

SUPPLEMENTAL MATERIALS

Single Cell Transcriptomic Profiling of Vascular Smooth Muscle Cell Phenotype Modulation in Marfan Syndrome Aortic Aneurysm

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Major Resources Table

In order to allow validation and replication of experiments, all essential research materials listed in the Methods should be included in the Major Resources Table below. Authors are encouraged to use public repositories for protocols, data, code, and other materials and provide persistent identifiers and/or links to repositories when available. Authors may add or delete rows as needed.

Genetically Modified Animals

	Species	Vendor or Source	Background Strain	Other Information	Persistent ID / URL
Parent - Male	Mouse	The Jackson Laboratory	C57BL/6J	Fbn1 ^{C1041G/+} Heterozygous	https://www.jax.org/strain/012885
Parent - Female	Mouse	The Jackson Laboratory	C57BL/6J	Littermate Controls	

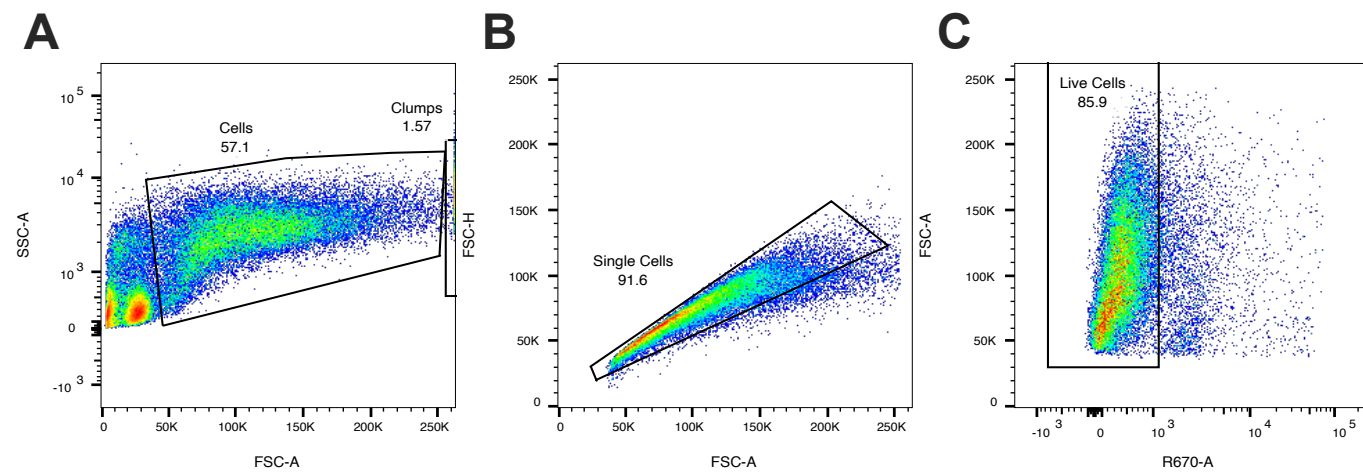
RNA *in-situ* hybridization Probes

Target Gene*	Vendor or Source	Catalog #	Persistent ID / URL
Hu TNFRSF11B	Advanced Cell Diagnostics	412291-C2	https://acdbio.com/catalog-probes
Hu SERPINE1	Advanced Cell Diagnostics	555961	https://acdbio.com/catalog-probes
Mm Tnfrsf11b	Advanced Cell Diagnostics	488961	https://acdbio.com/catalog-probes
Mm Mmp2	Advanced Cell Diagnostics	315931-C2	https://acdbio.com/catalog-probes

