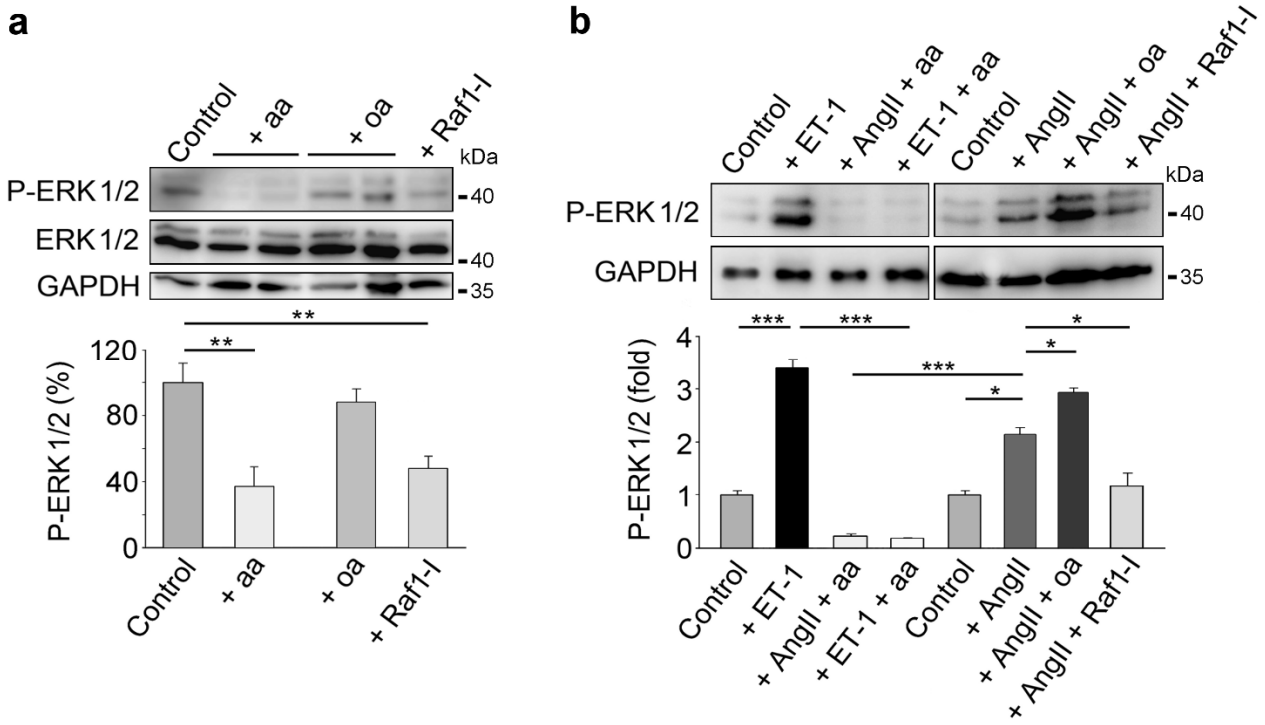
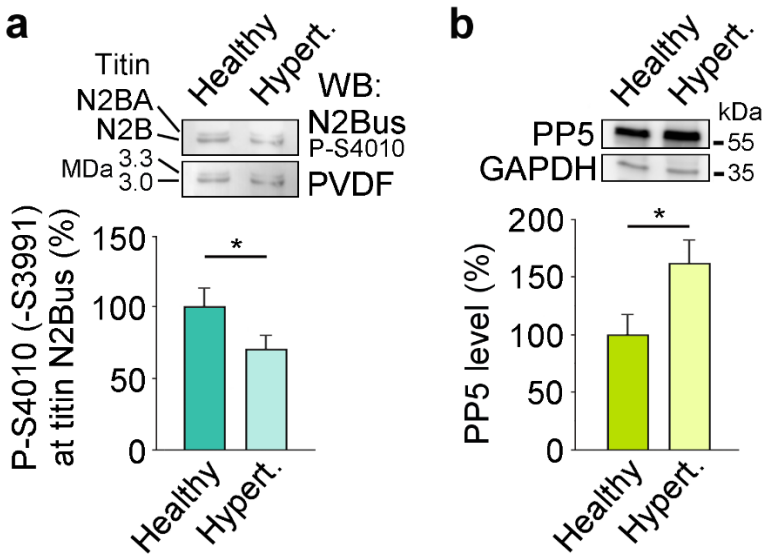
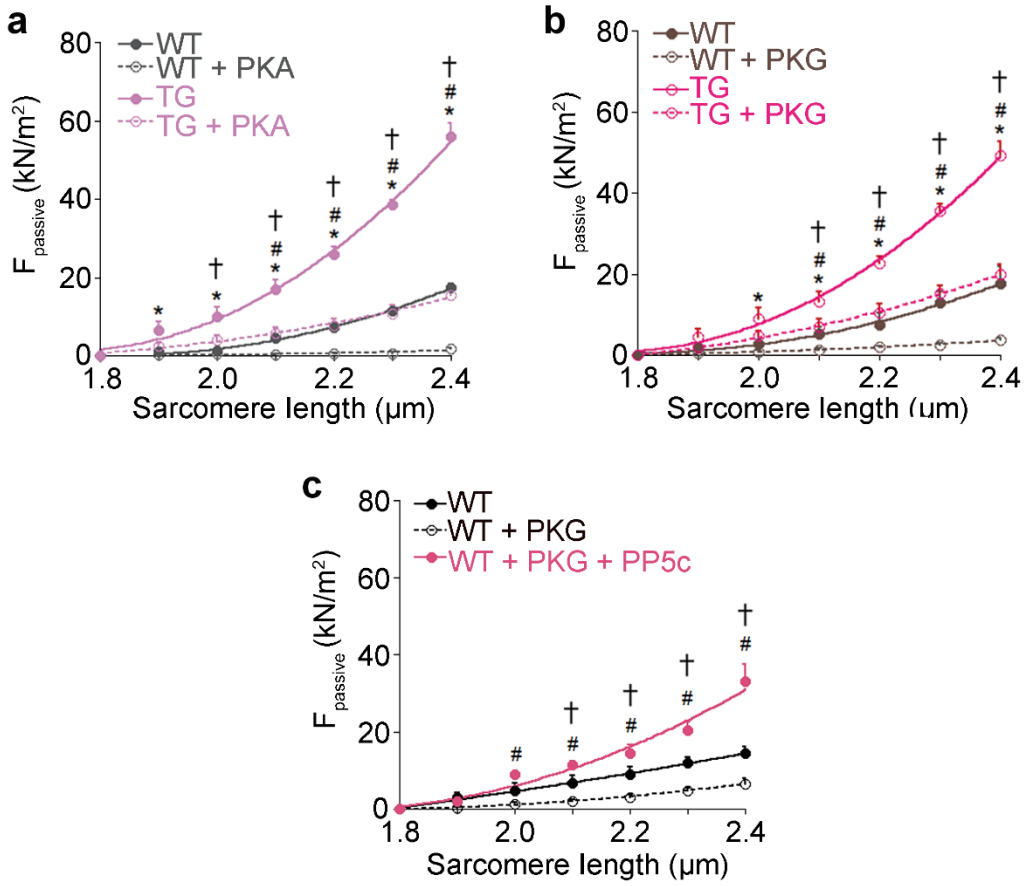


