Expanded View Figures

Figure EV1. Ligation of rBM in 2D versus 3D alters cellular gene expression profiles.

A Heatmap of microarray analysis of gene expression in HMT-3522 S-1 MECs cultured either as a 2D monolayer or as spheroids. The top 200 differentially expressed genes are shown ($n = 3$ independent biological replicates).

B Heatmap of RNA-seq experiment from MCF10A MECs ligated with rBM in 2D and 3D at 12 h post-plating. The top 200 genes differentially expressed between the 2D and 3D rBM conditions are shown ($n = 3$ independent biological replicates).

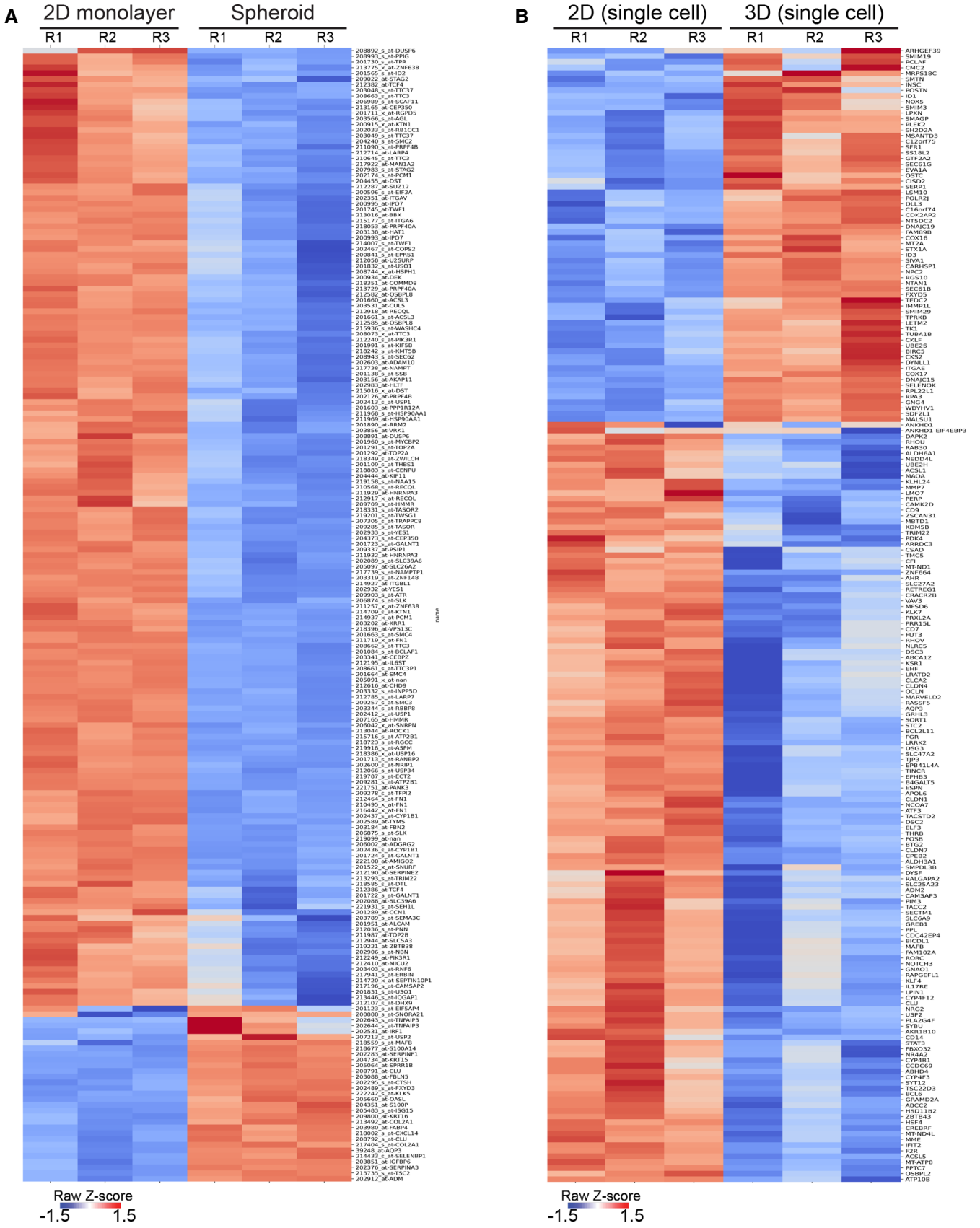

Figure EV1.

