



75th EAAP Annual Meeting 2024

Florence, Italy - 1/5 September 2024

Acetyl-CoA Carboxylase 1 Inhibition Enhances Cold Resistance and Reduces Fat Deposition in Pigs

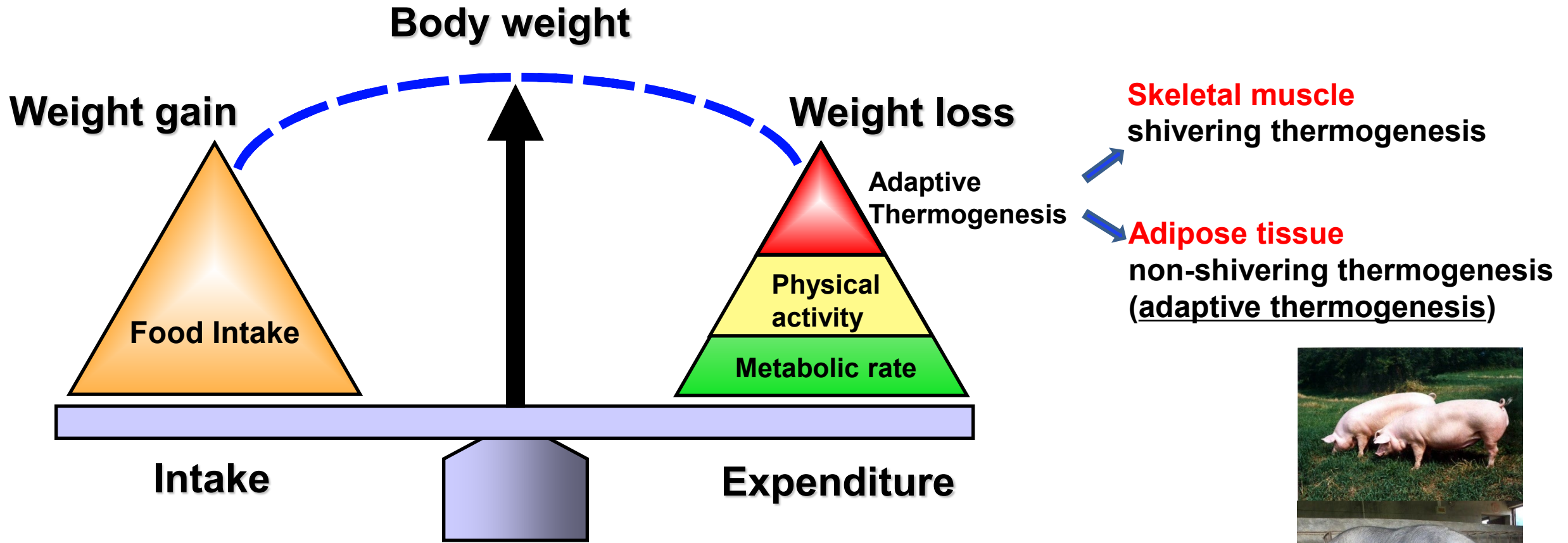
Cong Tao

Sep 4, 2024

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The adipose tissue is a central metabolic organ in the regulation of whole-body energy homeostasis

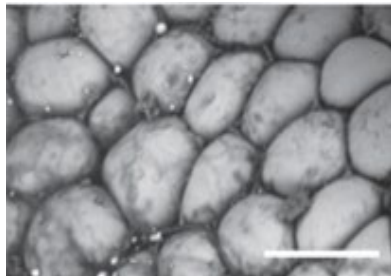
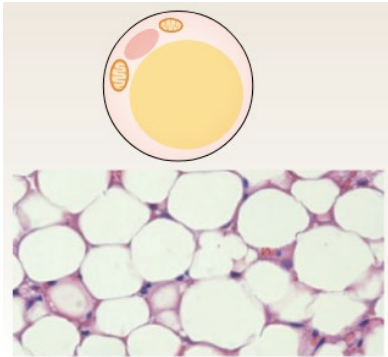


Characterizations of adipocytes

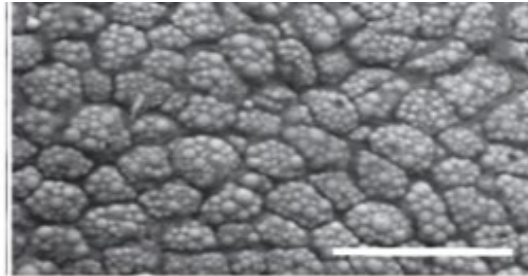
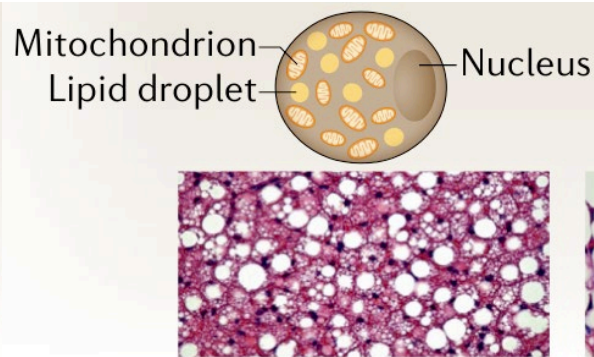
- Adipocytes are classified based on their thermogenic capacity.
- The heat production in adipocytes is mainly mediated by the uncoupling activity of the mitochondrial membrane protein UCP1.

Thermogenic capacity

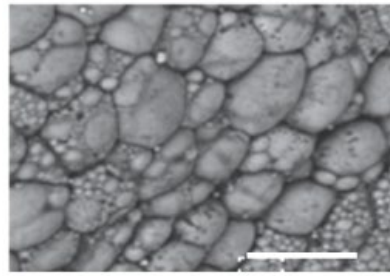
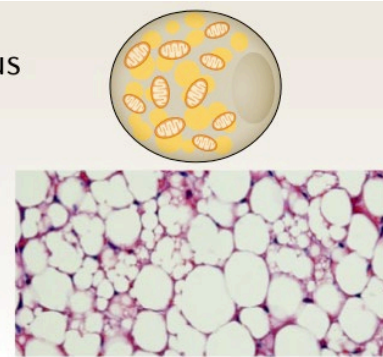
White adipocytes



Beige adipocytes



Brown adipocytes



Mitochondria Structural Features

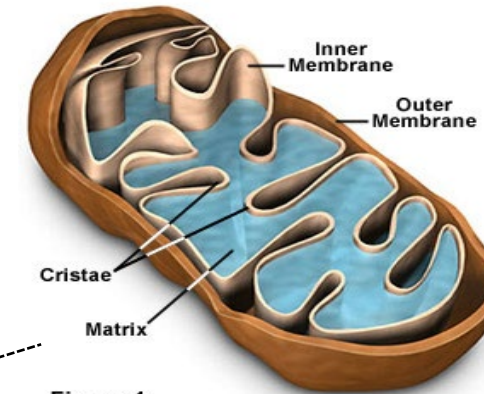
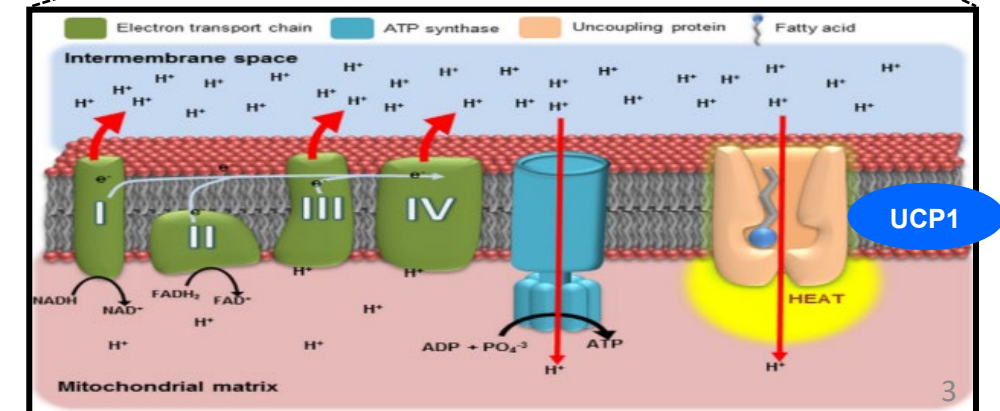


