Supplemental Material

Methods:

Primers used for quantitative rtPCR analysis:

TaqMan primers and probes for mouse Rcan1 (NM_001081549), Tgfb1 (NM_011577), Ctgf (NM_010217), Trpc6(NM_013838) and Gapdh (NM_008084) were purchased from Applied Biosystems. For SYBR green method, the following primers were used: Mouse Nppa(NM_008725) 5′-TCGTCTTGGCCTTTTGGCT-3′ (forward) and 5′-TCCAGGTGGTCTAGCAGGTTCT-3′ (reverse); Mouse Nppb(NM_008726) 5′-AAGTCC TAGCCAGTCTCCAGA -3′ (forward) and 5′-GAGCTGTCTCTGGGCCATTTC-3′ (reverse); Mouse Nos1(NM_008712) 5'-AGAAGCAGCGTCTGCTGGTCCTCAG-3' (forward), 5'-CTGTATCCGGTTGAGCCAGGAGGAG-3' (reverse); Mouse Nos2(NM_010927) 5'-CAGCTGGGCTGTACAAACCTT-3' (forward), 5'-CATTGGAAGTGAAGCGTTTCG-3' (reverse). Primers for mouse NOX2, forward 5' GGG CTA TTC AAT GCT TGT GGC TGT 3', reverse 5' TCT TCA CTG GCT GTA CCA AAG GGT 3. Primers for mouse NOX4, forward 5' TCA TGG ATC TTT GCC TCG AGG GTT 3', reverse 5' TCC AGG TCT GTG GGA AAT GAG CTT 3'.

Supplemental Figure Legends

S1) Resting heart function, and heart and lung weights normalized to tibia length for the four experimental groups. There was no difference in cardiac size, wall thickness, or function among models (n=3-7/group).

S2) Basal PKG activity in each of the four experimental models. Activity was much lower in the both models ($P5^+$ and P5/N3) with enhanced myocyte PDE5 expression (n=3-6/group). $P<0.0001$ for 1-way ANOVA, * $p<0.001$ versus CON and $N3^-$ models.

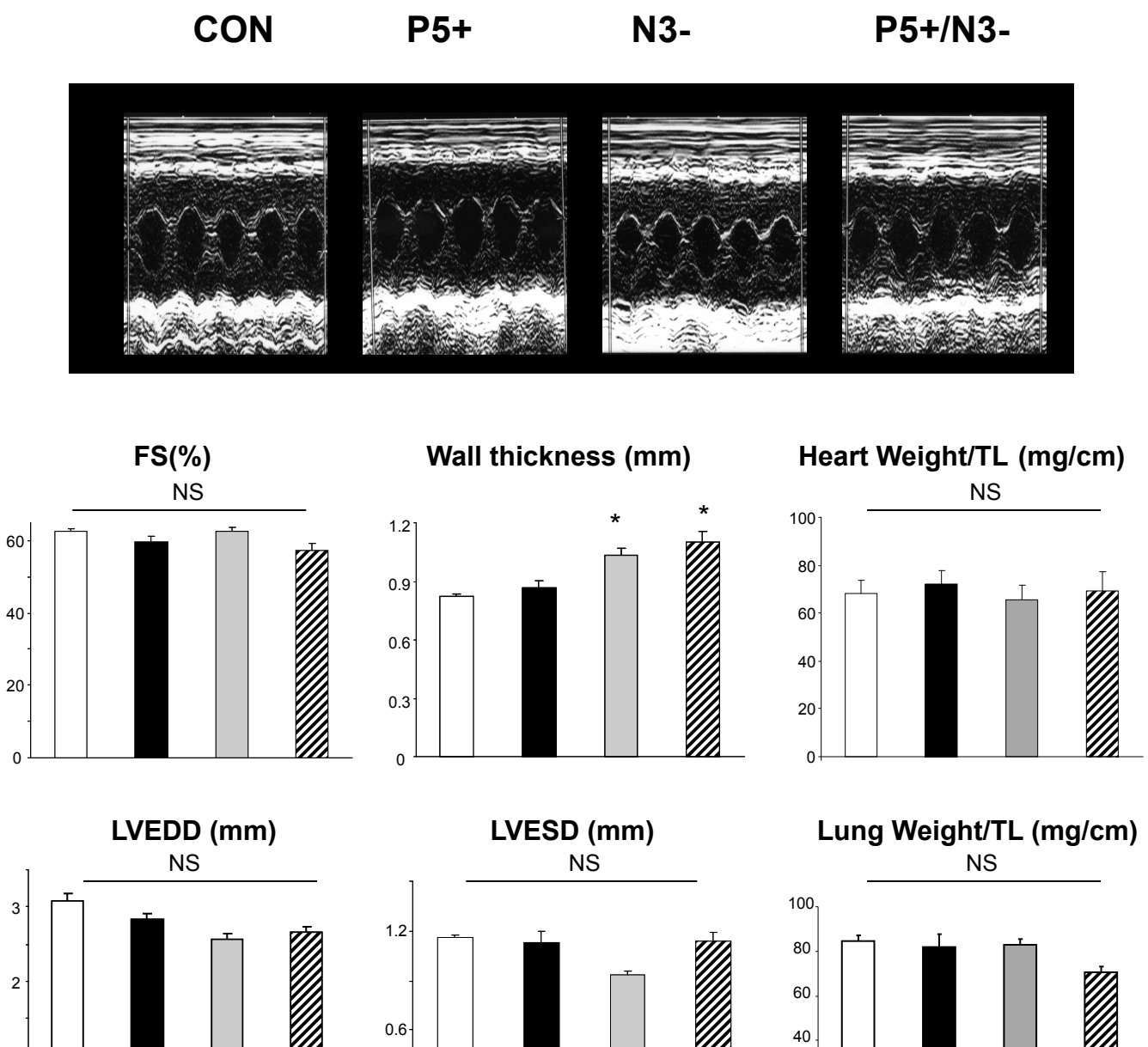
S3) Gene expression of NOS1 and NOS2 isoforms at baseline and after TAC in the four experimental groups. 1-way ANOVA within group (basal versus TAC) yielded no significant differences among experimental models (p value ranging 0.18 to 0.4). 3-way ANOVA (+/- TAC, +/-NOS3, +/- PDE5 over-expression) yielded a borderline significant effect of NOS3 expression on the TAC-induced increase in NOS2 ($p=0.047$), and overall effect of TAC on lowering NOS1 expression ($p=0.001$). n=3-9 per group.

