Supporting Information

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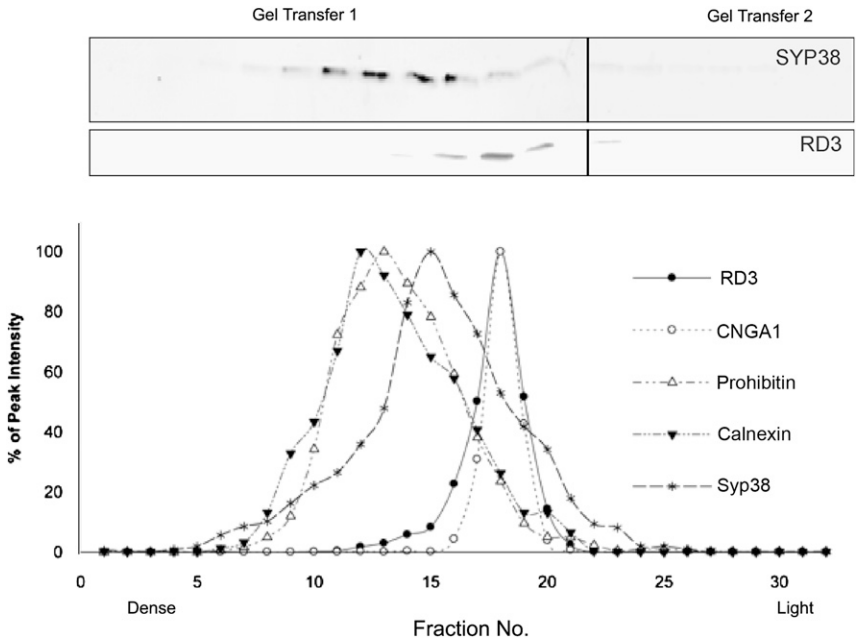

Fig. S1. Localization of RD3 to photoreceptor outer segments by subfractionation. A mouse retinal homogenate was subfractionated by equilibrium density centrifugation on a 20–60% sucrose gradient. The amount of protein in the fractions was quantified on western blots labeled with antibodies to various subcellular markers. Due to the large number of fractions, two gel transfers were used as indicated. Subcellular markers (antibodies): RD3 (Rd3 9D12 antibody); rod outer segment CNGA1 (PMc 2G11); mitochondria (prohibitin), endoplasmic reticulum (calnexin), synaptic vesicles (SYP 38). RD3 cosedimented with the rod outer segment marker CNGA1. Western blots labeled for synaptophysin (SYP38) and RD3 are shown on top.

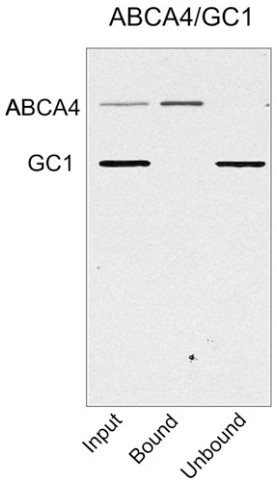

Fig. S2. GC1 does not bind nonspecifically to the immunoaffinity matrix. A detergent-solubilized mouse retinal extract (input lane) was incubated with a Rim3F4-Sepharose matrix specific for ABCA4. The unbound protein (unbound lane) and bound protein (bound fraction) were eluted with SDS for analysis on Western blots labeled with an antibody to ABCA4 (Rim3F4) and a polyclonal antibody to GC1. ABCA4 was present in the input and bound fractions but absent in the unbound fraction, whereas GC1 was present in the input and unbound fractions but absent in the bound fraction, indicating that GC1 does not nonspecifically bind to the immunoaffinity matrix used in coimmunoprecipitation studies.

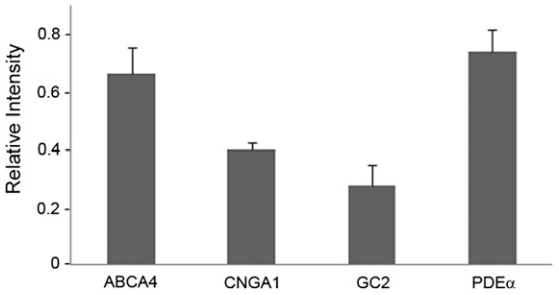

Fig. S3. Amounts of various outer segment proteins in 21-d-old *rd3* mouse retinal extracts relative to 21-d-old WT retinal extracts. Western blots of *rd3* and WT retinal extracts were labeled with antibodies to GC1, GC2, CNGA1, ABCA4, and PDE-α (Fig. 3*B*), and the band intensity was quantified on a LI-COR image analyzer. The relative intensity of the protein bands in the *rd3* and WT mouse retinal extracts are the average value of three different retinal preparations ± SD. GC1 produced no signal in the *rd3* retinal extract and thus is not shown in the bar graph.

