Supplemental Materials

Molecular Biology of the Cell

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SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure S1: Results supplemental to Figure 1. (A) Wide-field image of fixed control, nocodazole-, and latrunculin A-treated Huh-7 cells expressing Hsp47-GFP. Tubules (arrows) and sheets (arrowheads) are indicated. (B) Wide-field images and (C) relative fluorescence intensity measurements of fixed control and latrunculin A-treated cells stained with DNase I for monomeric actin show an increase in the fluorescence intensity after latrunculin A treatment. Error bars shown as sem. Bars 10 µm.

Supplemental Figure S2: Supplemental to Figures 1 and 4-7. The effects of nocodazole and latrunculin A treatments on ER morphology in HeLa cells can be seen from (A) wide-field images and (B) relative fluorescence intensity measurement of calreticulin of cells immunolabeled for calreticulin and/or FLAG, or stained with phalloidin. Magnified images of insets (a-c) were Gaussian filtered. See also Figure 1A. Error bars shown as ±sem. (C) Phalloidin staining and (D) the relative fluorescence intensity of phalloidin and DNaseI in control and latrunculin A-treated HeLa cells. Error bars shown as sem. See also Figures 1C and 1D and Supplemental Figure S1C. (E) Myo1c regulates actin filament arrays in HeLa cells. Phalloidin staining of cells expressing (a) CMVTag1 (control), (b) myo1c-2FLAG or (d) EGFP-myo1cΔABL (small inset) or (c) depleted with myo1c shRNAs. See also Figure 5. (F) Over-expression of myo1c in a HeLa cell. Cells expressing 2FLAG-C1 (control) or myo1c-2FLAG (myo1c over-expression) were immunolabeled for calreticulin and FLAG. See also Figure 6A. (G) Calreticulin immunolabeling in control (CMVTag1) and myo1c-depleted (shRNA) cells. See also Figure 6B. (H) Myo1c localizes to actin filament arrays in HeLa cells. Cells expressing myo1c-2FLAG (magenta) were immunolabeled for FLAG and stained with phalloidin (green). Longer filaments are not positive for myo1c whereas white signal indicate double positive shorter structures. See also Figure 4A. (I) Actin-binding domain of myo1c is crucial for proper ER phenotype in HeLa cells. A cell expressing EGFP-myo1cΔABL (small inset) was immunolabeled for calreticulin. Tubular network (arrows) is prevalent. See also Figure 7A. Tubules and sheets / interconnected sheet mass are indicated with arrows and arrowheads, respectively, in A, E, F and H. Long actin filaments, filaments shorter than 5 µm and foci are indicated with open arrowheads, arrows and arrowheads, respectively, in C, D and G. Bars 10 µm.

Supplemental Figure S3: Results supplemental to Figure 3. Representative confocal sections of Huh-7 cell expressing LifeAct-RFP, starting from the bottom of the cell and covering the whole depth of the cell. Long actin fibers i.e. stress fibers (open arrowheads) and actin arrays and foci (arrows) are found throughout the cell volume in cytosol and under the PM. z-step 0.285 µm. Bars 5 µm.

Supplemental Figure S4: Results supplemental to Figure 4. (A) Wide-field image of fixed and phalloidin stained (green) Huh-7 cell immunolabeled for cortactin (magenta). Boxed areas are shown at higher magnification portraying cortactin positive actin foci (inset a) and short actin filament (inset b). Occasionally actin filaments negative for cortactin were found (inset c). Long actin filaments i.e. stress fibers (open arrowheads) are not positive for cortactin. (B) Wide-field image of fixed Huh-7 cell expressing myo1c-2FLAG and immunolabeled for cortactin (green) and FLAG (magenta). Boxed areas are shown with higher magnification portraying structures positive for myo1c and cortactin (inset a) and

structures positive either for cortactin (inset b) or myo1c (inset c). Majority of the structures are double positive. Bars 10 µm.

Supplemental Figure S5: Results supplemental to Figure 5. (A) EGFP-myo1cΔABL-positive Huh-7 cell (same cell as in Figure 5D) immunolabeled for cortactin (green) and stained for phalloidin (magenta). Boxed area is shown at higher magnification showing that the remaining actin foci remain positive for cortactin (arrowheads). Wide-field images and corresponding mean fluorescence intensity measurements of fixed Huh-7 cells transfected with CMVTag1 or pooled myo1c shRNAs (myo1c depletion) and pulsed with (B and C) Dextran or (D and E) Transferrin for 7 min. Error bars shown as ±SD. Bars 10 µm.

