Rahman et al., http://www.jcb.org/cgi/content/full/jcb.201404118/DC1

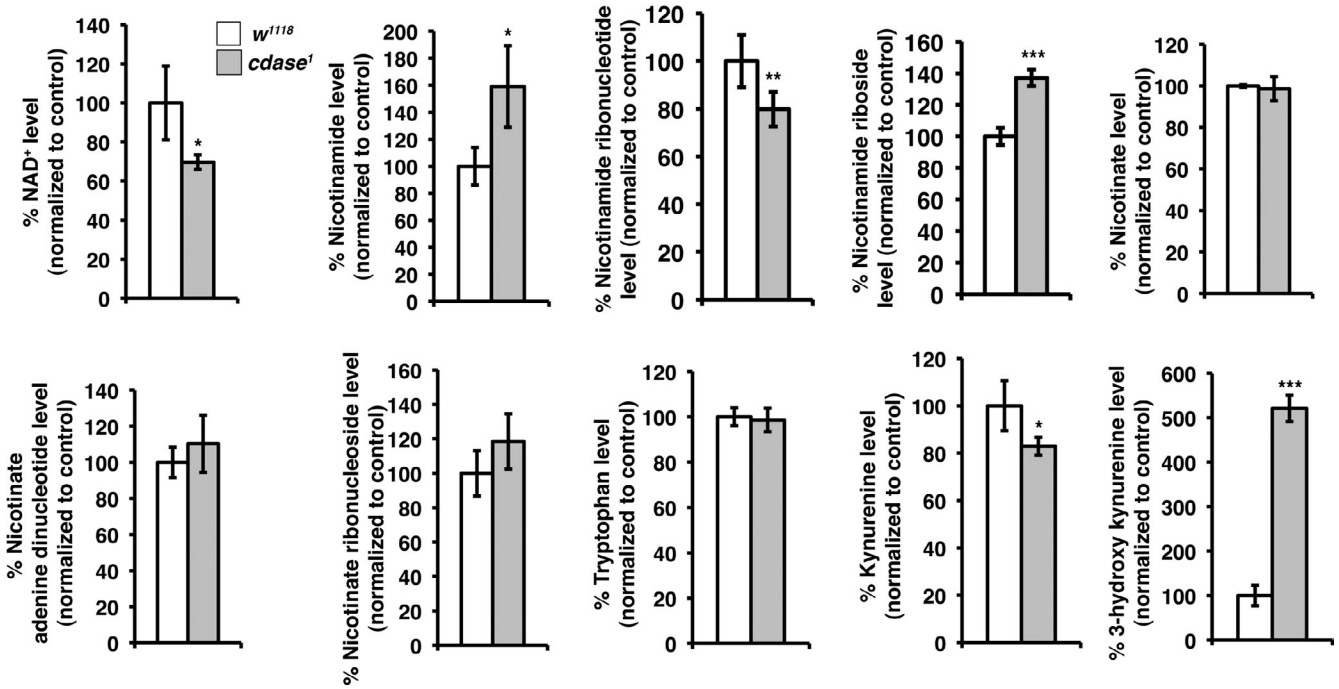

Figure S1. **MS measurements of NAD+ and metabolites involved in the salvage pathway and de novo synthesis of NAD+ from tryptophan in *cdase*[1] mutants NAD+ level is decreased in *cdase*[1] mutants.** The levels of nicotinamide riboside (in the salvage pathway) and 3-hydroxy kynurenine (synthesis from tryptophan) are increased. Extracts were prepared from 100 flies, and three replicates were performed. Error bars represent SDs, and statistical significance was determined using Student's *t* test; *, P ≤ 0.05–0.01; **, P ≤ 0.01–0.001; ***, P ≤ 0.001–0.0001.

