SUPPORTING INFORMATION

Two-Channel Compartmentalized Microfluidic Chip for Real-Time Monitoring of the Metastatic Cascade

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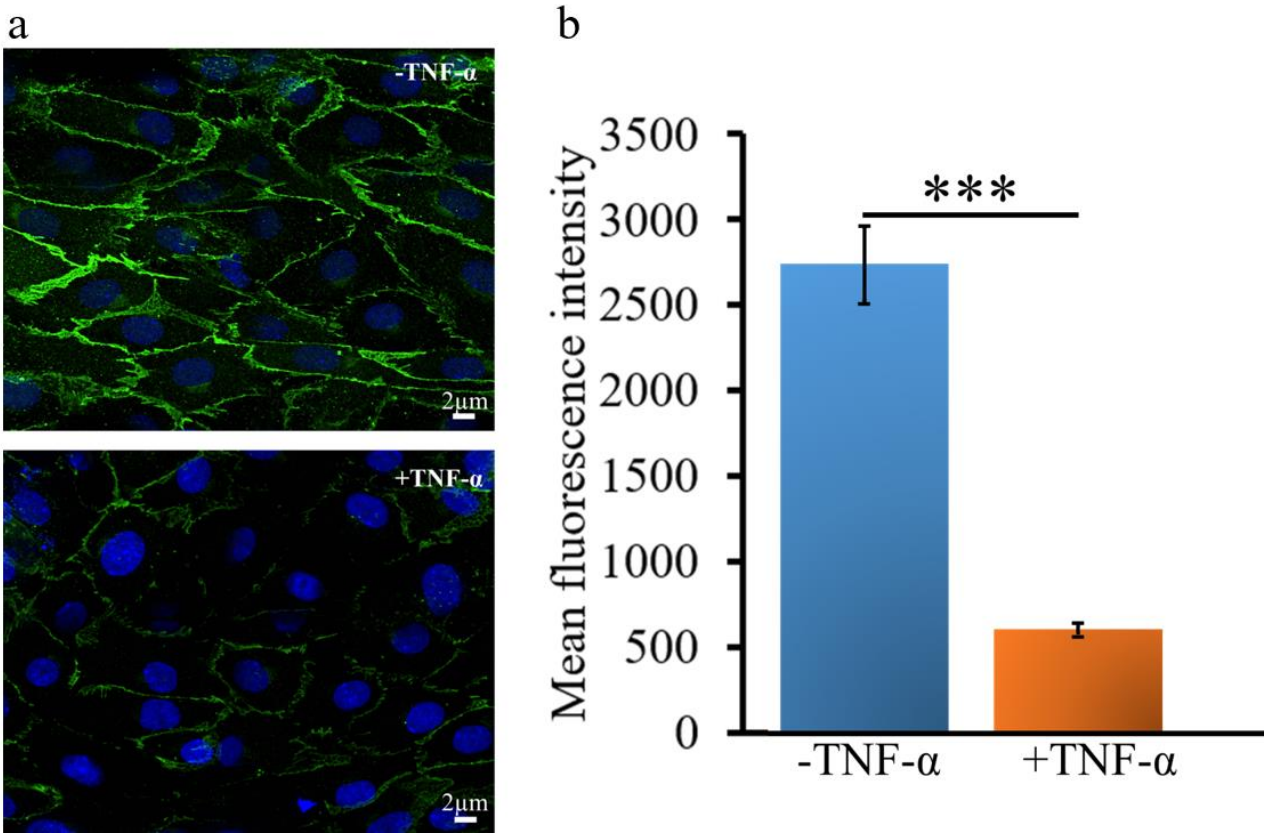
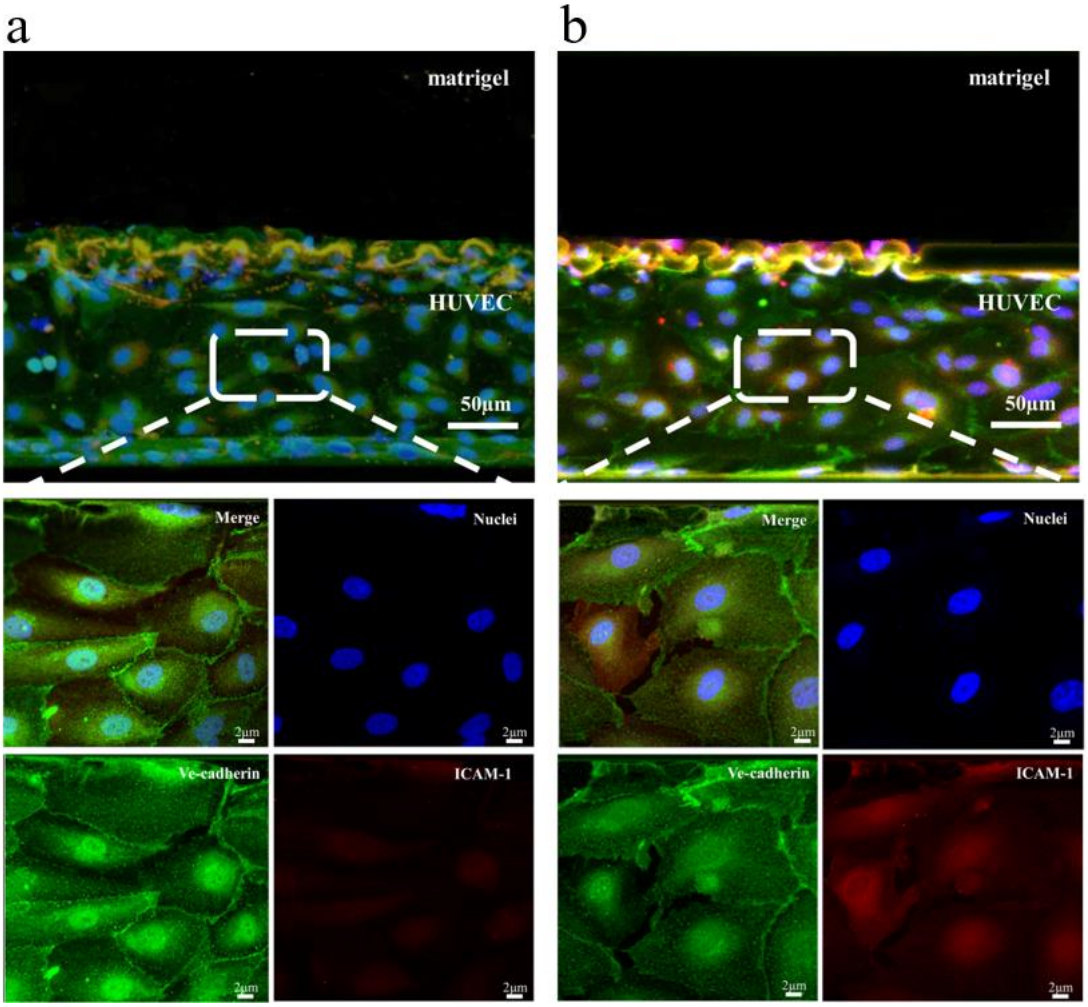
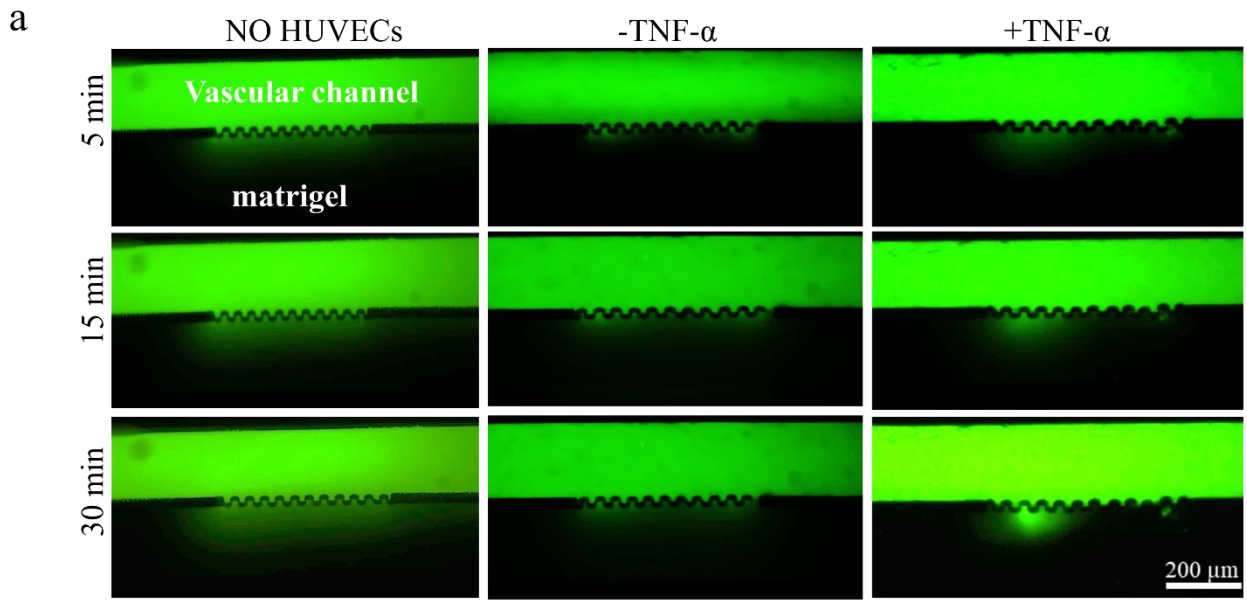
SUPPORTING DATA