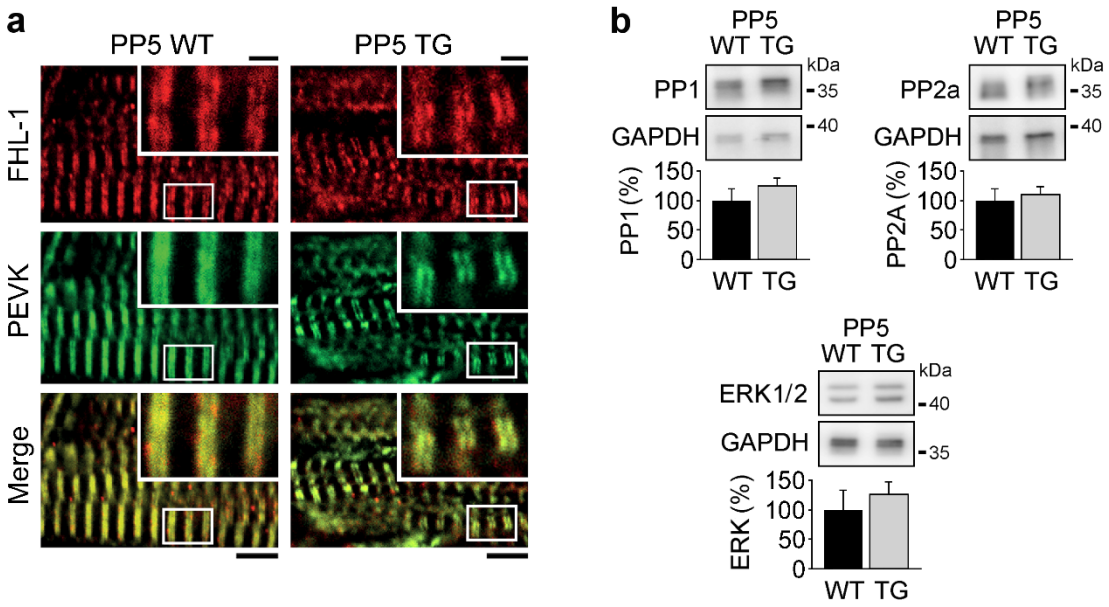

**Supplementary Figure 1. Pharmacological modulation of MAPK/ERK pathway activation in neonatal rat ventricular myocyte cultures.** (**a**) Expression of phosphorylated (activated) ERK1/2 and total ERK1/2 by Western blot under baseline conditions (control) and following treatment with arachidonic acid (aa; 200 μM, 2 h), okadaic acid (oa, 10 nM; 1 h), or inhibitor of Raf1 (Raf1-I, 20 μM; 1 h). P-ERK signals normalized to ERK signals (and indexed to GAPDH). (**b**) Phospho-ERK1/2 expression before and after treatment with endothelin-1 (ET-1, 10 nM; 24 h) or angiotensin-II (AngII, 1 μM; 24 h), and additional treatment with aa (200 μM; 2 h), oa (10 nM; 1 h), or Raf1-I (20 μM; 1 h). Bar graphs in (a) and (b) show phospho-ERK1/2 over ERK1/2 (indexed to GAPDH), relative to the mean expression level in control cells. Data are mean±SEM, n=3 different cell culture batches per condition; duplicate analysis per batch; *p<0.05, **p<0.01, and ***p<0.001, by Holm-Sidak method following ANOVA.




**Supplementary Figure 2. Expression of phospho-titin and PP5 in healthy and diseased dog left ventricular tissue.** (**a**) Site-specific N2Bus phosphorylation by Western blot in myocardial tissue from healthy canine hearts vs. hypertensive (Hypert.) hearts with diastolic dysfunction. Detection with phospho-specific antibody to human P-S4010. Bar graph shows the mean±SEM (n=5/group) phosphorylation level in hypertensive hearts relative to that in control hearts. (**b**) PP5 expression in healthy canine hearts vs. hypertensive hearts (n=5/group). Bar graph shows the mean±SEM (n=5/group) expression level in hypertensive hearts relative to that in control hearts. *p<0.05, by two-tailed Student's t-test.




**Supplementary Figure 3. Effect of PKA, PKG, and PP5c on passive tension of single permeabilized cardiomyocytes from WT and PP5 TG mouse hearts.** (**a**) Passive tension ($F_{passive}$) vs. SL curves of PP5 TG and WT cardiomyocytes in relaxing solution, before and after treatment with catalytic subunit of PKA. (**b**) $F_{passive}$-SL curves of PP5 TG and WT cardiomyocytes before and after treatment with cGMP-activated PKG. (**c**) $F_{passive}$-SL curves of WT cardiomyocytes before and after treatment with cGMP-activated PKG and additional exposure to PP5c. Data are mean±SEM, n=6 cells/condition (a and b), n=5 cells/condition (c). Curves are second-order polynomial fits to the means. *p<0.05, TG vs. WT; $^{\#}$p<0.05, WT+PKA vs. WT in (a) and WT+PKG vs. WT in (b) and (c); $^{\dagger}$p<0.05, TG+PKA vs. TG in (a), TG+PKG vs. TG in (b), and WT+PKG+PP5c vs. WT+PKG in (c); all by two-tailed Student's t-test.




**Supplementary Figure 4. Properties of signaling molecules in PP5 TG and WT mouse cardiomyocytes.** (**a**) Localization of FHL-1 in cardiomyocytes from PP5 TG and WT hearts by indirect immunofluorescence. Anti-FHL-1 antibody (secondary antibody: Cy3-conjugated IgG), counterstained with anti-PEVK (titin) antibody (secondary antibody: FITC-conjugated IgG). Bars, 5 μm (main) and 1 μm (insets). (**b**) Expression levels of PP1, PP2a, and ERK1/2 in PP5 TG mouse hearts, relative to those in WT hearts, by Western blot. Bar graphs show mean±SEM, n=4 hearts/group; duplicate analysis/heart.

