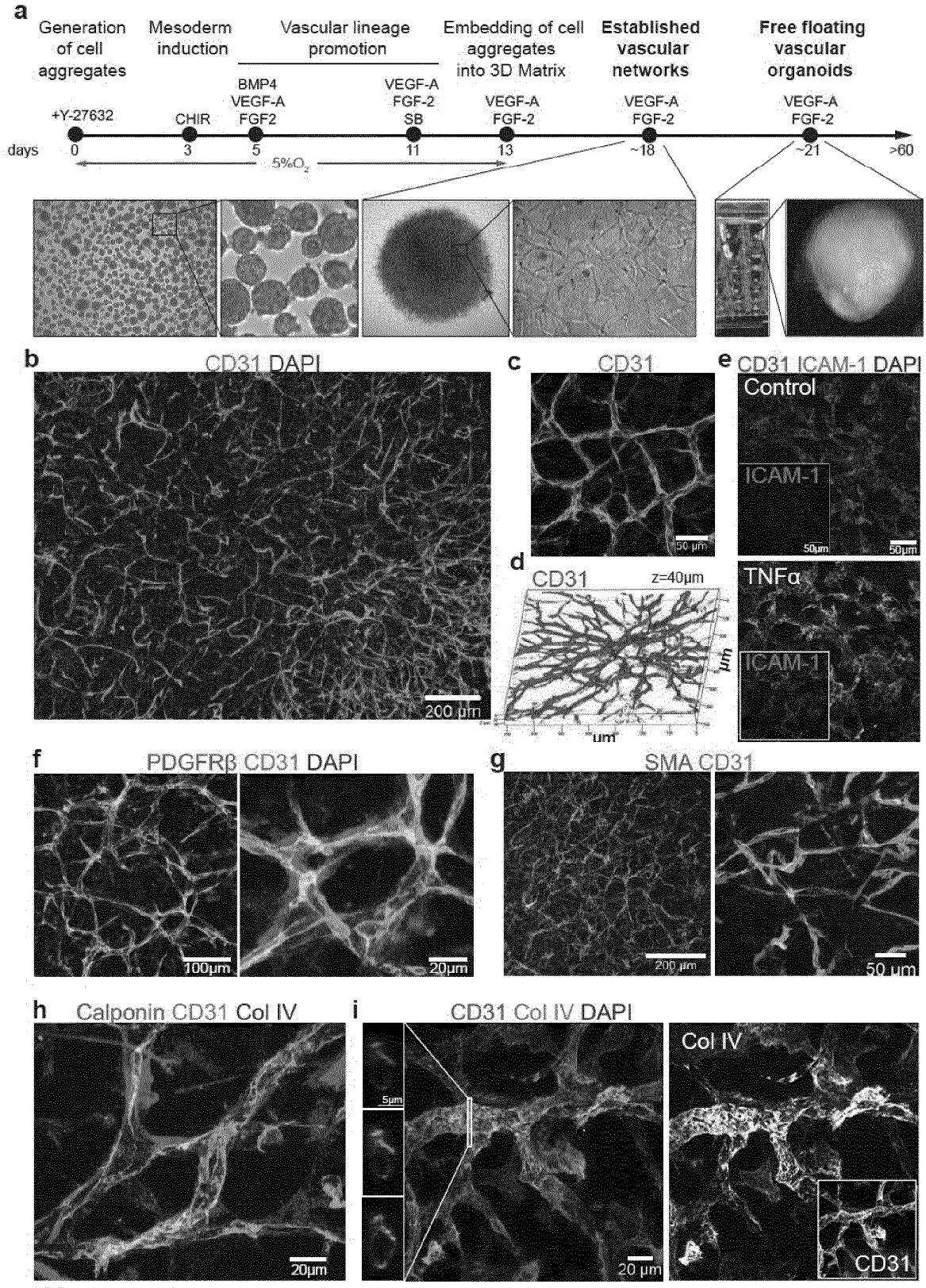

a
Generation of cell aggregates
Mesoderm induction
Vascular lineage promotion
Embedding of cell aggregates into 3D Matrix
Established vascular networks
Free floating vascular organoids
+Y-27632
CHIR
BMP4
VEGF-A
FGF2
VEGF-A
FGF-2
SB
VEGF-A
FGF-2
VEGF-A
FGF-2
VEGF-A
FGF-2
days
0
3
5
11
13
~18
~21
>60
5%O2
b
CD31 DAPI
200 µm
c
CD31
50 µm
d
CD31
z=40µm
µm
µm
e
CD31 ICAM-1 DAPI
Control
ICAM-1
50µm
50µm
TNFα
ICAM-1
f
PDGFRβ CD31 DAPI
100µm
20µm
g
SMA CD31
200 µm
50 µm
h
Calponin CD31 Col IV
20µm
i
CD31 Col IV DAPI
5µm
20 µm
Col IV
CD31

Figure 1

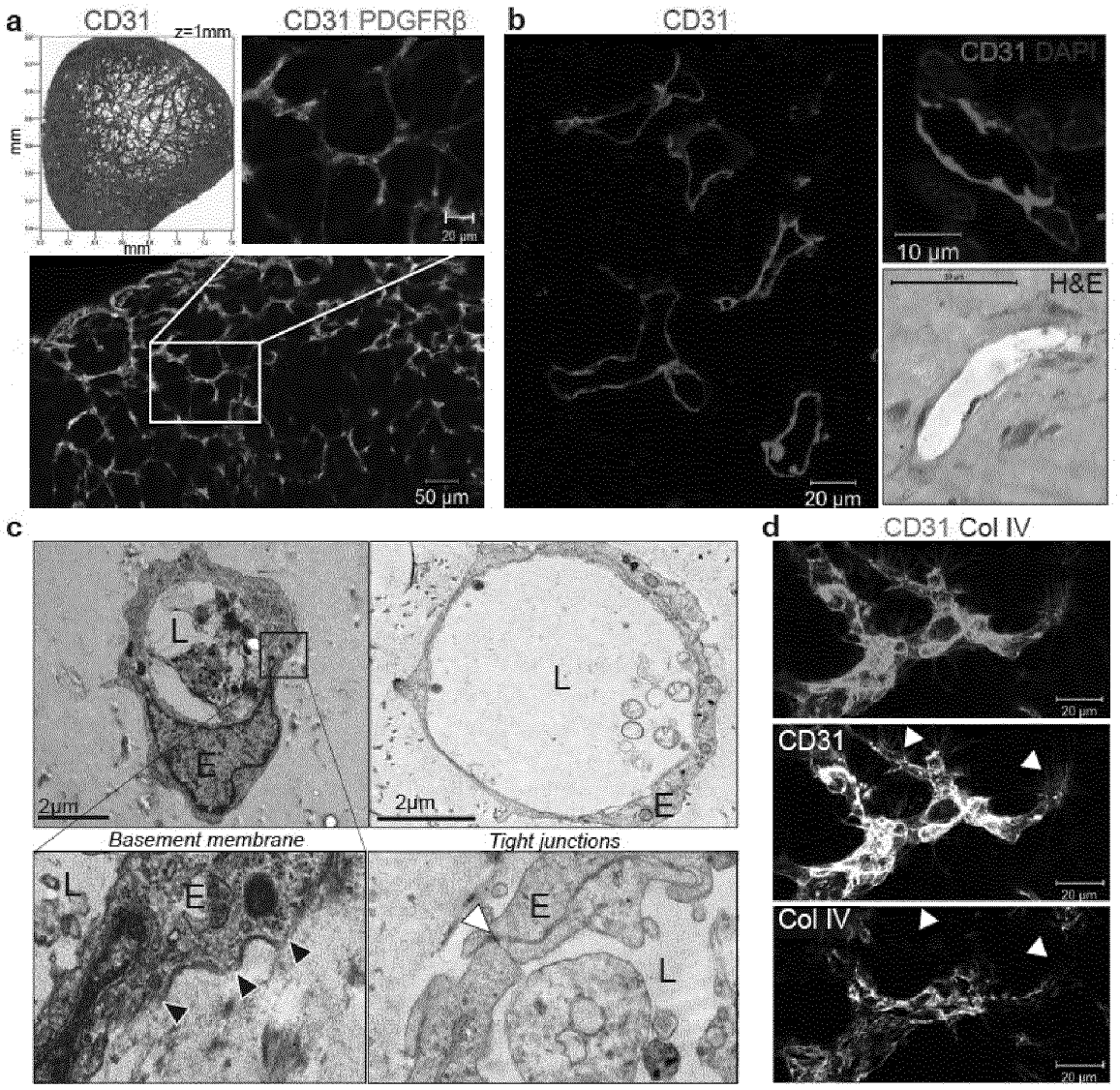
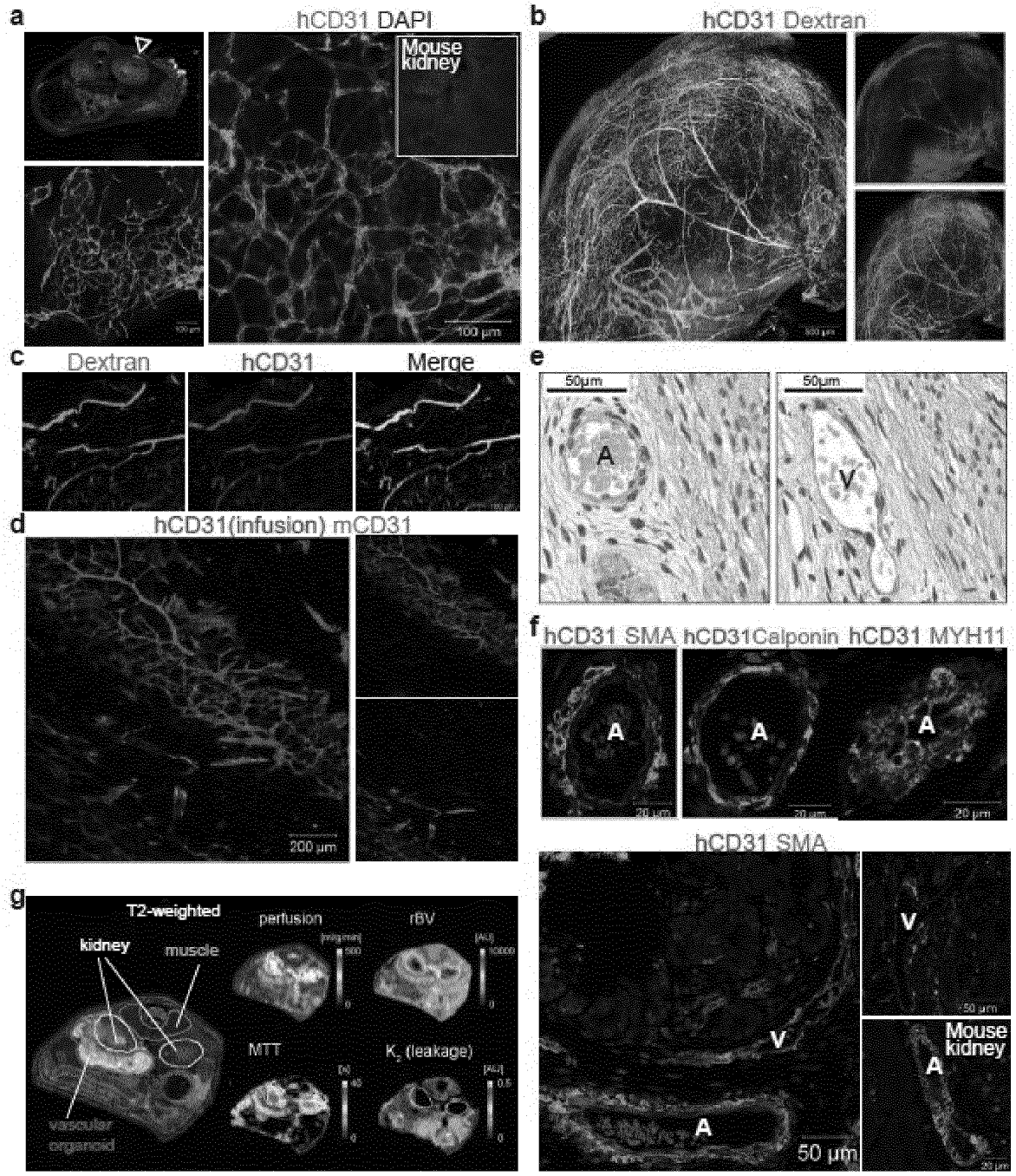

a
CD31
z=1mm
mm
mm
CD31 PDGFRβ
20 µm
50 µm
b
CD31
20 µm
CD31 DAPI
10 µm
H&E
c
L
E
2µm
L
2µm
E
Basement membrane
Tight junctions
L
E
E
L
d
CD31 Col IV
20 µm
CD31
20 µm
Col IV
20 µm

