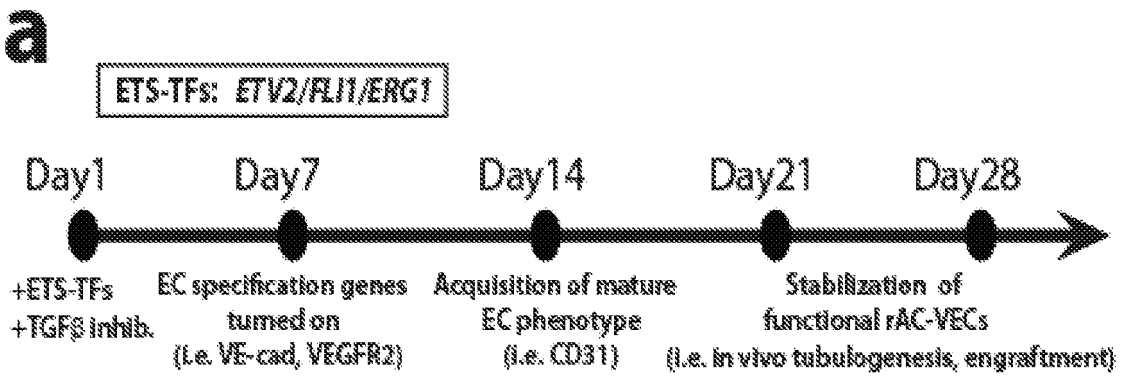
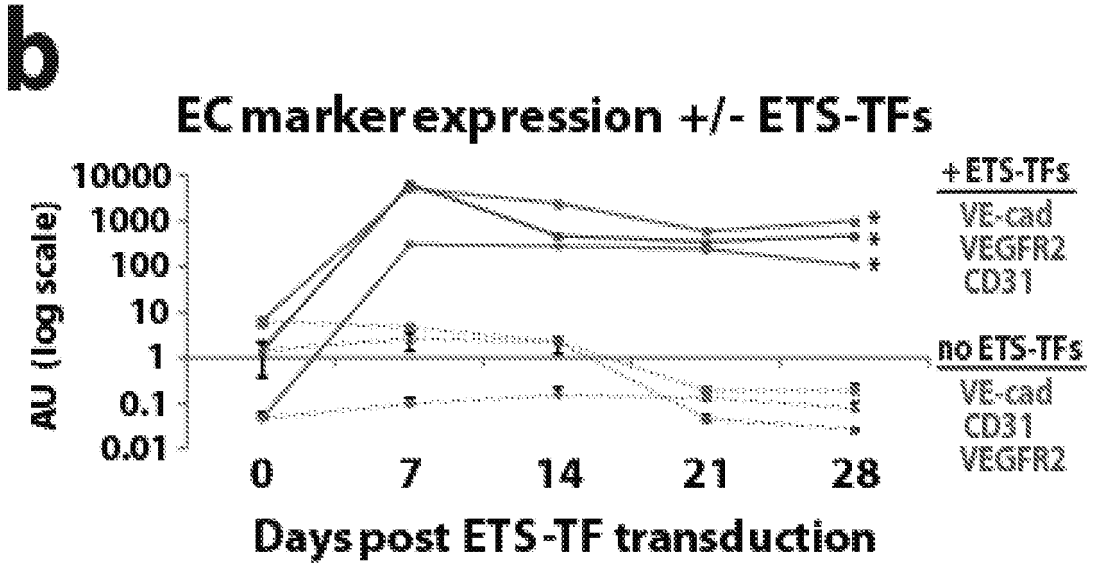
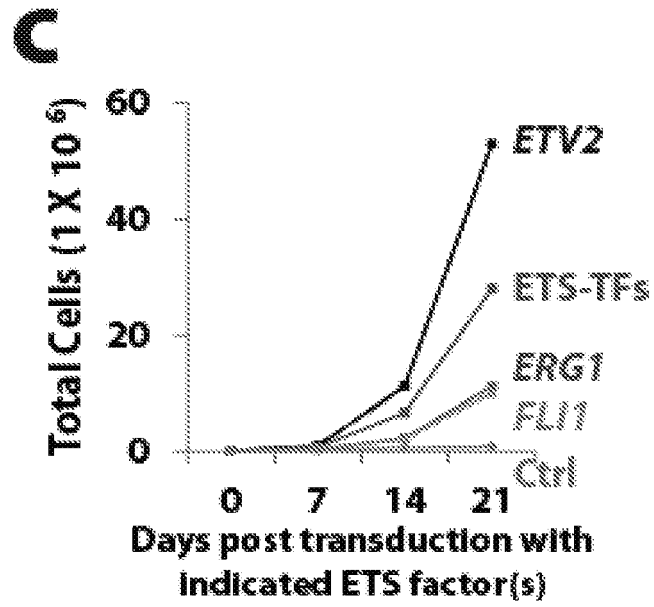
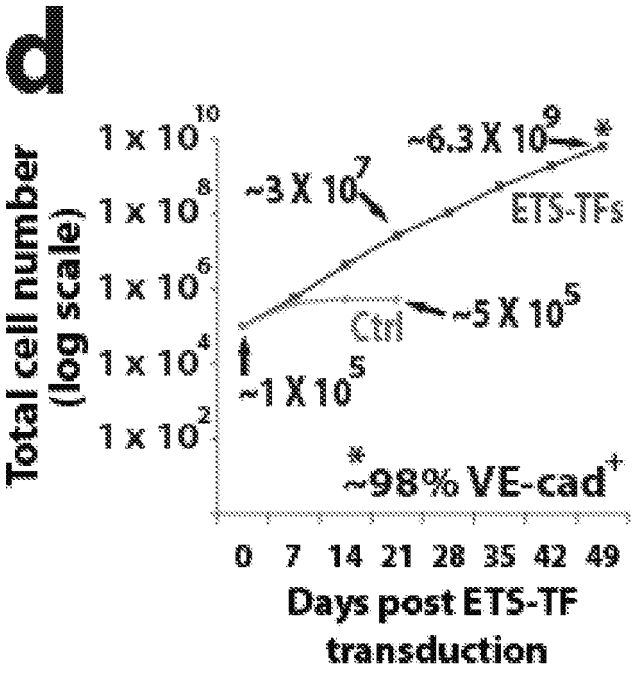

a
ETS-TFs: ETV2/FLI1/ERG1
Day1
Day7
Day14
Day21
Day28
+ETS-TFs
+TGFβ inhib.
EC specification genes turned on (i.e. VE-cad, VEGFR2)
Acquisition of mature EC phenotype (i.e. CD31)
Stabilization of functional rAC-VECs (i.e. in vivo tubulogenesis, engraftment)

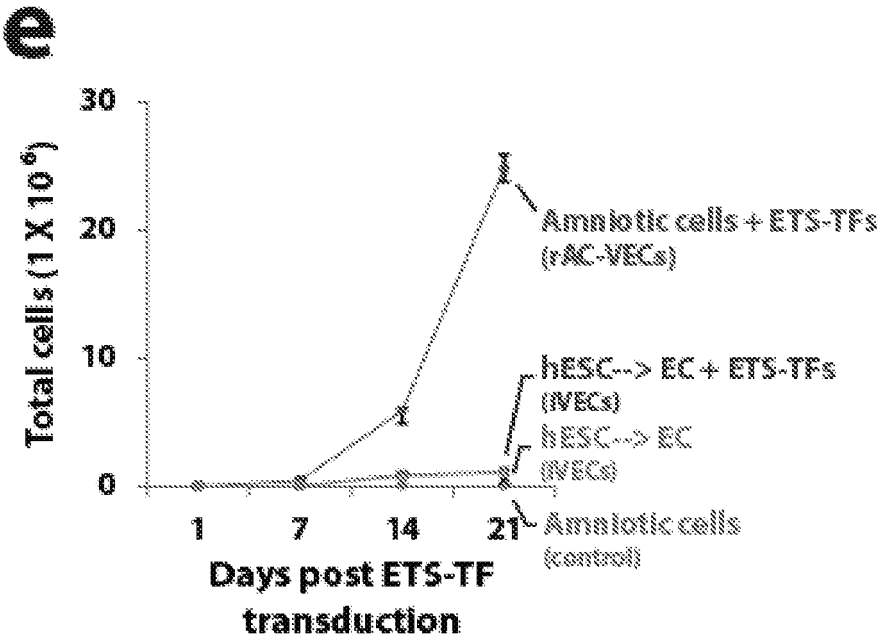

b
EC marker expression +/- ETS-TFs
AU (log scale)
10000
1000
100
10
1
0.1
0.01
0
7
14
21
28
Days post ETS-TF transduction
+ETS-TFs
VE-cad
VEGFR2
CD31
no ETS-TFs
VE-cad
CD31
VEGFR2

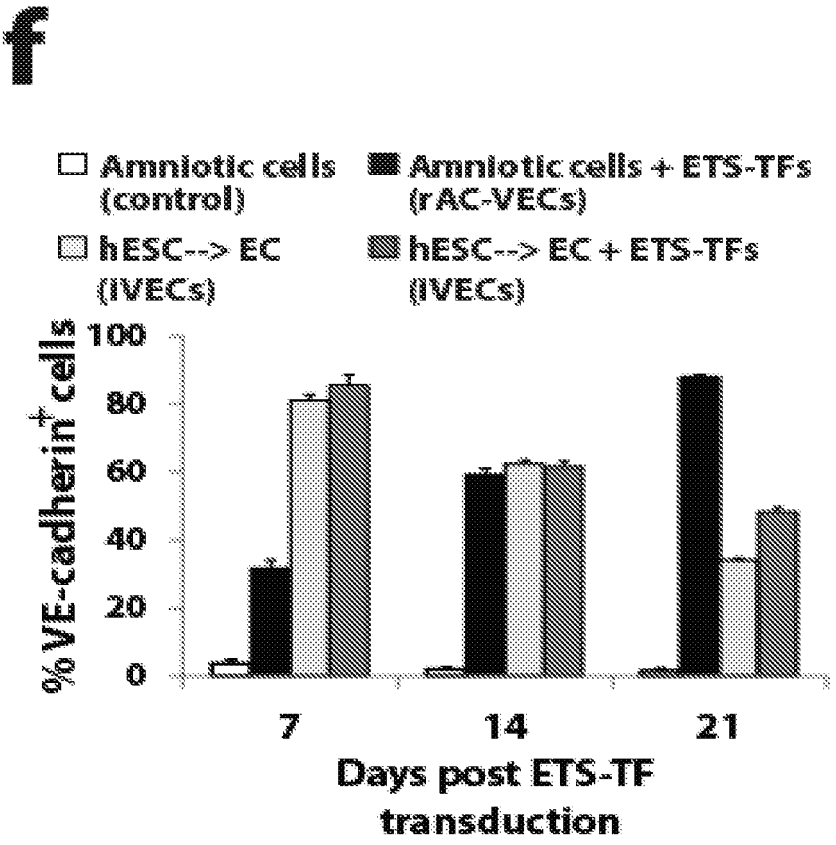
Figures 1a-b


c
Total Cells (1 X 10^6)
60
40
20
0
ETV2
ETS-TFs
ERG1
FLI1
Ctrl
0 7 14 21
Days post transduction with indicated ETS factor(s)


d
Total cell number (log scale)
1 x 10^10
1 x 10^8
1 x 10^6
1 x 10^4
1 x 10^2
~6.3 X 10^9
*
~3 X 10^7
ETS-TFs
Ctrl
~5 X 10^5
~1 X 10^5
*~98% VE-cad^+
0 7 14 21 28 35 42 49
Days post ETS-TF transduction

Figures 1c-d


e
Total cells (1 X 10^6)
30
20
10
0
1
7
14
21
Days post ETS-TF transduction
Amniotic cells + ETS-TFs (rAC-VECs)
hESC--> EC + ETS-TFs (iVECs)
hESC--> EC (iVECs)
Amniotic cells (control)


f
Amniotic cells (control)
Amniotic cells + ETS-TFs (rAC-VECs)
hESC--> EC (iVECs)
hESC--> EC + ETS-TFs (iVECs)
% VE-cadherin+ cells
100
80
60
40
20
0
7
14
21
Days post ETS-TF transduction