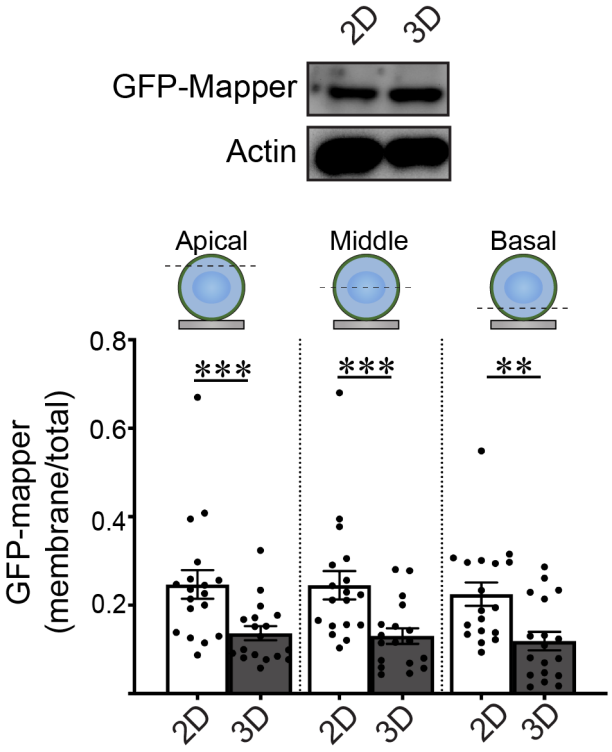

Figure EV2. rBM ligation in 3D decreases ER-PM contact site formation.

(Top) Expression levels of GFP-MAPPER in MCF10A MECs ligated with rBM in 2D or 3D was assessed by immunoblotting. Actin was used as the loading control. (Bottom) Quantification of levels of plasma membrane-proximal GFP-MAPPER in different focal planes (apical, middle, and basal) in MCF10A MECs ligated with rBM in 2D or 3D. The abundance of ER-PM contact sites in MECs was quantified as plasma membrane fluorescence relative to total fluorescence (mean ± SEM; 2D, $n = 18$; 3D, $n = 18$ cells from two independent experiments). Statistical analysis by one-way ANOVA followed by Uncorrected Fisher's LSD. Apical 2D versus 3D, ***$P = 0.0007$; middle 2D versus 3D, ***$P = 0.0004$; basal 2D versus 3D, **$P = 0.0011$.