Figure 2



	Perfusion (ml/g/min)	MTT (sec)	Leakage K2/muscle (a.u.)	Blood volume (a.u.)
Vessel transplant (Human)	103.1 ± 11.2	14.12 ± 1.43	0.786 ± 0.108	2983 ± 551
Adjacent kidney (Mouse)	390.3 ± 33.2	25.12 ± 1.15	0.036 ± 0.01	1396 ± 130
Adjacent muscle (Mouse)	36.1 ± 4.13	4.95 ± 0.41	1	2424 ± 524
				n=3

Figure 3

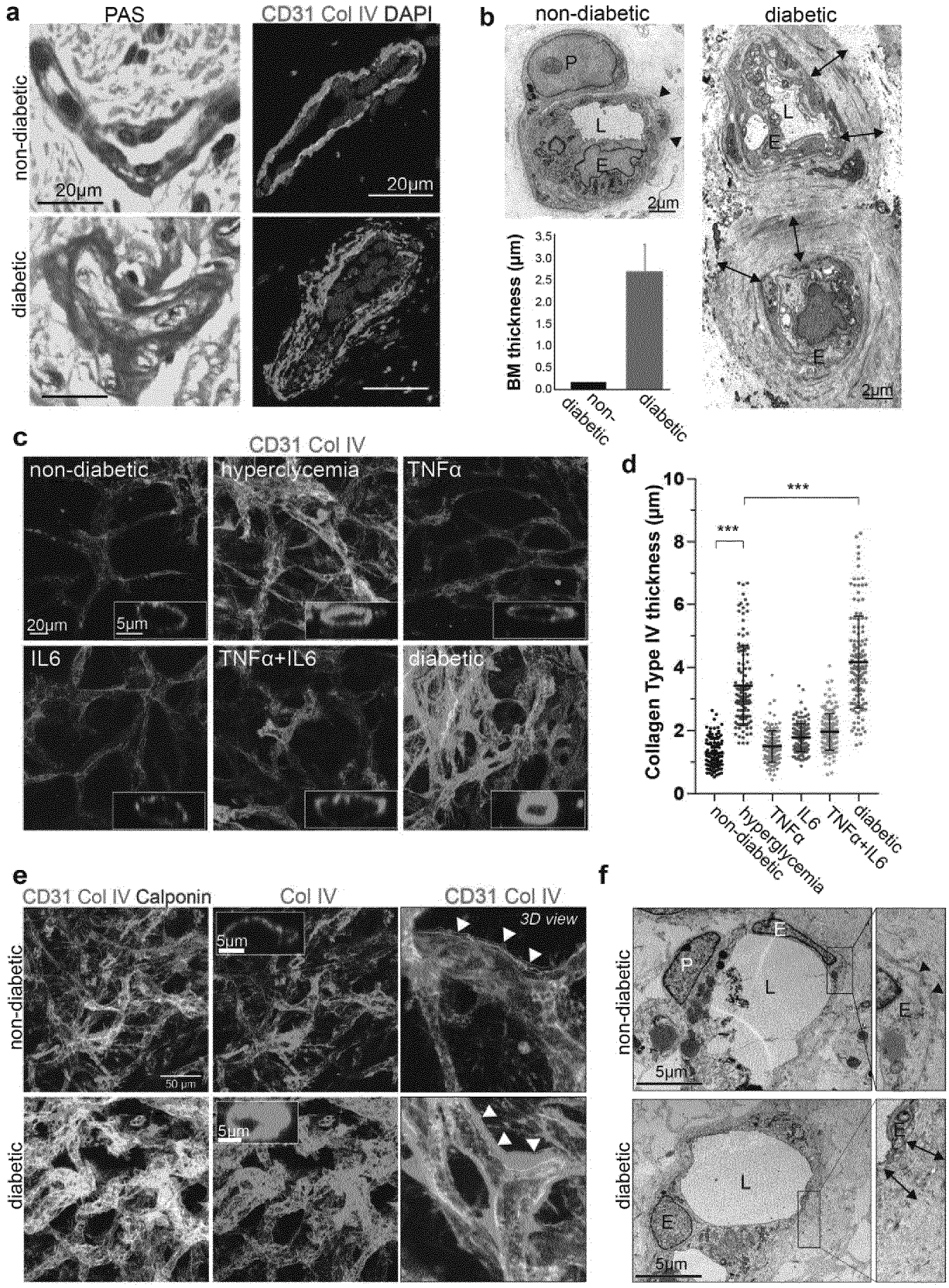

a
PAS
CD31 Col IV DAPI
non-diabetic
diabetic
20µm
b
non-diabetic
diabetic
P
L
E
2µm
BM thickness (µm)
non-diabetic
diabetic
c
CD31 Col IV
non-diabetic
hyperglycemia
TNFα
IL6
TNFα+IL6
diabetic
20µm
5µm
d
Collagen Type IV thickness (µm)
***
***
non-diabetic
hyperglycemia
TNFα
IL6
TNFα+IL6
diabetic
e
CD31 Col IV Calponin
Col IV
CD31 Col IV
3D view
50 µm
5µm
f
5µm

Figure 4

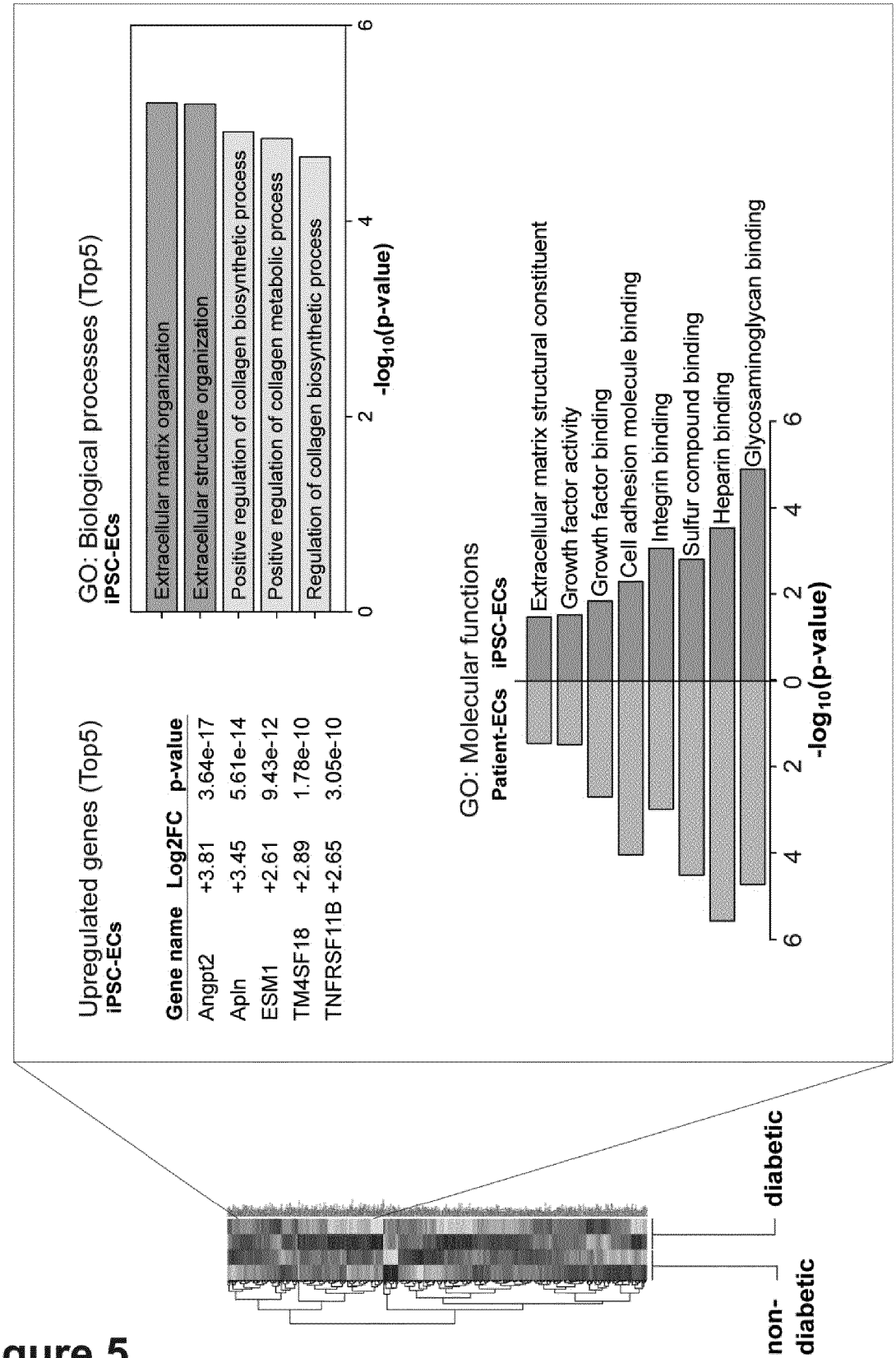

Upregulated genes (Top5)
iPSC-ECs
Gene name Log2FC p-value
Angpt2 +3.81 3.64e-17
Apln +3.45 5.61e-14
ESM1 +2.61 9.43e-12
TM4SF18 +2.89 1.78e-10
TNFRSF11B +2.65 3.05e-10
GO: Biological processes (Top5)
iPSC-ECs
Extracellular matrix organization
Extracellular structure organization
Positive regulation of collagen biosynthetic process
Positive regulation of collagen metabolic process
Regulation of collagen biosynthetic process
0 2 4 6
-log10(p-value)
GO: Molecular functions
Patient-ECs iPSC-ECs
Extracellular matrix structural constituent
Growth factor activity
Growth factor binding
Cell adhesion molecule binding
Integrin binding
Sulfur compound binding
Heparin binding
Glycosaminoglycan binding
6 4 2 0 2 4 6
-log10(p-value)
diabetic
non-diabetic
a
1 0.5 0 −0.5 −1

