Supplementary data movie 1 and 2:

Cancer cell recruitment to TNF-activated HUVEC was monitored using fluorescence timelapse microscopy (x4 objective, Olympus IX81). Cancer cells were labelled with 1µM green or orange Cell Tracker® (Invitrogen) fluorescent dyes so that they could be distinguished from endothelial cells and co-perfused at a density of $1x10^6$ cells per mL and at a shear rate of 1.25dynes/cm^2 for 10 (movie 1) or 30 minutes (movie 2). Note that cells labelled with Cell Tracker® orange appear red under fluorescence. The entire flow chamber was imaged after 10/20min of perfusion to visualize adherent cells and estimate the ratio of binding. The cell suspensions were also observed under fluorescent microscope to calculate the exact ratio of each cancer cell line in the mixture.

In movie 1, ZR-75-1 cells are dyed in green and BT-20 in red and account for 46% and 54% of the suspension respectively. Note that most red cells (BT-20) do interact with the HUVEC monolayer. Those that do interact are transient, with the red cell disappearing from the following frame in most of the cases. This is in accordance with a recent report that showed BT-20 rolling on HUVEC, using a slightly different protocol (i.e. different activation stimulus for HUVEC and lower shear force)(1). In contrast green cells (ZR-75-1) firmly adhered to activated HUVEC and accumulated with time. Imaging of the entire chamber at the endpoint of the experiment showed that more than 90% of adherent cells were ZR-75-1 (not shown).

In movie 2, ZR-75-1 cells are dyed in green and MDA-MB-231 in red and account for 41% and 59% of the suspension respectively. Red cells (MDA-MB-231) did not adhere at all to HUVEC, event after 20 minutes of perfusion, while green cells (ZR-75-1) behave as seen in movie 1. Imaging of the entire chamber at the endpoint of the experiment showed that all adherent cells were ZR-75-1 (not shown).

1. Shirure VS, Henson KA, Schnaar RL, Nimrichter L, Burdick MM. Gangliosides expressed on breast cancer cells are E-selectin ligands. Biochem Biophys Res Commun. 2011.