

**Adipocyte-specific modulation of KLF14 expression in mice leads to sex-dependent impacts on adiposity
and lipid metabolism**

Running title: KLF14 is sex-dimorphic in mouse metabolism

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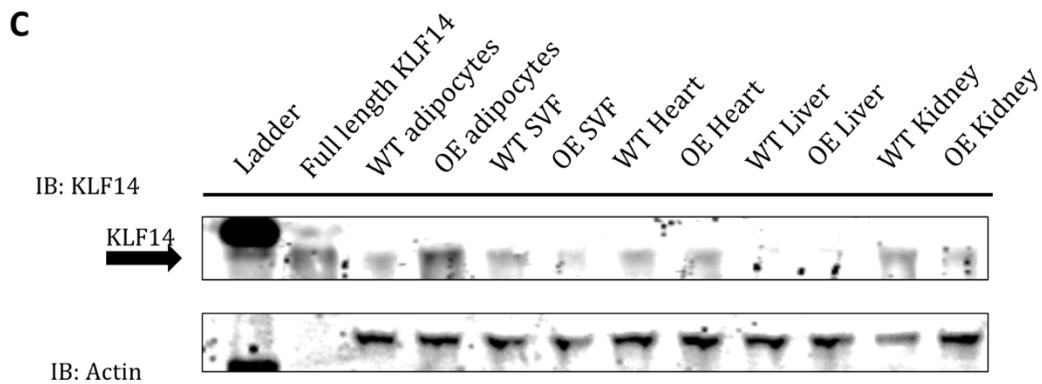
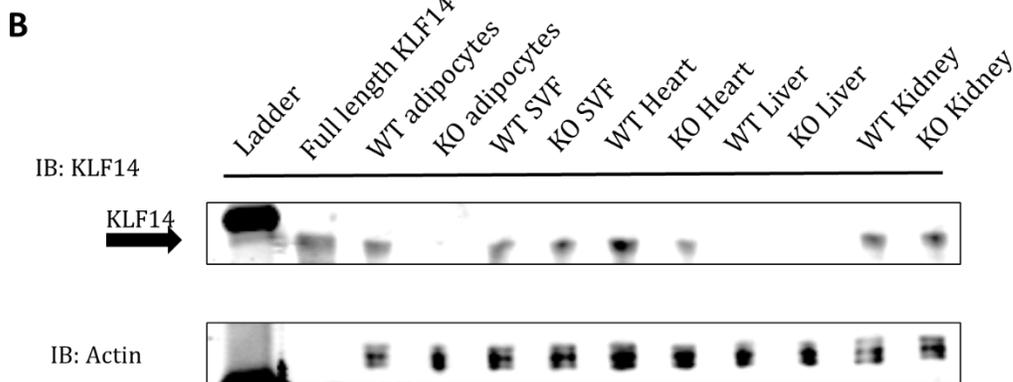
