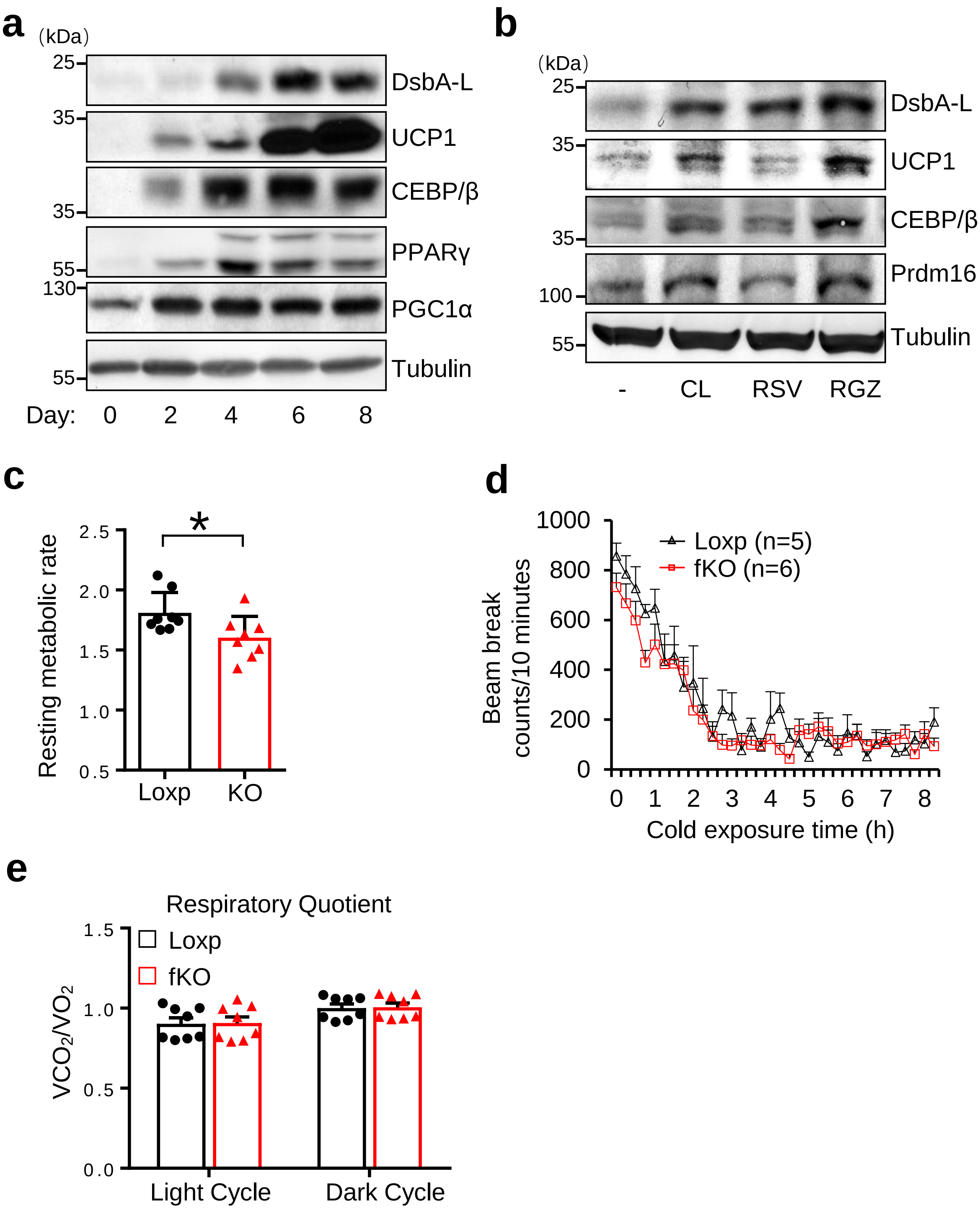
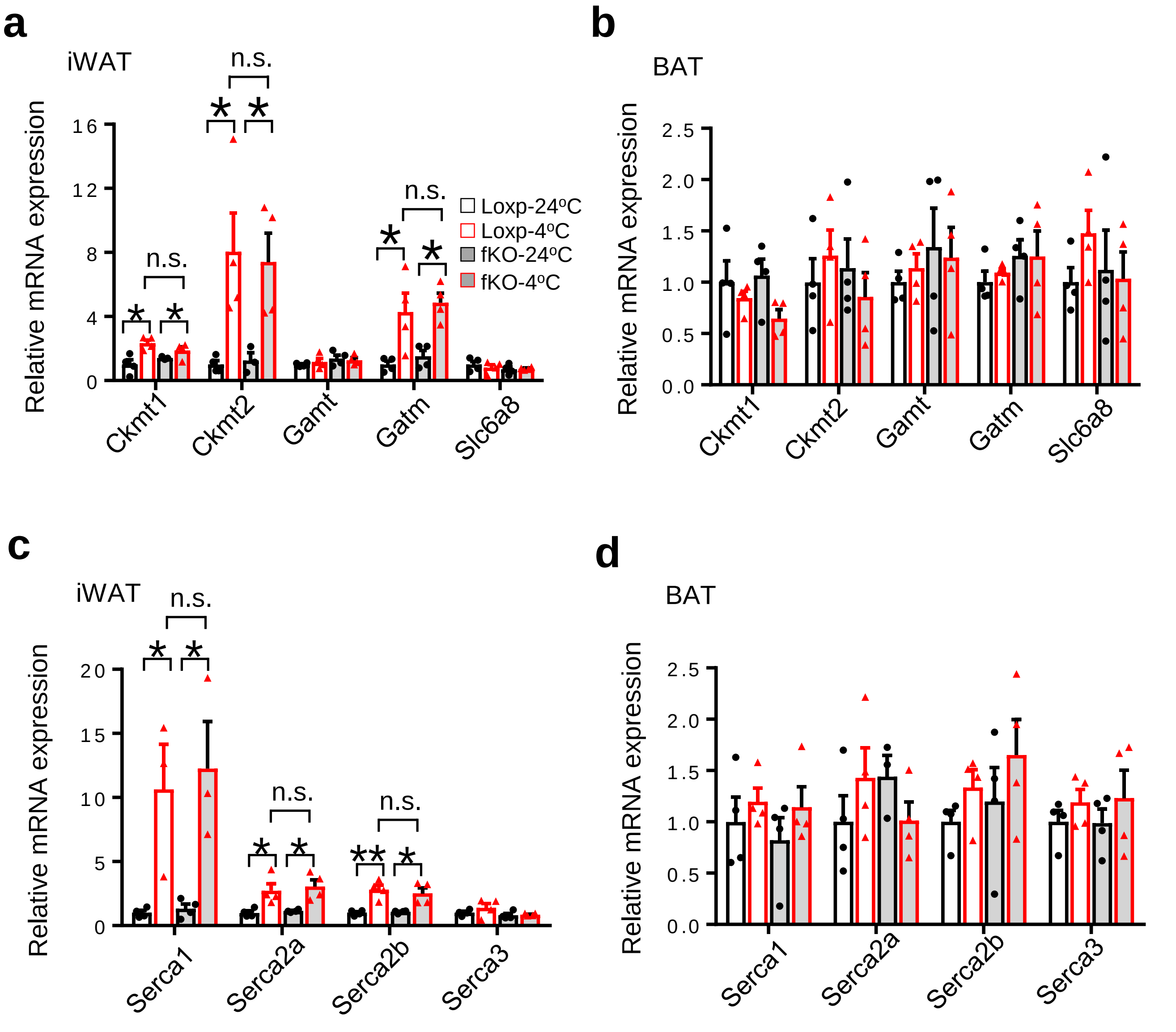
Supplementary Fig. 1.



Supplementary Figure 1. DsbA-L is positively correlated with thermogenic gene expression and resting metabolic rate.

a Immunoblot analysis of DsbA-L, UCP1, C/EBPβ, PPARγ and PGC1α expression during brown adipocyte differentiation. **b** Immunoblot analysis of DsbA-L, UCP1, C/EBPβ and Prdm16 expression in brown adipocytes treated with CL316243, resveratrol or rosiglitazone for 16 hr. **c** Resting metabolic rate of DsbA-L^{fKO} and loxp control mice. **d** The activities of DsbA-L^{fKO} and loxp control mice (n=8 for each group) exposed to cold (4^{o}C) under the feeding conditions at different time points as indicated. **e** The respiratory quotient of DsbA-L^{fKO} and loxp control mice (n=8 for each group) was measured during a 48 hr period, including two light/dark cycles. Data are presented as mean ± SEM of biologically independent samples, *p < 0.05 by unpaired two-tailed t-test.

