Supplementary Information

Intrinsic FGF2 and FGF5 promotes angiogenesis of human aortic endothelial cells in 3D microfluidic angiogenesis system

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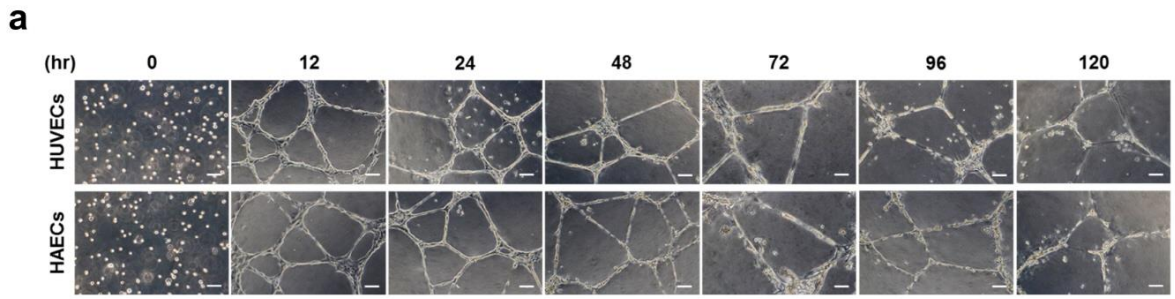
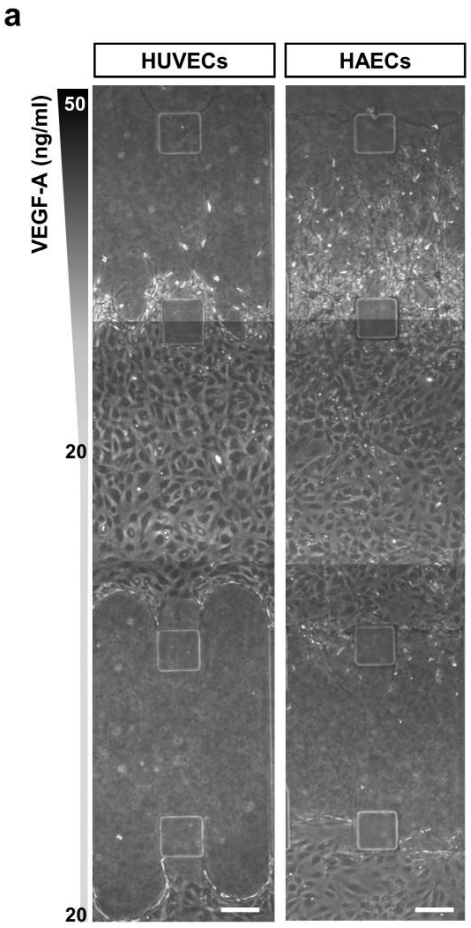
Supplementary Figure 1



