Figure 1

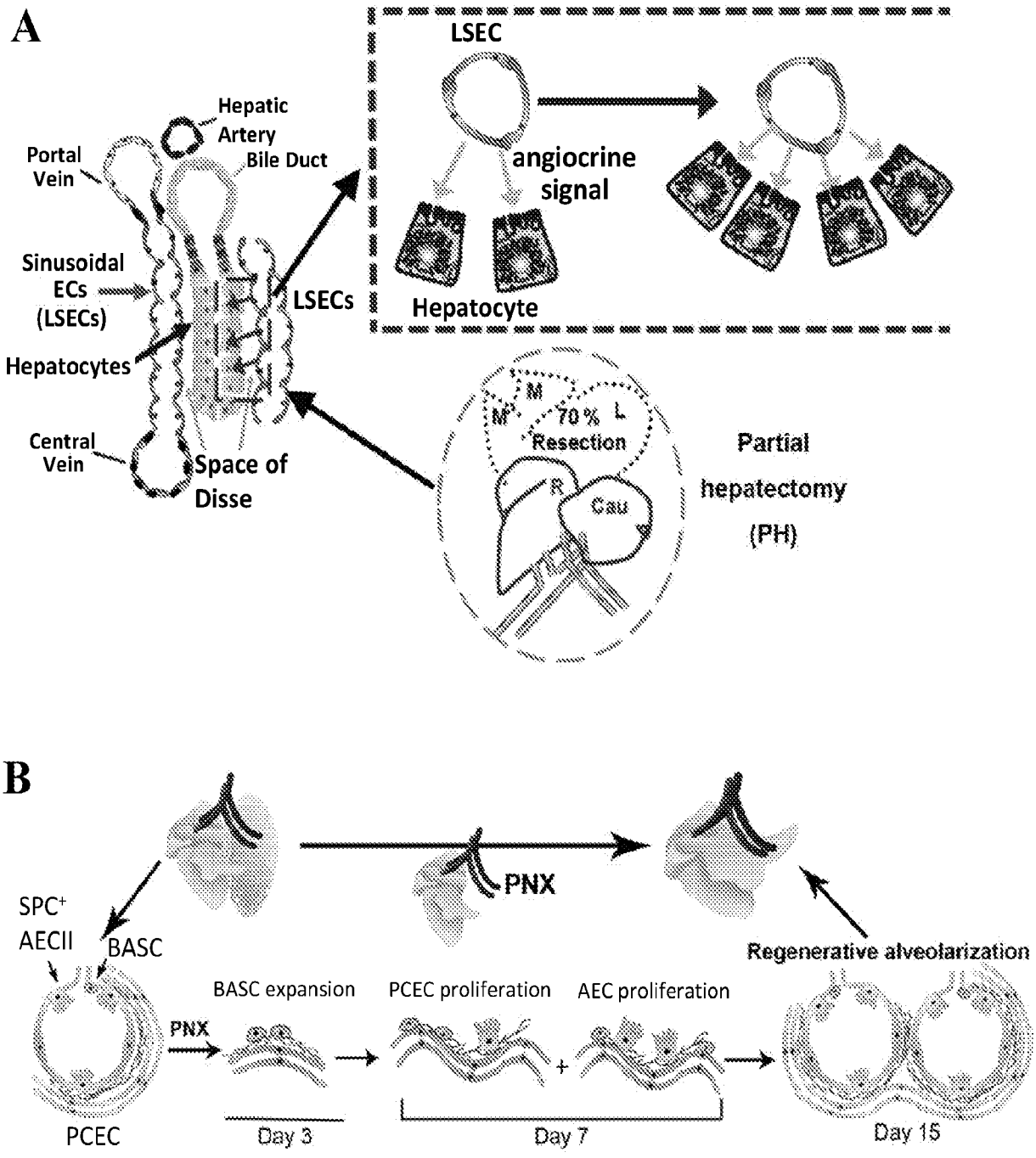

A
LSEC
angiocrine signal
Hepatocyte
Hepatic Artery
Portal Vein
Bile Duct
Sinusoidal ECs (LSECs)
LSECs
Hepatocytes
Central Vein
Space of Disse
M
M'
70 % Resection
L
R
Cau
Partial hepatectomy (PH)
B
SPC+ AECII
BASC
PNX
Regenerative alveolarization
BASC expansion
PCEC proliferation
AEC proliferation
PNX
PCEC
Day 3
Day 7
Day 15

Figure 2

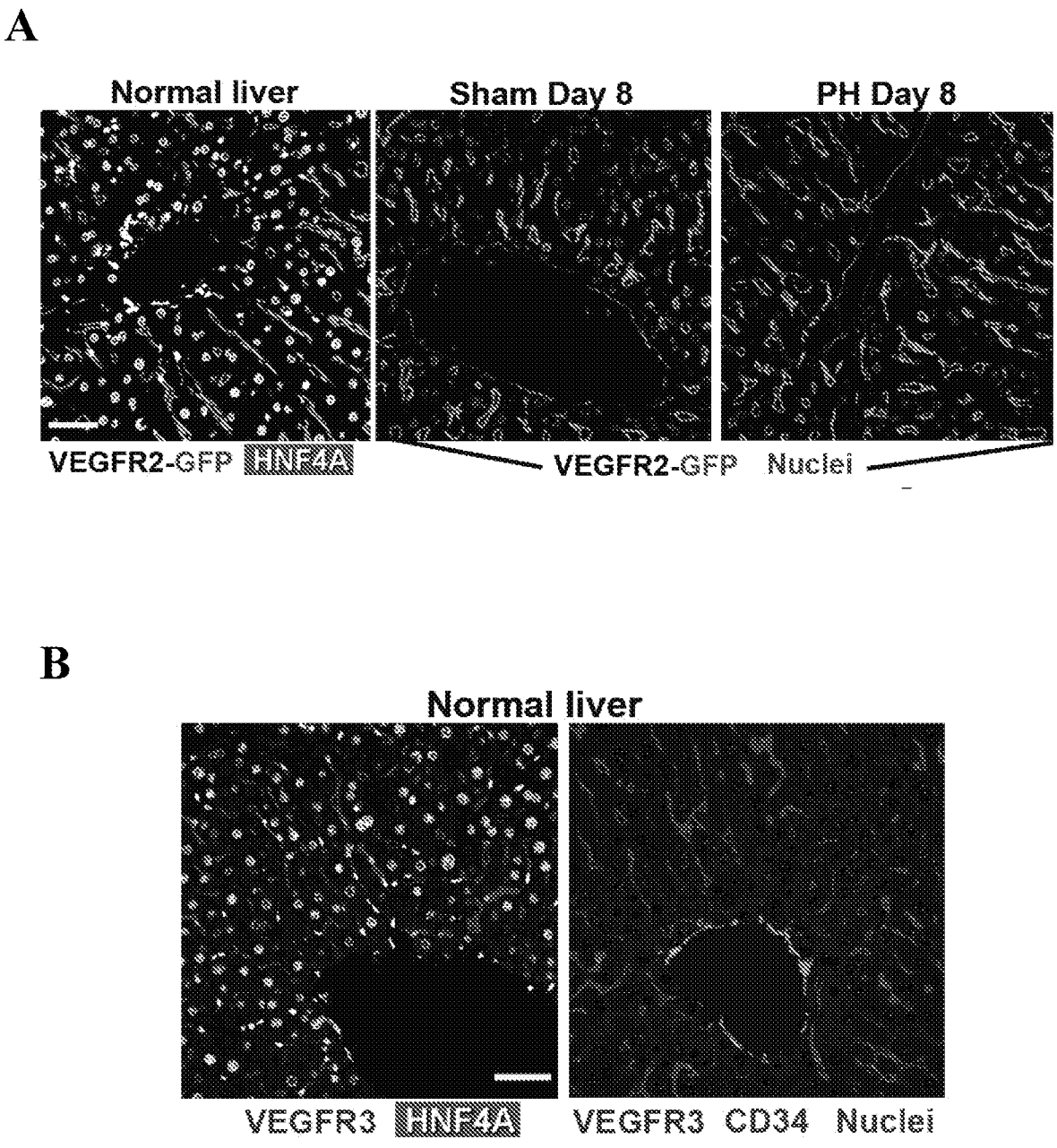

A
Normal liver
Sham Day 8
PH Day 8
VEGFR2-GFP HNF4A
VEGFR2-GFP Nuclei
B
Normal liver
VEGFR3 HNF4A
VEGFR3 CD34 Nuclei

Figure 2

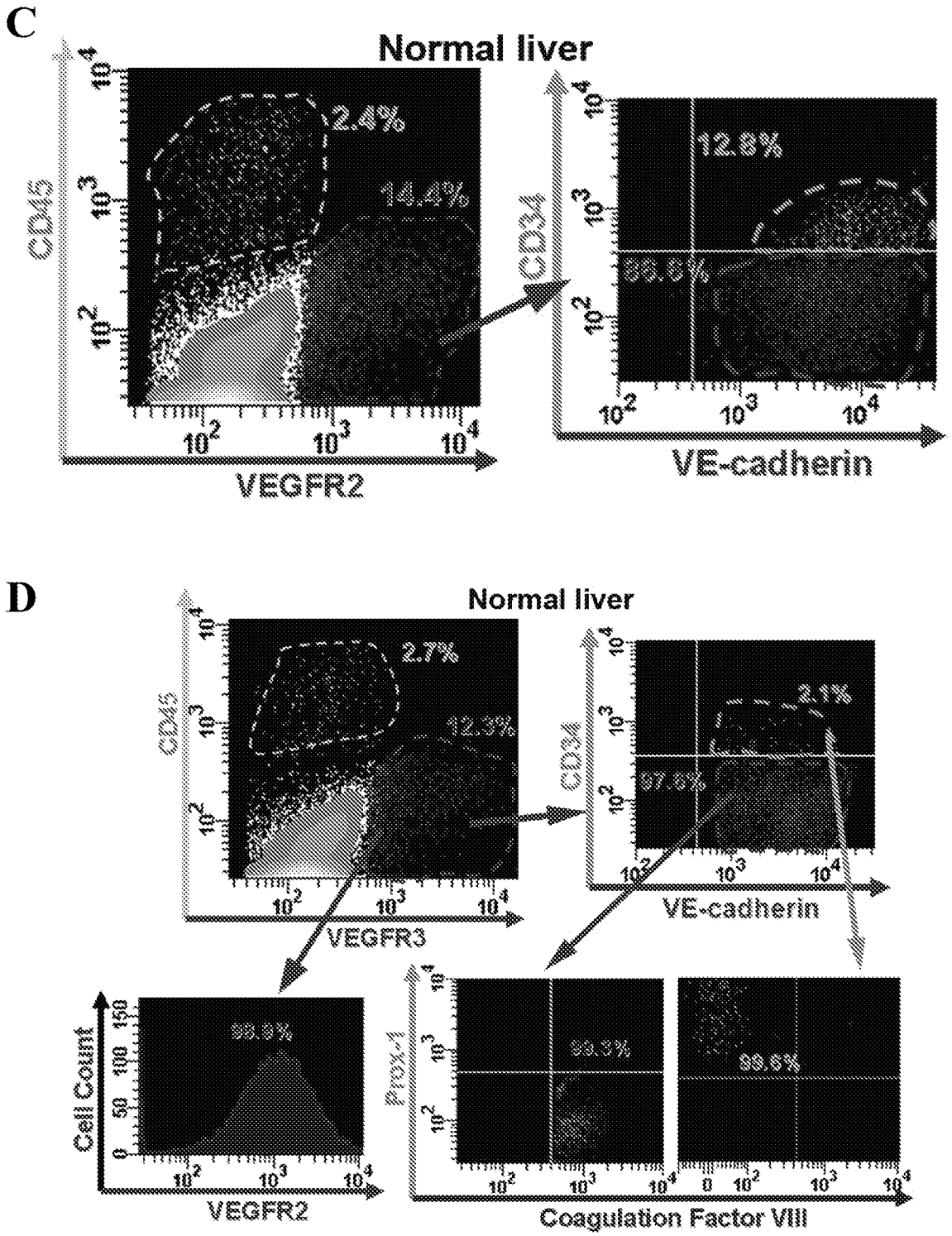

C
Normal liver
CD45
VEGFR2
2.4%
14.4%
CD34
VE-cadherin
12.8%
83.6%
D
Normal liver
CD45
VEGFR3
2.7%
12.3%
CD34
VE-cadherin
2.1%
97.6%
Cell Count
VEGFR2
98.6%
Prox-1
Coagulation Factor VIII
99.3%
99.6%

Figure 2

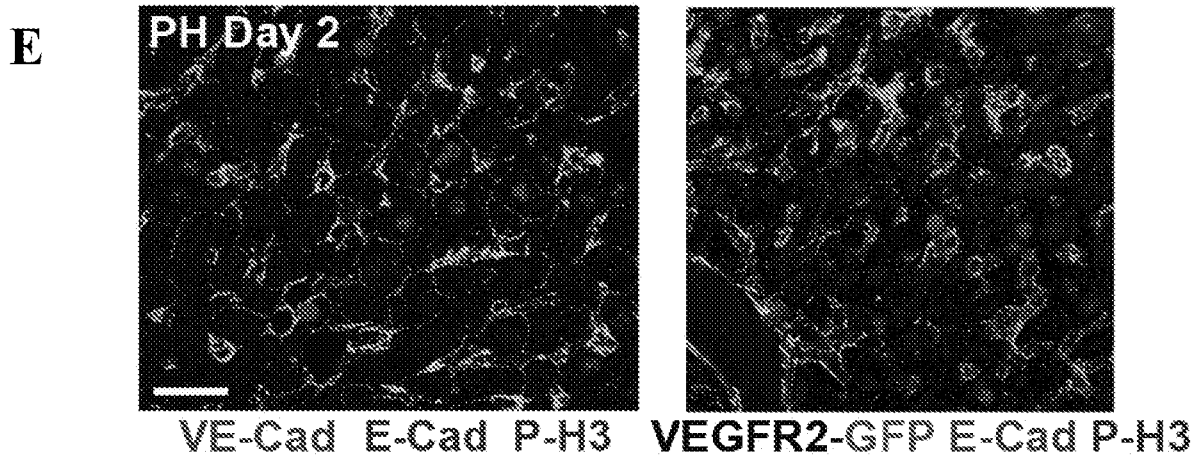

E
PH Day 2
VE-Cad E-Cad P-H3
VEGFR2-GFP E-Cad P-H3
F
VEGFR3+CD34- LSECs quantified per liver (x 1,000)
Inductive angiogenesis
Proliferative angiogenesis
Time (day)
G
P-H3+HNF4A+ hepatocytes per hpf
Inductive angiogenesis
Proliferative angiogenesis
Time (day)

Figure 3

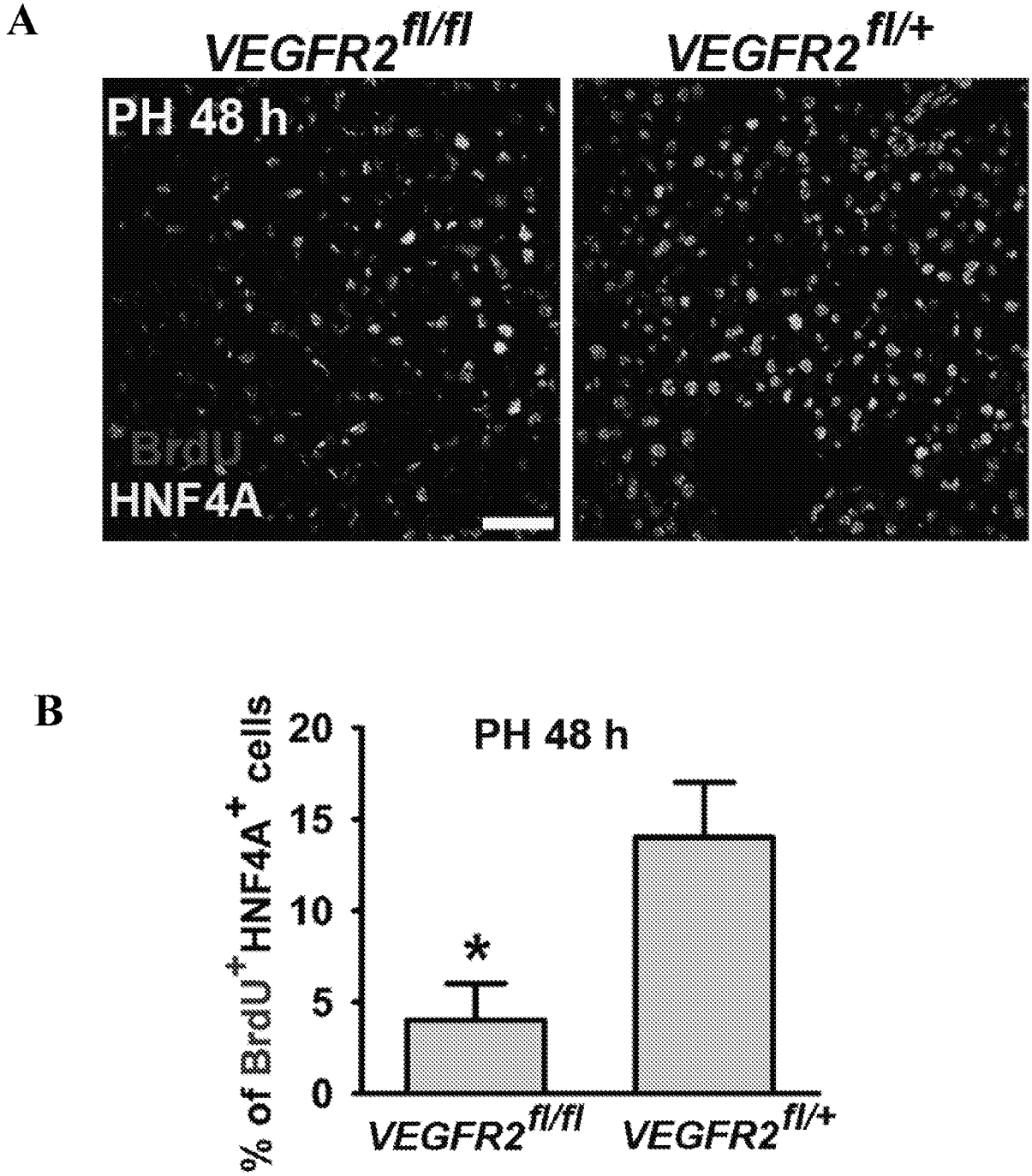
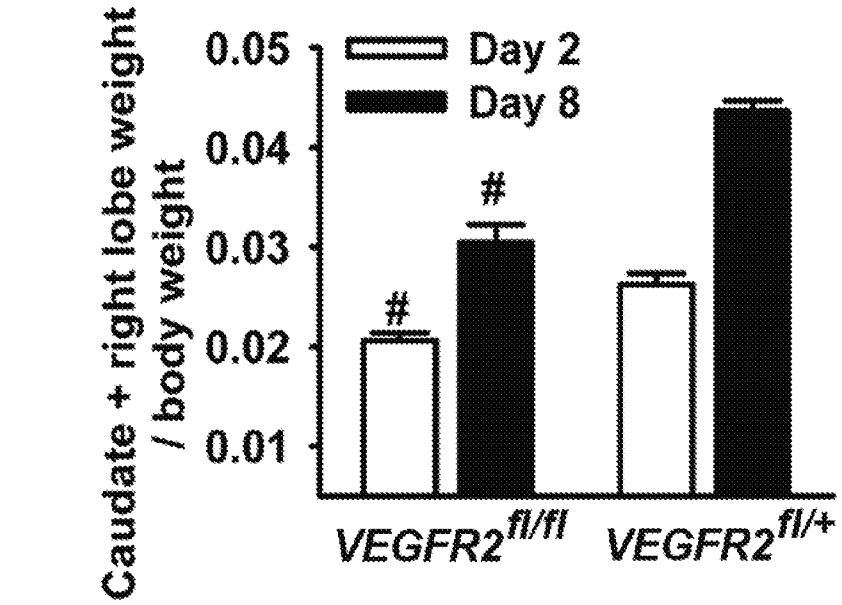

A
VEGFR2fl/fl
VEGFR2fl/+
PH 48 h
BrdU
HNF4A
B
PH 48 h
% of BrdU+HNF4A+ cells
20
15
10
5
0
*
VEGFR2fl/fl
VEGFR2fl/+

Figure 3


C
Caudate + right lobe weight / body weight
0.05
0.04
0.03
0.02
0.01
Day 2
Day 8
#
#
VEGFR2fl/fl
VEGFR2fl/+

