Supplementary Figures and Tables

Also see accompanying files: Figure S5.pdf, Table S2.xlsx, File S1.txt, File S2.m

Table S1 Yeast strains used in this study

Name	Genotype	Reference
yJW1200	S288C MATα his3Δ leu2Δ met15Δ ura3Δ LYS+ Δcan1::STE2pr-spHIS5 Δlyp1::STE3pr-LEU2 cyh2 Δura3::UPRE-GFP-TEF2pr-RFP-MET15-URA3	(Jonikas *et al.* 2009) Identical to yMJ003 but provided to us as "yJW1200"
CB008	W303 MATa, far1Δ, his3, trp1, leu2, ura3	(Bashor *et al.* 2008)
BS004	CB008 mfa2::KanMX-pFUS1-mCherry	This study
BS010	BS004 sst2::HygB	This study
BS011	BS010 ste2::TRP1	This study
BS017	BS011 gpa1::Gpa1-Gα_t(DCGLF)-LEU2	This study

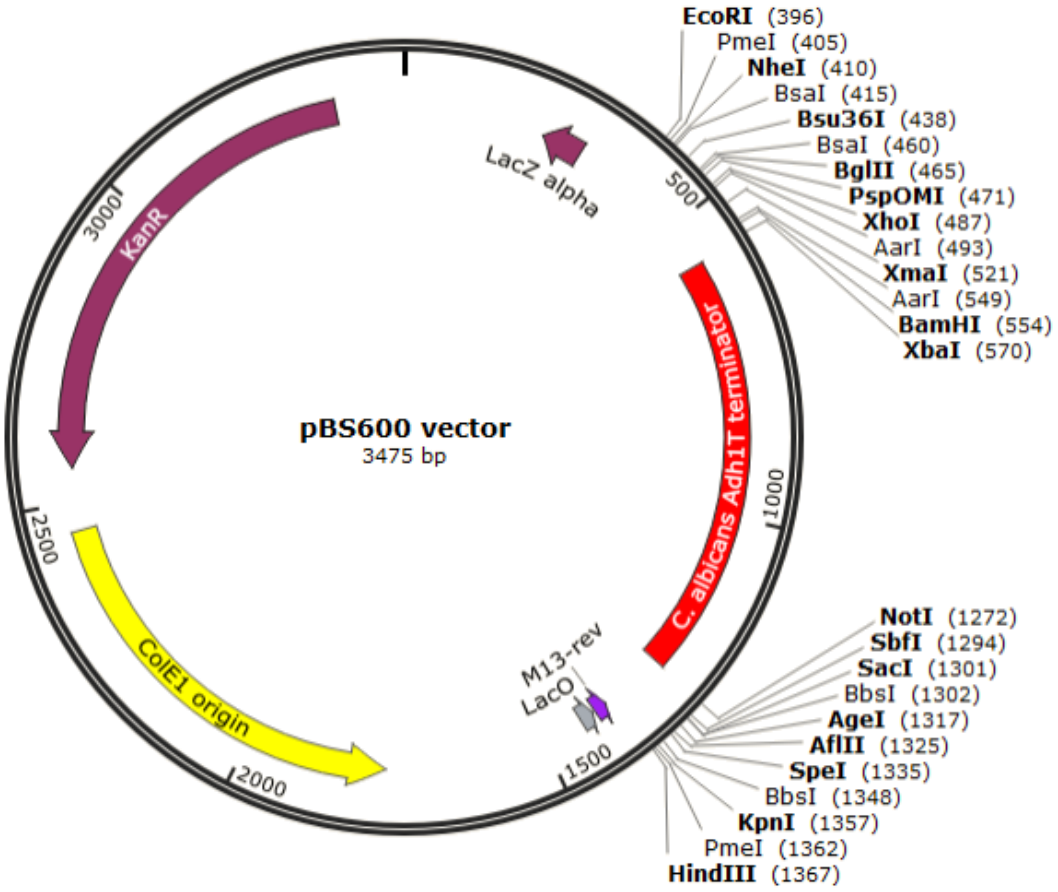

Figure S1 Plasmid Map of pBS600. Custom DNA insert synthesized by GenScript, inserted at EcoRI and HindIII sites in pUC57-Kan. Additional cloning steps not shown on plasmid map: Gpa1-$G\alpha_t$ gene inserted at AarI sites, LEU2 selection marker inserted at NotI/SacI sites, region homologous to the 800 bp 3' to natural Gpa1 gene inserted at SacI/KpnI sites. Cassette was linearized by digest with PmeI, then transformed into strain BS011 to generate strain BS017.

