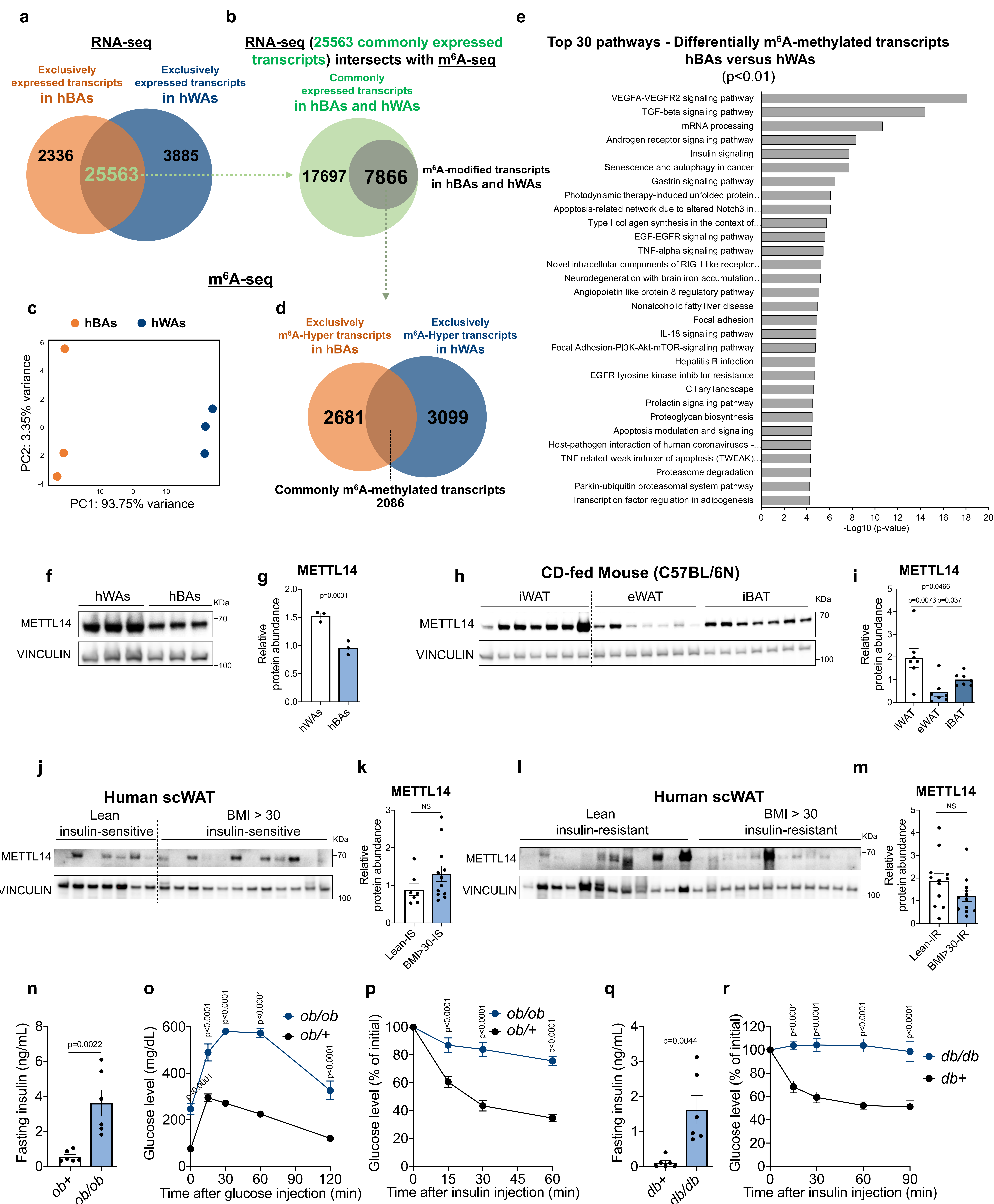


# **Divergent roles of m<sup>6</sup>A in orchestrating brown and white adipocyte transcriptomes and systemic metabolism**

**Xiao L et al.**



**Supplementary Figure 1, related to Figure 1. Brown and White Adipocytes Exhibit Distinct m<sup>6</sup>A Modification Patterns in Their mRNA Landscapes**

(a) Venn diagram representation of commonly or exclusively expressed transcripts in hBAs and hWAs (n = 3)..

