Supplementary Information

Optimized second generation CRY2/CIB dimerizers and photoactivatable Cre recombinase

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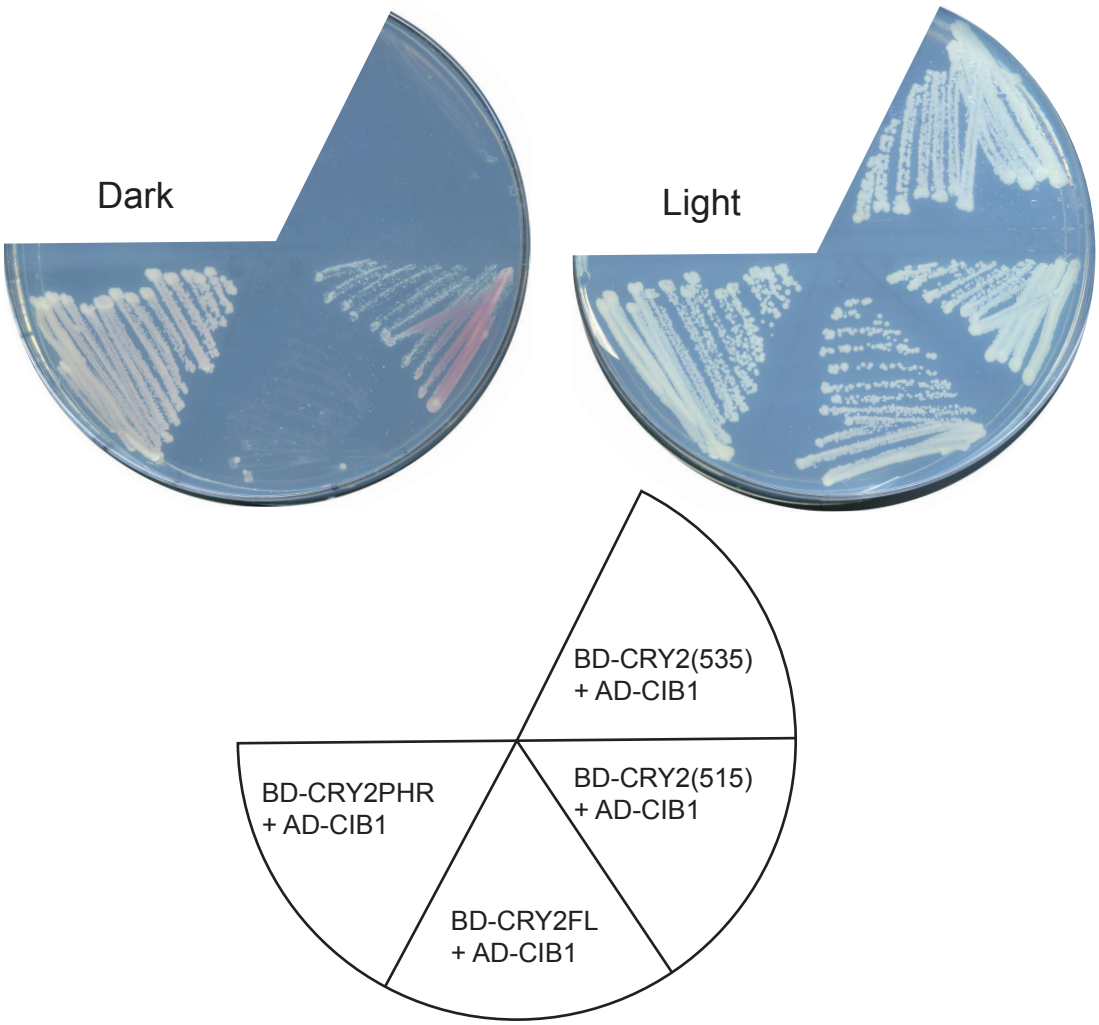
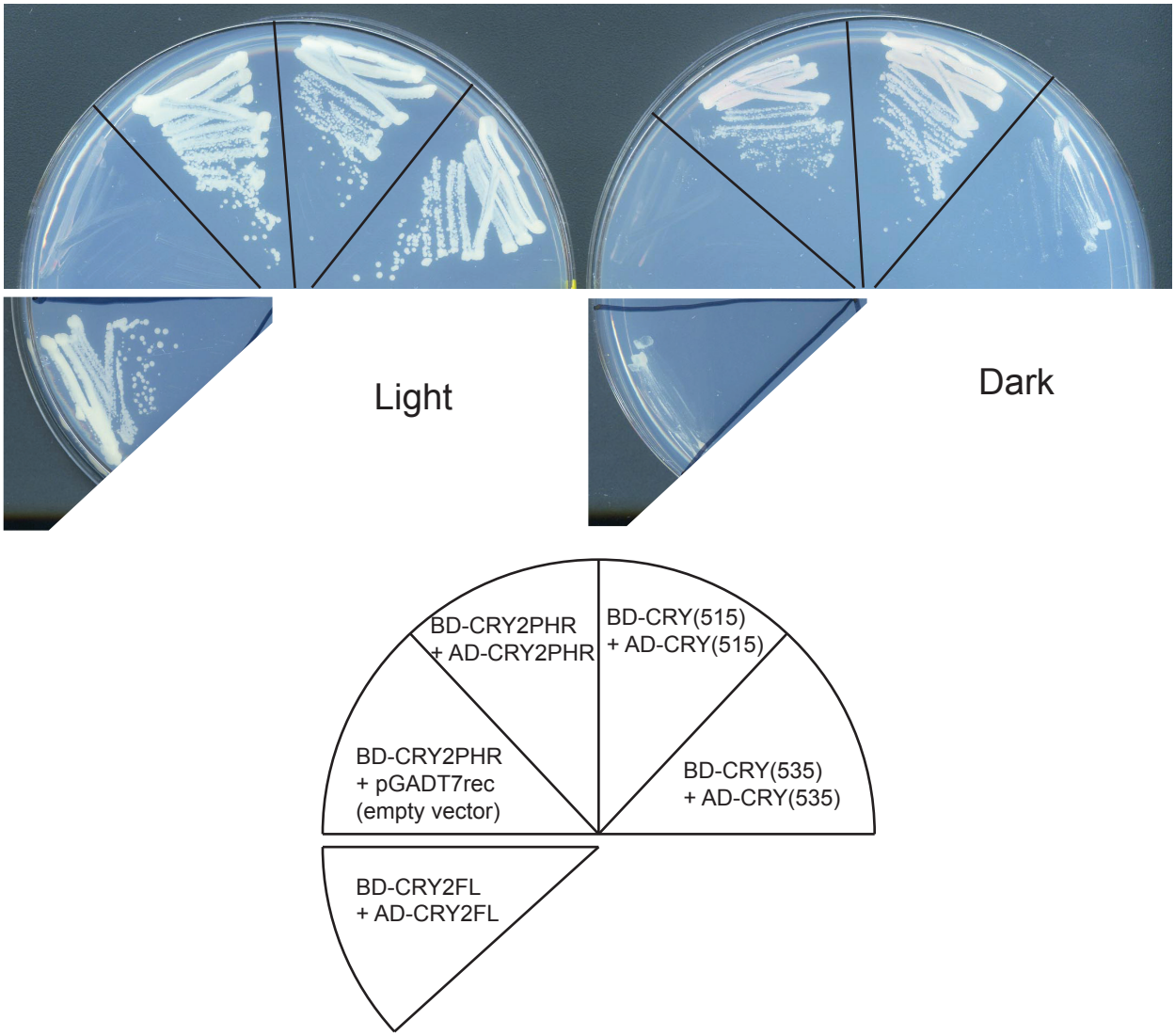
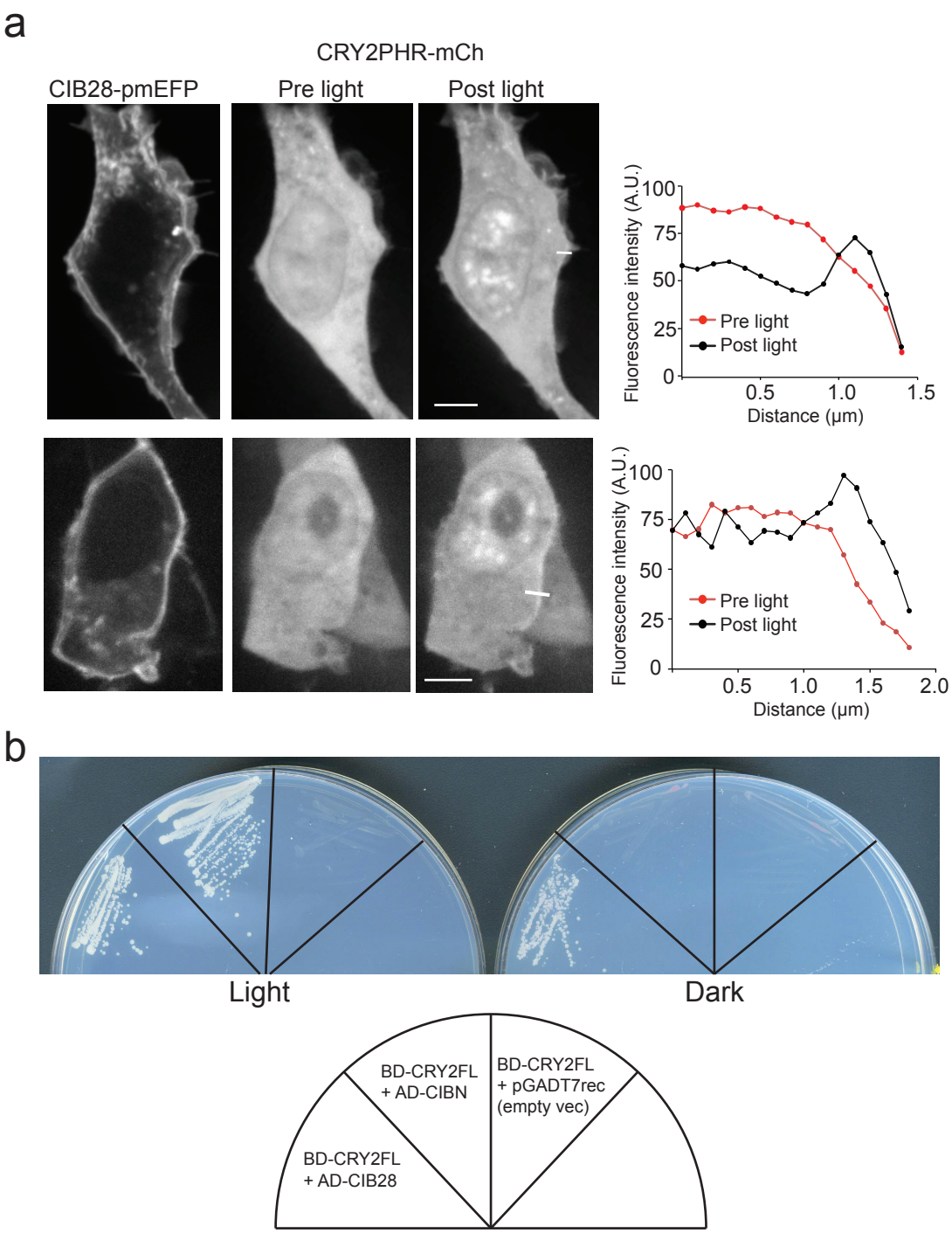
Supplementary Results



