

SUPPLEMENTAL MATERIAL

Tables

Table S1. Markers of hepatic and renal function in the plasma and urine from *Coq9^{+/+}* mice and *Coq9^{+/+}* mice under 0.33% of β -RA supplementation. GOT = glutamate-oxaloacetate transaminase; GPT = glutamate-pyruvate transaminase; AP = alkaline phosphatase.

PLASMA		
	<i>Coq9^{+/+}</i>	<i>Coq9^{+/+}</i> + 0.33 % β -RA
	Mean \pm SD	
GOT (U/L)	76.20 \pm 21.75	66.95 \pm 8.48
GPT (U/L)	41.70 \pm 19.63	25.20 \pm 9.81 *
AP (U/L)	24.58 \pm 17.86	18.77 \pm 15.49
Urea (mg/dl)	143.60 \pm 147.93	5.97 \pm 13.28 *
Creatinin (mg/dl)	0.50 \pm 0.17	0.41 \pm 0.086
Albumin (g/dl)	2.98 \pm 0.11	2.90 \pm 0.089
Bilirubin (mg/dl)	1.28 \pm 0.22	1.15 \pm 0.26
URINE		
	<i>Coq9^{+/+}</i>	<i>Coq9^{+/+}</i> + 0.33 % β -RA
	Mean \pm SD	
Albumin (g/dl)	0.06 \pm 0.026	0.03 \pm 0.021
Total Protein (g/dl)	0.80 \pm 0.46	0.72 \pm 0.21
Creatinin (mg/dl)	31.39 \pm 7.71	30.40 \pm 4.24
Urea (mg/dl)	78.96 \pm 125.65	3.72 \pm 1.54
Uric Acid (mg/dl)	1.80 \pm 1.70	1.72 \pm 0.82
Phosphorus (mg/ml)	66.63 \pm 26	77.02 \pm 18.14
Calcium (mg/dl)	7.83 \pm 4.83	6.79 \pm 3.07
Magnesium (mg/dl)	5.47 \pm 0.77	5.65 \pm 1.77

