Supporting Information Figure Legends

Supporting Information Figure S1. Glucose levels in metabolic test expressed as percentage of basal glucose.

(A) Circulating T4 levels in fed conditions. Age = 13 weeks. *n* = 7 untreated; *n* = 8 T4-treated. (B) Glucose concentration in blood during the OGTT expressed as the percentage of basal (time 0) glucose. *n* = 13 per group. (C) Glucose concentration in blood during the IPPTT expressed as the percentage of basal glucose. *n* = 7 untreated; *n* = 8 T4-treated. (D) Glucose concentration in blood during the ITT expressed as the percentage of basal glucose. *n* = 12 untreated; *n* = 13 T4-treated. (E) Glucose concentration in blood during a 24-hour fasting period expressed as the percentage of basal glucose. *n* = 7 untreated; *n* = 8 T4-treated. (F) Homeostatic assessment of insulin resistance (HOMA-IR) index. Age = 12 weeks. *n* = 7 untreated; *n* = 8 T4-treated. UT: Untreated; T4: T4-treated. Data are represented as the mean ± SEM. * $p < 0.05$ compared to untreated mice. A *t*-test two tailed was applied to panels A and F. A Mann-Whitney Rank Sum test was applied to panels B-E.

Supporting Information Figure S2. Energy intake and histology of the thyroid and the pituitary of in T4-treated mice.

(A) Energy intake divided by body weight. *n* = 7 untreated; *n* = 8 T4-treated. (B) Representative images of the histological analysis o the thyroid and pituitary of T4-treated and untreated mice. Thyroid; *n* = 6 untreated; *n* = 7 T4-treated. Pituitary; *n* = 5 untreated; *n* = 6 T4-treated. (C) Determination of the α-GSU of pituitary hormones in the serum of T4-treated and untreated mice. *n* = 7 untreated; *n* = 8 T4-treated. UT: Untreated; T4: T4-treated. Data are represented as the mean ± SEM. * $p < 0.05$ compared to untreated mice (t-test two tailed).

Supporting Information Figure S3. The subcellular localization of MAFA and FOXO1 is not affected by T4 supplementation.

(A) Pancreatic islets were isolated from animals treated or not with T4. Determination of messenger RNA (mRNA) levels of genes involved in pancreatic islet metabolism. Values were normalized to islets isolated from untreated mice. *n* = 6 untreated; *n* = 5 T4-treated. (B) Representative images of MAFA and insulin (INS) immunofluorescence staining in pancreases from mice treated or not with T4. Scale bar = 50 μm. *n* = 5 per group. (C) Representative images of FOXO1 and INS immunofluorescence staining in pancreases from mice treated or not with T4. Scale bar = 50 μm. *n* = 5 per group. UT:

Untreated; T3: T3-treated; T4: T4-treated. Data are represented as the mean ± SEM. * $p < 0.05$ compared to untreated mice (*t*-test two tailed).

Supplementary Figure 4. T4 induces a starvation-like state in the skeletal muscle.

(A) Western blots indicating activation of AKT in the skeletal muscle and the liver of T4-treated mice. Mice were fasted for 16 hours and then injected with insulin (0.75 $UI.kg^{-1}$ of body weight) 15 minutes prior euthanization. $n = 5$ untreated; $n = 6$ T4-treated. (B) Determination of mRNA levels of genes involved in energy metabolism in the skeletal muscle of mice treated or not with T4. Values were normalized to untreated mice. GLUT2; $n = 5$ untreated, $n = 6$ T4-treated. GK; $n = 5$ untreated, $n = 6$ T4-treated. G6Pase; $n = 5$ untreated, $n = 6$ T4-treated. LPK; $n = 5$ untreated, $n = 6$ T4-treated. PEPCK; $n = 5$ untreated, $n = 6$ T4-treated. GAPDH; $n = 6$. LDH; $n = 5$ untreated, $n = 6$ T4-treated. PGC1α; $n = 6$. PGC1β; $n = 6$. UCP2; $n = 5$. SREBP1c; $n = 5$ untreated, $n = 6$ T4-treated. (C) Determination of mRNA levels of genes involved in energy metabolism in the liver of mice treated or not with T4. Values were normalized to untreated mice. GLUT2; $n = 5$ untreated, $n = 6$ T4-treated. GK; $n = 5$. G6Pase; $n = 5$ untreated, $n = 6$ T4-treated. LPK; $n = 5$ untreated, $n = 6$ T4-treated. PEPCK; $n = 5$ untreated, $n = 6$ T4-treated. GAPDH; $n = 6$ untreated, $n = 5$ T4-treated. LDH; $n = 5$. PGC1α; $n = 5$ untreated, $n = 6$ T4-treated. PGC1β; $n = 6$. UCP2; $n = 6$ untreated, $n = 5$ T4-treated. SREBP1c; $n = 6$ untreated, $n = 5$ T4-treated. (D) Western blots indicating activation of AMPK in the skeletal muscle and the liver of T4-treated mice. Mice were fasted for 16 hours prior euthanization. $n = 5$ per group. (E) Densitometric analysis of the western blots using skeletal muscle extracts shown in Supporting Information Figure S4D. Values were normalized to untreated mice. (F) Densitometric analysis of the western blots using liver extracts shown in Supporting Information Figure S4D. Values were normalized to untreated mice. UT: Untreated; T4: T4-treated. Data are represented as the mean ± SEM. * $p < 0.05$ compared to untreated mice (*t*-test two tailed).

