Supplementary Data

Supplementary Material and Methods

Methioninase

Recombinant methionine γ-lyase [1] was kindly provided by Dr. Vadim Pokrovsky (N.N. Blokhin Cancer Research Center, Moscow, Russia).

NADP/NADPH assay

The NADP/NADPH ratio was measured using the NADP/NADPH Assay kit (Sigma-Aldrich #MAK038) according to the manufacturer's protocol. Cells were seeded overnight in 100 mm plates (4×10^6 cells/plate). The next day, the media was replaced with control or MR media, and cells were incubated for 0-72 hours. 4×10^6 cells have been used for NADP/NADPH extraction. To detect NADPH, NADP was decomposed via sample incubation at 60º C for 30 min. Total NADP (NADP and NADPH) or NADPH were measured by colorimetric assay at 450 nm.

Immunoblotting

Whole-cell lysates were immunoblotted using primary Abs against xCT (Cell Signaling Technology), CD98hc (Cell Signaling Technology) and β-actin (Sigma-Aldrich).

Cell viability and synergy analyses

Cell viability was measured using the CellTiter 96 AQueous One Solution Cell Proliferation (MTS) Assay (Promega) or CellTiter-Glo 2.0 Assay (Promega) according to the manufacturer's protocol. Briefly, cells were plated in 96-well plates (2.5×10^3 cells/well) overnight. The next day, the media was replaced with control or MR media, and the cells were incubated with the indicated

concentrations of drugs for the indicated number of hours. Cell viability was expressed as the percentage of viable cells compared to control vehicle-treated cells in complete medium. Synergy was assessed using the using the Excess over Bliss (EOB) method with positive EOB values indicating synergy and negative values indicating antagonism [2].

Histology

PDX TM00098 (Jackson Laboratory) mammary tumors were harvested at autopsy and fixed in 10% formalin. Tissue was paraffin-embedded, sectioned, and analyzed by H&E and immunohistochemistry staining for ER, PR and HER2 TRIP by the Laboratory of Translational Research Initiatives in Pathology at UW-Madison using standard methods with the respective antibodies (Ventana).

Supplementary Figure Legends

Fig. S1. Methionine restriction transiently increases the NADP/NADPH ratio and increases TXNRD activity in TNBC cells. **A** and **B**, GILM2 **(A)** or MDA-MB-468 **(B)** TNBC cells were grown in MR media for the indicated number of hours. The NADP/NADPH ratio and TXNRD activity (expressed as percentage of time t=0) was determined (mean ± SEM, n = 3). In all panels, *, $P < 0.05$, **, $P < 0.01$ and ***, $P < 0.001$ vs. t = 0.

Fig. S2. Methionine restriction increases expression of components of the glutamate-cystine antiporter xCT. Immunoblot of GILM2 and MDA-MB-468 TNBC cells grown in control or MR media for the indicated number of hours. These lysates were previously probed for TXNRD1, TXNRD2 expression (**Fig. 1C** and **1D** in the main body of the manuscript).

Fig. S3. Synergy analyses of methionine restriction and auranofin cytotoxicity.

A, GILM2 (black) and MDA-MB-468 (red) TNBC cells were incubated with auranofin (AU, 0.7 μM) and media containing varying concentrations (0-100 μM) of methionine for 24 hours and cell viability was measured by CellTiter-Glo 2.0 Assay (mean ± SEM, n = 3). **B**, Synergy analyses of the combination of MR and auranofin (AU) using the Excess over Bliss method [2]. Synergy is indicated by positive values (red).

Fig. S4. Methioninase sensitizes TNBC cells to auranofin. A and **B,** Crystal violet cell survival assay of GILM2 **(A)** or MDA-MB-468 **(B)** TNBC cells incubated with vehicle or methioninase (4 units/ml) for 72 hours and treated with vehicle or auranofin (1 μM) for the final 24 hours. Left panel: representative images. Middle panel: quantification of percentage confluence performed by counting cells in 3 fields of each well (mean ± SEM, n = 3). Right panel: GILM2 (**A**) and MDA-MB-468 (**B**) TNBC cells incubated with vehicle or methioninase (4 units/ml) for 72 hours, treated with vehicle or auranofin (1 μM) for the final 24 hours, and caspase-3/7 activity was measured (expressed as fold control, mean ± SEM, n=3). In all panels, **, $P < 0.01$, ***, $P < 0.001$ vs. vehicle control or the indicated comparison

