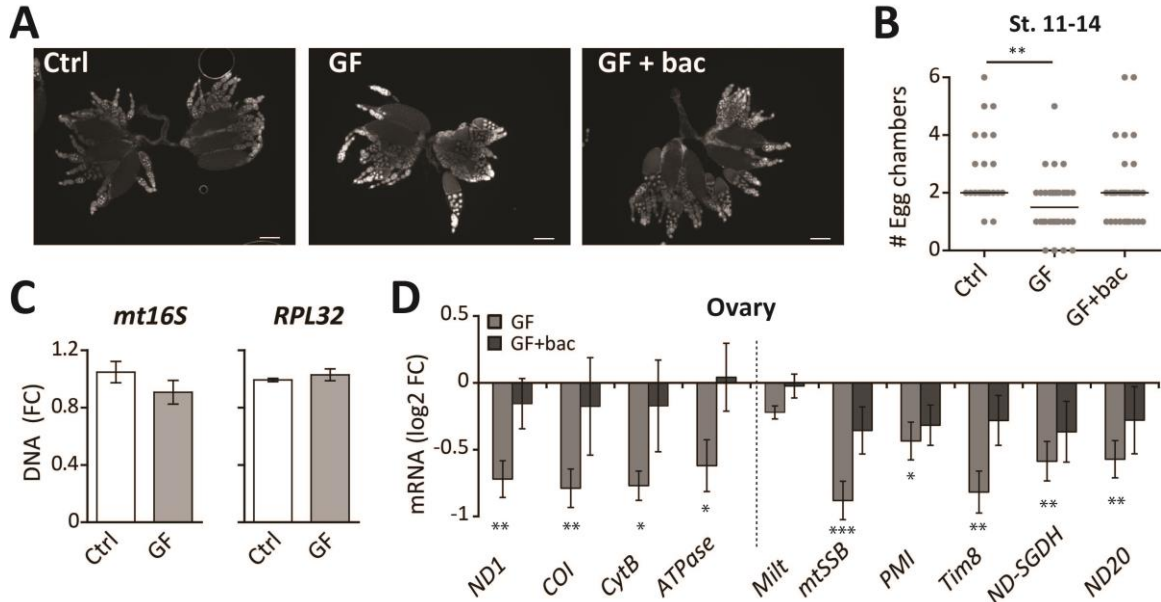


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**Supplemental Information**

**Systemic Regulation of Host Energy and Oogenesis  
by Microbiome-Derived Mitochondrial Coenzymes**

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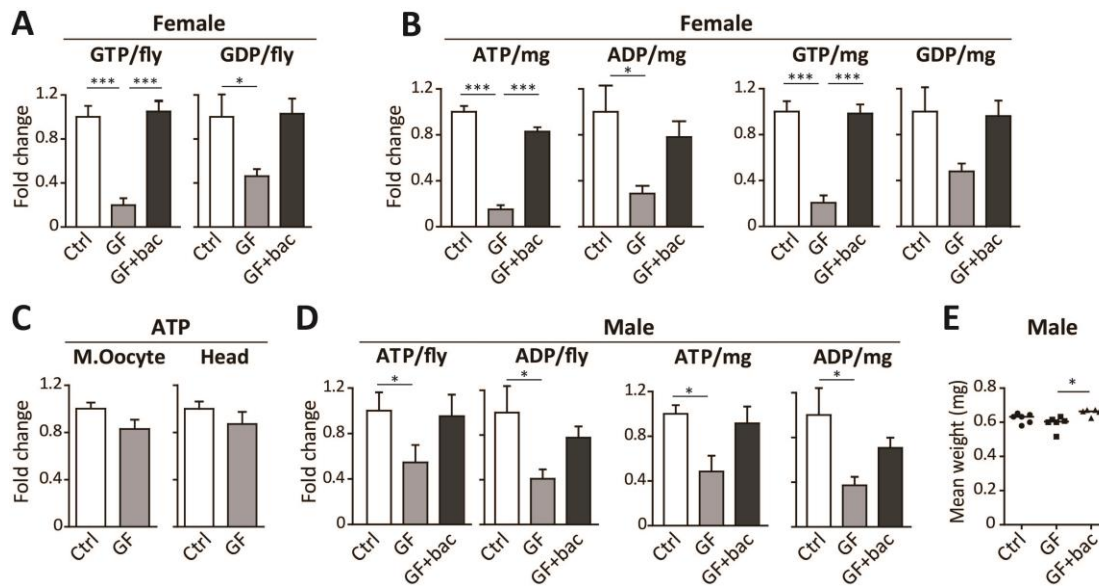
**Figure S1. Reduced mitochondrial DNA and RNA in ovaries of young GF females vs. control, Related to Figure 1**

