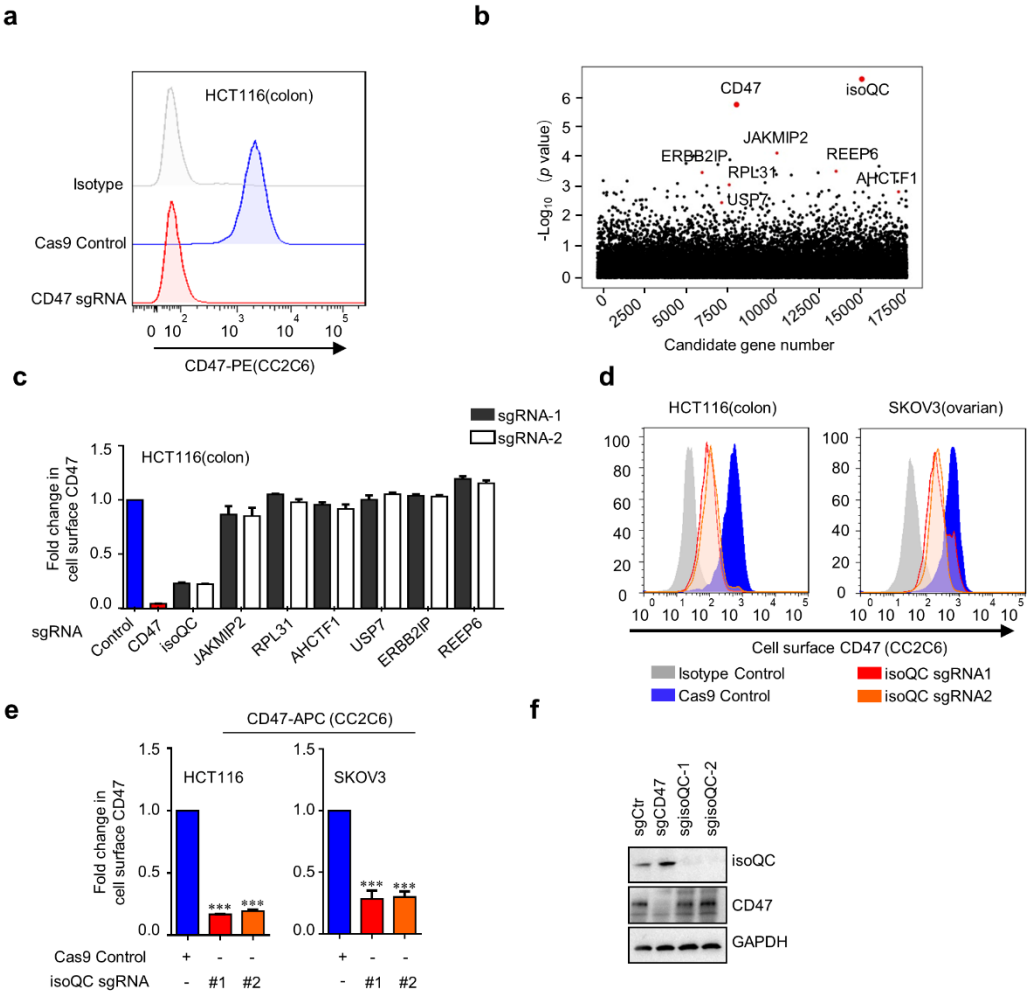

Figure S1. Identification of isoQC as a regulator of CD47. a, FACS analysis of cell surface CD47 using CD47 antibody (clone CC2C6) in the indicated knockout HCT116 cells. Shown is a representative experiment. Data represent n=3 biological replicates. b, Most enriched genes calculated by MAGeCK. Red dots show selected genes enriched in screening analysis ranked by *p* value. c, FACS analysis of surface CD47 in HCT116 cells stably expressing the indicated sgRNAs. The data were normalized using Cas9 control. Shown is a representative experiment. Data represent n=3 biological replicates. d-e, FACS analysis of surface CD47 in isoQC knockout HCT116 and SKOV3 cells. Shown is a representative experiment (d). Data represent three independent experiments with similar results, MFI values are shown in e. The data were normalized using Cas9 control. Shown is the mean ± SD of three independent experiments. t test, ***P<0.001. f, Western blot analysis of isoQC knockout efficiency in HCT116 cells. Shown is a representative experiment. Data represent three independent experiments with similar results.