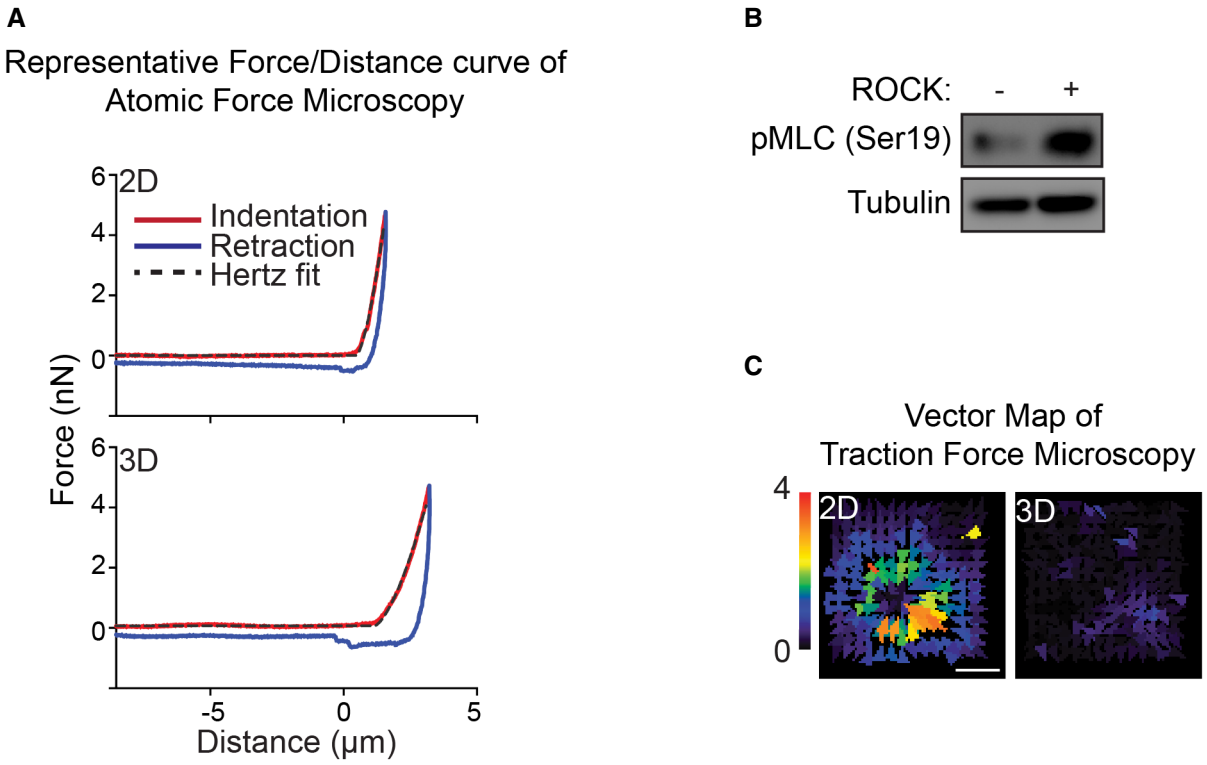

Figure EV3. Representative AFM force curve and TFM force map.

A Representative AFM force-distance curve of MECs interacting with either laminin-111 in 2D or 3D. The indentation curve (red), retraction curve (blue), and Hertz model fit of the indentation curve (black dash line) are shown. The indentation curve of MECs ligated to rBM in 3D exhibits a similar pattern of classical AFM indentation curve.

B ROCK activity in MCF10A MECs ligated to rBM in 3D for 18 h was assessed via immunoblot for Ser19 phosphorylated myosin light chain (pMLC) relative to alpha-tubulin loading control.

C Bead displacement field of MECs ligated to a rBM in 2D or 3D is displayed as a color-coded vector map. Scale bar, 10 μm.

