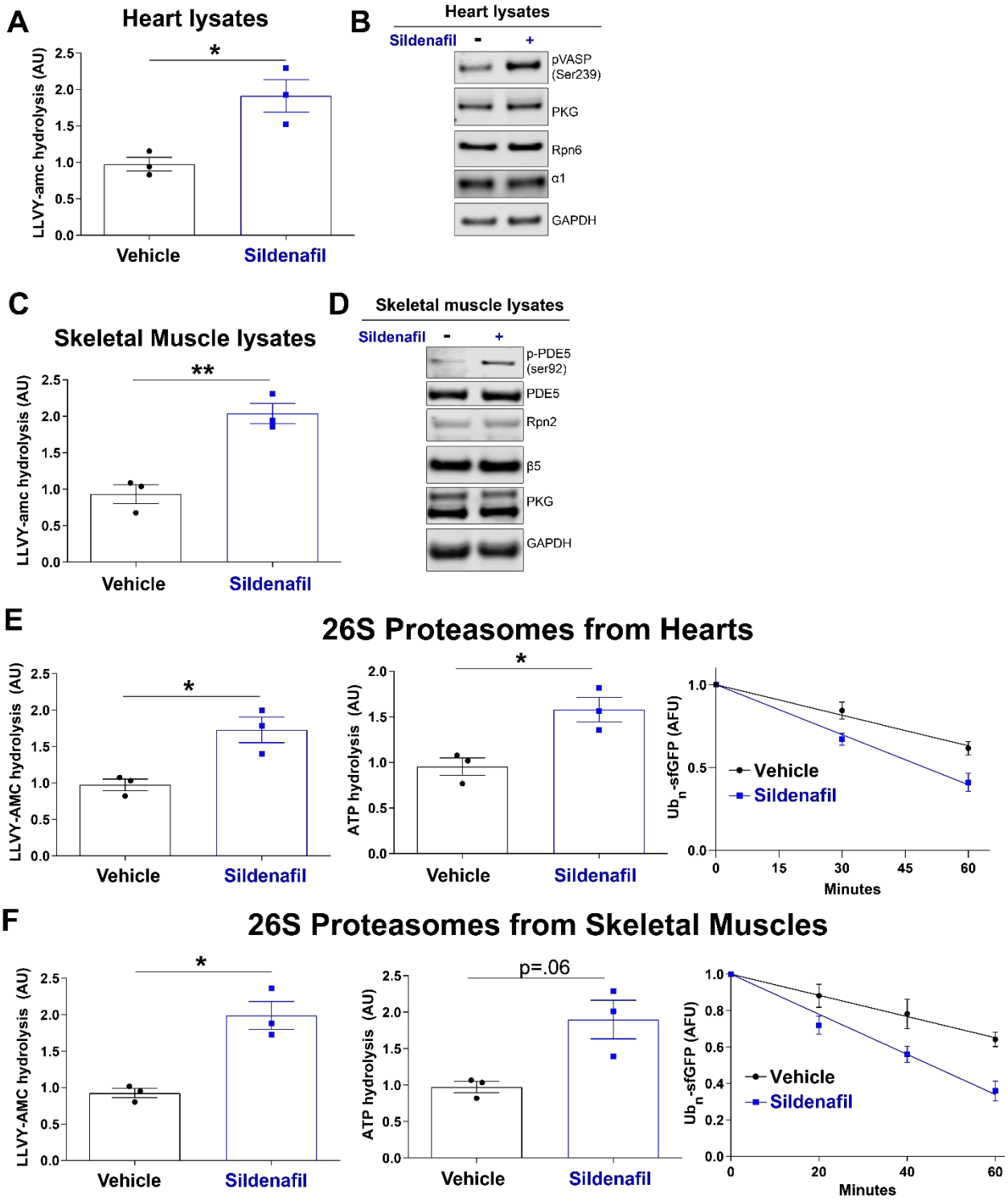
Supplemental Information

Supplemental Figure 1



Supplemental Figure 1: Sildenafil treatment for 5 days of wild type mice activates 26S proteasomes in hearts and skeletal muscles.

