

Bov-Rho_prot	1 MNGTEGPNFYVPFSNKTGVVRSPEAPQYYLAEPWQF SMLAAYMFL LIM	50
Pmax-RTC_prot	1 - - - - - MSSPTDTAVGMFSA - - - - - WE FRCVGIAYF IFGSA	30
Air-RTC_prot	1 - - - - - MSSPTDTAVGMFSV - - - - - WE FRFVGIA YF VFGSA	30
Tpac-RTC_prot	1 - - - - - MFGNPAMTGLHQFTM - - - - - WE HYFTGSIYLVLGCV	31
Bov-Rho_prot	51 GFPINFLTLVTVQHKKLRTPLNYILLNLAVADLFMVFGGFTTLYTSLH	100
Pmax-RTC_prot	31 GVLANAFTILTMRRESPISSPRHILQLNMAIANL-LVCAPFPFSGLSSFR	79
Air-RTC_prot	31 GVLANAFTILTMRRESPIGSPRHILQLNMAIANL-LVCAPFPFSGLSSFK	79
Tpac-RTC_prot	32 VFSLCGMC IIFLARQSPKPRRKYAILIHVLITAM-AVN GGDPAHASSSIV	80
Bov-Rho_prot	101 GFYFGPTGCNL EGFFATLGGEIALWSL VVLA IERYVVVCKPMNSNRFGE	150
Pmax-RTC_prot	80 GKWLFGDLGCQLYGTESFLLGMAATTFIPVVCIEHYLVSCRKDFYDTISS	129
Air-RTC_prot	80 GQWLFGELGCQLYGTESFLLGMAATTFIPVVCIEHYLASCKKDFYDTISS	129
Tpac-RTC_prot	81 GRWL YGSVG CQLMGFWGF FGGMSHIWMLFAFAMERYMAVCHREFYQQMPS	130
Bov-Rho_prot	†	
Pmax-RTC_prot	151 NHAIMGVAFTWVMALACAAPP LGW SRYIP EGMQCSCGIDYYTPHEETNN	200
Air-RTC_prot	130 GTWWTVAML CWMYASLWA ILPLFGWNSYDV ESSG IACG INWL R - - KDSNH	177
Tpac-RTC_prot	130 STWWTVAML CWMYAALWA ILPLFGWNSYDV ESSG IACG INWL K - - KDASH	177
131 VYYSIIVGLMYTFGT FWATMP LLGWASYGL E VHGT SCTINY SV - - SDES Y	178	
Bov-Rho_prot	201 E SFIVI YM FVVH F IIP L I VIFFCYGQL - - VFTVKEAAAQQQESATTQKAE	247
Pmax-RTC_prot	178 MTYLQAMVI-TWLILFAMA FYGLYQSRVYWE SVQT KADPKPDT-KNWFT E	225
Air-RTC_prot	178 ITYMQAMVI-TYLILFVMAFYGLYQSRVYWE SVQVKADPKPDA-KNWFT E	225
Tpac-RTC_prot	179 QSYVFFLAI F SFIF PMVSGWYAISKA - - WSGLSAIPDAEKEKD DILSE	225
Bov-Rho_prot	‡	
Pmax-RTC_prot	248 KEVTRMVI IMVIAFLICWL PYAGVAFY - IFT HQGSDFGP IFMT IPAFFAK	296
Air-RTC_prot	226 RQQAWICLAFMG IMCVGFGPYA ILGAWAALT-DSTTVSTLAI II IPSLACK	274
Tpac-RTC_prot	226 RQQAWICLAFMG IMCIGFGPYA ILGAWAALT-DSTTVSTLAI II IPSLACK	274
226 EQLTALAGAFIL ISLISWSGF GYVAI YSAL THGGAQLSHLRGHVPP IMSK	275	
Bov-Rho_prot	297 T SAVYNPVIYIMMNQFRNCMVTTLCCGKNPLGDDEASTVSKTETSQVA	346
Pmax-RTC_prot	275 ASSSLYPIPYIVASDRFRAAYL - - - - - GYRVSEQEAK - - A	307
Air-RTC_prot	275 ASSSLYPLPYIVASDKFRAAYL - - - - - GYRVSEQEAK - - A	307
Tpac-RTC_prot	276 TGCALFPLLIFLLTAR - - - - - SLPKSDTK - - K	300
Bov-Rho_prot	347 PA	348
Pmax-RTC_prot	- -	
Air-RTC_prot	308 N -	308
Tpac-RTC_prot	301 P -	301

