

## Supplementary materials

### **Vimentin knock-out results in increased expression of sub-endothelial basement membrane components and carotid stiffness in mice**

Benoit LANGLOIS<sup>1</sup>¶, Ekaterina BELOZERTSEVA<sup>1</sup>¶, Ara PARLAKIAN<sup>2</sup>, Mustapha BOURHIM<sup>1</sup>, Jacqueline GAO-LI<sup>2</sup>, Jocelyne BLANC<sup>2</sup>, Lei TIAN<sup>2</sup>, Dario COLETTI<sup>2</sup>, Carlos LABAT<sup>1</sup>, Zhor RAMDAME-CHERIF<sup>1</sup>, Pascal CHALLANDE<sup>3</sup>, Véronique REGNAULT<sup>1</sup>, Patrick LACOLLEY<sup>1</sup>, Zhenlin LI<sup>2\*</sup>

<sup>1</sup> Inserm, UMR\_S 1116, Université de Lorraine, Nancy, France.

<sup>2</sup> Sorbonne Universités, UPMC Univ Paris 06, CNRS, UMR 8256, INSERM ERL U1164, Institut Biologie Paris-Seine, Paris, France

<sup>3</sup> Sorbonne Universités, UPMC Univ Paris 06, CNRS, UMR 7190, Institut Jean Le Rond d'Alembert, Paris, France

¶ Equal contribution.

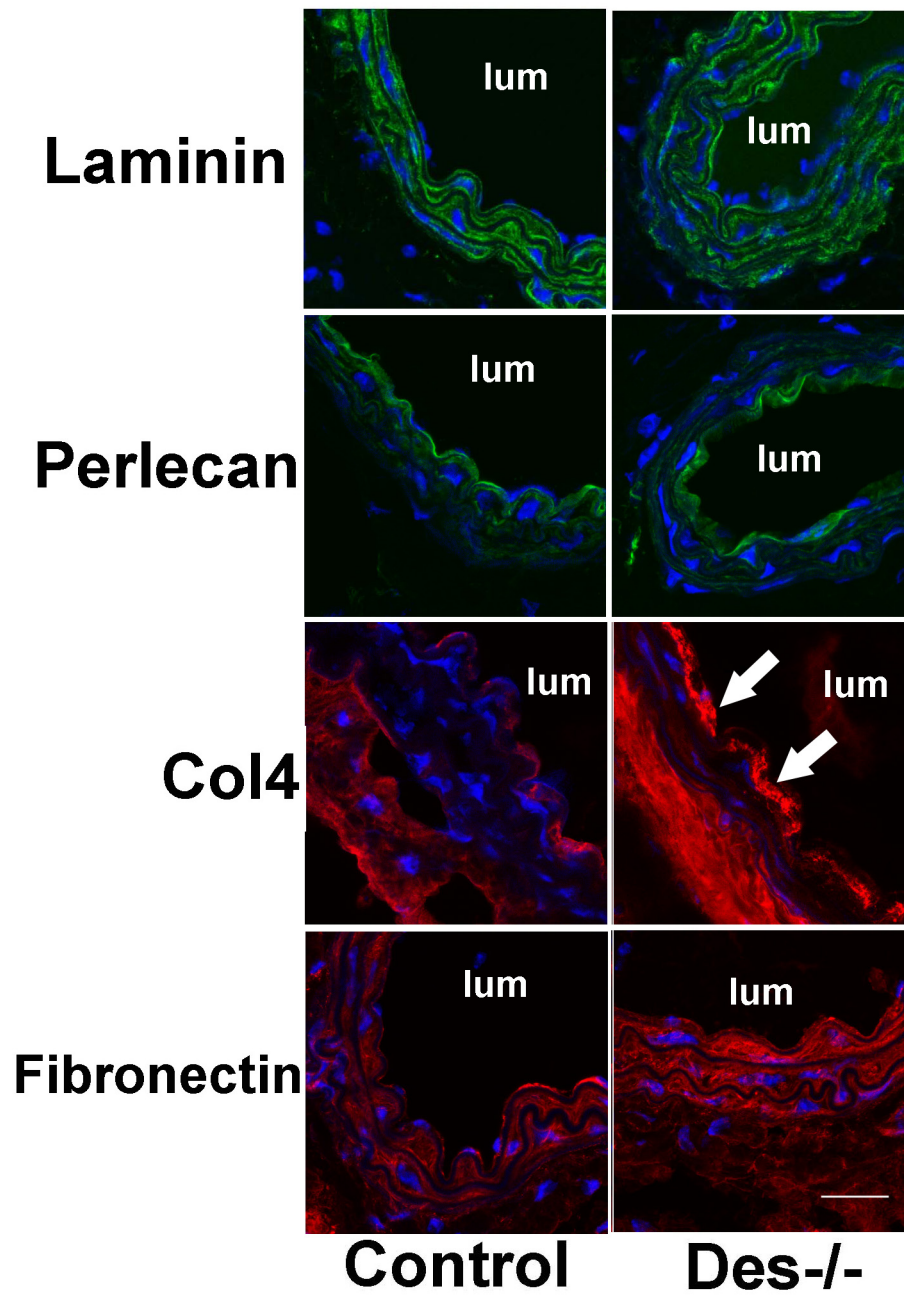
**Supplementary Table S1. Antibodies used for immunoblotting and immunofluorescence**