Figure 1b

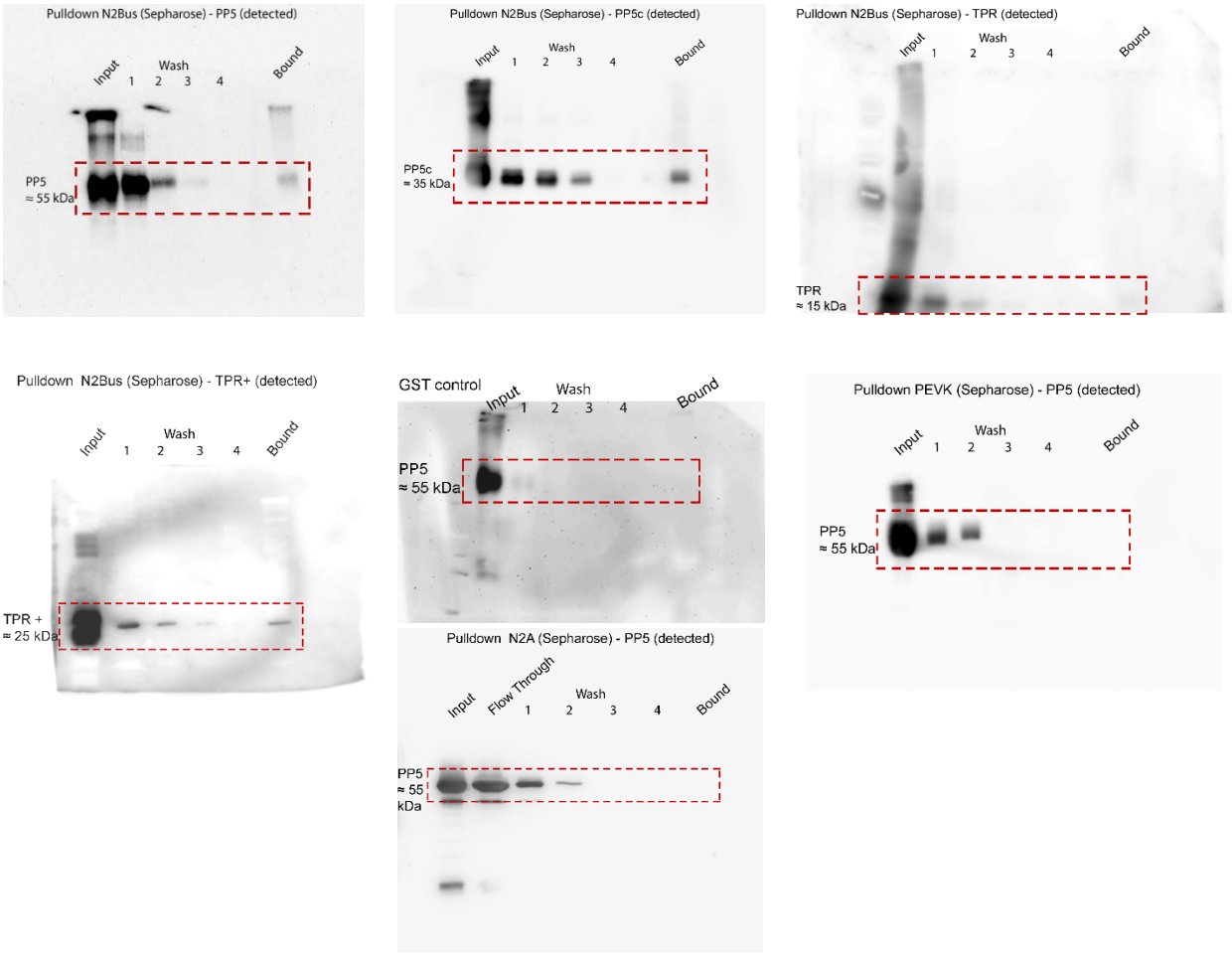



Figure 1c

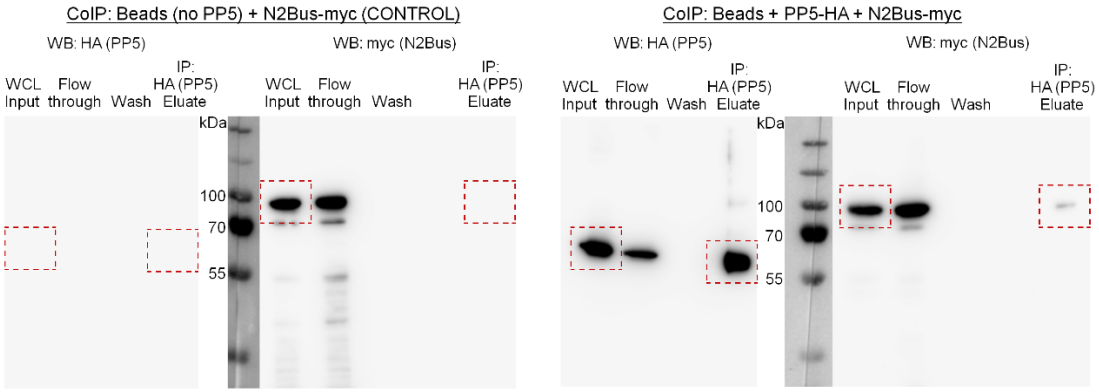


Figure 1e

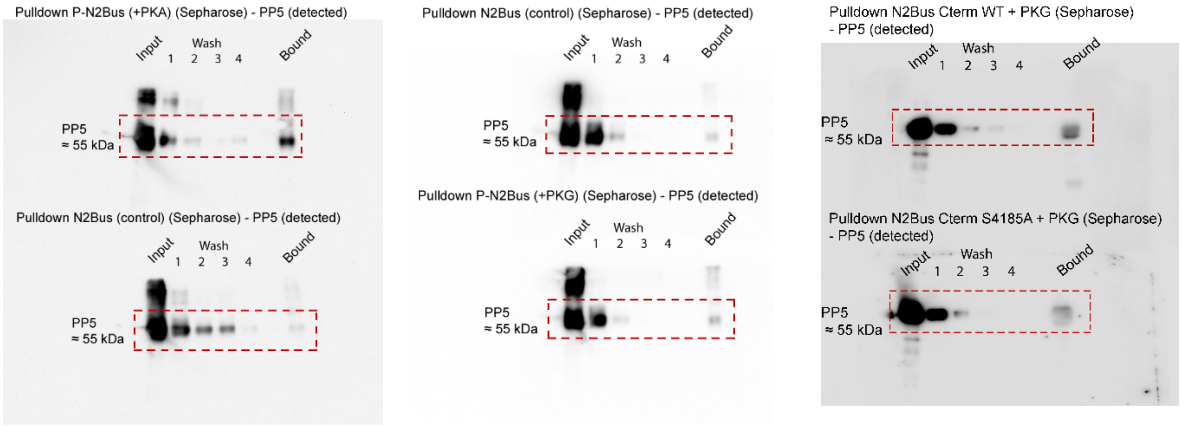
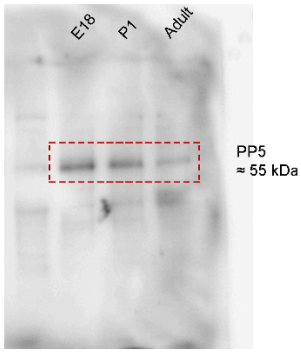



