

## Supplementary Materials for

### **Fibulin-4 is essential for maintaining arterial wall integrity in conduit but not muscular arteries**

Carmen M. Halabi, Thomas J. Broekelmann, Michelle Lin, Vivian S. Lee, Mon-Li Chu, Robert P. Mecham

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#### **This PDF file includes:**

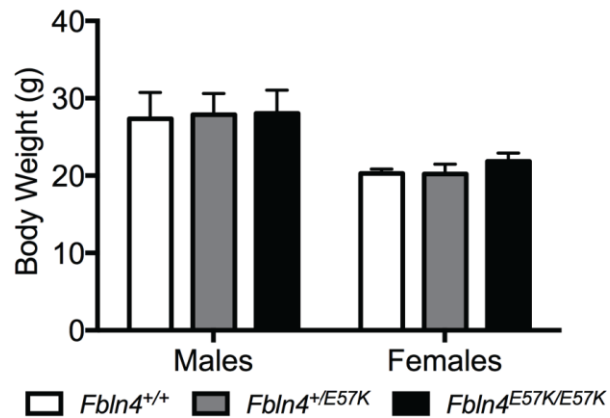
- table S1. Echocardiographic data of *Fbln4*<sup>E57K/E57K</sup> and littermate WT mice.
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**table S1. Echocardiographic data of *Fbln4*<sup>E57K/E57K</sup> and littermate WT mice.**

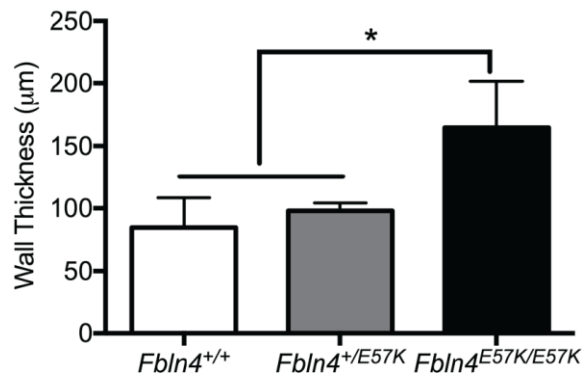
Mouse #	Genotype	Age	Body Wt (g)	AI (y/n)	LVM (mg)	FS (%)
1	WT	6 weeks	21.7	n	96.4	43
2	WT	6 weeks	20.4	n	81.8	42.2
3	WT	6 weeks	19.8	n	79.2	40.3
4	WT	6 months	33.3	n	103.6	43.6
5	WT	6 months	27.6	n	119.6	38.6
6	<i>Fbln4</i> <sup>E57K/E57K</sup>	6 weeks	20.9	n	99.5	40.8
7	<i>Fbln4</i> <sup>E57K/E57K</sup>	6 weeks	20	n	90.5	45.7
8	<i>Fbln4</i> <sup>E57K/E57K</sup>	6 weeks	17.9	y	121.6	48.4
9	<i>Fbln4</i> <sup>E57K/E57K</sup>	6 months	31.1	n	146.1	43.2
10	<i>Fbln4</i> <sup>E57K/E57K</sup>	6 months	30.8	y	249.3	31.8
11	<i>Fbln4</i> <sup>E57K/E57K</sup>	6 months	30.4	y	159	45.2
12	<i>Fbln4</i> <sup>E57K/E57K</sup>	6 months	29.6	n	88.2	48.5

Abbreviations: WT = wild-type, Wt = weight, AI = aortic insufficiency, y/n = yes/no, LVM = left ventricular mass, FS = fractional shortening.

