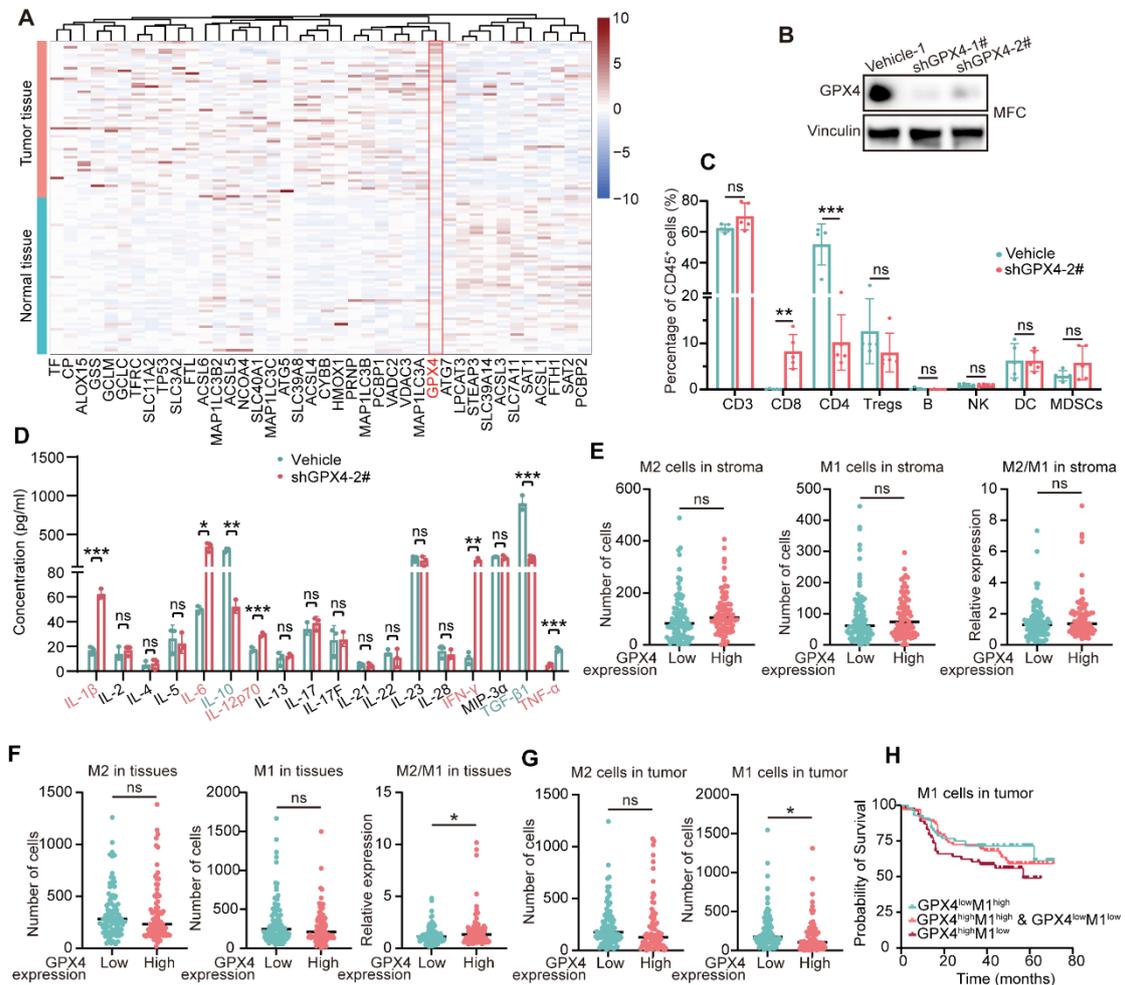
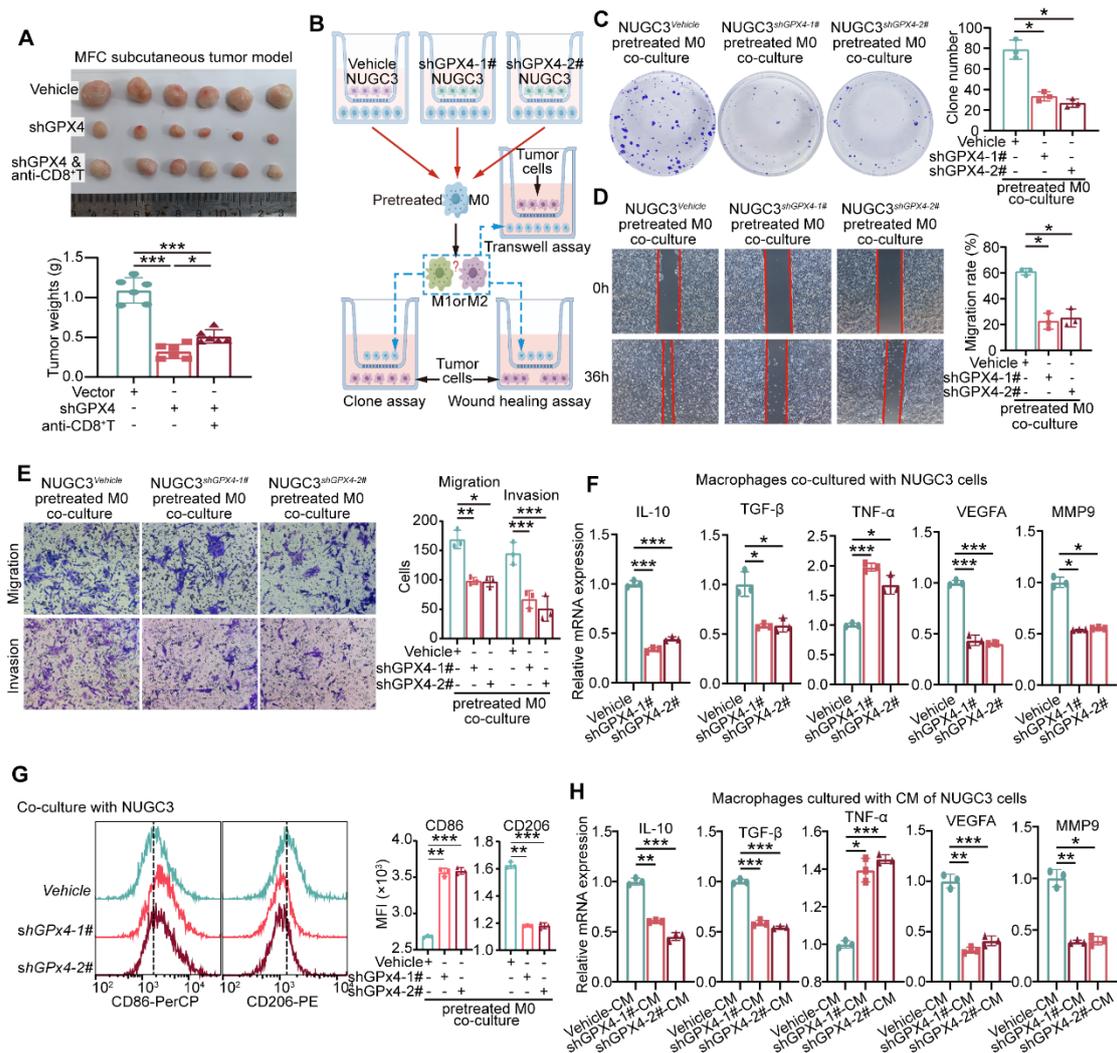


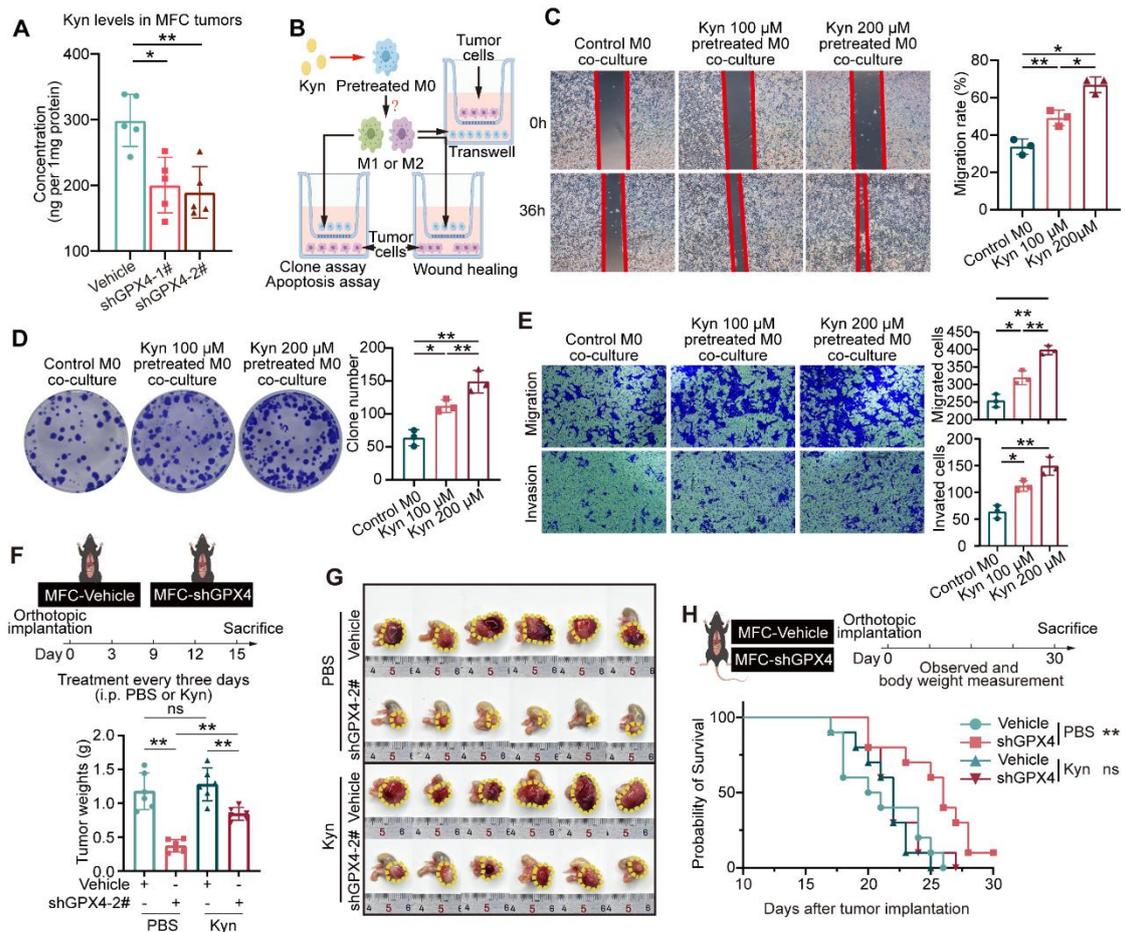
## Supplementary figures



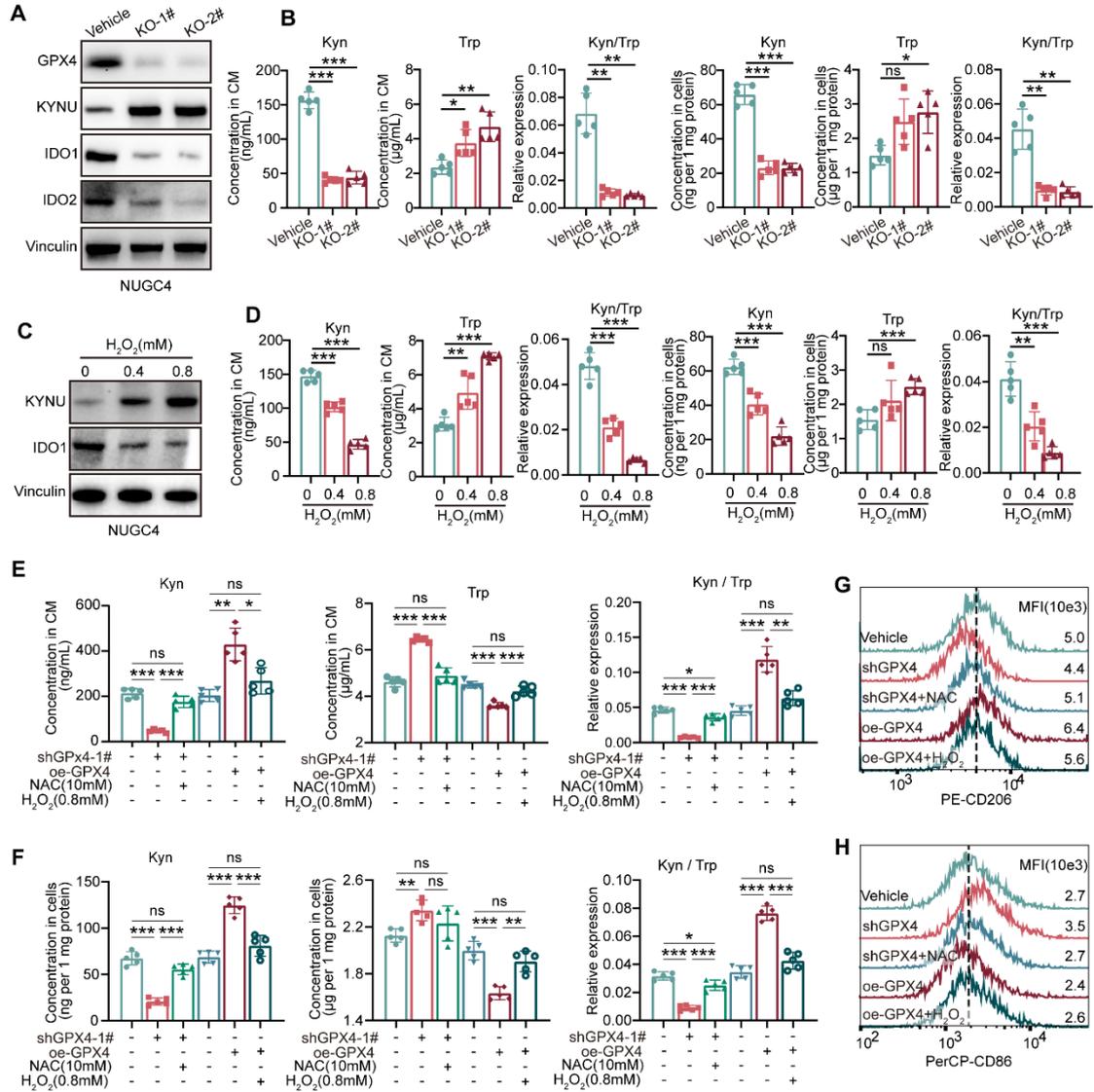
**Figure S1. GPX4 Expression, Macrophage Infiltration, and Prognostic Outcomes in Gastric Cancer.** (A) Heatmap of differentially expressed ferroptosis-related genes, including GPX4, in 60 pairs of GC and adjacent noncancerous tissue samples. (B) GPX4 knockdown efficiency in the murine GC cell line (MFC) assessed by western blot analysis. (C) Differences in immune cell populations, including CD8<sup>+</sup> T cells and CD4<sup>+</sup> T cells, between the GPX4-knockdown and vehicle groups, determined by spectral flow cytometry. (D) Expression differences of 18 cytokines in the peripheral blood of mice between the GPX4-knockdown and vehicle groups, detected by ELISA kit. (E-F) Associations of macrophage infiltration in the stromal compartment and entire tissue sections with tumor GPX4 expression levels, determined by tissue microarray analysis and multiplex immunohistochemistry. (G) Multiplex immunohistochemistry was performed to examine the relationship between GPX4 expression and M2 or M1 macrophage infiltration within the tumor tissue. (H) Prognostic analysis based on the associations of M1 macrophage levels with GPX4 expression in tumor tissues, assessing their combined impact on patient survival outcomes.



