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

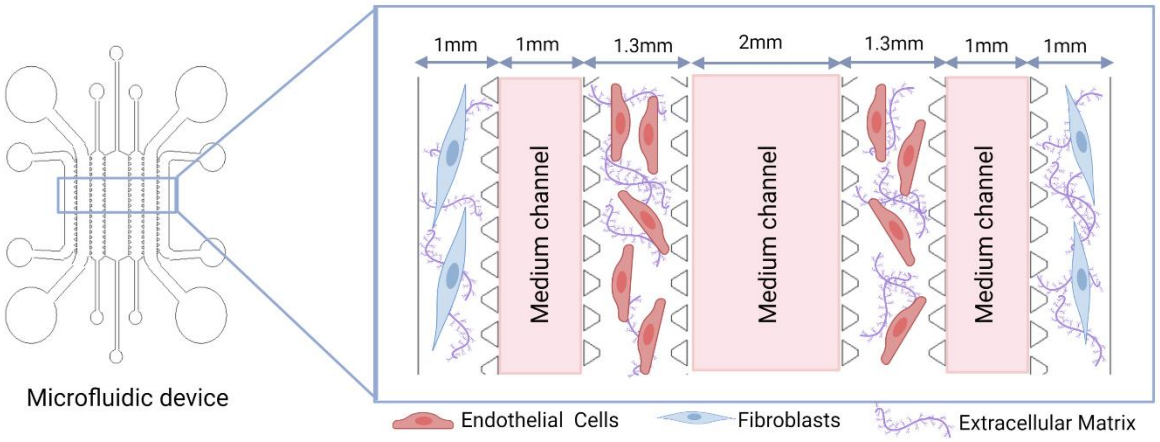

Fig. S1. Schematic showing the details of the microfluidic device.
The microfluidic device is designed to have seven channels. All the channels are partitioned by microposts to separate different cultures and prevent the leakage of the gels to other channels while allowing medium to flow through. An enlarged schematic gives a better view of the microposts and the dimensions of the channels. Channel height: 120 - 150µm. Pictures shown here are not scaled. (Created with BioRender.com.)

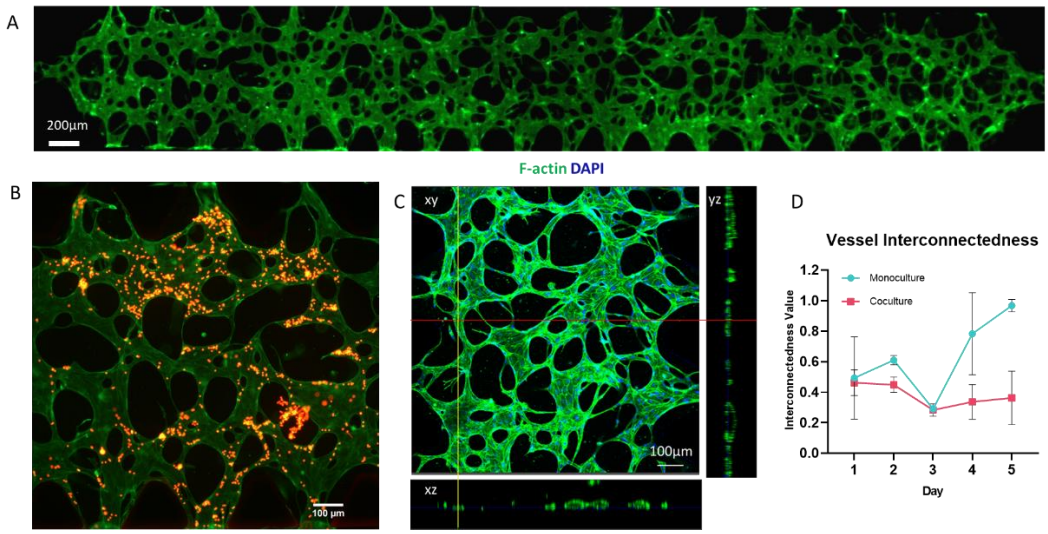

Fig. S2. Formation of 3D perfusable vascular network by coculturing with fibroblasts.
A. Stitching of images showing the vascular network spanning across the channel, scale bar =200um.

B. Perfusion of 5um fluorescent beads through the vascular network, scale bar = 100um.

C. Fluorescent images displaying the immunostaining of the vasculature formed. 2D fluorescent images projected from 3D z-stack showing the vascular network and hollow lumens formation at day 4; z-stack: 180µm, scale bar = 100µm.

D. Line graph tracking the interconnectedness of vascular network monocultured vs cocultured with FB from day 1 to day 5. Interconnectedness value defined as the number of vessel endpoints divided by the number of vessel junctions.