Supplemental Figure S6: Results supplemental to Figure 6. (A) Huh-7 cells transfected with either scrambled siRNAs or a pool of target-specific myo1c siRNAs were immunolabeled for calreticulin. Sheets (arrowheads) and tubules (arrows) are indicated. See Figure 6B for comparison. (B) Huh-7 cells transfected with scrambled siRNA or myo1c-spesific siRNA were analyzed by Western blotting with the indicated antibodies. An unknown band, which is not affected by myo1c depletion, is indicated (open arrow). (C) Myo1c depleted (pooled myo1c-spesific siRNAs) Huh-7 cell transiently expressing ssHRP-KDEL was cytochemically stained (dark precipitate) and imaged with SB-EM. Block face images of the dataset at 0 nm, +60 nm and +300nm are presented. Areas void of ER (asterisks) and the NE are indicated. See Figure 6Db for comparison. Bars 10 µm in A and 1 µm in C.

Supplemental Figure S7: Results supplemental to Figure 6. Myo1c depletion and actin depolymerization do not alter protein synthesis and secretion rates in Huh-7 cells. Comparison of (A) total cellular proteins (Instant Blue –stained gel) and (B) metabolically labeled newly synthesized proteins (20 minutes pulse) and (C) secreted proteins did not reveal statistical differences between Huh-7 cells transfected with the CMVTag1 (control), myo1c-spesific shRNAs, or, cells treated with latrunculin A at time points 0, 1 and 4 hours. Protein ladder molecular weights are given as kDa. Graphs show comparison of protein synthesis ratio (D) and secretion (E) adjusted to the total or newly synthesized protein amounts, respectively.

Supplemental Figure S8: Results supplemental to Figure 8. (A) Wide-field images of fixed control and trichostatin A-treated (TSA) Huh-7 cells immunostained for acetylated tubulin and (B) corresponding Western blot show the increased acetylation after TSA treatment. Bars 10 µm.

VIDEO LEGENDS

Video S1: 3D model of Huh-7 ER. The video shows the SB-EM dataset and the modeled ER (yellow) and NE (blue) of a control Huh-7 cell and a representative sheet at the end of the video (see Figure 2A). The cell was co-transfected with ssHRP-KDEL and CMVTag1 and cytochemically stained (dark precipitate). Images were acquired using FEG-SEM Quanta 250 (FEI) equipped with 3View system (Gatan Inc.). Voxel size is 14.3 x 14.3 x 30 nm and the dimensions of the bounding box are 14.9 x 14.8 x 2.5 µm. Bar 5µm.

Video S2: 3D model of ER in actin-depolymerized Huh-7 cell reveal the uneven ER network distribution and sheet remnants. The video shows the SB-EM dataset and the modeled ER (yellow) and NE (blue) of a Huh-7 cell treated with latrunculin A and a representative sheet remnant at the end of the video (see Figure 2B). Huh-7 cell expressing ssHRP-KDEL was cytochemically stained (dark precipitate). Images were acquired using FEG-SEM Quanta 250 (FEI) equipped with 3View system (Gatan Inc.). Voxel size is 17.3 x 17.3 x 40 nm and the dimensions of the bounding box are 24.3 x 23.1 x 2.0 µm. Bar 10 µm.

Video S3: Actin filament arrays localize to polygons defined by the surrounding ER sheets and tubules. Confocal frames of live Huh-7 cell expressing Hsp47-GFP (green) and mCherry-Actin (magenta) (see Figure 3A) showing short actin filament arrays and foci localizing to ER network polygons. While dynamic actin arrays localize in close proximity with ER, the longer actin filaments i.e. cortical actin and stress fibers do not have a clear interaction with ER. Images were acquired using an inverted TCS SP5II HCS A laser-scanning confocal microscope (Leica). The imaging frame rate was 1 frame/0.54 s and the playback frame rate is 10 frames/ s. The images were filtered with rotationally symmetric Gaussian lowpass filter, hsize is [3; 3], sigma is 0.6. Bar 5 µm.

