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Supplementary Information for

Phosphodiesterase 2 inhibition preferentially promotes NO-guanylyl cyclase-cGMP signaling to reverse the development of heart failure

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This PDF file includes:

Figures S1 to S8
Tables S1 to S4

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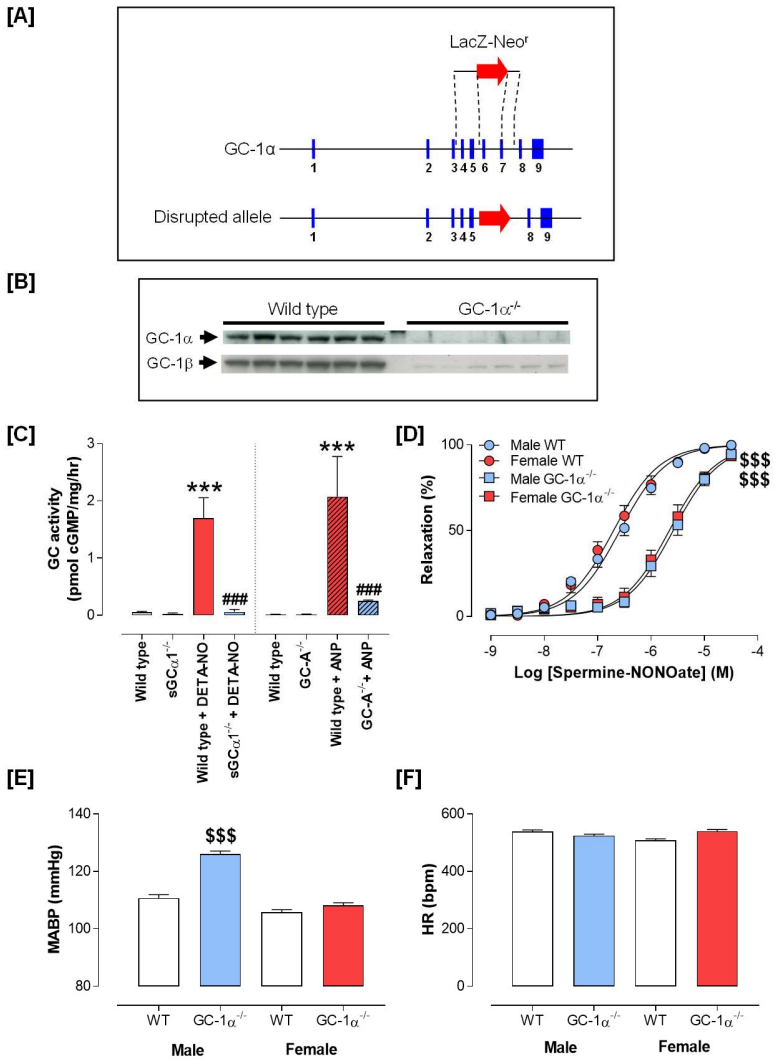

Figure S1. Design and verification of GC-1α and GC-A gene deletion. ***(A)*** The targeting vector comprised a LacZ-Neo^r cassette flanked by sequences on either side of exons 6 and 7 resulting in deletion of the cyclase domain. ***(B)*** Immunoblot of GC-1α and GC-1β subunits in soluble lung extracts showing complete deletion of the GC-1α protein (and significant reduction in GC-1β). ***(C)*** Guanylyl cyclase activity is markedly reduced in soluble lung extracts from sGC1α$^{-/-}$ and GC-A$^{-/-}$ mice stimulated with DETA-NONOate (100 μM) and ANP (1 μM), respectively. ***(D)*** The vasorelaxant potency of the NO-donor Spermine-NONOate is significantly reduced in aortic rings from male and female GC-1α$^{-/-}$ mice. ***(E)*** Mean arterial blood pressure (MABP) is increased in male, but not female, GC-1α$^{-/-}$ animals. ***(F)*** Heart rate (HR) is similar in WT and GC-1α$^{-/-}$ mice. Data are expressed as mean±sem with analysis by one-way ANOVA with Bonferroni post hoc test or two-way ANOVA. ***p<0.001 v basal, $^{###}$p<0.001 v WT + DETA-NONOate or ANP, $^{\$\$\$}$p<0.001 v corresponding WT. n=6.

