Supplementary Figures

Materials and Methods

Immunofluorescence using confocal microscopy. Confocal fluorescent images were obtained by a Zeiss LSM510NLO confocal scan head mounted on a Zeiss Axiovert 200M on an inverted-based microscope with a 63x objective. Sequential excitation at 488 nm and 543 nm was provided by argon and helium-neon gas lasers, respectively. Emission filters BP500-550 and LP560 were used for collecting green and red in channels one and two, respectively. After sequential excitation, green and red fluorescent images of the same cell were saved with Laser Sharp software. Images were analyzed by Zeiss software. The term colocalization refers to the coincidence of green and red fluorescence, as measured by the confocal microscope.

Time-lapse motion picture for *in vitro* scratch assay. RKO cells were seeded at confluency, transfected, and subjected to scratch wounding. A wound track was introduced by scraping the cell monolayer with a yellow pipette tip (200 μl). Cells cultured in Labtek chambers were placed on the stage of a phase-contrast/fluorescence microscope (Zeiss Axiovert 100M, Oberkochen, Germany) coupled to a CCD camera (Roper Scientific, Evry, France) to monitor wound closure. Acquisition was piloted with Metamorph software (Molecular Devices): phase-contrast images of two to eight selected fields were acquired every 5 min for at least 24 h. Images were analyzed using ImageJ software (NIH Image, Bethesda, MD). Videos were assembled using Image Ready (Adobe).

Supplementary Figure Legends

Supplementary Figure S1. *A*, RKO cells cotransfected with myc-liprin α1 and pcDNA-ING4 were seeded on glass coverslips and subjected to confocal microscopy analyses. myc-liprin α1 was detected by mouse anti-myc antibody (red) and ING4 by rabbit anti-ING4 antibody (green). *B*, ING4 colocalized with liprin α1 at the protruding membranes

in HEK-293 cells. HEK-293 cells were transfected with FLAG-ING4 and plated on glass coverslips for immunofluorescence microscopic analysis. Chicken anti-liprin α1 antibody was used to detect liprin α1 (green), and mouse anti-FLAG antibody was used to detect ING4 (red). *C*, U-87 MG cells cotransfected with myc-liprin α1 and FLAG-ING4 were seeded on glass coverslips and subjected to immunofluorescence microscopy. Myc-liprin α1 was detected by mouse anti-myc antibody (green) and FLAG-ING4 by rabbit anti-FLAG antibody (red).

Supplementary Figure S2. *A*, Supplementary images for Figure 3*A* (right). EGFP vector and EGFP-ING4 were used to transfect RKO cells. RKO cells were harvested and subsequently plated in serum-depleted medium at 2 x 10^5 cells per well on 6-well plates. Cells were allowed to adhere for 16 h, followed by 5% serum stimulation to initiate cell spreading. Cells were fixed and stained by rhodamine-phalloidin for visualizing F-actin (red) 1 h after serum stimulation. F-actin is shown in red and EGFP vector or EGFP-ING4-transfected cells are shown in green. Images were representative fields from the vector control and the ING4 transfection. Bar, 10 μm. *B*. Supplementary images for Figure 3*A* (left). RKO cells transfected with pFLAG vector or pFLAG-ING4 for 24 h were subjected to the cell spreading assay. Bar, 20 μm.

Supplementary Figure S3. The overexpression of ING4 suppressed cell migration in the *in vitro* scratch assay. RKO cells transfected with pcDNA vector (Vector) or pcDNA-ING4 (ING4) for 24 h were plated on cell culture dishes and grown to confluence. Still images (*A*) and a histogram (*B*) were derived from the time-lapse motion picture in Supplementary Video 1 (1 pixel = 0.645 μm).