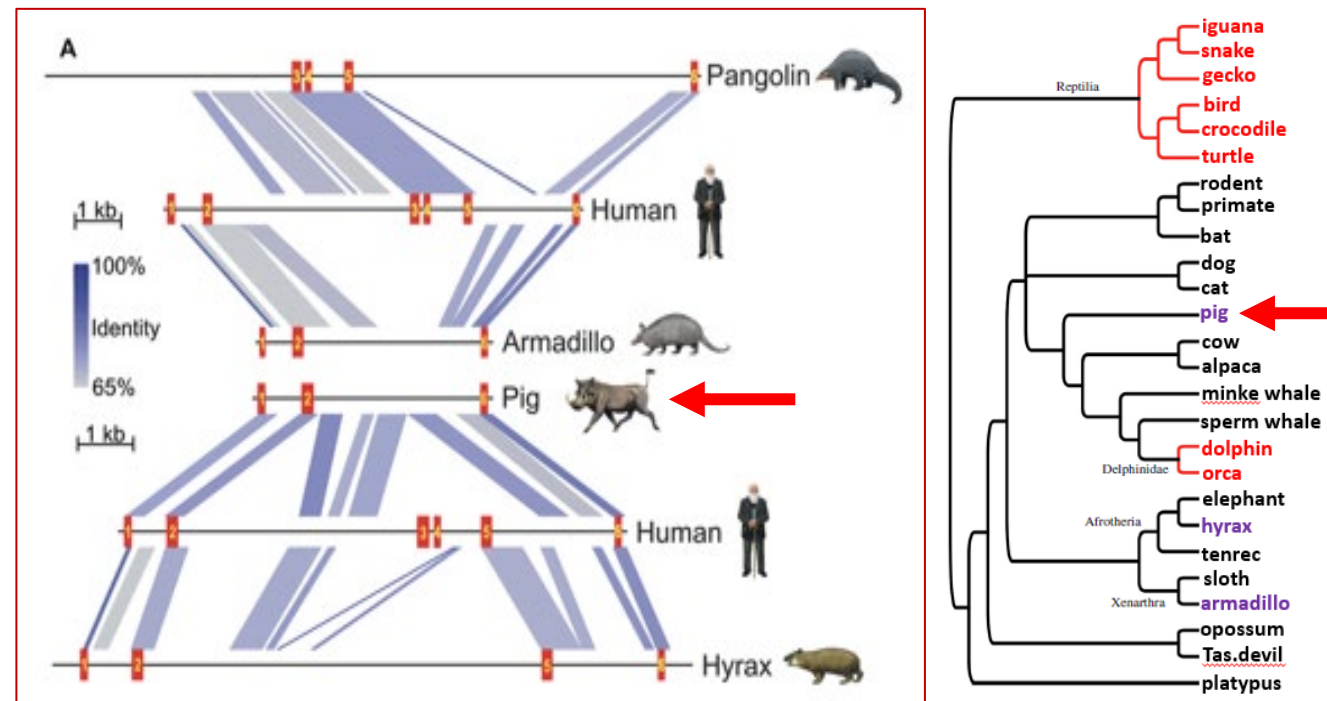
Figure 1



Loss of UCP1: a genetic explanation for poor thermoregulation in piglets

- Poor cold tolerance of newborn piglets in modern industrial production
- Genetic basis of cold intolerance linked to the lack of exons 3-5 in *UCP1*

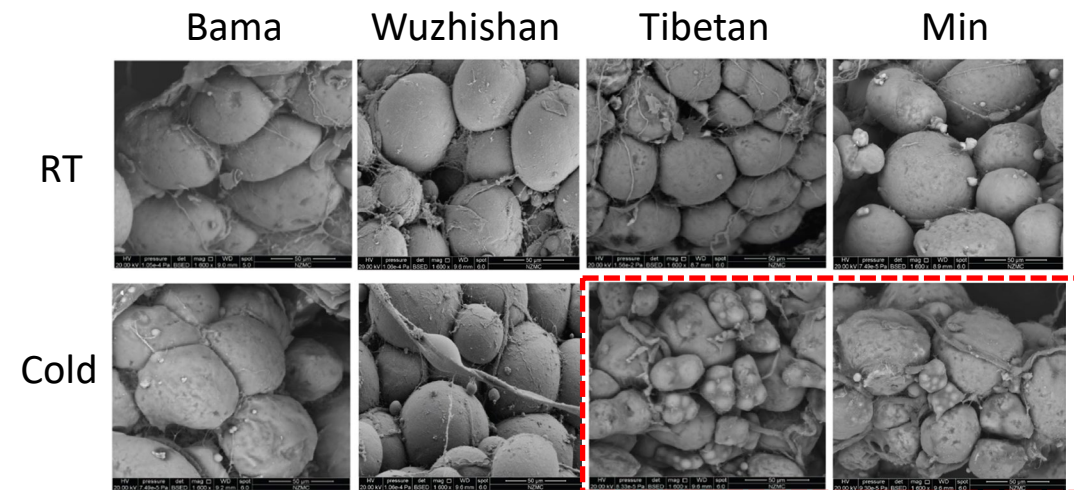
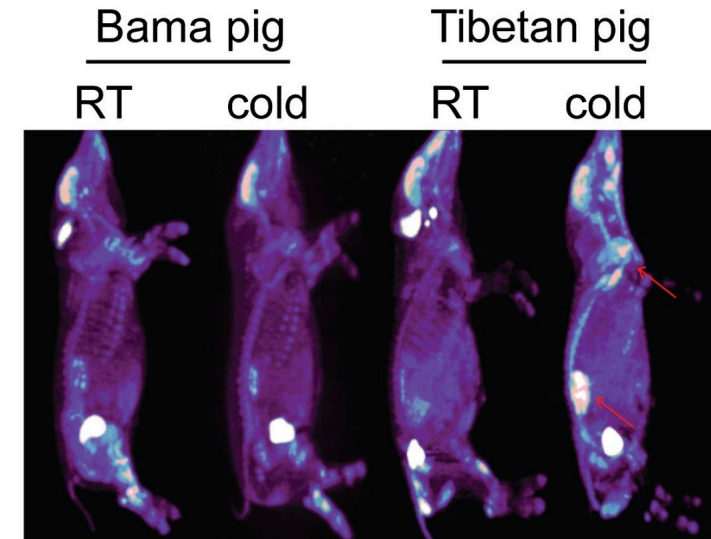
Poor thermoregulation leads to neonatal mortality



Michael J. Gaudry et al. Sci Adv 2017;

Cold adaption of cold-tolerant pig breeds relies on beige formation

- Cold tolerance variations among Chinese local pig breeds.
- Acute cold exposure induces beige adipocyte formation in cold-tolerant pig breeds.



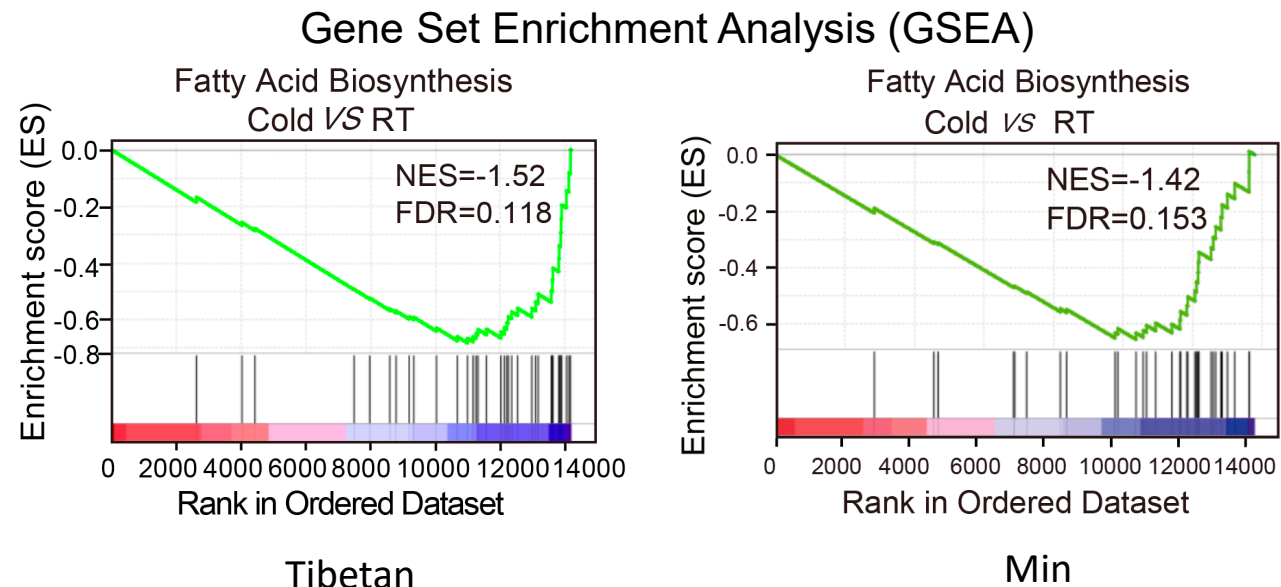
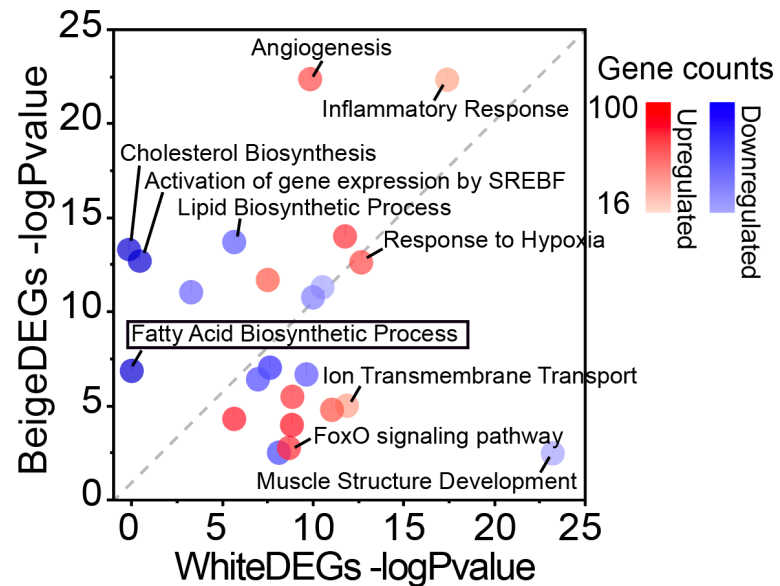
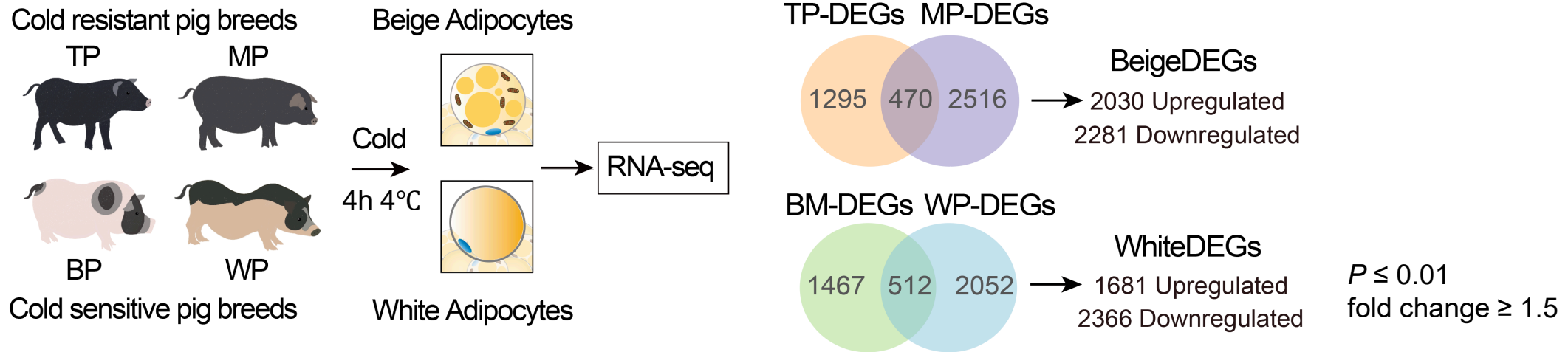
Biological questions

- What are the molecular mechanisms of beige adipocytes formation in cold-tolerant pig breeds?
- Can we reduce fat deposition in pigs by inducing beige formation?