Figures 1e-f

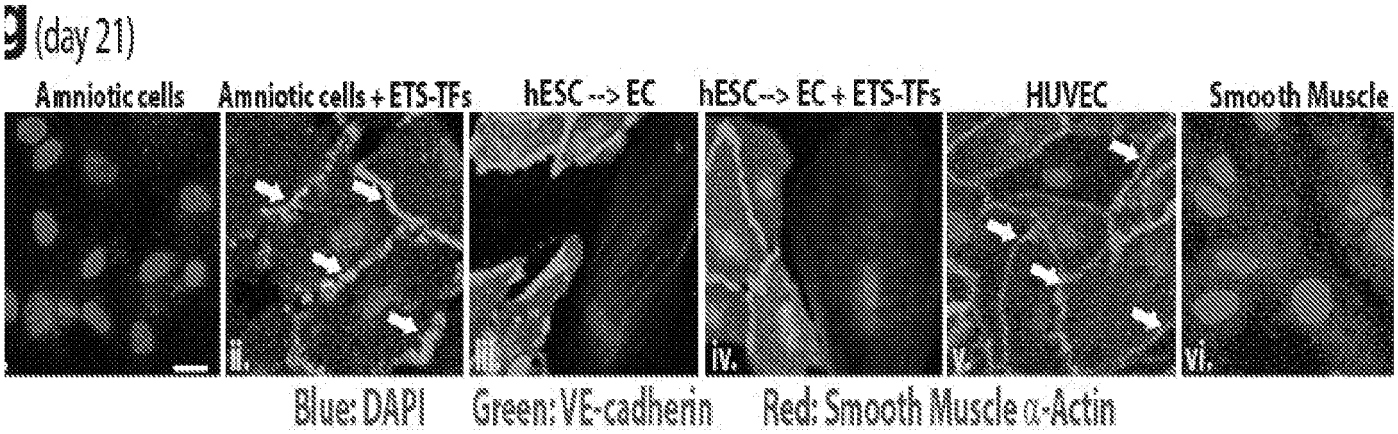

g (day 21)
Amniotic cells
Amniotic cells + ETS-TFs
hESC --> EC
hESC --> EC + ETS-TFs
HUVEC
Smooth Muscle
ii.
iii.
iv.
v.
vi.
Blue: DAPI
Green: VE-cadherin
Red: Smooth Muscle α-Actin

Figure 1g

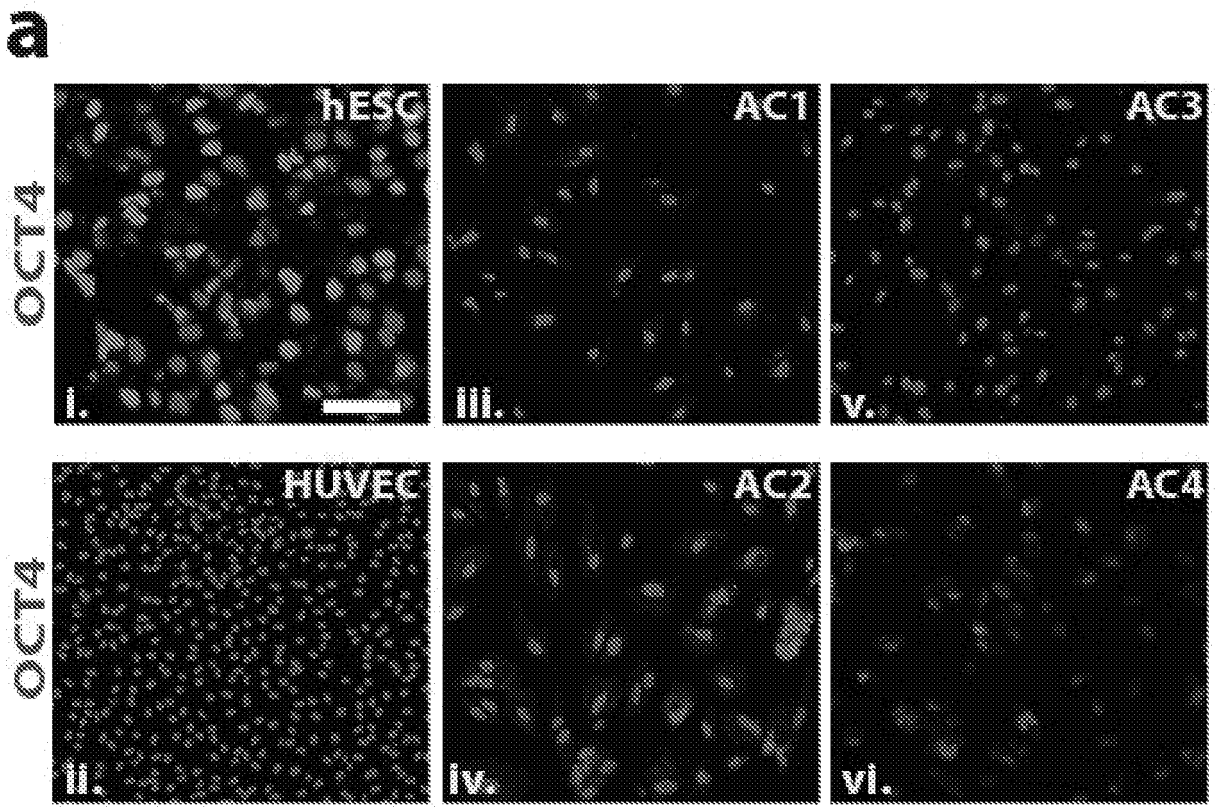


Figure 2a

b

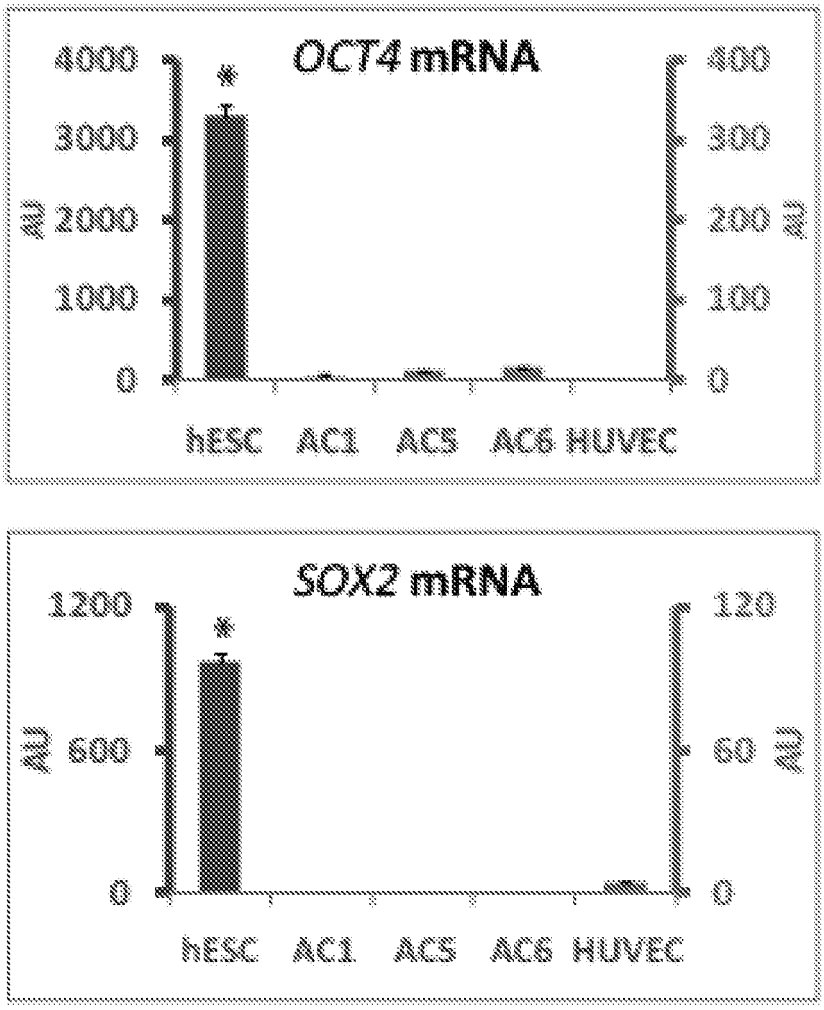
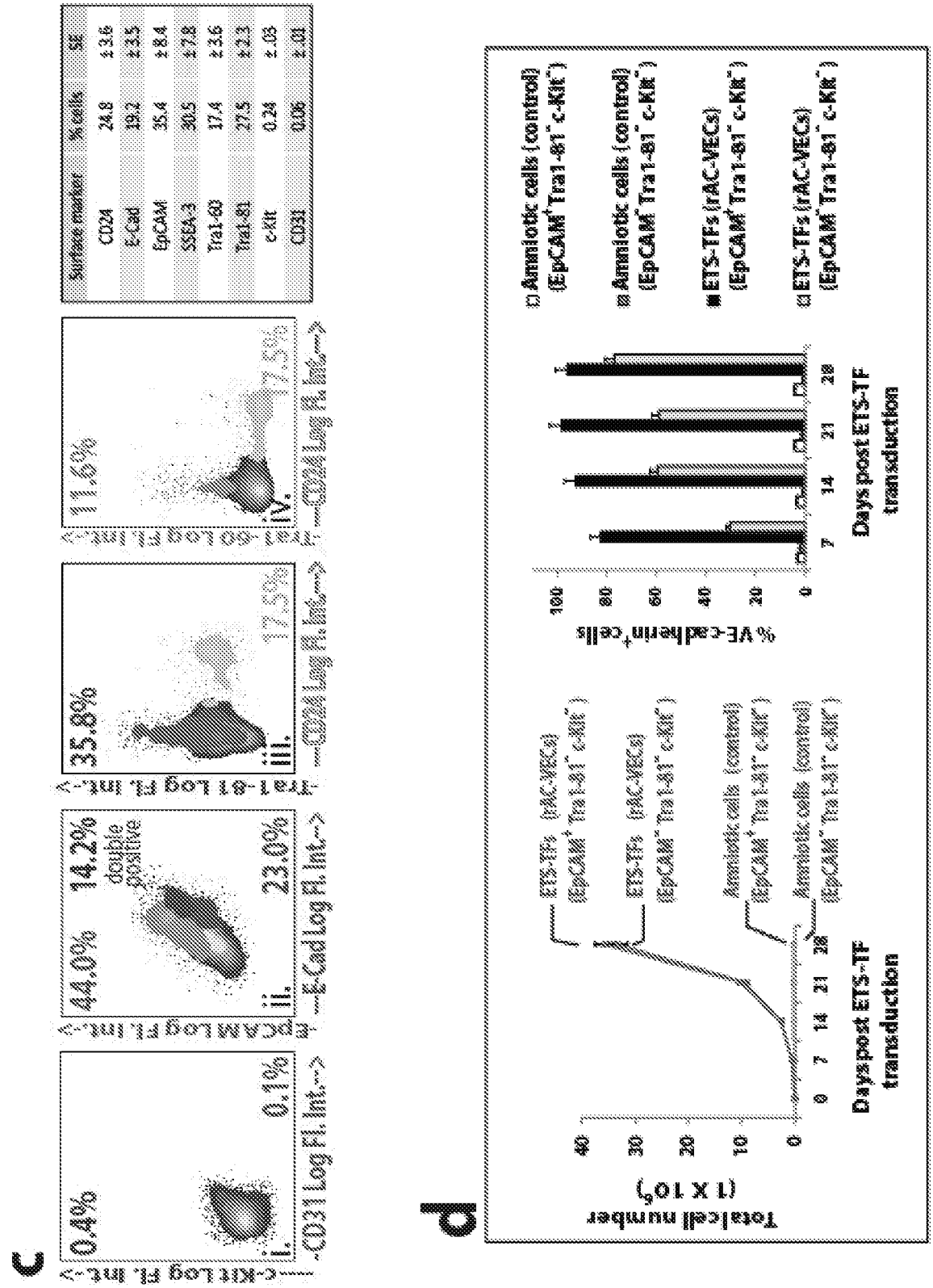

OCT4 mRNA
4000
3000
2000
1000
0
400
300
200
100
0
hESC
AC1
AC5
AC6
HUVEC
SOX2 mRNA
1200
600
0
120
60
0
hESC
AC1
AC5
AC6
HUVEC

