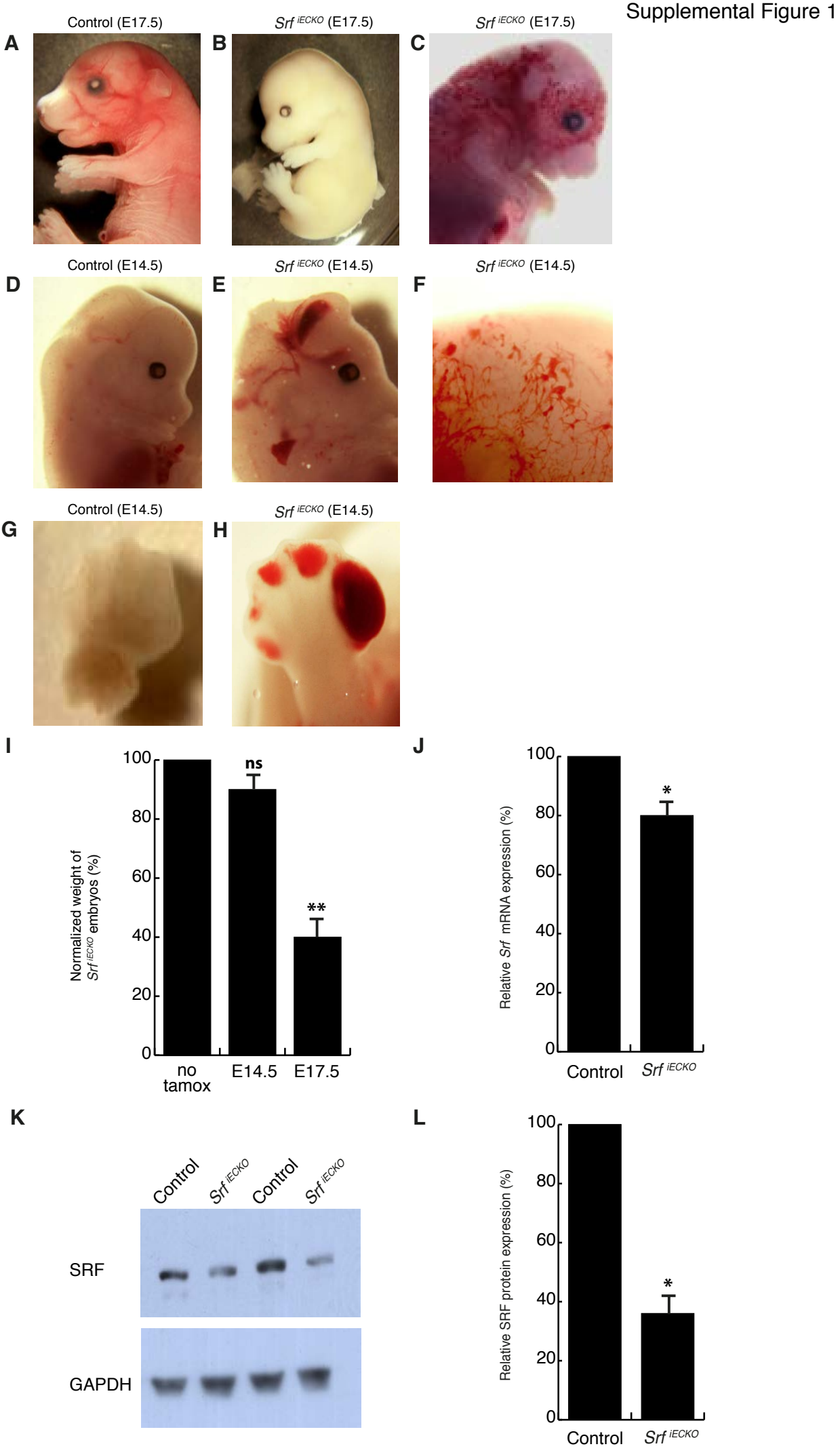
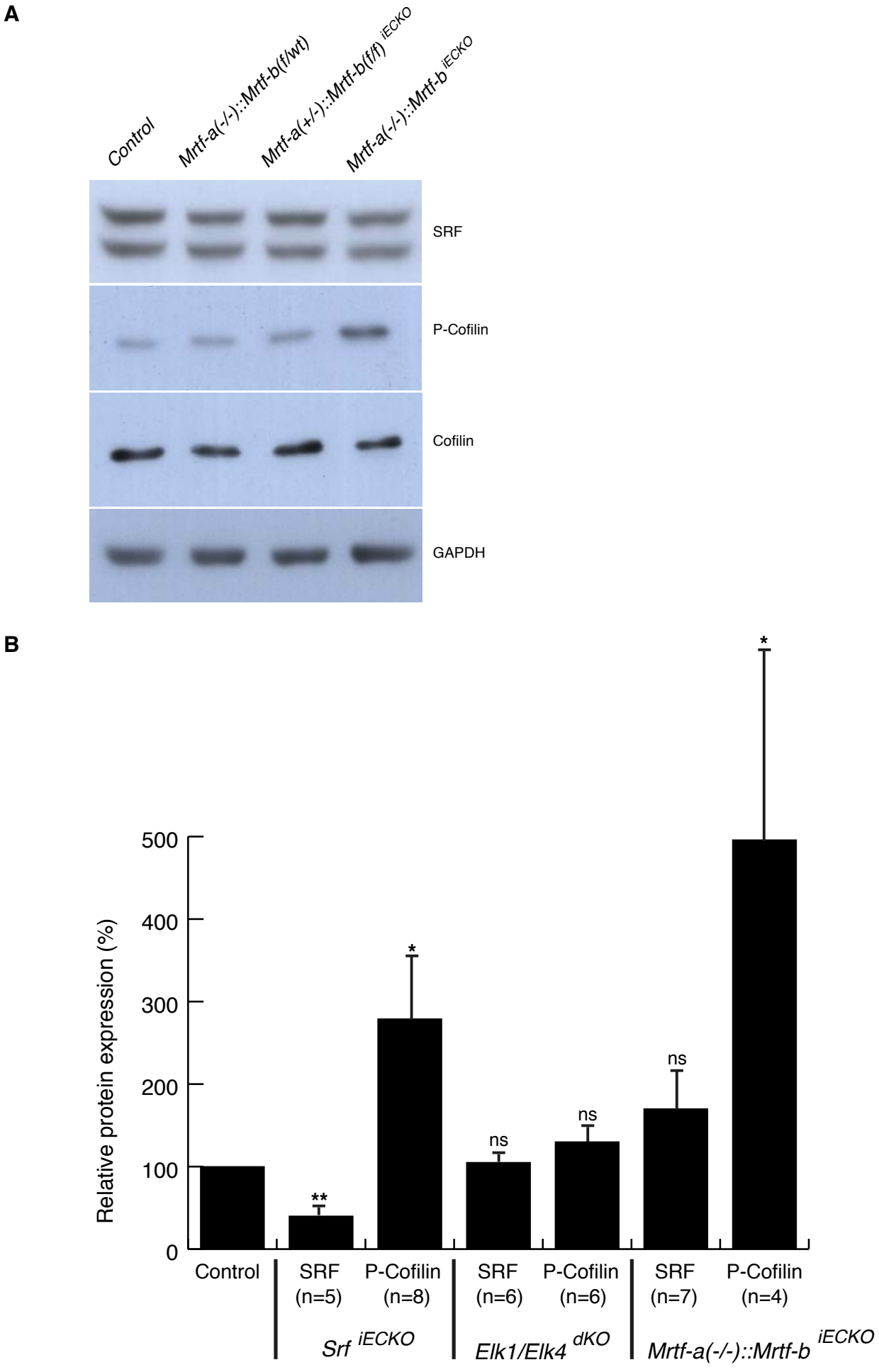
Supplemental Figure 1



