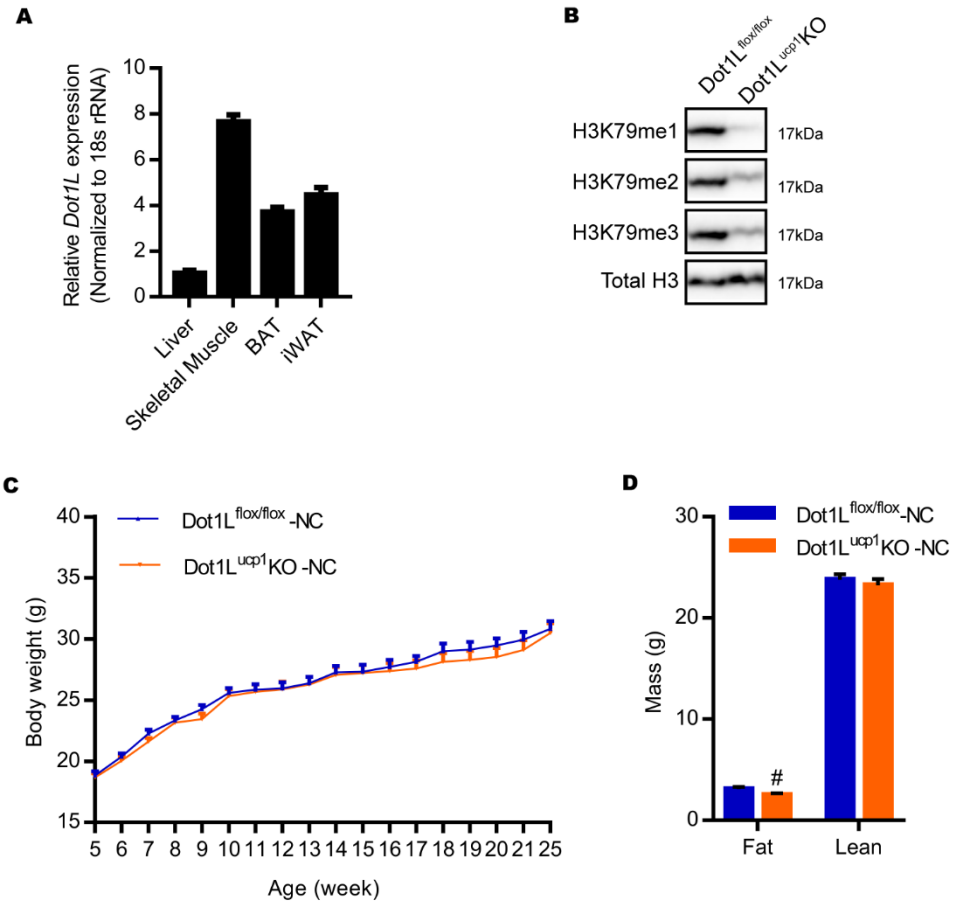
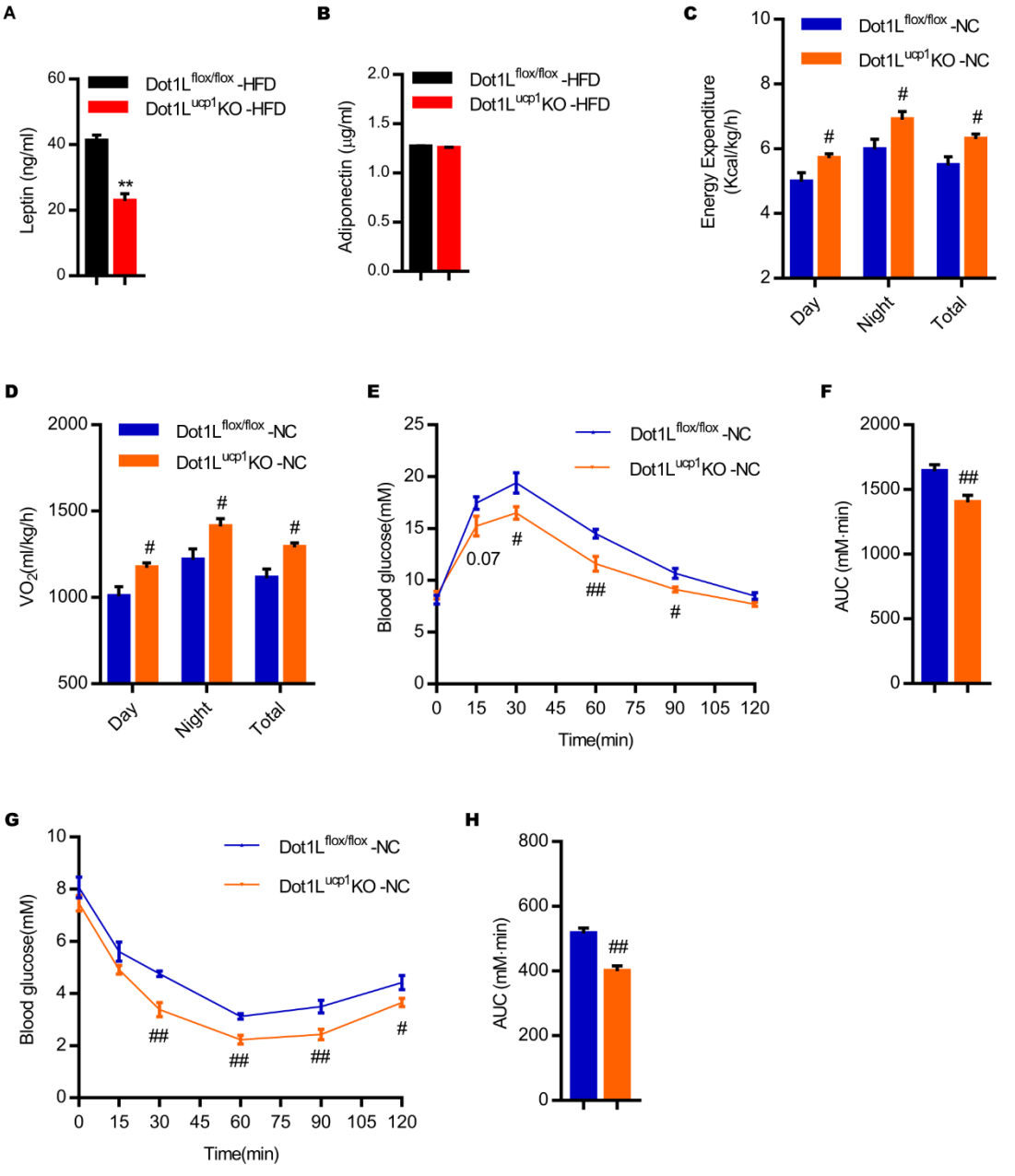
Antibodies. anti-PRDM16 (ab106410), and anti-UCP1 for immunohistochemistry (IHC) (ab10983) were from Abcam. Anti-PPARγ (sc-7273), anti-CEBPα (sc-61), and anti-CEBPβ (sc-7962) were from Santa Cruz. Anti-PGC-1α (ST1202) was from Calbiochem. Anti-UCP1 (ab5857) for Western blots was from Abclonal. β-ACTIN (AM1021B) was from Abgent. α-TUBULIN was from Cell Signaling. The anti-H3 (PTM-1002), anti-H3K79me1 (PTM-628), anti-H3K79me2 (PTM-629), and anti-H3K79me3 (PTM-630) for western blots were from PTM biolabs. ChIP grade antibodies: anti-H3K79me1 (ab2886), anti-H3K79me2 (ab3594), anti-H3K79me3 (ab2621) and anti-RNA polymerase II (ab5131) were from Abcam, and normal rabbit IgG (#2729) was from Cell Signaling technology.

