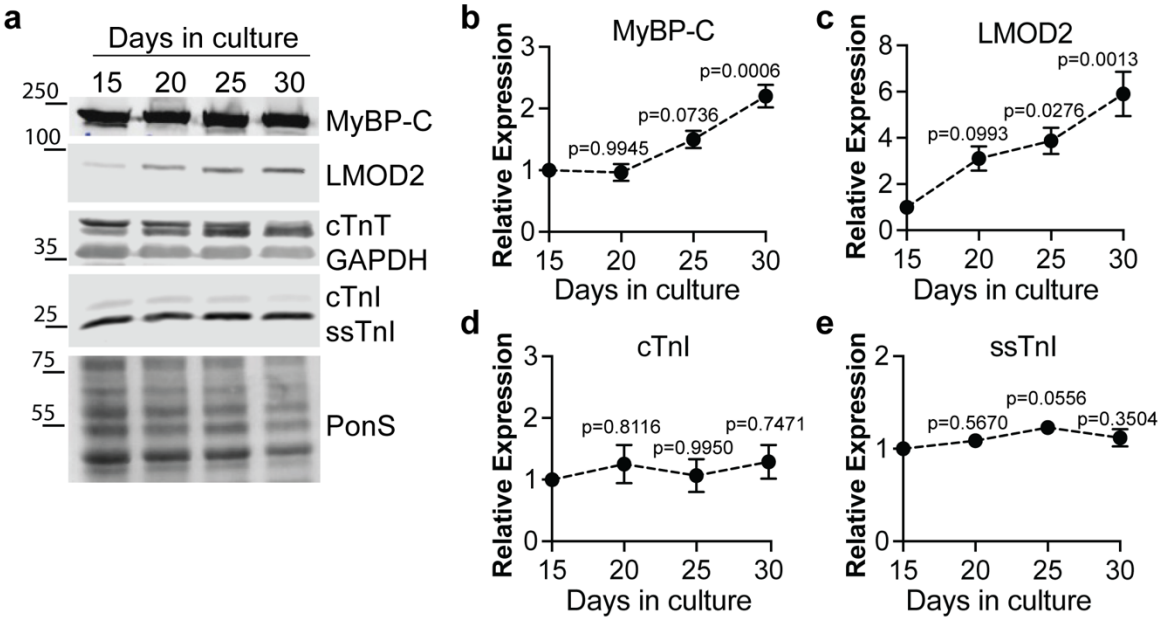

Supplemental Figure 1. Expression of Lmod2 increases over time in cell culture. a Immunoblot of whole cell lysate collected from cultured isogenic control hiPSC-CMs on days 15-30. **b-e** Quantification of immunoblots probed for MyBP-C as a marker of the thick filament, and LMOD2, showing increasing protein expression over time. Expression of cTnI and ssTnI were used as maturity markers, and cTnT was used as a canonical marker for cardiomyocytes. Relative expression levels were determined following normalization to Ponceau S (PonS). Values are mean ± s.e.m., N=3 independent cardiac differentiations, one-way ANOVA.