**(A)** Representative images of DAPI-stained ovaries in 2 day-old yw females, shown for the following cases: conventionally raised (Ctrl) and germ free females with and without supplementation of native gut bacteria to the female diet (GF+bac and GF, respectively). Scale bar 200μm.

**(B)** Number of egg chambers at stages 11-14 in ovary for the cases shown in (A). Scatter dot plot and median; n>22 ovaries; \*\*p<0.01 (Kruskal wallis with Dunn's multiple comparison test).

**(C)** Relative DNA levels (Fold-change, FC) of mitochondrial 16S (*mt16S*) and ribosomal protein L32 (*RPL32*) in ovaries of GF vs. control females, as determined by qPCR with *Drosophila* actin DNA as a reference. Mean ± SEM, n=6 with 10 ovaries per sample.

**(D)** mRNA fold change (log2 FC) of mitochondria-encoded (*ND1*, *COI*, *CytB*, *ATPase*) and nucleus-encoded mitochondrial genes (*Milt*, *mtSSB*, *Tim8*, *PMI*, *ND-SGDH*, *ND-20*) in ovaries of 2-day GF females vs. Ctrl. Mean ± SE, n>6 with 10 ovaries per sample; \*p<0.05, \*\*p<0.01, \*\*\*p<0.001 vs. Ctrl (t-test). *ND1*, NADH-ubiquinone oxidoreductase chain 1; *COI*, cytochrome C oxidase; *cytB*, cytochrome B; *Milt*, Milton; *mtSSB*, mitochondrial single stranded DNA-binding protein; *PMI*, protein of the mitochondrial inner membrane; *ND-SGDH*, NADH dehydrogenase (ubiquinone) SGD subunit; *ND-20*, NADH dehydrogenase (ubiquinone) 20kDa subunit.



**Figure S2. Reduced levels of GTP, GDP, ATP and ADP levels in GF females and males, Related to Figure 1**

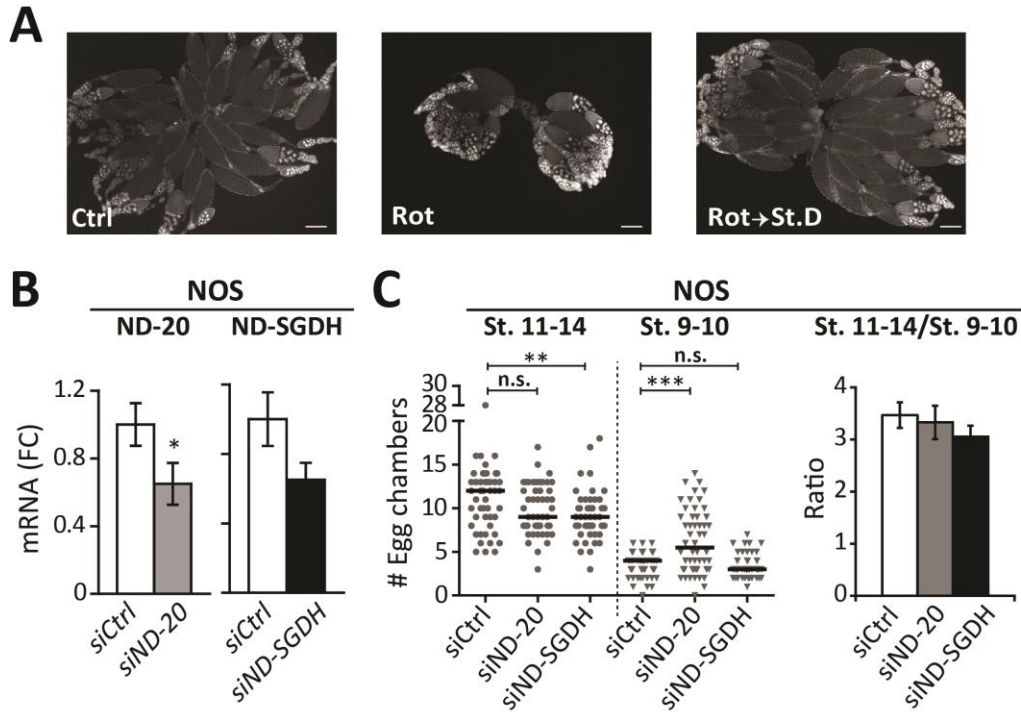
**(A)** Relative levels of GTP and GDP, measured by quantitative LC-MS in whole body of conventionally raised females (Ctrl) and GF females (day-6) with and without supplementation of native gut bacteria (GF + bac and GF, respectively). Mean  $\pm$  SEM,  $n=5$ , with  $\sim 50$  flies/sample; \*  $p<0.05$ , \*\*\*  $p<0.001$  (ANOVA, followed by Tukey's test).

