Supplementary material

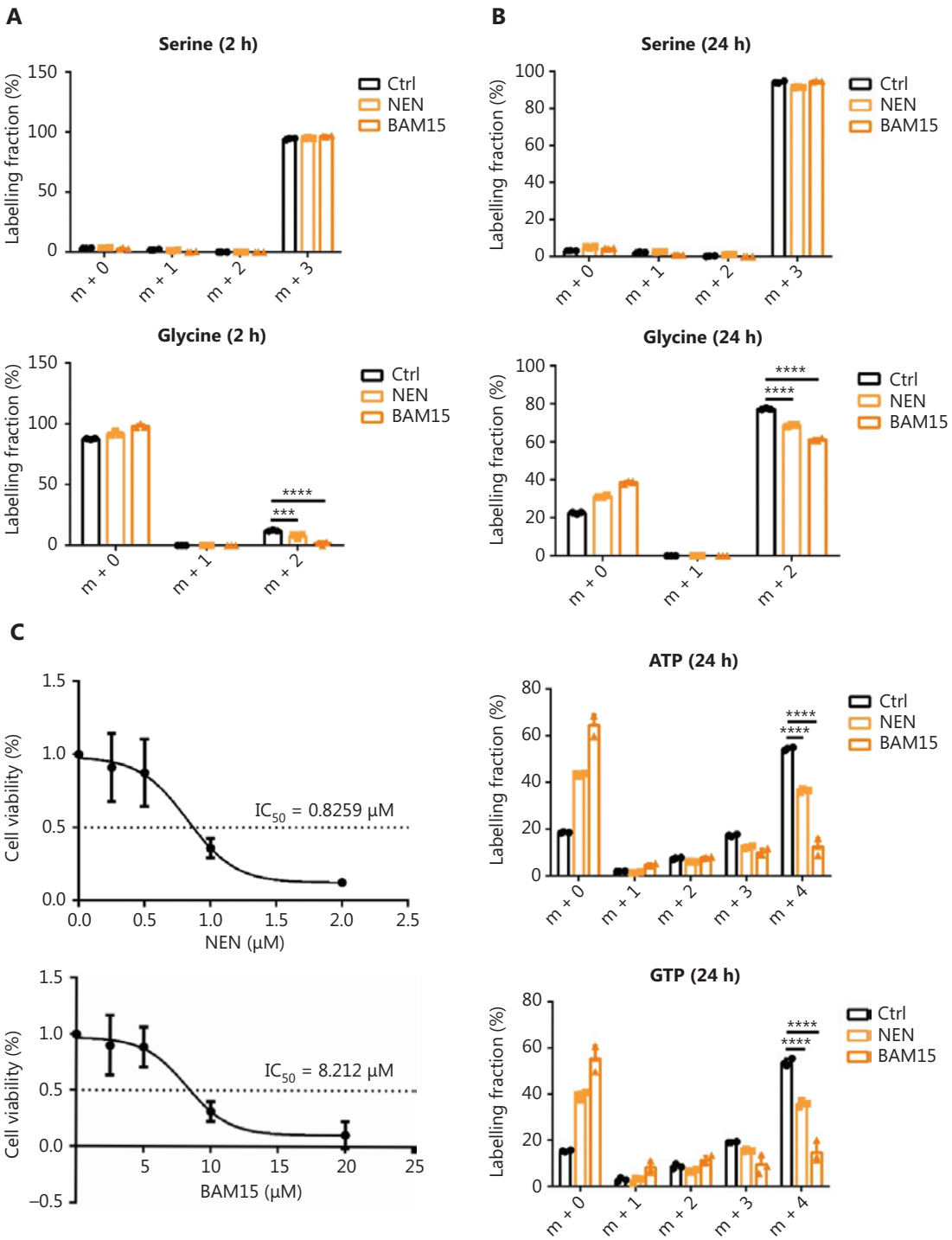


Figure S1 NEN and BAM15 treatment inhibits serine catabolism and *de novo* purine synthesis. 4175-LM2 cells were labeled with U-^{13}C-serine for 2 (A) or 24 h (B) and co-treated with DMSO, 1 μM NEN, or 10 μM BAM15. The relative isotopic labeling fraction of metabolites was measured using LC-MS (mean ± SD, n = 3, ***P < 0.001 ****P < 0.0001 by two-tailed Student's t-test). (C) Relative cell viability of 4175-LM2 cells after 3 days of NEN or BAM15 treatment and normalized to DMSO control.

