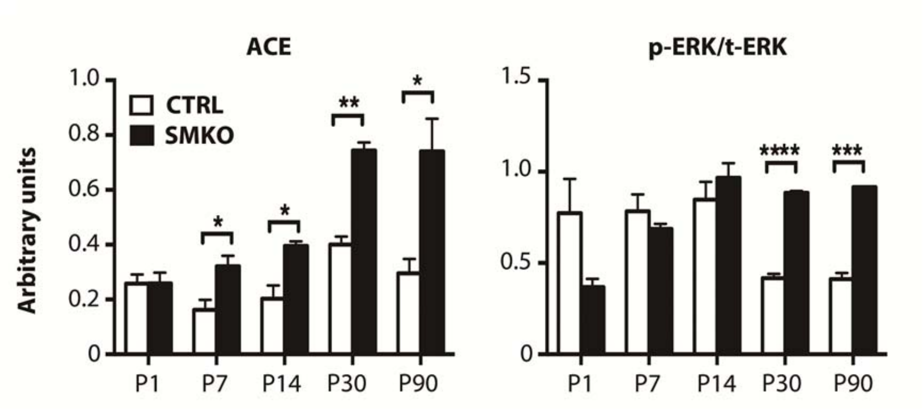
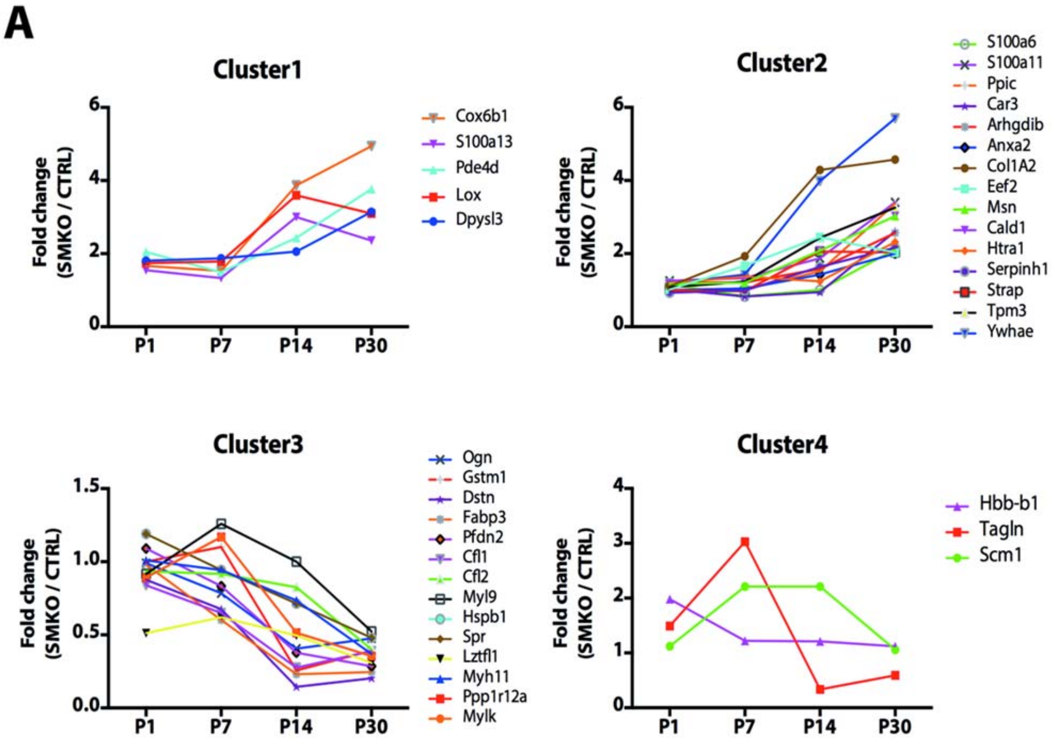


Fig. S1. ACE abundance and ERK phosphorylation in *Fbln4*^{SMKO} aortas after P7. Fig. 1A was performed three times with different pools of animals. The numbers of animals used per genotype in each experiment are: pooled P1, n=5-12; P7, n=5-15; P14, n=5-12; P30, n=3-7; P90, n=2-6. Quantification of proteins normalized to GAPDH is shown for ACE. Bars are means ± SEM. **P* < 0.05, ***P* < 0.01, ****P*<0.001, *****P* < 0.0001, unpaired *t*-test.



B

	Function	Score	Number	*P*-value	Protein Symbol
1	Actin cytoskeleton	6.58	14	2.80E-07	Cald1, Cfl1, Cfl2, Dstn, Msn, Myh11, Mylk, Tpm3 Anxa2, Arhgdib, Pde4d, Tagln, Ppp1r12a, Ywhae
2	ECM organization	3.02	7	8.20E-04	Col1a2, Serpinh1, Lox, Anxa2, Ctgf, Myh11, Ogn
3	Calcium ion binding	1.82	6	1.40E-02	S100a13, S100a6, S100a11, Mylk, Myl9
4	Metabolic process and oxidase activity	0.81	6	6.80E-02	Car3, Hbb-b1, Spr, Fabp3, Cox6b1, Lox
5	TGFβR signaling	0.49	2	3.00E-01	Htra1, Strap

Fig. S2. Ontology analysis and proteins that showed changes in abundance during aneurysm development. (A) The line graphs showing the fold-expression of proteins (SMKO/CTRL) in each cluster identified by proteomics screening (also see Fig. 1D). **(B)** Classification of identified proteins according to their biological functions using DAVID tools. Substantial numbers of genes are involved in actin cytoskeleton organization.