Supplementary Figure S4. Supplementary images for Figure 3*C*. The overexpression of liprin α1 enhanced cell spreading, and the coexpression of ING4 and liprin α1 suppressed spreading. RKO cells transfected with pcDNA-ING4 and/or myc-liprin α1 expression plasmids were subjected to the cell spreading assay. Cells were fixed and stained by rhodamine-phalloidin at 0 min (t_0) and 45 min (t_{45}) after serum stimulation. Cells at 0 min

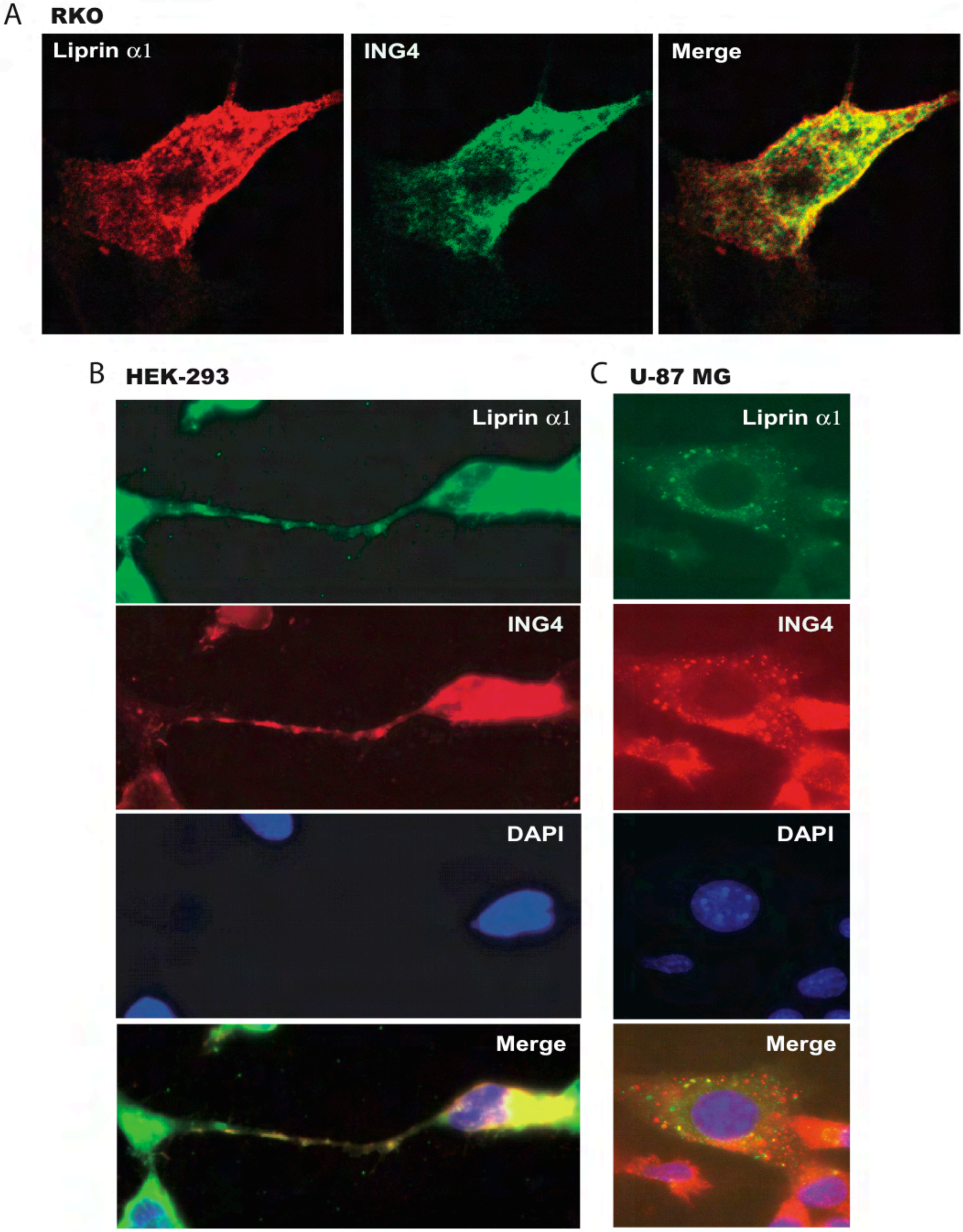
exhibited the same morphology, and thus, only the Control (t_0) was shown here as a representative. Bar, 20 μm.

