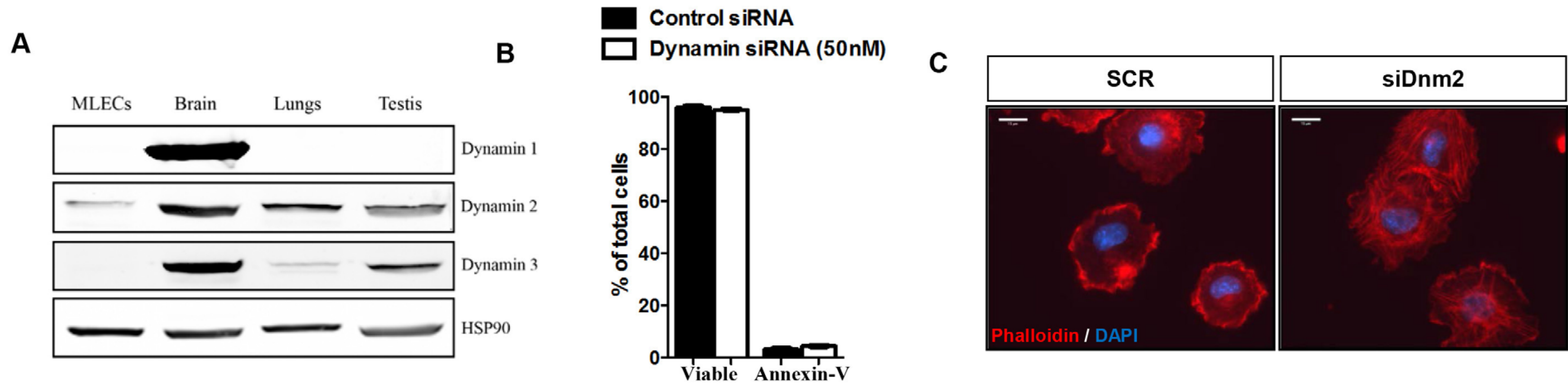
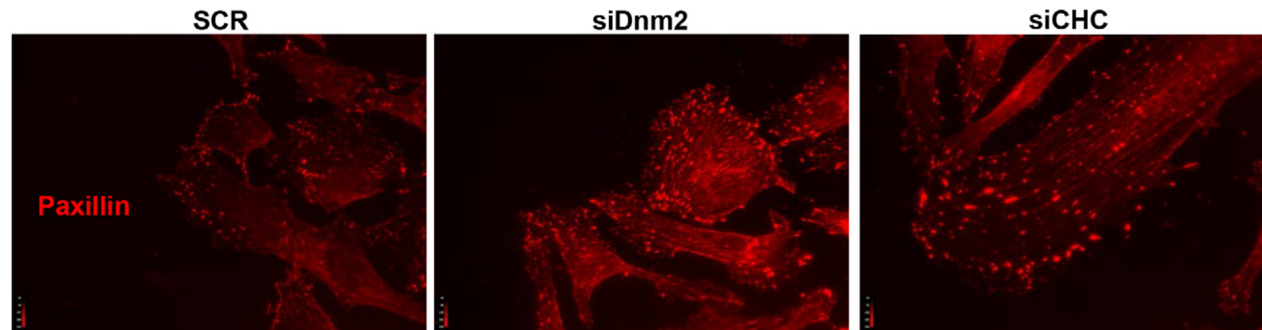
Supplemental Fig. 1