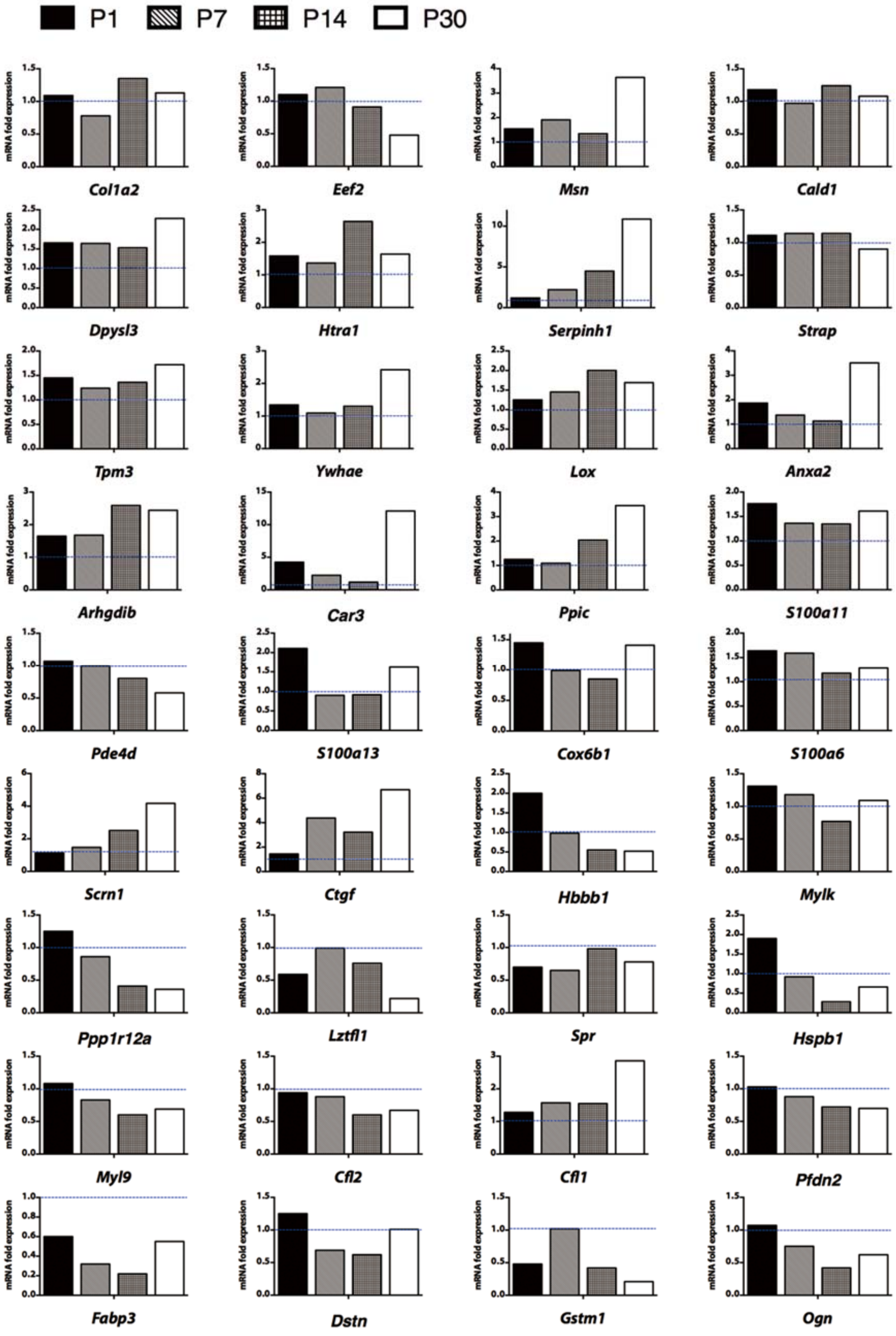


Fig. S3. qPCR analysis of genes encoding the proteins identified by 2D-DIGE.

mRNA were isolated from pooled ascending aortas from CTRL mice (P1, n=12, P7, n=18; P14, n=9; P30, n=12) and SMKO mice (P1, n=12; P7, n=18; P14, n=8; P30, n=11). Blue dotted line shows 1.0 fold expression (SMKO/CTRL).

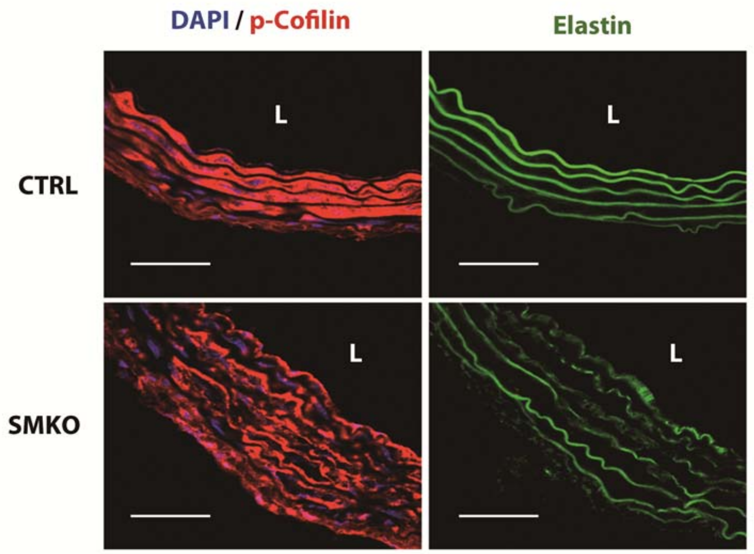


Fig. S4. Phosphorylation of cofilin in *Fbln4*^{SMKO} descending aortas.

Cross sections of the descending aorta from CTRL and SMKO mice (n=3 mice per genotype) at P30 immunostained with phosphorylated (p)-cofilin (red) and DAPI (blue). Elastic lamellae are green (autofluorescence). Bars are 50 □m. L: lumen.

