

Filamin A Regulates Caveolae Internalization and Trafficking in Endothelial Cells

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Supplemental Figure Legends:

Figure S1. Efficiency of FLN A, FLN B and Cav-1 siRNA mediated protein depletion in HLMVEC. HLMVEC were transfected with control, FLN A, FLN B or Cav-1 siRNA and the level of knockdown quantified by densitometric analysis of Western blots. Bar graph represents the level of expression of FLN A, FLN B or Cav-1 as a % of expression level in cells transfected with control siRNA (mean \pm SEM; n=3). The efficiency of knockdown was 76% for FLN A, 82% for FLN B and 67% for Cav-1.

Figure S2. Actin cytoskeletal staining in cells following reduction of FLN A or FLN B protein. (A) HLMVEC transfected with control, FLN A, or FLN B siRNA, and cells pretreated with 100 nM Latrunculin B or Jasplicinolide were subjected to F/G actin fractionation as described in Methods. An equal volume of each fraction was loaded and the amount of actin in F- and G fractions quantified by densitometry of Western blots. Bar graph represents F/G actin ratio (mean \pm SEM, n=3). Knockdown of FLN A or FLN B did not induce redistribution of actin between fractions. (B) HLMVEC were transfected with control, FLN A or FLN B siRNA (a, b, and c respectively) and stained with Alexa-568-Phalloidin or co-transfected with siRNA and actin-YFP (d, e, f) and fixed. Actin staining was analyzed by confocal microscopy. Bars = 10

μm . Localization and amount of polymerized actin in cells transfected with FLN A or FLN B siRNA was not different from that in cells transfected with control siRNA.

Figure S3. Analysis of Cav-1-GFP co-localization with markers of recycling organelles in M2 and A7 cells. M2 and A7 cells were transfected with Cav-1-GFP, and then 24h after transfection, uptake of Alexa-543 CTB (**A**) or immunostaining of early endosomal marker EEA-1 (**B**) or lysosomal marker Lamp-1 (**C**) assessed by confocal microscopy. In both cell lines, Cav-1-GFP partially co-localized with CTB and EEA1, but not with Lamp-1.