**(B)** Same as (A) for ATP, ADP, GTP and GDP levels, normalized by sample weight. Mean  $\pm$  SEM,  $n=5$  with  $\sim 50$  flies/sample; \*  $p<0.05$ , \*\*\*  $p<0.001$  (ANOVA with Tukey's multiple comparison test).

**(C)** ATP levels in mature oocytes (M.Oocyte) and heads of conventionally raised (Ctrl) and GF females. Mean  $\pm$  SEM,  $n=8$  for mature oocytes (30 oocytes/sample) and  $n=9$  for heads (10 heads/sample).

**(D)** Same as (B) for 6-day old males. Mean  $\pm$  SEM,  $n=5-6$ , with  $\sim 50$  flies/sample; \*  $p<0.05$  (ANOVA with Tukey's test).

**(E)** Average male weight (mg) for the cases in (D); \*  $p<0.05$  (Kruskal Wallis with Dunn's multiple comparison test).



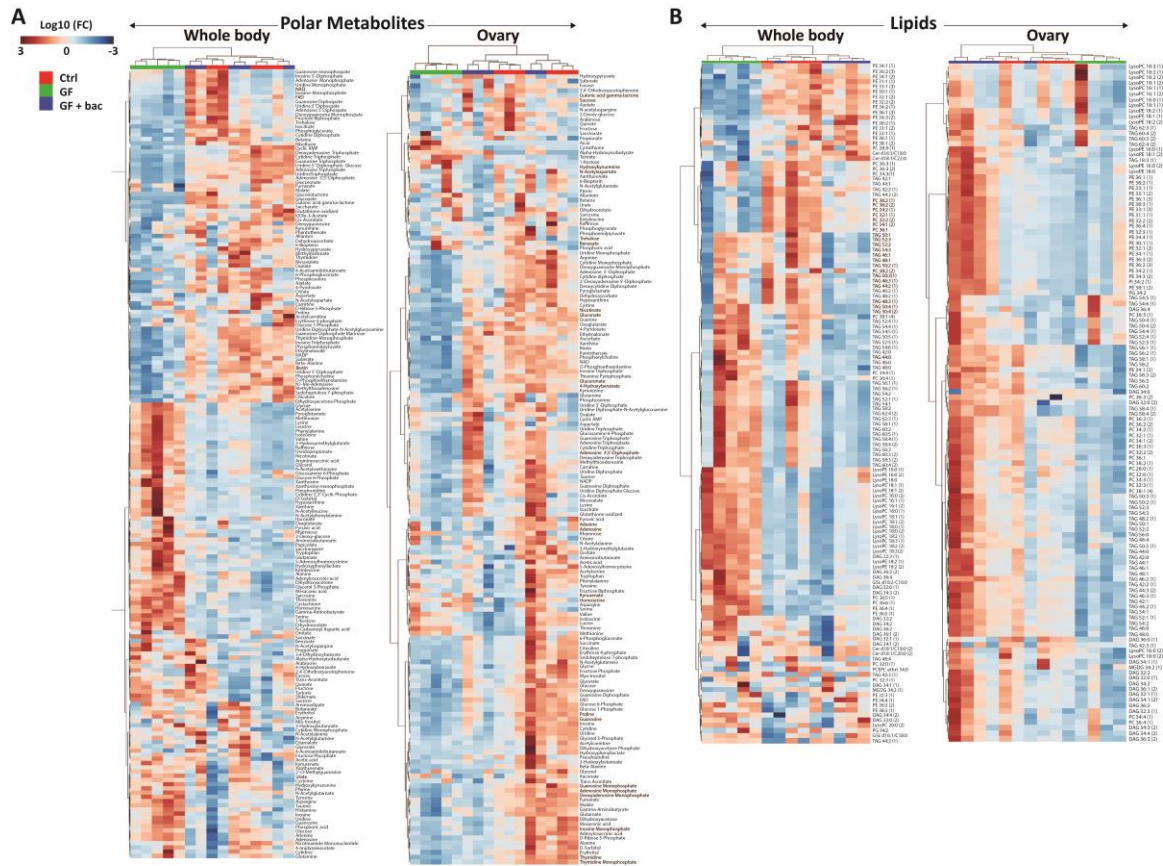
**Figure S3. Effect of global and germ-line specific inhibition of mitochondrial function on the ovaries, Related to Figure 2**

