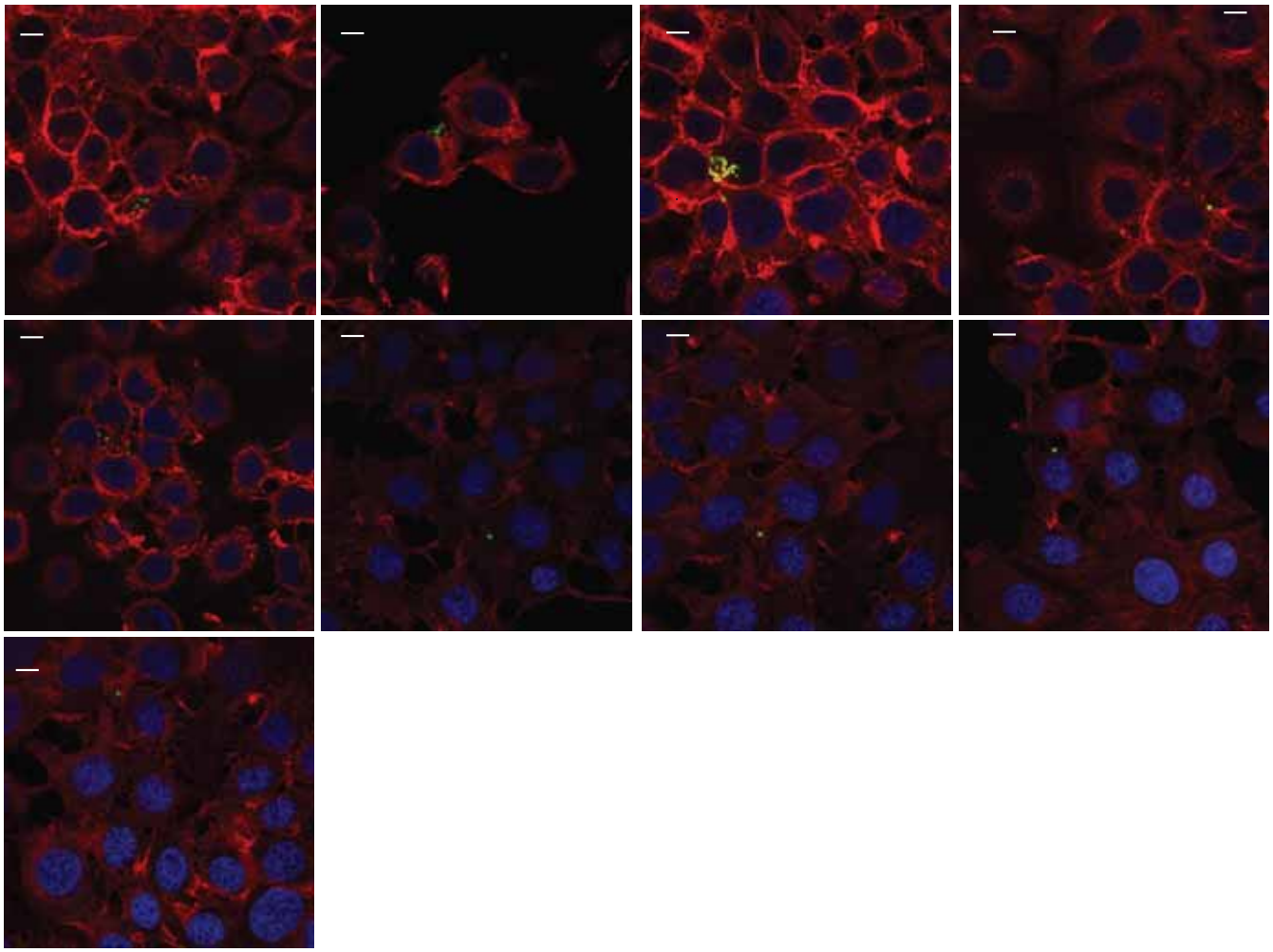
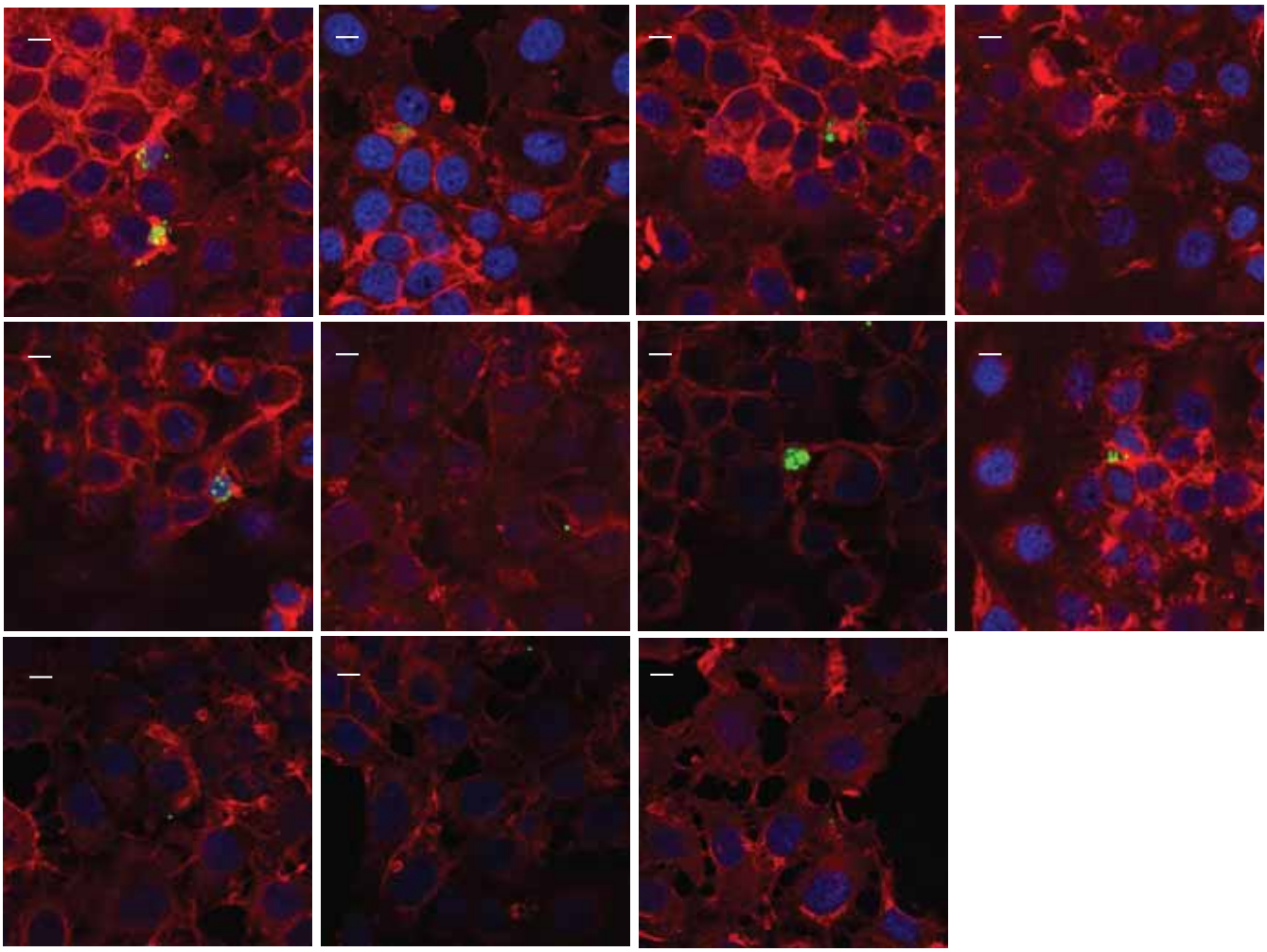
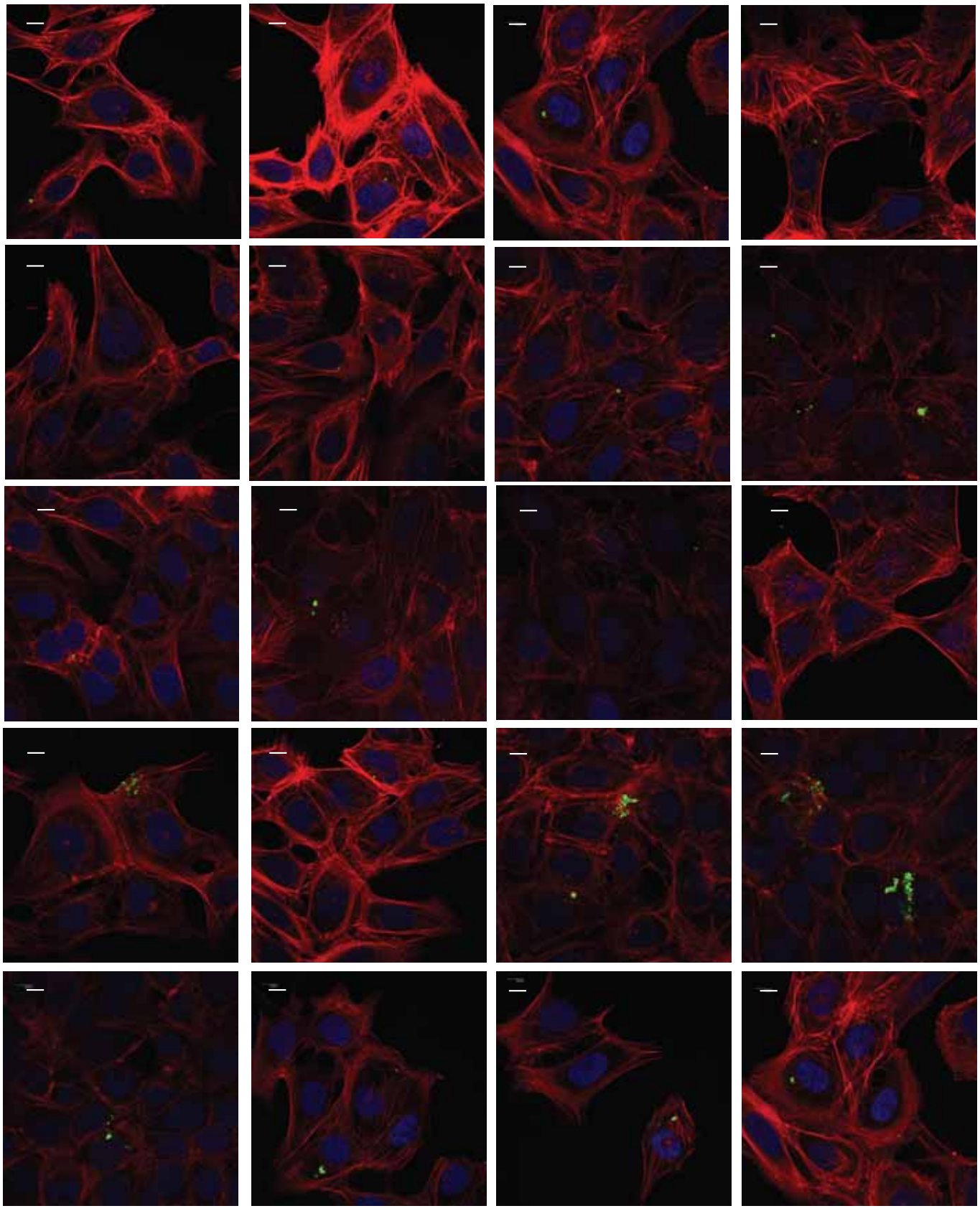
a MCF-7 cells without Prohep



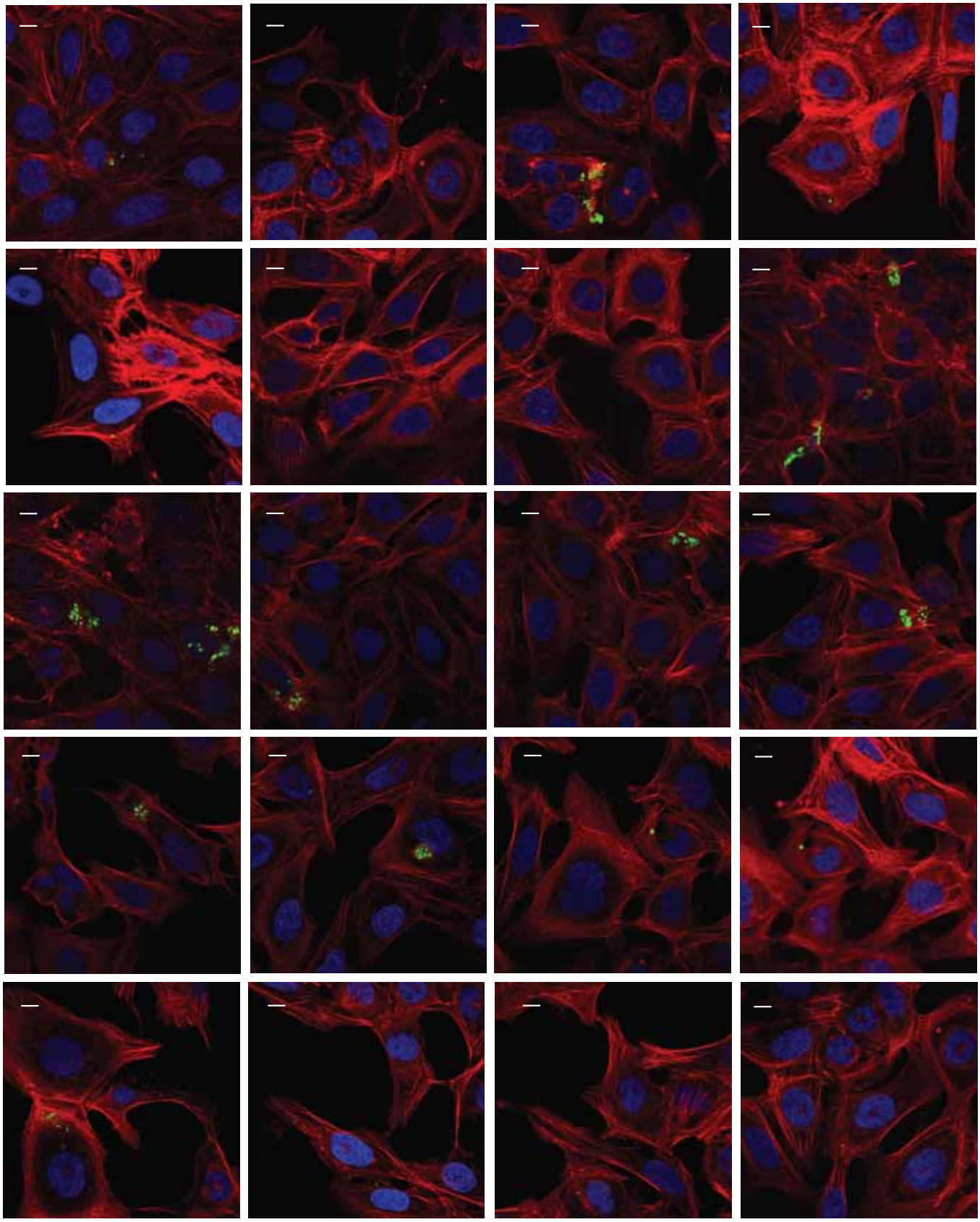