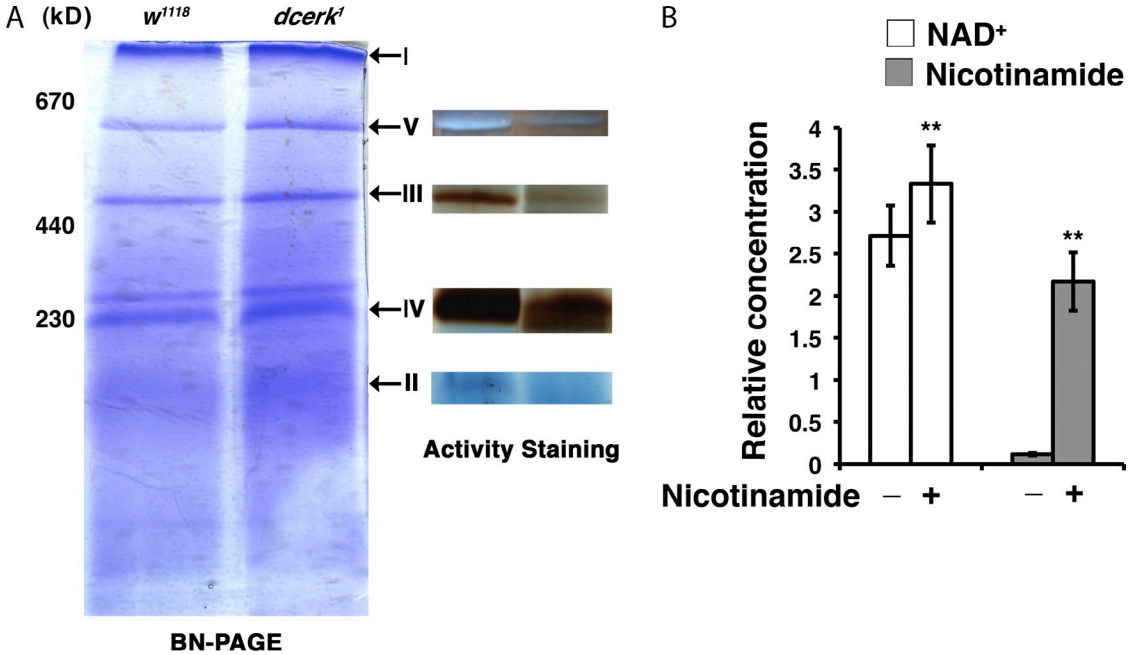

Figure S2. **Separation of OXPHOS complexes by BN-PAGE.** (A) Separation of mitochondrial OXPHOS complexes from w^{1118} and *dcerk*[1] by BN-PAGE and their activity staining. (B) Mass spectrometric measurements of NAD+ and nicotinamide in w^{1118} flies fed nicotinamide. Error bars represent SDs, and statistical significance was determined using Student's *t* test; **, P ≤ 0.01–0.001.

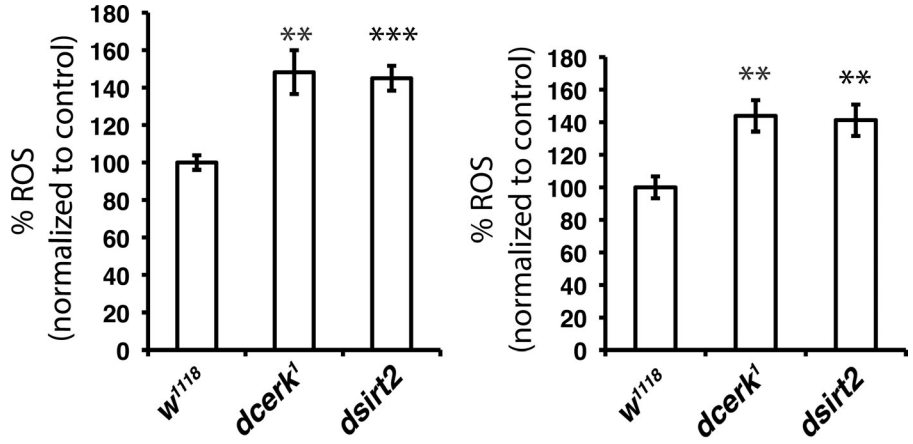

Figure S3. ***dsirt2* mutant flies show increased ROS levels.** Mitochondria were prepared from w^{1118}, *dcerk*^{1}, and *dsirt2* mutants, and ROS production was measured using pyruvate + proline (left) or *sn*-glycerol 3-phosphate (right) as a substrate. ROS levels are increased in *dcerk*^{1} and *dsirt2* compared with w^{1118} mitochondria. Mitochondria were prepared from ~1,000 flies for each batch. $n = 3$; error bars represent SDs. Statistical significance was determined using Student's *t* test: **, $P \leq 0.01$–0.001; ***, $P \leq 0.001$–0.0001.

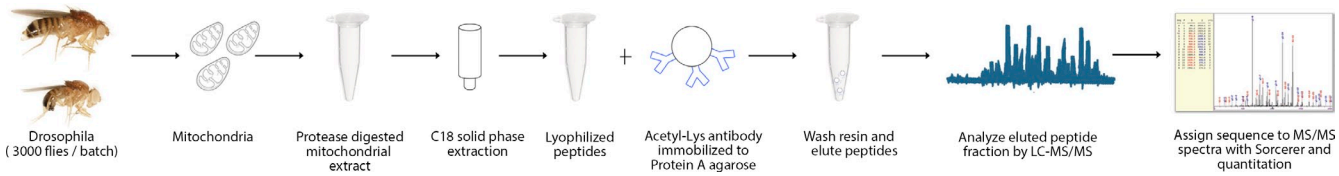

Figure S4. **Strategy for identification of *Drosophila* mitochondrial acetylome and dSirt2-regulated acetylome.** Mitochondria were isolated from 3,000 flies (w^{1118} or *dsirt2*) per batch. The mitochondrial extracts were digested with trypsin and desalted. Acetyl-Lys peptides were immunoprecipitated using an acetyl-Lys antibody. The enriched peptides were separated by LC-MS/MS. The MS/MS spectra were evaluated using SEQUEST 3G and SORCERER 2 platform, and label-free quantitation was performed from the acetyl-Lys peptide intensities. The experiment was performed in duplicate.

Table S1 shows details of acetyl-Lys peptides in the mitochondrial acetylome identified by MS and is provided in an Excel file.

Table S2 shows details of acetyl-Lys peptides that increase in *dsirt2* mutant mitochondrial acetylome identified by MS and is provided in an Excel file.