SUPPLEMENTAL MATERIAL

Aging alters the aortic proteome in health and thoracic aortic aneurysm

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Major Resources Table

Target	Vendor or Source	Catalog #	Working concentration	Persistent ID / URL
Parkin	Abcam	Ab77924	1 µg/mL	https://www.abcam.com/parkin-antibody-prk8-ab77924.html
PGC1α	Abcam	Ab54481	1 µg/mL	https://www.abcam.com/pgc1-alpha-antibody-bsa-and-azide-free-ab54481.html
STING	Cell Signaling Technology	13647	0.06 µg/mL	https://www.cellsignal.com/products/primary-antibodies/sting-d2p2f-rabbit-mab/13647
Phospho-STING	Cell Signaling Technology	19781	0.05 µg/mL	https://www.cellsignal.com/products/primary-antibodies/phospho-sting-ser366-d7c3s-rabbit-mab/19781
TLR9	Santa Cruz	52966	0.5 µg/mL	https://www.scbt.com/p/tlr9-antibody-26c593
IFNβ	Cell Signaling Technology	73671	1 µg/mL	https://www.cellsignal.com/products/primary-antibodies/ifn-b1-d1d7g-rabbit-mab/73671
LGALS9	Cell Signaling Technology	54330S	0.1 µg/mL	https://www.cellsignal.com/products/primary-antibodies/galectin-9-d9r4a-xp-rabbit-mab/54330
PHB2	Cell Signaling Technology	14085	0.1 µg/mL	https://www.cellsignal.com/products/primary-antibodies/phb2-e1z5a-rabbit-mab/14085
CDH13	Abcam	ABT121	2 µg/mL	https://www.sigmaaldrich.com/US/20en/product/mm/abt121
ORM2	ThermoFisher Scientific	PA5-37124	1 µg/mL	https://www.thermofisher.com/antibody/product/ORM2-Antibody-Polyclonal/PA5-37124
ACTBL2	ThermoFisher Scientific	PA5-72402	1 µg/mL	https://www.thermofisher.com/antibody/product/ACTBL2-Antibody-Polyclonal/PA5-72402
MGST1	Abcam	Ab131059	1 µg/mL	https://www.abcam.com/mgst1-antibody-epr7934-ab131059.html
β-Actin	Abcam	Ab8226	0.5 µg/mL	https://www.abcam.com/beta-actin-antibody-mabcam-8226-loading-control-ab8226.html
Anti-Rabbit HRP	Abcam	Ab205718	0.2 µg/mL	https://www.abcam.com/goat-rabbit-igg-hl-hrp-ab205718.html
Anti-Mouse HRP	Abcam	Ab205719	0.2 µg/mL	https://www.abcam.com/goat-mouse-igg-hl-hrp-ab205719.html
Anti-rodent HRP	Biocare	RMR622	N/A	https://biocare.net/product/rabbit-on-rodent-hrp-polymer/

Supplemental Tables
Table S1

	Young Healthy	Aged Healthy	Young TAA	Aged TAA
N	4	4	4	4
Age, mean (SD)	32.75 (6.34)	66.25 (2.99)**	46.5 (7.55)*	64.75 (5.19)$,#
Female, n (%)	0 (0)	0 (0)	0 (0)	0 (0)
Aneurysm diameter, mm (SD)	N/A	N/A	49.25 (4.35)	45.75 (6.99)†
Comorbidities				
BAV, n (%)	N/A	N/A	4 (100)	4 (100)†
CKD n, (%)	N/A	N/A	1 (25)	0 (0)†
ICM, n (%)	N/A	N/A	0 (0)	0 (0)†
NICM, n (%)	N/A	N/A	0 (0)	0 (0)†
Atrial Fibrillation, n (%)	N/A	N/A	0 (0)	2 (50)†
LV Assist Device, n (%)	N/A	N/A	0 (0)	0 (0)†
Hyperlipidemia, n (%)	N/A	N/A	0 (0)	1 (25)†
Type 2 Diabetes, n (%)	N/A	N/A	0 (0)	0 (0)†
Hypertension, n (%)	N/A	N/A	2 (50)	2 (50)†
Atherosclerosis, n (%)	N/A	N/A	1 (25)	2 (50)†

Table S1: Demographics of subjects in the proteomics study. BAV=bicuspid aortic valve, CKD=chronic kidney disease, LV=left ventricular, ICM=ischemic cardiomyopathy, NICM=non-ischemic cardiomyopathy, SD=standard deviation, TAA=thoracic aortic aneurysm. *=P<0.01 Young TAA vs. Young Healthy, $=P<0.01 Aged TAA vs. Young TAA, **=P<0.001 for Young Healthy vs. Aged Healthy, #=P-value is not significantly different between Aged TAA vs. Aged healthy, †=P-value is not significantly different between Aged TAA vs. Young TAA. P-value determined by Mann-Whitney U-test for age and aneurysm diameter and by Fisher's Exact test for comorbidities.

