

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. *Fbn1*^{C1039G/+} hearts display normal cardiac function in the absence of hemodynamic stress. A. Echocardiographic quantification of cardiac dimensions and function in *Fbn1*^{+/+} and *Fbn1*^{C1039G/+} mice with and without valvular regurgitation at 4 and 12 months of age. EDD, end-diastolic diameter; ESD, end-systolic diameter; FS, fractional shortening; LVM, left ventricular mass. n_≥14 per group. *p<0.05, **, p<0.01, ***p<0.001, 2w ANOVA, Tukey's correction. B. Representative PV loop of a 12mo *Fbn1*^{+/+} (blue line, blue fill) and *Fbn1*^{C1039G/+} mouse (red line, red fill) without valvular regurgitation. Summary data obtained from PV loop analysis shown in bar graphs: E_{es}, end-systolic elastance and PRSW, preload recruitable stroke work, reflect load-independent indices of ventricular contractility. Tau, relaxation time constant reflects a load-independent index of ventricular stiffness; n=3-6 per group. C. dP/dt_{max}, peak rate of left ventricular pressure rise, an index of contractility; dP/dt_{min}, peak rate of left ventricular pressure decline, an index of lusitropy. Invasive blood pressure measurements taken from PV loop analysis of *Fbn1*^{+/+} and *Fbn1*^{C1039G/+} mice without valvular regurgitation at 4 and 12 months of age. n=3-6 per group. D. Gross morphometric analysis of post-natal *Fbn1*^{+/+}, *Fbn1*^{C1039G/+}, *Fbn1*^{C1039G/C1039G} mice at 8do, 6mo and 12mo age, in mice without valvular regurgitation. HW/BW, heart weight/body weight ratio; HW/TL, heart weight/tibial length ratio. n_≥4 per group.

Supplemental Figure 2. *Fbn1*^{C1039G/+} hearts have normal TGFβ signaling in the absence of hemodynamic stress. A-C. Representative Western blot and summary quantification for phosphorylated/total (p/t), p/tSmad2, p/tERK1/2, and GAPDH using left ventricular tissue lysates. n=4 per group. D. mRNA expression normalized to *18S* and then to *Fbn1*^{+/+} data, assessed by real-time RT-PCR from left ventricular tissue of 3mo *Fbn1*^{+/+} and

Fbn1^{C1039G/+} hearts without regurgitation. *Ctgf*, connective tissue growth factor. *Serpine1*, plasminogen activator inhibitor, type 1. n>=4 per group.

Supplemental Figure 3. *Fbn1*^{C1039G/+} hearts develop hypertrophy and fibrosis in response to pressure overload. A. Baseline echocardiographic quantification of cardiac dimensions in 5mo *Fbn1*^{+/+} and *Fbn1*^{C1039G/+} mice without valvular regurgitation. EDD, end-diastolic diameter; ESD, end-systolic diameter; IVST, interventricular septum thickness; PWT, posterior wall thickness. n=4-6 per group. B. Myocyte hypertrophy, as assessed by cross-sectional area (CSA) in hematoxylin-eosin staining. Summary quantification of averaged data, >20 cells per heart. n=4 per group. C. Fibrotic area, as assessed by Masson's Trichrome stain and quantified by total number of blue pixels normalized to whole tissue area and then to *Fbn1*^{+/+}:SHAM data. n=4-6 per group. D. mRNA expression of hypertrophy genes, normalized to *Gapdh* and then to *Fbn1*^{+/+}:SHAM data, assessed by real-time RT-PCR. n=4-6 per group. *Myh7*, myosin heavy chain 7 (βMHC), *Nppa*, natriuretic peptide A (ANP), *Nppb*, natriuretic peptide B (BNP). **p<0.01, ***p<0.001, 1w ANOVA, Tukey's correction.

Supplemental Figure 4. *Fibrillin-1* protein fails to deposit in myocardium in load-induced heart failure in *Fbn1*^{C1039G/+} mice. Additional representative images of immunofluorescent fibrillin-1 staining (red) of heart sections from *Fbn1*^{+/+} and *Fbn1*^{C1039G/+} mice subjected to 4w TAC. Red, fibrillin-1 (interstitial space); blue, DAPI (nuclei); Green, lipofuscin (myocytes). Top 4 panels, Scale bar: 20 μm. Bottom 4 panels, 2x zoom. White arrow head points to an example nonmyocyte cell in the interstitial space.

Supplemental Figure 5. *Smad2* and *ERK1/2* activation are differentially increased in the myocyte and nonmyocyte compartments of failing *Fbn1*^{C1039G/+} hearts. A. Representative serial sections of pSmad2 (red, top panels) and pERK1/2 (red, bottom panels) immunostaining in *Fbn1*^{C1039G/+}

hearts subjected to 4w TAC. Far left panels, scale bar: 50 μ m. Middle and far right panels, zoom 2x. Blue, DAPI (nuclei); Green, lipofuscin (myocytes). White arrows, myocyte nuclei. Yellow arrows, nonmyocyte nuclei. B. Summary quantification of % of total pSmad2 and pERK1/2 immunofluorescence intensity localized to nonmyocyte (NM) and myocyte (M) cells per unit area. n=10-15 areas quantified. *p<0.05, **p<0.01, Student's t-test. C. Representative myocyte-enriched serial sections. Scale bar: 50 μ m. D. Representative co-immunostaining of serial sections with vimentin (yellow) and pSmad2 (left panel) and vimentin (yellow) and pERK1/2 (right panel). Scale bar: 25 μ m. Gray, lipofuscin (myocytes).

Supplemental Figure 6. *ERK1/2 activation, but not Smad2 activation, is significantly attenuated by MEK inhibition.* Immunoblot for pSmad2, pERK1/2 and GAPDH using cultured neonatal rat cardiac fibroblasts with TGF- β 3 (10 ng/ml) stimulation for 15 minutes with and without treatment with MEKi, RDEA119 (100nM) or PD98059 (50 μ M). n=4 per group. **p<0.01, ***p<0.001, 1w ANOVA, Tukey's correction.

Supplemental Figure 7. *Myocyte hypertrophy in $Fbn1^{C1039G/+}$ hearts is prevented with losartan and MEKi treatment.* A. Representative hematoxylin-eosin staining of formalin-fixed heart sections. White dotted line delineates myocyte diameter. Scale bars: 50 μ m. B. Myocyte hypertrophy as assessed by wheat germ agglutinin (WGA) staining. Scale bars: 25 μ m. C. Summary quantification of average cross-sectional area (CSA) data, >1000 cells per heart. n=4-7 per group. D. mRNA expression of hypertrophy genes, normalized to *Gapdh* and then to *Fbn1^{+/+}*:SHAM data, assessed by real-time RT-PCR. *Myh7*, myosin heavy chain 7 (β MHC), *Myh6*, myosin heavy chain 6 (α MHC), *Nppa*, natriuretic peptide A (ANP), *Nppb*, natriuretic peptide B (BNP). n=4-7 per group. *p<0.05, **p<0.01, ***p<0.001, 1w ANOVA, Tukey's correction.

