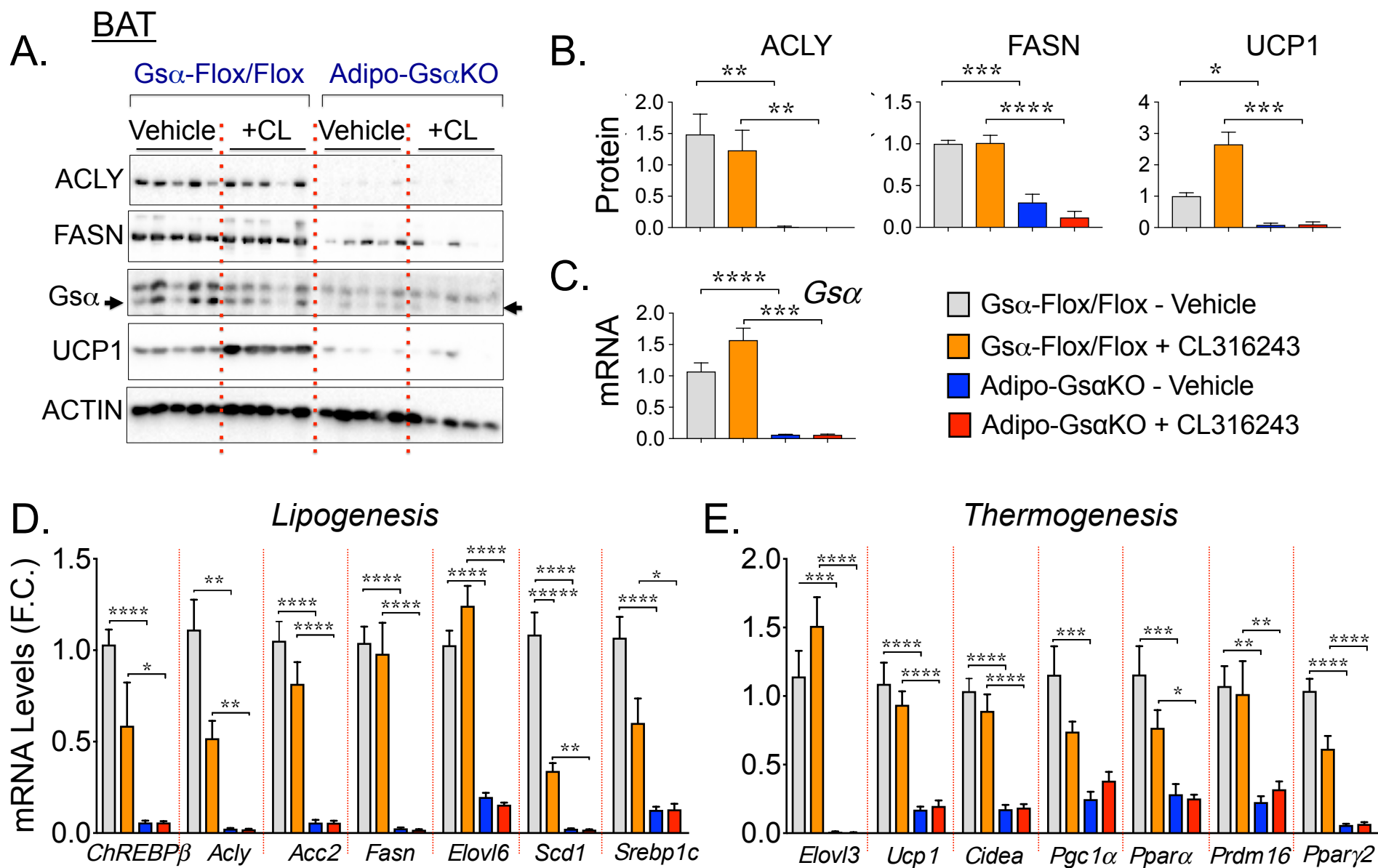


**Cell Reports, Volume 31**

**Supplemental Information**

**Control of Adipocyte Thermogenesis  
and Lipogenesis through  $\beta$ 3-Adrenergic  
and Thyroid Hormone Signal Integration**

