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Supplement figure legends

Supplement 1. (A) Live imaging of SAOS-α2β1 cells transfected with caveolin-GFP (green). Clustered α2β1 integrin (α2; red) was followed at different time points, right after binding of the clustering secondary antibody (anti-mouse Alexa 555; red; 0 h) up until 22 h after startup of integrin clustering. The fluorescent images are projections from 3D serial sections from confocal microscope. Colocalizing voxels are visualized below the projections. Bar, 10 µm. (B) Rhodamine dextran (0.25 mg/ml; red) was continuously internalized in SAOS-α2β1 and HeLa MZ cells during internalization of clustered integrin (α2; green) for 1 h. (C) **The effect of integrin clustering on the amount of uptake of dextran (rhodamine dextran 0.25 mg/ml; 1 h continuous uptake) was measured in SAOS-α2β1 cells (30 cells from three independent experiments). The amount of internalized vesicles have been quantified with the segmentation tool in the BiomageXD software.** (D) Confocal images of FITC-dextran (0.25 mg/ml) internalization in SAOS-α2β1 cells with or without integrin clustering for 2 h (pulse for 1 h and chase for 1 h in medium without dextran). **FITC signal was quantified from 10 large areas (200 µm x 200 µm; larger than the example confocal figures here) for both cases. The amount of fluorescent signal was quantified with the segmentation tool in the BiomageXD software. The experiment was done twice. Bars, 10 µm.**

Supplement 2. (A) **Confocal fluorescent control images of unclustered α2β1 integrin (α2) during GPI-GFP and CTxB internalization between 0 h and 2 h. Images are merged images of the integrin (red) and GPI-GFP (green) or CtXB (green). Here, α2β1 integrin was immunolabeled after fixation and permeabilization.** (B) Colocalization of flotillin 1 (green) and EV1 (red) at different time periods p.i. EV1 was first bound on the plasma membrane on ice, excess virus was washed away and EV1 was then internalized at 37ºC for different time periods.

EV1 and flotillin 1 were immunolabeled after fixation and permeabilization. **Colocalizing voxels are shown for each time point.** Bars, 10 μm.

Supplement 3. (A) Confocal images of internalized EV1 or clustered α2β1 integrin (α2) with classical endosomal markers after different time points: early endosome antigen (EEA1), cation independent mannose 6-phosphate receptor (CI-MPR) and CD63. (B) Live-imaging of clustered α2β1 integrin (green; clustered by goat anti mouse Alexa 488 antibody) with a labeled Lysotracker Red DND99. A time point after 2 h is shown. Bars, 10 μm.

Supplement 4. (A) **EV1 and α2β1 integrin were prelabeled with different sized protein A gold particles (5 nm and 10 nm, respectively) on ice before their internalization at 37°C for 5 and 30 min. In addition, rabbit anti mouse secondary antibody was used to cluster integrin on the plasma membrane before internalization. Here, EV1 and α2β1 integrin are found in similar endosomes after 5 and 30 min. Bars = 200 nm.** (B) The effect of the inhibition of macropinocytic regulators was studied by dextran uptake. Cells were treated with the inhibitors of the amiloride-sensitive Na+/H+ exchanger (EIPA), PI3K (LY294002) and phosphatidyl inositol specific phospholipase C (U73122) with a 30 min preincubation and followed by drug treatment and rhodamine dextran (0.25 mg/ml) uptake for further 1 hour. As EIPA and PLC seemed to affect the entry of dextran, quantification of their entry was studied. **The amount of fluorescent signal was quantified with the segmentation tool in the BiomageXD software.** (C) Evaluation of the toxic effects of these drugs on cell viability was tested using a commercial cell viability kit (Promega, see Materials and Methods). (D) **Ruthenium red was used to label endosomes that are still connected to the plasma membrane after control and EIPA treatment for 2 h. Clustered α2β1 integrin is labeled by protein A gold (10 nm). Plasma membrane is lined with dark ruthenium red (arrowheads) whereas integrin positive endosomes (arrows) are devoid of ruthenium red. Bars = 500 nm.**

