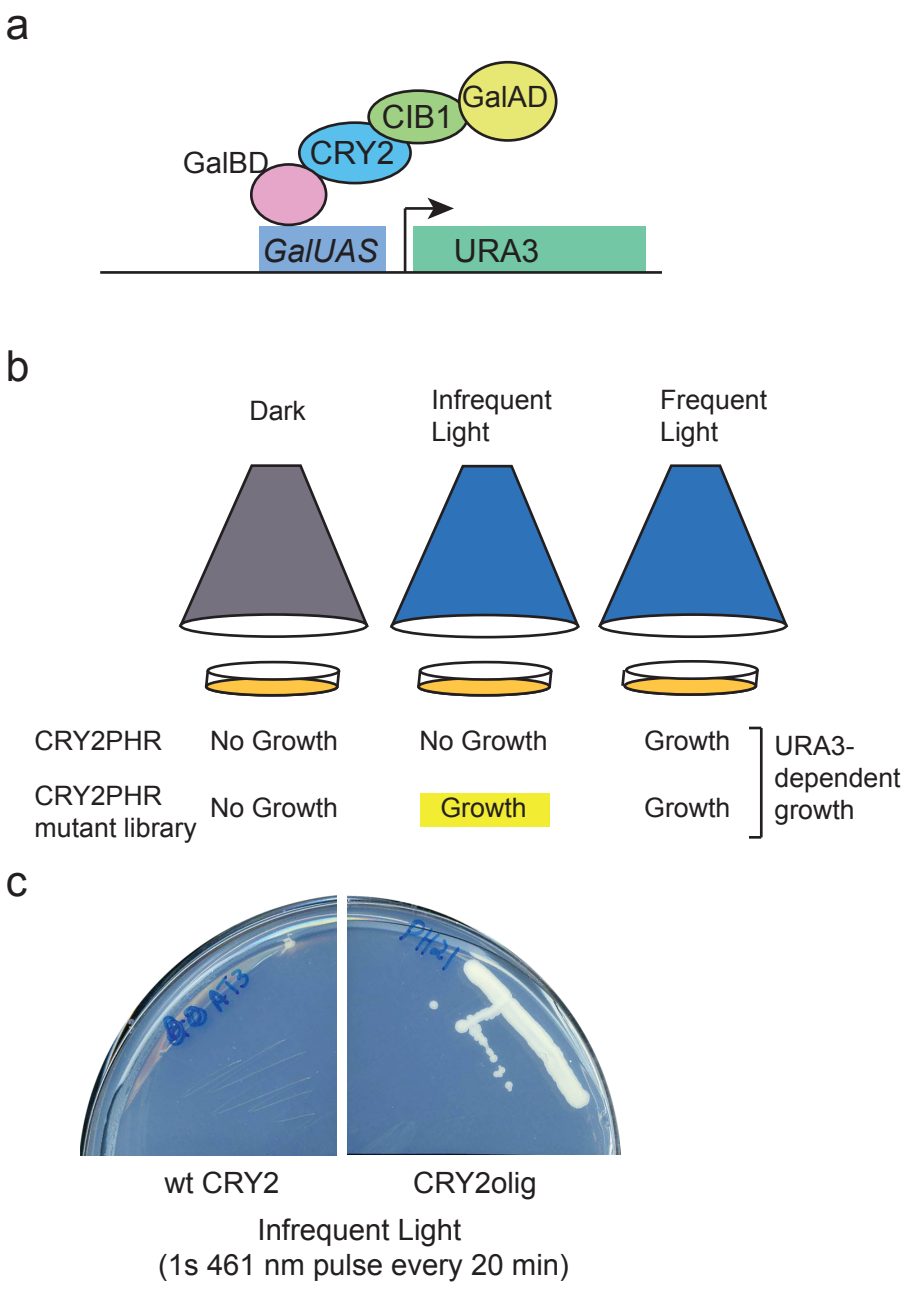
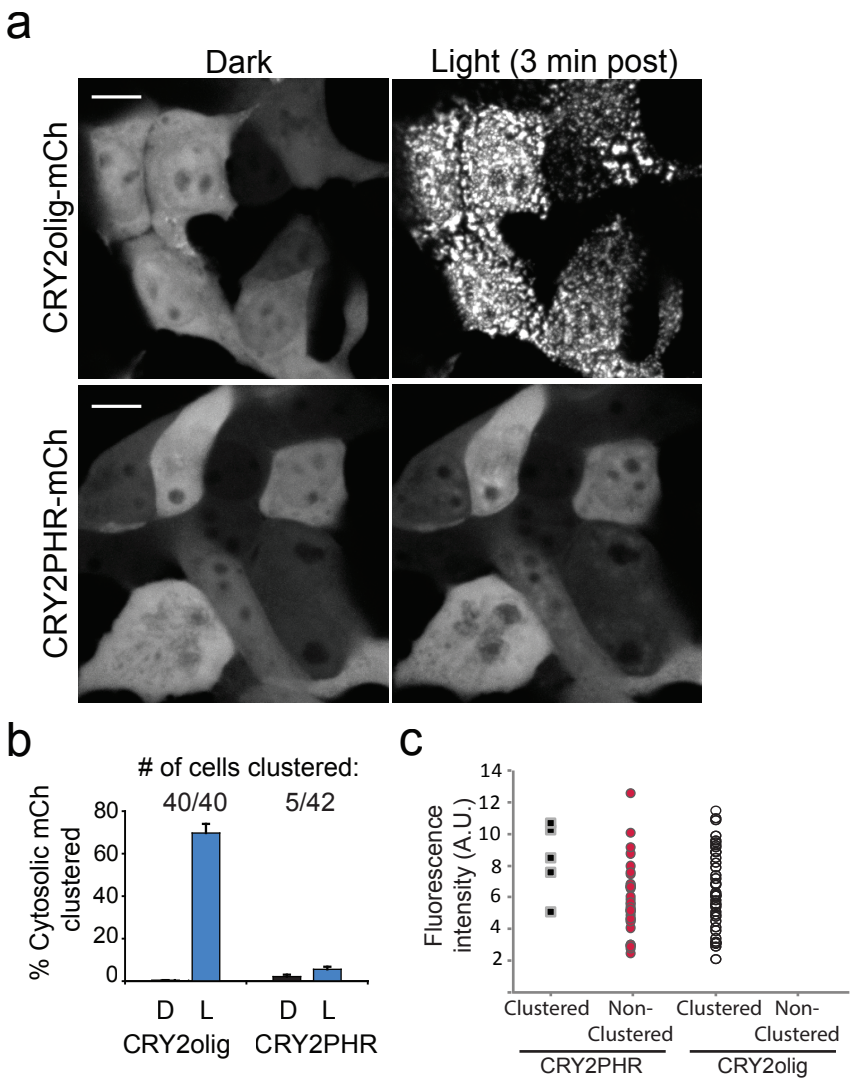
