Supplemental Table S1 and figures S1-S5.

Conscious echocardiography

	Ctrl (n=8)	Neb cKO (n=6)	p-value
Age (days)	60.25±0.7	59±1	0.31
BW (g)	19.3±0.73	19.1±1.6	0.92
HR (BPM)	677±16	675±31	0.96
LV-M mode protocol:			
LA (mm)	1.63±0.05	1.57±0.08	0.51
LVID;d (mm)	2.29±0.10	2.28±0.12	0.96
LVAW;d (mm)	1.14±0.02	1.05±0.05	0.07
LVPW; d	0.94±0.01	0.91±0.03	0.44
LVID;s (mm)	1.17±0.09	1.29±0.09	0.37
LVAW; s (mm)	1.54±0.03	1.40±0.05	0.08
LVPW, S	1.32±0.04	1.23±0.06	0.19
Eccentricity	2.14±0..08	2.29±0.18	0.45
LV Vol;d (μl)	18.5±1.9	18.3±2.42	0.94
LV Vol;s (μl)	3.0±0.6	3.2±0.8	0.87
FS (%)	52.9±3.1	50.9±2.7	0.64
EF (%)	84.5±2.5	83.5±2.7	0.77
Stroke volume (μl)	15.5±1.5	15.2±1.9	0.88
CO (ml/min)	10.5±1.1	10.3±1.5	0.88

Table S1. Conscious echocardiography of αMHC-Cre Neb cKO and Ctrl mice. M-mode echos were obtained in restrained mice using a parasternal short-axis view at the level of the papillary muscles.

Abbreviations: BW: body weight; HR: heart rate; BPM: beats per minute; LA: left atrium; LVIDd: left ventricular internal diastolic diameter; LVAWd: left ventricular anterior diastolic wall thickness; LVPWd: left ventricular posterior diastolic wall thickness; LVIDs; left ventricular internal systolic diameter; LVAWs: left ventricular anterior systolic wall thickness; LVPWd: left ventricular posterior systolic wall thickness; ; Eccentricity: LVIDd/((LVAWd+ LVPWd)/2)); LV Vold: left ventricular diastolic volume; LV Vols: left ventricular systolic volume; FS: fractional shortening; EF: ejection fraction; CO: cardiac output (stroke volume x HR).