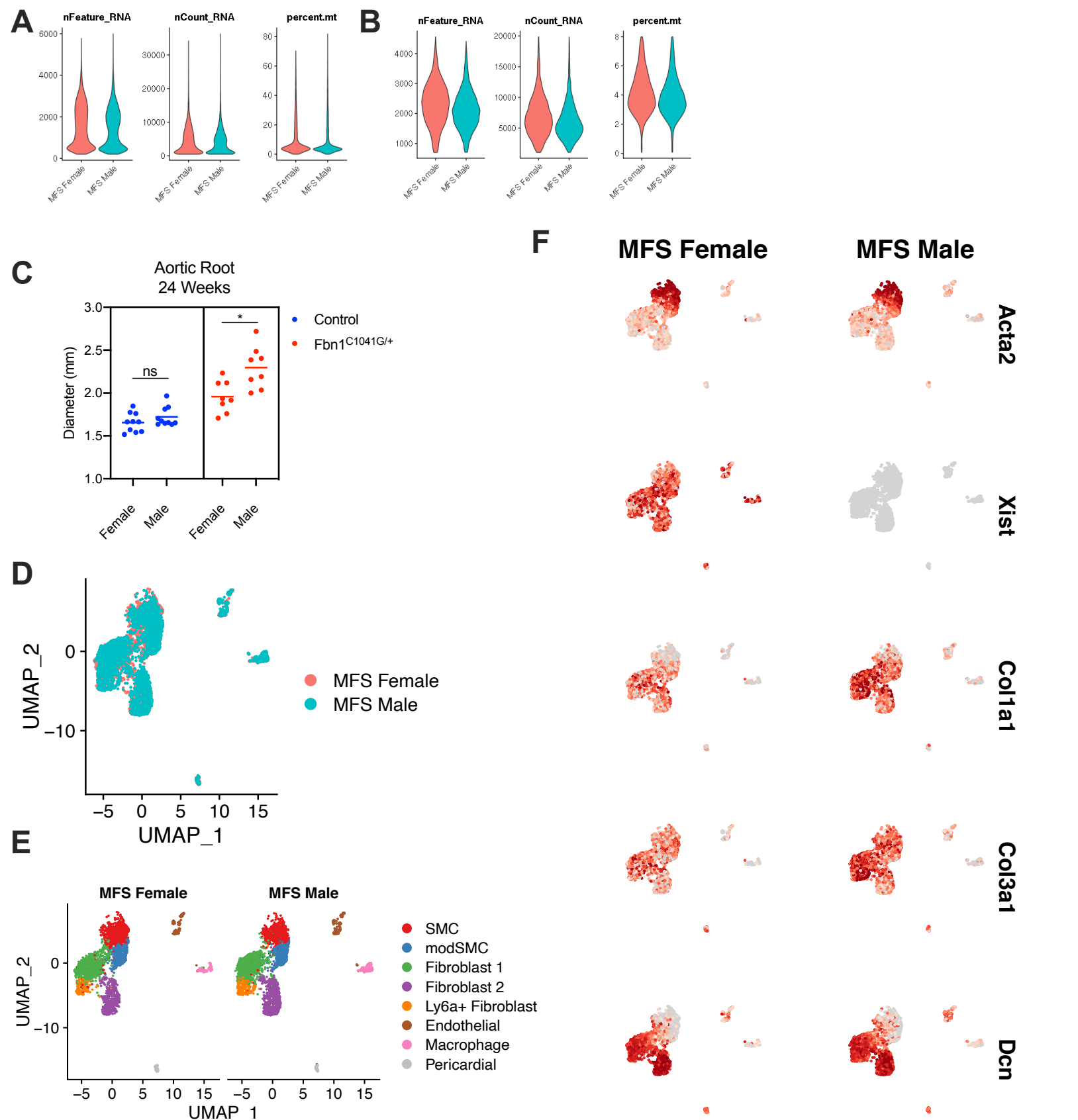
*Note: Probe sequences are proprietary

Data & Code Availability

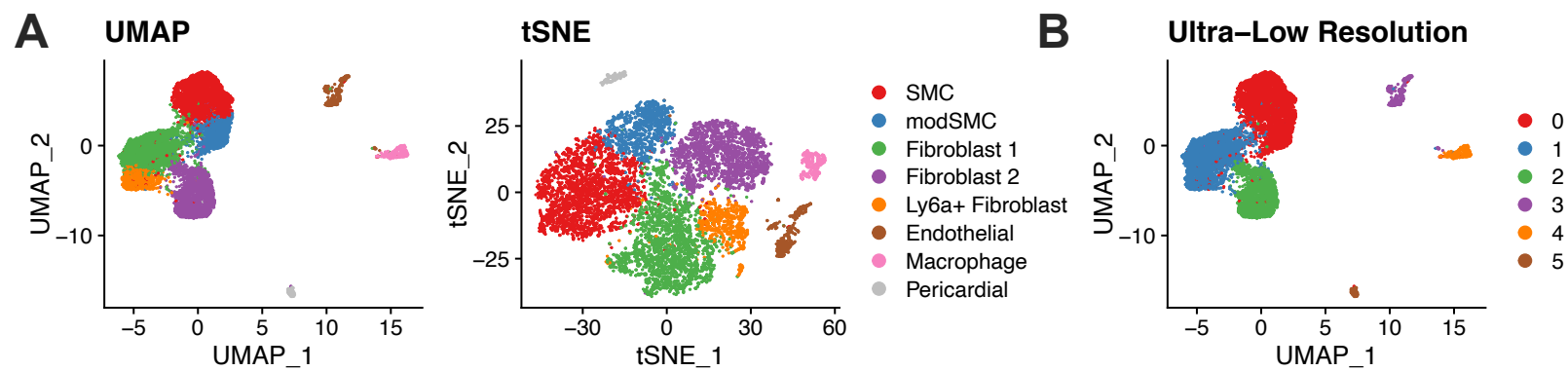
Description	Repository	Persistent ID / URL
Fbn1 ^{C1041G/+} and Control aortic scRNAseq FASTQ files and gene count matrices	NCBI Gene Expression Omnibus (GEO)	Accession Number Pending
Marfan Human patient aortic scRNAseq FASTQ file and gene count matrix	NCBI Gene Expression Omnibus (GEO)	Accession Number Pending



