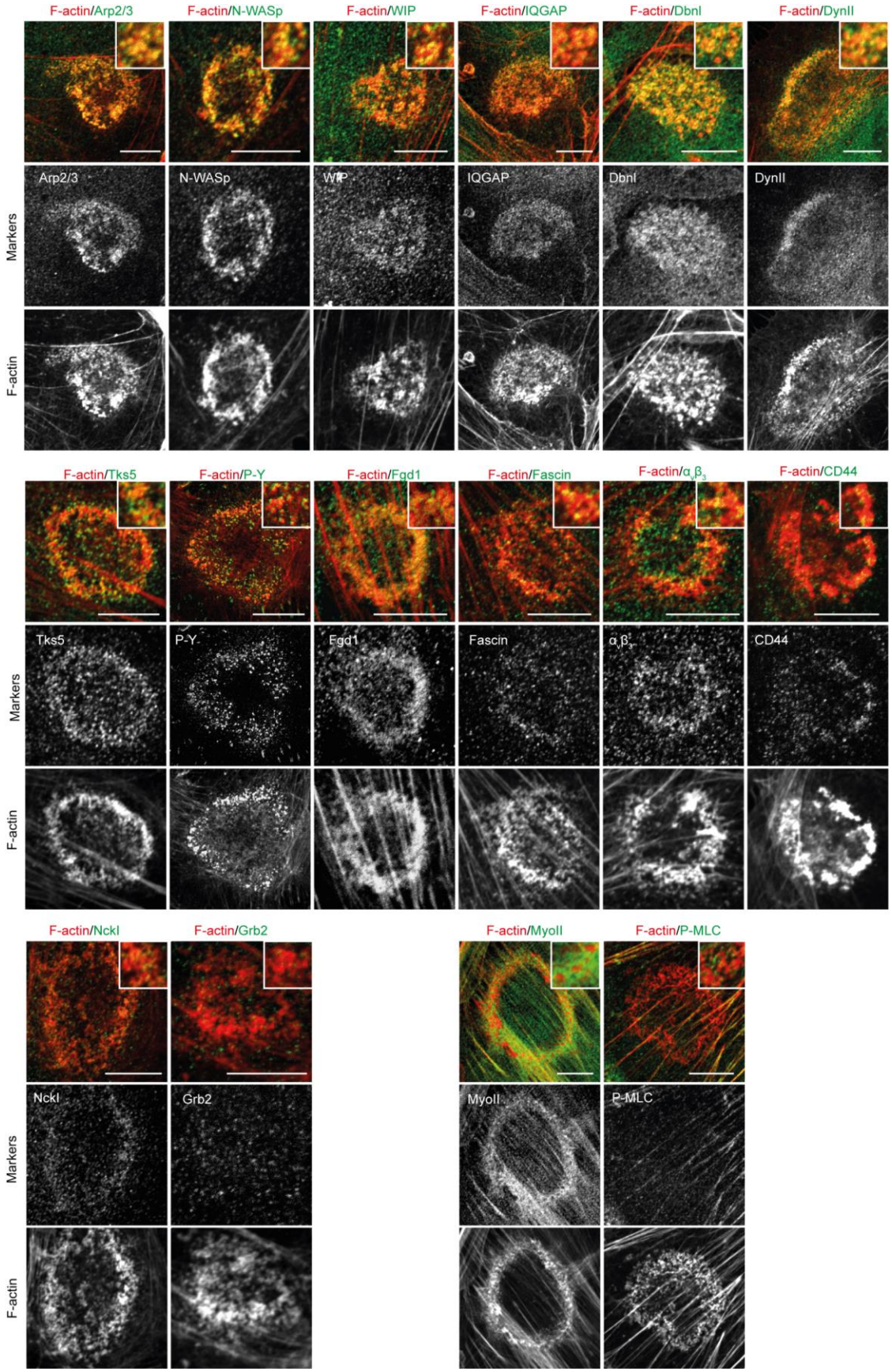

Fig. S1: Characterization of podosomes in HMVECs

Representative merge images of podosome rosettes in HMVECs double-stained with antibodies (green) against various podosomal proteins together with phalloidin (red). Scale bars, 10μm. The inset shows individual podosomes at a higher magnification in each panel. Images are representative of multiple cells from at least three similar experiments.

