nature cell biology

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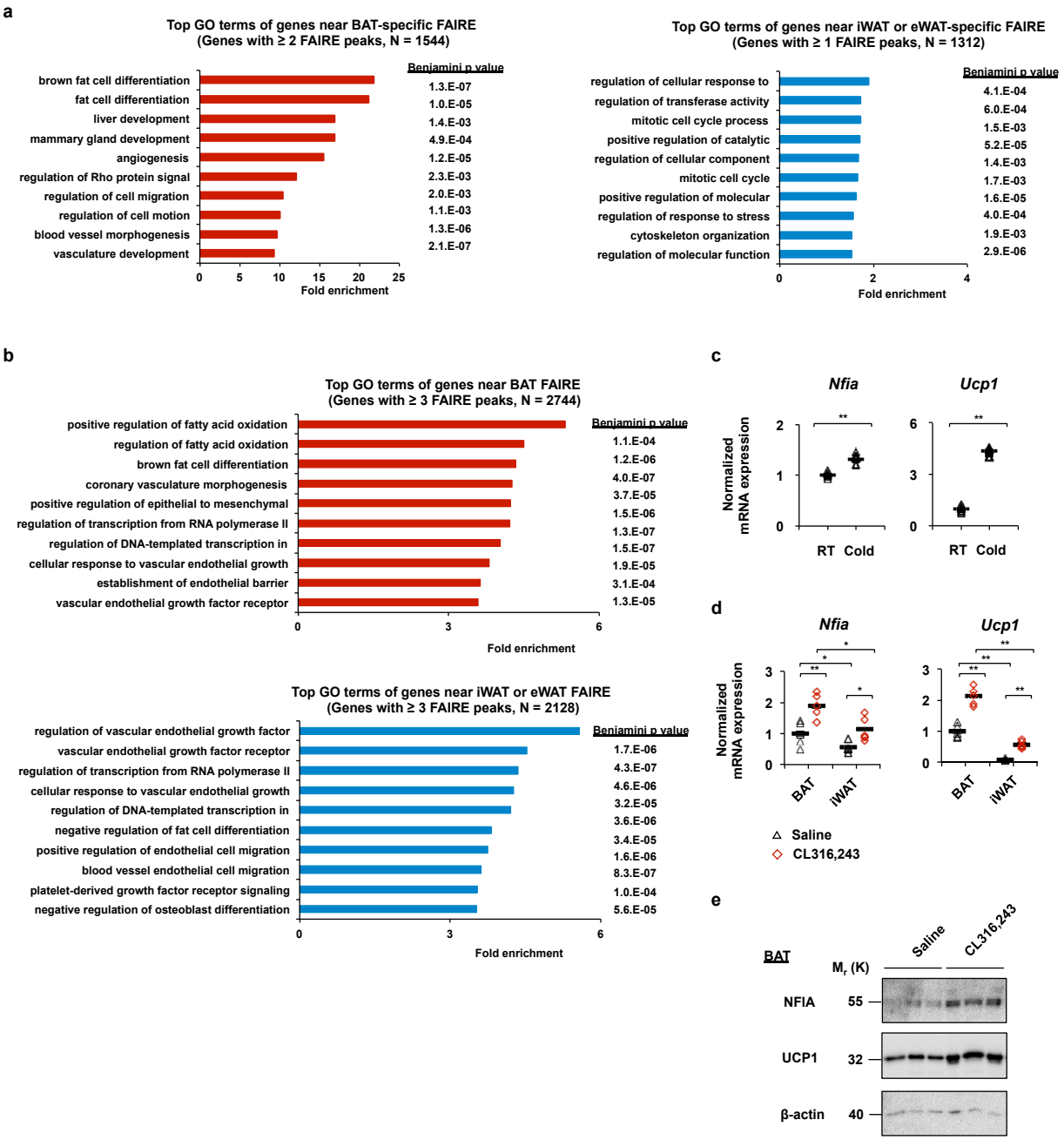
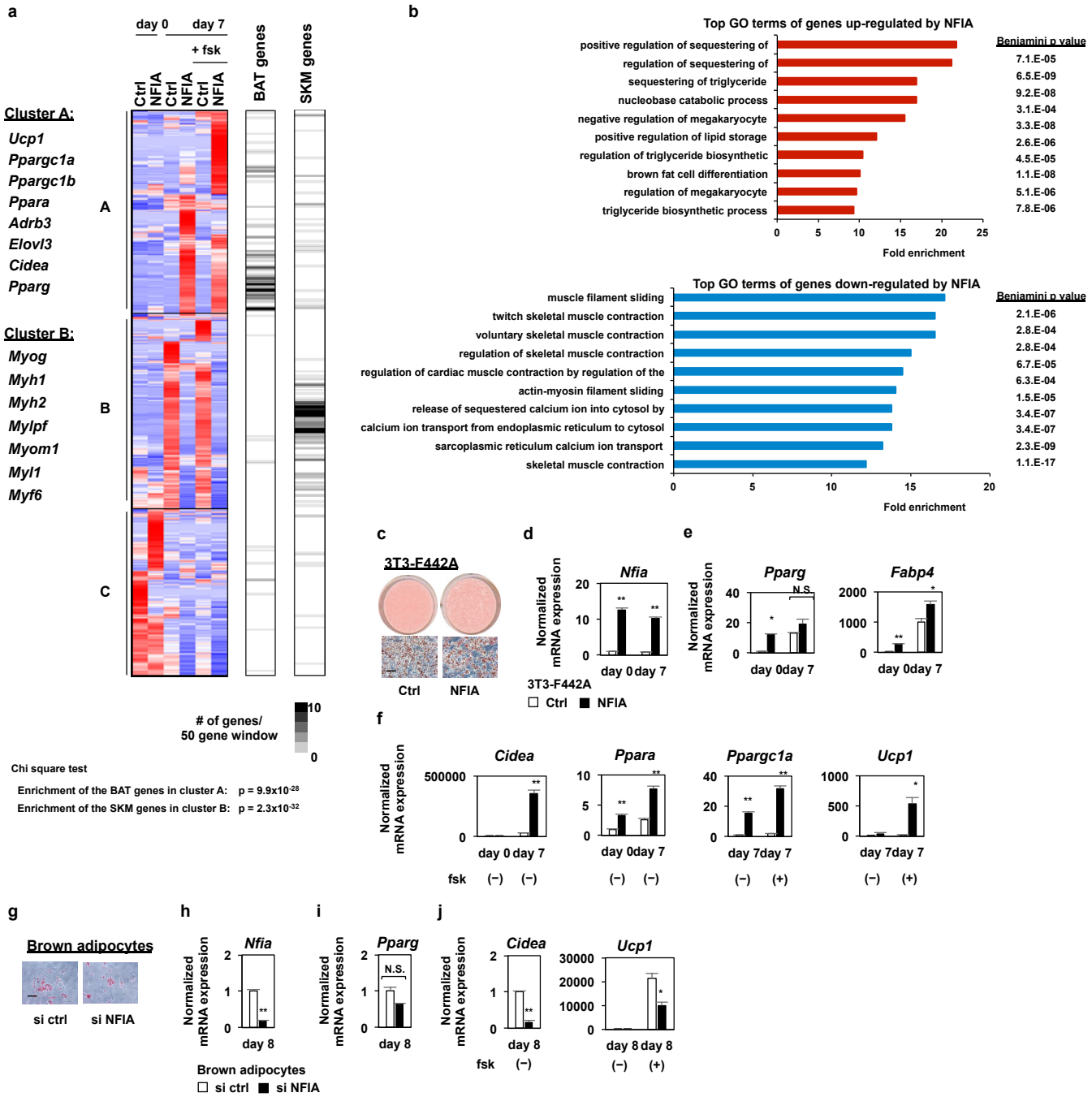
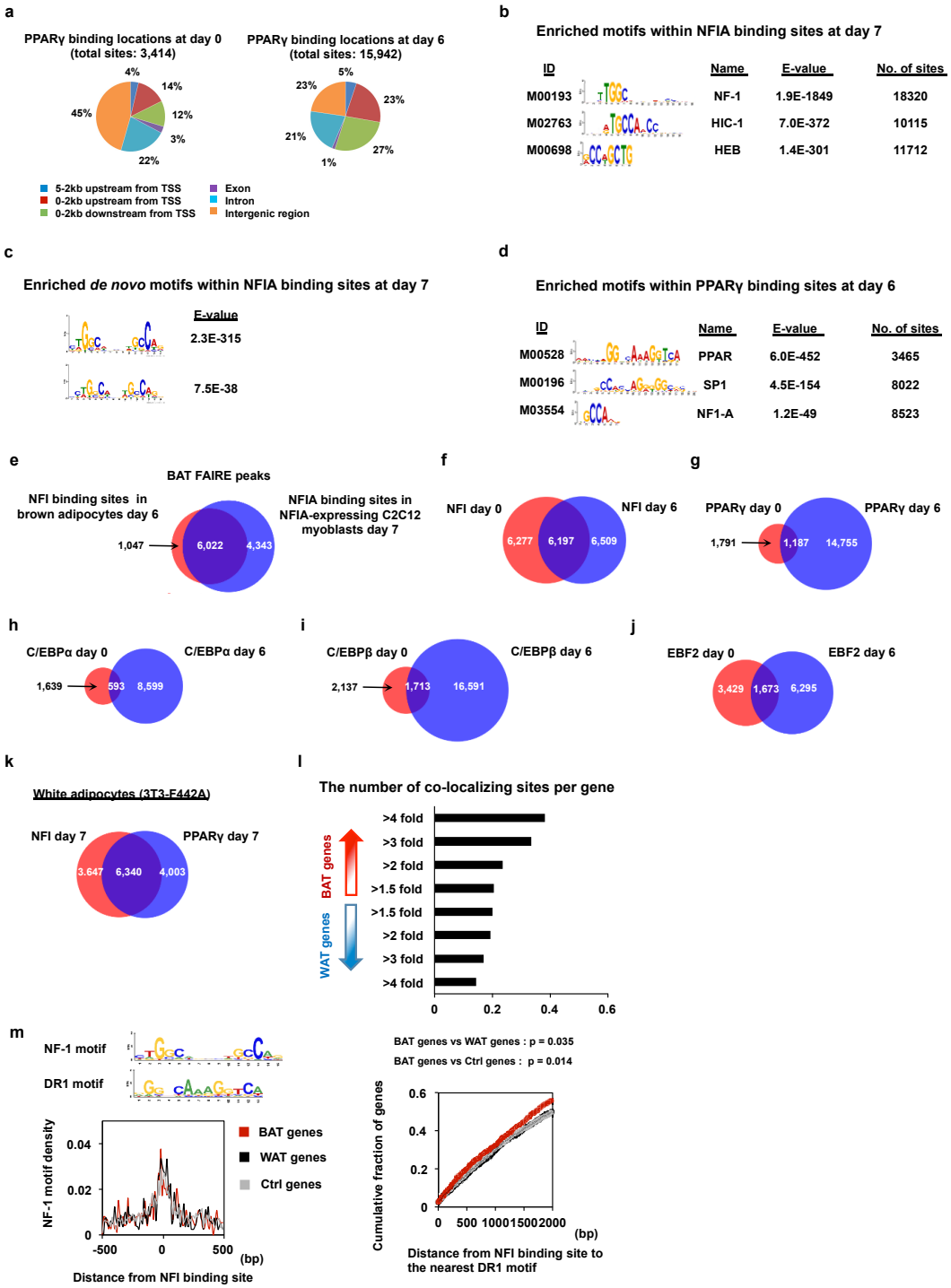


