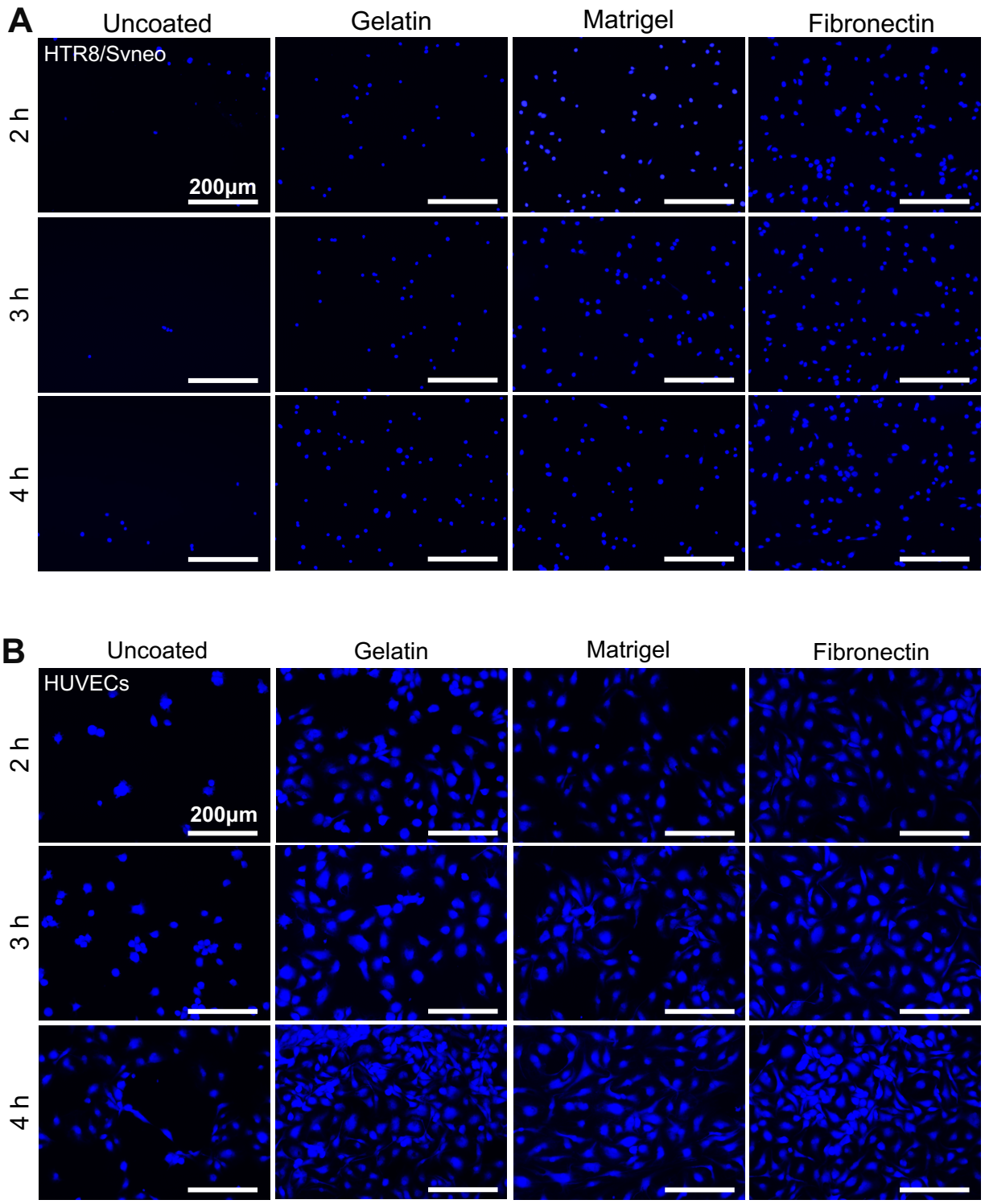
Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplemental Table 1

Primers for quantitative real time PCR.

