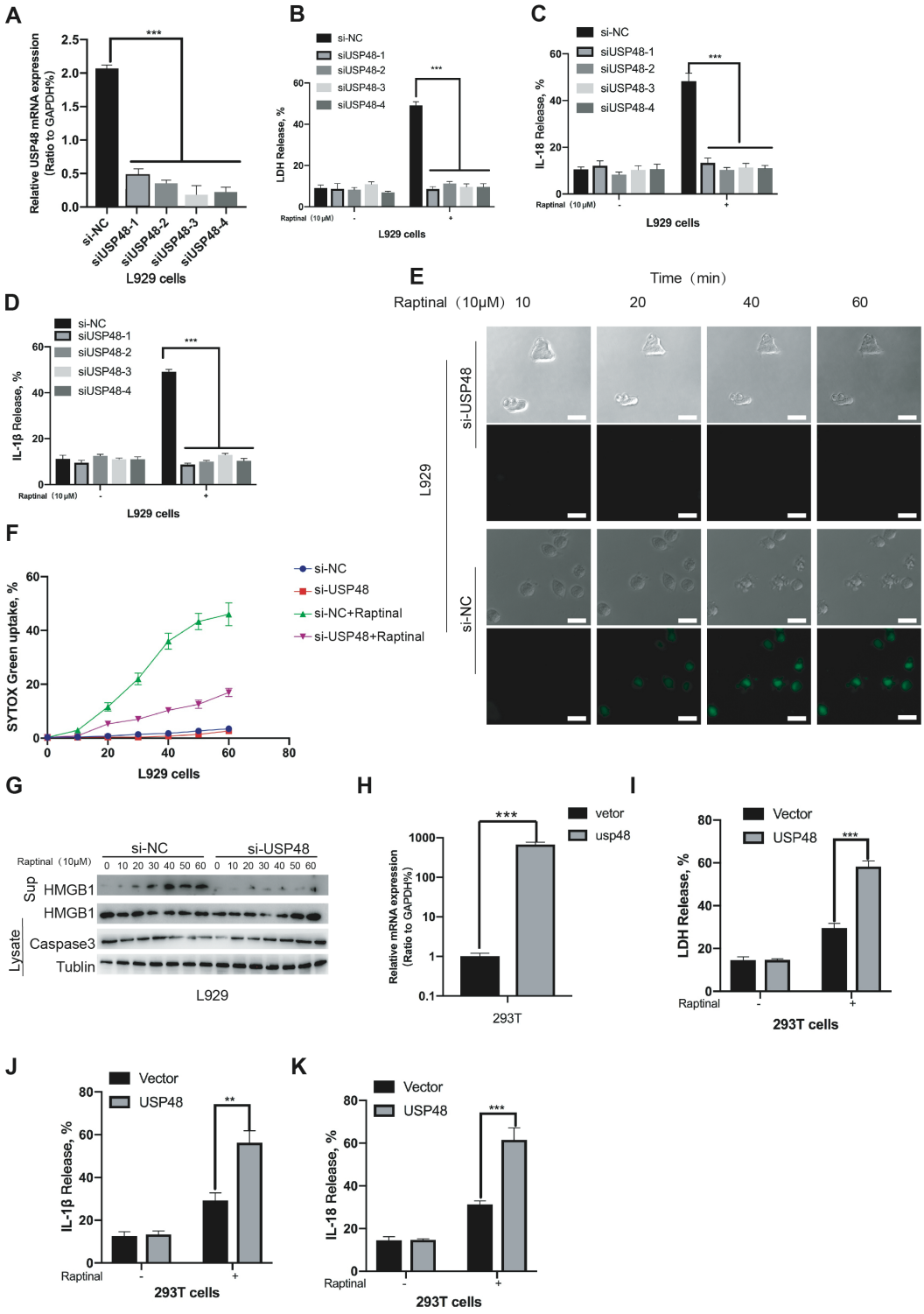
Supplementary Figures

Supplementary Figure 1

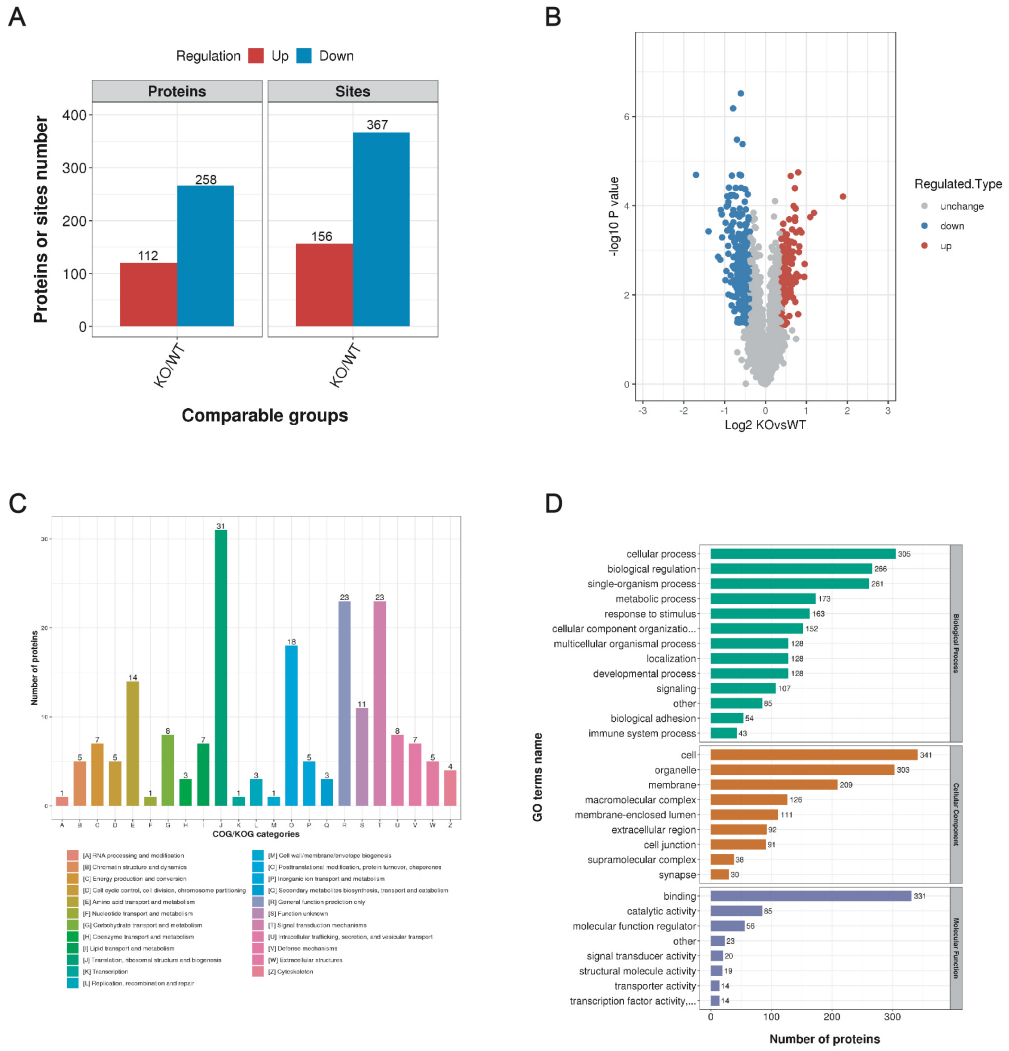


Supplementary Figure 1: USP48 has a regulatory effect on pyroptosis in L929 cells

(A) qRT-PCR verifies the transfection efficiency of si-NC and USP48 specific siRNA in L929 cells. (B) LDH detection kit was used to analyze the changes in LDH of L929 cells transfected with siRNA (si-NC) or siRNA specific to USP48 (si-USP48) after Raptinal treatment. (C) Elisa experiment was

