

Supplemental information

**MEDAG functions as an A-kinase-anchoring protein
in adipocytes**

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Supplementary figures and legends

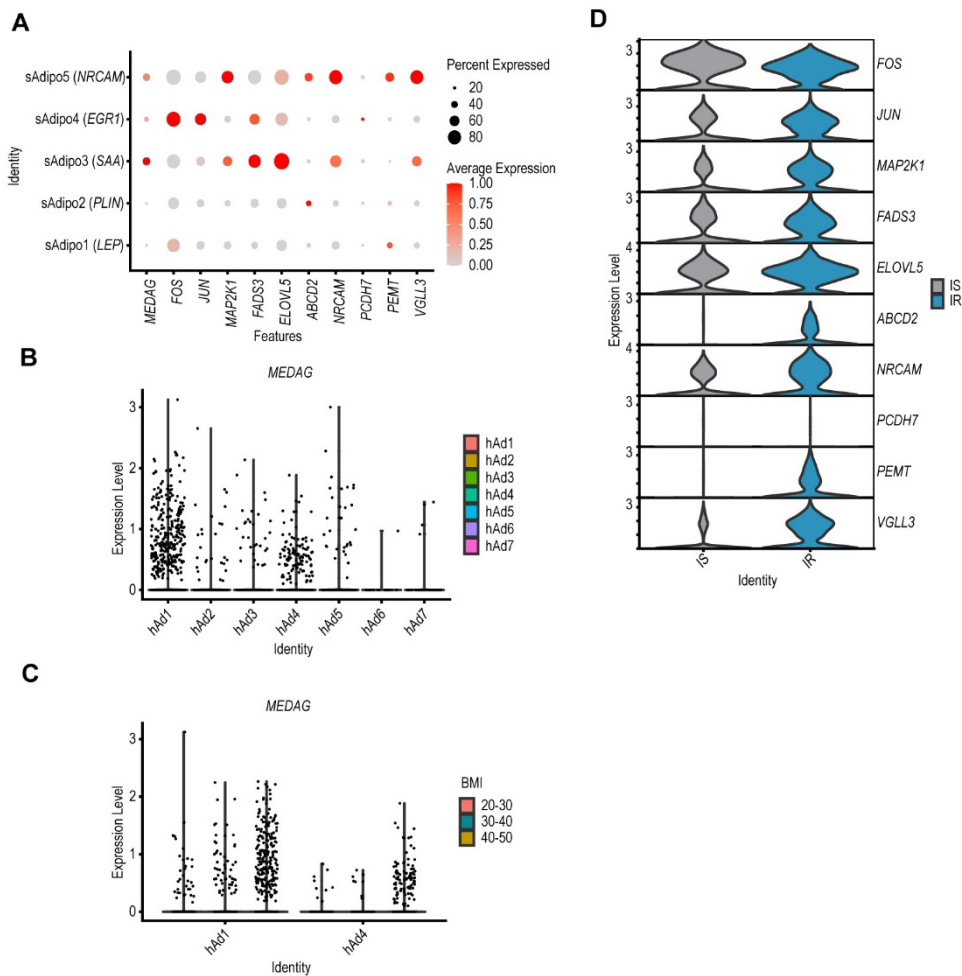


Figure S1. Adipose tissue *MEDAG* expression positively correlates with obesity and metabolic dysfunction in humans, related to Figure 1

(A) Dot plots showing expression of *MEDAG* and top marker genes within hAd1 and hAd4 subpopulation (Emont et al. dataset) in subcutaneous adipocyte subpopulations in human cohort 4.

(B) Expression of *MEDAG* in subcutaneous adipocyte subpopulations from Emont et al. dataset.

(C) Expression of *MEDAG* within hAd1 and hAd4 subpopulation (Emont et al. dataset) in individuals with different BMI.

(D) Violin plots comparing *MEDAG* expression and the top five markers of hAd1 and hAd4 subpopulation (Emont et al. dataset) between metabolically healthy (IS) versus unhealthy (IR) human subcutaneous WAT in human cohort 4.

