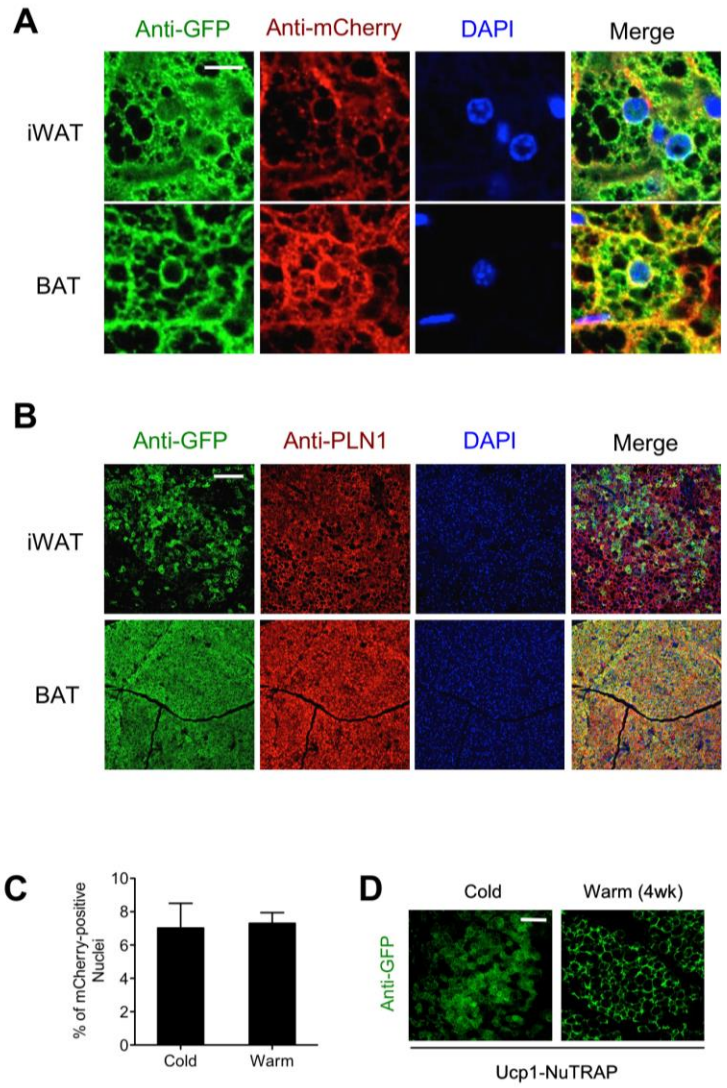

Figure S1. (Related to Figure 1)

Labeling and tracking of brown and beige adipocytes using Ucp1-NuTRAP

(a) Confocal immunofluorescence images of iWAT and BAT of Ucp1-NuTRAP mice exposed to 4°C for 1 week. GFP fluorescence shows ribosomal labeling, mCherry shows nuclear and cytoplasmic labeling by RanGAP1, and DAPI shows nuclei. Scale bar: 10μm

(b) Co-immunofluorescence staining images using anti-GFP and anti-PLIN1 antibodies. In iWAT, a subset of PLIN1-positive adipocytes population is co-stained with GFP, representing beige adipocytes. In BAT, nearly all adipocytes positive for PLIN1 are co-stained with GFP. Scale bar: 100μm

(c) Quantitative analysis of mCherry-labeled nuclei from cold exposed beige adipocytes and after 4wk of warming, as assessed by flow cytometry. The fraction of beige adipocytes among total nuclei from iWAT are shown. Bars indicate mean ± SEM (n=7 animals).