used to analyze the expression changes of IL-1β in L929 cells transfected with siRNA (si-NC) or siRNA specific to USP48 (si-USP48) after Raptinal treatment. (D) Elisa experiment was used to analyze the expression changes of L929 in 293T cells transfected with siRNA (si-NC) or siRNA specific to USP48 (si-USP48) after Raptinal treatment. (E) Time-lapse microscopy observed the morphological changes and the absorption of SYTOX green of L929 cells transfected with siRNA (si-NC) or USP48 specific siRNA (si-USP48) after Raptinal treatment. (F) Quantitative analysis chart of SYTOX green absorption. (G) Kinetics of caspase-3 and GSDME cleavage and HMGB1 release by immunoblot of cell lysates and culture supernatants.. (H)qRT-PCR verifies the transfection efficiency of USP48 in 293T cells. (I) LDH detection kit was used to analyze the changes in LDH of 293T cells transfected with USP48 or vector. (J) IL-1β detection kit was used to analyze the changes in LDH of 293T cells transfected with USP48 or vector. (K) IL-18 detection kit was used to analyze the changes in LDH of 293T cells transfected with USP48 or vector.

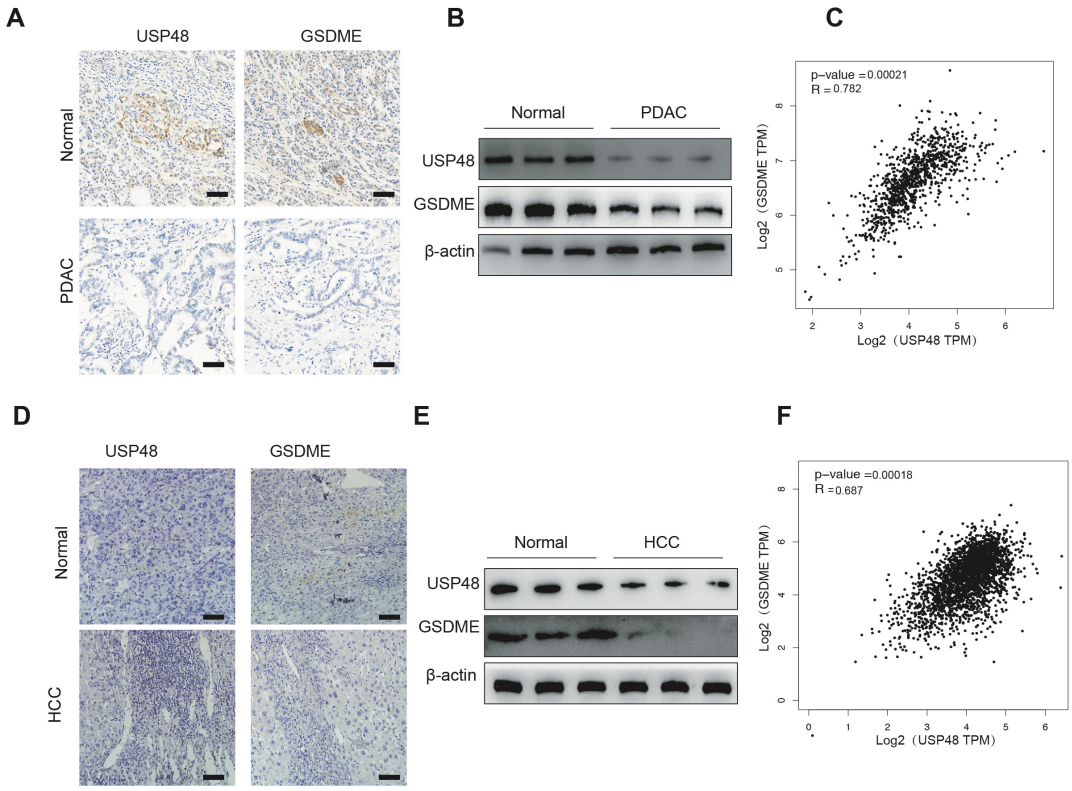
Supplementary Figure 2



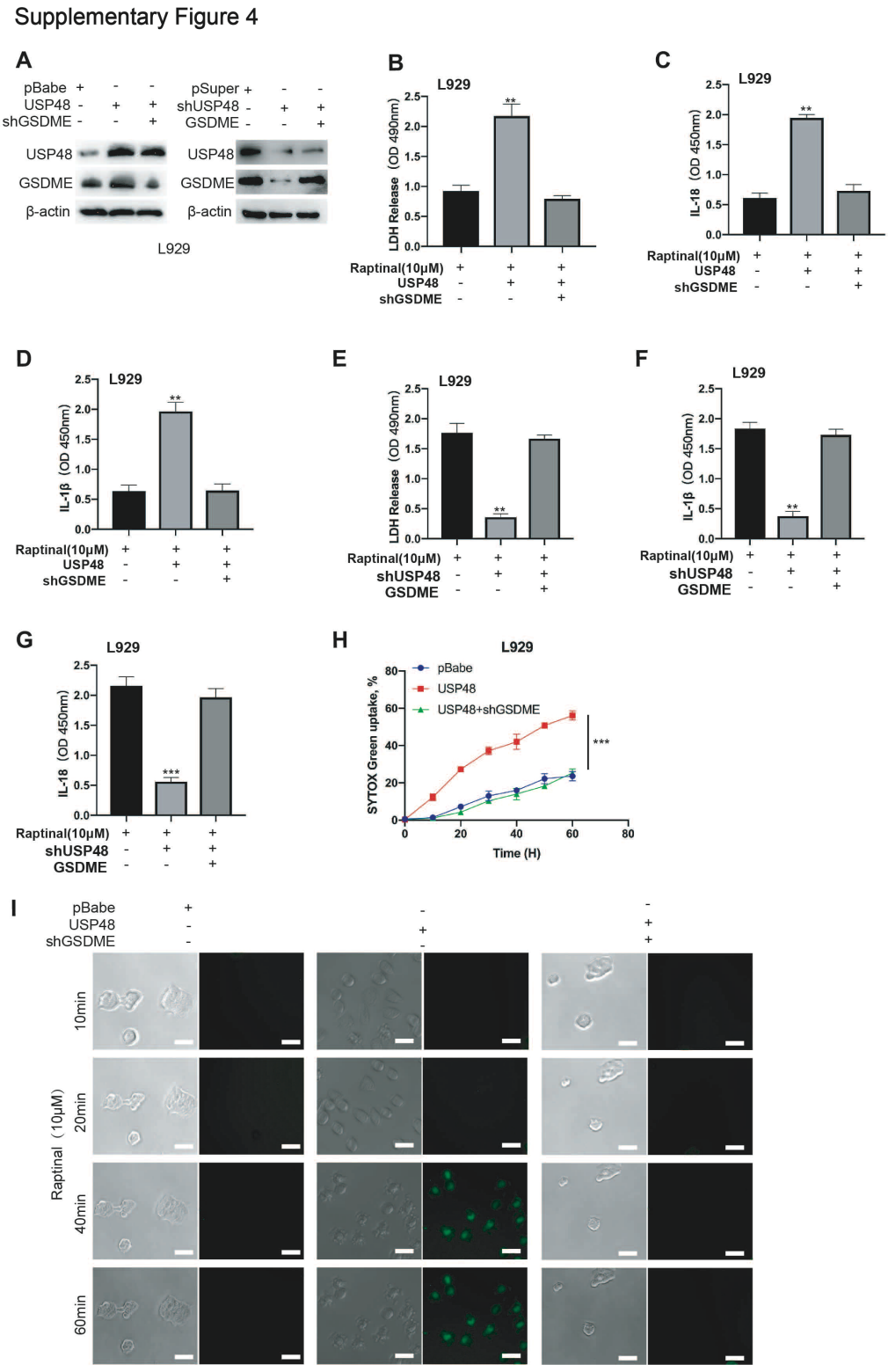
Supplementary Figure 2: Proteomics sequencing analysis

(A) Proteomic sequencing revealed 120 upregulated genes of USP48 and 266 downregulated genes. (B) Heat map of proteins regulated by USP48. (C-D) Functional enrichment analysis.

Supplementary Figure 3



Supplementary Figure 3: USP48 is positively correlated with the expression level of GSDME
(A) Immunohistochemistry to detect the expression of USP48 and GSDME in human liver cancer tissues and adjacent tissues. (B) Western blotting to detect the expression of USP48 and GSDME in human liver cancer tissues and adjacent tissues. (C) Correlation analysis of GSDME and USP48 expression in human liver cancer tissues. (D) Immunohistochemistry to detect the expression of USP48 and GSDME in human PDAC tissues and adjacent tissues.(E) Western blotting to detect the expression of USP48 and GSDME in human PDAC tissues and adjacent tissues. (F) Correlation analysis of GSDME and USP48 expression in human PDAC tissues and adjacent tissues