A

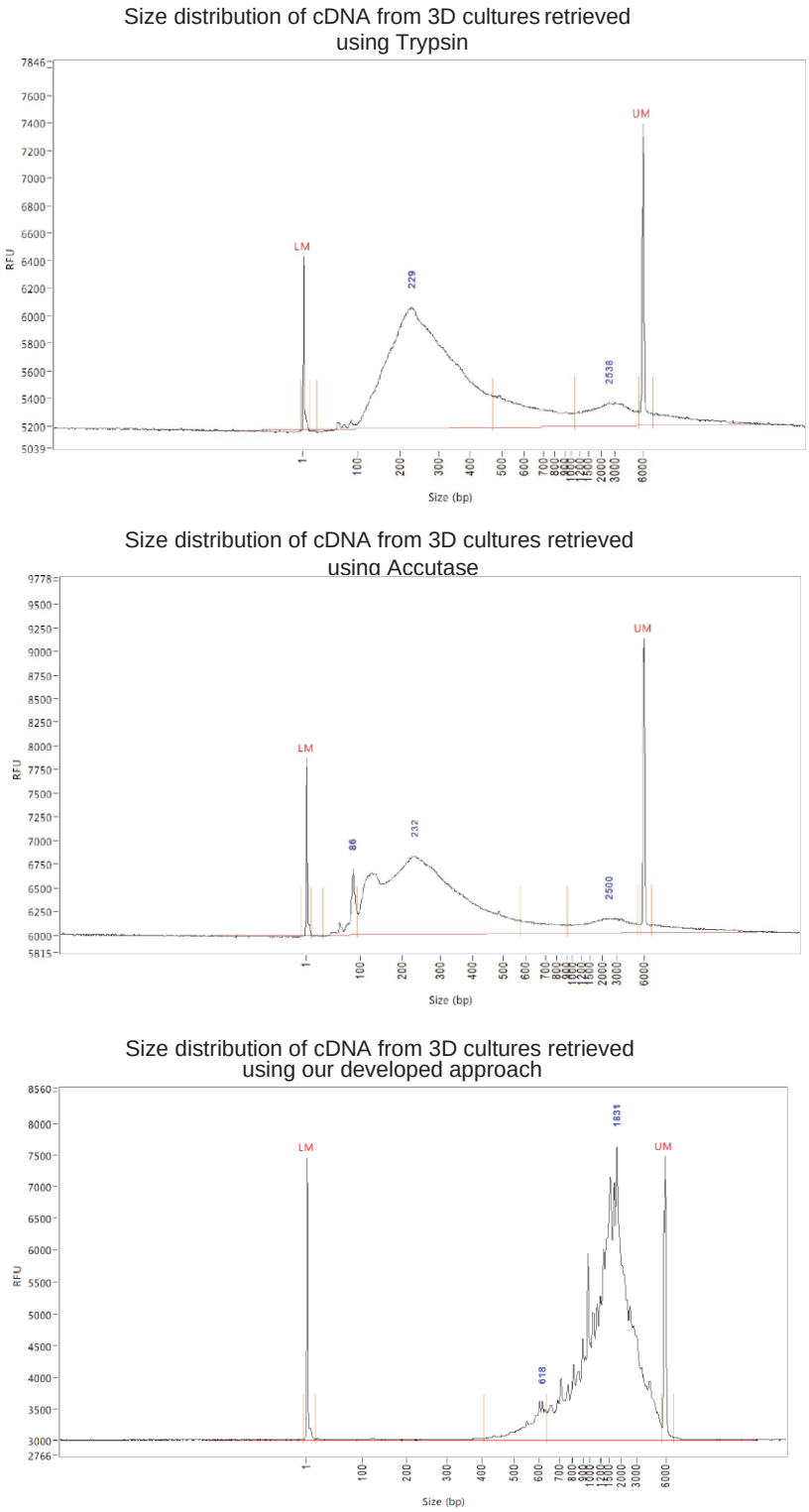

Fig. S3. Size distribution of cDNA from different enzymatic dissociation methods.
A. Representative example of the cDNA size distribution of 3D cultures processed using Trypsin, Accutase, and our developed workflow respectively.

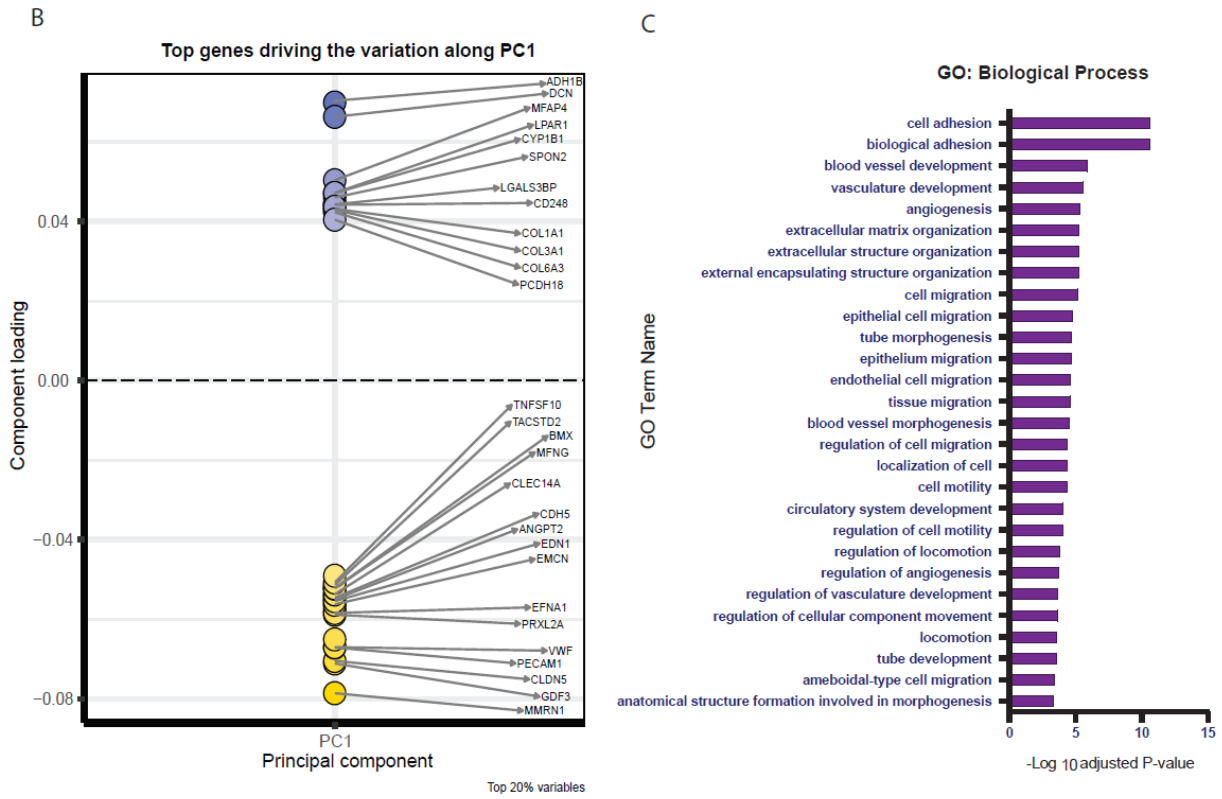

Fig. S3. Top gene loadings shows genes in PC1 and PC2.

B. Representative component loading plot displaying the top genes driving the variation along PC1 that separate EC and FB as two different cell types. The blue dots corresponds to genes related to FB identity while yellow dots corresponds to genes related to EC identity.

