

Supplementary Material

Basement Membrane Collagen IV Deficiency Promotes Abdominal Aortic Aneurysm Formation.

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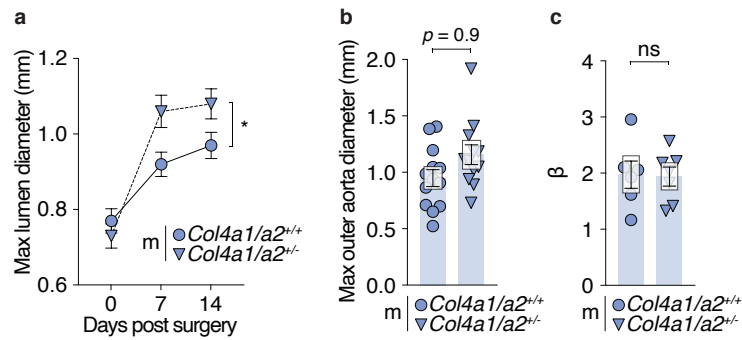
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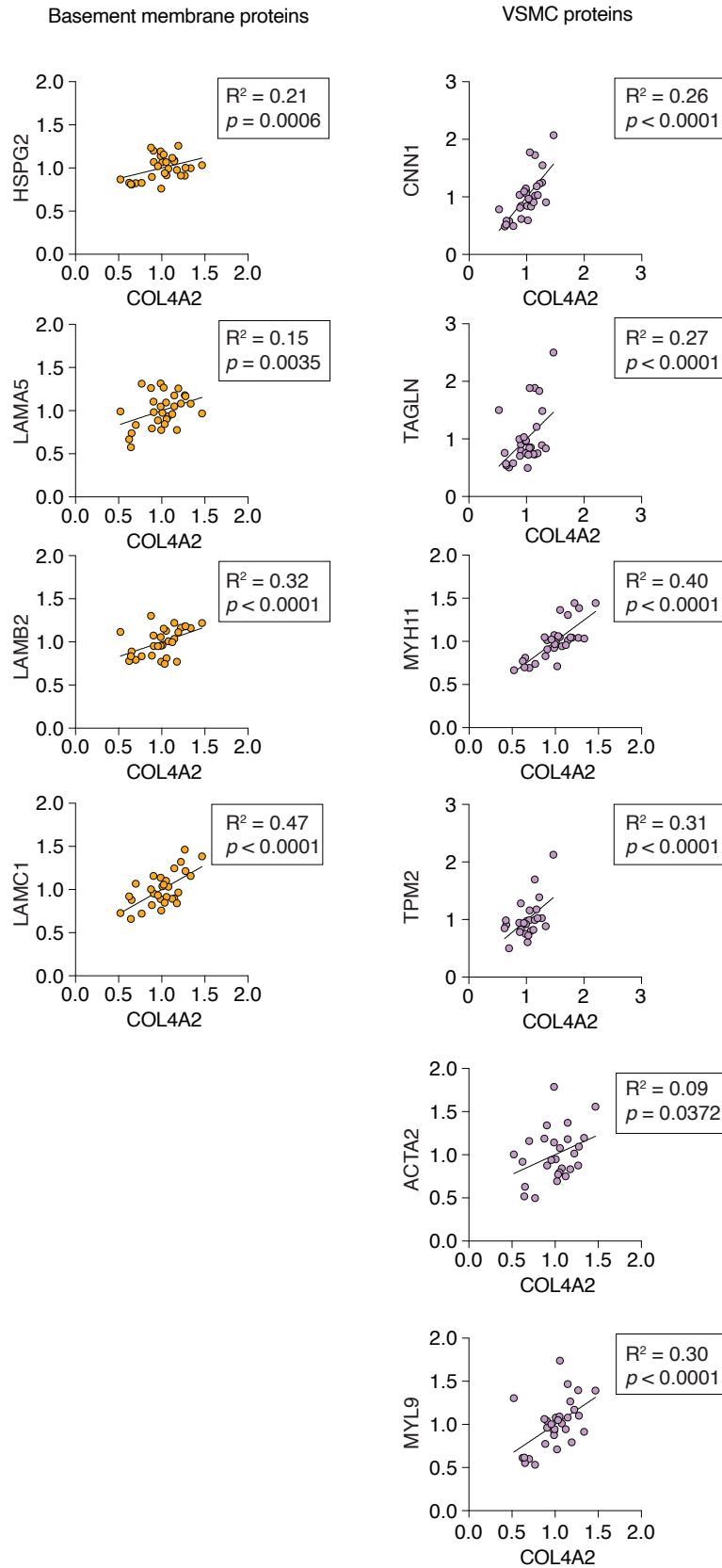
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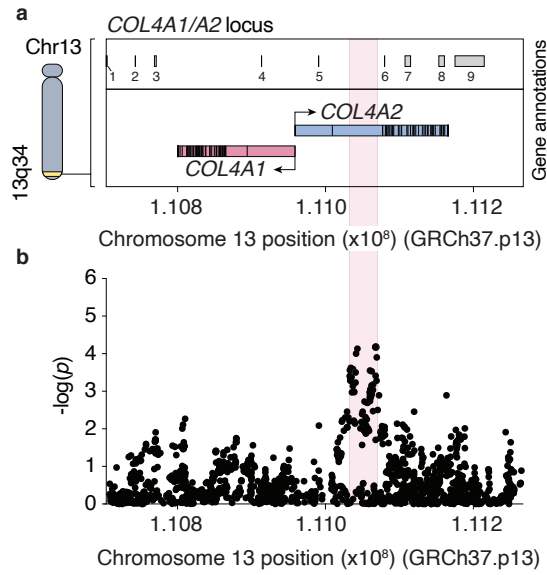
Running title: Reduced Collagen IV Promotes AAA Formation.



