

1 **Endoplasmic reticulum stress contributes to copper-induced**
2 **pyroptosis via regulating IRE1 α -XBP1 pathway in pig jejunal**
3 **epithelial cells**

4 Jianzhao Liao, Zhuoying Hu, Quanwei Li, Hongji Li, Weijin Chen, Haihua Huo,
5 Qingyue Han, Hui Zhang, Jianying Guo, Lianmei Hu, Jiaqiang Pan, Ying Li*, Zhaoxin
6 Tang*

7 College of Veterinary Medicine, South China Agricultural University, Guangzhou
8 510642, Guangdong, P.R. China.

9 * Corresponding author.

10 E-mail address: lying@scau.edu.cn (Y. Li); tangzx@scau.edu.cn (Z. Tang); Tel: 86-
11 20-8528 0248; Fax: 86-20-8528 0234

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13 **Supplementary materials**

14 **Table S1.** The ingredient and nutritional composition in the basic diet

Ingredient	%	Nutritional composition	
Corn	65.60	Digestible energy (MJ/kg)	13.75
Bean flour	22.00	Crude protein (%)	17.85
Wheat bran	11.00	Lysine (%)	0.95
Coarse fiber	9.00	Methionine +cystine (%)	0.38
Granulesten	1.50	Calciuml (%)	0.84
Fish flour	3.00	Phosphorus (%)	0.63
Meat flour	1.00		
Salt	0.40		
Limestone	0.80		
Dicalcium phosphate	0.80		
Bone meal	1.00		
Additive*	0.45		

15 * Per kilogram of diet: Cu (10 mg); Fe (150 mg); Zn (200 mg); Mn (10 mg); I (0.15 mg);
 16 Se (0.30 mg); vitamin A (4400 IU); vitamin D (440 IU); vitamin E (22 IU); vitamin K
 17 (1.1 mg); d-pantothenic acid (22 mg); niacin (22 mg); vitamin B₁₂ (22 mg); choline
 18 (0.61 g); d-biotin (0.14 mg) and folic acid (0.66 mg).

