SUPPLEMENTARY DATA

Supplementary Table 1. Antibodies used for immunoblotting and FACS analysis.

Antibodies	Supplier
pS^{473}Akt	Cell Signaling Technology #9271
Akt	Cell Signaling Technology #9272
pS^{79}-ACC	Cell Signaling Technology #3661
ACC	Cell Signaling Technology #3662
pT^{172}-AMPKα	Cell Signaling Technology #2531
AMPKα	Cell Signaling Technology #2532
pT^{202}/Y^{204}Erk1/2	Cell Signaling Technology #9101
Erk1/2	Cell Signaling Technology #9102
pT^{183}/Y^{185}JNK	Cell Signaling Technology #9255
JNK	Cell Signaling Technology #9258
pS^{536}NF-κB p65	Cell Signaling Technology #3033
NF-κB p65	Cell Signaling Technology #3034
Tubulin	Cell Signaling Technology #2128
Ucp1	Abcam ab10983
PerCP-Cyanine5.5-conjugated NK1.1	eBioscience #45-5941
PerCP-Cyanine5.5-conjugated CD3	eBioscience #45-036
PerCP-Cyanine5.5-conjugated CD19	eBioscience #45-0193
PerCP-Cyanine5.5-conjugated TER-119	eBioscience #45-5921
Allophycocyanin-eFluor 780-conjugated CD45	eBioscience#47-0451
Phycoerythrin-conjugated CD11c	eBioscience #12-0114
eFluor 450-conjugated Ly-6G (Gr-1)	eBioscience #48-5931
PE/Cy7-conjugated F4/80	Biolegend #123113
Alexa Fluor 647-conjugated CD206	Biolegend #141712
PE-Texas Red-conjugated CD11b	Invitrogen #RM2817

SUPPLEMENTARY DATA

Supplementary Table 2. Primers used for quantitative RT-PCR.

Gene/Primer	Forward	Reverse
Catalase	CCAGCGACCAGATGAAGCAG	CCACTCTCTCAGGAATCCGC
Ccl2	AGGTCCCTGTCATGCTTCTGG	CTGCTGCTGGTGATCCTCTTG
Ccr2	ATTCTCCACACCCTGTTTCG	GATTCCTGGAAGGTGGTCAA
CD11b	CATCAAGGGCAGCCAGATTG	GAGGCAAGGGACACACTGAC
CD11c	AAAATCTCCAACCCATGCTG	CACCACCAGGGTCTTCAAGT
CD68	CTTCCCACAGGCAGCACAG	AATGATGAGAGGCAGCAAGAGG
Cidea	TGCTCTTCTGTATCGCCCAGT	GCCGTGTTAAGGAATCTGCTG
Cpt1a	AAACCCACCAGGCTACAGTG	TCCTTGTAATGTGCGAGCTG
Dio2	CTTCTGAGCCGCTCCAAGTC	CACCCAGTTTAACCTGTTTGTAGG
Elovl3	TCCGCGTTCTCATGTAGGTCT	GGACCTGATGCAACCCTATGA
F4/80	CTTTGGCTATGGGCTTCCAGTC	GCAAGGAGGACAGAGTTTATCGTG
Fasn	AGAGACGTGTCACTCCTGGACTT	GCTGCGGAAACTTCAGAAAAT
Fabp4	GGGGCCAGGCTTCTATTCC	GGAGCTGGGTTAGGTATGGG
gp91^{phox}	TTGGGTCAGCACTGGCTCTG	TGGCGGTGTGCAGTGCTATC
Gpx1	TTCGGACACCAGGAGAATGG	TAAAGAGCGGGTGAGCCTTC
Hmox1	CAGAGCCGTCTCGAGCATAG	CAAATCCTGGGGCATGCTGT
Lbp	GTCCTGGGAATCTGTCCTTG	CCGGTAACCTTGCTGTTGTT
Nqo1	CTCTGGCCGATTCAGAGTGG	CTCCCAGACGGTTTCCAGAC
p22^{phox}	GTCCACCATGGAGCGATGTG	CAATGGCCAAGCAGACGGTC
p47^{phox}	GATGTTCCCCATTGAGGCCG	GTTTCAGGTCATCAGGCCGC
p67^{phox}	CTGGCTGAGGCCATCAGACT	AGGCCACTGCAGAGTGCTTG
Pparα	GAGGGTTGAGCTCAGTCAGG	GGTCACCTACGAGTGGCATT
Pparγ	GAAACTCTGGGAGATTCTCCT	CAGAGCTGATTCCGAAGTTGG
Ppargc1a	ATGTGTCGCCTTCTTGCTCT	ATCTACTGCCTGGGGACCTT
Prdm16	CAGCACGGTGAAGCCATTC	GCGTGCATCCGCTTGTG
Prdx1	TGTCCCACGGAGATCATTGC	GGGTGTGTTAATCCATGCCAG
Sod1	CAGCATGGGTTCCACGTCCA	CACATTGGCCACACCGTCCT
Srebf1	GGAGCCATGGATTGCACATT	GGCCCGGGAAGTCACTGT
Tnfa	CCCACACCGTCAGCCGATTT	GTCTAAGTACTTGGGCAGATTGACC
Ucp1	ACTGCCACACCTCCAGTCATT	CTTTGCCTCACTCAGGATTGG
Ucp2	ATGGTTGGTTTCAAGGCCACA	CGGTATCCAGAGGGAAAGTGAT
Ucp3	CTGCACCGCCAGATGAGTTT	ATCATGGCTTGAAATCGGACC

