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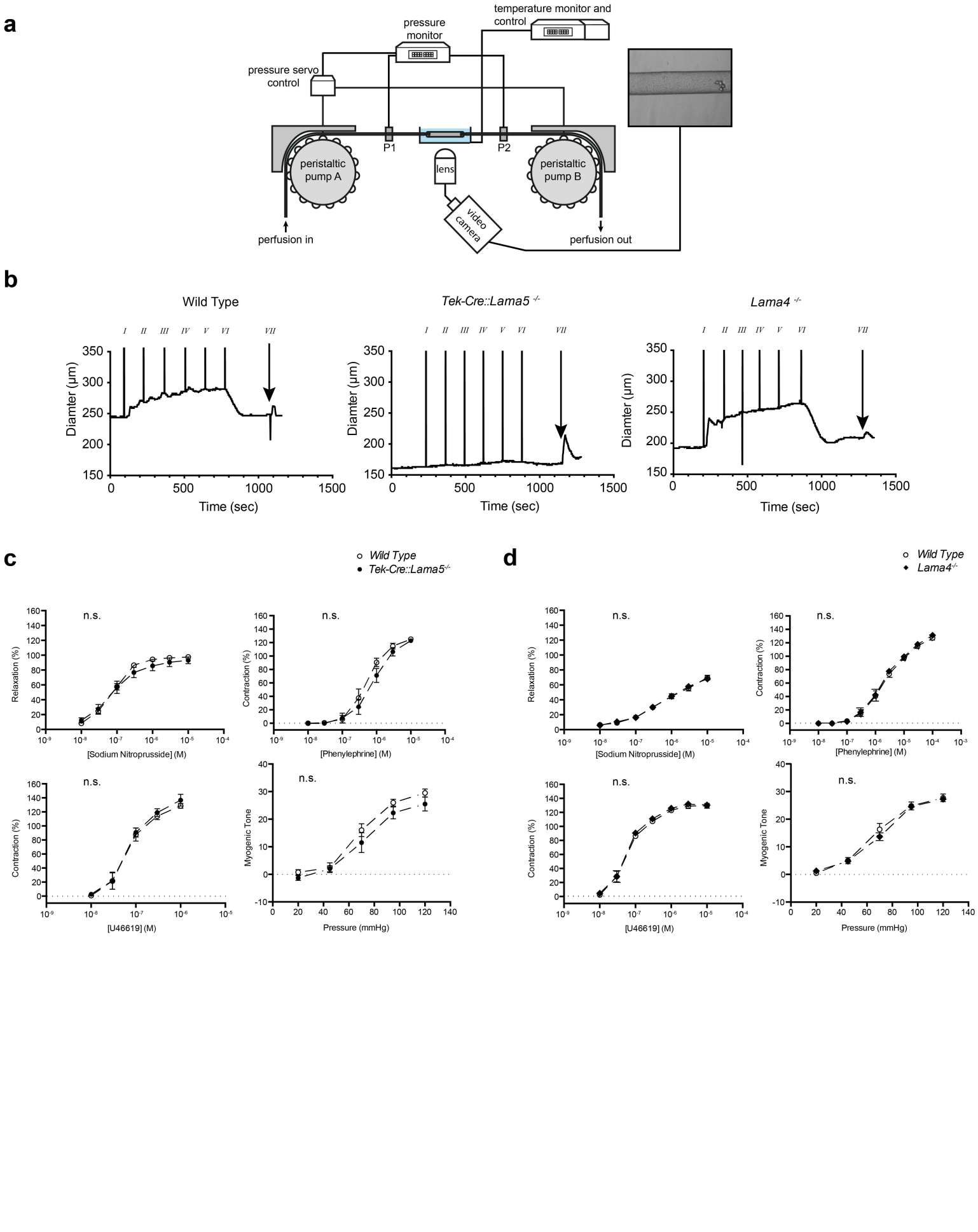

a
temperature monitor and control
pressure monitor
pressure servo control
P1
P2
peristaltic pump A
peristaltic pump B
lens
video camera
perfusion in
perfusion out
b
Wild Type
Tek-Cre::Lama5 -/-
Lama4 -/-
I II III IV V VI VII
Diamter (µm)
Time (sec)
c
Wild Type
Tek-Cre::Lama5-/-
d
Wild Type
Lama4-/-
n.s.
Relaxation (%)
[Sodium Nitroprusside] (M)
Contraction (%)
[Phenylephrine] (M)
[U46619] (M)
Myogenic Tone
Pressure (mmHg)