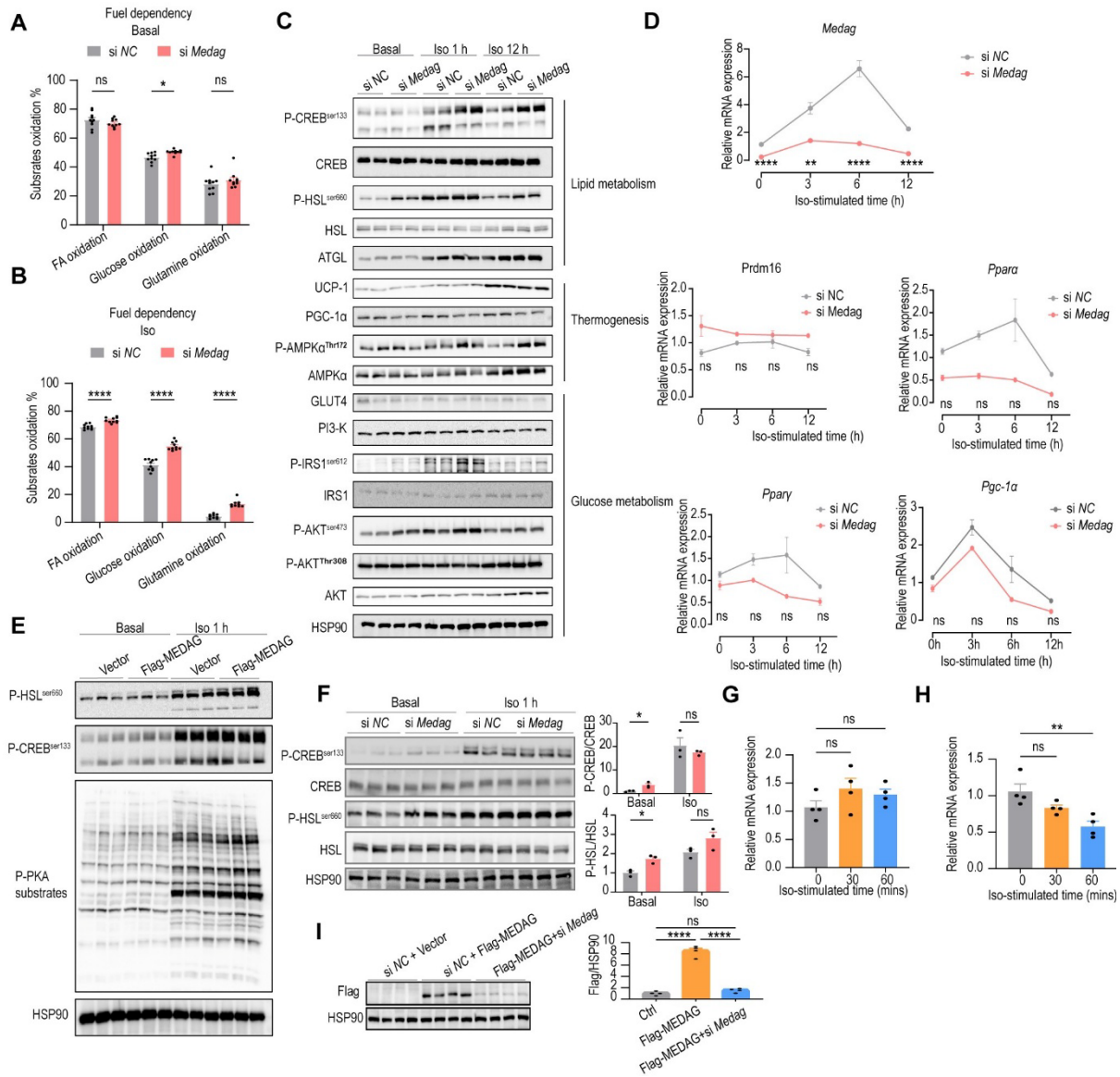


Figure S2. Medag regulates adipocyte respiration by PKA activity modulation, related to Figure 2

(A) Fuel dependency assay in iBAs with Medag knockdown (n=10).

(B) Fuel dependency assay in iBAs treated with 1 μ M isoproterenol for 1 h after Medag knockdown (n=9-10 for each group).

(C) Representative blots of markers linked to lipid metabolism and glucose/insulin signaling, in iBAs treated with 1 μ M isoproterenol for 1 h or 12 h after MEDAG knockdown (n = 2). Repeated independently three times with similar results.

(D) Real-time qPCR of thermogenesis markers in iBAs treated with 1 μ M isoproterenol for different timepoints (n = 4).

(E) Representative blots of P-HSL, P-CREB and P-PKA substrates protein levels (n = 3). Repeated independently three times with similar results.