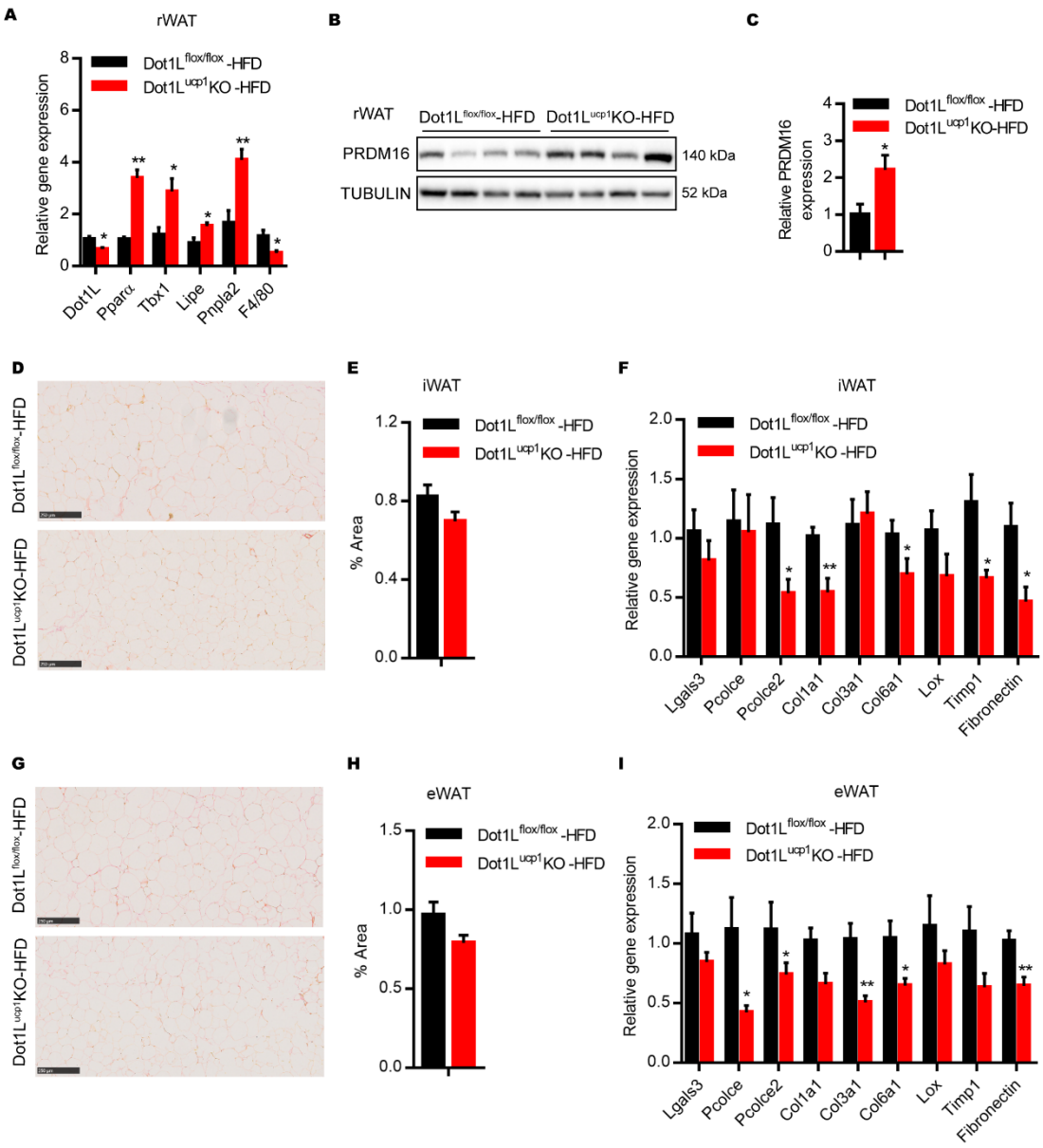
Supplementary figure and figure legends



Supplementary Figure 1. A-B Analysis of *Dot1L* expression and H3K79 methylations. **A** *Dot1L* expression levels in liver, skeletal muscle, BAT and iWAT from C57BL/6 mice. **B** H3K79 methylation expression levels in BAT from *Dot1L*$^{flox/flox}$ mice and *Dot1L*^{ucp1} knockout mice. **C** Body weight changes of *Dot1L*^{ucp1} knockout mice and control mice fed with normal chow diet. n=10. **D** Fat and lean mass content of *Dot1L*^{ucp1} knockout mice and control mice fed with normal chow diet. n=10. *P < 0.05, **P < 0.01, $^{\#}$P < 0.05, $^{\#\#}$P < 0.01.

