

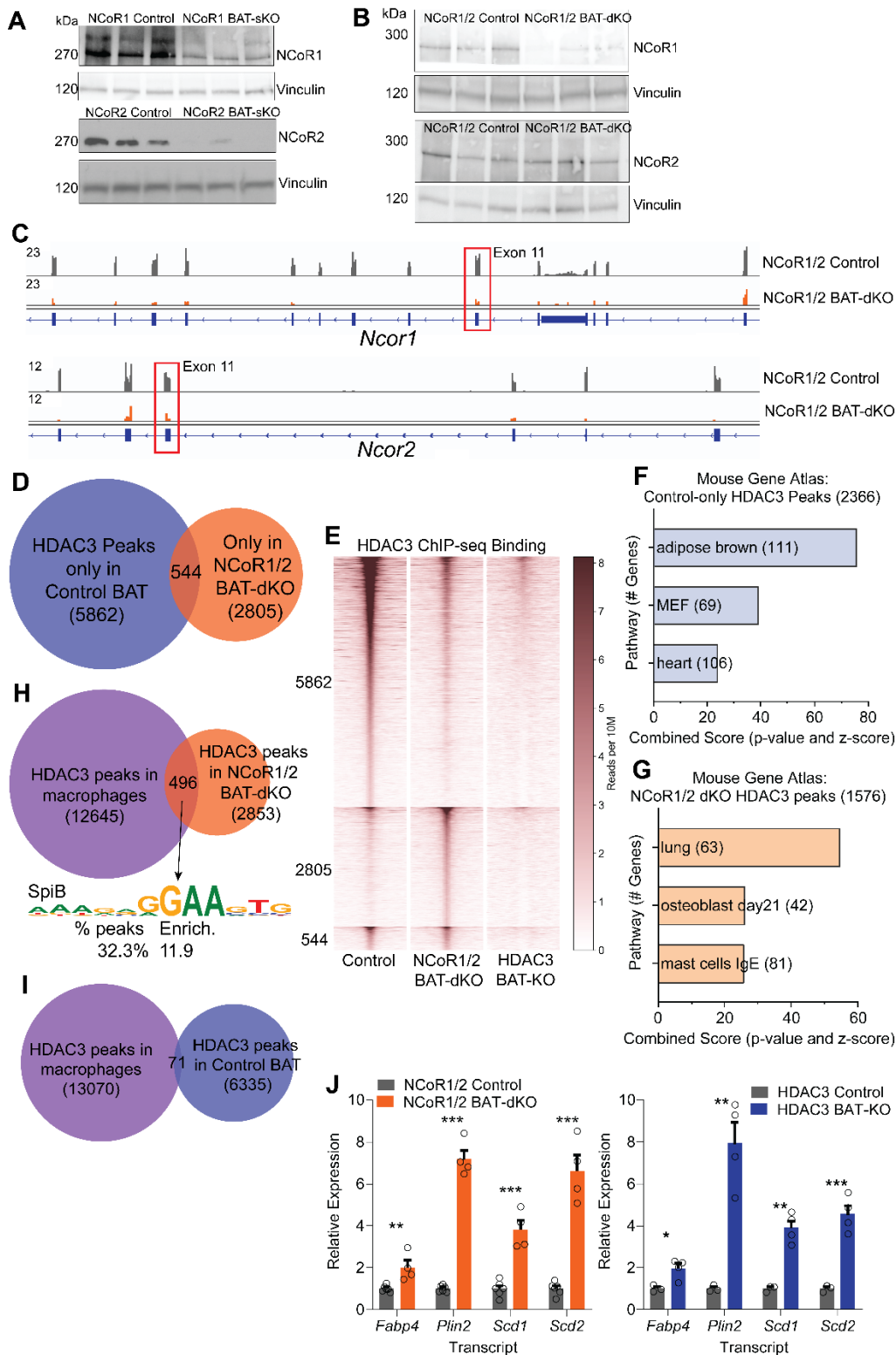
## **Supplementary Information for** Balanced Control of Thermogenesis by Nuclear Receptor Corepressors in Brown Adipose Tissue

