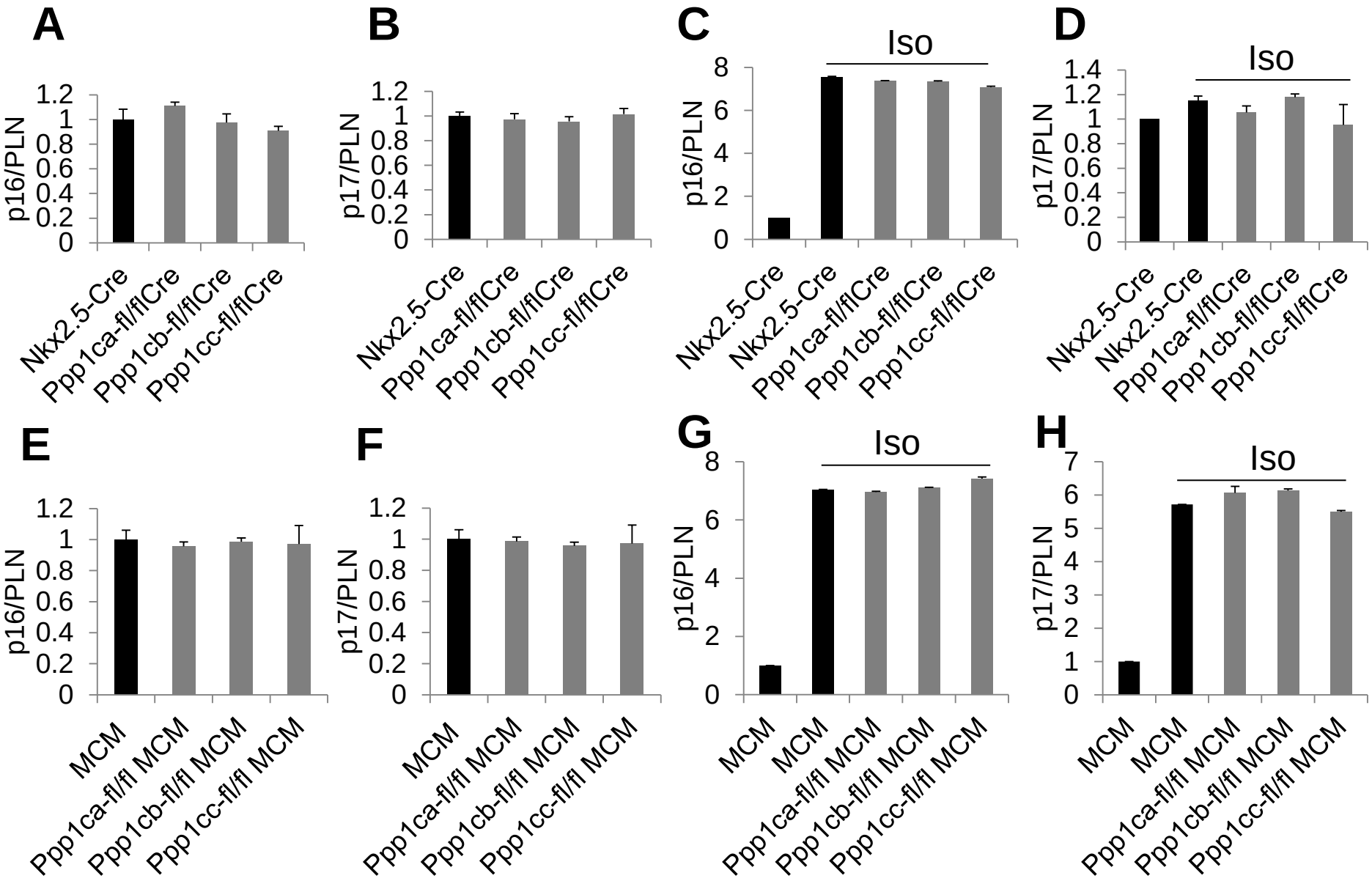
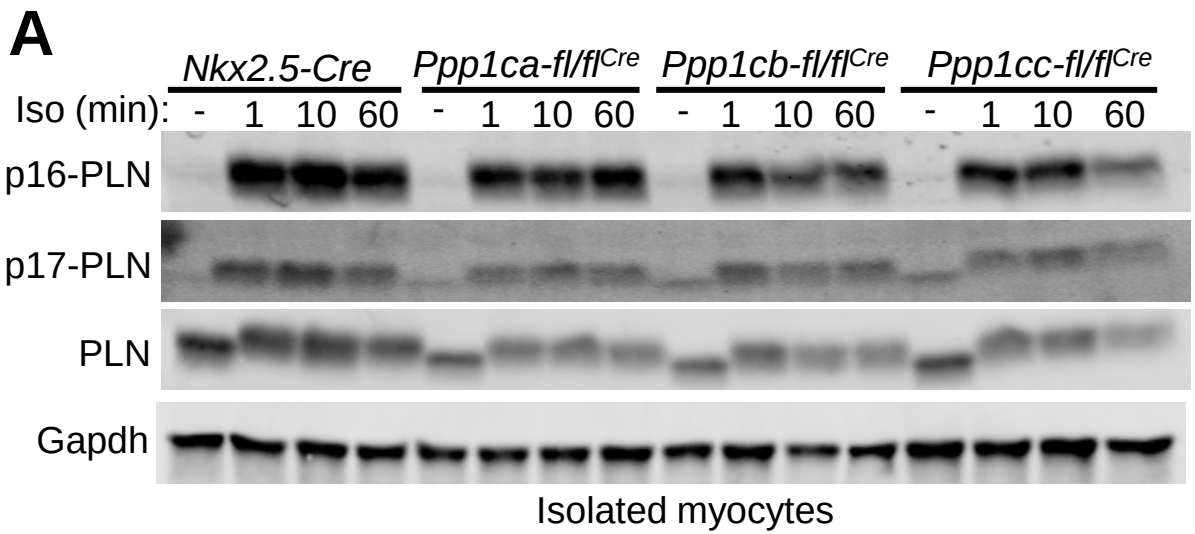
