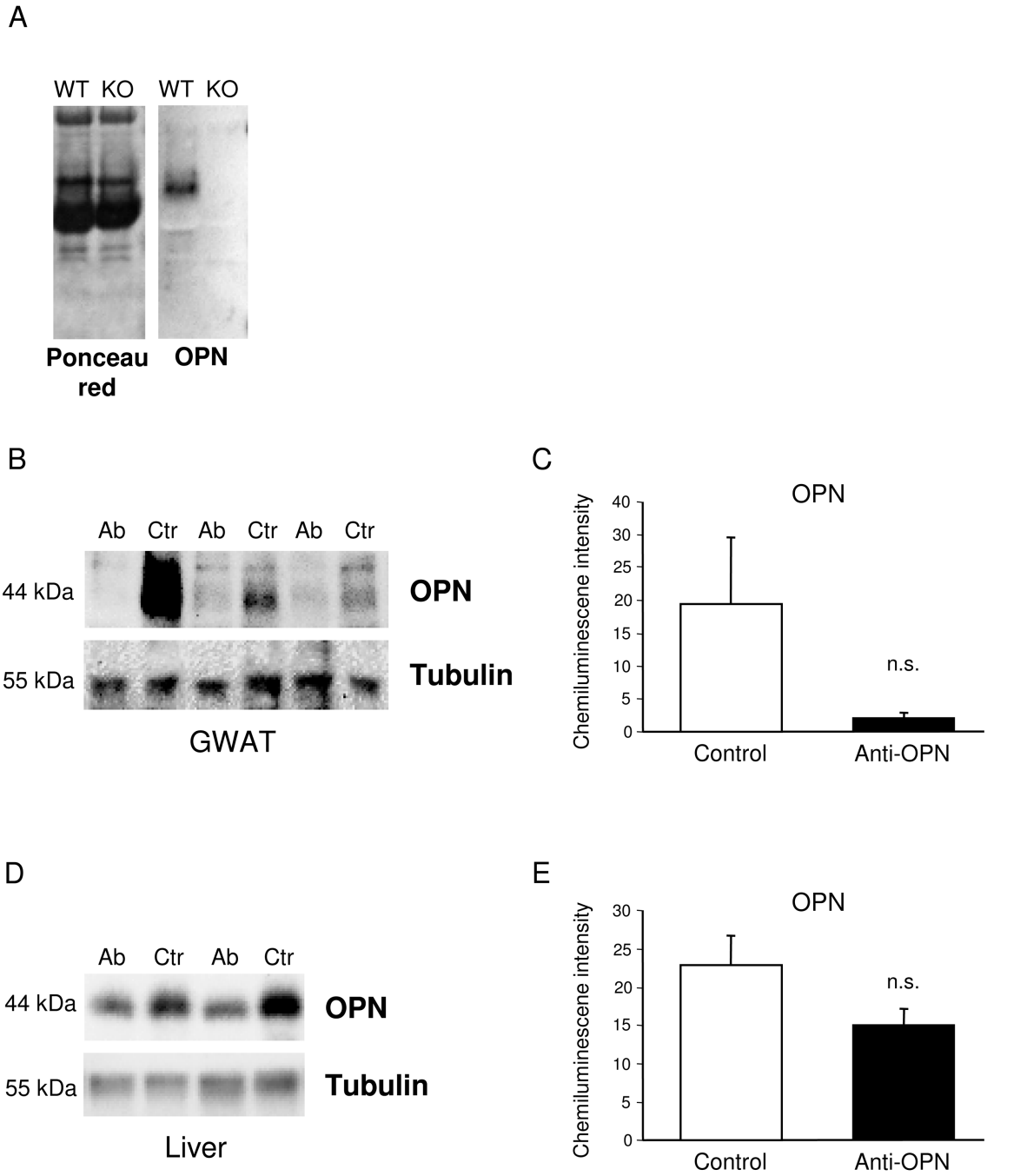
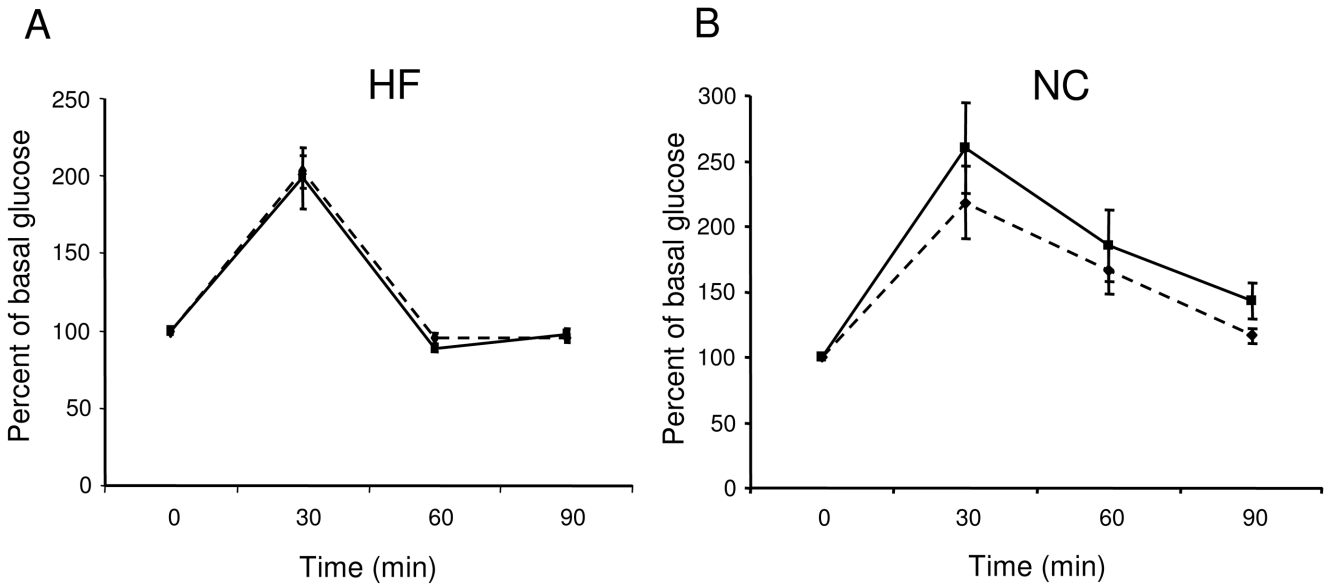
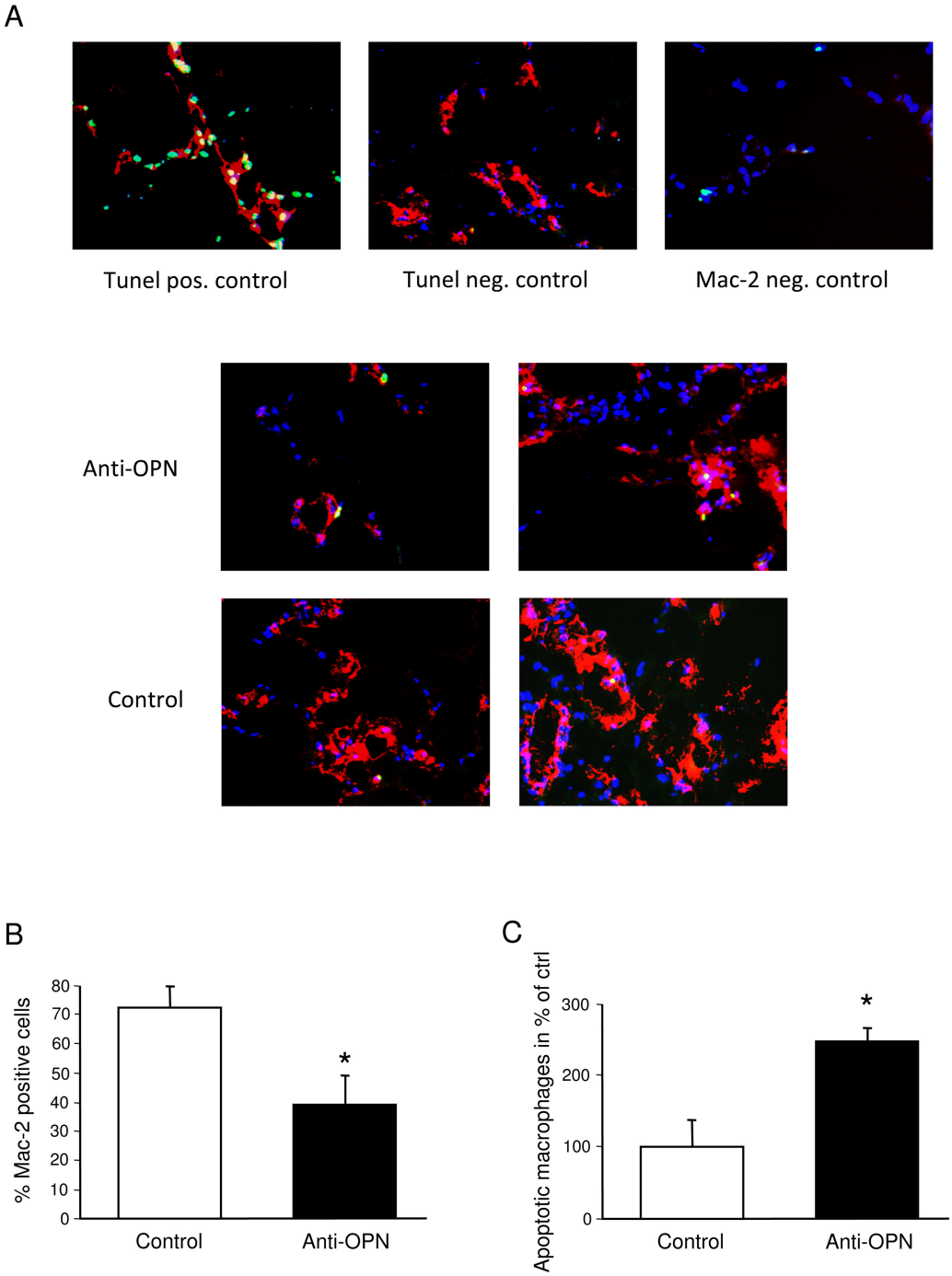
Supplemental Figure 1. Immunoblot analysis of OPN in plasma and GWAT. **(A)** Plasma of wild type and OPN deficient knockout mice (KO; Spp1-/-, purchased from Charles River Laboratories) was analyzed for OPN protein to test antibody specificity. A representative immunoblot and the respective Ponceau red control are given. **(B)** GWAT of HF-fed anti-OPN (Ab) and control antibody-treated mice (Ctr) was analyzed for OPN protein. A representative immunoblot is given together with a loading control (tubulin). **(C)** Quantification of OPN protein in GWAT. The diagram shows means of the chemiluminescence intensity. **(D)** Liver of HF-fed anti-OPN (Ab) and control antibody-treated mice (Ctr) was analyzed for OPN protein. A representative immunoblot is given together with a loading control (tubulin). **(E)** Quantification of OPN protein in liver. The diagram shows means of the chemiluminescence intensity. n.s. = not statistically significant.



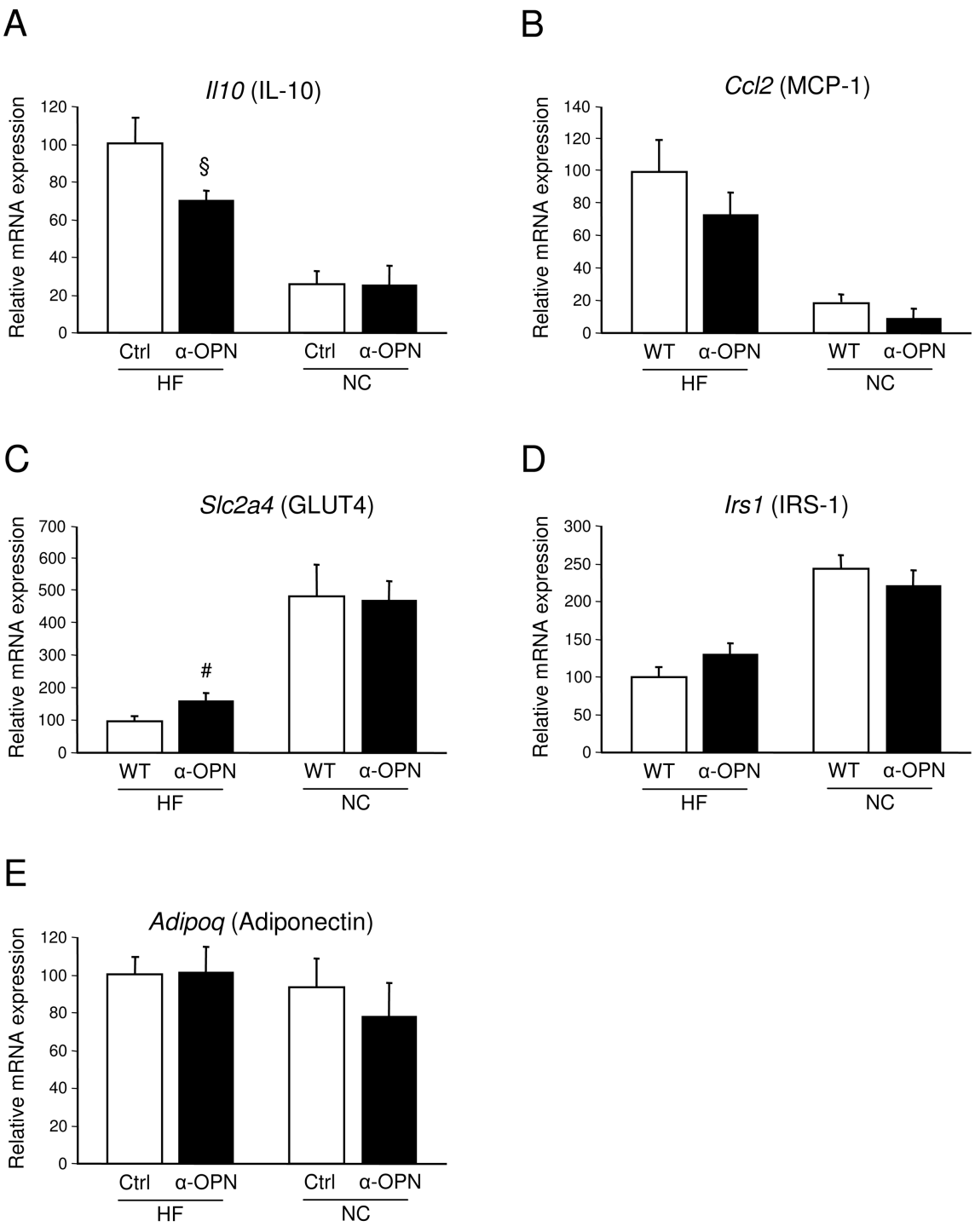
Supplemental Figure 2. Glucose tolerance is unaltered upon OPN neutralization. Mice were fed HF and NC, respectively, for 24 weeks and were treated