Legend to Supplemental Figure 1.

Characterization of embryos after EC-specific *Srf* deletion. **(A)** E17.5 control embryo. **(B)** E17.5 Srf^{iECKO} embryo, representing the majority (90%) of embryos being necrotic and smaller in size. **(C)** E17.5 Srf^{iECKO} embryo, displaying a subset (10%) with abnormal vascularization. **(D)** E14.5 control embryo. **(E)** E14.5 Srf^{iECKO} mutant embryo with hemorrhaging in the head region. **(F)** E14.5 mutant embryo with fragmented blood vessels in the head region. **(G)** E14.5 limb buds of a control embryo. **(H)** Higher magnification of blood pooling in limb buds of E14.5 Srf^{iECKO} embryos. **(I)** E17.5 *Srf* knockout embryos show a significant weight reduction. Data shown are means +/- s.e.m. **p<0,01 (n=6 each). **(J)** Semiquantitative RT-PCR analysis of *Srf* mRNA levels of embryonic limb buds (E14.5 control versus Srf^{iECKO} embryos). *Srf* mRNA levels were calculated in relation to *Gapdh* and normalized to the control (100%). The data shown are means +/- s.e.m. *p<0,05 (n=6 each). **(K)** Representative example of anti-SRF Western Blot analysis of embryonic limb bud tissues of pairs of control and Srf^{iECKO} E14.5 embryos. SRF was detected by 2C5 antibody, GAPDH served as loading control. **(L)** Quantitation of Western blots (embryonic limb bud tissue; control and Srf^{iECKO} E14.5). SRF protein levels were calculated relative to GAPDH and normalized to control (100%). Data shown are means +/- s.e.m. *p<0,05 (n=3 each).