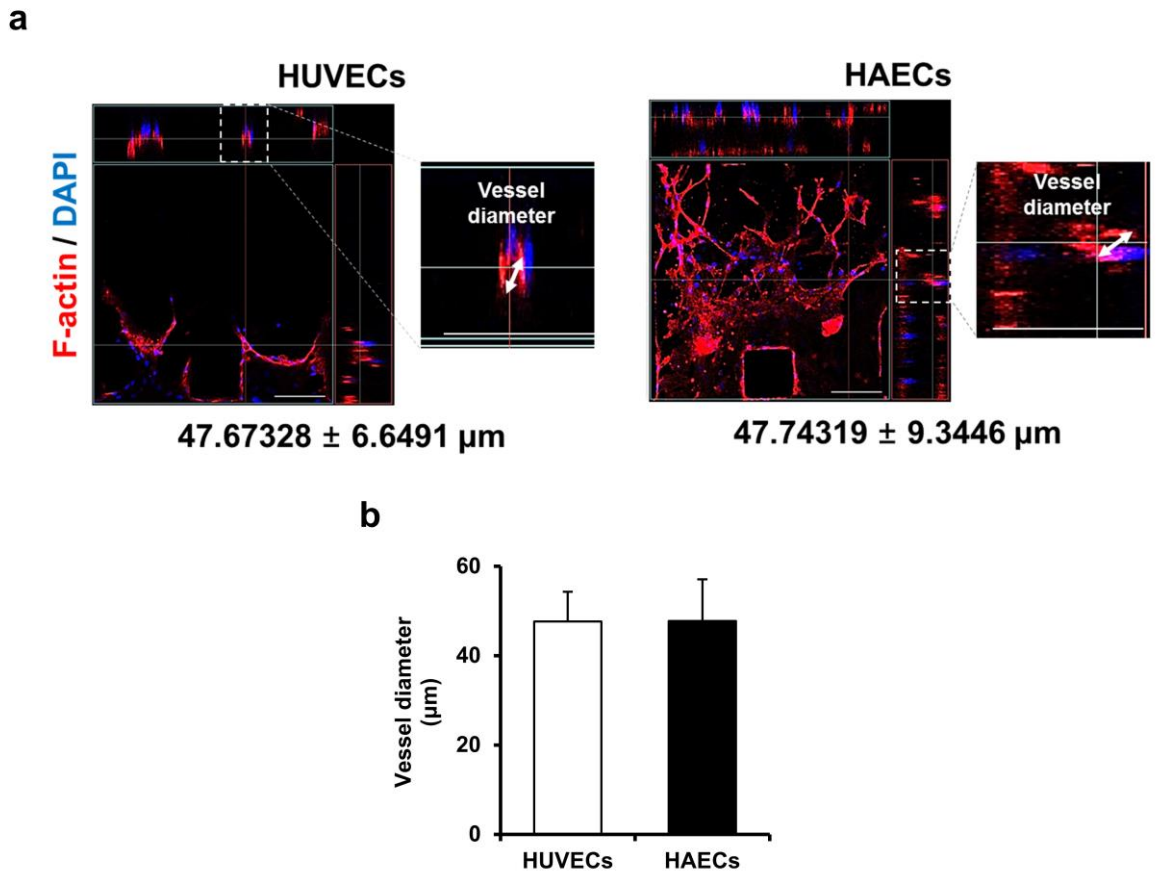
Supplementary Figure 1. *In vitro* Matrigel tube forming assay up to 120 hrs. (**a**) Phase contrast images obtained during network formation assay from 0–120 hrs between HUVECs and HAECs. Scale bar = 100 μm.