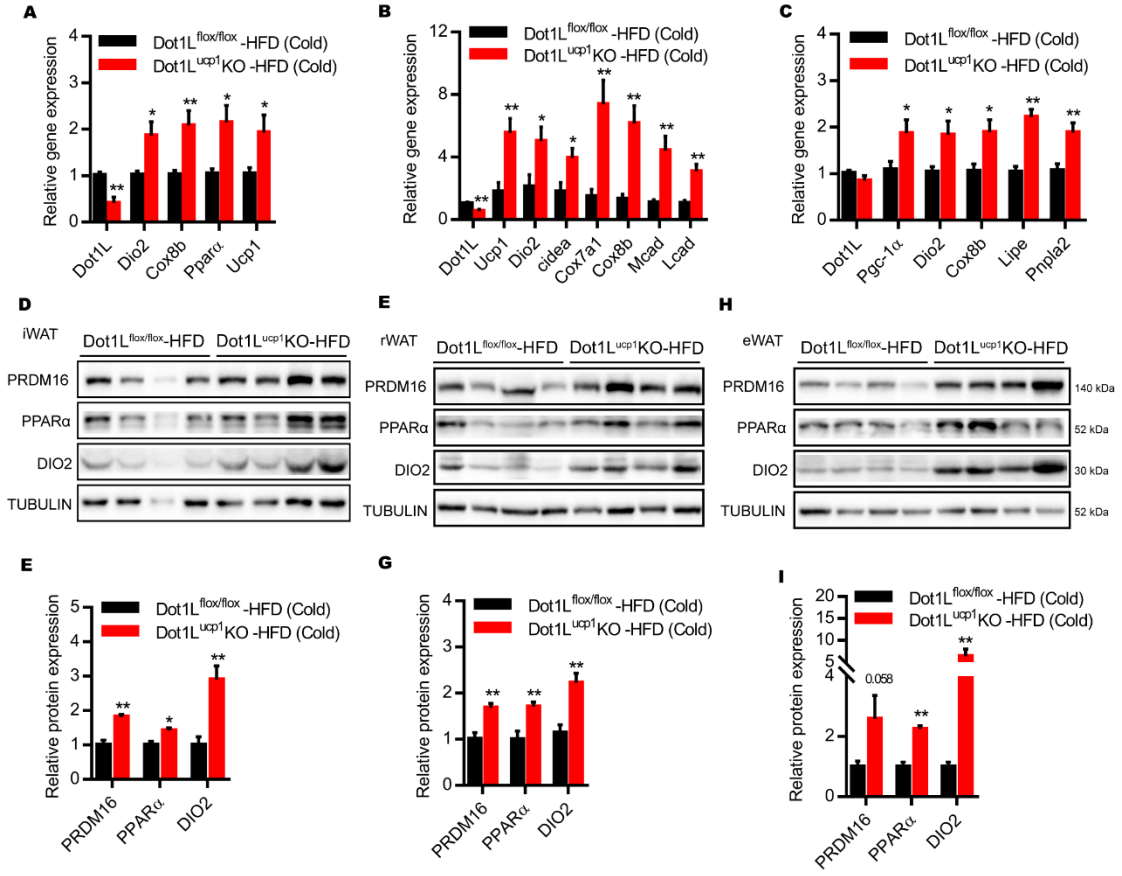


Supplementary Figure 2. A-B Plasma levels of leptin and adiponectin in *Dot1L*$^{flox/flox}$ mice and *Dot1L*^{ucp1} knockout mice. **A** Plasma leptin level. n=13 in each group. **B** Plasma adiponectin level. n=9 in each group. **C-H** Metabolic phenotypes on *Dot1L*^{ucp1} knockout mice fed with normal chow diet. **C** Energy expenditure, **D** Oxygen consumption of *Dot1L*^{ucp1} knockout mice and control littermates during a 24h period. 23 weeks old. n=6 in each group. **E**, **F** Glucose tolerance test in *Dot1L*^{ucp1} knockout mice and control littermates. Blood glucose levels at indicated time point and area under the curve. 18 weeks old. n=9 in each group. **G**, **H** Insulin tolerance test in *Dot1L*^{ucp1} knockout mice and control littermates. Blood glucose levels at indicated time points and area under the curve. 