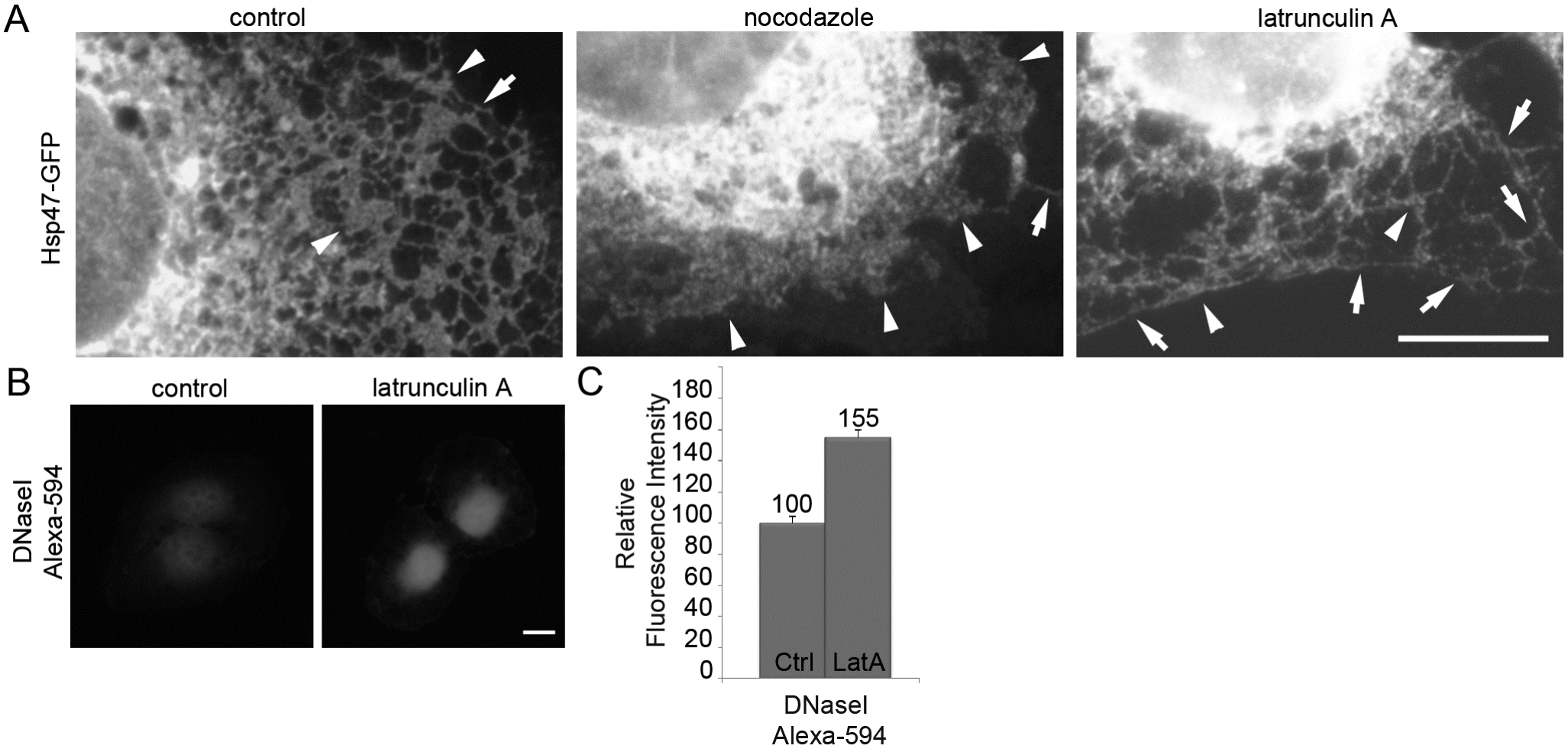
Video S4: Relocation or disappearance of actin arrays from ER network polygons precedes ER transformations. A cropped area of confocal acquisition of live Huh-7 cell expressing Hsp47-GFP (green) and mCherry-Actin (magenta). Images were acquired using an inverted TCS SP5II HCS A laser-scanning confocal microscope (Leica). The 00:00 time in Figure 3B corresponds to the first frame in the video. The imaging frame rate was 1 frame/0.50 s and the playback frame rate is 10 frames/s. The images were filtered with rotationally symmetric Gaussian lowpass filter, hsize is [3; 3], sigma is 0.6. Bar 5 µm. See also Video S5.

Video S5: Formation of short actin filament arrays leads to the subsequent opening of a polygon in ER sheets. A cropped area of confocal acquisition of live Huh-7 cell expressing Hsp47-GFP (green) and mCherry-Actin (magenta). Images were acquired using an inverted TCS SP5II HCS A laser-scanning confocal microscope (Leica). The 00:00 time in Figure 3C corresponds to the first frame in the video. The imaging frame rate was 1 frame/0.50 s and the playback frame rate is 10 frames/s. The images were filtered with rotationally symmetric Gaussian lowpass filter, hsize is [3; 3], sigma is 0.6. Bar 5 µm. See also Video S4.

Video S6: Actin filament arrays present a pool of transforming dynamic structures positive for myo1c. Confocal frames of live Huh-7 cell expressing myo1c-EGFP (green) and

mCherry-Actin (magenta) are shown in combination of merged channels. Images were acquired using an inverted TCS SP5II HCS A laser-scanning confocal microscope (Leica). Static (arrow) and dynamic (arrowhead) actin filaments are indicated. An actin filament array transforming into foci (circle) and *vice versa* (two dashed circles) are shown. The images were filtered with rotationally symmetric Gaussian lowpass filter, hsize is [3; 3], sigma is 0.6. The imaging frame rate was 1 frames/0.50 s and the playback frame rate is 10 frames/s. Bar 10 µm. See Figure 4B.

