Supplemental Data

Supplemental Figure 1. ESDN modulation in HUVECs. A-C) HA-tagged ESDN overexpression in HUVECs infected with ESDN (or control GFP) retrovirus, assessed by real time RT-PCR (A, n=3), Western blotting (B) and HA immunofluorescent staining (C). Nuclei are stained with DAPI in blue. Inset represents control staining. Scale bar: 30 μm. D, E) siRNA-mediated ESDN downregulation in HUVECs transfected with three different ESDN or control siRNA and analyzed by real time RT-PCR (D, n=3), showing that ESDN siRNA 2245 exerts the greatest downregulation of ESDN without affecting the stress response gene, 2'-5'-oligoadenylate synthetase (OAS1). Western blotting confirms siRNA 2245-mediated downregulation of ESDN protein (E).

Supplemental Figure 2. ESDN expression in mouse tissues and cells. A) Western blot analysis of ESDN expression in various WT mouse tissues, demonstrating high expression in the lungs, heart and aorta and no detectable expression in the blood. B) Immunohistochemical analysis of CD31, NG2, smooth muscle α-actin and CD68 expression in aortas from WT and *Esdn*$^{-/-}$ mice showing a similar expression pattern. Scale bar: 100 μm. C) Flow cytometric analysis of ESDN expression in VSMCs and fibroblasts of WT and *Esdn*$^{-/-}$ mice.

Supplemental Figure 3. Attenuation of adult angiogenesis and responses to exogenous VEGF in *Esdn* null mice. A-F) Hindlimb ischemia-induced neovascularization. A, B) Representative examples and quantification of NG2 (A, B),

smooth muscle α-actin (C, D) and PDGFR-β immunostaining (E, f) of calf blood vessels (n=3) 14 days after femoral artery ligation in WT or *Esdn*$^{-/-}$ mice. Scale bar: 100 μm. G-L) Ear angiogenesis. Examples and quantification of NG2 (G, H), smooth muscle α-actin (I, J) and PDGFR-β immunostaining (K, L) of ear blood vessels (G, n=6) 5 days following intradermal injection of Ad-VEGF or Ad-Lac Z into opposite ears of WT and *Esdn*$^{-/-}$ mice. N=3. Scale bar: 100 μm. M-P) Matrigel angiogenesis. Examples and quantification of smooth muscle α-actin (M, N) and PDGFR-β immunostaining (O, P) of matrigel plugs containing VEGF or control buffer implanted in WT and *Esdn*$^{-/-}$ mice. N=3. Scale bar: 100 μm.

Supplemental Figure 4. ESDN and developmental angiogenesis. A-D) Retinal angiogenesis in *Esdn*$^{-/-}$ mice. Examples and quantification of smooth muscle α-actin (A, B) and PDGFR-β immunostaining (C, D) of retinal blood vessels in WT and *Esdn*$^{-/-}$ mice on postnatal day 5. n= 6. Scale bar: 50μm. E) *esdn/dcbld2* expression detected by real time RT-PCR of duplicate samples in zebrafish *flk*+ (endothelial) and *flk*- cells. F) Downregulation of *esdn/dcbld2* expression in zebrafish embryos injected with *dcbld2* morpholinos, n=3.

Supplemental Figure 5. VEGF-induced cell proliferation and migration in MLECs. A) VEGF-induced MLEC growth monitored by MTT assay. The data represent % increase after 4 days relative to non-stimulated cells from three independent experiments. B) VEGF-induced [^{3}H] thymidine incorporation in WT and *Esdn*$^{-/-}$ MLECs. The data represent percent increase in [^{3}H] thymidine incorporation relative to un-

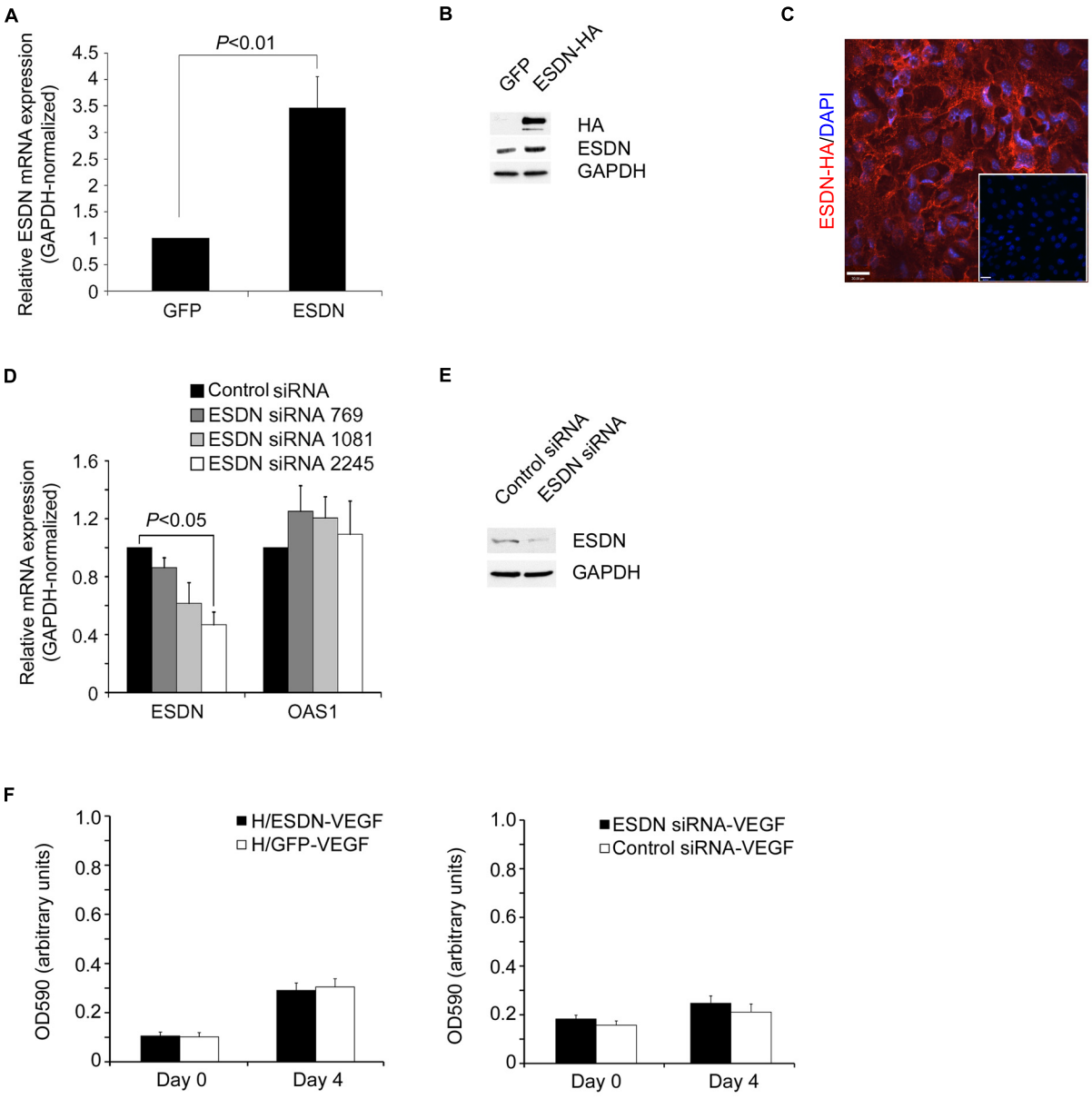
stimulated control cells from 3 independent experiments. C, D) VEGF-induced MLEC transwell migration. Data represent percent increase in migrated cells per microscopic field in VEGF-treated versus control cells from 3 independent experiments.

