Supplemental Information

An Optogenetic System to Control Membrane Phospholipid Asymmetry through Flippases activation in Budding Yeast.

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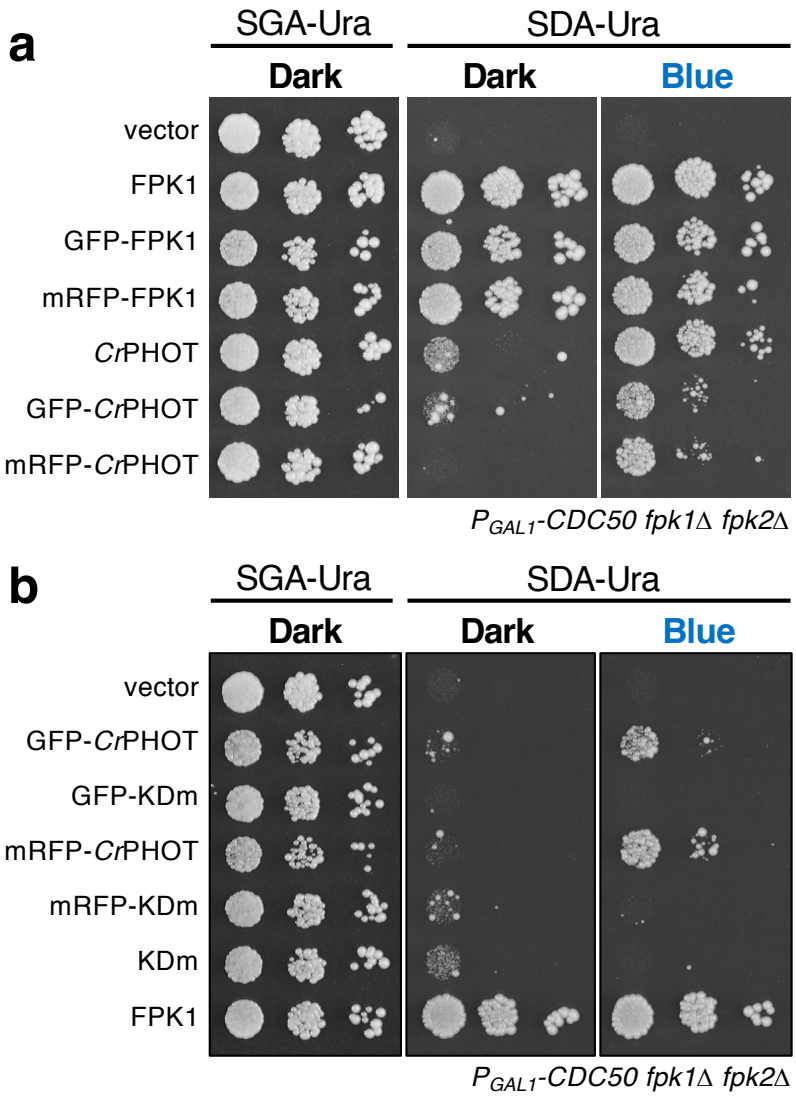
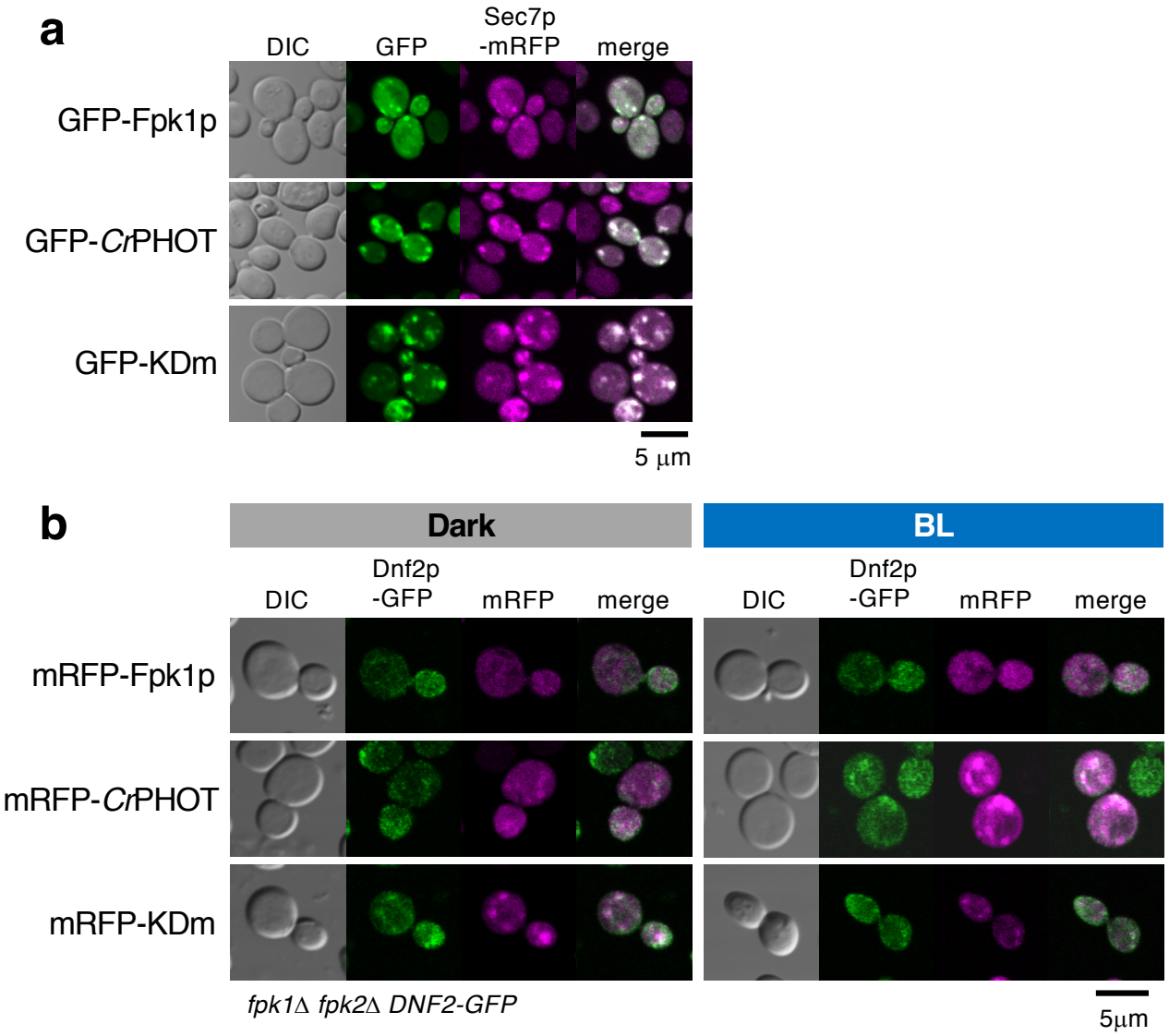
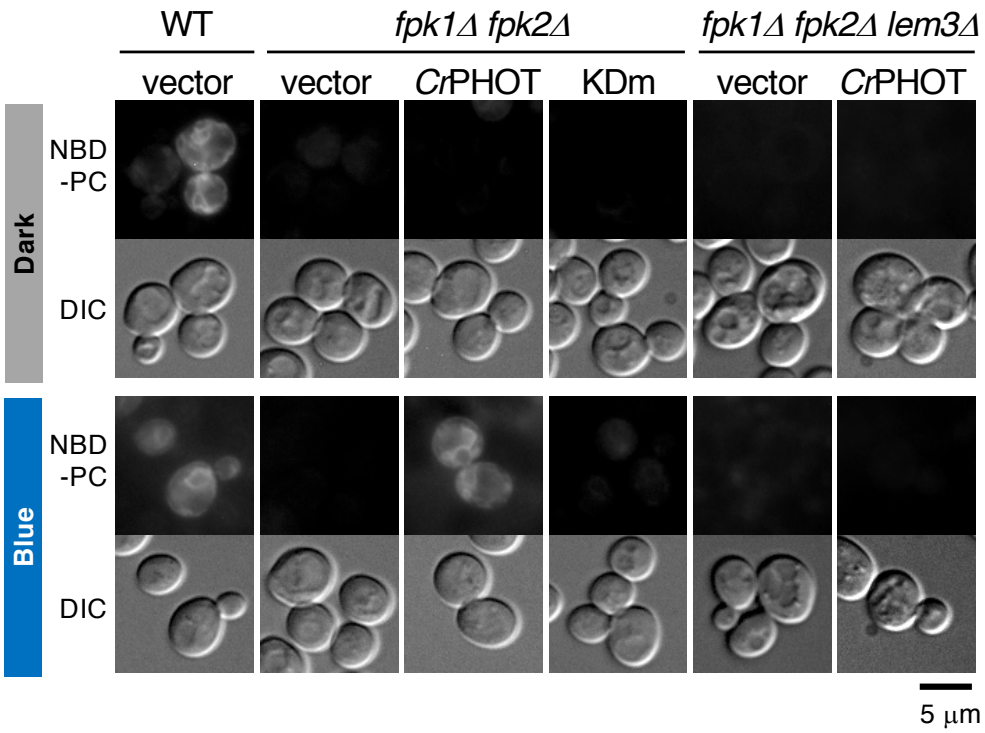
Supplementary Figures



