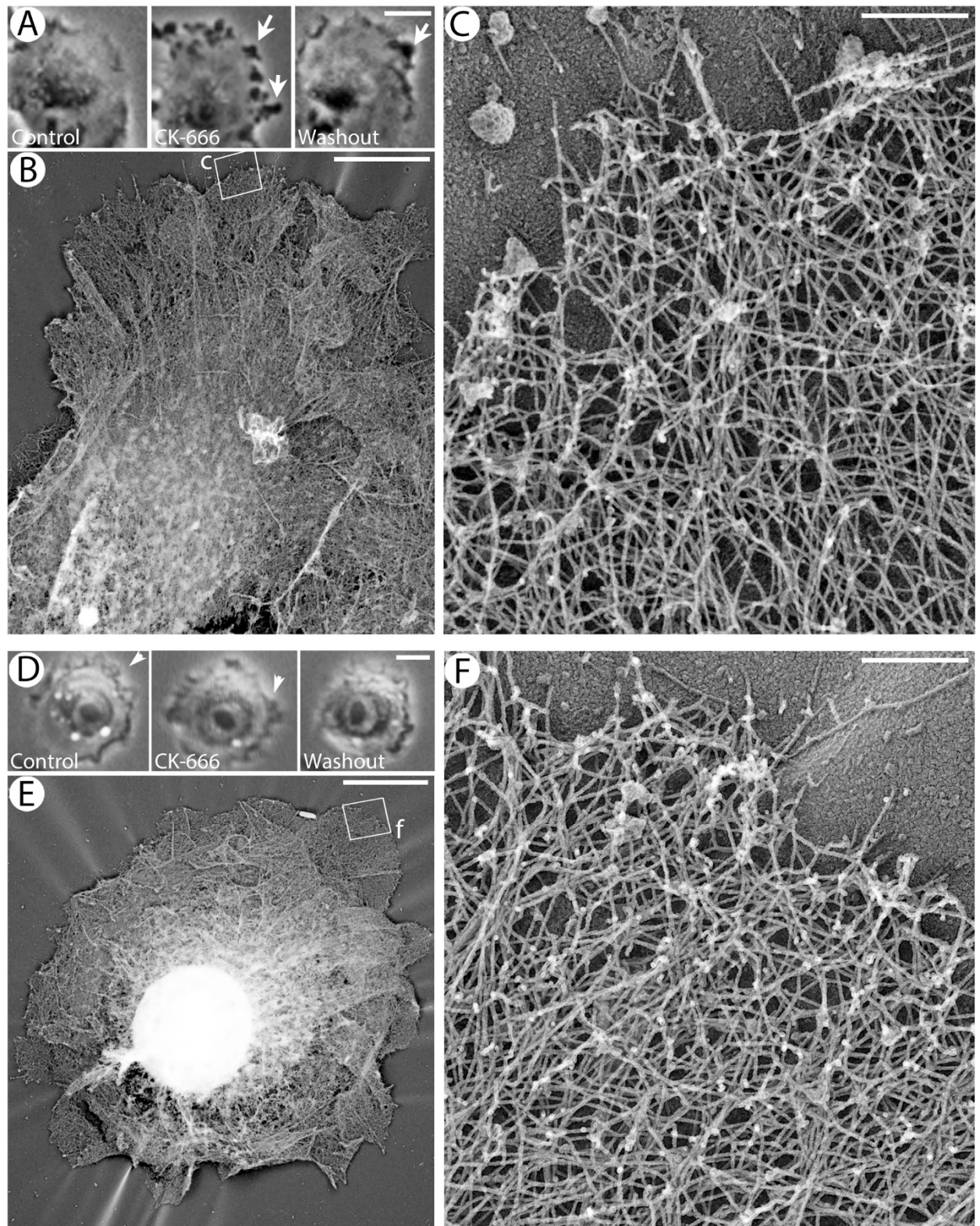
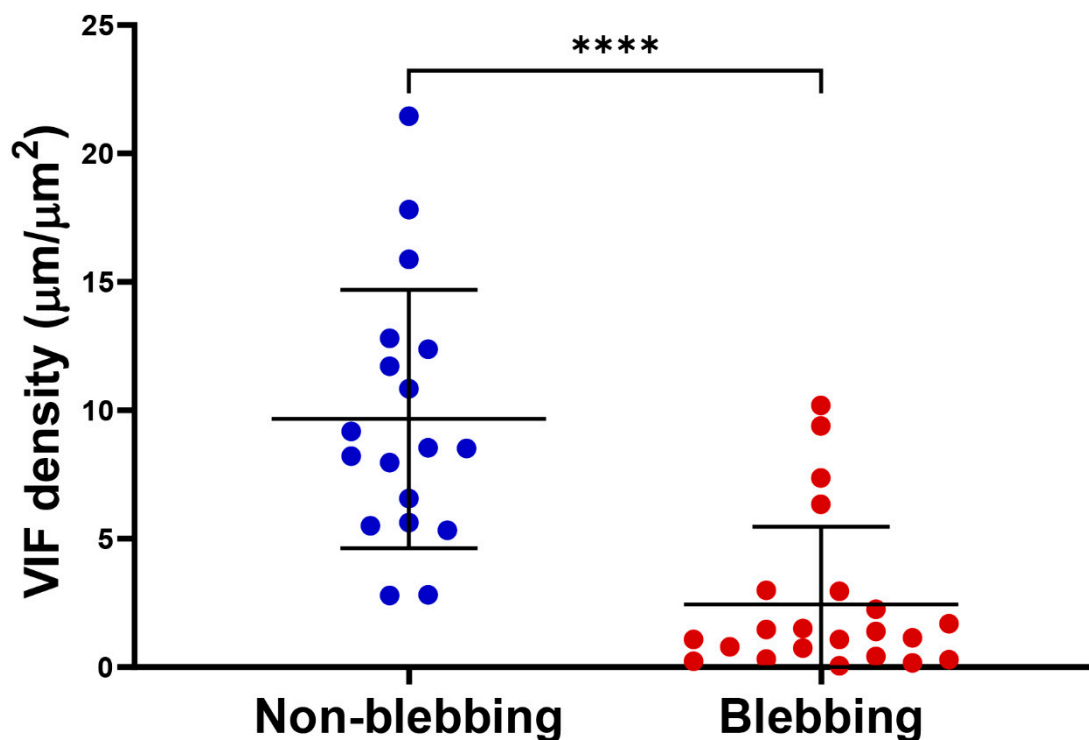