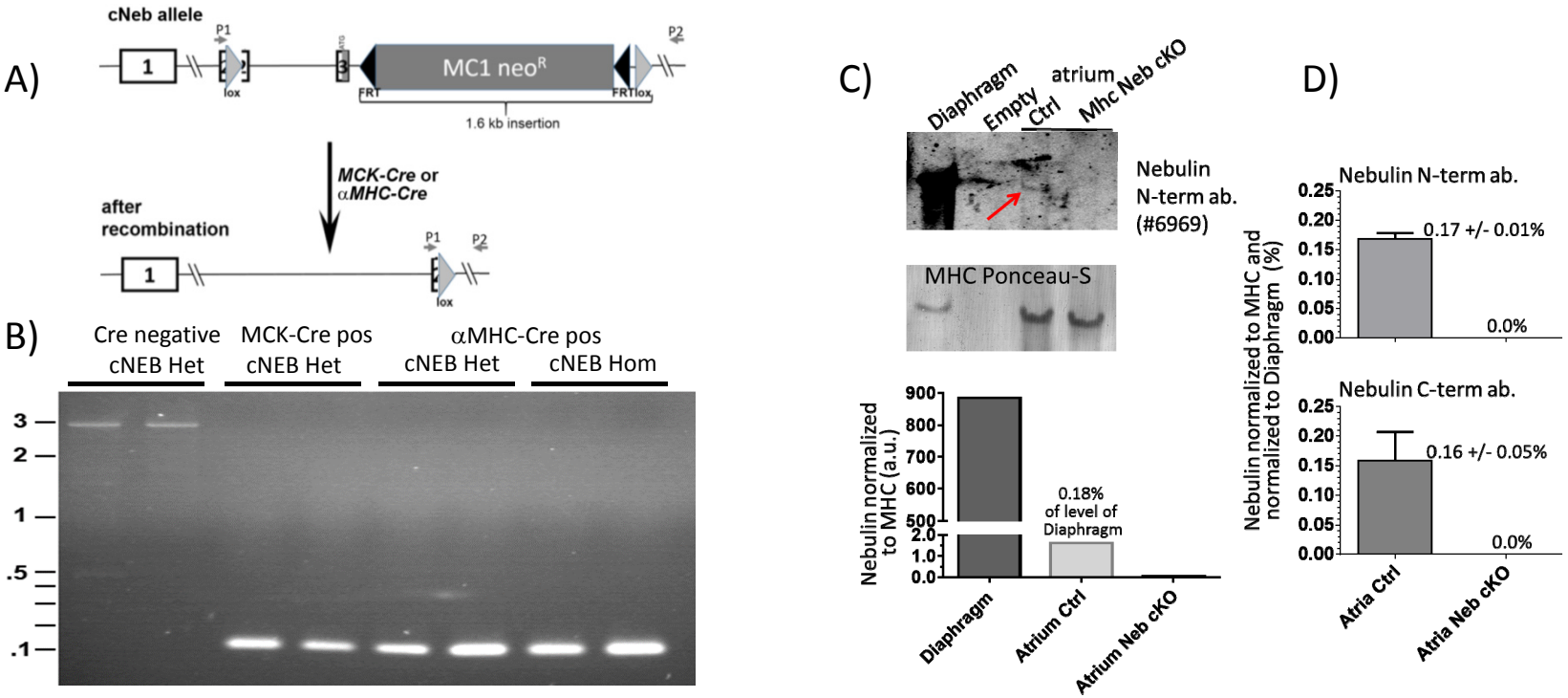
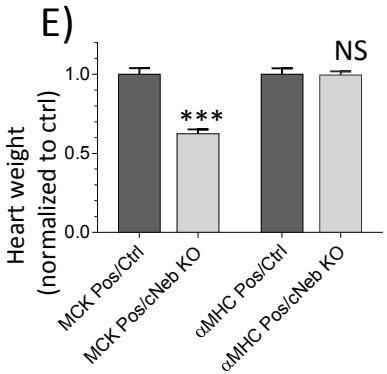


Figure S1. **Conditional deletion of nebulin in the heart.** A) The cNeb allele contains LoxP sites that flank exon 3 (containing the start codon) and was crossed with a model that expresses Cre-recombinase under control of the cardiac specific αMHC promotor or the muscle creatine kinase (MCK) promotor. B) PCR confirmation of recombination in LA of Cre positive cNeb Het or Hom mice. Primers P1 and P2 were designed to detect recombination. P1 sits in exon 2 just before the first loxP site. P2 sits in vector sequence at the edge of the second loxP site and is not present in the mouse genome. The genomic distance changes from 2916bp to 210bp upon recombination of the cNeb allele. We are able to specifically detect if recombination has occurred. C) Top. Western blot analysis with antibody to nebulin's N-terminus using atrial muscle lysate (right 2 lanes) from control and MHC Neb cKO mice. For comparison a diaphragm muscle lysate is also shown. Myosin heavy chain (MHC) was used as a loading control. The diaphragm was underloaded (on purpose) but still had a much strong WB signal. A just detectable band in present in the atrium of the control (see red arrow) but this band is absent in the Neb cKO mouse. Bottom: analysis shows that control atrium expresses nebulin at ~0.2% of that in the diaphragm. Note that to visualize the weak nebulin signal in the atrium the exposure time had to be very long and as a result minor unevenness in the membrane or contaminants became visible. D) Total nebulin content in control atria and atria from cNeb KO mice using the nebulin N-term (top) and nebulin C-term (bottom) antibodies. Control atria express ~0.17% nebulin compared to diaphragm and nebulin expression is undetectable in cNeb KO mice. Fig. S1 E and F, see next page.



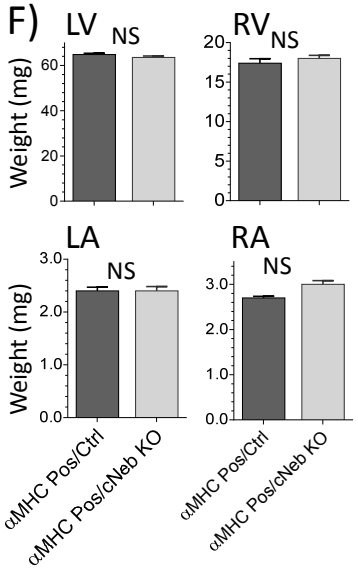


Figure S1 continued. E) Total heart weights of MCK-cre cNEB mice are reduced relative to control mice(left two bars) whereas heart weights of αMHC-cre cNEB mice (right two bars) are normal. F) Left ventricular (LV), right ventricular (RV), left atrial (LA) and right atrial (RA) weights of MHC6-cre cNEB mice are all normal. For additional details, see Text.

