Supplementary Data for

Muscarinic 2 Receptors Modulate Cardiac Proteasome Function in a Protein Kinase G-dependent Manner

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- Supplementary Table 1
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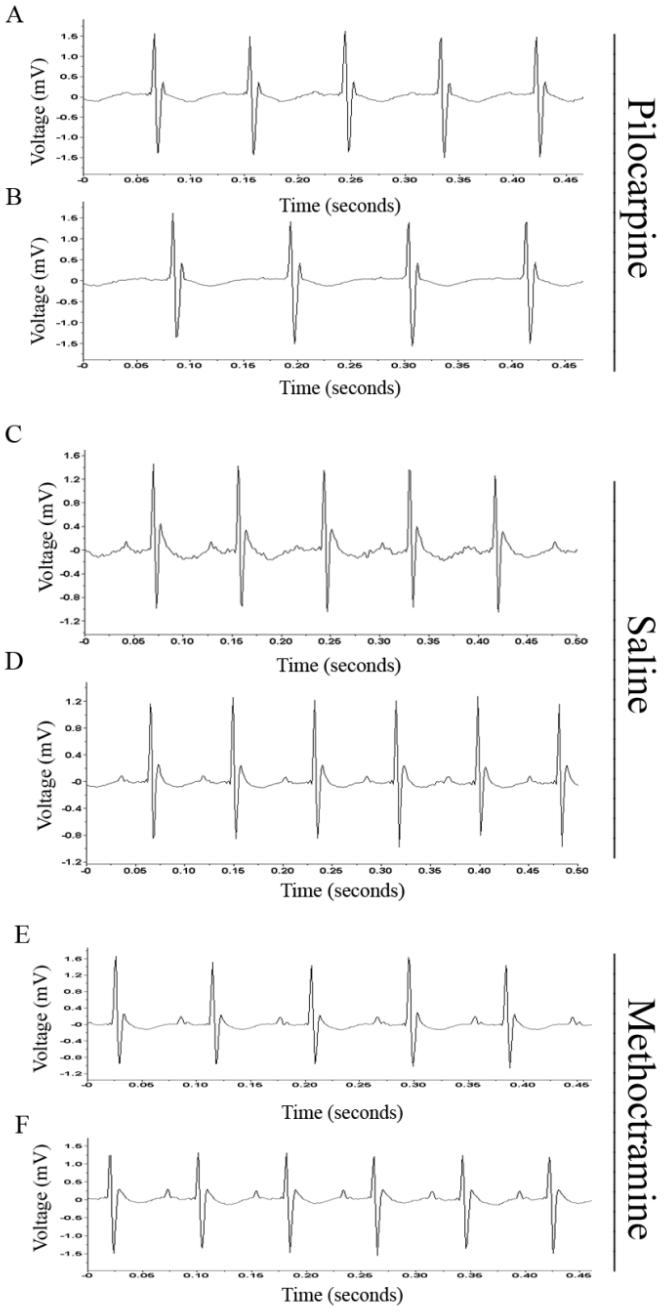
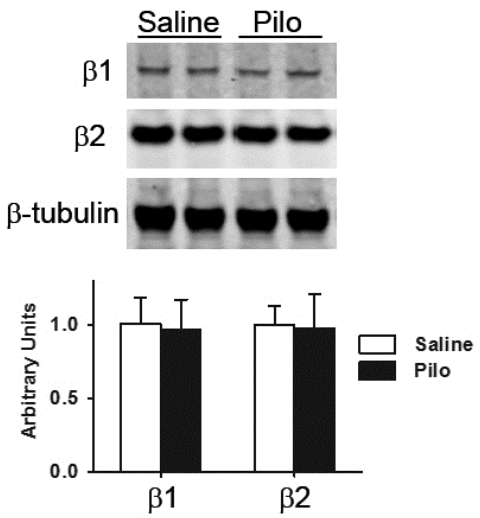
Supplementary Table 1. Gravimetric analysis of GFPdgn mice treated with muscarinic receptor modulators for 24 hours.

Treatment	N	BW (g)	HW (mg)	VW (mg)	TL (mm)	HW/BW (mg/g)	VW/BW (mg/g)	HW/TL (mg/mm)	VW/TL (mg/mm)
Pilocarpine	6	23.9± 2.1	108.2±10.0	90.3±7.2	18.7± 0.6	4.5± 0.6	3.8± 0.5	5.8± 0.6	4.8± 0.5
Saline	6	24.1± 1.9	112.3±12.8	93.0±8.2	19.5± 0.5	4.7± 0.7	3.9± 0.4	5.7± 0.6	4.8± 0.5
Methoctramine	6	24.7± 2.7	111.1±10.6	91.3±7.5	19.3± 0.4	4.5± 0.5	3.7± 0.6	5.7± 0.5	4.7± 0.4