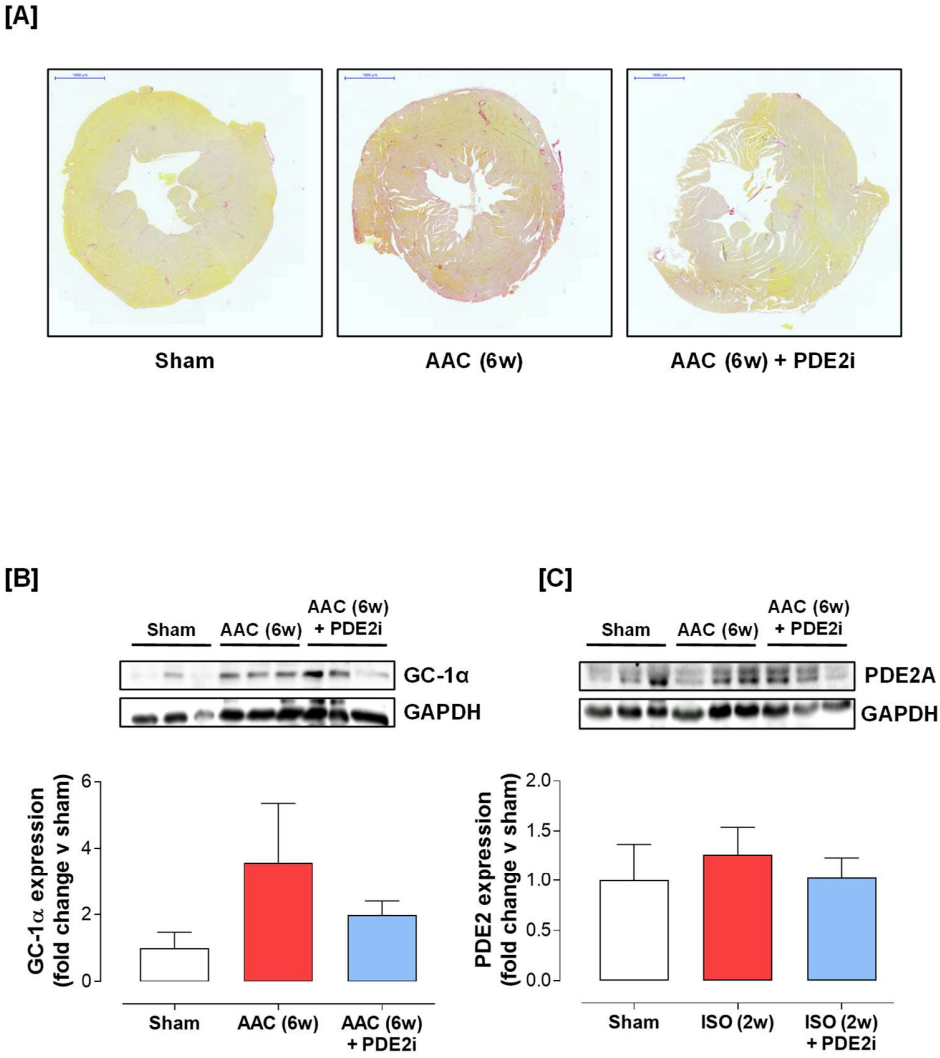

Figure S2. Effects of PDE2 inhibition on cardiac fibrosis, GC-1α and PDE2A expression. ***(A)*** Representative images of whole heart fibrotic burden and ***(B)*** GC-1α expression in sham mice and animals undergoing abdominal aortic constriction (AAC) for 6 weeks (6w) in the absence and presence of BAY 60-7550 (10 mg/kg/day; p.o.; initiated at 3w). ***(C)*** PDE2A expression in sham mice and animals administered isoproterenol (20mg/kg/day; s.c.) for 2 weeks in the absence and presence of BAY 60-7550 (10 mg/kg/day; p.o.; initiated at 1w). Data are expressed as mean±sem. n=3.