Table S1. Mass spectrometry of bound proteins from the anti-Rd3 immunoprecipitation experiment

Accession no./gene	Protein	Mass	Mascot score	No. of peptides
P52785/GUC2E_MOUSE	Retinal guanylyl cyclase 1 (GC-E)	121,675	2,397	89
Q62261/SPTB2_MOUSE	Spectrin beta chain, brain 1	274,908	1,080	32
P99024/TBB5_MOUSE	Tubulin beta-5 chain	50,095	989	31
P16546/SPTA2_MOUSE	Spectrin alpha chain, brain	285,221	964	34
P05213/TBA1B_MOUSE	Tubulin alpha-1B chain	50,804	798	19
P56480/ATPB_MOUSE	ATP synthase subunit beta	56,265	594	12
P63017/HSP7C_MOUSE	Heat-shock cognate 71 kDa protein	56,265	528	19
Q68FD5/CLH_MOUSE	Clathrin heavy chain 1	193,202	504	22
Q5SDA5/GUC2F_MOUSE	Retinal guanylyl cyclase 2 (GC-F)	125,315	458	22
P48962/ADT1_MOUSE	ADP/ATP translocase 1	33,111	442	20
Q6PIC6/AT1A3_MOUSE	Na/K ATPase subunit alpha-3	113,045	427	18
P46096/SYT1_MOUSE	Synaptotagmin-1	47,730	421	17
Q03265/ATPA_MOUSE	ATP synthase subunit alpha	59,830	398	14
P60710/ACTB_MOUSE	Actin, cytoplasmic 1	42,052	391	16
P17182/ENOA_MOUSE	Alpha-enolase	47,453	379	13
P20443/ARRS_MOUSE	S-arrestin	45130	376	15
P51881/ADT2_MOUSE	ADP/ATP translocase 2	33,138	365	17
Q8VDN2/AT1A1_MOUSE	Na/K ATPase subunit alpha-1	114,221	363	10
P15409/OPSD_MOUSE	Rhodopsin	39,572	328	8
P20612/GNAT1_MOUSE	Transducin G(t) subunit alpha-1	40,397	317	7
Q8BH59/CMC1_MOUSE	Ca-binding mitochondrial protein	74,922	297	10
P20029/GRP78_MOUSE	78 kDa glucose-regulated protein	72,492	279	11
P15105/GLNA_MOUSE	Glutamine synthetase	42,834	270	11
O70318/E41L2_MOUSE	Band 4.1-like protein	110,280	265	14
Q7TPR4/ACTN1_MOUSE	Alpha-actinin-1	103,631	259	11
P46460/NSF_MOUSE	Vesicle-fusing ATPase	83,131	248	9
Q8CJ40/CROCC_MOUSE	Rootletin	227,349	234	11
Q8BMK4/CKAP4_MOUSE	Cytoskeleton-associated protein 4	63,711	222	8
P16858/G3P_MOUSE	G-3-P-dehydrogenase	36,072	190	8
P27664/PDE6A_MOUSE	Rod phosphodiesterase subunit alpha	100,437	190	7
Q61595/KTN1_MOUSE	Kinectin	153,068	186	6
Q91VR2/ATPG_MOUSE	ATP synthase subunit gamma	32,979	170	8
Q01853/TERA_MOUSE	Transitional ER ATPase	89,950	157	7
Q9D6R2/IDH3A_MOUSE	Isocitrate dehydrogenase [NAD]	40,069	152	6
P27546/MAP4_MOUSE	Microtubule-associated protein 4	117,927	142	5
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Q07797/LG3BP_MOUSE	Galectin-3–binding protein	65,362	125	7
P15499/PRPH2_MOUSE	Peripherin-2	39,975	123	5
P29974/CNGA1_MOUSE	cGMP-gated cation channel alpha-1	79,809	116	6
P62874/GBB1_MOUSE	Transducin subunit beta-1	38,151	105	6
P20152/VIME_MOUSE	Vimentin	53,712	101	5
O35129/PHB2_MOUSE	Prohibitin-2	33,276	100	4
P10126/EF1A1_MOUSE	Elongation factor 1-alpha 1	50,424	94	5
O08553/DPYL2_MOUSE	Dihydropyrimidinase-related protein 2	62,638	94	4
Q8CAQ8/IMMT_MOUSE	Mitochondrial inner membrane protein	84,247	92	6
Q8BRE0/RD3_MOUSE	Protein RD3	22,826	91	4
Q925I1/ATAD3_MOUSE	ATPase family AAA	66,872	90	4
P38647/GRP75_MOUSE	Stress-70 protein	73,768	87	5

A detergent-solubilized mouse retinal membrane extract was applied to a Rd3-9D12 immunoaffinity matrix. After unbound protein was removed, the bound protein was eluted with SDS and digested with trypsin for analysis of peptides by LC/MS-MS. Data were searched using a Mascot server. Keratin, a common contaminant, and mouse Ig also were present as contaminating proteins, but are not included here. The table includes proteins identified from four or more peptides.