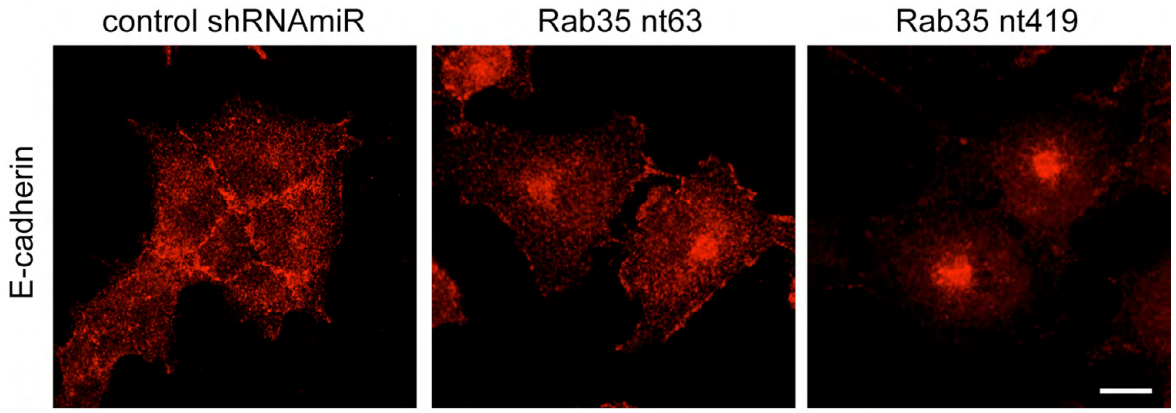

Fig. S1. Rab35 knock down inhibits recycling of E-cadherin. COS-7 cells were treated with control shRNAmiR or two different shRNAmiRs targeting Rab35 (Rab35 nt63 and Rab35 nt419) as indicated. Cells were transfected with Flag-tagged E-cadherin and then stained with an anti-Flag antibody. Scale bar = 10 μm.

