Electronic Supplementary Material (ESI) for Lab on a Chip
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Evaluation of intercellular communication between breast cancer cells and adipose-derived stem cells via passive diffusion in a two-layer microfluidic device

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Methods S1. Off-chip single culture experiments.

MDA-MB-231 cells were cultured on either tissue culture plastic (TCP) or 0.1mg/mL poly-d-lysine coated agarose in 100 mm dishes. For the agarose condition, the 100 mm petri dish was filled with 15 mL of agarose (total thickness of 2 mm) and treated with poly-d-lysine as described in the manuscript. For all experiments, 700,000 cells were added to the dish. Cells were imaged every 24 h for a total of 72 h using a Zeiss Primo Vert phase-contrast microscope equipped with an Axiocam105 color digital camera. All images were further processed in ImageJ (NIH) software. Cell growth was quantified by calculating the average number of ten images collected at each time point of the same positions in the dish using a 10x objective. Cell growth was normalized by dividing the number of cells of each time point by the 0-hour cell numbers. The equation is:

$$\text{Normalized cell growth} = \frac{Number\ of\ cells\ at\ 24h/48h/72\ h}{Number\ of\ cells\ in\ 0\ h}$$

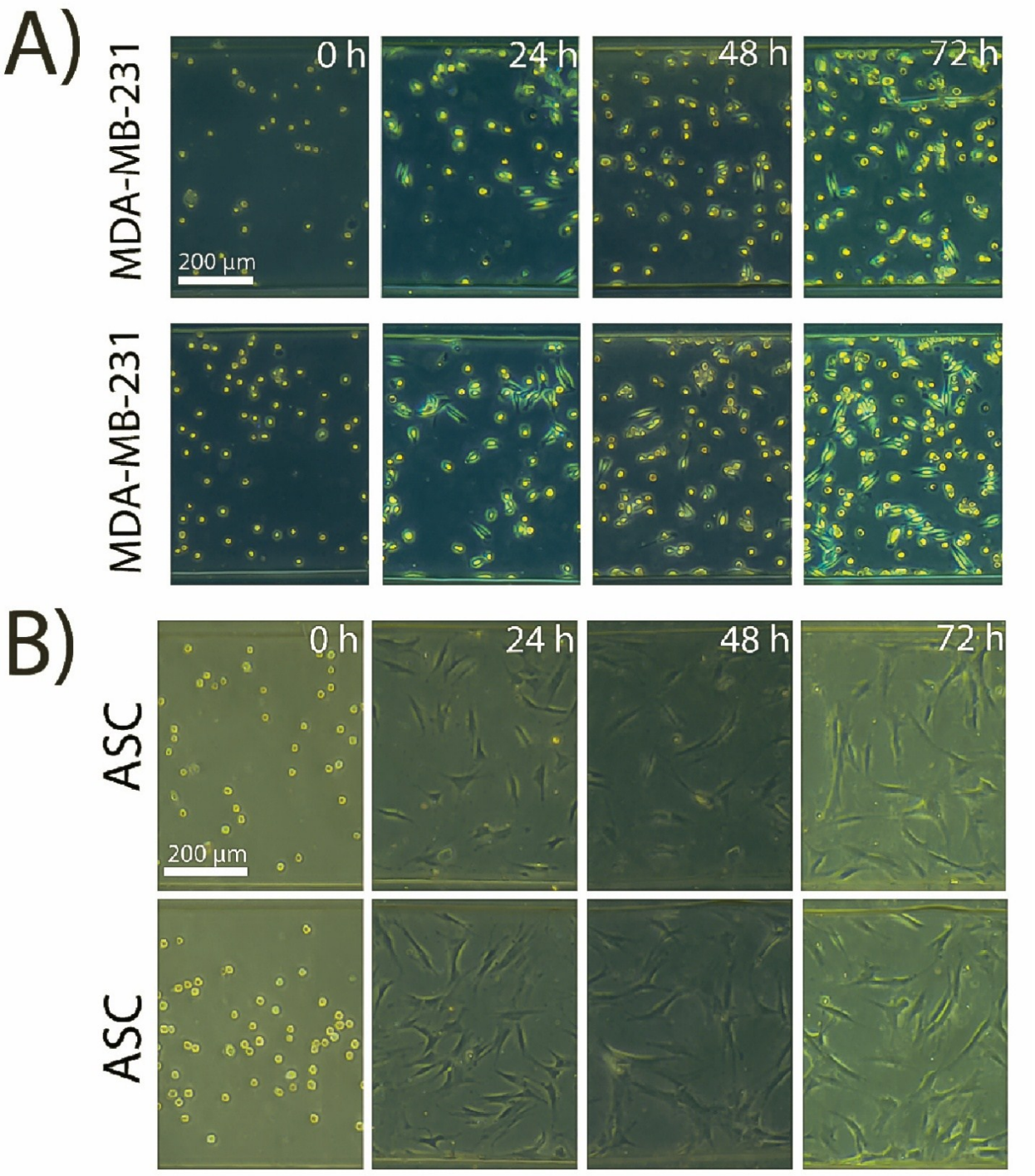

Figure S1. Single culture of MDA-MB-231 and adipose-derived stem cells (ASCs) in the microfluidic device. (A) MDA-MB-231 cells were cultured in both flow-free channels of the microfluidic device. The initial cell density was $5x10^5$ cells/mL (B) ASCs were cultured in both the center channel of the microfluidic device. The initial cell density was $1x10^6$ cells/mL. All images are representative cell growth across the entire channel and of triplicate experiments.