Figures

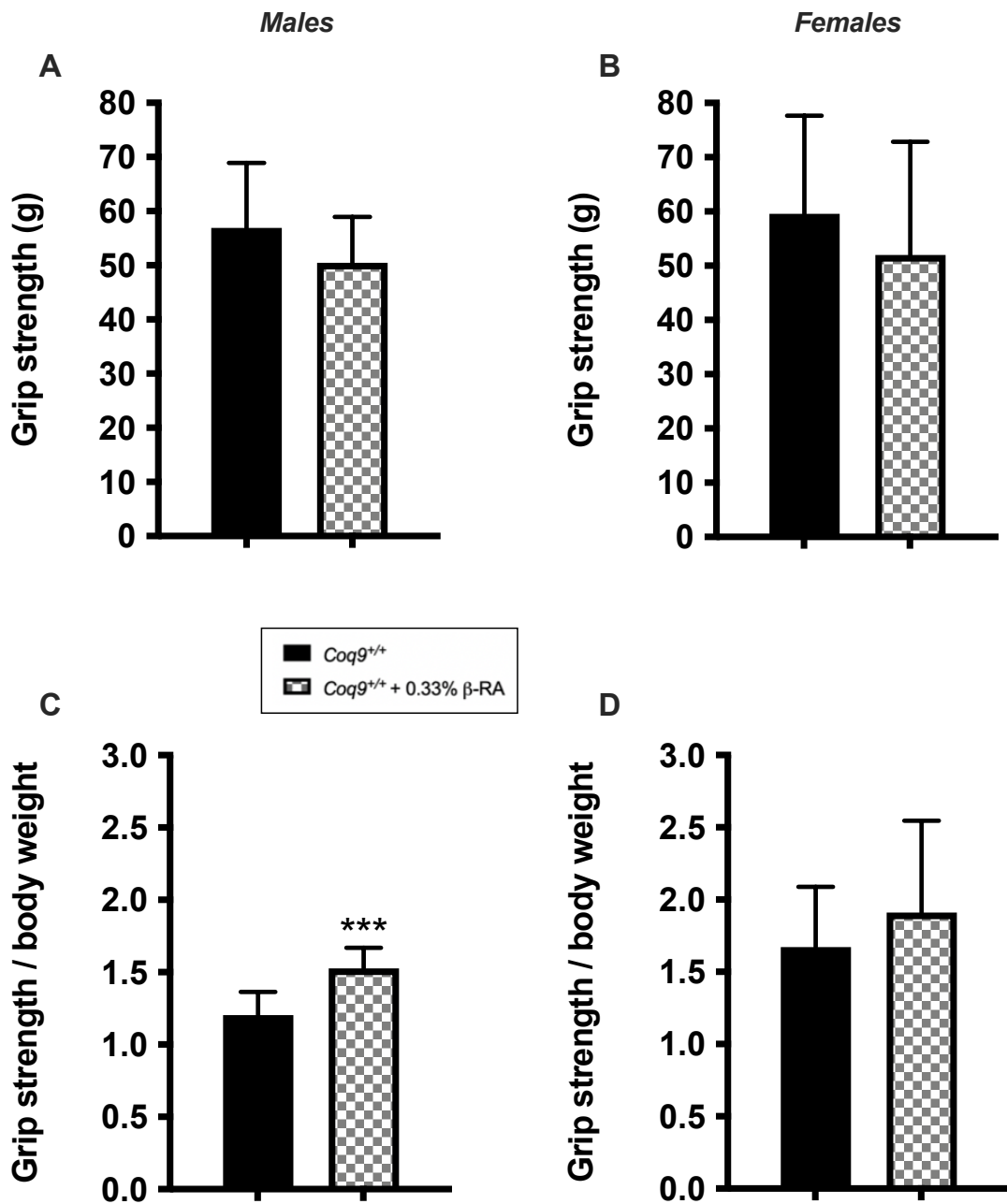


Figure S1. Muscle Strength.

(A to D) Grip test in the hind legs of male (A, C) and female (B, D) wild-type mice supplemented with 0.33% β -RA. Data are expressed as grip strength (A, B) and grip strength normalized by the body weight (C, D).

Data are expressed as mean \pm SD. *** $P < 0.001$, differences versus *Coq9*^{+/+} (*t*-test; $n = 6-9$ for each group).

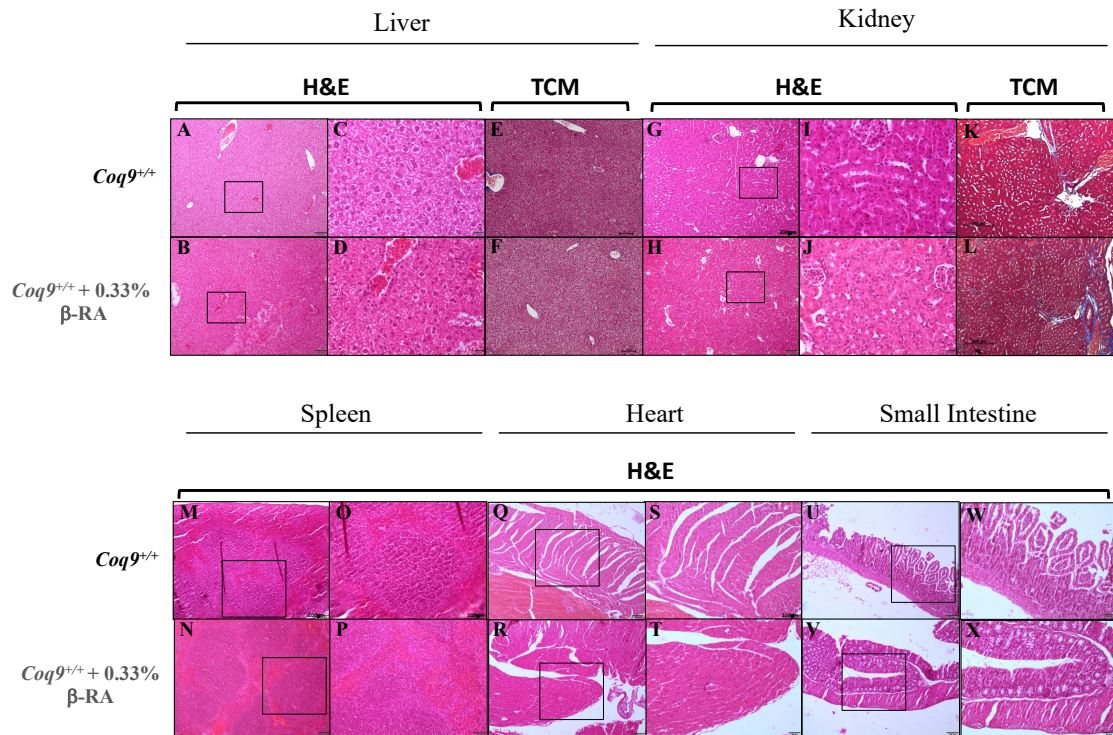


Figure S2. Morphological and histological features of the liver, kidney, spleen, heart and small intestine from *Coq9*^{+/+} and *Coq9*^{+/+} mice under 0.33% of β -RA treatment.

(A to D) H&E stain in the liver from *Coq9*^{+/+} mice and *Coq9*^{+/+} mice under 0.33% of β -RA treatment.

(E to F) Trichrome Gomori (TCM) stain in the liver from *Coq9*^{+/+} mice and *Coq9*^{+/+} mice under 0.33% of β -RA treatment.