S4) PDE5 cyclic G esterase activity assessed in WT versus $N3^-$ hearts before and after TAC. Activity was increased in WT after TAC, but this was significantly less so in the $N3^-$ heart. * $p<0.01$ versus sham, † $p<0.02$ interaction of genotype and TAC(n=4-12/group).

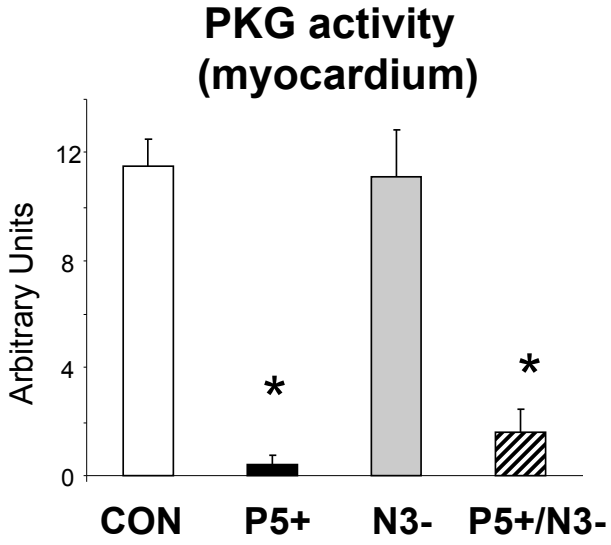
S5) Confocal immunohistochemistry of adult myocytes transfected in vivo with a DSred-PDE5 mutant fusion protein (S92A, and S92D). Cells were then isolated and infected cells

identified by fluorescence. Neither mutation (gain or loss of phosphorylation capacity) altered the localization of PDE5 to the sarcomere.

