## Supplementary Information

# Critical roles of nardilysin in the maintenance of body temperature homeostasis

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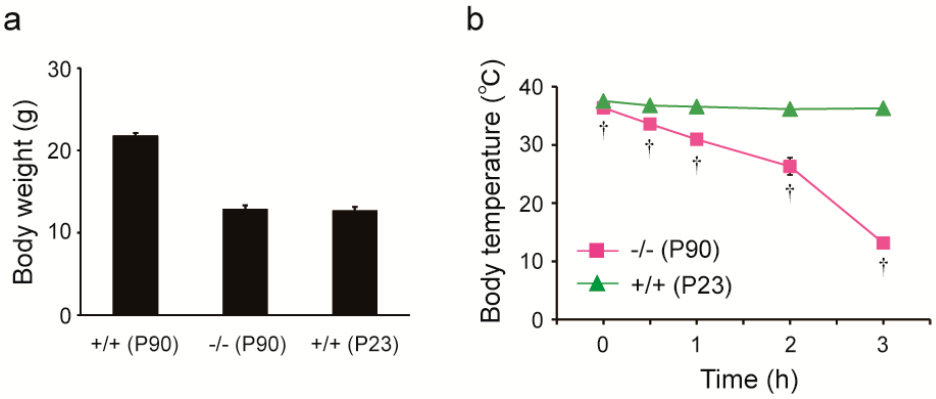
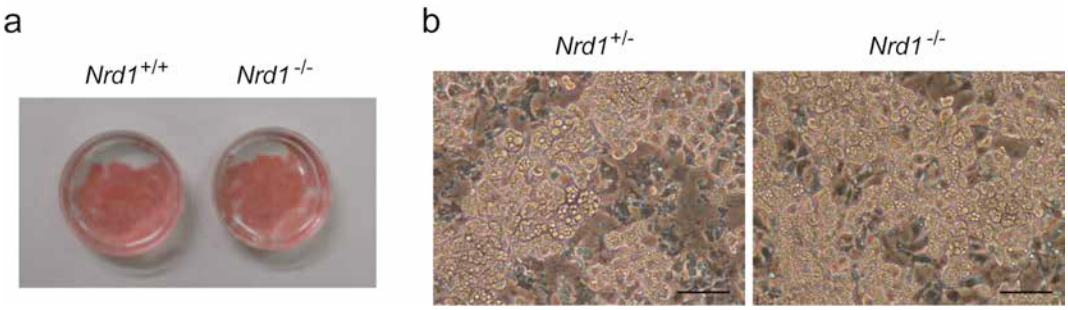
**Supplementary Figures 1-9**