(G to J) H&E stain in the kidney from *Coq9*^{+/+} mice and *Coq9*^{+/+} mice under 0.33% of β -RA treatment.

(K to L) TCM stain in the kidney from *Coq9*^{+/+} mice and *Coq9*^{+/+} mice under 0.33% of β -RA treatment.

(M to P) H&E stain in the spleen from *Coq9*^{+/+} mice and *Coq9*^{+/+} mice under 0.33% of β -RA treatment.

(Q to T) H&E stain in the heart from *Coq9*^{+/+} mice and *Coq9*^{+/+} mice under 0.33% of β -RA treatment.

(U to X) H&E stain in the gut from *Coq9*^{+/+} mice and *Coq9*^{+/+} mice under 0.33% of β -RA treatment.

Tissues from mice at 3 months of age.

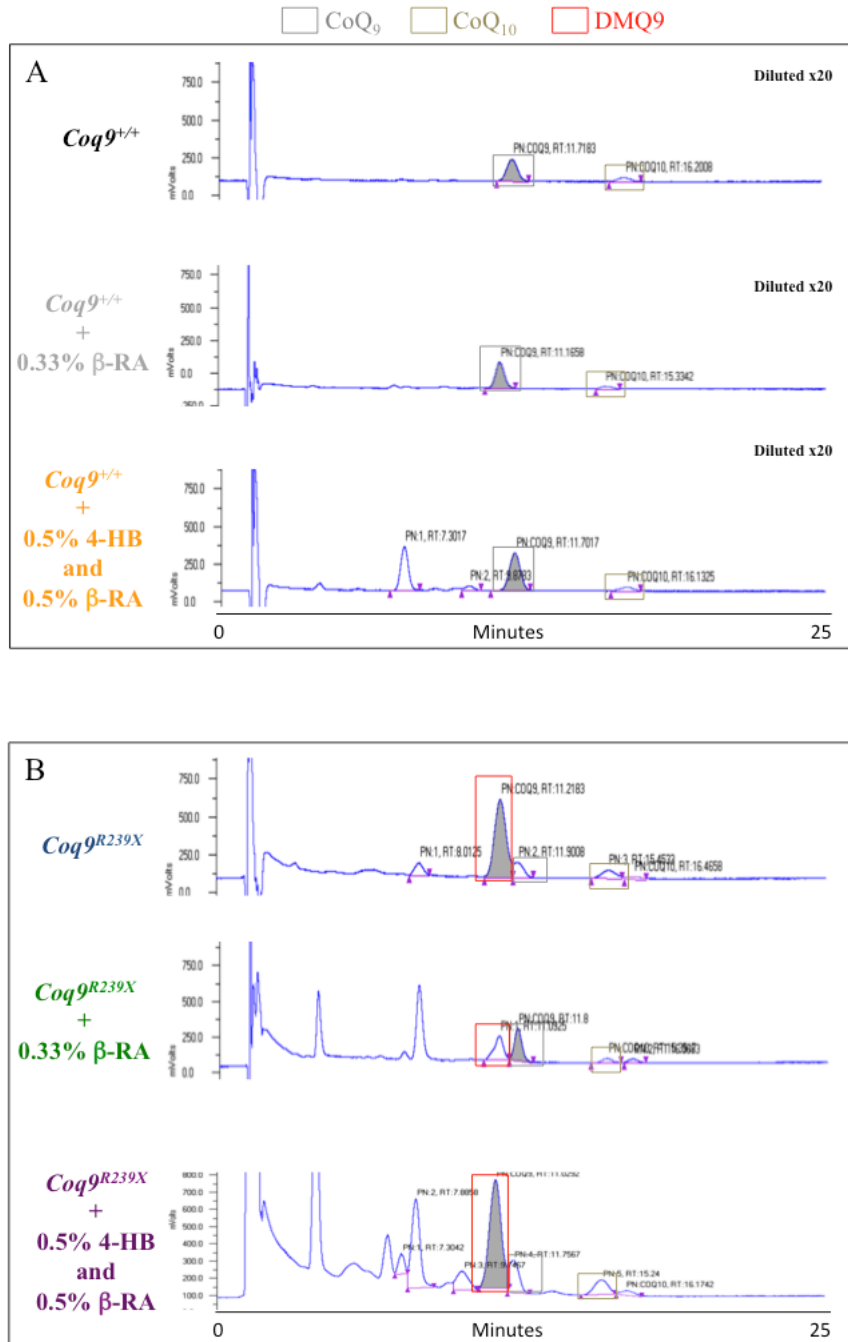


Figure S3. Representative chromatographs showing the peaks of CoQ₉ and DMQ₉ in the kidneys.