Fig S1 The set-up of flow-induced dilation experiments and functional analyses of laminin KO mesenteric resistance arteries. a) The pressure myograph setup employed to study flow-induced dilatory response of excised arteries. During the experiment the intraluminal pressure was monitored by two pressure transducers (P1 and P2) and kept constant through the use of a servo control system connected to peristaltic pump A. Intraluminal flow was applied through peristaltic pump B. The vessels behaviour was monitored continuously using a digital camera connected to an inverted microscope. b) Representative diameter-time relation of cannulated wild type, *Tek-Cre::Lama5*$^{-/-}$ and *Lama4*$^{-/-}$ arteries during the flow-induced dilation experiment. The vessels were subjected to 2.5 (I), 5 (II), 10 (III), 20 (IV), 40 (V) dyn/cm^2 of shear stress and set back to 0 (VI). After each experiment the viability of the endothelium was tested by the addition of 10 μM of acetylcholine to the bath (VII). Functional analyses of mesenteric resistance arteries from wild type and *Tek-Cre::Lama5*$^{-/-}$ mice (c) and wild type and *Lama4*$^{-/-}$ mice (d) in response to increasing doses of a vasodilatory drug (sodium nitropusside), vasoconstrictory drugs (U46619, phenylephrine) and intraluminal pressure. The analyses show comparable behaviour in wild type, *Tek-Cre::Lama5*$^{-/-}$ and *Lama4*$^{-/-}$ arteries. n.s = not significant. Data are expressed as percentage of the maximum force developed either in presence of 0.3 μM U46619 (relaxation %) or in KPSS buffer alone (contraction %). Data are mean responses ± s.e.m from 7 experiments with 1 wild type and 1 KO artery in each experiment.

