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Supplemental information

OGT/HIF-2α axis promotes the progression of clear cell renal cell carcinoma and regulates its sensitivity to ferroptosis

Zhou Yang, Xiyi Wei, Chengjian Ji, Xiaohan Ren, Wei Su, Yichun Wang, Jingwan Zhou, Zheng Zhao, Pengcheng Zhou, Kejie Zhao, Bing Yao, Ninghong Song, and Chao Qin

File list:

1. Figure S1
2. Figure S2
3. Figure S3

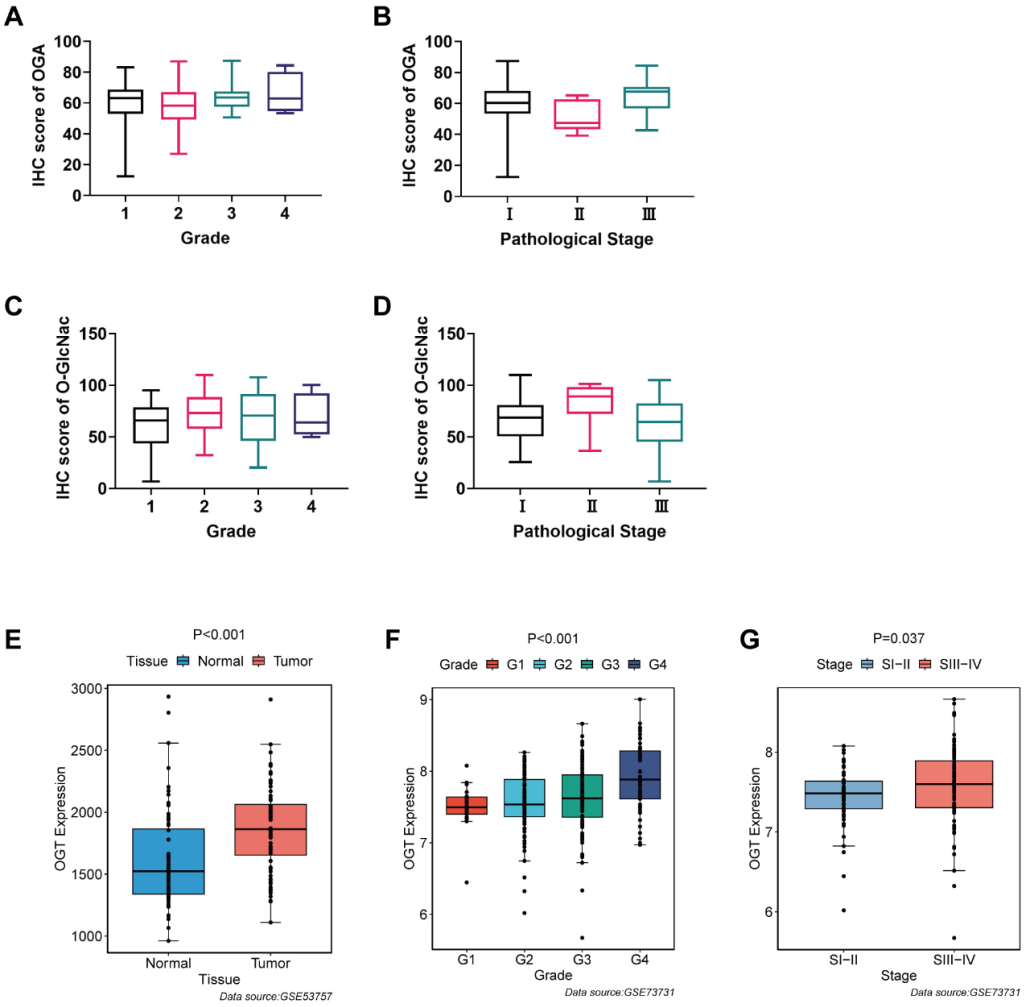

Figure S1. Expression of OGA and O-GlcNac in ccRCC, related to Figure 1. A. B. The expression of OGA in different grades (A) and pathological stages of ccRCC. C. D. The expression of O-GlcNac in different grades (C) and pathological stages (D) of ccRCC. E. The expression of OGT in ccRCC (n=72) and normal renal tissues (n=72) from GSE53757. F. G. The expression of OGT in different grades (F) and pathological stages (G) of ccRCC from GSE73731 (Grade 1: n=22, Grade 2: n=90, Grade 3: n=95, Grade 4: n=49); (Stage I-II: n=53, Stage III-IV: n=72). Statistical analysis of the data from 2 groups was performed using Student's t test. Comparisons among multiple groups were performed by one-way ANOVA followed by Fisher's LSD test. Data are represented as mean +/- SD.

