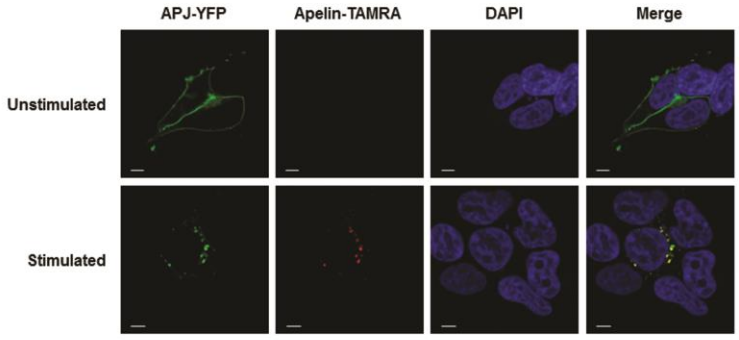
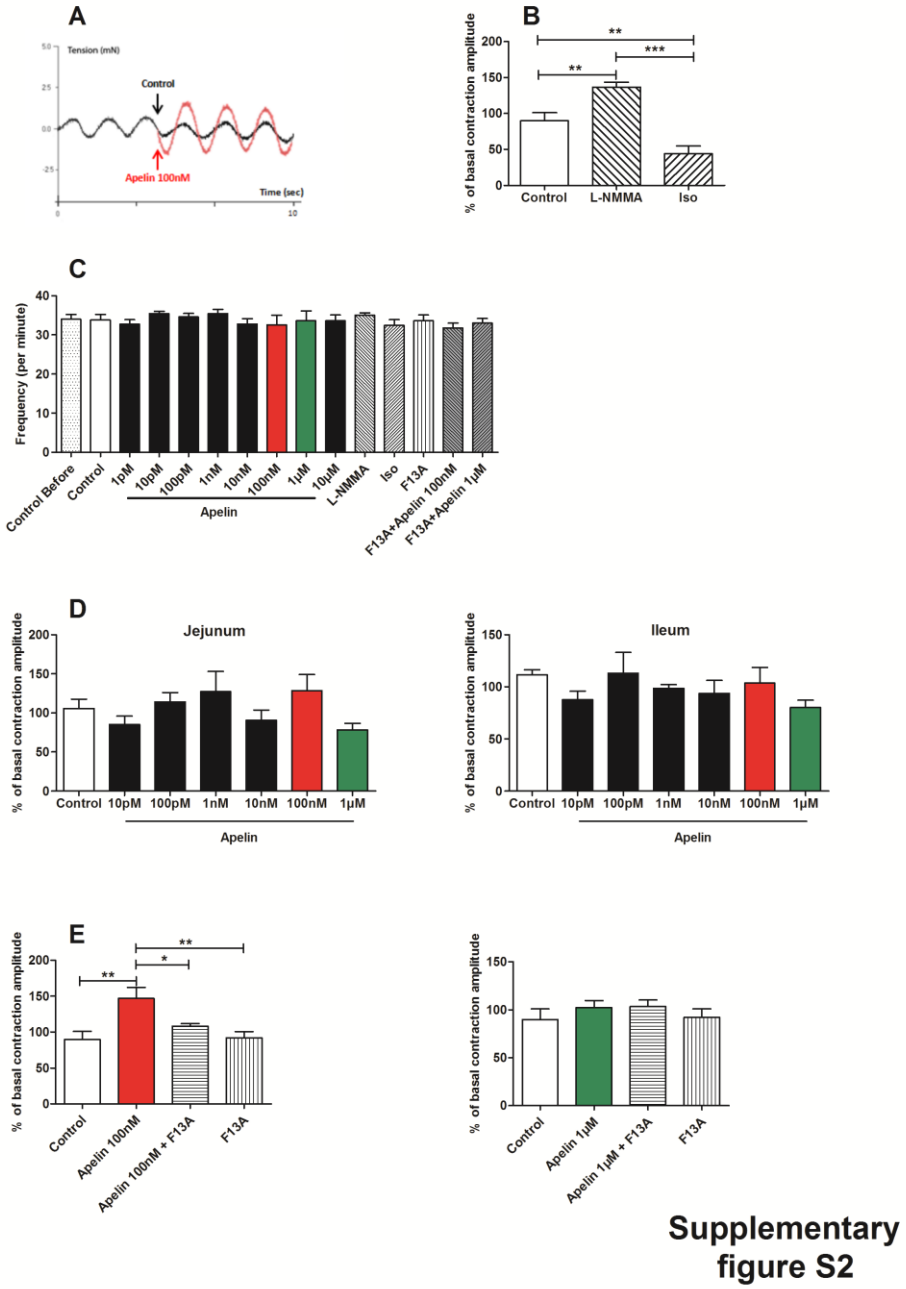
Supplementary legends