Supplemental Figure 6. ESDN modulation and VEGF signaling in ECs. A-F) $VEGF_{121}$ signaling in *Esdn*$^{-/-}$ MLECs. Examples of Western blots and quantification of $VEGF_{121}$-induced MAPK p44/42 Thr^{202}/Tyr^{204} phosphorylation (A,B) and Akt Ser^{473} phosphorylation (A,C) in WT and *Esdn*$^{-/-}$ MLECs. $VEGF_{121}$ dose response is shown in D-F. n= 3. G-I) Western blot analysis of $VEGF_{165}$ -induced MAPK p44/42 Thr^{202}/Tyr^{204} and p38 Thr^{180}/Tyr^{182} phosphorylation following siRNA-mediated ESDN downregulation in human ECs. The bar graphs represent the quantitative analysis of the ratio of phosphorylated/total MAPK from three independent experiments.

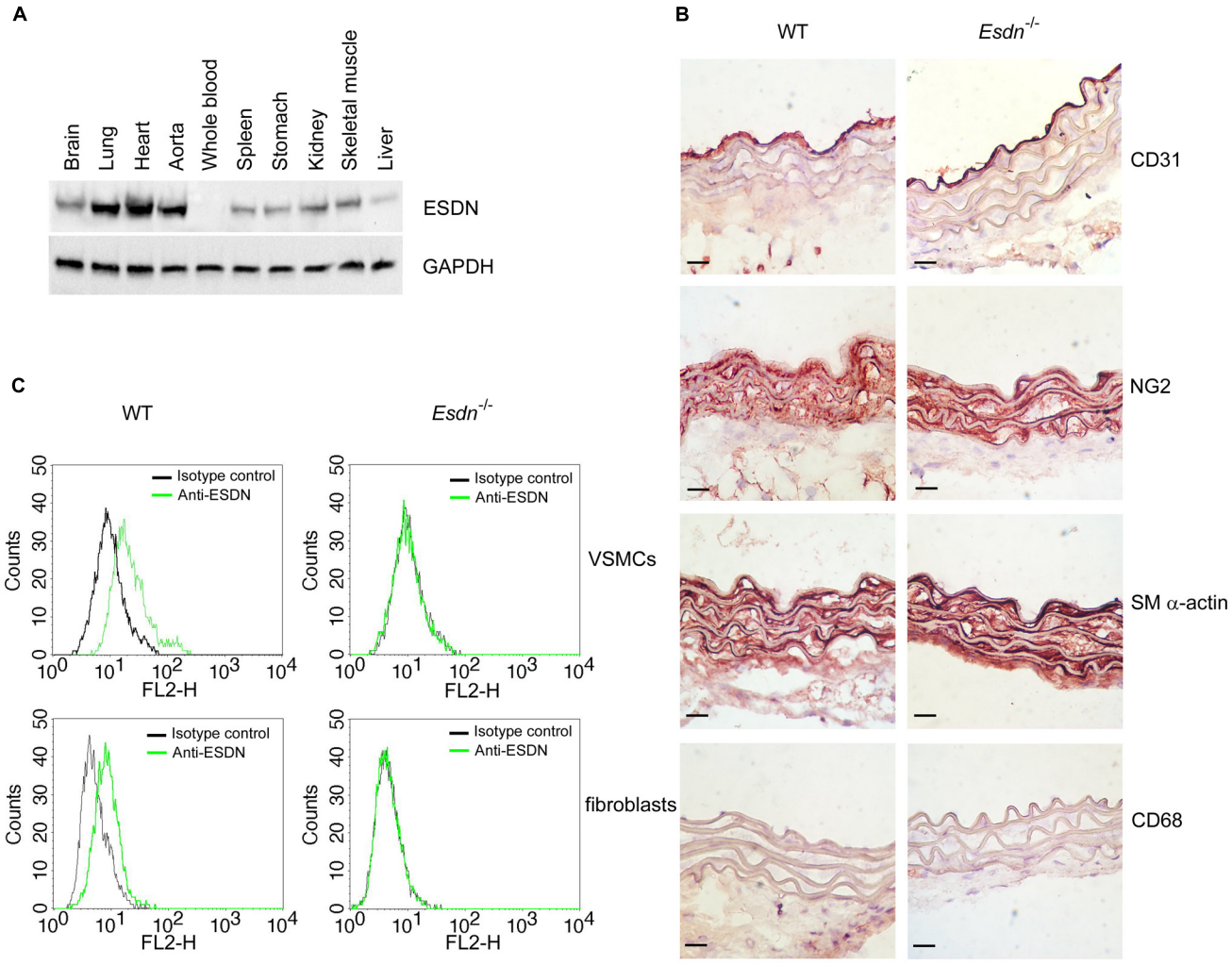
Supplemental Figure 7. ESDN and VEGFR-2 phosphorylation and complex formation in ECs. A,B) Western blot analysis of VEGF-induced VEGFR-2 Thr^{1054}/Tyr^{1059} phosphorylation in WT and *Esdn*$^{-/-}$ MLECs. The bar graph represents the quantitative analysis of the ratio of phosphorylated/total VEGFR-2 from three independent experiments. C, D) VEGFR expression assessed by Western blotting (C) and real time RT-PCR (D) following ESDN (or GFP) over expression, or siRNA-mediated downregulation in human ECs, and in WT and *Esdn*$^{-/-}$ MLECs. There is a similar level of gene expression between control and ESDN-modulated cells. Each panel represents the data from three independent experiments. E) Neuropilin-1 and -2 expression assessed by Western blotting and real time RT-PCR (n=3) demonstrating a

