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Figure S1:

- (A) Experimental strategy for the identification of known and unknown location for transgenes in Mouse Genome Informatics (MGI) site.
- (B) Proportions of all transgenes (left) and Cre-drivers (right) transgenic mice with known location in the genome.
- (C) Copy number assay of Cre of control, $1xUcp1-Cre^{Evd}$ and $2xUcp1-Cre^{Evd}$ mice. n= 5 per genotype.
- (D) Expected and observed offspring genotypes obtained from $1xUcp1-Cre^{Evd}$ to $1xUcp1-Cre^{Evd}$ crosses. N=251 pups from 46 litters. Statistical significance was calculated using Chi-square test. ***P < 0.001.
- (E) Expected and observed offspring sex distribution obtained from $1xUcp1-Cre^{Evd}$ to $1xUcp1-Cre^{Evd}$ crosses. N=251 pups from 46 litters. Statistical significance was calculated using Chi-square test.
- (F) Growth curves of control, $1xUcp1-Cre^{Evd}$ and $2xUcp1-Cre^{Evd}$ females. n= 17 controls, 41 $1xUcp1-Cre^{Evd}$, 6 $2xUcp1-Cre^{Evd}$. All $2xUcp1-Cre^{Evd}$ females analyzed in this growth curve survived up to week 6. \$ indicates significant differences between $2xUcp1-Cre^{Evd}$ and control, & indicates significant differences between $2xUcp1-Cre^{Evd}$ and $1xUcp1-Cre^{Evd}$.
- (G) Growth curves of control, $1xUcp1-Cre^{Evd}$ and $2xUcp1-Cre^{Evd}$ males. n= 23 controls, 46 $1xUcp1-Cre^{Evd}$, 14 $2xUcp1-Cre^{Evd}$. All $2xUcp1-Cre^{Evd}$ males analyzed in this growth curve survived up to week 6. \$ indicates significant differences between $2xUcp1-Cre^{Evd}$ and control, & indicates significant differences between $2xUcp1-Cre^{Evd}$ and $1xUcp1-Cre^{Evd}$.
- (H) Body weights of control, $1xUcp1-Cre^{Evd}$ and $2xUcp1-Cre^{Evd}$ males. n= 6 controls, 6 $1xUcp1-Cre^{Evd}$, 7 $2xUcp1-Cre^{Evd}$.
- (I) BAT weights of control, $1xUcp1-Cre^{Evd}$ and $2xUcp1-Cre^{Evd}$ males. n= 6 controls, 6 $1xUcp1-Cre^{Evd}$, 7 $2xUcp1-Cre^{Evd}$.
- (J) WAT weights of control, $1xUcp1-Cre^{Evd}$ and $2xUcp1-Cre^{Evd}$ males. n= 6 controls, 6 $1xUcp1-Cre^{Evd}$, 7 $2xUcp1-Cre^{Evd}$.
- (K) Liver weight of control, $1xUcp1-Cre^{Evd}$ and $2xUcp1-Cre^{Evd}$ males. n= 6 controls, 6 $1xUcp1-Cre^{Evd}$, 7 $2xUcp1-Cre^{Evd}$.
- (L) Other organs weight of control, $1xUcp1-Cre^{Evd}$ and $2xUcp1-Cre^{Evd}$ males. n= 6 controls, 6 $1xUcp1-Cre^{Evd}$, 7 $2xUcp1-Cre^{Evd}$.
- (M) Skeletal muscles weight of control, $1xUcp1-Cre^{Evd}$ and $2xUcp1-Cre^{Evd}$ males. n= 6 controls, 6 $1xUcp1-Cre^{Evd}$, 7 $2xUcp1-Cre^{Evd}$.
- (N) qPCR analysis of Cre in adipose tissue depots of control, $1xUcp1-Cre^{Evd}$ and $2xUcp1-Cre^{Evd}$ females. n= 6.
- (O) qPCR analysis of Cre in adipose tissue depots of control, $1xUcp1-Cre^{Evd}$ and $2xUcp1-Cre^{Evd}$ males. n= 6.
- (P) Representative H&E images of fat depots and liver from control, $1xUcp1-Cre^{Evd}$ and $2xUcp1-Cre^{Evd}$ males. n= 4. Scale bar, 50µm.

