

Figure S1. Light induced PIP₃ production with CIBN/CRY2-iSH2 module mimics insulin effect on downstream Akt phosphorylation. (A) Confocal images show dose dependent stimulation of Akt phosphorylation at Ser473 by insulin incubation in 3T3-L1 adipocytes. (B) Confocal images show dose dependent stimulation of Akt phosphorylation at Thr308 by insulin incubation in 3T3-L1 adipocytes. (C) Immunofluorescence staining of phosphorylated Akt (Thr308) in CIBN-pmGFP and CRY2-iSH2 expressed adipocytes after 488 nm light exposure. (D) Quantification of phosphorylated Akt (Thr308) levels in different treatment conditions as indicated. ($n=30$ cells, data are mean \pm SEM). Bars: (A-C) 10 μ m. (E) 3T3-L1 adipocytes were serum-starved, and pre-incubated with vehicle (0.1% DMSO), 100 nM Wortmannin or 10 μ M Akti for 10 min prior to stimulation (100 nM insulin; 10 min). Cells lysates were immunoblotted for proteins as indicated.

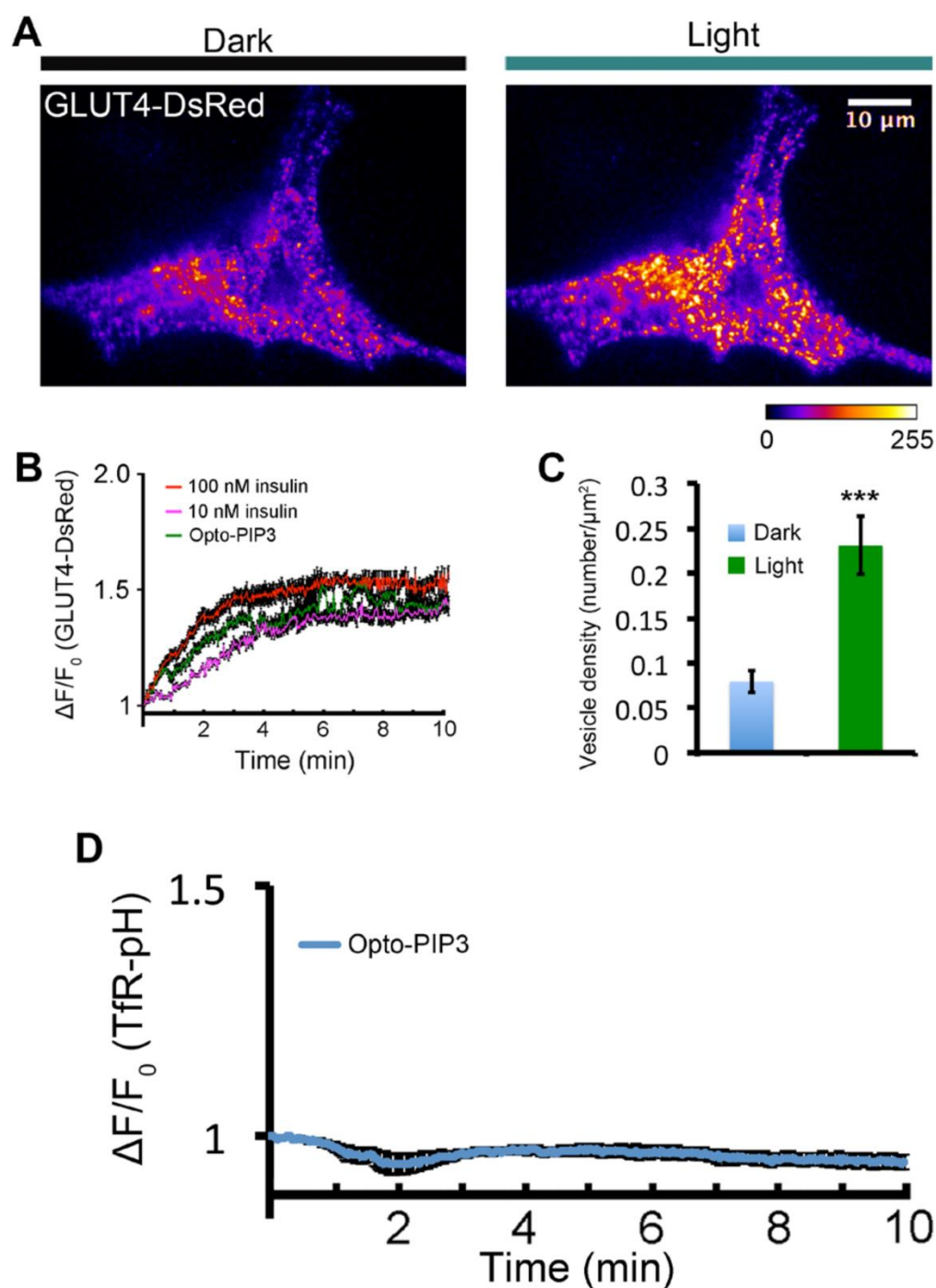


Figure S2. Light-induced PIP_3 production promotes GLUT4 vesicle docking and fusion with the PM, but not TfR containing endosomes. (A) 3T3-L1 adipocytes were electroporated with CIBN-CAAX, CRY2-iSH2 and GLUT4-DsRed plasmids. The cells were activated with 500-ms pulses of blue light (488 nm, 10 mW) at 5 s intervals under TIRF illumination. TIRF images showed GLUT4-DsRed signals in a cell before and after 10 min of light activation. (B) Quantification the dynamic increases of the footprint fluorescence signals of cells in the presence of light and different doses of insulin activation. ($n=3$ cells, data are mean \pm SEM) (C) Docking GLUT4 vesicle density before and 10 min after light activation. ($n=3$ cells, data are mean \pm SEM) (D) Quantification of Opto- PIP_3 induced TfR-pHluorin translocation. ($n=5$ cells, data are mean \pm SEM) Bar: (A) 10 μm .