Figure 5

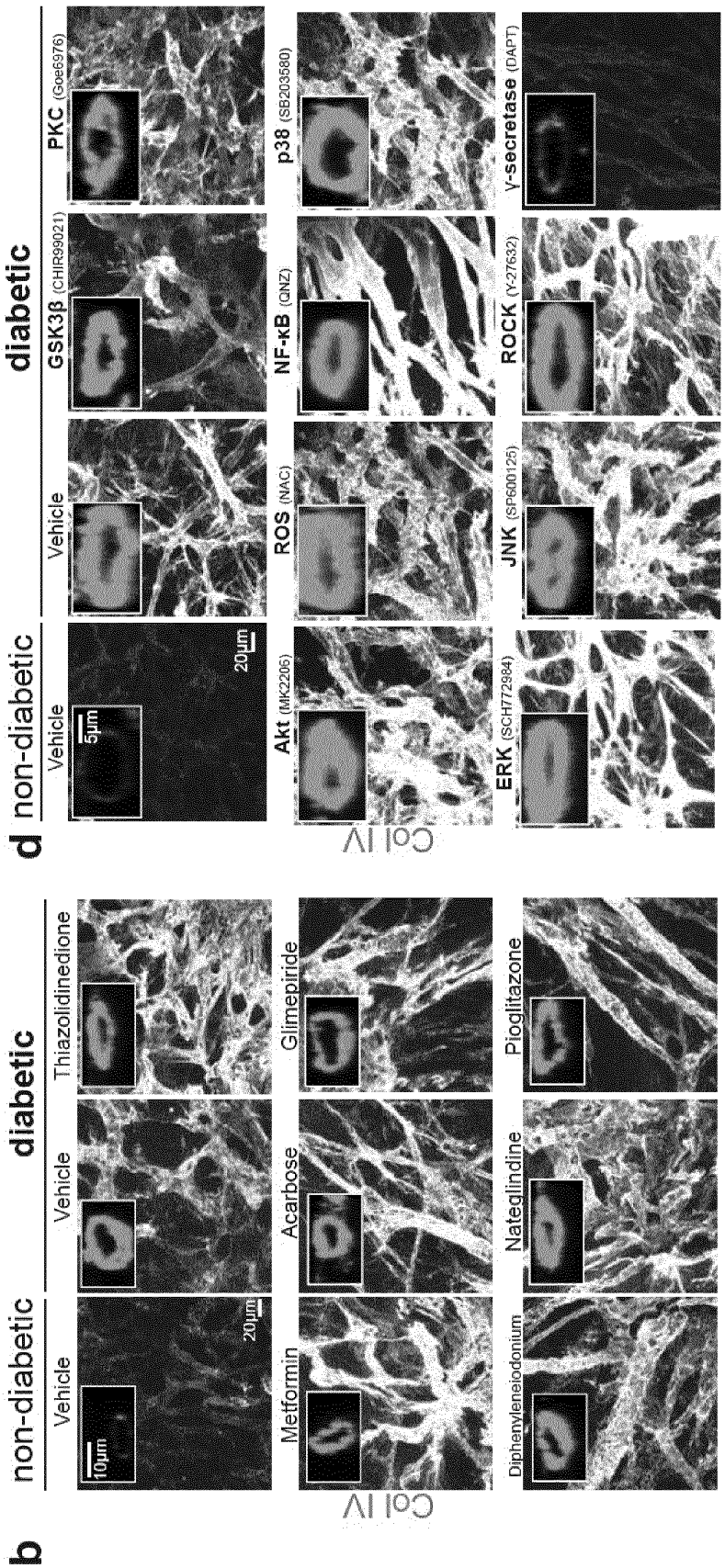

b
non-diabetic
diabetic
Vehicle
Vehicle
Thiazolidinedione
10μm
20μm
Metformin
Acarbose
Glimepiride
Diphenyleneiodonium
Nateglindine
Pioglitazone
Col IV
d
non-diabetic
diabetic
Vehicle
Vehicle
GSK3β (CHIR99021)
PKC (Goe6976)
5μm
20μm
Akt (MK2206)
ROS (NAC)
NF-κB (QNZ)
p38 (SB203580)
ERK (SCH772984)
JNK (SP600125)
ROCK (Y-27632)
γ-secretase (DAPT)
Col IV

Figure 5

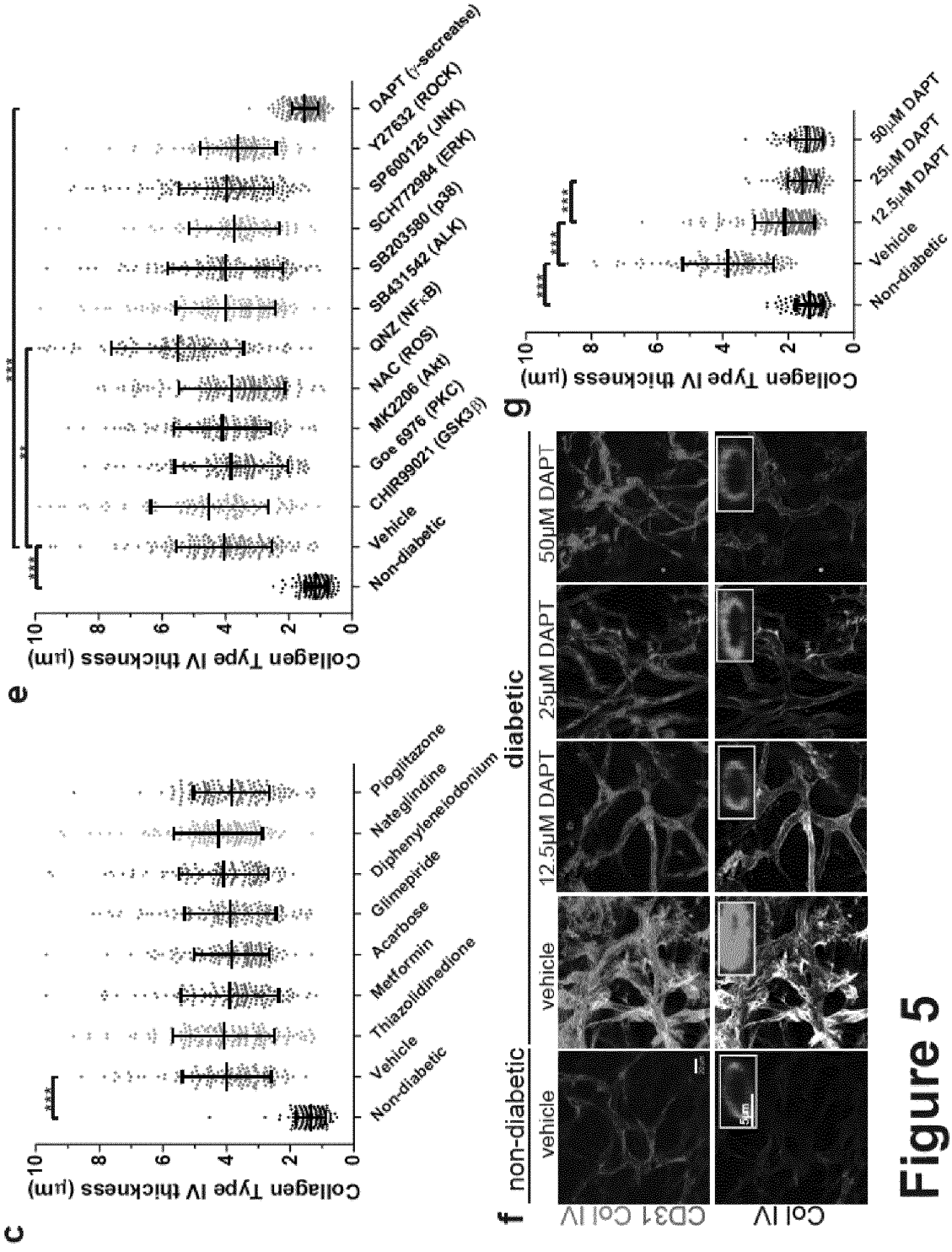

c
Collagen Type IV thickness (μm)
Pioglitazone
Nateglindine
Diphenyleneiodonium
Glimepiride
Acarbose
Metformin
Thiazolidinedione
Vehicle
Non-diabetic
e
Collagen Type IV thickness (μm)
DAPT (γ-secreatse)
Y27632 (ROCK)
SP600125 (JNK)
SCH772984 (ERK)
SB203580 (p38)
SB431542 (ALK)
QNZ (NFκB)
NAC (ROS)
MK2206 (Akt)
Goe 6976 (PKC)
CHIR99021 (GSK3β)
Vehicle
Non-diabetic
f
non-diabetic
diabetic
vehicle
vehicle
12.5μM DAPT
25μM DAPT
50μM DAPT
CD31 Col IV
Col IV
5μm
g
Collagen Type IV thickness (μm)
50μM DAPT
25μM DAPT
12.5μM DAPT
Vehicle
Non-diabetic