Fig S1. Protein alignment of scallop (Air-RTC, Pmax-RTC) and squid (Tpac-RTC) retinochrome with bovine rhodopsin (Bov-Rho). Alignment follows numbering system of Bov-Rho. Glutamate (E) counterion and the retinal-binding Lysine (K) in blue, indicated by † and ‡, respectively. Mutation sites in this study and their homologous positions in bovine and squid are shown in three boxes.

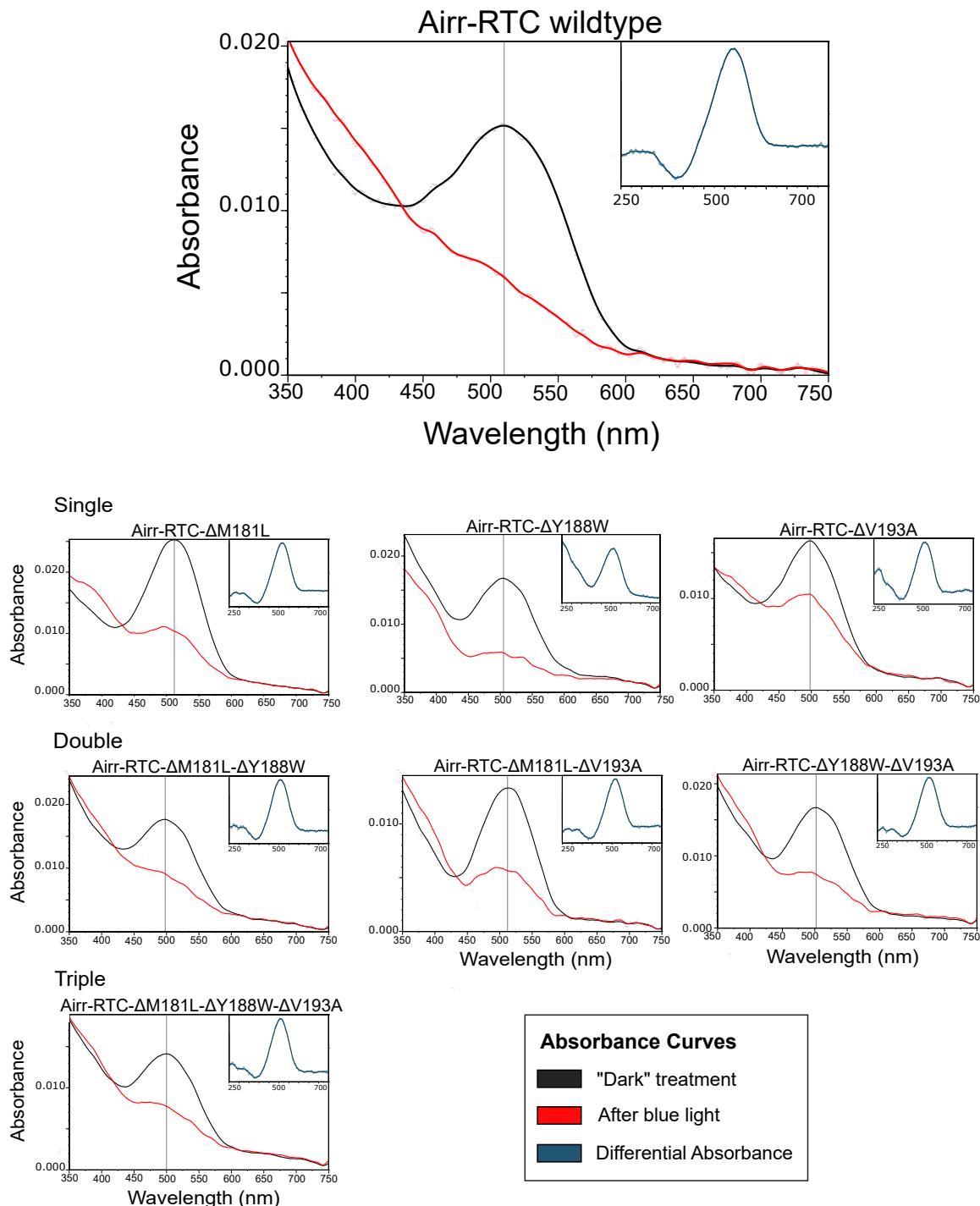


Fig S2. Unfitted spectral data of mutant *Argopecten irradians* retinochrome. Black curves plot of the average of five dark (unexposed) spectra and red curves show the average of five spectra after 3-minute exposure to blue light. Vertical black lines indicate maximum absorption peaks of unfitted data. Insets show the differential absorbance of the dark spectrum minus the spectrum recorded after irradiation with blue light.