Providing valuable resource

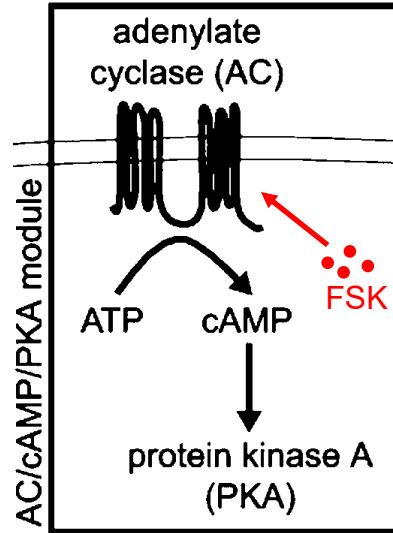
- agricultural purpose
- metabolic related disease (anti-obesity) biomedical research

Fatty acid Biosynthetic process pathway was suppressed in cold-resistant pig breeds

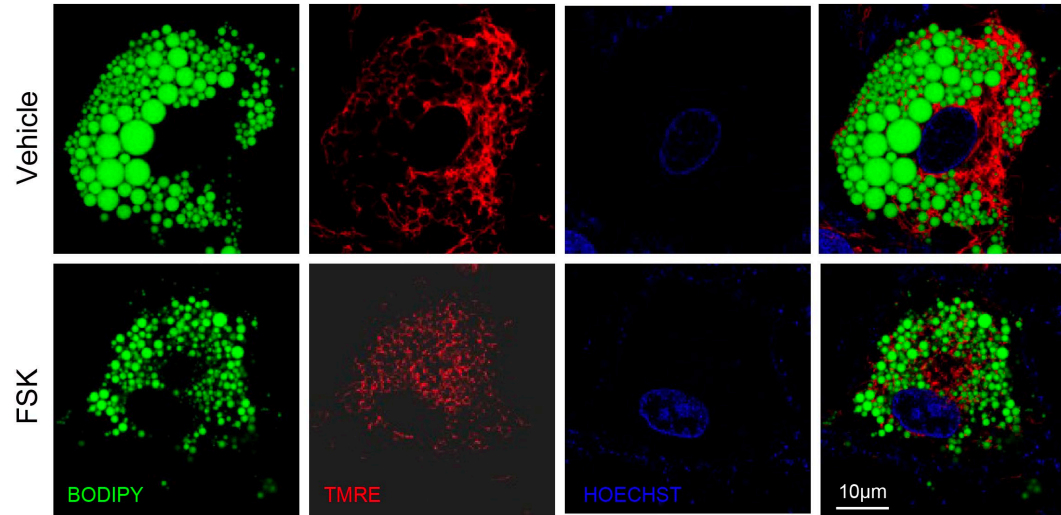


Establishment of an in vitro model of cold stimulation: forskolin (FSK) treatment

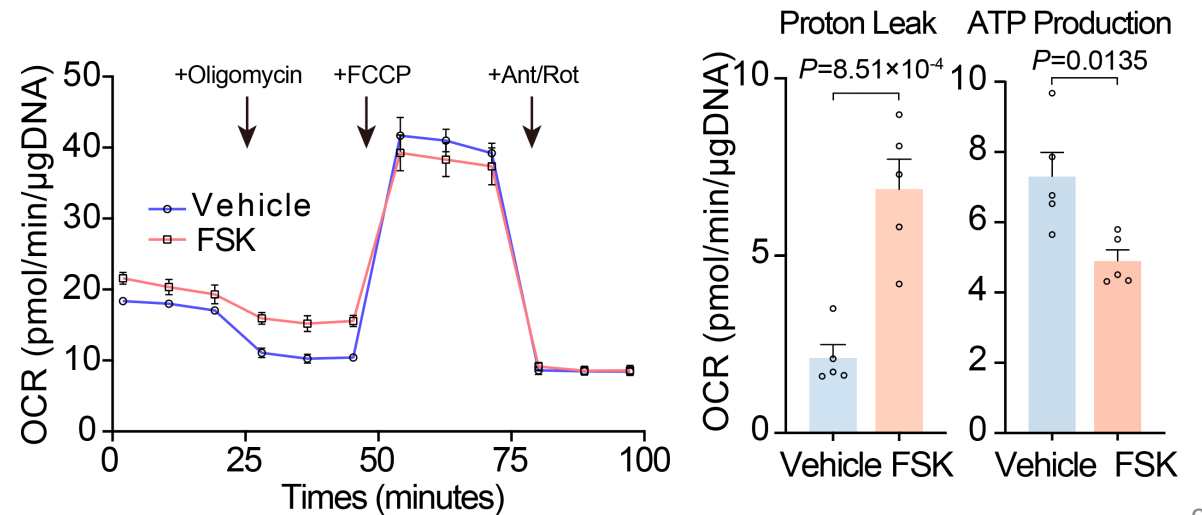
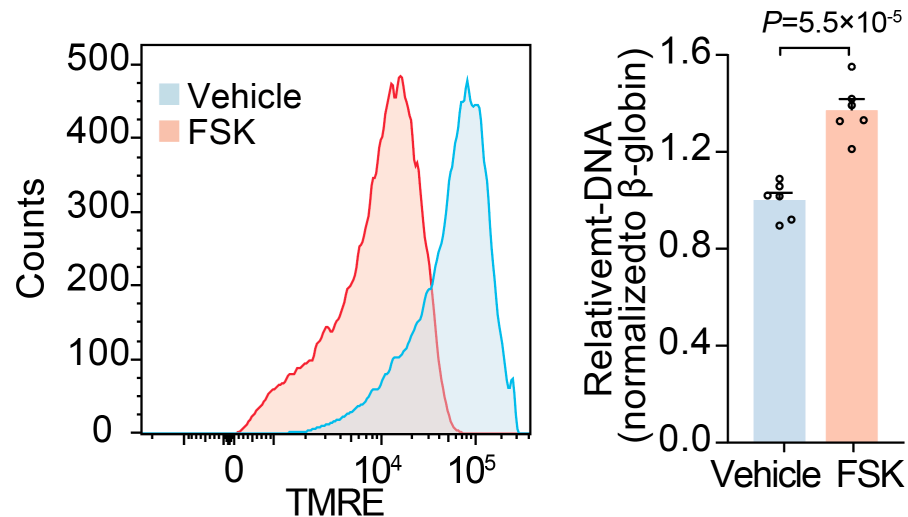
FSK (forskolin), an
adenylate cyclase
(AC) activator, a
cAMP enhancer