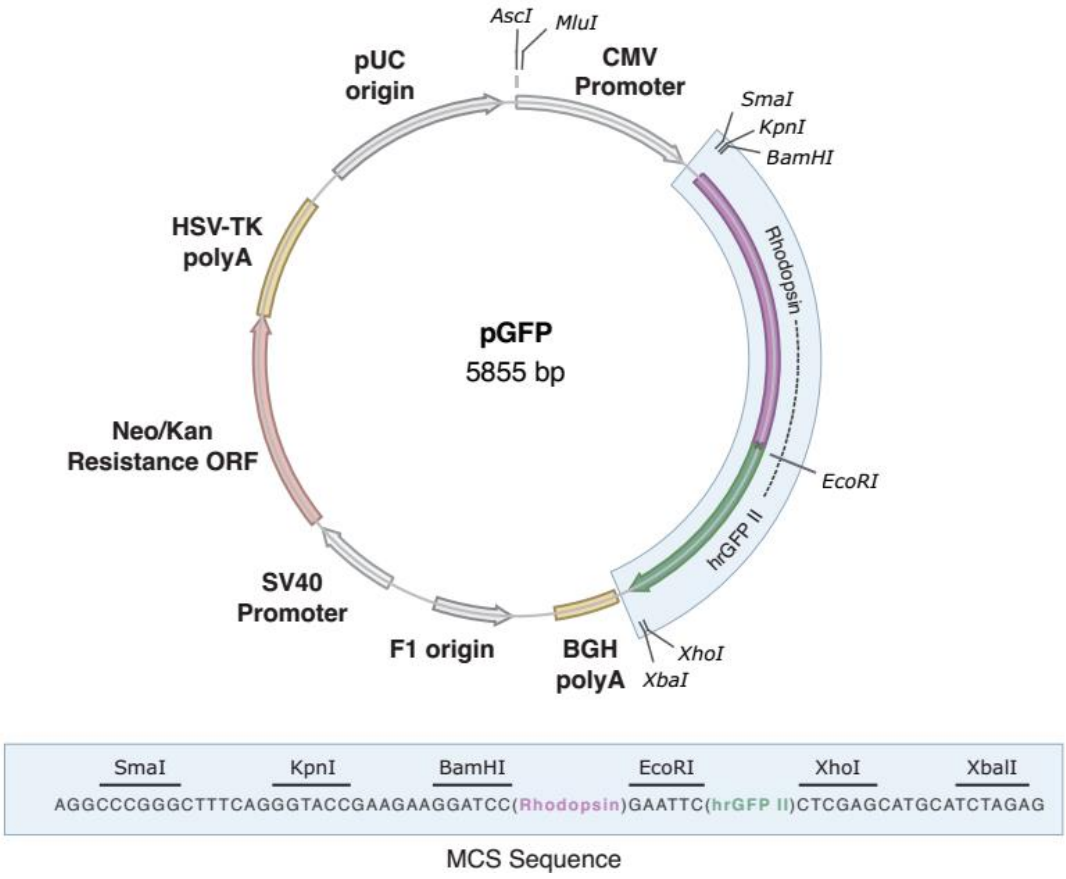

Figure S2 Map of the pGFP Expression Vector. Derived from p1D4-hrGFP II. Important features include the CMV promoter, and the C-terminal hrGFPII tag. Rhodopsin genes, or other genes of interest, can be ligated into the multiple cloning site between the unique BamHI and EcoRI restriction sites.

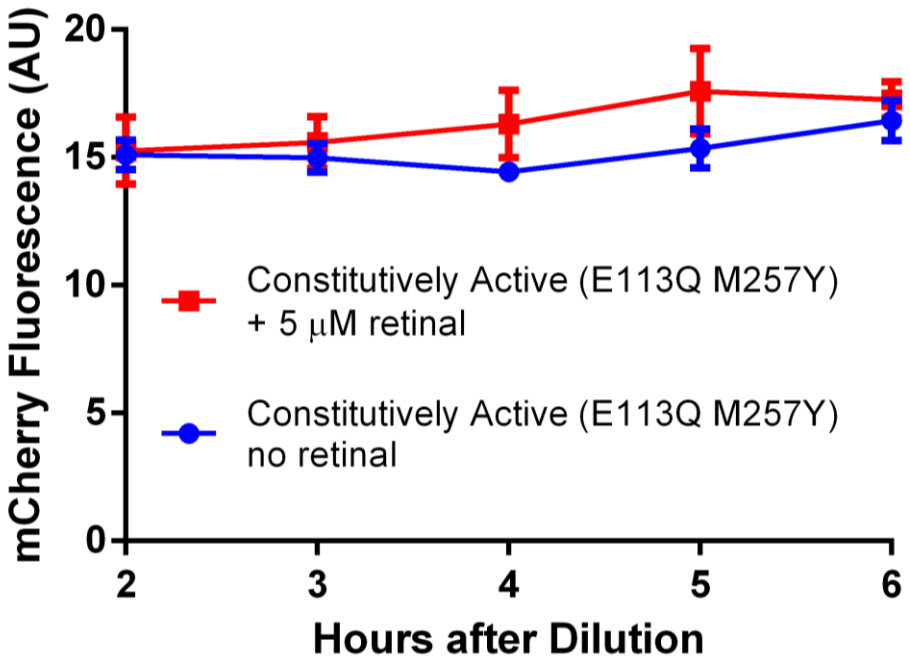

Figure S3 Functional Coupling of Constitutively Active Human Rhodopsin to the Mating Pathway. Constitutively active rhodopsin mutant E113Q M257Y resulted in high expression of the mCherry reporter protein, regardless of the presence of retinal, indicating productive functional coupling between human rhodopsin and the engineered mating pathway. Data points represent results of four individual colonies, each in a 5 mL culture. Error bars represent standard deviation.

