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Supplemental information

Endothelial Jagged1 levels and distribution are post-transcriptionally controlled by ZFP36 decay proteins

Hannah L. Sunshine, Andrew C. Cicchetto, Karolina Elżbieta Kaczor-Urbanowicz, Feiyang Ma, Danielle Pi, Chloe Symons, Martin Turner, Vipul Shukla, Heather R. Christofk, Thomas A. Vallim, and M. Luisa Iruela-Arispe

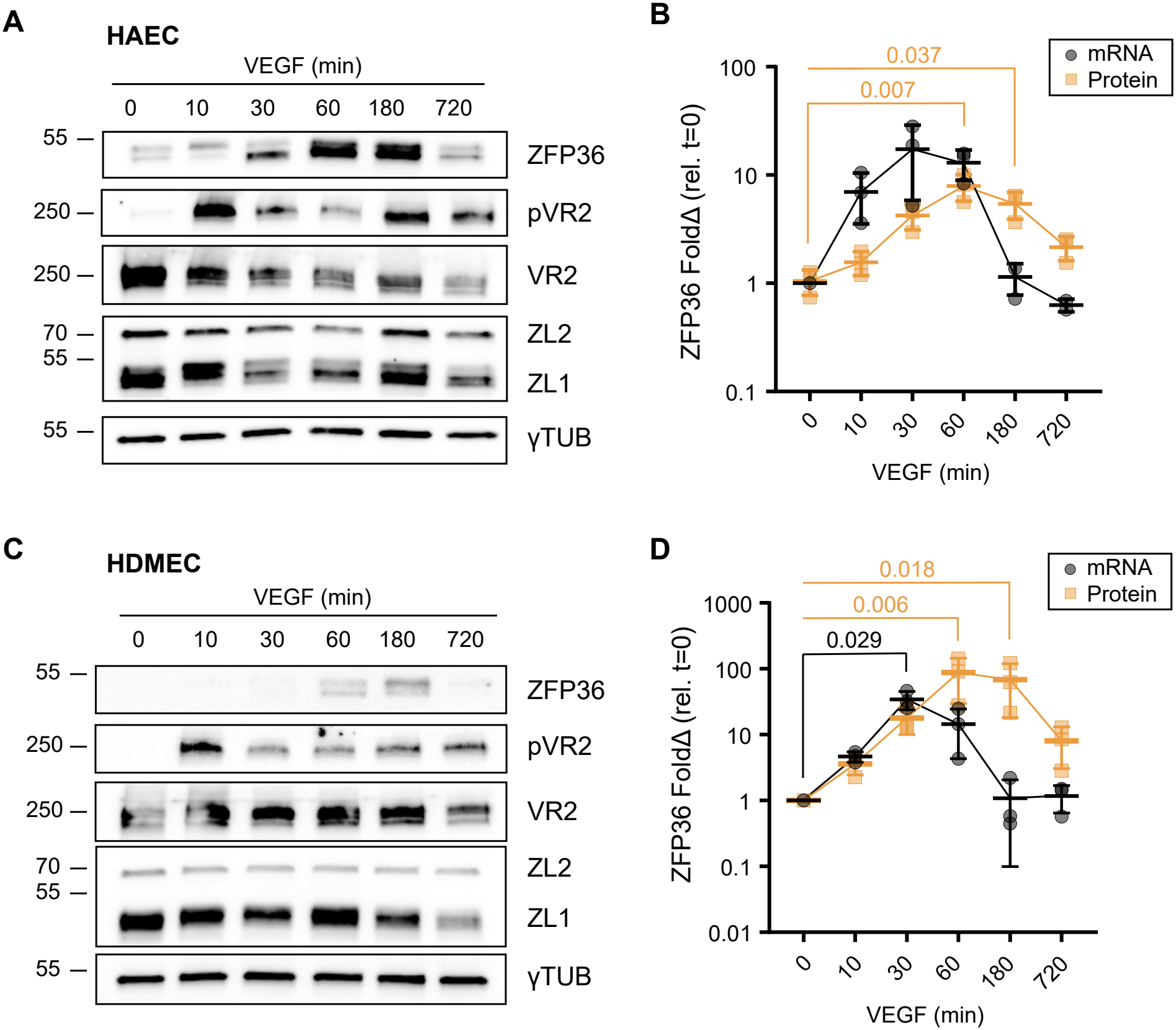
A
HAEC
VEGF (min)
0
10
30
60
180
720
55
250
250
70
55
55
ZFP36
pVR2
VR2
ZL2
ZL1
γTUB
B
mRNA
Protein
0.037
0.007
100
10
1
0.1
ZFP36 FoldΔ (rel. t=0)
0
10
30
60
180
720
VEGF (min)
C
HDMEC
VEGF (min)
0
10
30
60
180
720
55
250
250
70
55
55
ZFP36
pVR2
VR2
ZL2
ZL1
γTUB
D
mRNA
Protein
0.018
0.006
0.029
1000
100
10
1
0.1
0.01
ZFP36 FoldΔ (rel. t=0)
0
10
30
60
180
720
VEGF (min)