A.) Proteasomal chymotrypsin-like activity was greater in the lysates of the hearts from sildenafil-treated mice than in those of controls. Here and below, n=3 mice per condition, error bars represent SEM and * =p ≤.05; ** = p≤.01; *** = p≤.001. Student's T test.

B.) The levels of proteasome subunits in cardiac lysates was not changed by the sildenafil treatment. Increased phosphorylation of VASP on Ser239 indicates activation of PKG. Representative western blots from one mouse of each condition are shown.

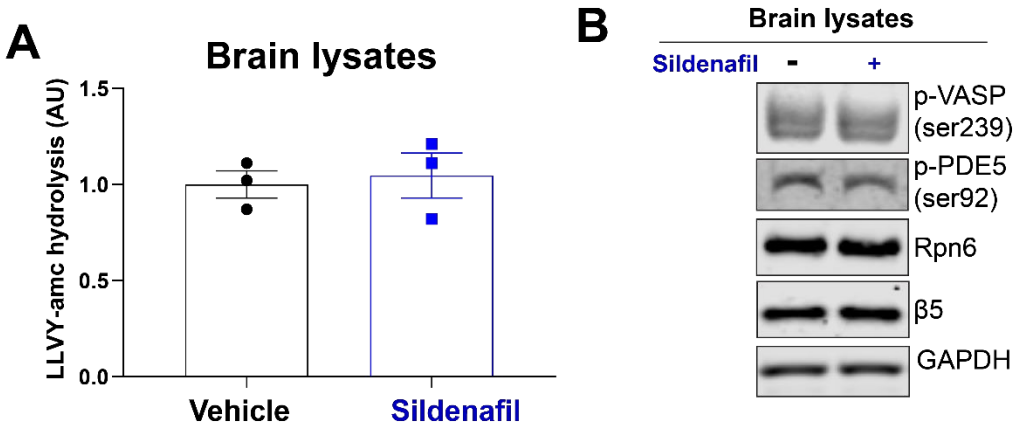
C.) Proteasomal chymotrypsin-like activity was increased in lysates of gastrocnemius muscle of sildenafil-treated mice over levels in controls.

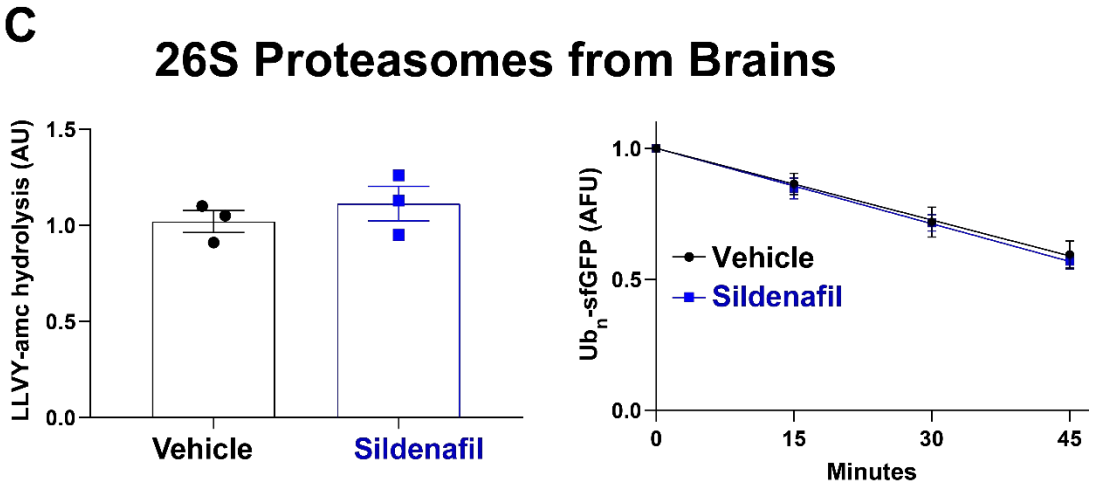
D.) The levels of proteasome subunits in skeletal muscle lysates were not changed by the sildenafil treatment. Increased phosphorylation of PDE5 on Ser92 indicates activation of PKG. Representative western blots from one mouse of each condition are shown.

