Supporting Information

A Smart 3D Microfluidic Tumor Spheroid-Vessel Co-Culture Model for Studying Exosomal HSP-Mediated Tumor Invasion and Angiogenesis

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Table of Content

Fig. S1 Chip design schematics

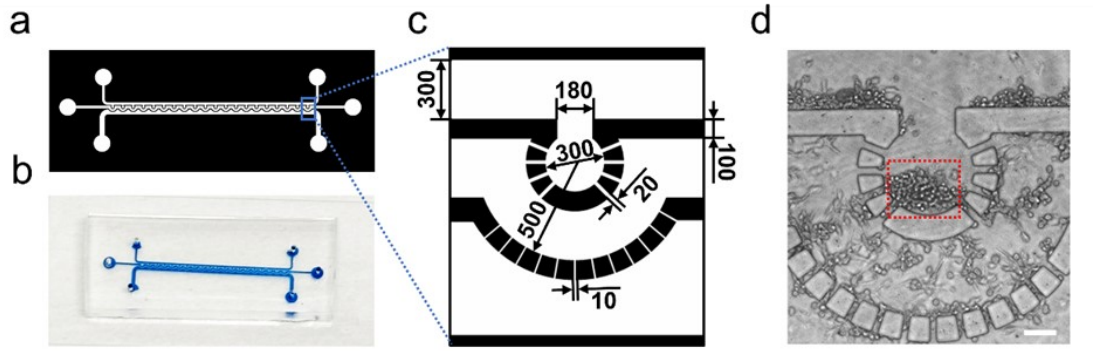

Fig. S1 Chip design schematics. (a) Photomask design of the chip: black regions are opaque while white areas are transparent. (b) Photograph of the fabricated chip. (c) Magnified view of the photomask: opaque black regions and transparent white regions are shown with dimensional units in μm. (d) Micrograph of the chip after cell loading: tumor spheroids are contained within the red square, scale bar: 100 μm.

Fig. S2 Schematic diagram of the on-chip cell loading process

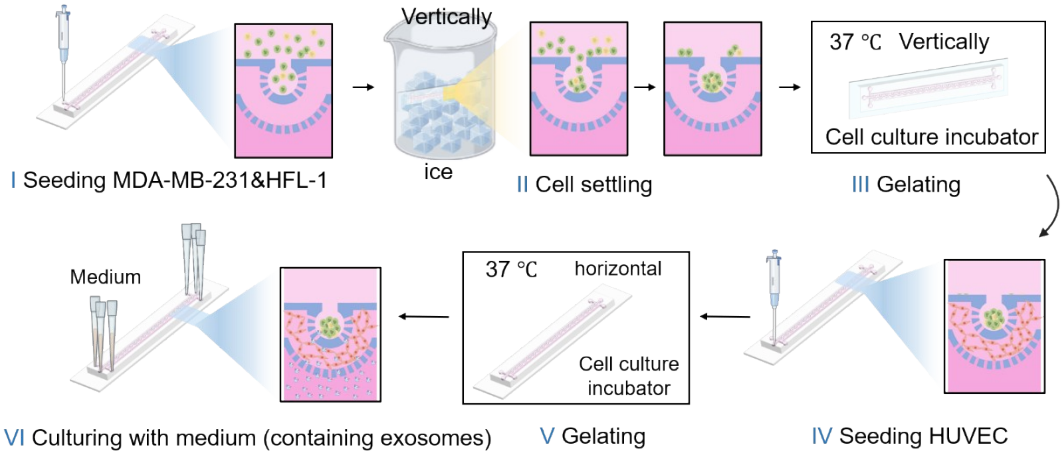

Fig. S2 Schematic diagram of the on-chip cell loading process.

