Supplementary Information File:

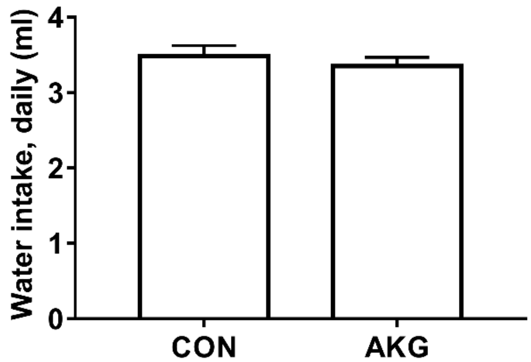
Dietary alpha-ketoglutarate promotes beige adipogenesis and prevents obesity in middle-aged mice

Supplementary Table S1. Primer sequences used for real-time quantitative PCR

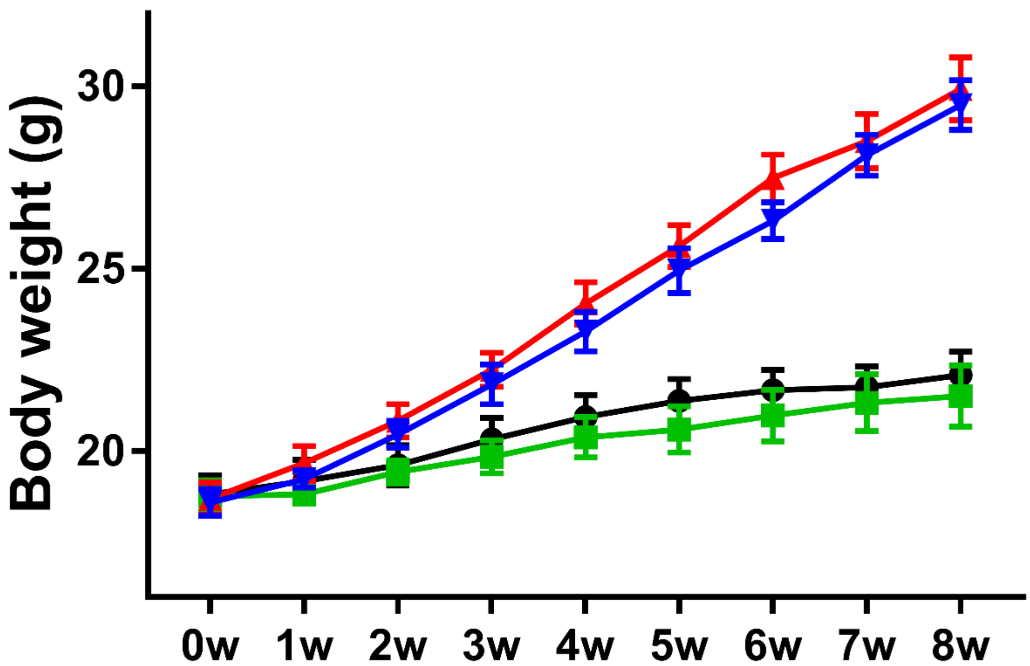
Gene	Forward (5′-3′)	Reverse (5′-3′)
18s	GTAACCCGTTGAACCCCATT	CCATCCAATCGGTAGTAGCG
Cytochrome C	TTTCAGGCTTCACCCTAGATGA	GAAGAATGTTATGTCCTACGAATATG
Ucp1	ACTGCCACACCTCCAGTCATT	CTTTGCCTCACTCAGGATTGG
Prdm16	CAGCACGGTGAAGCCATTC	GCGTGCATCCGCTTGTG
Pgc1α	CCCTGCCATTGTTAAGACC	TGCTGCTGTTCCTGTTTTC
Cidea	ATCACAACTGGCCTGGTTACG	TACTACCCGGTGTCCATTTCT
Elovl3	GATGGTTCTGGGCACCATCTT	CGTTGTTGTGTGGCATCCTT
Cox7a1	CAGCGTCATGGTCAGTCTGT	AGAAAACCGTGTGGCAGAGA
Idh2	CTAGAGTCCCCACCGCAC	ATCTCGTCACCGTCCATCTC
Pparγ	AGCTCCAAGAATACCAAAGTGCGAT	AGGTTCTTCATGAGGCCTGTTGTAGA
Zfp423	GTCACCAGTGCCCAGGAAGAAGAC	AACATCTGGTTGCACAGTTTACACTCAT
Cebpα	ATGAGAGAAGGAGGGGAGCAGG	AGGTGGGAGAGGCGTGGAACTA
Glut4	CTCTCAGGCATCAATGCTGTTTTCTA	CGAGACCAACGTGAAGACCGTATT
aP2	CGACAGGAAGGTGAAGAGCATCATA	CATAAACTCTTGTGGAAGTCACGCCT
TET1	CACCCTGTGACTGTGATGGAGGTA	ACTATCTTCTCAATCCGGATTGCCTT
TET2	AAGGATGCAATCCAGACAAAGATGAA	TTTAGCAATAGGACATCCCTGAGAGCT