E.) 26S proteasomes affinity-purified from the hearts of sildenafil-treated mice exhibited faster hydrolysis of a small peptide, ATP, and K48-linked ubiquitinated superfolder GFP than those from vehicle-treated controls. Here and in Fig. S1F, each dot on the graphs represents a 26S proteasome purification from an individual mouse.

F.) 26S proteasomes purified from gastrocnemius muscles of mice treated with sildenafil are more active than from those from vehicle-treated controls in the hydrolysis of a small peptide, ATP, and K48-linked ubiquitinated superfolder GFP.

Supplemental Figure 2





Supplemental Figure 2: Sildenafil treatment of mice does not activate PKG in the brain and does not increase proteasome activities in the brain.

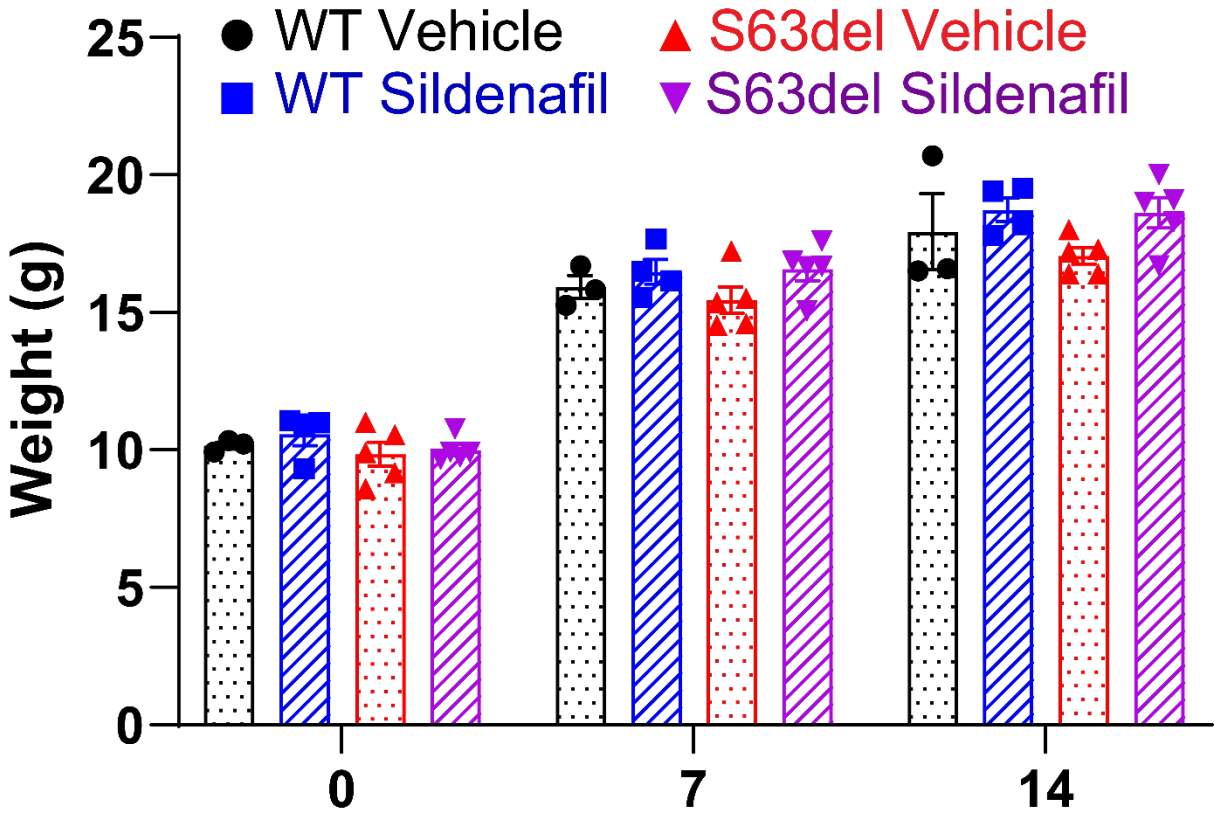
A.) Proteasomal chymotrypsin-like activity was similar in brain lysates from sildenafil- and vehicle-treated mice.