**(A)** Representative images of day-6 DAPI-stained ovaries of conventionally raised females (yw) for the following cases: no treatment (Ctrl), rotenone exposure (25 $\mu$ M) from day 3 to 6, with and without subsequent transfer to rotenone-free diet for 3 additional days (Rot and Rot  $\rightarrow$  Std. Diet, respectively). Scale bar 200 $\mu$ m.

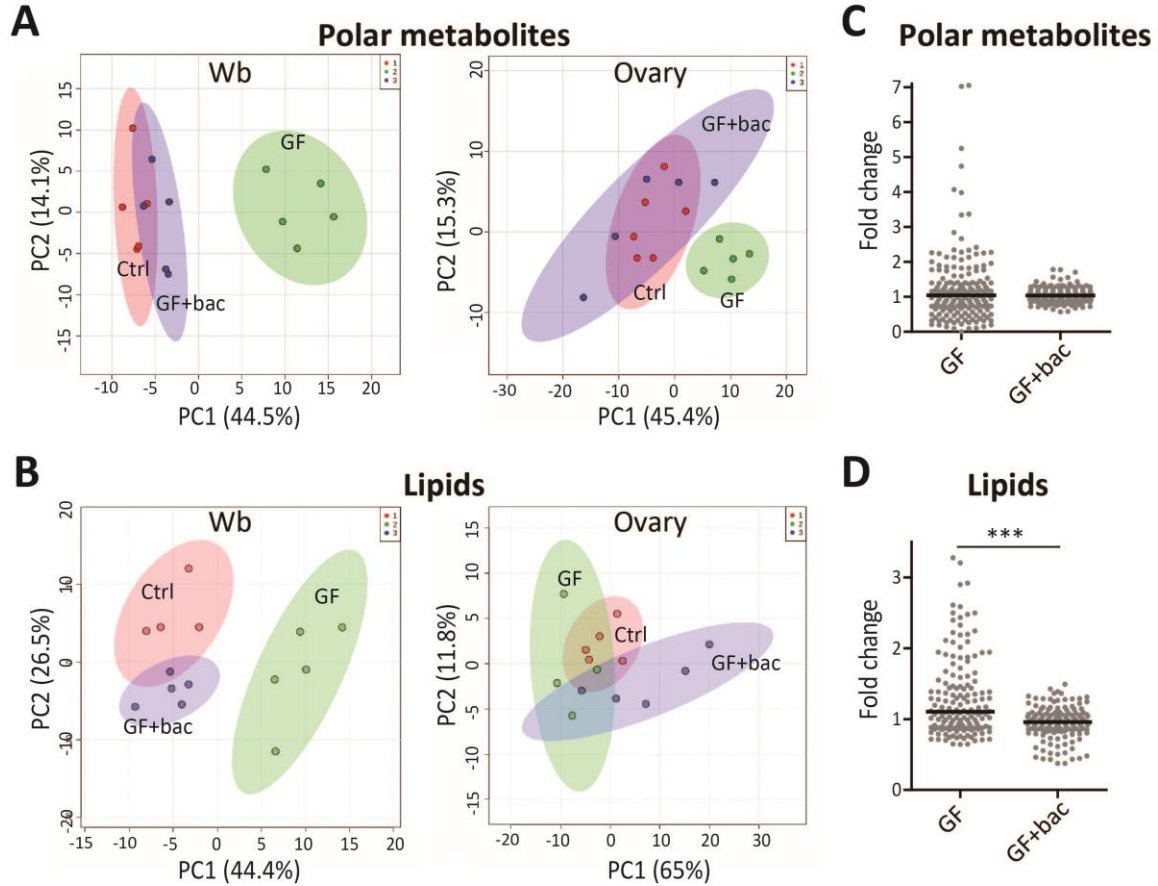
**(B)** mRNA fold change of ND-20 and ND-SGDH, in dechorionated mature oocytes of *NOS>siCtrl*, *NOS>siND-20* and *NOS>siND-SGDH* females. Mean  $\pm$  SEM,  $n \geq 4$  (~30 oocytes per sample);