Figure S1. VEGF triggered induction of ZFP36 is conserved in multiple EC types. **Related to Figure 1.**

(A) HAEC representative immunoblots of ZFP36, pVR2, VR2, ZFP36L1, ZFP36L2 and γTUB from VEGF stimulation time course experiment. Cultures were first incubated in serum-free medium overnight and stimulated with VEGF for the indicated times.

(B) HAEC immunoblot quantification of fold change (Δ) of ZFP36 protein relative to control normalized to γTUB (n = 3 technical replicates) and qRT-PCR of *ZFP36* transcripts of normalized to *HPRT* (n=3 technical replicates). Data for ZFP36 are individual replicates with line representing mean ± SD. Statistical analysis using Kruskal-Wallis with post-hoc Dunn's multiple comparison test.

(C) HDMEC representative immunoblots of ZFP36, pVR2, VR2, ZFP36L1, ZFP36L2 and γTUB from VEGF stimulation time course experiment. Cultures were first incubated in serum-free medium overnight and stimulated with VEGF for the indicated times.

(D) HDMEC immunoblot quantification of fold change (Δ) of ZFP36 protein relative to control normalized to γTUB (n = 3 technical replicates) and qRT-PCR of *ZFP36* transcripts normalized to *HPRT* (n=3 technical replicates). Data for ZFP36 are individual replicates with line representing mean ± SD. Statistical analysis using Kruskal-Wallis with post-hoc Dunn's multiple comparison test.

Abbreviations: Human aortic endothelial cell (HAEC), human dermal microvascular endothelial cell (HDMEC)