C. GO biological process analysis shows pathways related to the biological function of EC and FB.

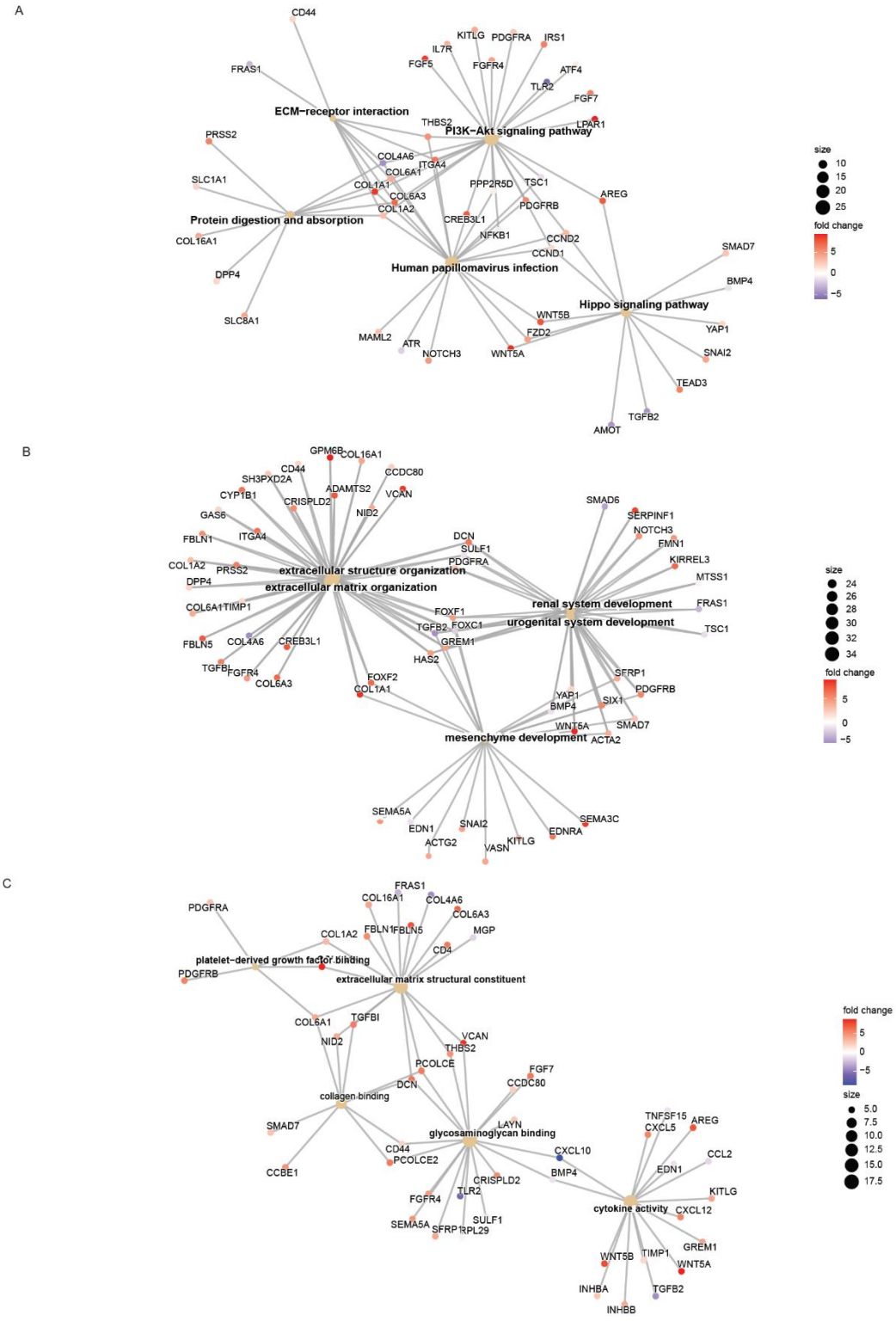

Fig. S4. Network plot shows the DEGs involved in A. KEGG pathway; B. GO biological processes and C. GO molecular functions, respectively.

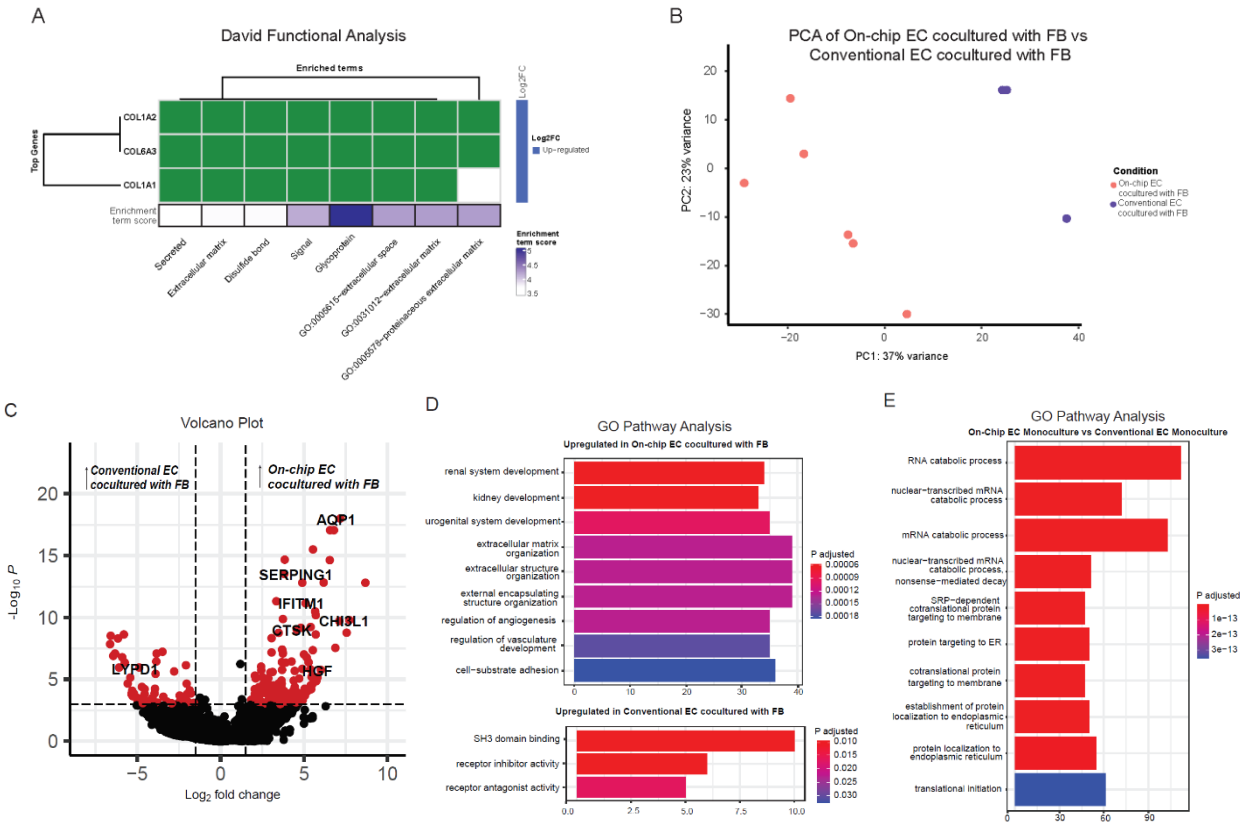

Fig. S5. Differentially expressed genes identified for conventional juxtacrine ECs cultures.
A. Clustering of DAVID gene enrichment reveals only 3 out of the 75 top DEGs from conventional cocultured ECs vs conventional monocultured ECs are consisted of secreated factors and are located at the extracellular space.