Supplementary Table S2. MeDIP primer sequences for *Prdm16, Ucp1* and *Zfp423*

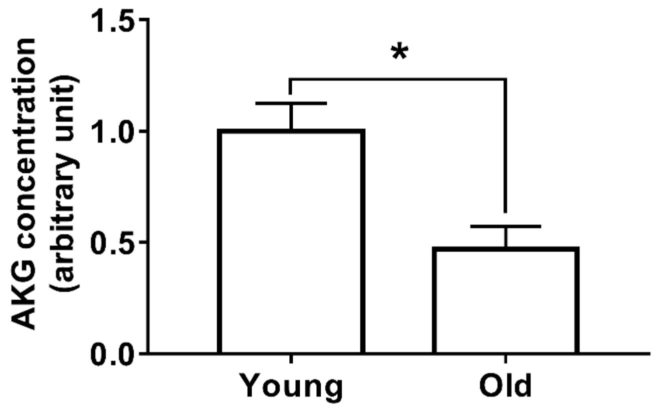
Gene	Forward (5′-3′)	Reverse (5′-3′)
Medip- Prdm16	GACACCGTGACAGCTCTGAA	CTGCGAGTACGGCTACGATT
Medip-Ucp1	GAGTGACAAAAGGCACCACG	GAGTGACAAAAGGCACCACG
Medip-Zfp423	GCACGGGCCTGTTATCTGT	AGGATGTGAGGAGCGGAGT



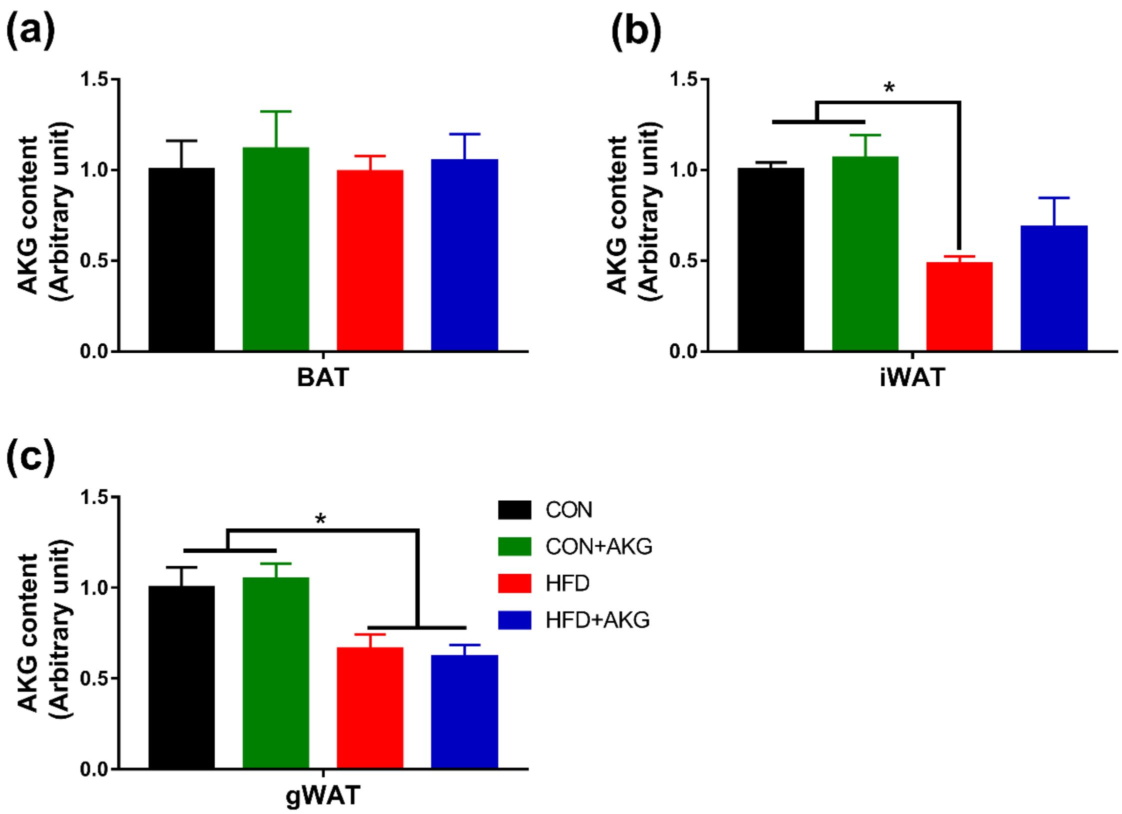
Supplementary Figure 1. Daily water intake of middle-aged mice supplemented with/without AKG. * $p < 0.05$ (n = 6, mean ± SEM).