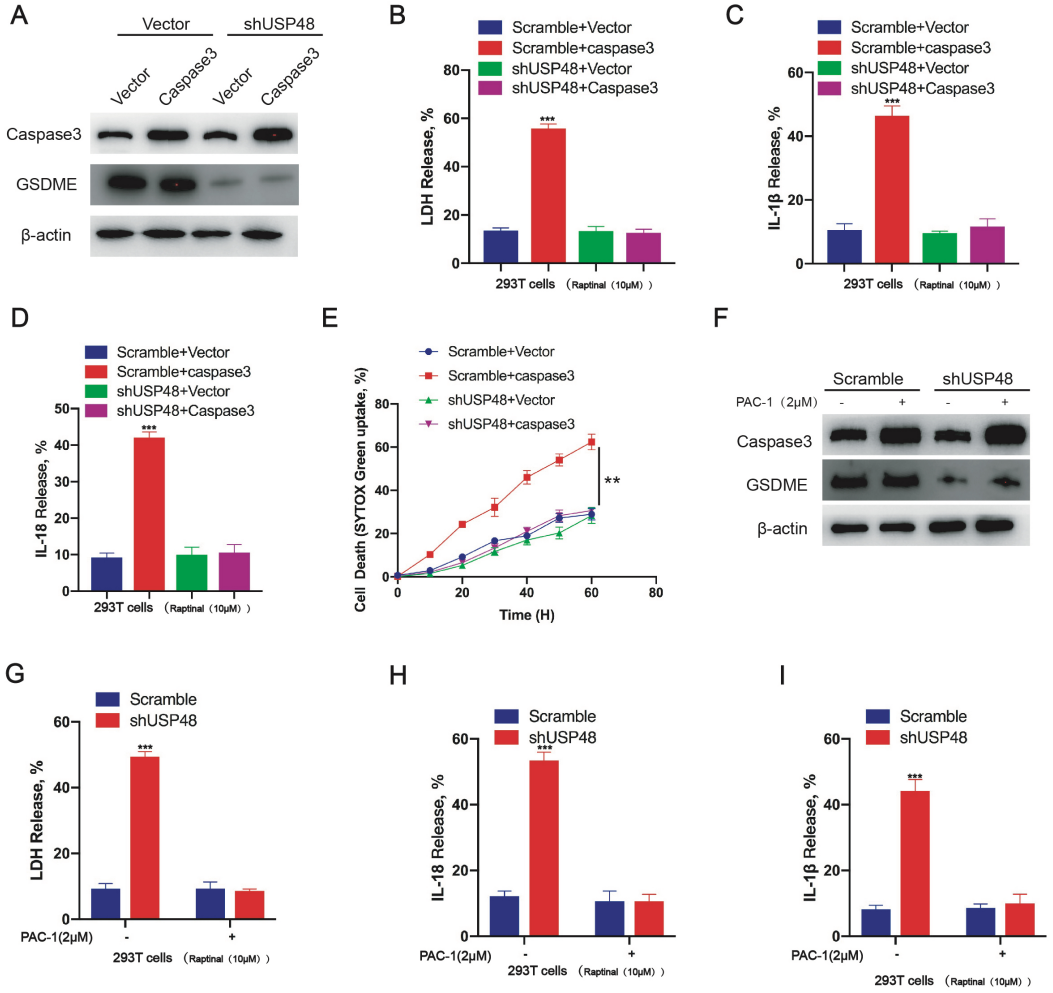


Supplementary Figure 4: Confirm the role of USP48-GSDME in pyroptosis in L929 cells

(A) Western blotting of USP48 and GSDME in L929 cells transfected with different combinations of control vector, USP48 vector, shUSP48 vector, sh-GSDME,and/or GSDME. (B) Measurement of LDH productionin transformed cells described in L929 cells transfected with different combinations of control vector, USP48 vector, and/or sh-GSDME. (C) Measurement of IL-18 productionin transformed cells in L929 cells transfected with different combinations of control

