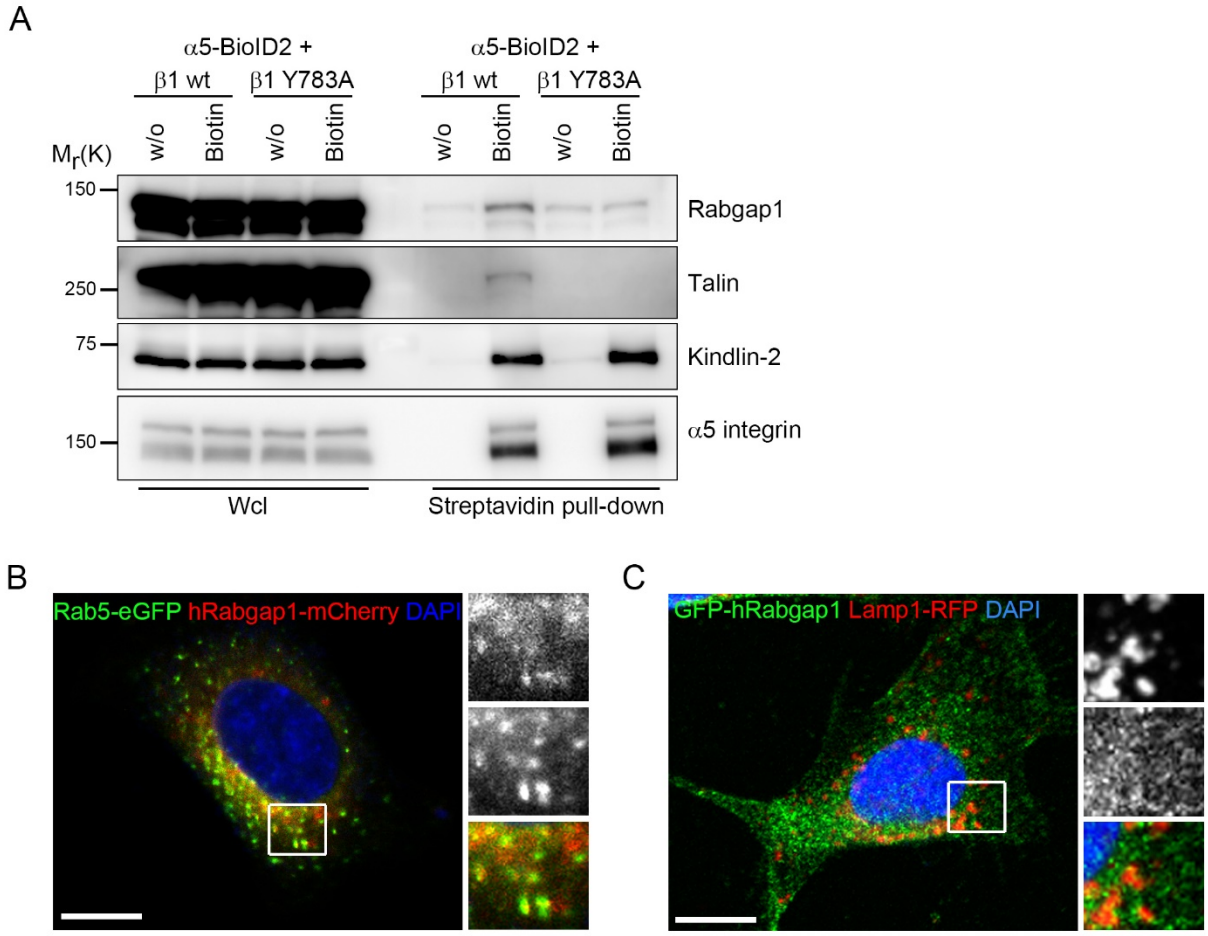

Figure S1. Rabgap1 co-staining with EEA1 and Lamp1. (A) Western blot analysis of proximity-biotinylation assays in wild-type or Y783A β1 integrin fibroblasts expressing α5 integrin fused to the promiscuous biotin ligase BioID2. Cells were left untreated (w/o) or incubated with 50 µM biotin for 16 hours before cell lysis and streptavidin bead pull-down. Wcl, whole cell lysate. **(B,C)** Confocal images of fibroblasts expressing Rabgap1-mCherry or GFP-hRabgap1 together with eGFP-tagged Rab5a (B) or RFP-tagged Lamp1 (C). DAPI (blue) was used to stain nuclei. Scale bars, 10 µm.