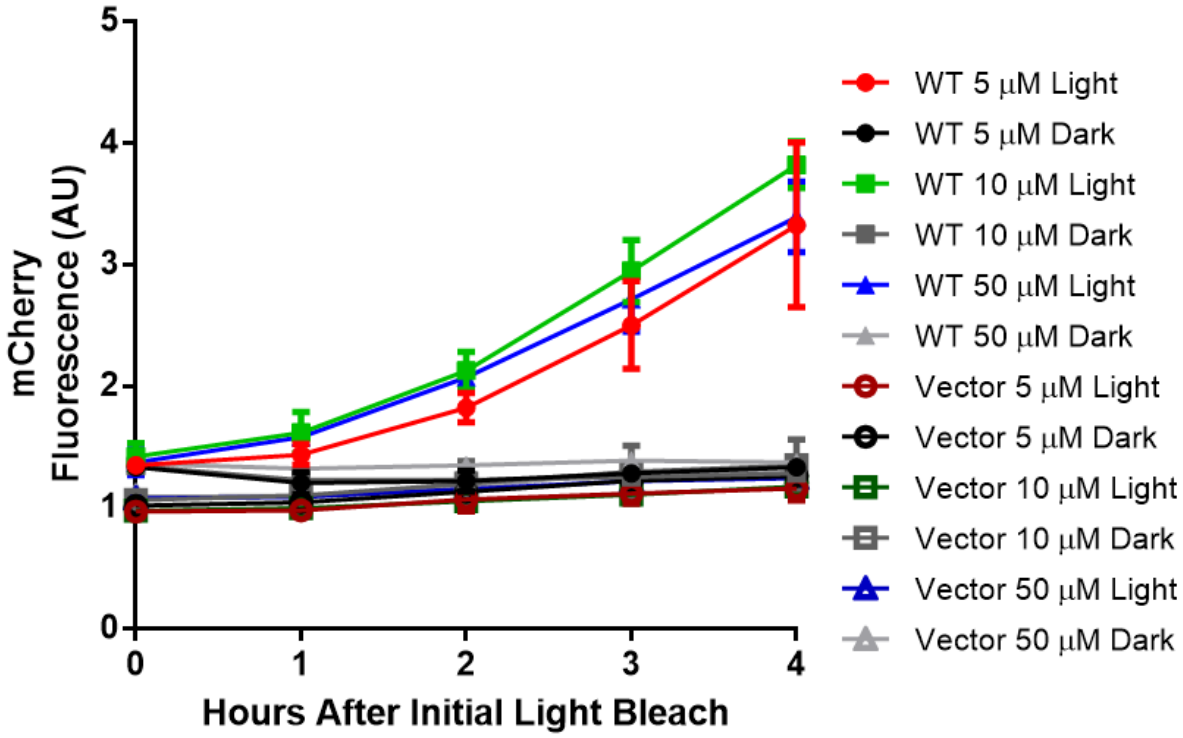

Figure S4 Various Retinal Concentrations' Effect on Light-Activated Signal Transduction. Modulating the retinal concentration did not improve the light-dependent activation of the mating pathway. Retinal was added only once, two hours prior to the first light exposure, followed by hourly light exposures. Incubating with the same concentration of retinal but keeping the culture in the dark did not result in mating pathway activation. "WT" denotes wild-type human rhodopsin. "Vector" denotes yeast transformed with a plasmid not containing the rhodopsin gene. Data points represent results of two individual colonies, each in a 5 mL culture. Error bars represent standard deviation.

