

**Electronic Supplementary Information:**

**The femtosecond-to-second photochemistry of red-shifted fast-closing  
anion channelrhodopsin *PsACR1***

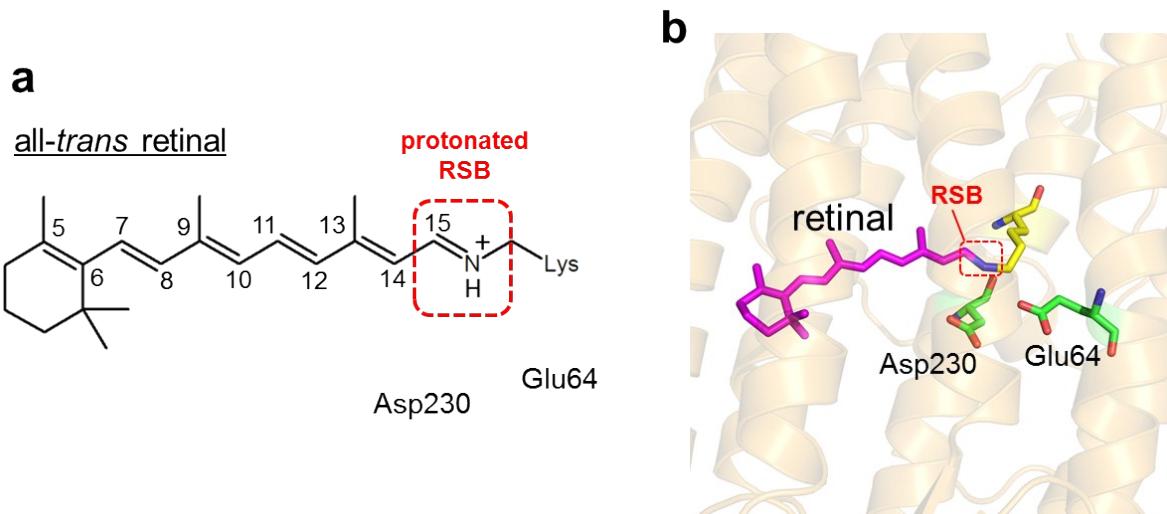
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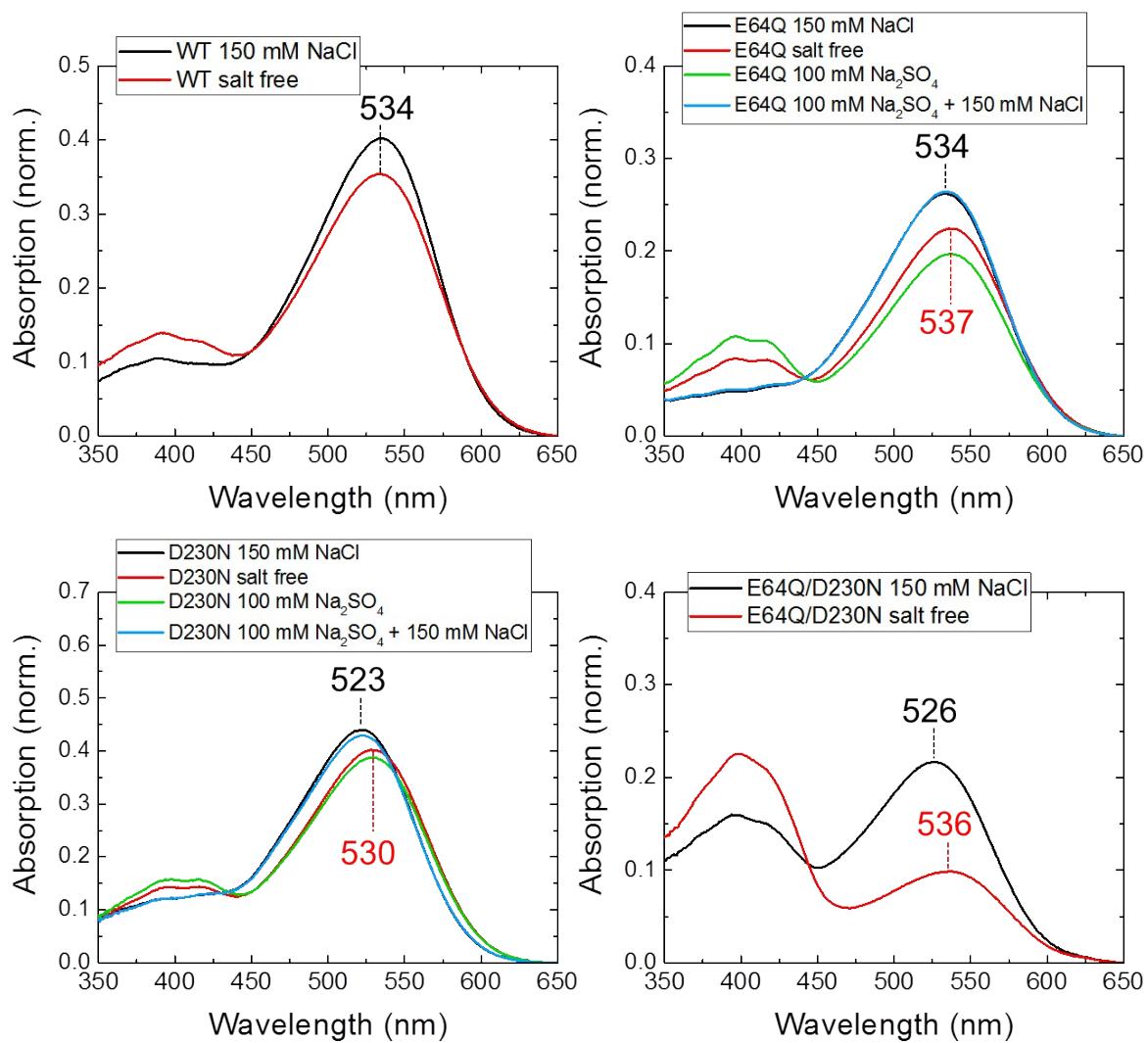
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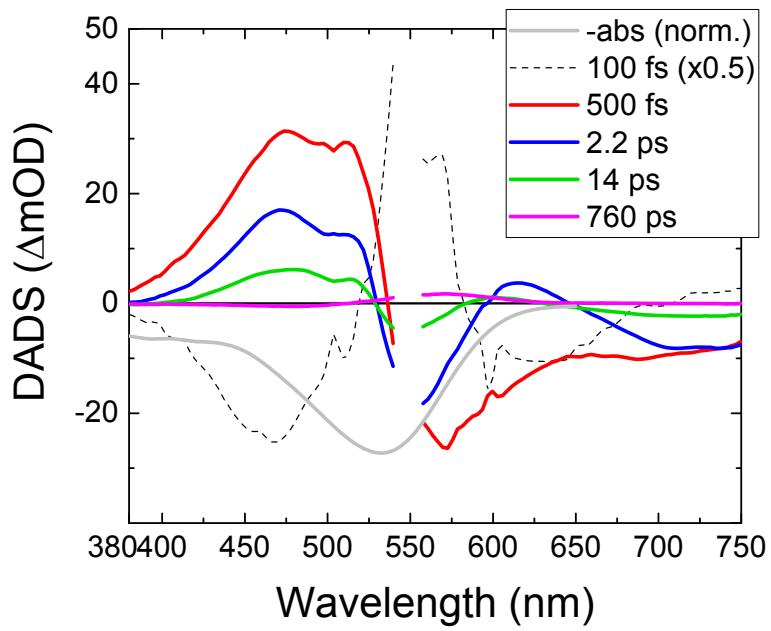
**Fig. S1. All-trans retinal structure and *PsACR1* homology model.** (a) Protonated retinal Schiff base (RSB) of all-*trans* retinal. (b) A homology model of *PsACR1* based on the crystal structure of cation channelrhodopsin C1C2<sup>1</sup> (PDB ID: 3UG9) built by the Robetta server ([robetta.bakerlab.org](http://robetta.bakerlab.org)). All-*trans* retinal is shown in magenta. Glu64 and Asp230 are shown in green sticks.



