

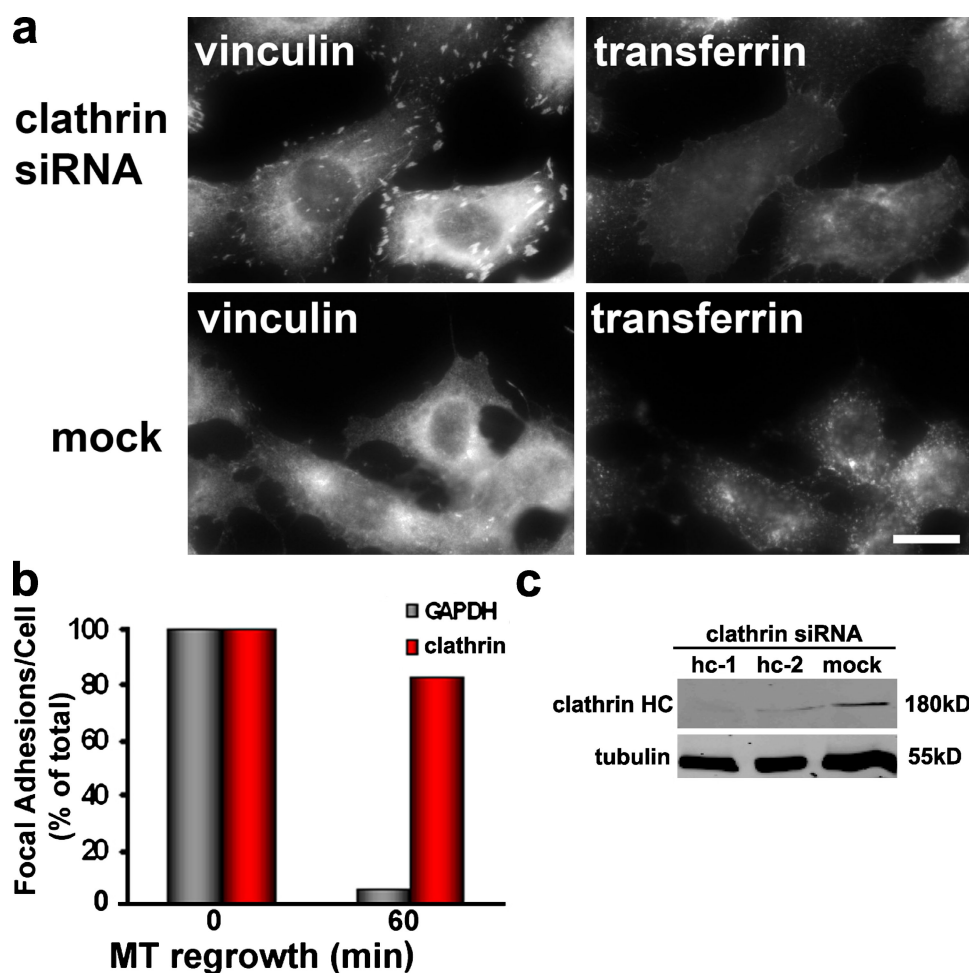
Ezratty et al., <http://www.jcb.org/cgi/content/full/jcb.200904054/DC1>

Figure S1. **Clathrin knockdown prevents focal adhesion disassembly and transferrin internalization compared with mock-transfected cells.** (a) NIH3T3 fibroblasts were transfected with clathrin siRNA or mock treated, and then the focal adhesion disassembly assay was performed in the presence of labeled transferrin. The panels show vinculin staining of focal adhesions and labeled transferrin. (b) Quantification of focal adhesion disassembly in clathrin heavy chain- or GAPDH-depleted NIH3T3 fibroblasts: the number of focal adhesions per cell in cells depleted of clathrin or GAPDH was determined (see Materials and methods). Data are the mean of two independent experiments in which hundreds of focal adhesions were analyzed for at least 30 cells/condition. (c) Western blot of clathrin levels from lysates of mock-treated cells or cells treated with two different clathrin siRNAs (hc-1 and hc-2). Tubulin is shown as a loading control. HC, heavy chain. Bar, 15 μ m.