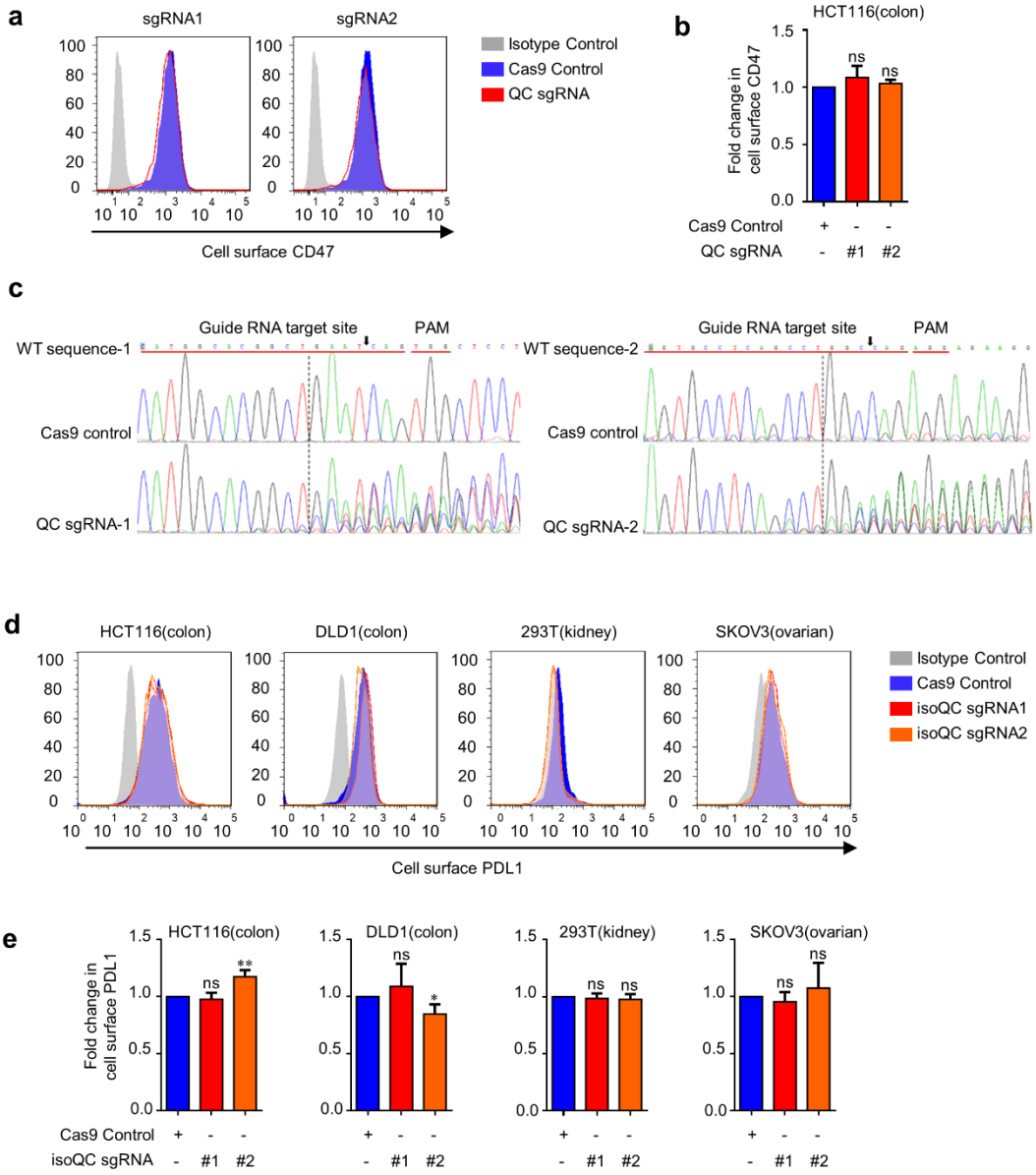

Figure S2. Specific regulation of surface expression of CD47 by isoQC. a-b, FACS analysis of surface CD47 in QC knockout HCT116 cells. Shown is a representative experiment (a). Data represent three independent experiments with similar results. MFI values are shown in b. The data were normalized using Cas9 control. Shown is the mean ± SD of three independent experiments. t test, ns, not significant. c, Sanger sequencing analysis of QC knockout HCT116 cell lines. d-e, FACS analysis of surface PD-L1 in isoQC knockout HCT116, DLD1, HEK293T, SKOV3 cells. Shown is a representative experiment (d). Data represent three independent experiments with similar results. MFI values are shown in e. The data were normalized using Cas9 control. Shown is the mean ± SD of three independent experiments. t test, **P*<0.05, ***P*<0.01, ns, not significant.