Supplementary Information

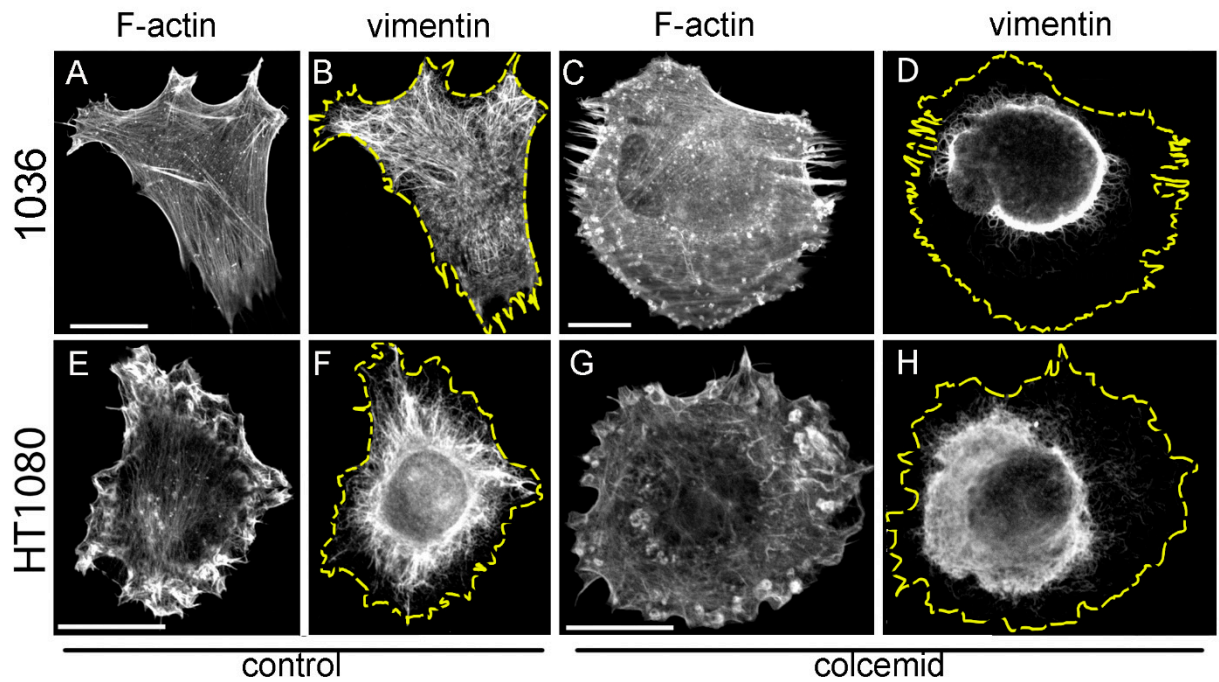


**Figure S1. Correlative phase-contrast microscopy and PREM of cells treated with 200  $\mu$ M CK-666 for 1 h and then allowed to restore lamellipodia after drug washout.**

