SUPPLENTAL FIGURE LEGENDS

Figure S1: Distribution of actin and MT networks in MDCK spread on Ecad-Fc.
Epithelial MDCK cells were spread for 2 hours on Ecad-Fc coated surfaces, fixed and immunostained for F-actin and tubulin. Scale bars: 10 μm.

Figure S2: Characterization of MT maturation in cells spread on Ncad-Fc and FN substrates.
Immunostaining for specific post-translationally modified forms of α-tubulin in cells plated on Ncad-Fc and FN. Tyrosinated tubulin (tyr) was the major form of tubulin found incorporated in MTs both on Ncad-Fc and Fn. More stable detyrosinated MTs (de-tyr; recognized by anti-Glu antibodies) were completely absent from cells plated on both Ncad-Fc and FN. Acetylated MTs (ace) were also absent from cells seeded on Ncad-Fc but detected in as small fragments in the center of cells seeded on FN. Scale bars: 10 μm.

Figure S3: Distribution of MT plus ends in cell monolayers and cells spread on Ncad-Fc substrates.
(A) C2C12 cell monolayers were either stained for β-catenin (red) and α-tubulin (green) on the left, or β-catenin (red) and EB1 (green) on the right. Cell-cell contacts are indicated with asterisks. **(B)** Cells expressing EB1-GFP were plated either on Ncad-Fc or FN for 2 hrs, then stained with anti-β-catenin (red) and anti-α-tubulin (green) antibodies. In cells spread on Ncad-Fc, MTs and comet-like EB1 accumulations decorating growing MT plus ends rarely populate the lamellipodium zone were are formed cadherin adhesions; the comets were frequently oriented tangential to the cell edge (top). In contrast, these comets at the tip of MTs were directed towards and reached the lamellipodium edge of cells spread on FN. Higher magnifications of boxed areas of cells spread on Ncad-Fc (b1) and FN (b2) are shown at the bottom. Scale bars: 10 μm.

Figure S4: Effect of the expression of the DN-Ncad mutant on MT penetration.
(A) Cells transfected with either WT Ncad (top) or DN Ncad (bottom) constructs plated on Ncad-Fc were immunostained for β-catenin and α-tubulin. Cadherin adhesion formation and actin treadmilling were not impaired in cells expressing low levels of DN Ncad, although their spreading was reduced (data not shown). **(B)** This moderate expression of DN Ncad did not

affect MT distribution either on Ncad-Fc or on FN. Scale bars 10 µm. Statistical analysis: One way ANOVA, ** p value <0.001; *** p value: <0.0001.

Figure S5: Effect of Cytochalasin D and blebbistatin treatments on F-actin and MT organization.

Cells spread on Ncad-Fc were treated with DMSO, cytochalasin D 100nM or blebbistatin 20 µM during 20 min, then fixed and stained for β-catenin, F-actin and MTs. Cytochalasin D treatment induced a disorganization of actin cytoskeleton in parallel of an extension of the MT networks towards the cell edges. The inhibition of myosin II activity by blebbistatin triggered similar although less drastic effects on actin and MT networks. The dashed lines on the right panels indicate the edge of the cells. Scale bar 10 µm.

Supplementary movie S1. **EB3-mCherry comet movements in C2 cells plated on fibronectin**.

One day post-transfection cells were dissociated in trypsin-free conditions and acquisitions were performed 2 hrs after plating on FN with a spinning disk. EB3 comets are numerous and generally travel towards the cell periphery. 20 frames/s, total duration 5 min.

Supplementary movie S2. **EB3-mCherry comet movements in C2 cells plated on Ncad-Fc.**

One day post-transfection cells were dissociated in trypsin-free conditions and acquisitions were performed 2 hrs after plating on Ncad-Fc with a spinning disk. EB3 comets can loop before entering the adhesion area and move towards the cell periphery, but few of them can reach the cell border. 20 frames/s, total duration 5 min.