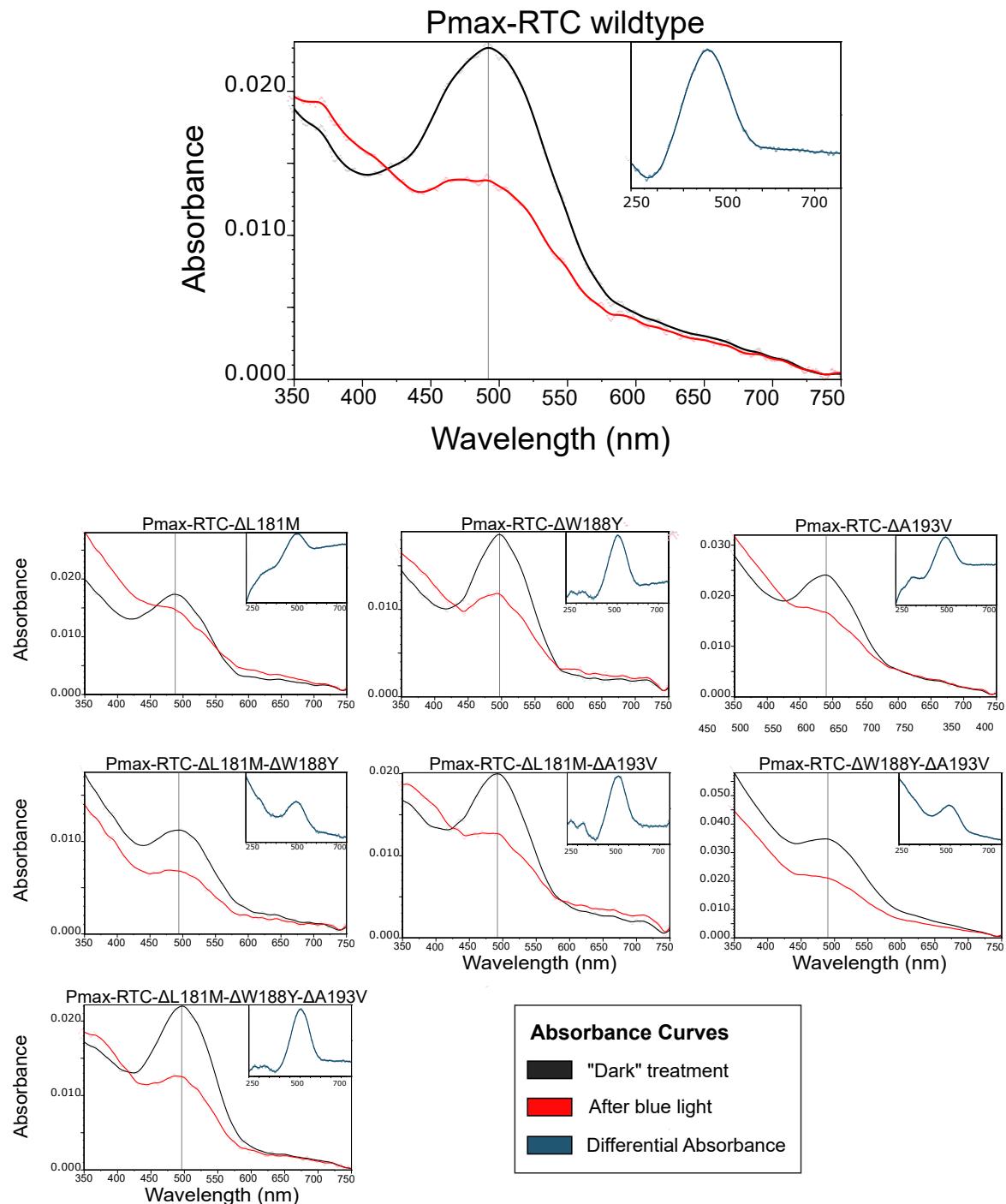


Fig S3. Unfitted spectral data of mutant *Pecten maximus* retinochrome. Black curves plot of the average of five dark (unexposed) spectra and red curves show the average of five spectra after 3-minute exposure to blue light. Vertical black lines indicate maximum absorption peaks of unfitted data. Insets show the differential absorbance of the dark spectrum minus the spectrum recorded after irradiation with blue light.

Table S1. Primer sequences and annealing temperatures used in cloning of wild-type retinochromes and the creation of the seven mutants.

Cloning Target	Sequence (5' to 3')	Annealing temp (°C)
<i>Airr-RTC</i> : Out-UTR_F Out-UTR_R In-UTR_F In-UTR_R BamHI site EcoR1 site	TGCATGGCAGTGGCTCGGAA ACGTCACTCGTTCTGTCTCAACA CACATTGATAGAATTGCTCTG CCTGACTGAAAATAGATAAAATCTCTG ATGCGGATCCCACCATGAGCTCCCTACAGATACCG GCATGAATTCTTGGCCTTGGCTTCCTGTC	Step up 49 – 54°C Step up 49 – 54°C Step up 49 – 54°C Step up 49 – 54°C 55°C 55°C
<i>Pmax-RTC</i> : Out-UTR_R In-UTR_F In-UTR_R BamHI site EcoR1 site	CCACGGACGCCGGGGTATTG GCACAGTGTAGATAGAGCTCGAGGG TGCCTGGCGGAGGACCTTC GCGGATCCCACCATGTCGTACCTACTGATAC GCATGAATTCTTGGCCTTGGCTTCCTGC	Step up 49 – 54°C Step up 49 – 54°C Step up 49 – 54°C 55°C 55°C
