Supplementary Information

Depletion of B cell-activating factor attenuates hepatic fat accumulation in a murine model of nonalcoholic fatty liver disease

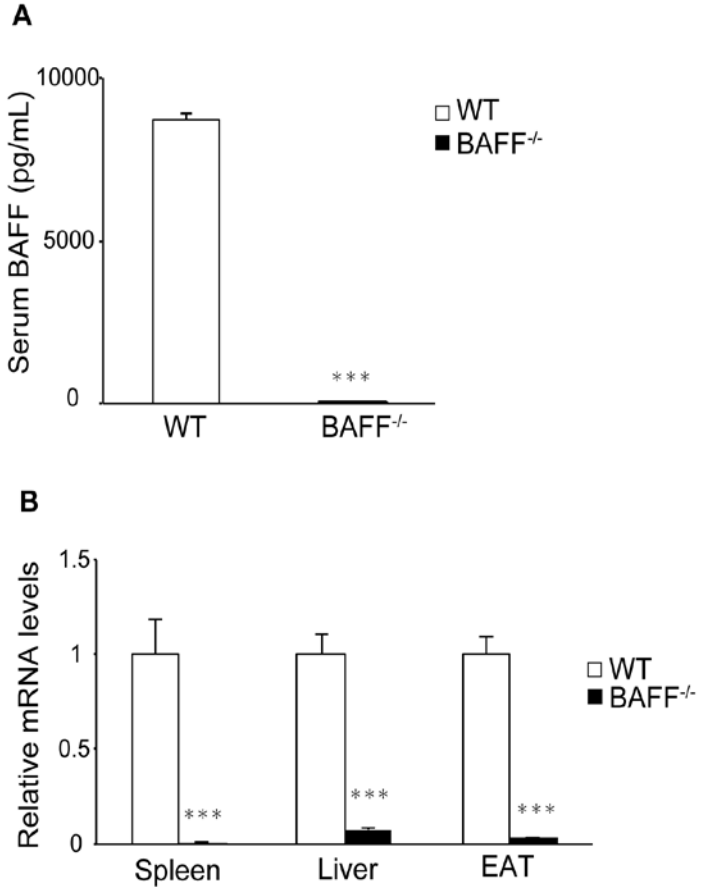
Yoshiko Nakamura, Masanori Abe, Keitarou Kawasaki, Teruki Miyake, Takao Watanabe, Osamu Yoshida, Masashi Hirooka, Bunzo Matsuura, Yoichi Hiasa

Table S1. Oligonucleotide sequences and annealing temperatures for quantitative real-time PCR.

Gene	GenBank Accession No.	Sequence (5′-forward-3′ 5′-reverse-3′)	Location	Annealing Temperature (°C)
HPRT1	NM_013556	tcctcctcagaccgcttt cctggttcatcatcgctaatc	nt104–122 nt173–193	60
F4/80	NM_010130	agtacgatgtggggcttttg ccccatctgtacatcccact	nt35–54 nt77–96	60
CD11c	NM_021334	atggagcctcaagacaggac ggatctgggatgctgaaatc	nt1725–1744 nt1768–1787	60
TNF-α	NM_013693	tcttctcattcctgcttgtgg ggtctgggccatagaactga	nt260–280 nt368–387	60
CD36	NM_016741	cgtctacccacccaacga agaaacagaggcgcacca	nt1148–1165 nt1220–1237	65
SREBP-1c	ENSMUST0000 0144942	catggattgcacatttgaaga cgggaagtcactgtcttggt	nt111–131 nt152–171	65
FAS	NM_007988	gctgctgttggaagtcagc agtgttcgttcctcggagtg	nt767–785 nt823–842	70
ACC	NM_133360	gcgtcgggtagatccagtt ctcagtggggcttagctctg	nt6434–6452 nt6484–6503	60
SCD-1	NM_009127	ttccctcctgcaagctctac cagagcgctggtcatgtagt	nt566–585 nt608–627	60
ACS	NM_007981	aaagatggctggttacacacg cgataatcttcaaggtgccatt	nt1818–1838 nt1863–1884	57
CD36	NM_001159555	ttgtacctatactgtggctaaatgaga cttgtgttttgaacatttctgctt	nt1689–1715 nt1737–1760	57
MTP	NM_008642	tgtcagaatgaaggctgcaa agtcctcccaggatcagctt	nt1395–1414 nt1440–1459	60
TGF-β1	NM_011577	tggagcaacatgtggaactc cagcagccggttaccaag	nt1358–1377 nt1411–1428	60
IL-6	NM_031168	acaaccacggccttccctactt cacgatttcccagagaacatgtg	nt92–113 nt198–220	63
IL-1β	NM_008361	ttgacggaccccaaaagat agctggatgctctcatcagg	nt142–160 nt195–214	60
IL-10	NM_010548	ggttgccaagccttatcgga acctgctccactgccttgct	nt302–321 nt473–492	63
IL-18	NM_008360	gacaacacgctttactttatacctga cagtgaagtcggccaaagtt	nt73–84 nt99–112	70
MCP-1	NM_011333	catccacgtgttggctca gatcatcttgctggtgaatgagt	nt139–56 nt192–214	70

