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Supplementary Materials for

Convergence of G Protein–Coupled Receptor and S-Nitrosylation Signaling Determines the Outcome to Cardiac Ischemic Injury

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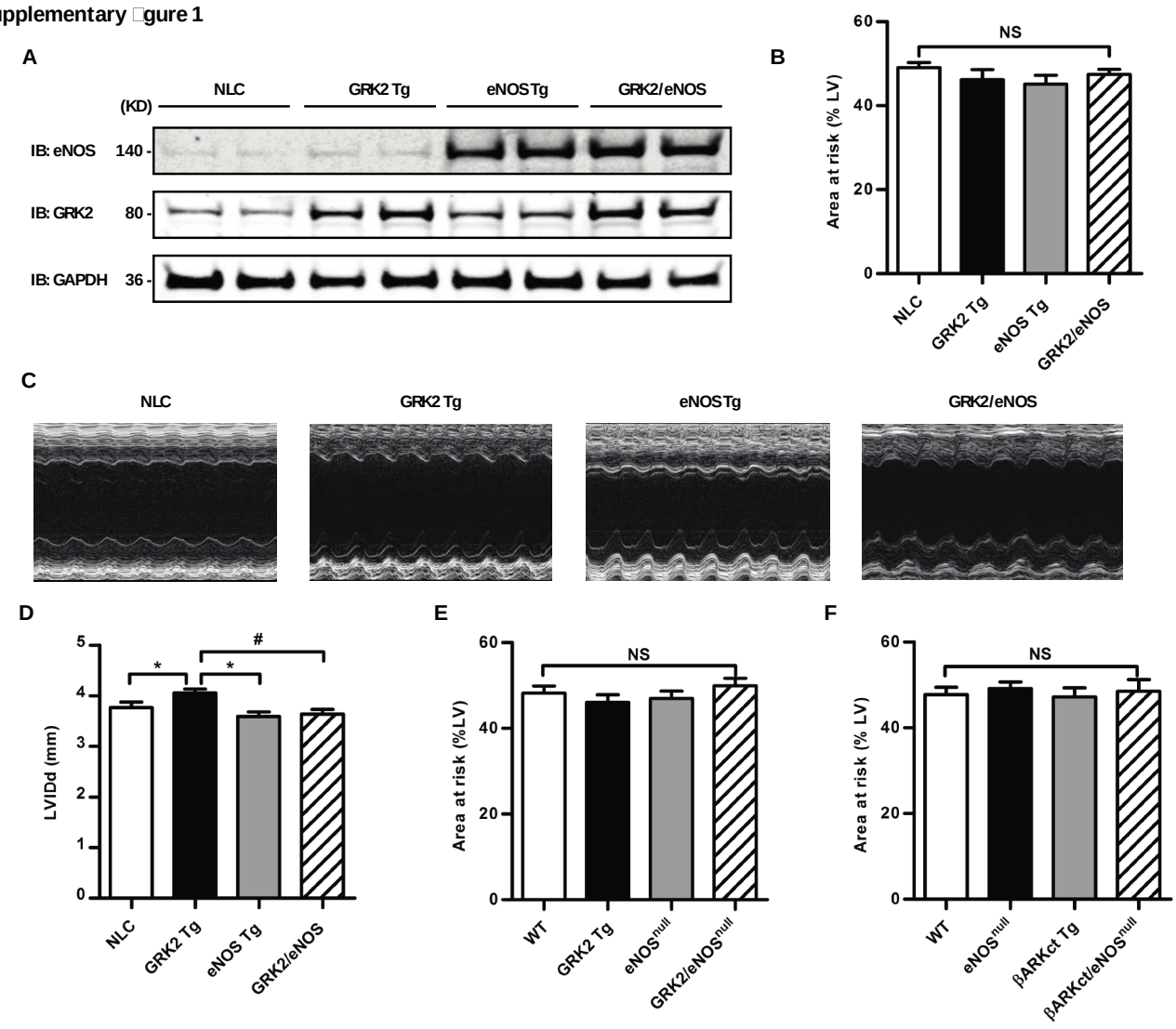
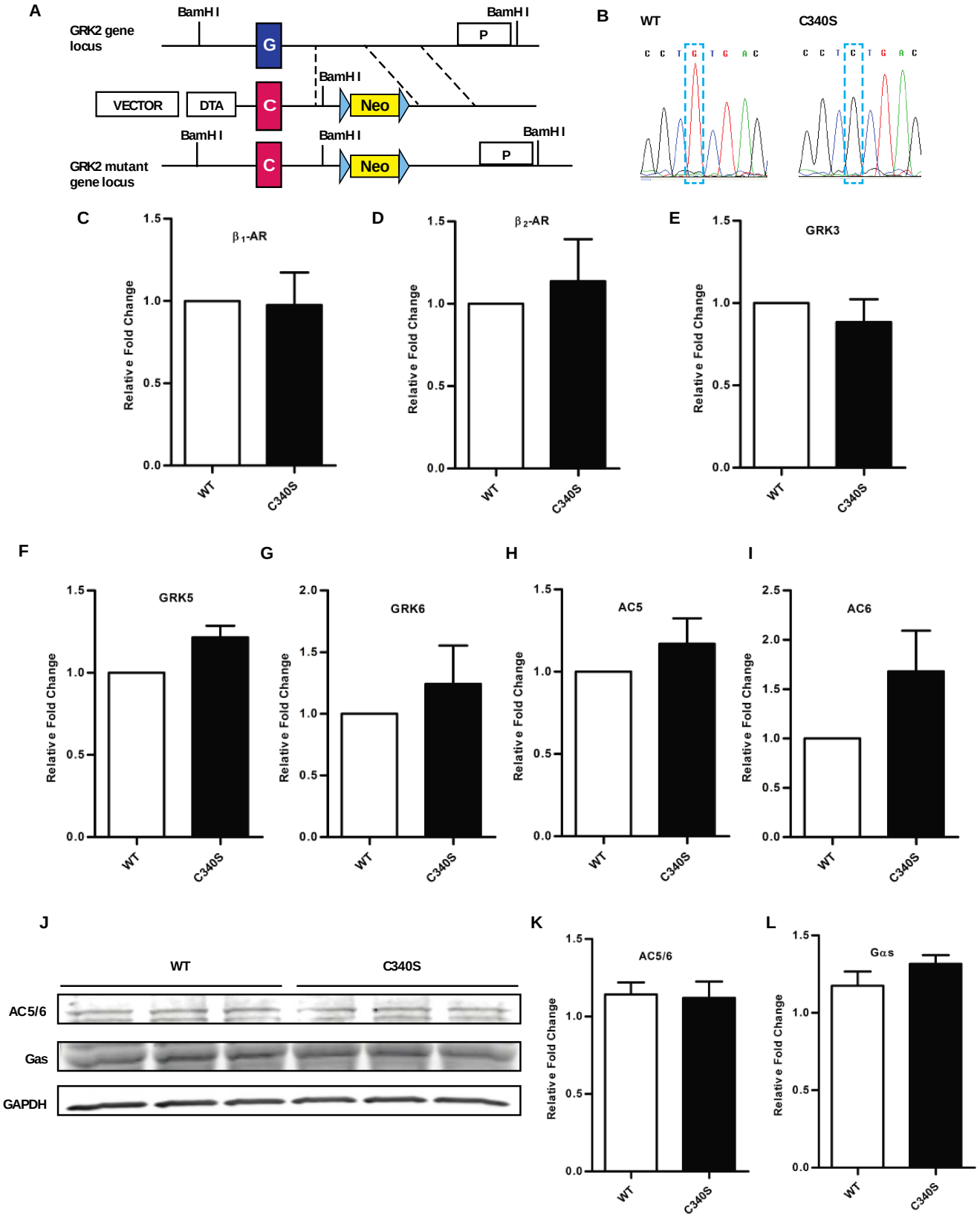
The PDF file includes:



Supplementary Figure 1. Ischemia/reperfusion injury in GRK2 Tg or βARKct Tg mice in which eNOS abundance was genetically manipulated. **A**, Representative Western blots showing the abundance of GRK2 and eNOS in non-transgenic littermate control (NLC), GRK2 Tg, eNOS Tg and GRK2/eNOS mouse hearts. GAPDH was used as a loading control. A Western blot representative of 4 (8 mice in each group) independent experiments is shown. **B**, LV ischemic AAR for NLC, GRK2 Tg, eNOS Tg and GRK2/eNOS mice following ischemia/reperfusion (n=8-12 mice/group). **C**, Representative M-Mode echocardiography images post-ischemia/reperfusion in NLC, GRK2 Tg, eNOS Tg and GRK2/eNOS mice. **D**, Left ventricular internal dimension during diastole (LVIDd) evaluated by echocardiography in in NLC, GRK2 Tg, eNOS Tg and GRK2/eNOS mice. *, $P<0.05$, #, $P<0.01$ (ANOVA, n = 8-

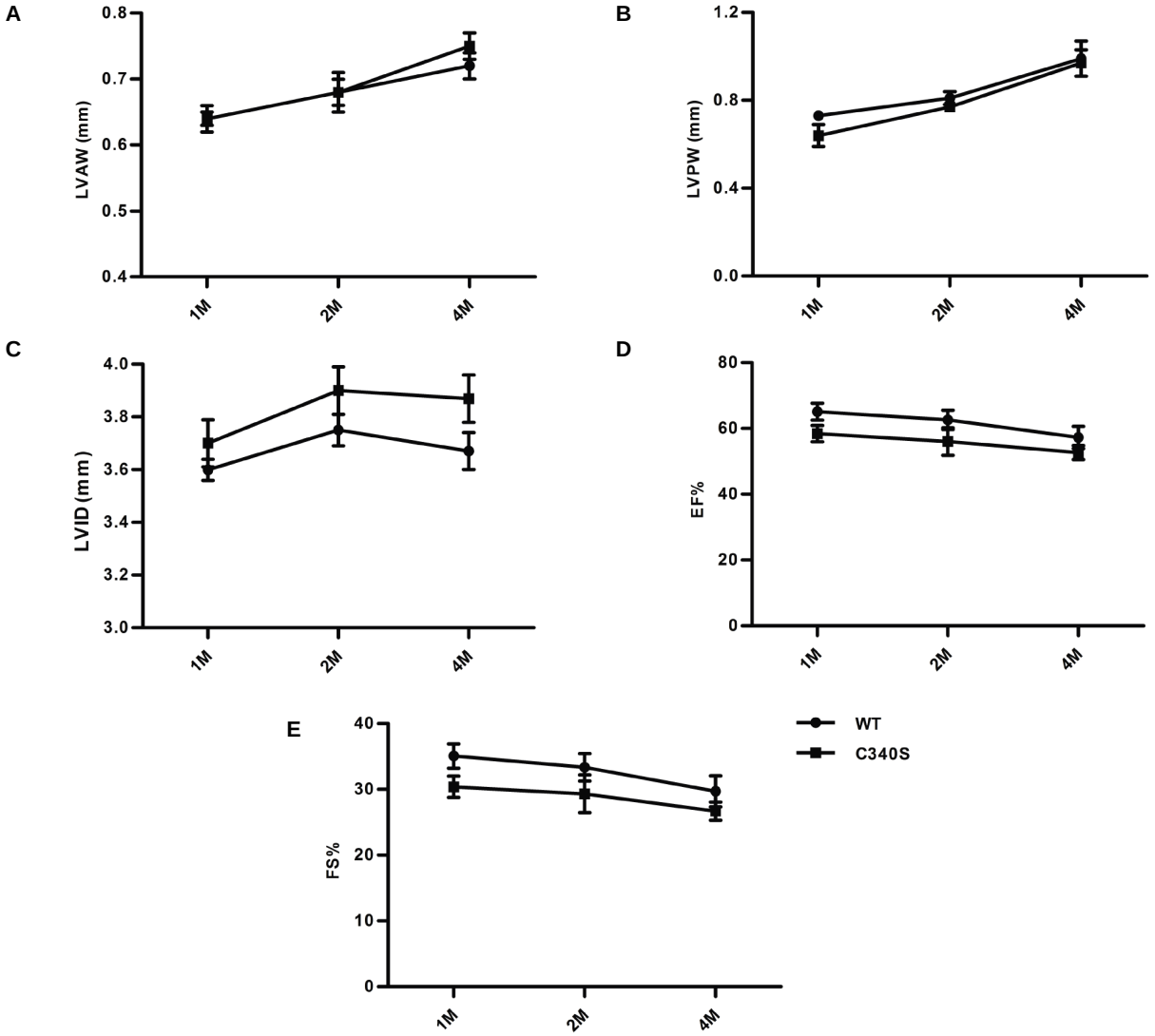
12mice/group). **E**, LV ischemic AAR for wild-type control, GRK2 Tg, eNOS^{null} and GRK2/eNOS^{null} mice following ischemia/reperfusion (n=10-12 mice/group). **F**, LV ischemic AAR for WT, eNOS^{null}, βARKct Tg and βARKct/eNOS^{null} mice following ischemia/reperfusion (n=6-8 mice/group).

