Microbiota-driven gut vascular barrier disruption is a prerequisite for non-alcoholic steatohepatitis development

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Supplementary methods

Special diet and treatments

Methionine choline deficient diet (MCDD, TD.90262) was used in parallel with CHOW diet in Fig S7. LPS was administered once via oral gavage at two different doses (1 $mg.kg^{-1}$ or 3 $mg.kg^{-1}$) as indicated in Fig S2.

Lung endothelial cells isolation and stimulation

Mouse lungs were removed from the thoracic cavity and placed in HBSS. Organs were digested with collagenase A (1,5 $mg.mL^{-1}$; Roche Diagnostic) and DNase I (8 $U.mL^{-1}$; Roche Diagnostic) for 75 min at 37°C. $CD45^{-}CD31^{+}$ endothelial cells were purified using specific MACS beads (Miltenyi Biotec). After selection, lung endothelial cells were cultured on 0.1% gelatin-coated plates in DMEM containing 20% FBS, 100 $\mu g.mL^{-1}$ heparin (Sigma-Aldrich) and 5 $\mu g.mL^{-1}$ endothelial cell growth supplement (Sigma-Aldrich). Confluent lung endothelial cells were treated with 10 μM OCA for 4h. Cells were then washed and lysed for the subsequent RNA analysis.

RNA extraction and Real Time-PCR

Isolated endothelial cells were homogenized in 500 µL of TRIzol (Invitrogen). RNA was extracted adding 100 µL of chloroform, precipitating the aqueous phase with 1 volume of 100% ethanol, and purified using Quick-RNA MiniPrep Kit (Zymo Research) following manufacturer's instructions. Reverse transcription of 1 µg of RNA was done using ImProm-II Reverse Transcriptase kit (Promega) with random

primers (Invitrogen). Real Time-PCR assay was performed with Fast Sybr Green Master Mix (Life Technologies). Primers used are listed in CTAT table. Expression levels were normalized to the 60S ribosomal protein gene expression Rpl32. Results were quantified using the 2-ΔCt method.

Fig S1 : Tight junction proteins expression in the intestine of 1 week HFD fed male mice

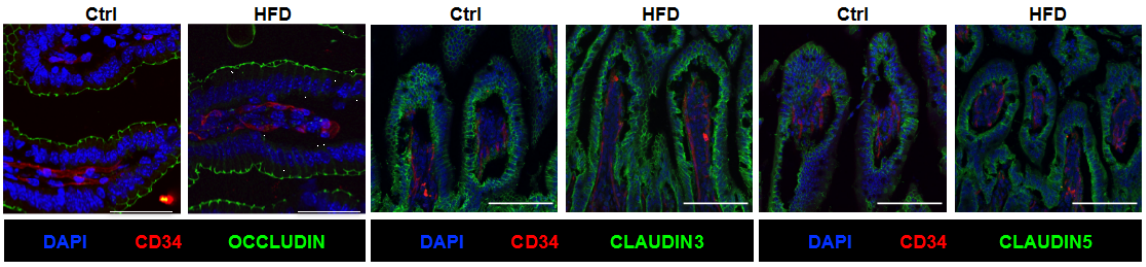

Fig. S1. Male mice were fed with Ctrl diet or HFD for 1 week, before ileum sections were stained for CD34 (red), DAPI (blue), and Occludin, Claudin-3 or Claudin-5 expression (green), as indicated.