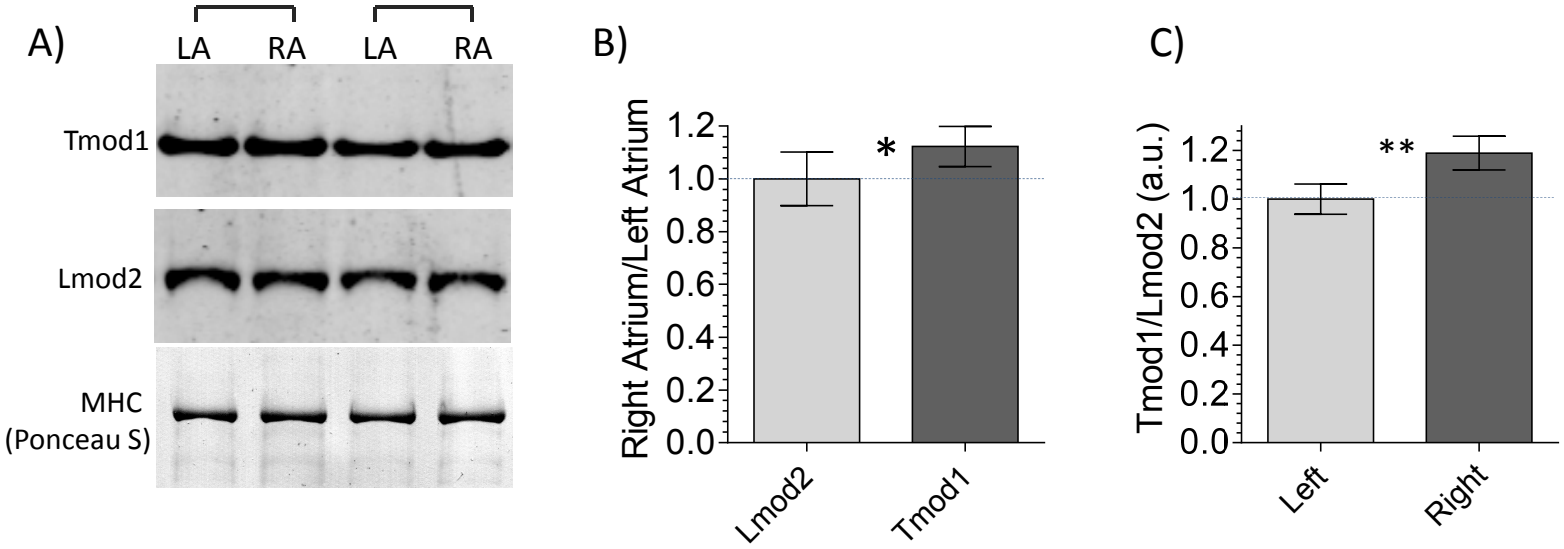


Figure S2. Expression levels of Tmod1 and Lmod2 in left and right atria of control mice (3 month old male). A) Western blot examples of left and right atrial lysates probed with Tmod1 and Lmod2 antibodies. LA and RA samples of the same heart were run side by side on the same gel (examples of 2 pairs are shown). MHC was used as a loading control. B) Total protein levels of Lmod2 and Tmod1 determined as a ratio in right vs left atria. C) Tmod1 vs Lmod2 expression ratio in right and left atria. Tmod1 expression is increased in the right atrium. Data from 13 mice were analyzed using a paired comparison (t-test). Expression levels were corrected for loading differences using myosin heavy chain expression (MHC) as a normalizer and ratios in C have been normalized to the mean ratio of the left atrium.

