Supplementary Information

Supplementary Figures Legends

Figure S1. Total protein levels and general protein synthesis is unaffected in cells deficient in NatA components. (A) HeLa cells were transfected with a pool of siRNAs targeting ARD1 or non-targeting control siRNA as indicated and treated with cyclohexamide (100ng/mL) for 5 hours. Caspase-2, -9, -3, and Chk1 but not Chk2 are substrates of NatA complex (Figure 2). This experiment is representative of 2 independent experiments. Asterisk indicates non-specific band. **(B)** HeLa cells were transfected as described in (A). Total protein lysates were analyzed by SDS PAGE, and the gel was stained with GelCode Blue (Pierce). Representative digital images were generated by an infrared imager (Li-Cor). **(C)** HeLa cells were transfected with siRNAs targeting ARD1 or NATH followed by 48h incubation to allow for protein turnover. Newly synthesized proteins were labeled with ^{35}S-Cys and ^{35}S-Met for 1h followed by a 24h chase with cold medium. Cells were lysed directly in SDS sample buffer and lysates were analyzed by SDS-PAGE and autoradiography. **(D)** The ability of NatA deficient cells to progress through the cell cycle normally is shown. Following RNAi treatment, cells were resuspended in PBS with propidium iodide for FACS analysis. Cells treated with RNAi against ARD1 or NATH do not show any appreciable change in the cell cycle compared to control. Altogether these results suggest that the defect in Chk1 activation is likely due to the decrease in acetylation of Chk1 in NatA deficient cells as assessed by subtiligase in Figure 1. Blots are representative of at least 3 independent experiments.

Figure S2. Caspase-2 is N-terminally acetylated. N-terminal peptides of WT, A2S, and A3P caspase-2 were identified by mass spectrometry. A2S substitution of caspase-2 remains N-alpha-acetylated, whereas N-alpha-acetylation of A3P caspase-2 is not

detected by mass spectrometry. Schematic of differential isotope labelling to detect acetylated or unacetylated N-terminal peptides of caspase-2 by mass spectrometry is shown in Figure 1C. (A) Analysis of differentially isotope labelled WT and A2S caspase-2 shows that the N-terminal Met is removed and the newly exposed residue is acetylated. N-terminal peptides from both WT and A2S mutant caspase-2 were found to co-elute and therefore shown in one MS spectrum. N-terminal peptide corresponding to acetylated wild type caspase-2 (heavy dimethyl modification) is A(Ac)APSGRSQSSLHRK(C_2D_6) (3+) at m/z= 519.96. N-terminal peptide corresponding to acetylated A2S caspase-2 (light dimethyl modification) is S(Ac)APSGRSQSSLHRK(C_2H_6) (3+) at m/z= 523.28. (B-C) Analysis of differentially isotope labeled wild type (B) and A3P caspase-2 (C) shows that only wild type caspase-2 is acetylated at the N-terminus. N-terminal peptide corresponding to acetylated wild type caspase-2 (heavy dimethyl modification) is A(Ac)APSGRSQSSLHRK(C_2D_6) (3+) at m/z= 519.96. The non-acetylated N-terminal mutant peptide (A3P) that is consequently dimethylated was identified at an earlier retention time on the reverse phase column. This is expected from the lower hydrophobicity of the molecule with a dimethyl group instead of an acetyl modification at the N-terminus for the A3P peptide. Thus the A3P and WT caspase-2 peptides are shown in two different MS spectra. N-terminal peptide corresponding to unacetylated A3P caspase-2 (light dimethyl modification) is A(C_2H_6)PPSGRSQSSLHRK(C_2H_6) (3+) at m/z= 521.96, and 391.71 (4+) (not shown). (D) FLAG tagged caspase-2 (C320G) was immunopurified in control or NATH knockdown cells. Peptides were generated by Lys-C followed by chemical modification using $NaCNBH_3$ or $NaCNBD_3$ and formaldehyde treatment (formaldehyde-H_2 or formaldehyde-D_2 respectively). Ratios for levels of N-terminal acetylation were determined by normalizing to 1:1 ratio of shared internal peptides. The following internal peptides were used for normalization: EELMK, VNALIK, NHTQSPGCEESDAGK.

Figure S3. Histone acetylation levels are unaffected in Bcl-xL expressing cells. Jurkat cells stably expressing Bcl-xL or GFP were lysed directly in SDS sample buffer. Lysates were analyzed by SDS-PAGE. Levels of acetylated histone-H3 and histone-H4 were analyzed by immunoblot.

