Supplementary Information file

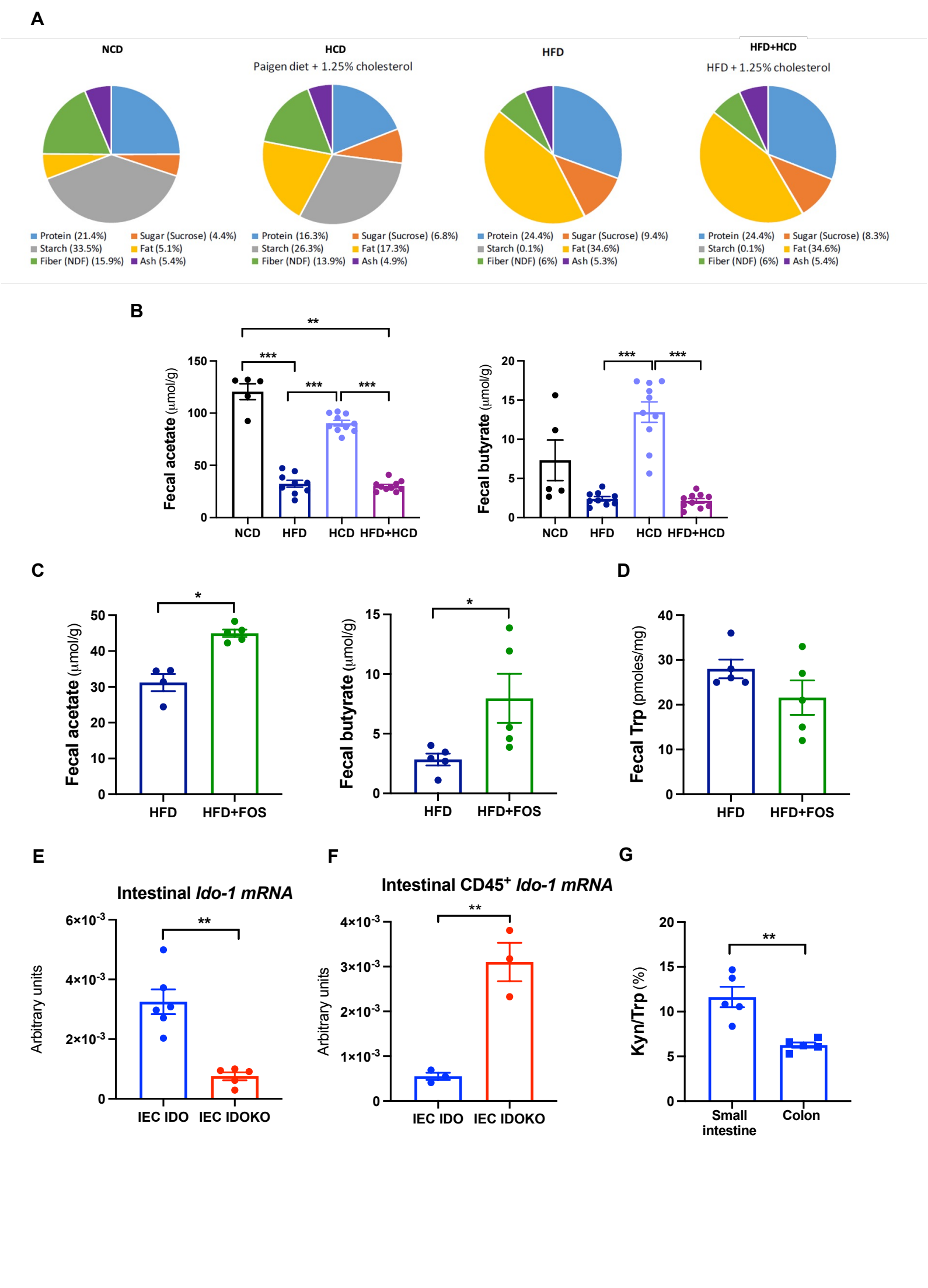
Harnessing intestinal tryptophan catabolism to relieve atherosclerosis in mice

Chajadine *et al.*

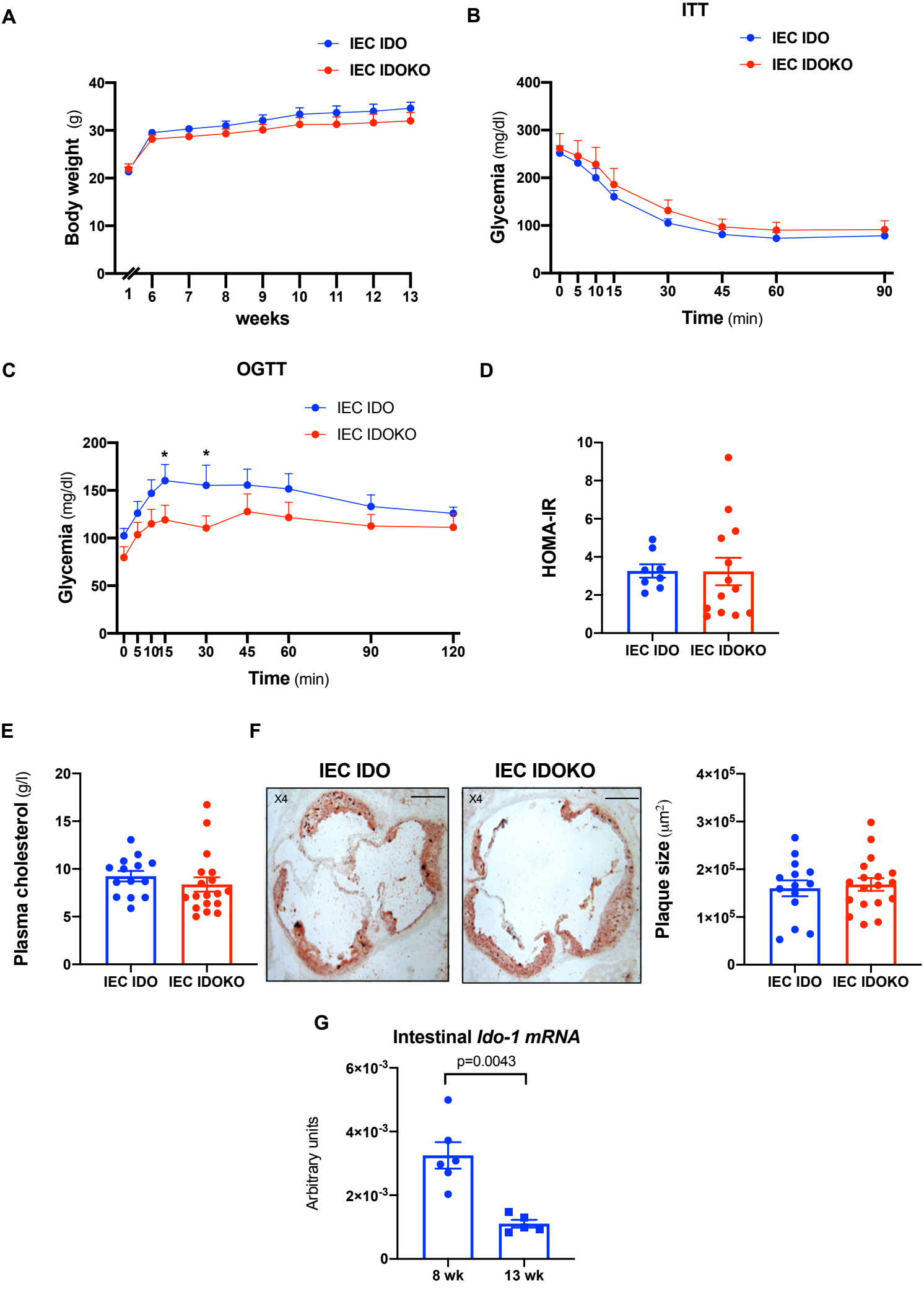
Test Date	Species	Submitter	Area	Profile
24 Aug 2023	Mouse	Inserm U970-PARCC	Zone 1-82 T16288	375M

Viruses	Test Methods	Latest Result
Ectromelia virus	Bead	0/1
Lymphocytic choriomeningitis virus	Bead	0/1
Minute virus of mice	Bead	0/1
Mouse adenovirus type 1 (MAd FL)	Bead	0/1
Mouse adenovirus type 2 (MAd K87)	Bead	0/1
Mouse hepatitis virus	Bead	0/1
Mouse norovirus	Bead	0/1
Mouse parvovirus	Bead	0/1
Mouse rotavirus (EDIM)	Bead	0/1
Pneumonia virus of mice	Bead	0/1
Reovirus type 3 (Reo 3)	Bead	0/1
Sendai Virus	Bead	0/1
Theiler's murine encephalomyelitis virus	Bead	0/1
Bacteria, Mycoplasma and Fungi	**Test Methods**	**Latest Result**
Streptobacillus moniliformis	qPCR	0/1
Helicobacter	qPCR	0/1
Citrobacter rodentium	Culture	0/1
Corynebacterium kutscheri	Culture	0/1
Pasteurella pneumotropica	Culture	0/1
Salmonella spp	Culture	0/1
Streptococci Beta-haemolytic (not group D)	Culture	0/1
Streptococcus pneumoniae	Culture	0/1
Clostridium piliforme	Bead	0/1
Mycoplasma pulmonis	Bead	0/1
Parasites	**Test Methods**	**Latest Result**
Ectoparasites	Microscopy	0/1
Endoparasites	Microscopy	0/1
Pathological Lesions	**Test Methods**	**Latest Result**
Pathological Lesions	Necropsy	0/1

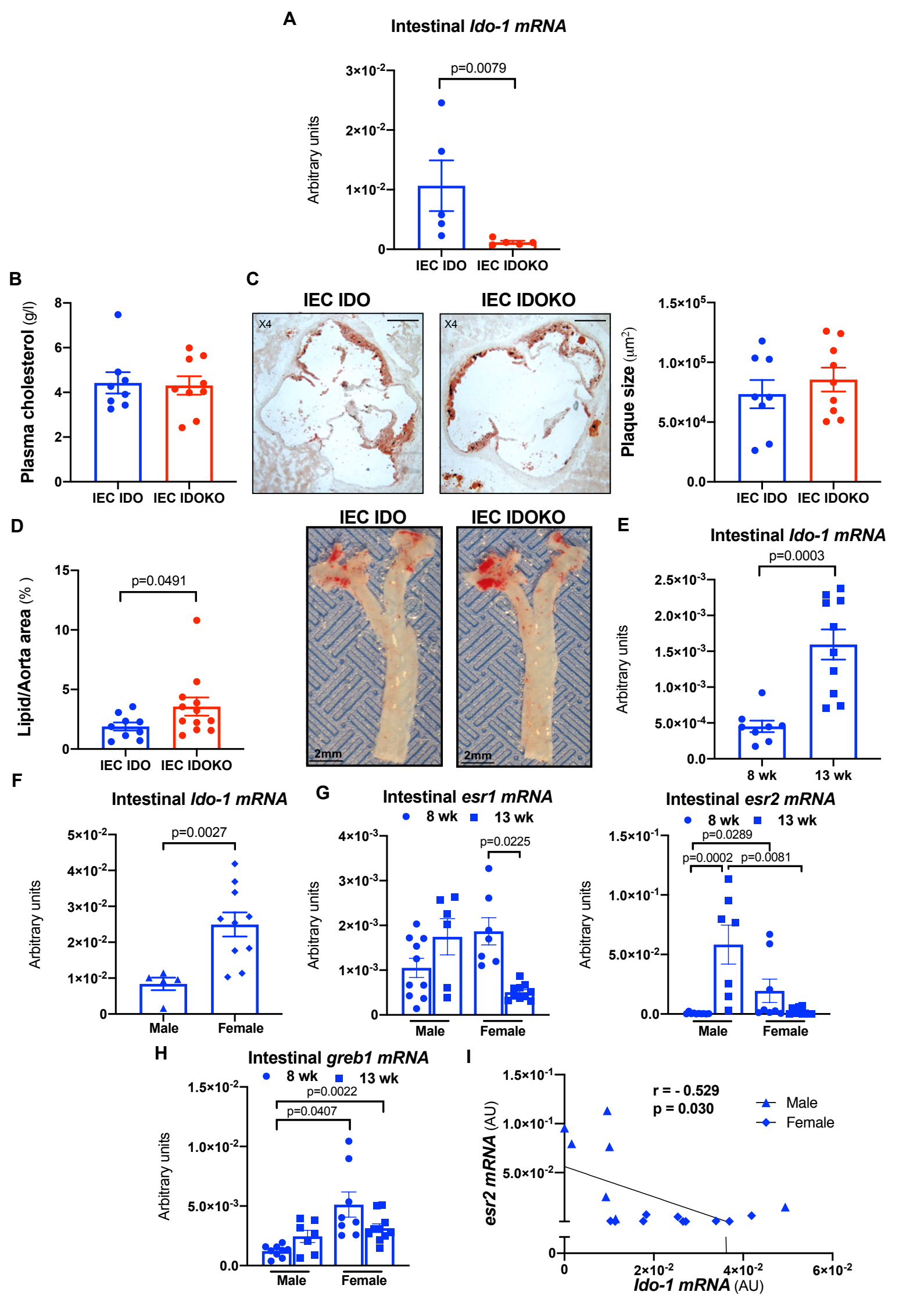
Supplementary Table 1: health certificate