Supplementary Figure S5. Supplementary images for Figure 4*B* and *C*. RKO cells transfected with ING4 siRNA-1, -2, -3 or -4 for 72 h were harvested and plated as described previously for the cell spreading assay. RKO cells were fixed and stained by rhodamine-phalloidin at 0 min (t_0) and 30 min (t_{30}) after serum stimulation. Cells at 0 min exhibit the same morphology, and thus, only the Control (t_0) is shown here as a representative. Bar, 20 μm.

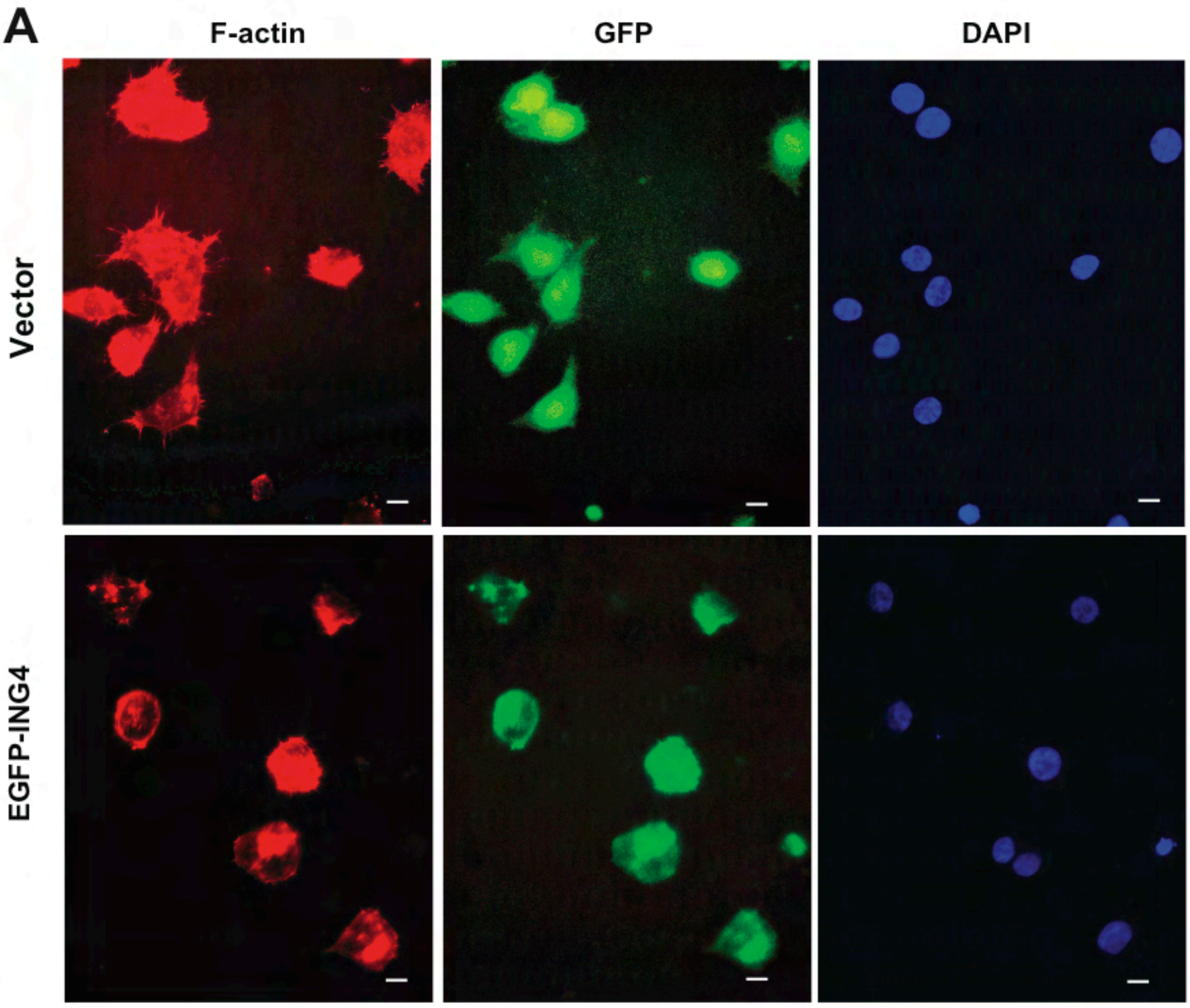
Supplementary Figure S6. Supplementary images for Figure 5*A*. The downregulation of liprin α1 reduced cell spreading capacity. RKO cells transfected with two different liprin α1 siRNAs for 72 h were subjected to the cell spreading assay. Immunofluorescence was performed at 0 h (t_0) and 1 h (t_{1h}) after serum stimulation. Cells at 0 h exhibited the same morphology and are shown here as a representative Control. Bar, 20 μm.