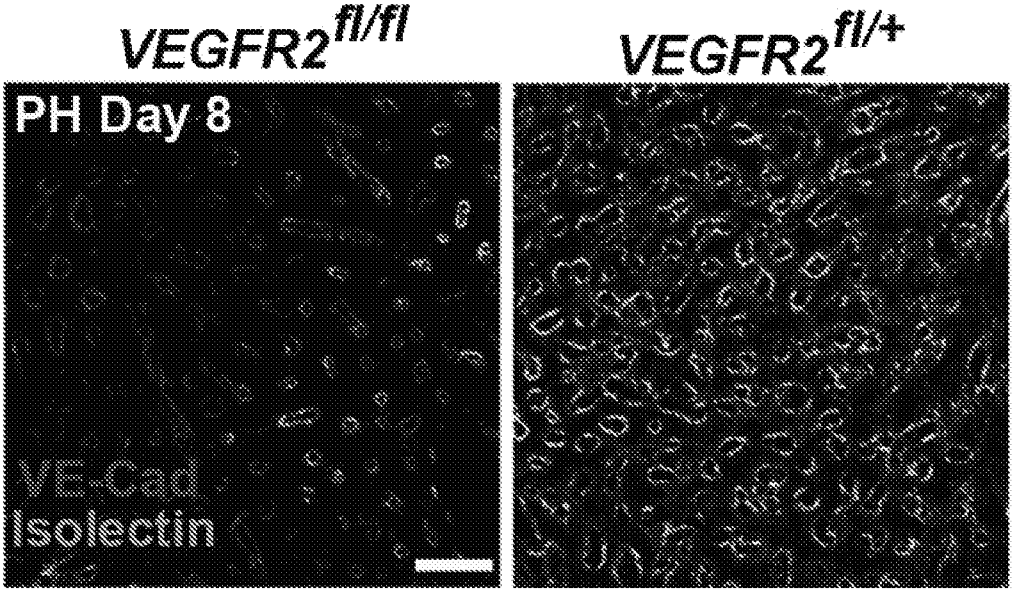

D
VEGFR2fl/fl
VEGFR2fl/+
PH Day 8
VE-Cad
Isolectin

Figure 3

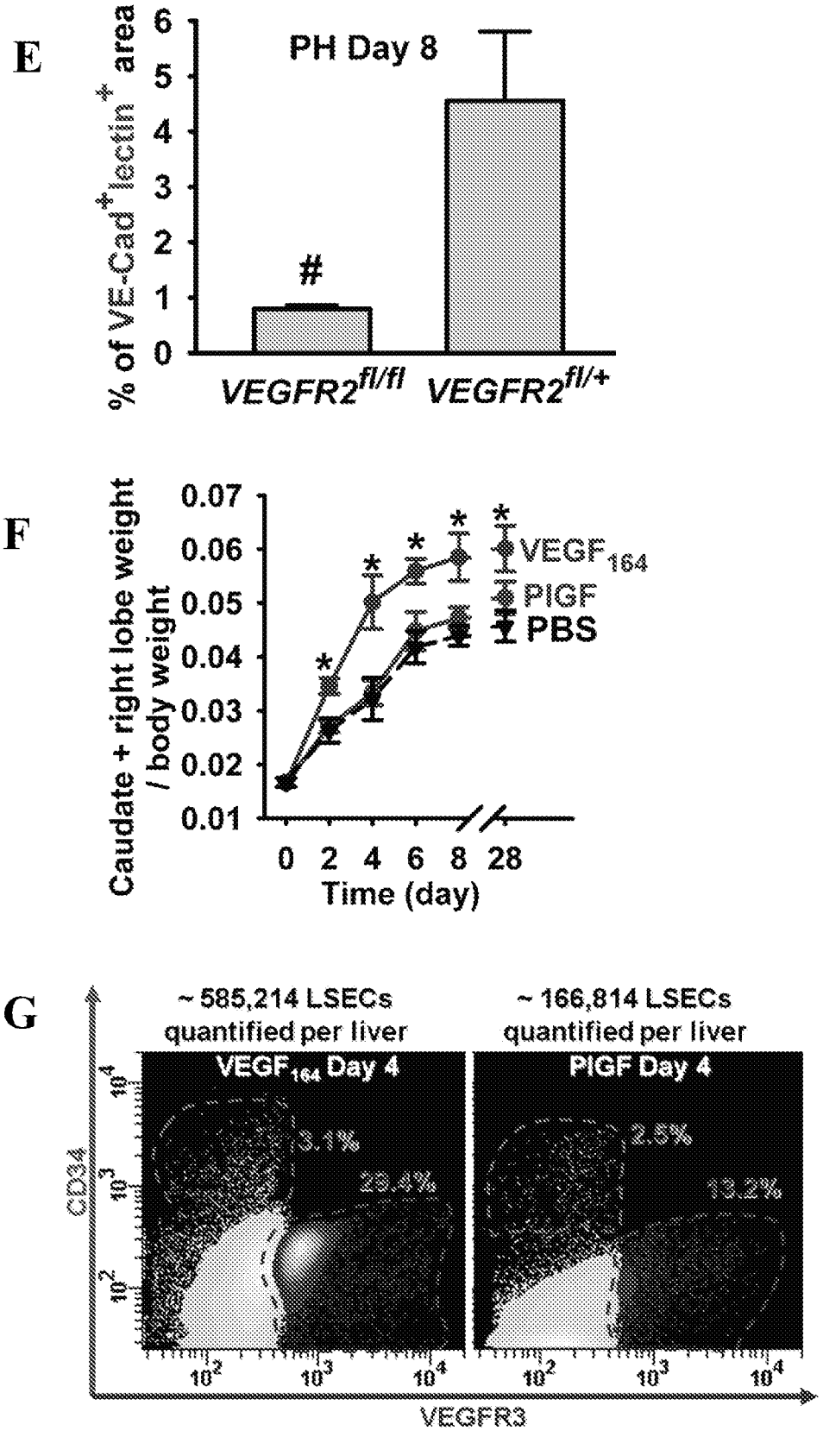
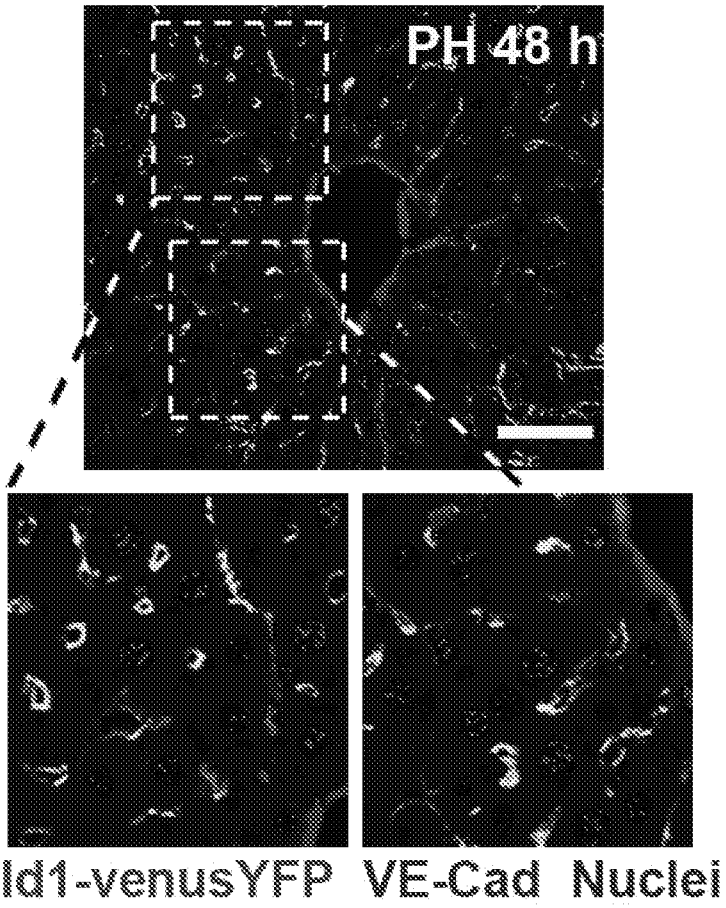

E
PH Day 8
% of VE-Cad+lectin+ area
#
VEGFR2fl/fl
VEGFR2fl/+
F
Caudate + right lobe weight / body weight
VEGF164
PIGF
PBS
Time (day)
G
~ 585,214 LSECs quantified per liver
~ 166,814 LSECs quantified per liver
VEGF164 Day 4
PIGF Day 4
3.1%
29.4%
2.5%
13.2%
CD34
VEGFR3

Figure 3

H


PH 48 h
Id1-venusYFP
VE-Cad
Nuclei

I

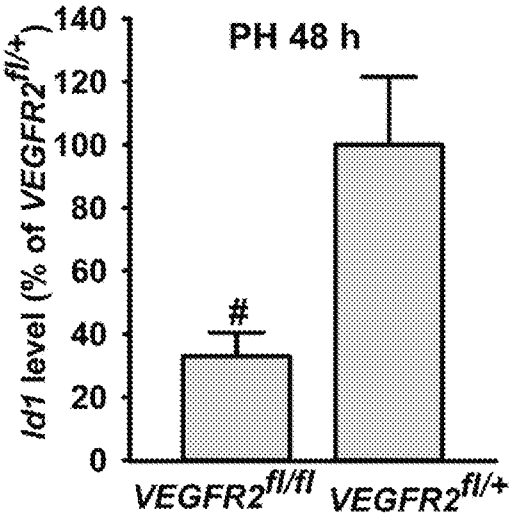
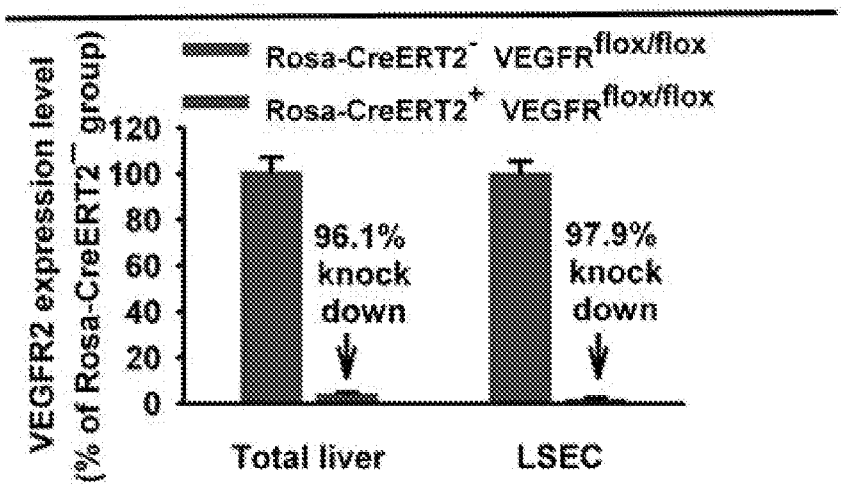

PH 48 h
Id1 level (% of VEGFR2fl/+)
140
120
100
80
60
40
20
0
#
VEGFR2fl/fl
VEGFR2fl/+

Figure 3

J


Rosa-CreERT2⁻ VEGFR flox/flox
Rosa-CreERT2⁺ VEGFR flox/flox
VEGFR2 expression level
(% of Rosa-CreERT2⁻ group)
120
100
80
60
40
20
0
96.1% knock down
97.9% knock down
Total liver
LSEC

K

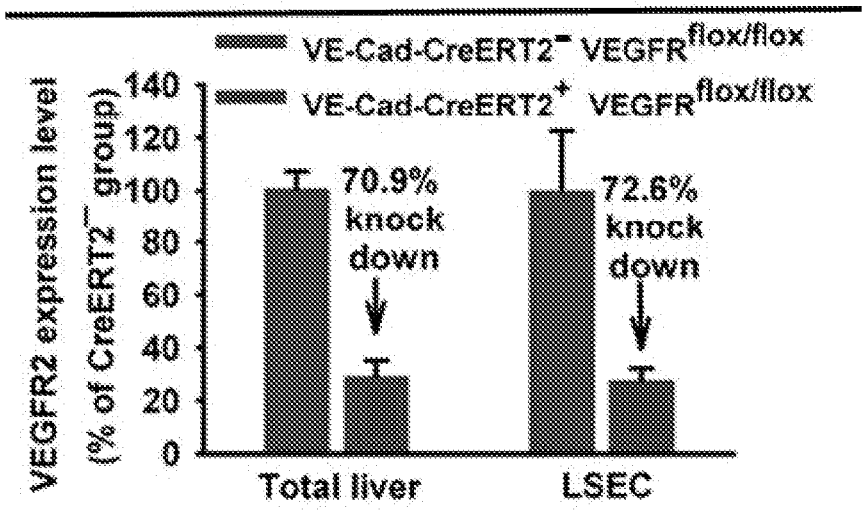

VE-Cad-CreERT2⁻ VEGFR flox/flox
VE-Cad-CreERT2⁺ VEGFR flox/flox
VEGFR2 expression level
(% of CreERT2⁻ group)
140
120
100
80
60
40
20
0
70.9% knock down
72.6% knock down
Total liver
LSEC

Figure 4

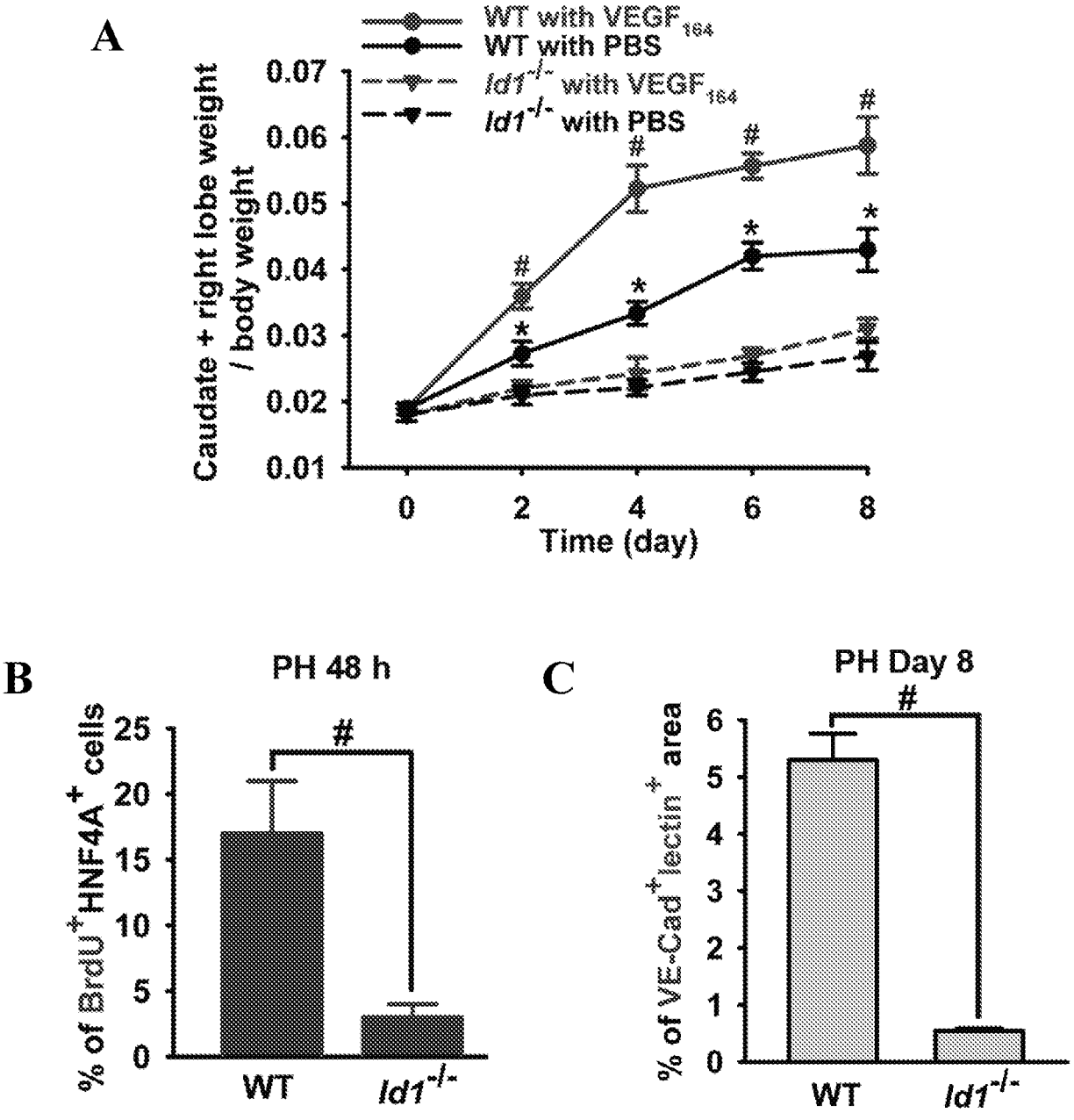
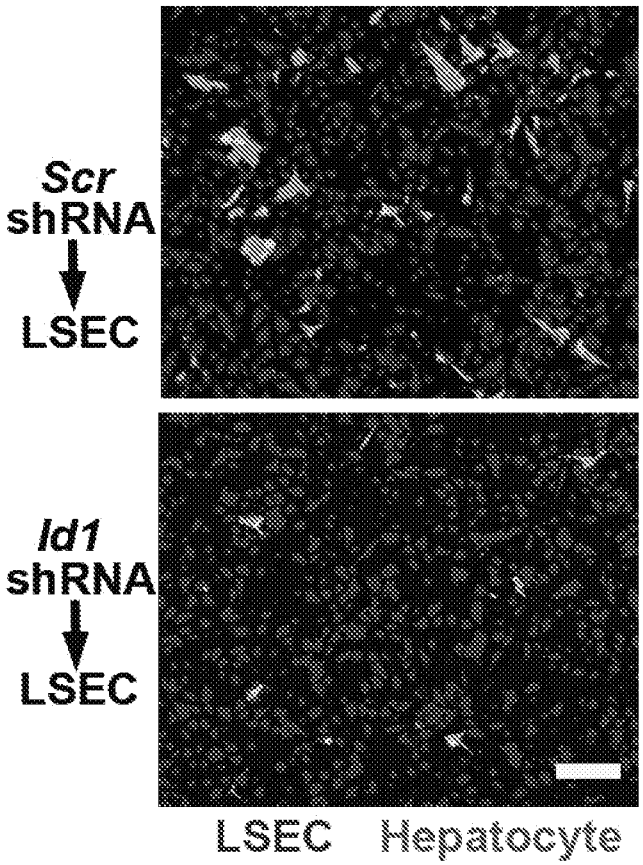

A
WT with VEGF164
WT with PBS
Id1-/- with VEGF164
Id1-/- with PBS
Caudate + right lobe weight / body weight
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
2
4
6
8
Time (day)
#
*
B
PH 48 h
% of BrdU+HNF4A+ cells
25
20
15
10
5
0
WT
Id1-/-
C
PH Day 8
% of VE-Cad+lectin+ area
6
5
4
3
2
1
0
WT
Id1-/-

Figure 4


D
Scr shRNA
LSEC
Id1 shRNA
LSEC
LSEC Hepatocyte

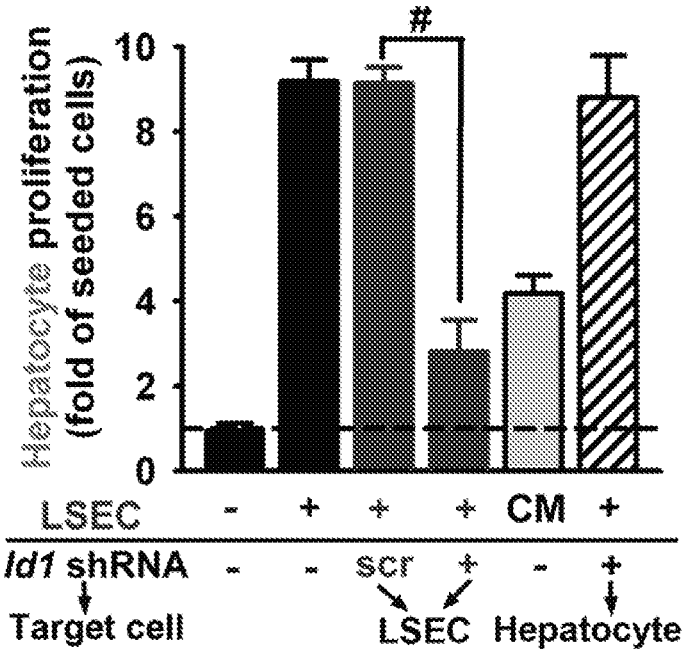
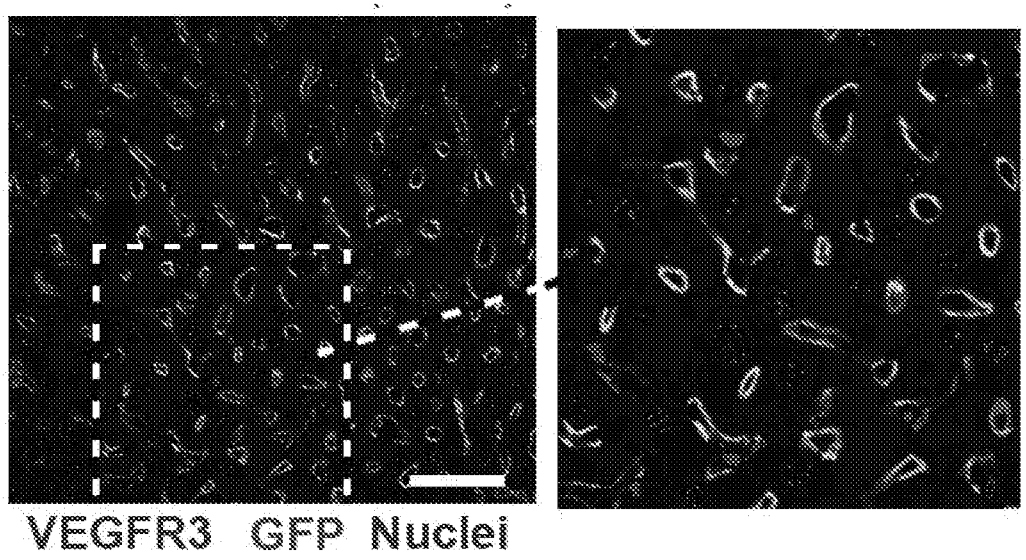

E
Hepatocyte proliferation (fold of seeded cells)
10
8
6
4
2
0
#
LSEC - + + + CM +
Id1 shRNA - - scr + - +
Target cell
LSEC Hepatocyte

Figure 4

F


VEGFR3 GFP Nuclei

G

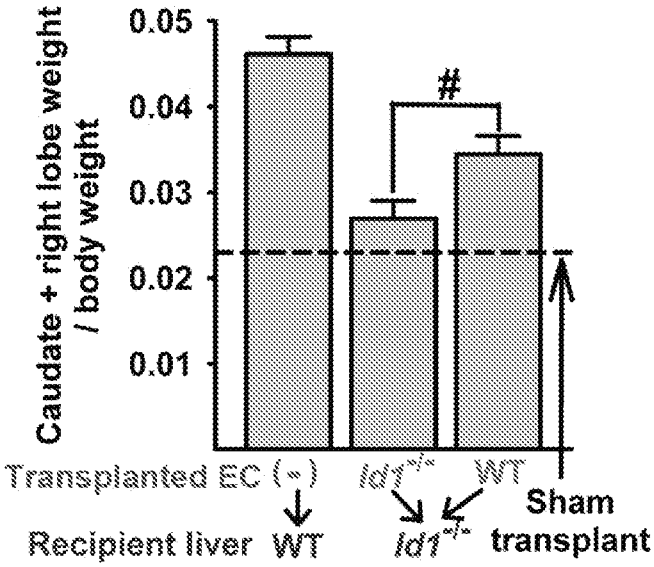
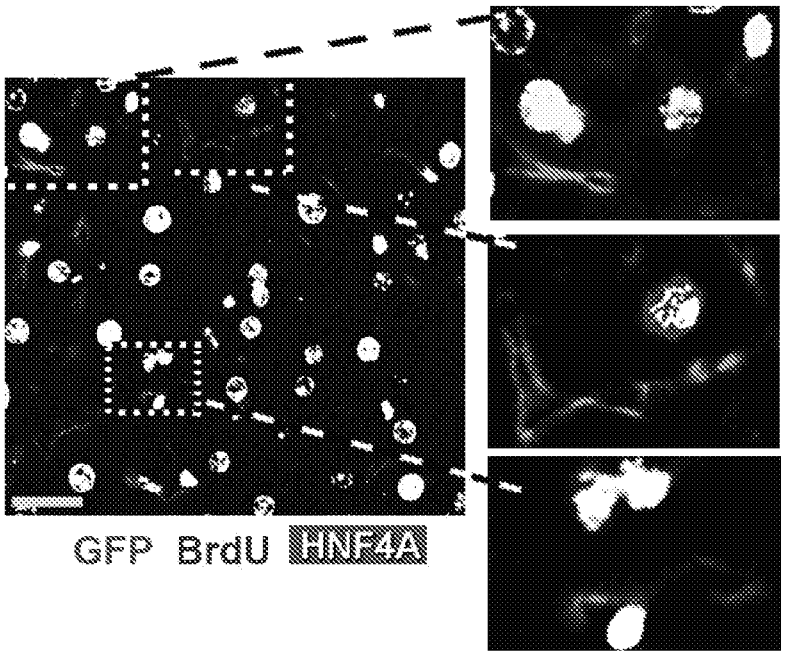

Caudate + right lobe weight / body weight
0.05
0.04
0.03
0.02
0.01
#
Transplanted EC (-)
Id1-/-
WT
Sham transplant
Recipient liver WT
Id1-/-