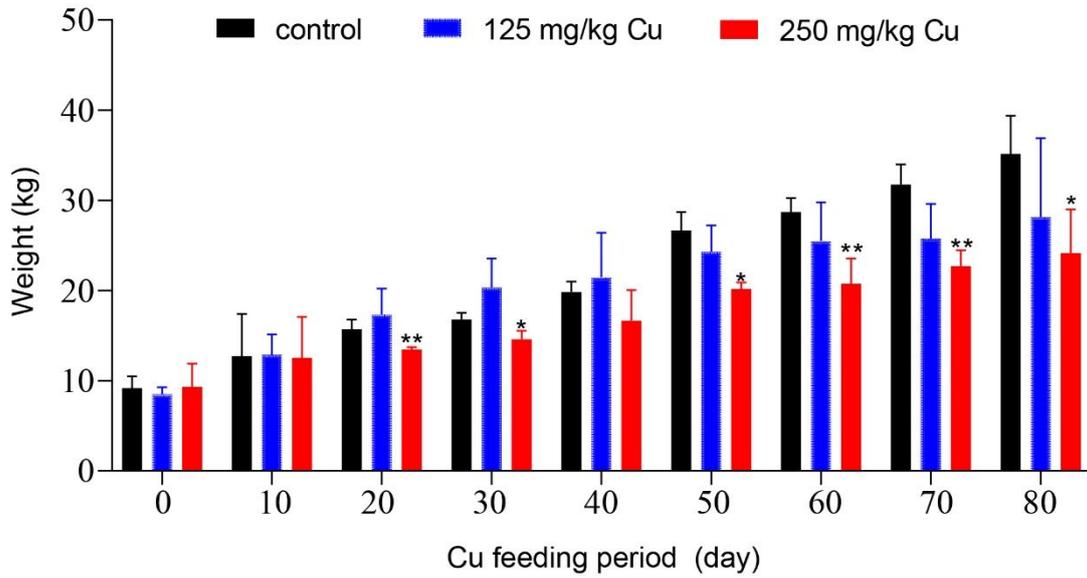
19 **Table S2.** Primer sequences

genes	Sense sequences (5'-3')	Antisense sequences (5'-3')
GRP78	TGCCAATGACCAGGGGAACC	GAGGGGTCATTCCAGGTGCG
GRP94	TGCCAAGGAAGGAGTGAAGT	GTTGCCTGACCATCCGTACT
IRE1 α	TCTATGCGTCGCCCTCAAT	ATTGAGGGCGACGCATAGA
XBP1	GGAGTTAAGACAGCGCTTGG	GAGATGTTCTGGAGGGGTGA
NLRP3	CAGCACGAACCAGAATCTCA	AGCAGCAGTGTGATGTGAGG
ASC	CATGAAGGAGGTGGCGGAG	TTTGGTGGGGTTGGTGTGC
Caspase-1	GAGAAAATCTCACCGCTTCG	TCACCTTGGGTTTGTCTTC
GSDMD	ACCTGTCCATCTGGGACATC	GTAGCGTGCACACGTTTCATC
IL-1 β	CAGCTGCAAATCTCTCACCA	CATTGCACGTTTCAAGGATG
IL-18	CTGCTGAACCGGAAGACAAT	CTCAAACACGGCTTGATGTC
GAPDH	GCCATCACAGCCACACAGAAGA	CGGCAGGTCAGGTCAACAACAG

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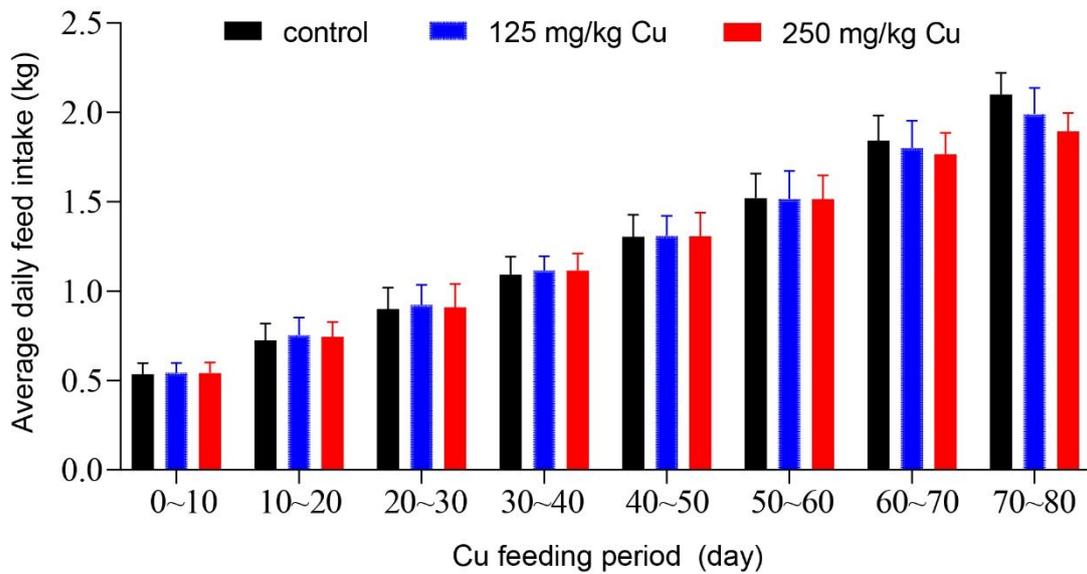
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22 **Supplementary figures**



