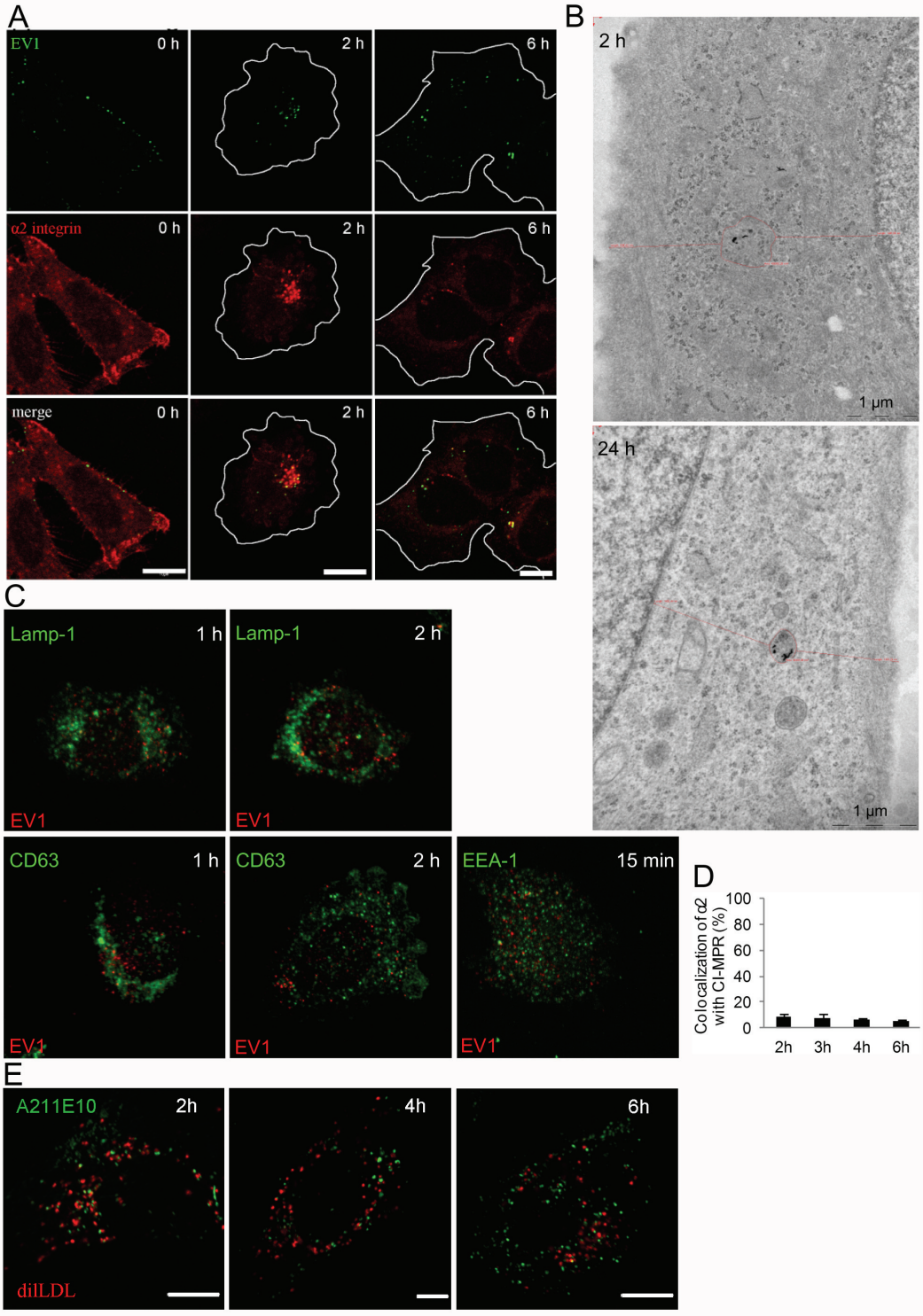
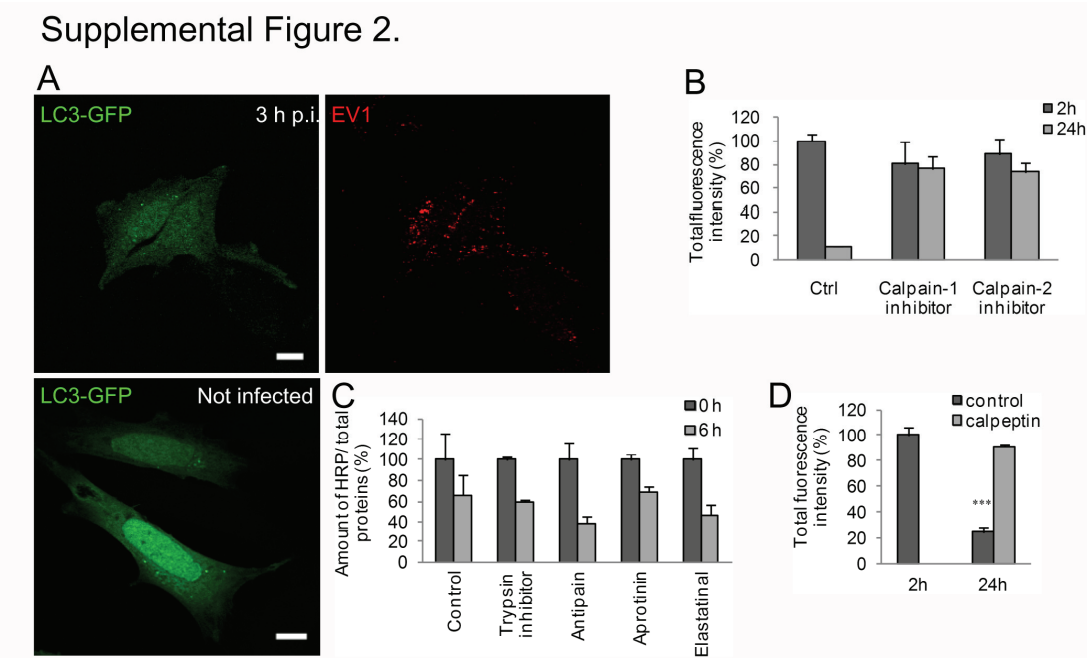
Supplemental Figure Legends

Supplemental Figure 1.

