

Glycolytic Reprogramming Mediated by the OTUD4–FXR1–HIF1A Axis Drives Gastric Cancer Progression

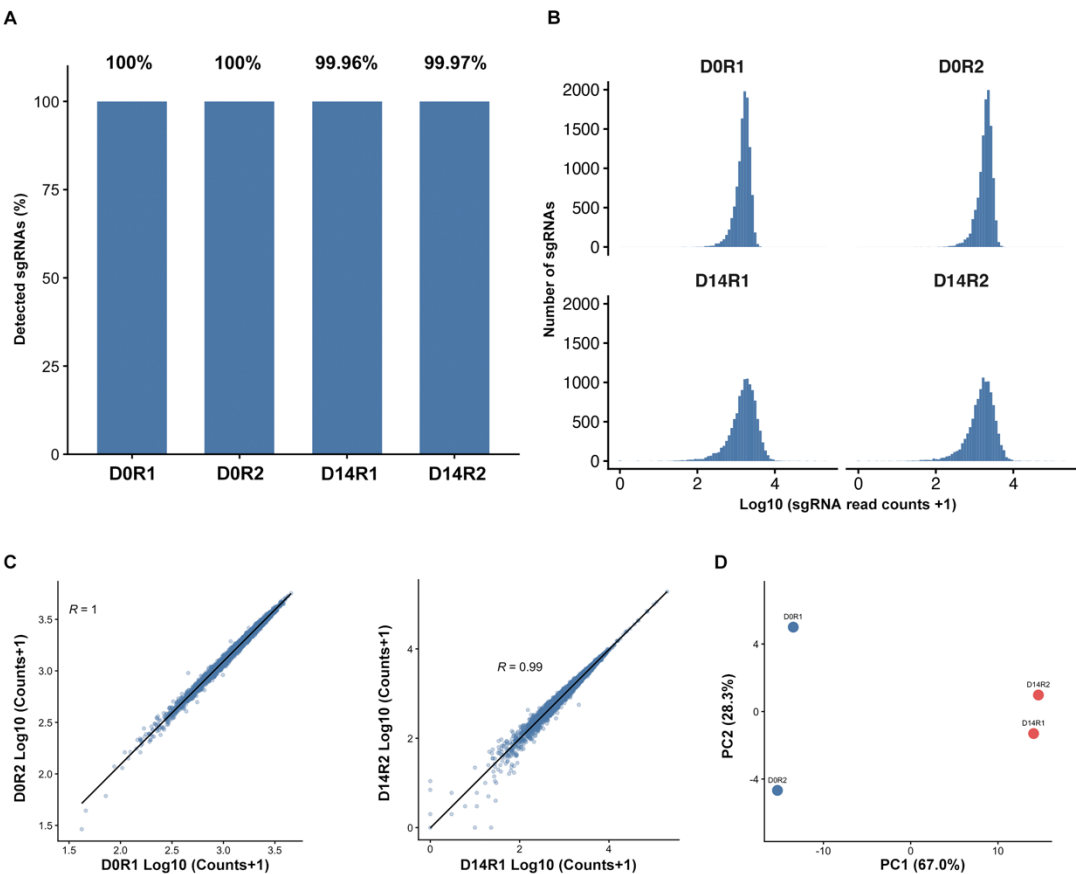


Fig. S1 Quality control of the CRISPR screening library. A Percentage of detected sgRNAs across samples collected at day 0 and day 14, including two biological replicates at each time point (D0R1, D0R2, D14R1, and D14R2). **B** sgRNA read count distribution across the indicated samples. **C** Correlation analysis between biological replicates. **D** Principal component analysis of the indicated samples.

