Supporting Information

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SI Materials and Methods

Photocurrent Measurements. The opsin domains of the wild-type *Gt*CCR2 or its mutants were cloned into the mammalian expression vector pcDNA3.1 (Life Technologies) in frame with an EYFP tag. Human embryonic kidney 293 (HEK293) cells were transfected using the ScreenFectA transfection reagent (Waco Chemicals). All-trans retinal (Sigma-Aldrich) was added as a stock solution in ethanol at a final concentration of 4 μM. Measurements were performed 48–72 h after transfection with an Axopatch 200B amplifier (Molecular Devices). The signals were digitized with a Digidata 1440A using pClamp 10 software (both from Molecular Devices) at the sampling rate of 250 kHz. Patch pipettes with resistances of 2–5 MΩ were fabricated from borosilicate glass and filled with the following standard solution (in millimoles): KCl 126, $MgCl_2$ 2, $CaCl_2$ 0.5, EGTA 5, Hepes 25, pH 7.4. The standard bath solution contained (in millimoles): NaCl 150, $CaCl_2$ 1.8, $MgCl_2$ 1, glucose 5, Hepes 10, pH was adjusted with *N*-methyl-D-glucamine (NMG^+) to the desired value. For analysis of intramolecular proton transfers, channel currents were eliminated by substituting NMG^+ for all permeable metal cations in the pipette and bath solutions, the pH of which was adjusted to the same value of 7.4. To correct IE dependencies of channel current for slow inwardly directed proton movement, its amplitude was subtracted from the amplitude of channel current. To correct for different expression levels, the amplitude of slow inwardly directed current was scaled according to the amplitude of fast outwardly directed proton transfer. A 4 M KCl bridge was used in all experiments. All measurements were carried out at room temperature (25 °C). Liquid junction potentials were calculated using the built-in tool of pClamp 10. Continuous light pulses were provided by a Polychrome V light source (T.I.L.L. Photonics GmbH) in combination with a mechanical shutter (Uniblitz model LS6, Vincent Associates; half-opening time 0.5 ms). Maximal quantum density at the focal plane of the 40× objective lens was 7.7 mW/mm^2 at 520 nm. Laser excitation was provided by a Minilite Nd:YAG laser (532 nm, pulsewidth 6 ns, energy 12 mJ; Continuum). A laser artifact was measured with a blocked optical path and digitally subtracted from the recorded traces. The signals were logarithmically averaged with a custom-created computer algorithm for further analysis. Curve fitting was performed using Origin 7 software (OriginLab).

Expression and Purification of *Gt*CCR2 from *P. pastoris*. The human-codon adapted opsin domain of *Gt*CCR2 (amino acid residues 1–300) was fused in frame with a C-terminal eight-His tag and subcloned into the pPIC9K vector (Invitrogen) between EcoRI and NotI sites. Mutations were introduced using a QuikChange XL site-directed mutagenesis kit (Agilent Technologies) and verified by DNA sequencing. *P. pastoris* strain SMD1168 (*his4*, *pep4*) was transformed by electroporation with the resultant plasmids linearized by SalI. The transformants were screened according to the company protocol for their ability to grow on histidine-deficient medium and geneticin resistance. For each construct, a single colony that grew on 4 mg/mL geneticin and yielded the brightest color in a small-scale test was used for large-scale protein production, which was carried out as follows: A starter culture was inoculated into buffered complex glycerol medium and grown until A_{600} reached two to five. The cells were harvested by centrifugation at 2,975 × *g* and transferred to buffered complex methanol medium supplemented with 5 μM all-trans retinal (Sigma-Aldrich). Expression was induced by the addition of 0.5% methanol. The cells were harvested after 24 h and disrupted in a bead beater (BioSpec Products) or French press in buffer A (20 mM sodium phosphate, pH 7.4, 100 mM NaCl, 1 mM EDTA, 5% glycerol). Cell debris and unbroken cells were removed by centrifugation at 2,975 × *g* for 20 min. Membrane fragments were collected by ultracentrifugation at 40,000 rpm for 1 h in a Ti45 rotor (Beckman Coulter), resuspended in buffer B (20 mM Hepes, pH 7.0, 300 mM NaCl, 5% glycerol) and solubilized by incubation with 1% dodecyl maltoside (DDM) for 1 h at 4 °C. After removing nonsolubilized material by ultracentrifugation at 50,000 rpm for 1 h in a TLA-100 rotor (Beckman Coulter), the supernatant was mixed with nickel-nitrilotriacetic acid agarose beads (Qiagen) for 1 h and loaded on a column. The column was thoroughly washed with buffer C (20 mM Hepes, pH 7.0, 300 mM NaCl, 5% glycerol, 0.02% DDM) containing 40 mM imidazole, after which the protein was eluted with buffer C containing 300 mM imidazole. The pigment was concentrated and imidazole removed by repetitive washing with imidazole-free buffer C using YM-10 centrifugal filters (Amicon).