(d) Confocal immunofluorescence images of GFP-labeled beige adipocytes in iWAT of Ucp1-NuTRAP mice after cold exposure and after 4wk of warming. Scale bar: 50μm

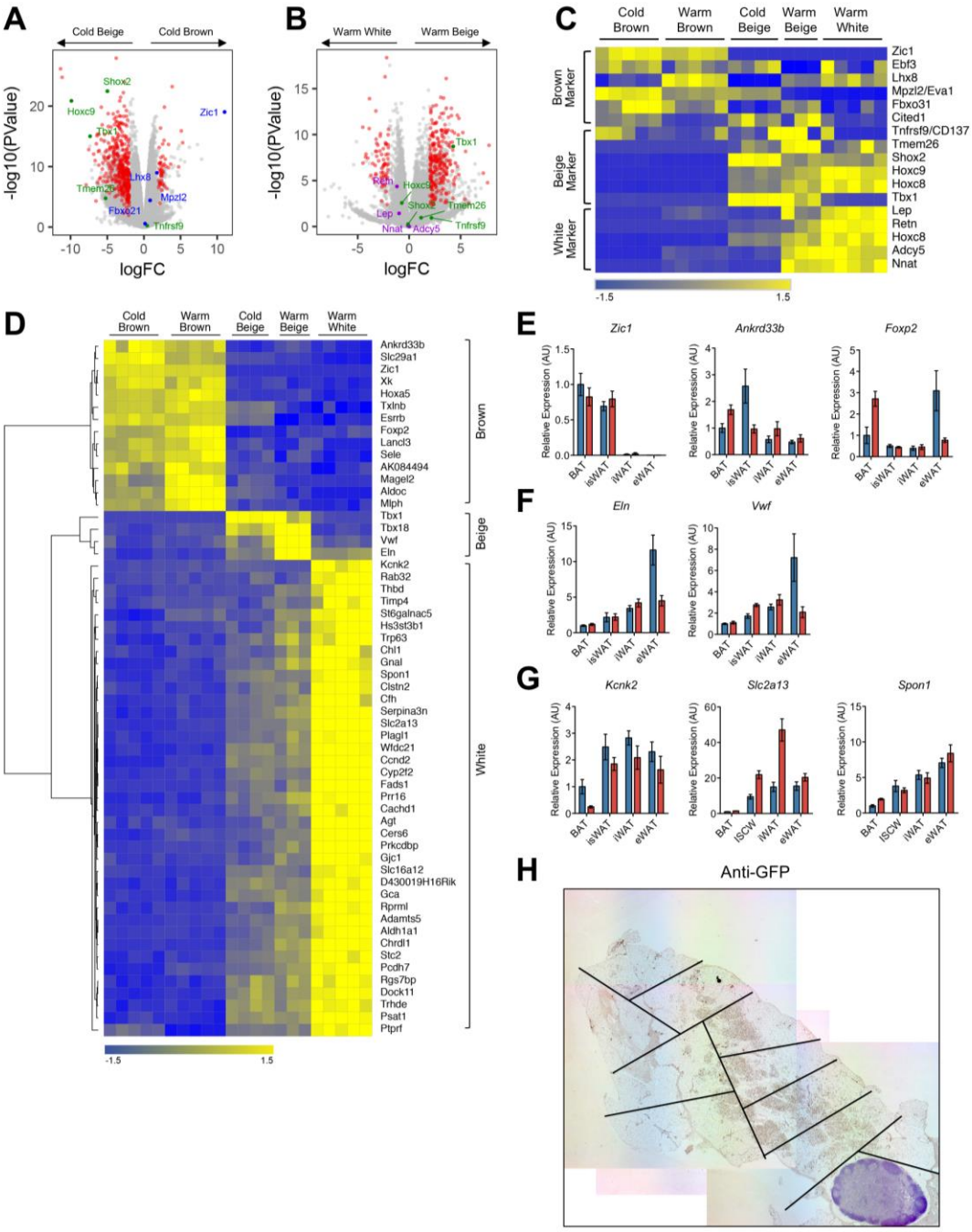

Figure S2. (Related to Figure 2)

Validation of adipocyte type-specific markers

(a) Volcano plot showing differentially expressed genes in cold beige and brown adipocytes. Traditional brown and beige adipocyte markers are highlighted in blue and green, respectively.

(b) Volcano plot showing differentially expressed genes in warm beige and white adipocytes. Traditional beige and white adipocyte markers are highlighted in green and purple, respectively.