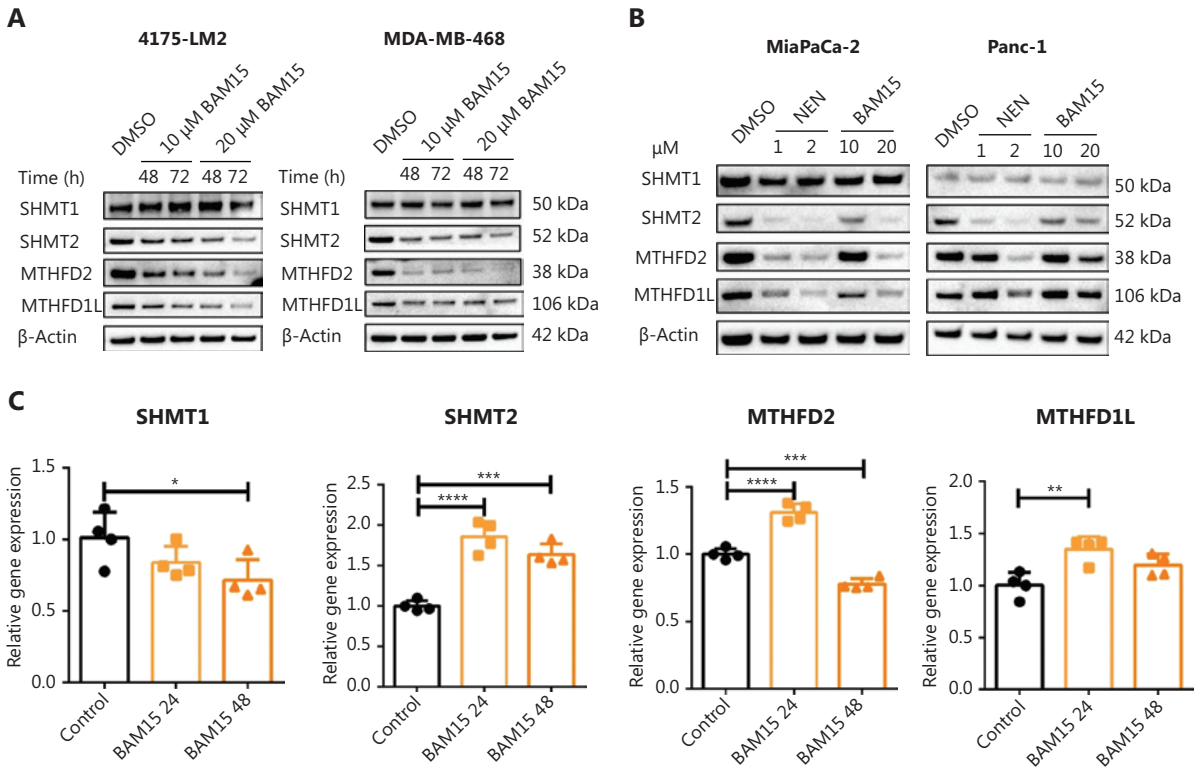


Figure S2 Mitochondrial uncoupling inhibits mitochondrial serine catabolic enzyme expression. (A) Immunoblots of the MSCP enzymes in 4175-LM2 and MDA-MB-468 cells treated with different concentrations of BAM15 for 48 or 72 h. (B) Immunoblots of the MSCP enzymes in MiaPaCa-2 and Panc-1 cells (PDAC cells) treated with different concentrations of NEN and BAM15 for 48 h. (C) RT-qPCR analysis of *SCP* gene expression in 4175-LM2 cells treated with 10 μM BAM15 for 24 and 48 h. Data represent the average ± SD (n = 4, *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001).

A

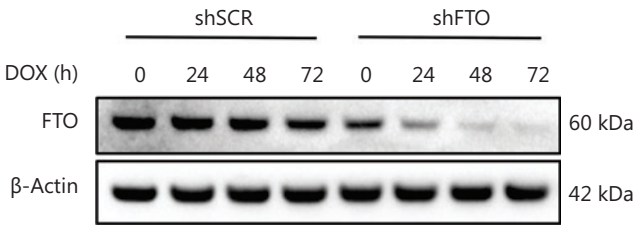
FTO

Comparison	Statistical significance
Normal-*vs.*-luminal	1.62447832963153E-12
Normal-*vs.*-HER2 positive	1.62436730732907E-12
Normal-*vs.*-TNBC	1.62447832963153E-12
Luminal-*vs.*-HER2 positive	2.813700E-04
Luminal-*vs.*-TNBC	4.215900E-03
HER2 positive-*vs.*-TNBC	7.181200E-01