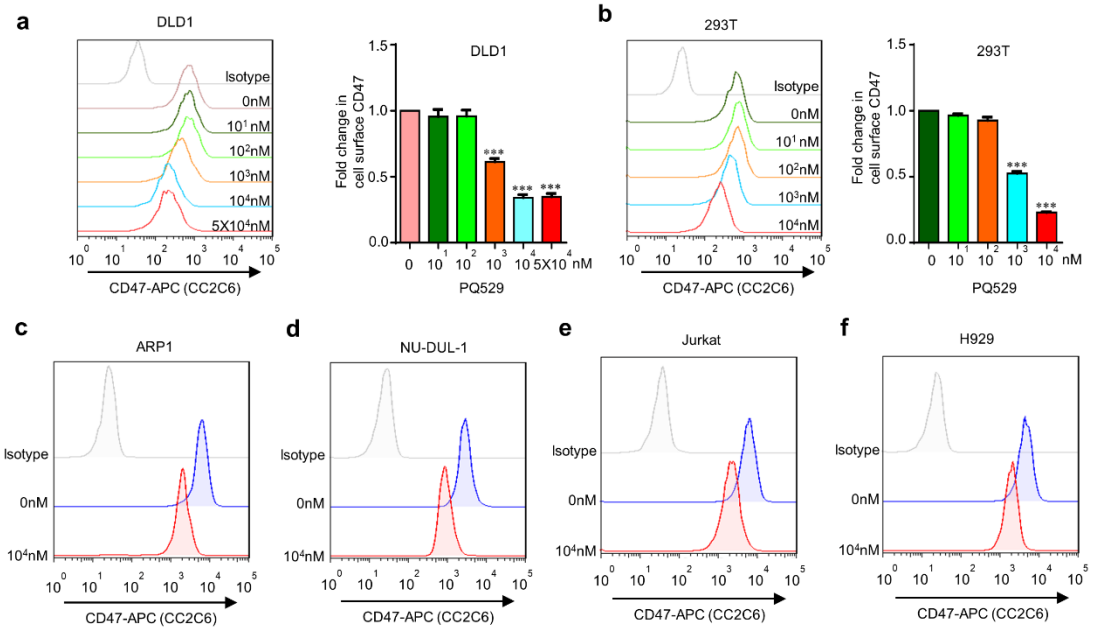

Figure S3. Regulation of CD47 by isoQC is enzymatic activity dependent. a, FACS analysis of cell surface CD47 after treated with increasing concentrations of isoQC inhibitor (PQ529) for 48 h in DLD1 cells. Shown is a representative experiment (left). Data represent three independent experiments with similar results. MFI values are shown in right. The data were normalized using DMSO control. Shown is the mean ± SD of three independent experiments. t test, ***P<0.001. b, FACS analysis of surface CD47 after treated with increasing concentrations of isoQC inhibitor (PQ529) for 48 h in HEK293T cells. Shown is a representative experiment (left). Data represent three independent experiments with similar results. MFI values are shown in right. The data were normalized using DMSO control. Shown is a mean ± SD of three independent experiments. t test, ***P<0.001. c-f, FACS analysis of surface CD47 after treated with isoQC inhibitor (PQ529, 10μM) or vehicle control for 48 h in ARP1 (c), NU-DUL-1 (d), Jurkat (e), H929 (f) cells. Shown is a representative experiment. Data represent three independent experiments with similar results.

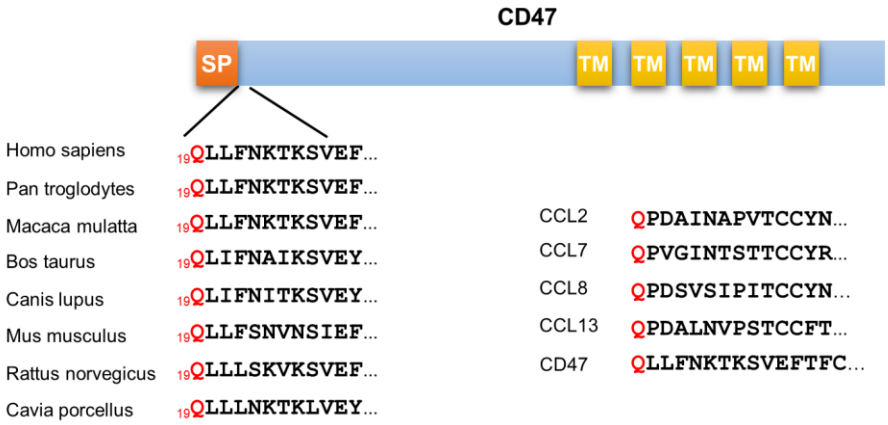

Figure S4. CD47 contains a conserved glutamine at the N-terminus. Sequence alignment of the putative pGlu modification site Q19 in CD47 from the indicated species (left). As similar to other pGlu modification substrates (human MCPs), CD47 also contains Glutamine (Q) at the N-terminus that is modified to pGlu in mature statement (right).