SUPPLEMENTARY DATA

Supplementary Table 3. Metabolic parameters of Nrf2$^{-/-}$ mice.

	NC (n = 8)	NC-GR (n =8)	HFD (n = 11)	HFD-GR (n = 7)
Plasma TG (mg/dL)	99 ± 11	102 ± 11	114 ± 11	131 ± 15
Plasma TC (mg/dL)	143 ± 25	152 ± 23	187 ± 18	199 ± 21
Plasma FFAs (mmol/L)	1.4 ± 0.1	1.3 ± 0.1	1.1 ± 0.1*	1.0 ± 0.1**
Blood glucose (mg/dL)				
Fed	158 ± 7	161 ± 6	191 ± 8**	176 ± 6
Fasted	65 ± 3	68 ± 4	100 ± 6**	98 ± 5**
Plasma insulin (ng/mL)				
Fed	2.2 ± 0.3	2.1 ± 0.3	9.6 ± 2.5	7.2 ± 2.1
Fasted	0.5 ± 0.1	0.5 ± 0.1	1.3 ± 0.1**	1.7 ± 0.2**
HOMA-IR	2.0 ± 0.4	2.0 ± 0.5	9.2 ± 1.1**	10.7 ± 1.7**
Plasma ALT (IU/L)	14.5 ± 1.8	13.8 ± 2.0	47.8 ± 6.6**	44.7 ± 4.7**
Plasma AST (IU/L)	52.7 ± 4.2	50.2 ± 3.9	105.4 ± 9.0**	105.1 ± 15.4**

Shown are blood glucose and plasma insulin levels of mice fed (ad libitum) or fasted for 16 h. Plasma triglyceride (TG), total cholesterol (TC), and free fatty acids (FFAs) were measured in fasting plasma. Measurements are reported as mean ± SEM. *P< 0.05, **P< 0.01 *vs.* NC. NC: normal chow, HFD: high-fat diet, GR: glucoraphanin, HOMA-IR: homeostasis model assessment of insulin resistance, ALT: alanine transaminase, AST: aspartate transaminase.

