

Supplementary Information for

RubyACRs, non-algal anion channelrhodopsins with highly red-shifted absorption

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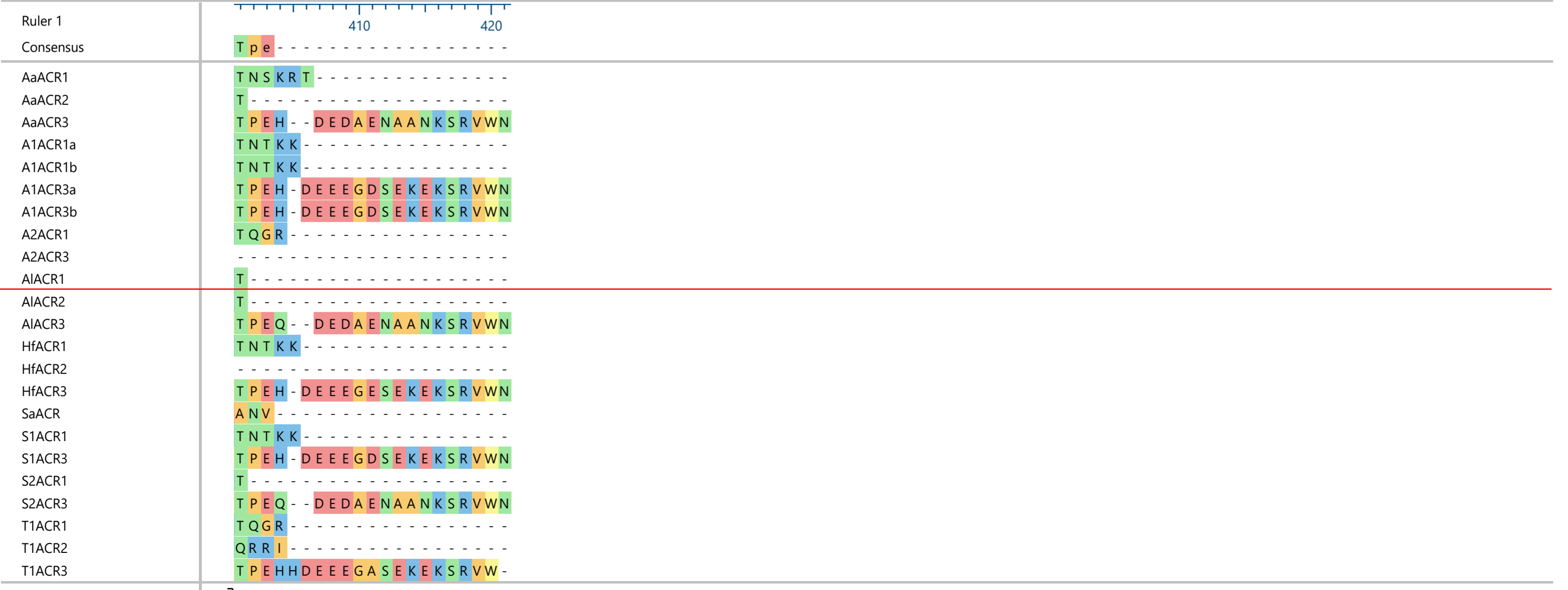
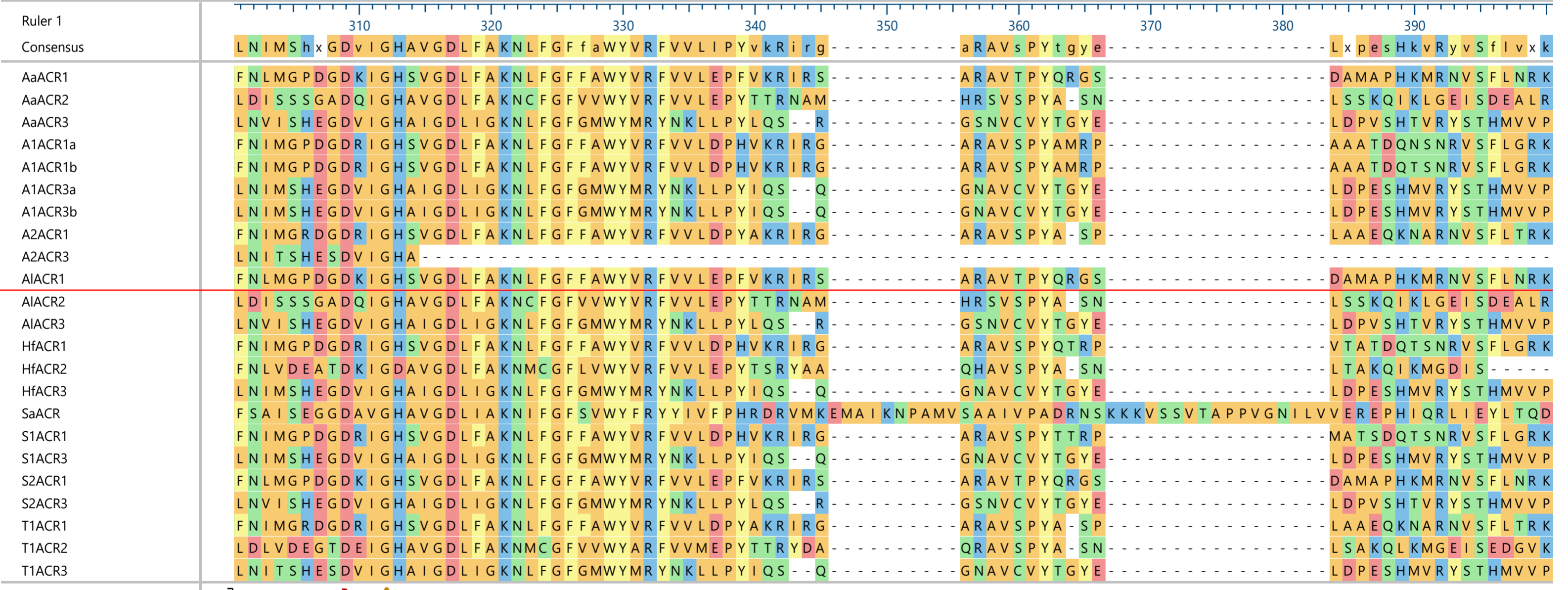
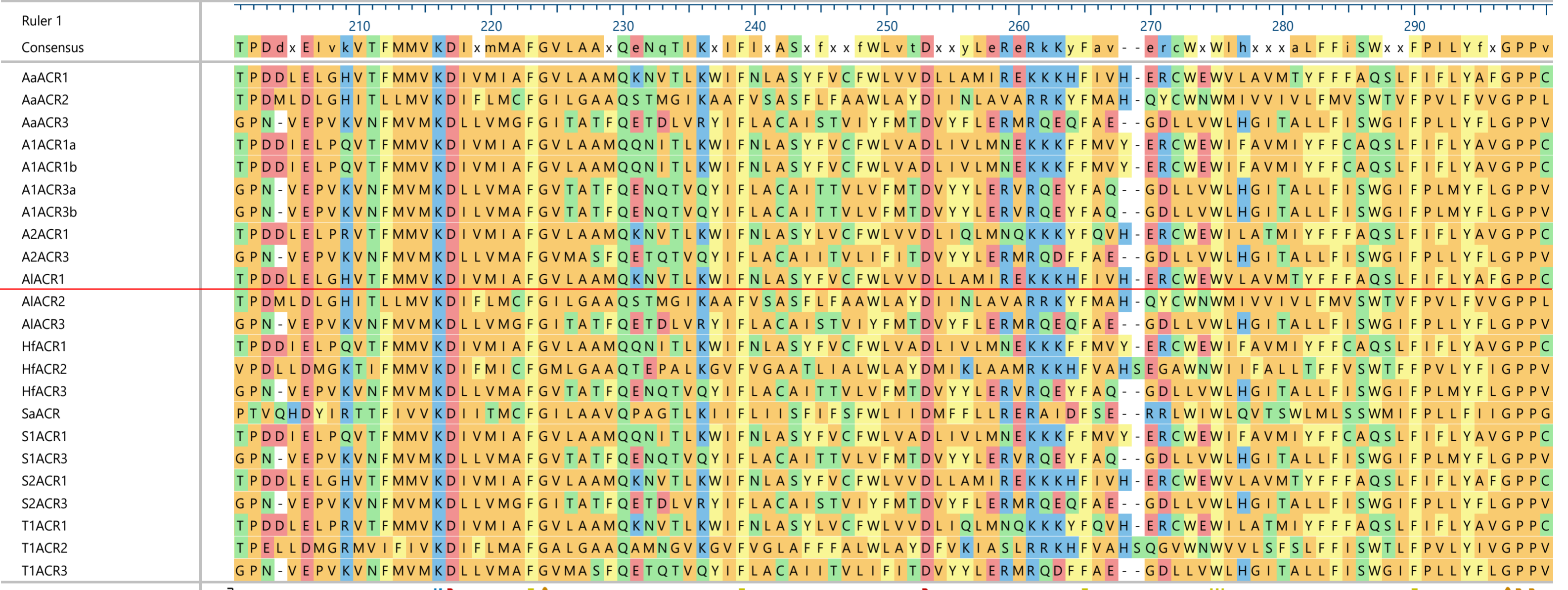