Supplement 5. **(A) The intensity of phospho Pak1 after overnight starvation (C) and after different periods with EV1 (between 5 min and 2 h) was quantified from 20 cells in confocal microscope images.** The signal was quantified with the segmentation tool in the BiomageXD software. The result stands for the number of segmented objects weighed with the object size in pixels ± SE. The experiment was done twice. (B) The effects of actin depolymerizing (cytochalasin D) and stabilizing (jasplakinolide) drugs on EV1 infection were quantified. For cytochalasin D (cyt D) various pre-incubation periods (0.5 h, 2 h, 4 h) were used whereas for jasplakinolide (jaspla) only 0.5 h preincubation time was used. **(C) The effects of different concentrations of cytochalasin D (1, 2.5, 5, 7.5 µg/ml) or jasplakinolide (0.1, 0.25, 0.5 and 0.75 µM) on infection were tested. Drugs were administered 30 min before EV1 attachment and the drugs were present through the whole 6 h for the infection. The results in C and D are mean values from calculations of 150 - 200 cells for each case ± SE.**

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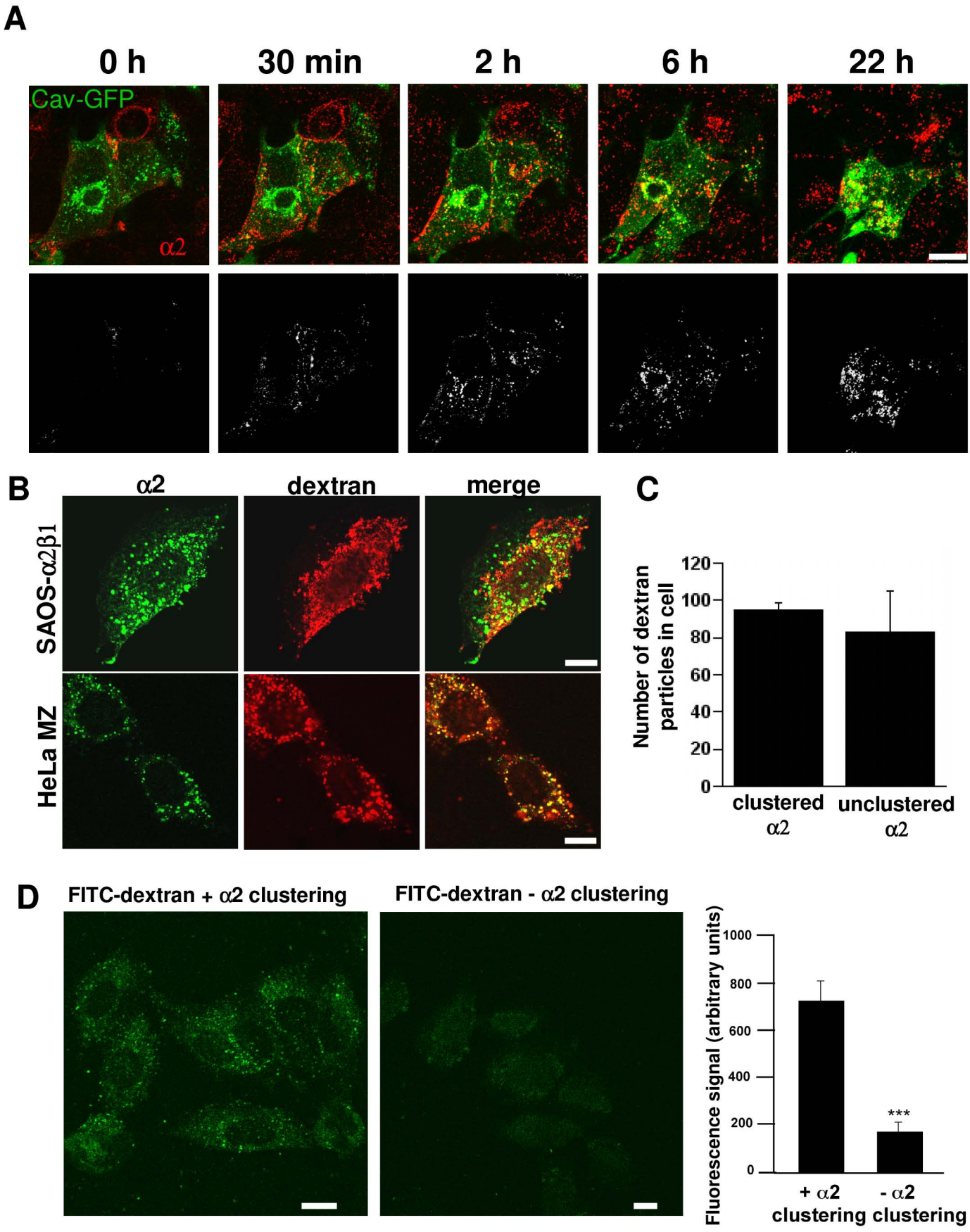