Fig S2 : LPS exposure induces disruption of IEB and subsequent damage of GVB

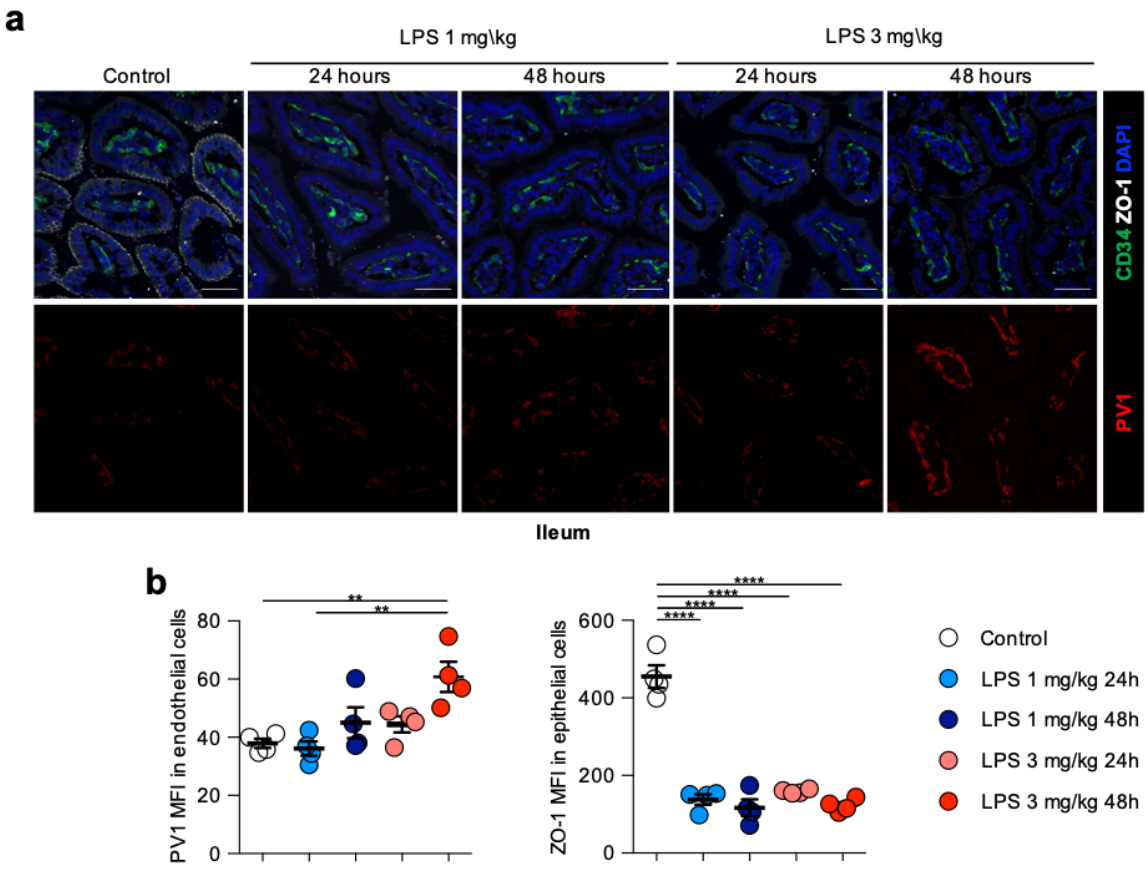

Fig. S2. LPS was administered via oral gavage at two different doses (1 mg/kg and 3 mg/Kg) and intestines harvested 24 and 48 hours later. (a) Sections from ileum were stained for CD34 (green), PV1 (red), ZO-1 (white) and DAPI (blue), as indicated. Scale bar indicates 50 µm. (b) Quantification of PV1 and ZO-1 MFI was performed on CD34+ and CD34- areas respectively.

**, p<0.005; ****, p<0.0001 unpaired two-tailed t-test.

Fig S3 : 1 week HFD fed male mice

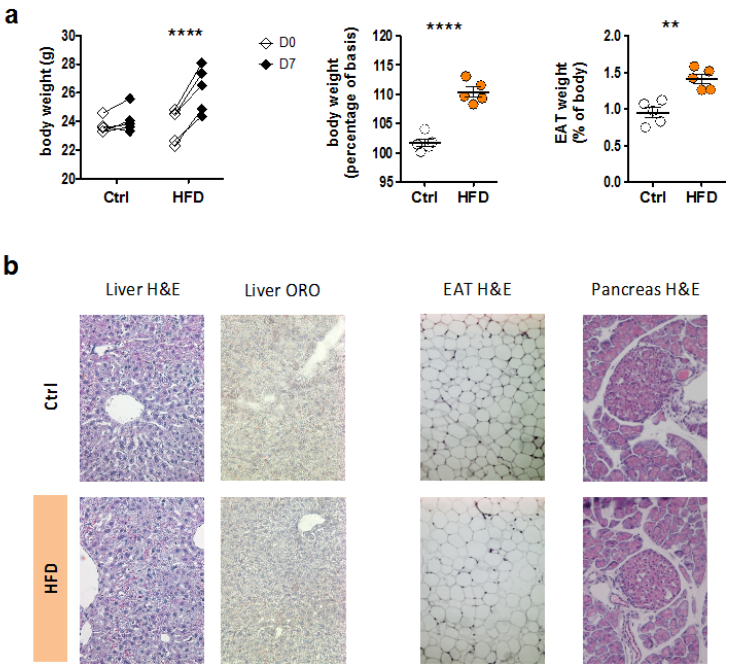

Fig. S3. Body and epididymal adipose tissue (EAT) weight was assessed in male mice were fed with Ctrl diet or HFD for 1 week (a). Liver, EAT and pancreas FFPE sections were stained with hematoxylin eosin (b). Liver OCT sections were stained with Oil Red O (ORO) staining (b).