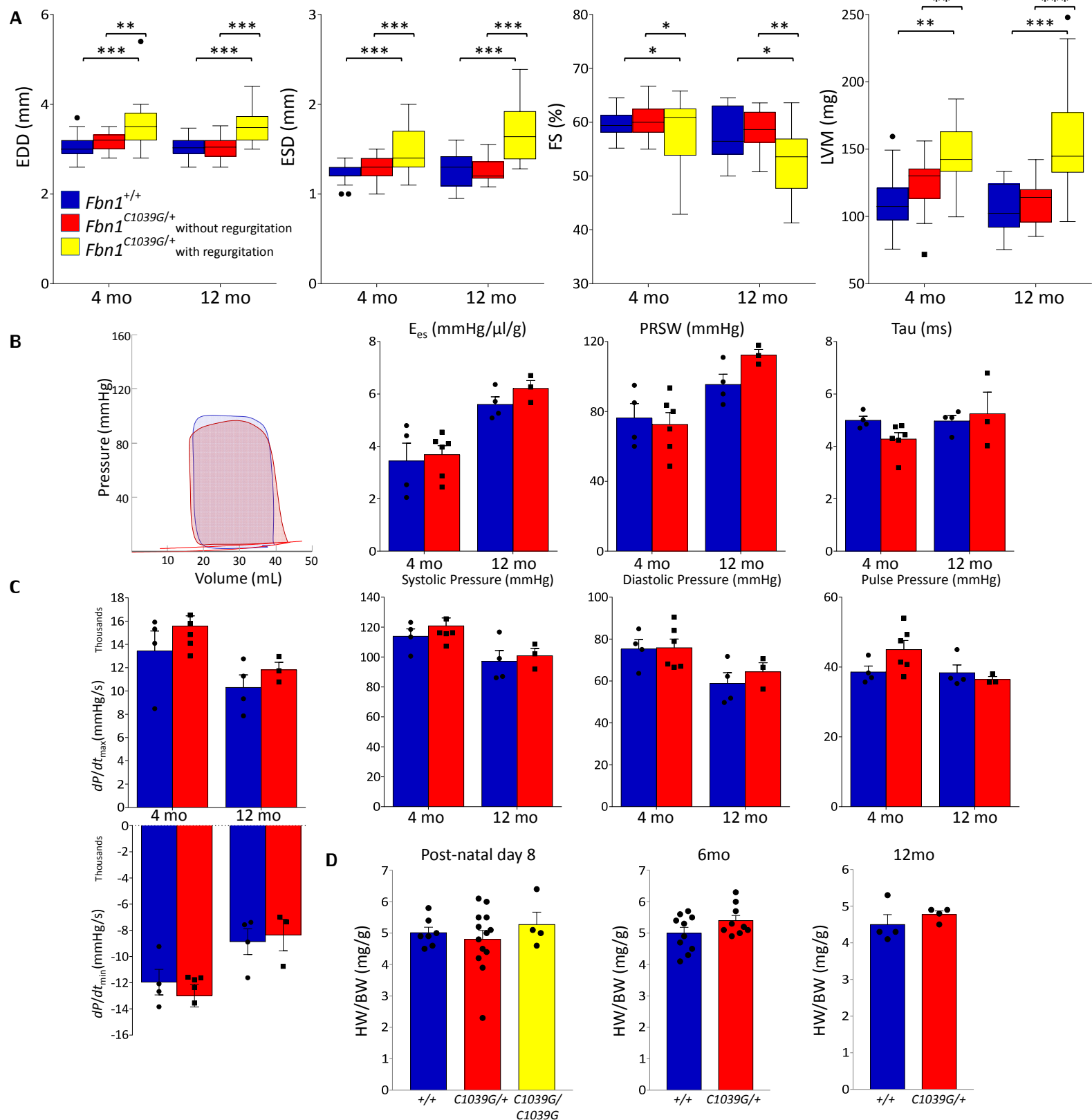
Supplemental Figure 8. *Smad2* and *ERK1/2* activation are differentially increased in response to *TGFβ* and angiotensin II in cardiac fibroblasts. Immunoblot for pSmad2, pERK1/2 and GAPDH using cultured neonatal murine cardiac fibroblasts with TGFβ1 (5 ng/ml) or AngII (5nM) stimulation for 24 hours. Lanes were run on the same gel but were noncontiguous. n>=3 per group. **p<0.01, 1w ANOVA, Tukey's correction.

Supplemental Figure 9. *Inhibition of ERK1/2 activation suppresses Smad2 activation in both nonmyocyte and myocyte compartments.* A-B. Labeling index (% positive cells) of pSmad2 or pERK1/2 immunofluorescence in the nonmyocyte and myocyte compartments. n>5 fields per group. C. Total number of nonmyocytes and myocytes normalized to *Fbn1*^{+/+}:SHAM data. n=15-30 fields per group. *p<0.05, **p<0.01, ***p<0.001, 1w ANOVA, Tukey's correction.

Supplemental Figure 10. *TGFβ3 is predominantly expressed in the nonmyocyte compartment.* A-C. Representative *in situ* hybridization of *Tgfb3*. A. *Fbn1*^{+/+}:TAC vs *Fbn1*^{C1039G/+}:TAC, *Tgfb3*, red. Left panel, scale bar: 10μm. Right panel, zoom 2x inset. Dotted line demarcates myocyte compartment. Red arrows point to examples of *in situ* RNA hybridization (red dots) found throughout the nonmyocyte compartment. B. Myocyte- vs nonmyocyte-enriched areas. Scale bar: 10μm. C. *Tgfb3* and *Vim*. Left panel, *Tgfb3*, red, left panel and *Vim*, red, right panel. Dotted-line outlines the cardiac myocytes. Scale bar: 10μm. D. mRNA expression of *Tgfb3* and *Vim* normalized to *Gapdh* and then to myocyte data. n=4-6 per group. ***p<0.001, Student's t-test.

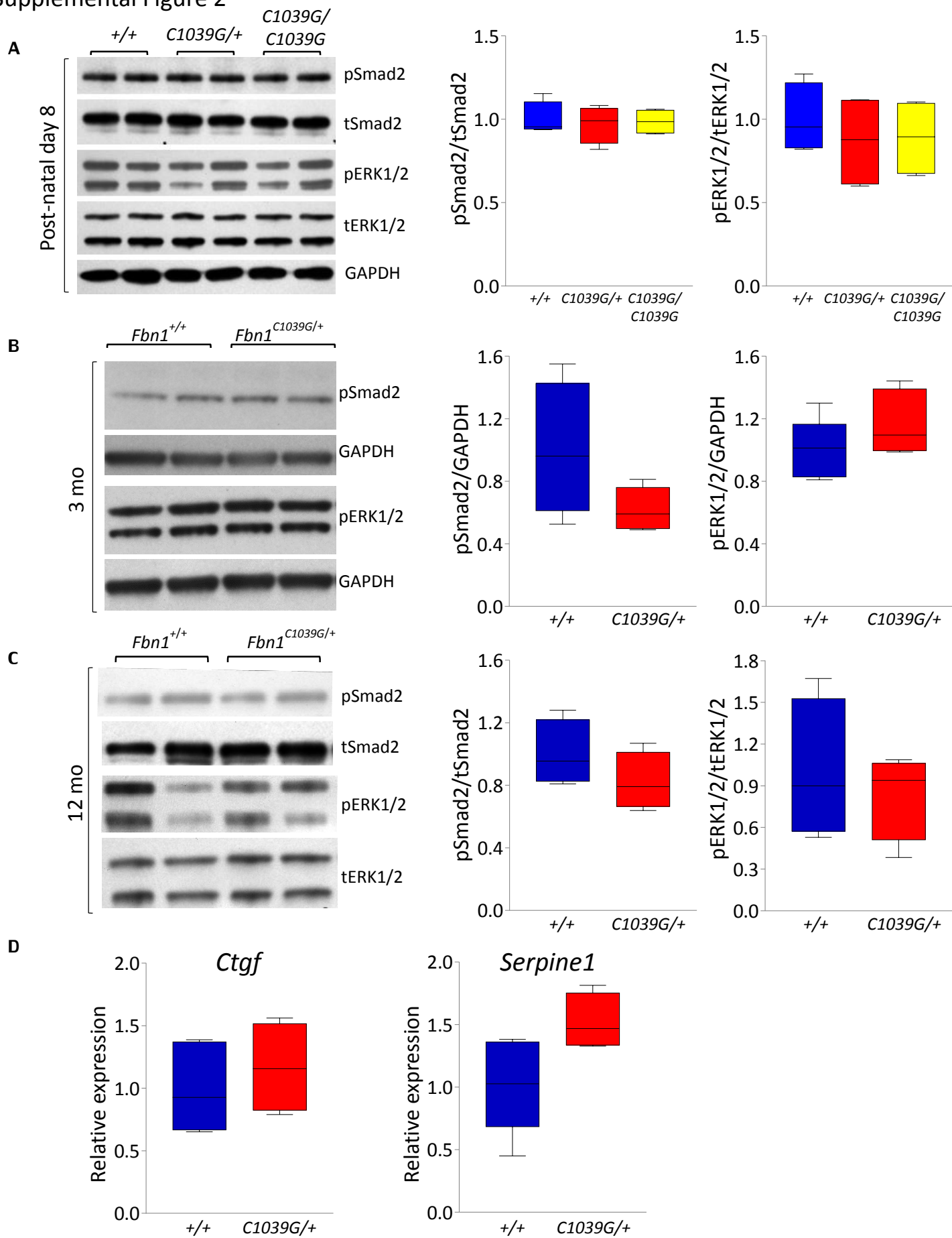
Supplemental Figure 11. *AngII-mediated ERK1/2 activation is differentially increased in Fbn1*^{C1039G/+} vs WT fibroblasts. Immunoblots for pSmad2, pERK1/2 and GAPDH using cultured neonatal murine cardiac fibroblasts from *Fbn1*^{C1039G/+} and *Fbn1*^{+/+} hearts with AngII (5nM) or TGFβ3 (5 ng/ml) stimulation for 12 hours. Lanes were run on the same gel but were noncontiguous. n>=3 per group. ***p<0.001, 1w ANOVA, Tukey's correction.