Figure S4. Metabolomic profiling of Bcl-xL expressing cells by NMR. Metabolite levels were compared between cells expressing Bcl-xL or green fluorescence protein (GFP). Principal components analysis (PCA) of 1D Proton NMR spectra of 10 Bcl-xl expressing, and 9 GFP expressing Jurkat cell extract samples using AMIX software (version 3.7.10, Bruker-BioSpin Corp.). On the left is a scores plot of PC1 vs. PC2 representing the corresponding loadings plot shown on the right. All spectra were corrected for dc offset, and calibrated to DSS at 0 ppm prior to binning. Spectra were binned using equally spaced buckets of 0.04 ppm width, covering the range from 0.5 to 10 ppm. The water region was excluded. Data were pareto scaled.

Supplementary Tables

Table S1. Profiling of glucose-derived metabolites in Bcl-xL expressing cells

Metabolite	Fold Change (BclxL/GFP)	*z* score*
5-phosphoribosyl-1-pyrophosphate	0.76	-0.93
trehalose-sucrose	0.91	-0.20
fructose-1,6-bisphosphate	1.25	1.48
geranyl-PP	1.39	2.18

N-acetyl-glucosamine-1-phosphate	1.00	0.23
1,3-diphopshateglycerate	0.88	-0.36
hexose-phosphate	0.90	-0.25
thymidine	0.87	-0.44
prephenate	0.96	0.02
ribose-phosphate	1.67	3.55
pantothenate	1.44	2.42
deoxyribose-phosphate	0.88	-0.38
D-erythrose-4-phosphate	1.18	1.14
D-gluconate	0.62	-1.64
citrate	0.77	-0.93
citrate-isocitrate	0.80	-0.77
3-phosphoglycerate	0.85	-0.51
myo-inositol	0.81	-0.73
shikimate	0.89	-0.33
allantoate	0.89	-0.29
sn-glycerol-3-phosphate	0.79	-0.82
dihydroxy-acetone-phosphate	0.89	-0.30
phosphoenolpyruvate	0.91	-0.22
dihydroorotate	0.96	0.029
allantoin	1.03	0.37
xanthine	0.88	-0.36
a-ketoglutarate	0.80	-0.78

acetylphosphate	0.71	-1.22
carbamoyl phosphate	0.78	-0.87
hypoxanthine	1.47	2.53
malate	0.73	-1.11
oxaloacetate	0.96	0.03
taurine	0.91	-0.21
succinate	0.82	-0.67
2-keto-isovalerate	0.82	-0.66
fumarate	0.88	-0.35
acetoacetate	0.81	-0.71
NADH	1.01	0.26
NAD+	1.22	1.33
S-adenosyl-L-methionine	0.86	-0.47
L-arginino-succinate	0.70	-1.23
biotin	1.21	1.28
cytidine	0.86	-0.46
tryptophan	1.25	1.47
glucosamine	1.00	0.24
N-carbamoyl-L-aspartate	0.89	-0.32
citrulline	0.98	0.11
pyridoxine	0.93	-0.14
phenylalanine	0.97	0.09
guanine	0.61	-1.71

methionine	1.02	0.33
glutamate	0.85	-0.50
lysine	0.92	-0.16
glutamine	0.94	-0.07
histidinol	1.12	0.82
leucine-isoleucine	0.95	-0.03
aspartate	1.06	0.54
asparagine	1.02	0.32
thymine	0.94	-0.05
nicotinamide	1.01	0.30
indole	1.24	1.41
proline	0.83	-0.64
cytosine	1.07	0.59
alanine	0.70	-1.27

*z score is defined as the standard deviation above or below the mean fold change (BclxL/GFP) of all glucose-derived metabolites

Extended Experimental Procedures

Antibodies.

The following antibodies were used for immunobloting: Caspase-9 (R&D systems), Caspase-2 (Alexis), Caspase-3 (Cell Signaling), cleaved Caspase-3 (Cell Signaling), ARD1 (Santa Cruz), NATH (provided by T. Arnesen and J. Lillehaug), γ-tubulin (Sigma), Bax (Santa Cruz), Bak (Santa Cruz), Msh2 (R&D Systems), Chk1 (Cell Signaling), pan-pyruvate kinase M (Abcam), Bcl-xL (provided by Craig Thompson), β-actin (Sigma), FLAG (Sigma), and 14-3-3β (Santa Cruz).

Cell Line Construction.