Supplemental Figures

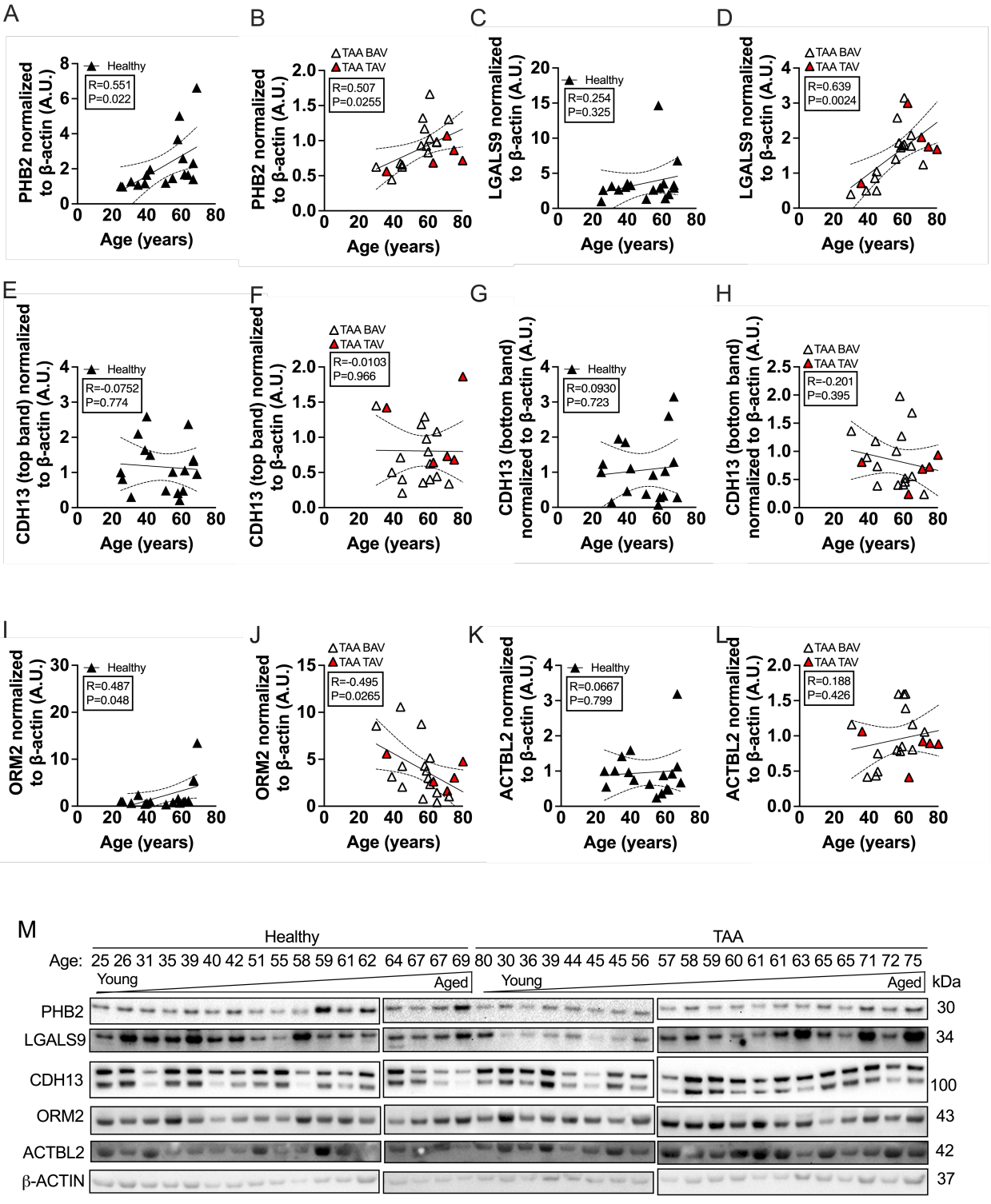

Figure S1. Expression of PHB2, LGALS9, CDH13, ORM2, and ACTBL2 in the aortas of healthy and TAA patients