**, $p<0.005$; ****, $p<0.0001$ unpaired two-tailed t-test.

Fig S4 : 6 weeks HFD fed female mice

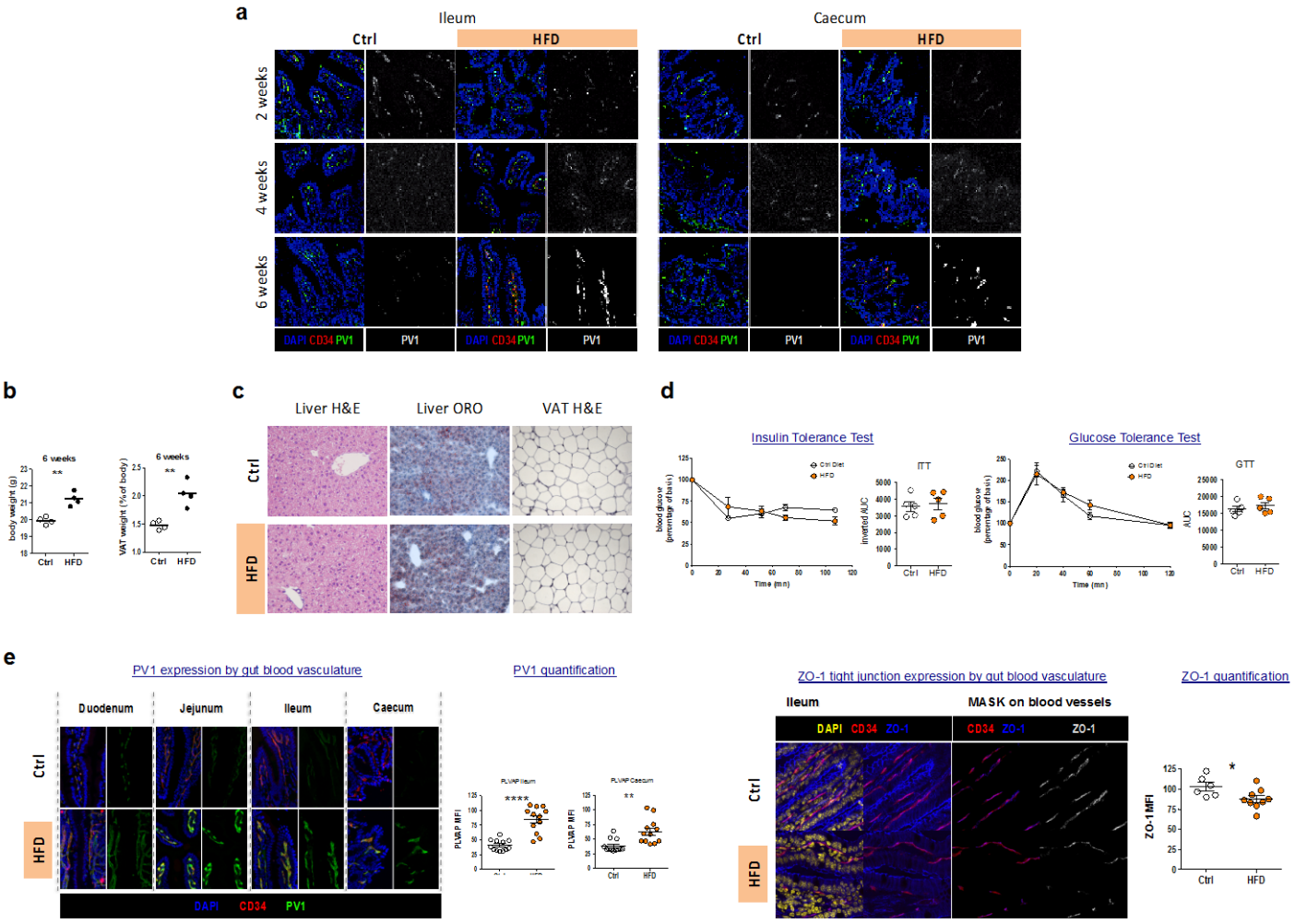

Fig. S4. Female mice were fed with Ctrl diet or HFD for 2, 4 and 6 weeks to assess the changes in the GVB. Sections from ileum and caecum were stained for CD34 (red), PV1 (green) and DAPI (blue), as indicated (a). Female mice were than fed with Ctrl diet or HFD for 6 weeks, before assessing body and visceral adipose tissue (VAT) weight (b). Liver and VAT FFPE sections were stained with hematoxylin eosin (c). Liver OCT sections were stained with ORO staining (c). ITT and GTT were performed as described in supplementary methods (d).

Intestinal sections were stained for CD34, DAPI, and PV1 (left panels) or ZO-1 (right panels) expression, as indicated. Quantification of PV1 and ZO-1 was performed on CD34+ areas (e).