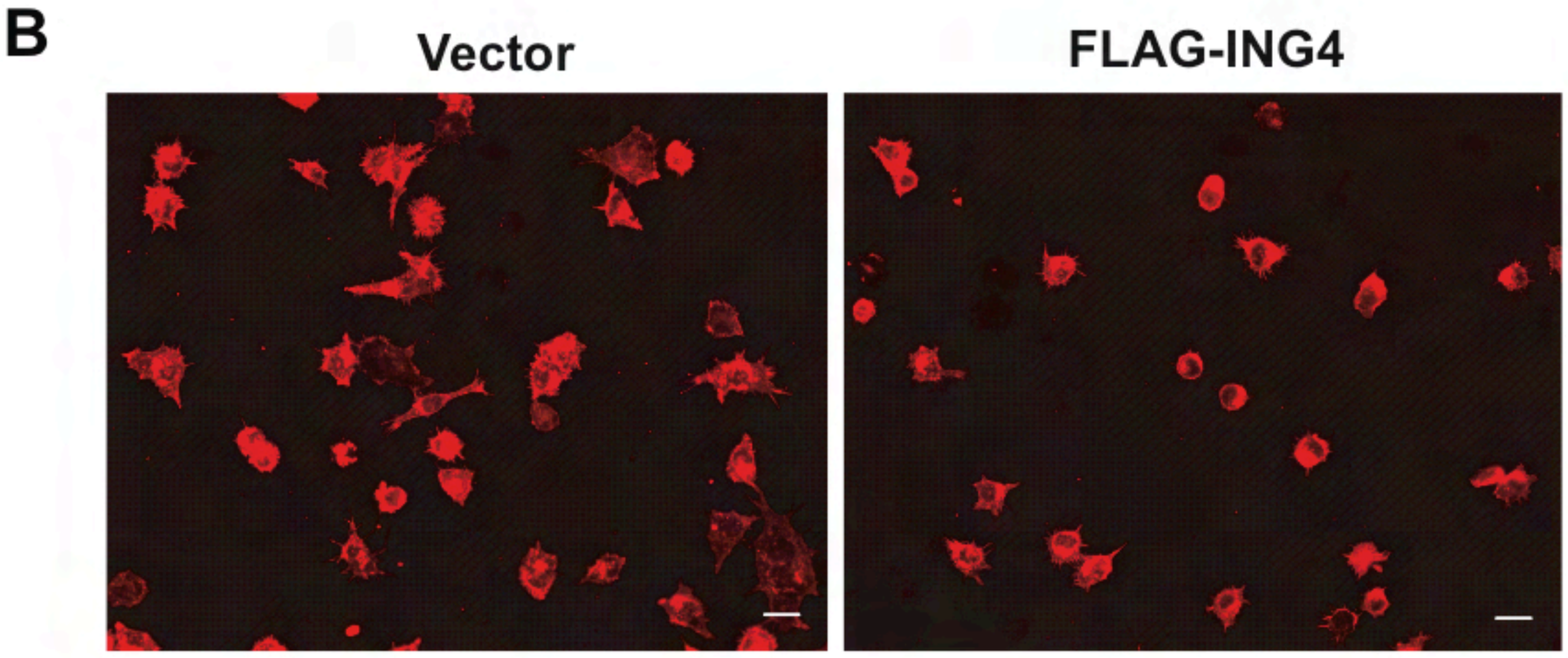
Supplementary Video 1. The suppression of cell migration by ING4 *in vitro* scratch assay. RKO cells transfected with pcDNA (top frame) or pcDNA-ING4 (bottom frame) were plated in a Labtek chamber to form a cell monolayer. A scratch on the monolayer was introduced and closure of the gap by cell migration was monitored by a phase contrast microscope coupled with a CCD camera. Picture frames were collected every 5 min for 24 h. The video displays at a rate of 10.3 frames/second. Still images at time 0, 12, and 24 h were taken for presentation in Supplementary Fig. S3*A*. Still images were also taken at time 0, 6, 12, 18, and 24 h for calculation of the gap widths, shown as a histogram in Supplementary Fig. S3*B*.