21 weeks old. n=6 in the control group; n=9 in the knockout group. *$P < 0.05$, **$P < 0.01$, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$.



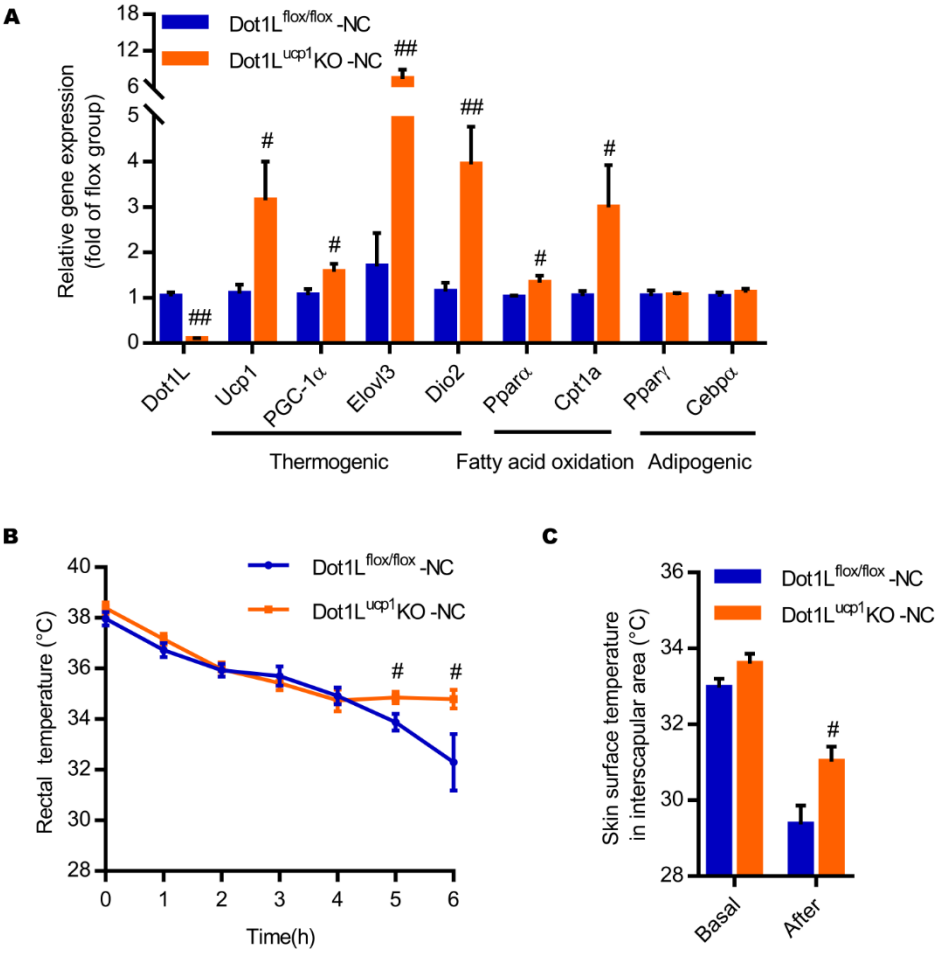
Supplementary Figure 3. A-C, Similar results were observed in rWAT, consistent with those in iWAT. **A** qRT-PCR analysis of the expression levels of the indicated gene, including *Dot1L*, thermogenic genes, beige adipocyte-specific genes, and inflammation genes, in rWAT. n=7 in the control group; n=6 in the knockout group. **B**, **C** Western blot analysis and quantification of PRDM16 expression in rWAT. n=5 in the control group; n=6 in the knockout group. **D-E** Sirius Red staining and analysis on the iWAT of DOT1L knockout mice and control mice. n=5 in each group**. F** Expression levels of pro-fibrosis genes in iWAT. n=6 in control group, n=7 in knockout mice group.