Fig. S3 Photograph of the medium loaded in the microfluidic chip

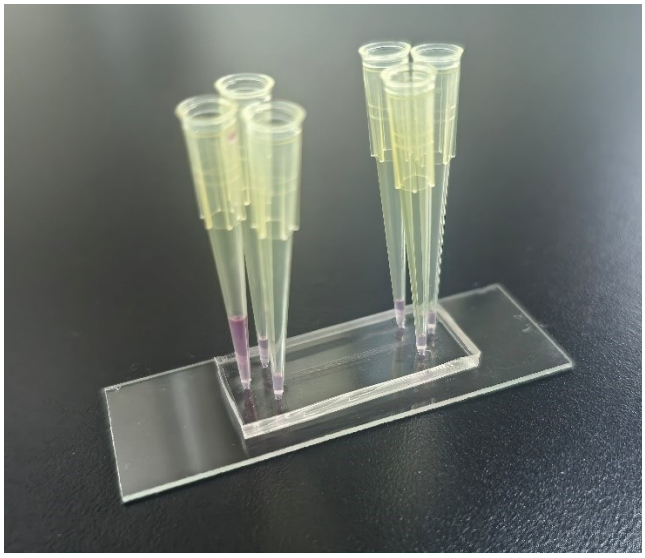

Fig. S3 Photograph of the medium loaded in the microfluidic chip.

Fig. S4 Optimization of the width of channels that physically connect the tumor spheroid and endothelial compartments

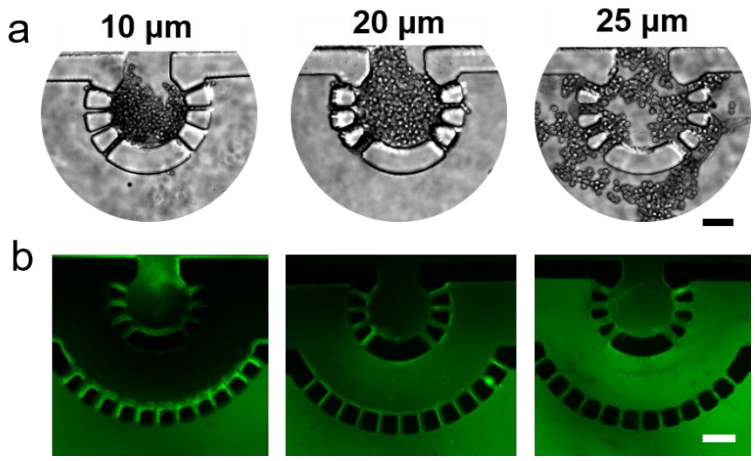

Fig. S4 Optimization of the width of channels that physically connect the tumor spheroid and endothelial compartments. (a) The distribution of cells within channels of different width (scale bar: 100 μm)**.** (b) The situation of molecular diffusion 20 minutes after being introduced into channels (scale bar: 150 μm).

Fig. S5 Spatial position of tumor spheroids within different microwells

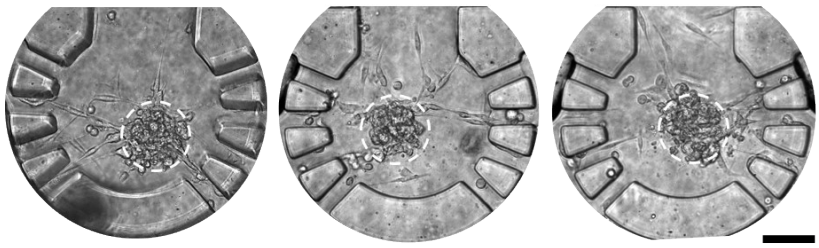

Fig. S5 Spatial position of tumor spheroids within different microwells (scale bar: 100 μm).