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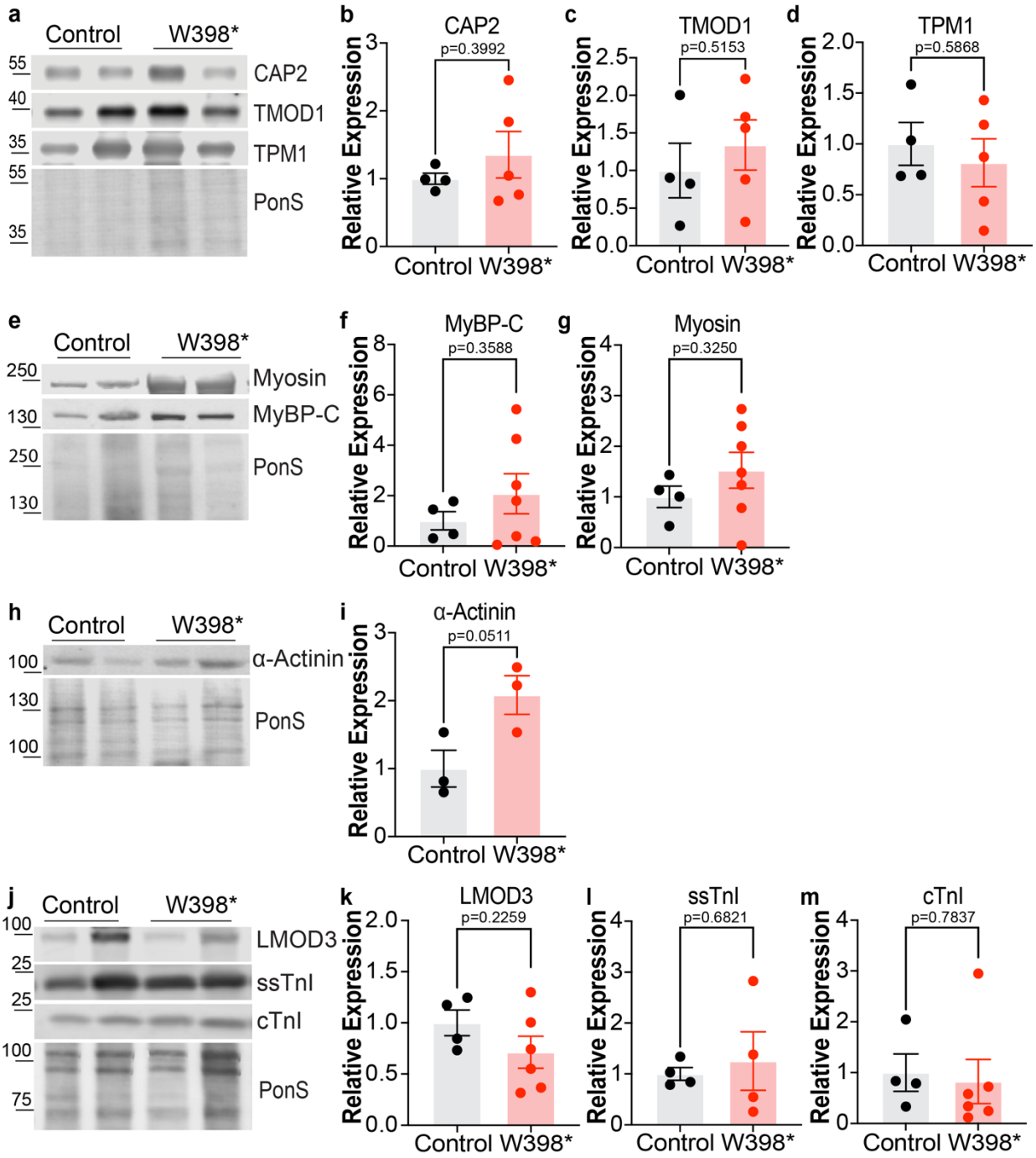
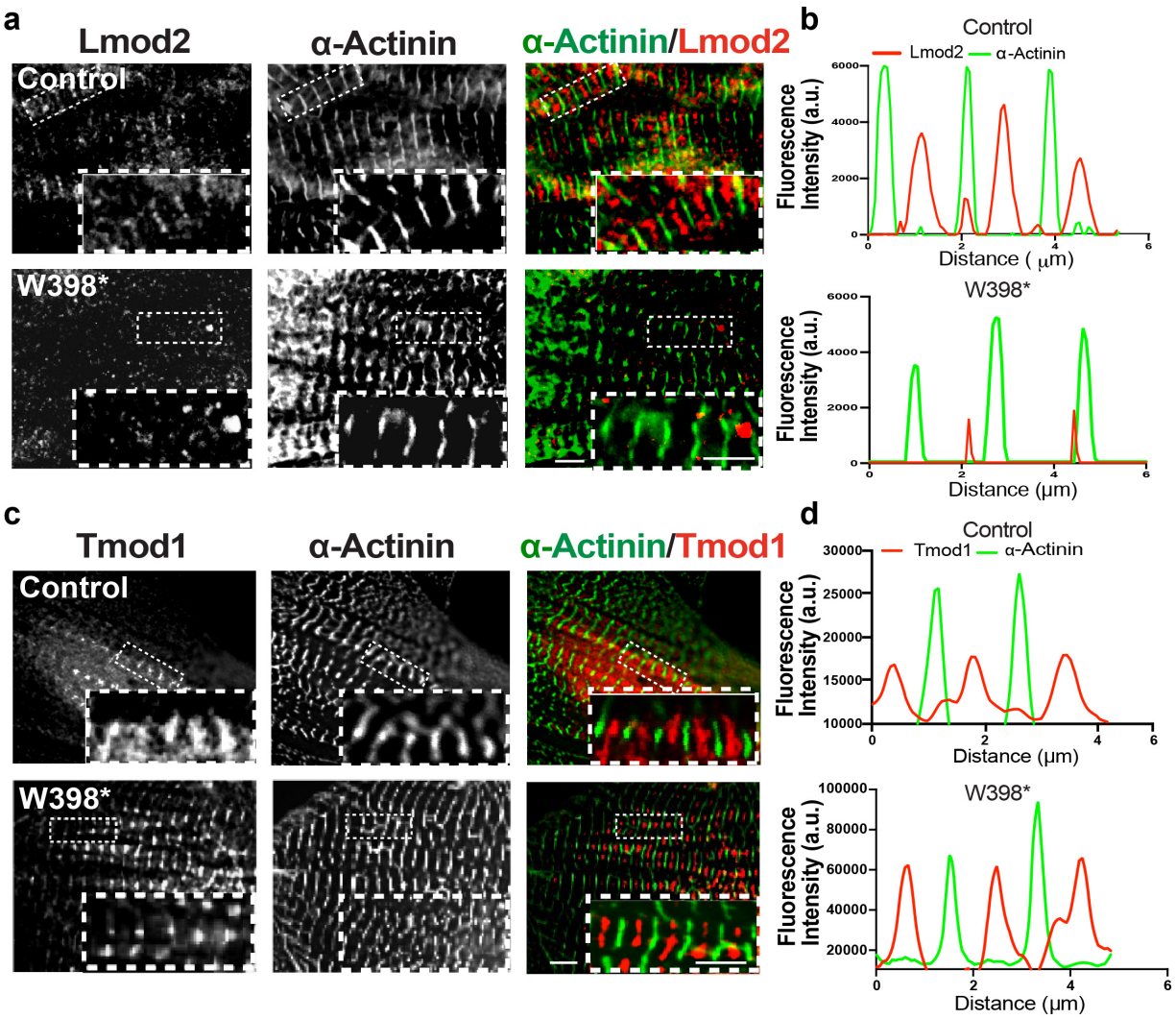
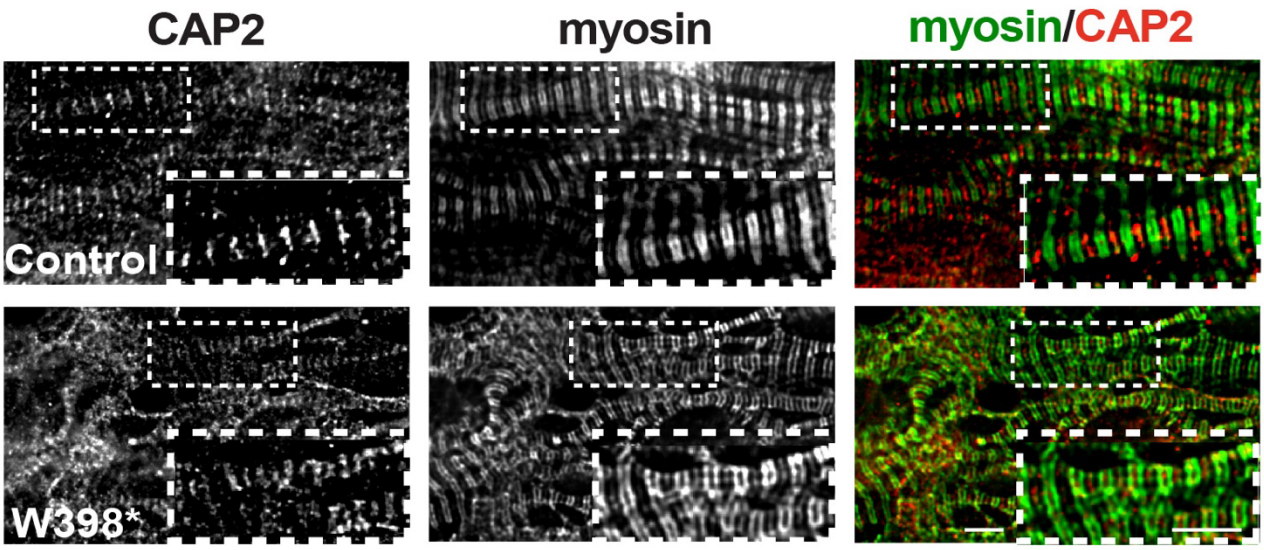


