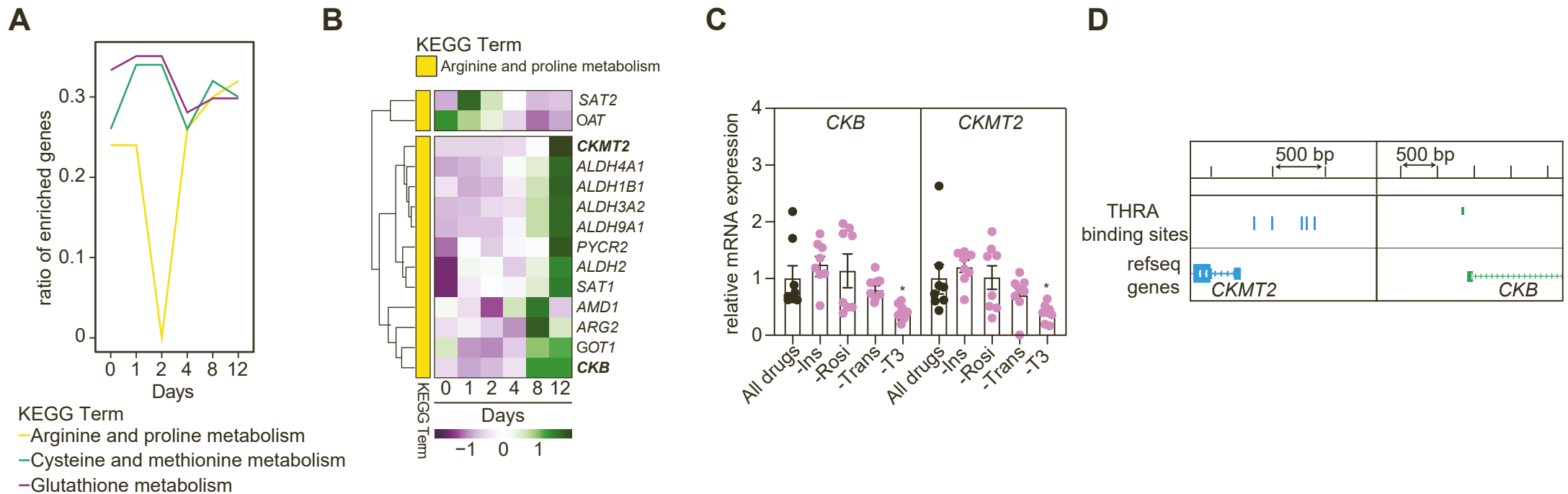


Supplemental information

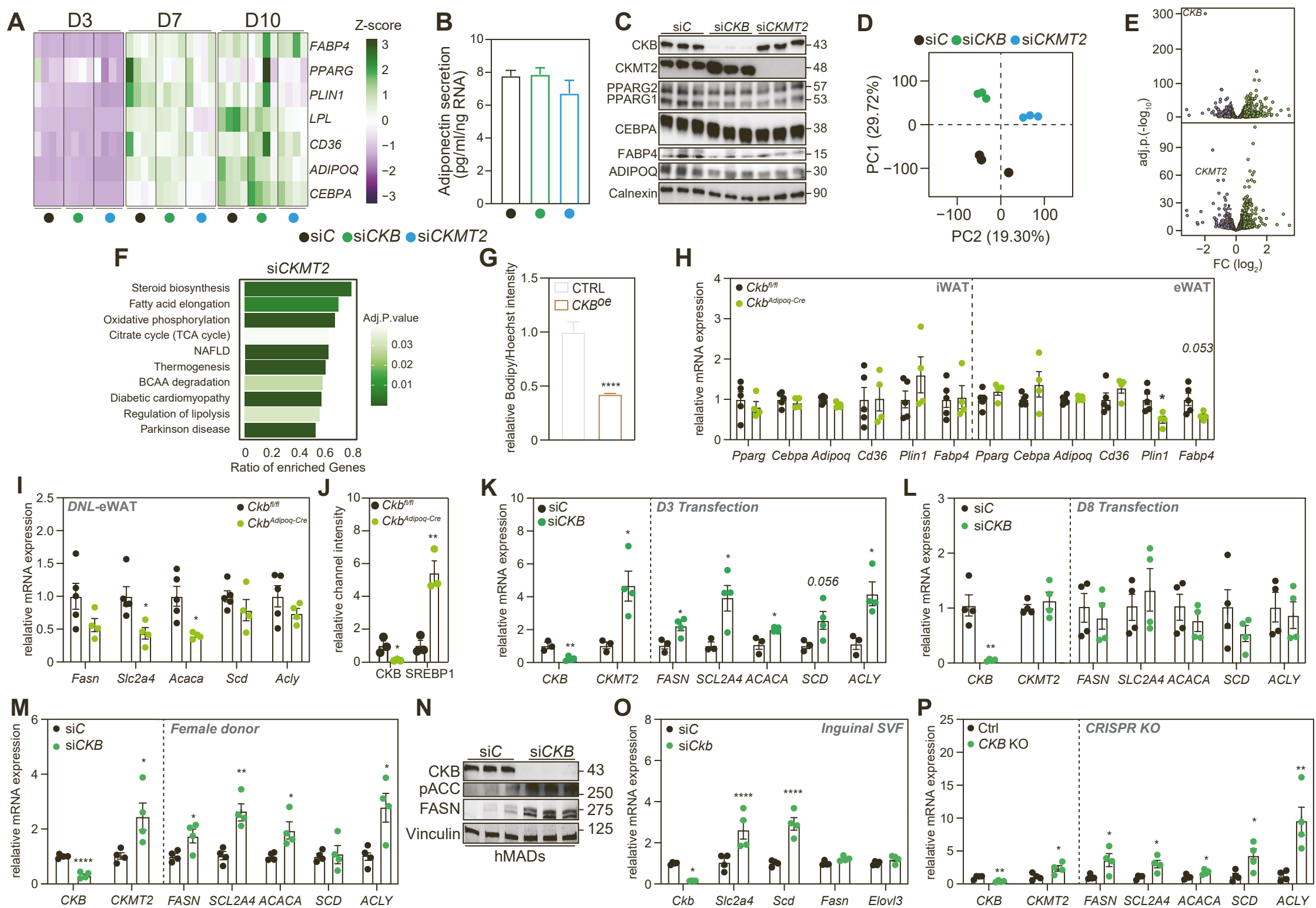
**Creatine kinase B regulates glycolysis and *de novo*
lipogenesis pathways to control lipid
accumulation during adipogenesis**

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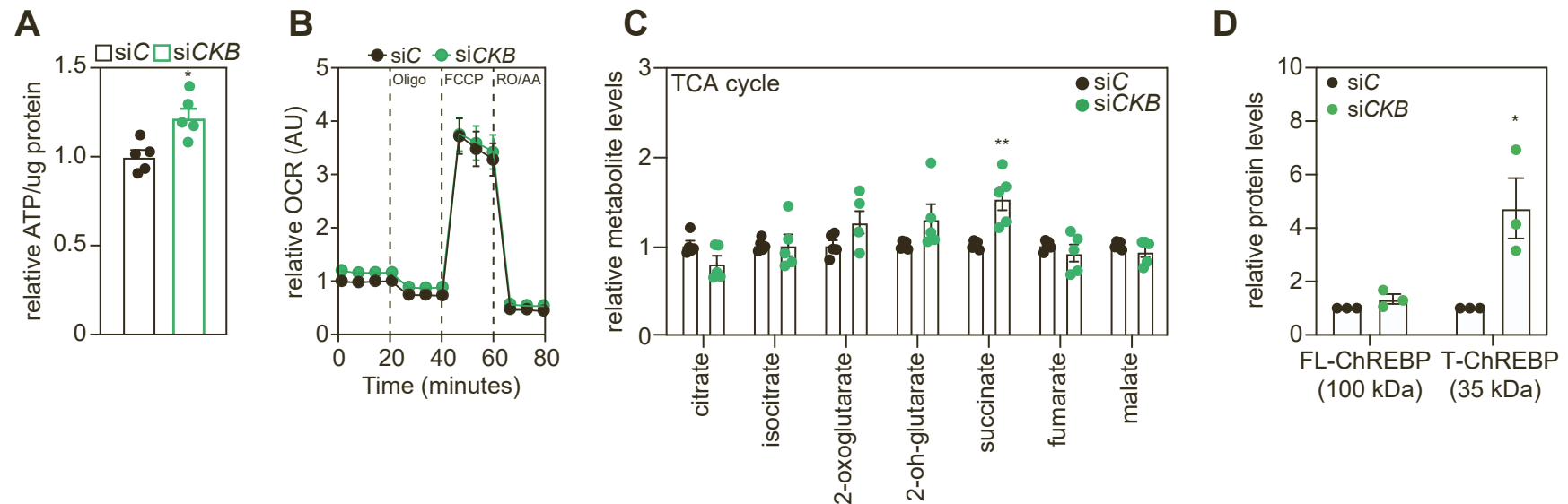
Supplementary Figure 1: Temporal induction of creatine kinases during adipogenesis is driven by thyroid hormone signaling.

- (A) Time course plot, representing trend of enrichment of amino acid related pathways during *in vitro* differentiation of human white preadipocytes from FANTOM5 data²⁷. On the y axis is displayed the enrichment ratio of genes/tot gene ontologies, on the x axis is specified the day of adipocyte differentiation.