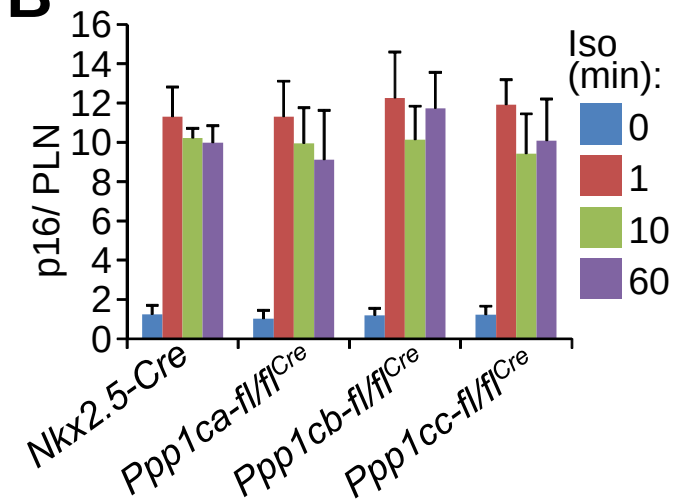
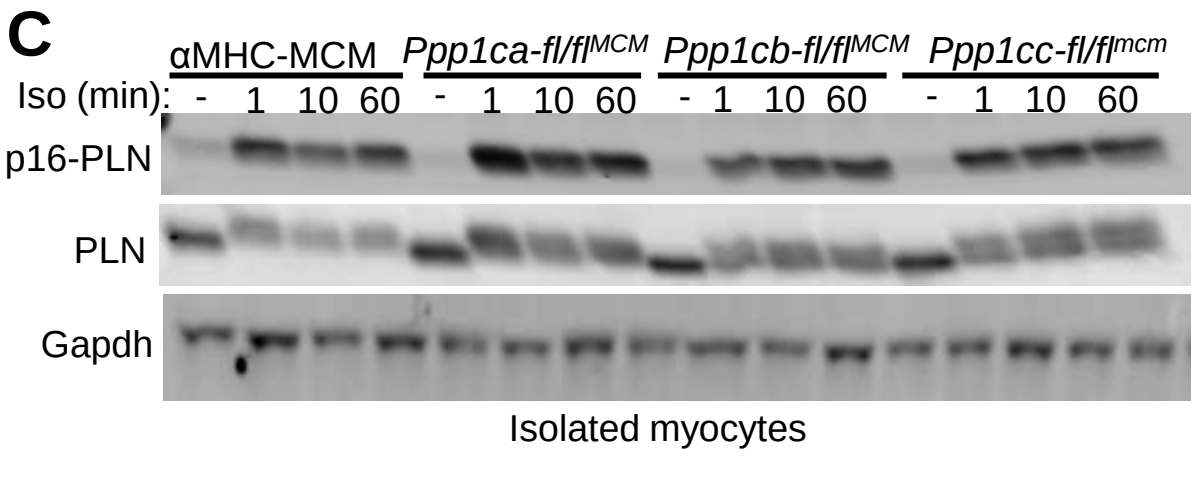
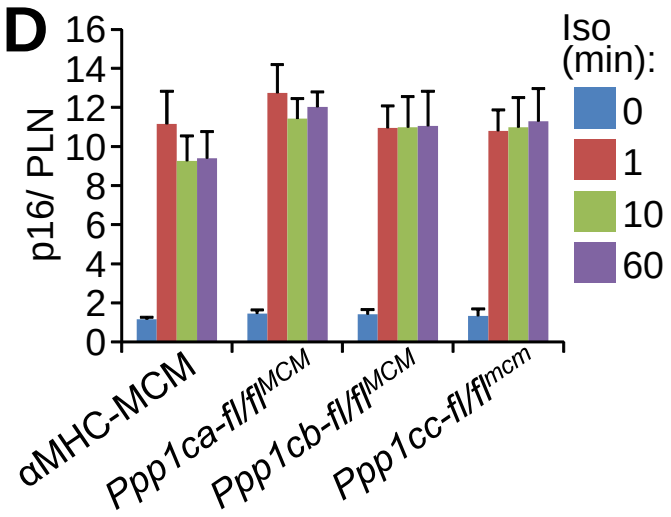


