Supplemental Figure 1. Functional cholinergic signaling pathway in mouse adipose tissue. (A) Tissue distribution of α7nAChR. (B) Expression of α7nAChR in isolated adipocytes and ATMs. (C) Tissue distribution of AChE and BChE. n=4 in A-C. (D)-(F) BChE mRNA levels in epididymal adipose tissue (WAT) of chronic nicotine-treated (D), high-fat (HF) diet-fed (E) and db/db (F) mice. n=5 in D and F, and 5 and 8 for chow and HF, respectively in E. (G-H) BChE protein levels in WAT of chronic nicotine-treated (G) and db/db (H) mice. The bar graphs in G and H show BChE protein levels normalized to β-actin. In D and G, male 2-month-old B6 mice on chow diet were treated with 400μg/kg nicotine intraperitoneally (i.p.) twice daily for 3 weeks. In E, 6-week-old male B6 mice were put on chow or HF diet for 16 weeks. In F and H, 11-week-old db/db and lean control mice (+/−) were used. Total RNA was isolated and α7nAChR, AChE and BChE expression was measured by regular RT-PCR in A and real-time RT-PCR in B-F. BChE protein levels was detected by immunoblotting using polyclonal antibodies that specifically recognizes BChE (1). n=4 in G and 3 in H. Data are expressed as mean±SE. * p<0.05 vs. saline in D and G, chow in E and Lean mice in F and H. AChE: acetylcholinesterase. BChE: butyrylcholinesterase. MΦ: macrophage.

Supplemental Figure 2. Body weight, food intake and serum insulin levels in db/db and lean control mice treated with saline or nicotine. 8-week-old db/db and lean control mice on chow diet were treated (i.p.) with 400 ug/kg nicotine twice daily for 3 weeks. Body weight (A), food intake (B) and fed insulin (C) were measured. n=5. Data are expressed as mean±SE. In Fig A, *p<0.05 for saline and nicotine-treated lean groups vs. saline and nicotine-treated db/db groups. In Fig C, statistical significance is indicated by the presence of different superscripts. Groups labeled with the same superscripts are not statistically different from each other. Groups labeled with different superscripts are statistically different from each other.

Supplemental Figure 3. Body weight, fasting glucose levels and insulin signaling events in liver and fat in DIO mice treated with saline or nicotine. 6-week-old male C57BL/6 mice were put on chow or HF diet for 11 weeks followed by saline or nicotine (400 μg/kg) injection (i.p.) twice daily for 3 weeks. Body weight (A), fasting glucose levels (B) were measured. (C-D), Insulin signaling study was conducted at the end of 3-week saline or nicotine treatment by i.p. injection of 10 U/kg insulin into overnight-fasted mice. Liver and epididymal fat were collected 10 minutes later and phosphorylation of insulin receptor (IR) at Tyr1162-Tyr1163 and Akt/PKB at ser473, and total IR and Akt/PKB were measured by immunoblotting analysis. Bar graphs in Fig C and D show phosphorylation levels of IR and Akt/PKB normalized to total IR and Akt/PKB levels. In A-B, n=10 for chow-saline, 5 for HF-saline and 7 for HF-nicotine. In C-D, n=5 each for
chow-saline and HF-saline groups and 6 for HF-nicotine group. Data are expressed as mean±SE. In Fig A, *p<0.05 vs.
other two groups. In Fig B-D, statistical significance is indicated by the presence of different superscripts. Groups labeled
with the same superscripts are not statistically different from each other. Groups labeled with different superscripts are
statistically different from each other. Nic: nicotine. Ins: insulin.

Supplemental Figure 4. Activation of the cholinergic signaling pathway by nicotine suppresses adipose tissue
inflammation in DIO mice. The expression of TNFα, F4/80, MCP1, IL6, IL1β and iNOS in epididymal adipose tissue of
saline- or nicotine-treated DIO mice was measured by real-time RT-PCR. RNA expression levels were normalized to
cyclophilin. n=10 for each group. Data are expressed as mean±SE. Statistical significance is indicated by the presence of
different superscripts. Groups labeled with the same superscripts are not statistically different from each other. Groups
labeled with different superscripts are statistically different from each other. Nic: nicotine.

Supplemental Figure 5. α7nAChR mediates nicotine’s anti-inflammatory effect in peritoneal macrophages. (A)
TNFα levels in peritoneal macrophages from WT mice treated with vehicle (bovine serum albumin, BSA) or 500uM each
of myristic acid (C14:0), palmitic acid (C16:0) and stearic acid (C18:0). (B) TNFα, (C) IL1β and (D) IL6 expression in
WT and α7KO peritoneal macrophages treated with TNFα (10ng/ml) in the presence or absence of nicotine. The
expression of target genes was measured by real-time RT-PCR and normalized to cyclophilin. n=4 for each group in A
and n=3-4 for treatments in WT groups and 5-6 for treatments in α7KO in KO groups in B-D. Data are expressed as
mean±SE. Statistical significance is indicated by the presence of different superscripts. Groups labeled with the same
superscripts are not statistically different from each other. Groups labeled with different superscripts are statistically
different from each other. Nic: nicotine.