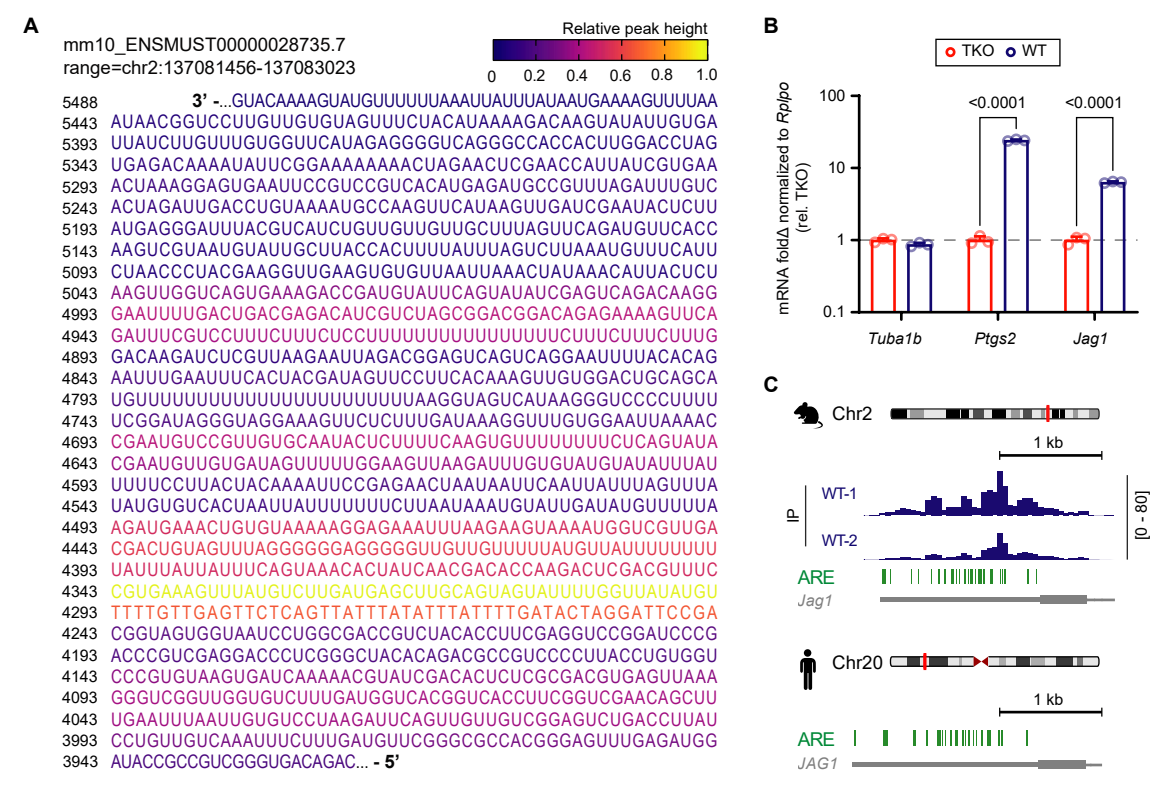
A
mm10_ENSMUST00000028735.7
range=chr2:137081456-137083023
Relative peak height
0 0.2 0.4 0.6 0.8 1.0
5488 3' -...GUACAAAAGUAUGUUUUUUAAAUUAUUUAUAAUGAAAAGUUUUAA
5443 AUAACGGUCCUUGUUGUAGUUUCUACAUAAAAGACAAGUAUAUUUGUGA
5393 UUAUCUUGUUUGUGGUUCAUAGAGGGGUCAGGGCCACCACUUGGACCUAG
5343 UGAGACAAAAUAUUCGGAAAAAAAACUAGAACUCGAACCAUUAUCGUGAA
5293 ACUAAAGGAGUGAAUUCCGUCCGUCACAUGAGAUGCCGUUUAGAUUUGUC
5243 ACUAGAUUGACCUGUAAAAUGCCAAGUUCAUAAGUUGAUCGAAUACUCUU
5193 AUGAGGGAUUUACGUCACUGUUGUUGUUCUUUAGUUCAGAUGUUCACC
5143 AAGUCGUAAUGUAUUGCUUACCACUUUUAUUUAGUCUUAAAUGUUUCAUU
5093 CUAACCCUACGAAGGUUGAAGUGUGUUAAUUAAACUAUAAACAUUACUCU
5043 AAGUUGGUCAGUGAAAGACCGAUGUAUUCAGUAUAUCGAGUCAGACAAGG
4993 GAAUUUUGACUGACGAGACAUCGUCUAGCGGACGGACAGAGAAAAGUUCA
4943 GAUUUCGUCCUUUCCUUCCUUUUUUUUUUUUUUUCUUUCUUUCUUUG
4893 GACAAGAUCUCGUUAAGAAUUAGACGGAGUCAGUCAGGAAUUUUACACAG
4843 AAUUUGAAUUUCACUACGAUAGUUCCUUCACAAAGUUGUGGACUGCAGCA
4793 UGUUUUUUUUUUUUUUUUUUUUUUUAAGGUAGUCAUAAGGGUCCCUUUU
4743 UCGGAUAGGGUAGGAAAGUUCUCUUUGAUAAAGGUUUGUGGAAUUAAAAC
4693 CGAAUGUCCGUUGUGCAAUACUCUUUUCAAGUGUUUUUUUUCUCAGUAUA
4643 CGAAUGUUGUGAUAGUUUUUGGAAGUUAAGAUUUGUGUAUGUAUAUUUAU
4593 UUUUCCUUACUACAAAAUUCCGAGAACUAAUAAUUCAAUUAUUUAGUUUA
4543 UAUGUGUCACUAAUUAUUUUUUUCUUAAUAAAUGUAUUGAUAUGUUUUUA
4493 AGAUGAAACUGUGUAAAAAGGAGAAAUUUAAGAAGUAAAAUGGUCGUUGA
4443 CGACUGUAGUUUAGGGGGGAGGGGGUUGUUGUUUUUAUGUUAUUUUUUUU
4393 UAUUUAUUAUUUCAGUAAACACUAUCAACGACACCAAGACUCGACGUUUC
4343 CGUGAAAGUUUAUGUCUUGAUGAGCUUGCAGUAGUAUUUUGGUUAUAUGU
4293 TTTTGTTGAGTTCTCAGTTATTTATATTTATTTTGATACTAGGATTCCGA
4243 CGGUAGUGGUAAUCCUGGCGACCGUCUACACCUUCGAGGUCCGGAUCCCG
4193 ACCCGUCGAGGACCCUCGGGCUACACAGACGCCGUCCCCUUACCUGUGGU
4143 CCCGUGUAAGUGAUCAAAAACGUAUCGACACUCUCGCGACGUGAGUUAAA
4093 GGGUCGGUUGGUGUCUUUGAUGGUCACGGUCACCUUCGGUCGAACAGCUU
4043 UGAAUUUAAUUGUGUCCUAAGAUUCAGUUGUUGUCGGAGUCUGACCUUAU
3993 CCUGUUGUCAAAUUUCUUUGAUGUUCGGGCGCCACGGGAGUUUGAGAUGG
3943 AUACCGCCGUCGGGUGACAGAC... - 5'
B
TKO WT
mRNA foldΔ normalized to Rplpo (rel. TKO)
100
10
1
0.1
<0.0001
<0.0001
Tuba1b
Ptgs2
Jag1
C
Chr2
1 kb
IP
WT-1
WT-2
[0 - 80]
ARE
Jag1
Chr20
1 kb
ARE
JAG1

