Supplemental Figures:

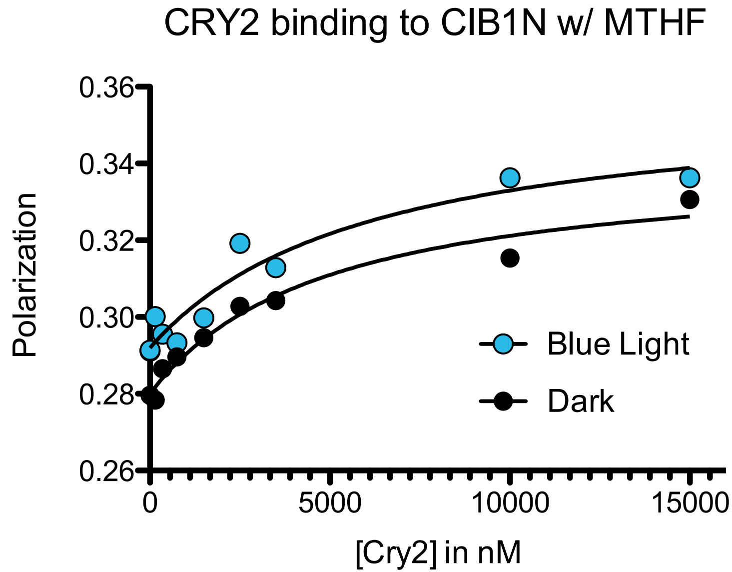

Figure S1: Addition of MTHF does not significantly change CRY2-CIB1N binding. Direct fluorescence polarization binding experiment of CRY2 to CIB1N with the addition of MTHF, a possible CRY2 cofactor.

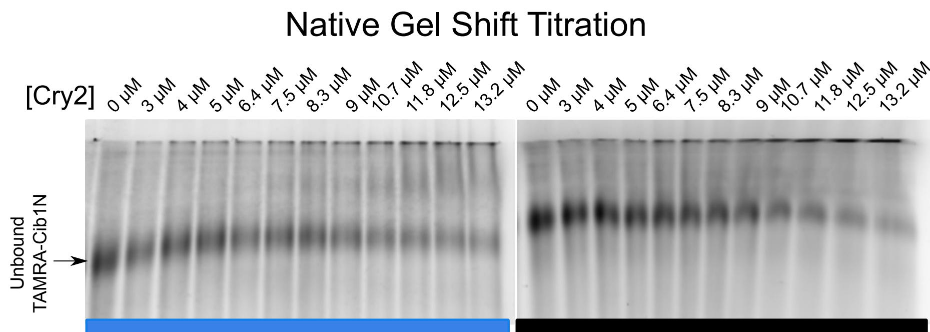

Figure S2: Binding measured by native gel shift indicates weak binding. Titration of TAMRA labeled CIB1N with CRY2 at indicated concentration. Samples were prepared identically and run on two native-PAGE gels, one in the presence of blue light and one in darkness. Binding is indicated by shift of TAMRA-CIB1N to higher molecular weight species. Binding affinity does not appear to change drastically with addition of blue light.