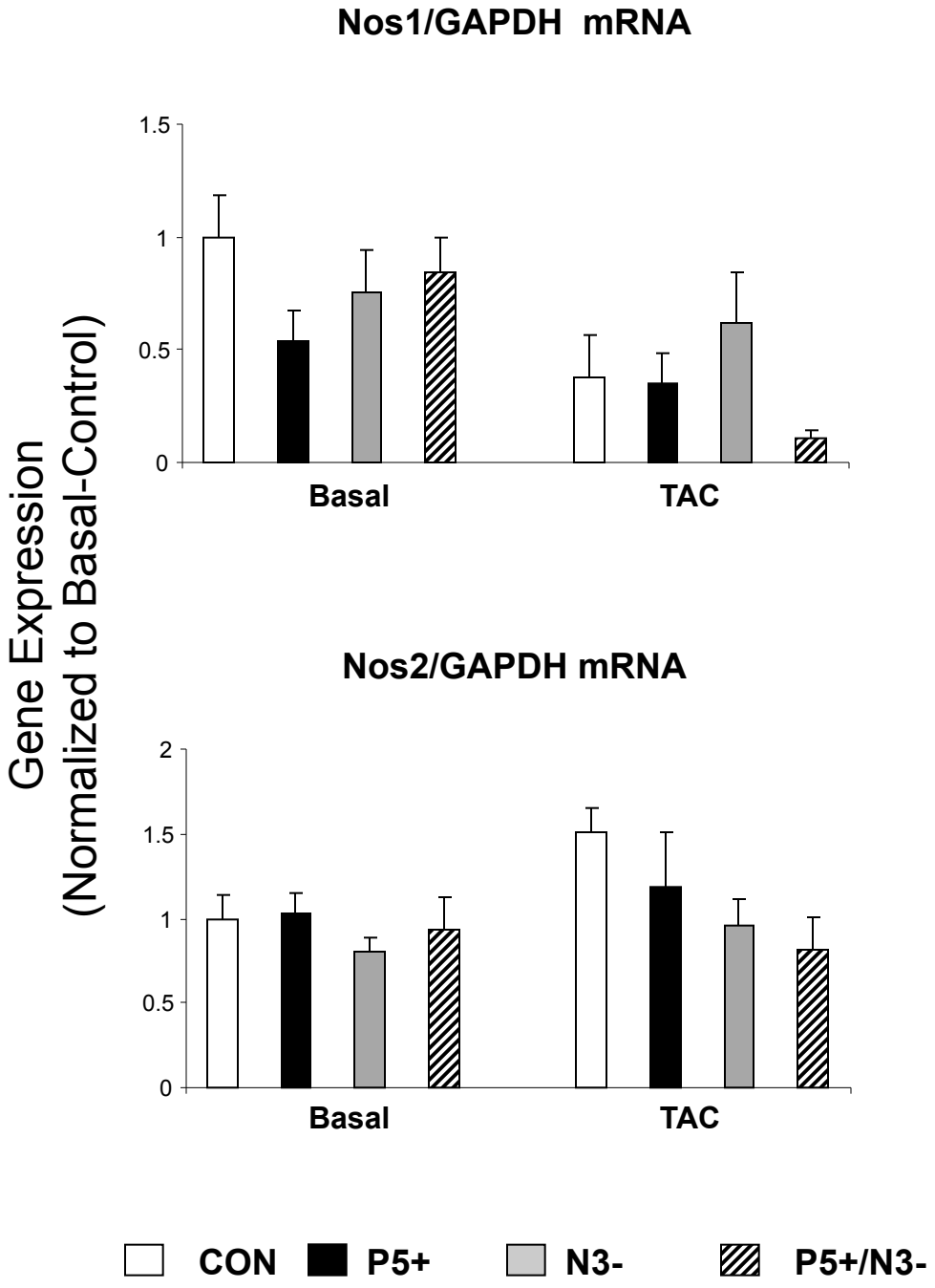
S6) NOX4 and NOX2 gene expression in the four experimental models – at baseline and after TAC. NOX2 expression rose in all four models similarly, whereas NOX4 increases most in the two models with enhanced PDE5 expression, and was lowest in $N3^-$ (n=3-11/group). P-values are for 2-way ANOVA. Top value reflects overall impact of TAC on expression, lower value is the interaction between TAC and experimental model. * $p<0.05$ versus P5+; † $p<0.05$ versus P5+ and P5+/N3-.