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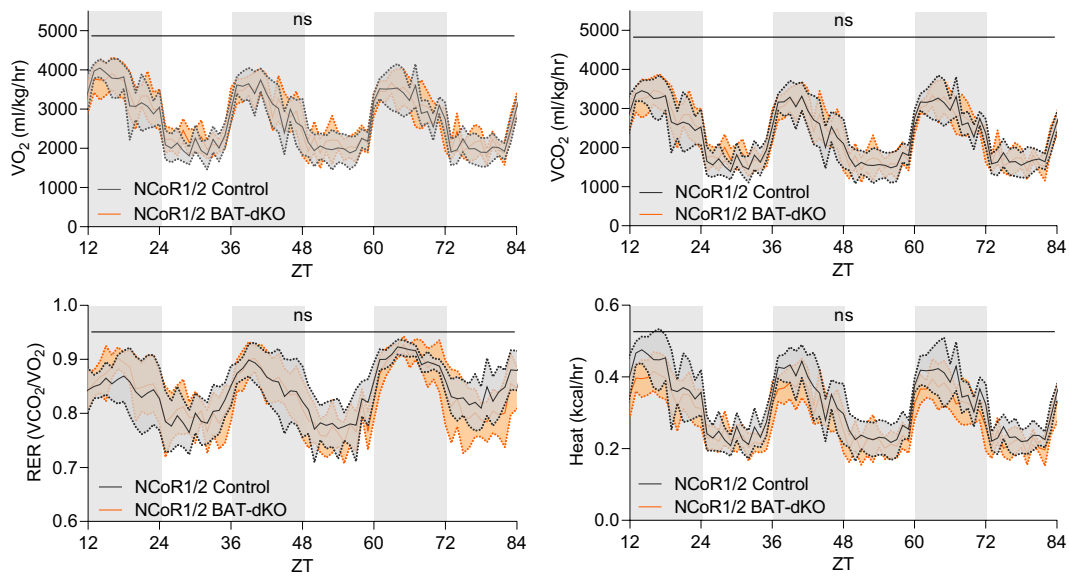
### **This PDF file includes:**

Figures S1 to S4  
Table S1

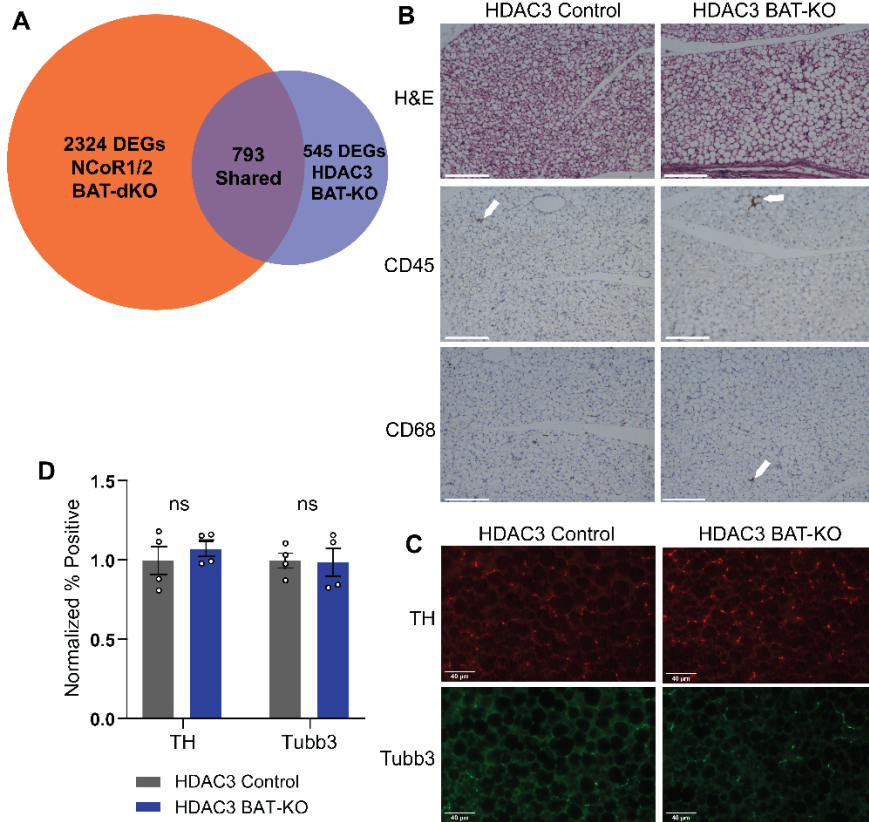


**Figure S1.** Validation of NCoR1/2 BAT-dKO and HDAC3 binding loss. **A**, Western blots showing loss of either NCoR1 or NCoR2 in brown adipose tissue from the appropriate BAT-sKO model with Vinculin loading control (n=3 per group). **B**, Western blots for NCoR1 and NCoR2 in Control or NCoR1/2 BAT-dKO whole BAT lysate, with Vinculin loading control (n=3,3). **C**, Tracks for RNA

quantity from Control and NCoR1/2 BAT-dKO RNA-seq, highlighting floxed exons. **D**, Venn diagram of all HDAC3 peaks called over HDAC3-KO in either Control or NCoR1/2 dKO BAT. **E**, Heat map of peaks from (E), grouped as in (E), and sorted by total signal within groups. **F**, Mouse Gene Atlas enrichment of genes expressed in NCoR1/2 RNA-seq with nearby control BAT HDAC3 peaks. **G**, Mouse Gene Atlas enrichment of genes expressed in NCoR1/2 RNA-seq with nearby HDAC3 peaks in NCoR1/2 BAT-dKO tissue. **H**, Venn diagram of HDAC3 peaks called in macrophages or NCoR1/2 BAT-dKO BAT with top motif identified by HOMER. **I**, Venn diagram of HDAC3 peaks called in macrophages or Control BAT. **J**, Gene expression in BAT for key lipid metabolism transcripts in either Control/NCoR1/2 BAT-dKO (n=6,4) or Control/HDAC3 BAT-KO tissue (n=3,4) housed at thermoneutrality.