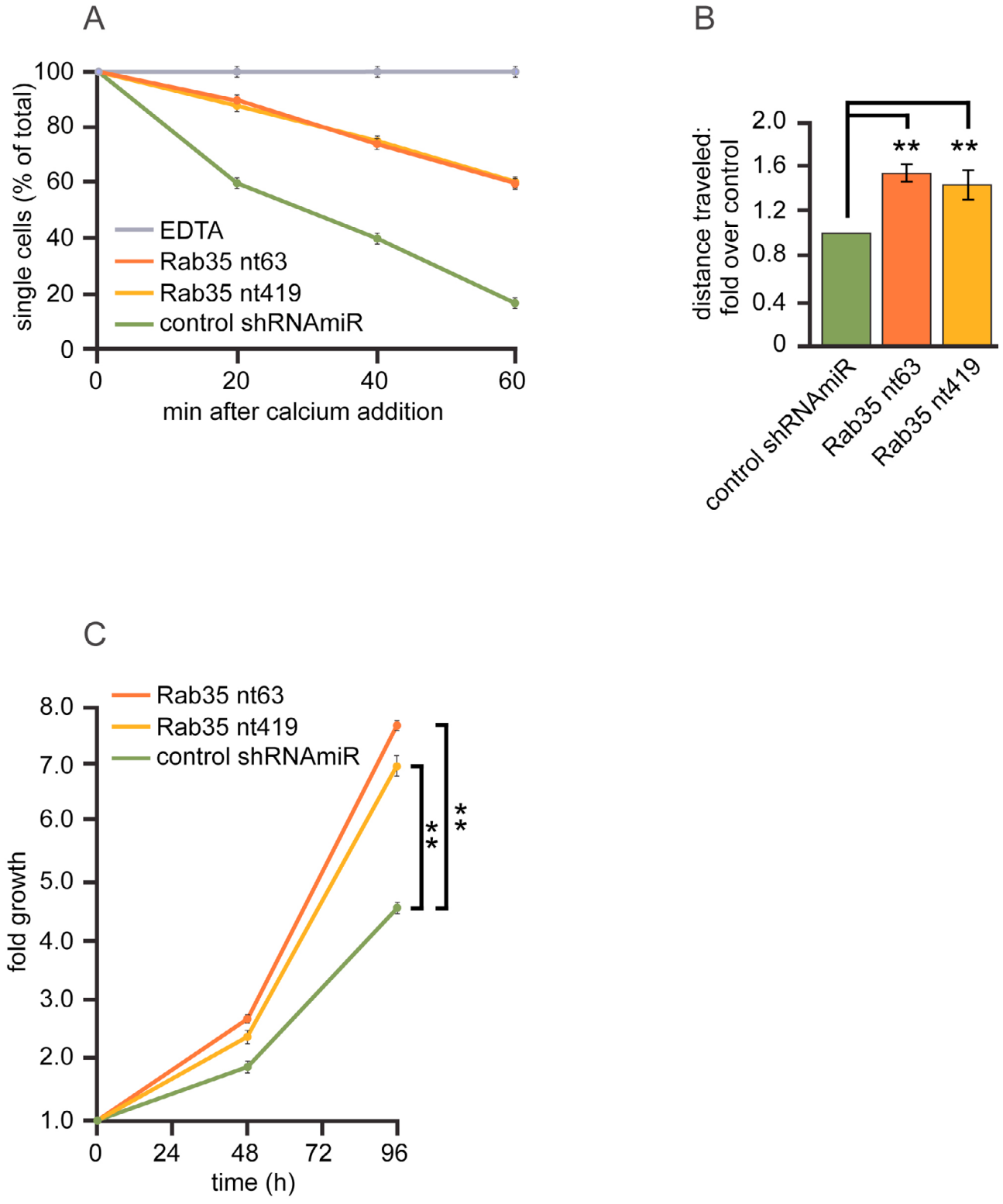

Fig. S2. Adhesion, migration and proliferation of glioblastoma derived U251 cells. (**A-C**) For all panels, U251 glioblastoma-derived cells were treated with control shRNAmiR or two different shRNAmiRs targeting Rab35 (Rab35 nt63 and Rab35 nt419) as indicated. **(A)** Cells were treated with 2 mM EDTA to dissociate monolayers into single cells. The cells were then pelleted, washed and resuspended in Ca^{2+}-containing culture media. Cells were agitated gently and 50 µl aliquots of cell suspension were analyzed at 20 min intervals. Four fields of approximately 200 cells were analyzed per time point from 3 duplicate aggregation assays. Statistical analysis employed a repeated-measure two-way ANOVA followed by a Bonferroni post-test. (**B**) A scratch was made in a confluent monolayer of cells and images were obtained immediately after the scratch and after 16 h. Cell migration into the scratch was determined from 3 duplicate experiments. Error bars represent standard error of the means and statistical analysis employed a repeated-measure one-way ANOVA followed by a Dunnet's post-test (**p<0.01). **(C)** Cells were plated in 96-well plates at 1000 cells per well. Cell growth at indicated time point represents fold change relative to time 0. The graph represents 8 repeats from 3 separate experiments. Error bars represent standard error of the means. Statistical analysis employed a repeated-measure two-way ANOVA followed by a Bonferroni post-test (*p<0.05).

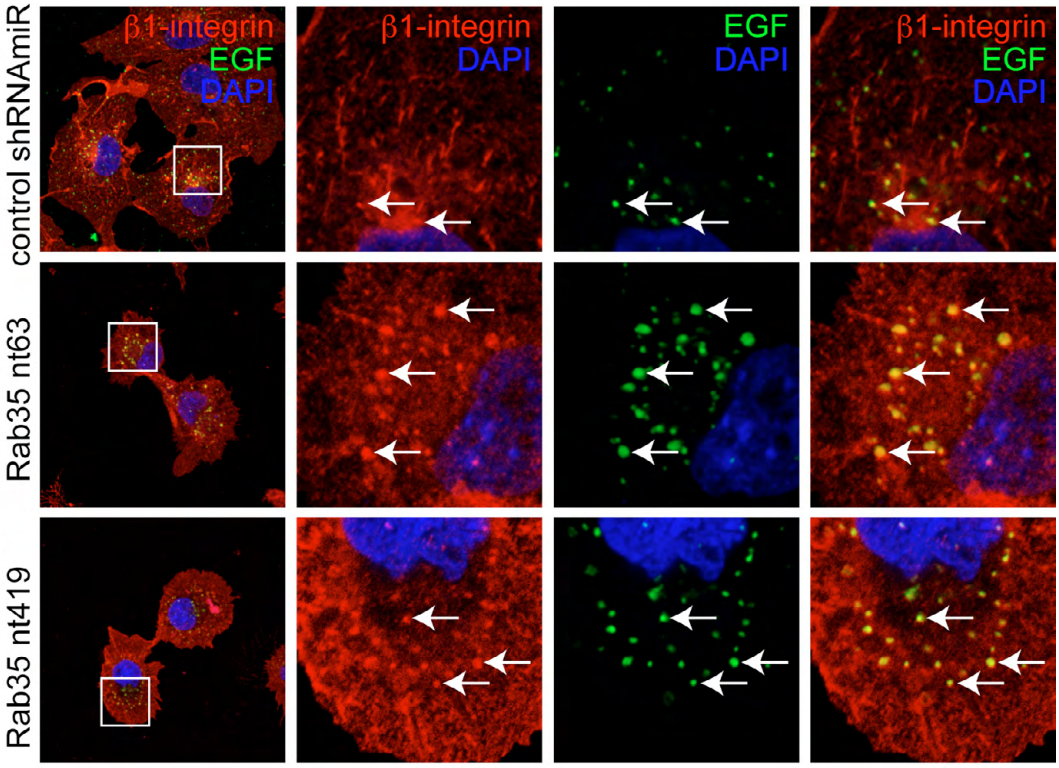

Fig. S3. β1-integrin and internalized EGF show enhanced co-localization following Rab35 knock down. COS-7 cells were treated with control shRNAmiR or two different shRNAmiRs targeting Rab35 (Rab35 nt63 and Rab35 nt419) as indicated. Cells were then serum-starved 2 h and then treated for 10 min with 2 ng/ml Alexa647-labeled EGF. Cells were then fixed and stained for endogenous β1-integrin along with DAPI to reveal the nucleus. Arrows point to enlarged intracellular vesicles containing EGF and β1-integrin.