Unless otherwise noted, data are mean + SEM and statistical significance was calculated using one-way ANOVA followed by Tukey's multiple comparisons test. *P < 0.05, **P < 0.01, ***P < 0.001.

1003 **Figure S2:**

1004 (A) Schematic representation of the location of the six probe pairs used for TLA
1005 analysis. See also Table S3.

1006 (B) Whole genome TLA mapping analysis of $1xUcp1-Cre^{Evdv}$ genome using probes
1007 surrounding the BAC 148M1 sequence.

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1009

1059 **Figure S3:**

- 1060 (A) RNA-seq comparing female $1xUcp1-Cre^{EvdR}$ and $2xUcp1-Cre^{EvdR}$ iBAT gene
1061 expression (left). Each dot represents one gene. Corresponding GO analysis
1062 (right). Genes and pathways significantly enriched in $1xUcp1-Cre^{EvdR}$ are labeled
1063 in red and those enriched in $2xUcp1-Cre^{EvdR}$ are labeled in brown.
- 1064 (B) RNA-seq comparing female $1xUcp1-Cre^{EvdR}$ and $2xUcp1-Cre^{EvdR}$ psWAT gene
1065 expression (left). Each dot represents one gene. Corresponding GO analysis
1066 (right). Genes and pathways significantly enriched in $1xUcp1-Cre^{EvdR}$ are labeled
1067 in red and those enriched in $2xUcp1-Cre^{EvdR}$ are labeled in brown.
- 1068 (C) qPCR analysis of iBAT of control, $1xUcp1-Cre^{EvdR}$ and $2xUcp1-Cre^{EvdR}$ males at 6
1069 weeks of age. n= 6.
- 1070 (D) qPCR analysis of psWAT of control, $1xUcp1-Cre^{EvdR}$ and $2xUcp1-Cre^{EvdR}$ males at
1071 6 weeks of age. n= 6.
- 1072 (E) qPCR analysis of pgWAT of control, $1xUcp1-Cre^{EvdR}$ and $2xUcp1-Cre^{EvdR}$ males at
1073 6 weeks of age. n= 6.
- 1074 (F) Body weight change and tissue weights of control and $1xUcp1-Cre^{EvdR}$ females
1075 after cold challenge. n= 3 control RT, 3 $1xUcp1-Cre^{EvdR}$ RT, 3 control cold, 4
1076 $1xUcp1-Cre^{EvdR}$ cold. Statistical significance was calculated using unpaired t-test
1077 between RT and cold samples.
- 1078 (G) Representative H&E images of fat depots from control, $1xUcp1-Cre^{EvdR}$ females
1079 after cold challenge. n= 4. Scale bar, 50 μ m.
- 1080 (H) qPCR analysis of iBAT of control and $1xUcp1-Cre^{EvdR}$ females after cold
1081 challenge or maintained at room temperature. n= 3. Statistical significance was
1082 calculated using unpaired t-test between RT and cold samples.
- 1083 (I) qPCR analysis of psWAT of control and $1xUcp1-Cre^{EvdR}$ females after cold
1084 challenge or maintained at room temperature. n= 3. Statistical significance was
1085 calculated using unpaired t-test between RT and cold samples.
- 1086 (J) qPCR analysis of pgWAT of control and $1xUcp1-Cre^{EvdR}$ females after cold
1087 challenge or maintained at room temperature. n= 3. Statistical significance was
1088 calculated using unpaired t-test between RT and cold samples.