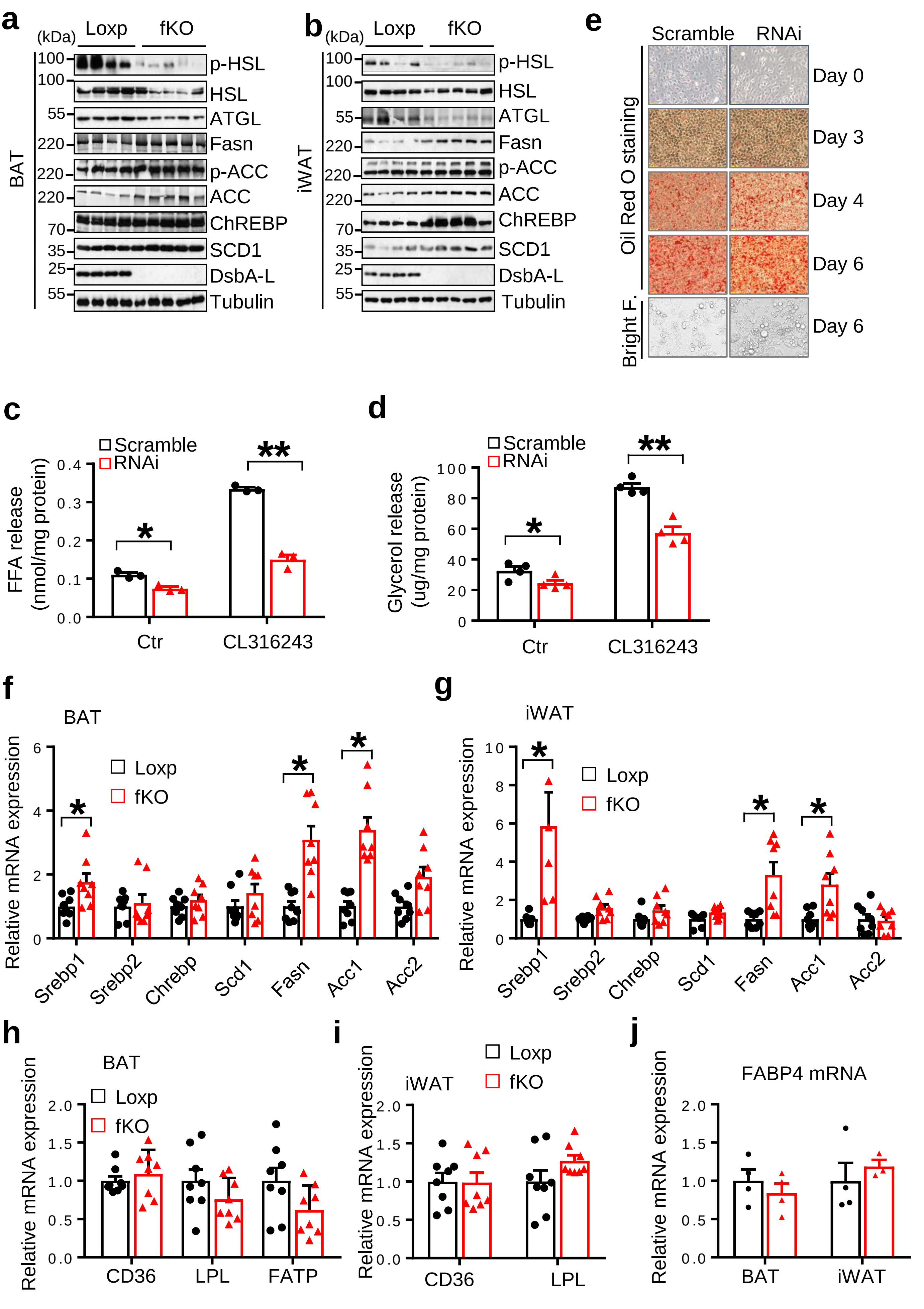
Supplementary Fig. 2.



Supplementary Figure 2. Fat-specific knockout of DsbA-L has no effect on creatine and calcium cycle-related gene expression in mice.

a-b The mRNA levels of creatine-metabolism-related genes in (**a**) iWAT and (**b**) BAT of DsbA-L^{fKO} and loxp control mice (n=4 for each group) were determined by qPCR and normalized to β-actin. **c-d** The mRNA levels of calcium cycle-related genes in (**c**) iWAT and (**d**) BAT of DsbA-L^{fKO} and loxp control mice (n=8 for each group) were determined by qPCR and normalized to β-actin. Data are presented as mean ± SEM of biologically independent samples, *p < 0.05 and **p < 0.01 by one-way ANOVA.

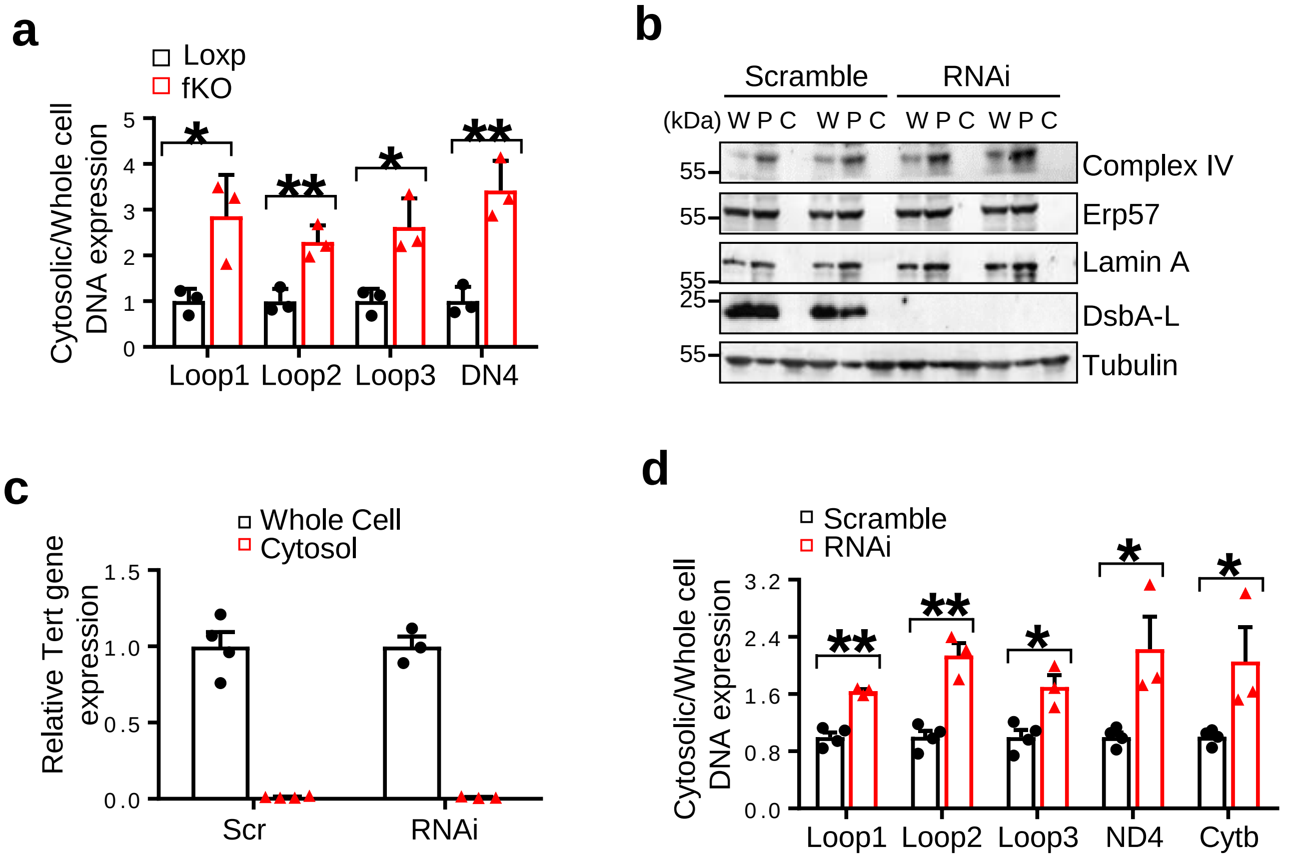
Supplementary Fig. 3.