similar level of gene expression in WT and *Esdn*$^{-/-}$ MLEC. F) Cell surface VEGFR-1 and VEGFR-2 expression assessed by flow cytometry in human ECs following siRNA-mediated ESDN downregulation. ConsiRNA: Control siRNA. G) PTP1B, TCPTP, and VE-cadherin mRNA expression assessed by real time RT-PCR demonstrating a similar level of gene expression in WT and *Esdn*$^{-/-}$ MLECs. n=3. H-M) siRNA-mediated PTP1B and TCPTP downregulation in MLECs. GAPDH-normalized PTP1B (H, J, L) and TCPTP (I,K,M) expression and their siRNA-mediated downregulation assessed by real time RT-PCR (H,I) and Western blotting (J-M) in WT and *Esdn*$^{-/-}$ MLECs. n=3. N-P) Western blot analysis of VEGF-induced MAPK p44/42 Thr^{202}/Tyr^{204} phosphorylation and Akt Ser^{473} phosphorylation following siRNA-mediated PTP1B or/and TCPTP downregulation in *Esdn*$^{-/-}$ MLECs in comparison with WT MLECs transfected with a control siRNA. Panels O and P represent the quantitative analysis of phosphorylated proteins from three independent experiments.

Supplemental Figure 1



Supplemental Figure 2



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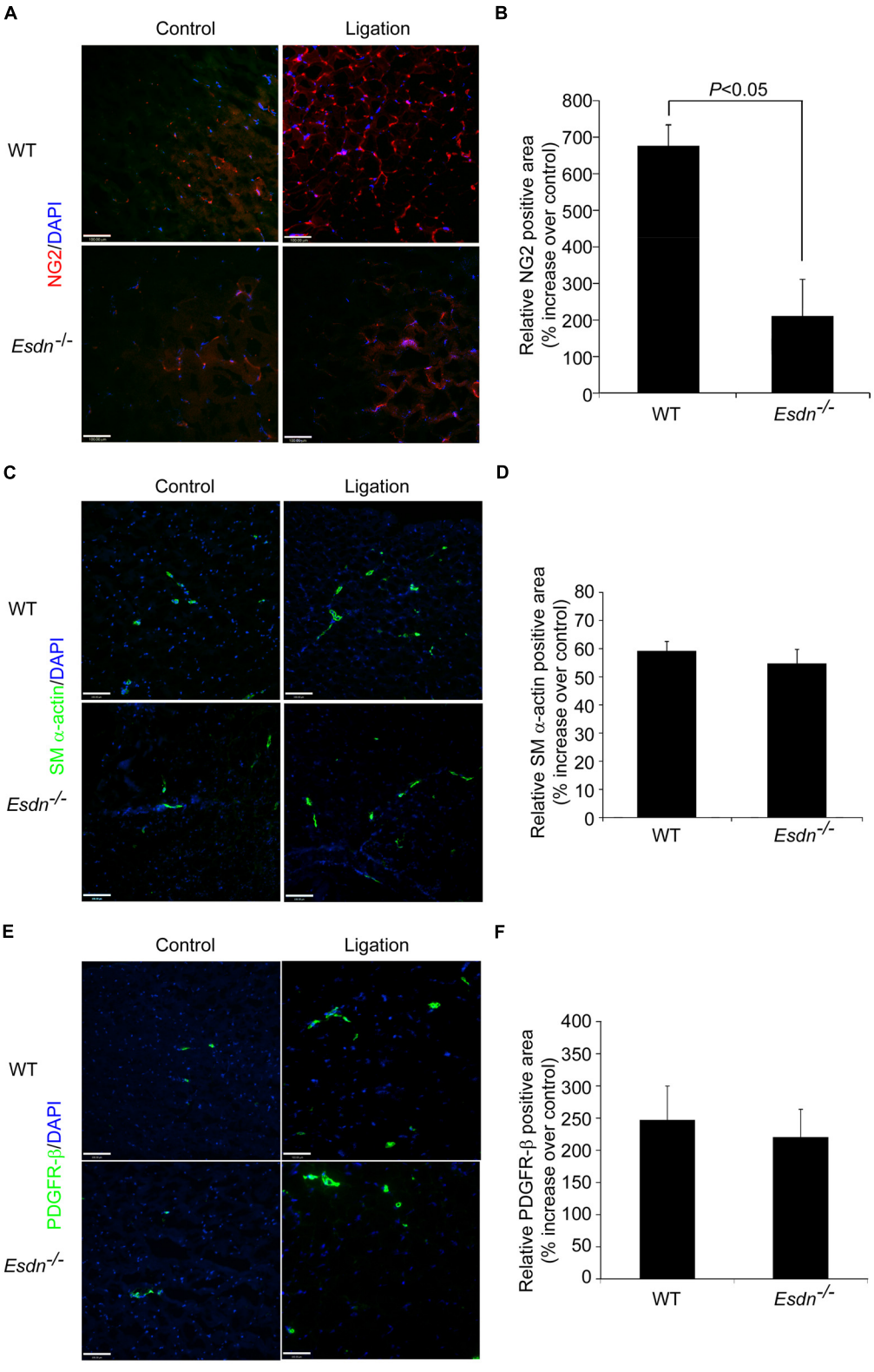
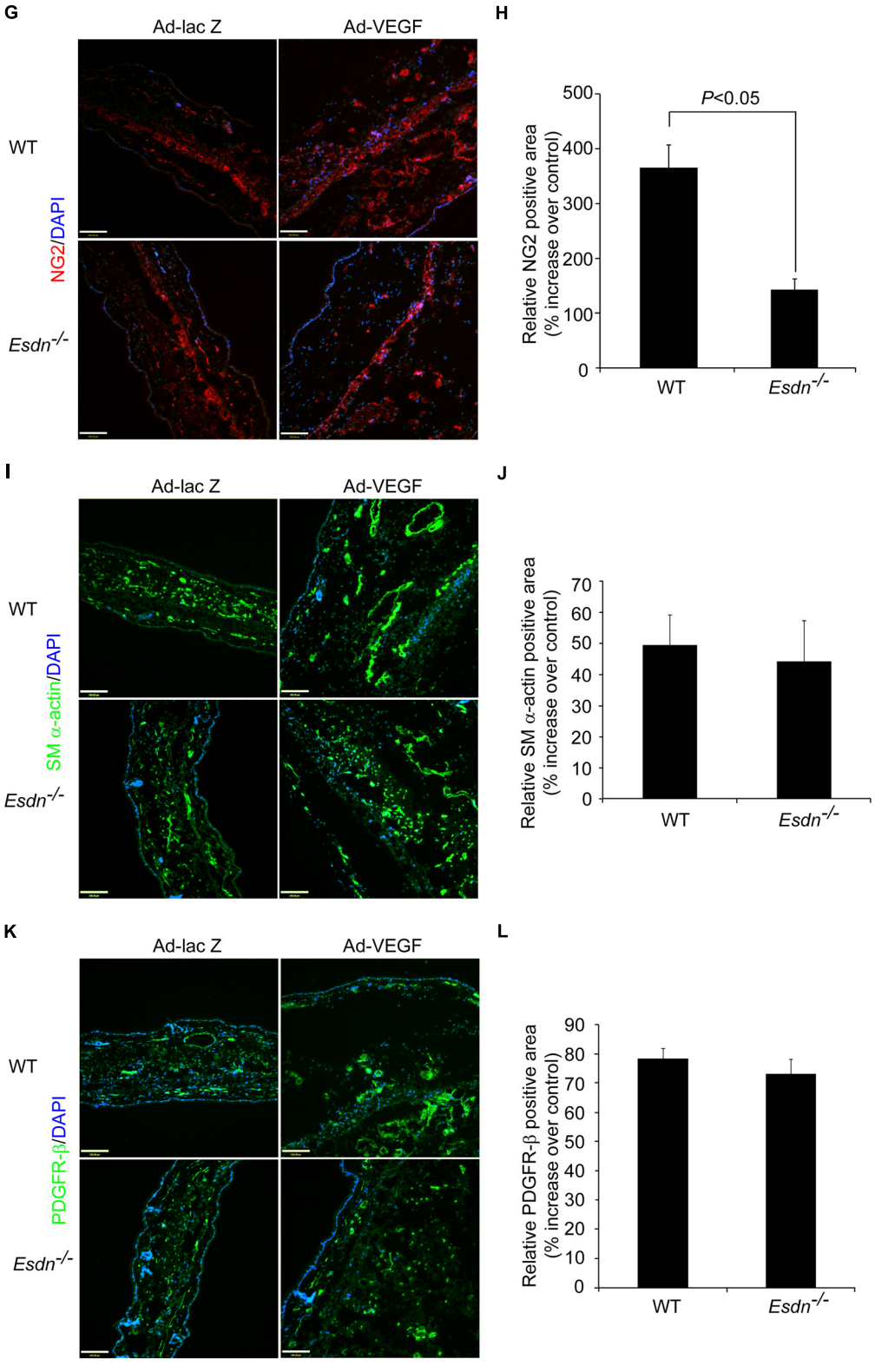
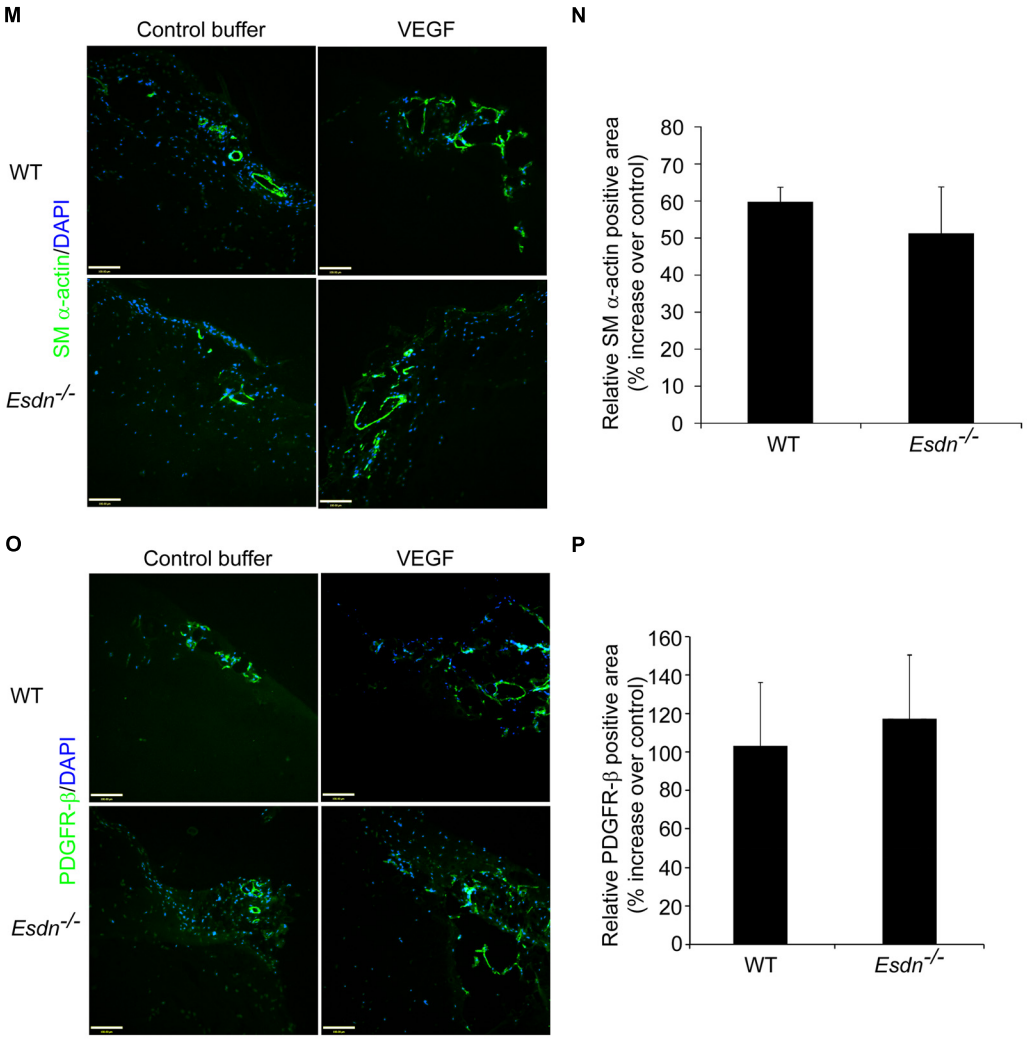
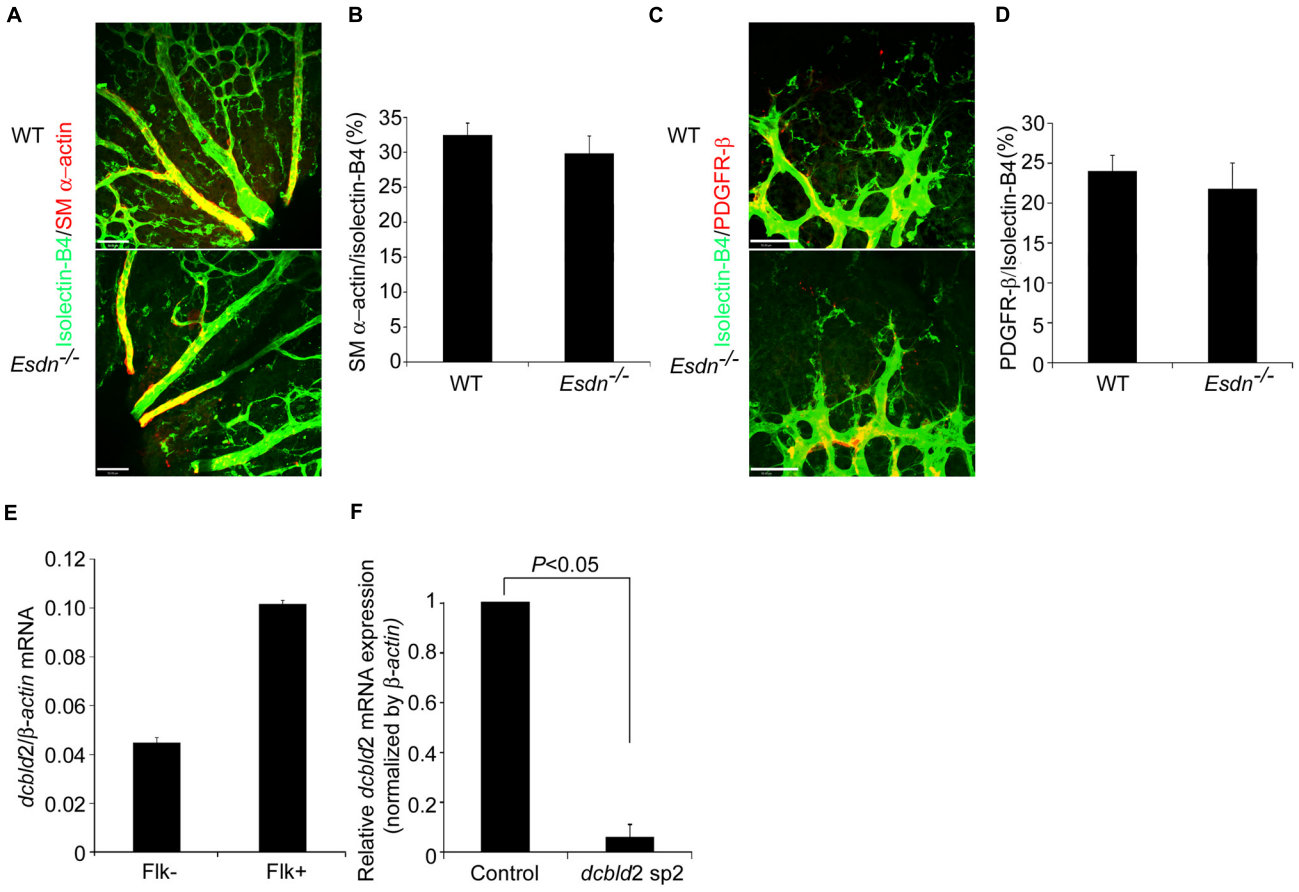