(c) Heatmap of traditional brown, beige and white adipocyte marker gene expression in cold and warm conditions. Columns represent biological replicates. Expression values (CPM) of each mRNA are represented by z-scores.

(d) Heatmap of hierarchical clustering for brown, beige and white adipocyte-selective genes identified in this study. Columns represent biological replicates. Expression values (CPM) of each mRNA are represented by z-scores.

(e-g) Gene expression analysis by qRT-PCR of brown (e), beige (f), and white (g) adipocyte-selective genes in different fat depots in response to cold (4°C, 1 week) and subsequent warming (30°C, 4 week). Bars indicate mean ± SEM (n=6 animals/group). BAT, interscapular brown adipose tissue; isWAT, interscapular white adipose tissue; iWAT, inguinal white adipose tissue; eWAT, epididymal white adipose tissue.

(h) Histology of a single iWAT pad stained with anti-GFP (generated by combining multiple images) from Ucp1-NuTRAP mice after 1 week of warming. Examples of cut lines are shown to describe how iWAT pads are processed; each piece has different amounts of GFP-labeled cells. iWAT from Ucp1-NuTRAP mice after 4 week of warming was subjected to this processing for marker gene analysis.

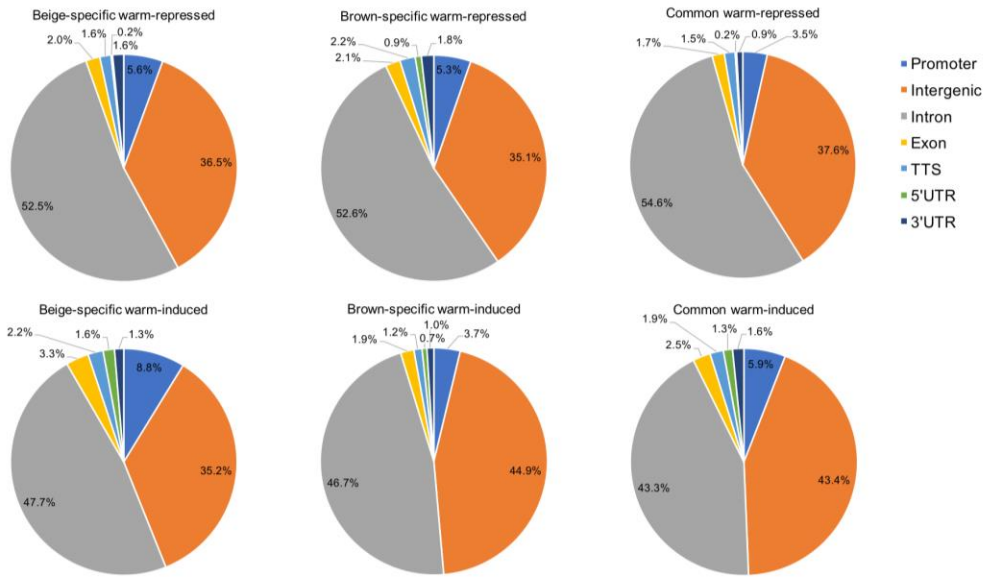

Figure S3. (Related to Figure 3)

Distribution of H3K27ac peaks of different types of adipocytes in cold and warm conditions

Distribution of H3K27ac peaks in different clusters, as indicated in Figure 3F. Peak locations are annotated as Promoter (+1kb to -100bp), TTS (transcription termination site, -100bp to +1kb), Exon (coding), 5' UTR Exon, 3' UTR Exon, Intronic, or Intergenic regions.