Supporting Information Figure S5. T4 supplementation increased circulating T4, alters organs weight and modulates insulin signaling in immunized RIP-B7.1 mice.

(A) Circulating T4 levels in fed conditions. $n = 9$ untreated; $n = 8$ T4-treated. (B) Organs weight divided by body weight. $n = 6$ untreated; $n = 9$ T4-treated. (C) Western blots showing the amount of phospho-tyrosine 632 IRS1, total IRS1 and GAPDH, used as loading control, in the skeletal muscle and the liver of T4-treated mice. Skeletal muscle $n = 6$ untreated, $n = 5$ T4-treated. Liver $n = 6$ per group. (D) Densitometric analysis of the western blots using skeletal muscle extracts shown in Supporting

Information Figure S5C. Values were normalized to untreated mice. (E) Densitometric analysis of the western blots using liver extracts shown in Supporting Information Figure S5C. Values were normalized to untreated mice. UT: Untreated; T4: T4-treated. Data are represented as the mean ± SEM. * $p < 0.05$ compared to untreated mice (*t*-test two tailed).

Supporting Information Figure S6. T4-treated and untreated immunized RIP-B7.1 mice show similar degree of insulitis. (A) Representative images of immune infiltrations in T4-treated and untreated immunized RIP-B7.1 mice. (B) Insulitis was scored as grade 0–4 according to the percentage of infiltrated islet area (0: 0%; 1: <10%; 2: >10% and <55%; 3: >55% and <75%; 4: >75%). Scale bar = 50 μm. $n = 5$ per group. UT: Untreated; T4: T4-treated. Data are represented as the mean ± SEM. * $p < 0.05$ compared to untreated mice (*t*-test two tailed).

Supporting Information Table S1. Antibodies used in this study.

Antibody	**Dilution**	**Vendor**	**Catalog number**
Anti-Ki67	1:150	Leica Microsystems	NCL-L-Ki67-MM1
Biotin-rabbit anti-mouse	1:300	Sigma-Aldrich	B8520
Alexa fluor 555 donkey anti-mouse	1:800	Life technologies	A31579
Alexa fluor 647 donkey anti-rabbit	1:800	Life technologies	A31573
Anti-insulin (H-86)	1:500	Santa Cruz Biotechnology	SC9168
Anti-insulin	1:500	Sigma-Aldrich	I2018
Anti-glucagon	1:150	Sigma-Aldrich	G2654
Anti-MAFA	1:200	Bethyl Laboratories	IHC-00352
Anti-GK	1:50	Santa Cruz Biotechnology	SC-7908
Anti-IR-β	1:200	Santa Cruz Biotechnology	SC-711
Anti-IRS1	1:200	Santa Cruz Biotechnology	SC-599
Anti-pTyr 632 IRS1	1:200	Santa Cruz Biotechnology	SC-17196
Anti-PI3K	1:1000	Cell Signaling	4292
Anti-pSer473 AKT	1:500	Cell Signaling	9271
Anti-AKT	1:1000	Cell Signaling	9272
Anti-pSer256 FOXO1	1:300	Cell Signaling	9461
Anti-FOXO1	1:1000 WB; 1:100 IHC	Cell Signaling	2880
Anti-GSK3β	1:200	Santa Cruz Biotechnology	SC-9166
Anti-pThr202/pTyr204 ERK	1:1000	Cell Signaling	9106
Anti-ERK	1:1000	Cell Signaling	9102