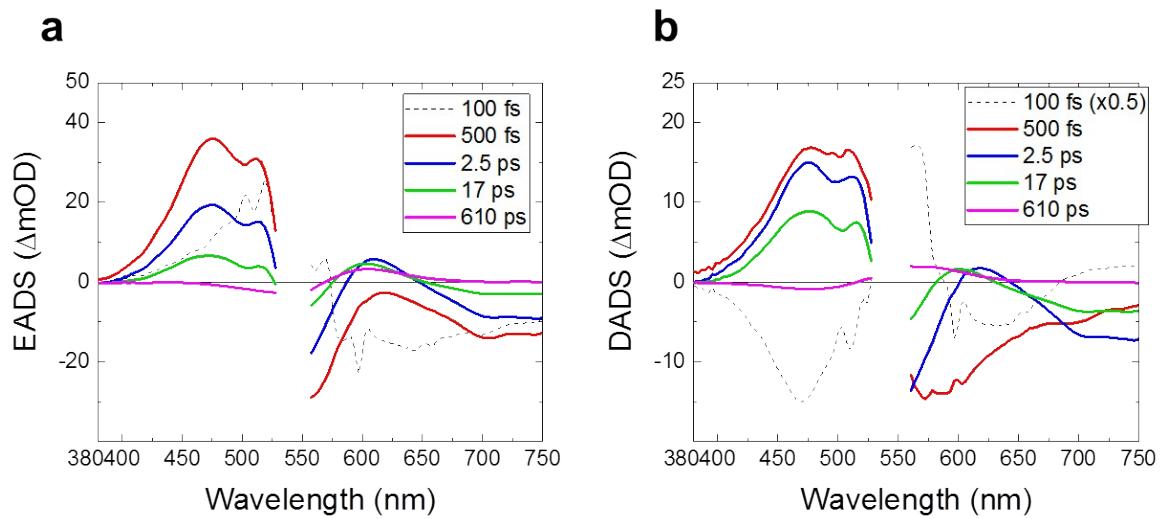
**Fig. S2. Dark-state absorption of *PsACR1* and its mutants in different anion conditions at pH 7.**