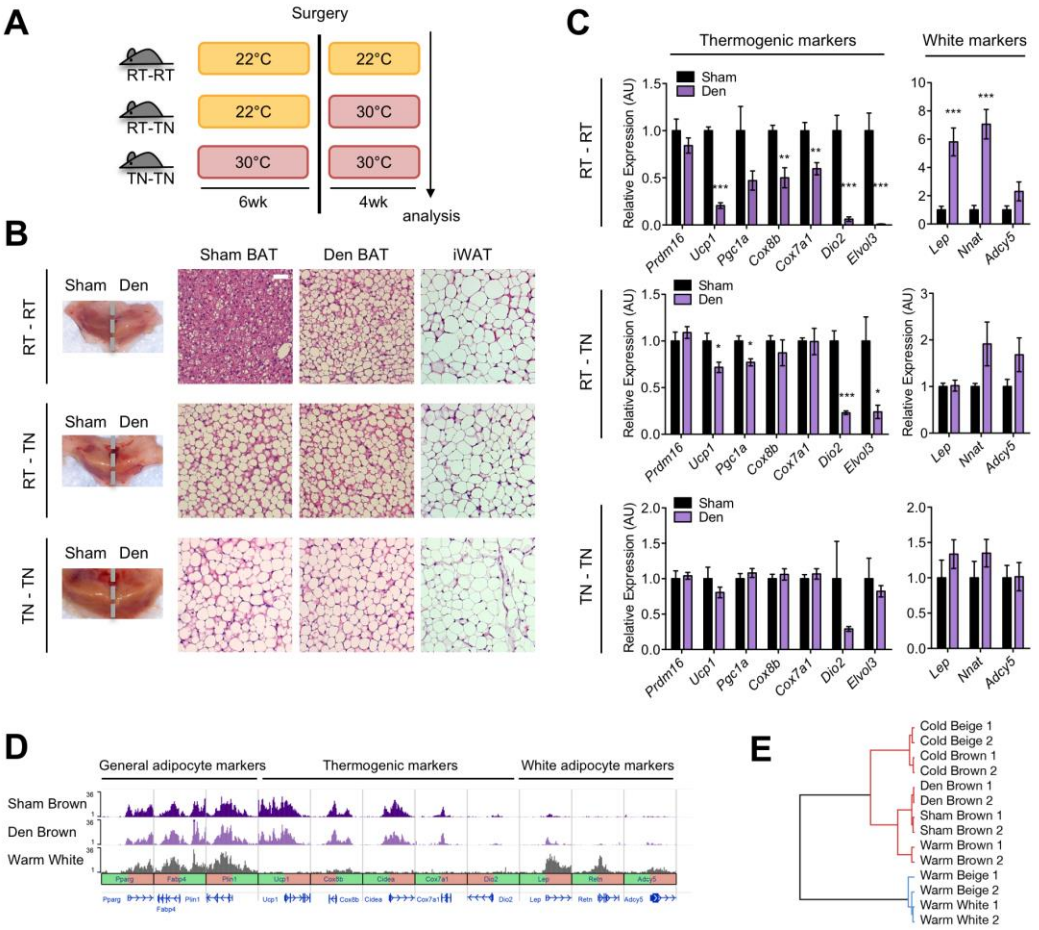

Figure S4. (Related to Figure 3)

Brown adipocyte chromatin state is maintained independently of sympathetic innervation.

(a) Scheme of the denervation experiments. Wild-type mice housed at RT (22˚C) are denervated unilaterally and subsequently incubated at RT (22˚C) (RT-RT) or thermoneutrality (30˚C) (RT-TN) for 4wk. Some wild-type mice are housed at thermoneutrality (30˚C) pre- and post-denervation (TN -TN).

(b) Morphology of BAT from different conditions described in (a). Gross morphology of BAT is shown on the left; the left pad is sham-operated (Sham), and the right pad is denervated (Den). H&E staining of BAT and iWAT sections is shown at right. iWAT from non-operated animals in the same temperature settings is shown for comparison with BAT. Scale bar: 20μm.

(c) Gene expression analysis of thermogenic and white adipocyte markers in denervated BAT by qRT-PCR. Bars indicate mean ± SEM (n=4 animals for RT-RT and TN-TN, n=3 animals for RT-TN) (*p<0.05; **p<0.01; ***p<0.005).

(d) H3K27ac peaks from Sham and Den brown adipocytes isolated from TN-TN mice are shown together with warm white adipocytes. Promoter regions (±5kb of TSS) of different marker genes are shown in each column.

(e) Dendrogram showing hierarchical clustering of H3K27ac peak profiles of Sham and Den brown adipocytes with beige, brown and white adipocytes in cold and warm conditions. Two biological replicates are shown. Brown and white adipocyte clusters are highlighted in red and blue, respectively.