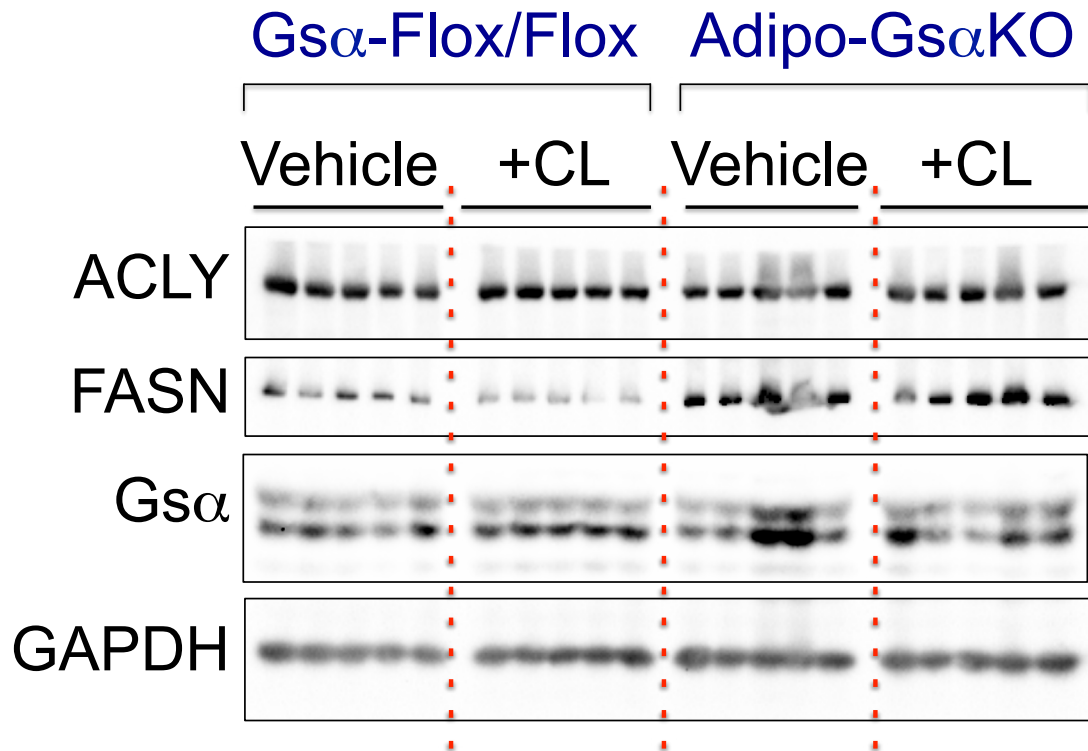
**Adilson Guilherme, Batuhan Yenilmez, Alexander H. Bedard, Felipe Henriques, Dianxin Liu, Alexandra Lee, Lauren Goldstein, Mark Kelly, Sarah M. Nicoloro, Min Chen, Lee Weinstein, Sheila Collins, and Michael P. Czech**



**Figure S1**

**FIGURE S1. Related to Figure 3: Gs $\alpha$ -protein inactivation in adipocytes suppresses thermogenic and DNL-related gene expressions in BAT. (A).** Depicted are representative Western blots for lipogenic (ACLY and FASN), thermogenic (UCP1) and Gs $\alpha$  protein expressions in BAT from Gs $\alpha$ -Flox/Flox control and adipocyte-specific Gs $\alpha$ KO mice (Adipo-Gs $\alpha$ KO) treated with vehicle or with CL316243 for 6 days. Actin was used for loading control. **(B)** Quantifications of proteins from Western blots depicted in **(A)**. **(C-E)** qRT-PCR analysis to quantify Gs $\alpha$  **(C)** lipogenic **(D)** and thermogenic **(E)** mRNA expressions in BAT from Gs $\alpha$ -Flox/Flox control and Adipo-Gs $\alpha$ KO mice treated or not with CL316243 for 6 days. Graphs depict the mean +/- SEM. N= 6 mice per group. \* P < 0.05; \*\* P < 0.01; \*\*\* P < 0.001; \*\*\*\* P < 0.0001 by one-way ANOVA followed by post-hoc Tukey's test for group comparisons.

A.  
Liver



B.