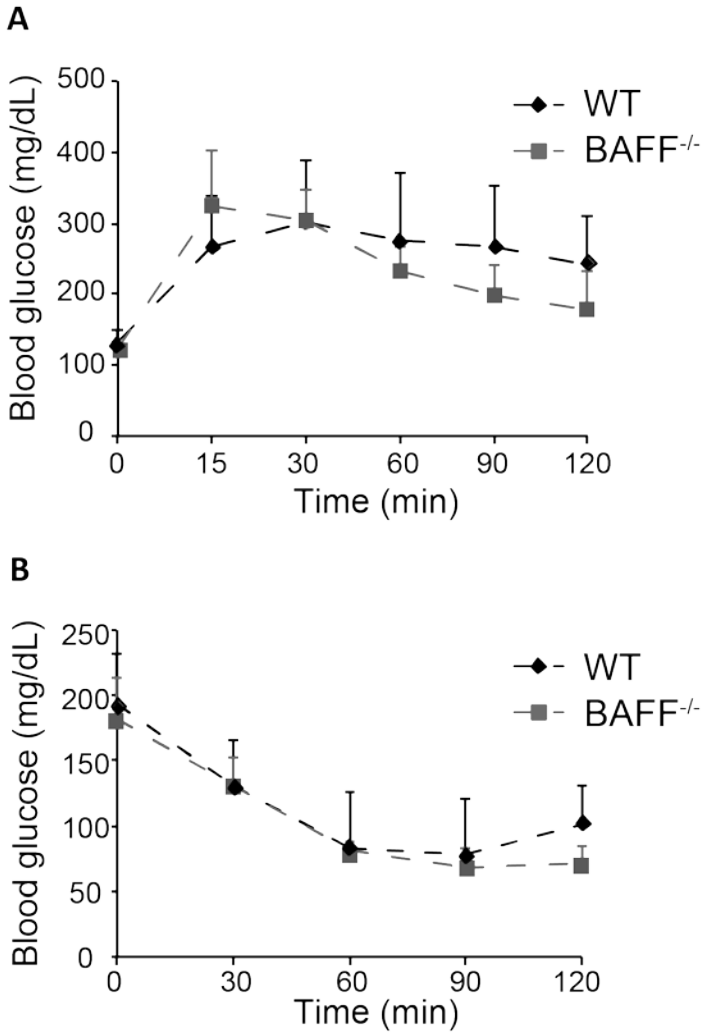
Resistin	ENSMUST0000 0012849	tgccagtgtgcaaggataga tggaaaccacgctcacttc	nt367–386 nt437–455	70
Adiponectin	NM_009605	ggagagaaaggagatgcaggt ctttcctgccaggggttc	nt319–339 nt410–427	67
α-SMA	NM_007392	cccacccagagtggagaa acatagctggagcagcgtct	nt9–26 nt55–74	65
Col-1a1	NM_007742	caggcaagcctggtgaac aacctctctcgcctcttgc	nt2030–2047 nt2089–2107	65
Col-1a2	NM_007743	gctgctcagtattctgacaaagg cctctgggtcccattaaacc	nt393–415 nt441–460	65
CD206	NM_008625.2	ggtctatggaaccacggatg tgcattgcccagtaaggagt	nt519–538 nt569–588	63
Arg1	NM_007482.3	gtctgtggggaaagccaat cttccaactgccagactgtg	nt315–333 nt403–422	60

HPRT1: hypoxanthine phosphoribosyltransferase 1, TNF: tumour necrosis factor; SREBP: sterol regulatory element-binding protein; FAS: fatty acid synthase; ACC: acetyl-CoA carboxylase; SCD: stearoyl-CoA desaturase; ACS: acyl-CoA synthetase, MTP: microsomal triglyceride transfer protein; TGF: transforming growth factor; IL: interleukin; MCP-1: monocyte chemotactic protein-1, SMA: smooth muscle actin, Col: collagen, Arg1: arginase 1



Supplementary Fig. S1. Verification of *BAFF* knockout. *BAFF* knockout was confirmed by (A) measurement of serum BAFF concentrations (n = 6) and (B) real-time PCR for quantitation of *BAFF* levels in samples from spleens, livers, and EAT (n = 8). $BAFF^{-/-}$ and C57BL/6J mice were fed an ND for 18 weeks. Data represent the mean ± SEM. ***P < 0.001, Mann–Whitney U test.