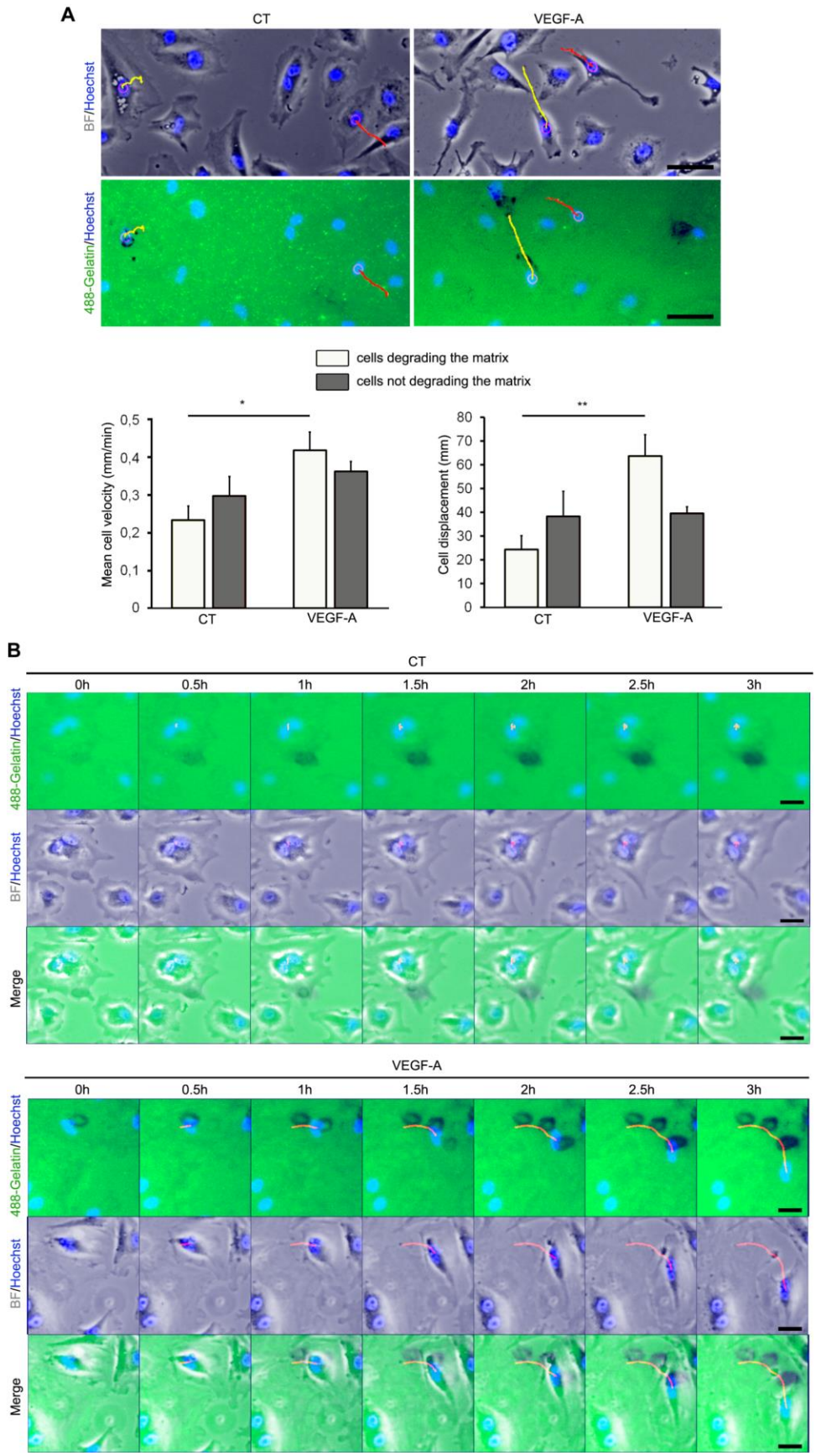

Fig. S2: Increased gelatin degradation by VEGF-A-stimulated cells is associated with high cell motility.

(A) Upper panels show representative bright field or fluorescent images of HMVECs stained for the nucleus and cultured on Oregon Green-488-gelatin. Cell motility was determined by manual tracking over a period of 3 consecutive hours in the second phase of the 24h response to VEGF-A (i.e., at least 3 hours after adhesion onset, Fig, 1 H and I). Yellow and red lines correspond to the trajectories of matrix degrading and non- degrading cells, respectively. Scale bars, 50µm. The graphs show the mean velocity and displacement of the cells during this time frame. n=3 individual experiments in which 9 matrix degrading and 9 non-degrading cells were tracked, mean ± s.e.m. is shown, one-way ANOVA Bonferroni multiple comparison test was used, $^{**}P<0.01$, $^{*}P<0.05$ versus control cells. (B) Representative images from time-lapse movies showing the trajectory (red line) of a cell degrading the matrix, under control conditions (upper panels) or after stimulation with VEGF-A (lower panels). Scale bars, 15µm.

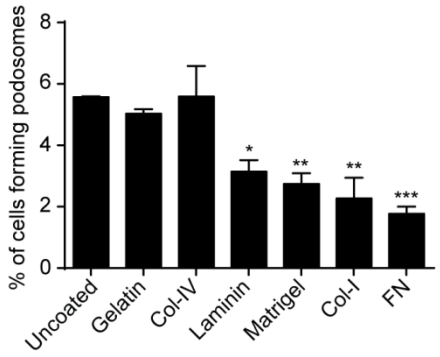

Fig. S3: A small fraction of the HMVEC population assembles podosomes regardless of the ECM protein coated on the substratum

Graph showing podosome formation (F-actin/cortactin staining) in HMVECs seeded on various ECM proteins as compared with uncoated substrata or gelatin. n= 3 independent experiments in which 300 cells were analysed per experimental point, mean ± s.d. is shown. Fold change compared with the CT in each group is shown. One-way ANOVA Bonferroni multiple comparison test was used, $^{***}P<0.001$, $^{**}P<0.01$, $^{*}P<0.05$, *compare with the uncoated substratum condition.

