## **Supporting Information**

## Acoustofluidic Engineering of Functional Vessel-on-a-Chip

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## Supporting figures



Figure S1. Morphology of HUVEC and fibroblast when in flask and in gel.



Figure S2. Various HUVEC self-assembly behavior under different *in vitro* culture conditions. A) The patterned HUVECs assemble into the straight thin vessel tube under static culture condition. B) The randomdistributed HUVECs assemble into the thin random vessel network under static culture condition. C) Red fluorescent beads were loaded into the vessel tube.



Figure S3. A) The immunostaining of the patterned vessel tube with the flow stimulation. The firbroblast is labeled with the  $\alpha$ -smooth muscle actin (green). The vessel tube is labeled with CD31 (red). Scale bar: 50 microns. B) The coculture of neural cells and the patterned vessel tube. The neural cells is labeled with the C-fos (green). The vessel tube is labeled with CD31 (red). Scale bar: 60 microns



Figure S4. The diameter of vessel tube with/without flow stimulation. A) The presentative image of the vessel tube with the flow stimulation. B) The presentative image of the vessel tube without the flow stimulation. C) The data collection of the diameter of the vessel tube with/without the flow stimulation. Three individual samples of each condition were measured to determine the average value. Vessel shape was labeled with FITC- cytoskeleton. Statistical significance between groups is denoted as \*P < 0.05 and \*\*\*P < 0.001.



Figure S5. Various acoustofluidic patterning of  $10-\mu$ m-polysterene beads.