Flash-Induced Absorption Changes. Laser flash-induced absorption changes were measured with a laboratory-constructed crossbeam apparatus. Excitation flashes (532 nm, 6 ns, up to 40 mJ) were provided by a Surelite I Nd-YAG laser (Continuum). Measuring light was from a 250-W incandescent tungsten lamp combined with a McPherson monochromator (model 272). A photomultiplier tube model R928 from Hamamatsu Photonics was protected from excitation laser flashes by a second monochromator of the same type and additionally with interference filters (12-nm bandwidth; Oriel Instruments). Signals were amplified by a low noise current amplifier (model SR445A, Stanford Research Systems) and digitized with a GaGe Octopus digitizer board (model CS8327, DynamicSignals), maximum sampling rate 50 MHz. The time interval between excitation flashes was 20 s, and up to 100 sweeps were averaged for each wavelength. Data analysis was performed with pClamp 10 (Molecular Devices) and OriginPro 7 (OriginLab) software. Logarithmic filtration of the data was performed using the GageCon program. Global fit of spectral transitions was performed using the FITEXP program provided by A. K. Dioumaev, University of California, Irvine, CA.

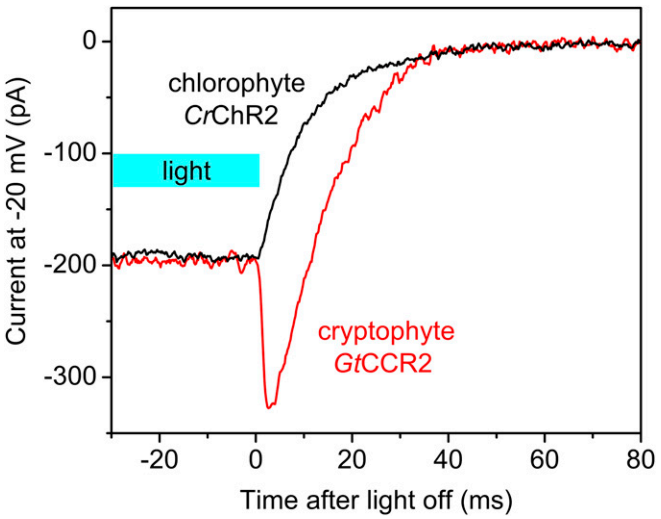

Fig. S1. Decay of photocurrents generated by *Gt*CCR2 (red line) and *Cr*ChR2 (channelrhodopsin 2 from the chlorophyte *Chlamydomonas reinhardtii*) at −60 mV after 1-s continuous illumination. The light pulse (500 and 470 nm, respectively) is shown as a cyan bar.

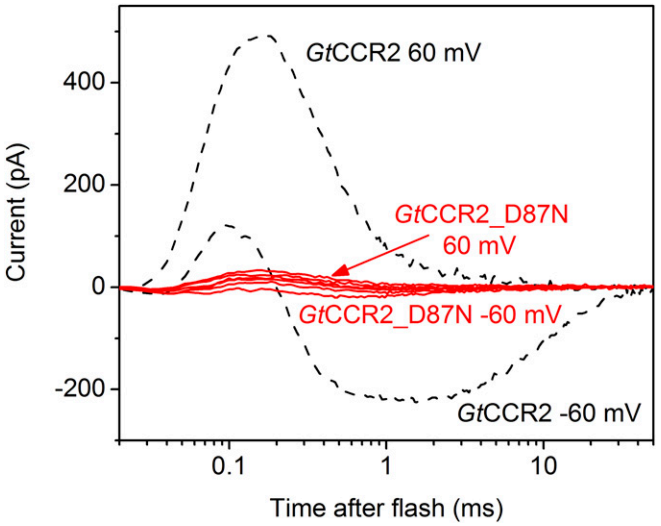

Fig. S2. The effect of the D87N mutation on photocurrent at pH 7.4. The red traces were recorded from *Gt*CCR2_D87N in 30-mV steps from −60 mV (*Bottom* trace). The traces of the wild-type *Gt*CCR2 at −60 mV and 60 mV are shown as black dashed lines for comparison.