Figure S2. Jag1 mRNA features facilitate ZFP36 mRNA binding. Related to Figure 2.
(A) Full mouse JAG1 3'UTR mRNA nucleotide sequence color coded based on relative eCLIP peak height.
(B) Quantification of *Tuba1b*, *Ptgs2*, and *Jag1* mRNA fold change normalized to *Rplpo* from CLIP-qPCR experiment post 40 min serum stimulation of WT relative to TKO *Zfp36/l1/l2* triple-floxed MEF clones (n = 3 technical replicates). Statistical analysis was performed using two-way ANOVA with Sidak's multiple comparison test.
(C) Integrative Genomics Viewer generated from ARESite2[68] identified adenosine-uridine rich element (ARE) motifs in mouse (mm10) and human (hg38) JAG1 3'UTR. Respective chromosome positions are marked with red line. Mouse 3'UTR alignment also includes binding peaks from WT-IP eCLIP-seq experiment with respective track height scale denoted in brackets.

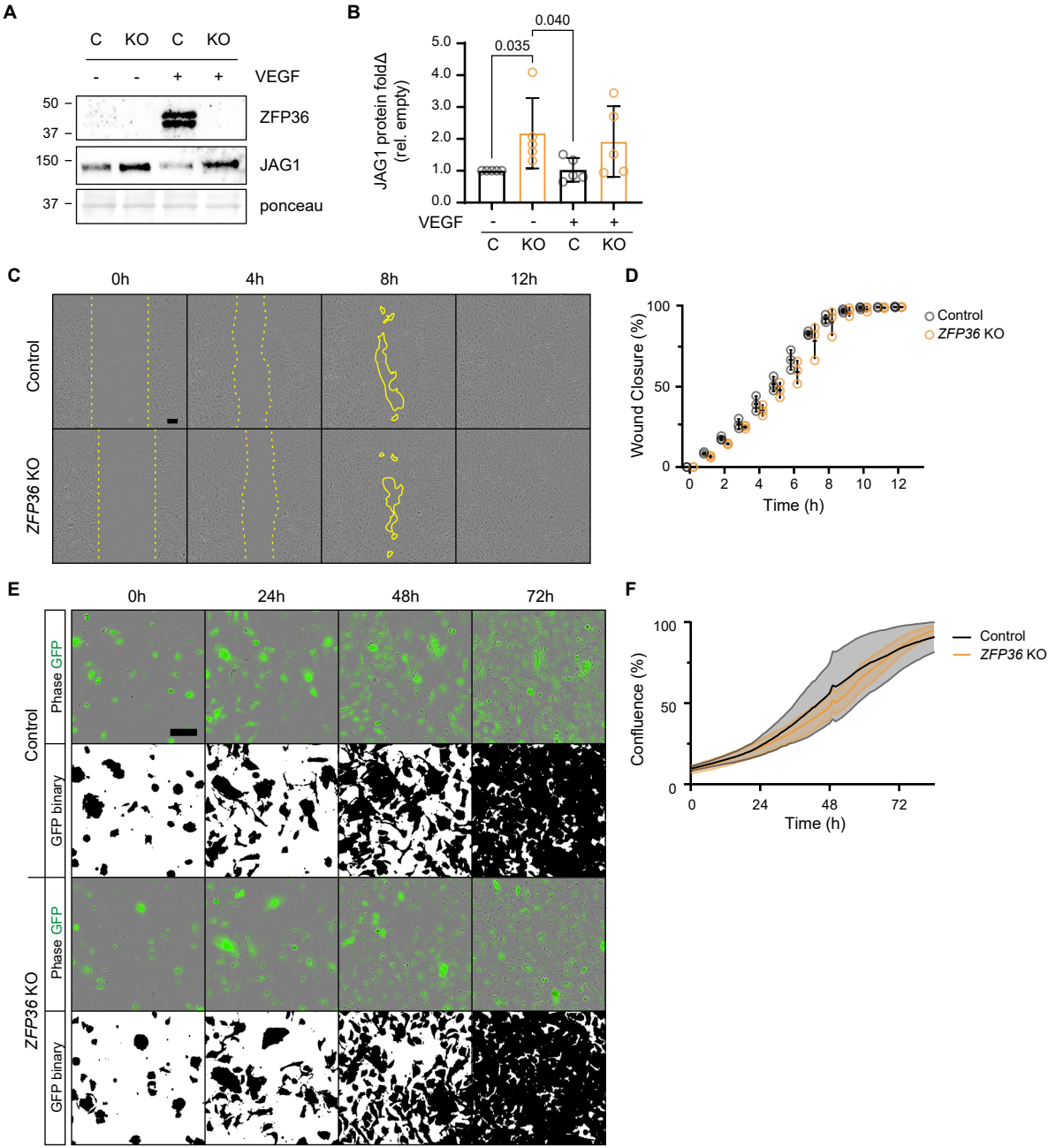
A
C
KO
C
KO
VEGF
- - + +
50
37
150
37
ZFP36
JAG1
ponceau
B
JAG1 protein foldΔ (rel. empty)
0.035
0.040
5.0
4.0
3.0
2.0
1.0
0.0
VEGF
- - + +
C KO C KO
C
0h
4h
8h
12h
Control
ZFP36 KO
D
Wound Closure (%)
100
50
0
0 2 4 6 8 10 12
Time (h)
Control
ZFP36 KO
E
0h
24h
48h
72h
Control
Phase GFP
GFP binary
ZFP36 KO
Phase GFP
GFP binary
F
Confluence (%)
100
50
0
0 24 48 72
Time (h)
Control
ZFP36 KO

