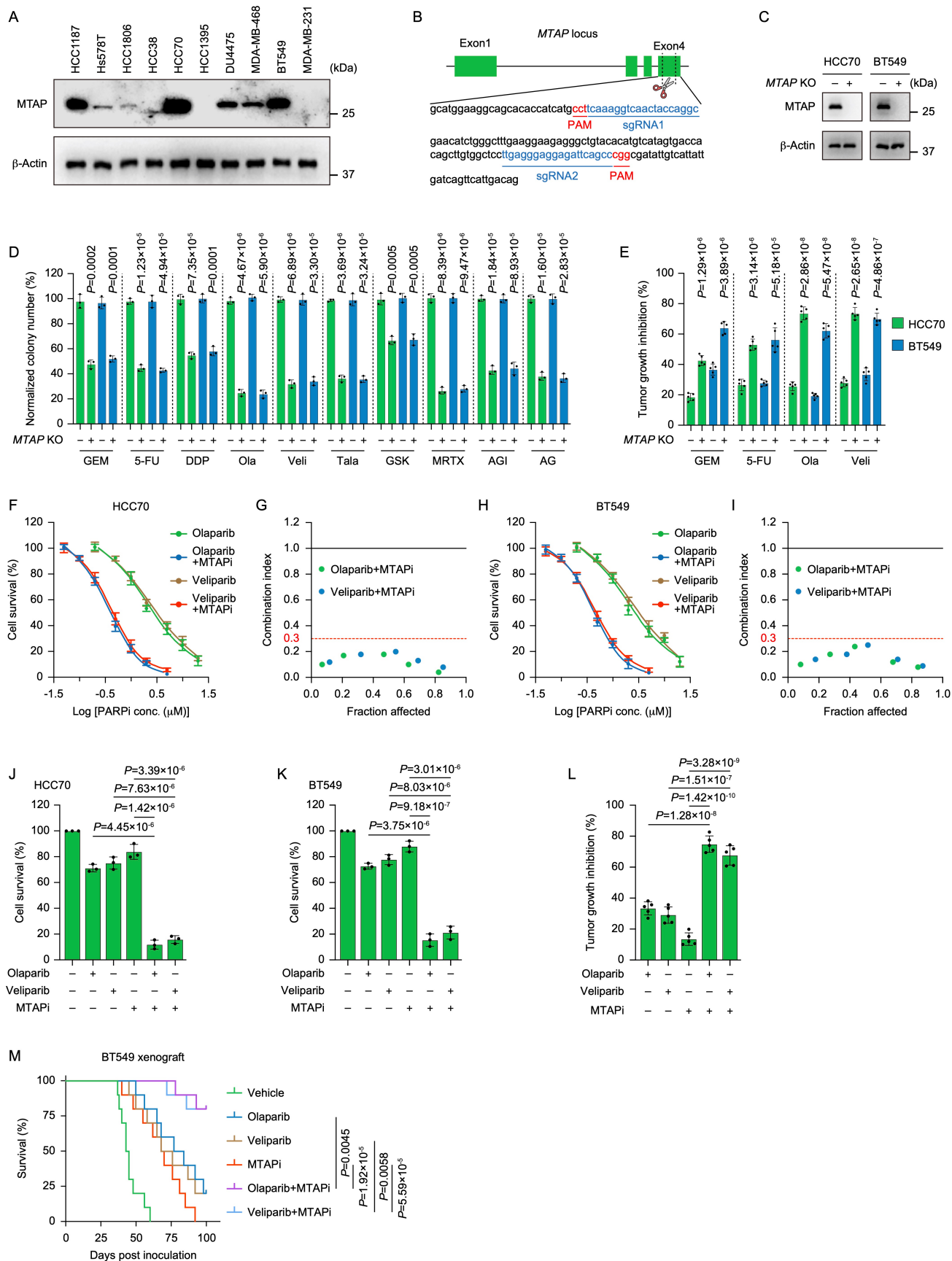
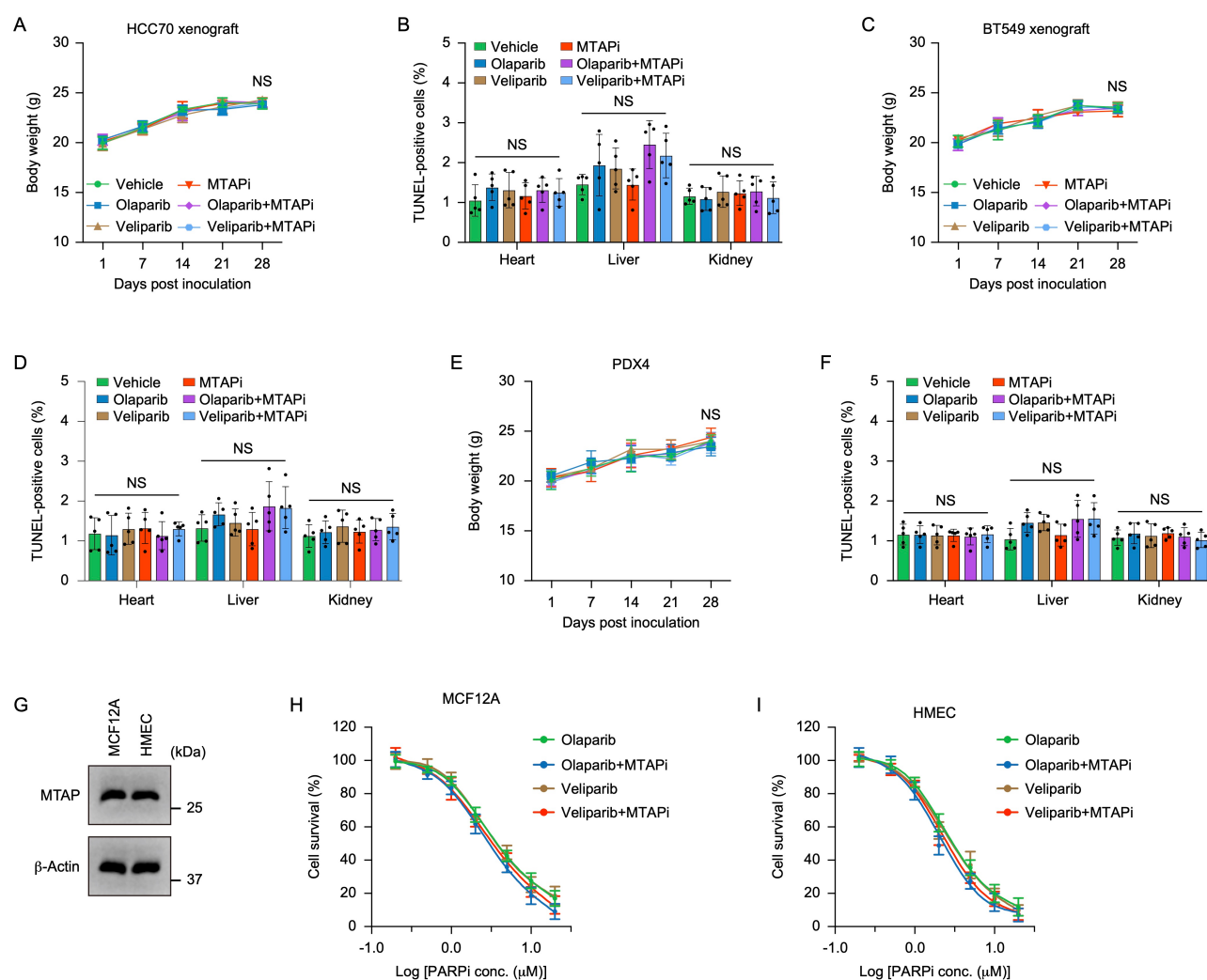
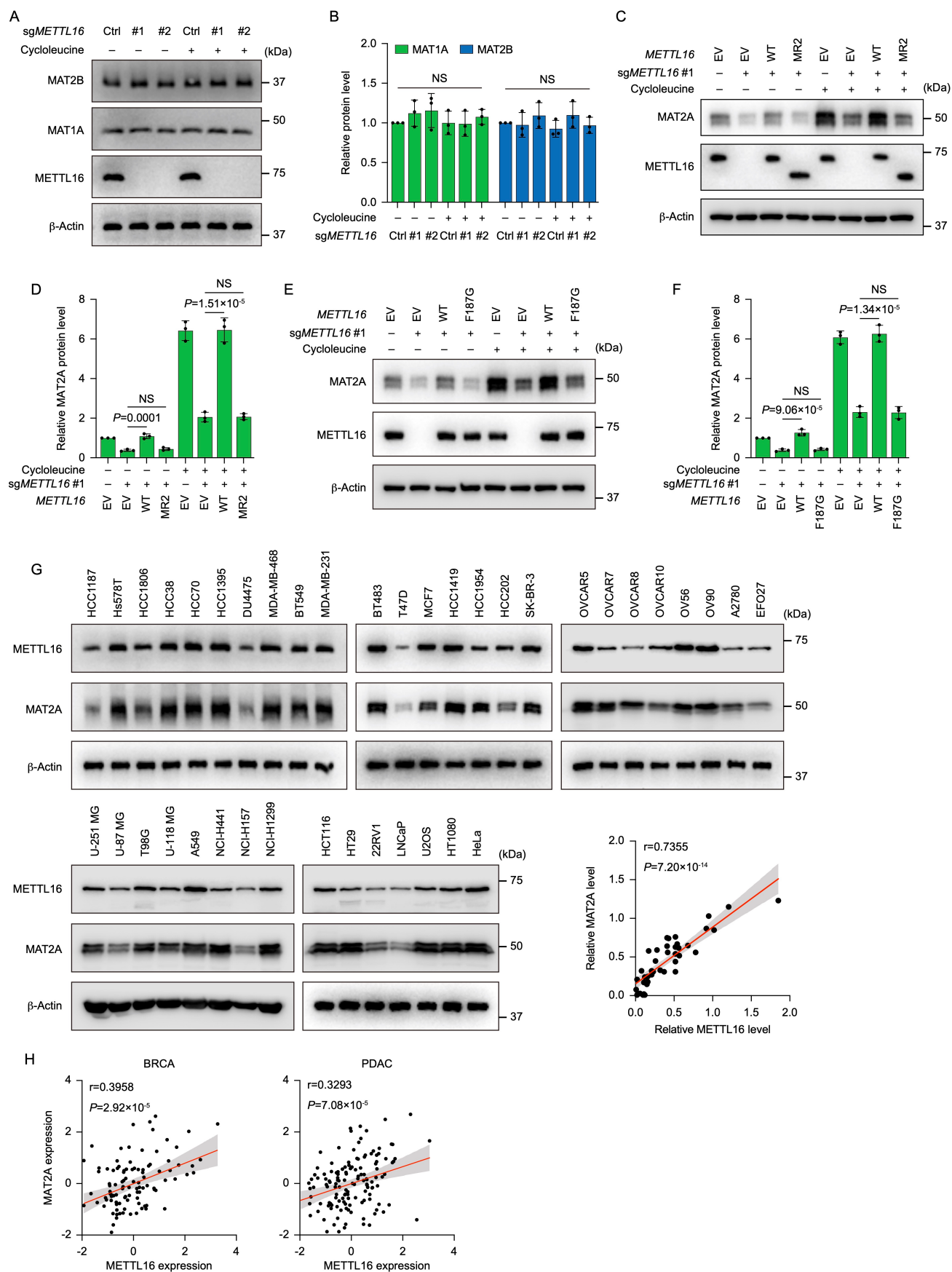




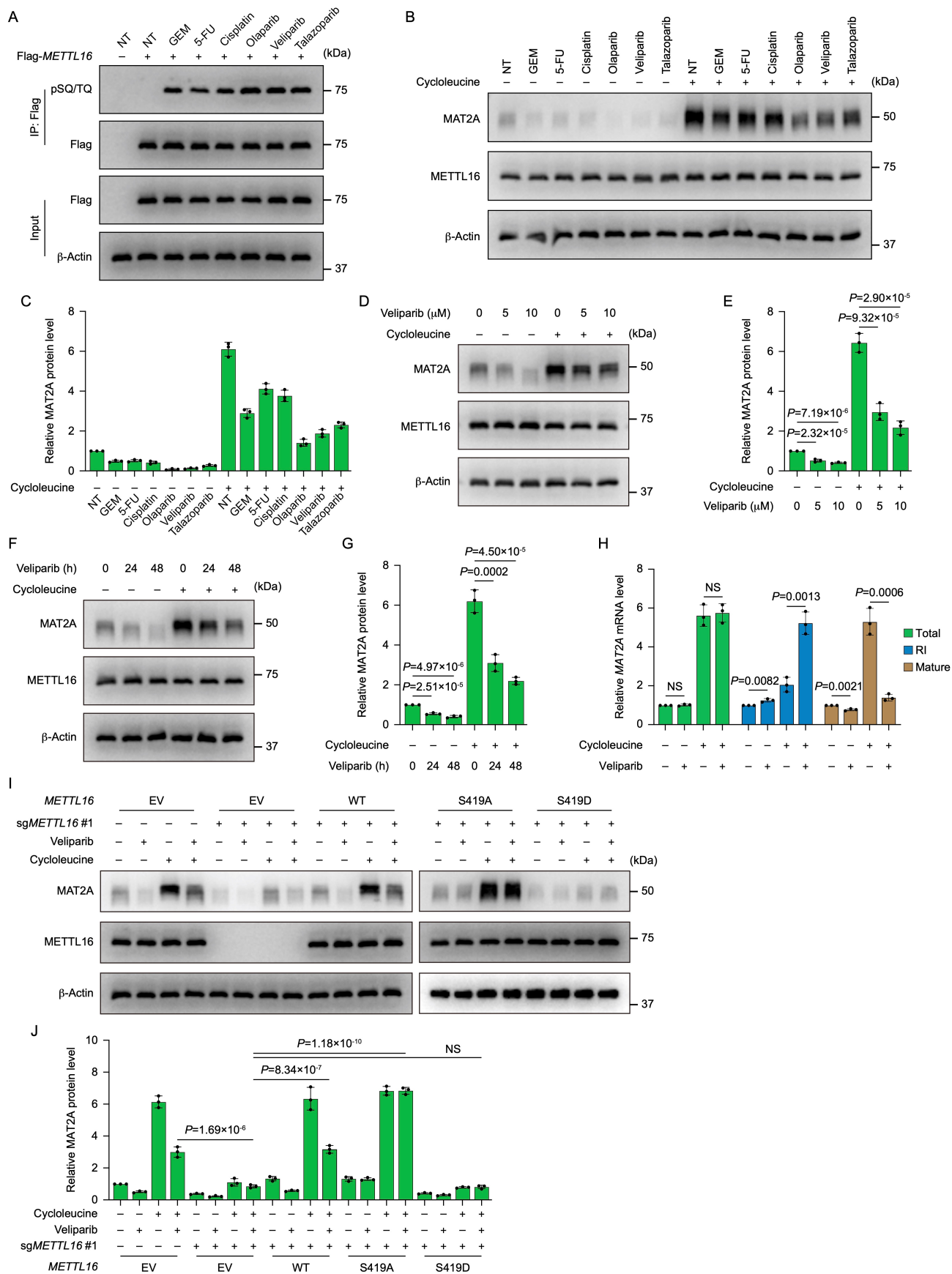
Supplemental Figure 1. Targeting MTAP combined with PARPi effectively kill tumor cells expressing MTAP. (A) Representative western blot of MTAP in diverse TNBC cell lines. (B) Schematic representation of paired sgRNAs designed for targeting *MTAP* exon 4. (C) Representative western blots of MTAP in HCC70 and BT549 cells with or without *MTAP* knock out. (D and E) Cell survival (D) and tumor growth inhibition (E) analysis of HCC70 and BT549 cells with WT or deleted *MTAP* treated with the indicated DNA damage-inducing agents. GEM, gemcitabine; DDP, cisplatin; Ola, olaparib; Veli, veliparib; Tala, talazoparib; GSK, GSK3326595; MRTX, MRTX1719; AGI, AGI-24512; AG, AG-270. (F-I) MTS assay and combination index (CI) values of HCC70 (F and G) and BT549 (H and I) cells treated