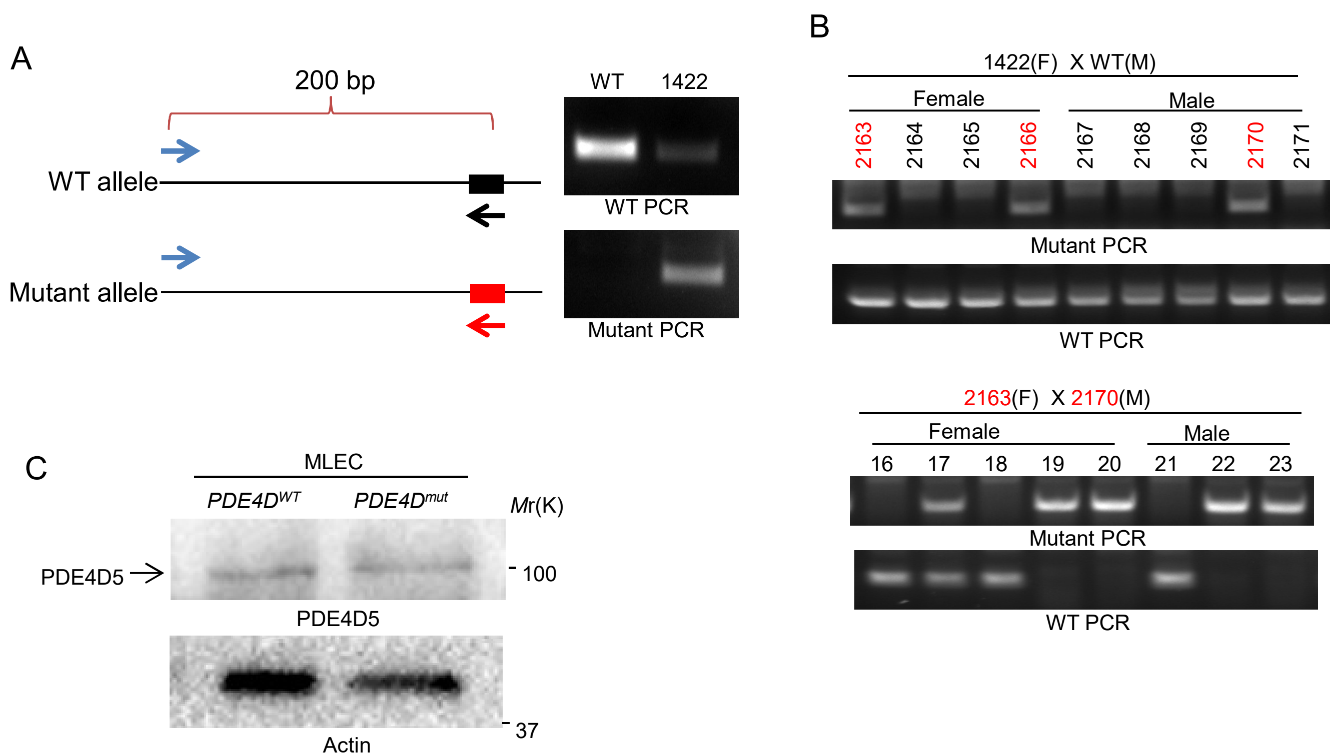