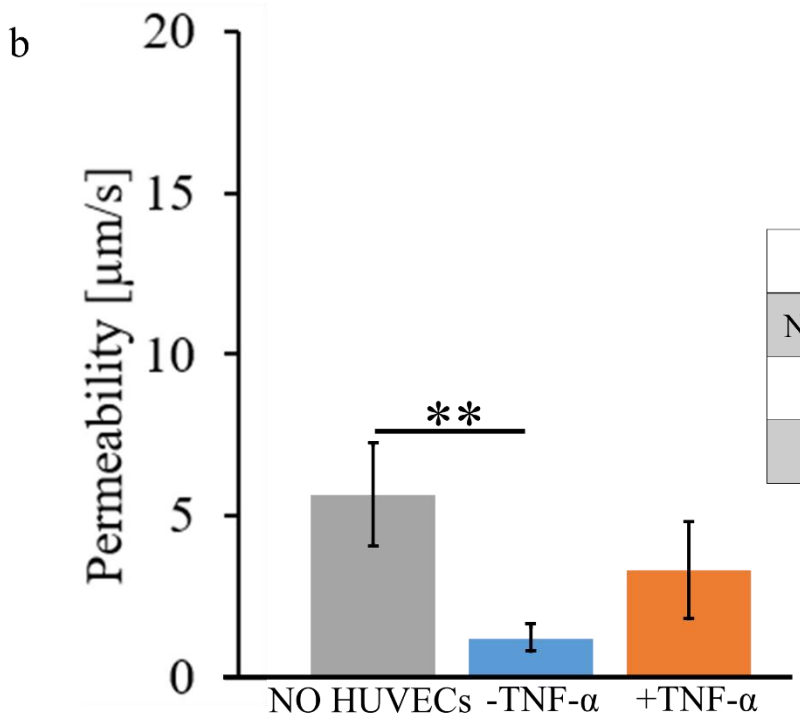


Supporting Figure 1. VE-cadherin molecules expression on HUVEC cells. a. VE-Cadherin immunostaining in unstimulated (-TNF-α - top image) and stimulated conditions (+TNF-α – bottom image). Nuclei were stained in blue using DAPI, VE-Cadherin adhesion molecule is shown in green. **b.** Mean fluorescence intensity of the VE-Cadherin expression for the two different conditions. Data are plotted as mean + SD, n = 4. Statistical analysis T test. *** denotes statistically significant difference $p<0.0001$.



Supporting Figure 2. Visualization of ICAM-1 adhesion molecules in the microfluidic chip. **a.** Confocal fluorescent microscopy images of the chip with a confluent layer of HUVECs, under unstimulated conditions (-TNF-α) (top). Confocal fluorescent images into different channels (60 x magnification) were obtained using Split-Channels and then recombining the images using Merge-Channels (bottom). Nuclei stained in blue with DAPI, VE-Cadherin immunostaining in green, ICAM-1 immunostaining in red. **b.** Representative confocal fluorescent microscopy image of chip with a confluent layer of HUVECs, under stimulated conditions (+ TNF-α,) (top). Confocal fluorescent images into different channels (60 x magnification) were obtained using *Split-Channels* and recombining the images using *Merge-Channels* (bottom). Nuclei stained in blue with DAPI, VE-Cadherin immunostaining in green, ICAM-1 immunostaining in red.