Fig. S6 On-chip cell density optimization

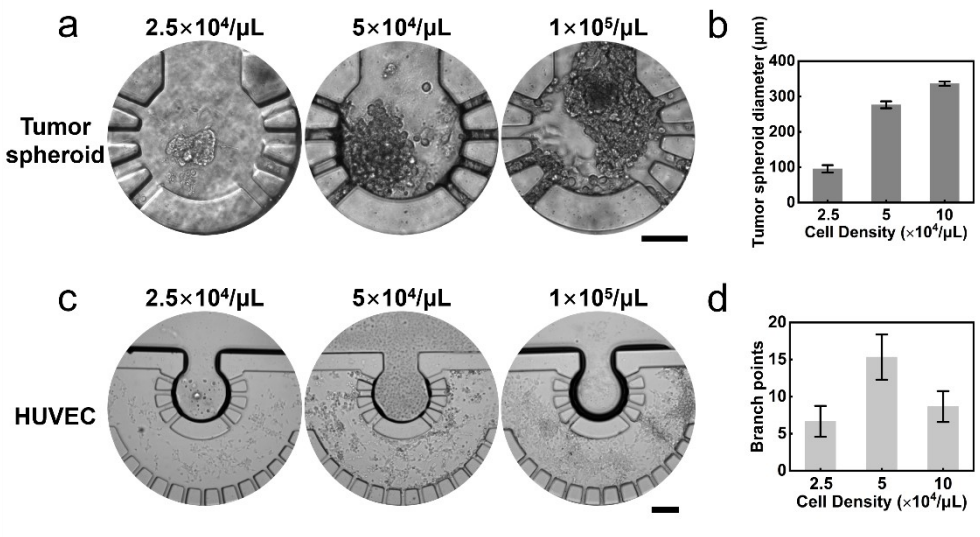

Fig. S6 On-chip cell density optimization. For tumor spheroids: (a) Bright-field image (scale bar: 100 μm). (b) Histogram of spheroid diameters under different cell densities (error bars represent triplicate experiments). For HUVECs: (c) Bright-field image (scale bar: 150 μm). (d) ImageJ-quantified branch points of microvascular networks across densities. Error bars represent triplicate experiments.

Fig. S7 Tumor spheres grown on the chip for different periods of time

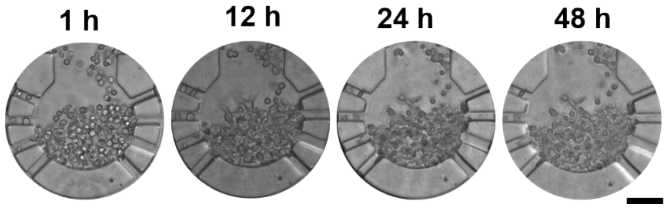

Fig. S7 Tumor spheres grown on the chip for different periods of time (scale bar: 100 μm).