Supplementary Figure 2



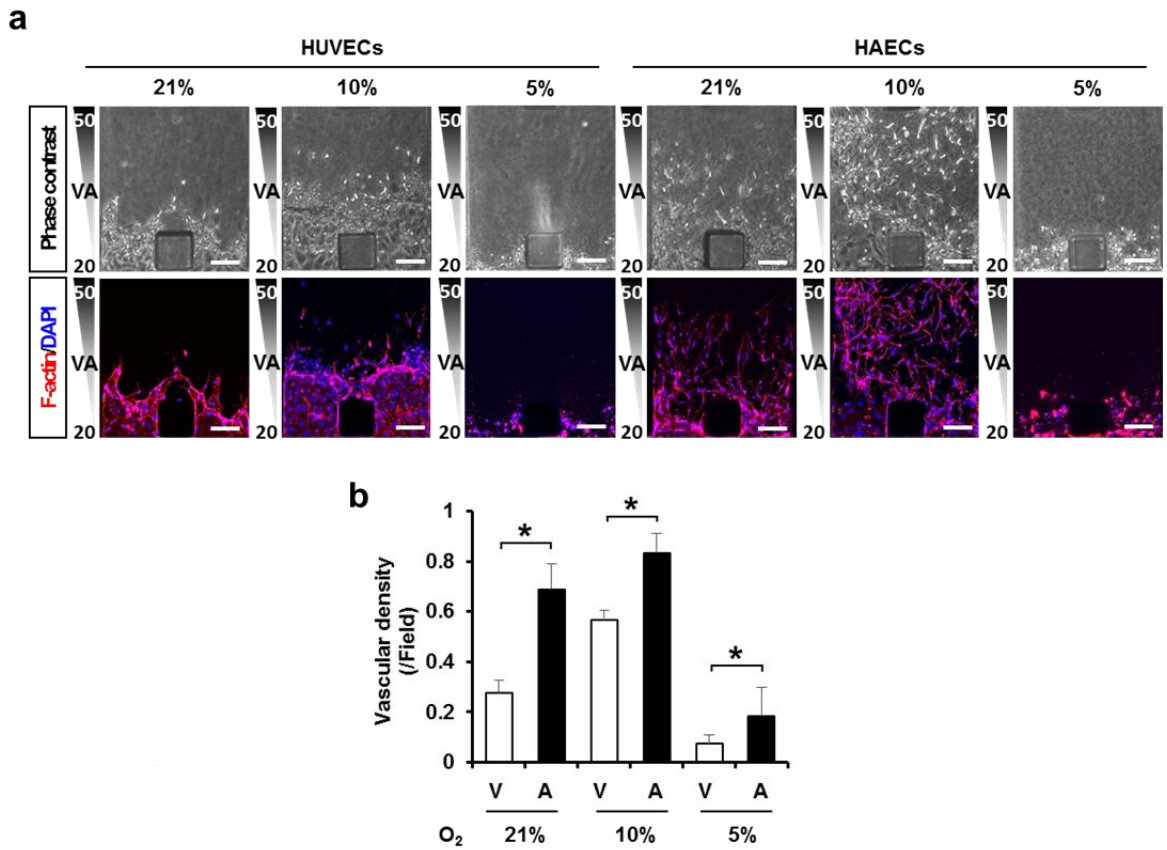
Supplementary Figure 2. Different sprouting patterns of HUVECs and HAECs in the 3D microfluidic angiogenesis system. (**a**) Phage contrast images of whole 3D microfluidic angiogenesis system channels under VEGF-A stimulation. Phase contrast image of the 3D microfluidic device culture stimulated with a VEGF-A gradient of HUVECs and HAECs. Scale bar = 150 μm.

Supplementary Figure 3



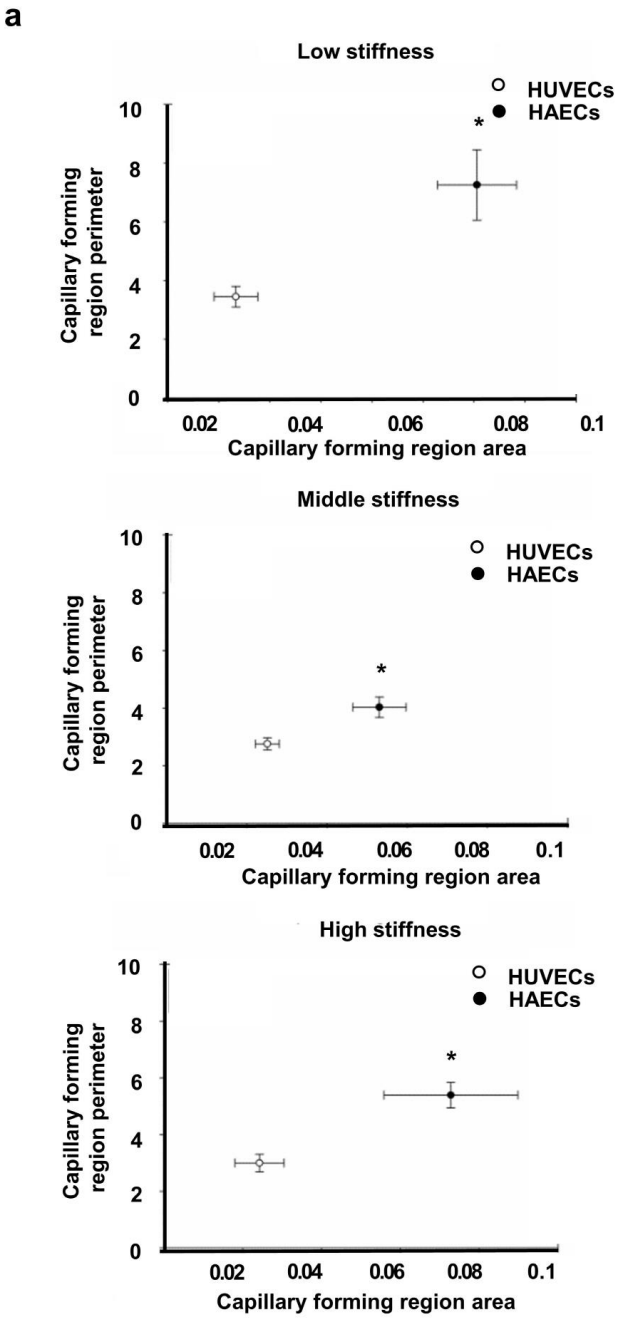
Supplementary Figure 3. Measurement of vessel diameter in HUVECs and HAECs. (**a**) Immunofluorescence staining with F-actin (red) and orthographic projection derived from a z-stack of HUVECs and HAECs. Nucleus were stained by DAPI (blue). Scale bar = 150 μm. (**b**) Quantification of vessel diameter between HUVECs and HAECs. *n* = 10.

