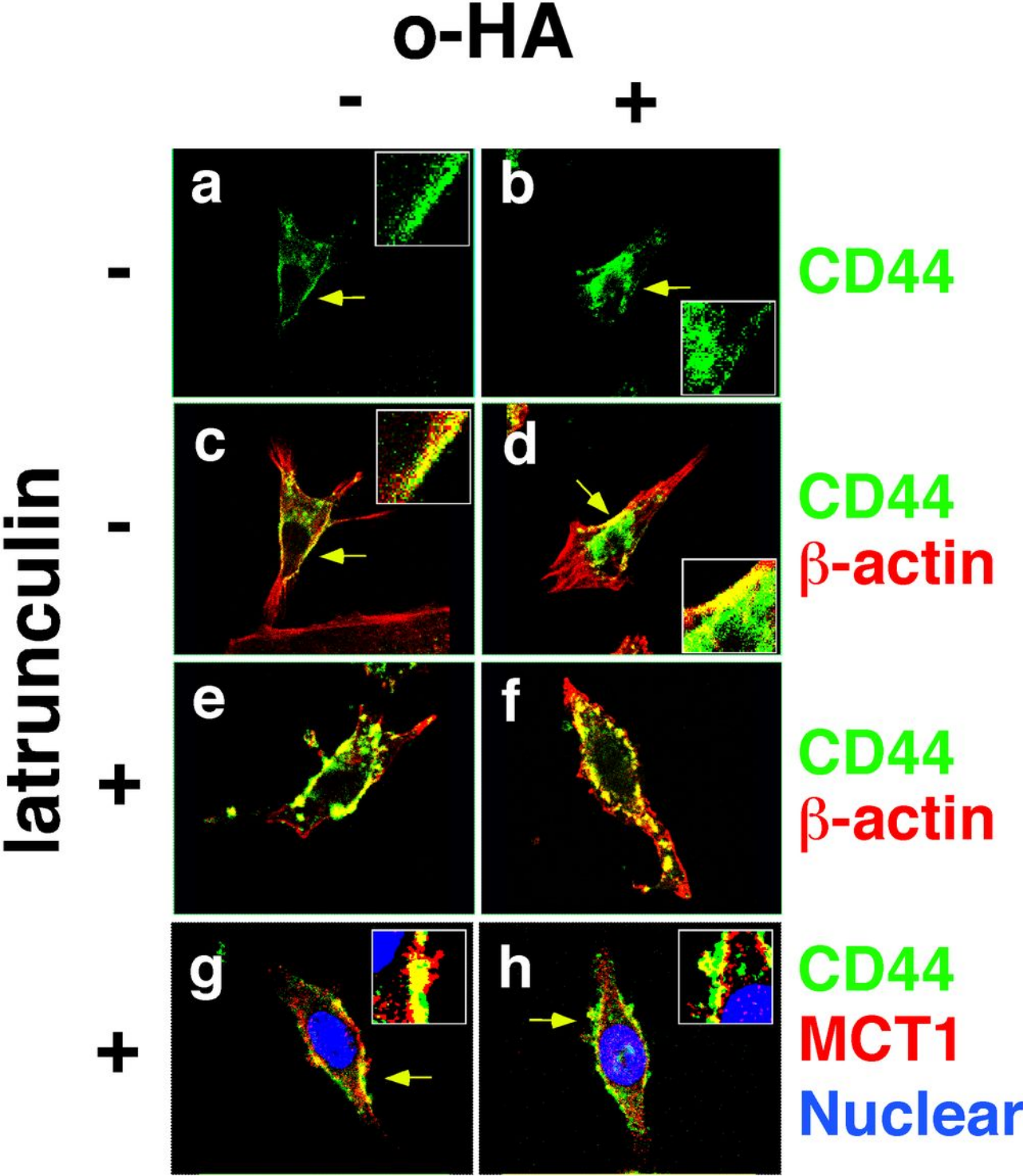
Supplementary Figure. Hyaluronan oligomers induce CD44 internalization in living cells. MB231 cells were pre-treated with and without latrunculin for 2 h before being treated in the presence or absence of latrunculin and hyaluronan oligomers for 1h in fresh medium containing an Alexa488-conjugated CD44 antibody (green). Following fixation, permeabilization, and phalloidin (actin) staining (red), cells were visualized by confocal microscopy at a Z-plane corresponding to the center of the cell. Arrows indicate areas shown at higher magnification in the insets. Note internalization of CD44 after treatment with hyaluronan oligomers alone (panel b), but co-localization of CD44 and actin (yellow) near the plasma membrane of untreated (panel c) and treated (panel d) cells. CD44 remained in association with actin in cell surface-associated clumps in latrunculin pre-treated cells whether or not they were also treated with hyaluronan oligomers (panels e an f). Panels g an h: In a separate experiment, MB231 cells were pre-treated for 2h -/+ latrunculin and 1h -/+ hyaluronan oligomers, and then processed in the same way as in Fig. 3. Cells were immuno-labeled for CD44 (green), MCT1 (red), and DRAQ5 nuclear stain (blue). Note localization of CD44 and MCT at the plasma membrane of latrunculin-treated cells whether or not they were also treated with hyaluronan oligomers. The figure is representative of three or more independent experiments.


o-HA
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