Supplemental Material to:
Sharma VP, Beaty BT, Patsialou A, Liu H, Clarke M, Cox D, et al. Reconstitution of in vivo macrophage-tumor cell pairing and streaming motility on one-dimensional micro-patterned substrates. IntraVital 2012; 1(1);
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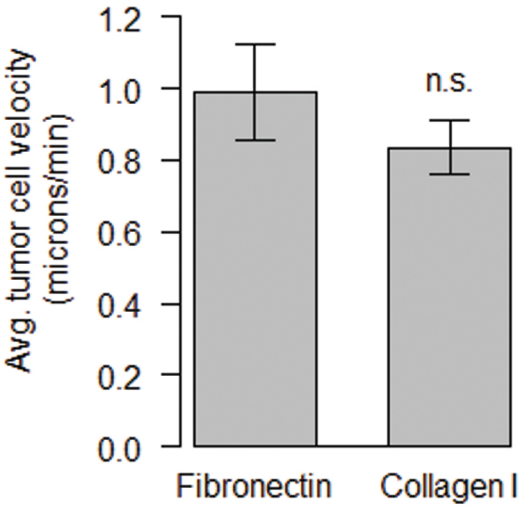
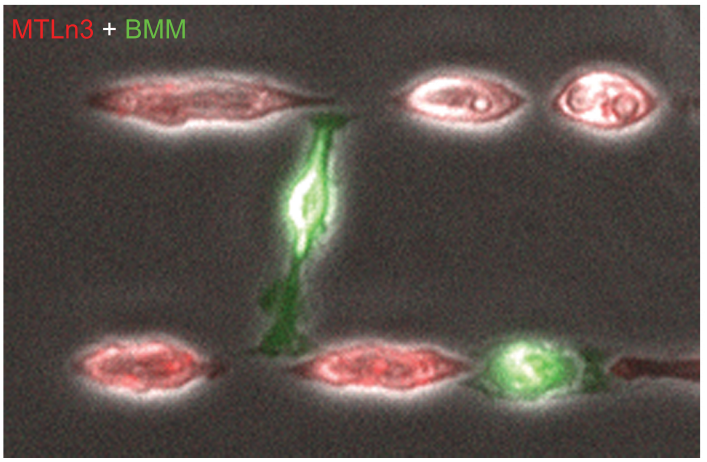
Supplementary Figure 1



Supplementary Figure 2



Video S1: Intravital microscopy within the patient primary cell-derived mammary tumor in a mouse. TN1 breast tumor cells, stably expressing GFP (green), were derived from the pleural effusion of a patient with triple-negative breast cancer. Host macrophages are identified as shadows in the tumor stream. Collagen fibers were visualized by second harmonic generation (blue). Images were taken using a 25x objective every 2 min for total 30 min. In this representative movie, TN1 tumor cells (open floating arrows) and host macrophages (solid floating arrow) can be seen moving rapidly in ordered streams (linear arrangement of multiple cells following each other) occurring on linear collagen fibers. Movie is played at 7 frames/sec.

Video S2: MTLn3 cells on 1D fibronectin stripes display contact inhibition of motility. MTLn3 cells stably expressing TagRFP-cortactin were plated for 3-4 hr on 2.5 μm-wide fibronectin stripes and time lapsed imaged for 6 hr. MTLn3 cells are highly contact-inhibited and rapidly changed their polarity and directionality upon contact with another cell on the stripe. MTLn3 cells were restricted to the adhesive stripe with no spreading or migration onto non-adhesive regions. Movie is a merge of 3 channels: phase, TagRFP-cortactin and 650 nm dye labeled fibronectin and is played at 10 frames/sec.

Videos S3, S4 and S5: The interaction between an MTLn3 cell and BMM on 1D fibronectin stripe. MTLn3 cells stably expressing TagRFP-cortactin were plated for 3-4 hr on 2.5 μm-wide fibronectin stripes. Cells were imaged for 1.5-2

hr before the addition of either GFP-BMMs or CellTracker™ green-labeled BMMs. Fluorescence and phase channels were merged and a montage of 6-7 consecutive fields of totaling approximately 2 mm of continuous stripe length was analyzed. Movies 3, 4, and 5 show representative examples of MTLn3 and BMM cell interactions. Movies are played at 10 frames/sec.

Video S6: Co-assembly of alternating MTLn3 cells and BMMs on 1D adhesive stripes. MTLn3 cells stably expressing TagRFP-cortactin were plated for 3-4 hr on 2.5 μm-wide unlabeled fibronectin stripes. Cells were imaged for 1.5-2 hr before the addition of CellTracker™ green-labeled BMMs. Cells were time lapsed for an additional 6 hr. Fluorescence and phase channels were merged and a montage of 6-7 consecutive fields of totaling approximately 2 mm of continuous stripe length was analyzed. Movie is played at 10 frames/sec.

Figure S1. The average 1D tumor cell velocities on fibronectin and type 1 collagen stripes are identical. MTLn3 cells stably expressing TagRFP-cortactin were plated for 3-4 hr on 2.5 μm-wide fibronectin or type I collagen stripes. Cells were time lapsed for 1.5-2 hr and average tumor cell velocity (μm/min) was calculated. Error bars represent the SEM.

Figure S2. Non-adhesive substrate can support BMM migration. A representative image of TagRFP-cortactin expressing MTLn3 cells and CellTracker™ green labeled BMMs in a 2.5 μm unlabeled fibronectin stripes region. Non-adhesive regions between the fibronectin stripes do not support tumor cell spreading or attachment, however, BMMs were capable of crossing over from a 1D stripe to the adjacent 1D stripe.