Supplementary Figure 3. Fat-specific knockout of DsbA-L impairs lipid metabolism.

a-b Immunoblot analysis of the expression of lipid metabolism-related genes such as ATGL, FASN, ChREBP, SCD1 and the phosphorylation of ACC and HSL in (**a**) BAT and (**b**) iWAT of DsbA-L^{fKO} (n=4) and loxp control (n=5) mice. **c** Free fatty acid and **d** glycerol release from DsbA-L-suppressed brown adipocytes treated with or without 1μM CL316243 (n=3 for each group) for 16 hr. **e** Representative Oil Red O stain or bright field photo during DsbA-L-suppressed brown adipocytes or scramble control cells differentiation. **f-g** The mRNA levels of lipid synthesis genes in (**f**) BAT and (**g**) iWAT of DsbA-L^{fKO} and loxp control mice (n=8 for each group). **h-i** The mRNA levels of lipid uptake-related genes in (**h**) BAT and (**i**) iWAT of DsbA-L^{fKO} and loxp control mice (n=8 for each group) were determined by qPCR and normalized to β-actin. **j** The mRNA levels of FABP4 in BAT and iWAT of DsbA-L^{fKO} and loxp control mice (n=4 for each group). Data are presented as mean ± SEM of biologically independent samples, $^*p < 0.05$ and $^{**}p < 0.01$ by unpaired two-tailed t-test.

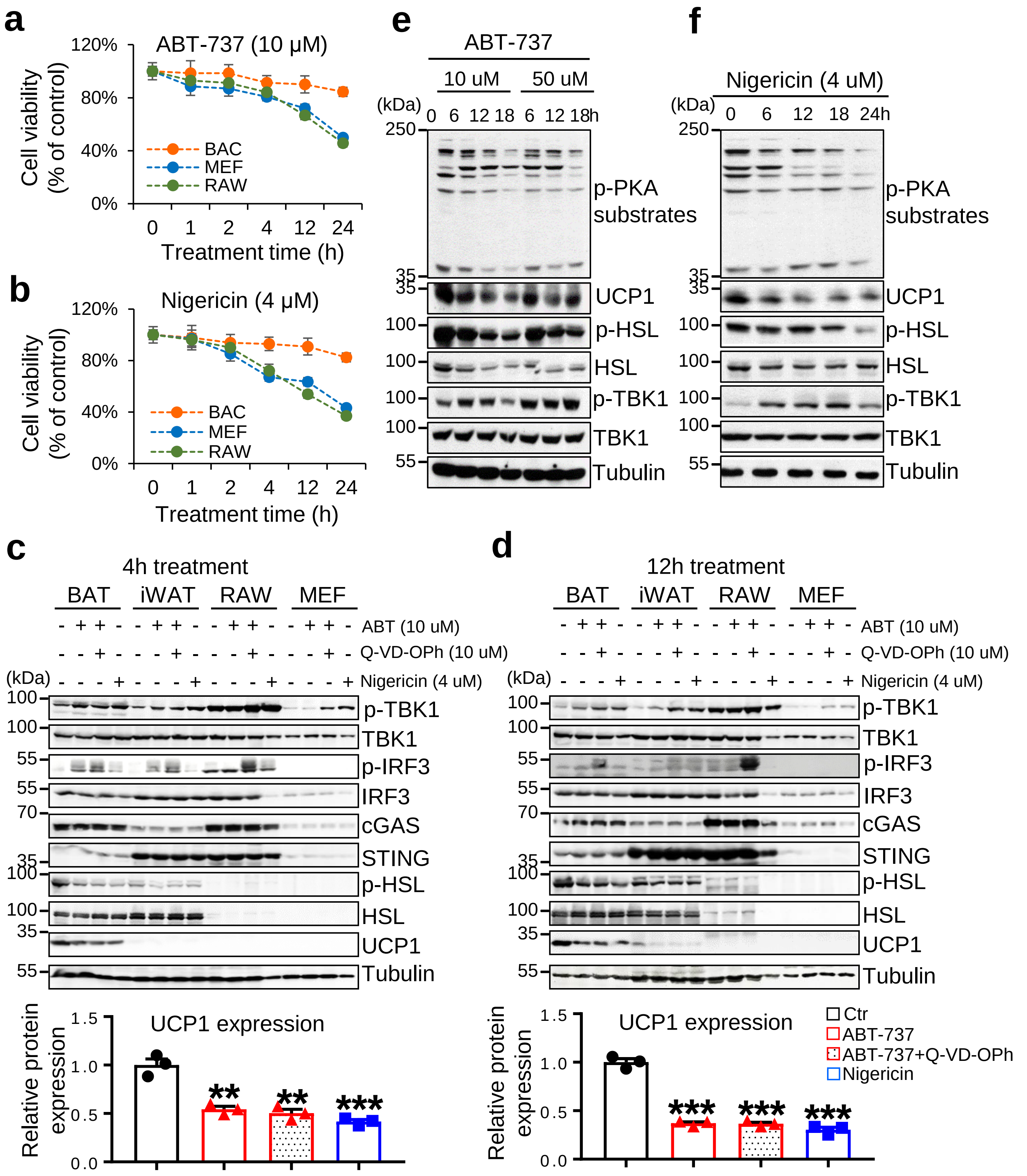
Supplementary Fig. 4.



Supplementary Figure 4. DsbA-L deficiency results in increased mtDNA release into the cytosol in brown adipocytes.

a Cytosolic mtDNA content was quantitated via qPCR using mtDNA primers (Dloop1-3 and mtND4) in primary brown adipocytes from DsbA-L^{fKO} and loxp control mice (n=3 for each group). **b** Scramble and DsbA-L-suppressed brown adipocytes were subjected to digitonin fractionation. Whole-cell extracts (W), pellets (P) and cytosolic extracts (C) were immunoblotted using indicated antibodies. **c** The expression of nuclear-encoded Tert gene in whole-cell and cytosolic extract was measured from scramble (n=4) and DsbA-L-suppressed (n=3) brown adipocytes, indicating the high purity of cytosol fraction. **d** Cytosolic mtDNA content was quantitated via qPCR using mtDNA primers (Dloop1-3, mtND4 and Cytb) in brown scramble (n=4) and DsbA-L-suppressed (n=3) adipocytes. Data are presented as mean ± SEM of biologically independent samples, *p < 0.05 and **p < 0.01 by unpaired two-tailed t-test.

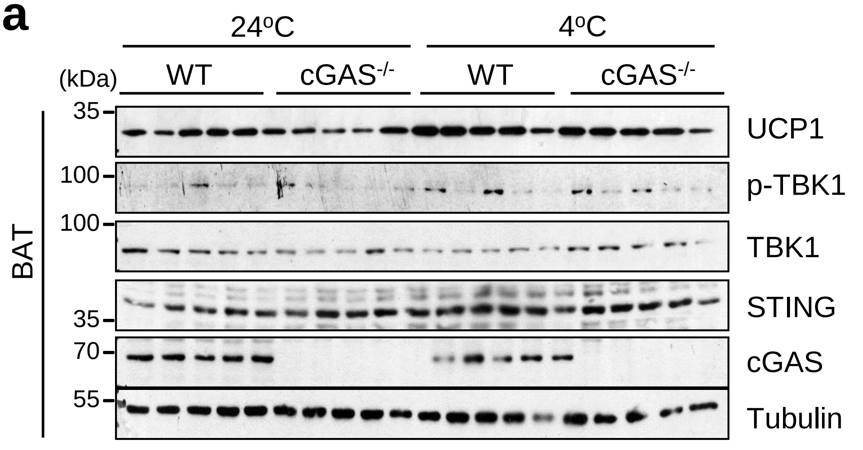
Supplementary Fig. 5.