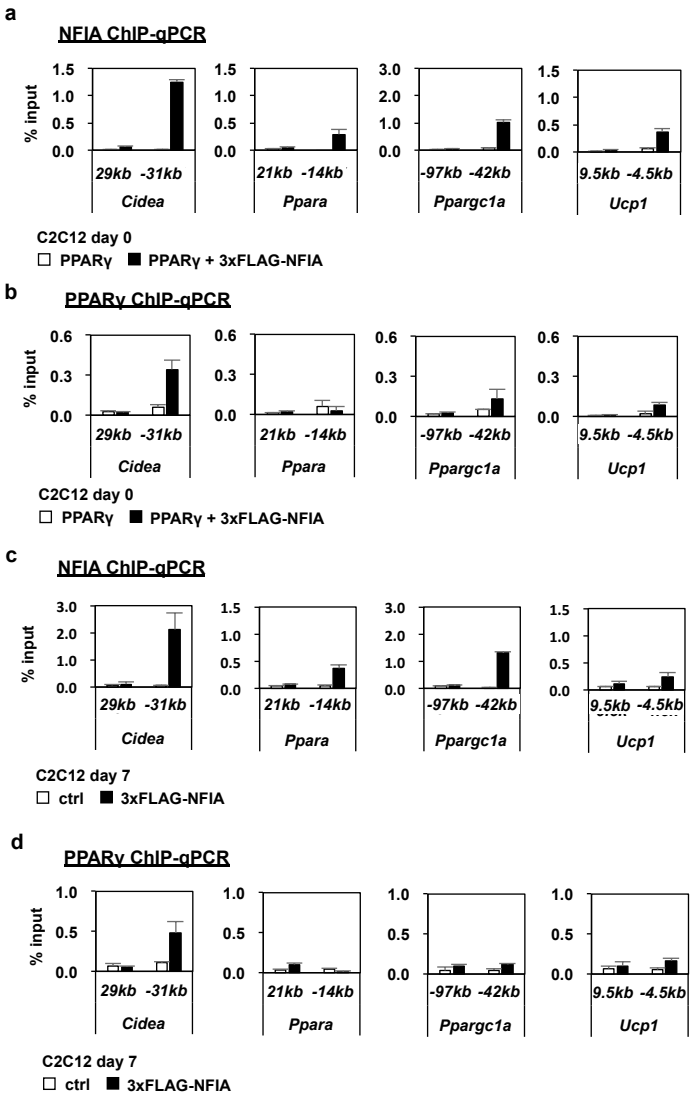
Supplementary Figure 1 GO analysis of the *in vivo* FAIRE peaks, and changes in expression levels of *Nfia* after challenge **a**, Top GO terms of genes near BAT-specific FAIRE peaks and iWAT- or eWAT- specific FAIRE peaks. **b,** Top GO terms of genes near BAT FAIRE peaks and iWAT or eWAT FAIRE peaks. The genome-wide analyses were performed once based on the FAIRE-seq dataset. **c,** RT-qPCR of *Nfia* and *Ucp1* before and after mice are challenged at 4°C for 4 hours. (mean +/- S.E.M.; N = 5 mice for room temperature and 6 mice for cold challenge, respectively; * p <0.05, ** p <0.01). The representative results of two independent experiments are shown. **d,** RT-qPCR analysis of *Nfia* and *Ucp1* of indicated tissues in mice treated with saline or β3 agonist CL316,243, 1 mg/kg body weight, intraperitoneal injection, for 7 days. (mean +/- S.E.M.; N = 5 mice per group; * p <0.05, ** p <0.01). The representative results of two independent experiments are shown. **e,** Western blot analysis of NFIA and UCP1. β-actin was used as a loading control. The representative images of two independent experiments are shown.