\* $p < 0.05$  (t-test).

**(C)** Left: Number of egg chambers at stages 11-14 and 9-10 in ovaries of *NOS>siCtrl*, *NOS>siND-20* and *NOS>siND-SGDH* females. Right: Ratio between the numbers of chambers in 11-14th vs. 9-10th stage. Mean  $\pm$  SEM;  $n = 46-52$  ovaries; \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  versus *NOS>siCtrl* (Mann Whitney test).



**Figure S4. Hierarchical clustering of polar and lipid metabolites, Related to Figure 4**  
**(A, B)** Relative abundance of polar metabolites (A) and lipids (B) measured by LC-MS in whole body and ovary of 6-day conventionally raised (Ctrl) and germ-free (GF), with and without supplementation of native gut bacteria (GF+bac and GF, respectively). Color code corresponds to log<sub>10</sub> fold-change versus Ctrl, with each row and column corresponding, respectively, to a specific metabolite and a particular sample.

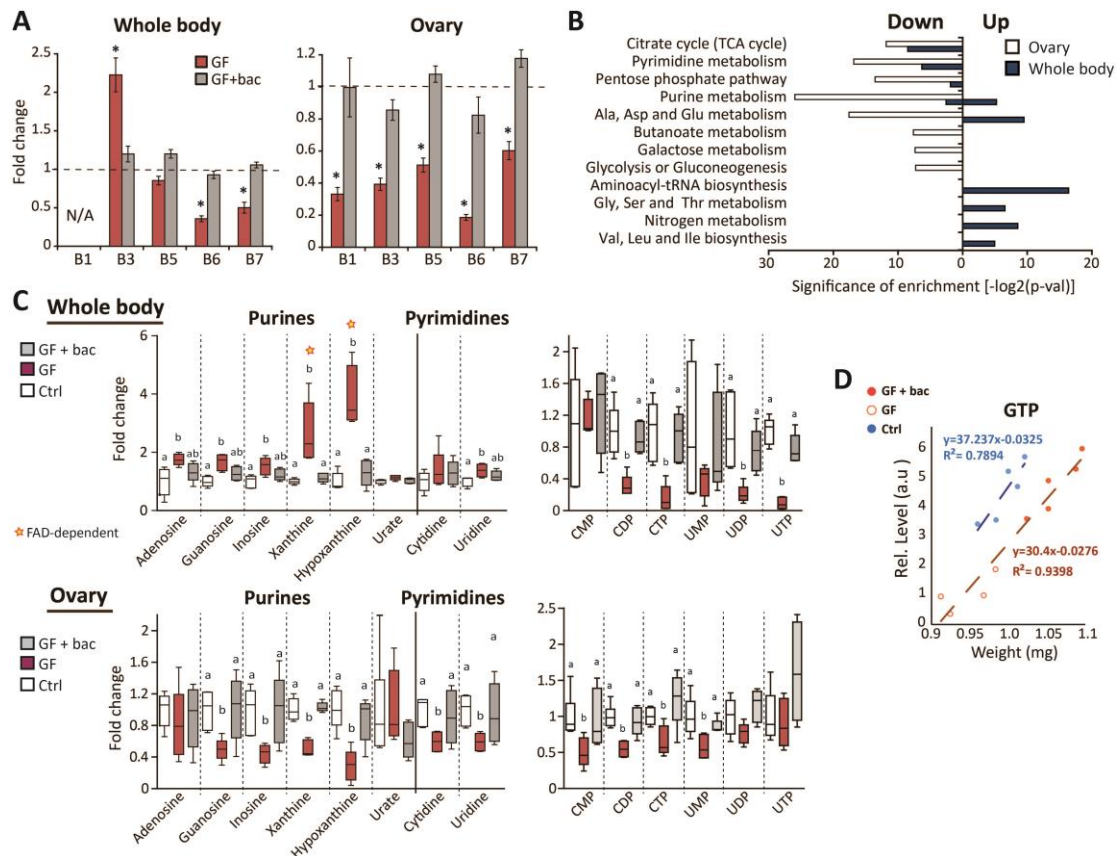


**Figure S5. Global analysis of metabolic changes, Related to Figure 4**

**(A, B)** Principle component analysis (PCA) of polar metabolites (A) and lipids (B), based on LC-MS measurements in whole body (Wb) and ovary samples of day-6 conventionally raised (Ctrl) and germ free females, with and without supplementation of native gut bacteria (GF+bac and GF, respectively). n=5-6; ~50 females/ovaries per sample.