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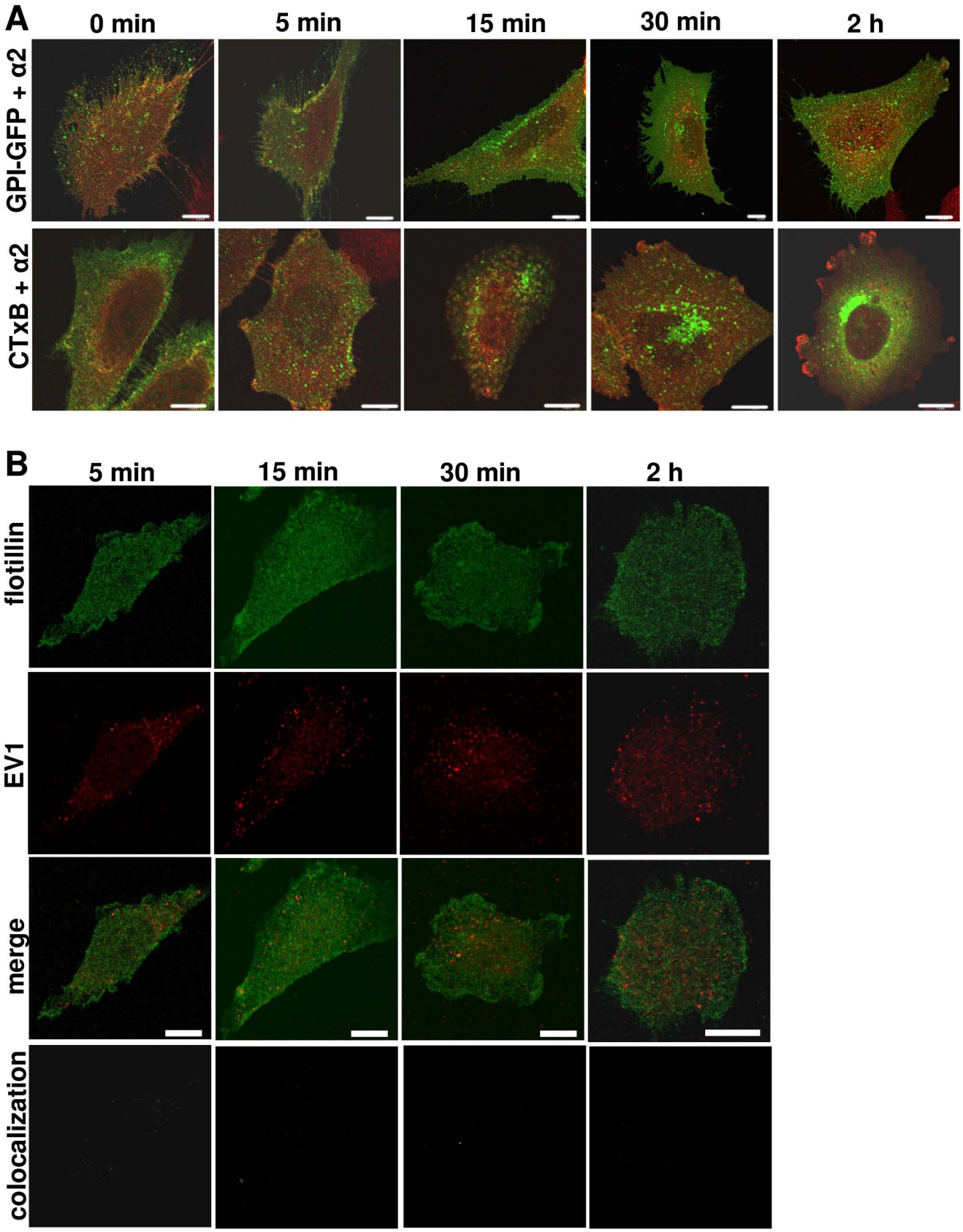