ALKBH5

Comparison	Statistical significance
Normal-*vs.*-luminal	7.115400E-01
Normal-*vs.*-HER2 positive	1.871860E-02
Normal-*vs.*-TNBC	9.644000E-01
Luminal-*vs.*-HER2 positive	1.281540E-02
Luminal-*vs.*-TNBC	9.211000E-01
HER2 positive-*vs.*-TNBC	6.612100E-02

B



C

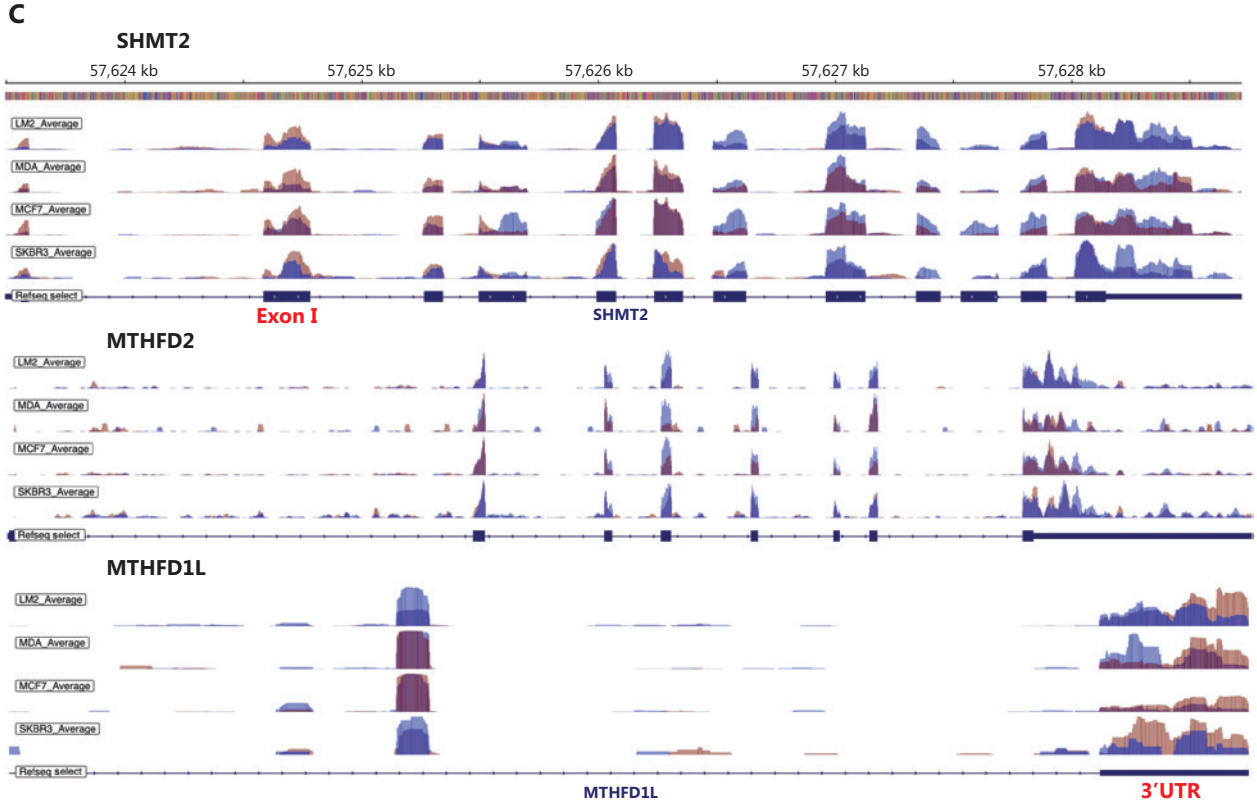


Figure S3 Continued

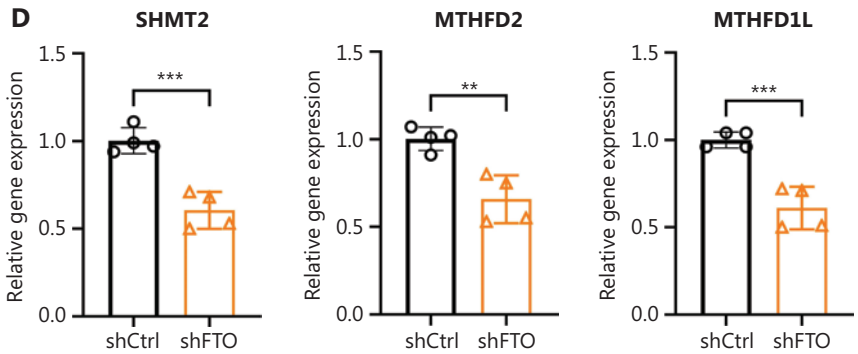


Figure S3 The m^{6}A demethylase, FTO, is responsible for MSCP mRNA demethylation. (A) The statistics for Figure 3B. (B) Immunoblot analysis was performed on 4175-LM2 Dox-inducible FTO knockdown or shSCR control cells treated with 0.5 μg/mL Dox for 24, 48, or 72 h. (C) m^{6}A peaks were identified in the exon I region of *SHMT2* and the 3′UTR of *MTHFD1L*. The tracks show m^{6}A IP (red) and input control (blue), where an m^{6}A peak is defined by an enrichment of the IP signal over the input. Only the 3′ end is displayed due to the length for the *MTHFD1L* gene. LM2, 4175-LM2; MDA, MDA-MB-231. (D) Real-time PCR analysis of MSCP gene expression in 4175-LM2 Dox-inducible shCtrl or shFTO cells. Data represent the average ± SD (n = 4, *P < 0.05, **P < 0.01 and ***P < 0.001).

A

Cox model for Clynes data

Term	Estimate	Conf.low	Conf.high	*P*.value
Fto_catlow	1.596	0.753	3.380	0.222
Grade	2.202	1.250	3.881	0.006
ER_status	0.589	0.318	1.091	0.092

Cox model for Bertucci data (stratified by ER_IHC)

Term	Estimate	Conf.low	Conf.high	*P*.value
Fto_catlow	1.982	0.982	3.997	0.056
Grade	1.491	1.050	2.117	0.026

B

FTO

Comparison	Statistical significance
Primary pretreatment-*vs.*-primary posttreatment	2.477324E-01
Primary pretreatment-*vs.*-metastasis brain	9.828360E-01