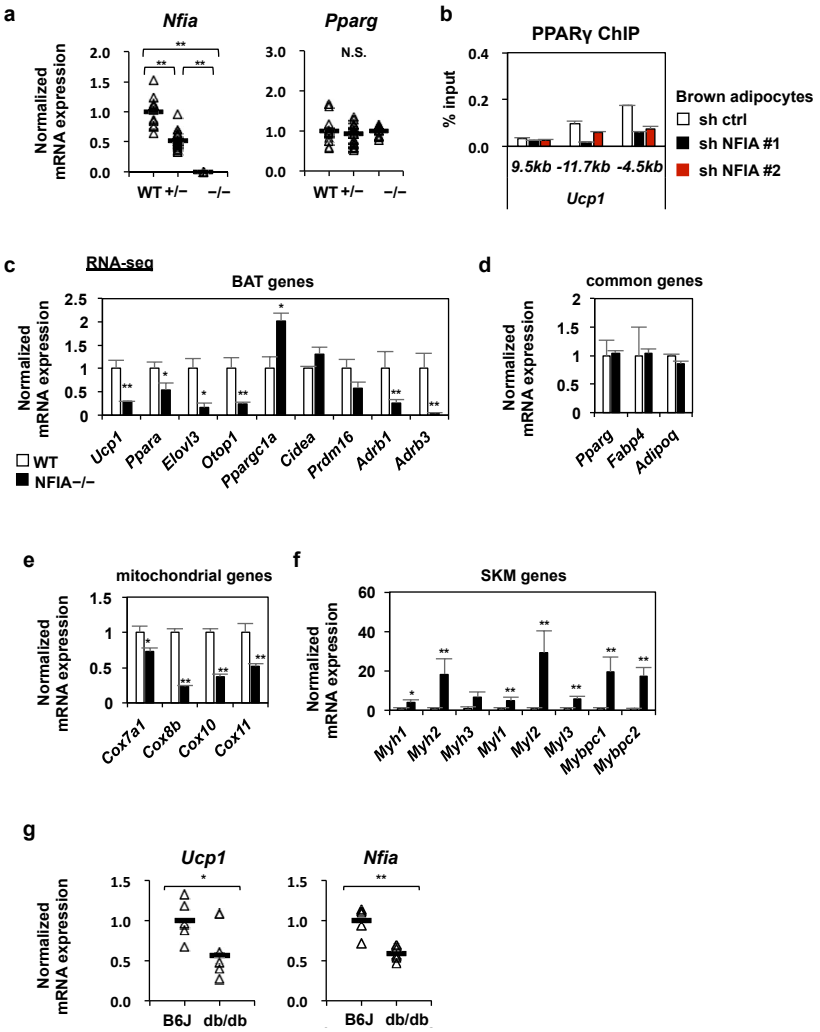
Supplementary Figure 2 Gain- and loss-of-function experiments showed the effect of NFIA on the brown fat gene program **a**, Hierarchal clustering analysis of genes up- or down-regulated by NFIA. For example, genes up-regulated NFIA (Cluster A) include *Ucp1*, *Ppargc1a*, and *Adrb3*—the vital components for the induction of thermogenesis in response to adrenergic stimulus, as well as *Pparg*—the master regulator of adipogenesis. In contrast, Cluster B represents genes down-regulated by NFIA, including critical myogenic genes such as *Myog*, *Myh1*, and *Myl1*. Note that the clusters of genes up-regulated by NFIA largely overlap with BAT-selective genes ($p = 9.9 \times 10^{-28}$), and the cluster of genes down-regulated by NFIA overlap with SKM -selective genes ($p = 2.3 \times 10^{-32}$). The definition of BAT- and SKM- selective genes (N = 254 and N = 312, respectively) are shown in the methods. The analyses were performed once based on the RNA-seq dataset. **b**, Top GO terms of genes up- or down-regulated by introduction of NFIA into C2C12 myoblasts. The analyses were performed once based on the RNA-seq dataset. **c**, Control and NFIA-expressing 3T3-F442A adipocytes were stained with Oil Red O seven days after inducing adipocyte differentiation. Scale bar, 50 μm. **d**-**f**, *Nfia* (**d**), general adipocyte genes (**e**) and the brown-fat-specific genes (**f**) were quantified by RT-qPCR at the indicated time course (mean +/- S.E.M.; N = 3 independent samples; * p <0.05, ** p <0.01). **g**, Immortalized, differentiated brown adipocytes were electroporated with a control siRNA or a siRNA for NFIA and stained with Oil Red O. Scale bar, 50 μm. **h**-**j**, *Nfia* (**h**), *Pparg* (**i**), *Cidea* and *Ucp1* (**j**) were quantified by RT-qPCR at the indicated time course (mean +/- S.E.M.; N = 3 independent samples; * p <0.05, ** p <0.01).



