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Supplementary Materials for

Restricting mitochondrial GRK2 post-ischemia confers cardioprotection by reducing myocyte death and maintaining glucose oxidation

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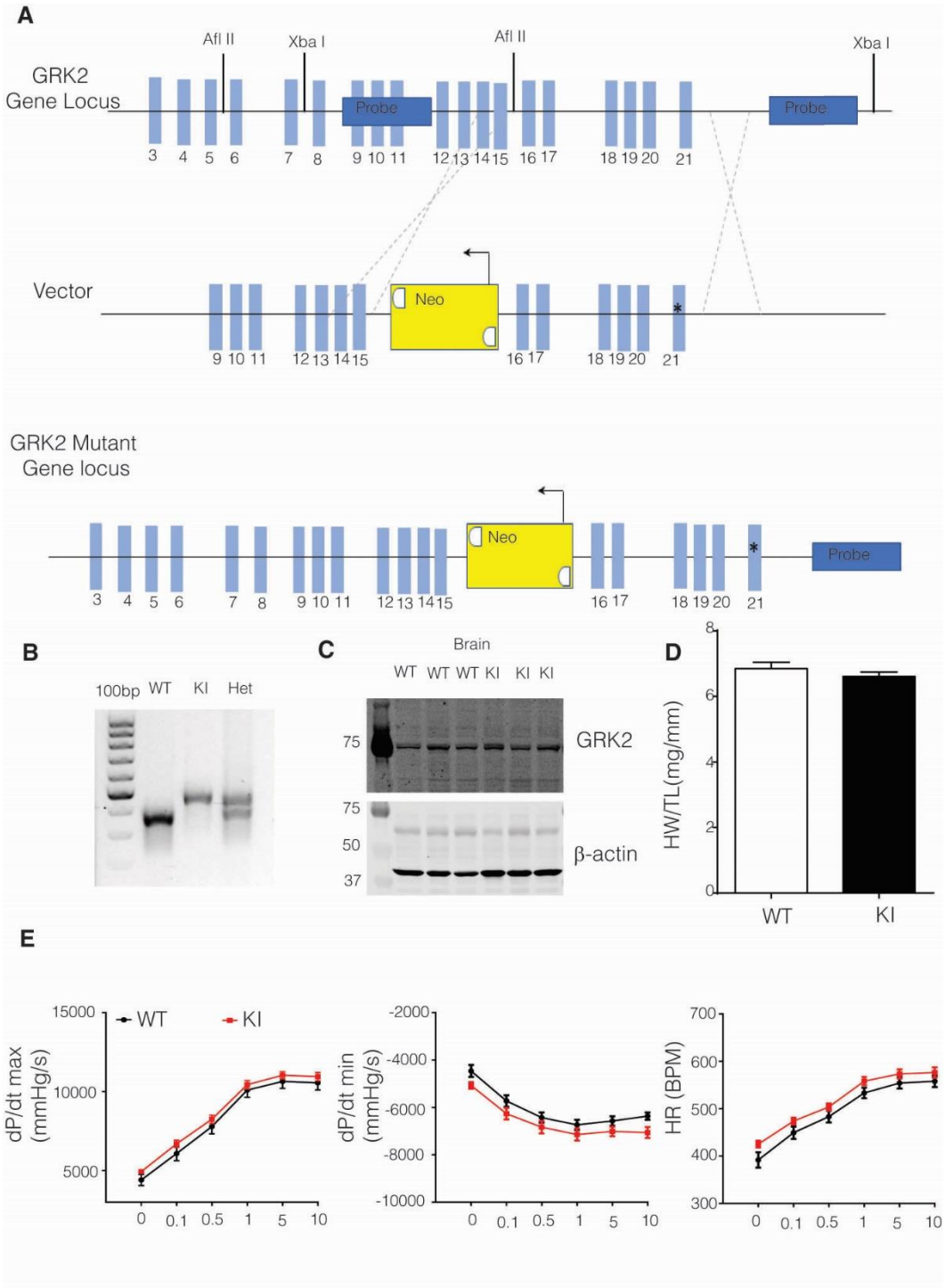


Fig. S1. Targeting strategy for the GRK2-S670A KI mice, screening, and baseline characteristics. (A) Vector targeting strategy for the construction of GRK2-S670A (KI) mice. Half moon shapes are FRT sites. Asterisk denotes location for the point mutation. (B) Genotyping screening for the KI mice. (C) Western blot for GRK2 from brain of WT and KI mice (n=3 animals/genotype). (D) Heart weight to tibia length (HW/TL) in WT and KI animals (n=14-20 hearts/genotype) (E) Mean maximal (dP/dt max), mean minimal (dP/dt min), and heart rate (BPM) at progressive isoproterenol doses up to 10ng (n=21 animals/genotype).