Supplementary Figure 1. Individual deletion of PP1 isoforms has no effect on phospholamban phosphorylation in vivo. (A-D) Quantitation of pSer16-PLN or pThr17-PLN relative to total PLN in Nkx2.5-Cre control and PP1 deleted mice at baseline (A-B) or following isoproterenol stimulation (C-D). N=4 for each group. (E-H) Quantitation of pSer16-PLN or pThr17-PLN relative to total PLN in αMHC-MCM control or PP1 deleted mice at baseline (E-F) or following stimulation (G-H). N=4 for each group.

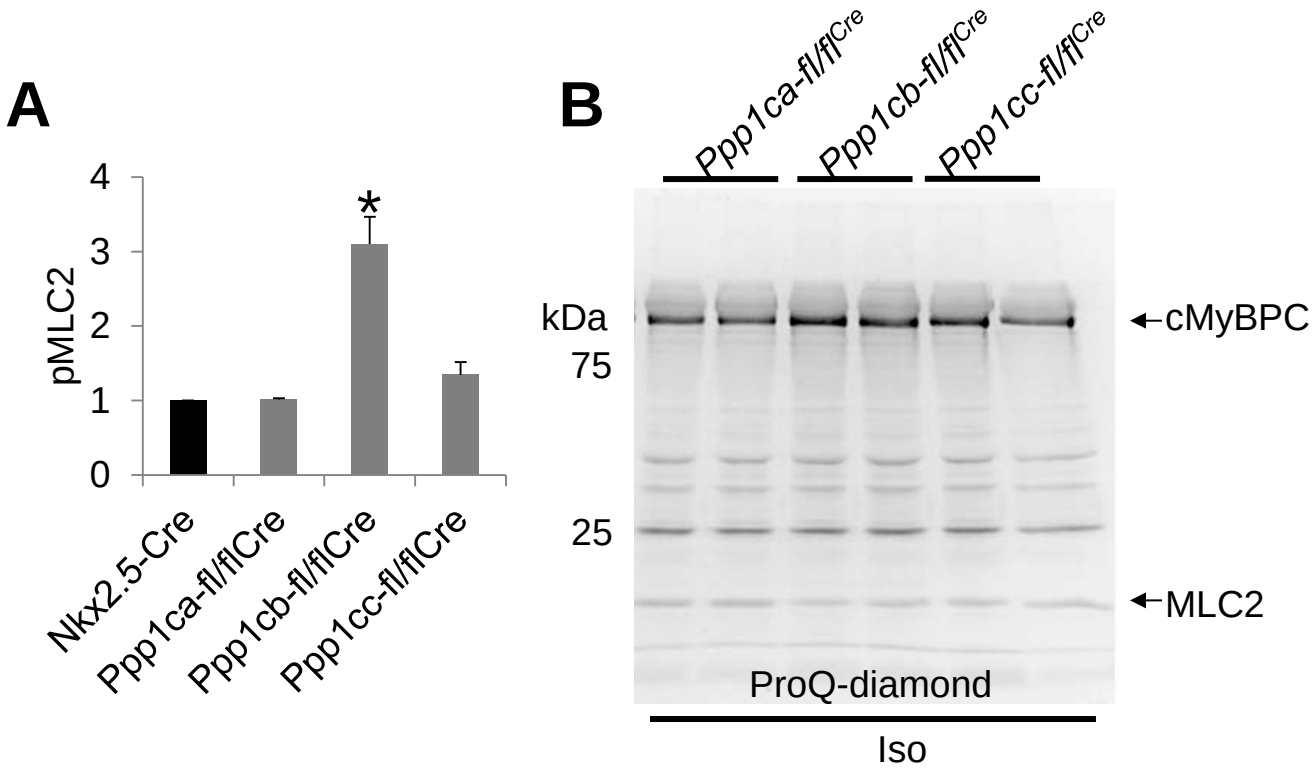




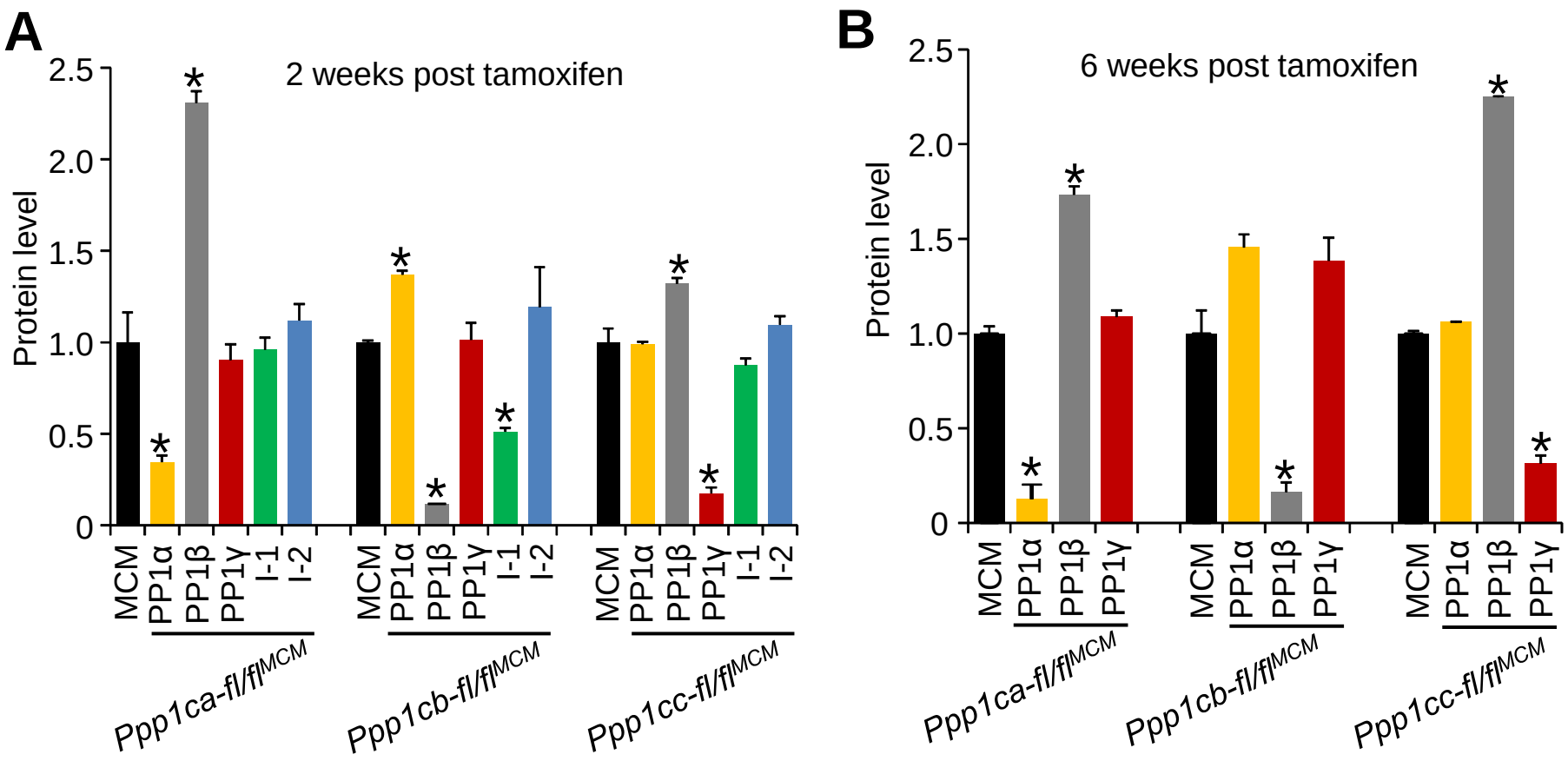




Supplementary Figure 2. Individual deletion of PP1 isoforms has no effect on phospholamban phosphorylation. (A,B) Western blotting and quantitation of phospho-PLN, total PLN and gapdh from adult cardiomyocytes isolated from Cre control mice or PP1 deleted mice driven by *Nkx2.5-Cre*. The quantitation in "B" is for phospho-16-PLN. (C,D) Western blotting and quantitation of phospho-PLN, total PLN and gapdh from adult cardiomyocytes isolated from MerCreMer (MCM) control mice, or PP1 deleted mice driven by the αMHC-MerCreMer transgene. The quantitation in "D" is for phospho-16-PLN. Tamoxifen was administered for 5 consecutive days and myocytes were isolated 6 weeks after treatment for the western blotting analysis. N=3 for each group.