Figure 2b



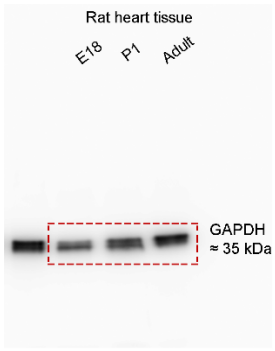




Figure 2b

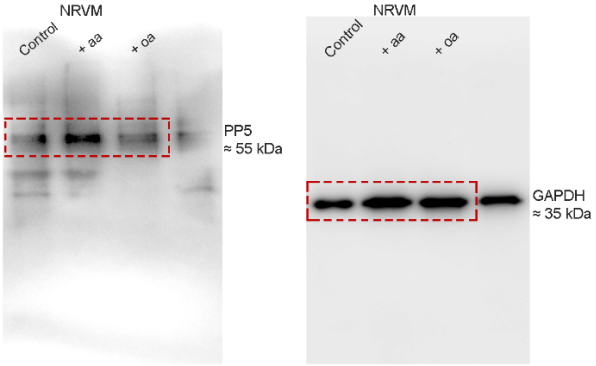



Figure 2e

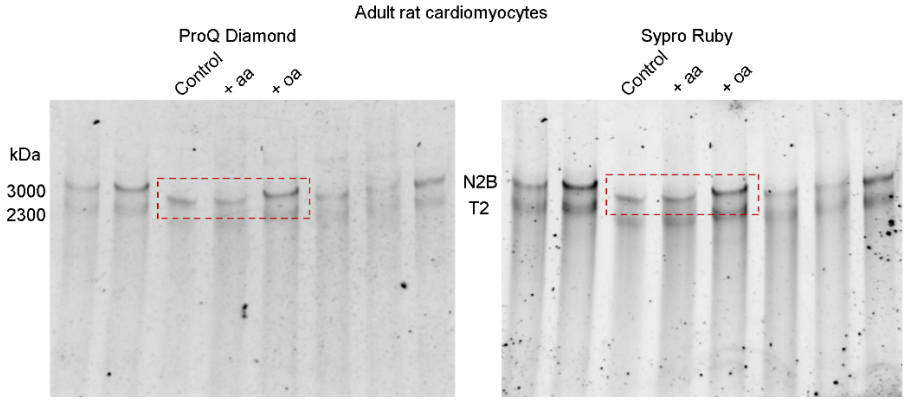



Figure 3a

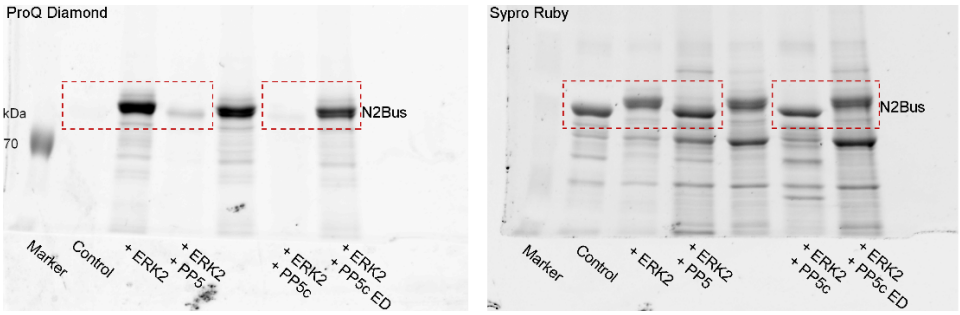


Figure 3b

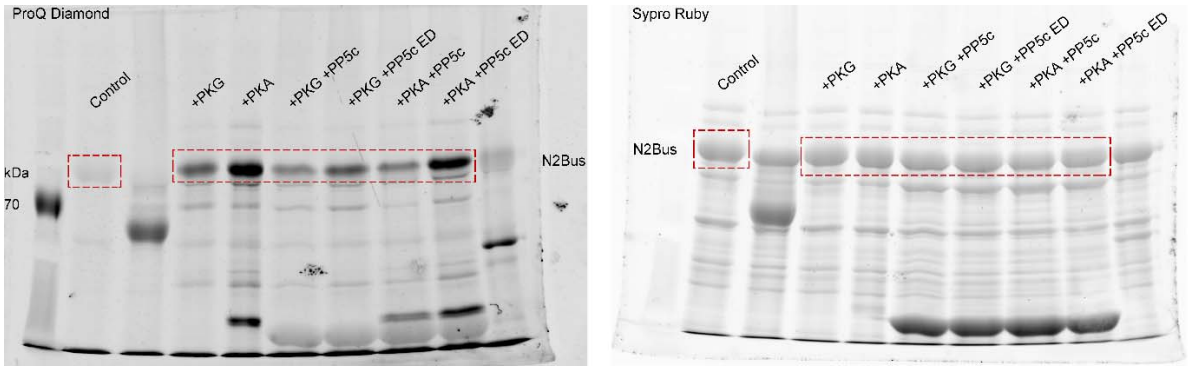



Figure 3c