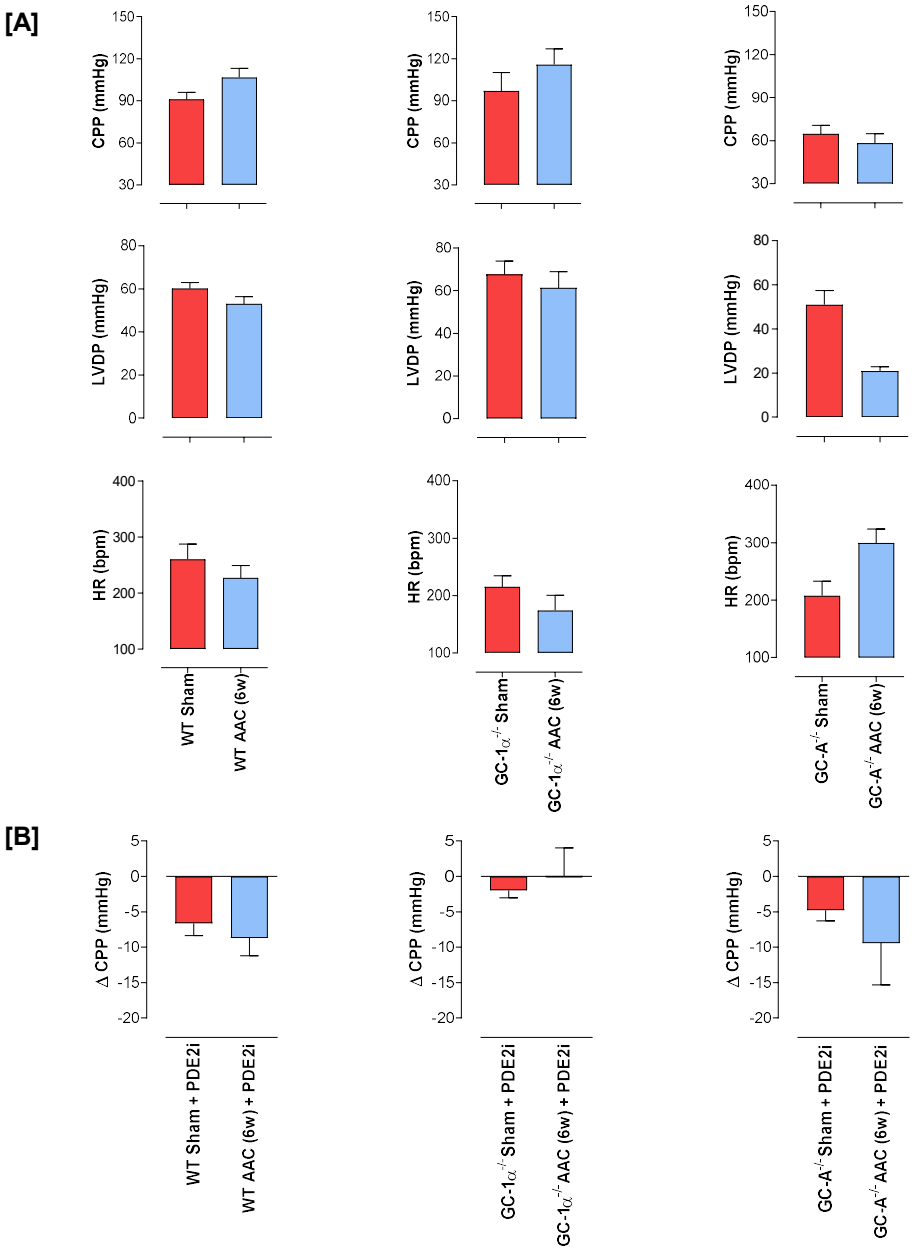

Figure S3. Characteristic cardiac dysfunction in Langendorff hearts from mice subjected to AAC. ***(A)*** Coronary perfusion pressure (CPP), left ventricular developed pressure (LVDP) and heart rate (HR) in WT, GC-1α$^{-/-}$ or GC-A$^{-/-}$ sham animals or mice subjected to 6 weeks of abdominal aortic constriction (AAC) in the absence and presence of BAY 60-7550 (10 mg/kg/day; p.o.; initiated at 3w). ***(B)*** Changes (Δ) in CPP in response to BAY 60-7550 (100 nM) in WT, GC-1α$^{-/-}$ or GC-A$^{-/-}$ sham animals or mice subjected to 6 weeks of abdominal aortic constriction (AAC). Data are expressed as mean±sem. n=6-12.

