Supplementary Materials: Therapeutic effect of endothelin-converting enzyme inhibitor on chronic kidney disease through the inhibition of endoplasmic reticulum stress and the NLRP3 inflammasome

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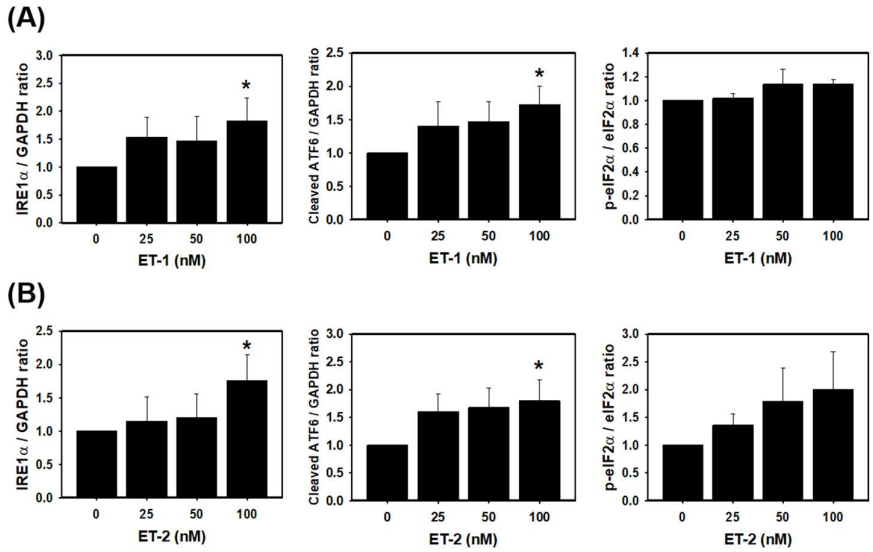


Figure S1. Effects of ET-1 (A) or ET-2 (B) exposure on the ER stress in human kidney cells. The ER stress-related proteins expression of histogram represent the average normalized densitometric values. GAPDH was used as the internal control. Data are presented as the means ± standard deviation of three independent experiments. *P < 0.05 compared with the control.

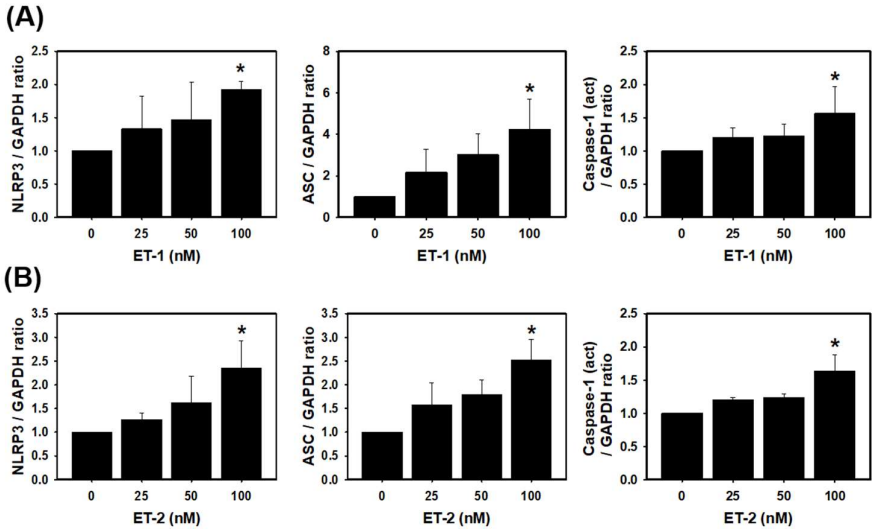


Figure S2. Effects of ET-1 (A) or ET-2 (B) exposure on the NLRP3 inflammasome in human kidney cells. The NLRP3 inflammasome-related proteins expression of histogram represent the average normalized densitometric values. GAPDH was used as the internal control. Data are presented as the means ± standard deviation of three independent experiments. *P < 0.05 compared with the control.