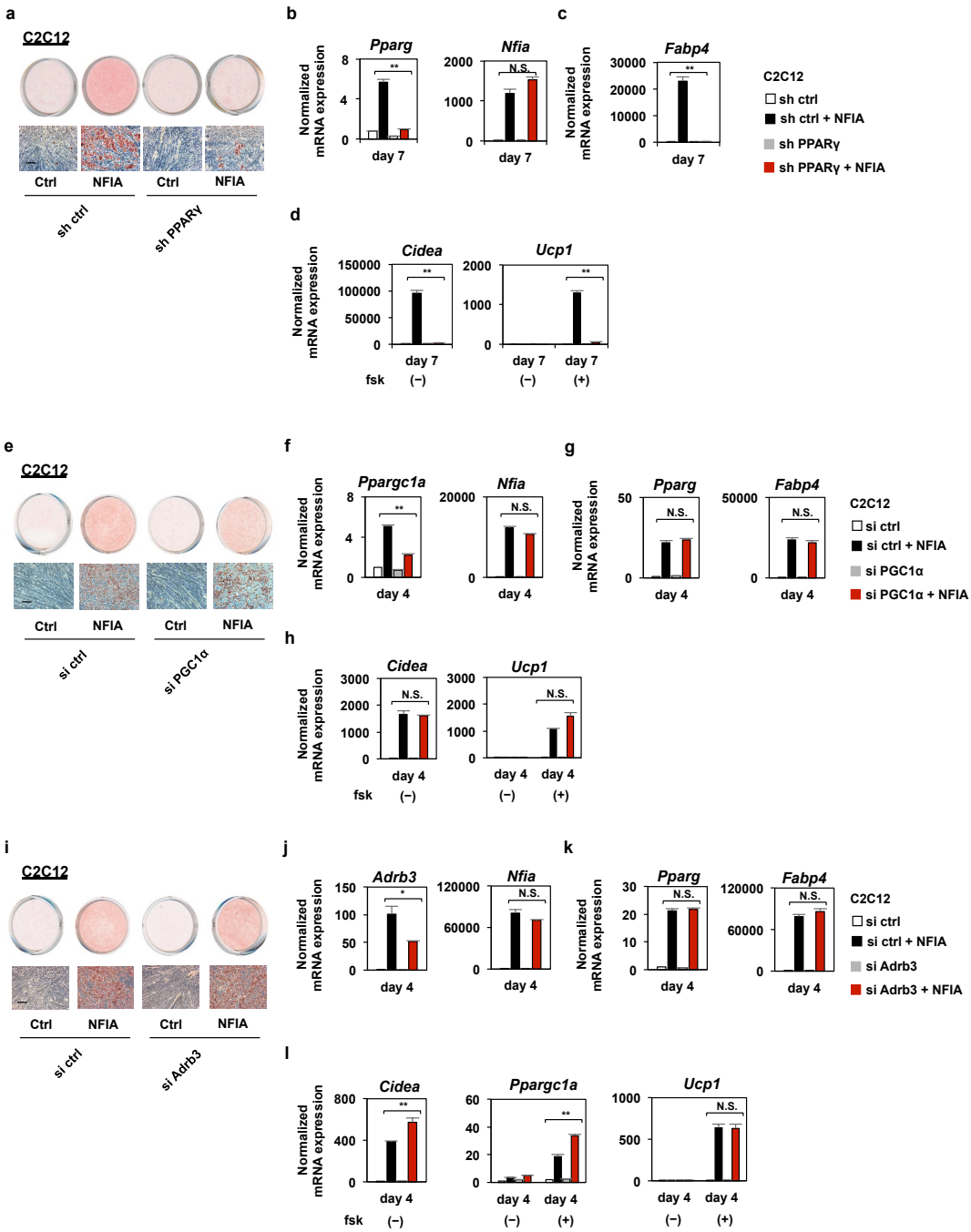
Supplementary Figure 3 ChIP-seq analysis of NFI, PPARγ and other transcription factors **a**, Genomic location of PPARγ binding sites in brown adipocytes at day 0 and day 6 of differentiation. **b,** Enriched known motifs within NFIA binding sites in NFIA-expressing myoblasts at day 7. **c,** Enriched de novo motifs within NFIA binding sites in NFIA-expressing myoblasts at day 7. **d,** Enriched known motifs within PPARγ binding sites in brown adipocytes at day 6. **e,** Venn diagram showing the overlap of NFI binding sites in brown adipocytes and NFIA binding sites in NFIA-expressing C2C12 myoblasts within BAT FAIRE peaks. **f-j.** Venn diagram showing the overlap of indicated transcription factors at day0 and day 6 of differentiation. **k,** Venn diagram showing the overlap of NFI and PPARγ ChIP-seq peaks in 3T3-F442A white adipocytes at day 6. **l,** Bar graph showing the number of co-localizing sites per gene within +/- 50 kb of BAT- and WAT-selective genes stratified by the fold changes of gene expression. **m,** To the left, NF-1 motif density around the NFI binding sites near BAT genes, WAT genes and control genes (genes with invariant expression between BAT and WAT, N = 2000 genes). The definition of BAT- and WAT- selective genes (N = 549 and N = 849, respectively) are shown in the methods. To the right, distance from NFI binding sites to the nearest DR1 motif is shown for binding sites near BAT genes, WAT genes and control genes. Statistical significance was determined by Mann–Whitney U test. The analyses were performed once based on the ChIP-seq dataset.