B. Principal component analysis (PCA) shows the data generated from conventional juxtacrine cocultured ECs versus on-chip cocultured ECs.

C. Volcano plot shows the DEGs in conventional juxtacrine cocultured ECs versus on-chip co-cultured ECs. Differential expression is defined by P-adjusted < 0.05 and abs(log2FC) >1.

D. GO pathway analysis shows the pathways related to upregulated DEGs in on-chip cocultured ECs and in conventional cocultured ECs, respectively.

E. GO pathway analysis demonstrates the pathways related to DEGs in on-chip monocultured ECs versus conventional monocultured ECs.

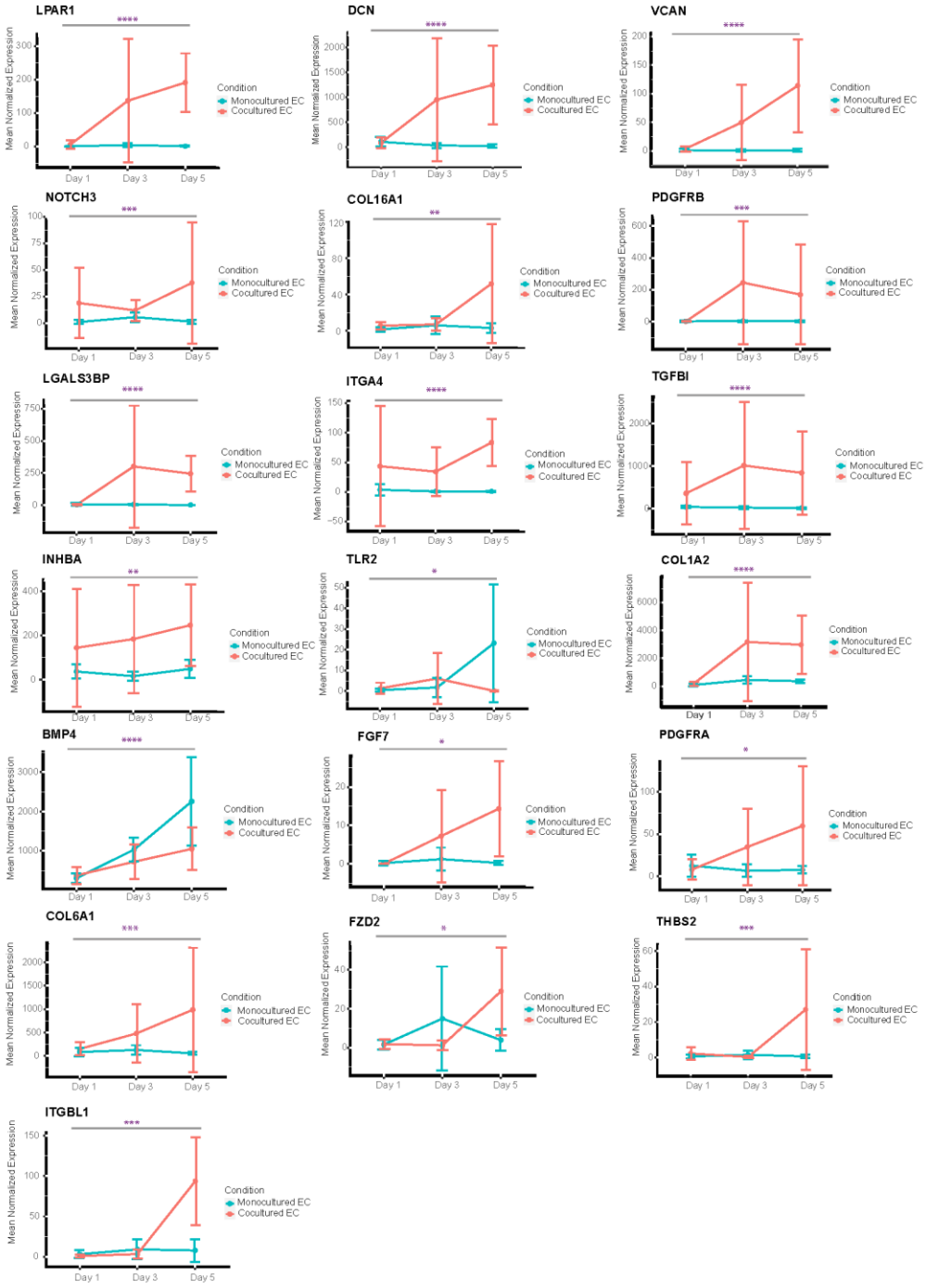

Fig. S6. Temporal gene expression patterns of EC genes encoding receptor or ligands. Line plots showing the trend of DEGs (P-adjusted <0.05 in temporal analysis) encoding ligands of ECs in both cocultured and monocultured condition. Likelihood ratio test are performed for all the graphs. *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001 across all condition.

