

Supplementary Information

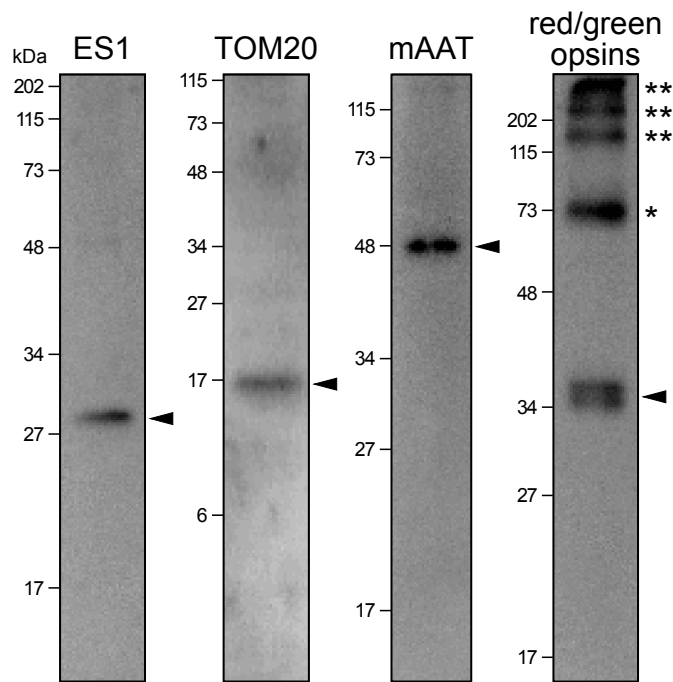
ES1 is a mitochondrial enlarging factor contributing to form
mega-mitochondria in zebrafish cones

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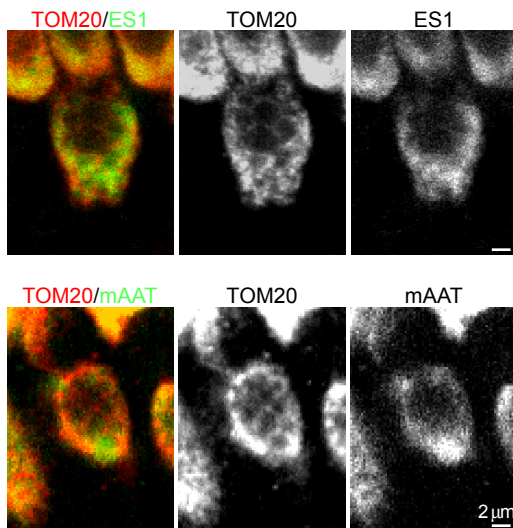
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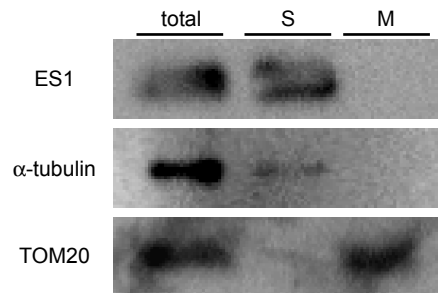


Supplementary Figure S1. Immuno-specificities of antibodies used for immunohistochemical analyses. Immunoblottings were performed with anti-ES1, TOM20, mAAT or red/green opsins antibodies on the lysate from the zebrafish eyes. Anti-ES1, TOM20 and mAAT antibodies showed only single bands (arrowheads), which correspond to the calculated molecular masses of each of the proteins. Anti-red/green opsins antibody showed multiple bands which correspond to monomers (arrowhead), dimers (single asterisk) or multimers (double asterisks) of red/green opsins as previously reported¹.

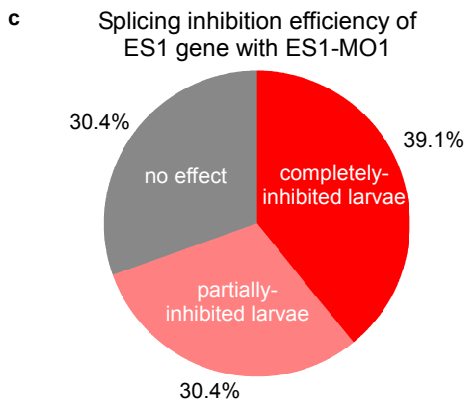
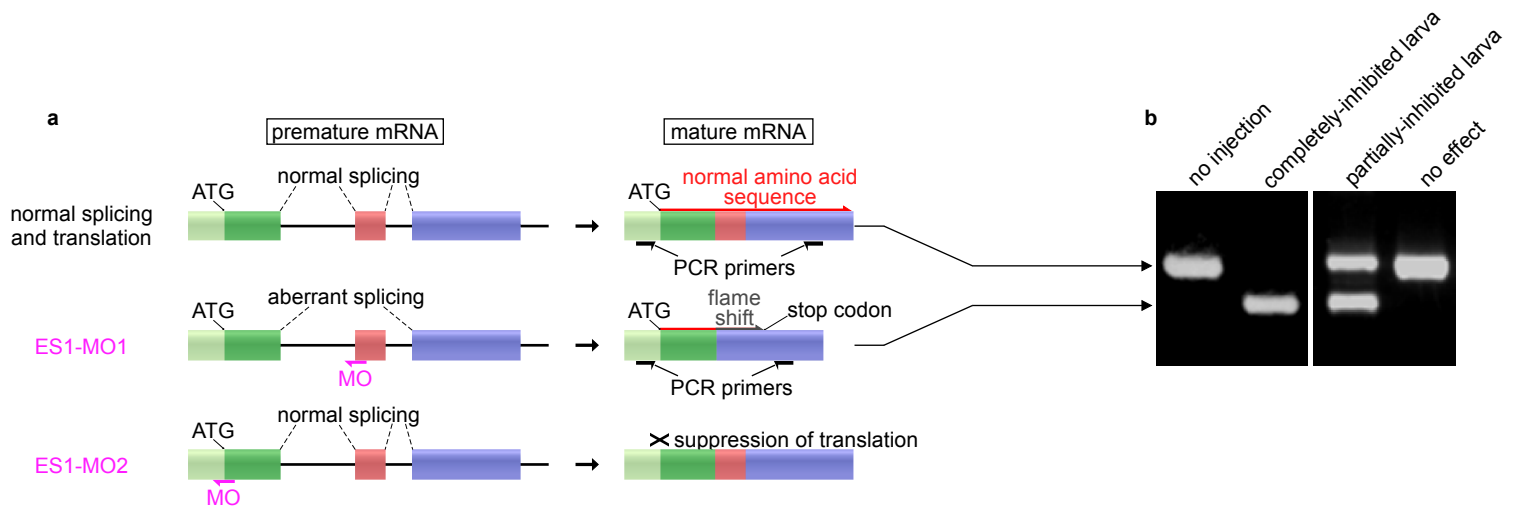
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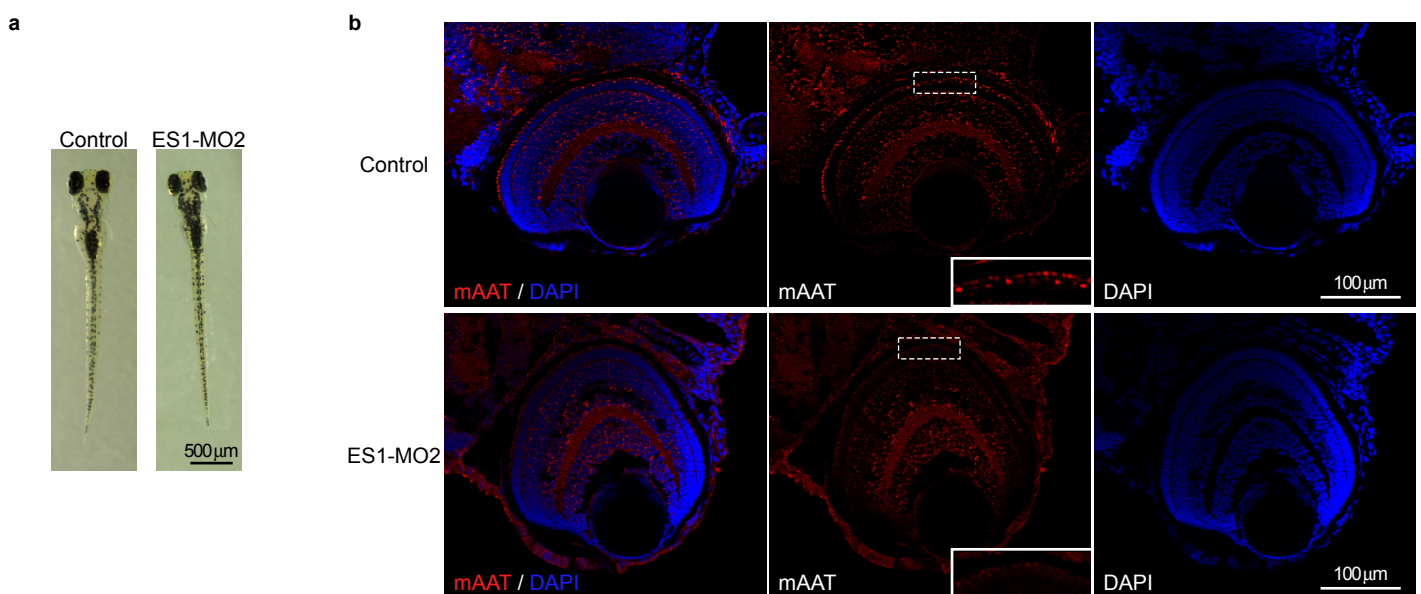
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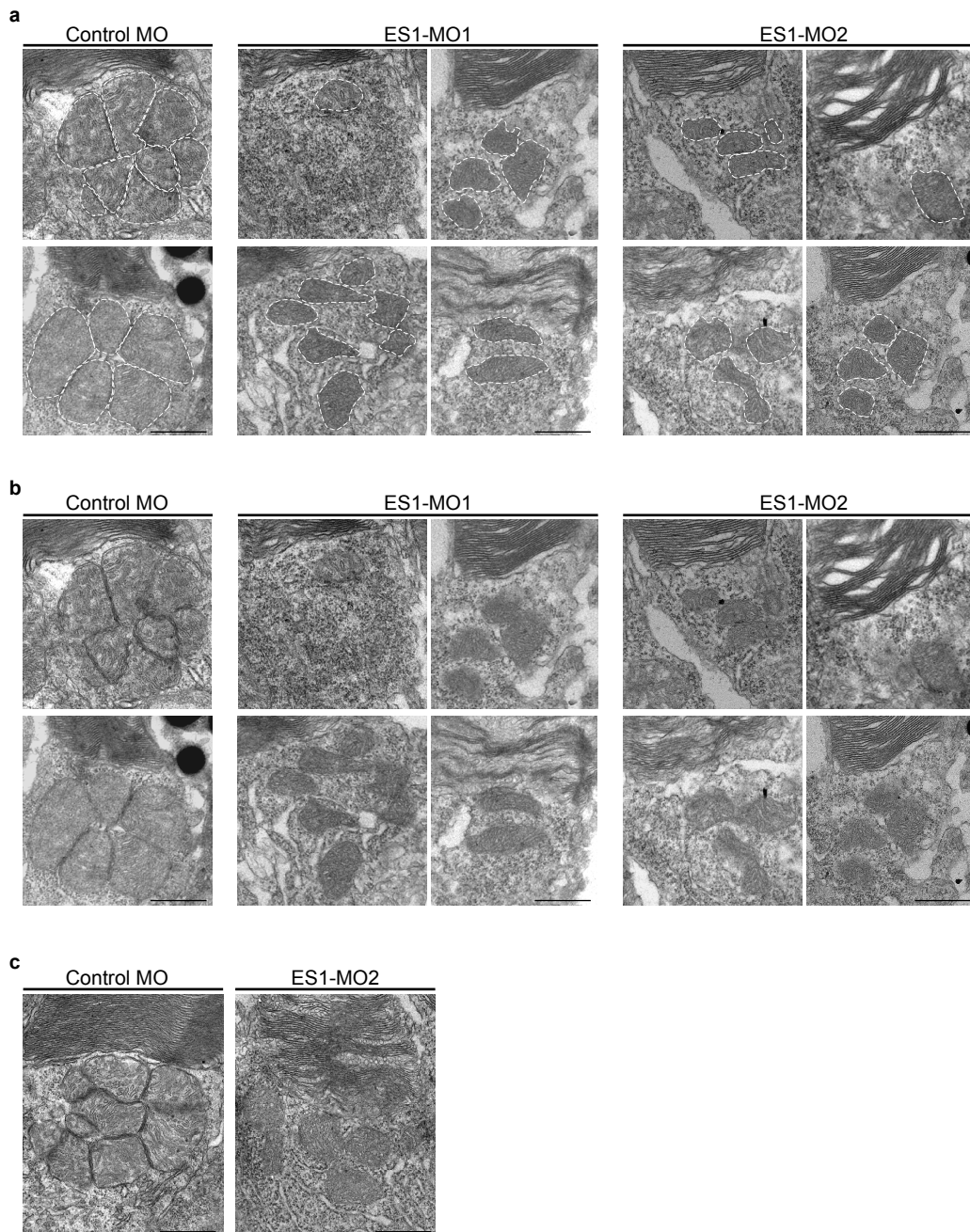
Supplementary Figure S2. Subcellular localization of ES1 in zebrafish cones. (a) Apparent heterogeneity of immunoreactivities in cone ellipsoid. High magnification views of double immunostainings of cone ellipsoids with TOM20/ES1 antibodies (upper panels) and TOM20/mAAT antibodies (lower panels). TOM20 antibody was used as a mitochondrial outer membrane marker to detect the outline of individual mitochondria. Both ES1- and mAAT-immunopositive signals were weaker at apical (upward direction) and central regions of the cone ellipsoids (right panels). In the same regions, TOM20 antibody depicted clear round patches >2 μm in diameter (middle panels), indicating the presence of mega-mitochondria. (b) Subcellular fractionation analysis for ES1. Immunoblotting analysis with anti-ES1 antibodies was performed using fractionated cells from retinas. Anti- α -tubulin and TOM20 antibodies were used as a marker for soluble fraction and membrane fraction, respectively. S: soluble fraction, M: membrane fraction.