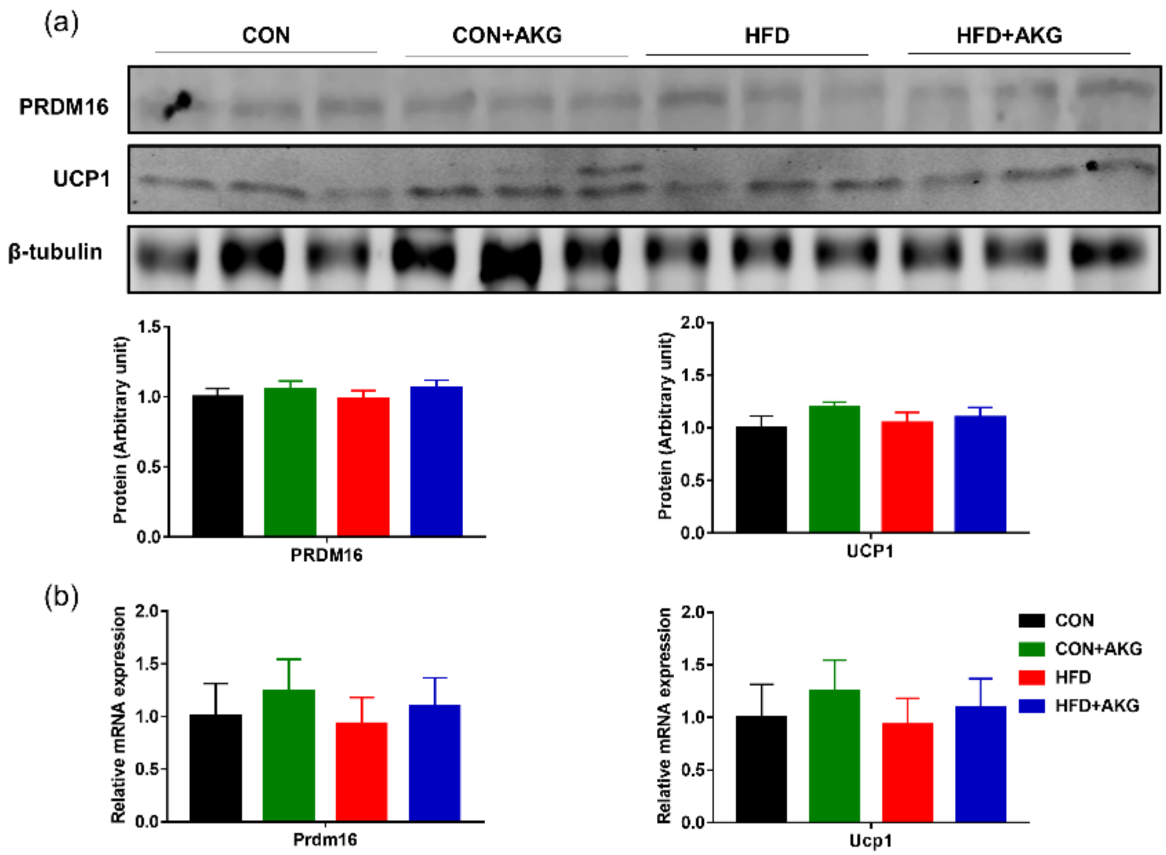
Supplementary Figure 2. Two-month-old C57BL6 mice were fed either control diet or HFD and supplemented with 0 or 1% (w/v) alpha-ketoglutarate for 2 mo. Body weight change during the treatment period. * $p < 0.05$ (n = 6, mean ± SEM).



