Supplementary information

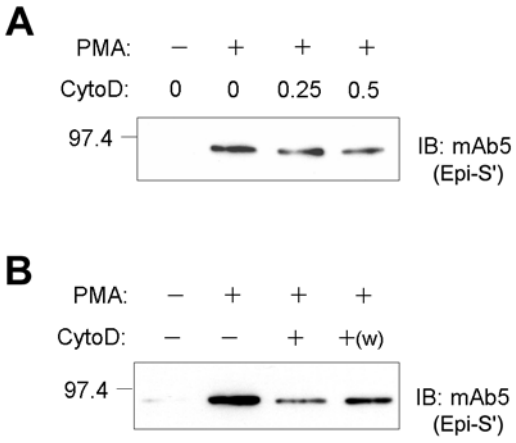

Figure S1. Cytochalasin D inhibits the epithin release in a dose-dependent and reversible manner.

427.1.86 cells were pretreated with cytochalsin D (0, 0.25, and 0.5 μM in panel A, and 0, and 0.5 μM in panel B) in serum-free medium for 1 h, and then incubated in the same medium supplemented with 1 μM PMA for 1 h as indicated. TCA-precipitated proteins in the medium were analyzed by Western blotting with mAb5. To examine the reversibility of cytochalasin D effect, cytochalasin D was washed out for the cells treated with 0.5 μM cytochalasin D (lane 4 in panel B, labeled by +(w)), and the cells were incubated in growth medium for 30 min before PMA treatment. Cytochalasin D exhibited the toxic effects in 427.1.86 cells at concentrations of 1.0 μM or above.

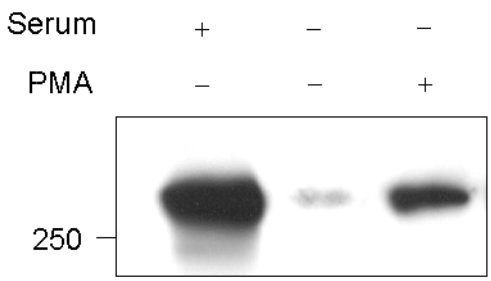

Figure S2. Serum enhances the interaction of epithin and filamin.

Interaction of epithin and filamin was tested in COS7 cells in growth medium containing 10% FBS, serum free medium, and serum free medium supplemented with 1 μM PMA. Both serum and PMA enhanced interaction of the two proteins.

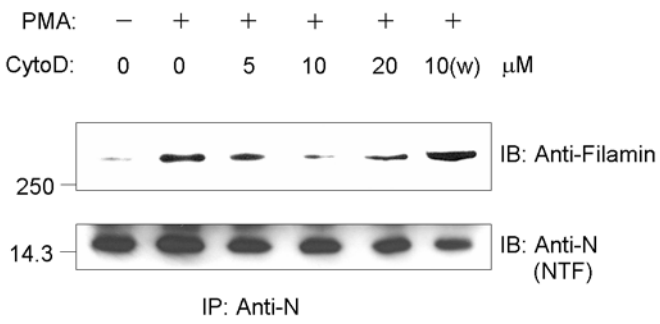

Figure S3. Cytochalasin D impairs the interaction of epithin and filamin.

COS7 cells were transfected with epithin cDNA. One day after transfection, cells were treated with increasing amounts of cytochalasin D and 1 μM PMA as indicated. The cell lysates were immunoprecipitated with anti-N antiserum and blotted with anti-filamin antibody or anti-N antiserum. To examine the reversibility of cytochalasin D's effect, cytochalasin D was washed out for the cells treated with 10 μM cytochalasin D (lane 6, labeled by 10(w)), and the cells were incubated in growth medium for 30 min before PMA treatment. COS7 cells were much more resistant to the cytochalasin D treatment than 427.1.86 cells. Cytochalasin D exhibited toxic effects in COS7 cells at concentrations of 20 μM or above.

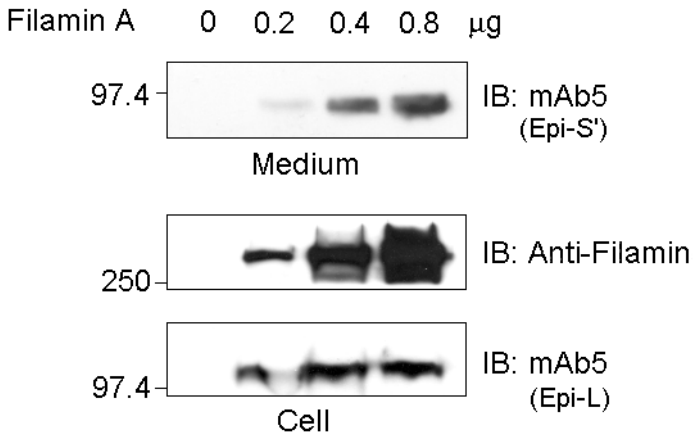

Figure S4. Epithin release in M2 cells is filamin A dose-dependent.