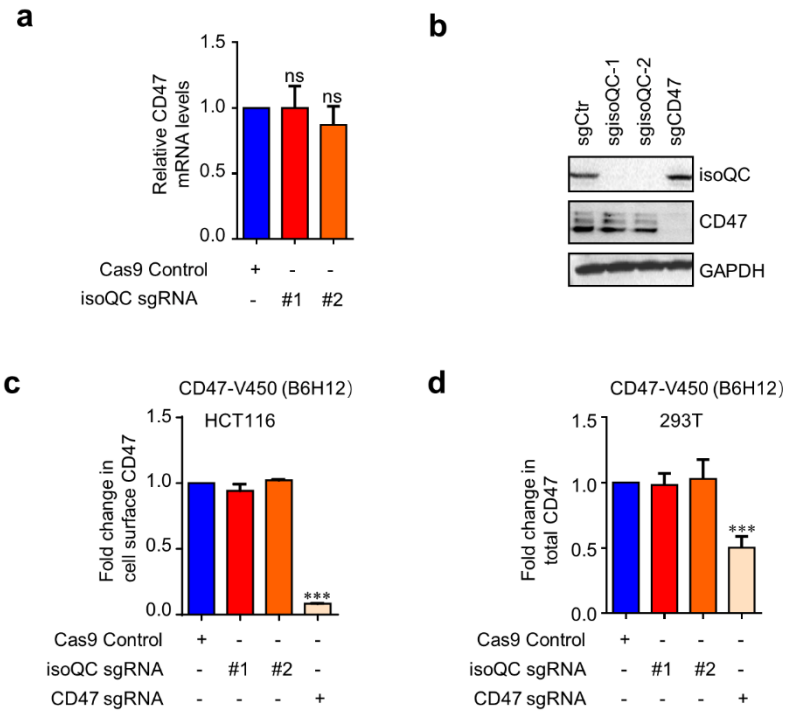

Figure S5. isoQC did not affect the protein level of CD47. a, qRT-PCR analysis of relative CD47 mRNA levels in isoQC knockout HCT116 cells. The data were normalized using Cas9 control. Shown is the mean ± SD of four independent experiments. t test, ns, not significant. b, Western blot analysis of total expression of CD47 in knockout HEK293T cells (CD47 antibody: CST 63000). Shown is a representative experiment. Data represent three independent experiments with similar results. c, FACS analysis of surface CD47 in isoQC knockout HCT116 cells detected with B6H12 antibody. The data were normalized using Cas9 control. Shown is the mean ± SD of three independent experiments. t test, ***P<0.001. d, FACS analysis of total expression of CD47 in isoQC knockout HEK293T cells detected with B6H12 antibody. The data were normalized using Cas9 control. Shown is the mean ± SD of three independent experiments. t test, ***P<0.001.