Jurkat cells stably expressing GFP or Bcl-xL were generated by retroviral-mediated expression of pMIG bicistronic vector containing GFP only or GFP and Bcl-xL (Addgene) using standard procedures. High GFP expressing cells were purified by fluorescence activated cell sorting. HeLa cells stably expressing GFP or Bcl-xL were generated using the same methods without sorting. Cells that showed greater than 90% infection efficiency were used for experiments described in the study. HeLa cells were maintained in DMEM with glutamine (Invitrogen) and 10% NCS. Jurkat cells were maintained in RPMI with glutamine and 10% NCS.

HEK 293T cells stably expressing shRNA against human ARD1 or NATH were generated by retroviral-mediated expression of pSRP-puro vector using standard procedures. The sequences used for the oligonucleotides inserted into the pSRP vector correspond to the individual siRNA sequences provided by Dharmacon (ARD1, J-009606-05; NATH, J-012847-05). Following puromycin selection, cells were passaged for no more than 1 week. Following puromycin selection, 293T cells were transiently transfected with full-length mouse caspase-2 containing active cysteine mutation (C320G) to reduce cell death using calcium phosphate.

HEK 293T cells were transiently transfected with pMIG GFP empty vector or pMIG GFP and Bcl-xL vector using calcium phosphate.

All cells described above were maintained in the presence of penicillin and streptomycin (Invitrogen) and passaged for no more than 4 weeks with exceptions noted.

Unlabeled NMR Sample Preparation.

10 replicates of Jurkat cells stably expressing GFP or Bcl-xL ($100x10^6$ for each replicate), and metabolites were extracted in 4mL 100% ice-cold methanol followed by addition of 4mL chloroform and 4mL water. Lysates were spun at 10,000g for 30 minutes at 4° to separate the aqueous and inorganic phases. Methanol was evaporated from aqueous fractions by nitrogen gas and lyophilized. Samples were prepared for NMR spectroscopy by dissolving the lyophilized cell extract pellets in 400 ml of sample buffer, containing 50mM HEPES (pH=7.0) and 2mM DSS (as a chemical shift reference). The samples were immediately transferred into 5mm Shigemi NMR tubes (Shigemi, Inc. Suite 21, 4790 Route 8, Allison Park, PA 15101) for data acquisition.

NMR Spectroscopy.

All NMR spectra were acquired on a Varian INOVA 600MHz spectrometer (Varian, Inc., Palo Alto, CA.) using a 5mm triple resonance (H, C, N) Salt Tolerant Cold Probe. The sample temperature was 25 degrees centigrade for all samples. One-dimensional proton spectra were acquired using a one pulse sequence with continuous-wave (CW) pre-saturation of the residual water signal during the relaxation delay (2 seconds), with an RF field of 50Hz. 16K complex data points were acquired, with 256 transients and 4 dummy transients. The spectral width was 8KHz.

Mass Spectrometry (MS) Analysis and Quantification of Caspase-2.

FLAG-tagged caspase-2 or mutant (A2S and A3P) was transiently expressed in HEK 293T cells. After lysis in 0.2% Tween 20 and 0.2% Triton X-100 buffer, FLAG-tagged caspase-2 was affinity purified with FLAG agarose beads (Sigma) and eluted with FLAG peptide. Eluants were resolved on SDS-PAGE and visualized by GelCode Blue stain. The band containing caspase-2 or mutant protein was excised and subjected to in-gel Endoproteinase Lys-C digestion. Peptides were extracted from the gel, differentially labeled with reductive dimethylation as previously described (Hsu et al., 2003; Khidekel et al., 2007), and heavy- and light-dimethylated peptides were combined 1:1 (wild type: mutant) for MS analysis.

The peptide mixture was analyzed by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Peptides were separated across a 37-min gradient ranging from 4% to 27% (v/v) acetonitrile in 0.1% (v/v) formic acid in a microcapillary (125 μm x 18 cm) column packed with C_{18} reverse-phase material (Magic C18AQ, 5 μm particles, 200 Å pore size, Michrom Bioresources) and on-line analyzed on an LTQ Orbitrap XL™ hybrid FTMS (Thermo Electron). For each cycle, one full MS scan acquired on the Orbitrap at high mass resolution was followed by ten MS/MS spectra on the linear ion trap XL from the ten most abundant ions. Single ion chromatograms from the Orbitrap MS scans were generated for N-terminal peptides. The area under the SIC curve was used to determine relative abundance between wild type and mutant. Internal peptides common to both wild type and mutant were used to normalize mixing ratio to 1:1. This isotope-labeling strategy allowed for a comparative analysis of a wild type and a mutant sample together in a single LC-MS/MS experiment.

References

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