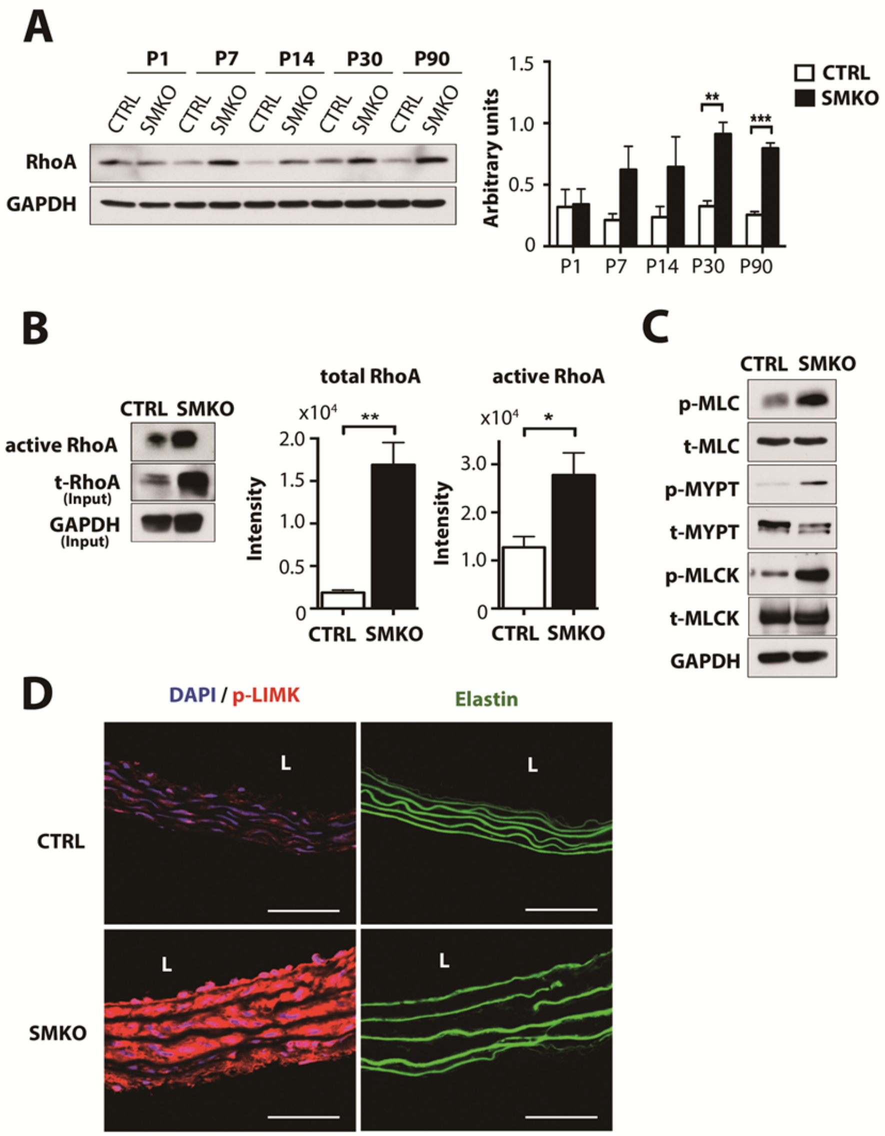


Fig. S5. Activated RhoA signaling in *Fbln4*^{SMKO} ascending aortas. (A) Representative Western blots showing RhoA abundance in ascending aortas from CTRL and SMKO at the indicated post-developmental time points. The experimente were performed three times with different pools of animals with similar results. N values as in fig. S1. Quantification of proteins, normalized to GAPDH is shown in the graphs. Bars are means ± SEM. $**P < 0.01$, $***P < 0.001$, unpaired *t*-test. **(B)** RhoA activation assays using aortas at P30 (n=3 mice per genotype). Quantification is shown in the graphs (right). Bars are means ± SEM. $*P < 0.05$, $**P < 0.01$, unpaired *t*-test. **(C)** Phosphorylated MLC, MLCK, and MYPT are increased in SMKO ascending aortas at P30 (n=3 mice per genotype). **(D)** Cross sections of the ascending aorta from CTRL and SMKO mice at P30 immunostained with p-LIMK (red) and DAPI (blue). Elastic lamellae are green (autofluorescence). Images are representative of 3 mice per genotype. Bars are 50 □m. L: lumen.