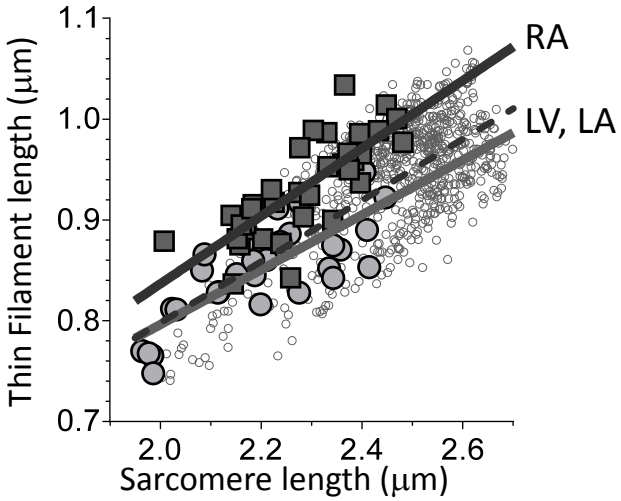
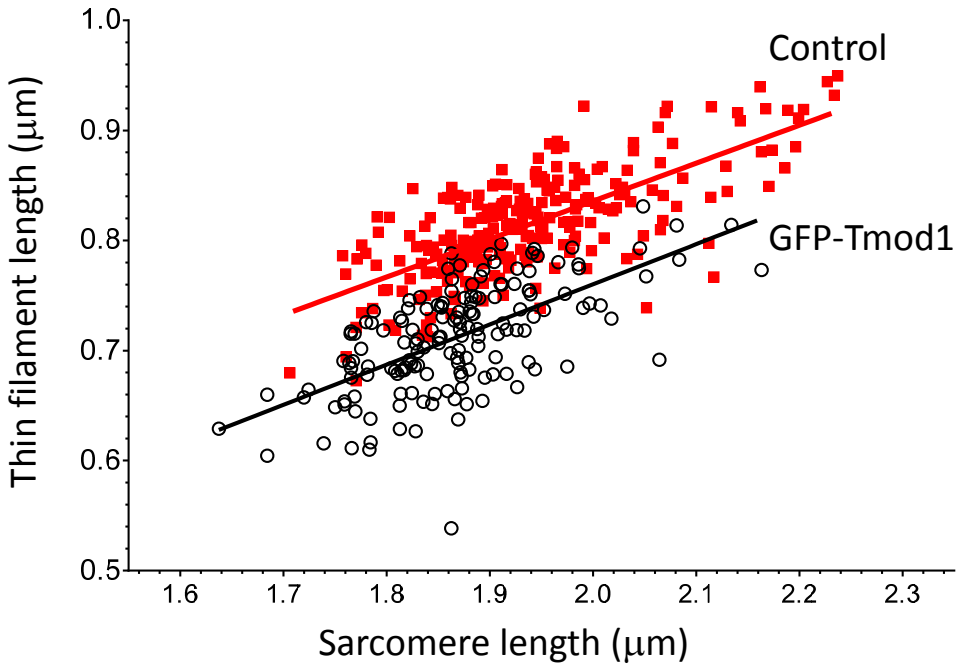


Figure S3. Comparison of TFLs in left ventricle (LV), left atrium (LA) and right atrium (RA). Results were fit with linear regression lines (RA solid black line; LV: broken line; LA solid grey line). Slopes of lines are the same ($p=0.74$) but the offset of RA regression line is significantly larger ($p<0.0001$). (For clarity we used Tmod results from the WT data set of the Rbm20$^{\Delta RRM}$ study (Fig. 5) and atrial result from both Ctrl and Neb cKO mice (from Fig. 3, bottom panels).



	Slope	slope different from zero?	slopes different from each other?	Offsets different from each other?
Control	0.34	Yes (p<0.0001)	No (p=0.6)	Yes (p<0.0001)
GFP-Tmod1	0.36	Yes (p<0.0001)		

Figure S4. TFL measurements in cultured neonatal rat cardiac myocytes. In both control myocytes and myocytes in which GPP-Tmod1 is expressed, TFL varies linearly with SL. The slopes of the linear regression lines are the same in the two data sets. However, the offset of the Tmod1 regression line is significantly less ($p<0.0001$) indicating that TFL is reduced. Note that if the two conditions (control and GFP-Tmod1) were compared without consideration given to SL, erroneous conclusions might result (e.g., if the control cells were to be at a much shorter SL than the GPP-Tmod1 cells). Clearly, when comparing TFL in different cell types, the TFL-SL curves need to be measured followed by a statistical comparison of the regression lines.

