Brown fat activation mitigates alcohol-induced liver steatosis and injury in mice

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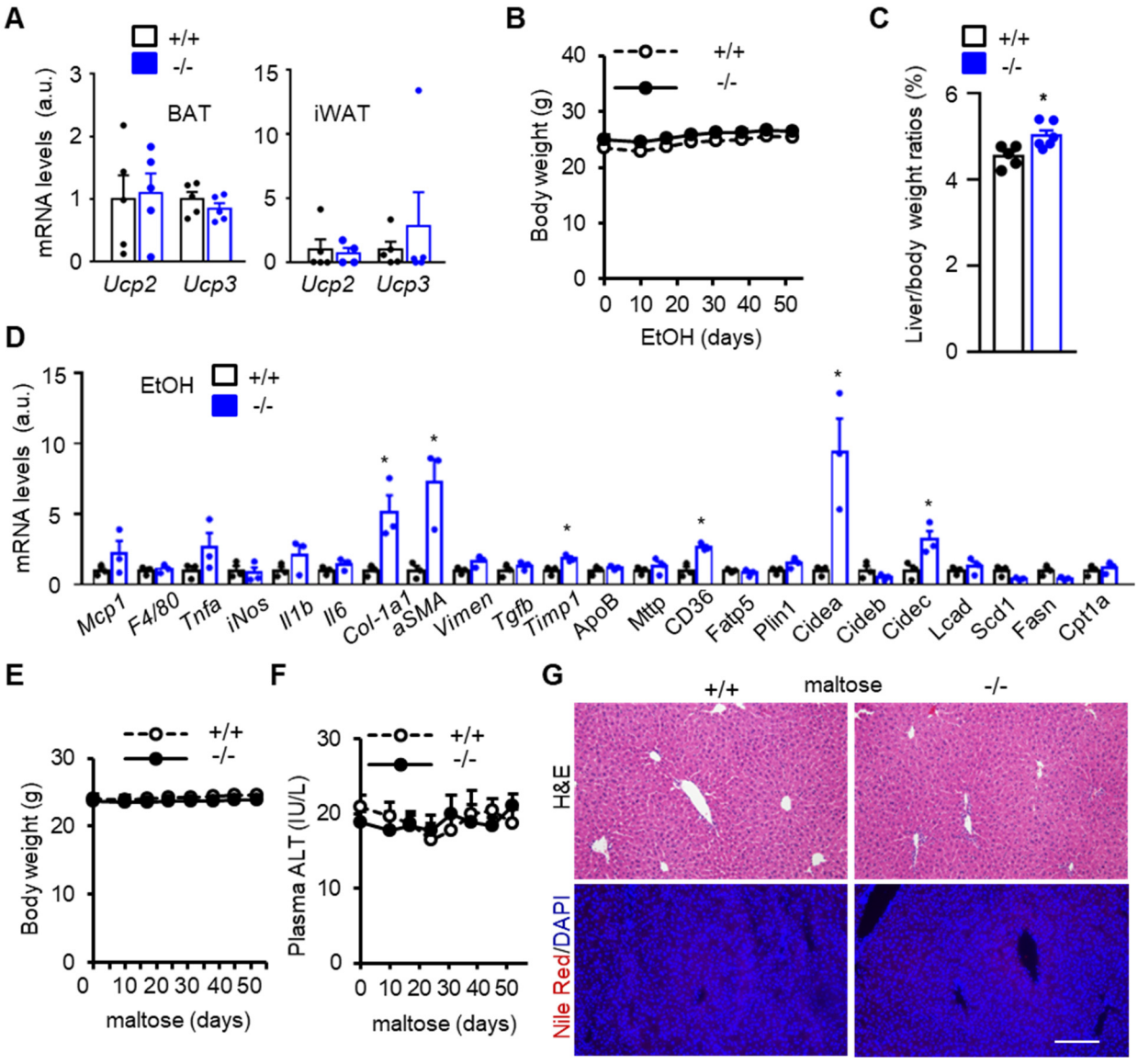
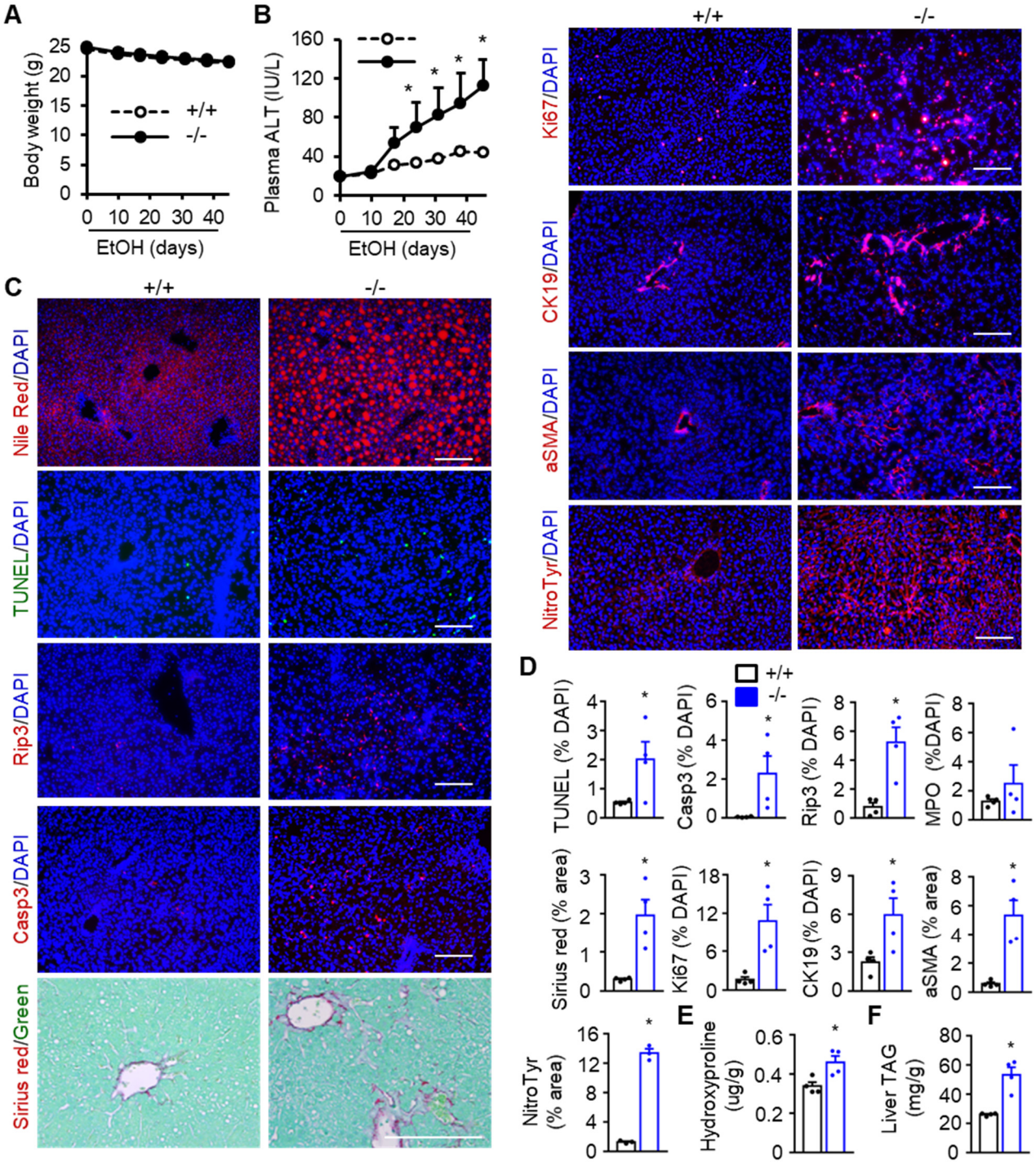
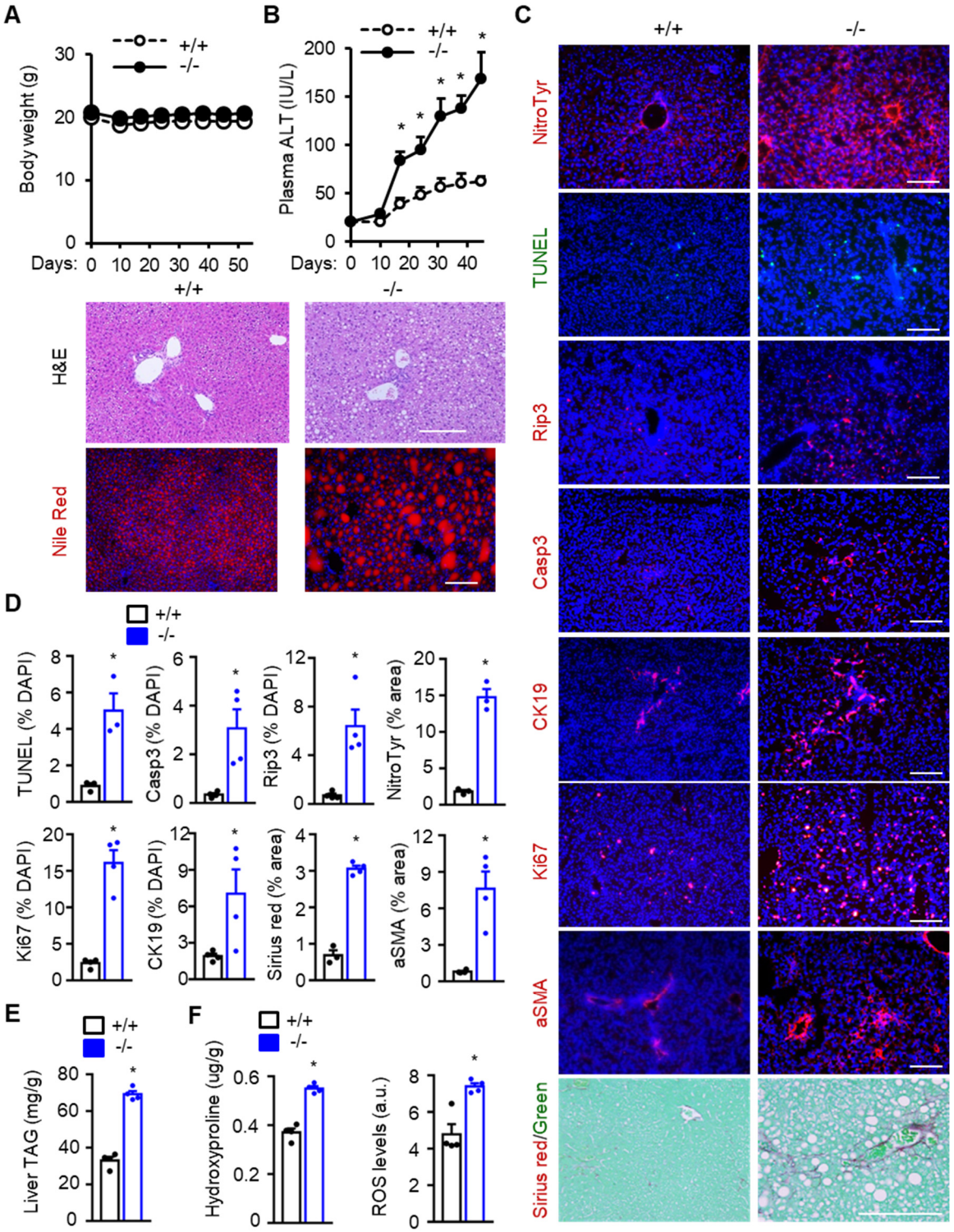
Supplemental Figures



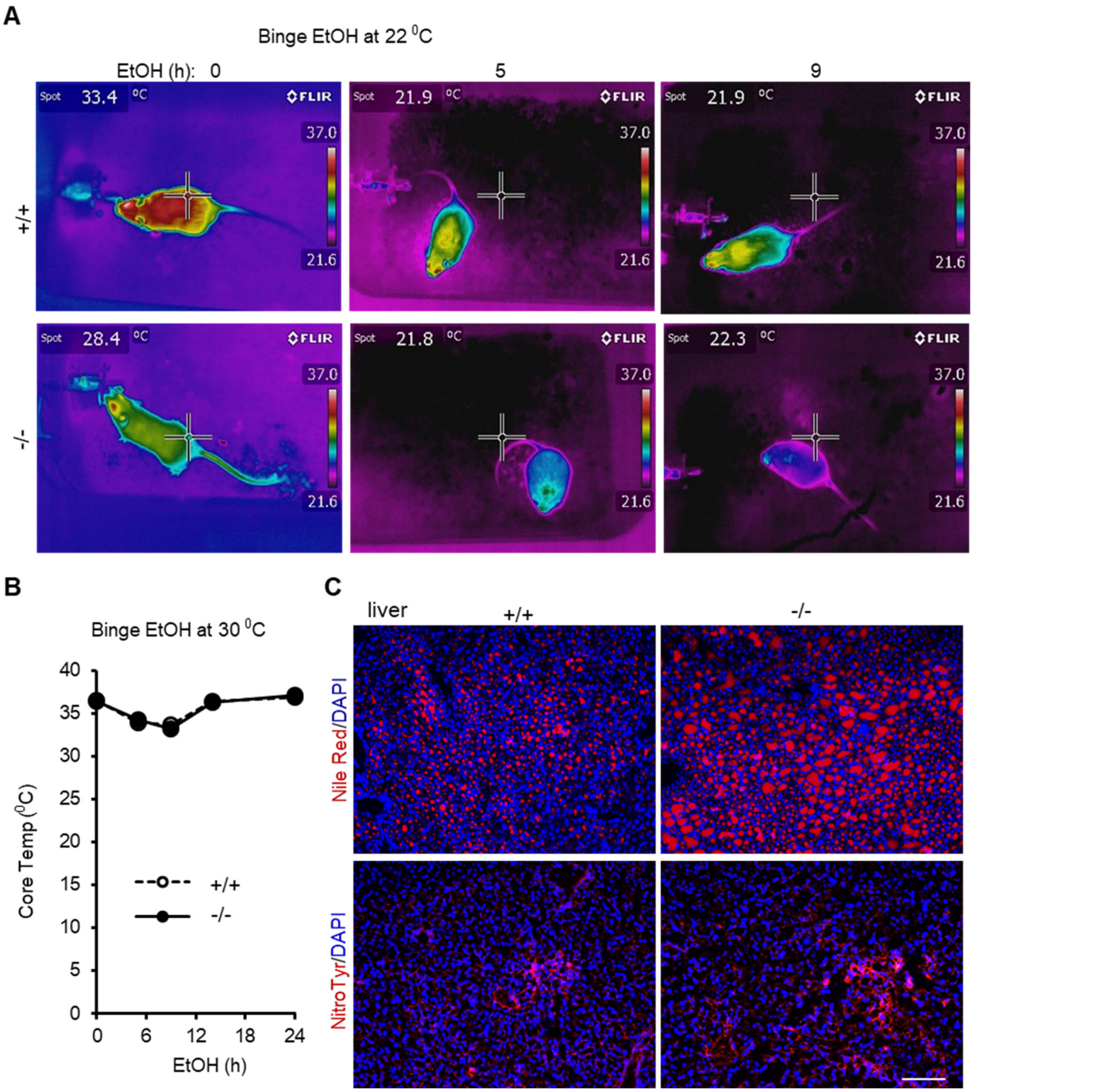
Supplemental Figure 1. Ucp1 deficiency worsens alcoholic liver inflammation. (A, E-G) *Ucp1*$^{+/+}$ (n=5) and *Ucp1*$^{-/-}$ (n=4) males were fed a maltose diet for 7 weeks. **(B-D)** *Ucp1*$^{+/+}$ and *Ucp1*$^{-/-}$ males were fed an alcoholic diet for 7 weeks (n=5-6 per group). **(A)** *Ucp2* and *Ucp3* mRNA abundances in BAT and iWAT (normalized to 18S levels, n=4-5 per group). **(B, E)** Growth curves. **(C)** Liver weight. **(D)** Liver gene expression was measured by qPCR and normalized to 18S levels (n=3 mice per group). **(F)** Plasma ALT activities. **(G)** Representative liver sections. Scale bar: 200 μm. Data are presented as mean ± SEM. *p<0.05, 2-tailed unpaired Student's *t* test.