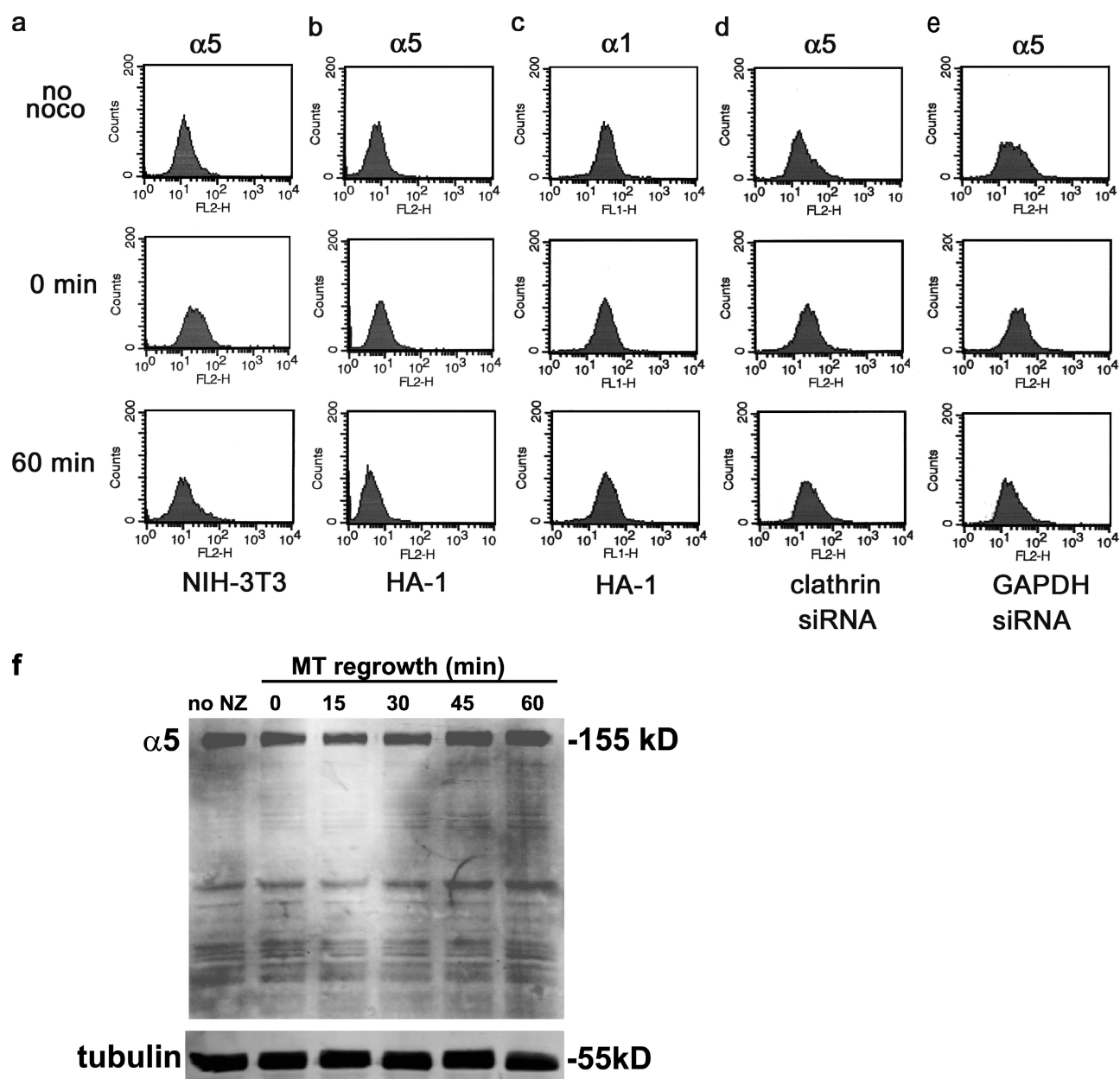


Figure S2. Total surface $\alpha 5$ or $\alpha 1$ integrin levels before and after MT-induced focal adhesion disassembly. (a–e) Representative flow cytometry profiles of surface $\alpha 5$ or $\alpha 1$ integrin (as indicated) in cells before nocodazole treatment (no noco), after nocodazole treatment but before MT regrowth (0 min), and 60 min after nocodazole washout to stimulate focal adhesion disassembly (60 min). (a) Surface $\alpha 5$ integrin in NIH3T3 cells. (b) Surface $\alpha 5$ levels in HA1 NIH3T3 cells that express both $\alpha 5$ and $\alpha 1$ integrins. (c) Surface $\alpha 1$ integrin in HA1 NIH3T3 cells grown on fibronectin. (d) Surface $\alpha 5$ integrin levels in NIH3T3 cells depleted of clathrin. (e) Surface $\alpha 5$ integrin levels in NIH3T3 cells depleted of GAPDH. (f) Blot of integrin $\alpha 5$ levels in cell lysates before nocodazole treatment (no NZ) or during various time points during MT-induced focal adhesion disassembly. Tubulin levels are shown as a loading control.

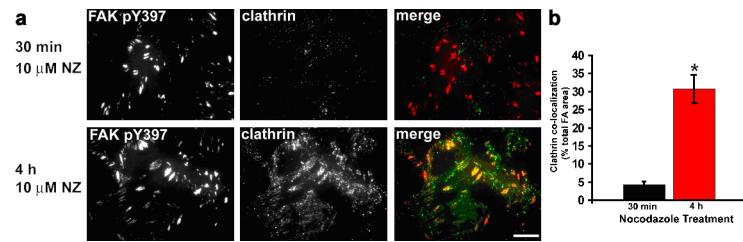


Figure S3. **Clathrin accumulates at focal adhesions during nocodazole treatment.** (a) NIH3T3 fibroblasts were serum starved and then treated with 10 μ M nocodazole (NZ) for the indicated amount of time before being fixed and stained for pY397 FAK and clathrin. The right panels are merged images of pY397 FAK (red) and clathrin (green). (b) Quantification of clathrin colocalization with focal adhesions (FA) in cells treated with nocodazole for 30 min or 4 h. *, $P \leq 0.001$ by Student's *t* test. Bar, 15 μ m.