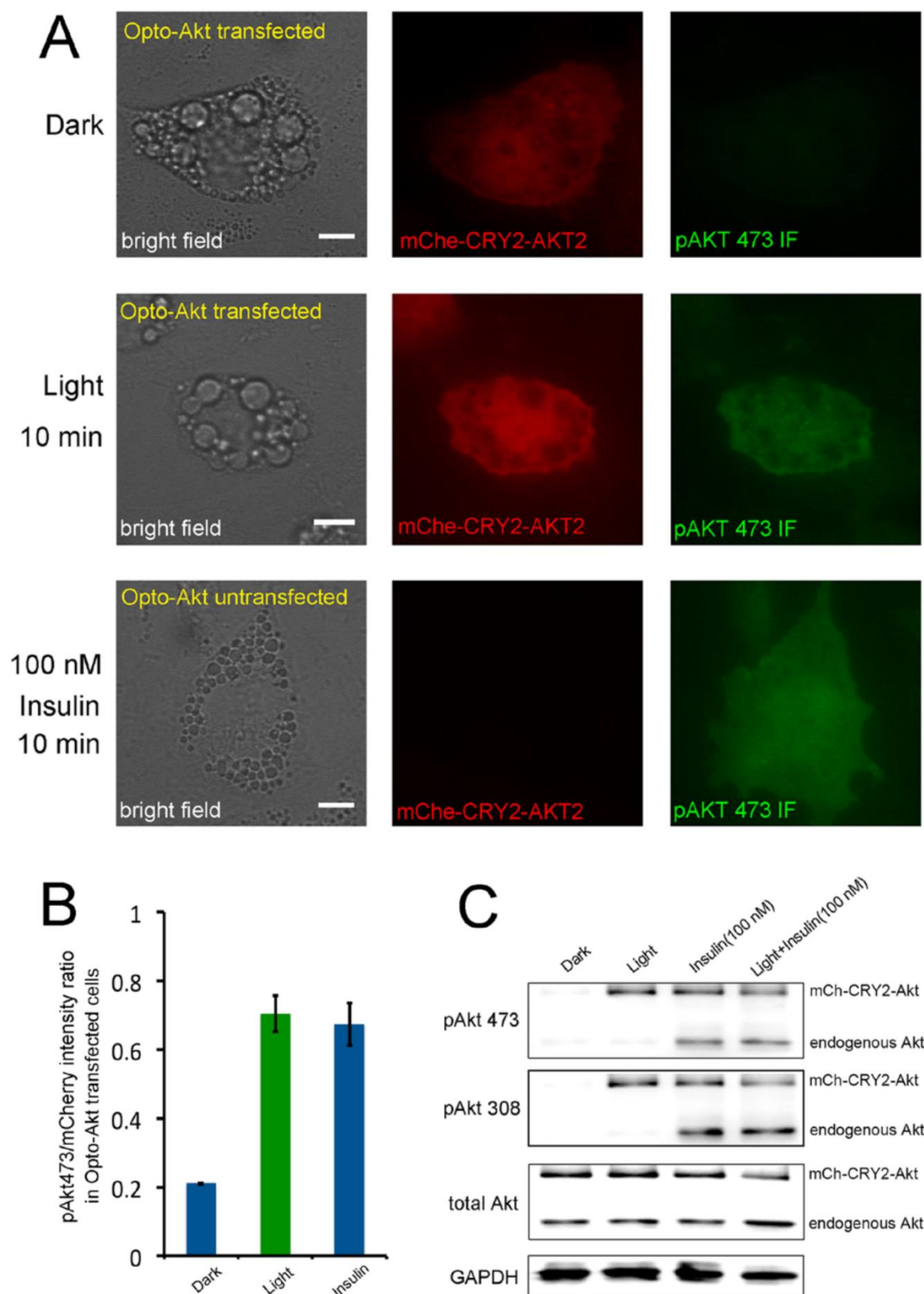


Figure S3. Opto-Akt2 induced Akt phosphorylation at Ser473 mimics maximal insulin effect. (A) 3T3-L1 adipocytes were electroporated with CIBN-CAAX, mCh-CRY2-Akt2 plasmids. The cells were activated either by a custom-made LED array or 100 nM insulin for 10 min. The cells were then fixed for immunofluorescence staining of

phosphorylated Akt (Ser473). Bright field and TIRF images of 3T3-L1 adipocytes expressing mCherry-CRY2-Akt2 and stained for pAkt473 were shown. (B) Quantification of the intensity ratio of phosphorylated Akt (Ser473) and mCherry near the plasma membrane of adipocytes in different treatment conditions as indicated. ($n=20$ cells per condition, mean \pm SEM). The insulin effect on exogenously expressed mCherry-CRY2-Akt2 was calculated as pAkt473 intensity from Opto-Akt transfected cells minus pAkt473 intensity on Opto-Akt un-transfected cells after, and was normalized to mCherry fluorescence intensity. (C) 3T3-L1 adipocytes transfected with CIBN-CAAX and mCh-CRY2-Akt2 plasmids were treated with insulin or exposure to 488 nm LED light, respectively. Cells lysates were immunoblotted as indicated. Bar: (A) 10 μ m.