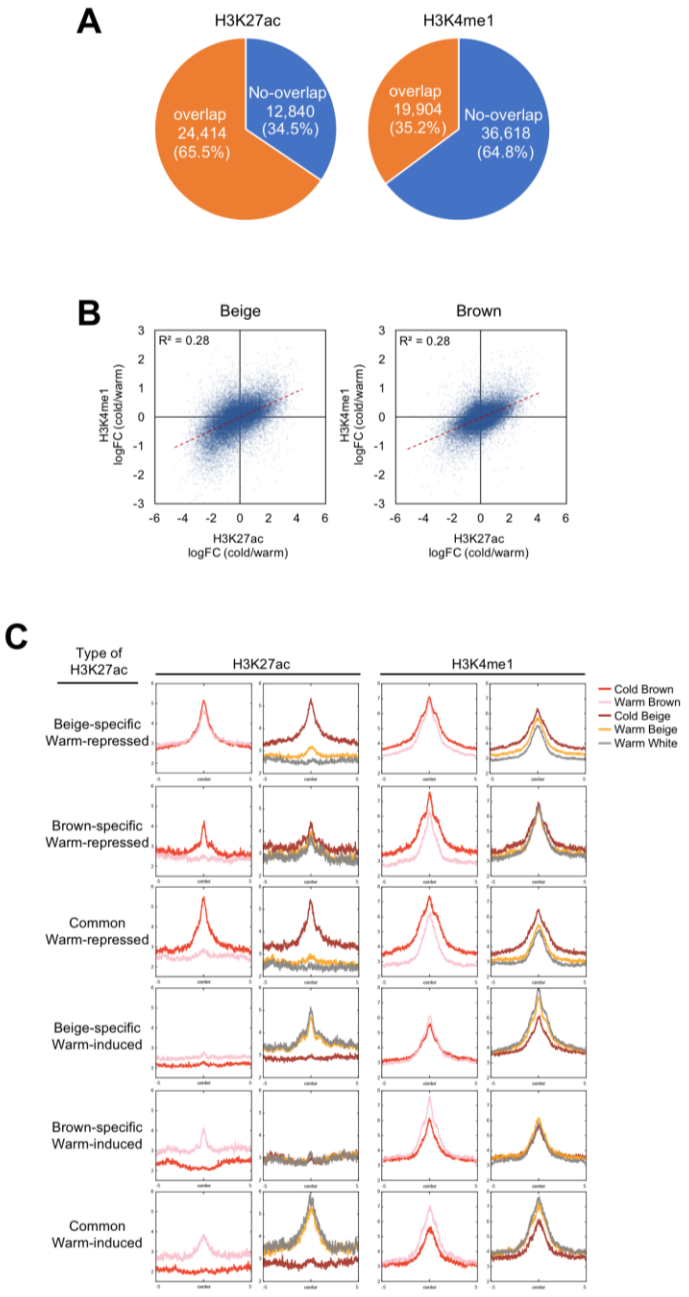

Figure S5. (Related to Figure 4)

Identification and characterization of poised enhancers in beige adipocytes

(a) Fractions of overlapping and non-overlapping H3K27ac (left) and H3K4me1 (right) peaks in all adipocytes.

(b) Scatter plots showing correlation of the changes of overlapping peak signals upon warming between H3K27ac and H3K4me1 in beige (left) and brown (right) adipocytes. The red dashed line is the trend line.

(c) Distribution plots of H3K27ac (left) and H3K4me1 (right) peaks in each cluster described in Figure 4A. Brown adipocyte samples are shown separately from beige and white adipocytes.