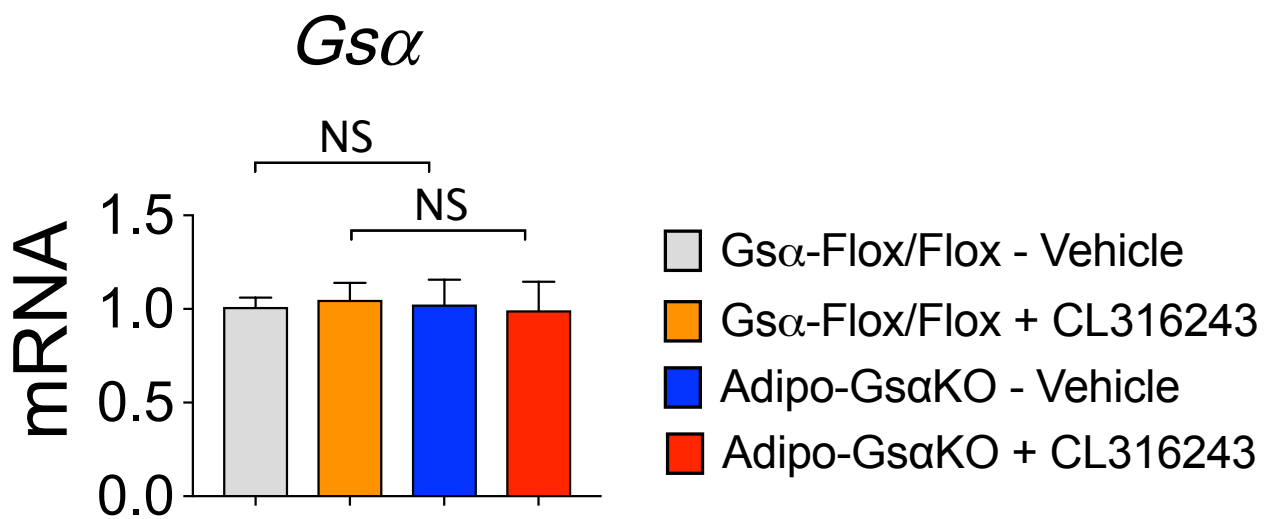
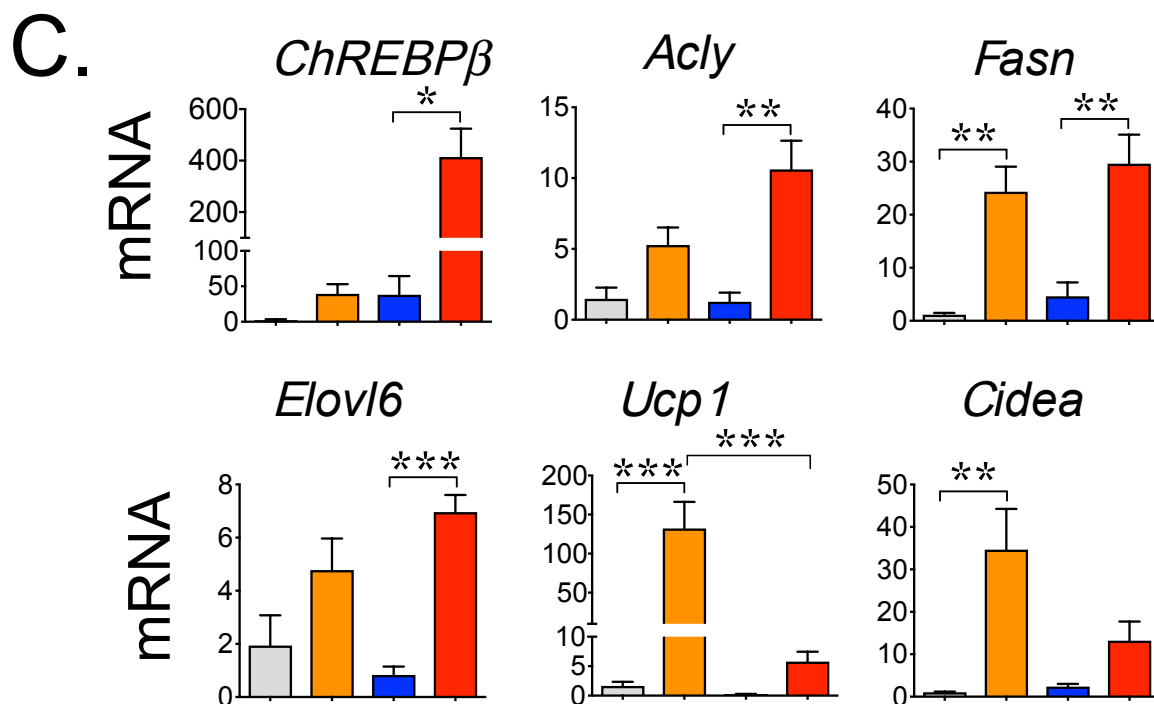