A
Control
Mrtf-a(-/-)::Mrtf-b(f/wt)
Mrtf-a(+/-)::Mrtf-b(f/f) iECKO
Mrtf-a(-/-)::Mrtf-b iECKO
SRF
P-Cofilin
Cofilin
GAPDH
B
Relative protein expression (%)
500
400
300
200
100
0
**
*
ns
ns
ns
*
Control
SRF (n=5)
P-Cofilin (n=8)
SRF (n=6)
P-Cofilin (n=6)
SRF (n=7)
P-Cofilin (n=4)
Srf iECKO
Elk1/Elk4 dKO
Mrtf-a(-/-)::Mrtf-b iECKO

Legend to Supplemental Figure 2.

Phosphorylation of cofilin is increased upon SRF and MRTF-A/B depletion.

(**A**) Representative Western blot of SRF and phospho-Cofilin (P-Cofilin) levels in *Mrtf-a(-/-)::Mrtf-b*^{iECKO} whole retinal tissue (P8). Cofilin and GAPDH served as loading controls. Extracts from: Control (*Mrtf-a(+/-)::Mrtf-b(f/wt)* (lane 1), *Mrtf-a(-/-)::Mrtf-b(f/wt)* (lane 2), *Mrtf-a(+/-)::Mrtf-b(f/f)*^{iECKO} (lane 3), *Mrtf-a(-/-)::Mrtf-b*^{iECKO} (lane 4). (**B**) Normalized quantification of Western blots of SRF and P-Cofilin levels in the different mutant mouse strains used in this study (*Srf*^{iECKO}*, Elk1/Elk4*^{dKO} and *Mrtf-a(-/-)::Mrtf-b(f/f)*^{iECKO} mice). SRF protein levels are reduced in *Srf*^{iECKO}, and unchanged in *Elk1/Elk4*^{dKO} and *Mrtf-a(-/-)::Mrtf-b(f/f)*^{iECKO} mice. P-Cofilin levels are increased in *Srf*^{iECKO} and *Mrtf-a(-/-)::Mrtf-b(f/f)*^{iECKO} mutants, but unchanged in *Elk1/Elk4*^{dKO} mutants.