with olaparib or veliparib with or without combining with MTAPi (0.1 μ M). CI < 0.3 indicates strong synergism. (J and K) Colony formation assay of HCC70 (J) and BT549 (K) cells treated with olaparib (0.2 μ M), veliparib (0.2 μ M), or MTAPi (0.1 μ M), or the indicated combination. (L and M) Tumor growth inhibition (L) and Kaplan-Meier survival curves (M) of BT549 xenograft tumor models treated with olaparib (50 mg/kg), veliparib (50 mg/kg), or MTAPi (10 mg/kg), or the indicated combination. (A and C) Experiment was repeated 3 times, and representative blots are presented. (D, F, H, J, and K) Data are shown as the mean $\pm$ SD from 3 independent experiments. (E and L) Data are shown as the mean $\pm$ SD from one representative experiment of 5 mice per group, n = 10 mice per group in M. *P* values are indicated. Significance determined using (D and E) unpaired *t*, (J-L) 1-way ANOVA, or (M) log-rank (Mantel-Cox) test.



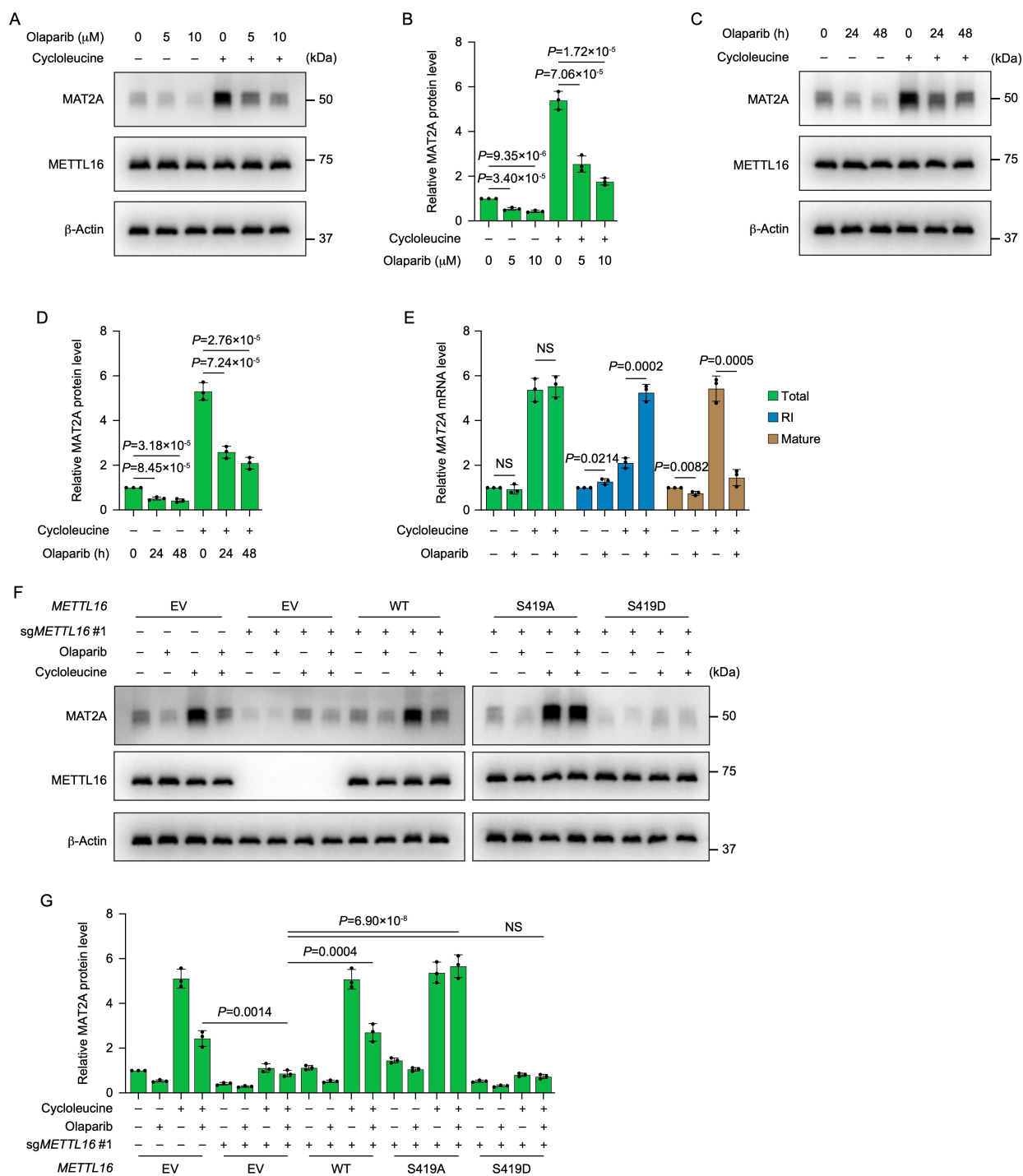
Supplemental Figure 2. Combination of PARPi and MTAPi is well tolerant. (A-F) body weight and quantification of TUNEL-positive cells in heart, liver, and kidney of HCC70 (A and B), BT549 (C and D), and PDX4 (E and F) xenograft tumor models treated with olaparib (50 mg/kg), veliparib (50 mg/kg), or MTAPi (10 mg/kg), or the indicated combination. Data are shown as the mean $\pm$ SD from one representative experiment of 5 mice per group. (G) Representative western blot showing levels of MTAP in the indicated cell lines. Experiment was repeated 3 times, and representative blots are presented. (H and I) MTS assay of MCF12A (H) and HMEC (I) cells treated with olaparib or veliparib with or without combining with MTAPi (0.1 μ M). Data are shown as the mean $\pm$ SD from 3 independent experiments. *P* values are indicated. Significance determined using (A, C, and E) 2-way ANOVA, (B, D, and F) 1-way ANOVA test.