- (B) Heatmap representing changes in gene expressions of the genes enriched in “Arginine and proline metabolism” KEGG pathway throughout preadipocyte differentiation.
- (C) mRNA expression of *CKB/CKMT2* in *in vitro* differentiating human white preadipocytes incubated with media lacking one of the components of the adipogenic cocktail from D5 to D8 of differentiation. Values are mean \pm SEM. *p* value was calculated with One-way ANOVA with Dunnett’s multiple-comparison test, * *p* < 0.05 when compared to the “all drugs” condition. Ins = Insulin; Rosi = Rosiglitazone; Trans = Transferrin.
- (D) Graphical visualization of genomic area of Chromosome 14 and Chromosome 5 with focus on *CKB/CKMT2* TSS and 1000 base pairs upstream to it on the first row, annotated thyroid receptor A (THRA) binding sites, on the second row the annotated gene.



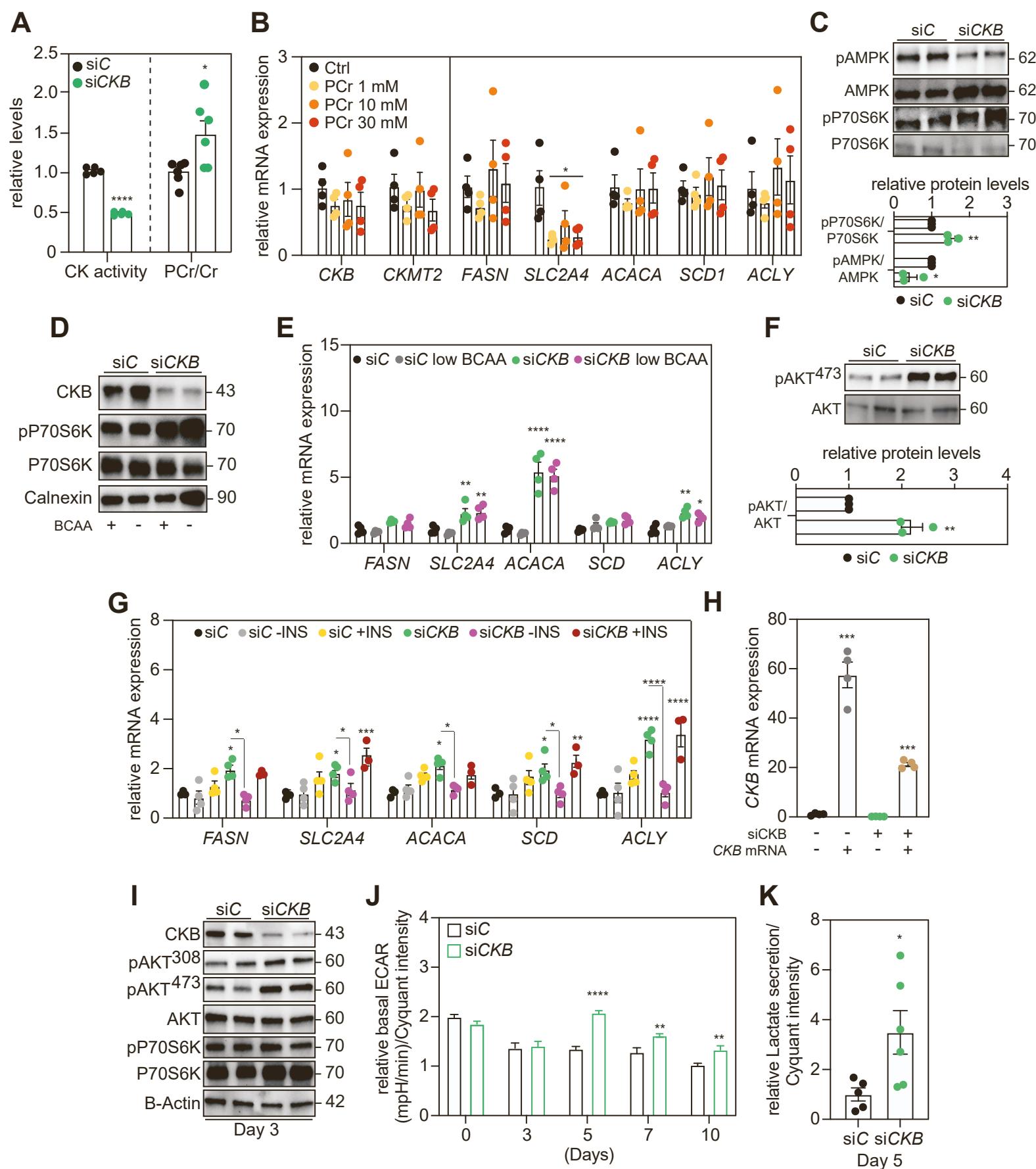
Supplementary Figure 2: Creatine kinase B regulates *de novo* lipogenesis without altering adipogenesis.

- (A) Heatmap representing mRNA expression quantified by qPCR for several markers of mature adipocytes in siC/siCKB/siCKMT2 cells transfected at D-1 and harvested at different days of differentiation (D3/D7/D10). Values are centered and scaled according to row mean.
- (B) Secretion of ADIPOQ in media harvested at D10 from siC/siCKB/siCKMT2 cells transfected with siRNA at preadipocyte level (D-1). Values are mean \pm SEM. $n > 10$.
- (C) Representative immunoblots of multiple mature adipocyte markers in siC/siCKB/siCKMT2 cells transfected at D-1 and harvested at D10 of differentiation.
- (D) Dimension 1 and 2 from principal component analysis of 9 samples, data were generated through RNA sequencing of siC/siCKB/siCKMT2 cells transfected at D-1 and harvested at D10 of adipocyte differentiation for analysis.
- (E) Volcano plots displaying differentially expressed genes of cells transfected with siC/siCKB/siCKMT2 at preadipocyte level and harvested D10 of differentiation for analysis.
- (F) KEGG pathways enriched in siCKMT2 compared to siC, color represents significance of enrichment and on the x axis is displayed the enrichment ratio of genes/tot gene ontologies.