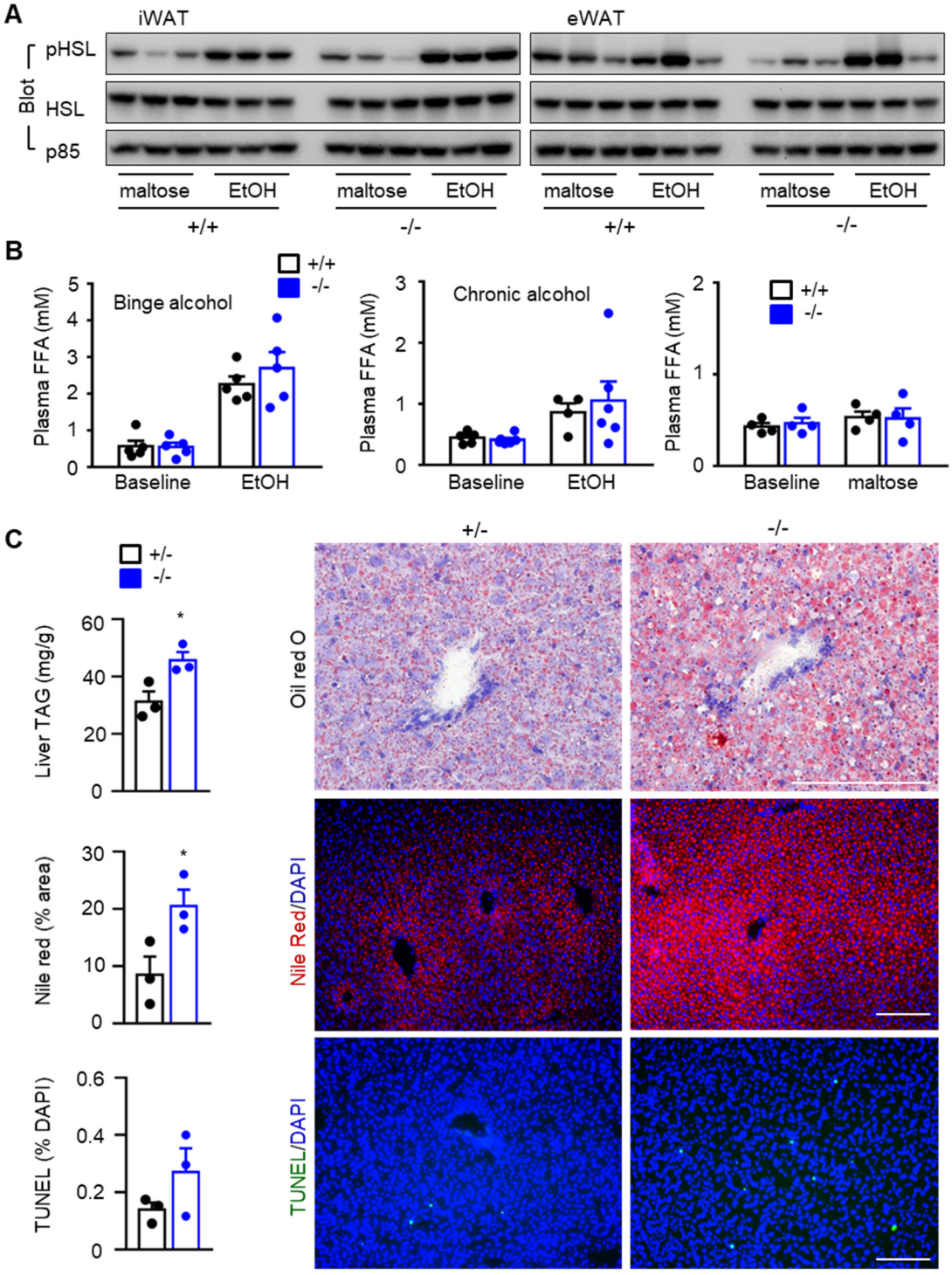
Supplemental Figure 2. Ucp1 deficiency worsens ALD in males. *UcpP1*$^{+/+}$ (n=4) and *Ucp1*$^{-/-}$ (n=4) male mice were fed an alcoholic diet for 6 weeks superimposed with binge alcohol intake (2.5 g/kg body weight, twice a week). **(A)** Growth curves. **(B)** Plasma ALT levels. **(C-D)** Liver sections were stained with the indicated reagents. Positive signals were quantified. Scale bar: 200 μm. Data are presented as mean ± SEM. *p<0.05, 2-tailed unpaired Student's *t* test.