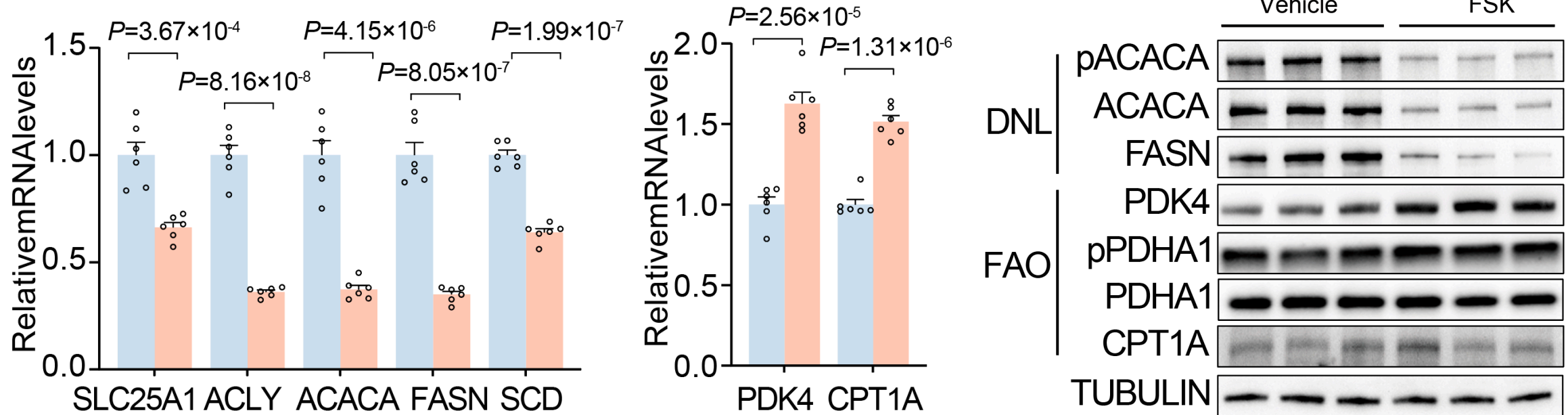
Differentiated Tibetan pig adipocytes



5 μ M
24 hours



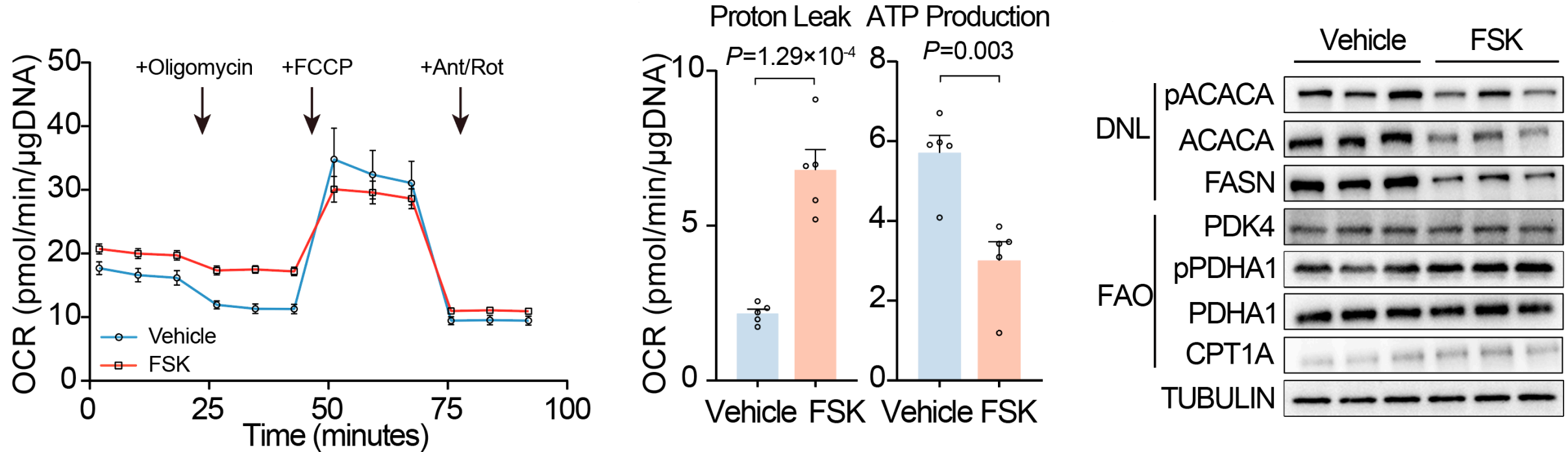
De Novo Lipogenesis (DNL) pathway was suppressed in FSK treated adipocytes from **Tibetan pigs**



Enhanced uncoupled respiration and the suppression of the DNL pathway in beige adipocytes from Tibetan pigs are likely mediated by the cAMP signaling pathway.

FSK also activated the thermogenesis program and suppressed DNL pathway in Bama adipocytes

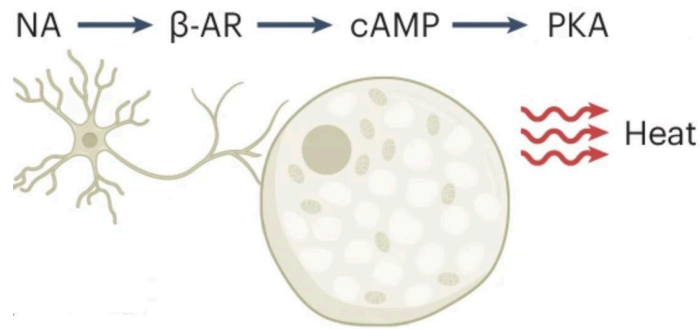
Differentiated Bama pig adipocytes



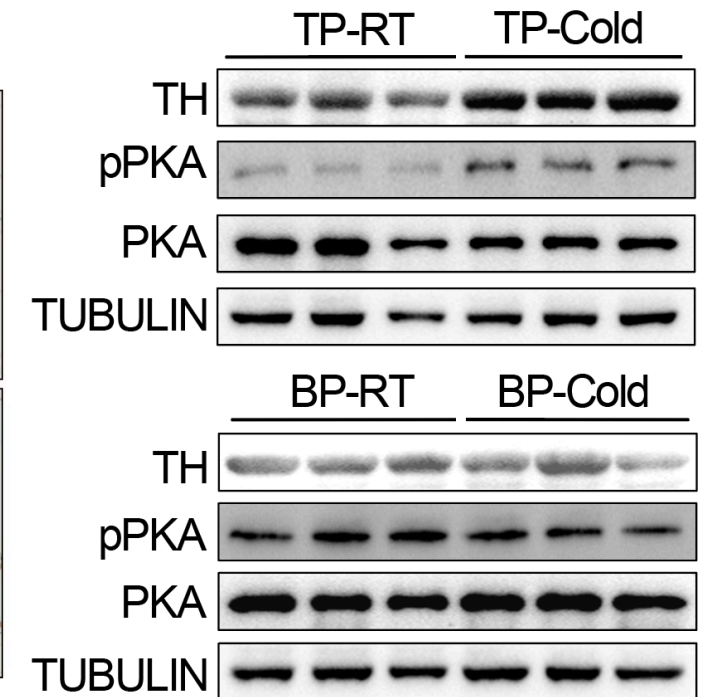
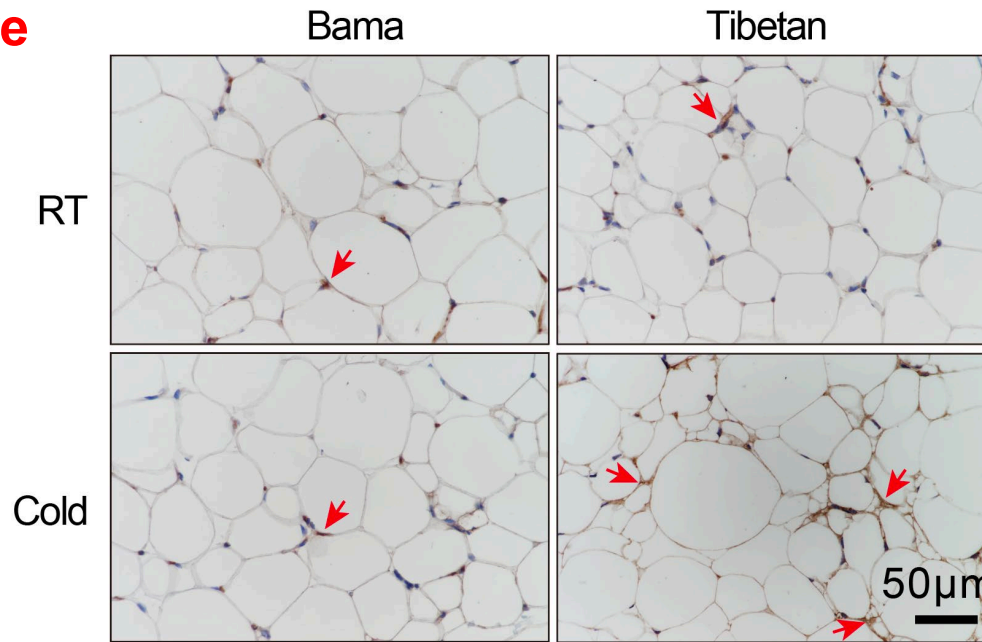
These results suggesting that thermogenesis in porcine adipocytes is **not a cell-autonomous effect.**

Sympathetic innervation plays a crucial role in determining beige formation in different pig breeds upon cold stimulation

Noradrenaline (NA) stimulates the β -adrenergic receptor (β -AR) to induce cAMP in mature thermogenic adipocytes.