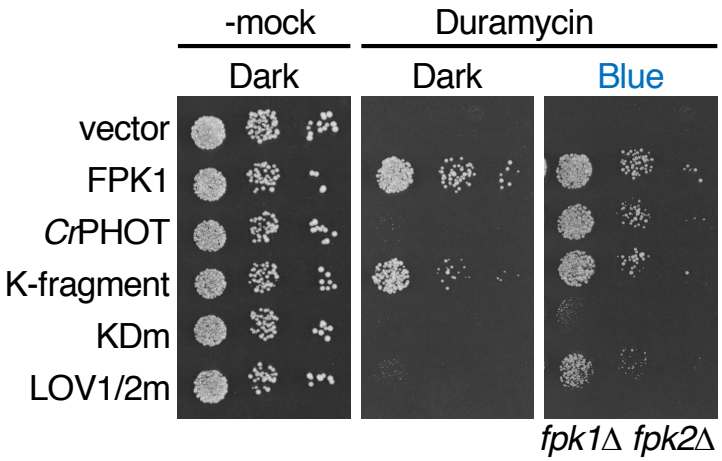
Supplementary Figure S1. The fluorescent-protein tagged *Cr*phot and KDm are also functional. Growth of the yeast conditional mutant P_{GAL1}-*CDC50 fpk1*Δ *fpk2*Δ expressing GFP or mRFP tagged *Cr*PHOT (a) and KDm, kinase-dead mutant with D549N (b). Yeast cells were serially diluted and spotted onto plates containing galactose (SGA-Ura) or glucose (SDA-Ura), which were incubated in darkness (Dark), or under 10 μmol m^{-2} s^{-1} BL irradiation (Blue) at 28 ˚C for 3 days.