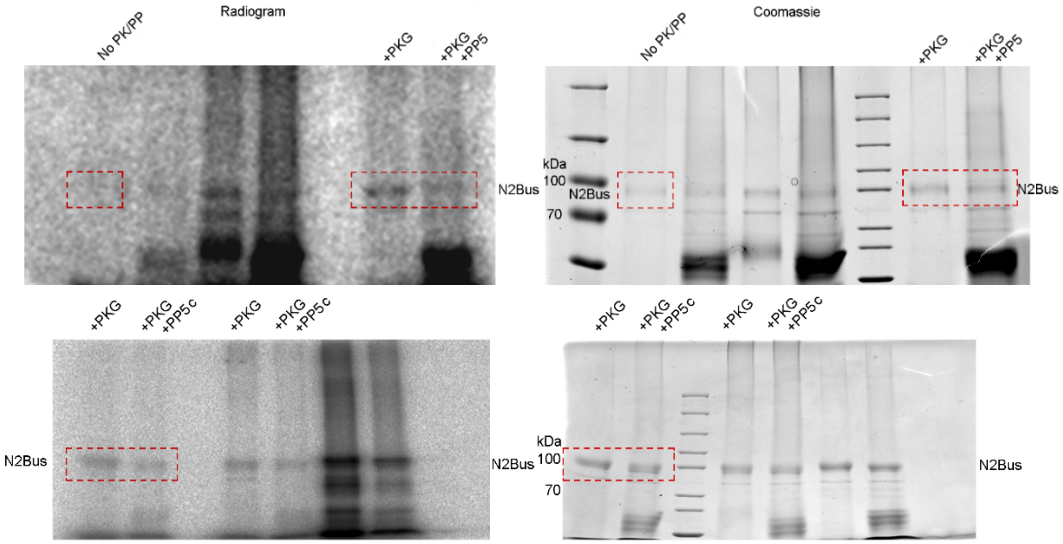



Figure 3d

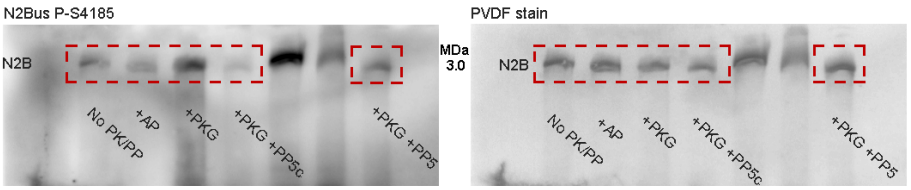


Figure 3e

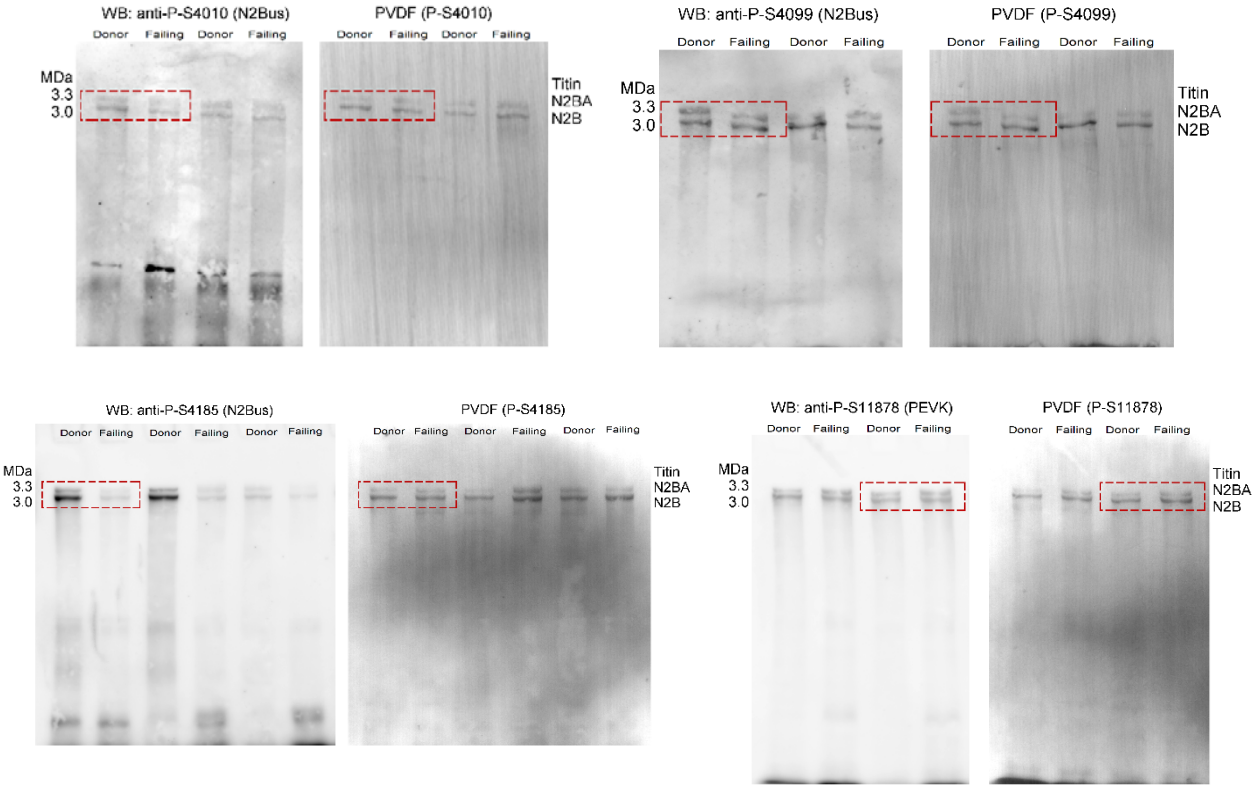



Figure 3f

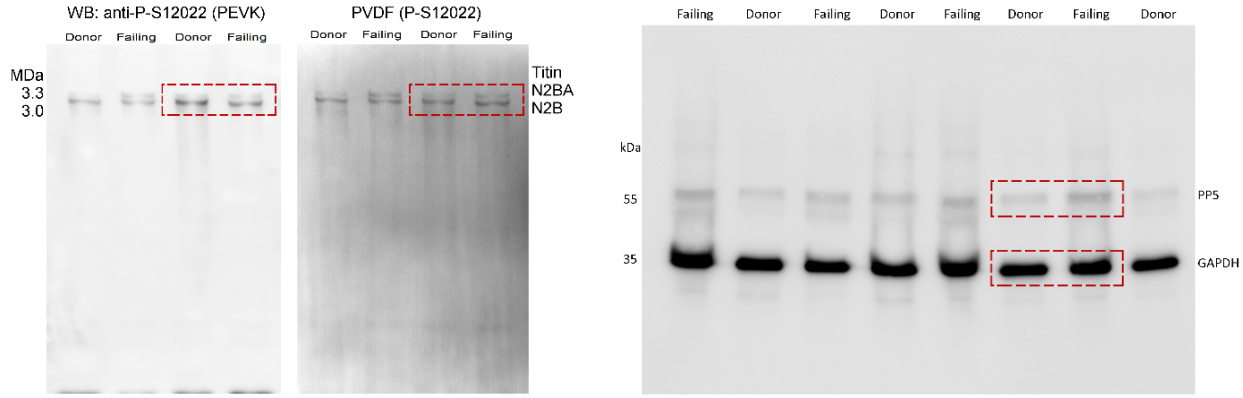
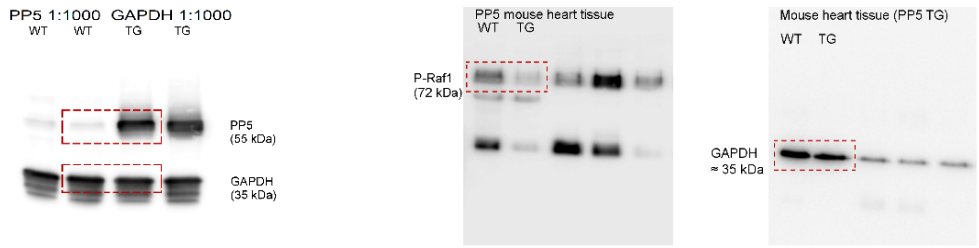
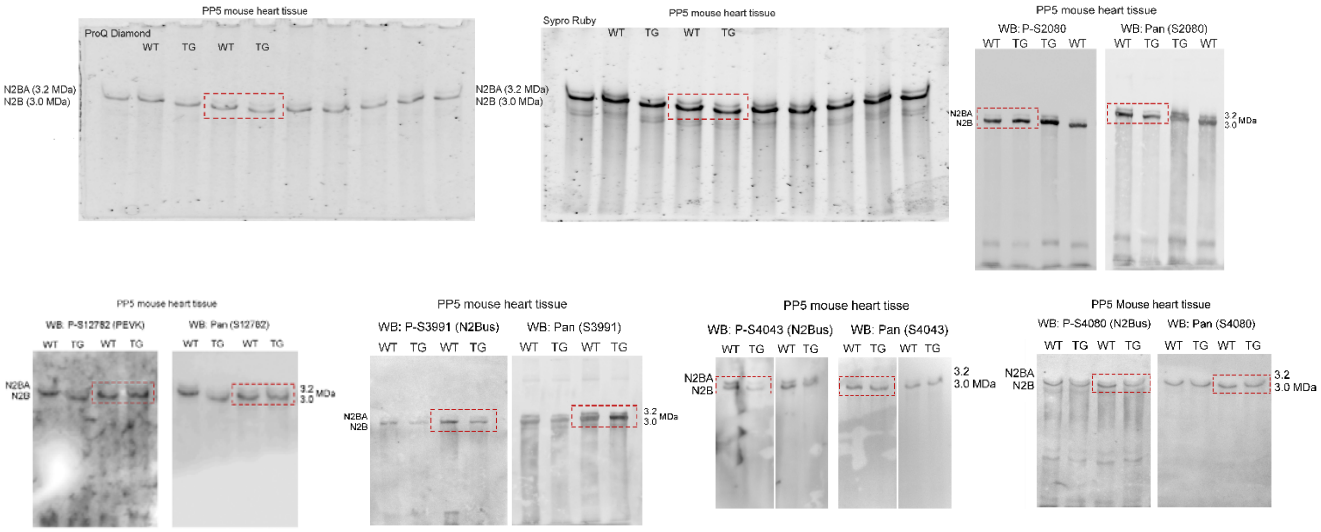