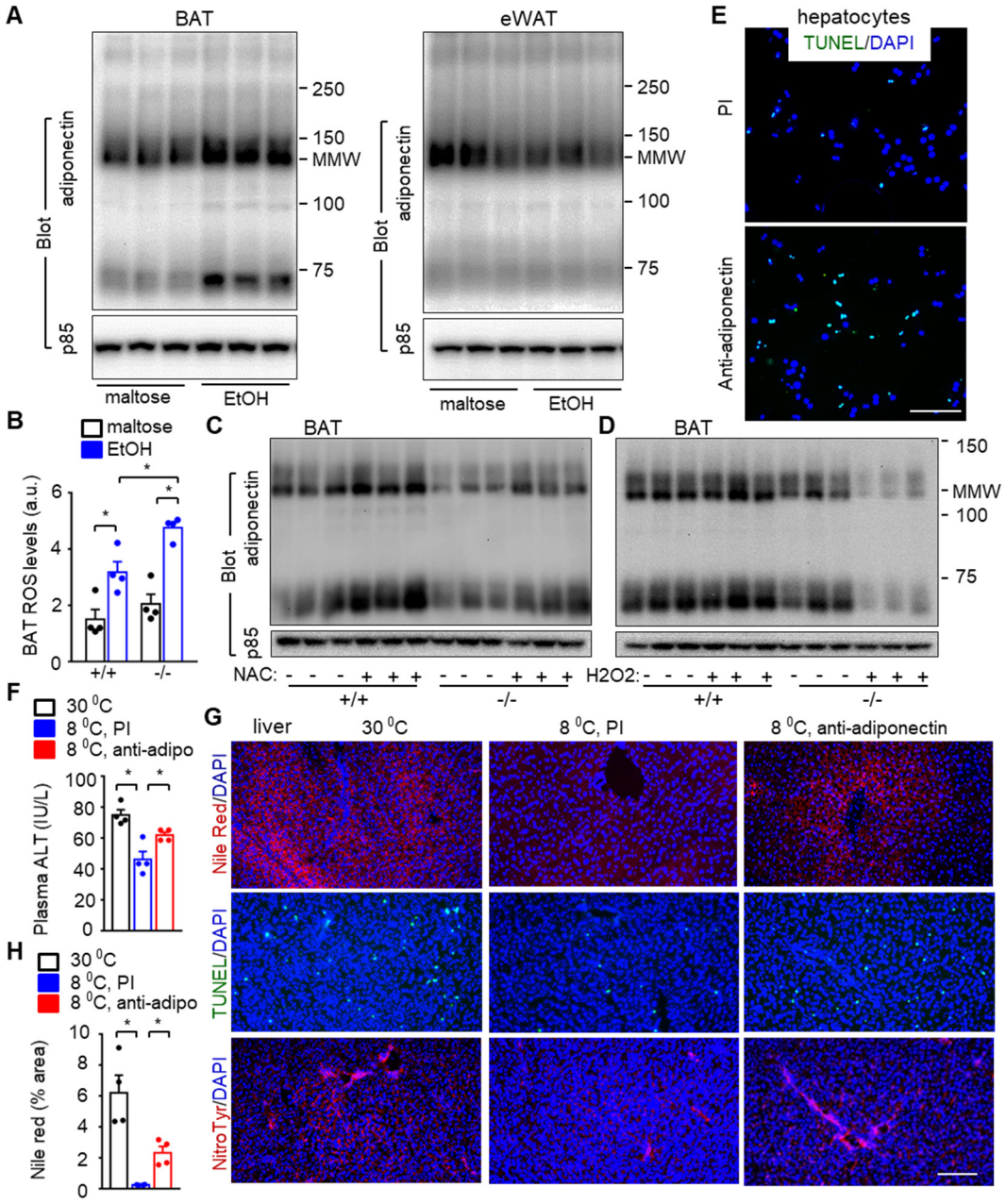
Supplemental Fig. 3. Ucp1 deficiency worsens ALD in females. (A) Body weight (n=4 per group). **(B-F)** *Ucp1*$^{+/+}$ (n=4) and *Ucp1*$^{-/-}$ (n=4) females were fed an alcohol diet for 6 weeks superimposed with multiple bouts of binge drinking (housed at 22 ^{0}C, 2.5 g/kg body weight, twice a week). **(B)** Plasma ALT activity. **(C-D)** Liver sections were stained with the indicated reagents. Positive signals were quantified. Scale bar: 200 μm. **(E)** Liver TAG levels (normalized to liver weight). **(F)** Liver hydroxyproline and ROS levels (normalized to liver weight). Data are presented as mean ± SEM. *p<0.05, 2-tailed unpaired Student's *t* test.



