Supplemental Experimental Procedures

Neuronal cultures. Briefly, 35-mm plastic tissue culture dishes (Falcon) or optical plastic dishes (Ibidi) were coated with 500 μg/ml of poly-DL-ornithine (Sigma Aldrich) and 10 μg/ml of natural mouse laminin (Life Technologies). Evenly spaced grooves were etched on the dishes before a silicone grease-coated tri-chamber (Tyler Research) was placed. SCGs were trypsinized and triturated before plating in the S compartment. Neurons were maintained in neurobasal medium (Gibco) supplemented with 100 ng/ml nerve growth factor 2.5S (Invitrogen), 2% B27 (Gibco) and 1% penicillin and streptomycin with 2 mM glutamine (Life Technologies). 48 h after plating, 1 mM cytosine-D-arabinofuranoside (Sigma-Aldrich) was added for 2 days to kill non-neuronal dividing cells. Neurons were cultured for 14-21 days prior to experiments.

Western blot analyisis. Dissociated neurons or cell bodies from the S compartment were lysed in radioimmunoprecipitation assay (RIPA) buffer without sodium deoxycholate, supplemented with 1 mM dithiothreitol (DTT) and protease inhibitor cocktail (Sigma-Aldrich). Lysates were kept on ice for 30 min, sonicated to shear genomic DNA, and centrifuged at 11,000 rpm at 4°C. Supernatants were transferred into new tubes and mixed with 5x Laemmli buffer. Axons from N compartment were directly lysed in 2x Laemmli buffer on dish. Samples were heated at 95°C for 10 min before resolved by 8% SDS polyacrylamide gel electrophoresis. Gels were transferred to nitrocellulose membranes (Whatman) using semidry transfer (Biorad). After transfer, membranes were incubated in 5% non-fat dry milk in tris-buffered saline (TBS) solution for 1 h at room temperature. Immunoblots were performed using primary and secondary antibodies in 1% milk TBS solution. Membranes were incubated with chemiluminescent substrates (Supersignal West

Pico or Dura, Thermo scientific). Protein bands were visualized by exposure on HyBlot CL (Denville scientific) blue X-ray films.

Immunofluorescence Staining. Samples were fixed with 4% paraformaldehyde (Electron Microscopy Sciences) in phosphate-buffered saline (PBS) for 10 min and permeablized with ice-cold methanol for 10 min at −20°C. Samples were then incubated in PBS containing 3% bovine serum albumin (BSA) (Sigma-Aldrich) for 1 h prior to staining with antibodies and DAPI diluted in 3% BSA in PBS. Teflon tri-chambers were removed before mounting with Aqua-Poly/Mount (Polysciences) and covering with glass coverslips (Fisher Scientific).

Quantitative Reverse Transcription-PCR. Primers are: rat Mx1 (Fw: CCTGCTTGGCAAAGAGACTGAC, Rv: TTGGCAGCGATTTACAGATGTGG); rat IFIT1 (Fw: CTGAGATGTCACTTCACATGGGG, Rv: GTGCATCCCCAGTGAGTTCT); rat GBP2 (Fw: CTGCATTATGTGACAGAGCTG, Rv: GAGCCCACACAAAGGTTGGAAA); rat β-actin (Fw: CATGAAACTACATTCAATTC, Rv: GTAACAGTCCGCCTAGAAGC).

Mass spectrometry-based proteomic sample preparation and data analysis. Briefly, axons were washed twice with PBS and collected in NuPAGE LDS sample buffer (Life Technologies). Samples were treated with 100 mM DTT at 70°C for 10 min and then with 100 mM iodoacetamide at room temperature for 30 min protected from light. One-fifth of each sample was resolved by SDS-PAGE (4 - 12% NuPAGE BisTris gels, Invitrogen) and stained with Coomassie blue. Coomassie stained gel lanes were excised, cut into 1 mm slices, and pooled into 8 fractions per lane. Proteins were digested in-gel with trypsin (20 µl of 12.5 ng/µl trypsin in 50

mM ammonium bicarbonate) overnight at 37 °C. Peptides were extracted in 0.5% formic acid/50% ACN, desalted, and half of each sample was analyzed by nanoliquid chromatography-tandem mass spectrometry using a Dionex Ultimate 3000 nanoRSLC coupled online to an LTQ Orbitrap XL mass spectrometer with an nanoelectrospray Flex ion source (Thermofisher Scientific). Peptides were separated by reverse phase chromatography (Acclaim PepMap RSLC C_{18}, 1.8 µm, 75 µm x 25 cm) over a 90 min linear gradient from 4-35% mobile phase B (mobile phase B = 97% acetonitrile containing 0.1% formic acid and mobile phase A = Water containing 0.1% formic acid). Peptide ions were selected for MS analysis using a data-dependent acquisition method that permitted label-free spectral counting analysis, as previously described (1) but with some modifications. Specifically, one MS acquisition cycle collected a single MS spectrum in the orbitrap detector (resolution = 60,000; Preview scan enabled) and up to ten MS/MS fragmentation scans on the most intense ions using dynamic exclusion. Acquisition cycles were repeated throughout the LC gradient.

MS/MS spectra were extracted, filtered, and searched using Proteome Discoverer/SEQUEST software (v1.4 ThermoFisher Scientific) against a protein sequence database containing rat and mouse compiled from the UniProt Swiss-Prot repository (2013-08), and common contaminants (total of 74,017 entries). Protein sequences were reversed for estimation of global peptide and protein false discovery rates (FDR). SEQUEST peptide-spectrum matches corresponding to the same biological replicate (minimum of 3 per condition) were combined and then analyzed in Scaffold (v4.1) (Proteome Software) using the X!Tandem refinement search strategy. Probabilities for peptide spectral matches (PSMs) were calculated using Scaffold's Bayesian-based local FDR scoring model, and then PSMs were assembled into protein groups. Peptide and

protein probability thresholds were defined empirically to achieve ≤ 1% peptide and protein FDR (minimum of 2 unique sequences). Filtered data were exported to mzIdentML format and imported into perSPECtives (v2.0.5) (Proteome Software) to perform spectral counting and statistical analysis. Proteins were clustered and weighted spectrum counts were calculated using the "shared evidence clustering" method and summarization at the biosample level. Weighted spectral counts were normalized by the average number of unique spectra across all samples divided by the number of unique spectra in each individual sample. Differential abundance was calculated separately for (1) IFNβ versus No Treatment and (2) IFNγ versus No Treatment, using weighted spectral counts and a permutation test with a Benjamini-Hochberg post-test ($p < 0.05$) to correct FDR for multiple comparisons. A protein cluster was considered significant if at least one member of the cluster achieved statistical significance. Data was exported to Excel for additional data processing.

Detection of L-AHA-Containing Proteins. Neurons in chambers were incubated in methionine free media (RPMI 1640) for 1 hour, followed by addition of 50 mM L-azidohomoalanine (L-AHA) and IFNβ or IFNγ in the N compartment for 2 h. Methionine in newly synthesized proteins was substituted with L-AHA, which contains an azide group that was subsequently covalently linked to alkyne-bearing Alexa Fluor 488 (Life Technologies). Click reaction was performed by using Click-IT Cell Reaction Buffer Kit (Life Technologies).

Supplemental Reference

1. **Tsai YC, Greco TM, Cristea IM.** 2014. Sirtuin 7 plays a role in ribosome biogenesis and protein synthesis. Mol Cell Proteomics **13:**73-83.