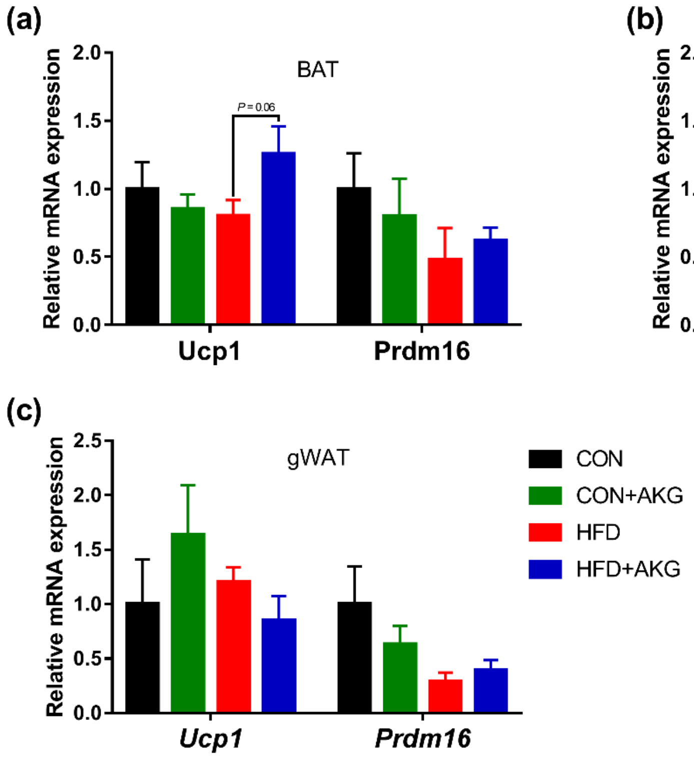
Supplementary Figure 3. Serum AKG content decreased in middle-aged mice. * $p < 0.05$ (n = 6, mean ± SEM).



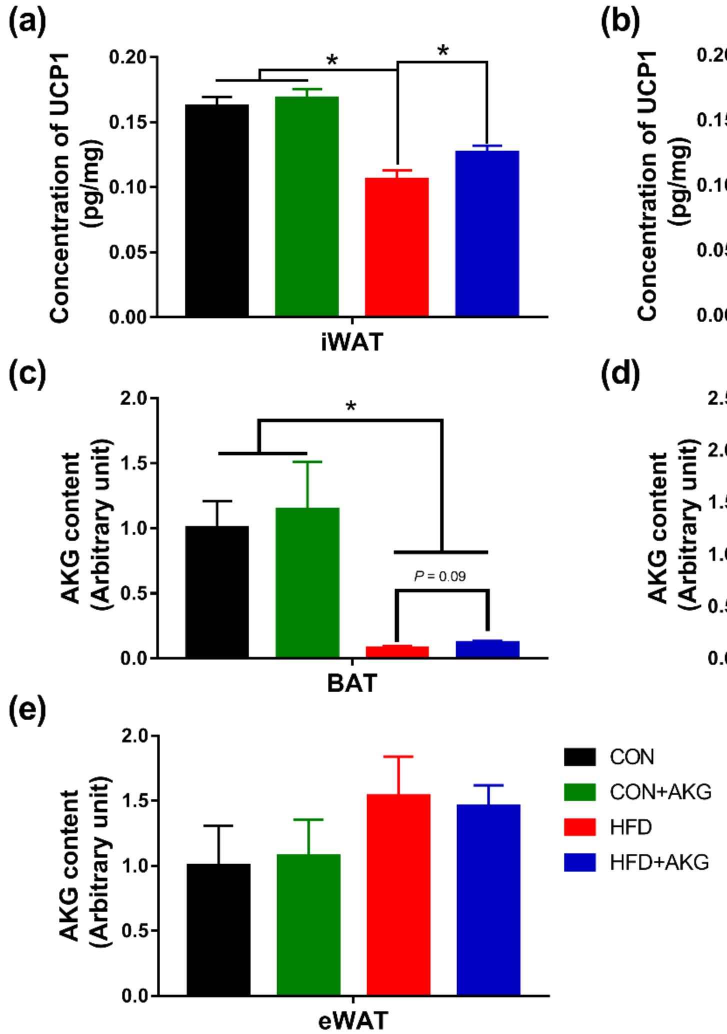
Supplementary Figure 4. AKG levels in BAT and WAT in young mice (2-month-old). (a) AKG concentration in BAT. (b) AKG concentration in iWAT. (c) AKG concentration in gWAT. * $p < 0.05$ (n = 6, mean ± SEM).