1089
1090 Unless otherwise noted, data are mean + SEM and statistical significance was
1091 calculated using one-way ANOVA followed by Tukey's multiple comparisons test.
1092 *P < 0.05, **P < 0.01, ***P < 0.001.
1093

1134 **Figure S4:**

- 1135 (A) Schematic representation of the genomic structure of the *Ucp1*-floxed
1136 (*Ucp1^{tm1a}(EUCOMM)Hmgu*) allele.
- 1137 (B) Portion of the genomic sequence used for development of a specific FRT copy
1138 number assay.
- 1139 (C) Body weight of control, *Ucp1-fl/+^{Ucp1-CreEvd}* and *Ucp1-fl/fl^{Ucp1-CreEvd}* males. n= 14
1140 control, 13 *Ucp1-fl/+^{Ucp1-CreEvd}*, 9 *Ucp1-fl/fl^{Ucp1-CreEvd}*.
- 1141 (D) Liver weight of control, *Ucp1-fl/+^{Ucp1-CreEvd}* and *Ucp1-fl/fl^{Ucp1-CreEvd}* males. n= 14
1142 control, 13 *Ucp1-fl/+^{Ucp1-CreEvd}*, 9 *Ucp1-fl/fl^{Ucp1-CreEvd}*.
- 1143 (E) Muscle weights of control, *Ucp1-fl/+^{Ucp1-CreEvd}* and *Ucp1-fl/fl^{Ucp1-CreEvd}* males. n=
1144 14 control, 13 *Ucp1-fl/+^{Ucp1-CreEvd}*, 9 *Ucp1-fl/fl^{Ucp1-CreEvd}*.
- 1145 (F) Other organs weight of control, *Ucp1-fl/+^{Ucp1-CreEvd}* and *Ucp1-fl/fl^{Ucp1-CreEvd}* males.
1146 n= 14 control, 13 *Ucp1-fl/+^{Ucp1-CreEvd}*, 9 *Ucp1-fl/fl^{Ucp1-CreEvd}*.
- 1147 (G) qPCR analysis of *Cre* in iBAT of control, *Ucp1-fl/+^{Ucp1-CreEvd}* and *Ucp1-fl/fl^{Ucp1-}*
1148 *CreEvd* males. n=8.
- 1149 (H) qPCR analysis of *Cre* in psWAT of control, *Ucp1-fl/+^{Ucp1-CreEvd}* and *Ucp1-fl/fl^{Ucp1-}*
1150 *CreEvd* males. Values are relative to those of iBAT control. n=8.
- 1151 (I) qPCR analysis of *Cre* in pgWAT of control, *Ucp1-fl/+^{Ucp1-CreEvd}* and *Ucp1-fl/fl^{Ucp1-}*
1152 *CreEvd* males. Values are relative to those of iBAT control. n=8.
- 1153 (J) qPCR analysis in iBAT of control, *Ucp1-fl/+^{Ucp1-CreEvd}* and *Ucp1-fl/fl^{Ucp1-CreEvd}*
1154 males. n=8.
- 1155 (K) qPCR analysis in psWAT of control, *Ucp1-fl/+^{Ucp1-CreEvd}* and *Ucp1-fl/fl^{Ucp1-CreEvd}*
1156 males. n=8.
- 1157 (L) qPCR analysis in pgWAT of control, *Ucp1-fl/+^{Ucp1-CreEvd}* and *Ucp1-fl/fl^{Ucp1-CreEvd}*
1158 males. n=8.
- 1159 (M) Liver weight of control and *Ucp1-fl/fl^{Ucp1-CreERT2Biat}* males. n= 7 control, 6 *Ucp1-*
1160 *fl/fl^{Ucp1-CreERT2Biat}*.
- 1161 (N) Muscles weight of control and *Ucp1-fl/fl^{Ucp1-CreERT2Biat}* males. n= 7 control, 6 *Ucp1-*
1162 *fl/fl^{Ucp1-CreERT2Biat}*.
- 1163 (O) Other organs weight of control and *Ucp1-fl/fl^{Ucp1-CreERT2Biat}* males. n= 7 control, 6
1164 *Ucp1-fl/fl^{Ucp1-CreERT2Biat}*.

