## Supporting Information

Includes Supplementary Figure Legends and Supplementary Figures S1 - S4.

## Supplementary Figure Legends

Supplementary Figure S1. Yeast expressing an AD-exPDZb1 fusion show reduced growth rates. Constructs containing a C-terminal extension after the ePDZb1 domain (exPDZb1) show normal growth in yeast as a Gal4BD fusion. When fused to Gal4AD in a higher-expressing vector, exPDZb1 retains some toxicity, indicated by a reduction in growth rate.

## Supplementary Figure S2. Comparison of TULIP variants for control of yeast

 transcription. (a) B-galactosidase reporter activity of yeast expressing BD-exPDZb1 and ADLOVpep variants (unmodified (wt), V416I, and 3xA (T406A, T407A, I532A). Samples were incubated in the dark (' $D$ ') or exposed to blue light pulses (1s 461 nm pulse every 45 s ) for 4 hours. (b) B-galactosidase reporter activity of yeast expressing BD-LOVpep and AD-exPDZb1. Samples were treated as in (a).
## Supplementary Figure S3. Examination of crossreactivity of blue and red light systems.

GalBD-CRY or TULIP bait constructs show no crossreactivity with other prey constructs and respond specifically to blue light. Shown are AH109 yeast expressing indicated bait (GalBDCRY2, GaIBD-exPDZb1, or vector control) and prey (GaIAD-CIB1, GaIAD-LOVpep, GaIADPIF3, or vector) constructs grown on SD -Trp/-Leu/-His +3mM 3-AT selective plates under dark, blue light, or red light conditions for three days. EV indicates empty vector control (pDBTrp or pGADT7rec for bait or prey, respectively).

## Supplementary Figure S4. Optimization of CRY2/CIB-mediated activation of yeast MAP

 kinase pathway. Yeast expressing indicated Mid2 and Ste5 $\Delta \mathrm{N}$ fusion constructs with various full length and truncated versions of CRY2 and CIB1 were evaluated for light-dependent activation of a PFUS1-DsRed reporter. Samples were kept in the dark or exposed to light for 5 hours before imaging.
## Supplementary Figure S1, Pathak et al



## Supplementary Figure S2, Pathak et al.




Figure S4, Pathak et al