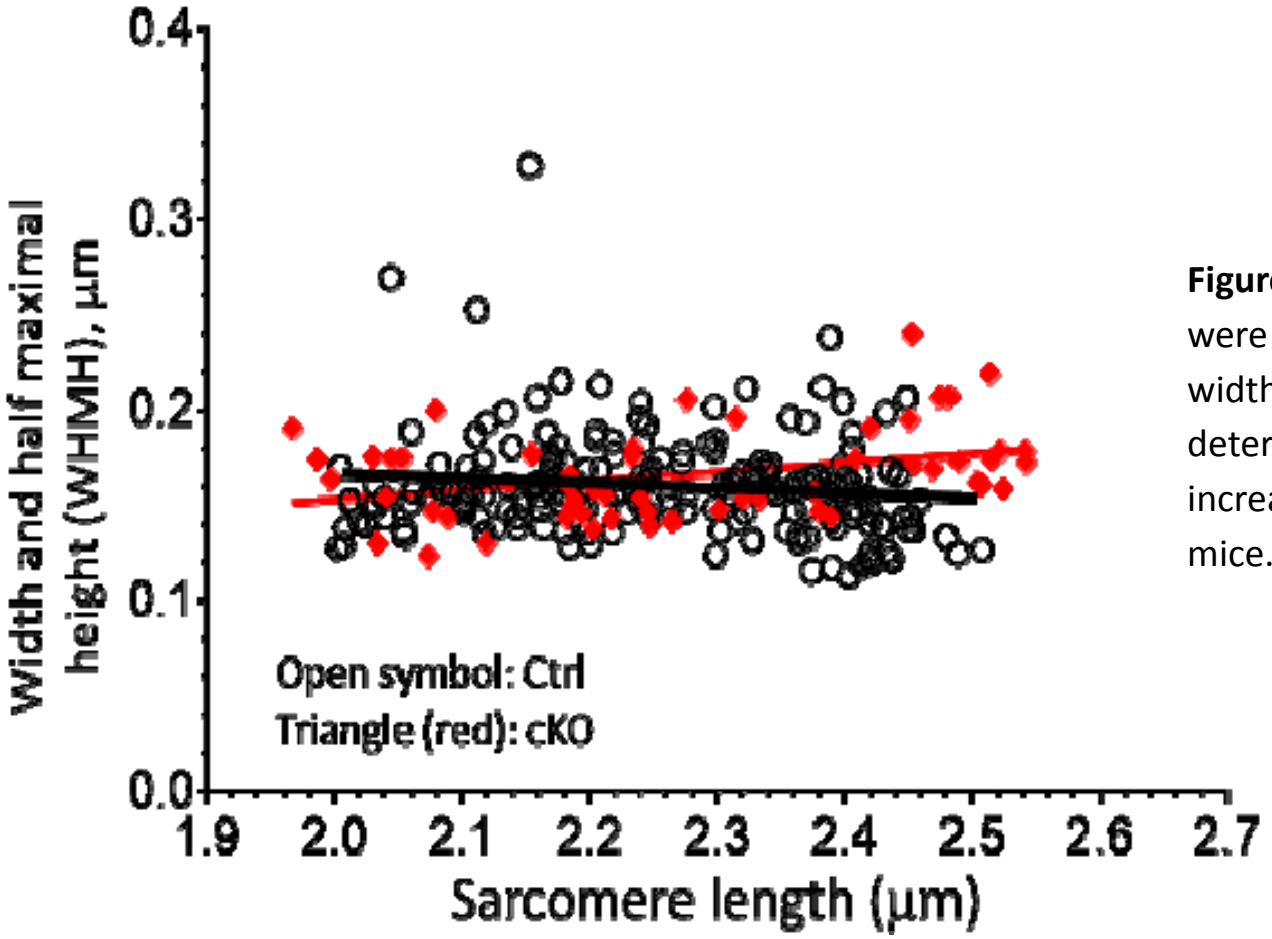


Figure S5. Tmod peak analysis. Tmod peaks were fit with Gaussian curves and the half width at half maximal height (HWHM) was determined as a function of SL. HWHM slightly increases/decreases with SL in Ctrl/Neb cKO mice. (See Discussion for details.)