Figure 2b


c
0.4%
i.
0.1%
44.0%
ii.
14.2%
double positive
23.0%
35.8%
iii.
17.5%
11.6%
iv.
17.5%
Surface marker % cells SE
CD24 24.8 ±3.6
E-Cad 19.2 ±3.5
EpCAM 35.4 ±8.4
SSEA-3 30.5 ±7.8
Tra1-60 17.4 ±3.6
Tra1-81 27.5 ±2.3
c-Kit 0.24 ±.03
CD31 0.06 ±.01
d
Amniotic cells (control) (EpCAM+Tra1-81+c-Kit+)
Amniotic cells (control) (EpCAM+Tra1-81+c-Kit-)
ETS-TFs (rAC-VECs) (EpCAM+Tra1-81+c-Kit+)
ETS-TFs (rAC-VECs) (EpCAM+Tra1-81+c-Kit-)
Total cell number (1 x 10^5)
% VE-cadherin+cells
Days post ETS-TF transduction

Figures 2c-d

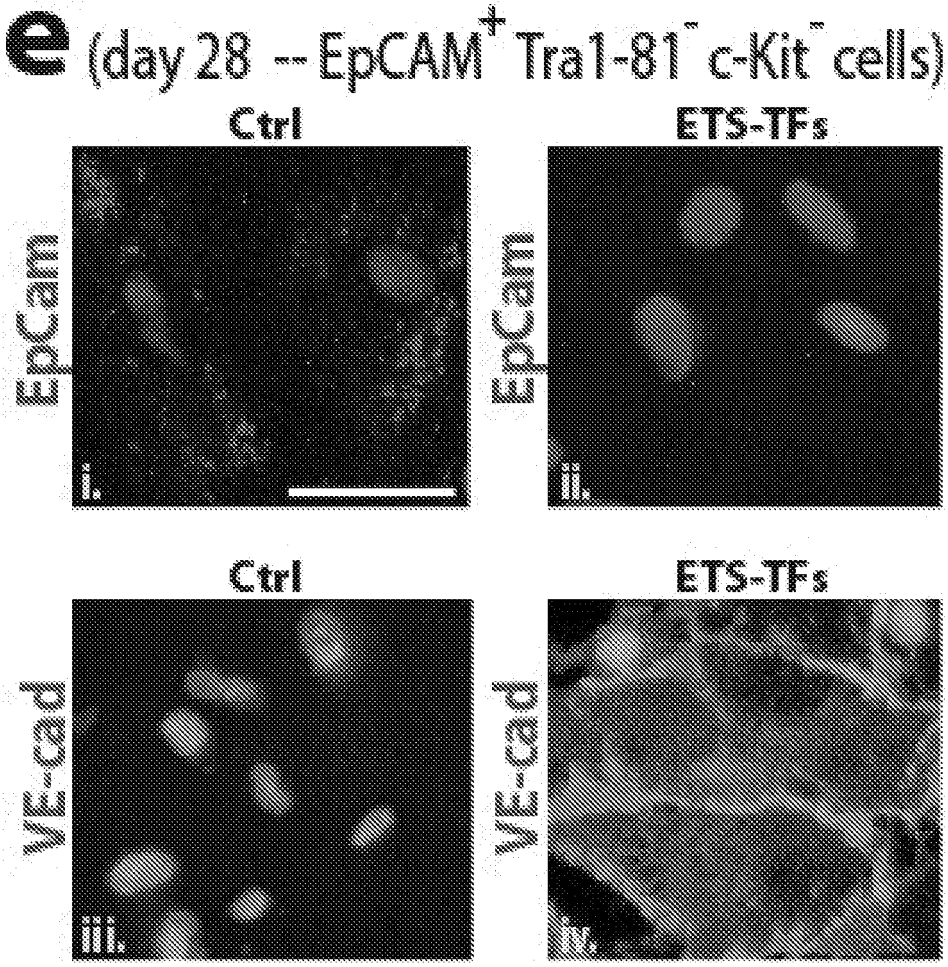

e (day 28 -- EpCAM+ Tra1-81- c-Kit- cells)
Ctrl
ETS-TFs
EpCam
EpCam
i.
ii.
Ctrl
ETS-TFs
VE-cad
VE-cad
iii.
iv.

Figure 2e

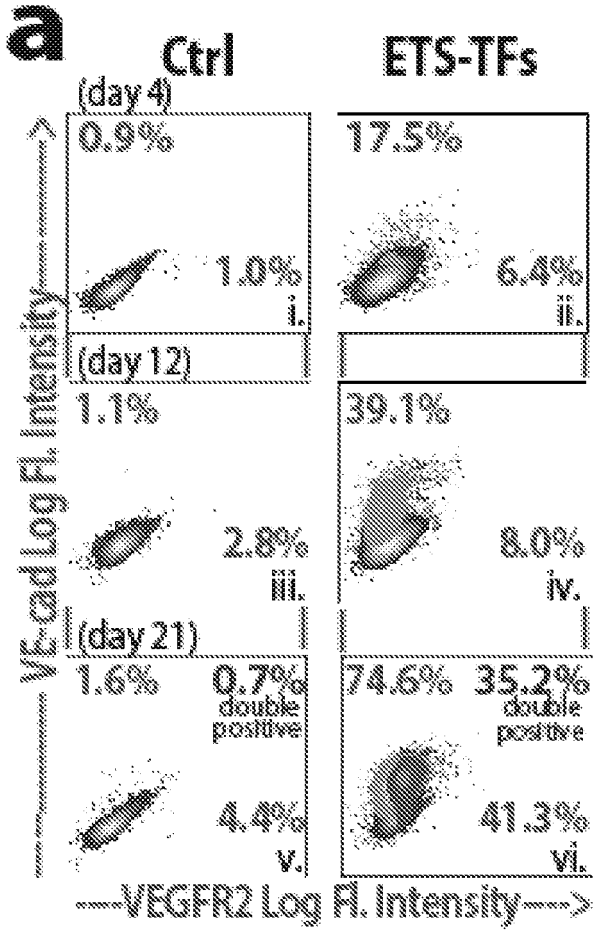

a
Ctrl
ETS-TFs
(day 4)
0.9%
1.0%
i.
17.5%
6.4%
ii.
(day 12)
1.1%
2.8%
iii.
39.1%
8.0%
iv.
(day 21)
1.6%
0.7%
double positive
4.4%
v.
74.6%
35.2%
double positive
41.3%
vi.
VE-cad Log Fl. Intensity
VEGFR2 Log Fl. Intensity

Figure 3a

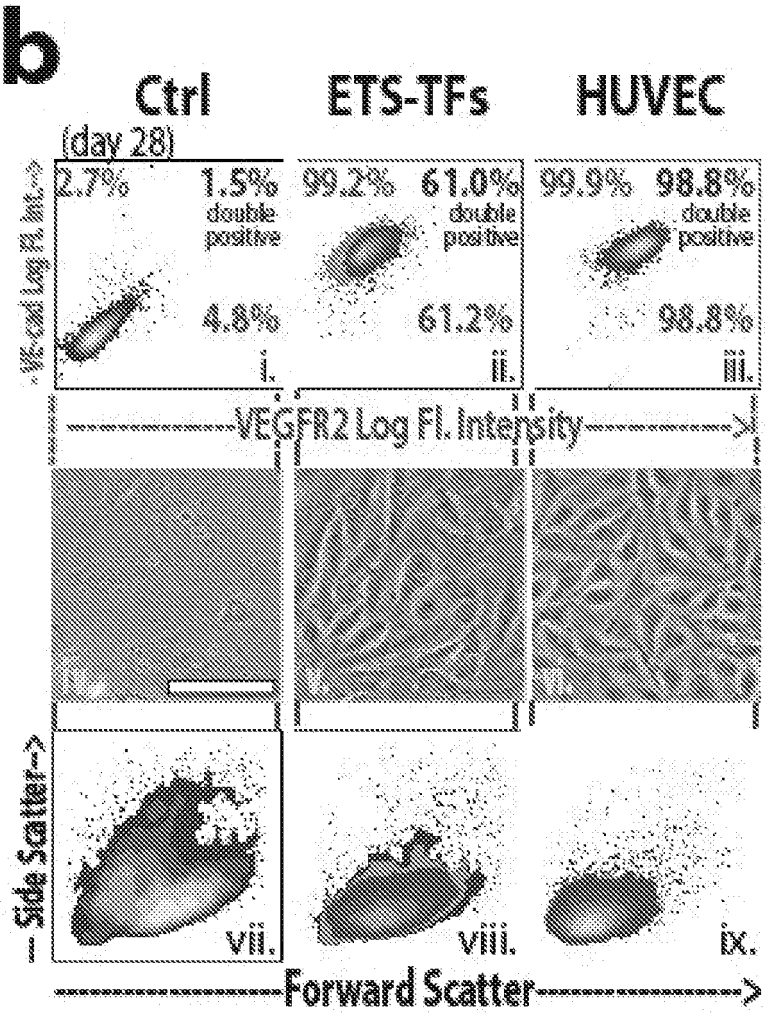
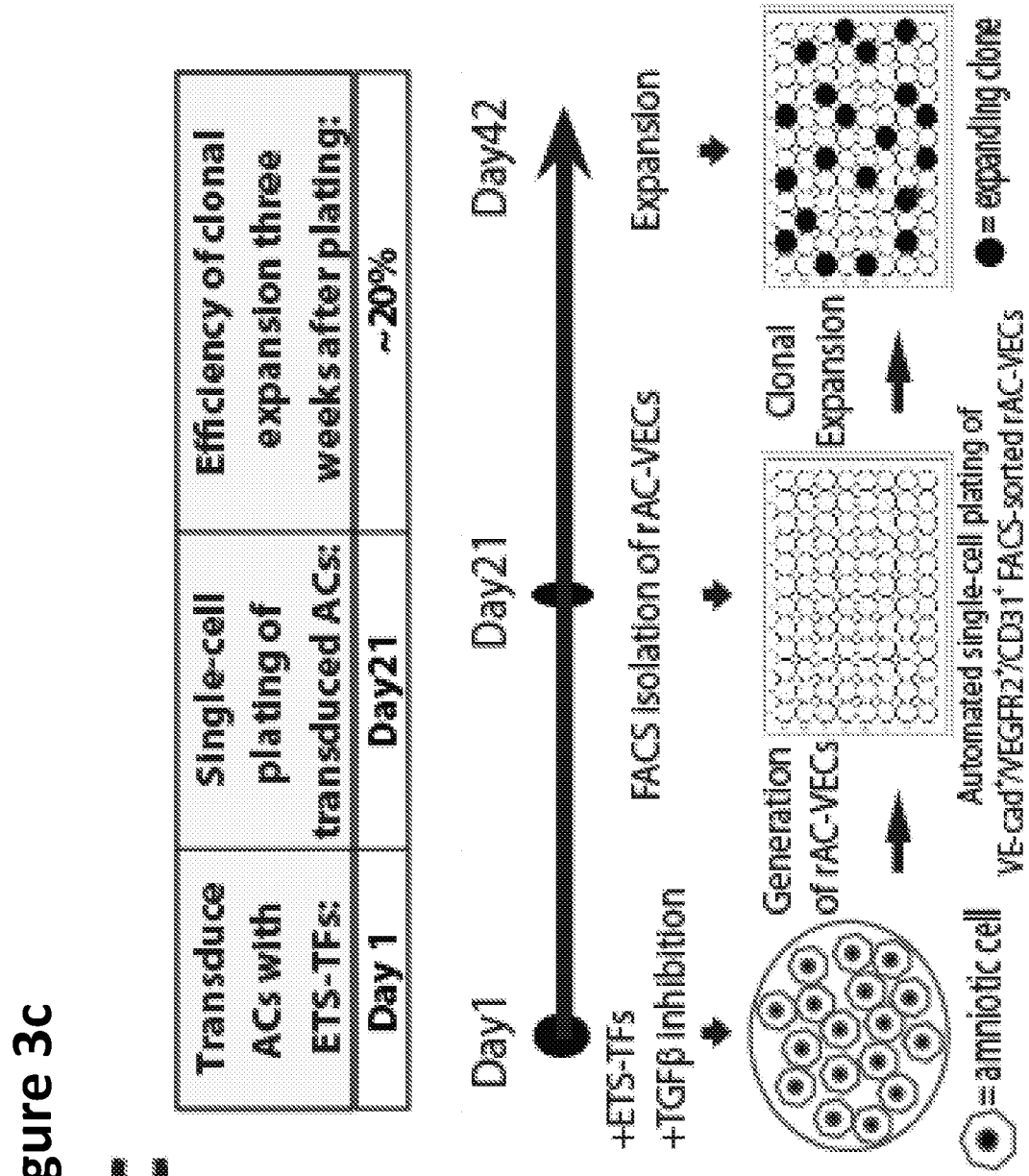

b
Ctrl
ETS-TFs
HUVEC
(day 28)
2.7%
1.5%
double positive
4.8%
i.
99.2%
61.0%
double positive
61.2%
ii.
99.9%
98.8%
double positive
98.8%
iii.
VE-cad Log Fl. Int.->
VEGFR2 Log Fl. Intensity
vii.
viii.
ix.
Side Scatter->
Forward Scatter