Primary pretreatment-*vs.*-metastasis lung	3.418596E-01
Primary pretreatment-*vs.*-metastasis liver	6.178915E-02
Primary pretreatment-*vs.*-metastasis others	1.798809E-01
Primary posttreatment-*vs.*-metastasis brain	2.586341E-01
Primary posttreatment-*vs.*-metastasis lung	1.170367E-01
Primary posttreatment-*vs.*-metastasis liver	6.646997E-02
Primary posttreatment-*vs.*-metastasis others	1.040857E-01
Metastasis brain-*vs.*-metastasis lung	3.960275E-01
Metastasis brain-*vs.*-metastasis liver	1.204096E-01
Metastasis brain-*vs.*-metastasis others	2.882213E-01
Metastasis lung-*vs.*-metastasis liver	6.925292E-01
Metastasis lung-*vs.*-metastasis others	9.275921E-01
Metastasis liver-*vs.*-metastasis others	4.092180E-01

SHMT2

Comparison	Statistical significance
Primary pretreatment-*vs.*-primary posttreatment	4.018019E-02
Primary pretreatment-*vs.*-metastasis brain	1.037726E-02
Primary pretreatment-*vs.*-metastasis lung	2.162846E-02
Primary pretreatment-*vs.*-metastasis liver	1.438933E-02
Primary pretreatment-*vs.*-metastasis others	9.16796795577478E-05
Primary posttreatment-*vs.*-metastasis brain	8.878106E-02
Primary posttreatment-*vs.*-metastasis lung	1.834319E-01
Primary posttreatment-*vs.*-metastasis liver	2.053360E-01
Primary posttreatment-*vs.*-metastasis others	5.509872E-01
Metastasis brain-*vs.*-metastasis lung	5.248360E-01
Metastasis brain-*vs.*-metastasis liver	4.601477E-01
Metastasis brain-*vs.*-metastasis others	1.319931E-01
Metastasis lung-*vs.*-metastasis liver	9.064304E-01
Metastasis lung-*vs.*-metastasis others	2.853729E-01
Metastasis liver-*vs.*-metastasis others	3.240387E-01