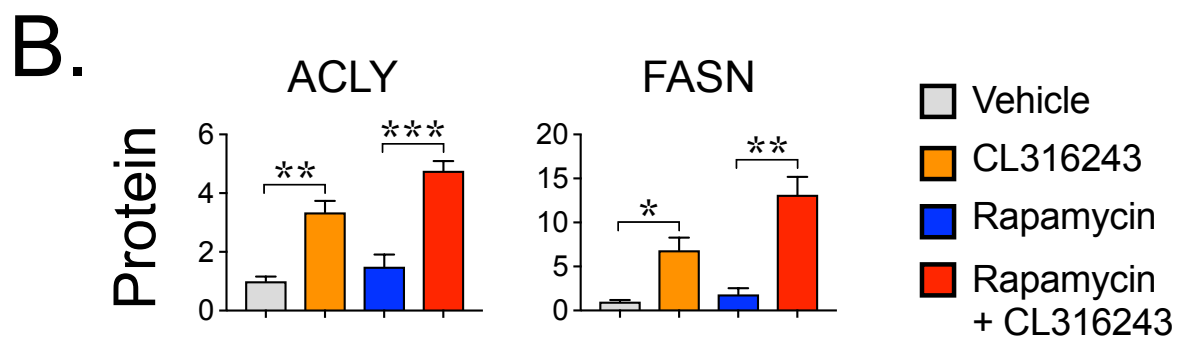
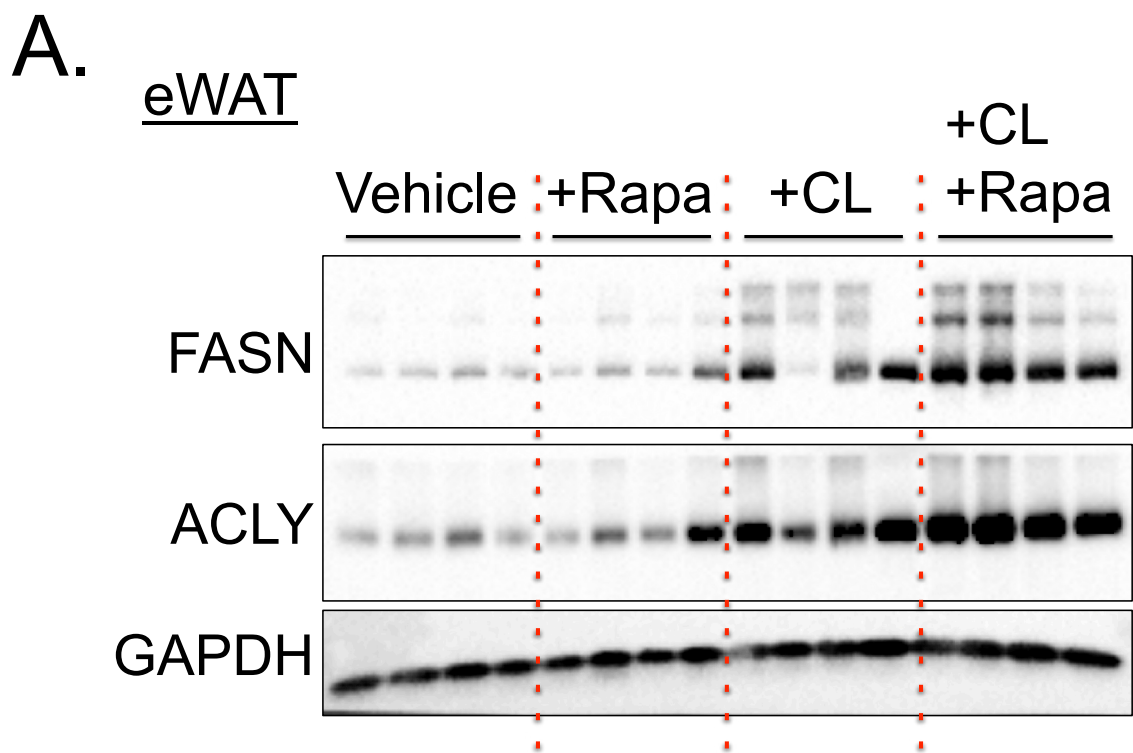