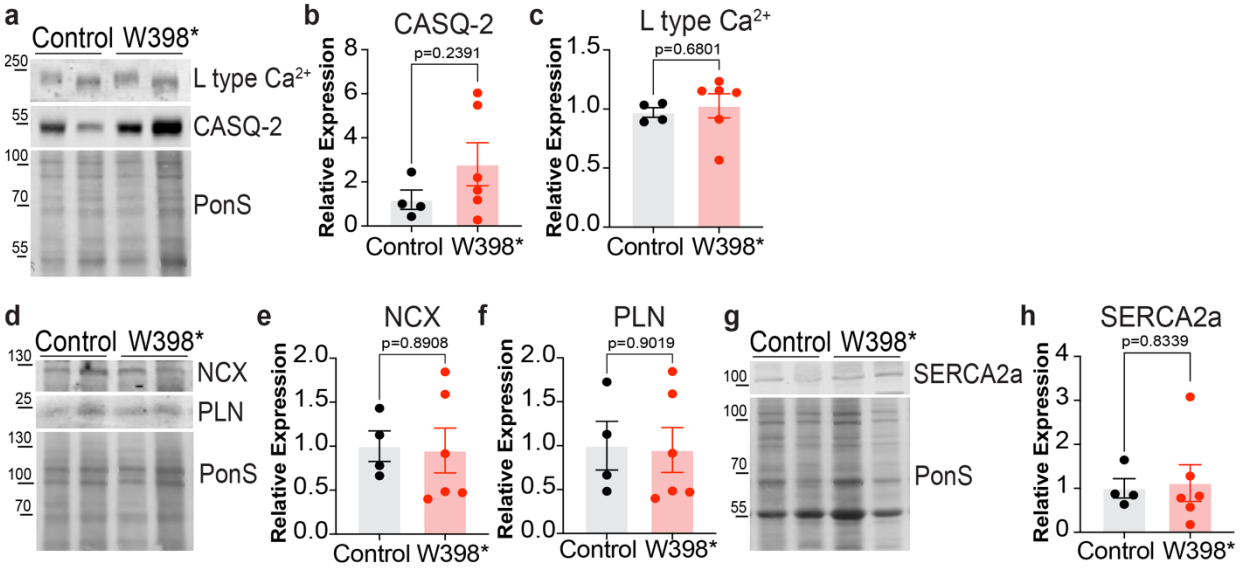
Supplemental Figure 2. *LMOD2* W398* hiPSC-CMs have no significant change in the expression of thick and thin filament proteins compared to isogenic controls. a Immunoblots of thin filament proteins. Quantification of thin filament-associated proteins CAP2 (**b**), TMOD1 (**c**), and tropomyosin 1 (TPM1) (**d**). **e** Immunoblots of thick filament proteins. Quantification of thick filament-associated proteins MyBP-C (**f**) and myosin (**g**). **h-i** Immunoblot and quantification of the Z-disc protein, α-actinin. **j** Immunoblots of LMOD3, ssTnI, and cTnI with corresponding quantification of the skeletal isoform, LMOD3 (**k**) and cardiac maturity markers, ssTnI (**l**) and cTnI (**m**). Relative expression levels were determined following normalization to Ponceau S (PonS). Values are mean ± s.e.m., N=3-6 independent cardiac differentiations, Student's t-test, two-tailed.



