**Supplemental Methods:**

**Cell Lines:**

The following human sarcoma cell lines were kindly provided: SK-LMS-1, MG-63, HTB-93 (ATCC, Manassas, VA), SYO-1 (Dr. Akira Kawai, National Cancer Centre Hospital, Tokyo, Japan), Fuji (Dr. Kazuo Nagashima, Hokkaido University School of Medicine, Sapporo, Japan), Aska-SS, Yamata-SS, HS-SY-II (purchased from Riken BRC, deposited by Dr. Naka Norifumi and Dr. Sonobe Hiroshi, Ibaraki, Japan) and MOJO (Dr. K. Jones, University of Utah, Salt Lake City, UT). HTB-93 was isolated in 1974 morphologically resembles SS but lacks the pathognomonic translocation that is classic for SS, making it a tissue type control that does not represent translocation dependent SS biology(1). SK-LMS-1, MG-63, Aska-SS, HS-SY-II, HTB-93, MOJO, SYO-1, and Yamata-SS cell lines were cultured at 37oC in 5% CO2, in Dulbecco’s Modified Eagle Medium (DMEM) (Life Technologies, Grand Island, NY) supplemented with 10% FBS and Penicillin-Streptomycin 100x (10,000U/mL) (Life Technologies, Grand Island, NY) Fuji was cultured in RPMI Medium 1640 (Life Technologies, Grand Island, NY) supplemented with 10% FBS and Penicillin-Streptomycin 100x (10,000U/mL) (Life Technologies, Grand Island, NY). Cell lines were tested for mycoplasma and confirmed negative with the MycoAlert Mycoplasma Detection kit (Lonza LT07-118).

**Microarray:**

Synovial sarcoma samples are from gene expression omnibus (GEO) accession GSE20196 (2), and other sarcoma samples are from GSE21050 (3), GSE23980(4), and GSE20559 (5). Baseline and insulin sensitive cases were selected from studies involving subcutaneous abdominal fat (adipose) and/or vastus lateralis muscle (skeletal muscle), from accessions GSE13070 (6), GSE20950 (7), GSE13506 (8), GSE35411 (9), GSE6798 (10) and GSE18583 (11). Raw CEL files were downloaded from GEO and processed using BioConductor packages within the R statistical software environment (<http://www.R-project.org>)(12). Array quality was assessed using the package simpleaffy (13), and outlying arrays were identified and excluded using the package mdqc (14). Log base 2 expression values were determined using the package gcrma (15), with Affymetrix probe set specifications. Data were median centered, such that the expression value for each gene is relative to the median value across all specimens in the cohort.

**ME1 Activity Assay:**

Cells were plated at 1x106 in 10cm dishes the day before the assay was performed. Cells were collected and a cytoplasmic fraction was produced (Cayman #10009277). The cytoplasmic fraction was then accessed according to the manufacturer’s protocol (Pyruvate Assay Kit, ab65342, abcam, Cambridge, United Kingdom). This protocol was adapted by substituting the pyruvate enzyme mix with malate enzyme mix composed of 67 mM triethanolamine, 3.3 mM L-malate, 0.3 mM β-NADP+, and 5 mM MgCl2(16,17). All other steps within the manufacturers protocol were followed.

**Lentiviral-mediated ME1 Expression:**

The ME1 overexpression vector ME1/pBABE-puro (Plasmid #49163) was purchased from Addgene as bacteria in an agar stab and subsequently transformed into competent XL10 bacteria and purified into plasmid DNA with Qiagen Plasmid Plus Midi Kit (12945Qiagen Inc, Valencia, CA). TransIT-LT1 Transfection reagent (Mirus, Madison, WI) was used for plasmid transfection of retro-VSVG and pLECO, plasmid into Lenti-X 293T cells (Takara, Mountain View, CA). Viral supernatants were harvested and cells were transduced at 40 MOI with viral particles. Transduced cells were selected for and maintained by culturing in puromycin. ME1 knockdown lentiviral particles (Mission shRNA – TRCN0000064730, TRCN0000064728, TRCN0000064732) were purchased from Millipore-Sigma (Saint Louis, MO). For the vector control MISSON pLKO.1-puro Non-Target shRNA control Transduction Particles were purchased from Millipore-Sigma (Saint Louis, MO) (SHC016V). Cells were transduced and selected per manufacturers protocol.