Supplemental figure S1. Apelin-TAMRA is functional and induces apelin receptor internalization. HEK-293T cells transiently expressing YFP tagged APJ were incubated for 1 hour with: no drug (Unstimulated) or 100nM Apelin-TAMRA (Stimulated). Representative images of each condition showing native YFP fluorescence (left panels, green), TAMRA fluorescence (middle left panels, red), DAPI (middle right panels, blue) and Merge (right panels). Representative pictures of each condition are shown (n=4). Bars = 10 µm.



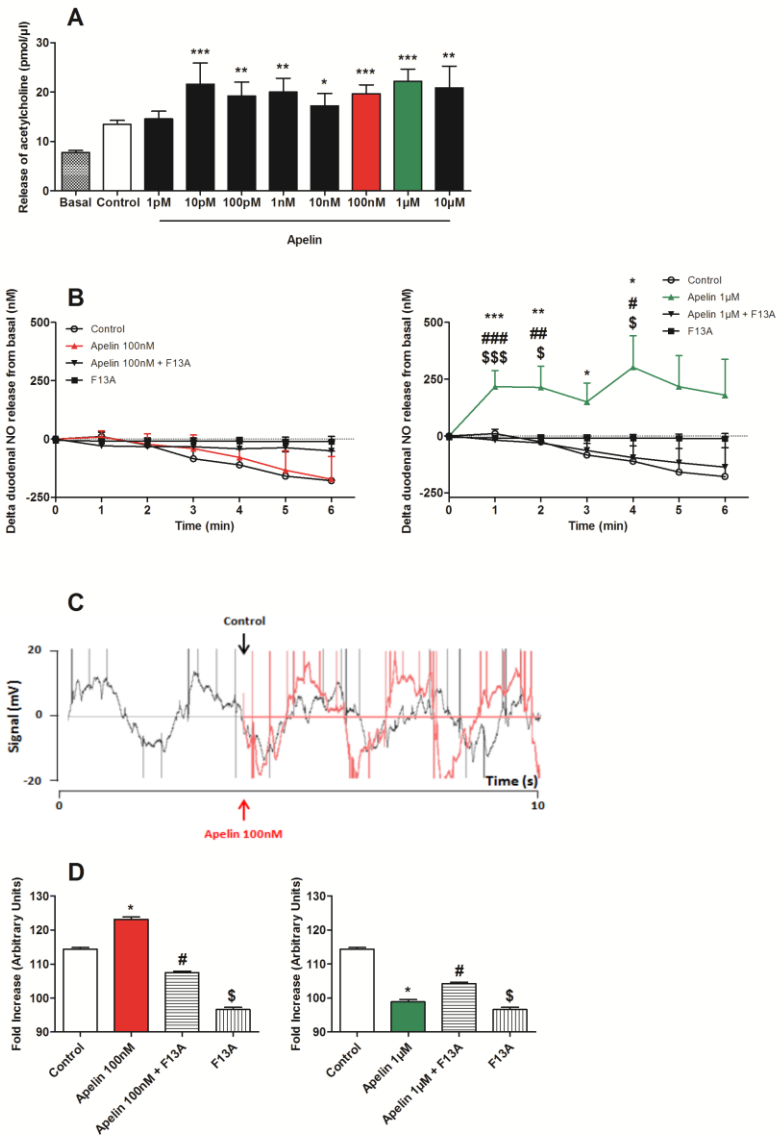
Supplementary figure S1

Supplemental figure S2. Apelin modulates duodenal contractions. (A) Representative recording of duodenal mechanical contractions in response to Krebs-Ringer (Control) or Apelin 100nM. (B) Ex vivo measurement of duodenal mechanical contractions amplitude in response to Krebs-Ringer (Control), L-NMMA or Iso (Isoproterenol). n=5-6 per group. **P<0.01, ***P<0,001. (C) Ex vivo measurement of duodenal mechanical contractions frequency before injection (Control Before) and in response to Krebs-Ringer (Control), increasing concentrations of Apelin (1pM-10µM), L-NMMA, Iso (Isoproterenol) and F13A alone or F13A plus Apelin (100nM or 1µM). n=4-6 per group. (D) Ex vivo measurement of jejunal (left panel) and ileal (right panel) mechanical contractions amplitude in response to Krebs-Ringer (Control) or increasing concentrations of Apelin (10pM-1µM). n=4-7 per group. (E) Ex vivo measurement of duodenal mechanical contractions amplitude in response to H20 (Control), Apelin 100nM (left panel) or 1µM (right panel), F13A or combination of Apelin plus F13A. n=5-6 per group. *P<0.05, **P<0.01.