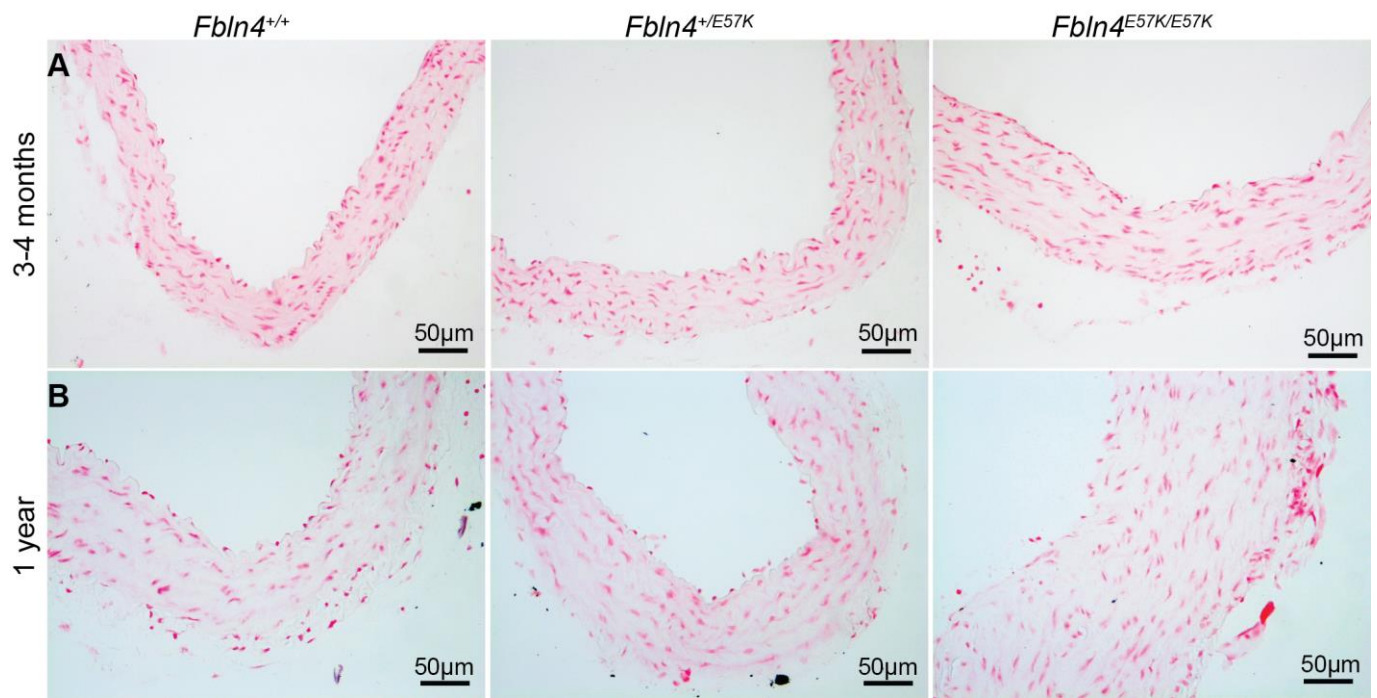
## Supplementary Figures



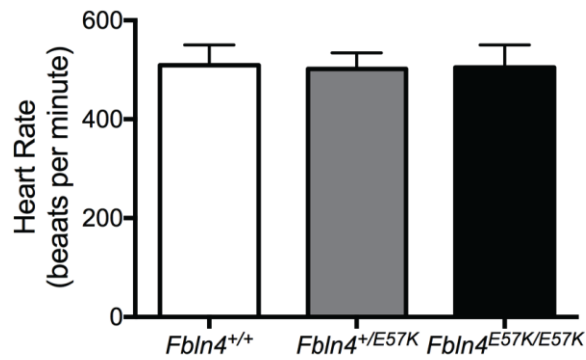
**fig. S1.  $Fbln4^{E57K/E57K}$  mice are similar in size to their littermates.** Body weight of 3-4 month-old  $Fbln4^{E57K/E57K}$  mice (closed bars) and littermates ( $Fbln4^{+/+}$ , open bars and  $Fbln4^{+/E57K}$ , grey bars) separated by gender. Data are presented as mean values  $\pm$  standard deviation and were compared using two-way ANOVA with Tukey's multiple comparison test. N = 8-9 for males, N = 3 for females.