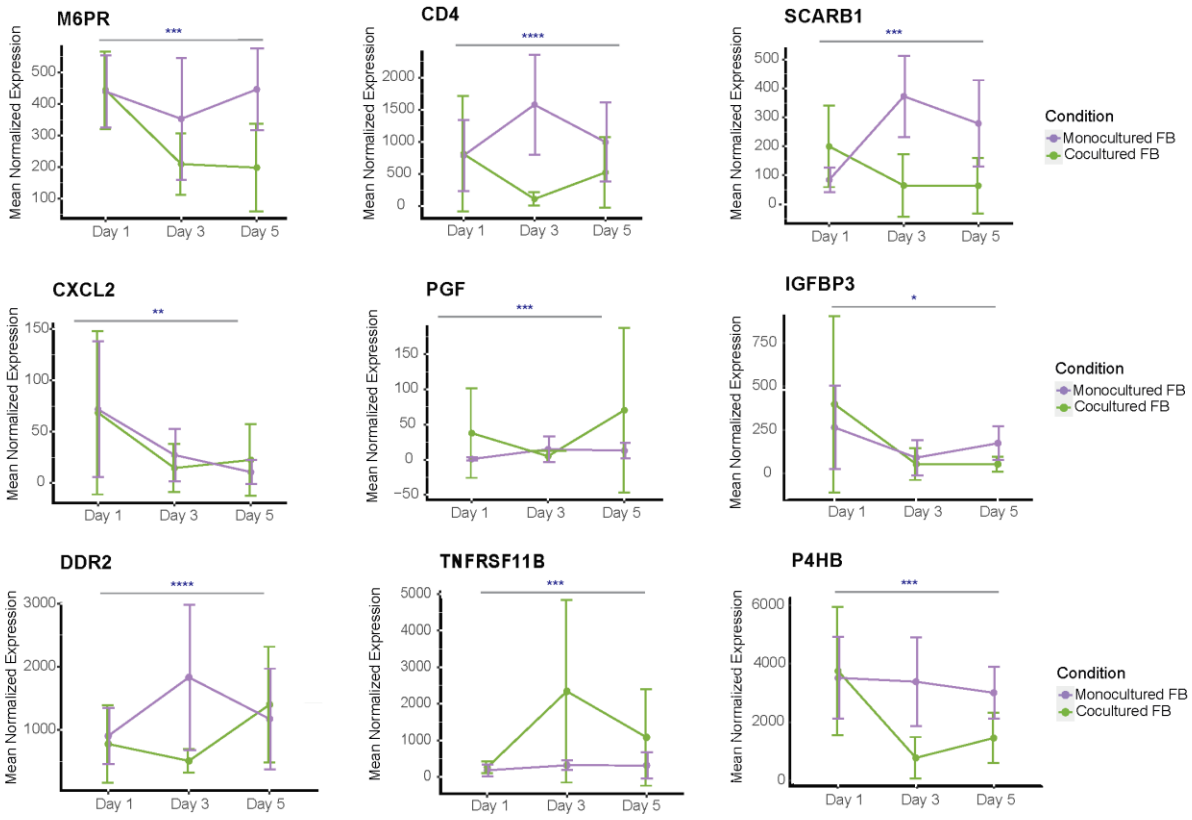

Fig. S7. Temporal gene expression patterns of FB genes encoding receptor or ligands. Line plots showing the trend of DEGs (P-adjusted <0.05 in temporal analysis) encoding ligands of FBs in both cocultured and monocultured condition. Likelihood ratio test are performed for all the graphs. *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001 across all condition.

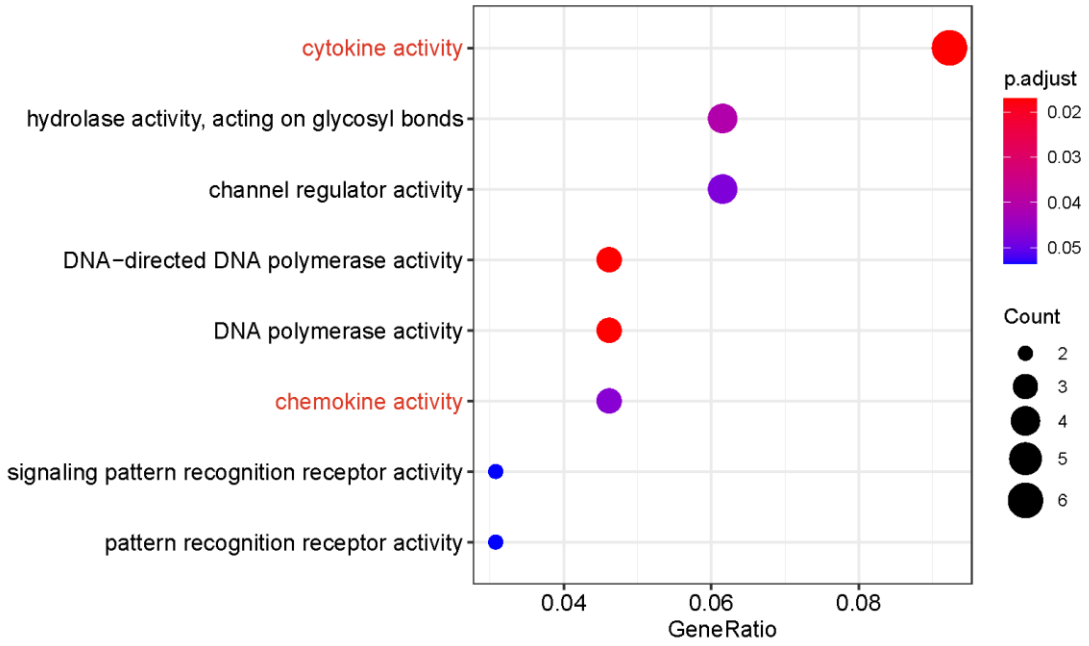

Fig. S8. GO analysis of cluster 4 genes showing transcriptomic changes for coculture versus monoculture cells across three time points.

The group of genes with higher expression at both day 1 and day 3 are shown to be associated with cytokines and chemokines related pathways (highlighted in red).

Table S1. A full list of genes found to be genes to be differentially expressed between the monocultured and cocultured ECs (P adjusted <0.05, log2 fold change >1).

Gene Symbol	Log2FoldChange	P-adjusted
LGALS3BP	7.94	4E-26
LPAR1	9.21	1E-25
CD248	10.73	2E-24
PTGER2	10.44	1E-23
ITGA4	6.31	9E-23
SPON2	10.06	8E-20
FBLN5	7.37	9E-18
SEMA3C	8.25	1E-15
COL6A3	6.79	6E-15
COL1A1	8.61	2E-14
PSG4	24.20	2E-13
AREG	7.35	3E-12
WNT5B	7.57	4E-12
SERPINF1	8.11	8E-12
TGFBI	5.71	1E-11
HOXB-AS3	6.97	1E-11
PCOLCE	5.79	3E-11
GPM6B	8.96	3E-11
DCN	6.09	1E-10
ADH1B	7.44	1E-09
NT5E	1.75	2E-09
FGF5	8.45	6E-09
SIK3	-2.78	1E-08
USP9Y	5.51	1E-08
CREB3L1	7.36	1E-08
SEMA5A	4.89	2E-08
MYH10	-1.02	2E-08
ACTA2	3.72	2E-08
AKR1C1	5.26	6E-08
PLEK2	1.73	6E-08
MT1X	2.11	8E-08
TRPA1	8.75	8E-08
WNT5A	8.81	2E-07
KCNMA1	4.08	2E-07
VWCE	-7.80	3E-07
DNAJC6	-7.45	4E-07