<b>Antibody</b>	<b>Source</b>	<b>Dilution</b>	<b>Society</b>
<b>Western Blot</b>			
Vim	Guinea pig	1/1000	Progen
Fibronectin	Rabbit	1/500	Sigma-Aldrich
Laminin	Rabbit	1/2000	Sigma-Aldrich
SM-MHC	Rabbit	1/1000	Abcam
Smoothelin	Rabbit	1/250	Santa Cruz
$\alpha_v$ integrin	Rabbit	1/1000	Abcam
talin	Mouse	1/1000	Sigma-Aldrich
vinculin	Mouse	1/1000	Sigma-Aldrich
pFAK (Tyr <sup>397</sup> )	Rabbit	1/1000	Cell Signaling
FAK	Rabbit	1/1000	Cell Signaling
pSrc(Tyr <sup>529</sup> )	Rabbit	1/1000	Biosource
Src	Rabbit	1/1000	Cell Signaling
pERK1/2(Thr <sup>202</sup> /Tyr <sup>204</sup> )	Rabbit	1/1000	Cell Signaling
ERK1/2	Rabbit	1/1000	Cell Signaling
Cofilin	Rabbit	1/1000	Cell Signaling
RhoA	Rabbit	1/1000	Cell Signaling
My19	Rabbit	1/1000	Cell Signaling
CPI-17	Rabbit	1/1000	Abcam
Calponin	Rabbit	1/1000	Milipore
MYPT1	Rabbit	1/1000	Cell Signaling
Caldesmon	Rabbit	1/250	Santa Cruz
GAPDH	Mouse	1/3000	Sigma-Aldrich
<b>Immunofluorescence</b>			
Fibronectin	Rabbit	1/100	Abcam
Vinculin	Mouse	1/100	Sigma-Aldrich
SM-MHC	Rabbit	1/300	Cell Signaling
Laminin	Rabbit	1/200	Sigma-Aldrich
$\alpha_v$ integrin	Rabbit	1/300	Abcam
Perlecan	Rat	1/200	Millipore
Collagen IV	Mouse	1/100	Dako
$\alpha$ -SM actin	mouse	1/100	Sigma-Aldrich
Smoothelin	Rabbit	1/100	Santa Cruz
Vimentin	Guinea pig	1/100	Progen
Synemin	Rabbit	1/500	Labo-made
CD31	Rat	1/100	BD Bioscience
VWF	Rabbit	1/300	Dako
VE-cadherin	Rabbit	1/100	BD Bioscience