Figure 3b

Figure 3c

C

Transduce ACs with ETS-TFs:	Single-cell plating of transduced ACs:	Efficiency of clonal expansion three weeks after plating:
Day 1	Day21	~20%



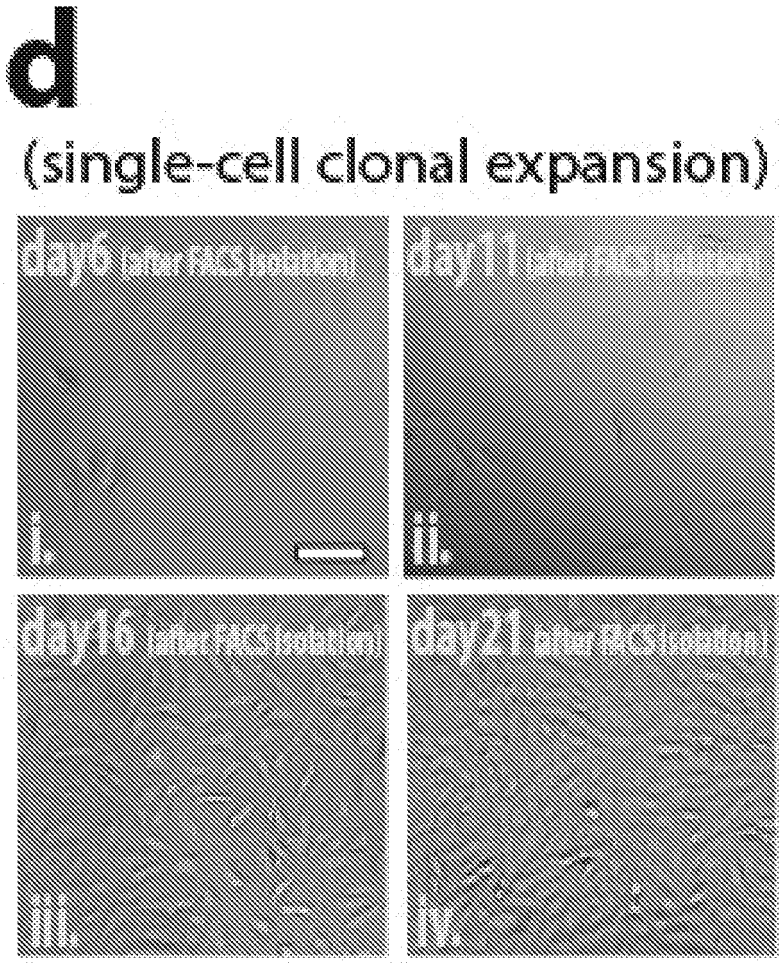

d
(single-cell clonal expansion)
day6 (after FACS isolation)
i.
day11 (after FACS isolation)
ii.
day16 (after FACS isolation)
iii.
day21 (after FACS isolation)
iv.

Figure 3d

Figure 3e

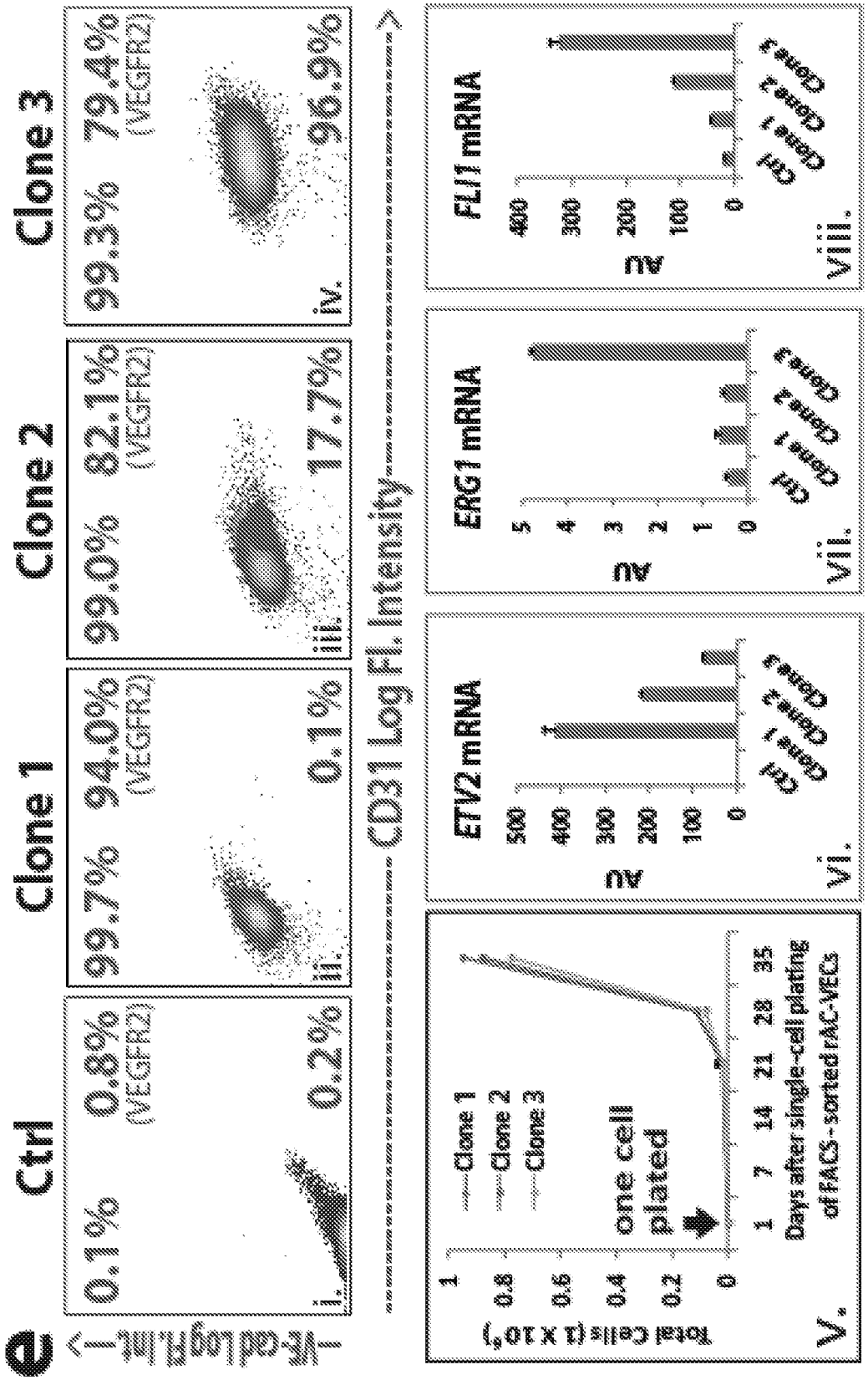
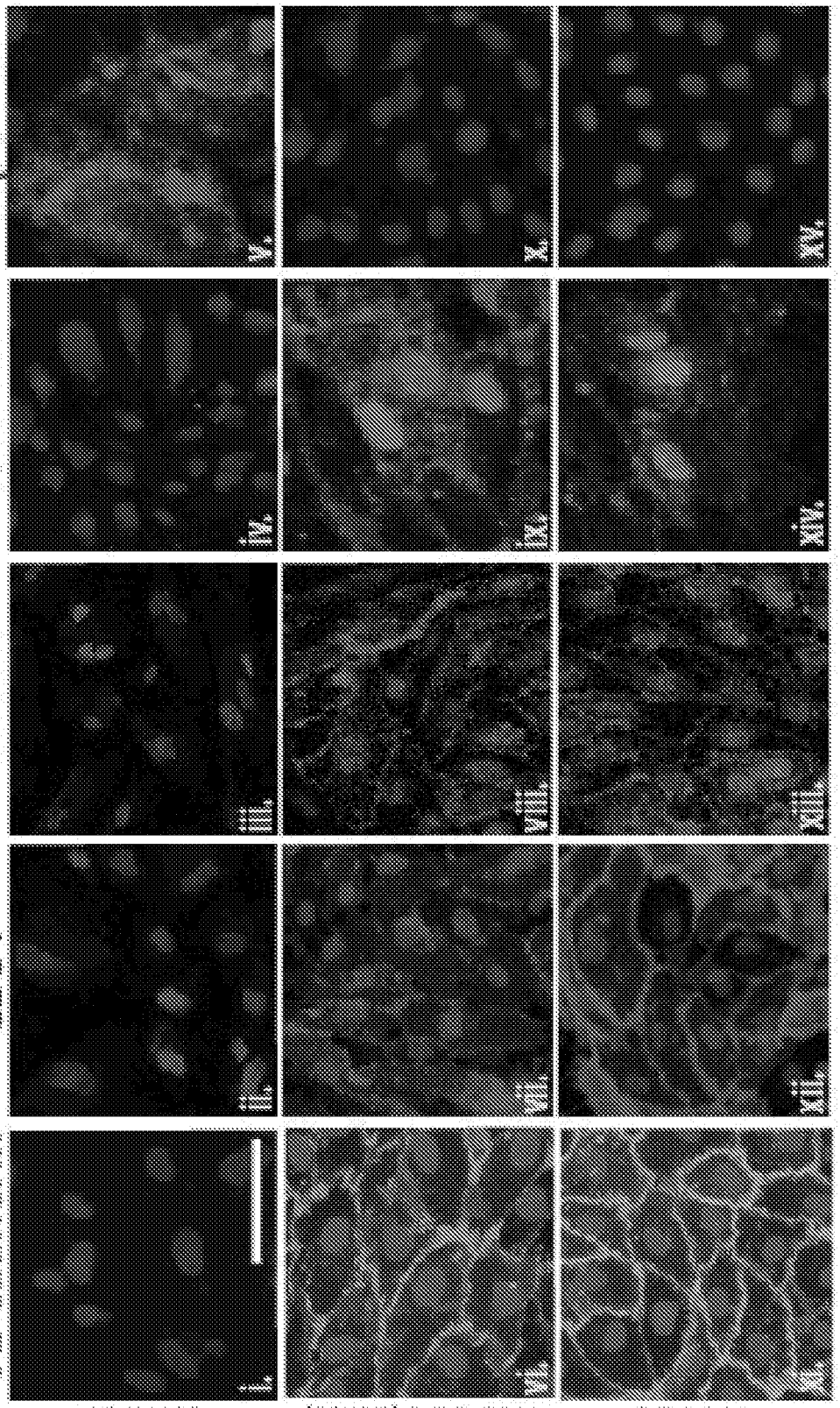

e
Ctrl
Clone 1
Clone 2
Clone 3
0.1%
0.8%
(VEGFR2)
99.7%
94.0%
(VEGFR2)
99.0%
82.1%
(VEGFR2)
99.3%
79.4%
(VEGFR2)
0.2%
0.1%
17.7%
96.9%
i.
ii.
iii.
iv.
VE-cad Log Fl. Intensity
CD31 Log Fl. Intensity
ETV2 mRNA
ERG1 mRNA
FLI1 mRNA
AU
Ctrl
Clone 1
Clone 2
Clone 3
vi.
vii.
viii.
Total Cells (1 x 10^5)
Clone 1
Clone 2
Clone 3
one cell plated
Days after single-cell plating of FACS-sorted rAC-VECs
v.

Figure 4a


a
VE-cadherin
CD31
ESAM
JAM-A
EpCAM
Control
rAC-VEC (clone 3)
HUVEC
i.
ii.
iii.
iv.
v.
vi.
vii.
viii.
ix.
x.
xi.
xii.
xiii.
xiv.
xv.