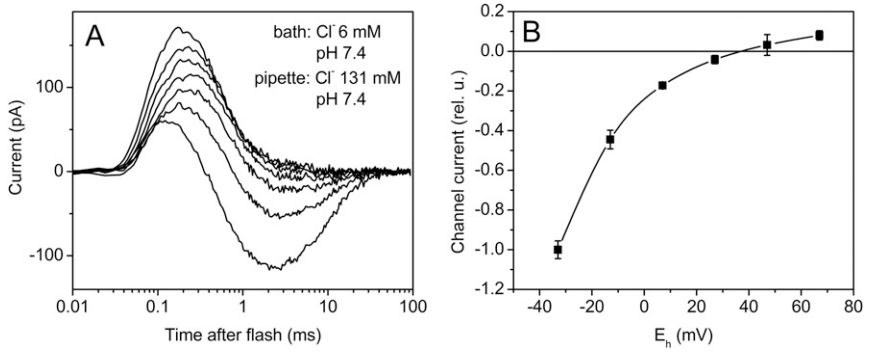

Fig. S3. *Gt*CCR2 does not conduct Cl^-. (*A*) Series of current traces recorded from *Gt*CCR2 in response to a 6-ns laser flash after replacement of Cl^- with Asp^- in the bath. The holding voltage at the amplifier output was changed in 20-mV steps from −60 mV (*Bottom* trace). (*B*) The IE curves for the channel current, the amplitude of which was derived by multiexponential fit to the traces, as explained in the main text. To calculate the error values, data obtained on different cells were normalized and plotted as relative units (rel. u.). The data points are the mean values ± SEM recorded from three cells.

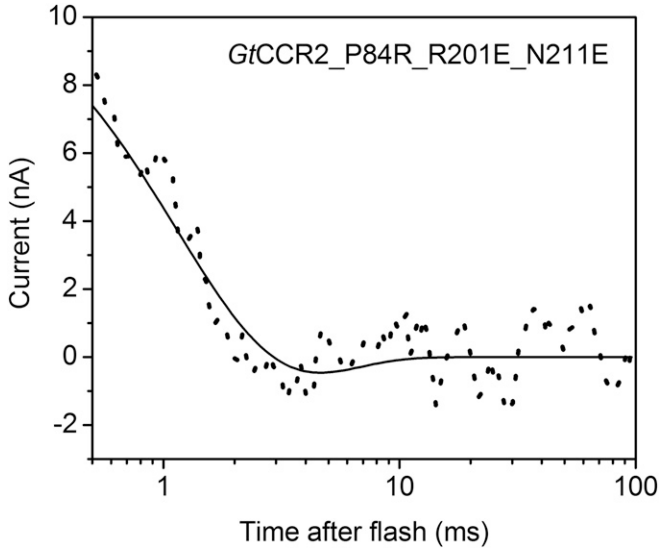

Fig. S4. Introducing the BR proton release group residues in *Gt*CCR2 does not result in proton pumping. Photocurrent in *Gt*CCR2_P84R_R201E_N211E triple mutant is shown. Experimental data are shown as dots and multiexponential fit, as solid line.

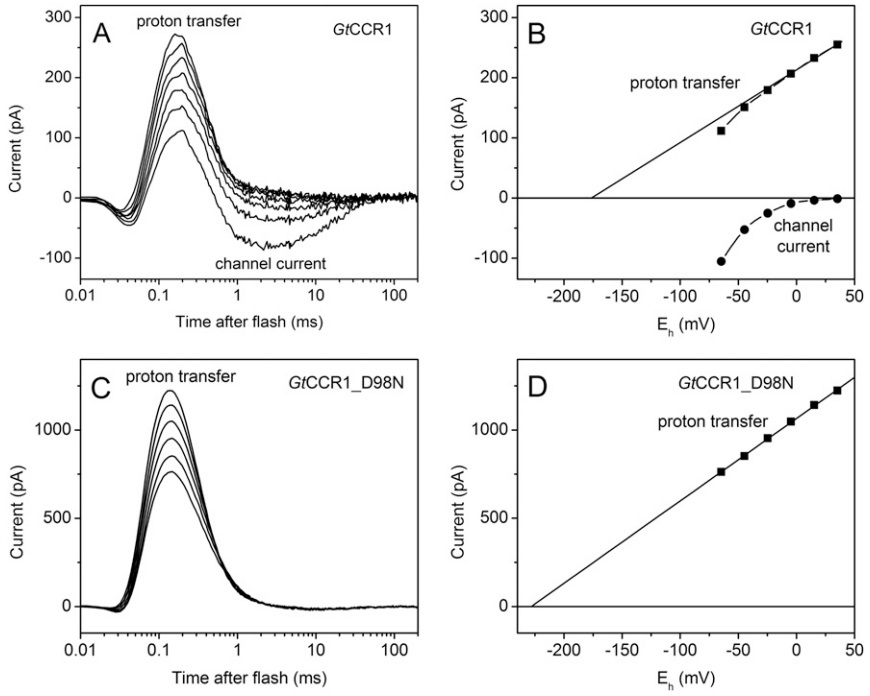

Fig. S5. Photocurrents recorded under single turnover conditions from wild-type *Gt*CCR1, a close homolog of *Gt*CCR2, and its D98N mutant. (*A* and *C*) Series of current traces recorded in response to a 6-ns laser flash at standard conditions from *Gt*CCR1 (*A*) and *Gt*CCR1_D98N (*C*). (*B* and *D*) Corresponding voltage dependencies of the peak amplitudes of proton transfer currents (squares) and, for the wild-type *Gt*CCR1, of the channel current derived by multiexponential fit, as described in the main text (circles).