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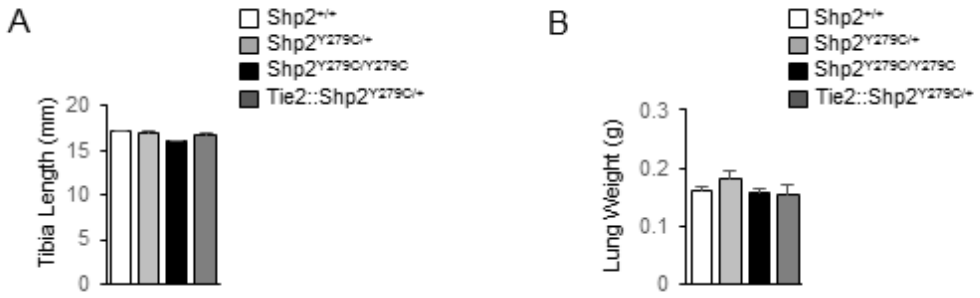
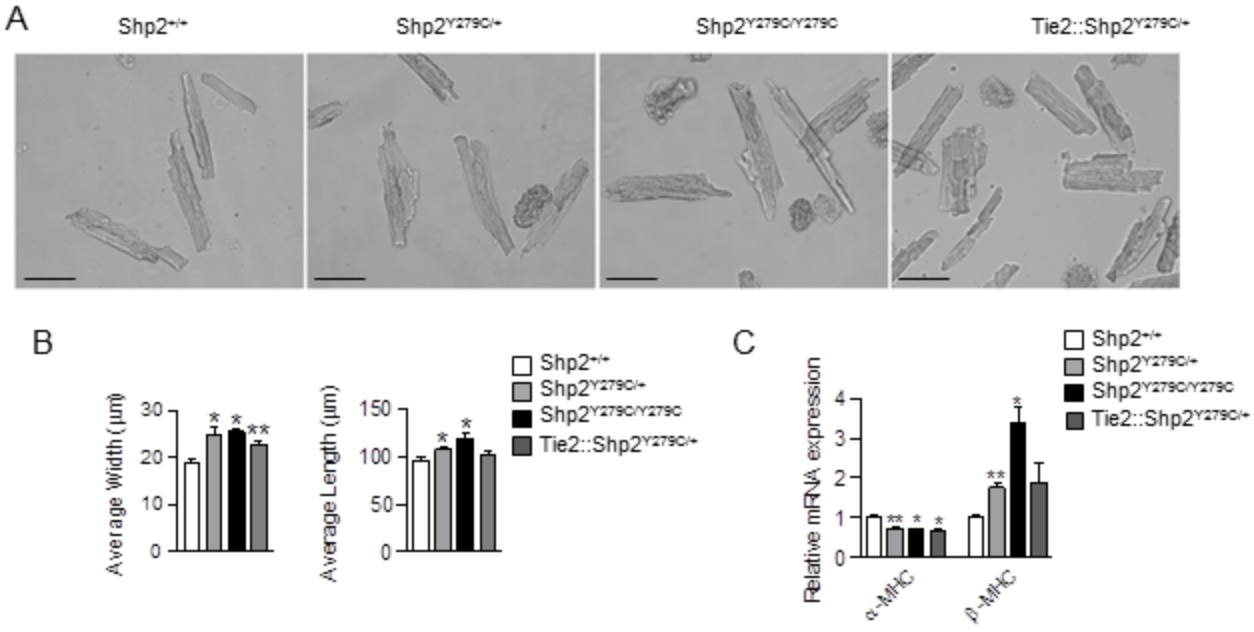
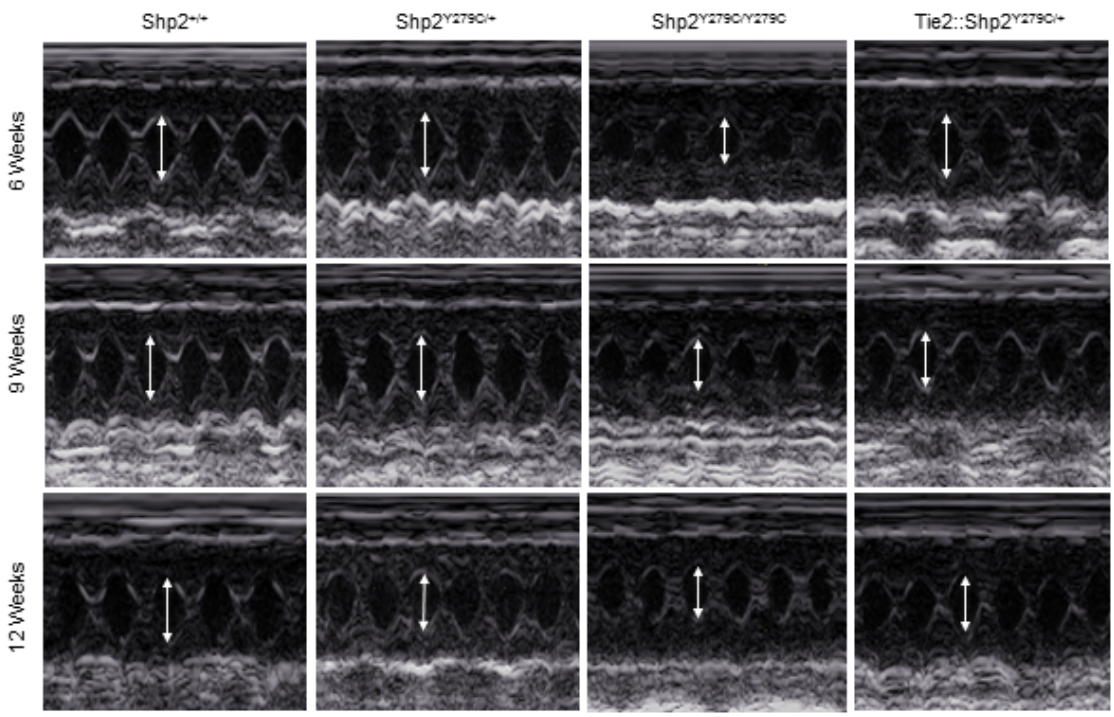