Shen et al., Figure S1.

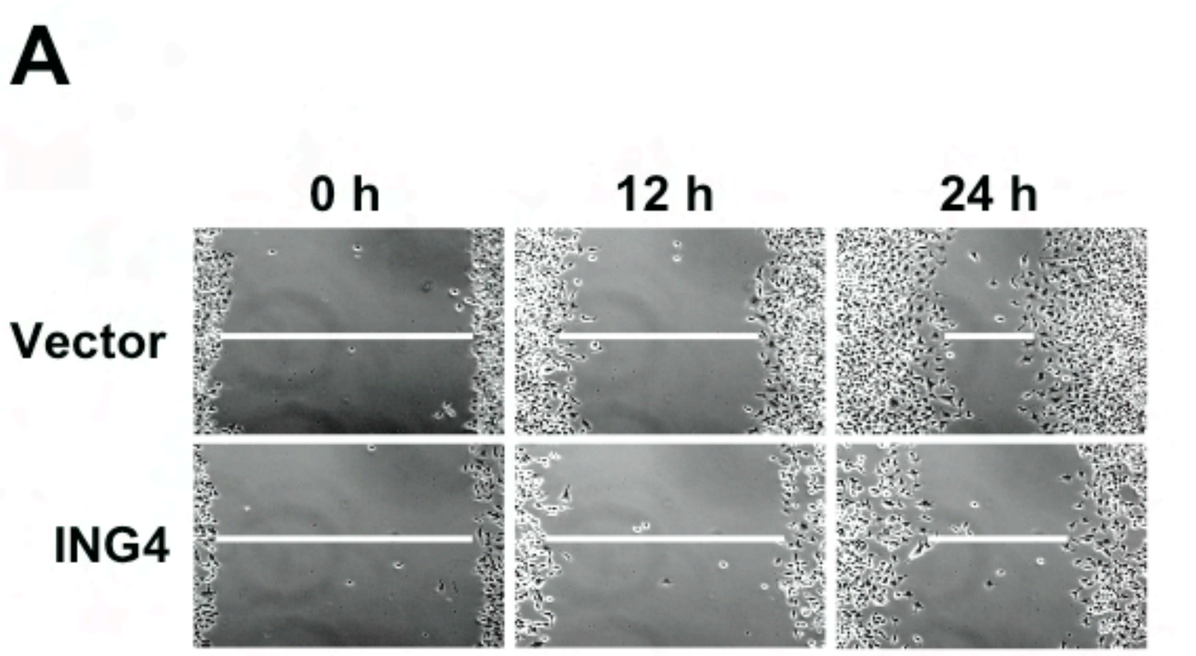