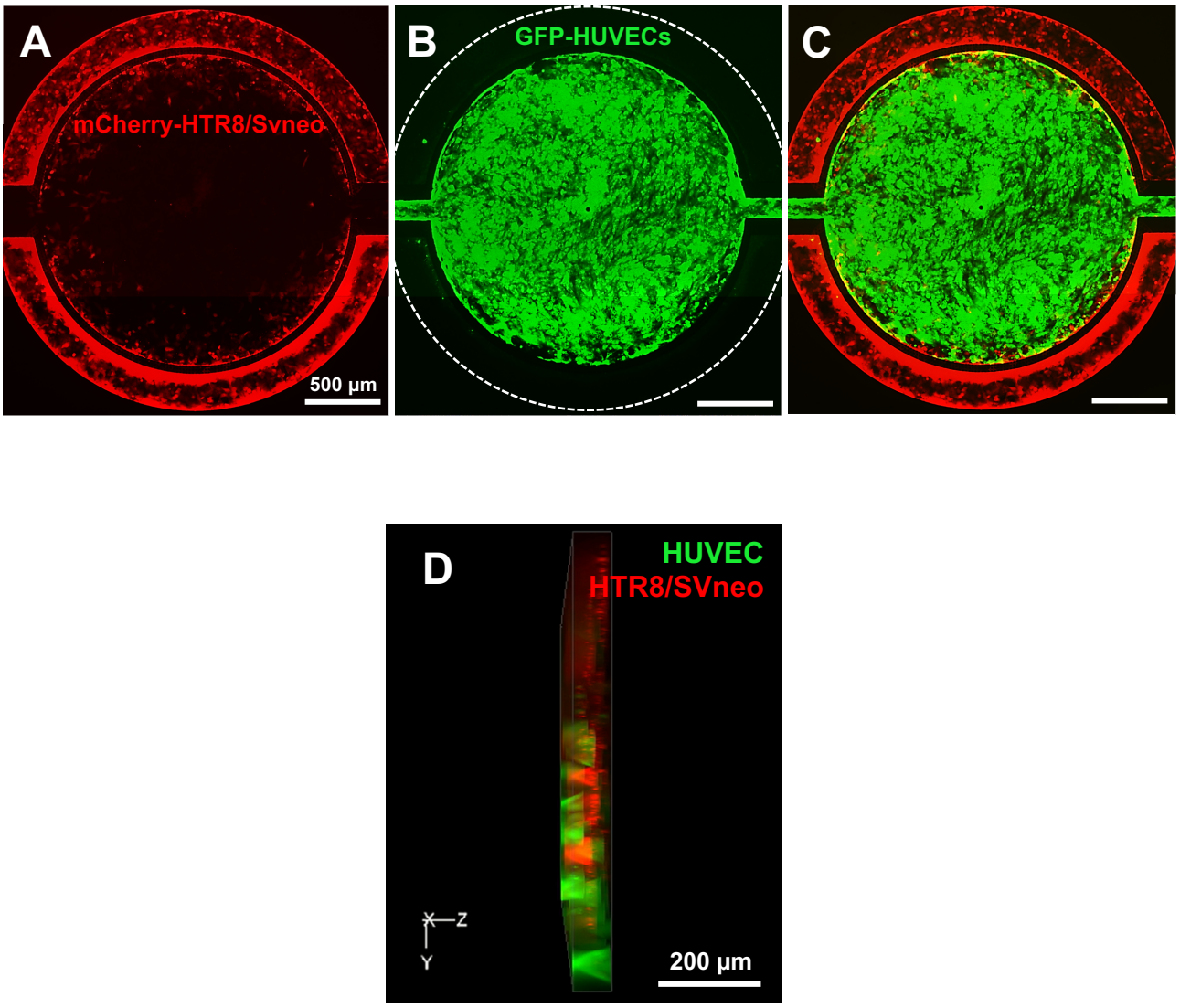
Gene	Primer sequences (5' - 3')	Product Size	Accession No.
MMP2	F: CCTGATGTCCAGCGAGTG	172 bp	NM_001127891
MMP2	R: CGGCATCCAGGTTATCG		
GAPDH	F: GGGAAGCTCACTGGCATGGCCTTCC	119 bp	NM_001256799
GAPDH	R: GCCTGCTTCACCACCTTCTTG		