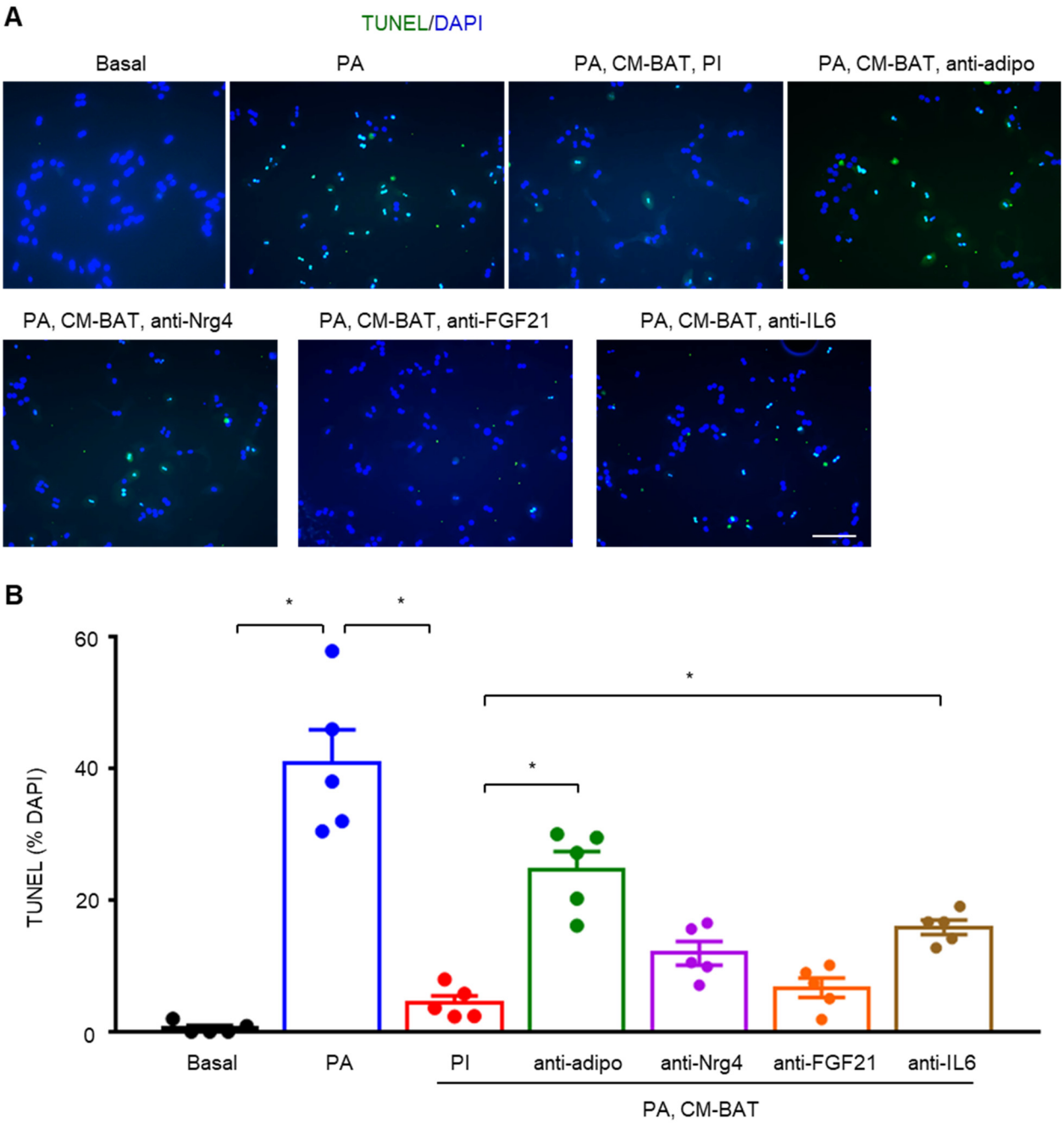
Supplemental Figure 4. Ucp1 deficiency exacerbates acute binge alcohol intake-induced liver steatosis at thermoneutrality. *Ucp1*$^{+/+}$ and *Ucp1*$^{-/-}$ male mice were fed an alcohol diet for 10 days, followed by a single bout of binge alcohol (5 g/kg body weight, via gavage) at 22 ^{0}C **(A)** or 30 ^{0}C **(B-C)**. **(A)** Representative mouse images taken by a thermal imaging camera (FLIR E60BX, Fluke Corporation, Everett, WA). **(B)** Rectal temperature and **(C)** Representative liver sections in 36 h post binge alcohol intake. Scale bar: 200 μm. N=3-4 per group.



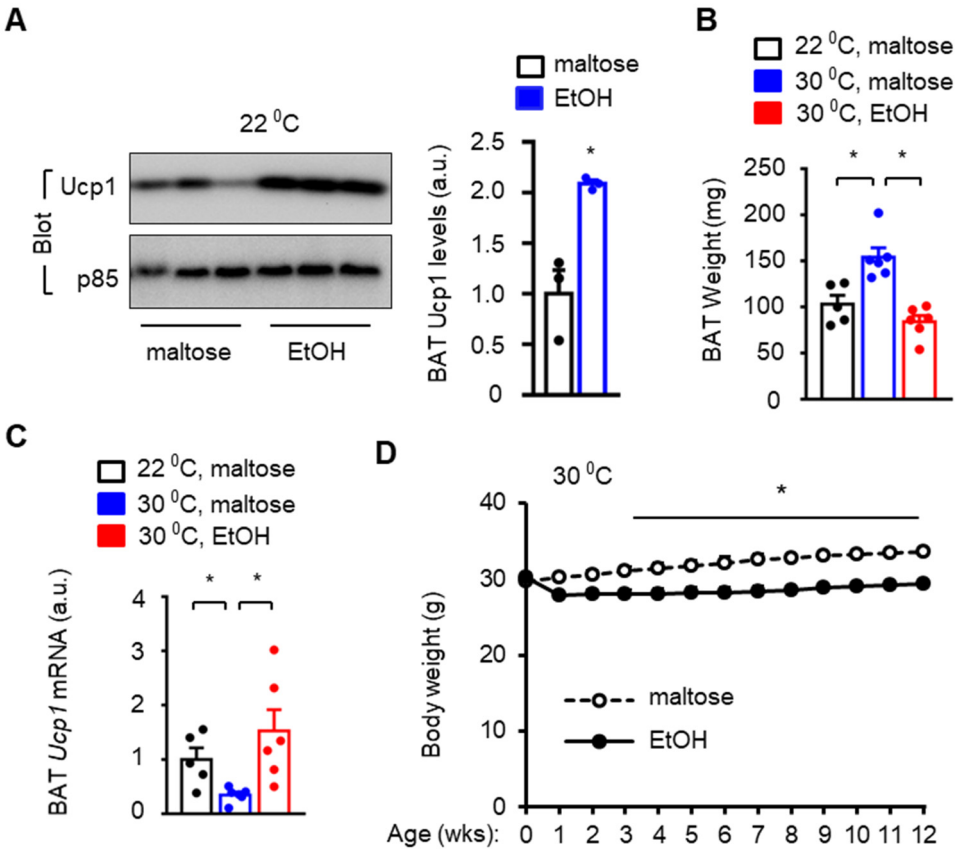
Supplemental Figure 5. BAT protects against β-adrenergic agonist-stimulated liver steatosis. (A-D) *Ucp1*$^{+/+}$ and *Ucp1*$^{-/-}$ male mice were fed an alcohol or maltose diet for 7 weeks. **(A)** eWAT and iWAT extracts were immunoblotted with anti-phospho-HSL, HSL, or p85 (loading control) antibodies. **(B)** Non-fasting plasma free fatty acid (FFA) levels under baseline, single bout of binge alcohol, 7-week alcohol, or 7-week maltose treatment conditions (n=4-6 per group). **(C)** *Ucp1*$^{+/-}$ (n=3) and *Ucp1*$^{-/-}$ (n=3) male mice were injected with CL316243 (5 mg/kg body weight, ip), and liver was harvested 9 h later. Liver TAG levels were normalized to liver weight. Liver sections were stained with the indicated reagents. Positive signals were quantified. Scale bar: 200 μm. Data are presented as mean ± SEM. *p<0.05, 2-tailed unpaired Student's *t* test.



Supplemental Figure 6. BAT-derived adiponectin protects against ALD. (A) C57BL/6 male mice were fed an alcohol or maltose diet for 7 weeks. BAT and epididymal WAT extracts were immunoblotted with anti-adiponectin and anti-p85 antibodies. **(B)** *Ucp1*$^{+/+}$ and *Ucp1*$^{-/-}$ males were fed an alcohol diet for 7 weeks. BAT ROS levels were measured using DCF dyes (normalized to BAT weight, n=4 per group). **(C-D)** SVF-differentiated brown adipocytes were treated with N-acetylcysteine (NAC, 100 μM) or H_2O_2 (100 μM) for 12 h in the presence of CL316243 (0.1 μM). Cell extracts were immunoblotted with the indicated antibodies. MMW: medium molecular weight