Supplemental table and figure legends

Table S1 Diets composition used in this study (related to Diet section of STAR Methods)

Figure S1 (related to fig 1&2). Impact of maternal lactation diet on intestine.

Figure S2 (related to fig 3&4). Impact of maternal lactation diet on offspring DIO proneness was alleviated by antibiotics or co-housing.

Figure S3 (related to fig 5). Impact of maternal lactation diet on 12-week-old offspring.

Figure S4 (related to fig 5). Food restriction largely normalized DIO severity in offspring of fiber deprived dams.

Figure S5 (related to fig 6). Enrichment of maternal diet with inulin ameliorated low graded inflammation in offspring.

Fig S6 (related to fig 7). Enrichment of maternal diet with inulin reduced offspring proneness to DIO.

Table S1. Diets composition used in this study.

Product #	**LabDiet 5001**		**D12450J**		**D13081108**		**D12492**	
	GBC		**LF-LFD**		**IE-LFD**		**LF-HFD**	
	gm%	kcal%	gm%	kcal%	gm%	kcal%	gm%	kcal%
Protein	24.1	28.672	19	20	18	20	26	20
Carbohydrate	48.7	57.9	67	70	74	70	26	20
Fat	5	13.384	4	10	4	10	35	60
Fiber	15-25		5		20		5	
Total		100		100		100		100
kcal/gm	3.36		3.8		3.5		5.2	
Ingredient			gm	kcal	gm	kcal	gm	kcal
Casein			200	800	200	800	200	800
L-Cystine			3	12	3	12	3	12
Corn Starch			506.2	2025	456.2	1825	0	0
Maltodextrin 10			125	500	125	500	125	500
Sucrose			68.8	275	68.8	275	68.8	275
Cellulose, BW200			50	0	0	0	50	0
Inulin			0	0	200	200	0	0
Soybean Oil			25	225	25	225	25	225
Lard			20	180	20	180	245	2205
Mineral Mix, S10026			10	0	10	0	10	0
DiCalcium Phosphate			13	0	13	0	13	0
Calcium Carbonate			5.5	0	5.5	0	5.5	0
Potassium Citrate, 1 H2O			16.5	0	16.5	0	16.5	0
Vitamin Mix, V10001			10	40	10	40	10	40
Choline Bitartrate			2	0	2	0	2	0
FD&C Yellow Dye #5			0.04	0	0.025	0	0	0
FD&C Red Dye #40			0	0	0	0	0	0
FD&C Blue Dye #1			0.01	0	0.025	0	0.05	0
Total			1055.1	4057	1155.1	4057	773.85	4057