Supplementary Figure 5. mtDNA release-induced activation of the cGAS-STING pathway inhibits PKA signaling in brown adipocytes.

a-b MTT assay indicated cell viability of brown adipocytes (BAC), MEF and RAW264.7 cells treated with (**a**) 10 μM ABT-737 or (**b**) 4 μM nigericin at different time points as indicated. **c-d** Immunoblot analysis of the phosphorylation of TBK1, IRF3, HSL and UCP1, cGAS, STING expression in primary brown adipocytes, primary inguinal adipocytes, RAW264.7 and MEF cells treated with 4 μM nigericin or 10 μM ABT-737 in the presence or absence of Q-VD-OPh for (**c**) 4h or (**d**) 12 hr (n=3 for each group). The data was semi-quantified by Image J program. **e** Immunoblot analysis of UCP1 expression and the phosphorylation of PKA substrates, HSL, TBK1 and IRF3 in brown adipocytes treated with 10 μM or 50 μM ABT-737 at different time points as indicated. **f** Immunoblot analysis of UCP1 expression and the phosphorylation of PKA substrates, HSL, TBK1 and IRF3 in brown adipocytes treated with 4 μM nigericin at different time points as indicated.

a



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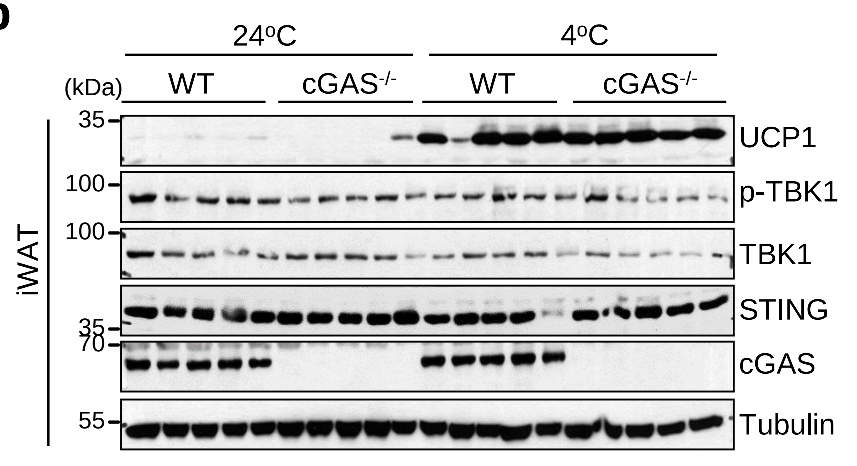


Supplementary Figure 6. Knockout of cGAS has no effect on UCP1 expression in mice. **a** Immunoblot analysis of UCP1, cGAS, STING expression and the phosphorylation of TBK1 in BAT from wild-type and cGAS knockout mice exposed to cold (4ºC) or housed at room temperature (24ºC) (n=5/group). **b** Immunoblot analysis of UCP1, cGAS, STING expression and the phosphorylation of TBK1 in iWAT from wild-type and cGAS knockout mice exposed to cold (4ºC) or housed at room temperature (24ºC) (n=5 for each group).