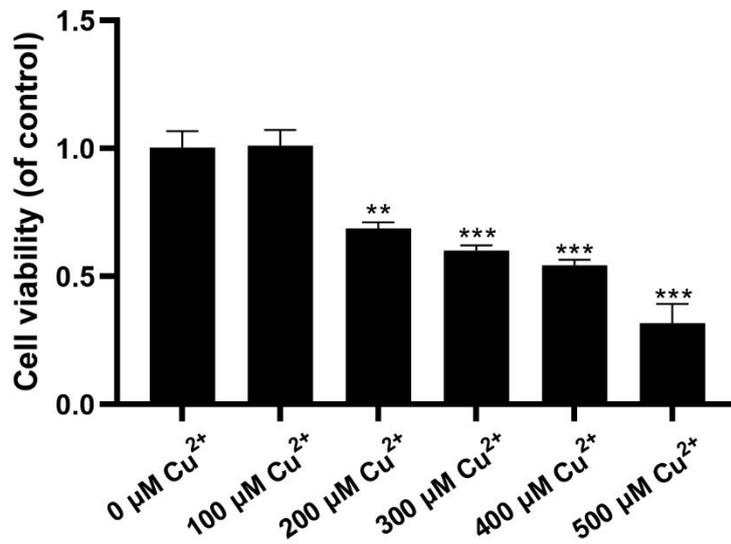
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24 **Figure S1** Body weight per pig during the experiments.



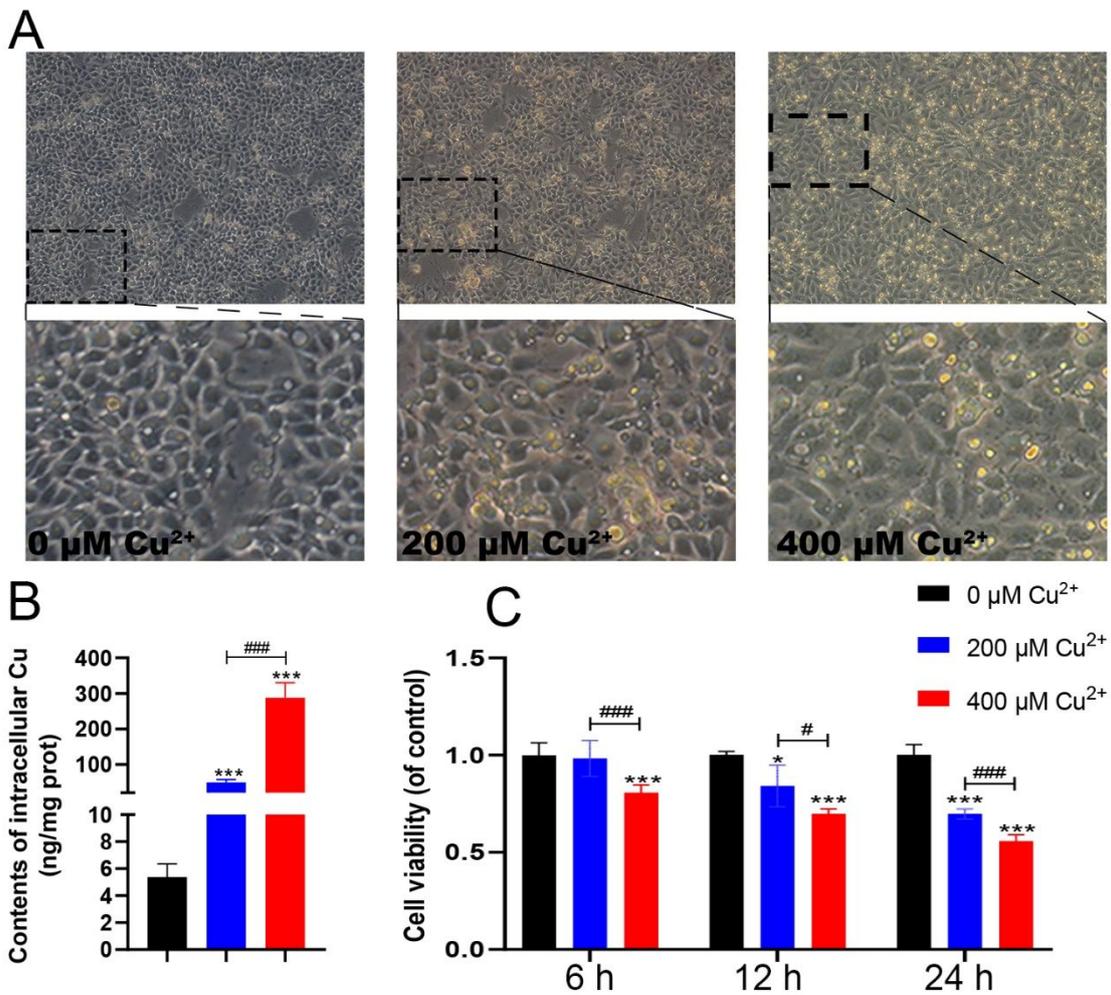
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26 **Figure S2** Average daily feed intake per pig during the experiments.



