Supplemental Figure 1: $G\alpha_{i2}$ expression analysis in adipocyte-specific *Gnai2*-deficient mice (*Gnai2*^{ako}). ***(A)*** schematic overview of the *Gnai2* gene and PCR strategy for recombination analyses. ***(B)*** genomic DNA analysis of isolated adipocytes and tails derived from control (genotypes: *Gnai2*$^{fl/fl}$, *Gnai2*$^{+/fl}$; AdipoqCreERT2$^{+/tg}$; *Gnai2*$^{+/fl}$) and *Gnai2*^{ako} (genotype: *Gnai2*$^{+/fl}$; AdipoqCreERT2$^{+/tg}$) animals. Recombination is restricted to gWAT, iWAT, and isolated adipocytes derived from *Gnai2*^{ako}., whereas brain (B), liver (L), pancreas (P), and tail (T) showed only the floxed band. M = marker. Statistical analysis of $G\alpha_{i1}$ expression levels in ctrl and *Gnai2*^{ako} ***(C)*** gWAT, ***(D)*** iWAT, and ***(E)*** primary white adipocytes (WA). Lack of $G\alpha_{i2}$ restricted to adipocytes does not influence $G\alpha_{i1}$ expression levels in CD- or HFD-fed *Gnai2*^{ako} animals. **(*F*)** Representative immunoblots of liver, skeletal muscle (SKM), and brain homogenates isolated from ctrl and *Gnai2*^{ako} mice. **(*G*)** Statistical analysis of $G\alpha_{i2}$ expression levels in liver, skeletal muscle and brain of HFD-fed *Gnai2*^{ako} and ctrl animals. ***(H-K)*** Statistical analysis of $G\alpha_{i2}$ expression levels in ctrl and *Gnai2*^{ako} brown and white adipose tissue depots and primary white adipocytes. $G\alpha_{i2}$ protein levels are reduced by 60% in CD-fed *Gnai2*^{ako} and 50% in HFD-fed *Gnai2*^{ako} adipose tissue homogenates and by 80% in primary white adipocytes. β-actin levels were used as reference to normalize the data. Brown and white adipose tissue homogenates from the animals were analyzed in ≥3 independent experiments. Data were analyzed by multiple comparison two-way ANOVA followed by post-hoc comparison with Bonferroni's multiple comparison ***(C, D, G-H)*** or the Student *t* test **(*E, K*)** and are presented as means ± SEMs. Statistical difference between the two genotypes is indicated: $^{**}P < 0.01$.

Supplemental Figure 2: (*A*) Representative H&E stained histological sections of CD- and HFD-fed ctrl and *Gnai2*^{ako} brown adipose tissue. Scale bar = 20 µm **(*B*)** Similar fecal energy content in ctrl and *Gnai2*^{ako} mice on a HFD

Supplemental Figure 3: Similar liver parameters in HFD-fed ctrl and *Gnai2*^{ako} mice. ***(A)*** Liver weight and ***(B)*** liver to body weight ratio in ctrl and *Gnai2*^{ako} mice fed an HFD. Representative H&E ***(C)*** and Oil Red O ***(D)*** stained liver histological sections of HFD-fed ctrl and *Gnai2*^{ako} mice. Scale bars = 20 µm **(*E*)** Liver triglyceride levels were slightly increased in HFD-fed *Gnai2*^{ako} mice compared to littermate ctrl. Data were analyzed by multiple comparison two-way ANOVA followed by post-hoc comparison with Bonferroni's multiple comparison ***(A,B)*** or the Student *t* test **(*E*)** and are presented as means ± SEMs. Statistical difference between the two genotypes is indicated: $^{**}P < 0.01$

