Supporting information

Supplemental figure legends

Fig. S1

Absorption spectra of amphioxus rhodopsin Ala-269 mutants.

A Absorption spectra of the purified amphioxus rhodopsin mutants A269L (red curve) and A269S (blue curve) in the dark. The absorption spectrum of WT (black dotted curve) is also shown. The λ_{max} values are indicated. All the absorbances at the λ_{max} were normalized to 1.0. ***B*** Difference absorption spectra of amphioxus rhodopsin mutants A269L (red curve) and A269S (blue curve) in the membrane preparations. The differnece spectrum of WT (black dotted curve) is also shown. The λ_{max} values are indicated. All the difference absorbances at the λ_{max} of active states were normalized to 1.0.

Fig. S2

Difference spectra of the amphioxus rhodopsin L208A/A269L mutant.

The normalized difference spectra of parapinopsin WT (black), A269L (red) and L208A/A269L (green) in the membrane preparations are shown. All the difference absorbances at the λ_{max} of active states were normalized to 1.0. It should be noted that the spectrum of L208A/A269L is different in shape at the shorter wavelengths region from that of the WT, indicating that subsequent L208A mutation does mostly but not completely recover the spectral properties of active state of amphioxus rhodopsin. The

spectra of WT and A269L mutant are the same as those in supplemental Fig. S1B.

Fig. S3

Absorption spectra of the purified parapinopsin mutants in the dark.

The absorption spectra of WT (black), the mutants A269L (red), F208A (orange), F208A/A269L (green), F212C (turquoise) and F212C/A269L (blue) are shown. The λ_{max} of these mutants are the same as that of WT. All the absorbances at the λ_{max} were normalized to 1.0.

Fig. S4

Absorption spectra of the Ala-269 mutant of bovine rhodopsin.

A Meta-I-rich difference absorption spectra of the difference spectra of bovine rhodopsin WT (curve 1), F208A (curve 2), and F212A (curve 3). ***B*** Meta-I-rich difference spectra of bovine rhodopsin WT (curve 1), A269L (curve 2), F208A/A269L (curve 3) and F212A/A269L (curve 4). All the difference absorbances at the positive peaks were normalized to 1.0, and all spectra were obtained under the conditions stabilizing meta-I by using membrane preparations.

Fig. S5

Comparison of amino acid residues at positions 208 and 269 in various rhodopsins.

Phylogenetic relationships of rhodopsins are schematically represented. Note that Leu-269 is conserved among long wave length absorbing type Gq-coupled rhodopsins,

and these rhodopsins have small amino acids at position 208 (indicated by box in contrast).

Table S1

Opsin shifts of parapinopsin and amphioxus rhodopsin

	Parapinopsin					Amphioxus rhodopsin				
	dark state		photoproduct			dark state		photoproduct	
mutation	λmax (nm)	opsin shift*1 (cm^{-1})	λmax (nm)	opsin shift*1 (cm^{-1})	mutation	λmax (nm)	opsin shift*1 (cm^{-1})	λmax (nm)	opsin shift*1 (cm^{-1})
WT	363	0	493	0	WT	485	0	551	0
A118L	ND*3	ND*3	ND*3	ND*3	F118A	471	-610	549	-70
A118S	365	+150	498	+200	F118Y	486	+40	552	+100
G121A	364	+80	490	-120	A121L	486	+40	556	+160
G121S	369	+450	496	+120	A121S	479	-260	554	+100
I122A	368	+370	491	-80	L122A	480	-210	551	0
I122E	364	+80	491	-80	L122E	482	-130	552	+30
Y207A	ND*3	ND*3	ND*3	ND*3	V207A	485	0	548	-100
Y207F	363	0	489	-170	V207E	485	0	553	+70
C211A	364	+80	490	-120	V211A	487	+80	552	+30
C211S	364	+80	493	0	V211E	487	+80	552	+30
F212C	364	+80	491	-80	Y212A	483	-90	551	0
F212Y	365	+150	492	-40	Y212F	484	-40	551	0
F261A	364	+80	502	+360	F261A	482	-130	549	-70
F261Y	364	+80	492	-40	F261Y	491	+250	552	+30
W265L	ND*3	ND*3	ND*3	ND*3	W265L	464	-930	521	-1050
W265F	363	0	485	-330	W265F	468	-750	534	-580
Y268A	ND*3	ND*3	ND*3	ND*3	Y268A	481	-170	541	-340
Y268F	363	0	479	-590	Y268F	485	0	545	-200
A269L	363	0	471	-950	A269L	485	0	*2	*2
A269S	363	0	496	+120	A269S	488	+130	553	+70

*1 To clarify the data, plus and minus values represent red-shift and blue-shift values, respectively.