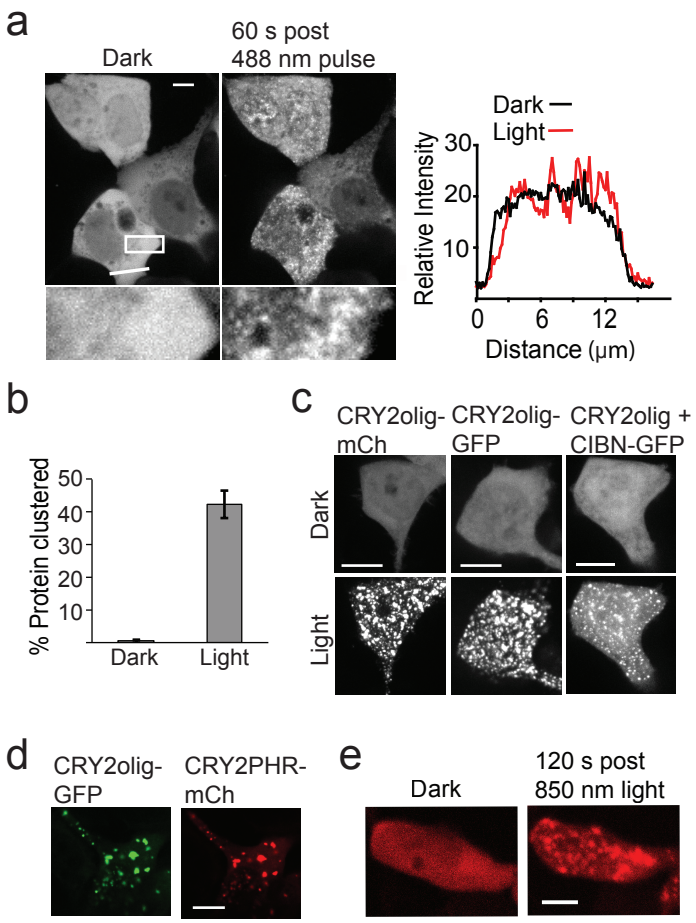


