
2	2493 1L	100	TTFA
2	2493 1R	127	TTFA
2	3570 2L	105	TTFA
2	4357 4L	93	TTFA
2	3139 2L	113	Antimycin
2	3139 2R	126	Antimycin
2	3590 2R	77	Antimycin
2	7702 0L	116	Antimycin
2	1350 0R	95	KCN
2	1350 1R	54	KCN
2	3159 0L	138	KCN
2	4357 3L	117	KCN
3	3139 0R	46	Str/Bic
3	3139 1L	121	Str/Bic
3	3139 4R	84	Str/Bic
3	3139 0L	47	NBQX/AP5
3	3139 1R	120	NBQX/AP6

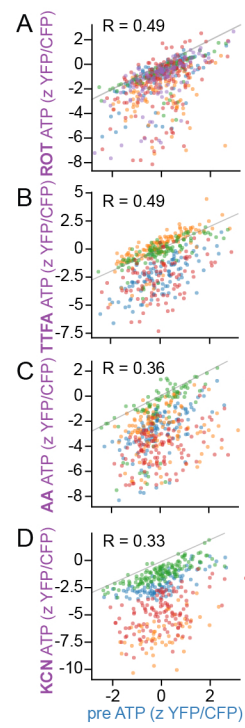
3	3139 3R	16	NBQX/AP7
3	3139 4L	99	NBQX/AP8
3	3590 0L	163	Stim
3	3590 0R	137	Stim
3	3590 2R	88	Stim
3	3590 4R	97	Stim
4	4071 0R	125	Rotenone
4	4083 1R	56	Rotenone
4	4071 0L	132	Rotenone/NBQX
4	4071 1R	34	Rotenone/NBQX
4	4071 2R	102	Rotenone/NBQX
4	4083 1L	81	Rotenone/NBQX
4	1410 0R	68	Rotenone stim
4	1410 2L	53	Rotenone stim
4	5507 1R	163	Rotenone stim
5	735 0L	50	Survival
5	735 0R	73	Survival
5	736 0L	52	Survival
5	736 1L	24	Survival
5	736 1R	65	Survival
5	1436 0L	41	Vehicle
5	1447 0R	39	Vehicle
5	1447 1L	44	Vehicle
5	1447 2R	85	Vehicle
5	3618 1R	94	Vehicle
5	3617 0R	151	NMN
5	3617 1L	33	NMN
5	3617 3L	50	NMN
5	3618 2L	54	NMN
S1	3184 0R	128	etomoxir
S1	856 1R	102	etomoxir
S1	1290 0L	143	etomoxir
S3	975 1L	156	2-deoxyglucose
S3	1914 1R	68	2-deoxyglucose
S3	1914 4L	139	2-deoxyglucose
S3	3083 3L	52	2-deoxyglucose
S5	3138 0L	147	Ca Str/Bic
S5	3138 0R	168	Ca Str/Bic
S5	3138 1R	128	Ca Str/Bic
S5	3138 2L	167	Ca Str/Bic
S5	3138 3L	219	Ca Str/Bic

S5	3138 3R	94	Ca Str/Bic
S5	3139 0L	47	Ca NBQX/AP5
S5	3139 1R	120	Ca NBQX/AP5
S5	3139 3R	16	Ca NBQX/AP6
S5	3139 4L	99	Ca NBQX/AP7
S5	2505 0L	155	Ca stim
S5	2505 0R	172	Ca stim
S5	2505 1R	120	Ca stim
S5	3138 0L	209	Ca stim
S5	3138 0R	190	Ca stim
S5	3138 1L	83	Ca stim
S5	3138 1R	139	Ca stim
S5	3138 2L	194	Ca stim
S5	3138 2R	113	Ca stim
S5	3138 3L	231	Ca stim
S5	3138 3R	125	Ca stim
S5	3138 4L	204	Ca stim
S5	3138 4R	120	Ca stim