Supplementary Figure 4



Supplementary Figure 4. Effect of hypoxic condition between HUVECs and HAECs in 3D microfluidic angiogenesis system. (**a**) Phase contrast image of HUVECs and HAECs in the 3D microfluidic angiogenesis system with different oxygen concentration (contained 21%, 10%, and 5% O2). Immunofluorescence staining using antibody followed by F-actin (red) and DAPI (blue). Scale bar = 150 μm. (**b**) Quantification of the rate of vascular density per field in different oxygen concentration between HUVECs and HAECs. 'V' was indicated HUVECs and 'A' was indicated HAECs. $n = 6$. $^{*}p < 0.05$ versus HUVECs group.

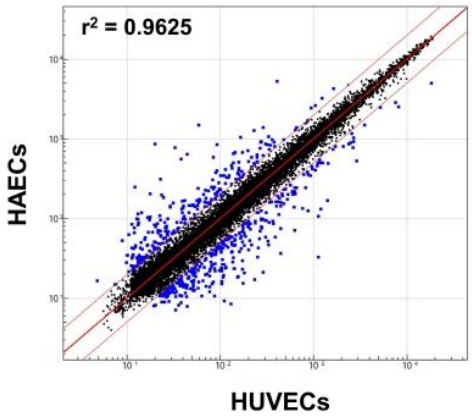
Supplementary Figure 5



Supplementary Figure 5. Differences in sprouting pattern and other parameters between HUVECs and HAECs according to collagen gel stiffness. (**a**) Comparison of the perimeter and area of the capillary-forming region in HUVECs and HAECs. $n = 4$. $^{*}p < 0.05$ *versus* HUVECs group.

Supplementary Figure 6

a



b

Gene	NCBI Accession Number	Fold Change (log 2)
GBX2	NM_001485.2	1.85508307
FGF2	NM_002006.3	1.83315735
FGF5	NM_004464.3	1.66399121
COL8A1	NM_020351.2	1.42960853
CXCL10	NM_001565.1	1.28796940
HMOX1	NM_002133.1	1.12995061
ID1	NM_181353.1	1.07554140
TSPAN12	NM_012338.3	-1.01980168
PLAU	NM_002658.2	-1.06941479
CCL2	NM_002982.3	-1.33719416
PTGS2	NM_000963.1	-1.58895890
APOLD1	NM_030817.1	-2.73906490
ANGPT2	NM_001118888.1	-2.78391438
HOXA5	NM_019102.2	-3.01344519

Supplementary Figure 6. mRNA expression scatter plot between HUVECs and HAECs. (**a**) mRNA expression correlation between HUVECs and HAECs. (**b**) List of typical angiogenesis-related factors sorted according to the "GO-term" document profile.