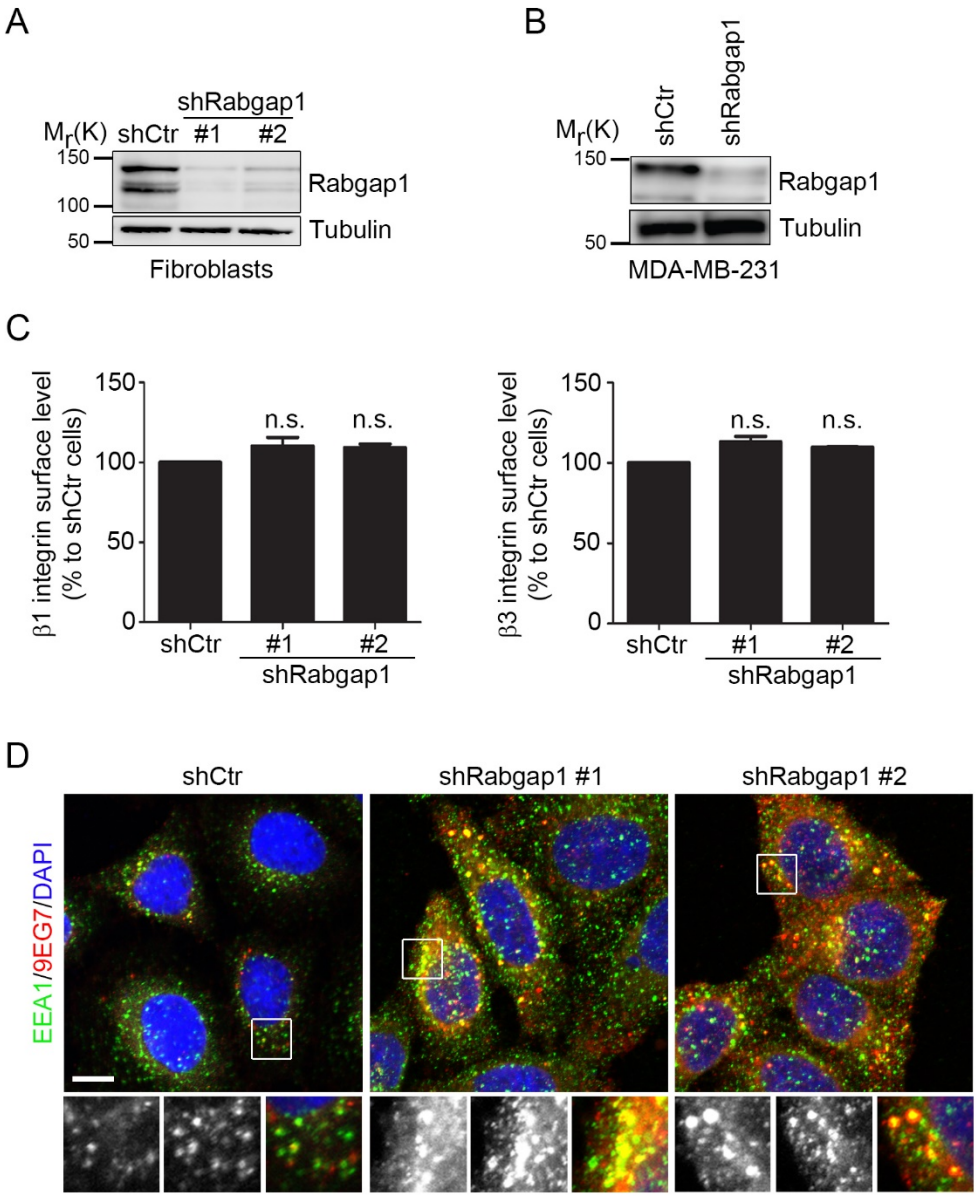

Figure S2. Rabgap1 depletion does not affect ß1 and ß3 integrin surface level. (A, B) Western blot analysis of control (shCtr) and Rabgap1-depleted (shRabgap1) mouse fibroblasts (A) and MDA-MB-231 cells (B). Tubulin served as loading control. **(C)** Quantification of ß1 and ß3 integrin surface levels in control and Rabgap1-depleted fibroblasts determined by FACS. Data are mean±s.d.; *n*=3; n.s., not significant (unpaired *t*-test). **(D)** Confocal images of control (shCtr) or Rabgap1-depleted (shRabgap1) fibroblasts after surface labeling of active ß1 integrins (9EG7, red) at 4°C for 30 min followed by integrin internalization at 37°C for 30 min and the induction of integrin recycling. After an acid wash to remove antibody from the cell surface the cells were co-stained with an antibody against EEA1 (green). DAPI (blue) was used to stain nuclei. Scale bar: 10 µm.

