Morselli et al., http://www.jcb.org/cgi/content/full/jcb.201008167/DC1

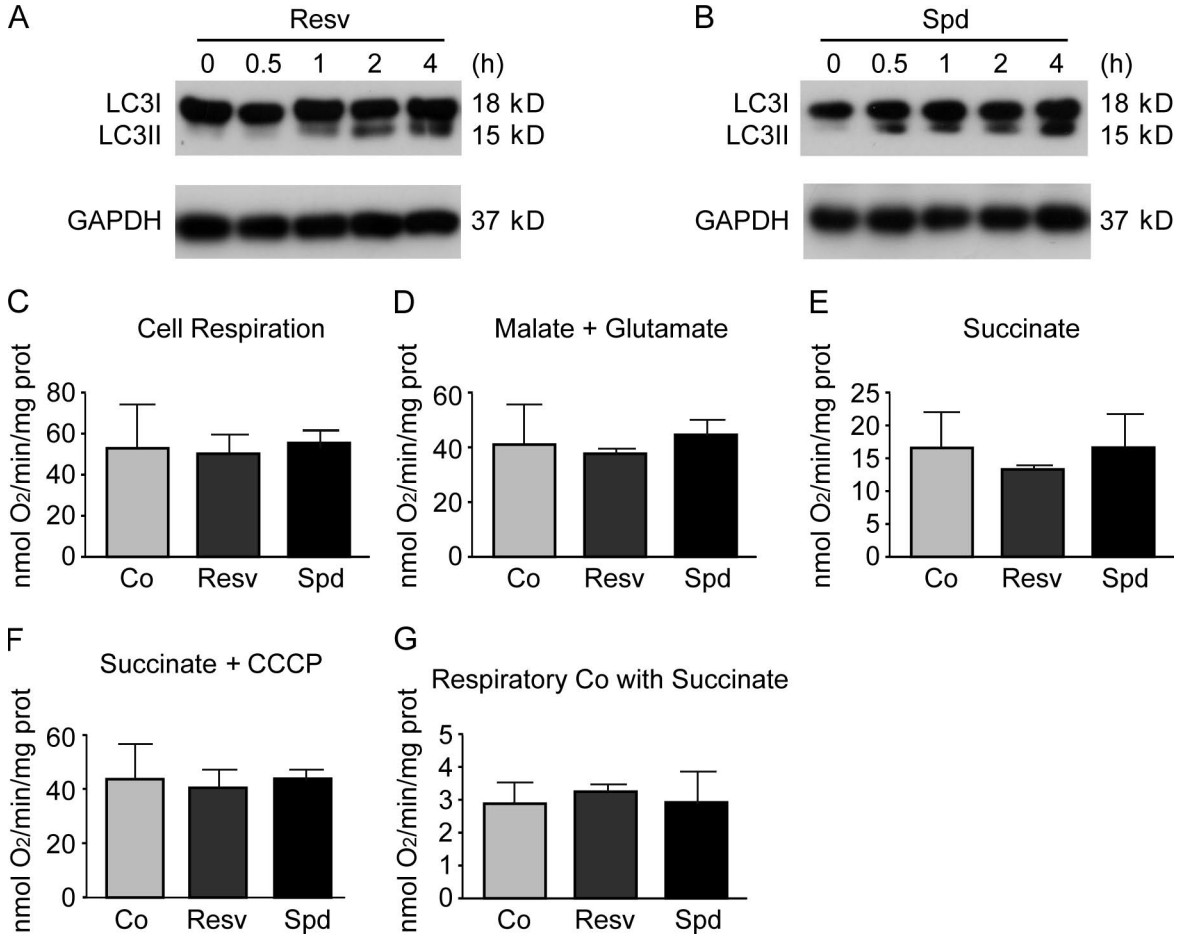

Figure S1. **The kinetics of autophagy induction after 100-µM resveratrol and spermidine treatment and measurements of cell respiration and mitochondrial substrate oxidation in the absence (control) or presence of 100-µM resveratrol or spermidine.** (A and B) Representative immunoblots showing endogenous LC3B lipidation in colon carcinoma HCT 116 cells treated with 100-µM spermidine (Spd) or resveratrol (Resv) for the indicated time. (C–G) HCT 116 cells were left untreated (Co, control) or treated with 100-µM resveratrol or spermidine for 4 h and then processed for the polarographic measurement of oxidative phosphorylation. Bars depict nanomoles of O_2 consumed per minute and milligrams of protein (prot; means ± SD; $n = 3$). CCCP, carbonyl cyanide m-chlorophenyl hydrazone. GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