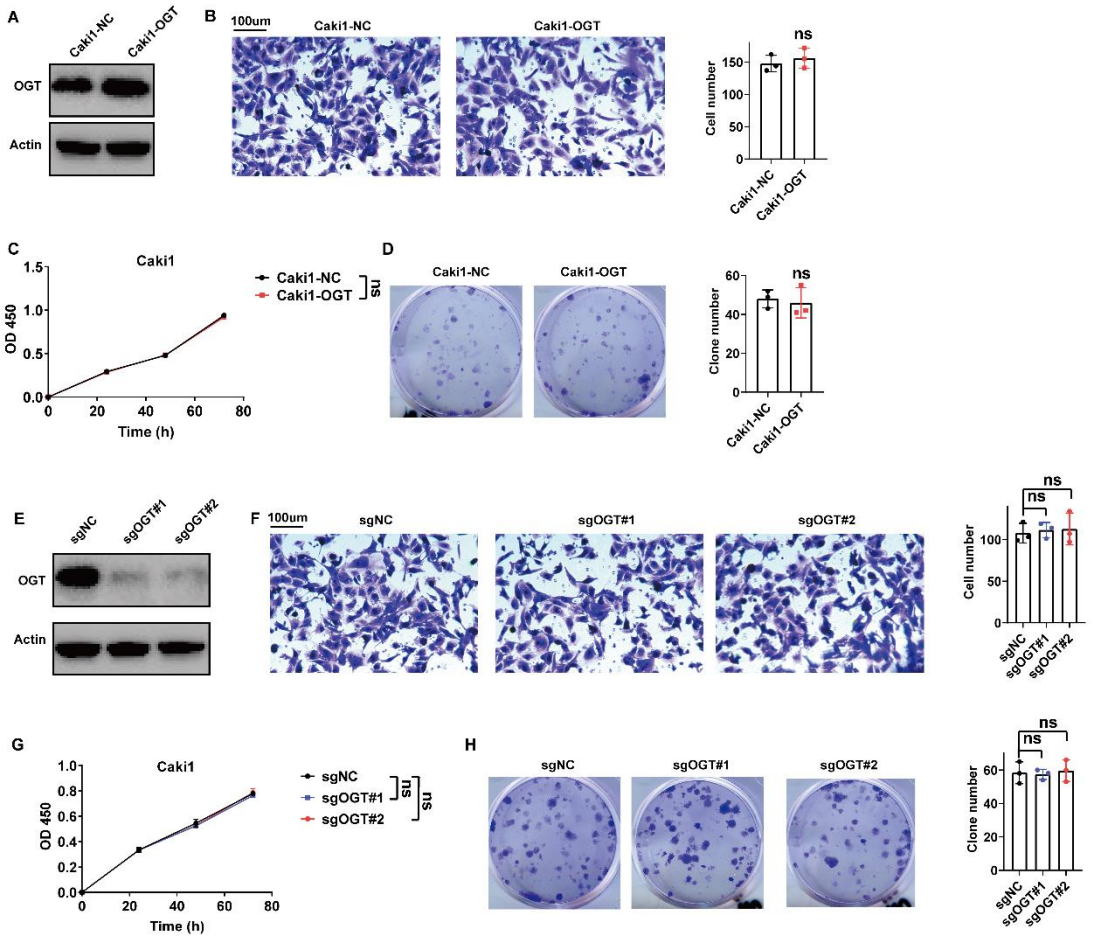

Figure S2. OGT shows no effect at Caki-1, related to Figure 2. A. Overexpression of OGT in Caki1 cells validated by Western Blotting analysis. B-D. The invasion (B), proliferation (C), and clone formation (D) of Caki1-NC (Caki1 negative control) and Caki1-OGT (OGT overexpressed Caki1). Scale bar, 100 μm. E. Knockout of OGT in Caki1 cells validated by Western Blotting analysis. F-H. The invasion (F), proliferation (G), and clone formation (H) of OGT-depleted and control Caki1 cells. Scale bar, 100 μm. Statistical analysis of the data from 2 groups was performed using Student's t test. Comparisons among multiple groups were performed by one-way ANOVA followed by Fisher's LSD test. Data are represented as mean +/- SD. (*ns*: no significance)

