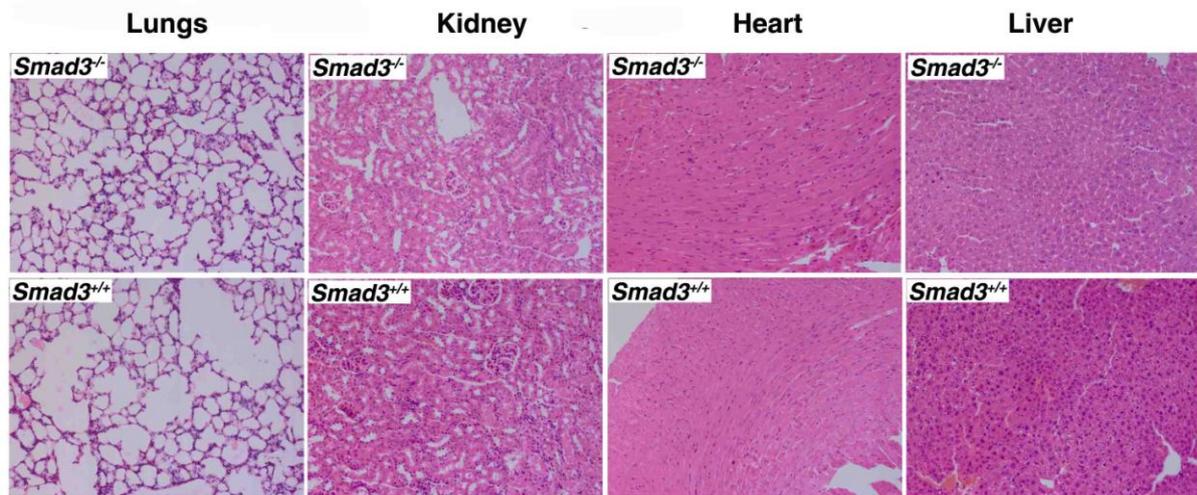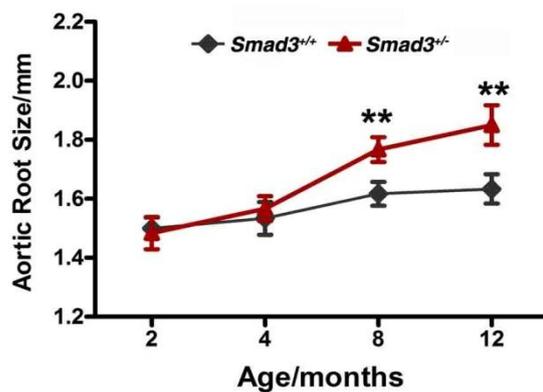# Supplemental Material

Supplemental Figure 1

Supplemental Figure 1. Representative HE staining of the lungs, kidney, heart and liver from 1-mo-old $Smad3^{+/+}$ and $Smad3^{-/-}$ which were showed normal. There are no inflammation was found in these organs. Original magnification, 200×.
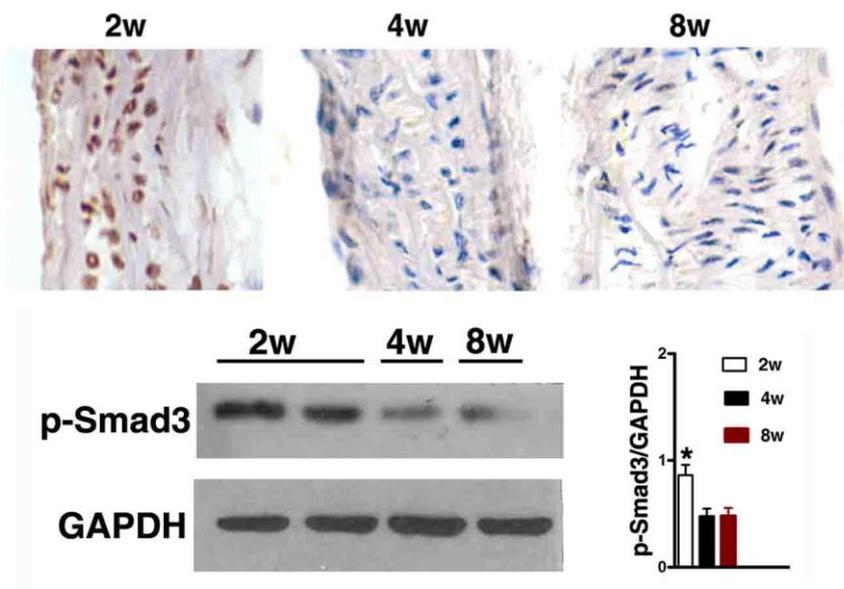
Supplemental Figure 2