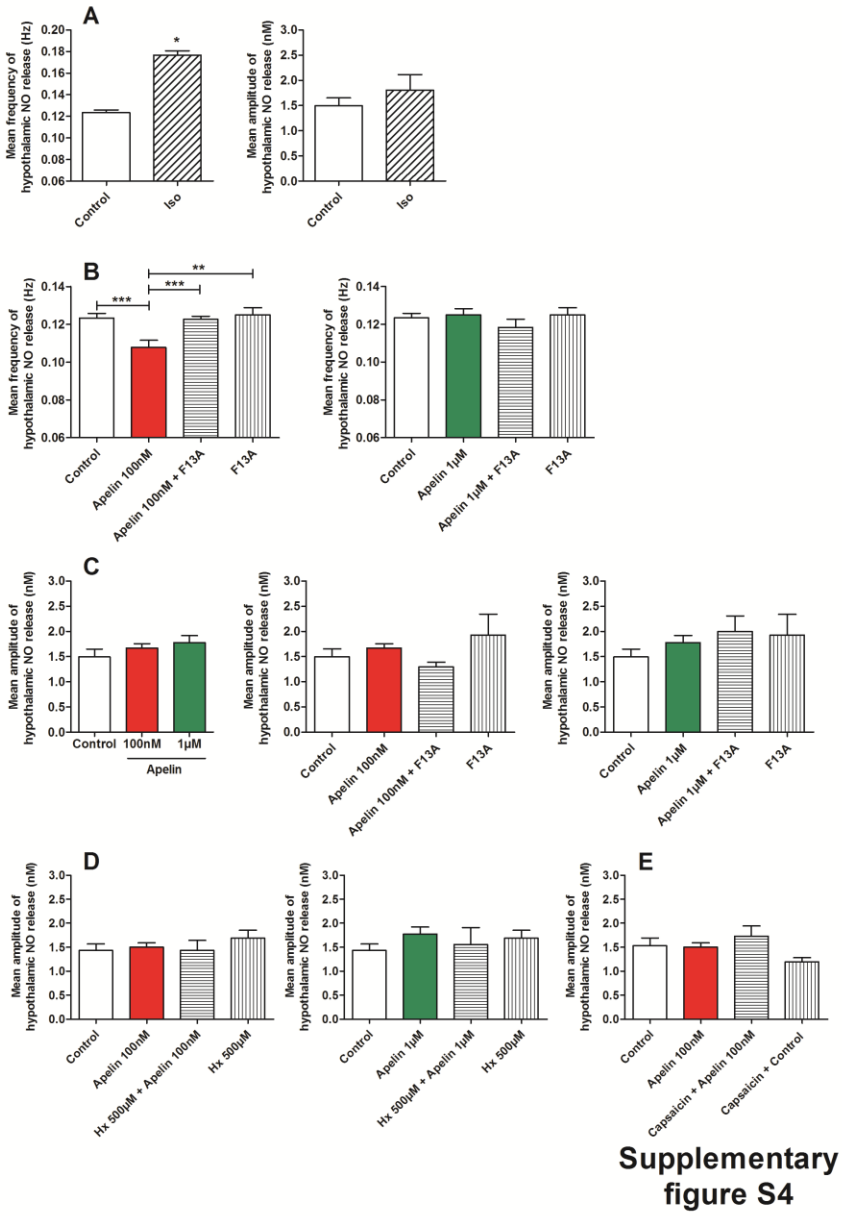
Supplementary figure S2

Supplemental figure S3. Apelin acts on the ENS to modulate duodenal contractions. (A) Ex vivo measurement of duodenal acetylcholine release in basal condition and in response to Krebs-Ringer (Control) or increasing concentrations of Apelin (1pM-10µM). n = 5-13 per group. *P < 0.05, **P < 0.01, ***P < 0.001 vs. Basal. (B) Duodenal NO release in response to Krebs-Ringer (Control), Apelin 100nM (left panel) or 1µM (right panel), F13A or combination of Apelin plus F13A. n=5-8 per group. *P<0.05, **P<0.01, ***P<0.001 vs. Control. #P<0.05, ##P<0.01, ###P<0.001 vs. F13A. §P<0.05, §§§P<0.001 vs. F13A + Apelin 1µM. (C) Representative recording of duodenal electrical activity in response to Krebs-Ringer (Control) or Apelin 100nM. (D) In vivo measurement of duodenal electrical activity in response to H20 (Control), Apelin 100nM (left panel) or 1µM (right panel), F13A or combination of Apelin plus F13A. n=4-7 per group. Left panel: *P<0,001 vs. Control, vs. Apelin 100nM + F13A and vs. F13A. #P<0.001 vs. Control, vs. Apelin 100nM and vs. F13A. $P<0.001 vs. Control, vs. Apelin 100nM and vs. Apelin 100nM + F13A. Right panel: *P<0,01 vs. Control, vs. Apelin 1µM + F13A and vs. F13A. #P<0.01 vs. Control, vs. Apelin 1µM and vs. F13A. $P<0.01 vs. Control, vs. Apelin 1µM and vs. Apelin 1µM + F13A.