Figure 5

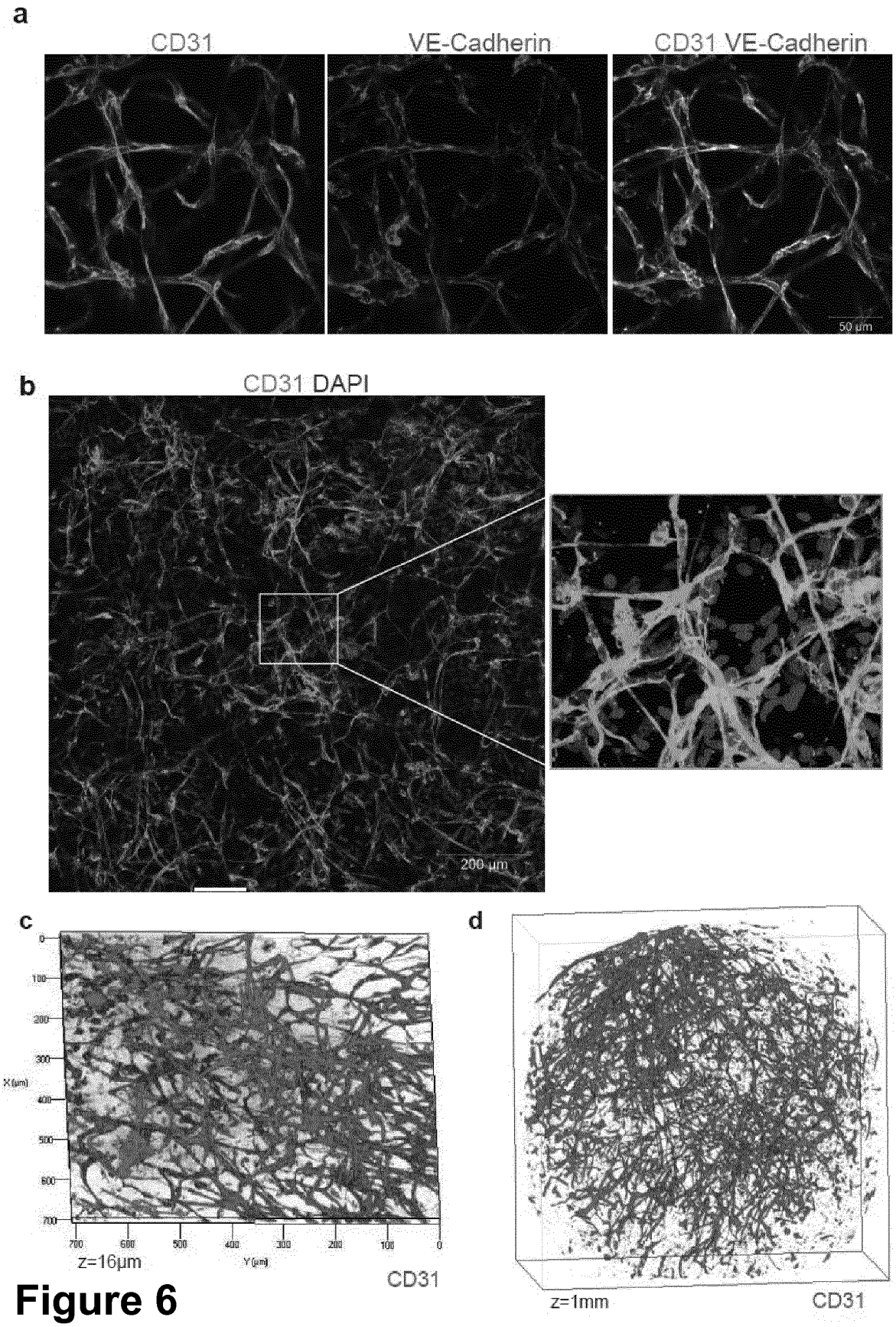
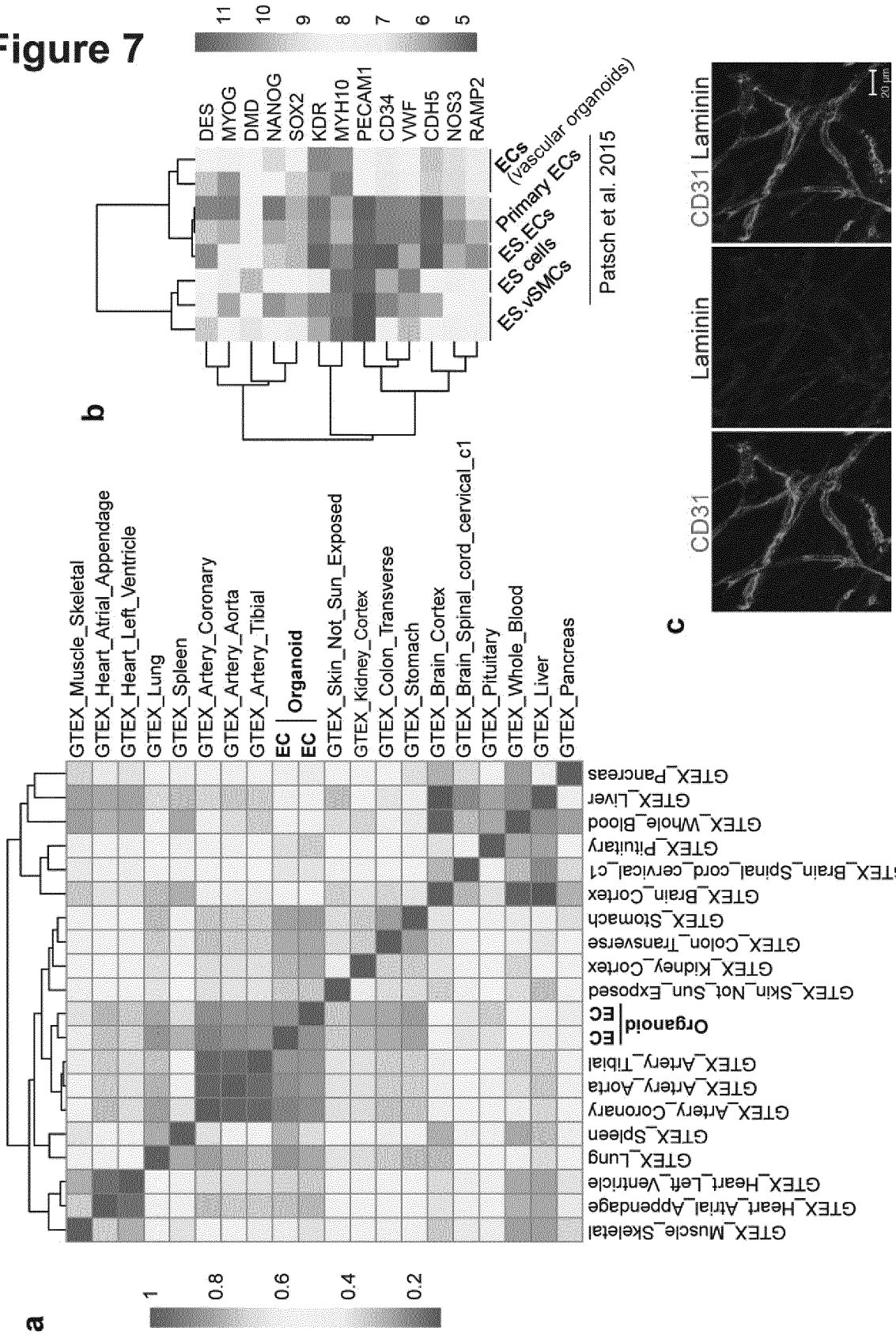

a
CD31
VE-Cadherin
CD31 VE-Cadherin
50 µm
b
CD31 DAPI
200 µm
c
X (µm)
Y (µm)
z=16µm
CD31
d
z=1mm
CD31

Figure 6

Figure 7


a
1
0.8
0.6
0.4
0.2
GTEX_Muscle_Skeletal
GTEX_Heart_Atrial_Appendage
GTEX_Heart_Left_Ventricle
GTEX_Lung
GTEX_Spleen
GTEX_Artery_Coronary
GTEX_Artery_Aorta
GTEX_Artery_Tibial
EC | Organoid
EC
GTEX_Skin_Not_Sun_Exposed
GTEX_Kidney_Cortex
GTEX_Colon_Transverse
GTEX_Stomach
GTEX_Brain_Cortex
GTEX_Brain_Spinal_cord_cervical_c1
GTEX_Pituitary
GTEX_Whole_Blood
GTEX_Liver
GTEX_Pancreas
GTEX_Muscle_Skeletal
GTEX_Heart_Atrial_Appendage
GTEX_Heart_Left_Ventricle
GTEX_Lung
GTEX_Spleen
GTEX_Artery_Coronary
GTEX_Artery_Aorta
GTEX_Artery_Tibial
Organoid | EC
EC
GTEX_Skin_Not_Sun_Exposed
GTEX_Kidney_Cortex
GTEX_Colon_Transverse
GTEX_Stomach
GTEX_Brain_Cortex
GTEX_Brain_Spinal_cord_cervical_c1
GTEX_Pituitary
GTEX_Whole_Blood
GTEX_Liver
GTEX_Pancreas
b
11
10
9
8
7
6
5
DES
MYOG
DMD
NANOG
SOX2
KDR
MYH10
PECAM1
CD34
VWF
CDH5
NOS3
RAMP2
ECs (vascular organoids)
Primary ECs
ES.ECs
ES cells
ES.vSMCs
Patsch et al. 2015
c
CD31
Laminin
CD31 Laminin
20 µm

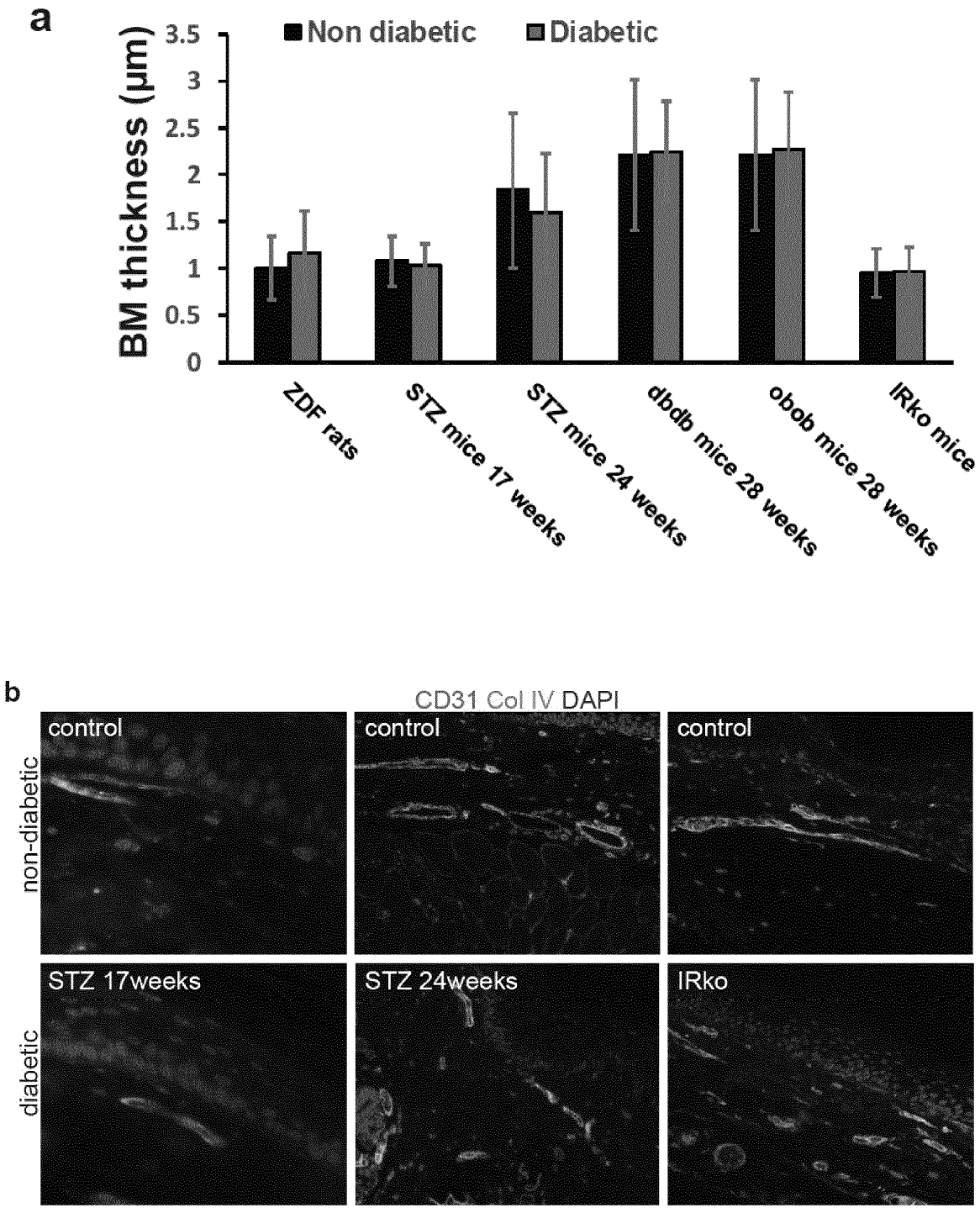

a
Non diabetic
Diabetic
BM thickness (µm)
3.5
3
2.5
2
1.5
1
0.5
0
ZDF rats
STZ mice 17 weeks
STZ mice 24 weeks
dbdb mice 28 weeks
obob mice 28 weeks
IRko mice
b
CD31 Col IV DAPI
control
control
control
non-diabetic
STZ 17weeks
STZ 24weeks
IRko
diabetic