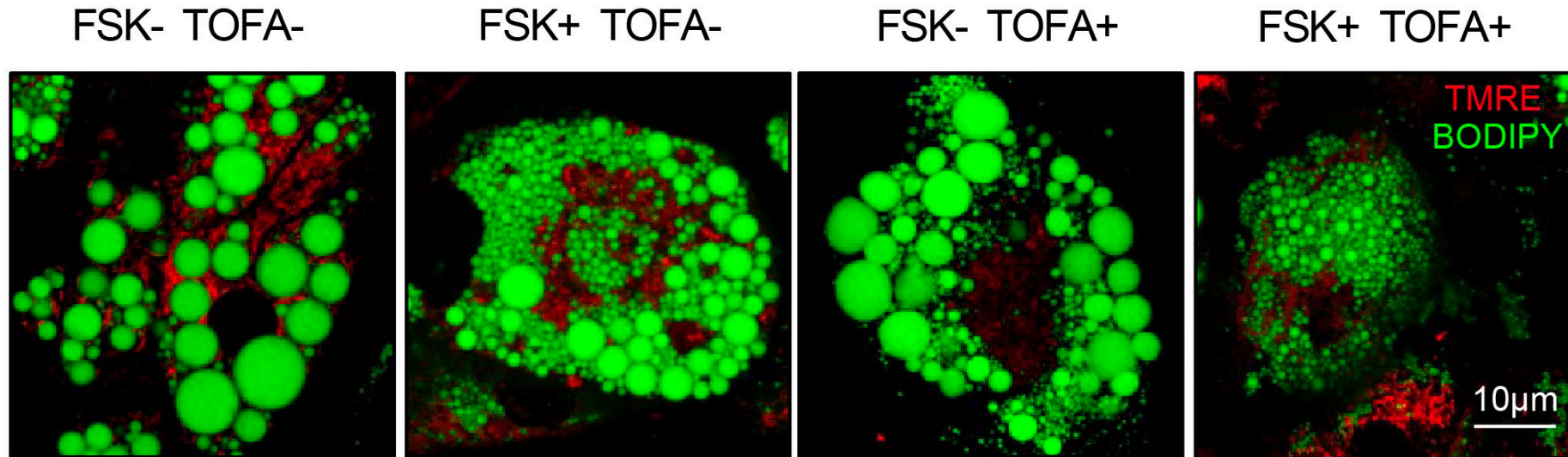


TH, tyrosine hydroxylase, a widely used marker for sympathetic nerve fibers



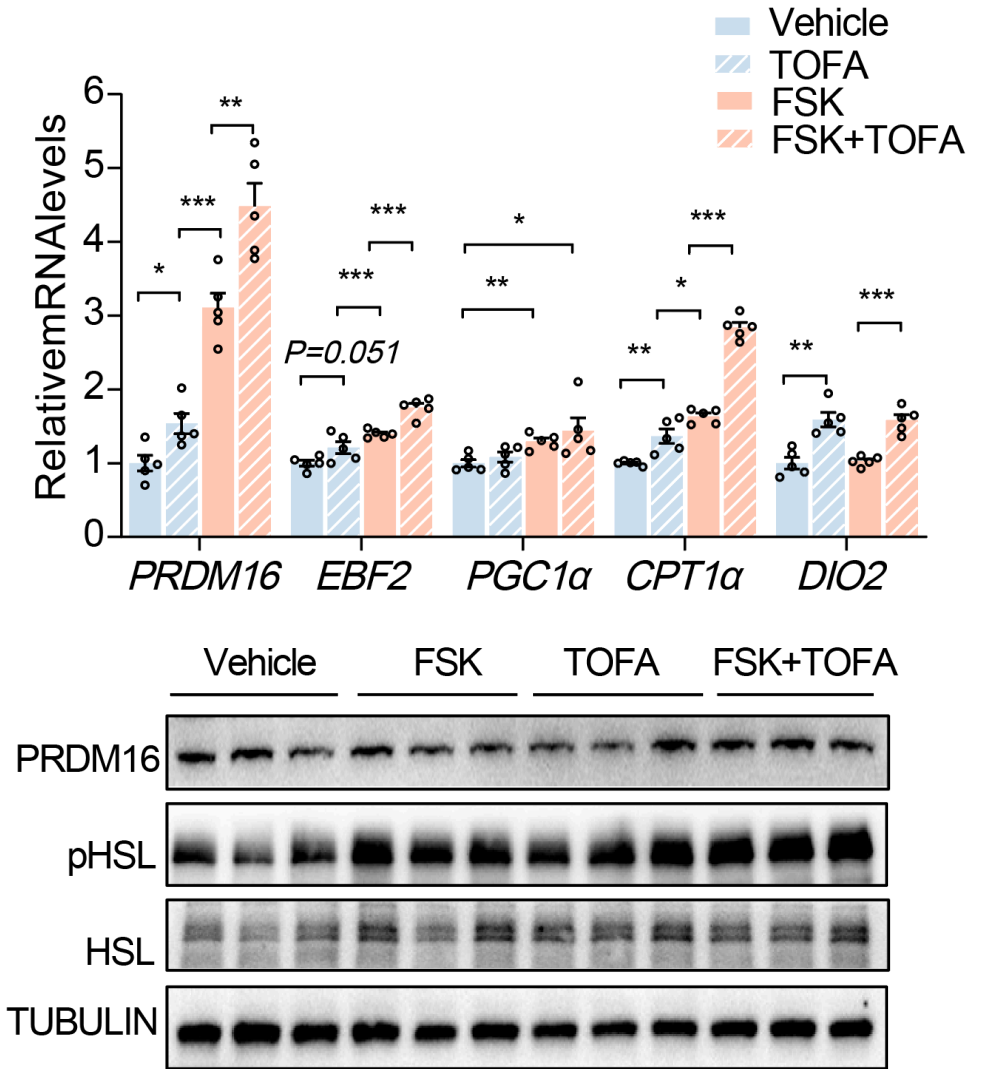
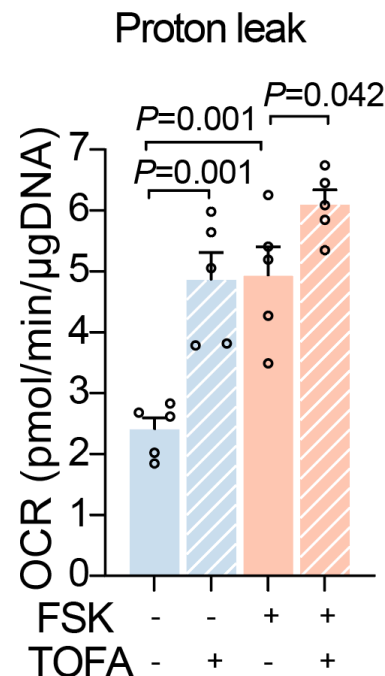
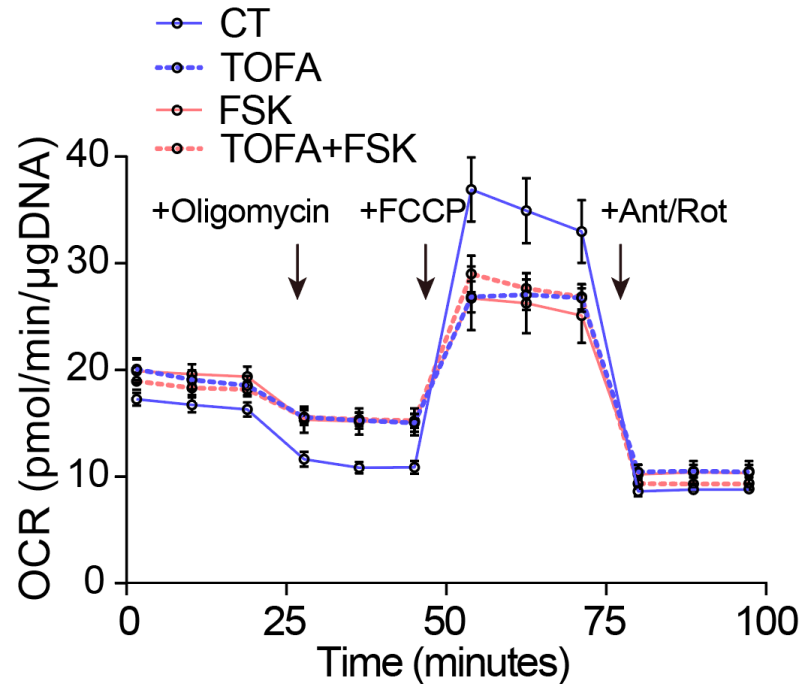
ACACA inhibitor, TOFA, enhanced FSK-induced thermogenesis of adipocytes from Tibetan pigs

- ACACA, the rate-limiting enzyme in DNL, catalyzing the carboxylation of acetyl-CoA to form malonyl-CoA in the cytoplasm.
- TOFA**: 5-(tetradecyloxy)-2-furoic acid, a cell-permeable allosteric ACACA inhibitor



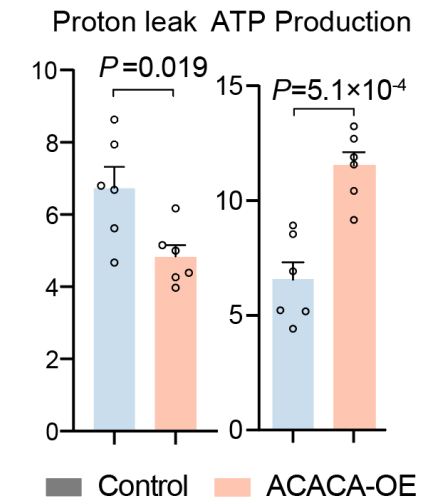
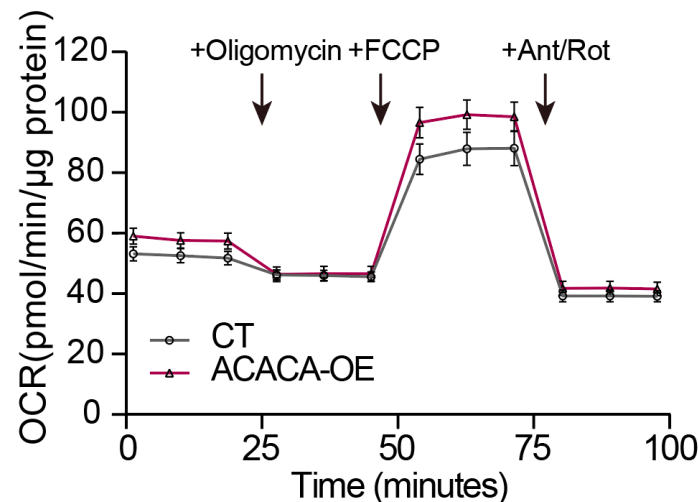
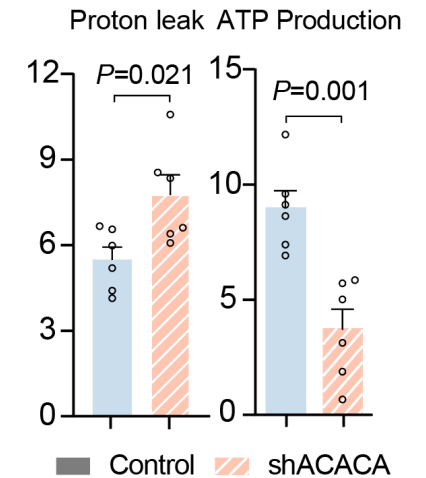
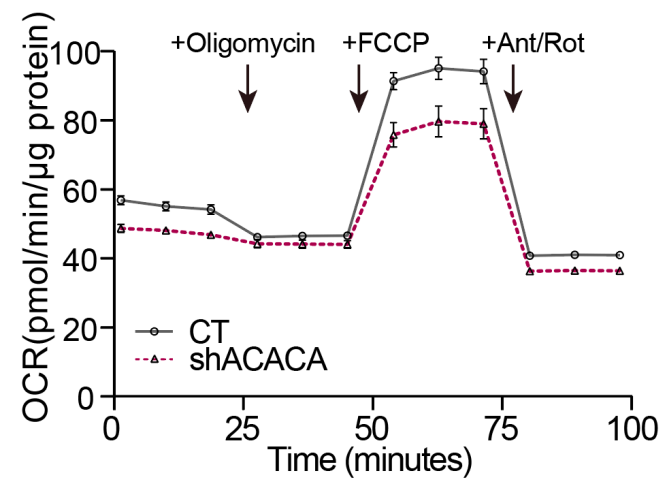
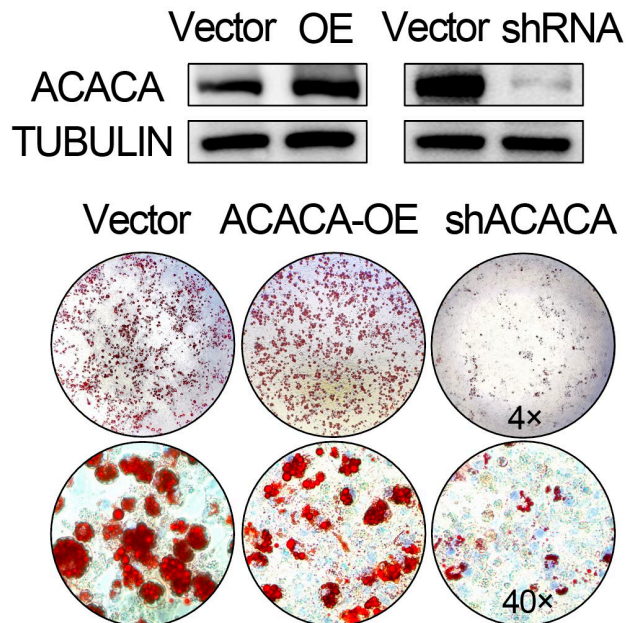
ACACA inhibitor, TOFA, enhanced thermogenesis of pig adipocytes and has a additive effect with FSK

- TOFA or FSK significantly increased proton leak respiration;
- TOFA and FSK elevated the expressions of beige marker and lipolysis genes.



