Li, J., Li, S., Hu, X., Nedergaard, J. (2026) Functional dissection of *Junb* in *Ucp1*-independent thermogenic regulation in pigs adipose tissue, *Proceedings 13th World Congress on Genetics Applied to Livestock Production* 23651: https://doi.org/10.31274/wcgalp.23651
©2026 Li, et al. CC BY-NC-ND

Functional dissection of *Junb* in *Ucp1*-independent thermogenic regulation in pigs adipose tissue

J. Li[1*], S. Li[1], X. Hu[1], and J. Nedergaard[2]

[1]*China Agricultural University, Beijing, China;* [2]*Stockholm University, Stockholm, Sweden;*
[*]*Corresponding author:huxx@cau.edu.cn, Jan.Nedergaard@su.se*

Introduction

In mammals, adipose tissue serves not only as an energy reservoir but also plays a crucial role in maintaining systemic energy homeostasis and regulating body temperature. White adipose tissue (WAT) primarily stores excess energy, and hyperplasia and hypertrophy of white adipocytes contribute to fat accumulation (Farmer, 2006). Brown adipose tissue (BAT) and beige adipocytes are specialized for thermogenesis, generating heat through uncoupling protein 1 (*UCP1*)-mediated non-shivering thermogenesis (NST) under cold exposure (Nedergaard and Cannon, 2014). During evolution, pigs have lost functional *UCP1* and lack BAT, resulting in thermogenic mechanisms that differ from those in rodents and humans.

Neonatal piglets, with thin hair, low subcutaneous fat, immature immunity, and absence of BAT, are highly susceptible to hypothermia, leading to elevated mortality rates of 10–20% during winter, particularly within the first 72 hours (Kirkden *et al*., 2013). Consequently, pig cold resistance largely depends on shivering thermogenesis in muscle, *UCP1*-independent thermogenesis in adipose tissue, and optimized husbandry conditions. Understanding the molecular basis of non-*UCP1* thermogenesis and identifying key regulatory factors is therefore essential for improving neonatal cold tolerance and for breeding cold-resilient pig lines.

In this study, single-cell RNA sequencing (scRNA-seq) of adipocytes from 45-day-old piglets under cold exposure revealed cell heterogeneity and identified *Junb* as a candidate cold-responsive gene. Functional assays demonstrated that *Junb* promotes long-chain fatty acid synthesis and lipid accumulation in pig adipocytes, whereas in *UCP1*-KO mouse white adipocytes, *Junb* activation enhances triglyceride hydrolysis and free fatty acid release, facilitating non-*UCP1*-dependent thermogenesis. These findings indicate species-specific regulatory mechanisms of *Junb* in naturally *UCP1*-deficient versus induced *UCP1*-deficient models.

Materials & Methods

Animals and cold exposure: Seven 2-month-old Xiangzhu minipigs were used. Four pigs were exposed to a cold room (4–10 °C) for 14 days, while three remained outdoors (26–31.5 °C). Samples were collected only after cold exposure, and no paired samples were obtained before cold stimulation. Ear root temperatures were measured daily at 10:00 using an infrared thermometer. Animals had ad libitum access to water and food.

Single-Cell suspension preparation: Stromal vascular fraction (SVF) cells were trypsinized, centrifuged, and resuspended. Cell debris and clumps were removed via a 40 μm strainer. Cell concentration was adjusted to 700–1200 cells/μl.

Single-Cell RNA sequencing and data analysis: Libraries were prepared using the 10xGenomics platform. Data were processed using Cell Ranger v6.1.1 and R packages for demultiplexing, alignment, Unique Molecular Identifier (UMI) counting, normalization, and clustering. Pseudo-time trajectories were analyzed with Monocle 2.4. Differentially expressed genes (DEGs) were defined as $P \leqslant 0.05$ and fold-change>2. GO enrichment analysis was by Metascape.

Results

Single-Cell analysis reveals *Junb*$^{+}$ cell expansion in minipig neck white adipose under cold exposure:To investigate non-shivering thermogenic responses in pigs under cold exposure, minipigs were subjected to long-term cold stimulation. Histological analysis revealed that neck adipose tissue from cold-exposed pigs exhibited a darker appearance and reduced lipid droplet size compared with controls (Fig. 1A). Consistently, the expression of beige adipocyte marker genes was significantly upregulated following cold exposure (Fig. 1B, C), indicating cold-induced beiging of neck white adipose tissue. Single-cell RNA sequencing further demonstrated increased cellular heterogeneity after cold exposure and revealed a marked expansion of *Junb*$^{+}$ cells within the stromal vascular fraction (Fig. 1D). In vitro validation confirmed that *Junb* expression was significantly upregulated in pig adipose tissue in response to cold stimulation (Fig. 2A, B).