Figure 4

H


GFP BrdU HNF4A

I

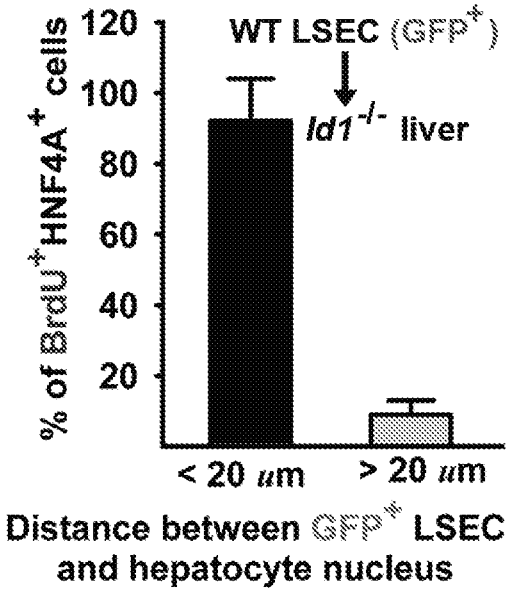
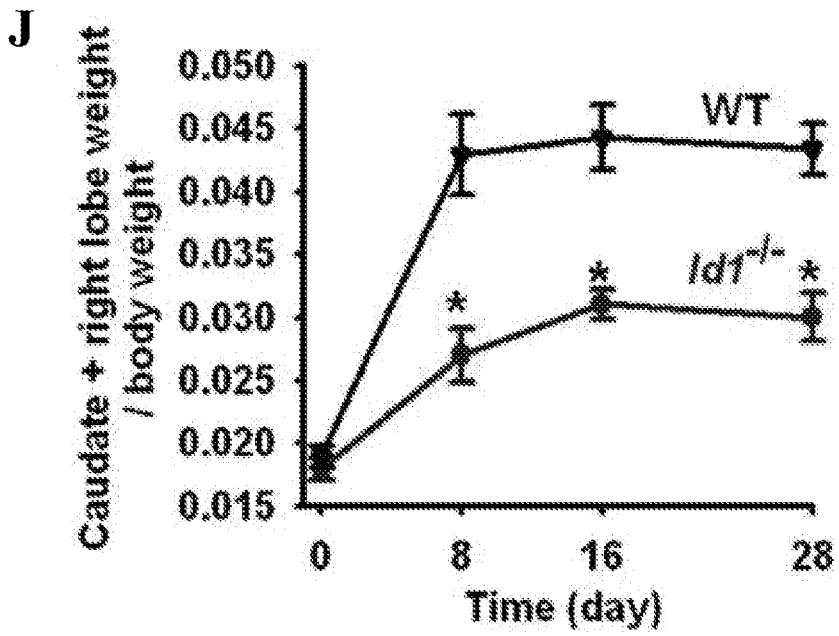

% of BrdU+HNF4A+ cells
120
100
80
60
40
20
WT LSEC (GFP+)
Id1-/- liver
< 20 um
> 20 um
Distance between GFP+ LSEC and hepatocyte nucleus

Figure 4


J
Caudate + right lobe weight / body weight
0.050
0.045
0.040
0.035
0.030
0.025
0.020
0.015
WT
Id1-/-
*
*
*
0
8
16
28
Time (day)

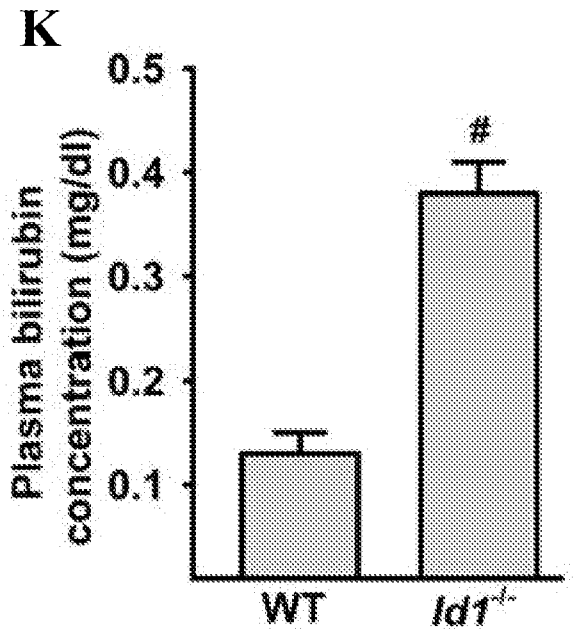

K
Plasma bilirubin concentration (mg/dl)
0.5
0.4
0.3
0.2
0.1
#
WT
Id1-/-

Figure 5

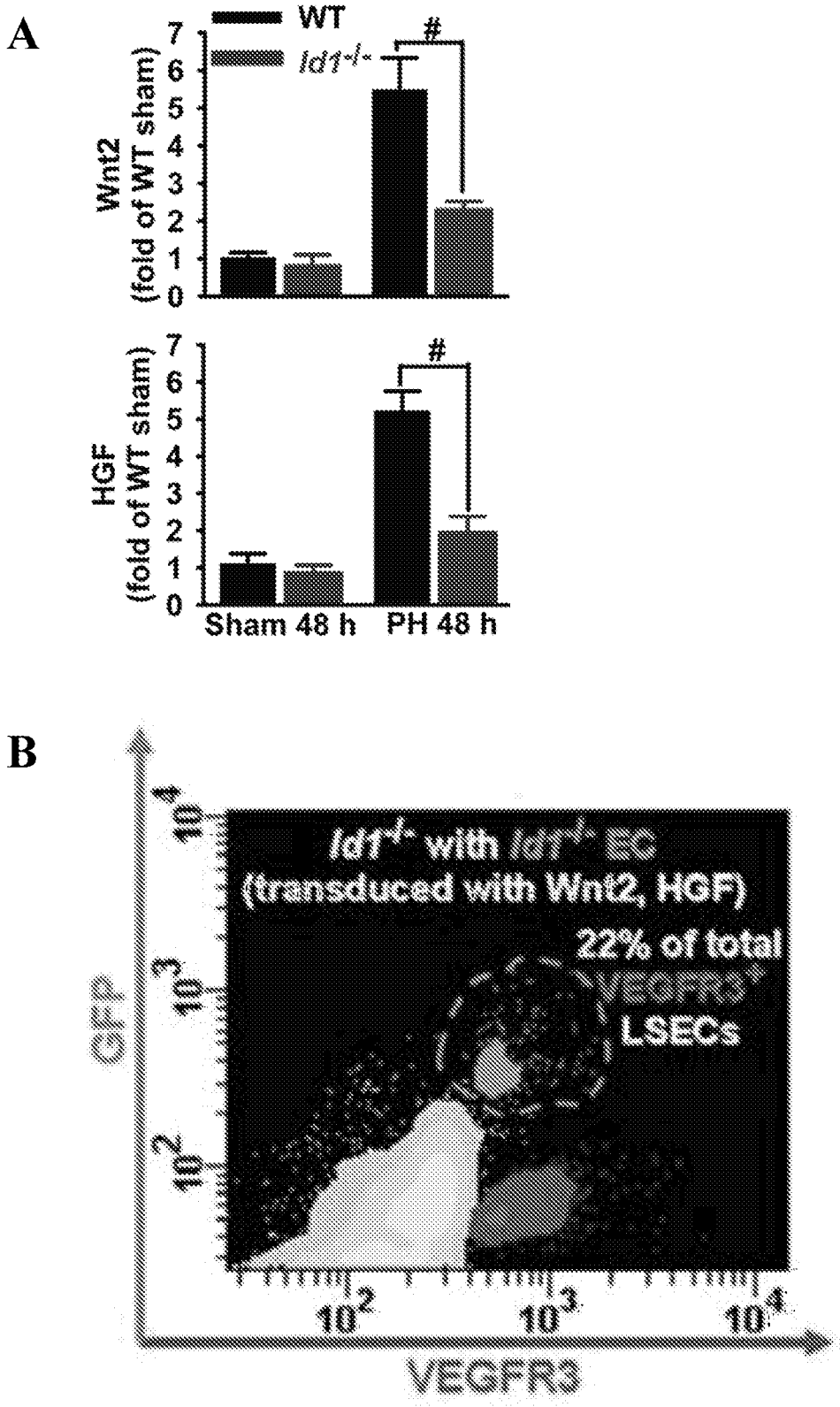
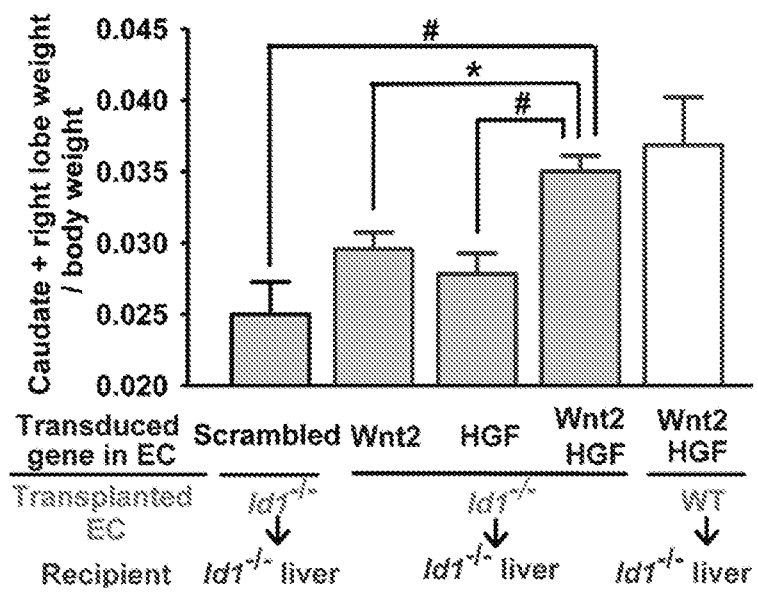

A
WT
Id1-/-
#
Wnt2
(fold of WT sham)
HGF
(fold of WT sham)
Sham 48 h
PH 48 h
B
Id1-/- with Id1-/- EC
(transduced with Wnt2, HGF)
22% of total
VEGFR3+
LSECs
GFP
VEGFR3

Figure 5


C
Caudate + right lobe weight / body weight
0.045
0.040
0.035
0.030
0.025
0.020
#
*
#
Transduced gene in EC
Scrambled
Wnt2
HGF
Wnt2 HGF
Wnt2 HGF
Transplanted EC
Id1-/-
Id1-/-
WT
Recipient
Id1-/- liver
Id1-/- liver
Id1-/- liver

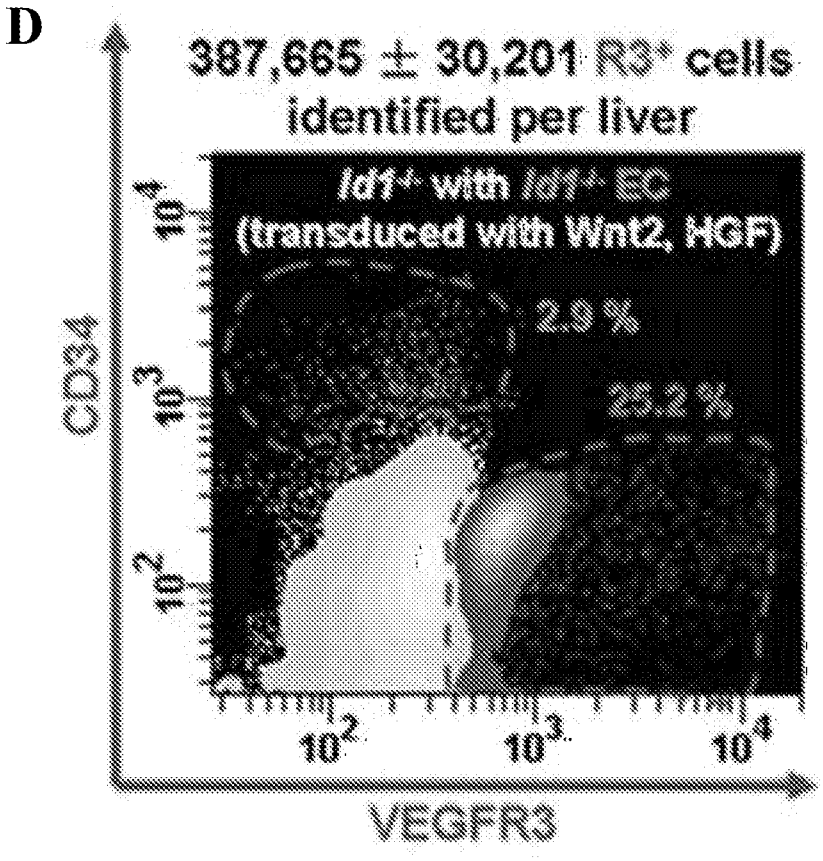
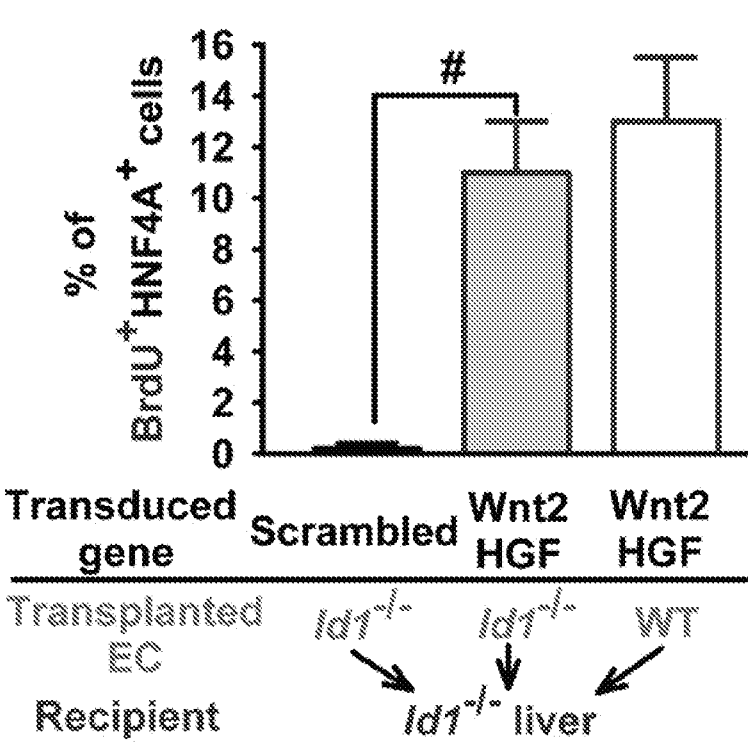

D
387,665 ± 30,201 R3+ cells identified per liver
Id1+/- with Id1-/- EC (transduced with Wnt2, HGF)
2.9 %
25.2 %
CD34
VEGFR3
10^2
10^3
10^4

Figure 5


E
% of BrdU+HNF4A+ cells
16
14
12
10
8
6
4
2
0
#
Transduced gene
Scrambled
Wnt2 HGF
Wnt2 HGF
Transplanted EC
Id1-/-
Id1-/-
WT
Recipient
Id1-/- liver

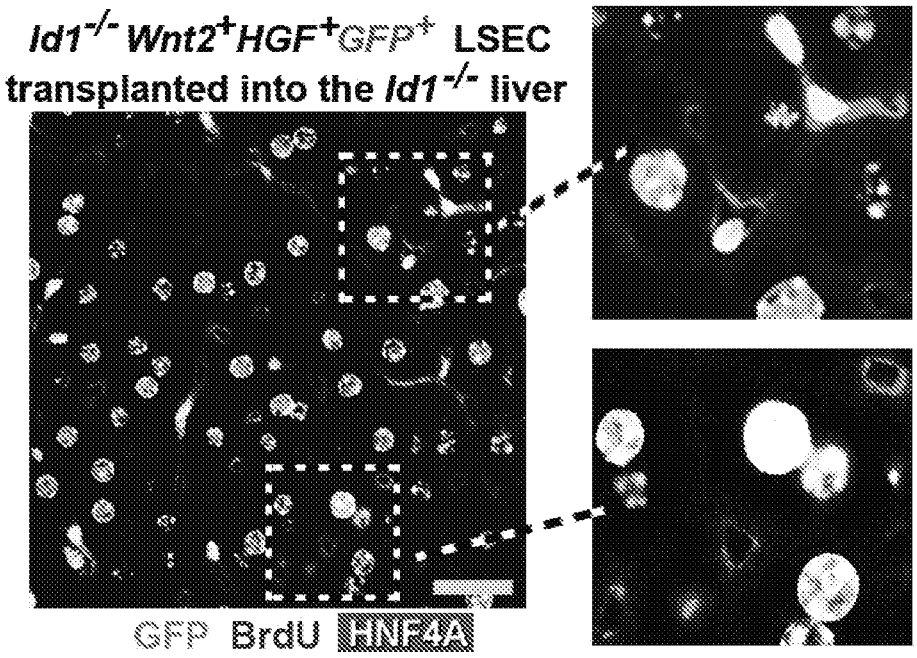

F
Id1-/- Wnt2+HGF+GFP+ LSEC
transplanted into the Id1-/- liver
GFP BrdU HNF4A

Figure 5

G

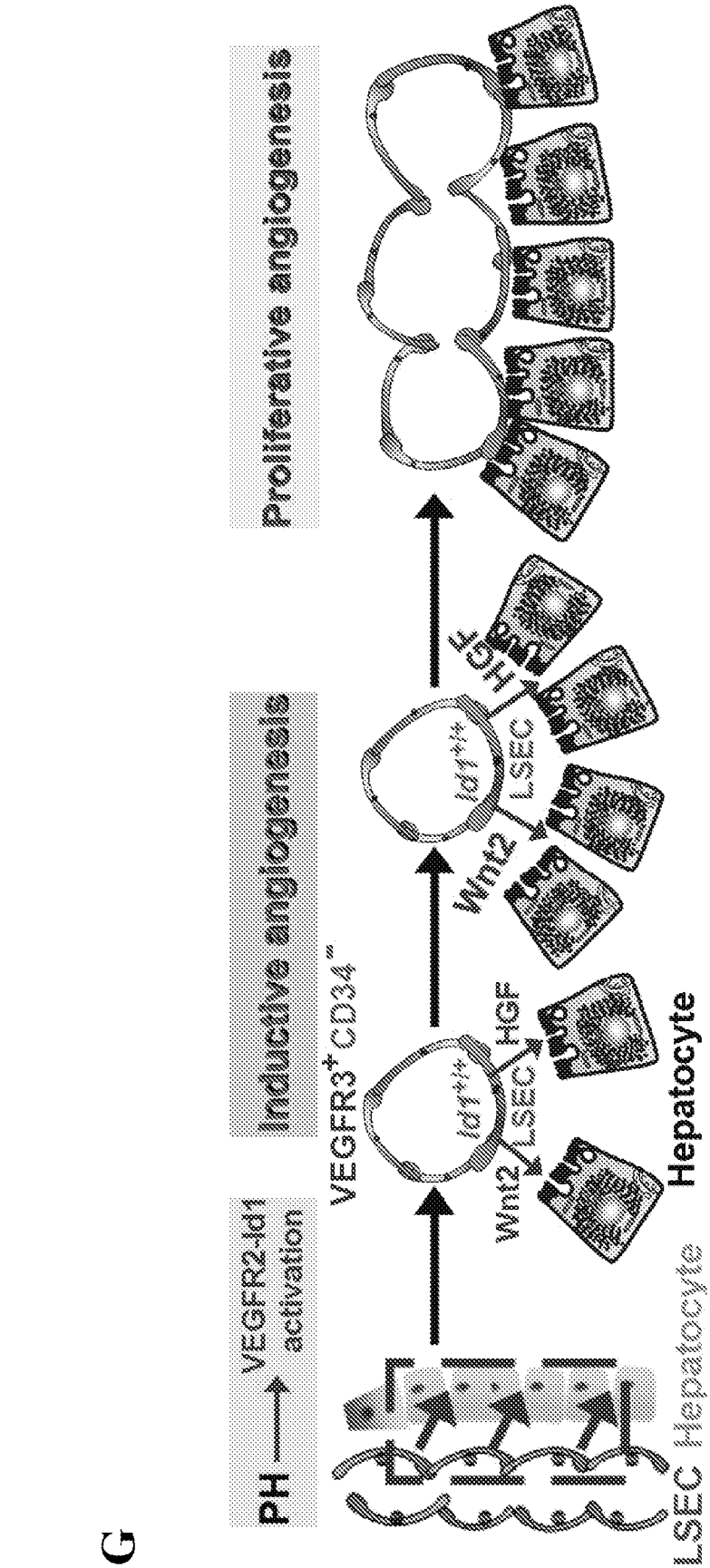
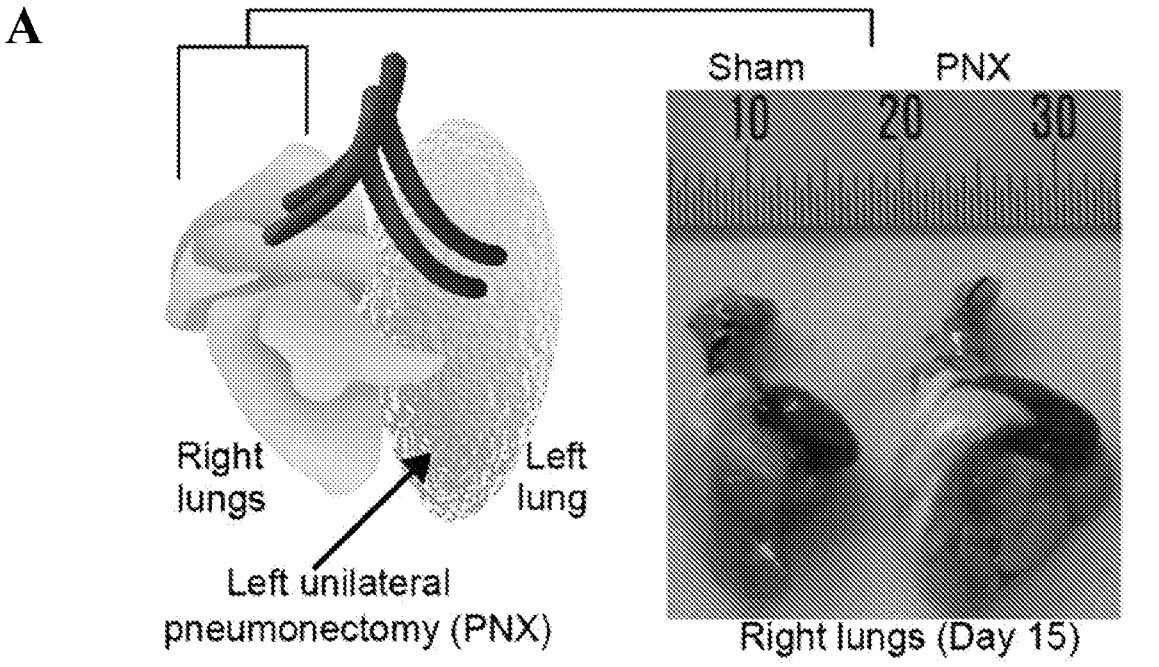

PH
VEGFR2-Id1 activation
Inductive angiogenesis
Proliferative angiogenesis
VEGFR3+CD34−
Id1+/+
Wnt2
LSEC
HGF
Hepatocyte
LSEC Hepatocyte

Figure 6


A
Sham
PNX
10
20
30
Right lungs
Left lung
Left unilateral pneumonectomy (PNX)
Right lungs (Day 15)