**Supplementary Table S2. Primer sequences**

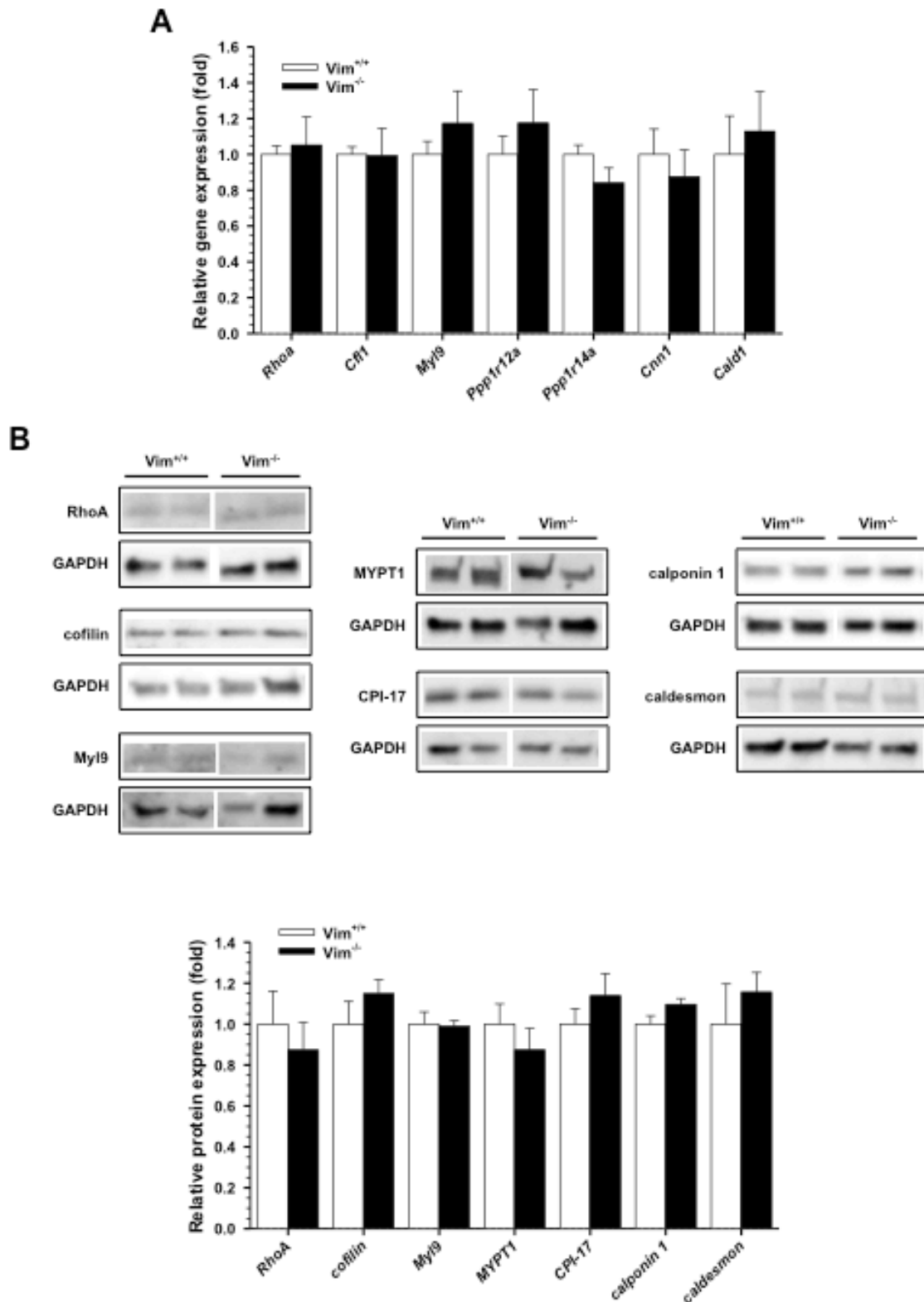
Gene family	Gene	Sense primer (5'-3')	Anti-sense primer (5'-3')
Extracellular matrix proteins	<i>Coll1a1</i>	actggtacatcagcccgaac	tactcgaacgggaatccatc
	<i>Col3a1</i>	accaaaggatgatgctggac	gacctcgtgctccagtttagc
	<i>Col8a1</i>	gaatgctggaatcaaagga	ggattcccataggtccgatt
	<i>Eln</i>	gacttctgggaacgtttgga	agctttataagccgcagcag
	<i>Fn1</i>	aagggtcgggaagaggtgt	ccgtgtaagggtcaaagcat
	<i>Lama2</i>	ccaagaaggaggctgcatag	ccagggttgggaagacact
	<i>Lama4</i>	gcctcctgcctgatgtagag	gacaagatgggttcttgggt
	<i>Lamb1</i>	gttcgagggaaactgctctg	gttcaggcctttggtgtgt
SMC markers	<i>Acta2</i>	tgtgctggactctggagatg	gaaggaaatagccacgctcag
	<i>Myh11</i>	ggcttcatttcttctcca	cgagcgtccatttcttctc
	<i>Smtn</i>	tctcaacagcgagaagctga	tggtaactctcagacatca
	<i>Cald1</i>	ccaagcctctgacttgagg	tgggttcttctccgattg
	<i>Cf12</i>	gccaagtgcatttgatctt	gggaccagtgaaaagggaat
SMC regulatory Proteins	<i>Cnn1</i>	ggccaagacaaaaggaaaca	ccatctgcagtccaatgatg
	<i>Ppp1r12a</i>	ccgcaaaggctatacagaa	tccactaaaatccgacatgct
	<i>Ppp1r14a</i>	gggacttctggaggcttgt	ctcaggctggggtcatctg
	<i>Myl9</i>	accgcaacagcgccgaggac	caaacatggcgaagacattg
	<i>Rhoa</i>	tccgctcggtttctctccatag	cttctcagatgcaaggctca
Focal adhesion proteins	<i>Itgav</i>	gggacagggagaaaggagtc	gattccacagcccaaagtgt
	<i>Itgb8</i>	gaggttttgatgccatgctt	tgccaatttctgtcaagag
	<i>Tln1</i>	gagaatgagacgggtggtgt	tccgaagcatctcttctctg
	<i>Vcl</i>	aggccttcttctggtgatt	aagaaatagggggagcctga
	<i>Ptk2</i>	gcgatcctattgggagatga	ttttggccttgacagaatcc
	<i>Tjp1</i>	agcaagccttctgcacatct	cagcatcagtttctgggtttt
	<i>Cldn1</i>	tggaagatgatgaggtgcag	ccactaatgtcgcagacct
	<i>F11r</i>	tatgatcctgggctctttgg	gggagaggagaagccagagt
	<i>Cdh1</i>	agccattgccaagtacatcc	aaagaccggctgggtaact
	<i>Cdh5</i>	ccatcttctctgcatctc	caactgctcgtgaatctcca
	<i>Kdr</i>	agagttggtggagcatttgg	taggcagggagagtccagaa
	<i>Ednrb</i>	caggaagaagagcggatgct	gaggaccaggcagaagactg
	<i>Tek</i>	gatcttacacggtgccgatt	ttagattggaaggccacag
	<i>Angpt1</i>	gatcttacacggtgccgatt	ttagattggaaggccacag
	<i>Angpt2</i>	tccaagagctcgggtgctat	agttggggaaggctcagtg
<i>Nr3c2</i>	ctagcacagtggggtccatt	aacgctgtgagcactttct	
Reference gene	<i>Gapdh</i>	aactttggcattgtggaagg	acacattgggggttaggaaca

Supplementary figure S1.



**Supplementary figure S1.** Confocal immunofluorescence images show the increased expression of only collagen IV (red) indicated by arrows, but not laminin (green), perlecan (green) and fibronectin (red) of basement membrane components in Des<sup>-/-</sup> mice compared to control mice. Nuclei are stained in blue. Lum: lumen. Scale bars = 25  $\mu$ m.

Supplementary figure S2.



**Supplementary figure S2.** (A) Relative mRNA levels (Vim<sup>-/-</sup> vs Vim<sup>+/+</sup>) of genes encoding RhoA, Cfl1 (Cofilin), Myl9, Ppp1r12a (MYPT1), Ppp1r14a (CPI-17), Cnn1 (calponin) and Cad11 (caldesmon) in the carotid artery by qRT-PCR. (B) Western blot analysis of RhoA, Cofilin, Myl9, MYPT1, CPI-17, calponin and caldesmon in the carotid artery of Vim<sup>-/-</sup> and Vim<sup>+/+</sup> mice. Results are expressed as means  $\pm$  SEM ( $n \geq 6$  in each group). No difference of expression of these genes between the Vim<sup>-/-</sup> and Vim<sup>+/+</sup> mice at both the mRNA and protein levels.