B.) The phosphorylation of VASP or PDE5 in the brain was not increased by sildenafil treatment. The levels of proteasome subunits in the brain were also not altered by this treatment.

C.) 26S proteasomes affinity-purified from the brains of mice treated with sildenafil or vehicle exhibited similar rates of hydrolysis of suc-LLVY-amc and the K48-linked polyubiquitinated superfolder GFP.

Supplemental Figure 3

A



Supplemental Figure 3: Sildenafil treatment of mice for 14 days does not cause weight loss

A.) The weights of WT or S63del mice were not reduced by the 14-day treatment with sildenafil. Mice were treated with sildenafil as in Figure 3. n=3 mice for WT vehicle, 4 for WT sildenafil, 5 for S63del vehicle, and 5 for S63del sildenafil.

Uncropped Western Blots

Figure 1B

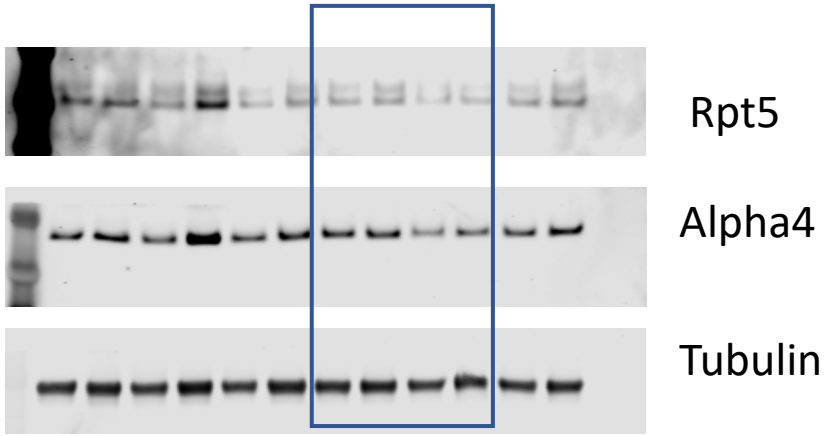


Figure 1C

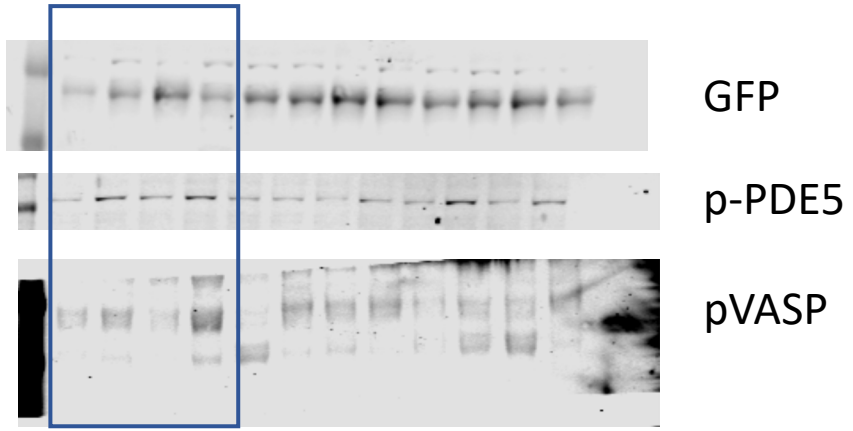
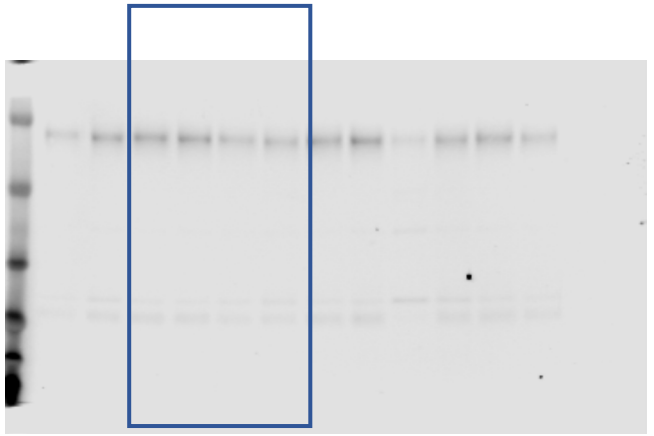


