Supplementary material

Deacetylation of MTHFD2 by SIRT4 senses stress signal to inhibit cancer cell growth by remodeling folate metabolism

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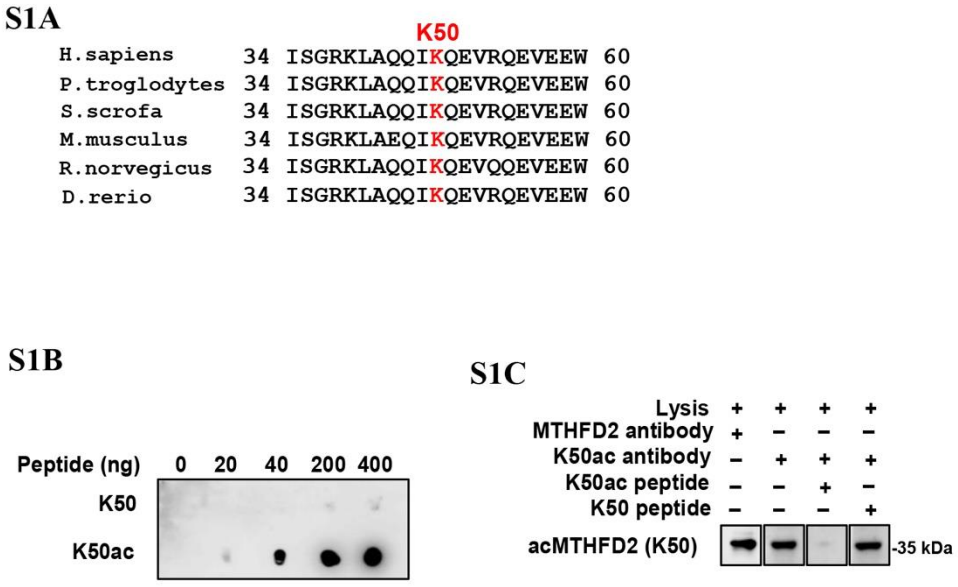
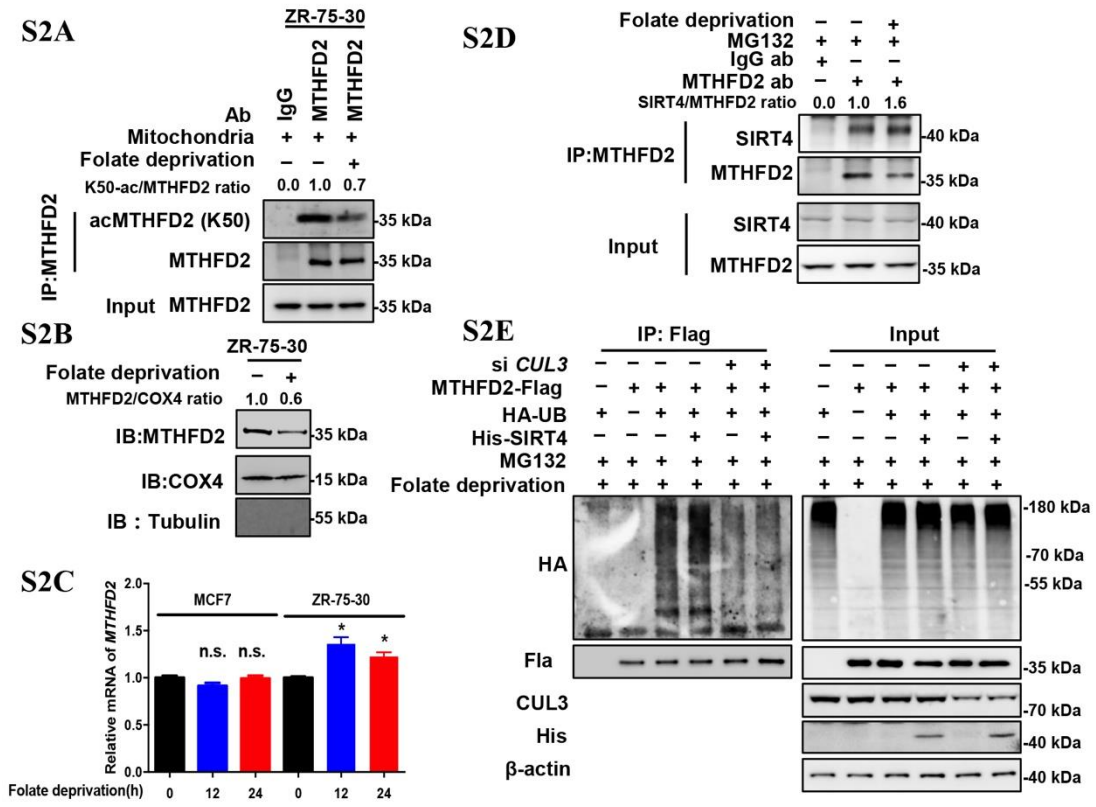
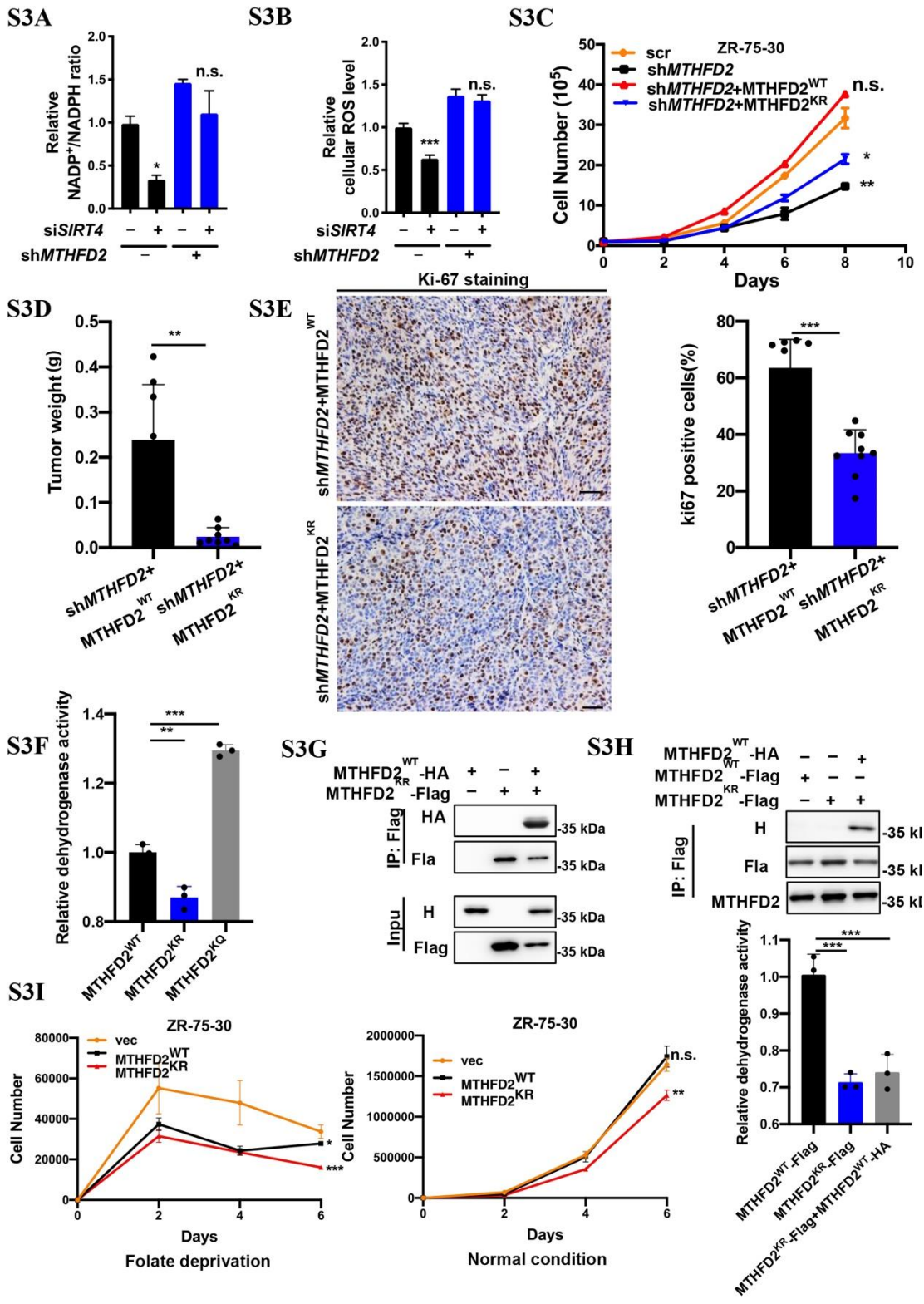