Western blot used in the figures

Figure 1C

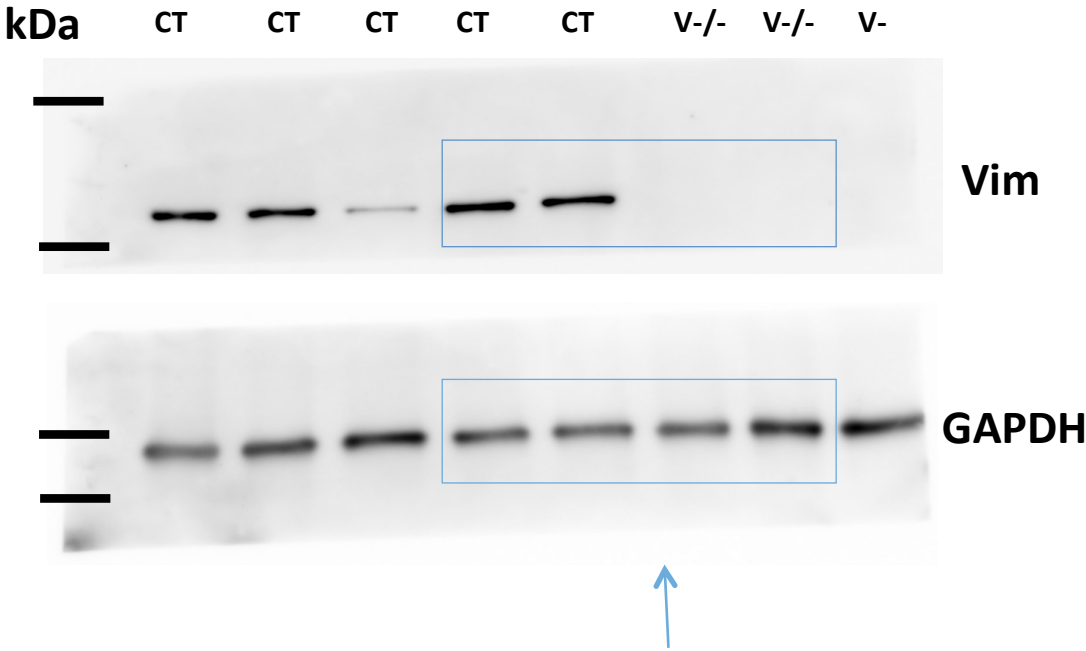




Figure 4C

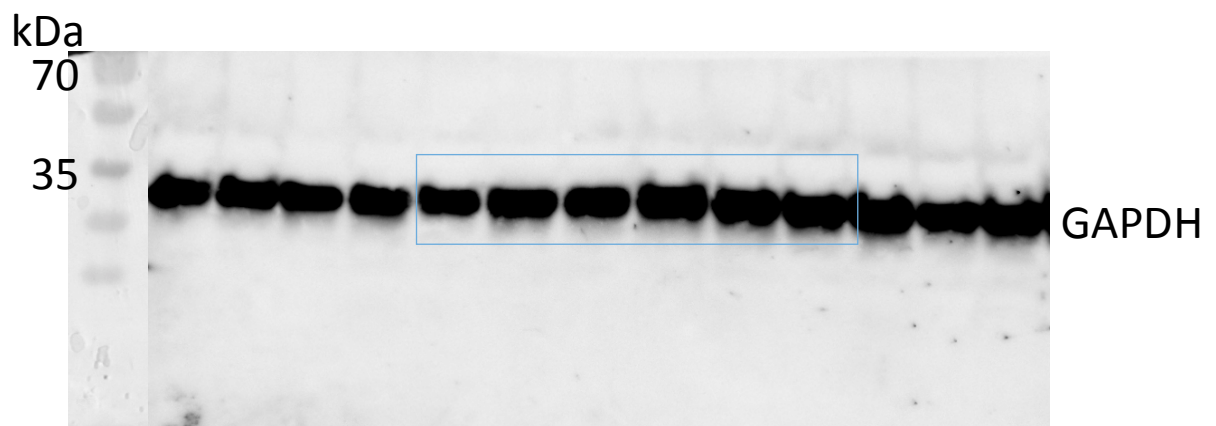
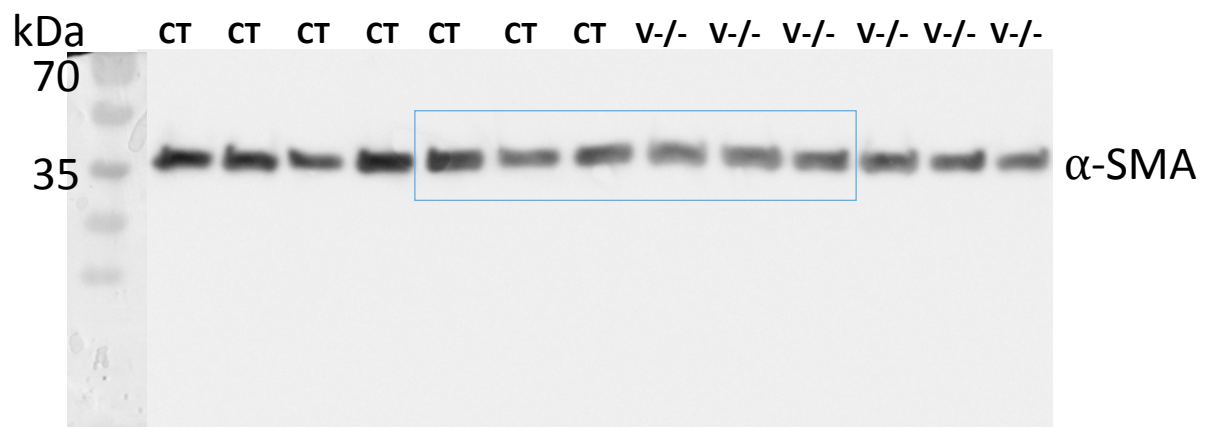