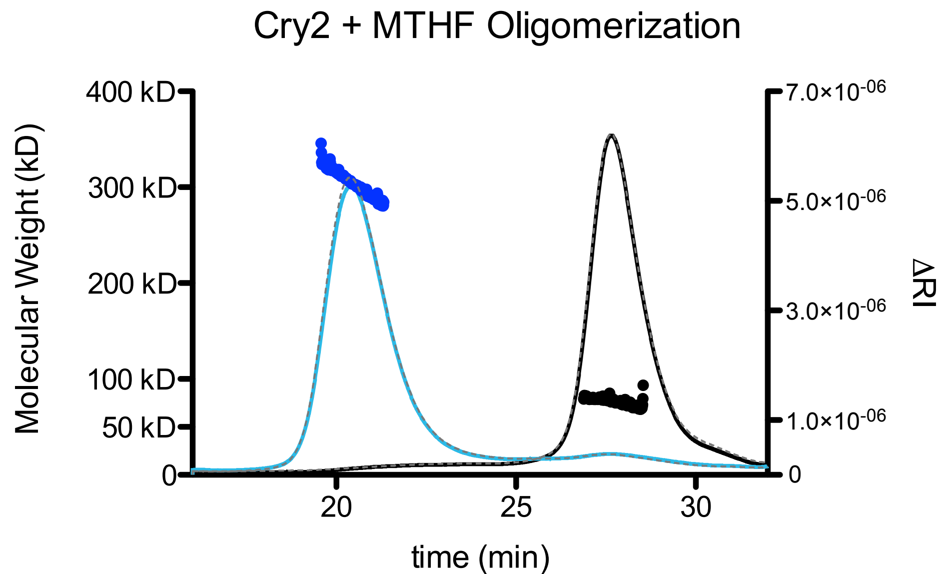

Figure S3: Addition of MTHF does not change CRY2 oligomerization. Size exclusion chromatography multi-angle light scattering traces for full length CRY2 with saturating MTHF cofactor added run under blue light (blue line) or darkness (black line). Fit molecular weight from MALS data for each peak is shown for lit (blue dots) and dark (black dots) peaks. Elution traces from Figure 3 are shown in grey dashed lines for comparison.

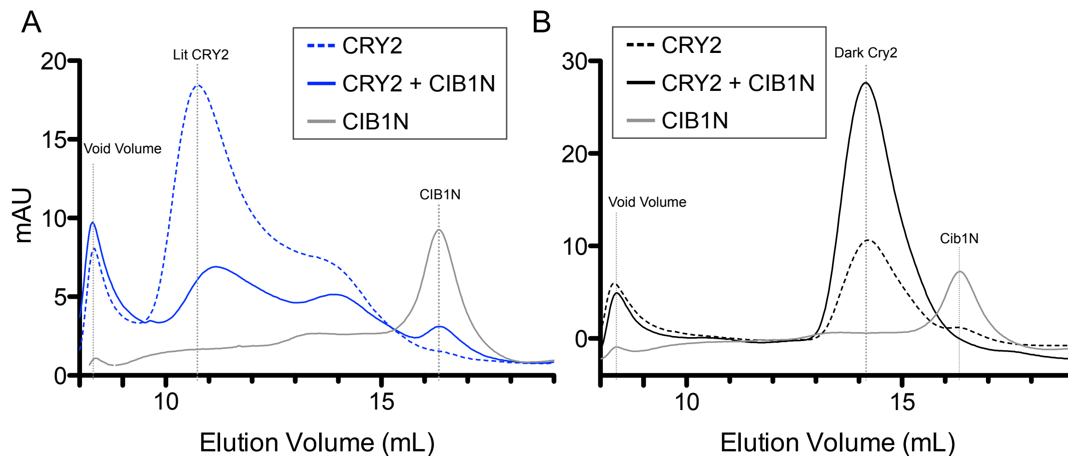

Figure S4: CRY2 and CIB1N do not fully co-elute in size exclusion chromatography. Analytical size exclusion chromatography traces of CRY2 alone (dashed line), CRY2+CIB1N (solid line), and CIB1N alone (grey line) while A) under blue light and B) in darkness. Vertical dashed lines indicate void volume and peaks of Lit or Dark CRY2 for comparison to CRY2+CIB1N traces.

