

## Research Advance

# Comments on ‘*FNIP1 regulates adipocyte browning and systemic glucose homeostasis in mice by shaping intracellular calcium dynamics*’

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Owing to its remarkable benefits on metabolic health and its demonstrated presence in adult humans, beige or ‘brite’ adipocytes hold great promise to combat obesity and metabolic diseases. Delineation of the mechanisms involved in adipocyte ‘beiging’ or ‘browning’ is thus of particular interest. Previous studies of the molecular regulatory pathways that drive the beiging of white adipose tissue (WAT) have centered on the cyclic adenosine monophosphate (cAMP)-related signaling (Wang and Seale, 2016; Cohen and Kajimura, 2021). Acting via the cAMP–protein kinase A pathway, norepinephrine dually stimulates adipocyte lipolysis and the expression of thermogenic genes, such as uncoupling protein 1 (*Ucp1*) and peroxisome proliferator-activated receptor- $\gamma$  coactivator-1 $\alpha$  (*Ppargc1a*) (Inagaki et al., 2016). While the second messenger calcium ( $\text{Ca}^{2+}$ ) is fundamental to a wide variety of cellular processes including, but not limited to, muscle contraction, gene transcription, exocytosis, and cellular metabolism in virtually all eukaryotic cells (Arruda and Hotamisligil, 2015), whether and how  $\text{Ca}^{2+}$  signals orchestrate WAT beiging remains largely unclear, in part

because of a limited understanding of the regulatory mechanism that controls intracellular  $\text{Ca}^{2+}$  dynamics in adipocytes.

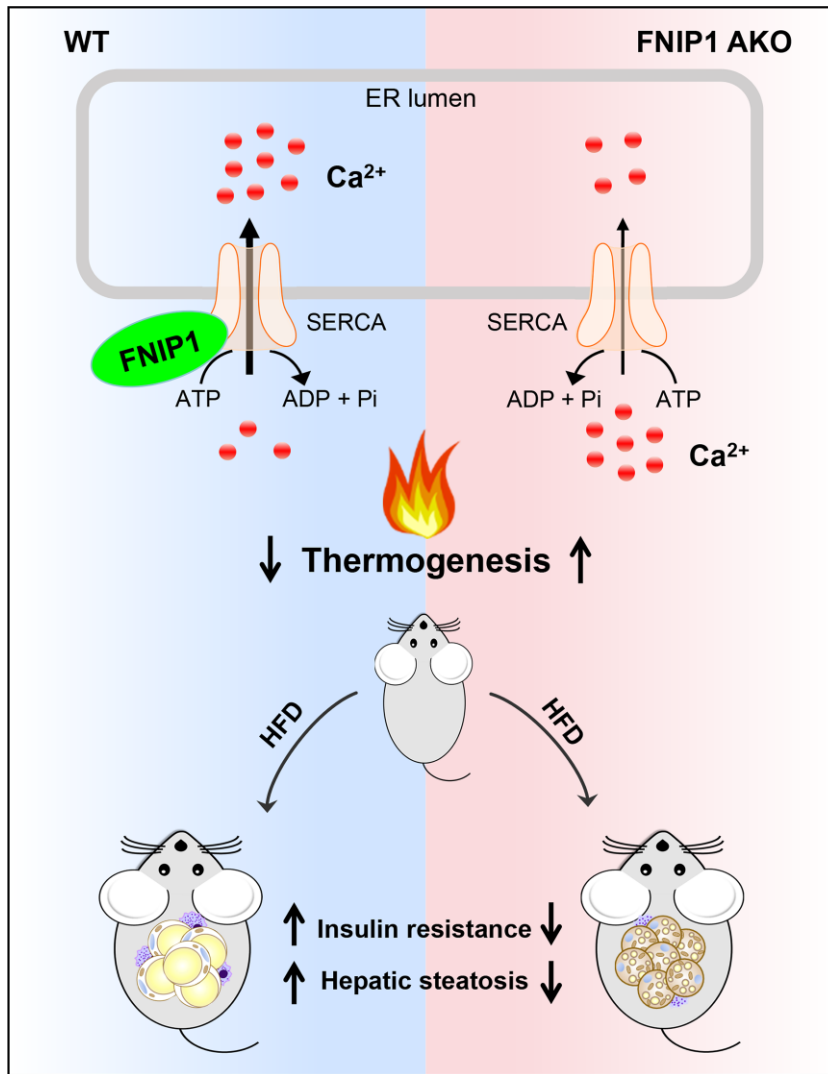
In our recent work (Yin et al., 2022), we discovered a previously unrecognized WAT beiging mechanism that targets the folliculin (FLCN) interacting protein 1 (FNIP1)–sarco/endoplasmic reticulum  $\text{Ca}^{2+}$ -ATPase (SERCA)–intracellular  $\text{Ca}^{2+}$  axis in adipocytes (Figure 1; commented in Bunk and Kazak, 2022). Using multiple  $\text{Ca}^{2+}$  imaging approaches, together with gain- and loss-of-function strategies, we provided clear data demonstrating that FNIP1 is a crucial regulator of intracellular  $\text{Ca}^{2+}$  dynamics and negatively regulates WAT beiging. We also uncovered the working mechanisms that FNIP1 binds to and promotes the activity of SERCA, a main  $\text{Ca}^{2+}$  pump responsible for cytosolic  $\text{Ca}^{2+}$  removal, thereby dampening  $\text{Ca}^{2+}$ -dependent thermogenic programs. Furthermore, we showed that adipocyte-specific ablation of FNIP1 leads to a broad thermogenic remodeling of WAT with strong effects on whole-body glucose homeostasis and hepatic steatosis.