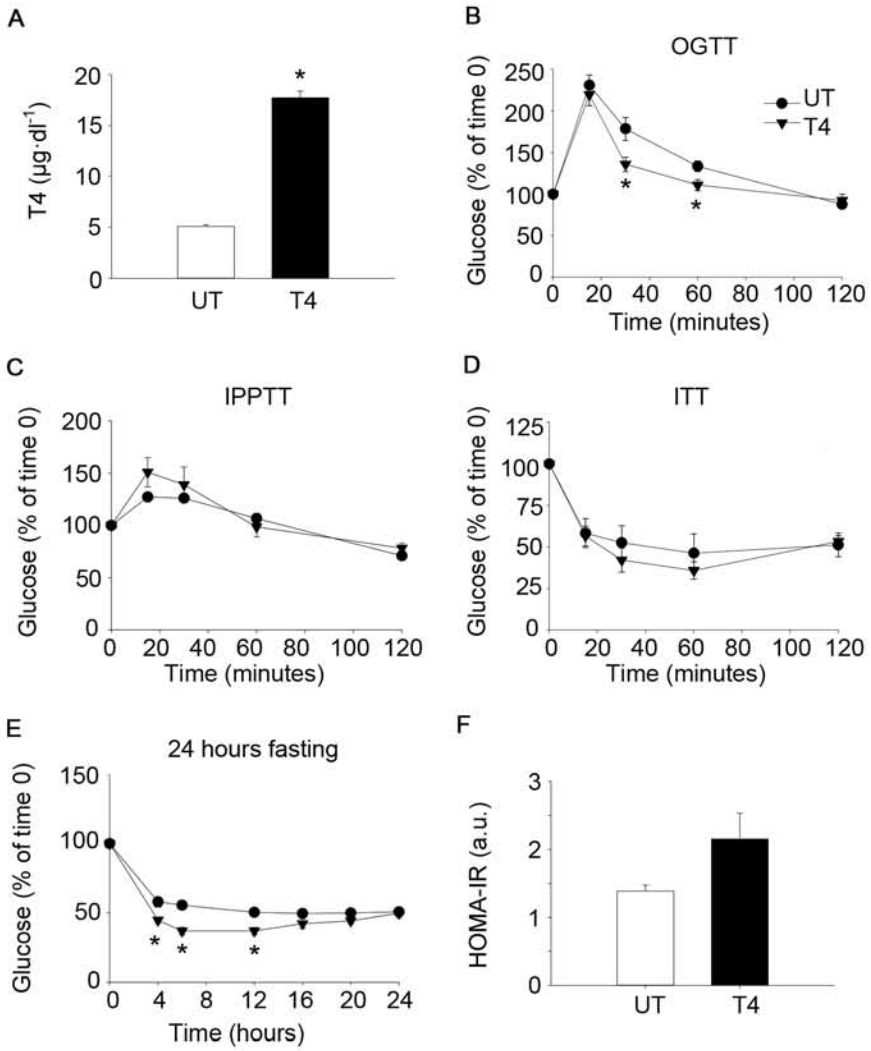
Anti-GAPDH	1:1000	Cell Signaling	2118
Anti-pThr172 AMPK	1:1000	Cell Signaling	2535
Anti-AMPK	1:1000	Cell Signaling	2532
HRP-goat anti-rabbit	1:5000	Sigma-Aldrich	A0545
HRP-rabbit anti-mouse	1:5000	Sigma-Aldrich	A9044
HRP-rabbit anti-goat	1:5000	Sigma-Aldrich	A5420

WB: Western blot; IHC: Immunohistochemistry.

Supporting Information Table S2. Primer pair sequences used for quantitative RT-PCR analysis.