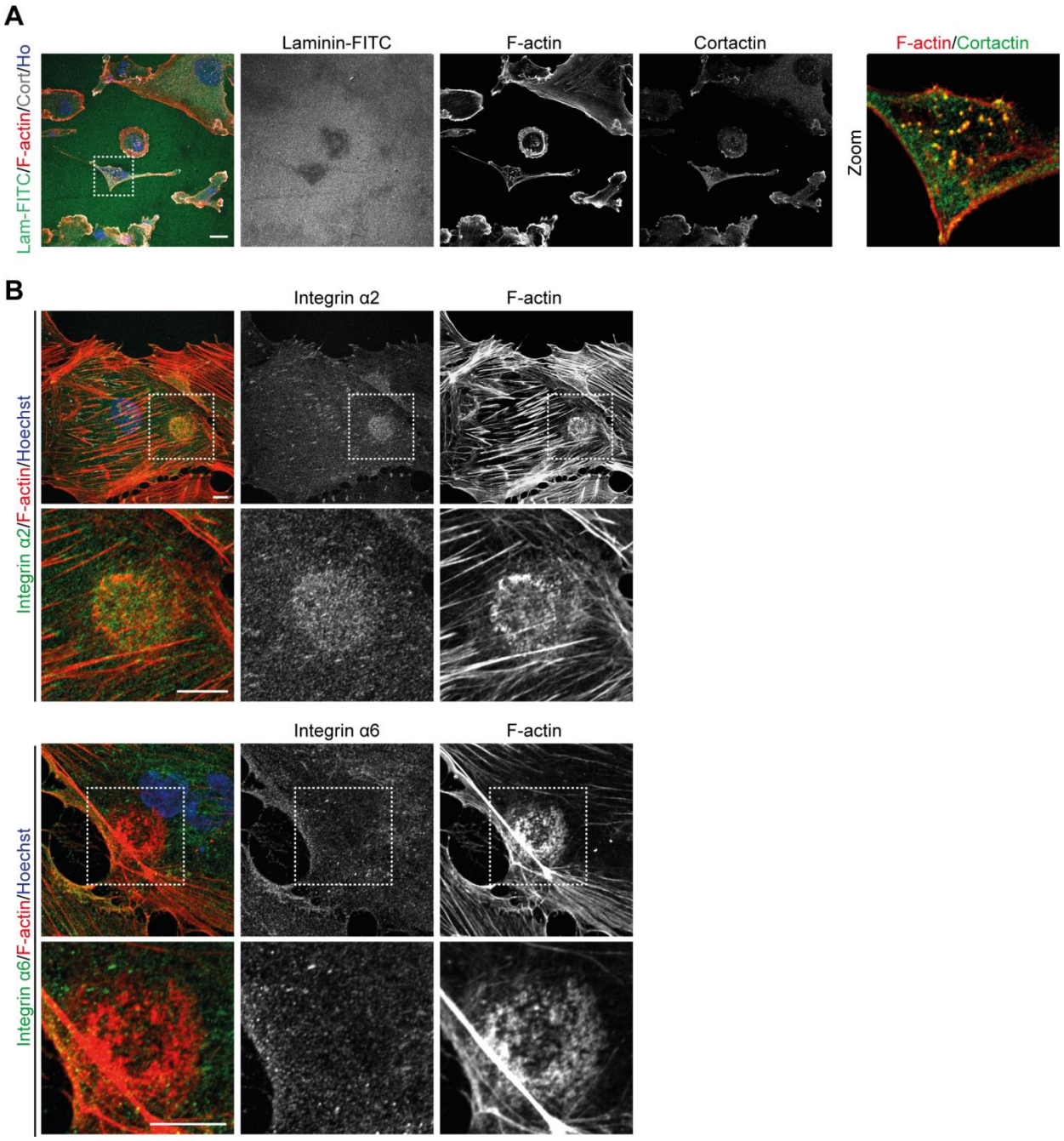

Fig. S4: Cell-dependent podosome specificities

(A) Representative images showing the merge image and individual stainings of MDA-MB-231 cells on a laminin-FITC coating, indicating that laminin-FITC is a suitable substrate that can be degraded by MDA-MB-231 podosome-like structures. In contrast to HMVEC podosomes, podosome-like structures in

tumour cells elicit laminin degradation. Scale bar, 20µm. Last panel shows a zoomed image of the F-actin/cortactin structures.

(B) Representative merge images of podosome rosettes in HMVECs seeded on either Col-IV (left panel) or laminin (right panel) and double-stained with antibodies (green) against α-subunit integrins (as indicated) together with phalloidin (red) and individual stainings. The boxed image is shown at higher magnification in the bottom panel. Podosome rosettes stained for α2 but not for α6 integrins. Scale bars, 10µm.