- (G) High-throughput quantification of the immunohistochemical Bodipy staining of cells transfected with mRNA encoding catalytically inactive Cas9 coupled to a VPR complex with/without guide RNA targeting *CKB* promoter, transfected at D-1 and harvested at D10 of differentiation. Values are normalized by Hoechst intensity and represented as mean \pm SEM. $n > 10$, p value was calculated by Student's t-test, **** $p < 0.0001$.
- (H) mRNA expression by qPCR of mature adipocyte markers from inguinal and epididymal WAT of *Ckb^{fl/fl}* and *Ckb^{Adipoq-Cre}* male mice. Values are mean \pm SEM. p value was calculated by Student's t-test, * $p < 0.05$.
- (I) mRNA expression of DNL-related genes in epididymal WAT of *Ckb^{fl/fl}* and *Ckb^{Adipoq-Cre}* male mice. Values are mean \pm SEM. p value was calculated by Student's t-test, * $p < 0.05$.
- (J) Relative immunofluorescence signal quantification normalized over Hoechst intensity of CKB/SREBP1 in paraffin embedded inguinal WAT of *Ckb^{fl/fl}* and *Ckb^{Adipoq-Cre}* male mice. Values are mean \pm SEM. p value was calculated by Student's t-test, * $p < 0.05$, ** $p < 0.01$.
- (K) mRNA expression of *CKB/CKMT2* and DNL-related genes in siC/siCKB preadipocytes transfected at D3 of differentiation, harvested at D10. Values are mean \pm SEM. p value was calculated by Student's t-test, * $p < 0.05$, ** $p < 0.01$.
- (L) mRNA expression of *CKB/CKMT2* and DNL-related genes in siC/siCKB cells transfected at D8 of differentiation, harvested at D10. Values are mean \pm SEM. p value was calculated by Student's t-test, ** $p < 0.01$.
- (M) mRNA expression of *CKB/CKMT2* and DNL-related genes in siC/siCKB early transfected (D-1) preadipocytes derived from a female donor, harvested at D10. Values are mean \pm SEM. p value was calculated by Student's t-test, * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$.
- (N) Representative immunoblot of siC/siCKB in hMADs transfected at D-1 and harvested at mature state. It displays the upregulation of multiple DNL-related proteins upon *CKB* knockdown.
- (O) mRNA expression of *Ckb* and DNL-related genes in siC/siCkb early transfected preadipocytes isolated from inguinal WAT of female mice and differentiated *in vitro*. Values are mean \pm SEM. p value was calculated by Student's t-test. * $p < 0.05$, **** $p < 0.0001$.
- (P) mRNA expression of *CKB/CKMT2* and DNL-related genes in siC/siCKB early transfected preadipocytes using CRISPR/Cas9 knockout technology, harvested at D10. Values are mean \pm SEM. p value was calculated by Student's t-test, * $p < 0.05$, ** $p < 0.01$.



Supplementary Figure 3: CKB depletion enhances ATP production without mitochondrial activation.

- (A) ATP levels in siC/siCKB cells transfected at D-1 and harvested at D10 of differentiation. Values are relative, normalized by protein concentration and represented as mean \pm SEM. p value was calculated by Student's t-test, * $p < 0.05$.
- (B) Representative Mito stress test demonstrating oxygen consumption rate (OCR) in siC/siCKB cells transfected at D-1 and analysed at D10 of differentiation. Values are represented as mean \pm SEM. Oligo = Oligomycin; FCCP = Carbonil cianuro-p-trifluorometossifenilidrazone; RO/AA = Rotenone/Antimycin.
- (C) Levels of targeted metabolites involved in the TCA cycle of siC/siCKB cells transfected at D-1 and harvested at D10 of differentiation. Values are mean \pm SEM. p value was calculated by Student's t-test, ** $p < 0.01$.