Supplemental Figure 1



Supplemental Figure 1. *Fbn1*^{C1039G/+} hearts display normal cardiac function in the absence of hemodynamic stress. A. Echocardiographic quantification of cardiac dimensions and function in *Fbn1*^{+/+} and *Fbn1*^{C1039G/+} mice with and without valvular regurgitation at 4 and 12 months of age. EDD, end-diastolic diameter; ESD, end-systolic diameter; FS, fractional shortening; LVM, left ventricular mass. $n \geq 14$ per group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, 2w ANOVA, Tukey's correction. B. Representative PV loop of a 12mo *Fbn1*^{+/+} (blue line, blue fill) and *Fbn1*^{C1039G/+} mouse (red line, red fill) without valvular regurgitation. Summary data obtained from PV loop analysis shown in bar graphs: E_{es} , end-systolic elastance and PRSW, preload recruitable stroke work, reflect load-independent indices of ventricular contractility. Tau, relaxation time constant reflects a load-independent index of ventricular stiffness; $n = 3-6$ per group. C. dP/dt_{max} , peak rate of left ventricular pressure rise, an index of contractility; dP/dt_{min} , peak rate of left ventricular pressure decline, an index of lusitropy. Invasive blood pressure measurements taken from PV loop analysis of *Fbn1*^{+/+} and *Fbn1*^{C1039G/+} mice without valvular regurgitation at 4 and 12 months of age. $n = 3-6$ per group. D. Gross morphometric analysis of post-natal *Fbn1*^{+/+}, *Fbn1*^{C1039G/+}, *Fbn1*^{C1039G/C1039G} mice at 8do, 6mo and 12mo age, in mice without valvular regurgitation. HW/BW, heart weight/body weight ratio; HW/TL, heart weight/tibial length ratio. $n \geq 4$ per group.

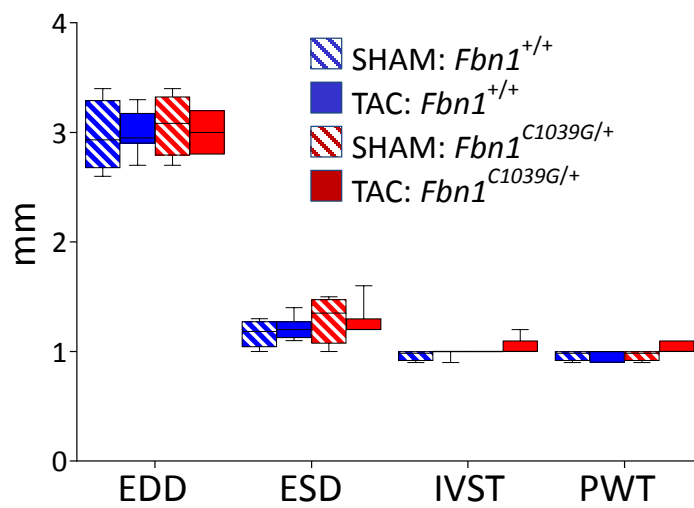
Supplemental Figure 2



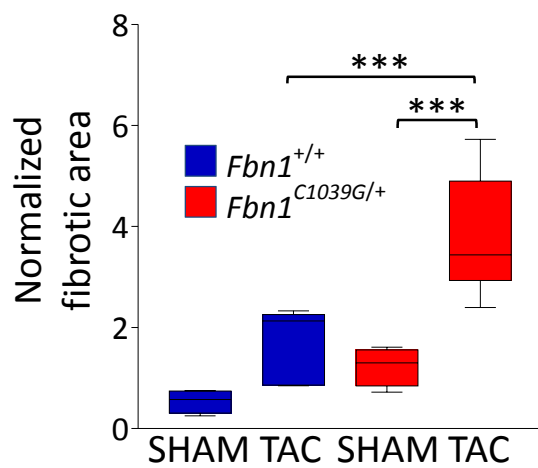
Supplemental Figure 2. *Fbn1*^{C1039G/+} hearts have normal TGF β signaling in the absence of hemodynamic stress. A-C. Representative Western blot and summary quantification for phosphorylated/total (p/t), p/tSmad2, p/tERK1/2, and GAPDH using left ventricular tissue lysates. n=4 per group. D. mRNA expression normalized to 18S and then to *Fbn1*^{+/+} data, assessed by real-time RT-PCR from left ventricular tissue of 3mo *Fbn1*^{+/+} and *Fbn1*^{C1039G/+} hearts without regurgitation. *Ctgf*, connective tissue growth factor. *Serpine1*, plasminogen activator inhibitor, type 1. n>=4 per group.