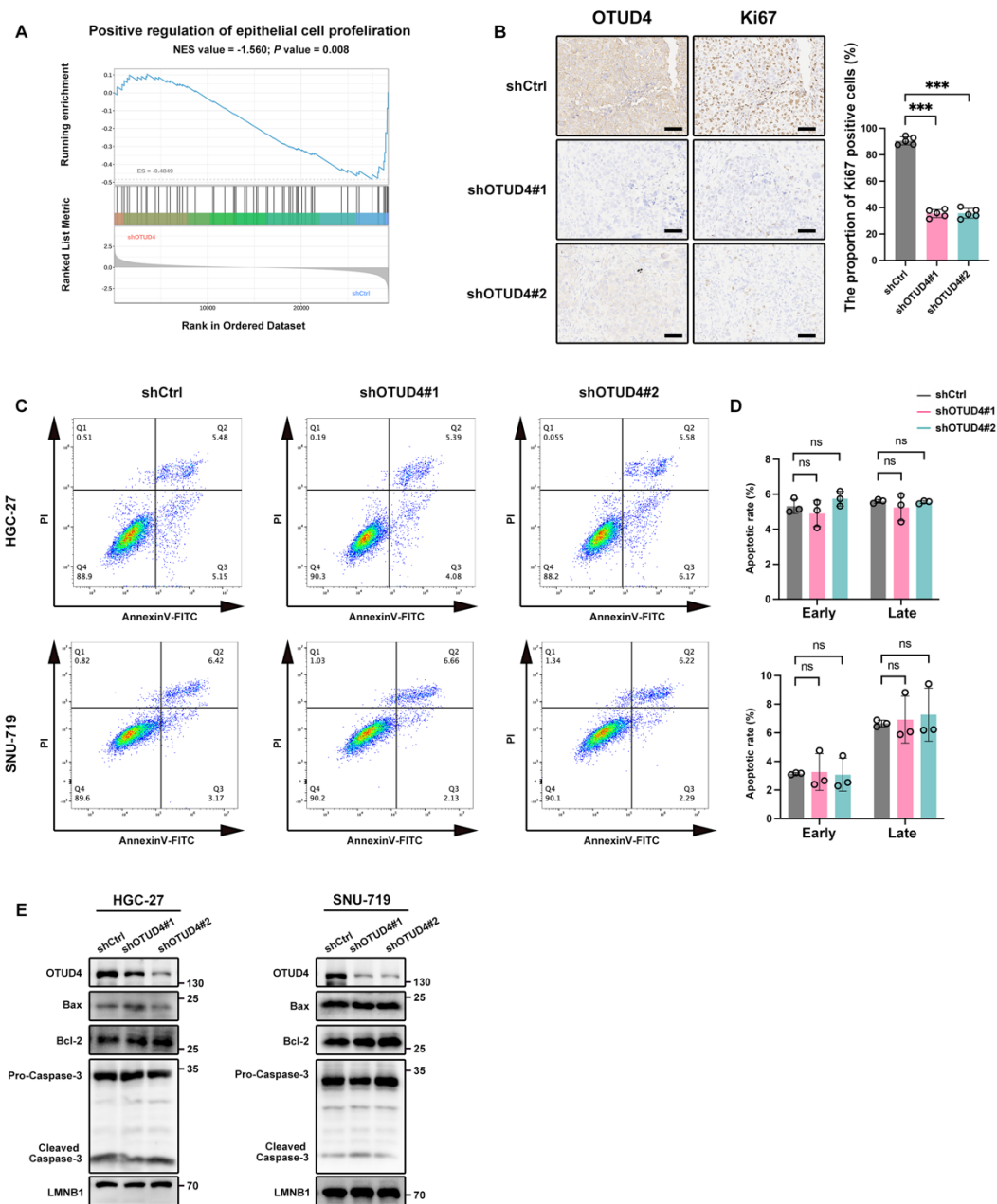
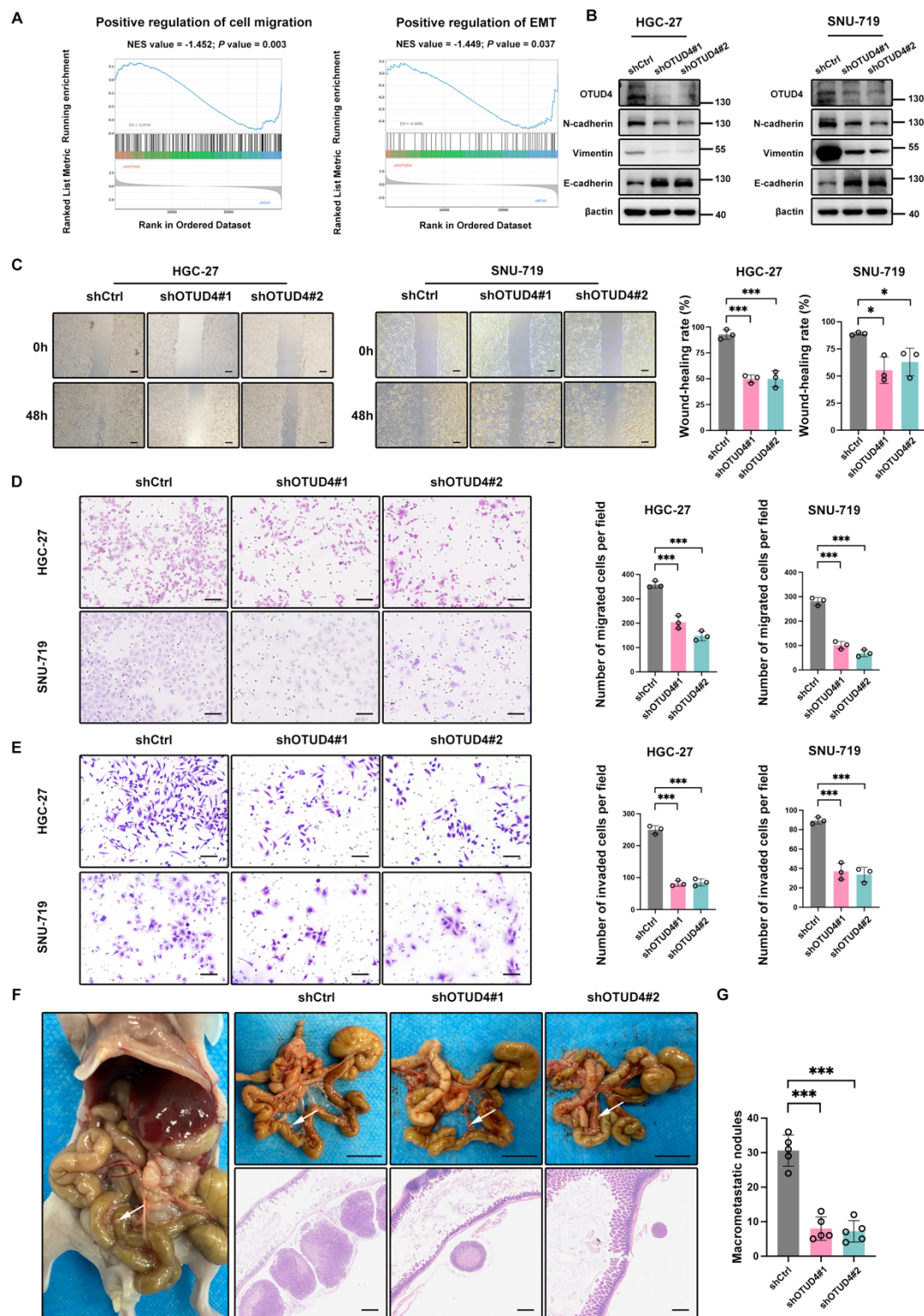