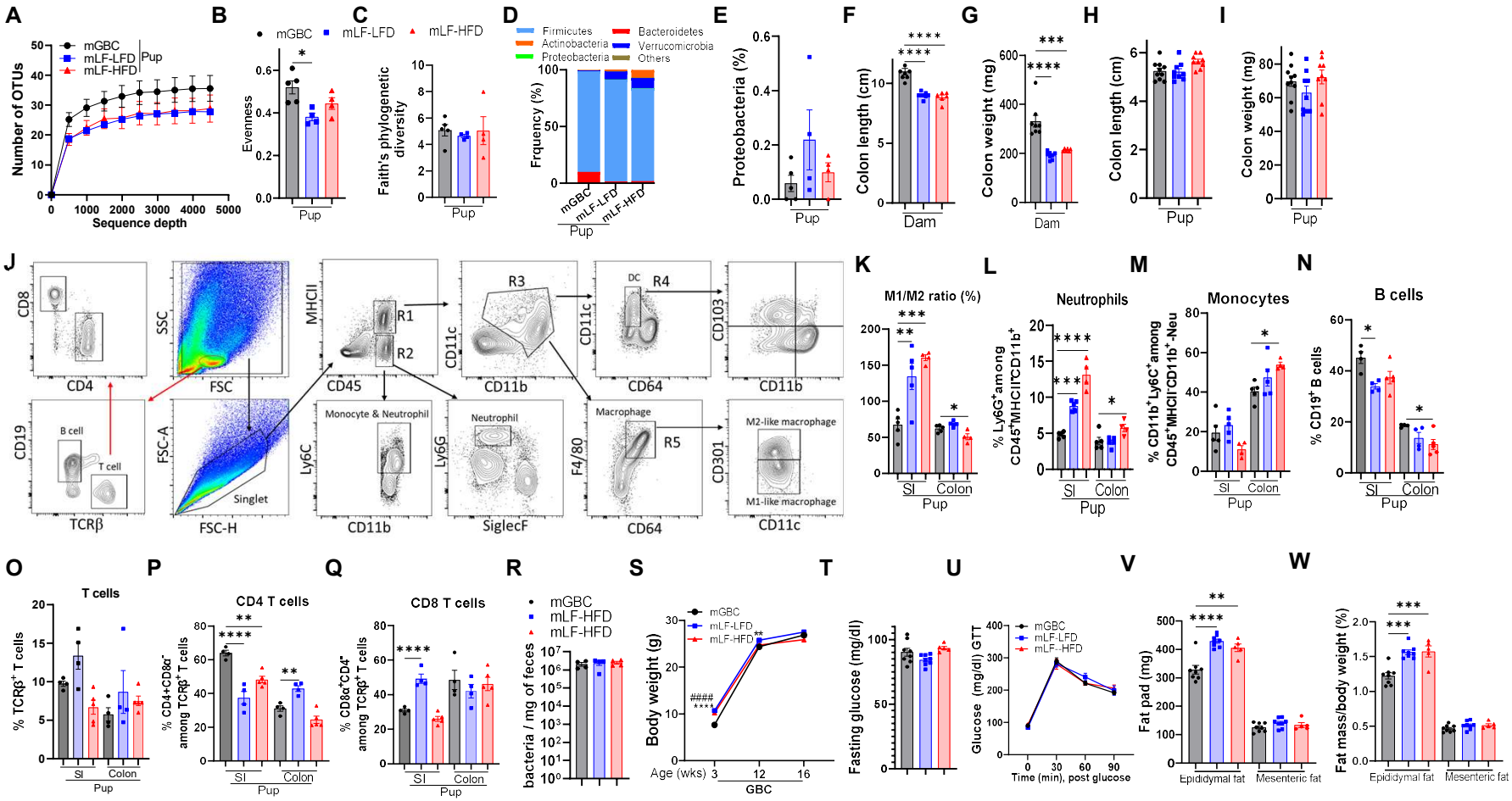
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P
Q
R
S
T
U
V
W
mGBC
mLF-LFD
mLF-HFD
Pup
Number of OTUs
Sequence depth
Evenness
Faith's phylogenetic diversity
Firmicutes
Actinobacteria
Proteobacteria
Bacteroidetes
Verrucomicrobia
Others
Frquency (%)
Proteobacteria (%)
Colon length (cm)
Colon weight (mg)
Dam
CD8
CD4
SSC
FSC
MHCII
R1
R2
CD11c
CD11b
R3
CD45
R4
DC
CD64
CD103
CD19
B cell
T cell
TCRβ
FSC-A
FSC-H
Singlet
Monocyte & Neutrophil
Ly6C
Neutrophil
Ly6G
SiglecF
Macrophage
R5
F4/80
M2-like macrophage
M1-like macrophage
CD301
M1/M2 ratio (%)
Neutrophils
Monocytes
B cells
% CD19+ B cells
SI
Colon
T cells
% TCRβ+ T cells
CD4 T cells
CD8 T cells
bacteria / mg of feces
Body weight (g)
Age (wks)
GBC
Fasting glucose (mg/dl)
Glucose (mg/dl) GTT
Time (min), post glucose
Fat pad (mg)
Epididymal fat
Mesenteric fat
Fat mass/body weight (%)

Figure S1 (related to fig 1&2). Impact of maternal lactation diet on intestine.

(**A-E**). Ileal microbiota composition of pups via 16S rRNA gene sequencing. Observed OTUs (A), Pielou's evenness (B), Faith's phylogenetic diversity (C); Overall relative phylogenetic abundance (D), and percent Proteobacteria (E).

(**F-I**) Colon length and weights of indicated dams (F&G) and their 3-week-old female offspring (H&I).

(J-Q) Lamina propria cells from small intestine and colon were analyzed by FACS as gated in (J). M1 and M2 macrophage, neutrophils, monocytes, B and T cells in small intestine (SI) and colon were quantitated (K-Q).

(R) qPCR measure of bacteria per mg feces of 12-week-old offspring mice.

(S-W) Metabolic measurements in 3-week-old male offspring weaned onto GBC. Body weight was monitored (S). At 16 weeks of age, overnight fasting glucose (T) and glucose tolerance (U) was measured. Epididymal, mesenteric fat including absolute value (V) and in relation to body weight (W) were measured after euthanasia. One-way ANOVA: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$; $^{\#\#\#\#}p < 0.0001$. In S, *indicated mLF-LFD vs mGBC; $^{\#}$ indicated mLF-HFD vs mGBC.