Supplementary movie S3. **EB3-GFP movements in cells plated on Ncad-Fc, before and after cytochalasin D treatment.**

Cells were co-transfected with N-cadherin-DsRed and EB3-GFP one day before live cell imaging. Image acquisitions were performed before treatment, and resumed after 20 minutes of incubation with cytochalasin D 100 nM. 20 frames/s, total duration 1 min before treatment and 1 min after treatment.

Supplementary movie S4. **EB3-GFP movements in cells plated on Ncad-Fc substrate, before and after blebbistatin treatment.**

The experimental conditions are the same as for supplementary movie S3, blebbistatin 20 µM replacing cytochalasin D treatment.

Supplementary movie S5. Actin dynamics in cells plated on Ncad-Fc substrate.

Cells were transfected with Lifeact-GFP one day before live cell imaging and plated on Ncad-Fc substrate. Interval acquisition 1 s, total duration 5 min.

Supplementary movie S6. Actin dynamics in cells plated on FN substrate.

Cells were transfected with Lifeact-GFP one day before live cell imaging and plated on FN substrate. Interval acquisition 1 s, total duration 5 min.

Figure S1

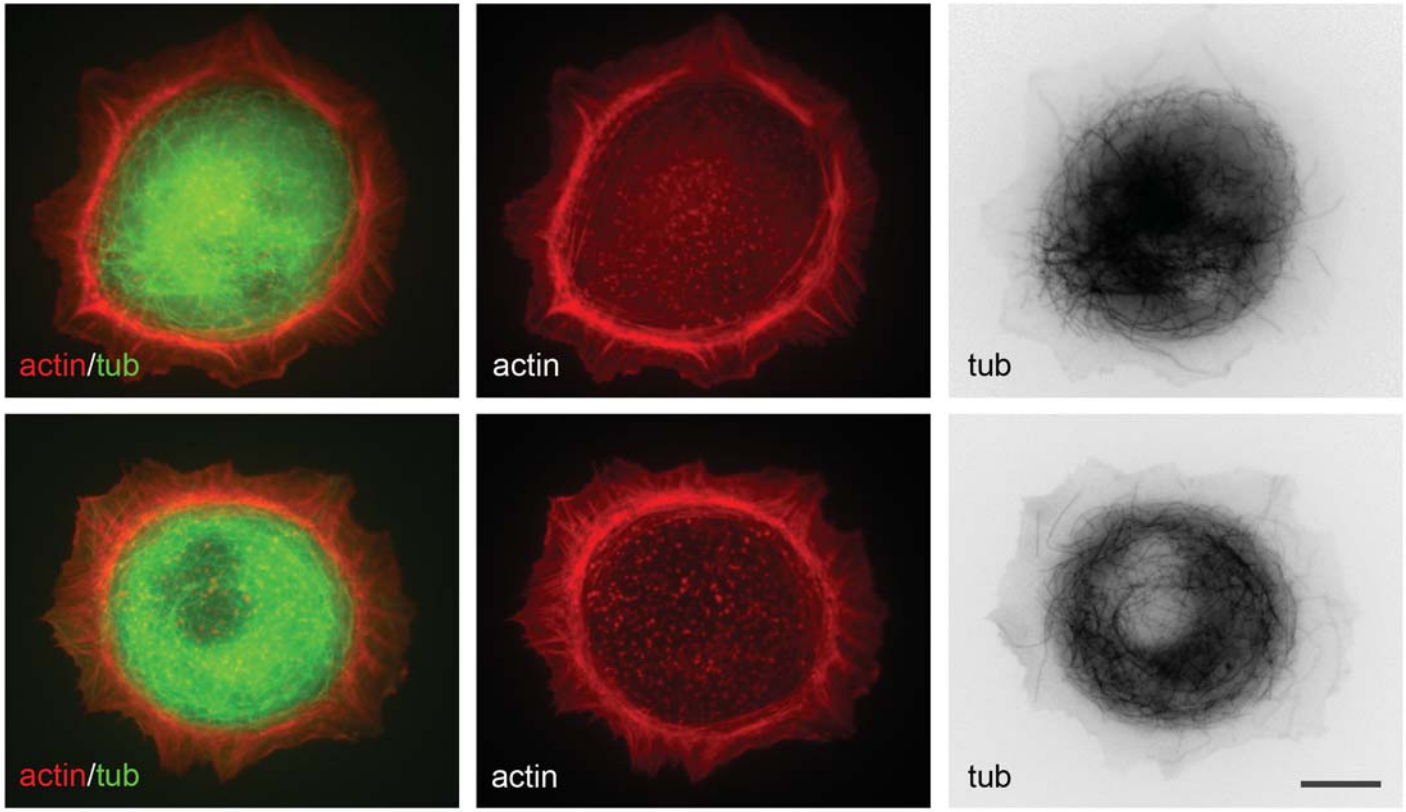


Figure S2

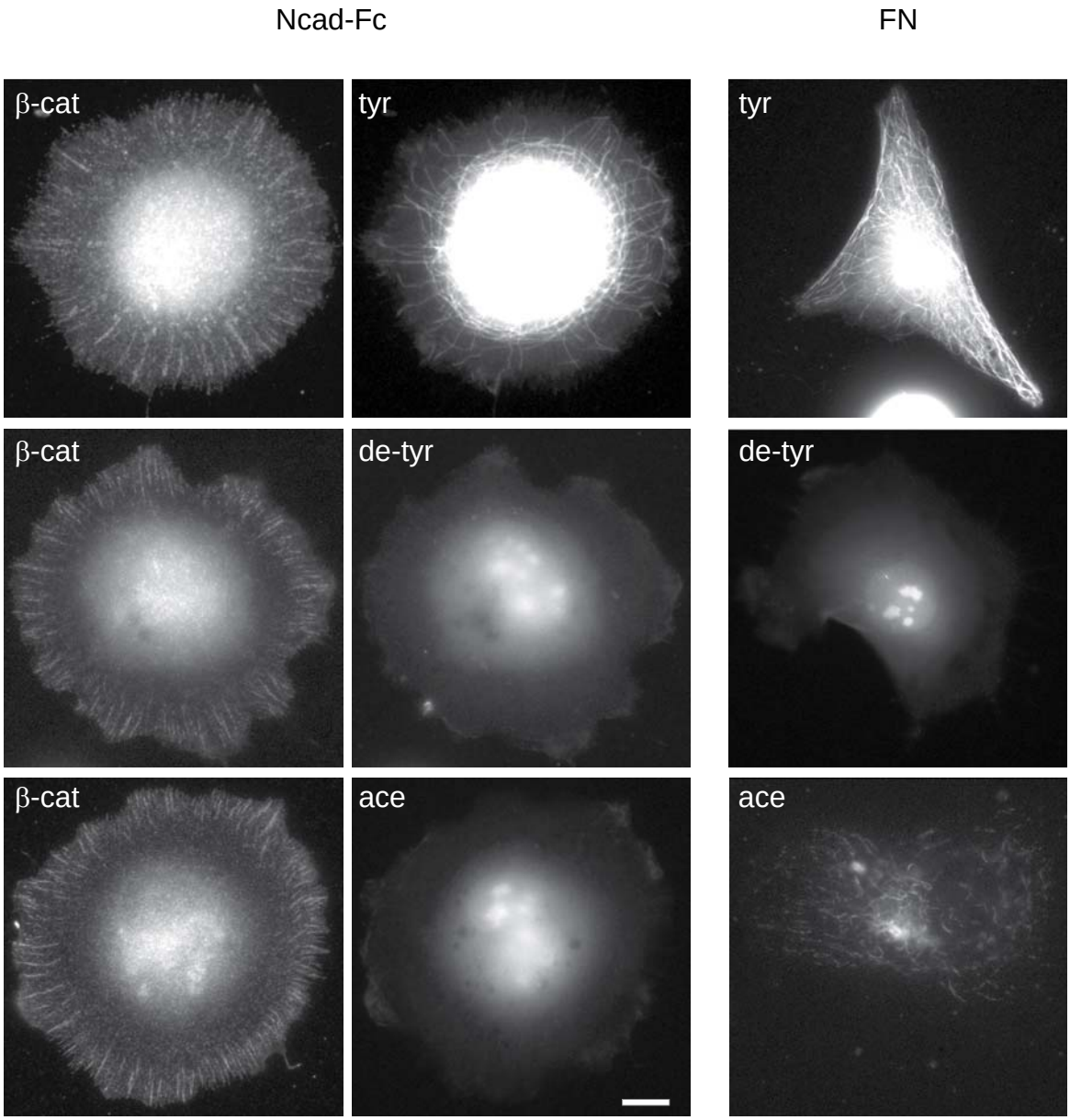


Figure S3

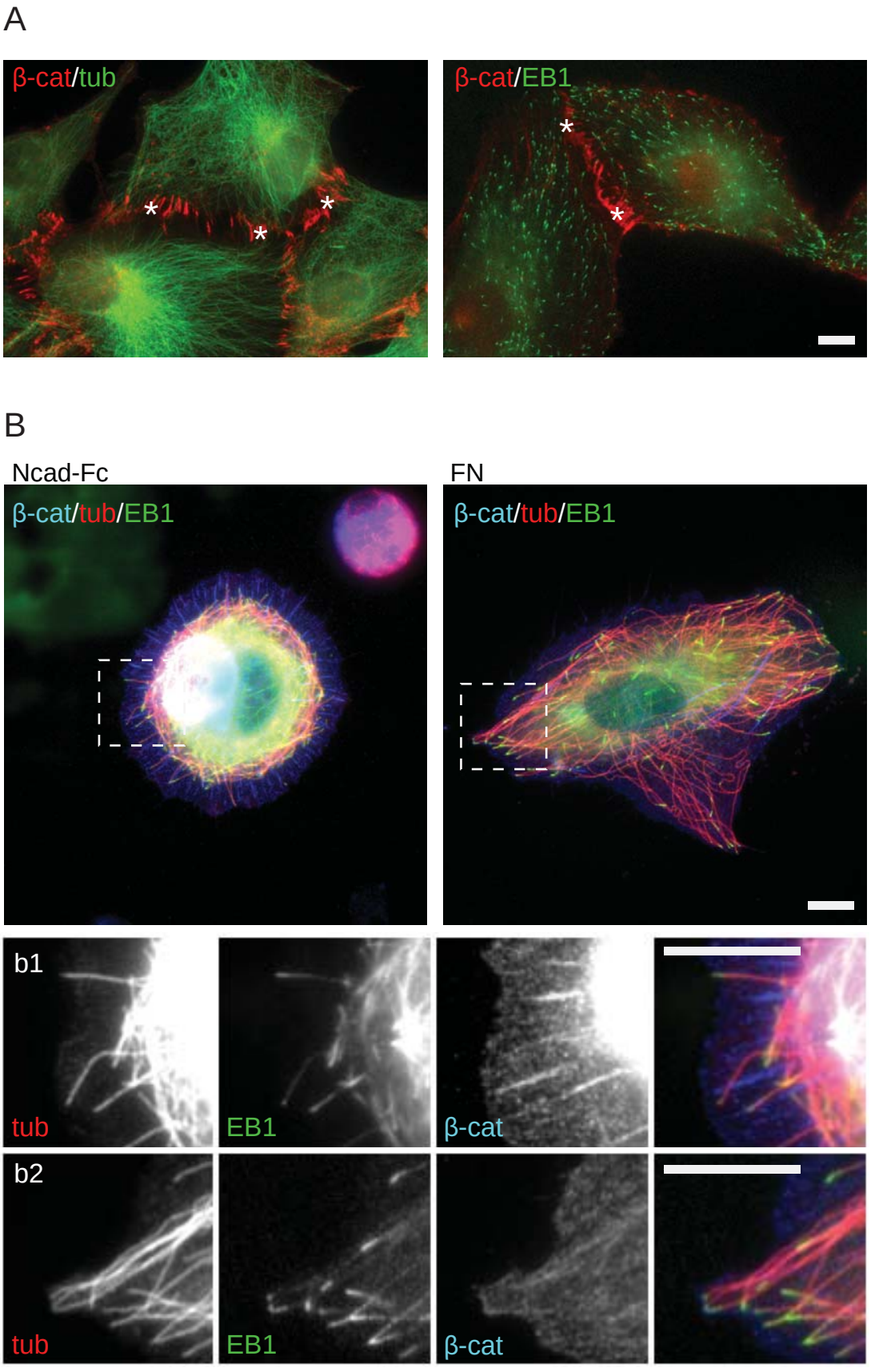
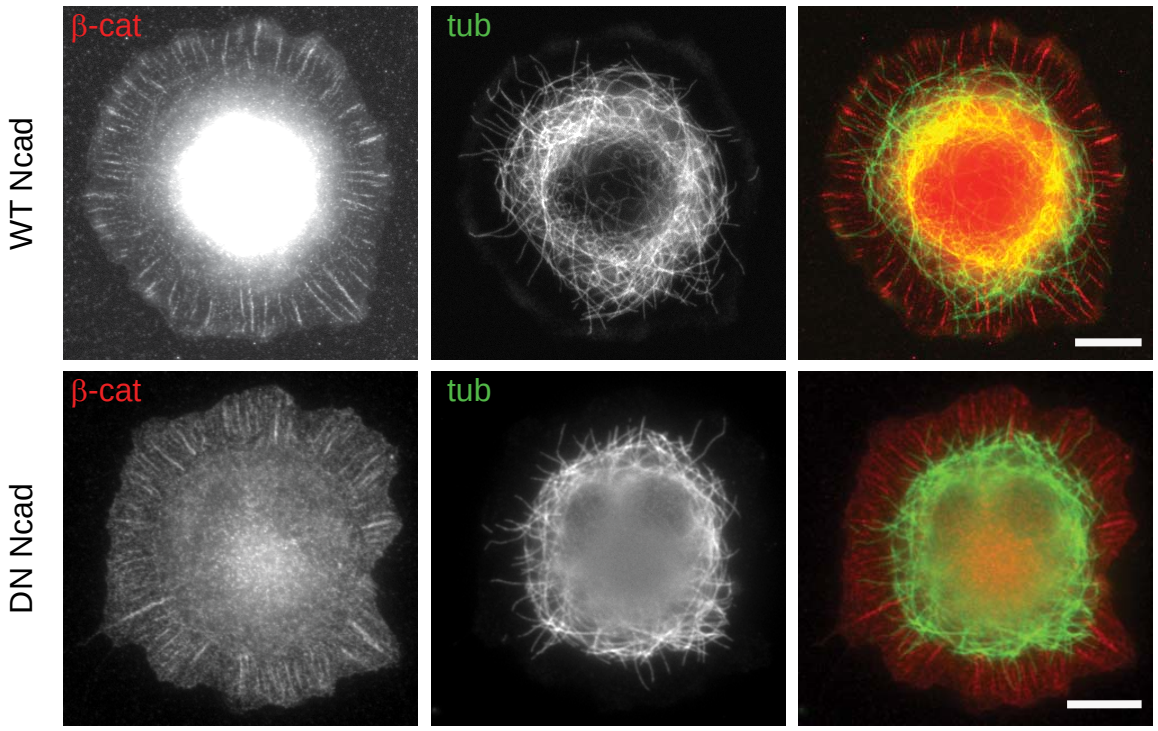
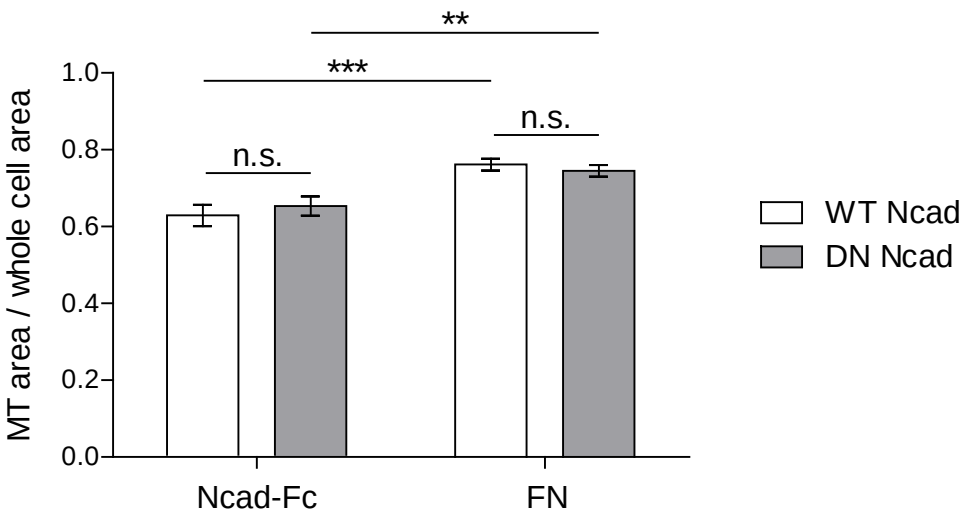