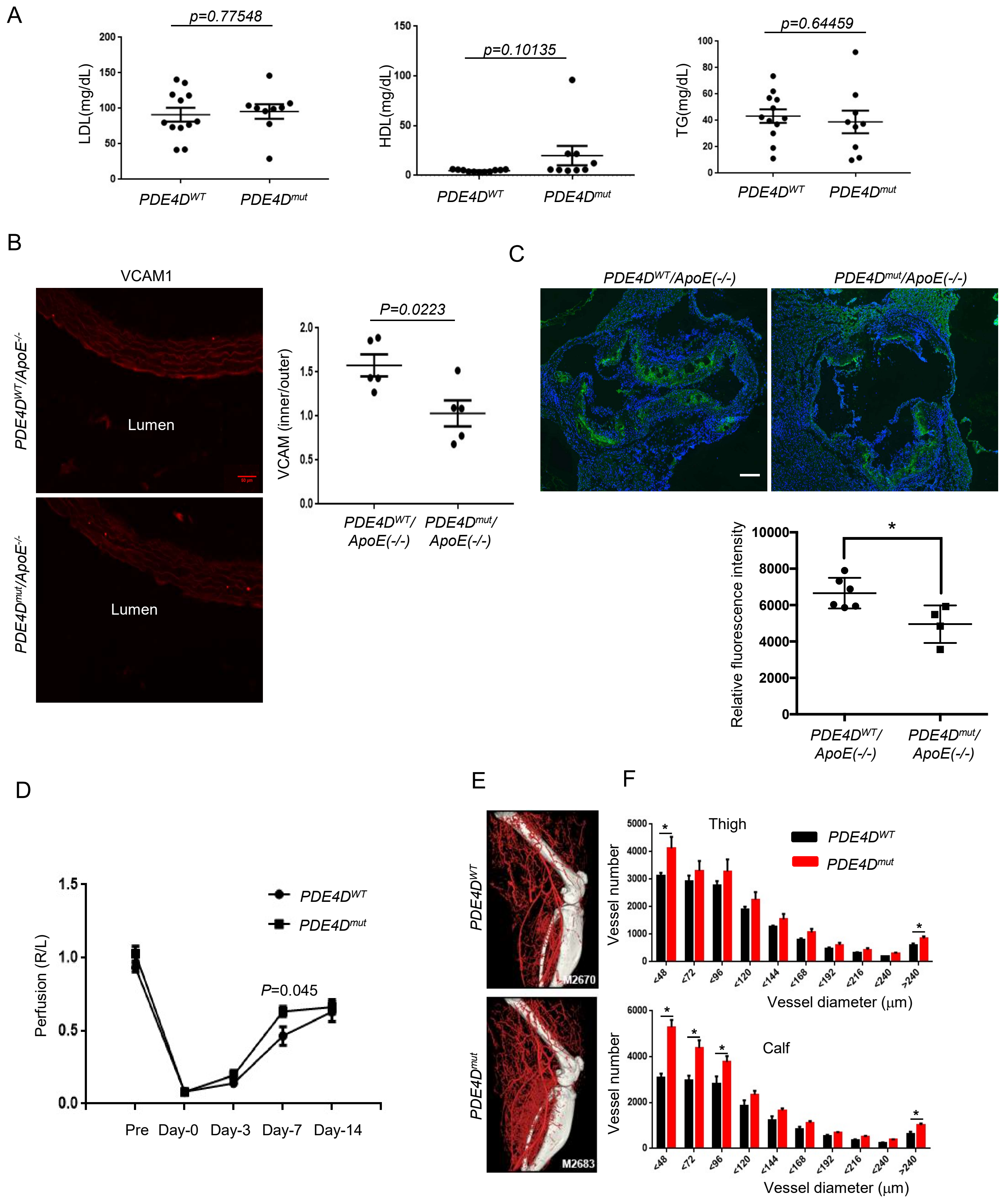