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28 **Figure S3** Effects of Cu²⁺ (0~500 μM) on the viability of IPEC-J2 cells.

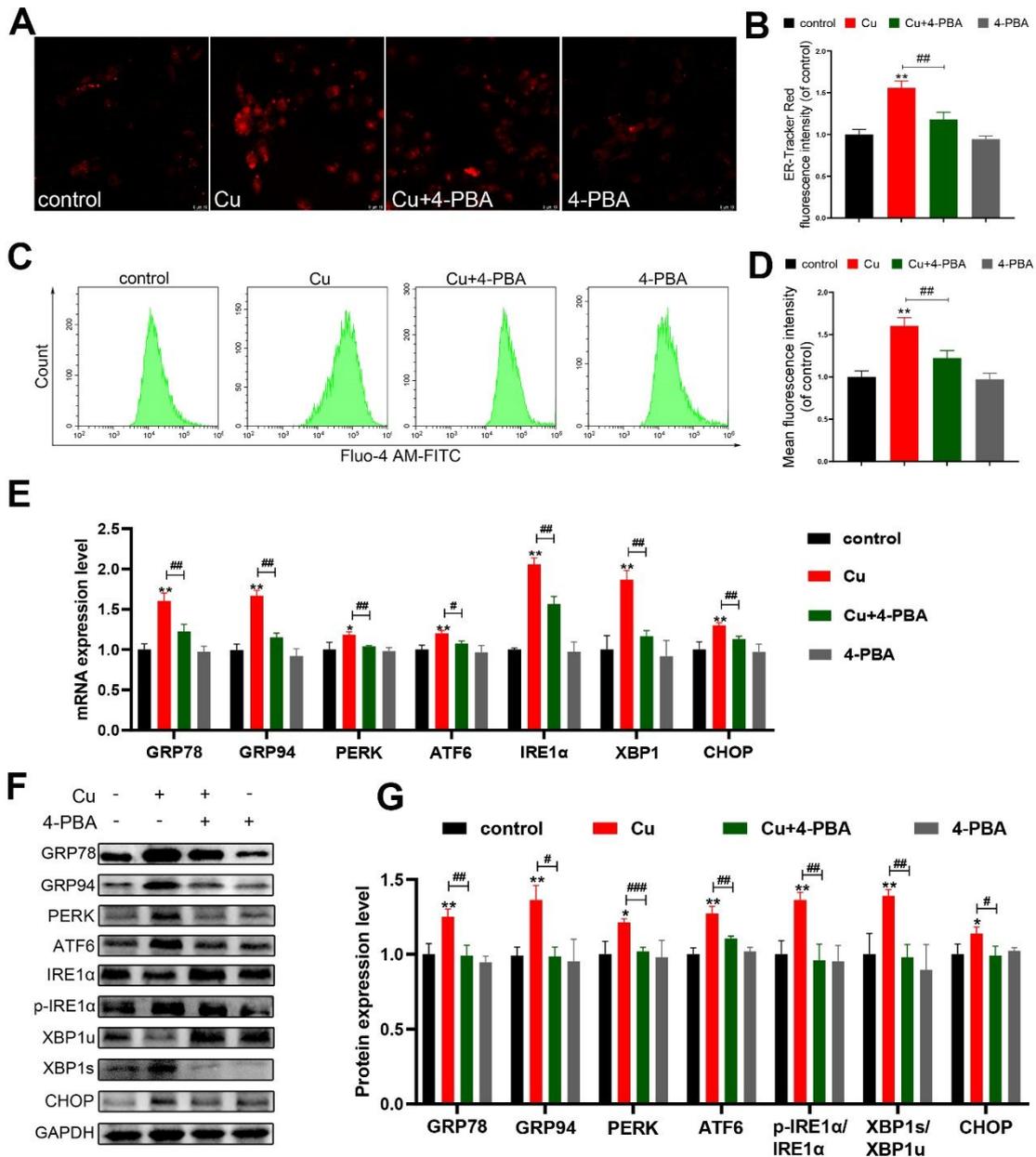


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30 **Figure S4** Effects of Cu²⁺ on the cell morphology, intracellular Cu contents, and the