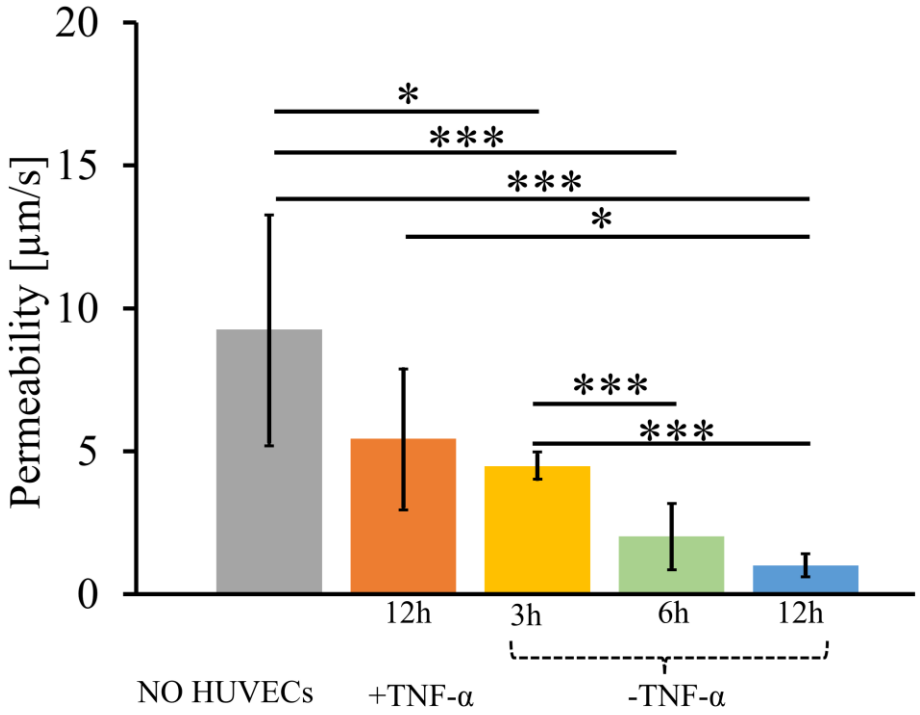




$$P = \frac{(I_f - I_i)w}{(I_i - Ib)\Delta t}$$

	P
NO HUVECs	5.77 ± 1.58
- TNF-α	1.21 ± 0.38
+ TNF-α	3.39 ± 1.53

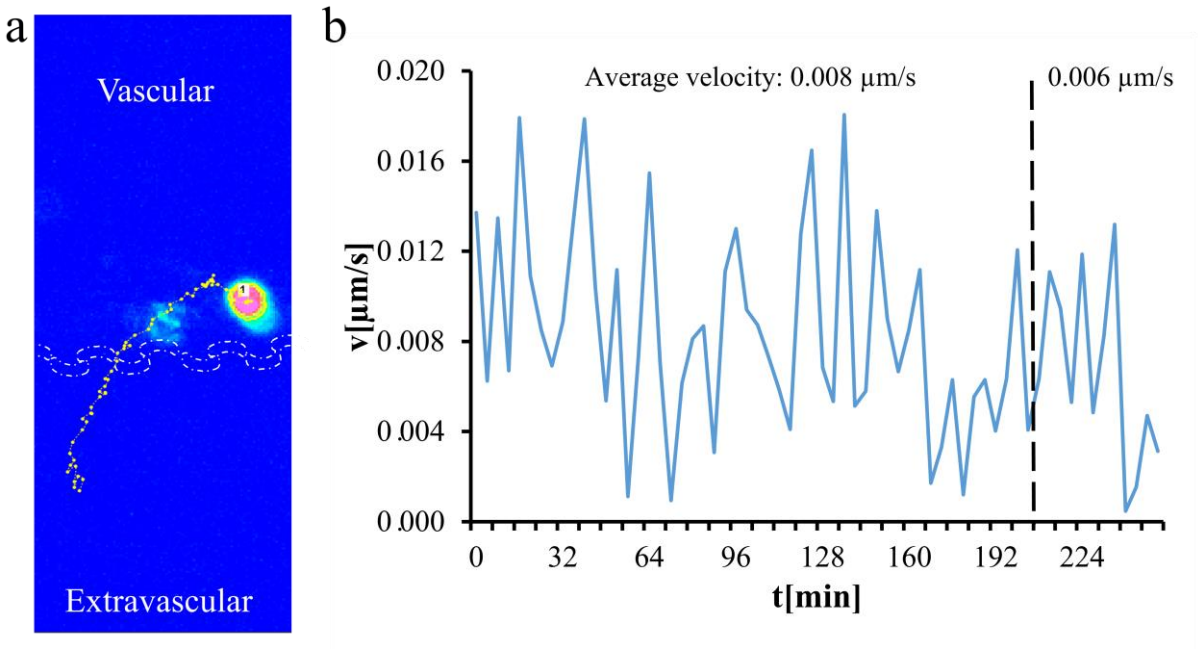
Supporting Figure 3. Vascular permeability at the micro-membrane at Q = 50 nL/min. a. Representative fluorescent images of free FITC-Dextran (40 kDa) diffusing (Q = 50 nL/min) in the vascular channel with an healthy (- TNF-α); inflamed endothelium (+ TNF-α) and with no endothelial cells (NO HUVECs) (TNF-α treatments were performed at 50 ng/mL for 12h). **b.** Vascular permeability coefficients. Formula for calculating the permeability (right). Data are plotted as mean + SD, n = 5. Statistical analysis ANOVA. ** denotes statistically significant difference $p<0.05$.



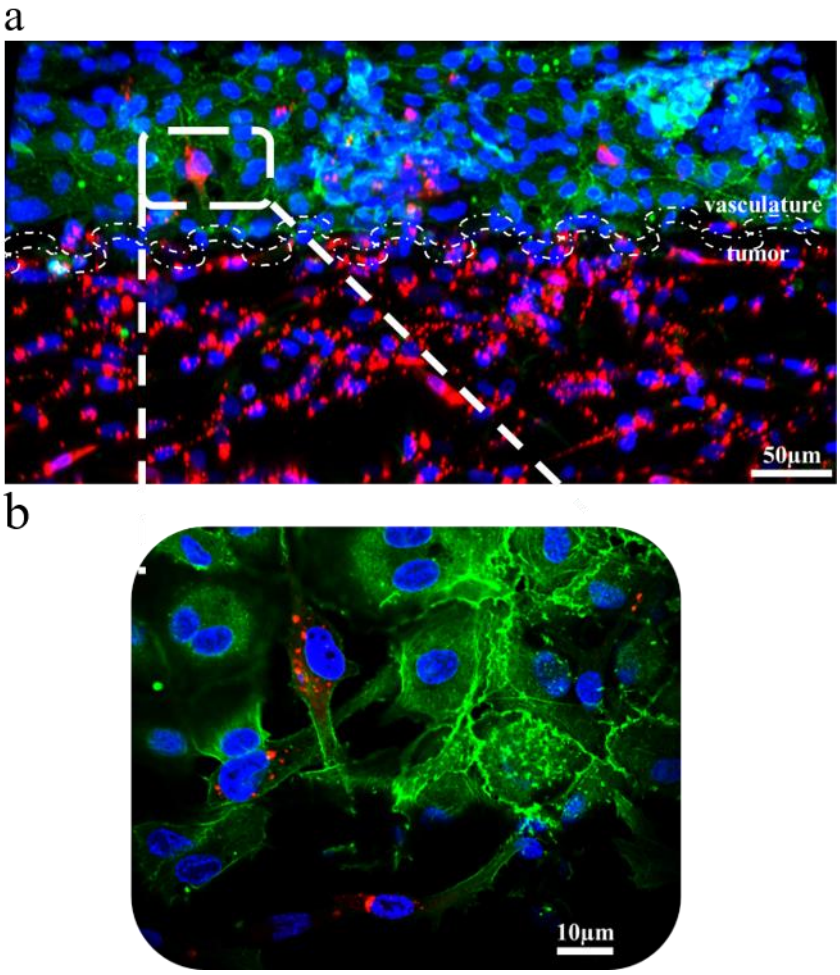
$$P = \frac{(I_f - I_i)w}{(I_i - Ib)\Delta t}$$