A
NCD
Protein (21.4%)
Sugar (Sucrose) (4.4%)
Starch (33.5%)
Fat (5.1%)
Fiber (NDF) (15.9%)
Ash (5.4%)
HCD
Paigen diet + 1.25% cholesterol
Protein (16.3%)
Sugar (Sucrose) (6.8%)
Starch (26.3%)
Fat (17.3%)
Fiber (NDF) (13.9%)
Ash (4.9%)
HFD
Protein (24.4%)
Sugar (Sucrose) (9.4%)
Starch (0.1%)
Fat (34.6%)
Fiber (NDF) (6%)
Ash (5.3%)
HFD+HCD
HFD + 1.25% cholesterol
Protein (24.4%)
Sugar (Sucrose) (8.3%)
Starch (0.1%)
Fat (34.6%)
Fiber (NDF) (6%)
Ash (5.4%)
B
Fecal acetate (µmol/g)
Fecal butyrate (µmol/g)
NCD
HFD
HCD
HFD+HCD
C
Fecal acetate (µmol/g)
Fecal butyrate (µmol/g)
HFD
HFD+FOS
D
Fecal Trp (pmoles/mg)
E
Intestinal Ido-1 mRNA
Arbitrary units
IEC IDO
IEC IDOKO
F
Intestinal CD45+ Ido-1 mRNA
G
Kyn/Trp (%)
Small intestine
Colon

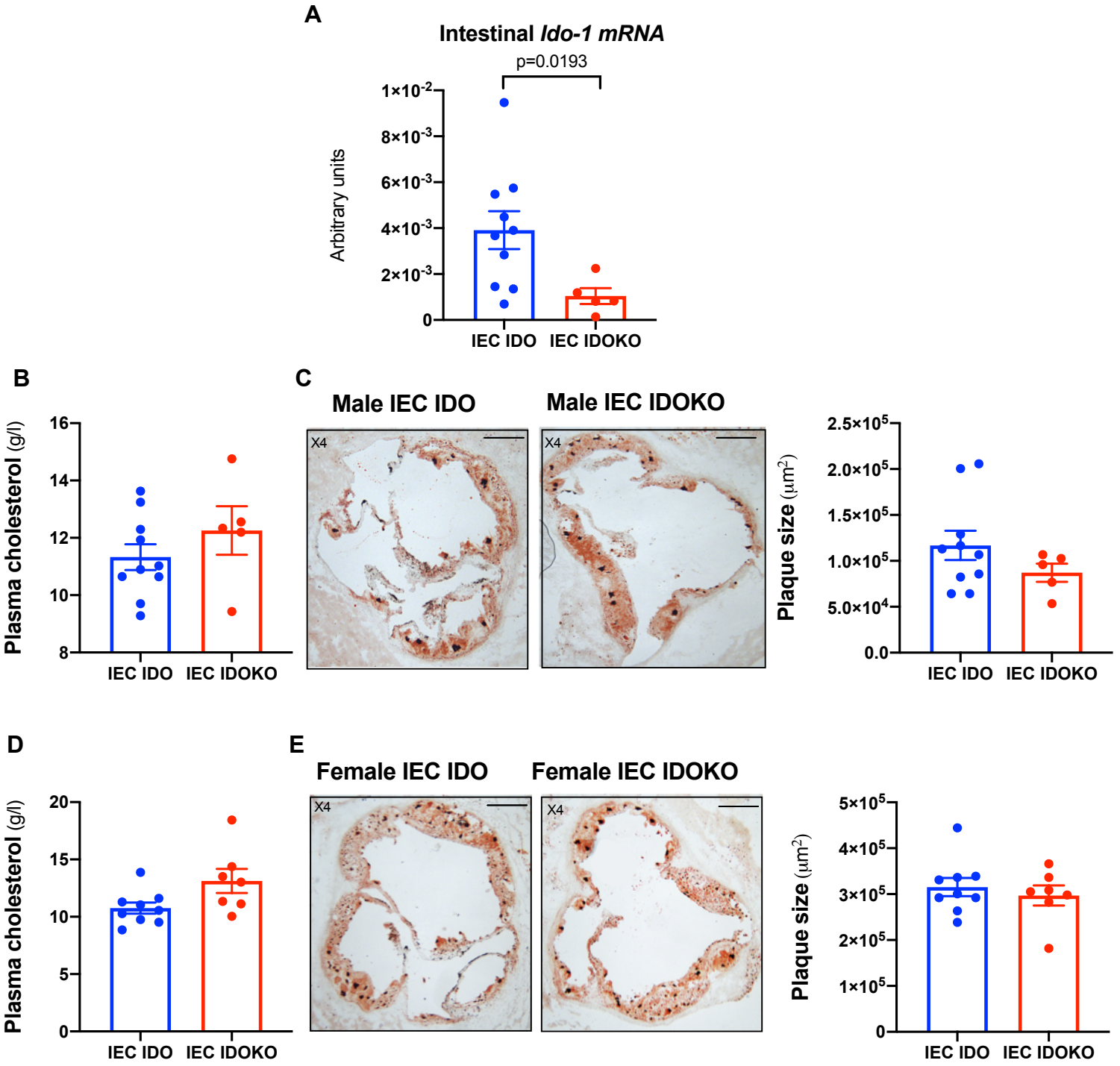
Supplementary Fig. 1: effects of diet on SCFAs and Trp metabolites. A. diet composition, **B.** acetate and butyrate levels in feces from male *Ldlr*$^{-/-}$ mice fed either normal chow diet (NCD) (n=5 mice), high-fat diet (HFD) (n=9 mice), or high-cholesterol diet (HCD) (n=10 mice) or the combination of both HFD+HCD (n=10 mice) for 13 weeks, **C**. short chain fatty acid (SCFA), acetate and butyrate, **D.** Trp levels in feces from *Ldlr*$^{-/-}$ mice fed HFD for 13 weeks supplemented or not with fructooligosaccharide (FOS) (n=5 mice/group). **E.** *Ido-1 mRNA* in the small intestines of male *Ldlr*$^{-/-}$ IEC IDO KO mice and littermate control *Ldlr*$^{-/-}$ IEC IDO mice fed HFD+HCD for 8 weeks (IEC IDO n=6 mice, IEC IDOKO n=5 mice). **F.** *Ido-1 mRNA* in CD45+ FACS-sorted cells from digested small intestines of male *Ldlr*$^{-/-}$ IEC IDO KO mice and littermate control *Ldlr*$^{-/-}$ IEC IDO mice fed HFD+HCD for 8 weeks (n=3 mice/group, pools of n=7-9 mice independent samples/group). **G**. Kyn/Trp % in the small intestine and colon extracts of *Ldlr*$^{-/-}$ mice at baseline (n=5 mice/group). Individual data are presented as scattered dot plots, with the mean and s.e.m. The p values were determined using Brown-Forsythe ANOVA test followed by Tukey's multiple comparison test for A, the two-tailed Mann-Whitney test for B, C, and two-tailed Unpaired T test for E. Source data are provided as a Source Data file.


A
IEC IDO
IEC IDOKO
Body weight (g)
weeks
B
ITT
IEC IDO
IEC IDOKO
Glycemia (mg/dl)
Time (min)
C
OGTT
IEC IDO
IEC IDOKO
Glycemia (mg/dl)
Time (min)
D
HOMA-IR
IEC IDO
IEC IDOKO
E
Plasma cholesterol (g/l)
IEC IDO
IEC IDOKO
F
IEC IDO
IEC IDOKO
X4
X4
Plaque size (µm²)
IEC IDO
IEC IDOKO
G
Intestinal Ido-1 mRNA
p=0.0043
Arbitrary units
8 wk
13 wk

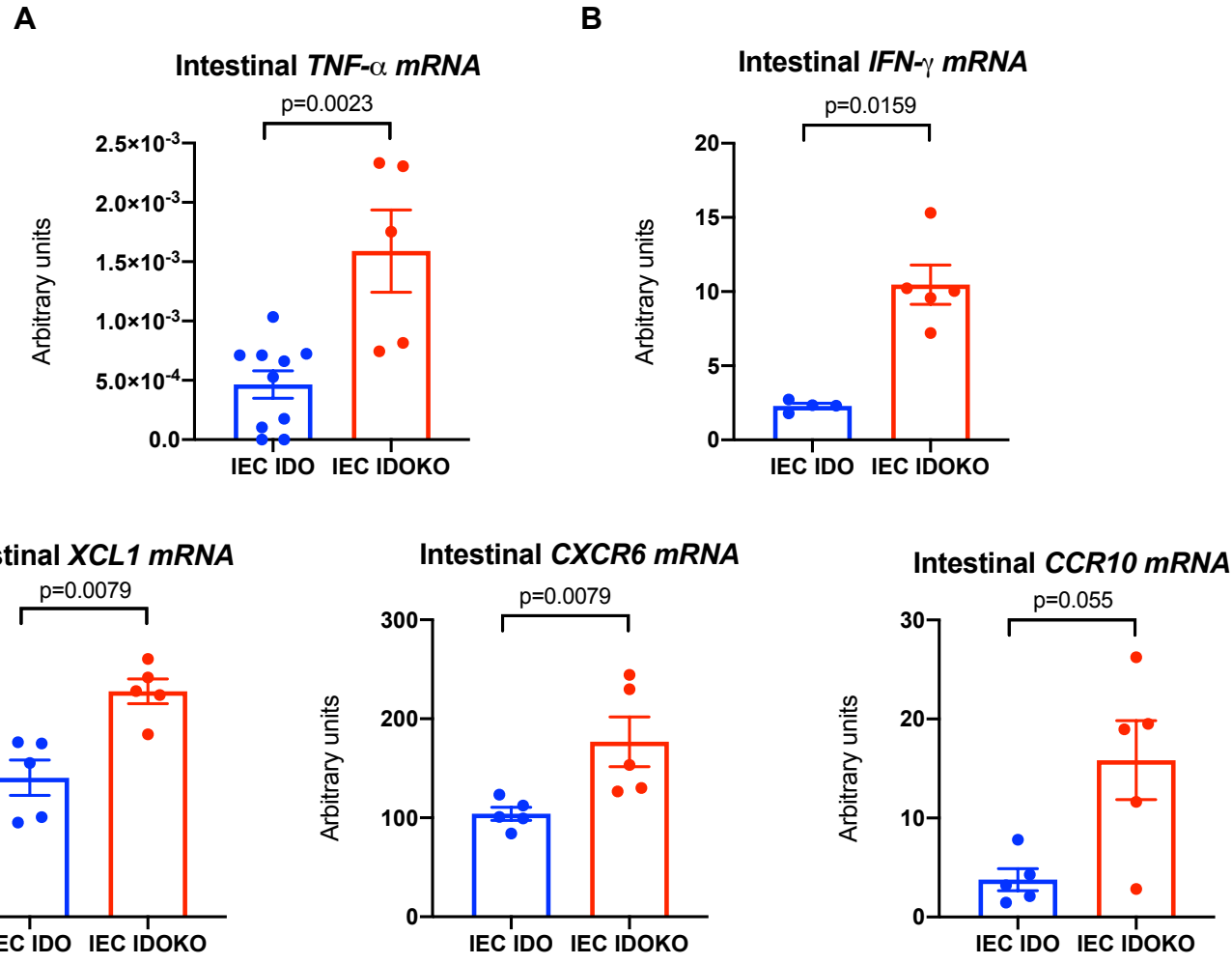
Supplementary Fig. 2: effects of intestinal IDO deletion on metabolic parameters and atherosclerosis. A. weight curves, **B.** ITT, **C.** OGTT, and **D.** HOMA-IR in male *Ldlr*$^{-/-}$ IEC IDO KO, and littermate control *Ldlr*$^{-/-}$ IEC IDO mice fed HFD+HCD for 13 weeks (IEC IDO n=8 mice, IEC IDOKO n=13 mice). **E.** plasma cholesterol, **F.** representative pictures and quantifications of plaque size in the aortic sinus of male *Ldlr*$^{-/-}$ IEC IDO KO, and littermate control *Ldlr*$^{-/-}$ IEC IDO mice fed HFD+HCD for 13 weeks (IEC IDO n=14 mice, IEC IDOKO n=18 mice); scale bar 200µm. **G.** *Ido-1 mRNA* in the small intestines of male *Ldlr*$^{-/-}$ mice fed HFD+HCD for 8 or 13 weeks (wk) (8wk n=6 mice, 13wk n=5 mice). Individual data are presented as scattered dot plots, with the mean and s.e.m. The p values were determined using the two-tailed Mann-Whitney test. Source data are provided as a Source Data file.