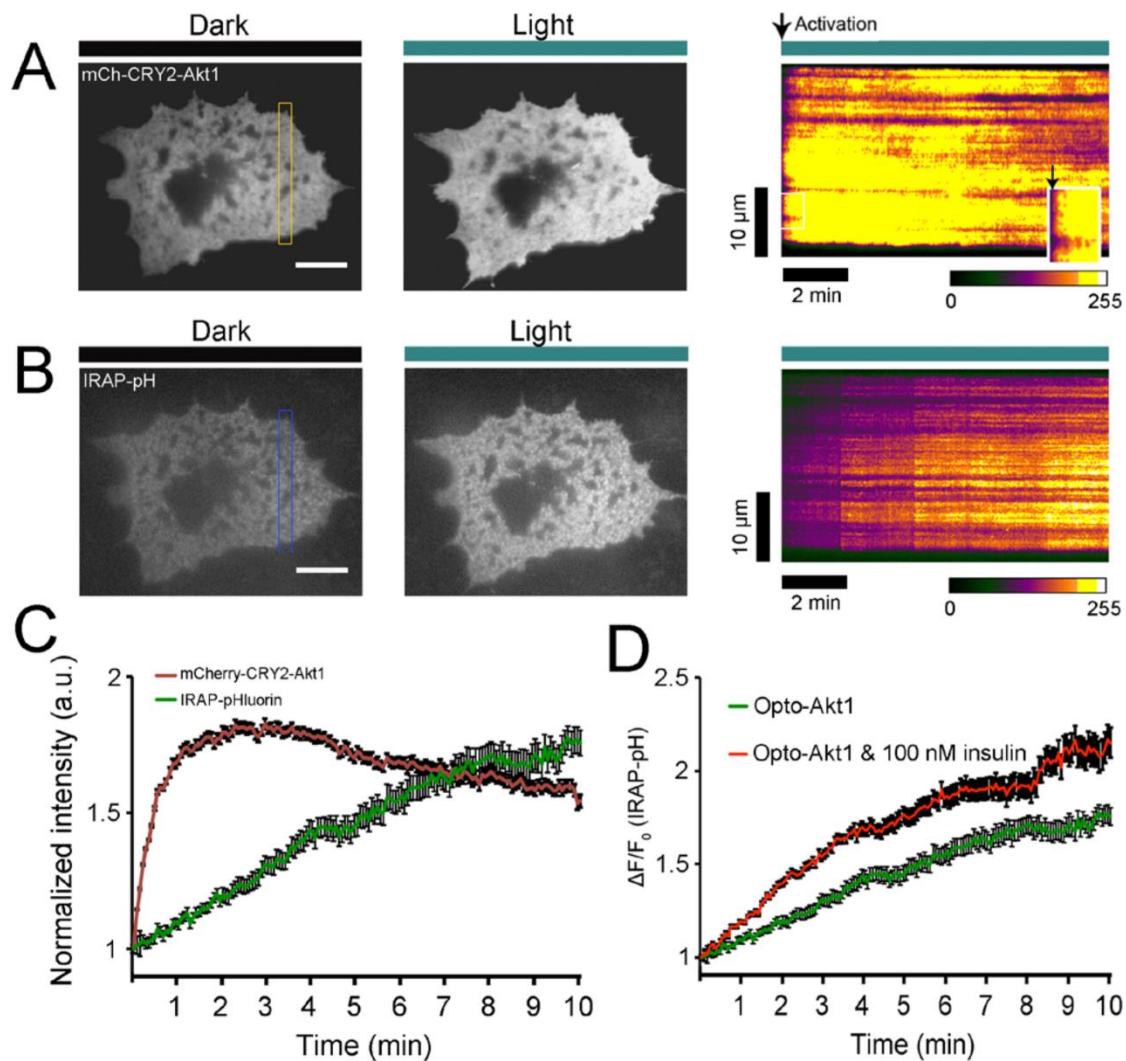
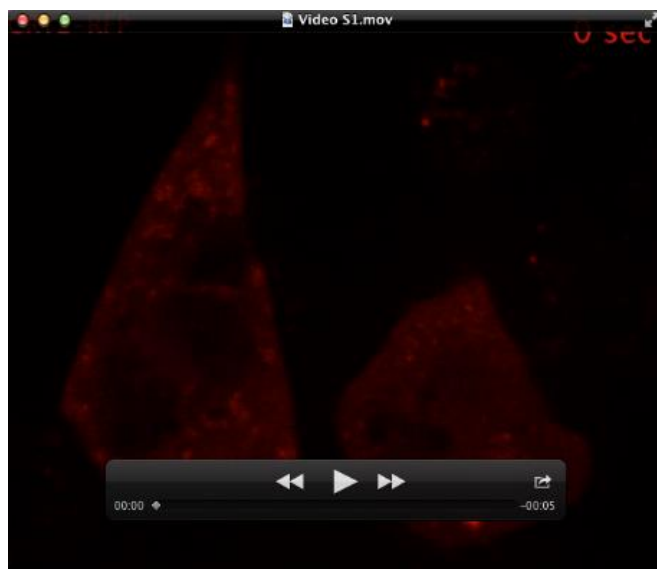