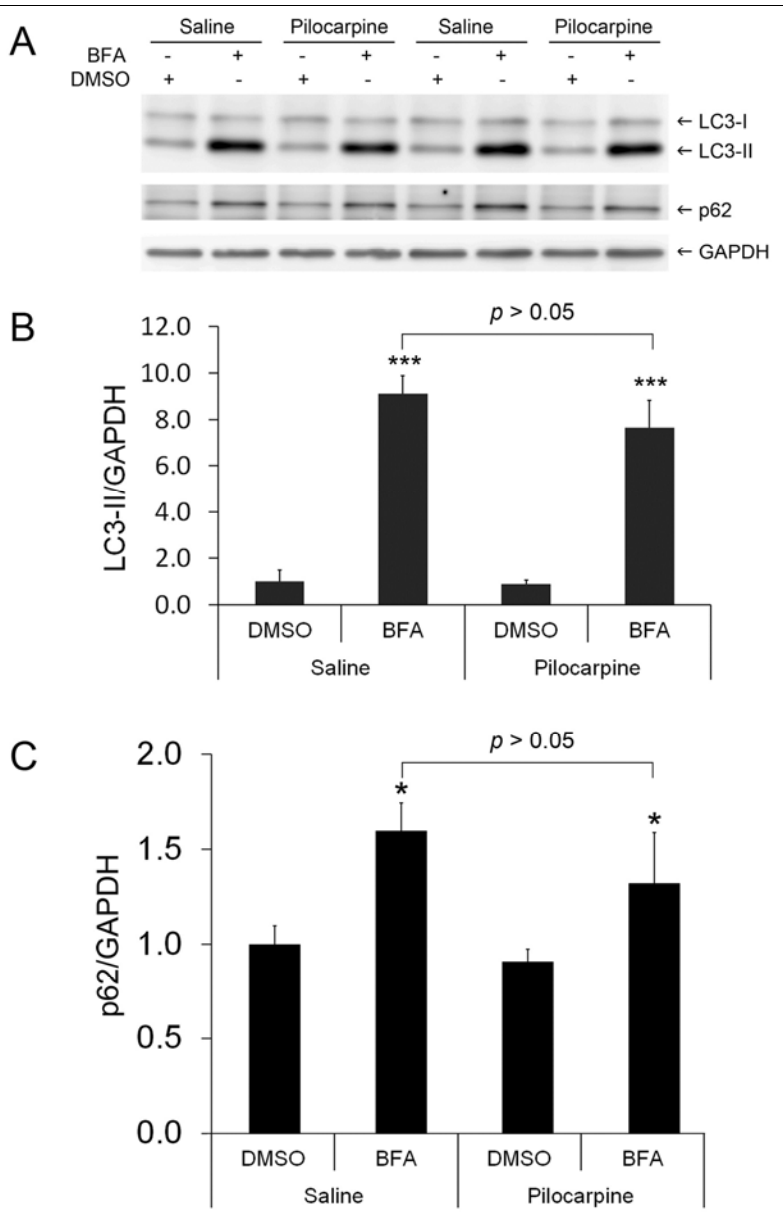
N: number of samples; BW: body weight; HW: heart weight; VW: ventricular weight; TL: tibial length; HW/BW: heart weight/body weight ratio, VW/BW: ventricular weight/body weight ratio; HW/TL: heart weight/tibial length ratio; No difference in any of the parameters among different groups achieved statistical significance. ANOVA and *post hoc* tests.



Supplementary Figure 1. Radiotelemetric ECG tracings of mice subjected to muscarinic receptor manipulation. Mice were subcutaneously implanted with ECG transmitters and monitored during muscarinic receptor modulation. Representative radiotelemetric lead II ECG tracings from a conscious mouse immediately before **(A)** and 1 hour after **(B)** pilocarpine treatment (i.p., 1 mg/kg), before **(C)** and 1 hour after **(D)** saline treatment (i.p., volume corrected), and before **(E)** and 1 hour after **(F)** methoctramine treatment (i.p., 1 mg/kg).



Supplementary Figure 2. Western blot analyses for the steady state protein levels of β1 and β2 subunits of the 20S proteasome in cultured neonatal rat ventricular myocytes (NRVMs) treated with pilocarpine (Pilo) or vehicle control (saline). The cell culture and Pilo treatment were described in **Figure 5** of the main text. Similar to β5 subunit, stimulation of muscarinic receptors by Pilo did not alter the protein abundance of 20S proteasome β1 and β2 subunits, the two subunits responsible for the caspase-like and trypsin-like activities, respectively. N=6 repeats/group.



Supplementary Figure 3. The effect of muscarinic stimulation via pilocarpine on autophagic flux in cultured cardiomyocytes. NRVMs are cultured and treated with pilocarpine (10 μM) or saline control as described in **Figure 4A** of the main text. The cells were harvested at 48h of pilocarpine treatment. At 6 hours prior to cell harvest, bafilomycin A1 (BFA, 50 nM) or vehicle control (DMSO) was administered to the cultures. Total protein extracts were used for western blot analyses of LC3, p62, and GAPDH. Representative image sare shown in Panel **A** and pooled densitometry data of LC3-II and p62 from 4 biological repeats are summarized in Panel **B** and **C**, respectively. *p<0.05, ***p<0.001, vs. the respective DMSO treated group.