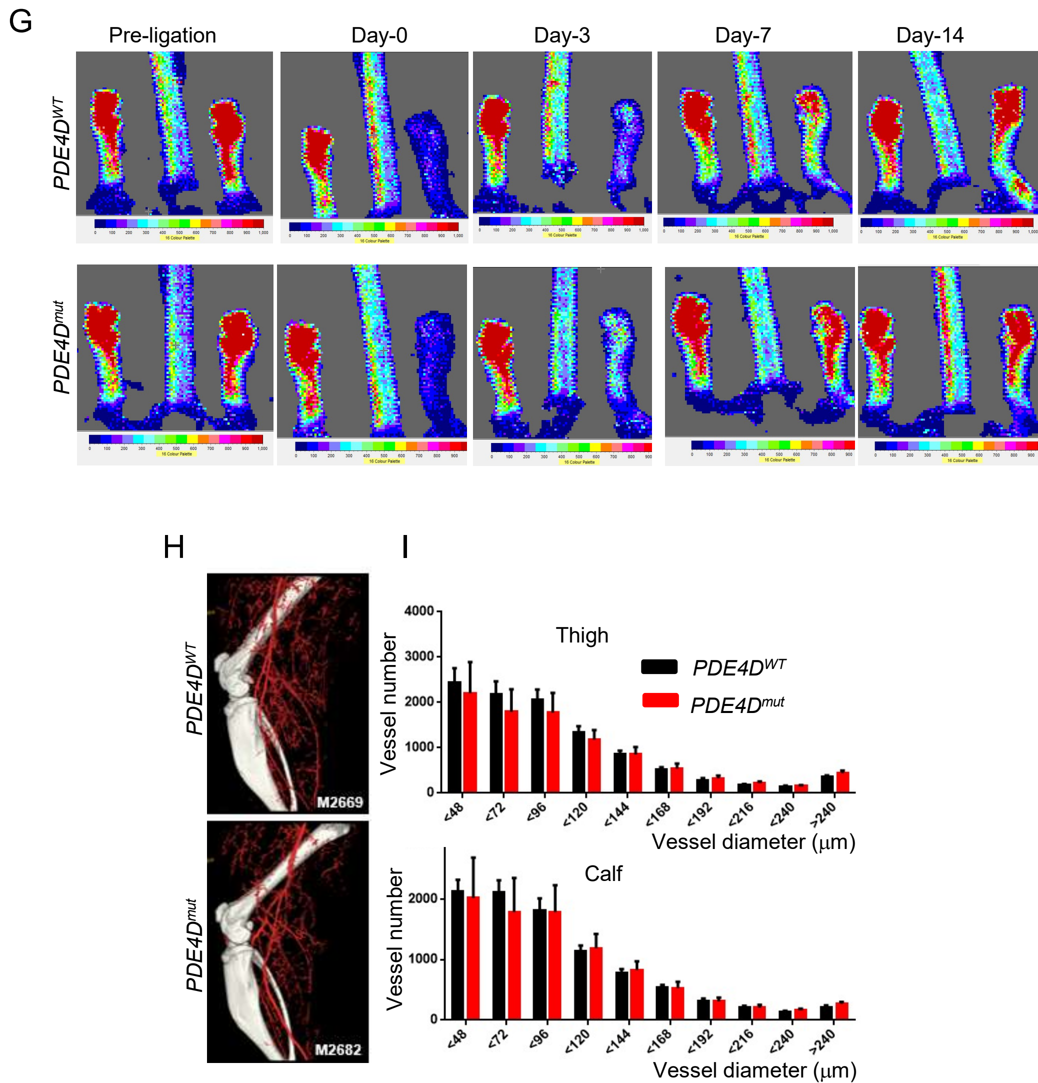
1. Generation of PDE4D^{mut} mice

A. Genotype was screened by PCR using primers that discriminate between wild type and mutant alleles. **B**. Germ-line transmission. The mutant allele was successfully inherited in F1 and F2 offspring, including homozygous F2 pups. **C.** PDE4D5 protein levels. Endothelial cells from each strain were lysed and immunoblotted for PDE4D5.

Supplemental Fig. 1



Supplemental Fig. 2



2. Characterization of *PDE4D*^{mut} mice

A. Serum lipid profiles of *PDE4D*^{mut}*; ApoE*$^{-/-}$ mice on high fat diets (n=9-12). **B**. Longitudinal sections of aortas from *WT* or *PDE4D*^{mut} *ApoE null* mice after 4 months on a high fat diet were stained for VCAM1. Images show the inner curvature of the aortic arch. **C**. MMP activity within the plaque was measured using in situ zymography. Scale bar: 200 μm. **D**. Blood flow recovery. Femoral artery ligation was performed on the right leg of three month old wild type or mutant mice and blood flow measured by laser Doppler at each time; values are means ±SEM, n=6. *p<0.05 by two-tailed t-test. **E, F**. MicroCT of ischemic legs 14 days after surgery. Results were quantified according to vessel diameter. * p< 0.05 in two-tailed t-tests. **G**. Representative Doppler images of hindlimb blood flow at the indicated times after femoral artery ligation. **H, I**. Vessel density in the control leg 14 days after surgery by MicroCT. Results were quantified according to vessel diameter. *p<0.05 by two-tailed t-test