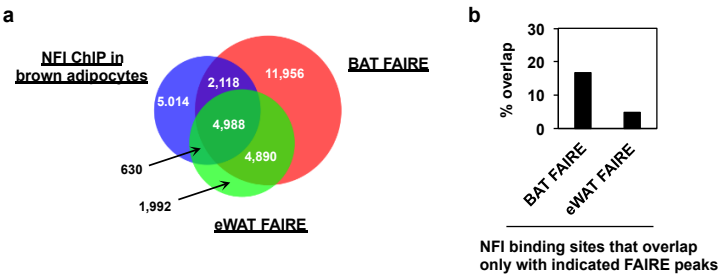
Supplementary Figure 4 Co-localization of NFIA facilitates PPARγ binding even before differentiation **a-b**, ChIP-qPCR analysis of NFIA (**a**) and PPARγ (**b**) in C2C12 cells with introduction of PPARγ alone or both PPARγ and NFIA, at day 0 of differentiation. *Cidea 29k*, *Ppara 21k*, *Ppargc1a -97k*, and *Ucp1 9.5k* are background sites. The representative result of two independent experiments is shown (N = 2 independent samples; mean +/- S.E.M.). **c-d**, ChIP-qPCR analysis of NFIA (**c**) and PPARγ (**d**) in C2C12 cells with or without introduction of NFIA, differentiation day 7. *Cidea 29k*, *Ppara 21k*, *Ppargc1a -97k*, and *Ucp1 9.5k* are background sites. The representative result of two independent experiments is shown (N = 2 independent samples; mean +/- S.E.M.)



Supplementary Figure 5 Deficiency of NFIA results in decreased expression of the brown-fat-specific genes and reciprocal elevation of muscle genes. **a**, RT-qPCR analysis *Nfia* and *Pparg* in WT, NFIA +/-, and NFIA -/- BAT (mean +/- S.E.M.; N = 11 mice for WT, 24 mice for NFIA +/-, and 15 mice for NFIA -/-, respectively; * $p < 0.05$, ** $p < 0.01$). **b**, ChIP-qPCR analysis of PPARγ in brown adipocytes with NFIA knockdown. *Ucp1 9.5k* is a background site. The representative result of three independent experiments is shown (N = 2 independent samples; mean +/- S.E.M.). **c-f**, RNA-seq analysis of representative BAT genes (**c**), common genes (**d**), mitochondrial genes (**e**) and SKM genes (**f**) (mean +/- S.E.M.; N = 3 independent samples, * $p < 0.05$, ** $p < 0.01$). **g**, RT-qPCR analysis of *Nfia* and *Ucp1* in C57BL/6J and db/db mice (mean +/- S.E.M.; N = 5 mice per group; * $p < 0.05$, ** $p < 0.01$). The representative result of two independent experiments is shown.



Supplementary Figure 6 PPARγ is indispensable, while PGC1α and Adrb3 are dispensable for the effect of NFIA **a**, Control sh RNA or sh RNA for PPARγ was introduced into control or NFIA-expressing C2C12 myoblasts, and stained with Oil Red O seven days after inducing adipocyte differentiation. Scale bar, 50 μm. **b-d**, *Pparg* and *Nfia* (**b**), general adipocyte marker *Fabp4* (**c**) and the brown-fat-specific genes *Cidea* and *Ucp1* (**d**) were quantified by RT-qPCR at the indicated time course (mean +/- S.E.M.; N = 3 independent samples; * p <0.05, ** p <0.01). **e**, Control si RNA or si RNA for PGC1α was introduced into control or NFIA-expressing C2C12 myoblasts, and stained with Oil Red O seven days after inducing adipocyte differentiation. Scale bar, 50 μm. **f-h,** *Ppargc1a* and *Nfia* (**f**), common adipocyte genes (**g**) and the brown-fat-specific genes (**h**) were quantified by RT-qPCR at the indicated time course (mean +/- S.E.M.; N = 3 independent samples; * p <0.05, ** p <0.01). The representative result of two independent experiments is shown. **i**, Control si RNA or si RNA for Adrb3 was introduced into control or NFIA-expressing C2C12 myoblasts, and stained with Oil Red O seven days after inducing adipocyte differentiation. Scale bar, 50 μm. **j-l,** *Adrb3* and *Nfia* (**j**), common adipocyte genes (**k**) and the brown-fat-specific genes (**l**) were quantified by RT-qPCR at the indicated time course (mean +/- S.E.M.; N = 3 independent samples; * p <0.05, ** p <0.01). The representative result of two independent experiments is shown.



Supplementary Figure 7 Overlap of NFI binding sites and BAT or eWAT FAIRE **a,** Venn diagram showing the overlap of NFI binding sites in brown adipocytes, FAIRE peaks in BAT and FAIRE peaks in eWAT. **b,** Bar graph showing the percentage of NFI binding sites that overlap only with BAT FAIRE peaks or eWAT FAIRE peaks. The analyses were performed once based on the ChIP-seq and FAIRE-seq dataset.