Supplementary Figure 7

a

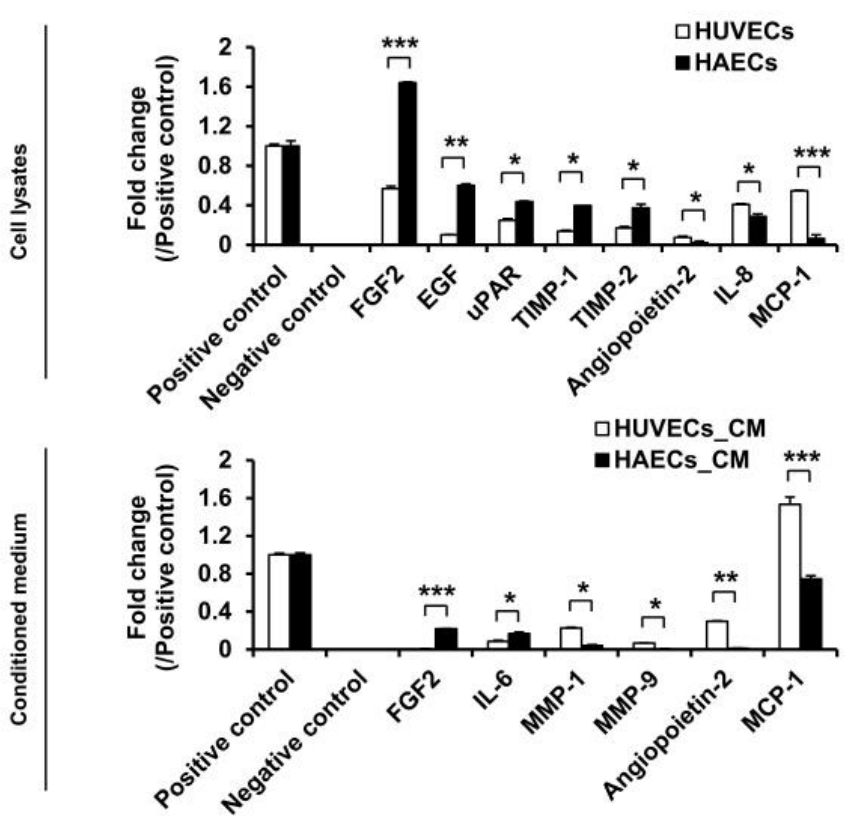
Array 1

POS	POS	NEG	NEG	Angiogenin	EGF	ENA-78	FGF2
POS	POS	NEG	NEG	Angiogenin	EGF	ENA-78	FGF2
GRO	IFN-γ	IGF-1	IL-6	IL-8	LEPTIN	MCP-1	PDGF-BB
GRO	IFN-γ	IGF-1	IL-6	IL-8	LEPTIN	MCP-1	PDGF-BB
PIGF	RANTES	TGF-β1	TIMP-1	TIMP-2	Thrombopoietin	VEGF	VEGF-D
PIGF	RANTES	TGF-β1	TIMP-1	TIMP-2	Thrombopoietin	VEGF	VEGF-D
						NEG	POS
						NEG	POS

