Supplemental materials and methods

Q-PCR

Total RNA was extracted using the RNeasy Plus Mini kit (Qiagen) according to the manufacturer's instructions. For reverse transcription, 1 μg total RNA/sample was used as a template for cDNA synthesis using Superscript III (Thermo Fisher Scientific) following the manufacturer's instructions. Q-PCR reactions were conducted on a Stratagene Mx3005P® (Agilent Technologies) using prevalidated TaqMan primer/probe sets purchased as Assays-on-Demand gene expression products from Applied Biosystems (Thermo Fisher Scientific). Real-time PCR conditions were 5 minutes at 95 °C and 40 cycles of 30 seconds at 95 °C, followed by 1 minute at 60 °C. Expression data were normalized to expression of 18S mRNA and were calculated by the $2^{-\Delta\Delta CT}$ method.

Extracellular flux analysis

Oxygen consumption rate (OCR) was analyzed using an XF-24 extracellular flux analyzer according to the manufacturer's instructions (Seahorse Bioscience). Forty-eight hours after transfection, cells were treated without or with M1 or M2 inducer for 24 hours, followed by seeding (5×10^4 cells/well) in 100 μL medium on cellTak-coated assay plates (BD Biosciences) and incubated overnight. The following day, cells were washed and media was replaced with 500 μl of temperature/CO2 pre-adjusted Seahorse XF Base medium RPMI. OCR measurements were taken before and after the sequential addition of electron transport chain modulators, including 1 μM oligomycin, 0.5 μM carbonyl cyanide-4-(trifluoromethoxy) phenylhydrazone (FCCP), and 0.5 μM rotenone plus 0.5 μM antimycin A. All reagents were obtained from Sigma-Aldrich. Normalization was performed using the number of living cells.

Mass spectrometry-based proteomics and analysis

Mass spectrometry analysis was performed in the Advanced Mass Spectrometry Core Facility of the National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health. Human THP-1 cells transfected with a GMFG siRNA or control siRNA for 48 hours were lysed in IP lysis buffer (Thermo Fisher Scientific) and immunoprecipitated with either monoclonal GMFG antibody (LifeSpan BioSciences) or control rabbit IgG overnight at 4 °C. Immunoprecipitates were incubated with protein G Dynabeads (Thermo Fisher Scientific) for 3

hours at 4 °C and protein complexes were washed with pre-urea washing buffer for 3 times. The samples were then eluted using urea elution buffer (8 M urea in 100 mM Tris/HCI pH 8.0) by incubating for 30 minutes at room temperature. In preparation for mass spectrometry, the protein samples were reduced by adding dithiothreitol (DTT) to a final concentration of 5 mM and incubated at 37 °C for 30 minutes and samples were cooled down to 23 °C, followed by alkylation using iodoacetamide (IAA) at a final concentration of 10 mM in the dark for 1 hour. Proteins were digested by adding 1 µg of LysC (Wako, 125-05061) for overnight incubation at room temperature, followed by the addition of 2 µg of trypsin (Promega, V5113) the next day for 4 hours. After digestion, samples were desalted and concentrated using a serial stack of self-packed Stage tips [1] with C8 and C18 Spin Columns. Each stack received a different labeling cocktail leading to the reductive dimethylation of lysine side chains and the N-termini of peptides with -CH3 (transfected cells immunoprecipitated with IgG antibody), -CD2H (GMFG siRNA-transfected cells immunoprecipitated with GMFG antibody) and 13C-D3 (control siRNA-transfected cells immunoprecipitated with GMFG antibody) using the approach described previously [2]. Then the sample was subdivided and applied to a set of 4 in house fabricated SCX Stage tips at 250G. The columns were then washed twice with 200 µL of 80% acetonitrile/0.4% formic acid prior to being eluted with 300 µL of 0.4% formic acid/20% acetonitrile/300 mM ammonium acetate. The eluates were combined and then dried down under a stream of nitrogen gas at 50 °C. The sample was next dissolved in 50 µL of 0.2% formic acid/2% acetonitrile heated to 50 °C for 10 minutes, subjected to sonication for 10 minutes and then spun down briefly at 800G to consolidate the liquid on the bottom of the container. Sample peptide (comprising three subsamples distinguished with different dimethyl labeling chemistry) were analyzed by LC-MS/MS system comprising of a micro-HPLC system (NanoAcquity, Waters) and a QE-Orbitrap mass spectrometer (ThermoFisher Scientific). The LC system was configured with a vented column comprising a trapping column (Symmetry C18 180 µm x 20 mm, 186003514) and an analytical column (BEH 130 C18 1.7 micron, 75 µm x 250 mm). The sample (10 µL) was injected onto the vented trap column at a flow rate of 6 µL/minute and 1% acetonitrile for 6 minutes and separation was conducted by a linear acetonitrile gradient from 1% to 32% acetonitrile over 510 minutes and then to 80% over the following 89 minutes. A minute after injection a contact closure signal began collection of data on the QE mass spectrometer.

In order to improve reliability, a two computer configuration was used with the NanoAcquity controlled by a computer running Xcalibur 2.2 SP1 and Waters Acquity 2.50 with a contact closure signal sent from the NanoAcquity to the mass spectrometer a minute after the gradient start. The Q-Exactive (running the early 32-bit system; Xcalibur 3.0 build 47, Q-Exactive Orbitrap MS 2.3 build 1765) then began a data dependent analysis with single MS1 spectra collected from 200-2000 M/Z with up to 8 signals targeted for fragmentation data collection with dynamic exclusion with a 10 second window. The raw data were analyzed using MaxQuant 1.4.1.2 [3] as a 3 channel dimethyl experiment and searched against the human proteome downloaded in February 2014.

References in Supplemental methods

1. Rappsilber J, Mann M, Ishihama Y. Protocol for micro-purification, enrichment, pre-fractionation and storage of peptides for proteomics using StageTips. *Nat Protoc*. 2007;2(8):1896-1906.

2. Boersema PJ, Raijmakers R, Lemeer S, Mohammed S, Heck AJ. Multiplex peptide stable isotope dimethyl labeling for quantitative proteomics. *Nat Protoc*. 2009;4(4):484-494.

3. Cox J, Mann M. MaxQuant enables high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide protein quantification. *Nat Biotechnol*. 2008;26(12):1367-1372.