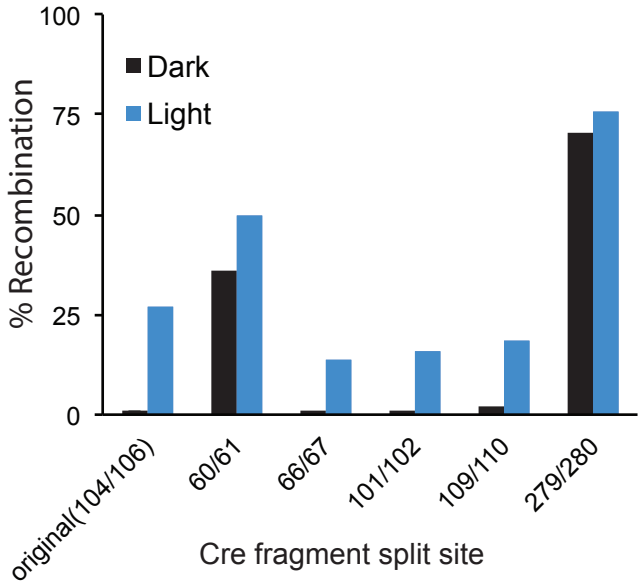
Supplementary Figure 1. Interaction of CRY2 truncations with CIB1 visualized by yeast two-hybrid. Shown are AH109/Y187 yeast expressing indicated Gal4BD-CRY2 truncation constructs tested for interaction with GalAD-CIB1. Positive interaction induces His3 reporter activity, allowing growth on SD -Trp/-Leu/-His +3mM 3AT plates. Dark samples were kept in the dark, while light samples were subjected to blue light pulses (1s pulse every 3 min, 461 nm, 5.8 mW/cm2) for 52 hrs. Growth assays were repeated twice with similar results.



