**Supplementary Material and Methods**

**Ki67 analysis**

To determine the number of Ki67-positive TNBC cells, cells were fixed with methanol for 5 minutes, permeabilized with 0.1% PBS-Tween 20 and incubated with Ki67 Ab (Abcam) for 30 minutes at room temperature. Cells were then washed 3 times with PBS, resuspended in PBS containing 4′,6-diamidino-2-phenylindole ( DAPI, 1 μg/mL), and analyzed by flow cytometry.

**Mass spectrometry analysis**

Cells were cultured in complete or 0% Met medium for 72 hours (three replicates per condition), trypsin-released, and 5 x 106 cells for each sample were spun down at 1200 rcf prior to further processing. The resulting pellet was lysed and the proteins digested as previously described ([1](#_ENREF_1)). Protein concentration was determined using BCA assay (Thermo Scientific), and 100 μg of each sample was processed and digested as described ([2](#_ENREF_2)). Resulting peptides were desalted using Sep-Pak solid phase extraction cartridges (C18; Waters) and samples were then isobarically tagged employing a Tandem Mass Tag (TMT) 6-plex strategy according to manufacturer’s specifications (Thermo Scientific). Tagged samples were then combined in 1:1 ratios (as determined from a preliminary experiment) prior to analysis by LC-MS/MS employing a Waters nanoAcquity nLC (Waters) coupled to an Orbitrap Velos Pro mass spectrometer (Thermo Scientific). Survey massspectra were acquired at 60,000 resolving power and a data-dependent top-10 method was employed, with each precursor ion being fragmented by higher-energy collisional dissociation (HCD) ([3](#_ENREF_3), [4](#_ENREF_4)). Resulting .raw data files were converted to .txt files compatible for searching with the Open Mass Spectrometry Search Algorithm (OMSSA) ([4](#_ENREF_4)) using in-house software ([5](#_ENREF_5), [6](#_ENREF_6)). Searches were run against a concatenated version of the International Protein Index human proteindatabase (ipi.HUMAN.v3.87) using OMSSA with the following parameters: peptide tolerance ±2 Da, fragment ion tolerance ±0.8 Da, a maximum of three missed cleavages, fixed modifications of carbamidomethyl on Cysteine and TMT 6-plex on Lysines and N-*termini,* and variable modifications of oxidation on Methionine and TMT 6-plex on Tyrosine. Search results were downloaded as .dat files and peptide identifications subsequently filtered to a < 1% false discovery rate (FDR) using the target-decoy strategy (7) and COMPASS software (6). Peptide and protein quantification were then performed using COMPASS, with a cut-off of 5% FDR for protein identifications.

**Supplementary Figure and Table Legends**

**Figure S1. Methionine restriction inhibits cell proliferation**. mCherry-labeled MDA-MB-231 (231), GILM2 or MDA-MB-468 (468) TNBC cells were grown in control or 0% Met media for 72 hours. The number of Ki67-positive cells was determined by flow cytometry using a Ki67 Ab (mean ± SEM, n = 3). \*, *P* < 0.05, \*\*, P < 0.01

**Figure S2. Methioninase sensitizes TNBC cells to lexatumumab. A,** Crystal violet cell survival assay of TNBC cells pretreated with vehicle or methioninase (0.2 units/ml, AntiCancer) for 24 hours, and treated for 48 hours with vehicle, lexatumumab (0.3 μg/ml) for MDA-MB-231 and GILM2 cells, or lexatumumab (0.4 μg/ml) for MDA-MB-468 cells. Left panel: representative images. Right panel: quantification performed by counting cells in 3 fields of each well (mean ± SEM, n = 3). \*\*, P < 0.01, \*\*\*, P < 0.001 **B,** TNBC cells were pretreated with vehicle or methioninase (0.4 units/ml) for 24 hours, and treated overnight with vehicle, lexatumumab (1.5 μg/ml) for MDA-MB-231 and GILM2 cells, or lexatumumab (2 μg/ml) for MDA-MB-468 cells. PARP and cleaved PARP were detected by immunoblotting. **C,** Cancer cells were treated with vehicle or methioninase (0.2 units/ml) for 48 hours followed by isolation of total RNA. Expression of TRAIL-R1 and TRAIL-R2 was assessed by real-time PCR and normalized to expression in TNBC cells grown in control media.

**Table S1. Composition of diets.** The nutrient and amino acid composition (g/kg) for the control and methionine-free diets is indicated.

**Table S2. Identification of proteins differentially expressed in response to methionine deprivation.** MDA-MB-231-mCherry and GILM2 TNBC cells were cultured in control of methionine-free media for 72 hours and differentially expressed proteins were identified by mass spectrometry. Identification of proteins was limited to those with a false discovery rate less than five percent. Proteins found to be significantly regulated (P < 0.05) by methionine deprivation in MDA-MB-231-mCherry cells (left columns) and GILM2 cells (right columns) are listed. Expression is indicated as log-transformed mass spectrometry intensities.

**Supplementary References**

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