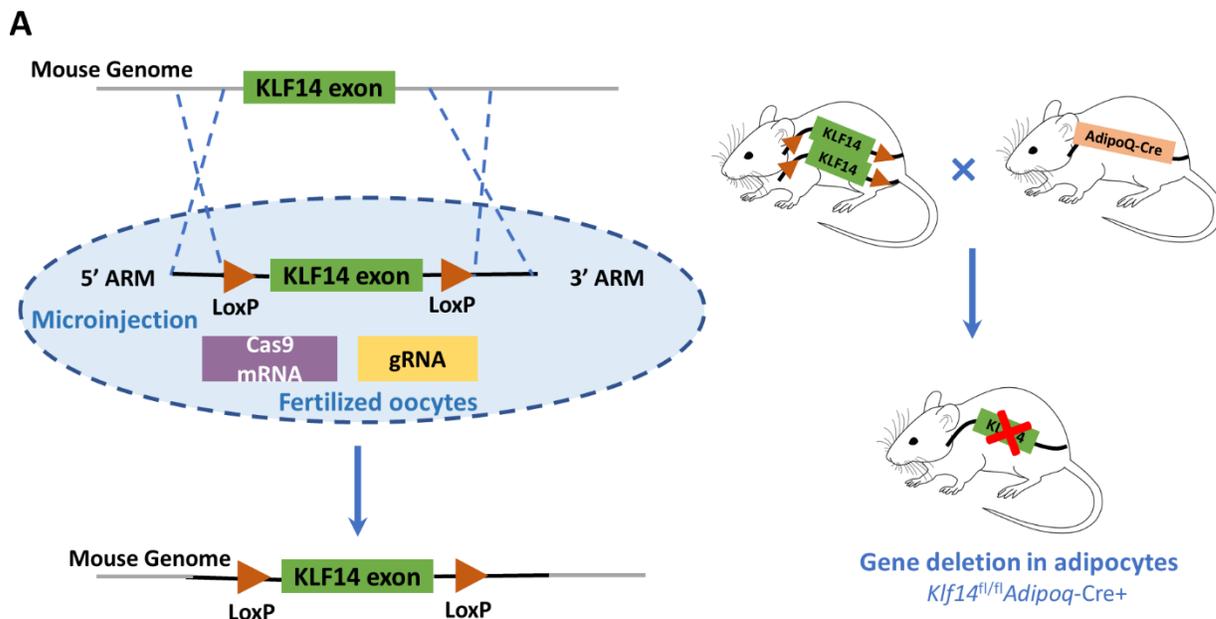
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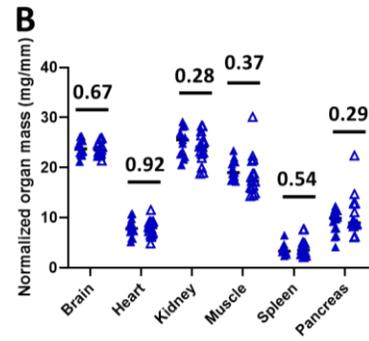
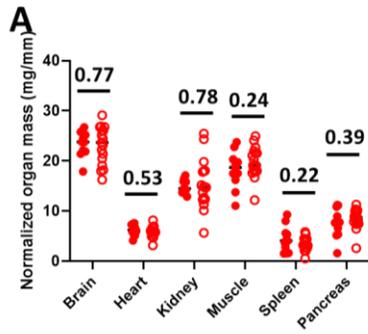
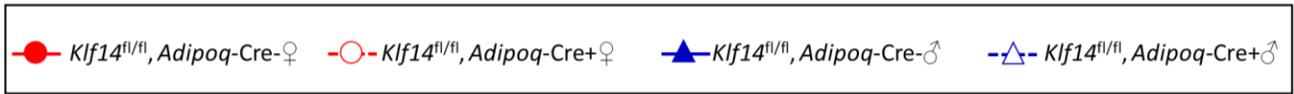
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SUPPLEMENTAL FIGURES:

Supplemental Figure 1

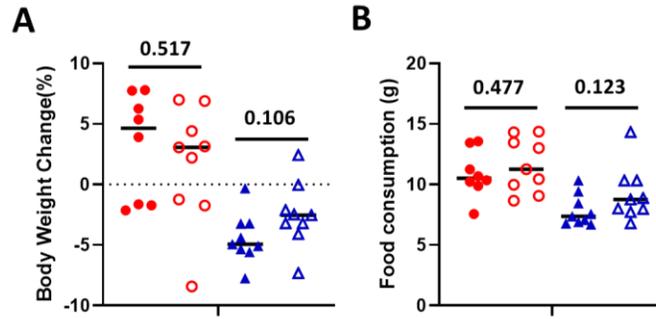


Supplemental Figure 2

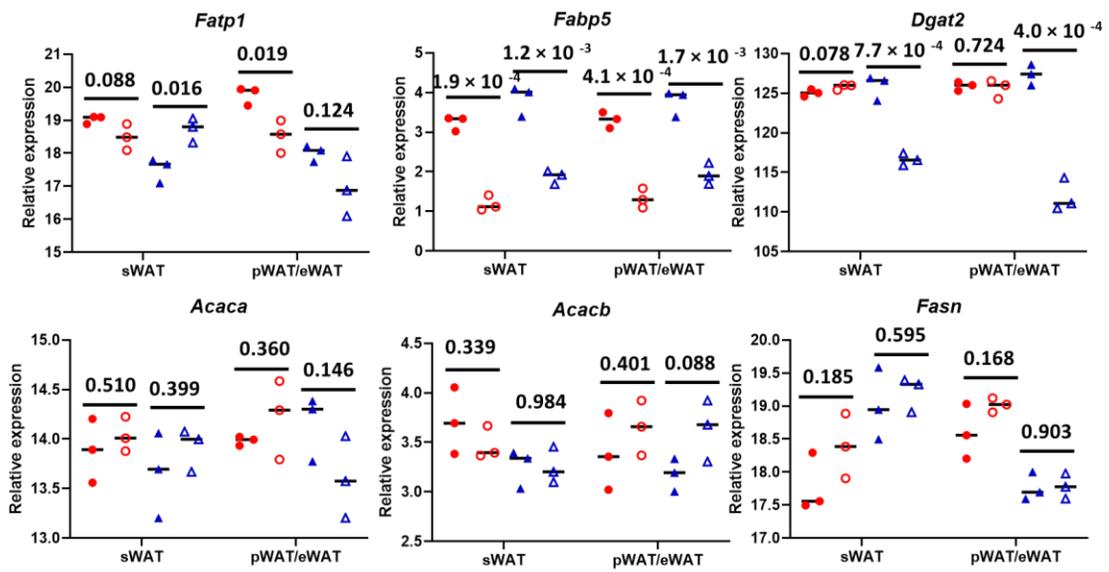


Supplemental Figure 3

● *Klf14^{fl/fl}, Adipoq-Cre⁻*♀ ○ *Klf14^{fl/fl}, Adipoq-Cre⁺*♀ ▲ *Klf14^{fl/fl}, Adipoq-Cre⁻*♂ △ *Klf14^{fl/fl}, Adipoq-Cre⁺*♂

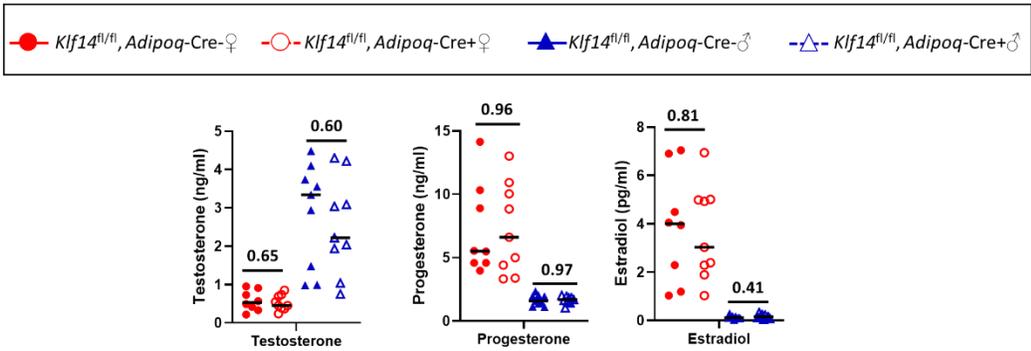


Supplemental Figure 4

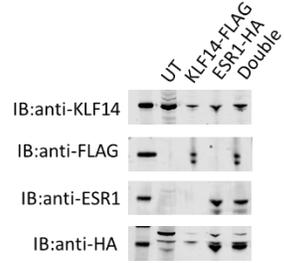


Supplemental Figure 5

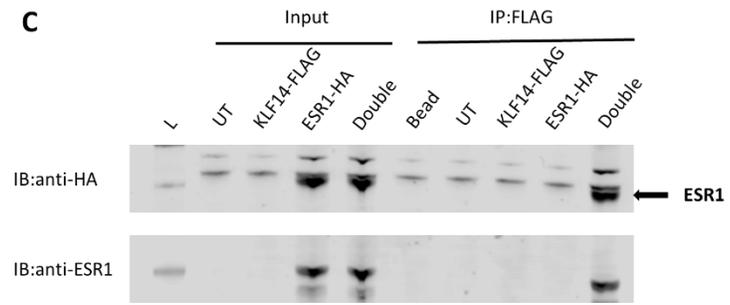
A



B



C



SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1: Characterization of transgenic mice. **(A)** Targeting strategy for deletion of *Klf14* in mouse adipocytes. **(B)** KLF14 protein level in adipocytes, stromal vascular fraction (SVF), heart, liver and kidney of wild-type (WT) and knockout (KO) mice. **(C)** KLF14 protein level in adipocytes, stromal vascular fraction (SVF), heart, liver and kidney of wild-type (WT) and overexpression (OE) mice.