Figure S3. *In vitro* migration and proliferation are unaffected by *ZFP36* KO. Related to Figure 2.

(A) Representative immunoblots of ZFP36 and JAG1 protein expression from CRISPR control and *ZFP36* KO HUVECs stimulated with and without VEGF for 1 h. Ponceau is used as loading control reference.

(B) Quantification of WB results fold change (Δ) in relative to empty control normalized to total protein (mean ± SD, n = 5 biological replicates). Statistical analysis using Kruskal-Wallis with post-hoc Dunn's multiple comparison test.

(C) Representative phase images from scratch assay using CRISPR control and *ZFP36* KO HUVECs at indicated times. Empty culture area indicated with yellow outlines (scale bar = 100 μm).

(D) Quantification of scratch assay wound closure (%) area fraction (mean ± SD) measured at 1h increments (n = 3 biological replicates, each averaged from at least 4 technical replicates).

(E) Representative overlay phase and fluorescence images from proliferation assay using CRISPR control and *ZFP36* KO HUVECs infected with CMV-GFP lentivirus. Binary GFP images were used for confluence quantification (scale bar = 100 μm).

(F) Quantification of mean confluence overtime (%) calculated from GFP binary area fraction (mean line ± SD) measured at 1h increments (n = 2 biological replicates, 4 technical replicates each).