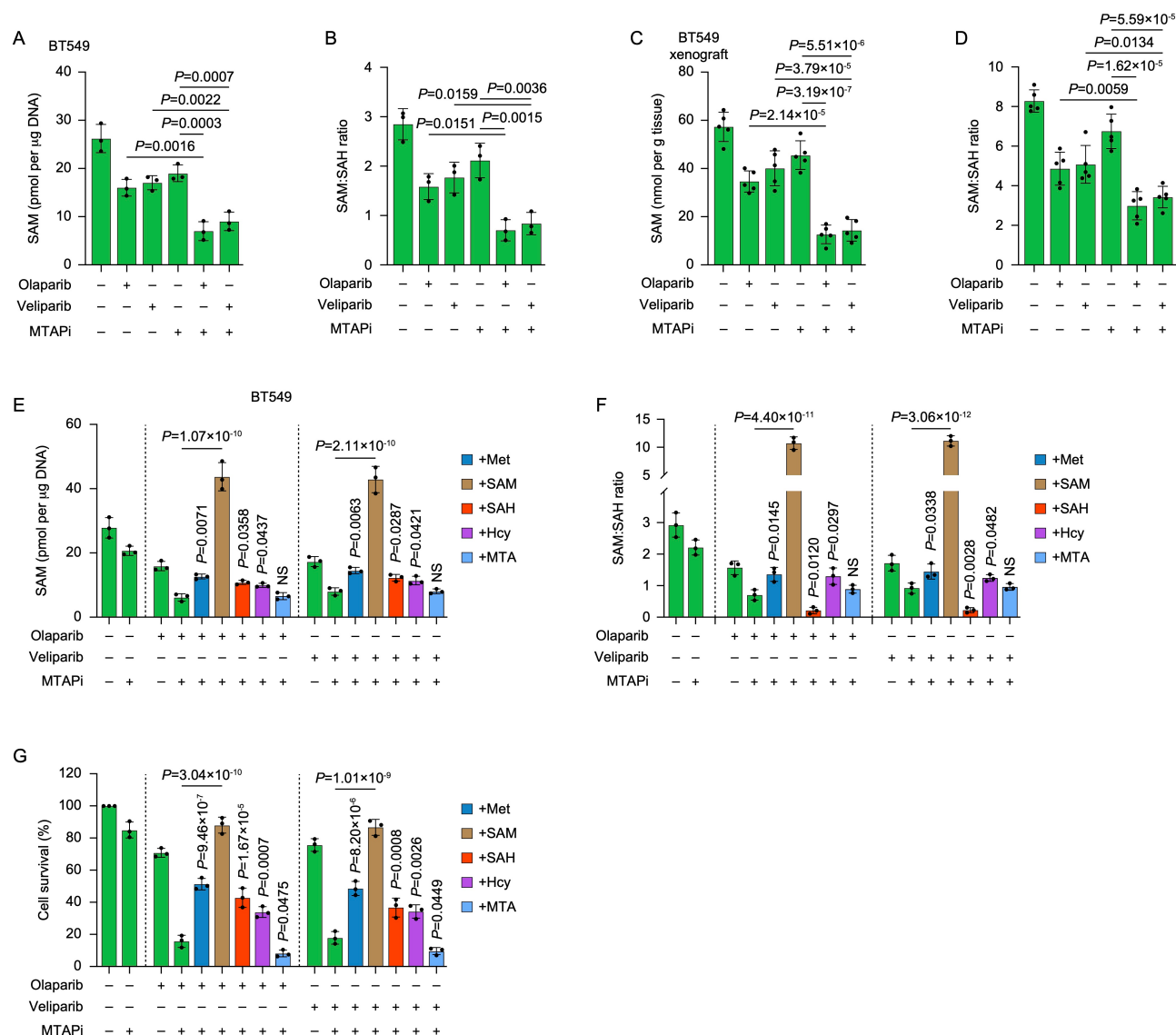
Supplemental Figure 3. METTL16 positively regulates MAT2A. (A and B) Representative western blots (A) and quantitation (B) showing levels of MAT2B, MAT1A, and METTL16 in cells expressing control or *METTL16* sgRNAs with or without cycloleucine (20 mM) treatment. (C-F) Representative western blots and quantitation showing levels of MAT2A and METTL16 in *METTL16*-null cells re-introduced *METTL16* WT, MR2 (C and D) or F187G (E and F) vectors with or without cycloleucine (20 mM) treatment. (G) Representative western blots showing METTL16 and MAT2A expression levels and Pearson correlation analysis in diverse cell lines. (H) Correlation of METTL16 and MAT2A protein expression in Reverse Phase Protein Array (RPPA) of breast cancer and pancreatic ductal adenocarcinoma. Data are from <https://www.cbioportal.org>. (A, C, E, and G) Experiment was repeated 3 times, and representative blots are presented. (B, D, and F) Data are shown as the mean $\pm$ SD from 3 independent experiments. *P* values are indicated. Significance determined using (B, D, and F) 1-way ANOVA, or (G and H) Pearson correlation test.