Fig. 1c

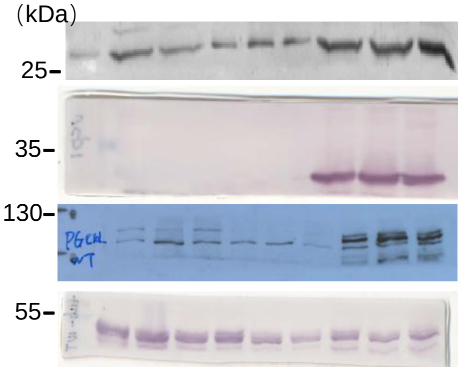


Fig. 2c

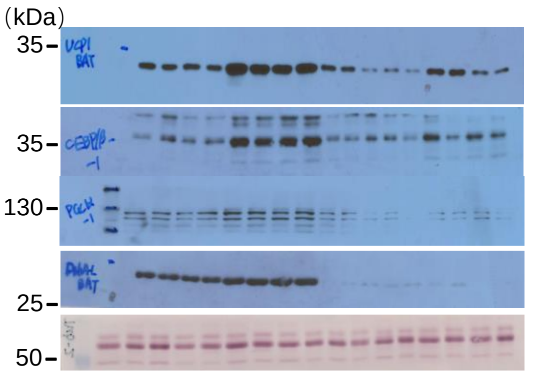


Fig. 2d

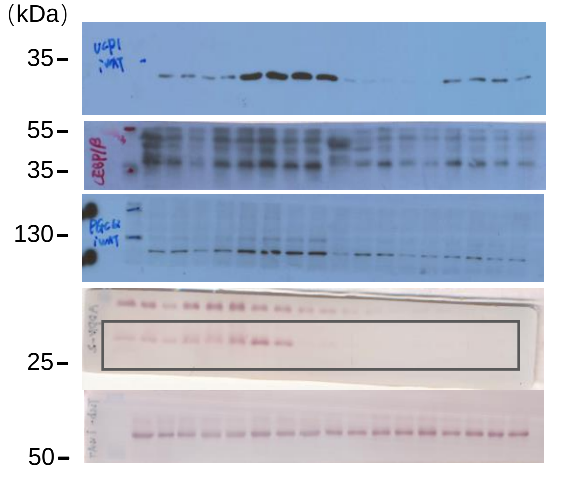


Fig. 2f

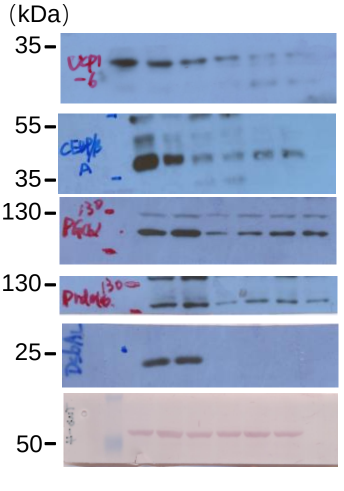


Fig. 2g

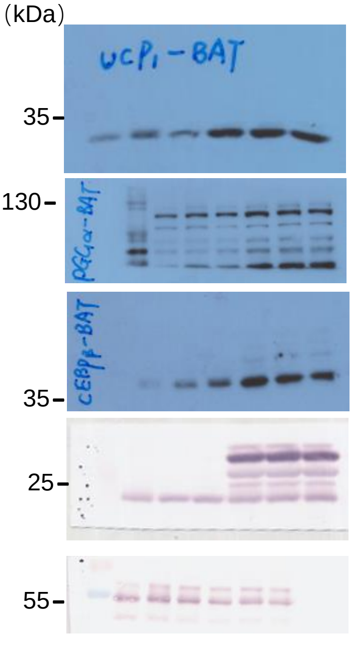


Fig. 2h

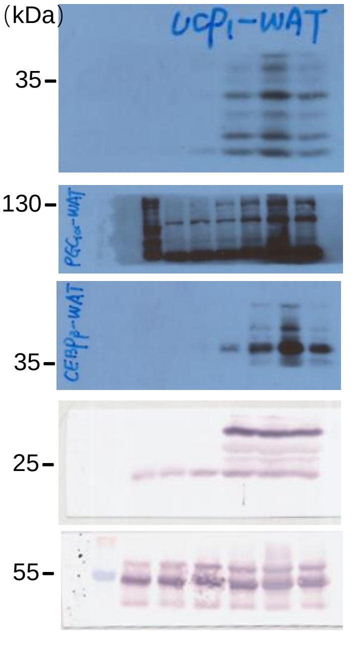


Fig. 4b

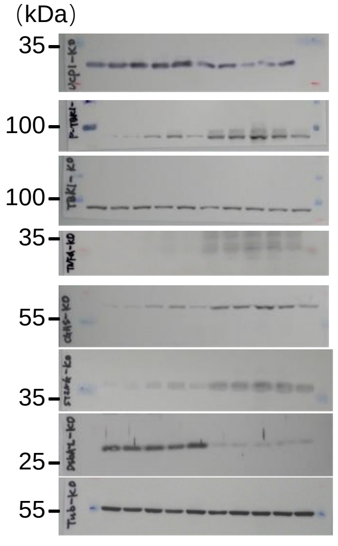


Fig. 4c

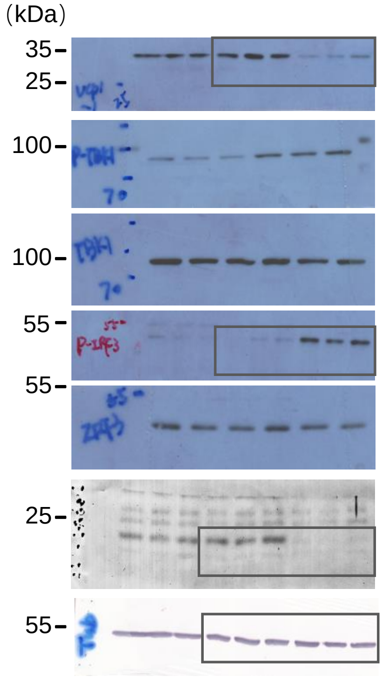


Fig. 4e

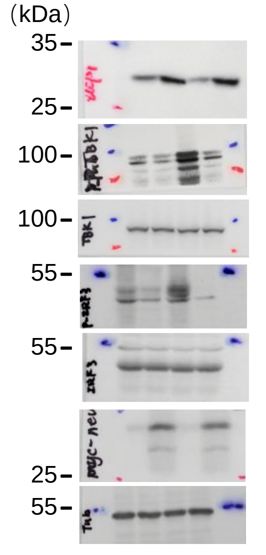


Fig. 4f & Fig. 4g

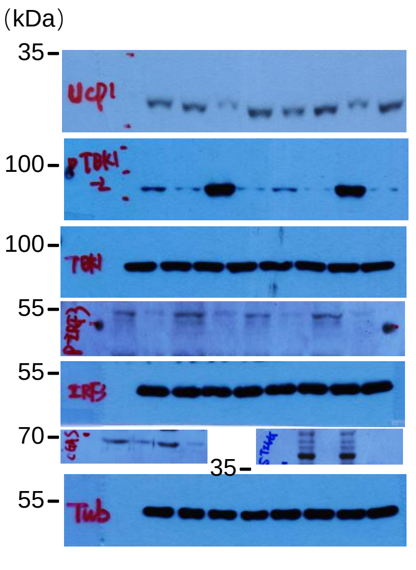


Fig. 4h

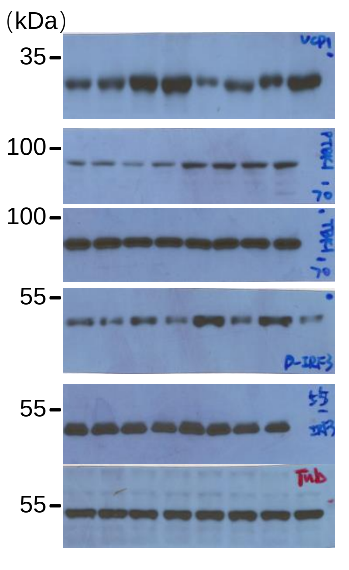


Fig. 4i

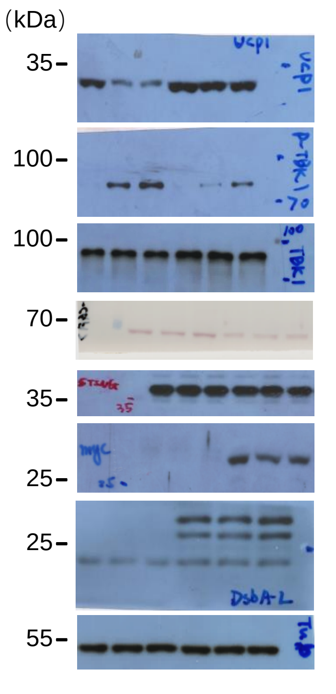


Fig. 5a

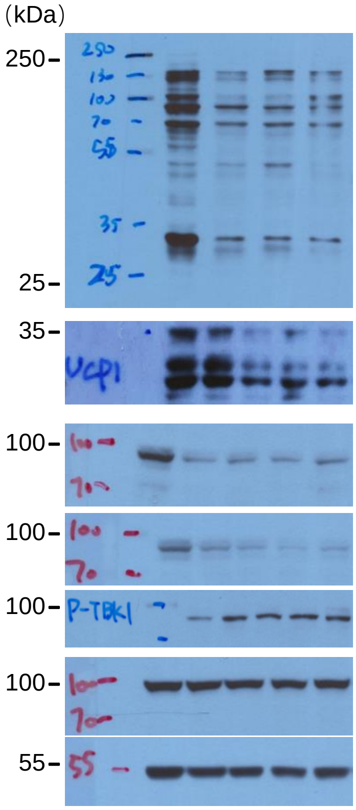


Fig. 5b

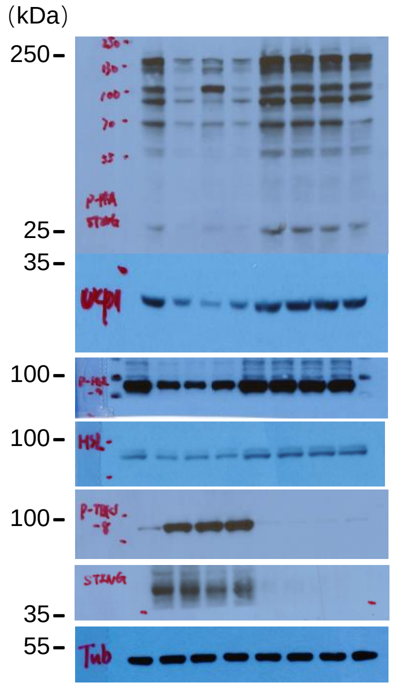


Fig. 5c

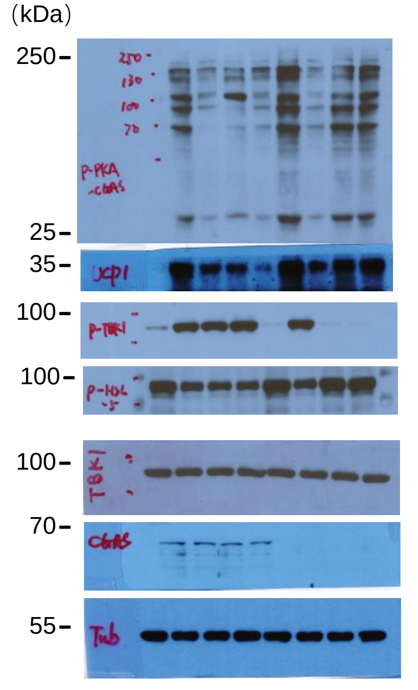


Fig. 5d & Fig. 5e

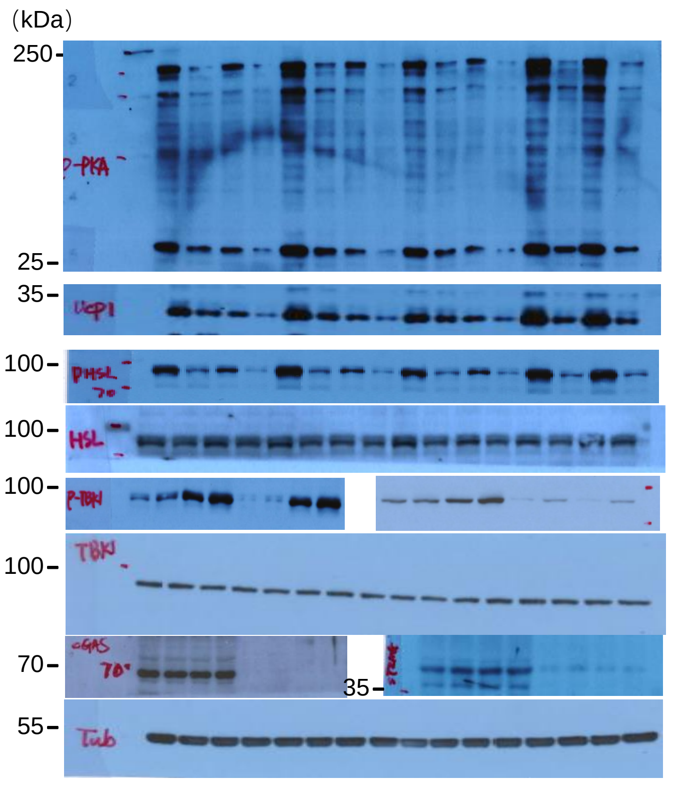


Fig. 5f

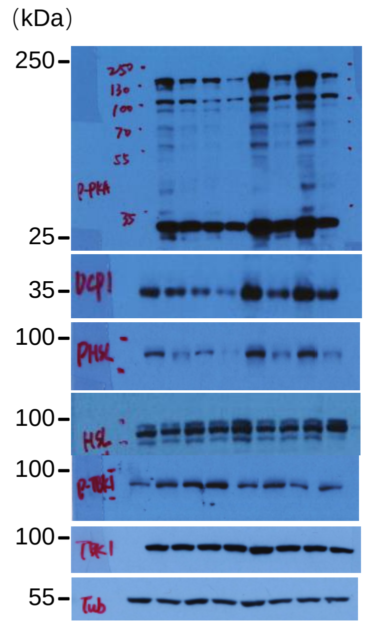
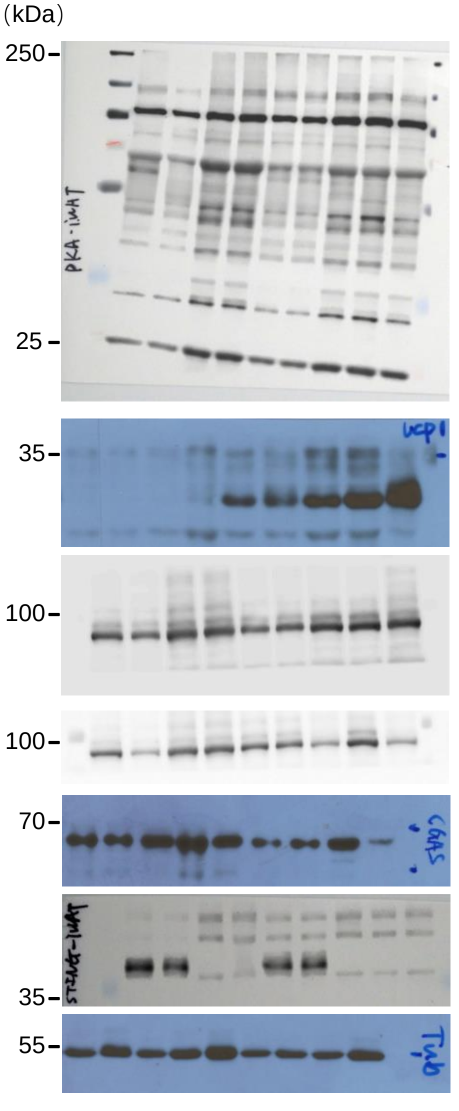