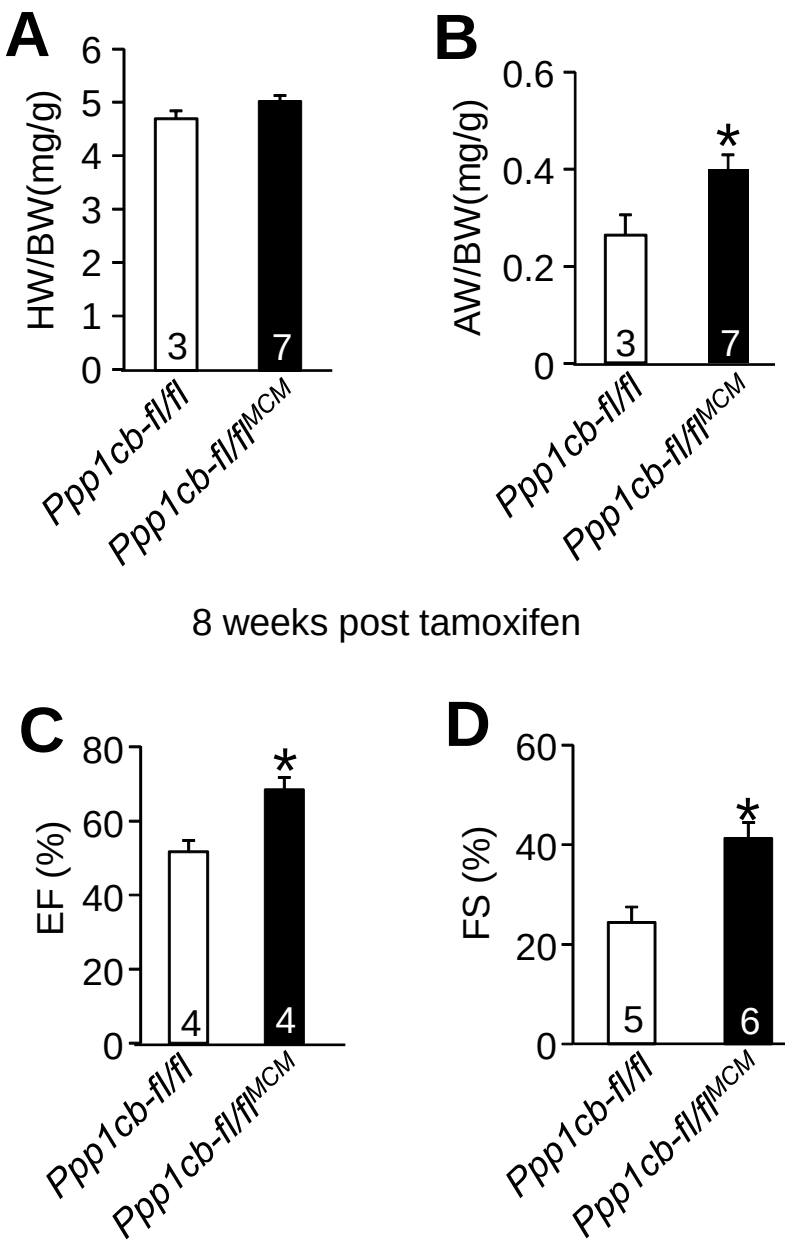


Supplementary Figure 3. Deletion of PP1β leads to enhanced phosphorylation of MLC2 at baseline and of cMyBPC upon isoproterenol challenge. (A) Quantification of MLC2 phosphorylation shown in Fig. 5E. N=6 for each group.*P<0.05 vs Nkx2.5-Cre. (B) ProQ-diamond staining for the phosphorylation status of myofilament proteins from hearts of the indicated mice challenged by isoproterenol for 10 minutes. An increase in signal for cMyBPC is observed in the 2 lanes of heart extracts from *Ppp1cb-fl/fl*$^{Nkx2.5\text{-}Cre}$ mice versus the other 2 isoform deleted mice.