Supplemental Figure 6. Body weight, epididymal fat pad mass and food intake in WT and α7KO mice fed either
LF or HF diet. (A-B) Body weight of WT and α7KO mice on LF (A) or HF (B) diet. In A, n=7 for WT-LF, 5 for α7KO-
LF. In B, n=7 for WT-HF and 6 for α7KO-HF. (C) Epididymal fat pad mass and (D) food intake in WT and α7KO mice
on LF or HF diet. In C, n=5 for WT-LF and α7KO-LF, 6 for WT-HF and 5 for α7KO HF. In D, n=4. Data are expressed
as mean±SE. Statistical significance is indicated by the presence of different superscripts. Groups labeled with the same
superscripts are not statistically different from each other. Groups labeled with different superscripts are statistically
different from each other.

Supplemental Figure 7. MCP1 expression in adipocytes and ATMs isolated from WT and α7KO mice on LF and
HF diet. Adipocytes and ATMs were isolated from WT and α7KO mice as described in Materials and Methods. MCP1
expression in adipocytes and ATMs from mice on LF (A) and HF (B) diet was measured by real-time RT-PCR and normalized to cyclophilin. n=4 for each group. Data are expressed as mean±SE. *p<0.05 vs. WT.

Supplemental Figure 8. α7KO mice develop insulin resistance on LF diet. Fed insulin levels (A), GTT (B) and ITT(C) in 6-month-old male WT and α7KO mice fed LF diet. GTT was performed in overnight fasted mice with intraperitoneal injection of glucose at 1.5 g/kg of body weight. ITT was performed 4 hours after food removal in mice with intraperitoneal injection of insulin at 1.5 units/kg of body weight. n=7 for WT and 5 for α7KO in A-C. Data are expressed as mean±SE. *p<0.05 vs WT mice.

Supplemental Figure 9. Insulin signaling events and gene expression in liver of WT and α7KO mice on HF diet. (A) Insulin signaling study was conducted in 6-month-old male WT and α7KO mice on HF diet by intraperitoneal injection of 10 U/kg insulin into overnight-fasted mice. Liver was collected 10 minutes later and phosphorylation of IR at Tyr^1162-Tyr^1163 and total IR were measured by Western blotting analysis. Bar graph shows tyrosyl phosphorylation levels of IR normalized to total IR levels. (B) Expression of G6P and PEPCK in liver of 6-month-old male WT and α7KO mice on HF diet under overnight fasted state. The expression of target genes was measured by real-time RT-PCR and normalized to cyclophilin. n=4 for each group. Data are expressed as mean±SE. *p<0.05 vs WT mice.


The PRiMA-linked cholinesterase tetramers are assembled from homodimers: hybrid molecules composed of acetylcholinesterase and butyrylcholinesterase dimers are up-regulated during development of chicken brain. J Biol Chem 285:27265-27278
Supplemental Fig 2

A  Body Weight

[Graph showing body weight over weeks of treatment with saline and nicotine treatment groups, marked with asterisks (*) indicating significant differences.]

B  Food Intake

[Bar chart showing grams/mouse/day for saline and nicotine treatment groups, with error bars.]

C  Fed insulin

[Bar chart showing ng/ml for saline and nicotine treatment groups, with letters indicating significant differences.]

Legend:
- Lean
- db/db
- Nic - +
- - +

Note: The figure illustrates the effect of saline and nicotine treatment on body weight, food intake, and fed insulin levels in lean and db/db mice.
Supplemental Fig 3

A  Body Weight

B  Fasting glucose

C  Liver

D  FAT

% of WT

Saline  Saline  Nic
Chow    HF

% of WT

Saline  Saline  Nic
Chow    HF

% of WT

Saline  Saline  Nic
Chow    HF

% of WT

Saline  Saline  Nic
Chow    HF
Supplemental Fig 6

A. Body weight

B. Body weight

C. WAT

D. Food Intake

Grams

Time (weeks)

Grams/mouse•day

Supplemental Fig 6
Supplemental Fig 7

A.

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B.

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* Significant difference compared to WT and α7KO.
Supplemental Fig 8

A. Fed insulin

B. GTT

C. ITT

Supplemental Fig 8
Supplemental Fig 9

A

Liver

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% of WT

WT α7KO

B

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