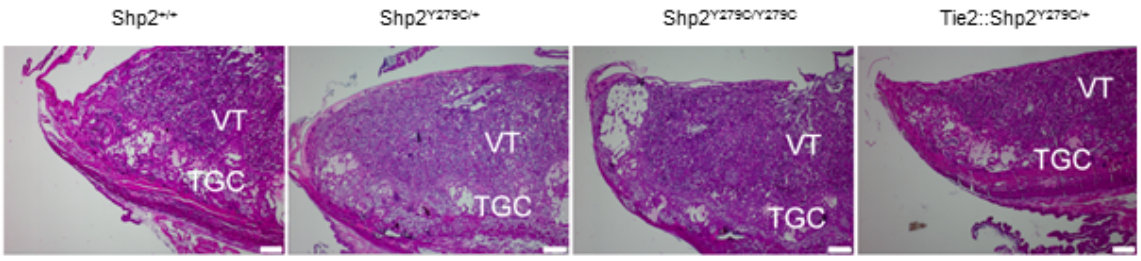
Supplemental Figure 1. **NSML mice have no differences in tibia lengths or lung weights at 12-weeks of age**. **A.** Tibia lengths and **B.** lung weights of NSML adult hearts. Note: N= 3-6 - mice/group. Data represent mean ± SEM; **P < 0.01, 2-tailed Student's t test.