Figure 1f

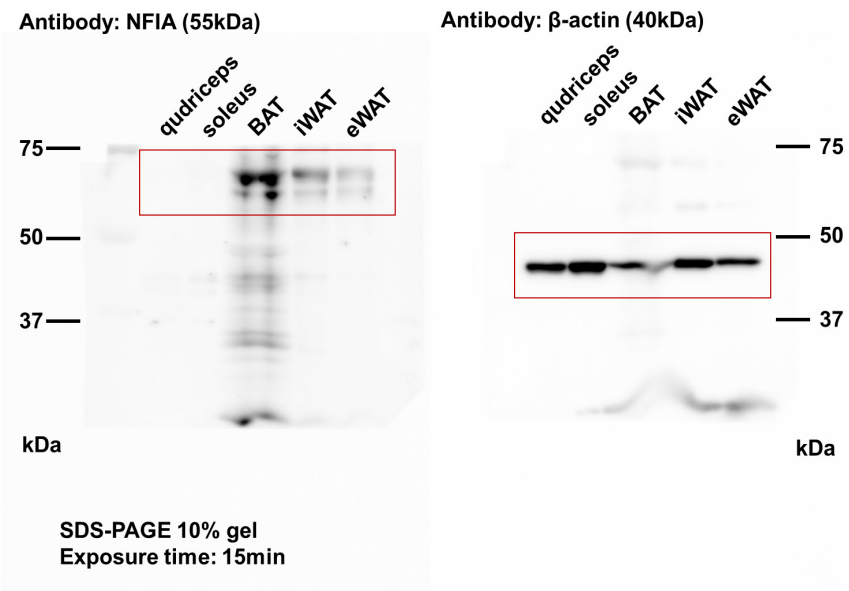
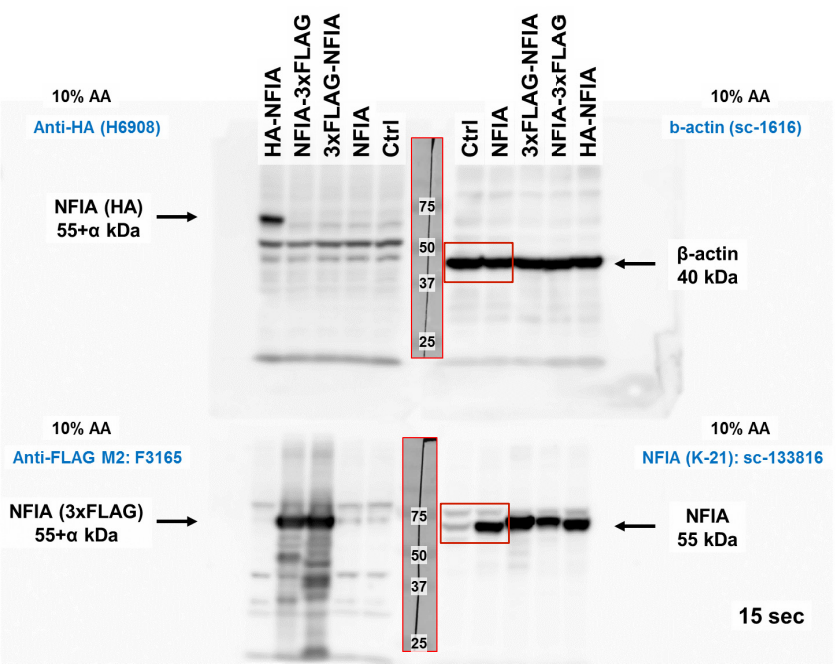


Figure 2a



Supplementary Figure 8 Uncropped pictures of the Western blot analysis

Figure 2k

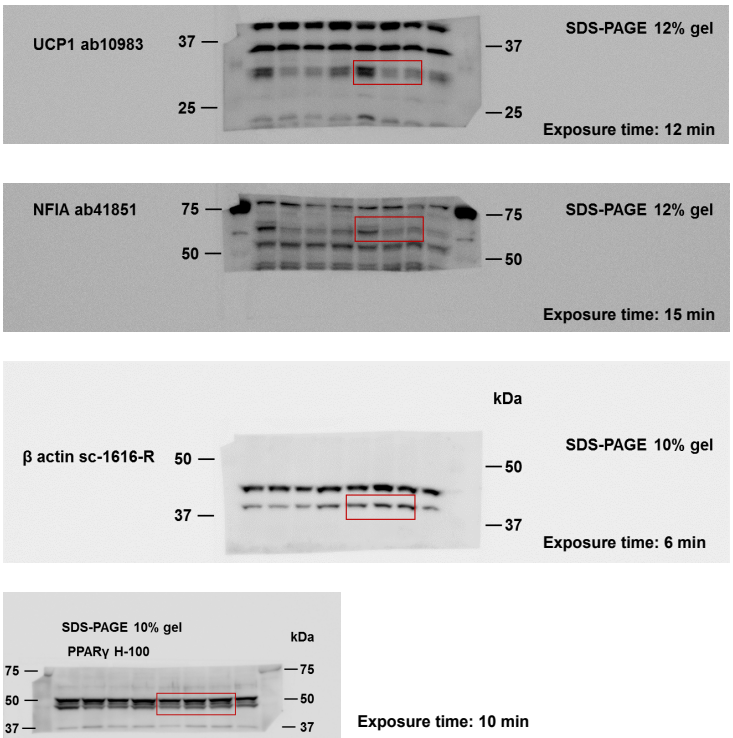
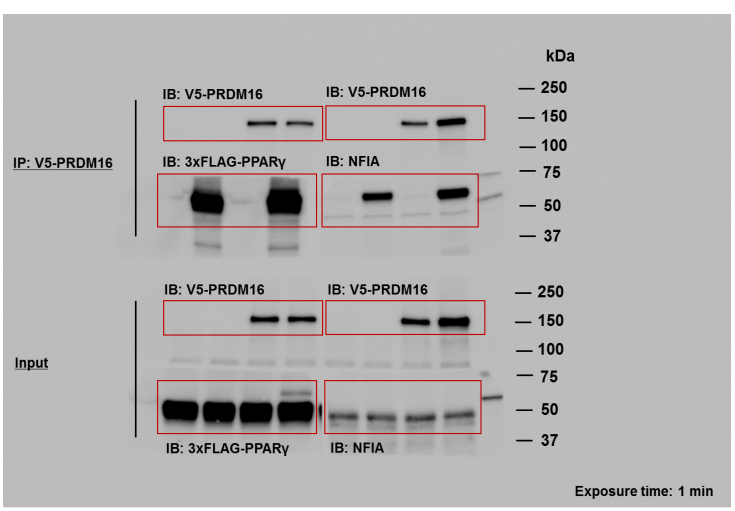