(F) Representative blots and quantification of Flag-MEDAG protein levels in iBAs with Flag-MEDAG overexpression following Medag knockdown (n = 4).

(G) Representative blots and quantification of P-HSL and P-CREB protein levels in 3T3 treated with 10 μ M isoproterenol for 1 h after Medag knockdown (n = 3).

(H) Real-time qPCR of *Medag* in iBAs treated with 1 μ M isoproterenol for different timepoints (n=4).

(I) Real-time qPCR of *Medag* in 3T3 treated with 10 μ M isoproterenol for different timepoints (n=4).

Data are presented as mean \pm SEM and analyzed using unpaired two-tailed t-test (A, B, D, G) and one-way ANOVA with Tukey's post hoc multiple comparison test (H, I). ns, not significant, **p < 0.01, ***p < 0.001, ****p < 0.0001.

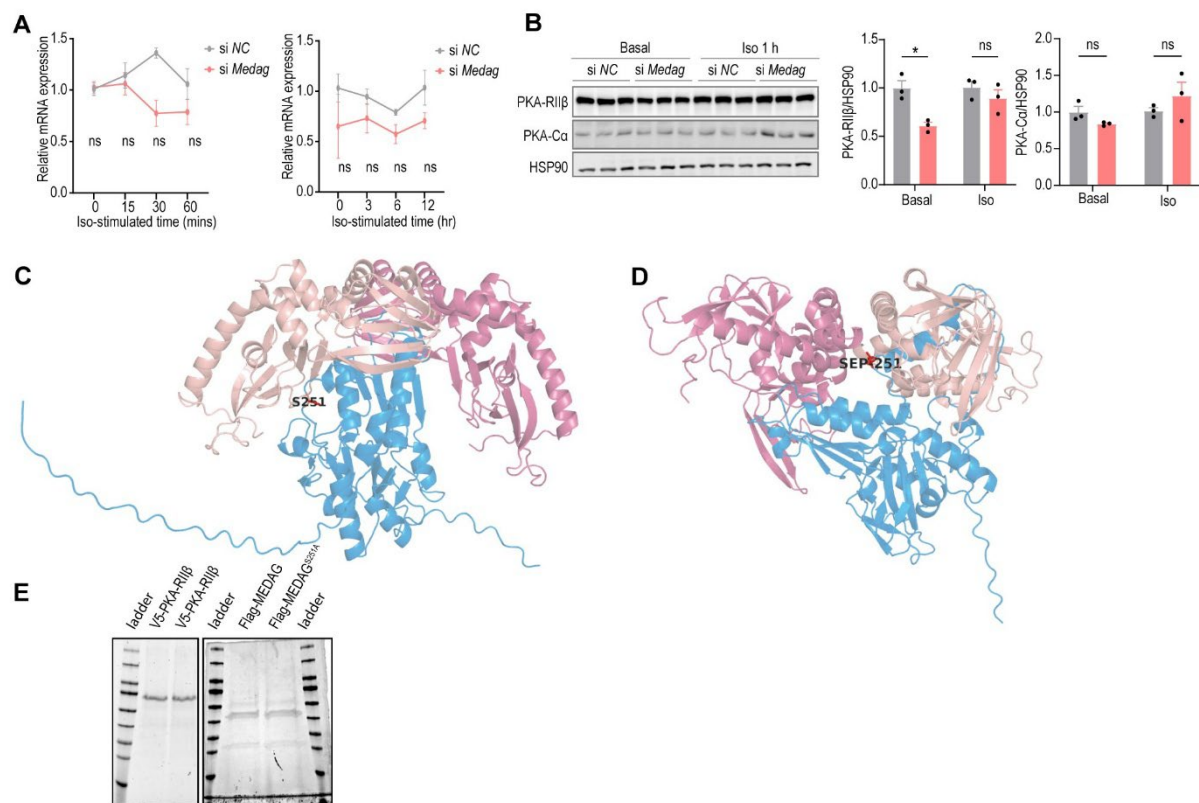


Figure S3. MEDAG regulates the stability of the PKA-RIIβ subunits, related to Figure 4

(A) Real-time qPCR of PKA-RIIβ in iBAs treated with 1 μM isoproterenol for different timepoints (n = 6).

(B) Representative blots and quantification of PKA-RIIβ protein levels in 3T3 under basal conditions and following 10 μM isoproterenol stimulation after Medag knockdown (n = 3).