Overexpression or knockdown of ACACA regulates lipid deposition and thermogenesis in porcine adipocytes

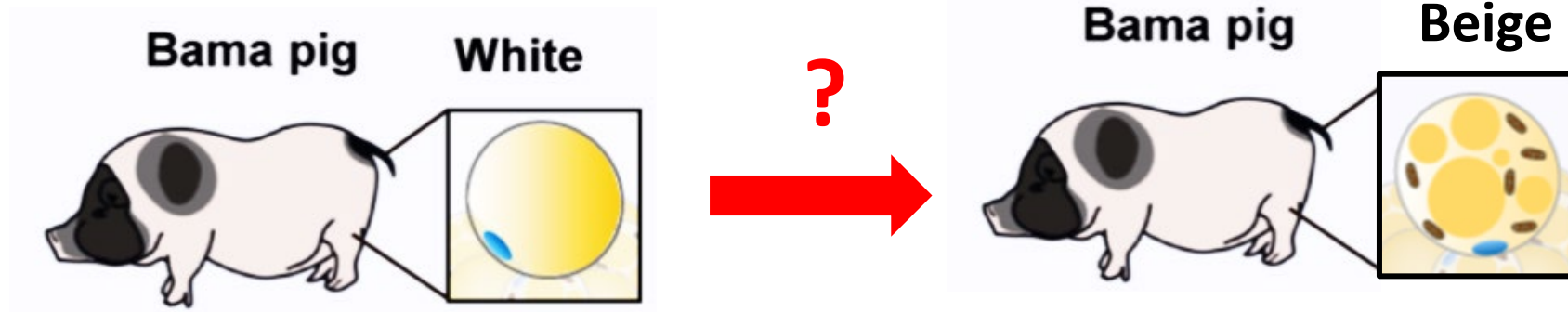
- To verify the specificity of the pharmacological effects of TOFA, we employed genetic methods to either overexpress or knock down ACACA expression.



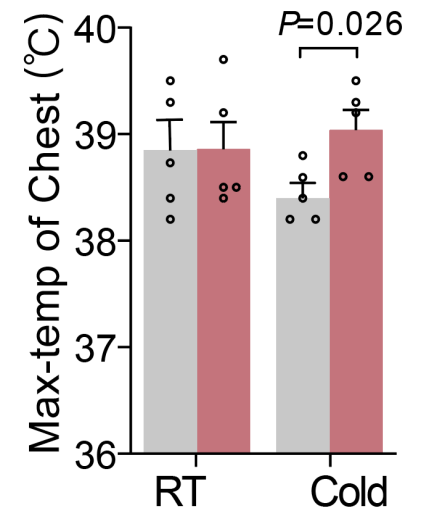
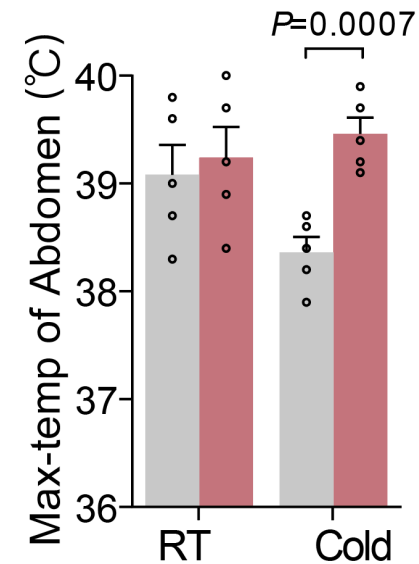
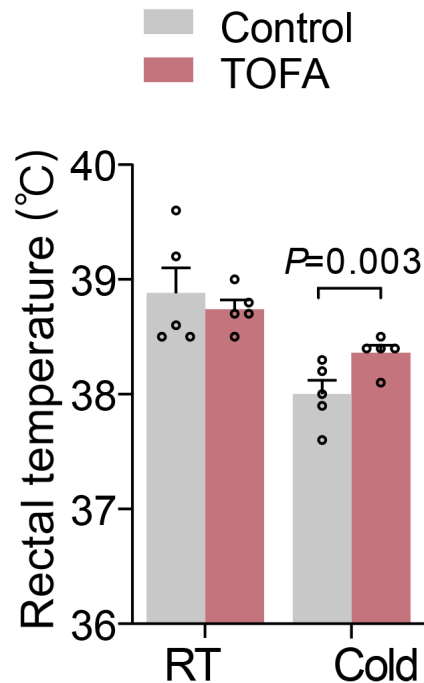
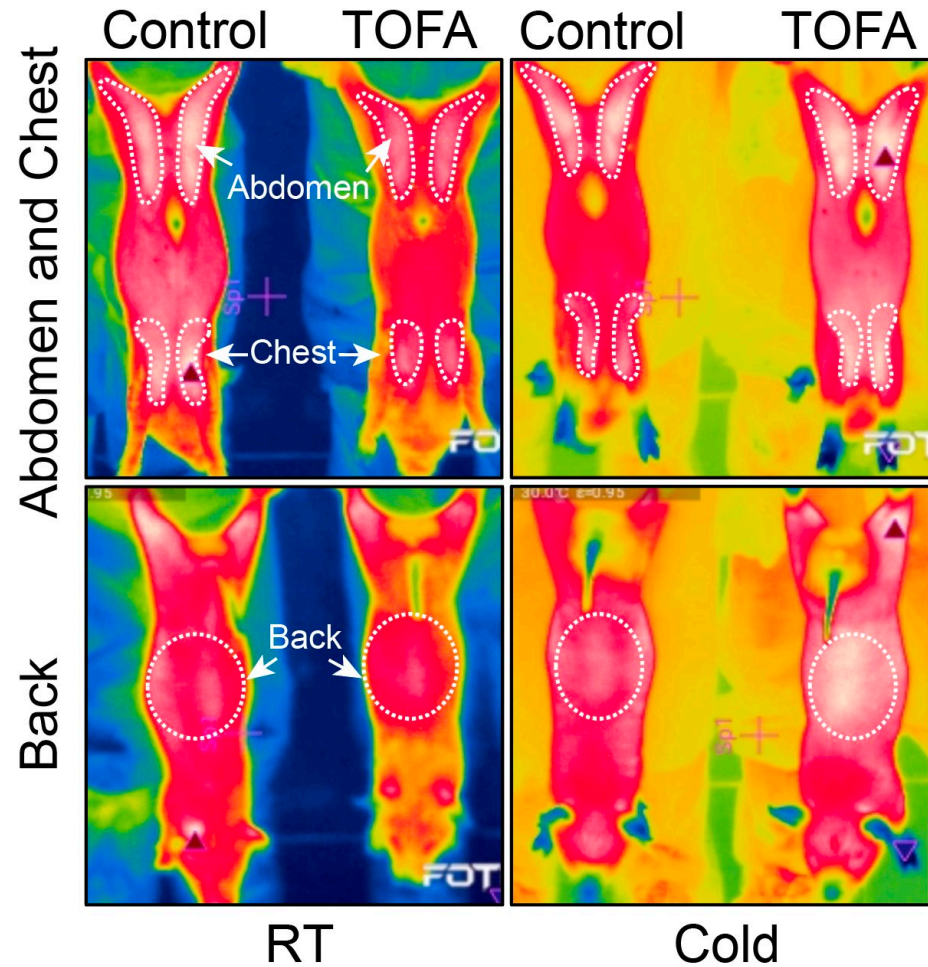
Biological question

- Does TOFA improve cold tolerance in cold-intolerant Bama pigs?