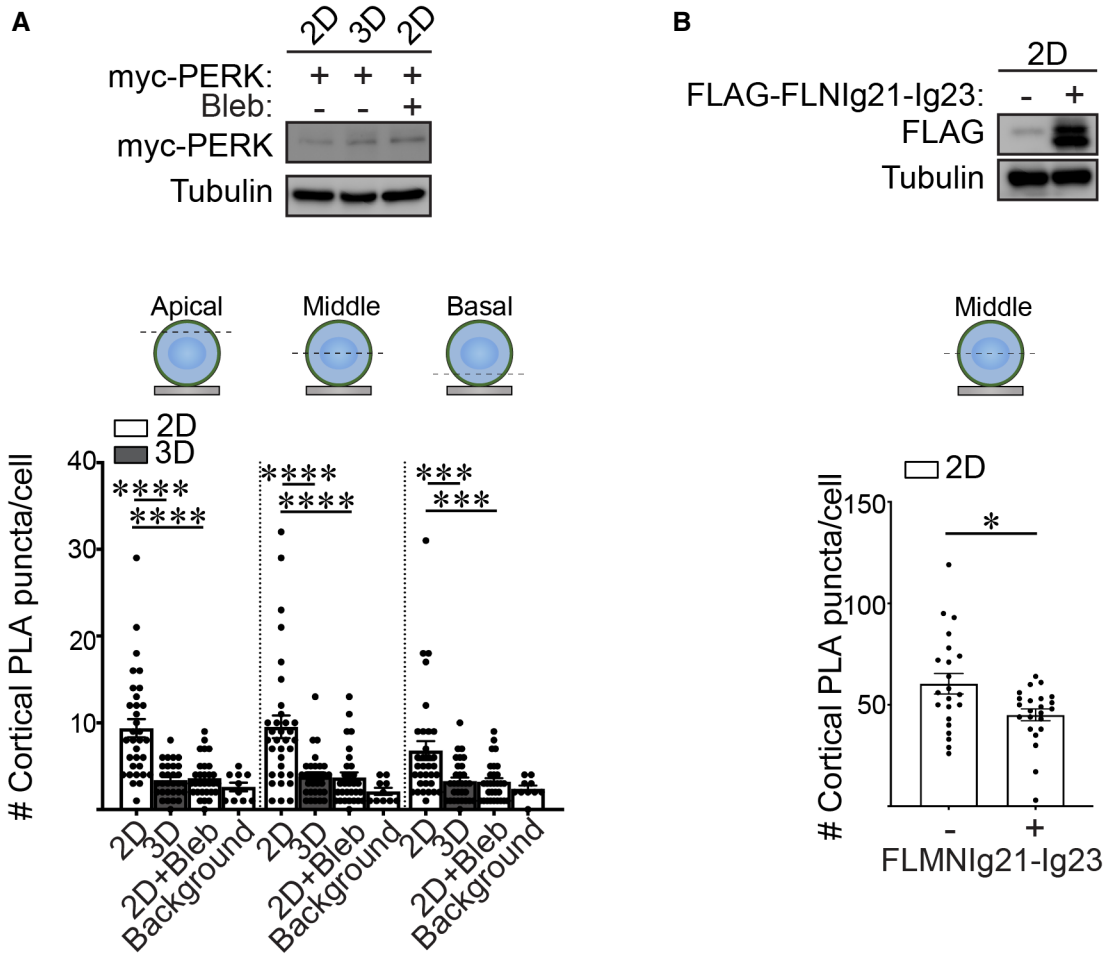

Figure EV4. FLMNIg21-Ig23 domain regulates PERK-filamin interaction at the cell cortex.

A (Top) Immunoblots of myc-PERK and alpha-tubulin (control) in lysate from MCF10A MECs harboring doxycycline-inducible myc-PERK. MCF10A MECs were ligated with rBM in 2D or 3D for 18 h and treated with doxycycline for 7 h. (Bottom) Quantification of PLA signals within the actin cortex (cortical PLA puncta) at different focal planes (apical, middle, and basal) in MCF10A MECs expressing doxycycline-inducible myc-PERK. MECs were ligated with rBM in 2D or 3D in the absence or presence of blebbistatin (2D + Bleb) for 18 h and myc-PERK was induced for 7 h prior to PLA. The number of PLA puncta per cell were quantified to measure of the levels of interaction between filamin and PERK. Background PLA signal was measured from cells that were stained in the absence of primary antibodies (mean ± SEM; 2D, $n = 33$; 3D, $n = 30$ cells; 2D + Bleb, $n = 30$; background, $n = 10$ cells from three independent experiments). Statistical analysis by one-way ANOVA followed by Uncorrected Fisher's LSD. ****$P < 0.0001$; basal 2D versus 3D, ***$P = 0.0010$; basal 2D versus 2D + Bleb, ***$P = 0.0008$.

B (Top) Immunoblots of FLAG-FLMNIg21-Ig23 and alpha-tubulin (control) in MCF10A MECs expressing doxycycline-inducible myc-PERK. (Bottom) Quantification of cortical PLA signals in the middle focal plane of myc-PERK expressing MCF10A MECs transduced with vector encoding FLAG alone (control) or FLAG-FLMNIg21-Ig23 domain (mean ± SEM; 2D, $n = 22$; 3D, $n = 23$ cells from two independent experiments). *$P = 0.0176$ (Student's t-test).

Figure EV5. Cortical actin tension modulates plasma membrane protein composition.

A Excess chemical potential required for the recruitment of positive curvature sensing domains in basal, annulus, and protrusion regions of the plasma membrane plotted as a function of A/Ap.

B Immunoblots of Exo70-GFP and alpha-tubulin (control) in MCF10A MECs ligated with rBM in 2D or 3D. Quantification of the levels of plasma membrane-proximal Exo70 at different focal planes (apical, middle, and basal) in MCF10A MECs ligated with rBM in 2D or 3D. The levels of plasma membrane-proximal Exo70 in MECs were quantified as plasma membrane fluorescence (GFP colocalization with farnesylated mCherry) relative to total cellular Exo70-GFP fluorescence (mean ± SEM; 2D, $n = 22$; 3D, $n = 23$ cells from two independent experiments). Statistical analysis by one-way ANOVA followed by Uncorrected Fisher's LSD. Apical and middle 2D versus 3D, ****$P < 0.0001$; basal 2D versus 3D, ***$P = 0.0007$.

C Representative fluorescence microscopy images of MCF10A MECs stably expressing recombinant V5-APEX2-CAAX and ligated to rBM in 2D in the absence and presence of blebbistatin. MECs were immunostained with antibodies targeting V5 (green) and counterstained with phalloidin (red). Scale bar, 10 μm.

D MECs ligated to rBM in 2D with and without 2 h of blebbistatin treatment were harvested for immunoblotting. The activity of APEX2-CAAX (V5) was examined via immunoblot for biotinylated proteins with streptavidin-HRP.