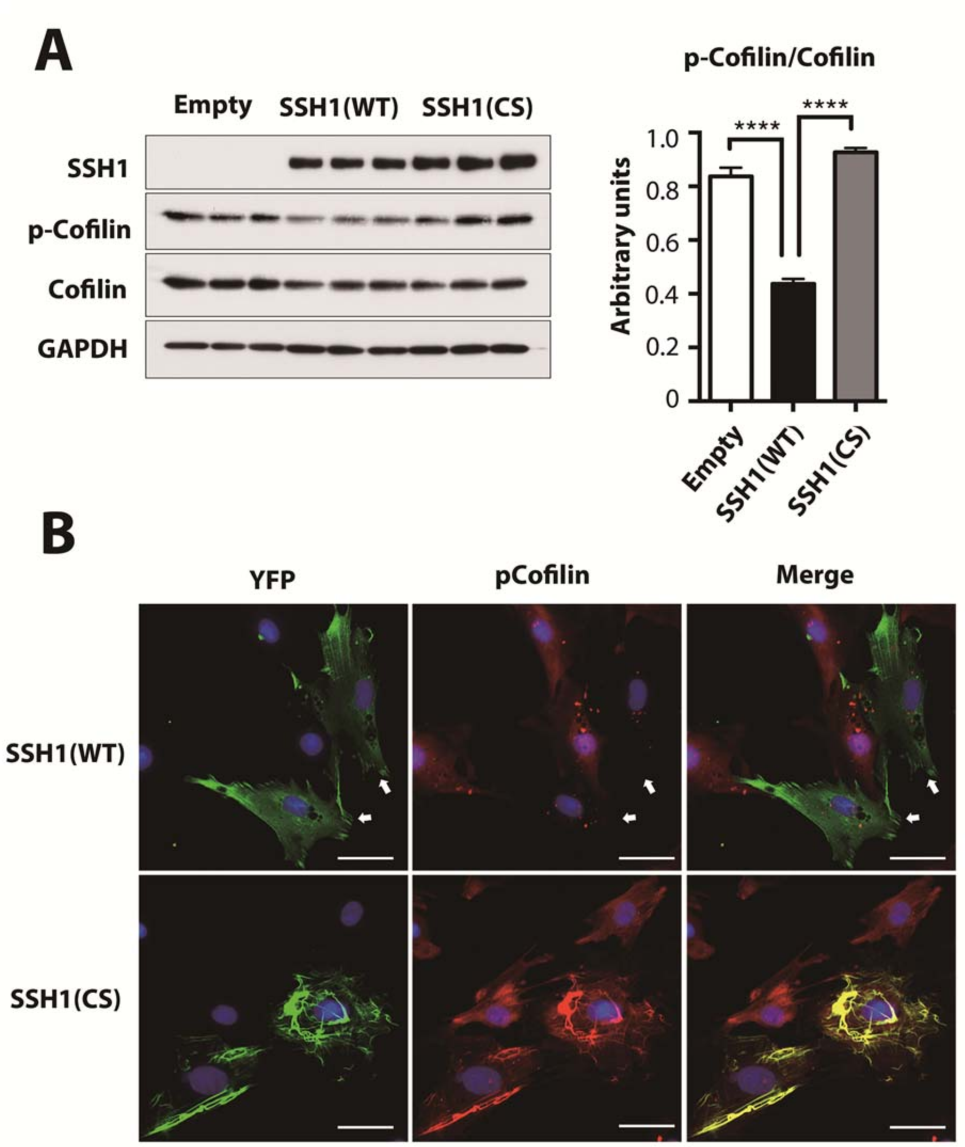


Fig. S6. Dephosphorylation of cofilin by SSH1 in rat vascular SMCs. (A) Western blots showing SSH1, phosphorylated (p)-cofilin and total cofilin abundance in extracts of cells transfected with empty vector (Empty), SSH1 (WT) or SSH1 (CS mutant). n=3 independent experiments. ****$P < 0.0001$, one-way ANOVA. **(B)** Immunostaining with DAPI (blue), YFP (SSH1 or SSH1CS mutant) and p-cofilin (red). Arrows indicate YFP positive SSH1-expressing cells with absence of p-cofilin (upper panel). Bars are 40 µm. n=3 independent experiments.

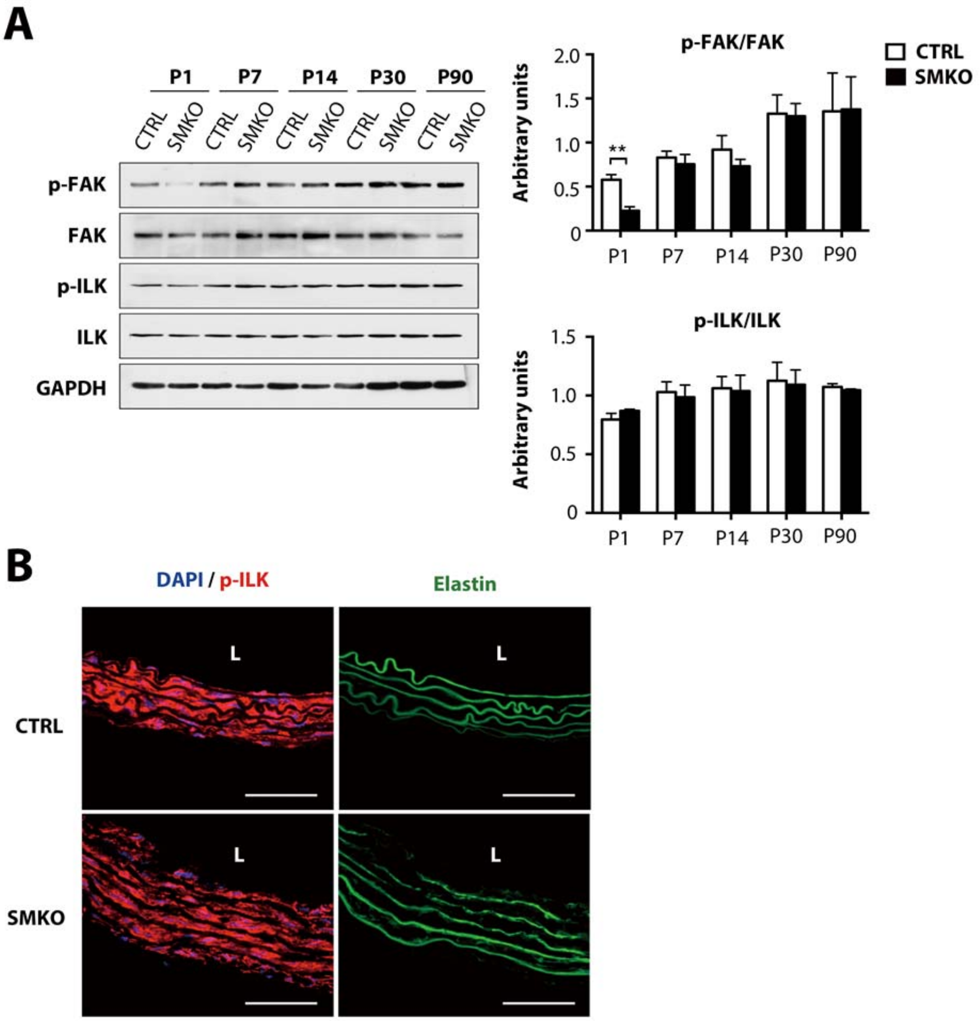


Fig. S7. Phosphorylation of FAK and ILK in ascending aortas. (A) Representative Western blots showing p-FAK, FAK, p-ILK and ILK amounts in ascending aortas from CTRL and SMKO mice at the indicated post-developmental time points. The experiment was repeated three times with different sets of animals with similar results. Quantification of proteins, normalized to GAPDH is shown in the graphs. N values as in fig. S1. Bars are means ± SEM. $^{**}P < 0.01$, unpaired *t*-test. **(B)** Representative images of cross sections of the P30 ascending aorta from CTRL (n=4) and SMKO (n=5) mice immunostained with p-ILK (red) and DAPI (blue). Elastic lamellae are green (autofluorescence). Bars are 50 μm.