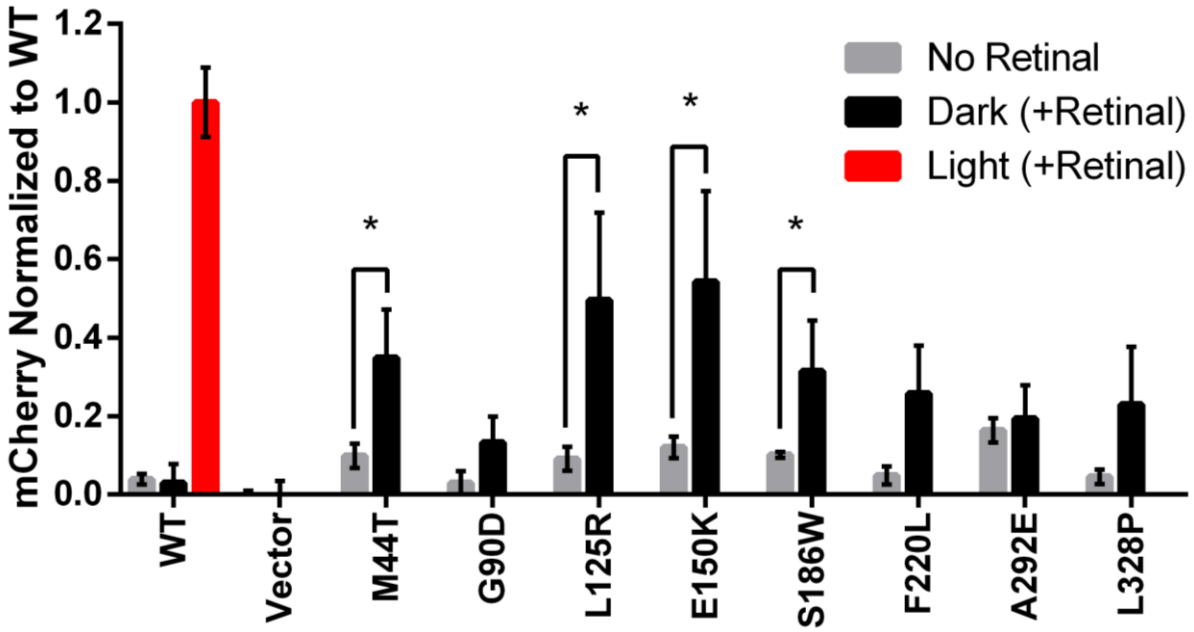

Figure S6 Signaling in Yeast in the Absence of Retinal for Rhodopsin Mutants with Increased Dark State Signaling. The indicated rhodopsin mutants were incubated without retinal, using an otherwise identical protocol to the "Yeast Light Activation Assay". "WT" denotes wild-type human rhodopsin. "Vector" denotes yeast transformed with a plasmid not containing the rhodopsin gene. Data points for "No Retinal" represent results of three individual colonies, each in a 5 mL culture, minus the mCherry fluorescence of Vector, and normalized to wild-type. Data for "Dark (+Retinal)" and "Light (+Retinal)" was reused from Figure 4. Error bars represent the 95% CI, * $P < 0.05$ for indicated comparison.

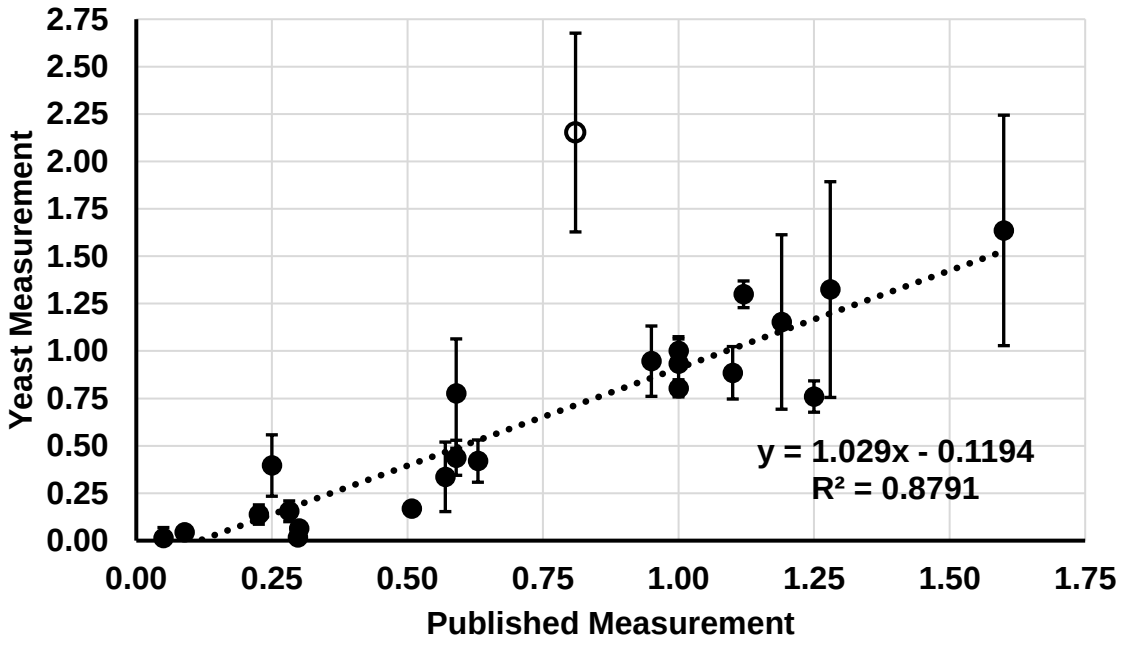

Figure S7 Comparison of Rhodopsin Activity in Yeast to Published Measurements of Rhodopsin Signaling. Yeast data points represent the mean response of nine measurements, normalized to wild-type rhodopsin. Error bars represent 95% CI of yeast measurement. Open circle (A292E) was found to be an outlier from the trend and was not included in the calculation of slope nor R^2. See Table S2 for complete data and references.