	P
NO HUVECs	9.25 ± 3.96
+ TNF-α (12h)	5.42 ± 2.42
- TNF-α (3h)	4.47 ± 0.43
- TNF-α (6h)	2.02 ± 1.11
- TNF-α (12h)	1.01 ± 0.34

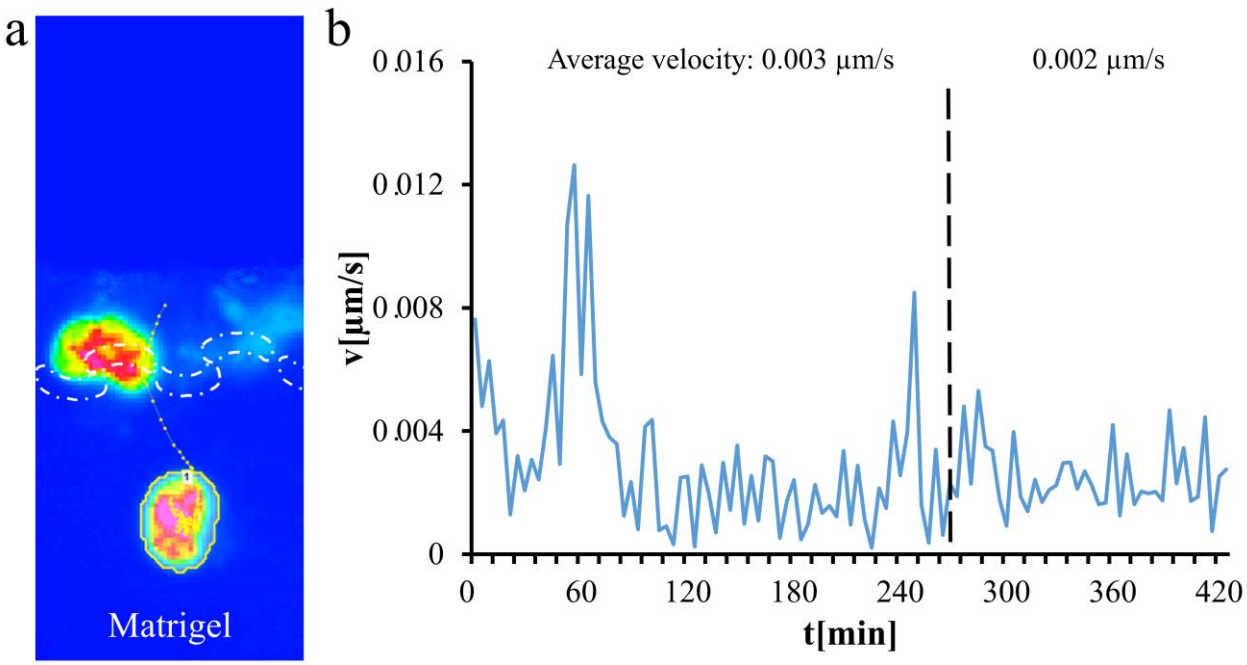
Supporting Figure 4. Vascular permeability at the micro-membrane at Q = 100 nL/min. Vascular permeability coefficients for FITC-Dextran (40 kDa) in the vascular channel on healthy HUVECs (- TNF-α) 12 h after seeding; 3h post HUVEC seeding; 6h post HUVEC seeding; on inflamed endothelium (+ TNF-α) 12h after seeding and with no endothelial cells (NO HUVECs) (TNF-α treatments were performed at 50 ng/mL for 12h). The formula for calculating the permeability coefficients (right). Data are plotted as mean + SD, n = 5. Statistical analysis ANOVA. * denotes statistically significant difference $p<0.05$. *** denotes statistically significant difference $p<0.001$ (Q = 100 nL/min).