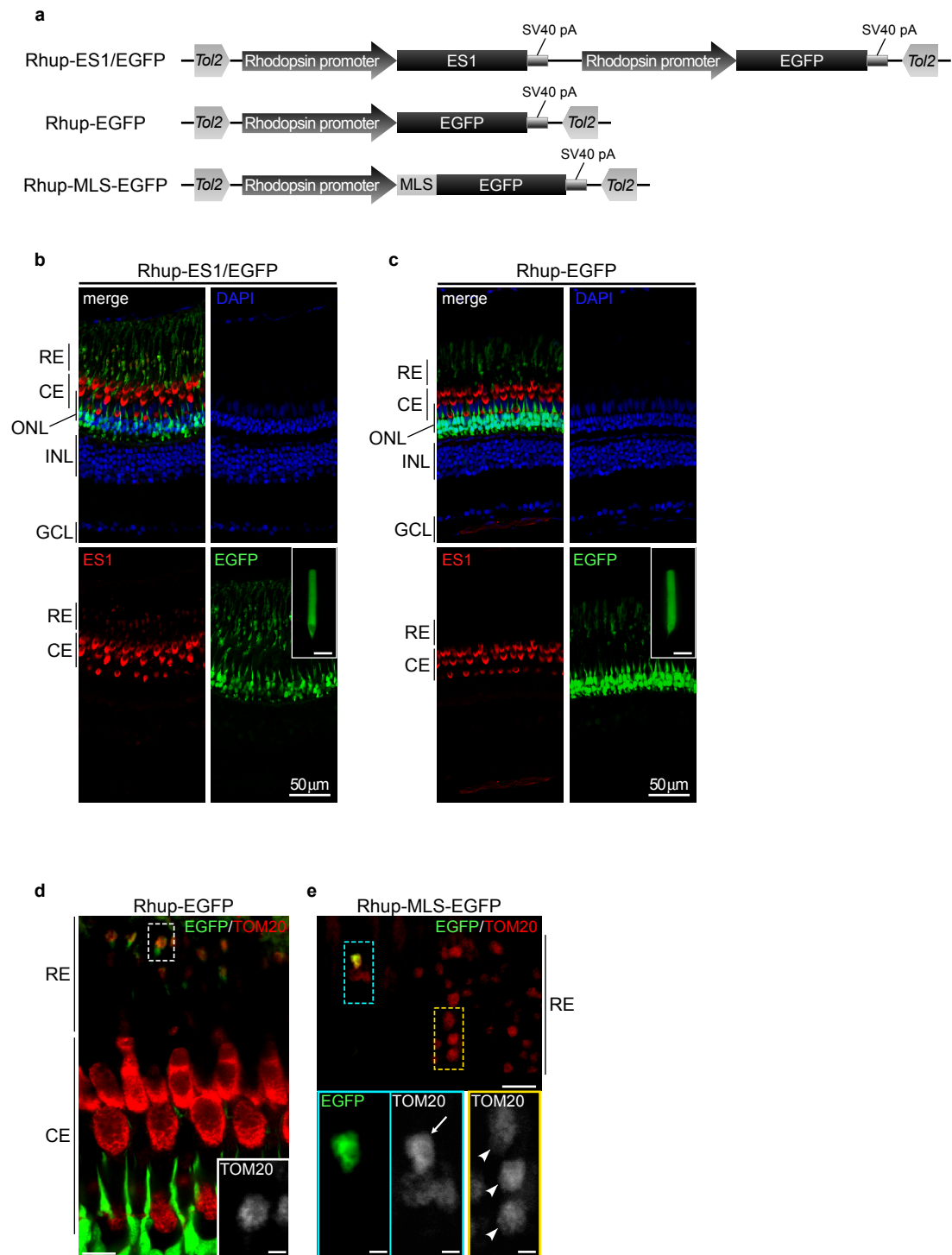
Supplementary Figure S3. Two knock-down strategies for ES1 by using MOs. (a) Schematic drawings of the exon-intron structure of the ES1 gene and expected effects of two MOs. Thick coloured bars and thin lines indicate exons and introns, respectively. ES1-MO1 was designed to hybridize to an intron-exon boundary, leading to an exon-skipping to form a frame-shifted mRNA coding only a short aberrant sequence (24 amino acids) following N-terminal mitochondrial localization signal (23 amino acids). ES1-MO2 was designed to hybridize in close proximity to the translation initiation site of ES1 mRNA and to suppress the translation. (b) Representative results of RT-PCR assay evaluating the effect of ES1-MO1. Template RNAs were extracted from larvae at 4 dpf stage. The upper band was derived from the normally-spliced mRNA and the lower band was derived from aberrantly-spliced mRNA. (c) Splicing inhibition efficiency of ES1 transcript with ES1-MO1. Splicing was completely inhibited (red) in 39%, partially inhibited (pink) in 30% and not inhibited (gray) in 30% of ES1-MO1-injected larvae. n=23. Dead or malformed larvae were eliminated from the calculation.



Supplementary Figure S4. Morphology of MO-injected larvae. (a) Representative whole body images of control MO- and ES1-MO2-injected 4 dpf larvae. (b) Immunohistochemistry of whole eye sections of control MO- and ES1-MO2-injected 4 dpf larvae with mAAT antibody (red). Nuclei were stained with DAPI (blue). Immunopositive signals for mAAT were reduced only in cone ellipsoid layer (see dashed boxes and their magnified views shown in insets) of ES1-MO2-injected larvae compared to the control larvae.

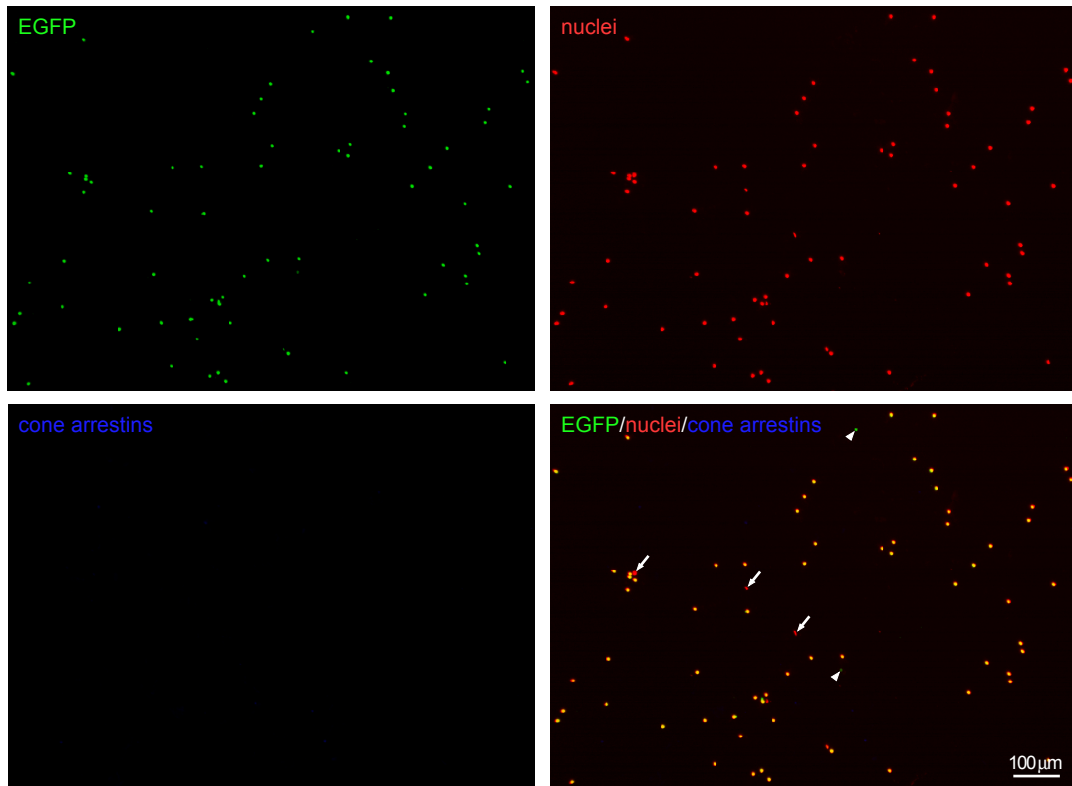


Supplementary Figure S5. Morphology of cone mitochondria of MO-injected larvae. (a) Representative electron microscopic images of cone mitochondria of control MO-injected (left panels), ES1-MO1-injected (center panels) or ES1-MO2-injected (right panels) larvae at 4 dpf. Dashed lines outline each mitochondrion. (b) Images of a without dashed lines. (c) Images of Fig. 2e without dashed lines. Scale bars, 1 μ m.