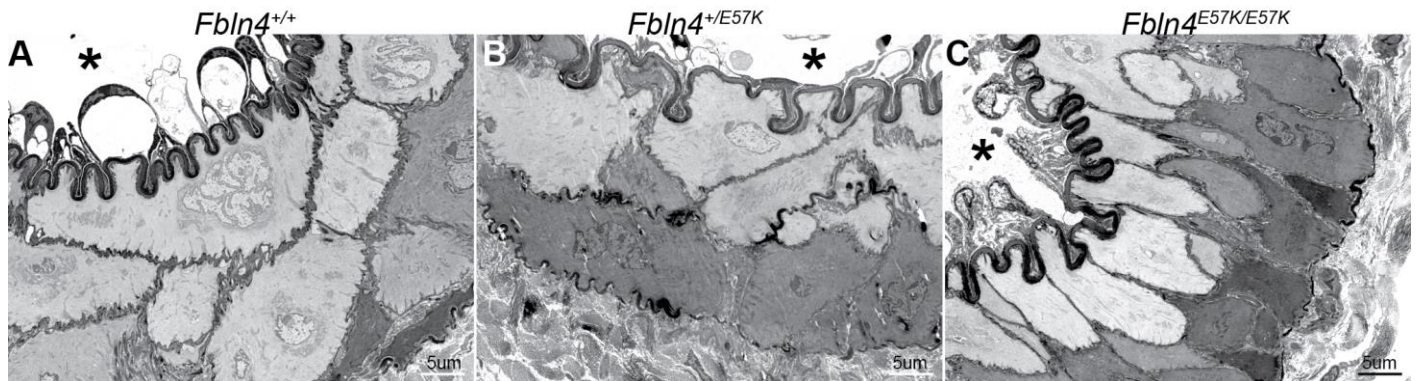
**fig. S2. *Fbln4*<sup>E57K/E57K</sup> ascending aortae have increased medial wall thickness.** Medial wall thickness of ascending aortae of *Fbln4*<sup>E57K/E57K</sup> and littermate control mice. 8-12 measurements per cross section were averaged for each mouse. N=3 per genotype. Thickness was compared among all genotypes using one-way ANOVA with Tukey's multiple comparison test. Data are presented as mean values ± standard deviation. Significant difference: \*P<0.05.



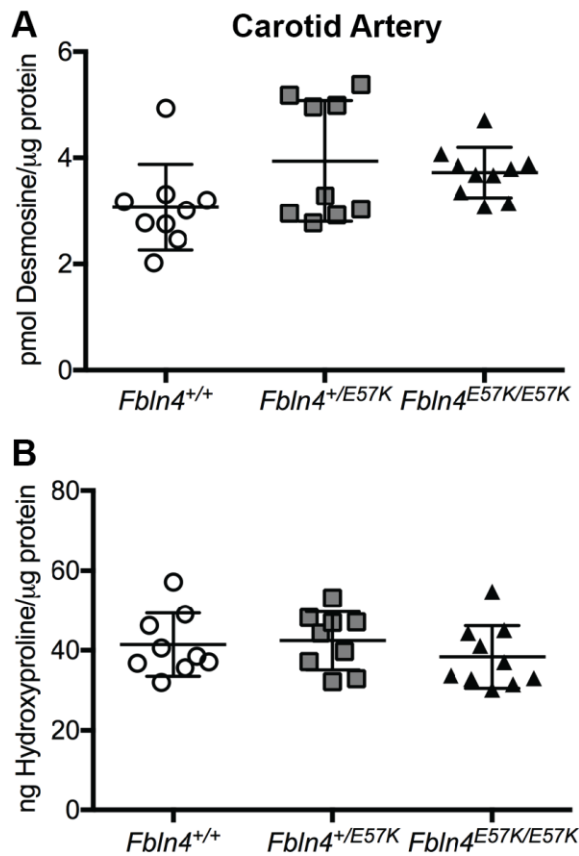
**fig. S3. *Fbln4*<sup>E57K/E57K</sup> ascending aortae do not develop calcification.** von Kossa staining of ascending aortae cross sections from 3-4 month-old (A) and 1 year-old (B) *Fbln4*<sup>E57K/E57K</sup> and littermate (*Fbln4*<sup>+/+</sup> and *Fbln4*<sup>+/E57K</sup>) mice.



