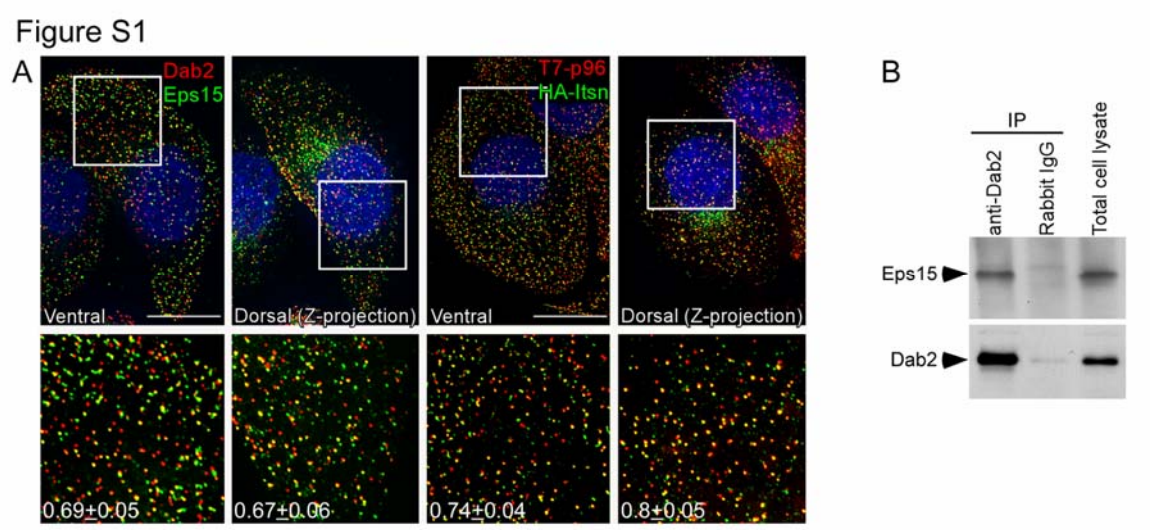
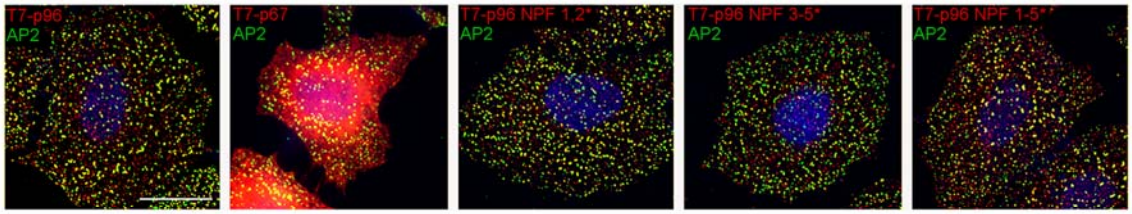



**Figure S1. Dab2 binds and colocalizes with Eps15 and HA-Itsn.**
(A) Untransfected or T7-p96 + HA-Itsn-expressing HeLa cells were grown on collagen IV-coated coverslips, fixed, permeabilized and stained with either anti-Dab2 and anti-Eps15 or anti-T7 and anti-HA. Single 0.2 μm sections at the ventral surface or Z-projections of the dorsal surface are shown. The white boxes indicate the enlarged images shown in the insets. The fraction of Dab2 that colocalizes with Eps15 or HA-Itsn at both surfaces was determined by ImageJ (Manders colocalization coefficient). In all cases random colocalization, obtained by flipping the red channel (Dab2), was <0.10. ~10 cells/condition from two independent experiments were analyzed. Bar,10 μm. (B) HeLa cell lysates containing the cross-linking agent DSP were immunoprecipitated with anti-Dab2 antibody or rabbit IgG and immunoblotted with anti-Eps15.

Figure S2




| | Fraction Dab2 colocalized with AP2 | Fraction AP2 colocalized with Dab2 | Fraction Dab2 colocalized with AP2 (random) | Fraction AP2 colocalized with Dab2 (random) |
|---|---|---|---|---|
| T7-p96 | 0.76 ± 0.14 | 0.73 ± 0.13 | 0.14 ± 0.06 | 0.11 ± 0.04 |
| T7-p67 | 0.16 ± 0.03 | 0.87 ± 0.1 | 0.07 ± 0.02 | 0.5 ± 0.12 |
| T7-p96 NPF 1-2* | 0.86 ± 0.15 | 0.69 ± 0.11 | 0.13 ± 0.05 | 0.1 ± 0.05 |
| T7-p96 NPF 3-5* | 0.68 ± 0.08 | 0.71 ± 0.1 | 0.13 ± 0.05 | 0.12 ± 0.06 |
| T7-p96 NPF 1-5* | 0.68 ± 0.1 | 0.78 ± 0.09 | 0.15 ± 0.05 | 0.1 ± 0.04 |

