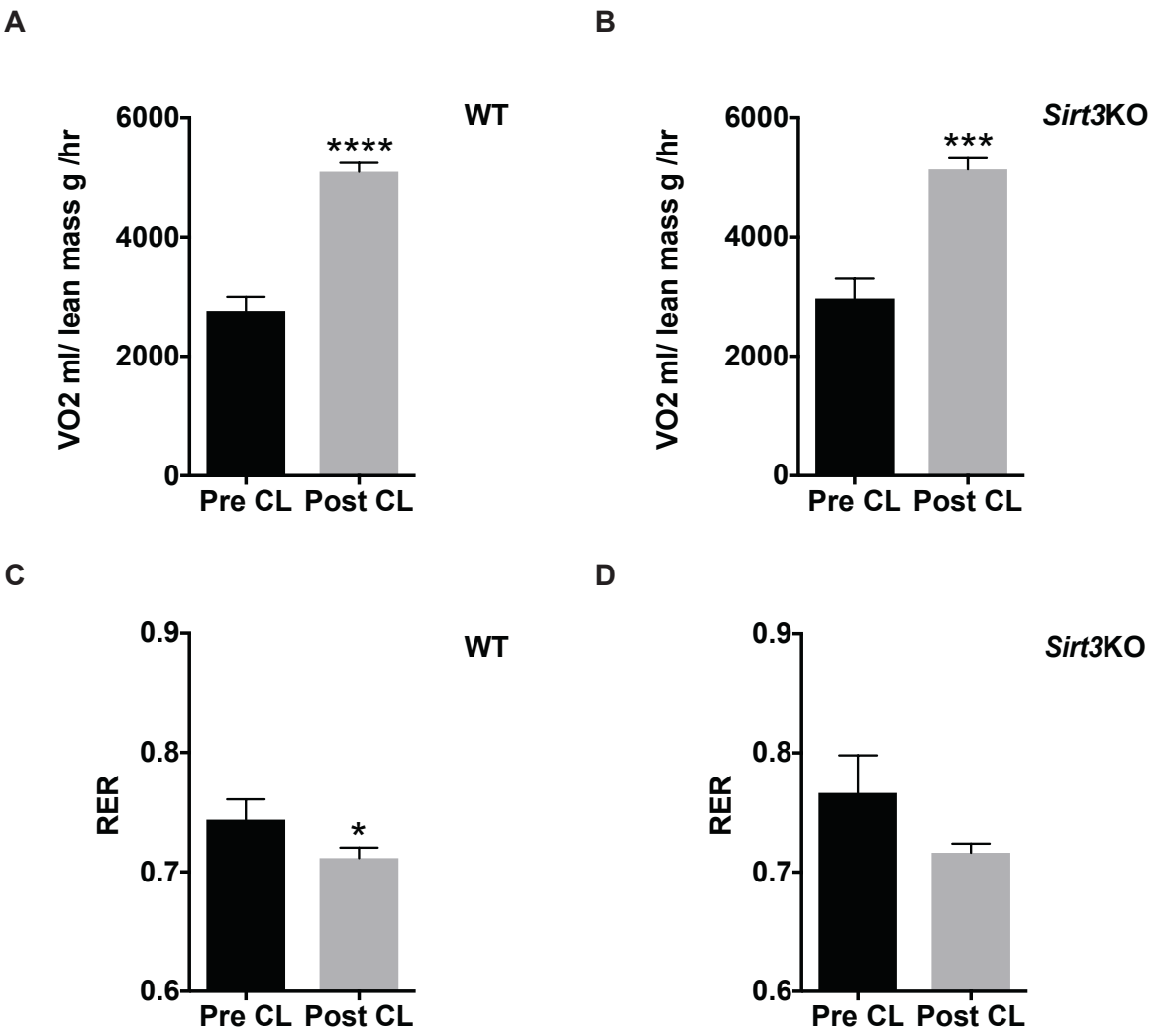
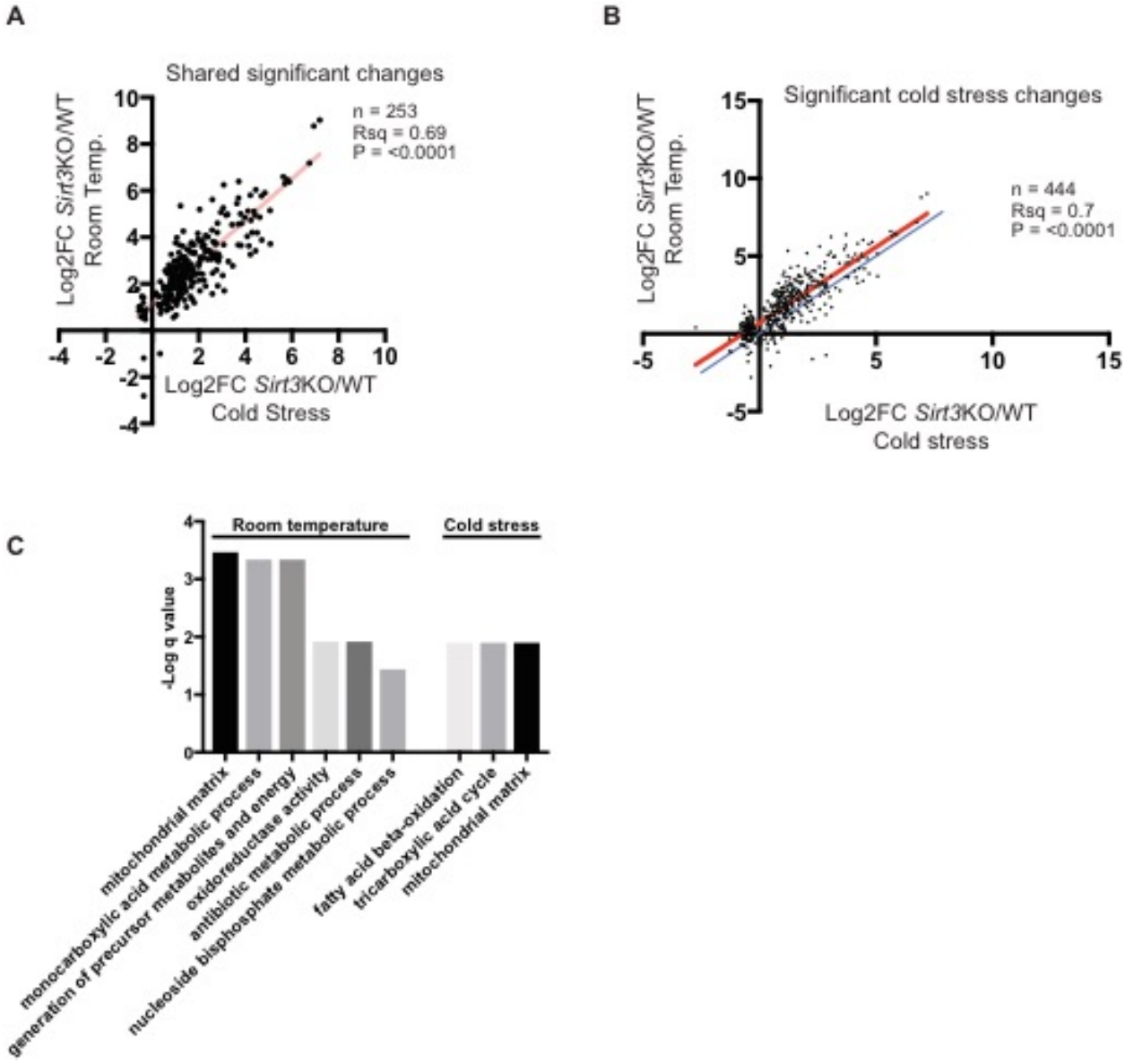
# Sebaa *et al.* 2019 - Supplementary Data