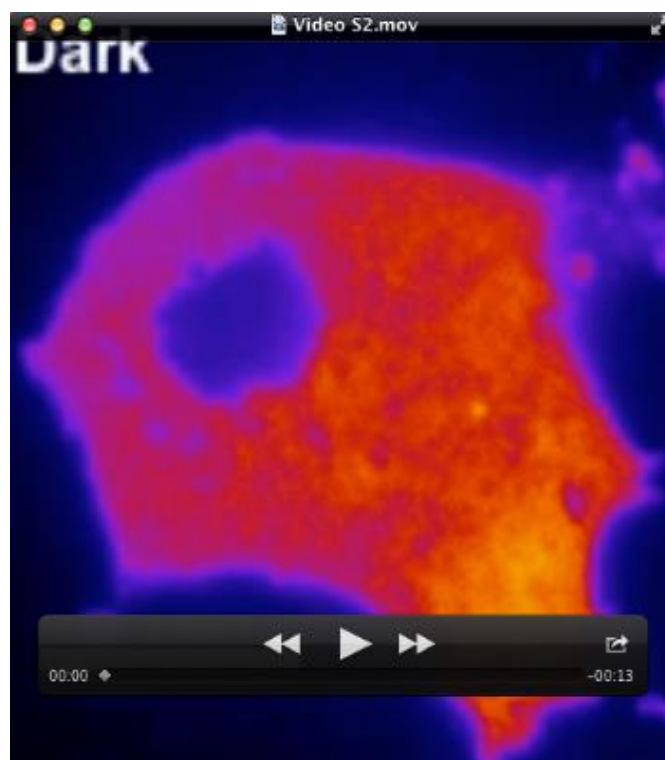