A
Intestinal Ido-1 mRNA
p=0.0079
Arbitrary units
IEC IDO
IEC IDOKO
B
Plasma cholesterol (g/l)
IEC IDO
IEC IDOKO
C
IEC IDO
IEC IDOKO
X4
Plaque size (µm²)
D
Lipid/Aorta area (%)
p=0.0491
2mm
E
Intestinal Ido-1 mRNA
p=0.0003
8 wk
13 wk
F
Intestinal Ido-1 mRNA
p=0.0027
Male
Female
G
Intestinal esr1 mRNA
Intestinal esr2 mRNA
p=0.0225
p=0.0289
p=0.0002
p=0.0081
H
Intestinal greb1 mRNA
p=0.0022
p=0.0407
I
r = - 0.529
p = 0.030
esr2 mRNA (AU)
Ido-1 mRNA (AU)

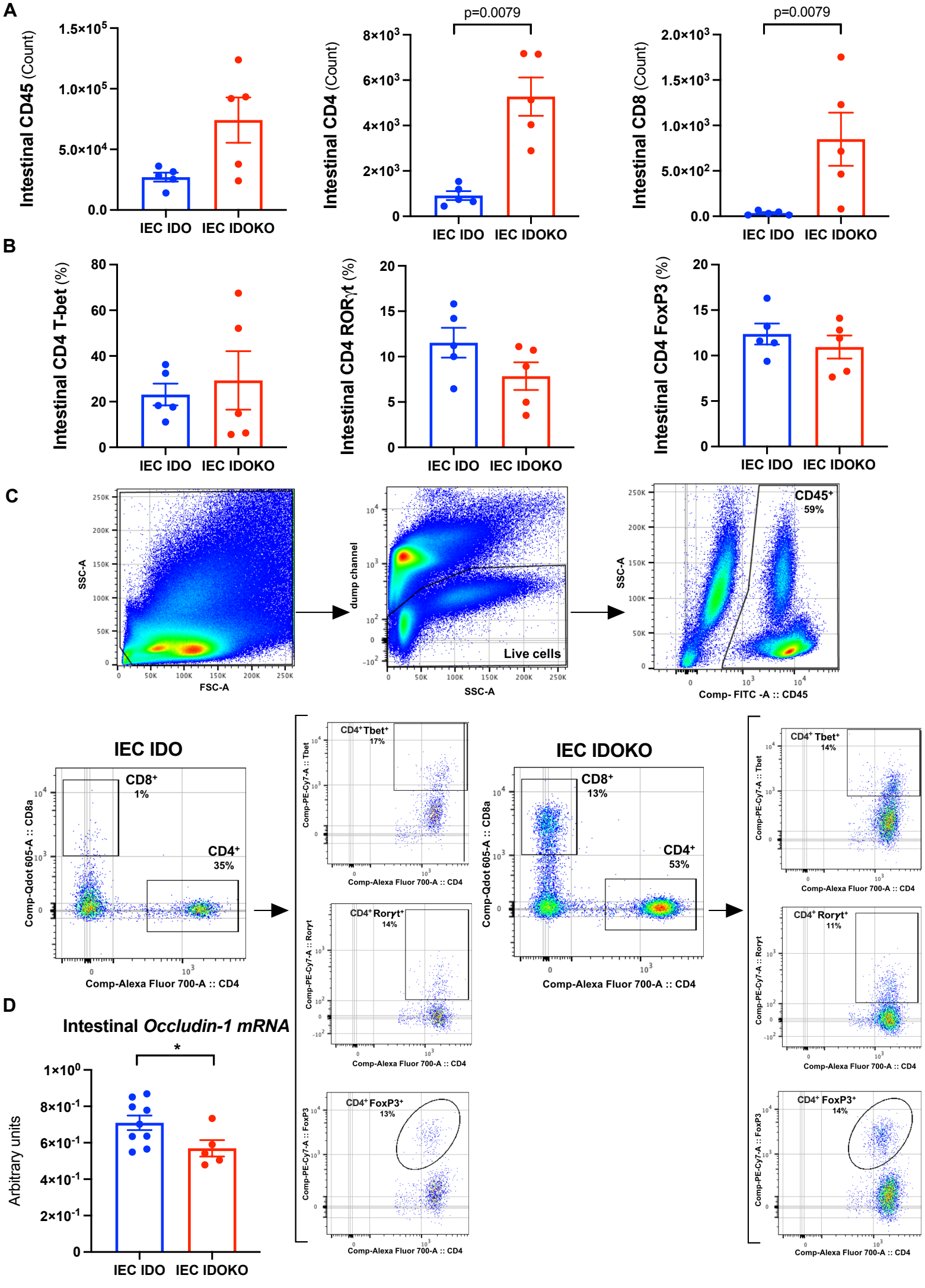
Supplementary Fig. 3: effects of intestinal IDO deletion on atherosclerosis and assessing IDO expression in males and females. A. *Ido-1 mRNA* in small intestines (n=5 mice/group), **B.** plasma cholesterol, **C.** representative pictures and quantifications of plaque size in the aortic sinus of female *Ldlr*$^{-/-}$ IEC IDO KO, and littermate control *Ldlr*$^{-/-}$ IEC IDO mice fed HFD+HCD for 8 weeks (IEC IDO n=8 mice, IEC IDOKO n=9 mice); scale bar 200µm. **D.** representative photomicrographs and lipid quantification with en-face staining in the thoracic aorta of female *Ldlr*$^{-/-}$ IEC IDO KO, and littermate control *Ldlr*$^{-/-}$ IEC IDO mice fed HFD+HCD for 13 weeks (IEC IDO n=9 mice, IEC IDOKO n=12 mice); scale bar 2mm. **E.** *Ido-1 mRNA* in the small intestines of female *Ldlr*$^{-/-}$ mice fed HFD+HCD for 8 or 13 weeks (8wk n=8 mice, 13wk n=10 mice). **F.** *Ido-1 mRNA* in the small intestines of male and female *Ldlr*$^{-/-}$ mice fed HFD+HCD for 13 weeks (male n=5 mice, female n=10 mice). **G-H.** *esr1, esr2, and greb1 mRNA* in the small intestines of male and female *Ldlr*$^{-/-}$ mice fed HFD+HCD for 8 or 13 weeks (n=6-10 mice/group). **I.** correlation between *esr2 mRNA* and *Ido-1 mRNA* in the small intestines of male and female *Ldlr*$^{-/-}$ mice fed HFD+HCD for 13 weeks (Spearman correlation) (male n=7 mice, female n=10 mice). Individual data are presented as scattered dot plots, with the mean and s.e.m. The p values were determined using the two-tailed Mann-Whitney test for A, D, E, F, and Brown-Forsythe ANOVA test followed by Tukey's multiple comparison test for G (esr1) and H and Kruskal-Wallis, followed by post-Hoc Dunn’s test for G (esr2). Source data are provided as a Source Data file.


A
Intestinal Ido-1 mRNA
p=0.0193
Arbitrary units
1×10-2
8×10-3
6×10-3
4×10-3
2×10-3
0
IEC IDO
IEC IDOKO
B
Plasma cholesterol (g/l)
16
14
12
10
8
IEC IDO
IEC IDOKO
C
Male IEC IDO
Male IEC IDOKO
X4
X4
Plaque size (µm2)
2.5×105
2.0×105
1.5×105
1.0×105
5.0×104
0.0
IEC IDO
IEC IDOKO
D
Plasma cholesterol (g/l)
20
15
10
5
0
IEC IDO
IEC IDOKO
E
Female IEC IDO
Female IEC IDOKO
X4
X4
Plaque size (µm2)
5×105
4×105
3×105
2×105
1×105
0
IEC IDO
IEC IDOKO

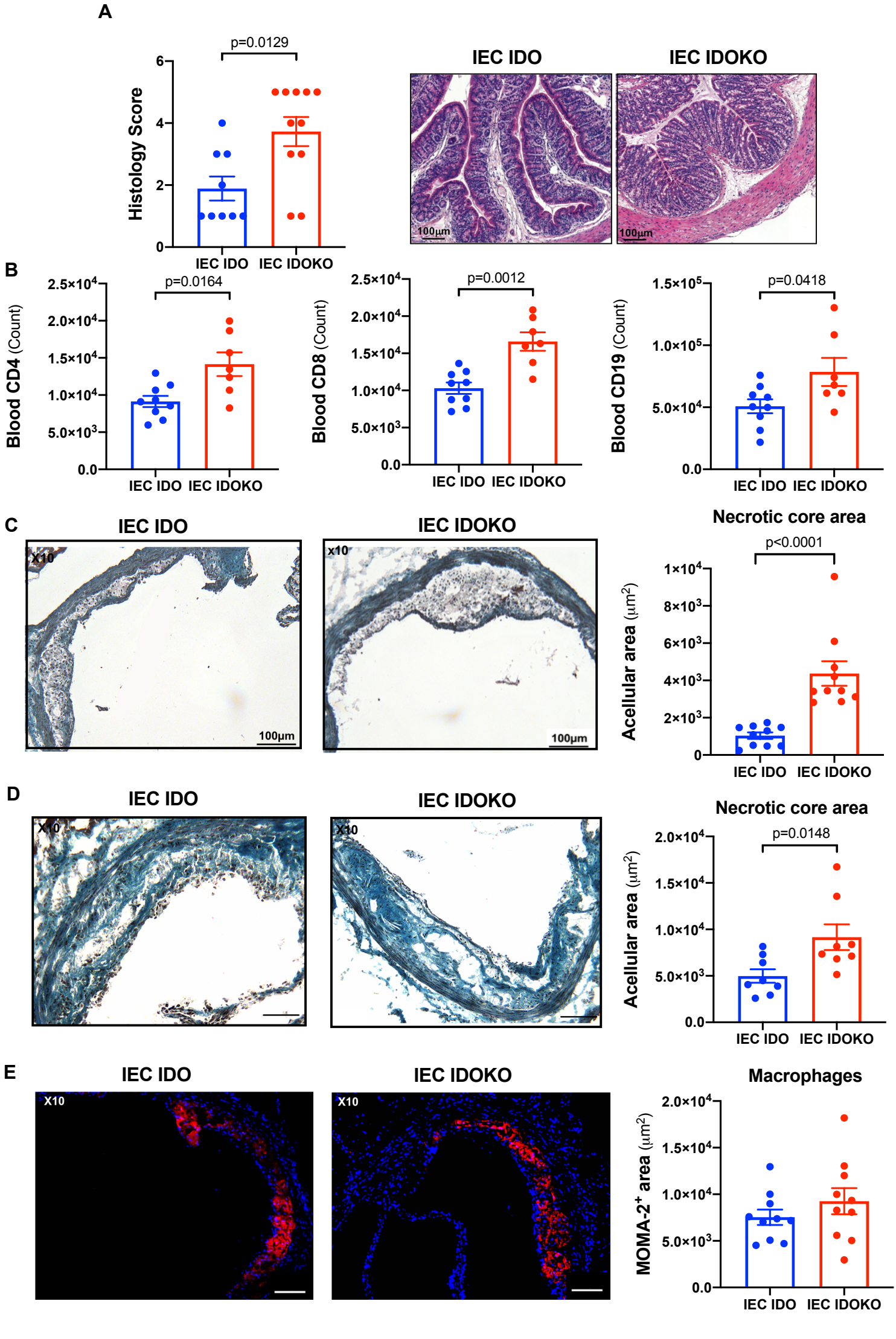
Supplementary Fig.4: effects of intestinal IDO deletion on atherosclerosis under HCD. A. *Ido-1 mRNA* in small intestines, **B.** plasma cholesterol, **C.** representative pictures and quantifications of plaque size in the aortic sinus of male *Ldlr*$^{-/-}$ IEC IDO KO, and littermate control *Ldlr*$^{-/-}$ IEC IDO mice fed a high-cholesterol diet (HCD) for 8 weeks (IEC IDO n=10 mice, IEC IDOKO n=5 mice); scale bar 200µm. **D.** plasma cholesterol, **E.** representative pictures and quantifications of plaque size in the aortic sinus of female *Ldlr*$^{-/-}$ IEC IDO KO, and littermate control *Ldlr*$^{-/-}$ IEC IDO mice fed HCD for 8 weeks (IEC IDO n=9 mice, IEC IDOKO n=7 mice); scale bar 200µm. Individual data are presented as scattered dot plots, with the mean and s.e.m. The p values were determined using the two-tailed Mann-Whitney test. Source data are provided as a Source Data file.