form. **(E)** BAT conditioned medium was pretreated with anti-adiponectin antibody or pre-immune serum (PI), and then used to treat primary hepatocytes in the presence of PA. TUNEL assays were performed 24 h later. **(F-H)** C57BL/6 males were housed at 30 °C or 8 °C for 10 days and then subjected to a single bout of binge alcohol treatment and assessed 9 h later. The mice were also treated with PI or anti-adiponectin (5 μg/mouse) twice (12 h and 1 h prior to binge alcohol) (n=4 per group). **(F)** Plasma ALT levels (n=4 per group). **(G)** Representative liver sections. **(H)** Nile red areas of the liver sections (n=4 per group). Scale bar: 200 μm. Data are presented as mean ± SEM. *p<0.05, one-way ANOVA after Greenhouse-Geisser correction with subsequent Bonferroni's multiple comparisons.



Supplemental Figure 7. BAT-derived adipokines protect primary hepatocytes from death. BAT-conditioned medium (CM-BAT) was precleared with preimmune serum (PI) or the indicated antibodies (2 µl/ml). Primary hepatocytes were cultured in pretreated CM-BAT in the presence of palmitic acid (PA, 200 µM) for 24 h, and were then subjected to TUNEL assays. **(A)** Representative TUNEL staining of hepatocytes. **(B)** Quantifications of TUNEL$^+$ hepatocytes (n=5 per group). Scale bar: 200 µm. Data are presented as mean ± SEM. *p<0.05, one-way ANOVA after Greenhouse-Geisser correction with subsequent Bonferroni's multiple comparisons.



Supplemental Figure 8. Alcohol intake stimulates BAT thermogenesis. (A) C57BL/6 male mice were raised at 22 ^{0}C and fed an alcohol or maltose diet for 13 weeks. BAT extracts were immunoblotted with antibodies against Ucp1 and p85. Ucp1 levels were normalized to p85 levels. **(B-D)** C57BL/6 male mice (n=5-7 per group) were raised at 22 ^{0}C or 30 ^{0}C and fed an alcohol or maltose diet for 13 weeks. **(B)** BAT weight. **(C)** *Ucp1* mRNA levels (normalized to 18S expression). **(D)** Growth curves at 30 ^{0}C. Data are presented as mean ± SEM. Difference was analyzed by 2-tailed unpaired Student's *t* test (between two groups) or one-way ANOVA after Greenhouse-Geisser correction with subsequent Bonferroni's multiple comparisons (between multiple groups). *p<0.05.