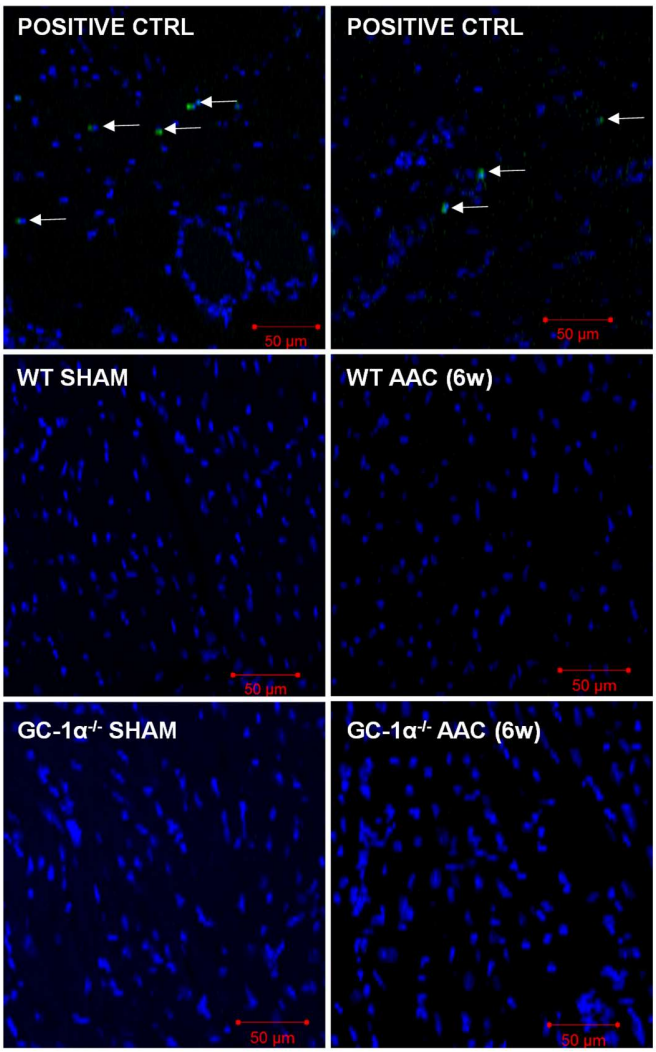

Figure S4. The increased cardiomyocyte size in GC-1α$^{-/-}$ mice is not due to apoptosis. Representative images depicting terminal deoxynucleotidyl transferase (TdT) dUTP Nick-End Labeling (TUNEL)-staining of heart sections from wild type (WT) and GC-1α$^{-/-}$ sham mice and animals undergoing abdominal aortic constriction (AAC) for 6 weeks. Positive apoptotic control shown in the upper panel.

