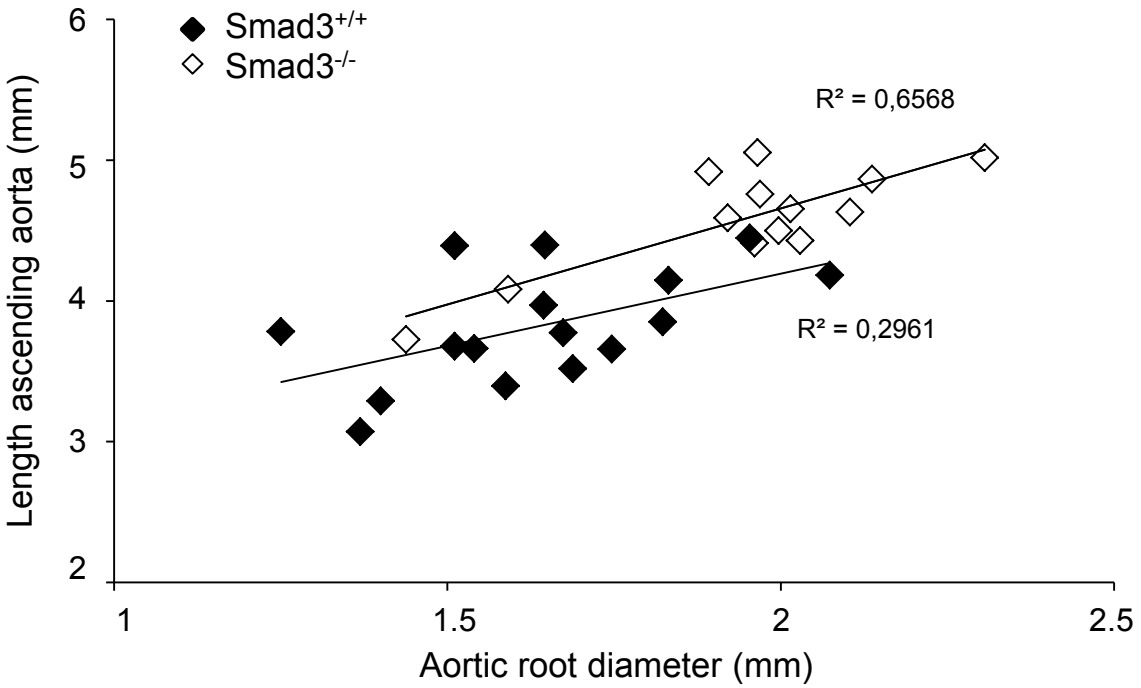
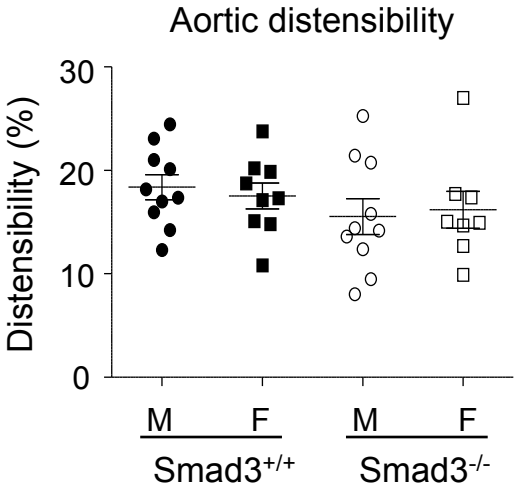
Supplemental Material belonging to manuscript ‘Defective connective tissue remodeling in Smad3 mice leads to accelerated aneurysmal growth through disturbed downstream TGF-β signaling’, van der Pluijm et al.