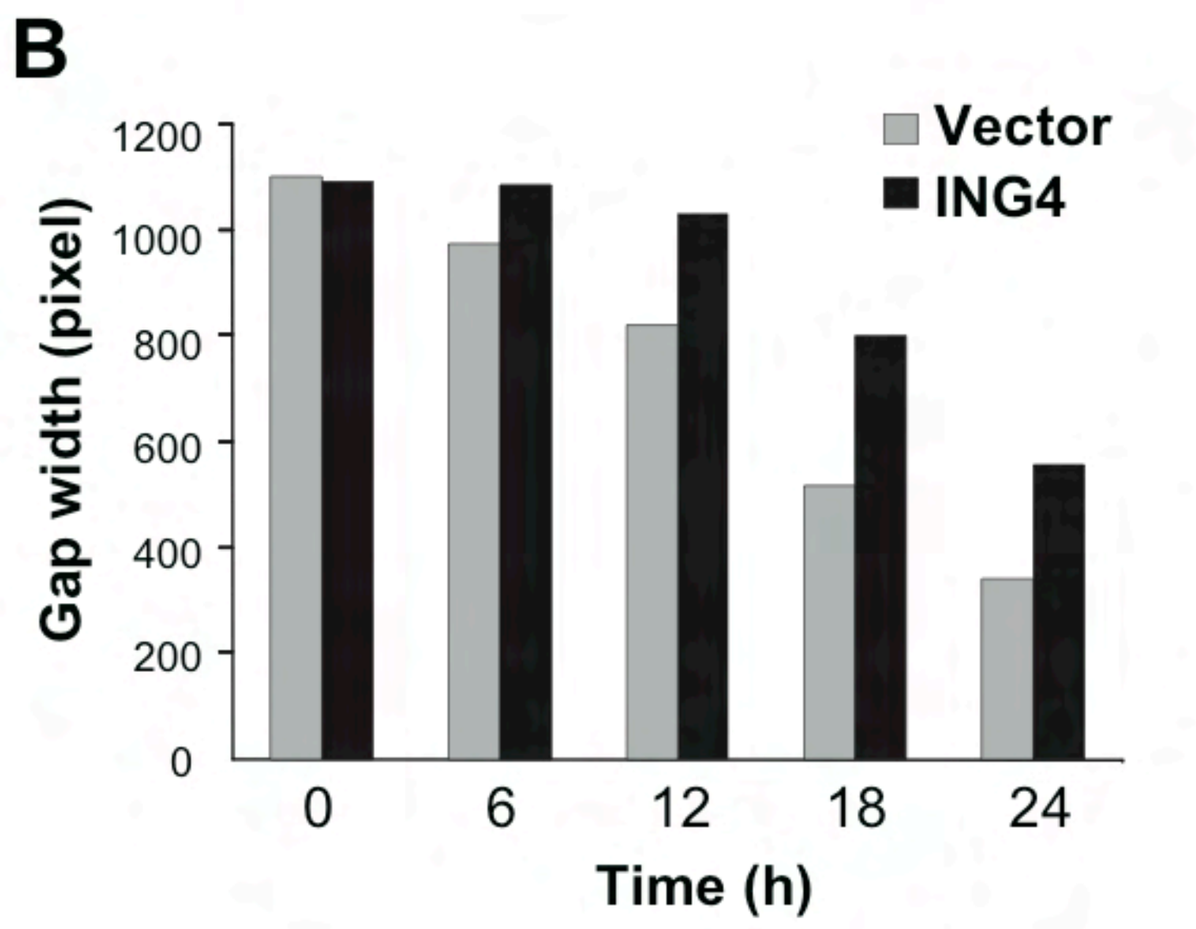
Shen et al., Figure S2



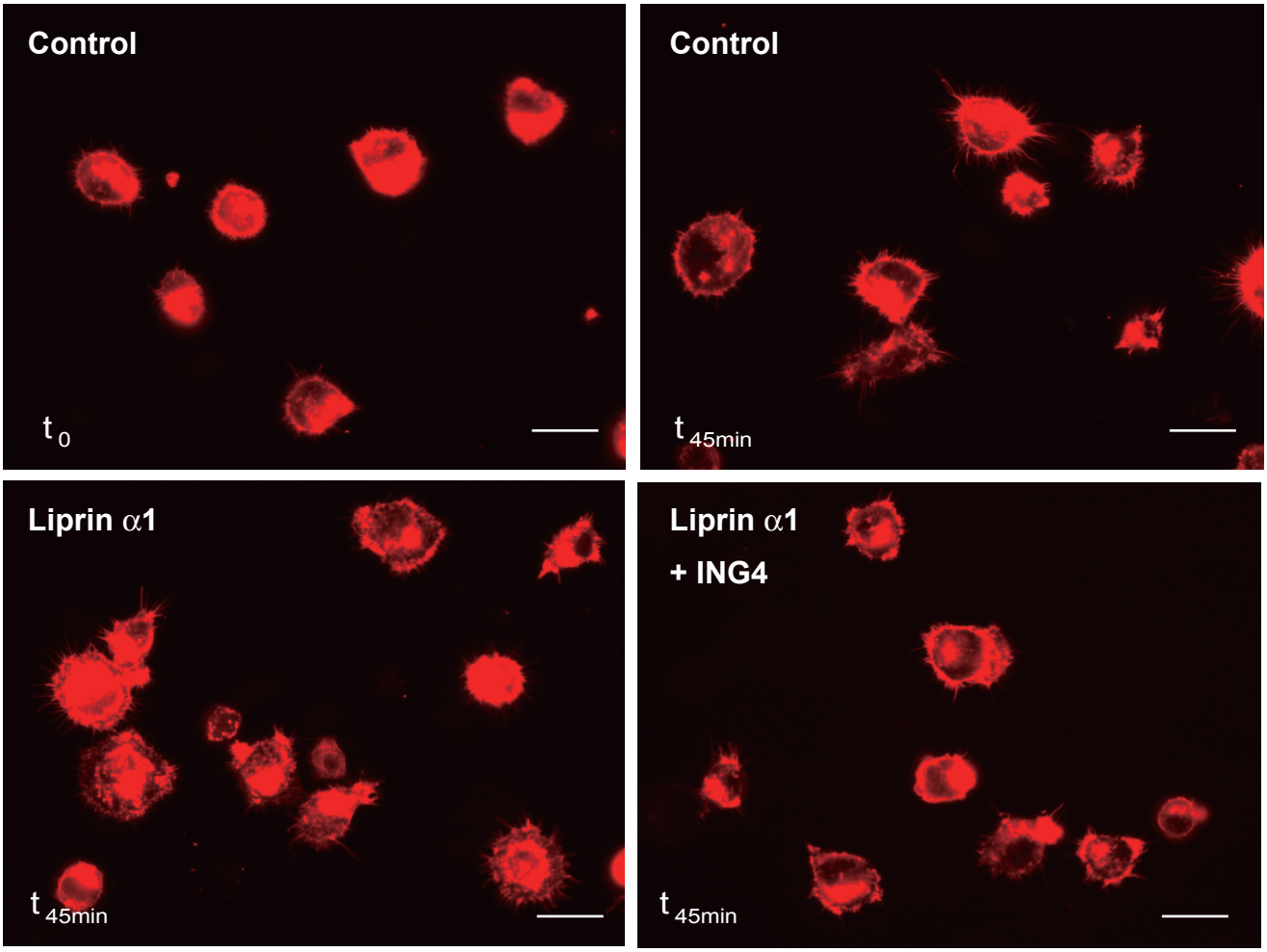