Fig.S2 OTUD4 enhances gastric cancer cell proliferation without influencing apoptosis. **A** GSEA analysis shows a reduction in epithelial cell proliferation following OTUD4 knockdown. **B** Representative IHC images and quantitative analysis of Ki67 expression in OTUD4 knockdown and control subcutaneous xenografts tumor (n=5). Scale bar = 50µm. **C** Flow cytometric analysis of apoptosis in the OTUD4 knockdown

16 compared with control cells (n=3). **D** Quantification of apoptotic rates in the indicated
17 groups by flow cytometry (n=3). **E** WB analysis of apoptotic-related protein Bax, BCL-
18 2 and Caspase-3 in the indicated groups.
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21 **Fig.S3 OTUD4 promotes gastric cancer migration and invasion.** A GSEA analysis

22 indicated decreased cell migration (left) and EMT level (right) in SNU-719 cells

23 following OTUD4 knockdown. B WB analysis revealed altered expression of EMT-

24 related proteins, including N-cadherin, Vimentin, and E-cadherin, in OTUD4-
25 knockdown HGC-27 and SNU-719 cells compared with control cells. **C** Representative
26 images and quantification of OTUD4 knockdown and control GC cells in the wound-
27 healing migration assays (n=3). Scale bar = 1mm. **D** Representative images and
28 quantification of the indicated groups in the Transwell migration assays (n=3). Scale
29 bar = 100μm. **E** Representative images and quantification of the indicated groups in the
30 Transwell invasion assays (n=3). Scale bar = 100μm. **F** Representative images (upper)
31 and hematoxylin and eosin (HE) staining (lower) of mesenteric metastatic nodules
32 (arrows). Upper scale bar = 10mm, Lower scale bar = 500μm. **G** Quantitative analysis
33 of macro metastatic nodules in the indicated groups (n=5).

