Nakatsu et al., http://www.jcb.org/cgi/content/full/jcb.201005018/DC1

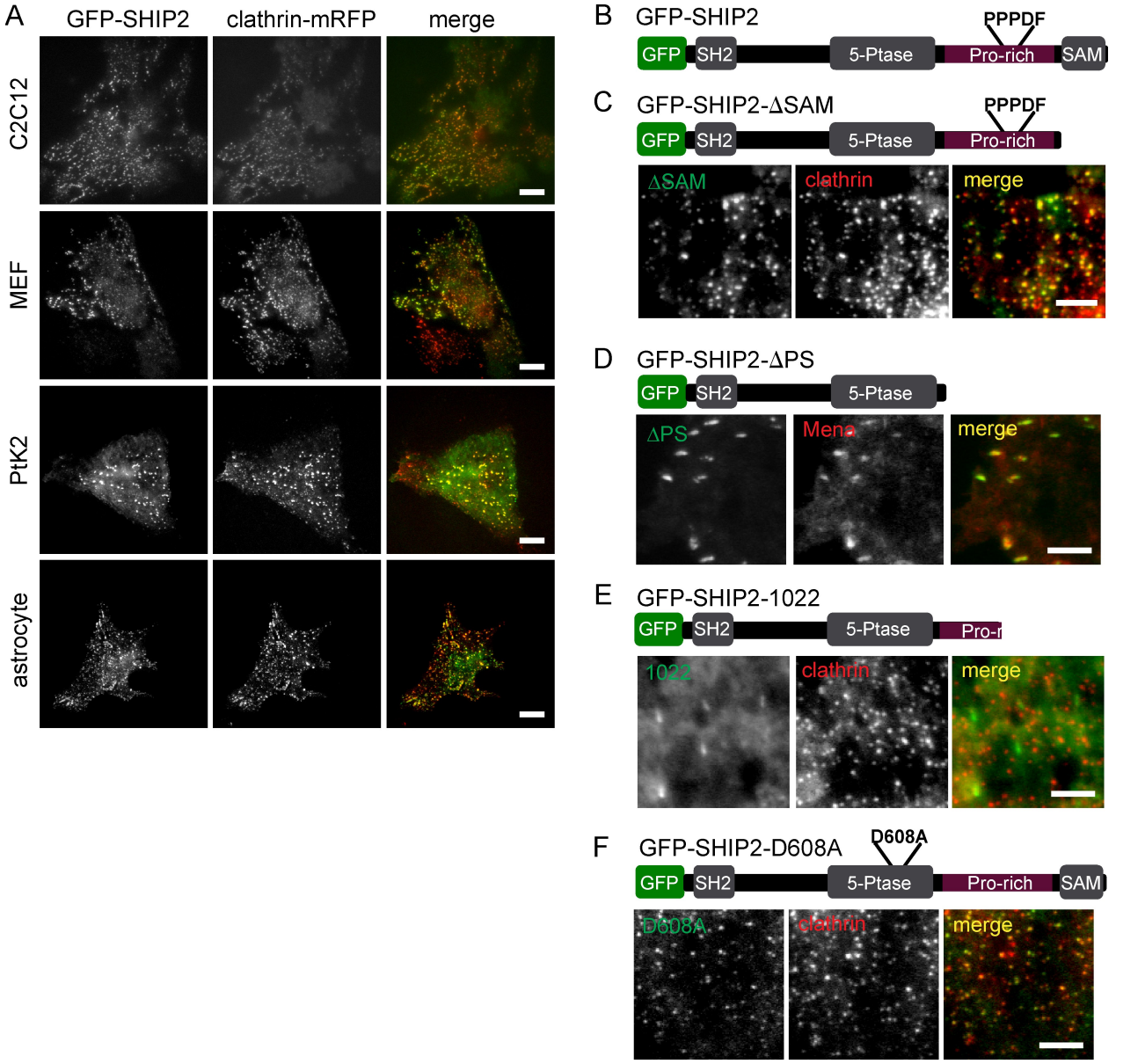


Figure S1. **TIRFM images (from live cells) showing the localization of GFP-SHIP2 and GFP-SHIP2 mutants in various cell types.** (A) Colocalization of GFP-SHIP2 with clathrin-mRFP in myoblastic cells (C2C12), PtK2 cells, mouse embryonic fibroblasts (MEF), and mouse astrocytes in primary culture. (B) Schematic representation of the domain structure of full-length GFP-SHIP2. (C–F) COS-7 cells transiently expressing GFP fusions of the SHIP2 mutant constructs indicated (C-terminal deletion constructs or a catalytically inactive point mutant) together with either clathrin-mRFP or RFP-Mena. SHIP2 constructs lacking the PPPDF sequence do not colocalize with clathrin and are instead localized at focal adhesions, as indicated by colocalization with Mena. 5-Ptase, 5-phosphatase. Bars: (A) 10 µm; (C–F) 5 µm.