*, p<0.05; **, p<0.005; ****, p<0.0001 unpaired two-tailed t-test.

Fig S5 : HFD fed male GOF mice

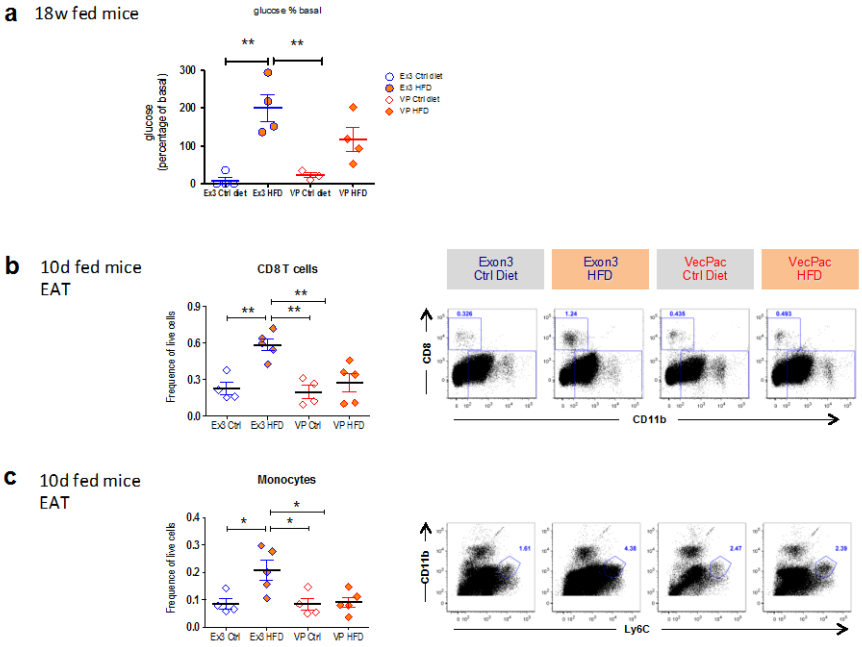

Fig. S5. Male mice of the indicated genotype were fed Ctrl or HFD for 18 weeks before measuring blood glucose levels (a).

Male mice of the indicated genotype were fed Ctrl or HFD for 10 days before harvesting EAT. EAT tissue was digested in Collagenase D, as described in supplementary methods, to obtain cell suspensions. After surface staining, cells were analyzed by FACS, then CD8 T cells (b) and monocytes (c) were gated as shown in the plots.

Fig S6 : ZO-1 expression in endothelial CD34+ cells is increased upon OCA treatment in HFD fed mice