Array 2

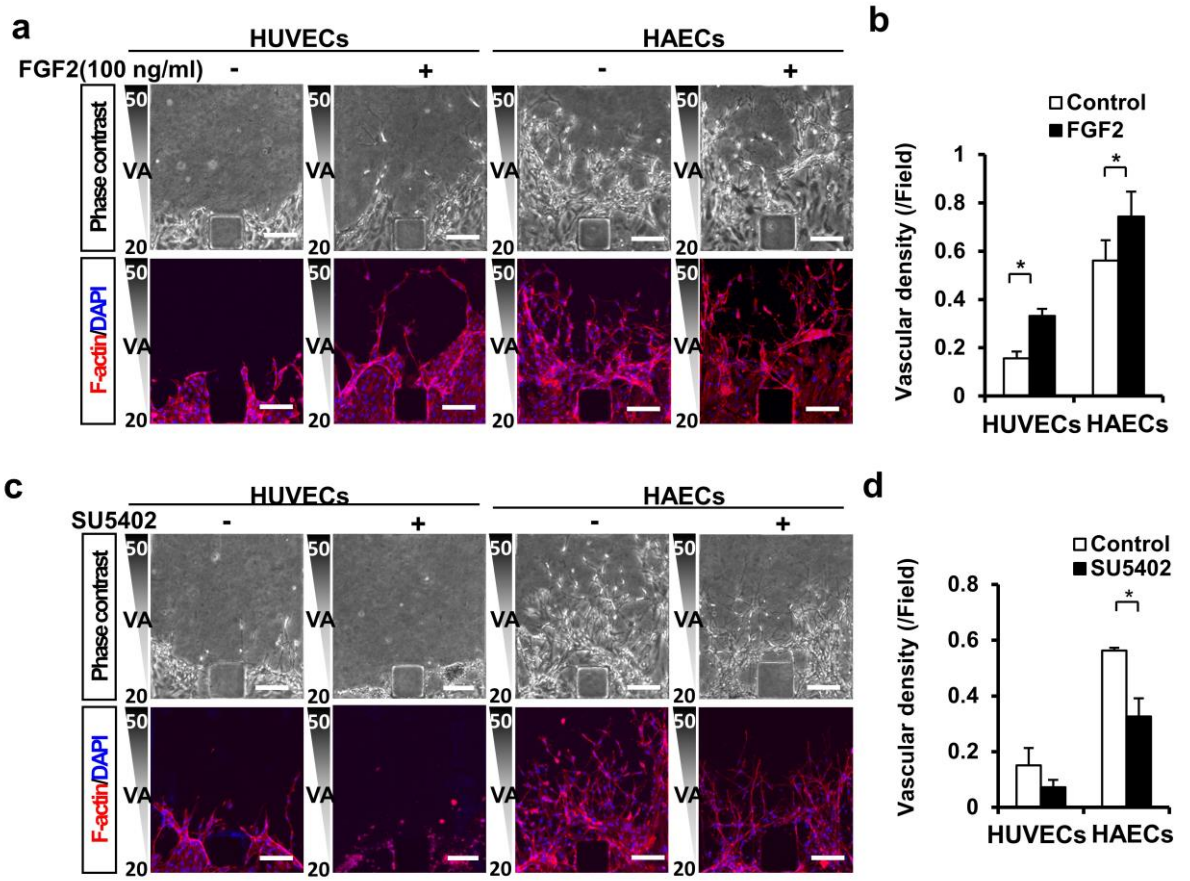
POS	POS	NEG	NEG	Angiopoietin-1	Angiopoietin-2	Angiostatin	Endostatin
POS	POS	NEG	NEG	Angiopoietin-1	Angiopoietin-2	Angiostatin	Endostatin
G-CSF	GM-CSF	I-309	IL-10	IL-1α	IL-1β	IL-2	IL-4
G-CSF	GM-CSF	I-309	IL-10	IL-1α	IL-1β	IL-2	IL-4
I-TAC	MCP-3	MCP-4	MMP-1	MMP-9	PECAM-1	Tie-2	TNF-α
I-TAC	MCP-3	MCP-4	MMP-1	MMP-9	PECAM-1	Tie-2	TNF-α
uPAR	VEGFR2	VEGFR3					POS
uPAR	VEGFR2	VEGFR3					POS