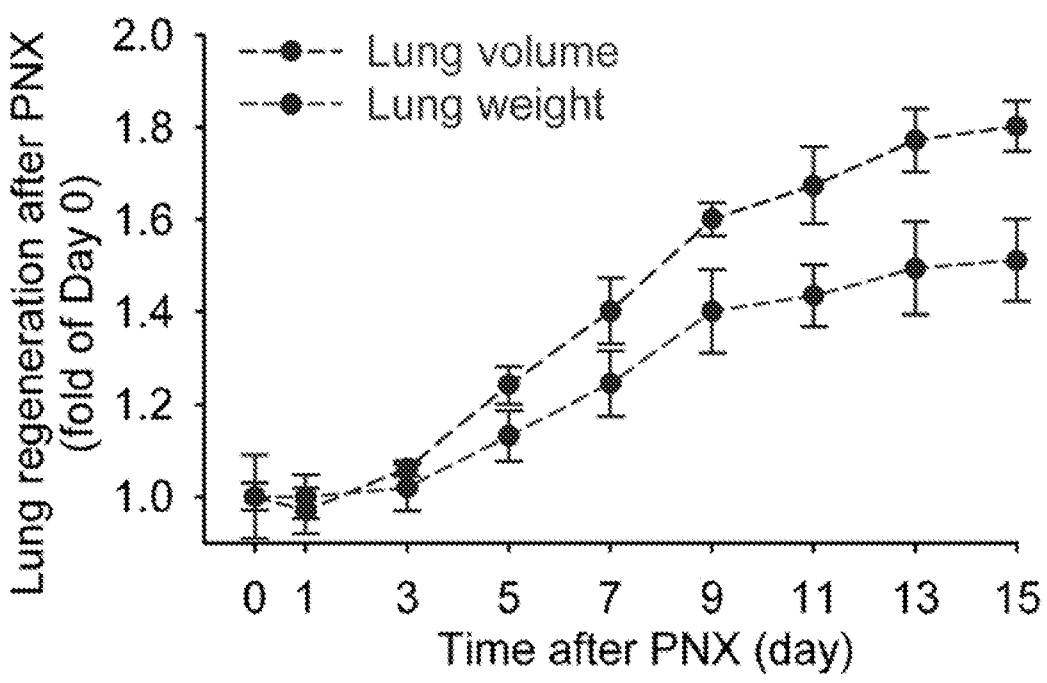

B
Lung volume
Lung weight
Lung regeneration after PNX (fold of Day 0)
2.0
1.8
1.6
1.4
1.2
1.0
0 1 3 5 7 9 11 13 15
Time after PNX (day)

Figure 6

C

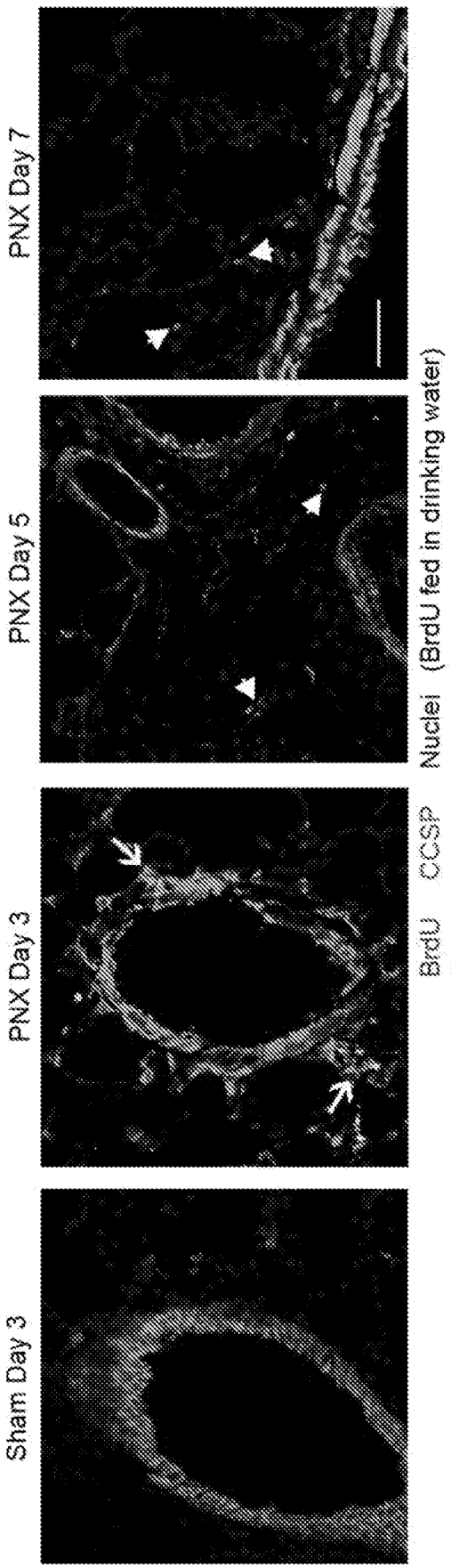
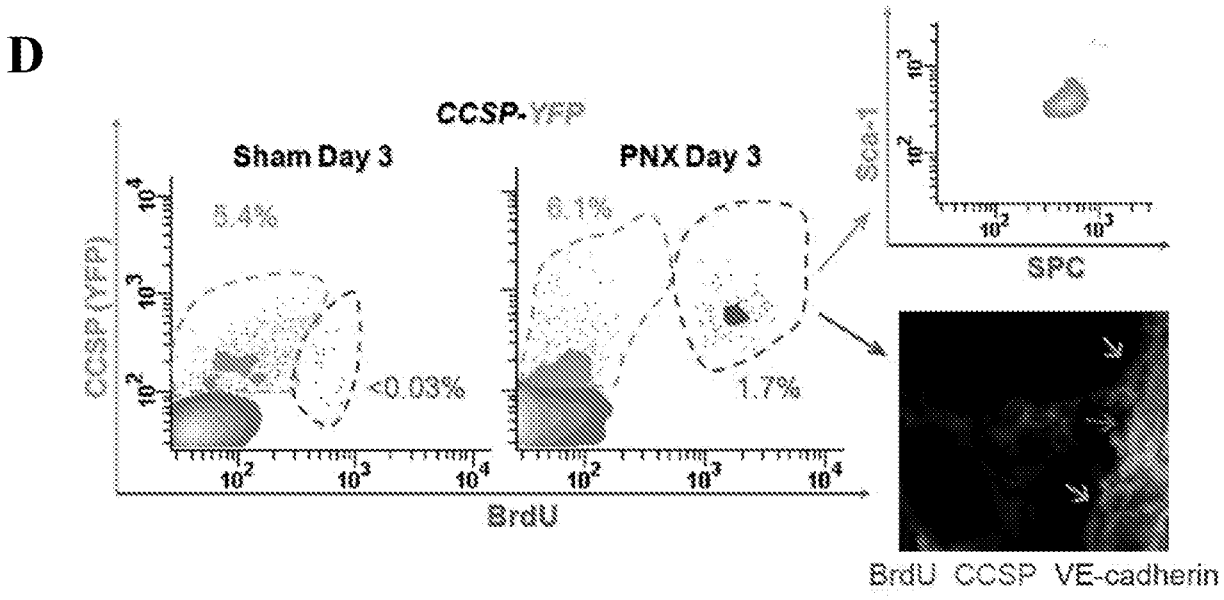

Sham Day 3
PNX Day 3
PNX Day 5
PNX Day 7
BrdU CCSP Nuclei (BrdU fed in drinking water)

Figure 6


D
CCSP-YFP
Sham Day 3
PNX Day 3
5.4%
<0.03%
6.1%
1.7%
CCSP (YFP)
BrdU
Sca-1
SPC
BrdU CCSP VE-cadherin

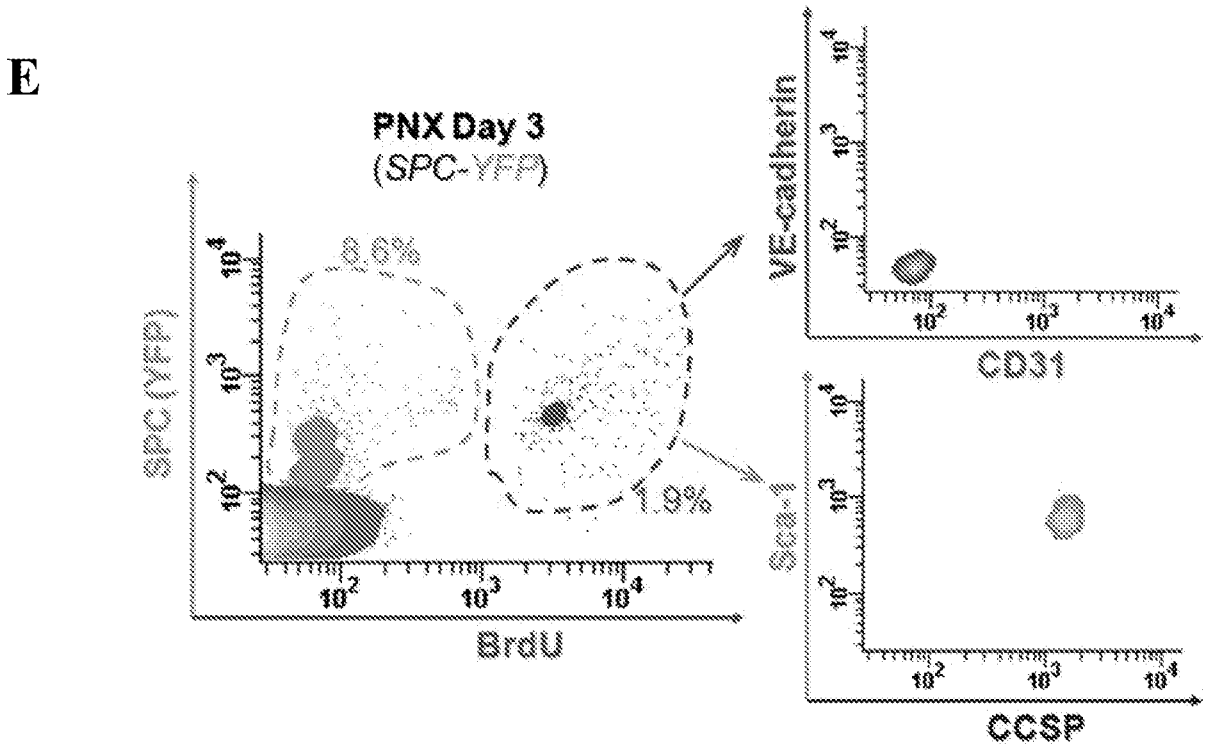

E
PNX Day 3
(SPC-YFP)
8.6%
1.9%
SPC (YFP)
BrdU
VE-cadherin
CD31
Sca-1
CCSP

Figure 7

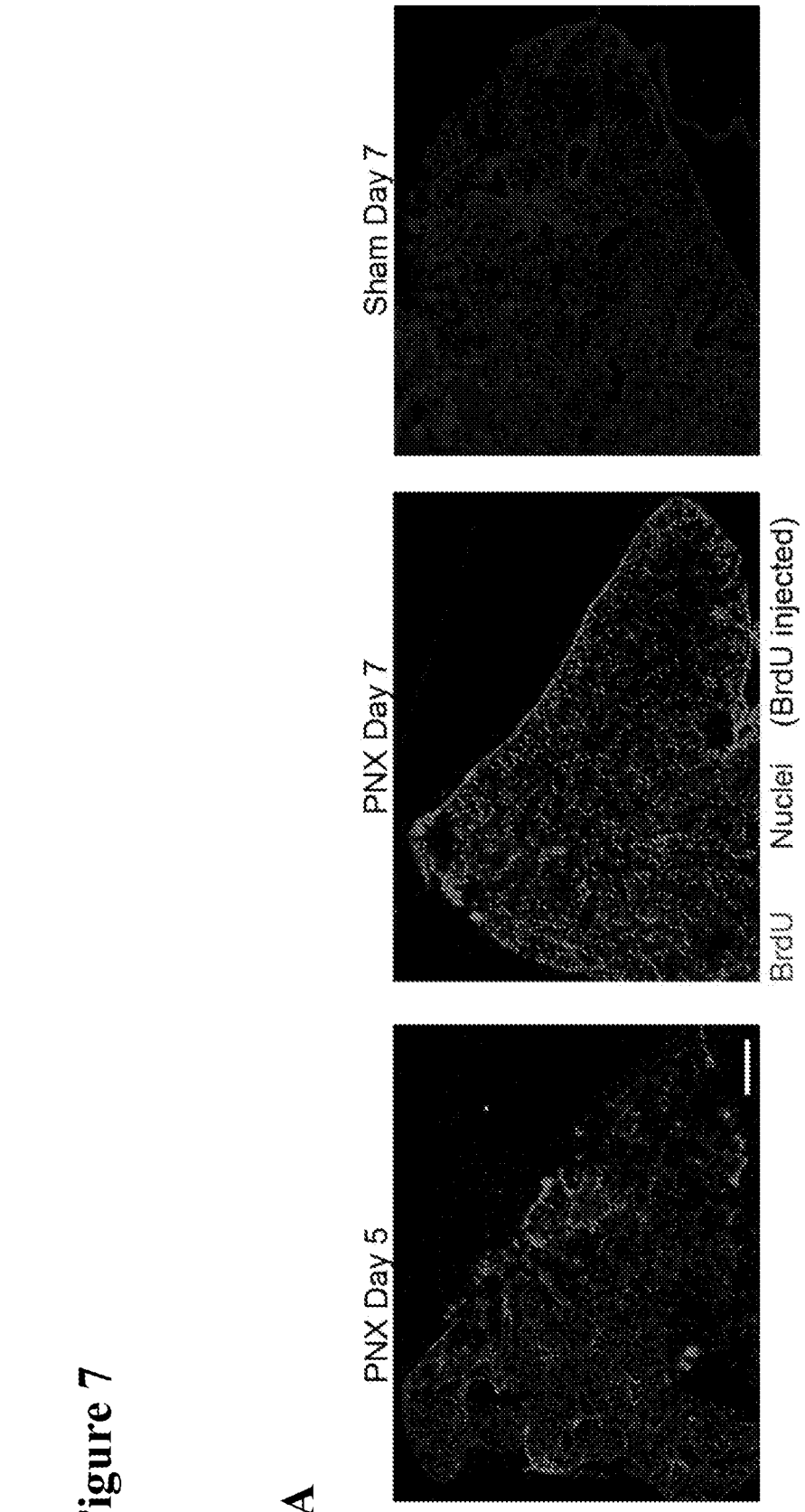


Figure 7

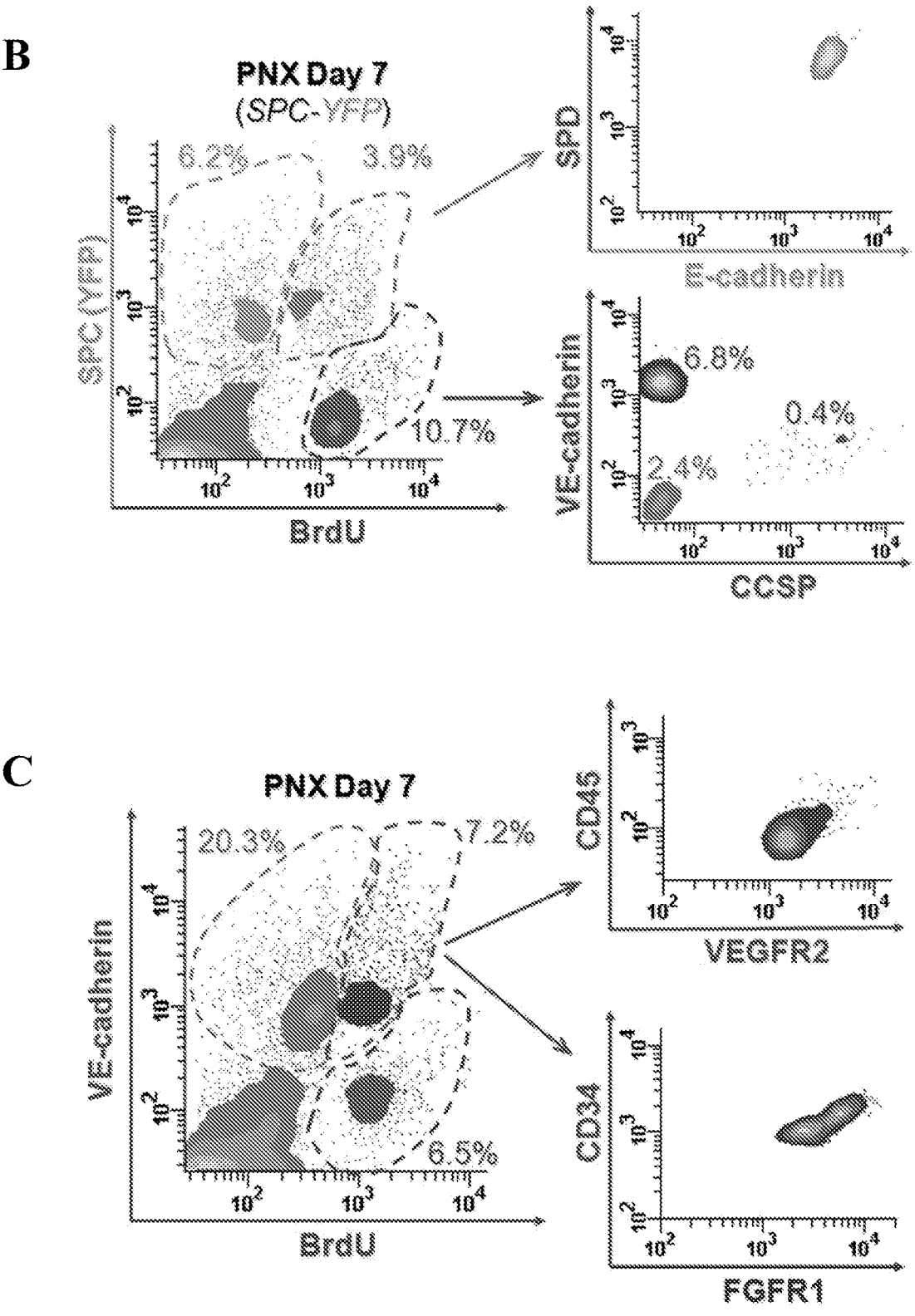
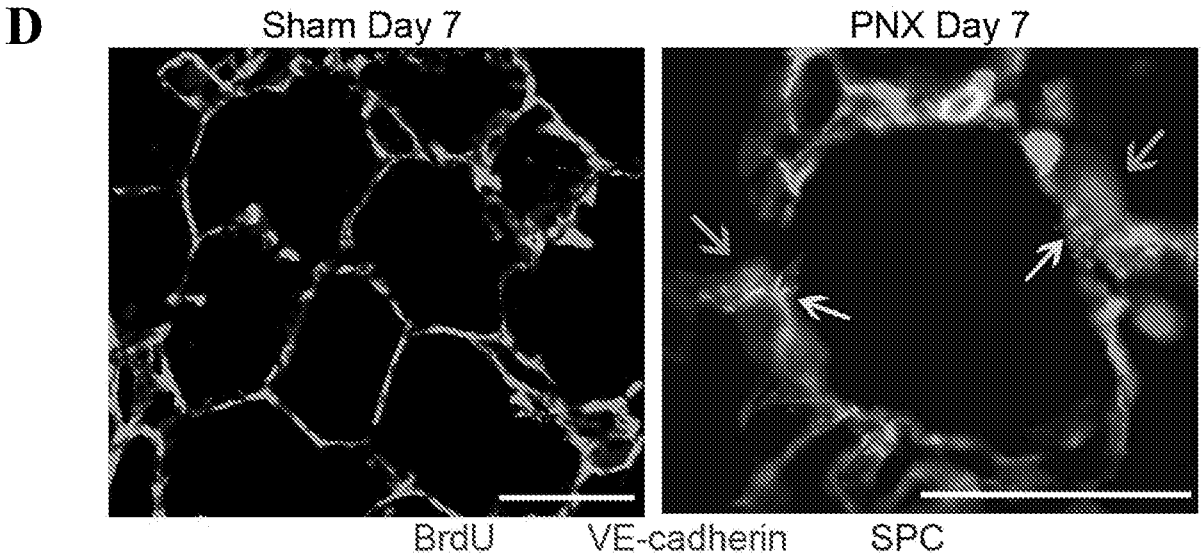

B
PNX Day 7
(SPC-YFP)
6.2%
3.9%
10.7%
SPC (YFP)
BrdU
SPD
E-cadherin
VE-cadherin
6.8%
0.4%
2.4%
CCSP
C
PNX Day 7
20.3%
7.2%
6.5%
VE-cadherin
BrdU
CD45
VEGFR2
CD34
FGFR1

Figure 7


D
Sham Day 7
PNX Day 7
BrdU
VE-cadherin
SPC

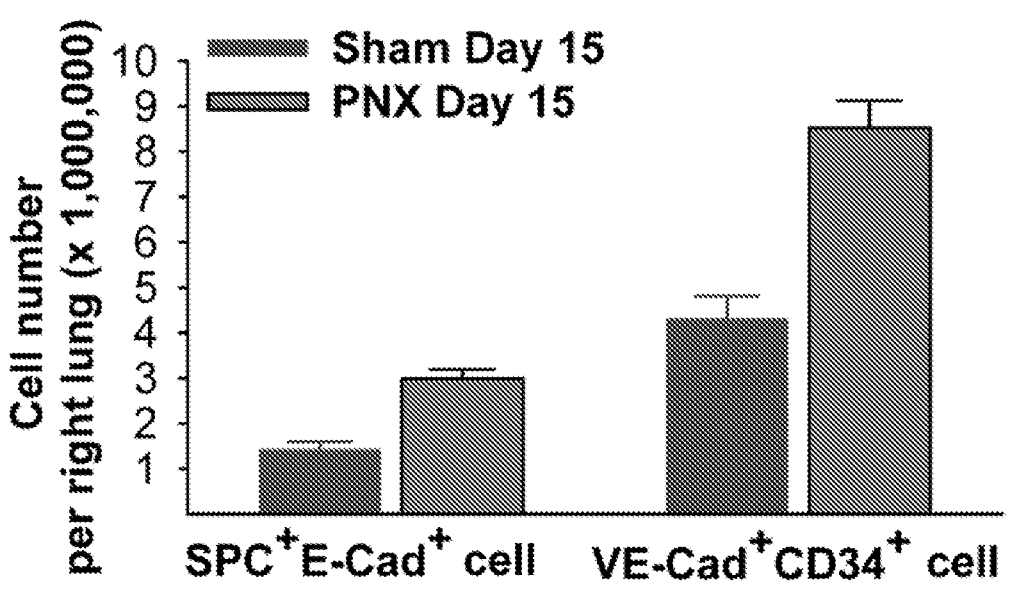
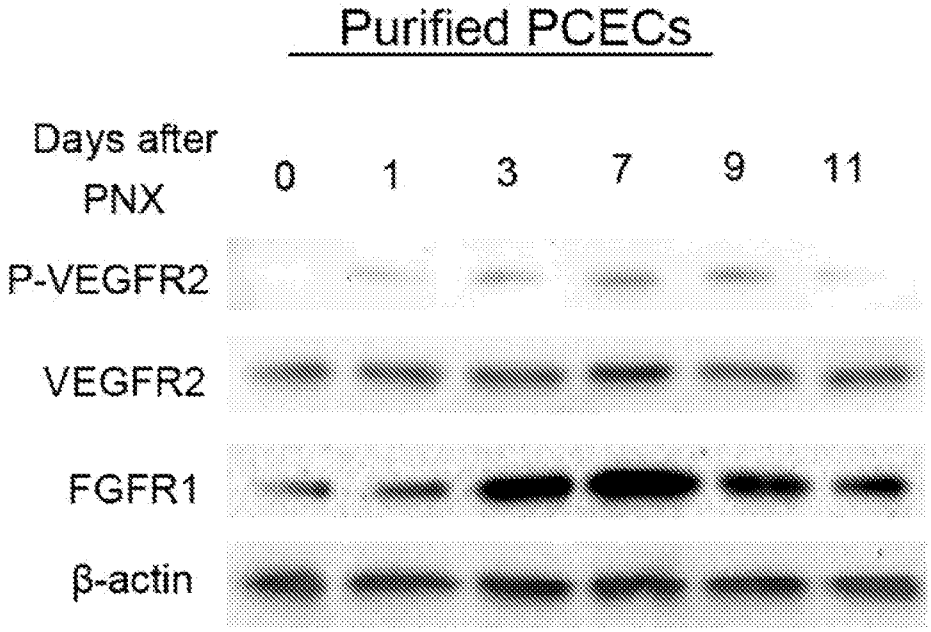