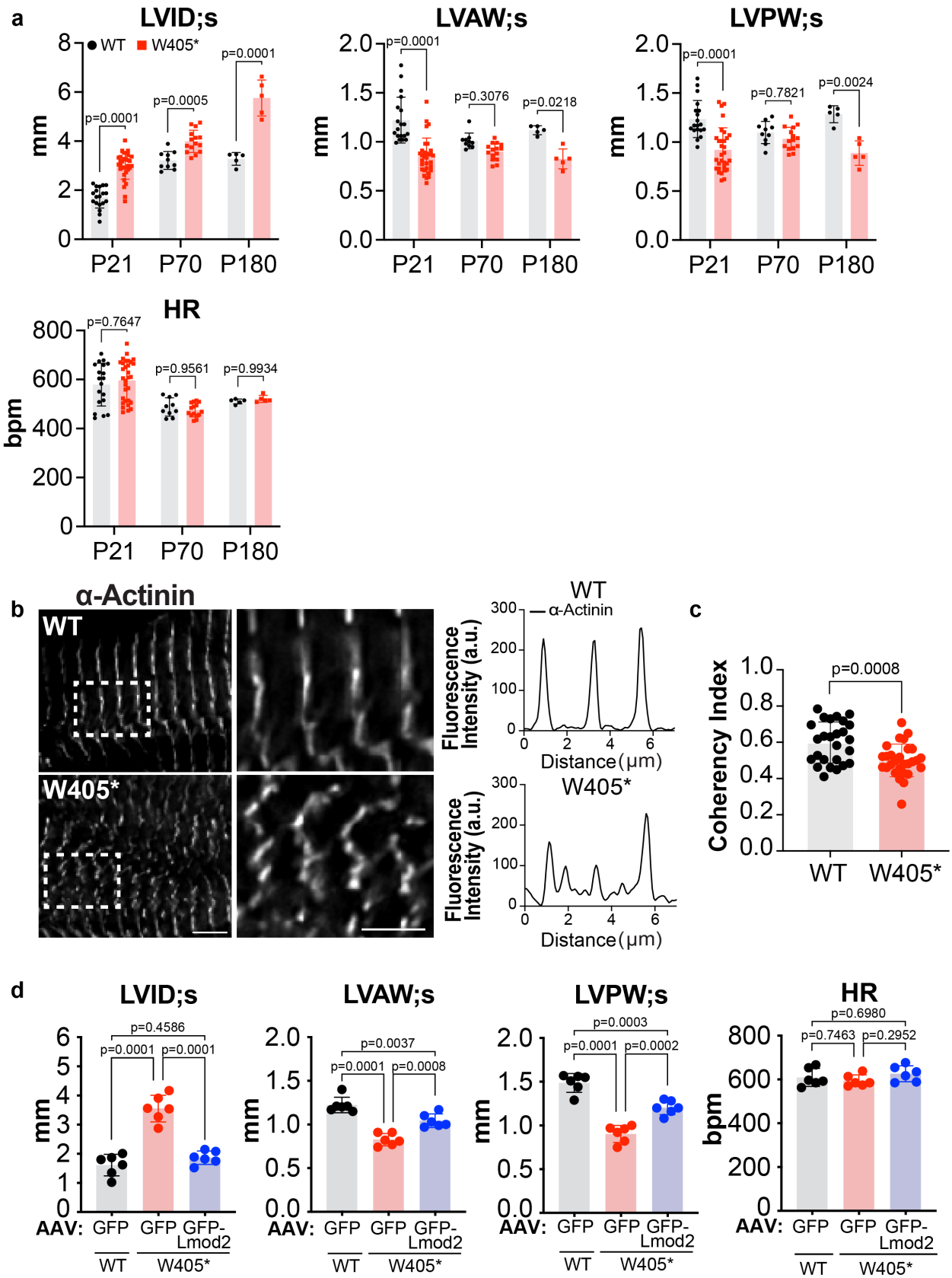
Supplemental Figure 3. *LMOD2* W398* hiPSC-CMs have loss of Lmod2 pointed-end localization in cardiac thin filaments. a Representative immunofluorescence image stained with anti-Lmod2 and anti-α-actinin (a Z-disc marker). **b** Accompanying line scan analysis of Lmod2 with α-actinin showing the distribution of Lmod2 in relation to the Z-disc. **c** Representative immunofluorescence images stained with anti-Tmod1 and anti-α-actinin. **d** Accompanying line scan analysis of Tmod1 with α-actinin showing the distribution of Tmod1 in relation to the Z disc. Scale bar=1.5μm. Immunofluorescence images are representative of a minimum of 3 independent cardiac differentiations for *LMOD2* W398* and isogenic hiPSC-CMs.



