



### **Supplementary Information Appendix**

UDP-glucose pyrophosphorylase 2, a regulator of glycogen synthesis and glycosylation, is critical for pancreatic cancer growth

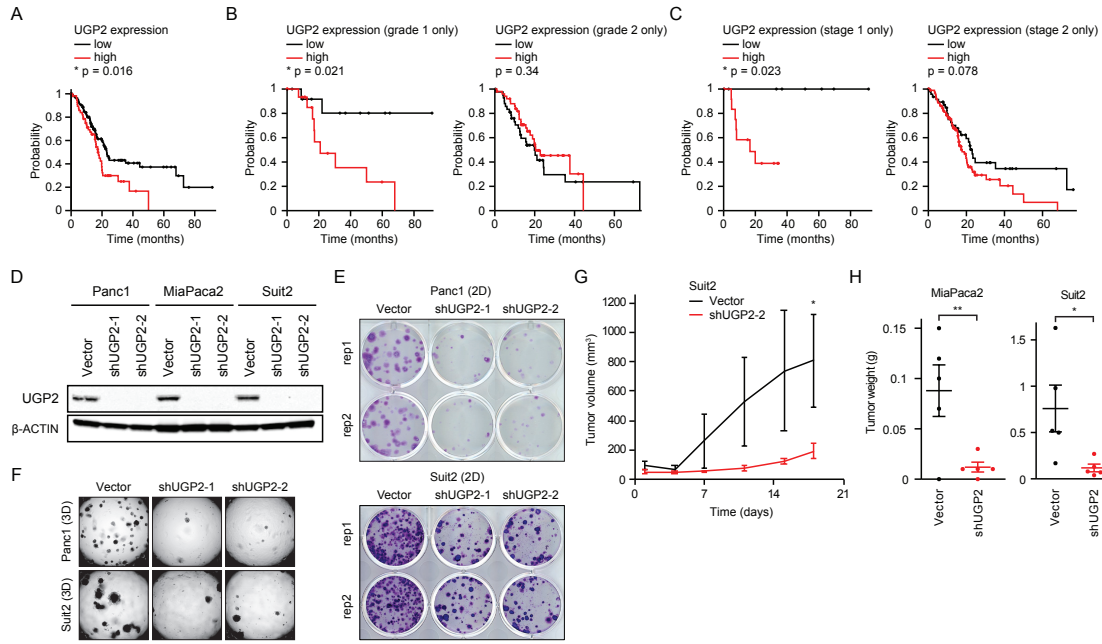
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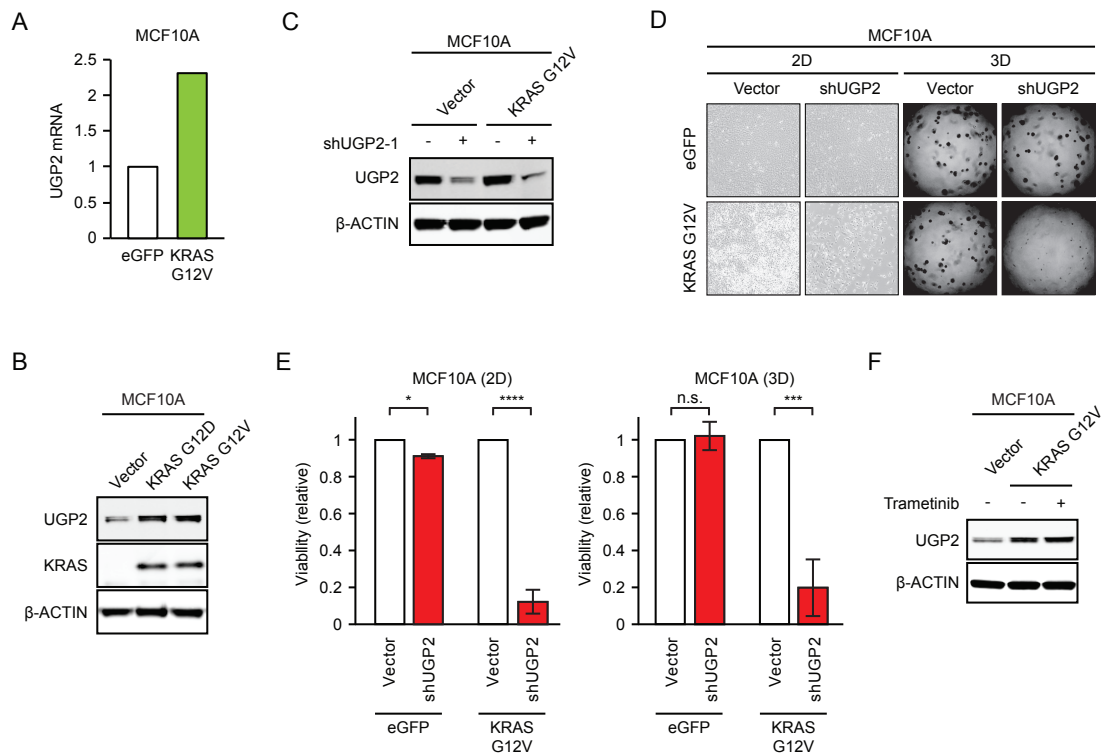
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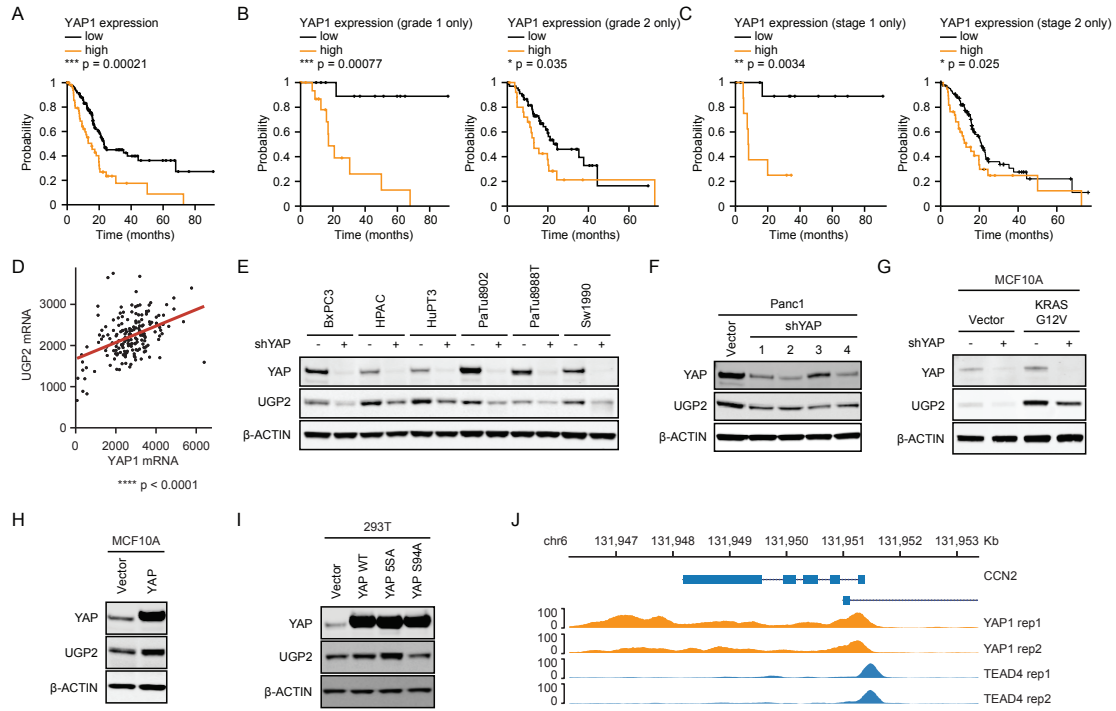
This file includes Supplementary Figures S1-S5, Table S1, and legends.



