Intestinal PPARδ protects against diet-induced obesity, insulin resistance and dyslipidemia

Marcela Doktorova[1*], Irene Zwarts[1*], Tim van Zutphen[1], Theo H. van Dijk[2], Vincent W. Bloks[1], Liesbeth Harkema[3], Alain de Bruin[1,3], Michael Downes[4], Ronald M. Evans[4], Henkjan J. Verkade[1] and Johan W. Jonker[1#]

[1]Section of Molecular Metabolism and Nutrition, Department of Pediatrics and [2]Department of Laboratory Medicine, University of Groningen, University Medical Center Groningen, Hanzeplein 1, 9713 GZ Groningen, The Netherlands. [3]Dutch Molecular Pathology Center, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 1, 3584 CL Utrecht, The Netherlands. [4]Howard Hughes Medical Institute and Gene Expression Laboratory, Salk Institute for Biological Studies, 10010 North Torrey Pines Road, La Jolla, California 92037, USA. [#]To whom correspondence should be addressed. E-mail: j.w.jonker@umcg.nl. [*]These authors contributed equally to this work.

Supplementary data

Supplementary Table 1

	Proximal		Middle		Distal	
LFD	wild-type	*PPARδ*$^{IEC\text{-}KO}$	wild-type	*PPARδ*$^{IEC\text{-}KO}$	wild-type	*PPARδ*$^{IEC\text{-}KO}$
Villus length (µm)	398 ± 14	412 ± 73	202 ± 26	224 ± 27	197 ± 13	190 ± 22
Crypt depth (µm)	58 ± 5	64 ± 5	69 ± 8	62 ± 3	71 ± 11	67 ± 10
Inflammation scoring	0	0	0	0	0	0
HFD	wild-type	*PPARδ*$^{IEC\text{-}KO}$	wild-type	*PPARδ*$^{IEC\text{-}KO}$	wild-type	*PPARδ*$^{IEC\text{-}KO}$
Villus length (µm)	410 ± 35	383 ± 66	266 ± 70	329 ± 79	212 ± 66	242 ± 40
Crypt depth (µm)	71 ± 9	64 ± 8	69 ± 13	69 ± 4	67 ± 12	73 ± 14
Inflammation scoring	0	0	0	0	0	0

Supplementary Table 1. Histopathologic analysis of HE stained intestinal sections of wild-type and *PPARδ*$^{IEC\text{-}KO}$ mice after a LFD (n = 3) or a HFD (n = 8). Values are presented as mean ± SD. Inflammation scoring: 0 = no inflammation, 1 = mild inflammation, 2 = moderate inflammation, 3 = severe inflammation.

Supplementary Table 2

HFD	wild-type	*PPARδ*$^{IEC\text{-}KO}$
Sum of steatosis	0.5 [0-2]	2 [0-3] *
Lobular inflammation	0 [0-1]	0.75 [0-2]
Ballooning	0 [0-1]	0 [0-1]
NAFLD activity score	0.5 [0-3.5]	3 [0-5] *

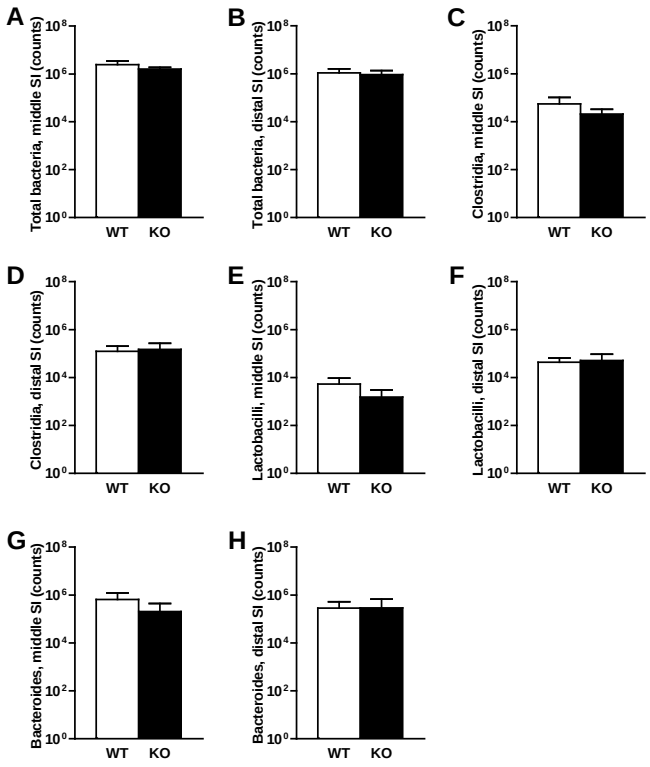
Supplementary Table 2. Liver composition of wild-type and *PPARδ*$^{IEC\text{-}KO}$ mice fed a HFD. Values are presented as median; [range] (n = 8). Steatosis grade 0= <5%; 1 = 5-33%; 2 = 33-66%; 3 = >66%. Lobular inflammation 0= none; 1 = <2 foci per 200x; 2 = 2-4 foci. Ballooning 0 = none; 1 = few; 2 = prominent ballooning. NAS = NAFLD activity score (sum of steatosis + lobular inflammation + ballooning). *Significant difference between wild-type and *PPARδ*$^{IEC\text{-}KO}$ mice ($p<0.05$).