Supplemental Figure 3

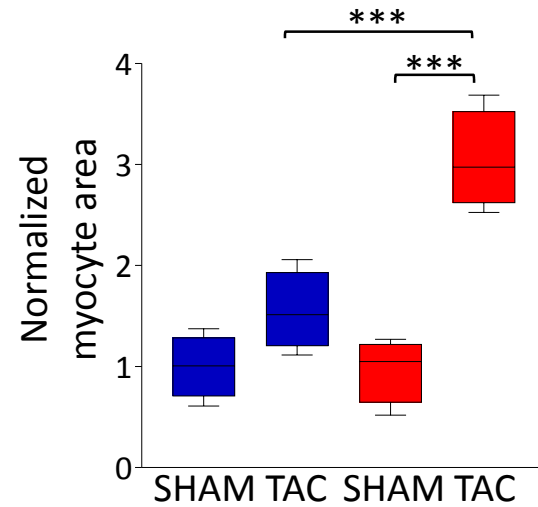
A



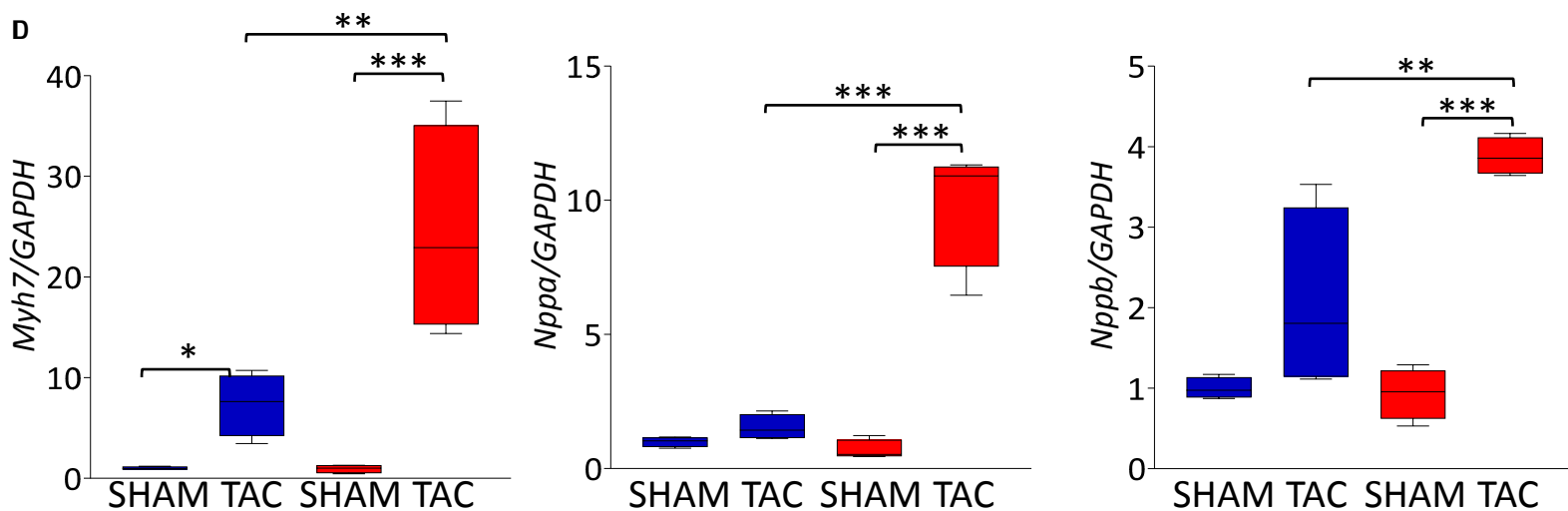
B



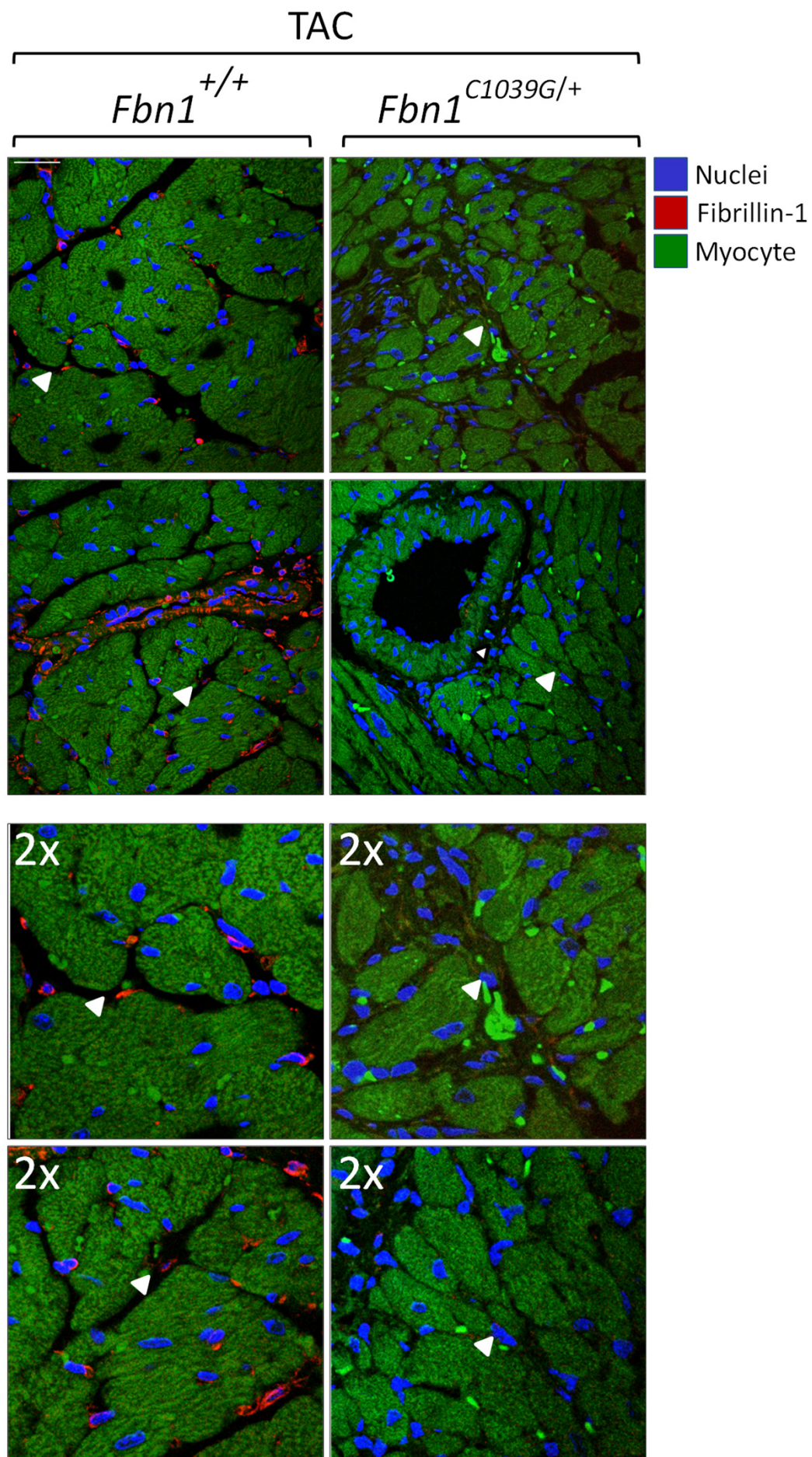
C



D

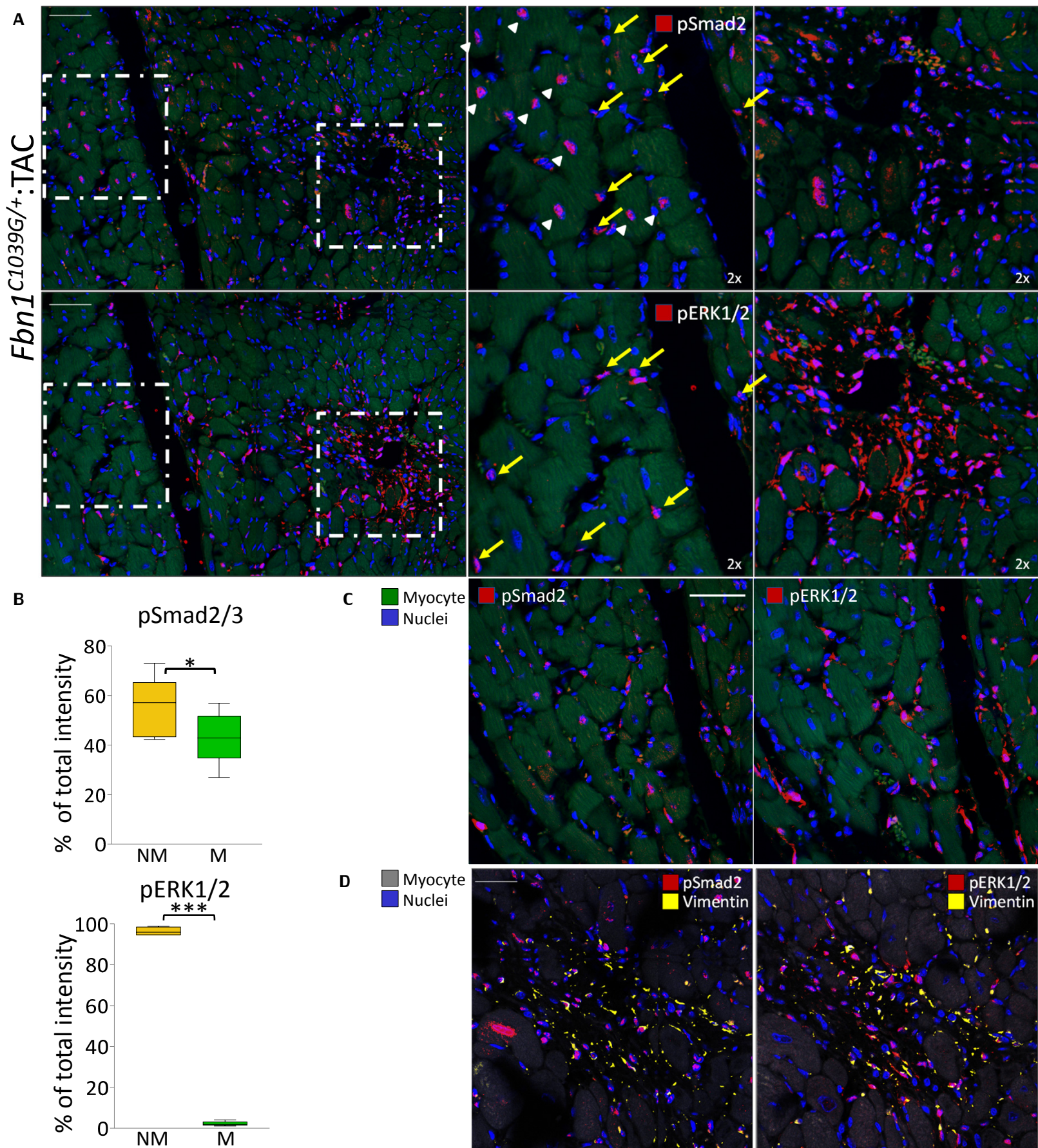


Supplemental Figure 3. *Fbn1*^{C1039G/+} hearts develop hypertrophy and fibrosis in response to pressure overload. A. Baseline echocardiographic quantification of cardiac dimensions in 5mo *Fbn1*^{+/+} and *Fbn1*^{C1039G/+} mice without valvular regurgitation. EDD, end-diastolic diameter; ESD, end-systolic diameter; IVST, interventricular septum thickness; PWT, posterior wall thickness. n=4-6 per group. B. Myocyte hypertrophy, as assessed by cross-sectional area (CSA) in hematoxylin-eosin staining. Summary quantification of averaged data, >20 cells per heart. n=4 per group. C. Fibrotic area, as assessed by Masson's Trichrome stain and quantified by total number of blue pixels normalized to whole tissue area and