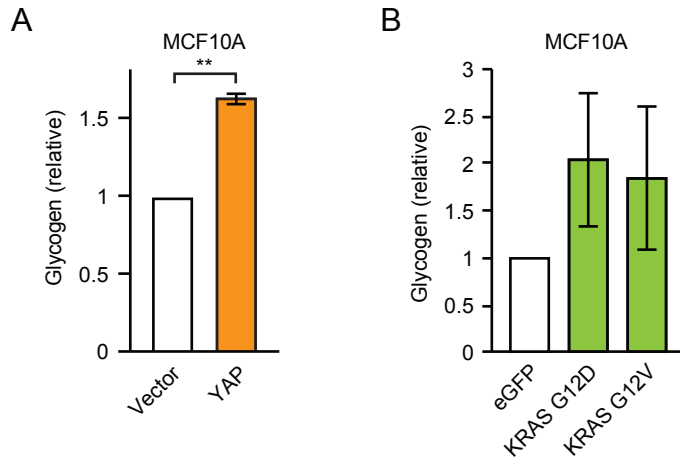
**Figure S1. A-C**, Cumulative probability of survival of 177 patients with pancreatic ductal adenocarcinoma based on expression level of UGP2 for all patients (A), grade 1 (B, left), grade 2 (B, right), stage 1 (C, left), and stage 2 (C, right). Data adapted from cBioPortal and KM Plotter (12). **D**, Immunoblots for shRNAs knocking down UGP2 in Panc1, MiaPaca2, and Suit2 cells, probed as indicated. **E**, Representative crystal violet staining of Panc1 and Suit2 cells with two independent shRNAs against UGP2 or empty vector control grown in two-dimensional culture for 10 days. **F**, Representative images of Panc1 and Suit2 cells stably expressing shUGP2 or empty vector control grown in three-dimensional matrigel for 14 days. **G**, Tumor volumes of Suit2 cells with a second shRNA against UGP2 or empty vector control xenografted on opposite flanks of nude mice,  $n = 5$ ,  $* p < 0.05$ . Error bars represent SEM. **H**, Tumor weights at endpoint of MiaPaca2 and Suit2 cells with shUGP2 or empty vector control xenografted on opposite flanks of nude mice,  $n = 4$ ,  $* p < 0.05$ ,  $** p < 0.01$ .