Figure 8

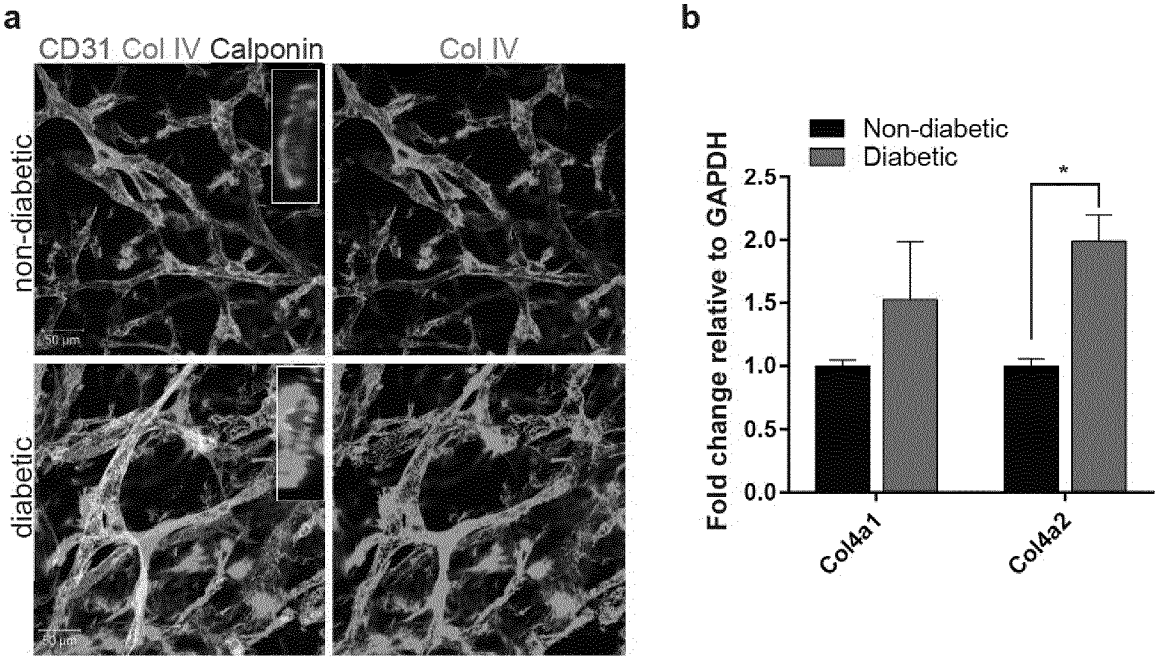

a
CD31 Col IV Calponin
Col IV
non-diabetic
diabetic
50 µm
50 µm
b
Non-diabetic
Diabetic
Fold change relative to GAPDH
2.5
2.0
1.5
1.0
0.5
0.0
*
Col4a1
Col4a2

Figure 9

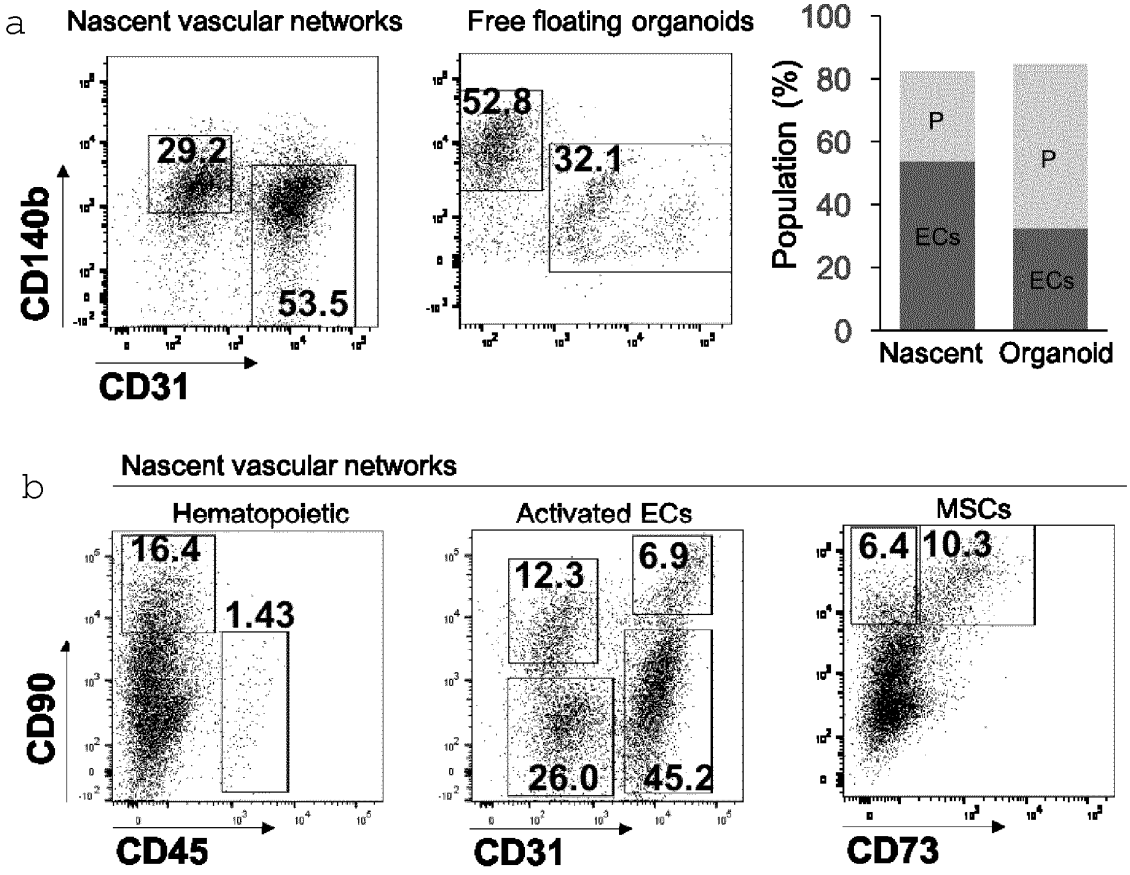

a
Nascent vascular networks
Free floating organoids
CD140b
CD31
29.2
53.5
52.8
32.1
Population (%)
100
80
60
40
20
0
P
ECs
P
ECs
Nascent
Organoid
b
Nascent vascular networks
Hematopoietic
Activated ECs
MSCs
CD90
CD45
CD31
CD73
16.4
1.43
12.3
6.9
26.0
45.2
6.4
10.3