Acute cold exposure (4°C, 4h)+TOFA treatment

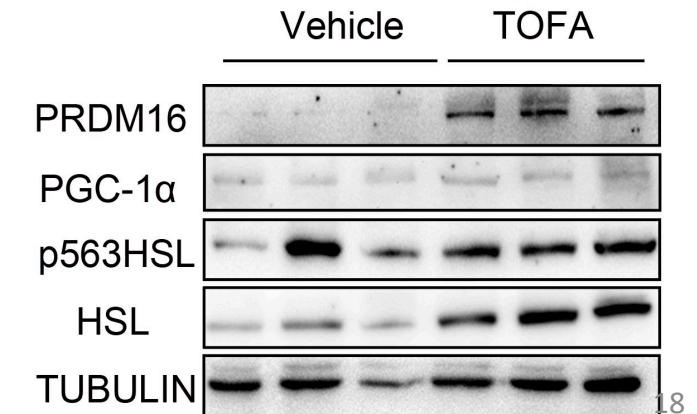
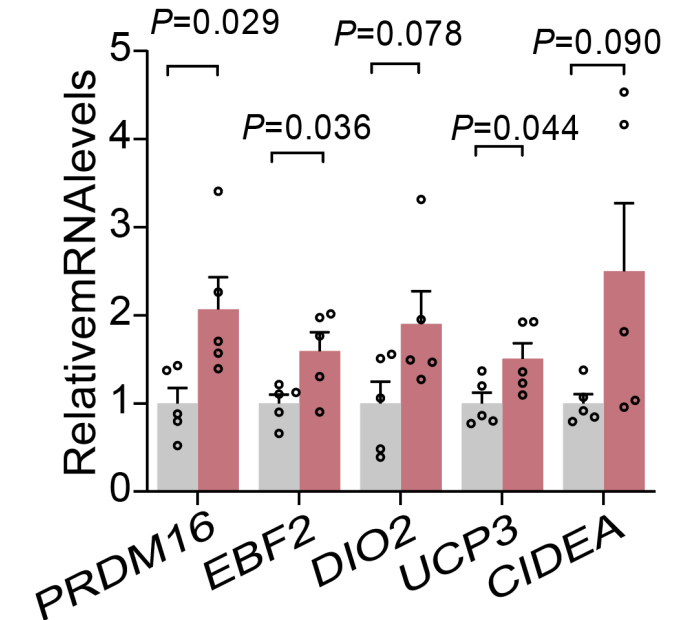
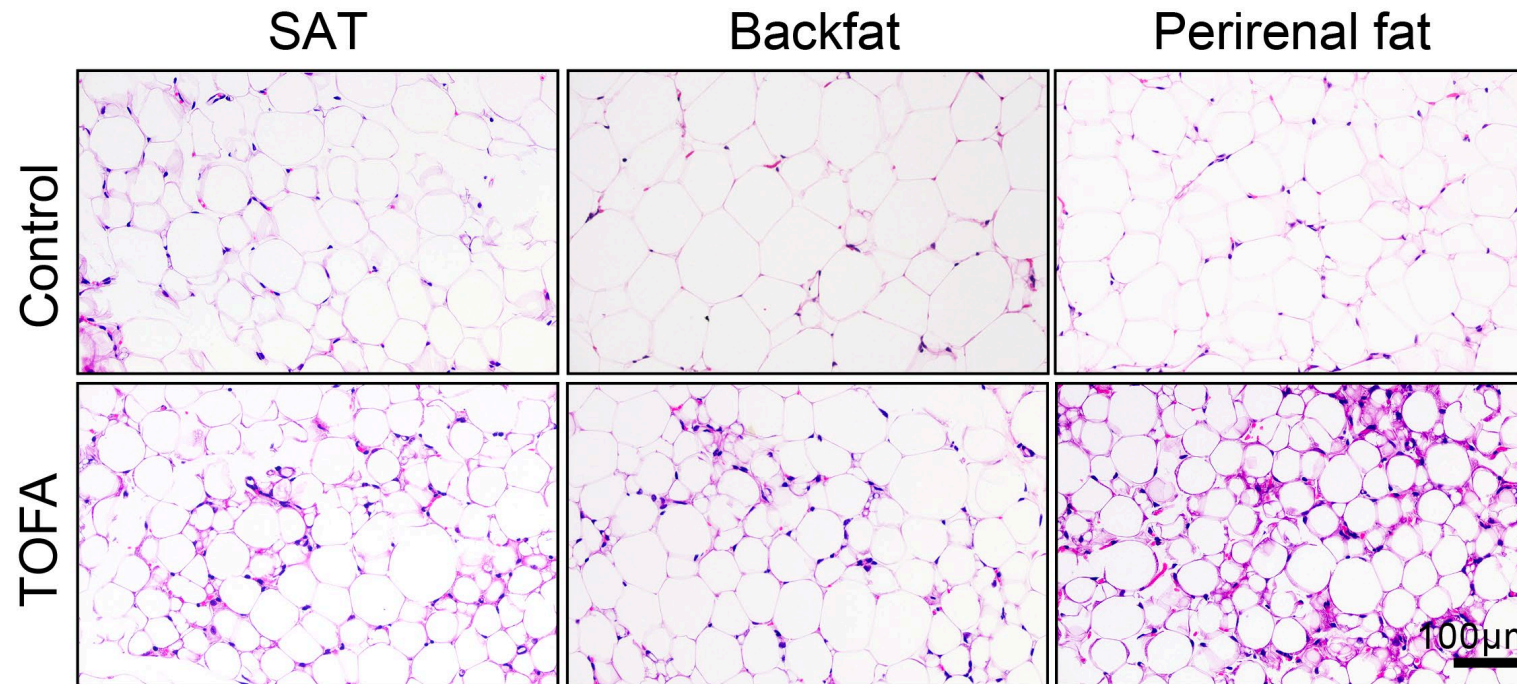


TOFA and acute cold treatment improved thermoregulation in Bama pigs



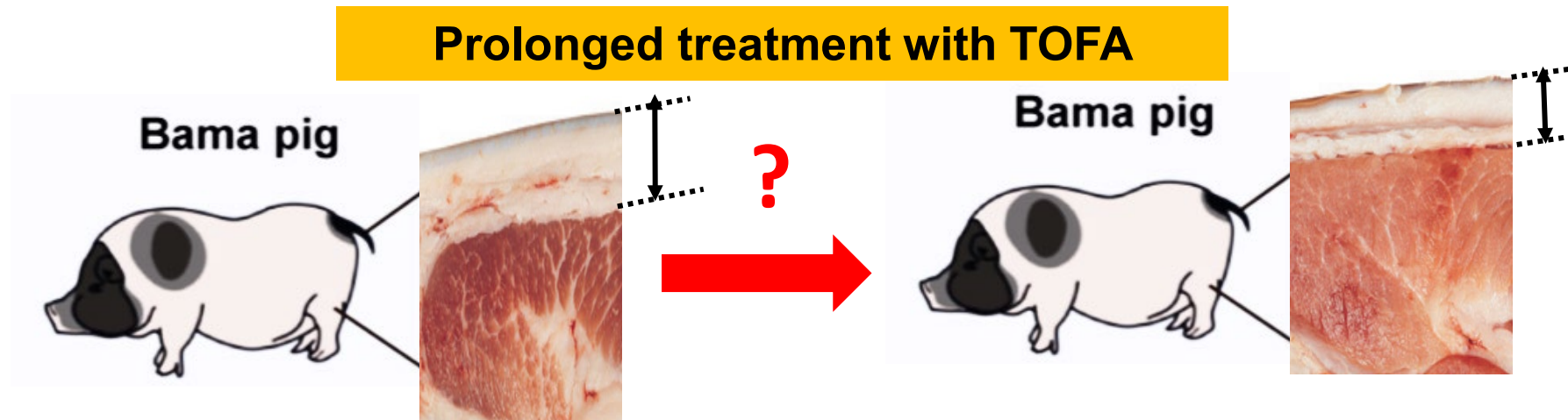
Beige was induced in TOFA treated Bama pigs

- Uni-locular adipocytes in control pigs and multilocular adipocytes in TOFA treatment pigs.
- Beige marker genes were significantly induced in TOFA and acute cold treated Bama pigs.

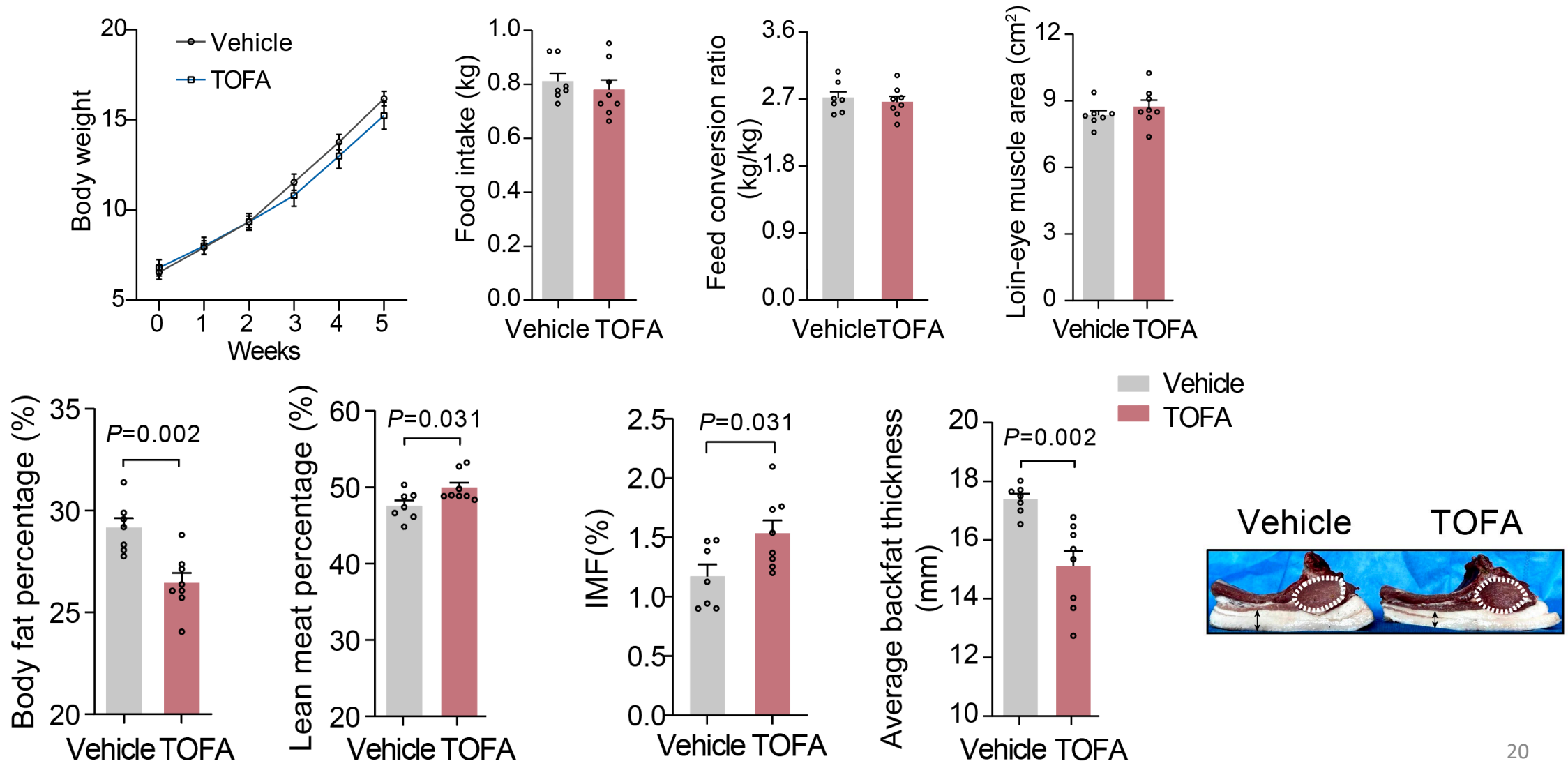


Biological question

- Can TOFA reduce fat deposition by inducing the formation of beige adipocytes in pigs?