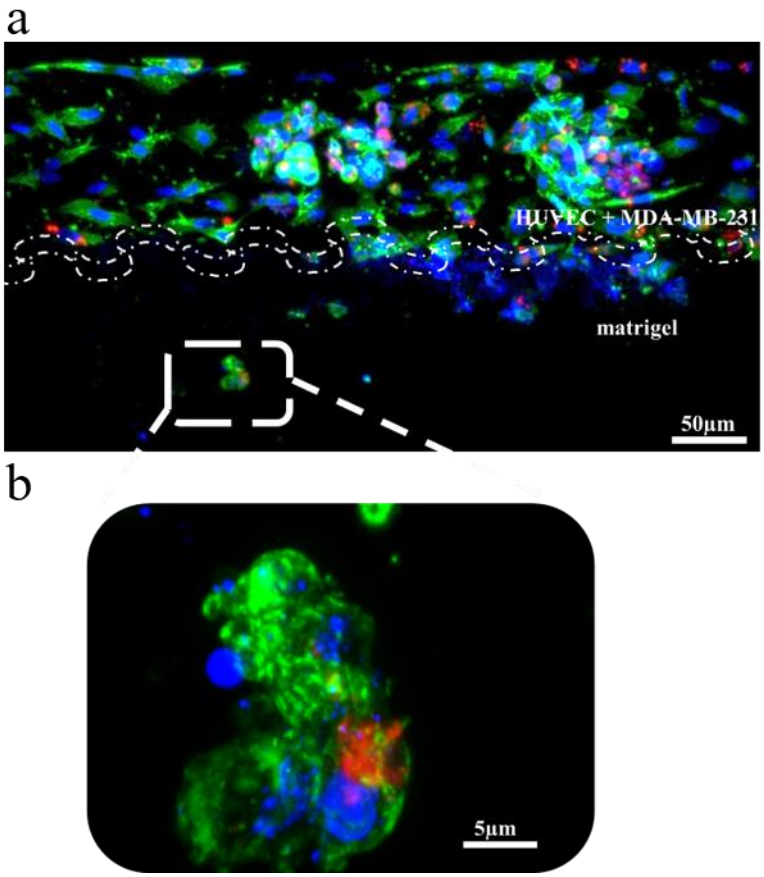
Supporting Figure 5. Cancer cell intravasation: single cell tracking and instantaneous velocity **a.** Trajectory of a breast cancer cell MDA-MB-231 moving from the extravascular compartment, filled with a matrigel matrix, across the micro-membrane, to the vascular compartment filled with a confluent monolayer of HUVECs. The tracking is performed via time lapse microscopy. **b.** Cancer cell velocity over time during intravasation calculated by time lapse microscopy. The dashed black line indicates when the micro-membrane is crossed by the cell.



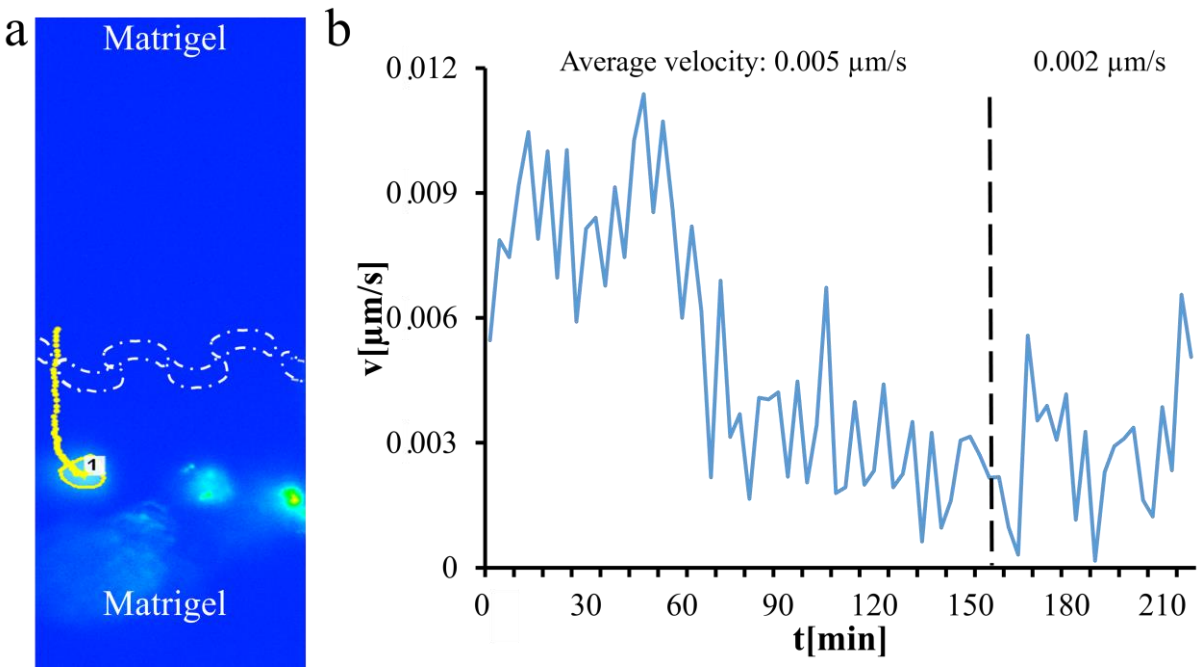
Supporting Figure 6. Cancer cell intravasation a. Confocal fluorescent microscopy image showing breast cancer cells (MDA-MB-231, red) intravasating from the extravascular compartment, filled with a matrigel matrix, to the vascular compartment, filled with a confluent HUVEC monolayer. **b.** Magnified image at 60 x showing tumor cells (red) in an elongated shape, infiltrating the HUVEC monolayer. (Breast cancer cells have the membrane labeled in red with CM-DiI. Nuclei are stained in blue with DAPI. F-Actin is stained in green).