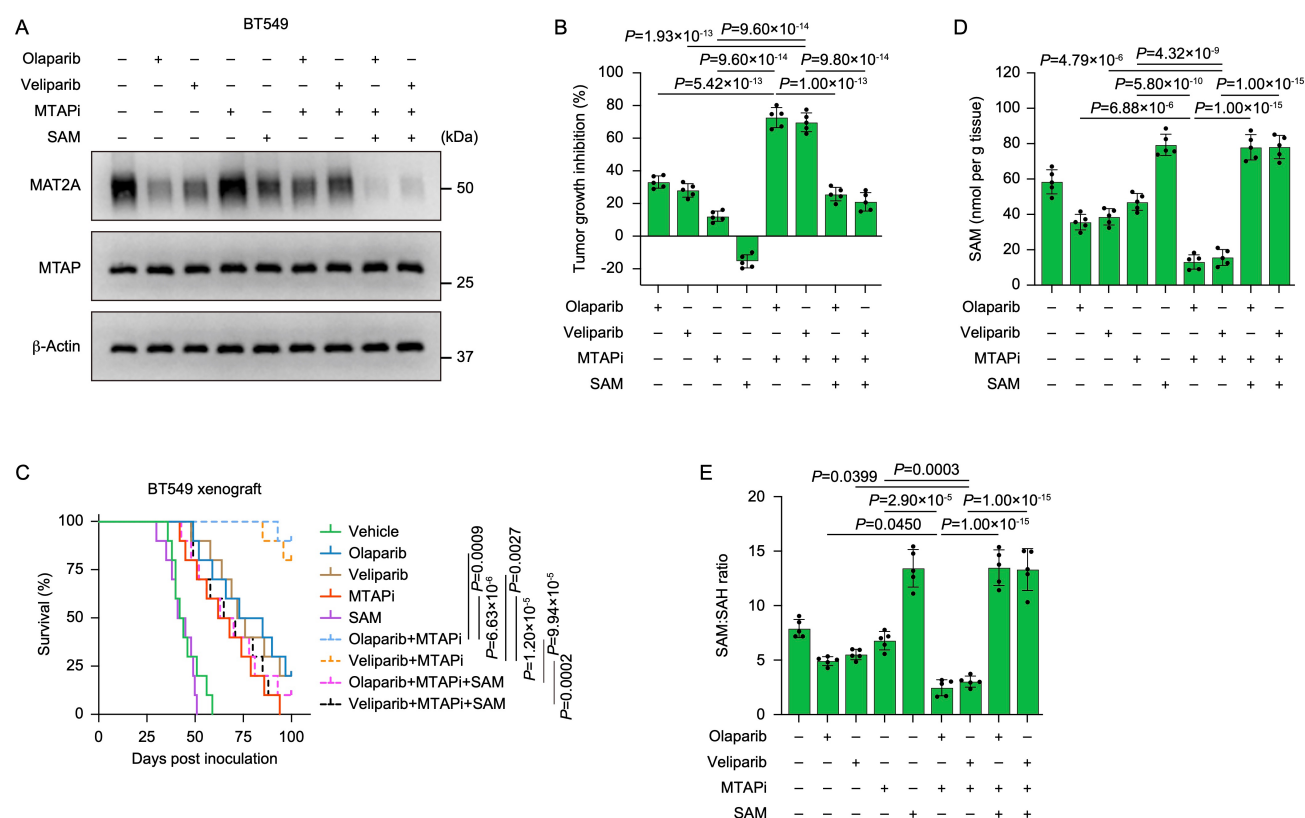
Supplemental Figure 4. PARPi attenuates MAT2A expression by suppressing METTL16 facilitated mRNA intron retention. (A) Representative western blots showing levels of METTL16 pSQ/TQ with the indicated treatment after transfection with Flag-*METTL16* and immunoprecipitated with Flag. (B and C) Representative western blots (B) and quantitation (C) showing levels of MAT2A and METTL16 with the indicated treatment. (D-G) Representative western blots and quantitation showing levels of MAT2A and METTL16 in HCC70 cells treated with the indicated dose of veliparib for 24 h (D and E) or the indicated time period of veliparib (5 μ M) (F and G). (H) RT-qPCR analysis of total, intron retention, and mature *MAT2A* mRNA levels in HCC70 cells treated with veliparib (5 μ M), or cycloleucine (20 mM), or their combo. (I and J) Representative western blots (I) and quantitation (J) showing levels of MAT2A and METTL16 in HCC70 cells stably expressing the indicated vectors treated with veliparib (5 μ M), or cycloleucine (20 mM), or their combo. GEM (10 μ M), 5-FU (10 μ M), cisplatin (10 μ M), olaparib (5 μ M), veliparib (5 μ M), and talazoparib (2 μ M) were used in A and B. (A, B, D, F, and I) Experiment was repeated 3 times, and representative blots are presented. (C, E, G, H, and J) Data are shown as the mean $\pm$ SD from 3 independent experiments. *P* values are indicated. Significance determined using (E, G, H, and J) 1-way ANOVA test. NT, not treatment.



