

Supplementary materials

Ion Channel Properties of a Cation Channelrhodopsin, *Gt_CCR4*

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Table S1. Amino acid alignments of bacteriorhodopsin (BR), *Cr_ChR2* and *Gt_CCR4*. The characteristic amino acids were detected in BR and *Cr_ChR2*. In addition, amino acid numbers of each protein and transmembrane helix (TM) numbers are indicated.

No. in BR	44	45	52	59	63	85	89	90	96	118	214	216
No. in <i>Cr_ChR2</i>	82	83	90	97	101	123	127	128	134	156	253	257
No. in <i>Gt_CCR4</i>	75	76	84	90	94	116	120	121	127	149	242	246
TM number	2	2	2	2	2	3	3	3	3	4	7	7
BR (H ⁺ pump)	A	I	I	S	G	D	T	H	D	M	D	K
<i>Cr_ChR2</i>	E	E	E	E	E	E	T	C	H	D	D	K
<i>Gt_CCR4</i>	E	E	N	T	R	D	T	C	D	A	D	K

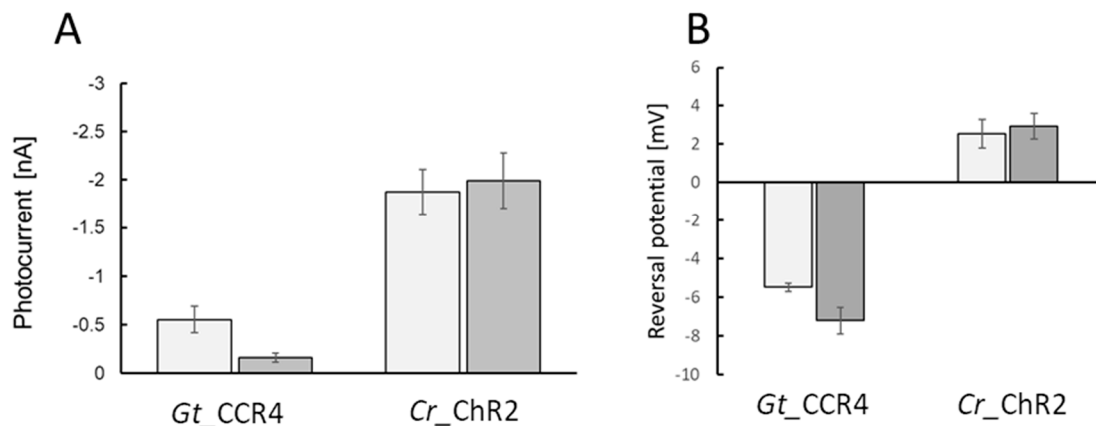


Figure S1. Ca²⁺ photocurrents of *Gt_CCR4* and *Cr_ChR2*. **A**, Comparison of current amplitude of *Gt_CCR4* and *Cr_ChR2* in the presence of two Ca²⁺ concentrations at -60 mV. Each channelrhodopsin expressed in ND7/23 cells was stimulated by 530 nm (*Gt_CCR4*) and 480 nm (*Cr_ChR2*) LED light (6.8 mW/mm²). A standard pipette solution was used. The bath solution contained (in mM) 60 NaCl, 1 KCl, 2 (white bar) or 40 (grey bar) CaCl₂, 2 MgCl₂, 76 (white bar) or 0 (grey bar) *N*-methyl D-glucamine, 10 HEPES, at pH 7.2. **B**, Comparison of reversal potentials of *Gt_CCR4* and *Cr_ChR2* in the presence of two Ca²⁺ concentrations. Solution in each condition is the same as in **A**. (n = 3-6 cells).

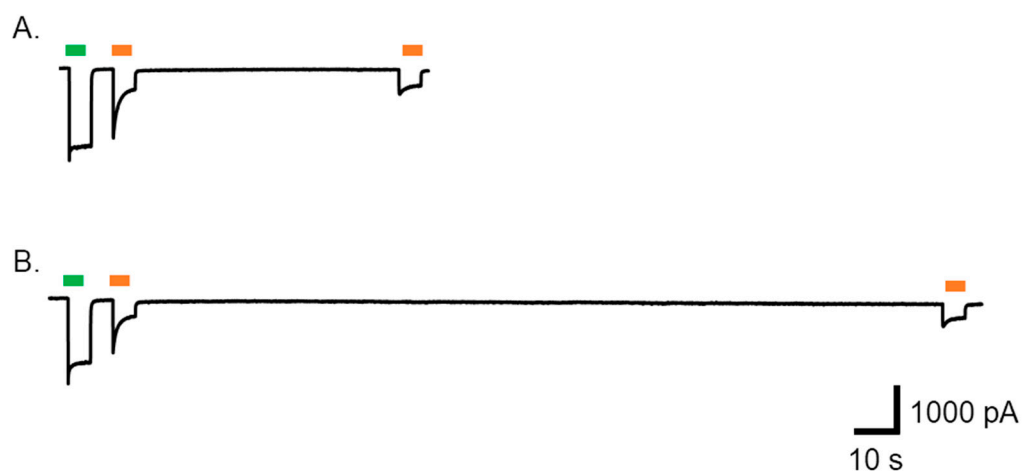


Figure S2. *Gt_CCR4* has a long-lived and long wavelength-absorbing inactivated state. Standard solutions were used. Photocurrents at -60 mV are shown. **A**, After activation by 530 nm light shown in a green bar, 590 nm light (in yellow bar) reduced the current amplitude. After a 60 s dark period, the second 590 nm was applied. **B**, Dark period was prolonged to 3 min. The second 590 nm light still reduced the current, indicating a very stable inactivated state.