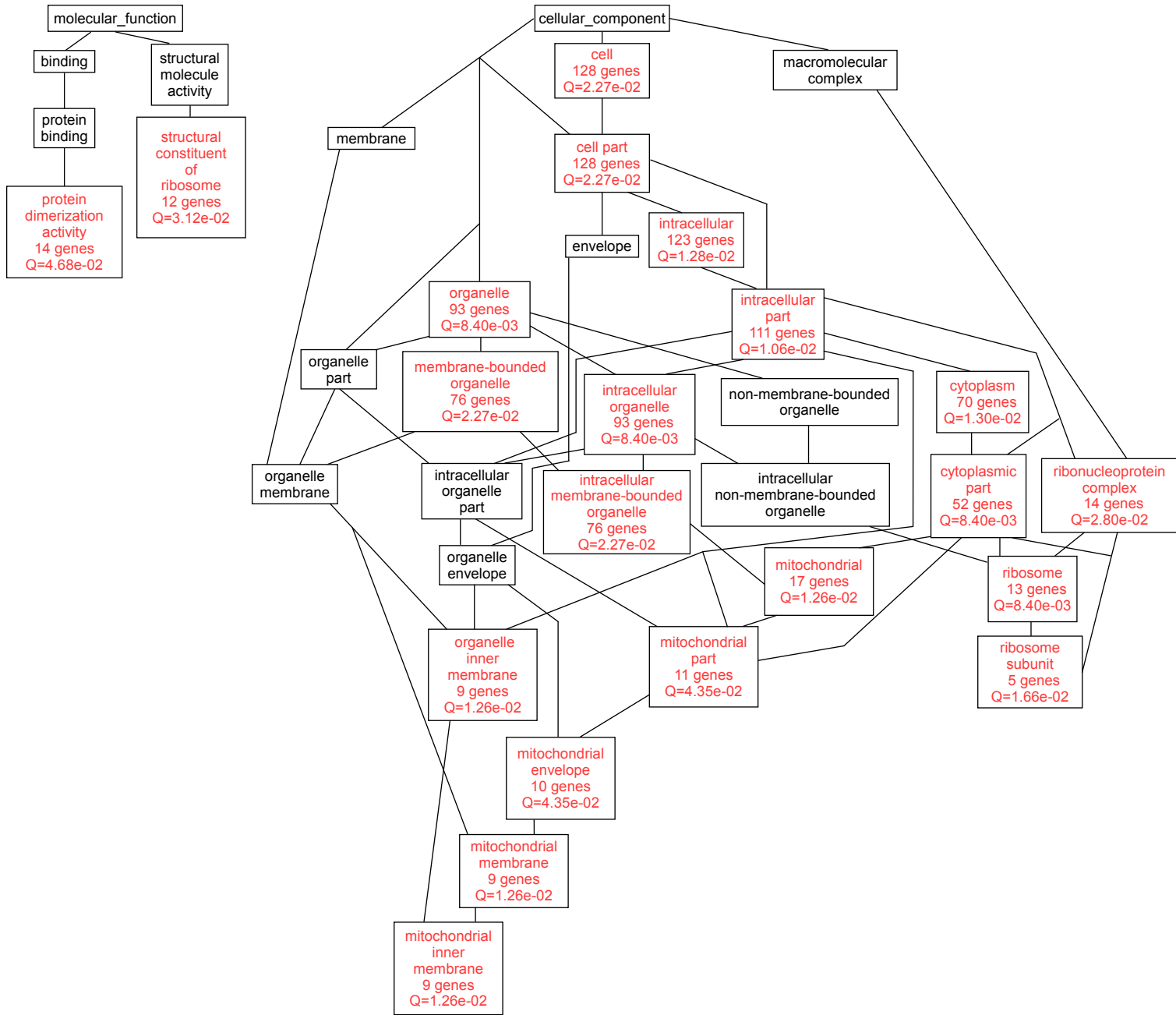
Supplementary Figure S6. Ectopic expression of transgenes in zebrafish rods with the aid of *Tol2* transposon system.

(a) Schematic drawings of *Tol2*-based expression constructs. Coding sequences of ES1, EGFP and MLS-EGFP were driven by a sequence upstream 1,084 bp of rhodopsin gene². Each construct was flanked by inverted repeats of *Tol2* transposon. Rhup-ES1/EGFP construct was used for the expression of ES1 and a reporter, EGFP, in rods. Rhup-EGFP and Rhup-MLS-EGFP constructs were used as controls. MLS-EGFP contains MLS of COX8 at the N-terminus³. (b and c) Immunohistochemistries of retinal sections of TG zebrafish harboring Rhup-ES1/EGFP (b, F₀ mosaic fish of the ES1-TG) or Rhup-EGFP (c, F₁ non-mosaic fish of the EGFP-TG) with ES1 antibody (red). EGFP signals are represented in green. Nuclei were stained with DAPI (blue). An isolated rod outer segment and ellipsoid from each retina is shown in each inset. Scale bars, 10 μm in the insets. (d and e) Immunohistochemistries of retinal sections of EGFP-TG (d) or TG harboring Rhup-MLS-EGFP (MLS-EGFP-TG; F₀ generation; mosaic, e) with TOM20 antibody (red). The inset in d and the lower panels in e are magnified views of areas surrounded by dashed lines in d and in the top panel in e, respectively. In panel e, an ellipsoid of MLS-EGFP-TG rod and ellipsoids of wild-type rods are indicated by an arrow and arrow heads, respectively. Scale bars, 10 μm in the main panel of d and the upper panel of e, and 2 μm in the inset of d and the lower panels of e. RE: rod ellipsoid, CE: cone ellipsoid, ONL: outer nuclear layer, INL: inner nuclear layer, GCL: ganglion cell layer.

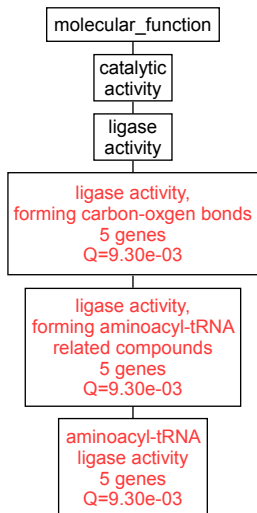


Supplementary Figure S7. Purity of FACS-purified rods. Representative microscopic images of a purified rod fraction from ES1-TG. Rod cell bodies were identified by signals for both EGFP (green) and nuclei (red, stained with DAPI). Approximately 97% of nuclei-containing dots were EGFP-positive. Few dots showed signal only for either EGFP (arrow heads) or nuclei (arrows), which were derived from rod outer segment or other cells, respectively. Possible contamination of cones was also investigated by immunostaining with antibodies against cone arrestins. Population of cone arrestins-immunopositive cells was only 0.28%.

a Up-regulated genes



b Down-regulated genes



Supplementary Figure S8. Directed acyclic graph of the enriched GO categories of differentially expressed genes between the ES1- and EGFP-expressing rods. GO analyses based on the RNA-seq data were performed on more than 1.5 fold up-regulated (a) or down-regulated (b) genes. In multiple test adjustment performed by Benjamini and Hochberg procedure, significantly enriched ($Q > 0.05$) and not enriched GO categories are represented in red and black, respectively. Minimum number of genes for a GO category was five.

Supplementary Table S1

Up-regulated genes in the ES1-expressing rods. Genes were sorted in descending order according to the value of fold change.

Genbank accession number	Gene symbol	Gene description	fold change	P value	Q value
NM_131039	<i>es1</i>	es1 protein	476.41	5.0E-5	0.005
NM_001006039	<i>samsn1b</i>	SAM domain, SH3 domain and nuclear localisation signals, 1b	49.90	5.0E-5	0.005
NM_131256	<i>nr6a1a</i>	nuclear receptor subfamily 6, group A, member 1a	8.60	5.0E-5	0.005
NM_001113659	<i>zgc:198419</i>	<i>zgc:198419</i>	6.80	0.002	0.084
NM_001085494	<i>hoxa13a</i>	homeo box A13a	5.19	5.0E-5	0.005
NM_131249	<i>evx1</i>	even-skipped homeobox 1	4.35	5.0E-5	0.005
NM_001244736	<i>cnbpa</i>	CCHC-type zinc finger, nucleic acid binding protein a	4.05	2.5E-4	0.017
NM_001109854	<i>zgc:173594</i>	<i>zgc:173594</i>	3.94	5.0E-5	0.005
NM_001077315	<i>zgc:153911</i>	<i>zgc:153911</i>	3.80	5.0E-5	0.005
NM_001002190	<i>rgcc</i>	regulator of cell cycle	3.80	5.0E-5	0.005
NR_036645	<i>rpph1</i>	ribonuclease P RNA component H1	3.77	0.010	0.206
NM_001037573	<i>actn2</i>	actinin, alpha 2	3.20	5.0E-5	0.005
NM_200303	<i>zgc:56676</i>	<i>zgc:56676</i>	3.16	0.031	0.332
NM_001020476	<i>dnmt3b</i>	DNA (cytosine-5-)-methyltransferase 3 beta	3.00	5.0E-5	0.005
NM_131295	<i>cpla2</i>	cytosolic phospholipase a2	2.90	5.0E-5	0.005
NM_001162502	<i>ncapd2</i>	non-SMC condensin I complex, subunit D2	2.90	5.0E-4	0.028
NM_001098760	<i>mul1b</i>	mitochondrial E3 ubiquitin ligase 1b	2.88	0.006	0.146
NM_001114720	<i>coq4</i>	coenzyme Q4 homolog (<i>S. cerevisiae</i>)	2.72	0.019	0.271
NM_213180	<i>zgc:77112</i>	<i>zgc:77112</i>	2.69	5.0E-5	0.005
NM_001126421	LOC565395	novel protein similar to DIRAS family, GTP-binding RAS-like 1 (<i>diras1</i>)	2.62	0.008	0.182
NM_131368	<i>smad5</i>	MAD homolog 5 (<i>Drosophila</i>)	2.60	5.0E-5	0.005

Genbank accession number	Gene symbol	Gene description	fold change	P value	Q value
NM_001003533	<i>bag3</i>	BCL2-associated athanogene 3	2.60	5.0E-5	0.005
NM_001076749	<i>zgc:154164</i>	<i>zgc:154164</i>	2.56	2.0E-4	0.015
NM_130936	<i>klfd</i>	Kruppel-like factor d	2.53	5.0E-5	0.005
NM_001128780	<i>zgc:194423</i>	<i>zgc:194423</i>	2.52	0.007	0.171
NM_200172	<i>mycb</i>	myelocytomatosis oncogene b	2.52	0.004	0.125
NM_001039144	<i>slc8a1b</i>	solute carrier family 8 (sodium/calcium exchanger), member 1b	2.42	5.0E-5	0.005
NM_199580	<i>hmmr</i>	hyaluronan mediated motility receptor	2.41	1.5E-4	0.012
NM_001002075	<i>ccne2</i>	cyclin E2	2.40	5.0E-5	0.005
NM_001045854	<i>pex11b</i>	peroxisomal biogenesis factor 11 beta	2.40	0.027	0.312
NM_001122611	<i>magi2</i>	membrane associated guanylate kinase, WW and PDZ domain containing 2	2.36	5.0E-5	0.005
NM_001077545	<i>gfm2</i>	G elongation factor, mitochondrial 2	2.33	7.5E-4	0.039
NM_001008644	<i>bmp2k</i>	BMP2 inducible kinase	2.31	5.0E-5	0.005
NM_199850	<i>c20orf24</i>	c20orf24 homolog (H. sapiens)	2.29	3.0E-4	0.019
NM_200167	<i>cpne3</i>	copine III	2.25	2.0E-4	0.015
NM_001014343	<i>sh2d5</i>	SH2 domain containing 5	2.17	0.002	0.067
NM_131237	<i>mxi1</i>	max interacting protein	2.16	5.0E-5	0.005
NM_200701	<i>wdr21</i>	WD repeat domain 21	2.13	0.032	0.336
NM_001110833	<i>ccdc94</i>	coiled-coil domain containing 94	2.12	0.018	0.267
NM_001201561	LOC555548	cardiac phospholamban-like	2.12	0.007	0.163
NM_199987	<i>jun</i>	jun proto-oncogene	2.10	5.0E-5	0.005
NM_200802	<i>clu</i>	clusterin	2.08	5.0E-5	0.005
NM_001020735	<i>pcyt1ab</i>	phosphate cytidylyltransferase 1, choline, alpha b	2.06	4.5E-4	0.026
NM_001007340	<i>ppm1db</i>	protein phosphatase 1D magnesium-dependent, delta isoform b	2.06	0.009	0.190