Supplemental Figure 1



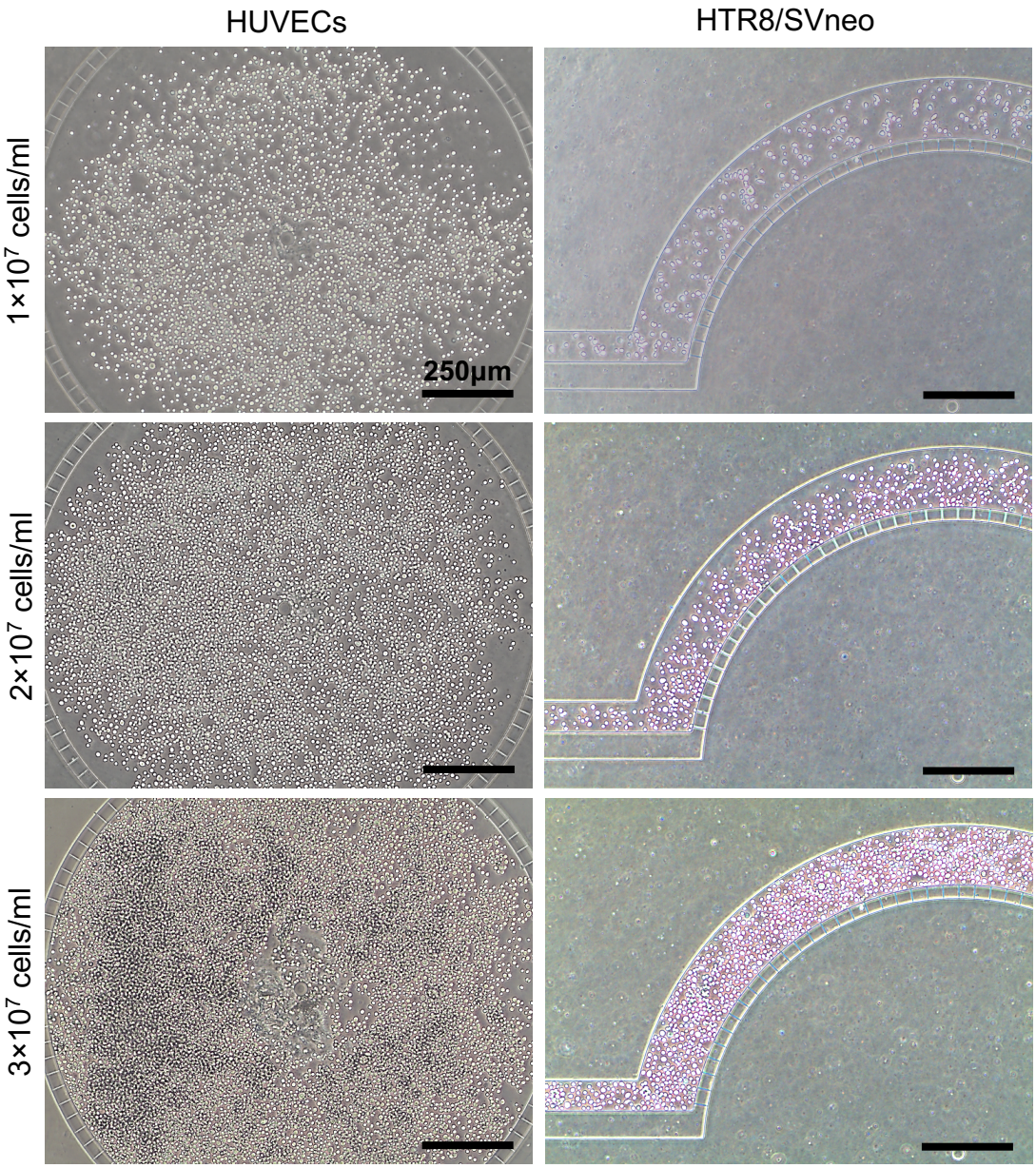
Supplemental Figure 1. Testing cell adhesion in extracellular matrix coated dish. Cell adhesion for HTR8/SVneo trophoblast cells (**A**) and human umbilical vein endothelial cells (HUVECs) (**B**) after gelatin, Matrigel and fibronectin coating on petri dishes 2, 3, and 4 h after seeding compared to uncoated dishes.