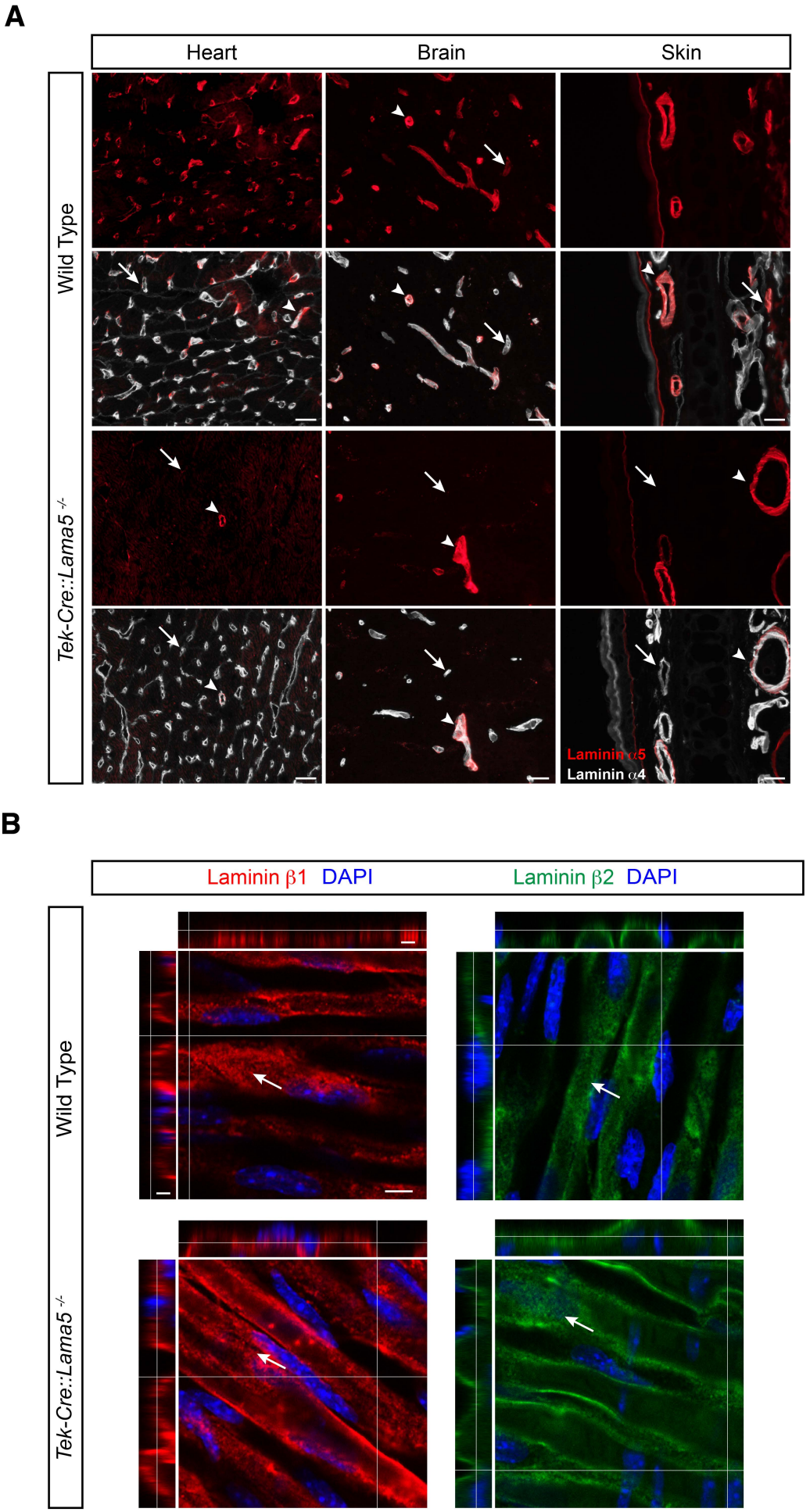

A
Heart
Brain
Skin
Wild Type
Tek-Cre::Lama5 -/-
Laminin α5
Laminin α4
B
Laminin β1 DAPI
Laminin β2 DAPI
Wild Type
Tek-Cre::Lama5 -/-

Fig S2 The characterization of endothelial basement membrane components in *Tek-Cre::Lama5*$^{-/-}$ mouse did not reveal major differences. A) Immunofluorescence staining for laminin α5 (red) and laminin α4 (white) in 5μm sections of heart, brain and skin from wild type and *Tek-Cre::Lama5*$^{-/-}$ mice, showing the absence of laminin α5 in endothelial basement membranes of *Tek-Cre::Lama5*$^{-/-}$ tissues (arrows), while the presence of laminin α4 was not altered. Laminin α5 is however detectable in the smooth muscle layers of larger vessels (arrow heads) of both wild type and *Tek-Cre::Lama5*$^{-/-}$ mice. Scale bars are 20 μm. B) Orthogonal view of confocal images of wild type and *Tek-Cre::Lama5*$^{-/-}$ mesenteric resistance arteries stained with laminin β1 and laminin β2. The comparison of the stainings shows no difference in pattern and intensity in endothelial basement membranes (arrows). Scale bar are 5μm in XY plane and 2.5 μm in ZY/ZX planes.