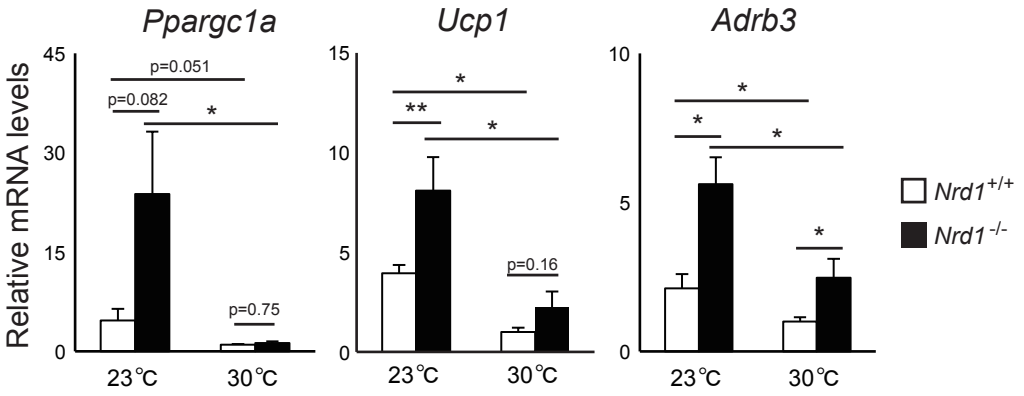
**Supplementary Tables 1-2**



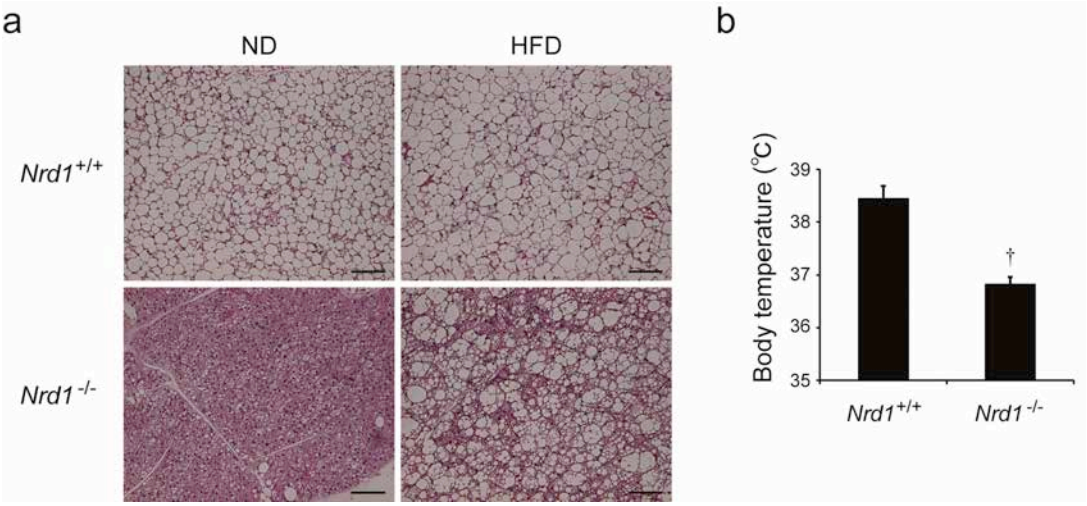

**Supplementary Figure 1. Cold-tolerance of younger *Nrd1*$^{+/+}$ mice with similar body size as *Nrd1*$^{-/-}$ mice (P90).** (**a**) Average of body weight of *Nrd1*$^{+/+}$ (P23 and P90) and *Nrd1*$^{-/-}$ (P90) mice. (**b**) *Nrd1*$^{+/+}$ mice (P23), which have the similar body weight as P90 *Nrd1*$^{-/-}$ mice, kept their body temperature during 3 hours-cold exposure. n = 6 per genotype. All data represent means ± s.e.m. $^{\dagger}P < 0.0001$.