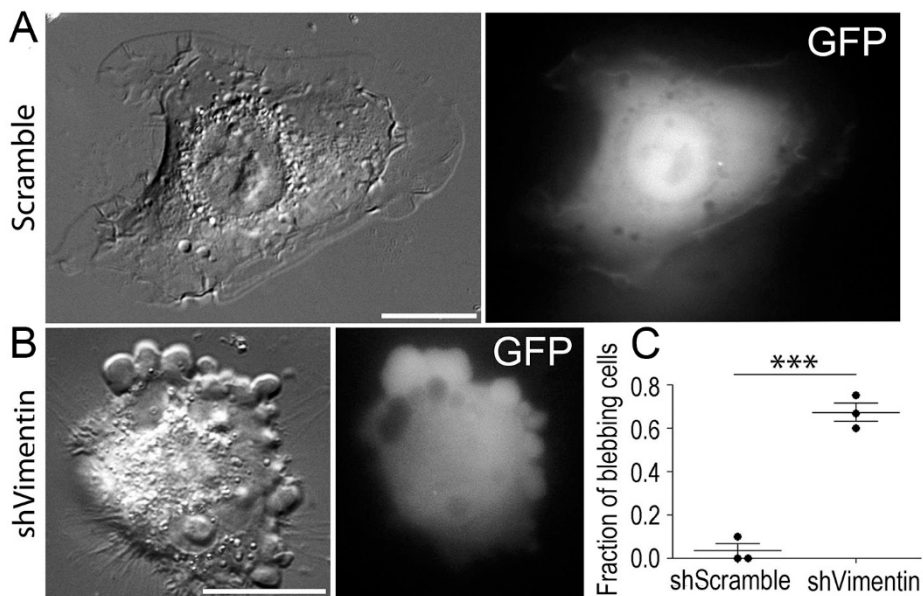
(A-C) Example of a cell that switched to blebbing under CK-666 treatment. (D-F) Example of a cell that did not switch to blebbing under CK-666 treatment. (A, D) Phase contrast images taken before CK-666 application (left), under CK-666 treatment (middle) and after CK-666 washout (right). (B, E) Correlative PREM of the cells shown in A and D, respectively, after detergent extraction, but without gelsolin treatment. (C, F) Enlarged boxed regions from B and E, respectively. Fibrillar structures correspond to actin filaments. Scale bars, 5  $\mu\text{m}$  (A, B, D, E) and 500 nm (C, F).



**Figure S2. Quantification of VIF density in PREM samples.** Each data point indicates area-normalized total VIF length within an individual cell region located adjacent to the leading edge; n=18 regions from 11 cells with total area of 134.36  $\mu\text{m}^2$  for non-blebbing cells and 22 regions from 6 cells with total area of 183.21  $\mu\text{m}^2$  for blebbing cells; \*\*\*\*,  $p < 0.0001$ , Mann-Whitney test.

