Supplementary Information

Intestinal AMPK modulation of microbiota mediates cross-talk with brown fat to control thermogenesis

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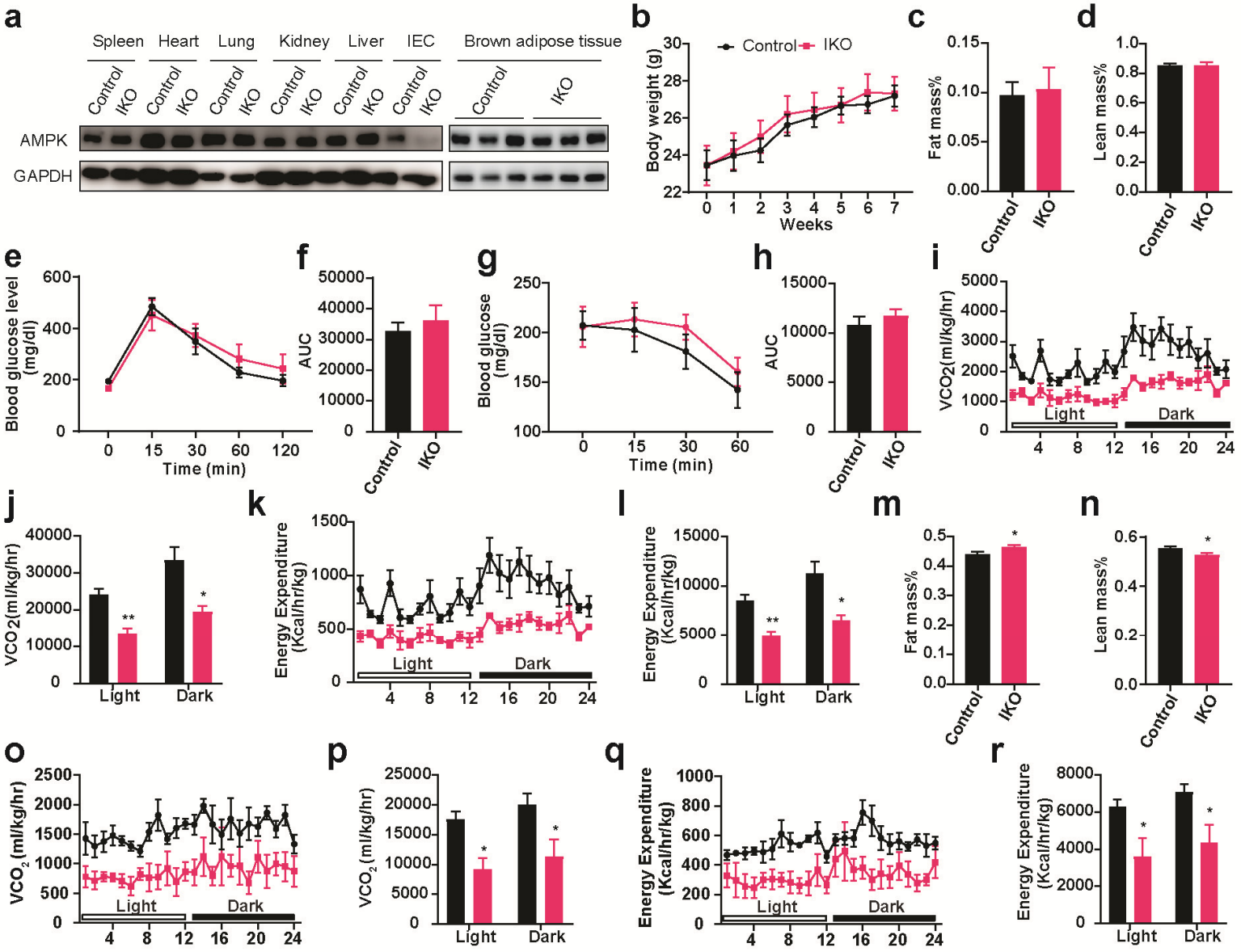
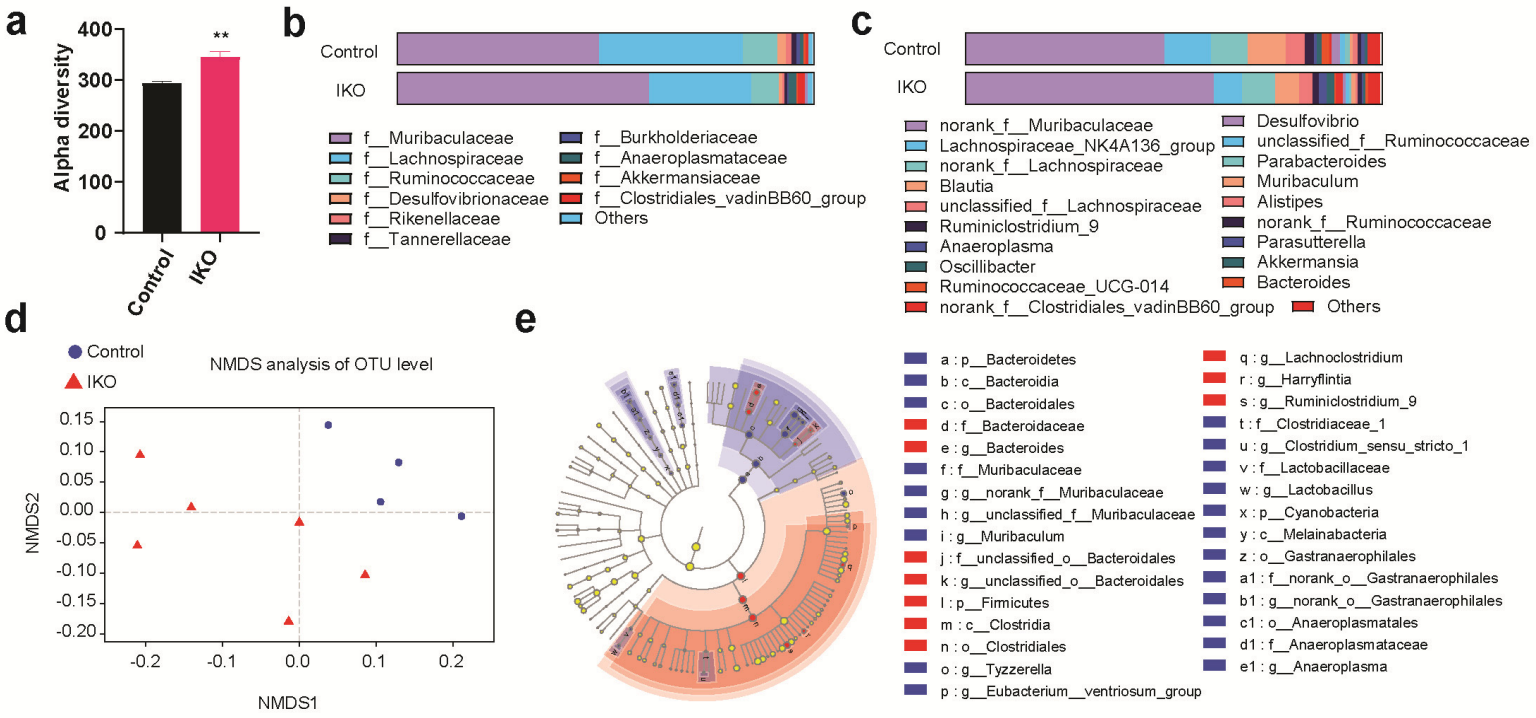
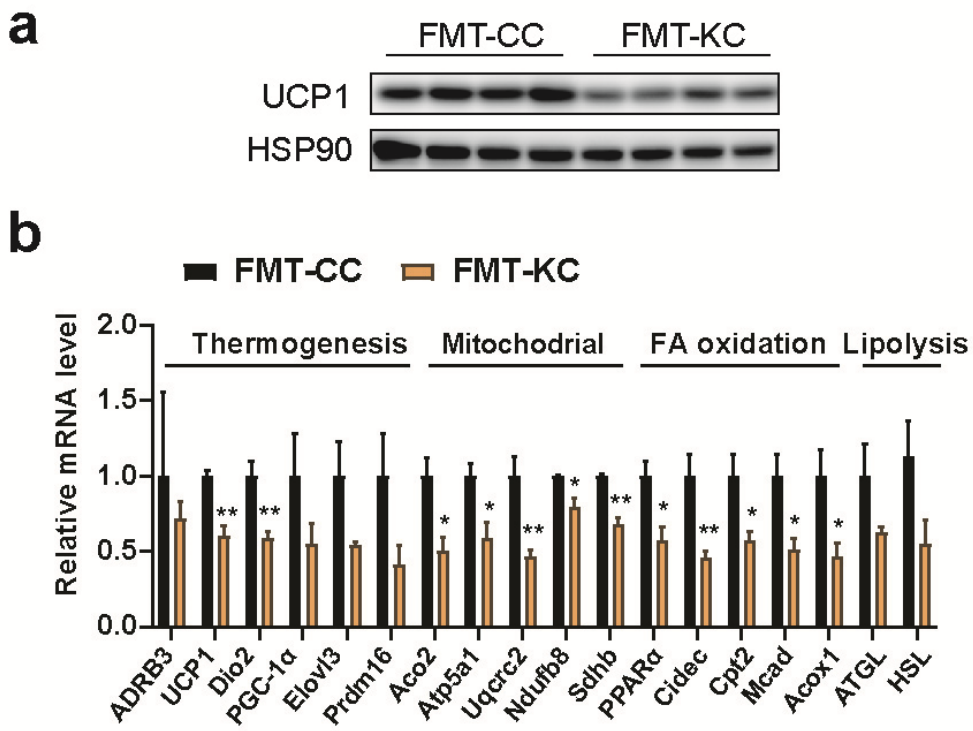