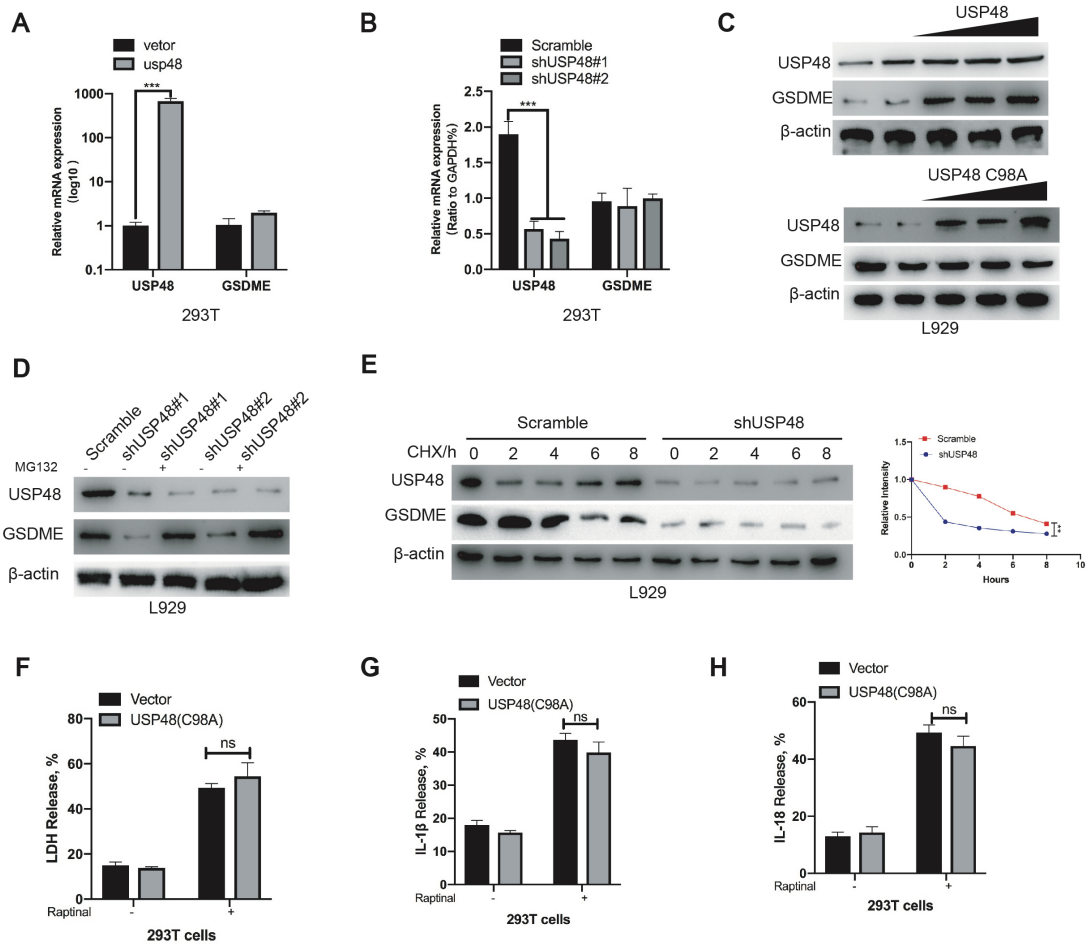
vector, USP48 vector, and/or sh-GSDME. (D) Measurement of IL-1β productionin transformed cells in L929 cells transfected with different combinations of control vector, USP48 vector, and/or sh-GSDME. (E) Measurement of LDH productionin transformed cells in L929 cells transfected with different combinations of control vector, shUSP48 vector, and/or GSDME. (F) Measurement of IL-1β productionin transformed cells in L929 cells transfected with different combinations of control vector, shUSP48 vector, and/or GSDME. (G) Measurement of IL-18 productionin transformed cells in L929 cells transfected with different combinations of control vector, shUSP48 vector, and/or GSDME. (H-I) Time-lapse microscopy observed the morphological changes and the absorption of SYTOX green of L929 cells transfected with different combinations of control vector, USP48 vector, and/or sh-GSDME.