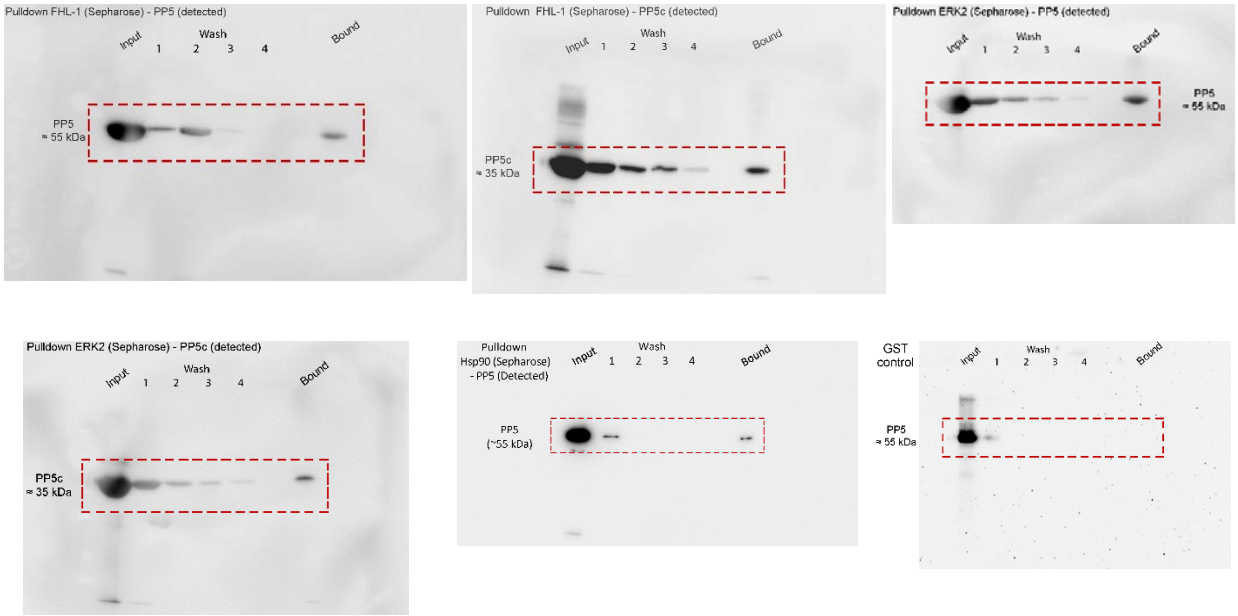

Figure 4a



## Figure 4d




## Figure 6a




## Figure 6b

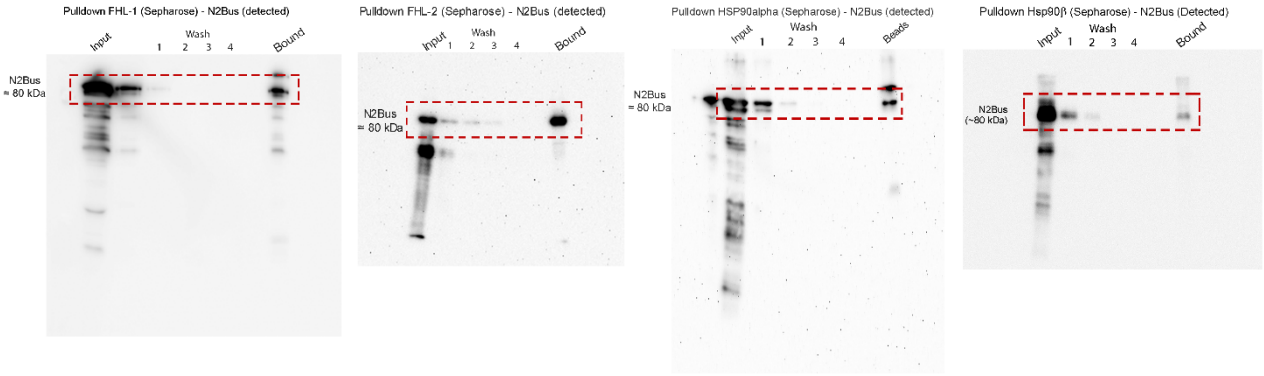


Figure 6c

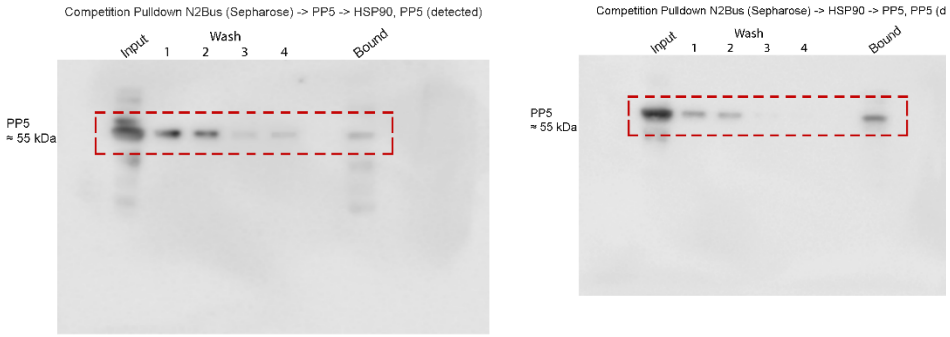
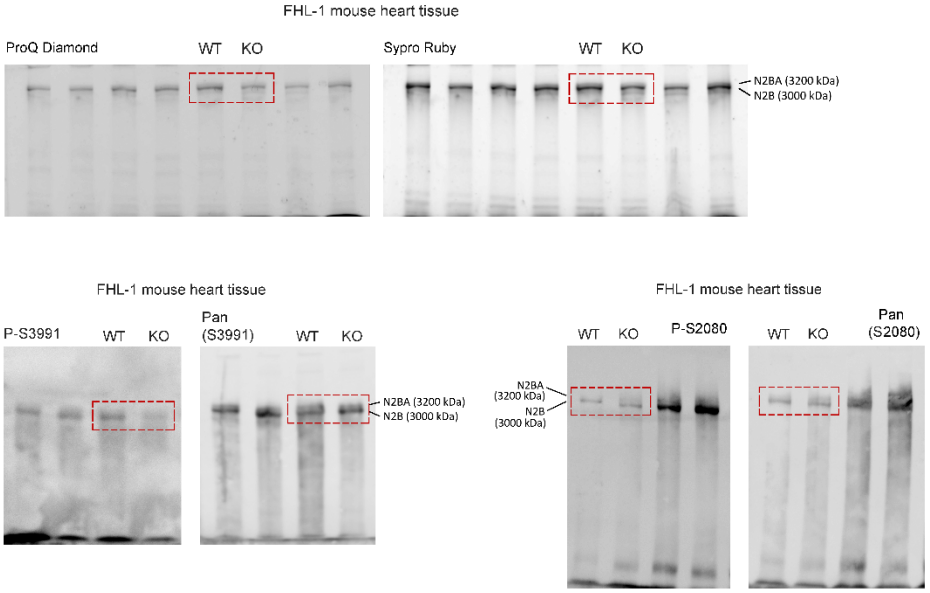
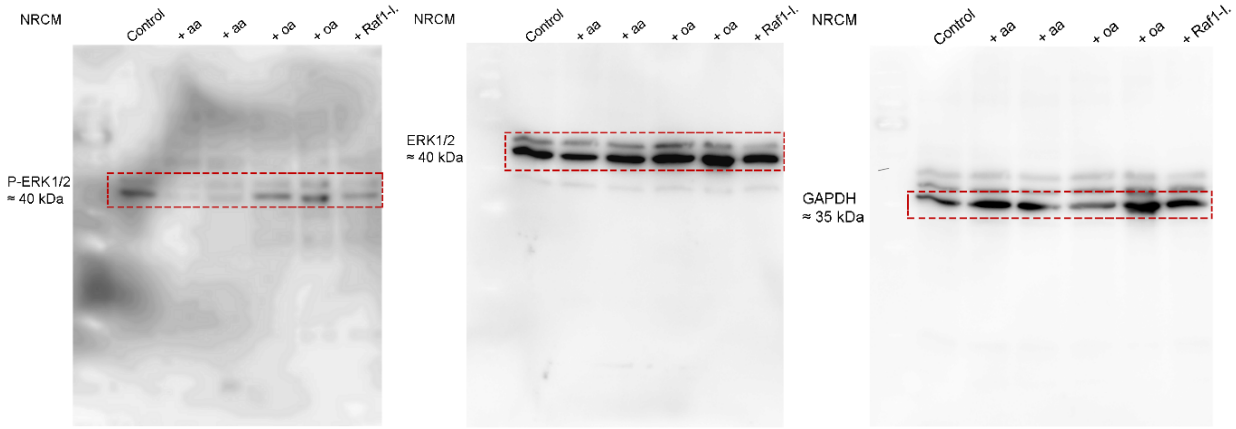



Figure 7a