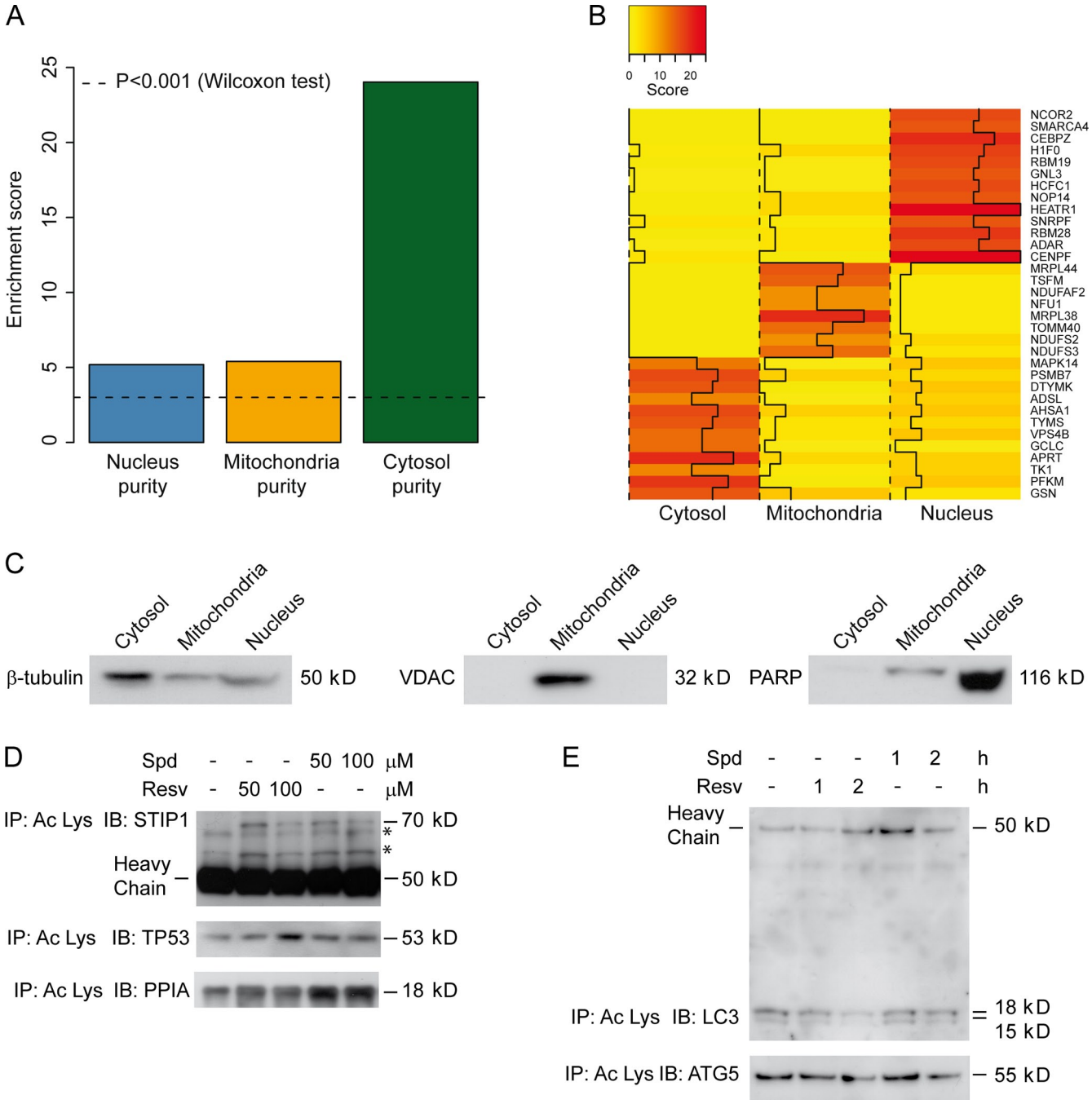

Figure S2. **Measurement of SILAC fraction purity and analysis of the acetylation status of autophagy essential proteins after spermidine and/or resveratrol treatment.** (A–C) Determination of the purity and quality of the organelle fractionation. (A) Fraction purity is measured by an enrichment score calculated from Wilcoxon testing for distribution location shift in compartment-specific peptides classified by GO cellular component terms compared with all identified peptides within the corresponding organelle fractions. The dashed line indicates a high significant score corresponding to $P < 0.001$ (Wilcoxon test). (B) Using a Western blot–like approach, 33 organelle-specific markers were selected and used to determine the purity of organelle fractions. The color key is a visualization of the fraction-specific number of identified peptides associated with the protein whose gene name is indicated as the row name. The fraction-wise relative number is indicated in the heat map trace histogram (black line). (C) Control immunoblots on the subcellular fractions used for the proteomic experiments. Cytoplasmic, mitochondrial, and nuclear fractions were blotted with antibodies specific for β-tubulin, voltage-dependent anion channel (VDAC), and histone H3. PARP, poly-(ADP-ribose) polymerase. (D–E) Human colon carcinoma HCT 116 cells were treated with resveratrol (Resv) or spermidine (Spd) at the indicated concentrations for up to 2 h, processed for immunoprecipitation (IP) with an anti–acetyl lysine (Ac-Lys) antibody, and then subjected to immunoblotting (IB) with the depicted antibodies. Asterisks represent nonspecific bands detected by the antibody.