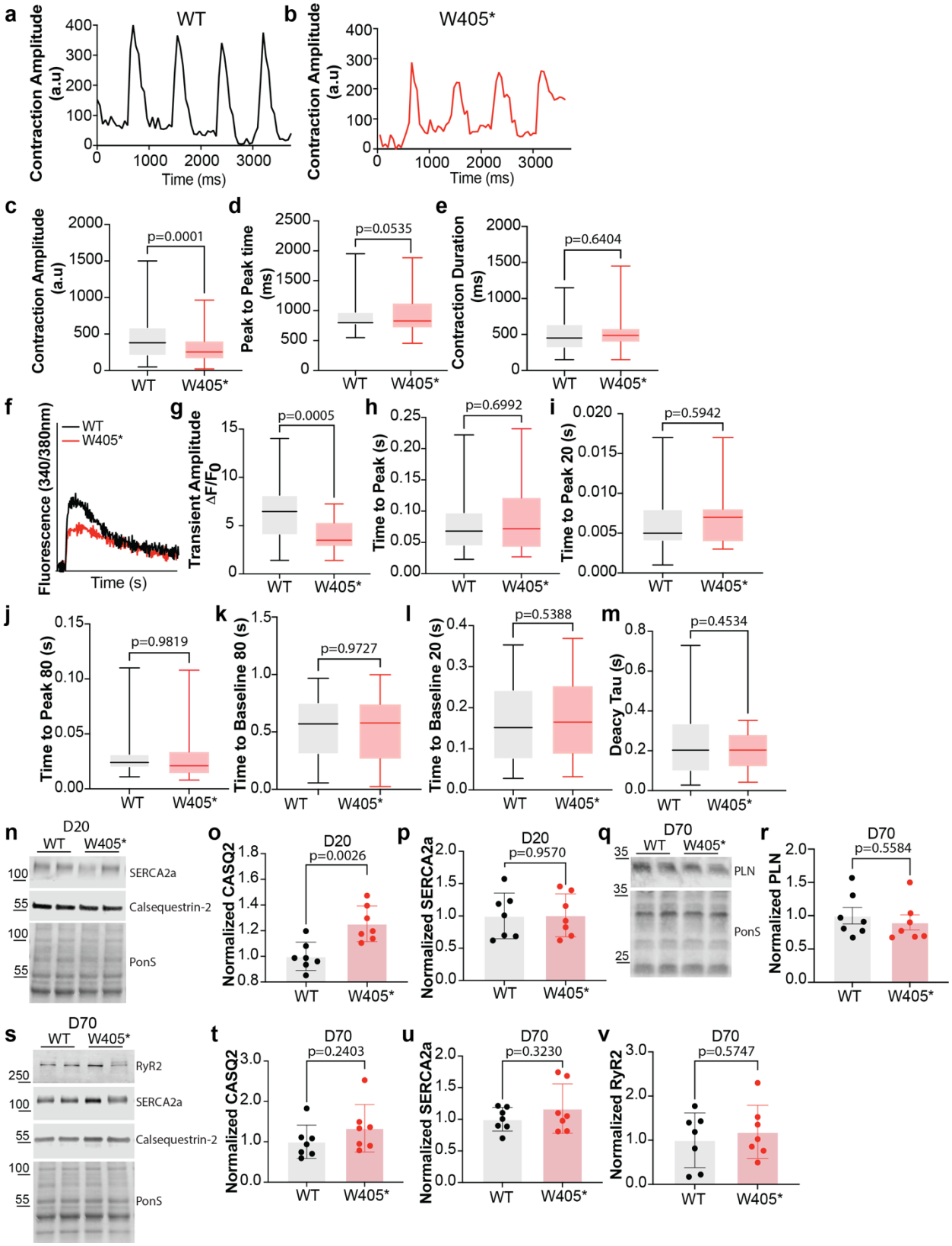
Supplemental Figure 4. *LMOD2* W398* hiPSC-CMs display proper localization of CAP2 and myosin in cardiac thin filaments. Representative immunofluorescence images of CAP2 and myosin. Scale bar=1.5μm. Images are representative of a minimum of 3 independent cardiac differentiations for *LMOD2* W398* and isogenic hiPSC-CMs.



Supplemental Figure 5. Expression of calcium handling proteins is unaltered in *LMOD2* W398* hiPSC-CMs. **a** Immunoblot of calcium handling proteins, L-type Ca^{2+} channel and Calsequestrin-2 in isogenic control and *LMOD2* W398* hiPSC-CMs. **b-c** Quantification of calsequestrin-2 (CASQ-2) and L-type Ca^{2+} channel. **d** Immunoblot of sodium-calcium exchanger (NCX) and phospholamban (PLN). **e-f** Quantification of NCX and PLN in hiPSC-CMs. **g** Immunoblot of SERCA2a in control and *LMOD2* W398* hiPSC-CMs. **h** Quantification of SERCA2a. Relative expression levels were determined following normalization to Ponceau S (PonS). Values are mean ± s.e.m., N= 4-6 independent cardiac differentiations, Student's t-test, two-tailed.