E
Sham Day 15
PNX Day 15
Cell number per right lung (x 1,000,000)
10
9
8
7
6
5
4
3
2
1
SPC+E-Cad+ cell
VE-Cad+CD34+ cell

Figure 8

A


Purified PCECs
Days after PNX
0
1
3
7
9
11
P-VEGFR2
VEGFR2
FGFR1
β-actin

B

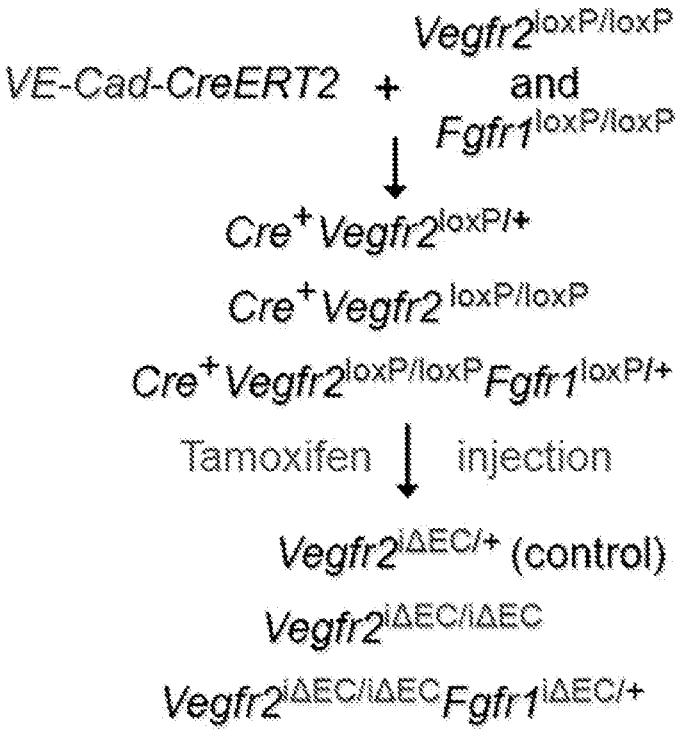

VE-Cad-CreERT2 + Vegfr2loxP/loxP and Fgfr1loxP/loxP
Cre+Vegfr2loxP/+
Cre+Vegfr2loxP/loxP
Cre+Vegfr2loxP/loxPFgfr1loxP/+
Tamoxifen injection
Vegfr2iΔEC/+ (control)
Vegfr2iΔEC/iΔEC
Vegfr2iΔEC/iΔECFgfr1iΔEC/+

Figure 8

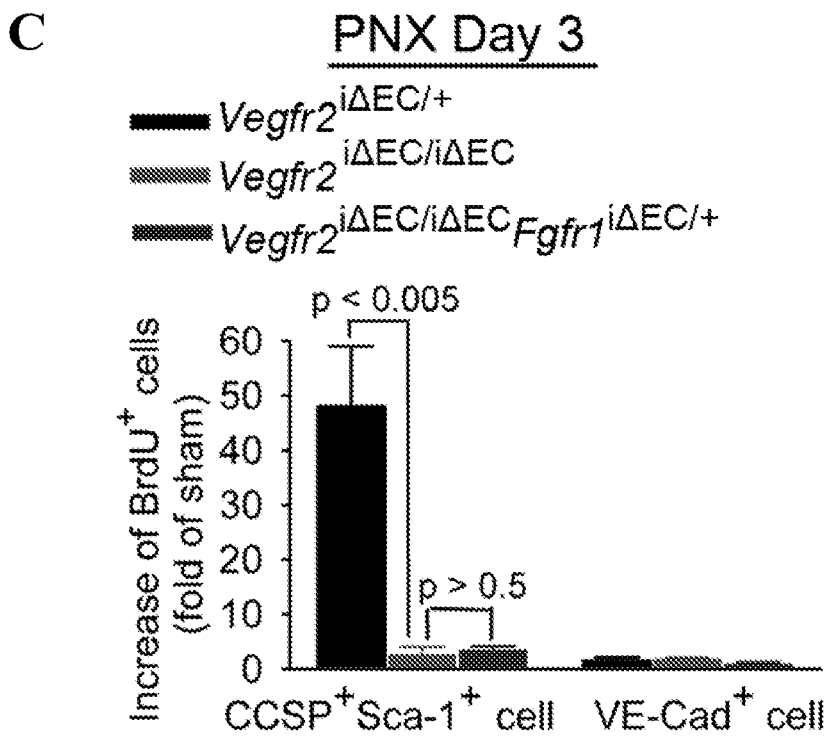

C
PNX Day 3
Vegfr2iΔEC/+
Vegfr2iΔEC/iΔEC
Vegfr2iΔEC/iΔECFgfr1iΔEC/+
p < 0.005
p > 0.5
Increase of BrdU+ cells (fold of sham)
60
50
40
30
20
10
0
CCSP+Sca-1+ cell
VE-Cad+ cell

Figure 8

D

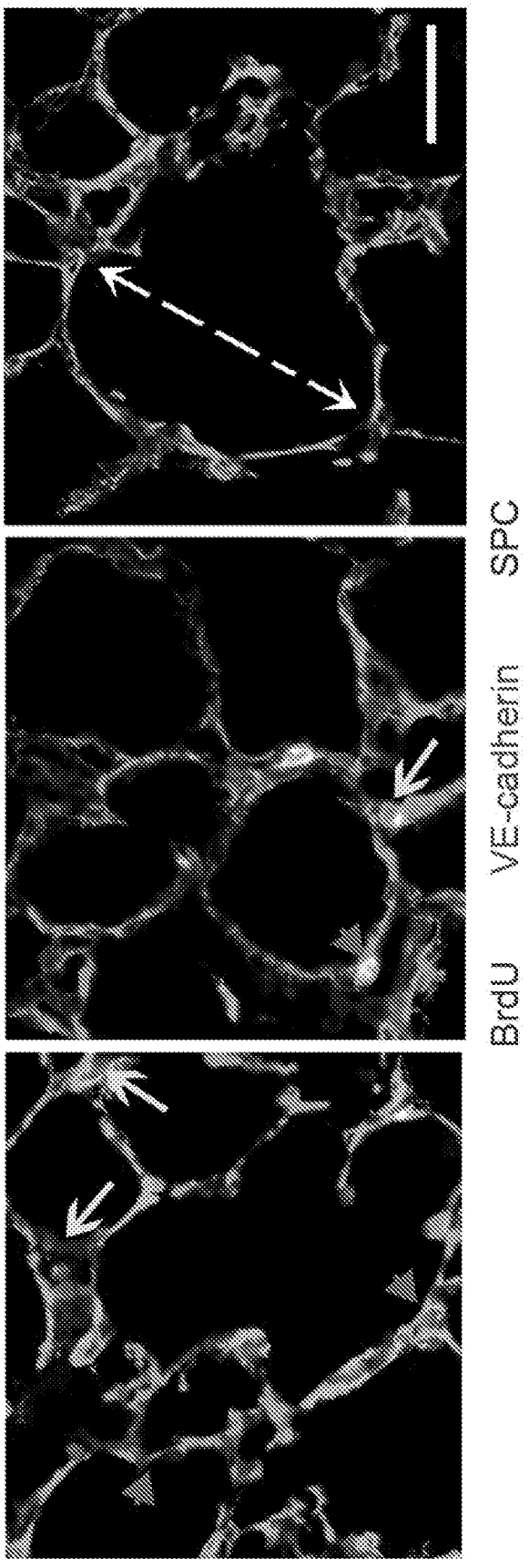

PNX Day 7
Vegfr2iΔEC/+
Vegfr2iΔEC/iΔEC
Vegfr2iΔEC/iΔECFgfr1iΔEC/+
BrdU
VE-cadherin
SPC

Figure 8

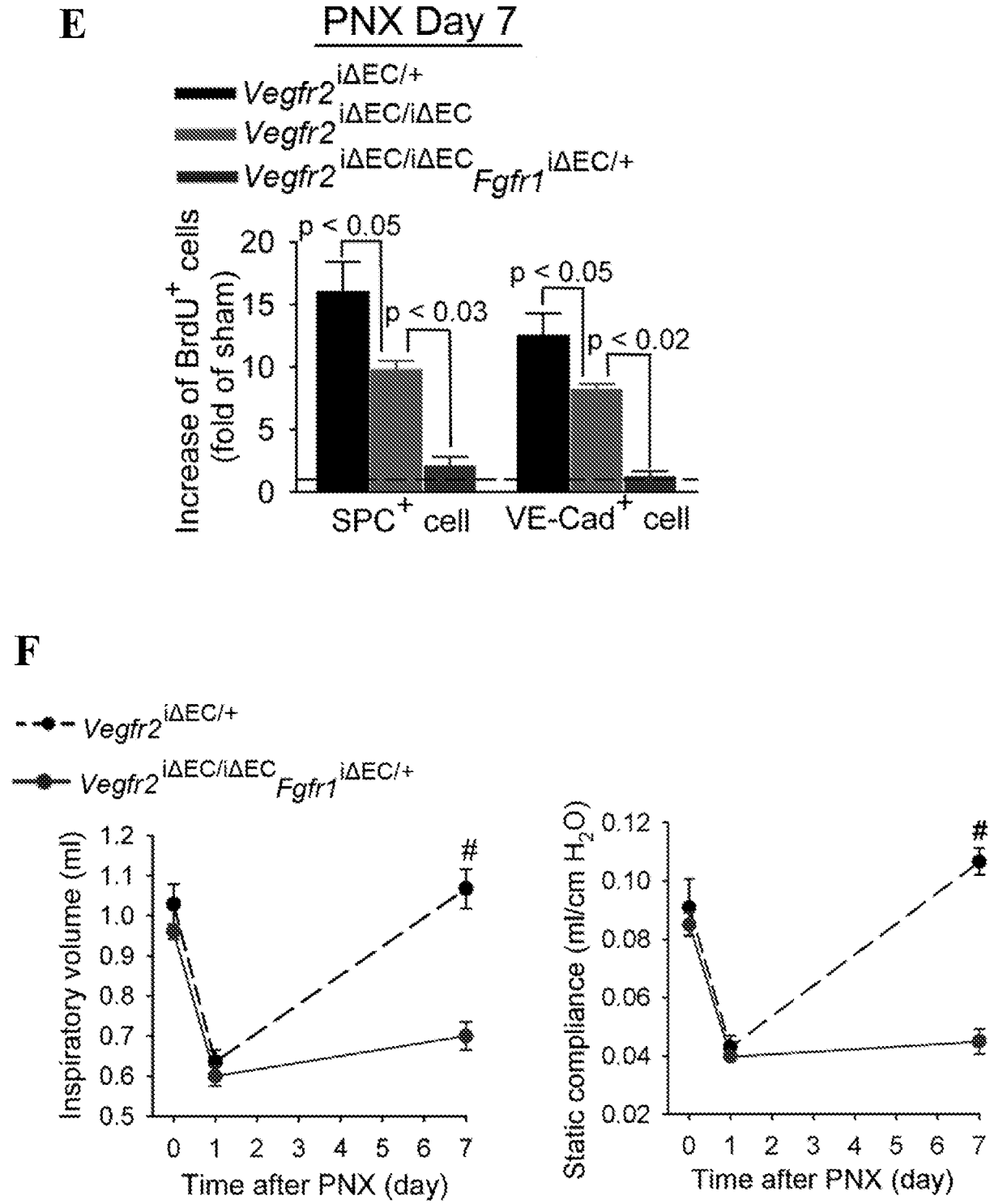

E
PNX Day 7
Vegfr2iΔEC/+
Vegfr2iΔEC/iΔEC
Vegfr2iΔEC/iΔEC Fgfr1iΔEC/+
Increase of BrdU+ cells (fold of sham)
p < 0.05
p < 0.03
p < 0.05
p < 0.02
SPC+ cell
VE-Cad+ cell
F
Vegfr2iΔEC/+
Vegfr2iΔEC/iΔEC Fgfr1iΔEC/+
Inspiratory volume (ml)
Time after PNX (day)
Static compliance (ml/cm H2O)
Time after PNX (day)

Figure 8

G

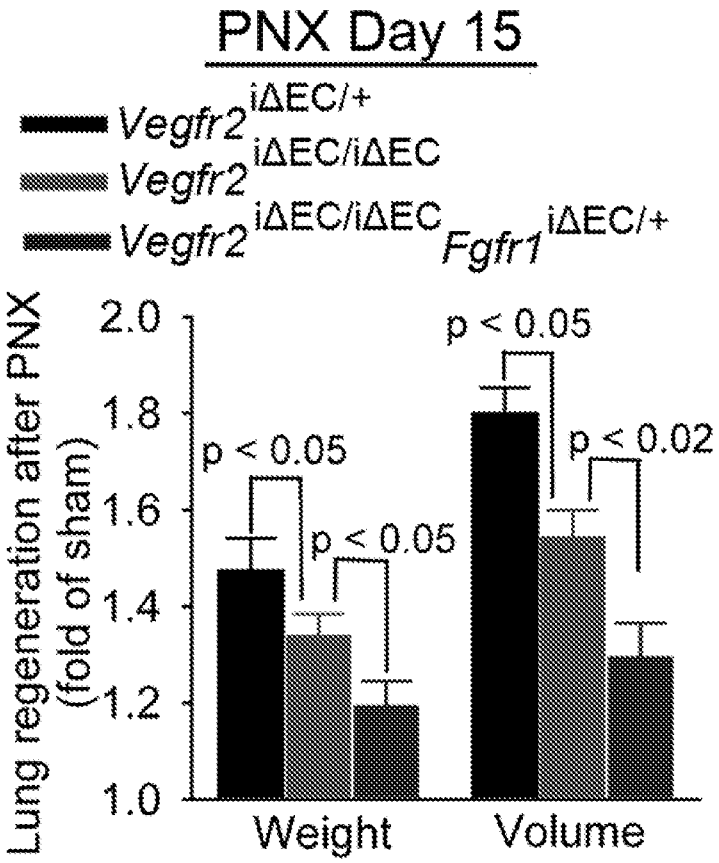
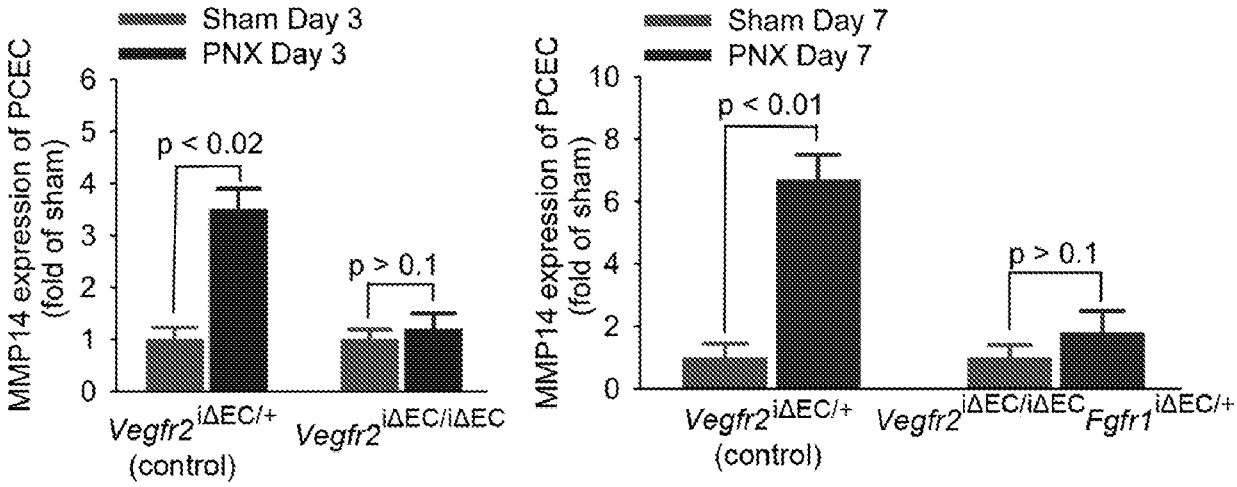

PNX Day 15
Vegfr2 iΔEC/+
Vegfr2 iΔEC/iΔEC
Vegfr2 iΔEC/iΔEC
Fgfr1 iΔEC/+
Lung regeneration after PNX (fold of sham)
2.0
1.8
1.6
1.4
1.2
1.0
p < 0.05
p < 0.05
p < 0.05
p < 0.02
Weight
Volume

Figure 9

A


Sham Day 3
PNX Day 3
MMP14 expression of PCEC (fold of sham)
p < 0.02
p > 0.1
Vegfr2iΔEC/+ (control)
Vegfr2iΔEC/iΔEC
Sham Day 7
PNX Day 7
p < 0.01
p > 0.1
Vegfr2iΔEC/+ (control)
Vegfr2iΔEC/iΔEC
Fgfr1iΔEC/+

B

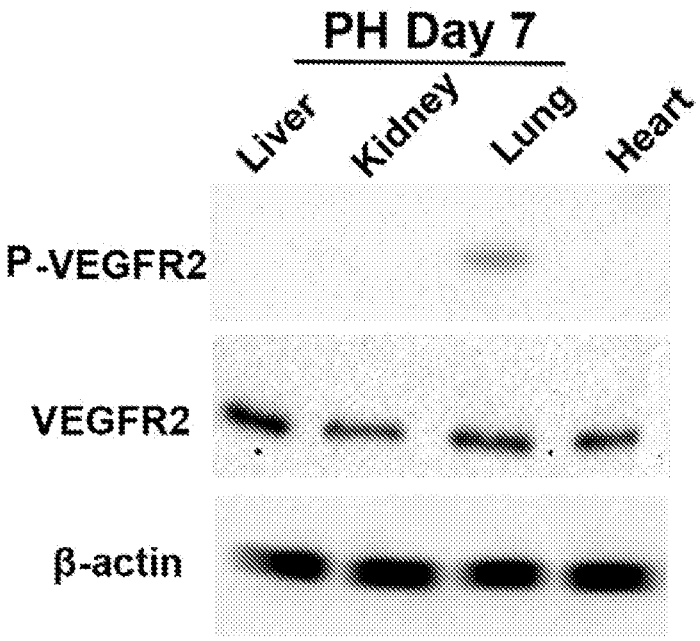

PH Day 7
Liver
Kidney
Lung
Heart
P-VEGFR2
VEGFR2
β-actin

Figure 9

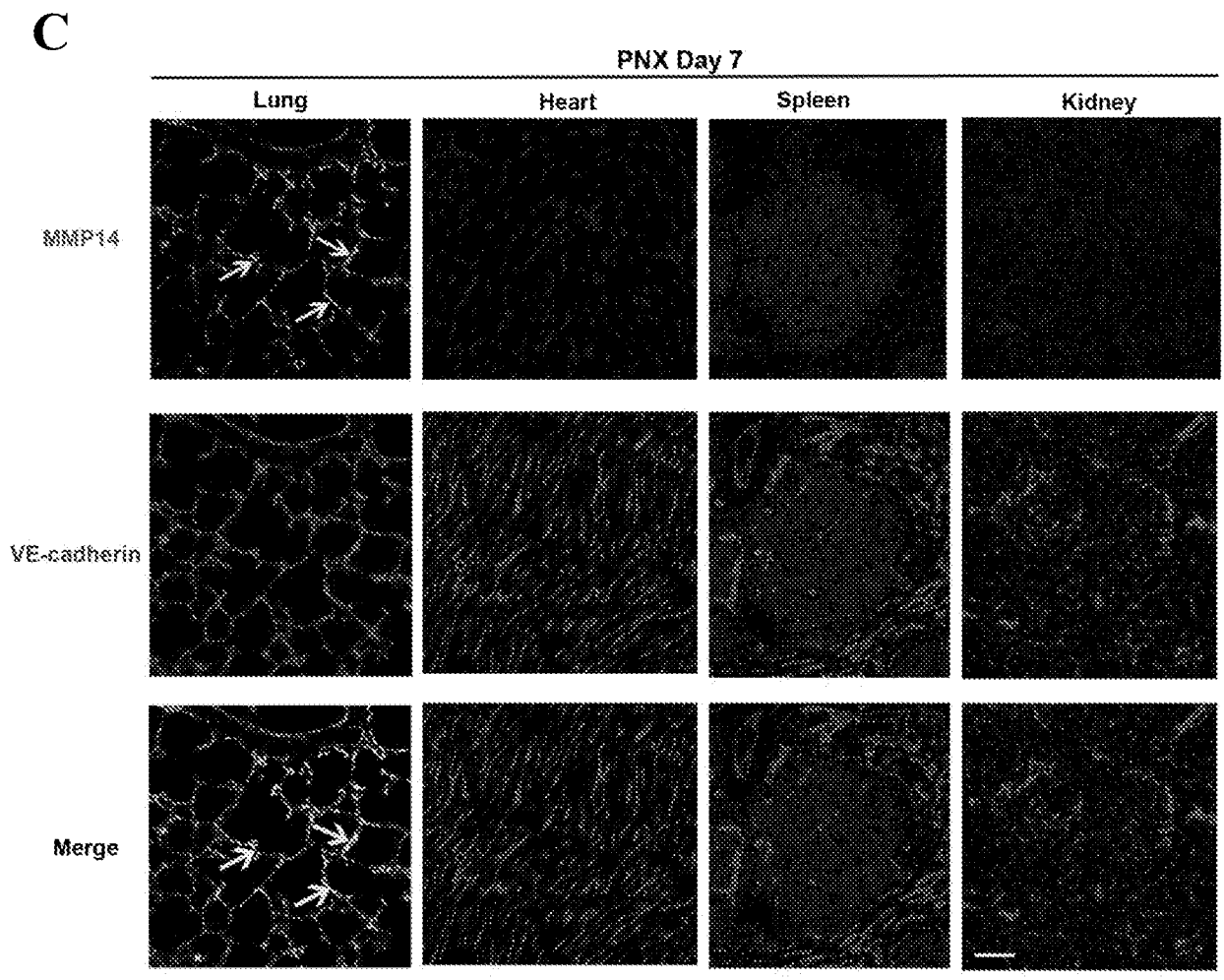
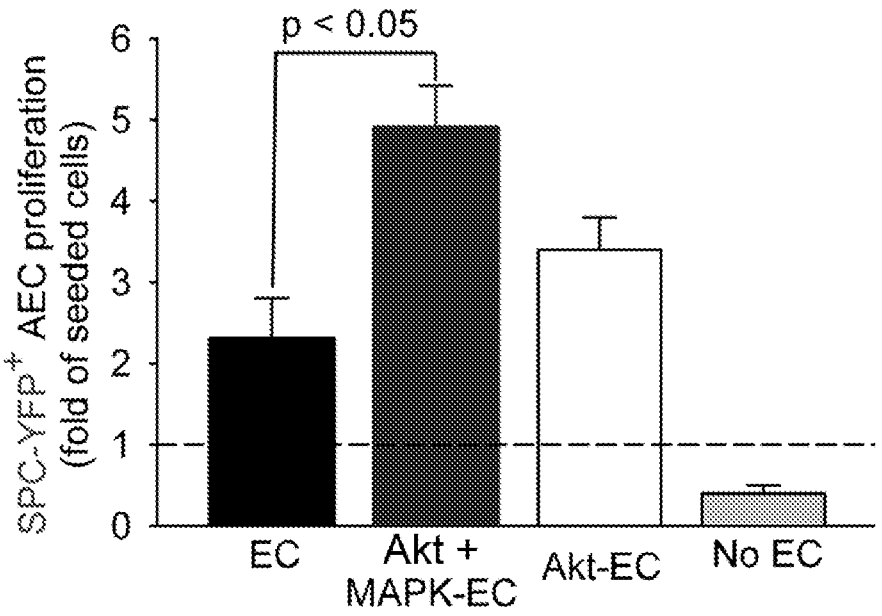