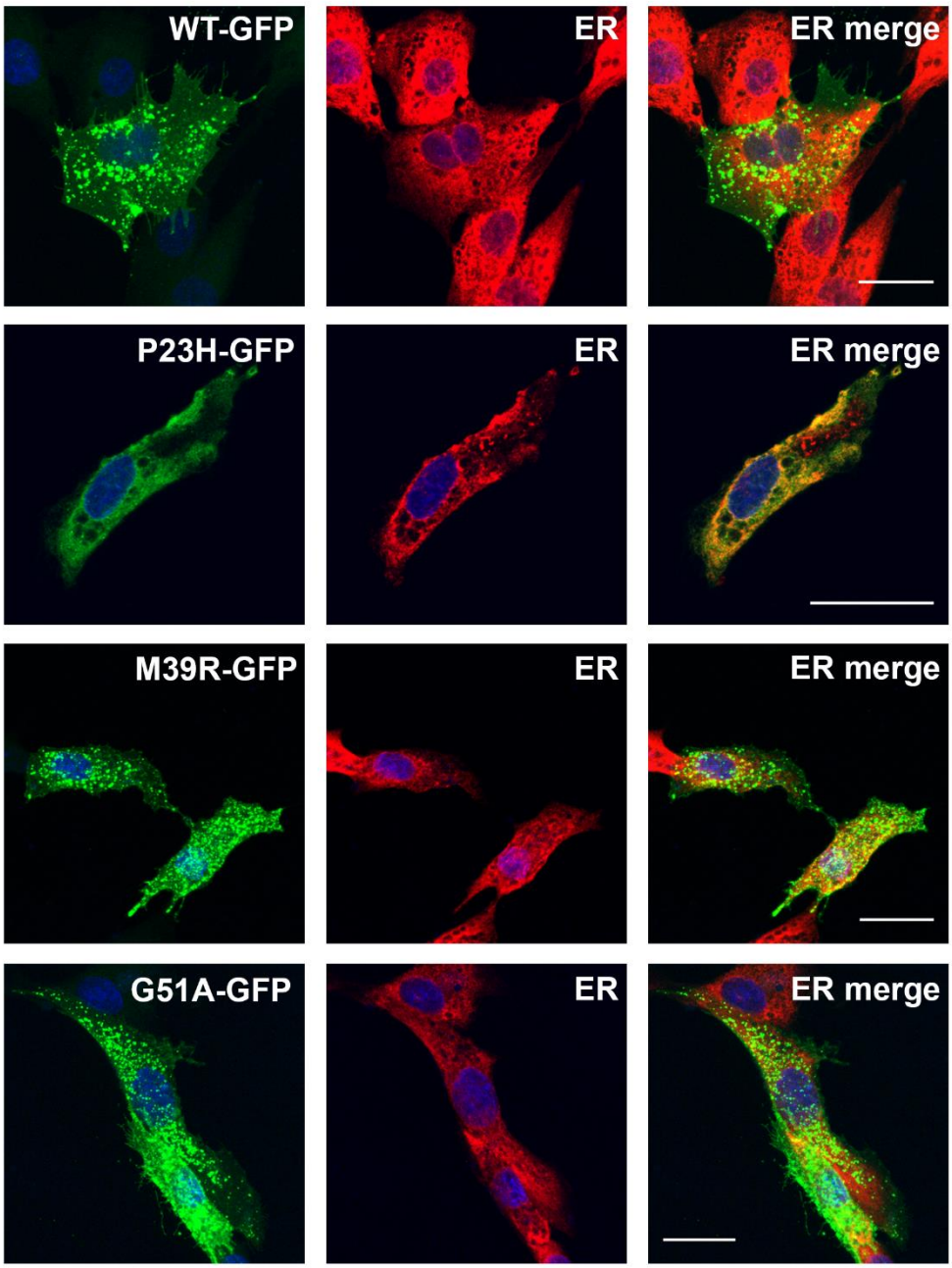

Figure S8 Representative Subcellular Localization of Rhodopsin Mutants. Comparative subcellular localization of rhodopsin mutants in SK-N-SH. “ER merge” indicates fluorescence of a ER specific marker, merged with GFP-tagged rhodopsin. Scale bars are 30 μm.