Video S7: 3D model of ER in myo1c-depleted Huh-7 cell reveal ER network distribution defect and sheet remnants. The video shows the SB-EM dataset and the modeled ER (yellow) and NE (blue) of myo1c depleted Huh-7 cell and a representative sheet remnant at the end of the video (Video S1 for comparison). Huh-7 cell expressing ssHRP-KDEL was cytochemically stained (dark precipitate). The model consists of ER membranes (yellow) and NE (blue). See Figure 6D. Images were acquired using FEG-SEM Quanta 250 (FEI) equipped with 3View system (Gatan Inc.). Voxel size is 14.6 x 14.6 x 30 nm and the dimensions of the bounding box are 20.14 x 19.03 x 1.71 µm. Bar 5 µm.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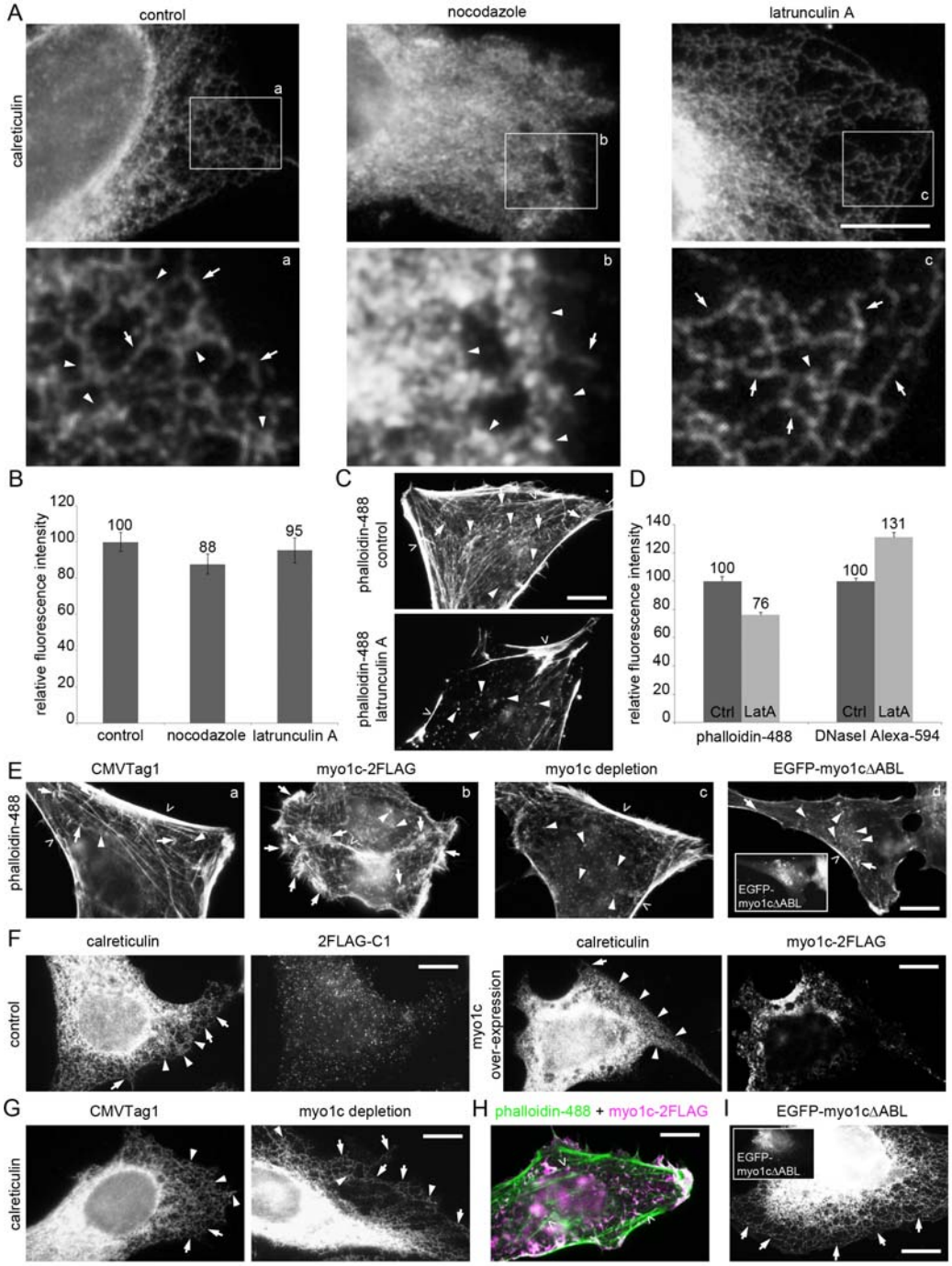
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G
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LifeAct-RFP
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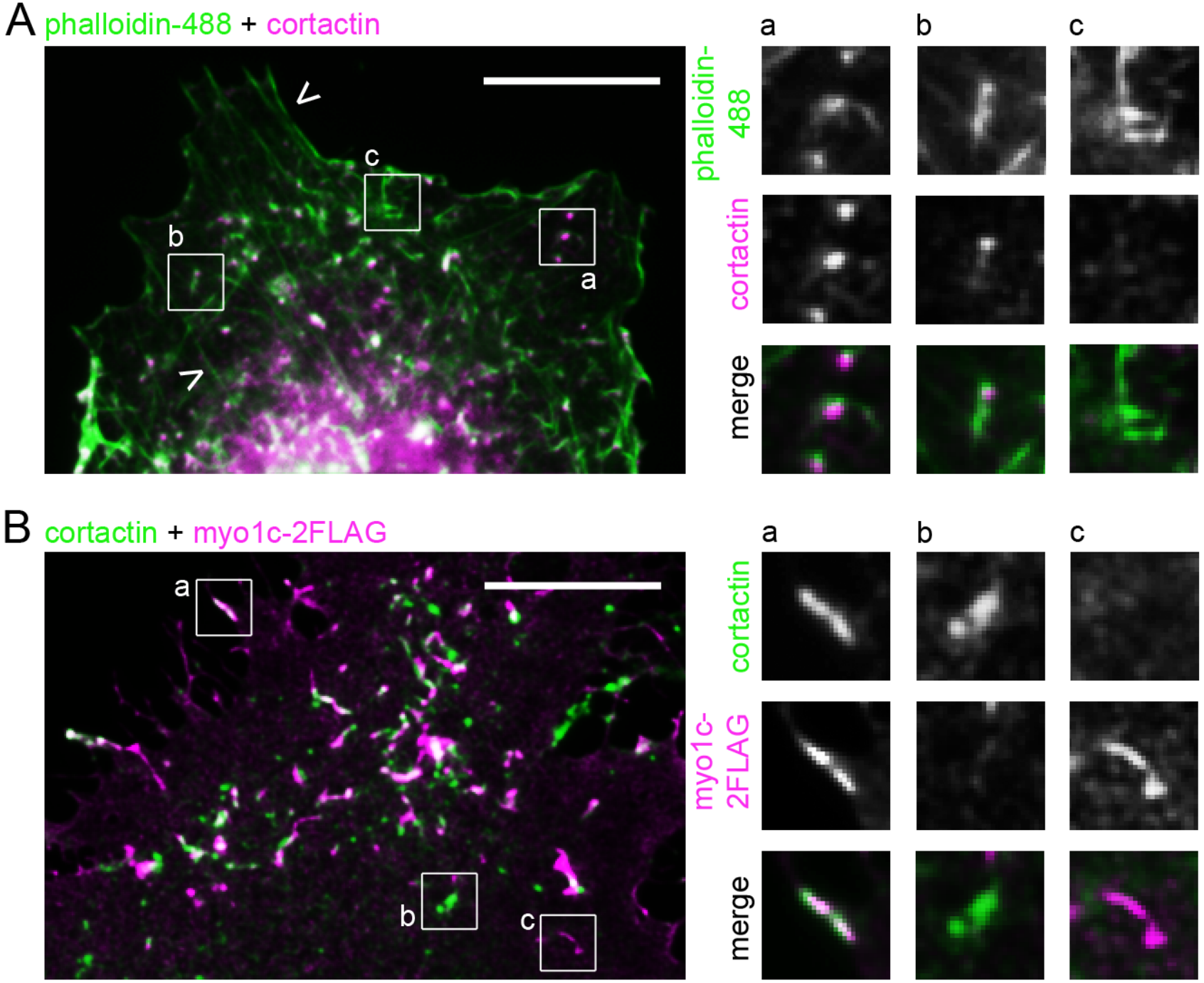
cell top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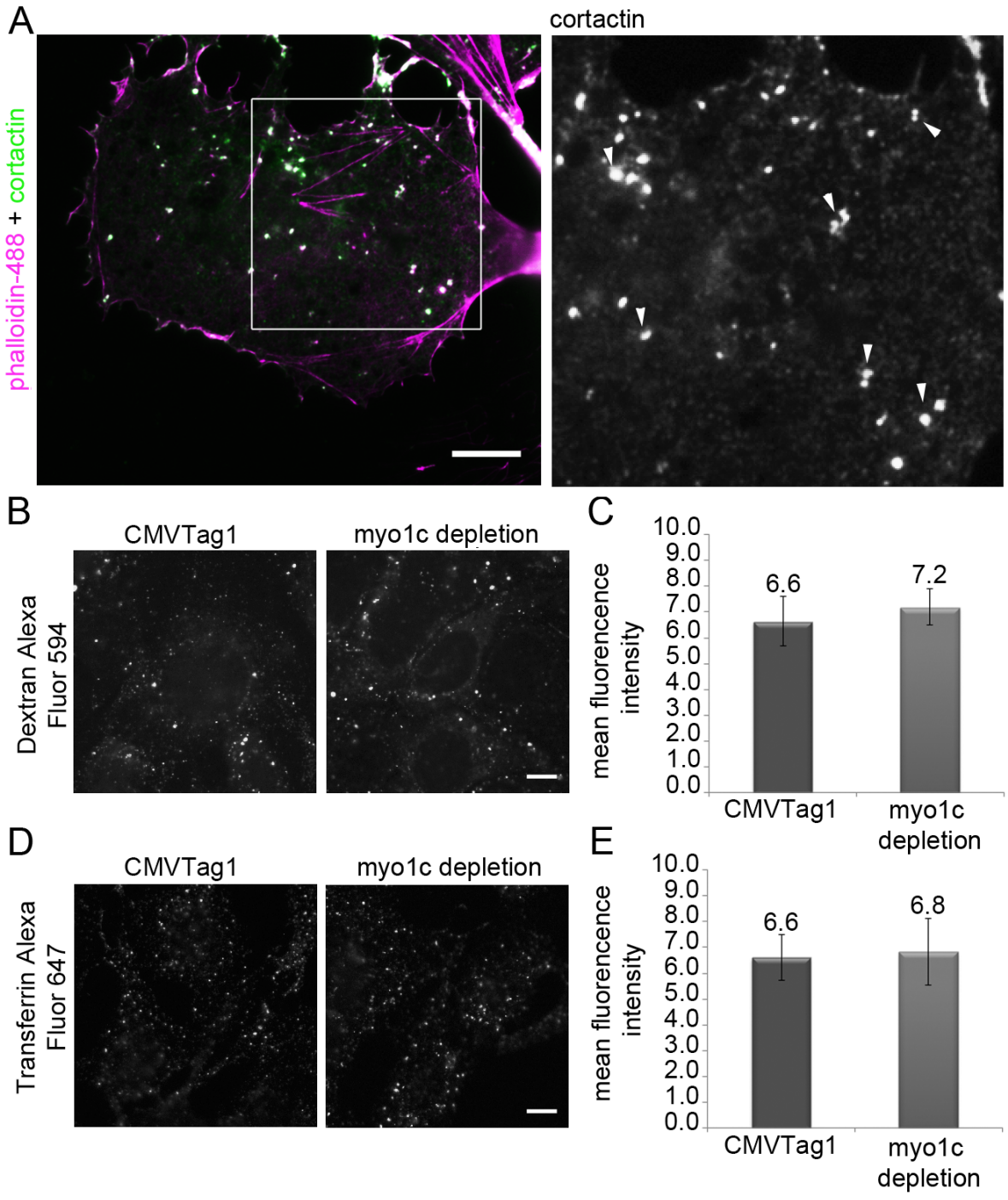