Supplemental Figure 2. Aortic root diameter, measured by echocardiography, at different ages of WT ($Smad3^{+/+}$) (n=6/time points) and $Smad3^{+/-}$ (n=7/time points) mice. **$P < 0.001$ $Smad3^{+/-}$ versus WT at the same age.

Supplemental Figure 3
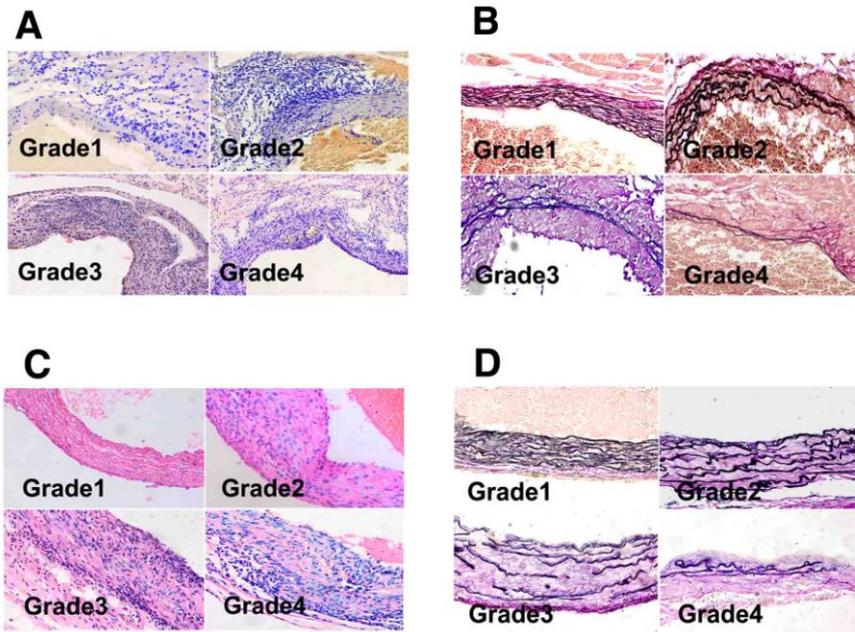
Supplemental Figure 3. Elastin degradation–grading (4 grades) keys (**B, D**) and inflammatory cell infiltration-grading (4 grades) (**A, C**) of aortic root (**A, B**) and ascending aortas (**C, D**) were shown. Original magnification, 400×.
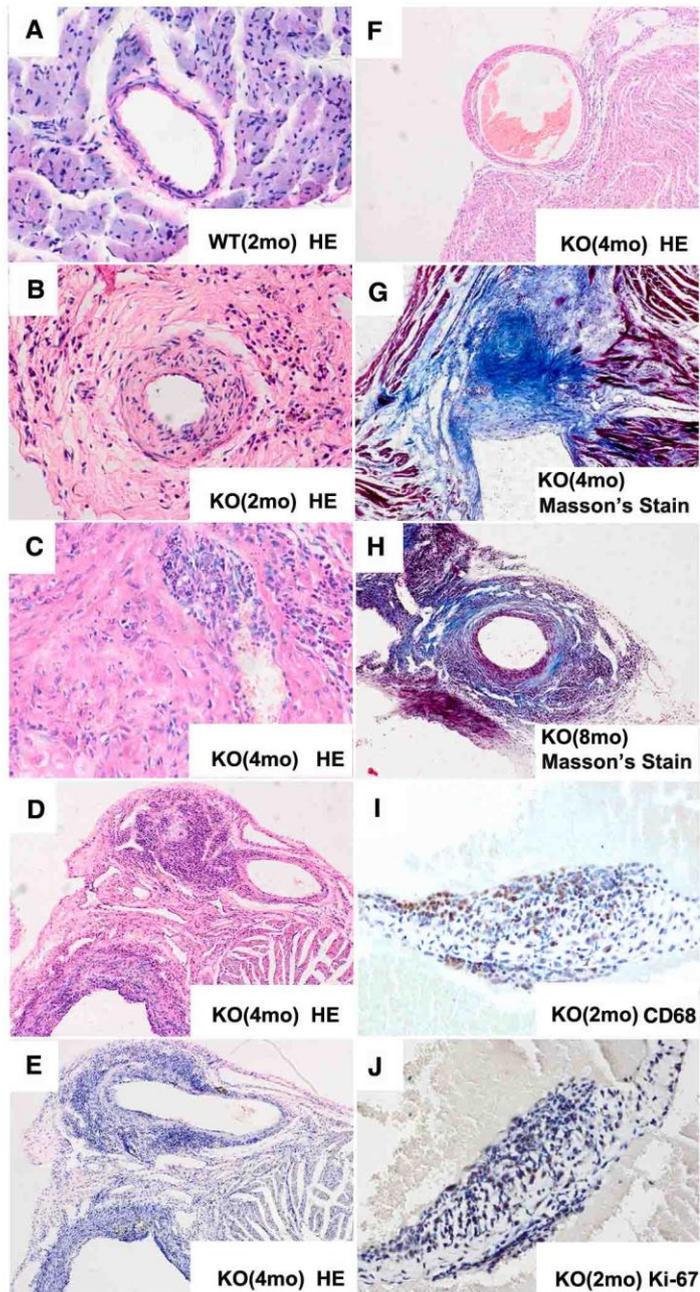
Supplemental Figure 4