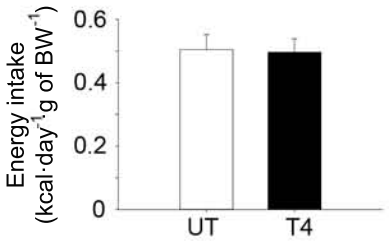
Gene	Forward primer sequence	Reverse primer sequence
MAFA	5′-CAGCAGCGGCACATTCTG-3′	5′-GCCCGCCAACTTCTCGTAT-3′
IR-β	5'–ACCCTGGACCCAATACGC–3'	5'–CCATTGGGGTCAGAGGGG–3'
IRS1	5'–GGCAGGGGAGGACTTGAG–3'	5'–CTGCCTCGGAGTTCAGCT–3'
AKT	5'–AGGTAGCTGTCAACAAGGCA–3'	5'–CTTGCCGAGGAGTTTGAGA–3'
FOXO1	5'–GAGAAGAGGCTCACCCTGTC–3'	5'–ACAGATTGTGGCGAATTGAA–3'
GSK3-β	5'–CCGGCTAACACCACTGGA–3'	5'–GTCCACGGTCTCCAGCAT–3'
ERK	5'–GCTCACCCTTACCTGGAACA–3'	5'–GGACCAGATCCAAAAGGACA–3'
GLUT2	5'–TTGACTGGAGCCCTCTTGATG–3'	5'–CACTTCGTCCAGCAATGATGA–3'
GK	5'–CTTCACCTTCTCCTTCCCTG–3'	5'–ATCTCAAAGTCCCCTCTCCT–3'
G6Pase	5'–TCTTGTGGTTGGGATTCTGG–3'	5'–CGGATGTGGCTGAAAGTTTC–3'
LPK	5'–AGTCTTCCCCTTGCTCTACC–3'	5'–AATCACCAGATCACCAACTCG–3'
PEPCK	5'–CCATCCCAACTCGAGATTCTG–3'	5'–CTGAGGGCTTCATAGACAAGG–3'
GAPDH	5'–CACCAACTGCTTAGCCCC–3'	5'–TCTTCTGGGTGGCAGTGATG–3'
LDH	5'–AGTCTCCCGTGCATCCTCAA–3'	5'–AGGGTGTCCGCACTCTTCCT–3'
PGC1α	5'–GGGTCAGAGGAAGAGATAAAGTTG–3'	5'–CACCAAACCCACAGAAAACAG–3'
PGC1β	5'–GTGATAAAACCGTGCTTCTGG–3'	5'–GGTGTTCGGTGAGATTGTAGAG–3'
UCP2	5'–GCTTGGGATCCTGGAACGT–3'	5'–GGCAGCCATTAGGGCTCTTT–3'
SREBP1c	5'–GCATGCCATGGGCAAGTAC–3'	5'–AGCATCTCCTGCGCACTCA–3'
β-actin	5'–GGACCAGATCCAAAAGGACA–3'	5'–GCTCACCCTTACCTGGAACA–3'

Suporting Information Figure S1



Supporting Information Figure S2

A



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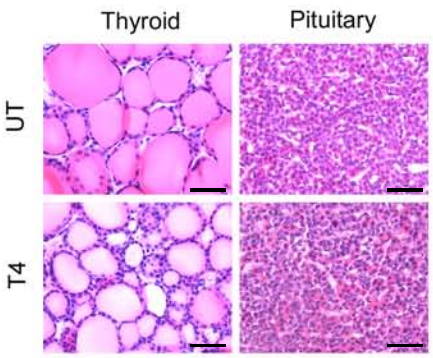

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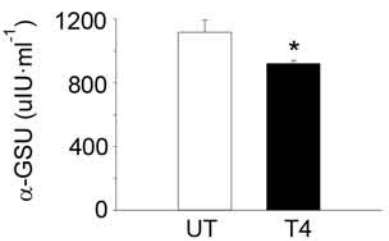