then to *Fbn1*^{+/+}:SHAM data. n=4-6 per group. D. mRNA expression of hypertrophy genes, normalized to *Gapdh* and then to *Fbn1*^{+/+}:SHAM data, assessed by real-time RT-PCR. n=4-6 per group. *Myh7*, myosin heavy chain 7 (β MHC), *Nppa*, natriuretic peptide A (ANP), *Nppb*, natriuretic peptide B (BNP). **p<0.01, ***p<0.001, 1w ANOVA, Tukey's correction.



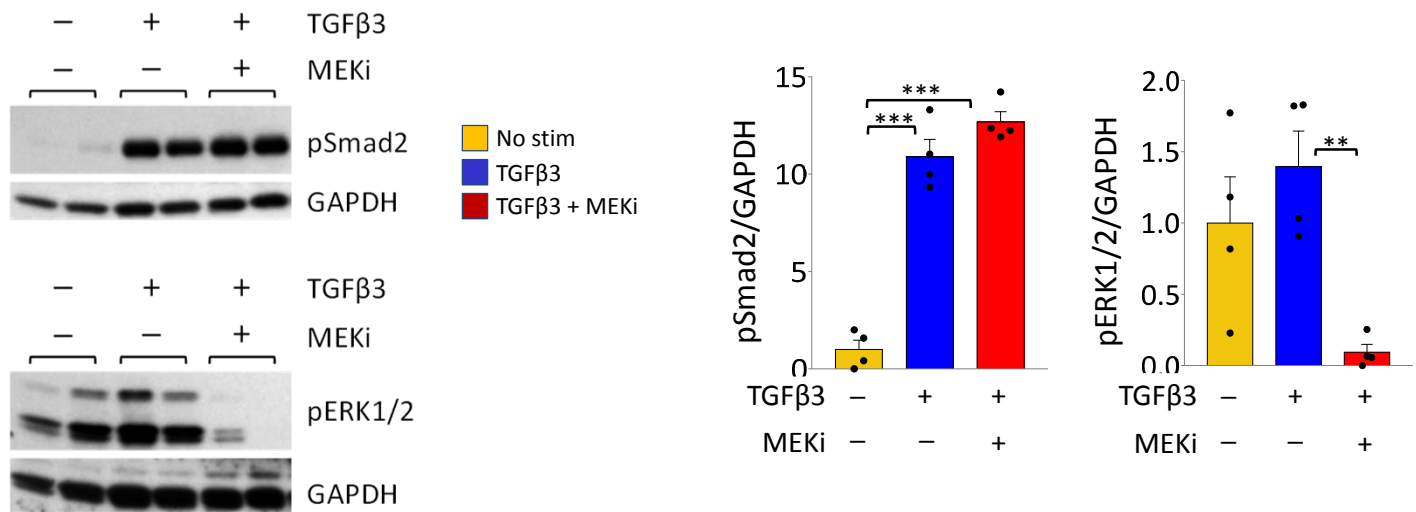
Supplemental Figure 4. *Fibrillin-1* protein fails to deposit in myocardium in load-induced heart failure in $Fbn1^{C1039G/+}$ mice. Additional representative images of immunofluorescent fibrillin-1 staining (red) of heart sections from $Fbn1^{+/+}$ and $Fbn1^{C1039G/+}$ mice subjected to 4w TAC. Red, fibrillin-1 (interstitial space); blue, DAPI (nuclei); Green, lipofuscin (myocytes). Top 4 panels, Scale bar: 20 μ m. Bottom 4 panels, 2x zoom. White arrow head points to an example nonmyocyte cell in the interstitial space.