b



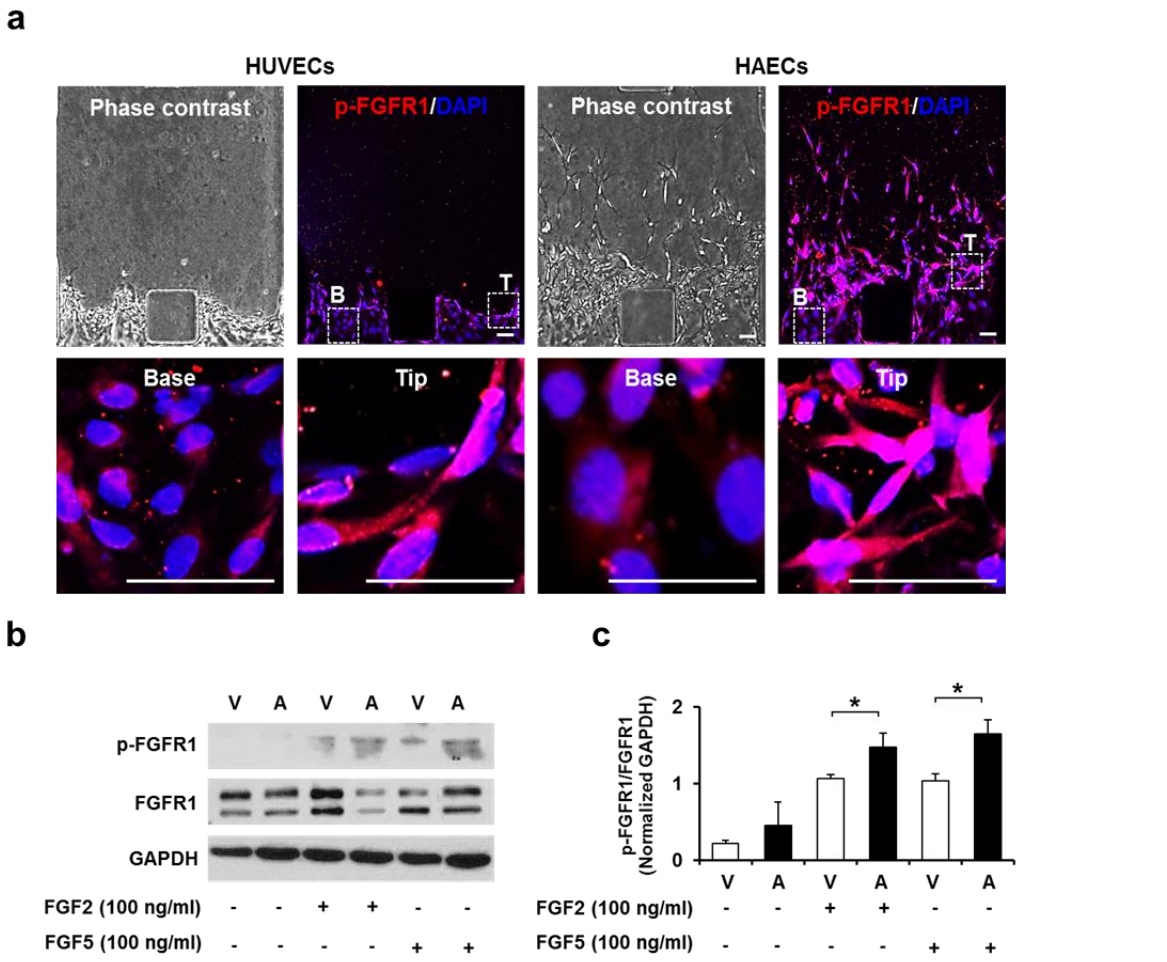
Supplementary Figure 7. Quantification of various protein levels between HUVEC or HAEC lysates and conditioned media. (**a**) Template showing the location of cytokines and growth factors on an angiogenesis cytokine array membrane. (**b**) Quantification of relative protein production levels in HUVECs and HAECs. $n = 3$. $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ *versus* HUVECs or the HUVECs_CM group.

Supplementary Figure 8



Supplementary Figure 8. FGF2 is a crucial factor for angiogenesis via VEGF-A stimulation. (**a**) Phase contrast image of HUVECs and HAECs in the 3D microfluidic angiogenesis system with the addition of FGF2 (100 ng/ml). Immunofluorescence staining using antibody followed by F-actin (red) and DAPI (blue) staining. Scale bar = 150 μm. (**b**) Quantification the rate of vascular density per field between HUVECs and HAECs. $n = 6$. (**c**) Phase contrast image of HUVECs and HAECs in the 3D microfluidic angiogenesis system with addition of the FGFR inhibitor SU5402 (10 nM). Immunofluorescence staining using antibody followed by F-actin (red) and DAPI (blue) staining. Scale bar = 150 μm. (**d**) Quantification the rate of vascular density per field between HUVECs and HAECs with the addition of SU5402. $n = 6$. $^{*}p < 0.05$ *versus* Control group.

