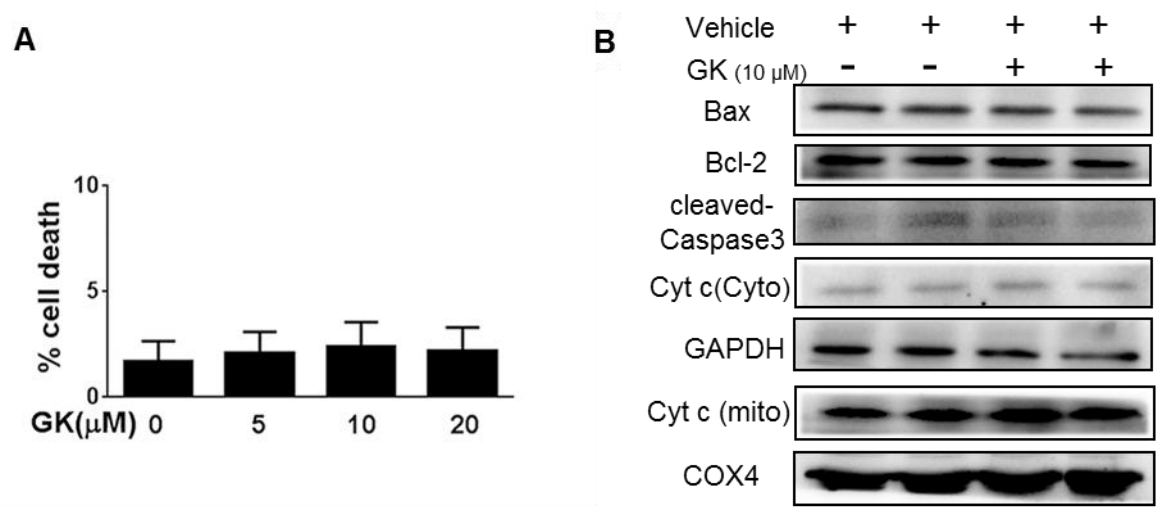
Supporting information

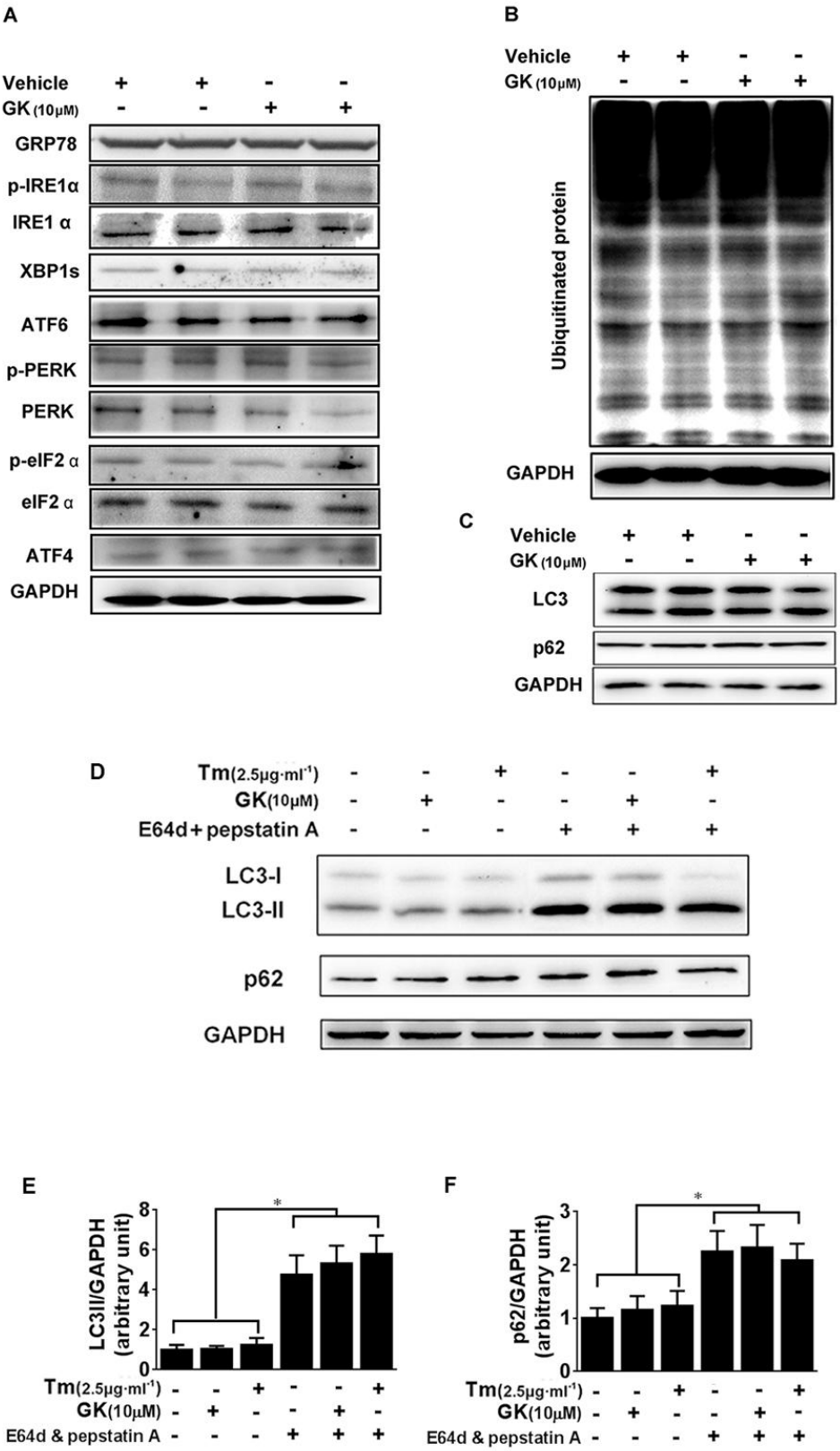
Supplementary figure S1



Supplementary Fig. S1. The effects of GK alone on cell death and apoptosis signalling pathways in NRCMs.

