

SUPPLEMENT

Table S1. List of the experimental methods and references for retrieving the data, which were utilized for the pathway enrichment analysis

Method/Source	References
Multiple analytical methods [26]	[1-22]
LC/MS-MS; liver mitochondrial proteins; wild type (wt) vs. SIRT3 KO mice	[23]
Nano reverse-phase LC/MS; liver mitochondrial proteins; wt mice vs. SIRT3 KO	[24]
SILAC, LC/MS-MS; SIRT3 KO vs. wt murine embryonic fibroblasts cells; U2OS cells with the upregulated SIRT3 vs. cells with downregulated SIRT3	[25]

REFERENCES

1. Vassilopoulos, A., Pennington, J. D., Andresson, T., Rees, D. M., Bosley, A. D., et al. (2013) SIRT3 deacetylates ATP synthase F1 complex proteins in response to nutrient- and exercise-induced stress, *Antioxid. Redox Signal.*, **21**, 551-564, doi: 10.1089/ars.2013.5420.
2. Poulou, N., and Raju, R. (2015) Sirtuin regulation in aging and injury, *Biochim. Biophys. Acta*, **1852**, 2442-2455, doi: 10.1016/j.bbadis.2015.08.017.
3. Hallows, W. C., Lee S., and Denu, J. M. (2006) Sirtuins deacetylate and activate mammalian acetyl-CoA synthetases, *Proc. Natl. Acad. Sci. USA*, **103**, 10230-10235, doi: 10.1073/pnas.0604392103.
4. Shimazu, T., Hirschey, M. D., Hua, L., Dittenhafer-Reed, K. E., Schwer, B., et al. (2010) SIRT3 deacetylates mitochondrial 3-hydroxy-3-methylglutaryl CoA synthase 2 and regulates ketone body production, *Cell Metab.*, **12**, 654-661, doi: 10.1016/j.cmet.2010.11.003.
5. Bharathi, S. S., Zhang, Y., Mohsen, A. W., Uppala, R., Balasubramani, M., et al. (2013) Sirtuin 3 (SIRT3) protein regulates long-chain acyl-CoA dehydrogenase by deacetylating conserved lysines near the active site, *J. Biol. Chem.*, **288**, 33837-33847, doi: 10.1074/jbc.M113.510354.
6. Finley, L. W. S. S., Carracedo, A., Lee, J., Souza, A., Egia, A., et al. (2011) SIRT3 opposes reprogramming of cancer cell metabolism through HIF1 α destabilization, *Cancer Cell*, **19**, 416-428, doi: 10.1016/j.ccr.2011.02.014.
7. Sundaresan, N. R., Samant, S. A., Pillai, V. B., Rajamohan, S. B., and Gupta, M. P. (2008) SIRT3 is a stress-responsive deacetylase in cardiomyocytes that protects cells from stress-mediated cell death by deacetylation of Ku70, *Mol.*