Supplementary Figure 9



Supplementary Figure 9. HAECs have more FGFR1 sensitivity than HUVECs. (**a**) Phase contrast and immunofluorescence image of HUVECs and HAECs in the 3D microfluidic angiogenesis system. Immunofluorescence staining using antibody followed by p-FGFR1 (red) and DAPI (blue). Scale bar = 50 μm. 'B' indicates base part and 'T' indicates tip part of sprouted vessel. (**b**) HUVECs and HAECs were stimulated for 30 min with basal medium (lane 1 and 2), basal medium containing 100 ng/ml FGF2 (lane 3 and 4) or FGF5 (lane 5 and 6). Total FGFR1 and GADPH were evaluated as a loading control. 'V' indicates HUVECs and 'A' indicates HAECs. (**c**) Activation of FGFR1 was evaluated by quantification of western blot intensity and blotting for p-FGFR1. $n = 3$. $^{*}p < 0.05$ versus HUVECs group.

Supplementary Table 1. List of primers and product sizes

Gene name	Forward sequence (5'-3')	Reverse sequence (5'-3')	Product (bp)
GAPDH	ACCACCATGGAGAAGGC	GGCATGGACTGTGGTCATGA	119
FGF1	TGAGAAGAAGACACCAAGTGGA	TTGTGGCGCTTTCAAGACTA	110
FGF2	AGCGGCTGTACTGCAAAAAC	GCTTGAAGTTGTAGCTTGATGTG	109
FGF3	CTACTGCGCCACGAAGTACC	TCCACTGCCGTTATCTCCA	102
FGF4	CGGCTCTACTGCAACGTG	GCCGAAGATGCTCACCAC	138
FGF5	ACTGGCCAATTTTTGAAATAAGAT	CTGAGACTTTCAAATAGGGCAGA	96
FGF6	GGACCCACGAGGAGAACC	TCACTCCAAAGAGACTCACCAC	78
FGFR1	CCACCTACTTCTCCGTCAATG	GGGTTTGGTTTGGTGTTATCTG	112
FGFR2	TCTGCGTTTGGAGTTGCTC	GCTGCTGCTGCAGTCACTT	124
FGFR3	TGGGTTTTCTCATCACTCTGC	CCACCAGGATGAACAGGAAG	134
FGFR4	ATTCCATCGGCCTCTCCTAC	TAGCAAAGTGGGAGACTTGGT	121
VEGFR2	ATGACATTTTGATCATGGAGC	CCCAGATGCCGTGCATGAG	193
CXCR4	CCTGCCTGGTATTGTCATCC	AGGATGACTGTGGTCTTGAGG	105
GBX2	AAAGAGGGCTCGCTGCTC	ATCGCTCTCCAGCGAGAA	144
COL8A1	CATCTCAAGAACAAAAGACAACTGA	TTGCTGGTGCCTTCCTGT	107
CXCL10	GAAAGCAGTTAGCAAGGAAAGGT	GACATATACTCCATGTAGGGAAGTGA	132
HMOX1	TGAACTCCCTGGAGATGACTC	AGCTCCTGCAACTCCTCAAA	103
ID1	GAATCATGAAAGTCGCCAGTG	ACAGACAGCGCACCACCT	111
TSPAN12	CAATGGCCAGAGAAGATTCC	CTGCCAACACACTGATGAC	93
PLAU	TTGCTCACCACAACGACATT	GGCAGGCAGATGGTCTGTAT	94
CCL2	AGTCTCTGCCGCCCTTCT	GTGACTGGGGCATTGATTG	93
PTGS2	CTTCACGCATCAGTTTTTCAAG	TCACCGTAAATATGATTTAAGTCCAC	96
APOLD1	CCAGGGGTACTCGGAAGG	AGCAGCAGTCCCTGGAAG	136
ANGPT2	TGCCACGGTGAATAATTCAG	TTCTTCTTTAGCAACAGTGGG	123
HOXA5	GCGCAAGCTGCACATAAG	CGGTTGAAGTGGAACTCCTT	114