A
Intestinal TNF-α mRNA
p=0.0023
Arbitrary units
2.5×10-3
2.0×10-3
1.5×10-3
1.0×10-3
5.0×10-4
0.0
IEC IDO
IEC IDOKO
B
Intestinal IFN-γ mRNA
p=0.0159
Arbitrary units
20
15
10
5
0
IEC IDO
IEC IDOKO
Intestinal XCL1 mRNA
p=0.0079
Arbitrary units
80
60
40
20
0
IEC IDO
IEC IDOKO
Intestinal CXCR6 mRNA
p=0.0079
Arbitrary units
300
200
100
0
IEC IDO
IEC IDOKO
Intestinal CCR10 mRNA
p=0.055
Arbitrary units
30
20
10
0
IEC IDO
IEC IDOKO

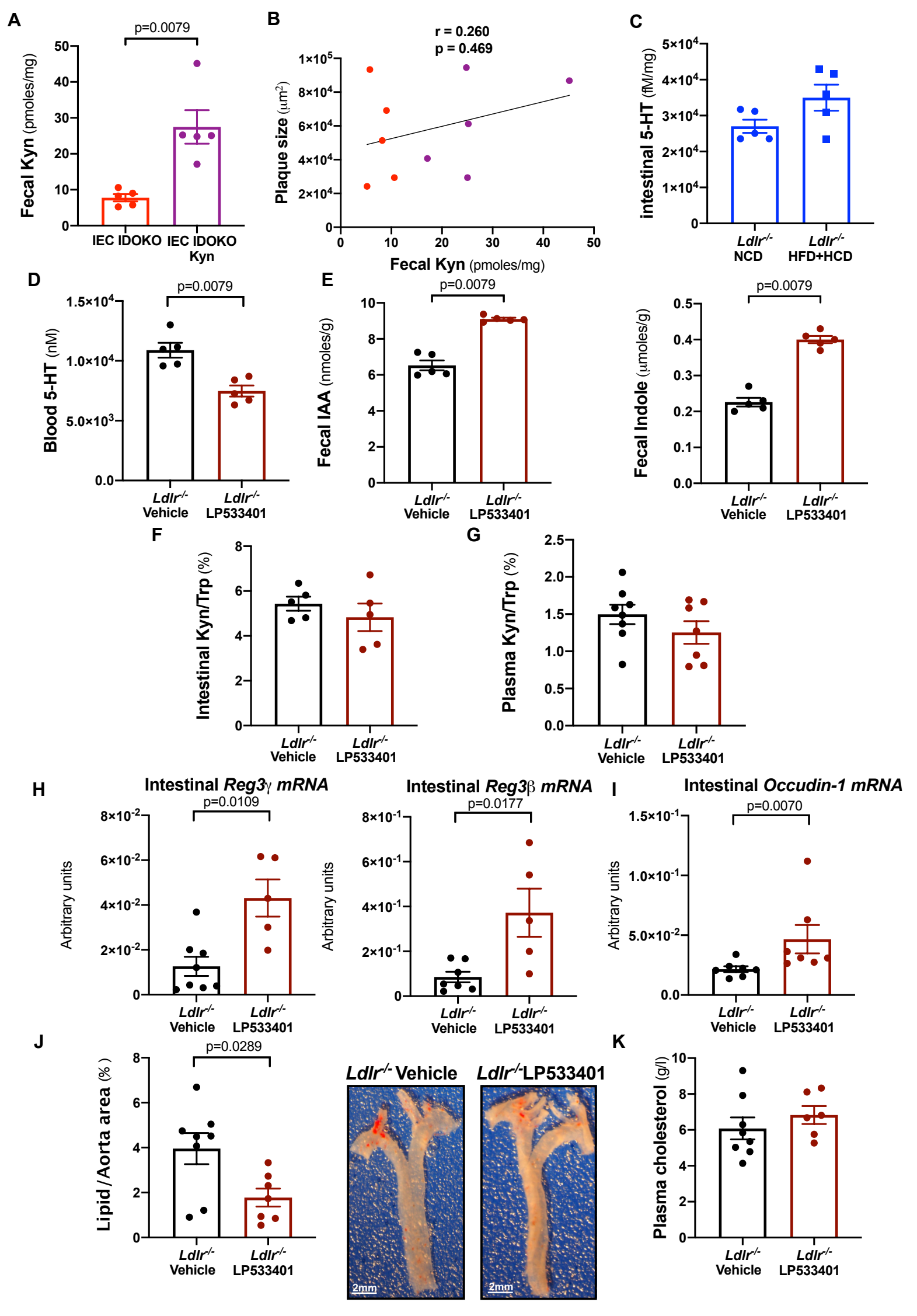
Supplementary Fig. 5: effects of intestinal IDO deletion on inflammation. A. *TNF-α mRNA* by qPCR in the small intestine of male *Ldlr*$^{-/-}$ IEC IDO KO, and littermate control *Ldlr*$^{-/-}$ IEC IDO mice fed HFD+HCD for 8 weeks (IEC IDO n=10 mice, IEC IDOKO n=5 mice). **B.** *Ifn-γ, XCL1, CCR10*, and *CXCR6 mRNA* by nanostring in the small intestine of male *Ldlr*$^{-/-}$ IEC IDO KO, and littermate control *Ldlr*$^{-/-}$ IEC IDO mice fed HFD+HCD for 8 weeks (n=5 mice/group). Individual data are presented as scattered dot plots, with the mean and s.e.m. The p values were determined using the two-tailed Mann-Whitney test. Source data are provided as a Source Data file.


A
Intestinal CD45 (Count)
1.5×10^5
1.0×10^5
5.0×10^4
0.0
IEC IDO
IEC IDOKO
Intestinal CD4 (Count)
p=0.0079
8×10^3
6×10^3
4×10^3
2×10^3
0
Intestinal CD8 (Count)
p=0.0079
2.0×10^3
1.5×10^3
1.0×10^3
5.0×10^2
B
Intestinal CD4 T-bet (%)
80
60
40
20
Intestinal CD4 RORγt (%)
Intestinal CD4 FoxP3 (%)
C
SSC-A
FSC-A
dump channel
Live cells
CD45+
59%
Comp- FITC -A :: CD45
IEC IDO
CD8+
1%
CD4+
35%
Comp-Qdot 605-A :: CD8a
Comp-Alexa Fluor 700-A :: CD4
CD4+ Tbet+
17%
Comp-PE-Cy7-A :: Tbet
CD4+ Rorγt+
14%
Comp-PE-Cy7-A :: Rorγt
CD4+ FoxP3+
13%
Comp-PE-Cy7-A :: FoxP3
IEC IDOKO
CD8+
13%
CD4+
53%
CD4+ Tbet+
14%
CD4+ Rorγt+
11%
CD4+ FoxP3+
14%
D
Intestinal Occludin-1 mRNA
*
Arbitrary units
1×10^0
8×10^-1
6×10^-1
4×10^-1
2×10^-1
0

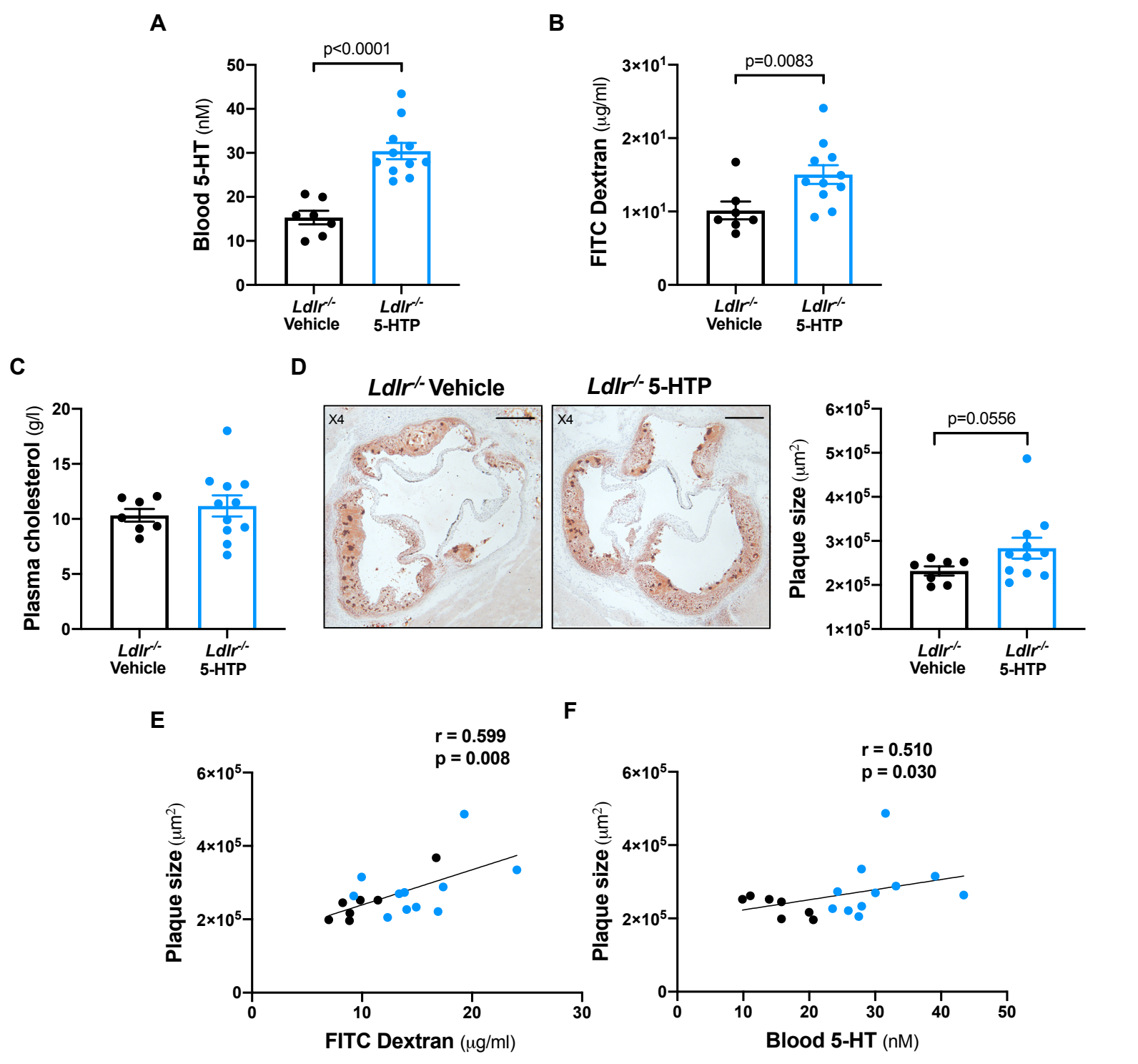
Supplementary Fig. 6: effects of intestinal IDO deletion on immune cells by flow cytometry. A. flow cytometry analysis of CD45+, CD4+, and CD8+ cells (count) in the lamina propria of the small intestines of male *Ldlr*$^{-/-}$ IEC IDO KO, and littermate control *Ldlr*$^{-/-}$ IEC IDO mice fed HFD+HCD for 8 weeks (n=5 mice/group), **B.** Th subset (%)**, C.** Pseudocolor plot examples show the gating strategy. A dump channel represents dead cells as well as CD11b+ and CD11c+ cells, which were excluded. T cells were gated as single cells dump^{-} CD45^{+} CD4^{+}. The different T cell subsets were identified according to the T-bet, ROR-γt, and FoxP3 expression. **D.** *Occludin-1 mRNA* in the small intestines of male *Ldlr*$^{-/-}$ IEC IDO KO, and littermate control *Ldlr*$^{-/-}$ IEC IDO mice fed HFD+HCD for 8 weeks (IEC IDO n=9 mice, IEC IDOKO n=5 mice). Individual data are presented as scattered dot plots, with the mean and s.e.m. The p values were determined using the two-tailed Mann-Whitney test. Source data are provided as a Source Data file.