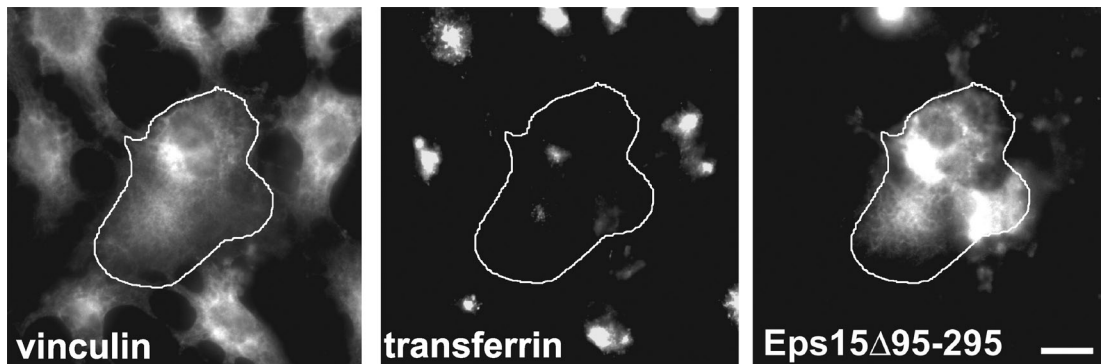


Figure S4. **Expression of Eps15 Δ 95-295 inhibits transferrin uptake but not focal adhesion disassembly.** NIH3T3 fibroblasts transiently expressing Eps15 Δ 95-295 were treated with 10 μ M nocodazole and stimulated for focal adhesion disassembly before being fixed and processed for immunofluorescence. Fluorescent transferrin was added during MT regrowth. The cell expressing Eps15 Δ 95-295 is outlined in white to show that it does not endocytose transferrin. Bar, 15 μ m.

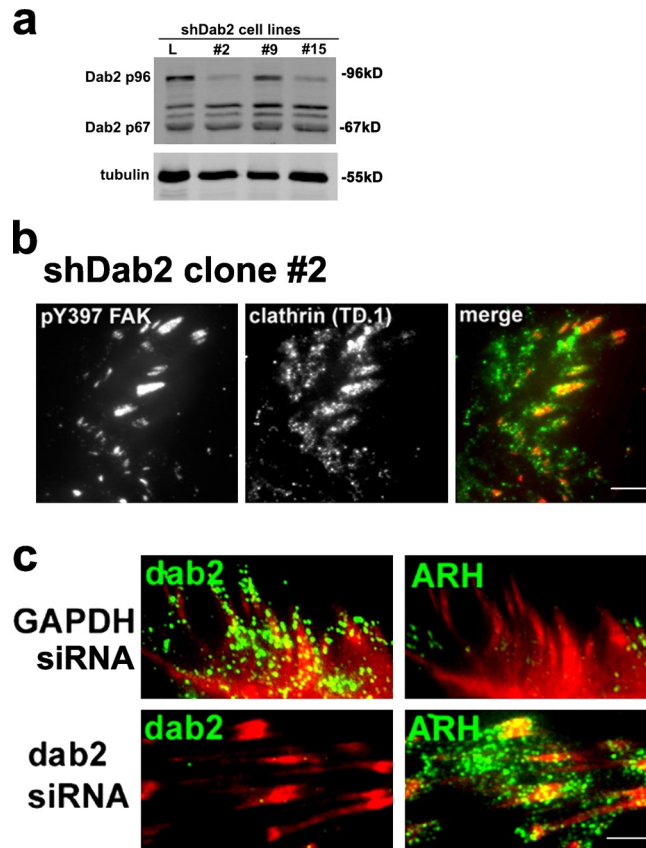


Figure S5. **Localization of clathrin and clathrin adaptor proteins in cells depleted of Dab2.** (a) Western blot of total cell lysates derived from three different NIH3T3 clonal cell lines (#2, #9, and #15) in which Dab2 was stably depleted using an shDab2 hairpin construct. L is lysate from untreated cells. Tubulin is shown as a loading control. Note that only the larger p96 isoform was targeted by the short hairpin RNAs. (b) The shDab2 clone #2 cell line was treated with 10 μ M nocodazole before being fixed and stained for pY397 FAK and clathrin. The right panel is a merged image of pY397 FAK (red) and clathrin (green). (c) Images of an NIH3T3 cell treated with siRNA against GAPDH or Dab2 before being fixed and stained for pY397 FAK (red) and ARH or Dab2 (green), as indicated. ARH is not localized at focal adhesions in the GAPDH-depleted cell but redistributes to focal adhesions when Dab2 is depleted. Bars: (b) 15 μ m; (c) 2 μ m.