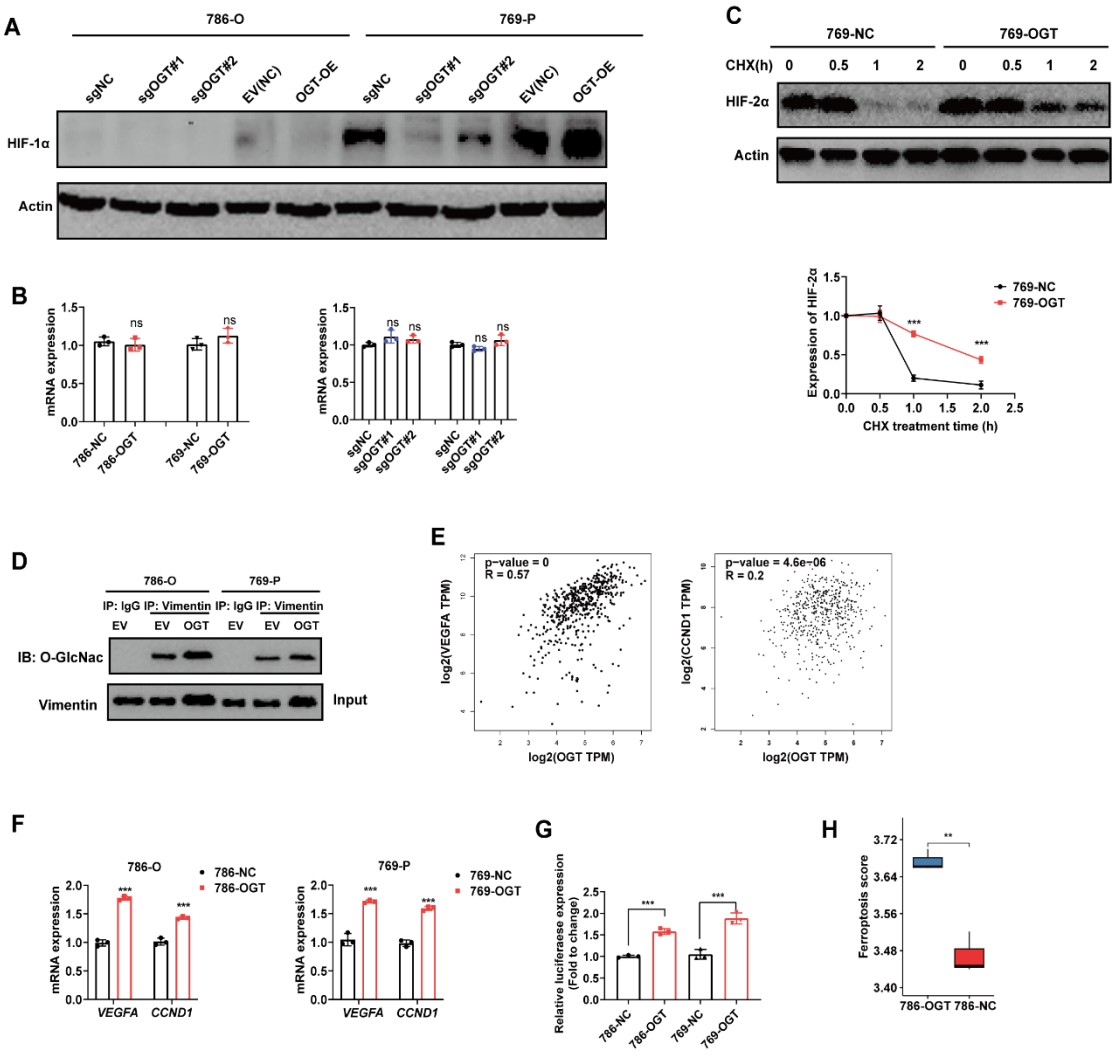

Figure S3. OGT regulates proteasomal degradation of HIF-2α by direct binding, related to Figure 3. A. The expression of HIF-1α in 786-O and 769-P cells. B. The mRNA level of HIF-2α measured by RT-qPCR. C. Overexpression of OGT increased the protein stability of HIF-2α. Protein synthesis inhibitor Cycloheximide (CHX, 20μg/ml). D. O-GlcNac modification of Vimentin in 786-O cells detected by Co-IP analysis. E. Correlation between OGT and HIF-2α targeted genes at the transcriptional level in the TCGA database (TPM: transcripts per million). F. Overexpression of OGT increased the mRNA level of HIF-2α targeted genes (*VEGFA* and *CCND1*). G. Overexpression of OGT enhanced the transcripti onal activity of HIF-2α. H. Ferroptosis

score of 786-OGT and 786-NC cells analyzed by ssGSEA algorithm. Data are represented as mean +/- SD. Statistical analysis of the data from 2 groups was performed using Student's t test. Comparisons among multiple groups were performed by one-way ANOVA followed by Fisher's LSD test. (***P<0.001, *ns*: no significance)