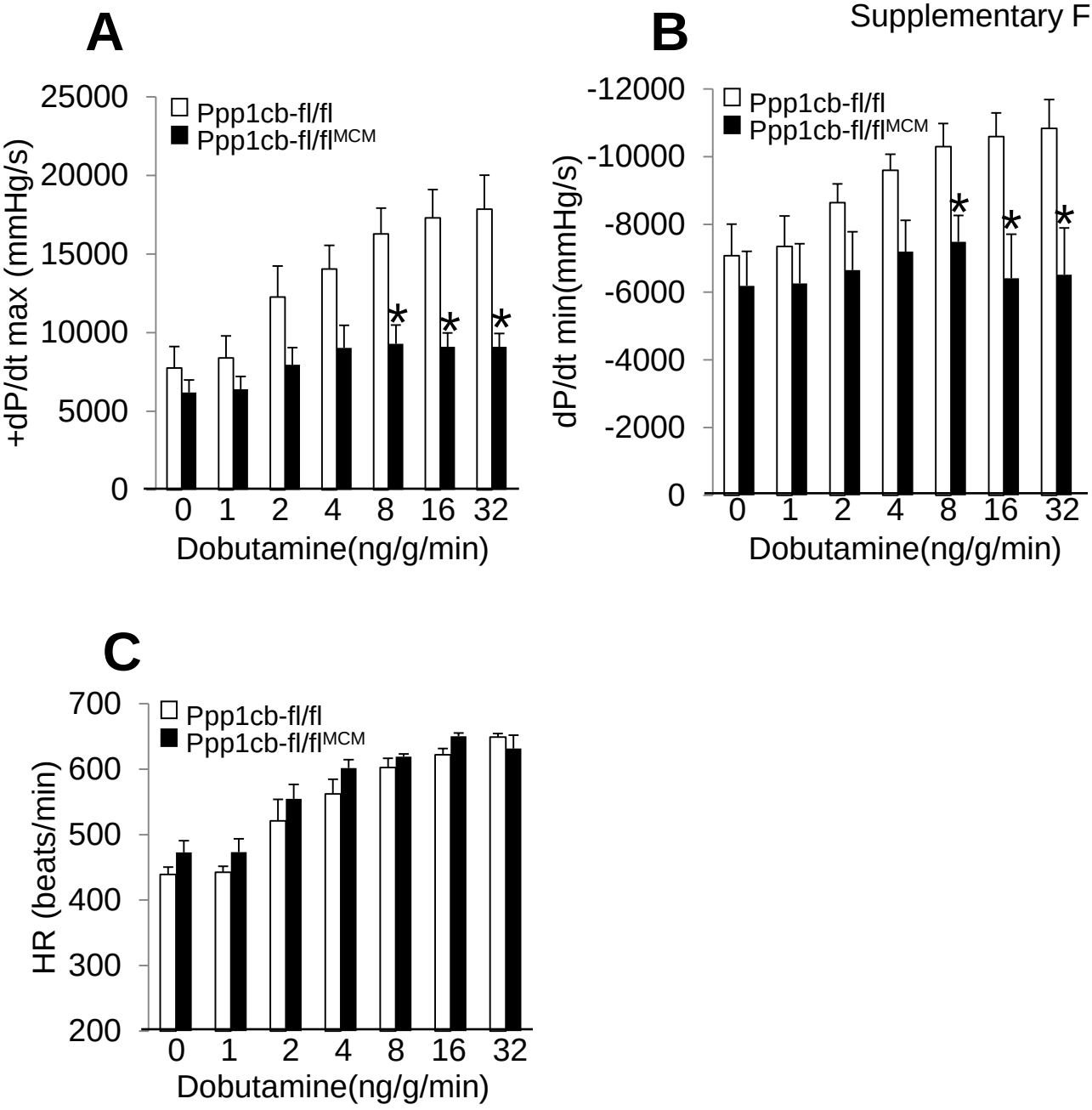


Supplementary Figure 4. Quantitation of the effect of individual PP1 deletion on the expression of other PP1 isoforms and inhibitor proteins. (A) Quantification of protein levels of PP1α, PP1β, PP1γ, I-1 and I-2 in the hearts of αMHC-MerCreMer (MCM) or each of the shown PP1 isoform deficient mice 2 weeks post tamoxifen treatment. (B) Quantification of protein levels of PP1α, PP1β and PP1γ in the hearts of MCM or each of PP1 isoform deficient mice 6 weeks post tamoxifen treatment.. *p<0.05 *vs* MCM. N=4 for each group.

Supplementary Fig. 5



Supplementary Figure 5. PP1β deletion leads to atria enlargement and enhanced cardiac function. (A,B) Measurement of heart weight/body weight (HW/BW) and atria weight/body weight (AW/BW) in *Ppp1cb-fl/fl* or *Ppp1cb-fl/fl*^{MCM} mice 8 weeks after tamoxifen treatment. *p<0.05 *vs Ppp1cb-fl/fl*. (C,D) Measurement of ejection fraction (EF) percentage and fractional shortening (FS) percentage by echocardiography in *Ppp1cb-fl/fl* or *Ppp1cb-fl/fl*^{MCM} mice 8 weeks after tamoxifen treatment. *p<0.05 *vs Ppp1cb-fl/fl*. Number of mice analyzed is shown in the bars of each of the graphs