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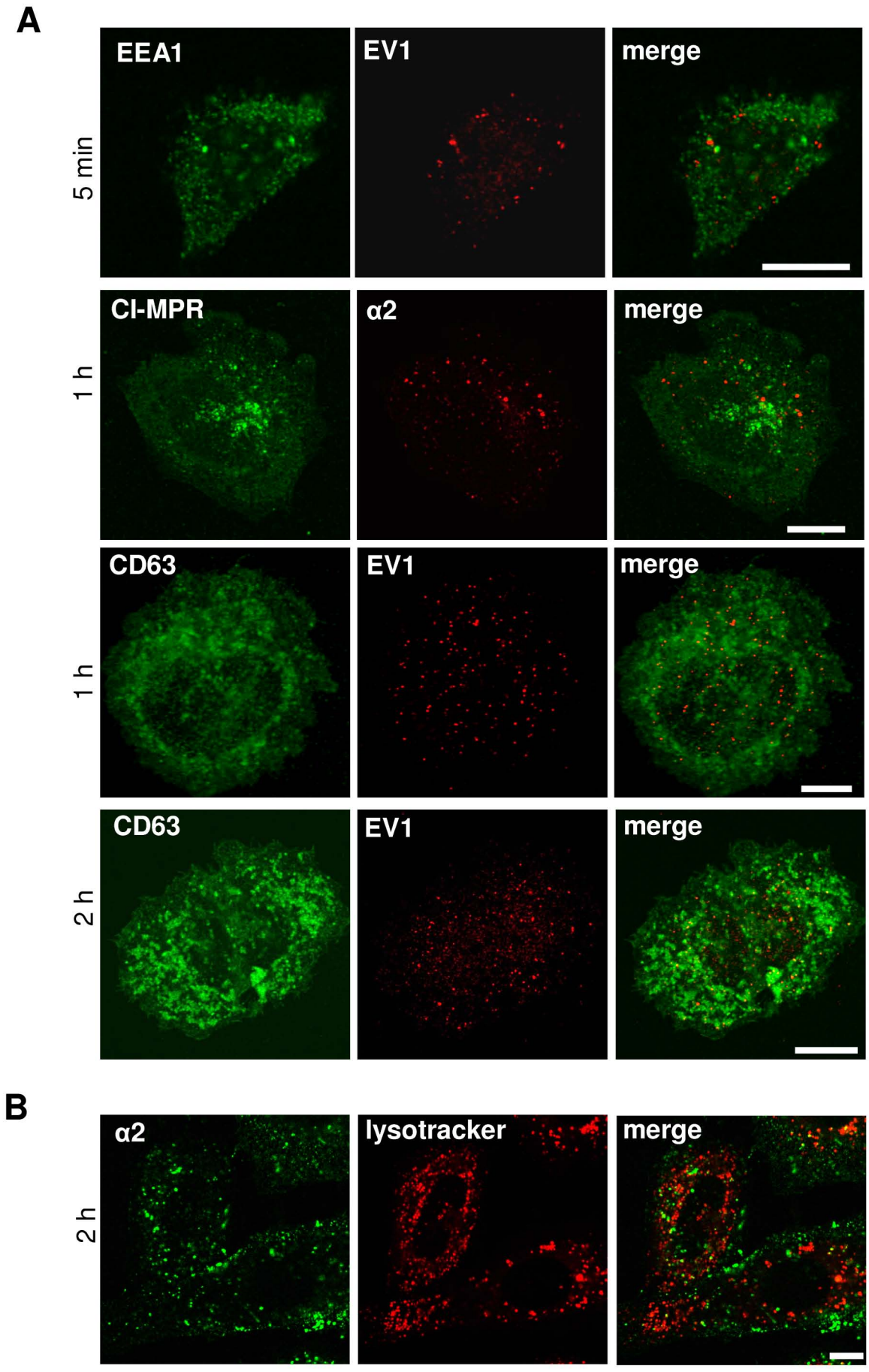