Supplementary Figure 5



Supplementary Figure 5: USP48 regulates pyroptosis in a way that is independent of Caspase3
(A) Western blotting detects the changes of GSDME in cell lysates expressing shUSP48 and Caspase3. (B) Detect the secretion of LDH in 293T cells expressing shUSP48 and Caspase3. (C) Detect the secretion of IL-1β in 293T cells expressing shUSP48 and Caspase3. (D) Detect the secretion of IL-18 in 293T cells expressing shUSP48 and Caspase3. (E) Time-lapse microscopy observed the morphological changes and the absorption of SYTOX green of 293T cells transfected with shUSP48 and Caspase3. (F) Western blotting was used to detect the expression of GSDME in USP48 overexpressed 293T cells treated with Caspase3 inhibitor (PAC1). (G) Detect the secretion of LDH in USP48 overexpressed 293T cells treated with PAC1. (H) Detect the secretion of IL-1β in USP48 overexpressed 293T cells treated with PAC1. (I) Detect the secretion of IL-18 in USP48 overexpressed 293T cells treated with PAC1

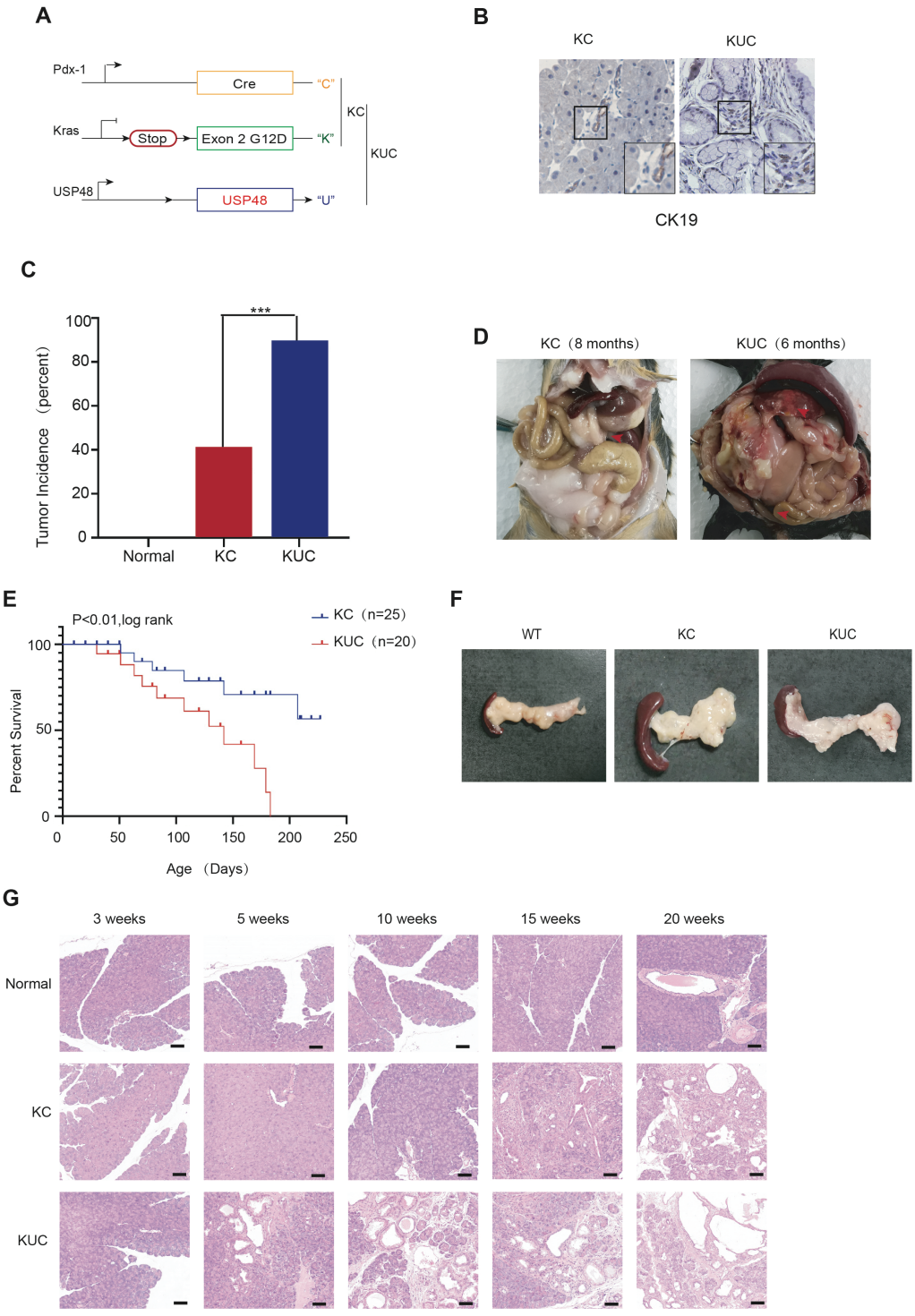
Supplementary Figure 6



Supplementary Figure 6: Clarify the deubiquitination effect of USP48 on GSDME in L929 cells

(A-B) qRT-PCR detection of USP48 expression in 293T cells on the regulation of GSDME. (C) Western blotting to measure USP48 and GSDME protein levels in L929 cells with Dox-inducible expression of wild-type USP48 (USP48/WT) and a catalytically inactive mutant of USP48 (USP48/C98A). (D) In the absence or presence of the proteasome