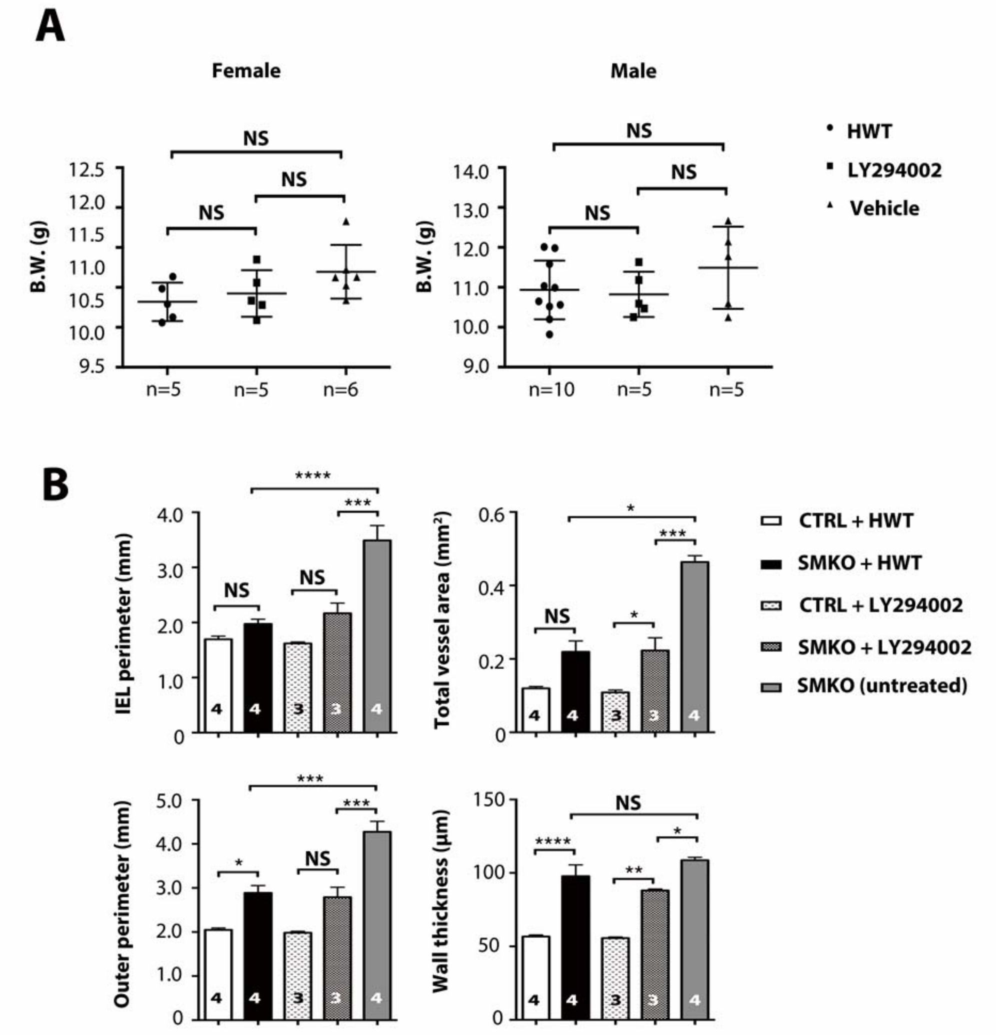


Fig. S8. The effects of PI3K inhibitors on general growth and aneurysm development. (A) Comparison of body weight (B.W.) between vehicle-, Wortmannin (HWT)-, or LY294002-treated SMKO at P30. Each point indicates a different animal. NS, not significant, one-way ANOVA. **(B)** Morphometric analysis of cross sections of the ascending aorta from HWT-treated CTRL, SMKO, LY294002-treated CTRL, SMKO and untreated SMKO. IEL perimeter, outer perimeter, total vessel area and wall thickness are shown. Bars are means ± SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$. Kruskal-Wallis tests. Number of animals is indicated on each bar.