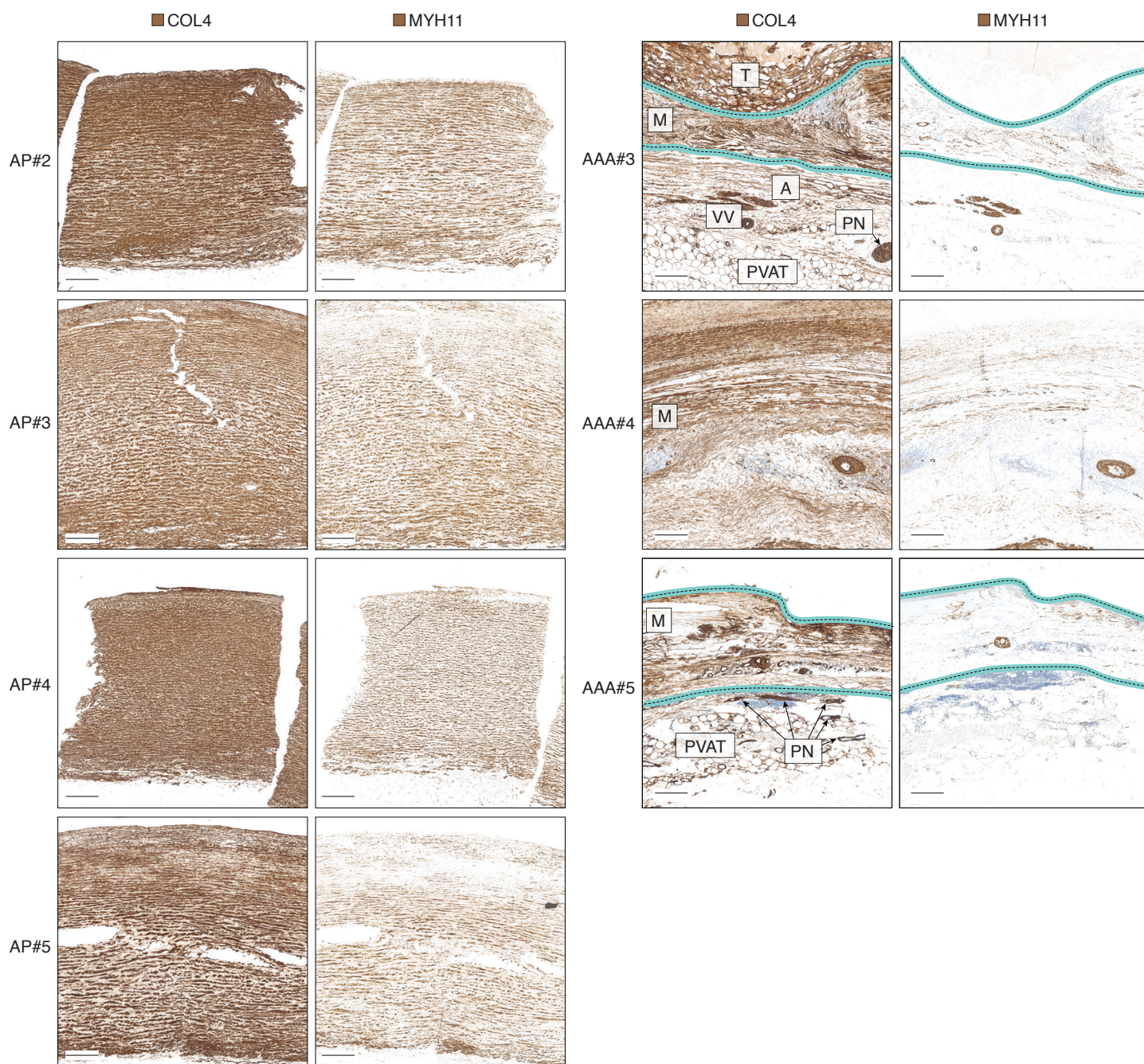
Supplementary Figure 2 | **a.** Maximal lumen diameter measured by ultrasound in absolute values (as an alternative representation of data shown in Figure 2c). **b.** Maximal outer aorta diameter measured directly using images exemplified in Figure 2d (as an alternative representation of data shown in Figure 2e). **c.** β -value derived from exponential fitting of the stress-strain relationship in Figure 2h. The β -value is proportional to the intrinsic wall stiffness. Data are presented as mean \pm SEM. *: $p \leq 0.05$, ns = non-significant. Two-way ANOVA was used in a. Unpaired t-test was used in b-c. n per group (PPE experiment) = 14-15. n per group (wire myography experiment) = 6-9. m = male.