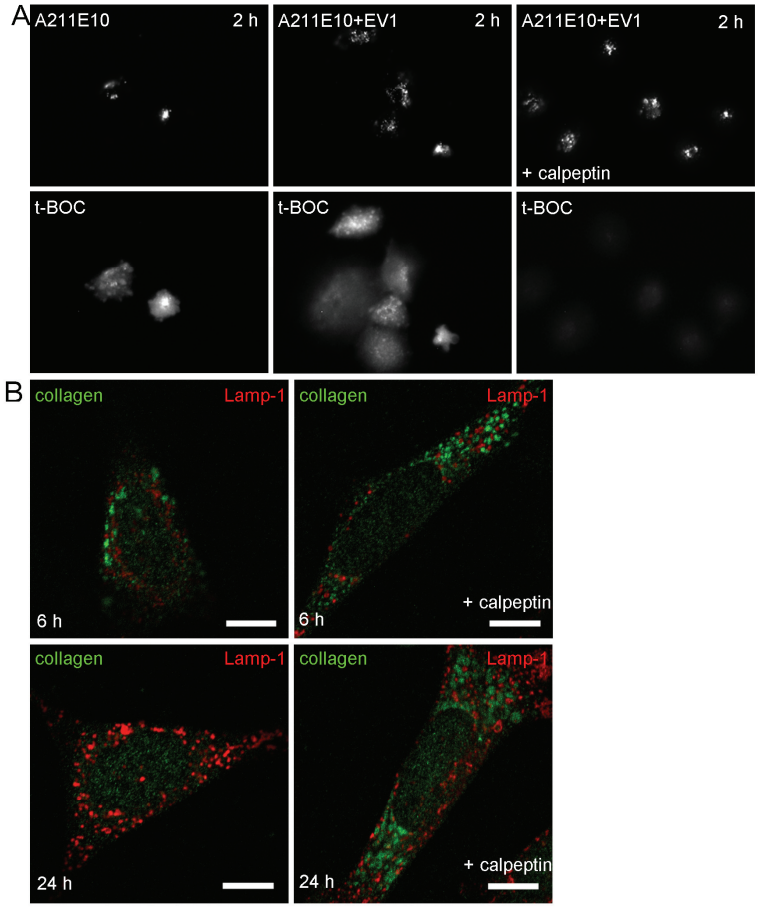


Supplemental Figure 1. A) EV1 binding to α2 integrin causes redistribution of both signals into α2-MVBs. α2 integrin was labeled with anti-α2 integrin antibody and fluorescent unclustering Fab fragment (red) simultaneously with binding and prelabeling of EV1 (green) with primary and secondary antibodies on ice. Bars 10 μm. B) Example EM images from calculations of the size and location of α2-MVBs after 2 and 24 h. Bar, 1μm. C) Labeling of EV1 together with Lamp-1 and CD63 after 1 and 2 h internalization, as well as with EEA1 after 15 min internalization. D) α2-MVBs are not colocalized with CI-MPR. Colocalization of late endosomal marker, CI-MPR with internalized α2 integrin was determined at 2, 3, 4, and 6 h after clustering. Quantification was done from confocal single stacks with colocalization tool in the BioImageXD software. Altogether 30 cells from three independent experiments were analysed +/- S.E. Bars 10 μm. 1E) Deconvoluted example images of o/n labeled Dil-LDL (red) cells clustered with integrin antibodies (green). Bars 10 μm.



Supplemental Figure 2. A) Distribution of LC3-GFP (green) after expression for 24 h in non-infected cells and after EV1 (red) internalization for 3 h, during a time when replication has already started in the cytoplasm. Bar, 10 µm. B) Calpain-1 and calpain-2 inhibitors block the degradation of integrin. The effect of calpain-1 and calpain-2 inhibitors on the fluorescence intensities of α2-MVBs was tested. Both inhibitors were used at 130 µM concentrations and preincubated for 1 h. The drugs were also present after internalization. Results are averages of three independent tests (+/- S.E.), altogether 30 single stacks of cells were quantified with segmentation tools of BioImageXD. C) Elastatinal, aprotinin, antipain or soybean trypsin inhibitor does not inhibit integrin degradation. The effect of elastatinal (250 µM), aprotinin (1 µM), antipain (500 µM) and soybean trypsin inhibitor (75 µM) on degradation of HRP conjugate of secondary antibody was tested. Cells were pretreated for 1 h and the drugs were also present after internalization. All the results are mean values from three separate experiments (+/- SE). D) Calpeptin can inhibit integrin degradation also after α2-MVBs are fully formed. 50 µM calpeptin was added on cells 2 h after clustering. Intensities of α2-MVBs were measured from confocal single stacks with BioimageXD segmentation tools. Altogether 30 cells from three independent experiments were quantified (+/- S.E.).

Supplemental Figure 3.



Supplemental Figure 3. A) Labeling of clustered integrin by antibodies (A211E10) or with EV1 present (A211E10 + EV1) for 2 h with or without calpeptin. t-BOC was added on cells 20 min before fixation. B) Labeling of collagen type I (green) and Lamp-1 (red) after plating the cells on collagen in the presence or absence of calpeptin for 6 and 24 h. Bars, 10 μm.