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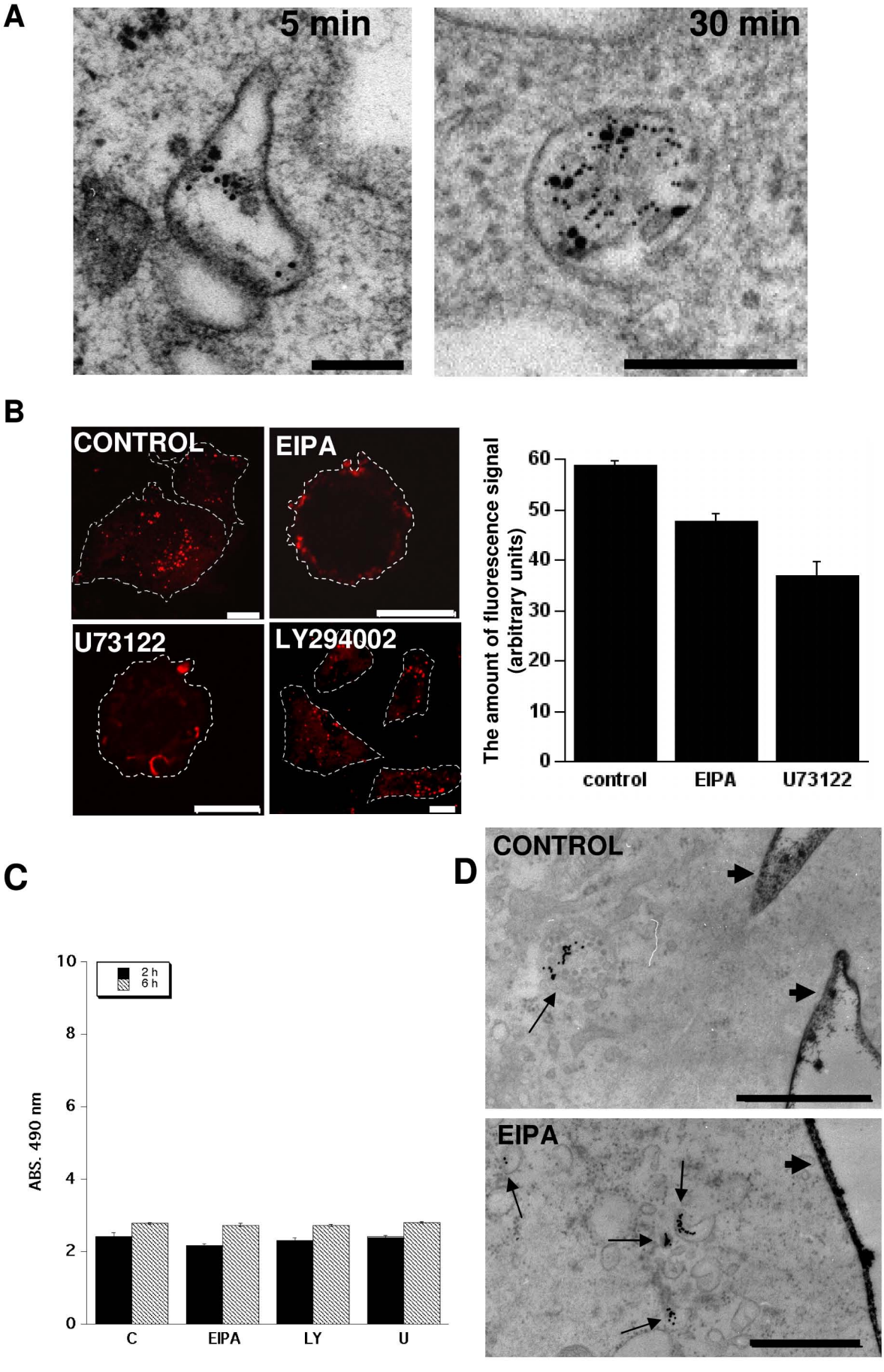

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