G, **H** Sirius Red staining and analysis on the eWAT of DOT1L knockout mice and control mice. n=6 in each group. **I** Expression levels of pro-fibrosis genes in eWAT. n=6 in control group, n=7 in knockout mice group. *P < 0.05, **P < 0.01.

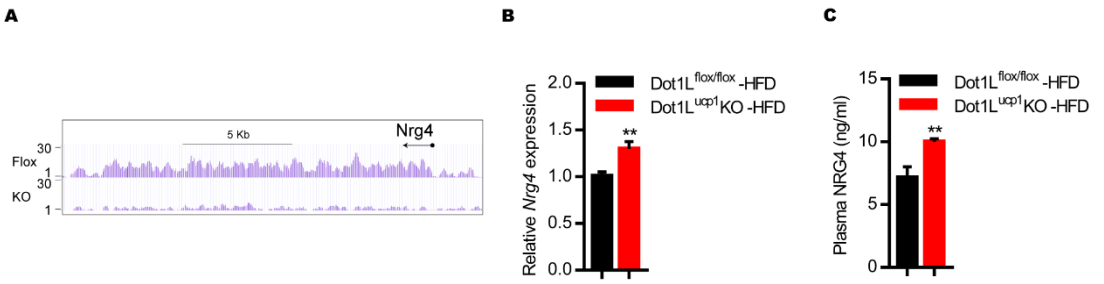


Supplementary Figure 4. Loss of DOT1L in beige adipocytes promotes an overall browning in WATs under cold challenge. **A-C** qRT-PCR analysis of expression levels of indicated gene, including *Dot1L*, thermogenic genes, and lipid metabolism genes, in iWAT (**A**, n=7 in the control group; n=6 in the knockout group), rWAT (**B**, n=7 in each group), and eWAT (**C**, n=7 in each group except *Dio2*: n=7 in the control group; n=6 in the knockout group). **D**, **E** Western blot analysis and quantification of PRDM16, PPARα and DIO2 in iWAT. n=6 in the control group, n=7 in the knockout group. **F**, **G** Western blot analysis and quantification of PRDM16, PPARα and DIO2 in

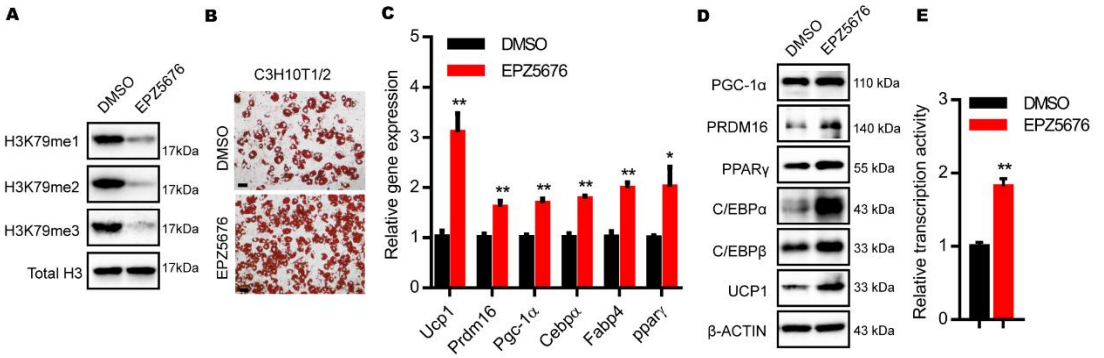
rWAT. n=5 in each group. **H**, **I** Western blot analysis and quantification of PRDM16, PPARα and Dio2 in eWAT. n=6 in the control group, n=5 in the knockout group. *P < 0.05, **P < 0.01.



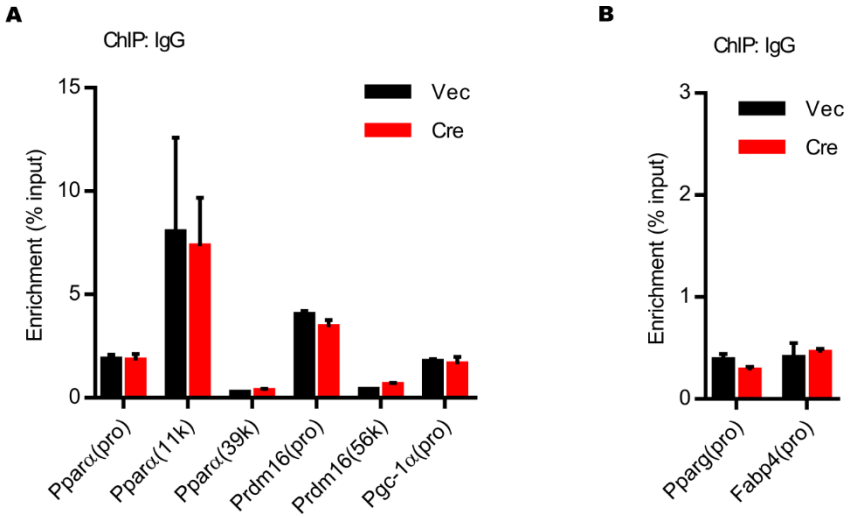
Supplementary Figure 5. Loss of DOT1L in brown and beige adipocytes facilitates adaptive thermogenesis. **A** qRT-PCR analysis of expression levels of indicated genes in BAT of *Dot1L*^{ucp1} knockout mice and control littermates fed with normal chow diet. **B, C** 22-week-old *Dot1L*^{ucp1} knockout mice and control littermates fed with normal chow diet were placed at 4 °C. **B** Rectal temperature changes following a 6 h cold challenge. **C** Statistics of skin surface temperature in interscapular areas before and after 6 h cold exposure, respectively. n=6 in control group, n=7 in knockout mice group. $^{\#}$P < 0.05, $^{\#\#}$P < 0.01.