SUPPLEMENTARY DATA

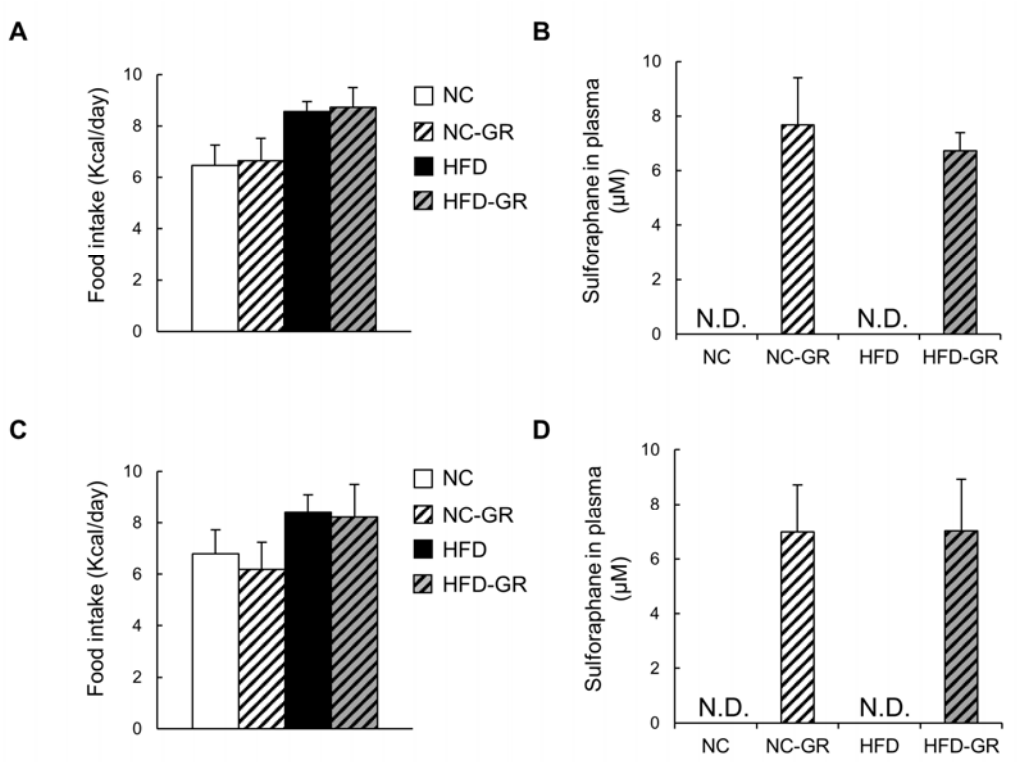
Supplementary Table 4. Metabolic endotoxemia positively correlated with mRNA expression of genes involved in inflammation, oxidative stress, and macrophage infiltration in the liver.

		Inflammation				Oxidative stress				Macrophage infiltration		
	LPS	Tnf-α	Il-1β	Ccl2	Ccr2	gp91^{phox}	p22^{phox}	p47^{phox}	p67^{phox}	F4/80	Cd11b	Cd68
LPS	1											
Tnf-α	0.44*	1										
Il-1β	0.47*	0.55**	1									
Ccl2	0.64**	0.76**	0.54**	1								
Ccr2	0.55**	0.56**	0.65**	0.75**	1							
gp91^{phox}	0.59**	0.66**	0.74**	0.80**	0.69**	1						
p22^{phox}	0.47*	0.70**	0.64**	0.72**	0.53*	0.94**	1					
p47^{phox}	0.58**	0.61**	0.84**	0.55**	0.58**	0.89**	0.86**	1				
p67^{phox}	0.65**	0.67**	0.72**	0.72**	0.69**	0.83**	0.87**	0.85**	1			
F4/80	0.51*	0.53**	0.67**	0.51*	0.51*	0.85**	0.90**	0.87**	0.81**	1		
Cd11b	0.57**	0.60**	0.60**	0.70**	0.50*	0.88**	0.84**	0.71**	0.77**	0.78**	1	
Cd68	0.53*	0.68**	0.64**	0.79**	0.50*	0.91**	0.90**	0.67**	0.69**	0.63**	0.90**	1