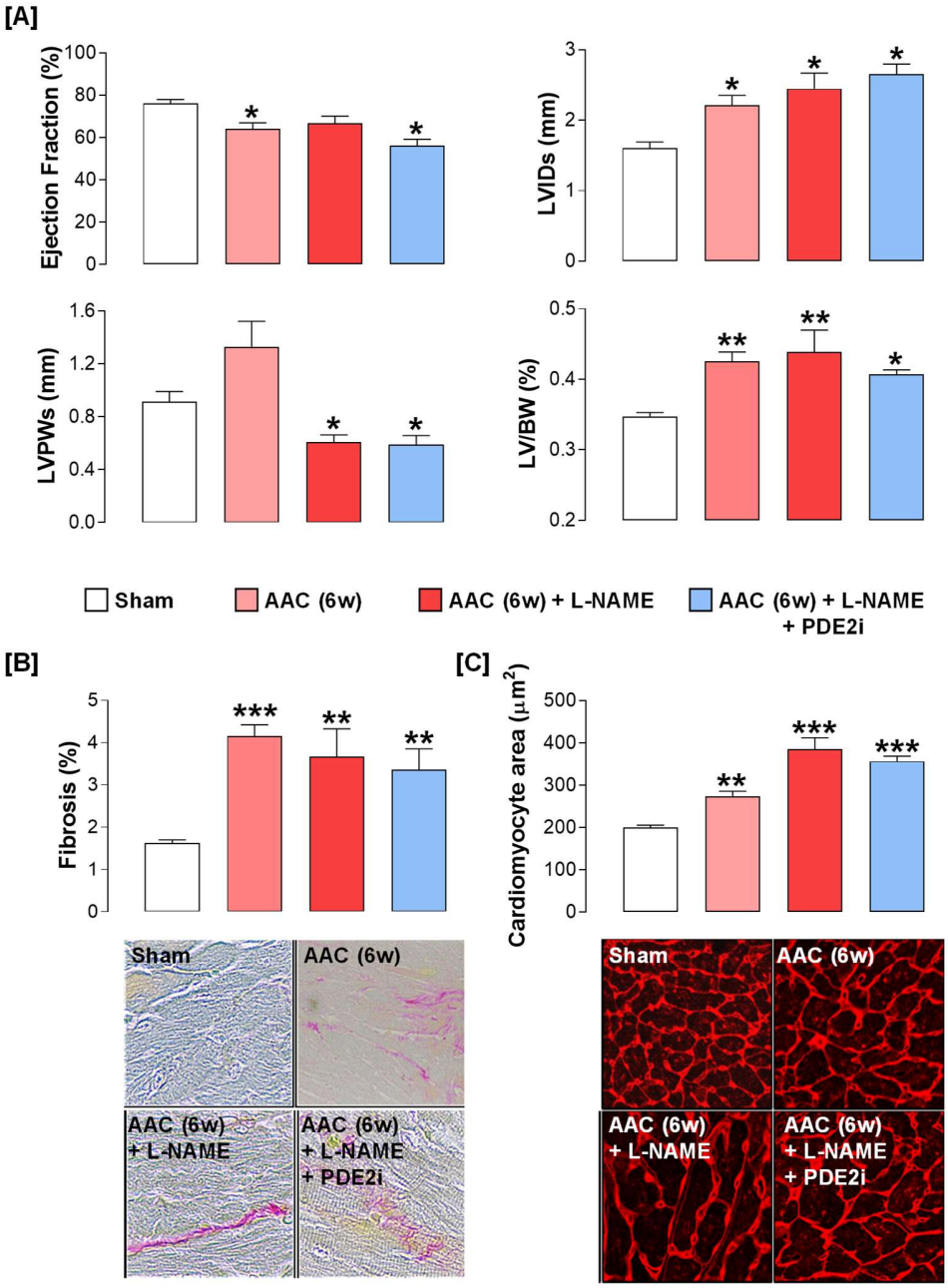

Figure S5. Treatment with a NOS inhibitor ameliorates the beneficial effects of PDE2 inhibition in HF. ***(A)*** Echocardiographic indices of heart structure & function, ***(B)*** cardiac fibrosis, and ***(C)*** cardiomyocyte size in sham mice and animals undergoing abdominal aortic constriction (AAC) for 6 weeks (6w) with administration of the NOS inhibitor L-N^G-nitroarginine methylester (L-NAME; 100 mg/kg/day; p.o.) in the absence and presence of BAY 60-7550 (10 mg/kg/day; p.o.; initiated at 3w). Data are expressed as mean±sem and analyzed by one-way ANOVA with Bonferroni post hoc test. *p<0.05, **p<0.01, ***p<0.001 v sham. n=6-12.