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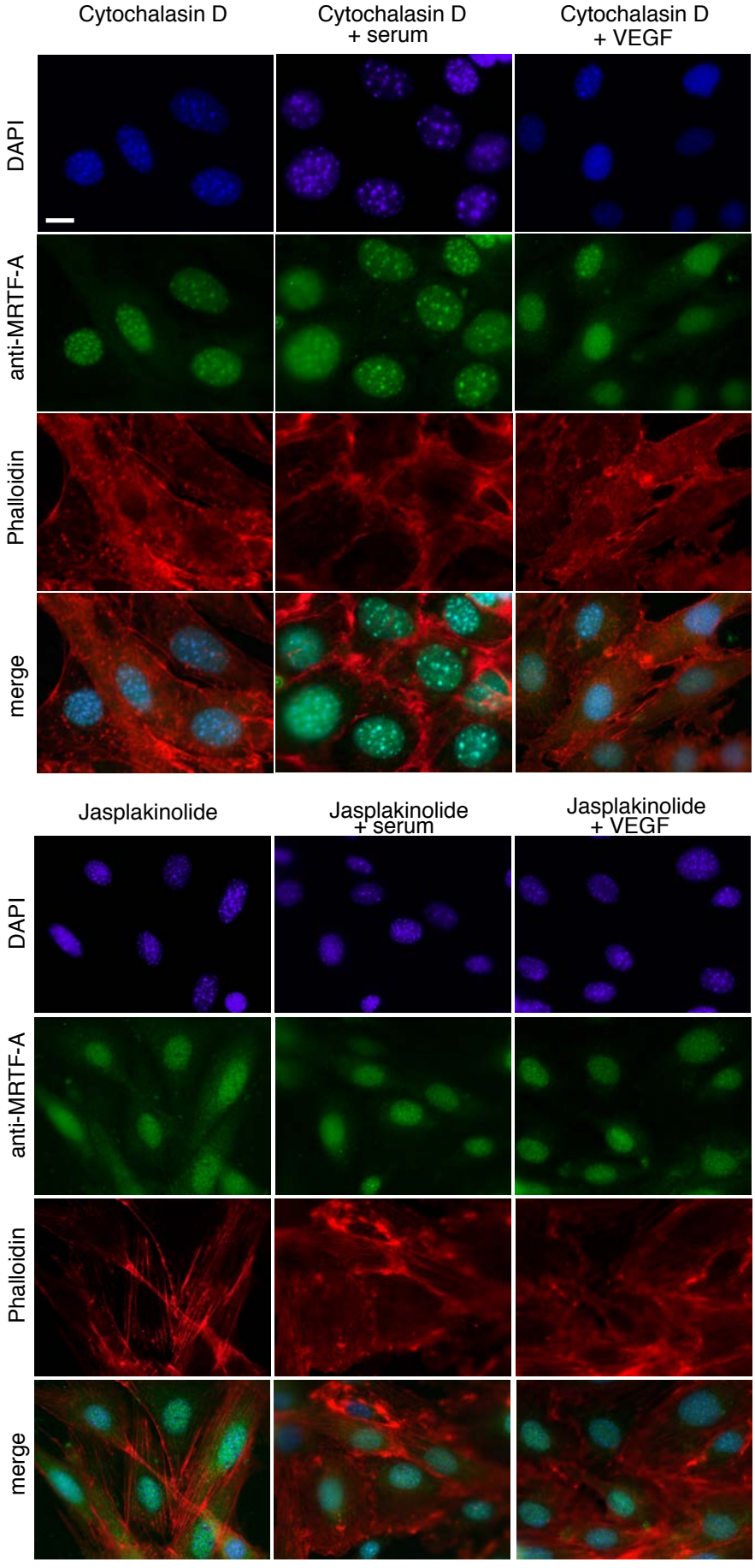
Genotype Comparison	t-test (p value)	Significance
1 versus 2	0,040	*
1 versus 3	0,0000044	***
1 versus 4	0,000047	***
1 versus 5	0,00068	***
1 versus 6	0,00017	***
2 versus 3	0,069	ns
2 versus 4	0,094	ns
2 versus 5	0,057	ns
2 versus 6	0,00035	***
3 versus 4	0,84	ns
3 versus 5	0,65	ns
3 versus 6	0,0033	**
4 versus 5	0,58	ns
4 versus 6	0,0025	**
5 versus 6	0,0041	**

Legend to Supplemental Figure 3.

Statistical analysis of radial outgrowth of P8 retinae from Figure 9K. Genotypes analysed:

Genotype 1:	*Mrtf-a(+/-)::Mrtf-b(f/x)*	n=8
Genotype 2:	*Mrtf-a(-/-)::Mrtf-b(f/x)*	n=7
Genotype 3:	*Mrtf-a(+/-)::Mrtf-b(f/wt)*^{iECKO}	n=5
Genotype 4:	*Mrtf-a(-/-)::Mrtf-b(f/wt)*^{iECKO}	n=6
Genotype 5:	*Mrtf-a(+/-)::Mrtf-b(f/f)*^{iECKO}	n=5
Genotype 6	*Mrtf-a(-/-)::Mrtf-b*^{iECKO}	n=6

x denotes either the *floxed* or the *wt* allele of *Mrtf-b*. Shown is the Student´s t-test analysis including t-test p-values and significance levels for each genotype compared to each other.


Cytochalasin D
Cytochalasin D
+ serum
Cytochalasin D
+ VEGF
DAPI
anti-MRTF-A
Phalloidin
merge
Jasplakinolide
Jasplakinolide
+ serum
Jasplakinolide
+ VEGF
DAPI
anti-MRTF-A
Phalloidin
merge

Legend to Supplemental Figure 4.

Nuclear localization of MRTF-A in mECs upon treatment with Cytochalasin D (30 minutes) and Jasplakinolide (2 hours). Subsequently, cells were additionally treated with serum (15% FCS for 1h) or VEGF-A (100 ng/ml for 1h), fixed and stained for cell nuclei (DAPI, blue), MRTF-A (green), and actin filaments (Phalloidin, red). Scale bar: 10 μm.