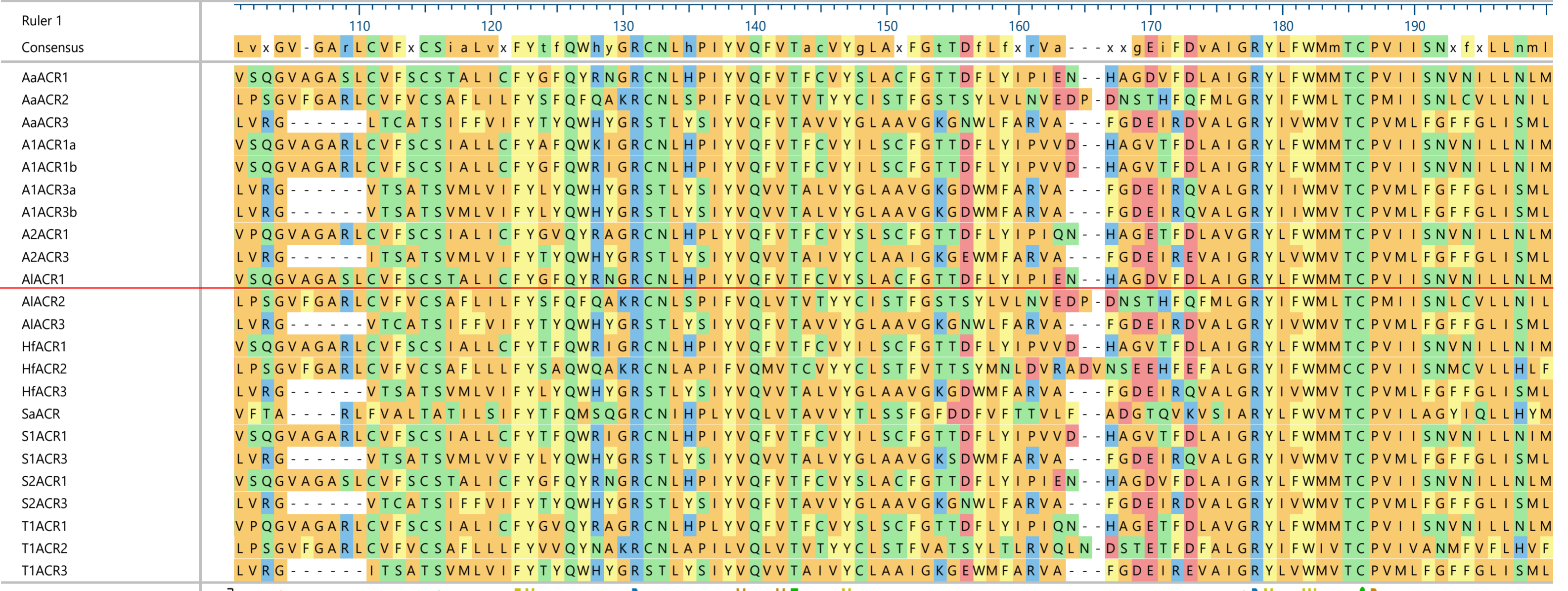
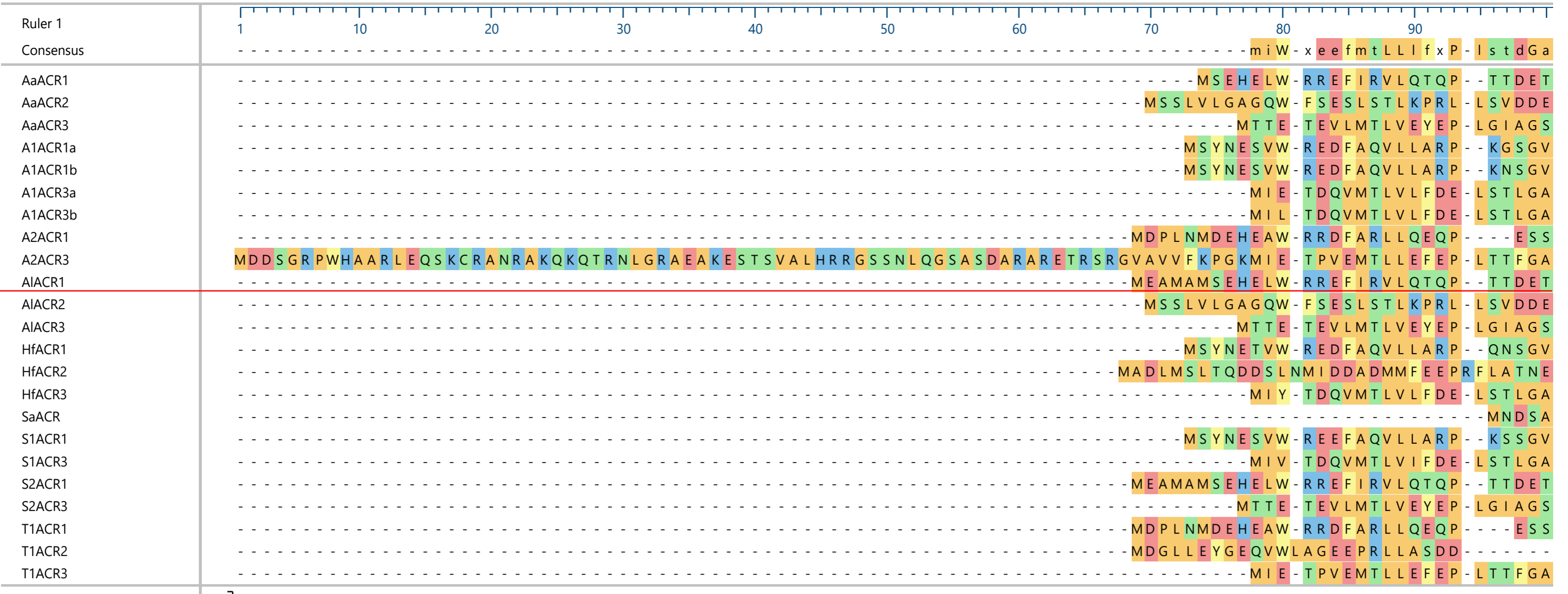