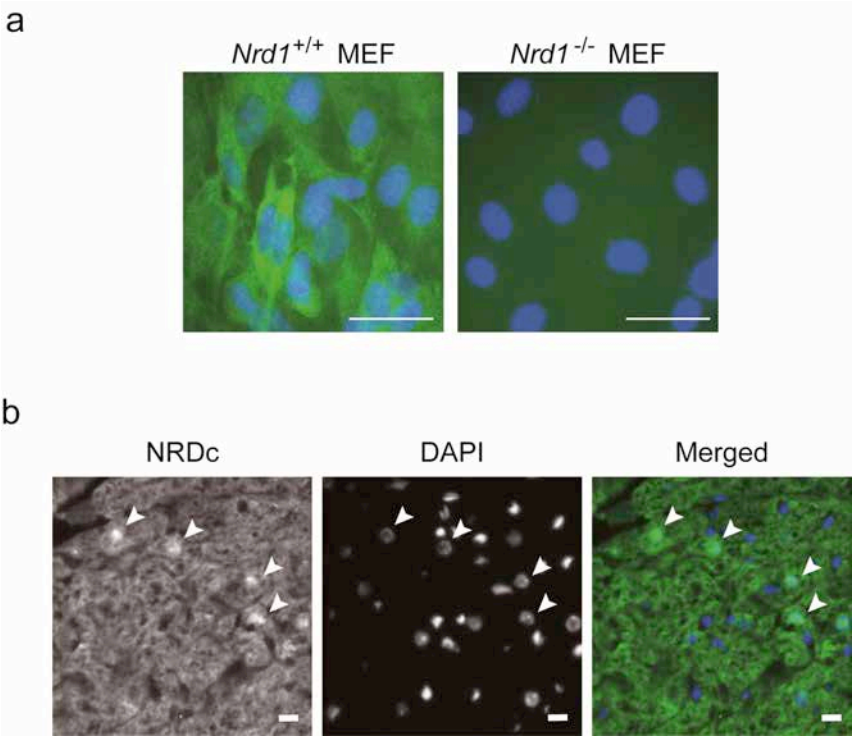

**Supplementary Figure 2. NRDc has no effect on adipogenic differentiation..** (**a**) Brown preadipocytes derived from neonatal *Nrd1*$^{+/+}$ and *Nrd1*$^{-/-}$ BAT were differentiated for 8 days and stained with oil red O. (**b**) Brown preadipocyte derived from neonatal *Nrd1*$^{+/-}$ and *Nrd1*$^{-/-}$ BAT were immortalized by stable introduction of large T antigen. Those cells were differentiated for 6 days and observed by phase contrast microscopy. Scale bars represent 20 μm.



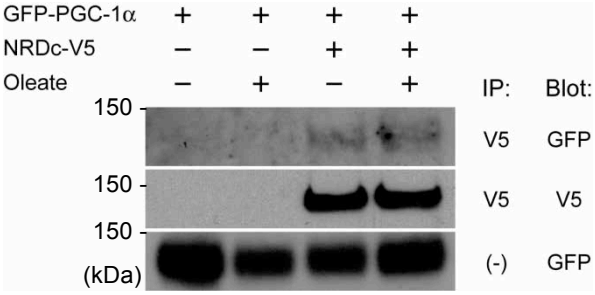

**Supplementary Figure 3. Expression of thermogenic genes at different ambient temperature.** Quantitative reverse transcription PCR (qRT-PCR) analysis for thermogenic genes in BAT from 6-month-old *Nrd1*$^{+/+}$ and *Nrd1*$^{-/-}$ mice housed at 23 °C or 30 °C for 10 days. The mRNA level is normalized by β-actin mRNA, and the level is arbitrarily set at 1 in *Nrd1*$^{+/+}$ BAT at 30 °C. n = 6 per genotype. All data represent means ± s.e.m. **P* < 0.05, ***P* < 0.001, n.s.; not significant.