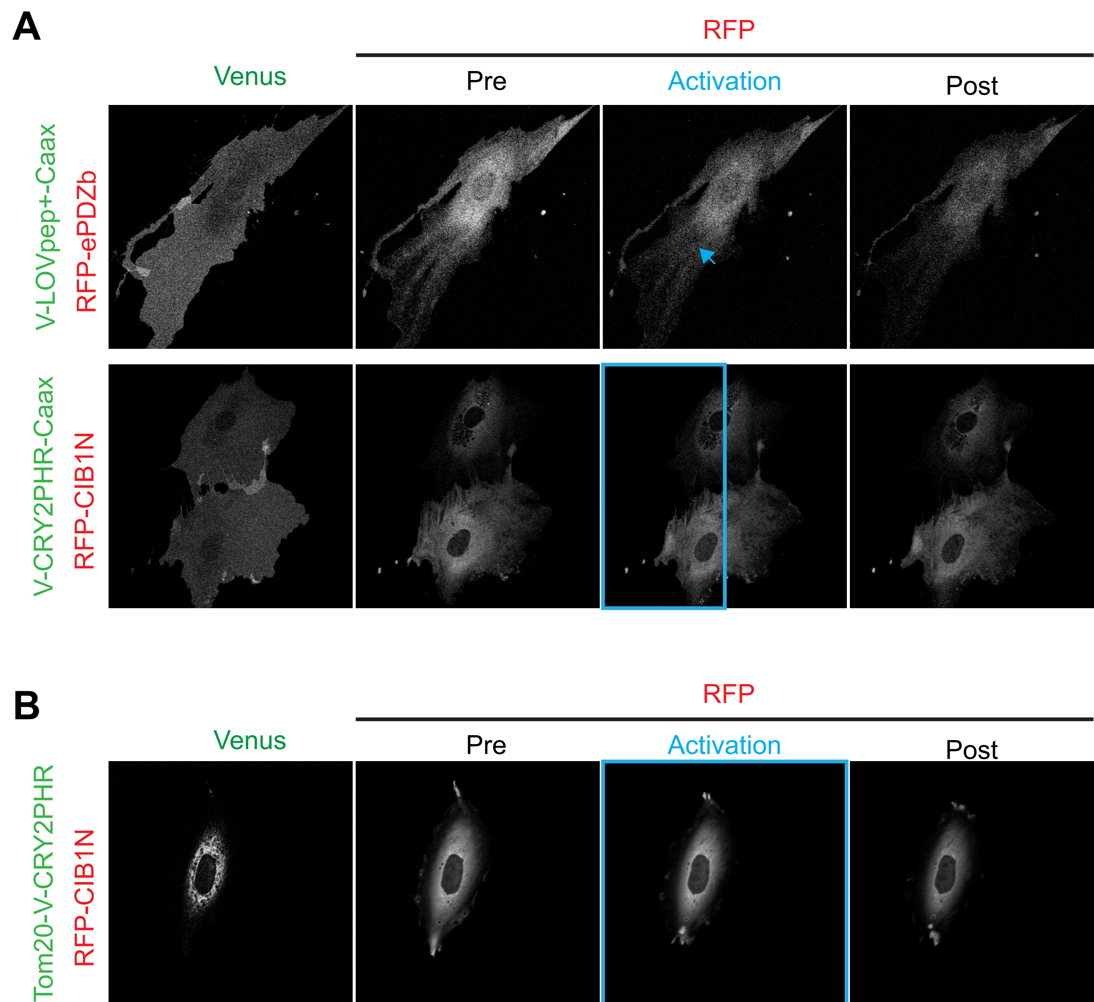

Figure S5: For CRY2PHR/CIB1N and TULIPs, orientation matters for function. a) CRY2 and TULIP switches do not function in particular orientations in respect to the plasma membrane localization. b) CRY2PHR anchored to the mitochondria is toxic to cells and does not function in binding CIB1N upon activation. (Bar = 50 µm)