intravenously with an OPN neutralizing (Anti-OPN) or control antibody three times during five days at the end of the feeding period. **(A-B)** A GTT was performed in lean and obese OPN antibody (dashed lines) and control antibodytreated mice (solid lines) two days after the last antibody application (n = 8 per group for HF and n = 5 per group for NC) and glucose concentrations were expressed as percent of basal levels.



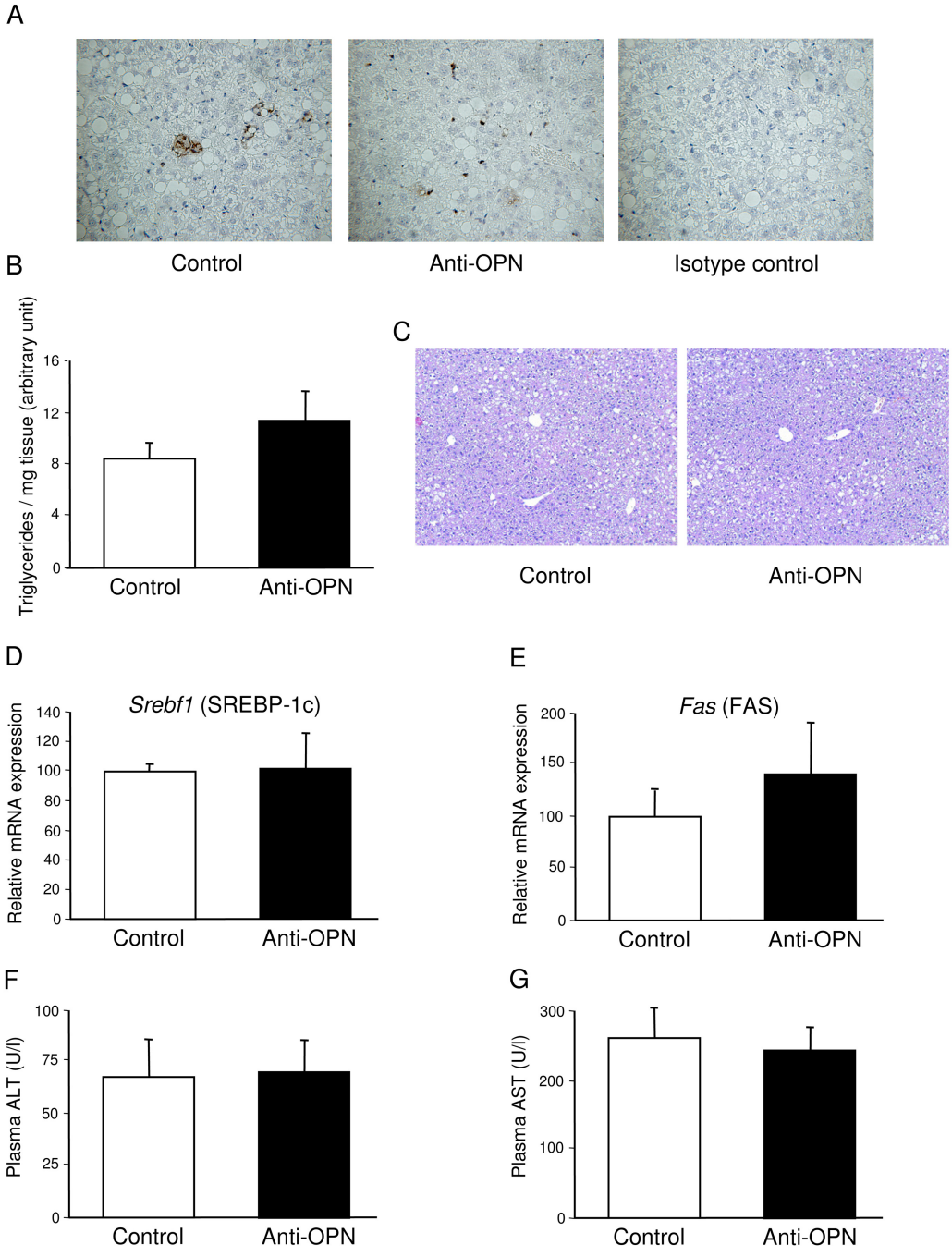
Supplemental Figure 3. Adipose tissue macrophage accumulation and apoptosis following OPN neutralization in obese mice. Obese HF-fed mice were treated with OPN neutralizing (Anti-OPN) or control antibody (n = 8 per group). **(A)** As an alternative to F4/80 staining (Fig. 2) adipose tissue macrophage accumulation was determined by immunofluorescence of Mac-2+ macrophages in GWAT isolated from HF-fed mice after anti-OPN or control treatment. Apoptotic cells were determined by tunel staining (green), macrophages were stained red by immunoflourescence using anti-Mac-2 monoclonal antibody on frozen sections. **(A)** Representative pictures and respective positive and negative control stainings are given in 60-fold magnification. **(B)** Adipose tissue macrophages were counted as Mac-2+ cells relative to total number of cells. **(C) Q**uantification of apoptotic macrophages (Tunel and Mac-2 doublepositive cells per Mac-2-positive cells). *P _ 0.05.



Supplemental Figure 4. Adipokine expression in anti-OPN (_-OPN) treated mice. Obese HF- and lean NC-fed mice were treated with OPN neutralizing (Anti-OPN) or control antibody (n = 8 per group for HF and n = 5 per group for NC). **(A-C)** mRNA expression of the adipokines IL-10 **(***Il10*; **A),** MCP-1 **(***Ccl2*; **B)**, GLUT4 (*Slc2a4;* **C**), IRS-1 (*Irs1;* **D**) and adiponectin (*Adipoq*; **E**) was analyzed in GWAT. The mean of Ctrl HF was set to 100%. §P = 0.06, #P = 0.08.



