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# Stromal cell-derived NGF controls sympathetic innervation in subcutaneous fat

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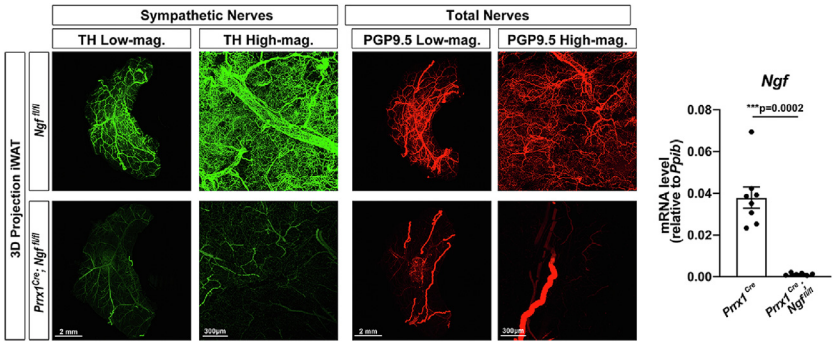


The densely distributed sympathetic nerves in the mouse subcutaneous inguinal white adipose tissues (iWAT) promote the thermogenic capacity to counteract the cold environment. The sympathetic innervation within iWAT is established during early development and persists throughout adulthood (1). Previous studies have indicated that the sympathetic nerve outgrowth could be modulated by neurotrophic factors, resulting in altered parenchymal sympathetic density at the adult stage (2, 3). Intriguingly, expression profiles examining the iWAT-resident cells reveal abundant expression of nerve growth factor (*Ngf*) in the stromal cells, in contrast to other cell types including adipocytes (2). To determine whether stromal cell-derived NGF may play a significant role in sympathetic innervation, we bred $Ngf^{fl/fl}$ mice generated in the previous study (2) with $Prrx1^{Cre}$ mice to achieve *Ngf* deletion in stromal cells. Transcript analysis verified that *Ngf* expression reduced drastically in iWAT, in agreement with a predominant contribution of NGF from stromal cells. Next, we performed whole-mount immunostaining and volume-fluorescence imaging using antibodies against tyrosine hydroxylase (TH) and protein gene product 9.5 (PGP9.5) to visualize sympathetic nerves and total peripheral nerves, respectively. Remarkably, at the whole tissue level, sympathetic nerves immunolabeled by TH were largely abrogated in the iWAT parenchyma of $Prrx1^{Cre}$; $Ngf^{fl/fl}$ mice compared to the control $Ngf^{fl/fl}$ mice (supplemental Movie S1 for $Ngf^{fl/fl}$ and supplemental Movie S2 for $Prrx1^{Cre}$; $Ngf^{fl/fl}$). Consistent with the predominant distribution of sympathetic nerve type in the iWAT, total nerves immunolabeled by PGP9.5 also showed defective branching in $Prrx1^{Cre}$; $Ngf^{fl/fl}$ mice, with only a few by-passing nerve bundles visible (supplemental Movie S5 for $Ngf^{fl/fl}$ and supplemental Movie S6 for $Prrx1^{Cre}$; $Ngf^{fl/fl}$). Moreover, the fine nerve fibers imaged at high magnification within inguinal regions proximal to the lymph node presented a similar result (supplemental Movies S3 and S4 for TH, supplemental Movies S7 and S8 for PGP9.5). Taken together, these data suggest that stromal cell-derived NGF plays a dominant role in the establishment of intra-adipose sympathetic innervations.

**EQUIPMENT:** Ultramicroscope II with a 1.1x 0.1 NA objective lens (Miltenyi Biotec, 1x zoom) or 4x 0.35 NA objective lens (Miltenyi Biotec, 2x zoom).

**REAGENTS AND ANIMALS:** The whole-mount immunostaining and volume-fluorescence imaging were carried out as previously described (2) with the following antibodies: rabbit anti-TH (Millipore catalog no. AB152, RRID: AB_390204), rabbit anti-PGP9.5 (Proteintech catalog no. 14730-1-AP, RRID: AB_2210497), and Alexa Fluor 647-goat anti-rabbit (Thermo Fisher Scientific). $Prrx1^{Cre}$ mice were obtained from Jackson Laboratory (JAX 005584, RRID: IMSR_JAX:005584).

**SOFTWARE:** Imaris (Oxford Instruments).

*Supplemental data*

This article contains supplemental data.

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*Author contributions*

X. M. and J. C. methodology; X. M. validation; X. M. and W. Z. writing–original draft; J. C. and W. Z. funding acquisition; W. Z. conceptualization.

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*Conflict of interest*
The authors declare that they have no conflicts of interest with the contents of this article.

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