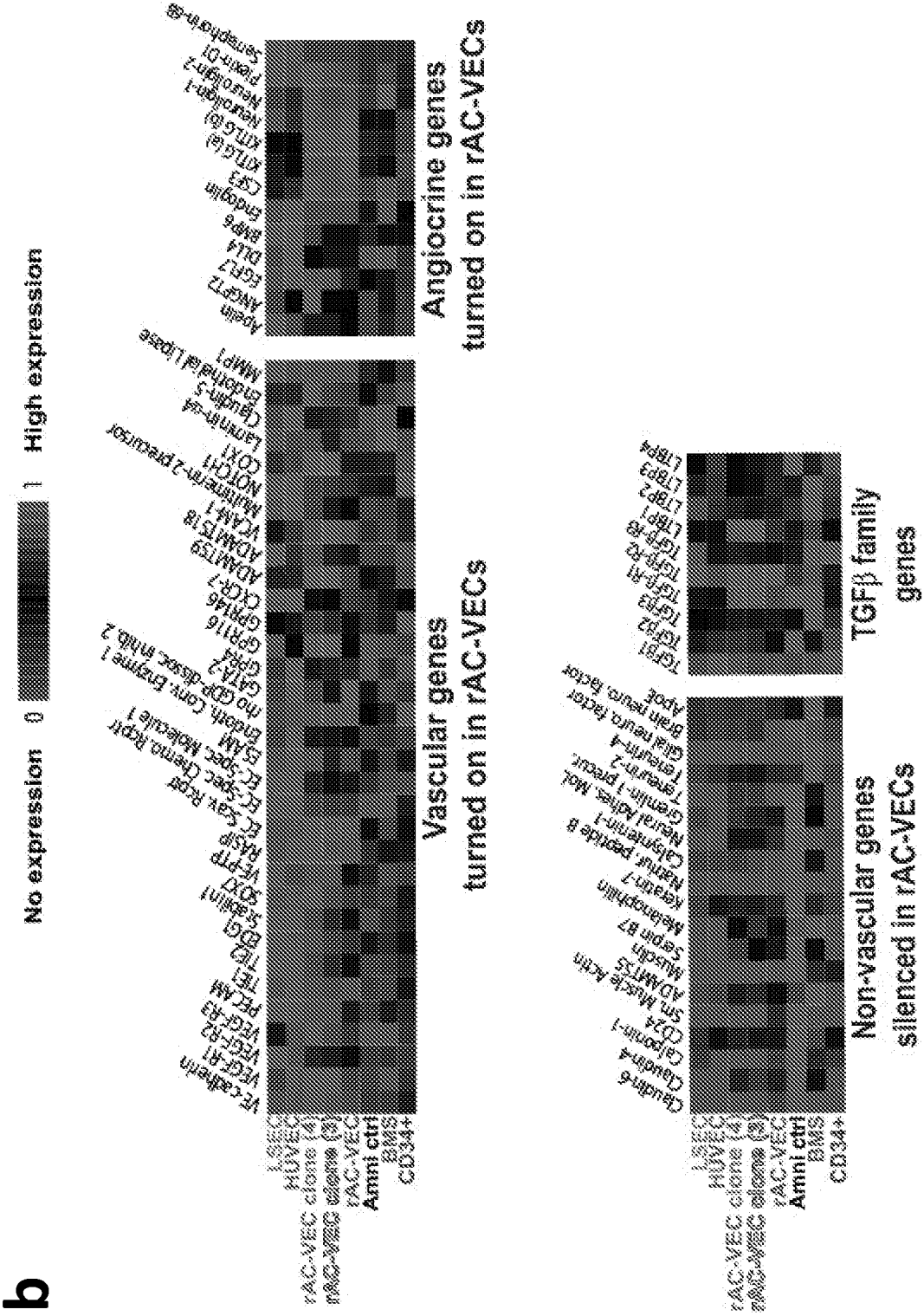
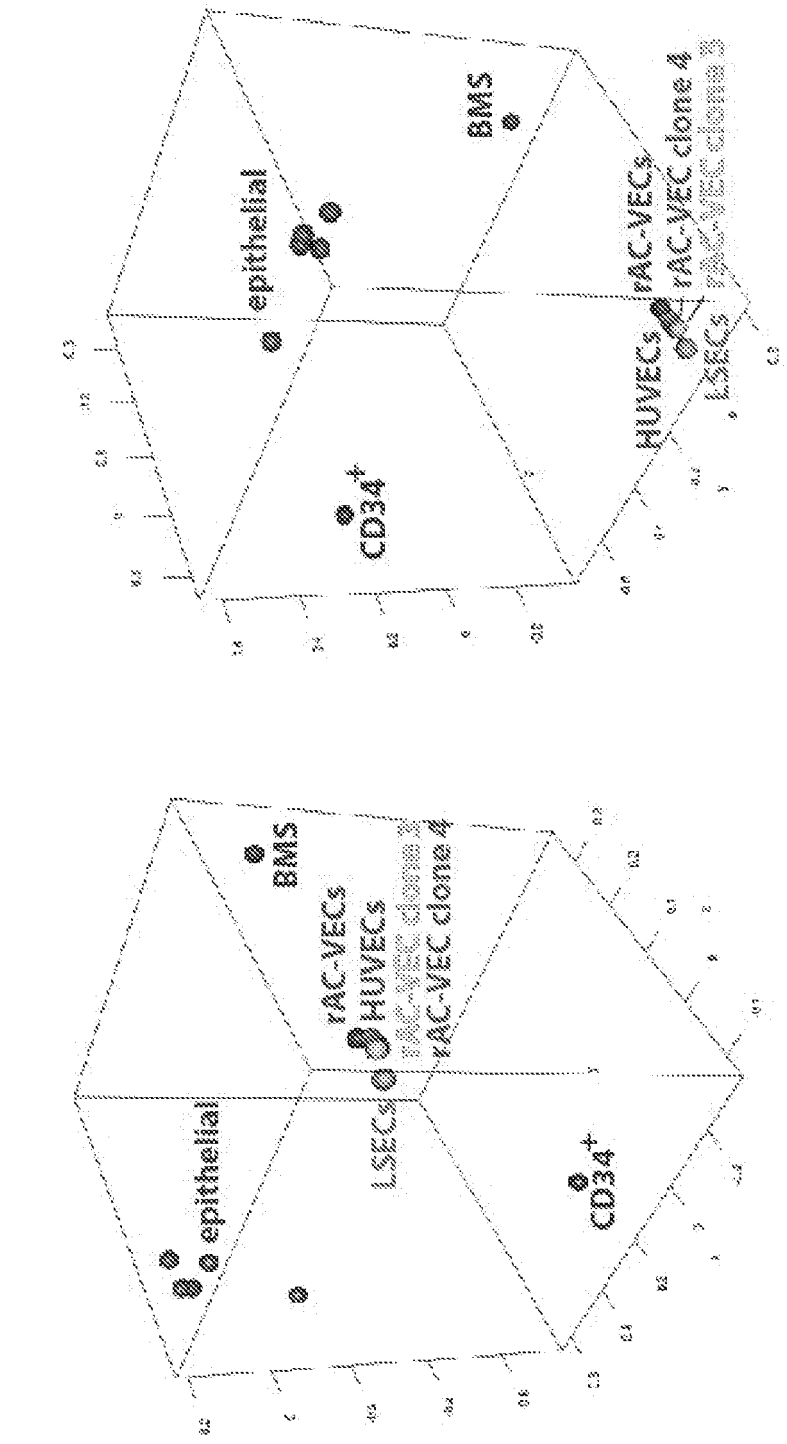
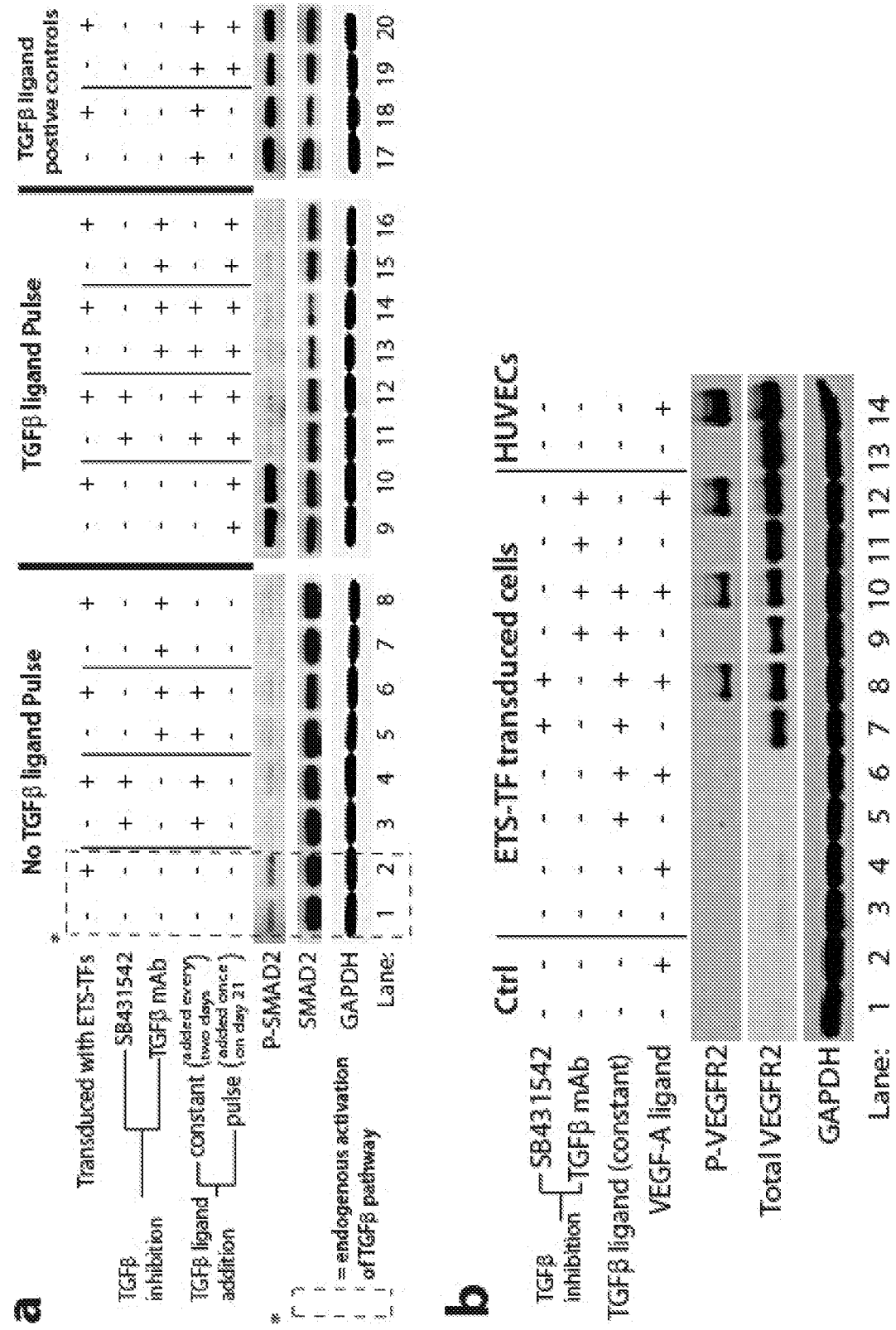

b
No expression
0
1
High expression
LSEC
HUVEC
rAC-VEC clone (14)
rAC-VEC clone (9)
rAC-VEC
Amni ctrl
BMS
CD34+
VE-cadherin
VEGF-A1
VEGF-A2
VEGF-A3
PECAM
TIE1
TIE2
5-alkylin
SCKY7
VE-PTP
RASIP
EC-Cave Rcdr
EC-Spec Chemo. Repr
EC-Spec Molecule 1
SAM
Endoth Conv Enzyme 1
mo GDP-dissoc inhib 2
GATA-2
GPR116
GPR146
CXCR7
ADAMTS9
ADAMTS18
VCAM-1
Multimerin-2 precursor
NOTCH1
COX1
Laminin-a4
Cyclophilin 3
Endothelial Lipase
MMP1
Adelin
ANGPT2
EGFL7
DLL4
ERP6
Endoglin
CXP3
KITLG (a)
KITLG (b)
Neuroblastin-1
Neuroligin-2
Plexin-D1
Semaphorin-4a
Angiocrine genes
turned on in rAC-VECs
Vascular genes
turned on in rAC-VECs
Claudin-6
Claudin-4
Calponin-1
CD24
Sm Muscle Actin
ADAMTS5
Mucsdin
Serpin B7
Melanophilin
Keratin-7
Nephur. peptide B
Cytospherin-1
Neural Adhes. Mol.
Grenilin-1 precur.
Tenascin-2
Tenascin-4
Glia neuro. factor
Brain neuro. factor
Apoe
TGFB1
TGFB2
TGFB3
TGFR-A1
TGFR-B
TGFBP1
LTBP2
LTBP3
LTBP4
Non-vascular genes
silenced in rAC-VECs
TGFβ family
genes