Fig. S5. BSO does not sensitize TNBC cells to methionine restriction. A, GILM2 or MDA-MB-468 TNBC cells were grown in control or MR media for 72 hours and treated with vehicle or the indicated concentrations (μM) of BSO for the final 24 hours. Cell viability (expressed as the percentage of control-treated cells) was measured by CellTiter-Glo 2.0 Assay (mean ± SEM, n = 3). **B**, GILM2 TNBC cells were grown in control or MR media for 72 hours and treated with

vehicle or BSO (400 μM) for the final 24 hours. Left panel: representative images. Middle panel: quantification of percentage confluence performed by counting cells in 3 fields of each well (mean ± SEM, n = 3). Right panel: GILM2 TNBC cells were grown in control or MR media for 72 hours, treated with vehicle or BSO (400 μM) for the final 24 hours, and caspase-3/7 activity was measured (expressed as fold control, mean ± SEM, n=3). In all panels, *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$ vs. vehicle control.

Fig. S6. Body weights of tumor-bearing female NSG mice in each treatment group.

Mouse body weights on the last day of treatment for GILM2-mCherry **(A)** and TM00098 PDX **(B)** models. In all panels, **, $P < 0.01$, ***, $P < 0.001$ vs. vehicle control

Fig. S7. Immunohistochemistry analyses of TM00098 PDX mammary tumors.

Representative images of primary PDX tumors stained with H&E and analyzed by immunohistochemistry for ER, PR and HER2 expression.

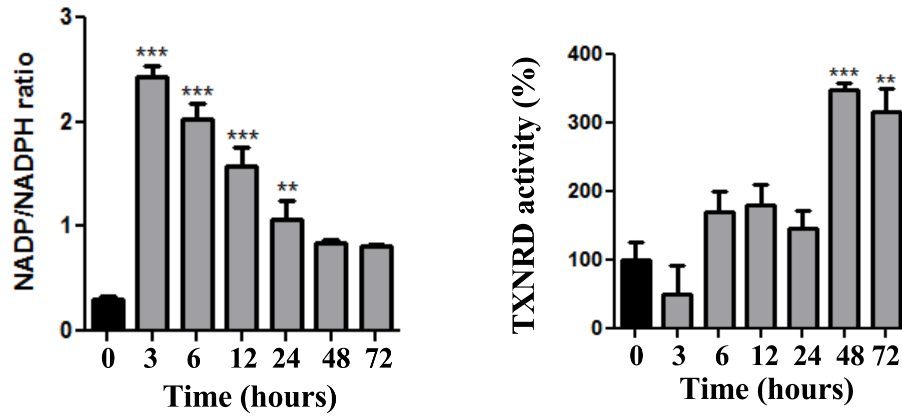
Supplementary References

1. Pokrovsky VS, Yu Anisimova N, Zh Davydov D, Bazhenov SV, Bulushova NV, Zavilgelsky GB, et al. (2019) Methionine gamma lyase from Clostridium sporogenes increases the anticancer effect of doxorubicin in A549 cells and human cancer xenografts. Invest New Drugs 37:201-209.

2. Liu Q, Yin X, Languino LR, Altieri DC. (2019) Evaluation of drug combination effect using a Bliss independence dose-response surface model. Stat Biopharm Res 10:112-122.