Supplementary Figure 1. CRY2PHR E490G shows prolonged interaction with CIB. (a) Schematic showing the *GalUAS*-HIS3 reporter and yeast two-hybrid activation domain and binding domain fusion constructs used to identify CRY2 signaling state mutants. **(b)** Schematic of selection screen to identify CRY2 signaling mutants, based on URA3-dependent growth of yeast expressing GalAD-CIB1 and a GalBD-CRY2 mutant library under infrequent light pulse conditions. **(c)**. Plates showing growth of yeast expressing GalAD-CIB1 and wild-type or E490G CRY2 Gal4BD constructs as in (b) subjected to infrequent light pulses (1 s pulse every 20 min, 461nm, 1.1 mW cm^{-2}) for 72 hours at 30 C°.

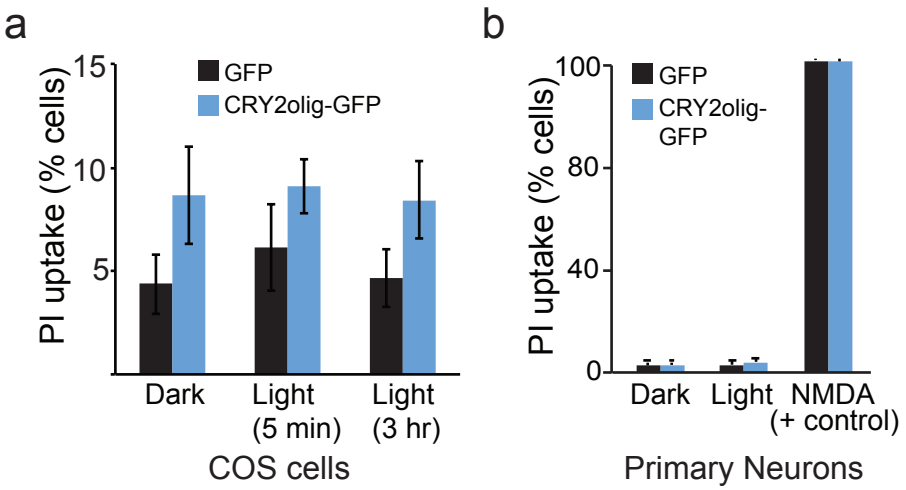


Supplementary Figure 2 Comparison of CRY2olig and CRY2PHR clustering.