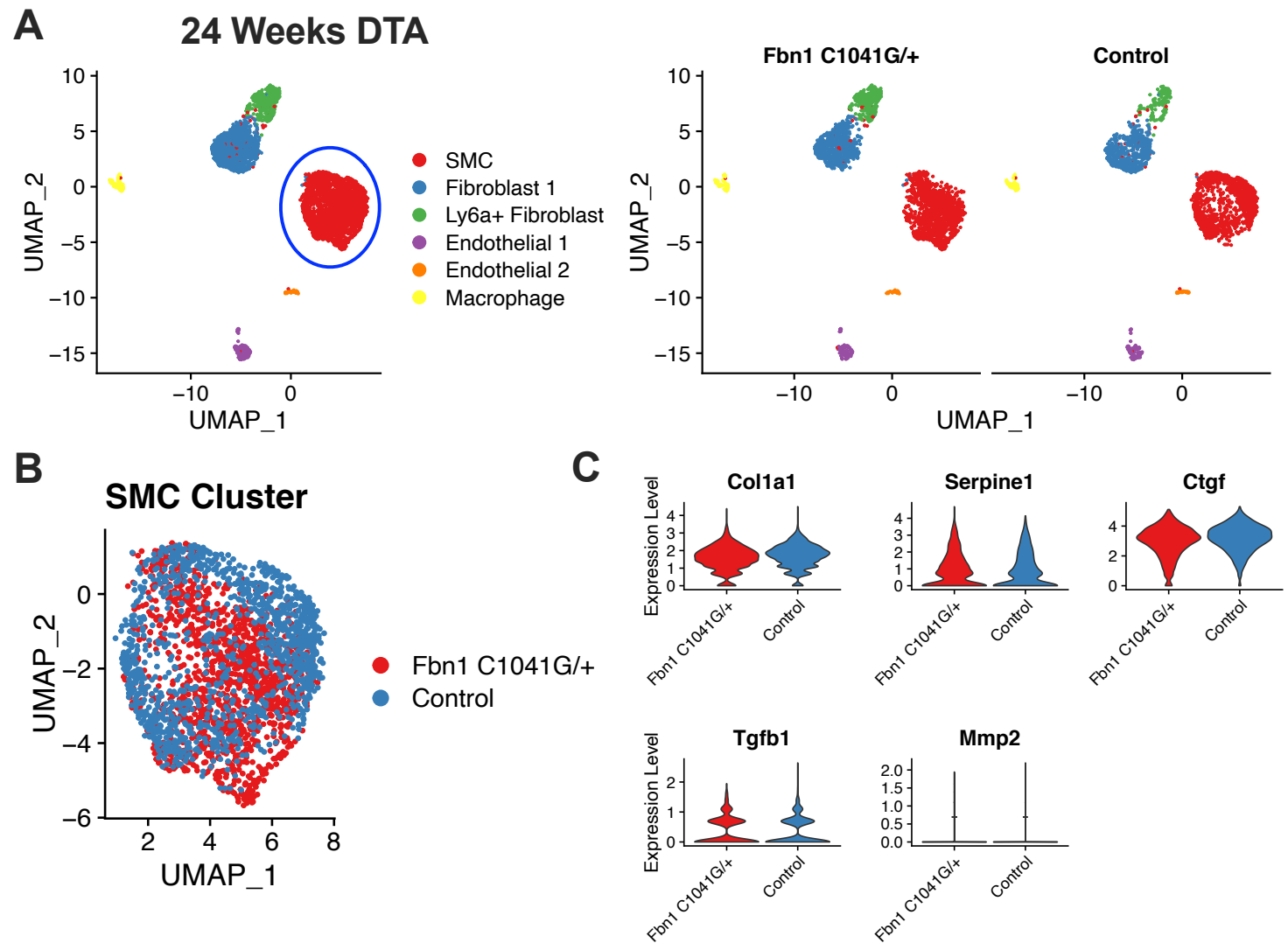
Supplemental Figure I: FACS gating strategy. **A** Representative scatter from enzymatically dispersed aortic root tissue. Largest events on upper forward scatter margin excluded to reduce doublets and clumps **B** Secondary gating for doublet exclusion **C** Live/dead discrimination gate using 7AAD as nonviable cell marker. Plots representative of all samples used for scRNAseq.