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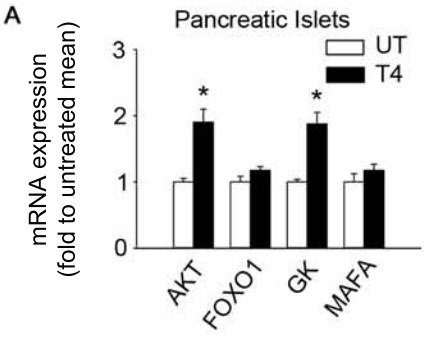
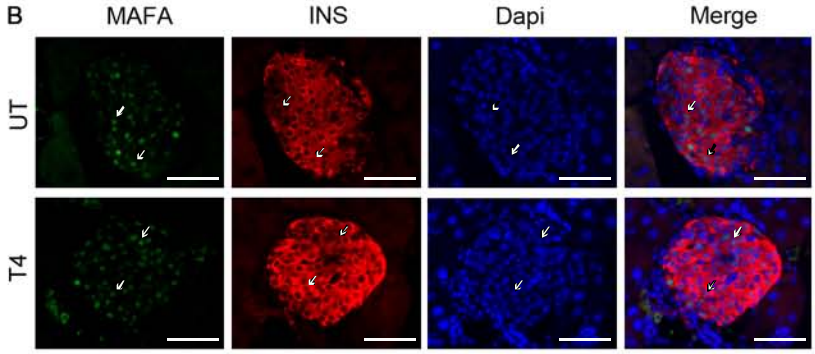
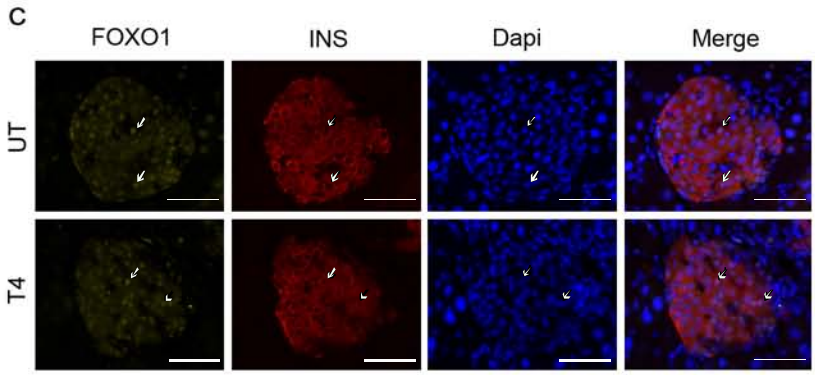
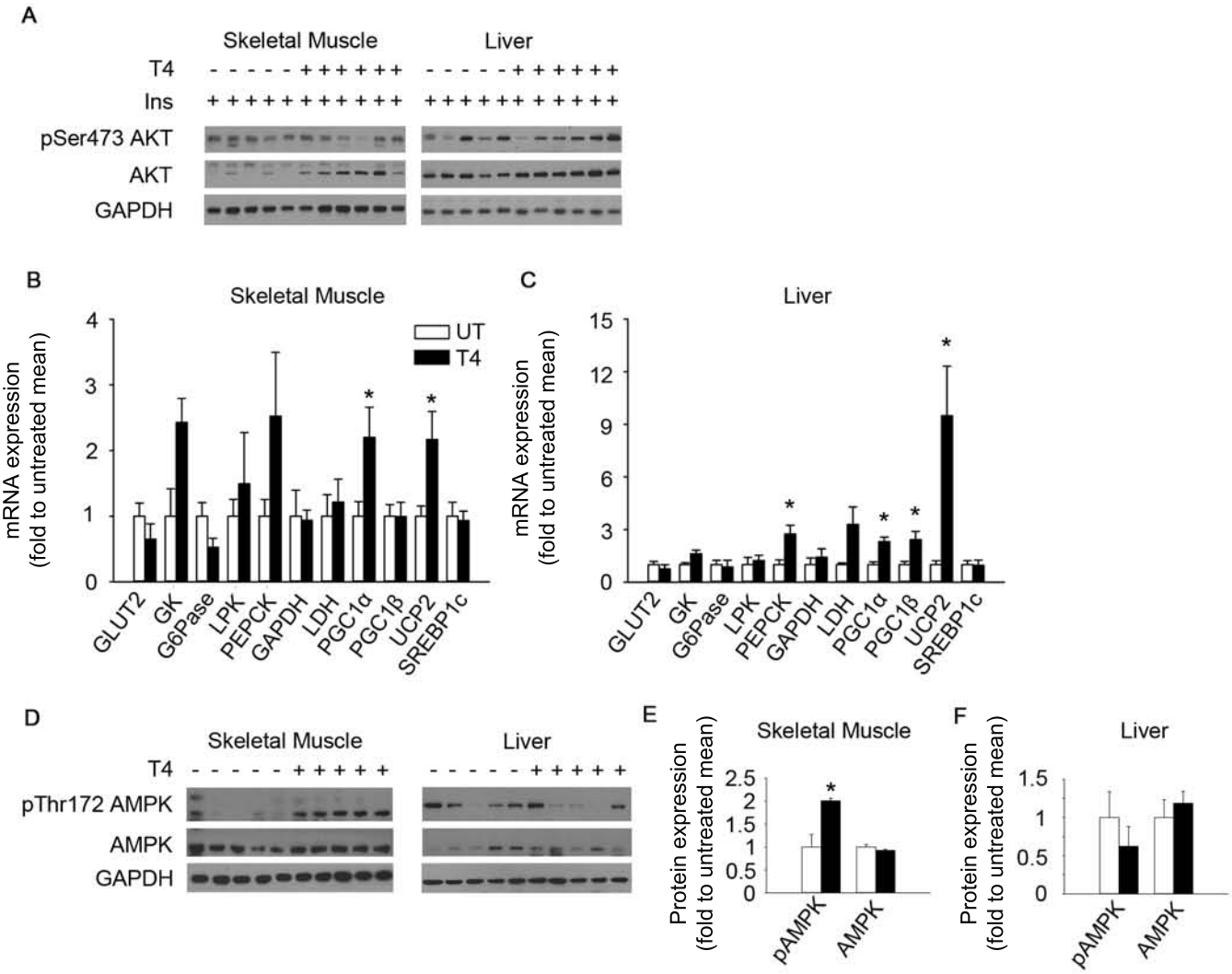


