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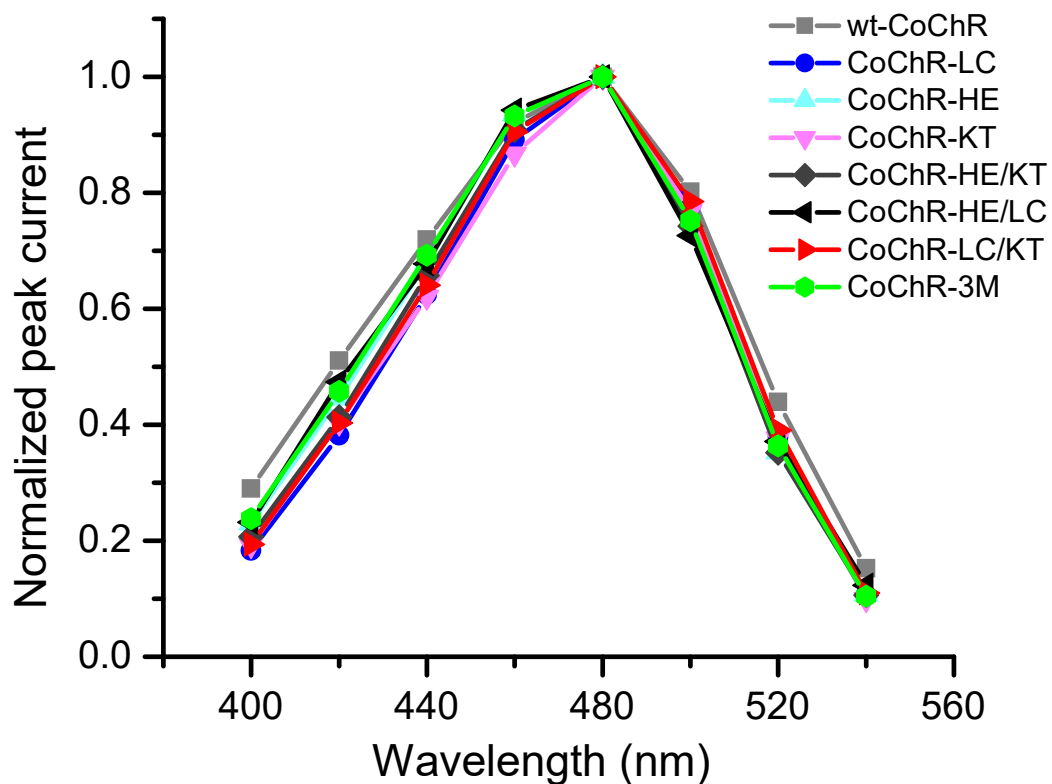
## **Supplemental Information**

### **Improved CoChR Variants Restore Visual Acuity and Contrast Sensitivity in a Mouse Model of Blindness under Ambient Light Conditions**

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			1	10	20	30
CoChR	(1)	-----	MLGNGSAIVPIDQCFCLAWTDSLGS	DT	EQ	L
Chr2	(1)		MDYGGALSAVGRELLFVTNPVVVNGSVLVPEDQCYCAGWIESRGTNGAQT			
			31	40	50	60
CoChR	(31)		VANILQWF	AFGFSILILMFYAYQTWRATCGWEEVYVCCVELTKVII	IEFFH	
Chr2	(51)		ASNVLQWLAAGFSILLLMFYAYQTKSTCGWEEIYVCAIEMVKVILEFFF			
			81	90	100	110
CoChR	(81)		EFDDPSMLYLANGHRVQWLRYAEWLLT	CPVIL	LIHLSNLTGLKDDYSKRTM	
Chr2	(101)		EFKNPSMLYLATGHRVQWLRYAEWLLT	CPVIL	LIHLSNLTGLSNDYSRRTM	
			131	140	150	160
CoChR	(131)		RLLVSDVGTIVWGATSAMSTGYVKVIFVVLGCIYGANTFFHAAKVYIESY			
Chr2	(151)		GLLVSDIGTIVWGATSAMATGYVKVIFVFLGLCYGANTFFHAAKAYIEGY			
			181	190	200	210
CoChR	(181)		HVVPKGRPRTVVRIMAWLFFLSWGMFPVLFVVGPEGFDAISVYGSTIGHT			
Chr2	(201)		HTVPKGRCRQVVTGMAWLFFVSWGMFPILFILGPEGFGVLSVYGSTVGH			
			231	240	250	260
CoChR	(231)		IIDLMSKNCWGLLGHYLRVLIHQHIIIIYGD	IRK	TKINVAGE	EMEVETMV
Chr2	(251)		IIDLMSKNCWGLLGHYLRVLIHEHILIHGDIRKTTKLNIGGTEIEVETLV			
			281			
CoChR	(281)		DQ	EE	ETV	-----
Chr2	(301)		EE	EA	EAGAVNKGTGK	

**Figure S1. Alignment of the amino acid sequences of Chr2 and CoChR**  
The labeled amino acids are for CoChR. The conserved amino acids are highlighted in yellow. The mutated amino acids in CoChR reported in this study are highlighted in red.