Figure 1D



GFP

Figure 3B

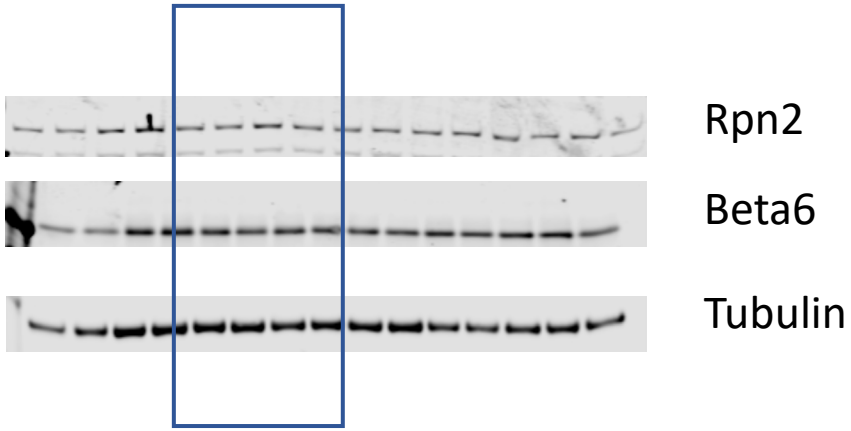


Figure 3C

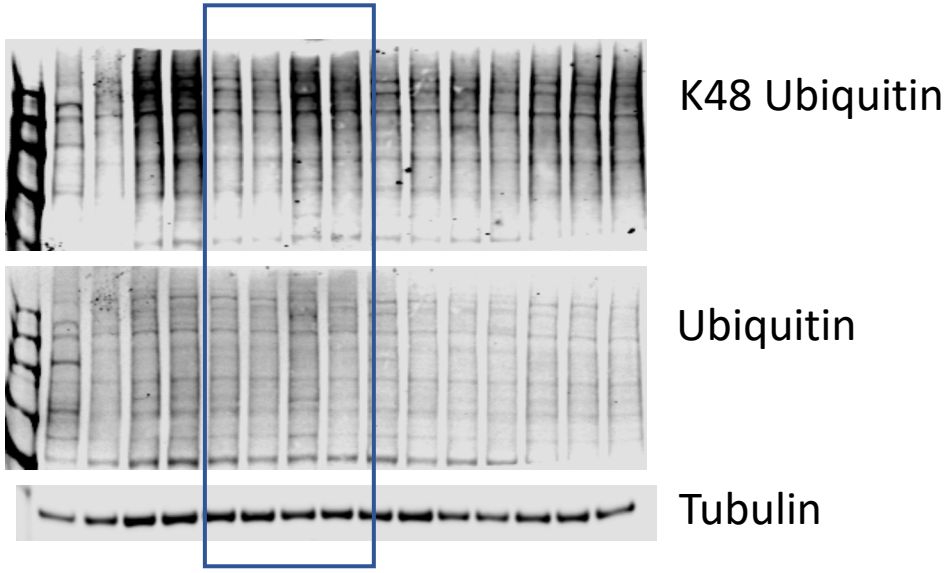


Figure 3D