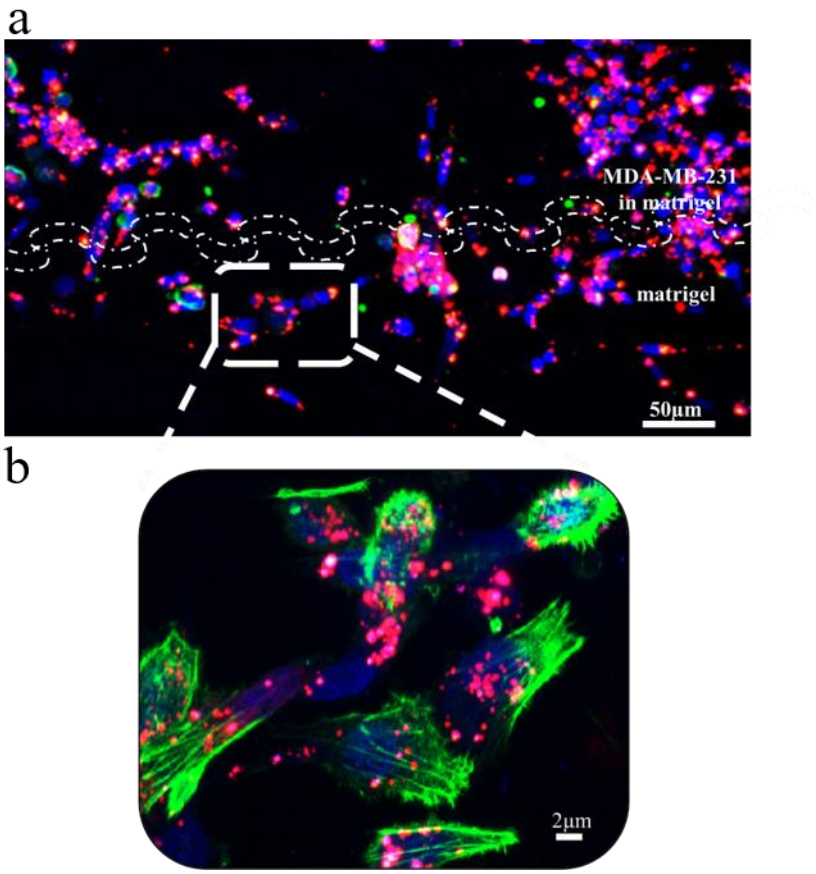
Supporting Figure 7. Cancer cell extravasation: single cell tracking and instantaneous velocity **a.** Trajectory of a breast cancer cell MDA-MB-231 moving from the vascular compartment, filled with a confluent HUVEC monolayer, across the micro-membrane, to the extravascular compartment, filled with a matrigel matrix. The tracking is performed via time lapse-microscopy. **b.** Cancer cell velocity over time during extravasation calculated by time lapse-microscopy. The dashed black line indicates when the micro-membrane is crossed by the cell.



Supporting Figure 8. Cancer cell extravasation. a. Confocal fluorescent microscopy image showing breast cancer cells (MDA-MB-231, red) extravasating from the vascular compartment, filled with a confluent HUVEC monolayer, to the extravascular compartment filled with a matrigel matrix. **b.** Magnified image at 60 x showing tumor cells (red), infiltrating the extravascular compartment. Breast cancer cells have the membrane labeled in red with CM-DiI. Nuclei are stained in blue with DAPI. F-Actin is stained in green.



Supporting Figure 9. Cancer cell invasion: single cell tracking and instantaneous velocity a. Trajectory of a breast cancer cell MDA-MB-231 moving from a compartment, filled with a matrigel matrix, across the micromembrane, to the other compartment, filled with a matrigel matrix. The tracking is performed via time lapse-microscopy. **b.** Cancer cell velocity over time during invasion calculated by time lapse-microscopy. The dashed black line indicates when the micromembrane is crossed by the cell.



Supporting Figure 10. Cancer cell invasion. a. Confocal fluorescent microscopy image showing breast cancer cells (MDA-MB-231, red) migrating from a compartment, to the other compartment, both filled with a matrigel matrix. **b.** Magnified image at 60 x showing tumor cells (red) in an elongated shape, infiltrating the other compartment. Breast cancer cells have the membrane labeled in red with CM-DiI. Nuclei are stained in blue with DAPI. F-Actin is stained in green.

	Invasion Velocity (μm/h)	Extravasation Velocity (μm/h)	Intravasation Velocity (μm/h)	
MDA-MB-231 cells[1-3]	86.4		–	*in vivo*
	13-31	–	–	*in vitro*
	37.5-72	–	_	*in vitro*
MDA-MB-435 cells[4]	–	75.6	_	*in vivo*
Murine pancreatic cells[5]	14.04	–	–	*in vivo*
MTLn3 cells[6]	–	–	204	*in vivo*
MTC cells[6]	–	–	222	*in vivo*
HEp3 cells[7]	–	–	20-40	*in vitro*
Present work	18	7.2	28.8	*in vitro*

Supporting Table 1. Velocity of different cancer cells during the metastatic steps. Average velocity for invasion, extravasation and intravasation of MDA-MB-231 cells, MDA-MB-435, murine pancreatic, MTLn3, MTC and HEp3 cancer cells, in vitro and in vivo.

SUPPORTING MOVIES

Supporting Movie 1. Permeability studies with healthy endothelium. Fluorescent microscopy movie of free FITC-Dextran 40 kDa diffusing under dynamic conditions (Q = 100 nL/min) in the vascular channel covered by a confluent layer of HUVECs.