Supplemental Figure 2



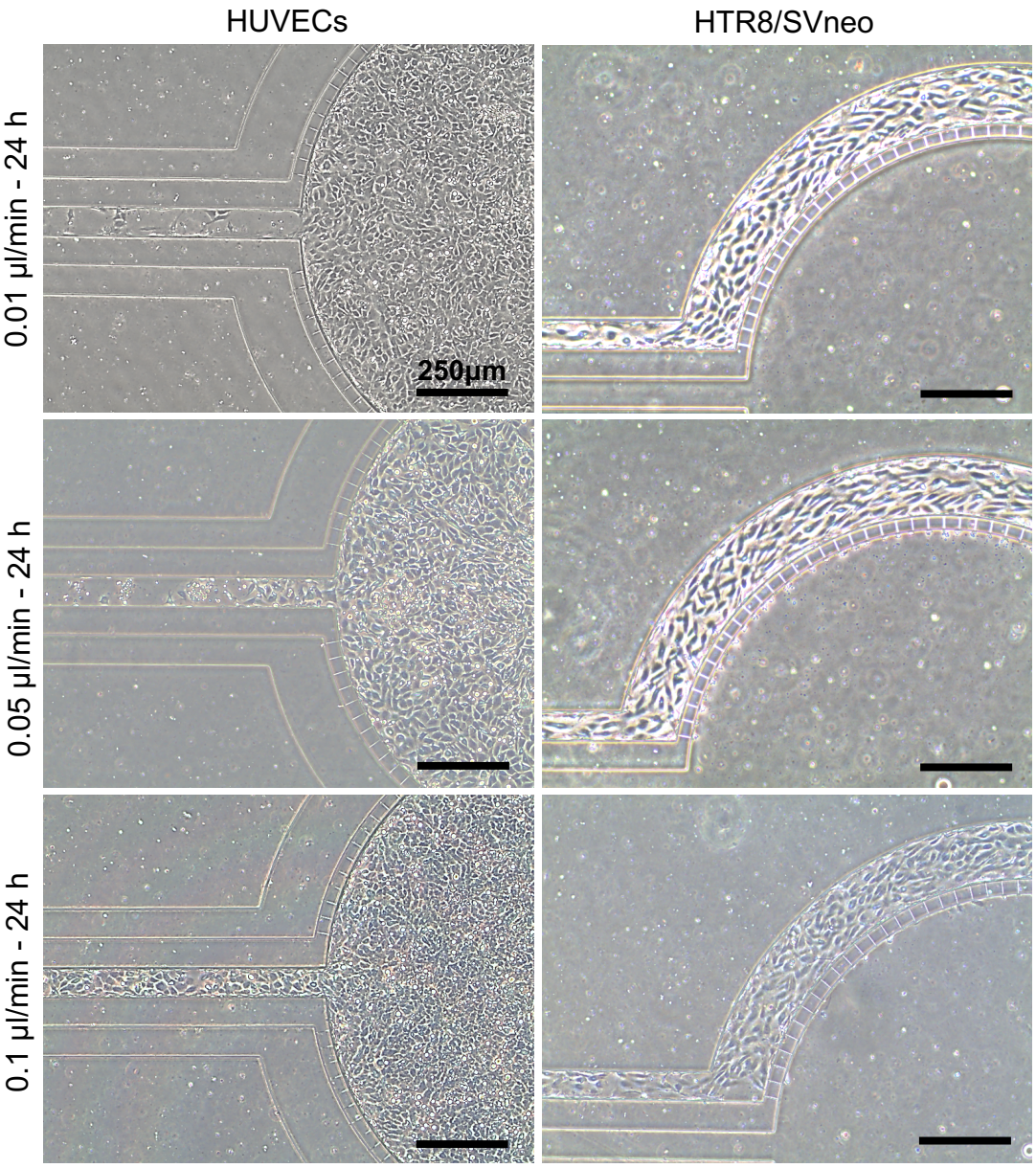
Supplemental Figure 2. Fluorescence tagged HTR8/Svneo cells and HUVECs cells after 3 days of culture in the microfluidic chip (culture conditions: fibronectin, 30 million cells/ml, and 0.01 µl/min flow speed). (**A**) mCherry tagged HTR8/SVneo cells (red), (**B**) GFP tagged HUVECs cells (green), (**C**) merged. (**D**) Z-stack image of cell-cell interaction between GFP tagged HUVECs and mCherry tagged HTR8/SVneo cells.