Figure 4b



C

Figure 4c


a
No TGFβ ligand Pulse
TGFβ ligand Pulse
TGFβ ligand positive controls
Transduced with ETS-TFs
TGFβ inhibition
SB431542
TGFβ mAb
TGFβ ligand addition
constant (added every two days)
pulse (added once on day 21)
P-SMAD2
SMAD2
GAPDH
= endogenous activation of TGFβ pathway
Lane:
b
Ctrl
ETS-TF transduced cells
HUVECs
TGFβ inhibition
SB431542
TGFβ mAb
TGFβ ligand (constant)
VEGF-A ligand
P-VEGFR2
Total VEGFR2
GAPDH
Lane:

Figures 5a-b

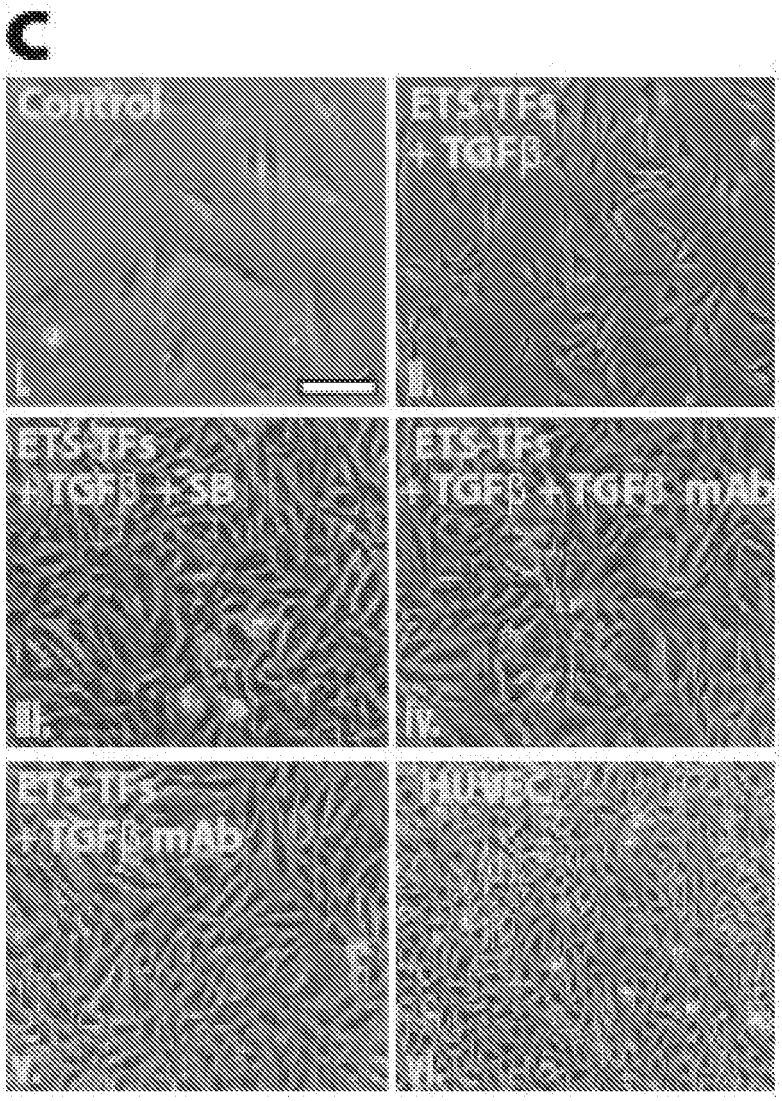
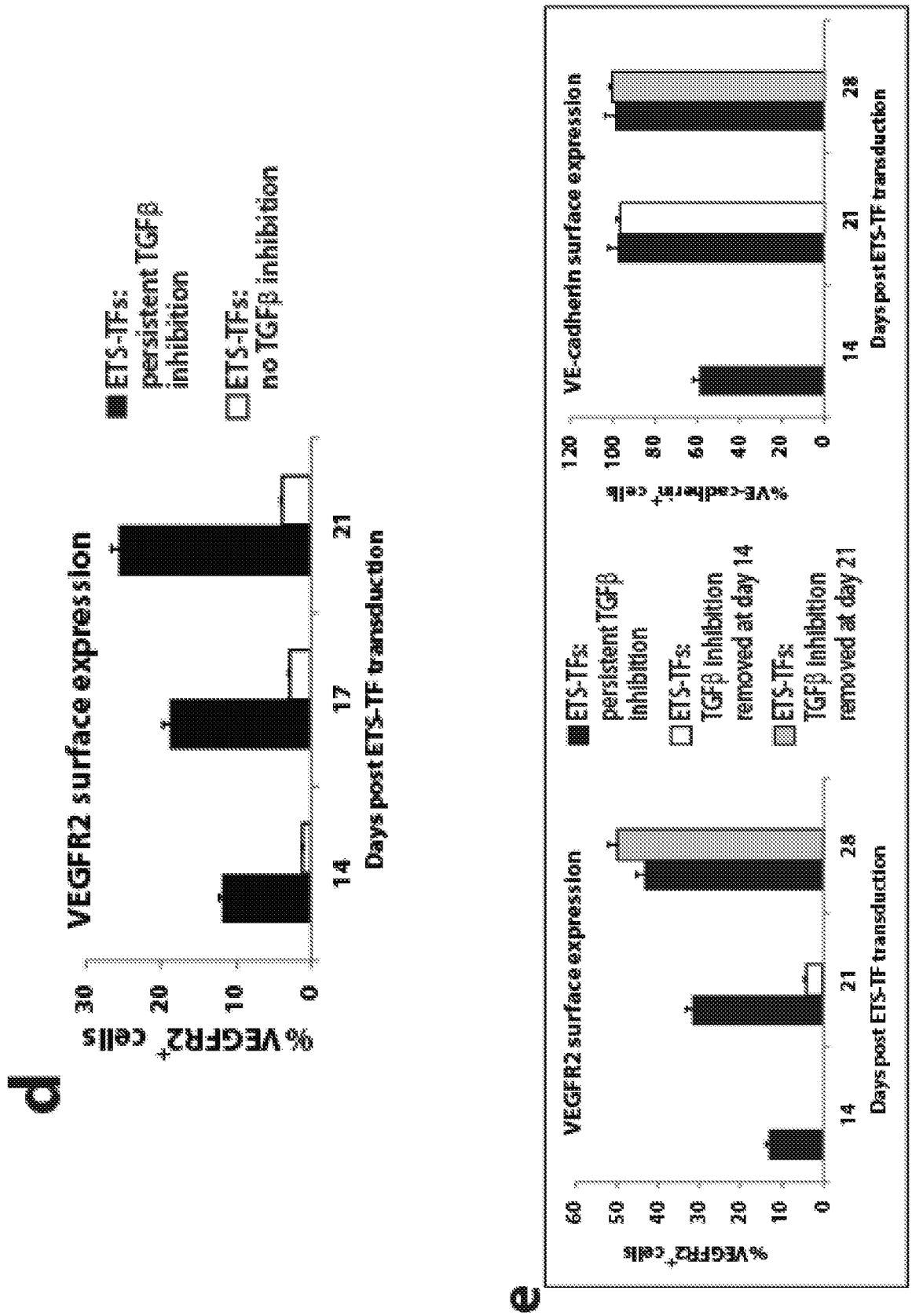
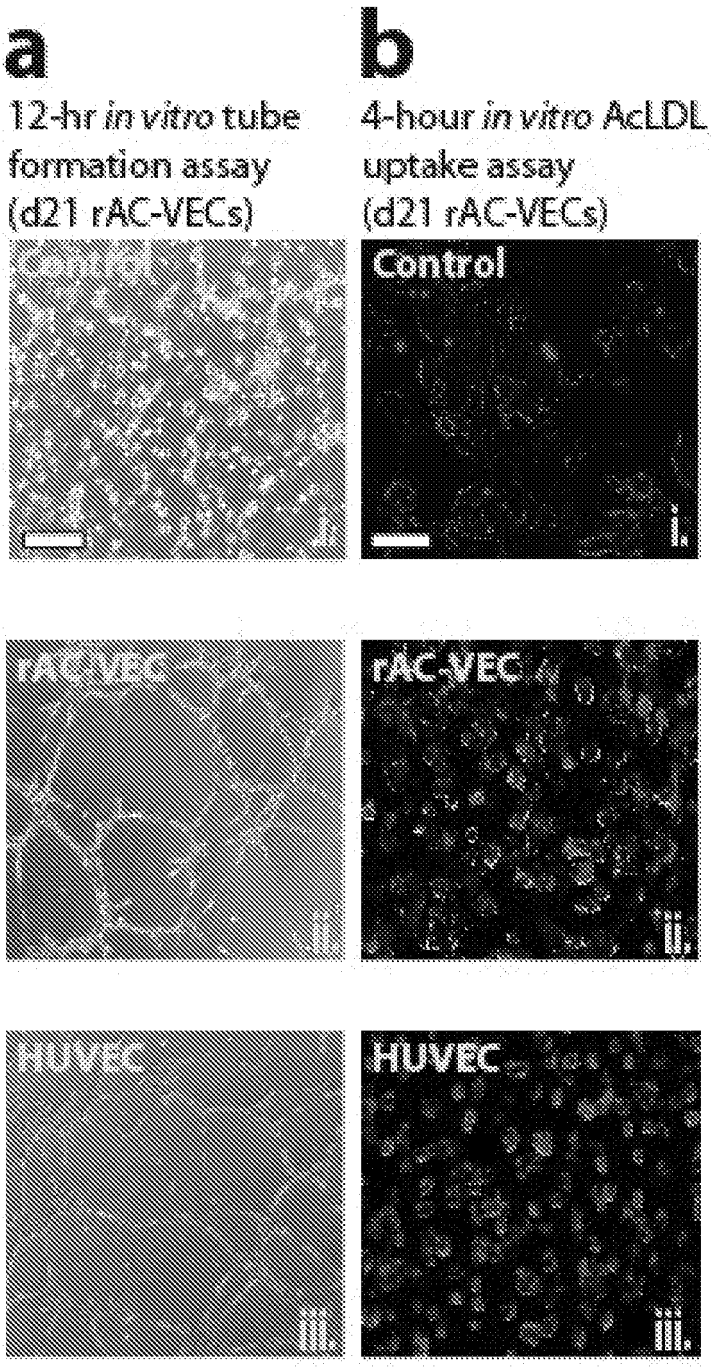

C
Control
ETS-TFs + TGFβ
ETS-TFs + TGFβ + SB
ETS-TFs + TGFβ + TGFβ mAb
ETS-TFs + TGFβ mAb
HUVEC

Figure 5c


d
VEGFR2 surface expression
% VEGFR2+ cells
30
20
10
0
14
17
21
Days post ETS-TF transduction
ETS-TFs: persistent TGFβ inhibition
ETS-TFs: no TGFβ inhibition
e
VEGFR2 surface expression
% VEGFR2+ cells
60
50
40
30
20
10
0
14
21
28
Days post ETS-TF transduction
ETS-TFs: persistent TGFβ inhibition
ETS-TFs: TGFβ inhibition removed at day 14
ETS-TFs: TGFβ inhibition removed at day 21
VE-cadherin surface expression
% VE-cadherin+ cells
120
100
80
60
40
20
0
14
21
28
Days post ETS-TF transduction

Figures 5d-e


a
12-hr *in vitro* tube formation assay (d21 rAC-VECs)
b
4-hour *in vitro* AcLDL uptake assay (d21 rAC-VECs)
Control
i.
rAC-VEC
ii.
HUVEC
iii.