- Cell. Biol.*, **28**, 6384-6401, doi: 10.1128/mcb.00426-08.
8. Qiu, X., Brown, K., Hirschey, M. D., Verdin, E., and Chen, D. (2010) Calorie restriction reduces oxidative stress by SIRT3-mediated SOD2 activation, *Cell Metab.*, **12**, 662-667, doi: 10.1016/j.cmet.2010.11.015.
 9. Yu, W., Dittenhafer-Reed, K. E., and Denu, J. M. (2012) SIRT3 protein deacetylates isocitrate dehydrogenase 2 (IDH2) and regulates mitochondrial redox status, *J. Biol. Chem.*, **287**, 14078-14086, doi: 10.1074/jbc.M112.355206.
 10. Schlicker, C., Gertz, M., Papatheodorou, P., Kachholz, B., Becker, C. F. W., et al. (2008) Substrates and regulation mechanisms for the human mitochondrial sirtuins Sirt3 and Sirt5, *J. Mol. Biol.*, **382**, 790-801, doi: 10.1016/j.jmb.2008.07.048.
 11. Pillai, V. B., Sundaresan, N. R., Kim, G., Gupta, M. M. P., Rajamohan, S. B., et al. (2010) Exogenous NAD blocks cardiac hypertrophic response via activation of the SIRT3-LKB1-AMP-activated kinase pathway, *J. Biol. Chem.*, **285**, 3133-3144, doi: 10.1074/jbc.M109.077271.
 12. Yang, Y., Cimen, H., Han, M. J., Shi, T., Deng, J. H., et al. (2010) NAD⁺-dependent deacetylase SIRT3 regulates mitochondrial protein synthesis by deacetylation of the ribosomal protein MRPL10, *J. Biol. Chem.*, **285**, 7417-7429, doi: 10.1074/jbc.M109.053421.
 13. Hirschey, M. D., Shimazu, T., Goetzman, E., Jing, E., Schwer, B., et al. (2010) SIRT3 regulates mitochondrial fatty-acid oxidation by reversible enzyme deacetylation, *Nature*, **464**, 121-125, doi: 10.1038/nature08778.
 14. Xue, L., Xu, F., Meng, L., Wei, S., Wang, J., et al. (2012) Acetylation-dependent regulation of mitochondrial ALDH2 activation by SIRT3 mediates acute ethanol-induced eNOS activation, *FEBS Lett.*, **586**, 137-142, doi: 10.1016/j.febslet.2011.11.031.
 15. Wang, Z., Inuzuka, H., Zhong, J., Liu, P., Sarkar, F. H., et al. (2012) Identification of acetylation-dependent regulatory mechanisms that govern the oncogenic functions of Skp2, *Oncotarget*, **3**, 1294-1300, doi: 10.18632/oncotarget.740.
 16. Tseng, A. H. H., Shieh, S. S., and Wang, D. L. (2013) SIRT3 deacetylates FOXO3 to protect mitochondria against oxidative damage, *Free Radic. Biol. Med.*, **63**, 222-234, doi: 10.1016/j.freeradbiomed.2013.05.002.
 17. Jing, E., O'Neill, B. T., Rardin, M. J., Kleinridders, A., Ilkeyeva, O. R., et al. (2013) Sirt3 regulates metabolic flexibility of skeletal muscle through reversible enzymatic deacetylation, *Diabetes*, **62**, 3404-3417, doi: 10.2337/db12-1650.
 18. Cheng, Y., Ren, X., Gowda, A. S. P., Shan, Y., Zhang, L., et al. (2013) Interaction of Sirt3 with OGG1 contributes to repair of mitochondrial DNA and protects from apoptotic cell death under oxidative stress, *Cell Death Dis.*, **4**, 1-11, doi: 10.1038/cddis.2013.254.
 19. Samant, S. A., Zhang, H. J., Hong, Z., Pillai, V. B., Sundaresan, N. R., et al. (2014) SIRT3 deacetylates and activates OPA1 to regulate mitochondrial dynamics during stress, *Mol. Cell. Biol.*, **3**, 807-819, doi: 10.1128/mcb.01483-13.
 20. Lu, Z., Chen, Y., Aponte, A. M., Battaglia, V., Gucek, M., et al. (2015) Prolonged fasting identifies heat shock protein 10 as a sirtuin 3 substrate: Elucidating a new mechanism linking mitochondrial protein acetylation to fatty acid oxidation enzyme folding and function, *J. Biol. Chem.*, **290**, 2466-2476, doi: 10.1074/jbc.M114.606228.
 21. Rauh, D., Fischer, F., Gertz, M., Lakshminarasimhan, M., Bergbrede, T., et al. (2013) An acetylome peptide microarray reveals specificities and

- deacetylation substrates for all human sirtuin isoforms, *Nat. Commun.*, **4**, 2327-2337, doi: 10.1038/ncomms3327.
22. Yang, H., Zhou, L., Shi, Q., Zhao, Y., Lin, H., et al. (2015) SIRT 3-dependent GOT2 acetylation status affects the malate-aspartate NADH shuttle activity and pancreatic tumor growth, *EMBO J.*, **34**, 1110-1125, doi: 10.15252/emj.201591041.
 23. Rardin, M. J., Newman, J. C., Held, J. M., Cusack, M. P., Sorensen, D. J., et al. (2013) Label-free quantitative proteomics of the lysine acetylome in mitochondria identifies substrates of SIRT3 in metabolic pathways, *Proc. Natl. Acad. Sci. USA*, **110**, 6601-6606, doi: 10.1073/pnas.1302961110.
 24. Hebert, A. S., Dittenhafer-Reed, K. E., Yu, W., Bailey, D. J., Selen, E. S., et al. (2013) Calorie restriction and SIRT3 trigger global reprogramming of the mitochondrial protein acetylome, *Mol. Cell.*, **49**, 186-199, doi: 10.1016/j.molcel.2012.10.024.
 25. Sol, E. M., Wagner, S. A., Weinert, B. T., Kumar, A., Kim, H. S., et al. (2012) Proteomic investigations of lysine acetylation identify diverse substrates of mitochondrial deacetylase Sirt3, *PLoS One*, **7**, 1-9, doi: 10.1371/journal.pone.0050545.
 26. Oti, M. (2006) Predicting disease genes using protein–protein interactions, *J. Med. Genet.*, **43**, 691-698, doi: 10.1136/jmg.2006.041376.