Values correspond to Spearman's r correlation. *P< 0.05, ** P< 0.01

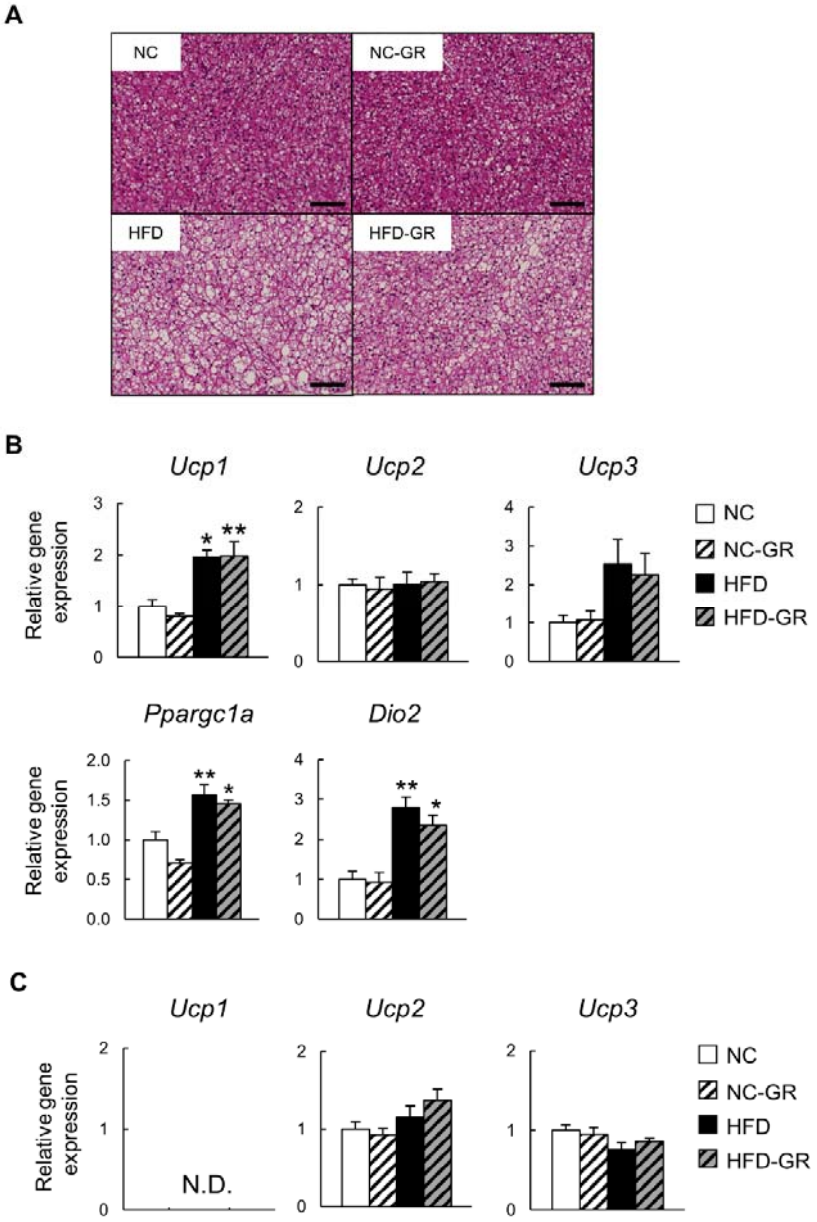
SUPPLEMENTARY DATA

Supplementary Figure 1—*A.* Food intake of wild-type mice fed normal chow (NC), chow with glucoraphanin (NC-GR), a high-fat diet (HFD), or HFD with GR (HFD-GR) was measured after 3 weeks of feeding. Data are presented as the mean ± SEM (n = 9/group). *B*: Plasma concentration of sulforaphane in wild-type mice fed the indicated diet for 6 weeks. Data are presented as the mean ± SEM (n = 3/group). *C:* Food intake of $Nrf2^{-/-}$ mice fed the indicated diet after 3 weeks. Data are presented as the mean ± SEM (n = 7–11/group). *D*: Plasma concentration of sulforaphane of $Nrf2^{-/-}$ mice fed the indicated diet for 6 weeks. Data are presented as the mean ± SEM (n = 5–6/group).