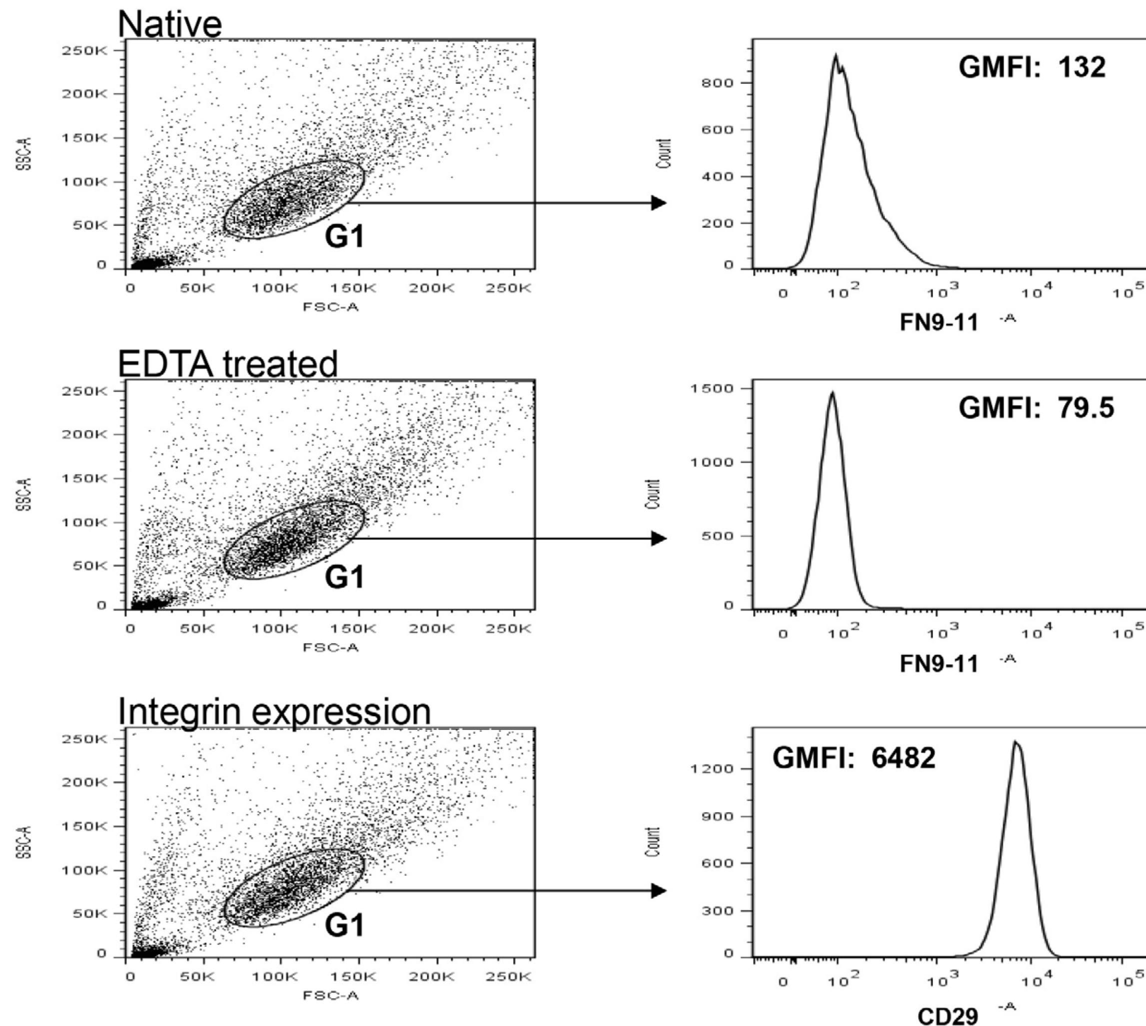
A. Dnm isoforms in mouse lung endothelial cells (MLEC) and tissues. Hsp 90 is a loading control. **B.** Loss of Dnm2 in HUVEC had no effect on cell viability or apoptosis 48hrs after siRNA transfection. Data from representative experiment, repeated 2 additional times. **C.** Loss of Dnm2 in HUVEC increases appearance of F-actin stress fibers.. Scale bar: **A**: 15µm.

Supplemental Fig. 2



Paxillin distribution in HUVEC treated with scramled (SCR), Dnm2 or CHC siRNAs. Both the loss Dnm2 or CHC increase paxillin positive, focal adhesion size. Representive images are shown from at least three experiments.