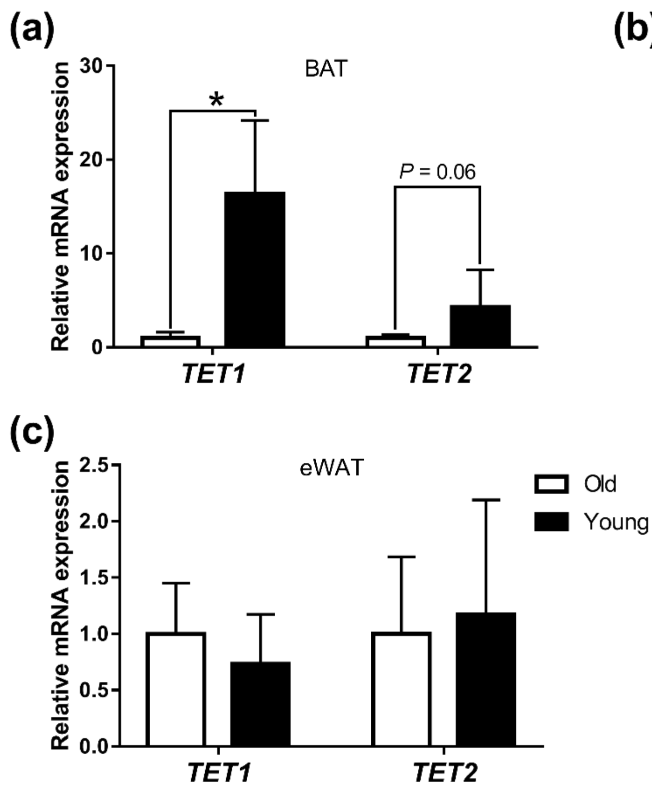
Supplemental Figure 5. Effect of alpha-ketoglutarate supplementation on metabolic activity of gWAT. (a) Protein contents of PRMD16 and UCP1 in BAT analyzed by western. (b) Brown adipose gene mRNA levels in BAT analyzed by q-PCR. * $p < 0.05$ ($n = 6$, mean ± SEM).



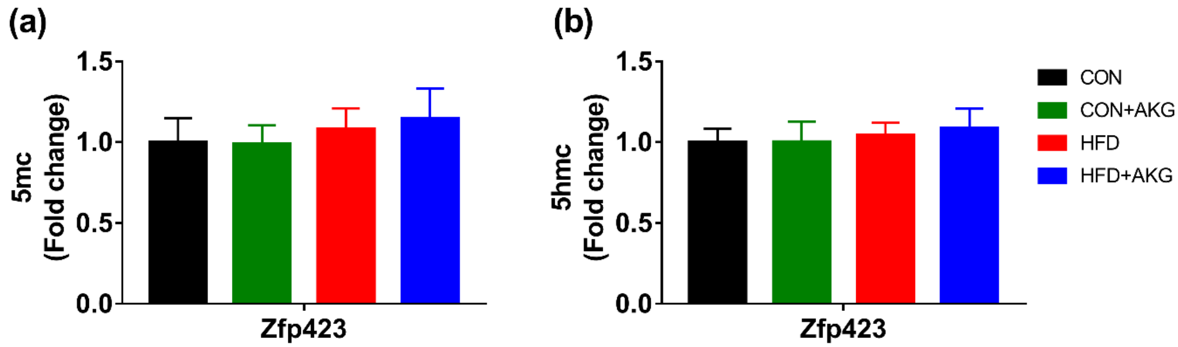
Supplementary Figure 6. Brown adipose gene mRNA levels in young mice. (a) Brown adipose gene mRNA levels in BAT analyzed by q-PCR. (b) Brown adipose gene mRNA levels in iWAT analyzed by q-PCR. (c) Brown adipose gene mRNA levels in gWAT analyzed by q-PCR. **p* < 0.05 (n = 6, mean ± SEM).



Supplementary Figure 7. UCP1 levels and AKG content in different adipose depots after cold exposure. (a) UCP-1 content in iWAT analyzed by q-PCR. (b) UCP-1 content in eWAT analyzed by q-PCR. (c-e) AKG content in BAT (c), iWAT (d) and eWAT (e). *$p < 0.05$ (n = 6, mean ± SEM).



Supplementary Figure 8. Comparison of TET expression levels between young mice and middle-aged mice. (a) TET1 and TET2 mRNA expression in BAT analyzed by q-PCR. (b) TET1 and TET2 mRNA expression in iWAT analyzed by q-PCR. (c) TET1 and TET2 mRNA expression in eWAT analyzed by q-PCR. *$p < 0.05$ (n = 6, mean ± SEM).



Supplementary Figure 9. DNA methylation of the *Zfp423* promoter after cold exposure. (a) Enrichment of 5mC at *Zfp423* promoter region relative to input normalized by the positive control in iWAT after cold exposure. (b) Enrichment of 5hmC at *Zfp423* promoter region relative to input normalized by the positive control in iWAT after cold exposure.