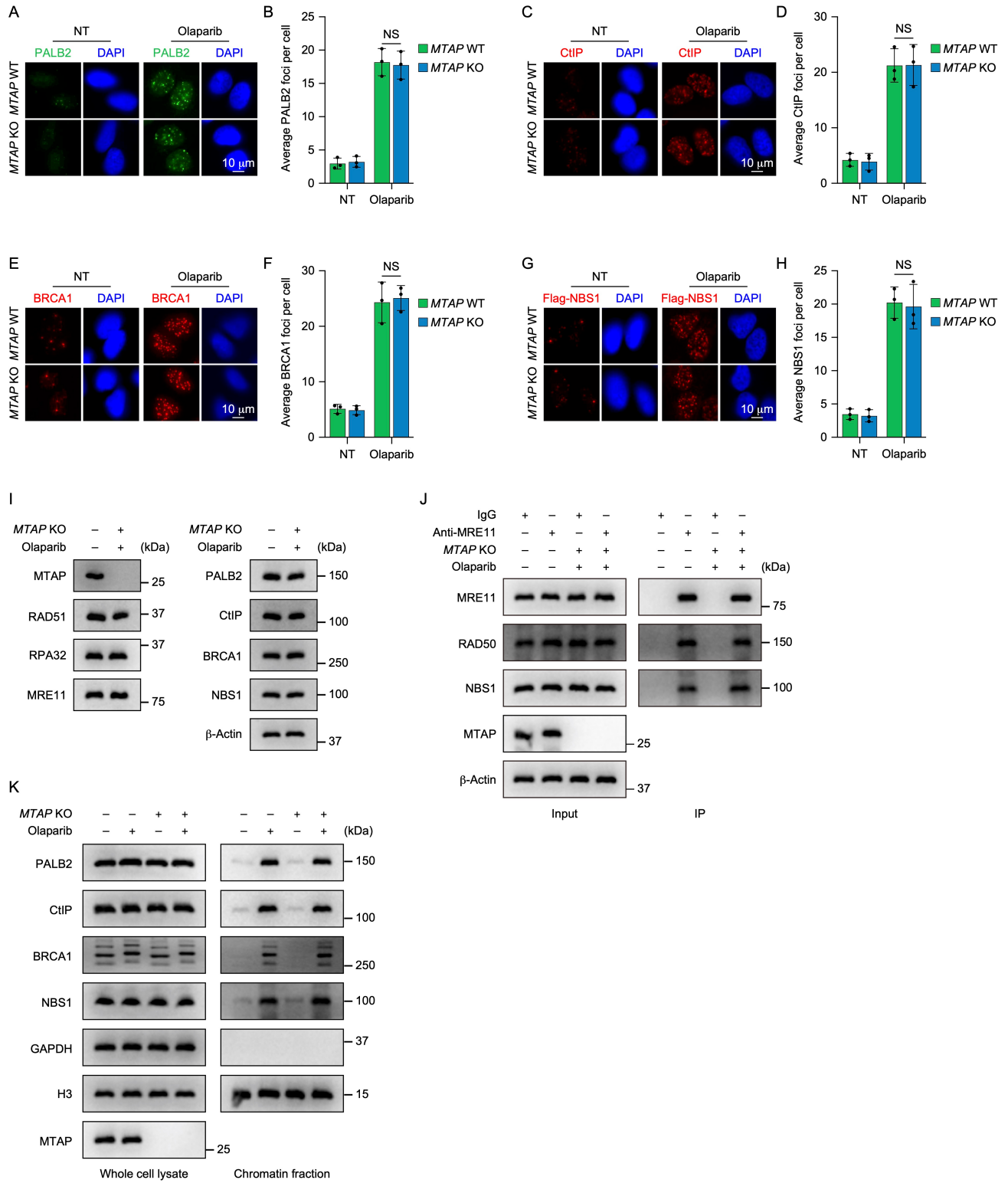
Supplemental Figure 5. PARPi reduces MAT2A expression via repressing METTL16 mediated mRNA intron retention. (A-D) Representative western blots and quantitation showing levels of MAT2A and METTL16 in BT549 cells treated with the indicated dose of olaparib for 24 h (A and B) or the indicated time period of olaparib (5 μ M) (C and D). (E) RT-qPCR analysis of total, intron retention, and mature *MAT2A* mRNA levels in BT549 cells treated with olaparib (5 μ M), or cycloleucine (20 mM), or their combo. (F and G) Representative western blots (F) and quantitation (G) showing levels of MAT2A and METTL16 in BT549 cells stably expressing the indicated vectors treated with olaparib (5 μ M), or cycloleucine (20 mM), or their combo. (A, C, and F) Experiment was repeated 3 times, and representative blots are presented. (B, D, E, and G) Data are shown as the mean $\pm$ SD from 3 independent experiments. *P* values are indicated. Significance determined using (B, D, E, and G) 1-way ANOVA test.



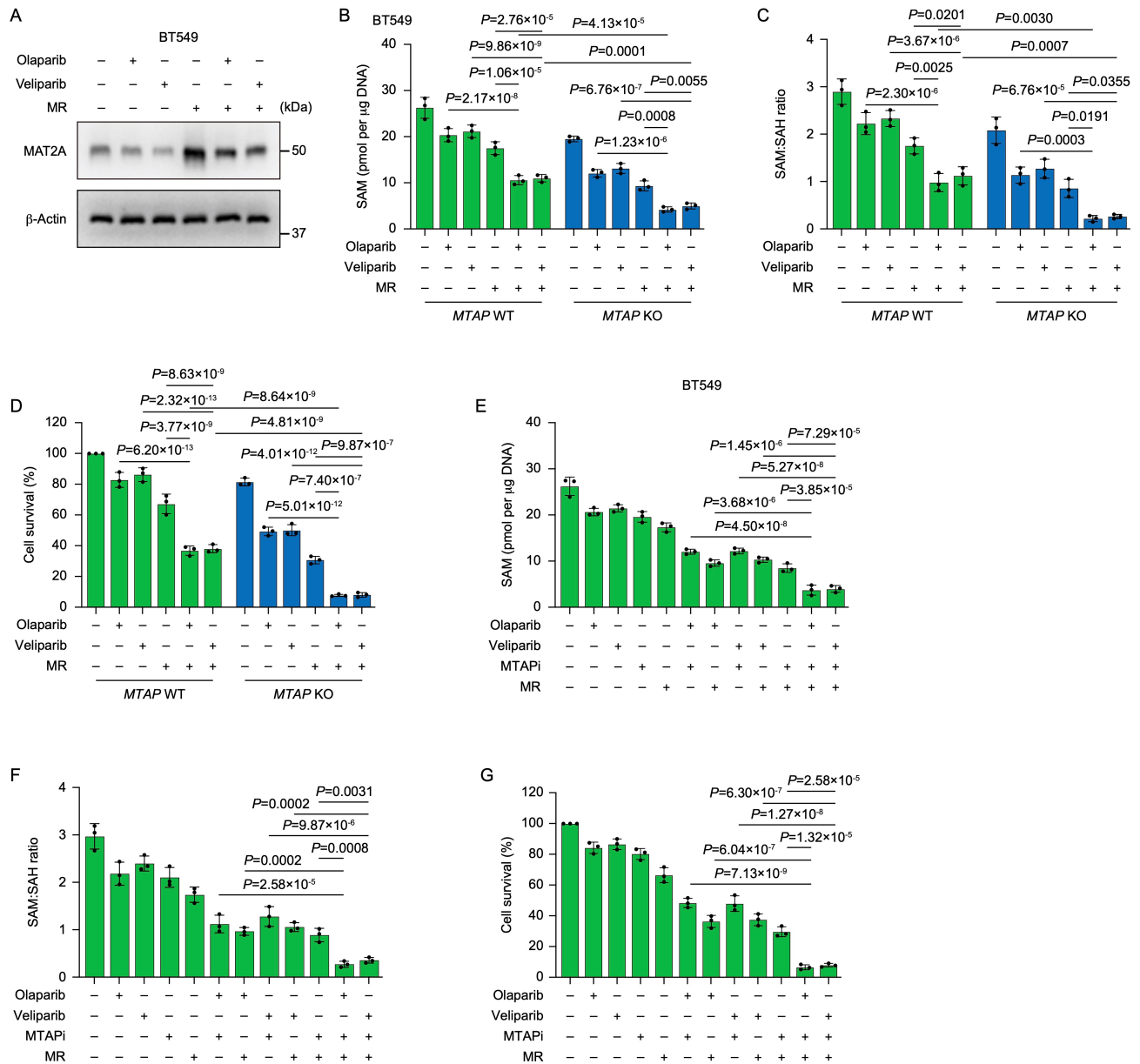
Supplemental Figure 6. Inhibition of MTAP and PARP synergistically eliminates tumor cells by depleting SAM. (A and B) Intracellular SAM (A) and SAM:SAH ratio (B) of BT549 cells treated with olaparib (2 μ M), veliparib (2 μ M), or MTAPi (1 μ M), or the indicated combination. (C and D) Intratumor SAM (C) and SAM:SAH ratio (D) of BT549 xenograft tumors treated with olaparib (50 mg/kg), veliparib (50 mg/kg), or MTAPi (10 mg/kg), or the indicated combination. Data are shown as the mean $\pm$ SD from one representative experiment of 5 mice per group. (E-G) Intracellular SAM (E), SAM:SAH ratio (F), and colony formation assay (G) of BT549 cells treated with olaparib, veliparib, or MTAPi, or the indicated combination, and were supplemented with 100 μ M Met, SAM, SAH, Hcy, or MTA. (A, B, and E-G) Data are shown as the mean $\pm$ SD from 3 independent experiments. *P* values are indicated. Significance determined using (A-G) 1-way ANOVA test.