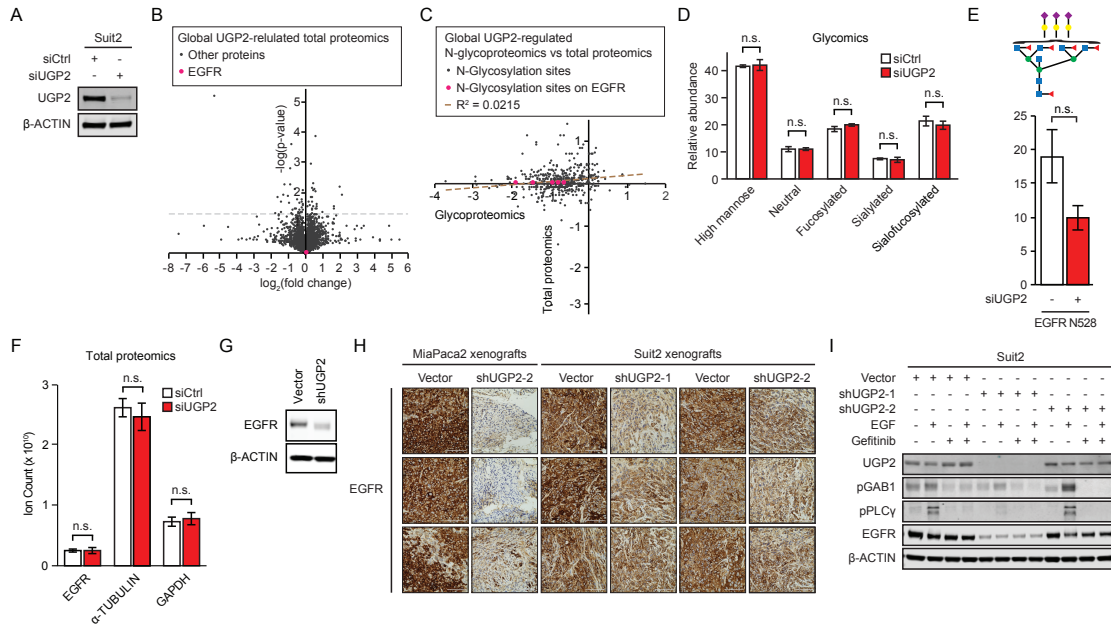
**Figure S2. A**, mRNA expression of UGP2 from RNAseq on MCF10A cells stably expressing KRAS G12V or empty vector. **B**, Immunoblot of lysates from MCF10A cells stably expressing KRAS G12D, KRAS G12V, or empty vector. **C**, Immunoblot of lysates from MCF10A cells expressing KRAS G12V or empty vector with or without knockdown of UGP2. **D-E**, Representative images (D) and relative viability (E) of MCF10A cells expressing KRAS G12V or empty vector with or without UGP2 knockdown grown in 2D or 3D matrigel for 14 days,  $n = 3$ . Error bars represent SEM,  $n = 3$ . \*  $p < 0.05$ , \*\*\*  $p < 0.001$ , \*\*\*\*  $p < 0.0001$ . **F**, Immunoblot of lysates from MCF10A cells stably expressing KRAS G12V or empty vector treated with 100 nM trametinib for 48 hours, probed as indicated.