Fig. S8 Viability assessment of on-chip tumor spheroids

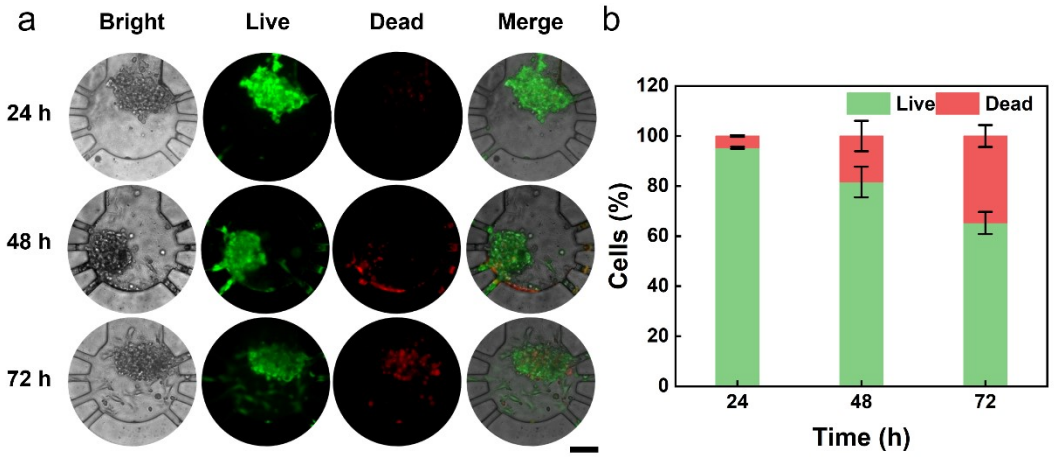

Fig. S8 Viability assessment of on-Chip tumor spheroids. (a) Microscopic images after 24 h, 48 h, and 72 h culture (green: live cells; red: dead cells. Scale bar: 100 μm). (b) Histogram of cell viability rates. Error bars represent triplicate experiments.

Fig. S9 Viability assessment of on-chip HUVECs

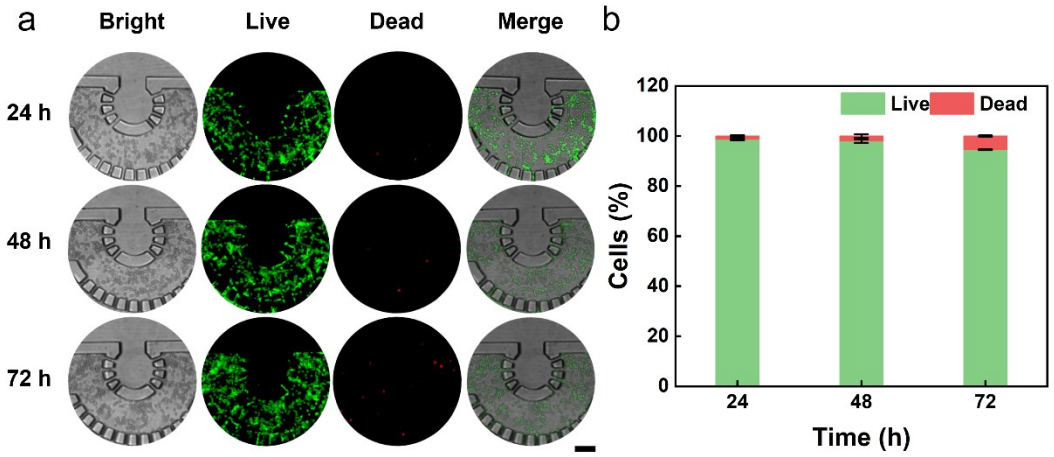

Fig. S9 Viability assessment of on-chip HUVECs. (a) Microscopic images after 24 h, 48 h, and 72 h culture (green: live cells; red: dead cells. Scale bar: 150 μm). (b) Histogram of cell viability rates. Error bars represent triplicate experiments.

Fig. S10 Effects of MDA-MB-231-derived exosomes on tumor invasion and angiogenesis under different incubation times