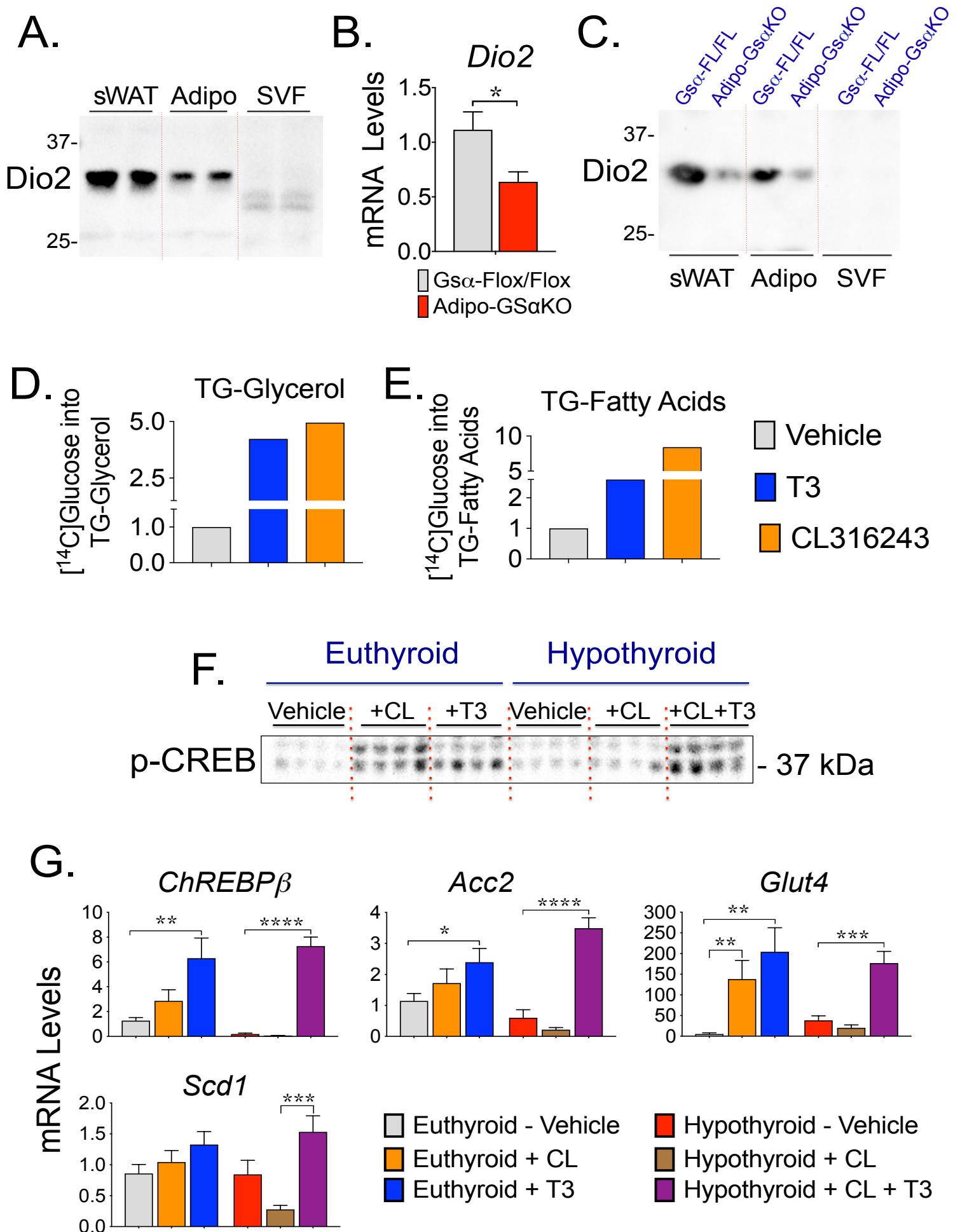
Figure S2

**FIGURE S2. Related to Figure 3: Deletion of adipocyte  $Gs\alpha$  does not affects ACLY, FASN or  $Gs\alpha$  protein expressions in liver. (A)** Liver tissue lysates from  $Gs\alpha$ -Flox/Flox and Adipo- $Gs\alpha$ KO mice treated or not with CL316243 were immunoblotted for ACLY, FASN,  $Gs\alpha$  and GAPDH as indicated. Depicted are representative Westerns of 5 mice per group (B) qRT-PCR was performed for quantifications of  $Gs\alpha$  mRNA in liver from  $Gs\alpha$ -Flox/Flox and Adipo- $Gs\alpha$ KO mice. N = 6 per group. Graphs depict the mean  $\pm$  SEM. N= 6 mice per group. NS = not significant ( $P > 0.05$ ) by one-way ANOVA.



**Figure S3**

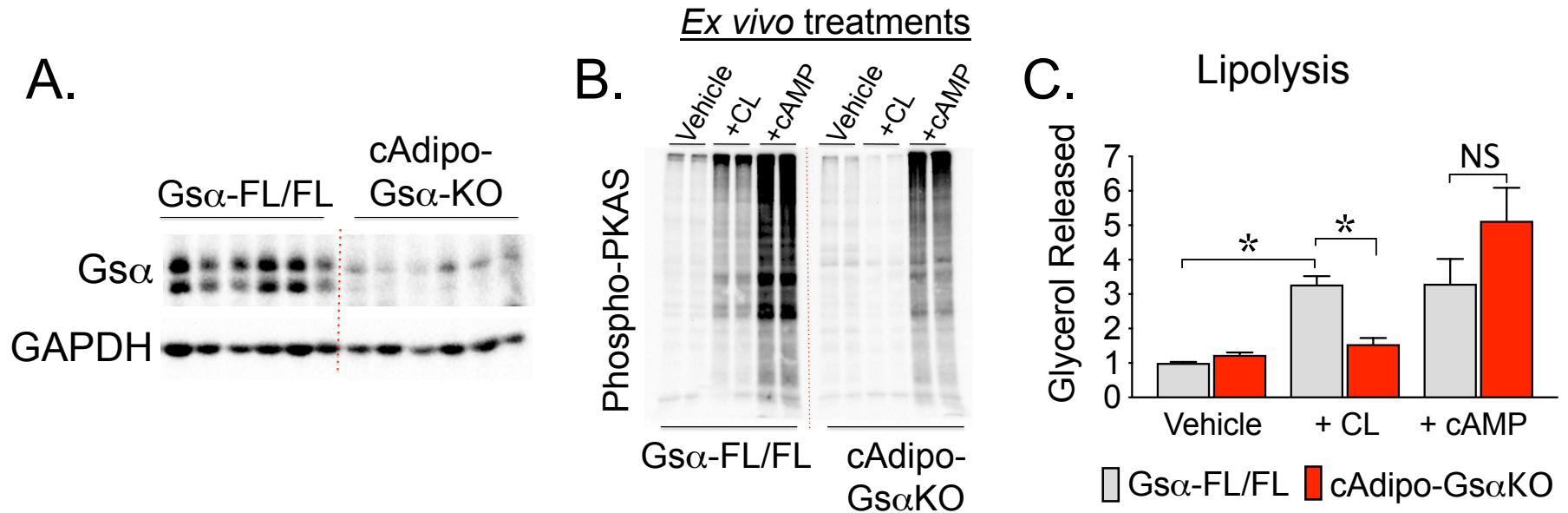
**FIGURE S3. Related to Figure 4: Rapamycin treatment fails to block the  $\beta$ 3-AR-stimulated DNL gene expression in eWAT. (A)** Depicted are representative Westerns to detect FASN, ACLY and GAPDH (loading control) proteins in epididymal adipose tissue (eWAT) from control and CL316243-treated mice, co-treated or not with rapamycin. **(B)** Quantification of protein bands shown in Western blots from **(A)**. **(C)** qRT-PCR analysis of indicated DNL and thermogenic gene expression in eWAT from CL-treated mice, with or without rapamycin. Graphs shown the mean  $\pm$  SEM. N= 6 mice per group. \* P < 0.05; \*\* P < 0.01; \*\*\* P < 0.001 by one-way ANOVA followed by post-hoc group comparisons.