Supplemental Figure 3



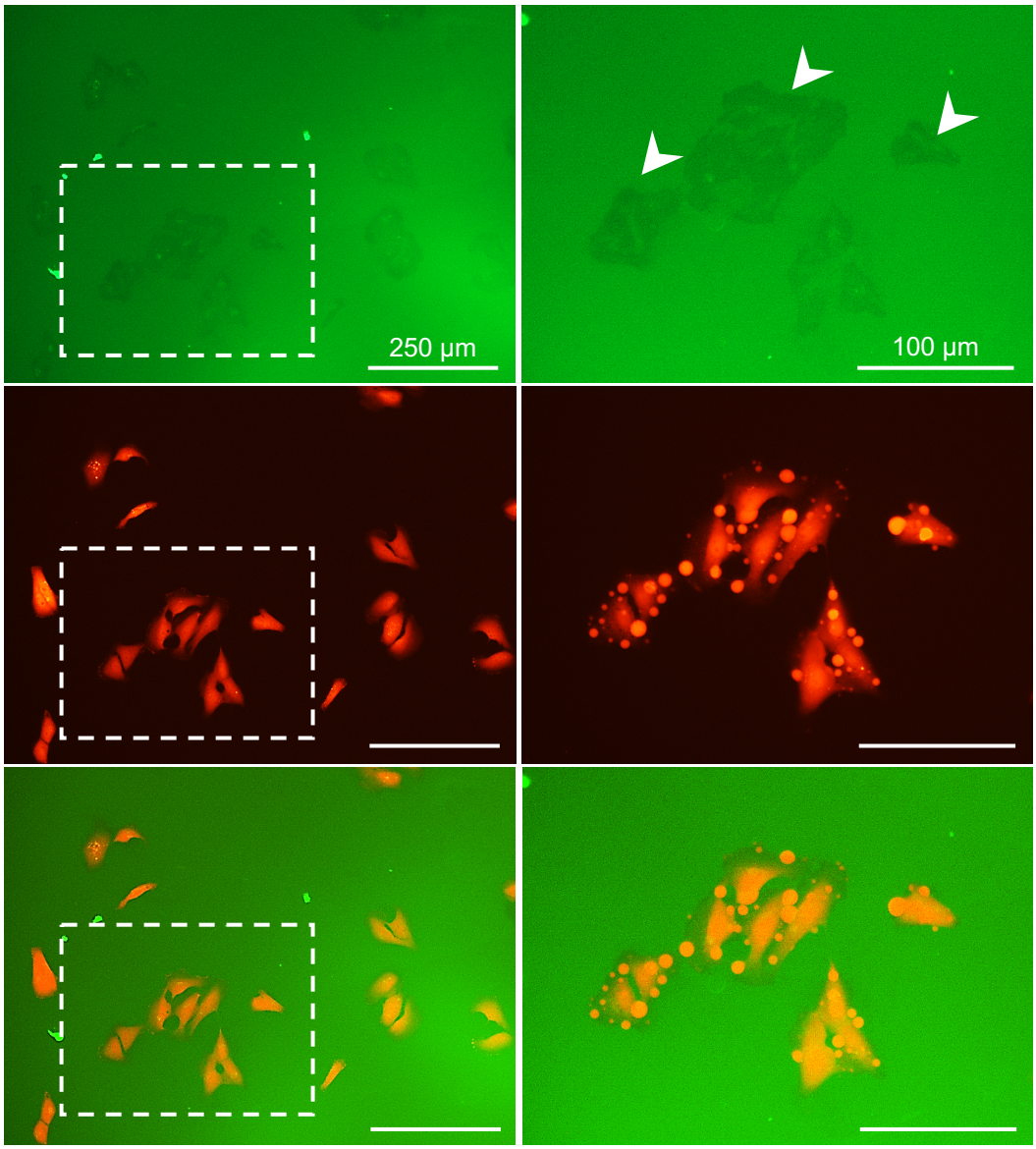
Supplemental Figure 3. Testing cell seeding density. HUVECs (*left panel*) and HTR8/SVneo (*right panel*) cells were seeded into the central compartment and outer channels, respectively, in cell densities of 1, 2, or 3 x 10^7 cells/ml.