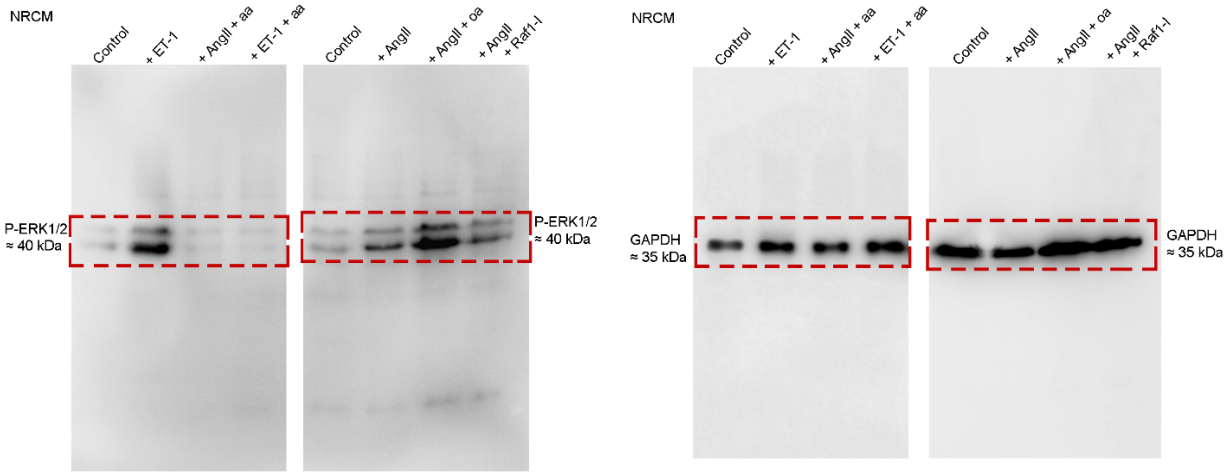



**Supplementary Figure 5. Uncropped images of blots and gels used in main figures.** The number of the corresponding figure is shown above the panels. Regions used for figures are boxed. Position of molecular mass marker is shown on the side (kDa or MDa).