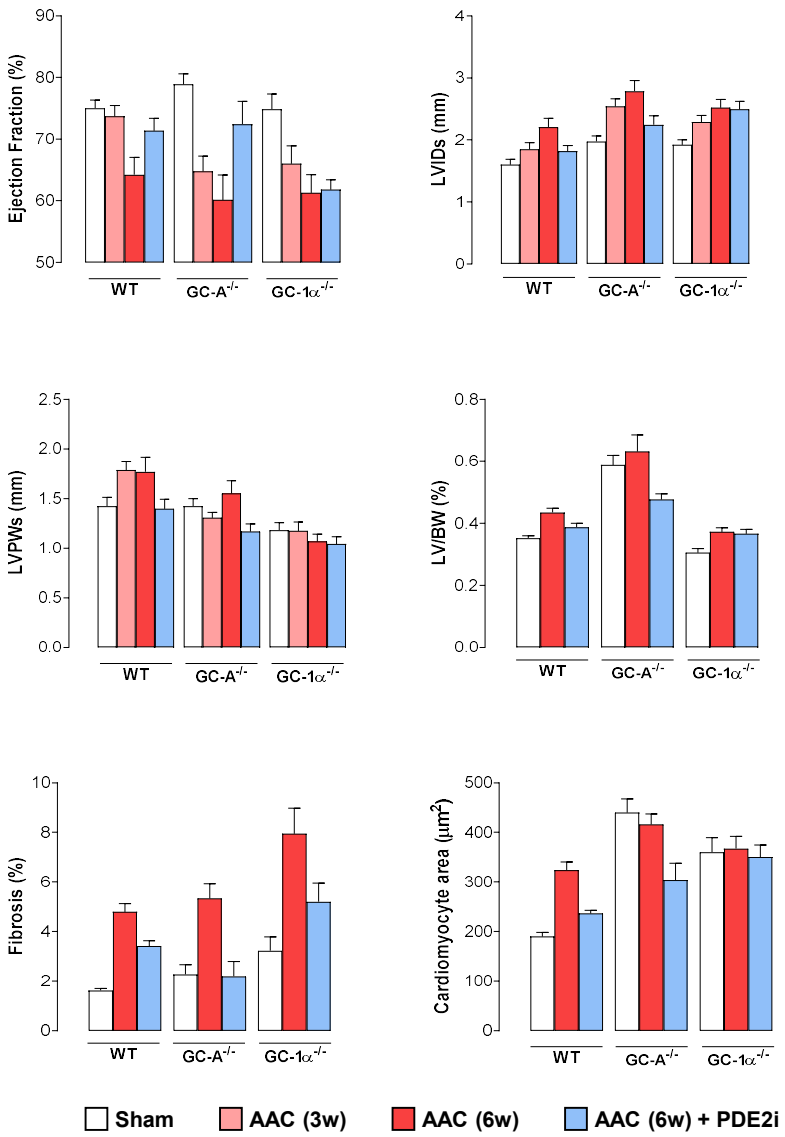

Figure S6. Comparison of the effects of BAY 60-7550 across genotypes following pressure overload. Echocardiographic indices of heart structure & function, cardiac fibrosis, and cardiomyocyte size in sham mice and animals undergoing abdominal aortic constriction (AAC) for 3 weeks (3w) or 6 weeks (6w) in the absence and presence of BAY 60-7550 (10mg/kg/day; p.o.; initiated at 3w). Data are expressed as mean±sem. n=6-12.

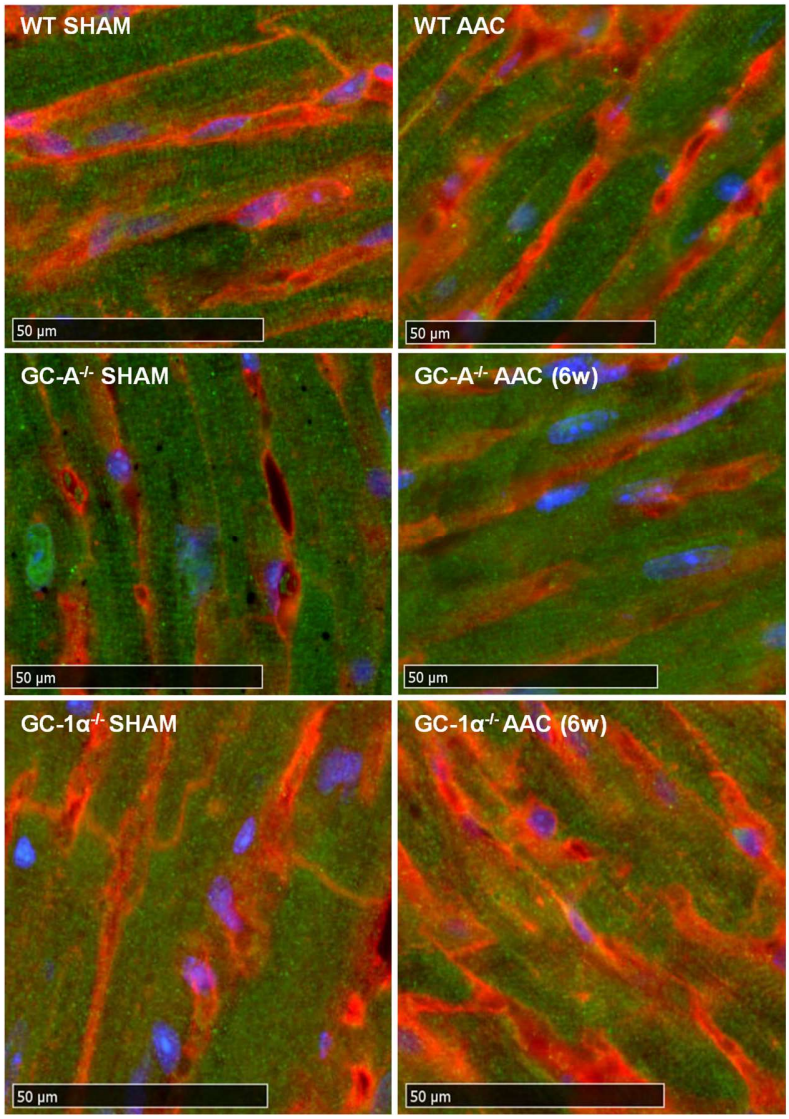

Figure S7. The cellular localization of PDE2 does not change overtly in response to pressure overload. Representative images of wheatgerm agglutinin (cell membrane, red) and PDE2 (green) stained heart sections from wild type (WT), GC-A$^{-/-}$ and GC-1α$^{-/-}$ sham mice and animals undergoing abdominal aortic constriction (AAC) for 6 weeks.