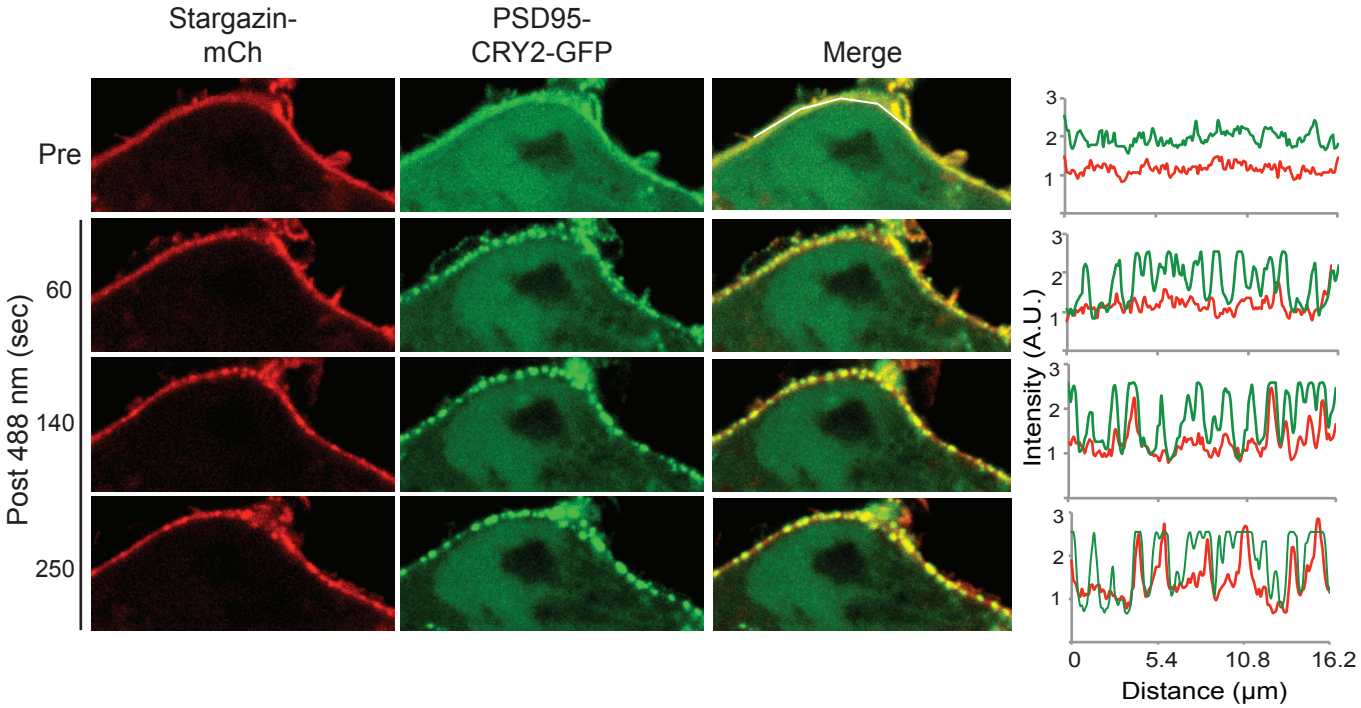
(a) HEK293 cells expressing CRY2olig-mCh or CRY2PHR-mCh before and after blue light exposure (25 ms pulse, 488 nm). Scale bar, 7.5 μm. **(b)** Graph showing percent cytosolic CRY2 distributed in clusters in cells exposed to dark vs light (1 min post) expressing CRY2olig-mCh or CRY2PHR-mCh. Results are shown only from cells that clustered with light (100% of CRY2olig-mCh expressing cells; 12% of CRY2PHR-mCh expressing cells). **(c)** Correlation of expression level (A.U., arbitrary fluorescence units) for cells expressing CRY2PHR-mCh or CRY2olig-mCh that clustered or did not cluster in light. Note that cells expressing CRY2olig-mCh clustered at all measurable expression levels.



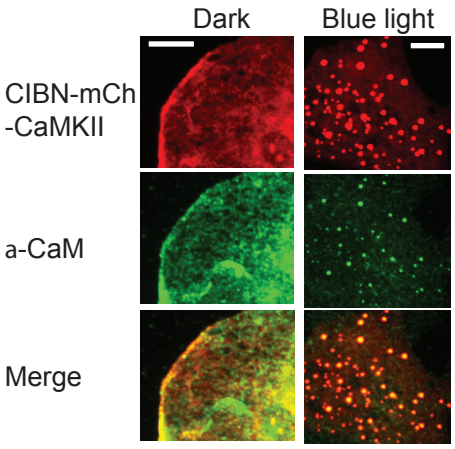
Supplementary Figure 3. Characterization of CRY2olig clustering properties. (a) CRY2oligFL clusters with light, but forms smaller clusters than CRY2olig. HEK293 cells expressing CRY2oligFL pre or post blue light (100 ms pulse, 488 nm). Graph at right shows the relative fluorescence intensity under the white line. Area indicated by square is shown at bottom. **(b)** Quantification of CRY2oligFL-mCh clustered in dark vs light in HEK293 cells. **(c)** HEK293 cells expressing indicated constructs before or after blue light. **(d)** When coexpressed with CRY2olig-GFP, CRY2PHR-mCh is recruited into CRY2olig-GFP clusters upon photostimulation. **(e)** Two-photon excitation of CRY2olig-mCh at 850 nm. All scale bars, 7.5 μm.