Supplemental Figure 4



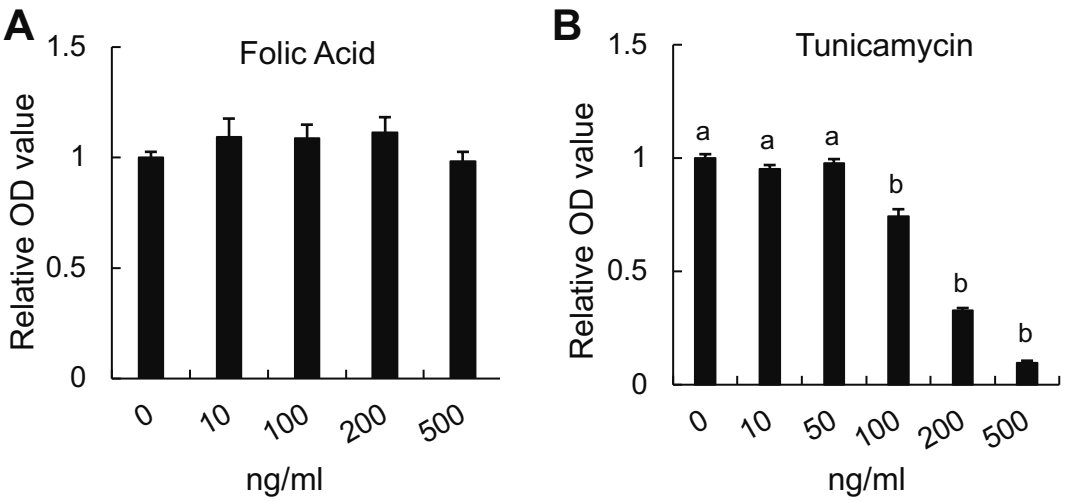
Supplemental Figure 4. Testing media flow speeds. HUVECs (*left panel*) and HTR8/SVneo cells (*right panel*) were seeded (density of 3×10^7 cells/ml) into the central compartment and outer channels, respectively. After overnight adhesion, cells were flushed with medium for 24 h at 0.01, 0.05, or 0.1 µl/min flow speeds.