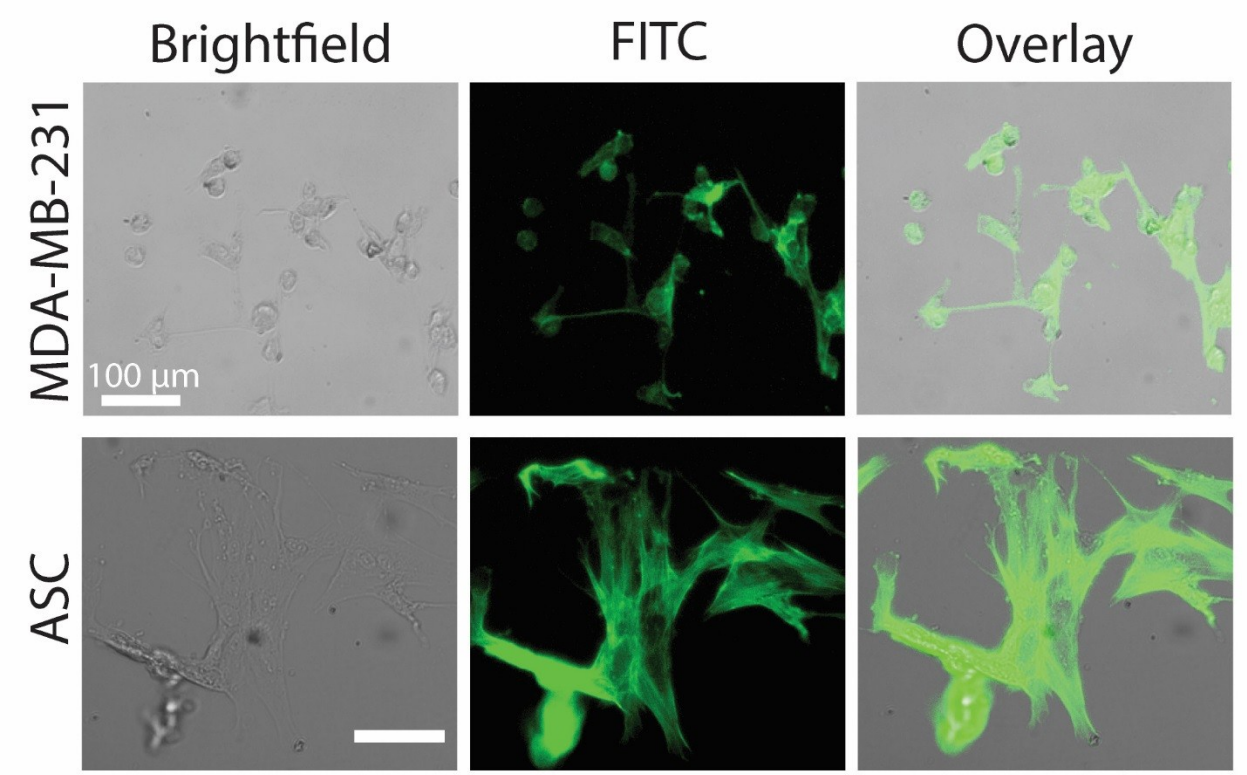

Figure S2. Actin staining of the cytoskeleton of adipose-derived stem cells (ASCs) and MDA-MB-231 cells. After 72 h of co-culture in the microfluidic device, both MDA-MB-231 and ASCs were stained using fluorescently-labeled phalloidin (Alexa Fluor 488) to confirm the structure of the actin cytoskeleton. Immunostaining was performed at first fixing the cells using 4% paraformaldehyde in PBS and then permeabilizing the cells using 0.5 %Triton X-100 in PBS and 0.1% BSA in BSA at room temperature. The BSA- Phalloidin solution was incubated in the device for 6 hours by continuous pumping using a syringe pump at room temperature. For actin cytoskeleton staining. The fluorescence image was taken using both transmitted light (brightfield) and FITC filter (green). Both scale bars are 100 µm.

