Supplemental Material

Legends for supplementary Videos:

Video 1: Membrane exocytosis occurs during cell spreading.

After preloading with the lipid dye FM1-43 (see "FM1-43 before spreading" protocol in the Methods section and in Figure 2A), RPTP α cell was recorded at 1 image/ 10 sec during the cell spreading process. Bright field (left) and TIR-FM (right) acquisitions of the cell are presented. The video is presented at 10 frames per sec.

Video 2: "FM1-43 during spreading" experiments reveal that an exocytic burst added PM area during cell spreading.

RPTP α cells were recorded at 1 image/15sec to 30 sec during the cell spreading process. The lipid dye FM1-43 was added during the spreading to record the total exocytic rate of the cell (see "FM1-43 during spreading" protocol in the Methods section and in Figure 3A). After 30 min of spreading, cells were washed to measure the amount of endocytosis. DIC and epifluorescence acquisitions of the cells are presented in the top and bottom pictures respectively. The video is presented at 6 frames per sec.

Video 3: FM1-43 uptake reveals an exocytic burst proportional to PM area in NIH-3T3 cell.

NIH-3T3 cells were recorded at 1 image/120 sec during the cell spreading process. The lipid dye FM1-43 was added during the spreading to record the total exocytic rate of the

cell (see “FM1-43 during spreading” protocol in the Methods section and in Figure 5 A). DIC and epifluorescence acquisitions of the cells are presented (in the top and bottom pictures respectively). The video is presented at 6 frames per sec.

Video 4: Post-mitotic spreading induces exocytosis.

RPTP α cell was recorded at 1 image/60 sec during mitosis and post-mitotic spreading. The lipid dye FM1-43 was added during the spreading to record the total exocytic rate of the cell (see “FM1-43 during spreading” protocol in the Methods section and in Figure 5 A). DIC and epifluorescence acquisitions of the cells are presented (in the top and bottom pictures respectively). The video is presented at 12 frames per sec.

Video 5: Golgi exocytosis occurs during cell spreading.

YFP-golgi transfected RPTP α cell was recorded with TIR-FM at 4 images per sec during the cell spreading process. The video presents 5 min of spreading and plays at 30 frames per sec.

Video 6: LY exocytosis occurs during cell spreading.

GFP-Lamp1 transfected RPTP α cell was recorded with TIR-FM at 3 images per sec during the cell spreading process. The video presents 5 min of spreading and plays at 23 frames per sec**.**

Video 7: Golgi exocytosis occuring during cell spreading is microtubule dependent.

YFP-golgi transfected and nocodazole treated RPTP α cell was recorded with TIR-FM at 4 images per sec during the cell spreading process. The video presents 5 min of spreading and plays at 30 frames per sec.

Video 8: LY exocytosis occuring during cell spreading is microtubule dependent.

GFP-Lamp1 transfected and nocodazole treated RPTP α cell was recorded with TIR-FM at 3.5 images per sec during the cell spreading process. The video presents 5 min of spreading and plays at 26 frames per sec.

Video 9: Golgi exocytosis occuring during cell spreading is BFA sensitive

YFP-golgi transfected and BFA treated RPTP α cell was recorded with TIR-FM at 4 images per sec during the cell spreading process. The video presents 5 min of spreading and plays at 30 frames per sec.

Video 10: ER exocytosis is not measurable during cell spreading

RFP-ER transfected RPTP α cell was recorded with TIR-FM at 3 images per sec during the cell spreading process. The video presents 5 min of spreading and plays at 23 frames per sec**.**

Video 11: Tfr exocytosis is not measurable during cell spreading

RPTP α cell loaded with Alexa 568 coupled transferrin was photobleached with TIF-FM and then recorded with TIR-FM at 4 images per sec during the cell spreading process. The video presents 5 min of spreading and plays at 30 frames per sec**.**

Video 12: GPI anchored protein labeled vesicle are exocytosed during cell spreading.

GFP-GPI transfected RPTP α cell was recorded with TIR-FM at 7 images per sec during the cell spreading process after irreversible acid fluorescence quenching (pH 4) of the extracellular construct. The video presents 5 min of spreading and plays at 60 frames per sec.

Video 13: GPI anchored protein labeled vesicle are exocytosed by a microtubule independent process during cell spreading.

GFP-GPI transfected and nocodazole treated RPTP α cell was recorded with TIR-FM at 7 images per sec during the cell spreading process after irreversible acid fluorescence quenching (pH 4) of the extracellular construct. The video presents 5 min of spreading and plays at 60 frames per sec.