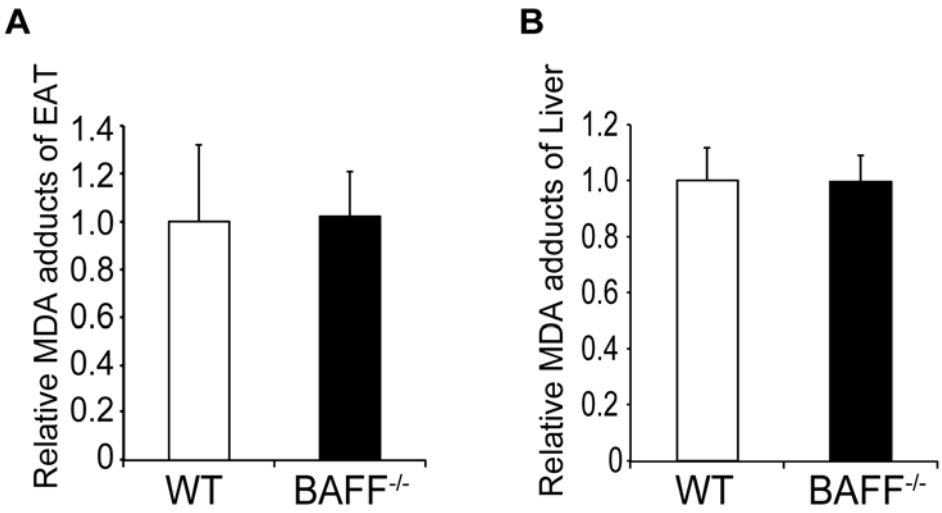
BAFF, B cell-activating factor; EAT, epididymal adipose tissue; ND, normal diet.



Supplementary Fig S2. Insulin sensitivity in *BAFF*$^{-/-}$ mice fed with an ND

(A) Glucose- and (B) insulin-tolerance tests in ND-fed WT and *BAFF*$^{-/-}$ mice (n = 5/group). For all bar plots, data are expressed as the mean ± SD.

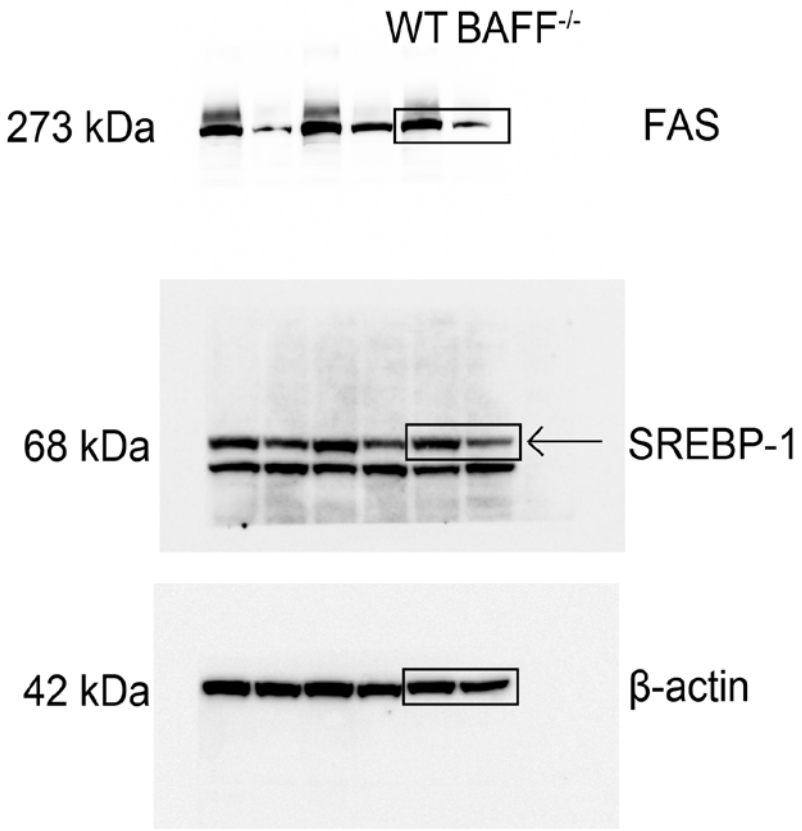
BAFF, B cell-activating factor; ND, normal diet; WT, wild-type



Supplementary Fig. 3 MDA adduct levels in the EAT and liver

MDA adduct levels of lysate of (A) EAT and (B) liver from *BAFF*$^{-/-}$ and WT mice fed with an HFD for 24 weeks, were estimated by ELISA (*n* = 7/group). Data are expressed as the mean ± SD and expressed as a ratio to values obtained for the WT mice.

MDA, malondialdehyde; BAFF, B cell-activating factor; EAT, epididymal adipose tissue; ND, HFD, high-fat diet; WT, wild-type



Supplementary Fig. S4. Original blots for Figure 6B.