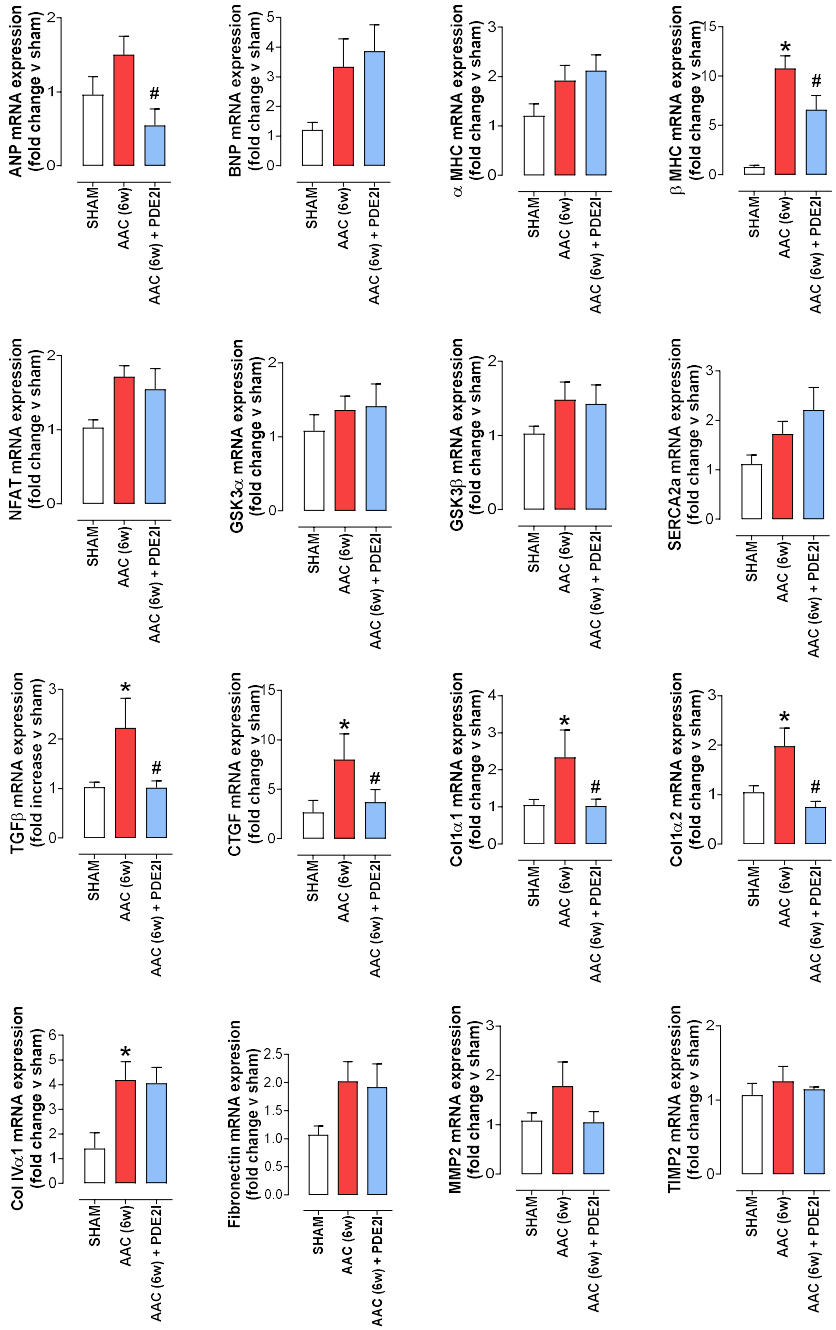

Figure S8. Expression of pro-hypertrophic and pro-fibrotic markers is diminished by PDE2i following AAC. Quantitate PCR analysis of an array of established pro-hypertrophic and pro-fibrotic markers in whole heart homogenates from sham mice or animals subjected to abdominal aortic constriction (AAC) for 6 weeks in the absence and presence of BAY 60-7550 (10 mg/kg/day; p.o.; initiated at 3w). Data are expressed as mean±sem with analysis by one-way ANOVA with Bonferroni post-hoc test. *p<0.05 v sham, $^{\#}$p<0.05 v AAC (6w). n=6.

