## **Supporting Information**

Includes Supplementary Figure Legends and Supplementary Figures S1 – S4.

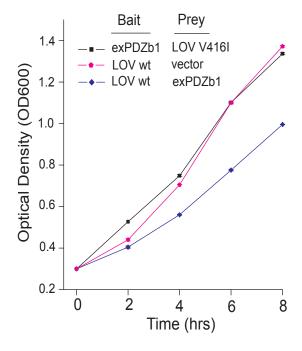
## **Supplementary Figure Legends**

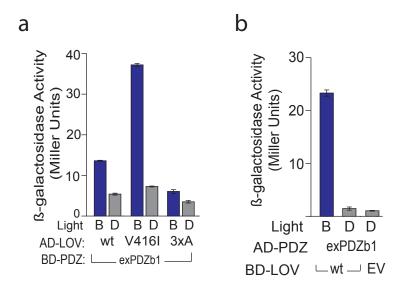
**Supplementary Figure S1. Yeast expressing an AD-exPDZb1 fusion show reduced growth rates.** Constructs containing a C-terminal extension after the ePDZb1 domain (exPDZb1) show normal growth in yeast as a Gal4BD fusion. When fused to Gal4AD in a higher-expressing vector, exPDZb1 retains some toxicity,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

Supplementary Figure S2. Comparison of TULIP variants for control of yeast transcription. (a) ß-galactosidase reporter activity of yeast expressing BD-exPDZb1 and AD-LOVpep 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 45s) for 4 hours. (b) ß-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 (GalBD-CRY2, GalBD-exPDZb1, or vector control) and prey (GalAD-CIB1, GalAD-LOVpep, GalAD-PIF3, 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∆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.





## Supplemental Figure S3, Pathak et al.