Genbank accession number	Gene symbol	Gene description	fold change	P value	Q value
NM_001014330	<i>mtmr1b</i>	myotubularin related protein 1b	2.05	7.5E-4	0.039
NM_001002743	<i>tk2</i>	thymidine kinase 2, mitochondrial	2.01	0.011	0.216
NM_001122705	<i>abl2</i>	v-abl Abelson murine leukemia viral oncogene homolog 2	2.01	0.010	0.208
NM_001001846	<i>pura</i>	purine-rich element binding protein A	2.00	0.003	0.104
NM_001077605	<i>zgc:153381</i>	<i>zgc:153381</i>	1.99	0.013	0.233
NM_001020551	<i>hmg20b</i>	high-mobility group 20B	1.99	0.020	0.278
NM_200117	<i>hist2h2l</i>	histone 2, H2, like	1.98	5.0E-5	0.005
NM_001102675	<i>pard6b</i>	par-6 partitioning defective 6 homolog beta (C. elegans)	1.98	0.048	0.385
NM_001017678	<i>ppie</i>	peptidylprolyl isomerase E (cyclophilin E)	1.97	0.014	0.244
NM_001113610	<i>si:ch211-163121.7</i>	<i>si:ch211-163121.7</i>	1.95	4.0E-4	0.024
NM_001005587	<i>crata</i>	carnitine O-acetyltransferase a	1.95	0.008	0.187
NM_001017840	<i>rasl11a</i>	RAS-like, family 11, member A	1.95	0.003	0.107
NM_001130625	<i>zgc:194737</i>	<i>zgc:194737</i>	1.95	0.005	0.131
NM_001077389	<i>tfam</i>	transcription factor A, mitochondrial	1.93	0.029	0.320
NM_212612	<i>ddx5</i>	DEAD (Asp-Glu-Ala-Asp) box polypeptide 5	1.91	5.0E-5	0.005
NM_200102	<i>pcd8</i>	programmed cell death 8 (apoptosis-inducing factor)	1.91	1.5E-4	0.012
NM_212750	<i>junbb</i>	jun B proto-oncogene b	1.90	1.0E-4	0.008
NM_001109835	<i>zgc:171937</i>	<i>zgc:171937</i>	1.88	0.034	0.344
NM_199636	<i>rpl10a</i>	ribosomal protein L10a	1.87	2.5E-4	0.017
NM_200233	<i>hif1ab</i>	hypoxia-inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor) b	1.86	5.0E-5	0.005
NM_001007765	<i>ndufs1</i>	NADH dehydrogenase (ubiquinone) Fe-S protein 1	1.86	2.0E-4	0.015
NM_199629	<i>nfkbiab</i>	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha b	1.84	1.0E-4	0.008
NM_001044985	<i>si:dkey-72114.3</i>	<i>si:dkey-72114.3</i>	1.84	0.002	0.065

Genbank accession number	Gene symbol	Gene description	fold change	P value	Q value
NM_213256	<i>ttyh2l</i>	tweety homolog 2, like	1.83	0.020	0.273
NM_200789	<i>zgc:73324</i>	<i>zgc:73324</i>	1.83	0.017	0.262
NM_001110286	<i>prkaa1</i>	protein kinase, AMP-activated, alpha 1 catalytic subunit	1.83	0.004	0.118
NM_001004542	<i>zgc:91887</i>	<i>zgc:91887</i>	1.82	0.008	0.187
NM_001098389	<i>hexim1</i>	hexamethylene bis-acetamide inducible 1	1.82	0.022	0.283
NM_200407	<i>ergic2</i>	ERGIC and golgi 2	1.82	0.044	0.379
NM_131073	<i>jak1</i>	Janus kinase 1	1.80	0.005	0.134
NM_199776	<i>ppp2r2d</i>	protein phosphatase 2, regulatory subunit B, delta isoform	1.80	0.005	0.137
NM_001002304	<i>twf1b</i>	twinfilin, actin-binding protein, homolog 1b	1.79	0.018	0.267
NM_001020543	<i>zgc:109982</i>	<i>zgc:109982</i>	1.79	0.011	0.210
NM_001083123	<i>ar</i>	androgen receptor	1.79	0.026	0.309
NM_200845	<i>rps17</i>	ribosomal protein S17	1.78	0.041	0.370
NM_213021	<i>glrx5</i>	glutaredoxin 5 homolog (<i>S. cerevisiae</i>)	1.78	0.035	0.349
NM_153662	<i>st8sia2</i>	ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 2	1.78	7.5E-4	0.039
NM_213408	<i>slc25a22</i>	solute carrier family 25 (mitochondrial carrier: glutamate), member 22	1.78	0.023	0.293
NM_205553	<i>zgc:55936</i>	<i>zgc:55936</i>	1.78	0.023	0.292
NM_001017755	<i>ndufs3</i>	NADH dehydrogenase (ubiquinone) Fe-S protein 3, (NADH-coenzyme Q reductase)	1.76	0.009	0.191
NM_001045015	<i>ap3d1</i>	adaptor-related protein complex 3, delta 1 subunit	1.76	0.001	0.055
NM_201511	<i>cyp2p10</i>	cytochrome P450, family 2, subfamily P, polypeptide 10	1.75	0.047	0.384
NM_001045109	<i>furinb</i>	furin (paired basic amino acid cleaving enzyme) b	1.75	0.032	0.336
NM_199272	<i>esrp2</i>	epithelial splicing regulatory protein 2	1.75	2.0E-4	0.015
NM_001029956	<i>u2af2a</i>	U2 small nuclear RNA auxiliary factor 2a	1.74	0.005	0.145
NM_001089480	<i>zgc:162310</i>	<i>zgc:162310</i>	1.73	0.026	0.308

Genbank accession number	Gene symbol	Gene description	fold change	P value	Q value
NM_001077257	<i>foxo1a</i>	forkhead box O1 a	1.73	0.020	0.276
NM_001130603	<i>tmtc2</i>	transmembrane and tetratricopeptide repeat containing 2	1.73	0.002	0.070
NM_001082839	<i>wdr85</i>	WD repeat domain 85	1.72	0.002	0.067
NM_199992	<i>sema6a</i>	sema domain, transmembrane domain (TM), and cytoplasmic domain, (semaphorin) 6A	1.72	0.019	0.271
NM_001177744	<i>aqp9b</i>	aquaporin 9b	1.70	0.005	0.131
NM_001002512	<i>zgc:92818</i>	<i>zgc:92818</i>	1.70	0.008	0.187
NM_199674	<i>stub1</i>	STIP1 homology and U-Box containing protein 1	1.69	0.031	0.332
NM_001103125	<i>fam46a</i>	family with sequence similarity 46, member A	1.69	0.011	0.211
NM_001033091	<i>si:ch211-23714.6</i>	<i>si:ch211-23714.6</i>	1.69	0.003	0.109
NM_001020663	<i>bach1</i>	BTB and CNC homology 1, basic leucine zipper transcription factor 1	1.68	0.019	0.269
NM_200179	<i>cdc14b</i>	CDC14 cell division cycle 14 homolog B	1.68	0.003	0.096
NM_001167826	<i>zgc:73329</i>	<i>zgc:73329</i>	1.68	0.028	0.314
NM_200989	<i>pacsin3</i>	protein kinase C and casein kinase substrate in neurons 3	1.68	0.008	0.181
NM_001002722	<i>ttc33</i>	tetratricopeptide repeat domain 33	1.67	0.018	0.267
NM_001003493	<i>suds3</i>	suppressor of defective silencing 3 homolog (SDS3, <i>S. cerevisiae</i>)	1.67	0.035	0.349
NM_001007778	<i>gpsm1b</i>	G-protein signaling modulator 1b	1.66	0.037	0.353
NM_001003479	<i>rasgef1bb</i>	RasGEF domain family, member 1Bb	1.66	0.020	0.278
NM_201153	<i>rps3</i>	ribosomal protein S3	1.66	0.004	0.128
NM_199777	<i>sec23b</i>	Sec23 homolog B (<i>S. cerevisiae</i>)	1.66	0.017	0.265
NM_130968	<i>odz3</i>	odd Oz/ten-m homolog 3	1.66	0.007	0.165
NM_213415	<i>zbtb2b</i>	zinc finger and BTB domain containing 2b	1.66	0.021	0.281
NM_201315	<i>abcf2a</i>	ATP-binding cassette, sub-family F (GCN20), member 2a	1.65	0.005	0.137
NM_200724	<i>guk1b</i>	guanylate kinase 1b	1.65	0.002	0.075

Genbank accession number	Gene symbol	Gene description	fold change	P value	Q value
NM_198363	<i>tob1a</i>	transducer of ERBB2, 1a	1.64	0.003	0.101
NM_001080036	<i>tp53inp1</i>	tumor protein p53 inducible nuclear protein 1	1.64	0.014	0.240
NM_001256204	<i>elac2</i>	elaC homolog 2 (E. coli)	1.64	0.032	0.334
NM_001030234	<i>zgc:113372</i>	<i>zgc:113372</i>	1.64	0.045	0.381
NM_001045485	<i>pi4k2b</i>	phosphatidylinositol 4-kinase type 2 beta	1.64	0.005	0.131
NM_212645	<i>hnmpa0a</i>	heterogeneous nuclear ribonucleoprotein A0a	1.63	0.003	0.101
NM_200949	<i>zfxand5b</i>	zinc finger, AN1-type domain 5b	1.63	0.010	0.199
NM_199215	<i>ca2</i>	carbonic anhydrase II	1.63	0.002	0.066
NM_001002355	<i>zgc:92139</i>	<i>zgc:92139</i>	1.63	0.008	0.182
NM_001077379	<i>sec23ip</i>	SEC23 interacting protein	1.63	0.021	0.280
NM_001126454	<i>fam222a</i>	family with sequence similarity 222, member A	1.62	0.041	0.370
NM_001002708	<i>pde6d</i>	phosphodiesterase 6D, cGMP-specific, rod, delta	1.62	0.002	0.072
NM_200276	<i>ncor1</i>	nuclear receptor co-repressor 1	1.62	0.003	0.088
NM_001006016	<i>atl3</i>	atlastin 3	1.62	0.046	0.382
NM_001110123	<i>oscp1a</i>	organic solute carrier partner 1a	1.62	0.002	0.066
NM_001082998	<i>fosl2</i>	fos-like antigen 2	1.62	0.012	0.222
NM_001044859	<i>si:ch211-15d5.5</i>	<i>si:ch211-15d5.5</i>	1.62	0.029	0.318
NM_001044848	<i>arhgef1b</i>	Rho guanine nucleotide exchange factor (GEF) 1b	1.61	0.020	0.273
NM_212963	<i>dip2ba</i>	DIP2 disco-interacting protein 2 homolog Ba (Drosophila)	1.61	0.007	0.163
NM_001080651	<i>creb3l2</i>	cAMP responsive element binding protein 3-like 2	1.61	0.007	0.163
NM_131081	<i>cdh2</i>	cadherin 2, neuronal	1.61	0.013	0.232
NM_213198	<i>orc5</i>	origin recognition complex, subunit 5	1.61	0.002	0.079
NM_001077768	<i>zranb1b</i>	zinc finger, RAN-binding domain containing 1b	1.61	0.012	0.228