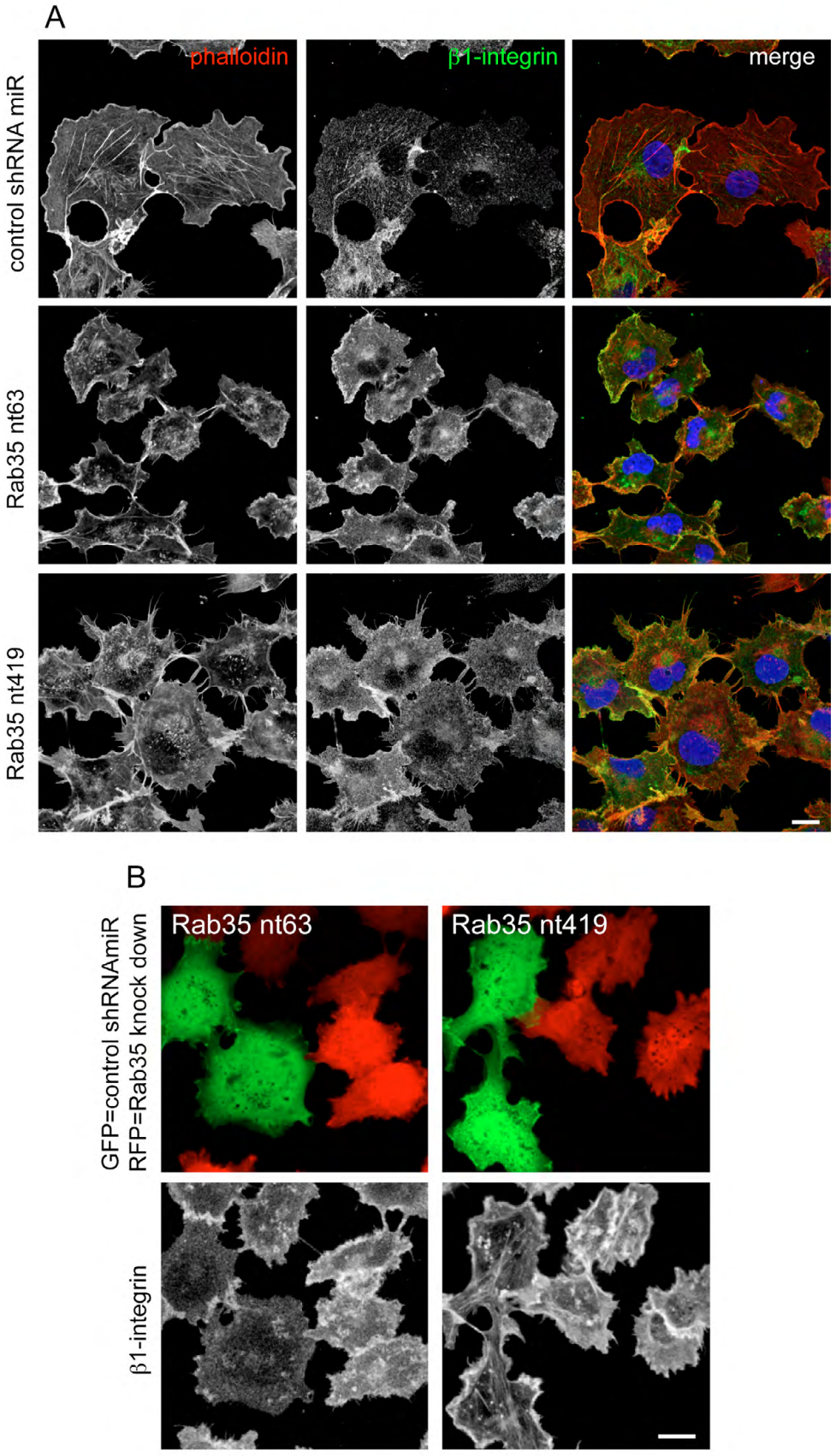

Fig. S4. Rab35 knock down cells demonstrate alterations in cell morphology. **(A)** COS-7 cells were treated with control shRNAmiR or two different shRNAmiRs targeting Rab35 (Rab35 nt63 and Rab35 nt419) as indicated. Cells were then stained with phalloidin to reveal F-actin and with an antibody recognizing β1-integrin. Scale bar = 10 μm. **(B)** COS-7 cells were treated with control shRNA driven from a plasmid also driving GFP expression or with shRNAs targeting Rab35 (Rab35 nt63 and Rab35 nt419) and driven from a plasmid also driving RFP expression. The cultures were then mixed, fixed and stained for β1-integrin. Scale bar = 10 μm.