Supplemental Figure 4: Unaltered glucose handling in 10-week-old HFD-fed *Gnai2*^{ako} mice and CD-fed *Gnai2*^{ako} mice. ***(A)*** Basal blood glucose levels of young (10-week-old) HFD-fed ctrl and *Gnai2*^{ako} mice. Basal blood glucose levels were similar in HFD-fed *Gnai2*^{ako} mice compared to HFD-ctrl mice. ***(B)*** Blood glucose levels were similar in 10-week-old HFD-fed ctrl and *Gnai2*^{ako} mice following i.p. injection of 2 mg/g body weight glucose after overnight fasting ($ctrl_{HFD}$ n = 7; *Gnai2*$^{ako}{}_{HFD}$ n = 7). **(*C*)** Blood glucose levels were similar in 28-week-old CD-fed ctrl and *Gnai2*^{ako} mice following i.p. injection of 2 mg/g body weight glucose after overnight fasting ($ctrl_{CD}$ n = 12; *Gnai2*$^{ako}{}_{HFD}$ n = 12). ***(D)*** Similar plasma insulin levels in CD-fed ctrl and *Gnai2*^{ako} animals during the glucose tolerance test ($ctrl_{CD}$ n = 12; *Gnai2*$^{ako}{}_{HFD}$ n = 12). ***(E)*** Unaltered insulin tolerance tests in CD-fed *Gnai2*^{ako} mice compared to CD-fed ctrls receiving 1 mU/g body weight insulin following a 4-h-fasting period ($ctrl_{CD}$ n = 12; *Gnai2*$^{ako}{}_{HFD}$ n = 12). Unaltered resistin ***(F)*** and leptin ***(G)*** levels in the plasma of HFD-fed *Gnai2*^{ako} compared to HFD-fed ctrl mice. **(*H*)** mRNA expression levels of adiponectin were significantly higher in gWAT and iWAT of HFD-fed *Gnai2*^{ako} mice compared to HFD-ctrl mice. Data were analyzed using multiple comparison two-way ANOVA followed by post-hoc comparison with Bonferroni's multiple comparison **(*B-H*)** or the Student *t* test ***(A)*** and are presented as means ± SEMs. Statistical difference between the two genotypes is indicated: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Supplemental Figure 5: Unaltered intracellular cAMP levels and *ex vivo* lipolysis in CD-fed *Gnai2*^{ako} mice. cAMP accumulation was measured under basal and stimulated (10 µM isoproterenol) conditions in gWAT ***(A)*** and iWAT ***(B)*** of CD-fed ctrl and *Gnai2*^{ako} mice for 15 minutes in the presence of 1 mM IBMX. Glycerol release was measured in gWAT **(*C*)** and iWAT **(*D*)** of CD-fed ctrl and *Gnai2*^{ako} mice. No

significantly differences were detectable under CD-feeding. ***(E)*** Plasma triglyceride content was similar in HFD-fed *Gnai2*^{ako} compared to HFD-fed ctrl mice. Data were analyzed using multiple comparison two-way ANOVA followed by post-hoc comparison with Bonferroni's multiple comparison **(*A*-*E*)** and are presented as means ± SEMs. Statistical difference between the two genotypes is indicated: **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

Supplemental Figure 6: Mitochondria function of primary brown and white adipocytes derived from HFD-fed *Gnai2*^{ako} and control mice. Oxygen consumption rate (OCR) of brown **(*A*)** and white **(*B*)** adipocytes was measured before and after the addition of inhibitors to determine several parameters of mitochondrial respiration. Initially, baseline and NE-stimulated OCR were measured. Next oligomycin, a complex V inhibitor, was added to derive ATP-linked respiration following carbonyl cyanide-p-trifluoromethoxyphenyl-hydrazon (FCCP), a protonophore, addition to collapse the inner membrane gradient. Lastly, antimycin A and rotenone, inhibitors of complex III and I, were added to reveal non-mitochondrial respiration. **(*C*)** Oxygen consumption rate (OCR) is measured before and after the addition of norepinephrine (NE) (1 µM). NE-stimulated OCR was significantly decreased in primary white adipocytes isolated from *Gnai2*^{ako} mice compared to ctrl mice. Data were analyzed using multiple comparison two-way ANOVA followed by post-hoc comparison with Bonferroni's multiple comparison **(*A*-*C*)** and are presented as means ± SEMs. Statistical difference between the two genotypes is indicated: **P* < 0.05, ***P* < 0.01, ****P* < 0.001.