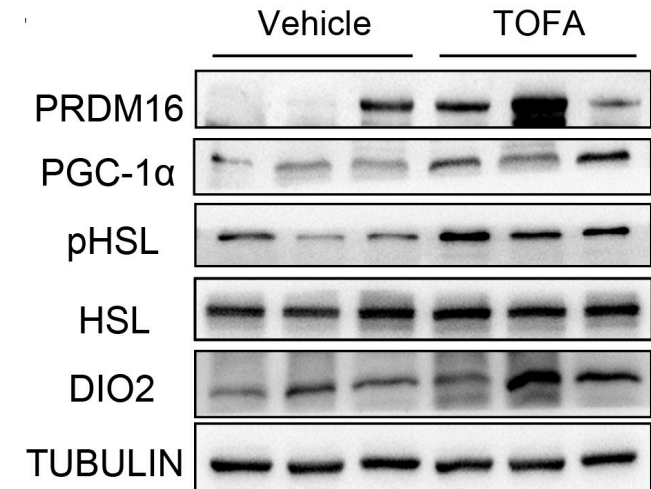
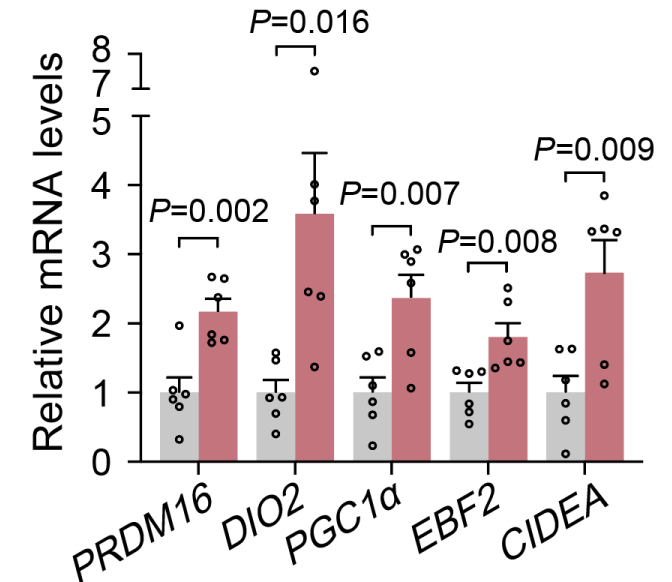
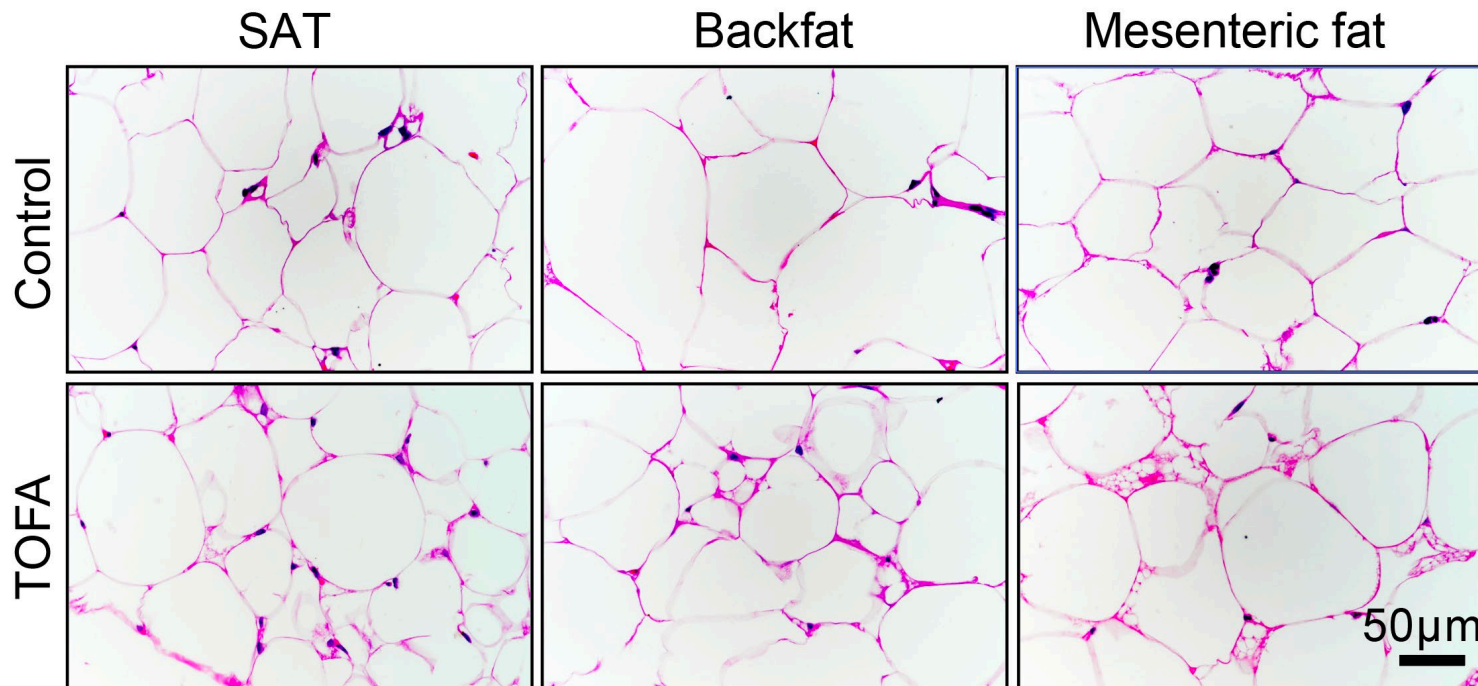


Prolonged treatment with TOFA significantly reduced fat deposition



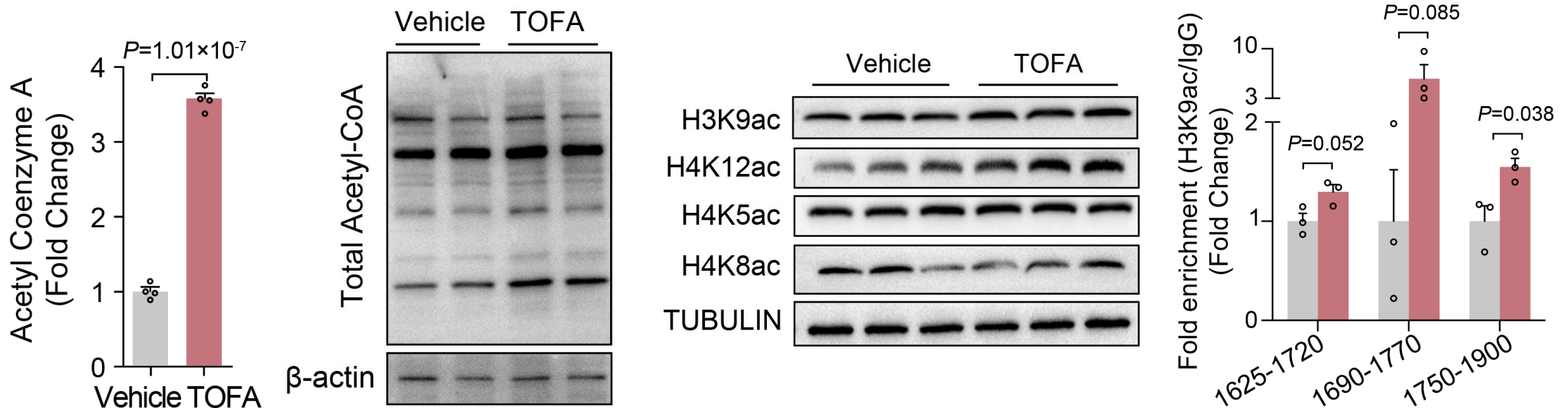
Prolonged treatment with TOFA significantly reduced fat deposition

- Multilocular adipocytes were observed in various depot fats of the TOFA-treated pigs
- TOFA administration substantially upregulated the expression level of beige marker genes



ACACA inhibition activate a transcriptional program of *PRDM16* for beige formation by augmenting histone acetylation

- TOFA treatment led to an increase in both acetyl-CoA and total protein acetylation in porcine adipocytes
- Levels of H3K9ac and H4K12ac were substantially upregulated following TOFA treatment
- TOFA enhances H3K9ac enrichment at the promoter region of the *PRDM16* gene



Conclusions

- Acute cold stimulation represses the de novo lipogenesis (DNL) pathway in the adipocytes of cold-tolerant pig breeds;
- Differences in cold tolerance among pig breeds may depend on the intensity of neurotransmitter activity;
- Inhibition of ACACA not only enhanced thermogenesis in cold-sensitive Bama pigs during acute cold stimulation, but also significantly increased lean body mass.

Acknowledgments

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