

Figure S1. Detection of transmigrated tumor cells. Left, schematic of the cells in the insert, showing the orientation of the cells relative to the membrane. Right, three representative confocal images showing tumor cells (TCs, red) relative to LECs (stained with PECAM-1, green) after 12h. The insert membrane is indicated by dotted line.

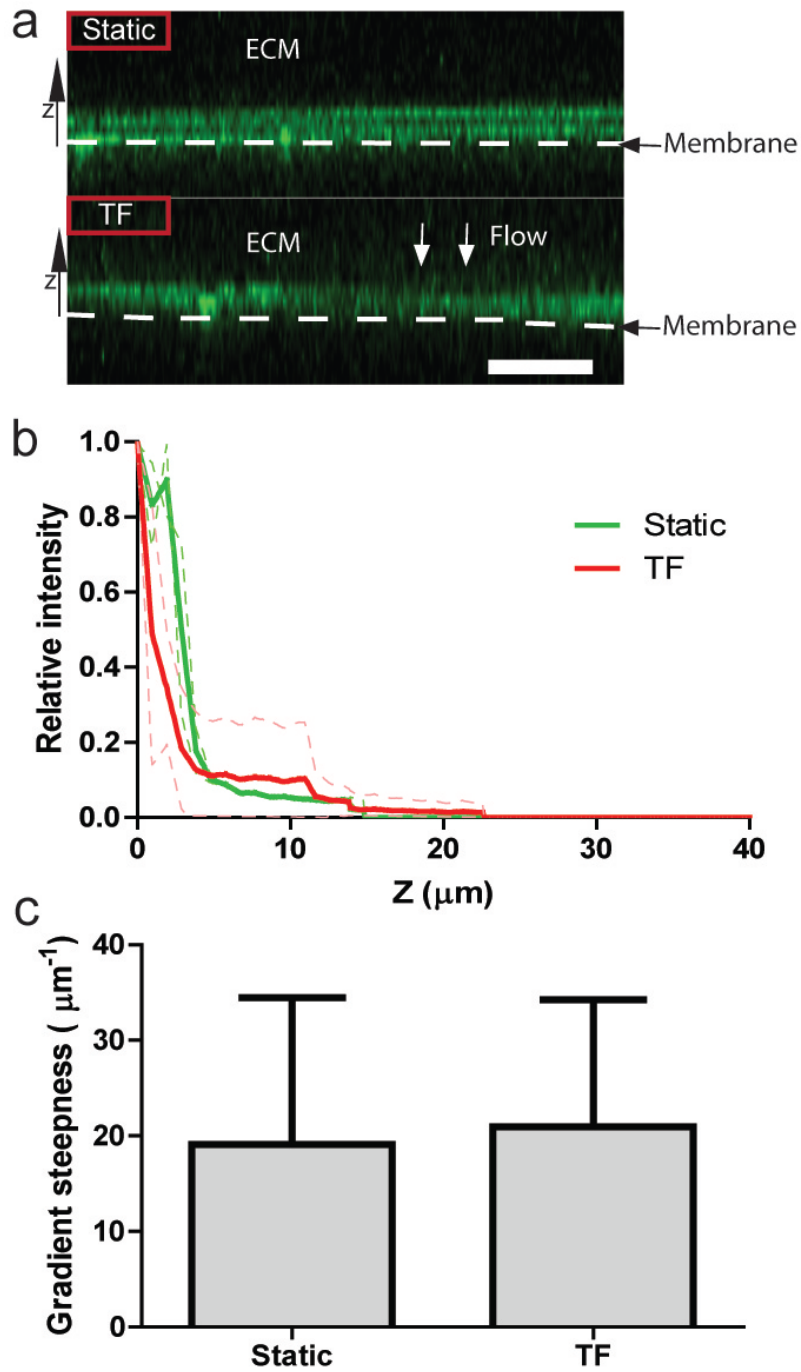


Figure S2. CCL21 gradients in the extracellular matrix are dominated by binding to the extracellular matrix rather than by transmural flow. (a) Representative confocal images of exogenous CCL21 gradients in the collagen-matrigel composite extracellular matrix (ECM) in static vs. flow conditions. CCL21 was added to the insert membrane before the ECM was incorporated. The samples were incubated for 12 h in static vs. transmural flow ($1\mu\text{m/s}$) conditions and subsequently stained with an antibody against CCL21. Scale bar: $50\mu\text{m}$. (b) Average CCL21 concentration, assumed linearly related to intensity, as a function of distance from the cell source. Data are representative from 3 independent experiments. Dotted lines represent standard deviations. (c) Quantification of the steepness of the CCL21 gradient in the matrix, calculated as described in the method section. Each column represents 3 independent experiments.