Supplementary Figure 6. Adult cardiac-specific deletion of PP1β leads to reduced β-adrenergic responsiveness. (A,B) Invasive hemodynamic measurement of cardiac contractility assessed as maximum and minimum rate of pressure change in the left ventricle over time (dP/dt max and dP/dt min, respectively) upon 1 minute of dobutamine stimulation in *Ppp1cb-fl/fl* mice or *Ppp1cb-fl/fl*^{MCM} mice, 2 weeks after tamoxifen treatment. N=3 for each group. *P<0.05 versus *Ppp1cb-fl/fl.* (C) Heart rate(HR) for each group at baseline and upon dobutamine challenge.

Supplementary Table 1
Echocardiographic parameters in Ppp1cb-fl/fl and Ppp1cb-fl/fl^{MCM} mice

	Ppp1cb-fl/fl	Ppp1cb-fl/fl^{MCM}	P value
2 weeks post tamoxifen	N=13	N=21	
LVIDd(mm)	3.87±0.12	3.87±0.09	0.98
LVIDs(mm)	2.63±0.08	2.46±0.15	0.15
LVPWd(mm)	0.95±0.02	1.00±0.01	0.12
LVPWs(mm)	1.12±0.03	1.18±0.01	0.09
IVSd(mm)	0.76±0.03	0.80±0.04	0.52
IVSs(mm)	1.14±0.03	1.24±0.03	0.04*
HR(bpm)	401.4±17.3	411.5±10.7	0.76
6 weeks post tamoxifen	N=12	N=22	
LVIDd(mm)	3.95±0.09	3.97±0.0.6	0.87
LVIDs(mm)	2.70±0.10	2.58±0.0.6	0.32
LVPWd(mm)	1.00±0.02	1.02±0.01	0.65
LVPWs(mm)	1.23±0.04	1.23±0.02	0.71
IVSd(mm)	0.76±0.01	0.76±0.01	0.99
IVSs(mm)	1.10±0.03	0.23±0.01	0.02*
HR(bpm)	395.2±9.4	410.1±10.6	0.43

Data are presented as mean±SE. LVIDd and LVIDs: left ventricular end-diastolic and end-systolic diameters; LVPWd and LVPWs: left ventricular end-diastolic and end-systolic posterior wall thickness; IVSd and IVSs: end-diastolic and end-systolic intraventricular septal thickness. HR: heart rate. *$p<0.05$.

Supplementary Table 2
Cardiac hemodynamic parameters in Ppp1cb-fl/fl and Ppp1cb-fl/fl^{MCM} mice

	Ppp1cb-fl/fl(N=6)	Ppp1cb-fl/fl^{MCM}(N=7)	P value
dP/dt max(mmHg/s)	7933.5±496.6	7567.1±695.1	0.68
dP/dt min(mmHg/s)	-7257.5±470.4	-7443.5±763.6	0.84
HR(bpm)	464.6±13.2	475.1±17.0	0.67
SV(µl)	22.7±1.6	25.6±1.2	0.16
CO(µl/min)	10506.3±695.1	12161.4±671.6	0.11
Tau(ms)	7.1±0.2	8.8±0.6	0.02*
EDP(mmHg)	12.4±0.7	11.6±0.8	0.49
ESP(mmHg)	88.2±3.2	85.5±2.4	0.51
VED(µl)	29.7±1.9	40.1±2.4	0.008**
VES(µl)	12.1±1.1	20.0±1.8	0.005**
Vmax(µl)	33.7±2.1	43.3±2.1	0.01*
Vmin(µl)	10.9±1.0	17.6±1.9	0.01*

Data are presented as mean±SE. HR, heart rate; SV, stroke volume; CO, cardiac output; EDP, end diastolic pressure; ESP, end systolic pressure; VED, end-diastolic volume; VES, end-systolic volume; Vmax, maximum volume; Vmin, minimal volume. *p<0.05; ** p<0.01.