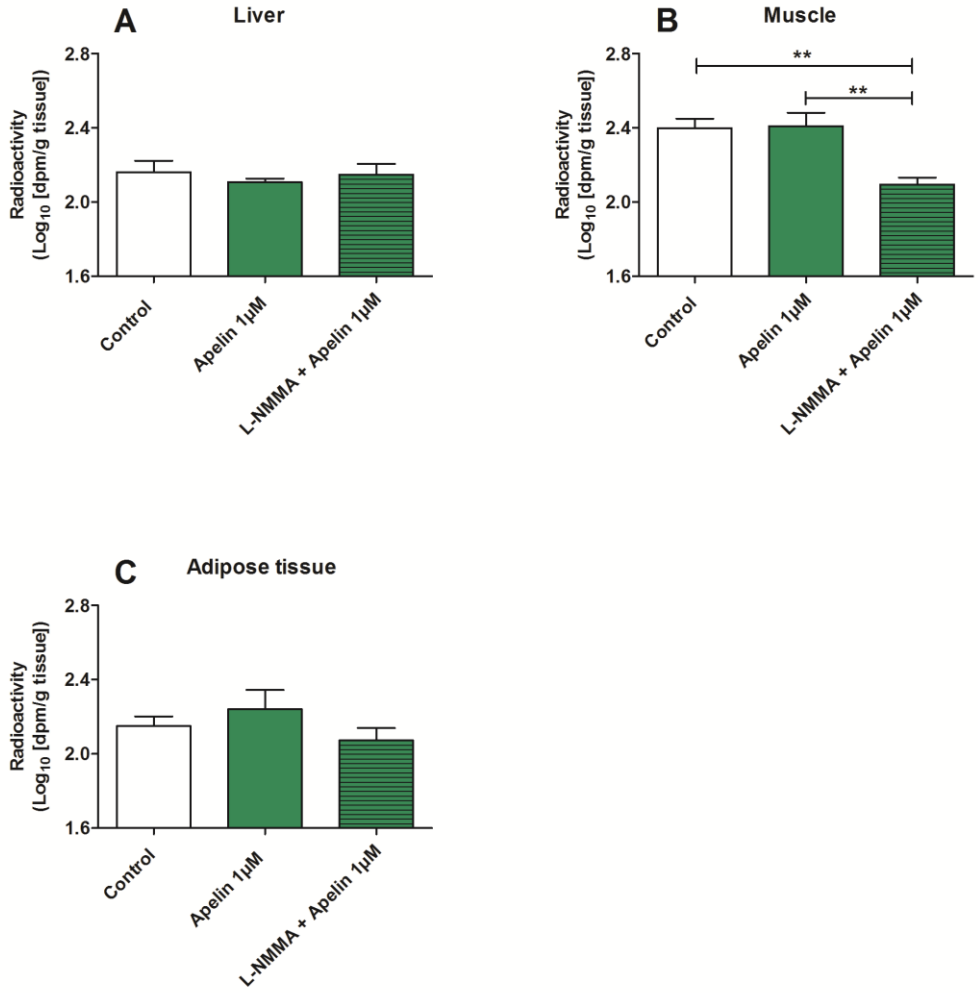
Supplementary figure S3

Supplemental figure S4. Duodenal apelin modulates NO release in the hypothalamus. (A) In vivo effect of intragastric perfusion of H20 (Control) or Isoproterenol (Iso) on NO hypothalamic release frequency (left panel) and amplitude (right panel). n=5-7 per group. *P<0.05 vs. Control. (B) In vivo effect of intragastric perfusion of H20 (Control), Apelin 100nM (left panel) or 1µM (right panel), F13A or combination of Apelin plus F13A. n=4-7 per group. **P<0,01, ***P<0,001. (C) In vivo effect of intragastric perfusion of H20 (Control), Apelin 100nM alone or with F13A, Apelin 1µM alone or with F13A, or F13A alone, on hypothalamic NO release amplitude. n=4-7 per group. (D) In vivo effect of intragastric perfusion of H20 (Control), Apelin 100nM (left panel) or 1µM (right panel), Hexamethonium 500µM (Hx 500µM) or combination of Apelin plus Hexamethonium 500µM, on hypothalamic NO release amplitude. n=5-8 per group. (E) In vivo effect of intragastric perfusion of H20 (Control) and Apelin 100nM in mice pretreated or not by Capsaicin on hypothalamic NO release amplitude. n=4-7 per group.