A
p=0.0129
Histology Score
IEC IDO
IEC IDOKO
100µm
B
p=0.0164
Blood CD4 (Count)
p=0.0012
Blood CD8 (Count)
p=0.0418
Blood CD19 (Count)
C
X10
100µm
Necrotic core area
p<0.0001
Acellular area (µm²)
D
p=0.0148
E
Macrophages
MOMA-2⁺ area (µm²)

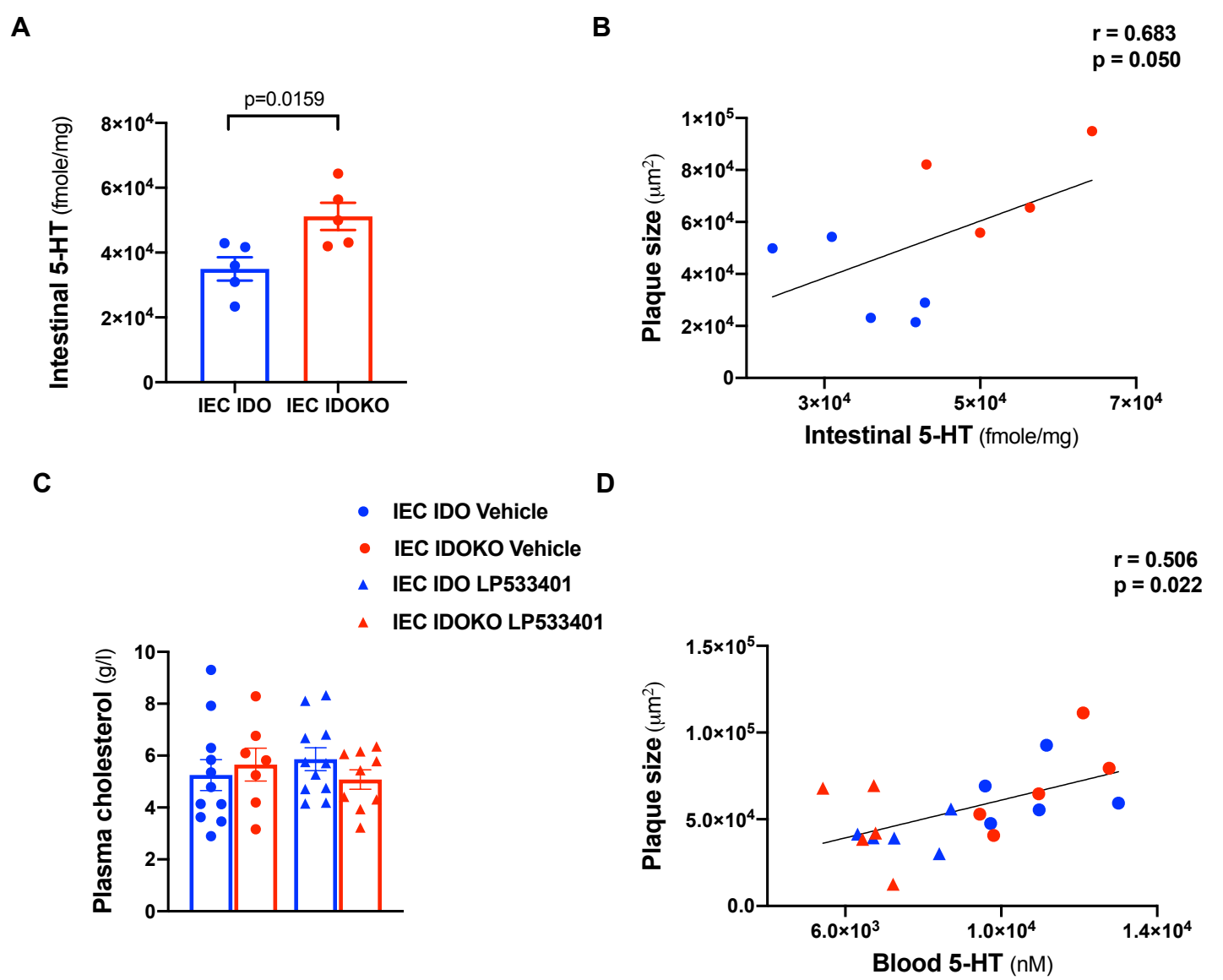
Supplementary Fig. 7: effects of intestinal IDO deletion on the gut, blood immune cells, and lesional necrotic cores. A. colon pathohistological scoring in female *Ldlr*$^{-/-}$ IEC IDO KO and littermate control *Ldlr*$^{-/-}$ IEC IDO mice fed HFD+HCD for 13 weeks (IEC IDO n=9 mice, IEC IDOKO n=11 mice); scale bar 100µm. **B.** flow cytometry analysis of blood CD4+, CD8+, and CD19+ cells (count) in male *Ldlr*$^{-/-}$ IEC IDO KO, and littermate control *Ldlr*$^{-/-}$ IEC IDO mice fed HFD+HCD for 8 weeks (IEC IDO n=9 mice, IEC IDOKO n=7 mice). **C.** representative pictures and quantifications of necrotic cores visualized by Masson's Trichrome staining in the aortic sinus of male *Ldlr*$^{-/-}$ IEC IDO KO and littermate control *Ldlr*$^{-/-}$ IEC IDO mice fed HFD+HCD for 8 weeks (n=10 mice/group); scale bar 100µm. **D.** representative pictures and quantifications of necrotic cores visualized by Masson's Trichrome staining in the aortic sinus of female *Ldlr*$^{-/-}$ IEC IDO KO and littermate control *Ldlr*$^{-/-}$ IEC IDO mice fed HFD+HCD for 13 weeks (n=8 mice/group); scale bar 100µm. **E.** representative pictures and quantifications of macrophages (MOMA2+ in red) accumulation within plaques in the aortic sinus of male *Ldlr*$^{-/-}$ IEC IDO KO, and littermate control *Ldlr*$^{-/-}$ IEC IDO mice fed HFD+HCD for 8 weeks (n=10 mice/group); scale bar 100µm. Individual data are presented as scattered dot plots, with the mean and s.e.m. The p values were determined using the two-tailed Mann-Whitney test. Source data are provided as a Source Data file.


A
p=0.0079
Fecal Kyn (pmoles/mg)
IEC IDOKO
IEC IDOKO Kyn
B
r = 0.260
p = 0.469
Plaque size (µm²)
Fecal Kyn (pmoles/mg)
C
intestinal 5-HT (fM/mg)
Ldlr-/- NCD
Ldlr-/- HFD+HCD
D
p=0.0079
Blood 5-HT (nM)
Ldlr-/- Vehicle
Ldlr-/- LP533401
E
p=0.0079
Fecal IAA (nmoles/g)
Ldlr-/- Vehicle
Ldlr-/- LP533401
p=0.0079
Fecal Indole (µmoles/g)
Ldlr-/- Vehicle
Ldlr-/- LP533401
F
Intestinal Kyn/Trp (%)
Ldlr-/- Vehicle
Ldlr-/- LP533401
G
Plasma Kyn/Trp (%)
Ldlr-/- Vehicle
Ldlr-/- LP533401
H
Intestinal Reg3γ mRNA
p=0.0109
Arbitrary units
Ldlr-/- Vehicle
Ldlr-/- LP533401
Intestinal Reg3β mRNA
p=0.0177
Arbitrary units
Ldlr-/- Vehicle
Ldlr-/- LP533401
I
Intestinal Occudin-1 mRNA
p=0.0070
Arbitrary units
Ldlr-/- Vehicle
Ldlr-/- LP533401
J
p=0.0289
Lipid / Aorta area (%)
Ldlr-/- Vehicle
Ldlr-/- LP533401
Ldlr-/- Vehicle
Ldlr-/- LP533401
2mm
2mm
K
Plasma cholesterol (g/l)
Ldlr-/- Vehicle
Ldlr-/- LP533401

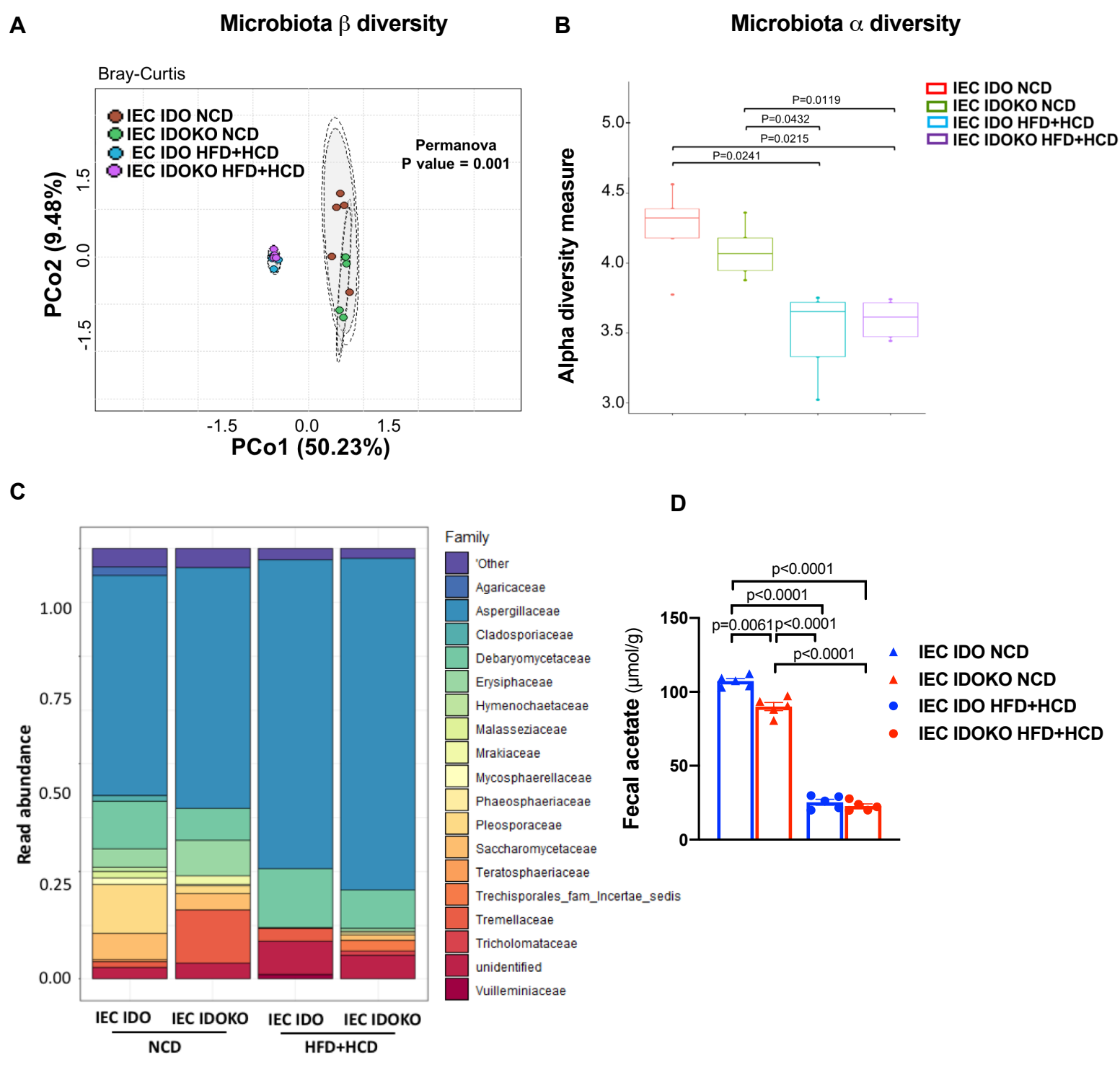
Supplementary Fig. 8: effects of Kyn supplementation and the inhibition of 5-HT production. A. Kynurenine (Kyn) levels in feces of male *Ldlr*$^{-/-}$ IEC IDO KO mice supplemented or not with Kyn and fed high-fat and high-cholesterol diet (HFD+HCD) for 8 weeks (n=5 mice/group), **B.** correlation between plaque size and fecal Kyn in *Ldlr*$^{-/-}$ IEC IDO KO mice supplemented or not with Kyn during the 8 weeks of HFD+HCD (Spearman correlation). **C.** 5-hydroxytryptamine (HT) levels in the small intestine extracts of male *Ldlr*$^{-/-}$ mice fed NCD or HFD+HCD for 8 weeks (n=5 mice/group). **D.** 5-HT levels in blood, **E.** fecal IAA and indole levels, **F.** Kyn/Trp ratio (%) in the small intestine extracts of male *Ldlr*$^{-/-}$ mice treated with LP533401 or vehicle and fed HFD+HCD for 8 weeks (n=5 mice/group). **G.** Kyn/Trp ratio (%) in the plasma of male *Ldlr*$^{-/-}$ mice treated with LP533401 or vehicle and fed HFD+HCD for 8 weeks (*Ldlr*$^{-/-}$ Vehicle n=8 mice, *Ldlr*$^{-/-}$ LP533401 n=7 mice). **H.** *Reg3g, Reg3b mRNA* in the small intestines of male *Ldlr*$^{-/-}$ mice treated or not with LP533401 during the 8 weeks of HFD+HCD (*Ldlr*$^{-/-}$ Vehicle n=8 mice, *Ldlr*$^{-/-}$ LP533401 n=5 mice), **I.** *Occludin-1 mRNA* in the small intestines of male *Ldlr*$^{-/-}$ mice treated or not with LP533401 during the 8 weeks of HFD+HCD (n=7 mice/group). **J.** representative photomicrographs and lipid quantification with en-face staining in the thoracic aorta; scale bar 2mm, **K.** plasma cholesterol levels in male *Ldlr*$^{-/-}$ treated with LP533401 or vehicle and fed HFD+HCD for 8 weeks (*Ldlr*$^{-/-}$ Vehicle n=8 mice, *Ldlr*$^{-/-}$ LP533401 n=7 mice). Individual data are presented as scattered dot plots, with the mean and s.e.m. The p values were determined using the two-tailed Mann-Whitney test. Source data are provided as a Source Data file.