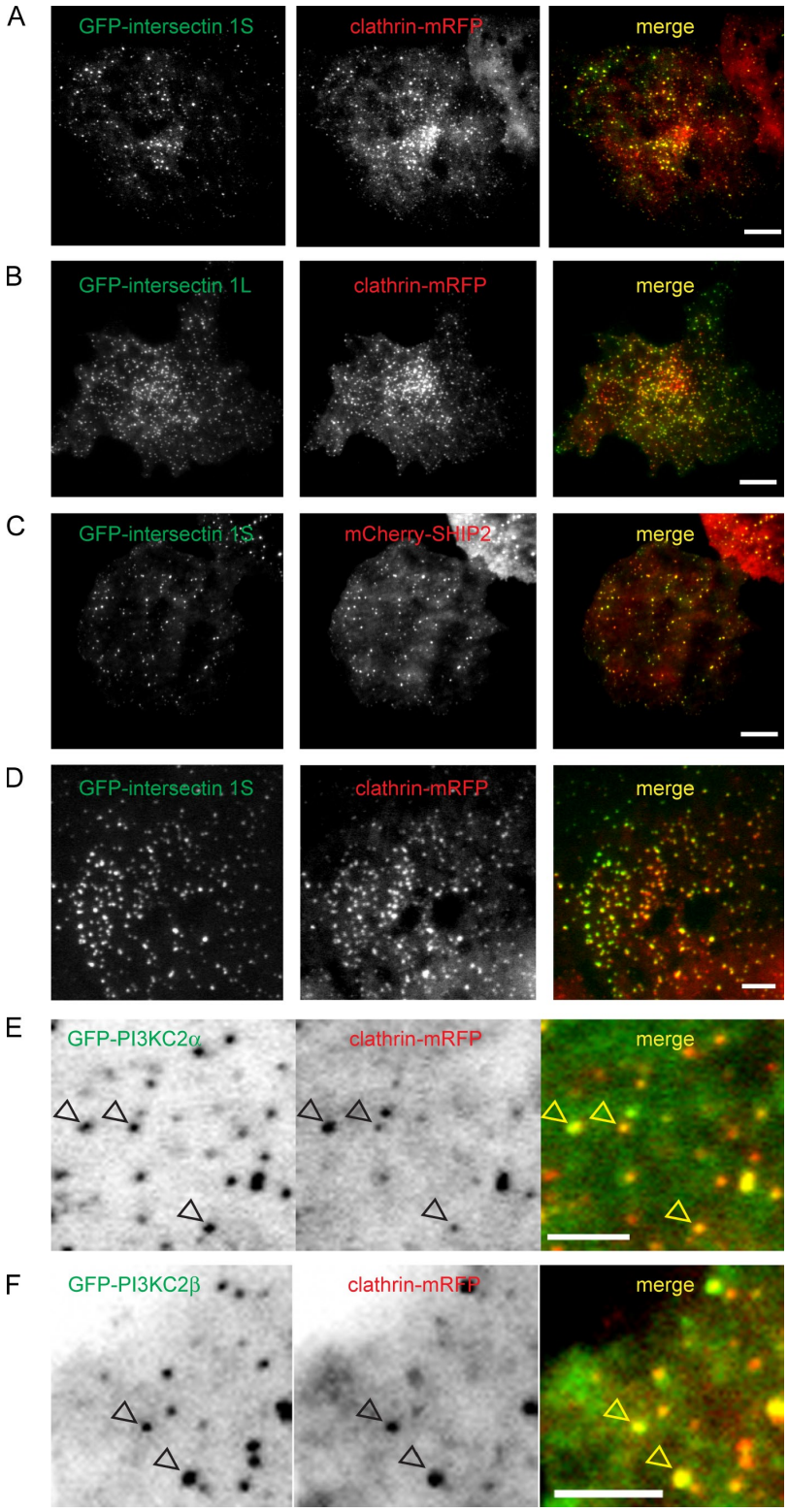


Figure S2. **TIRFM images (from live cells) showing localization of intersectin, SHIP2, and PI3KC2-α and PI3KC2-β at CCPs.** (A–C) Intersectin-S and intersectin-L colocalize with clathrin and SHIP2 in control COS-7 cells. (D) GFP-intersectin colocalizes with clathrin at endocytic CCPs