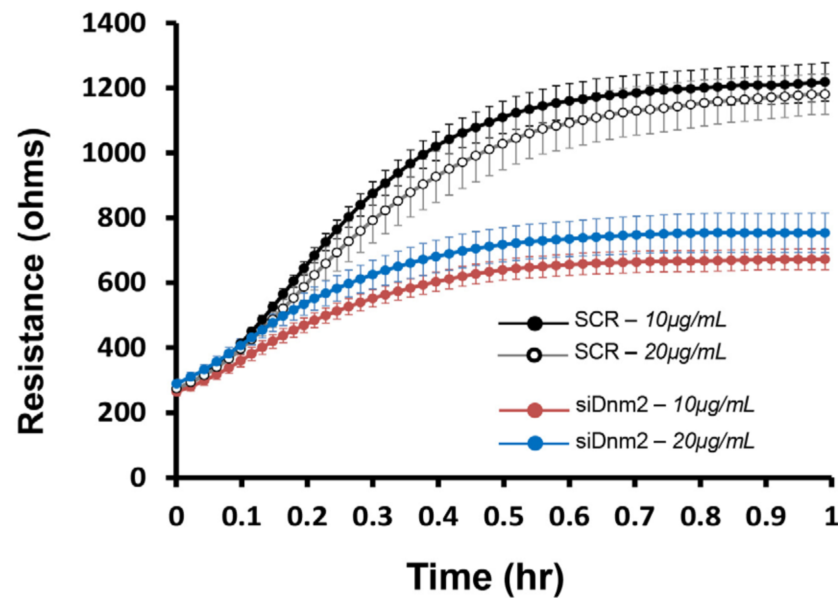
Supplemental Fig. 3



Assessment of b1 integrin activation and surface integrin expression in HUVECs

Cells were stained and data were acquired as described in Methods. Data analysis was performed using FlowJo software. A plot of forward scatter (FSC-A) vs. side scatter (SCA-A) was prepared and the gate G1 was drawn around the live cell population based on its homogenous size and granularity; debris and dead cells that scatter differently were excluded. The same G1 gate was set for each condition within an experiment, then frequency histograms for G1 were plotted showing FN9-11 binding or integrin expression (b1, aVb3 or avb5) within the G1 gate. Activation index was calculated using geometric mean fluorescence intensity (GMFI) as : (Native - EDTA) / b1 Integrin expression.

Supplemental Fig. 4



ECIS measurement of resistence in HUVEC treated with control versus Dnm2 siRNA. Transfected cells were plated onto wells coated with 10 and 20 ug/ml of fibronection and changes in electrical resistance Monitored over 60 min after plating. HUVEC lacking Dnm2 had reduced adhesion and spreading.

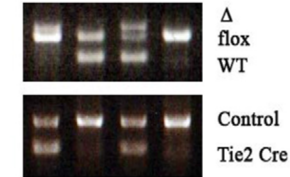
Supplemental Fig. 5

A

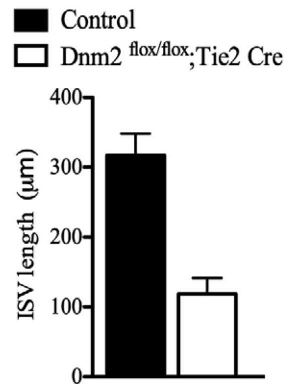
Age	Dnm2 $^{+/flox}$	Dnm2 $^{flox/flox}$	Dnm2 $^{+/flox}$ Tie2Cre	Dnm2 $^{flox/flox}$ Tie2Cre
P0	10	12	12	0
E11.5	6	10	5	0
E9.5	12	9	8	12 (1 dead)
E8.5	13	10	8	8
E7.5	3	3	1	1