Genbank accession number	Gene symbol	Gene description	fold change	P value	Q value
NM_001128534	<i>arvcfb</i>	armadillo repeat gene deleted in velocardiofacial syndrome b	1.60	0.004	0.116
NM_213497	<i>crip2</i>	cysteine-rich protein 2	1.60	0.011	0.216
NM_212684	<i>atxn2l</i>	ataxin 2-like	1.60	0.020	0.276
NM_001130663	si:ch211-160i2.3	si:ch211-160i2.3	1.59	0.048	0.385
NM_199830	<i>klhl18</i>	kelch-like 18 (Drosophila)	1.58	0.015	0.248
NM_001002495	<i>uqcrcq</i>	ubiquinol-cytochrome c reductase, complex III subunit VII	1.58	0.043	0.375
NM_001017800	zgc:110331	zgc:110331	1.58	0.003	0.102
NM_200813	<i>ca7</i>	carbonic anhydrase VII	1.58	0.028	0.314
NM_001128259	<i>uck11a</i>	uridine-cytidine kinase 1-like 1a	1.58	0.004	0.124
NM_199480	<i>lnpa</i>	limb and neural patterns a	1.58	0.019	0.269
NM_001165918	wu:fb15g10	wu:fb15g10	1.58	0.023	0.293
NM_001083075	<i>znf865</i>	zinc finger protein 865	1.57	0.012	0.219
NM_199541	<i>id2b</i>	inhibitor of DNA binding 2, dominant negative helix-loop-helix protein, b	1.57	0.019	0.269
NM_001044786	<i>shoc2</i>	soc-2 suppressor of clear homolog (C. elegans)	1.57	0.018	0.267
NM_001170739	<i>mga</i>	MAX gene associated	1.57	0.005	0.139
NM_212974	<i>tob1b</i>	transducer of ERBB2, 1b	1.56	0.003	0.091
NM_212955	<i>esrra</i>	estrogen-related receptor alpha	1.56	0.041	0.370
NM_001034976	<i>prkacbb</i>	protein kinase, cAMP-dependent, catalytic, beta b	1.56	0.032	0.336
NM_001044918	<i>znf704</i>	zinc finger protein 704	1.56	0.026	0.306
NM_001144784	<i>inaa</i>	internexin neuronal intermediate filament protein, alpha a	1.55	0.003	0.109
NM_201470	<i>ip6k2</i>	inositol hexaphosphate kinase 2	1.55	0.015	0.251
NM_194380	<i>dup6</i>	dual specificity phosphatase 6	1.55	0.004	0.112
NM_001127515	si:dkey-110k5.6	si:dkey-110k5.6	1.55	0.018	0.267

Genbank accession number	Gene symbol	Gene description	fold change	P value	Q value
NM_131900	<i>cacna1c</i>	calcium channel, voltage-dependent, L type, alpha 1C subunit	1.55	0.009	0.198
NM_201149	<i>cdc14aa</i>	CDC14 cell division cycle 14 homolog A, a	1.54	0.010	0.206
NM_200064	<i>bcat1</i>	branched chain aminotransferase 1, cytosolic	1.54	0.012	0.222
NM_001012378	<i>dlg2</i>	discs, large (Drosophila) homolog 2	1.54	0.027	0.312
NM_213149	<i>fkbp5</i>	FK506 binding protein 5	1.54	0.037	0.353
NM_212759	<i>cyfip1</i>	cytoplasmic FMR1 interacting protein 1	1.53	0.009	0.198
NM_001159971	<i>spty2d1</i>	SPT2, Suppressor of Ty, domain containing 1 (S. cerevisiae)	1.53	0.019	0.269
NM_001098177	<i>myh9a</i>	myosin, heavy polypeptide 9a, non-muscle	1.53	0.005	0.144
NM_001110279	<i>mll</i>	myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila)	1.53	0.011	0.210
NM_001007330	<i>cbl</i>	Cas-Br-M (murine) ecotropic retroviral transforming sequence	1.53	0.042	0.374
NM_194399	<i>cflar</i>	CASP8 and FADD-like apoptosis regulator	1.52	0.028	0.318
NM_001145089	<i>camk2n1a</i>	calcium/calmodulin-dependent protein kinase II inhibitor 1a	1.52	0.016	0.256
NM_001030136	<i>daam1b</i>	dishevelled associated activator of morphogenesis 1b	1.52	0.016	0.261
NM_001001940	<i>copb2</i>	coatamer protein complex, subunit beta 2	1.52	0.020	0.276
NM_212732	<i>mdm4</i>	transformed 3T3 cell double minute 4 homolog (mouse)	1.51	0.048	0.385
NM_001012304	<i>rbm39a</i>	RNA binding motif protein 39a	1.51	0.007	0.168
NM_001044911	<i>nrg1</i>	neuregulin 1	1.51	0.012	0.222
NM_001007032	<i>ncor2</i>	nuclear receptor co-repressor 2	1.51	0.042	0.371
NM_213442	<i>srrm1</i>	serine/arginine repetitive matrix 1	1.50	0.044	0.380
NM_001089366	<i>morc3a</i>	MORC family CW-type zinc finger 3a	1.50	0.048	0.385
NM_200595	<i>fbxo45</i>	F-box protein 45	1.50	0.046	0.382
NM_001114926	<i>c3orf58b</i>	C3orf58 chromosome 3 open reading frame 58, b	1.49	0.019	0.269
NM_199672	<i>stk3</i>	serine/threonine kinase 3 (STE20 homolog, yeast)	1.49	0.033	0.341

Genbank accession number	Gene symbol	Gene description	fold change	P value	Q value
NM_001020660	<i>cyb5r4</i>	cytochrome b5 reductase 4	1.49	0.023	0.293
NM_001004553	<i>ppp3r1b</i>	protein phosphatase 3 (formerly 2B), regulatory s1ubunit B, alpha isoform, b	1.49	0.010	0.201
NM_131183	<i>nr2f2</i>	nuclear receptor subfamily 2, group F, member 2	1.48	0.026	0.308
NM_213489	<i>prpf38b</i>	PRP38 pre-mRNA processing factor 38 (yeast) domain containing B	1.48	0.043	0.374
NM_131411	<i>aanat2</i>	arylalkylamine N-acetyltransferase	1.48	0.012	0.219
NM_194362	<i>ube4b</i>	ubiquitination factor E4B, UFD2 homolog (<i>S. cerevisiae</i>)	1.48	0.018	0.267
NM_194427	<i>slc7a4</i>	solute carrier family 7 (cationic amino acid transporter, y+ system), member 4	1.48	0.046	0.382
NM_001002681	<i>zgc:86841</i>	<i>zgc:86841</i>	1.47	0.045	0.382
NM_001024440	<i>spc24</i>	SPC24, NDC80 kinetochore complex component, homolog (<i>S. cerevisiae</i>)	1.47	0.044	0.379
NM_001246660	<i>rpgr11</i>	RPGRIP1-like	1.47	0.048	0.385
NM_001197074	<i>u2surp</i>	U2 snRNP-associated SURP domain containing	1.47	0.022	0.284
NM_001110371	<i>ppp4r2b</i>	protein phosphatase 4, regulatory subunit 2b	1.47	0.042	0.372
NM_001044914	<i>trim8</i>	tripartite motif-containing 8	1.47	0.031	0.328
NM_213379	<i>got2a</i>	glutamic-oxaloacetic transaminase 2a, mitochondrial (aspartate aminotransferase 2)	1.46	0.014	0.245
NM_001082840	<i>prkaca</i>	protein kinase, cAMP-dependent, catalytic, alpha, genome duplicate a	1.46	0.037	0.353
NM_001089401	<i>ubtd1a</i>	ubiquitin domain containing 1a	1.46	0.030	0.325
NM_131406	<i>raraa</i>	retinoic acid receptor, alpha a	1.46	0.047	0.382
NM_199273	<i>zgc:66433</i>	<i>zgc:66433</i>	1.45	0.012	0.228
NM_001045073	<i>hsp90aa1.2</i>	heat shock protein 90, alpha (cytosolic), class A member 1, tandem duplicate 2	1.45	0.020	0.278
NM_200697	<i>ckmt2</i>	creatine kinase, mitochondrial 2 (sarcomeric)	1.45	0.032	0.336
NM_131031	<i>actb1</i>	actin, beta 1	1.45	0.027	0.313
NM_001029966	<i>trna1apl</i>	tRNA selenocysteine 1 associated protein 1, like	1.44	0.041	0.370
NM_173274	<i>dag1</i>	dystroglycan 1	1.44	0.035	0.349

Genbank accession number	Gene symbol	Gene description	fold change	P value	Q value
NM_001007152	<i>dpf2</i>	D4, zinc and double PHD fingers family 2	1.44	0.046	0.382
NM_001030116	<i>scm2</i>	secernin 2	1.44	0.036	0.351
NM_200472	<i>pygmb</i>	phosphorylase, glycogen (muscle) b	1.44	0.020	0.275
NM_001044982	<i>cited4a</i>	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 4a	1.44	0.029	0.322
NM_200867	<i>srsf5a</i>	serine/arginine-rich splicing factor 5a	1.44	0.044	0.377
NM_212644	<i>dennd5b</i>	DENN/MADD domain containing 5B	1.44	0.026	0.308
NM_001045108	<i>fam65a</i>	family with sequence similarity 65, member A	1.43	0.034	0.345
NM_001128539	<i>zbtb10</i>	zinc finger and BTB domain containing 10	1.43	0.025	0.304
NM_199983	<i>mylipa</i>	myosin regulatory light chain interacting protein a	1.43	0.028	0.314
NM_131015	<i>mafba</i>	glutamate receptor, ionotropic, N-methyl D-aspartate 1b	1.43	0.026	0.309
NM_001017618	<i>icmt</i>	isoprenylcysteine carboxyl methyltransferase	1.42	0.031	0.332
NM_001045049	si:ch211-69g19.2	si:ch211-69g19.2	1.42	0.050	0.388
NM_212749	<i>rhoab</i>	ras homolog gene family, member Ab	1.42	0.038	0.356
NM_199333	<i>pkma</i>	pyruvate kinase, muscle, a	1.42	0.028	0.318
NM_001007287	<i>fhla</i>	four and a half LIM domains a	1.42	0.027	0.314
NM_001089363	<i>ccn1a</i>	cyclin L1a	1.41	0.040	0.365
NM_213397	<i>pfkfb3</i>	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3	1.41	0.046	0.382
NM_001003612	<i>boka</i>	BCL2-related ovarian killer a	1.40	0.026	0.308
NM_001004552	<i>pdxdc1</i>	pyridoxal-dependent decarboxylase domain containing 1	1.40	0.027	0.313
NM_213333	<i>sltm</i>	SAFB-like, transcription modulator	1.39	0.034	0.344
NM_200704	<i>aanat1</i>	arylalkylamine N-acetyltransferase 1	1.39	0.042	0.373
NM_001044993	<i>nfs1</i>	NFS1 nitrogen fixation 1 (S. cerevisiae)	1.38	0.041	0.370
NM_207072	<i>clasp2</i>	cytoplasmic linker associated protein 2	1.38	0.041	0.369

Genbank accession number	Gene symbol	Gene description	fold change	P value	Q value
NM_001007295	<i>zgc:92107</i>	<i>zgc:92107</i>	1.36	0.039	0.361
NM_001005402	<i>cdhr1a</i>	cadherin-related family member 1a	1.36	0.041	0.370
NM_205762	<i>traf4a</i>	tnf receptor-associated factor 4a	1.36	0.049	0.386
NM_200150	<i>ankrd12</i>	ankyrin repeat domain 12	1.36	0.045	0.382
NM_001123253	LOC560226	novel protein similar to vertebrate breakpoint cluster region (BCR)	1.35	0.044	0.377

Values were calculated with Cuffdiff, n=3.