Supplementary Figure 2

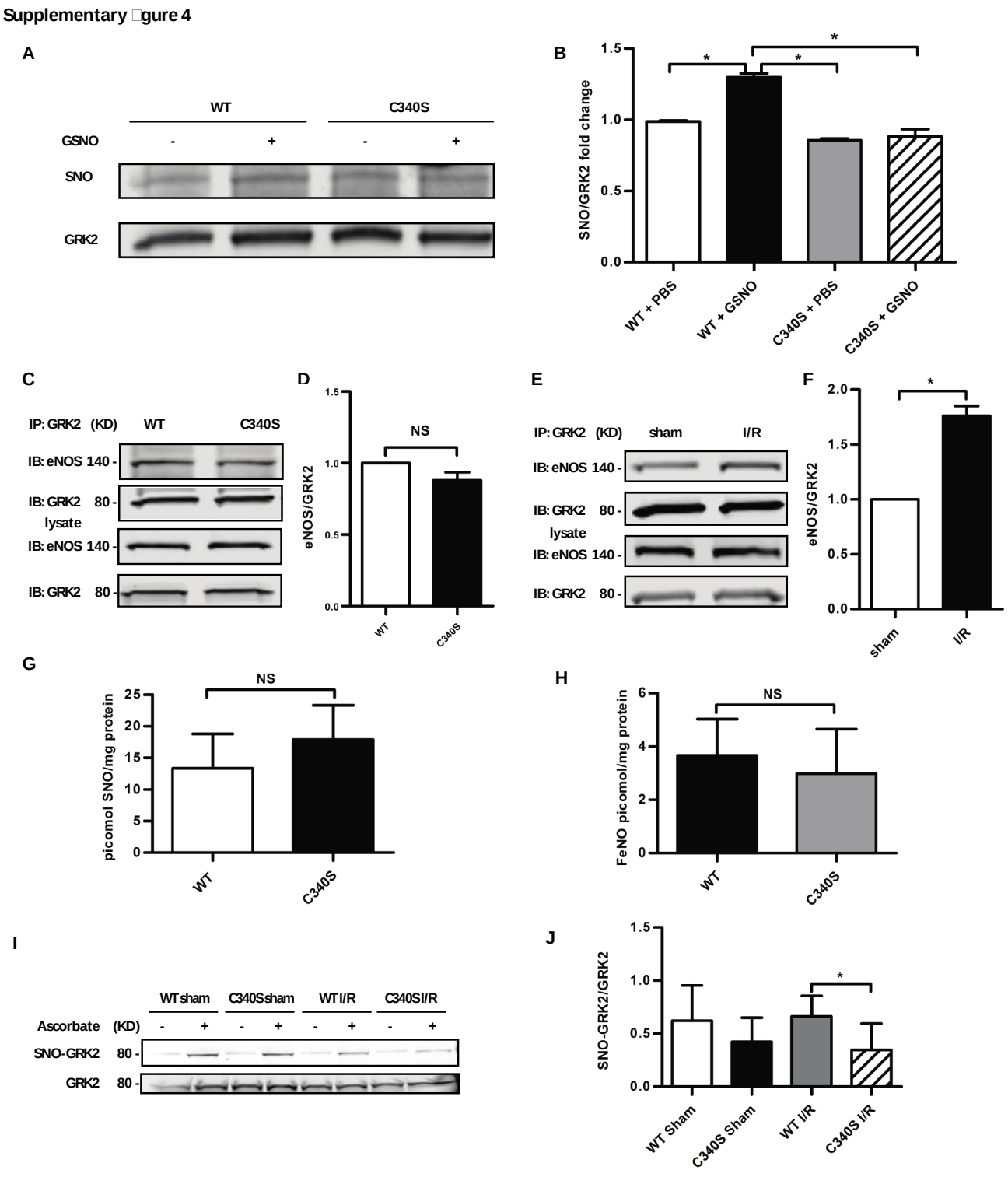


Supplementary Figure 2. Generation of GRK2-C340S knock-in mouse and real-time PCR and Western blot measurements of the abundance of βAR signaling pathway components in wild-type and GRK2-C340S knock-in mouse hearts. **A**, Map of the WT GRK2 locus, the targeting vector, and the C340S GRK2 locus after homologous recombination. **B**, Sequencing of PCR screening products showing mutation of G in WT to C in the mutant conferring the Cys to Ser replacement in GRK2 residue 340. **C-I**, Quantitative RT-PCR to measure the relative abundance of mRNAs encoding β_1AR, β_2AR, GRK3, GRK5, GRK6, AC5, and AC6 in 2 month old wild-type and GRK2-C340S knock-in mouse hearts. (NS, n=4 mice/group, Mann-Whitney test). **J**, The abundance of AC5/6 and Gαs was tested by Western blot in 2 month old WT and GRK2-C340S knock-in mouse hearts. Western blot representative