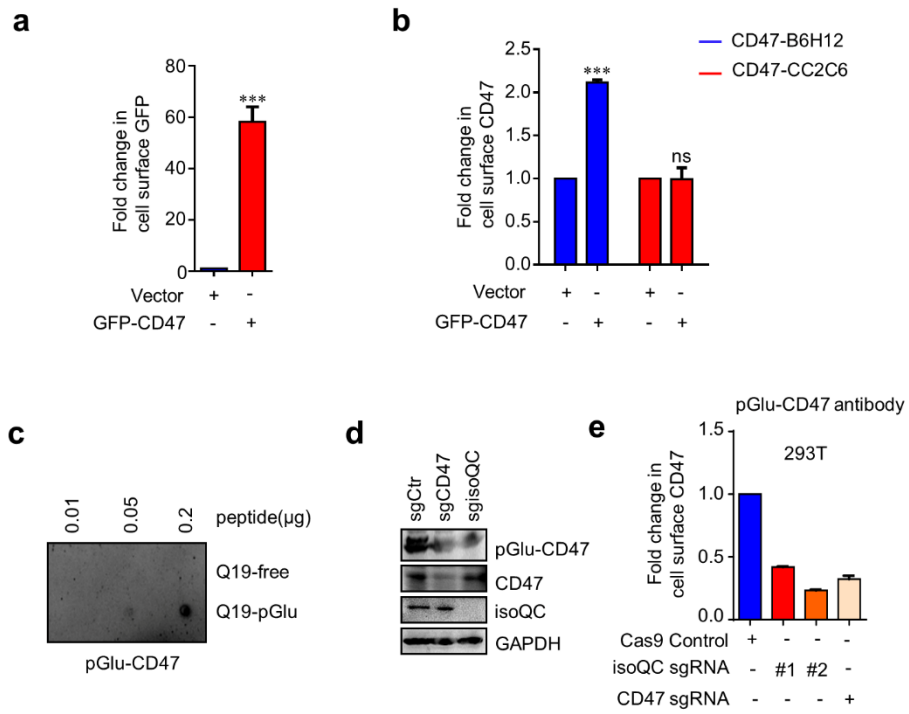

Figure S6. isoQC mediated pGlu formation of CD47. a, Analysis of the expression level of GFP-CD47 in HEK293T cells. The data were normalized using vector control. Shown is the mean ± SD of three independent experiments. t test, ***P<0.001. b, Analysis of cell surface CD47 in HEK293T cells by using different FACS antibodies. The data were normalized using vector control. Shown is a mean ± SD of three independent experiments. t test, ***P<0.001, ns, not significant. c, Dot blot analysis of CD47 pGlu modification antibody using Q19 unmodified (Q19-free) and Q19 pGlu-modified (Q19-pGlu) peptides. Shown is a representative experiment. Data represent two independent experiments with similar results. d, Western blot analysis of CD47 pGlu modification antibody in knockout HEK293T cells. Shown is a representative experiment. Data represent two independent experiments with similar results. e, FACS analysis of cell surface CD47 in HEK293T cells performed in duplicate with CD47 pGlu modification antibody. The data were normalized using Cas9 control. Shown is a representative experiment. Data represent two independent experiments with similar results.

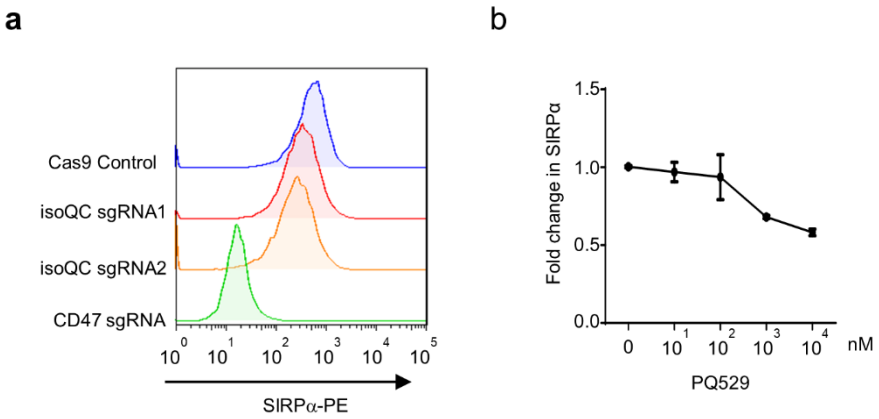