Q-values represent adjusted P values with Benjamini and Hochberg procedure for multiple testing.

Supplementary Table S2

Down-regulated genes in the ES1-expressing rods. Genes were sorted in ascending order according to the value of fold change.

Genbank accession number	Gene symbol	Gene description	fold change	P value	Q value
NM_001256251	<i>msnb</i>	moesin b	0.08	5.0E-5	0.005
NM_001003987	<i>epb413a</i>	erythrocyte membrane protein band 4.1-like 3a	0.16	2.5E-4	0.017
NM_001168350	<i>adcy1b</i>	adenylate cyclase 1b	0.17	0.015	0.249
NM_001128759	<i>zgc:194989</i>	<i>zgc:194989</i>	0.20	0.002	0.066
NM_001076593	<i>zgc:153394</i>	<i>zgc:153394</i>	0.20	0.003	0.097
NM_001017846	<i>rgs16</i>	regulator of G-protein signaling 16	0.21	0.006	0.151
NM_001003629	<i>rgs3</i>	regulator of G-protein signaling 3	0.21	4.5E-4	0.026
NM_001004671	<i>uxt</i>	ubiquitously-expressed transcript	0.26	0.012	0.226
NM_212680	<i>hmga2</i>	high mobility group AT-hook 2	0.29	5.0E-5	0.005
NM_213398	<i>vamp1</i>	vesicle-associated membrane protein 1	0.30	0.042	0.372
NM_131098	<i>apoeb</i>	apolipoprotein Eb	0.30	5.0E-5	0.005
NM_001025464	<i>pdcb</i>	phosducin b	0.31	5.0E-5	0.005
NM_001113589	<i>hsp70l</i>	heat shock cognate 70-kd protein, like	0.32	5.0E-5	0.005
NM_001045173	<i>ndrg4</i>	N-myc downstream regulated gene 4	0.33	5.0E-5	0.005
NM_001005924	<i>tmem56b</i>	transmembrane protein 56b	0.34	5.0E-5	0.005
NM_200199	<i>smu1</i>	smu-1 suppressor of mec-8 and unc-52 homolog (C. elegans)	0.35	2.5E-4	0.017
NR_029934	<i>mir204-1</i>	microRNA 204-1	0.35	3.0E-4	0.019
NM_131397	<i>hsp70</i>	heat shock cognate 70-kd protein	0.35	5.0E-5	0.005
NM_001007351	<i>btg3</i>	B-cell translocation gene 3	0.35	0.007	0.170
NM_001020550	<i>tp53rk</i>	TP53 regulating kinase	0.36	0.003	0.108
NM_200455	<i>zgc:63568</i>	<i>zgc:63568</i>	0.37	0.013	0.236

Genbank accession number	Gene symbol	Gene description	fold change	P value	Q value
NM_001083843	<i>comtb</i>	catechol-O-methyltransferase b	0.37	5.0E-5	0.005
NM_001045138	<i>pdcd2</i>	programmed cell death 2	0.38	0.012	0.223
NM_001034968	<i>slit1a</i>	slit homolog 1a (Drosophila)	0.38	5.0E-5	0.005
NM_001007282	<i>gpx4a</i>	glutathione peroxidase 4a	0.38	1.0E-4	0.008
NM_201093	<i>ttni2a.2</i>	troponin I, skeletal, fast 2a.2	0.38	3.0E-4	0.019
NM_199569	<i>eif2s1l</i>	eukaryotic translation initiation factor 2, subunit 1 alpha, like	0.38	0.007	0.163
NM_001080020	<i>grma</i>	glutamate receptor, metabotropic a	0.38	5.0E-5	0.005
NM_001089391	<i>slc5a5</i>	solute carrier family 5 (sodium iodide symporter), member 5	0.39	2.5E-4	0.017
NM_001076667	<i>dync1i2a</i>	dynein, cytoplasmic 1, intermediate chain 2a	0.40	0.048	0.385
NM_152961	<i>fabp3</i>	fatty acid binding protein 3, muscle and heart	0.40	5.5E-4	0.030
NM_001144131	<i>grin1b</i>	glutamate receptor, ionotropic, N-methyl D-aspartate 1b	0.40	1.0E-4	0.008
NM_131869	<i>gnat2</i>	guanine nucleotide binding protein (G protein), alpha transducing activity polypeptide 2	0.41	5.0E-5	0.005
NM_001006093	<i>scp2b</i>	sterol carrier protein 2b	0.41	9.0E-4	0.044
NM_001002875	<i>gnl3l</i>	guanine nucleotide binding protein-like 3 (nucleolar)-like	0.41	0.010	0.200
NM_131055	<i>flh</i>	floating head	0.41	0.001	0.051
NM_213373	<i>znf330</i>	zinc finger protein 330	0.43	0.018	0.267
NM_001003516	<i>zgc:100908</i>	<i>zgc:100908</i>	0.43	0.041	0.371
NM_200871	<i>pde6c</i>	phosphodiesterase 6C, cGMP-specific, cone, alpha prime	0.43	2.0E-4	0.015
NM_200698	<i>cabp5a</i>	calcium binding protein 5a	0.43	0.003	0.108
NM_173284	<i>tsr2</i>	TSR2, 20S rRNA accumulation, homolog (S. cerevisiae)	0.44	0.026	0.306
NM_131070	<i>mdka</i>	midkine-related growth factor	0.44	0.005	0.138
NM_201072	<i>rtm3</i>	reticulon 3	0.44	5.5E-4	0.030
NM_205645	<i>pigf</i>	phosphatidylinositol glycan, class F	0.45	0.022	0.287

Genbank accession number	Gene symbol	Gene description	fold change	P value	Q value
NM_001089466	<i>sreb2</i>	sterol regulatory element binding transcription factor 2	0.45	5.0E-5	0.005
NM_001004529	<i>echs1</i>	enoyl Coenzyme A hydratase, short chain, 1, mitochondrial	0.45	0.001	0.062
NM_001007329	<i>slc7a3a</i>	solute carrier family 7 (cationic amino acid transporter, y+ system), member 3a	0.45	5.0E-5	0.005
NM_201059	<i>waslb</i>	Wiskott-Aldrich syndrome-like b	0.46	3.5E-4	0.021
NM_001045078	zk13a21.14	coiled-coil domain containing 120-like	0.46	0.003	0.098
NM_001130612	<i>atxn10</i>	ataxin 10	0.46	0.019	0.272
NM_001037112	zgc:123238	zgc:123238	0.46	0.007	0.163
NM_198374	<i>pik3ip1</i>	phosphoinositide-3-kinase interacting protein 1	0.46	0.003	0.098
NM_205653	<i>znf410</i>	zinc finger protein 410	0.46	0.009	0.189
NM_200837	zgc:73359	zgc:73359	0.46	0.033	0.340
NM_001115051	si:dkey-5i22.6	si:dkey-5i22.6	0.47	0.006	0.150
NM_001020696	<i>alg3</i>	asparagine-linked glycosylation 3 homolog (S. cerevisiae, alpha-1,3-mannosyltransferase)	0.47	0.018	0.267
NM_001033749	<i>sagb</i>	S-antigen; retina and pineal gland (arrestin) b	0.47	0.008	0.176
NM_198357	<i>tcerg1a</i>	transcription elongation regulator 1a (CA150)	0.48	5.0E-5	0.005
NM_001144792	<i>cept1a</i>	choline/ethanolamine phosphotransferase 1a	0.48	0.003	0.109
NM_200692	<i>ndrg1b</i>	N-myc downstream regulated gene 1b	0.48	0.006	0.153
NM_001077611	<i>dars</i>	aspartyl-tRNA synthetase	0.48	0.005	0.137
NM_001002094	<i>msrb3</i>	methionine sulfoxide reductase B3	0.48	0.006	0.154
NM_001025502	<i>h2afy2</i>	H2A histone family, member Y2	0.48	0.008	0.182
NM_001044856	<i>ociad2</i>	OCIA domain containing 2	0.48	2.5E-4	0.017
NM_001037239	zgc:123177	zgc:123177	0.48	0.017	0.265
NM_153666	<i>tbx24</i>	T-box 24	0.48	0.006	0.146
NM_001030251	zgc:114103	zgc:114103	0.49	3.5E-4	0.021

Genbank accession number	Gene symbol	Gene description	fold change	P value	Q value
NM_200095	<i>anssd1</i>	asparagine synthetase domain containing 1	0.49	0.025	0.303
NM_198070	<i>tkt</i>	transketolase	0.49	3.0E-4	0.019
NM_001105129	<i>srebfl</i>	sterol regulatory element binding transcription factor 1	0.49	5.0E-5	0.005
NM_200783	<i>efna1b</i>	ephrin A1b	0.49	0.018	0.267
NM_001005601	<i>tmem18</i>	transmembrane protein 18	0.49	0.008	0.176
NM_001039808	<i>slc2a1a</i>	solute carrier family 2 (facilitated glucose transporter), member 1a	0.50	9.0E-4	0.044
NM_001032366	<i>dcun1d4</i>	DCN1, defective in cullin neddylation 1, domain containing 4 (<i>S. cerevisiae</i>)	0.50	5.0E-5	0.005
NM_213126	<i>b2ml</i>	beta-2-microglobulin, like	0.50	5.0E-5	0.005
NM_199529	<i>mthfd1b</i>	methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 1b	0.50	0.002	0.066
NM_131397	<i>hsp70</i>	heat shock cognate 70-kd protein	0.51	5.0E-5	0.005
NM_213205	<i>m6pr</i>	mannose-6-phosphate receptor (cation dependent)	0.51	0.018	0.267
NM_001145564	<i>cyp2u1</i>	cytochrome P450, family 2, subfamily U, polypeptide 1	0.52	0.015	0.251
NM_199727	<i>serinc1</i>	serine incorporator 1	0.52	5.0E-5	0.005
NM_205700	<i>cfl2</i>	cofilin 2 (muscle)	0.52	0.009	0.198
NM_001003566	<i>phpt1</i>	phosphohistidine phosphatase 1	0.52	0.009	0.198
NM_001045293	<i>flj11011</i>	hypothetical protein FLJ11011-like (<i>H. sapiens</i>)	0.52	0.006	0.163
NM_001004530	<i>fuom</i>	fucose mutarotase	0.52	0.039	0.364
NM_001126485	LOC100006564	novel NACHT domain containing protein	0.53	0.020	0.274
NM_199745	<i>esf1</i>	ESF1, nucleolar pre-rRNA processing protein, homolog (<i>S. cerevisiae</i>)	0.53	0.022	0.283
NM_213202	<i>gnb3b</i>	guanine nucleotide binding protein (G protein), beta polypeptide 3b	0.53	0.001	0.062
NM_001076569	zgc:153980	zgc:153980	0.53	0.002	0.076
NM_213527	<i>hiat1b</i>	hippocampus abundant transcript 1b	0.53	0.003	0.087
NM_213104	<i>pgrmc2</i>	progesterone receptor membrane component 2	0.53	0.006	0.156