Fig. 6a



(kDa)
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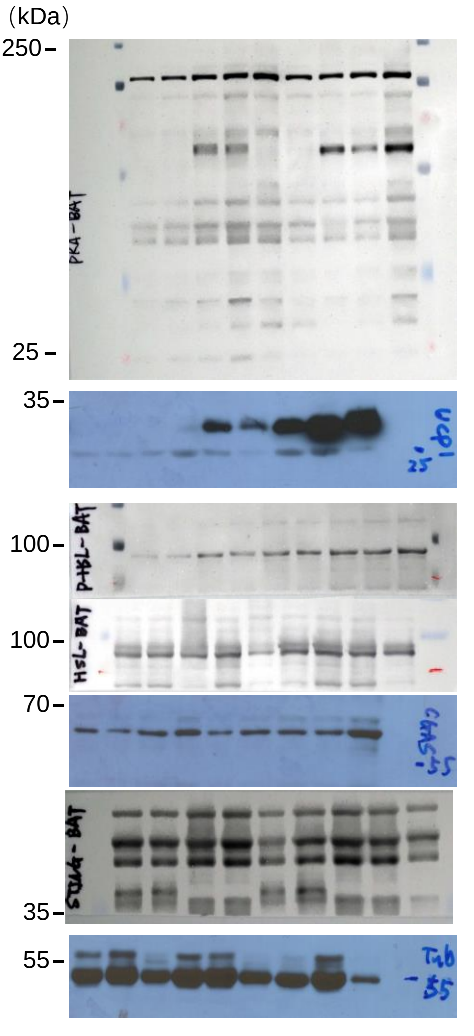
25
35
100
100
70
35
55
PKA-iWAT
UCP1
CGAS
STING-iWAT
Tub

Fig. 6b



(kDa)
250
25
35
100
100
70
35
55
PKA-BAT
UCP1
P-HSL-BAT
HSL-BAT
CGAS
STING-BAT
Tub

Fig. 6d

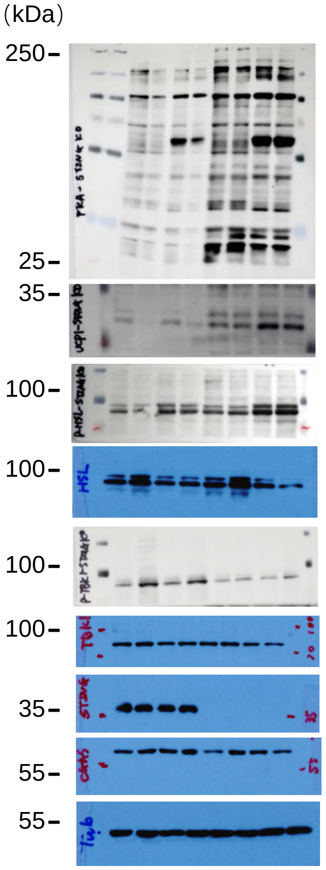


Fig. 6e

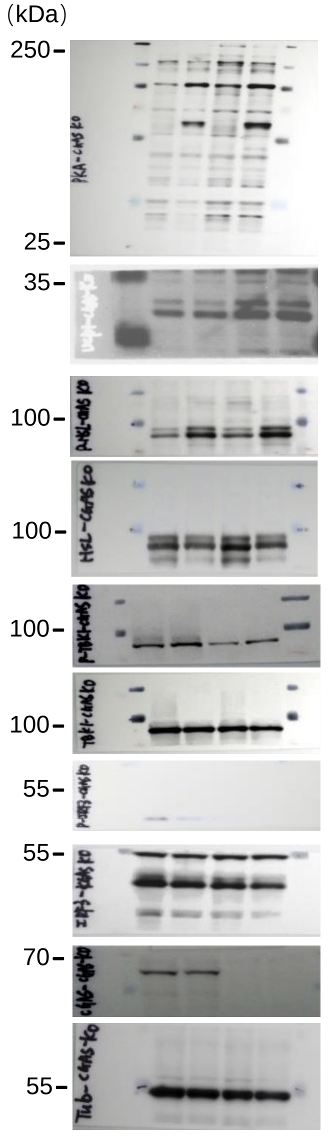
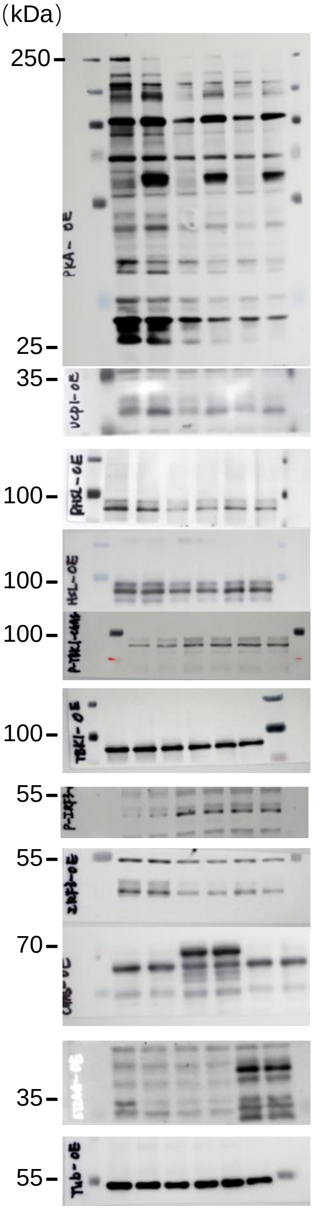
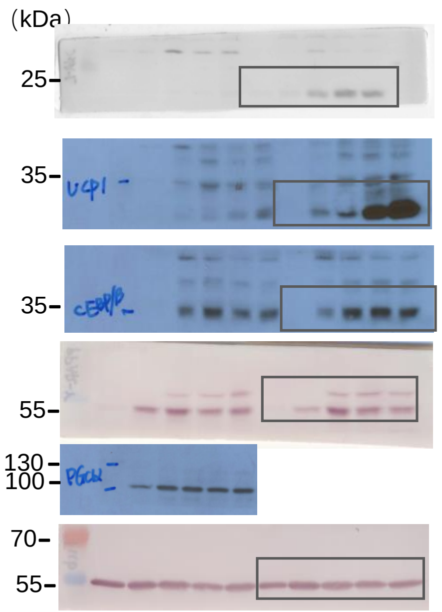