- (D) Relative protein quantification of ChREBP isoforms in siC/siCKB cells transfected at D-1 and harvested at D10 of differentiation (calculated from two independent experiments). Values are mean \pm SEM. p value was calculated by Student's t-test, * $p < 0.05$. FL = full length; T = transcriptionally active isoform of ChREBP.



Supplementary Figure 4: CKB modulates glycolysis and DNL by regulating AKT phosphorylation.

- (A) On the left, creatine kinase activity of isolated cytosolic fraction at D10 of siC/siCKB cells transfected at D-1. On the right, metabolite levels of phosphocreatine/creatinine ratio at D10 of differentiation in siC/siCKB cells. Values are mean \pm SEM. p value was calculated by Student's t-test, $*p < 0.05$, $****p < 0.0001$.
- (B) mRNA expression of *CKB/CKMT2* along with DNL-related genes in *in vitro* differentiated adipocytes incubated with sequential doses of phosphocreatine for 24 hours at D10 of differentiation. Values are mean \pm SEM. p value was calculated with One-way ANOVA and Dunnett's multiple-comparison test comparing to control, $*p < 0.05$.
- (C) Representative immunoblot of siC/siCKB cells transfected at D-1, showcasing AMPK/pAMPK and P70S6K/pP70S6K with relative quantification on the bottom (quantification from two independent experiments). Values are mean \pm SEM. p value was calculated by Student's t-test, $*p < 0.05$, $**p < 0.01$. Please note that these blots are revealed from the same proteins as the blot on Figure 3C, where CKB and a B-Actin are presented.
- (D) Representative immunoblot of siC/siCKB cells transfected at D-1 incubated with media containing normal or low BCAA concentration for 48 hours at D8 and harvested afterward.
- (E) mRNA expression by qPCR of DNL-related genes of siC/siCKB cells transfected at D-1 incubated with media containing normal or low BCAA concentration for 48 hours at D8 and harvested afterward. Values are mean \pm SEM. p value was calculated with Two-way ANOVA and Tukey's multiple-comparison test, comparing all conditions to control, $*p < 0.05$, $**p < 0.01$, $****p < 0.0001$.
- (F) Representative immunoblot of siC/siCKB cells, showcasing pAKT/AKT with relative quantification on the bottom (quantified from two independent experiments). Values are mean \pm SEM. p value was calculated by Student's t-test, $**p < 0.01$. Please note that these blots are revealed from the same proteins as the blot on Figure 3C, where CKB and a B-Actin are presented.
- (G) Representative DNL-gene expression by qPCR of siC/siCKB cells transfected at D-1. At D10, cells were starved from insulin for 48 hours (-INS) and consequently stimulated for 2 hours with insulin (+INS), post-starvation. Values are mean \pm SEM. p value was calculated with Two-way ANOVA and Tukey's multiple-comparison test comparing to siC unless indicated otherwise, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$.
- (H) mRNA expression by qPCR of *CKB* of early transfected (at D-1) siC/siCKB cells, re-transfected at D8 with mRNA encoding *CKB* or a non-coding sequence. Values are mean \pm SEM. p value was calculated with Two-way ANOVA and Tukey's multiple-comparison test comparing to siC, $***p < 0.001$.
- (I) Representative immunoblot of early siC/siCKB transfected cells (D-1) harvested at D3 of adipocyte differentiation.
- (J) Representative basal ECAR of Seahorse (Glycostress) analysis during preadipocyte differentiation in siC/siCKB cells transfected at D-1. The analyses were performed on different days of differentiation (from D0 to D10). Values are mean \pm SEM. p value was calculated by a Student's t-test for each day. $n > 10$, $**p < 0.01$, $****p < 0.0001$.
- (K) Lactate secretion in siC/siCKB cells transfected at D-1, measured at D5 of differentiation. Values are mean \pm SEM and normalized by Cyquant intensity. p value was calculated by Student's t-test, $*p < 0.05$.