**Figure S3. A-C**, Cumulative probability of survival of 177 patients with pancreatic ductal adenocarcinoma based on expression level of YAP1 for all patients (A), grade 1 (B, left), grade 2 (B, right), stage 1 (C, left), and stage 2 (C, right). Data adapted from cBioPortal and KM Plotter (12). **D**, Correlation between UGP2 and YAP1 mRNA level in PDAC patient samples. Analysis performed using cBioPortal,  $n = 178$ ,  $R^2 = 0.18$ , Pearson = 0.43,  $p = 3 \times 10^{-9}$ . **E**, Immunoblots on a panel of PDAC cell lines cells with shRNAs against YAP or vector control, probed as indicated. **F**, Immunoblots of Panc1 with four different YAP shRNAs, probed as indicated. **G**, Immunoblots of MCF10A stably expressing KRAS G12V or control vector with knockdown of YAP, probed as indicated. **H-I**, Immunoblots on lysates from MCF10A (H) and 293T (I) cells stably expressing wild-type YAP, YAP 5SA, YAP S94A, or empty vector, probed as indicated. **J**, ChIP-seq for the control YAP1/TEAD4 binding site in the CCN2 genomic region.



**Figure S4. A**, Relative glycogen levels in MCF10A cells expressing YAP or empty vector control, \*\*  $p < 0.01$ . **B**, Relative glycogen levels in MCF10A cells expressing KRAS G12D or G12V or empty vector control.



**Figure S5.** **A**, Immunoblots on lysates of Suit2 cells transfected with siUGP2 or non-targeting control siRNAs for 48 hours, probed as indicated. **B**, Global total proteomic analysis in Suit2 cells upon knockdown of UGP2 relative to non-targeting control siRNAs after 48 hours, red dots represent total EGFR. **C**, Shown are  $\log_2$  of the fold change in specific modifications plotted against  $\log_2$  of the fold change in total protein upon knockdown of UGP2 in Suit2 cells.  $R^2 = 0.0215$ , red dots represent EGFR modifications. **D**, Global glycomic comparison in Suit2 cells with siUGP2 of non-targeting control siRNAs at 48 hours, n.s. not significant at a threshold of  $p < 0.05$ . **E**, Quantification of glycan modifications on EGFR N528 in Suit2 cells with siUGP2 or non-targeting control siRNAs at 48 hours. n.s. not significant at a threshold of  $p < 0.05$ , blue squares represent N-acetylglucosamines, green circles represent mannoses, yellow circles represent galactoses, red triangles represent fucoses, and purple diamonds represent N-acetylneuraminic acid. **F**, Quantification of total EGFR,  $\alpha$ -TUBULIN, and GAPDH proteins by mass spectrometry in Suit2 cells upon knockdown of UGP2 or non-targeting control siRNAs. n.s. not significant at a threshold of  $p < 0.05$ . **G**, Immunoblot of lysates from Panc1 cells stably expressing shUGP2 or empty vector, probed as indicated. **H**, Representative immunohistochemical staining of EGFR in three opposite-flank pairs of MiaPaca2 and Suit2 tumors with shUGP2-1, shUGP2-2, or vector control at endpoint. Scale bars represent 100  $\mu\text{m}$ . **I**, Immunoblot of Suit2 cells stably expressing shUGP2-1, shUGP2-2, or empty vector, pretreated with 10  $\mu\text{M}$  gefitinib for 1 hour and/or stimulated with 100 ng/mL EGF for 10 minutes, probed as indicated.

**Table S1.** N-glycoproteomics and total proteomics. A-B, Changes in N-glycan modifications (A) and total protein (B) in Suit2 cells treated with siUGP2 or siCtrl pools for 48 hours, n = 3.