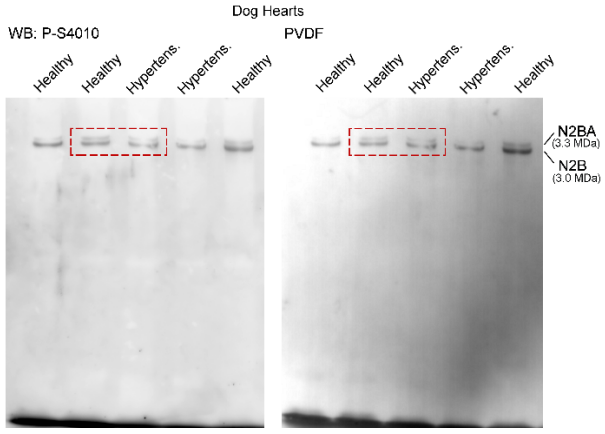
## Supplementary Figure 1a



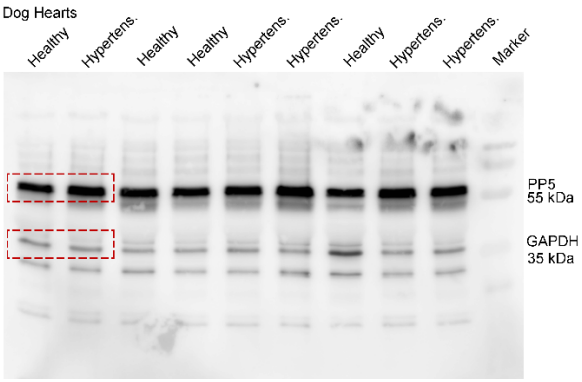

## Supplementary Figure 1b



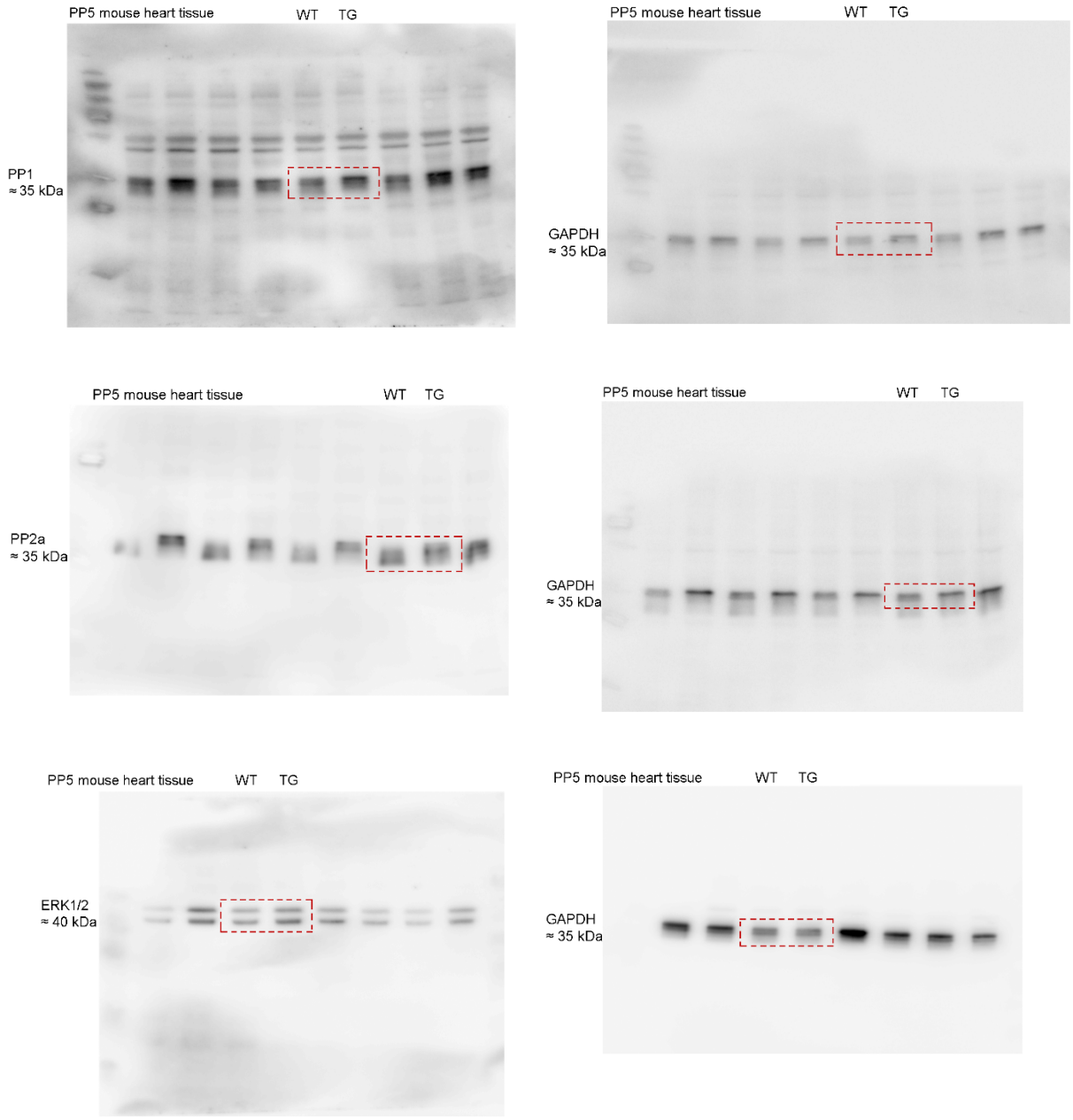

## Supplementary Figure 2a




## Supplementary Figure 2b



Supplementary Figure 4b




**Supplementary Figure 6. Uncropped images of blots and gels used in supplementary figures.** The number of the corresponding figure is shown above the panels. Regions used for figures are boxed. Position of molecular mass marker is shown on the side (kDa or MDa).

**Supplementary Table 1.** Primers for PCR used to generate recombinant WT constructs.

| Name | Restriction site | Sequence |
|---|---|---|
| Titin N2Bus 5‘ | *Nde*I (CATATG) | GACATGACTGATACCCCCTGC |
| Titin N2Bus 3‘ | *Sal*I (GTCGAC) | GCCATCCTCTTTGATTAAGC |
| Titin N2Bus 5‘ | *Bam*HI GGATCC) | GACATGACTGATACCCCCTGC |
| Titin N2Bus 3‘ | *Xho*I (CTCGAG) | GCCATCCTCTTTGATTAAGC |
| N2Bus C-Term 5‘ | *EcoR*I (GAATTC) | CTTGTTACTTCGGCAAAGTCTGTAACAG |
| N2Bus C-Term 3‘ | *Xho*I (CTCGAG) | GCCATCCTCTTTGATTAAGCCACCCTCAG |
| PP5 full-length 5‘ | *EcoR*I (GAATTC) | ATGGCGATGGCGGA |
| PP5 full-length 3‘ | *Xho*I (CTCGAG) | TCACATCATTCCTAGCTGCA |
| PP5 catalytic 5‘ | *EcoR*I (GAATTC) | AGCATGACCATTGAGGAT |
| PP5 catalytic 3‘ | *Xho*I (CTCGAG) | CATCATTCCTAGCTGCAG |
| PP5 TPR 5‘ | *EcoR*I (GAATTC) | GCAGAGGAGC |
| PP5 TPR 3‘ | *Xho*I (CTCGAG) | GTCATGGGGCT |
| PP5 TPR+ 5‘ | *EcoR*I (GAATTC) | ATGGCGATGGCGGA |
| PP5 TPR+ 3‘ | *Xho*I (CTCGAG) | GTCTTCAAGCTTGGGTCC |
| Hsp90 5‘ | *EcoR*I (GAATTC) | ATGCCCCCGTGTTCGGGC |
| Hsp90 3‘ | *Xho*I (CTCGAG) | GTCTACTTCTTCCATG |
| FHL-2 5‘ | *EcoR*I (GAATTC) | ATGACTGAGCGCTTTGAC |
| FHL-2 3‘ | *Xho*I (CTCGAG) | TCAGATGTCTTTCCCACAG |
| Titin N2A 5‘ | *EcoR*I (GAATTC) | GCTGGCAGGGAAATAAAGC |
| Titin N2A 3‘ | *Sal*I (GTCGAC) | GGTGGCACTGCTCTGATAGG |
| Titin PEVK 5‘ | *EcoR*I (GAATTC) | CCTGGAGGTGAAAAGAAAG |
| Titin PEVK 3‘ | *Xho*I (CTCGAG) | GGTGAACGGGGCTT |