(C) Computational predicted structure complex of WT-MEDAG with PKA-RIIβ dimer using AlphaFold 3. The two dimers of PKA-RIIβ are labeled in Salmon and Warmpink while MEDAG is labeled in Marine.

(D) Computational predicted structure complex of Ser251 phospho-MEDAG with PKA-RIIβ dimer using AlphaFold 3. The two dimers of PKA-RIIβ are labeled in Salmon and Warmpink while MEDAG is labeled in Marine.

(E) Coomassie Blue-stained SDS-PAGE showing immunoaffinity-purified V5-PKA-RIIβ, WT-MEDAG and Flag-MEDAG^{S251A}.

Data are presented as mean ± SEM and analyzed using unpaired two-tailed t-test (A, B). ns, not significant, *p < 0.05.

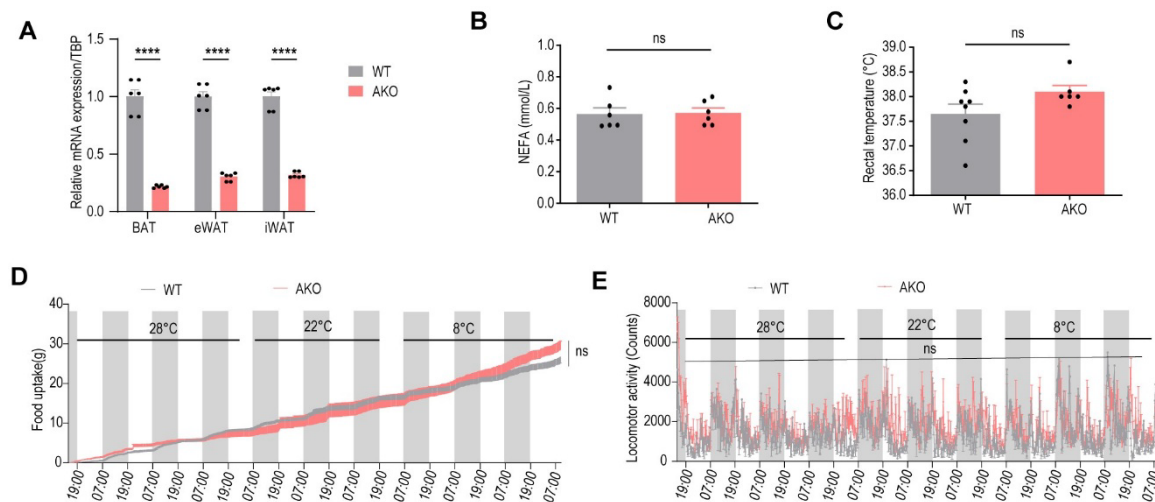


Figure S4. *Medag* AKO mice exhibit increased EE and are resistant to HFD-induced obesity, related to Figure 5

(A) *Medag* knockout efficiency in different adipose depots in AKO mice (n = 6).

(B) Plasma non-esterified fatty acids (NEFA) after 12 weeks of HFD feeding (n = 6).

(C) Rectal temperature of mice housed at 22°C (n = 8 for WT and 6 for AKO).

(D) Accumulative food uptake in mice throughout different temperature exposure at 28°C (S), 22°C (T), 8°C (U) (n = 5).

(E) Locomotor activity in mice throughout different temperature exposure at 28°C (S), 22°C (T), 8°C (U) (n = 5).

Data are presented as mean \pm SEM. Each data point represents an individual animal. Statistical analysis was performed using unpaired two-tailed t-test (A-E). ns, not significant, ****p < 0.0001.