BICC1	8.11	4E-07
COL1A2	3.09	5E-07
CRISPLD2	5.24	7E-07
WDFY3-AS2	-4.55	8E-07
MTERF4	1.18	8E-07
ENGASE	-5.65	2E-06
AOX1	7.02	2E-06
PLAC9	3.55	3E-06
COL6A1	3.84	3E-06
CRYAB	4.74	5E-06
EIF1AY	5.02	5E-06
VCAN	8.30	5E-06
LIFR-AS1	-8.37	5E-06
HSPB6	4.63	6E-06
MT1E	1.95	6E-06
MARK1	-6.05	7E-06
PSAT1	4.26	7E-06
NID2	3.89	8E-06
TAGLN	3.70	8E-06
ITGBL1	4.69	1E-05
TBX2-AS1	7.64	1E-05
MAP9	4.05	1E-05
CD4	6.00	1E-05
APCDD1	6.69	2E-05
WDR90	-4.86	2E-05
SERINC2	3.40	3E-05
PRSS2	5.80	4E-05
ADAMTS2	7.33	4E-05
GALNT5	6.55	4E-05
PHGDH	2.26	5E-05
AC123023.1	-6.87	5E-05
PDLIM1P4	-6.89	5E-05
MRGPRF	6.73	6E-05
KIF21B	-7.07	6E-05
IGFBP6	4.07	8E-05
CPA4	7.42	8E-05
AC244669.1	4.87	9E-05
FRMD4B	-1.15	9E-05
ATP1A1-AS1	-6.19	9E-05
FOXF2	6.71	1E-04
CCDC80	2.20	1E-04
Z97192.3	-7.10	1E-04

FGF7	5.88	1E-04
INHBA	2.59	1E-04
AC064836.3	-6.71	2E-04
RPS4Y1	3.99	2E-04
SLITRK2	4.04	2E-04
SNAI2	4.23	2E-04
ARHGAP11A	3.74	2E-04
FOXF1	4.41	2E-04
TRNP1	4.70	2E-04
YAP1	1.95	2E-04
PCAT6	-5.37	2E-04
ATF4	1.02	3E-04
TIGD5	-3.52	3E-04
UACA	1.37	3E-04
LMOD1	7.30	3E-04
MXRA8	8.57	3E-04
AC097103.2	-6.23	3E-04
TMEM231	1.60	4E-04
APPL2	1.12	4E-04
ELL2	1.72	4E-04
CHST6	-5.85	4E-04
SWAP70	-1.01	5E-04
CCND1	1.80	5E-04
EBF1	3.10	6E-04
MAPKAPK3	1.50	6E-04
TXLNGY	8.22	6E-04
EPHX4	-1.05	7E-04
NEK10	-4.17	7E-04
CARMN	4.53	7E-04
OPHN1	-2.94	7E-04
ZFP3	-7.37	7E-04
ANKRD7	-6.66	7E-04
PRRG4	-5.72	7E-04
NUP155	2.02	8E-04
AC016205.1	-4.44	8E-04
SOCS2	1.47	8E-04
PDGFRB	5.56	8E-04
LINC02407	1.68	8E-04
DDX3Y	6.26	8E-04
SPDL1	1.26	8E-04
LINC01270	-7.14	9E-04
MT1L	2.69	9E-04

CCDC157	-2.44	9E-04
DKK3	1.98	1E-03
AC024337.2	-6.96	1E-03
LMCD1	2.95	1E-03
AC009549.1	-7.63	1E-03
KIRREL3	6.66	1E-03
DNAJC3-AS1	-4.04	1E-03
GPR89A	1.04	1E-03
GPR89A	1.04	1E-03
KRT7	4.40	1E-03
NOTCH3	4.81	1E-03
LRRC42	1.86	1E-03
SNHG18	6.32	1E-03
FMN1	4.63	1E-03
KITLG	3.98	1E-03
AC010969.2	-3.18	1E-03
PTGFR	6.83	1E-03
IRS1	5.82	1E-03
CADM4	-6.24	2E-03
ZNF347	-4.83	2E-03
NBEA	-1.88	2E-03
ACTG2	5.59	2E-03
MGP	-1.64	2E-03
PAMR1	7.21	2E-03
QPRT	5.45	2E-03
QPRT	5.45	2E-03
MAP2K3	2.03	2E-03
CCDC9	-4.30	3E-03
COL16A1	4.06	3E-03
KSR2	-5.90	3E-03
GPC2	-7.21	3E-03
SPRY4	2.34	3E-03
ABCA9	-3.03	3E-03
PDLIM2	1.20	3E-03
LINC00960	5.83	3E-03
WDR91	3.11	3E-03
RHOD	1.28	3E-03
C4orf32	-1.41	3E-03
HS3ST3B1	5.13	3E-03
CCL2	-1.50	3E-03
AC058791.1	-3.89	3E-03
AC092747.4	4.92	3E-03

RBM38	-3.63	3E-03
PDGFRA	2.74	3E-03
FBLN1	5.17	4E-03
ABLIM3	2.10	4E-03
LAYN	2.35	4E-03
XPOT	2.35	4E-03
AP001528.3	5.42	4E-03
USP8	-1.19	4E-03
PRRX1	5.31	4E-03
SOCS7	-3.43	5E-03
HOXB6	1.64	5E-03
PCDH18	5.14	5E-03
MACROD2	-5.17	5E-03
KIAA1147	-2.26	5E-03
DGAT2	-4.72	5E-03
CCND2	3.29	5E-03
FER1L5	-5.28	5E-03
AC005154.1	-1.56	5E-03
CLEC2D	-2.84	5E-03
WFDC1	5.44	5E-03
DTX4	5.59	5E-03
LINC01154	-5.08	5E-03
SH2D4A	4.95	5E-03
UNC5B	6.95	5E-03
PCOLCE2	5.83	6E-03
FOXD2-AS1	-6.85	6E-03
CPM	3.81	6E-03
AL358115.1	-5.69	6E-03
THY1	2.94	6E-03
CKAP2L	1.47	6E-03
SUSD2	5.49	6E-03
LIN9	3.56	6E-03
PCDHB16	-6.69	6E-03
KIF26A	-3.19	6E-03
MMAB	1.02	6E-03
ABHD8	3.16	6E-03
SIX1	5.49	6E-03
ALDH3B1	5.23	6E-03
ARRDC4	4.96	6E-03
FGFR4	4.46	6E-03
PNMA6A	-6.77	6E-03
TUBA1C	1.19	6E-03