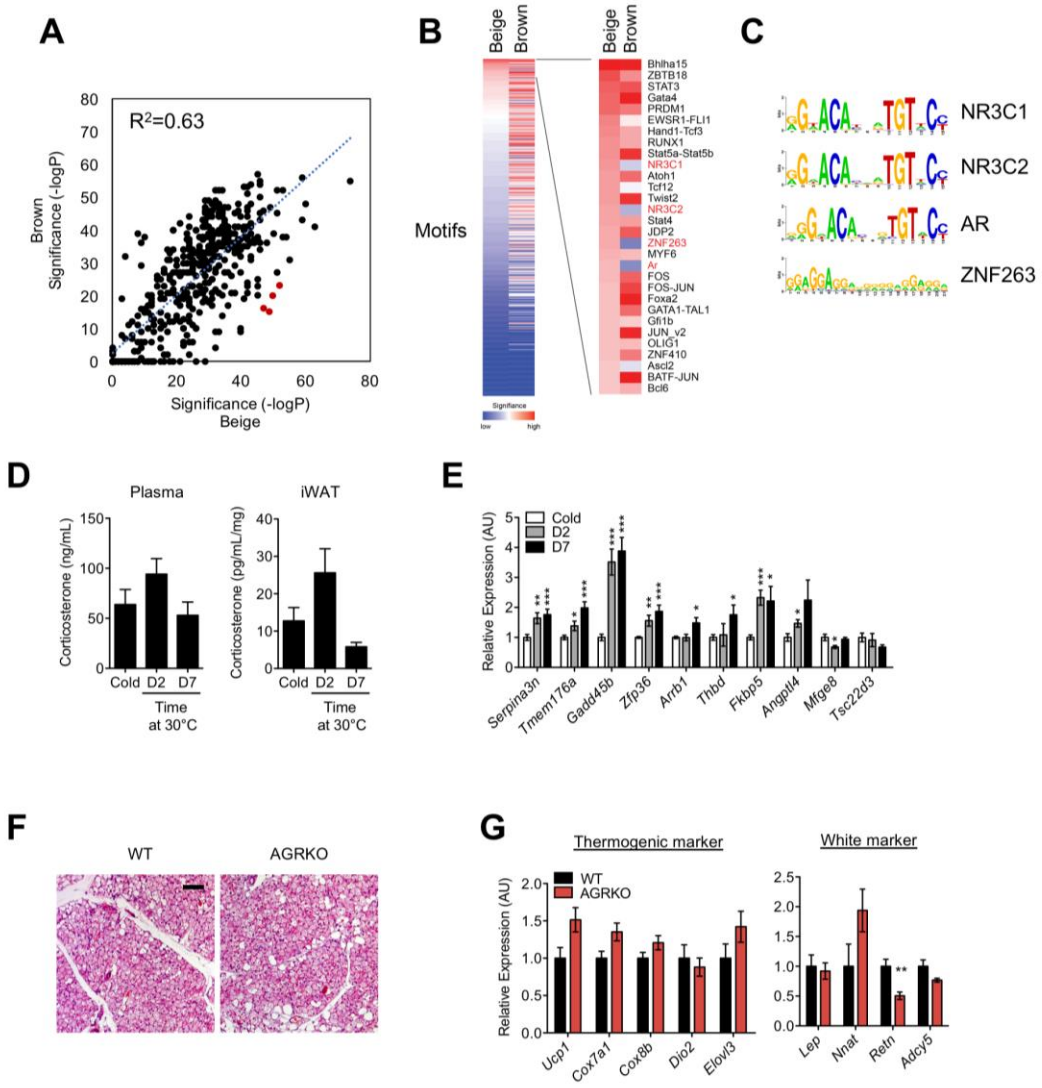

Figure S6. (Related to Figure 6)

Identification of glucocorticoid receptor as a beige adipocyte whitening factor

(a) Scatter plot showing the correlation of significance of motifs enriched by warming between brown and beige adipocytes. Blue dotted line is the trend line. Four motifs preferentially enriched in beige adipocytes are highlighted in red.

(b) Heatmap of significance of motifs (rows) enriched by warming in brown and beige adipocytes (columns). Ranks of motifs within each cell type are represented by color scale as shown. Motifs are sorted by their significance in beige adipocytes. Top 30 motifs from beige adipocytes are shown on the right with the beige-preferentially enriched motifs highlighted in red.

(c) Position weight matrix (PWM) logos of beige-enriched motifs.