b MCF-7 cells with Prohep



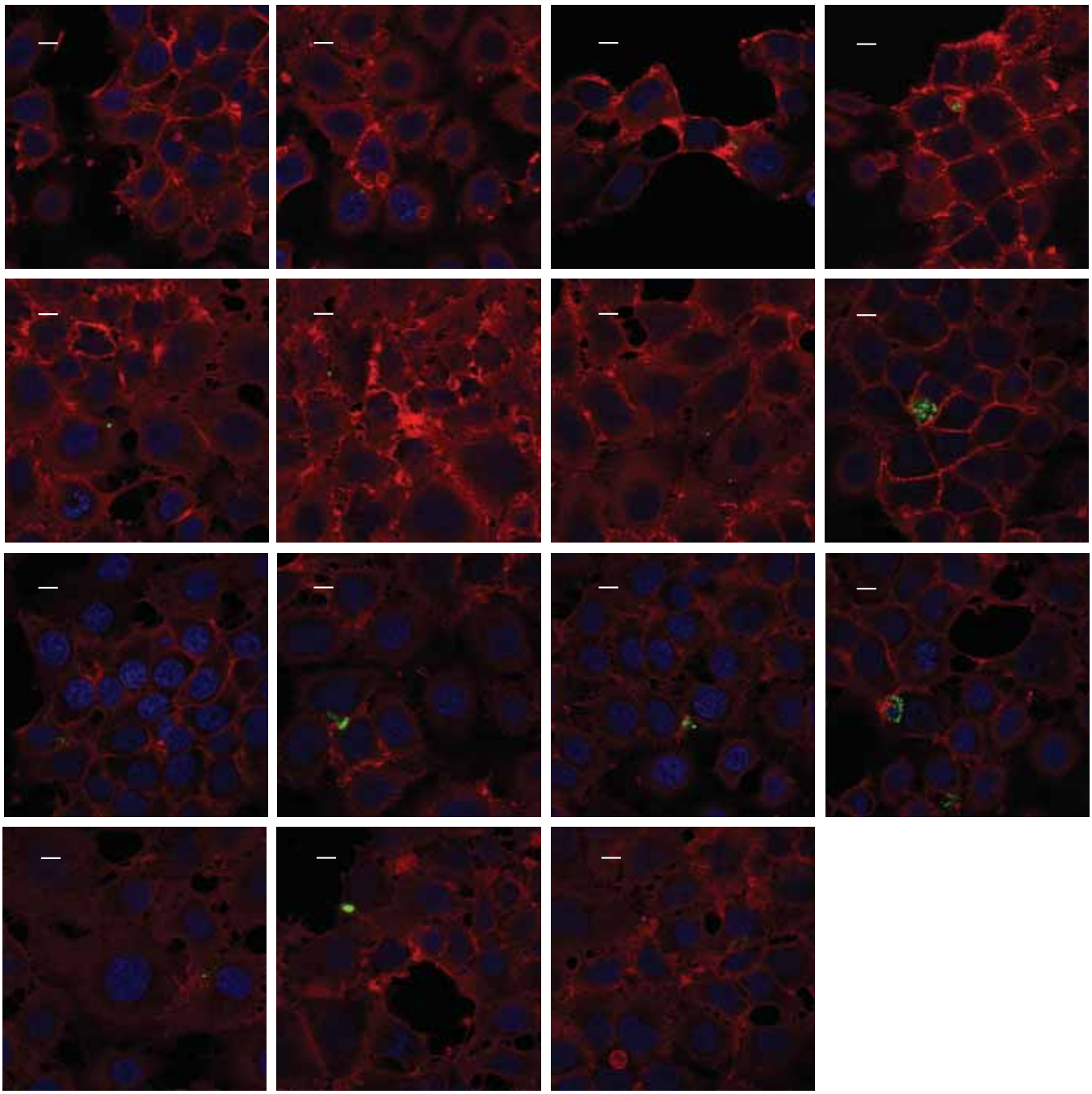
c U-2 OS cells without Prohep



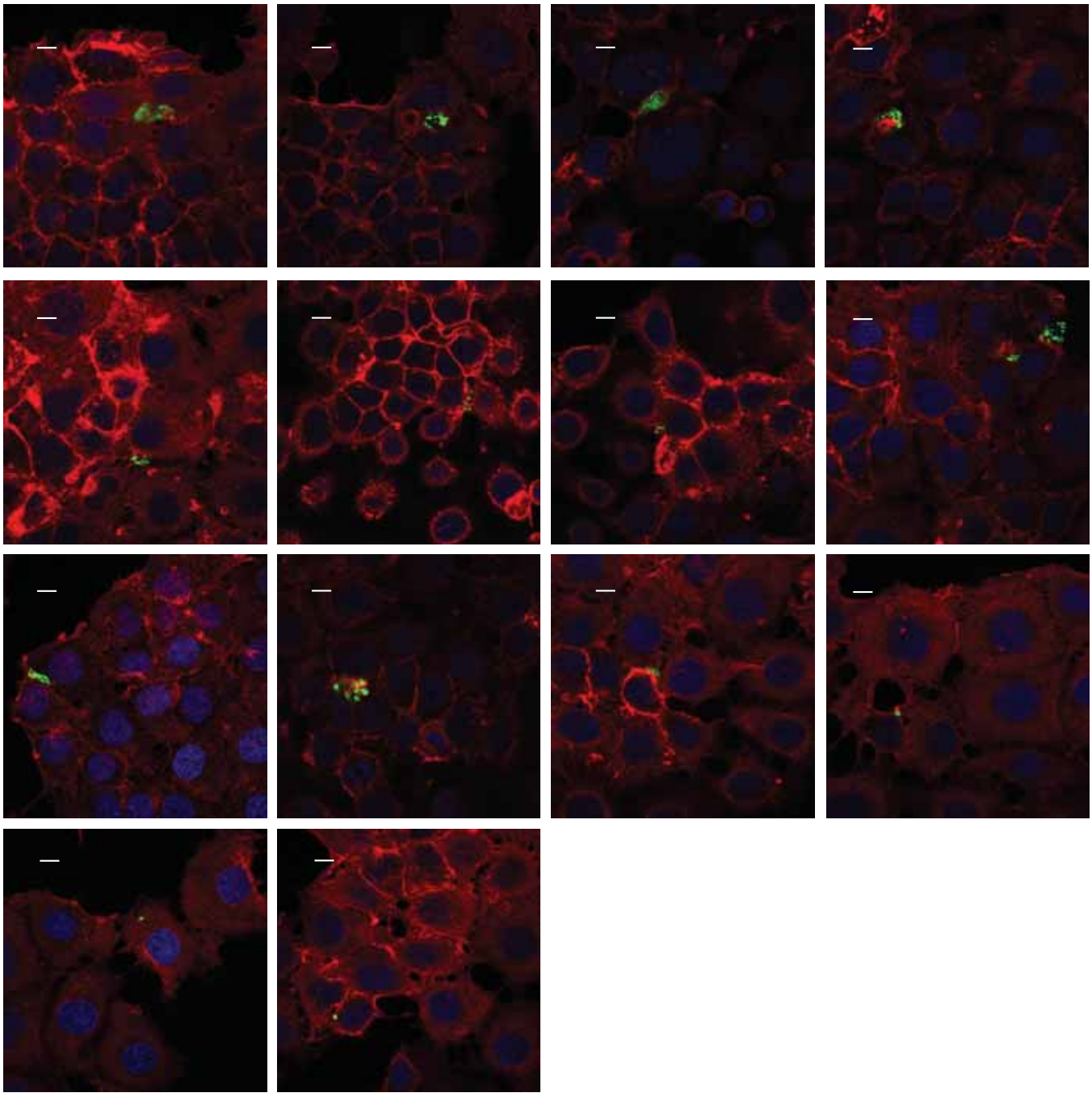
d U-2 OS cells without Prohep



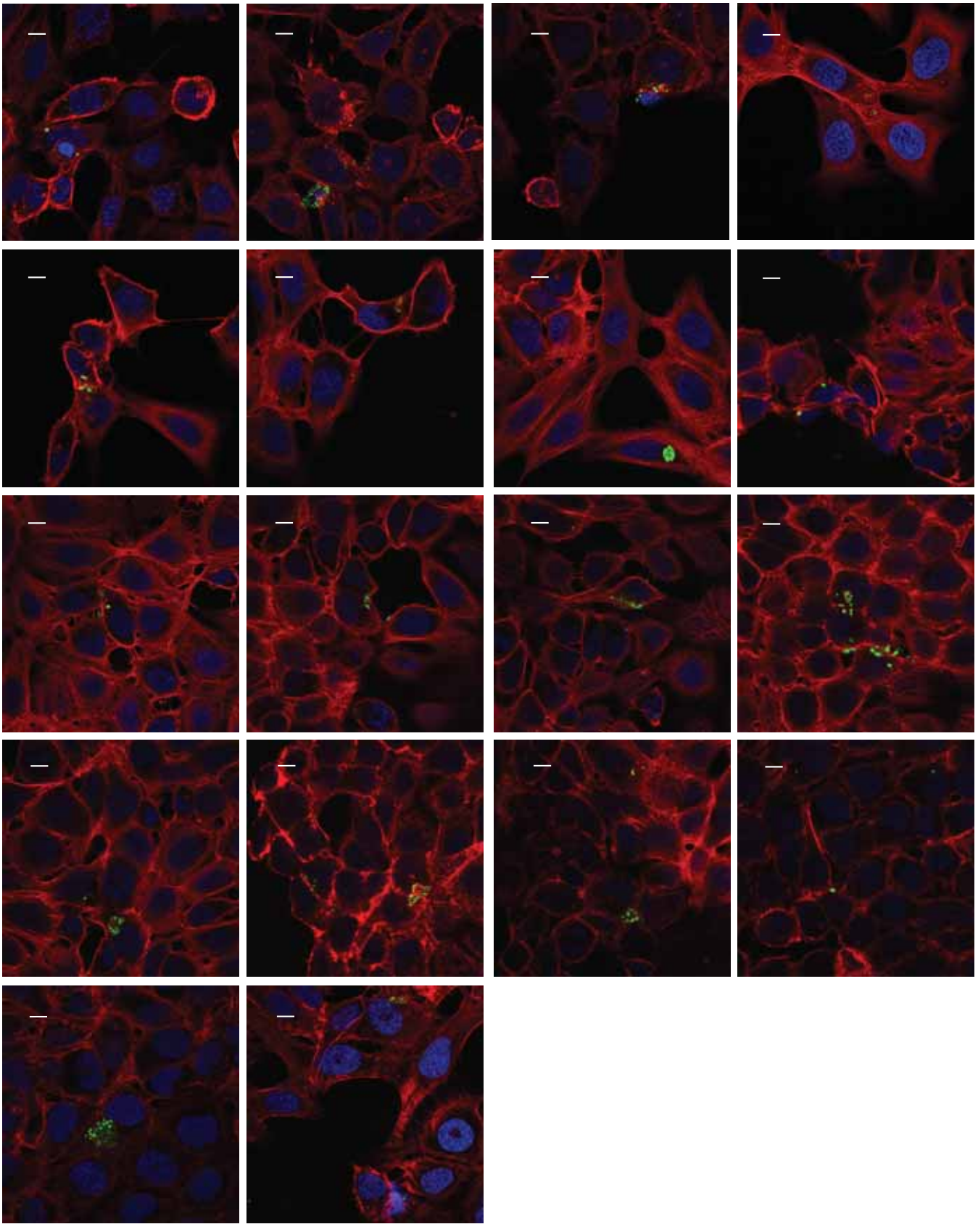