Figures 6a-b

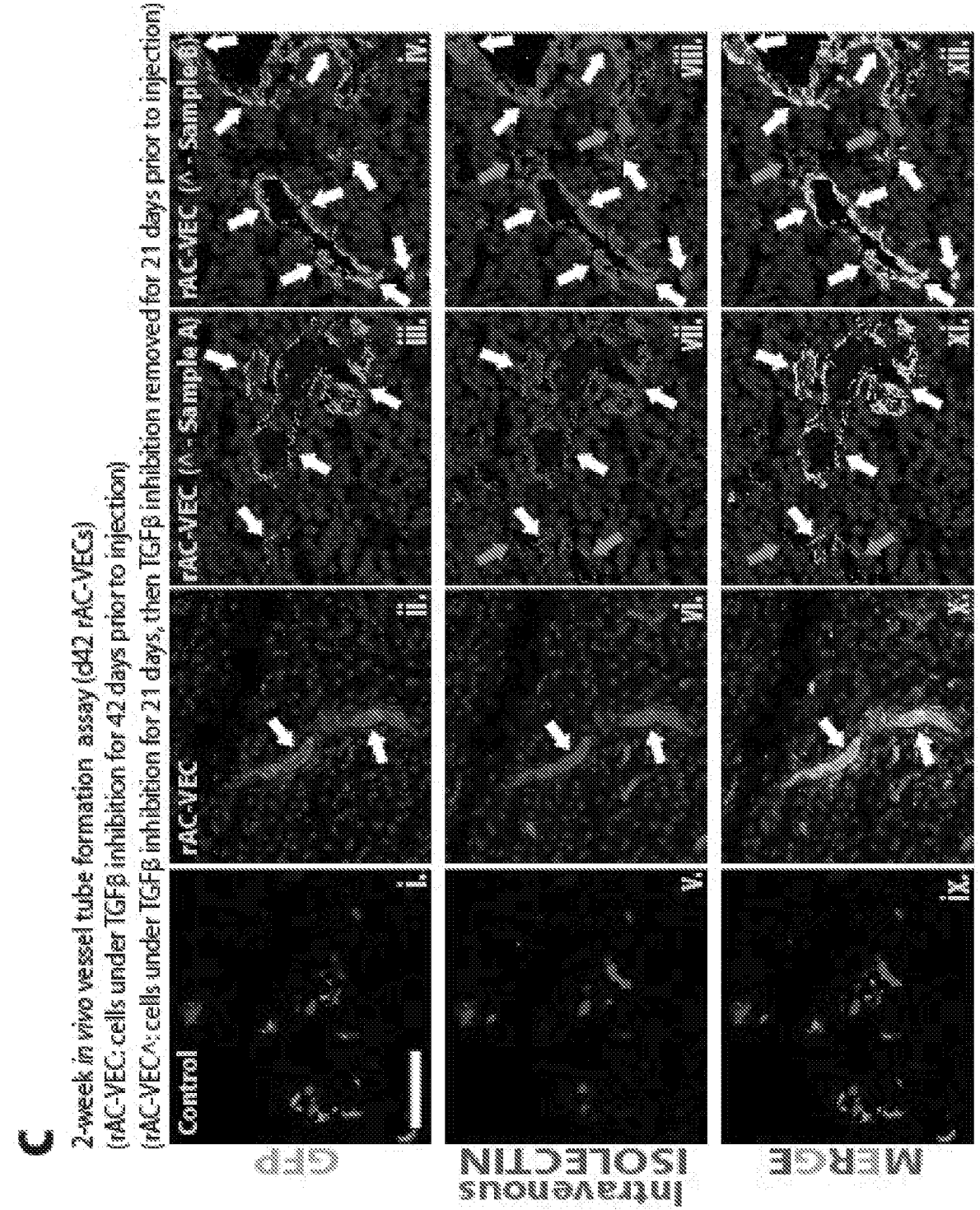
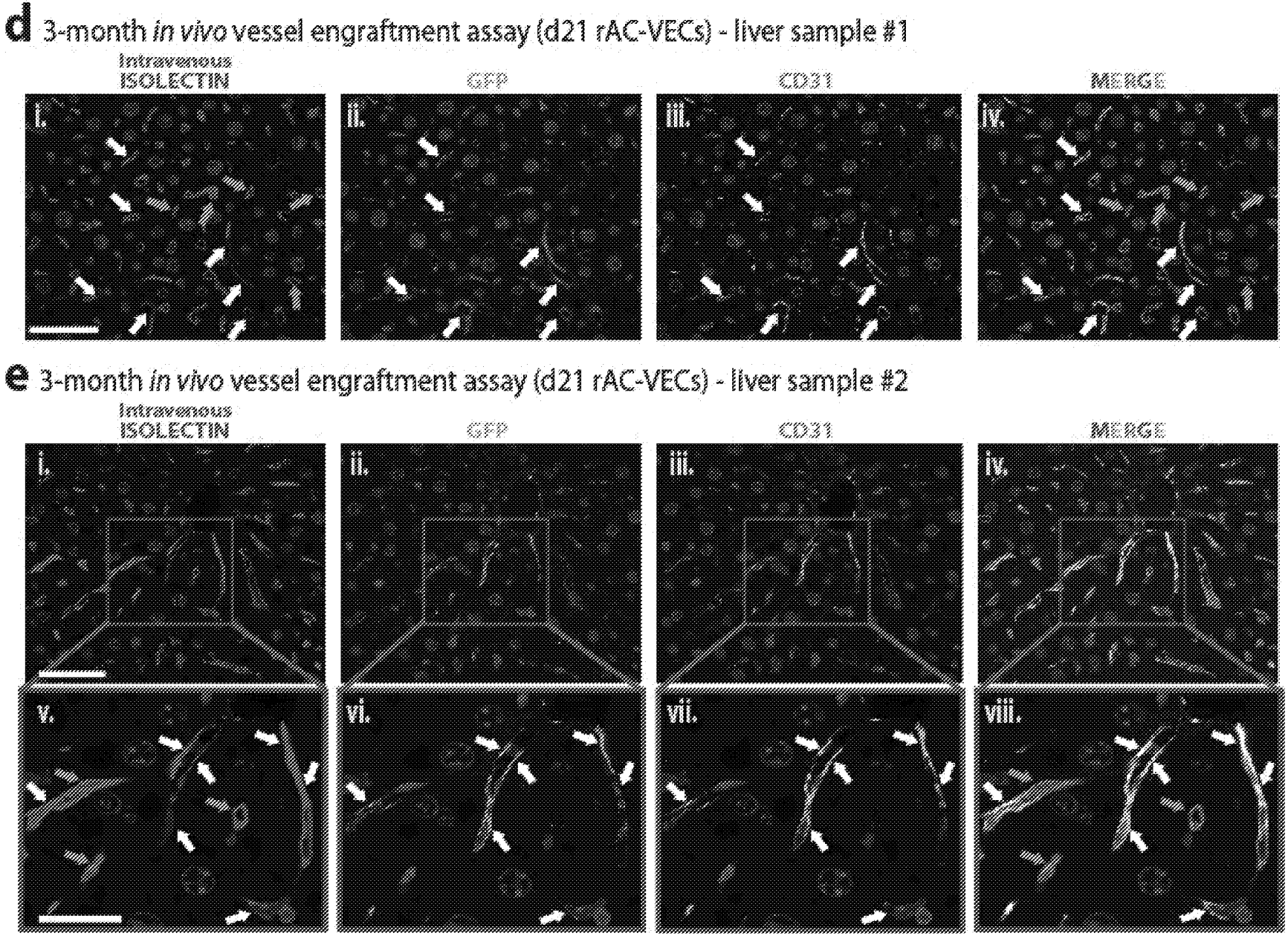

C
2-week in vivo vessel tube formation assay (d42 rAC-VECs)
(rAC-VEC: cells under TGFβ inhibition for 42 days prior to injection)
(rAC-VEC Δ: cells under TGFβ inhibition for 21 days, then TGFβ inhibition removed for 21 days prior to injection)
Control
rAC-VEC
rAC-VEC (Δ - Sample A)
rAC-VEC (Δ - Sample B)
GFP
Intravenous ISOLECTIN
MERGE
i.
ii.
iii.
iv.
v.
vi.
vii.
viii.
ix.
x.
xi.
xii.

Figure 6c

Figures 6d-e


d 3-month *in vivo* vessel engraftment assay (d21 rAC-VECs) - liver sample #1
Intravenous ISOLECTIN
GFP
CD31
MERGE
i.
ii.
iii.
iv.
e 3-month *in vivo* vessel engraftment assay (d21 rAC-VECs) - liver sample #2
Intravenous ISOLECTIN
GFP
CD31
MERGE
i.
ii.
iii.
iv.
v.
vi.
vii.
viii.

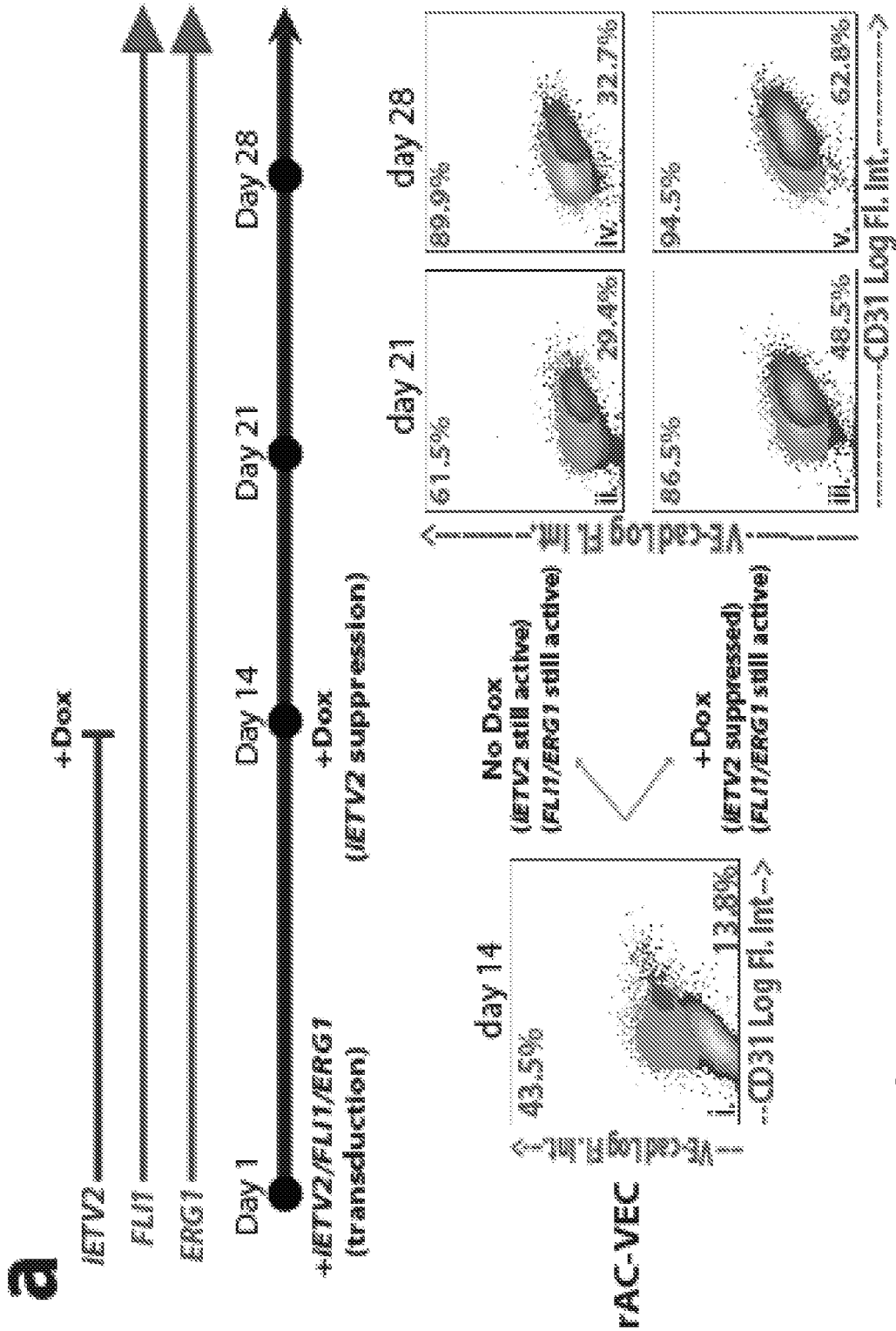
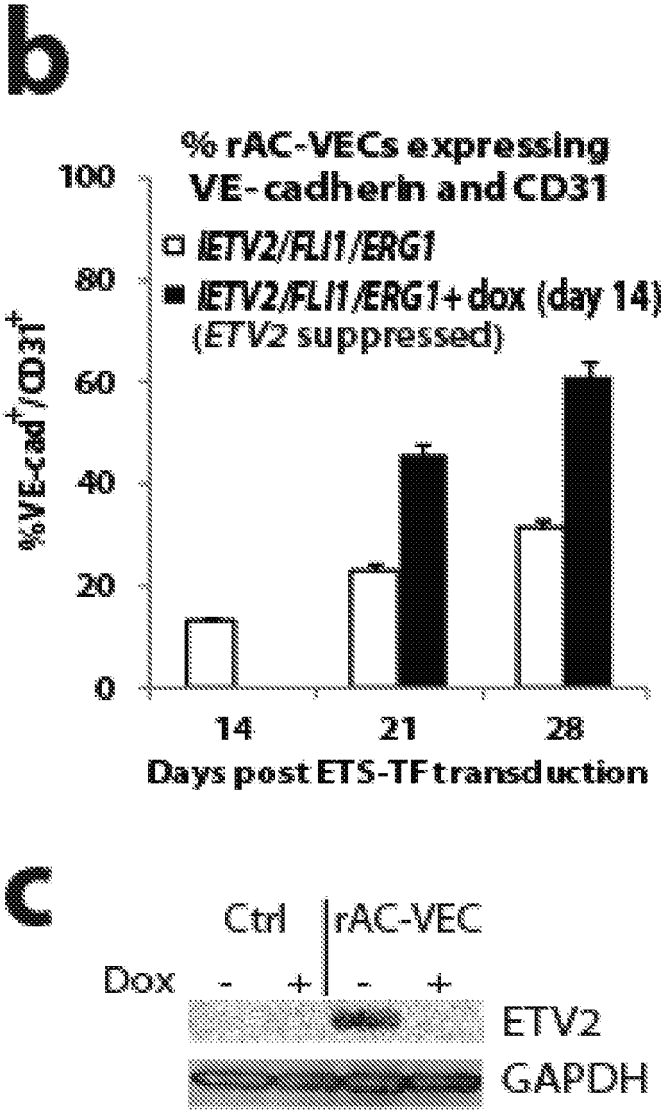

