

Fig. S1 QARS glutaminylates FBW7 K604 (related to Fig. 2). (A), Co-immunoprecipitation showing interactions between ectopically expressed c-Myc and QARS. (B), Ubiquitination levels of ectopically expressed c-Myc in HCT116^{+/+}, *FBW7*-silenced HCT116^{+/+} cells with or without 4 mmol/L glutamine. (C), MS/MS spectra confirming the identification of FBW7 Gln-K604 (upper) compared with synthetic Gln-K604 peptide (lower). (D and E), Characterization of site-specific Gln-K604 antibody. Antibody specificity tested against Gln-K604-containing and non-glutaminylated peptides. Peptide sequences are shown below (D). Reactivity of the antibody with wild-type FBW7, FBW7 K604R, and FBW7 K604Q mutants (E). (F), Intracellular glutamine levels in glutamine-starved HCT116^{+/+} cells transferred to glutamine-free or 4 mmol/L glutamine-containing media for 2 h (n=3).

Fig. S2 Gln-K604 specifically induces c-Myc and Mcl-1 (related to Fig. 3). (A), Endogenous Mcl-1 levels in untreated HCT116^{+/+} cells and cells treated with methyl- α -ketoglutarate, glutamate, or glutamine. (B), Effects of glutamine supplementation on other cell cycle regulators levels in HCT116^{+/+} cells. (C), DNA sequencing confirming successful generation of K604Q KI and K604R KI cells, using HCT116^{+/+} cells as a reference. (D), Levels of Rb, Notch1, Jun, Cyclin E1 and Cdh1 in HCT116^{+/+}, K604Q KI and K604R KI cells.

Fig. S3 Gln-K604 disrupts the binding of c-Myc and Mcl-1 to FBW7 (related to Fig. 4). (A-C), Ubiquitination levels of ectopically expressed Cyclin E1 (A), Notch (B), and Jun (C) in glutamine-starved HCT116^{+/+} and K604Q KI cells with or without 4 mmol/L glutamine. (D), Schematic representation of FBW7 (upper) and its Δ 6th WD40 deletion mutant FBW7 ^{Δ 6thWD40} (lower). (E-G), Ubiquitination levels of ectopically expressed Cyclin E1 (E), Notch (F) and Jun (G) in HCT116^{+/+}, FBW7-overexpressing HCT116^{+/+}, and FBW7 ^{Δ 6thWD40}-overexpressing HCT116^{+/+} cells. (H), Co-immunoprecipitation showing the effects of glutamine supplementation on FBW7-c-Myc and FBW7-Mcl-1 interactions in HCT116^{+/+} cells. (I), Structural analysis of interaction between FBW7^{WT}/FBW7^{Gln-K604} and c-Myc (upper) and interaction between FBW7^{WT}/FBW7^{Gln-K604} and Mcl-1 (lower). Close-up view of the FBW7-c-Myc/Mcl-1 interacting amino acids are shown on the right. (J and K), Binding of c-Myc and Mcl-1 to FBW7 in HCT116^{+/+} and K604R KI cells under 0 mmol/L or 4 mmol/L glutamine (J) or with QARS overexpression (K).

Fig. S4 Cell cycle-dependent Gln-K604 promotes anabolism and apoptosis resistance (related to Fig. 5). (A), EdU incorporation into DNA of HCT116^{+/+}, K604Q KI, and K604R KI cells, analyzed via fluorescence-activated cell sorting. (B), Cell cycle distribution of ALS-arrested HCT116^{+/+} and K604R KI cells in the presence or absence of 4 mmol/L glutamine, quantified by flow cytometry (n=3). (C and D), Apoptotic rate of ALS-treated HCT116^{+/+} and K604R KI cells with QARS overexpression (C) or glutamine supplementation (D). (E and F), Effects of *QARS* silencing on apoptosis of ALS-(E) and HU-treated (F) HCT116^{+/+} and K604R KI cells. (G and H), Apoptotic rates of ALS-treated and *MCL-1*-silenced HCT116^{+/+} cells with QARS overexpression (G) or glutamine supplementation (H), assayed by FCM.

Fig. S5 SIRT1 removed Gln-K604 and reversed Gln-K604 effects in vitro (related to Fig. 6). (A and B), Cell cycle distribution of untreated or HU-treated (A) and ALS-treated (B) HCT116^{+/+}, *SIRT1*-silenced HCT116^{+/+}, K604R KI and *SIRT1*-silenced K604R KI cells, assayed by FCM (n=3).

Fig. S6 Precise chemotherapy-sensitizing strategies for Gln-K604-intact and Gln-K604-null cancers (related to Fig. 7). (A), Overview of cancer types with different FBW7 and Gln-K604 statuses. (B and C), Responses of FBW7 substrates to glutamine starvation (B) and *QARS* silencing (C) in Gln-K604-intact and Gln-K604-null cells. (D), Apoptotic rates in Gln-K604-intact and Gln-K604-null cells with glutaminol, ALS, or both.

Fig. S7 FBW7 glutamine signaling (related to the Discussion). Schematic representation of the FBW7 signaling network. *QARS* glutaminylates FBW7 K604 to form Gln-K604, which specifically activates c-Myc to promote glutamine uptake and anabolism. Gln-K604 also enhances Mcl-1 activity, conferring apoptosis resistance. SIRT1 reverses Gln-K604 and its effects.