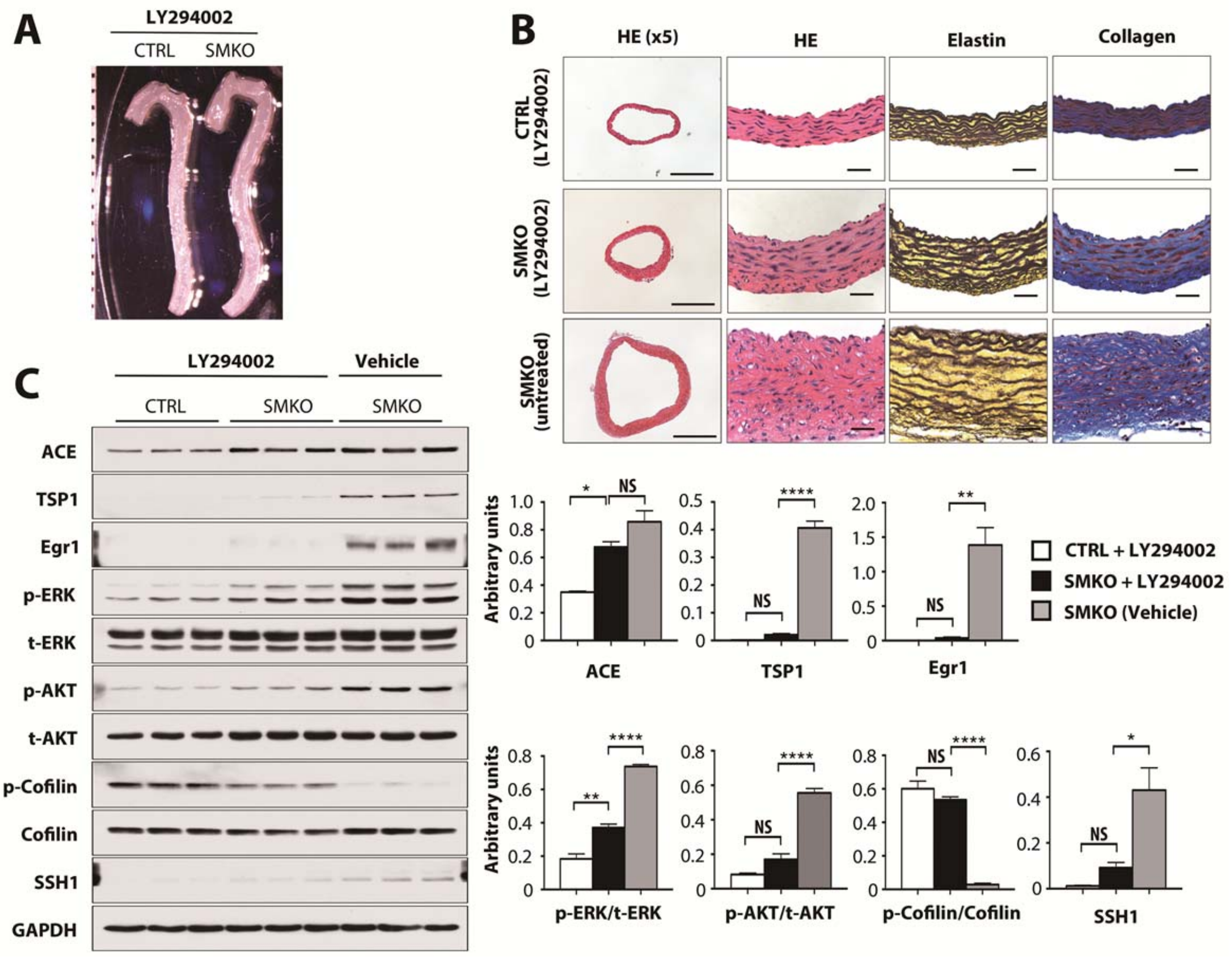


Fig. S9. The effect of LY294002 on aneurysm development in *Fbln4*^{SMKO} mice. (A) Gross photos of CTRL and SMKO aortas treated with LY294002. Images are representative of 10 mice per genotype. **(B)** Histological images of cross sections of ascending aortas from LY294002-treated CTRL, SMKO and untreated-SMKO mice (n=3 per genotype) stained with hematoxylin and eosin (HE), Hart's (elastin) and Masson-Trichrome (collagen). Scale bars are 500 μm (x5) and 20 μm. **(C)** The effect of LY294002 treatment from P7 to P30 in SMKO aortas, analyzed immunoblotting with antibodies as indicated. N=6 per genotype and treatment. Quantification of proteins, normalized to GAPDH is shown in the graph. Bars are means ± SEM. *$P < 0.05$, ** $P < 0.01$, ****$P < 0.0001$, one-way ANOVA.