Figure S4 Continued

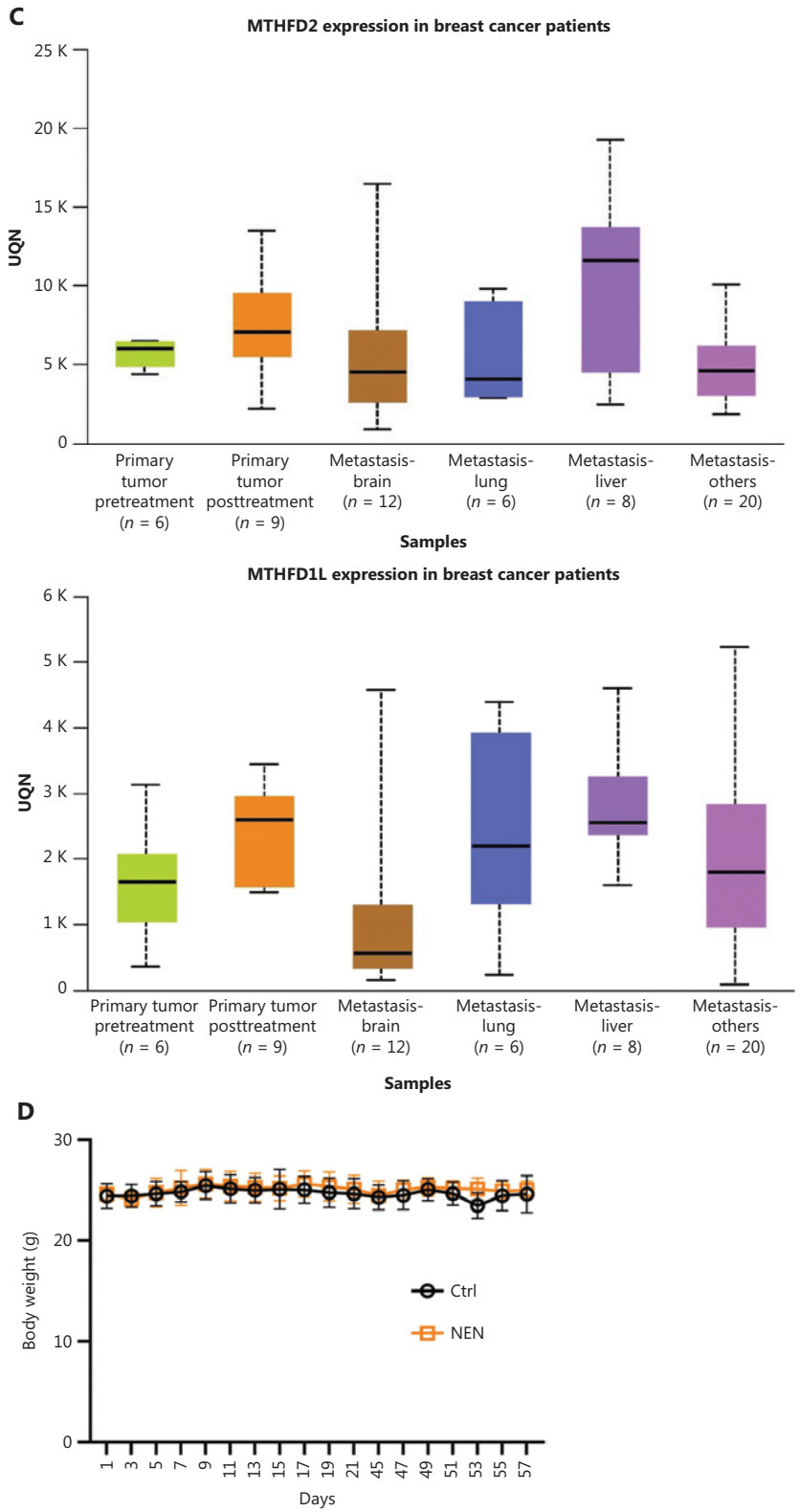


Figure S4 Levels of FTO and MSCP gene expression in primary and metastatic breast cancer. (A) Cox model for patient survival data in Figure 5A and B. (B) The statistics for Figure 5C. (C) Levels of MTHFD2 and MTHFD1L RNA expression across different stages of breast cancer. (D) Body weights of mice in the control and NEN treatment groups were recorded at the indicated time points throughout the study. Data are presented as the mean ± SEM.