## LEGENDS

**Figure 1. Palmitoleic acid restores basal and glucose-stimulated insulin sensitivity in HFD-fed mice.** Body weight change during high fat diet (HFD)-feeding (A), adipose depot weights (B), fasting glucose, insulin levels and HOMA-IR (C), glucose levels during insulin tolerance test (ITT) and respective glucose clearance constant (KITT) (D), glucose changes during glucose tolerance test (GTT) and respective area under curve (AUC) (E). Wild-type (WT) mice fed with a standard diet (SD) or high-fat diet treated with oleic acid (HFD OA) or palmitoleic acid (HFD POA). Data are presented as the mean ± SEM. *p<0.05, **p<0.01, ***p<0.001 ****p<0.0001 *vs.* WT SD; #p<0.05 HFD POA *vs.* HFD OA. (One-way ANOVA followed by Bonferroni correction).

**Figure 2. Palmitoleic acid induces AMPK activation and reduces lipid accumulation in liver in HFD-fed mice.** Glucose production on pyruvate tolerance test (PTT) (A), liver weight and blood levels of aspartate transaminase (AST) and alanine aminostrasferase (ALT) (B), histological slices of livers stained with hematoxylin and eosin at 40 x magnification (C), phosphorylation of AMPK (p-thr172) normalized by the respective total protein (AMPK) and by α-actin (D). Wild-type (WT) mice fed with a standard diet (SD) or high-fat diet treated with oleic acid (HFD OA) or palmitoleic acid (HFD POA). Data are presented as the mean ± SEM. *p<0.05, **p<0.01, ***p<0.001 *vs.* WT SD; #p<0.05 HFD POA *vs.* HFD OA. (One-way ANOVA followed by Bonferroni correction).

**Figure 3. POA was successfully incorporated into liver but lipidomics does not show a clear segregation of the HFD-fed mice with or without POA treatment.** Incorporation of fatty acids into the liver measured by gas chromatography (A) and lipidomic profile (B) of liver from wild-type (WT) mice fed with a standard diet (SD) or high-fat diet treated with oleic acid (HFD OA) or palmitoleic acid (HFD POA). The data are presented as the mean ± SEM. *p<0.05, **p<0.01, ***p<0.001 ****p<0.0001 *vs.* indicated groups. (One-way ANOVA followed by Bonferroni correction).

**Figure 4. Palmitoleic acid promotes anti-inflammatory effects in liver of HFD-fed mice by modulation of the liver macrophages population.** Monocyte chemoattractant protein **(**MCP)-1, interleukin (IL)-1β and tumor necrosis factor (TNF)-α levels in liver, hepatocytes and liver macrophages (LM) (A), flow cytometry gate strategy (B) and % F4/80, CD11c, CD86 and CD206 positive LM (C), relative mRNA expression of inflammation-related genes in LM. Wild-type (WT) mice fed with a standard diet (SD) or high-fat diet treated with oleic acid (HFD OA) or palmitoleic acid (HFD POA). Ct were normalized to B2M. The data are presented as the mean ± SEM. *p<0.05 *vs.* indicated groups. (One-way ANOVA followed by Bonferroni correction).

**Figure 5. Palmitoleic acid increases PPAR-γ in liver and restores HFD-related diabetes only in WT mice, not in macrophage-specific PPAR-γ KO mice.** Liver protein levels of peroxisome proliferator activated receptor (PPAR)-α, PPAR-β, PPAR-γ normalized by the respective a-actin levels (A), scheme for generation of specific myeloid cell PPAR-γ knockout mice showing that PPAR-γ Flox mice were crossed with Lysozyme

M-Cre (LysCre) mice (B), body weight change during high fat diet (HFD) feeding and adipose tissue weight (C), glucose levels during glucose tolerance test (GTT) and respective area under curve (AUC) (D), glucose levels during insulin tolerance test (ITT) and respective glucose clearance constant (KITT) (E). Wild-type (WT) mice fed with a standard diet (SD) or high-fat diet treated with oleic acid (HFD OA) or palmitoleic acid (HFD POA) (A), WT(Cre-) or PPAR-γ KO (Cre+) mice fed with high-fat diet and treated with oleic acid (HFD OA) or palmitoleic acid (HFD POA) (C, D and E). Data are presented as the mean ± SEM. *p<0.05 *vs.* indicated groups. (One-way ANOVA (A) or two-way ANOVA (C, D and E) followed by Bonferroni correction).

**Figure 6. Palmitoleic acid reduces liver inflammation by modulation of the liver macrophages population and independently of macrophage-specific PPAR-γ knockout.** Interleukin (IL)-6, monocyte chemoattractant protein (MCP)-1 and tumor necrosis factor (TNF)-α levels in liver and hepatocytes (A), flow cytometry gate strategy and % F4/80, CD86 and CD206 positive liver macrophages (LM) (C), relative mRNA expression of inflammation-related genes in LM normalized by B2M. Wild-type (WT) (Cre-) or PPAR-γ KO (Cre+) mice fed with high-fat diet treated with oleic acid (HFD OA) or palmitoleic acid (HFD POA). Data are presented as the mean ± SEM. *p<0.05 *vs.* indicated groups. (Two-way ANOVA followed by Bonferroni correction).

**Figure 7. Palmitoleic acid reduces TLR4 activation in liver of HFD-fed mice and the structural prediction model indicates palmitoylation plays a role in that interaction.** Hepatic protein levels of toll-like receptor (TLR)-4 normalized by the respective α-actin

levels (A), Docking analysis of TRL-4/MD-2 complex with (9Z)-hexadec-9-enoic acid and derivates (B) and energy of binding (ΔG) to the disruptive potential of different fatty acids and TLR-4 antagonist to block TLR4-MD2 dimer formation and interaction. Livers of WT mice fed with a standard diet (SD) or high-fat diet treated with oleic acid (HFD OA) or palmitoleic acid (HFD POA). Data are presented as the mean ± SEM. $^{\#}p<0.05$ *vs.* indicated groups. (One-way ANOVA followed by Bonferroni correction).