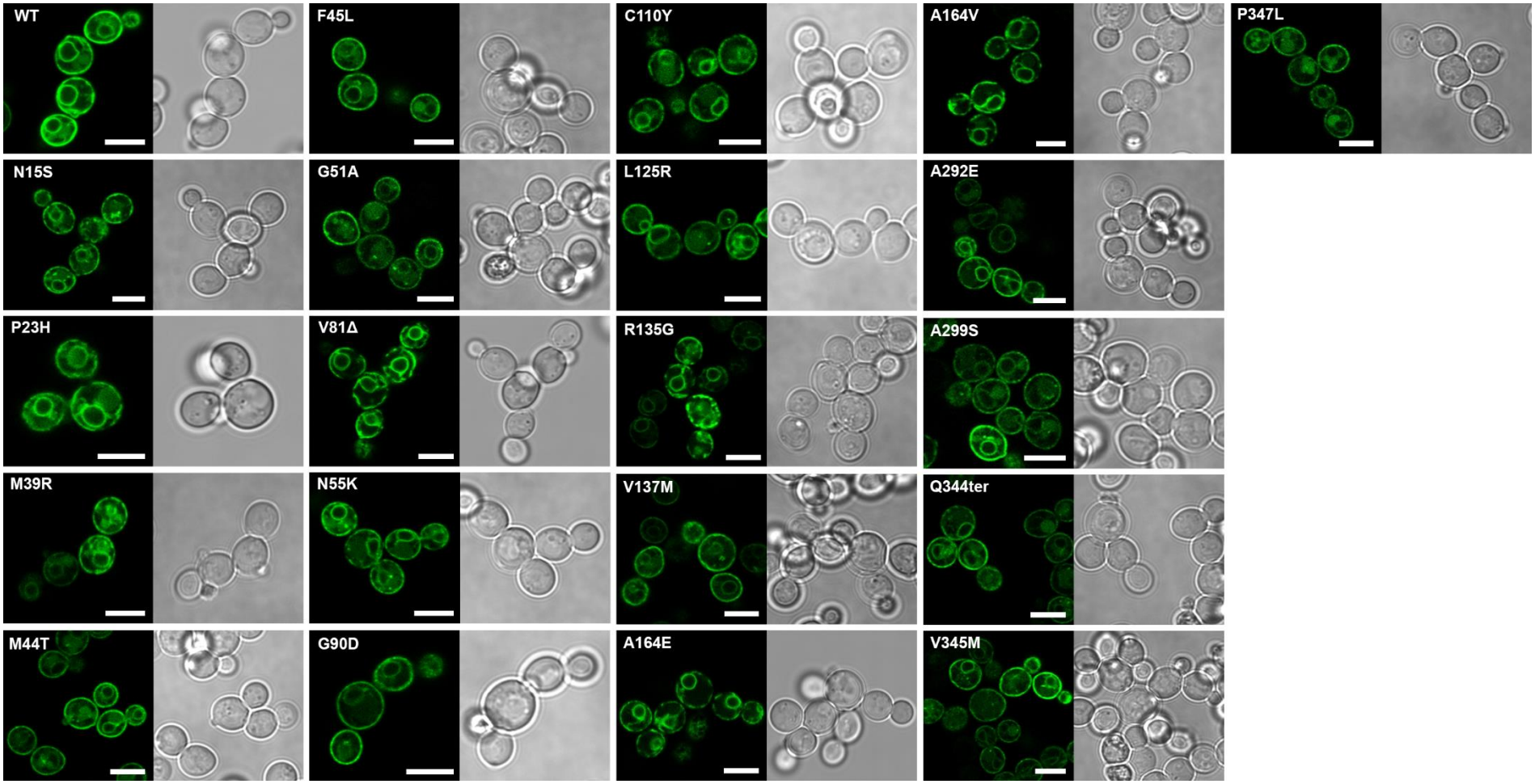

Figure S9 Representative Images of GFP-tagged Rhodopsin Expressed by Yeast. GFP was fused to the C-terminus of human rhodopsin, and expressed using the BS017 strain transformed with the appropriate pRS316 centromere plasmid containing the strong constitutive promoter pTDH3. Scale bars are 5 μm.

Table S3 Patient Phenotype Summary

	Case 1 (V81Δ)	Case 2 (A164V)	Case 3 (A164E)
Onset symptoms	10 yo	Teenage years	10 yo
VA (age)	20/60 (34) 20/80 (36) 20/50 (38)	20/25 (63) 20/40 (67)	20/20 (40) 20/25 (44) 20/30 (47) 20/30 (53)
Color vision	Mild defect	Normal	Normal
GVF (age)	10 degrees (34) 5 degrees (39)	Paracentral scotoma (60) denser paracentral scotoma (64)	Paracentral scotoma (47) annular Scotoma (53)
ERG amplitude (age)	rod: ND (26) Rod/cone: ⇓99% Cone: ⇓60%	Rod: LLNormal (63) Rod/cone: Mild decrease	rod: ND (45) Rod/cone: ⇓98% Cone: ⇓30%

Legend: Ref sequence NM_000539.3. VA: visual acuity, ND: response not detected, LL: lower limit.