Video 14: LY exocytosis occurs during cell spreading but can still be further stimulated by a calcium ionophore

GFP-Lamp1 transfected RPTP α cell was recorded with TIR-FM at 4 images per sec during the cell spreading process, the calcium ionophore A23187 was then added to the extracellular medium enriched in CaCl2 (4mM instead of 1mM for normal Ringer). The video presents 20 min of spreading and plays at 120 frames per sec**.**

Video 15: LY exocytosis occuring during cell spreading is microtubule dependent but can still be stimulated by a calcium ionophore

GFP-Lamp1 transfected and nocodazole treated RPTP α cell was recorded with TIR-FM at 4 images per sec for 2 min after spreading the calcium ionophore A23187 was then added to the extracellular medium enrichied in CaCl2 (4mM instead of 1mM for normal Ringer). The video presents 10 min of recording and plays at 120 frames per sec**.**

Legends for supplementary Figures:

Supplementary Figure 1: Microcontact printing of patterned circles coated with fibronectin (FN) onto which cells have been spread for 45 min. The pictures present a reconstruction of several images taken of the same pattern in order to present the half part of a patterned region. The left reconstructed image present the specific coating of the circles with alexa-546 coupled fibronectin (Baneyx and Vogel 1999).

Supplementary Figure 2: (A) Total internal reflection fluorescence microscopy (TIR-FM) analysis of the cells was performed, revealing FM1-43 efficiently labelled the PM area facing the coverslip during the acquisition using the "FM1-43 during spreading" protocol. (B) NIH 3T3 cells during spreading using the “FM1-43 during spreading” protocol. The arrow shows the perinuclear region brightening. Scale bar 10 µm. (C) FM1-43 fluorescence intensity plotted as a function of time for each cells presented in panel B. (D) FM1-43 fluorescence intensity fold increases (compared with the initial FM1-43 fluorescence intensity at t=0”) for each cells plotted as a function of time. (E) Schematic representation of the fibronectin (FN) coating on a single coverslip. (F) NIH-3T3 cells analyzed using the “FM1-43 during spreading” protocol, after 60 min of spreading on either uncoated or fibronectin-coated regions of the same coverslip. (G) Examples of possible problems occurring during recording using the “FM1-43 during spreading” protocol. The cells could be dead (left panel white arows) inducing a PM disruption and a very bright FM1-43 staining, beyond the threshold of the measurements (PM represents only a small portion of the total cell membranes). The cell could be

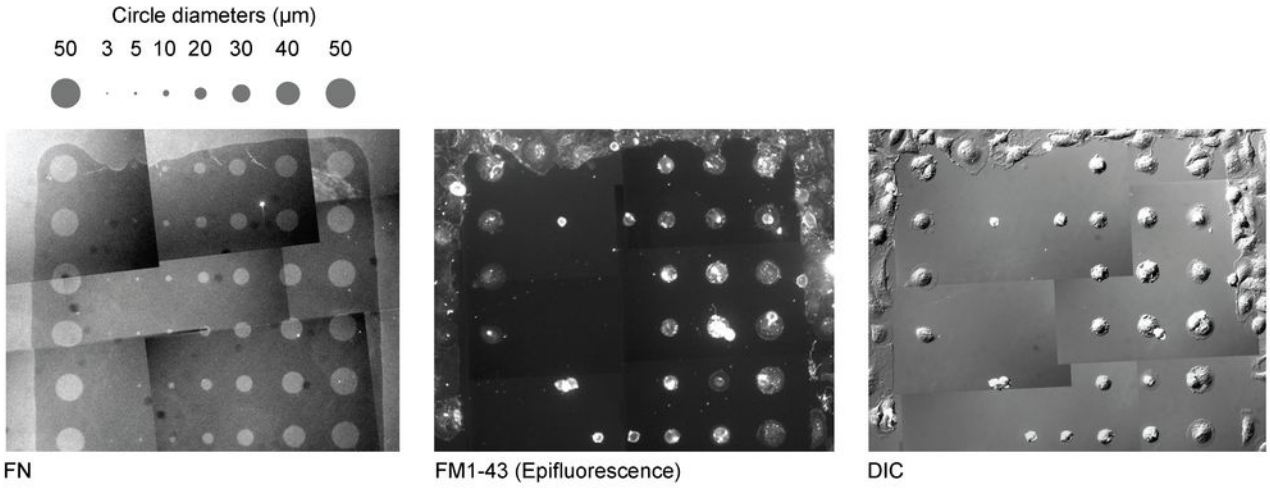
spread but also present a membrane disruption and again a very bright FM1-43 staining (middle panel, white arrows). Finally, on silanizated uncoated glass, some cells could present a bright halo indicating that part of the membrane has been absorbed by the slightly hydrophobic coverslip (right panel, white arrows). The three cases presented were always excluded from measurements and only single individual cells were taken in account (red arrows in each panel).