A
β-cat/tub
β-cat/EB1
B
Ncad-Fc
FN
β-cat/tub/EB1
β-cat/tub/EB1
b1
tub
EB1
β-cat
b2
tub
EB1
β-cat

Figure S4

A


β-cat
tub
WT Ncad
β-cat
tub
DN Ncad

B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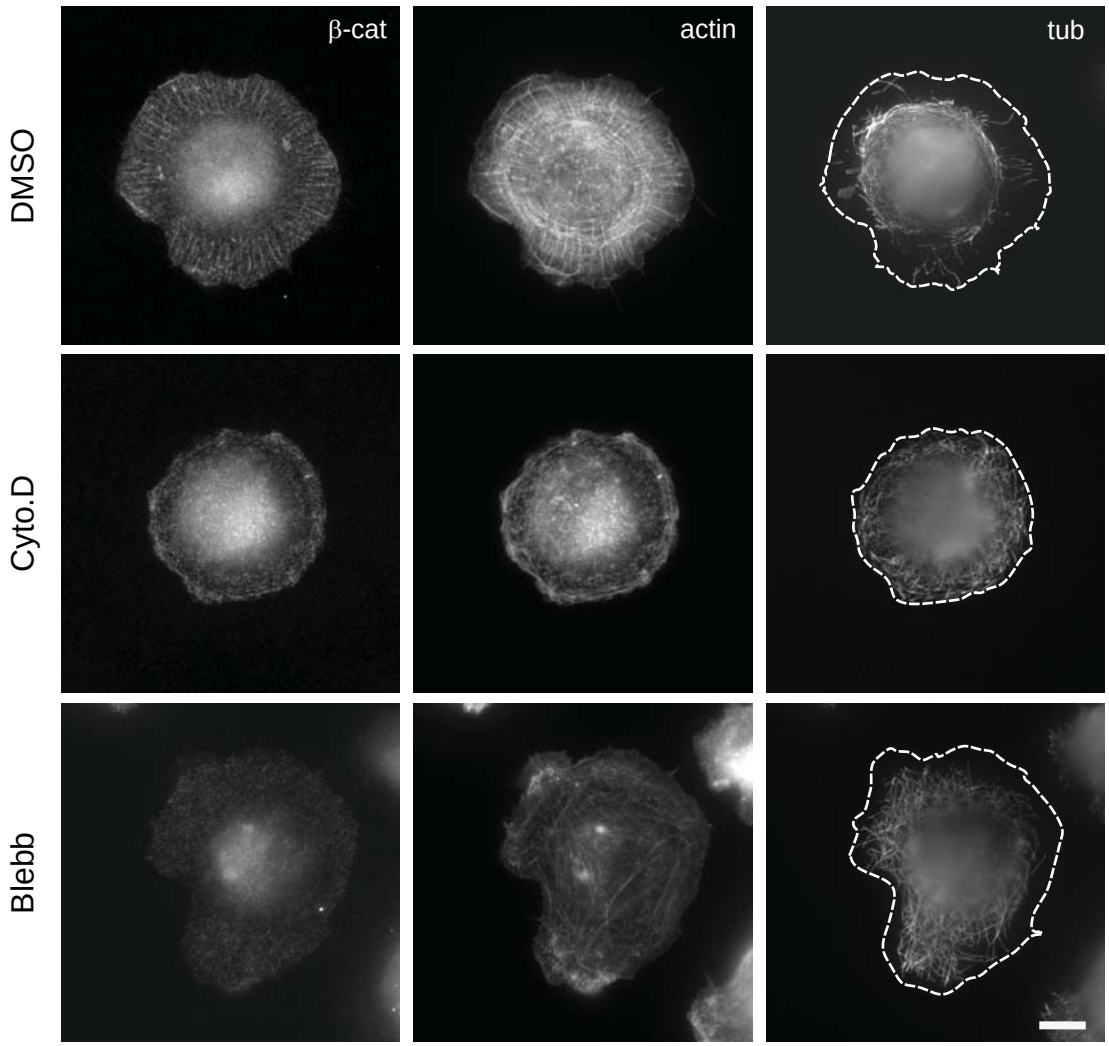