Figure 10

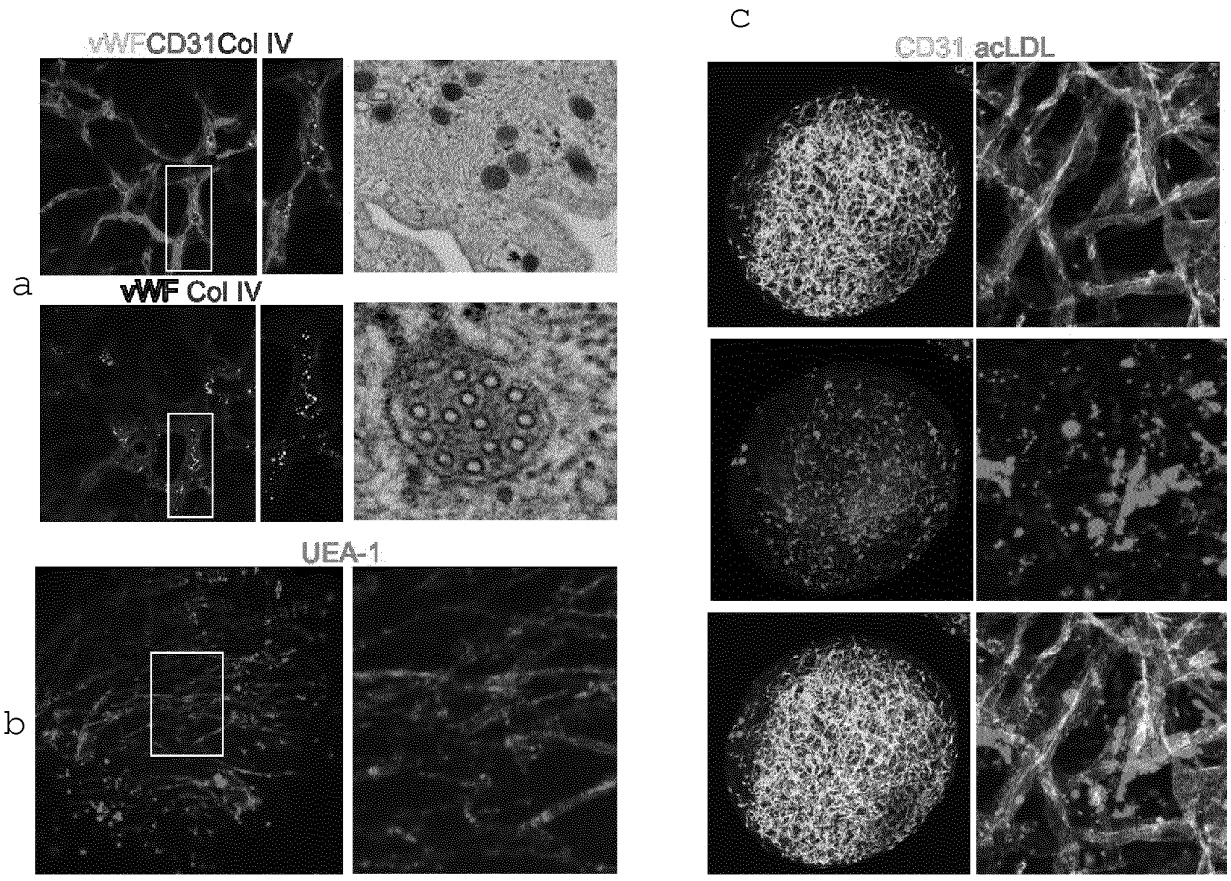


Figure 11

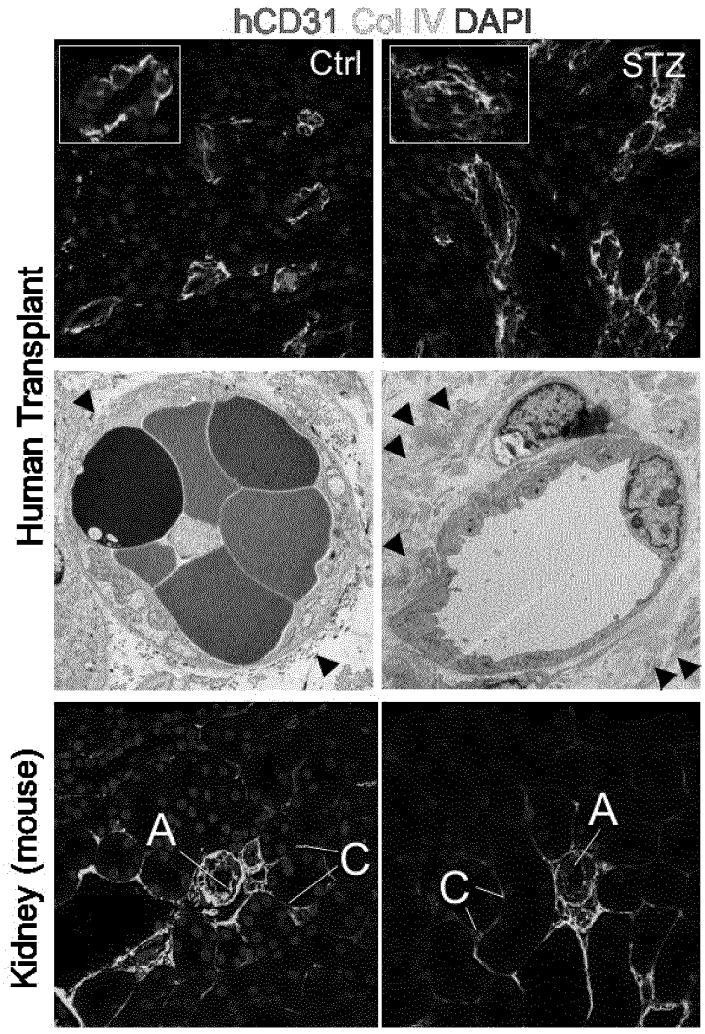

hCD31 Col IV DAPI
Ctrl
STZ
Human Transplant
Kidney (mouse)
A
C
A
C

Figure 12

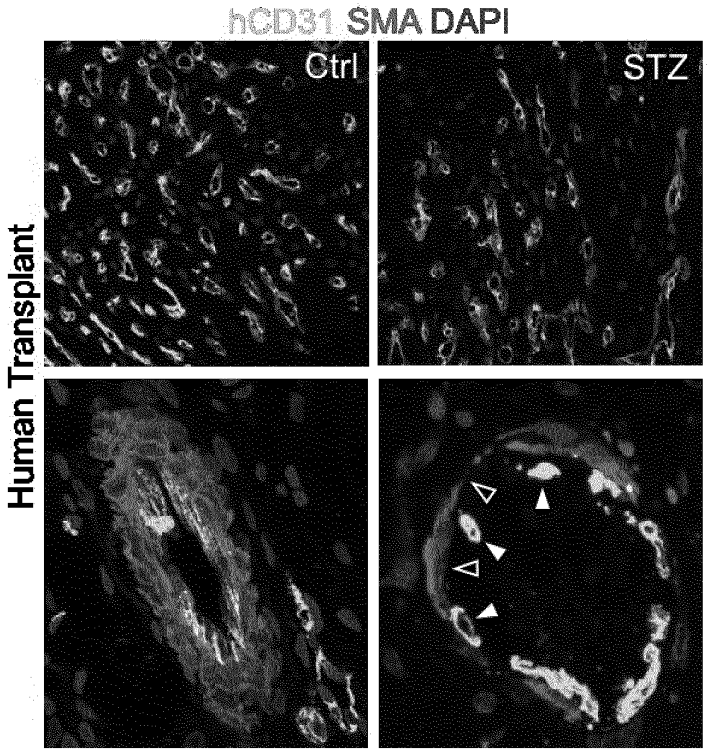


Figure 13

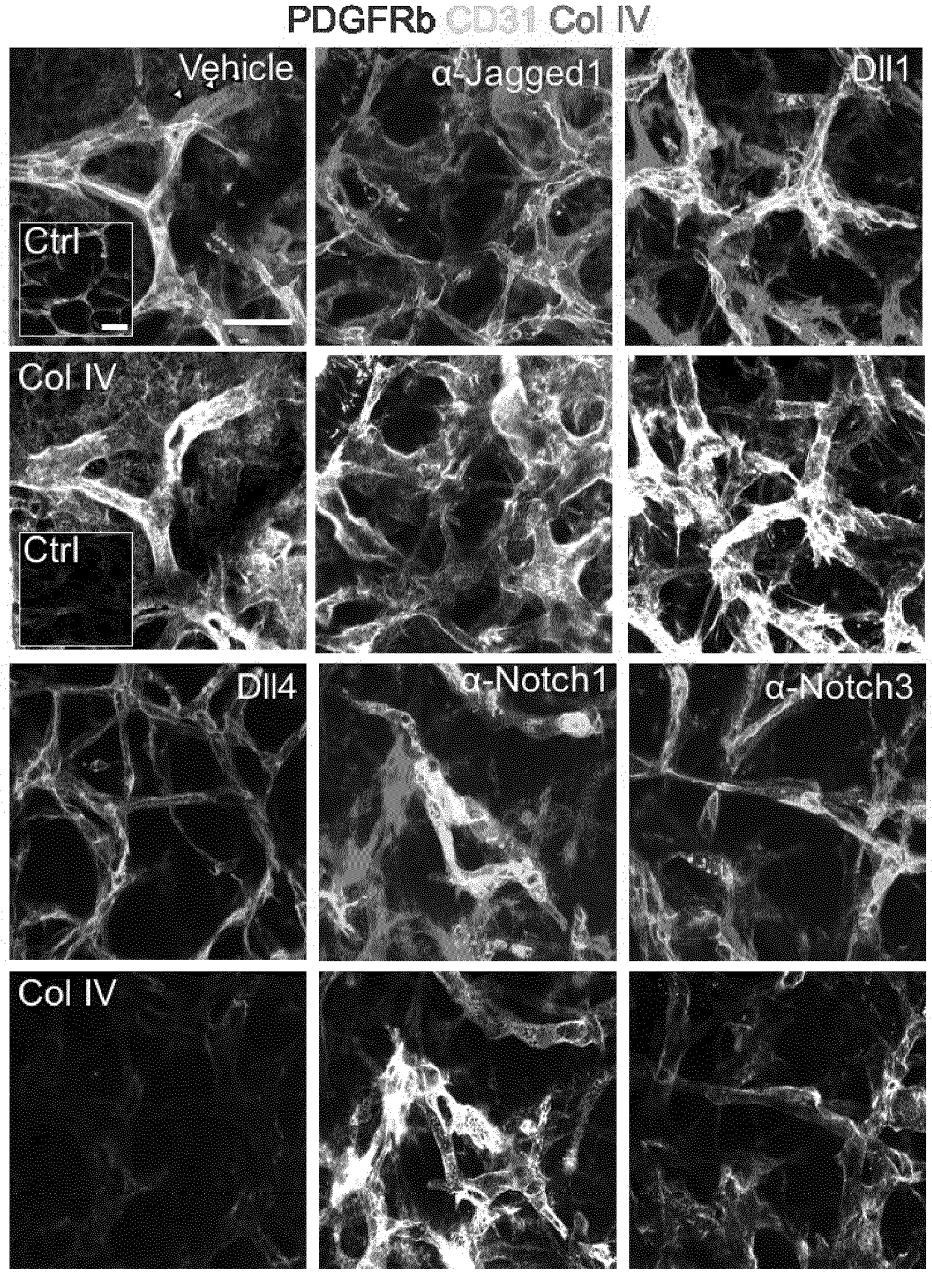

PDGFRb CD31 Col IV
Vehicle
α-Jagged1
Dll1
Ctrl
Col IV
Ctrl
Dll4
α-Notch1
α-Notch3
Col IV

Figure 14

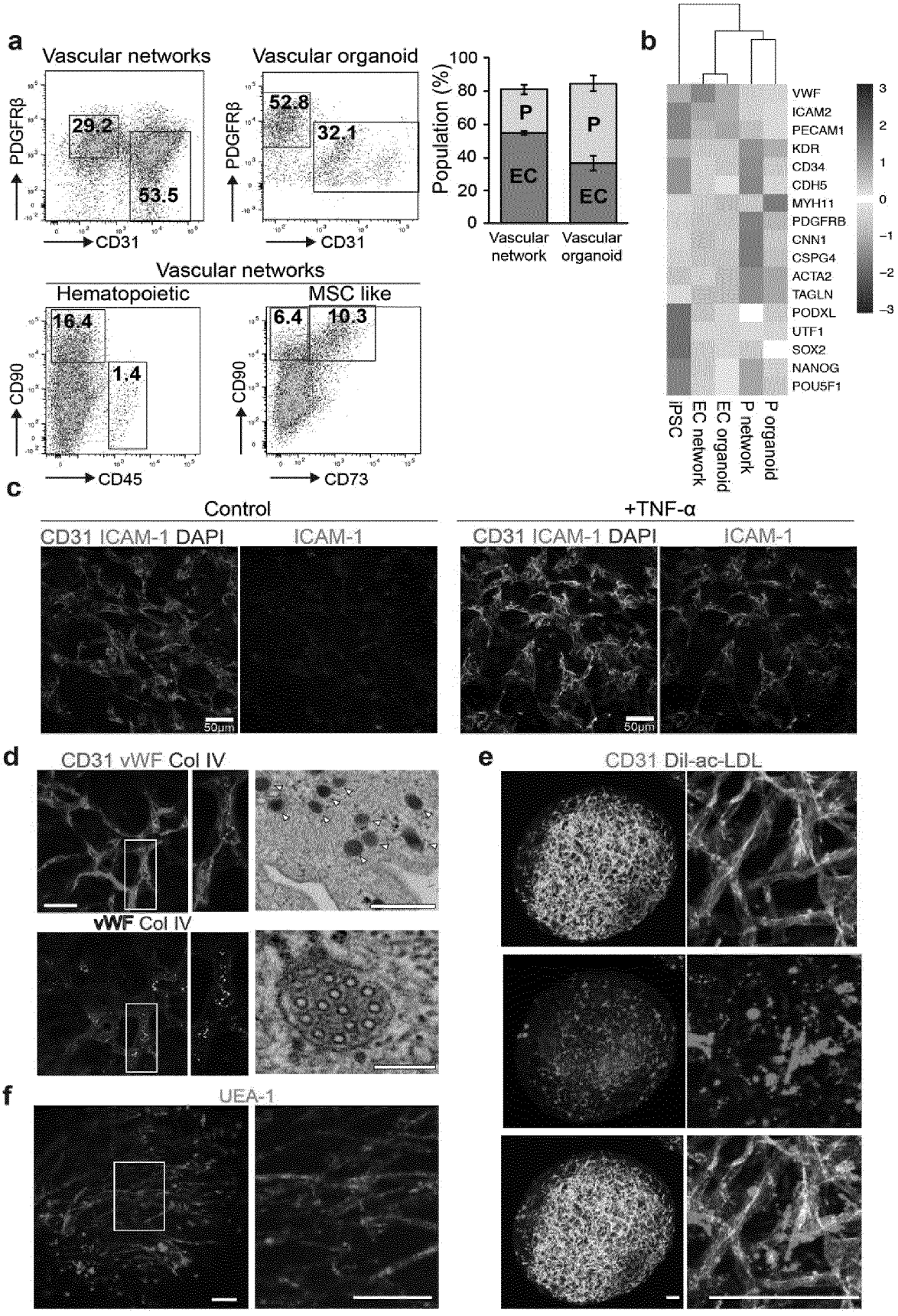

a
Vascular networks
Vascular organoid
PDGFRβ
CD31
29.2
53.5
52.8
32.1
Population (%)
P
EC
Vascular network
Vascular organoid
Vascular networks
Hematopoietic
MSC like
CD90
CD45
CD73
16.4
1.4
6.4
10.3
b
VWF
ICAM2
PECAM1
KDR
CD34
CDH5
MYH11
PDGFRB
CNN1
CSPG4
ACTA2
TAGLN
PODXL
UTF1
SOX2
NANOG
POU5F1
iPSC
EC network
EC organoid
P network
P organoid
c
Control
+TNF-α
CD31 ICAM-1 DAPI
ICAM-1
50µm
d
CD31 vWF Col IV
vWF Col IV
e
CD31 DiI-ac-LDL
f
UEA-1