of 3 (5 mice in each group) independent experiments is shown. **K-L**, Quantification of the blots described in (J) (NS, n=5 mice/group, Mann-Whitney test).

Supplementary Figure 3



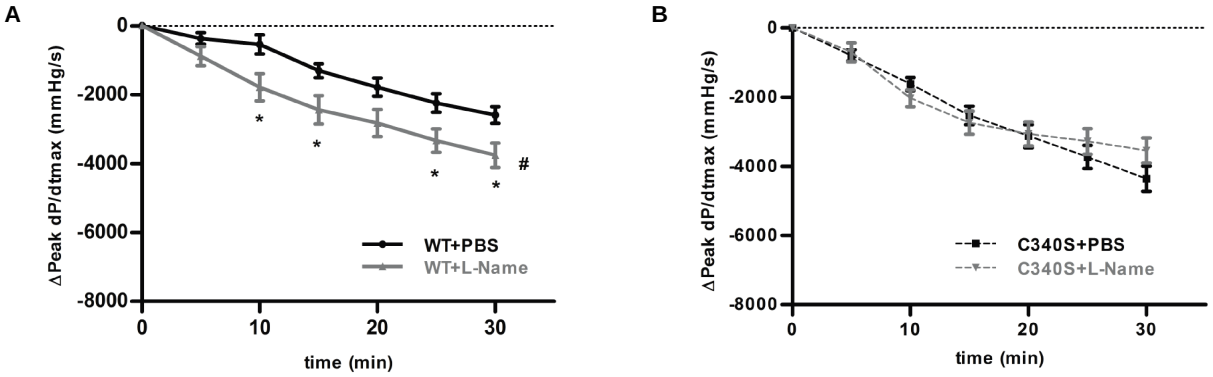
Supplementary Figure 3. Echocardiography measurements in wild-type and GRK2-C340S mice. Measurements were determined at 1, 2, and 4 months of age (NS, n=5-7 mice/group, Mann-Whitney test). Values for (**A**) LV anterior wall thickness (LVAW), (**B**) LV posterior wall thickness (LVPW), (**C**) LV internal diameter (LVID), (**D**) ejection fraction (EF%), (**E**) fractional shortening (FS%) are shown.



Supplementary Figure 4. Biochemical characteristics of GRK2 in wild-type and GRK2-C340S knock-in mouse hearts. **A**, Representative Western blot of cardiac GRK2 immunoprecipitations showing S-nitrosylated GRK2 using an SNO-Cys specific antibody following 7 days of GSNO infusion. A