Supplementary Figure 3 | Graphical representation of correlations shown in Table 1. Only correlations between COL4A2 (and not COL4A1) and the panel of proteins are shown for simplicity.



Supplementary Figure 4 | a. The *COL4A1/A2* locus (defined here as the *COL4A1/A2* genes (357 kb) flanked by 100 kb border regions) in chromosomal band 13q34. Besides the *COL4A1/A2* genes, the locus harbors nine genes/pseudogenes (shown in grey): 1. *RP11-403A3-3*, 2. *RN7SL783P*, 3. *RP11-472K17.3*, 4. *RP11-212E4.1*, 5. *MIR8073*, 6. *RP11-291I6.2*, 7. *COL4A2-AS2*, 8. *COL4A2-AS1*, 9. *RAB20*. Exons of *COL4A1/A2* are shown as vertical black lines. **b.** LocusZoom plot of AAA GWAS metadata showing associations between SNPs and AAA in the *COL4A1/A2* locus.



Supplementary Figure 5 | COL4 and MYH11 immunohistochemical staining of four non-lesioned aortas and three of AAA lesions complementing stainings shown in Figure 4. To assist assessment of AAA lesions, the partially degraded medial (M) and adventitial (A) layers are labeled on overview images of COL4 stainings, and the media borders are marked with cyan/dotted lines. In addition, if visible, the mural thrombus (T), vaso vasorum (VV), peripheral nerves (PN), and perivascular adipose tissue (PVAT) are labeled. Scalebars = 1 mm. #2-#5 refer to sample IDs.

SUPPLEMENTARY TABLES

Supplementary Table 1 | Dichotomous and continuous variables between AAA patients and controls and their associations with plasma C4M and PRO-C4 levels.