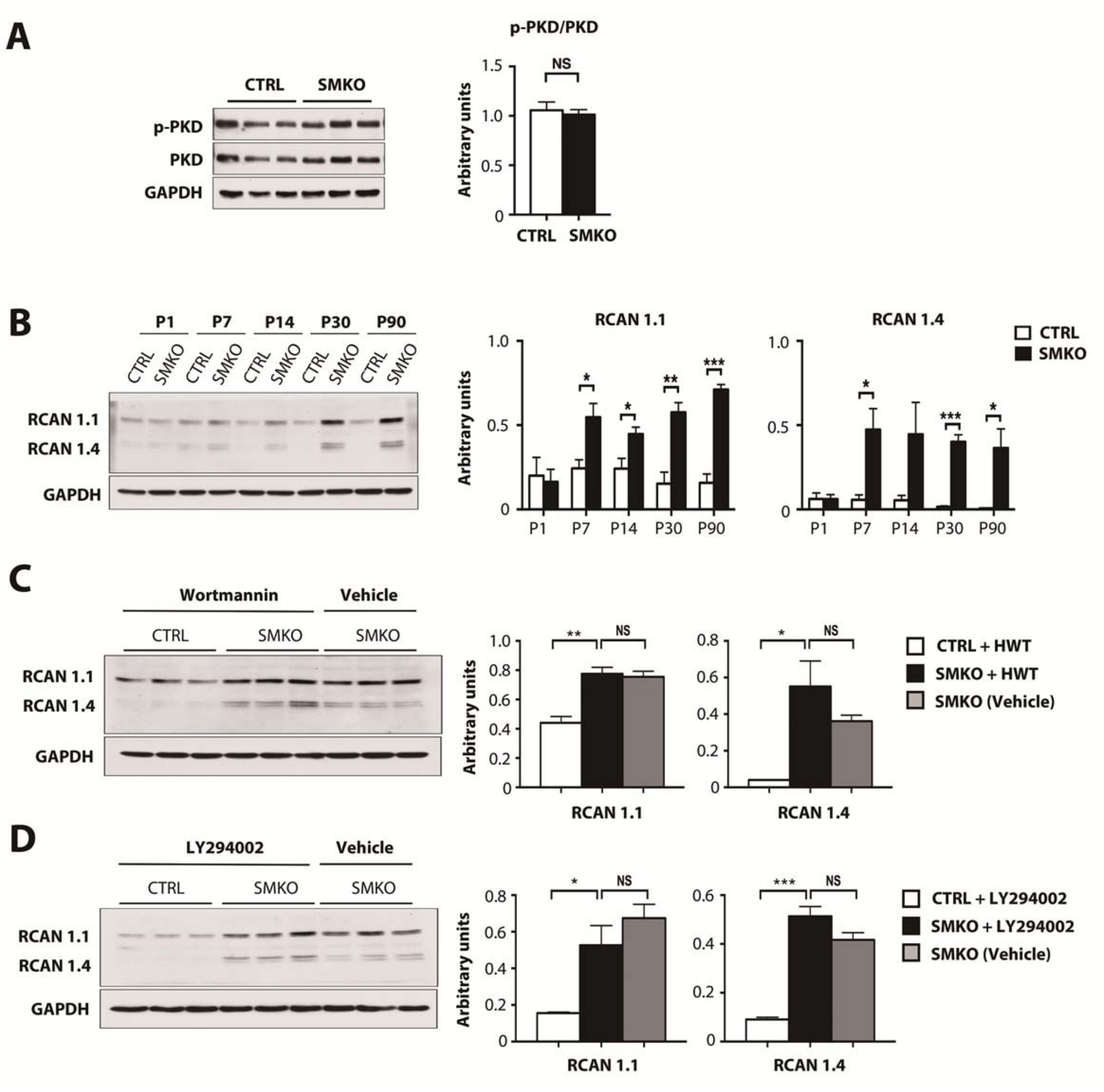


Fig. S10. Evaluation of PKD phosphorylation and calcineurin activity in ascending aortas. **(A)** PKD phosphorylation is unchanged between CTRL and SMKO mice at P30. N values as in fig. S5C. Unpaired *t*-test. **(B-D)** Western blots showing calcineurin activity (as assessed by the abundance of the inhibitor RCAN1.4) in the aortas from P1 to P90 in CTRL and SMKO mice (B) and after treatment with Wortmannin (HWT; C) or LY294002 (D). N values as in fig. S1 (B), Fig. 6C (C), and fig. S9C (D). Quantification of proteins, normalized to GAPDH is shown in the graphs. Bars are means ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001, unpaired *t*-test (B), one-way ANOVA (C, D).

Table S1. Antibodies used in this study.

Western blot analysis

Antibody	**Dilution**	**Source**	**Catalog Number**
GAPDH	1:3000	Cell Signaling	#2118L
ACE	1:1000	Santa Cruz Biotechnology	sc-20791
p-MEK1/2	1:500	Cell Signaling	#9154
p-ERK1/2	1:500	Cell Signaling	#4376S
t-ERK	1:1000	Cell Signaling	#9102S
p-Cofilin (Ser3)	1:500	Cell Signaling	#3313S
t-Cofilin	1:1000	Cell Signaling	#5175S
SSH1	1:500	ECM Biosciences	#SP1711
SSH1	1:500	Gifted from Dr. Kensaku Mizuno	
SSH2	1:500	Santa Cruz Biotechnology	sc-160843
SSH3	1:500	Santa Cruz Biotechnology	sc-390058
PKD	1:1000	Cell Signaling	#2052P
p-PKD(Ser744/748)	1:500	Cell Signaling	#2054P
CIN	1:1000	Cell Signaling	#4686S
RhoA	1:1000	Cell Signaling	#2117S
p-MLC 2 (Thr18/Ser19)	1:500	Cell Signaling	#3674S
p-MYPT 1 (Thr696)	1:500	Cell Signaling	#5163P
p-MLCK	1:500	Life technologies	44-1085G
MLCK	1:1000	Sigma	M7905
p-FAK (Y397)	1:500	Cell Signaling	#8556P
t-FAK	1:1000	Cell Signaling	#3285P
ILK	1:1000	Gifted from Dr. Chuanyue Wu	
p-ILK (Ser246)	1:500	Millipore	#AB1076
TSP1	1:1000	NeoMarkers	MS-421-P1
Egr1	1:500	Cell Signaling	#4154S
p-Akt (Thr308)	1:500	Cell Signaling	#4056S
Akt	1:1000	Cell Signaling	#9272S
MLC2	1:1000	Cell Signaling	#3672S

Immunostaining

Antibody	Dilution	Source	Catalog Number
p-Cofilin 1 (hSer3)	1:200	Santa Cruz Biotechnology	sc-12912
Rhodamine Phalloidin	1:500	Cytoskeleton	#PHDR1
p-ILK (Ser246)	1:200	Millipore	#AB1076
p-LIMK1/2	1:200	Santa Cruz Biotechnology	sc-28409-R

Table S2. QPCR primer sequences

Gene	Forward primer 5' -3'	Reverse primer 5' -3'
	For all qPCR data	
B2m	ccgagcccaagaccgtcta	aactggatttgtaattaagcaggttca
Gapdh	tgacgtgccgcctggagaaa	agtgtagcccaagatgcccttcag
	Fig. 1B	
Acta2	atcgtccaccgcaaatgc	aaggaactggaggcgctg
Cnn1	aacttcatggatggcctcaaa	acccggctgcagcttgt
Myh11	tcaacgccaaccgcaggaagctg	tgctaagcagtctgctgggct
Sm22	gcgcctgggcttcca	caggctgttcaccaatttgct
Myocd	caccccacgacatcaaatcc	tgcatcattcttgtcactttctga
Srf	gtctccctctcgtgacagcag	cagttgtgggtacagacgacgt
	Fig. 5B	
Fbln4	gcactgccgggatgtca	gcatttcatctcacccttgca
	Fig. S3	
Col1a1	gagcggagagtactggatcg	gcttcttttccttggggttc
Eef2	gtggtggactgtgtgtctgg	cgctggaaggtctggtagag
Msn	gataagttgcttccccaaagagt	ctcatagatgttgagacccaagg
Cald1	agagcctctaacatttccaatcc	tgcagtcttagtggcttttcttc
Dpysl3	tgccttacaagggaatgacc	ggctctgtacctcctgcttg
Htra1	ttggaagccccttttctctt	atggaattgcgaaggagatg
Serpinh1	aagggagacaagatgcgaga	tctgggctccatgtcctaac
Strap	tcagtcctgatggggaactc	tctggaaatccgatctttgg
Tpm3	gggcctcatgcatgttagtt	gagggcgatgagatgatgtt
Ywhae	gtgattggagccagaagagc	cctgtggtagtcccctttca
Lox	tgccaacacacagaggagag	ccaggtagctggggtttaca
Anxa2	caccaacttcgatgctgaga	caaaatcaccgtctccaggt
Arhgdi2	ggactggcatgagagtggat	aggtgaggtggtcctgtttg
Car3	cttgatgccctggacaaaat	agctcacagtcatgggctct
Ppic	tgattggcctctttggaaac	cctccagtgccatctctagc
S100a11	gcattgagtccctgattgct	atctagctgcccgtcacagt
Pde4d	gaaagccagcctgaaacttg	tcctggcagatgacagtgag
S100a13	ctttcccggtcaatctgtgt	ctagagagcccacgtccttg