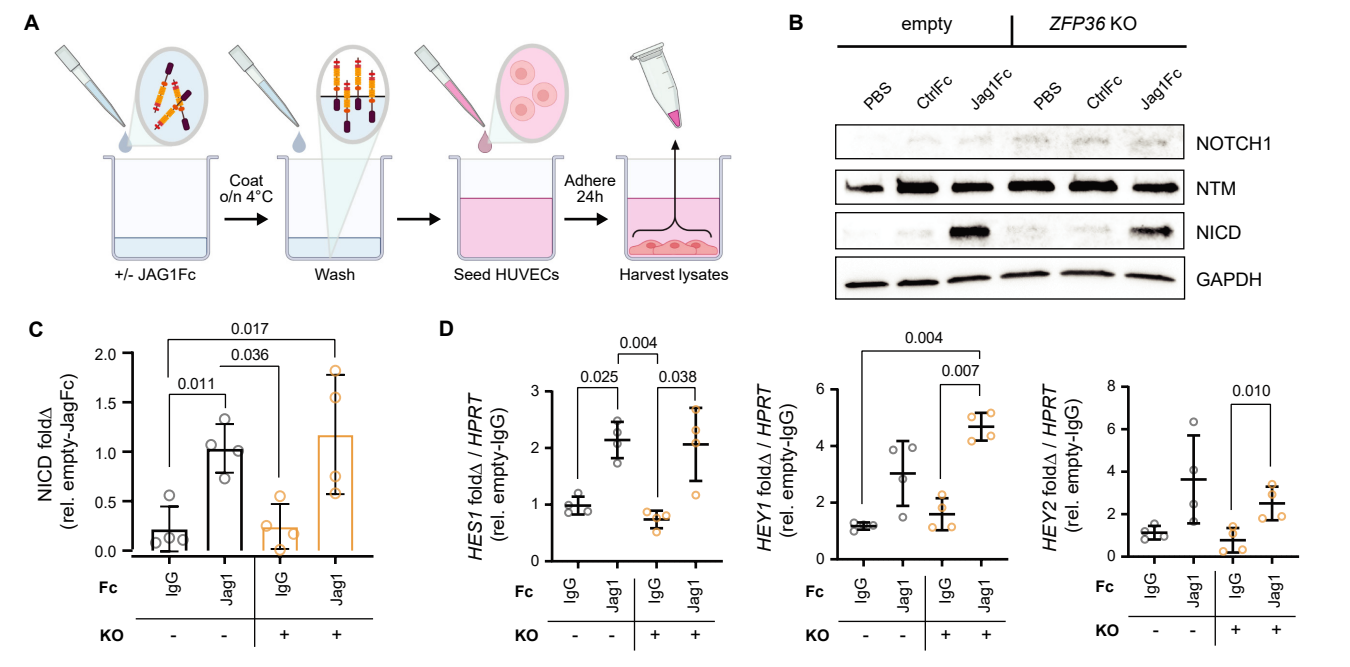
A
Coat
o/n 4°C
Adhere
24h
+/- JAG1Fc
Wash
Seed HUVECs
Harvest lysates
B
empty
ZFP36 KO
PBS
CtrlFc
Jag1Fc
PBS
CtrlFc
Jag1Fc
NOTCH1
NTM
NICD
GAPDH
C
NICD foldΔ
(rel. empty-JagFc)
0.017
0.036
0.011
Fc
IgG
Jag1
IgG
Jag1
KO
-
-
+
+
D
HES1 foldΔ / HPRT
(rel. empty-IgG)
0.004
0.025
0.038
HEY1 foldΔ / HPRT
(rel. empty-IgG)
0.004
0.007
HEY2 foldΔ / HPRT
(rel. empty-IgG)
0.010

Figure S4. Trans-endothelial Notch activation is preserved in *ZFP36* KO cells when presented exogenous ligand. Related to Figure 5.

(A) Schematic diagramming experimental design of JAG1Fc-coated plate assay. After coating human recombinant JAG1Fc overnight on tissue culture plate, HUVECs are seeded on top to confluence and allowed to adhere for 24 h before harvesting for Notch signaling analysis.

(B) Representative immunoblots of full-length NOTCH1, NOTCH1 transmembrane fragment (NTM), cleaved NOTCH1 (NICD), and GAPDH from CRISPR empty or *zfp36*KO HUVECs post-24 h culture on JAG1-Fc coated plates.

(C) Quantification of fold change (Δ) of ZFP36 protein relative to control normalized to housekeeping protein GAPDH or γTUB (n = 4 biological replicates). Statistical analysis using Kruskal-Wallis with post-hoc uncorrected Dunn's test.

(D) Quantification of downstream NOTCH1 target genes *HES1, HEY1,* and *HEY2* mRNA mean fold change ± SD by qRT-PCR from HUVEC JAG1-Fc experiments relative to CRISPR empty IgG cultured cells. Cq-values normalized to *HPRT* (n = 4 biological replicates). Statistical analysis performed using Kruskal-Wallis with post-hoc Dunn's multiple comparison test.