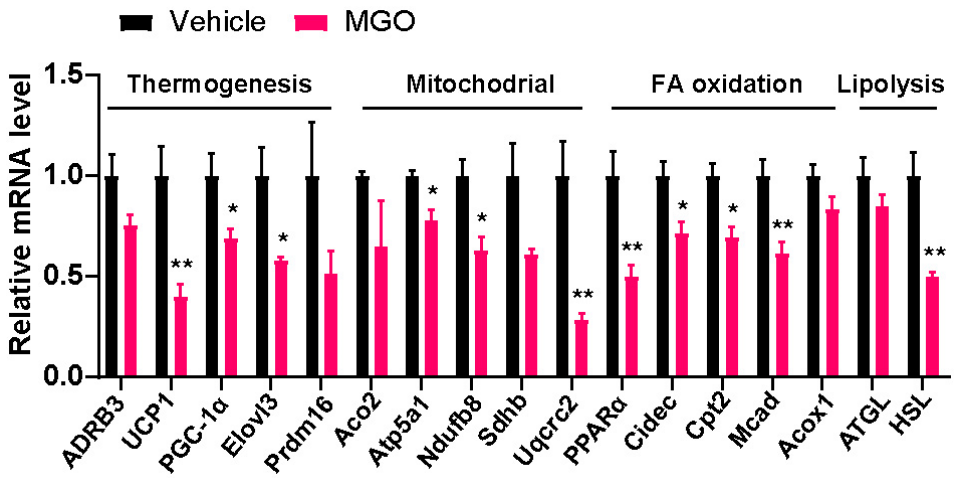
Supplementary Fig. 1 Metabolic phenotypes of AMPK-IKO mice. For a-l, mice were fed ND chow. (a) Western blot analysis of AMPKα1 protein in various tissues of AMPK$^{fl/fl}$ (Control) and AMPK-IKO mice. (b) Body weight of mice from 6-week-old (n=7 for control group and n=6 for IKO group). (c-d) The ratio of fat mass (c) and lean mass (d) to the body weights of mice (n=4). (e-h) Glucose tolerance test (e, f) and insulin tolerance test (g, h) results, including blood glucose levels and the area under the curve (AUC) for 12-week-old mice (n=5). (i-l) VCO2 (i-j) and energy expenditure (k-l) of mice over 24 h (n=5 for control mice and n=4 for IKO mice). For m-t, mice were fed HFD. (m-n) The ratio of fat mass (m) and lean mass (n) to the body weights of mice. (n=11 for control mice and n=9 for IKO mice). (o-r) VCO2 (o-p) and energy expenditure (q-r) of mice (n=4). (s) GLP-1 secretion test in mice at 11 weeks of HFD feeding. (n=4). (t) Gene expression in the livers of mice. (n=8). Values are means ± s.e.m. *P < 0.05, and **P < 0.01 cf. Control mice by two-tailed Student's t-tests.