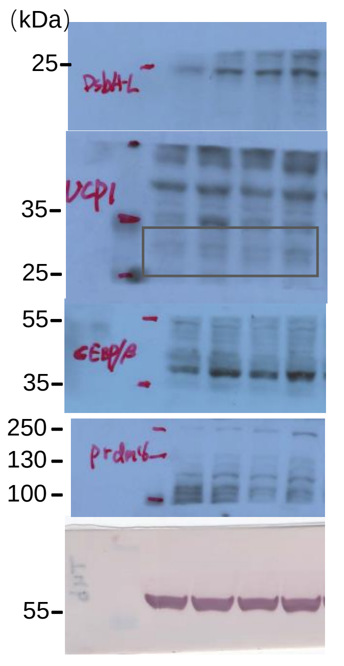
Fig. 6h



Suppl Fig.1a



Suppl Fig.1b



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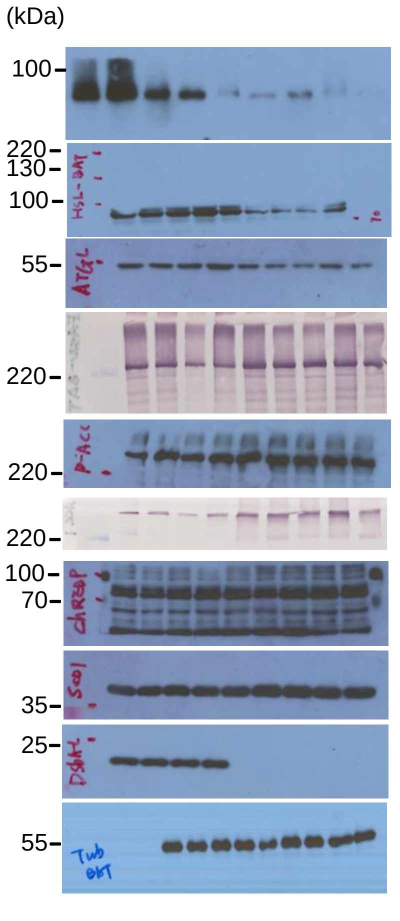
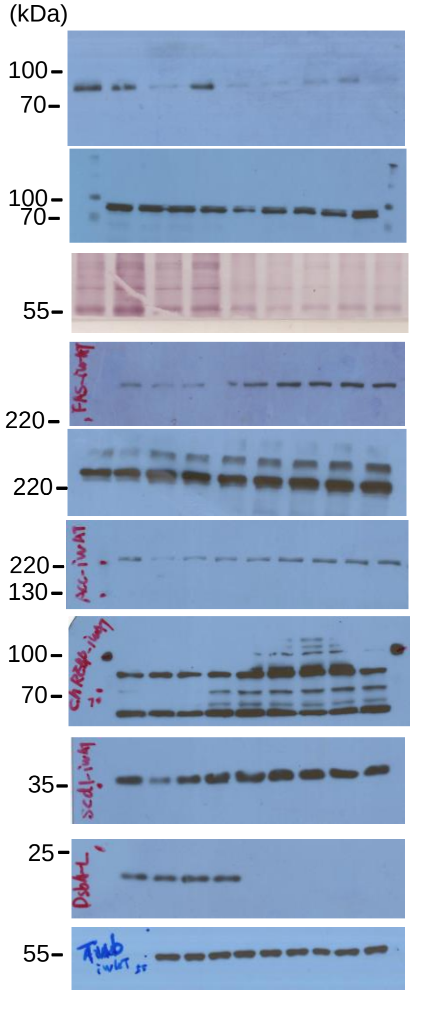


Suppl Fig.3b



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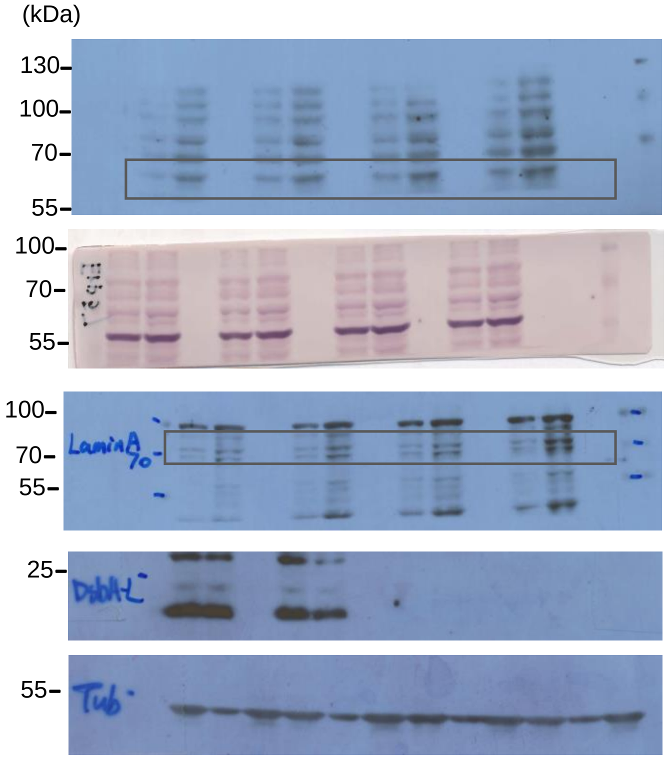

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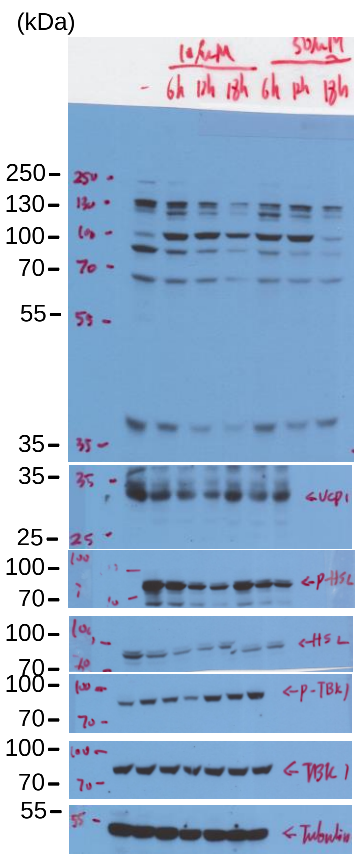

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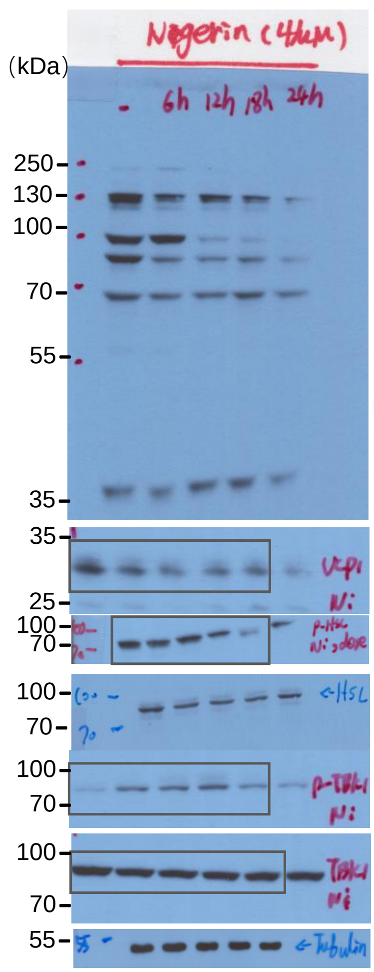

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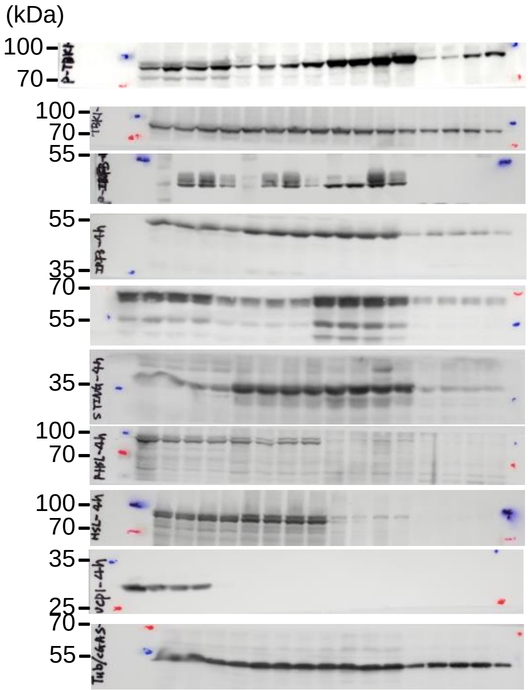

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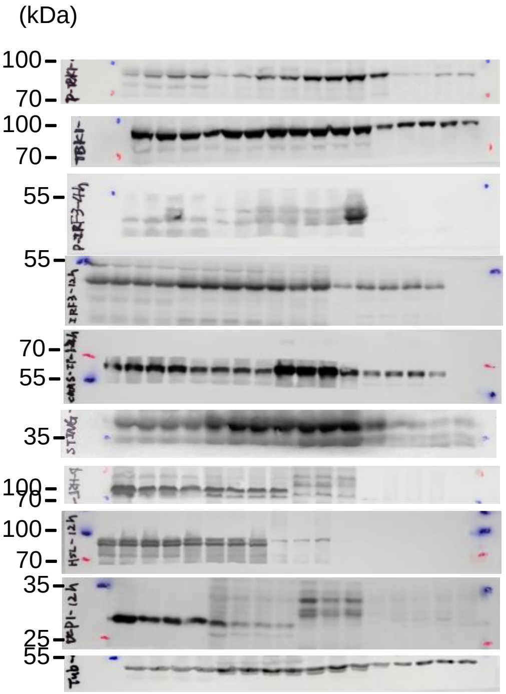