Supplementary Table 3

Gene	Forward primer 5' --- 3'	Reverse primer 5' --- 3'
36b4	CTG TTG GCC AAT AAG GTG CC	GGA GGT CTT CTC GGG TCC TA
Abca1	CCC AGA GCA AAA AGC GAC TC	GGT CAT CAT CAC TTT GGT CCT TG
Abcg5	CTC CTC GCC TAC GTG CTA CA	GAT ACA AGC CCA GAG TCC AAT AAC A
Apoa1	CCC AGT CCC AAT GGG ACA	CAG GAG ATT CAG GTT CAG CTG TT
Cre	GCA TTA CCG GTC GAT GCA ACG AGT G	GAA CGC TAG AGC CTG TTT TGC ACG TTC
Gcg	CAA GAG GAA CCG GAA CAA CAT T	CCT GGC CCT CCA AGT AAG AA
Npc1l1	GAG AGC CAA AGA TGC TAC TAT CTT CA	CCC GGG AAG TTG GTC ATG
Pdk4	GCA TTT CTA CTC GGA TGC TCA TG	CCA ATG TGG CTT GGG TTT CC
Ppard	CTC AAT GGG GGA CCA GAA CA	AAG GGG AGG AAT TCT GGG AGA
Tnf	GTA GCC CAC GTC GTA GCA AAC	AGT TGG TTG TCT TTG AGA TCC ATG

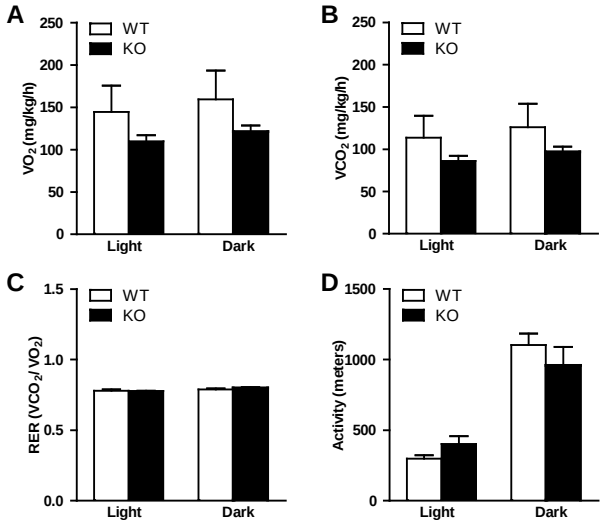
Supplementary Table 3: qPCR primer sequences.

Supplementary Figure 1



Supplementary Figure 1. Intestinal PPARδ does not regulate bacterial colonization of small intestine. Bacterial counts of **(A,B)** total bacteria and **(C,D)** bacterial groups of Clostridium; **(E,F)** Lactobacilli and **(G,H)** Bacteroides in middle and distal part of the intestine of wild-type and *PPARδ*$^{IEC\text{-}KO}$ mice. (n = 4-5)

Supplementary Figure 2



Supplementary Figure 2. Effect of a HFD on metabolic parameters in *PPARδ*$^{IEC\text{-}KO}$ and wild-type mice. (A) Oxygen consumption (VO_2); **(B)** Carbon dioxide production (VCO_2); **(C)** Respiratory exchange ratio (RER) and **(D)** Activity in *PPARδ*$^{IEC\text{-}KO}$ mice and wild-type littermates (n = 4-6) after a HFD challenge.