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# Altered thermogenic and behavioral responses to cold stress in mice carrying a camel-derived Nrap mutation

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## Introduction

Adaptation to extreme environments necessitates a complex integration of physiological, metabolic, and behavioural adjustments. Species inhabiting harsh climates, such as the dromedary camel (*Camelus dromedarius*), exhibit remarkable resilience to severe thermal fluctuations and limited resource availability (Gebreyohanes and Assen, 2017). While the camel's ability to maintain homeostasis under extreme heat and cold is well-documented, the underlying molecular and genetic mechanisms remain largely elusive. Recent genomic studies have identified unique genetic variants in camels that are absent in other mammals (Almathen and Salim, 2025), suggesting these as candidates for their extraordinary environmental adaptability. However, functional validation of these variants in the native species is hindered by significant ethical, logistical, and technical constraints associated with large-animal experimentation. To circumvent these challenges, transgenic mouse models have emerged as an indispensable tool for dissecting the functional impacts of specific, camel-derived genetic variants. Among the candidates for muscular and metabolic adaptation is the Nebulin-related anchoring protein (*NRAP*). *NRAP* is a critical myofibrillar protein primarily located at the intercalated discs of cardiac muscle and the myotendinous junctions of skeletal muscle, where it facilitates force transmission and structural stability (Carroll *et al*., 2000; Zhang *et al*., 2023). Building upon our laboratory's previous findings regarding camel-specific *NRAP* exons, the present study focuses on a novel variant: *Nrap*c.255ins78 (Lee *et al*., 2025). We hypothesize that this insertion influences systemic thermoregulation and motor activity via altered cardiac or skeletal muscle function. In this study, we evaluate the thermogenic capacity and behavioral profiles of *Nrap*$^{c.255ins78}$ knock-in mice under both normothermic and cold-stressed conditions to elucidate the role of this variant in environmental adaptation.

## Materials & Methods

**Animal Models and Ethics.** All experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC MS-2022-01). A "camelized" mouse model was generated by introducing the camel-specific exon 4 into the murine *Nrap* locus using CRISPR/Cas9-mediated genome editing. To establish the experimental cohorts, mice were selectively bred based on their specific genotypes and intercrossed to produce homozygous mutant (*Nrap*$^{c.255ins78}$) and wild-type (WT) littermates. For the physiological and behavioral assays, five mice were assigned to each experimental group (n = 5 per group), and these individuals were used for all subsequent experiments.

**Cold Exposure Protocol.** Adult male mice (12 weeks old) from both genotypes were randomly assigned to either room temperature (normothermic, 24 ± 1°C) or cold exposure groups. For the cold stress challenge, mice were transferred to a temperature-controlled chamber maintained at 4°C for a total duration of 6 hours. During this period, mice had *ad libitum* access to water and food to focus on endogenous thermogenic responses.

**Physiological and Behavioral Measurements.** Rectal temperature and body weight were recorded at three time points: 0 and 6 hours post-exposure. Behavioral parameters were quantified using an automated video tracking system. Parameters included: total distance traveled (mm) to assess overall locomotor activity; average speed (mm/s) to evaluate movement intensity; freezing time (s) to measure anxiety-like or stress-induced immobility; and exploration rate based on zone preference and movement frequency.

**Tissue Collection and Reverse Transcription-PCR (RT-PCR).** Following the experimental period, mice were euthanized, and various tissues including cardiac muscle, skeletal muscle, liver, and adipose tissue were harvested. Total RNA was extracted using TRIzol reagent. The expression levels of the *Nrap* gene across tissues were quantified via semi-quantitative RT-PCR to determine the tissue-specific expression patterns influenced by the c.255ins78 variant.

**Statistical Analysis.** Data are presented as mean ± standard error of the mean (SEM). Statistical significance was determined using two-way analysis of variance (ANOVA) to evaluate the effects of genotype, time, and their interaction. Bonferroni's post-hoc test was subsequently applied for multiple comparisons between groups at specific time points. All statistical analyses were performed using GraphPad Prism 9.0, with p-values $< 0.05$ considered statistically significant.