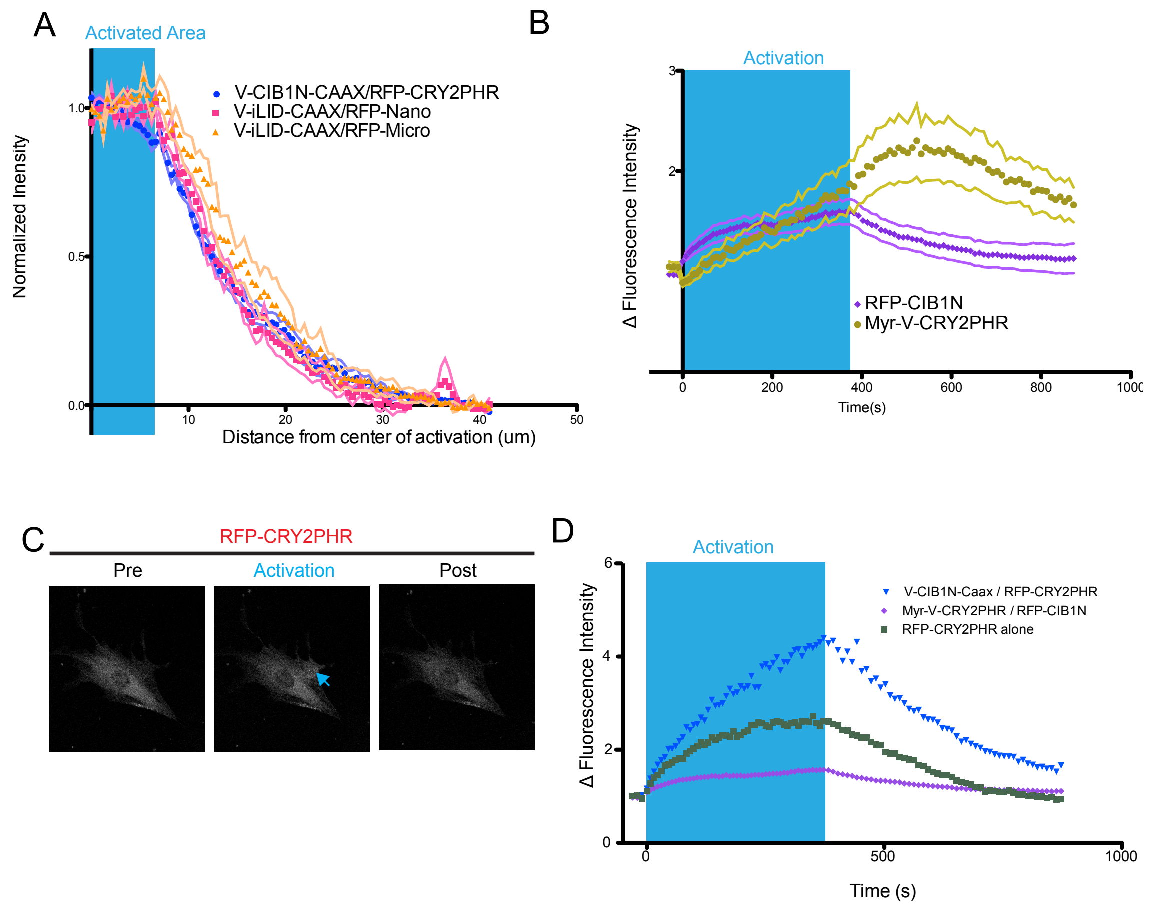

Figure S6: CRY2PHR oligomerization affects observed binding in cells. A) CRY2PHR in the cytoplasm has similar spatial resolution of activation to the iLID switches anchored to the membrane. B) Myr-Venus-CRY2PHR clusters form slower than CIB1N binding occurs and continues to form after stimulation. C) Representative images of tgRFPt-CRY2PHR alone signal increase upon activation. (Bar = 50 μm) D) Quantification of tgRFPt-CRY2PHR alone signal increase upon activation as compared to CRY2PHR in combination with CIB1N.