Cox6b1	gcgtctttgctaagggtcac	accactcacacacggagaca
S100a6	aagctgcaggatgctgaaat	ctggatttgaccgagagagg
Scrn1	gagtgacgaggagcaaggtc	tagtcgggtgggaagttttg
Ctgf	caaagcagctgcaaatacca	ggccaaatgtgtcttccagt
Hbb-b1	gctggttgtctacccttgga	acgatcatattgcccaggag
Mylk	atatccgaggaagagaggaagg	acttcttcttgccacctagcac
Ppp1r12a	ttgcgtatgtcacccctaca	ttggtgttgatgtggtgctt
Lztfl1	gtttcacgtagggcttgcat	cttcctggccttcaactgag
Spr	tagaggtgggaagagcagga	ccagtgaacaccacaggatg
Hspb1	cctcttccctatcccctgag	caaaagagcgcacagattga
Myl9	atgaggaggtggacgagatg	cacggggagggtagagtgta
Cfl2	atacacctcacctgccttgg	catcgggaaaccaaagacat
Cfl1	cgcaagtcttcaacaccaga	ttgtctggcagcatcttgac
Pfdn2	ggagcggactgtcaaagaag	cttctcatcttcccccatga
Fabp3	catcgagaagaacggggata	tgccatgagtgagagtcagg
Dstn	cagtgcagacaaaaagtgcatag	acttttcagaggtgcttgttctg
Gstm1	tagggtctgctgctctggtt	ctggtgctgtggtcttctca
Ogn	cctggaatctgtgcctccta	agctgcactgatggggttag

Table S3. List of links to raw data for mass spectrometry.

File name	Spot #	Protein	Accession no.
PRJ11-OTV1_C00072_spot1.raw	**1**	Collagen 1 alpha-2 chain	NP_031769
PRJ12-QEX1_C00096_120413164131.raw	**2**	Elongation factor 2	NP_031933
PRJ17-QEX1_C00178.raw	**3**	Moesin	NP_034963
		Caldesmon-1	NP_663550
PRJ12-QEX1_C00103_120414045615.raw	**4**	Dihydropyrimidinase-like 3	NP_001129558
PRJ17-QEX1_C00180.raw	**5**	Serine protease HTRA1	NP_062510
PRJ11-OTV1_C00073_spot7.raw	**6**	Serpin H1	NP_001104514
PRJ17-QEX1_C00183.raw	**7**	Serine-threonine kinase receptor-associated protein	NP_035629
PRJ21-QEX1_C00282.raw	**8**	Tropomyosin 3	NP_071709
PRJ18-QEX1_C00190_Spot12.raw	**9**	14-3-3 protein epsilon	NP_033562
PRJ21-QEX1_C00284.raw	**10**	Protein-lysine6-oxidase	NP_034858
PRJ21-QEX1_C00290.raw	**11**	Annexin A2	NP_031611
PRJ11-OTV1_C00075_spot15.raw	**12**	Rho dissociation inhibitor 2	NP_031512
PRJ11-OTV1_C00076_spot16.raw	**13**	Carbonic anhydrase 3	NP_031632
PRJ8-OTV1_C00049_17_120320080634.raw	**14**	Peptidyl-prolyl cis-trans isomerase C	NP_032934
PRJ17-QEX1_C00174.raw	**15**	Protein S100-A11	NP_058020
PRJ17-QEX1_C00175.raw	**16**	Phosphodiesterase 4D, cAMP-specific (Fragment)	NP_035186
PRJ8-OTV1_C00051_20.raw	**17**	Protein S100-A13	NP_033139
PRJ8-OTV1_C00051_22.raw	**18**	Cytochrome c oxidase subunit 6B1	NP_079904
PRJ8-OTV1_C00051_23.raw	**19**	Protein S100-A6	NP_035443
PRJ18-QEX1_C00194_spot60.raw	**20**	Secernin-1	NP_081544
PRJ11-OTV1_C00080_spot31.raw	**21**	Connective tissue growth factor	NP_034347
PRJ14-QEX1_C00137_spot54B.raw	**22**	Transgelin (SM-22)	NP_035656
PRJ21-QEX1_C00291.raw	**23**	Hemoglobin subunit beta-1	NP_032246
PRJ12-QEX1_C00097_120413182628.raw	**24**	Myosin, light polypeptide kinase	NP_647461
		Protein phosphatase 1 regulatory subunit 12A	NP_082168
PRJ12-QEX1_C00098_120413201126.raw	**25**	Myh11 protein	NP_038635
PRJ11-OTV1_C00080_spot31.raw	**26**	Leucine zipper transcription factor-like 1	NP_201579
PRJ12-QEX1_C00099_120413215624.raw	**27**	Sepiapterin reductase	NP_035597
		Heat shock protein beta-1	NP_038588
PRJ12-QEX1_C00100_120413234123.raw	**28**	Myosin regulatory light polypeptide 9	NP_742116
PRJ14-QEX1_C00130_spot36.raw	**29**	Cofilin-2	NP_031714

PRJ14-QEX1_C00131_spot37.raw	**30**	Cofilin-1	NP_031713
PRJ14-QEX1_C00132_spot38.raw	**31**	Prefoldin subunit 2	NP_035200
PRJ17-QEX1_C00179.raw	**32**	Fatty acid-binding protein, heart	NP_034304
PRJ8-OTV1_C00051_41.raw	**33**	Destrin	NP_062745
PRJ8-OTV1_C00051_42.raw	**34**	Glutathione S-transferase Mu	NP_034488
PRJ11-OTV1_C00077_spot44.raw	**35**	Mimecan (Osteoglycin)	NP_032786
MassIVE ID: MSV000079171 http://massive.ucsd.edu/ProteoSAFe/status.jsp?task=53d969d6ef0840c69e30261ee6220238			