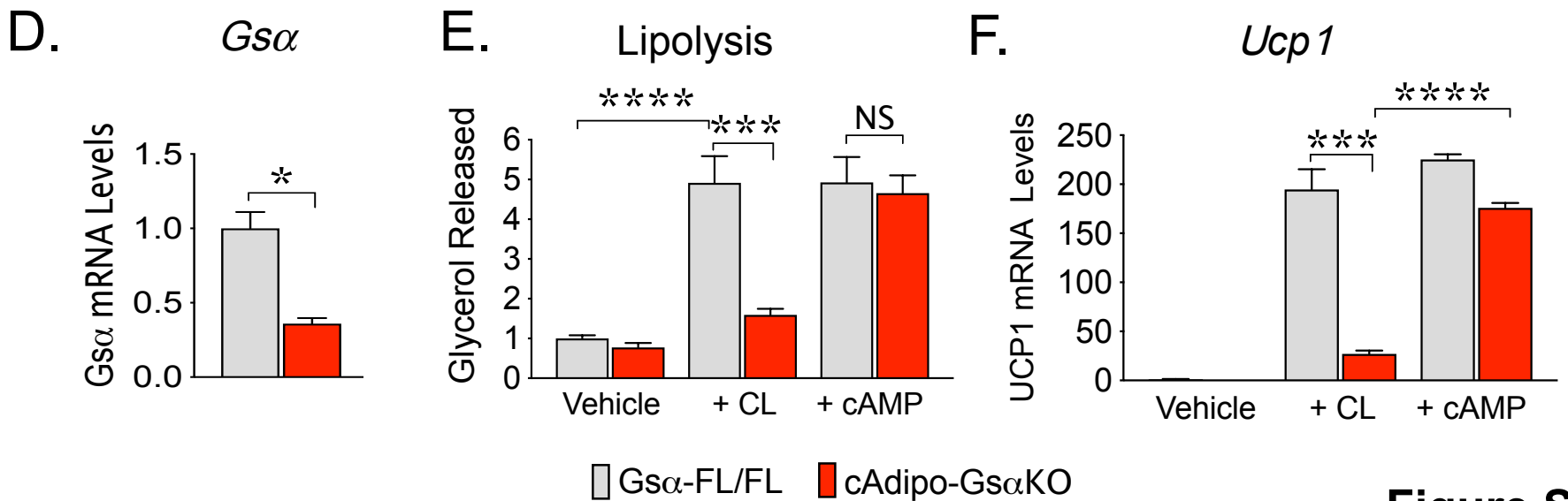


**FIGURE S4. Related to Figure 6:** (A) Dio2 protein expression is higher in isolated subcutaneous adipocytes than SVF. sWAT, isolated adipocytes and SVF lysates were immunoblotted for Dio2. (B, C) Deletion of adipocyte Gs $\alpha$  suppresses Dio2 expression in adipose tissues. (B) qRT-PCR quantification to measure *Dio2* mRNA expression in BAT and (C) Western blot to detect Dio2 protein levels in sWAT, isolated adipocytes and SVF from Gs $\alpha$ -FL/FL control or Adipo-GS $\alpha$ KO mice. (D, E) [<sup>14</sup>C]-glucose conversions into (D) triglyceride-glycerol (TG-Glycerol) and into (E) triglyceride-fatty acids (TG-Fatty acids) were measured in sWAT explants from vehicle, T3 and CL316243 treated mice. Depicted are the relative values of [<sup>14</sup>C]-TG-Fatty Acids and [<sup>14</sup>C]-TG-Glycerol produced by sWAT explants from 5 mice per conditions. Samples from each condition were pooled together to obtain enough CPM counts per conditions. (F) Representative Western blots to detect phospho-CREB protein in sWAT from euthyroid and hypothyroid mice treated with vehicle or with CL316243 or T3 as indicated in Figure 6. (G) qRT-PCR quantifications to determine the expression levels of *ChREBP $\beta$* , *Acc2*, *Glut4*, and *Scd1* mRNAs in sWAT from euthyroid and hypothyroid mice treated or not with the indicated agonists. Graphs depict the mean  $\pm$  SEM. N= 5-6 mice per group. \* P < 0.05; \*\* P < 0.01; \*\*\* P < 0.001; \*\*\*\*P <0.0001 by Student's *t*-test (B) or one-way ANOVA followed by post-hoc group comparisons (G).