E Schematic of the strategy used for the two-state SILAC experiment. MECs expressing APEX-CAAX were treated with biotin-phenol overnight followed by 1 min of H_2O_2 exposure. MECs labeled with heavy isotope amino acids were treated with blebbistatin to reduce myosin II activity, whereas those labeled with light amino acids were treated with DMSO (vehicle). Cells were lysed and excess biotin phenol trapped in the polyacrylamide gels was removed using acetone precipitation. The resuspended protein was purified using streptavidin beads and identified by mass spectrometry. For each protein, the H/L SILAC ratio reflects the extent of its biotinylation by APEX2-CAAX in the presence/absence of blebbistatin.

F GO Cellular Component analysis of proteins enriched at the plasma membrane of cells with low cortical tension (Fig 7I) using PANTHER online database.

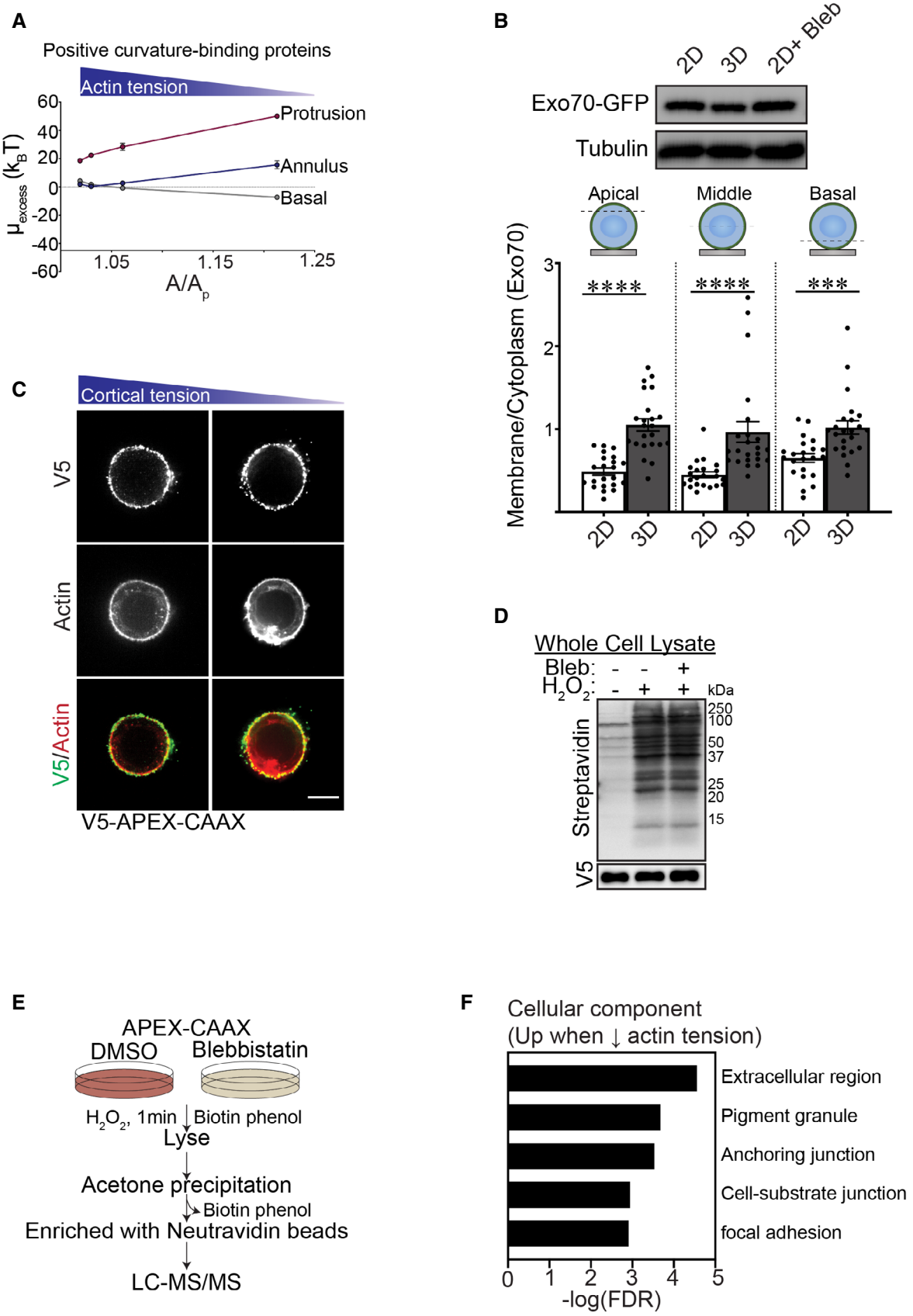

Figure EV5.