A
p<0.0001
Blood 5-HT (nM)
50
40
30
20
10
0
Ldlr-/- Vehicle
Ldlr-/- 5-HTP
B
p=0.0083
FITC Dextran (µg/ml)
3×10^1
2×10^1
1×10^1
0
Ldlr-/- Vehicle
Ldlr-/- 5-HTP
C
Plasma cholesterol (g/l)
20
15
10
5
0
Ldlr-/- Vehicle
Ldlr-/- 5-HTP
D
Ldlr-/- Vehicle
Ldlr-/- 5-HTP
X4
X4
p=0.0556
Plaque size (µm2)
6×10^5
5×10^5
4×10^5
3×10^5
2×10^5
1×10^5
Ldlr-/- Vehicle
Ldlr-/- 5-HTP
E
r = 0.599
p = 0.008
Plaque size (µm2)
6×10^5
4×10^5
2×10^5
0
0
10
20
30
FITC Dextran (µg/ml)
F
r = 0.510
p = 0.030
Plaque size (µm2)
6×10^5
4×10^5
2×10^5
0
0
10
20
30
40
50
Blood 5-HT (nM)

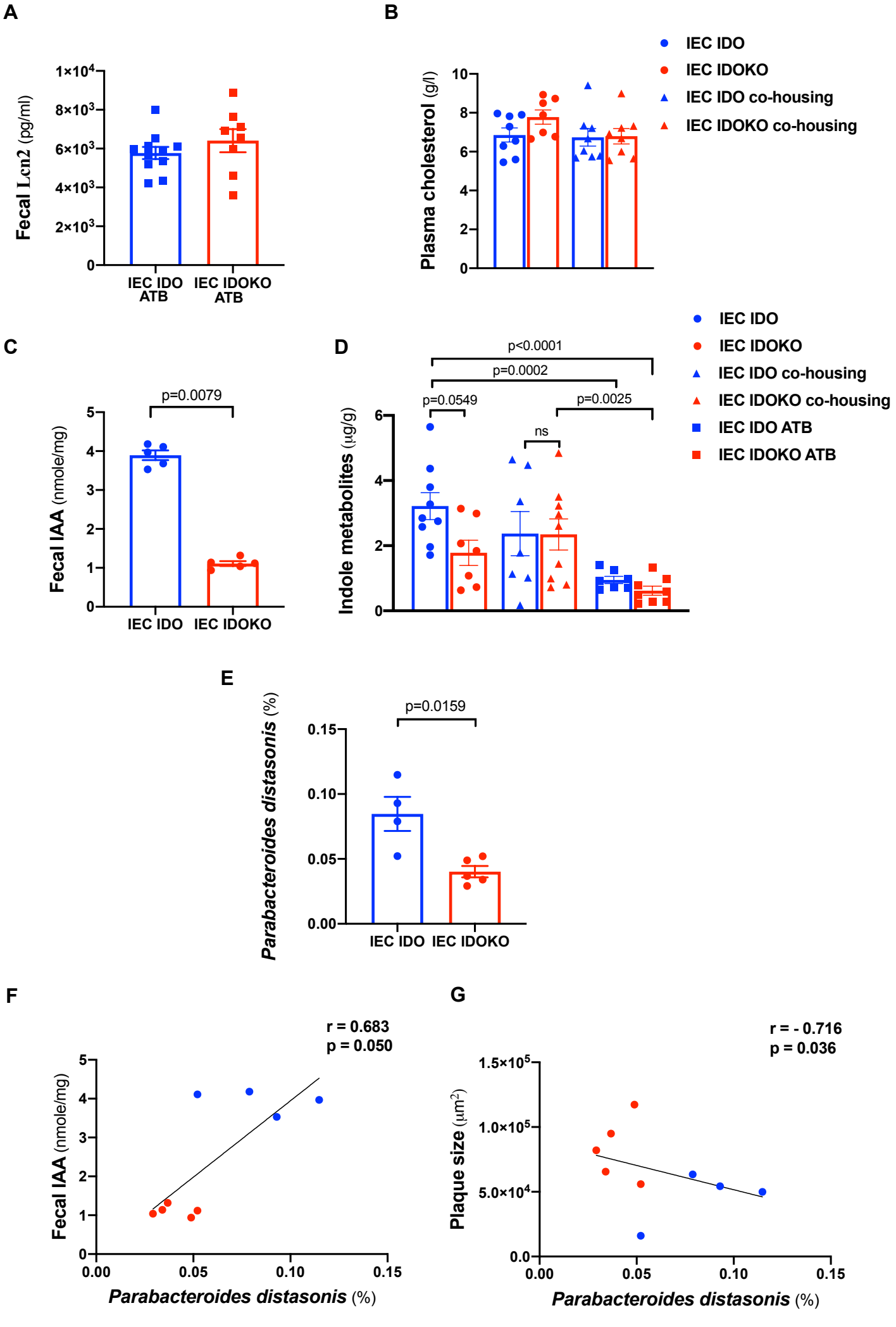
Supplementary Fig. 9: effects of serotonin supplementation. A. blood 5-hydroxytryptamine (HT) levels, **B.** serum FITC-dextran levels, **C.** plasma cholesterol, **D.** representative images and plaque quantifications in the aortic sinus of male *ldlr*$^{-/-}$ fed high-cholesterol diet (HCD) supplemented or not with 5-hydroxytryptophan (HTP) (*Ldlr*$^{-/-}$ Vehicle n=7 mice, *Ldlr*$^{-/-}$ 5-HTP n=11 mice); scale bar 200µm**. E-F.** correlations between plaque size with serum FITC-dextran and blood 5-HT (Spearman correlation). Individual data are presented as scattered dot plots, with the mean and s.e.m. The p values were determined using the two-tailed Mann-Whitney test. Source data are provided as a Source Data file.


A
p=0.0159
Intestinal 5-HT (fmole/mg)
8×10^4
6×10^4
4×10^4
2×10^4
0
IEC IDO
IEC IDOKO
B
r = 0.683
p = 0.050
Plaque size (µm^2)
1×10^5
8×10^4
6×10^4
4×10^4
2×10^4
0
3×10^4
5×10^4
7×10^4
Intestinal 5-HT (fmole/mg)
C
IEC IDO Vehicle
IEC IDOKO Vehicle
IEC IDO LP533401
IEC IDOKO LP533401
Plasma cholesterol (g/l)
10
8
6
4
2
0
D
r = 0.506
p = 0.022
Plaque size (µm^2)
1.5×10^5
1.0×10^5
5.0×10^4
0.0
6.0×10^3
1.0×10^4
1.4×10^4
Blood 5-HT (nM)

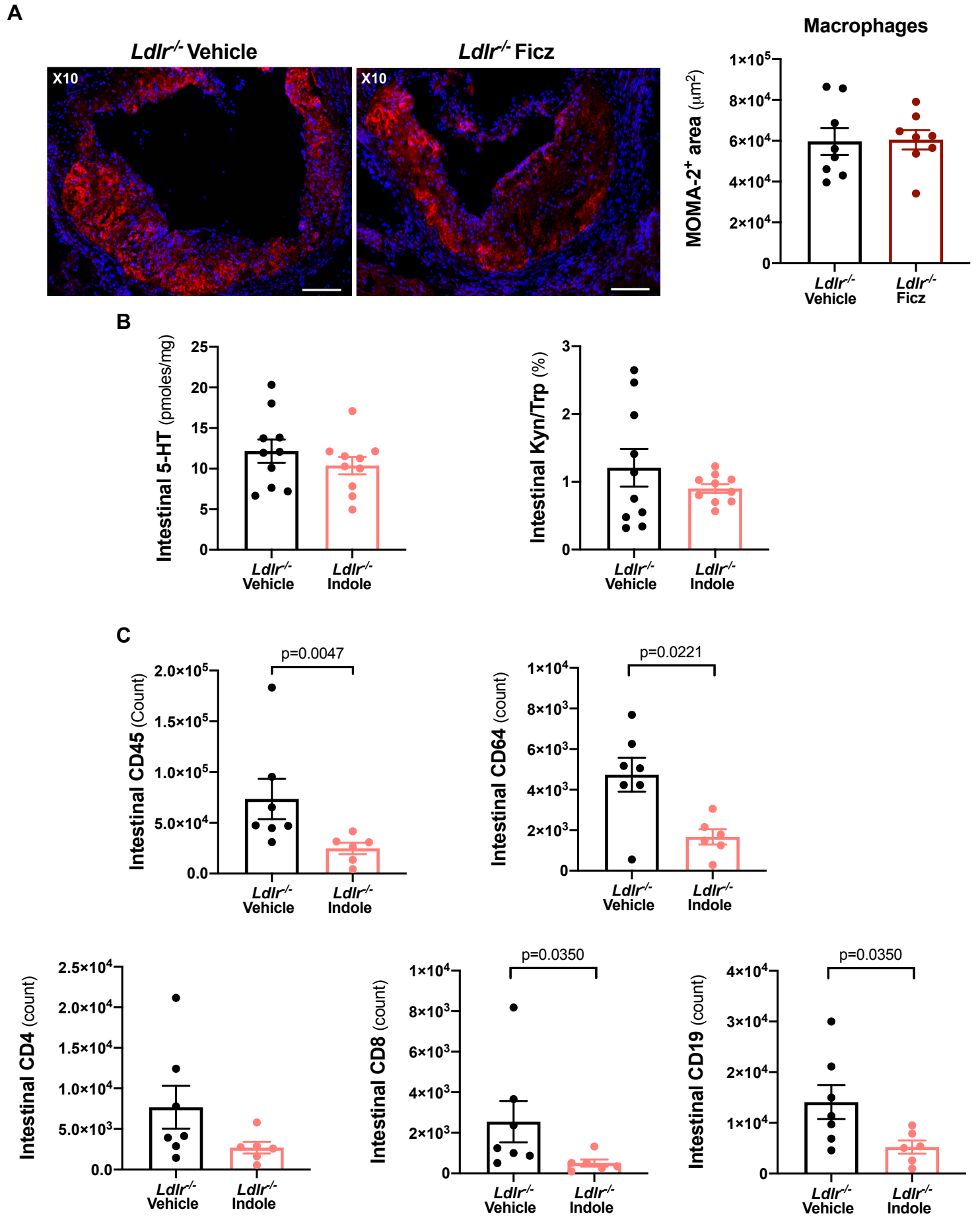
Supplementary Fig. 10: characterization of serotonin production in the model with intestinal IDO deletion. A. 5- (hydroxytryptamine) HT levels in the intestines in male *Ldlr*$^{-/-}$ IEC IDO KO and littermate control *Ldlr*$^{-/-}$ IEC IDO mice fed high-fat and high-cholesterol diet (HFD+HCD) for 8 weeks (n=5 mice/group), **B.** correlation between plaque size and 5-HT levels in the gut (Spearman correlation). **C.** plasma cholesterol levels in male *Ldlr*$^{-/-}$ IEC IDO KO and littermate control *Ldlr*$^{-/-}$ IEC IDO mice treated with LP5333401 or vehicle and fed HFD+HCD for 8 weeks (IEC IDO Vehicle n=11 mice, IEC IDO KO Vehicle n=7 mice, IEC IDO LP533401 n=11 mice, IEC IDO KO LP533401 n=9 mice). **D.** correlation between plaque size and blood 5-HT (Spearman correlation). Individual data are presented as scattered dot plots, with the mean and s.e.m. The p values were determined using the two-tailed Mann-Whitney test for A. Source data are provided as a Source Data file.