1165

1166 Unless otherwise noted, data are mean + SEM. Statistical significance was
1167 calculated using unpaired t-test or one-way ANOVA followed by Tukey's multiple
1168 comparisons test. *P < 0.05, **P < 0.01, ***P < 0.001.

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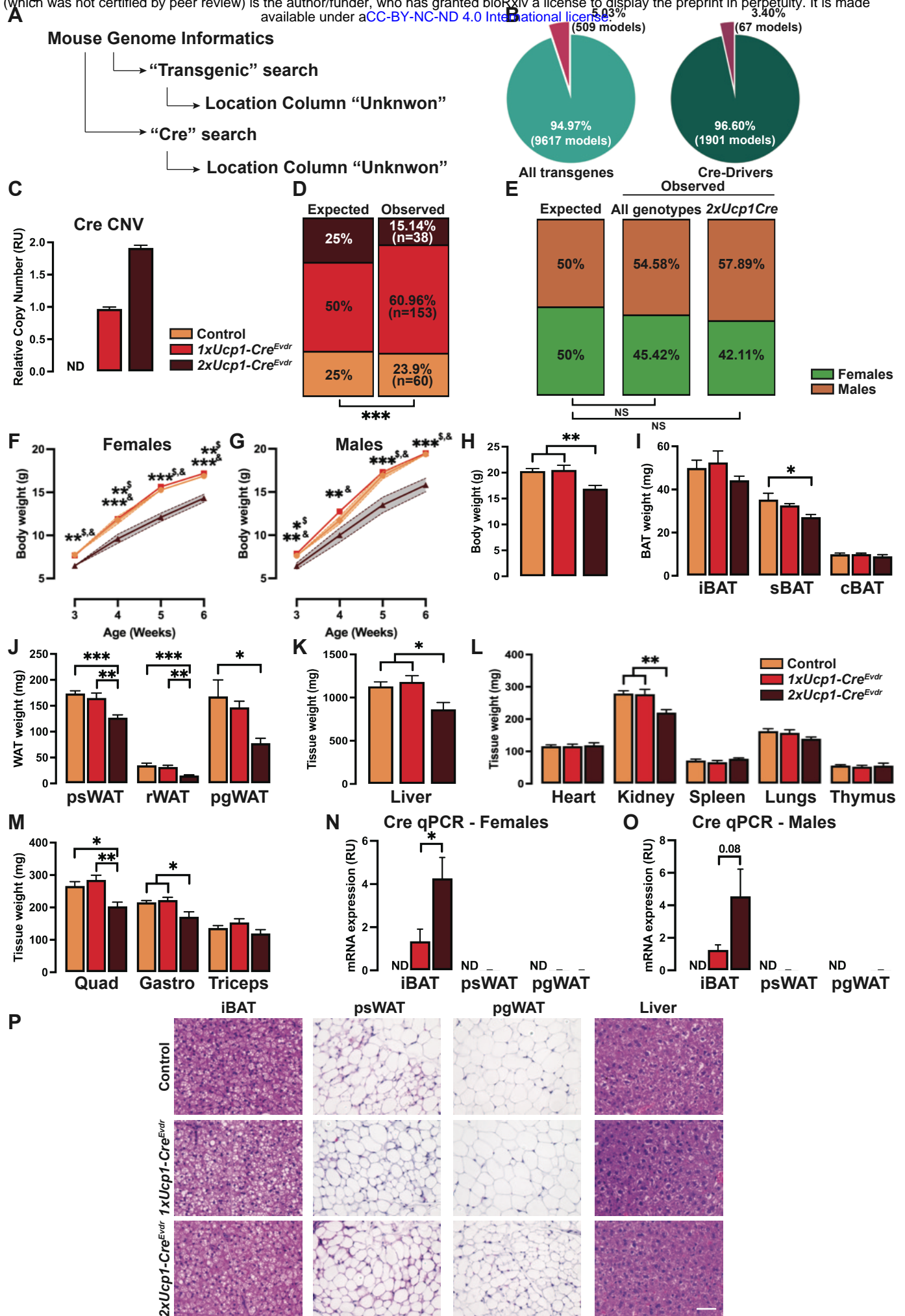
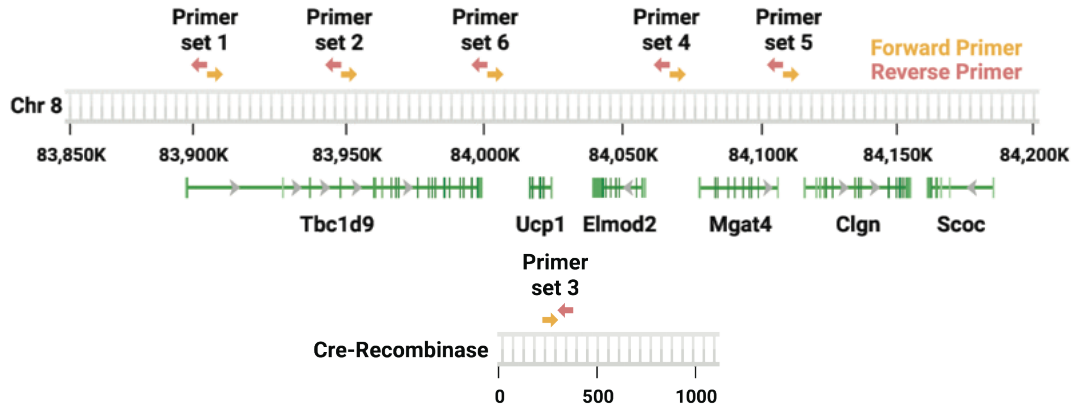