ANTIBODY	SOURCE	Cat#	lot#	clone#	DILUTION
UCP-1	EMD Millipore	662045	D00157127	N/A	1:10,000
Oxphos	Abcam	ab110413	N/A	N/A	1:10,000
TH	Santa Cruz	sc-14007	12909	N/A	1:500
p-HSL (Ser660)	Cell Signaling Technology	4126	3	N/A	1:2,000
HSL	Cell Signaling Technology	4107	2	N/A	1:1,000
c-Fos	Cell Signaling Technology	2250	6	N/A	1:5,000
RIP3	Cell Signaling Technology	14401	1	N/A	1:1,000
Caspase-3	Cell Signaling Technology	9661	2	N/A	1:1,000
Myeloperoxidase	Thermo Fisher	RB-373-A0	373A1709E	N/A	1:1,000
Ki67	Cell Signaling Technology	9129	3	N/A	1:1,000
α-SMA	Sigma	A5228	129k4819	1A4	1:5,000
Keratin-19	DSHB (University of Iowa)	Troma-III	N/A	N/A	1:100
Nitrotyrosine	EMD Millipore	05-233	2552274	1A6	1:1,000
Adiponectin	Sigma	A6354	028M4757V	N/A	1:2,000
IL-6	Biolegend	504508	B232722	MP5-20F3	1:500
FGF21	R&D	AF3057	N/A	N/A	1:500
Nrg4	home made	N/A	N/A	N/A	1:500
p85	home made	N/A	N/A	N/A	1:10,000

Table 1. Antibody list

Genes	Forward	Reverse
Ucp-1	ATACTGGCAGATGACGTCCC	GTACATGGACATCGCACAGC
Ucp-2	GCCACTTCACTTCTGCCTTC	GAGCATGGTAAGGGCACAGT
Ucp-3	GTGGATGTGGTAAAGACCCG	AAAGGAGGGCACAAATCCTT
*IL-1*β	GCCTTGGGCCTCAAAGGAAAGAATC	GGAAGACACAGATTCCATGGTGAAG
IL6	AGCCAGAGTCCTTCAGA	GGTCCTTAGCCACTCCT
iNos	CAGGGCCACCTCTACATTTG	TGCCCCATAGGAAAAGACTG
Tnfα	CATCTTCTCAAAATTCGAGTGACAA	TGGGAGTAGACAAGGTACAACCC
F4/80	CTTTGGCTATGGGCTTCCAGTC	GCAAGGAGGACAGAGTTTATCGTG
Mcp1	ACTGAAGCCAGCTCTCTCTTCCTC	TTCCTTCTTGGGGTCAGCACAGAC
vimentin	GACCTCACTGCTGCCCTGCG	GACTCCTGCTTGGCCTGGCG
*Tgf-*β*1*	TTGCTTCAGCTCCACAGAGA	TGGTTGTAGAGGGCAAGGAC
Timp-1	GCTAAATTCATGGGTTCCCCAG	GAGAAAGCTCTTTGCTGAGCAG
αSMA	GTTCAGTGGTGCCTCTGTCA	ACTGGGACGACATGGAAAAG
Colla 1a1	TCACCTACAGCACCCTTGTG	GGTGGAGGGAGTTTACACGA
ApoB	CCAGAGTGTGGAGCTGAATGT	TTGCTTTTTAGGGAGCCTAGC
Mttp	CTCCACAGTGCAGTTCTCACA	AGAGACATATCCCCTGCCTGT
CD36	GGAGTGGTGATGTTTGTTGCT	GCACACACCACCATTTCTTCT
Fatp5	CGAGCTACGATTAAGTGGAAATC	GCACGTTGCTCACTTGTATGA
Plin1	CACTCTCTGGCCATGTGGAT	AGAGGCTGCCAGGTTGTG
Cidea	ATCACAACTGGCCTGGTTACG	TACTACCCGGTGTCCATTTCT
Cideb	GACCCTTCCGTGTCTGTGAT	GTAGCAGCAAGGTCTCCAGG
Cidec	CCTATGACCTGCACTGCTACAAG	CATGTAGCTGGAGGTGCCAAG
Lcad	CACTCAGATATTGTCATGCCCT	TCCATTGAGAATCCAATCACTC
Scd1	AGGTGCCTCTTAGCCACTGA	CCAGGAGTTTCTTGGGTTGA
Fasn	TTGACGGCTCACACACCTAC	CGATCTTCCAGGCTCTTCAG
Cpt1a	CTGATGACGGCTATGGTGTTT	GTGAGGCCAAACAAGGTGATA
*Pgc-1*α	TGGACGGAAGCAATTTTTCA	TTACCTGCGCAAGCTTCTCT
TH	CCTCACCTATGCACTCACCC	GAACCAGTACACCGTGGAGA
Dio2	GCACGTCTCCAATCCTGAAT	TGAACCAAAGTTGACCACCA
Elolv3	TCCGCGTTCTCATGTAGGTCT	GGACCTGATGCAACCCTATGA
Prdm16	AGCAGCTGAGGAAGCATTT	GCGTGGAGAGGAGTGTCTTC
18S	CGCTTCCTTACCTGGTTGAT	GAGCGACCAAAGGAACCATA

Table 2. Primer list.