(A) Chromatographs for CoQ₉ in the kidney of a *Coq9*^{+/+} mouse, *Coq9*^{+/+} mouse under 0.33% of β-RA treatment, and *Coq9*^{+/+} mouse under 0.5% of 4-HB + 0.5% of β-RA treatment.

(B) Chromatographs for CoQ₉ and DMQ₉ in the kidney of a *Coq9*^{R239X} mouse, *Coq9*^{R239X} mouse under 0.33% of β-RA treatment, and *Coq9*^{R239X} mouse under 0.5% of 4-HB + 0.5% of β-RA treatment.

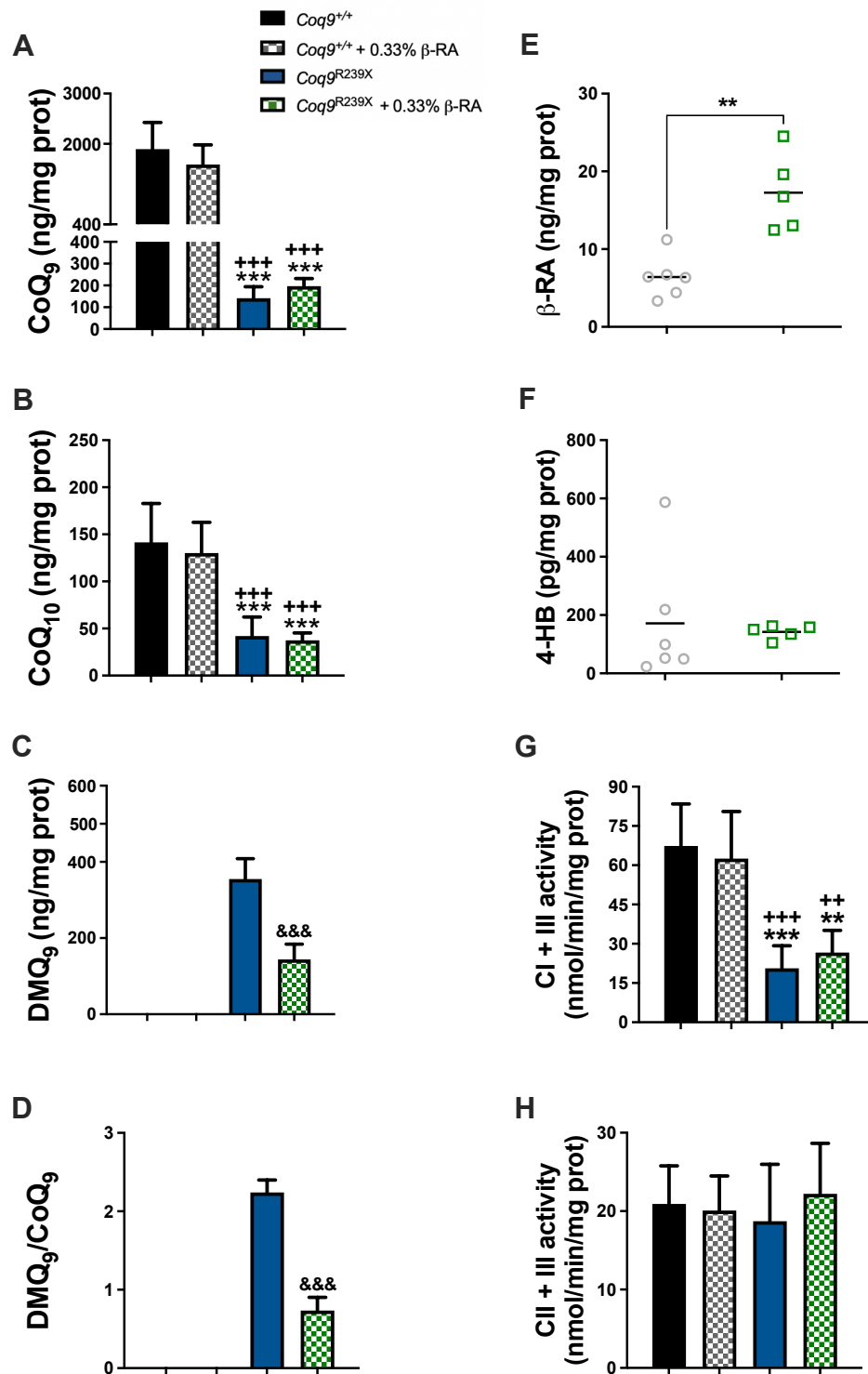


Figure S4. CoQ metabolism and mitochondrial function in the heart from *Coq9*^{+/+} mice, *Coq9*^{+/+} mice under the supplementation with 0.33% β-RA, *Coq9*^{R239X} mice and *Coq9*^{R239X} mice under the supplementation with 0.33% β-RA.