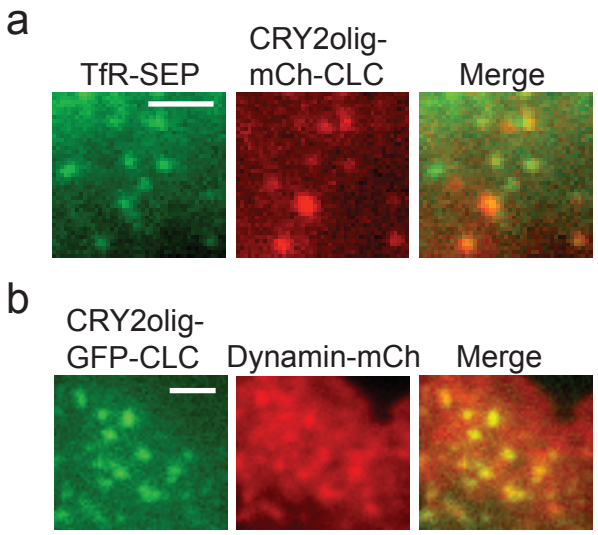
Supplementary Figure 4. Testing of CRY2olig toxicity. (a) COS-7 cells were treated with blue light (461 nm) for indicated times, then propidium iodide uptake was measured 24 hours after the start of light treatments (1 s pulse, 461 nm, every 1 min) to assay toxicity. Data represents average and standard deviation. **(b)** Primary neurons expressing CRY2olig-GFP show no toxicity compared to a GFP control. Data represents average and standard deviation. No significant difference between dark vs light treated CRY2olig-GFP or light treated GFP vs light treated CRY2olig-GFP was observed in either COS-7 cells or neurons ($p > 0.05$).



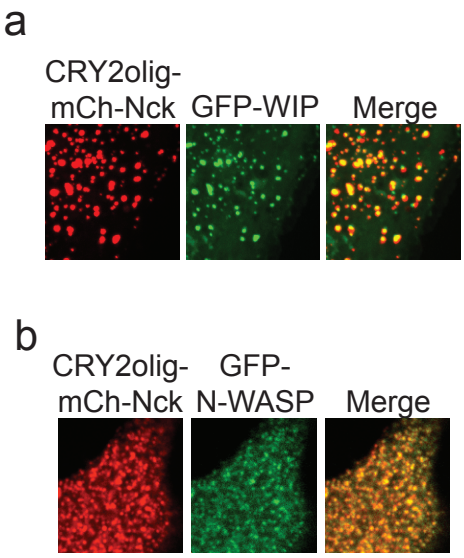
Supplementary Figure 5. LINC assay with membrane proteins. Interacting proteins stargazin (stargazin-mCh), a transmembrane protein, and palmitoylated PSD95-eGFP-CRY2olig co-cluster at the plasma membrane of COS-7 after light (100 ms pulse, 488 nm), where they move together in time-lapse frames. At right is a line scan of the area along the membrane indicated by the white line (indicated in merged photo at top right).



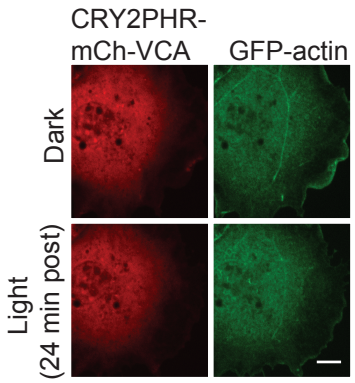
Supplementary Figure 6. LINC assay with endogenous proteins. LINC assay showing co-clustering of CaMKII and endogenous CaM visualized by immunofluorescence. COS-7 cells expressing CIBN-mCh-CaMKIIα and CRY2olig were treated with ionomycin for 1 min then exposed to blue light for 5 min (1 s pulse every 1 min, 461 nm, 1.1 mW cm^{-2}) or kept in the dark. Cells were fixed and probed with an α-CaM antibody. In the presence of light but not in dark, endogenous CaM co-clusters with CaMKII. Scale bars, 7.5 μm.



Supplementary Figure 7. Colocalization of CRY2olig-CLC with endocytic markers. (a) TIRF imaging showing colocalization of CRY2olig-mCh-CLC puncta at the plasma membrane with a pH sensitive superecliptic phlorin-transferin receptor fusion (TfR-SEP). **(b)** Colocalization of CRY2olig-GFP-CLC with dynamin-mCh. All scale bars, 2 μm.



Supplementary Figure 8. Co-clustering of CRY2olig-mCh-Nck and actin-associated proteins. Shown are images of COS-7 cells expressing CRY2olig-mCh-Nck and WIP-GFP (a) or N-WASP-GFP (b) after blue light-induced clustering of CRY2olig-mCh-Nck.



Supplementary Figure 9. Clustering of VCA requires use of CRY2olig.

COS-7 cells expressing CRY2PHR-mCh-VCA, CRY2PHR-mCherry, and GFP-actin in dark or 6 min post blue light (500 ms pulse, 488 nm, every 3 min). No change is observed in cells expressing wild-type CRY2PHR-mCh-VCA fusions with light.