Supplemental Fig. 2

LCMS Peptides

Protein ID 2ABA_PIG

Protein Name Serine/threonine-protein phosphatase 2A 55 kDa regulatory subunit B alpha isoform (Fragment) OS=Sus scrofa GN=PPP2R2A PE=2 SV=1

Percent Coverage 22.8

5 peptides identified with score greater than identity score

Score	Expectation	Peptide Sequence	Start	End	M/Z	Ion Mass	Ion Mass(calc)	Delta	ppm	Charge
86.58	1.1E-7	R.VVIFQQEQENK.I	31	41	681.3606	1360.7066	1360.6987	0.008	5.9	2
77.63	6.8E-7	R.SFFSEIISSISDVK.F	258	271	779.9067	1557.7989	1557.7926	0.0062	4	2
70.9	0.0000039	K.NAAQFLLSTNDK.T	85	96	661.3403	1320.666	1320.6674	-0.0014	-1.1	2
51.35	0.00034	R.DITLEASR.E	355	362	452.741	903.4674	903.4661	0.0013	1.4	2
41.53	0.0023	R.INLWHLEITDR.S	179	189	470.5894	1408.7462	1408.7463	-0.0001	-0.1	3

Export options: CSV | Excel

One peptide identified with score between homology score and identity score

Score	Expectation	Peptide Sequence	Start	End	M/Z	Ion Mass	Ion Mass(calc)	Delta	ppm	Charge
15.85	0.41	R.GEYNVYSTFQSHEPEFDYLK.S	48	67	818.3667	2452.0782	2452.0859	-0.0077	-3.1	3

Export options: CSV | Excel

2 peptides identified with score less than homology score

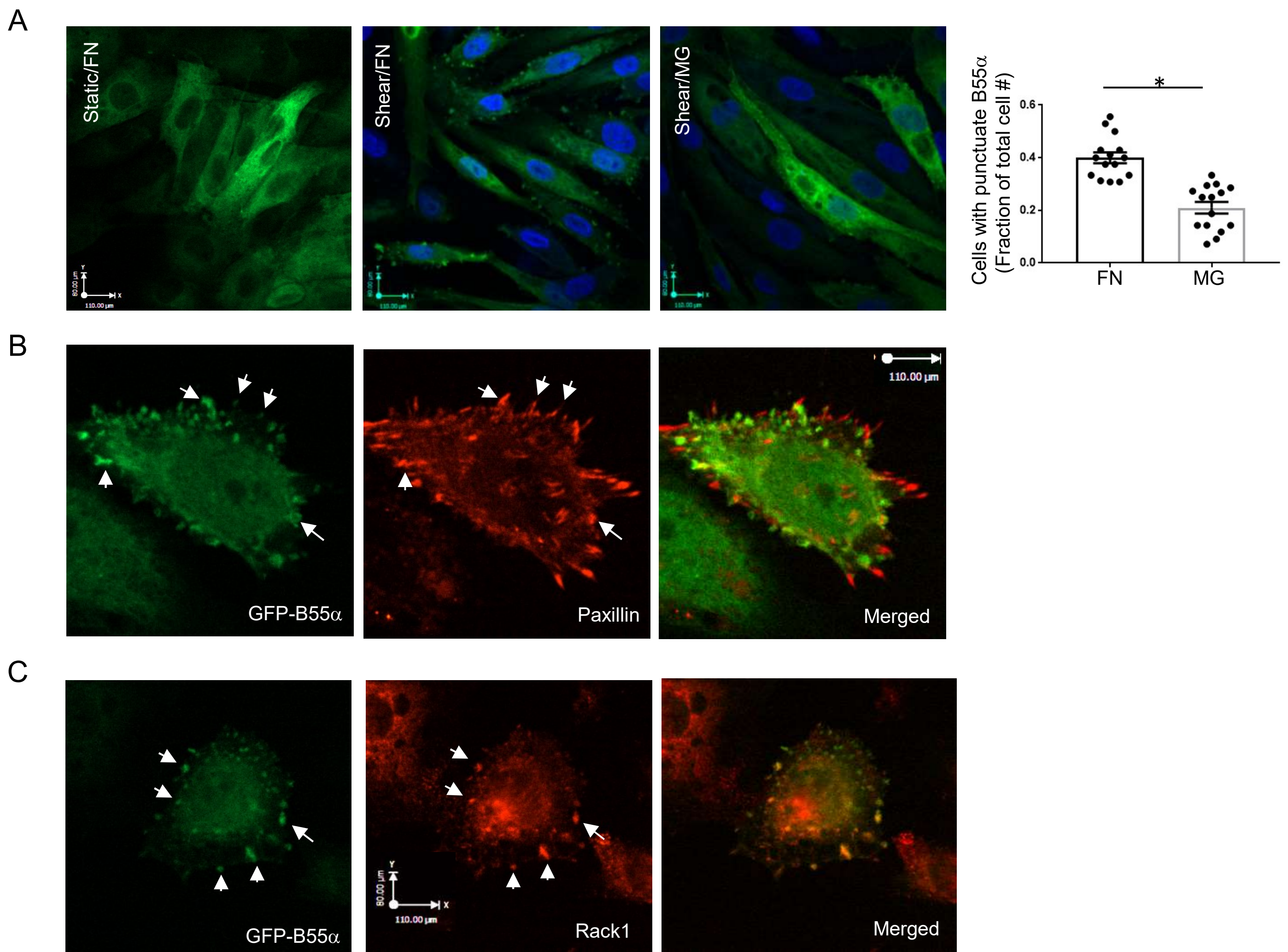
Score	Expectation	Peptide Sequence	Start	End	M/Z	Ion Mass	Ion Mass(calc)	Delta	ppm	Charge
8.63	6.6	R.DKRPEGYNLK.E	107	116	407.2202	1218.6389	1218.6357	0.0032	2.6	3
6.53	6.2	R.PMDLMVEASPR.R	138	148	623.3024	1244.5903	1244.5893	0.001	0.8	2