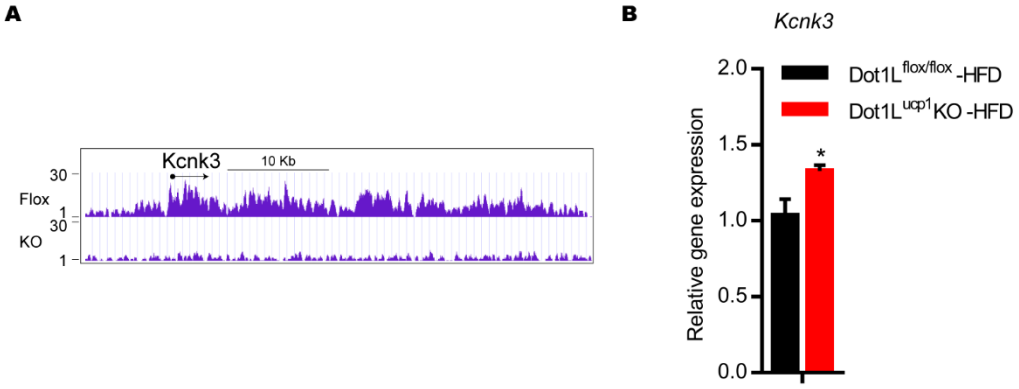
Supplementary Figure 6. DOT1L-mediated H3K79 methylation is associated with a BAT-enriched secreted factor *Nrg4* expression. **A** Signal tracks of H3K79me2 in *Nrg4* loci. **B** Expression of *Nrg4* in BAT of *Dot1L*^{ucp1} knockout mice and control littermates. n=8 in each group. **C** Plasma NRG4 levels. n=8 in each group. *P < 0.05, **P < 0.01.



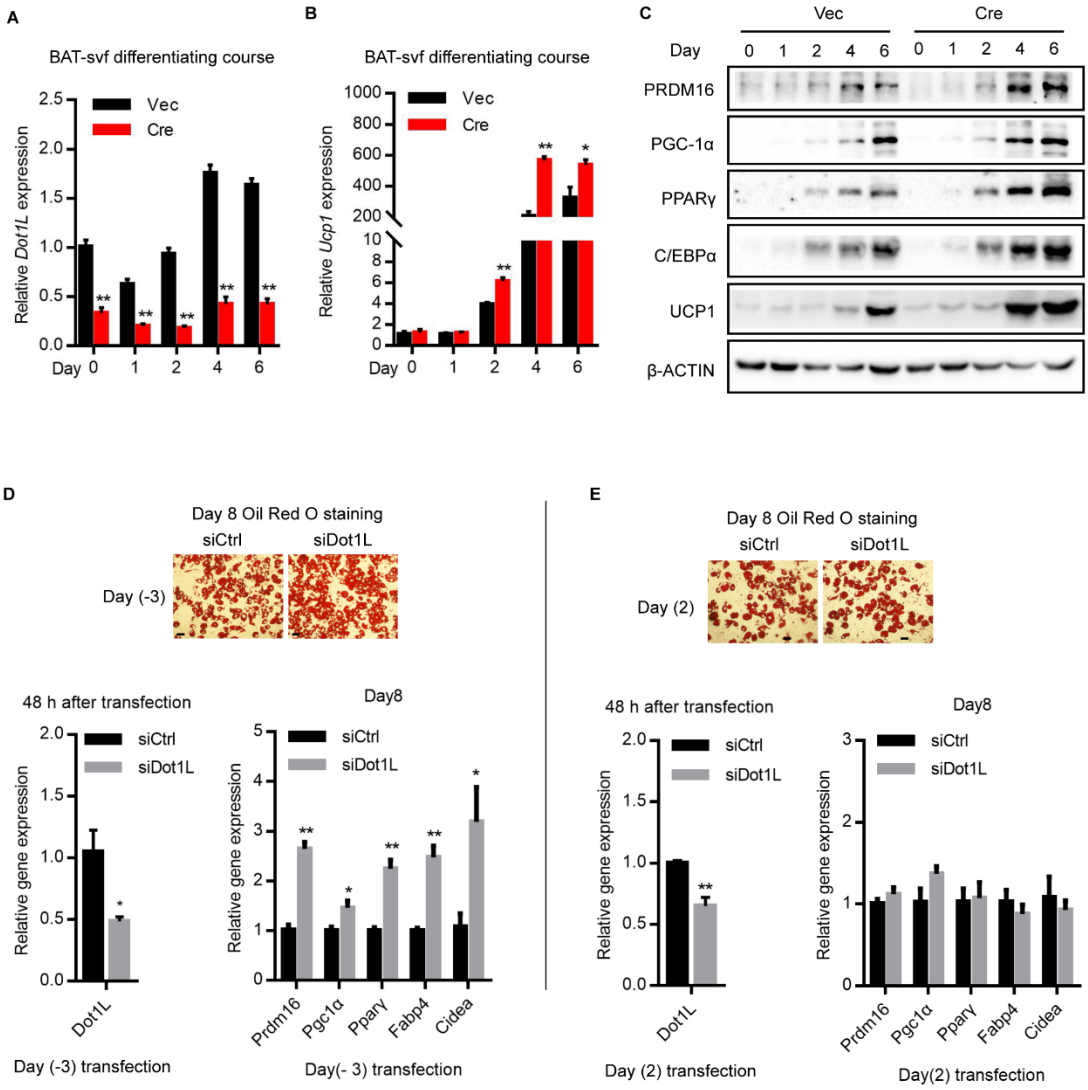
Supplementary Figure 7. Inhibition of DOT1L by EPZ5676 promotes brown adipocyte differentiation and *Ucp1* transcription. **A** Western blot analysis of H3K79 methylation levels. **B** Oil red O staining of differentiated C3H10T1/2 cells. **C** qRT-PCR analysis of the expression of adipogenic and thermogenic genes in differentiated C3H10T1/2 cells. n=4 in each group. **D** Western blot analysis of adipogenic and thermogenic markers in differentiated C3H10T1/2 cells. **E** Luciferase assay using a reporter construct containing the *Ucp1* promoter in HEK293 cells. n=8 in each group. *P < 0.05, **P < 0.01.



