SUPPLEMENTARY DATA

Supplementary Figure 1. A) List of antibody clones used in various flow cytometry experiments. B) List of primer sequences used to probe osteoblast gene expression.

A

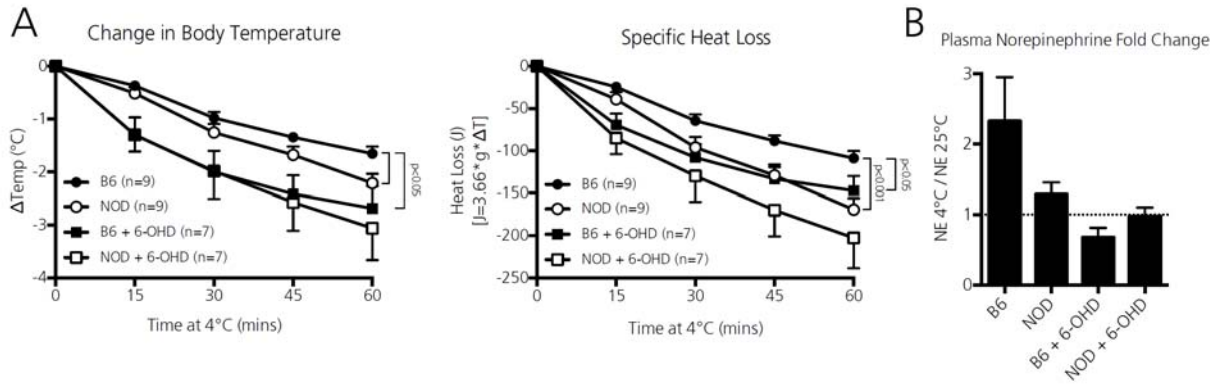
Antibody Target	Clone	Company
B220	RA3-6B2	BD Biosciences
CD3e	145-2C11	BD Biosciences
CD11b	M1/70	BD Biosciences
CD45RB	C363.16A	BioLegend
c-Kit	2B8	BD Biosciences
CXCR4	2B11	eBioscience
Ly6C/G	RB6-8C5	BD Biosciences
Osteocalcin	Rabbit Polyclonal	Bioss Antibodies
Rank-L	IK22/5	eBioscience
Sca-1	D7	BD Biosciences
TER-119	TER-119	BD Biosciences

B

Gene	Forward (5′ to 3′)	Reverse (5′ to 3′)
CXCL12	TGCATCAGTGACGGTAAACCA	GTTGTTCTTCAGCCGTGCAA
Osteocalcin	CCGGGAGCAGTGTGAGCTTA	TAGATGCGTTTGTAGGCGGTC
Rank-L	CACCATCAGCTGAAGATAGT	CCAAGATCTCTAACATGACG
Alkaline Phosphatase	ATCTTTGGTCTGGCTCCCATG	TTTCCCGTTCACCGTCCAC
Osteopontin	GATGATGATGACGATGGAGACC	CGACTGTAGGGACGATTGGAG
Collagen 1a	GAGCGGAGAGTACTGGATCG	GTTCGGGCTGATGTACCAGT
GAPDH	TCACCACCATGGAGAAGGC	GCTAAGCAGTTGGTGGTGCA

SUPPLEMENTARY DATA

Supplementary Figure 2. *Pre-diabetic NOD mice demonstrate impaired global sympathetic nervous system activity.* A) Age (8 week) and sex matched (female) non-diabetic B6 and pre-diabetic NOD mice were left untreated or chemically sympathectomized with 6-OHD and housed a 4°C for one hour (12). Rectal temperatures were measured. Plasma Norepinephrine (NE) levels were measured at the end of the hour (4°C) and one day after mice had acclimated to room temperature (25°C) by HPLC at the Vanderbilt Hormone Assay Core. Within untreated mice, pre-diabetic NOD mice (white circles) demonstrated a significantly greater loss of absolute body temperature and specific heat (formula displayed on graph y-axis) as compared to B6 mice (black circles) (upper panels, p values at 60 minutes determined by One-way ANOVA followed by Tukey's multiple comparisons post-test). B) Whereas plasma NE increased over 2-fold in untreated B6 mice in response to cold (p=0.03 vs baseline, t-test), plasma NE levels did not significantly increase in NOD mice. 6-OHD treated, SNS deficient B6 (black squares) and NOD (white squares) mice demonstrated the greatest loss of body temperature. 6-OHD treatment in B6 mice abrogated the NE response to cold. Data is pooled from two independent experiments to include a total of n=7-9 mice per group.



SUPPLEMENTARY DATA

Supplementary Figure 3. *Bone marrow sympathetic nervous system innervation is relatively similar between non-diabetic B6 and pre-diabetic NOD mice.* Top Panels) To evaluate sympathetic innervation within bone marrow, femurs from age (8 week) and sex-matched (female) non-diabetic B6 and pre-diabetic NOD mice were fixed in 10% formalin, decalcified with Calrite (ThermoFisher, Waltham, MA), mounted in paraffin, sectioned at 6μm, and stained with anti-Tyrosine Hydroxylase (TH) (AB152; Rabbit polyclonal, EMD Millipore, Billarica, MA) followed by development with a Leica Bond Refine Kit (Newcastle, UK) (13). IHC samples were imaged by a Leica Biosystems Aperio ScanScope CS at 20x resolution. Although bone marrow resident TH+ fibers were sparse, we observed no significant differences in TH+ fiber morphology/number within the bone marrow of either strain. Bottom Panels) Sterna were obtained from age (8 week) and sex matched (female) non-diabetic B6 and pre-diabetic NOD mice, sectioned coronally, and stained for endothelial CD31 (RM0032-1D12, Rat IgG2a, Abcam, Cambridge, MA), anti-Tyrosine Hydroxylase (AB152; Rabbit polyclonal, EMD Millipore, Billarica, MA) and then counterstained with anti-Rat-Alexa488 and anti-Rabbit-Alexa647 antibodies (Cell Signaling, Danvers, MA). Fluorescently stained samples were imaged by a Nikon LSM710 META Inverted Confocal Microscope at 10x resolution housed by the Vanderbilt Cell Imaging Shared Resource (CISR) Core. At 10x, both strains demonstrate significant positive staining for vessels (magenta) surrounded by TH+ nerve fibers (yellow).