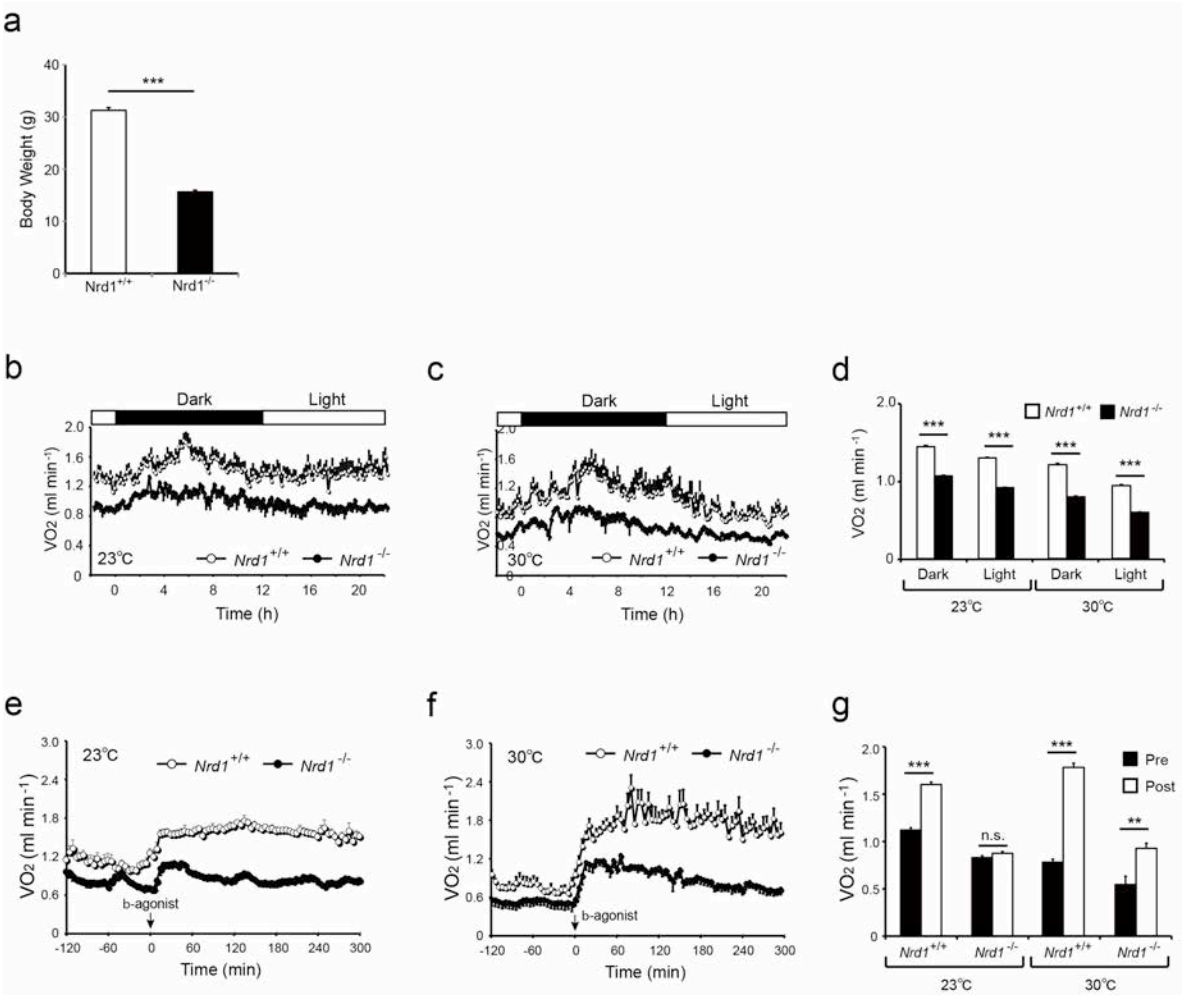

**Supplementary Figure 4. Increased lipid accumulation in BAT of *Nrd1*$^{-/-}$ mice fed a high fat diet.** (**a**) Haematoxylin and eosin stained sections of BAT from 6-month-old *Nrd1*$^{+/+}$ and *Nrd1*$^{-/-}$ mice fed a normal diet (ND) or a high fat diet (HFD). Scale bars represent 250 μm. (b) Body temperature of 6-month-old *Nrd1*$^{+/+}$ and *Nrd1*$^{-/-}$ mice fed a HFD at room temperature. n = 6 per genotype. All data represent means ± s.e.m. $^{\dagger}P <$ 0.0001.




**Supplementary Figure 5. Immunostaining analysis of mouse embryonic fibroblast (MEF) or BAT.** (**a**) MEF derived from *Nrd1*$^{+/+}$ and *Nrd1*$^{-/-}$ mice were fixed with 4% paraformaldehyde (PFA, wt/vol) and immunostained with anti-mouse NRDc antibody, established in our laboratory. Note that no staining was detected in *Nrd1*$^{-/-}$ MEF. Scale bars represent 50 μm. (**b**) Immunohistochemical analysis of BAT with anti-NRDc antibody. Sections of *Nrd1*$^{+/+}$ BAT were immunostained with anti-NRDc antibody and counterstained with DAPI. Arrowheads indicate the nuclear expression of NRDc. Scale bars represent 50 μm.