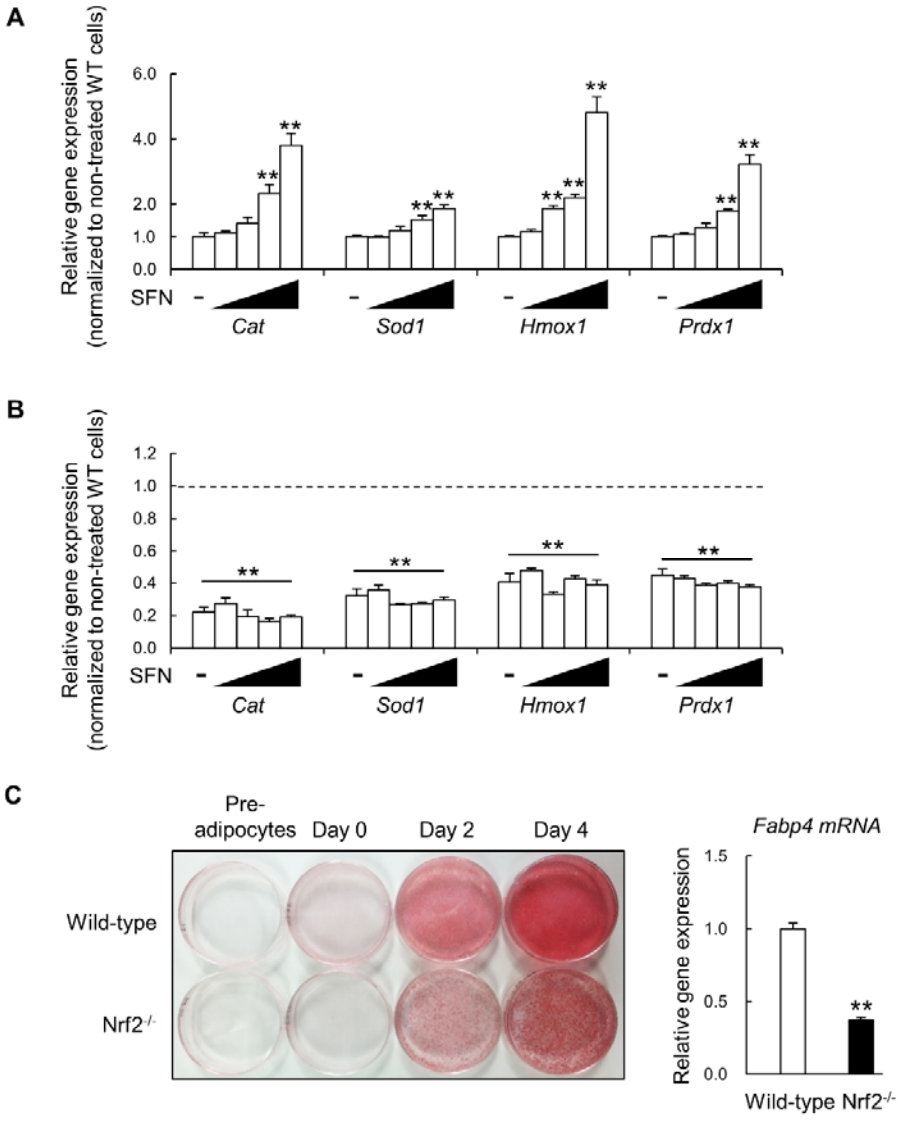
SUPPLEMENTARY DATA

Supplementary Figure 2—*A*. H&E staining of brown adipose tissue of mice fed the indicated diet for 14 weeks (original magnification, ×200; scale bars, 100 μm). *B*: Relative mRNA expression of genes implicated in uncoupling proteins (*Ucp-1*, *Ucp-2*, and *Ucp-3*), PGC-1α (*Ppargc1a*), and Deiodinase 2 (*Dio2*) in the brown adipose tissue of mice fed the indicated diet for 14 weeks (n = 8/group). *C*: Relative mRNA expression of genes implicated in uncoupling proteins in quadriceps muscle of mice fed the indicated diet for 14 weeks. Results represent the mean ± SEM (n = 8/group). **P* < 0.05, ***P* < 0.01 *vs.* NC. N.D., not detected.