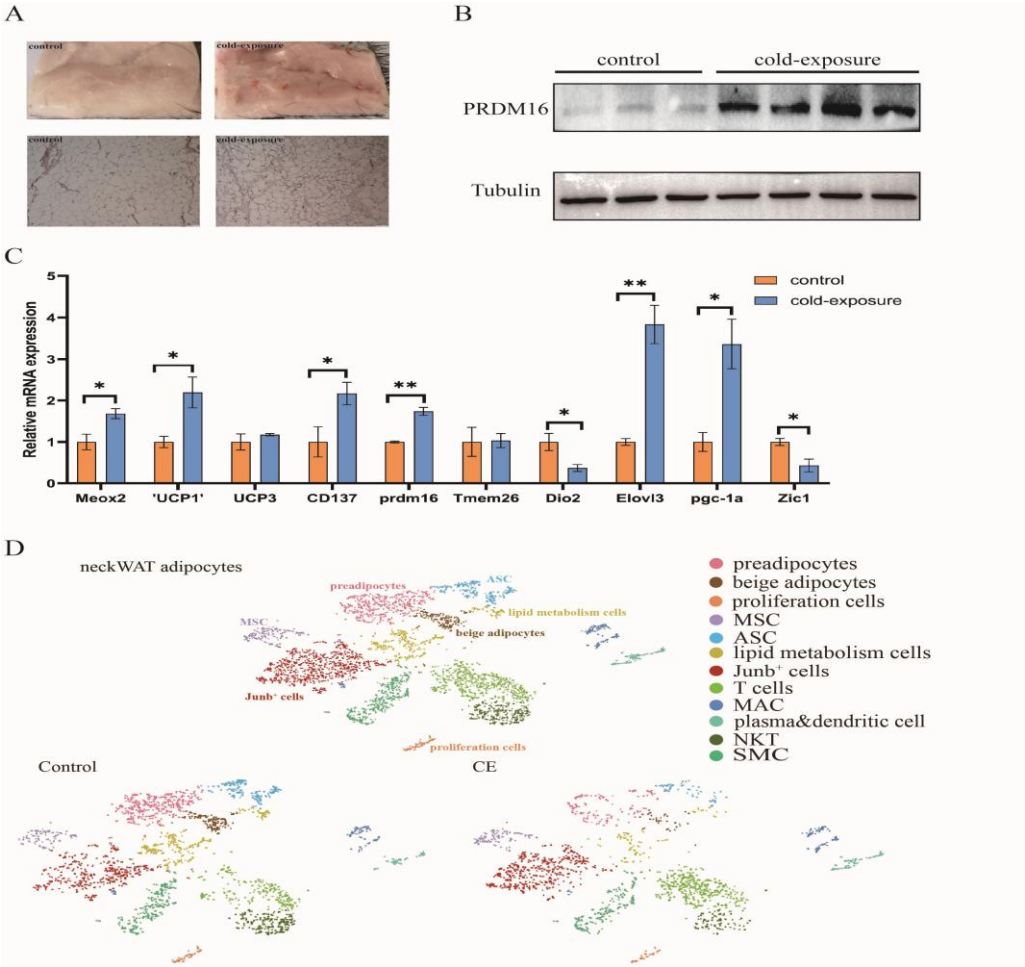

Figure 1. Cold exposure induces *Junb* expression in porcine neck white adipose tissue.**(**A) H&E staining of neck adipose tissue from control and cold-exposed pigs. (B) Immunoblot analysis of the thermogenic marker PRDM16 in neck adipose tissue. (C) qPCR analysis of thermogenic gene expression in neck adipose tissue. (D) Single-cell RNA-seq analysis reveals cellular heterogeneity from porcine neck adipose tissue.

***Junb* promotes lipid accumulation by regulating long-chain fatty acid synthesis:**To further investigate the role of *Junb* under cold conditions, immortalized porcine adipocyte cell lines with stable *Junb* overexpression or knockout were established. *Junb* overexpression significantly enhanced adipogenesis, as evidenced by increased lipid accumulation (Fig. 2C, D), accompanied by upregulation of the fatty acid synthase gene *Fasn* (Fig. 2E). In contrast, the expression of thermogenic genes *Pgc1a* and *Cidea* was markedly reduced (Fig. 2F).