Aortic lysates from healthy (N = 17) and TAA (N = 20) subjects were immunoblotted against prohibitin 2 (PHB2), galectin 9 (LGALS9), T-cadherin (CDH13), alpha-1 acid glycoprotein 2 (ORM2), Actin beta-like 2 (ACTBL2), and β-actin. The expression of PHB2 (**A-B**), LGALS9 (**C-D**), CDH13 top band (**E-F**), CDH13 bottom band (**G-H**), ORM2 (**I-J**), and ACTBL2 (**K-L**) in the healthy and TAA groups were plotted against age and regression analysis was performed. **M:** All immunoblots for PHB2, LGALS9, CDH13 top band, CDH13 bottom band, ORM2, ACTBL2, and β-Actin along with subject age are shown. Note that the 80-yr old TAA sample was erroneously place out of order. BAV = bicuspid aortic valve (open triangle), TAV = triscuspid aortic valve (red triangle). P values and correlation coefficients represent all data points within each graph.

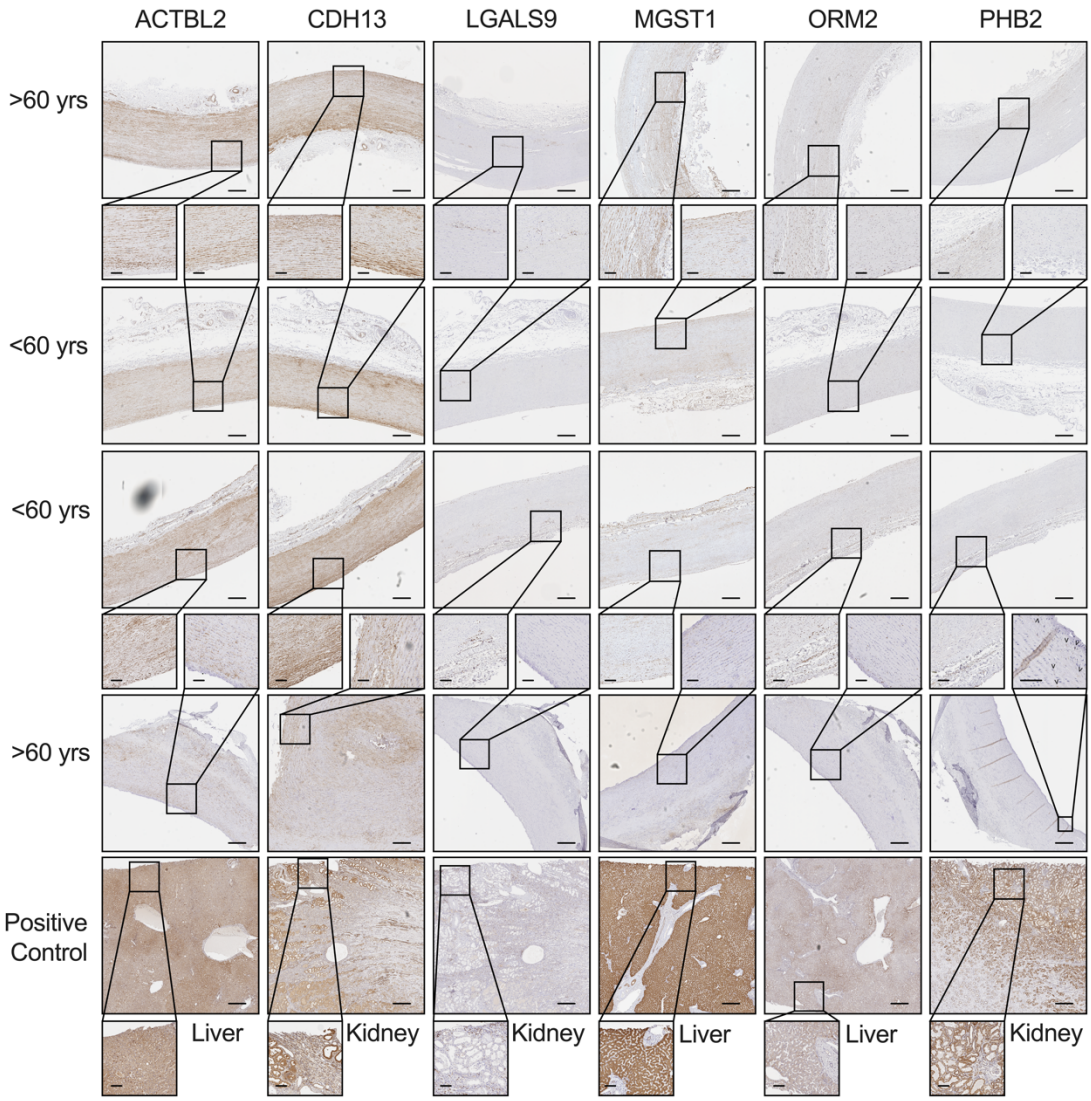

Figure S2. Immunohistochemistry of ACTBL2, CDH13, LGALS9, MGST1, ORM2, and PHB2 in aortas of young and aged TAA patients

