Fig. S1. Aortic dissection in Nos3<sup>−/−</sup> mice is associated with BAV.

Additional histological staining of wild type and Nos3<sup>−/−</sup> mice presented in figure 1 stained with a combination of collagen (red) and elastin (pink) showing the ascending aorta (A-B) and aortic root (C-D). This case of aortic dissection developed in conjunction with a bicuspid aortic valve (D). Aortic dissection is apparent in the aortic vessel wall of the Nos3<sup>−/−</sup> mouse (arrow heads). Ao: Aorta, NC: Non-coronary leaflet, RC: Right coronary leaflet, LC: Left coronary leaflet, R: Right leaflet, L: Left leaflet, Scale bar: 100 µm
Fig. S2. Temporal distribution of spontaneous death events.
Wild type (n=103) and Nos3−/− (n=133) in which spontaneous death was observed were examined for the chronologic distribution of death events. No significant (P >0.05) difference was observed between wild type and mutant populations using Mantel-Cox comparison of survival curves.
Fig. S3. Collagen deposition is not affected in the ascending aortic wall of Nos3<sup>−/−</sup> mice. A-B: Transverse sections of the aortic wall of adult (A) wild type and (B) Nos3<sup>−/−</sup> mice stained with Sirius red to show collagen deposition in the media and adventitia of the ascending aorta. C-D: Sirius red staining of the embryonic aortic wall of (C) wild type and (D) Nos3<sup>−/−</sup> mice at stage E17.5. E-H: Volumetric quantification of collagen staining within the medial (E,G) as well as adventitial layers (F,H) of the adult and embryonic ascending aortic wall show no difference (P > 0.05) in the deposition of collagen between wild type and Nos3<sup>−/−</sup> mice. Ao: Aorta. Data are mean ± s.d. for n ≥ 3 mice per group. Scale bar: 50 µm
Fig. S4. Neural crest derived smooth muscle cells populate the inner media of the ascending aortic vessel wall.

A-B: Transversal sections of the ascending aorta of Wnt1Cre;mTmG and Nos3^{−/−};Wnt1Cre;mTmG embryos at E12.5. Neural crest derived vascular smooth muscle cells (VSMCs) express both Wnt1Cre-GFP (green) and ACTA2 (magenta). C-D: Fluorescent images similar to A and B, but showing embryos of developmental age E17.5. Note that expression of ACTA2 is more pronounced in neural crest derived VSMCs than VSMCs of different origin at E12.5 in both wild type and Nos3^{−/−} embryos. Nuclear staining: DAPI (grey). Scale bars: 50µm. Ao: Aorta.
Fig. S5. Differential gene expression profiles among VSMC clusters.
A-B: The differential expression profile of VSMCs affected by the Nos3 mutation. Astrix indicates downregulated genes associated with aneurysm formation. Bold gene names correspond to upregulated genes.
Fig. S6. Extended qPCR evaluation normalized to Rpl32 and Gapdh.
A-F: qPCR expression results of 6 month old wild type (N=5) and Nos3−/− (N=5) mice using 
Rpl32 as well as Gapdh as reference genes. Statistical analysis were performed using a two-
tailed student T-test, * and ** indicate P<0.05 and P<0.01 respectively. A.U: Arbitrary Units.
Data are mean ± sd.
| Primers       | Sequences                                      |
|--------------|------------------------------------------------|
| ELN_FWD      | CCC ACC TCT TTG TGT TTC GC                    |
| ELN_REV      | CCC AAA GAG CAC ACC AAC AAT                   |
| FBLN5_FWD    | GTG CTT GGG GTT GTT TTT GA                    |
| FBLN5_REV    | TCA GTT CCC CAT CTT TTG CCA                   |
| ACTA2_FWD    | GCT ACG AAC TGC CTG ACG G                     |
| ACTA2_REV    | TAG GTG GTT TCG TGG ATG CC                    |
| RPL32_FWD    | CAC CAC TCA GAC CGA TAT GTG AAA A            |
| RPL32_REV    | TGT TGT CAA TGC CTC TGG GTT T                |
| GAPDH_FWD    | TTG ATG GCA ACA ATC TCC AC                   |
| GAPDH_REV    | CGT CCC GTA GAC AAA ATG GT                    |

**Table S1. Primers used for qPCR**