(b) Venn diagram representation of m<sup>6</sup>A-modified commonly expressed transcripts in hBAs and hWAs (n = 3)..

(c) Principal component analysis (PCA) of m<sup>6</sup>A-seq data from hBAs and hWAs.

(d) Venn diagram representation of the differentially m<sup>6</sup>A-hypermethylated transcripts in hBAs and hWAs, statistical analyses were performed using the Benjamin-Hochberg procedure and genes were filtered for  $p < 0.01$  and  $FDR < 0.1$ .

(e) Top 30 enriched pathways of differentially m<sup>6</sup>A methylated transcripts in hBAs versus hWAs.

(f and g) Western blots (f) and quantification (g) of METTL14 and VINCULIN in fully differentiated hWAs and hBAs (n = 3).

(h and i) Western blots (h) and quantification (i) of Mettl14 and Vinculin in inguinal white adipose tissue (iWAT), epididymal white adipose tissue (eWAT), and interscapular brown adipose tissue (iBAT) from CD-fed C57BL/6N male mice (n = 7).

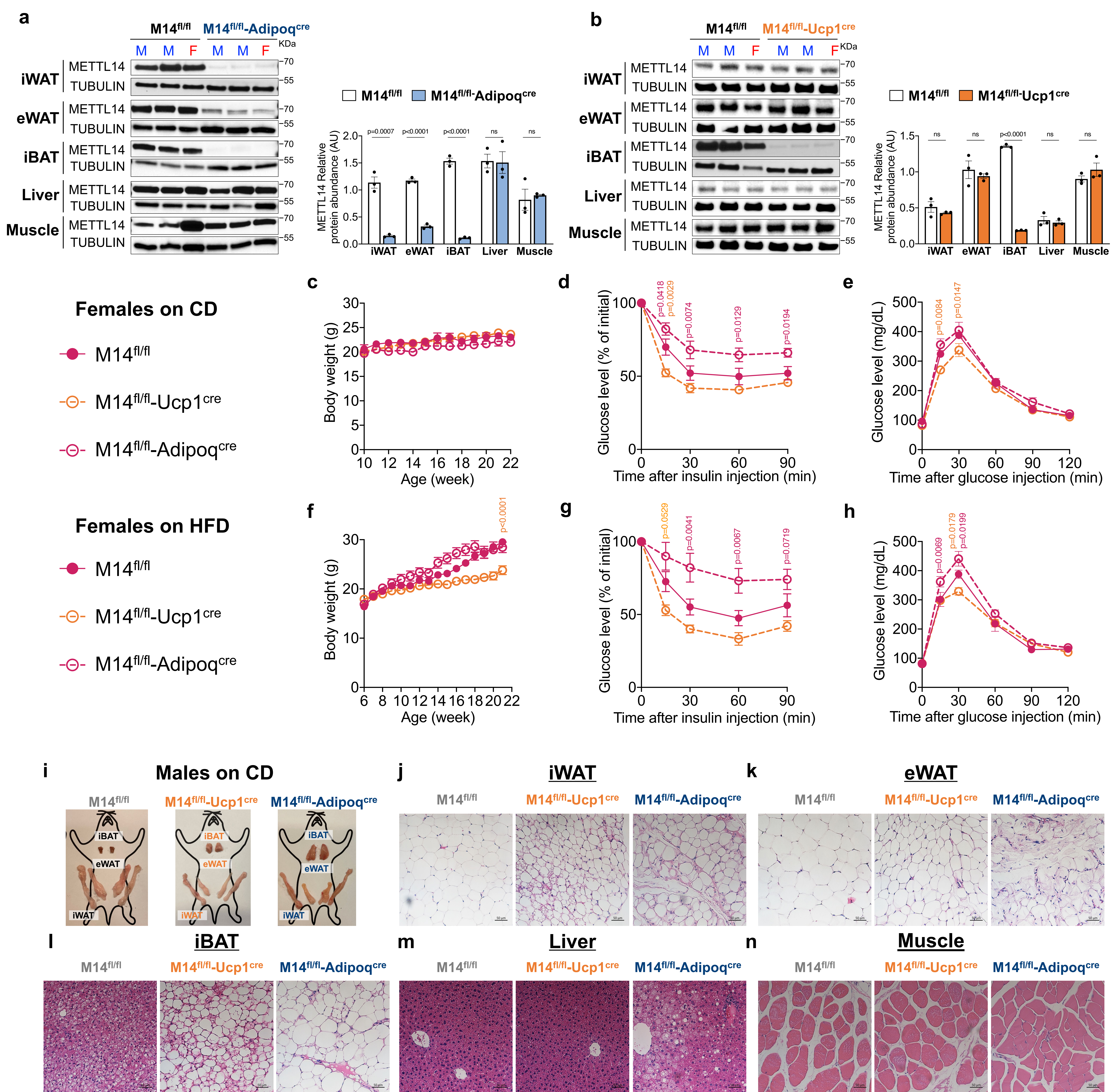
(j-m) Western blots (j and i) and quantification (k and m) of METTL14 and VINCULIN in subcutaneous white adipose tissue (scWAT) from human subjects classified by insulin sensitivity and BMI. The groups include lean and insulin-sensitive (Lean-IS), individuals with obesity and insulin-sensitive (OB-IS), lean and insulin-resistant (Lean-IR), and individuals with obesity and insulin-resistant (OB-IR). Sample sizes are as follows: lean and insulin-sensitive scWAT (n = 7), insulin-sensitive individuals with obesity scWAT (n = 12), lean and insulin-resistant scWAT (n = 12), and insulin-resistant individuals with obesity scWAT (n = 12).