Figure S7. isoQC-mediated pGlu modification of CD47 is required for SIRPα binding. a, FACS analysis of the interaction between SIRPα and CD47 in the indicated knockout HEK293T cells. Shown is a representative experiment. Data represent three independent experiments with similar results. b, FACS analysis of the interaction between SIRPα and CD47 in HEK293T cells. Cells were treated with increasing concentrations of isoQC inhibitor (PQ529) for 48 h. The data were normalized using DMSO control. Shown is the mean ± SD of three independent experiments.

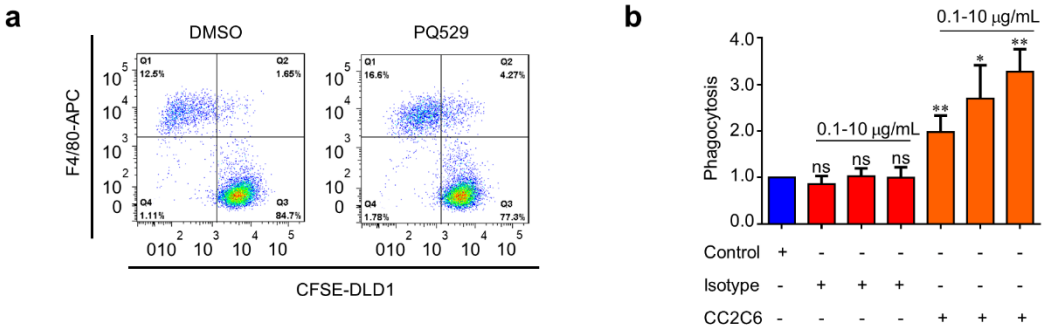

Figure S8. pGlu modification of CD47 is required for its inhibition of macrophage-mediated phagocytosis. a, FACS analysis of the phagocytosis of the indicated DLD1 cells treated with isoQC inhibitor (PQ529, 10μM) or vehicle control for 48 h before co-culture. Shown is a representative experiment. Data represent at least three independent experiments with similar results. b, FACS analysis of the phagocytosis of the indicated DLD1 cells treated with isotype, CC2C6 (0.1-10 mg/mL) or vehicle control during co-culture. The data were normalized using vehicle control. Shown is the mean ± SD of three independent experiments. t test, **P*<0.05, ***P*<0.01, ns, not significant.

