

Table S6. Substitutions between amino acids with different physical properties that were located in the transmembrane region. Retinal binding pocket sites are shaded grey. Sites that have been previously demonstrated to tune opsin spectral sensitivity and that vary among cichlids are marked at the bottom of the table. In addition, two sites that correspond with measured differences in cichlid sensitivity are highlighted. Numbers correspond to bovine rhodopsin. Abbreviations for the amino acid physical properties are as follows nonpolar hydrophobic (NPH), polar uncharged (PU), polar acidic (PA), and polar basic (PB). The top middle and bottom panels correspond to Malawi rock, Malawi sand, and Victoria groups.

	37	114	160	166	204	217	248	269	39	124	107	151	218	RH2Ab	RH2Aa		LWS	217	261	262	42	158	163	Rh1	166	213	298	299			
<i>Aulonocara huaseri</i>	Y	S	T	G	T	S	K	A	A	S	A	T	V	T	S/A	T/A	A	A	Y	C	A	A	A	S	T	A	A				
<i>Aulonocara baenschi</i>	F	G	A	G	I	S	K	A	A	S	S	T	T/I	A	S	T	A	A	Y	A	F/Y	C	A	A	A	S	T	A	A		
<i>Cynotilapia afra</i>	F	S	T	G	T	S	K	A	A	S	S	A	V	A	S/A	T	A	A	Y	A	F	C	A	A	A	S	T	A	A		
<i>Labeotropheus fuelleborni</i>	F	S	T	G	T	S	K	A	A	S	S	A	I	A	A	A	A	A	Y	A	Y	C	A	A	A	S	T	A	A		
<i>Labidochromis chisumulae</i>	F/Y	S	T	G	T	S	K	A	A	S	P	A	I	A	A	A	A	A	Y	A	Y	C	A	A	A	S	T	A	A		
<i>Melanochromis auratus</i>	Y	S	T	G	T	F	K	A	A	S	S	A	T	A	A	A	A	A	S	Y	A	Y	C	A	A	A	S	T	A	A	
<i>Melanochromis vittimaculatus</i>	F	S	T	G	T	S	K	A	A	S	S	A	V	A	A	A	A	A	S	Y	A	Y	C	A	A	A	A	T	S	S	
<i>Metriaclima zebra</i>	F	S ¹	T	G	T	S	K	A	A	T	S	S	A	I	A	A	A	A	Y	A	Y	C	A	A	A	S	T	A	A		
<i>Pseudotropheus acei</i>	F	A ²	A	A	I	S	K	A	T	S	S	A	V/I	A	A	A	A	S	Y	A	Y	C	A	A	A	A	T	S	S		
<i>Copadichromis borleyi</i>	Y	S	T	G	T	S	K	A	A	S	T	I		A	A	A	A	Y	A	Y	C	A	A	A	A	A	I	S	S		
<i>Dimidiochromis compressiceps</i>	F	S	T	G	T	S	K	T	A	S	S	A	I	A	A	A	A	Y	A	Y	C ³	A	A	A	A	A	I	S	S		
<i>Lethrinops parvidens</i>	F	S/T	T/A	G	T/I	S	K	A	A	A	P	A	I	A	S	A	A	S	Y	A	Y	C	A	A	A	A	A	I	S	S	
<i>Mylochromis lateristriga</i>	F	S	T	G	T	S	K	A	A	A	S	T	I	A	A	A	A	A	Y	A	Y	C	A	A	A	A	A	T	S	S	
<i>Stigmatochromis modestus</i>	F	S	T	G	T	S	E	A	A	A	A	T	T	A	S	T	A	A	Y	A	Y	C	A	A	A	A	A	T	A	A	
<i>Tramitichromis intermedius</i>	F	S	T	G	T	S	K	T	A	S	P	A	I	A	A	A	A	A	Y	A	Y	C ³	A	A	A	A	S	T	A	A	
<i>Tyrranochromis maculatus</i>	F	S	T	G	T	S	K	A	A	A	S	T	I	A	A	A	A	Y	A	Y	C	A	A	A	A	I	A	A			
<i>Lipochromis melanopterus</i>	F	A	A	G	I	S	K	T	A	S	P	A	I	A	A	A	A	A	Y	A	Y	C	A	A/G	A	S	?	A	A		
<i>Neochromis greenwoodi</i>	F	A	A	G	I	S	K	T	A	S	P	A	I	A	A	A	A	S	Y	A	Y	I	C	C	G	G	S	L	A	A	
<i>Neochromis omnicaeruleus</i>	F	A	A	S	I	S	K	T	A	S	P	A	I	A	A	A	A	A	Y	A	Y	C	C	C	G	G	S	L	A	A	
<i>Paralabidochromis chilotes</i>	F	A	A	G	I	S	K	A	A	S	P	A	I	A	A	A	A	A	F	T	Y	I	C	C	G	G	S	L	A	A	
<i>Paralabidochromis cyaneus</i>	F	A	A	G	I	S	K	A	A	S	P	A	I	A	A	A	A	G	S	Y	A	Y	C	A	A	A	A	S	L	A	A
<i>Pundamilia azurea</i>	F	A	A	S	I	S	K	T	A	S	P	A	I	A	A	A	A	A	Y	T	Y	I ⁴	C	C	G	G	S	L	A	A	
<i>Pundamilia luanso</i>	F	A	A	S	I	S	K	T	A	S	P	A	I	A	A	A	A	A	F	T	Y	I ⁴	C	C	G	G	S	L	A	A	
<i>Pundamilia nyererei</i>	F	A	A	G	I	S	K	T	A	S	P	A	I	A	A	A	A	A	Y	A	Y	C ³	C	C	G	G	S	L	A	A	
<i>Pundamilia nyererei</i>																						C	C	G	G	S	L	A	A		
<i>Pundamilia nyererei</i>																						C	G	G	S	L	A	A			
<i>Pundamilia pundamilia</i>	F	A	A	G	I	S	K	T	A	S	P	A	I	A	A	A	A	A	F	T	Y	I ⁴	C	C	G	G	S	L	A	A	
<i>Pundamilia redhead</i>	F	A	A	G	I	S	K	T	A	S	P	A	I	A	A	A	A	Y	A	Y	C ³	A	A	A	A	S	L	A	A		