Figure 5B

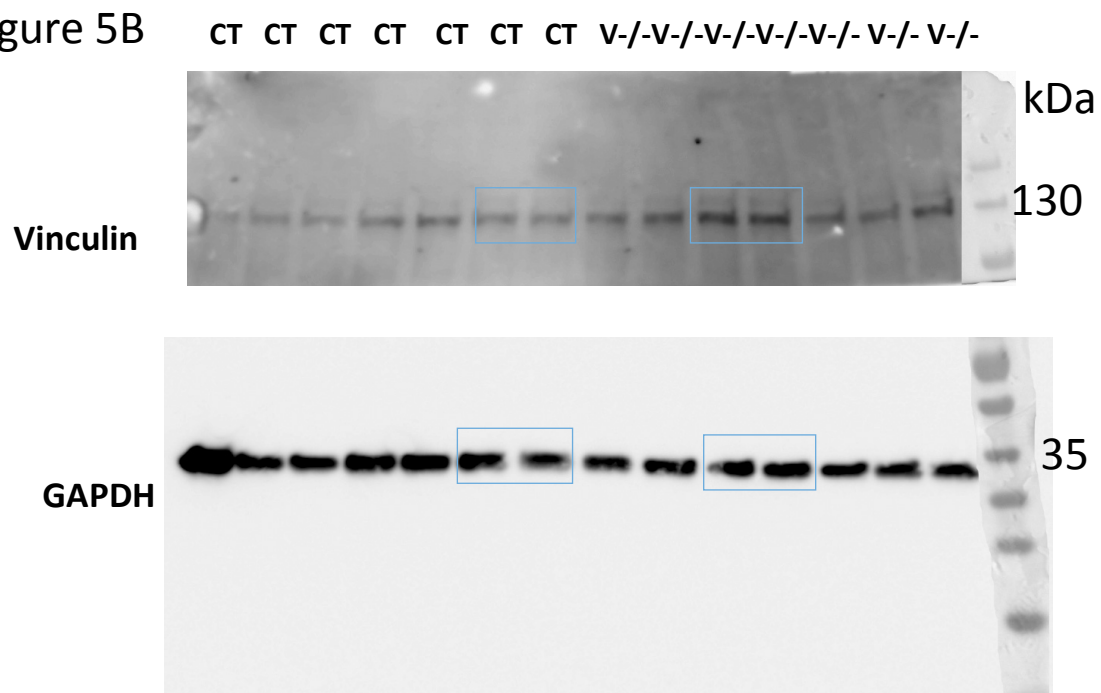


Fig 5D

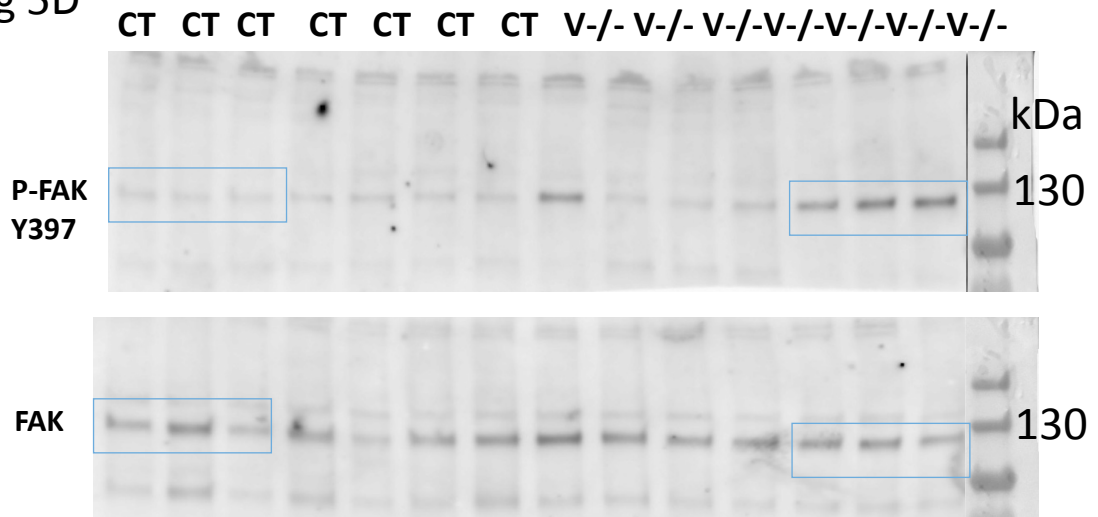


Figure 5D