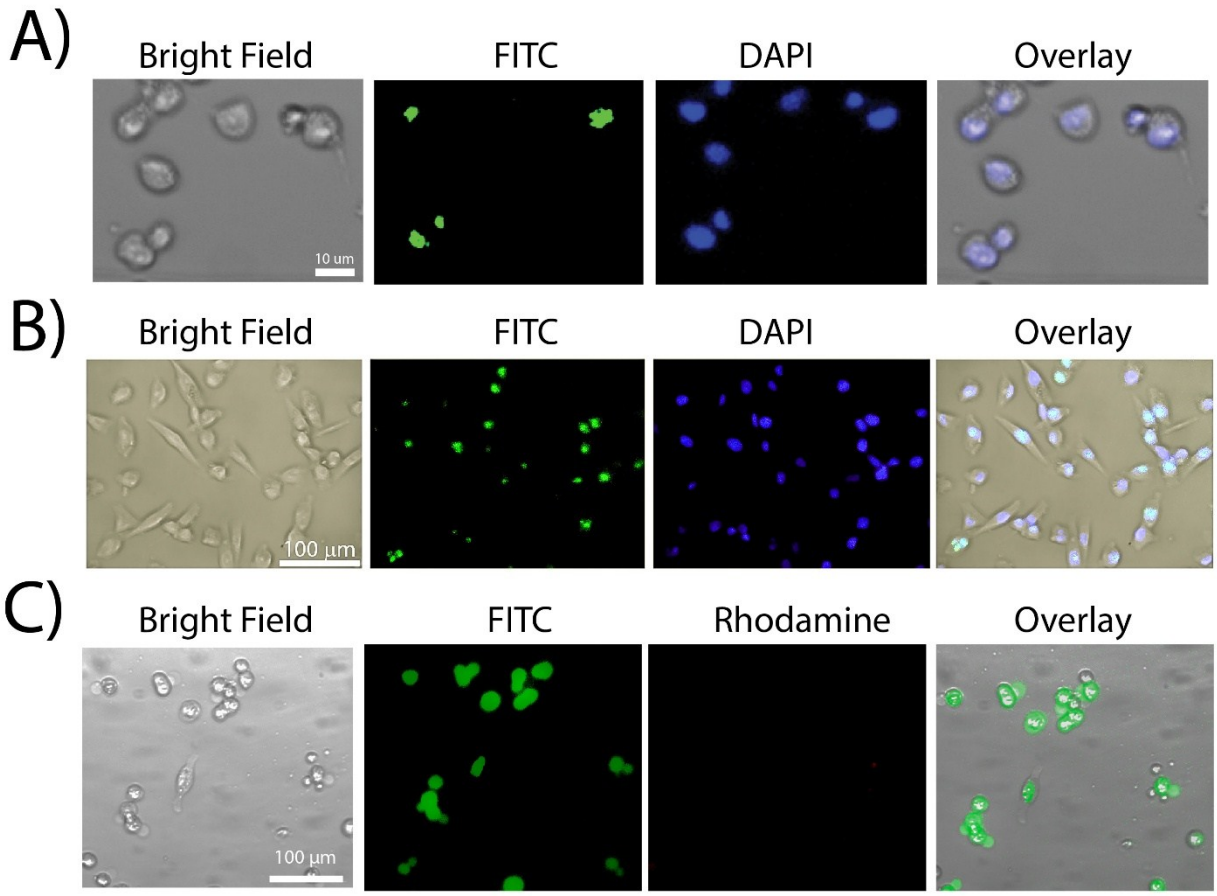

Figure S3. The proliferation of MDA-MB-231 cells in single culture and co-culture experiments and viable cells in co-culture. Proliferation staining of MDA-MB-231 cells in the microfluidic device using anti Ki-67 antibodies in FITC filter (green color) and DAPI (blue color) for nuclear staining after three days of (A)single culture (B) Co-culture. Ki-67 was diluted at 1:20 in the 0.1% BSA solution and incubated in the device for 6 hours by continuous infusion through the media channel using a syringe pump at room temperature. (C) Viability of MDA-MB-231 cells after three days of co-culture with ASCs in the microfluidic device. At the end of 72h of co-culture, MDA-MB-231 cells were incubated