Export options: CSV | Excel

3. Liquid chromatography-mass spec identification of B55α

The fibronectin-specific 55 kDa band from Fig 3A was submitted for mass analysis. Multiple peptides identify it as the PP2A B55α subunit.

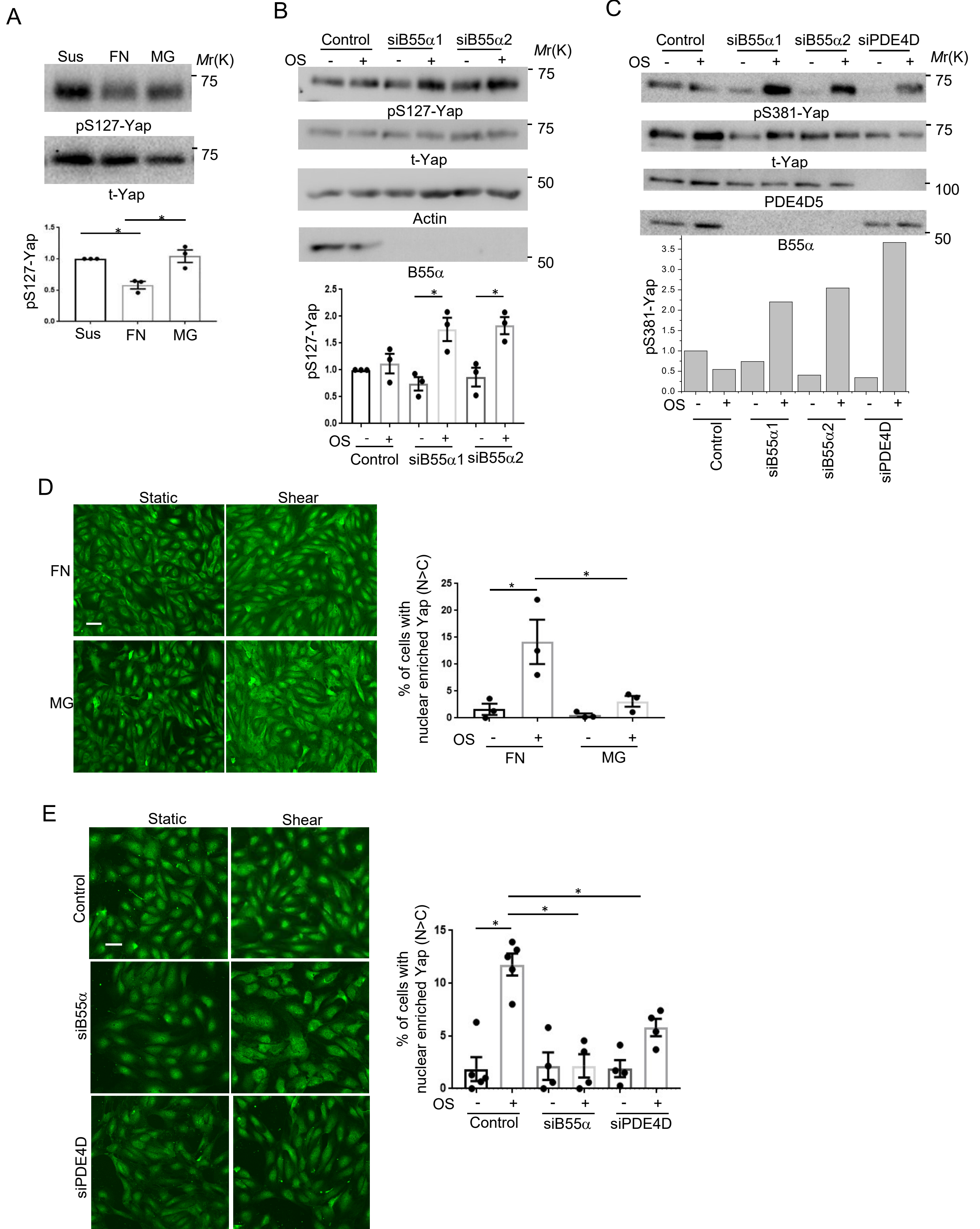
Supplemental Fig. 3



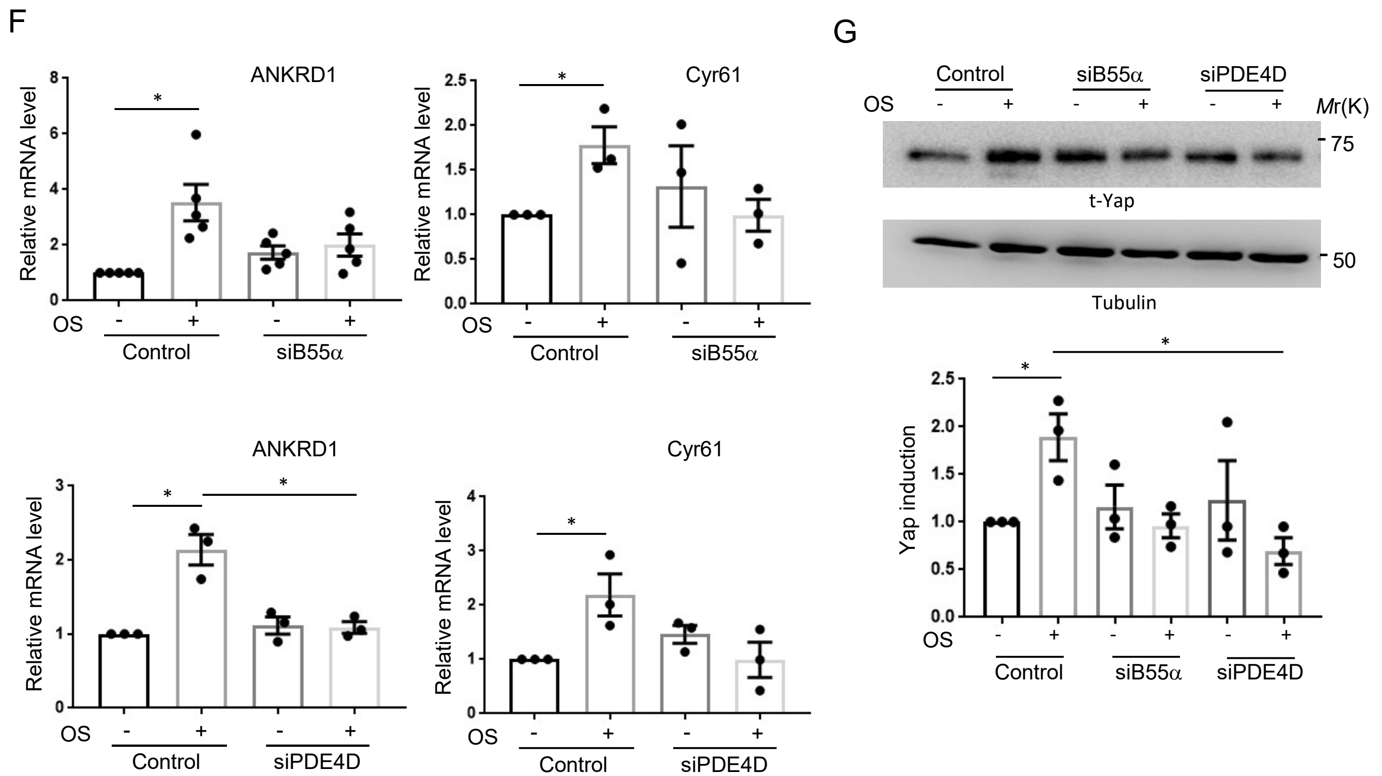
4. ECM-dependent PP2A-B55α localization

A. B55α localization. BAECs expressing GFP-B55α were plated on FN or MG and exposed to laminar shear for 30 min. After fixation, GFP fluorescence was imaged using confocal microscopy. The number of cells with punctuate B55α was compared between FN and MG (n=15 images pooled across three independent experiments). *$p<0.05$ by two-tailed t-test. **B**. BAECs expressing GFP-B55α and RFP-paxillin were plated on FN and subject to shear for 30 min. **C**. BAECs expressing GFP-B55α were plated on FN and exposed to shear for 30 min. After fixation, the cells were stained for RACK1 and imaged.