Supplementary Figures

Figure S1

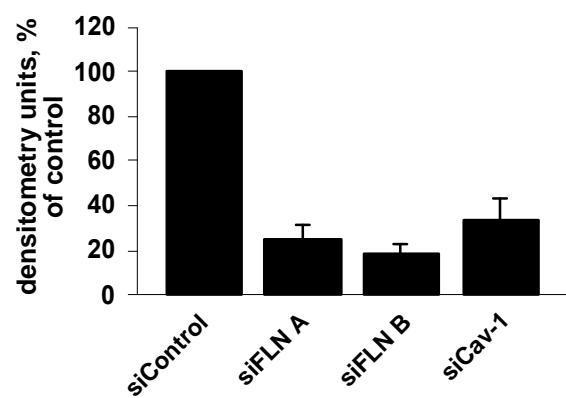


Figure S2

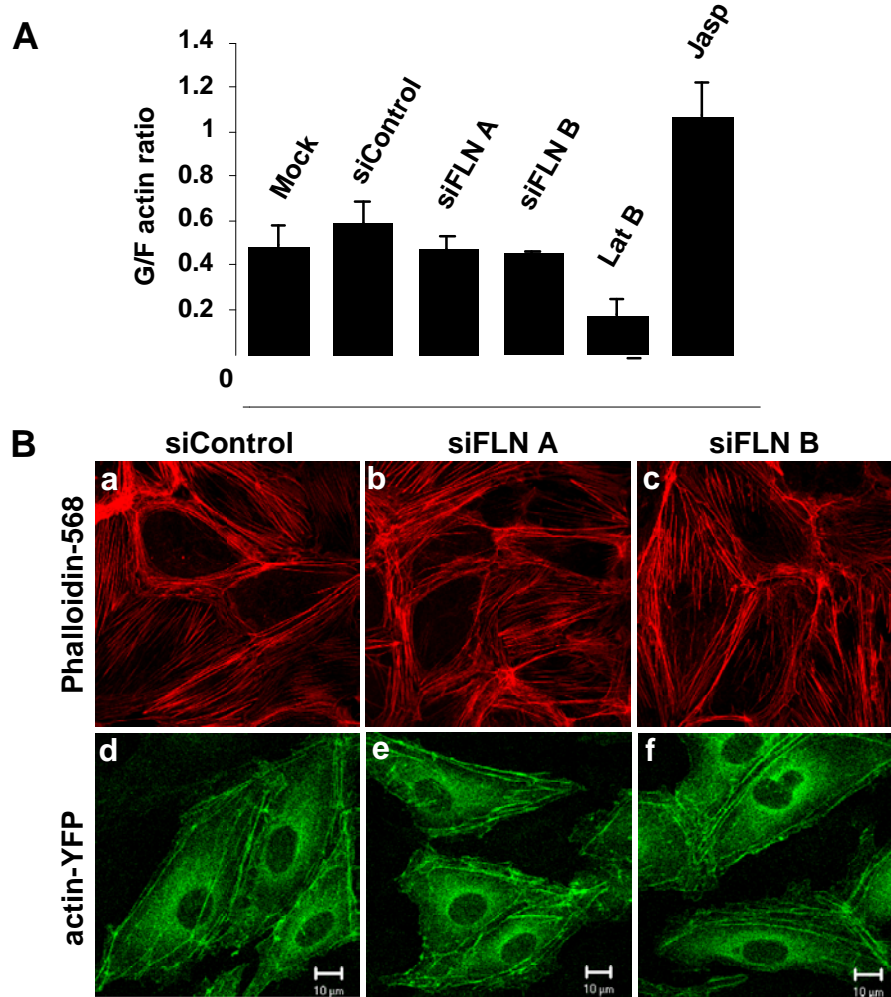
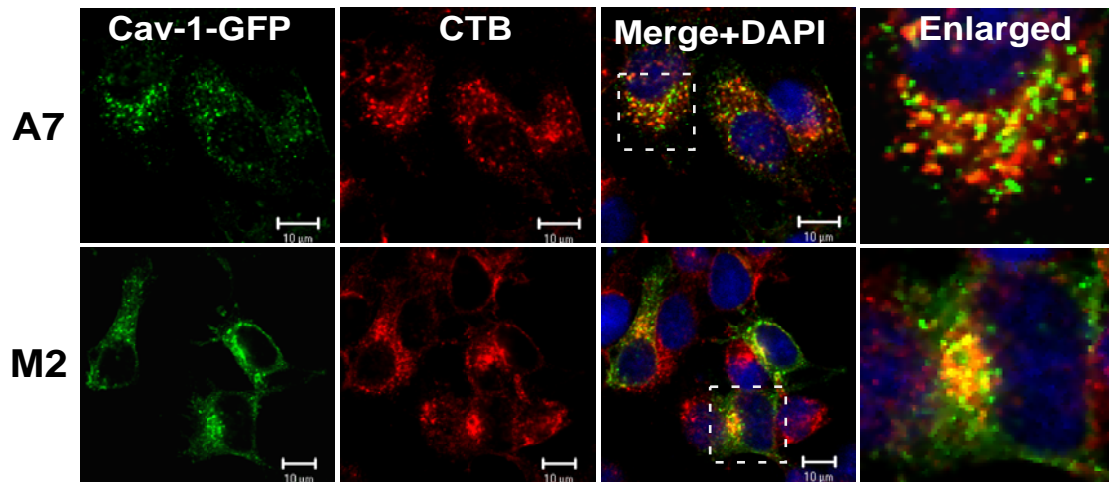
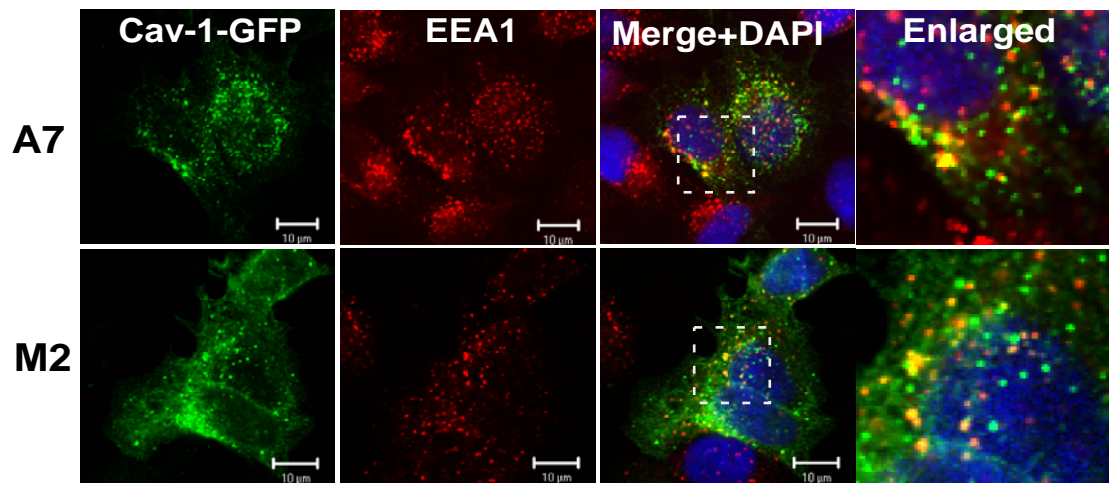


Figure S3

A



B



C