M2 cells were transfected with 0.8 μg of epithin cDNA and increasing amounts of filamin-A cDNA as indicated. Proteins in the media were precipitated with TCA and analyzed by Western blotting with mAb5.

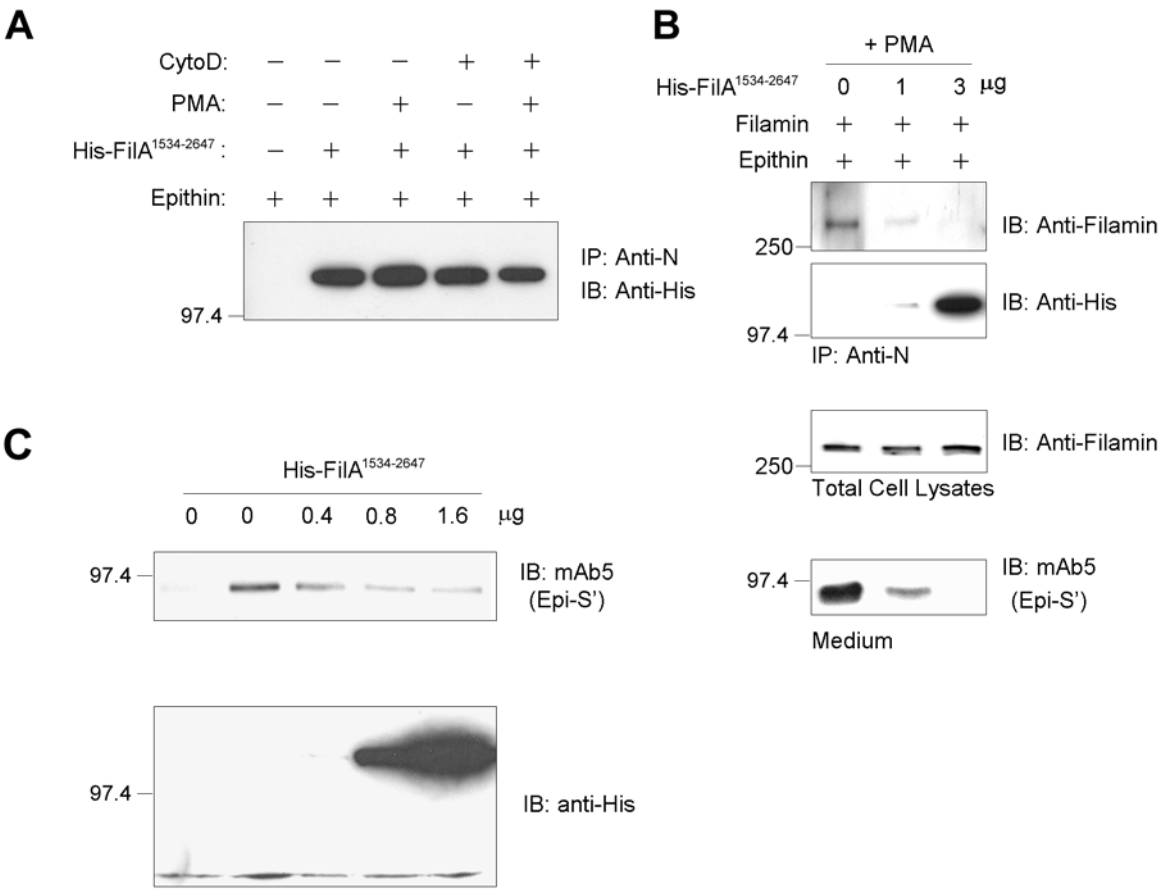

Figure S5. Interaction of filamin with epithin and release of epithin are inhibited by actin binding-deficient filamin A.

A, epithin and His-tagged human filamin $A^{1534-2647}$ constructs were co-transfected in M2 cells, and the cells were treated with PMA and/or cytochalasin D as indicated. Cell lysates were immunoprecipitated with anti-N antiserum, and blotted with anti-His antibody. B, M2 cells

were transfected with the indicated cDNAs and treated with PMA. Cell lysates were immunoprecipitated with anti-N antiserum, and blotted with anti-filamin antiserum for filamin A and anti-His antibody for His-filamin $A^{1534-2647}$ (upper panels). The amounts of filamin A in the cell lysates are shown (middle panel). The corresponding medium was analyzed with mAb5 for epithin release (lower panel). C, release of endogeneous epithin in 427.1.86 cells was examined after transfection of increasing amounts of His-filamin $A^{1534-2647}$ cDNAs as indicated.