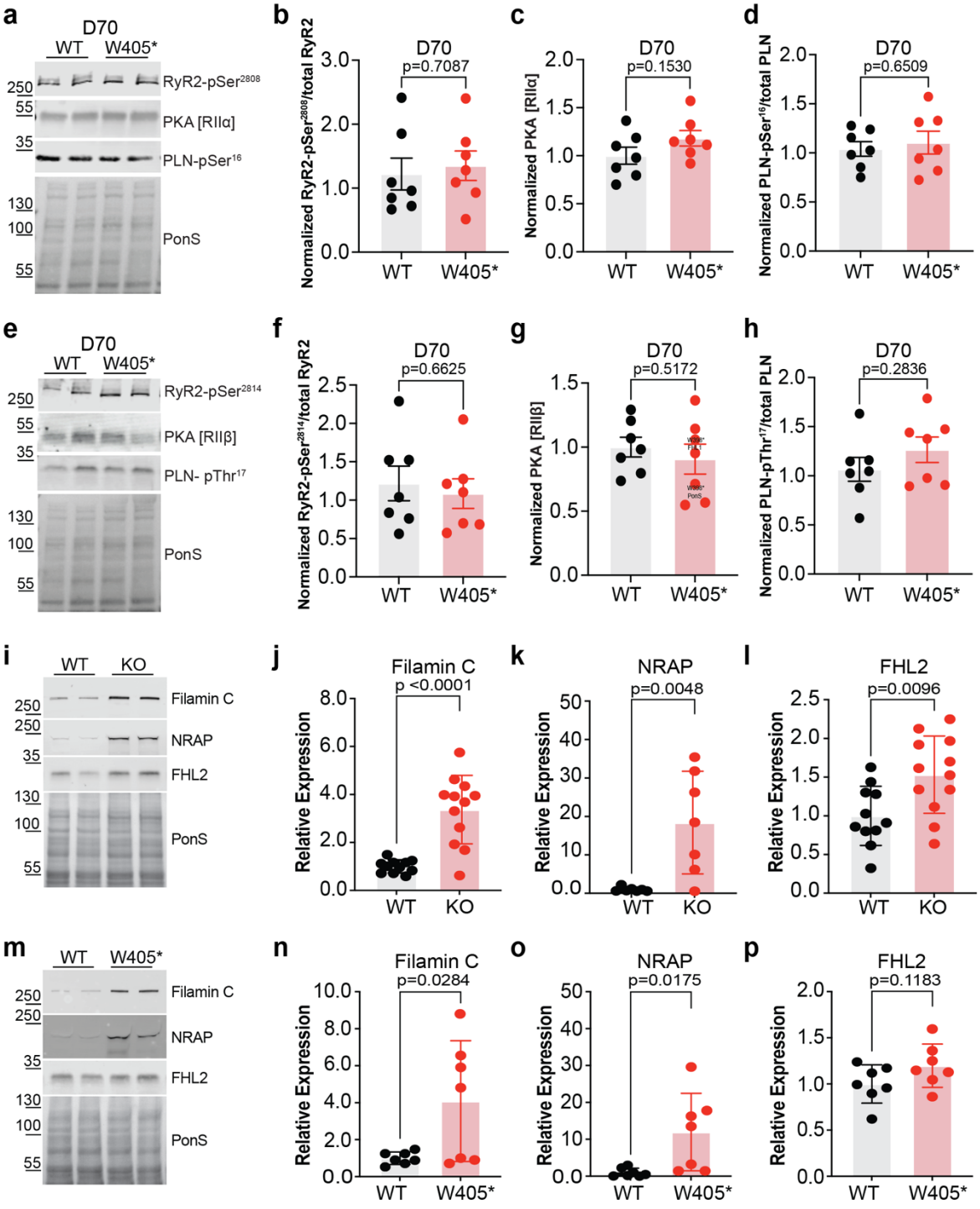

a
LVID;s
WT
W405*
mm
p=0.0001
p=0.0005
p=0.0001
P21
P70
P180
LVAW;s
p=0.0001
p=0.3076
p=0.0218
LVPW;s
p=0.0001
p=0.7821
p=0.0024
HR
bpm
p=0.7647
p=0.9561
p=0.9934
b
α-Actinin
WT
W405*
Fluorescence Intensity (a.u.)
α-Actinin
Distance (μm)
c
Coherency Index
p=0.0008
d
LVID;s
p=0.4586
p=0.0001
p=0.0001
LVAW;s
p=0.0037
p=0.0001
p=0.0008
LVPW;s
p=0.0003
p=0.0001
p=0.0002
HR
p=0.6980
p=0.7463
p=0.2952
AAV:
GFP
GFP
GFP-Lmod2
WT
W405*

Supplemental Figure 6. Additional echocardiography results demonstrating cardiac dilation in *Lmod2* W405* mice is prevented upon expression of wild-type GFP-Lmod2. a Echocardiography of wild-type (black) and *Lmod2* W405* (red) mice at postnatal days 21, 70 and 180. LVID;s, left ventricle internal diameter in systole; LVAW;s, left ventricle anterior wall thickness in systole, LVPW;s, left ventricle posterior wall thickness in systole; HR, heart rate in beats per minute. Values are means ± s.d., n=19-31 (P21), 10-15 (P70), and 5 (P180), one-way ANOVA with Sidak's multiple comparison test. **b** Representative immunofluorescence images of wild-type (top) and *Lmod2* W405* (bottom) LV tissue at P60 stained for α-actinin (Z-disc marker) (scale bar= 2μm). Line scan analysis showing pattern for α-actinin distributions (y-axis, fluorescence intensity in arbitrary units; x-axis, distance in μm). **c** Quantification of sarcomeric alignment using anti-α-actinin antibody staining to measure the average coherency index. WT and *Lmod2* W405*, n= ~10 measurements from each animal, N= 3, Student's t-test, two-tailed, values are means ± s.d. **d** Echocardiography of wild-type mice expressing GFP (black), *Lmod2* W405* mice expressing GFP (red) and *Lmod2* W405* mice expressing GFP-Lmod2 (blue) at postnatal day 21. LVID;s, left ventricle internal diameter in systole; LVAW;s, left ventricle anterior wall thickness in systole, LVPW;s, left ventricle posterior wall thickness in systole; HR, heart rate in beats per minute. Values are means ± s.d., n= 6, one-way ANOVA with Tukey's multiple comparison test.