G
Ad-lac Z
Ad-VEGF
WT
NG2/DAPI
Esdn-/-
H
P<0.05
Relative NG2 positive area
(% increase over control)
500
400
300
200
100
0
WT
Esdn-/-
I
Ad-lac Z
Ad-VEGF
WT
SM α-actin/DAPI
Esdn-/-
J
Relative SM α-actin positive area
(% increase over control)
70
60
50
40
30
20
10
0
WT
Esdn-/-
K
Ad-lac Z
Ad-VEGF
WT
PDGFR-β/DAPI
Esdn-/-
L
Relative PDGFR-β positive area
(% increase over control)
90
80
70
60
50
40
30
20
10
0
WT
Esdn-/-


M
Control buffer
VEGF
WT
SM α-actin/DAPI
Esdn-/-
N
Relative SM α-actin positive area
(% increase over control)
80
70
60
50
40
30
20
10
0
WT
Esdn-/-
O
Control buffer
VEGF
WT
PDGFR-β/DAPI
Esdn-/-
P
Relative PDGFR-β positive area
(% increase over control)
160
140
120
100
80
60
40
20
0
WT
Esdn-/-

Supplemental Figure 4



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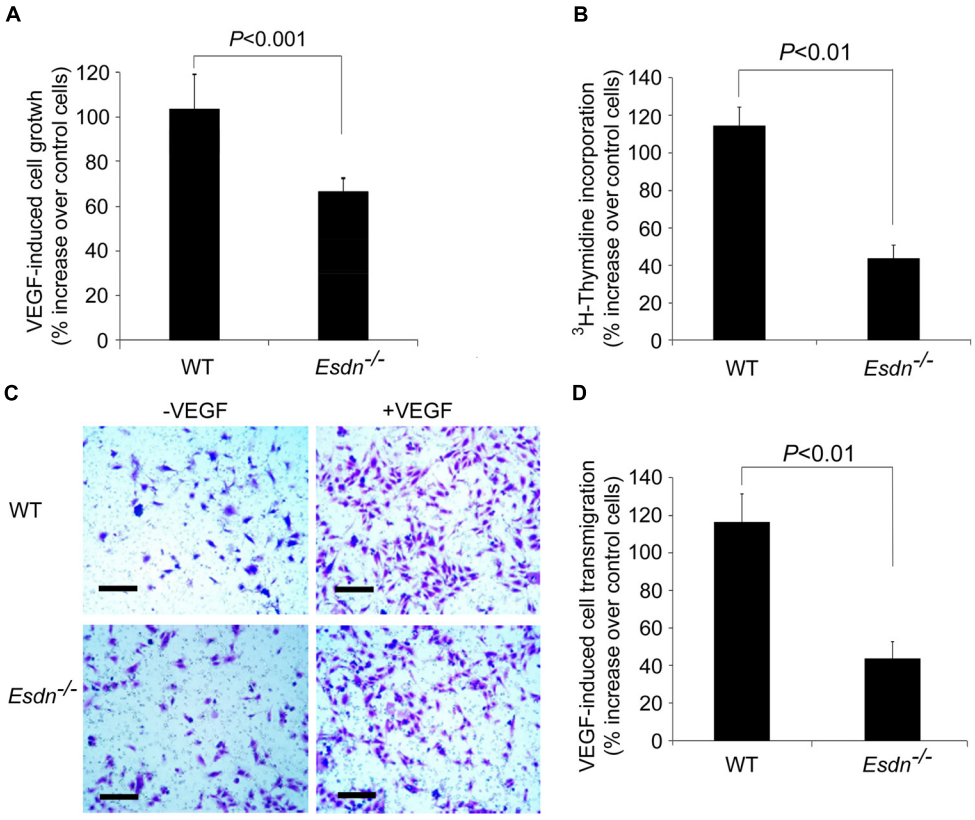
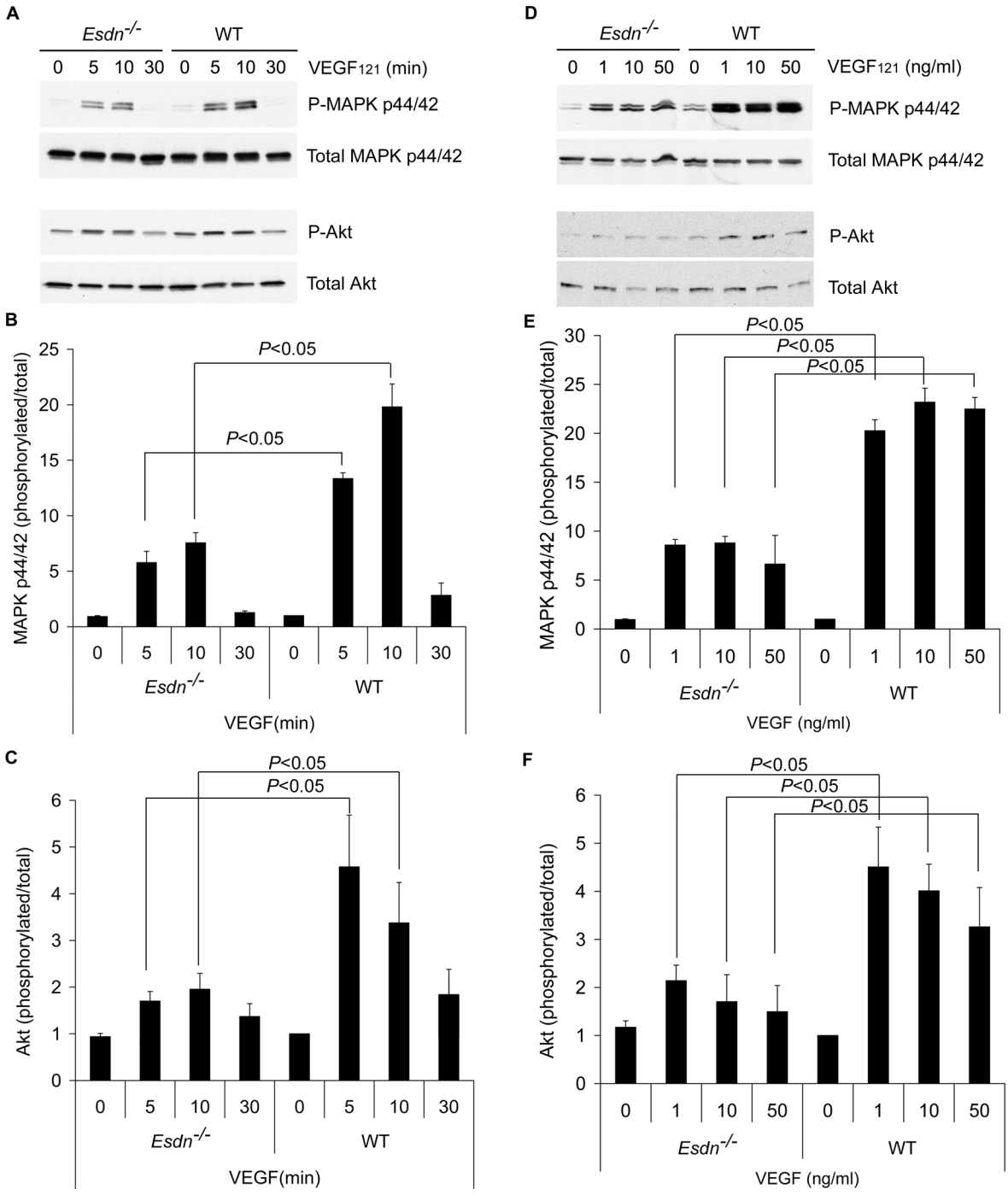
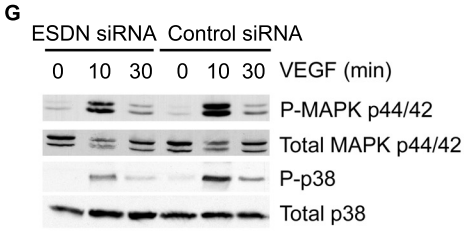
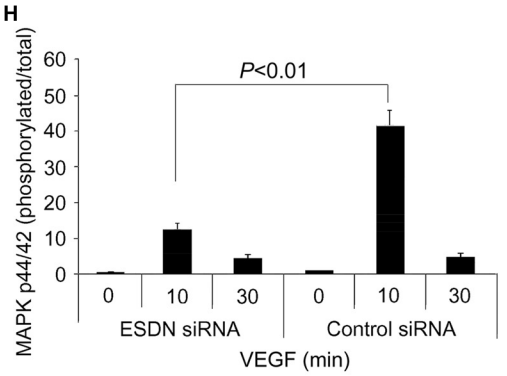
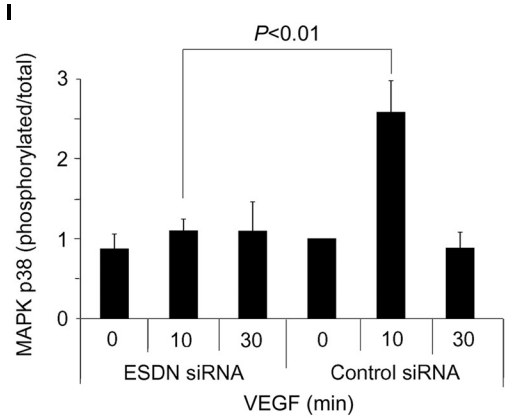