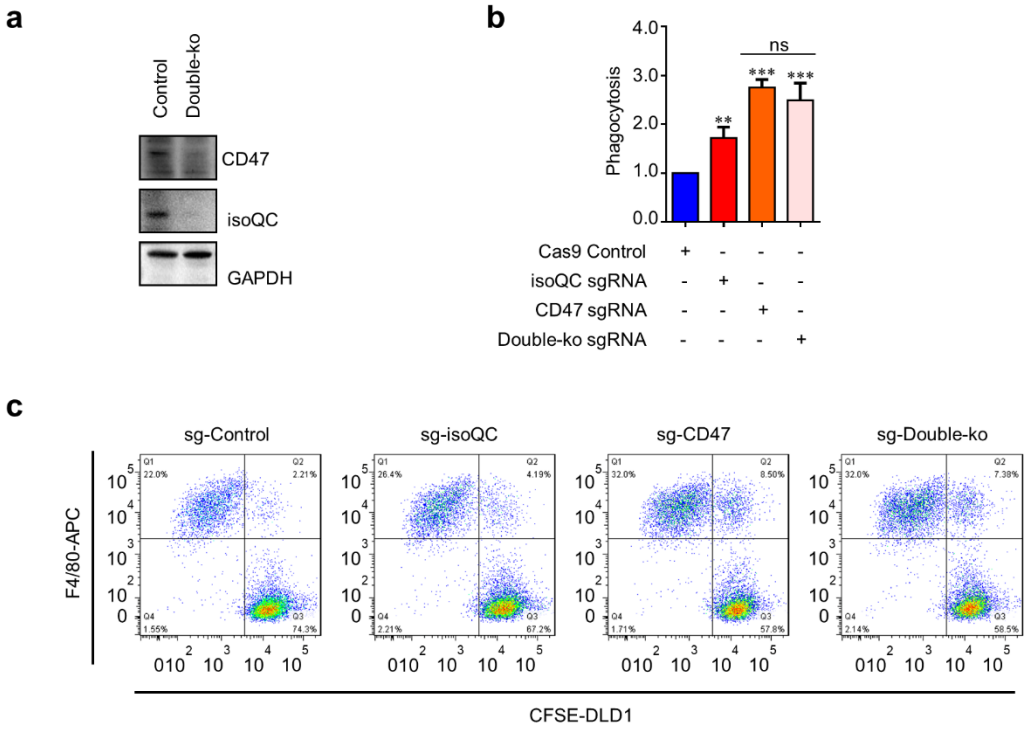

Figure S9. isoQC inhibits the phagocytosis of cancer cells by macrophages in a CD47-dependent manner. a, Western blot analysis of double-knockout efficiency in DLD1 cells. Shown is a representative experiment. Data represent three independent experiments with similar results. b-c, Phagocytosis of knockout DLD1 cells by macrophages was analyzed via FACS. The data were normalized using Cas9 control. Shown is the mean ± SD of four independent experiments (b). One-way ANOVA, $^{**}P<0.01$, $^{***}P<0.001$, ns, not significant. Phagocytosis was evaluated as a sum of the $CFSE^{+}$ macrophages, expressed as a percentage of the total macrophages, as depicted in the representative FACS shown in (c).