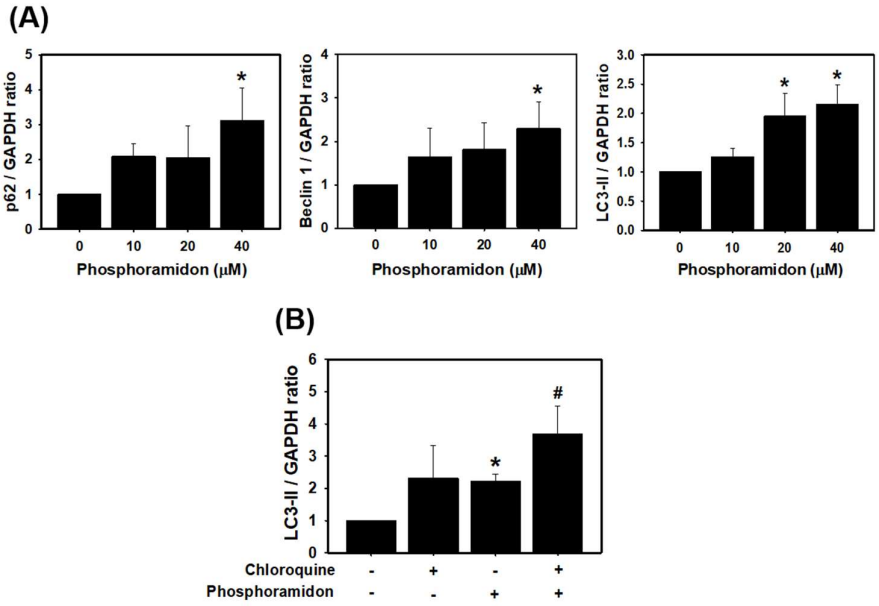


Figure S3. Effects of phosphoramidon treatment on autophagy in human kidney cells. (A) The autophagy-related proteins expression of histogram represent the average normalized densitometric values. *P < 0.05 compared with the control. (B) Quantification of LC3-II in HK-2 cells treated with chloroquine and phosphoramidon. The cells were pretreated with chloroquine

(5 μM) for 1 h and then treated with phosphoramidon (20 μM) for 24 h. GAPDH was used as the internal control. Data are presented as the means ± standard deviation of three independent experiments. *P < 0.05 compared with the control. *P < 0.05 compared with the control. # p < 0.05 compared with the phosphoramidon.

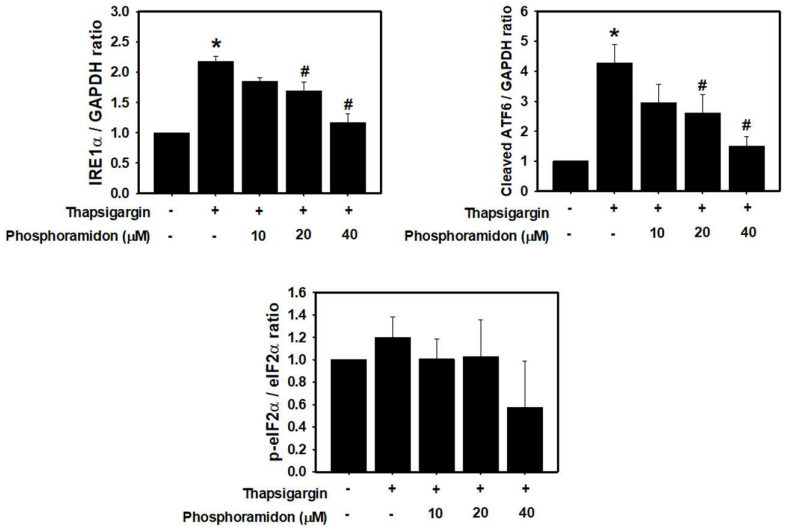


Figure S4. Effects of phosphoramidon treatment on the thapsigargin-induced ER stress in human kidney cells. The ER stress-related proteins expression of histogram represent the average normalized densitometric values. GAPDH was used as the internal control. Data are presented as the means ± standard deviation of three independent experiments. *P < 0.05 compared with the control. # p < 0.05 compared with thapsigargin.

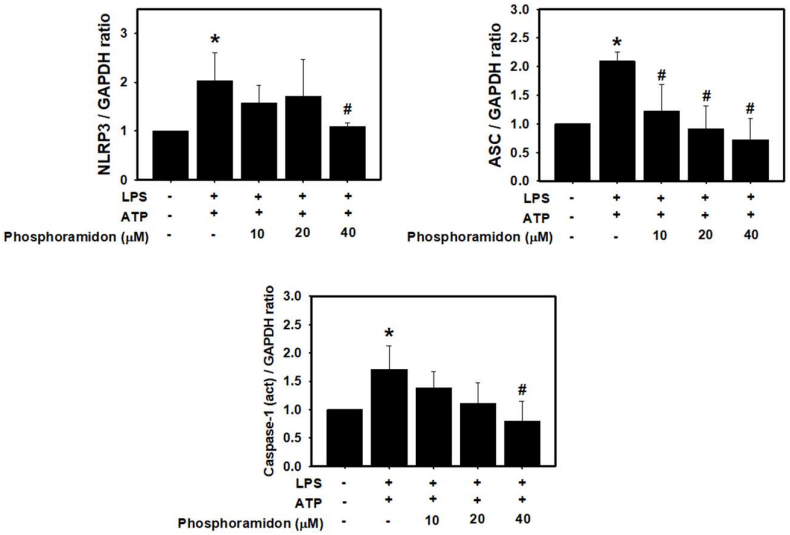


Figure S5. Effects of phosphoramidon treatment on the LPS+ATP-induced NLRP3 inflammasom in human kidney cells. The NLRP3 inflammasom-related proteins expression of histogram represent the average normalized densitometric values. GAPDH was used as the internal control. Data are presented as the means ± standard deviation of three independent experiments. *P < 0.05 compared with the control. # p < 0.05 compared with the LPS+ATP group.