**fig. S4. *Fbln4*<sup>E57K/E57K</sup> mice have similar heart rates to their heterozygous and WT littermates.** Heart rate of 3-4 month-old male and female *Fbln4*<sup>E57K/E57K</sup> (closed bars) mice and littermates (*Fbln4*<sup>+/+</sup>, open bars and *Fbln4*<sup>+/E57K</sup>, grey bars) as measured by arterial catheterization. Data are presented as mean values  $\pm$  standard deviation and were compared using one-way ANOVA with Tukey's multiple comparison test. N = 10-12 per genotype.

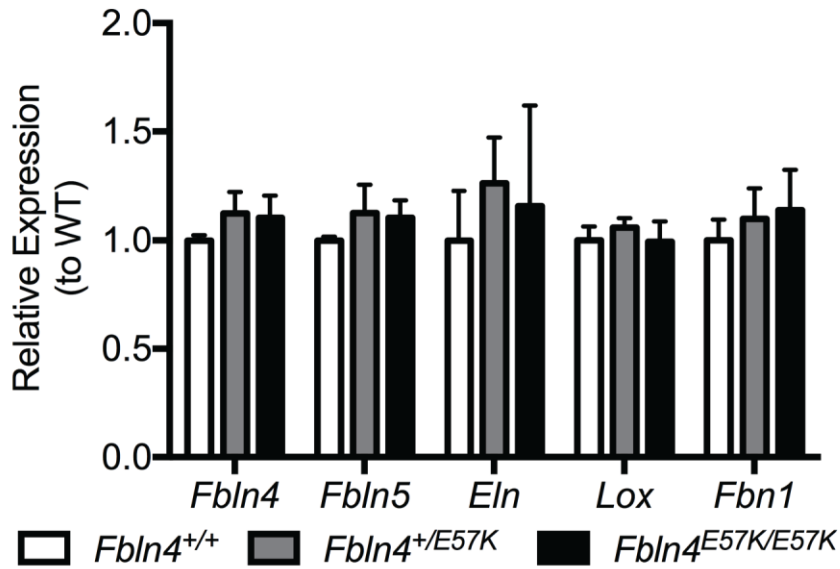


**fig. S5. Internal elastic lamina is unaffected in *Fbln4*<sup>E57K/E57K</sup> saphenous arteries.** Transmission electron micrographs of saphenous arteries from 3-4 month-old *Fbln4*<sup>E57K/E57K</sup> mice (C) and littermate controls (*Fbln4*<sup>+/+</sup> - A and *Fbln4*<sup>+/E57K</sup> - B). Asterisks indicate vessel lumen.