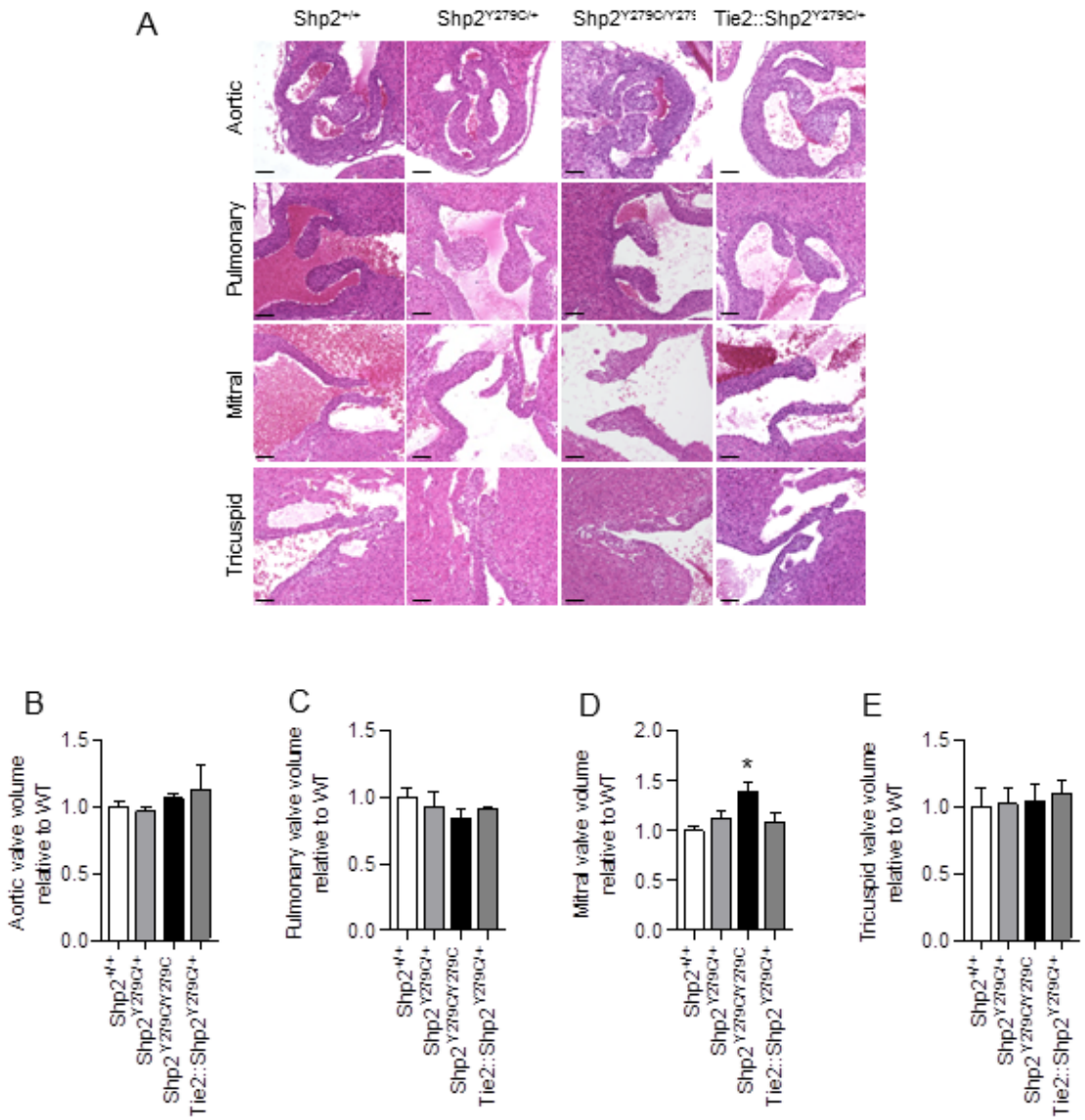
Supplemental Figure 2. **Isolated individual cardiomyocytes are larger and fetal gene expression profile is upregulated in adult NSML mice. A.** Representative images from cardiomyocytes isolated from 12-week-old NSML hearts. Scale bar: 50μm. **B.** Average cardiomyocyte width and length measurements for NSML hearts. N=3 mice/group, with 100–300 cardiomyocytes measured/group. Data represent mean ± SEM; *p<0.05, 2-tailed Student's t test. **C.** Cardiac fetal gene expression of alpha- (αMHC) and beta-myosin heavy chain (βMHC), where total RNA from NSML mice (n = 3–6 mice/group) was used to perform quantitative RT-PCR (each sample in triplicate). The ratio of ΔΔCT was analyzed using GAPDH as a control. Data represent mean ± SEM; *p<0.01, 2-tailed Student's t test.