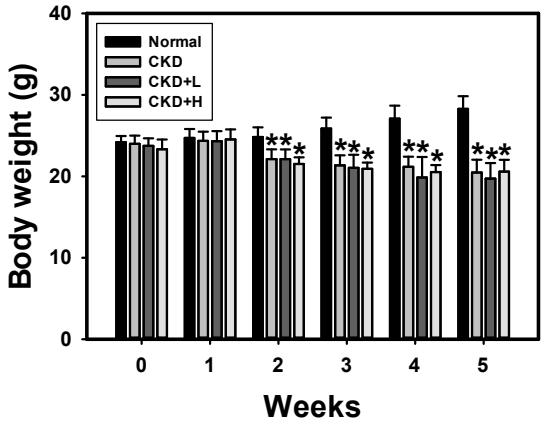
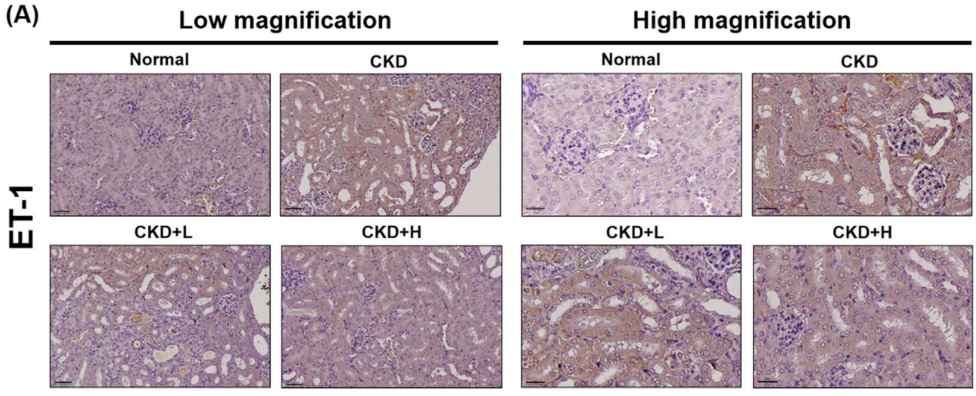


Figure S6. Measurement of body weights of C57BL/6 mice in various groups (5 mice per group). * p < 0.05 compared with the normal group. Data are presented as the means ± standard deviation.



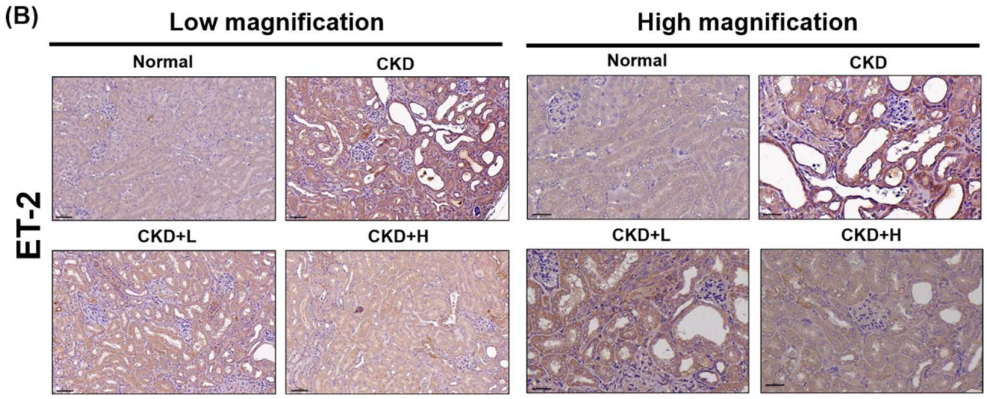


Figure S7. The ET-1 and ET-2 expression of kidneys after phosphoramidon treatment in the adenine diet-induced CKD model. IHC was used to determine the expression levels of ET-1 (A) and ET-2 (B) in kidney tissues. The scale bar of low magnification=60 μm. The scale bar of high magnification=30 μm.