(n) Fasting insulin levels in the plasma from *db*<sup>+</sup> or *db/db* mice, n = 6/group.

(o) Insulin sensitivity tolerance test of *db*<sup>+</sup> or *db/db* mice, n = 6/group.

All samples in each panel are biologically independent. Data are presented as means  $\pm$  SEM from two independent experiments by Two-tailed unpaired t-test (g, i, k, n, n, and q), or by Two-way ANOVA (o, p, and r).







**Supplementary Figure 2, related to Figure 2. Ablation of *Mettl14* in BAT or WAT Results in Opposite Systemic Metabolic Phenotypes in Female Mice**

(a) Western blots analysis and quantification of METTL14 protein abundance in the indicated metabolic tissues from M14<sup>fl/fl</sup> or M14<sup>fl/fl</sup>-Adipoq<sup>cre</sup> mice. TUBULIN was used as a loading control (n = 3/group).

(b) Western blots analysis and quantification of METTL14 protein abundance in the indicated metabolic tissues from M14<sup>fl/fl</sup> or M14<sup>fl/fl</sup>-Ucp1<sup>cre</sup> mice. TUBULIN was used as a loading control (n = 3/group).

(c) Body weight trajectories of CD-fed female mice (n = 6/group).

(d and e) Insulin (d) and glucose tolerance tests (e) of CD-fed female mice (n = 6/group for intraperitoneal insulin tolerance test (IPITT); n = 4 for intraperitoneal glucose tolerance test (IPGTT)).

(f) Body weight trajectories of HFD-fed female mice (n = 7 for M14<sup>fl/fl</sup> group, n = 6 for M14<sup>fl/fl</sup>-Ucp1<sup>cre</sup> group, and n = 7 for M14<sup>fl/fl</sup>-Adipoq<sup>cre</sup> group).

(g) Insulin tolerance tests of HFD-fed female mice (n = 7 for M14<sup>fl/fl</sup> group, n = 6 for M14<sup>fl/fl</sup>-Ucp1<sup>cre</sup> group, and n = 7 for M14<sup>fl/fl</sup>-Adipoq<sup>cre</sup> group).