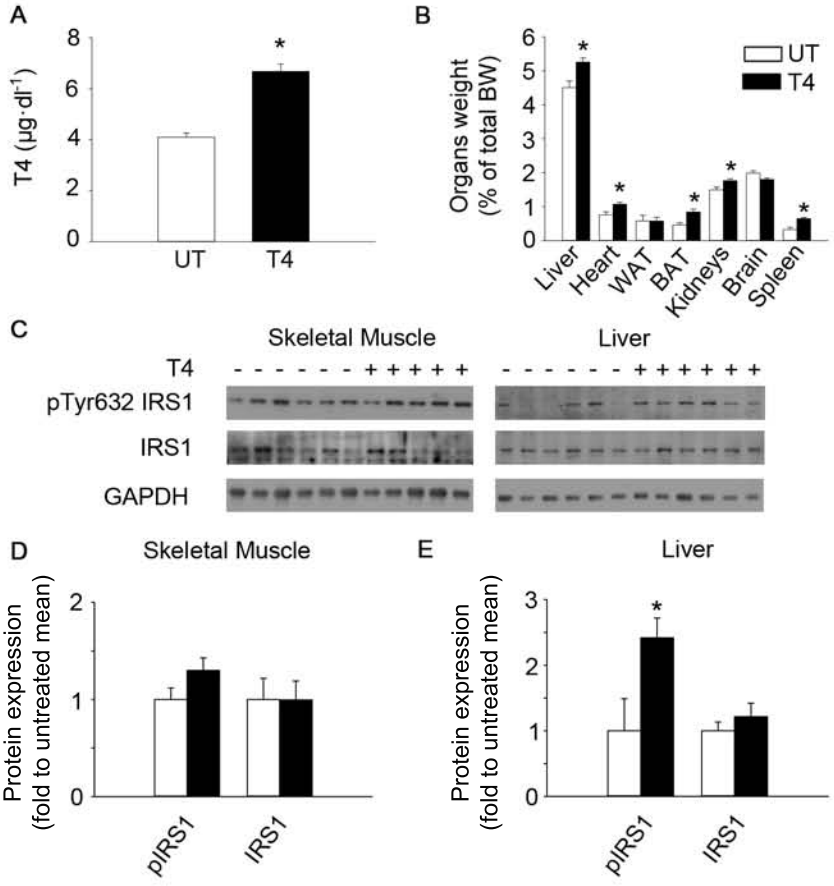




Supporting Information Figure S4



Supporting Information Figure S5



Supporting Information Figure S6