B

Dnm2 $^{flox/flox}$ x Dnm2 $^{flox/+}$; Tie2 Cre

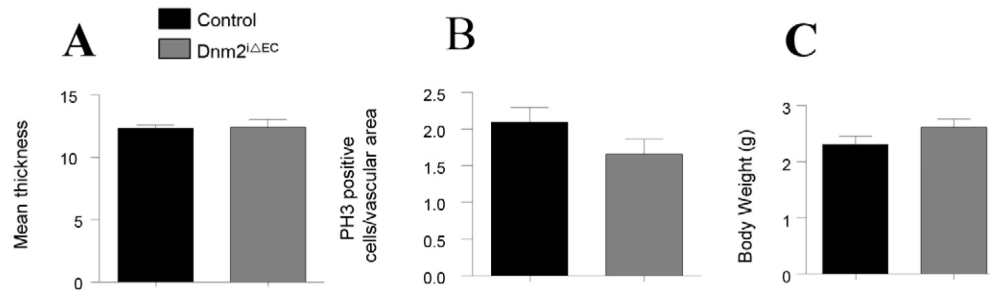


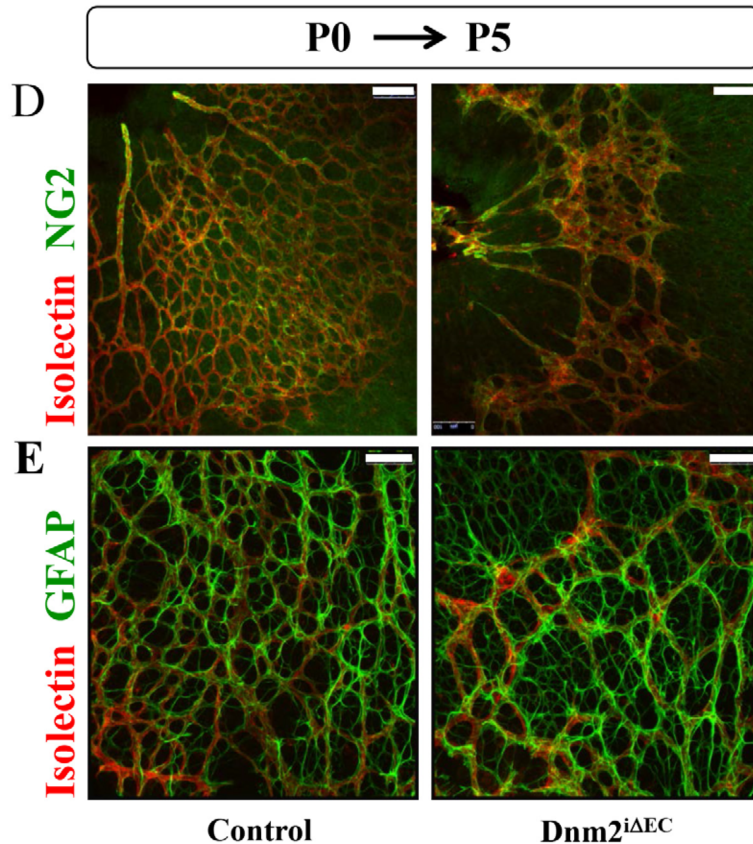
C

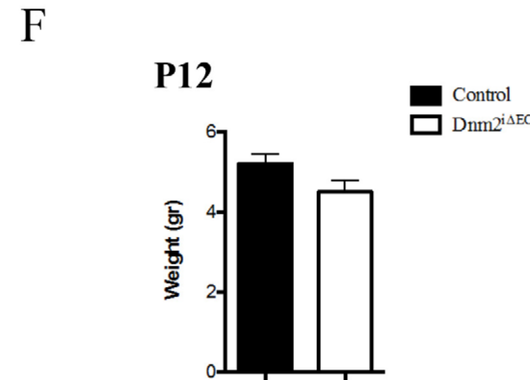


A. Number of embryos at different embryonic stages (E7.5, E8.5, E9.5 and E11.5) recovered from crossing *Dnm2*$^{flox/flox}$ females with *Dnm2*$^{+/flox}$*; Tie2Cre* male mice. **B.** Yolk sac DNA genotyping derived from *Dnm2*$^{flox/flox}$ females crossed with *Dnm2*$^{+/flox}$*; Tie2Cre* male mice. WT=192bp, flox=258bp and Δ=326bp for Dnm2 genotyping and Control=324bp, Tie2 Cre=100bp for Tie2 genotyping. **C.** Tail ISV length quantification from Control and *Dnm2*$^{flox/flox}$*; Tie2Cre* embryos at E9.0 (n=4).

Supplemental Fig. 6







Vessel thickness (**A**), PH3 (**B**) and body weights (**C**) were not different between the strains. **D** Whole mount retina (P5) from Control and $Dnm2^{i\Delta EC}$ pups (50µg TMX intragastric injection from P0-P3) stained with isolectin GS-IB4 (endothelial cell marker) and NG2 (pericytes marker). Scale bar: 100µm and **E.** GFAP (astrocytes marker). Scale bar: 75µm. **F.** Body weight at P12 of Control and $Dnm2^{i\Delta EC}$ pups (IP Tamoxifen injection, 100µgr from P5-P9, n=4-12).