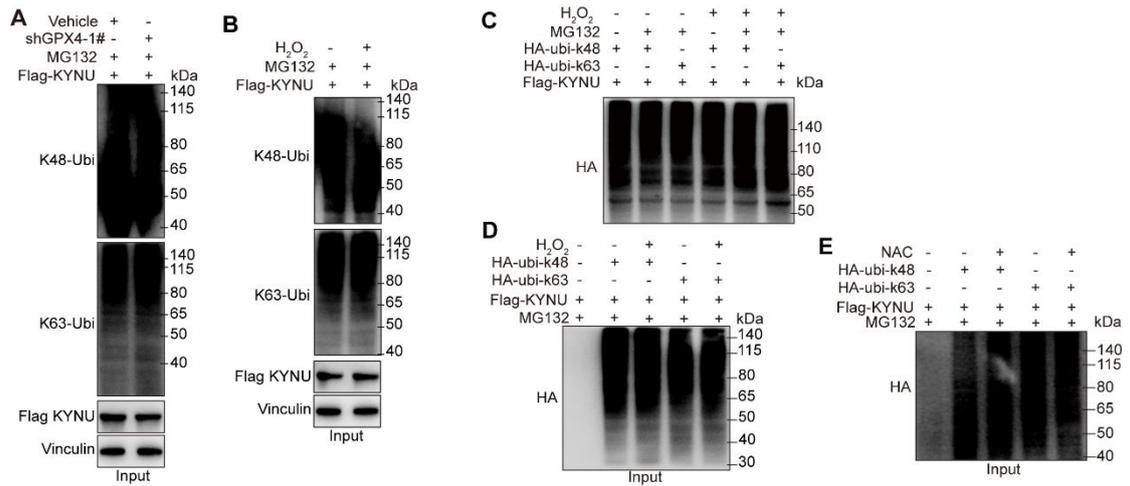
**Figure S2. Impacts of GPX4 Knockdown on Macrophage Polarization and Tumor Cell Behavior in Gastric Cancer.** (A) The MFC subcutaneous tumor model displays representative images and tumor weights from the vehicle group, GPX4 knockdown group, and GPX4 knockdown combined with anti-CD8<sup>+</sup> T antibody treatment group. (B) Schematic diagram for investigating macrophage polarization changes and their functional impact. THP-1 cells, pretreated with 320 nM PMA for 6 h, were cultured with vehicle or GPX4-knockdown NUGC3 cells for 48 h. (C-E) Colony formation (C), wound-healing (D), and Transwell (E) assays in pretreated THP-1 cells co-cultured with GC cells. (F-G) RT-qPCR and flow cytometry analyses of IL-10, TGF- $\beta$ , TNF- $\alpha$ , VEGFA, MMP9, as well as CD86 and CD206 expression in THP-1-derived macrophages after 48 h of co-culture with control NUGC3 cells and GPX4-knockdown NUGC3 cells. (H) RT-qPCR analysis of IL-10, TGF- $\beta$ , TNF- $\alpha$ , VEGFA, and MMP9 expression levels in THP-1-derived macrophages after 48 h of culture with conditioned media from control NUGC3 cells and GPX4-knockdown NUGC3 cells.



**Figure S3. Effects of Kynurenine on Macrophage Function and Tumor Growth Dynamics.** (A) Kynurenine levels in tumor tissues from GPX4-knockdown and vehicle MFC tumor-bearing mice. (B) Schematic diagram illustrating the investigation of macrophage polarization changes and their functional impact. THP-1 cells, pretreated with 320 nM PMA for 6 h, were exposed to varying concentrations of kynurenine for 48 h. (C-E) Wound-healing (C), colony formation (D), and Transwell (E) assays in pretreated THP-1 cells subsequently co-cultured with NUGC3 cells. (E) Schematic representation of kynurenine treatment in mice to evaluate its role in GPX4 knockdown-induced tumor suppression. (F-G) Experimental setup showing the effects of GPX4 knockdown combined with kynurenine on tumor growth, as assessed by tumor weight and representative images, in an orthotopic GC mouse model. (H) Design of the orthotopic mouse model used to assess the survival benefits of GPX4 knockdown and the impact of kynurenine on survival outcomes.



**Figure S4. Role of ROS in GPX4-mediated kynurenine metabolism regulation and macrophage polarization.** (A-B) GPX4 was knocked out in gastric cancer NUGC4 cells using sgRNA. Subsequently, the expression levels of KYNU, IDO1, and IDO2 proteins were measured, along with tryptophan, kynurenine, and the kynurenine/tryptophan ratio in both the culture supernatant and within the cells. (C-D) After treating NUGC4 cells with H<sub>2</sub>O<sub>2</sub> for 6 h, the expressions of KYNU and IDO1, as well as the levels of tryptophan, kynurenine, and their ratio, were analyzed. (E-F). After treating GPX4-knockdown NUGC3 cells with NAC (10nM) for 24 h and GPX4-overexpression NUGC3 cells with H<sub>2</sub>O<sub>2</sub> (0.8mM) for 6 h, levels of kynurenine, tryptophan, and the kynurenine/tryptophan ratio were measured in conditioned medium and cells. (G-H) PMA-differentiated THP1 cells were treated with the above conditioned medium for 48 h, and flow cytometry was used to analyze CD206 and CD86 expressions.



**Figure S5. Co-IP Input Corresponding to the Experiments in Panels G (A), H (B), J (C), K (D), and L (E) of Figure 6.**