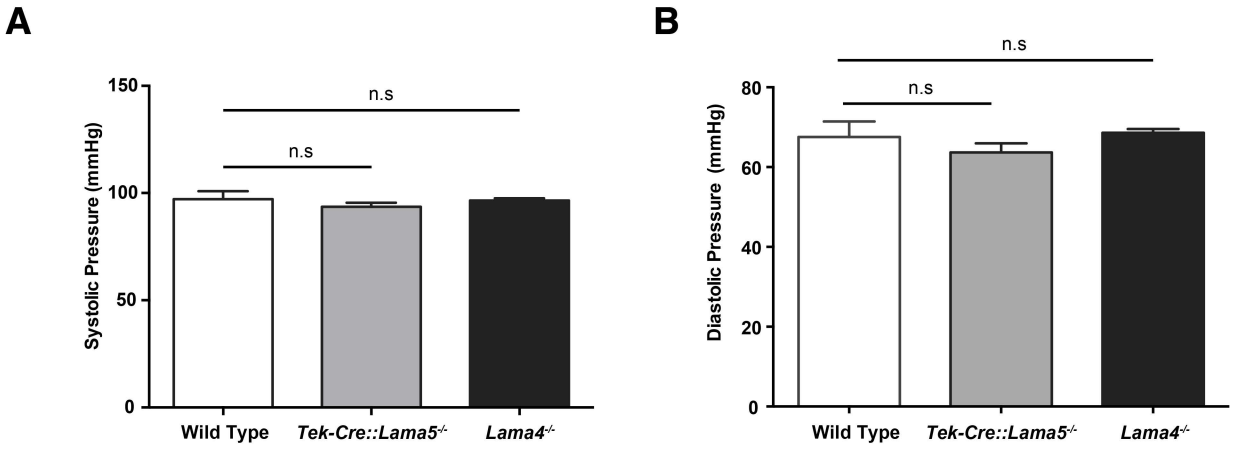
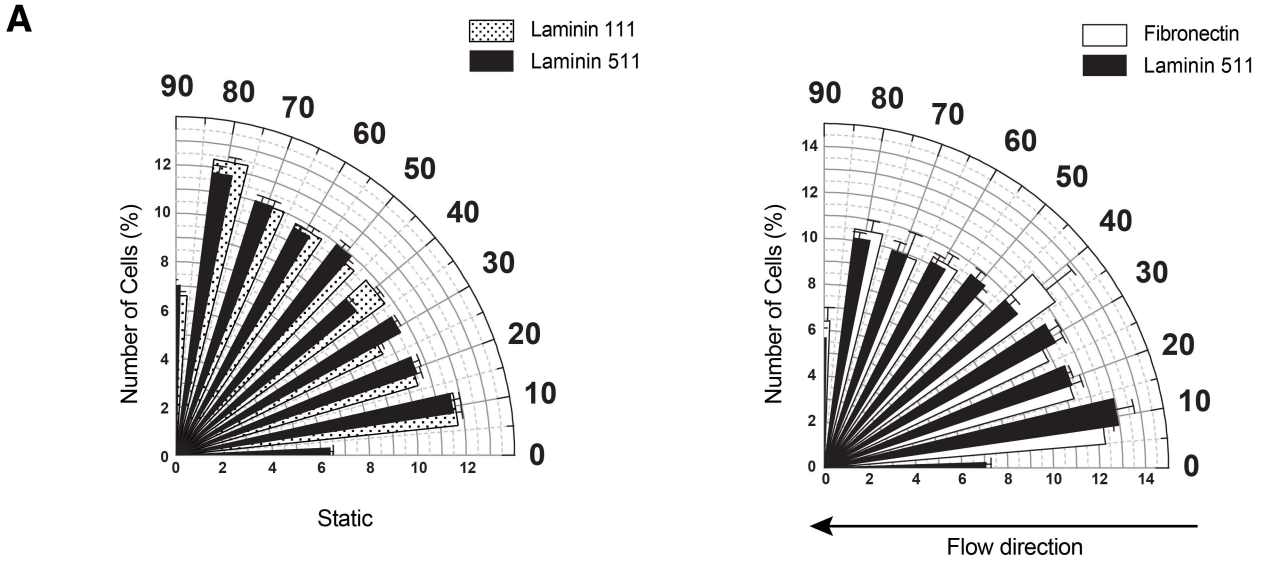

A
150
100
50
0
Systolic Pressure (mmHg)
n.s
n.s
Wild Type
Tek-Cre::Lama5-/-
Lama4-/-
B
80
60
40
20
0
Diastolic Pressure (mmHg)
n.s
n.s
Wild Type
Tek-Cre::Lama5-/-
Lama4-/-

Fig S3 Comparable systolic and diastolic mean blood pressure between wild type and laminin KO mice. Systolic (A) and diastolic (B) mean blood pressure measurements in anesthetized mice, show no detectable differences between wild type, *Tek-Cre::Lama5*$^{-/-}$ and *Lama4*$^{-/-}$ mice. Data are means ± s.e.m from 10 mice each genotype. n.s. = not significant.


A
Laminin 111
Laminin 511
Number of Cells (%)
Static
Fibronectin
Laminin 511
Number of Cells (%)
Flow direction