Supplemental Figure 4. The expression of p-Smad3 in the *Smad3$^{+/+}$* mice. he image showed Immunostaining for p-Smad3 on slides of aortic root from *Smad3$^{+/+}$* aged at 2 , 4 and 8 weeks. Representative Western blot showing p-Smad3 levels in

proximal ascending aortas from *Smad3*$^{+/+}$ mice aged at 2 , 4 and 8 weeks. The ratio between p-Smad3 to GAPDH levels is shown. *$P < 0.01$,vs 4 or 8 weeks. Original magnification, $400\times$.

Supplemental Figure 5

Supplemental Figure 5. Inflammation in the coronary arteries and aortic valves from *Smad3*^{-/-} mice. **A,** HE staining showing normal coronary arteries from *Smad3*^{+/+} mice. **B,** Intimal thickening. C, Inflammatory cells accumulating in the vascular space. **D,** Complete occlusion of the coronary artery. **E, F,** Coronary artery ectasia. **G, H,** Varying degrees of vascular fibrosis. **I, J,** Adhesion of CD68^{+} cells to the aortic valve and expression of Ki-67, a cell proliferation marker. Original magnification, (D, E, F, H):100×; (A,B,C,G): 400×.
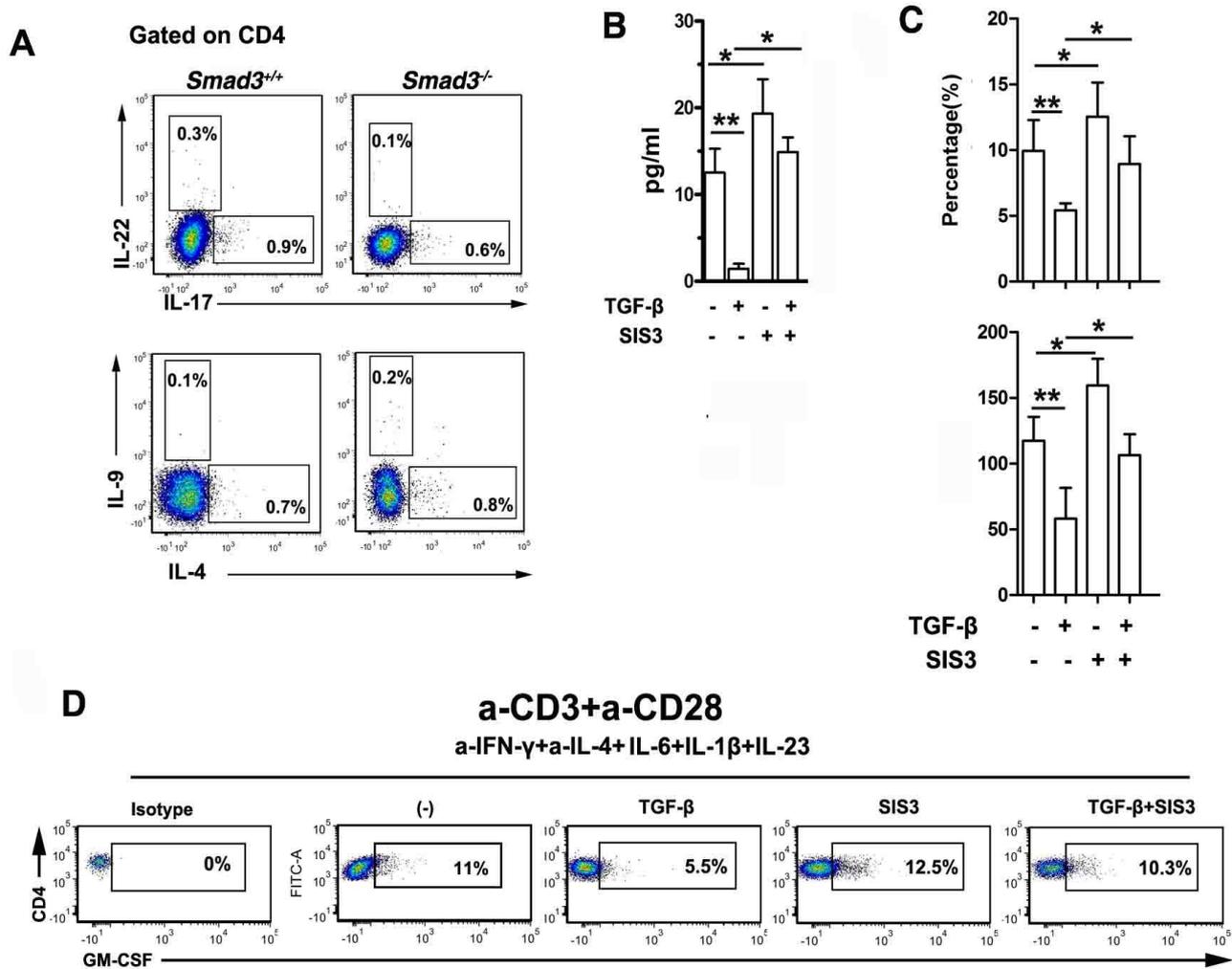
Supplemental Figure 6
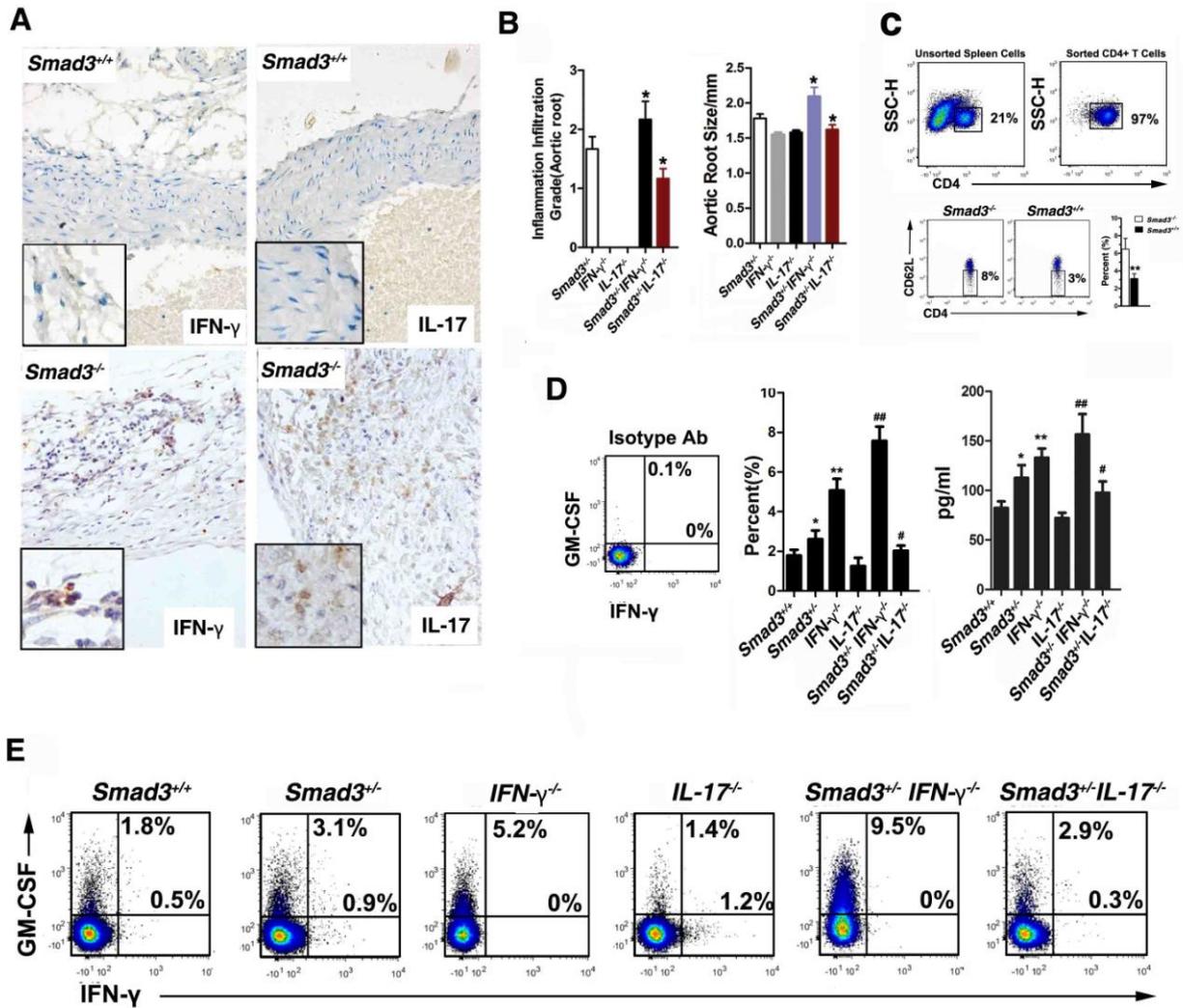
Supplemental Figure 6. IFN-γ and IL-17 play opposite role on regulation of GM-CSF secretion by CD4^{+} T cells, which impact the aortic pathological changes of *Smad3*^{+/-} mice. A, Immunostaining with the IFN-γ and IL-17 antibody revealed an accumulation of IFN-γ- and IL-17-producing cells in the aortas of *Smad3*^{-/-} mice, and no obvious positive staining was observed in the aortas of *Smad3*^{+/+} mice. Original magnification, 400×; Magnified panels, 1000×. **B,** The histograms show different inflammation severity and aortic root size in the aortic roots from 6 genetically engineered mice, which exhibited worse inflammation severity in *Smad3*^{+/-} *IFN-γ*^{-/-} mice, while *Smad3*^{+/-} *IL-17*^{-/-} mice showed slightly better than the *Smad3*^{+/-} mice. *$P < 0.01$.vs *Smad3*^{+/-} mice. **C,** Flow cytometry analysis of the sorting efficiency of CD4^{+}T cells. *Smad3*^{-/-} mice contains more CD4^{+}CD62L^{-} cells in the spleen than *Smad3*^{+/+} mice, which showed an activated phenotype. **D,** IFN-γ deficiency promoted GM-CSF secretion in CD4^{+} T cells from WT and *Smad3*^{+/-} mice, while IL-17 deficiency had a slight