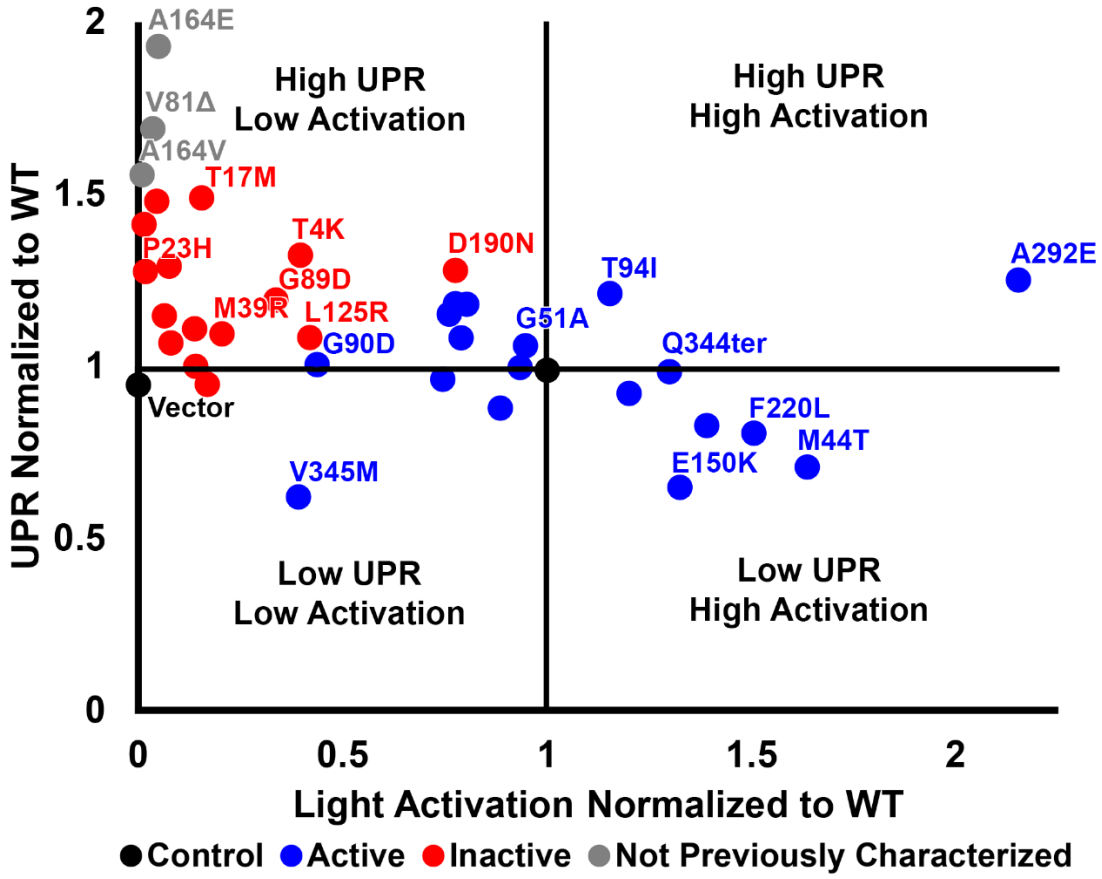

Figure S10 Combined Yeast Assays of UPR Upregulation versus Light Activation. "Inactive" refers to mutants in Figure 4 A, "Active" refers to mutants in Figure 4 B. Data points represent the mean of nine measurements for each of the UPR and light activation experiments.

Supplementary References

Andres, A., P. Garriga, and J. Manyosa, 2003. "Altered functionality in rhodopsin point mutants associated with retinitis pigmentosa." *Biochem Biophys Res Commun* 303 (1):294-301.

Athanasiou, D., M. Aguila, J. Bellingham, W. Li, C. McCulley, P. J. Reeves *et al.,* 2018. "The molecular and cellular basis of rhodopsin retinitis pigmentosa reveals potential strategies for therapy." *Prog Retin Eye Res* 62:1-23. doi: 10.1016/j.preteyeres.2017.10.002.

Bashor, C. J., N. C. Helman, S. Yan, and W. A. Lim, 2008. "Using engineered scaffold interactions to reshape MAP kinase pathway signaling dynamics." *Science* 319 (5869):1539-43. doi: 10.1126/science.1151153.

Bosch, L., E. Ramon, L. J. Del Valle, and P. Garriga, 2003. "Structural and functional role of helices I and II in rhodopsin. A novel interplay evidenced by mutations at Gly-51 and Gly-89 in the transmembrane domain." *J Biol Chem* 278 (22):20203-9. doi: 10.1074/jbc.M301319200.

Davies, W. I., S. M. Downes, J. K. Fu, M. E. Shanks, R. R. Copley *et al.,* 2012. "Next-generation sequencing in health-care delivery: lessons from the functional analysis of rhodopsin." *Genet Med* 14 (11):891-9. doi: 10.1038/gim.2012.73.