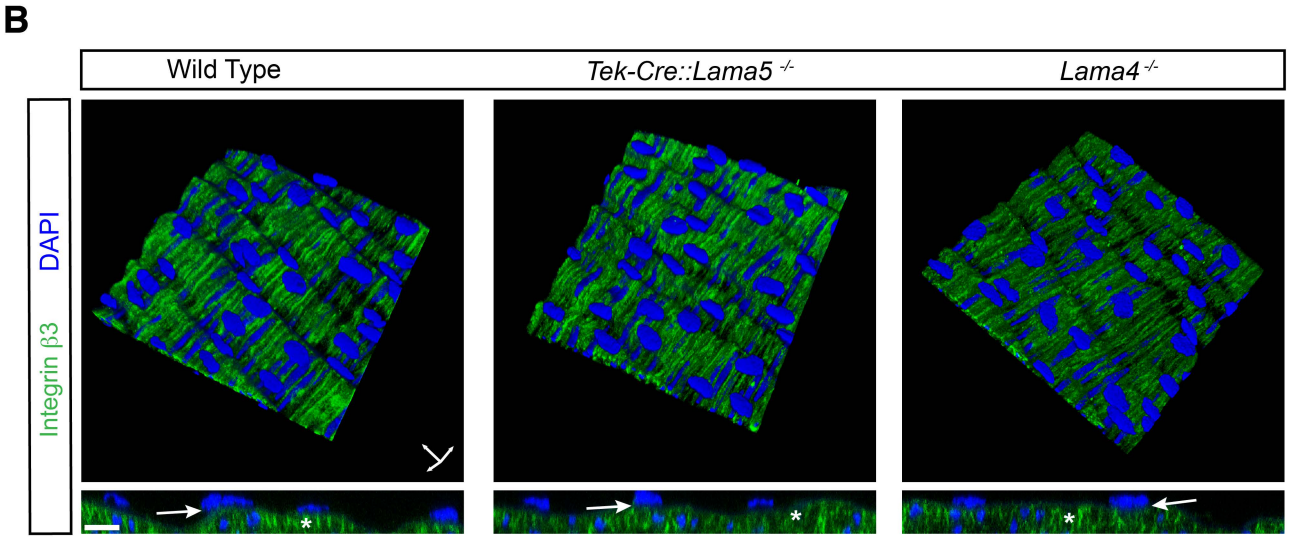

B
Wild Type
Tek-Cre::Lama5 -/-
Lama4 -/-
Integrin β3
DAPI

Fig S4 HUAECs align under shear stress and integrin β3 absence in endothelial cells of mesenteric resistance arteries. A) Angle histogram of HUAECs orientation after 120 min of static culture condition or 10dyn/cm^2 shear . Comparisons were made between cells plated on the same molar concentrations (25 nM) of laminin 511 and 111, or laminin 511 and fibronectin. Data are means ± s.e.m of 6 independent experiments. B) Whole mount staining of arteries from wild type, *Tek-Cre::Lama5*$^{-/-}$ and *Lama4*$^{-/-}$ mice show the absence of integrin β3 staining on endothelial cells (arrow), but its presence in the smooth muscle layer (asterisk). Scale bars are 10μm.