(A) Levels of CoQ₉; (B) Levels of CoQ₁₀; (C) Levels of DMQ₉; (D) DMQ₉/CoQ₉ ratio; (E) Levels of β-RA; (F) Levels of 4-HB; (G) Complex I + III (CI + III) activities; (H) Complex II+ III (CII + III) activity.

Tissues from mice at 3 months of age. Data are expressed as mean \pm SD. **P < 0.01; ***P < 0.001; differences versus *Coq9*^{+/+}. ++P < 0.01; +++P < 0.001, differences versus *Coq9*^{+/+} under 0.33% β -RA treatment (one-way ANOVA with a Tukey's post hoc test or t-test; n = 5–8 for each group). Note that DMQ₉ is not detected in samples from *Coq9*^{+/+} mice.

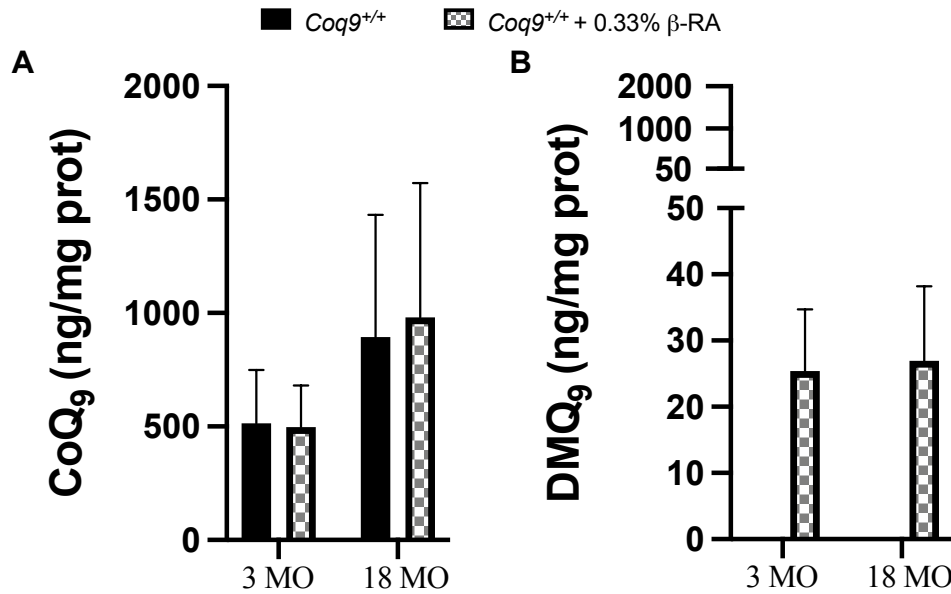


Figure S5. CoQ levels in WAT from *Coq9*^{+/+} mice and *Coq9*^{+/+} mice under the supplementation with 0.33% β -RA.

(A) Levels of CoQ₉; (B) Levels of DMQ₉.

Tissues from mice at 3 or 18 months of age (MO). Note that DMQ₉ is not detected in samples from *Coq9*^{+/+} mice.

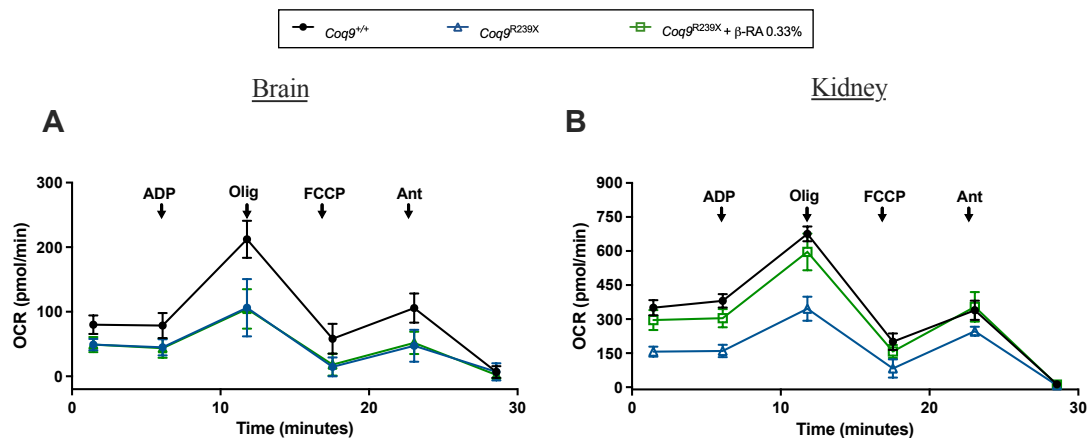


Figure S6. Mitochondrial oxygen consumption rate (represented as State 3_o, in the presence of ADP and substrates) in brain (A) and kidneys (B).