Supplementary Figure 3: (A) A RPTPα cell expressing YFP-microtubule (depicted in blue) and RFP-actin (depicted in green) was recorded with TIR-FM and DIC during spreading. The arrows outline the boundaries between actin and microtubule network during spreading. Scale bar: 10µm. (B) Dmso or nocodazole treated NIH-3T3 cells images after 60 min of spreading using the "FM1-43 during spreading" protocol. Cells were analyzed with two chambers on the same coverslip. (C) Averages of FM1-43 fluorescence intensity and area per NIH 3T3 cell after 60 min of spreading. Error bars represent s.d. between 40 cells from one typical experiment. (D) Comparison of Dmso and nocodazole (5 µM) treated RPTP α cells after 30 min of spreading. The imges presented show that microtubules were efficiently depolymerized under nocodazole and that the cells presented smallest substrate contact areas and more "spiky" edges than DMSO-treated control cells. Microtubules were stained with an antibody against alpha tubulin and a secondary antibody coupled with Alexa 568 (Sigma-Aldrich Co, Saint-Louis, MO). Actin was stained with Alexa 488-coupled phalloïdin (Sigma-Aldrich Co). (E) Large scale phase contrast microscopy observations of RPTP α cells after 30 min of

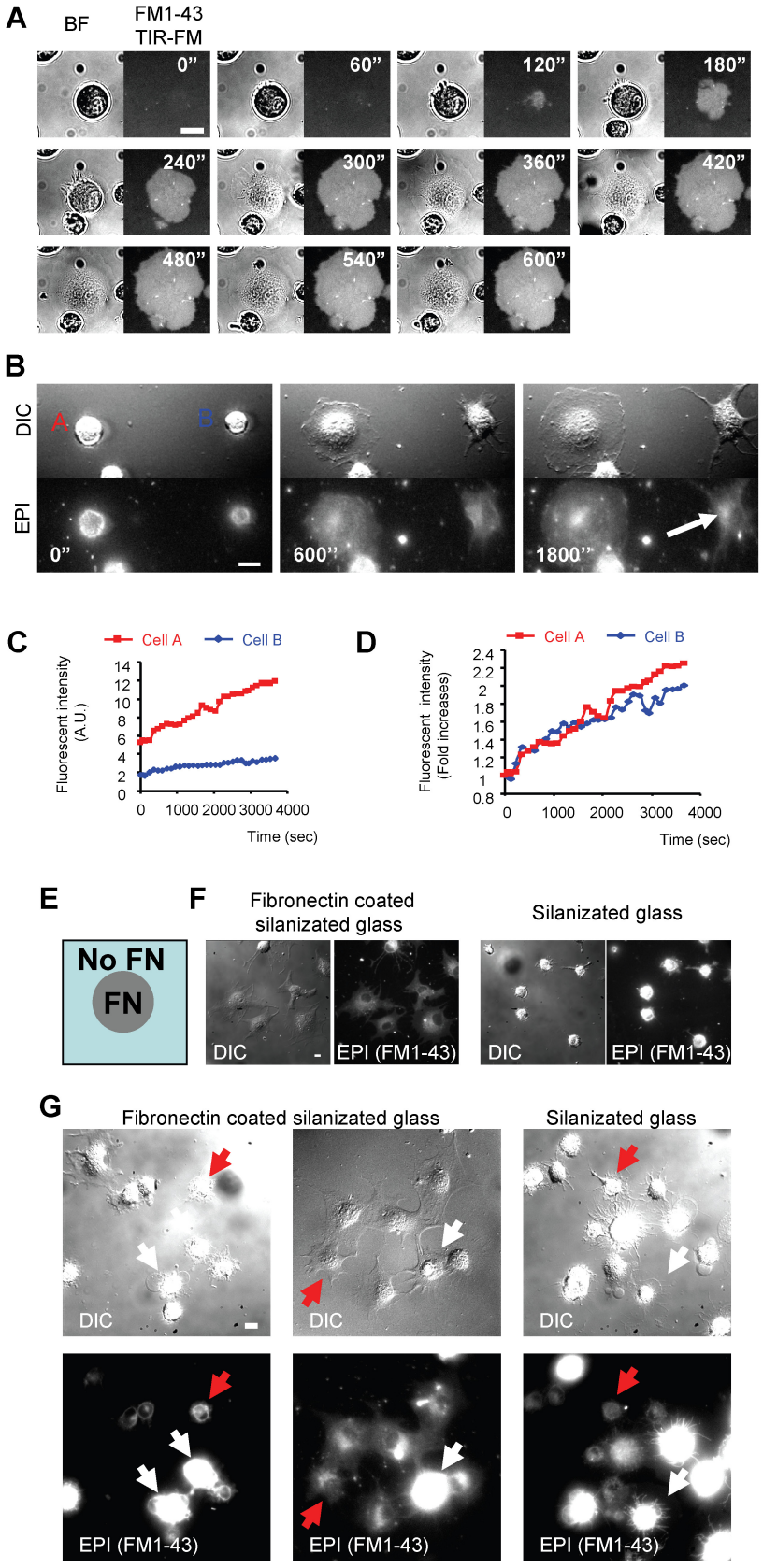
spreading, further supporting that under nocodazole, cells presented smallest substrate contact areas and more "spiky" edges. Scale bars 10 μm.