FIGURE 15

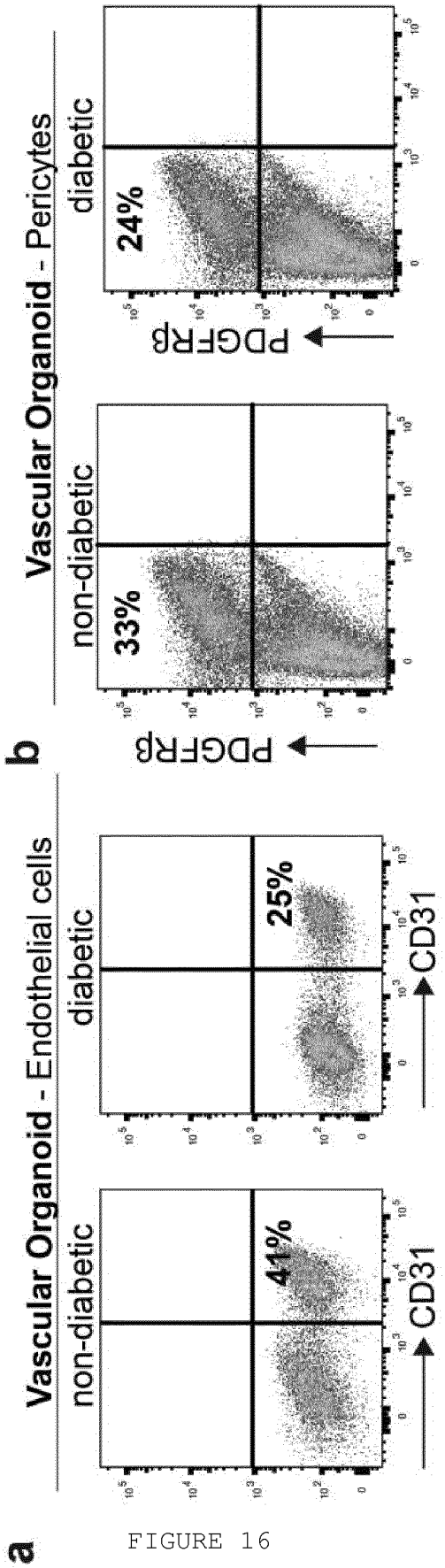

a
Vascular Organoid - Endothelial cells
non-diabetic
41%
CD31
diabetic
25%
CD31
b
Vascular Organoid - Pericytes
non-diabetic
33%
PDGFRβ
diabetic
24%
PDGFRβ

FIGURE 16

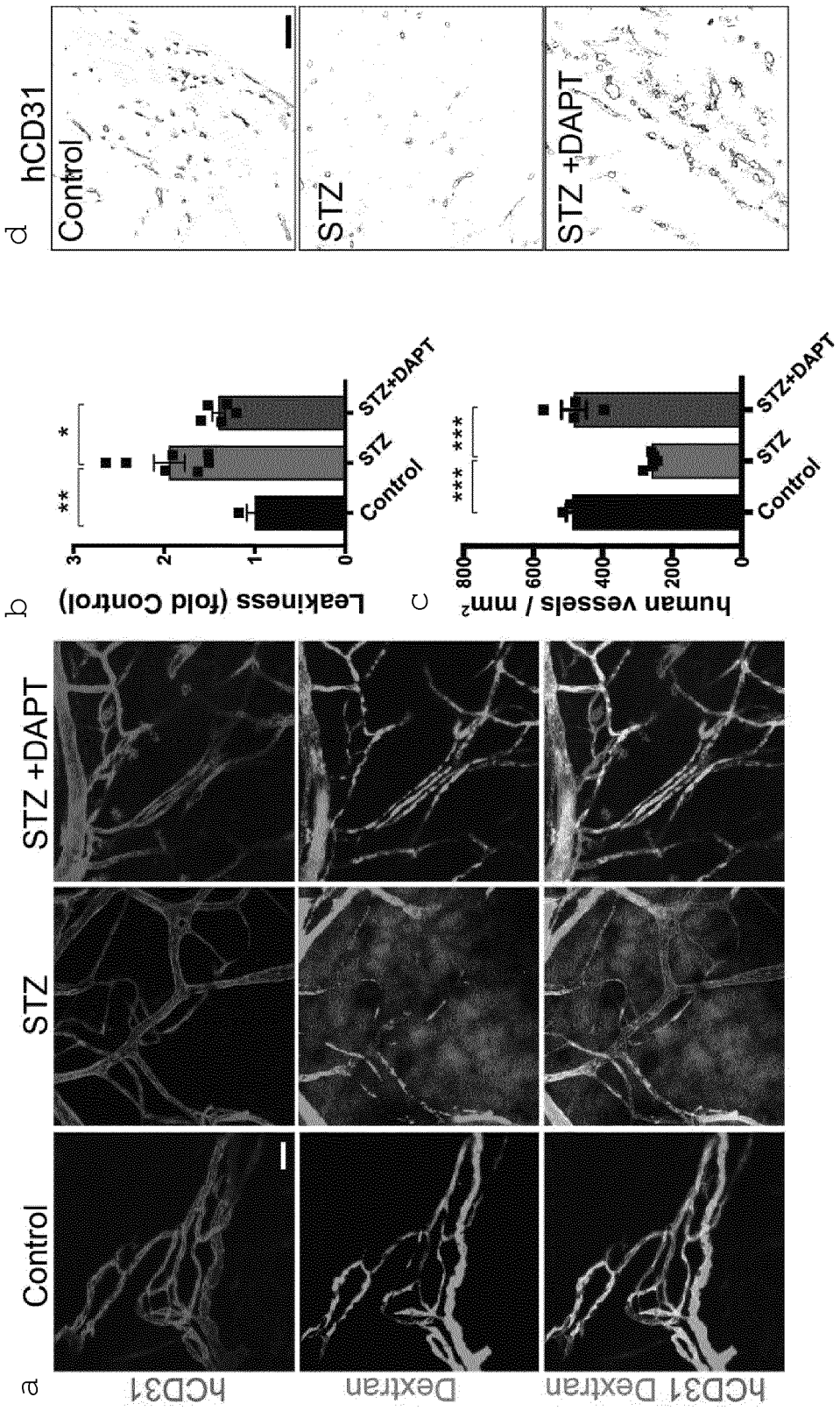

a
Control
STZ
STZ +DAPT
hCD31
Dextran
hCD31 Dextran
b
Leakiness (fold Control)
Control
STZ
STZ+DAPT
c
human vessels / mm²
Control
STZ
STZ+DAPT
d
hCD31
Control
STZ
STZ +DAPT

FIGURE 17

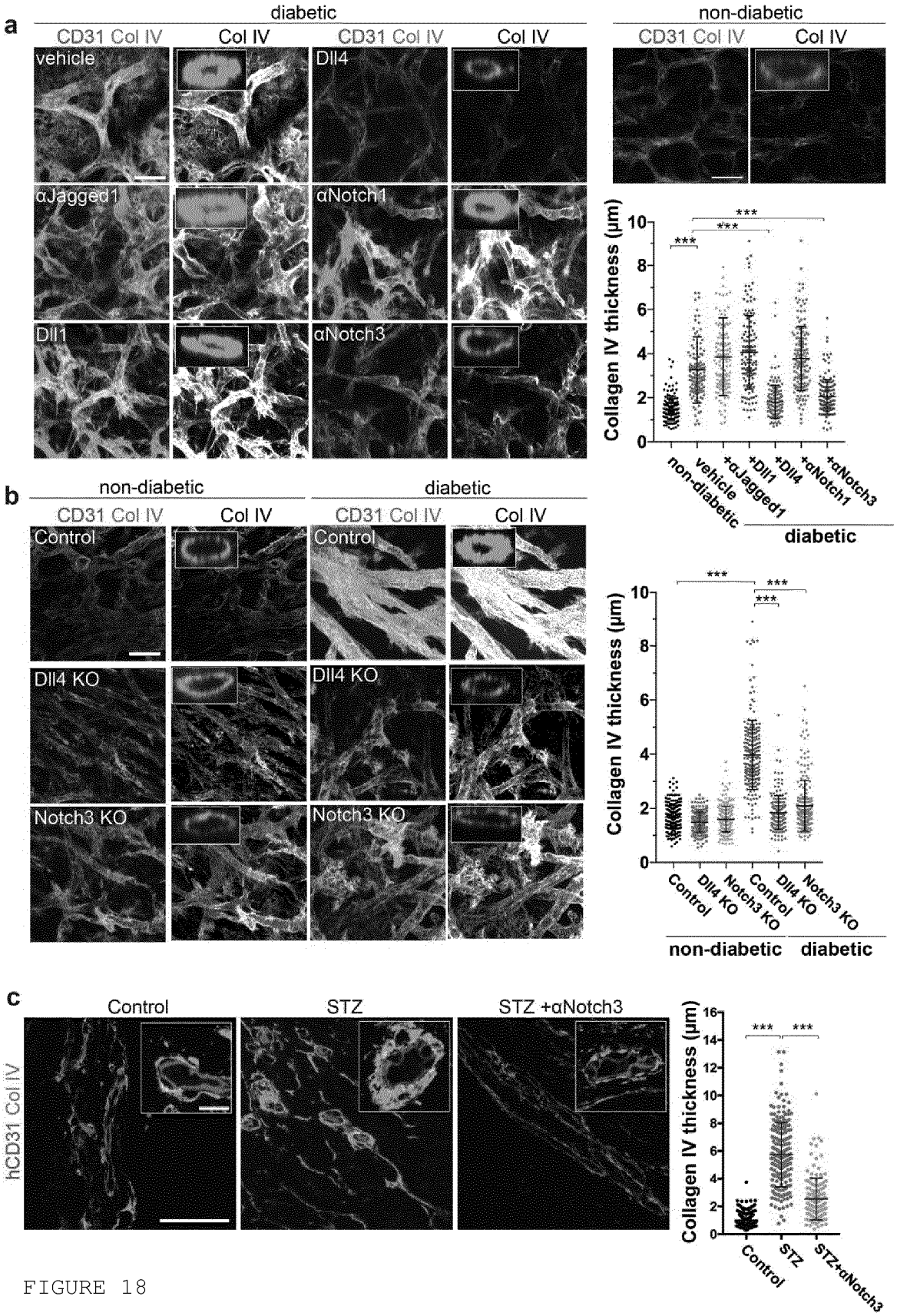

a
diabetic
non-diabetic
CD31 Col IV
Col IV
vehicle
Dll4
αJagged1
αNotch1
Dll1
αNotch3
Collagen IV thickness (µm)
non-diabetic
vehicle
+αJagged1
+Dll1
+Dll4
+αNotch1
+αNotch3
diabetic
b
non-diabetic
diabetic
Control
Dll4 KO
Notch3 KO
Control
Dll4 KO
Notch3 KO
Collagen IV thickness (µm)
non-diabetic
diabetic
c
Control
STZ
STZ +αNotch3
hCD31 Col IV
Collagen IV thickness (µm)
Control
STZ
STZ+αNotch3

