

Supplemental Information:

Supplemental Information 1:

In the following, we address the classification of cancer cells into invasive and non-invasive cell lines due to their capacity to invade into 3D ECMs. Data is shown for 8 cell lines, with data for the remaining cell lines available in the literature. [18,24]

We used 2.4 mg/ml collagen type I gels (a 1:1 mixture of rat tail collagen R and bovine collagen G) to analyze the ability of cancer cells to invade these gels within three days when seeded on top of them. [18,24] All cancer cell lines were able to adhere to these collagen matrices. The experiments were performed several times over a time span of three years, and for each experiment, the classification of the cell lines into invasive and non-invasive cancer cell lines was the same. After three days the adherent cells on top and invasive cells inside the collagen matrices were Glutaraldehyde-fixed (2.5 %). Then, the number of invasive cells was determined as well as the invasion depths of each cell in a field of view of in total at least 12 fields of view.

MDA-MB-231, Me180, Haca1 and the subcell line $\alpha 5\beta 1^{\text{high}}$ cells were more invasive compared to the MCF-7, MS751, SW480 and the subcell line $\alpha 5\beta 1^{\text{low}}$ cells (Fig. S1). The former are classified as invasive cells as they have a 3.4-fold to 10.3-fold higher number of cells that were able to invade into 3D ECMs compared to the other group that are classified as non-invasive cells (Fig. S1A). This classification is supported by the invasion profiles of the cancer cells lines that showed higher invasion depth of MDA-MB-231, Me180, Haca1 and the subcell line $\alpha 5\beta 1^{\text{high}}$ cells compared to their counterparts (regarding the tissue) MCF-7, MS751, SW480 and the subcell line $\alpha 5\beta 1^{\text{low}}$ cells (Fig. S1B).