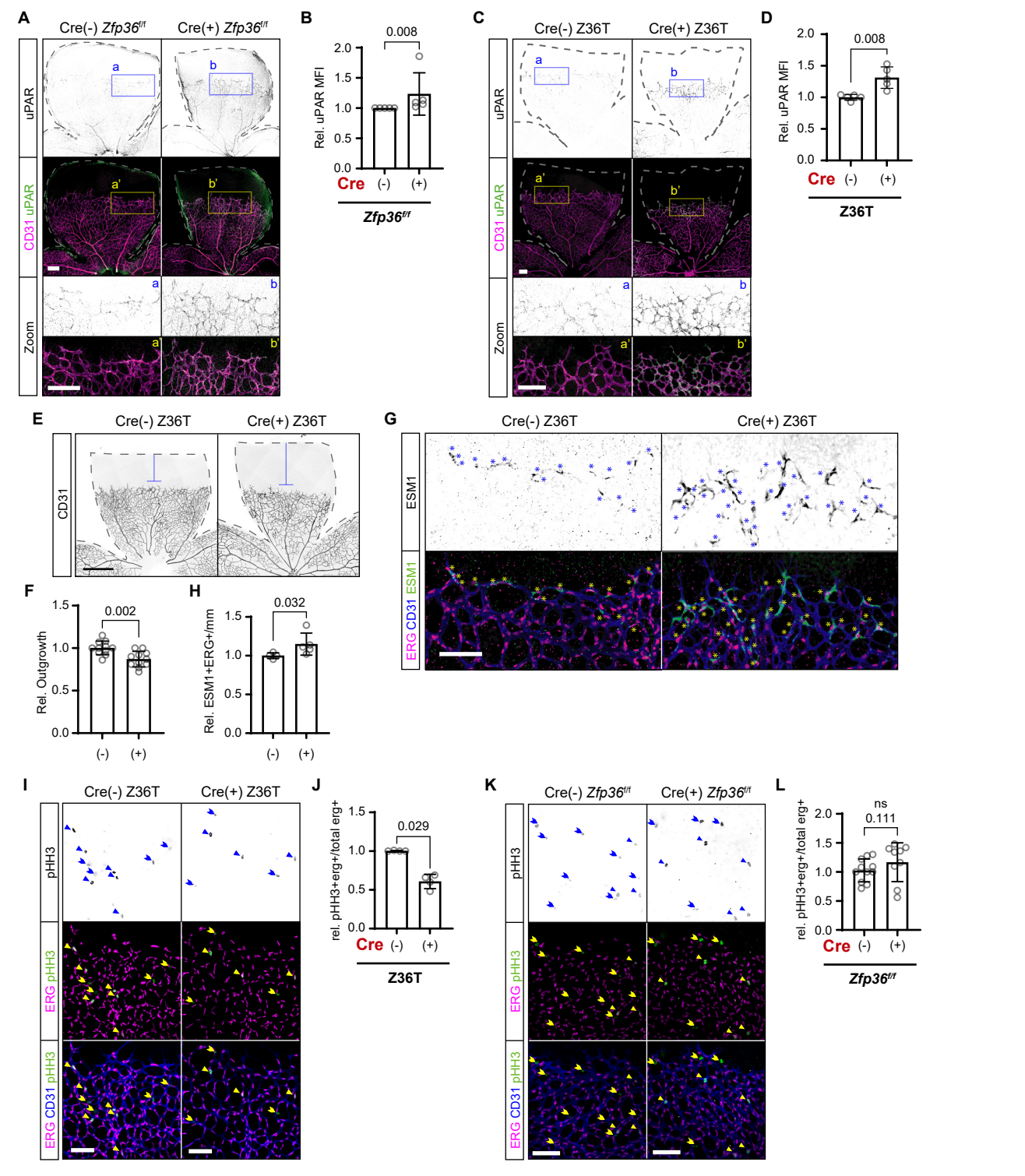
A
Cre(-) Zfp36f/f
Cre(+) Zfp36f/f
uPAR
CD31 uPAR
Zoom
a
b
a'
b'
B
0.008
Rel. uPAR MFI
Cre (-) (+)
Zfp36f/f
C
Cre(-) Z36T
Cre(+) Z36T
D
0.008
Rel. uPAR MFI
Cre (-) (+)
Z36T
E
Cre(-) Z36T
Cre(+) Z36T
CD31
F
0.002
Rel. Outgrowth
(-) (+)
G
Cre(-) Z36T
Cre(+) Z36T
ESM1
ERG CD31 ESM1
H
0.032
Rel. ESM1+ERG+/mm
(-) (+)
I
Cre(-) Z36T
Cre(+) Z36T
pHH3
ERG pHH3
ERG CD31 pHH3
J
0.029
rel. pHH3+erg+/total erg+
Cre (-) (+)
Z36T
K
Cre(-) Zfp36f/f
Cre(+) Zfp36f/f
pHH3
ERG pHH3
ERG CD31 pHH3
L
ns
0.111
rel. pHH3+erg+/total erg+
Cre (-) (+)
Zfp36f/f

Figure S5. Increased severity of ZFP family member triple EC-KO involves additional changes in cell proliferation. Related to Figure 7.