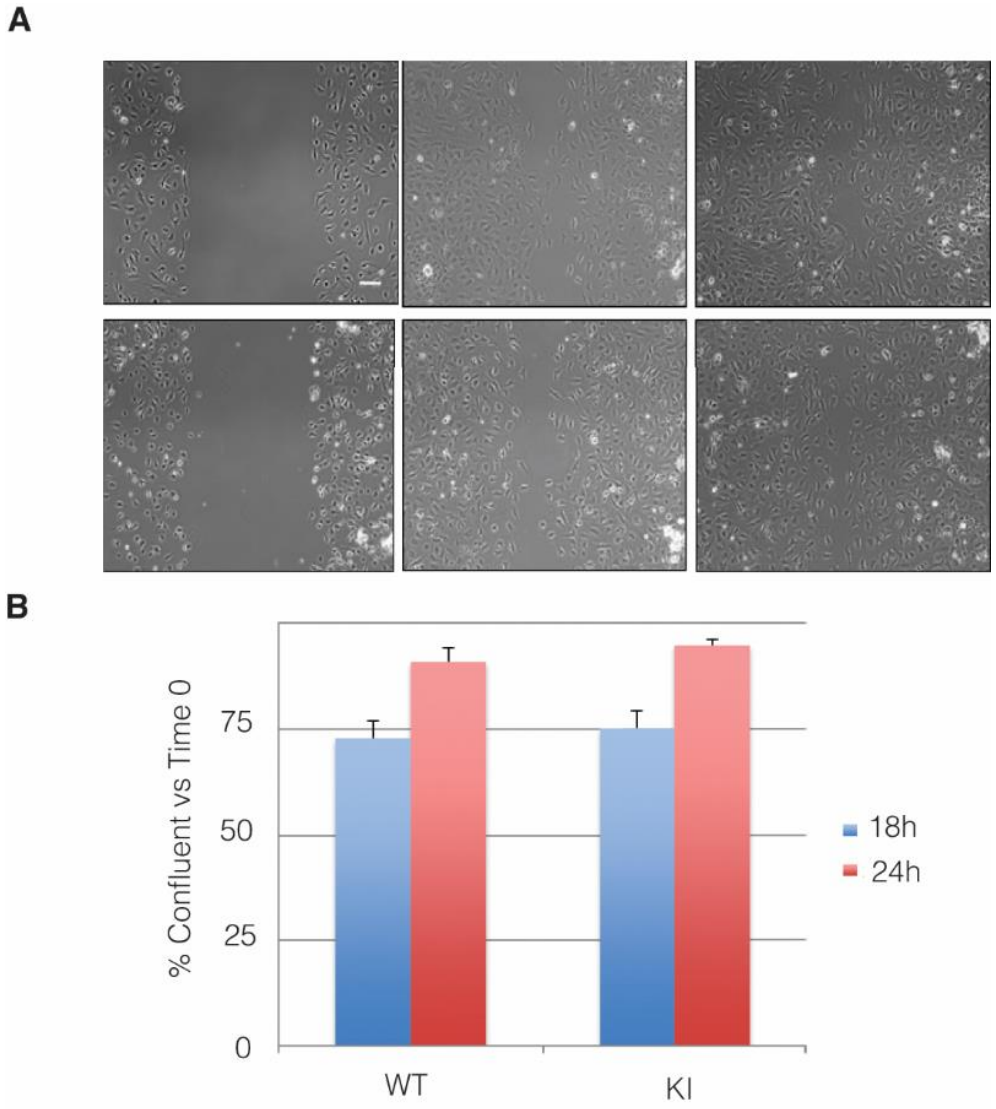


Fig. S2. Adult fibroblast migration is not affected by the S670A mutation in GRK2. (A) Representative images taken at time 0, 18h, and 24h post removal of the insert. Scale bar is 100μm. (B) Bar Graph showing confluency as a function of time 0. Experiment was conducted in technical triplicate for 3 independent hearts/genotype, $p>0.05$ Student's t-test