31 cell viability in IPEC-J2 cells. (A) Cell morphology at 24 h. (B) Intracellular Cu

32 contents at 24 h. (C) Cell viability at 6, 12, and 24 h.



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34 **Figure S5** Effects of 4-PBA on the Cu-induced ER stress in jejunal epithelial cells. (A)

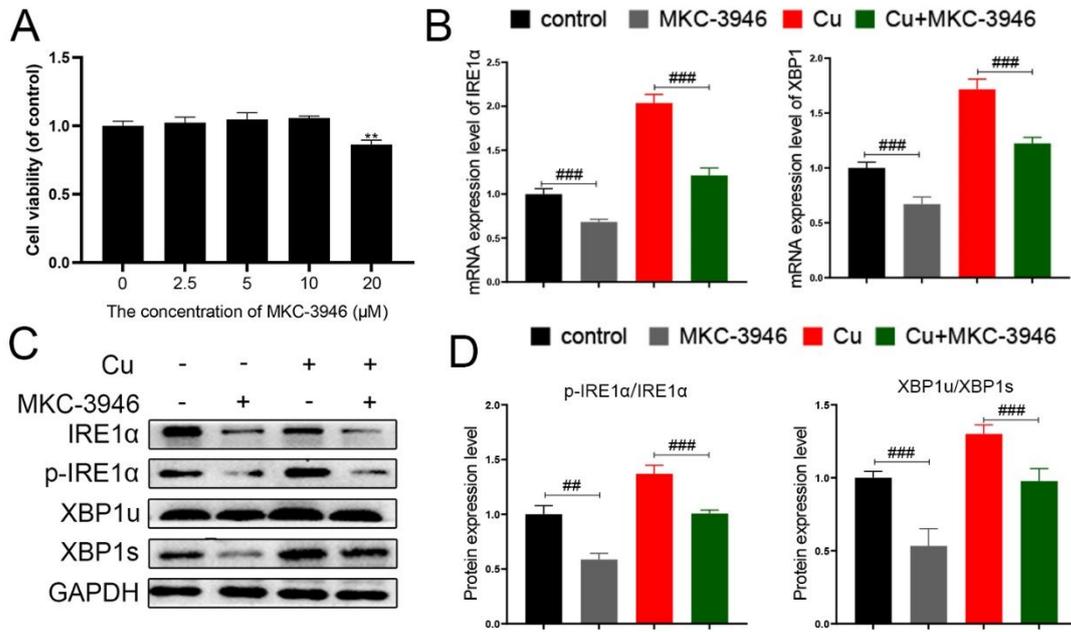
35 ER-Tracker Red staining. (B) Fluorescence intensity of ER-Tracker Red. (C) Ca²⁺ was

36 detected with Fluo-4 AM by flow cytometry. (D) Quantitative analysis of the Ca²⁺

37 fluorescence intensity. (E) The mRNA levels of ER stress-related genes. (F) Protein

38 bands of ER stress-related proteins. (G) Quantitative analysis revealed the expression

39 levels of ER stress-related proteins.



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41 **Figure S6** Effects of MKC-3946 on IRE1 α -XBP1 signal pathway in jejunal epithelial

42 cells. (A) Effects of different concentration of MKC-3946 on the viability of IPEC-J2

43 cells. (B) mRNA expression levels of IRE1 α and XBP1. (C) Protein bands of IRE1 α ,

44 p-IRE1 α , XBP1u, and XBP1s. (D) Quantitative analysis of the protein expression of p-

45 IRE1 α /IRE1 α and XBP1u/XBP1s.