Supplemental Figure 5



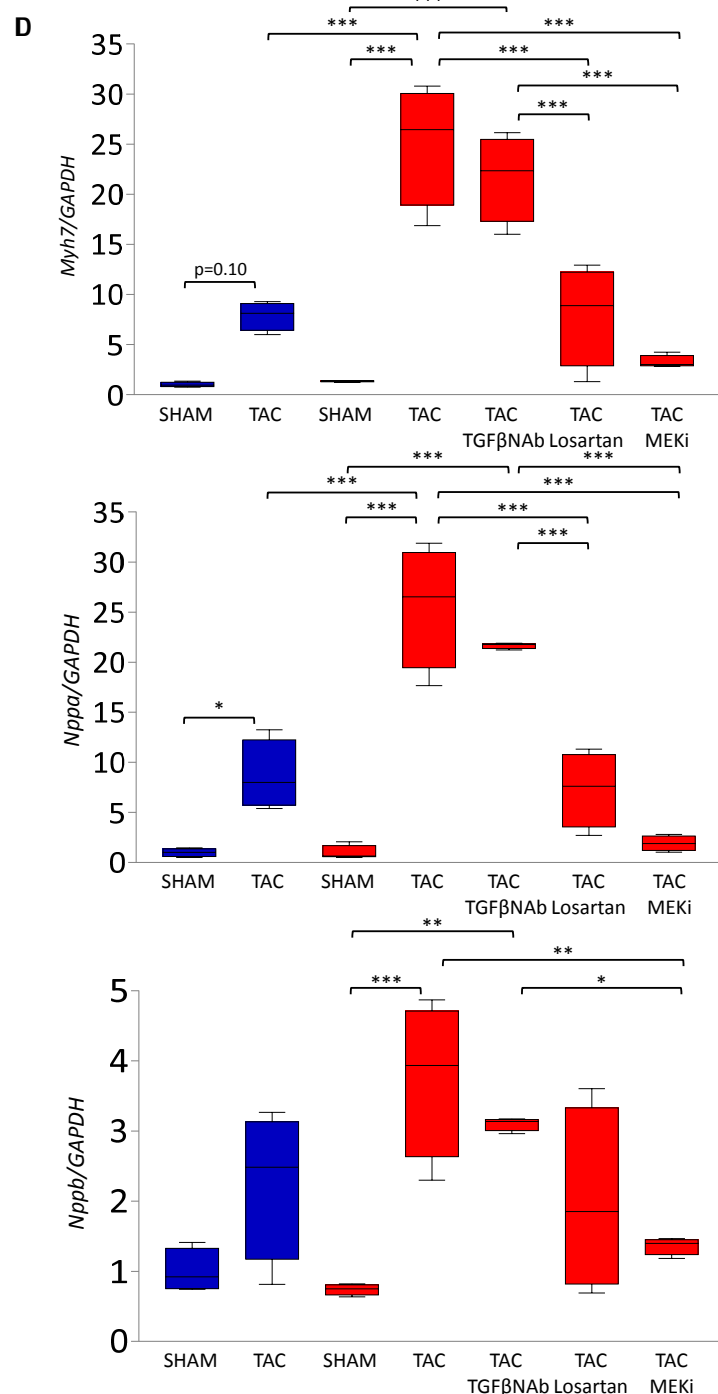
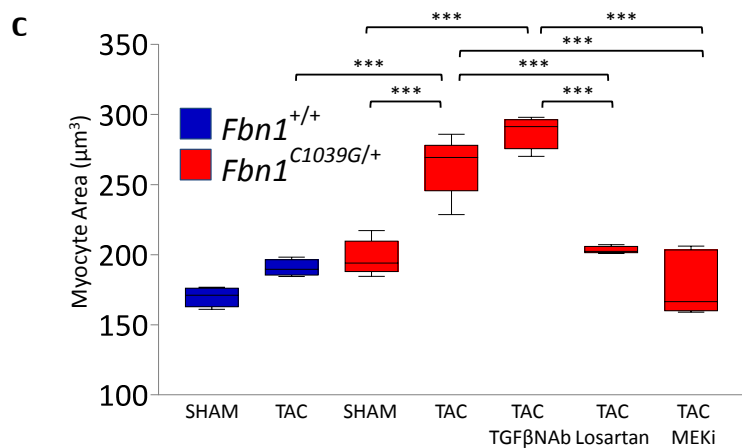
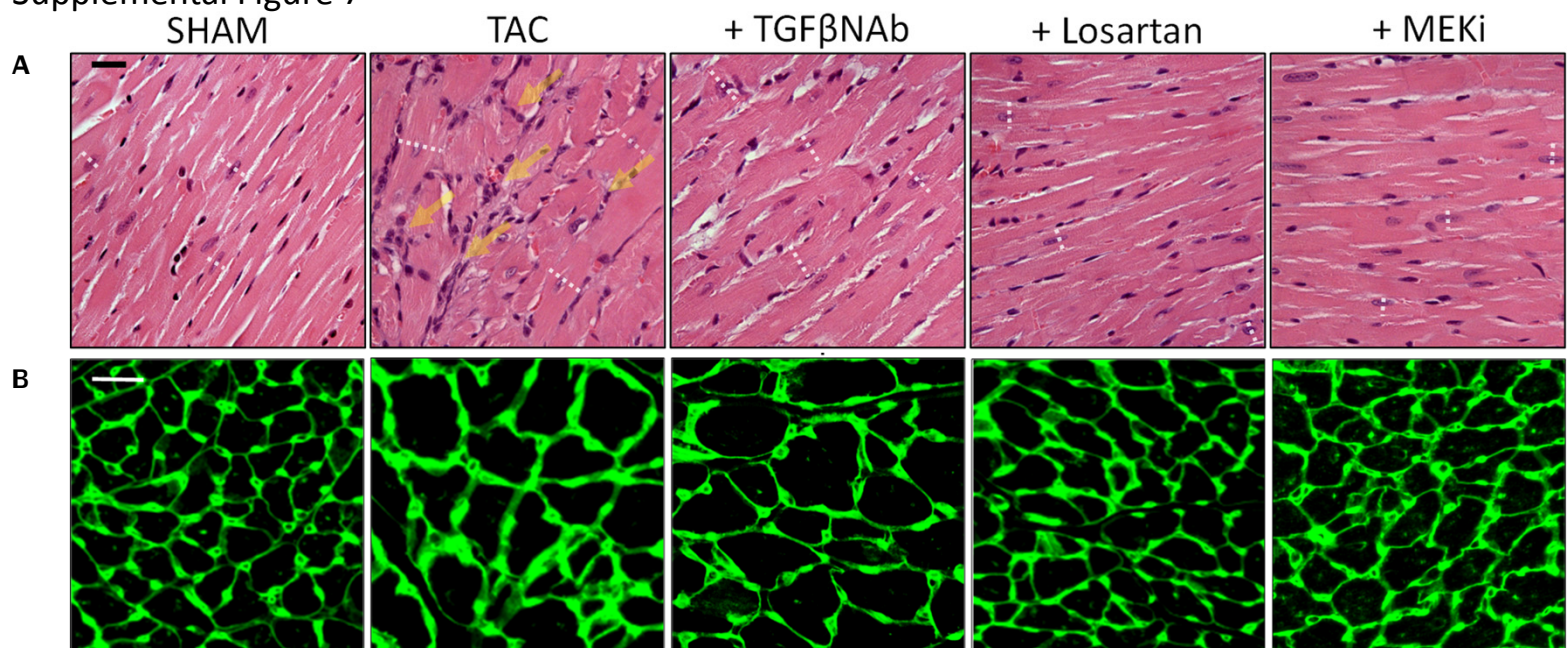
Supplemental Figure 5. *Smad2* and *ERK1/2* activation are differentially increased in the myocyte and nonmyocyte compartments of failing *Fbn1^{C1039G/+}* hearts. A. Representative serial sections of pSmad2 (red, top panels) and pERK1/2 (red, bottom panels) immunostaining in *Fbn1^{C1039G/+}* hearts subjected to 4w TAC. Far left panels, scale bar: 50 μ m. Middle and far right panels, zoom 2x. Blue, DAPI (nuclei); Green, lipofuscin (myocytes). White arrows, myocyte nuclei. Yellow arrows, nonmyocyte nuclei. B. Summary quantification of % of total pSmad2 and pERK1/2 immunofluorescence intensity localized to nonmyocyte (NM) and myocyte (M) cells per unit area. n=10-15 areas quantified. * $p < 0.05$, *** $p < 0.01$, Student's t-test. C. Representative myocyte-enriched serial sections. Scale bar: 50 μ m. D. Representative co-immunostaining of serial sections with vimentin (yellow) and pSmad2 (left panel) and vimentin (yellow) and pERK1/2 (right panel). Scale bar: 25 μ m. Gray, lipofuscin (myocytes).

Supplemental Figure 6



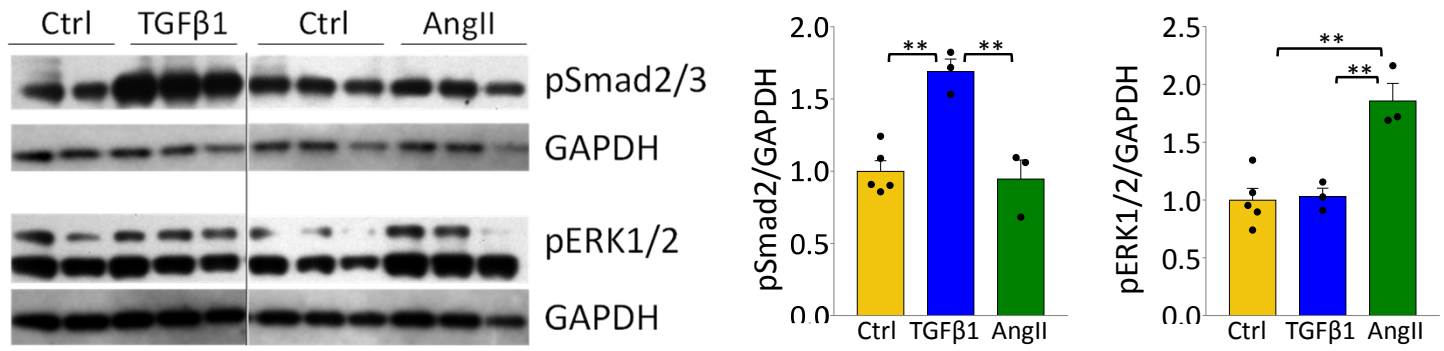
Supplemental Figure 6. *ERK1/2* activation, but not *Smad2* activation, is significantly attenuated by MEK inhibition. Immunoblot for pSmad2, pERK1/2 and GAPDH using cultured neonatal rat cardiac fibroblasts with TGF-β3 (10 ng/ml) stimulation for 15 minutes with and without treatment with MEKi, RDEA119 (100nM) or PD98059 (50μM). n=4 per group. **p<0.01, ***p<0.001, 1w ANOVA, Tukey's correction.