Genbank accession number	Gene symbol	Gene description	fold change	P value	Q value
NM_201464	<i>impdh2</i>	IMP (inosine monophosphate) dehydrogenase 2	0.54	0.025	0.303
NM_131205	<i>etv5b</i>	ets variant 5b	0.54	0.006	0.155
NM_198368	<i>taf12</i>	TAF12 RNA polymerase II, TATA box binding protein (TBP)-associated factor	0.54	0.018	0.267
NM_001020733	<i>lin7b</i>	lin-7 homolog B (<i>C. elegans</i>)	0.54	0.025	0.301
NM_178297	<i>sepp1a</i>	selenoprotein P, plasma, 1a	0.54	0.002	0.064
NM_001171587	<i>prox1b</i>	prospero-related homeobox gene 1b	0.54	0.008	0.176
NM_001115122	<i>dpp6b</i>	dipeptidyl-peptidase 6b	0.55	0.002	0.082
NM_200745	<i>edf1</i>	endothelial differentiation-related factor 1	0.55	0.026	0.308
NM_205570	<i>zgc:73226</i>	<i>zgc:73226</i>	0.55	2.5E-4	0.017
NM_205759	<i>eepl1</i>	endonuclease/exonuclease/phosphatase family domain containing 1	0.55	0.032	0.336
NM_001034988	<i>cx41.8</i>	connexin 41.8	0.55	0.018	0.267
NM_001089338	<i>zgc:162958</i>	<i>zgc:162958</i>	0.55	0.010	0.208
NM_001007422	<i>zgc:100824</i>	<i>zgc:100824</i>	0.56	0.038	0.356
NM_001023581	<i>tmem167a</i>	transmembrane protein 167A	0.56	0.021	0.281
NM_001014313	<i>tmem35</i>	transmembrane protein 35	0.56	0.005	0.131
NM_001114702	<i>zgc:174710</i>	<i>zgc:174710</i>	0.56	1.1E-3	0.050
NM_001083544	<i>rbx1</i>	ring-box 1	0.56	0.010	0.207
NM_001190382	<i>sell</i>	selenoprotein L	0.57	0.040	0.365
NM_131400	<i>tefa</i>	thyrotroph embryonic factor a	0.57	0.002	0.066
NM_212629	<i>ubr7</i>	ubiquitin protein ligase E3 component n-recognin 7	0.57	0.007	0.163
NM_199582	<i>tpd52l2b</i>	tumor protein D52-like 2b	0.57	0.046	0.382
NM_199869	<i>insig1</i>	insulin induced gene 1	0.57	0.041	0.370
NM_001005990	<i>kansl2</i>	KAT8 regulatory NSL complex subunit 2	0.57	0.043	0.375

Genbank accession number	Gene symbol	Gene description	fold change	P value	Q value
NM_152886	<i>appb</i>	amyloid beta (A4) precursor protein b	0.58	4.0E-4	0.024
NM_212990	<i>ef3i</i>	eukaryotic translation initiation factor 3, subunit I	0.58	0.036	0.352
NM_001039981	<i>zgc:136866</i>	<i>zgc:136866</i>	0.58	0.047	0.382
NM_001007291	<i>oxct1a</i>	3-oxoacid CoA transferase 1a	0.58	0.030	0.325
NM_001082997	<i>si:dkey-23c22.6</i>	<i>si:dkey-23c22.6</i>	0.58	0.003	0.097
NM_001004648	<i>nudt4b</i>	nudix (nucleoside diphosphate linked moiety X)-type motif 4b	0.58	0.018	0.267
NM_200766	<i>paip2b</i>	poly(A) binding protein interacting protein 2B	0.58	0.011	0.218
NM_001020775	<i>zgc:113201</i>	<i>zgc:113201</i>	0.59	0.050	0.389
NM_001077391	<i>zgc:153997</i>	<i>zgc:153997</i>	0.59	0.002	0.084
NM_001089335	<i>bri3bp</i>	bri3 binding protein	0.59	0.023	0.290
NM_001002634	<i>nipal3</i>	NIPA-like domain containing 3	0.59	0.028	0.314
NM_199793	<i>dazap2</i>	DAZ associated protein 2	0.59	0.003	0.095
NM_001006087	<i>ormd13</i>	ORM1-like 3 (<i>S. cerevisiae</i>)	0.59	0.007	0.163
NM_199481	<i>ccng1</i>	cyclin G1	0.59	0.009	0.196
NM_212575	<i>mtx2</i>	metaxin 2	0.59	0.035	0.346
NM_001079969	<i>spoplb</i>	speckle-type POZ protein-like b	0.60	0.040	0.365
NM_201036	<i>vti1b</i>	vesicle transport through interaction with t-SNAREs homolog 1B (yeast)	0.60	0.027	0.312
NM_001002707	<i>zgc:92606</i>	<i>zgc:92606</i>	0.60	0.005	0.139
NM_001004605	<i>arrdc3b</i>	arrestin domain containing 3b	0.60	0.006	0.156
NM_201067	<i>rorab</i>	RAR-related orphan receptor A, paralog b	0.60	0.010	0.203
NM_214819	<i>rcan2</i>	regulator of calcineurin 2	0.60	0.017	0.265
NM_001020714	<i>zgc:113162</i>	<i>zgc:113162</i>	0.60	0.048	0.385
NM_198978	<i>pdc4b</i>	programmed cell death 4b	0.60	0.004	0.120

Genbank accession number	Gene symbol	Gene description	fold change	P value	Q value
NM_214770	<i>rab11ba</i>	RAB11B, member RAS oncogene family, a	0.60	0.038	0.356
NM_001005932	<i>tars</i>	threonyl-tRNA synthetase	0.60	0.030	0.325
NM_001080079	<i>mapkapk3</i>	mitogen-activated protein kinase-activated protein kinase 3	0.60	0.015	0.249
NM_001024221	<i>wdr33</i>	WD repeat domain 33	0.60	0.045	0.381
NM_001045564	<i>psmb7</i>	proteasome (prosome, macropain) subunit, beta type, 7	0.60	0.039	0.364
NM_213407	<i>zranb2</i>	zinc finger, RAN-binding domain containing 2	0.61	0.006	0.146
NM_001014306	<i>lifra</i>	leukemia inhibitory factor receptor alpha a	0.61	0.009	0.196
NM_199681	<i>phb2</i>	prohibitin 2	0.61	0.015	0.251
NM_212708	<i>slc16a3</i>	solute carrier family 16 (monocarboxylic acid transporters), member 3	0.61	0.006	0.156
NM_001017732	<i>prkar1ab</i>	protein kinase, cAMP-dependent, regulatory, type I, alpha (tissue specific extinguisher 1) b	0.61	0.007	0.163
NM_212850	<i>glcea</i>	glucuronyl C5-epimerase a	0.61	0.022	0.283
NM_001003875	<i>snmp70</i>	small nuclear ribonucleoprotein 70 (U1)	0.61	0.002	0.066
NM_205745	<i>wdfy2</i>	WD repeat and FYVE domain containing 2	0.61	0.046	0.382
NM_201171	<i>dusp4</i>	dual specificity phosphatase 4	0.62	0.008	0.176
NM_131065	<i>nr1d2b</i>	nuclear receptor subfamily 1, group D, member 2b	0.62	0.025	0.304
NM_201111	<i>hook2</i>	hook homolog 2 (Drosophila)	0.62	0.009	0.196
NM_001044840	<i>tmem181</i>	transmembrane protein 181	0.62	0.024	0.297
NM_001029968	<i>nif3l1</i>	NIF3 NGG1 interacting factor 3-like 1 (S. cerevisiae)	0.62	0.037	0.353
NM_001025538	<i>tspan3a</i>	tetraspanin 3a	0.62	0.002	0.069
NM_001256646	<i>flrt3</i>	fibronectin leucine rich transmembrane 3	0.62	0.010	0.209
NM_001080074	<i>zgc:158316</i>	<i>zgc:158316</i>	0.62	0.027	0.312
NM_213435	<i>zgc:55683</i>	<i>zgc:55683</i>	0.63	0.033	0.337
NM_131758	<i>stom</i>	stomatin	0.63	0.005	0.137

Genbank accession number	Gene symbol	Gene description	fold change	P value	Q value
NM_001013312	<i>zgc:113324</i>	<i>zgc:113324</i>	0.63	0.042	0.374
NM_001128811	<i>zgc:194176</i>	<i>zgc:194176</i>	0.63	0.024	0.300
NM_001201344	<i>slc43a3a</i>	solute carrier family 43, member 3a	0.63	0.007	0.163
NM_212904	<i>chmp2bb</i>	chromatin modifying protein 2Bb	0.63	0.046	0.382
NM_001110479	<i>trpm1b</i>	transient receptor potential cation channel, subfamily M, member 1b	0.63	0.021	0.281
NM_001089562	<i>rab8a</i>	RAB8A, member RAS oncogene family	0.63	0.020	0.278
NM_001089384	<i>zgc:162698</i>	<i>zgc:162698</i>	0.63	0.009	0.191
NM_001003731	<i>zgc:91910</i>	<i>zgc:91910</i>	0.63	0.048	0.385
NM_001045295	<i>farsa</i>	phenylalanyl-tRNA synthetase, alpha subunit	0.64	0.047	0.382
NM_213308	<i>stk25b</i>	serine/threonine kinase 25b	0.64	0.019	0.269
NM_001007424	<i>dhrs13a.3</i>	dehydrogenase/reductase (SDR family) member 13a.3	0.64	0.032	0.336
NM_001003623	<i>naa50</i>	N(alpha)-acetyltransferase 50, NatE catalytic subunit	0.64	0.039	0.361
NM_001044942	<i>parp1</i>	poly (ADP-ribose) polymerase family, member 1	0.64	0.027	0.312
NM_199919	<i>ap2b1</i>	adaptor-related protein complex 2, beta 1 subunit	0.65	0.013	0.234
NM_213262	<i>EIF5A2</i>	eukaryotic translation initiation factor 5A2	0.65	0.008	0.174
NM_001079977	<i>hmgcra</i>	3-hydroxy-3-methylglutaryl-Coenzyme A reductase a	0.65	0.009	0.191
NM_182967	<i>calm3a</i>	calmodulin 3a (phosphorylase kinase, delta)	0.65	0.014	0.244
NM_001008617	<i>ttc26</i>	tetratricopeptide repeat domain 26	0.65	0.040	0.369
NM_213087	<i>sdf4</i>	stromal cell derived factor 4	0.65	0.013	0.228
NM_201327	<i>ssr1</i>	signal sequence receptor, alpha	0.65	0.030	0.328
NM_001170599	<i>tbx21</i>	T-box 21	0.65	0.030	0.328
NM_001037699	<i>golp3l</i>	golgi phosphoprotein 3-like	0.65	0.048	0.385
NM_199555	<i>hmgbl1a</i>	high-mobility group box 1a	0.65	0.011	0.214