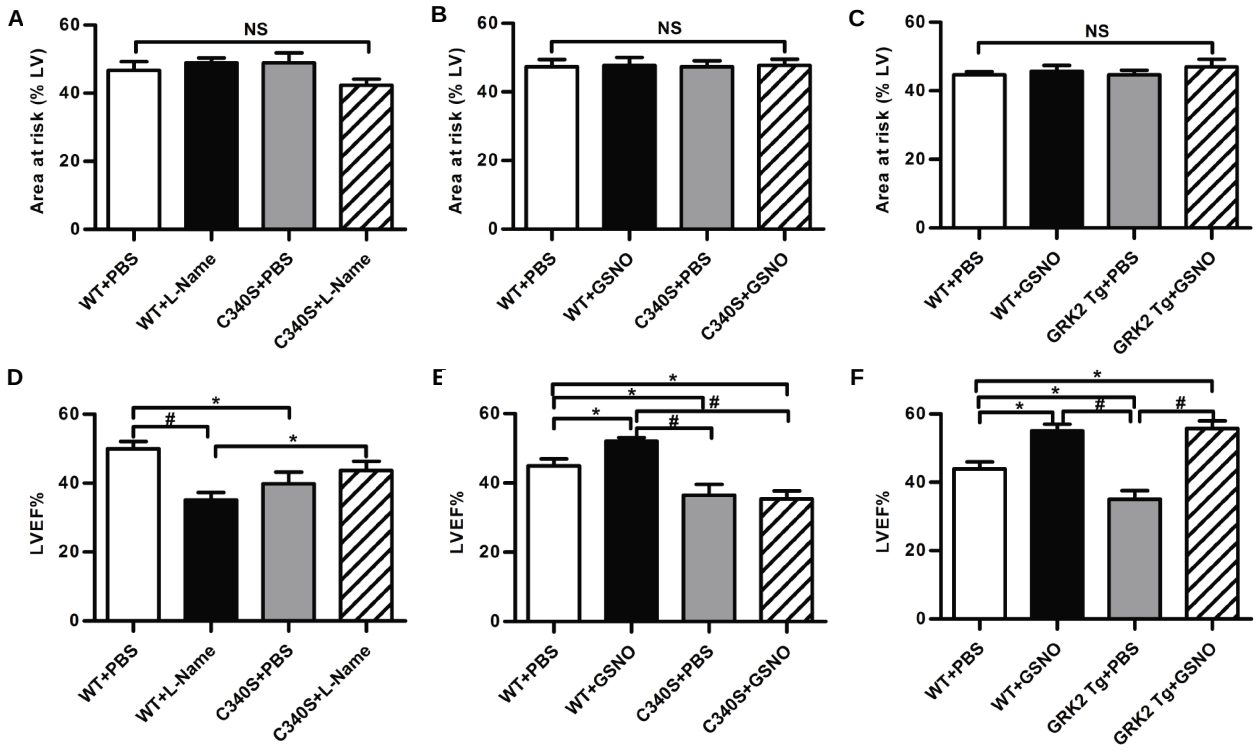
representative of 3 independent experiments is shown. **B**, Relative change of SNO-GRK2/GRK2 ratio in WT or C340S mouse hearts after 7 days of PBS or GSNO infusions. *, $P<0.001$ (Kruskal-Wallis test, n=4 mice/group). **C**, Representative Western blot of cardiac GRK2 and eNOS immunoprecipitations in WT and GRK2-C340S mouse hearts. **D**, Quantification of the amount of cardiac eNOS immunoprecipitated by GRK2 in WT mice and GRK2-C340S knock-in mice with eNOS/GRK2 (mean±SEM) values shown. (Mann-Whitney test, n=3 mice/group). **E**, Representative Western blot of cardiac GRK2 and eNOS immunoprecipitations in sham or ischemia/reperfusion-treated GRK2-C340S mouse hearts. **F**, Quantification of the amount of cardiac eNOS immunoprecipitated in GRK2-C340S mice by GRK2 in sham treated and ischemia/reperfusion-treated mice with eNOS/GRK2 (mean±SEM) values shown. *, $P<0.05$ (Mann-Whitney test, n=3 mice/group). **G-H**, Global S-nitrosothiol abundances in WT and GRK2-C340S hearts under basal conditions (t-test, n=4 mice/group). **I**, Representative Western blot of cardiac S-nitrosylated GRK2 using SNO-RAC assay following sham or ischemia/reperfusion treatment in both wild-type and GRK2-C340S knock-in mice. **J**, Relative change of SNO-GRK2/GRK2 ratio in WT or C340S mouse hearts with sham or ischemia/reperfusion treatment. *, $P<0.05$ (n=6 mice/group, Mann-Whitney test).