## Results

**Physiological Stability Under Cold Stress.** To evaluate the impact of the *Nrap*$^{c.255ins78}$ mutation on physiological resilience, body weight and rectal temperature were monitored during 6 hours of cold exposure (4°C). Both WT and *Nrap*$^{c.255ins78}$ mice showed a slight decrease in body weight after 6 hours. However, there was no significant difference in weight loss trajectories between the genotypes ($p = 0.1712$), suggesting that the mutation does not adversely affect overall energy balance during acute cold stress. While WT mice experienced a significant drop in rectal temperature after 6 hours ($p = 0.0083$), the Nrap c.255ins78 mice maintained a stable body temperature with no statistically significant decline ($p = 0.9045$). Comparing the rectal temperatures at the two genotypes at the 6-hour mark revealed that the mutant mice had significantly higher core temperatures than their WT counterparts ($p = 0.0227$), confirming superior thermoregulatory stability (Figure 1).

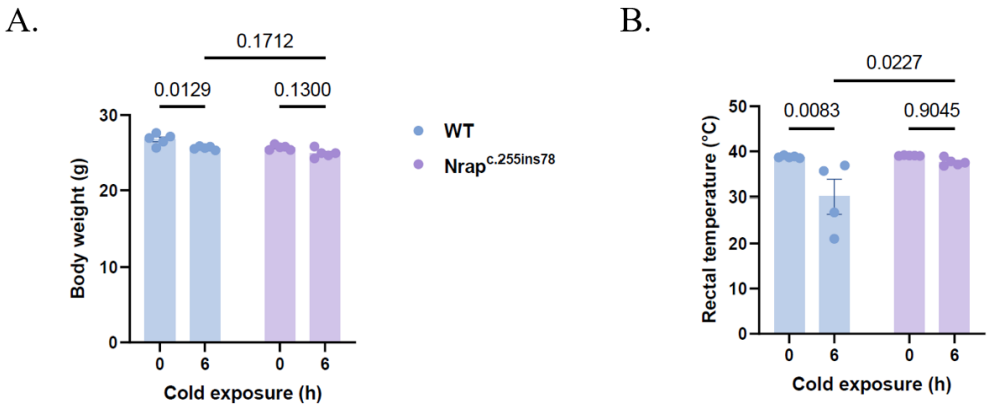


**Figure 1.** The change in body temperature between WT and NRAPc.255ins78 mice during cold exposure.

**Behavioral and Locomotor Responses.** Behavioral tracking during cold exposure revealed marked differences in locomotor activity between genotypes. *Nrap*$^{c.255ins78}$ mice exhibited significantly higher total distance traveled ($p = 0.0196$) and average speed ($p = 0.0204$) compared to WT mice. The mobility rate was significantly elevated in mutant mice ($p =$

0.0197). Conversely, the total time frozen was drastically reduced in the *Nrap*$^{c.255ins78}$ group (p = 0.0210), indicating a proactive behavioral response to the cold environment rather than stress-induced immobility (Figure 2). No significant difference was observed in the exploration rate (p = 0.5657).