**(C, D)** Fold change (versus control) shown for all the polar (C) and lipid metabolites (D) in whole-body of GF females, with and without bacterial supplementation (GF+bac and GF, respectively). Each dot corresponds to the average fold-change of a specific metabolite; Scatter dot plot with medians; \*\*\* p<0.001 (Mann Whitney test).





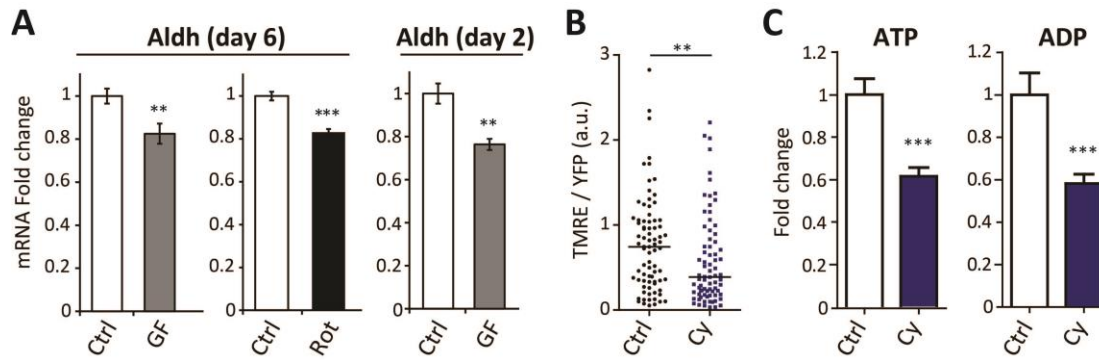
**Figure S6. Impact of gut bacteria on B-type vitamins, purines and pyrimidines, Related to Figure 4**

**(A)** Fold change of B-type vitamins in whole body and ovary of day 6 GF females with and without bacterial supplementation (GF+bac and GF), relative to conventionally raised females (Ctrl, dashed line). Mean  $\pm$  SEM;  $n=5-6$ ;  $\sim 50$  females/ovaries per sample. \*FDR<0.05 (ANOVA with Tukey's multiple comparison test).

**(B)** Pathway enrichments in sets of polar metabolites that increase (Up) and decrease (Down) in whole body and ovary of GF vs. conventionally raised females. Significance of enrichment is displayed as  $-\log_2$  of FDR determined by hypergeometric test.

**(C)** Fold change of purines and pyrimidines in whole body (upper panels) and ovary (lower panels) for the cases in (A). Median  $\pm$  quartiles; medians sharing a letter are not significantly different (ANOVA with Tukey's multiple comparison test, FDR<0.05).