**fig. S6. Homozygous E57K mutation in *Fbln4* does not alter arterial elastin or collagen content.**

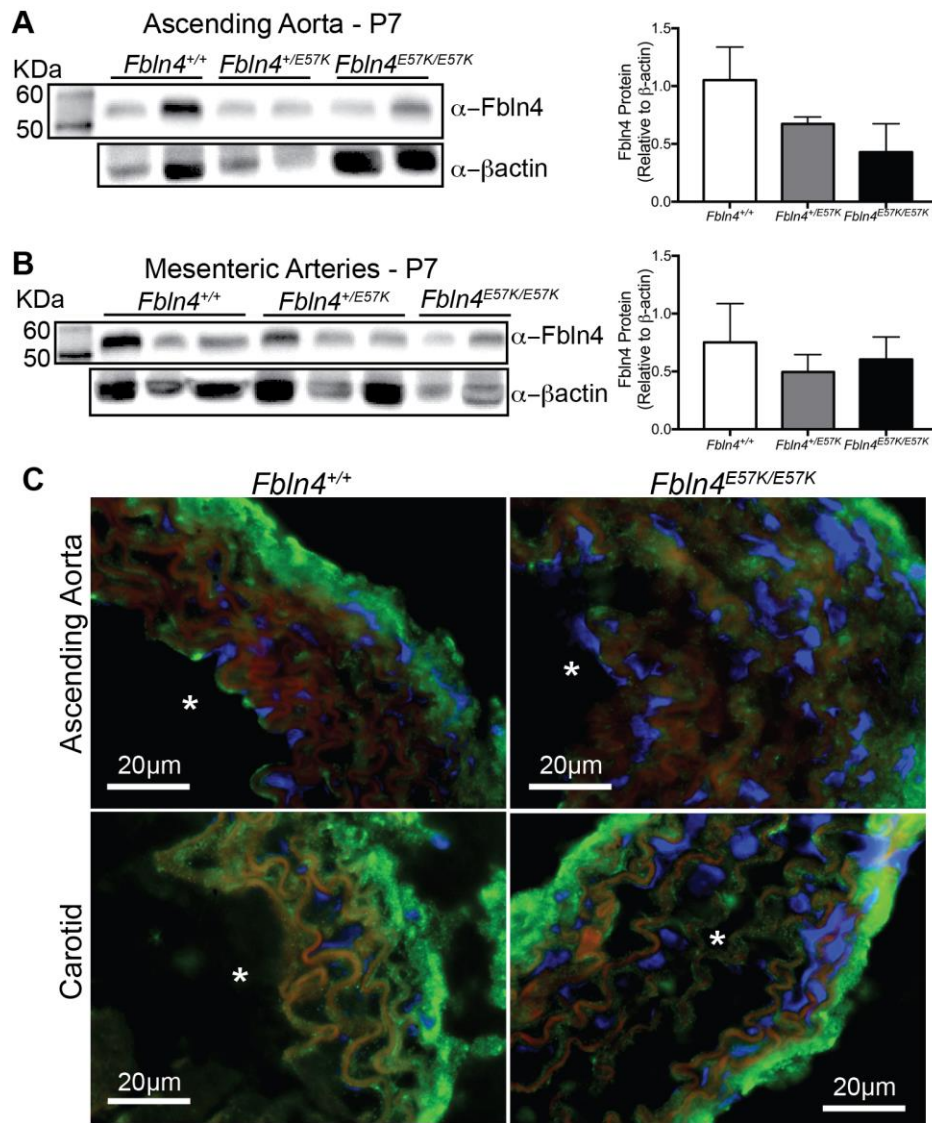
Desmosine (A) and hydroxyproline (B) content were determined in carotid arteries of 3-4 month-old *Fbln4*<sup>E57K/E57K</sup> (closed triangles) male and female mice and littermates (*Fbln4*<sup>+/+</sup> - open circles and *Fbln4*<sup>+/E57K</sup> - grey squares). Data are presented as mean values  $\pm$  standard deviation and were compared using one-way ANOVA with Tukey's multiple comparison test.