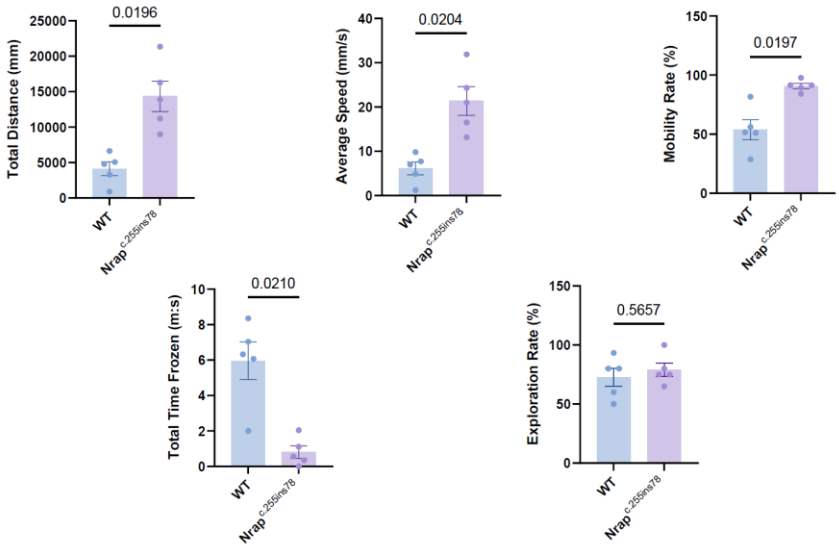


**Figure 2.** The mobility alteration between WT and NRAPc.255inc78 mice during cold exposure.

**Tissue-Specific Expression of *Nrap*.** RT-PCR analysis was conducted to determine the expression profile of the *Nrap* variant across various tissues. In all genotypes (WT, Homozygous, and Heterozygous), *Nrap* mRNA expression was exclusively and robustly detected in the heart muscle. No detectable expression was found in the brain, lung, liver, kidney, or small intestine. This cardiac-specific expression pattern confirms that the phenotypic changes observed are likely mediated through altered cardiac function (Figure 3)

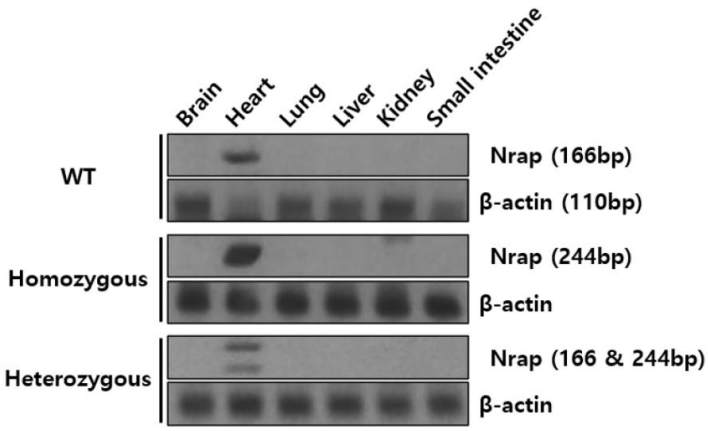


**Figure 3.** A comparison of the *Nrap* mRNA expression level between cardiac muscle (heart) and non-muscle tissues (brain, lung, kidney and small intestine) through RT-PCR.

## Discussion

The present study demonstrates that the camel-derived *Nrap*c.255ins78 allele confers a significant physiological advantage under cold stress when introduced into a murine model. The most striking finding is the mutation's ability to stabilize core body temperature. While

WT mice struggled to maintain thermal homeostasis, the "camelized" mice showed remarkable resilience, mirroring the metabolic efficiency seen in *Camelus dromedarius*. The behavioral data suggests that this thermal stability is supported by enhanced locomotor vigor. The significant increase in average speed and reduced freezing time in mutant mice implies that the *Nrap* variant facilitates active thermogenesis, possibly through improved muscular efficiency or cardiovascular support. Unlike the previous *Nrap* exon 4 model (Lee et al., 2025), which focused on cold-induced thermogenesis, the *Nrap*c.255ins78 variant appears to drive a more robust behavioral adaptation. The heart-specific expression of *Nrap* provides a crucial mechanistic clue. Since *Nrap* is essential for myofibrillar assembly and force transmission at the intercalated discs, the 78 bp insertion likely optimizes cardiac output or structural integrity. Under cold stress, enhanced cardiac performance is vital to meet the increased metabolic demand required for heat production. The absence of *Nrap* expression in non-muscle tissues further supports the hypothesis that the heart is the primary organ driving this systemic environmental adaptation.

## Conclusions

Using mouse models to overcome the limitations of direct experimentation in camels, we demonstrate that the *Nrap*c.255ins78 allele strengthens thermal homeostasis during cold exposure while enhancing locomotor activity under normothermic conditions. These findings offer new insight into camel-inspired environmental adaptation mechanisms and highlight the value of transgenic mouse systems for experimentally validating adaptive genes from extreme-environment species.

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