A
Microbiota β diversity
Bray-Curtis
IEC IDO NCD
IEC IDOKO NCD
EC IDO HFD+HCD
IEC IDOKO HFD+HCD
Permanova
P value = 0.001
PCo2 (9.48%)
PCo1 (50.23%)
B
Microbiota α diversity
IEC IDO NCD
IEC IDOKO NCD
IEC IDO HFD+HCD
IEC IDOKO HFD+HCD
P=0.0119
P=0.0432
P=0.0215
P=0.0241
Alpha diversity measure
C
Family
'Other
Agaricaceae
Aspergillaceae
Cladosporiaceae
Debaryomycetaceae
Erysiphaceae
Hymenochaetaceae
Malasseziaceae
Mrakiaceae
Mycosphaerellaceae
Phaeosphaeriaceae
Pleosporaceae
Saccharomycetaceae
Teratosphaeriaceae
Trechisporales_fam_Incertae_sedis
Tremellaceae
Tricholomataceae
unidentified
Vuilleminiaceae
Read abundance
IEC IDO
IEC IDOKO
IEC IDO
IEC IDOKO
NCD
HFD+HCD
D
p<0.0001
p<0.0001
p=0.0061
p<0.0001
p<0.0001
Fecal acetate (µmol/g)
IEC IDO NCD
IEC IDOKO NCD
IEC IDO HFD+HCD
IEC IDOKO HFD+HCD

Supplementary Fig. 11: characterization of gut microbiota in mice expressing or deficient for intestinal IDO. A. PCoA plot based on bacterial 16S rDNA gene relative abundance using the Bray-Curtis dissimilarity in fecal contents of male *Ldlr*$^{-/-}$ IEC IDO KO and littermate control *Ldlr*$^{-/-}$ IEC IDO mice fed with either normal chow diet (NCD) or high-fat and high-cholesterol diet (HFD+HCD) for 8 weeks (n=5 mice/group). Axes correspond to principal coordinate axis 1 (x-axis) and 2 (y-axis). Ellipses were drawn around the centroids of each emerging community (PERMANOVA: F = 6.4231, df = 3, P = 0.001) at 95% (inner) and 97% (outer) confidence intervals. **B.** bacterial diversity based on the Shannon index in the fecal samples (n=5 mice/group). **C.** Barplots at the family level in the fecal samples of male *Ldlr*$^{-/-}$ IEC IDO KO and littermate control *Ldlr*$^{-/-}$ IEC IDO mice fed either NCD or HFD+HCD for 8 weeks (n=5 mice/group). **D.** fecal acetate levels in male *Ldlr*$^{-/-}$ IEC IDO KO and littermate control *Ldlr*$^{-/-}$ IEC IDO mice fed with either NCD or HFD+HCD for 8 weeks (n=5 mice/group). Individual data are presented as scattered dot plots, with the mean and s.e.m. The p values were determined using Brown-Forsythe ANOVA test followed by Tukey's multiple comparison for B and D. Source data are provided as a Source Data file.

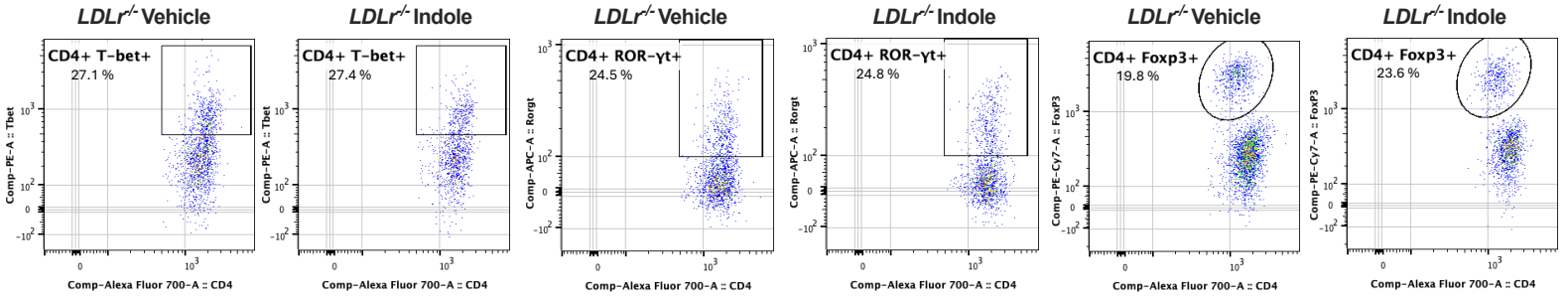

A
Fecal Lcn2 (pg/ml)
1×10^4
8×10^3
6×10^3
4×10^3
2×10^3
0
IEC IDO ATB
IEC IDOKO ATB
B
Plasma cholesterol (g/l)
10
8
6
4
2
0
IEC IDO
IEC IDOKO
IEC IDO co-housing
IEC IDOKO co-housing
C
Fecal IAA (nmole/mg)
p=0.0079
5
4
3
2
1
0
IEC IDO
IEC IDOKO
D
Indole metabolites (µg/g)
p<0.0001
p=0.0002
p=0.0549
p=0.0025
ns
6
4
2
0
IEC IDO
IEC IDOKO
IEC IDO co-housing
IEC IDOKO co-housing
IEC IDO ATB
IEC IDOKO ATB
E
Parabacteroides distasonis (%)
p=0.0159
0.15
0.10
0.05
0.00
IEC IDO
IEC IDOKO
F
r = 0.683
p = 0.050
Fecal IAA (nmole/mg)
5
4
3
2
1
0
0.00
0.05
0.10
0.15
Parabacteroides distasonis (%)
G
r = - 0.716
p = 0.036
Plaque size (µm2)
1.5×10^5
1.0×10^5
5.0×10^4
0.0
0.00
0.05
0.10
0.15
Parabacteroides distasonis (%)

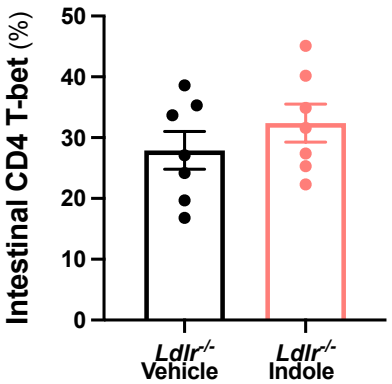
Supplementary Fig. 12: effects of antibiotics and co-housing in mice expressing or deficient for intestinal IDO A. lipocalin-2 (Lcn2) levels in feces of male *Ldlr*$^{-/-}$ IEC IDO KO and littermate control *Ldlr*$^{-/-}$ IEC IDO mice treated with antibiotics (ATB) during the 8 weeks of HFD+HCD feeding period (IEC IDO ATB n=11 mice, IEC IDO KO ATB n=8 mice. **B.** plasma cholesterol levels in male *Ldlr*$^{-/-}$ IEC IDO KO and littermate control *Ldlr*$^{-/-}$ IEC IDO mice either separated by the genotype or mixed (co-housing) in the same cages from the weaning. The mice were fed HFD+HCD for 8 weeks (IEC IDO n=8 mice, IEC IDO KO n=7 mice, IEC IDO co-housing n=8 mice, and IEC IDO KO co-housing n=8 mice). **C.** IAA levels in feces from male *Ldlr*$^{-/-}$ IEC IDO KO and littermate control *Ldlr*$^{-/-}$ IEC IDO mice after 8 weeks of HFD+HCD feeding period (n=5 mice/group). **D.** fecal AhR agonist (IAA, IAld, and tryptamine) levels from male *Ldlr*$^{-/-}$ IEC IDO KO and littermate control *Ldlr*$^{-/-}$ IEC IDO mice after 8 weeks of HFD+HCD feeding period. The mice were either separated according to the genotypes, or mixed (co-housing), or treated with ATB (IEC IDO n=9 mice, IEC IDOKO n=7 mice, IEC IDO co-housing n=7 mice, IEC IDO KO co-housing n=9 mice, IEC IDO ATB n=7 mice, IEC IDO KO ATB n=8 mice). **E.** *Parabacteroides distasonis* relative abundance (%) in feces of male *Ldlr*$^{-/-}$ IEC IDO KO and littermate control *Ldlr*$^{-/-}$ IEC IDO mice fed HFD+HCD for 8 weeks (IEC IDO n=4 mice, IEC IDOKO n=5 mice). **F-G**. correlation between *Parabacteroides distasonis* relative abundance and fecal IAA and plaque size (Spearman correlation). Individual data are presented as scattered dot plots, with the mean and s.e.m. The p values were determined using the two-tailed Mann-Whitney test for C, E, and two-tailed Unpaired T test for D. Source data are provided as a Source Data file.

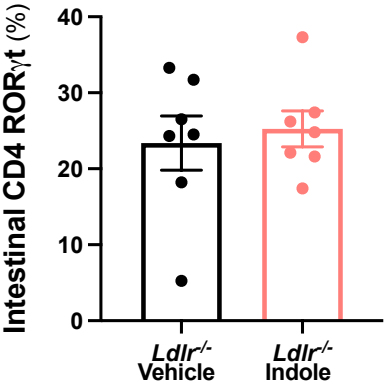

A
Ldlr-/- Vehicle
Ldlr-/- Ficz
X10
X10
Macrophages
MOMA-2+ area (µm2)
1×10^5
8×10^4
6×10^4
4×10^4
2×10^4
0
Ldlr-/- Vehicle
Ldlr-/- Ficz
B
Intestinal 5-HT (pmoles/mg)
25
20
15
10
5
0
Ldlr-/- Vehicle
Ldlr-/- Indole
Intestinal Kyn/Trp (%)
3
2
1
0
Ldlr-/- Vehicle
Ldlr-/- Indole
C
Intestinal CD45 (Count)
p=0.0047
2.0×10^5
1.5×10^5
1.0×10^5
5.0×10^4
0.0
Ldlr-/- Vehicle
Ldlr-/- Indole
Intestinal CD64 (count)
p=0.0221
1×10^4
8×10^3
6×10^3
4×10^3
2×10^3
0
Ldlr-/- Vehicle
Ldlr-/- Indole
Intestinal CD4 (count)
2.5×10^4
2.0×10^4
1.5×10^4
1.0×10^4
5.0×10^3
0.0
Ldlr-/- Vehicle
Ldlr-/- Indole
Intestinal CD8 (count)
p=0.0350
1×10^4
8×10^3
6×10^3
4×10^3
2×10^3
0
Ldlr-/- Vehicle
Ldlr-/- Indole
Intestinal CD19 (count)
p=0.0350
4×10^4
3×10^4
2×10^4
1×10^4
0
Ldlr-/- Vehicle
Ldlr-/- Indole

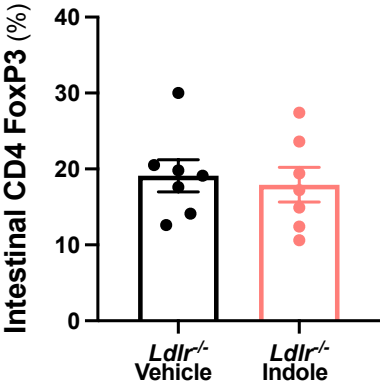
Supplementary Fig. 13: characterization of the effects of aryl hydrocarbon receptor (AhR) agonists. A. representative pictures and quantifications of macrophages (MOMA2+ in red) accumulation within plaques in the aortic sinus of male *Ldlr*$^{-/-}$mice treated with 6-Formylindolo(3,2-b)carbazole (Ficz) or vehicle (n=8 mice/group) during the 8 weeks of high-cholesterol diet (HCD) feeding period); scale bar 100µm. **B.** 5-HT and Kyn/Trp (%) in the small intestine of female *Ldlr*$^{-/-}$mice supplemented or not with indole derivatives (IAA, IPA, and tryptamine) (n=10 mice/group). **C.** flow cytometry analysis of CD45+, CD64+, CD4+, CD8+, and CD19+ cells (count) in the lamina propria of the small intestines of female *Ldlr*$^{-/-}$mice supplemented or not with indole derivatives (IAA, IPA, and tryptamine) (*Ldlr*$^{-/-}$ Vehicle n=7 mice, *Ldlr*$^{-/-}$ Indole n=6 mice). Individual data are presented as scattered dot plots, with the mean and s.e.m. The p values were determined using the two-tailed Mann-Whitney test. Source data are provided as a Source Data file.

