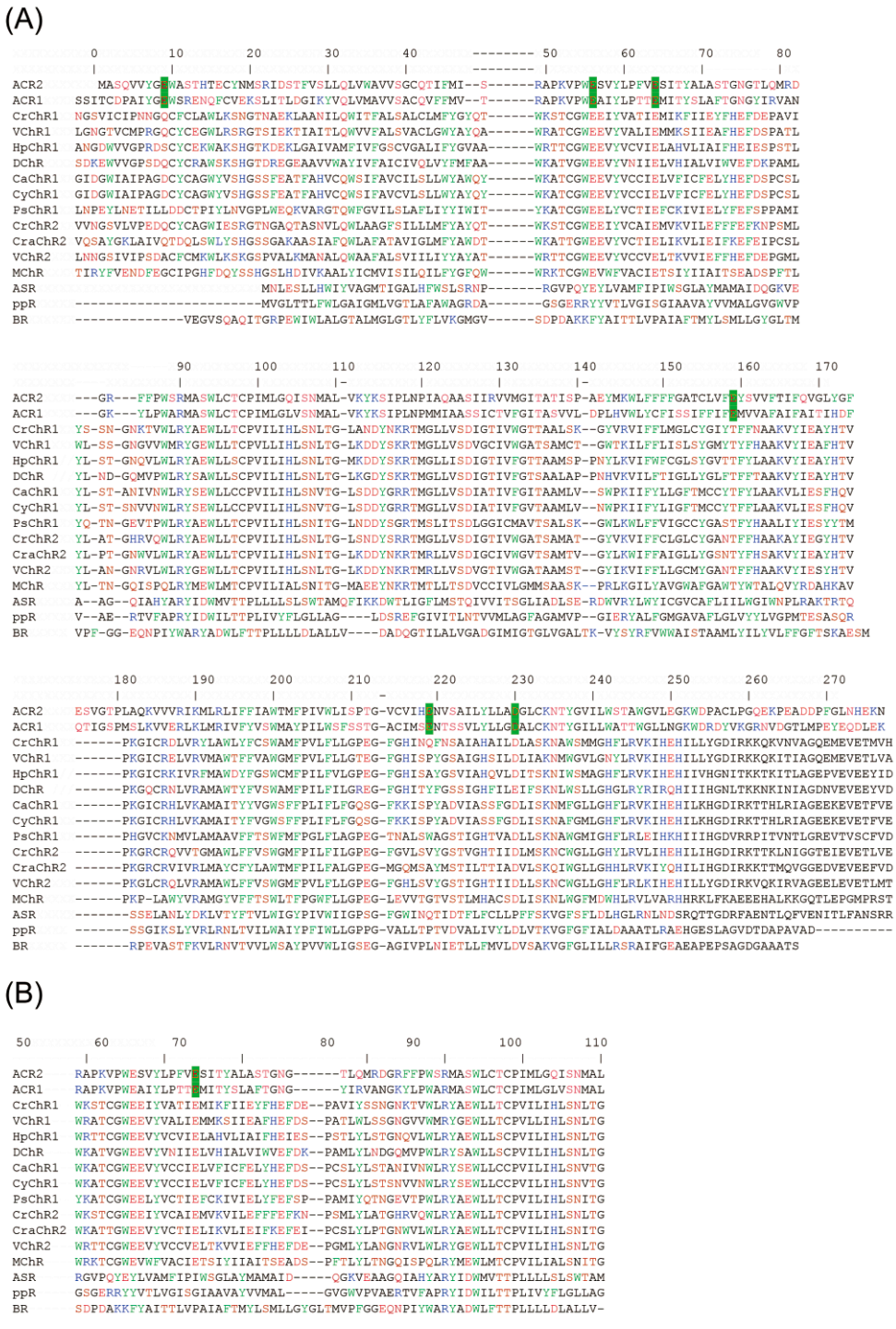
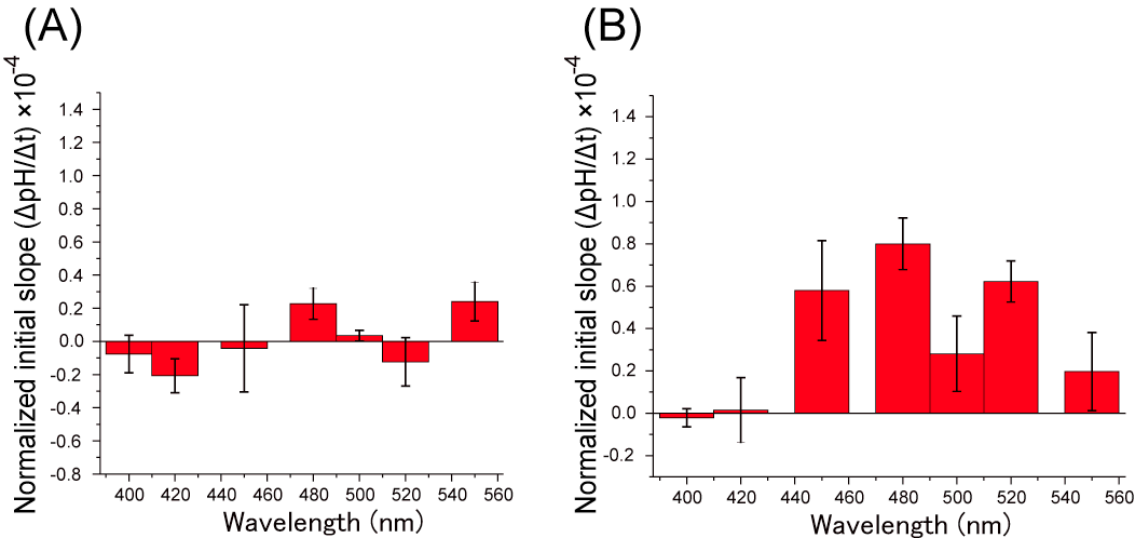


Supplementary Figure S1. **Amino acid sequence alignment of microbial rhodopsins including ACRs. (A)** The region containing 7-transmembrane (TM) helices in ACR2 is shown. The green colored background indicates the conserved carboxylates. Positively and negatively

charged residues are colored blue and red, respectively. Aromatic amino acids are colored green. **(B)** An alternative sequence alignment for MD simulations, in which ACR has a β-sheet in the BC-loop and did not show chloride binding (Fig. 5). NCBI accession numbers used in this figure are as follows; ACR2 (KP171709), ACR1 (KP171708), CrChR1 (AF508965), VChR1 (ABZ90901), HpChR1 (JN596950), DChR (JX983144), CaChR1 (JN596951), CyChR1 (JN596948), PsChR1 (JX983143), CrChR2 (AF508966), CraChR2 (JN596949), VChR2 (ABZ90903), MChR (JF922293), ASR (WP_010997316), ppR (WP_011324120) and BR (WP_010903069).



Supplementary Figure S2. Dependency on wavelength of light for anion transport in the E159Q and D230N mutants of ACR2. The normalized initial slope amplitudes of the light-induced pH changes of E159Q **(A)** and D230N **(B)** were plotted against the wavelength of light in a solution containing 300 mM NaCl. The samples were illuminated with a Xenon lamp (Asahi Spectra Co. Ltd., Tokyo, Japan) through a series of band-pass filters (400 ± 10 nm, 418.5 ± 9 nm, 450 ± 10 nm, 480 ± 10 nm, 500 ± 10 nm, 520 ± 10 nm and 550 ± 10 nm), where the light intensity was measured and adjusted to 8–10 mW/cm^2 using an optical power meter with an optical sensor. All error bars represent the SEM of more than three independent measurements.