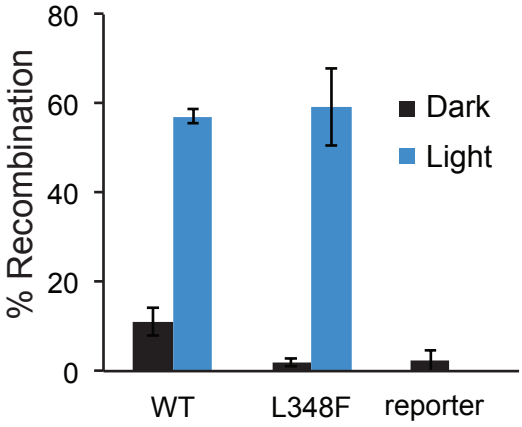
Supplementary Figure 2. CRY2 truncation constructs tested for self-interaction. Shown are AH109/Y187 yeast expressing indicated CRY2 truncation constructs. Positive interaction induces His3 reporter activity, allowing growth on SD –Trp/-Leu/-His +3mM 3AT plates. Dark samples were kept in the dark, while light samples were subjected to blue light pulses (1s pulse every 3 min, 461 nm, 5.8 mW/cm2) for 48 hrs. CRY2FL and CRY2(535) show a substantial reduction in dark self-interaction, compared with CRY2PHR and CRY2(515). Experiments were repeated twice with similar results



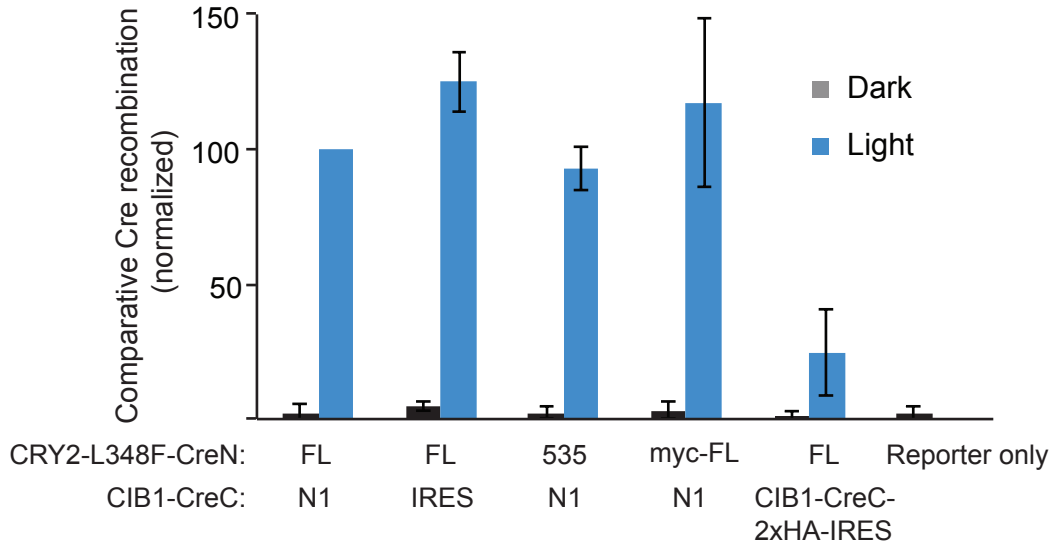
Supplementary Figure 3. Functional tests of interaction of CRY2PHR with CIB28.
(a) Plasma membrane recruitment of CRY2PHR-mCherry to CIB28 following a pulse of light. Shown are images of HEK293T cells expressing CRY2PHR-mCherry and membrane-anchored CIB28-pmGFP (containing a CaaX motif at the C-terminus of EGFP) before and 30 s post blue light exposure. CRY2PHR-mCherry is weakly recruited to the plasma membrane by CIB28. Graphs at right show quantification of fluorescence intensity at membrane at indicated areas. Scale bars, 5 μm. (b) Yeast two-hybrid interaction analysis of AH109/Y187 yeast expressing indicated constructs. Yeast expressing Gal4BD-CRY2FL and Gal4AD-CIB28 show substantial growth in dark, compared with Gal4BD-CRY2FL + Gal4AD-CIBN. Yeast were grown in dark or subjected to blue light pulses (1s pulse every 3 min, 461 nm, 5.8 mW/cm2) for 48 hrs. Experiments were repeated three times with similar results.