Supplementary Figure 4: Test that the GPI-GFP constructs expressed in RPTPα cell is pH sensitive. Almost 80% of the cell fluorescence is quenched by the acidic pH, in a reversible manner (top sequence and left graph). However at pH 4 the GFP fluorescence is quenched in a non-reversible manner (bottom sequence and right graph). Scale bars 10μm.

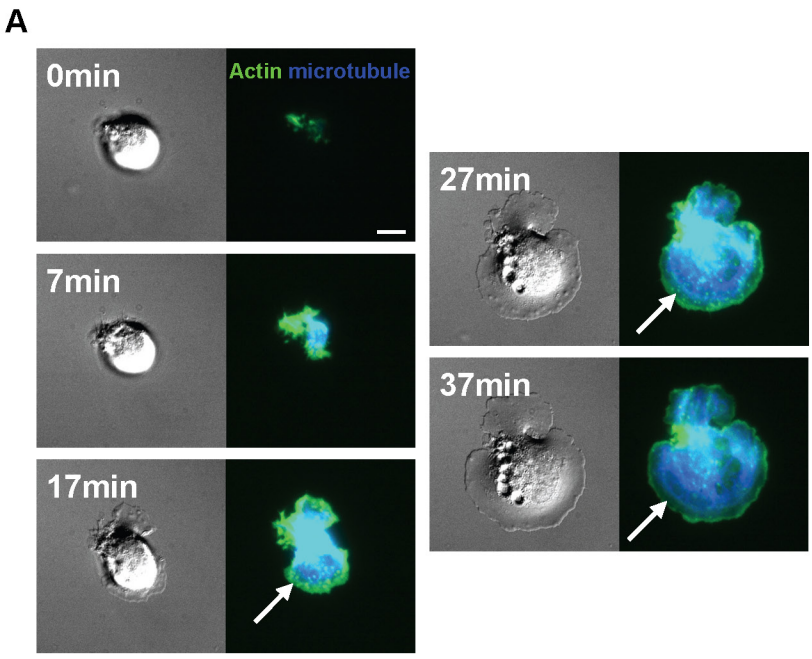
Supplementary Figure 5: Images of Hela cell analysed with the "FM1-43 during spreading" protocol during mitosis between the end of metaphases (note the chromosomal plaque in DIC) and cytokynesis. Scale bar 10 μm. In the little model, V is for volume and S is for surface.