Supplemental Figure 6

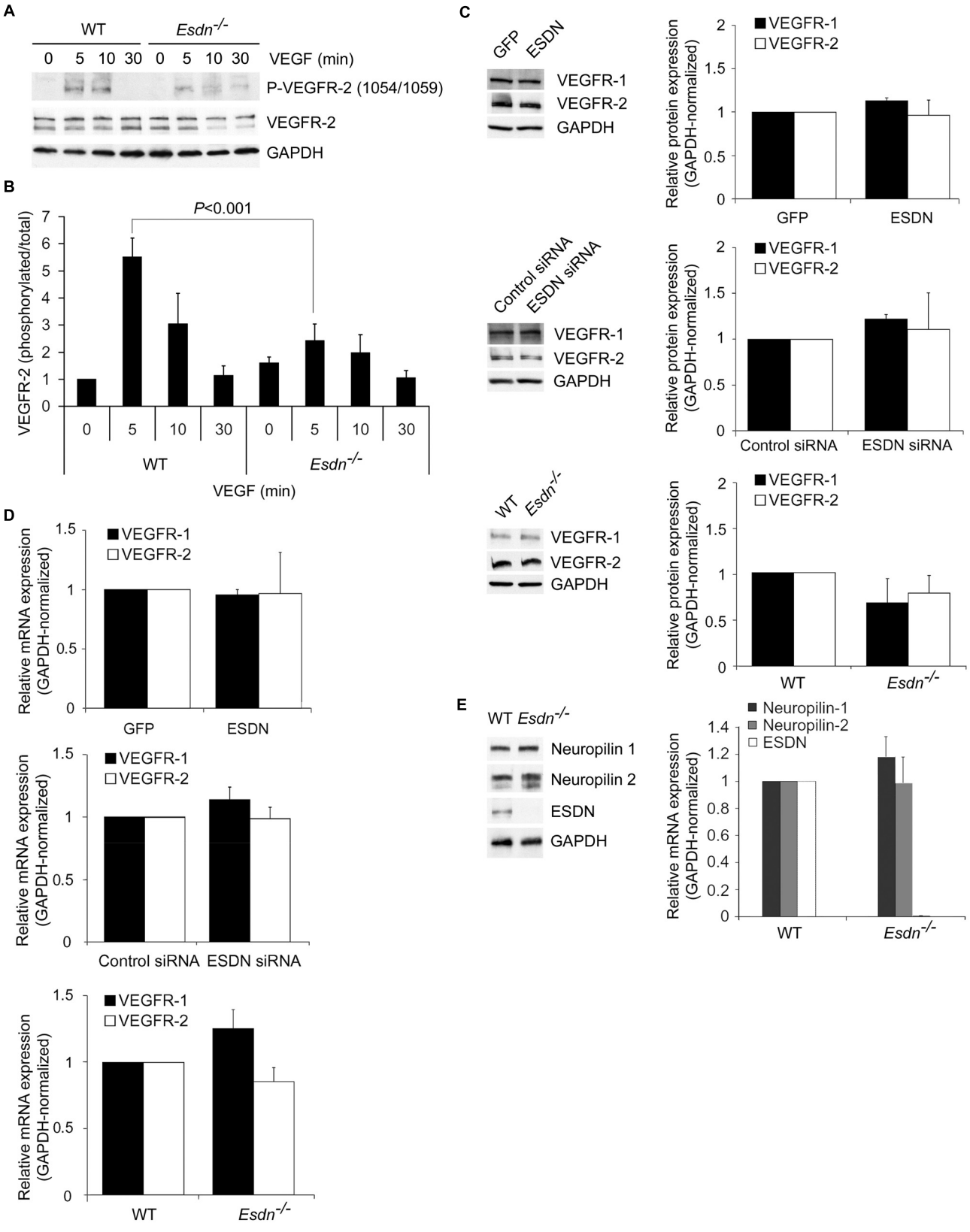


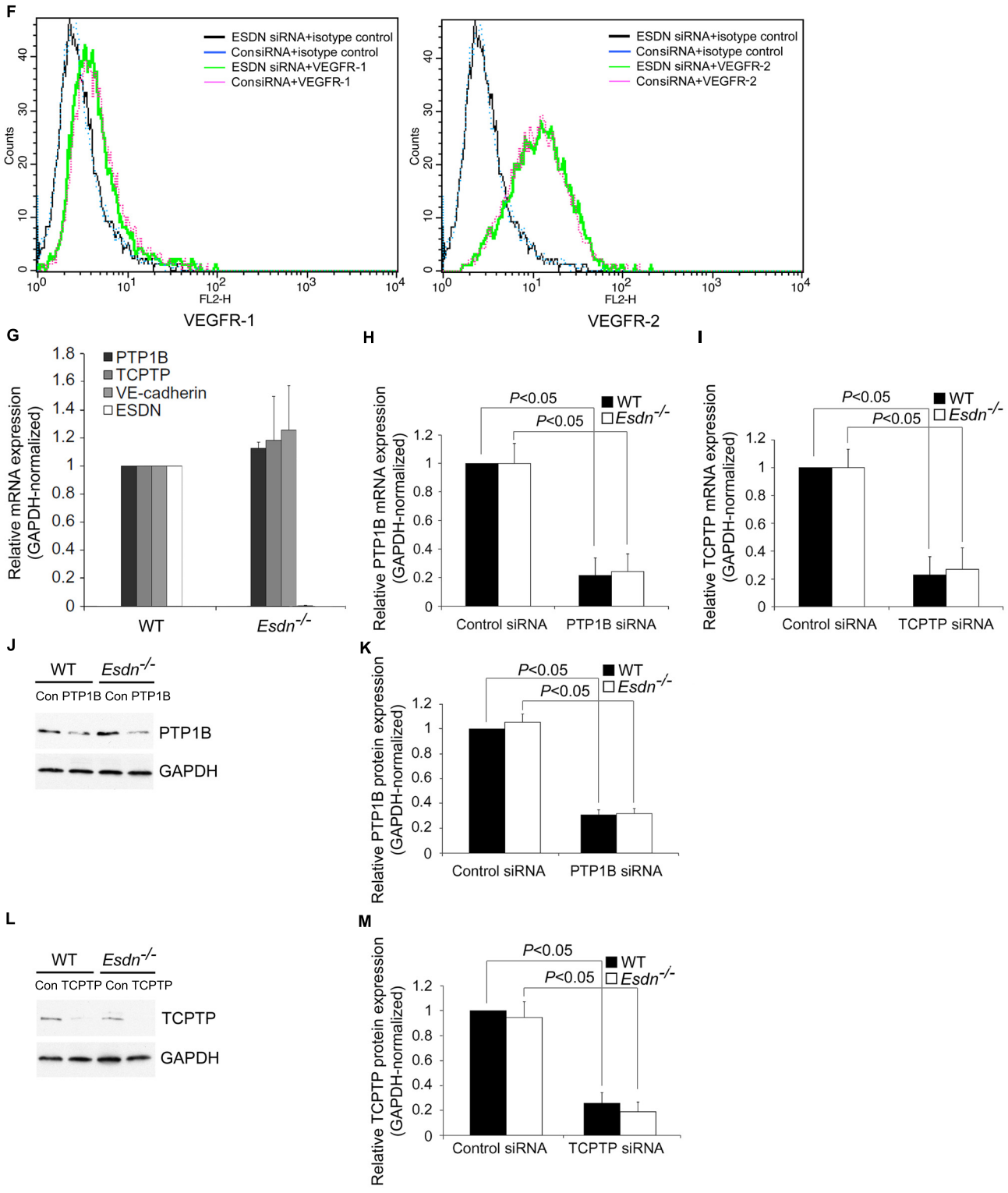

G
ESDN siRNA
Control siRNA
0 10 30 0 10 30 VEGF (min)
P-MAPK p44/42
Total MAPK p44/42
P-p38
Total p38