Genbank accession number	Gene symbol	Gene description	fold change	P value	Q value
NM_001003861	<i>rpl9</i>	ribosomal protein L9	0.66	0.017	0.264
NM_131451	<i>irbp</i>	interphotoreceptor retinoid-binding protein	0.66	0.030	0.325
NM_199965	<i>rbp4l</i>	retinol binding protein 4, like	0.66	0.019	0.271
NM_001040313	<i>zgc:136739</i>	<i>zgc:136739</i>	0.66	0.017	0.266
NM_001082872	<i>nat15</i>	N-acetyltransferase 15 (GCN5-related, putative)	0.66	0.048	0.385
NM_001076663	<i>rabggt</i>	Rab geranylgeranyltransferase, alpha subunit	0.66	0.045	0.381
NM_001128358	LOC797250	novel protein similar to H.sapiens solute carrier family 7 (cationic amino acid transporter, y+ system), member 5	0.66	0.011	0.216
NM_001083002	<i>arid3b</i>	AT rich interactive domain 3B (Bright like)	0.66	0.012	0.219
NM_001030102	<i>brms1la</i>	breast cancer metastasis-suppressor 1-like a	0.67	0.040	0.365
NM_001103111	<i>mntb</i>	MAX binding protein b	0.67	0.027	0.312
NM_194417	<i>sepw2a</i>	selenoprotein W, 2a	0.67	0.011	0.215
NM_213529	<i>tmem50a</i>	transmembrane protein 50A	0.67	0.040	0.367
NM_001045402	<i>cbx3a</i>	chromobox homolog 3a (HP1 gamma homolog, Drosophila)	0.67	0.048	0.385
NM_001114585	<i>zgc:174944</i>	<i>zgc:174944</i>	0.67	0.020	0.274
NM_173254	<i>atp6v1e1b</i>	ATPase, H+ transporting, lysosomal, V1 subunit E isoform 1b	0.67	0.015	0.251
NM_001013337	<i>zgc:113200</i>	<i>zgc:113200</i>	0.67	0.029	0.319
NM_001045052	<i>tmem106bb</i>	transmembrane protein 106Bb	0.67	0.013	0.231
NM_131710	<i>ctsd</i>	cathepsin D	0.68	0.024	0.296
NM_001130192	<i>pnrc2</i>	proline-rich nuclear receptor coactivator 2	0.68	0.020	0.273
NM_212912	<i>tmed7</i>	transmembrane emp24 protein transport domain containing 7	0.68	0.029	0.319
NM_001077376	si:ch211-133n4.4	si:ch211-133n4.4	0.68	0.023	0.290
NM_131465	<i>pcmt</i>	l-isoaspartyl protein carboxyl methyltransferase	0.69	0.023	0.291
NM_001002353	<i>dnajb6a</i>	DnaJ (Hsp40) homolog, subfamily B, member 6a	0.69	0.045	0.381

Genbank accession number	Gene symbol	Gene description	fold change	P value	Q value
NM_001077568	<i>ctsf</i>	cathepsin F	0.69	0.016	0.256
NM_201166	<i>dctn2</i>	dynactin 2 (p50)	0.69	0.037	0.356
NM_178306	<i>tph1a</i>	tryptophan hydroxylase 1 (tryptophan 5-monoxygenase) a	0.69	0.028	0.314
NM_200009	<i>eef1a1a</i>	eukaryotic translation elongation factor 1 alpha 1a	0.69	0.015	0.248
NM_199666	<i>ndfip1</i>	Nedd4 family interacting protein 1	0.70	0.019	0.271
NM_200362	<i>tmem106ba</i>	transmembrane protein 106Ba	0.70	0.029	0.320
NM_001025475	<i>idi1</i>	isopentenyl-diphosphate delta isomerase 1	0.70	0.041	0.371
NM_001099448	<i>wwp2</i>	WW domain containing E3 ubiquitin protein ligase 2	0.70	0.048	0.385
NM_001144040	<i>cog3</i>	component of oligomeric golgi complex 3	0.70	0.038	0.356
NM_199957	<i>ppiab</i>	peptidylprolyl isomerase Ab (cyclophilin A)	0.70	0.050	0.389
NM_001167570	<i>pvr11a</i>	poliovirus receptor-related 1a	0.70	0.050	0.389
NM_200925	<i>stau2</i>	staufen, RNA binding protein, homolog 2 (Drosophila)	0.71	0.035	0.350
NM_201112	<i>arl6ip1</i>	ADP-ribosylation factor-like 6 interacting protein 1	0.71	0.031	0.332
NM_178290	<i>selt1a</i>	selenoprotein T, 1a	0.71	0.034	0.346
NM_001164027	<i>npepps</i>	aminopeptidase puromycin sensitive	0.71	0.037	0.356
NM_001252649	si:ch211-117m20.5	si:ch211-117m20.5	0.72	0.037	0.353
NM_213250	<i>csnk1db</i>	casein kinase 1, delta b	0.73	0.048	0.385
NM_199587	<i>lztfl1</i>	leucine zipper transcription factor-like 1	0.73	0.049	0.387
NM_001002298	<i>asna1</i>	arsA arsenite transporter, ATP-binding, homolog 1 (bacterial)	0.73	0.046	0.382

Values were calculated with Cuffdiff, n=3.

Q-values represent adjusted P values with Benjamini and Hochberg procedure for multiple testing.

Genes coding cone opsins were excluded because they can be derived from contamination of cones or misalignment of rhodopsin transcripts which are expressed in rods the most abundantly.

Supplementary Methods

Antibodies.

Antigens used for production of each antibody prepared in our laboratory or raised commercially were purified carp ES1 (AB794660)⁴, a peptide fragment of carp mAAT (Ser28-Lys428; AB793727), purified carp rhodopsin, a partial peptide of the sequence conserved between carp red and green opsins (STSKTEVSSVAPA; AB055656.1 and AB110602.2, respectively)⁵, and peptide fragments of carp cone-type arrestin-1 and -2 (Arg66-Lys102; AB794065 and Arg68-Ala110; AB794066, respectively)⁵. Each antibody was used at the following dilutions for immunoblotting; anti-ES1 (1:1,000), anti-alpha-tubulin (1:1,000), anti-mAAT (1:1,000), anti-TOM20 (1:1,000), anti-red/green opsins (1:3,000), anti-rhodopsin (1:3,000), anti-AMPK α (1:300) and anti-phospho-AMPK α (1:300), and for immunohistochemistry; anti-ES1 (1:500), anti-mAAT (1:300), anti-TOM20 (1:300) and anti-red/green opsins (1:500) and for immunocytochemistry; anti-cone-type arrestin-1 (1:300) and -2 (1:300).

Subcellular fractionation

Cells dissociated from retinas were suspended in a potassium gluconate buffer (115 mM potassium gluconate, 2.5 mM KCl, 2 mM MgCl₂, 10 mM HEPES, 0.2 mM EGTA, 0.1 mM CaCl₂, pH 7.5). After freeze-thawed three times, the cells were centrifuged at 100,000 x g for 10 min three times and fractionated into supernatant as a soluble fraction and precipitation as a membrane fraction.

In situ hybridization.

In situ hybridization was performed using 10- μ m frozen ocular sections prepared from light-adapted zebrafish as previously described⁶. Complementary RNA probes against ES1 mRNA at 372-755 bases of open reading frame and rhodopsin mRNA at 718-936 bases were synthesized by using DIG RNA Labeling Kit (Roche Diagnostics K.K.).

Immunohistochemical studies.

Eyecups of adult zebrafish were isolated and fixed at a time point of 4 h after light onset (i.e. 4 h light adaptation). And whole embryos were fixed at various time points within 3-10 h after light onset. Immunohistochemistry was performed using 10- μ m frozen sections as previously described⁶. Secondary antibodies used are anti-mouse IgG and anti-rabbit IgG which are conjugated with either Alexa Fluor 488

(1:300 dilution, Molecular probes), Alexa Fluor 568 (1:300 dilution, Molecular probes) or ATTO 655 (1:200 dilution, Sigma-Aldrich). Immunostained sections were observed by using a BX-51 fluorescence microscope (Olympus) and a confocal fluorescence microscope (FluoView FV1000, Olympus). Signal intensities in the confocal images were determined by the aid of software imageJ.

Electron microscopic studies.

Light conditions before tissue fixations were described above (see **Immunohistochemical studies**). Eyecups from adult zebrafish or whole embryos were fixed with Ringer's solution containing 1% (w/v) paraformaldehyde and 2.5% (w/v) glutaraldehyde for 2 h at 4°C, followed by treatment with 0.5% osmium tetroxide in Ringer's solution for 30 min at 4°C. After dehydration through a graded ethanol series, the tissue fragments were embedded either in epoxy resin Quetol 812 (Nissin EM) or LR-White (for immunoelectron microscopy, London Resin Company), and were cut into ultrathin sections (80-100 nm) by using an ultramicrotome (EM-Ultracut UCT; Leica). After immunostaining process described in Methods or without the immunostaining, the sections were post-stained with a 4% uranyl acetate solution and a lead staining solution (Sigma-Aldrich), and observed by using a transmission electron microscope (JEM-1011; JEOL).

Read mapping and estimation of gene expression on RNA-seq data.

Short reads with less than 40 bases and low-quality reads, more than 30% of which bases showed a Phred score less than 17, were excluded by FastX-Tool kit (http://hannonlab.cshl.edu/fastx_toolkit/), and the remaining reads were mapped to the zebrafish reference genome (Zv9) from Ensembl database (http://www.ensembl.org/Danio_reio/Info/Index) using TopHat2 software (v2.0.11)⁷. We subsequently quantified gene expression levels by Cufflinks software (v2.2.1) and determined differentially expressed genes by Cuffdiff software⁸. Three replicates for each transgenic line were subjected to these analyses. Expression levels of some important genes were validated by quantitative real-time PCR assays as described in Methods.

GO and pathway enrichment analyses.

Based on the Cuffdiff result, more than 1.5 fold up- or down-regulated genes were subjected to GO analysis using WEB-based Gene Set Analysis Toolkit (WebGestalt) database⁹. Pathway enrichment analysis for these genes was performed by

Wikipathways included in WebGestalt. All genes (10,633 genes) whose expressions were detected in ES1-TG and/or EGFP-TG were used as reference for these statistical analyses.

Quantification of mtDNA content.

Retinas were isolated from the ES1-TG and EGFP-TG at a time point of 8 h after light onset (i.e. 8 h light adaptation). Rods composed of the outer segment and the ellipsoid were purified from the retinas as described in “Estimation of cellular energy states of rods” in Methods section. The purified rods were treated with lysis buffer (50 mM Tris-HCl pH 8.5, 1 mM EDTA, 0.5% Tween-20, 200 µg/ml proteinase K) at 55°C for 2 h, and then proteinase K was denatured at 95°C for 10 min. Then DNAs were purified with Fast Gene Gel/PCR Extraction Kit (NIPPON Genetics Co. Ltd., Tokyo, Japan). Mitochondrial DNA contents were quantified by real-time PCR using a primer set for mtDNA encoded NADH dehydrogenase 1 as described in ref. 10 in two technical replicates. The content of mtDNA was normalized by the number of cells.

References for Supplementary Information

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