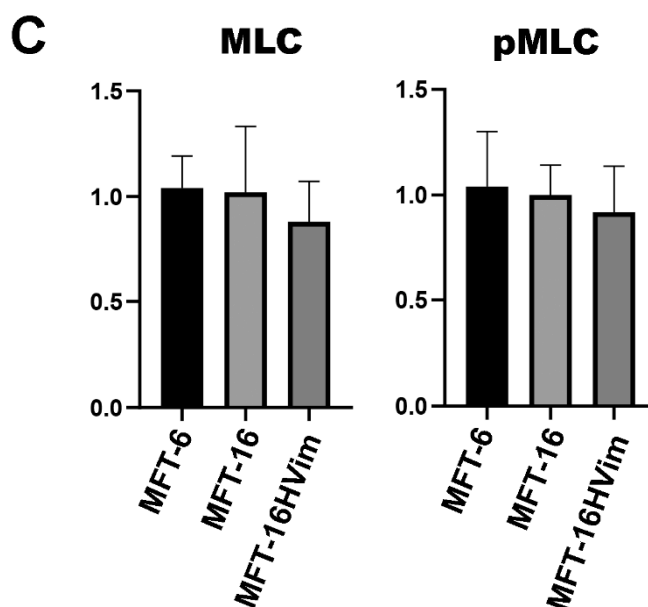
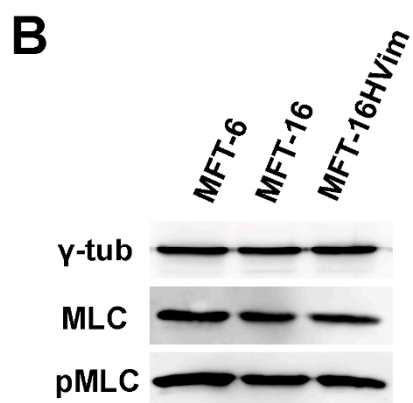
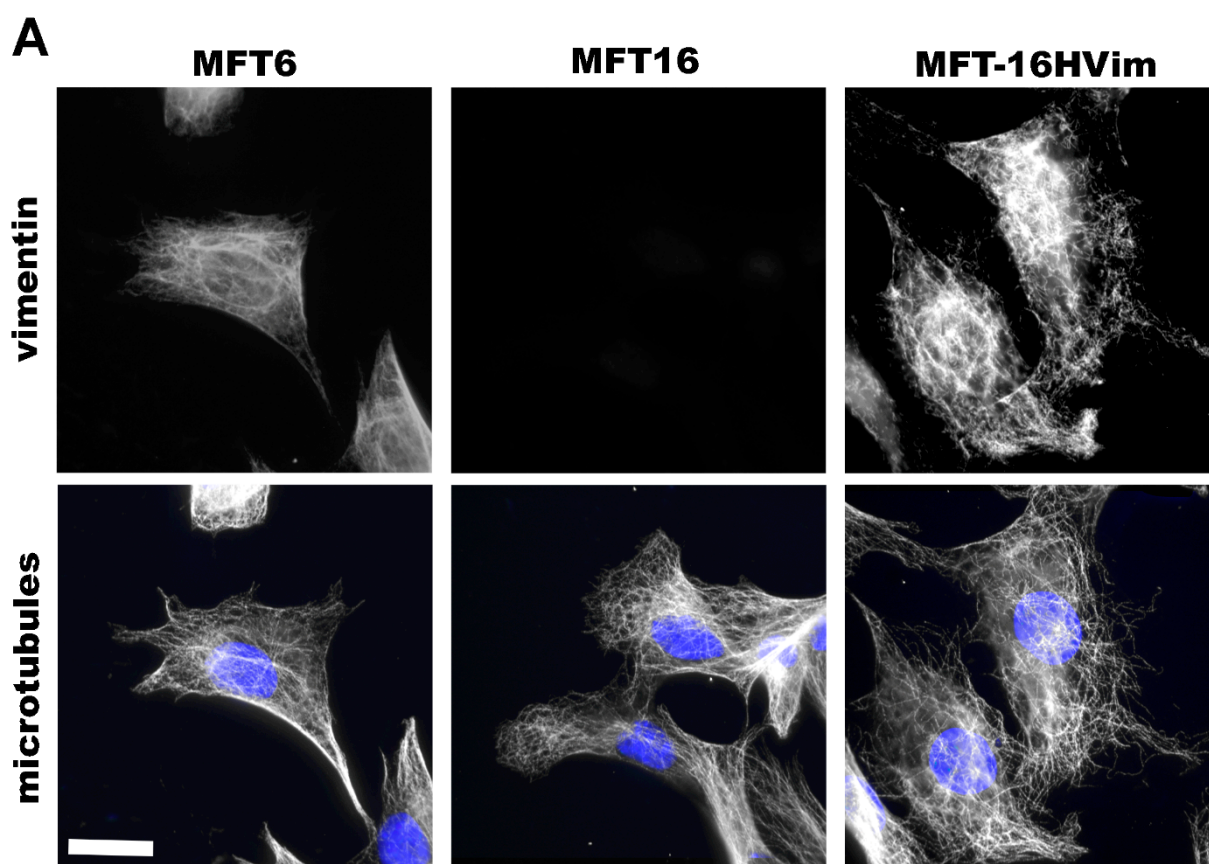


**Figure S3.** Fluorescence staining of 1036 cells (A-D) and HT1080 (E-H) with phalloidin (F-actin) and vimentin antibody in the presence or absence of 0.2  $\mu$ M colcemid. Colcemid treatment for 1 h induced a collapse of VIFs. Cell contours in vimentin-stained images (B, D, F, H) are outlined with yellow.



**Figure S4.** Transient transfection of HT1080 cells with scrambled (A) or vimentin-targeting (B) shRNAs together with EGFP-expressing plasmid. Blebbing is induced in cells transfected with vimentin but not scrambled shRNA even in the absence of CK-666. EGFP panels confirm successful transfection with shRNAs. (C) Fractions of

blebbing cells expressing indicated shRNAs. Data points show individual experiments; lines indicate mean  $\pm$  SD; n=19, \*\*\* p=0.0003, unpaired t-test.