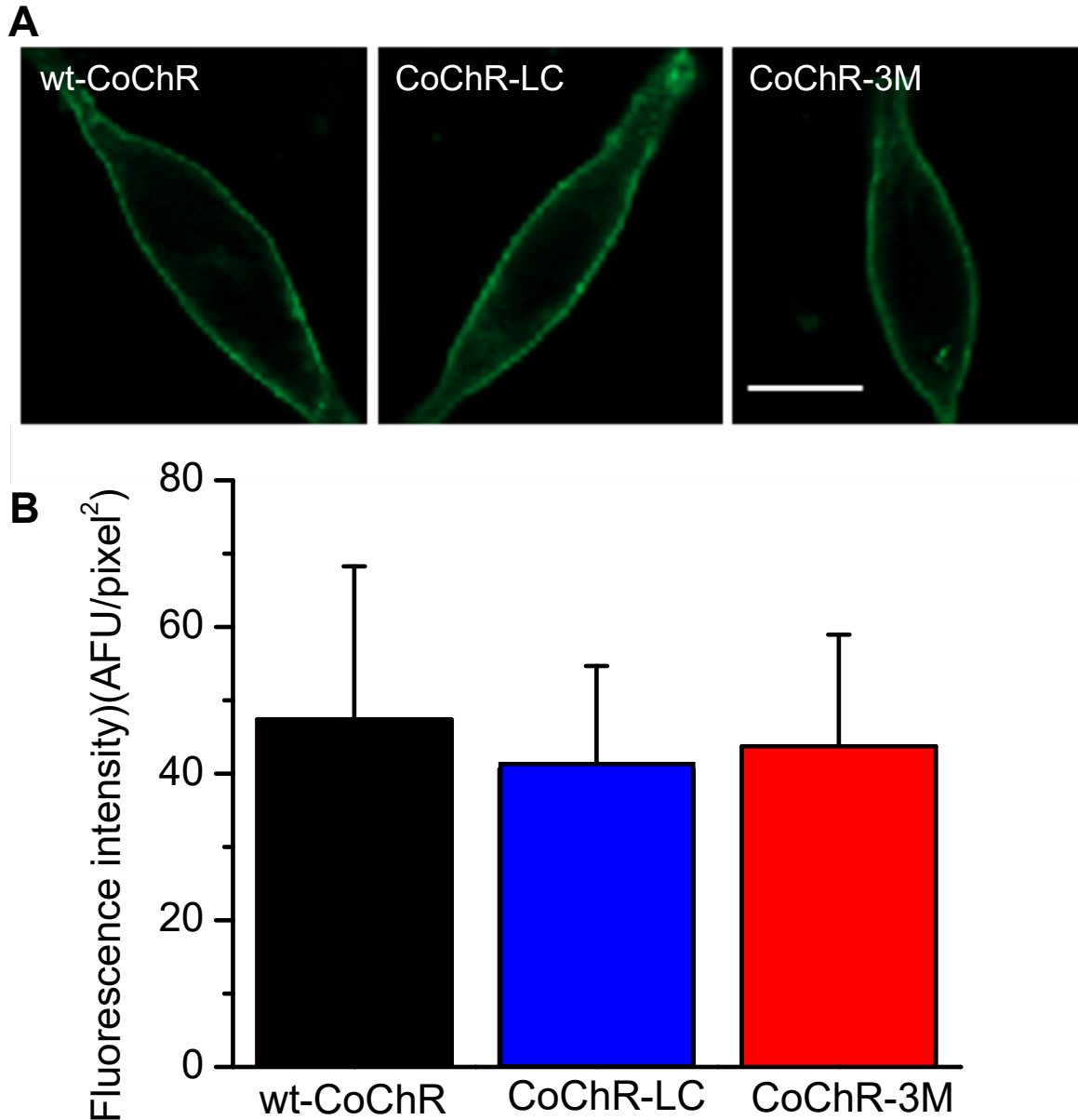
**Figure S2. Comparison of the spectral properties of wt-CoChR and CoChR mutants in HEK cells**

Photocurrents were elicited by 1-s light pulses of different wavelengths with a ND filter of 2.5. The spectral curves are normalized to the peak photocurrents. The data for each mutant are the average of 3 cells.