Supplementary □gure 5

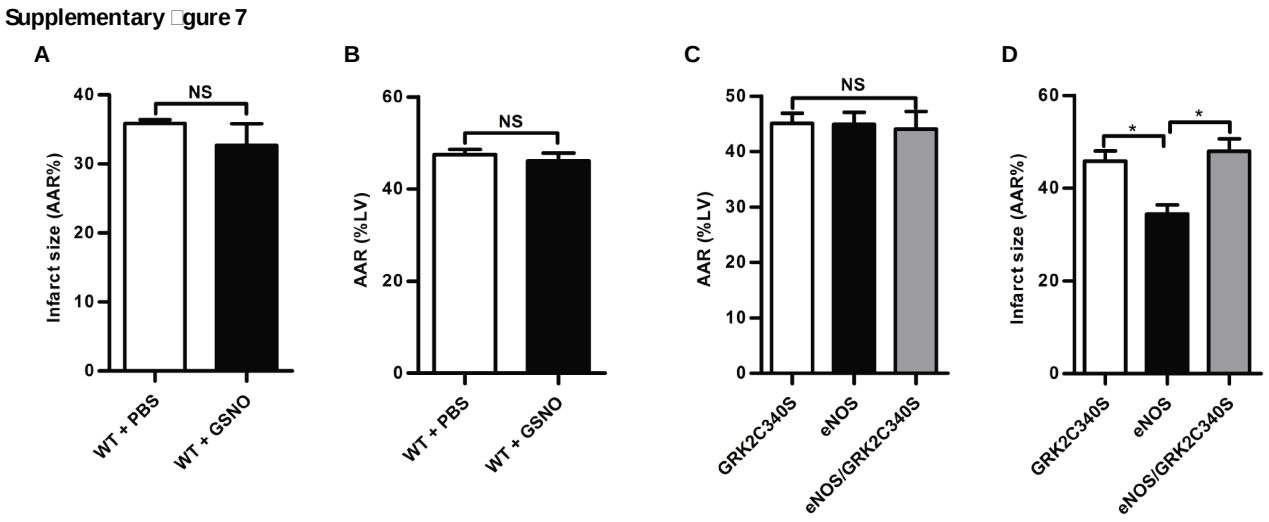


Supplementary Figure 5. Decline in the in vivo LV contractility of wild-type and GRK2-C340S mice during isoproterenol infusion. LV contractility, as assessed by the change in LV peak dP/dt_{max}, was determined in wild-type and GRK2-C340S mice during an infusion of ISO maintained over 30 min, with or without L-NAME pretreatment. **A**, Decline of contractility in wild-type mice with and without L-NAME pretreatment. *, $P<0.05$, #, $P<0.01$ WT with L-NAME curve vs WT with PBS (two way ANOVA, n=7 mice/group). **B**, Decline of contractility in GRK2-C340S knock-in mice with and without L-NAME pretreatment (two way ANOVA, n=7 mice/group).