Supplemental Figure 7. Dual inhibition of MTAP and PARP suppresses tumor growth by decreasing SAM. (A) Representative western blots showing levels of MAT2A and MTAP in BT549 cells treated with olaparib (2 μ M), veliparib (2 μ M), MTAPi (1 μ M), or SAM (100 μ M), or the indicated combination. Experiment was repeated 3 times, and representative blots are presented. (B-E) Tumor growth inhibition (B), Kaplan-Meier survival curves (C), intratumor SAM (D), and SAM:SAH ratio (E) of BT549 xenograft tumors treated with olaparib, veliparib, or MTAPi, or the indicated combination. Olaparib and veliparib were used at 50 mg/kg and MTAPi was used at 10 mg/kg, intraperitoneally, 5 times per week. SAM was administered at 10 mg/kg subcutaneously per day. (B, D, and E) Data are shown as the mean $\pm$ SD from one representative experiment of 5 mice per group, $n = 10$ mice per group in C. P values are indicated. Significance determined using (B, D, and E) 1-way ANOVA, or (C) log-rank (Mantel-Cox) test.

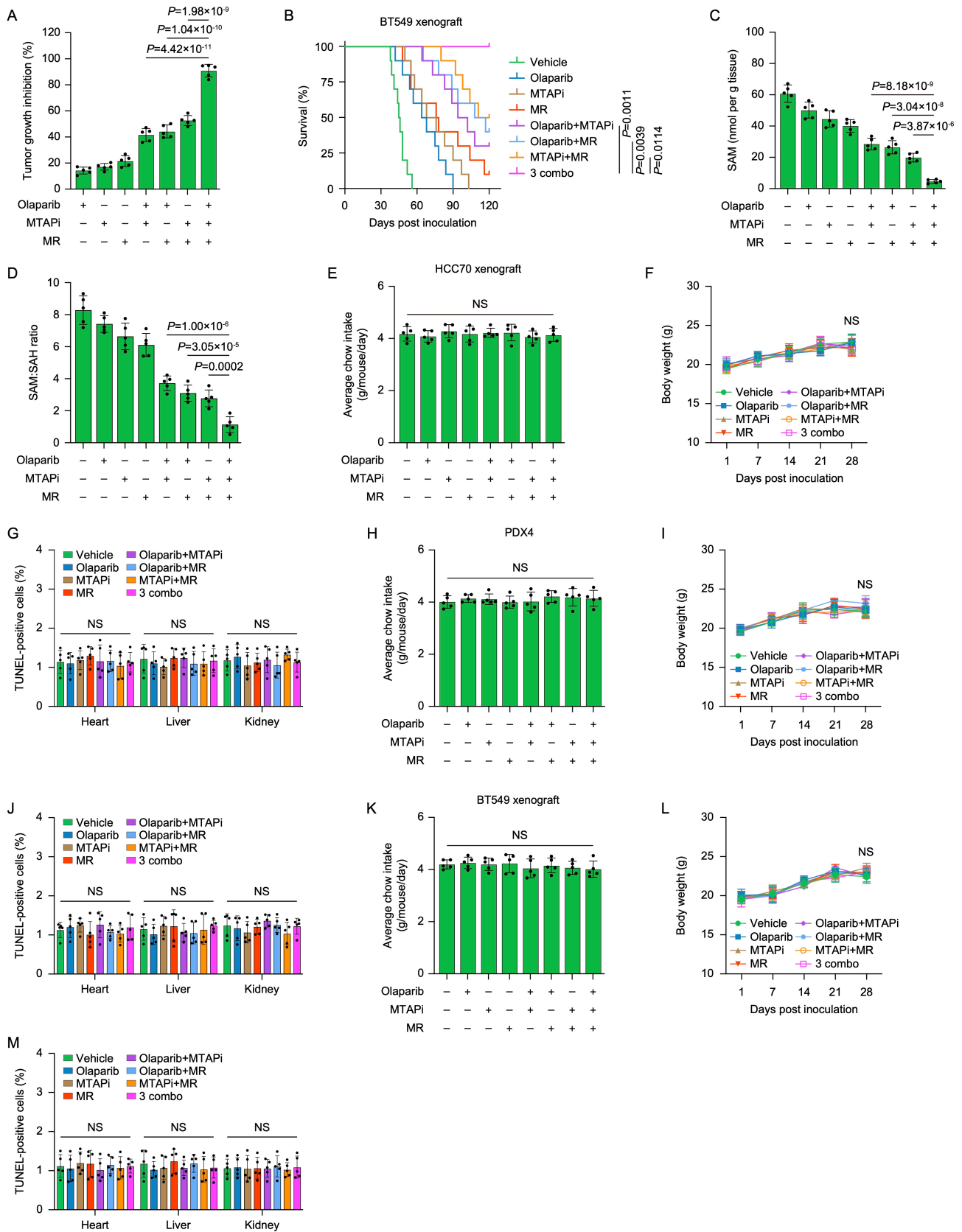


Supplemental Figure 8. Low SAM blocks DNA repair by inhibiting methylation of MRE11. (A-H) Representative micrographs and quantitation for PALB2 (A and B), CtIP (C and D), BRCA1 (E and F), or NBS1 (G and H) foci formation in the indicated cells without treatment, or recovery at 1 h after olaparib (20 μ M) treatment. (I) Representative western blots immunoblotted with the indicated antibodies in cells with WT or depletion *MTAP* treated with or without olaparib. (J) Endogenous IP between MRE11 and RAD50 or NBS1 in cells with WT or depletion *MTAP* treated with or without olaparib (5 μ M). (K) Representative western blots immunoblotted with the indicated antibodies in whole cell lysate and chromatin fraction of the indicated cells without treatment, or recovery at 1 h after olaparib (20 μ M) treatment. (A, C, E, G, and I-K) Experiment was repeated 3 times, and representative micrographs/blots are presented. (B, D, F, and H) Data are shown as the mean $\pm$ SD from 3 independent experiments. *P* values and scale bars are indicated. Significance determined using (B, D, F, and H) unpaired *t* test.