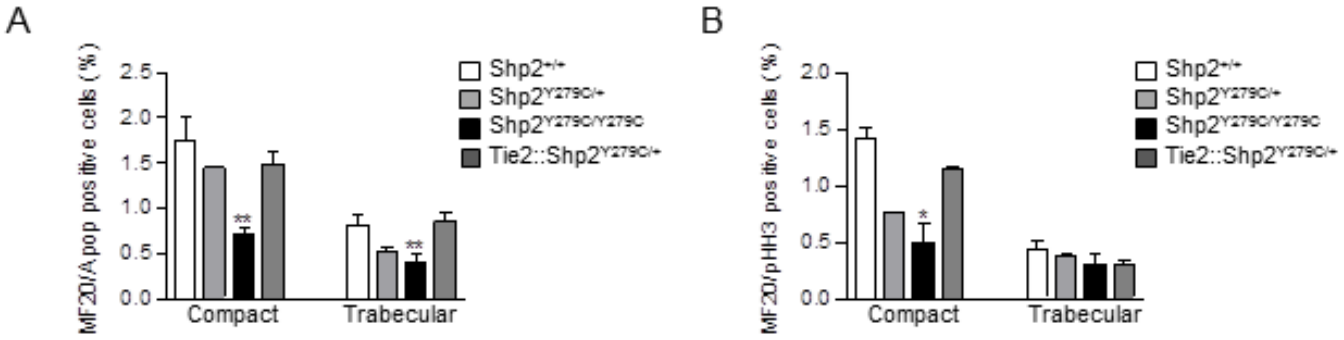
Supplemental Figure 3. **NSML mice have enlarged chamber dimensions, indicative of hypertrophy**. Representative echocardiographs of $Shp2^{+/+}$, $Shp2^{Y279C/+}$, $Shp2^{Y279C/Y279C}$, and Tie2::Cre;$Shp2^{Y279C/+}$ mouse hearts at 6, 9, and 12 weeks of age. Two-headed arrows indicate left ventricular chamber dimension (LVDd). Note, the chamber is noticeably decreased in the $Shp2^{Y279C/Y279C}$ hearts as early as 6 weeks, whereas the $Shp2^{Y279C/+}$ and Tie2::Cre;$Shp2^{Y279C/+}$ hearts show decreased chambers by 12 and 9 weeks, respectively.



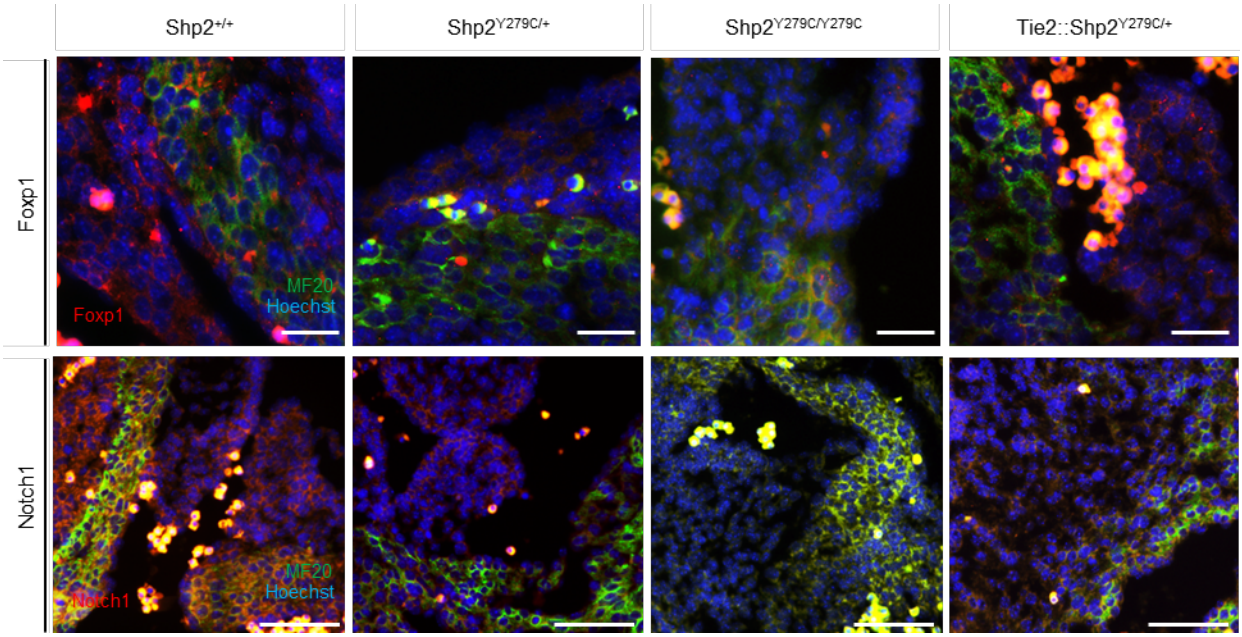
Supplemental Figure 4. **NSML mice have normal placentae histology**. Representative sections of E14.5 placentae from $Shp2^{+/+}$, $Shp2^{Y279C/+}$, $Shp2^{Y279C/Y279C}$, and Tie2::Cre;$Shp2^{Y279C/+}$ mice. Note: no abnormalities are observed. Scale bar: 20μm VT: villous trophoblast TGC: trophoblast giant cell.