Conversely, *Junb* knockout resulted in decreased *Fasn* expression (Fig. 2G) and a concomitant increase in the thermogenic gene *Pgc1a* (Fig. 2H). These results indicate that *Junb* regulates lipid utilization and thermogenic responses in porcine adipocytes by modulating long-chain fatty acid synthesis.

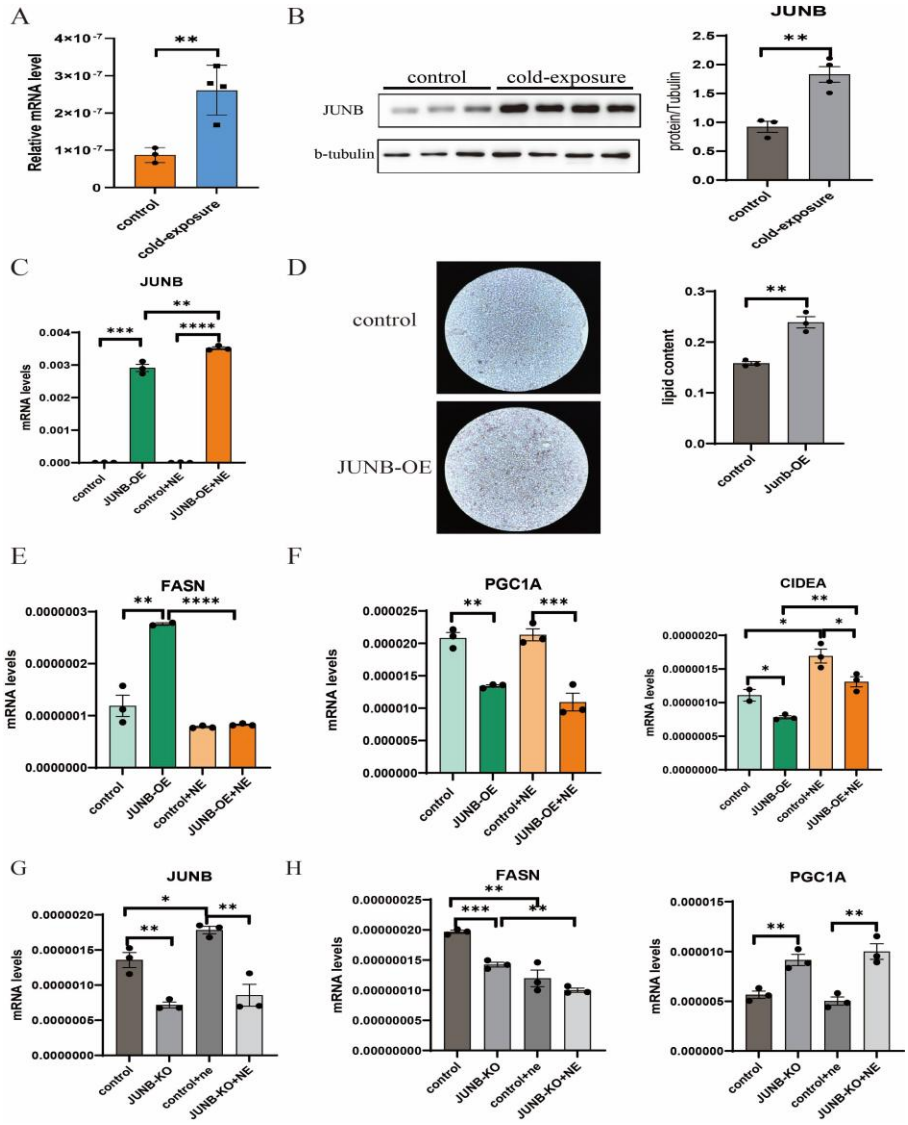

Figure 2. *Junb* regulates lipid metabolism in porcine adipocytes. (A–B) *Junb* expression in pig neck adipose tissue measured by qPCR (A) and western blot (B). (C–F) Effects of *Junb* overexpression in immortalized pig adipocytes: (C) *Junb* mRNA, (D) lipid droplet formation by Oil Red O staining, (E) *Fasn* expression, (F) thermogenic genes *Pgc1a* and *Cidea.* (G–H) Effects of *Junb* knockout in pig adipocytes: (G) *Junb* mRNA, (H) *Fasn* and *Pgc1a* expression.