Fig. S1. Protein sequence alignment of the rhodopsin domains of Laby channelrhodopsins. Residues are color-coded according to their chemical properties. The arrows point to the positions of the residues known to be functionally important in *GtACR1*.

Fig. S2. Protein sequence alignment of the rhodopsin domains of Hapto channelrhodopsins. Residues are color-coded according to their chemical properties. The arrows point to the positions of the residues known to be functionally important in *Gt*ACR1.

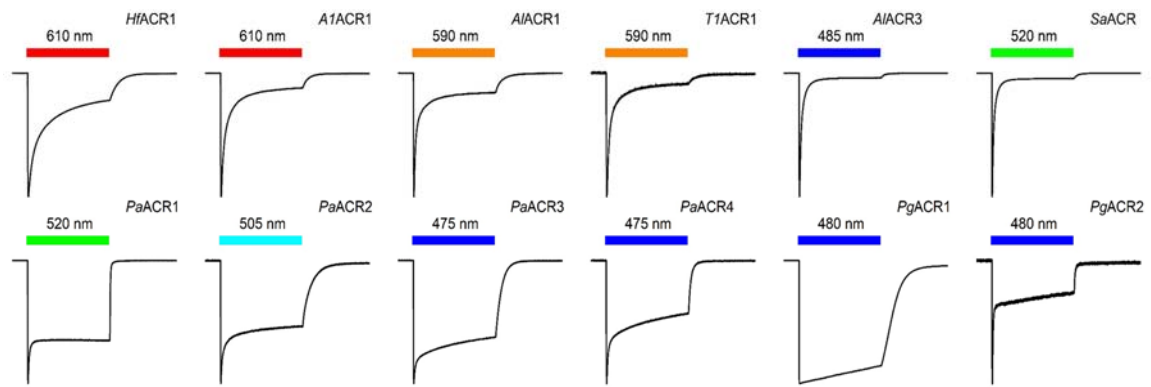


Fig. S3. Photocurrent traces recorded in response to the first 1-s light pulse at -60 mV at the amplifier output, normalized at their peak value. The duration of illumination is showed as a colored bar on top.

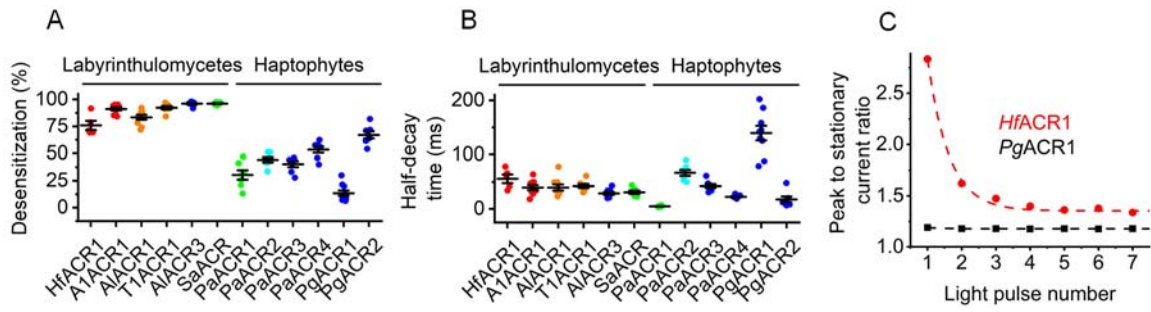


Fig. S4. (A and B) The magnitude of desensitization during continuous illumination (A) and half-decay of photocurrent after switching the light off (B). The black lines show the mean values and s.e.m. ($n = 5-10$ cells for each variant); colored circles, the individual data points. (C) The ratio of the peak amplitude to that of the stationary current (measured at the end of a 1-s light pulse) in a series of pulses applied with 30-s time interval. The lines are single exponential fits.