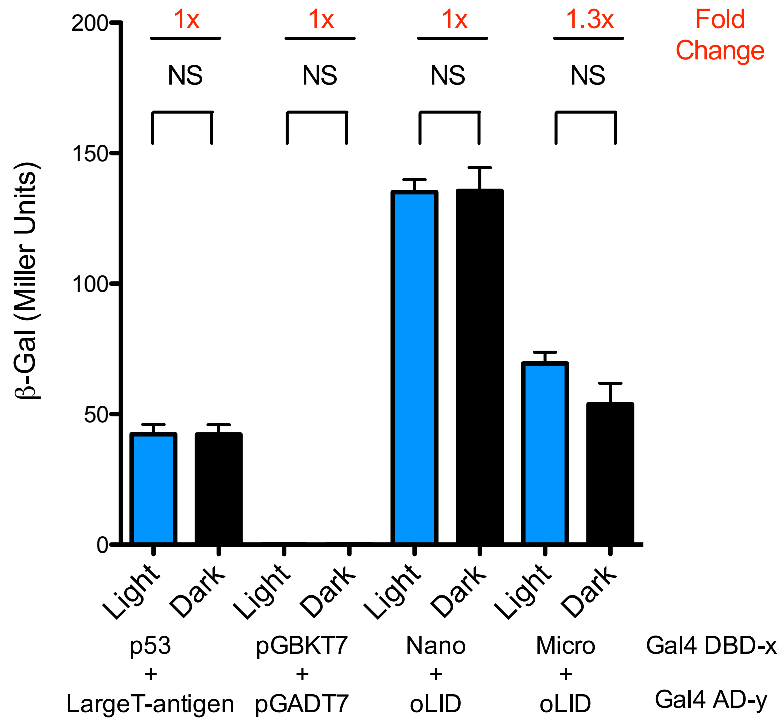

Figure S7: oLID does not allow light dependent transcription in yeast ß-galactose expression under blue light or dark for oLID paired with Nano or Micro (n = 9 each, mean reported ± SEM and statistical significance is calculated with unpaired two-tailed t-student's test). NS – not significant.