Figure 3a



Supplementary Figure 8 Continued

Figure 6a

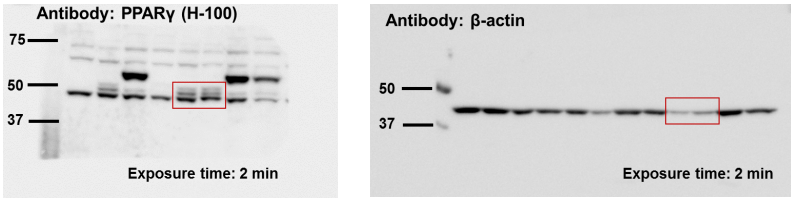
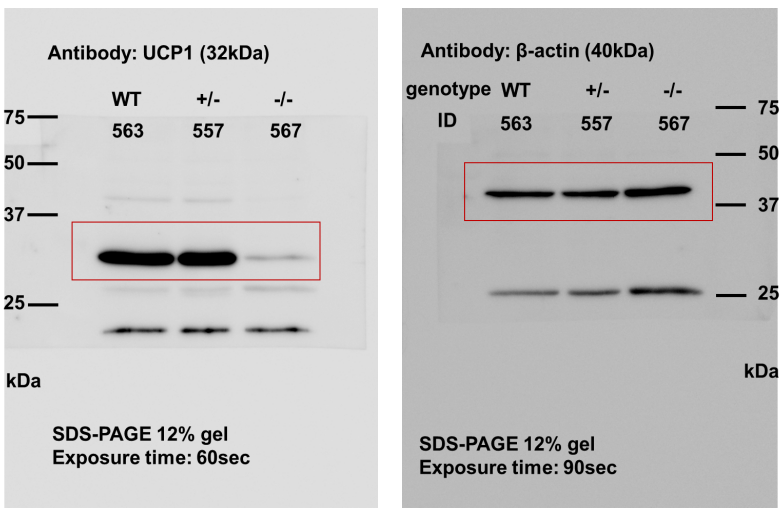
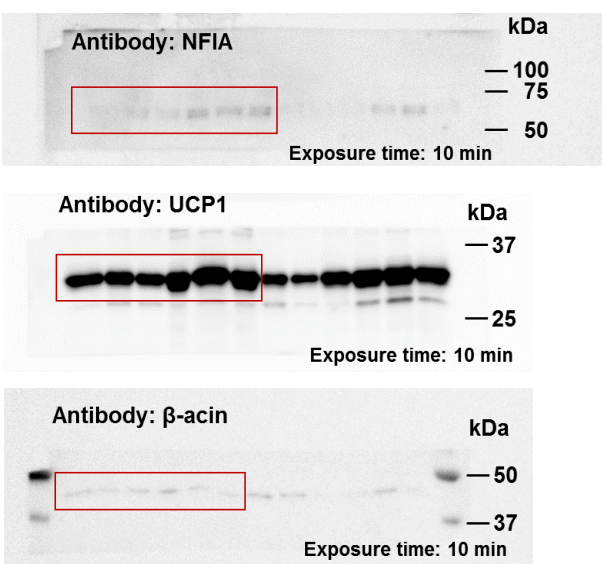


Figure 7c



Supplementary figure 1e



Supplementary Figure 8 Continued

SUPPLEMENTARY INFORMATION

Supplementary Table Legends

Supplementary Table 1 Primer sequences used for the qPCR analysis

Supplementary Table 2 Source data for Fig. 6d and 6e

nature research

Corresponding author(s): Takashi Kadowaki

☐ Initial submission ☐ Revised version ☒ Final submission

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size

Describe how sample size was determined.

At least five mice were used for all the animal studies, and this group size was based on our previous studies.

2. Data exclusions

Describe any data exclusions.

No mice, tissues nor cells were excluded for analysis.

3. Replication

Describe whether the experimental findings were reliably reproduced.

All attempts at replication were successful. For experiments using human samples, experiments were performed once because the samples were highly limited.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

Randomization was not performed.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

The investigators were not blinded to mouse genotype

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒	A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	A statement indicating how many times each experiment was replicated
☐	☒	The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☒	☐	A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒	The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals noted
☐	☒	A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒	Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.

High-throughput sequencing was performed by using the Illumina Genome Analyzer System or HiSeq 2500. Demultiplex and base calling were performed with the CASAVA 1.8.2 software (Illumina).

For ChIP-seq, FAIRE-seq and ATAC-seq data processing, the sequence reads were mapped to UCSC build mm9 (NCBI Build 37) assembly of the mouse genome using the ELAND mapping program. Peak calling was performed using the MACS version 1.4 with default parameters. Peaks of ChIP-seq, FAIRE-seq and ATAC-seq were visualized by a GenomeJack browser (version 3.1, Mitsubishi Space Software). Galaxy cistrome was used for genomic region handling. Motif analysis was performed using CentriMo version 4.10.2 with default parameters. We used the licensed version of TRANSFAC database. Gene ontology annotation analysis was performed using DAVID. Biological process terms "GO_BP_FAT" were used. A heat map representation was generated using in-house software.

For RNA-seq data processing, the sequence reads were mapped to the mm9 mouse genome using TopHat. Fragments per kilobase of exon per million fragment mapped (FPKM) values were calculated for each gene using CuffLinks. Differentially expressed genes were analyzed using DeSeq2. A heat map representation was generated using GenePattern online software.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

Human brown adipose tissues and human brown adipocytes are highly limited. Other materials are readily available from standard commercial sources, or available from the authors on reasonable request.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

The antibodies used for ChIP experiments were FLAG M2 (Sigma F3165), NFI (Santa Cruz Biotechnology, sc-30198), PPARγ (mix of Santa Cruz Biotechnology, sc-7273, and Perseus Proteomics, A3409A), C/EBPα (Santa Cruz Biotechnology, sc-61), C/EBPβ (Santa Cruz Biotechnology, sc-150), EBF2 (R&D Systems, AF7006) and H3K27Ac (Abcam, ab4729). All the antibodies were validated by the manufacturers.

The antibodies used for Western blot analyses were anti-NFIA (Santa Cruz Biotechnology, sc-133816), anti-UCP1 (Abcam, ab10983), anti-PPARγ (Santa Cruz Biotechnology, sc-7196) and anti-β actin (Santa Cruz Biotechnology, sc-1616). All the antibodies were validated by the manufacturers.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

Immortalized brown adipocytes, C2C12, 3T3-L1, 3T3-F442A, Plat E, and HEK293 cells were used.

b. Describe the method of cell line authentication used.

Immortalized brown adipocyte cell lines were developed and authenticated in Ohno et al. Nature 2013. Other cell lines were readily available from standard commercial sources, and authenticated in scientific community.

c. Report whether the cell lines were tested for mycoplasma contamination.

Immortalized brown adipocytes and C2C12 were negative for mycoplasma contamination. Other cell lines were low passage and not tested for mycoplasma.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

No commonly misidentified cell lines were used.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

NFIA-KO mice (stock number: 010318-UNC) were purchased from MMRRC (Mutant Mouse Regional Resource Center). The founder of this stock was 129S6 and subsequently was backcrossed to C57BL6/J for more than 20 generations. All the experiments were performed using male mice. Age of the mice for each experiment was indicated in the main text or figure legends.
Randomization was not performed and the investigators were not blinded to mouse genotype.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

Participants of prerenal BAT analysis were described in Nagano et al. PLoS One. 2015. Participants of brown and white adipocyte differentiation experiments were described in Jespersen et al. Cell Metabolism 2013. Participants of human neck BAT and WAT analysis were described in Cypess et al. Nature Medicine 2013.
Randomization was not performed and the investigators were not blinded to the type of the samples.

nature research

Corresponding author(s): Takashi Kadowaki

☐ Initial submission ☐ Revised version ☒ Final submission

ChIP-seq Reporting Summary

Form fields will expand as needed. Please do not leave fields blank.