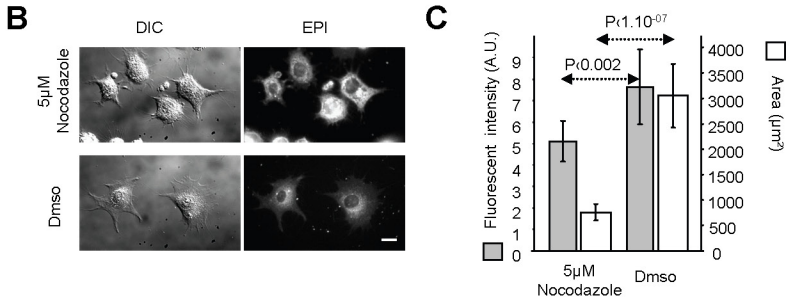


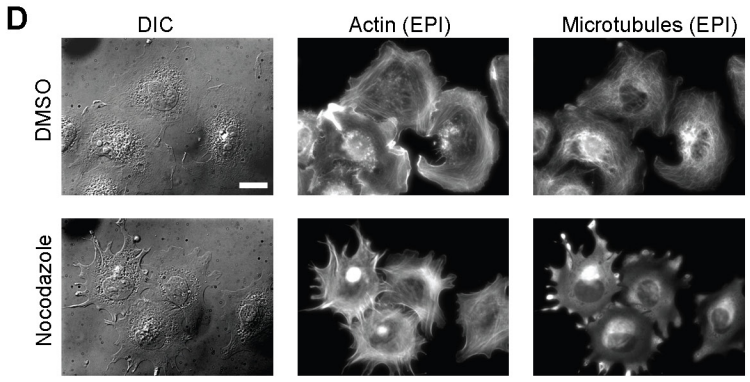
Supplementary Figure 1

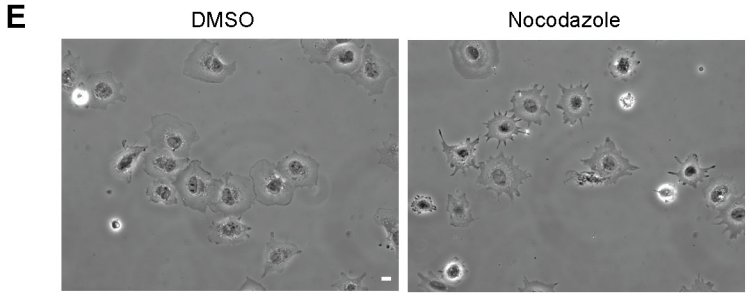