Figure 9

D


p < 0.05
SPC-YFP+ AEC proliferation
(fold of seeded cells)
0
1
2
3
4
5
6
EC
Akt + MAPK-EC
Akt-EC
No EC

E

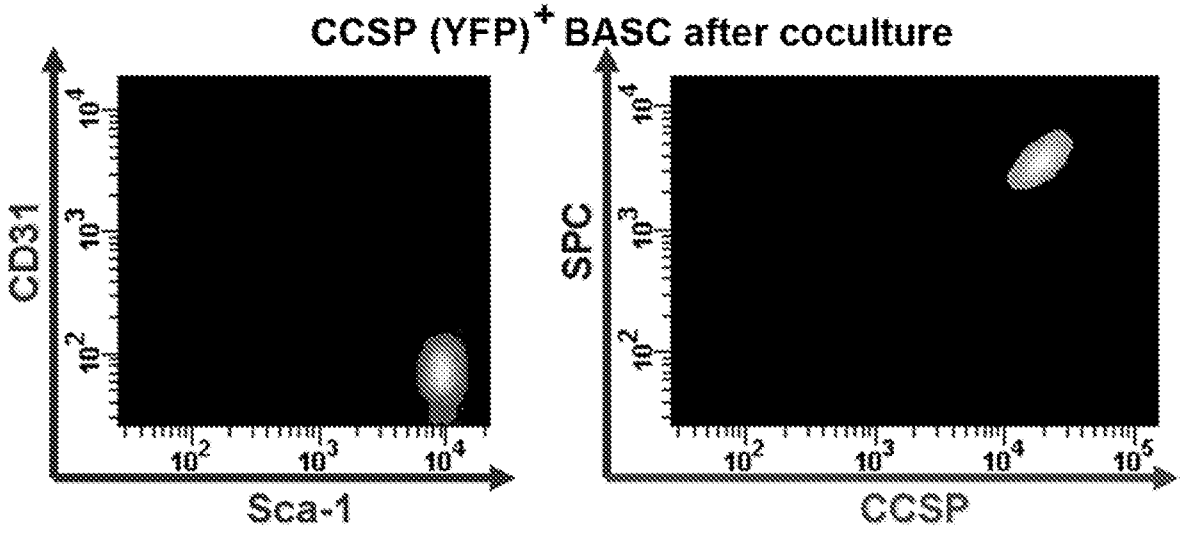
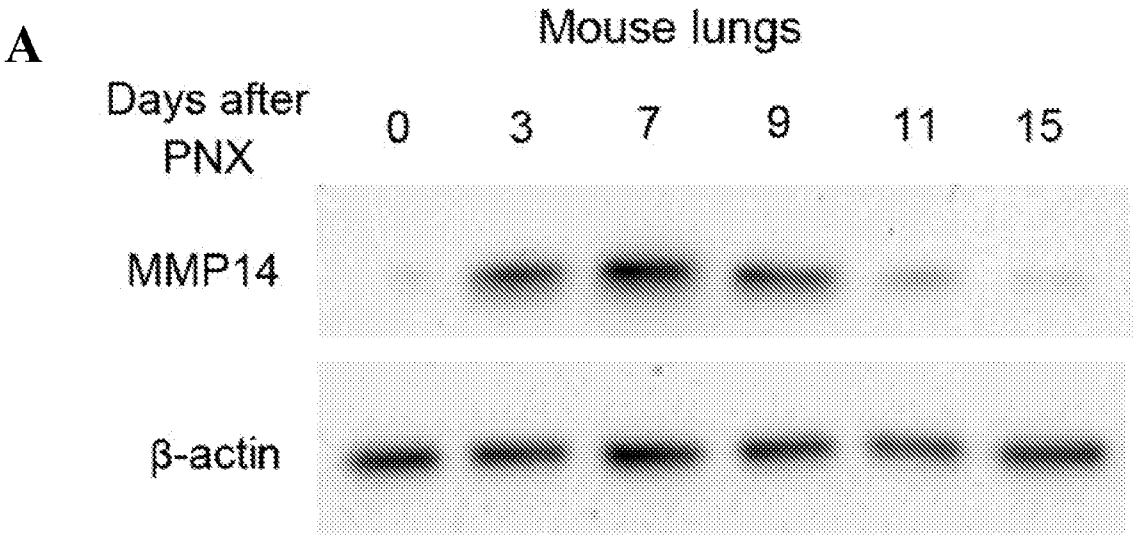

CCSP (YFP)+ BASC after coculture
CD31
Sca-1
SPC
CCSP

Figure 10


A
Mouse lungs
Days after PNX
0
3
7
9
11
15
MMP14
β-actin

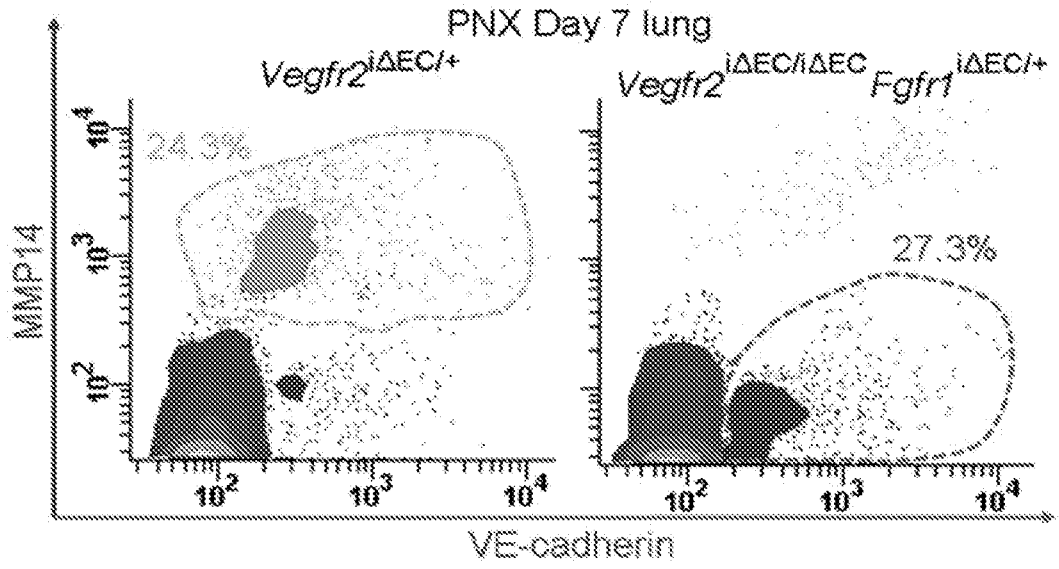

B
PNX Day 7 lung
Vegfr2iΔEC/+
Vegfr2iΔEC/iΔEC Fgfr1iΔEC/+
24.3%
27.3%
MMP14
VE-cadherin
10^2
10^3
10^4

Figure 10

C

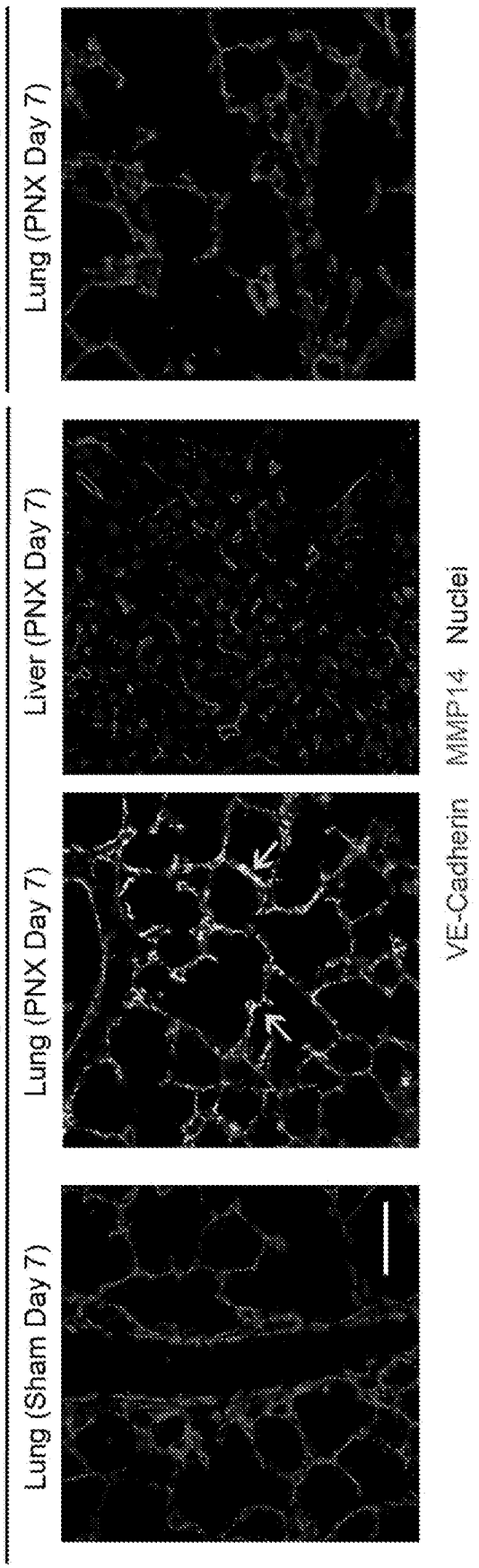
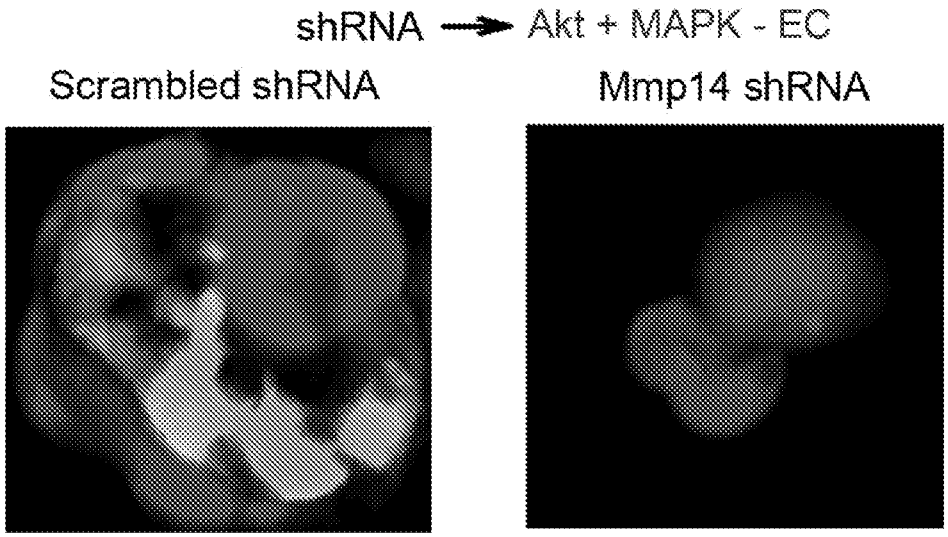

Vegfr2iΔEC/+
Vegfr2iΔEC/iΔECFgfr1iΔEC/+
Lung (Sham Day 7)
Lung (PNX Day 7)
Liver (PNX Day 7)
Lung (PNX Day 7)
VE-Cadherin
MMP14
Nuclei

Figure 10

D


shRNA → Akt + MAPK - EC
Scrambled shRNA
Mmp14 shRNA
Coculture of Akt + MAPK - EC and SPC (YFP)+ AEC

E

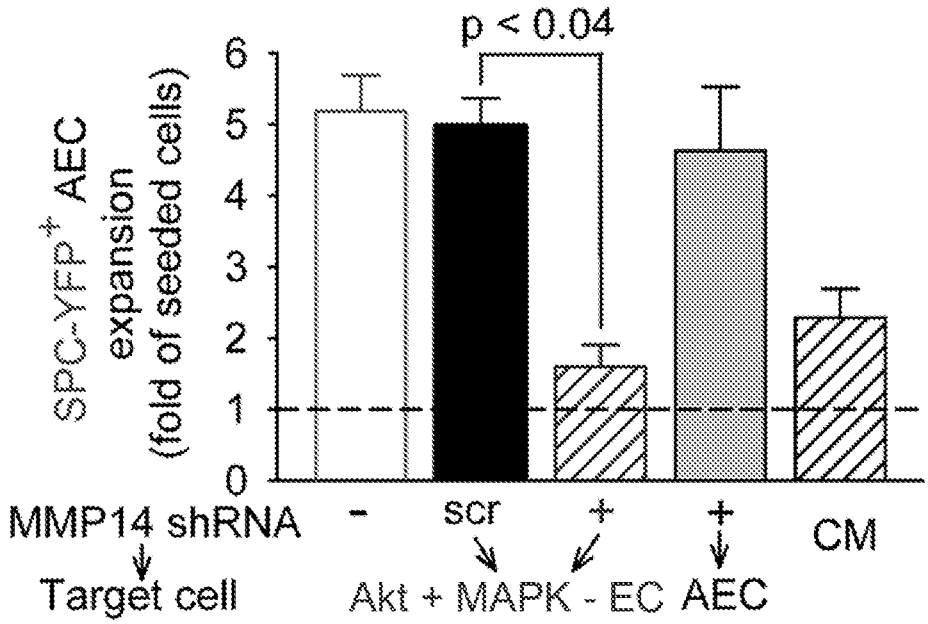
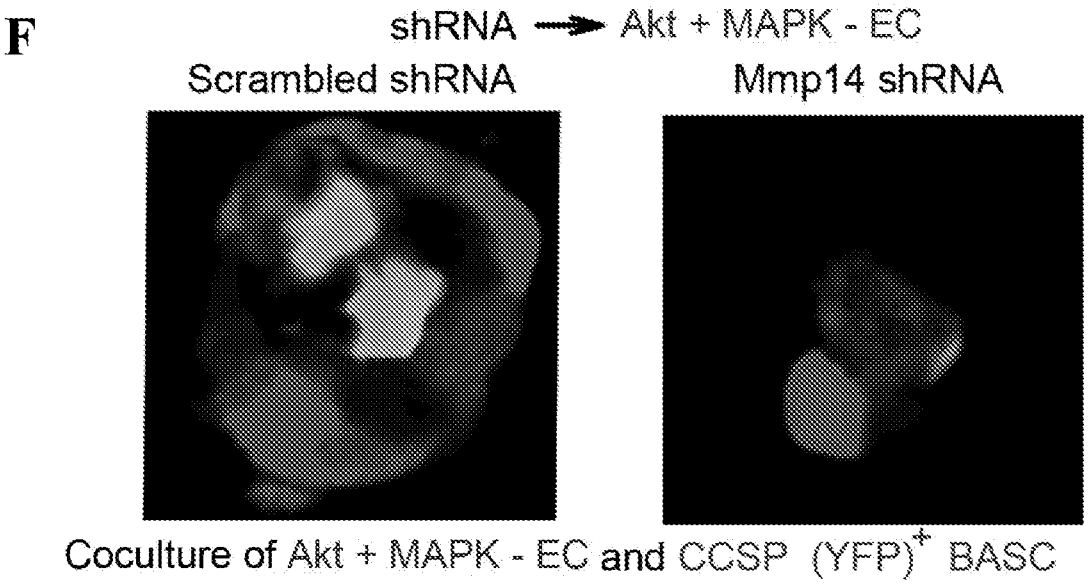

p < 0.04
SPC-YFP+ AEC expansion (fold of seeded cells)
0
1
2
3
4
5
6
MMP14 shRNA
-
scr
+
+
CM
Target cell
Akt + MAPK - EC AEC

Figure 10


F
shRNA → Akt + MAPK - EC
Scrambled shRNA
Mmp14 shRNA
Coculture of Akt + MAPK - EC and CCSP (YFP)+ BASC

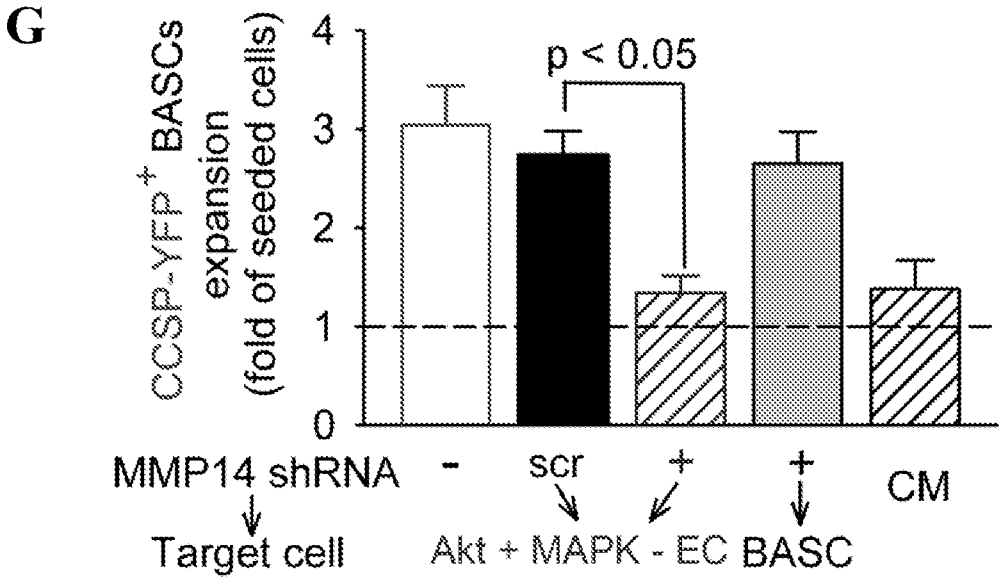
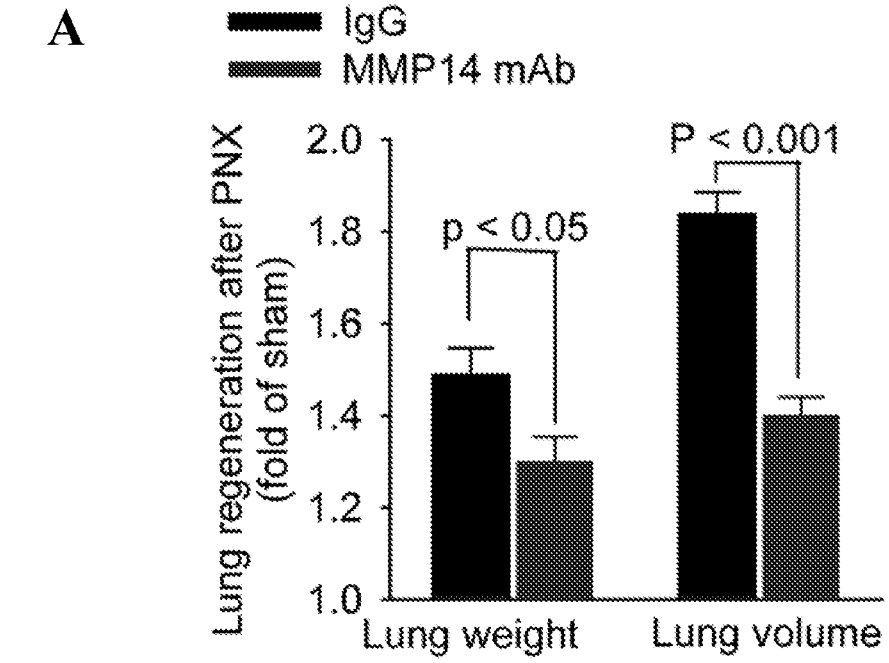

G
CCSP-YFP+ BASCs expansion (fold of seeded cells)
4
3
2
1
0
p < 0.05
MMP14 shRNA
-
scr
+
+
CM
Target cell
Akt + MAPK - EC BASC

Figure 11


A
IgG
MMP14 mAb
Lung regeneration after PNX
(fold of sham)
2.0
1.8
1.6
1.4
1.2
1.0
p < 0.05
P < 0.001
Lung weight
Lung volume

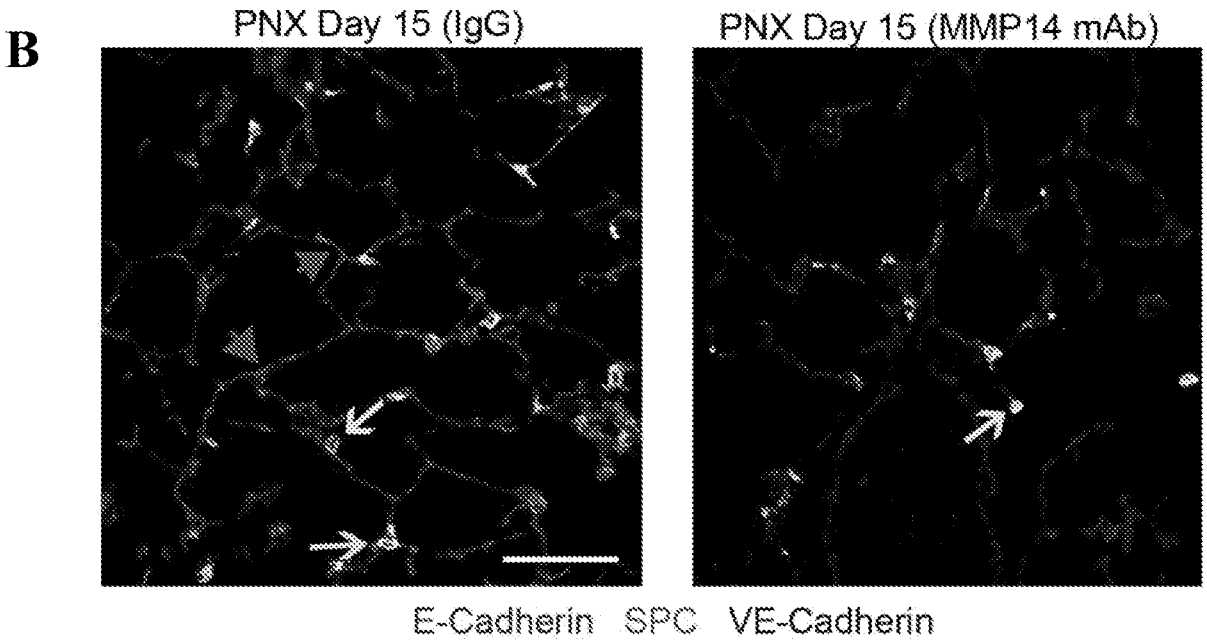
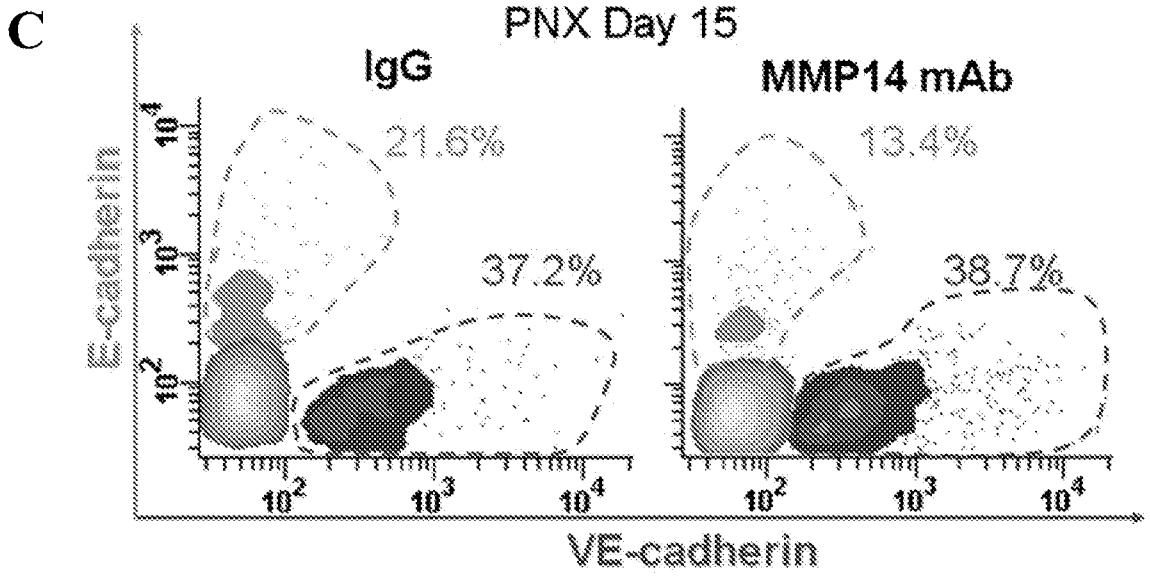