**Figure S2.** NCoR1/2 in BAT do not regulate basal respiration.  $VO_2$ ,  $VCO_2$ , RER and heat over 72 hours as measured by the CLAMS in Control and NCoR1/2 BAT-dKO animals at thermoneutral conditions ( $n=6,6$ ). 95% confidence interval is displayed in shaded area between dashed lines and dark periods indicated by grey shaded background. Statistical testing done with 2-way repeated measures ANOVA, ns=no significance between groups. ZT=Zeitgeber time.



**Figure S3.** HDAC3 BAT-KO does not show increased inflammation. **A**, Venn diagram of all DEGs from either NCoR1/2 BAT-dKO or HDAC3 BAT-KO. **B**, H&E staining, CD45 and CD68 IHC in HDAC3 Control BAT and HDAC3 BAT-KO, 20x magnification, arrows indicating positive staining, scale bar set to 200 $\mu$ M, **C**, TH and Tubb3 IF staining of HDAC3 Control BAT and HDAC3 BAT-KO, 40x magnification, scale bar set to 40 $\mu$ M, **D**, Quantification of TH and Tubb3 fluorescent signal from four images per group from two biological replicates.



**SYBR RT-qPCR**

Gene	Forward	Reverse
<i>18s</i>	5'-AGTCCCTGCCCTTTGTACACA-3'	5'-CGATCCGAGGGCCTCACTA-3'
<i>Ncor1</i>	5'-TCTGAACAGGAGAATAATGAGAAGCA-3'	5'-GATCCTCCATCAGCCCATTATAT-3'
<i>Ncor2</i>	5'-TGAGATCATTGATGGCTTGCTGA-3'	5'-CTTGATCCTCTGCTGGTCCG-3'
<i>Hdac3</i>	5'-CCTGGAACAGGTGACATGTATGA-3'	5'-CGTAAGGGCACATTGAGACAATAG-3'
<i>Gps2</i>	5'-ACCAGCTTCTCGGACTCATCTTCT-3'	5'-GAGGGTGGGCTGGAGCTCTCT-3'
<i>Cd4</i>	5'-AGTTGTGGGTGTTCAAAGTGACCT-3'	5'-GTGTTTGCACCTCTGTCAAGGGG-3'
<i>Ccl2</i>	5'-CTGCCCTAAGGTCTTCAGCAC-3'	5'-AGGCATCACAGTCCGAGTCA-3'
<i>Il17a</i>	5'-CCCTGGACTCTCCACCGCA-3'	5'-GTGGTCCAGCTTCCCTCCG-3'
<i>Il17f</i>	5'-CCCCTGAAACAGCCATGGTCA-3'	5'-CCAGGGGAGGACAGTCCCA-3'
<i>Il17ra</i>	5'-CATCACACGTGCAGGTCCA-3'	5'-TGCAACTGGCTTGGAACTGT-3'
<i>Il17rc</i>	5'-TCGAGGCTAGTCTTGGGGCT-3'	5'-AAGACCCTGCAGTCAGGCA-3'
<i>Mmp9</i>	5'-AGCTCTGCTGCCCTTACCA-3'	5'-TAGCGGTACAAGTATGCCTCTGC-3'
<i>Ucp1</i>	5'-ACTGCCACACCTCCAGTCATT-3'	5'-CTTTGCCTCACTCAGGATTGG-3'
<i>Ebf2</i>	5'-GCTGCGGGAAACCGGAACGAGA-3'	5'-ACACGACCTGGAACCGCCTCA-3'
<i>Adiponectin</i>	5'-GCACTGGCAAGTTCTACTGCA-3'	5'-GTAGGTGAAGAGAACGGCCTTG-3'
<i>Prdm16</i>	5'-CAGCACGGTGAAGCCATTC-3'	5'-GCGTGCATCCGCTTGTG-3'

**Genotyping**

Allele	Forward	Reverse
<i>Ncor1 loxp</i>	5'-TCTGAACAGGAGAATAATGAGAAGCA-3'	5'-GATCCTCCATCAGCCCATTATAT-3'
<i>Ncor2 loxp</i>	5'-TGAGATCATTGATGGCTTGCTGA-3'	5'-CTTGATCCTCTGCTGGTCCG-3'
<i>Hdac3 loxp</i>	5'-GCAGTGGTGGTGAATGGCTT-3'	5'-CCTGTGTAACGGGAGCAGAACTC-3'
<i>Ucp1-Cre</i>	5'-GCATTACCGGTCGATGCAACGAGTGATGAG-3'	5'-GAGTGAACGAACCTGGTCGAAATCAGTGCG-3'
<i>Rosa26-CreER</i>	5'-AAAGTCGCTCTGAGTTGTTAT-3'	5'-GGAGCGGGAGAAATGGATATG-3'
<i>Rosa26-CreER, cont.</i>	5'-CCTGATCCTGGCAATTCG-3'	
<i>Fabp</i> (pos. control)	5'-TGCCATGTGAGGCGTTAGGTTATCT-3'	5'-GAGCTTTGCCACATCACAGGTCATTC-3'

**Table S1.** Primer sequences for RT-qPCR and genotyping