NRCMs were treated with GK at the indicated concentrations for up to 36h, then cell death was detected by LDH methods and the apoptosis marker proteins were examined by Western blot. (A) GK alone did not affect the viability of NRCMs. (B) GK alone had no effect on levels of apoptosis relevant proteins Bax, Bcl-2, cytochrome c and cleaved Caspase 3 in NRCMs. Data are presented as means ± SEM (n = 5). Cyt c, cytochrome c; cyto, cytosolic; mito, mitochondrial.

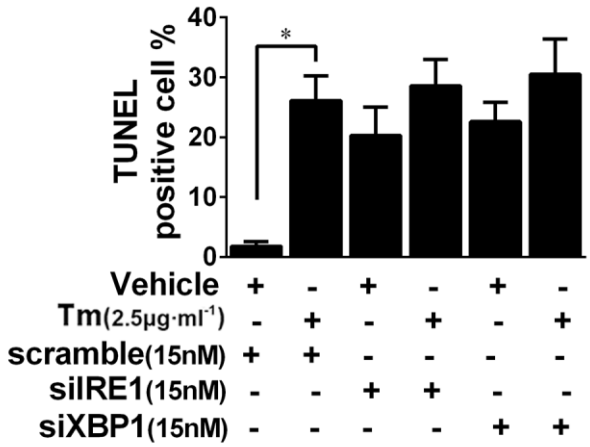
Supplementary figure S2



Supplementary Fig. S2. The effects of GK alone on ER stress, autophagy and ERAD signalling pathways.

(A-C) NRCMs were pre-treated with or without 10 mM GK for 36 h. Western blot showed that GK alone had no effects on ER stress (A), ERAD (B) and autophagy(C) signalling. (D) NRCMs were pre-treated with E64d and pepstatin A for 2 h to inhibit lysosomal proteases followed by incubation with or without 10 μM GK for 12 h and then with or without Tm for additional 24 h incubation. At the end point, LC3-II and p62 levels were analysed by Western blot. (D-F) Quantitative analysis of LC3 (D) and p62 (F). Data are presented as means ± SEM (n = 5); **P*<0.05.

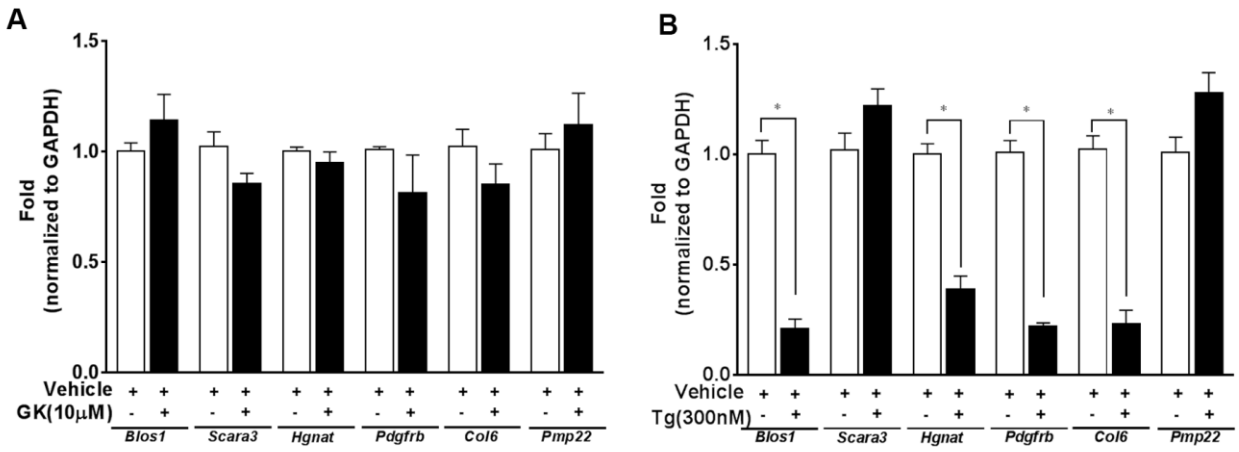
Supplementary figure S3



Supplementary Fig. S3. The effects of siIRE1 and siXBP1 on apoptosis in NRCMs.

NRCMs were transfected with negative control siRNA (scramble), specific siRNA of IRE1α (siIRE1) and XBP1 (siXBP1), respectively, for 72 h, and then stimulated with or without Tm for 24 h. Apoptosis was detected using the TUNEL assay. Quantitative analysis showed that siIRE1 or siXBP1 increased TUNEL positive cells in the presence or absence of Tm. Data are presented as means ± SEM (n = 5); **P*<0.05.

Supplementary figure S4



Supplementary Fig. S4. The effect of GK alone or Tg on RIDD in NRCMs.

NRCMs were pre-treated with or without 10 mM GK for 36 h. (A) qPCR results showed that GK alone had no effect on the transcription of Blos1, Scara3, Hgnat, Pdgfrb, Col6 and Pmp22. (B) Blos1, Hgnat, Pdgfrb, and Col6 were reduced in Tg treated NRCMs.