B
PNX Day 15 (IgG)
PNX Day 15 (MMP14 mAb)
E-Cadherin SPC VE-Cadherin

Figure 11


C
PNX Day 15
IgG
MMP14 mAb
21.6%
37.2%
13.4%
38.7%
E-cadherin
VE-cadherin
10^2
10^3
10^4

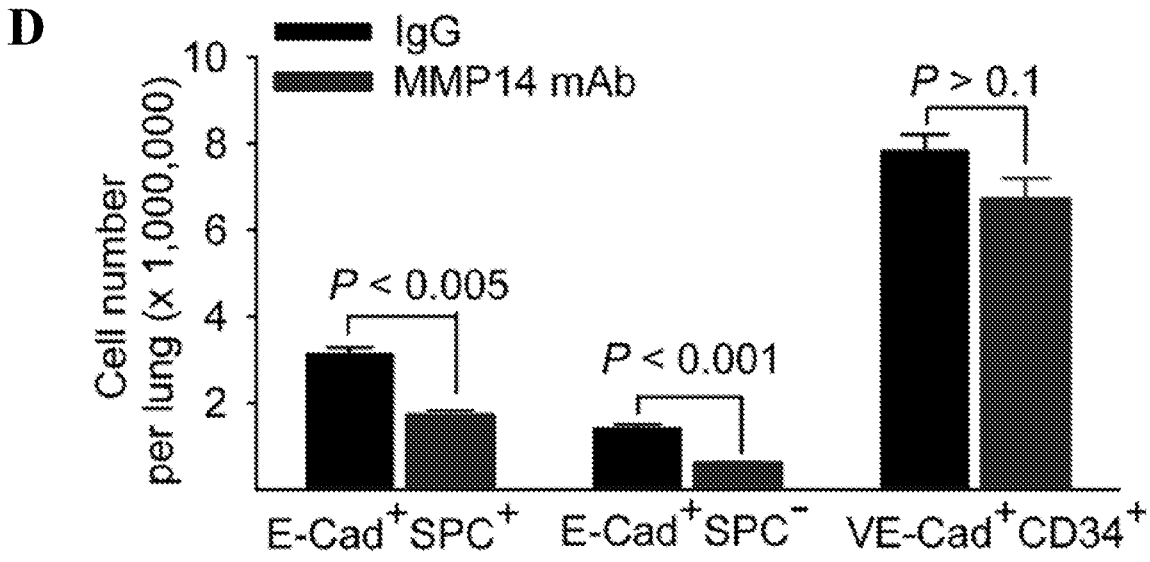

D
IgG
MMP14 mAb
Cell number per lung (x 1,000,000)
10
8
6
4
2
P < 0.005
P < 0.001
P > 0.1
E-Cad+SPC+
E-Cad+SPC−
VE-Cad+CD34+

Figure 11

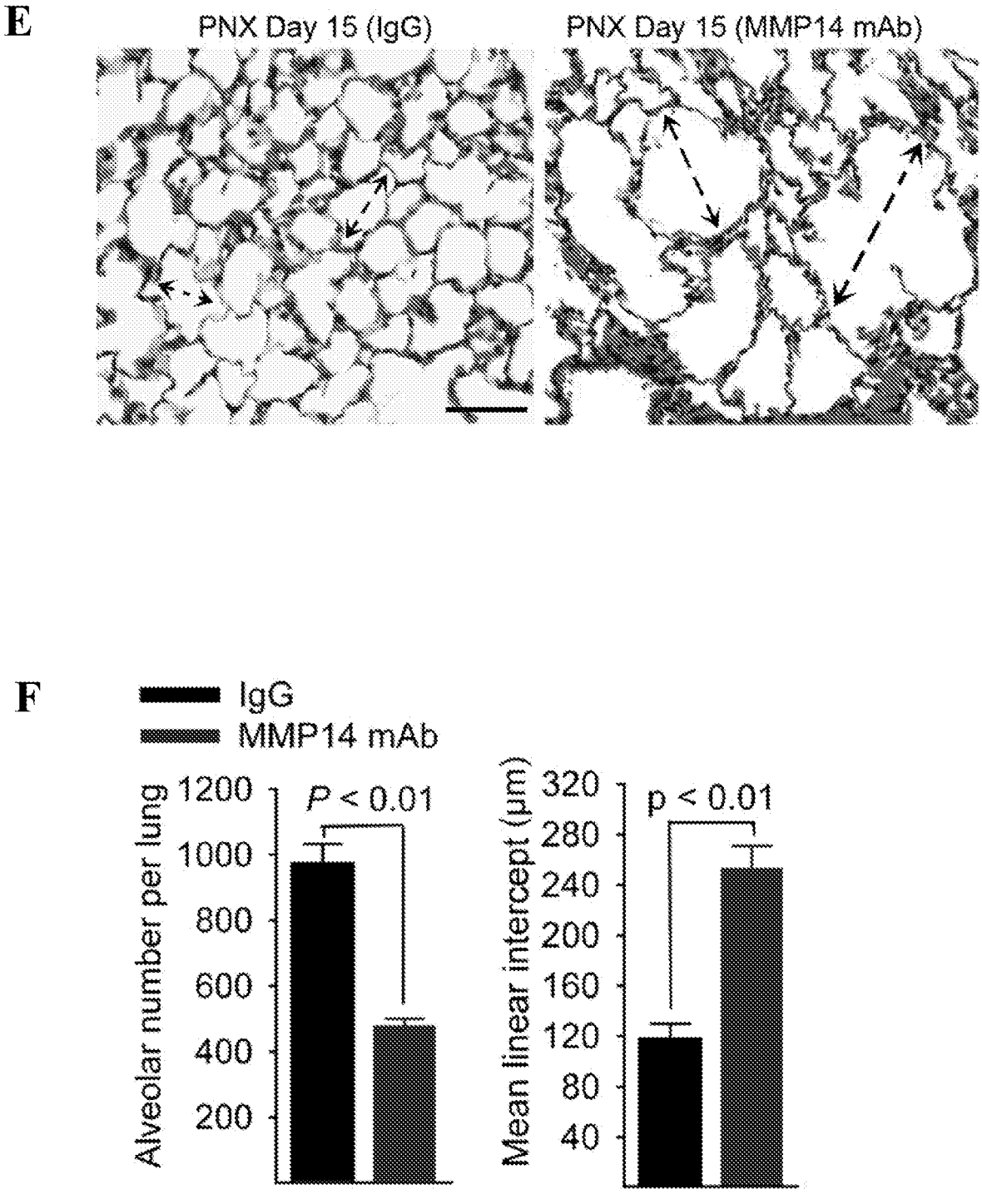
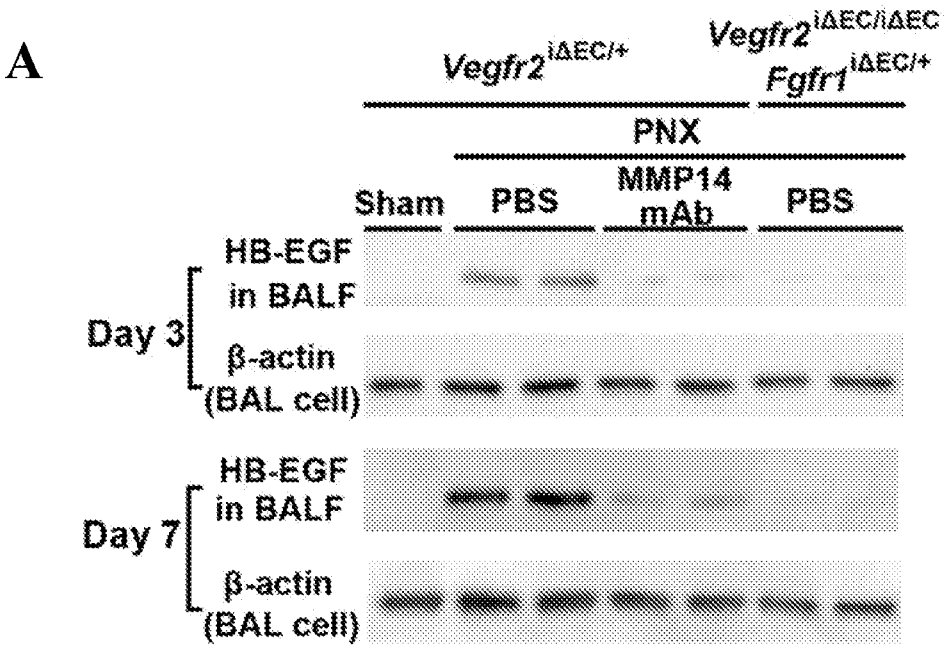

E
PNX Day 15 (IgG)
PNX Day 15 (MMP14 mAb)
F
IgG
MMP14 mAb
Alveolar number per lung
1200
1000
800
600
400
200
P < 0.01
Mean linear intercept (μm)
320
280
240
200
160
120
80
40
p < 0.01

Figure 12


A
Vegfr2iΔEC/+
Vegfr2iΔEC/iΔEC
Fgfr1iΔEC/+
PNX
Sham
PBS
MMP14 mAb
PBS
HB-EGF in BALF
Day 3
β-actin (BAL cell)
HB-EGF in BALF
Day 7
β-actin (BAL cell)

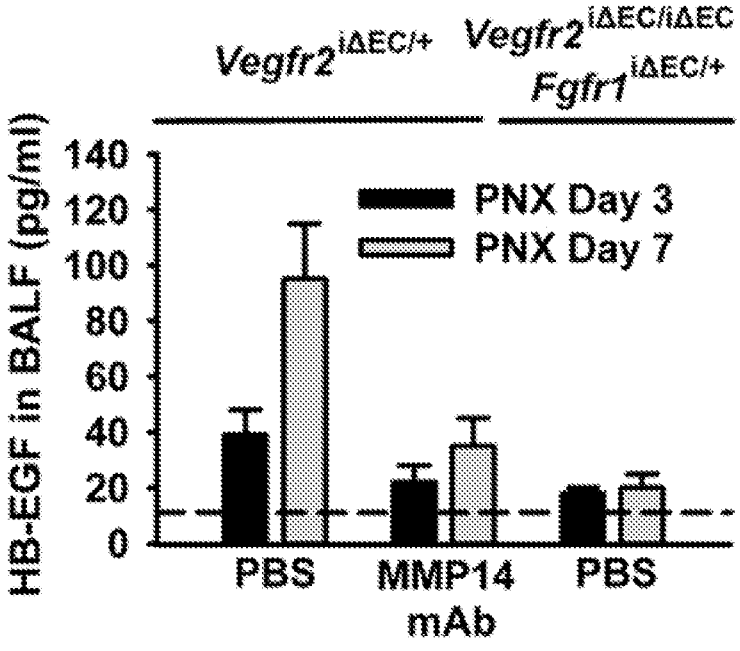
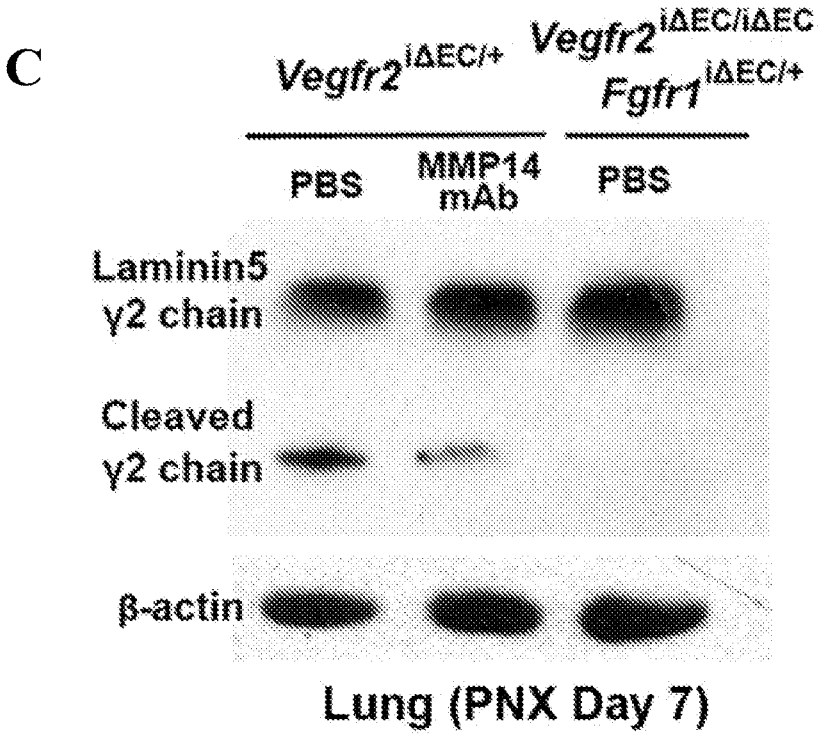

B
Vegfr2iΔEC/+
Vegfr2iΔEC/iΔEC
Fgfr1iΔEC/+
HB-EGF in BALF (pg/ml)
140
120
100
80
60
40
20
0
PNX Day 3
PNX Day 7
PBS
MMP14 mAb
PBS

Figure 12


C
Vegfr2iΔEC/+
Vegfr2iΔEC/iΔEC Fgfr1iΔEC/+
PBS
MMP14 mAb
PBS
Laminin5 γ2 chain
Cleaved γ2 chain
β-actin
Lung (PNX Day 7)

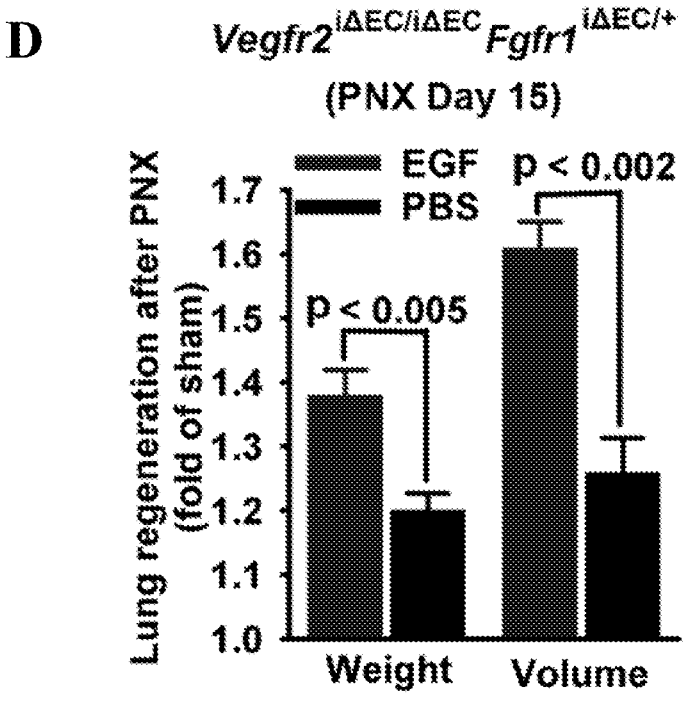
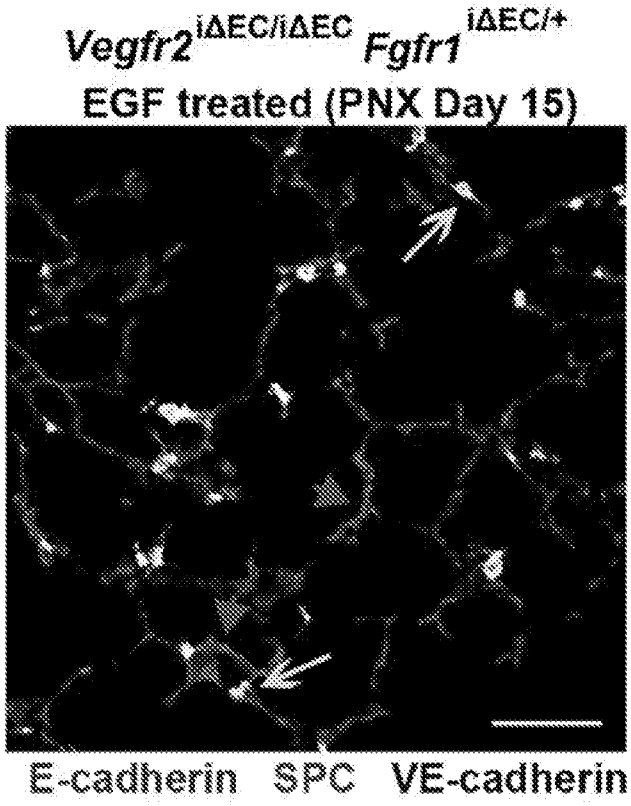

D
Vegfr2iΔEC/iΔEC Fgfr1iΔEC/+
(PNX Day 15)
EGF
PBS
p < 0.002
p < 0.005
Lung regeneration after PNX (fold of sham)
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
Weight
Volume

Figure 12

E


Vegfr2iΔEC/iΔEC Fgfr1iΔEC/+
EGF treated (PNX Day 15)
E-cadherin SPC VE-cadherin

F

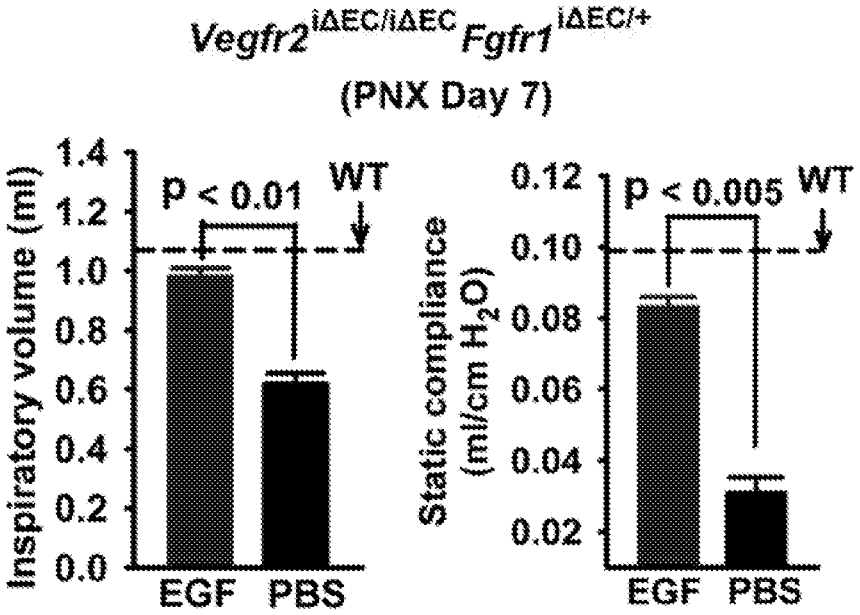
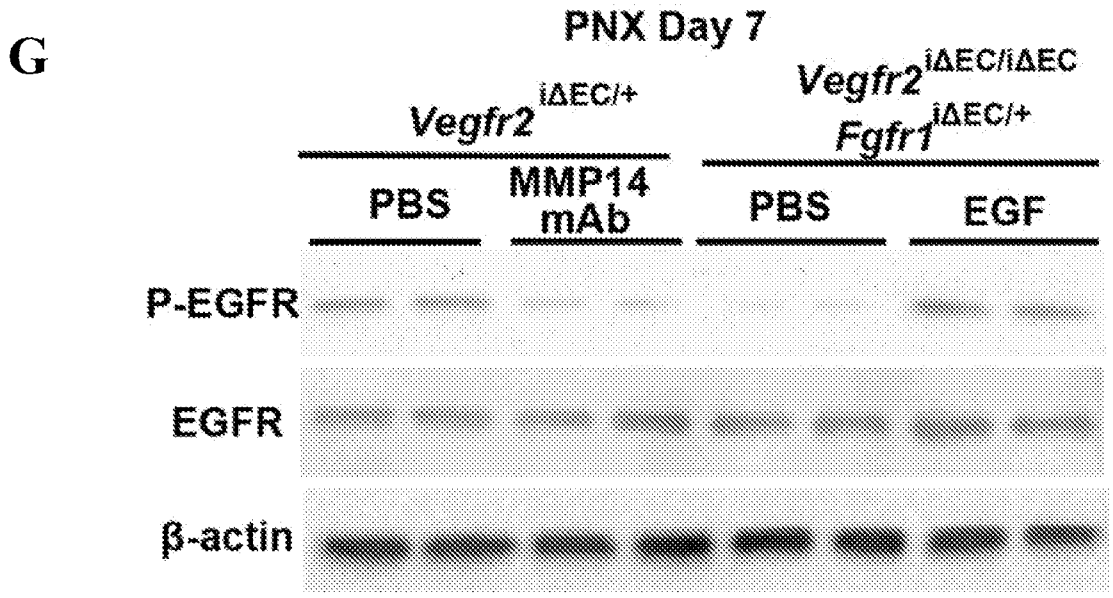

Vegfr2iΔEC/iΔEC Fgfr1iΔEC/+
(PNX Day 7)
Inspiratory volume (ml)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
P < 0.01
WT
EGF
PBS
Static compliance (ml/cm H2O)
0.12
0.10
0.08
0.06
0.04
0.02
P < 0.005
WT
EGF
PBS

Figure 12


G
PNX Day 7
Vegfr2 iΔEC/+
Vegfr2 iΔEC/iΔEC Fgfr1 iΔEC/+
PBS
MMP14 mAb
PBS
EGF
P-EGFR
EGFR
β-actin