Supplemental Fig. 4



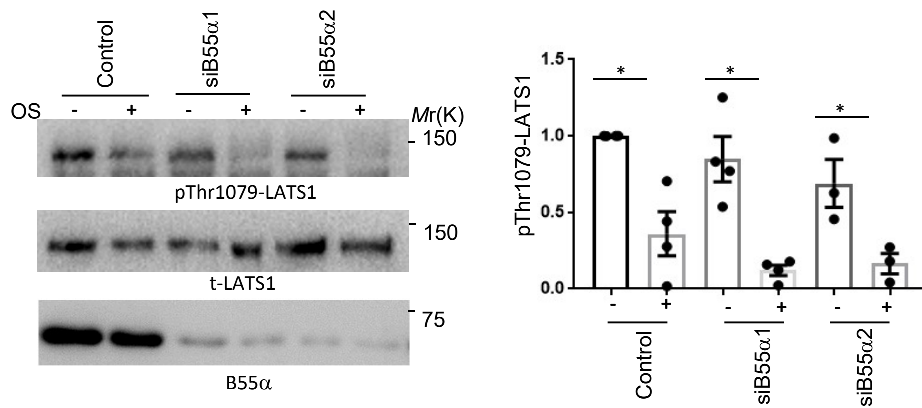
Supplemental Fig. 5



5. Role of PDE4D and B55α on FN-induced Yap activation

A. BAECs were detached and kept in suspension (Sus) or replated on the indicated matrix protein for 1 hour. pS^{127}-Yap was measured by immunoblotting (n=3). **B**. BAECs transfected with B55α siRNA were detached and replated on FN, then exposed to OS for 2 hr. Yap phosphorylation was assayed as in A (n=3). **C**. HUVECs transfected with B55α siRNA or PDE4D siRNA were detached and replated on FN, then sheared for 2 hr. Lysates were analyzed by Western blotting for pS^{381}-Yap. n=3 independent experiments. **D**. HUVECs were replated on FN or MG for 6 hr and exposed to oscillatory shear for 2 hr, fixed and stained for Yap. Cells with nuclear enriched Yap (N>C) were counted from 10 random fields for each condition (n=3 independent experiments). Scale bar: 50 μm. **E**, HUVECs were transfected with B55α siRNA or PDE4D siRNA and replated on FN, were exposed to oscillatory shear for 2 hr and Yap localization assayed as (D) (n=4-5 independent experiments). Scale bar: 50 μm. **F.** BAECs transfected with B55α siRNA or PDE4D siRNA were subject to oscillatory shear for 18 hr. mRNA was isolated and transcript level of Cyr61 and ANKRD1 was measured using qPCR (n=3). **G**. HUVECs transfected with B55α siRNA or PDE4D siRNA were replated on FN and subjected to oscillatory shear for 18 hr. The cells were lysed and probed for Yap expression (n=3). *$p<0.05$ by two-tailed t-test (B, C, F) or one way ANOVA (A, D, E, G).