A

GILM2



B

MDA-MB-468

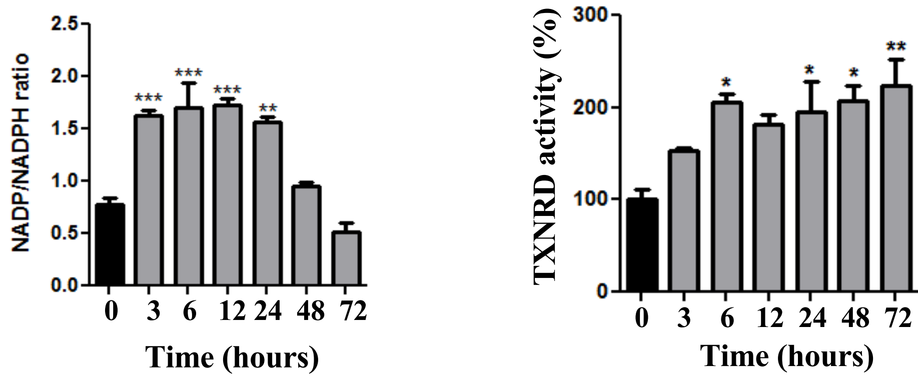


Fig. S1

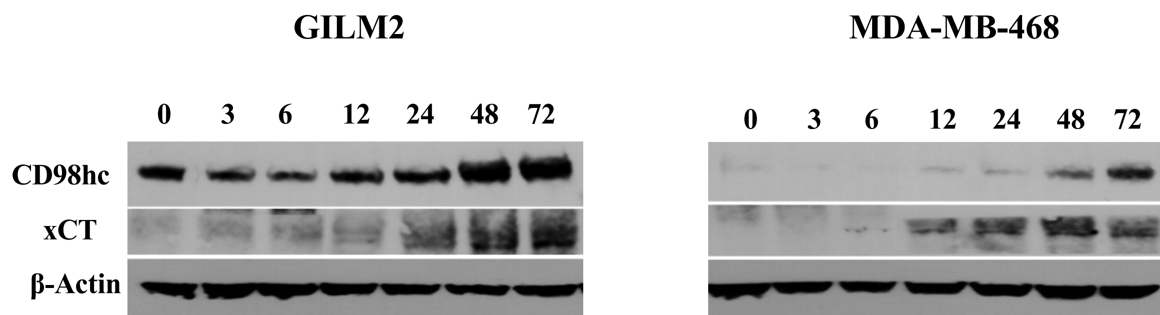
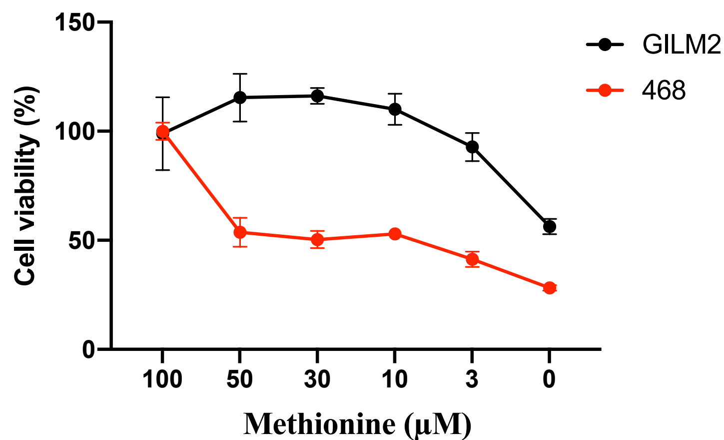