Figure S4. Opto-Akt1 promotes the exocytosis of IRAP-pHluorin in 3T3-L1 adipocytes. (A) Rapid relocation of Akt fusion proteins to the cell surface after light activation. TIRF images show mCherry tagged Akt1 fusion proteins before and after one pulse of blue light (488 nm, 10 mW) activation in a 3T3-L1 adipocyte. (B) Opto-Akt1 stimulates IRAP-pHluorin translocation. 3T3-L1 adipocytes were electroporated with CIBN-CAAX, mCherry-CRY2-Akt1 and IRAP-pHluorin plasmids. The cells were activated with 500-ms pulses of blue light (488 nm, 10 mW) at 5 s intervals under TIRFM illumination, and IRAP translocation were evaluated by pHluorin signals on the cell surface. TIRF images show IRAP-pHluorin signals before and 10 min after light activation. (C-D) Quantification of light-induced redistribution of mCherry tagged Akt1 fusion proteins, and Opto-Akt1 or Opto-Akt1 plus insulin induced IRAP-pHluorin to the cell surface. ($n=5$ cells, data are mean \pm SEM). Bar: (A-B) 10 μ m.



Movie 1. Blue light induced redistribution of mCherry-CRY2-iSH2 from the cytosol to the cell surface where CIBN-pmGFP resides.



Movie 2. Light-induced production of PI(3,4,5)P₃ (visualized by PH-AKT-mRFP signal), its reversibility and sensitivity to PI3K inhibitor wortmannin.



Movie 3. Light-induced PI(3,4,5)P₃ production promotes IRAP-pHluorin exocytosis in 3T3-L1 adipocytes.