on-chip with 2.5 µM Calcein AM (live, FITC channel, green fluorescence) and 4 µM Ethidium homodimer-1 (dead, Rhodamine channel, red) in PBS for 2 h. Both live and dead stains were introduced into the device via flow, at the rate of 15 µL/min, through the outer media channels at room temperature.

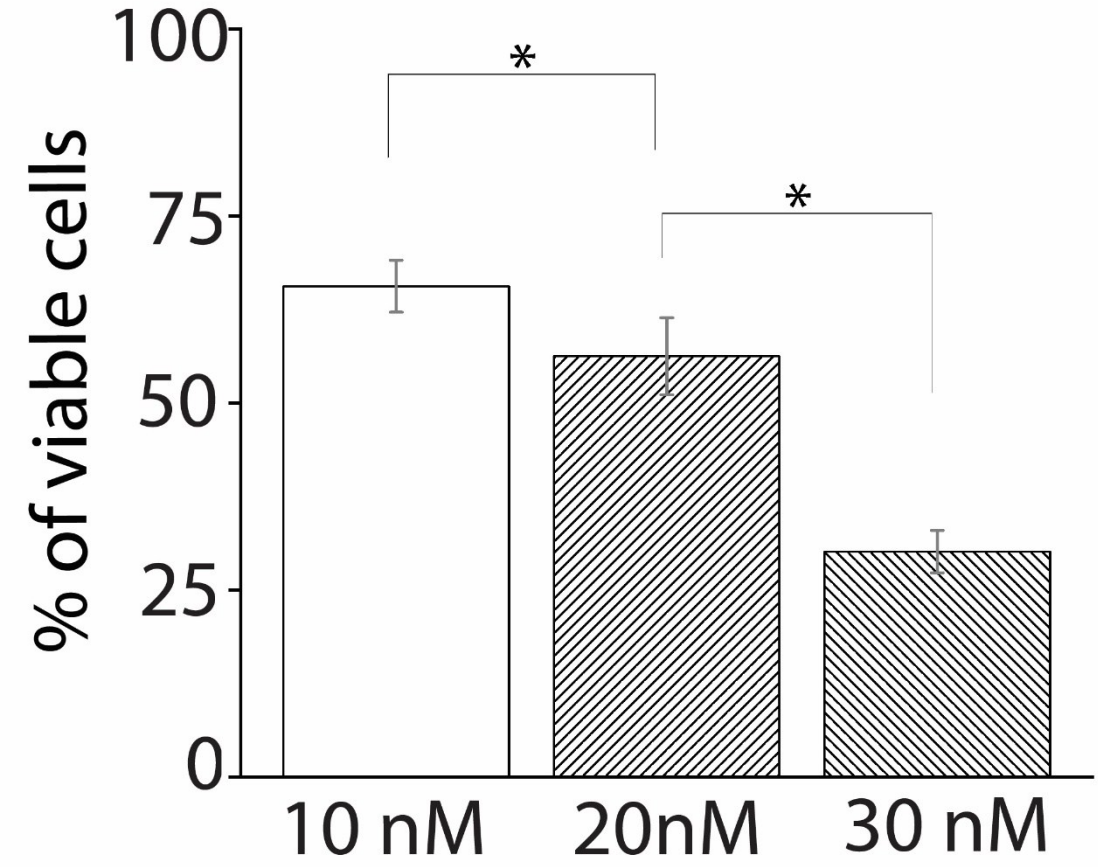

Figure S4. Paclitaxel dose optimization for viability on single cultured MDA-MB-231 cells. The viability of MDA-MB-231 cells was measured after three days of a single culture experiment in the microfluidic device. Experiments were conducted in duplicate and the statistical differences between two groups were determined by the student's t-test with a statistical confidence interval value set at $p<0.05$