Supplementary □gure 6

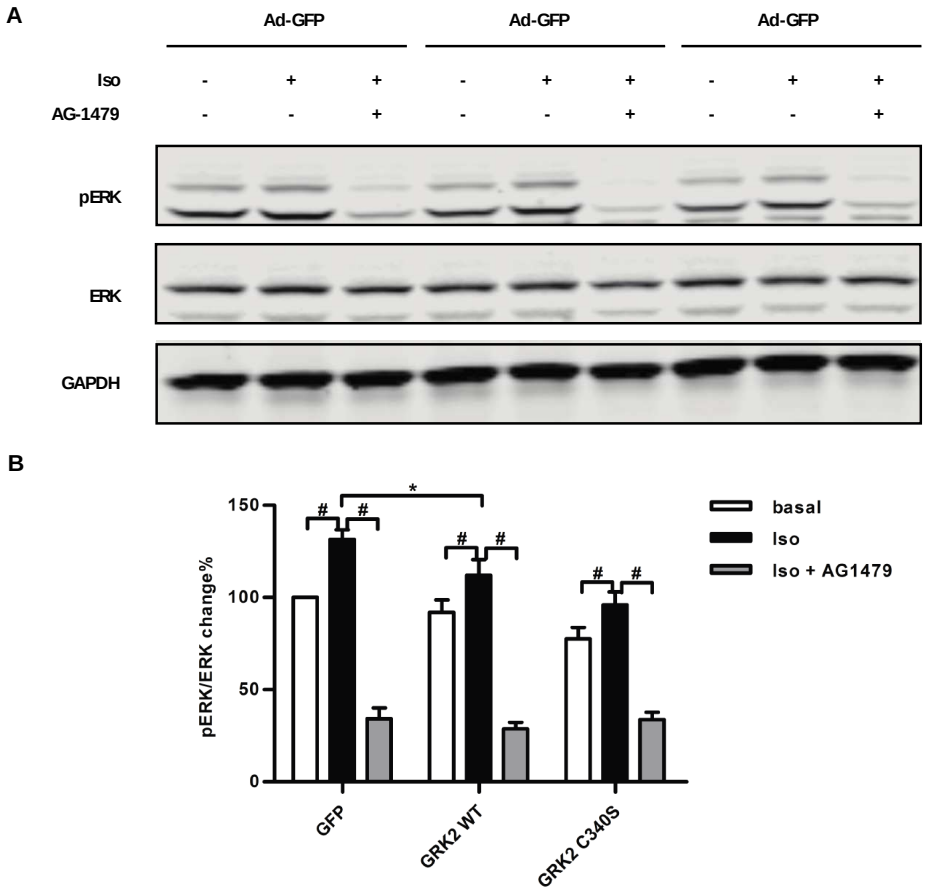


Supplementary Figure 6. Characteristics of ischemia/reperfusion injury in wild-type and GRK2-C340S knock-in mice after GSNO treatment. **A**, LV ischemic AAR for wild-type and GRK2-C340S mice with or without L-NAME pretreatment (n= 6-9 mice/group). **B**, LV ischemic AAR for wild-type and GRK2-C340S mice with or without GSNO pretreatment (n=6-7 mice /group). **C**, LV ischemic AAR for wild-type and GRK2 Tg mice with or without GSNO pretreatment (n=6 mice/group). **D**, LVEF% determined by echocardiography in wild-type and GRK2-C340S mice with and without L-NAME treatment following ischemia/reperfusion injury. *, $P<0.05$, #, $P<0.01$ (ANOVA, n=6-9 mice/group). **E**, LVEF% in post- ischemia/reperfusion wild-type and GRK2-C340S mice with or without a GSNO infusion. *, $P<0.05$, #, $P<0.001$ (ANOVA, n=6-7 mice/group). **F**, LVEF% in post-ischemia/reperfusion wild-type and GRK2 Tg mice with or without a GSNO infusion. *, $P<0.01$, #, $P<0.001$ (ANOVA, n=6 mice/group).



Supplementary Figure 7. Characteristics of ischemia/reperfusion injury in eNOS/GRK2-C340S knock-in mice and wild-type mice acutely infused with GSNO. **A**, LV infarct size for GRK2-C340S, eNOS Tg, and eNOS/GRK2-C340S mice. *, P<0.05 (ANOVA, n= 6-9 mice/group). **B**, LV ischemic AAR for GRK2-C340S, eNOS Tg, and eNOS/GRK2-C340S knock-in mice (ANOVA, n= 6-9 mice/group)**. C**, LV infarct size for wild-type mice with or without 24 hrs of GSNO pretreatment (ANOVA, n=6-8 mice/group). **D**, LV ischemic AAR for WT mice with or without 24 hours of GSNO pretreatment (ANOVA, n=6-8 mice/group).

Supplementary Figure 8



Supplementary Figure 8. Activation of the mitogen-activated protein kinase p42/p44 ERK in NRVMs treated with isoproterenol or isoproterenol and EGFR inhibitor. Activation of p42/p44 ERK was assessed by phosphorylation status in NRVMs treated with Ad-GFP, Ad-GRK2 or Ad-GRK2-C340S after stimulation with isoproterenol (ISO) or ISO and the EGFR inhibitor, AG1479. Mean±SEM from 9 individual experiments in which myocyte lysates were prepared after ISO treatment and Western blotted for pERK and total ERK. A, Representative Western blot. B, The pERK/total ERK ratio is normalized to that of GFP-treated control myocytes at baseline. *, $P<0.05$, #, $P<0.01$(two way ANOVA, n=9).