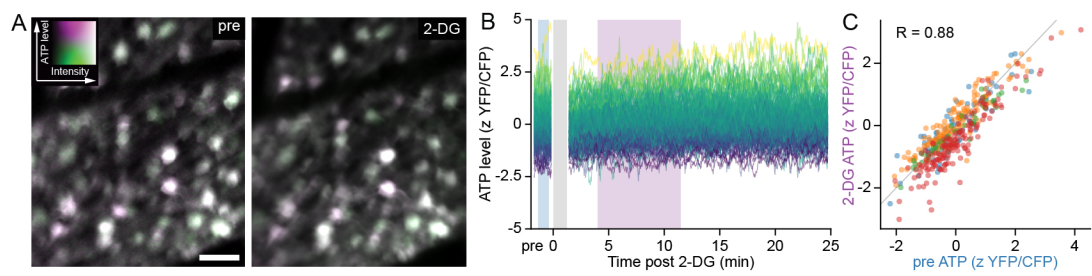
Supplementary Table 1. Catalogue of mice used for experiments. List of samples used to generate *in vivo* imaging data used in this manuscript. Figure, specific experiment and number of RGCs that passed inclusion criteria are listed.



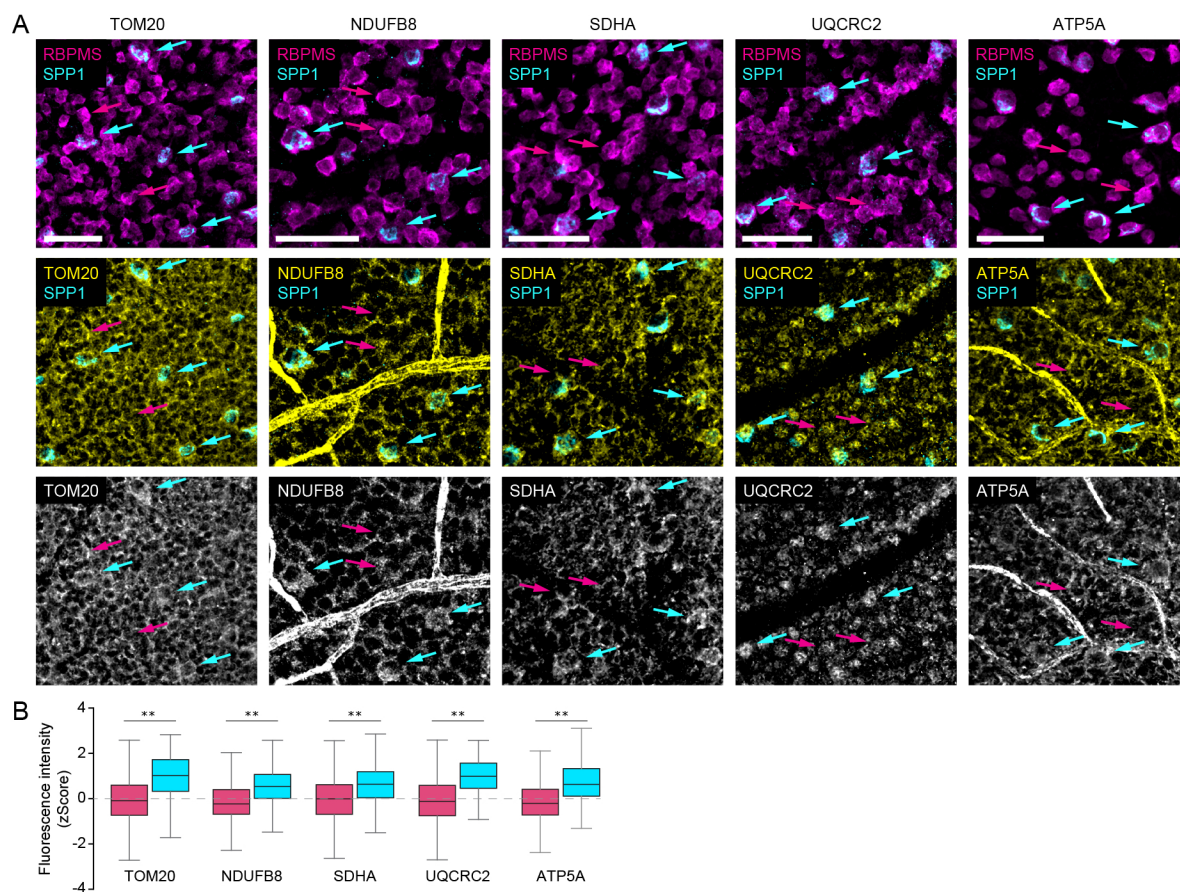
Supplementary Figure 1. The Complex I inhibitor Etomoxir induces ATP reductions that are most prevalent in α RGCs. (a) *In vivo* 2-photon aips of ATeam in the same retina imaged before and after intravitreal injection of 10 mM etomoxir. Scale bar = 50 μ m. (b). Traces of single RGC ATP levels tracked in an example retina in response to etomoxir. Each line indicates an individual RGC. zScores are pseudocolored from minimal (blue) to maximal (yellow) according to the values in the pre trace. Blue region indicates the 'pre' time series acquired before drug injections, gray region indicates the time between injection and start of imaging, and purple region indicates the time frame for post-drug injection measurements analyzed in following graphs. (c) Example traces of ATP levels in (b) displayed by RGC type according to *post hoc* immunostaining for SPP1⁺ (cyan) or lack of SPP immunostaining (gray). (d) Scatterplot of ATP levels in RGCs comparing pre and post etomoxir. Each point represents an RGC colored by RGC type (n=373 RGCs from 3 retinas and 3 mice). (e) Scatter and interquartile range boxplots of post injection changes in ATP levels from pre after injection of etomoxir (measured from blue and purple boxed regions in (b)). (f) Scatterplots of post injection changes in ATP levels represented by sample means of the RGCs graphed in (e). Two-sided Mann Whitney U test (e) or student's T-test (f). Source data are provided as a Source Data file.