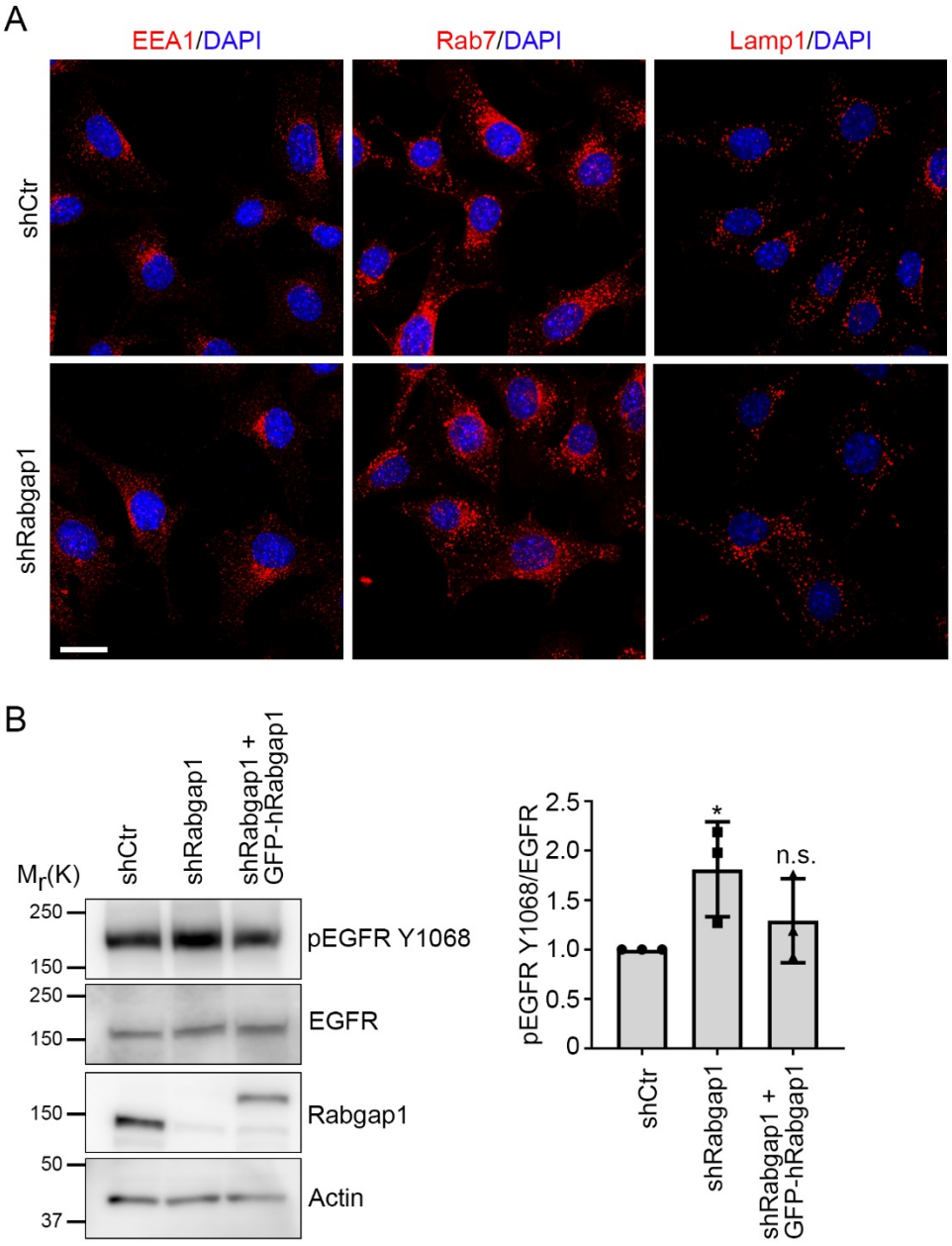

Figure S3. Effect of Rabgap1-depletion on endosomal marker localization and EGFR activation. (A) Confocal images of control (shCtr) and Rabgap1-depleted (shRabgap1) fibroblasts stained with antibodies against EEA1, Rab7 or Lamp1. DAPI (blue) was used to stain nuclei. Scale bar, 20 µm. (B) Western blot and densitometric analysis of control (shCtr), Rabgap1-depleted (shRabgap1) and Rabgap1-depleted fibroblasts after re-expression of wild-type GFP-tagged Rabgap1 after starvation and EGF treatment for 10 min at 37°C. Data are mean ±s.d. n=3; *P<0.05; n.s., not significant (unpaired t-test).