also in SHIP2 KD COS-7 cells. (E and F) Both GFP–PI3KC2-α (E) and –PI3KC2-β (F) colocalize with clathrin-mRFP at endocytic CCPs. Arrowheads point to CCPs positive for PI3KC2-α or PI3KC2-β. Bars, 10 µm.

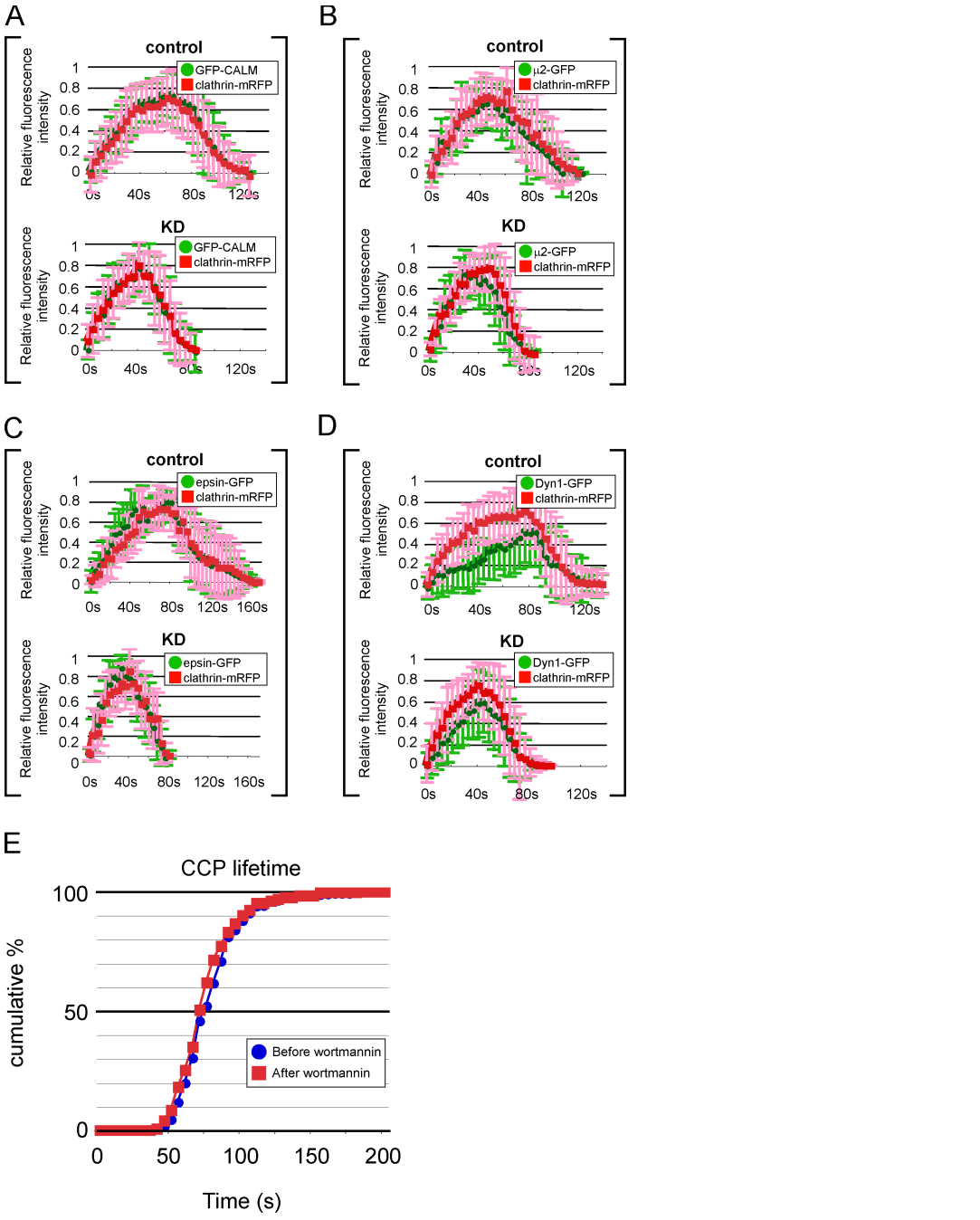
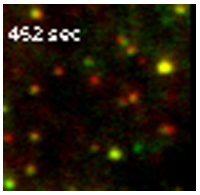
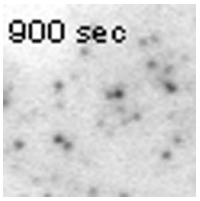