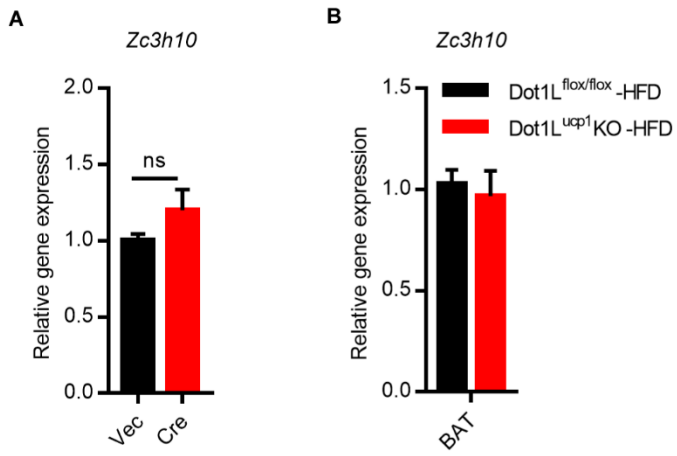
Supplementary Figure 8. Enrichment of IgG on genomic regions of BAT-selective genes (*Pparα*, *Prdm16*, and *Pgc-1α*) or common fat genes (*Pparg* and *Fabp4*). n=3. Pro indicated promoter region, within 1 kb of the TSS. ~ kb indicated around ~ kb distance to the TSS.



Supplementary Figure 9. DOT1L-mediated H3K79 methylation is associated with *Kcnk3* expression. **A** Signal tracks of H3K79me2 in *Kcnk3* loci. **B** Expression of *Kcnk3* in BAT of *Dot1L*^{ucp1} knockout mice and control littermates. n=7 in control group, n=8 in knockout mice group. *P < 0.05, **P < 0.01.



Supplementary Figure 10. Loss of DOT1L affected brown adipocyte differentiation, and functions indispensably in the early phase. **A-C** Gene and protein expression analysis during the time course of brown adipocyte differentiation. **D** Results as indicated from *Dot1L* knockdown by transfected with siRNA targeting *Dot1L* at day -3 before initiation of differentiation. **E** Results as indicated from *Dot1L* knockdown by transfected with siRNA targeting *Dot1L* at day 2 of differentiation. n=3-4 in each group. *P < 0.05, **P < 0.01.



Supplementary Figure 11. The expression of *Zc3h10* in differentiated BAT-SVF cells and BAT of DOT1L knockout mice. **A** in differentiated BAT-SVF cells. n=3 in the Vec group. n=3 in the Cre group. **B** in BAT of knockout mice and control mice. n=11 in each group. *P < 0.05, **P < 0.01.