*2 Spectral properties of the photoproduct of the A269L mutant of amphioxus rhodopsin is quite different from that of WT (see Fig. S1B).

*3 These mutants were expressed to very low level and the λmax values could not be determined.

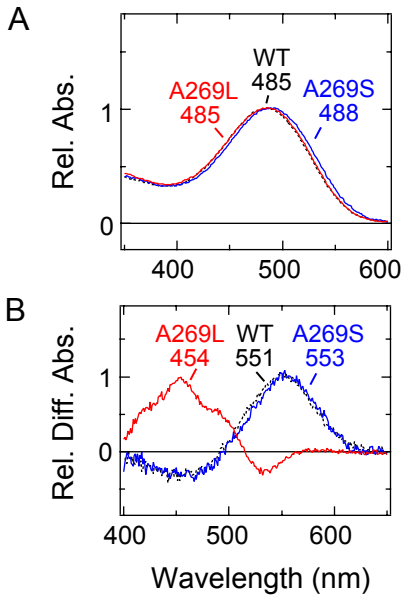

Fig. S1 Tsukamoto et al.

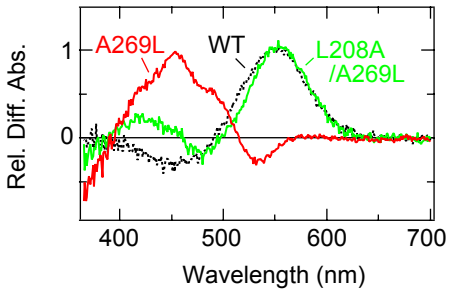

Fig. S2 Tsukamoto et al.

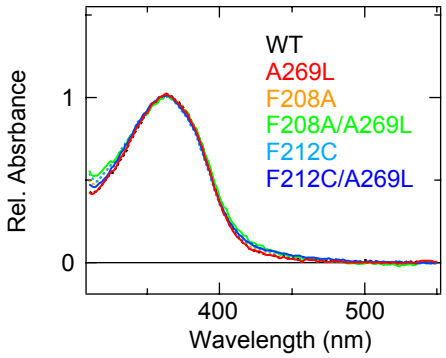

Fig. S3 Tsukamoto et al.

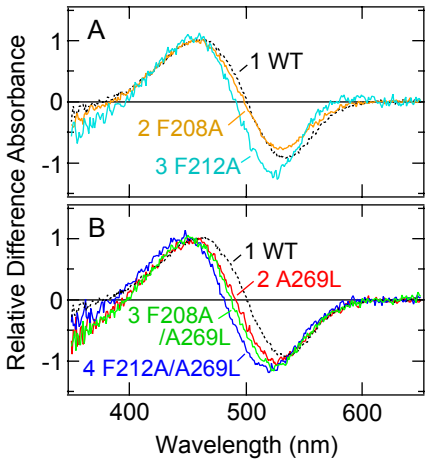

Fig. S4 Tsukamoto et al.

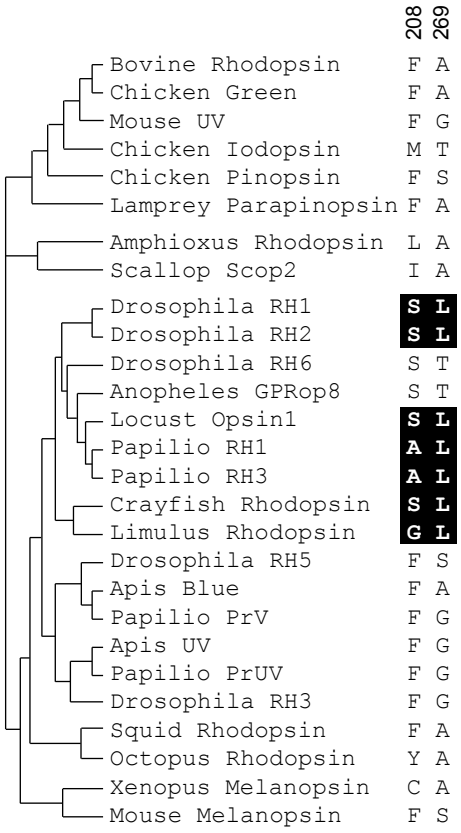

Fig. S5 Tsukamoto et al.