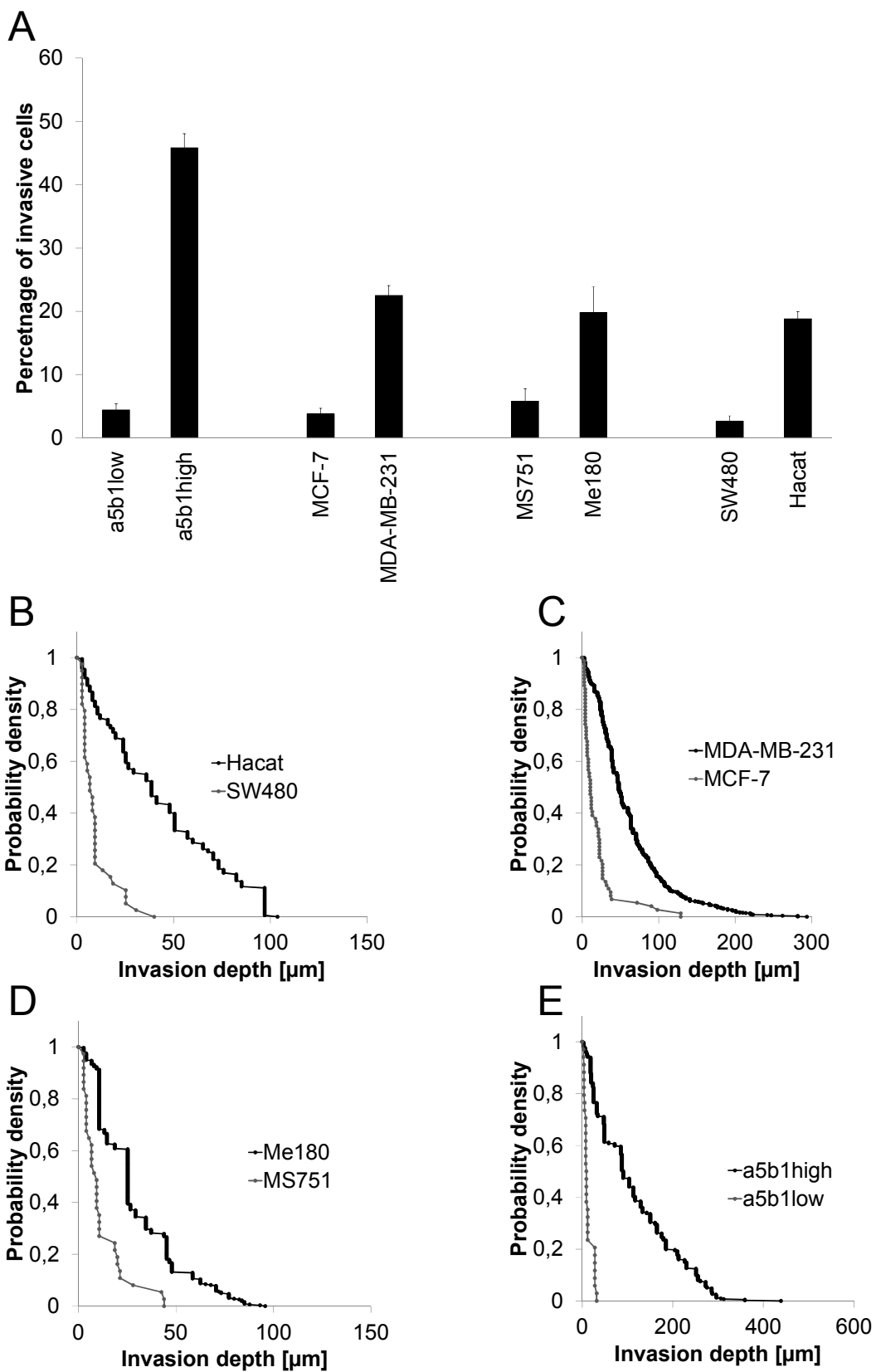


Figure S1: Invasiveness of cancer cell lines into 3D collagen matrices. (A) The number of invasive cells of cancer cell lines and cancer subcell lines into 3D collagen matrices were determined after three days of culture. (B) In parallel, the invasion depth of each invasive cell was determined. The probability density was plotted over the invasion depth for each pair of invasive and non-invasive cell lines, and is presented as an invasion profile.

Supplemental Information 2:

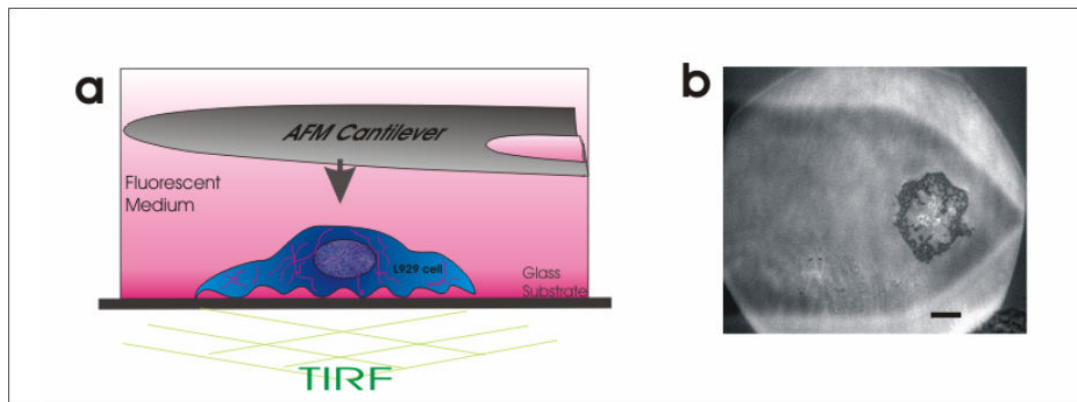


Figure S-3: (a) Schematic showing an adherent cell which is subject to a compressive force by a flat SFM cantilever at the apical membrane and simultaneous TIRF imaging at the basal membrane. (b) Combined Bright-field microscopy and TIRF showing both the adherent cell and the shadow outline of the SFM cantilever used for force application. Scale bar is 10 μm . Figure adapted from Jonas et al. [17]

Supplemental Information 3:

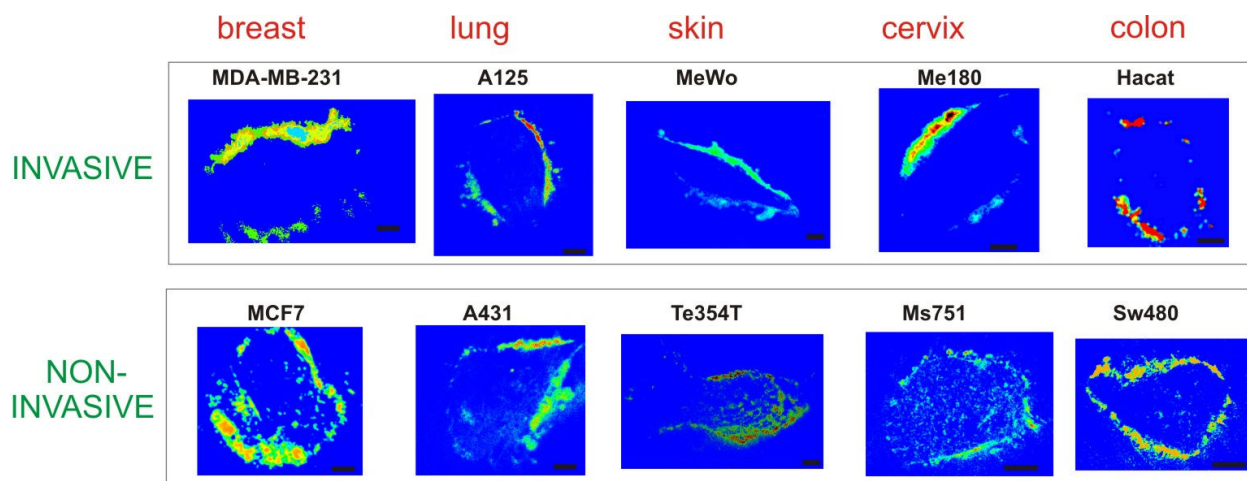


Figure S-4 : Series of force images during retraction of all cell types tested. Each image is created by subtracting a basal membrane topography of a single cell immediately before loading force is decreased from 80 nN loading (maximal) to 0 nN, from the same cell after 90 sec without loading. Red represents the greatest upward changes in the distance between basal membrane and substrate (ca. 30 nm), blue represents no changes. All invasive cell lines are shown in the top half, and all non-invasive cell lines are shown in the bottom half. All scale bars are 5 μ m.