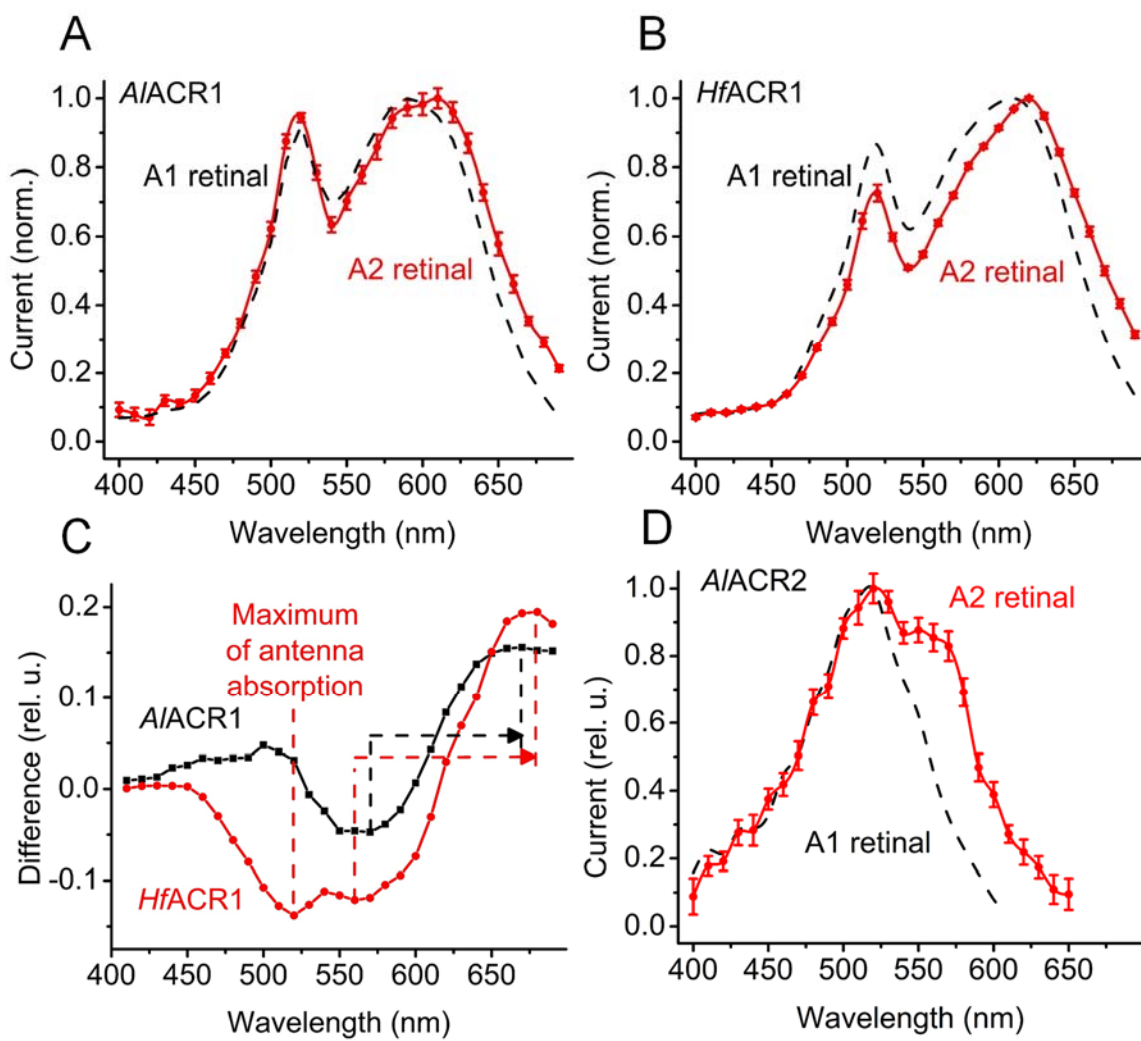


Fig. S5. (A, B and D) The action spectra of photocurrents generated by indicated proteins reconstituted with A1 (black) and A2 (red) retinal. (C) The difference spectra (A2-A1 retinal).

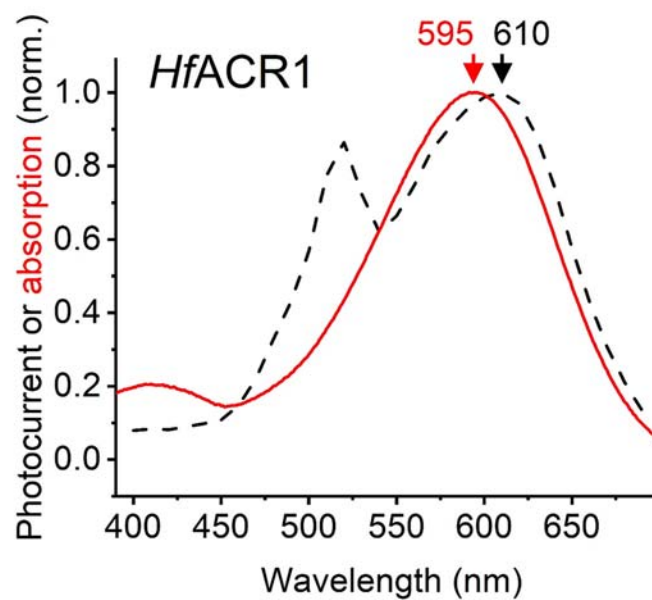


Fig. S6. The absorption spectrum of *HfACR1* detergent-purified from *Pichia* (red solid line) compared to the action spectrum of photocurrents generated upon its expression in HEK293 cells from Fig. 1B (black dashed line).