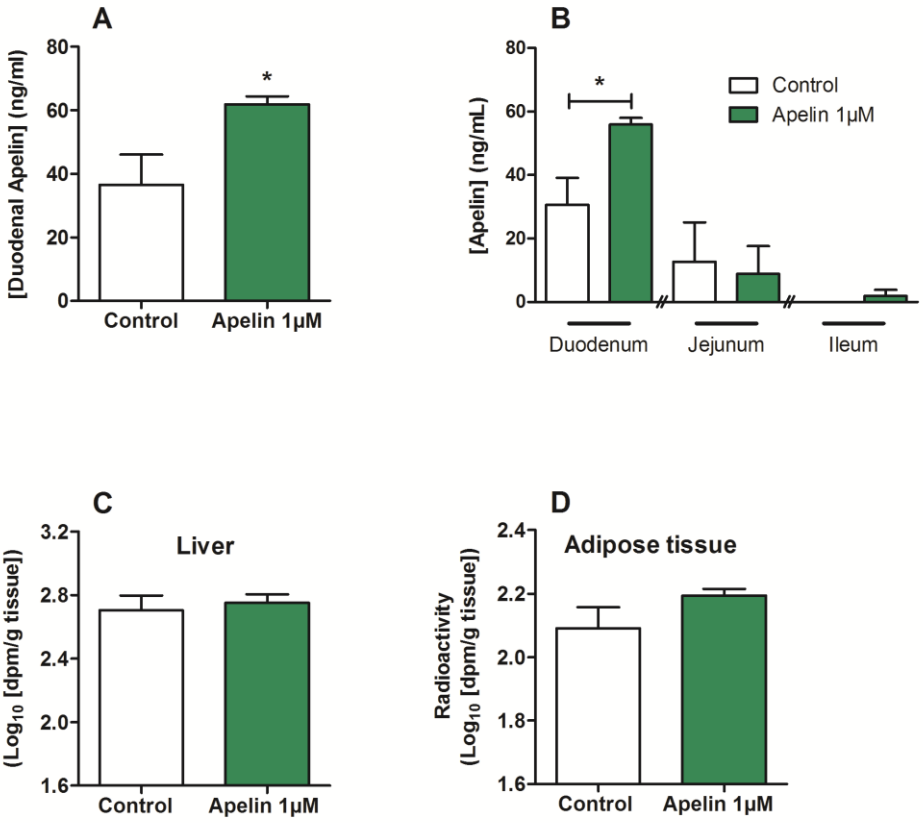
Supplementary figure S4

Supplemental figure S5. Hypothalamic NO mediates the effects of intestinal Apelin in muscle glucose utilization. In vivo measurement of glucose entry in liver (A), muscle (B) and adipose tissue (C) in response to oral gavage of radioactive glucose in combination with H20 (Control) or Apelin 1µM after an icv injection of aCSF (Control) or L-NMMA. n=8-12 per group. **P<0.01.



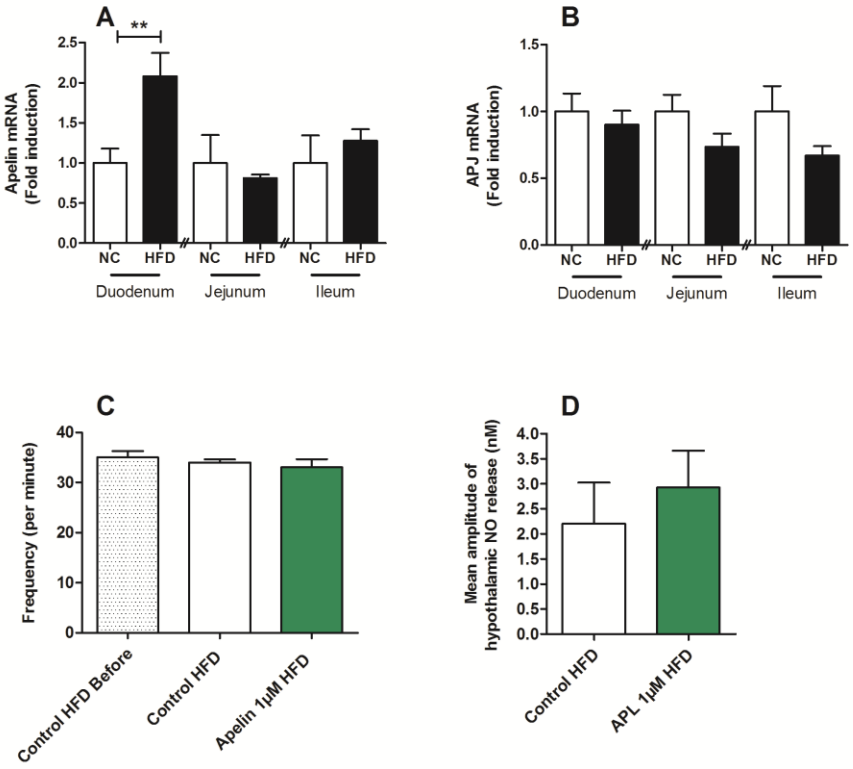
Supplementary figure S5

Supplemental figure S6. (A) Ex vivo measurement of duodenal Apelin of everted duodenal sacs incubated in Krebs-Ringer with 10g/L of glucose alone (Control), or with Apelin 1µM. n=5 per group. *P<0.05 vs. Control. (B) In vivo measurement of duodenal, jejunal and ileal Apelin in response to oral gavage of glucose with H2O (Control) or Apelin 1µM. n=4 per group. *P < 0.05. In vivo measurement of glucose entry in liver (C) and adipose tissue (D) in response to oral gavage of radioactive glucose, in mice orally gavaged for one week with H20 (Control) or Apelin 1µM. n=7 per group. *P<0.05 vs. Control.



Supplementary figure S6

Supplemental figure S7. Relative expression of Apelin (A) and APJ (B) mRNA in duodenum, jejunum and ileum of NC or HFD mice. n=5-6 per group. **P<0.01. (C) Ex vivo measurement of duodenal mechanical contraction frequency before injection (Control HFD Before) and in response to Krebs-Ringer (Control HFD) or Apelin 1µM in HFD mice. n=5-6 per group. (D) In vivo effect of intragastric perfusion of H20 (Control HFD) or Apelin 1µM, on NO hypothalamic release amplitude in HFD mice. n=4-6 per group.



Supplementary figure S7