## Adipose Tissue Explants



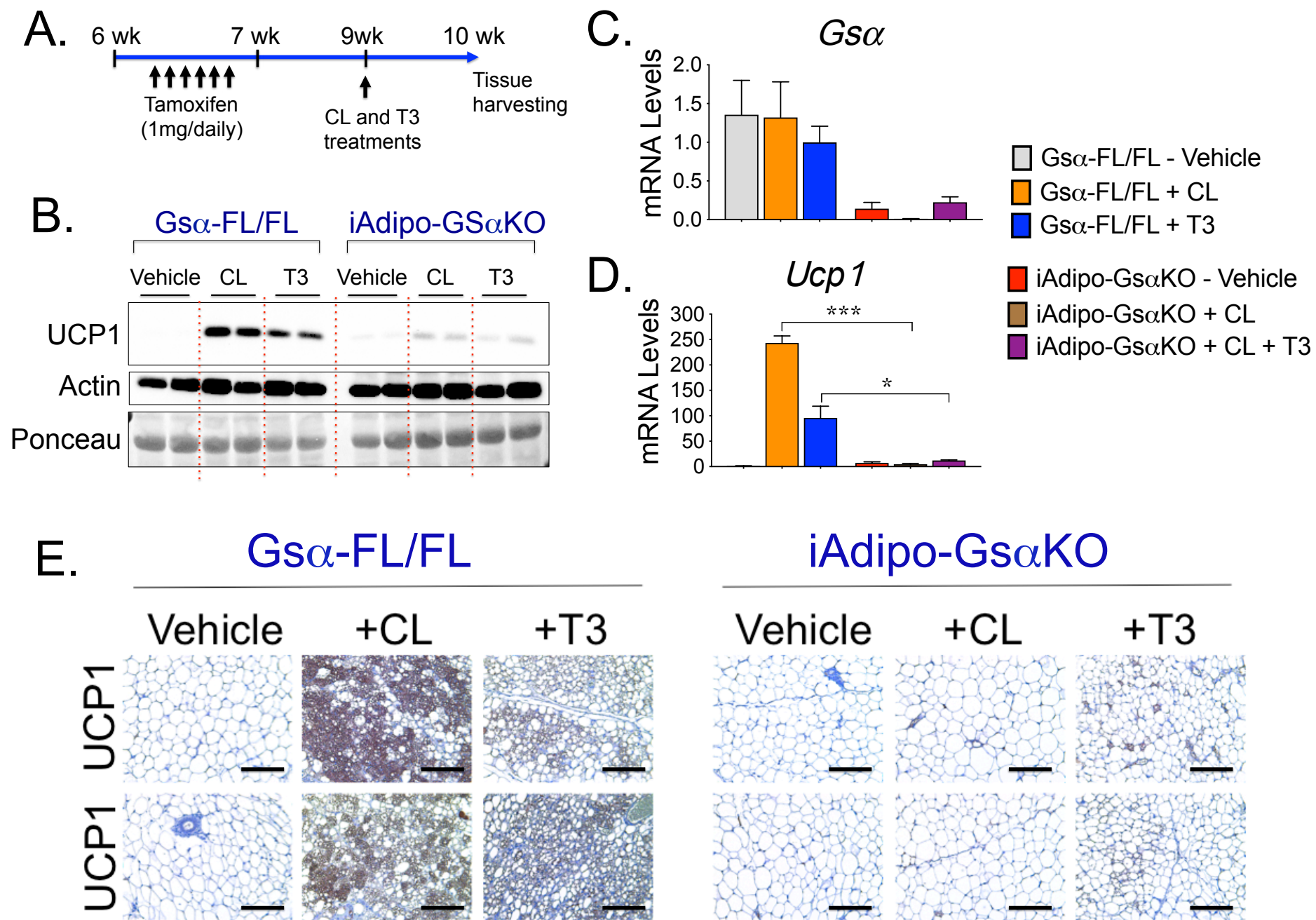
## Subcutaneous Adipocytes



**Figure S5**

**FIGURE S5. Related to Figure 7: Dibutyryl-cAMP treatment rescues PKA signaling to promote lipolysis and UCP1 expression in Adipo-Gs $\alpha$ KO mice.**

**(A)** Lysates of sWAT from Gs $\alpha$ -Flox/Flox and Adipo-Gs $\alpha$ KO mice were immunoblotted for Gs $\alpha$  and GAPDH proteins. **(B,C)** sWAT explants were incubated at 37°C with media containing vehicle, 2  $\mu$ M CL316243 or 1 mM dibutyryl-cAMP for 2.5 h. Tissue explants were then processed and lysates immunoblotted to detect levels of phospho-PKA substrate in sWAT explants treated with indicated agonist **(B)**. Media from the sWAT explants were collected to determine the levels of glycerol released by the explants treated with indicated agonist **(C)**. Graphs depict the mean  $\pm$  SEM. N= 6 mice per group. \*P < 0.05, by one-way ANOVA followed by post-hoc group comparisons. **(D)** Subcutaneous preadipocytes in the SVF of sWAT from Gs $\alpha$ -Flox/Flox and Adipo-Gs $\alpha$ KO mice were differentiated in vitro into mature adipocytes. Seven days post-differentiation, the mature adipocytes were treated with vehicle or 0.2 nM CL316243 or 1mM dibutyryl-cAMP. Depicted in **(D)** is the qRT-PCR quantification of Gs $\alpha$  mRNA in Gs $\alpha$ -Flox/Flox and Adipo-Gs $\alpha$ KO adipocytes. Graphic shown mean  $\pm$  SEM. N = 3 experiments. \*P , 0.05 by Mann Whitney U-Test. **(E)** Media from cells incubated with indicated agonist were collected after 2.5 h of incubation to determine the levels of glycerol released from adipocytes. **(F)** mRNA levels of *Ucp1* in Gs $\alpha$ -Flox/Flox and Adipo-Gs $\alpha$ KO adipocytes incubated with indicated agonist for 5 h. Graphics in **(C, E, F)** depict mean  $\pm$  SEM from 3 experiments. \*P < 0.05; \*\*\*P < 0.001; \*\*\*\*P < 0.0001 by one-way ANOVA followed by post-hoc group comparisons. NS = not statistically significant (P > 0.05).