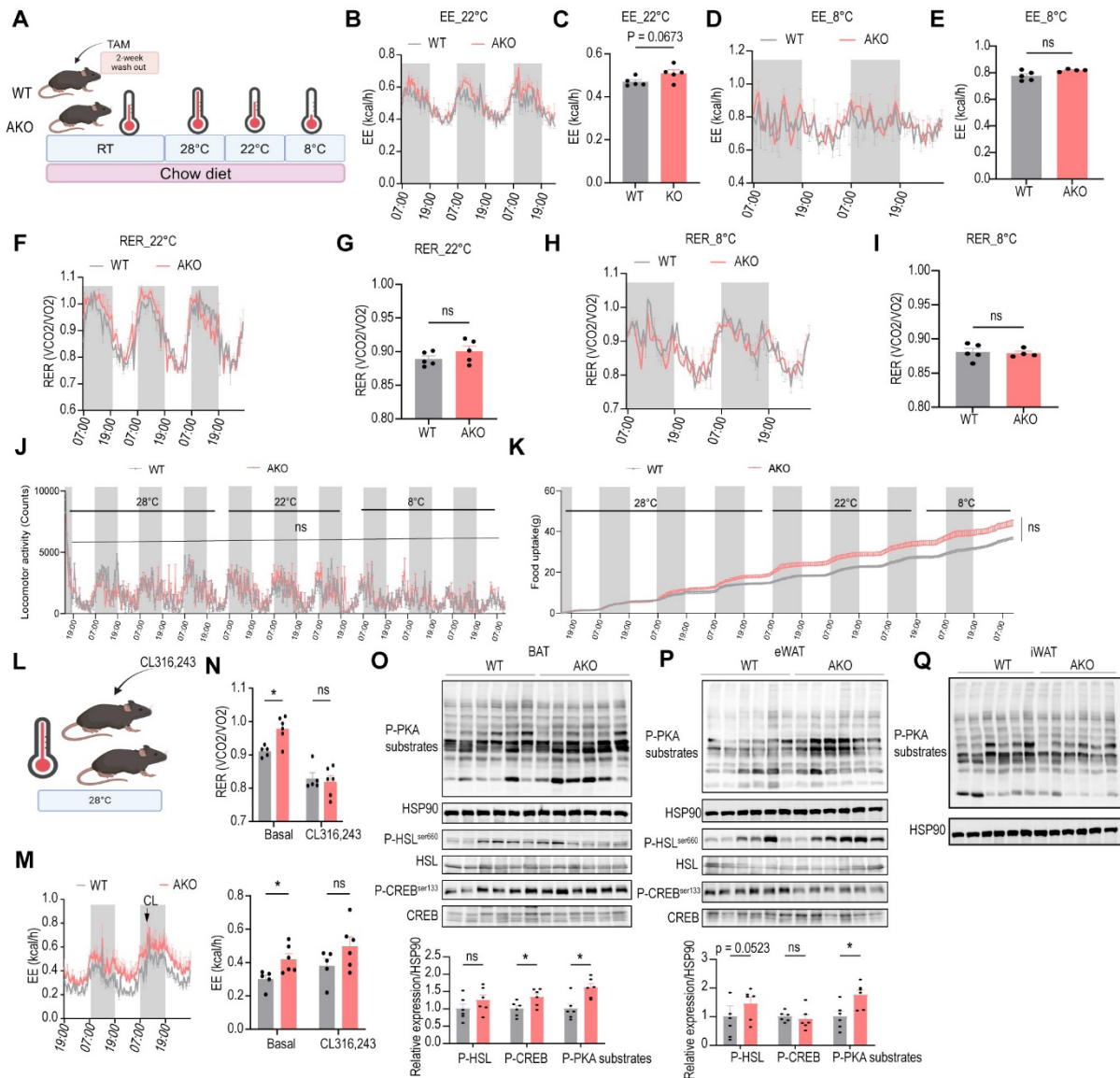


Figure S5. PPAR γ /GLUT signaling acts downstream of PKA activation in *Medag* AKO mice, related to Figure 6

(A) Schematic illustration of experimental design: thermoneutral (28°C), room temperature (22°C), and cold exposure (8°C).

(B-C) Energy expenditure curve (B) and daily energy expenditure (C) of mice housed at 22°C (n = 5).

(D-E) Energy expenditure curve (D) and daily energy expenditure (E) of mice housed at 8°C (n = 5 for WT and 4 for AKO).

(F-G) RER curve (F) and daily RER (G) of mice housed at 22°C (n = 5).

(H-I) RER curve (H) and daily RER (I) of mice housed at 8°C (n = 5 for WT and 4 for AKO).

(J) Locomotor activity in mice housed at 28°C (S), 22°C (T), 8°C (U) (n = 5 and n=4 for AKO when housed at 8°C).

(K) Accumulative food uptake in mice housed at 28°C (S), 22°C (T), 8°C (U) (n = 5 and n=4 for AKO when housed at 8°C).

(L) Scheme of experimental design.

(M) Energy expenditure mice housed at 28°C before and after CL316,243 injection (n = 5).