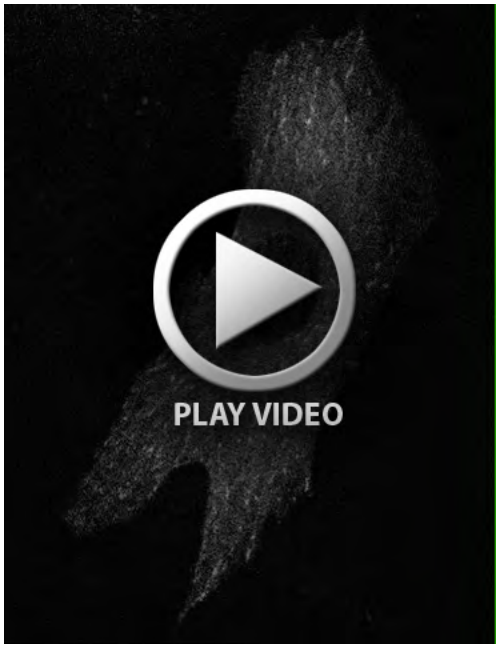
n.s.
n.s.
1.0
0.8
0.6
0.4
0.2
0.0
MT area / whole cell area
Ncad-Fc
FN
WT Ncad
DN Ncad

Figure S5





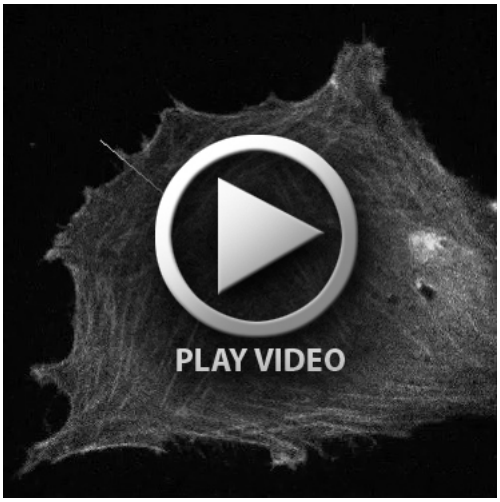
Movie 1.

Movie 2.

Movie 3.

Movie 4.

Movie 5.



Movie 6.