**Seahorse Assays:**

Both the Seahorse cell energy phenotype and fuel flex assays were performed per manufacturers protocol (Agilent, Santa Clara, CA). Cells were seeded in a Seahorse 96 well plate: SYO and FUJI at 40,000/well and HTB-93 at 30,000/well 12hrs prior to assay. After performance of the Seahorse assay cell counts were measured using YOYO-1 on the IncuCyte Zoom live cell imaging system and the Seahorse results were normalized to cell count.

**Enzyme Activity Assays:**

SYO-WT, SYO-OE, Fuji-WT, and Fuji-OE cells were seeded at 2 million cells per 10 cm dish one day prior to collection. The day of collection cells were harvested, pelleted, and homogenized. G6PD and 6PGD activity was assayed on the homogenized pellets using commercial kits according to manufacturer’s instructions (BioVision K757-100; BioVision K540). G6PD and 6PGD concentration was measured in cell lysates using the Simple Protein Wes and calculated from a standard curve of human recombinant G6PD and 6PGD (BioVision 7532; BioVision P1051). Enzyme activity was normalized to protein concentration.

**NADPH Measurements:**

SYO-WT, SYO-OE, HTB93-WT, HTB93-KD3 cells were seeded at 3 million cells per 10 cm dish one day prior to collection. Samples were treated with physiological glucose levels (5mM) or without glucose for 3hr. On the day of collection samples were washed briefly with ice cold sterile water twice. After two washes cells were flash frozen on the 10cm dish in liquid nitrogen prior to extraction.

Extraction buffer was prepared by adding 2:2:1 methanol/acetonitrile/water, 0.1 M formic acid, and internal standards at 1 μg/ml each (D4-Citric Acid, 13C5-Glutamine, 13C5-Glutamic Acid, 13C6-Lysine, 13C5-Methionine, 13C3-Serine, D4-Succinic Acid, 13C11-Tryptophan, and D8-Valine; Cambridge Isotope Laboratories) to lyophilized cell samples. Cells were scraped for 20 seconds and collected into Eppendorf tubes, flash-frozen in liquid nitrogen, and sonicated for 10 minutes. Samples were then placed on a rotating platform at –20°C for 1 hour and centrifuged at 4ºC for 10 minutes at 21,000 x g. 300 µL of the cleared metabolite extracts were transferred to fresh tubes for additional processing. An equal volume of each extract was pooled to serve as a quality control (QC) sample, which was analyzed at the beginning, end, and at regular intervals throughout the instrument run. Extraction buffer alone was analyzed as a processing blank sample. Metabolite extracts, the quality control sample, and the processing blank were evaporated to dryness using a speed-vacuum.

For liquid chromatography mass spectrometry (LC-MS), the dried metabolite extracts, QC sample, and processing blank sample were reconstituted in 30 μL of acetonitrile/ water (1:1, V/V), vortexed, and centrifuged at 4ºC for 10 minutes at 21,000 x g. Samples were then placed on a rotating platform at –20°C for 2 hours and centrifuged at 4ºC for 2 minutes at 21,000 x g. Samples were then transferred to autosampler vials for analysis.

For LC chromatographic separation, 2 µL of reconstituted metabolite extracts, QC sample, and processing blank were run on a Millipore SeQuant ZIC-pHILIC (2.1 X 150 mm, 5 µm particle size) column with a ZIC-pHILIC guard column (20 x 2.1 mm) attached to a Thermo Vanquish Flex UHPLC. Mobile phase comprised Buffer A – 20 mM (NH4)2CO3, 0.1% NH4OH and Buffer B: acetonitrile. The chromatographic gradient was run at a flow rate of 0.150 mL/min as follows: 0–21 min-linear gradient from 80 to 20% Buffer B; 20-20.5 min-linear gradient from 20 to 80% Buffer B; and 20.5–28 min-hold at 80% Buffer B. Data were acquired using a Thermo Q Exactive MS operated in full-scan, polarity-switching mode with a spray voltage set to 3.0 kV, the heated capillary held at 275°C, and the HESI probe held at 350°C. The sheath gas flow was set to 40 units, the auxiliary gas flow was set to 15 units, and the sweep gas flow was set to 1 unit. MS data acquisition was performed in a range of m/z 70–1,000, with the resolution set at 70,000, the AGC target at 10e6, and the maximum injection time at 200 ms.