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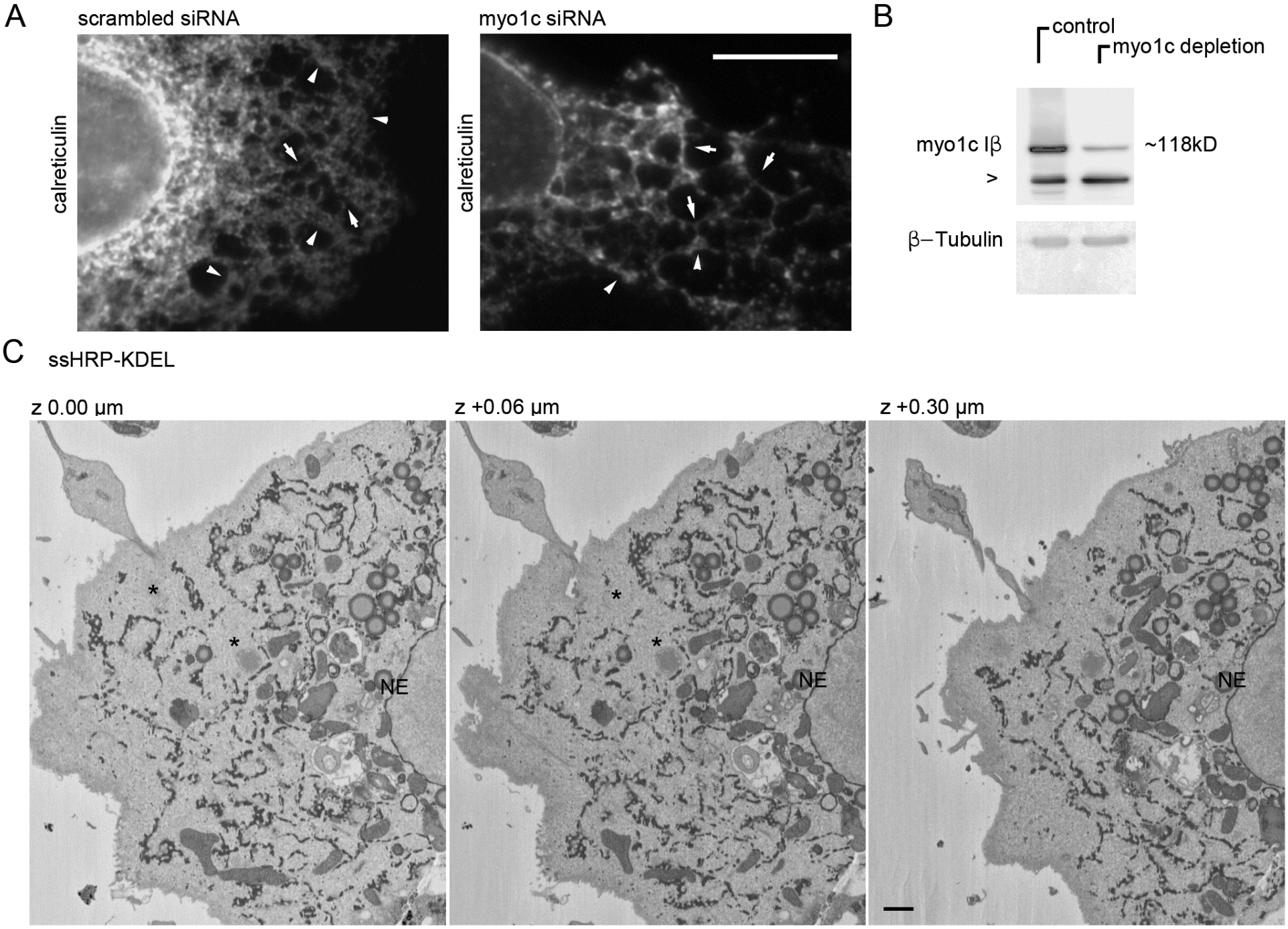
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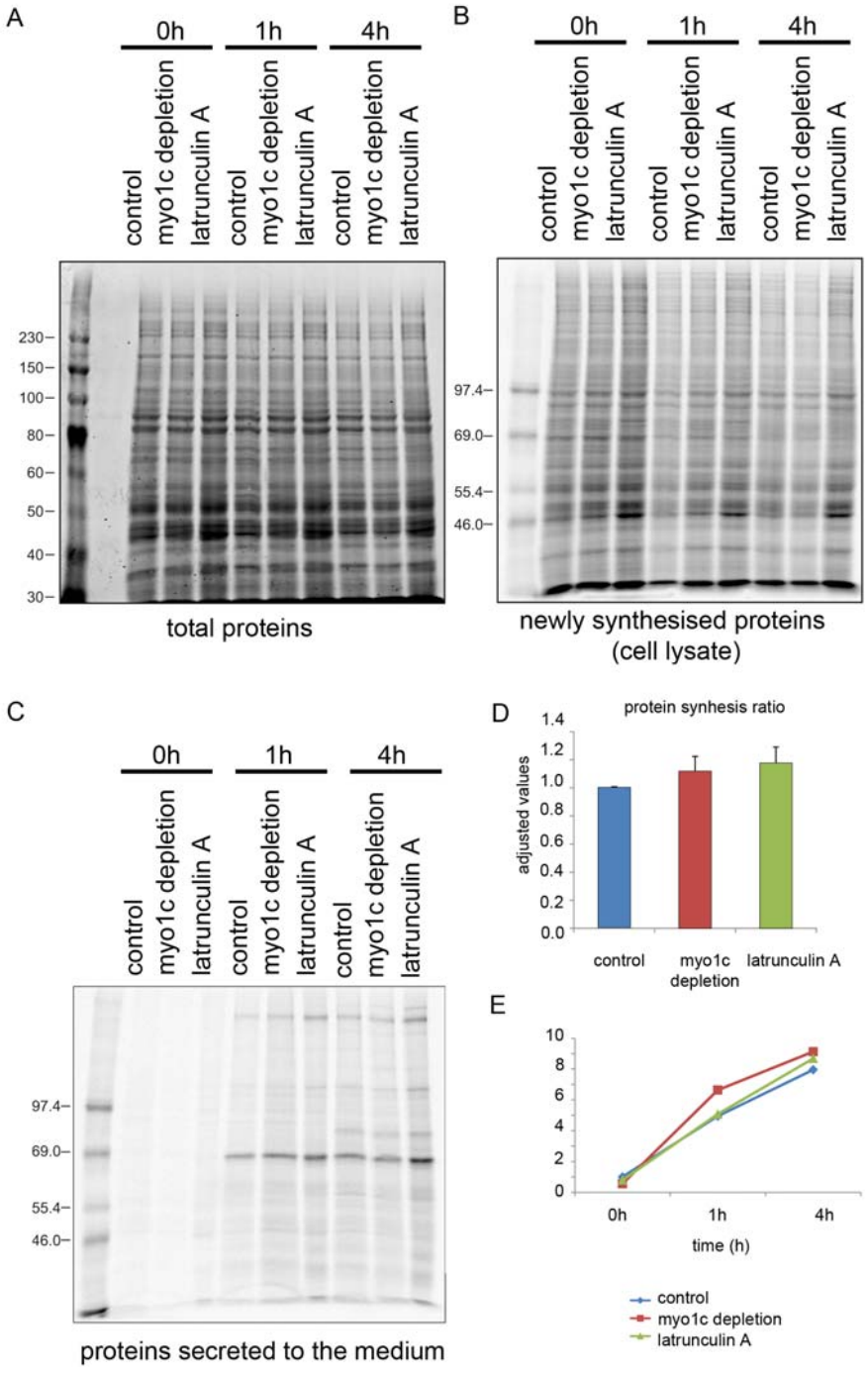
NE
*
*
*
*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69.0
55.4
46.0
newly synthesised proteins
(cell lysate)
C