**Figure S5. Microtubule distribution and expression levels of MLC and pMLC in MFT-6, MFT-16, and MFT-16HVim cells.**

(A) Immunofluorescence staining of VIFs (top) and microtubules (bottom). Scale bar 20  $\mu\text{m}$ . (B, C) Analysis of total MLC and active (phosphorylated) pMLC expression in MFT-6, MFT-16, and MFT-16HVim cells. (B) Western blot of MLC and pMLC. Gamma-tubulin stained by GTU-88 serves as loading control. (C) Quantification of Western blots by densitometry. Mean  $\pm$  SEM from 5 independent experiments.

**Video S1. Treatment of HT1080 cells with 0.2  $\mu\text{M}$  colcemid induces blebbing even without CK-666 treatment.** DIC live-cell imaging before and after colcemid treatment. Process is reversible after colcemid washout. Scale bar, 10  $\mu\text{m}$ . Time in min:sec.

**Video S2. Treatment with 0.2  $\mu\text{M}$  colcemid induces blebbing in 1036 cells.** DIC live-cell imaging before and after colcemid treatment. Scale bar, 10  $\mu\text{m}$ . Time in min:sec.

**Macros for image analysis for quantification of center-to-periphery distribution of the mean immunofluorescence intensity of vimentin in cells.**

Analysis was done using ImageJ 1.51h version, 64-bit, with Java 1.8.0\_66

1. Macro to make mask

```
/// Macro to make Mask

dir=getDirectory("Choose folder with images to analyse");

Extension="_ch01.tif";

dirMask=dir+"Mask"+File.separator();

File.makeDirectory(dirMask);

list=getFileList(dir);

for(file=0;file<lengthOf(list);file++) {

    if(endsWith(list[file],Extension)){

        open(dir+list[file]);
```

```

shortName=substring(list[file],0,lengthOf(list[file])-lengthOf(Extension));
run("Duplicate...", "title=temp");
run("Mean...", "radius=4");
run("Auto Threshold", "method=Triangle white");
run("Analyze Particles...", "size=5000-Infinity pixel exclude show=Masks clear");
run("Maximum...", "radius=10");
run("Minimum...", "radius=10");
run("Grays");
run("Fill Holes");
saveAs("Tiff",dirMask+shortName+"_Mask.tif");
run("Close All");
    }
}

```

## 2. Macro to analyze

```

/// Macro to analyze

dir=getDirectory("Choose folder with images to analyse");

Extension="_ch00.tif";

dirMask=dir+"Mask"+File.separator();

nbErosionStep=10;

list=getFileList(dir);

run("Set Measurements...", "area mean min centroid center integrated median stack redirect=None decimal=3");

for(file=0;file<lengthOf(list);file++) {
    if(endsWith(list[file],Extension)){
        roiManager("Reset");
        run("Clear Results");
        open(dir+list[file]);
        shortName=substring(list[file],0,lengthOf(list[file])-lengthOf(Extension));
        open(dirMask+shortName+"_Mask.tif");
        run("Duplicate...", "title=distMap");
        run("Distance Map");
        run("Select All");
        run("Measure");
        MaxDist=getResult("Max",0);
        run("Clear Results");

        selectWindow(shortName+"_Mask.tif");
        run("Invert");
        run("Create Selection");
        roiManager("Add");
        step=round(MaxDist/nbErosionStep);
        for(i=0;i<nbErosionStep;i++) {
            run("Enlarge...", "enlarge="+step+" pixel");
        }
    }
}

```

```

        roiManager("Add");
    }

    for(i=0;i<nbErosionStep;i++) {
        selectWindow(list[file]);
        roiManager("Select",i);
        run("Measure");
    }

    MeanInt=newArray(nbErosionStep);
    MeanIntNorm=newArray(nbErosionStep);
    for(i=0;i<(nbErosionStep-1);i++) {
        MeanInt[i]=(getResult("IntDen",i)-
getResult("IntDen",i+1))/(getResult("Area",i)-getResult("Area",i+1));
    }
    MeanInt[9]=getResult("Mean",9);

    Array.getStatistics(MeanInt, minMeanInt, maxMeanInt, meanMeanInt,
stdDevMeanInt);

    sum=meanMeanInt*nbErosionStep;
    for(i=0;i<(nbErosionStep);i++) {
        MeanIntNorm[i]=MeanInt[i]/sum;
    }

    Array.print(MeanIntNorm);

    run("Close All");
}
}

```