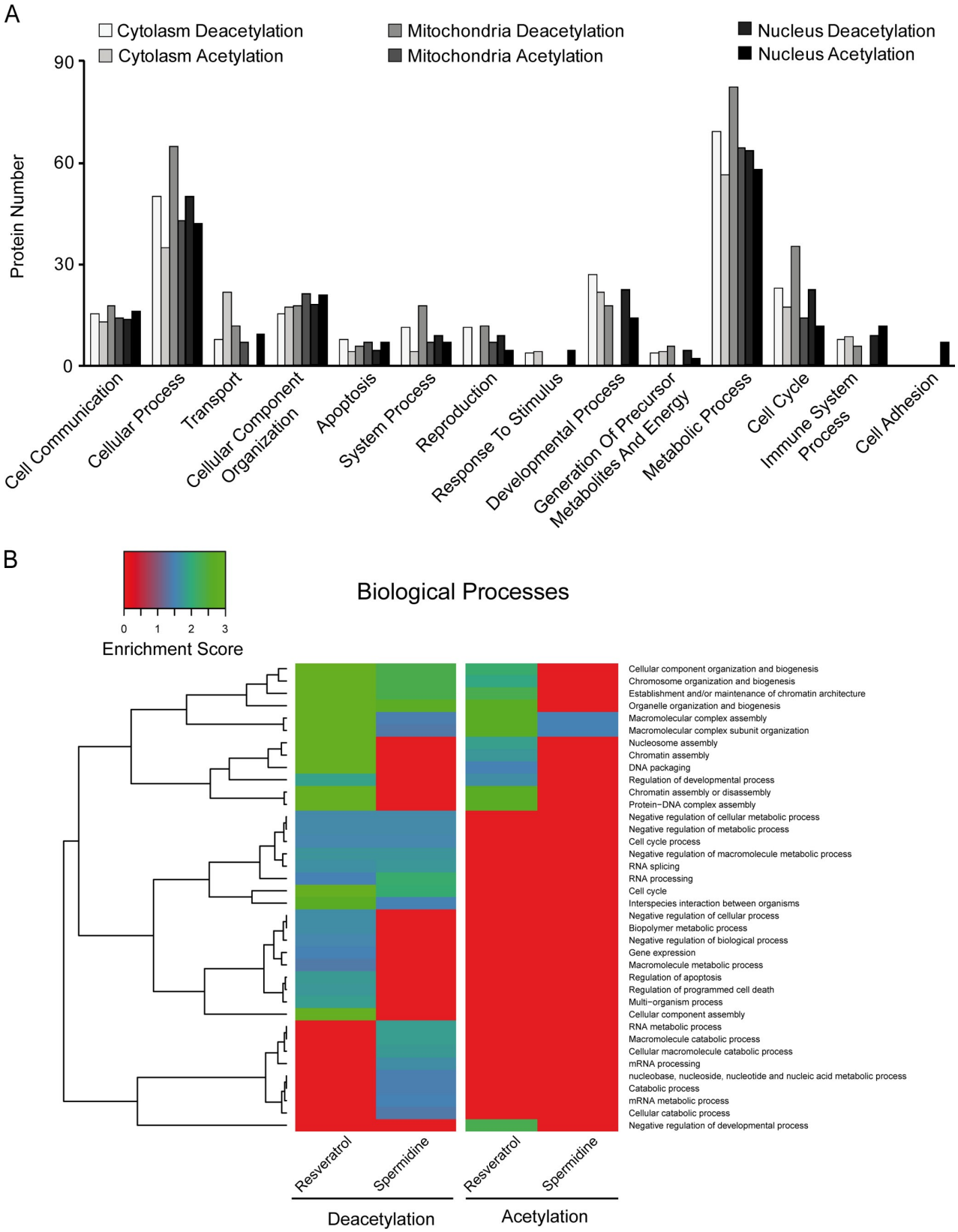

Figure S3. **The biological processes associated with the differentially acetylated proteins in response to resveratrol and spermidine treatment by means of GO enrichment.** (A) Summary of the GO biological processes found to be associated with the different protein groups subjected to convergent (de)acetylation in the different subcellular fractions after spermidine or resveratrol treatment. *n* = 375. (B) Fisher's exact test was applied to identify significant ($P < 0.05$) biological processes associated with proteins regulated by >1.5-fold acetylation and deacetylation during treatment with resveratrol or spermidine. The enrichment score calculated based on the adjusted p-value is indicated in the color key, in which a score >3 corresponds to $P < 0.001$.

Table S1 is provided as an Excel file and shows a list of detected acetyl lysine–containing motifs in colon carcinoma HCT 116 cells upon SILAC and spermidine or resveratrol treatment.

Table S2 is provided as an Excel file and shows a list of proteins belonging to the human autophagy network interacting with the proteins shown in Table S1.