inhibitory effect. The amount of GM-CSF in the supernatants was measured using a specific ELISA kit and was consistent with flow cytometry results. *$P < 0.05$, **$P < 0.01$ versus WT. [#]$P < 0.05$, [##]$P < 0.01$ versus *Smad3^{+/-}*. **E,** Flow cytometry analysis of the secretion of GM-CSF and IFN-γ by CD4^{+} T cells. Isotype control Abs were added for gating.

Supplemental Figure 7

Supplemental Figure 7. A, Under neutral priming conditions in spleen cells, *Smad3^{-/-}*CD4^{+} T cells appeared to have the same ability to secrete IL-4, IL-9, IL-22, and IL-17. Representative flow cytometry image were shown. **B,** GM-CSF concentration in the suspension were compared when isolated spleen CD4^{+}T cells were under neutral priming conditions with or without the addition of TGF-β/SIS3. *$P < 0.05$, **$P < 0.01$. C. Isolated spleen CD4^{+}T cells were transformed under GM-CSF priming conditions with or without the addition of TGF-β/SIS3. The percentage of CD4^{+} T cells producing GM-CSF and GM-CSF concentration in the suspension were compared between different groups. *$P < 0.05$, **$P < 0.01$. D. Intracellular cytokine staining was performed to determine the percentage of CD4^{+} T cells producing GM-CSF. Representative flow cytometry image were shown.