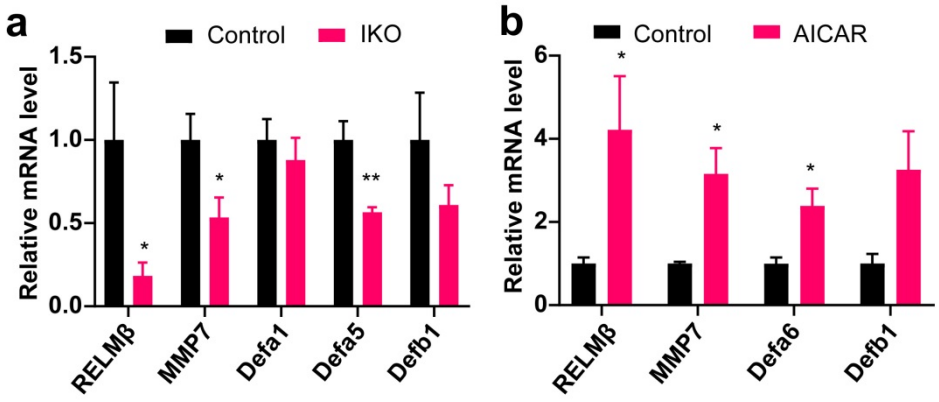
Supplementary Fig. 2 The gut microbiota profile of AMPK-IKO mice is altered. (a) Alpha diversity analysis of gut microbiota in AMPK$^{fl/fl}$ (Control) and AMPK-IKO mice fed ND chow (n=3). (b, c) Bacterial taxon-based analysis at the family level (b) and genus level (c) in mice fed ND chow. (d) NMDS analysis of OTU levels in Control and AMPK-IKO mice fed HFD. (e) Cladogram analysis from the phylum to genus level in Control and AMPK-IKO mice fed HFD. The different color nodes represent the microbial groups with significant enrichment in the corresponding groups. Circles indicate phylogenetic levels from domain to genus. The diameter of each circle is proportional to the abundance of the group. The yellow nodes represent the microbial groups that have no significant difference in different groups Values are means ± s.e.m. $^{**}P < 0.01$ by two-tailed Student's t-test.



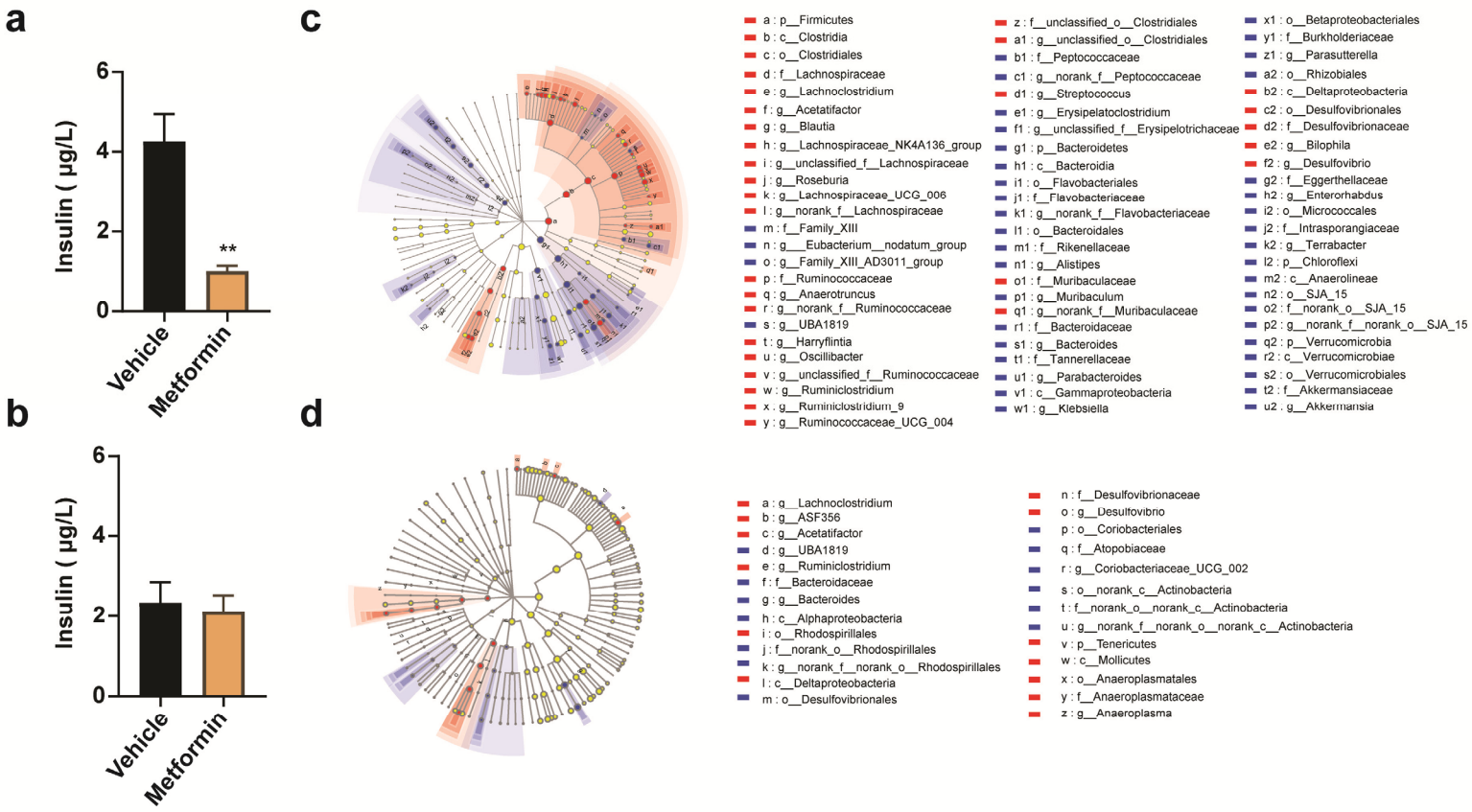
Supplementary Fig. 3 Gut microbiota alterations in AMPK-IKO mice are sufficient to impair BAT thermogenesis. Fresh feces from AMPK$^{fl/fl}$ and AMPK-IKO mice were transplanted to WT recipient mice. FMT-CC, FMT from AMPK$^{fl/fl}$ mice to WT mice; FMT-KC, FMT from AMPK-IKO mice to WT mice. (a) Western blot analysis of UCP1 protein levels in the BAT of recipient mice exposed to cold for 6 h. (b) Relative mRNA levels of genes expressed in the BAT of recipient mice exposed to cold for 6 h (n=3 for FMT-CC group and n=4 for FMT-KC group). Values are means ± s.e.m. **P* < 0.05, ***P* <0.01 by two-tailed Student's t-tests.