**(D)** Scatter plot of GTP (arbitrary units, a.u. of peak intensity) against weight of females for the cases in (A).



**Figure S7. Crosstalk between *Aldh* and mitochondrial functions, Related to Figure 1**

(A) Left panel: Changes in *Aldh* mRNA levels in ovaries of conventionally raised females (Ctrl), rotenone-treated females (Rot, 25 $\mu$ M) and GF females. Right panel: *Aldh* fold-change in young (day-2) conventionally raised (Ctrl) and germ free (GF) females. Mean  $\pm$  SE; n>6; \*\* p<0.01, \*\*\* p<0.001 (t-test).

(B) TMRE/EYFP intensity ratio (arbitrary units, a.u.) in follicle cells of 6-8 stage egg chambers of conventionally raised and cyanamide-treated females. Scatter dot plot with medians; n>77; 3 independent experiments. \*\*p<0.01 (Man Whitney test).

(C) ATP and ADP fold-change, measured by quantitative LC-MS in ovaries of day 6 conventionally raised (Ctrl) and 3mM cyanamide-treated females (CY). Mean  $\pm$  SE; 4 independent experiments; n $\geq$ 13; \*\*\* p<0.001 (t-test).



**Table S1. Primers used in this study, Related to Star Methods**

Target gene	Forward primer	Reverse primer	Related to Figures
<i>mtDNA 16S</i>	AAAAAGATTGCGACCTCGAT	AAACCAACCTGGCTTACACC	1D and S1C
<i>RPL-32</i>	AGGCCCAAGATCGTGAAGAA	TGTGCACCAGGAACTTCTTGAA	S1C
<i>Actin</i>	GGAAACCACGCAAATTCTCAGT	CGACAACCAGAGCAGCAACTT	1D and S1C
<i>Actin5C</i>	CCCTCGTTCTTGGGAATGG	CGGTGTTGGCATAACAGATCCT	1F,1G and S1D
<i>Col</i>	TGAACAGTACCTGCTTTAGGAGT	TGACCATAAAATAAACCCGGTCG	1F,1G and S1D
<i>ND1</i>	CCTCAACCTTTTTGTGATGCG	GACGACCAACCAGCTACTATAAC	1F,1G and S1D
<i>ATPase6</i>	TTTTCTGTATTGACCCCTTAGC	GATCCATTATGACCTGATGGTCC	1F,1G and S1D
<i>cytB</i>	ACTCCTTTAGTAACACCTGCCC	TGGTCGAGCTCCAATTCAAGT	1F,1G and S1D
<i>mtSSB</i>	GTCACCTTTTCGGTTGCTACA	GCTTGAACACCACTACACGATG	1F,1G and S1D
<i>Milt</i>	GCAGACGATGGCACAGATACT	CGTCGAGCAGGGAGTTGAC	1F,1G and S1D
<i>PMI</i>	CTGGACAAGGCGCTGGAA	CAAATCCAACGACCAGTTTCG	1F,1G and S1D
<i>Tim8</i>	AACCTTTCCGGCAATGACAAG	CCGATGCACTTCTCCCAGC	1F,1G and S1D
<i>ND-20<sup>a</sup></i>	CGTGGCTGCGATAGGATAAT	ACCACATCTGGAGCGTCTTC	1F,1G, 2I, S1D, S3B
<i>ND-SGDH<sup>a</sup></i>	AGTCACCGCATTGGTTCTCT	GAGATGGGGTGCTTCTCGTA	1F,1G, 2I, S1D, S3B

<sup>a</sup>Primer sequences from Copeland et al. (Copeland et al., 2009)

**Table S2. Parameters for quantitative LC-MS/MS based analysis of ATP and ADP levels, Related to Star methods**

Name of the compound	Retention time, min	Transition, m/z	Cone voltage, V	Collision energy, eV	LOQ (10ng/ml)
ADP	1.33	428.0 > 136.1	25	25	10
		428.0 > 348.1	25	14	
ATP	1.54	507.9 > 136.0	14	35	10
		507.9 > 410.0	14	18	