TraceFinder 5.1 was utilized to identify metabolites based on m/z and retention times that were determined by an in-house library of standards. After attaining metabolite identification and peak area integration by TraceFinder, the NOREVA software (http://idrblab.cn/noreva2017) was used for signal drift correction on a metabolite-to-metabolite basis using multiple analyses of the QC sample throughout the instrument run. Metabolite peak intensities were then normalized to total ion signal.

**Iron and hydrogen peroxide Assay:**

Nuclight Red cell lines were seeded at 20,000/well (SYO-WT, SYO-OE, FUJI-WT and FUIJI-OE) in a 96 well plate. Cell lines were incubated with each probe per the manufactures protocol. Fe3+ (Ferrum 430TM, Ursa BioScience), Fe2+ (FeRhoNoxTM-1, Goryo Chemical), and H2O2(HYDROPTM, Goryo Chemical). Wells were imaged every 15 minutes over 1 hour, and analyzed using IncuCyte image analysis software (Sartorius, Ann Arbor, MI). Probe excitation was quantified using and IncuCyte FLR imaging system (Sartorius, Ann Arbor, MI).

**Lipid peroxidation assay:**

Cells were plated at 10,000 cells/well (SYO-1 WT, SYO-1 ME1 OE, Fuji WT, Fuji ME1 OE, HTB93 WT, and HTB93 KDs) the day before treatment with erastin. Cells were treated with erastin (1µM, 4µM, or 8µM) and incubated for 18hr at 37˚C. After incubation the media was removed and cells were treated with 10µM Image-IT (Invitrogen C10445) for 30min. Cells were washed with PBS and fluorescence was then measured on the plate reader (Ex/Em: 488/510 nm). After completion of fluorescent measurement, cell counts were measured using YOYO-1 on the IncuCyte S3 live cell imaging system and the fluorescent measurements were normalized to cell count.

**Cystine Uptake Assay:**

Cells were plated at 15,000 cells per well in a 96 well plate the day prior to the experiment. Cells were stained with NucLight Rapid Red (1:1000) for 45 min. The media was removed and the wells were washed with PBS. BioTracker Cystine-FITC live cell probe (Cat. No. SCT047, EMD Millipore) was added at 5µM and cells were imaged on the incucyte S3 every 15min for 3hr. NucLight Rapid Red positive cells were counted using IncuCyte S3 software and used for cell count normalization in the final analysis. Cystine-FITC intensity was measured using IncuCyte S3 software and normalized to the cell count per well.

**Statistical analysis**

Data were analyzed using GraphPad Prism 7 Software. *In vitro* protein expression, cell death, activity assay, uptake assays, lipid peroxidation, and labile iron data are expressed as mean ± standard deviation and Student *t* test for the individual group comparison. Statistical analysis for *in vivo* study data was performed by analysis of variance for multiple-group comparison and two-way ANOVA for the individual group comparison with data expressed as standard error of the mean. \*, *P* < 0.05; \*\*, *P* < 0.01; \*\*\*, *P* < 0.001; \*\*\*\*, P <0.0001 were considered to represent statistically significant group differences.

References:

1. Kawano S, Grassian AR, Tsuda M, Knutson SK. Preclinical Evidence of Anti-Tumor Activity Induced by EZH2 Inhibition in Human Models of Synovial Sarcoma. PLoS One. 2016;11:1–22.

2. Nakayama R, Mitani S, Nakagawa T. Gene Expression Profiling of Synovial Sarcoma : Distinct Signature of Poorly Differentiated Type. Am J Surg Pathol. 2010;34:1599–607.