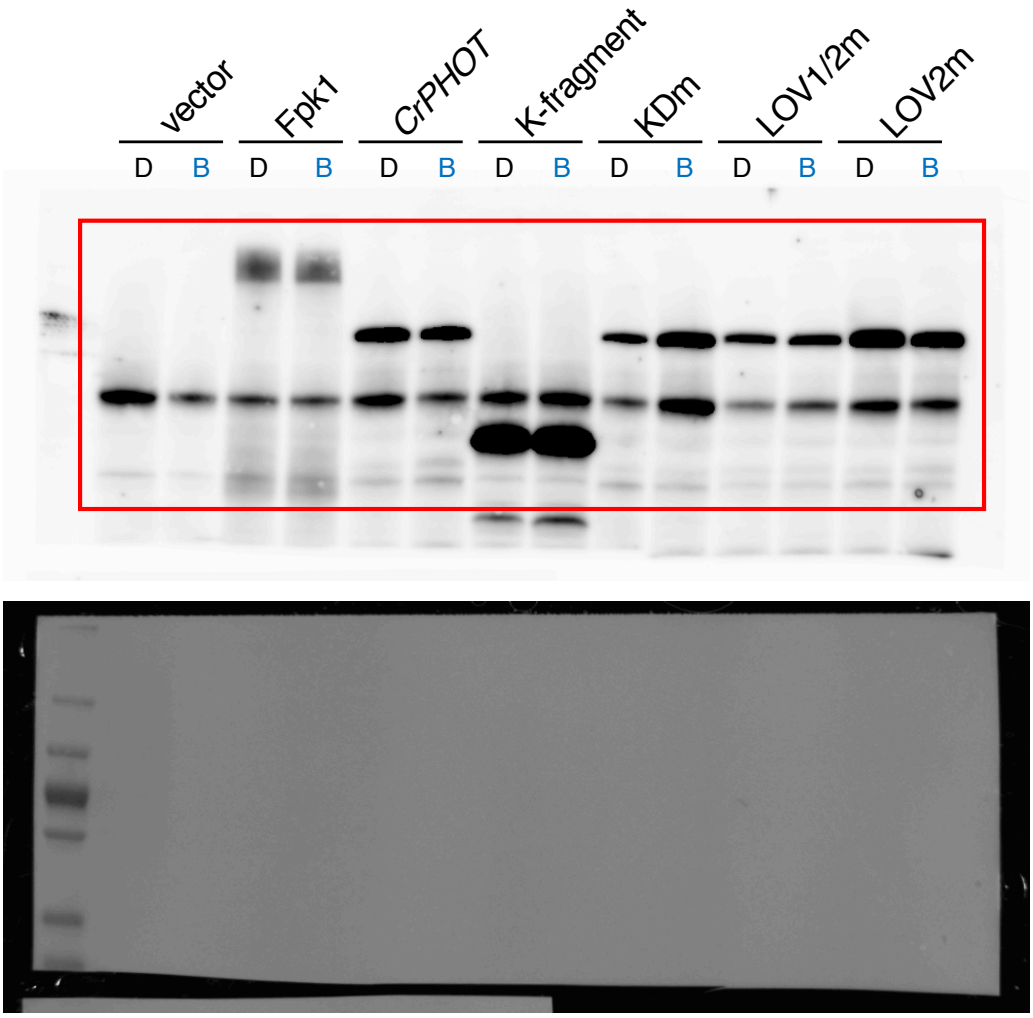
Supplementary Figure S2. Colocalization of *Cr*PHOT with Dnf2p or Sec7p. a Confocal images of YKT905 (*SEC7-mRFP*) cells expressing GFP tagged Fpk1p, *Cr*PHOT and KDm proteins under room light. **b** Confocal images of KKT336 (*fpk1*Δ *fpk2*Δ, *DNF2-GFP*) cells expressing mRFP tagged Fpk1p, *Cr*PHOT and KDm proteins. Yeast cells cultured in darkness (Dark) or under BL irradiation (Blue). Images merged to compare the two signal patterns. Scale bars = 5 μm.



Supplementary Figure S3. NBD-PC internalization by *Cr*PHOT under BL. Wild-type (WT) harbouring vector plasmids, *fpk1*Δ *fpk2*Δ mutant harboring vector, *Cr*PHOT or KDm plasmids, and KKT274 (*fpk1*Δ *fpk2*Δ *lem3*Δ) harboring vector or *Cr*PHOT plasmids were grown in SC medium in the dark (Dark) or under BL (Blue) at 30 ˚C, and treated with NBD-PC. A representative cell image by confocal microscopic observation in each condition is shown. Scale bar = 5 μm.



Supplementary Figure S4. Optical control of PE amount in the outer leaflet of the plasma membrane in a kinase-dependent-manner. Growth sensitivity of *fpk1*Δ *fpk2*Δ cells carrying pRS416-CrPHOT or its derivatives to PE-specific binding antibiotics, duramycin. Yeast cells were cultured in YPDA medium at 28 ˚C, serially diluted and spotted on to YPDA plate with or without 20 μM duramycin, which were incubated in darkness (Dark), or under BL irradiation (Blue) 28 ˚C for 2 days.



Supplementary Figure S5. The original unprocessed images of Western blots for Figure 1. Raw data of Western blots (upper panel) with corresponding membrane (lower panel). The area used in Fig. 1 is indicated by a red frame.