Supplemental Material

Adipocyte-specific deficiency of NADPH oxidase 4 delays the onset of insulin resistance and attenuates adipose tissue inflammation in obesity



Supplemental Figure I. Adipocyte-specific deficiency of NOX4 initially lowers oxidative stress in adipose tissue during the development of obesity.

A; Epididymal fat isolated from Adipoq-Cre/+;NOX4^{+/+} and Adipoq-Cre/+;NOX4^{Flox/Flox} mice fed a HFHS diet for 8,12, and 16 weeks was analyzed by immunohistochemistry using a 4-HNE antibody which detects lipid peroxidation product (n=5). Tissues were photographed using microscopy (original magnification ×60), and quantified using Image Pro Plus software. *P < 0.05 vs. Adipoq-Cre/+;NOX4^{+/+} in HFHS. ANOVA and Bonferroni post-hoc test.



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B; Adjacent sections from EWAT isolated from Adipoq-Cre/+;NOX4^{+/+} and Adipoq-Cre/+;NOX4^{Flox/Flox} mice fed a HFHS diet for 8,12, and 16 weeks were also stained using 4-HNE, Mac-2 and perlipin-I antibodies. Tissues were photographed using microscopy (original magnification \times 60)



Supplemental Figure II. NOX activity is transiently increased in control mice and unchanged in Adipoq-Cre/+;NOX4^{Flox/Flox} mice during the development of obesity.

A; Adipoq-Cre/+;NOX4^{+/+} and Adipoq-Cre/+;NOX4^{Flox/Flox} mice were fed HFHS for the indicated time periods (n=5). At sacrifice, the AE fraction from EWAT was harvested and analyzed for NOX activity. Data are representative of at least 3 independent experiments. *P<0.005 vs Adipoq-Cre/+;NOX4 +/+. ANOVA and Bonferroni post-hoc test. B; The AE and SVC fractions from EWAT of Adipoq-Cre/+;NOX4^{+/+} were isolated and analyzed by Western blotting using F4/80 and GAPDH antibody (n=3).