3. Chibon F, Lagarde P, Salas S, Pérot G, Brouste V, Tirode F, et al. Validated prediction of clinical outcome in sarcomas and multiple types of cancer on the basis of a gene expression signature related to genome complexity. Nat Med. Nature Publishing Group; 2010;16:781–8.

4. Terrier P, Bonnin S, Lagarde P. From PTEN loss of expression to RICTOR role in smooth muscle differentiation : complex involvement of the mTOR pathway in leiomyosarcomas and pleomorphic sarcomas. Mod Pathol. 2012;25:197–211.

5. Doyle KR, Mitchell MA, Roberts CL, James S, Johnson JE, Zhou Y, et al. Validating a gene expression signature proposed to differentiate liposarcomas that use different telomere maintenance mechanisms. Oncogene. Nature Publishing Group; 2012;265–6.

6. Sears DD, Hsiao G, Hsiao A, Yu JG, Courtney CH, Ofrecio JM, et al. Mechanisms of human insulin resistance and thiazolidinedione-mediated insulin sensitization. PNAS. 2009;106:18745–50.

7. Hardy OT, D M, Perugini RA, D M, Nicoloro SM, S B, et al. Body mass index-independent inflammation in omental adipose tissue associated with insulin resistance in morbid obesity. SOARD [Internet]. Elsevier Inc.; 2011;7:60–7. Available from: http://dx.doi.org/10.1016/j.soard.2010.05.013

8. Plaisier CL, Kytta M, Weissglas-volkov D, Sinsheimer JS, Huertas-vazquez A, Riba L, et al. Galanin Preproprotein Is Associated With Elevated Plasma Triglycerides. Arter Thromb Vasc Biol. 2008;29:147–52.

9. Johansson LE, Danielsson APH, Parikh H, Klintenberg M, Norstro F, Groop L. Differential gene expression in adipose tissue from obese human subjects during weight loss and weight maintenance 1 – 5. Am J Clin Nutr. 2012;96:196–207.

10. Skov V, Glintborg D, Knudsen S, Jensen T, Kruse TA, Tan Q, et al. Reduced Expression of Nuclear-Encoded Genes Involved in Mitochondrial Oxidative Metabolism in Skeletal Muscle of Insulin-Resistant Women With Polycystic Ovary Syndrome. Diabetes. 2007;56:2349–55.

11. Keller P, Vollaard NBJ, Gustafsson T, Gallagher IJ, Sundberg CJ, Rankinen T, et al. A transcriptional map of the impact of endurance exercise training on skeletal muscle phenotype. J Appl Physiol. 2021;110:46–59.

12. Gentleman RC, Carey VJ, Bates DM, Bolstad B, Dettling M, Dudoit S, et al. Bioconductor : open software development for computational biology and bioinformatics. Genome Biol. 2004;5:R80.

13. Wilson CL, Miller CJ. Simpleaffy : a BioConductor package for Affymetrix Quality Control and data analysis. Bioinformatics. 2005;21:3683–5.

14. Freue GVC, Hollander Z, Shen E, Zamar RH, Balshaw R, Scherer A, et al. Gene expression MDQC : a new quality assessment method for microarrays based on quality control reports. Bioinformatics. 2007;23:3162–9.

15. Wu Z, Irizarry RA, Gentleman R, Martinez-murillo F, Wu Z, Irizarry RA, et al. A Model-Based Background Adjustment for Oligonucleotide Expression Arrays A Model-Based Background Adjustment for Oligonucleotide Expression Arrays. J Am Stat Assoc. 2004;99:909–17.

16. Jiang P, Du W, Mancuso A, Wellen KE, Yang X. Reciprocal regulation of p53 and malic enzymes modulates metabolism and senescence. Nature [Internet]. Nature Publishing Group; 2013;493:689–93. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3561500&tool=pmcentrez&rendertype=abstract

17. Zhang YJ, Wang Z, Sprous D, Nabioullin R. In silico design and synthesis of novel malic enzyme inhibitors. Bioorg Med Chem Lett. 2006;16:525–8.