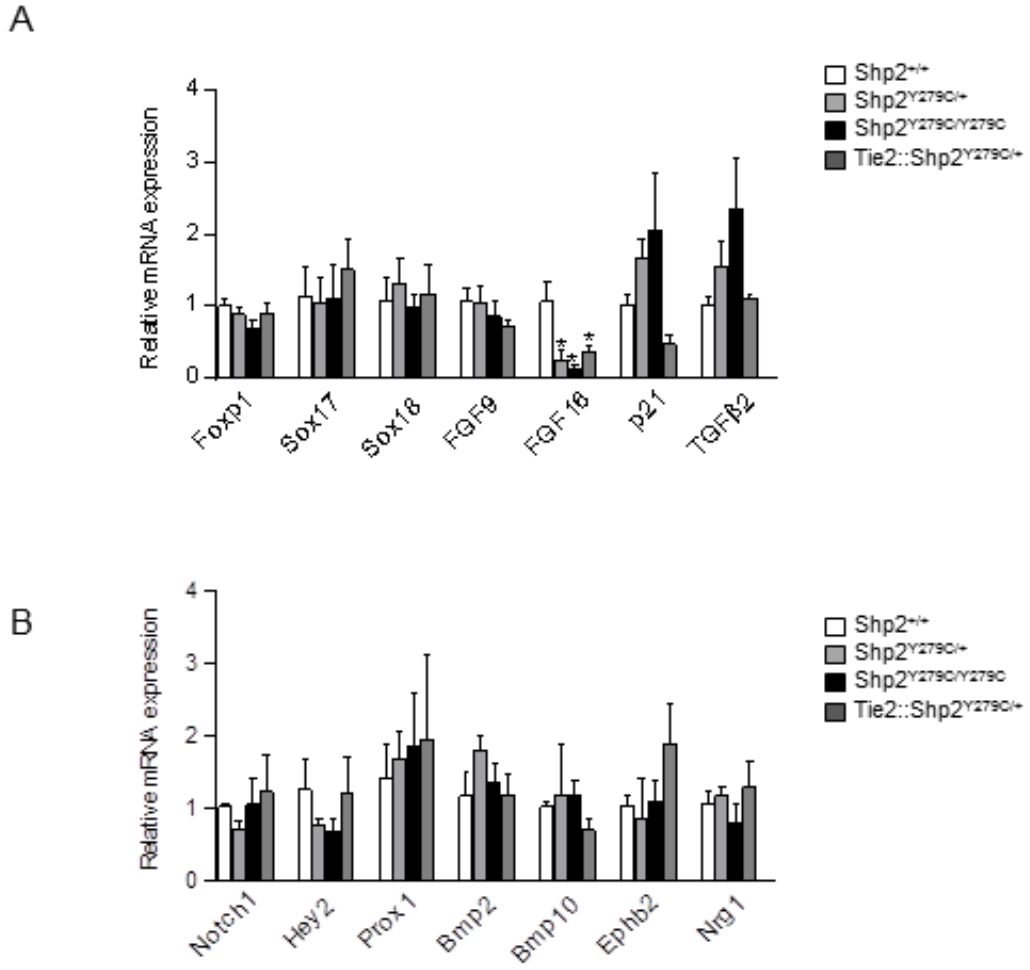
Supplemental Figure 5. **NSML mice have normal valve volumes at P1. A.** Representative images of NSML P1 aortic, pulmonary, mitral and tricuspid valves. Scale bar: 50µm. **B.** Quantification of serial-section reconstructions of the entire heart, representing **B.** aortic, **C.** pulmonary, **D.** mitral and **E.** tricuspid valve leaflet total volumes at P1. n=3 mice per group. Data represent mean ± SEM. *$p<0.05$, where p values were derived from one-way ANOVA with Bonferroni post-test when ANOVA was significant.