H
MAPK p44/42 (phosphorylated/total)
P<0.01
60
50
40
30
20
10
0
0 10 30 0 10 30
ESDN siRNA
Control siRNA
VEGF (min)

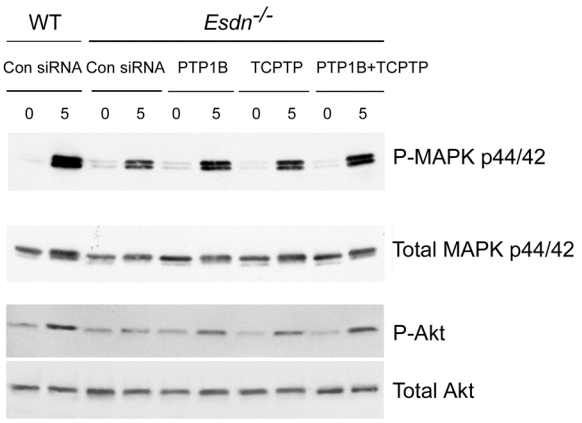

I
MAPK p38 (phosphorylated/total)
P<0.01
3
2
1
0
0 10 30 0 10 30
ESDN siRNA
Control siRNA
VEGF (min)

Supplemental Figure 7

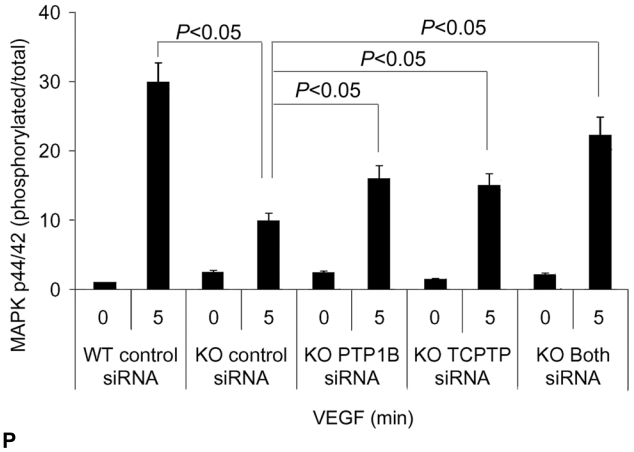



F
ESDN siRNA+isotype control
ConsiRNA+isotype control
ESDN siRNA+VEGFR-1
ConsiRNA+VEGFR-1
Counts
FL2-H
VEGFR-1
ESDN siRNA+isotype control
ConsiRNA+isotype control
ESDN siRNA+VEGFR-2
ConsiRNA+VEGFR-2
Counts
FL2-H
VEGFR-2
G
PTP1B
TCPTP
VE-cadherin
ESDN
Relative mRNA expression (GAPDH-normalized)
WT
Esdn-/-
H
P<0.05
P<0.05
WT
Esdn-/-
Relative PTP1B mRNA expression (GAPDH-normalized)
Control siRNA
PTP1B siRNA
I
P<0.05
P<0.05
WT
Esdn-/-
Relative TCPTP mRNA expression (GAPDH-normalized)
Control siRNA
TCPTP siRNA
J
WT
Esdn-/-
Con PTP1B Con PTP1B
PTP1B
GAPDH
K
P<0.05
P<0.05
WT
Esdn-/-
Relative PTP1B protein expression (GAPDH-normalized)
Control siRNA
PTP1B siRNA
L
WT
Esdn-/-
Con TCPTP Con TCPTP
TCPTP
GAPDH
M
P<0.05
P<0.05
WT
Esdn-/-
Relative TCPTP protein expression (GAPDH-normalized)
Control siRNA
TCPTP siRNA

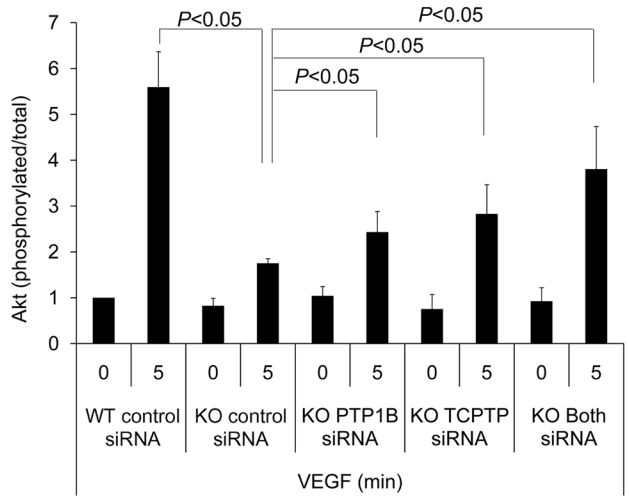
N


WT
Esdn-/-
Con siRNA
Con siRNA
PTP1B
TCPTP
PTP1B+TCPTP
0 5 0 5 0 5 0 5 0 5
P-MAPK p44/42
Total MAPK p44/42
P-Akt
Total Akt

O


MAPK p44/42 (phosphorylated/total)
40
30
20
10
0
P<0.05
P<0.05
P<0.05
P<0.05
0 5 0 5 0 5 0 5 0 5
WT control siRNA
KO control siRNA
KO PTP1B siRNA
KO TCPTP siRNA
KO Both siRNA
VEGF (min)

P


Akt (phosphorylated/total)
7
6
5
4
3
2
1
0
P<0.05
P<0.05
P<0.05
P<0.05
0 5 0 5 0 5 0 5 0 5
WT control siRNA
KO control siRNA
KO PTP1B siRNA
KO TCPTP siRNA
KO Both siRNA
VEGF (min)