A

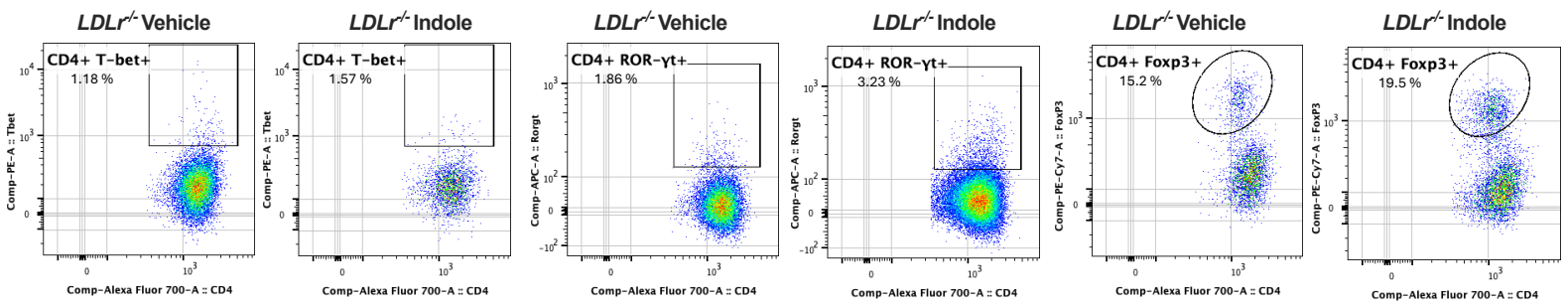

LDLr-/- Vehicle
CD4+ T-bet+
27.1 %
LDLr-/- Indole
CD4+ T-bet+
27.4 %
LDLr-/- Vehicle
CD4+ ROR-γt+
24.5 %
LDLr-/- Indole
CD4+ ROR-γt+
24.8 %
LDLr-/- Vehicle
CD4+ Foxp3+
19.8 %
LDLr-/- Indole
CD4+ Foxp3+
23.6 %
Comp-PE-A :: Tbet
Comp-APC-A :: Rorgt
Comp-PE-Cy7-A :: FoxP3
Comp-Alexa Fluor 700-A :: CD4

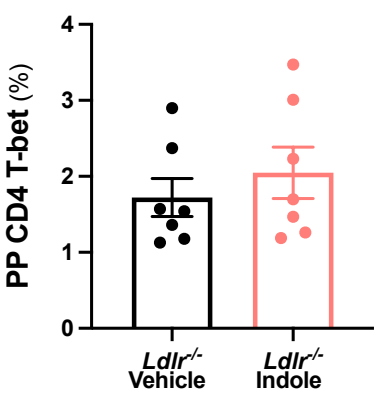

Intestinal CD4 T-bet (%)
Ldlr-/- Vehicle
Ldlr-/- Indole

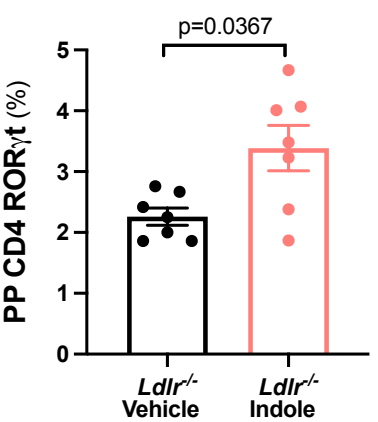

Intestinal CD4 RORγt (%)
Ldlr-/- Vehicle
Ldlr-/- Indole

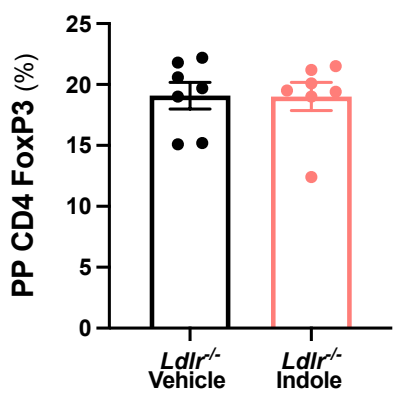

Intestinal CD4 FoxP3 (%)
Ldlr-/- Vehicle
Ldlr-/- Indole

B

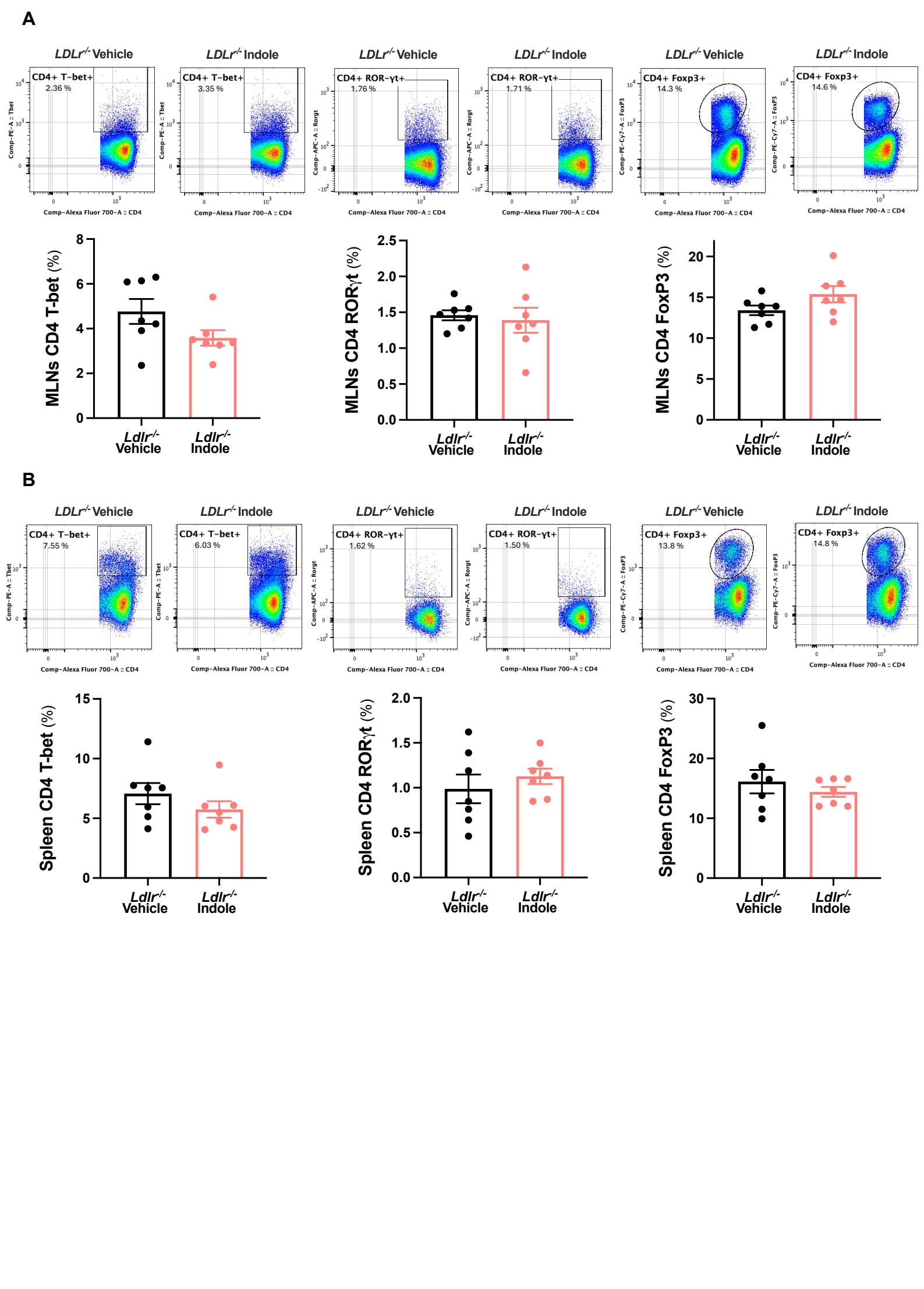

LDLr-/- Vehicle
CD4+ T-bet+
1.18 %
LDLr-/- Indole
CD4+ T-bet+
1.57 %
LDLr-/- Vehicle
CD4+ ROR-γt+
1.86 %
LDLr-/- Indole
CD4+ ROR-γt+
3.23 %
LDLr-/- Vehicle
CD4+ Foxp3+
15.2 %
LDLr-/- Indole
CD4+ Foxp3+
19.5 %
Comp-PE-A :: Tbet
Comp-APC-A :: Rorgt
Comp-PE-Cy7-A :: FoxP3
Comp-Alexa Fluor 700-A :: CD4


PP CD4 T-bet (%)
Ldlr-/- Vehicle
Ldlr-/- Indole


p=0.0367
PP CD4 RORγt (%)
Ldlr-/- Vehicle
Ldlr-/- Indole


PP CD4 FoxP3 (%)
Ldlr-/- Vehicle
Ldlr-/- Indole

Supplementary Fig. 14: characterization of effects of indole supplementation on Th subsets by flow cytometry in small intestines and PPs. Representative plots and flow cytometry analysis of Th subset (as % of CD4+). The different T cell subsets were identified according to the T-bet, RORγt and FoxP3 expression in the lamina propria of small intestines **(A)** and Peyer's Patches (PP) **(B**), of female *Ldlr*$^{-/-}$mice supplemented or not with indole derivatives (IAA, IPA, and tryptamine) (n=7 mice/group). Individual data are presented as scattered dot plots, with the mean and s.e.m. The p values were determined using the two-tailed Mann-Whitney test. Source data are provided as a Source Data file.


A
LDLr-/- Vehicle
LDLr-/- Indole
LDLr-/- Vehicle
LDLr-/- Indole
LDLr-/- Vehicle
LDLr-/- Indole
CD4+ T-bet+
2.36 %
CD4+ T-bet+
3.35 %
CD4+ RORγt+
1.76 %
CD4+ RORγt+
1.71 %
CD4+ Foxp3+
14.3 %
CD4+ Foxp3+
14.6 %
Comp-PE-A :: Tbet
Comp-APC-A :: Rorgt
Comp-PE-Cy7-A :: FoxP3
Comp-Alexa Fluor 700-A :: CD4
MLNs CD4 T-bet (%)
MLNs CD4 RORγt (%)
MLNs CD4 FoxP3 (%)
Ldlr-/- Vehicle
Ldlr-/- Indole
B
LDLr-/- Vehicle
LDLr-/- Indole
LDLr-/- Vehicle
LDLr-/- Indole
LDLr-/- Vehicle
LDLr-/- Indole
CD4+ T-bet+
7.55 %
CD4+ T-bet+
6.03 %
CD4+ RORγt+
1.62 %
CD4+ RORγt+
1.50 %
CD4+ Foxp3+
13.8 %
CD4+ Foxp3+
14.8 %
Comp-PE-A :: Tbet
Comp-APC-A :: Rorgt
Comp-PE-Cy7-A :: FoxP3
Comp-Alexa Fluor 700-A :: CD4
Spleen CD4 T-bet (%)
Spleen CD4 RORγt (%)
Spleen CD4 FoxP3 (%)
Ldlr-/- Vehicle
Ldlr-/- Indole

Supplementary Fig. 15: characterization of effects of indole supplementation on Th subsets by flow cytometry in MLNs and spleens. Representative plots and flow cytometry analysis of Th subset (as % of CD4+). The different T cell subsets were identified according to the T-bet, RORγt and FoxP3 expression in the lamina propria of Mesenteric Lymph Nodes (MLNs) **(A)**, and spleens (**B**) of female *Ldlr*$^{-/-}$mice supplemented or not with indole derivatives (IAA, IPA, and tryptamine) (n=7 mice/group). Individual data are presented as scattered dot plots, with the mean and s.e.m. Source data are provided as a Source Data file.