Dichotomy variables	Control vs. AAA (%)	p	C4M (No vs. Yes)	p	PRO-C4 (No vs. Yes)	p
Familiar disposition	3.1 7.1	0.037	14.3 (8.1) 16.2 (8.51)	0.246	150.3 (99.7) 182.8 (213.1)	0.337
Current smoking	17.6 40.4	≤ 0.001	13.7 (0.74) 13.4 (0.68)	≤ 0.001	144.9 (103.9) 166.1 (118.4)	0.021
Diabetes Mellitus	14.9 11.2	0.225	14.7 (8.54) 13.4 (6.50)	0.102	154.1 (112.6) 137.4 (83.3)	0.102
Hypertension	46.3 53.4	0.069	14.1 (9.19) 14.9 (7.40)	0.204	143.0 (98.0) 160.9 (119.1)	0.031
Previous stroke	1.0 3.7	0.056	14.5 (8.37) 14.6 (5.97)	0.963	52.3 (110.3) 145.7 (73.5)	0.701
Previous AMI	1.5 6.3	0.008	14.8 (8.37) 15.3 (7.0)	0.524	152.7 (111.2) 140.3 (63.8)	0.300
Previous/current angina pectoris	5.4 10.0	0.055	14.6 (8.51) 13.4 (5.75)	0.128	153.2 (112.6) 140.4 (66.0)	0.186
Previous/current PAD	0.5 1.2	0.386	14.5 (8.32) 15.8 (7.47)	0.666	151.7 (109.7) 190.1 (67.4)	0.184
Previous ischemic event	10.3 21.1	0.001	14.7 (8.75) 13.8 (5.82)	0.194	154.3 (116.9) 141.8 (64.1)	0.099
Use of bronchodilator	7.9 10.7	0.198	14.5 (8.48) 14.3 (6.45)	0.851	151.1 (112.1) 155.8 (79.1)	0.662
Use of ACE-inhibitor	22.2 27.3	0.138	14.2 (8.67) 15.4 (7.20)	0.085	150.2 (118.0) 156.8 (82.9)	0.417
Use of calcium-blocker	17.2 23.6	0.075	14.5 (8.69) 14.5 (7.00)	0.979	148.6 (103.3) 164.1 (132.0)	0.187
Use of beta-blocker	22.7 29.5	0.057	14.6 (9.07) 14.1 (5.64)	0.407	152.5 (120.4) 147.8 (71.0)	0.531
Use of anti-blood platelets	24.6 47.3	≤ 0.001	14.2 (7.36) 14.8 (9.53)	0.393	151.4 (112.5) 152.4 (105.5)	0.900
Use of statins	36.5 52.5	≤ 0.001	14.4 (9.75) 14.2 (6.36)	0.372	159.6 (132.9) 143.5 (76.3)	0.049
Continuous variables	Mean (SD)	p	C4M (Pearson's r)	p	PRO-C4 (Pearson's r)	p
Age (Years)	69.7 (2.9) 70.0 (2.8)	0.178	-0.200	0.559	-0.015	0.692
Body mass index (kg/m ²)	26.2 (3.4) 27.2 (3.6)	≤ 0.001	0.053	0.168	0.028	0.458
Systolic blood pressure (mmHg)	148 (19.4) 155 (21.4)	≤ 0.001	0.057	0.136	0.039	0.314
Diastolic blood pressure (mmHg)	81.0 (10.5) 88.1 (12.2)	≤ 0.001	0.064	0.095	0.045	0.241
Max. aortic diameter (mm)	18.3 (2.8) 40.9 (11.9)	≤ 0.001	0.146	≤ 0.001	0.079	0.037
AAA-growth rate	- 3.06 (2.57)	-	0.029	0.585	-0.006	0.902
ILT (intraluminal thrombus) (prop. of max area)	- 0.274 (0.315)	-	0.051	0.262	0.052	0.251

Potential confounders associated with a $p \leq 0.1$ for both AAA and a COL4A1/A2 fragments: Smoking, hypertension, previous ischemic event, use of statins, and diastolic blood pressure.

Supplementary Table 2 | Univariate and multiple Cox regression analyses of plasma C4M and PRO-C4 levels as independent risk factors of need for AAA repair and death (95% C.I. in parenthesis).

	Need for repair		Death	
	HR	Adjusted HR**	HR	Adjusted HR*
C4M	1.009 (0.996; 1.022) $p = 0.160$	1.001 (0.983; 1.020) $p = 0.907$	1.020 (1.007; 1.032) $p = 0.002$	1.018 (1.004; 1.032) $p = 0.012$
PRO-C4	1.000 (0.999; 1.001) $p = 0.609$	1.000 (0.998; 1.001) $p = 0.797$	1.002 (1.001; 1.002) $p = 0.001$	1.001 (1.000; 1.002) $p = 0.008$

*Adjusted for smoking, hypertension, previous ischemic event, statins, and diastolic blood pressure.

** : Adjusted as above and baseline maximal AAA diameter