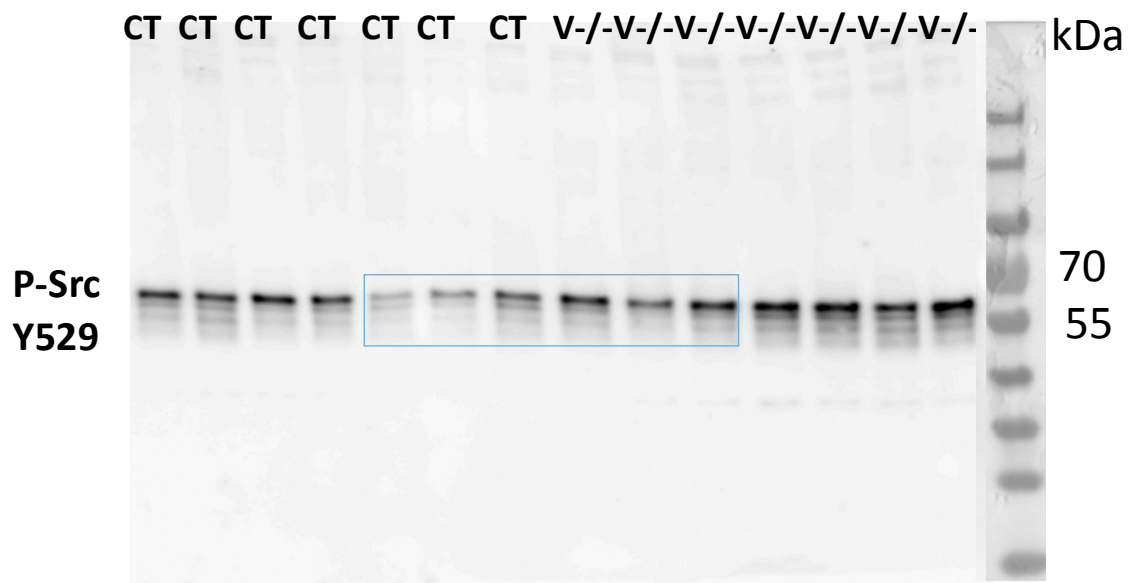
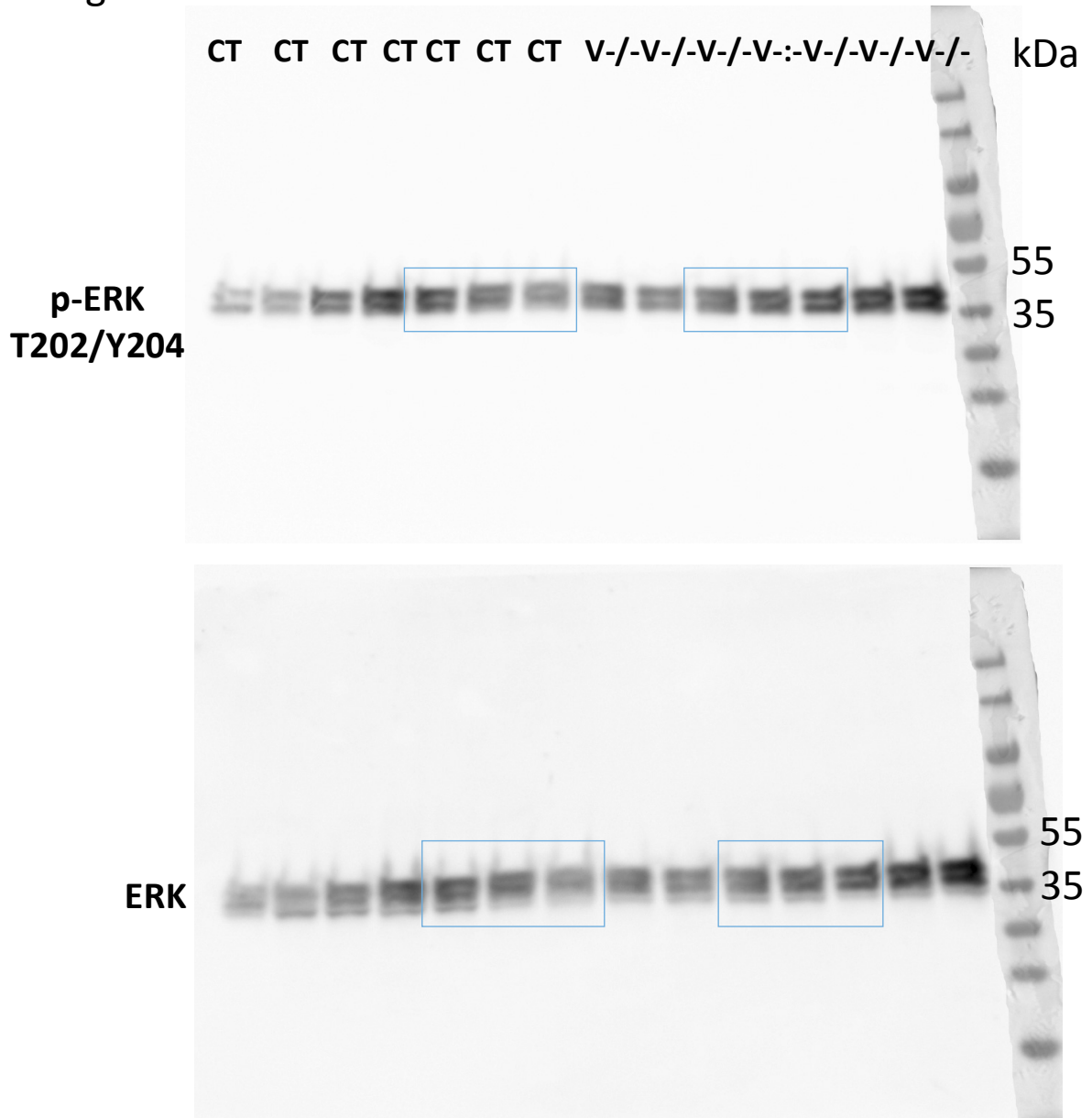
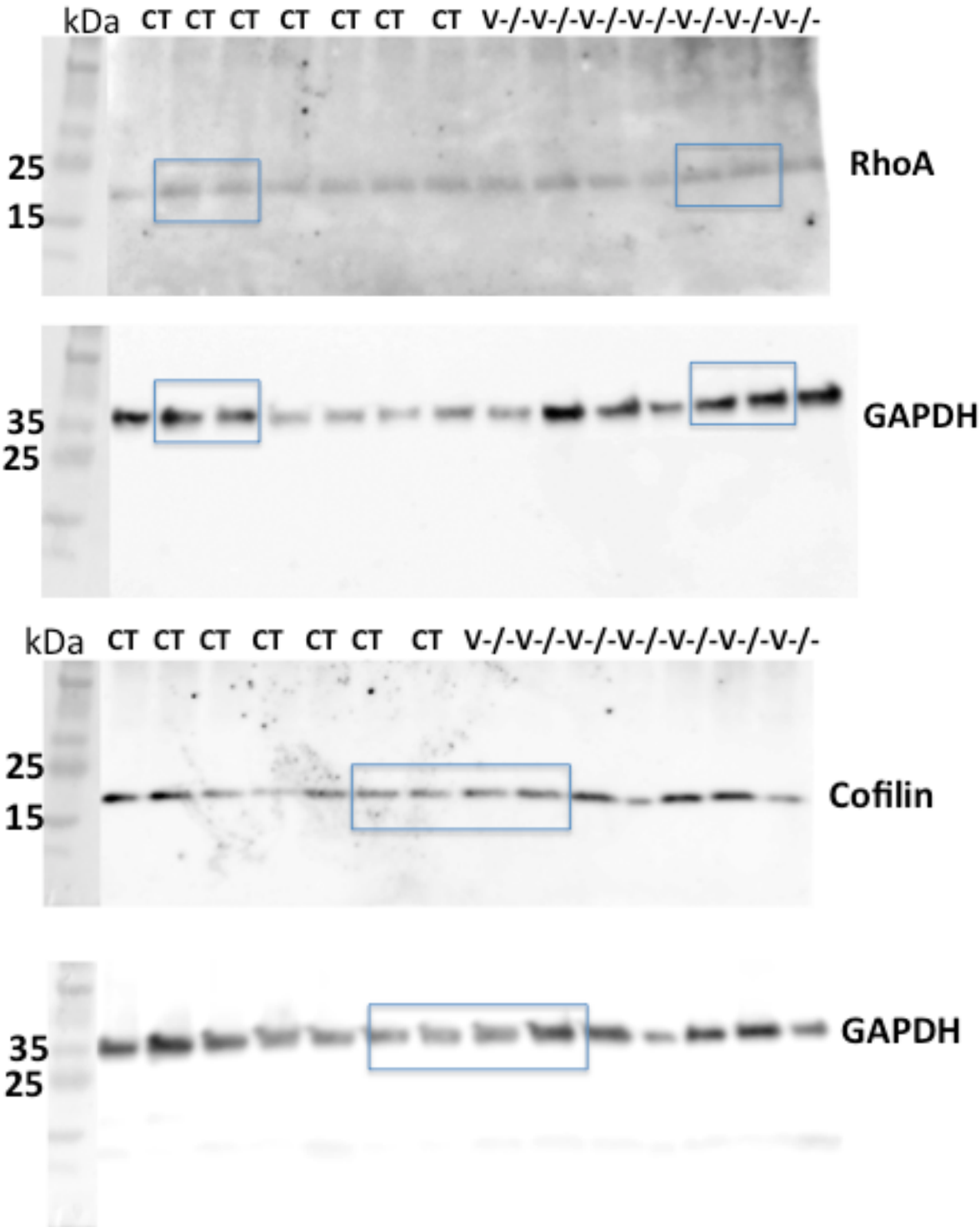