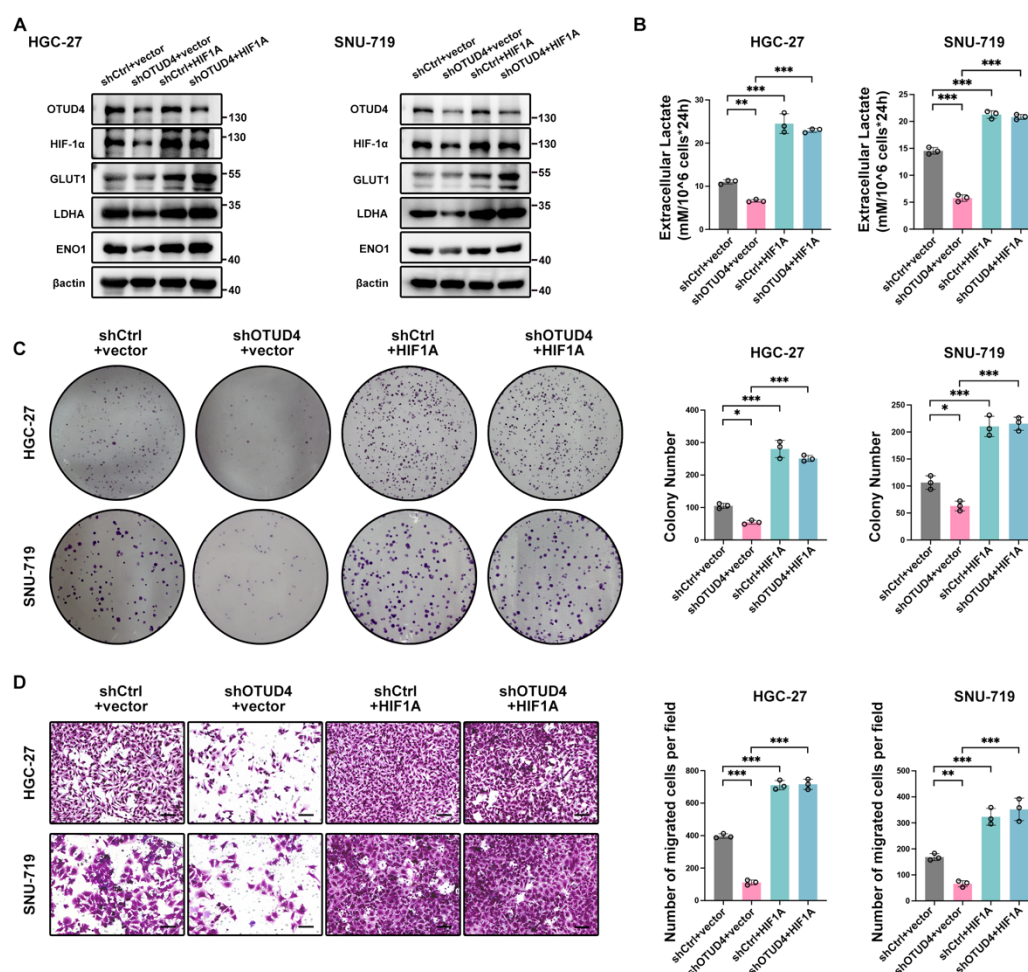
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36 **Fig.S4 OTUD4 enhances glycolysis in GC.** KEGG analysis of top 30 *P* value of GSEA

37 enrichment.

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40 **Fig.S5 HIF1A overexpression rescues glycolysis and malignant phenotypes**

41 **suppressed by OTUD4 knockdown. A** WB analysis of glycolysis related protein

42 GLUT1, LDHA and ENO1 in OTUD4-knockdown HGC-27 and SNU-719 cells

43 following transfection with HIF1A constructs or empty vector. **B** Extracellular lactate

44 level in the indicated groups. **C** Representative images of colony formation assays and

45 quantification of colony numbers in the indicated groups. **D** Representative images and

46 quantification of Transwell migration assays in the indicated groups (n=3). Scale bar =

47 100μm.

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49 **Fig.S6 OTUD4 stabilizes FXR1 by mediating its deubiquitination. A** Co-IP assays
50 showed that OTUD4 did not interact with HIF-1 α in HGC-27 and SNU-719 cells. **B**
51 Immunoblotting revealed that the N-terminal region (amino acids 1–245) of OTUD4
52 mediates its interaction with FXR1.