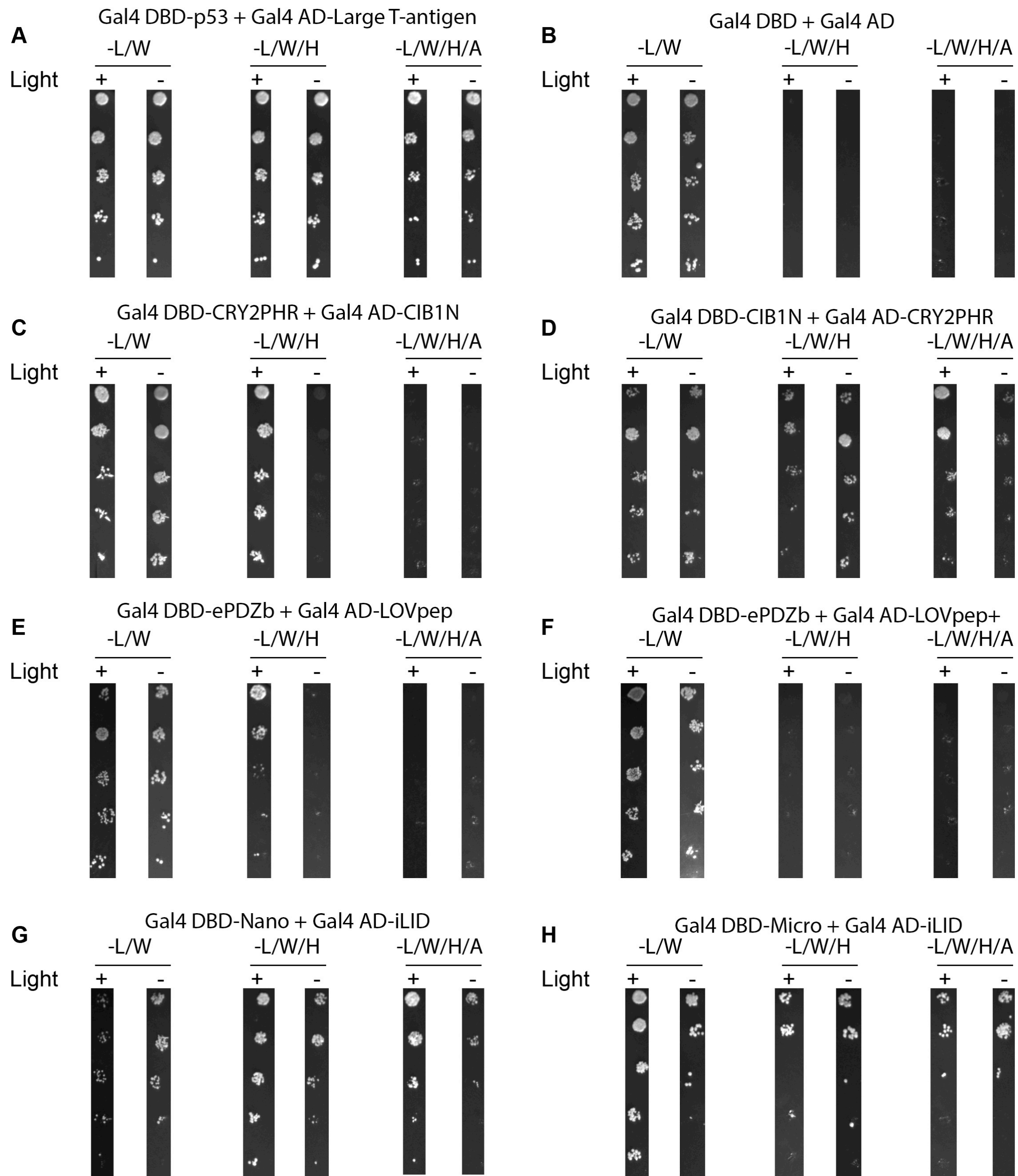

Figure S8: Light dependent growth on dropout plates demonstrates that low-level transcription is achieved for ePDZb-LOVpep and CRY2PHR-CIB1N A) Y2H positive control p53-DBD paired with Large T-antigen-AD, B) Y2H negative control empty pGBKT7 and pGADT7 vectors, C) CRY2PHR-DBD paired with CIB1N-AD, D) CIB1N-DBD paired with CRY2PHR-AD, E) ePDZb-DBD paired with LOVpep, F) ePDZb-DBD paired with LOVpep+, G) Nano-DBD paired with iLID-AD, H) Micro-DBD paired with iLID-AD.

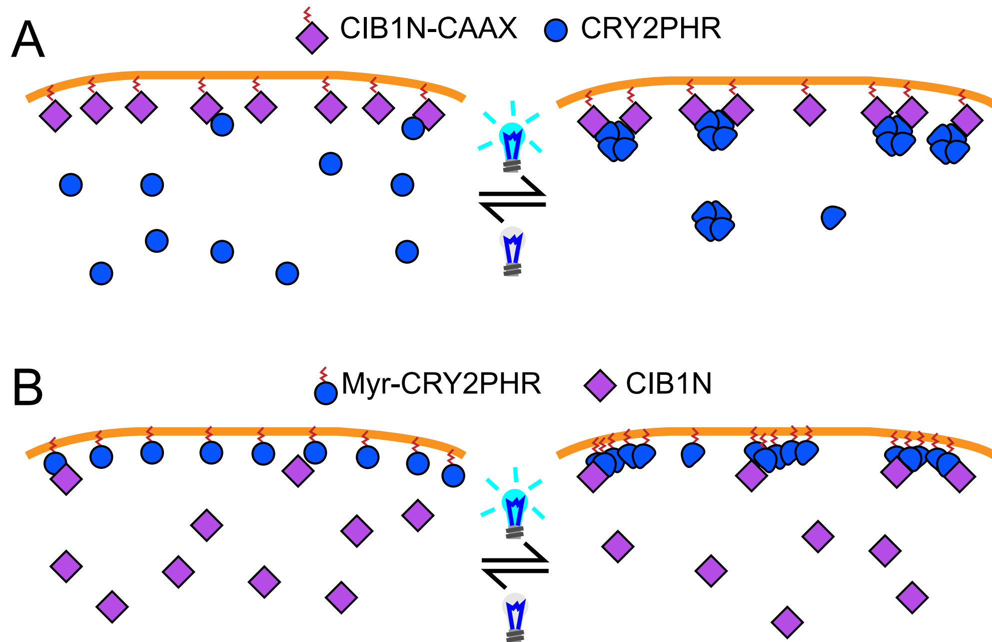

Figure S9: Model of how CRY2PHR oligomerization enhances CIB1N binding in an orientation dependent manner. A) CIB1N-CAAX gains multivalency through localization at the membrane. Upon light stimulation CRY2PHR oligomerizes, enhancing affinity for CIB1N through avidity. B) CRY2PHR-CAAX forms oligomers at the membrane upon light stimulation. However, monomeric CIB1N binds without the avidity affect modeled in A.