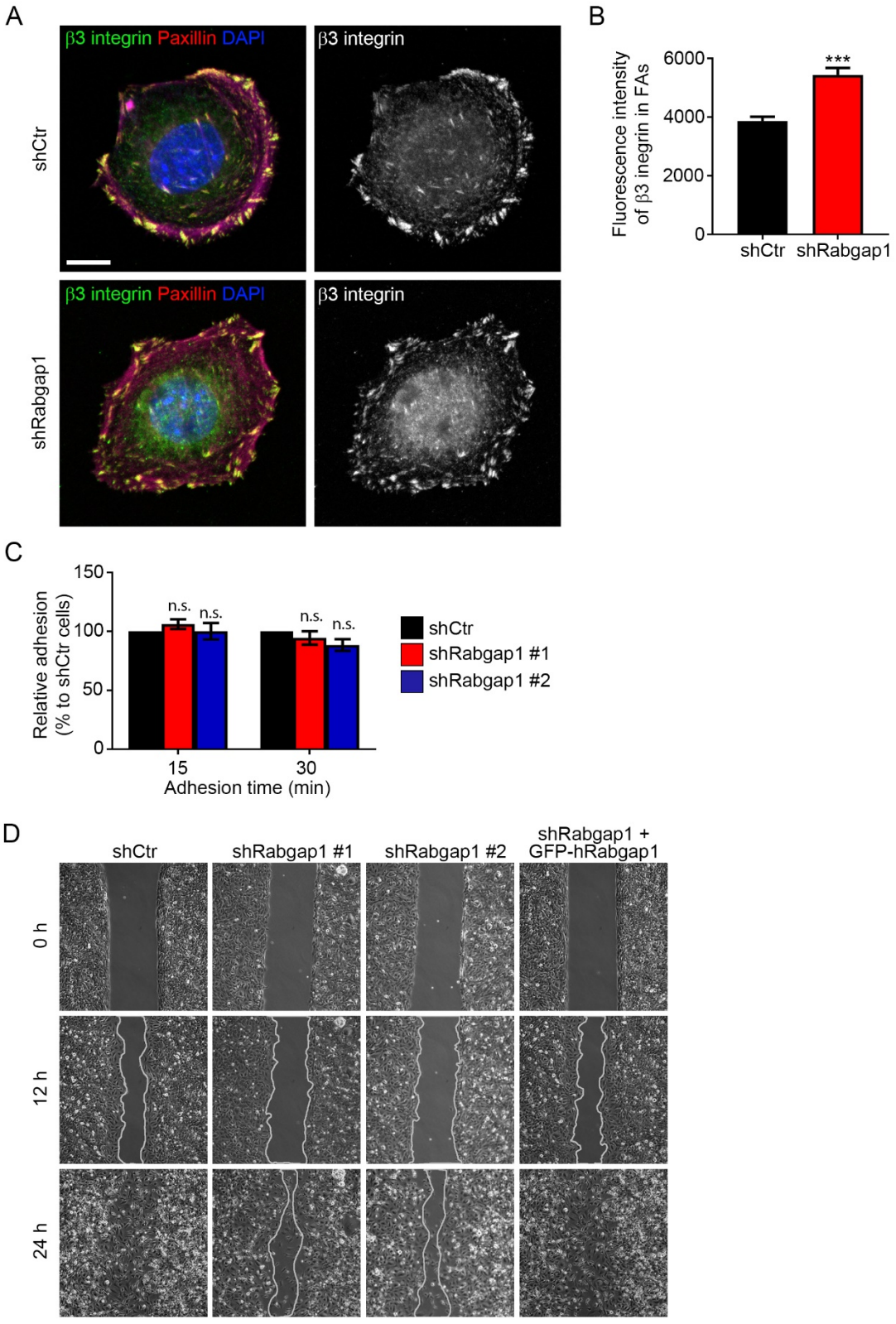

Figure S4. Rabgap1 depletion increases β3 integrin levels in focal adhesions. (A) Confocal microscopy images of control (shCtr) and Rabgap1-depleted (shRabgap1) mouse fibroblasts plated

for 90 min on fibronectin with antibodies against paxillin (red) and β3 integrin (green). DAPI (blue) was used to stain nuclei. Scale bar: 10 µm. **(B)** Quantification of β3 integrin immunofluorescence intensity with Image J (integrate density) within the focal adhesions. Data are mean+s.e.m. of n=46 cells; ***P<0.0001 (unpaired t-test). **(C)** Adhesion assay of control (shCtr) and Rabgap1-depleted (shRabgap1) fibroblasts on fibronectin substrates for 15 and 30 minutes. Data are mean±s.e.m. n=5; n.s., not significant (unpaired t-test). **(D)** Migration analysis of the indicated cell lines in a scratch wound assay. Pictures of representative wounds from time-lapse videos at the indicated times are shown.