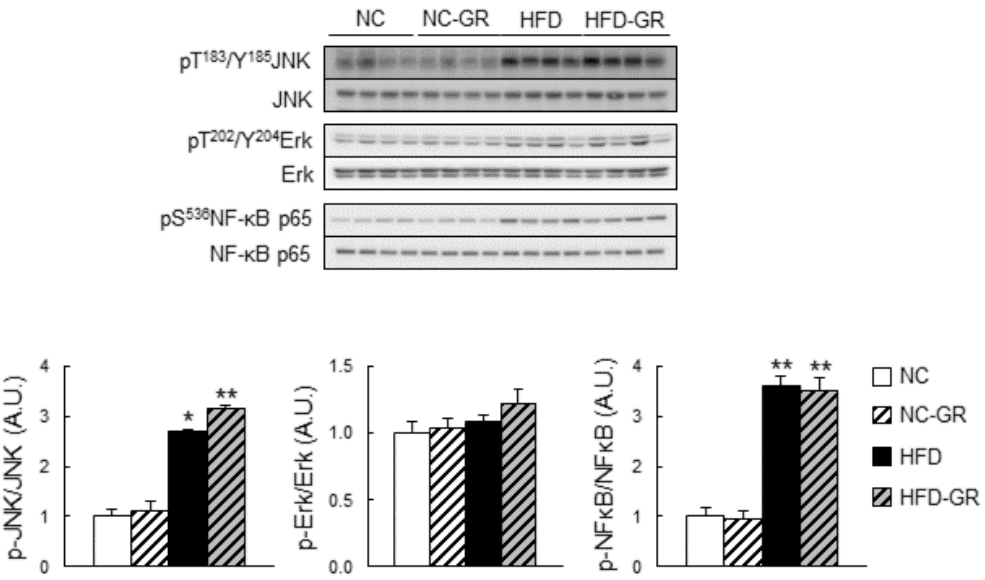
SUPPLEMENTARY DATA

Supplementary Figure 3. Stromal vascular fraction cells were grown to confluence, then differentiation was induced as described in Methods. *A*: Quantitative real-time PCR determination of mRNA levels of Nrf2 target antioxidant genes in the absence or presence of sulforaphane (SFN; 0.2, 1, 2, or 5 μM) for 48 h in primary beige adipocytes isolated from inguinal WAT of wild-type mice (normalized against 36B4). *B*: The same experiment as in (*A*) was repeated with primary beige adipocytes isolated from inguinal WAT of Nrf2$^{-/-}$ mice. Bar graphs represent mean ± SEM (n = 6/group). **P* < 0.05, ***P* < 0.01 *vs.* DMSO-treated adipocytes from wild-type mice. The difference was determined using a one-way ANOVA. Post hoc analysis was performed using Dunnett's test. Cat: catalase, Sod1: superoxide dismutase 1, Hmox1: heme oxygenase 1, Prdx1: peroxiredoxin 1. *C* (left panel): At various stages of differentiation, cells were fixed and stained with oil red O. *C* (right panel): Quantitative real-time PCR determination of mRNA levels of adipocyte differentiation marker, fatty acid binding protein 4 (*Fabp4*), in wild-type and Nrf2-deficient beige adipocytes after 7 days of culture with maintenance medium. Bar graphs represent mean ± SEM (n = 6/group). ***P* < 0.01 *vs.* wild-type adipocytes.