HUNK	-5.96	6E-03
CELSR1	-3.08	6E-03
INHBB	3.78	7E-03
POPDC2	5.54	7E-03
TSC1	-1.33	7E-03
ORC1	6.72	7E-03
CELF2-AS2	-5.86	7E-03
CXCL5	5.38	7E-03
SSBP2	1.35	7E-03
FAM129A	3.29	7E-03
RCHY1	-1.04	7E-03
PARP8	4.98	7E-03
CAMK2N1	1.68	8E-03
AC010247.1	-4.79	8E-03
CCDC144A	-3.37	8E-03
GMFG	1.06	8E-03
NEXN	2.18	8E-03
FOXL1	5.71	8E-03
THBS2	4.97	9E-03
SMAD6	-3.53	9E-03
EWSAT1	5.58	9E-03
SLC20A1	1.63	9E-03
HAS2	4.45	9E-03
SLC35F2	-1.17	9E-03
CDKN2A	2.40	9E-03
ZNF432	4.81	9E-03
TPM2	1.65	9E-03
CCBE1	4.87	9E-03
MINDY4	1.26	9E-03
DPP4	1.89	1E-02
FDFT1	1.14	1E-02
CCDC8	6.96	1E-02
TIMP1	1.76	1E-02
RPS6KA1	-3.40	1E-02
AC139795.1	6.28	1E-02
ZNF211	2.77	1E-02
EPS8	1.34	1E-02
CECR2	4.59	1E-02
AC007038.2	-2.80	1E-02
SLC1A1	2.05	1E-02
BPGM	-1.26	1E-02
AL117329.1	-4.34	1E-02

HDAC7	-1.11	1E-02
TMEM97	1.83	1E-02
AL031666.3	-5.14	1E-02
SLC16A13	-4.15	1E-02
CPED1	2.72	1E-02
CBR3	-1.02	1E-02
ANKRD1	-1.54	1E-02
SYT16	-5.94	1E-02
LURAP1L-AS1	-5.08	1E-02
LINC00924	-4.01	1E-02
TLR2	-5.93	1E-02
PIF1	-5.27	1E-02
PTGIR	3.58	1E-02
IDI1	1.26	1E-02
SPDYE21P	-2.57	1E-02
CAPN11	-5.66	1E-02
RAMP1	5.74	1E-02
CKB	5.42	1E-02
CASC15	-1.99	1E-02
TBX5-AS1	4.91	1E-02
CXCL12	5.49	1E-02
AC100821.2	-6.33	1E-02
TRPV2	3.98	1E-02
PGAP1	1.97	1E-02
HSD52	-5.12	1E-02
GREM1	4.26	1E-02
SLC8A1	4.35	1E-02
ALPK2	5.66	1E-02
VEPH1	-1.01	1E-02
GAS6	1.95	1E-02
PYGL	1.14	2E-02
SVEP1	3.13	2E-02
TBX3	4.59	2E-02
MAML2	2.97	2E-02
ACSL3	1.18	2E-02
LPCAT4	2.34	2E-02
LURAP1	3.21	2E-02
DIRAS3	2.96	2E-02
HDDC2	1.07	2E-02
SH3PXD2A	2.48	2E-02
BAIAP2L2	4.98	2E-02

INSC	6.58	2E-02
LINC01436	6.05	2E-02
CENPI	-5.32	2E-02
KCNN2	-4.46	2E-02
ZNF554	-2.88	2E-02
IL1R1	1.62	2E-02
CSPG4	5.85	2E-02
DAAM1	-1.45	2E-02
HFE	3.50	2E-02
NPR3	6.14	2E-02
BOP1	1.83	2E-02
PDE4DIP	1.10	2E-02
PDE4DIP	1.10	2E-02
CXXC5	1.73	2E-02
CATSPERE	-5.41	2E-02
AC027097.2	-4.49	2E-02
KIAA1211L	-5.39	2E-02
TAF1	1.65	2E-02
MYO1B	1.12	2E-02
DNAJC27	-6.03	2E-02
BCL2A1	-6.15	2E-02
AP4S1	-2.04	2E-02
MT-TY	1.85	2E-02
AL133243.3	4.65	2E-02
PRSS12	6.07	2E-02
SLC25A21	-5.19	2E-02
EDNRA	6.16	2E-02
AC026471.4	-4.44	2E-02
INTU	-2.40	2E-02
TFAP4	5.43	2E-02
FOXC1	-1.74	2E-02
B3GALNT1P1	-6.61	2E-02
LINC00342	-2.06	2E-02
SDC2	2.70	2E-02
AL606834.2	-6.04	2E-02
DOCK5	1.95	2E-02
ARHGEF10L	4.10	2E-02
SLC29A1	-1.01	2E-02
ADHFE1	-5.76	2E-02
ISPD	4.13	2E-02
C12orf75	2.17	2E-02
AC127024.6	-5.61	2E-02

CATSPERG	-4.24	2E-02
ZNF667	2.98	2E-02
GLA	-1.04	2E-02
HELLPAR	-3.06	2E-02
PDE8A	1.31	2E-02
PRR16	8.03	2E-02
CFAP46	-3.30	2E-02
COLEC12	-1.15	2E-02
MT-TA	3.01	2E-02
MGAT4A	-1.34	2E-02
SMIM3	2.42	2E-02
CRACR2A	-2.54	2E-02
PRR34	-6.46	2E-02
FZD2	4.40	2E-02
PAQR3	2.76	2E-02
AC099522.2	-5.67	2E-02
GDPD1	-4.62	2E-02
DAAM2	4.34	2E-02
CYP1B1	6.27	2E-02
FKTN	1.98	2E-02
AC118344.2	-4.46	3E-02
BAG2	1.78	3E-02
AMOT	-4.13	3E-02
AL133485.1	-6.00	3E-02
AL158209.1	-4.91	3E-02
SLC44A5	3.62	3E-02
SNIP1	2.02	3E-02
MOXD1	6.41	3E-02
CXCL10	-8.65	3E-02
ZNF100	-2.53	3E-02
PDE10A	-3.92	3E-02
IRF2BP1	2.52	3E-02
AC005498.2	-4.57	3E-02
BUB1B	2.88	3E-02
CES1	4.96	3E-02
BMS1P1	-1.81	3E-02
NR2F2	-1.07	3E-02
TET3	-3.07	3E-02
AC010127.1	-5.86	3E-02
AC010127.1	-5.86	3E-02
SMAD7	2.76	3E-02
PLEKHH3	-3.18	3E-02