(A) Representative uPAR and CD31 IHC of TAM fed inducible *Cdh5*-Cre *Zfp36*$^{f/f}$ mice. Dashed lines outline retina area (low magnification scale bar = 200 μm; zoom scale bar = 100 μm).

(B) Quantification of uPAR MFI ± SD within CD31+ area of TAM fed inducible *Cdh5*-Cre *Zfp36*$^{f/f}$ mice relative to respective littermate Cre(-) controls (n = 5 replicates each; pairs from 5 independent litters). Statistical analysis performed using Mann-Whitney test.

(C) Representative uPAR and CD31 IHC of TAM fed Z36T mice. Dashed lines outline retina area (low magnification scale bar = 300 μm; zoom scale bar = 100 μm).

(D) Quantification of uPAR MFI ± SD within CD31+ area of TAM fed Z36T mice relative to respective littermate Cre(-) controls (n = 4 animals each). Statistical analysis performed using Mann-Whitney test.

(E) Representative CD31 IHC of TAM fed Z36T mice. Dashed lines outline retina area and blue bracket indicates angiogenic outgrowth based on remaining retina area (scale bar = 500 μm).

(F) Quantification of outgrowth (CD31+ area/total area) averaged per animal of TAM Z36T mice retina relative to average littermate control (mean ± SD; n = 11 animals each, derived from 5 independent litters).

(G) Representative CD31, ESM1, and ERG immunohistochemistry of TAM fed Z36T mice. Asterisks indicate ESM1+ ERG (scale bar = 100 μm).

(H) Quantification of ESM1+ ERG normalized to width of angiogenic front (mm) of TAM fed Z36T mice relative to average littermate control (mean ± SD; n = 4 Cre(-) and 5 Cre(+) animals, derived from 3 independent litters).

(I) Representative IHC of pHH3, ERG, and CD31 from TAM fed Z36T mice. pHH3^{+}ERG^{+}(arrowheads) and pHH3^{+}ERG^{-} (chevrons) are indicated (scale bar = 100 μm).

(J) Quantification of pHH3^{+} ERG normalized to total ERG of TAM fed Z36T mice relative to average littermate control (mean ± SD; n = 4 animals each, derived from 3 independent litters). Statistical analysis performed using Mann-Whitney test.

(K) Representative IHC of pHH3, ERG, and CD31 from TAM fed CDH5-Cre *zfp36*$^{f/f}$ mice. pHH3^{+}ERG^{+}(arrowheads) and pHH3^{+}ERG^{-} (chevrons) cells are indicated (scale bar = 100 μm).

(L) Quantification of pHH3^{+} ERG normalized to total ERG of TAM fed CDH5-Cre *zfp36*$^{f/f}$ mice relative to average littermate control (mean ± SD; n = 11 Cre(-) and 9 Cre(+) animals, derived from >3 independent litters). Statistical analysis performed using Mann-Whitney test.

TABLE S1 - SUPPLEMENTARY TABLE 1 RELATED TO STAR METHODS - qPCR primers

qPCR primers

Gene	Forward (5' to 3')	Reverse (5' to 3')	Species	Source
ZFP36	GACTGAGCTATGTCGGACCTT	GAGTTCCGTCTTGTATTTGGGG	Human	This paper
ZFP36L1	GATGACCACCACCCTCGT	TGGGAGCACTATAGTTGAGCATC	Human	This paper
ZFP36L2	CTGCTGCTGACTGCGGTA	ATCCAGACCCACAACTTTGC	Human	This paper
JAG1	GACTCATCAGCCGTGTCTCA	TGGGGAACACTCACACTCAA	Human	This paper
HPRT	GCCCTGGCGTCGTGATTAGT	AGCAAGACGTTCAGTCCTGTC	Human	Mack et al[56]
Jag1	CGTAGAGTACACTGCCTGCC	CAAGTATCTCCCCAGTCCCG	Mouse	Cicchetto et al[26]
Tuba1b	GAGACCCGGTGTCTGCTTC	GAGATGCACTCACGCATGATA	Mouse	Cicchetto et al[26]
Ptgs2	TGAGTACCGCAAACGCTTCT	CAGCCATTTCCTTCTCTCCTGT	Mouse	Cicchetto et al[26]
Rplpo	CACTGGTCTAGGACCCGAGAAG	GGTGCCTCTGGAGATTTTCG	Mouse	Cicchetto et al[26]