Supplemental Figure 8
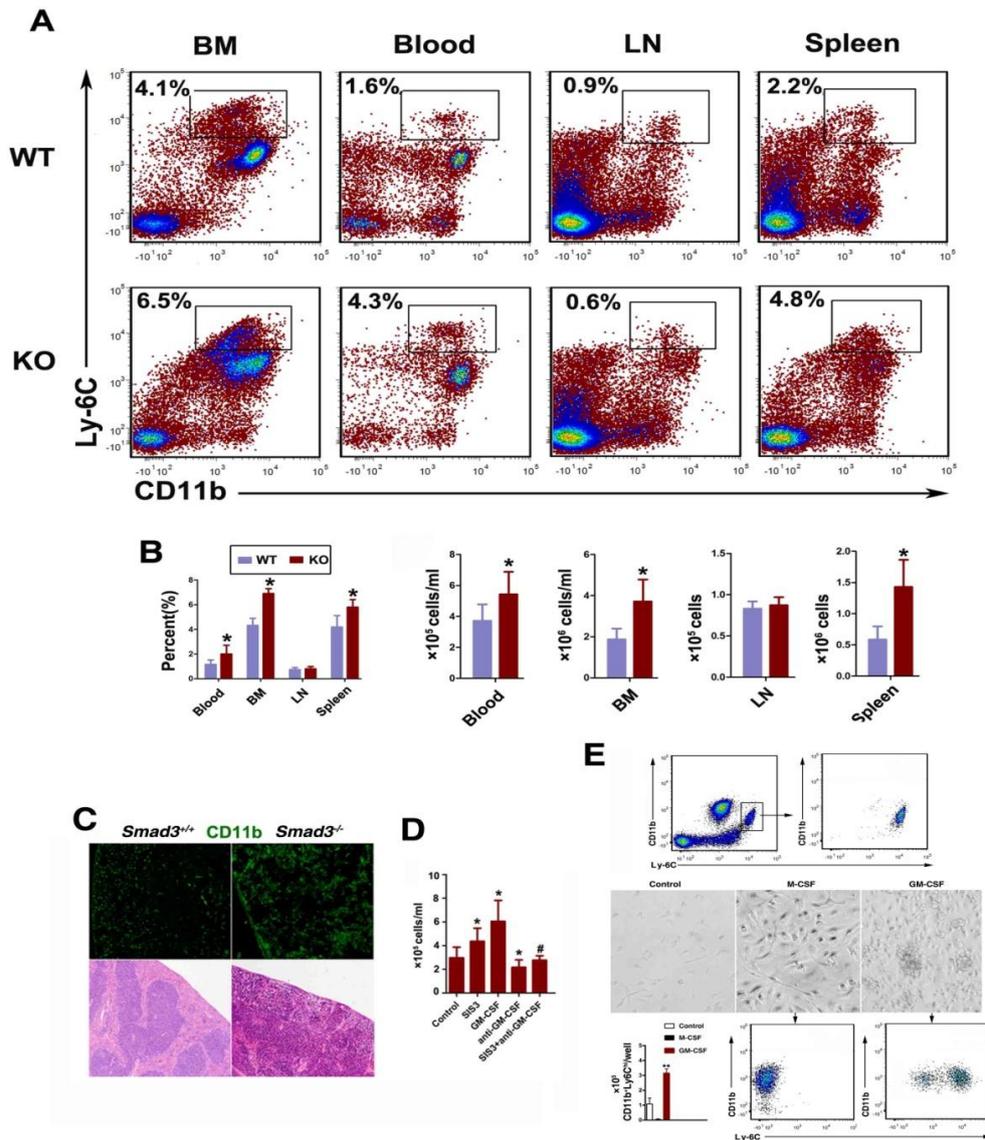
Supplemental Figure 8. Smad3 inhibition induced GM-CSF-dependent bone marrow and extramedullary hematopoiesis, which induced a relatively high number of CD11b^{+}Ly-6C^{hi} cells. **A,** Representative flow cytometry image of blood, spleen, LN and BM cells gated on Ly-6C and CD11b. **B,** *Smad3^{-/-}* mice contained more CD11b^{+}Ly-6C^{hi} inflammatory monocytes in the blood, BM and spleen. *$P < 0.05$. **C.** Representative HE staining of spleen cells and CD11b immunofluorescencestaining showed altered structure of spleen cells and arelatively increased number of CD11b^{+} cells in *Smad3^{-/-}* mice than that in *Smad3^{+/+}* mice. Original magnification, 200×. **D,** The percentage and number of CD11b^{+}Ly-6C^{hi} in 1ml blood from WT mice received different agents were compared. *$P < 0.05$ versus control. #$P < 0.05$ versus SIS3. **E,** Isolated the inflammatory monocytes from the blood of WT mice by flow cytometry, and cultured them with or without GM-CSF or M-CSF. M-CSF could effectively induce the maturation of inflammatory monocytes and their transformation into fusiform wall-adherent cells and they no longer express Ly-6C. GM-CSF maintained the most traits of the cells and promoted their proliferation. Without addition of any cytokines, the inflammatory monocytes hardly proliferated. **$P < 0.01$ versus control. Original magnification, 400×.

Supplemental Table 1. Clinical information of study participants

	Case1	Case2
Age(years)	35	40
Gender	F	M
Smoking	NO	NO
Hypertension	NO	NO
Atrial fibrillation	YES	NO
Thoracic aortic aneurysm	YES	YES
Diameter of aneurysm(cm)	6.4	5.4
Abdominal aortic aneurysm	NO	NO
Aortic dissection/rupture	NO	NO
Aortic tortuosity	YES	NO
Ventricular hypertrophy	NO	YES
Mitral valve anomalies	YES	NO
Congenital heart malformation	NO	NO
Osteoarthritis of $\geqq$ 1 joint	YES	YES
Joint laxity	NO	NO
Painful joints	YES	YES
Skeletal anomalies	NO	YES
Hypertelorism	NO	YES
Abnormal palate or uvula	NO	NO
Velvety skin	NO	NO