Supplementary Fig. 4 Methylglyoxal treatment reduces the expression of functional genes in brown adipocyte. Gene expression in mature HIB1B cell line treated with 50 μM methylglyoxal (n=4). Values are means ± s.e.m. *P < 0.05 by two-tailed Student's t-tests.



Supplementary Fig. 5 Intestinal AMPK regulates the expression of AMPs. Relative mRNA levels of AMPs in (a) the jejunum of AMPK$^{fl/fl}$ (Control) and AMPK-IKO mice fed ND chow (n=6) and (b) HT-29 cells treated with 500 μM of AICAR (n=4). Values are means ± s.e.m. **P* < 0.05, ***P* < 0.01, ****P* < 0.001 by two-tailed Student's t-tests.



Supplementary Fig. 6 Intestinal AMPK is required for the metabolic benefits of metformin. DIO AMPK$^{fl/fl}$ and AMPK-IKO mice were orally garaged with metformin (100 mg/kg) once daily for 8 weeks. (a, b) Fasting serum levels of insulin in AMPK$^{fl/fl}$ mice (a) and AMPK-IKO mice (b) (n=5). (c, d) Cladogram analysis from the phylum to genus level in AMPK$^{fl/fl}$ mice (c) and AMPK-IKO mice (d) (n=5). The different color nodes represent the microbial groups with significant enrichment in the corresponding groups. Circles indicate phylogenetic levels from domain to genus. The diameter of each circle is proportional to the abundance of the group. The yellow nodes represent the microbial groups that have no significant difference in different groups. Values are means ± s.e.m. ***P* <0.01 by two-tailed Student's t-tests.

Supplementary Table 1 Characteristics of patients who provided experimental samples.

	Age	Gender	BMI	HbA1c (%)
Non-obese Non-diabetic	53	M	28.0	5.3
	52	M	20.7	5.0
	54	M	24.0	5.8
	47	F	22.4	5.5
	50	F	23.0	5.6
Obese with T2D	50	M	36.6	8.7
	46	F	33.0	10.7
	50	F	35.0	9.9
	51	M	37.5	6.6
	58	M	40.0	7.0

Supplementary Table 2. Sequences of primers for qPCR.