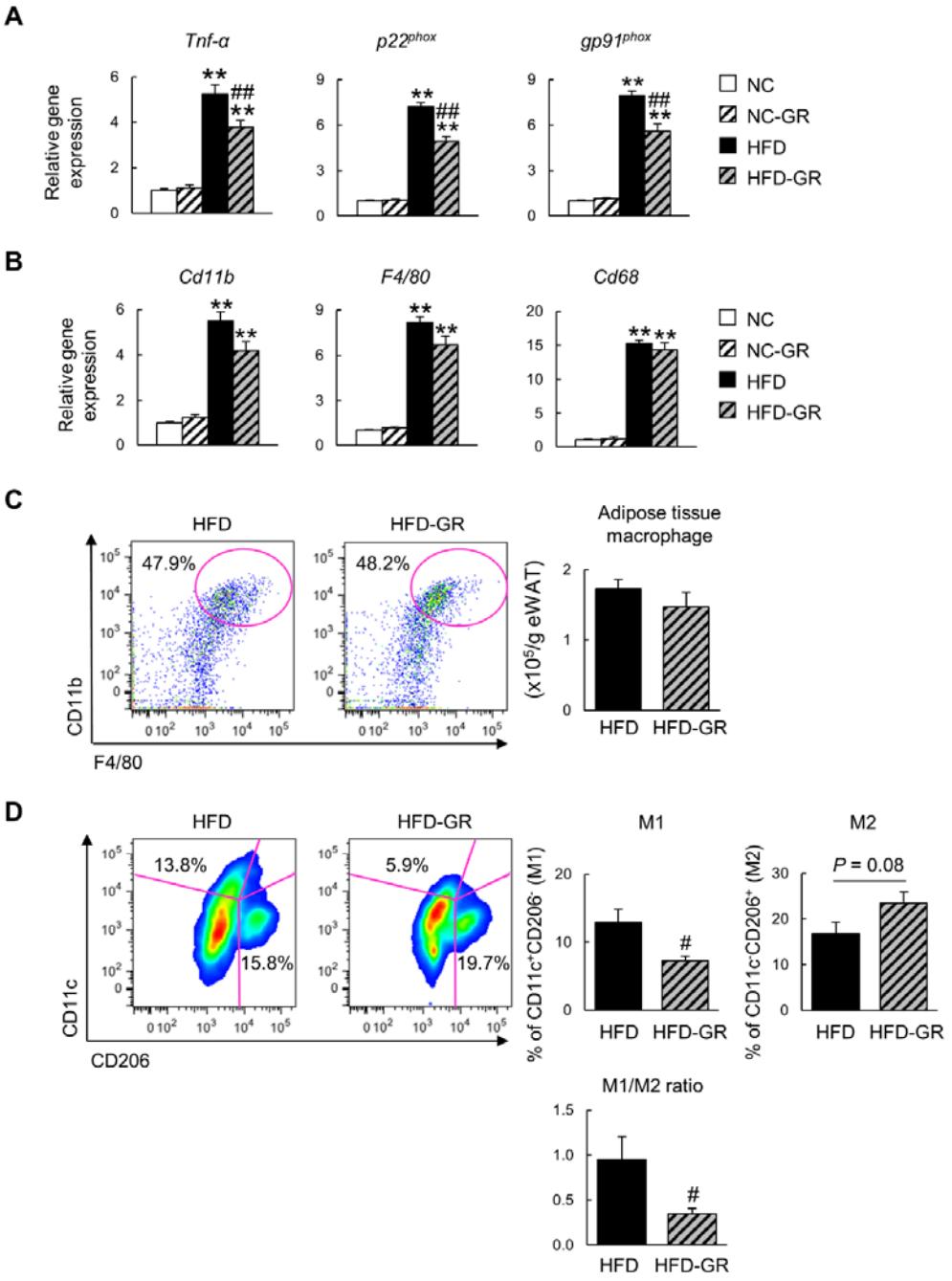
SUPPLEMENTARY DATA

Supplementary Figure 4. Immunoblot analysis of p-JNK (Thr183/Tyr185), JNK, p-ERK (Thr202/Tyr204), ERK, p-NF-κB p65 (Ser536), and NF-κB p65 using liver lysates of $Nrf2^{-/-}$ mice fed the indicated diet for 14 weeks. Each lane represents a liver lysate from a different animal (n = 8/group). Bar graphs represent normalized data of p-JNK/JNK, p-Erk/Erk, and p-NF-κB p65/ NF-κB p65 from two independent experiments, presented as mean ± SEM. **P* < 0.05, ***P* < 0.01 *vs.* NC. A.U.: arbitrary unit.



SUPPLEMENTARY DATA

Supplementary Figure 5. Relative mRNA expression of genes implicated in (*A*) *Tnf-α*, *p22^{phox}*, and *gp91^{phox}*, and (*B*) cell surface markers for macrophage, including *Cd11b*, *F4/80*, and *Cd68* in the epididymal white adipose tissue (eWAT) of mice fed the indicated diet for 14 weeks. *C*: FACS analysis of macrophage in eWAT of mice fed HFD or HFD-GR diet. Macrophages are defined as propidium iodide-CD45+NK1.1-CD3-CD19-TER119-CD11b+F4/80+ cells. Bar graphs show the number of macrophages in eWAT. *D*: M1-like and M2-like macrophages are defined as CD11c+CD206- and CD11c-CD206+ cells, respectively. Bar graphs show the percentage of M1- and M2-like macrophages, and the M1/M2 ratio. Data are presented as mean ± SEM (n = 8/group). *P < 0.05, **P < 0.01 vs. NC; #P < 0.05, ##P < 0.01 vs. HFD.



SUPPLEMENTARY DATA

Supplementary Figure 6. Immunoblot analysis of p-AMPKα (Thr172), AMPKα, p-ACC (Ser79), and ACC using lysates of liver, quadriceps muscles, intrascapular brown adipose tissue (BAT), inguinal white adipose tissue (ingWAT), and epididymal WAT (eWAT) from mice on the indicated diet for 14 weeks. Bar graphs represent normalized data of p-AMPK/AMPKα and p-ACC/ACC from two independent experiments, presented as mean ± SEM (n = 8/group). ***P* < 0.01 vs. NC. A.U., Arbitrary unit.