ADP = Adenosine diphosphate; Olig = Oligomycin, a mitochondrial ATP synthase inhibitor; FCCP = Carbonyl cyanide-p-trifluoromethoxyphenylhydrazone, an uncoupler of mitochondrial respiration and ATP synthesis; Ant = Antimycin A, a mitochondrial Complex III inhibitor.

Skeletal Muscle

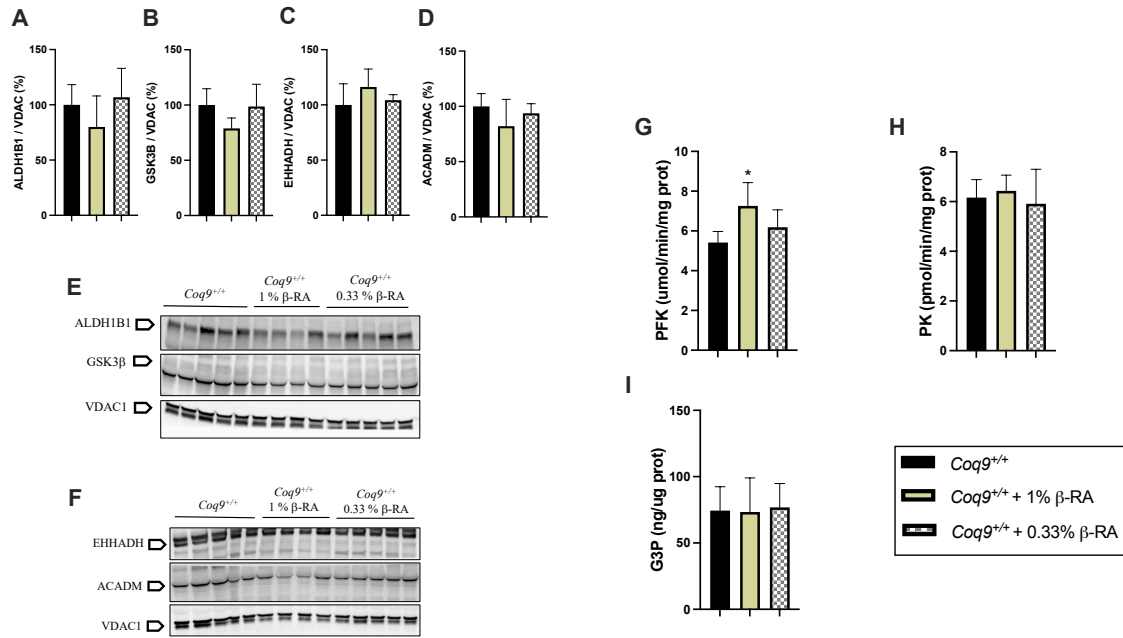


Figure S7. Metabolic characterization of the skeletal muscle after the treatment with β -RA in *Coq9*^{+/+} mice.

(A to F) Levels of the proteins ALDH1B1 (A, E), GSK3 β (B, E), EHHADH (C, F) and ACADM (D, F) in the skeletal muscle of *Coq9*^{+/+} mice treated with β -RA at 1% and 0.33%. VDAC1 was used as loading control. The experiments were performed in tissue homogenate.

(G to I) Activities of the glycolytic enzymes Phosphofructokinase (PFK) (G) and Pyruvate Kinase (PK) (H) in the skeletal muscle; levels of Glycerol-3-Phosphate (G3P) (I) in the skeletal muscle (S).

Tissues from mice at 3 months of age. Data are expressed as mean \pm SD. *P < 0.05; differences versus *Coq9*^{+/+} (one-way ANOVA with a Tukey's post hoc test or t-test; n = 5–7 for each group).