Supplemental Figure 7

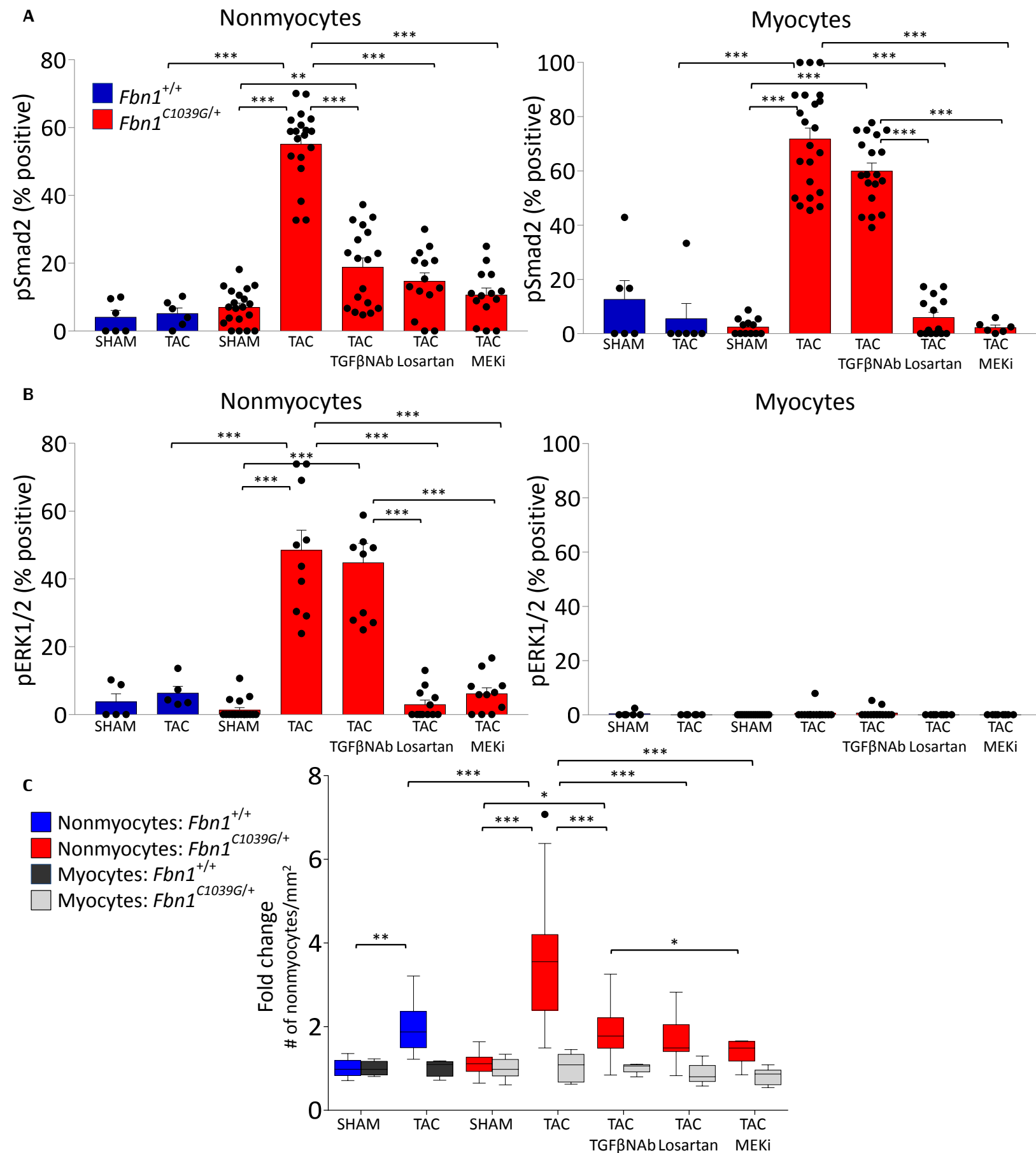


Supplemental Figure 7. Myocyte hypertrophy in *Fbn1*^{C1039G/+} hearts is prevented with losartan and MEKi treatment. **A**. Representative hematoxylin-eosin staining of formalin-fixed heart sections. White dotted line delineates myocyte diameter. Scale bars: 50 μm. **B**. Myocyte hypertrophy as assessed by wheat germ agglutinin (WGA) staining. Scale bars: 25 μm. **C**. Summary quantification of average cross-sectional area (CSA) data, >1000 cells per heart. n=4-7 per group. **D**. mRNA expression of hypertrophy genes, normalized to *Gapdh* and then to *Fbn1*^{+/+}:SHAM data, assessed by real-time RT-PCR. *Myh7*, myosin heavy chain 7 (βMHC), *Myh6*, myosin heavy chain 6 (αMHC), *Nppa*, natriuretic peptide A (ANP), *Nppb*, natriuretic peptide B (BNP). n=4-7 per group. *p<0.05, **p<0.01, ***p<0.001, 1w ANOVA, Tukey's correction.