Name	Forward primer (5'-3')	Reverse primer (5'-3')
m_Ucp1	GTACACCAAGGAAGGACCGA	TTTATTCGTGGTCTCCCAGC
m_ADRB3	GGCCCTCTCTAGTTCCCAG	TAGCCATCAAACCTGTTGAGC
m_IRF4	TCCGACAGTGGTTGATCGAC	CCTCACGATTGTAGTCCTGCTT
m_Dio2	AATTATGCCTCGGAGAAGACCG	GGCAGTTGCCTAGTGAAAGGT
m_PGC-1α	TATGGAGTGACATAGAGTGTGCT	CCACTTCAATCCACCCAGAAAG
m_Elovl3	TTCTCACGCGGGTTAAAAATGG	GAGCAACAGATAGACGACCAC
m_Cox8	TGTGGGGATCTCAGCCATAGT	AGTGGGCTAAGACCCATCCTG
m_Prdm16	CCAAGGCAAGGGCGAAGAA	AGTCTGGTGGGATTGGAATGT
m_Aco2	ATCGAGCGGGGAAAGACATAC	TGATGGTACAGCCACCTTAGG
m_Atp5a1	TCTCCATGCCTCTAACACTCG	CCAGGTCAACAGACGTGTCAG
m_Ndufb8	TGTTGCCGGGGTCATATCCTA	AGCATCGGGTAGTCGCCATA
m_Sdhb	AATTTGCCATTTACCGATGGGA	AGCATCCAACACCATAGGTCC
m_Uqcrc2	AAAGTTGCCCCGAAGGTTAAA	GAGCATAGTTTTCCAGAGAAGCA
m_Pparα	AGAGCCCCATCTGTCCTCTC	ACTGGTAGTCTGCAAAACCAAA
m_Cidec	ATGGACTACGCCATGAAGTCT	CGGTGCTAACACGACAGGG
m_Cpt2	CAGCACAGCATCGTACCCA	TCCCAATGCCGTTCTCAAAAT
m_Acox1	TAACTTCCTCACTCGAAGCCA	AGTTCCATGACCCATCTCTGTC
m_Mcad	AGGGTTTAGTTTTGAGTTGACGG	CCCCGCTTTTGTCATATTCCG
m_ATGL	GGATGGCGGCATTTCAGACA	CAAAGGGTTGGGTTGGTTCAG
m_Scd1	TTCTTGCGATACACTCTGGTGC	CGGGATTGAATGTTCTTGTCGT
m_36B4	AGATTCGGGATATGCTGTTGGC	TCGGGTCCTAGACCAGTGTTC
m_Reg3γ	ATGCTTCCCCGTATAACCATCA	GGCCATATCTGCATCATACCAG
m_Reg3β	ACTCCCTGAAGAATATACCCTCC	CGCTATTGAGCACAGATACGAG
m_RELMβ	AAGCCTACACTGTGTTTCCTTTT	GCTTCCTTGATCCTTTGATCCAC
m_Defb1	AGGTGTTGGCATTCTCACAAG	GCTTATCTGGTTTACAGGTTCCC
m_MMP7	CTGCCACTGTCCCAGGAAG	GGGAGAGTTTTCCAGTCATGG
m_Defa1	TCAAGAGGCTGCAAAGGAAGAGAAC	TGGTCTCCATGTTCAGCGACAGC
m_Defa5	TCAAAAAAGCTGATATGCTATTG	AGCTGCAGCAGAATACGAAAG
h_Reg3a	AGCTACTCATACGTCTGGATTGG	CACCTCAGAAATGCTGTGCTT
h_RELMβ	CCGTCCTCTTGCCTCCTTC	CTTTTGACACTAGCACACGAGA
h_Defb1	ATGAGAACTTCCTACCTTCTGCT	TCTGTAACAGGTGCCTTGAATTT
h_Defa6	CTGAGCCACTCCAAGCTGAG	GTTGAGCCCAAAGCTCTAAGAC
h_MMP7	GAGTGAGCTACAGTGGGAACA	CTATGACGCGGGAGTTTAACAT
h_36B4	AACATGCTCAACATCTCCCC	CCGACTCCTCCGACTCTTC