Table S1. Primer sequences for genotyping

Gene target	Primer sequence (5'-3')
WT (GC-1α$^{+/+}$)	Forward: TTACCCCAGTAGCCATTTCC Reverse: CTTCAGCTTCCCCAACCTCT
GC-1α$^{-/-}$	Forward: AGGTAGCCGGATCAAGCGTAT Reverse: GATGGATTGCACGCAGGTTCT
WT (GC-A$^{+/+}$)	Forward: GCATGGTTCAGCTCTAAGAC Reverse: CCTTCAGTTATCTACATCTGC
GC-A$^{-/-}$	Forward: CTAACCCTGTGAACTGTAAGC Reverse: CCTTCAGTTATCTACATCTGC

Table S2. Primer sequences for qPCR analysis

Gene target	Primer sequence (5'-3')
PDE2	Forward: ATCTTTGCCTTGTTTATTTCCTG Reverse: CAGCCAGCACAGATTTCG
ANP	Forward: GGATTTCAAGAACCTGCTAGACC Reverse: GCAGAGCCCTCAGTTTGCT
BNP	Forward: TGGGCTGTAACGCACTGAA Reverse: TGTTGTGGCAAGTTTGTGCTT
αMHC	Forward: GCCCAGTACCTCCGAAAGTC Reverse: GCCTTAACATACTCCTCCTTGTC
βMHC	Forward: ACTGTCAACACTAAGAGGGTCA Reverse: TTGGATGATTTGATCTTCCAGGG
Collagen Type 1 α1	Forward: TCTGACTGGAAGAGCGGAGAG Reverse: AGACGGCTGAGTAGGGAACA
Collagen Type 1 α2	Forward: TGGATACGCGGACTCTGTTG Reverse: CCCTTTCGTACTGATCCCGATT
Collagen Type 4 α1	Forward: CTGGAGAAAAGGGCCAGAT Reverse: TCCTTAACTTGTGCCTGTCC
CTGF	Forward: GGGCCTCTTCTGCGATTC Reverse: ATCCAGGCAAGTGCATTGGTA
Fibronectin	Forward: CCGGTGGCTGTCAGTCAGA Reverse: CCGTTCCCACTGCTGATTTATC
GSK3α	Forward: GGAGAAGAAGGACGAGCTGTA Reverse: TGTCAGGGTCCACAAGCAAA
GSK3β	Forward: CAAAAGGAGTGAAAAGCCAAGAG Reverse: TTGCTGCCATCTTTATCTCTGCT
MMP-2	Forward: GACAAGTTCTGGAGATACAATGAAGTG Reverse: CAGGTTATCAGGGATGGCATTC
NFATc3	Forward: AACCCTATCGAGTGTTCCCA Reverse: TCCCGGTCAGTCTTTGCTTC
NF-κB	Forward: CCTACGGAACTGGGCAAATGT Reverse: TCCCCTCTGTTTTGGTTGCT
RPL-19	Forward: GCTTGCCTCTAGTGTCCTCC Reverse: TTGGCGATTTCATTGGTCTCA
SERCA-2	Forward: TGGAACCTTTGCCGCTCATTT Reverse: CAGAGGCTGGTAGATGTGTT
TGF-β	Forward: TCAGACATTCGGGAAGCAGT Reverse: GCCCTGTATTCCGTCTCCTTG
TIMP-2	Forward: GATTCAGTATGAGATCAAGCAGATAAAGA Reverse: GCGAGACCCCGCACACT
αSMA	Forward: ACTACTGCCGAGCGTGA Reverse: ATAGGTGGTTTCGTGGATGC
β-actin	Forward: CACAGCTTCTTTGCAGCTCCTT Reverse: TCAGGATACCTCTCTTGCTCT

Table S3. Effect of BAY 60-7550 (10 mg/kg/day) on mean arterial blood pressure (MABP) in pressure overload (AAC; 6w)-induced HF.

	WT			**GC-A$^{-/-}$**			**GC-1α$^{-/-}$**		
	Sham	***AAC (6w)***	***AAC (6w) + PDE2i***	***Sham***	***AAC (6w)***	***AAC (6w) + PDE2i***	***Sham***	***AAC (6w)***	***AAC (6w) + PDE2i***
MABP (mmHg)	97.7 *±1.9*	*114.1 *±3.4*	*113.1 *±1.5*	101.5 *±2.9*	*118.7 *±3.9*	*119.3 *±1.3*	107.4 *±3.7*	*117.1 *±2.0*	*115.5 ±2.8

*p<0.05 v sham, n=6-12

Table S4. Effect of BAY 60-7550 (10 mg/kg/day) on heart rate (HR) in pressure overload (AAC; 6w)- and isoproterenol (ISO; 2w)- induced HF.

	Sham	***AAC (6w)***	***AAC (6w) + PDE2i***	***Sham***	***ISO (2w)***	***ISO (2w) + PDE2i***
HR (bpm)	529±48	524±11	514±28	515±32	631±25*	663±17*

*p<0.05 v sham, n=6-12