(d) Corticosterone measured by ELISA in the plasma and iWAT from WT animals exposed to cold and subsequent warming for 2 and 7 days at 30˚C. Bars indicate mean ± SEM (n=8 animals/group).

(e) Expression of GR target genes in iWAT at the indicated different time points during warming after cold exposure. Bars indicate mean ± SEM (n=8 animals/group) (*p<0.05; **p<0.01; ***p<0.005).

(f) H&E stained sections of iWAT from WT and AGRKO mice after cold exposure. Scale bar: 20μm.

(g) Expression of thermogenic and white adipocyte genes in iWAT from WT and AGRKO mice after cold exposure. Bars indicate mean ± SEM (n=5 animals/group) (*p<0.05; **p<0.01; ***p<0.005).

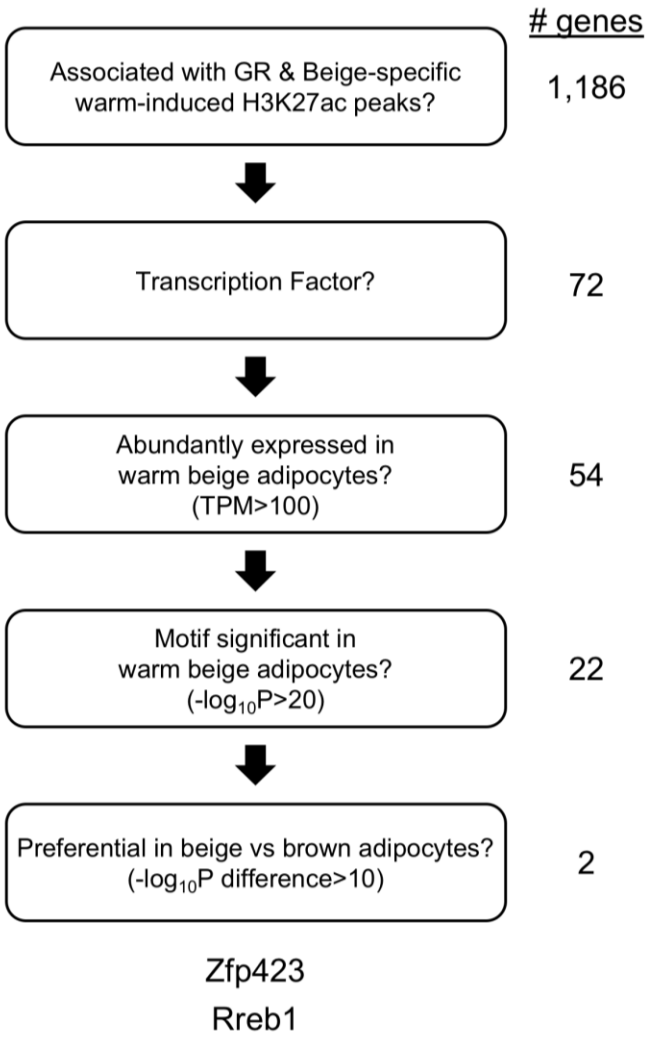

Figure S7. (Related to Figure 7)

Identification of Zfp423 as a direct target of GR in beige adipocyte whitening

Transcription factors downstream of GR that potentially mediate beige adipocyte whitening were identified using a series of filtering steps: (1) co-localization of beige-specific warm-induced H3K27ac peaks and GR binding sites, (2) transcription factor, (3) transcript abundance, (4) significance of the motifs in warm-induced H3K27ac in beige adipocytes, (5) motif preference for beige vs brown adipocytes. Numbers of genes remaining after each filtering step are shown at right. Two transcription factors, Zfp423 and Rreb1, meet all criteria.