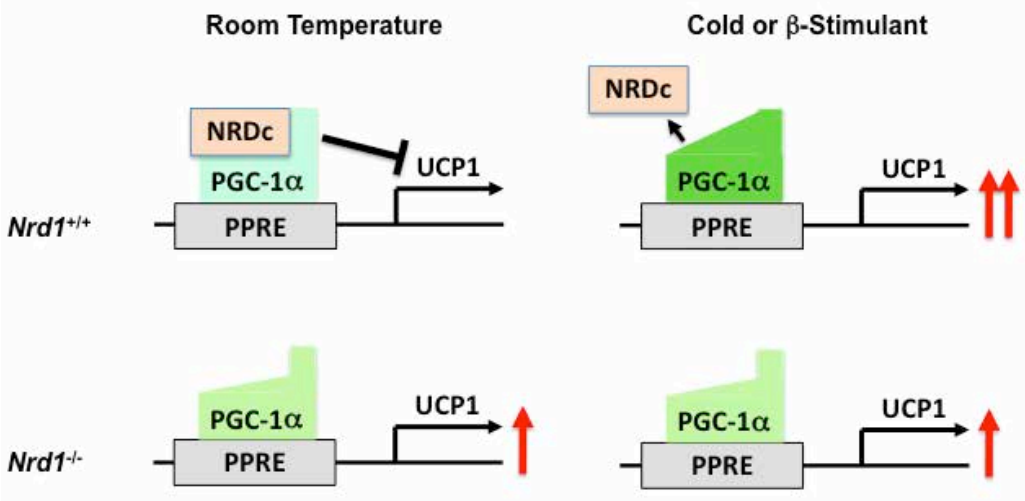



**Supplementary Figure 6. Association of NRDc and PGC-1α shown by co-immunoprecipitation experiments.** COS7 cells were transfected with the indicated expression vectors, followed by the treatment with or without oleate for 24 h. Immunoprecipitation with anti-V5 (NRDc) antibody revealed a complex of NRDc and PGC-1α. Twenty % of input was blotted with anti-GFP (PGC-1α) in the bottom lane.



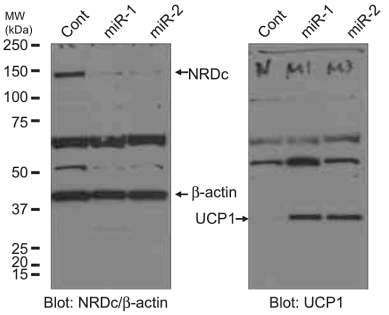

**Supplementary Figure 7. Oxygen consumption per animal at different ambient tenperature.** (**a**) Average of body weight of *Nrd1*$^{+/+}$ and *Nrd1*$^{-/-}$ (P180) mice. n = 6 per genotype. (**b,c**) Oxygen consumption per animal of *Nrd1*$^{+/+}$ and *Nrd1*$^{-/-}$ mice were monitored at 23 °C (**b**) or 30 °C (**c**). n = 6 per genotype and temperature. (**d**) The average oxygen consumption per animal during dark and light period of *Nrd1*$^{+/+}$ and *Nrd1*$^{-/-}$ mice housed at 23 °C or 30 °C. n = 6 per genotype and temperature. (**e,f**) Oxygen consumption per animal induced by β3-agonist (BRL37344: 5 mg kg$^{-1}$) in *Nrd1*$^{+/+}$ and *Nrd1*$^{-/-}$ mice

housed at 23 °C (**e**) or 30 °C (**f**). n = 6 per genotype and temperature. (**g**) The average oxygen consumption per animal of *Nrd1*$^{+/+}$ and *Nrd1*$^{-/-}$ mice before (Pre) and after (Post) β3-agonist injection at 23 °C or 30 °C. n = 6 per genotype and temperature. All data represent means ± s.e.m. $^{**}P < 0.005$, $^{***}P < 0.001$, n.s.; not significant (two-tailed Student's *t*-test).

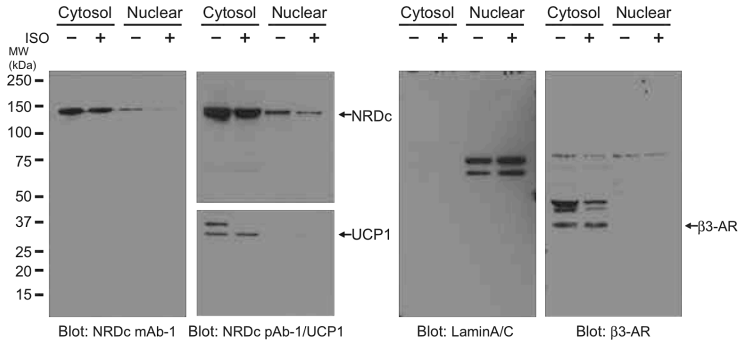



**Supplementary Figure 8. Role of NRDc in the derepression of PGC-1α activity.** NRDc represses PGC-1α activity and UCP1 transcription by binding directly to PGC-1α. Upon activation, removal of NRDc from the complex derepresses the coactivator function of PGC-1α, allowing it to activate UCP1 transcription (Upper panel). In the absence of NRDc, the basal levels of PGC-1α and UCP1 activation are upregulated. However, further activation of those genes by β-stimulant is impaired without the derepression process by NRDc (Lower panel).