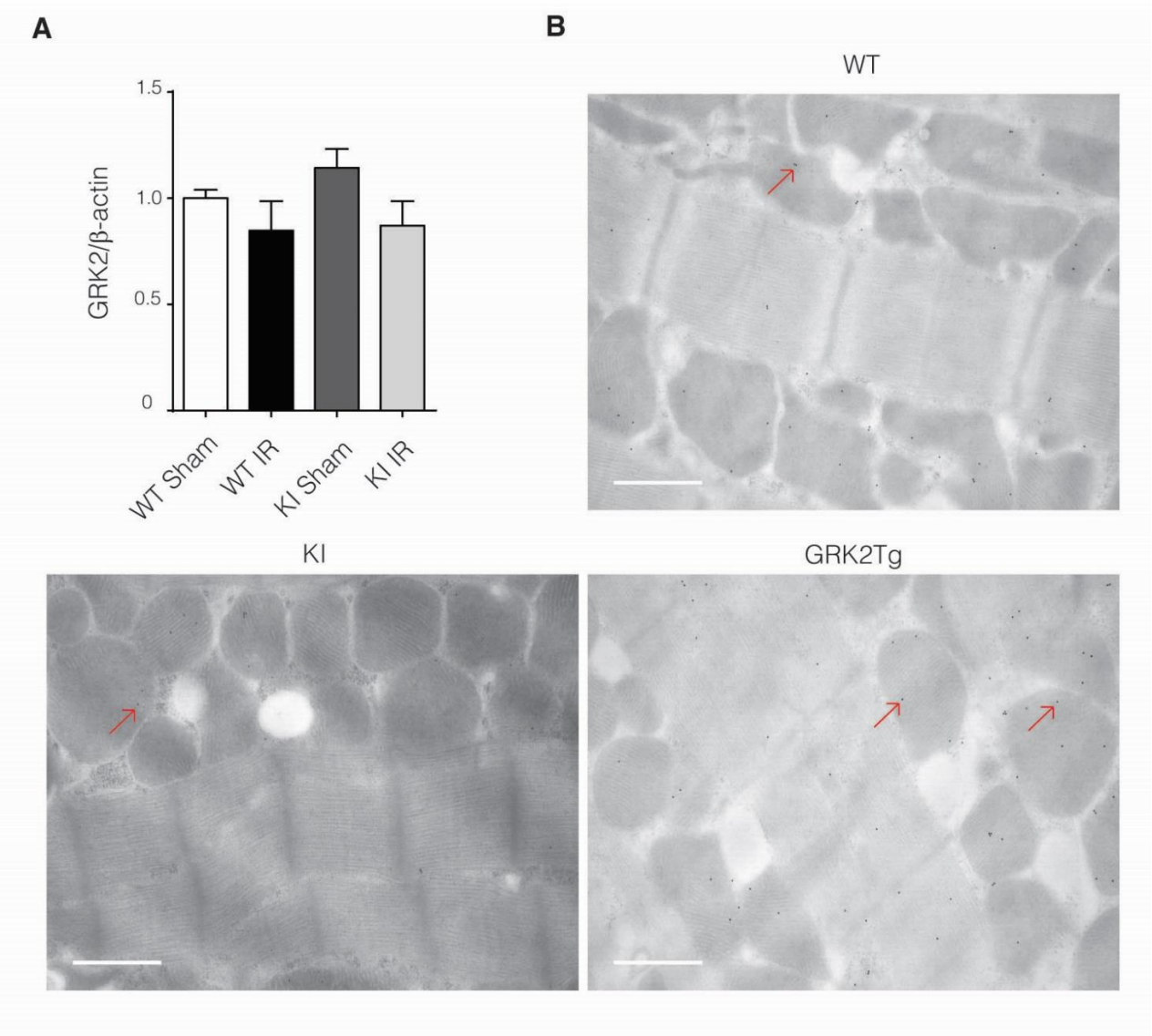
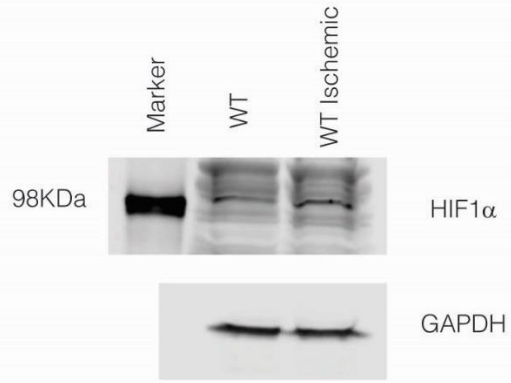


Fig. S3. Total GRK2 levels in the AAR are not altered post-IR injury, and immunogold electron microscopy suggests a role for phosphorylation of Ser^{670} in GRK2 for mitochondrial translocation. (A) GRK2 Western blot analysis of total lysates from the AAR from sham- or IR-operated animals (n=4-6 hearts/group, p>0.05). (B) Representative GRK2 immunogold EM of the AAR in WT, KI, and GRK2Tg mice. Arrows indicate mitochondrial GRK2. Scale bar is 1μm (n=3 hearts/genotype). Statistical significance was determined by ANOVA.



	WT	WT Ischemic	KI	KI Ischemic
Experiment 1	205 +/- (5,21)	147 +/- (2,10)	239 +/- (5,22)	198 +/- (4, 15)
Experiment 2	268 +/- (4,17)	221 +/- (5,20)	365 +/- (6,27)	338 +/- (9,37)
Experiment 3	200 +/- (7,31)	131 +/- (3,11)	239 +/- (9,36)	171 +/- (5,20)

Fig. S4. Raw baseline OCR recordings from adult cardiomyocytes derived from WT and GRK2-S670A KI mice subjected to an in vitro ischemia protocol. Ischemic stress in adult myocytes increases HIF1α levels and raw baseline OCR (pmol/min) recordings from the Seahorse experiments from Fig. 3 are shown as mean±SEM/SD. Data are from 3 hearts/genotype.