**Figure S2. The Dab2 NPFs are not required for localization to CCSs.**
HeLa cells were transfected with T7-p96, T7-p67 or T7-p96 NPF mutants. Cells were grown on collagen IV-coated coverslips, fixed, permeabilized and stained with anti-T7 and anti-AP2. Single 0.2 μm sections at the ventral surface are shown. The fraction of T7-Dab2 that colocalized with AP2 (Manders colocalization co-efficient, M1) and the fraction of AP2 that colocalized with T7-Dab2 (Manders colocalization co-efficient, M2) were determined with ImageJ. The red channel (T7-Dab2) was flipped to determine random colocalization. Mean values and standard errors are shown for ~5 cells/treatment. Bar, 10 μm.

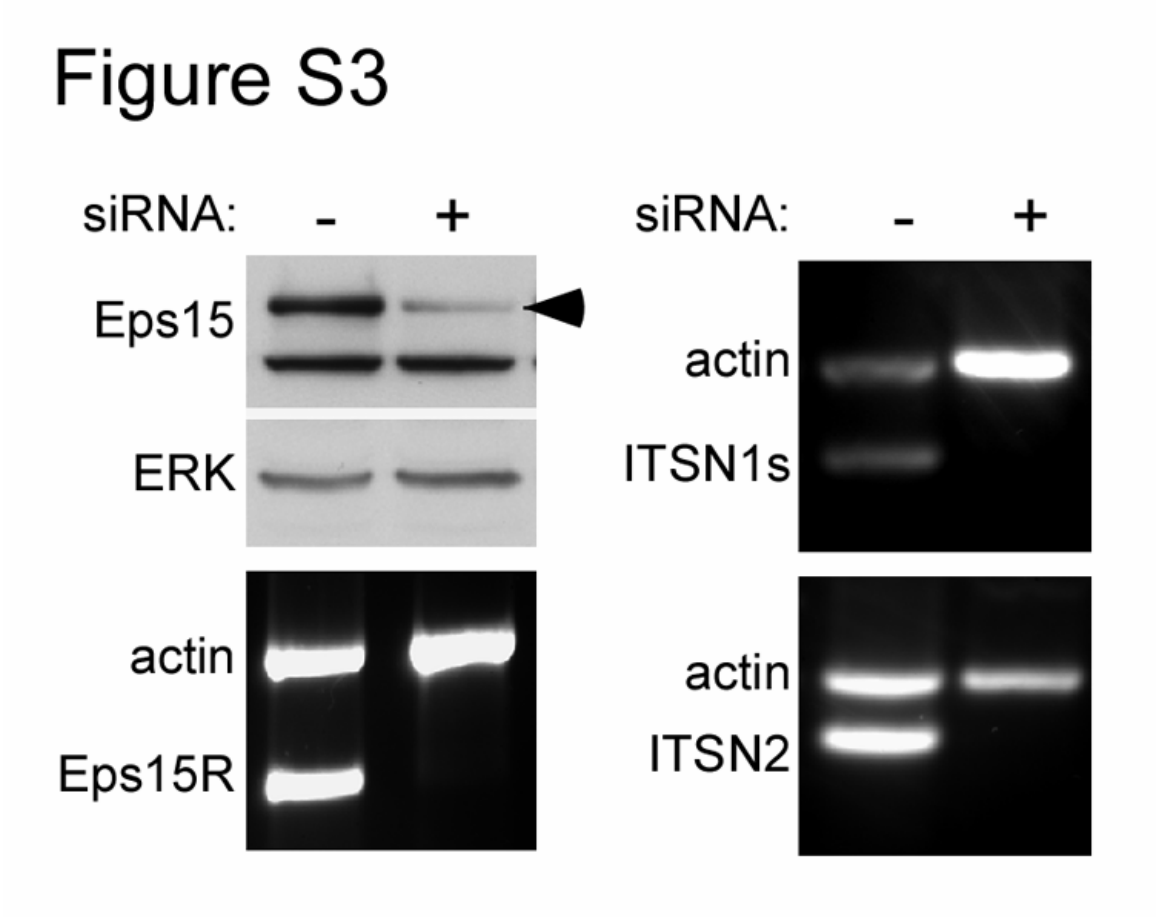
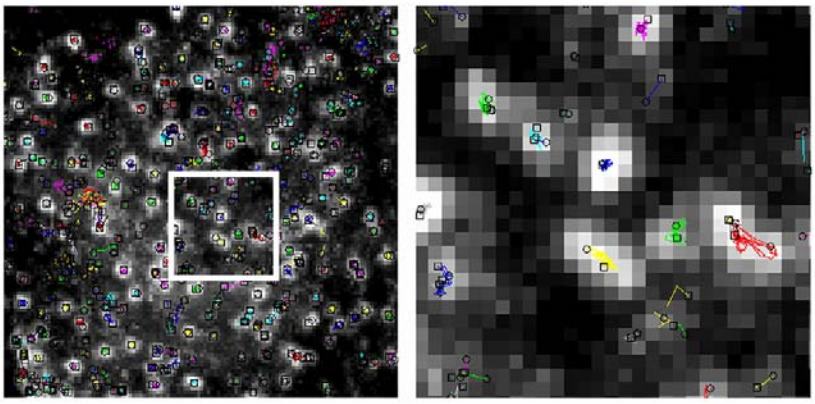