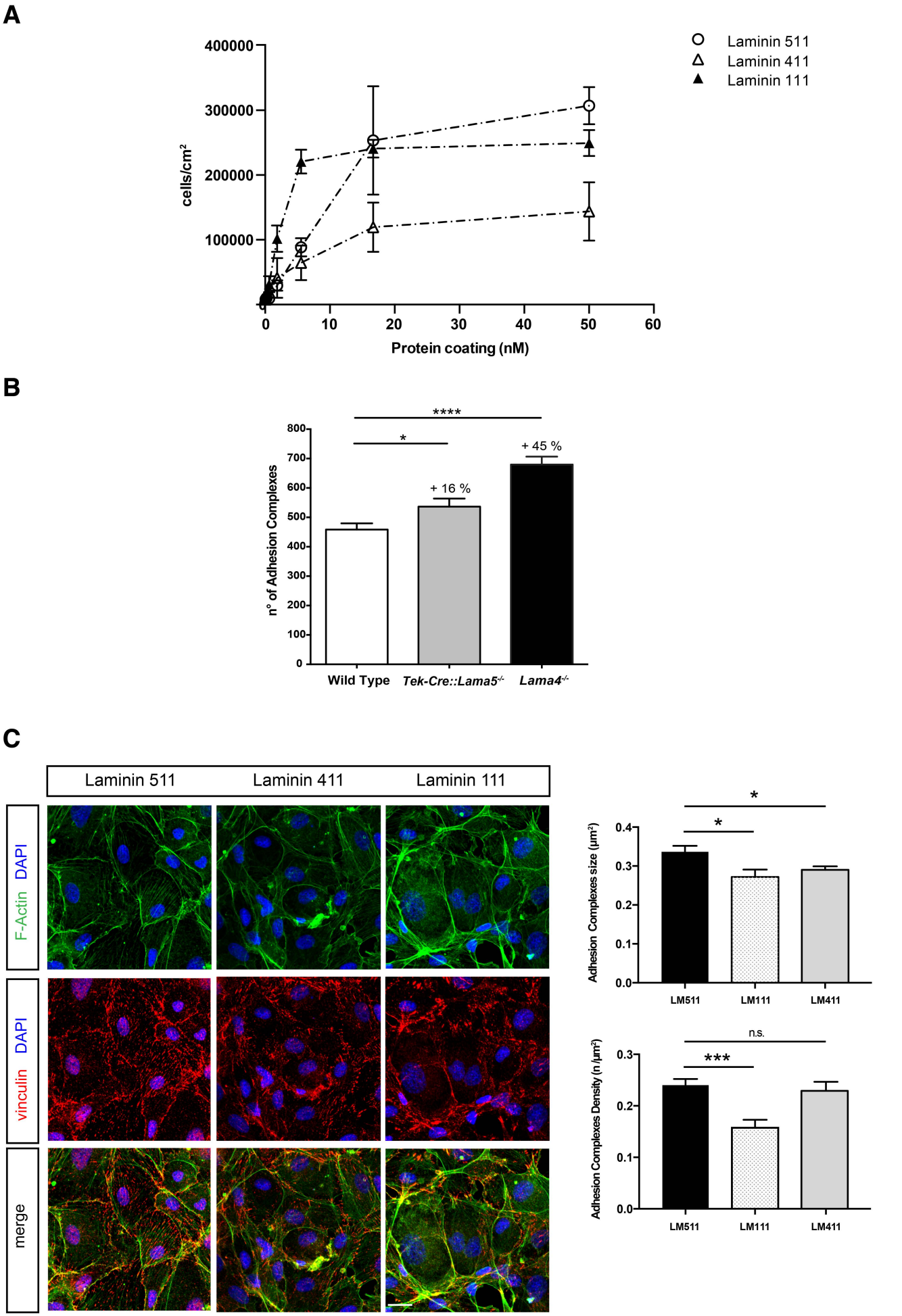
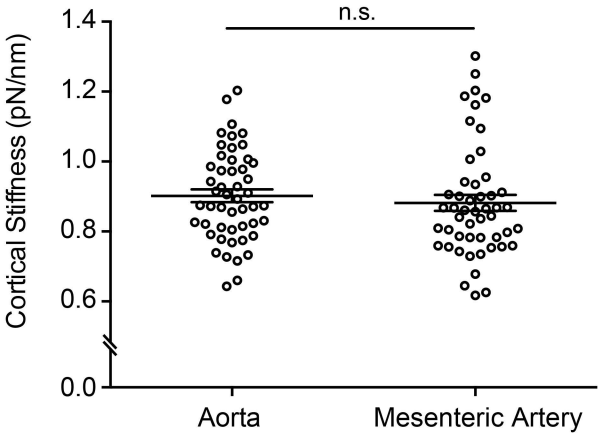

A
Laminin 511
Laminin 411
Laminin 111
cells/cm²
400000
300000
200000
100000
0
10
20
30
40
50
60
Protein coating (nM)
B
****
*
+ 45 %
+ 16 %
n° of Adhesion Complexes
800
700
600
500
400
300
200
100
0
Wild Type
Tek-Cre::Lama5-/-
Lama4-/-
C
Laminin 511
Laminin 411
Laminin 111
F-Actin DAPI
vinculin DAPI
merge
*
*
Adhesion Complexes size (µm²)
0.4
0.3
0.2
0.1
0.0
LM511
LM111
LM411
n.s.
***
Adhesion Complexes Density (n /µm²)
0.3
0.2
0.1
0.0
LM511
LM111
LM411