Table S2. (Related to STAR Methods) Primers used for qRT-PCR

Gene	Forward	Reverse
Adcy5	CATCTCTCTGCACACCAACT	TGCAGGAGAAGATGAGGACA
Agt	AGCACCCTACTTTTCAACACC	GTTGTCCACCCAGAATTCATG
Angptl4	GGACCTTAACTGTGCCAAGAG	CGTGGGATAGAGTGGAAGTATTG
Ankrd33b	GGAGACTGACCGCAACG	AGGCGAATCCATTTCCAGATC
Arrb1	TGTCTCTTCAACACAGCTCAG	AGGAAGGGAGTCAGCGTATAG
Cidea	TGCTCTTCTGTATCGCCCAGT	GCCGTGTTAAGGAATCTGCTG
Cox7a1	GCTCTGGTCCGGTCTTTTAG	CTTTCAAGTGTACTGGGAGGTC
Cox8b	GAACCATGAAGCCAACGACT	GCGAAGTTCACAGTGGTTCC
Dio2	CAGTGTGGTGCACGTCTCCAATC	TGAACCAAAGTTGACCACCAG
Eln	GGTGGCTTTCCTGGCTATG	CTCCAGCACCATACTTAGCAG
Elovl3	ATGCAACCCTATGACTTCGAG	ACGATGAGCAACAGATAGACG
Fabp4	AAGGTGAAGAGCATCATAACCCT	TCACGCCTTTCATAACACATTCC
Fkbp5	GTAGAATCAAACGGAAAGGCG	CCACATCTCGGCAATCAAATG
Foxp2	ACATCGACAGCAATGGGAAC	ATTGGACAGTCTTCATCCTCTG
Gadd45b	GCGGCCAAACTGATGAATG	GCAGAACGACTGGATCAGG
Hoxa5	CAAGCTGCACATTAGTCACG	GGTAGCGGTTGAAGTGGAAT
Hsd11b1	AGGGATTGGAAGAGAAATGGC	TTCAAGGCAGCGAGACAC
Kcnk2	TCTTTGGCTTTCTACTGGCTG	CTTCGTCTGACTAACATTCCACT
Lep	GAGACCCCTGTGTCGGTTC	CTGCGTGTGTGAAATGTCATTG
Mfge8	AGCTGTACCCTGTTTCGTG	TCTGGCTGTCAGGAATTGTG
Nnat	ACCCACTTTCGGAACCATG	CAGCTTCTGCAGGGAGTAC
Pdk4	GCAGCCCGCTTCGTGATG	CACAGGCATTTTCTGAACCAAAG
Pgc1a	CCCTGCCATTGTTAAGACC	TGCTGCTGTTCCTGTTTTC
Prdm16	CAGCACGGTGAAGCCATTC	GCGTGCATCCGCTTGTG
Retn	ACAAGACTTCAACTCCCTGTTTC	TTTCTTCACGAATGTCCCACG
serpina3n	AGGACATTGATGGTGCTGG	CCGCGTAGAACTCAGACTTG
Slc16a12	TGTAGACTGTATGACAATGCTCTG	TGAAACCAGTAGATGCCAGC
Slc29a1	CAGAGCAGGAGACCAAGTTG	GTATGGCTTTGATAGACTGGTTTC
Slc2a13	GCATCAGGAACAGTATCGAGG	ACATCCCACAACTAACGCTC
Spon1	CTCCTACTTCAGAGGTTTCACG	ACTGGGTCTCTTCCTCATCTATG
Tbp	ACCCTTCACCAATGACTCCTATG	TGACTGCAGCAAATCGCTTGG
Tbx1	GGCAGGCAGACGAATGTTC	TTGTCATCTACGGGCACAAAG
Thbd	CATACGGAAACCCCTCTGC	CCAGCACACCCAGAAAGA
Tmem176a	CATGCCTAGAACCACTCCAG	CCCAGATACCCAAGAGCATAG
Tsc22d3	TGGTTCTGCGGTGTAAGTG	TCCACATGAGATGACGCTTG
Ucp1	ACTGCCACACCTCCAGTCATT	CTTTGCCTCACTCAGGATTGG
Vwf	GCCAGAGCCTGTCTATCAATG	ACACATCGGTCTTCATCCAAG
Zfp36	TGGATCTCTCTGCCATCTACG	ATGGAGTCCGAGTTTATGTTCC
Zic1	AACCTCAAGATCCACAAAAGGA	CCTCGAACTCGCACTTGAA