Supporting Movie 2. Permeability studies with inflamed endothelium. Fluorescent microscopy movie of free FITC-Dextran 40 kDa diffusing under dynamic conditions (Q = 100 nL/min) in the vascular channel covered by a confluent layer of inflamed HUVECs (+ TNF-α).

Supporting Movie 3. Permeability studies with NO HUVECs. Fluorescent microscopy movie of free FITC-Dextran 40 kDa diffusing under dynamic conditions (Q = 100 nL/min) in the vascular channel with no endothelial cells.

Supporting movie 4. Intravasation of cancer cells. Time-lapse microscopy movie showing the intravasation of breast cancer cells (MDA-MB-231), embedded in the matrigel, from the extravascular compartment to the vascular compartment, covered by a confluent layer of HUVECs. MDA-MB-231 cells are stained in red with CM-DiI, nuclei of both HUVECs and MDA-MB-231 cells are stained in blue with DAPI. Merged channels and fluorescence signal in rainbow scale are respectively reported on the left and on the right of the screen area.

Supporting movie 5. Vascular adhesion of breast cancer cells on healthy endothelium (100 nL/min). Bright field microscopy movie showing the rolling and adhesion of breast cancer cells (MDA-MB-231) over a confluent monolayer of HUVECs, which were not treated with TNF-α. The imposed flow rate was of 100 nL/min. Cells were observed to move through the channel, transported by the flow, without any adhesion to the vascular walls.

Supporting Movie.6 Vascular adhesion of breast cancer cells on inflamed endothelium (100 nL/min). Bright field microscopy movie showing the rolling and adhesion of breast cancer cells (MDA-MB-231) over a confluent monolayer of HUVECs, which were treated with TNF-α (50 ng/mL). The imposed flow rate was of 100 nL/min. Multiple cells are observed to firmly adhere to the endothelium, at the micromembrane. Other cells, debris, and a few cell clusters are clearly observed to move downstream transported by the flow.

Supporting movie 7. Extravasation of cancer cells. Time-lapse microscopy movie showing the extravasation of breast cancer cells (MDA-MB-231), originally adhering on a confluent HUVEC monolayer, from the vascular to the extravascular compartment. MDA-MB-231 cells are stained in red with CM-DiI, nuclei of both HUVECs and MDA-MB-231 cells are stained in blue with DAPI. Merged channels and fluorescence signal in rainbow scale are respectively reported on the left and on the right of the screen area.

Supporting movie 8. Invasion of cancer cells. Time-lapse microscopy movie shows the invasion of breast cancer cells (MDA-MB-231) from a compartment to the other of the microfluidic device. MDA-MB-231 cells are stained in red with CM-DiI. Merged channels and fluorescence signal in rainbow scale are respectively reported on the left and on the right of the screen area.

For the generation of these data same conditions indicated in the main text were used.

References:

1. Ghaffari, A., V. Hoskin, G. Turashvili, S. Varma, J. Mewburn, G. Mullins, P.A. Greer, F. Kiefer, A.G. Day, and Y. Madarnas, *Intravital imaging reveals systemic ezrin inhibition impedes cancer cell migration and lymph node metastasis in breast cancer.* Breast Cancer Research, 2019. **21**(1): p. 12 DOI: 10.1186/s13058-018-1079-7.
2. Sun, W., C.T. Lim, and N.A. Kurniawan, *Mechanistic adaptability of cancer cells strongly affects anti-migratory drug efficacy.* Journal of The Royal Society Interface, 2014. **11**(99): p. 20140638 DOI: 10.1098/rsif.2014.0638.
3. Kurniawan, N.A., P.K. Chaudhuri, and C.T. Lim, *Concentric gel system to study the biophysical role of matrix microenvironment on 3D cell migration.* JoVE (Journal of Visualized Experiments), 2015(98): p. e52735 DOI: 10.3791/52735.
4. Stoletov, K., H. Kato, E. Zardouzian, J. Kelber, J. Yang, S. Shattil, and R. Klemke, *Visualizing extravasation dynamics of metastatic tumor cells.* J Cell Sci, 2010. **123**(13): p. 2332-2341 DOI: 10.1242/jcs.069443. Epub 2010 Jun 8.

5. Beerling, E., I. Oosterom, E. Voest, M. Lolkema, and J. van Rheenen, *Intravital characterization of tumor cell migration in pancreatic cancer*. Intravital, 2016. **5**(3): p. e1261773 DOI: 10.1080/21659087.2016.1261773. eCollection 2016.

6. Wyckoff, J.B., J.G. Jones, J.S. Condeelis, and J.E. Segall, *A critical step in metastasis: in vivo analysis of intravasation at the primary tumor.* Cancer research, 2000. **60**(9): p. 2504-2511.

7. Zijlstra, A., J. Lewis, B. DeGryse, H. Stuhlmann, and J.P. Quigley, *The inhibition of tumor cell intravasation and subsequent metastasis via regulation of in vivo tumor cell motility by the tetraspanin CD151*. Cancer cell, 2008. **13**(3): p. 221-234 DOI: 10.1016/j.ccr.2008.01.031.