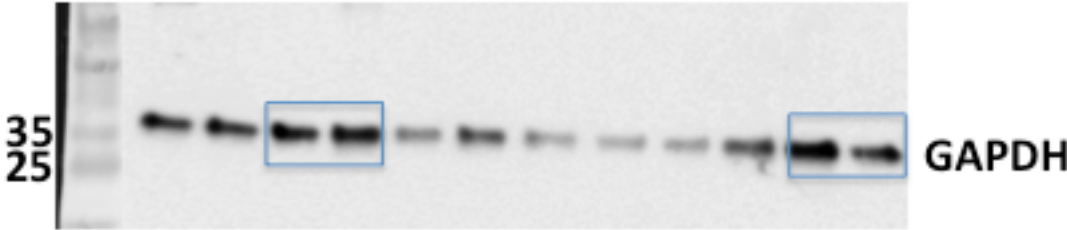
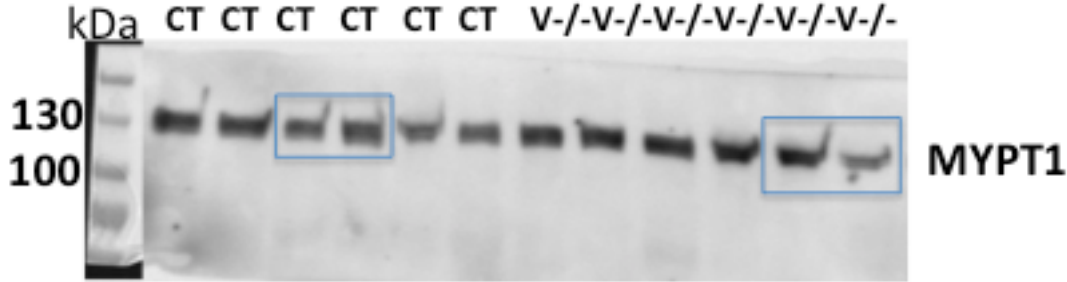
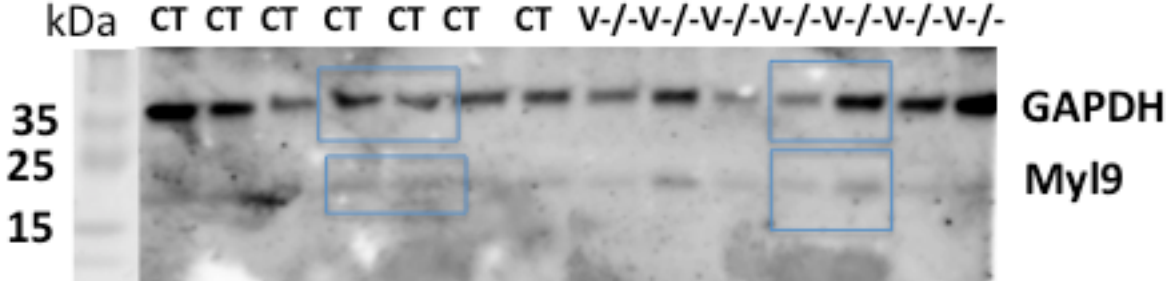
Figure 5D