Supplemental Figure 5



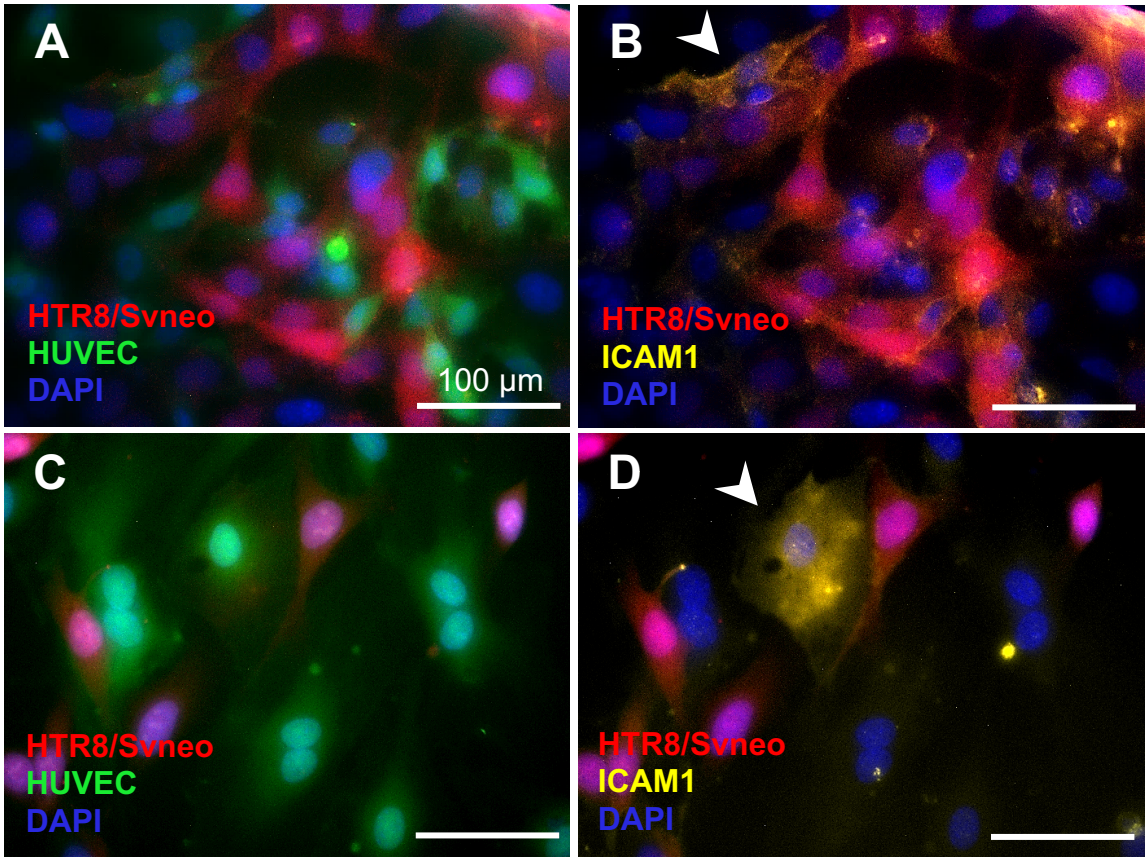
Supplemental Figure 5. Extracellular matrix degradation by HTR8/SVneo cells. 96-well plates were precoated with gelatin-FITC (60 µl, 1 mg/ml) overnight at 4 ºC. Thereafter, plates were pre-warmed (37 ºC and 5% CO_2) for 30 min and seeded with HTR8/Svneo-mCherry cells. Fluorescence micrographs show degradation of the gelatin-FITC layer after 24 h incubation (dark areas, *arrows*). Right panels are higher magnification indents (*white stripped lines*) of left panel images.

Supplemental Figure 6



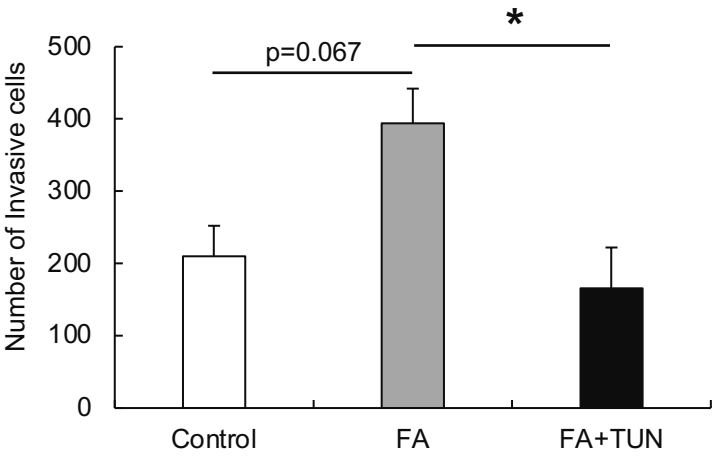
Supplemental Figure 6. Cytotoxicity effect of folic acid (0, 10, 100, 200 and 500 ng/ml) (**A**) and tunicamycin (0, 10, 50, 100, 200 and 500 ng/ml) (**B**) on HTR8/SVneo cells was assessed with MTT assay and quantified by optical density (OD) value after 5 days of exposure. Different letters denote differences between the control and each of the treatment groups ($a \neq b$ represent $P < 0.05$).