e MCF-7 without Prohep, trypsin treated



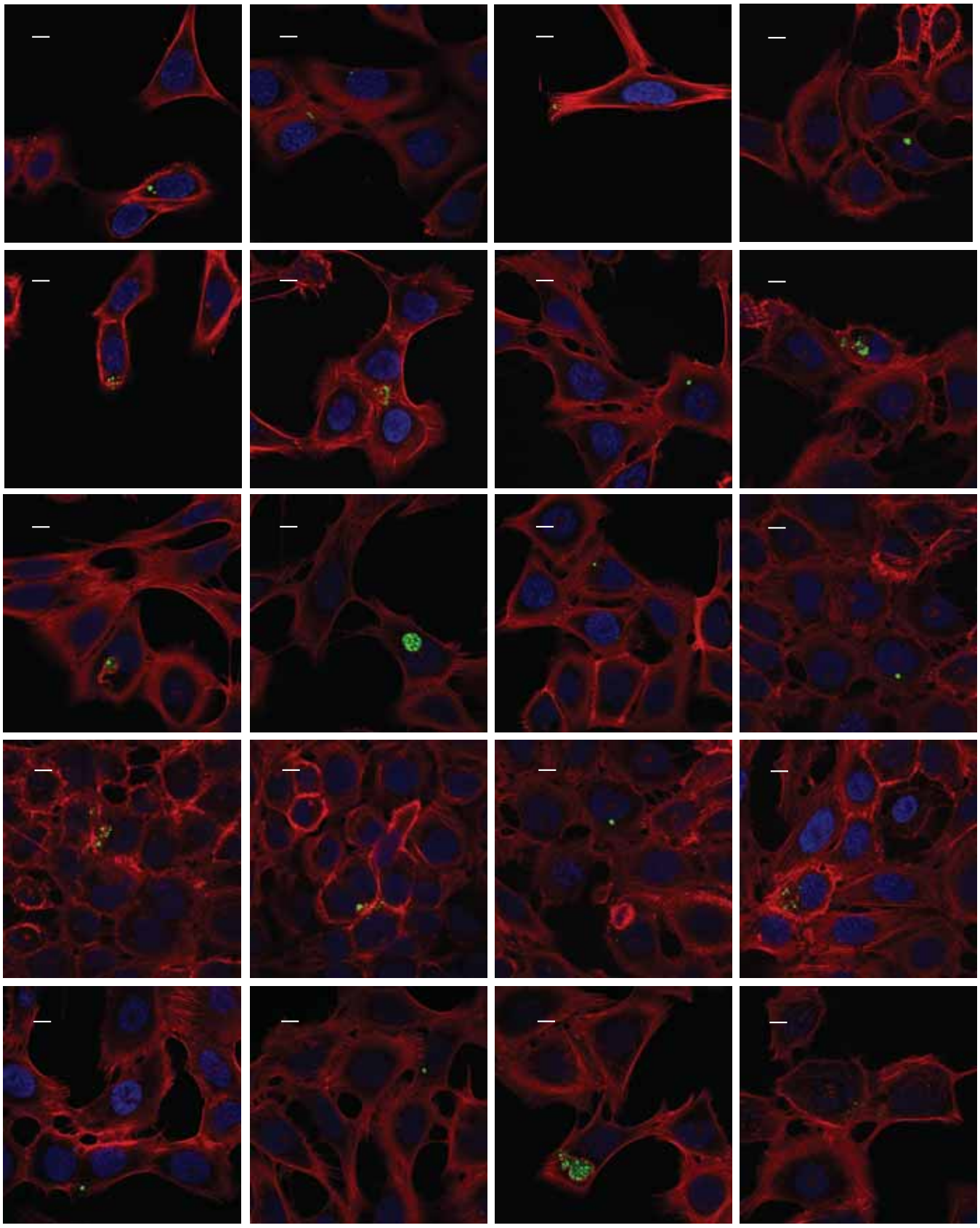
f MCF-7 cells with Prohep, trypsin treated



G U-2 OS cells without Prohep, trypsin treated



H U-2 OS cells with Prohep, trypsin treated



Supplementary information, Figure S5 Heparanase does not influence syntenin-exosome binding and uptake.

Gallery of confocal images used for quantification of uptake/binding of eGFP-syntenin-1-exosomes. MCF-7 or U-2 OS cells (plated at a density of 20,000 cells per well) were pre-treated with 10 nM proheparanase, or were left untreated, and were then incubated for 2 hours with conditioned media derived from MCF-7 cells expressing eGFP-syntenin-1. To distinguish between exosomes bound to the cell surface and internalized exosomes, at the end of the incubations, cells were treated with TPCK-treated trypsin (12,5 mg/ml). Before being prepared for microscopy cells were washed twice with PBS containing Soybean trypsin inhibitor (125 mg/ml). DAPI (cyan) and Alexa Fluor 594-Phalloidin (red) were used to visualize the nucleus and F-actin, respectively. Every cell containing eGFP was considered positive for syntenin-exosome binding/uptake.