**Supplementary figure S2B.**

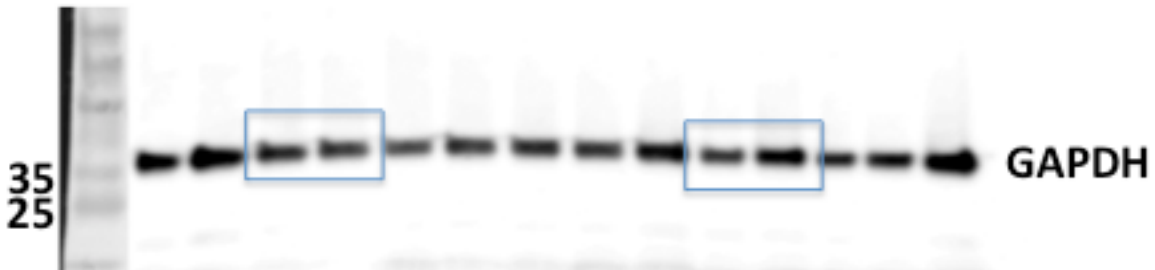
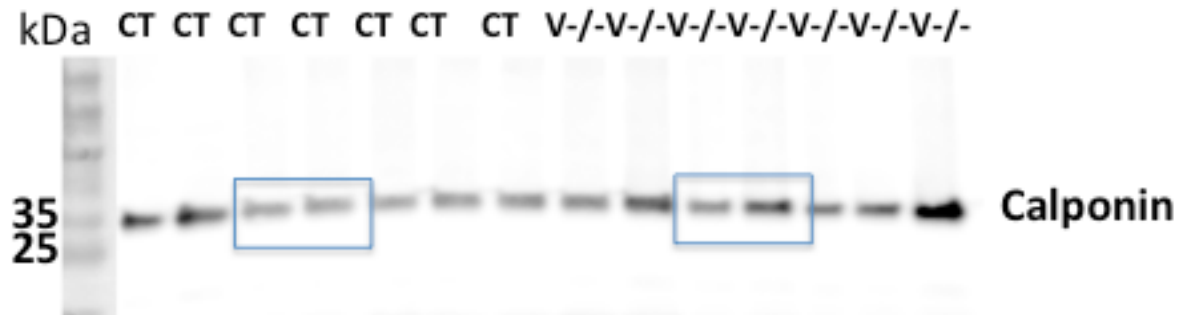
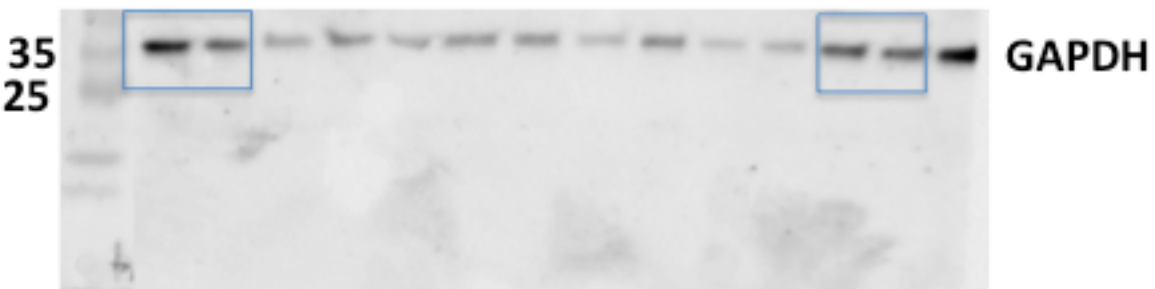
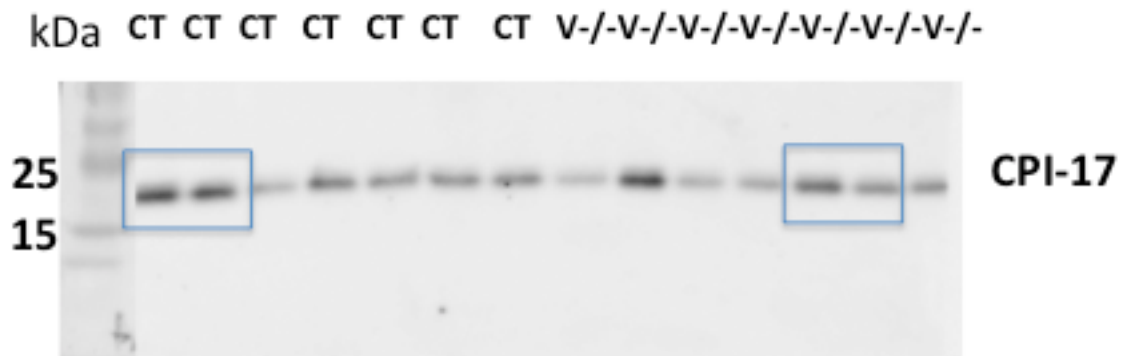


**Supplementary figure S2B.**





**Supplementary figure S2B.**



**Supplementary figure S2B.**