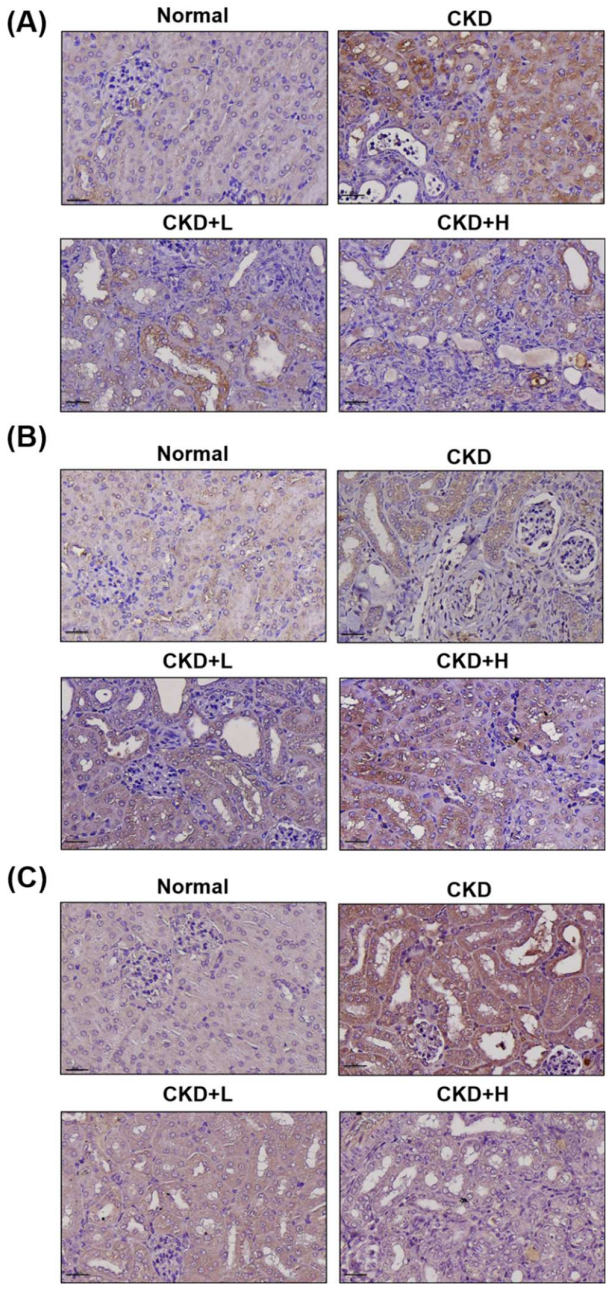


Figure S8. The high magnification of IHC images. Phosphoramidon regulates ER stress, autophagy and the NLRP3 inflammasome in the adenine diet-induced CKD model. The protein expression of IRE1α (A), LC3 (B) and NLRP3 (C) in kidney sections in the indicated groups. Scale bar=30 μm.

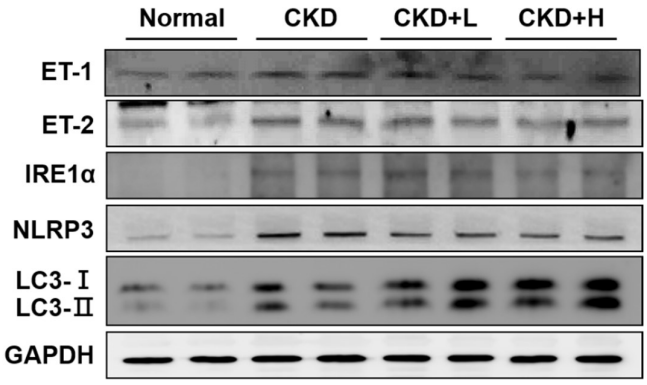


Figure S9. Western blot analysis of ET-1, ET-2, IRE1α, LC3 and NLRP3 in kidney tissue sections in the indicated groups.

Table S1. The quantification of immunohistochemical (IHC) and masson staining.

Item / Group	Normal	CKD	CKD+L	CKD+H
IRE1α area (%)	1.11±1.20	7.39±1.37*	6.25±1.72	3.25±1.91#
LC3 area (%)	2.10±1.62	5.56±1.66*	7.23±1.47#	8.90±1.35#
NLRP3 area (%)	1.91±1.06	26.19±5.87*	14.48±5.86#	10.75±2.97#
ET-1 area (%)	3.87±1.39	18.75±5.34*	15.96±5.18	9.10±3.57#
ET-2 area (%)	3.50±1.61	35.64±4.09*	21.99±3.19#	10.08±2.33#
Masson staining positive area (%)	0.2±0.13	5.8±1.86*	4.21±1.19#	2.02±0.61#

*p<0.05, CKD compared with normal.

#p<0.05, CKD+L or CKD+H compared with CKD.