**fig. S7. Homozygous E57K mutation in *Fbln4* does not alter ECM gene expression in lungs.**

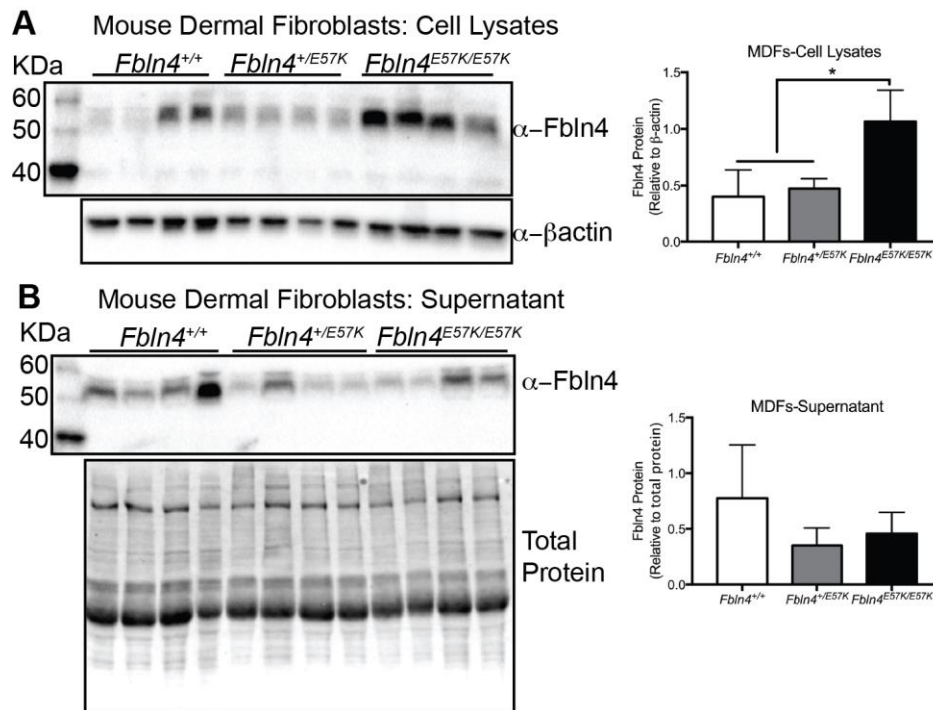
Expression of elastic fiber associated genes (*Fbln4*: Fibulin-4, *Fbln5*: Fibulin-5, *Eln*: Elastin, *Lox*: Lysyl oxidase, *Fbn1*: Fibrillin1) was compared in lungs of 3-4 month-old *Fbln4*<sup>E57K/E57K</sup> (closed bars) and littermate mice (*Fbln4*<sup>+/+</sup> - open bars and *Fbln4*<sup>+/E57K</sup> - grey bars). Gene expression was normalized to the expression of *Gapdh* and *cyclophilin*. Data are presented as mean values  $\pm$  standard deviation. N = 4 per genotype.





**fig. S8. FBLN4(E57K) protein level is reduced in P7 ascending aorta, but not in mesenteric arteries.**

Western blot analysis of urea extracts from ascending aorta (A) and mesenteric arteries (B) of P7 *Fbln4*<sup>*E57K/E57K*</sup> mice and littermate controls using a polyclonal rabbit anti-FBLN4 antibody. FBLN4 level was normalized to that of beta-actin. The normalized data is represented in the bar graphs on the right. N= 2-3 per genotype, therefore statistical comparison was not performed. Data are presented as mean ± standard deviation. C. Immunofluorescence of ascending aorta and carotid artery sections of P7 *Fbln4*<sup>*E57K/E57K*</sup> and wild type control mice. Green = FBLN4, Red = autofluorescence of elastic fibers, Blue = DAPI. \* indicates vessel lumen.



**fig. S9. FBLN4(E57K) protein secretion is impaired compared to WT FBLN4.** Western blot analysis of primary mouse dermal fibroblasts cell lysates (**A**) and supernatant (**B**) from *Fbln4*<sup>*E57K/E57K*</sup> and littermate control mice using anti-FBLN4 antibody. For cell lysates, FBLN4 protein level was normalized to  $\beta$ -actin, while for supernatant, data were normalized to the top band of the total protein gel. Normalized data are shown in the bar graphs on the right. Data are presented as mean  $\pm$  standard deviation and were compared using one-way ANOVA with Tukey's multiple comparison test. Significant difference: \*( $P < 0.05$ ). N=4 per genotype.