inhibitor MG132 (50μg/ml), the levels of USP48 and GSDME proteins in L929 cells transfected with control or USP48 shRNA were detected by western blotting. (E) Within the specified hours, in the absence or presence of the protein synthesis inhibitor cycloheximide (CHX; 50μg/ml), western blotting was used to detect USP48 and GSDME in L929 cells transfected with control or USP48 shRNA Protein levels. (F) Measurement of LDH productionin transformed cells in 293T cells transfected with vector and USP48(C98A). (G) Measurement of IL-1β productionin transformed cells in 293T cells transfected with vector and USP48(C98A). (H) Measurement of IL-18 productionin transformed cells in 293T cells transfected with vector and USP48(C98A)

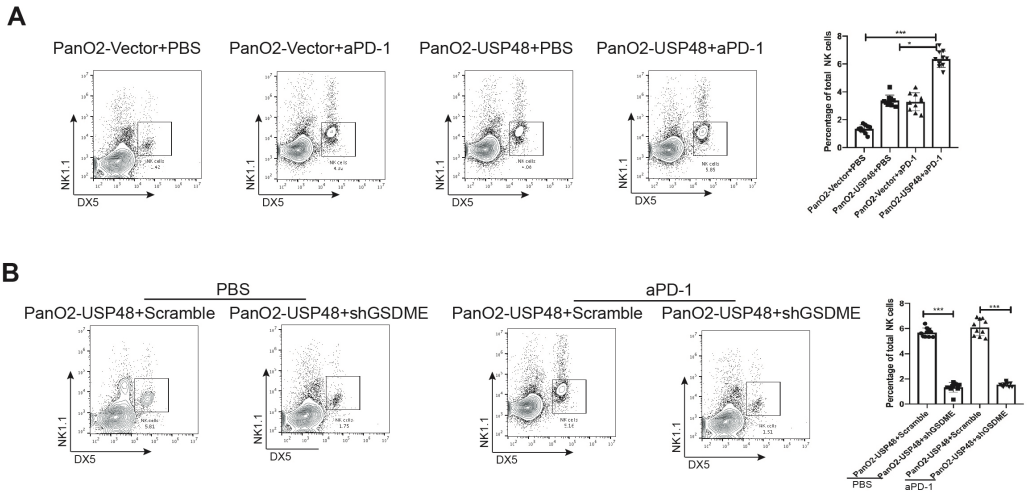
Supplementary Figure 7



Supplementary Figure 7: Loss of USP48 promotes pancreatic cancer in mice

(A) Schematic diagram of PDX-1 Cre; LSL Kras G12D; USP48-/-(KUC) mice. (B) Detect the expression of CK19 by immunohistochemistry. (C) Statistics of tumor incidence in KC and KUC mice. (D) Anatomical pictures of KC and KUC mice. (E) Statistical chart of survival period of KC and KUC mice. (F) Photographs of pancreas in KC, KUC, and wild-type mice. (G) H&E staining of pancreas in KC and KUC mice of different ages.

Supplementary Figure 8



Supplementary Figure 8: USP48-GSDME regulates the proportion of NK cells in tumor tissues

(A-B) Flow cytometry to detect the proportion of NK cells in tumor tissues of mice