**Table S1. Visible absorption maxima of *PsACR1* and the mutants in different salt conditions at pH 7.**

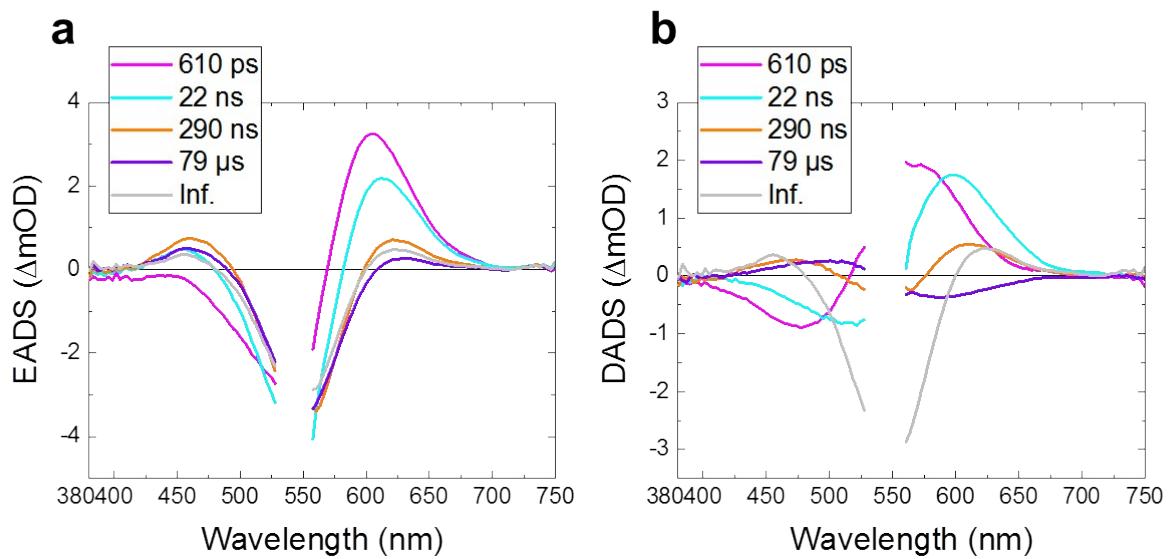
	$\lambda_{\max}$ (150 mM NaCl)	$\lambda_{\max}$ (<1 mM NaCl)	$\Delta\lambda_{\max}$	$\lambda_{\max}$ (100 mM Na <sub>2</sub> SO <sub>4</sub> )
WT	534 nm	534 nm	<b>0 nm</b>	---
E64Q	534 nm	537 nm	<b>3 nm</b>	537 nm
D230N	523 nm	530 nm	<b>7 nm</b>	530 nm
E64Q/D230N	526 nm	536 nm	<b>10 nm</b>	---