Supplementary Table. Primer sequences used in qPCR and ChIP-qPCR.

qPCR	Forward 5'-3'	Reverse 5'-3'
Ucp1	ACTGCCACACCTCCAGTCATT	CTTTGCCTCACTCAGGATTGG
Cebpa	AGTACCGGGTACGGCGGGAAC	GCGTGTCCAGTTCACGGCTCA
Cebpβ	TCGGGACTTGATGCAATCC	AAACATCAACAACCCCGC
Fabp4	ACACCGAGATTTCCTTCAAACTG	CCATCTAGGGTTATGATGCTCTTCA
Pparγ	TTCCGAAGAACCATCCGATTG	TGGCATTGTGAGACATCCCCAC
Prdm16	CAGCACGGTGAAGCCATTC	GCGTGCATCCGCTTGTG
Pgc-1α	TATGGAGTGACATAGAGTGTGCT	CCACTTCAATCCACCCAGAAAG
Pgc-1β	GTCCCTGGCTGACATTCACT	GCACGGATCTCATGGTCTCT
Dio2	AATTATGCCTCGGAGAAGACCG	GGCAGTTGCCTAGTGAAAGGT
Cidea	TGCTCTTCTGTATCGCCCAGT	GCCGTGTTAAGGAATCTGCTG
Cox5b	GCTGCATCTGTGAAGAGGACAAC	CAGCTTGTAATGGGTTCCACAGT
Cox7a1	CTCTTCCAGGCCGACAATGA	GCCCAGCCCAAGCAGTATAA
Cox8b	GAACCATGAAGCCAACGACT	GCGAAGTTCACAGTGGTTCC
Pparα	GCGTACGGCAATGGCTTTAT	GAACGGCTTCCTCAGGTTCTT
Acox1	TAACTTCCTCACTCGAAGCCA	AGTTCCATGACCCATCTCTGTC
Lcad	TCACCAACCGTGAAGCTCGA	CCAAAAAGAGGCTAATGCCATG
Mcad	AGCTGCTAGTGGAGCACCAAG	TCGCCATTTCTGCGAGC
Eva1	CCACTTCTCCTGAGTTTACAGC	GCATTTTAACCGAACATCTGTCC
Fbxo31	AAACTGCTTCACCGATACAGAC	ACCACGACGTTCAGCAATCC
Oplah	CTTCACGCACGTCTCCTTGT	GCATCTGCACAGGCCGTAT
Elovl3	TTCTCACGCGGGTTAAAAATGG	GAGCAACAGATAGACGACCAC
Cpt1a	CTCCGCCTGAGCCATGAAG	CACCAGTGATGATGCCATTCT
Slc27a1	CTGGGACTTCCGTGGACCT	TCTTGCAGACGATACGCAGAA
Foxc2	CACTCTGAACGGCATCTACCAG	TGAGTGACAGGTTGTGGCGGAT
Lipe	CCGCTGACTTCCTGCAAGAG	CTGGGTCTATGGCGAATCGG
Pnpla2	GGTGACCATCTGCCTTCCAG	TGCAGAAGAGACCCAGCAGT
Me1	GTCGTGCATCTCTCACAGAAG	TGAGGGCAGTTGGTTTTATCTTT
Acc1	TCTACGGCAGCAGTTACACCACAT	TCTCTTCATTACCTCAATCTCAGCATAG
Zic1	CTGTTGTGGGAGACACGATG	CCTCTTCTCAGGGCTCACAG
Sp100	TGATGGAGGGAACCCAAACTC	CTTCCTTGAGAATAGCTGGCAC
Tbx1	GGCAGGCAGACGAATGTTC	TTGTCATCTACGGGCACAAAG
Mcp-1	GCAGTTAACGCCCCACTCA	CCCAGCCTACTCATTGGGATCA
F4/80	AACTCTGTCCTCCTTGCCTGG	CAGCAACCTCGTGTCCTTGAG
IL-1β	CAACCAACAAGTGATATTCTCCATG	GATCCACACTCTCCAGCTGCA
Nrg4	CACGCTGCGAAGAGGTTTTTC	CGCGATGGTAAGAGTGAGGA
36b4	GAAACTGCTGCCTCACATCCG	GCTGGCACAGTGACCTCACACG
ChIP-qPCR	Forward 5'-3'	Reverse 5'-3'
Pparα-39k	CCCTGGTCTGTGGGAATAAA	TCGTGGTATCAGCCATGAAG
Pparα-11k	AAGAGCATGGGACAGTGGCCG	TGGCCAGCTGAAGGTCACCAC
Pparα-pro	CTGTCCGCCACTTCGAGTC	GAACACCAATGTTCGGAGCC
Prdm16-56k	ACTGATTGGCCTCAGCAAAC	CCTGAAGGCCTGACTCACTC
Prdm16-pro	AAGTGAGGTGAAGACCGAGAAGG	GAATCTCTCTCCTCCTCCTTGAGCC

Pgc-1α-pro	AGGGCTCCGGTTTAGAGTTG	TGCCTCAGTGAAGTAACGCTT
Pparγ-pro	CCCTCACAGAACAGTGAATGTGT	TGCTTTGGCAAGACTTGGTACAT
Fabp4-pro	CAGGAAAGGGACACAGCACT	GAGTGGAACTGGCAACCGTA