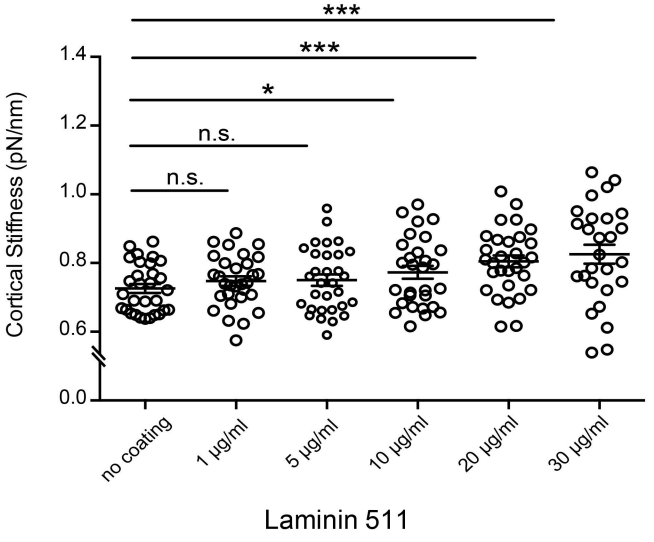
Fig S5 *In vitro* adhesion of mouse sEND.1 cells and HUAECs to different laminin isoform. A) *In vitro* cell adhesion assays employing mouse sEND.1 cells plated on increasing concentrations of purified laminin 411 and 511, compared to the non-endothelial cell laminin 111, showing high levels of sEND.1 adhesion to laminin 511 and low binding to laminin 411, comparable to the data obtained with HUAECs. Data are means ± s.e.m from 3 experiments with triplicates/experiments. B) Quantification of the number of adhesion complexes per endothelial cell in wild type, *Tek-Cre::Lama5*$^{-/-}$ and *Lama4*$^{-/-}$ mesenteric arteries. Data are means ± s.e.m from 300 cells from 9 wild type and 9 KO arteries isolated from 3 mice/genotype. C) Double immunofluorescence staining of vinculin and F-actin in HUAECs seeded on laminins 511, 111 and 411. DAPI reveals nuclei. Bar graphs show the quantification of sizes and density of cellular adhesion complexes. Data are means ± s.e.m from at least 24 cells each condition from 3 independent experiments. *P<0.05, ***P<0.001, ****P<0.0001, unpaired t-test. Scale bar is 10 μm.

A


n.s.
Cortical Stiffness (pN/nm)
1.4
1.2
1.0
0.8
0.6
0.0
Aorta
Mesenteric Artery

B

sEND.1


***
***
*
n.s.
n.s.
Cortical Stiffness (pN/nm)
1.4
1.2
1.0
0.8
0.6
0.0
no coating
1 µg/ml
5 µg/ml
10 µg/ml
20 µg/ml
30 µg/ml
Laminin 511

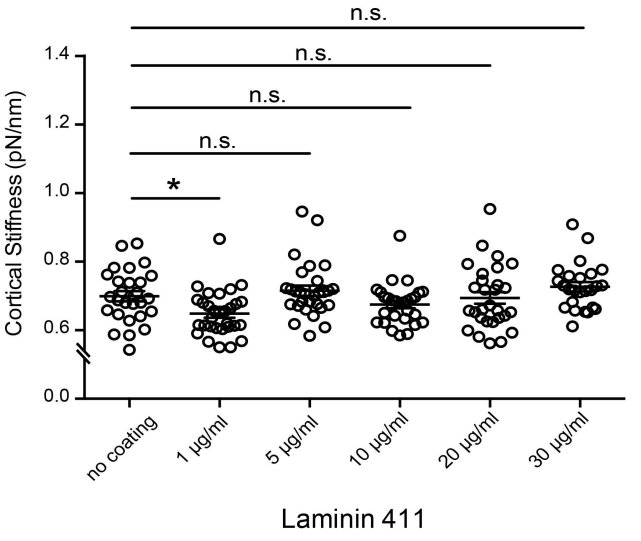

n.s.
n.s.
n.s.
n.s.
*
Cortical Stiffness (pN/nm)
1.4
1.2
1.0
0.8
0.6
0.0
no coating
1 µg/ml
5 µg/ml
10 µg/ml
20 µg/ml
30 µg/ml
Laminin 411

Fig S6 AFM measures of endothelial cell cortical stiffness *in vivo* and *in vitro*. A) Endothelial cell cortical stiffness on excised wild type aorta and mesenteric resistance artery shows no differences between the two vessel types. B) The cortical stiffness of sEND.1 seeded on increasing concentrations of purified laminins 511 and 411 show a concentration dependent increase in stiffness on laminin 511 and minimal effect in cells plated on laminin 411. Data shown are mean values ± s.e.m from 3 experiments with triplicates/experiments n.s. = not significant, *P<0.05, ***P<0.001, unpaired t-test.