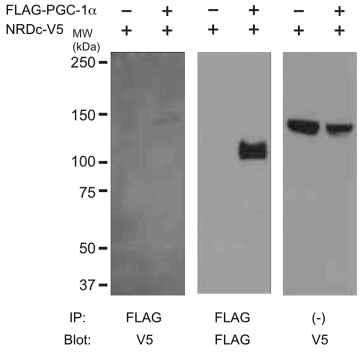
Full blots for Figure-6b



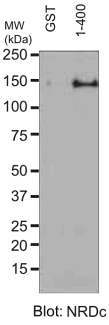

Full blots for Figure-7a



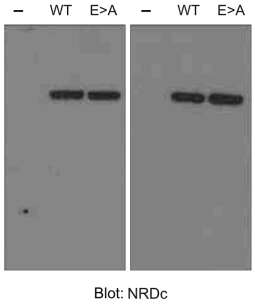

Full blots for Figure-8a




Full blots for Figure-8b




Full blots for Figure-8d




**Supplementary Figure 9. Full blots of Figures presented in the main paper.**

| | *Nrd1*$^{+/+}$ | *Nrd1*$^{-/-}$ | Unpaired *t*-test |
|---|---|---|---|
| $\Delta T_{skin}$ (°C) | -10.0 ± 1.7 | -11.1 ± 1.9 | n.s. |
| $\Delta T_{BAT}$ (°C) | 0.6 ± 0.1 | -0.2 ± 0.1 | P < 0.001 |
| ΔHR (bpm) | 39 ± 10 | 33 ± 25 | n.s. |
| $\Delta T_{rec}$ (°C) | -1.9 ± 0.5 | -2.0 ± 0.5 | n.s. |
| $\Delta T_{tail}$ (°C) | -2.3 ± 0.5 | -4.4 ± 0.7 | n.s. |

n.s.; not significant

**Supplementary Table 1. Cooling-evoked changes in physiological variables (n=4 for *Nrd1*$^{+/+}$, n=7 for *Nrd1*$^{-/-}$ mice, mean ± s.e.m.)**

| Gene | Forward (5’ -> 3’) | Reverse (5' -> 3') |
|---|---|---|
| *Nrd1* | ATGGATGGCCTTTCCCTTG | CGCGAAGTTCAGCTTGTCAA |
| *Ppargc1a* | AGCCGTGACCACTGACAACGAG | GCTGCATGGTTCTGAGTGCTAAG |
| *Ucp1* | GGCATTCAGAGGCAAATCAGCT | CAATGAACACTGCCACACCTC |
| *Adrb3* | CCACTCCGGGAACACCG | GGCAGTAGATGACCGGGTTG |
| *Cpt1a* | GGGCACCTCTGGGAGTTTGT | TTGGCTCACCCACACAGTGT |
| *Acadm* | GCCAAGATCTATCAGATTTATGAAGGT | AGCTATGATCAGCCTCTGAATTTGT |
| *Acadvl* | GCCAGGGCAGAATCGAAGT | TGGTAAGCTGGCCTTTGAACAT |
| *Pdk4* | GCAGTAGTCCAAGATGCCTTTGA | AATACTGGTCGCAGAGCATCTTT |
| *Acadl* | CCCTCCGCCCGATGTT | AAGGAGTTTCTAGACGCGCTTCT |
| *Esrra* | AGCAAGCCCCGATGGA | GAGAGGCCTGGGATGCTCTT |
| *Actb* | CTGACTGACTACCTCATGAAGATCCT | CTTAATGTCACGCACGATTTCC |

**Supplementary Table 2. Primer sequences used for RT-PCR**