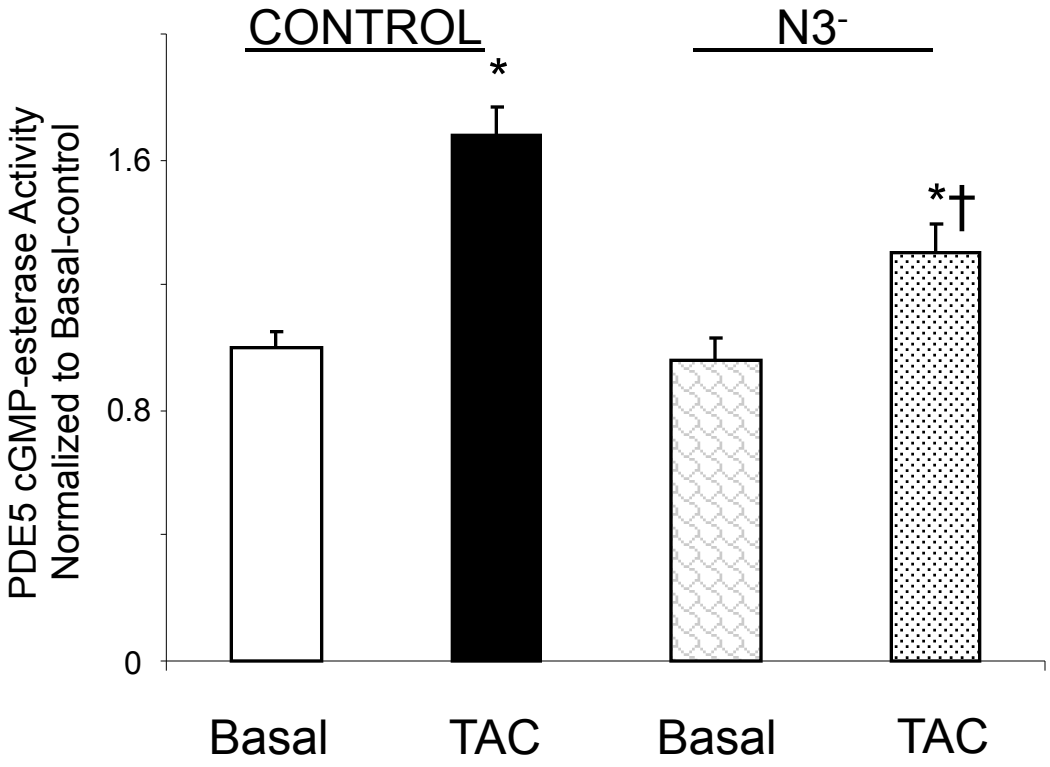
Supplemental Figure S1



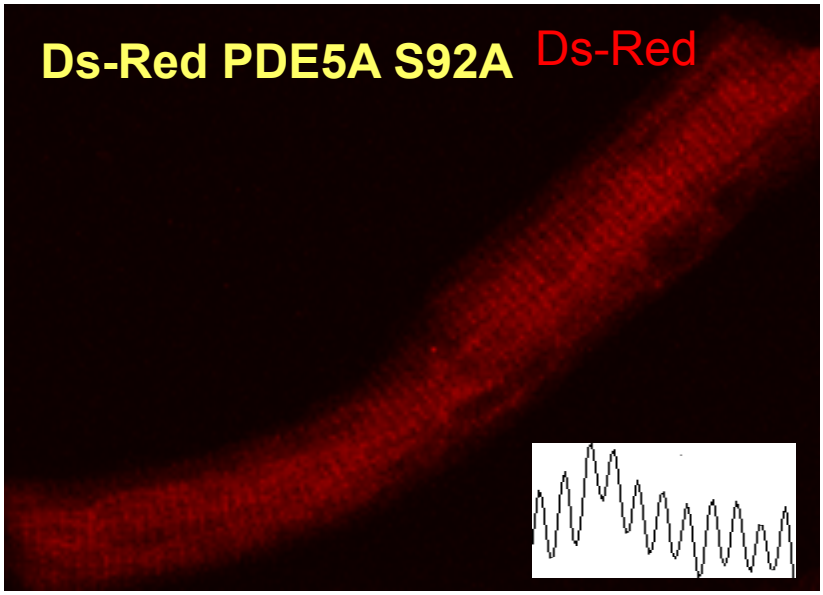
Supplemental Figure S2

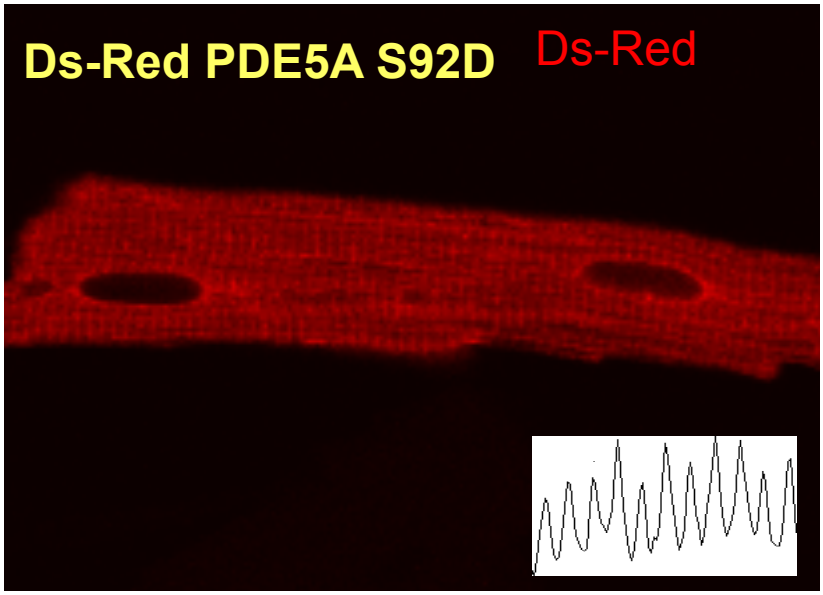