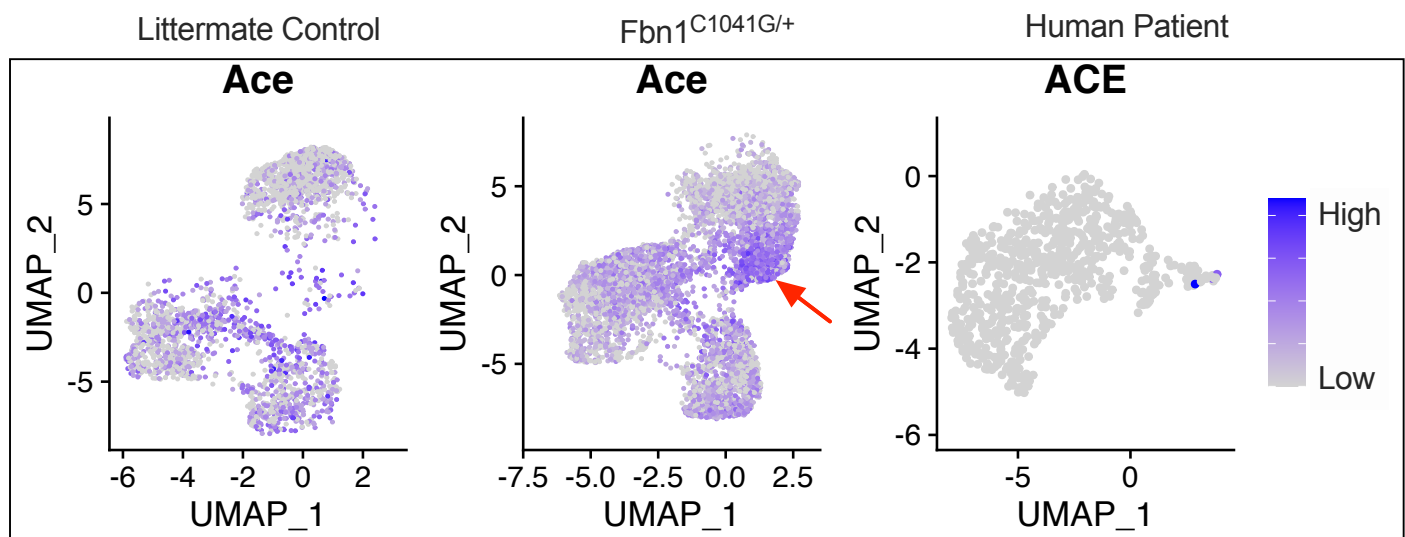
Supplemental Figure II: scRNAseq dataset quality control. A Violin plots for raw (unfiltered) dataset. Raw cells were filtered to exclude low feature counts (free RNA), high feature counts (doublets), and high mitochondrial RNA content ('percent.mt', representing dead/apoptotic cells). **B** Post QC datasets **C** Aortic root measurements by transthoracic echocardiography in 24 week-old mice. Male *Fbn1*^{C1041G/+} mice display significantly greater aneurysm size. *Denotes $p < 0.05$ by Mann-Whitney U non-parametric test. **D** Overlaid UMAP projection of male/female 24-week aortic root/ascending samples demonstrating consistent distribution within non-linear reduction between samples **E** UMAP projection of technical replicate samples revealing grossly equal contribution of each sample to every cell cluster within the projection. **F** Expression plots for SMC marker *Acta2*, representative sex-specific gene (*Xist*), and genes enriched in MFS male samples (*Col1a1*, *Col3a1*, *Dcn*).