Formalin-fixed, paraffin-embedded aortic tissues from TAA (N = 4) subjects, and liver and kidney of control subjects were sectioned and stained against actin beta-like 2 (ACTBL2), prohibitin 2 (PHB2), galectin 9 (LGALS9), T-cadherin (CDH13), alpha-1 acid glycoprotein 2 (ORM2), Actin beta-like 2 (ACTBL2), Microsomal glutathione S-transferase 1 (MGST1). Scale bars on large images are all 500μm and scale bars on all insets are 100μm. Please note that specimen 4 has non-specific PHB2 staining where the tissue has folded over. The inset on specimen 4, PHB2 is more enlarged than the others and has arrowheads to demonstrate true positive staining, and the scale bar is 100μm. Besides positive controls, all samples came from ascending thoracic aortic aneurysms in patients with bicuspid aortic valves.

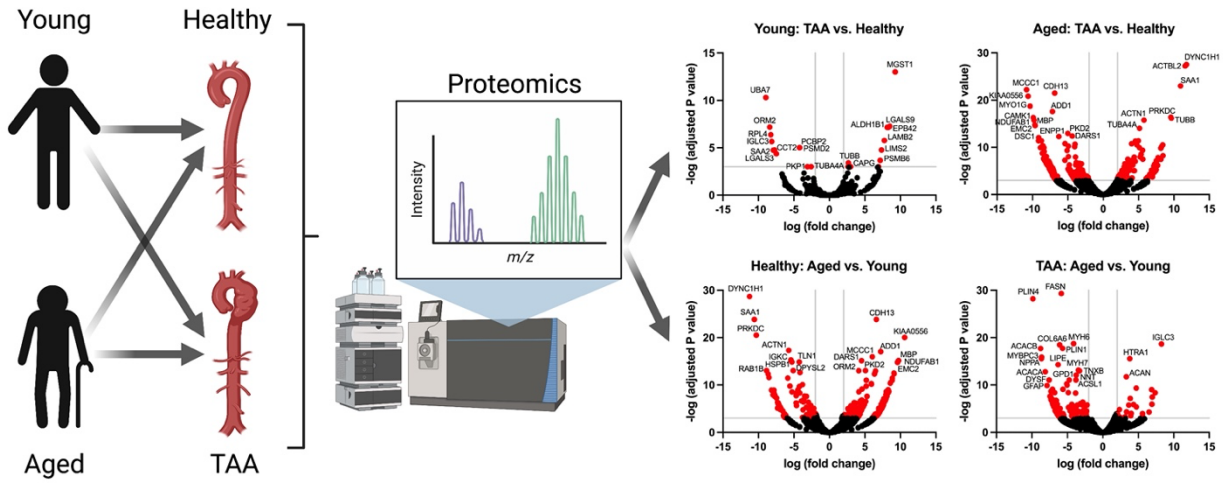

Figure S3. Graphical Abstract
We included aortic samples from young and aged individuals and samples from healthy and specimens with thoracic aortic aneurysm (TAA). We performed proteomics analysis of the aortic tissues and performed 4 differential comparisons, generating differential protein expression data and gene ontology and STRING analyses for each, which demonstrated that aging quantitatively and qualitatively impacts the aorta in health and in TAA.