Supplemental Figure 7



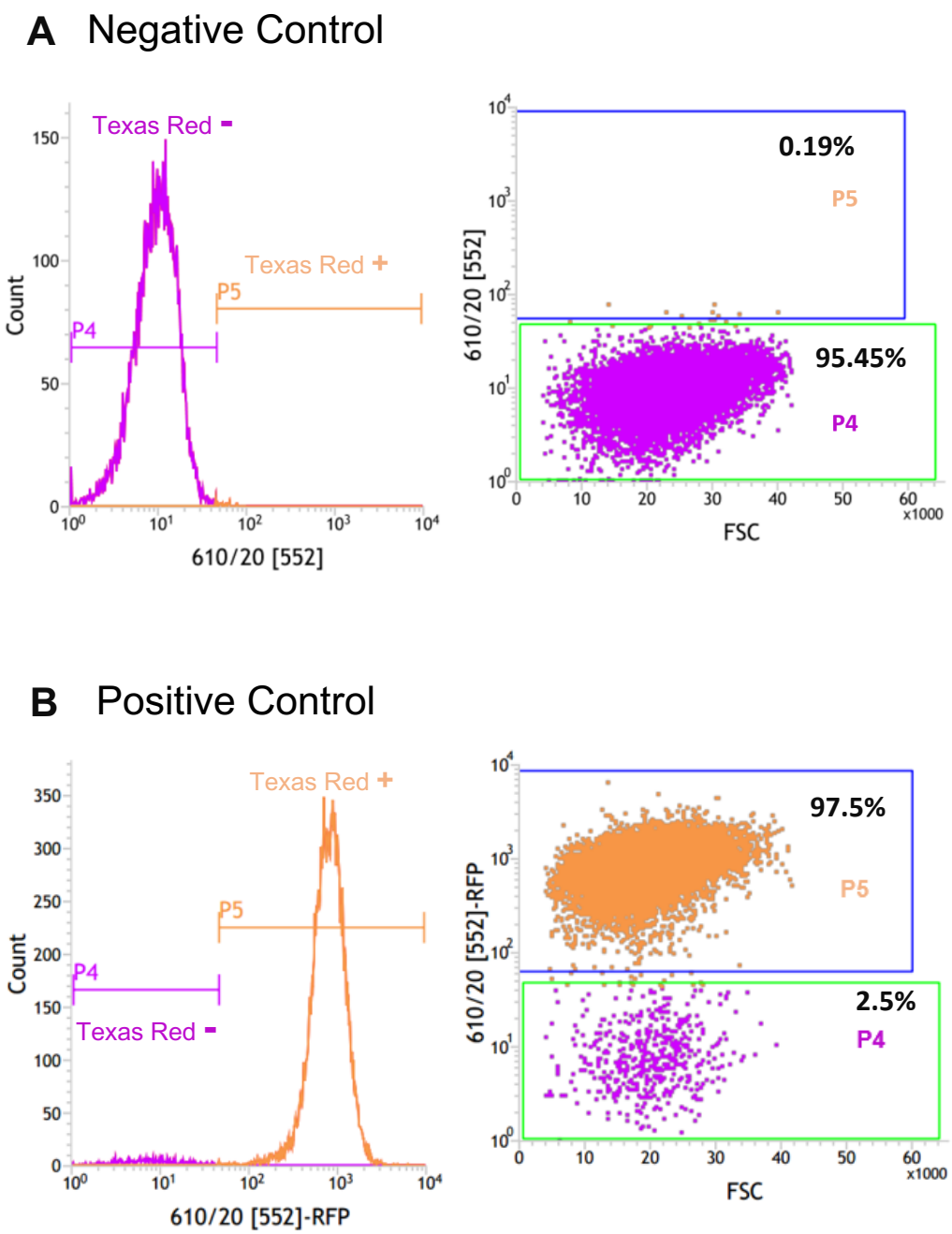
Supplemental Figure 7. Cell-to-cell interaction between GFP tagged HUVECs and mCherry tagged HTR8/SVneo cells. ICAM1 expression is enriched (*white arrows*) in HUVEC cells upon interaction with HTR8/SVneo cells after 72 h and 24 h incubation in the 3D microfluidic chip (A, B) and cell culture dish (C, D).

Supplemental Figure 8



Supplemental Figure 8. Quantification of invasive cells into the central compartment of the microfluidic chip after 72 h exposure to folic acid (FA) or folic acid + tunicamycin; FA + TUN). Asterisks denote differences among treatments ($P < 0.05$).

Supplemental Figure 9



Supplement Figure 9. Flow cytometry analysis. Wild type (WT) and mCherry tagged HTR8/SVneo cells were analyzed by flow cytometry to optimize the parameters for cell sorting. **A.** Negative control: WT HTR8/SVneo cells. **B.** Positive control: mCherry tagged HTR8/SVneo cells.