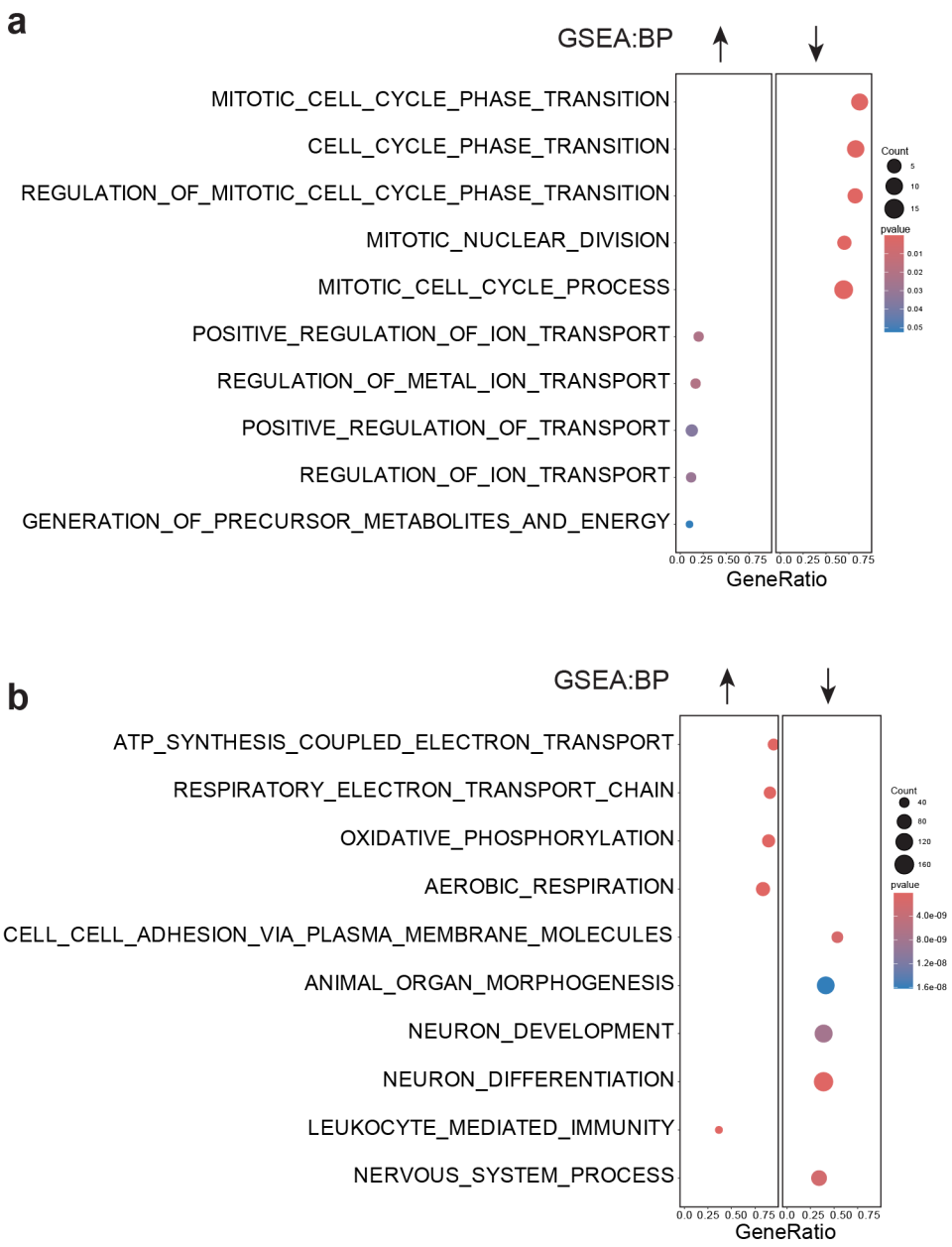
Supplemental Figure 7. Isolated neonatal cardiomyocytes from *Lmod2* W405* mice exhibit altered contractile properties and calcium handling dynamics. a-b Representative

contractility traces of wild-type and *Lmod2* W405* isolated neonatal cardiomyocytes following electrical pacing (1Hz). **c-e** Contractile parameters; contraction amplitude (a.u), peak to peak time (ms), and contraction duration (ms). N=3 independent mouse cultures, average of 40-50 cells sampled per culture, Student's t-test, two-tailed. **f** Representative calcium transients in electrically paced (1Hz) *Lmod2* W405* and wild-type isolated neonatal cardiomyocytes. **g-m** Calcium transient measurements at 1Hz pacing comparing the transient amplitude, time to peak (ms), time to 20% (T_{20}) and 80% (T_{50}) of Ca^{2+} release, time to 80% (T_{80}) and 20% (T_{50}) Ca^{2+} reuptake, and decay tau in wild-type and *Lmod2* W405* isolated mouse cardiomyocytes. N= 3 independent mouse cultures, average of 40-50 cells sampled per culture, Student's t-test, two-tailed. Box and whisker plots show median (horizontal line), standard deviation (box) and maximum and minimum values (whiskers), ms= milliseconds; a.u= arbitrary units). Immunoblot and quantification of calcium handling protein relative expression in wild-type and *Lmod2* W405* left ventricular tissue at day 20 (**n-p**) and day 70 (**q-v**). Relative expression levels were determined following normalization to Ponceau S. Values are means ± s.d., n= 7, Student's t-test, two-tailed. CASQ-2= calsequestrin-2; RyR2= ryanodine receptor-2; PLN= phospholamban.