Fig. S2

A



B

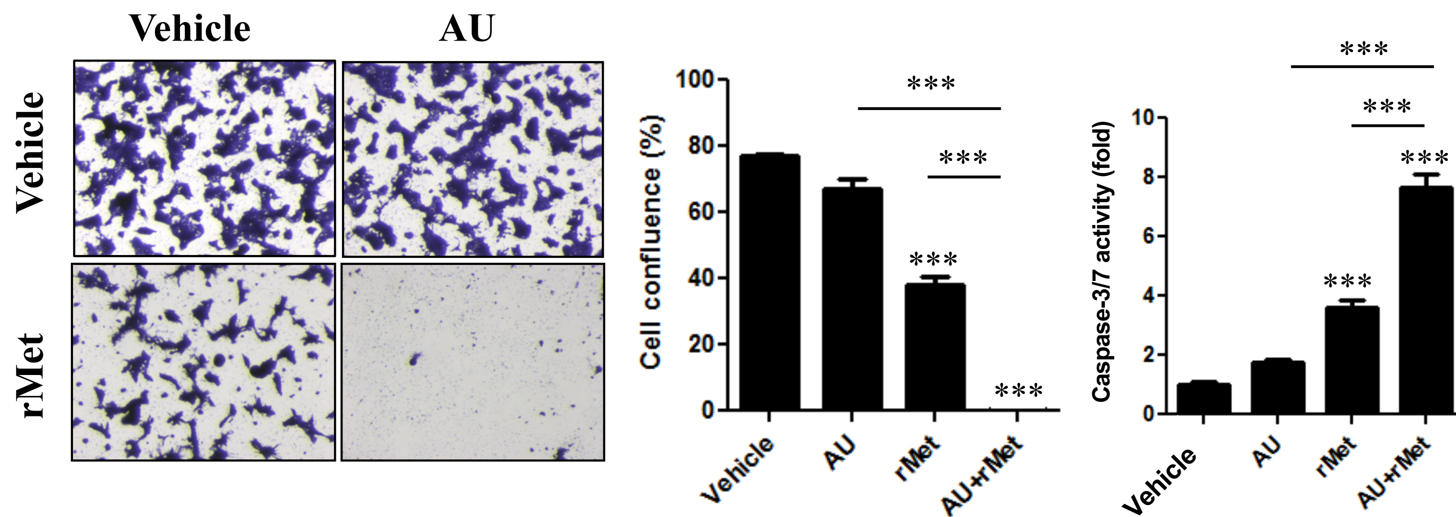
GILM2

Methionine	AU µM		
	0.3	0.7	1.5
50 µM	-49.186005	-17.820743	**5.21231669**
30 µM	-51.499007	-19.963788	**26.0385979**
10 µM	-35.66212	-15.040414	**28.7320459**
3 µM	-21.798497	**20.3319895**	**52.4061904**
0 µM	-36.600642	**16.8726478**	**31.9354671**

MDA-MB-468

Methionine	AU µM		
	0.3	0.7	1.5
50 µM	-30.956672	**20.4426004**	**27.5221547**
30 µM	-28.928369	**19.8328596**	**27.4603359**
10 µM	-11.186122	**10.554843**	**23.201678**
3 µM	-8.7779949	**14.8525974**	**10.5562869**
0 µM	-1.211922	**7.71897248**	**7.65470489**

Fig. S3



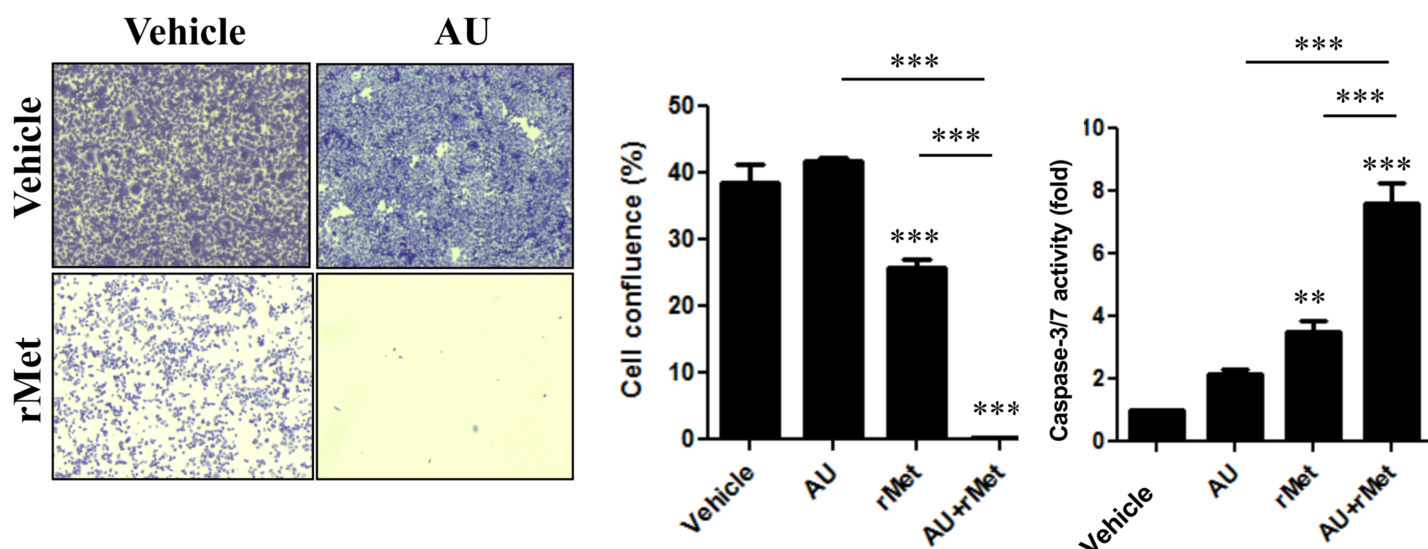
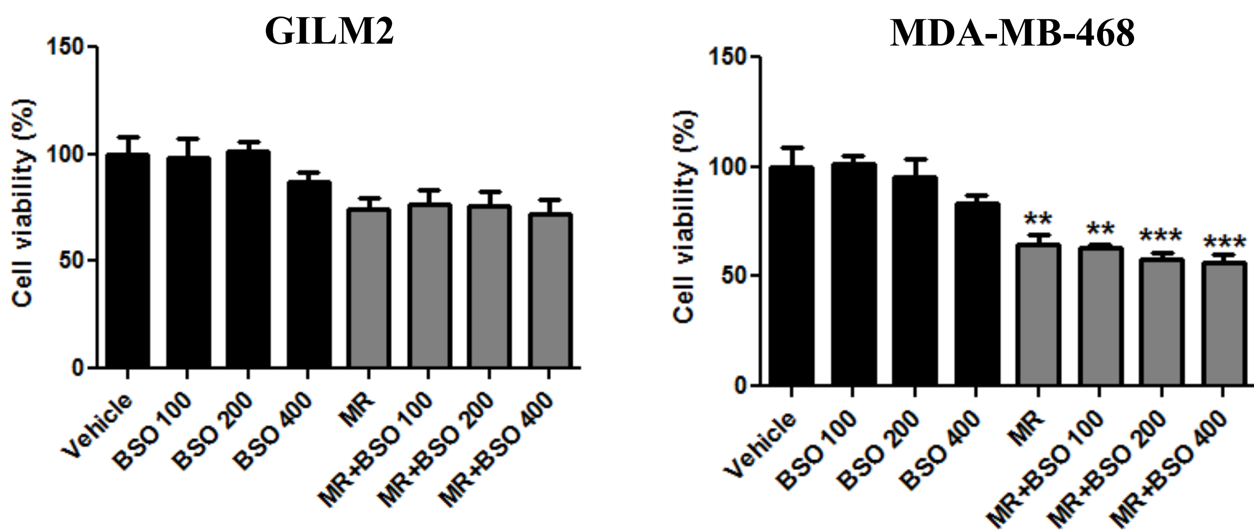