proteins secreted to the medium
D
protein synhesis ratio
adjusted values
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1.2
1.0
0.8
0.6
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4
2
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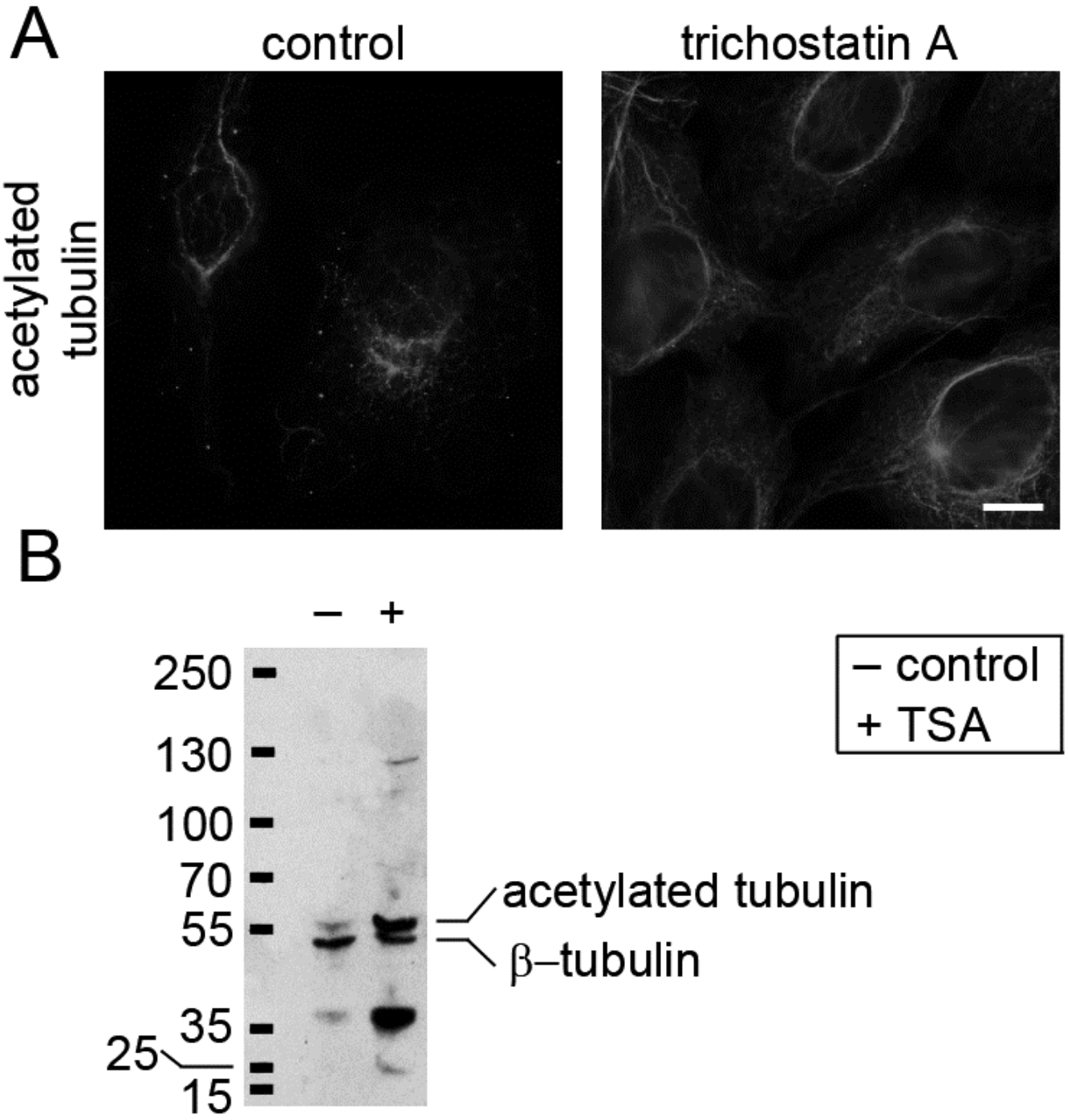
time (h)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