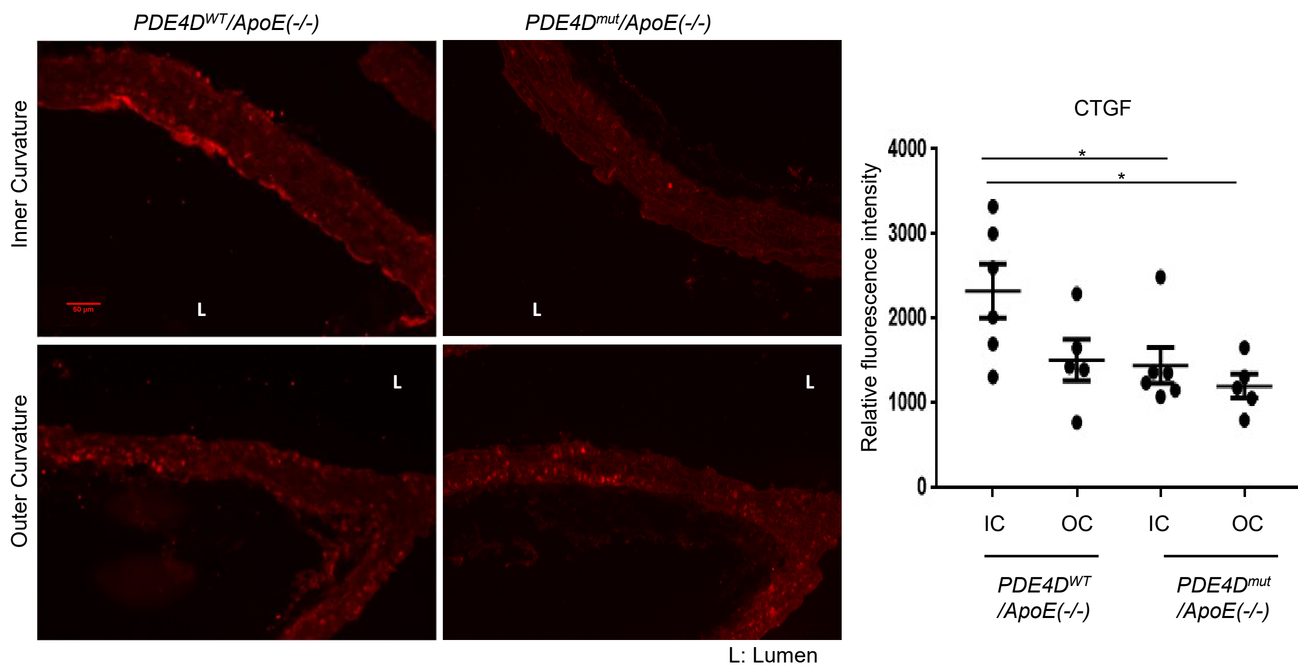
Supplemental Fig. 5



6. B55α regulation of the Hippo pathway

BAECs transfected with B55α siRNAs were plated on FN and subjected to oscillatory shear for 2 hr. Flow-dependent LATS1 phosphorylation was measured using pT^{1079}-LATS1 antibody (n=4). *$p<0.05$ by two-tailed t-test.

Supplemental Fig. 6



7. Effect of PDE4D mutation on Yap target gene expression

Aortas from *PDE4D^WT/ApoE null* mice or *PDE4D^mut/ApoE* on normal chow were fixed, longitudinally sectioned and stained for Yap target gene, CTGF. Mean intensity in the endothelial layer of the inner curvature (IC) and outer curvature (OC) was quantified (arbitrary units). *$p<0.05$ by one way ANOVA with Dunnett's multiple comparisons test.

Supplemental Fig. 7