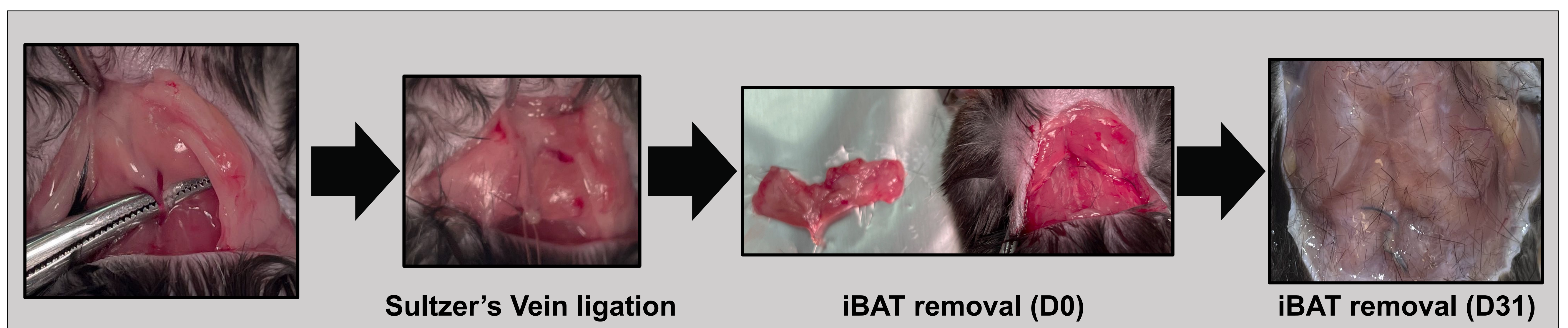
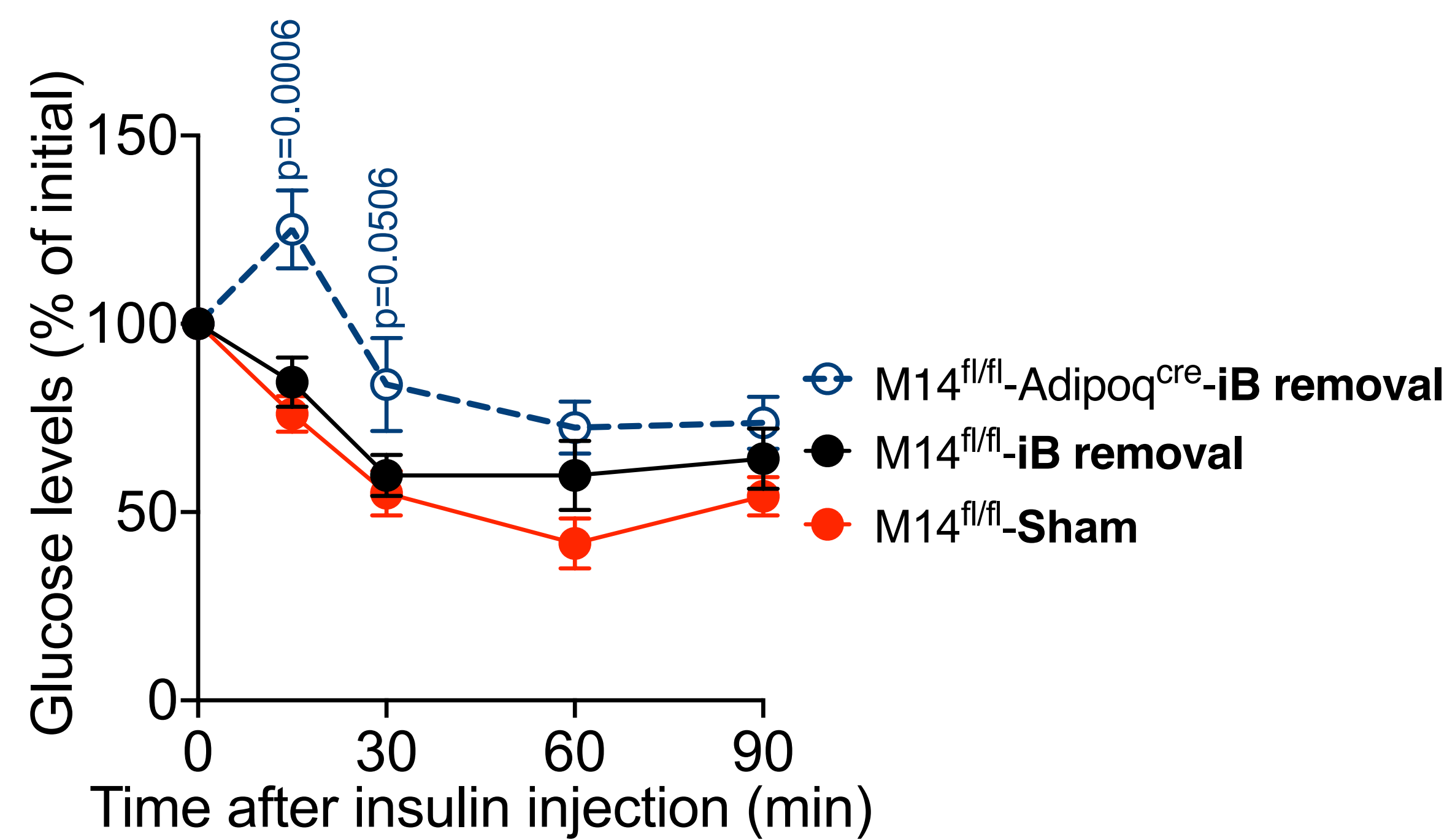
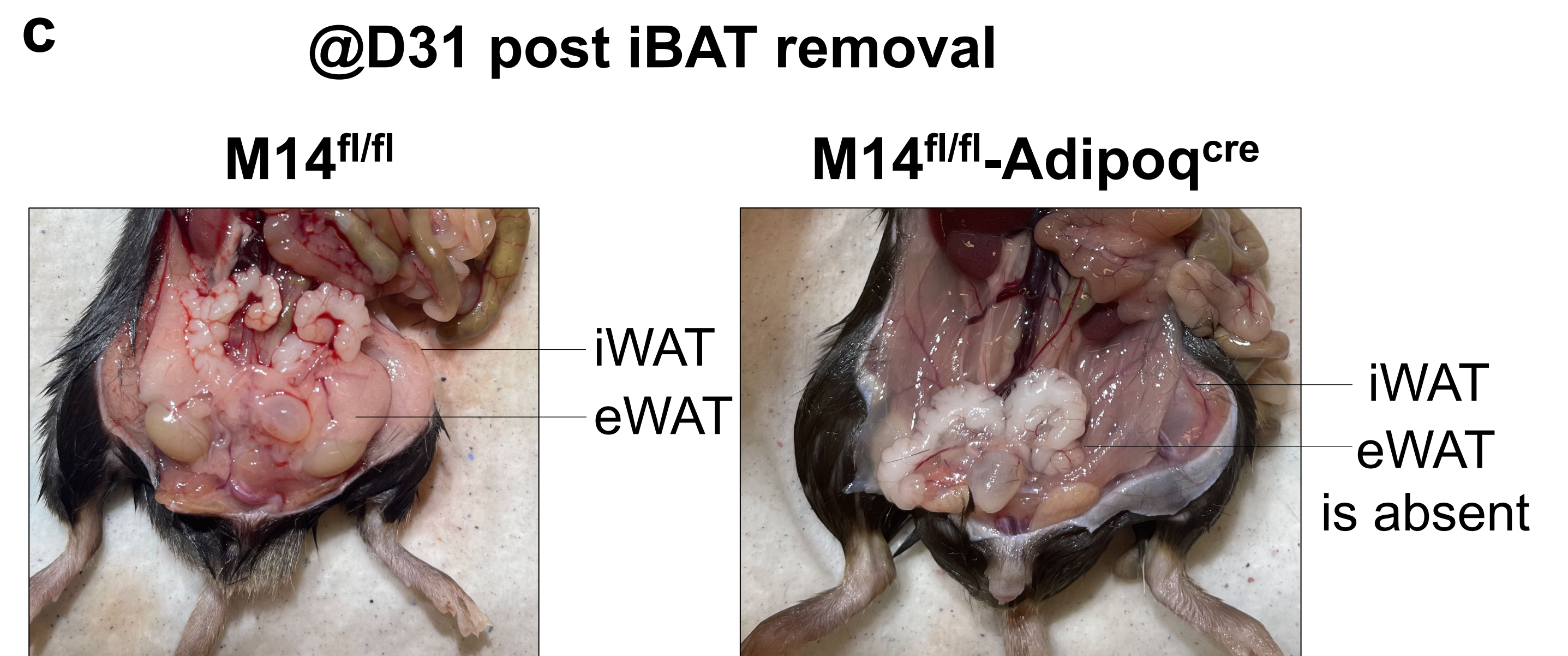
(h) Glucose tolerance tests of HFD-fed female mice (n = 7 for M14<sup>fl/fl</sup> group, n = 6 for M14<sup>fl/fl</sup>-Ucp1<sup>cre</sup> group, and n = 7 for M14<sup>fl/fl</sup>-Adipoq<sup>cre</sup> group).