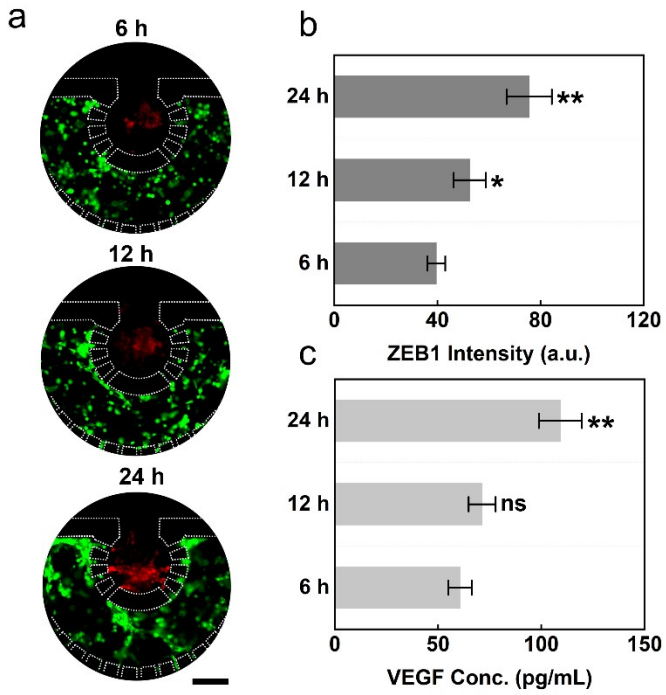

Fig. S10 Effects of MDA-MB-231-derived exosomes on tumor invasion and angiogenesis under different incubation times. (a) Immunofluorescence images of on-chip co-culture with exosomes (10^{12} particles/mL with 10 μM gw4869) under different incubation times. Red: ZEB1 (tumor invasion marker), Green: CD31 (vascular endothelial marker). Scale bar: 150 μm. (b) Tumor invasion assessment: Quantitative analysis of ZEB1 fluorescence intensity. Error bars represent standard deviations from triplicate experiments. (c) Angiogenesis evaluation: VEGF concentration measured by ELISA. Error bars indicate standard deviations from triplicate experiments. Statistical significance (two-tailed t-test): In (b) and (c), *p values compare 12 h and 24 h groups vs. 6 h group: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ns = not significant.

Fig. S11 Construction of Exosome$^{HSP\ del}$

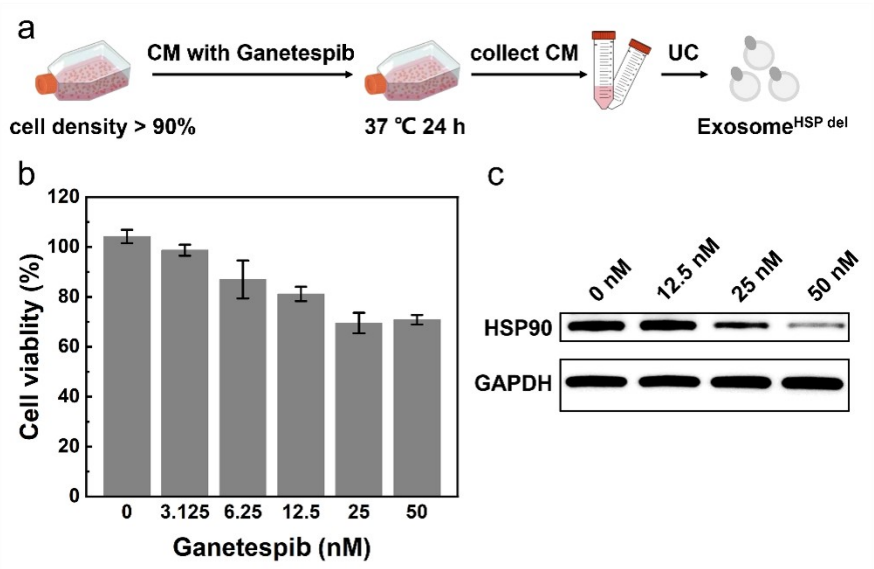

Fig. S11 Construction of Exosome$^{HSP\ del}$. (a) Schematic diagram of Exosome$^{HSP\ del}$ construction. CM: Conditioned medium; UC: Ultracentrifugation. (b) Cell viability of MDA-MB-231 cells after 24-hour incubation with different concentrations of Ganetespib at 37 °C. Error bars represent standard deviations from three independent experiments. (c) Western blot analysis of HSP90 protein expression in MDA-MB-231 cells treated with varying concentrations of Ganetespib.

Fig. S12 The variation trend of Absorbance versus HSP concentration.