Supplementary Figure S1 K50 is conserved and acMTHFD2 (K50) antibody is specific, related to Figure 1. S1A, K50 of MTHFD2 is conserved evolutionarily. MTHFD2 protein sequences of different species were aligned, and K50 is conserved across indicated species. **S1B,** acMTHFD2 (K50) antibody detects the acetylated, but not the unmodified peptide. Different amounts of either K50ac peptide or unmodified peptide were probed with acMTHFD2 (K50) antibody. **S1C,** acMTHFD2 (K50) antibody is specific to MTHFD2 K50 acetylation. HEK293T cell lysate was loaded repetitively for western blotting analysis. Four lanes were cut and exposed to the indicated antibodies separately. For peptide block, acetylated or non-acetylated K50 peptide were mixed with the diluted acMTHFD2 (K50) antibody incubating at 37 ℃ for 1 h and then added to membranes. Four membranes were exposed together under the same condition.



Supplementary Figure S2 Folate deprivation induces MTHFD2 deacetylation, related to Figure 4. S2A, K50 acetylation of MTHFD2 is decreased upon folate deprivation. Mitochondria of ZR-75-30 cells treated with or without folate were purified. Endogenous MTHFD2 was immunoprecipitated, K50 acetylation was detected by western blotting. **S2B,** MTHFD2 protein level is decreased upon folate deprivation. ZR-75-30 cells were treated with normal or folate-deprived medium for 24 h. Protein level of MTHFD2 was detected by western blotting and normalized against COX4 which was a mitochondrial protein. Tubulin is detected to make sure that mitochondria is not contaminated with cytoplasm contents. **S2C,** MTHFD2 mRNA level is not reduced upon folate deprivation. MCF7 and ZR-75-30 cells were treated with folate deprivation for 12 and 24 h. mRNA levels were measured by RT-qPCR. *** represents $P<0.001$, ** represents $P<0.01$, * represents $P<0.05$, and n.s. denotes no significance. **S2D,** Folate deprivation promotes the endogenous binding between MTHFD2 and SIRT4 in ZR-75-30 cells. **S2E,** MTHFD2 ubiquitylation level is increased by SIRT4 overexpression and decreased by *CUL3* silencing. Flag-tagged MTHFD2 was co-expressed with HA-tagged ubiquitin in HEK293T cells. Cells were treated with MG132 for 6 h and treated folate deprivation for 24h. Ubiquitylation of MTHFD2 was determined by western blotting.



Supplementary Figure S3 KR mutant suppresses tumor growth *in vivo* and *in vitro* by interfering NADPH and ROS, related to Figure 5. S3A and S3B, Knockdown of SIRT4 increases NADPH production (S3A) and reduces ROS level (S3B) of control cells, but not MTHFD2-knockdown ZR-75-30 cells. Control cells and stable MTHFD2-knockdown ZR-75-30 cells were transfected with scramble or siRNA targeting SIRT4. NADP+/NADPH ratio (S3A) and ROS levels (S3B) were determined. **S3C,** Re-introduction of WT MTHFD2, but not the KR mutant, rescues proliferation of

MTHFD2-depleting ZR-75-30 cells. Numbers of *MTHFD2*-knockdown ZR-75-30 cells re-expressing WT MTHFD2 or KR mutant at indicated time points were counted. **S3D,** KR mutant significantly reduces tumor growth by xenograft analysis. **S3E,** Re-introduction of KR mutant into cells significantly attenuates the staining intensity of cell proliferation marker Ki-67, scale bar: 50um. **S3F,** KR mutant significantly decreases while KQ mutant increases the enzymatic activity. **S3G and S3H,** KR mutant forms a heterodimer with WT MTHFD2 (S3G), which significantly inhibits the enzymatic activity of the WT MTHFD2 (S3H). Flag-tagged KR mutant was co-expressed with HA-tagged WT MTHFD2 in HEK293T. **S3I,** Ectopic expression of KR mutant significantly reduces viability of ZR-75-30 cells with endogenous MTHFD2. Data shown represent the results obtained from triplicate independent experiments with standard errors of the mean (mean ± SD). *** represents $P<0.001$, ** represents $P<0.01$, * represents $P<0.05$, and n.s. denotes no significance.