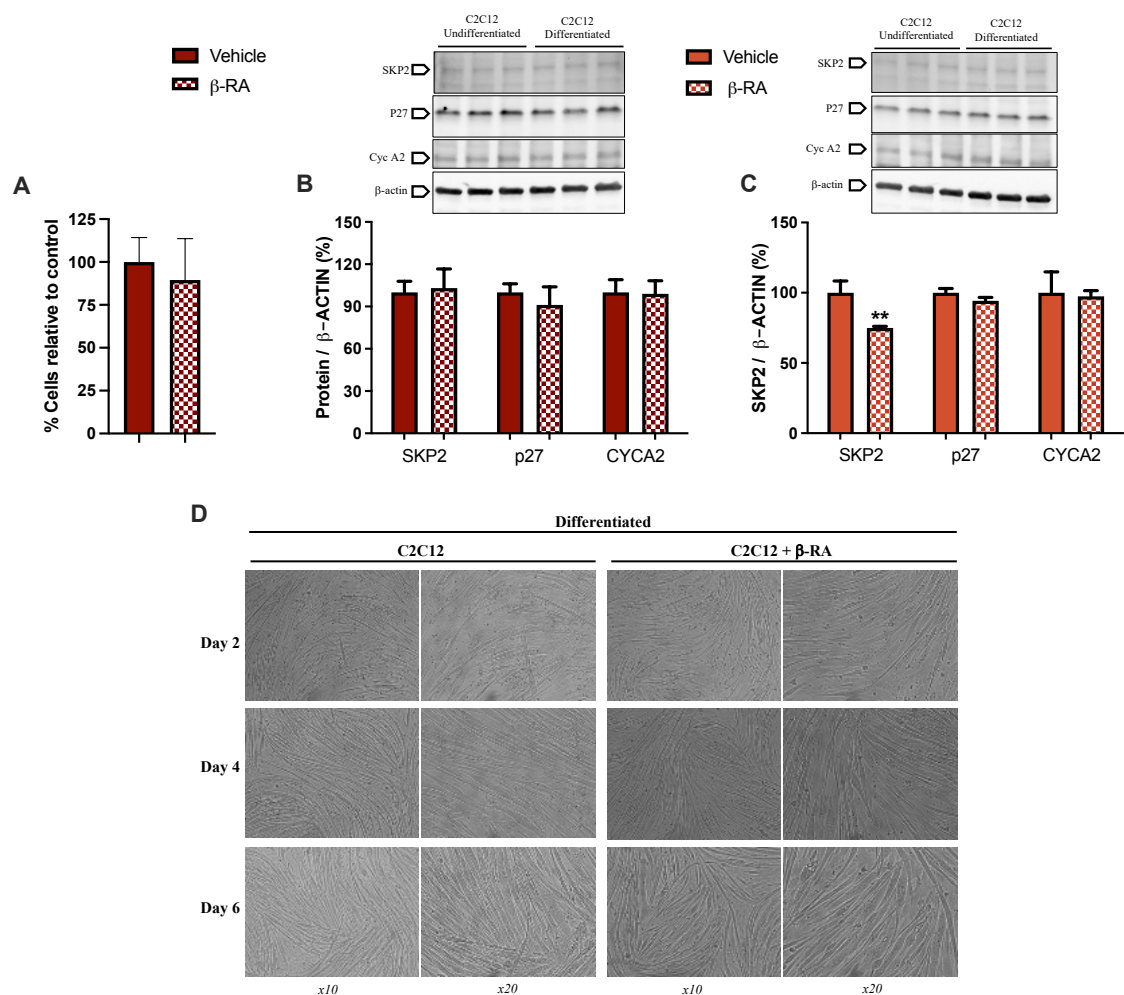


Figure S8. Effects of β -RA in the proliferation and differentiation of C2C12 myoblasts.

(A) Percentage of C2C12 cells after the treatment with 1mM β -RA, relative to the number of untreated C2C12 cells. Cells cultured in proliferative conditions.

(B) Levels of the proteins SKP2, p27 and CYCA2, which are involved in the control of the cell cycle. C2C12 cells were treated for seven days with 1mM β -RA in proliferative conditions.

(C) Levels of the proteins SKP2, p27 and CYCA2, which are involved in the control of the cell cycle. C2C12 cells were treated for seven days with 1mM β -RA in differentiative conditions.

(D) Images of the C2C12 cells under the microscope in differentiative conditions. C2C12 cells were treated with 1mM β -RA and the images were taken in different days (2, 4 and 6).

Data are expressed as mean \pm SD. ** $P < 0.01$, differences *versus* untreated cells (t-test; $n = 6$ for each group).