Supplemental Figure 5. Short-term OPN neutralization does not affect hepatic steatosis. Obese HF- and lean NC-fed mice were treated with OPN neutralizing (Anti-OPN) or control antibody (n = 8 per group for HF and n = 5 per group for NC). **(A)** Paraffin-embedded liver sections were immunohistochemically stained with the macrophage marker Mac-2 (brown). Representative pictures are given in 40-fold magnification. **(B)** Hepatic triglyceride content was determined by an enzymatic test following lipid extraction. **(C)** Hematoxylin-eosin staining was performed in paraffin sections of livers isolated from HF-fed mice after anti-OPN or control treatment. Representative pictures are given in 10-fold magnification. **(D-E)** Hepatic mRNA expression of the lipogenic makers SREBP-1c (*Srebf1*; **D),** FAS (*Fas*; **E)** was analyzed by real-time RT-PCR. The mean of Control was set to 100%. **(F-G)** Serum concentrations of the hepatic enzymes ALT and AST were measured in obese antibody- and control-treated mice.



Supplemental Table 1. Body characteristics

Parameter	Control NC	Anti-OPN NC	Control HF	Anti-OPN HF
Body weight before (g)	33.0 ± 0.8	32.6 ± 0.5	51.8 ± 1.8	51.5 ± 0.7
Body weight after (g)	32.2 ± 0.4	32.0 ± 0.6	50.1 ± 1.7	50.4 ± 0.7
Liver weight (g)	1.84 ± 0.12	1.66 ± 0.1	2.02 ± 0.21	2.32 ± 0.25
Fat pad weight (g)	0.27 ± 0.06	0.25 ± 0.06	1.83 ± 0.23	1.62 ± 0.11
Food intake/mouse/day (g)	3.55 ± 0.23	3.25 ± 0.33	2.75 ± 0.14	2.86 ± 0.2

After feeding NC or HF for 24 weeks mice were treated with OPN neutralizing IgG or control IgG. Body weight was determined before and after treatment. Liver and omental fat pad weight were measured immediately after sacrifice. Food intake was monitored during treatment and is given in gram per mouse per day. (n = 5 – 8 per group). Data are expressed as mean ± SEM.

Supplemental Table 2. Plasma measurements

Parameter	Control NC	Anti-OPN NC	Control HF	Anti-OPN HF
Glucose (mg/dl)	175 ± 5.3	170.7 ± 11.7	332.9 ± 18.5	304.0 ± 23.7
Glucose (overnight, mg/dl)	101.8 ± 5.5	109.4 ± 6.2	171.4 ± 7.9	187.1 ± 7.4
Insulin (µU/ml)	2.46 ± 1.8	1.7 ± 0.7	48.4 ± 9.6	30.5 ± 5.3
Cholesterol (mg/dl)	1.1 ± 0.8	0.7 ± 0.3	98.1 ± 5.5	96.0 ± 7.8
Triglycerides (mg/dl)	60.2 ± 4.4	50.2 ± 3.0	62.6 ± 11.5	42.4 ± 1.9$^{\#}$
Free fatty acids (µmol/l)	162.6 ± 12.5	131.6 ± 15.4	309.7 ± 39.7	282.3 ± 17.9
hs CRP (ng/ml)	n.d.	n.d.	118.9 ± 3.5	104.0 ± 5.7$^{\#}$
Adiponectin (µg/ml)	n.d.	n.d.	38.8 ± 9.1	41.9 ± 10.6
Leptin (ng/ml)	n.d.	n.d.	43.3 ± 4.8	38.0 ± 3.9
IL-6 (pg/ml)	n.d.	n.d.	17.2 ± 6.2	12.2 ± 3.2
TNF-α (pg/ml)	n.d.	n.d.	3.1 ± 0.5	4.1 ± 2.8
Serum amyloid P (ng/ml)	36.5 ± 6.4	29.6 ± 5.8	86.0 ± 24.4	30.9 ± 8.1*
OPN (ng/ml)	180.5 ± 23.1	156.7 ± 13.0	180.5 ± 22.7	171.3 ± 11.9

After feeding a NC or HF for 24 weeks mice were treated with OPN neutralizing IgG or control IgG. Blood samples were obtained after a three hour fasting period (or after overnight fasting if indicated) and analyzed for depicted plasma parameters (n = 5 – 8 per group). Data are expressed as mean ± SEM. *P ≤ 0.05, $^{\#}$P = 0.09 compared to Control HF.