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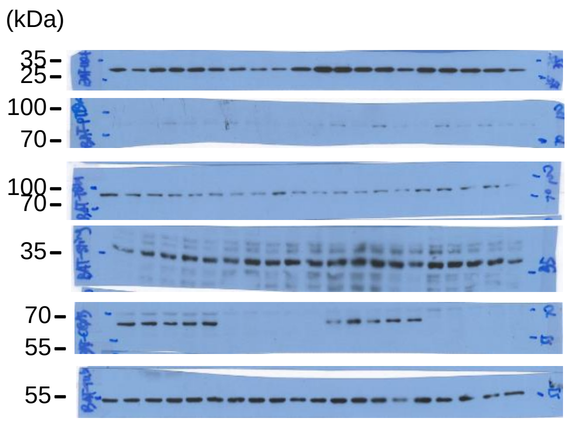

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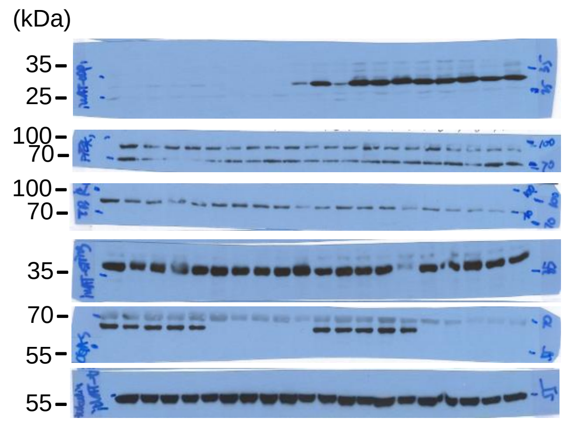

Supplementary Table 1: Primer pair sequences

Gene	Primer	Sequence	Gene	Primer	Sequence
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	Reverse	5'-CAACAGTGGTGGGTAGCG-3'		Reverse	5'-TTGCGATGTCTCCAGAAGTG-3'
UCP1	Forward	5'-AAGACAGAAGAGCATAGCATTCAC-3'	SREBP2	Forward	5'-AAGCTGGGCGATGGATGAG-3'
	Reverse	5'-CCAGTCATACACTCCCACCTC-3'		Reverse	5'-ATCTCGTCGATGTCCCCG-3'
C/EBPβ	Forward	5'-TTCCTCTCCGACCTCTTC-3'	ChREBP	Forward	5'-CTGGGGACCTAAACAGGAGC-3'
	Reverse	5'-GCTCACGTAACCGTAGTC-3'		Reverse	5'-GAAGCCACCCTATAGCTCCC-3'
PGC1α	Forward	5'-CCGAAGACACTACAGGTTCCATAG-3'	SCD1	Forward	5'-CGCTGGCACATCAACTTCAC-3'
	Reverse	5'-GGGAGGGAGAGAGGAGAGAGG-3'		Reverse	5'-CCCTGTGTGTACTGGCCTTT-3'
Prdm16	Forward	5'-TGAGGAAGCATTTGAAGTTAAAG-3'	FASN	Forward	5'-TCGTCTATACCACTGCTTACTAC-3'
	Reverse	5'-GTTCTTAGCCTGCCTGTAC-3'		Reverse	5'-ACACCACCTGAACCTGAG-3'
PPARγ	Forward	5'-TGTGGACCTCTCCGTGATGG-3'	ACC1	Forward	5'-TGCCACCACCTTATCACTATGTA-3'
	Reverse	5'-GGTTCTACTTTGATCGCACTTTGG-3'		Reverse	5'-CCTGCCTGTCTCCATCCA-3'
Tbx-1	Forward	5'-GCGGAAGGAAGTGGTATT-3'	ACC2	Forward	5'-CCAACAGTAAGGTGGAAGCC-3'
	Reverse	5'-CTCTCTCGGTCGTCTACA-3'		Reverse	5'-CAGGGAGTTTCCTCTGCTGAC-3'
Tmem26	Forward	5'-GTCTCTACAACCTCCTGCTCTG-3'	CD36	Forward	5'-ATTCCCTTGGCAACCAACCA-3'
	Reverse	5'-TGTGCTATGCCGTTCTGTCTAC-3'		Reverse	5'-TACGTGGCCCGGTTCTACTA-3'
Cidea	Forward	5'-CCAAGGTCGGGTCAAGTCGTC-3'	LPL	Forward	5'-GGCTGACACTGGACAAACAAA-3'
	Reverse	5'-CGTAGTCCCTGGCGGTCTCC-3'		Reverse	5'-CCTGGGTTAGCCACCGTTTA-3'
β-actin	Forward	5'-GTTGGTTGGAGCAAACATC-3'	FATP5	Forward	5'-AGGACCAGCTGCATCCTTC-3'
	Reverse	5'-CTTATTTCATGGATACTTGGAATG-3'		Reverse	5'-TCTCCTACGCGTCGTACATTC-3'
HSL	Forward	5'-TGTGTCAGTGCCTATTCAG-3'	mt-DNA Loop1	Forward	5'-AATCTACCATCCTCCGTGAAACC-3'
	Reverse	5'-GAACAGCGAAGTGTCTCT-3'		Reverse	5'-TCAGTTTAGCTACCCCCAAGTTTAA-3'
ATGL	Forward	5'-GCTGTGGAATGAGGACATAGGA-3'	mt-DNA Loop2	Forward	5'-CCCTTCCCCATTTGGTCT-3'
	Reverse	5'-GCATAGTGAGTGGCTGGTGAA-3'		Reverse	5'-TGGTTTCACGGAGGATGG-3'
CPT1	Forward	5'-ACTCCGCTCGCTCATTCCG-3'	mt-DNA Loop3	Forward	5'-TCCTCCGTGAAACCAACAA-3'
	Reverse	5'-CACACCCACCACCACGATAA-3'		Reverse	5'-AGCGAGAAGAGGGGCATT-3'
MCAD	Forward	5'-GATCGCAATGGGTGCTTTTGATAGAA-3'	mt-ND4	Forward	5'-AACGGATCCACAGCCGTA-3'
	Reverse	5'-AGCTGATTGGCAATGTCTCCAGCAAA-3'		Reverse	5'-AGTCCTTCGGGCCATGATT-3'
HMGCS2	Forward	5'-ATACCACCAACGCCTGTTATGG-3'	CYTB	Forward	5'-GCTTTCCACTTCATCTTACCATTTA-3'
	Reverse	5'-CAATGTCACCACAGACCAG-3'		Reverse	5'-TGTTGGGTTGTTTGATCCTG-3'
PPARα	Forward	5'-TCGCTATCCAGGCAGAAG-3'	Tert	Forward	5'-CTAGCTCATGTGTCAAGACCCTCTT-3'
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Ckmt1	Forward	5'-TGAGGAGACCTATGAGGTATTTGC-3'	Ckmt2	Forward	5'-GCATGGTGGCTGGTGATGAG-3'
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Gamt	Forward	5'-GCAGCCACATAAGGTTGTTCC-3'	Gatm	Forward	5'-GACCTGGTCTTGTGCTCTCC-3'
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Slc6a8	Forward	5'-TGCATATCTCCAAGGTGGCAG-3'	Serca1	Forward	5'-TGTTTGTCCTATTTCGGGGTG-3'
	Reverse	5'-CTACAAACTGGCTGTCCAGA-3'		Reverse	5'-AATCCGCACAAGCAGGTCTTC-3'
Serca2a	Forward	5'-GCTCATTTTCCAGATCACACCG-3'	Serca2b	Forward	5'-ACCTTTGCCGCTCATTTTCCAG-3'
	Reverse	5'-GTTACTCCAGTATTGCGGGTTG-3'		Reverse	5'-AGGCTGCACACACTCTTTACC-3'
Serca3	Forward	5'-GGAGCAGTTTGAGGACCTCTT-3'	Fabp4	Forward	5'-AAG GTG AAG AGC ATC ATA ACC CT-3'
	Reverse	5'-GGCCACGAGAATTAGCATGATG-3'		Reverse	5'-TCA CGC CTT TCA TAA CAC ATT CC-3'