Data deposition

1. For all ChIP-seq data:

☒ a. Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☒ b. Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

2. Provide all necessary reviewer access links.
The entry may remain private before publication.

ChIP-seq data has been deposited at the Gene Expression Omnibus (GEO) under the accession number GSE83764. The secure token to allow review of record GSE83764 while it remains in private status is: yhaxcsyojbsnzqr

3. Provide a list of all files available in the database submission.

For samples listed below, fastq, bigWig and bed files are available.

FAIRE_BAT
FAIRE_eWAT
FAIRE_iWAT
NFI_ChIP-seq_brown_adipocytes_day0
NFI_ChIP-seq_brown_adipocytes_day6
PPARg_ChIP-seq_brown_adipocytes_day0
PPARg_ChIP-seq_brown_adipocytes_day6
ATAC-seq_brown_adipocytes_day6
NFIA_ChIP-seq_NFIA-expressing_C2C12_myoblasts_day0
NFIA_ChIP-seq_NFIA-expressing_C2C12_myoblasts_day7
ATAC-seq_brown_adipocytes_day0
H3K27Ac_ChIP-seq_brown_adipocytes_day0
CEBPalpha_ChIP-seq_brown_adipocytes_day0
CEBPbeta_ChIP-seq_brown_adipocytes_day0
H3K27Ac_ChIP-seq_brown_adipocytes_day6
CEBPalpha_ChIP-seq_brown_adipocytes_day6
CEBPbeta_ChIP-seq_brown_adipocytes_day6
NFI_ChIP-seq_white_adipocytes_day7
PPARg_ChIP-seq_white_adipocytes_day7

4. If available, provide a link to an anonymized genome browser session (e.g. UCSC).

N/A

Methodological details

5. Describe the experimental replicates.

ChIP, FAIRE and ATAC experiments were independently performed at least three times, and representative samples were used for high-throughput sequencing.

6. Describe the sequencing depth for each experiment.

Sequencing depth (Numer of total reads) were listed below.

FAIRE_BAT: 51M
FAIRE_eWAT: 21M
FAIRE_iWAT: 24M
NFI_ChIP-seq_brown_adipocytes_day0: 38M
NFI_ChIP-seq_brown_adipocytes_day6: 27M
PPARg_ChIP-seq_brown_adipocytes_day0: 47M
PPARg_ChIP-seq_brown_adipocytes_day6: 43M

ATAC-seq_brown_adipocytes_day6: 52M
NFIA_ChIP-seq_NFIA-expressing_C2C12_myoblasts_day0: 59M
NFIA_ChIP-seq_NFIA-expressing_C2C12_myoblasts_day7: 69M
ATAC-seq_brown_adipocytes_day0_rep1: 100M
H3K27Ac_ChIP-seq_brown_adipocytes_day0: 51M
CEBPalpha_ChIP-seq_brown_adipocytes_day0: 40M
CEBPbeta_ChIP-seq_brown_adipocytes_day0: 51M
H3K27Ac_ChIP-seq_brown_adipocytes_day6: 34M
CEBPalpha_ChIP-seq_brown_adipocytes_day6: 25M
CEBPbeta_ChIP-seq_brown_adipocytes_day6: 31M
NFI_ChIP-seq_white_adipocytes_day7: 32M
PPARg_ChIP-seq_white_adipocytes_day7: 34M

7. Describe the antibodies used for the ChIP-seq experiments.

The antibodies used for ChIP-seq experiments were FLAG M2 (Sigma F3165), NFI (Santa Cruz Biotechnology, sc-30198), PPARγ (mix of Santa Cruz Biotechnology, sc-7273, and Perseus Proteomics, A3409A), C/EBPα (Santa Cruz Biotechnology, sc-61), C/EBPβ (Santa Cruz Biotechnology, sc-150), EBF2 (R&D Systems, AF7006) and H3K27Ac (Abcam, ab4729).

8. Describe the peak calling parameters.

Peak calling was performed using the MACS version 1.4 with default parameters.

9. Describe the methods used to ensure data quality.

We inspected the peak-called tracks using Genomejack browser (version 3.1, Mitsubishi Space Software) and confirmed the binding signal at previously known sites.

For ChIP-seq of NFI, we confirmed that most of the NFI binding sites in brown adipocytes and NFIA binding sites in NFIA-expressing C2C12 myoblasts overlapped each other at BAT FAIRE peaks (Supplementary Fig. 3e)

The number of called peaks are listed below.

FAIRE_BAT: 24585
FAIRE_eWAT: 12901
FAIRE_iWAT: 10235
NFI_ChIP-seq_brown_adipocytes_day0: 12486
NFI_ChIP-seq_brown_adipocytes_day6: 12748
PPARg_ChIP-seq_brown_adipocytes_day0: 3414
PPARg_ChIP-seq_brown_adipocytes_day6: 15942
ATAC-seq_brown_adipocytes_day6: 32425
NFIA_ChIP-seq_NFIA-expressing_C2C12_myoblasts_day0: 31992
NFIA_ChIP-seq_NFIA-expressing_C2C12_myoblasts_day7: 32374
ATAC-seq_brown_adipocytes_day0: 37311
H3K27Ac_ChIP-seq_brown_adipocytes_day0: 13570
CEBPalpha_ChIP-seq_brown_adipocytes_day0: 2451
CEBPbeta_ChIP-seq_brown_adipocytes_day0: 4273
H3K27Ac_ChIP-seq_brown_adipocytes_day6: 13068
CEBPalpha_ChIP-seq_brown_adipocytes_day6: 9192
CEBPbeta_ChIP-seq_brown_adipocytes_day6: 18034
NFI_ChIP-seq_white_adipocytes_day7: 10047
PPARg_ChIP-seq_white_adipocytes_day7: 10389

10. Describe the software used to collect and analyze the ChIP-seq data.

The sequence reads were mapped to UCSC build mm9 (NCBI Build 37) assembly of the mouse genome using the ELAND mapping program. Peak calling was performed using the MACS version 1.4 with default parameters.