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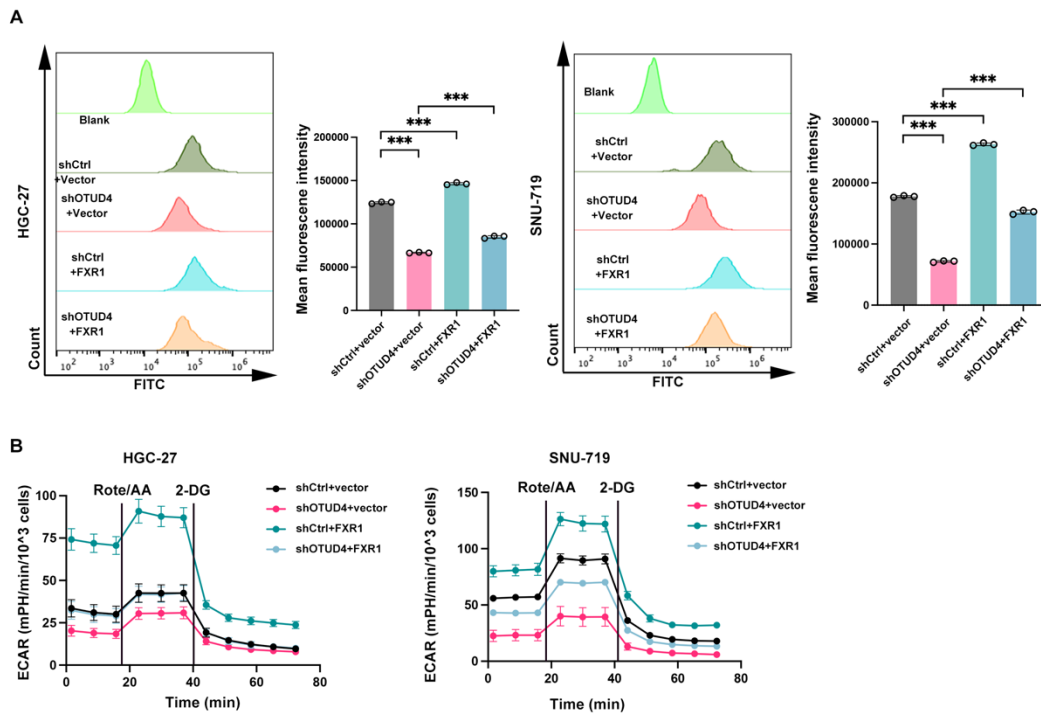


Fig.S7 OTUD4 enhances glycolysis in a FXR1-dependent manner. **A** Flow cytometric analysis of glucose uptake level in OTUD4-knockdown HGC-27 and SNU-719 cells following transfection with FXR1 constructs or empty vector, quantified as mean fluorescence intensity (MFI, n=3). **B** Seahorse analysis of ECAR in the indicated groups.