**Table S1. Off-rates and peak current amplitudes for wt-CoChR and CoChR mutants examined in HEK cells**

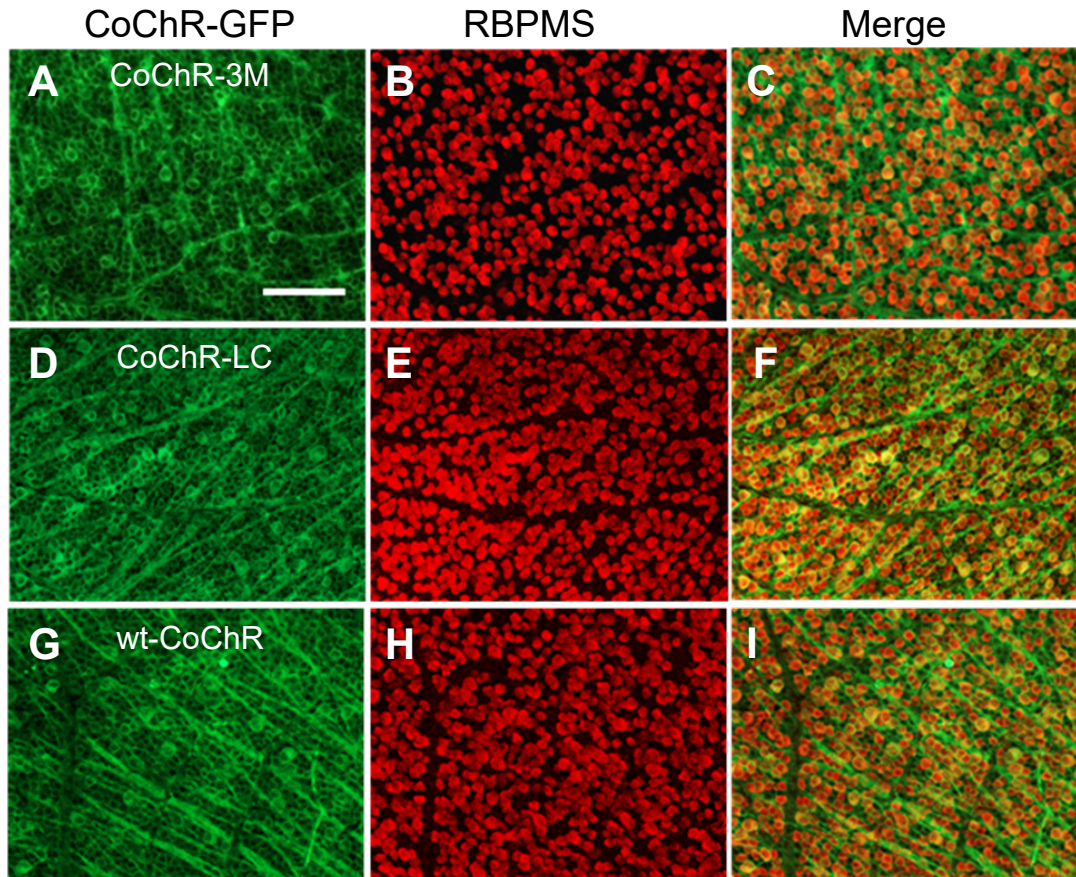
<b>CoChR mutants</b>	<b>Off rate (ms) mean <math>\pm</math> SEM</b>	<b>Current (pA) mean <math>\pm</math> SEM</b>	<b><i>n</i></b>
Wild type	112 $\pm$ 11	368 $\pm$ 44	10
L112C	372 $\pm$ 56	593 $\pm$ 91	10
L112A	1429 $\pm$ 170	707 $\pm$ 183	6
L112D*	nd	33 $\pm$ 7	5
L112S	1433 $\pm$ 96	474 $\pm$ 71	3
T139C	292 $\pm$ 33	393 $\pm$ 77	7
T139A	2109 $\pm$ 449	452 $\pm$ 131	7
T139S	671 $\pm$ 59	354 $\pm$ 118	6
L112C-T139C	751 $\pm$ 102	577 $\pm$ 123	6
C108A*	No deactivation	152 $\pm$ 4	3
C108T*	903 $\pm$ 180	52 $\pm$ 13	3
D136A*	nd	nd	3
D136C*	nd	39 $\pm$ 25	3
D136T*	nd	nd	2
K264T	186 $\pm$ 18	640 $\pm$ 90	10
H94E	174 $\pm$ 12	388 $\pm$ 42	10
H95E-K264T	133 $\pm$ 12	212 $\pm$ 35	11
H94E-L112C	614 $\pm$ 46	618 $\pm$ 80	10
L112C-K264T	376 $\pm$ 36	516 $\pm$ 65	13
H94E-L112C-K264T	723 $\pm$ 71	955 $\pm$ 87	10

Mutants noted with an asterisk exhibited notable aggregation. nd: not determined.



**Figure S3. Comparison of the membrane expression efficiencies of CoChRs in HEK cells**

(A) Representative images showing the expression of ChR-GFP for wt-CoChR, CoChR-LC, and CoChR-3M. Scale bar, 10  $\mu\text{m}$ . (B) Fluorescence intensities measured in the plasma membrane region. The data are shown in arbitrary fluorescence units (AFU/pixel<sup>2</sup>) (means  $\pm$  SD; n = 29 – 38 cells).



**Figure S4. Expression of CoChR-GFP in TKO mouse retinas *in vivo***  
 Representative immunofluorescence images showing co-labeling of GFP with RBPMS in retinal whole mounts for CoChR-3M (A–C), CoChR-L112C (D–F), wt-CoChR (G–I). Scale bar, 100  $\mu$ m.