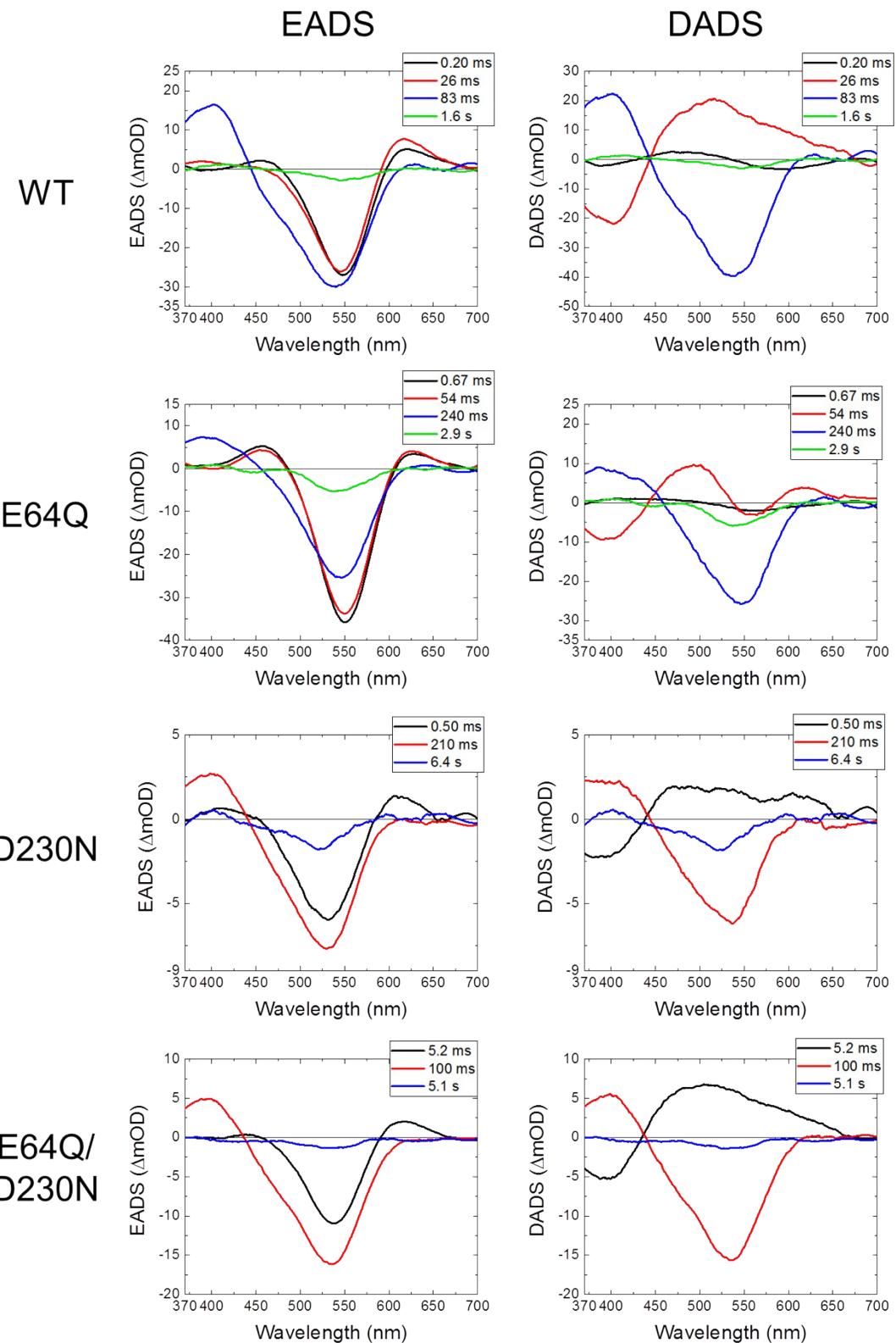
**Fig. S3. Decay-associated difference spectra (DADS) of fs-ps transient absorption for wild-type *PsACR1* at pH 8.** Normalized dark-state absorption is shown in the gray line, which is inverted for comparison with the GSB. The spectral region of 540–560 nm is omitted because of the strong pump-light scattering.



**Fig. S4. EADS and DADS of fs-ps transient absorption spectra of *PsACR1/E64Q* at pH 8.** The spectral region of 540–560 nm is omitted because of the strong pump-light scattering.



**Fig. S5. EADS and DADS of picosecond-submillisecond transient absorption spectra of *PsACR1/E64Q* at pH 8.** The spectral region of 540–560 nm is omitted because of the strong pump-light scattering.



**Fig. S6. Global-fitted flash photolysis results for WT *PsACR1* and the E64Q, D230N and E64Q/D230N mutants at pH 8.**

## References

1. H. E. Kato, F. Zhang, O. Yizhar, C. Ramakrishnan, T. Nishizawa, K. Hirata, J. Ito, Y. Aita, T. Tsukazaki, S. Hayashi, P. Hegemann, A. D. Maturana, R. Ishitani, K. Deisseroth and O. Nureki, *Nature*, 2012, **482**, 369-374.