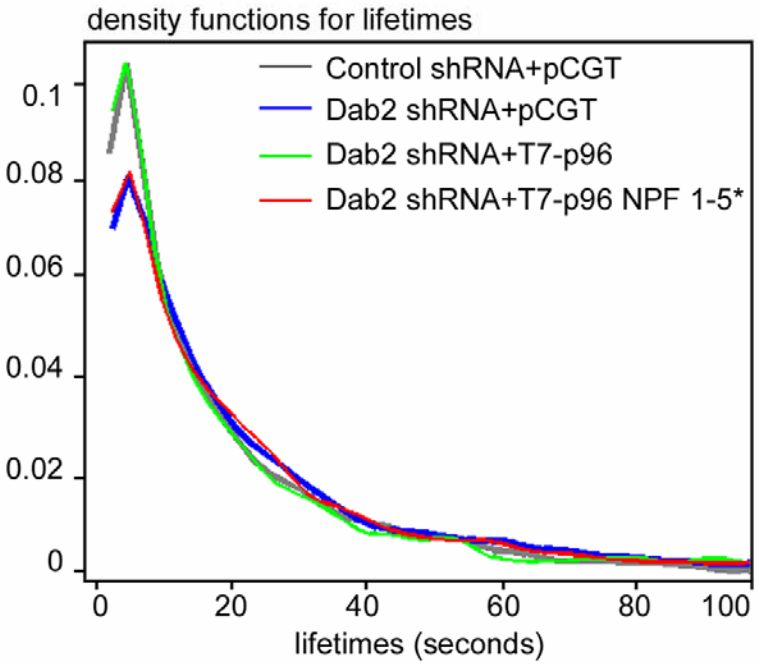

**Figure S3. EH domain protein KD by siRNA.**
EH domain proteins were efficiently depleted from HeLa cells following 2 rounds of transfection with siRNA. Eps15 protein levels were analyzed by Western blot. The arrow indicates the Eps15 band. Eps15R and Itsn mRNA levels were analyzed by RTPCR.

Figure S4

A



B




C

| p values for the Logrank test of difference between the cumulative distribution functions for the lifetimes | |
|---|---|
| Control shRNA vs Dab2 shRNA | 0.00023 * |
| Control shRNA vs Dab2 shRNA+T7-p96 | 0.92612 |
| Control shRNA vs Dab2 shRNA+T7-p96 NPF 1-5* | 0.00335 * |
| Dab2 shRNA vs Dab2 shRNA+T7-p96 | 0.00193 * |
| Dab2 shRNA vs Dab2 shRNA+T7-p96 NPF 1-5* | 0.62784 |
| Dab2 shRNA+T7-p96 vs Dab2 shRNA+T7-p96 NPF 1-5* | 0.01175 * |

**Figure S4. Lifetime distribution for CCSs.**

To track CCSs, ventral surfaces of control and Dab2-deficient HeLa cells re-expressing vector alone, T7-p96 or T7-p96 NPF1-5* and LCa-EGFP were imaged every 2 s for 4 min. (A) A single frame from a video overlaid with all observed trajectories. The white box indicates the enlarged image shown in the inset. (B) Lifetime distribution for CCSs was determined from the elapsed time between the appearance and disappearance of LCa-EGFP puncta. All structures appearing after the first frame and disappearing before the last frame are included. ~80% of larger clathrin structures (size > 0.032$\mu m^2$) persisted for longer than 4 min so were excluded. Also highly motile clathrin structures were not used to calculate lifetimes. (C) P values calculated by log-rank test. ~2000 puncta from two independent experiments were analyzed for each condition.