Figure S2

A



B

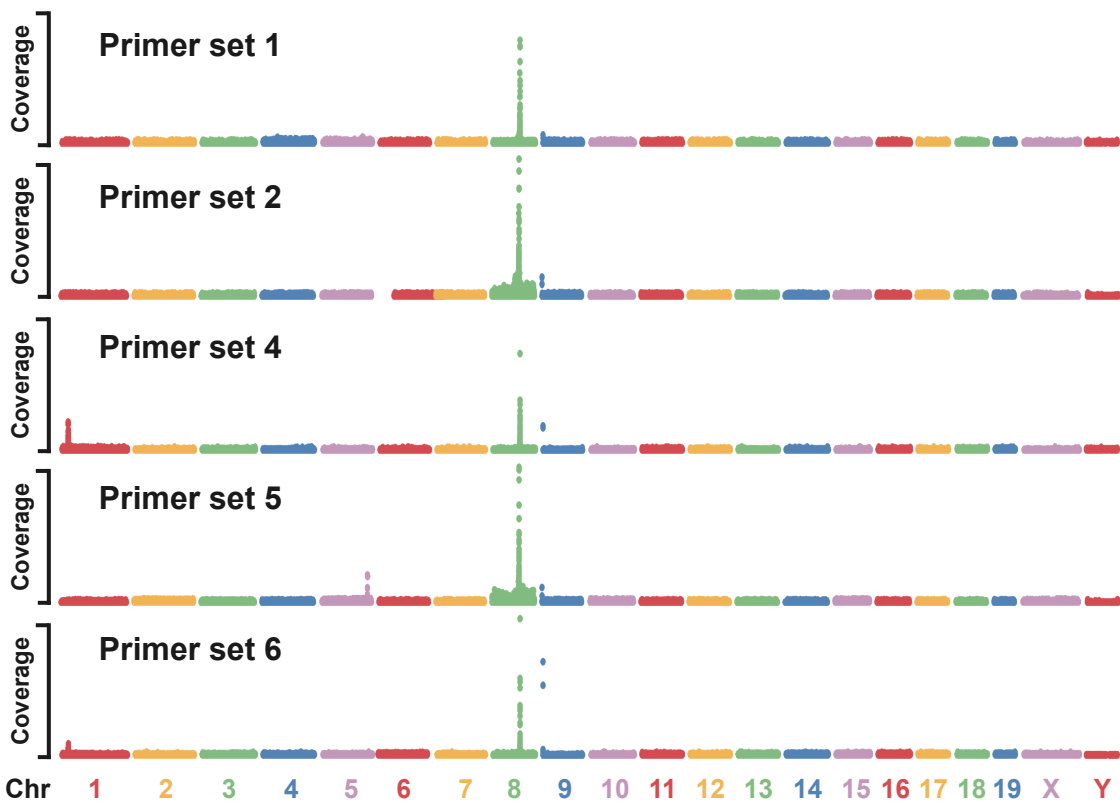


Figure S3

1xUcp1-Cre^{Evdr} vs 2xUcp1-Cre^{Evdr}

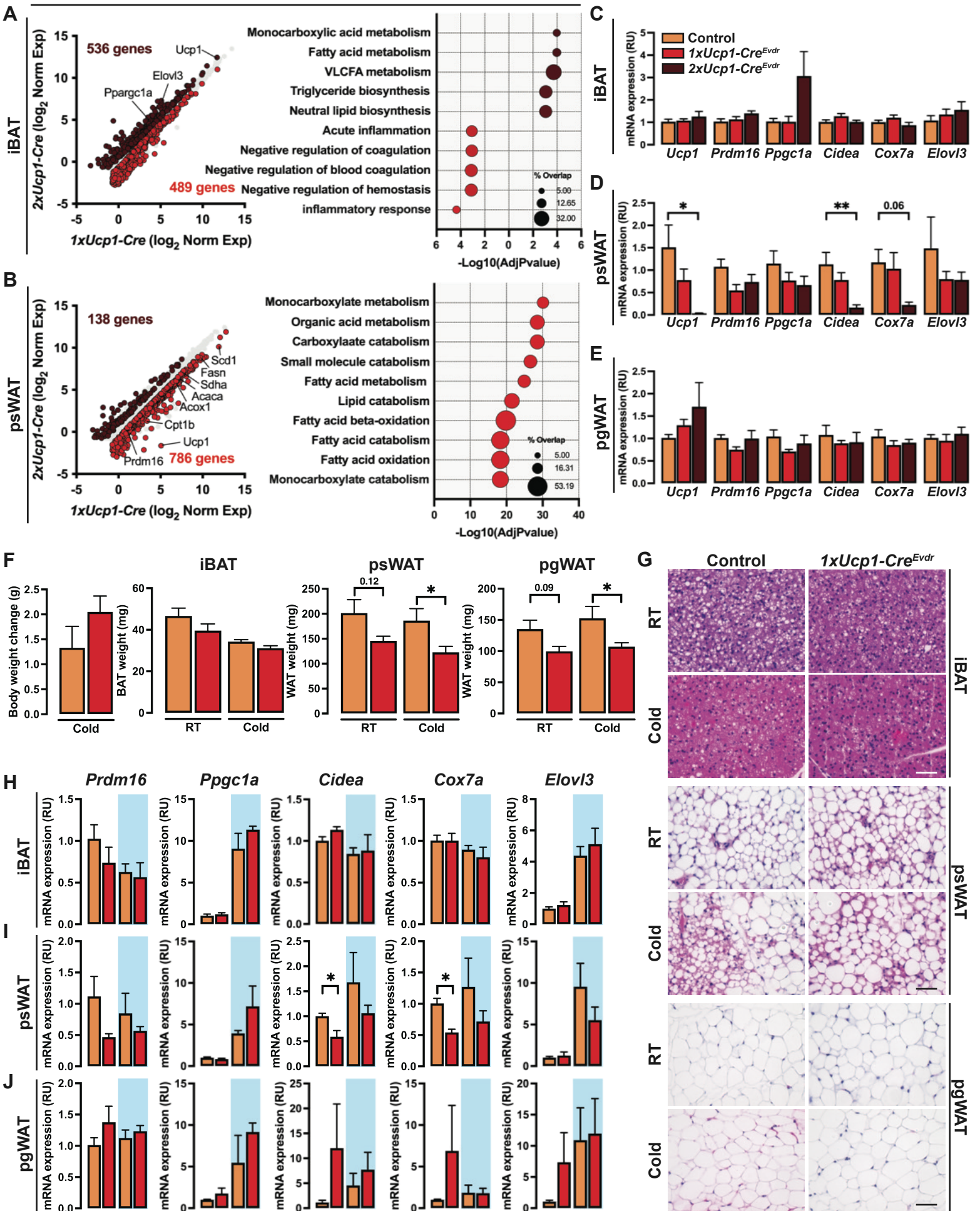


Figure S4

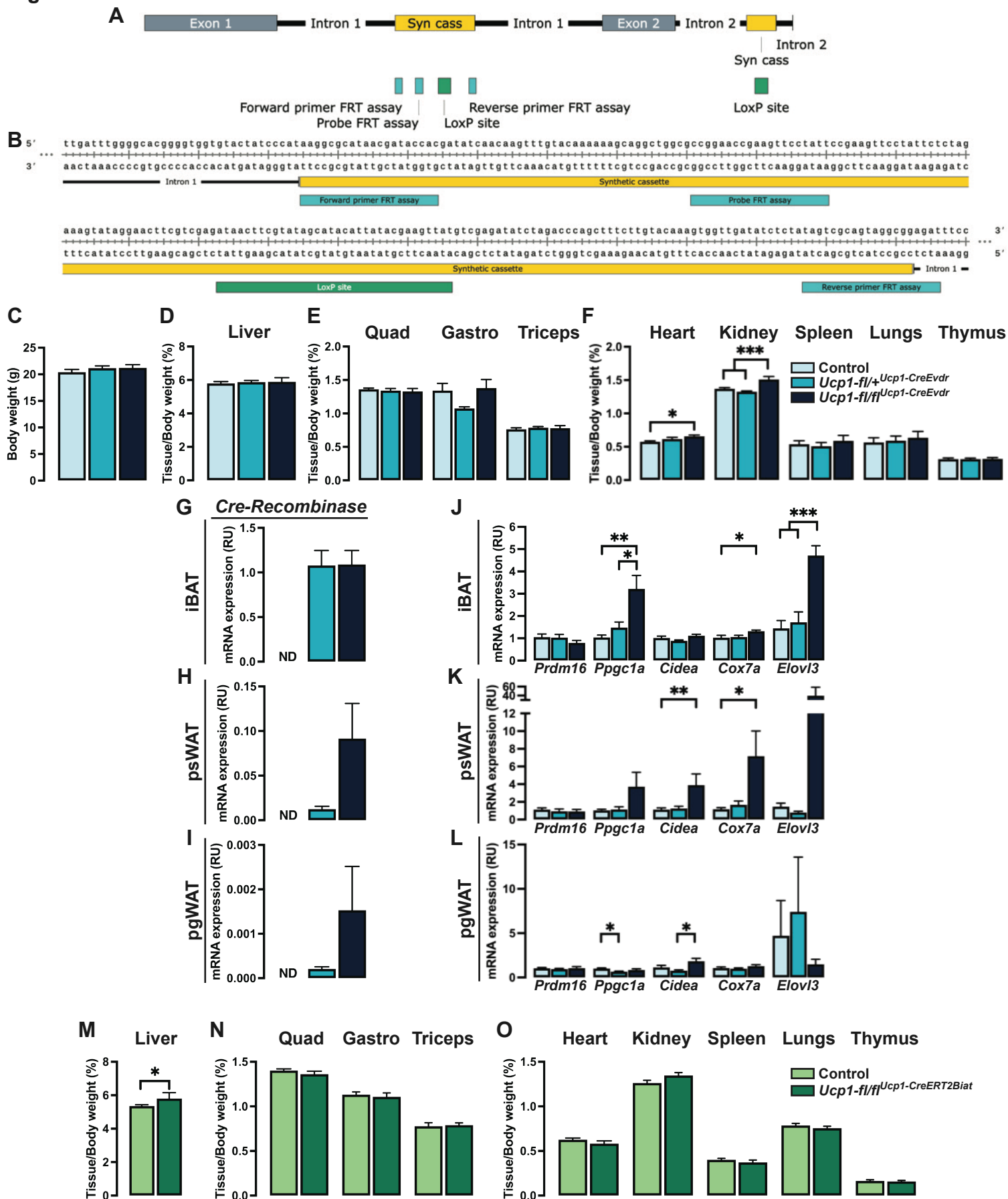


Table S1. Mouse primers

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
<i>Tbp</i>	ACCTTCACCAATGACTCCTATG	TGACTGCAGCAAATCGCTTGG
<i>Elovl3</i>	TCCGCGTTCTCATGTAGGTCT	GGACCTGATGCAACCCTATGA
<i>Prdm16</i>	GACATTCCAATCCCACCAGA	CACCTCTGTATCCGTCAGCA