(i) Representative images of the gross appearance of iBAT, iWAT, and eWAT from M14<sup>fl/fl</sup>, M14<sup>fl/fl</sup>-Ucp1<sup>cre</sup>, and M14<sup>fl/fl</sup>-Adipoq<sup>cre</sup> male mice on CD.

(j-n) Representative images of H&E stained iWAT (j), eWAT (k), iBAT (l), liver (m), and muscle (n) from M14<sup>fl/fl</sup>, M14<sup>fl/fl</sup>-Ucp1<sup>cre</sup>, and M14<sup>fl/fl</sup>-Adipoq<sup>cre</sup> male mice on CD (n = 4/group).

All samples in each panel are biologically independent. Data are presented as means ± SEM from two independent experiments by Two-tailed unpaired t-test (a and b), Two-way ANOVA (c-h).



**a****b****c**

**Supplementary Figure 3, related to Figure 2. Surgical Removal of iBAT Exacerbates the Insulin Resistance of M14<sup>fl/fl</sup>-Adipoq<sup>cre</sup> Male Mice**

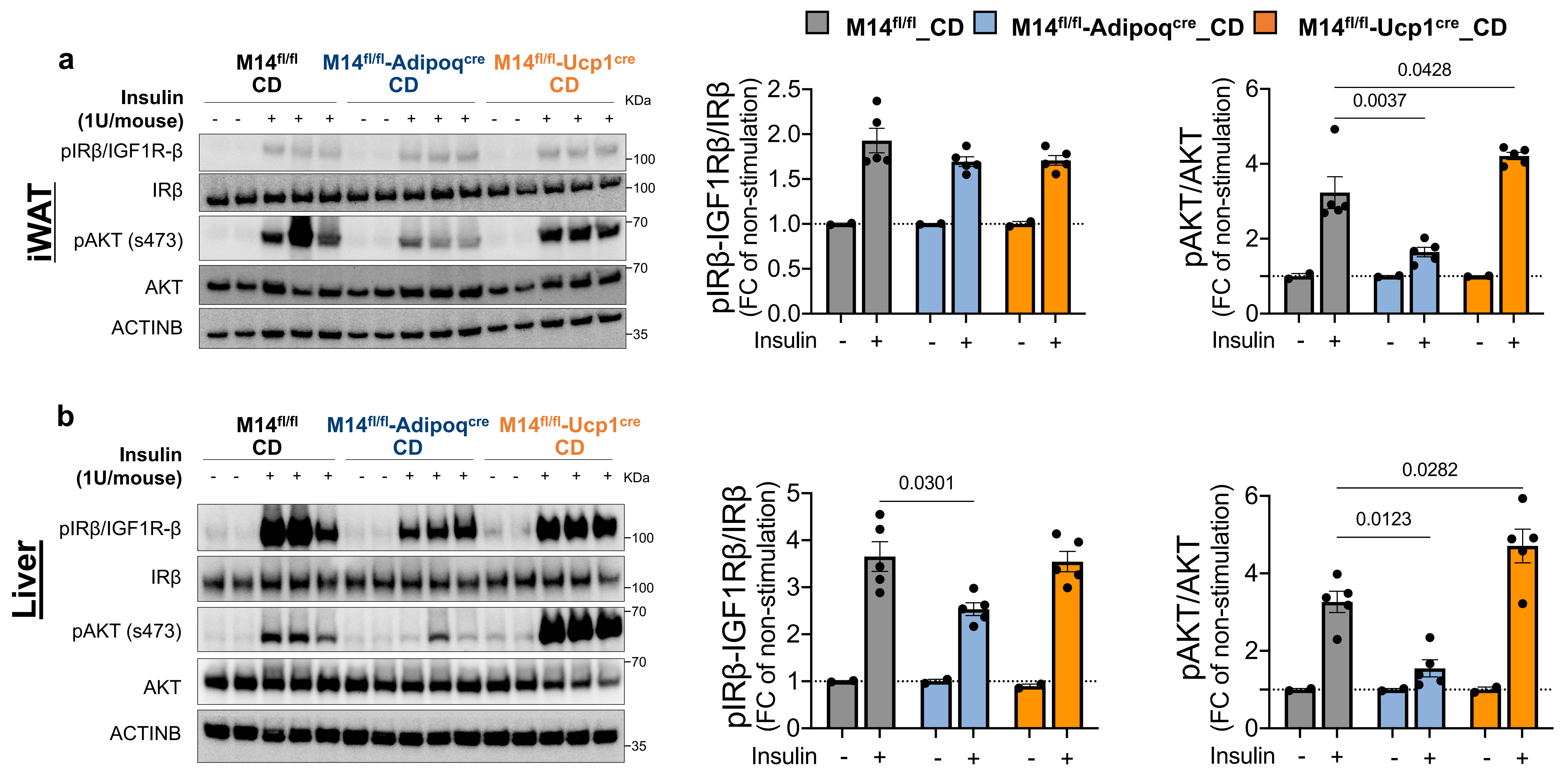
(a) Representative pictures of the iBAT removal procedure.