(N) RER of mice housed at 28°C before and after CL316,243 injection (n = 5).

(O) Representative blots and quantification of P-PKA substrates, P-HSL, and P-CREB protein levels in BAT from mice housed at 28°C for 1 week (n = 6).

(P) Representative blots and quantification of P-PKA substrates, P-HSL, and P-CREB protein levels in eWAT from mice housed at 28°C for 1 week (n = 6).

(Q) Representative blots of P-PKA substrates in iWAT of mice housed at 28°C for 1 week (n = 6).

Data are presented as mean \pm SEM. Each data point represents an individual animal. Statistical analysis was performed using unpaired two-tailed t-test (C, E, G, I-P). ns, not significant, *p < 0.05.

Table S1. Sequence of siRNA pools used to knockdown genes in immortalized brown adipocytes (Mouse), related to STAR Methods

Gene symbol	Sense siRNA Sequence (5'-3')
Medag	#1 GGGAACGATACTGAGCAA #2 GCAAACCTATGGTATTCTT #3 GCAGAATTCTCATTTCCAA
Praja2	#1 GUA GUA AGG CCC AAA GUU Aβ #2 CGG CAA GAA UCU CGG GAC A #3 UGG AAG AAG ACA UGC GUA U

Table S2. Sequence of qPCR primers used in this study, related to STAR Methods

Gene symbol	Species	Primer Sequence (5'-3')
Medag	Mouse	Forward: ATCGCCTCAGCAGCTACATC Reverse: TGTCTTCTTGGTCTGCACG
Prdm16	Mouse	Forward: CGCTTCGAATGTGAAACTG Reverse: AAGGTCTTGCCACAGTCAGG
Cox7A1	Mouse	Forward: CAGCGTCATGGTCAGTCTGT Reverse: AGAAAACCGTGTGGCAGAGA
Elovl	Mouse	Forward: GGCATAATTGTTACCTGATTGAGG Reverse: GATGGTTCTGGGCACCATCTT
Ppara	Mouse	Forward: TCGGCGAACTATTCGGCTG Reverse: GCACTTGTGAAACGGCAGT
Pparγ	Mouse	Forward: GTGGGGATAAAGCATCAGGC Reverse: CCGGCAGTTAAGATCACACCTA
Ucp-1	Mouse	Forward: CAGCCGGCTTAATGACTGGA Reverse: TGATCCCATGCAGATGGCTC
Pgc-1α	Mouse	Forward: CTCTCAGTAAGGGGCTGGTTG Reverse: CGAATGACGCCAGTCAAGC
Pkm2	Mouse	Forward: GTGGTGACCTGGGCATTGAG Reverse: GCACAGATGACAGGCTTCCC
Pepck	Mouse	Forward: CGGATGGGCATATCTGTGCT Reverse: AGGCCAGTTGTTGACCAA
Glut4	Mouse	Forward: GACGGACACTCCATCTGTTG Reverse: GCCACGATGGAGACATAGC
PKA-R11β	Mouse	Forward: CTTTATCGAGTCCCTGCCGT Reverse: CAGACGCTGCTCTTGGTTTG

Table S3. Sequence of primers used for molecular cloning, related to STAR Methods

Plasmid	Primer Sequence (5'-3')
Flag-MEDAG	Forward: GGACTAGTATGGCCACTGCAGCGTGC Reverse: CGACGCGTTCACTTATCGTCGTCATCCTTGTAAATCGATAAGTTGGCTGGATGCT
HA-PKA-Cα	Forward: GGACTAGTGCCACCATGGGCAACGCCGCCGCCG Reverse: CGACGCGTCTAGGCGTAGTCAGGCACGTCGTAAGGATAAACTCAGTAACTCCTTGCC
V5-PKA-R11β	Forward: GGACTAGTATGAGCATCGAGATCCCC Reverse: CGACGCGTTCAAGTAGAATCGAGACCGAGGAGAGGGTTAGGGATAGGCTTACCTCATGCAGTGGGCTCAAC
Flag-MEDAG ^{S251A}	Forward: CTAATACGAAGGAGTGCTTTCTCTGATCGGAAA Reverse: TTTCCGATCAGAGAAACGACTCCTTCGTATTAG
Flag-MEDAG ^{S251D}	Forward: CTAATACGAAGGAGTGATTTCTCTGATCGGAAA Reverse: TTTCCGATCAGAGAAAAGACTCCTTCGTATTAG