<i>Ppargc1alpha</i>	CCCTGCCATTGTTAAGACC	TGCTGCTGTTCCCTGTTTTTC
<i>cox7a</i>	GCTGCTGAGGAGGCAAATGAGG	CCATTCCCCCGCCTTTCAAG
<i>Cidea</i>	TGCTCTTCTGTATCGCCCAGT	GCCGTGTTAAGGAATCTGCTG
<i>UCP1</i>	GGATTGGCCTCTACGACTCA	TGCCACACCTCCAGTCATTA
<i>Cre</i>	GCGGTCTGGCAGTAAAACTATC	GTGAAACAGCATTGCTGTCACT

Table S2. Antibodies

Antigen	Source	Concentration
<i>UCP1</i>	Abcam (ab10983)	1:1000
<i>Tubulin</i>	Cell Signaling (cs2125)	1:1000

Table S3. TLA Primer Sets		
Primers Set		Sequence (5'-3')
1	<i>Rv</i>	ACAACAGGAAGCACATACAT
	<i>Fw</i>	GGTATATGTAGTGCGTGTGT
2	<i>Rv</i>	GACACAGATGAGCAACAAAG
	<i>Fw</i>	CCCAGGTTAATCTGAGTTCC
3	<i>Rv</i>	GTTCGAACGCACTGATTTTC
	<i>Fw</i>	AACCAGTGAAACAGCATTG
4	<i>Rv</i>	AGAGATACAGCAGAGTGACT
	<i>Fw</i>	TATCCACACTTGTCTGAAGC
5	<i>Rv</i>	GTGTCAGAGTAACAAAGAGTG
	<i>Fw</i>	CTGACCCTGCTATTCTTCC
6	<i>Rv</i>	GGAGCAAGGACTTTAGAGTT
	<i>Fw</i>	GACTGACTCAATTGCACATG

Table S4. Copy Number Assays

<i>Premade</i>		
Target	Location	Assay ID
<i>UCP1</i>	Intron 3	Mm00260416_cn
<i>CRE</i>	Unavailable	Mr00635245_cn
<i>Tfrc</i>	Exon 17	4458366

<i>Custom designed</i>				
Target	Forward Primer	Reverse Primer	Probe	Assay ID
FRT	aaggcgcataacgataccac	atctcgcctactgcgacta	ccggaaccgaagtctctatt	FRT_CNVD2W9GY

ddPCR Assay

Target	Location	Probe Fluorophore	Assay ID
<i>Tfrc</i>	mm10 chr16:32608778-32608900:+	HEX	dMmuCNS420644255
<i>Cre</i>	16-138 bp of <i>Cre</i> sequence	FAM	dCNS325197214