<i>Airr-RTC ΔM181L</i> : Forward Reverse	GAGTCACATTACGTACCTGCAGGCGATGGTAATC TAATGGTAGCGGACGTCCATGCATTACACTGAGC	60°C 60°C
<i>Airr-RTC ΔW188Y</i> : Forward Reverse	AGGCGATGGTAATCACGTGGCTCATTCTCTTGATGG GTACTGTTCTCTACTCGGTGCACTAATGGTAGCGGAC	60°C 60°C
<i>Airr-RTC ΔV193A</i> : Forward Reverse	GTACCTCATTCTCTTGCCATGGCGTTTACGGAC CAGGCATTGCGGTACCGTTCTTACTCCATG	60°C 60°C
<i>Airr-RTC ΔW188Y+V193A</i> : Forward Reverse	CACGTGGCTCATTCTCTTGCCATGGCGTTTAC GTAAAACGCCATGGCAAAGAGAATGAGCCACGTG	60°C 60°C
<i>Pmax-RTC ΔL181M</i> : Forward Reverse	AACCACATGACCTACATGCAGGCAATGGTAATCACGT CGTGATTACCATTGCCTGCATGTAGGTATGTGGTTC	60°C 60°C
<i>Pmax-RTC ΔY188W</i> : Forward Reverse	GCAGGCAATGGTAATCACGTACCTCATTCTCTTGCC GCAAAGAGAATGAGGTACGTGATTACCATTGCCTGCA	60°C 60°C
<i>Pmax-RTC ΔA193V</i> : Forward Reverse	GTCCGTAGAAAGCCATGACAAAGAGAATGAGCCAC GTGGCTCATTCTCTTGTCATGGCTTCTACGGAC	60°C 60°C
<i>Pmax-RTC ΔY188W+A193V</i> : Forward Reverse	CACGTACCTCATTCTCTTGTCATGGCTTCTACGGACT GTCCGTAGAAAGCCATGACAAAGAGAATGAGGTACGTGA	60°C 60°C