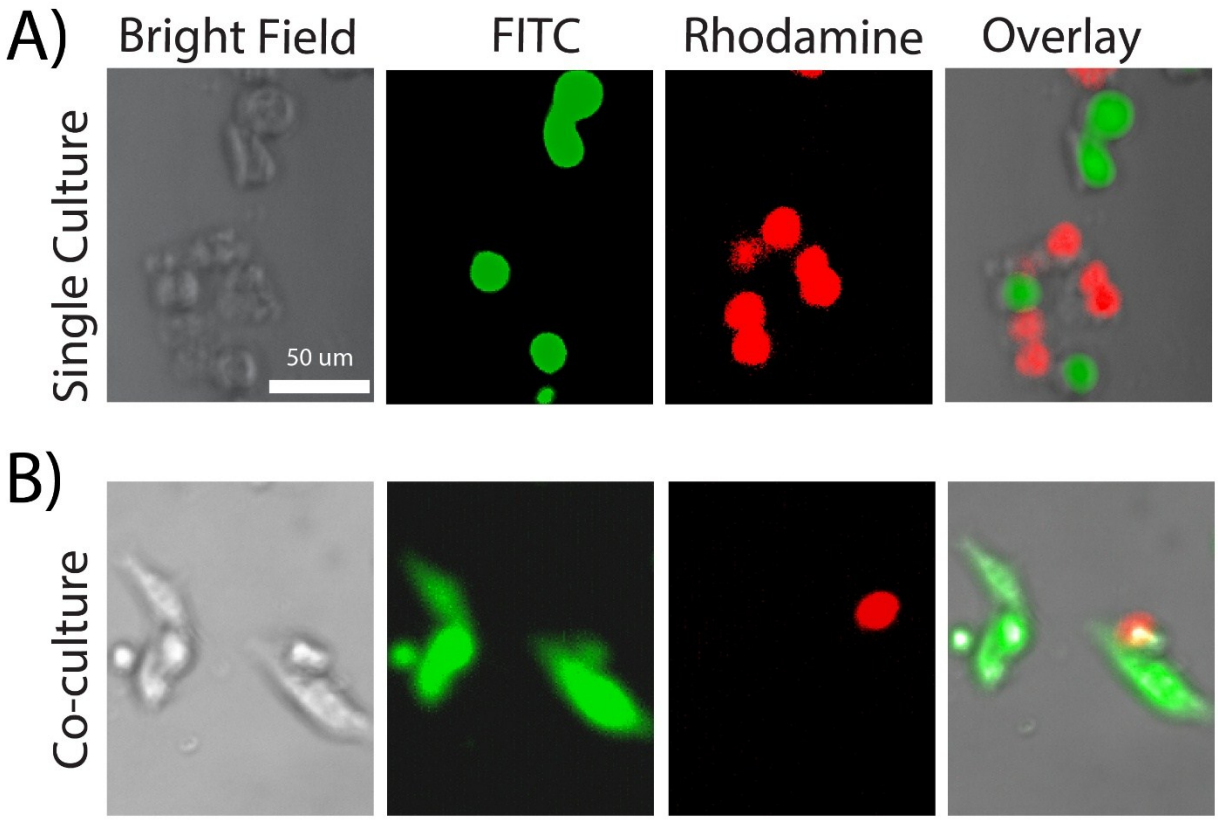

Figure S5. Viability of MDA-MB-231 cells after Paclitaxel treatment. The cell viability was measured on-chip after 3 days of 20 nM Paclitaxel treatment while incubated for one day in single cultured and co-cultured experiments. After termination of the experiment, MDA-MB-231 cells were incubated on-chip with 2.5 µM Calcein AM (live, FITC channel, green fluorescence) and 4 µM Ethidium homodimer-1 (dead, Rhodamine channel, red) in PBS for 2 h. Both live and dead stains were introduced into the device via flow, at the rate of 15 µL/min, through the outer media channels at room temperature. (A) Cell viability in single culture experiment (B) co-culture experiment