Table S1. A list of Laby ACR homologs (in bold – synthesized and tested by patch clamp in this study)

	GenBank accession number	Abbreviated protein name	Source organism	JGI gene model name	Total CDS length	λ_{\max} (nm)
1.	QDJC01000532	<i>Aa</i> ACR1	<i>Aurantiochytrium acetophilum</i> HS399	identical to <i>A</i> /ACR1	691	
2.	QDJC01003161	<i>Aa</i> ACR2		identical to <i>A</i> /ACR2	635	
3.	QDJC01000037	<i>Aa</i> ACR3		only two mismatches with <i>A</i> /ACR3	683	
4.	MT002467	<i>A</i>/ACR1	<i>Aurantiochytrium limacinum</i> ATCC MYA-1381	fgenesh1_pg.12_#_284	696	590
5.	MT002473	<i>A</i>/ACR2		gm1.7690_g	635	545
6.	MT002476	<i>A</i>/ACR3		estExt_Genemark1.C_1_t20010	678	485
7.	MT002468, BGKB01000037	<i>A</i>1ACR1	<i>Aurantiochytrium</i> sp. KH105		646	610
8.	BGKB01000105	<i>A</i> 1ACR1			645	
9.	BGKB01000099	<i>A</i> 1ACR3			680	
10.	BGKB01000102	<i>A</i> 1ACR3			680	
11.	LNGJ01004228	<i>A</i> 2ACR1		<i>Aurantiochytrium</i> sp. T66	identical to <i>T</i> 1ACR1	649
12.	LNGJ01002066	<i>A</i> 2ACR3			759	
13.	MT002469, GBG24568	<i>Hf</i>ACR1	<i>Hondaea fermentalgiana</i> FCC1311		646	610
14.	GBG24569	<i>Hf</i> ACR2			663	
15.	GBG23965	<i>Hf</i> ACR3			680	
16.	MT002463	<i>Sa</i>ACR	<i>Schizochytrium aggregatum</i> ATCC 28209	fgenesh1_pg.3_#_476	546	520
17.	JTFK01000019	<i>S</i> 1ACR1	<i>Schizochytrium</i> sp. CCTCC M209059		645	
18.	JTFK01000324	<i>S</i> 1ACR3			680	
19.	SMSO01000032	<i>S</i> 2ACR1	<i>Schizochytrium</i> sp. TIO01	Identical to <i>A</i> /ACR1	696	
20.	SMSO01000014	<i>S</i> 2ACR3			583	
21.	MT002470, MUFY01006470	<i>T</i>1ACR1	<i>Thraustochytrium</i> sp. ATCC 26185		649	590
22.	MUFY01006469	<i>T</i> 1ACR2			666	
23.	MUFY01009420	<i>T</i> 1ACR3			682	

Table S2. A list of Hapto ACR homologs tested in this study

	GenBank accession number	Abbreviated protein name	Source organism	JGI gene model name	Total CDS length	λ_{\max} (nm)
1.	MT002471	<i>Pa</i> ACR1	<i>Phaeocystis antarctica</i> CCMP1374	Phant.0066s0015.1	1682	520
2.	MT002474	<i>Pa</i> ACR2		Phant.0011s0329.1	647	505
3.	MT002477	<i>Pa</i> ACR3		Phant.0016s0461.1, Phant.0016s0462.1, Phant.0016s0464.1	427	475
4.	MT002464	<i>Pa</i> ACR4		Phant.0060s0074.1	469	475
5.	MT002465	<i>Pa</i> ACR5		Phant.0086s0086.1	312	N.A.
6.	MT002466	<i>Pa</i> ACR6		Phant.0001s0932.1	471	N.A.
7.	MT002472	<i>Pg</i> ACR1	<i>Phaeocystis globosa</i> Pg-G	Phglo.0395s0005.1	327	480
8.	MT002475	<i>Pg</i> ACR2		Phglo.0149s0014.1	435	480
9.	MT002478	<i>Pg</i> ACR3		Phglo.0128s0040.1	505	N.A.