(b) IPITT of M14<sup>fl/fl</sup> and M14<sup>fl/fl</sup>-Adipoq<sup>cre</sup> male mice at day 11 post iBAT removal (n = 7 for M14<sup>fl/fl</sup> group, two M14<sup>fl/fl</sup> mice served as sham mice for iBAT removal; and n = 8 for M14<sup>fl/fl</sup>-Adipoq<sup>cre</sup> group).

(c) Presentative pictures of iWAT and eWAT from M14<sup>fl/fl</sup> and M14<sup>fl/fl</sup>-Adipoq<sup>cre</sup> male mice at day 31 post iBAT removal, eWAT is absent in the M14<sup>fl/fl</sup>-Adipoq<sup>cre</sup> mice (n = 3/group).

All samples in each panel are biologically independent. Data are presented as means ± SEM from two independent experiments by Two-way ANOVA (b).





**Supplementary Figure 4, related to Figure 3. Ablation of *Mettl14* in BAT or WAT Differentially Impacts Insulin Sensitivity of iWAT and Liver from CD-fed Male mice.**

(a) Representative Western blot analysis and quantification of insulin-stimulated phosphorylation of IRβ/IGF1Rβ and AKT<sub>S473</sub> in iWAT from CD-fed male mice after injection of 1U insulin into the *vena cava* (n = 2 for non-stimulated groups, and n = 5 for insulin-stimulate groups).

(b) Representative Western blot analysis and quantification of insulin-stimulated phosphorylation of IRβ/IGF1Rβ and AKT<sub>S473</sub> in the liver from CD-fed male mice after injection of 1U insulin into the *vena cava* (n = 2 for non-stimulated groups, and n = 5 for insulin-stimulate groups).

All samples in each panel are biologically independent. Data are presented as means ± SEM from two independent experiments by Two-way ANOVA (a and b).



Supplementary Table 1. related to Figure 1. Clinical characteristics of human subjects.

Human study	n=72
Male/Female	32/40
Age	23-73
BMI	20.5–54.5
Fasting glucose (mmol/L)	3.98–17.1
Fasting insulin (pmol/L)	12.2–844.1
HOMA-IR	0.4–48.5
Type 2 diabetes %	59.7%

Supplementary Table 2. List of primers and sequences.