Supplemental Figure 8

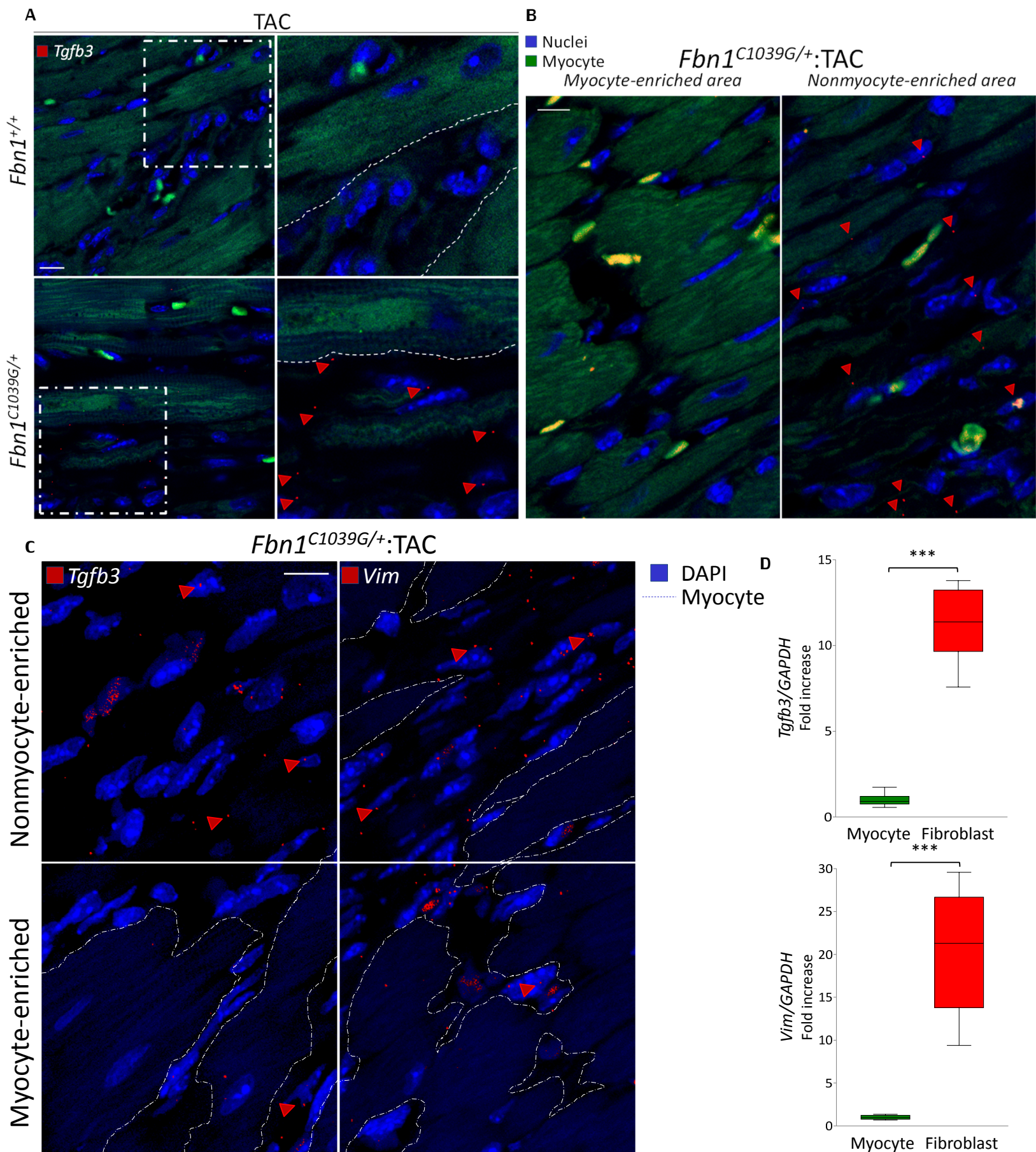


Supplemental Figure 8. *Smad2* and *ERK1/2* activation are differentially increased in response to *TGFβ* and angiotensin II in cardiac fibroblasts. Immunoblot for pSmad2, pERK1/2 and GAPDH using cultured neonatal murine cardiac fibroblasts with TGFβ1 (5 ng/ml) or AngII (5nM) stimulation for 24 hours. Lanes were run on the same gel but were noncontiguous. n>=3 per group. **p<0.01, 1w ANOVA, Tukey's correction.



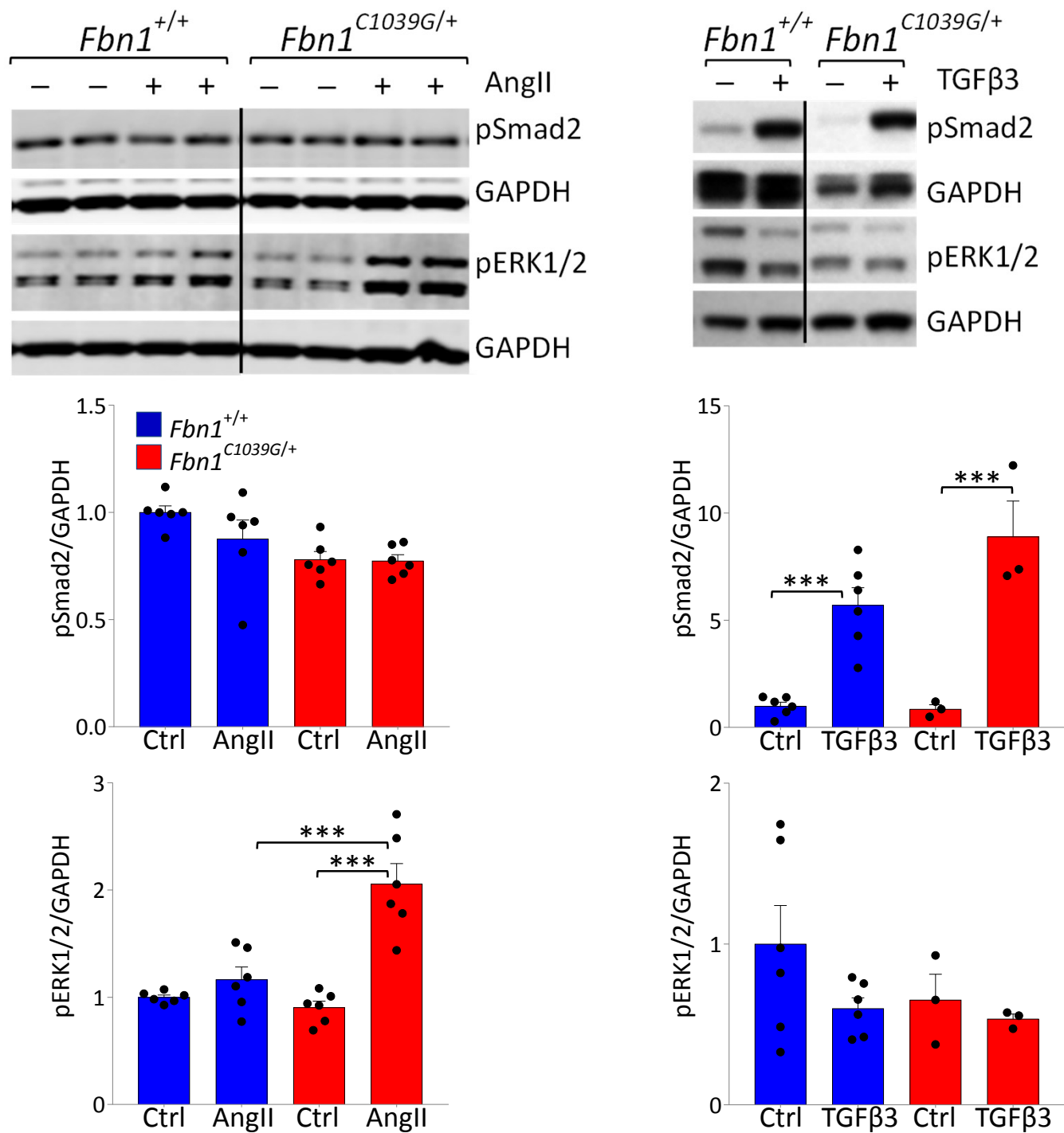
Supplemental Figure 9. Inhibition of ERK1/2 activation suppresses Smad2 activation in both nonmyocyte and myocyte compartments. A-B. Labeling index (% positive cells) of pSmad2 or pERK1/2 immunofluorescence in the nonmyocyte and myocyte compartments. n>5 fields per group. C. Total number of nonmyocytes and myocytes normalized to *Fbn1*^{+/+}:SHAM data. n=15-30 fields per group. *p<0.05, **p<0.01, ***p<0.001, 1w ANOVA, Tukey's correction.

Supplemental Figure 10



Supplemental Figure 10. *TGFβ3* is predominantly expressed in the nonmyocyte compartment. A-C. Representative *in situ* hybridization of *Tgfb3*. A. *Fbn1*^{+/+}:TAC vs *Fbn1*^{C1039G/+}:TAC, *Tgfb3*, red. Left panel, scale bar: 10μm. Right panel, zoom 2x inset. Dotted line demarcates myocyte compartment. Red arrows point to examples of *in situ* RNA hybridization (red dots) found throughout the nonmyocyte compartment. B. Myocyte- vs nonmyocyte-enriched areas. Scale bar: 10μm. C. *Tgfb3* and *Vim*. Left panel, *Tgfb3*, red, left panel and *Vim*, red, right panel. Dotted-line outlines the cardiac myocytes. Scale bar: 10μm. D. mRNA expression of *Tgfb3* and *Vim* normalized to *Gapdh* and then to myocyte data. n=4-6 per group. ***p<0.001, Student's t-test.

Supplemental Figure 11



Supplemental Figure 11. *AngII-mediated ERK1/2 activation is differentially increased in $Fbn1^{C1039G/+}$ vs WT fibroblasts.* Immunoblots for pSmad2, pERK1/2 and GAPDH using cultured neonatal murine cardiac fibroblasts from $Fbn1^{C1039G/+}$ and $Fbn1^{+/+}$ hearts with AngII (5nM) or TGFβ3 (5 ng/ml) stimulation for 12 hours. Lanes were run on the same gel but were noncontiguous. n>=3 per group. ***p<0.001, 1w ANOVA, Tukey's correction.