Primer Sequences for Genotyping

SRF
5‘ GCTCGCAGCGGCGGCCAGATCTATAAC 3‘
5‘ GCCTGCATTACCGGTCGATGCAACGA 3‘
5‘ CTTCGCGCACACCAGGACACAGAGGAT 3‘

Elk4
5’ GTGATGATGGTGGGATAGTGTATATCTGCAGGAGTG 3’
5’ CCAGAGCCACTGAAGCTGTTAAGTAAACTAGACTAAAC 3’
5’ CTGCAGGAATTCGATATCAAGCTTGAACTCC 3’

mTmG
5‘ CTCTGCTGCCTCCTGGCTTCT 3‘
5’ CGAGGCGGATCACAAGCAATA 3’
5’ TCACTGGGCGGGGGTCGTT 3’

CRE
5’ GCCTGCATTACCGGTCGATGCXAACGA 3’
5’ GTGGCAGATGGCGCGGCAACACC 3’

MRTF-A
5’ GTTGCTCAGTCATGTGACACCTGTACAG 3’
5’ GGCTTCAGTACCTTCCTAAGCTCTGCAG 3’
5’ CATGGTGGATCCTGAGACTGGCGAAT 3’

MRTF-B
5’ GGCTTAGACAAGATGGTTGGTCTGGCACTGCC 3’
5’ CCAGTGGTGTCCAGTCTTACTGAACAGCTCACTGAG 3’
5’ CATGGCGACTTCCTTCTCCTCTTCTCAAGGCTG 3’

siRNA Sequences

siGL2 siRNA (control)
sense: CGU ACG CGG AAU ACU UCG AdTdT
antisense: UCG AAG UAU UCC GCG UAC GdTdT

siSRF siRNA
sense: GAU GGA GUU CAU CGA CAA CAA
antisense: GUU GUC GAU GAA CUC CAU CUU

Primer Sequences for semiquantitative RT-PCR
Gapdh fw: TGG ATC TGA CGT GCC GC Gapdh rev: TGC CGT CTT CAC CAC CTT C
Srf fw: CAC GAC CTT CAG CAA GAG GAA Srf rev: CAA AGC CAG TGG CAC TCA TTC
Thrombospondin1 fw: TCG GCC TTT AAC GAA TGA GAA Thrombospondin1 rev: AGC GGG CAC CTT CCT AGT G
Cofilin fw: TCT GGG CCC CCG AGA AT Cofilin rev: TTG ATG GCA TCC TTG GAG C
ß-actin fw: AGA GAG GTA TCC TGA CCC TGA AGT ß-actin rev: CAC GCA GCT CAT TGT AGA AGG TGT
c-fos fw CTT GCC CCT TCT CAA CGA c-fos rev: GCT CCA CGT TGC TGA TGC T
Opsin-1 fw: CAA GCC CTT TGG CAA TGT GA Opsin-1 rev: GCT CCA ACC AAA GAT TGG TGG
Rhodopsin fw: TCA TGG TCT TCG GAG GAT TCA C Rhodopsin rev: TCA CCT CCA AGT GTG GCA AAG
Vegf-r2 fw: GAT GCC CGA CTC CCT TTG A Vegf-r2 rev: CGA AAG ACC ACA CAT CGC TCT
Vegf-r1 fw: AGC CTA CCT CAC CGT GCA AG Vegf-r1 rev: AAA AGA GGG TCG CAG CCA C
VE-Cadherin fw: CCA TCT TCC TCT GCA TCC TC VE-Cadherin rev: CAA CTG CTCGTG AAT CTC CA
Vegf-a fw: TCA CCA AAG CCA GCA CAT AG Vegf-a rev: TTG ACC CTT TCC CTT TCC TC
Pak1 fw: GCCCTAACTGTTAAAGGTCCTACCA Pak1 rev: GCCCATGTGTTAAACGCCTG

Amplification Protocols for Genotyping		
SRF	**Elk4**	**CRE**
94°C, 2 min 94°C, 30 sec 60°C, 30 sec 72°C, 30 sec 33 cycles 72°C, 7 min	95°C, 3 min 95°C, 1 min 69°C, 1 min 72°C, 1 min 30 cycles 72°C, 10 min	94°C, 1 min 94°C, 45sec 70°C, 45sec 72°C, 1min 35 cycles 72°C, 10min
MRTF-A	**MRTF-B**	**mTmG**
94°C, 2 min 94°C, 30sec 62°C, 30 sec 72°C, 50 sec 40 cycles 72°C, 7 min	94°C, 2 min 94°C, 30sec 70°C, 40 sec 72°C, 50 sec 35 cycles 72°C, 7 min	94°C, 3 min 94°C, 30sec 61°C, 1 min 72°C, 1min 35 cycles 72°C, 2 min

Amplification Protocol for semiquantitative RT-PCR	
Segment 1:	50°C, 2 min
Segment 2:	95°C, 10 min
Segment 3:	95°C, 15 sec
	60°C, 1 min
	40 cycles

Legend to Supplemental Table.

Primer sequences and PCR amplification protocols.