To further elucidate the role of *Junb* under cold exposure, *UCP1*-KO mice were subjected to long-term cold stimulation. *Junb* expression was significantly upregulated in white adipose tissue following cold exposure (Fig. 3A, B). Primary stromal vascular fraction cells were then isolated from white adipose tissue and induced to differentiate into adipocytes. Norepinephrine stimulation markedly increased *Junb* expression in differentiated adipocytes (Fig. 3C, D). *Junb* knockdown significantly reduced the expression of thermogenic genes *Pgc1a* and *Elovl3* (Fig. 3C), whereas *Junb* overexpression led to significant upregulation of *Elovl3* and the triglyceride lipase gene *Pnpla2* (Fig. 3D).

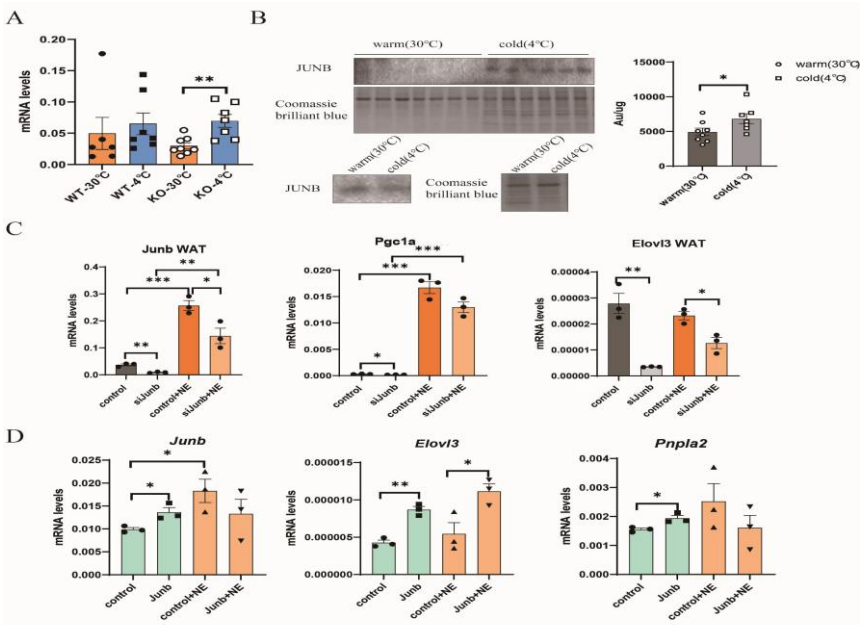

Figure 3. *Junb* regulates thermogenesis in *UCP1*-KO mouse white adipose tissue. (A–B) *Junb* expression in mouse white adipose tissue after chronic cold exposure, measured by qPCR (A) and western blot (B). (C–D) Effects of *Junb* manipulation in primary mouse white adipocytes: (C) *Junb* knockdown reduces *Pgc1a* and *Elovl3* expression, (D) *Junb* overexpression increases *Elovl3* and *Pnpla2* expression.

Discussion

Pigs lack functional *UCP1* and therefore rely on *UCP1*-independent mechanisms for cold adaptation. We show that cold exposure induces a beiging-like remodeling of porcine neck white adipose tissue, accompanied by robust induction of *Junb*. Functional analyses indicate that *Junb* enhances cold tolerance in pigs primarily by promoting lipid accumulation, suggesting a protective, lipid-centered strategy distinct from canonical thermogenesis. In mice, where *UCP*1-dependent thermogenesis predominates, *Junb* expression in white adipose tissue is unchanged by cold in wild-type animals but markedly induced in *UCP1*-deficient mice, recapitulating the porcine response. Thus, *Junb* facilitates cold adaptation by promoting triglyceride hydrolysis and fatty acid mobilization to support compensatory heat production. Together, these findings identify *Junb* as a conserved regulator of *UCP1*-independent thermogenesis that modulates lipid metabolism in a species- and context-dependent manner.

Conclusions

In conclusion, these findings suggest that *Junb* exerts distinct regulatory effects on non-*Ucp1*-dependent thermogenesis between *Ucp1*-deficient species (pigs) and *Ucp1*-knockout models (mice). Specifically, while *Junb* in pigs primarily facilitates lipid storage and thermal insulation under cold stress, it contributes to free fatty acid mobilization within compensatory thermogenic pathways in *Ucp1*-KO mice.

Acknowledgements

Supported by National Transgenic Breeding Project and Natural Science Foundation of China.

References

Armer S.R. 2006 Cell Metab. 4**:**263-73.
Kirkden R.D., Broom D.M. and Andersen I.L. 2013 Anim. Reprod. Sci. 138**:**14-24.
Nedergaard J. and Cannon B. 2014 Cell. Metab. 20**:**396-407.