Supplemental Figure 6. **NSML mutant expression differentially affects apoptosis and proliferation in compact vs. trabecular myocardium**. Quantification of myocardial **A.** apoptosis and **B.** proliferation in the compact versus trabecular regions in E14.5 hearts. n=3 embryos per group per analysis, with counts >1000 cells/embryo/defined area. All data are expressed as mean ± SEM. *$p < 0.05$ and **$p<0.01$. p values were derived from ANOVA with Bonferroni post-test when ANOVA was significant.



Supplemental Figure 7. **Loss-of-function mutations in PTPN11 lead to down-regulation of endocardial Foxp1 and Notch1 expression.** Immunofluorescence staining of E14.5 embryo hearts representative of endocardial Foxp1 (upper) and Notch1 (lower) expression (red) at low magnification (Scale bar: 25μm). Additional double-staining for MF20 was used to identify areas with myocardium (green) and Hoechst stain was used to mark the nuclei (upper and lower panels) and to demarcate the valves (upper panels).



Supplemental Figure 8. **Quantification of Foxp1 and Notch1 mRNA levels in NSML embryos**. mRNA expression in embryo hearts at E10.5 quantified by qPCR of **A.** Foxp1 and **B**. Notch1 signaling pathways. N=3-6 embryos per group. Data represent mean ± SEM; *p<0.05. p values were derived from one-way ANOVA with Bonferroni post-test when ANOVA was significant.

Viability of embryos from NSML heterozygous matings				
	Number of litters analyzed	Genotype of live embryos (%)		
Age	(Number of embryos/pups)	$Shp2^{+/+}$	$Shp2^{Y279C/+}$	$Shp2^{Y279C/Y279C}$
E10.5	5 (42)	26	54	20
E14.5	20 (153)	29	52	19
E15.5	3 (19)	21	63	11*
Birth	37(185)	28	65	7*

Supplemental Table 1. **Distribution table of embryonic viability from heterozygote $Shp2^{Y279C/+}$ timed-mating crosses.** N=3-6 embryos per group. Data represent mean ± SEM; *p<0.05. p values were derived from one-way ANOVA with Bonferroni post-test when ANOVA was significant.

	Anatomic and functional parameters, as assessed by echocardiography, in NSML Mice at the indicated ages				
	Parameters	$Shp2^{+/+}$	$Shp2^{Y279C/+}$	$Shp2^{Y279C/Y279C}$	$Tie2::Shp2^{Y279C/+}$
6 Weeks	HR (bpm)	709.90±9.52	740.43±16.38	611.41±5.01	669.83±16.62
	LVPW-th (mm)	1.07±0.07	1.06±0.05	1.16±0.04	1.06±0.07
	LVID-d (mm)	2.20±0.08	2.40±0.13	1.74±0.15**	2.09±0.17
	FS (%)	59.70±1.30	61.12±1.48	58.53±5.50	59.1±3.86
	EF (%)	93.03±0.71	93.72±0.67	92.22±3.00	92.49±1.86
9 Weeks	HR (bpm)	727.70±6.69	747.10±18.92	699.76±0.94	709.09±22.22
	LVPW-th (mm)	1.08±0.09	1.27±0.13	1.75±0.09*	1.44±0.02*
	LVID-d (mm)	2.22±0.12	2.09±0.12	1.64±0.05*	1.90±0.03
	FS (%)	64.5±1.75	59.43±1.76	54.86±4.05	51.12±1.60
	EF (%)	95.15±0.72	92.87±0.94	87.8±1.17	87.80±1.17
12 Weeks	BM (g)	25.48±0.79	26.6±0.80	15.21±5.09	22.38±1.36
	HR (bpm)	734.35±8.42	754.69±16.81	622.72±3.38	729.31±15.02
	LVPW-th (mm)	1.09±0.09	1.41±0.04*	1.71±0.13**	1.65±0.01*
	LVID-d (mm)	2.24±0.11	2.11±0.02**	1.93±0.08*	2.10±0.06**
	FS (%)	59.63±1.94	52.82±2.83	52.10±3.06	58.22±0.41
	EF (%)	91.97±1.02	88.80±1.89	87.16±3.41	92.36±0.41