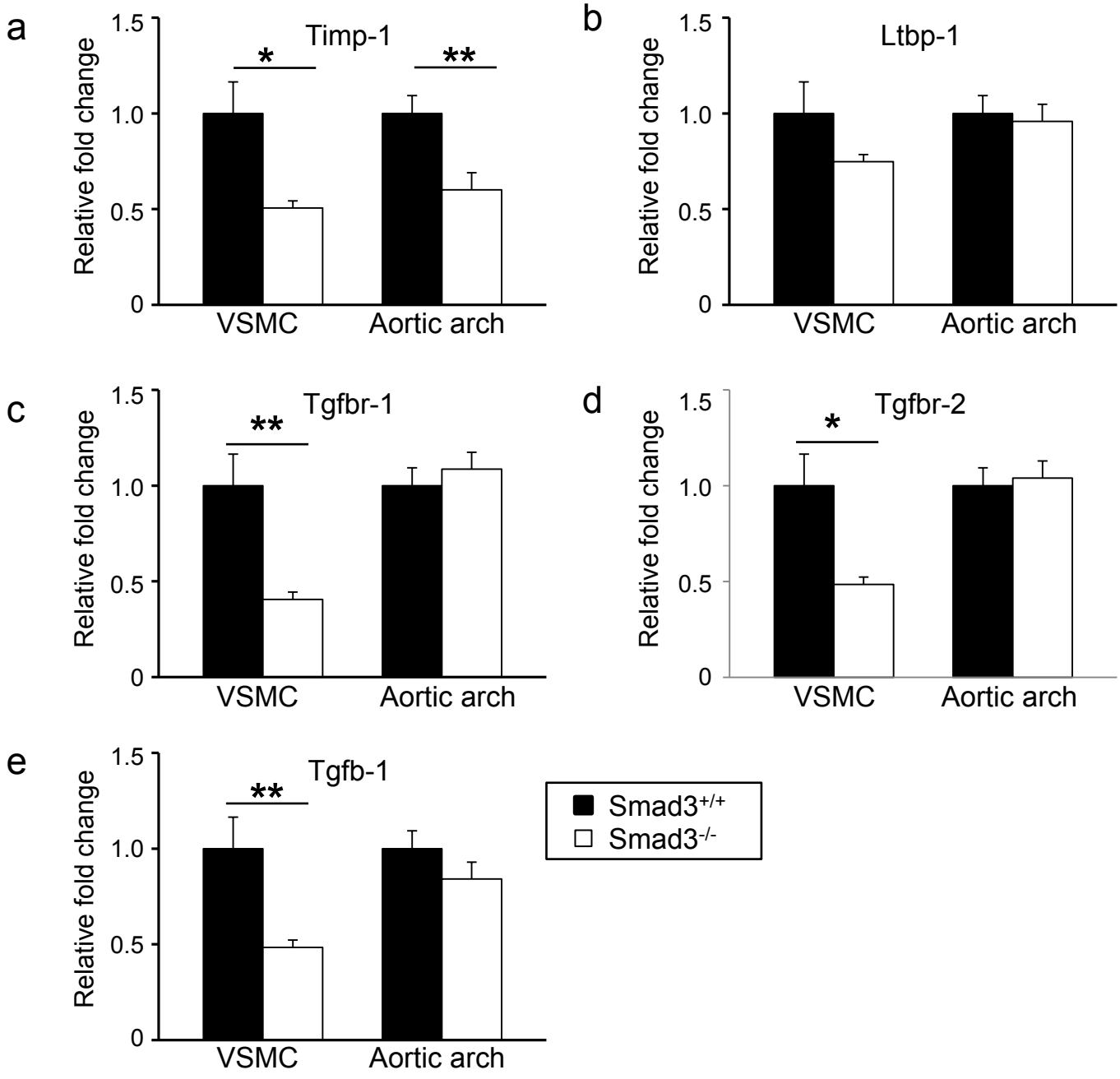
- Supplemental Figure 1
- Supplemental Figure 2
- Legends of supplemental figures and videos

a


Smad3+/+
Smad3-/-
R² = 0,6568
R² = 0,2961
Length ascending aorta (mm)
Aortic root diameter (mm)
6
5
4
3
2
1
1.5
2
2.5

b


Aortic distensibility
Distensibility (%)
30
20
10
0
M
F
M
F
Smad3+/+
Smad3-/-


a
Timp-1
Relative fold change
1.5
1.0
0.5
0
*
**
VSMC
Aortic arch
b
Ltbp-1
Relative fold change
1.5
1.0
0.5
0
VSMC
Aortic arch
c
Tgfbr-1
Relative fold change
1.5
1.0
0.5
0
**
VSMC
Aortic arch
d
Tgfbr-2
Relative fold change
1.5
1.0
0.5
0
*
VSMC
Aortic arch
e
Tgfb-1
Relative fold change
1.5
1.0
0.5
0
**
VSMC
Aortic arch
Smad3+/+
Smad3-/-

Supplemental Figure 1. Aortic root diameter and aortic length correlation in *Smad3*$^{-/-}$ animals. Aortic root diameter and aortic length measurements from all *Smad3*$^{+/+}$ and *Smad3*$^{-/-}$ animals (Figure 1) are depicted on the x- and y-axis, respectively. R^2 indicates the slope of simultaneous increase in aortic diameter and length. **B)** Quantification of aortic distensibility for *Smad3*$^{+/+}$males (n=10), females (n=9) and *Smad3*$^{-/-}$males (n=10), females (n=8) at age 6 weeks, showing no significant difference between the two groups.

Supplemental Figure 2. Dysregulated TGF-β signaling in *Smad3-/-* VSMC and aortas Real-time PCR analysis in *Smad3*$^{-/-}$ VSMC and aortic extracts compared to *Smad3*$^{+/+}$ shows downregulated mRNA levels of Timp-1 **A)**, and no significant change in **B)** Ltbp-1. mRNA levels of **C)** Tgfbr-1, **D)** Tgfbr-2 and **E)** Tgfb-1 were down regulated in *Smad3*$^{-/-}$ VSMCs compared to Smad3$^{+/+}$, but not in aortic extracts. Fold changes are shown for *Smad3-/-* relative to *Smad3*$^{+/+}$, *p <0.05, **p<0.01. The mean of three independent experiments are shown, n=9-12 per group.

Video File Legends

Video files supplement to Figure 2D. Rapid aneurysmal growth in *Smad3*$^{-/-}$ mice, not restricted to the aorta. D) Representative µCT video files of CT pictures of a *Smad3*$^{-/-}$ female (3D CT rendering Smad3ko mouse) and its littermate *Smad3*$^{+/+}$ control (3D CT rendering WT mouse) in 3D rotating view.