Supplemental Figure S3

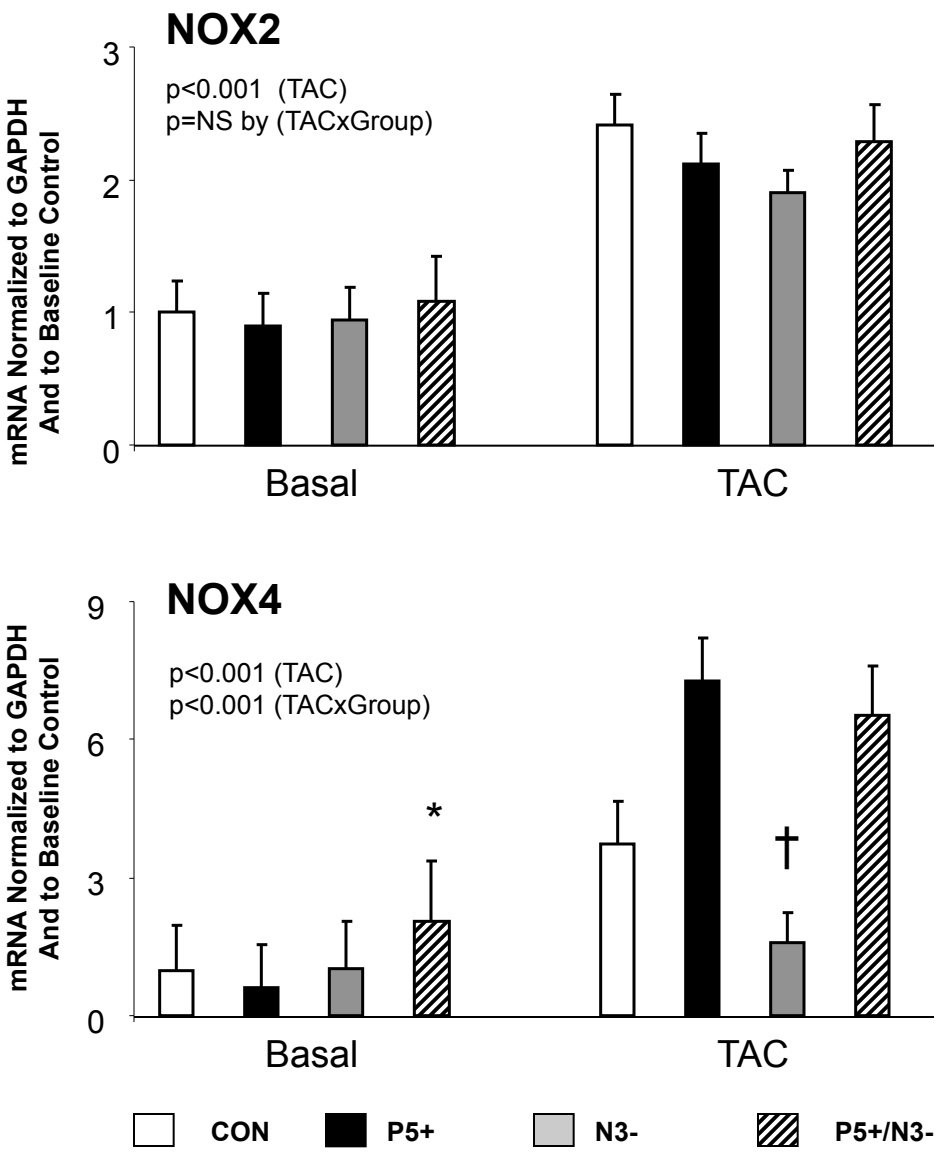


Supplemental Figure S4





Supplemental Figure S5



Supplemental Figure S6