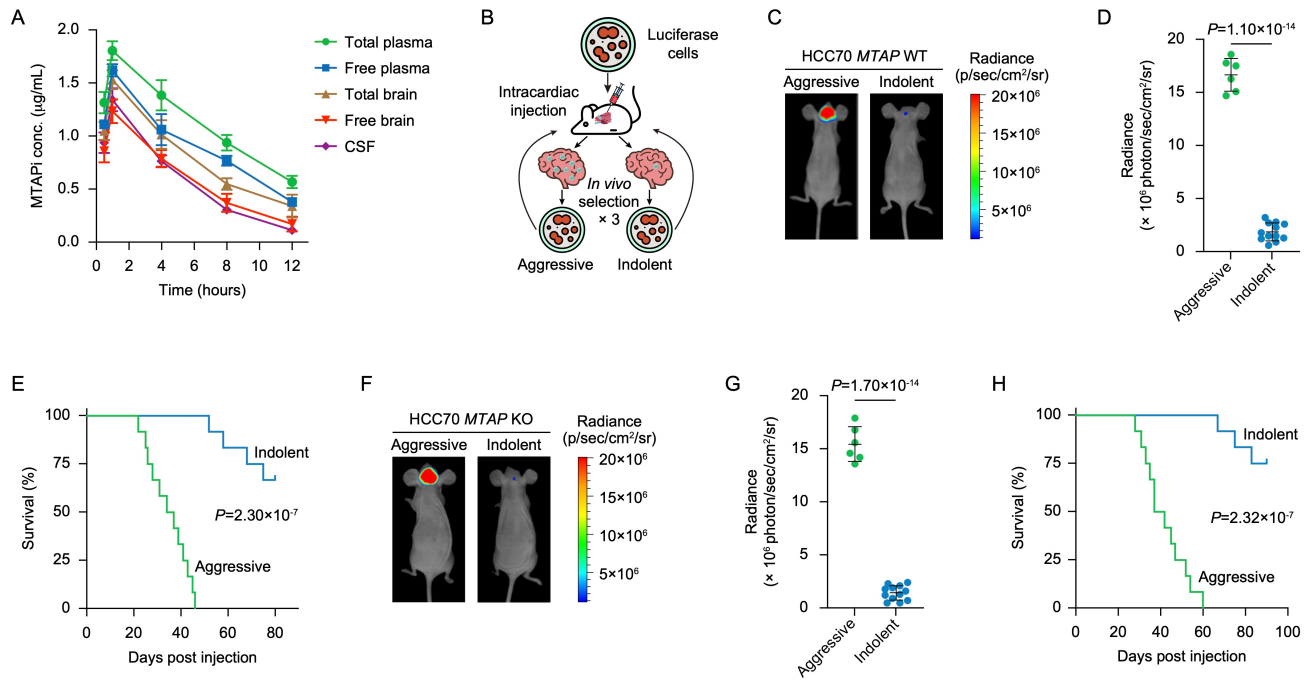


Supplemental Figure 9. MR enhances anti-tumor effect of *MTAP* deficiency/inhibition

combining PARPi by promoting SAM reduction. (A) Representative western blot showing levels of MAT2A in BT549 cells treated with olaparib (2 μ M), veliparib (2 μ M), or methionine restriction (MR) (20%), or the indicated combination. Experiment was repeated 3 times, and representative blots are presented. (B-D) Intracellular SAM (B), SAM:SAH ratio (C), and colony formation assay (D) of BT549 cells with or without *MTAP* deletion treated with olaparib, veliparib, or MR, or the indicated combination. (E-G) Intracellular SAM (E), SAM:SAH ratio (F), and colony formation assay (G) of BT549 cells treated with olaparib, veliparib, MTAPi, or MR, or the indicated combination. (B-G) Data are shown as the mean $\pm$ SD from 3 independent experiments. *P* values are indicated. Significance determined using (B-G) 1-way ANOVA test.



Supplemental Figure 10. Triple combination of MR and *MTAP* deficiency/inhibition combining PARPi is well tolerant. (A-D) Tumor growth inhibition (A), Kaplan-Meier survival curves (B), intratumor SAM (C), and SAM:SAH ratio (D) of BT549 xenograft tumors treated with olaparib, MTAPi, MR, or the indicated combination. All drugs were used at 10 mg/kg, intraperitoneally, 5 times per week. The control diet contained 0.86% methionine and methionine restriction diet contained 0.12% methionine. (E-M) Average chow intake, body weight, and TUNEL staining of heart, liver, and kidney of HCC70 (E-G), PDX4 (H-J), and BT549 (K-M) xenograft tumors with the indicated treatment. (A and C-M) Data are shown as the mean $\pm$ SD from one representative experiment of 5 mice per group, $n = 10$ mice per group in B. *P* values are indicated. Significance determined using (A, C-E, G, H, J, K, and M) 1-way ANOVA, (B) log-rank (Mantel-Cox), or (F, I, and L) 2-way ANOVA test.



Supplemental Figure 11. Establishment of aggressive BrM HCC70 cells with WT or deleted *MTAP*. (A) *In vivo* pharmacokinetic study of MTAPi. (B) Schematic representation of *in vivo* selection for aggressive brain metastatic HCC70 cells with WT or deleted *MTAP*. (C-H) Representative *in vivo* bioluminescence imaging and quantification of radiance at day 35 post injection, and Kaplan-Meier survival curves of HCC70 aggressive or indolent BrM intracardiac injection mouse models with WT (C-E) or deleted *MTAP* (F-H). (A) Data are shown as the mean $\pm$ SD from 3 independent experiments, (D and G) data are shown as the mean $\pm$ SD from one representative experiment, $n = 12$ mice per group in E and H. P values are indicated. Significance determined using (D and G) unpaired t , or (E and H) log-rank (Mantel-Cox) test.