Fig. S4



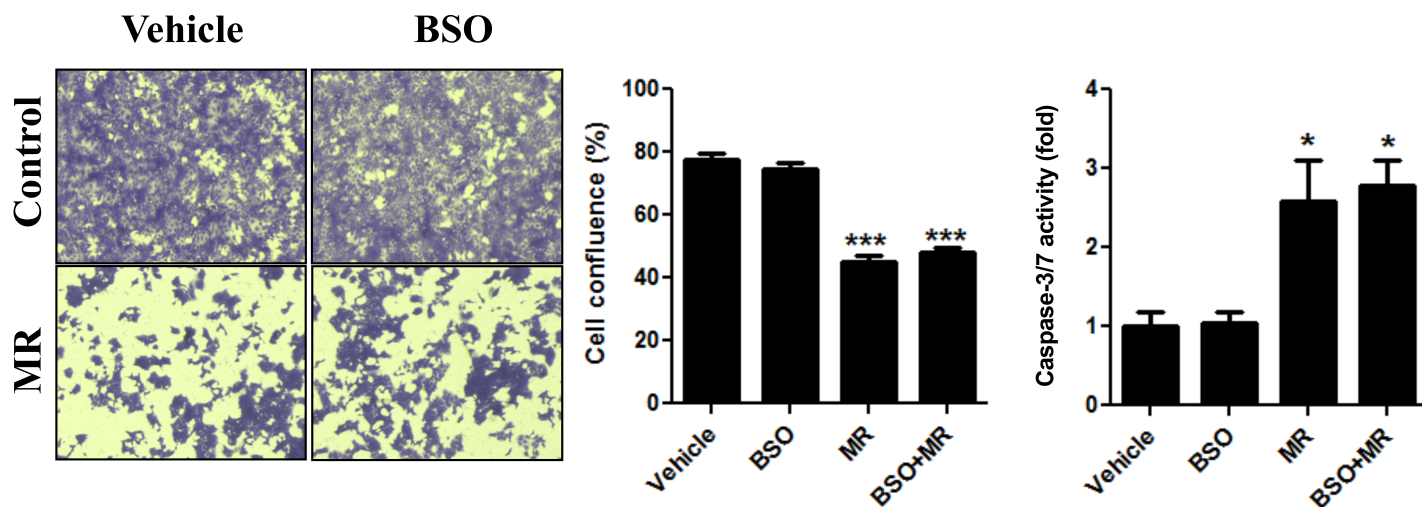
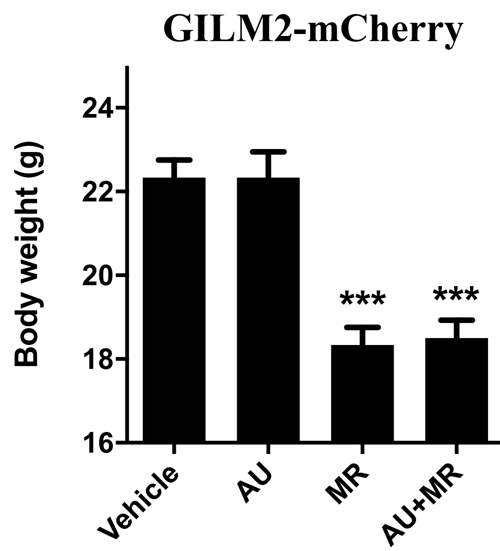