Supplementary Figure 2

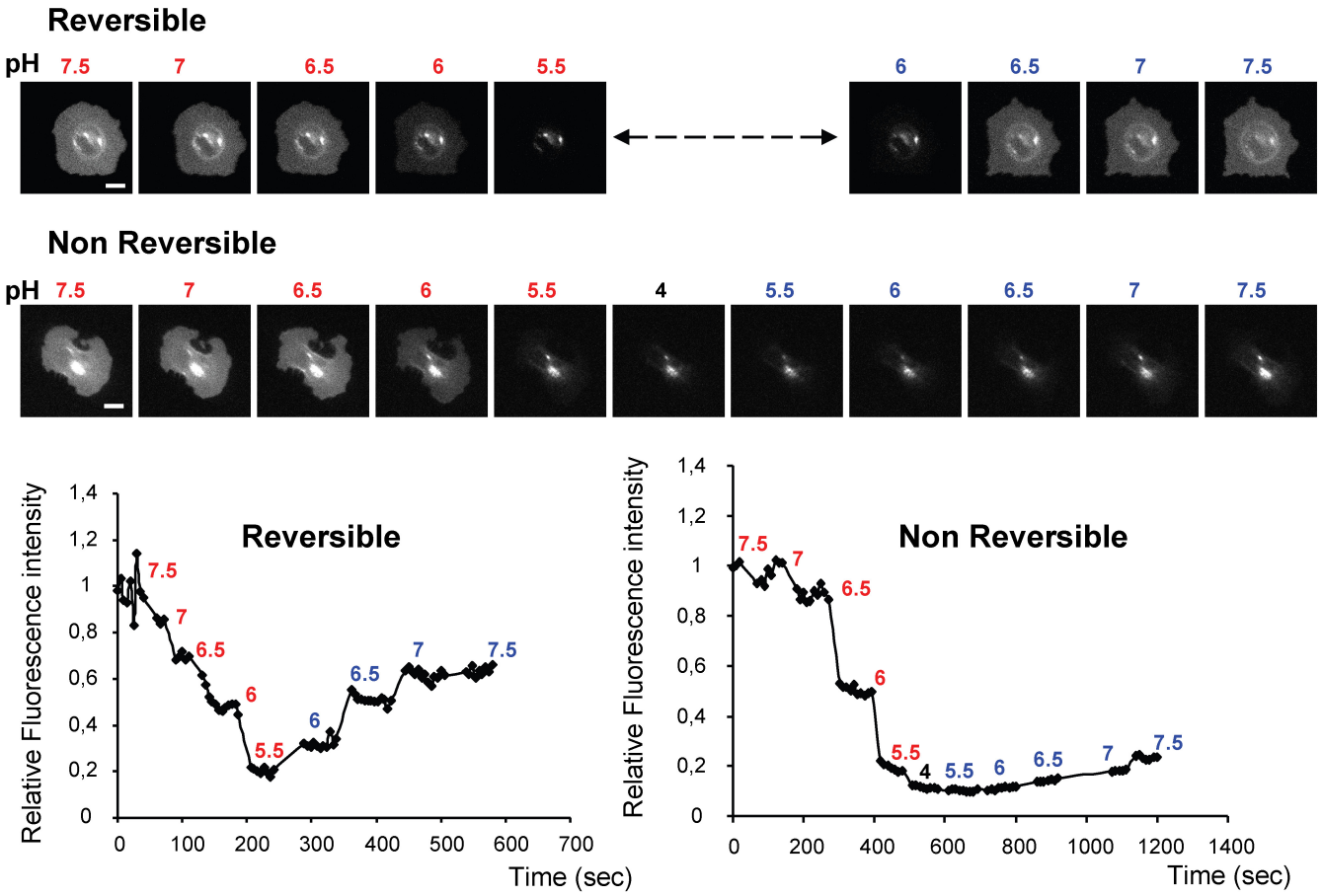




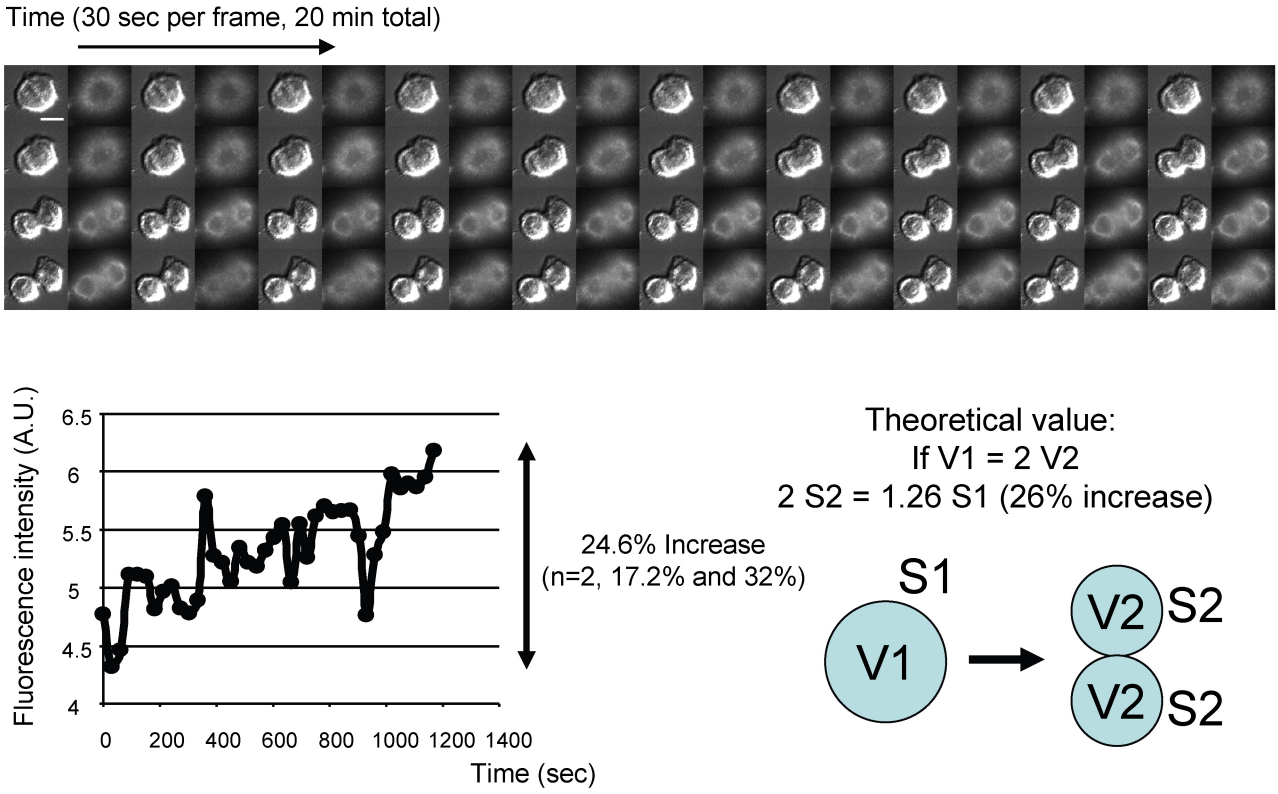




Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5