**Figure S6**

**FIGURE S6. Related to Figure 7: Inducible deletion of  $Gs\alpha$  in adipocytes from adult mice inhibits both  $\beta$ 3-agonist and T3 stimulations of UCP1 expression in sWAT. (A)**  $Gs\alpha$ -Flox/Flox and iAdipo- $Gs\alpha$ KO mice were treated with tamoxifen to induce  $Gs\alpha$  deletion in adipocytes from mature animals. Mice were then treated or not with CL316243 or T3 for 8 days to evaluate adipose  $\beta$ 3-AR and TR signaling to induce UCP1 expression in sWAT. Depicted is the diagram representing the experimental design. **(B)** Western blots to detect UCP1 and  $\beta$ -actin (loading control) proteins in sWAT from  $Gs\alpha$ -Flox/Flox and iAdipo- $Gs\alpha$ KO mice treated or not with CL316243 or T3 as indicated. Nitrocellulose membrane was also stained with Ponceau S to confirm equal loading of total protein. Shown in **(C,D)** are qRT-PCR quantifications to determine the expression levels of  $Gs\alpha$  and  $Ucp1$  mRNAs in sWAT from  $Gs\alpha$ -Flox/Flox and iAdipo- $Gs\alpha$ KO mice treated or not with the indicated agonists. Graphics show the mean  $\pm$  SEM. N = 3-4 mice per group. \*  $P < 0.05$ ; \*\*\*  $P < 0.001$  by one-way ANOVA followed by Tukey's test. **(E)** Representative IHC analyses for detection of UCP1 and proteins in sWAT from  $Gs\alpha$ -Flox/Flox and iAdipo- $Gs\alpha$ KO mice treated or not with the indicated agonist. Scale bar, 100  $\mu$ m.

<b>Gene</b>	<b>Forward</b>	<b>Reverse</b>
<i>Fasn</i>	GGAGGTGGTGTATAGCCGGTAT	TGGGTAATCCATAGAGCCCAG
<i>Ucp1</i>	ACTGCCACACCTCCAGTCATT	CTTTGCCTCACTCAGGATTGG
<i>Cidea</i>	ATCACAACCTGGCCTGGTTACG	TACTACCCGGTGTCCATTTCT
<i>Ppargc1a</i>	AGCCGTGACCACTGACAACGAG	GCTGCATGGTTCTGAGTGCTAAG
<i>Prdm16</i>	CAGCACGGTGAAGCCATTC	GCGTGCA TCCGCTTGTG
<i>Ppara</i>	TATGGAGTGACATAGAGTGTGCT	CCACTTCAATCCACCCAGAAAG
<i>Pdk4</i>	AGGGAGGTCGAGCTGTTCTC	GGAGTGTTCACTAAGCGGTCA
<i>Dio2</i>	AATTATGCCTCGGAGAAGACCG	GGCAGTTGCCTAGTGAAAGGT
<i>Rplp0</i>	TCCAGGCTTTGGGCATCA	CTTTATCAGCTGCACATCACTCAGA
<i>Hprt</i>	TCAGTCAACGGGGGACATAAA	TCAGTCAACGGGGGACATAAA
<i>Gapdh</i>	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA
<i>Acly</i>	ACCCTTTCACTGGGGATCACA	GACAGGGATCAGGTATTCCTTG
<i>Acaca</i>	TGTACAAGCAGTGTGGGCTGGCT	CCACATGGCCTGGCTTGGAGGG
<i>Acacb</i>	GGAGGCTGCATTGAACACAAGT	TGCTCCAAAGCGAGTGACAAA
<i>ChREBP<math>\beta</math></i>	TCTGCAGATCGCGTGGAG	CTTGTCCC GG CATAGCAAC
<i>Scd1</i>	TTCTTGC GATACTCTGGTGC	CGGGATTGAATGTTCTTGTCG
<i>Slc2a4</i>	GTGACTGGAACACTGGTCCTA	CCAGCCACGTTGCATTGTAG
<i>Pck1</i>	CTGCATAACGGTCTGGACTTC	CAGCAACTGCCCGTACTCC
<i>Ppar<math>\gamma</math>2</i>	ATGGGTGAAACTCTGGGAG	GTGGTCTTCCATCACGGAGA
<i>Sreb1c</i>	GGCCCGGAAGTCACTGT	GGAGCCATGGATTGCACATT
<i>Elovl6</i>	TCAGCAAAGCACCCGAAC	AGCGACCATGTCTTTGTAGGAG
<i>Elovl3</i>	TTCTCACGCGGGTAAAAATGG	GAGCAACAGATAGACGACCAC
<i>Gnas</i>	ACAAGCAGGTCTACCGGGCC	CTCCGTTAAACCCATTAACATGCA
<i><math>\beta</math>2m</i>	CATGGCTCGCTCGGTGAC	CAGTTCAGTATGTTCCGGCTTCC
<i>18S</i>	CGAACGTCTGCCCTATCAACTT	CCGGAATCGAACCCCTGATT
<i>Mcad</i>	CAAGTTT GCCAGAGAGGAGATTATC	AACGGG TACTCCCGCTTT

**Table S1. Related to STAR Methods:** Primer sequences used in qRT-PCR analysis.