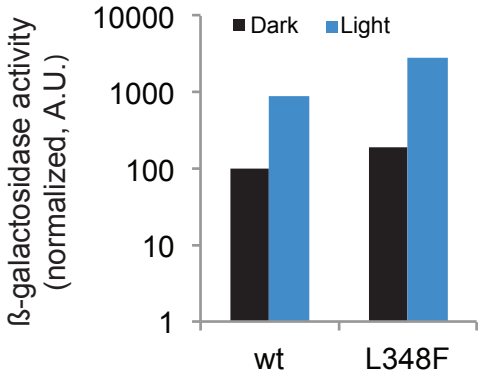
Supplementary Figure 4. Functional testing of different Cre recombinase split sites. N- and C-terminal fragments of Cre recombinase were generated at indicated split sites, and fused to CRY2 or CIB1, respectively. HEK293 cells were transfected with split Cre variants and a loxP-STOP-loxP-EGFP reporter, incubated for 24 hrs in dark, then exposed to 24 hrs blue light pulses (5.8 mW/cm2, 461 nm, 1s pulse every 3 min) before quantification of Cre reporter activity by manual cell count. Data shows average of two independent experiments (triplicate replicates).



Supplementary Figure 5. Activity of second generation PA-Cre constructs with extended illumination. HEK293 cells transfected with a loxP-STOP-loxP-EGFP reporter, CRY2-L348F-CreN, and CIB1-CreC(N1) were incubated in the dark, then kept in dark or exposed to 24 hrs of light treatments (2s pulse every 3 min, 461 nm, 5.8 mW/cm2) beginning 24 hrs after transfection. Cre reporter activity was assayed 48 hrs after transfection by manual count. Data represent mean values ± s.d. (n=3) from one experiment, and experiments were repeated two times with similar results.



Supplementary Figure 6. Activity of tagged and truncated versions of second generation PA-Cre constructs. HEK293 cells were cotransfected with a loxP-STOP-loxP-EGFP reporter and indicated CreN and CreC constructs and incubated in dark for 24 hrs. Cells were either kept in dark or exposed to a single 4 s pulse of blue light, then incubated in dark an additional 24 hrs before manual reporter quantification. Graph shows normalized mean values ± s.d. (n = 6 for FL/N1, n=3 for others) for three independent experiments. Data was normalized to results with CRY2-L348F-CreN + CIB1-CreC(N1) for comparison.



Supplementary Figure 7. Comparison of wild-type and L348F CRY2 with a LexA-VP16 transcriptional system. W303-1A cells were coexpressed with wild-type or L348F LexA-CRY2(535), VP16-CIB1, and a pSH18-34 transcriptional reporter. Shown is β-galactosidase reporter activity assayed in cells kept in dark, or 2 hr after treatment with a single flash of blue light (2s pulse, 461 nm, 5.8 mW/cm2). Graph shows normalized mean values from two independent experiments (n=3 replicates each).

Other Supplementary Information

Supplementary Video 1. Recruitment and dissociation of CRY2PHR-mCh with CIB81-pmEGFP. Shown are timelapse images of HEK293T cells expressing CRY2PHR-mCh and membrane localized CIB81-pmEGFP (containing a CaaX motif at C-terminus of EGFP) pre and post exposure to a single pulse of 488nm light (indicated by white dot on image stack). A time counter (mins) is indicated at top left.

Supplementary Table 1. Sequences of constructs used in studies (Excel spreadsheet).