a
+Dox
iETV2
FLI1
ERG1
Day 1
Day 14
Day 21
Day 28
+iETV2/FLI1/ERG1 (transduction)
+Dox (iETV2 suppression)
day 14
day 21
day 28
rAC-VEC
No Dox (iETV2 still active) (FLI1/ERG1 still active)
+Dox (iETV2 suppressed) (FLI1/ERG1 still active)
43.5%
13.8%
61.5%
29.4%
89.9%
32.7%
86.5%
48.5%
94.5%
62.8%
i.
ii.
iii.
iv.
v.
CD31 Log Fl. Int.
CD41 Log Fl. Int.

Figure 7a


b
% rAC-VECs expressing
VE-cadherin and CD31
ETV2/FLI1/ERG1
ETV2/FLI1/ERG1+dox (day 14)
(ETV2 suppressed)
%VE-cad+/CD31+
100
80
60
40
20
0
14
21
28
Days post ETS-TF transduction
c
Ctrl
rAC-VEC
Dox
-
+
-
+
ETV2
GAPDH

Figures 7b-c

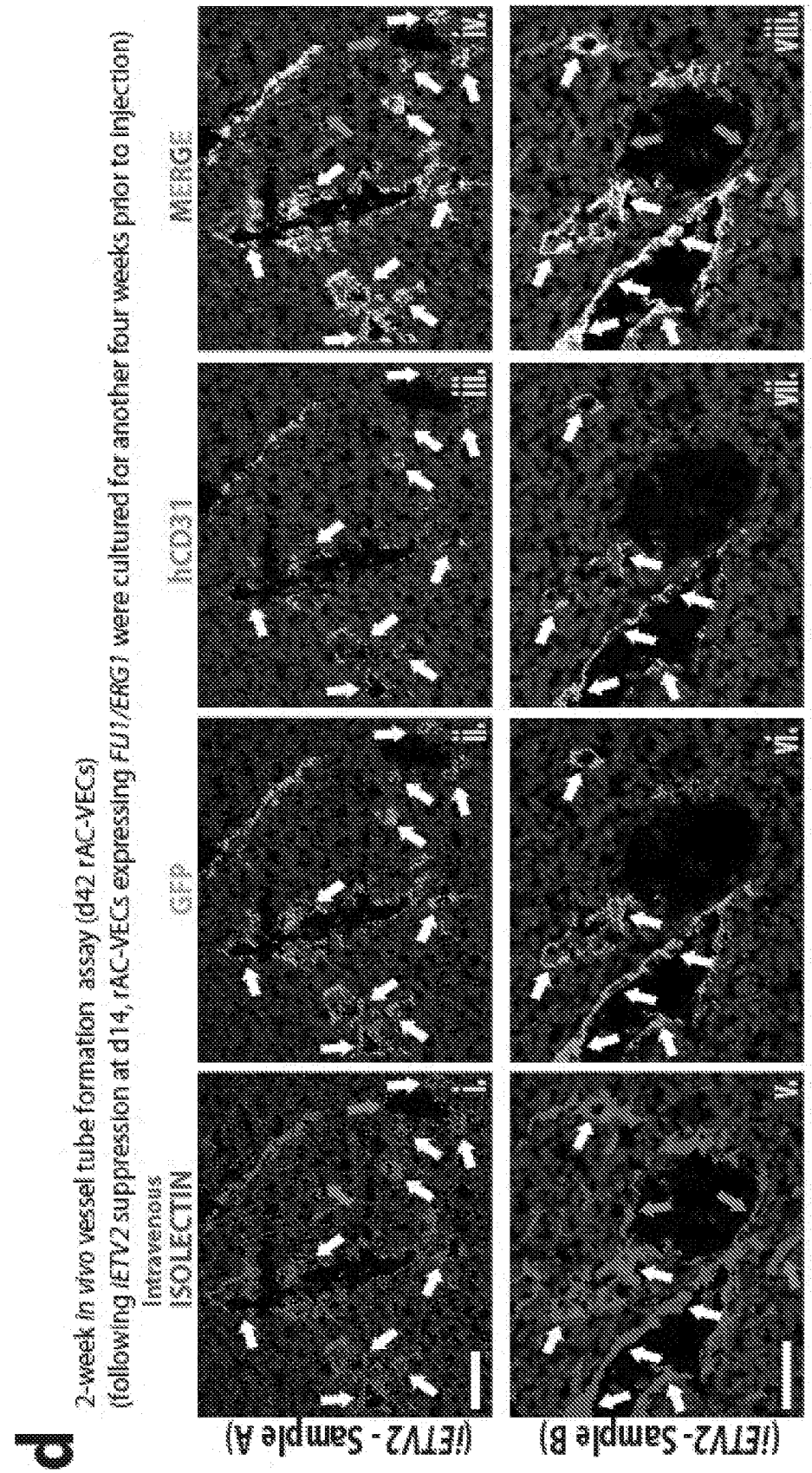

d
2-week in vivo vessel tube formation assay (d42 rAC-VECs)
(following iETV2 suppression at d14, rAC-VECs expressing FLI1/ERG1 were cultured for another four weeks prior to injection)
Intravenous
ISOLECTIN
GFP
hCD31
MERGE
(iETV2 - Sample A)
(iETV2 - Sample B)
I.
II.
III.
IV.
V.
VI.
VII.
VIII.

Figure 7d

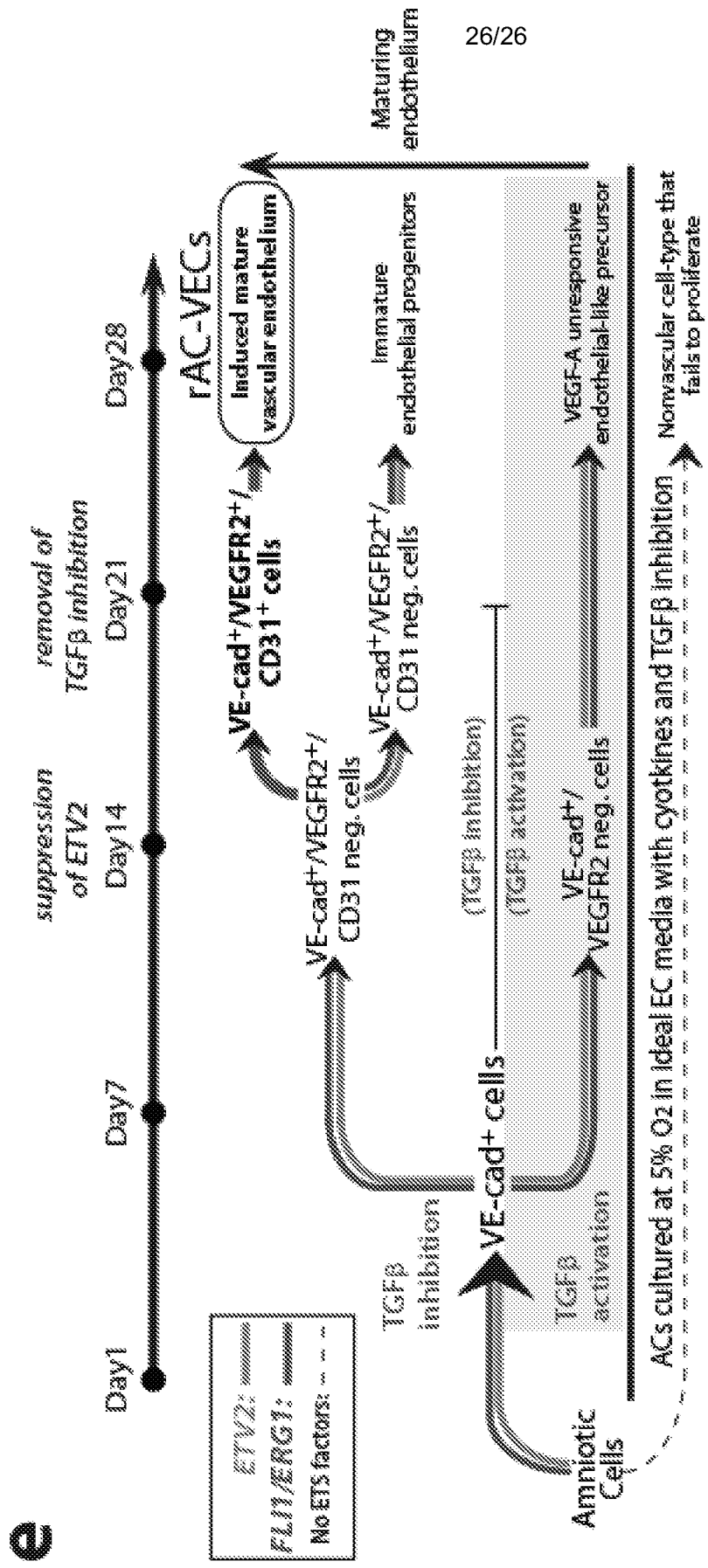

e
Day1
Day7
Day14
Day21
Day28
suppression of ETV2
removal of TGFβ inhibition
rAC-VECs
ETV2:
FLI1/ERG1:
No ETS factors:
TGFβ inhibition
VE-cad+ cells
Amniotic Cells
TGFβ activation
VE-cad+/ VEGFR2 neg. cells
(TGFβ activation)
(TGFβ inhibition)
VE-cad+/VEGFR2+/ CD31 neg. cells
VE-cad+/VEGFR2+/ CD31+ cells
VE-cad+/VEGFR2+/ CD31 neg. cells
Induced mature vascular endothelium
Immature endothelial progenitors
VEGF-A unresponsive endothelial-like precursor
Maturing endothelium
Nonvascular cell-type that fails to proliferate
ACs cultured at 5% O2 in ideal EC media with cyotkines and TGFβ inhibition

Figure 7e