WAT

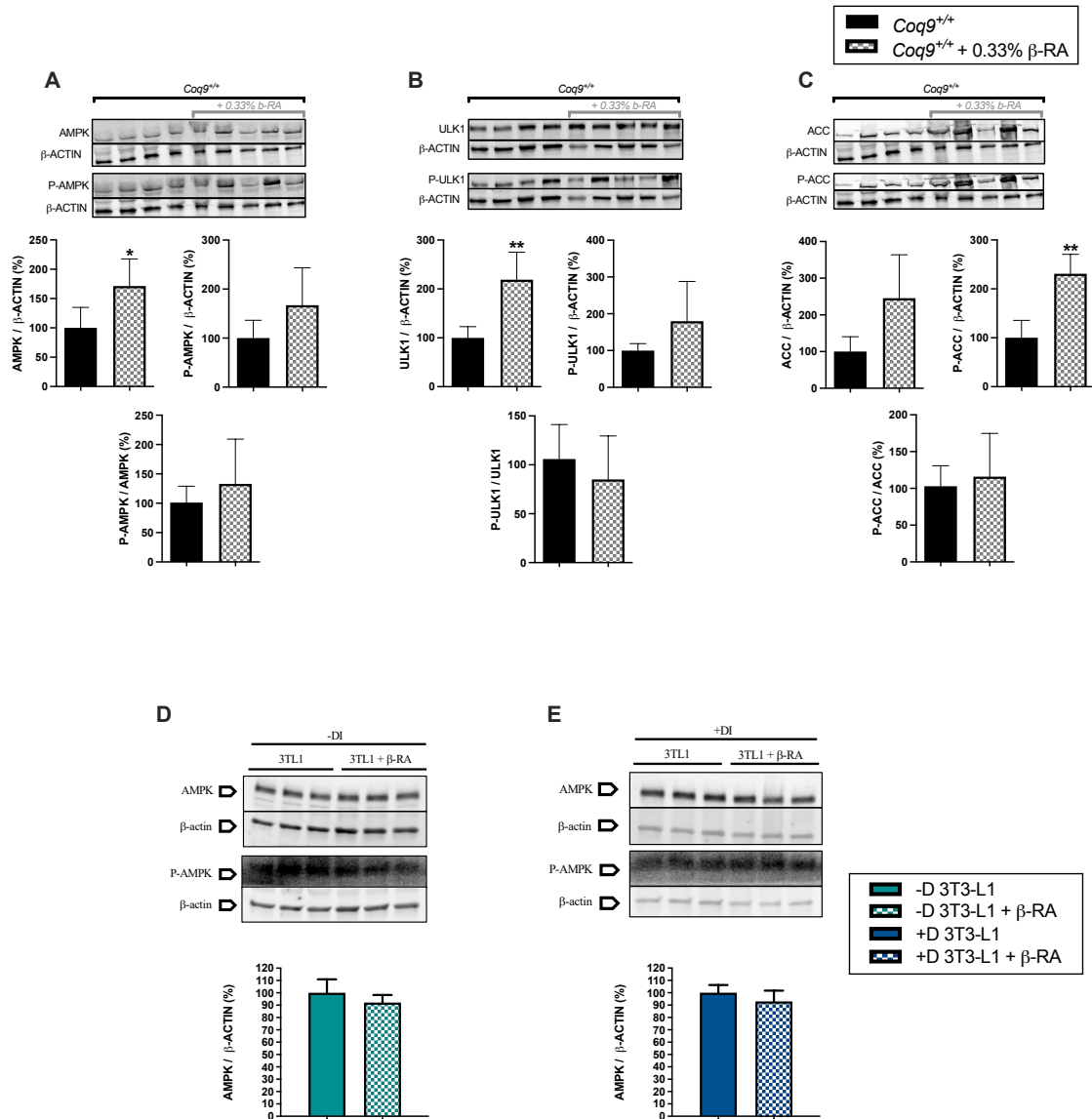


Figure S9. Analysis of the AMPK pathway in white adipose tissues and 3T1L1 cells.
(A) Levels of AMPK and p-AMPK, and p-AMPK/AMPK ratio in epididymal WAT from β -RA-treated and untreated wild-type mice.
(B) Levels of ULK1 and p-ULK1, and p-ULK1/ULK1 ratio in epididymal WAT from β -RA-treated and untreated wild-type mice.
(C) Levels of ACC and p-ACC, and p-ACC/ACC ratio in epididymal WAT from β -RA-treated and untreated wild-type mice.
(D) Levels of AMPK and p-AMPK, and p-AMPK/AMPK ratio in 3T1L1 cells treated with 1mM β -RA in proliferative conditions for seven days.
(E) Levels of AMPK and p-AMPK, and p-AMPK/AMPK ratio in 3T1L1 cells treated with 1mM β -RA in differentiative conditions for seven days.
Tissues from mice at 3 months of age (A to C). Data are expressed as mean \pm SD. * $P < 0.05$; ** $P < 0.01$, differences *versus* *Coq9*^{+/+} mice (t-test; $n = 4-6$ for each group).

Legends to Supplementary Videos

Movie S1. Video that shows the difference between a *Coq9*^{+/+} mouse and a *Coq9*^{R239X} mouse under 0.33% β -RA supplementation, both males at 20 months of age. Both animals have a healthy appearance, although the treated *Coq9*^{R239X} mouse is smaller, as previously reported.

Movie S2. Video that shows the difference between a *Coq9*^{R239X} mouse and a *Coq9*^{R239X} mouse under 0.33% β -RA treatment, both males at 3 months of age. The untreated *Coq9*^{R239X} mouse has developed a paralysis in the legs, although the treated *Coq9*^{R239X} mouse has a healthy appearance.

Movie S3. Video that shows a *Coq9*^{+/+} mouse and a *Coq9*^{+/+} mouse under 0.33% β -RA supplementation, both males at 20 months of age. The appearance of both animals is similar.