A-G=10 nm⁵

T-A=11 nm³

S-A=7 nm⁷

F-Y=10 nm⁷

¹*M. zebra* SWS1 has a λmax of 368 nm (Carleton et al. 2000. Vision Res 40: 879-890).

²*P. acei* SWS1 has a λmax 378 nm (Parry et al. 2005. Curr Biol 15: 1-6).

³These taxa have LWS λmax values that range from 567-569 nm (Carleton et al. 2005. Mol Ecol 14: 4341-4353; Parry et al. 2005. Curr Biol 15: 1-6).

⁴These taxa have LWS λmax values that range from 562-565 nm (Carleton et al. 2005. Mol Ecol 14: 4341-4353; Parry et al. 2005. Curr Biol 15: 1-6).

⁵Shi et al. 2001. Proc Natl Acad Sci USA 98: 11731-11736.

⁶Cowing et al. 2002. Biochemistry 41: 6019-6025.

⁷Asenjo et al. 1994. Neuron 12: 1131-1138.

F-NPH	S-PU	T-PU	GS-PU	T-PU	S-PU	K-PB	A-NPH	S-PU	A-NPH	V-I-NPH	T-PU	A-NPH	Y-PU	I-NPH	A-NPH	Y-PU	I-NPH	F-NPH	C-PU	A-NPH	A-NPH	S-PU	T-PU	A-NPH	A-NPH	
Y-PU	A-NPH	A-NPH	A-NPH	I-NPH	F-NPH	TM5	E-PA	T-PU	A-NPH	AP-NPH	T-PU	A-NPH	TM I	TM3	A-NPH	TM4	A-NPH	TM3	A-NPH	TM4	A-NPH	TM5	A-NPH	I,NPH	TM5	S-PU
TM 1	TM3	TM4				TM6	TM6	TM1	TM3		TM4	TM5	TM4	TM4	TM1	TM3	TM5	TM5	TM6	TM6	TM1	TM4	I,L-NPH	TM5	TM7	TM7