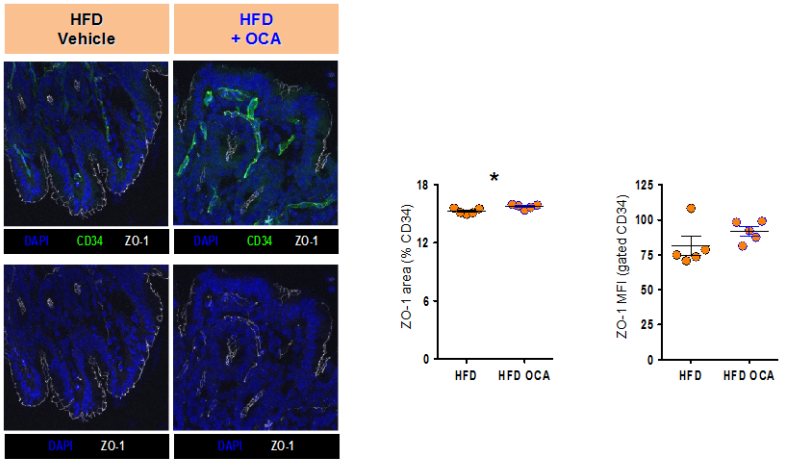

Fig. S6. Mice were fed with Ctrl diet or HFD supplemented or not with OCA for 1 week. Caecum sections were analyzed for ZO-1 expression (white), CD34 (green), DAPI (blue). Quantification of ZO-1 Mean Fluorescence Intensity (MFI) was performed on $CD34^{+}$ area.

Fig S7 : OCA protects from GVB disruption in the methionine-choline deficient diet (MCDD) model

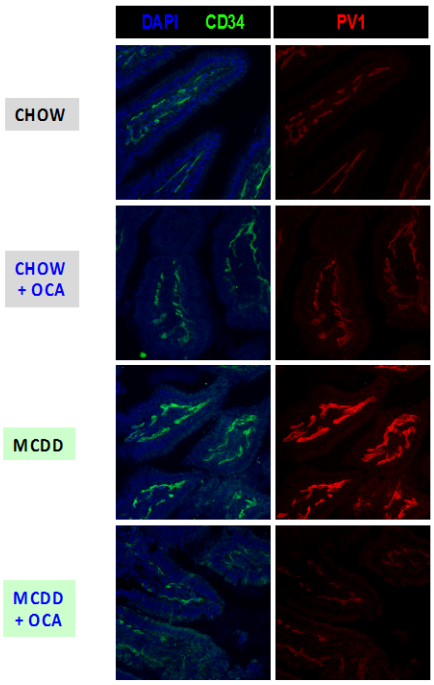

Fig. S7. Male mice were fed with CHOW diet or MCDD, with or without OCA oral administration (30 mg.kg^{-1}) for 1 week, before harvesting intestine. Ileum sections were stained for CD34, DAPI, and PV1 expression, as indicated.

Fig S8 : OCA drives β-catenin activation

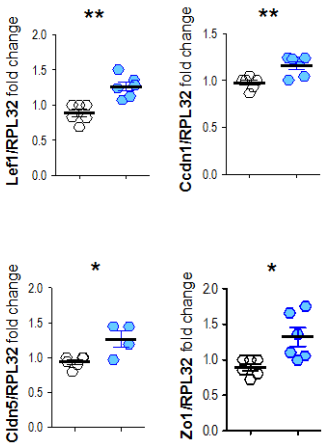

Fig. S8. Lung endothelial cells were isolated as described in Materials and Methods. They were then stimulated with 10 μM of OCA and gene expression was measured by RT-PCR. **, $p<0.005$; unpaired two-tailed t-test.

Table S1. qPCR primers

Gene	Forward primer (5'→3')	Reverse primer (5'→3')
Rpl32	AAGCGAAACTGGCGGAAAC	TAACCGATGTTGGGCATCAG
Lef1	AACACCTCAGGTCAAACAGGA	TGCTCCTTTCTCTGTTCGTG
Cldn5	AAATTCTGGGTCTGGTGCTG	GTCACGATGTTGTGGTCCAG
Zo1	CGCGGAGAGAGACAAGATGT	CCTGTGAAGCGTCAGTGTGT
Ccdn1	AGTGCGTGCAGAAGGAGATT	CTCTTCGCACTTCTGCTCCT
IFNγ	GCGTCATTGAATCACACCTG	CTGGACCTGTGGGTTGTTG
IL33	CCCGCCTTGCAAAATAAGA	CTTATGGTGAGGCCAGAACG
CCL2	AGGTCCCTGTCATGCTTCTG	TCATTGGGATCATCTTGCTG
PPIA	Quantitect	Catalog number: QT00248709

Table S2. Antibodies for immunofluorescence staining

Name	Clone	Cat. number
Anti-occludin	OC-3F10	Invitrogen
Anti-claudin-3	clone Z23.JM	Invitrogen
Anti-claudin-5	clone Z43.JK	Invitrogen