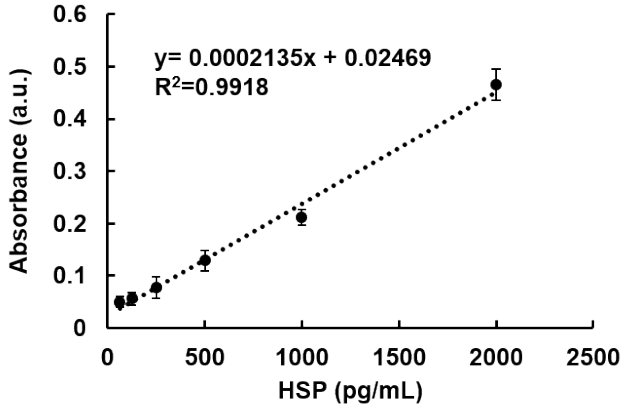

Fig. S12 The variation trend of Absorbance versus HSP concentration. The linear correlations inset can be described as $Absorbance = 0.0002135\ C_{HSP\ (pg/mL)} + 0.02469$. Error bars were derived from three parallel experiments.

Fig. S13 Effects of HSP90 concentration on tumor invasion and angiogenesis

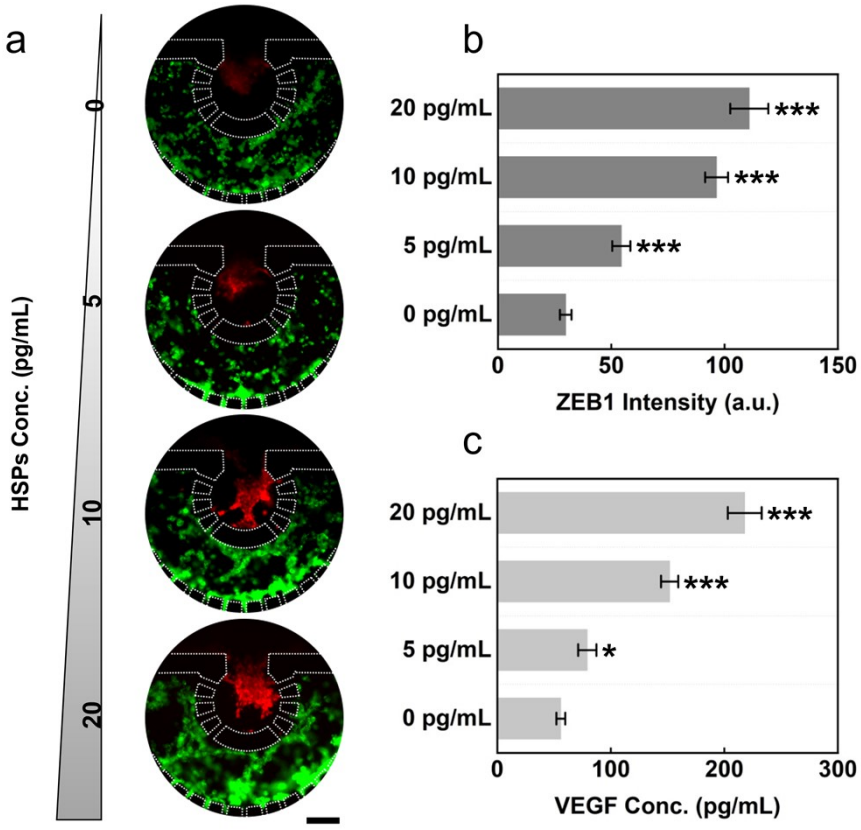

Fig. S13 Effects of HSP90 concentration on tumor invasion and angiogenesis. (a) Immunofluorescence images after 24-hour on-chip co-culture with different concentrations of HSP90. Red: ZEB1 (tumor invasion marker), Green: CD31 (vascular endothelial marker). Scale bar: 150 μm. (b) Tumor invasion assessment: Quantitative analysis of ZEB1 fluorescence intensity. Error bars represent standard deviations from triplicate experiments. (c) Angiogenesis evaluation: VEGF concentration measured by ELISA. Error bars indicate standard deviations from triplicate experiments. Statistical significance (two-tailed t-test): In (b) and (c), *p values compare 5 pg/mL, 10 pg/mL and 20 pg/mL groups vs. 0 pg/mL group: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ns = not significant.