Includes 4 Figures and associated legends, and legends for 3 supplementary tables provided as excel files.

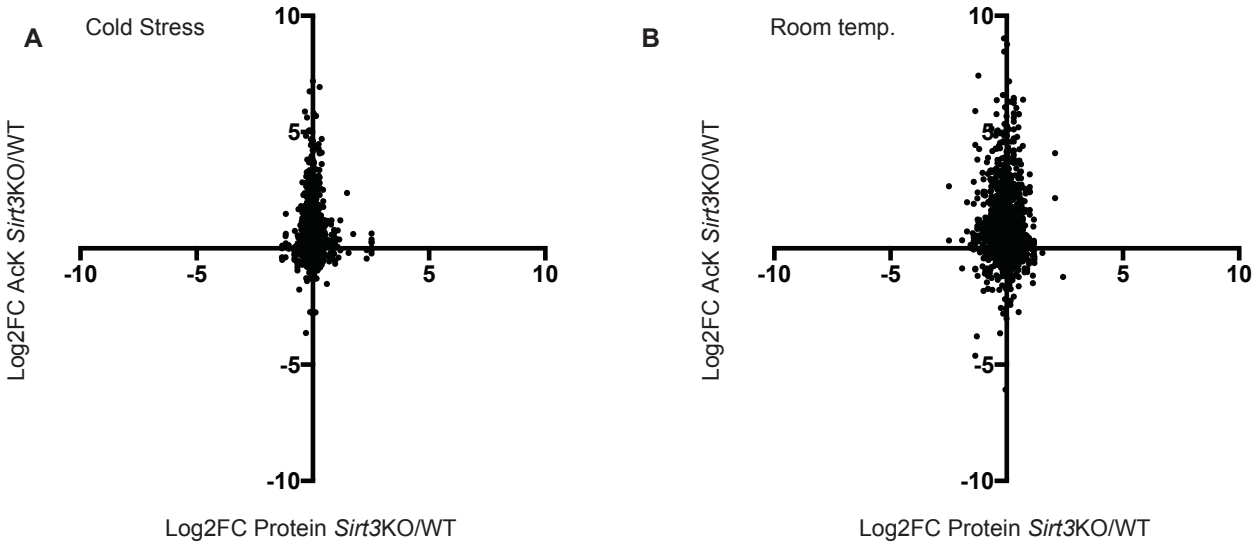



***Supplementary Figure 1 - related to Figure1:***
$VO_2$ was assessed in (A) WT mice and (B) *Sirt3*KO mice that were kept at 28 °C and were injected with CL316,243 (1mg/kg; *i.p.*). $VO_2$ values were assessed before and after CL316, 243 injection. N=6/group. RER values associated with the selected $VO_2$ values were assessed in (C) WT mice and (D) *Sirt3*KO mice. N=6/group. Data are represented as mean ± SEM. Student's t-test; Two-tailed, **p<0.05, ***p<0.001, ****p<0.0001*.