Garriga, P., X. Liu, and H. G. Khorana, 1996. "Structure and function in rhodopsin: correct folding and misfolding in point mutants at and in proximity to the site of the retinitis pigmentosa mutation Leu-125-->Arg in the transmembrane helix C." *Proc Natl Acad Sci U S A* 93 (10):4560-4.

Gross, A. K., V. R. Rao, and D. D. Oprian, 2003. "Characterization of rhodopsin congenital night blindness mutant T94I." *Biochemistry* 42 (7):2009-15. doi: 10.1021/bi020613j.

Hwa, J., P. J. Reeves, J. Klein-Seetharaman, F. Davidson, and H. G. Khorana. 1999. "Structure and function in rhodopsin: further elucidation of the role of the intradiscal cysteines, Cys-110, -185, and -187, in rhodopsin folding and function." *Proc Natl Acad Sci U S A* 96 (5):1932-5.

Jonikas, M. C., S. R. Collins, V. Denic, E. Oh, E. M. Quan *et al.,* 2009. "Comprehensive characterization of genes required for protein folding in the endoplasmic reticulum." *Science* 323 (5922):1693-7. doi: 10.1126/science.1167983.

Kaushal, S., and H. G. Khorana, 1994. "Structure and function in rhodopsin. 7. Point mutations associated with autosomal dominant retinitis pigmentosa." *Biochemistry* 33 (20):6121-8.

Liu, M. Y., J. Liu, D. Mehrotra, Y. Liu, Y. Guo *et al.,* 2013. "Thermal stability of rhodopsin and progression of retinitis pigmentosa: comparison of S186W and D190N rhodopsin mutants." *J Biol Chem* 288 (24):17698-712. doi: 10.1074/jbc.M112.397257.

Min, K. C., T. A. Zvyaga, A. M. Cypess, and T. P. Sakmar, 1993. "Characterization of mutant rhodopsins responsible for autosomal dominant retinitis pigmentosa. Mutations on the cytoplasmic surface affect transducin activation." *J Biol Chem* 268 (13):9400-4.

Opefi, C. A., K. South, C. A. Reynolds, S. O. Smith, and P. J. Reeves, 2013. "Retinitis Pigmentosa Mutants Provide Insight into the Role of the N-terminal Cap in Rhodopsin Folding, Structure, and Function." *J Biol Chem* 288 (47):33912-26. doi: 10.1074/jbc.M113.483032.

Ploier, B., L. N. Caro, T. Morizumi, K. Pandey, J. N. Pearring *et al.,* 2016. "Dimerization deficiency of enigmatic retinitis pigmentosa-linked rhodopsin mutants." *Nat Commun* 7:12832. doi: 10.1038/ncomms12832.

Ramon, E., A. Cordomi, M. Aguila, S. Srinivasan, X. Dong *et al.,* 2014. "Differential light-induced responses in sectorial inherited retinal degeneration." *J Biol Chem* 289 (52):35918-28. doi: 10.1074/jbc.M114.609958.

Rao, V. R., G. B. Cohen, and D. D. Oprian, 1994. "Rhodopsin mutation G90D and a molecular mechanism for congenital night blindness." *Nature* 367 (6464):639-42. doi: 10.1038/367639a0.

Rohatgi, A, 2017. "WebPlotDigitizer Version 4.0." Last Modified October, 2017. https://automeris.io/WebPlotDigitizer.

Sancho-Pelluz, J., J. Tosi, C. W. Hsu, F. Lee, K. Wolpert *et al.,* 2012. "Mice with a D190N mutation in the gene encoding rhodopsin: a model for human autosomal-dominant retinitis pigmentosa." *Mol Med* 18:549-55. doi: 10.2119/molmed.2011.00475.

Stojanovic, A., I. Hwang, H. G. Khorana, and J. Hwa, 2003. "Retinitis pigmentosa rhodopsin mutations L125R and A164V perturb critical interhelical interactions: new insights through compensatory mutations and crystal structure analysis." *J Biol Chem* 278 (40):39020-8. doi: 10.1074/jbc.M303625200.

Sung, C. H., C. Makino, D. Baylor, and J. Nathans, 1994. "A rhodopsin gene mutation responsible for autosomal dominant retinitis pigmentosa results in a protein that is defective in localization to the photoreceptor outer segment." *J Neurosci* 14 (10):5818-33.

Zhang, N., A. V. Kolesnikov, B. Jastrzebska, D. Mustafi, O. Sawada *et al.,* 2013. "Autosomal recessive retinitis pigmentosa E150K opsin mice exhibit photoreceptor disorganization." *J Clin Invest* 123 (1):121-37. doi: 10.1172/JCI66176.

Zvyaga, T. A., K. Fahmy, F. Siebert, and T. P. Sakmar, 1996. "Characterization of the mutant visual pigment responsible for congenital night blindness: a biochemical and Fourier-transform infrared spectroscopy study." *Biochemistry* 35 (23):7536-45. doi: 10.1021/bi960391n.