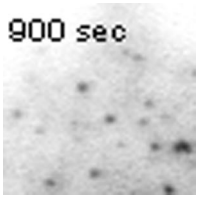
Figure S3. **Time course of coat protein fluorescence at endocytic CCPs and lack of effect of wortmannin on endocytic CCP lifetime.** (A–D) Time course of relative fluorescence intensity of mRFP and GFP fluorescence in cells double transfected with clathrin-mRFP and with GFP fusions of clathrin adaptors and dynamin as indicated. The graphs represent a different representation of the same data shown in Fig. 3 (D–G). Error bars show mean ± SD. (E) Cumulative histograms of CCP lifetime (GFP–clathrin light chain signal) in COS-7 cells before (n = 448) and after (n = 370) the addition of wortmannin.



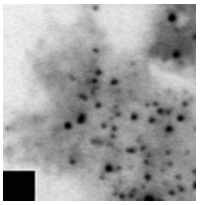
Video 1. **Dynamics of GFP-SHIP2 at CCPs examined by TIRFM COS-7 cells coexpressing GFP-SHIP2 and clathrin-mRFP.** Note that the GFP-SHIP2 signal disappears before clathrin disappearance. GFP-SHIP2 is shown in green, and clathrin-mRFP is shown in red. White lines circle example CCPs. This video is shown at 20 frames/s.



Video 2. **Dynamics of clathrin-GFP in SHIP2 KD COS-7 cells examined by TIRFM.** This video is shown at 50 frames/s.



Video 3. **Dynamics of clathrin-GFP in control COS-7 cells examined by TIRFM.** This video is shown at 50 frames/s.



Video 4. **Dynamics of clathrin-GFP in a ruffle of a FKBP-iSH2–expressing COS-7 cell examined by TIRFM.** This video is shown at 100 frames/s.