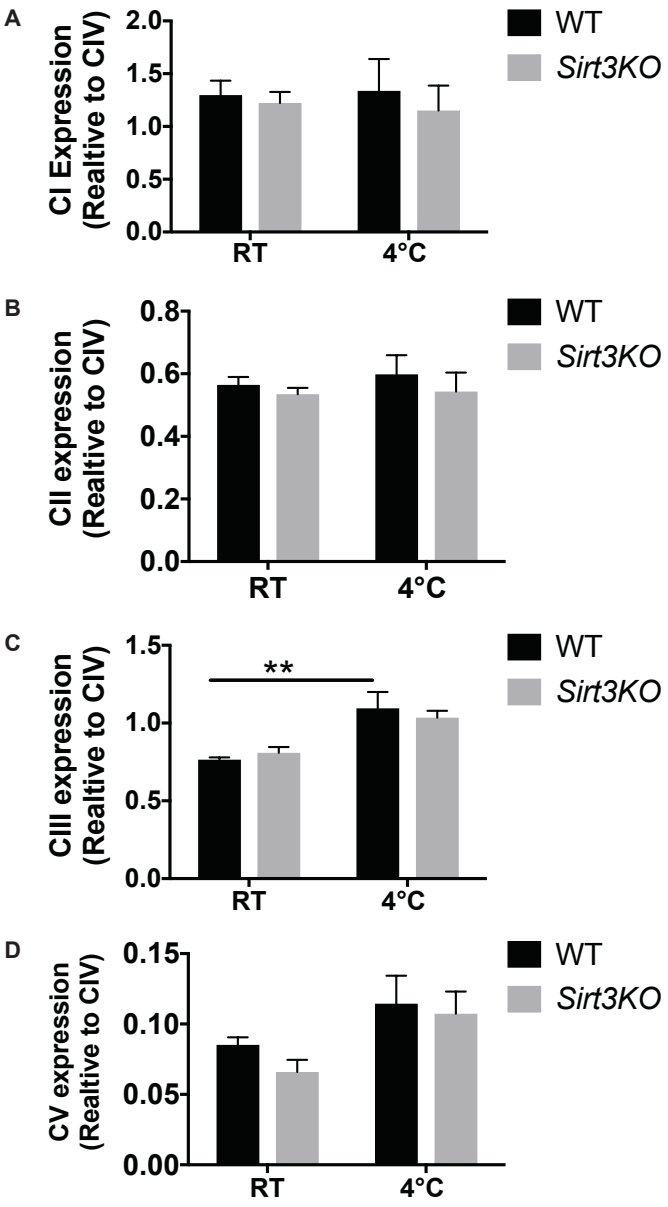



***Supplementary Figure 2 - related to Figure 3:***
(A) Measured Log2FC *Sirt3*KO/WT was plotted for acetylated lysines detected via MS for room temperature versus cold treated mice. Log2FC computed using MSStats as described in the experimental procedures. Linear regression calculated using GraphPad Prism. This analysis includes only measured changes that were deemed to be statistically significant (adjusted p value < 0.05) in both room temperature and cold stressed conditions. This graphs contains a subset of the data plotted in Figure 3B. (B) Plotted are significantly-regulated sites detected in samples from cold stressed animals, regardless of fold-change versus corresponding fold-change detected in room temperature animals, regardless of significance. Red line indicates the best fit line of linear regression. The blue line represents slope = 1. The majority of datapoints above blue line suggests more

dramatic changes in room temperature housed animals. This analysis is complementary to that descried in Figure 3E and contains a subset of the data plotted in Figure 3B.
(C) GO-term enrichment of SIRT3-regulated lysine acetylations sites calculated using Metascape for room temperature and cold regulated sites (log2 *Sirt3*KO/WT >1; adjusted $p<0.05$). Included as regulated sites were those detected in 6 biological replicates of *Sirt3*KO and not detected in any WT samples, for which no p value could be calculated. q values shown represent p values of GO-term analyses following adjustment for multiple-testing.




***Supplementary* Figure 3 - *related to Figure 3*:**
Comparison of Log2FC (*Sirt3* KO/WT) for acetylated peptides versus estimated protein abundance Log2FC (*Sirt3 KO*/WT) for mice housed under cold stress (A) or room-temperature (B) conditions. Protein abundance is based on intensities of non-acetylated peptides bound to beads used in anti-acetyllysine immunoprecipitations.




***Supplementary Figure 4 - related to Figure 6:***
Quantification of western blotting analysis of OXPHOS protein expression in isolated mitochondria from BAT of room temperature housed or cold exposed WT and *Sirt3*KO mice. N=3/group.

(A) Quantification results of CI expression.
(B) Quantification results of CII expression.
(C) Quantification results of CIII expression.
(D) Quantification results of CV expression.
Data are represented as mean ± SEM. Two-way ANOVA with Sidak's test was used, ***p<0.01.*

**Supplementary tables provided as excel files:**

**Supplementary Table 1:** This table contains Log2FC AND corrected P values (q values) for acetylome profiling experiments conducted at room temperature or during cold stress. Full Metascape analyses and data plotted in figures 4-6 are indicated in individual tabs.

**Supplementary Table S2:** Comparison of Log2FC (*Sirt3* KO/WT) for acetylated peptides versus estimated protein abundance Log2FC (*Sirt3 KO*/WT) for mice housed under cold stress (A) or room-temperature (B) conditions.

**Supplementary Table S3:** Sequence context and annotations for all acetylation sites mapped in this work. Analysis was completed with the SLiMSearch tool (see Materials and Methods).