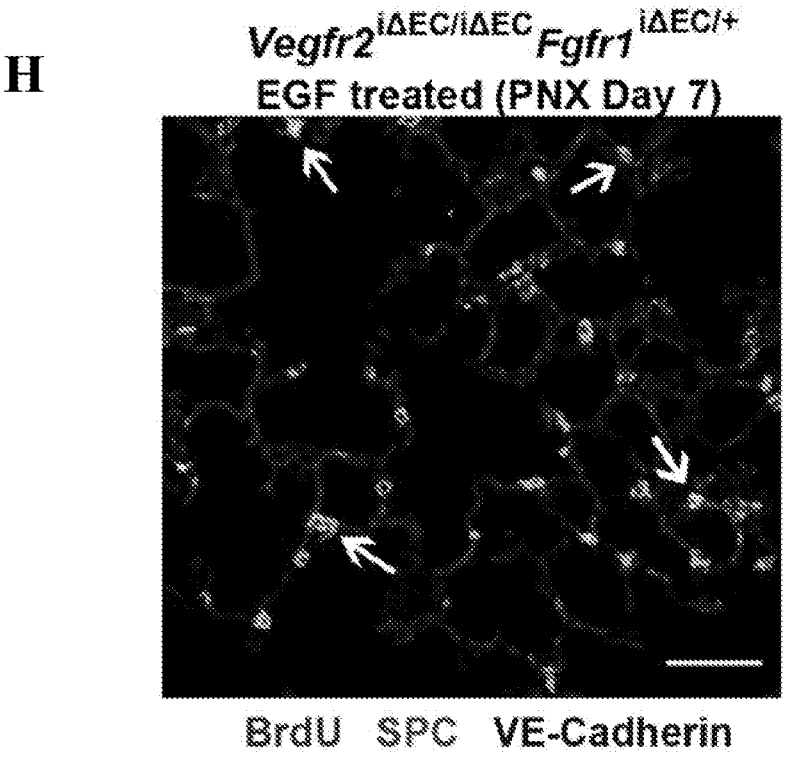
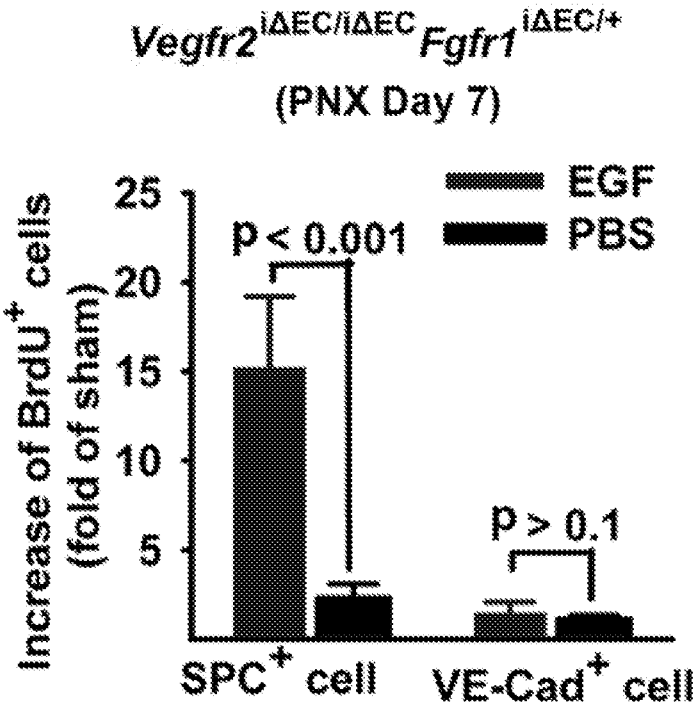

H
Vegfr2 iΔEC/iΔEC Fgfr1 iΔEC/+
EGF treated (PNX Day 7)
BrdU SPC VE-Cadherin

Figure 12

I


Vegfr2iΔEC/iΔEC Fgfr1iΔEC/+
(PNX Day 7)
EGF
PBS
P < 0.001
P > 0.1
Increase of BrdU+ cells (fold of sham)
25
20
15
10
5
SPC+ cell
VE-Cad+ cell

J

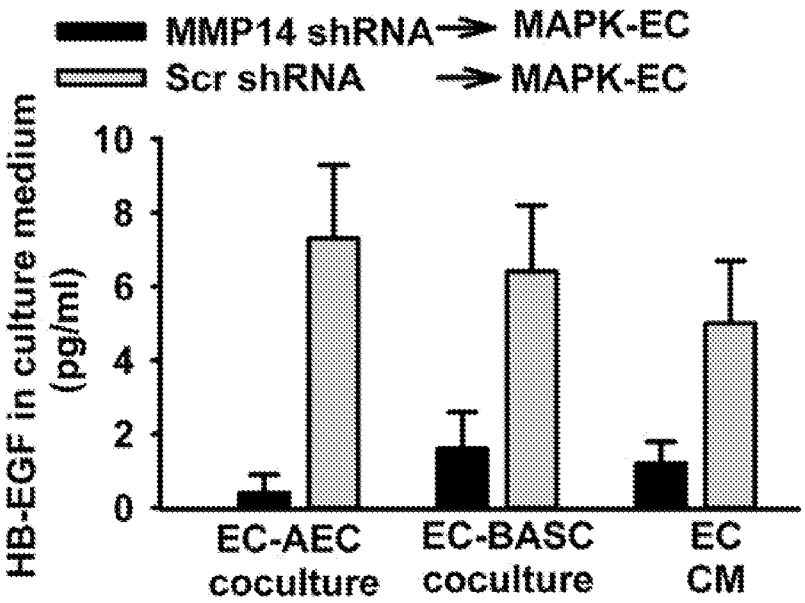

MMP14 shRNA → MAPK-EC
Scr shRNA → MAPK-EC
HB-EGF in culture medium (pg/ml)
10
8
6
4
2
0
EC-AEC coculture
EC-BASC coculture
EC CM

Figure 12

K

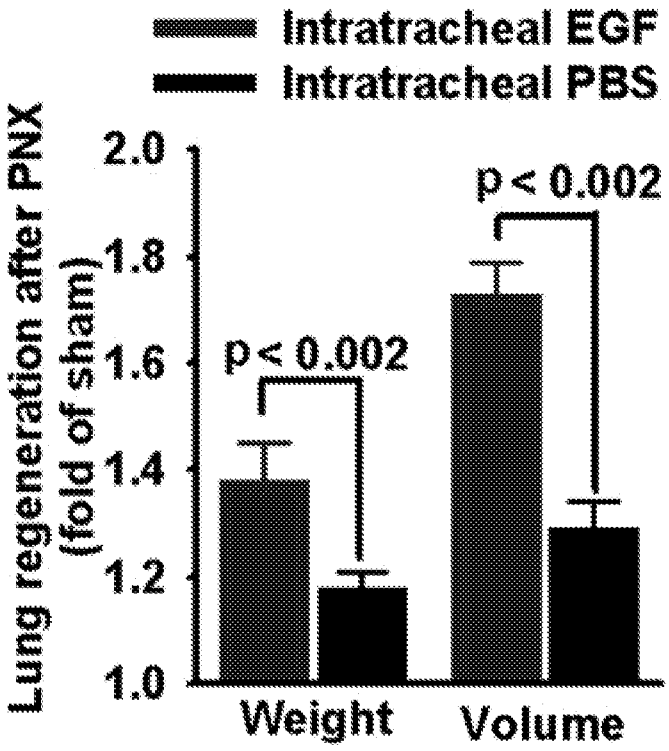
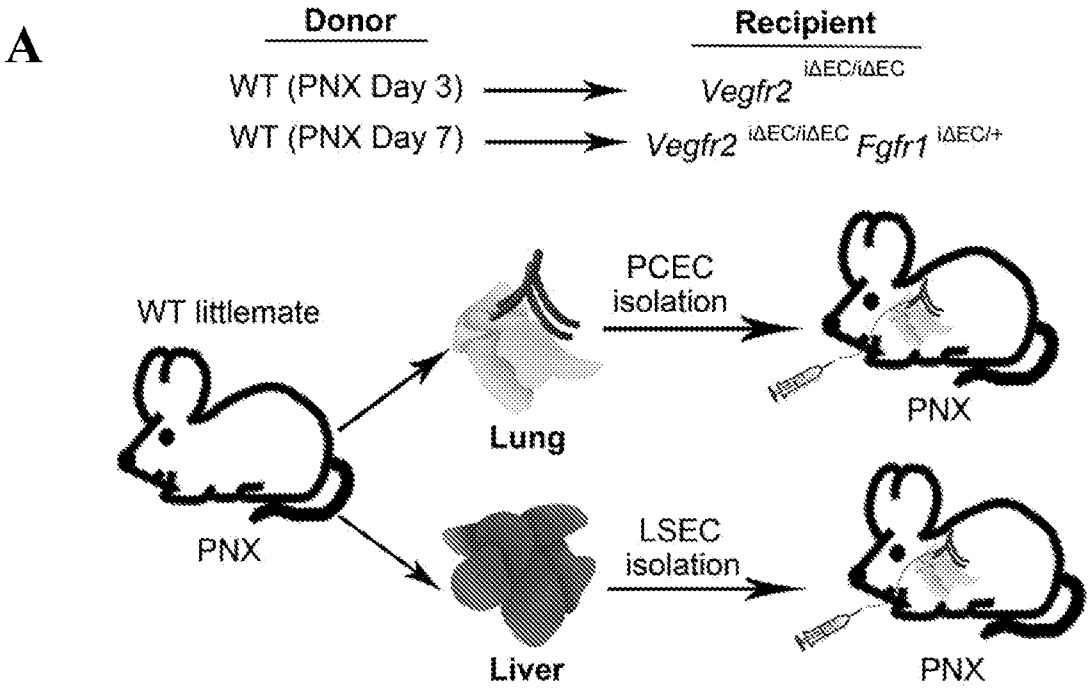

Intratracheal EGF
Intratracheal PBS
Lung regeneration after PNX
(fold of sham)
2.0
1.8
1.6
1.4
1.2
1.0
p< 0.002
p< 0.002
Weight
Volume

Figure 13


A
Donor
Recipient
WT (PNX Day 3)
Vegfr2 iΔEC/iΔEC
WT (PNX Day 7)
Vegfr2 iΔEC/iΔEC Fgfr1 iΔEC/+
WT littlemate
PCEC isolation
PNX
Lung
PNX
LSEC isolation
Liver
PNX

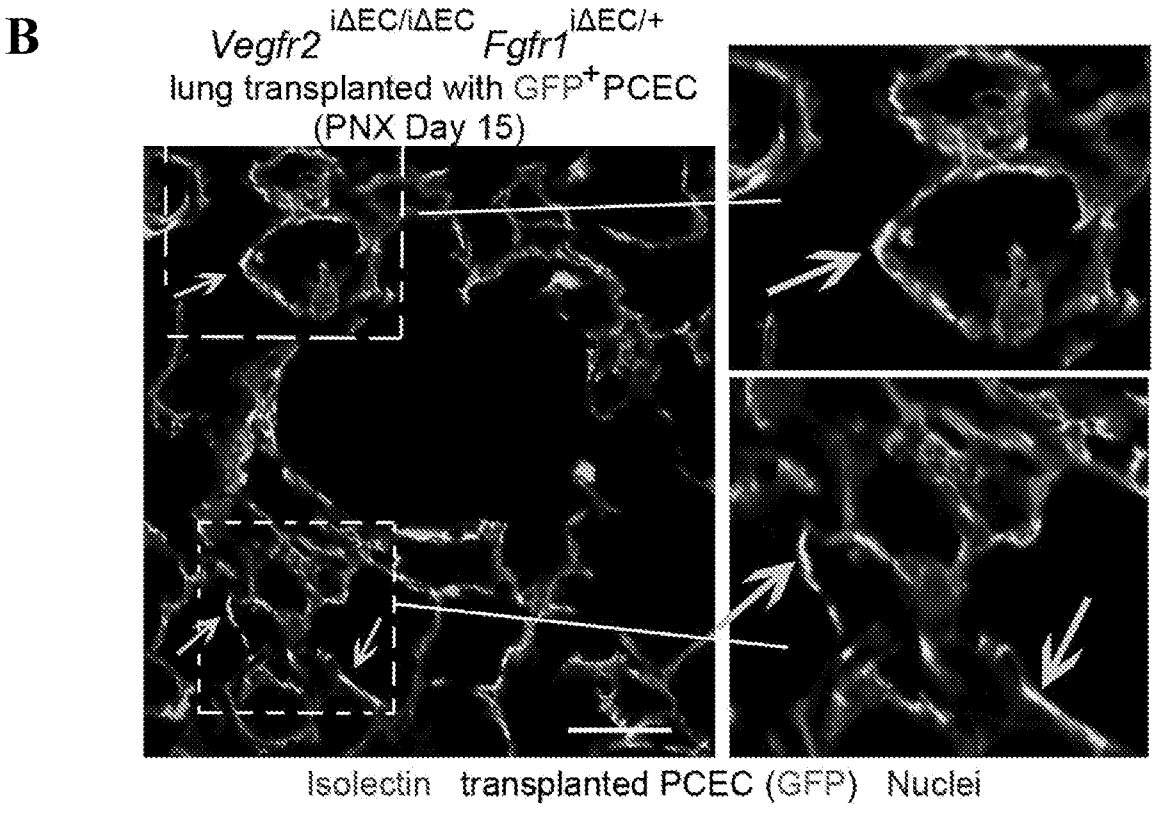
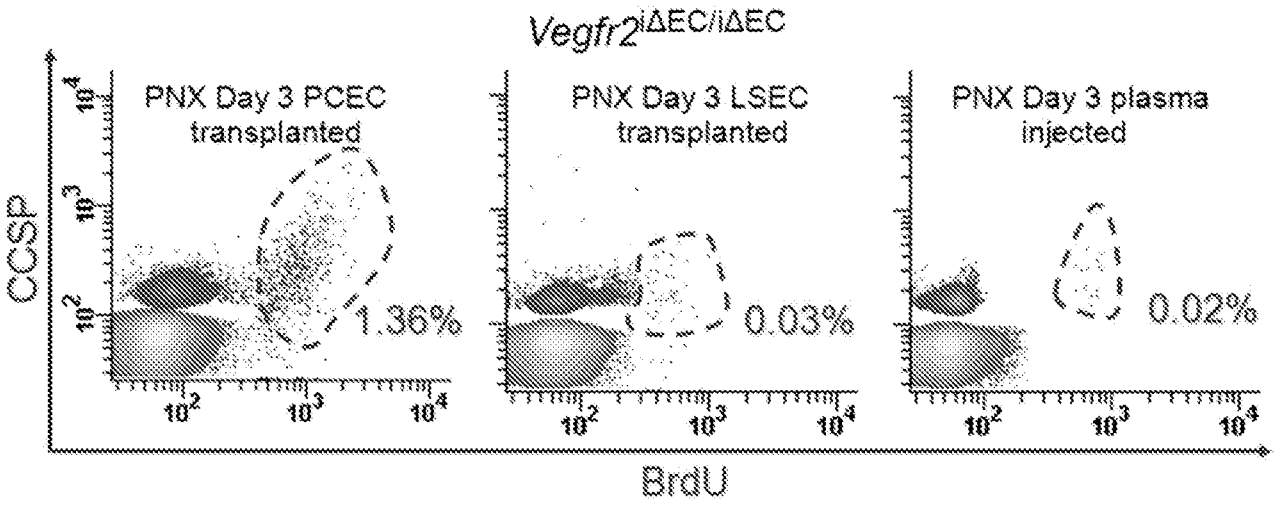

B
Vegfr2 iΔEC/iΔEC Fgfr1 iΔEC/+
lung transplanted with GFP+PCEC
(PNX Day 15)
Isolectin transplanted PCEC (GFP) Nuclei

Figure 13

C


Vegfr2iΔEC/iΔEC
PNX Day 3 PCEC transplanted
PNX Day 3 LSEC transplanted
PNX Day 3 plasma injected
CCSP
BrdU
1.36%
0.03%
0.02%

D

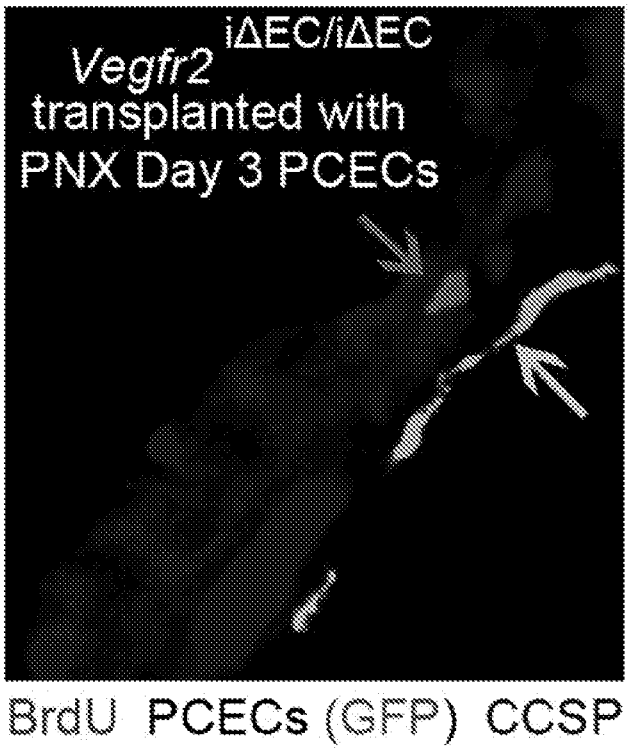
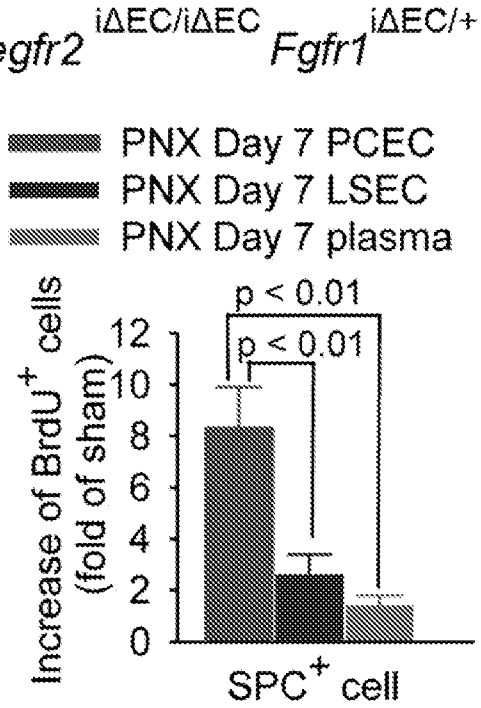

Vegfr2iΔEC/iΔEC transplanted with PNX Day 3 PCECs
BrdU PCECs (GFP) CCSP

Figure 13

E


Vegfr2 iΔEC/iΔEC Fgfr1 iΔEC/+
PNX Day 7 PCEC
PNX Day 7 LSEC
PNX Day 7 plasma
p < 0.01
p < 0.01
Increase of BrdU+ cells (fold of sham)
12
10
8
6
4
2
0
SPC+ cell

F

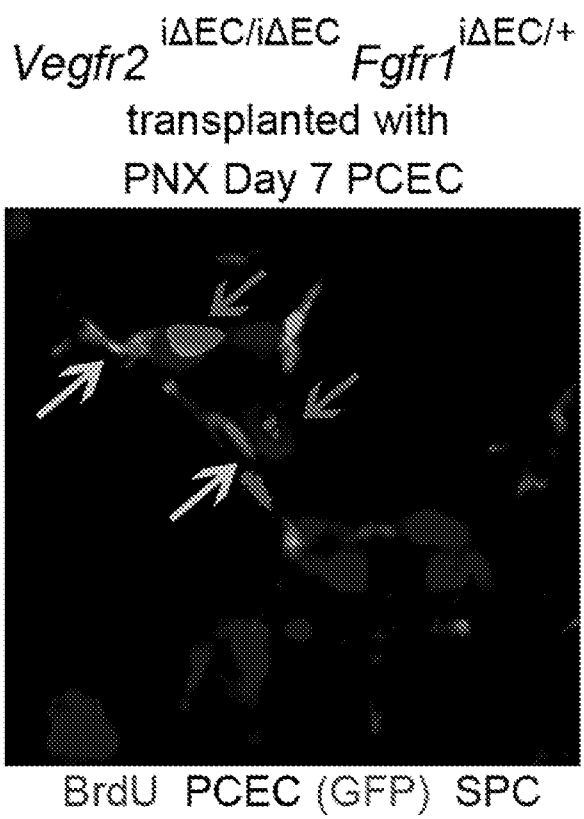
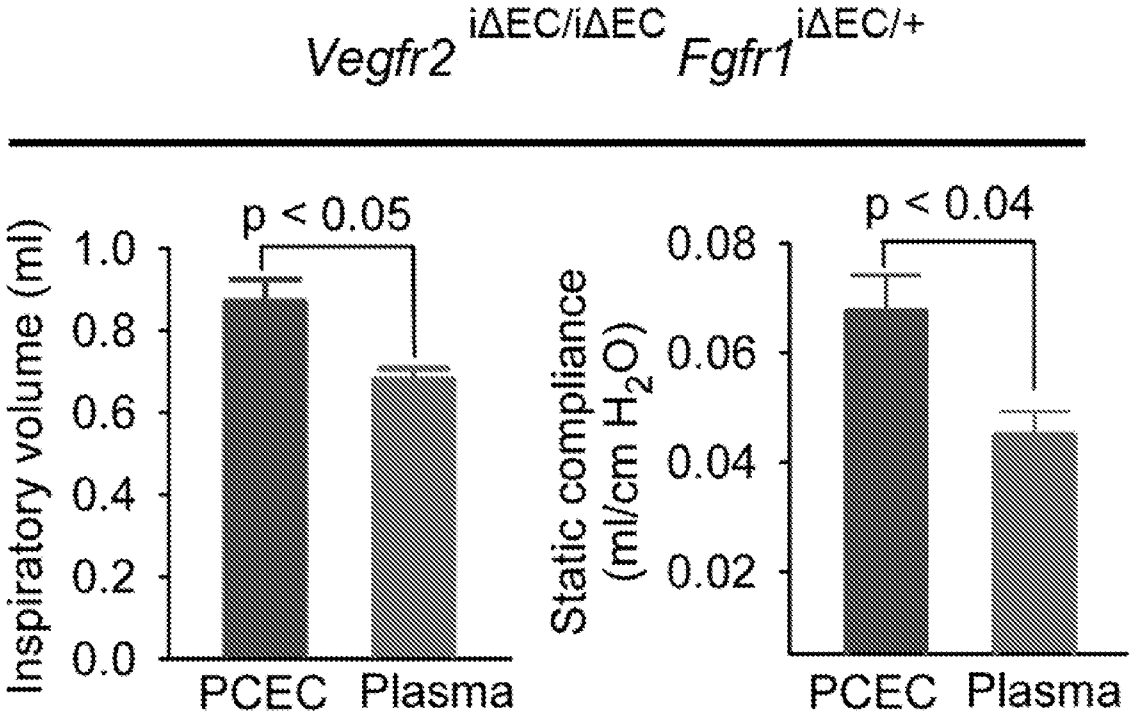

Vegfr2 iΔEC/iΔEC Fgfr1 iΔEC/+
transplanted with
PNX Day 7 PCEC
BrdU PCEC (GFP) SPC

Figure 13

G


Vegfr2 iΔEC/iΔEC Fgfr1 iΔEC/+
p < 0.05
Inspiratory volume (ml)
1.0
0.8
0.6
0.4
0.2
0.0
PCEC
Plasma
p < 0.04
Static compliance (ml/cm H2O)
0.08
0.06
0.04
0.02
PCEC
Plasma