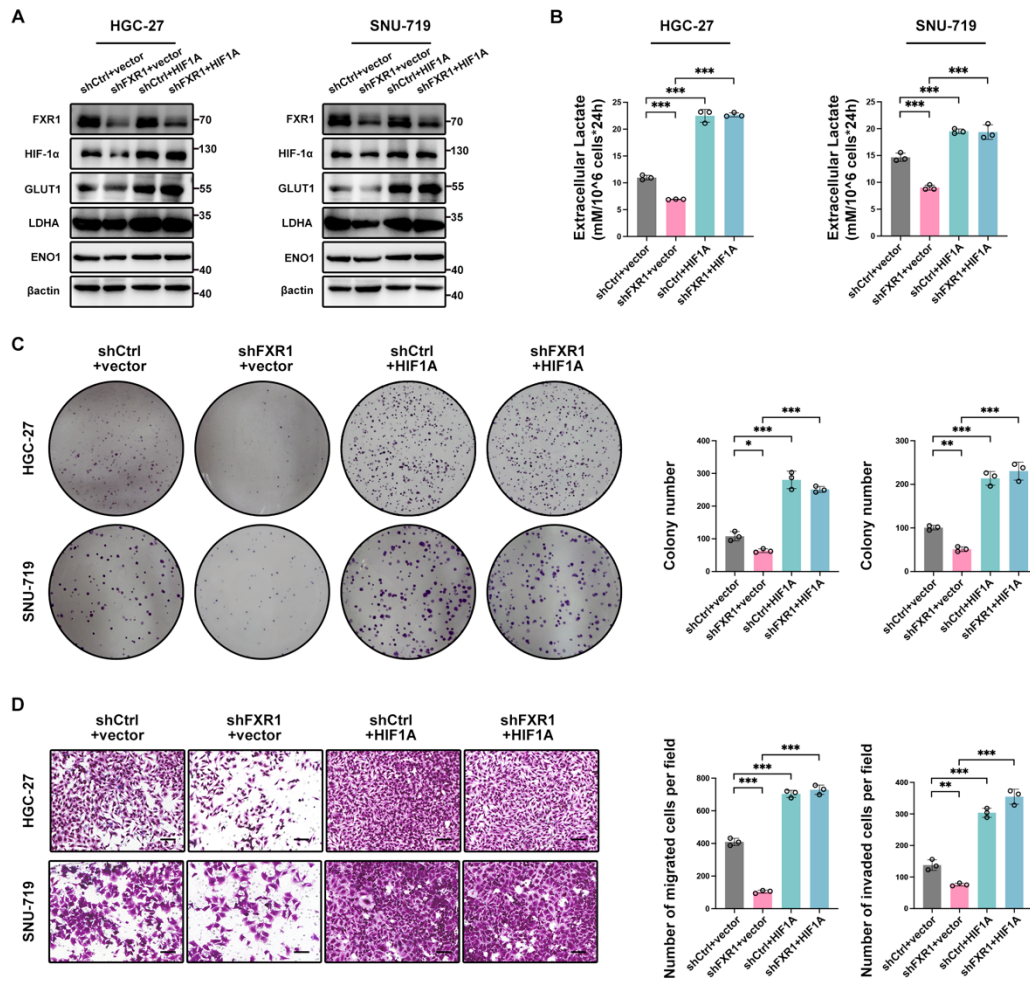
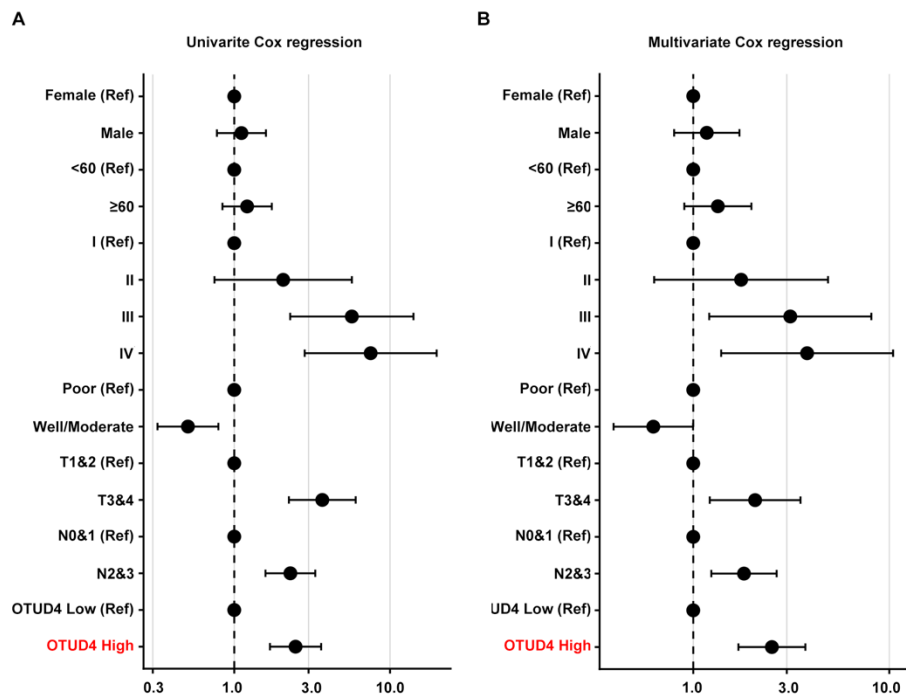


Fig.S8 HIF1A overexpression rescues glycolysis and malignant phenotypes suppressed by FXR1 knockdown. **A** WB analysis of glycolysis related protein GLUT1, LDHA and ENO1 in FXR1-knockdown HGC-27 and SNU-719 cells following transfection with HIF1A constructs or empty vector. **B** Extracellular lactate level in the indicated groups. **C** Representative images of colony formation assays and quantification of colony numbers in the indicated groups. **D** Representative images and quantification of Transwell migration assays in the indicated groups (n=3). Scale bar = 100μm.



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72 **Fig.S9. Univariate and multivariate Cox regression analyses of overall survival in**
73 **patients with gastric cancer. A** Univariate Cox regression analysis of OTUD4
74 expression and clinicopathological variables. **B** Multivariate Cox regression analysis of
75 OTUD4 expression and clinicopathological variables. Forest plots show hazard ratios
76 (HRs), 95% confidence intervals (CIs).