Gene Name	Forward Primer	Reverse Primer
<i>Human</i>		
<i>METTL3</i>	CTATCTCCTGGCACTCGCAAGA	GCTTGAACCGTGCAACCACATC
<i>METTL14</i>	CTGAAAGTGCCGACAGCATTGG	CTCTCCTTCATCCAGATACTTACG
<i>WTAP</i>	GTACAAGCTTTGGAGGGCAAGT	TGGACTTGCTTGAGGTACTGGA
<i>YTHDF2</i>	TAGCCAGCTACAAGCACACCAC	CAACCGTTGCTGCAGTCTGTGT
<i>YTHDF3</i>	GCTACTTTCAAGCATACCACCTC	ACAGGACATCTTCATACGGTTATTG
<i>PPAR<math>\gamma</math></i>	AGCCTGCGAAAGCCTTTTGGTG	GGCTTCACATTCAGCAAACCTGG
<i>PPARGC1<math>\alpha</math></i>	CCAAAGGATGCGCTCTCGTTCA	CGGTGTCTGTAGTGGCTTGACTION
<i>PPARGC1<math>\beta</math></i>	CGCTTTGAAGTGTTTGGTGAGATTG	GCTGGAAGGAGGGCTCGTTG
<i>PTGES2</i>	CCTCTATGAGGCTGCTGACAAG	ATCACACGCAGCACGCCATACA
<i>CBR1</i>	GCAAGTCAAAAGACATTGTTCTGG	TTGCCAAGGCACAAAGGACTGG
<i>AKR1B10</i>	GAGGACCTGTTCATCGTCAGCA	CGTCCAGATAGCTCAGCTTCAG
<i>15-PGDH</i>	TGGAGGTGAAGGCGGCATCATT	GAGCGTGTGAATCCAACATGCC
<i>PTGDS</i>	AGCCCAACTTCCAGCAGGACAA	CAGACTTGCACATGGACAACGC
<i><math>\beta</math>-ACTIN</i>	AGAGCTACGAGCTGCCTGAC	AGCACTGTGTTGGCGTACAG
<i>TNFSF10</i>	TGGCAACTCCGTCAGCTCGTTA	AGCTGCTACTCTCTGAGGACCT
<i>TNFR1A</i>	CCGCTTCAGAAAACCACCTCAG	ATGCCGGTACTGGTTCTTCCTG
<i>TNFRSF10B</i>	AGCACTCACTGGAATGACCTCC	GTGCCTTCTTCGCACTGACACA
<i>RIP</i>	TATCCCAGTGCCTGAGACCAAC	GTAGGCTCCAATCTGAATGCCAG
<i>CASP3</i>	GGAAGCGAATCAATGGACTIONCTGG	GCATCGACATCTGTACCAGACC
<i>CASP7</i>	CGGAACAGACAAAGATGCCGAG	AGGCGGCATTTGTATGGTCCTC
<i>DFFA</i>	GAGAGGAAGTGCGTCAGTCCAA	GTGTCTACTGCATCCACCTCCT
<i>TNFA</i>	CTCTTCTGCCTGCTGCACTTTG	ATGGGCTACAGGCTTGTCACTC
<i>NFKB1</i>	GCAGCACTACTTCTTGACCACC	TCTGCTCCTGAGCATTGACGTC
<i>Mouse</i>		
<i>Mettl3</i>	CAGTGCTACAGGATGACGGCTT	CCGTCCTAATGATGCGCTGCAG
<i>Mettl14</i>	AGAGTGCGGATAGCATTGGTGC	CTCCTTCATCCAGACACTTCCG
<i>Wtap</i>	AGTGCCTGGAAGTTTACGCCTG	GCTTCAAGCTGTGCAATACGGC
<i>Ppar<math>\gamma</math></i>	GTACTIONGTGCGTTTCAGAACTIONGCC	ATCTCCGCCAACAGCTTCTCCT
<i>Ppargc1<math>\alpha</math></i>	GAATCAAGCCACTACAGACACCG	CATCCCTCTTGAGCCTTTCGCTG
<i>Ppargc1<math>\beta</math></i>	GGAGAAACCCCTTTCCAGG	ACCTGAAGGTGCATCTGCTT
<i>Ptges2</i>	GGTAGACCTCTATGAAGCAGCC	CATCACTIONCGCAGCACACCATACTION
<i>Cbr1</i>	CCTTCCACATTCAAGCAGAGGTG	CTGAGACTIONCACCATGCTGGACA
<i>Akr1b10</i>	GAGGACCTCTTCATCGTCAGCA	GCCAGTGGATTAGATACAGGTCC
<i>15-pgdh</i>	AAGCAAAACGGAGGTGAAGGCG	GAGCGTGTGAATCCGATGATGC
<i>Ptgds</i>	TCGCCTCCACTIONCAAGCTGGTT	CCATGATCTTGGTCTCACACTIONGG
<i><math>\beta</math>-actin</i>	CATTGCTGACAGGATGCAGAAGG	TGCTGGAAGGTGGACAGTGAGG
<i>Tnfsf10</i>	GGAAGACCTCAGAAAGTGGCAG	TTTCCGAGAGGACTIONCCCAGGAT
<i>Tnfr1a</i>	GTGTGGCTGTAAGGAGAACCAG	CACACGGTGTCTCTGAGTCTCCT
<i>Tnfrsf10b</i>	TGTGTGCGATGCAAACCAGGCAC	GCCGTTTTTGGAGACACTIONTCC
<i>Rip</i>	TGTGTGCGATGCAAACCAGGCAC	GCCGTTTTTGGAGACACTIONTCC
<i>Casp3</i>	GGAGTCTGACTIONGGAAAGCCGAA	CTTCTGGCAAGCCATCTCCTCA
<i>Casp7</i>	CCGTCCACAATGACTIONGCTCTTG	CCCGTAAATCAGGTCTCTTCC
<i>Dffa</i>	GCTGTGAGAAGAGGACCTCCAA	TCTCTCTGGTCAAGCACCTGCT
<i>Nfkb1</i>	GCTGCCAAAGAAGGACACGACA	GGCAGGCTATTGCTCATCACAG
<i>Tnfa</i>	CATCTTCTCAAAATTGAGTGACAA	TGGGAGTAGACAAGGTACAACCC