Shen et al., Figure S3

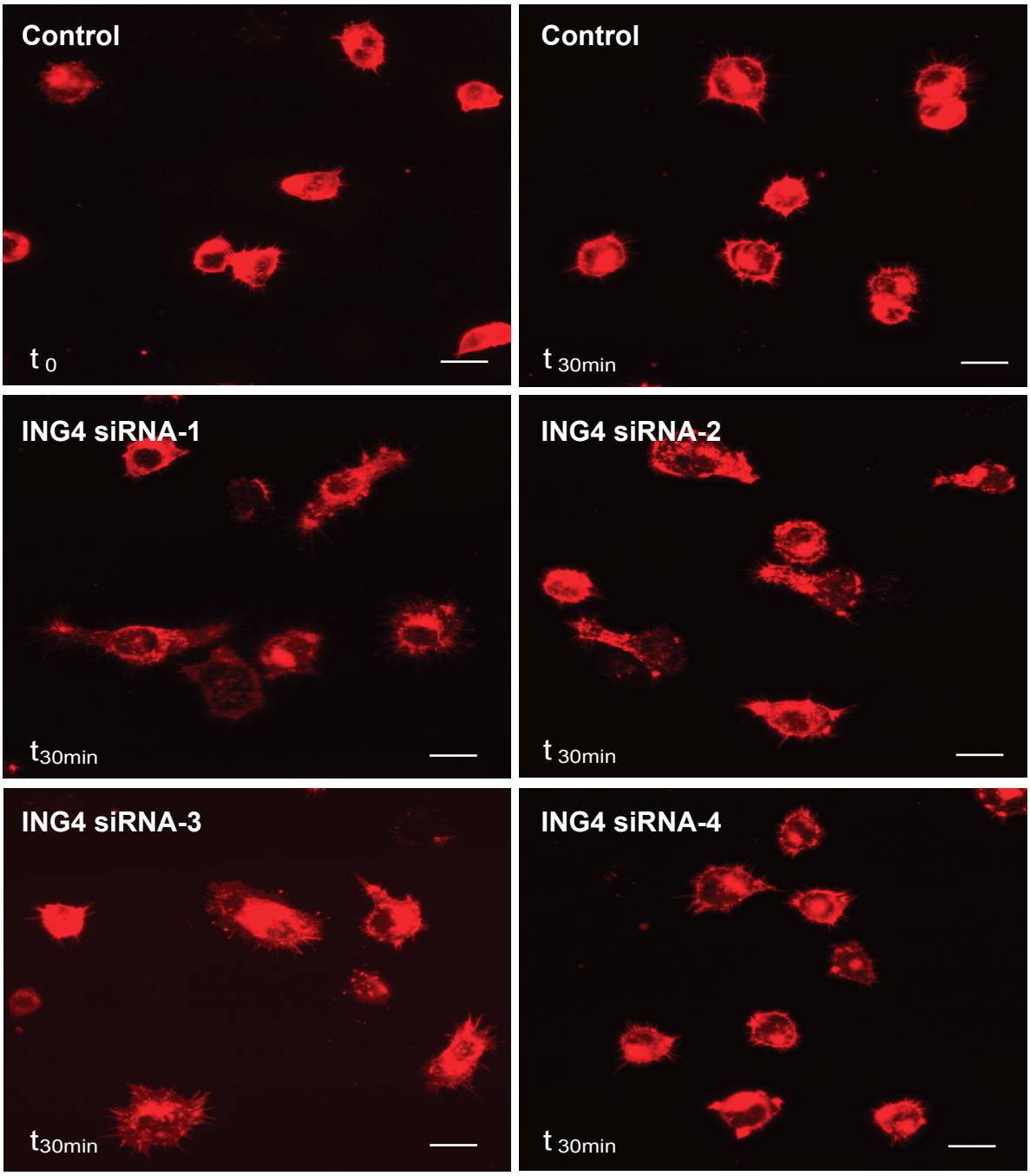




Shen et al., Figure S4



Shen et al., Figure S5



Shen et al., Figure S6