

SUPPLEMENTARY FIGURE LEGENDS

Figure S1. Representative immunoblots of RNAi-mediated silencing efficiency for Arf6 and BRAG2. HeLa cells were treated with siRNAs as indicated and lysates were probed with antibody to BRAG2 (top) or Arf6 (bottom) to demonstrate silencing efficiency of experiments in Figure 2.

Figure S2. BRAG2 depletion affects activation of endogenous Arf6. HeLa cells were either mock-depleted or depleted of BRAG2 with two different siRNAs. Lysates were precipitated with GST-GGA3 to measure activation of endogenous Arf1 and Arf6. Values are normalized to mock-depleted controls (t-test denotes * n=3, p<0.05).

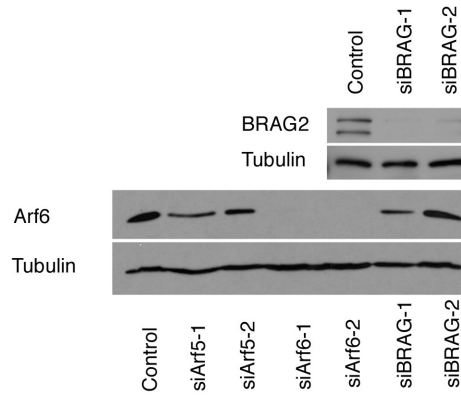
Figure S3. Effects of BRAG2 depletion on expression of endogenous Arfs. (A) Lysates from HeLa cells treated with siBRAG2-1 or siBRAG2-2 were resolved by SDS-PAGE and probed with anti-Arf1, a pan-Arf antibody (1D9), or anti-Arf6 antibodies to detect total Arf expression. (B) Densitometry was used to quantify the amount of Arf normalized to control for each siRNA condition (t-test denotes * n=4, p<0.05).

Figure S4. BRAG2 activates Arf4, but Arf4 does not regulate integrin internalization. Arf4-GFP was co-transfected with either empty vector or HA-BRAG2b into COS-7 cells. (A) Lysates were incubated with GST-GGA3 to precipitate Arf4-GTP and precipitates were probed using an antibody to GFP (B) Quantitation of Arf4 activation (* n=7 p<0.05 +/-SEM). (C) Internalization of β 1 integrin antibody by cells treated with control (non-target), Arf4, or Arf5 siRNAs, was measured using the flow cytometry assay as described in Fig. 4. (D) The amount of β 1 integrin antibody bound to the cell surface at time 0 (prior to internalization) was measured by flow cytometry. (t-test denotes * n=3, p<0.05).

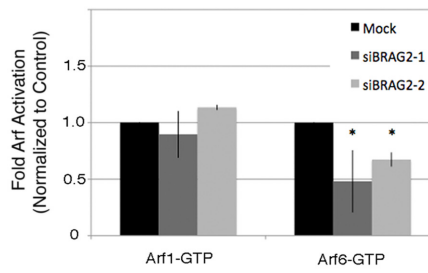
Figure S5. BRAG2b colocalization with endogenous AP-2. (A-C) HeLa cells were transfected with BRAG2b-GFP (A), fixed, and immunostained with an antibody to AP-2 (B). Fixed cells were then imaged by TIR-FM. (D-F) Control cells expressing unfused GFP show diffuse staining with no concentration at clathrin-coated structures.

Figure S1-S3

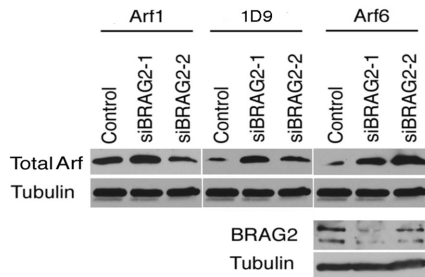
S1.



S2.



S3A.



S3B.

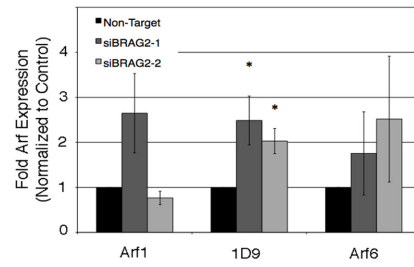
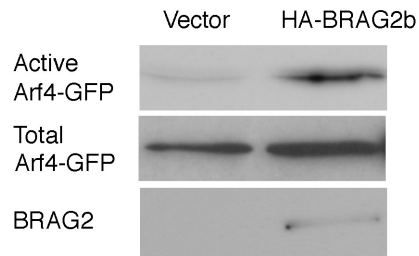
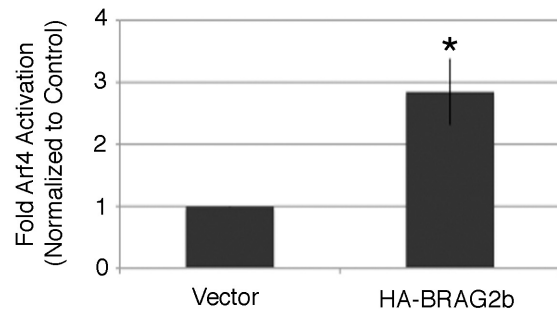


Figure S4

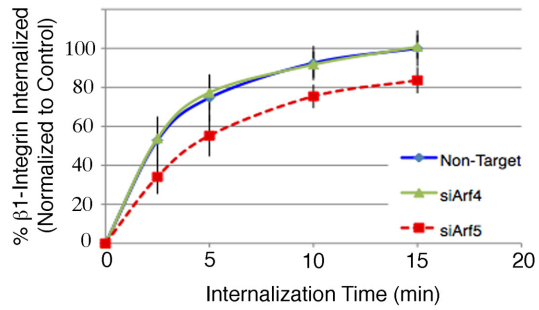
S4A.



S4B.



S4C.



S4D.

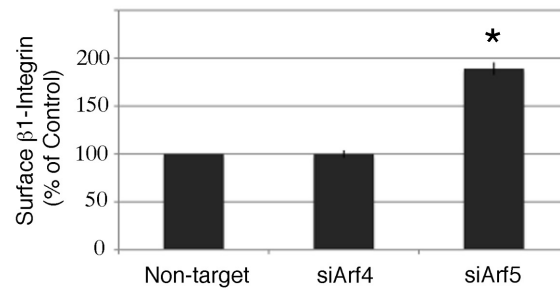


Figure S5

S5A-F