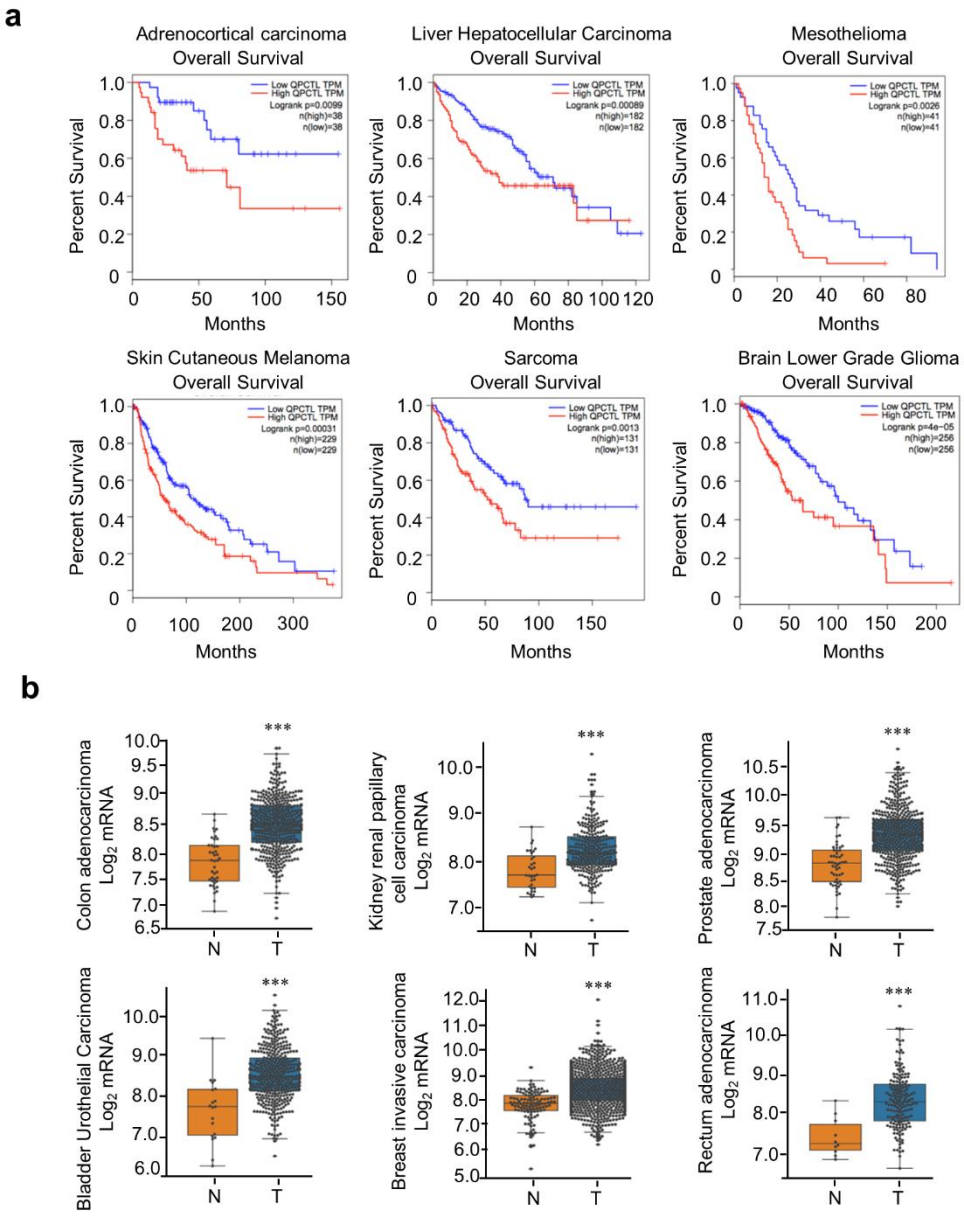

Figure S10. Expression of isoQC in cancers. a, Correlation of isoQC expression with prognosis in multiple types patients. b, Upregulation of isoQC in cancers. t test, ****P*<0.001. "N" represents normal tissue, "T" represents tumor tissue.