In this study, we set to investigate the intracellular  $\text{Ca}^{2+}$  dynamics in association with WAT beiging by the employment of GCaMP6f reporter mice, a mouse model that allowed us to monitor  $\text{Ca}^{2+}$  activity in adipose tissue *in vivo*. We found that dynamic changes of intracellular  $\text{Ca}^{2+}$  are required for the thermogenic gene program in white

adipocytes upon thermogenic stimuli. The endoplasmic reticulum (ER) serves as the main  $\text{Ca}^{2+}$  store that determines intracellular  $\text{Ca}^{2+}$  homeostasis. Among the ER membrane-localized  $\text{Ca}^{2+}$  channels, SERCA is the solely  $\text{Ca}^{2+}$  influx pump that has been shown to fine-tune intercellular  $\text{Ca}^{2+}$  dynamics. By performing immunoprecipitation and mass spectrometry analysis, we identified that SERCA2 is able to interact and precipitate with FNIP1, an adaptor protein originally identified through its interaction with FLCN and AMP-activated protein kinase (AMPK) (Baba et al., 2006). We then performed a series of cellular assays to demonstrate that FNIP1 acts as a positive regulator of SERCA in adipocytes, and binding of FNIP1 promotes both ATP hydrolysis and  $\text{Ca}^{2+}$  transport of the SERCA pump, leading to a faster removal of cytosol  $\text{Ca}^{2+}$ . As such, in FNIP1 knockout (KO) adipocytes, the reuptake of cytosolic  $\text{Ca}^{2+}$  into the ER lumen mediated by SERCA is reduced, resulting in elevated  $\text{Ca}^{2+}$  levels and an increased duration of  $\text{Ca}^{2+}$  transients in the cytosol upon external stimuli. More importantly, we showed that, functionally, loss of adipocyte FNIP1 in mice results in enhanced thermogenic remodeling in WAT. This improves systemic glucose homeostasis and prevents hepatic steatosis when mice are fed a high-fat diet (HFD).

We observed that FNIP1 deficiency in adipocytes triggers a robust UCP1<sup>+</sup>

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**Figure 1** The model of FNIP1 in control of beige adipocyte thermogenesis and metabolic health through modulating SERCA. FNIP1 binds to SERCA to enhance its  $\text{Ca}^{2+}$  pump activity. This limits intracellular  $\text{Ca}^{2+}$  dynamics and dampens  $\text{Ca}^{2+}$ -dependent thermogenic programs. Mice with adipocyte-specific ablation of FNIP1 showed enhanced thermogenic remodeling of WAT and were protected against HFD-induced insulin resistance and hepatic steatosis.

beige adipocyte formation and mitochondrial biogenesis in the absence of cold exposure or  $\beta$ -adrenergic stimulation. Therefore, FNIP1 acts as an endogenous ‘brake’ to inhibit adipocyte beiging. Intriguingly, our results also indicate that FNIP1 suppression of thermogenic programs occurs in a depot-selective manner, the reason for which is not clear. This could reflect the intrinsic differences between beige adipocytes and brown adipocytes that affect the adaptive range of thermogenic remodeling.

While we have demonstrated previously that FNIP1 acts through AMPK but not mammalian target of rapamycin (mTOR) complex 1 signaling to regulate mitochondrial function in the skeletal muscle (Xiao et al., 2021), interestingly, we did not observe a change in either AMPK or mTOR signaling in both FNIP1 KO primary adipocytes and FNIP1 adipocyte-specific KO (AKO) adipose tissues. These results suggest that the regulations of metabolic regulators by FNIP1 are context-specific or cell

type-specific. We clearly showed that FNIP1 binds to and promotes the activity of SERCA. We also examined the endogenous FNIP1 and SERCA interaction in adipose tissue after adrenergic stimulation. The results were interesting and consistent with the proposed mechanistic model. We found that CL316,243 stimulation significantly reduced the interaction between endogenous FNIP1 and SERCA in WAT.

Previously published studies have revealed that FNIP1 could actually act as a co-chaperone that regulates the chaperone function of Hsp90 (Woodford et al., 2016). We found that the N-terminal region of FNIP1 binds to the ATP hydrolysis site of the SERCA pump. Moreover, we observed that SERCA protein was stabilized in cells expressing FNIP1. Based on our data and the published literatures, it is possible that FNIP1 also functions as a co-chaperone of Hsp90 that regulates SERCA protein stability in adipocytes.

Notably, a recent study has shown that enhanced  $\text{Ca}^{2+}$  cycling in beige fat via the SERCA2–RyR pathway can promote a UCP1-independent thermogenesis (Ikeda et al., 2017). Whereas UCP1 and mitochondria were strongly induced by FNIP1 deletion, thus suggesting a UCP1-dependent thermogenesis in FNIP1 AKO inguinal WAT, our results did not determine whether this FNIP1 regulatory pathway is involved in UCP1-dependent or UCP1-independent beige adipocyte thermogenesis. Obviously, these are not mutually exclusive roles.

In summary, our recent work suggests that FNIP1 acts as a negative regulator of beige adipocyte thermogenesis to control systemic glucose homeostasis through modulating SERCA (Yin et al., 2022). Linking intracellular  $\text{Ca}^{2+}$  dynamics to adipocyte beiging is of interest, given that it could be an appealing strategy for the treatment of metabolic disorders. This raises several interesting questions. (i) What are the mechanisms by which the intracellular  $\text{Ca}^{2+}$  signals activate the thermogenic program? (ii) How is the interaction between FNIP1 and SERCA regulated under the physiological setting? (iii) Does this FNIP1–SERCA–intracellular  $\text{Ca}^{2+}$  pathway repre-

sent a broader metabolic regulatory network in other organs? (iv) Will it be possible to exploit such FNIP1–intracellular Ca<sup>2+</sup> signals as treatment strategies?

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