Supplementary Figure 2. Variation in response to ETC inhibitors is influenced by injection. (a-d) Scatterplot of ATP levels in RGCs comparing pre and post injection of the indicated ETC inhibitor. Each point represents an RGC, each color indicates a retina (ROT n=264 RGCs from 5 retinas and 5 mice, TTFA n = 212 RGCs from 4 retinas and 3 mice, AA n=220 RGCs from 4 retinas and 3 mice, KCN n=218 RGCs from 4 retinas and 4 mice). Grey reference line plots $y = x$. Source data are provided as a Source Data file.

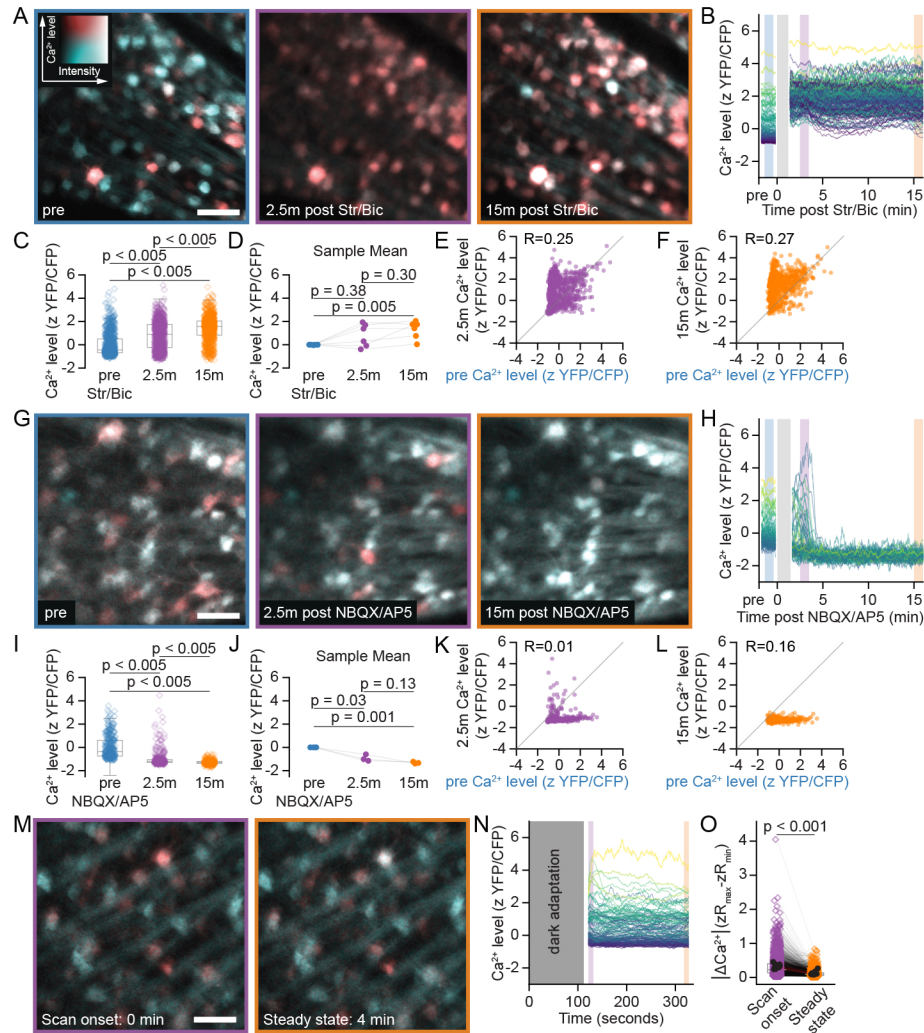


Supplementary Figure 3. Glycolysis inhibitor 2-deoxyglucose does not lead to reductions in RGC ATP levels. (a) *In vivo* 2-photon aips of ATeam in the same retina imaged before and at the indicated time points after intravitreal injection of 2-DG. (b) Traces of single RGC ATP levels tracked in an example retina in response to 2-DG. Each line indicates an individual RGC pseudocolored to represent pre-injection ATP levels. (C) Scatterplot of ATP levels in RGCs comparing pre and post injection of 2-DG. Each point represents an RGC, and each unique color indicates a retina (n=414 RGCs from 4 retinas and 4 mice). Grey reference line plots $y = x$. Source data are provided as a Source Data file.



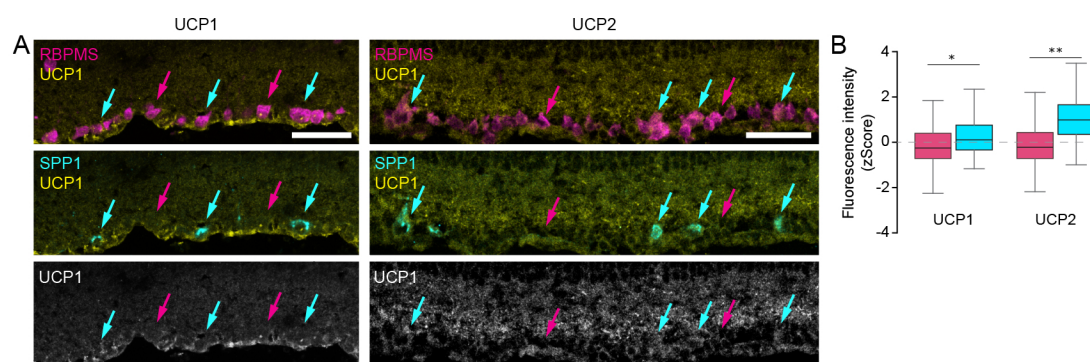
Supplementary Figure 4. Mitochondrial protein expression is higher in α RGCs. (a)