Supplemental Table 2. **Anatomic and functional parameters, as assessed by echocardiography, in NSML Mice at the indicated ages**. Note the increased posterior wall thickness and smaller left ventricular chamber dimension in NSML hearts. Note that the fractional shortening calculation includes the papillary muscle. Data represent mean ± SEM; $p<0.05$, 2-tailed Student's t test. HR, heart rate; LVPWth, left ventricular posterior wall thickness; LVDd, left ventricular chamber dimension in diastole; FS%, fractional shortening; EF, ejection fraction.

Aortic Measurements in NSML mice			
Genotype	Aortic Insufficiency	Aortic Regurgitation	Aortic Dilation
$Shp2^{+/+}$	60%	0%	None
$Shp2^{Y279C/+}$	100%	20%	None
$Shp2^{Y279C/Y279C}$	100%	0%	None
$Tie2::Shp2^{Y279C/+}$	66.70%	0%	None

Supplemental Table 3. **Aortic measurements in NSML mice.** N=3-5 mice per group. Note: no significant differences in any NSML mice were noted.

Primer names	Sequence 5'-3'
αMHC-F	CGCATCAAGGAGCTCACC
αMHC-R	CCTGCAGCCGCATTAAGT
βMHC-F	GAGCAAGGCCGAGGAGACGCAGCGT
βMHC-R	GAGCCTTCTCGTCCAGCTGCCGG
Foxp1-F	ACAAATGCAGCAGATCCTCC
Foxp1-R	CTTGAAGCTGCTGTTGAAGC
Sox17-F	GCTAGGCAAGTCTTGGAAGG
Sox17-R	CTTGTAGTTGGGGTGGTCCT
Sox18-F	CACAACGCAGTACTGAGCAAG
Sox18-R	GGCCGGTACTTGTAGTTGGG
Fgf9-F	CTTCCGGTGTCCACATGTTT
Fgf9-R	ACGAGAAGGGGGAGCTGTAT
Fgf16-F	GAGACAGTATTATGTGGCCCTGAA
Fgf16-R	CTACTGGCCTTGGTAAAAAGTGAGT
P21-F	CCTGGTGATGTCCGACCTG
P21-R	CCATGAGCGCATCGCAATC
TGFβ2-F	GAAGACCCCACATCTCCTGC
TGFβ2-R	ATTTCCATCCAAGATCCCTC
Notch1-F	CTGAGGCAAGGATTGGAGTC
Notch1-R	GAATGGAGGTAGGTGCGAAG
Hey2-F	TTCTGTCTCTTTCGGCCACT
Hey2-R	TTTGTCCCAGTGCTTGTCTG
Prox1-F	TTGACTCGGGACACAACAAG
Prox1-R	TGATGAGCTGCGAGGTAATG
Bmp2-F	TGCGCAGCTTCCATCACGAA
Bmp2-R	CACTCATCTCTGGAAGTTCCT
Bmp10-F	ACATCATCCGGAGCTTCAAGAACG
Bmp10-R	AACCGCAGTTCAGCCATGACG
Ephb2-F	CTGTGCCAGACCAGACCAAGA
Ephb2-R	CAGCAGAACTTGCATCTTGTC
Nrg1-F	AACAGCAGGCACAGCAGCCC
Nrg1-R	AGGGGAGCTTGGCGTGTGGA
Gapdh-F	GTACATGTTCCAGTATGATTCTACC
Gapdh-R	CAGTGGACTCCACAACATACTCA
Tbp-F	TTCTGGGAAAATGGTGTGC
Tbp-R	CCCACCATGTTCTGGATCTT
RPL13-F	CCTGCTGCTCTCAAGGTTGT
RPL-13-R	GGTACTTCCACCCGACCTC

Supplemental Table 4. **Primer sequences**. All qPCR experiments were performed according to the manufacturer instructions with an annealing temperature of 60°C. Melting curves were used to assess specificity.