Supplemental Figure 2: Tissue weights normalized to tibia length in adipocyte-specific *Klf14* knockout and wild type **(A)** female and **(B)** male mice. $N_{\text{Female, Adipoq-Cre-}} = 11$, $N_{\text{Female, Adipoq-Cre+}} = 17$, $N_{\text{Male, Adipoq-Cre-}} = 14$, $N_{\text{Male, Adipoq-Cre+}} = 14$.

Supplemental Figure 3: **(A)** Body weight change and **(B)** food consumption during the three-day period in metabolic cages. $N_{\text{Female, Adipoq-Cre-}} = 8$, $N_{\text{Female, Adipoq-Cre+}} = 9$, $N_{\text{Male, Adipoq-Cre-}} = 9$, $N_{\text{Male, Adipoq-Cre+}} = 9$.

Supplemental Figure 4: mRNA expression of fatty acid uptake and metabolism genes *Fatp1*, *Fabp5*, *Dgat2*, *Acaca*, *Acacb*, *Fasn* and in isolated mature adipocytes. $N = 3$ mice per genotype and sex. Relative gene expression, normalized to GAPDH levels, was calculated using the $2^{-\Delta\Delta\text{CT}}$ method. *P*-values were calculated using two-tailed unpaired Student's *t* -test.

Supplemental Figure 5: **(A)** Sex hormone Testosterone, Progesterone and Estradiol levels in serum of adipocyte *Klf14*-deficient female and male mice and control littermates at euthanasia after 21 weeks of HFD. $N_{\text{Female, Adipoq-Cre-}} = 8$, $N_{\text{Female, Adipoq-Cre+}} = 9$, $N_{\text{Male, Adipoq-Cre-}} = 9$, $N_{\text{Male, Adipoq-Cre+}} = 9$. **(B)** KLF14, FLAG, ESR1 and HA levels were measured in HEK293 cells that are untransfected (UT) or transfected with either *KLF14-FLAG* or *ESR1-HA* or both. **(C)** Abovementioned HEK293 cells were immunoprecipitated with FLAG antibody-conjugated magnetic beads. Samples were immunoblotted with anti-ESR1 or anti-HA antibodies.

Supplemental table 1: Mouse genotyping primer list.

Name	Forward	Reverse	Amplicon
Adipoq_Klf14_Cre	GAACCTGATGGACATGTTCAGG	AGTGCGTTCGAACGCTAGAGCCTGT	250bp
Adipoq_Klf14_OE	GGCCTACTACAAGTCGTCGC	CCGGGCTGCAGGAATTCGAT	489bp

Supplemental table 2: qPCR primer list.

Gene	Forward	Reverse	Accession ID	Reference
Fatp1	GGCTCCTGGAGCAGGAACA	ACGGAAGTCCCAGAAACCAA	NM_011977.4	(1)
Fatp4	ACGATGTTTCCTGCTGAGTGGTA	CTCTCCGACCTGCCACAGA	NM_011989.5	(1)
Fabp4	ATGTGCGACCAGTTTGTG	TTTGCCATCCCCTTCTG	NM_024406.3	(2)
Fabp5	GCTGATGGCAGAAAACTCAGA	CCTGATGCTGAACCAATGCA	NM_001272098.1	(3)
Acc1	CCTCCGTCAGCTCAGATACA	TTTACTAGGTGCAAGCCAGACA	NM_133360.2	(4)
Fasn	GCTGCGGAAACTTCAGGAAAT	AGAGACGTGTCACTCCTGGACTT	NM_007988.3	(5)
Acc2	ACAGAGATTT CACCGTTGCGT	CGCAGCGATGCCATTGT	NM_133904	(6)
Dgat1	ACCGCGAGTTCTACAGAGATTGGT	ACAGCTGCATTGCCATAGTTCCT	NM_010046.3	(7)
Dgat2	TGGGTCCAGAAGAAGTTCCAGAAGTA	ACCTCAGTCTCTGGAAGGCCAAAT	NM_026384.3	(7)
Hsl	GCTGGGCTGTCAAGCACTGT	GTAAGTGGGTAGGCTGCCAT	NM_001039507.2	(8)
Atgl	TGTGGCCTCATTCTCTCTAC	TCGTGGATGTTGGTGGAGCT	NM_001163689.1	(8)
Gapdh	CTCCCACTCTTCCACCTTCG	GCCTCTCTTGCTCAGTGTCC	NM_001289726.1	(9)

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