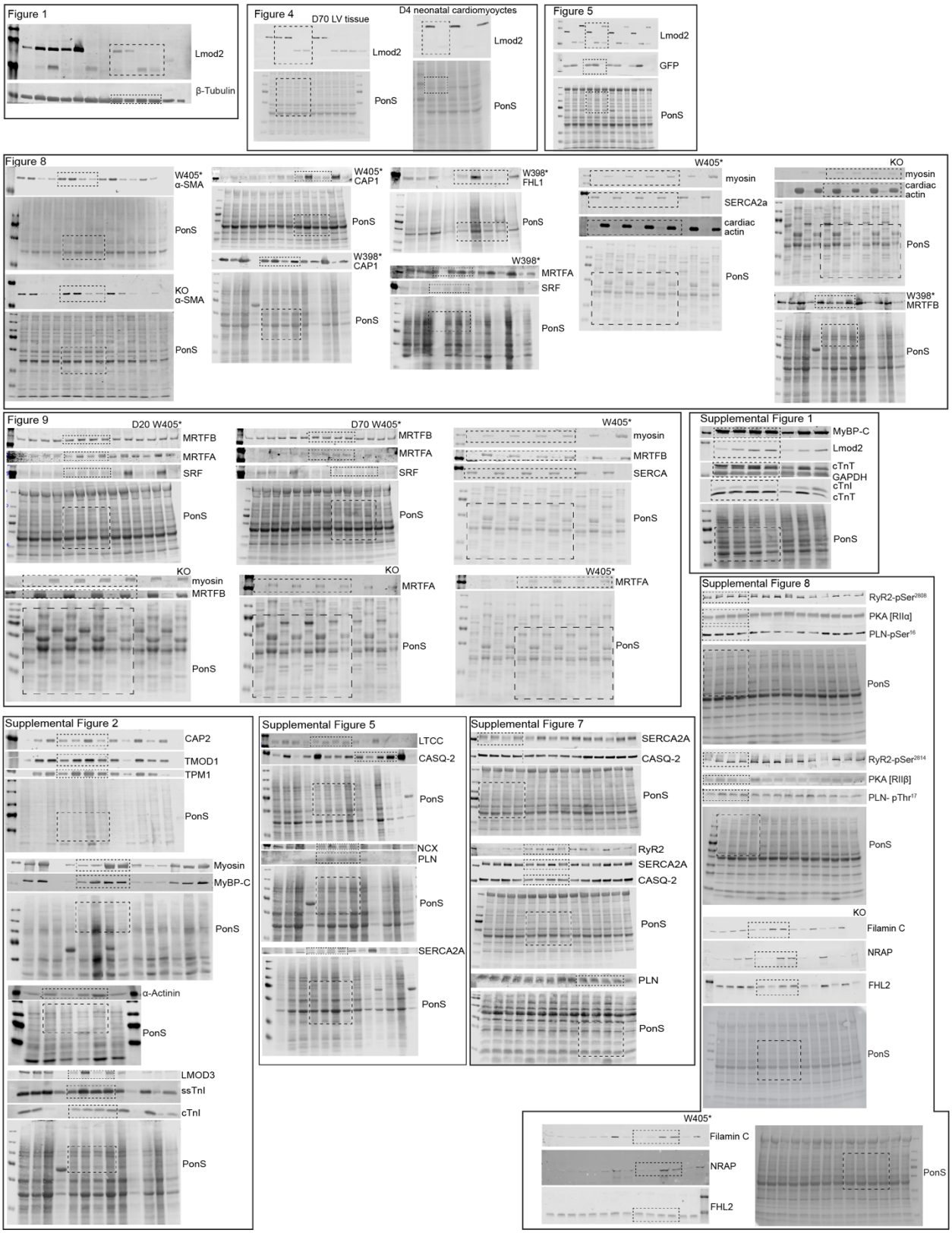


Supplemental Figure 8. Expression of phospholamban, ryanodine, and PKA phosphorylation sites are not altered however Z-disc expression is increased in mutant mouse tissue. a, e Immunoblot of calcium handling protein phosphorylation sites in wild-type and *Lmod2* W405* left ventricular tissue at day 70. Quantification of RyR2-pSer^{2808} (**b**), PKA [RIIα] (**c**), PLN-pSer^{16} (**d**), RyR2-pSer^{2814} (**f**), PKA [RIIβ] (**g**), and PLN-pThr^{17} (**h**). Immunoblot of Z-disc and intercalated disc proteins, as well as FHL2 in *Lmod2*-KO (**i**) and *Lmod2* W405* (**m**) left ventricular (LV) tissue at day 20. Quantification of Filamin-C, NRAP, and FHL2 in *Lmod2*-KO (**j-l**) and *Lmod2* W405* (**n-p**) LV tissue**.** Relative expression levels were determined following normalization to

Ponceau S. Values are means ± s.d., n= 7-12, Student's t-test, two-tailed. CASQ2= calsequestrin-2; RyR2= ryanodine receptor-2; PLN= phospholamban.



Supplemental Figure 9. Various biological processes are dysregulated in mutant mouse heart tissue and *LMOD2* W398* hiPSC-CMs. Gene set enrichment analysis (GSEA) demonstrating significant upregulated and downregulated biological process (BP) pathways identified in significant overlapping genes between **a** *Lmod2* W405* and *Lmod2*-KO mice left ventricular tissue, n= 3 (P14) and **b** control and *LMOD2* W398* hiPSC-CMs, N= 4-6 independent cardiac differentiations. P-values are represented by their respective biological process using colored conditional formatting.



Supplemental Figure 10. Uncropped blots from all figures. Areas represented in figures are surrounded by a dashed box.