FIGURE 18

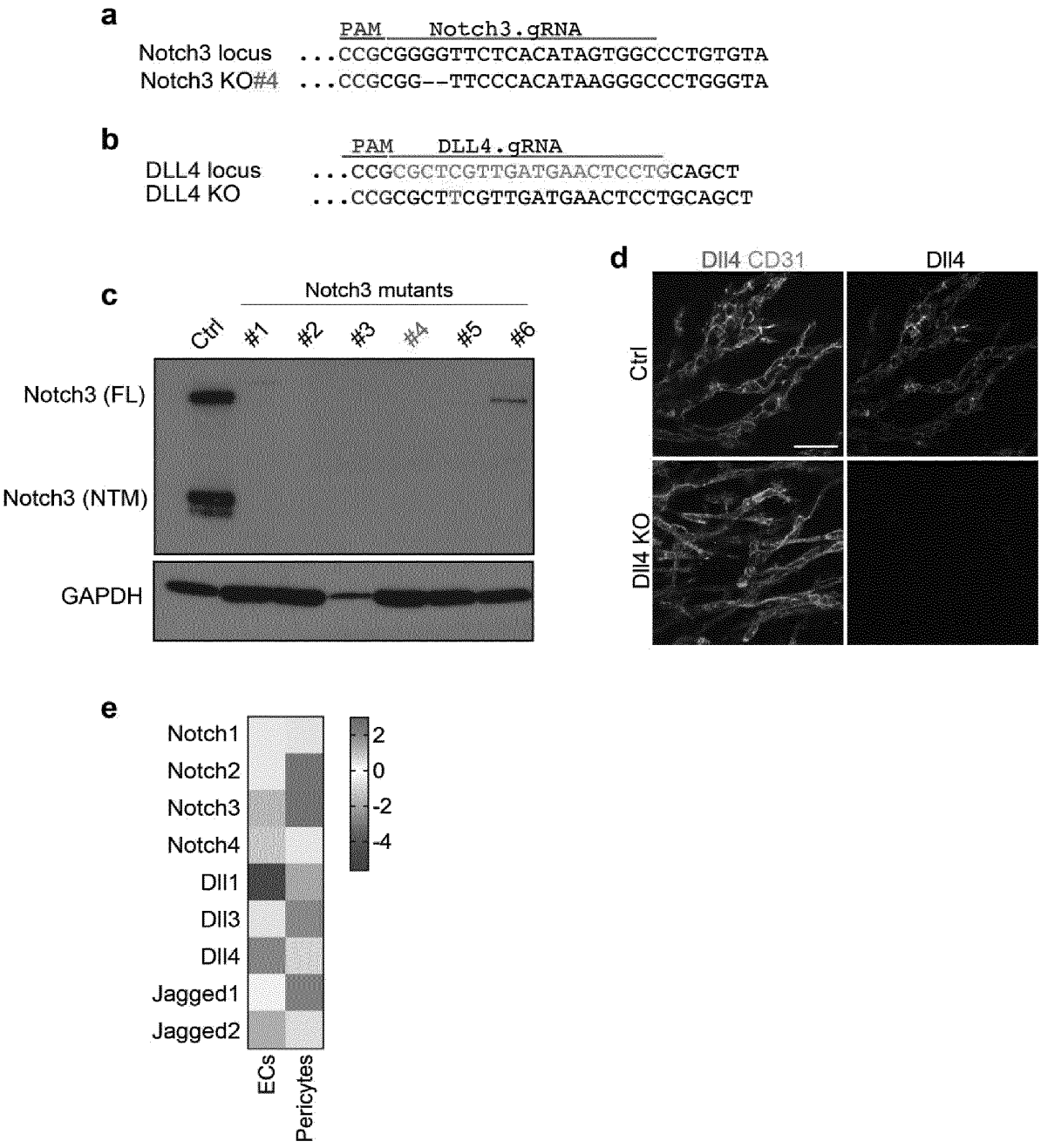

a
PAM Notch3.gRNA
Notch3 locus ...CCGCGGGGTTCTCACATAGTGGCCCTGTGTA
Notch3 KO#4 ...CCGCGG--TTCCCACATAAGGGCCCTGGGTA
b
PAM DLL4.gRNA
DLL4 locus ...CCGCGCTCGTTGATGAACTCCTGCAGCT
DLL4 KO ...CCGCGCTTCGTTGATGAACTCCTGCAGCT
c
Notch3 mutants
Ctrl
#1
#2
#3
#4
#5
#6
Notch3 (FL)
Notch3 (NTM)
GAPDH
d
Dll4 CD31
Dll4
Ctrl
Dll4 KO
e
Notch1
Notch2
Notch3
Notch4
Dll1
Dll3
Dll4
Jagged1
Jagged2
ECs
Pericytes
2
0
-2
-4

FIGURE 19

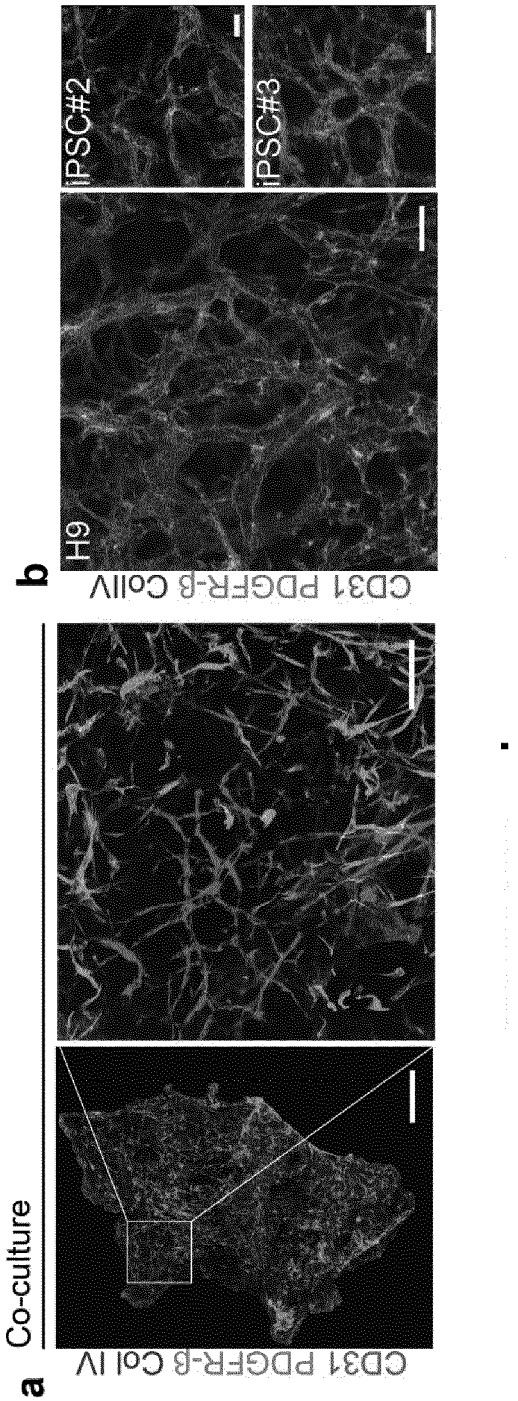

a
Co-culture
CD31 PDGFR-β Col IV
b
CD31 PDGFR-β ColIV
H9
iPSC#2
iPSC#3

FIGURE 20

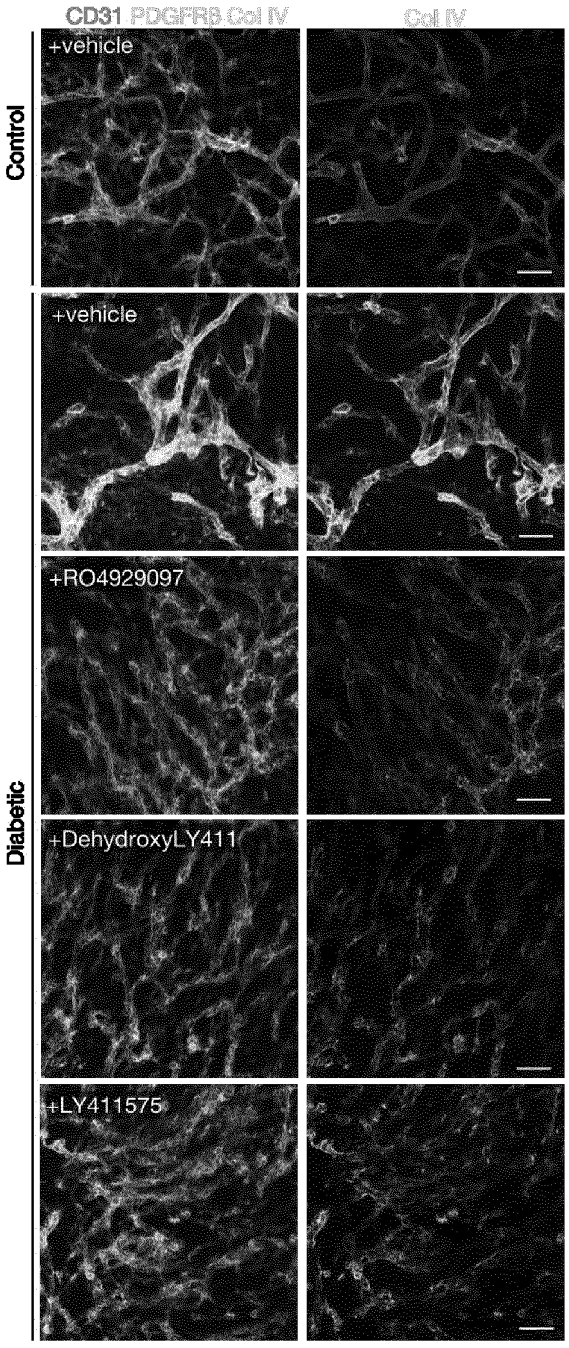

CD31 PDGFRB Col IV
Col IV
Control
Diabetic
+vehicle
+vehicle
+RO4929097
+DehydroxyLY411
+LY411575

FIGURE 21