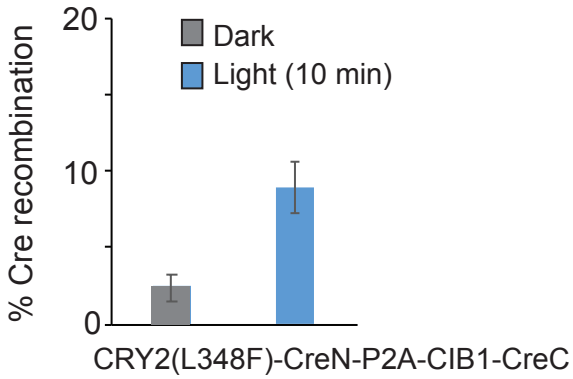
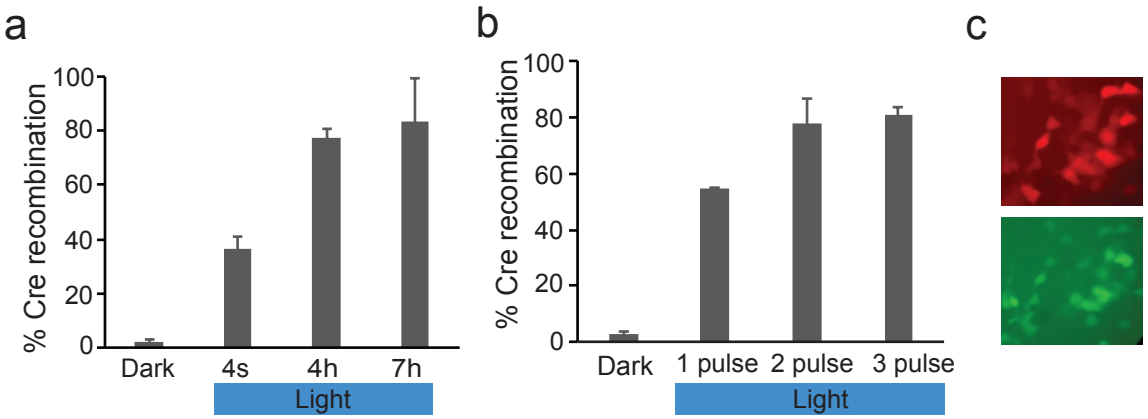
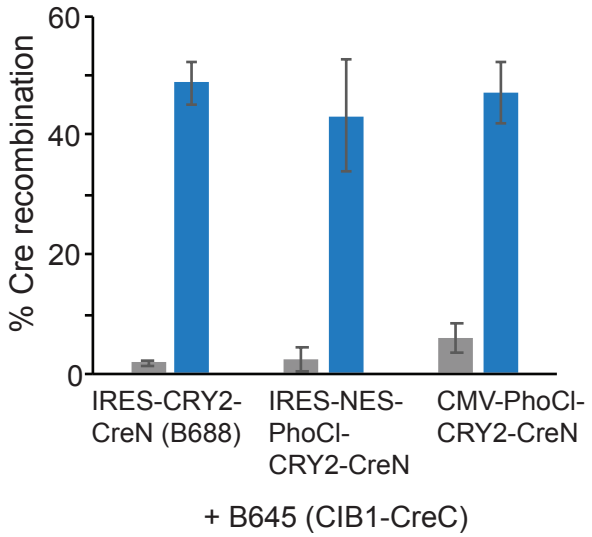


Supplementary Figure 1. Addition of P2A sequence at end of CRY2(L348F)-CreN reduces Cre recombinase activity. Shown are results from HEK293T cells transfected with CRY2(L348F)-CreN-P2A-CIB1-CreC and a loxP-stop-loxP-EGFP Cre reporter, kept in dark or exposed to light (10 min, 24 hrs after transfection), harvested at 48 hrs. Data represents average and range of two independent experiments.



Supplementary Figure 2. Light-dependence of combined PA-Cre2.0 construct KM10. (a) Quantification of Cre recombinase activity in HEK293T cells expressing KM10 and a loxP-STOP-loxP-EGFP reporter exposed to different light treatments (2s 461nm pulse every 3 min for extended treatments). Cells were treated with light starting at 24 hrs, then quantified at 48 hrs. Data represents average and s.d., three independent experiments. (b) KM10 shows enhanced activity with three interval-spaced light treatments. Cells were treated as in (a), but exposed to 1, 2, or 3 single 4s pulses of light, spaced 45 min apart. Graph shows average and range, two independent experiments. A typical result (3 4s pulses/45 min apart) is shown in (c), with most mCherry-expressing cells showing reporter expression.



Supplementary Figure 3. Addition of a NES-PhoCl tag to CRY2(L348F)-CreN does not improve background activity. HEK293 cells were transfected with indicated constructs and a loxP-STOP-loxP-EGFP Cre reporter (1 µg each). 24 hours post transfection, cells were illuminated with a 4 min pulse of 405 nm light, followed by a 4s pulse of blue light one hour later. Percent transfected cells showing Cre reporter activity were quantified 48 hours post transfection. All versions of CRY2-CreN contained the L348F mutation. Data represents average and error (*s.d., n=3-4 independent experiments*).