Supplemental Figure III: 24-week scRNAseq dataset from control and *Fbn1*^{C1041G/+} mouse aortic root/ascending aortas. **A** Modulated SMC cluster is positioned directly between SMC and fibroblast clusters in both UMAP projection and tSNE dimensional projections. **B** Under ultra-low cell clustering resolution, modSMCs cluster with SMCs (Cluster '0').



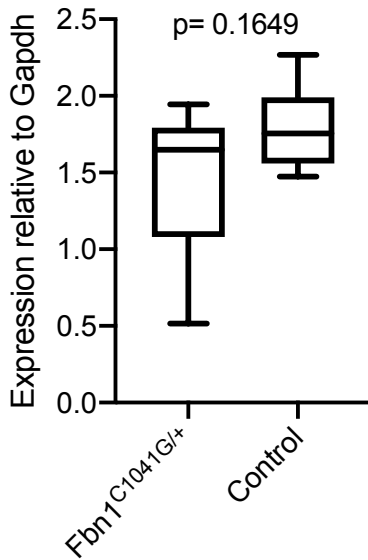
Supplemental Figure IV: scRNAseq from descending thoracic aorta (DTA) in 24 week-old *Fbn1*^{C1041G/+} and control mice. **A** UMAP dimensional reduction of >4,700 cells in merged dataset (n=3 each genotype) and split by genotype. Circled cluster indicates SMCs subsampled for analysis. **B** Overlaid UMAP projection of DTA SMCs color-coded by genotype. **C** Violin plots showing no significant differences in gene expression of *Col1a1*, *Serpine1*, *Ctgf*, *Tgfb1* and trivial expression of *Mmp2* in either sample.



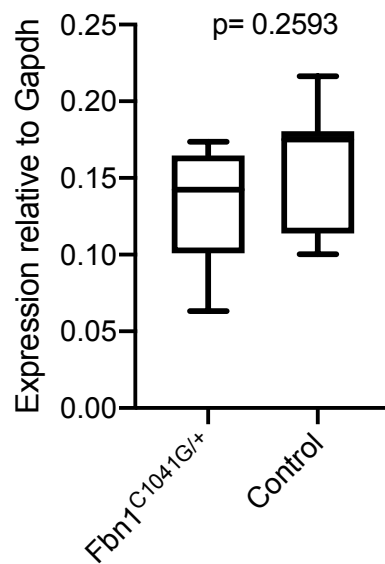
Supplemental Figure V: Angiotensin converting enzyme (*Ace*) expression in murine and human scRNAseq datasets. SMC/modSMC and fibroblast clusters depicted for each sample. Red arrow indicates enriched expression in Fbn1^{C1041G/+} modSMC cluster, while *ACE* expression is not detectable in human aortic root tissue dataset.

Aortic Root/Ascending Bulk RT-PCR

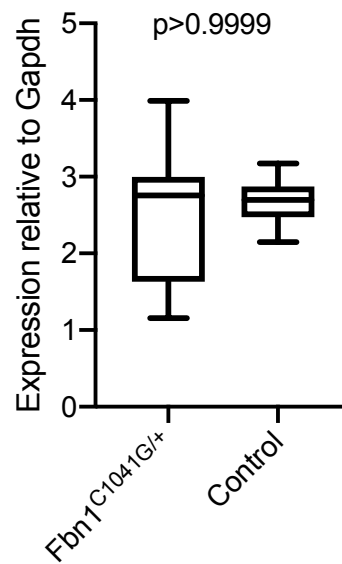
Myh11



Acta2



Tagln



Supplemental Figure VI: Bulk tissue RT-PCR for contractile genes *Myh11*, *Acta2*, *Tagln* in 24-week-old *Fbn1^{C1041G/+}* and control mice (n=7 animals each genotype).