Fig. S5



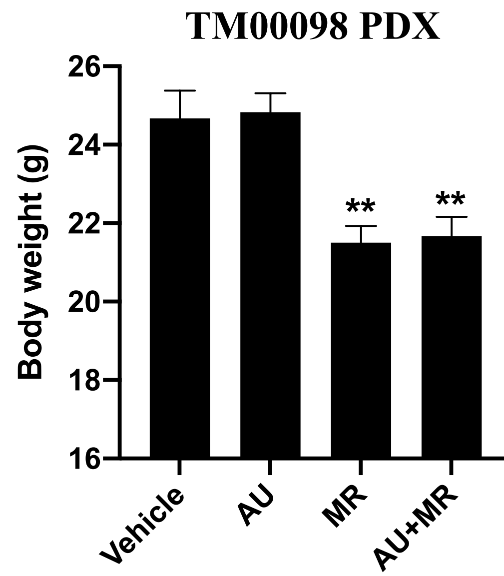
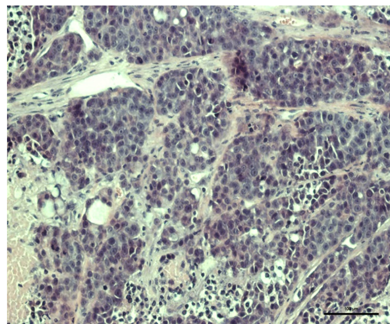
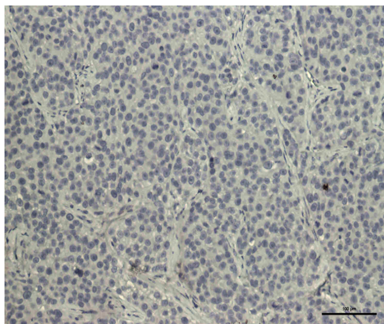


Fig. S6

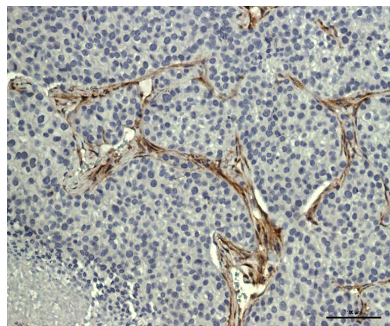
H&E



Estrogen Receptor (ER)



Progesterone Receptor (PR)



HER2

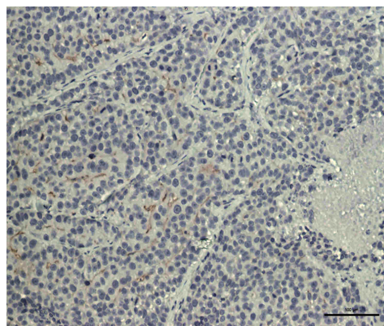


Fig. S7