Confocal maximum intensity projections (mip) of retina wholemounts immunostained for RBPMS (magenta), SPP1 (cyan) and the indicated mitochondrial component (yellow). Cyan arrows indicate SPP1⁺ α RGCs, magenta arrows indicate SPP1⁻ RGCs. Scale bars = 50 μ m. (b) Interquartile range boxplots of relative mitochondrial component expression intensity in SPP1⁻ (magenta) and SPP1⁺ (cyan) RGCs (TOM20 n = 2218 RGCs from 3 retinas and 3 mice, NDUFB8 n = 2204 RGCs from 3 retinas and 3 mice, SDHA n = 2604 RGCs from 4 retinas and 4 mice, UQCRC n = 2063 RGCs from 3 retinas and 3 mice, ATP5A n = 2170 RGCs from 3 retinas and 3 mice). ** p < 0.001, two-sided Student's t-test. Source data are provided as a Source Data file.



Supplementary Figure 5. Activity modulation induces Ca²⁺ changes in RGCs *in vivo*. (a) *In vivo* 2-photon aips of Twitch2b in the same retina imaged before and at the indicated time points after intravitreal Str/Bic injection. YFP and CFP pseudocolored red and cyan respectively throughout. (b) Traces of RGC Ca²⁺ levels tracked in an example retina after Str/Bic. Each line indicates an RGC pseudocolored to represent pre-injection Ca²⁺ levels. (c-d) Scatter and interquartile range boxplots of individual RGC Ca²⁺ levels (c) and sample means (d) before and at indicated time points after intravitreal Str/Bic injection. Colors indicate time points boxed in (b). One-way ANOVA with posthoc Tukey's test. (e-f) Scatterplots of Ca²⁺ levels before and at indicated time points after intravitreal Str/Bic injection (n = 923 RGCs from 6 retinas and 5 mice). Grey reference line plots y = x. (g) *In vivo* 2-photon aips of the same retina imaged before and at the indicated time points after intravitreal NBQX/AP5 injection. (h) Traces of RGC Ca²⁺ levels from an example retina after NBQX/AP5. (i-j) Scatter and interquartile range boxplots of RGC Ca²⁺ levels (i) and sample means (j) before and at indicated time points after intravitreal NBQX/AP5 injection. (k-l) Scatterplots of Ca²⁺ levels before and at indicated time points after intravitreal NBQX/AP5 injection (n = 334 RGCs from 3 retinas and 3 mice). (m) *In vivo* 2-photon aips of a retina dark-adapted for 2 min and then imaged immediately after scanning onset (left) or 2 min later after reaching steady state (right). (n) Traces of RGC Ca²⁺ levels tracked in a dark-adapted example retina from the time point immediately after scanning onset. (o) Scatterplot of the change in Ca²⁺ during the timeframe immediately after scanning onset or after reaching steady state indicated by purple and orange boxes (n) respectively. Black points indicate sample means. Two-sided Kruskal-Wallis test with posthoc Dunn's test. (n = 2055 RGCs from 13 retinas and 7 mice). Scale

bars = 50 μm . Str/Bic = strychnine / bicuculline, NBQX/AP5 = 2,3-dioxo-6-nitro-7-sulfamoyl-benzoquinoline / (2R)-amino-5-phosphonovaleric acid. Source data are provided as a Source Data file.



Supplementary Figure 6. Mitochondrial uncoupling proteins are expressed at higher levels in α RGCs. (a) Maximum intensity projections of confocal image stacks of retinal cross-sections immunostained for RBPMS (magenta), SPP1 (cyan) and either UCP1 (left) or UCP2 (right) (yellow/grayscale). Cyan arrows indicate SPP1⁺ RGCs and magenta arrows indicate SPP1⁻ RGCs. Scale bar = 50 μ m. (b) Interquartile range boxplots of UCP1 or UCP2 immunofluorescence intensity in SPP1⁻ (magenta) compared to SPP1⁺ (cyan) RGCs. For UCP1 n = 848 RGCs from 3 retinas and 3 mice p = 0.02. For UCP2 n = 966 RGCs from 3 retinas and 3 mice p < 0.001. Two-sided Mann Whitney U test. Source data are provided as a Source Data file.