ACLY	1.11	3E-02
SLC6A15	4.34	3E-02
MID1IP1	2.45	3E-02
GAS6-AS2	-6.15	3E-02
UBA6-AS1	1.43	3E-02
CAVIN4	-5.28	3E-02
LINC02363	-5.66	3E-02
TGFB2	-4.24	3E-02
AC090114.2	-3.64	3E-02
RALGPS1	-2.36	3E-02
SEC14L1P1	-4.78	3E-02
FAM89A	2.21	3E-02
SPDYE2	-2.20	3E-02
PRKCQ	-5.41	3E-02
RAPGEF4	1.77	3E-02
TOM1L1	3.60	3E-02
FRAS1	-2.85	3E-02
ACOX2	-3.86	3E-02
TNFSF15	-1.12	3E-02
AC060780.1	-2.18	3E-02
SYNJ2	-1.09	3E-02
ALKBH5	1.58	3E-02
PTPRJ	-2.75	3E-02
KIF13A	-1.24	4E-02
TEAD3	5.80	4E-02
CROCCP3	-5.90	4E-02
AC023051.1	-3.79	4E-02
FBXL6	-2.56	4E-02
SSC5D	3.60	4E-02
AC108449.2	-2.19	4E-02
CASC18	-4.77	4E-02
AC005911.1	-4.56	4E-02
ZNF596	3.58	4E-02
AL138756.1	-3.18	4E-02
AP001189.5	-2.98	4E-02
AC012360.3	-4.84	4E-02
SCN9A	2.47	4E-02
LEKR1	5.59	4E-02
CEACAM1	-3.90	4E-02
PEG13	5.43	4E-02
ATR	-1.80	4E-02
AP003486.1	6.14	4E-02

AC105411.1	-5.24	4E-02
ZNF138	1.31	4E-02
GPHN	2.06	4E-02
METTL7B	6.51	4E-02
AC091173.1	5.99	4E-02
AC003965.1	-5.56	4E-02
RLIM	1.02	4E-02
AC023790.2	-4.77	4E-02
CASC4	1.43	4E-02
CD44	1.93	4E-02
SCD	1.56	4E-02
TSGA10	-3.25	4E-02
SFRP1	3.92	4E-02
RPL23	1.06	4E-02
HMGCR	1.70	4E-02
PRPS2	1.09	4E-02
NIPAL3	1.06	4E-02
IL7R	3.70	4E-02
TSPAN8	-5.25	4E-02
HMGCS1	1.81	4E-02
AC068152.1	-1.32	4E-02
EDN1	-1.21	4E-02
PLA2G4C	-1.79	4E-02
SMPDL3B	-2.49	4E-02
SULF1	-1.15	4E-02
SH2D5	5.70	4E-02
SLC25A1	1.34	4E-02
AL109811.2	-3.95	4E-02
COL4A6	-4.33	4E-02
BTK	-6.47	4E-02
LINC00520	-4.07	4E-02
AC092653.2	-4.89	4E-02
VASN	4.03	4E-02
GATS	-3.46	4E-02
ZNF655	1.28	4E-02
RFLNA	-2.62	4E-02
RFLNA	-2.62	4E-02
B4GALT6	-2.17	4E-02
INMT	4.54	4E-02
DSEL	1.79	4E-02
MICAL2	1.48	4E-02
KCNQ5	3.92	4E-02

TNFRSF9	-4.72	5E-02
ENPP2	2.27	5E-02
ARRDC1-AS1	-1.95	5E-02
CRYZ	1.24	5E-02
AC069185.1	4.58	5E-02
FNDC4	-1.40	5E-02
RABGAP1L	1.21	5E-02
CDC6	1.43	5E-02
AC079174.2	-6.04	5E-02
TBC1D12	1.78	5E-02
LEAP2	3.93	5E-02
GPR35	-6.38	5E-02
ARPP21	-5.61	5E-02
HOXA6	-2.34	5E-02
AP003356.1	-4.81	5E-02
NFKBIE	-1.85	5E-02
NRDE2	-1.15	5E-02
HMBS	1.21	5E-02
SLC16A14	-5.99	5E-02
AC132872.1	-5.88	5E-02
AC016773.1	-5.40	5E-02
CHN1	1.19	5E-02
AP000962.1	-4.95	5E-02
MTBP	3.41	5E-02
CFAP58-AS1	5.44	5E-02
IARS	1.01	5E-02
LINC00882	3.81	5E-02
BMP4	-1.13	5E-02
HAAO	-6.10	5E-02
AP002807.1	-5.42	5E-02
DHTKD1	1.23	5E-02
PLXDC2	2.92	5E-02
CXCR4	1.58	5E-02
C2CD4A	-3.04	5E-02
RHOBTB3	2.17	5E-02

Table S2. List of receptors and ligands from the interactions of ECs and FBs in conventional juxtacrine cocultures.

EC receptors	**EC ligands**	**FB receptors**	**FB ligands**
HSPG2	COL1A1	CXCR4	CDH5
ITGA6	THBS2	CD93	VWF
KDR	S100A4	TINAGL1	APLN
PTPRB	COL6A2	ACVRL1	EFNA1
ANXA2	IGFBP2	PTPRJ	ICAM2
ITGB1	LTB	NR4A1	VWA1
ITGA5	COL1A2	EPHB2	IGFBP2
TGFBR2	NENF	TMED1	THBS2
ENG	MFAP2	ANXA2	TINAGL1
PLSCR4	SDF4	RPSA	MFAP2
BMPR2	HMGB2	AHR	COL4A2
ITGAV	HSPG2	CD46	ICAM1
IL6	STC2	LOXL4	INHBA
NPTN	PDGFB	ITGB1	FST
EPHA5	BMP2	TGFBR2	PGF
	PSAP	DPP4	CES1
	JAG1		CLU
	COL5A2		ANXA1
	FSTL1		FN1
	TGFB2		EFNA5
	EFNA5		IGFBP1
	COL4A1		
	LAMA2		
	SERPINE1		
	NPTN		
	FN1		
	MAMDC2		
	CXCL12		