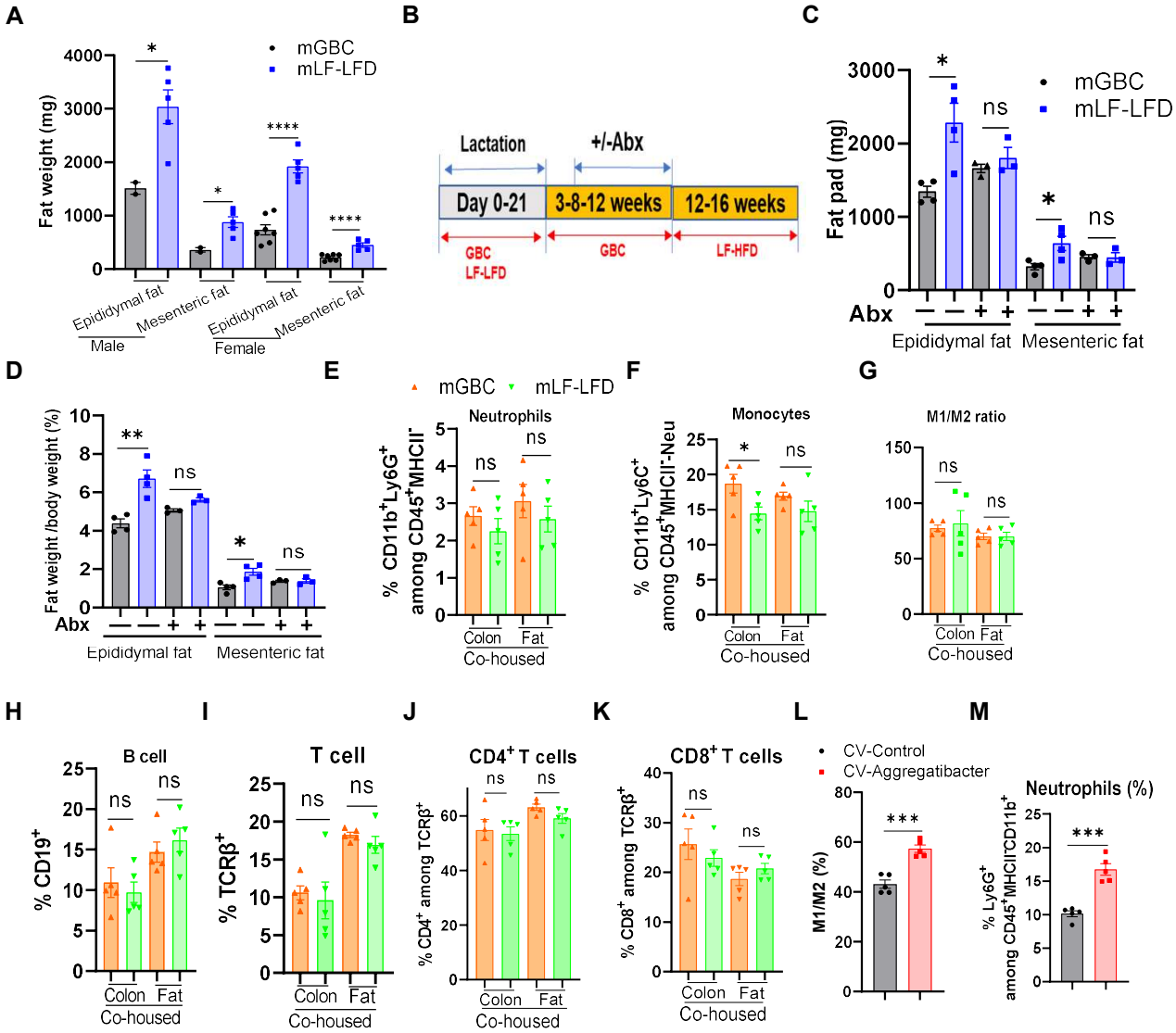
A
mGBC
mLF-LFD
Fat weight (mg)
Epididymal fat
Mesenteric fat
Male
Female
B
Lactation
+/-Abx
Day 0-21
3-8-12 weeks
12-16 weeks
GBC
LF-LFD
GBC
LF-HFD
C
Fat pad (mg)
ns
Abx
Epididymal fat
Mesenteric fat
D
Fat weight /body weight (%)
E
Neutrophils
% CD11b+Ly6G+ among CD45+MHCII-
F
Monocytes
% CD11b+Ly6C+ among CD45+MHCII-Neu
G
M1/M2 ratio
Colon
Fat
Co-housed
H
B cell
% CD19+
I
T cell
% TCRβ+
J
CD4+ T cells
% CD4+ among TCRβ+
K
CD8+ T cells
% CD8+ among TCRβ+
L
CV-Control
CV-Aggregatibacter
M1/M2 (%)
M
Neutrophils (%)
% Ly6G+ among CD45+MHCII+CD11b+

Figure S2 (related to fig 3&4). Impact of maternal lactation diet on offspring DIO proneness was alleviated by antibiotics or co-housing.

(A) Two dams nursing 9-10 mice in one cage were fed indicated diets during lactation. Offspring were weaned at 3 weeks of age onto GBC then, at 12 weeks of age, fed LF-HFD. Epididymal and mesenteric fat mass at the end of experiment.

(**B-D**) Scheme**:** Some offspring were subjected to microbiota ablation via administration of drinking water containing broad-spectrum antibiotics starting 8 weeks of age and ending before initiation of LF-HFD feeding at 12 weeks of age (B). Epididymal and mesenteric fat mass, gross (C) and calculated as percentage of body weight (D)**.**

(**E-K**) FACS analysis of colons and epididymal adipose tissue from co-housed offspring mice following a 4-week course of LF-HFD consumption.

(L&M) FACS analysis of colon following 4 weeks of Aggregatibacter strain administration. Student's t test: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$, ns, not significant.

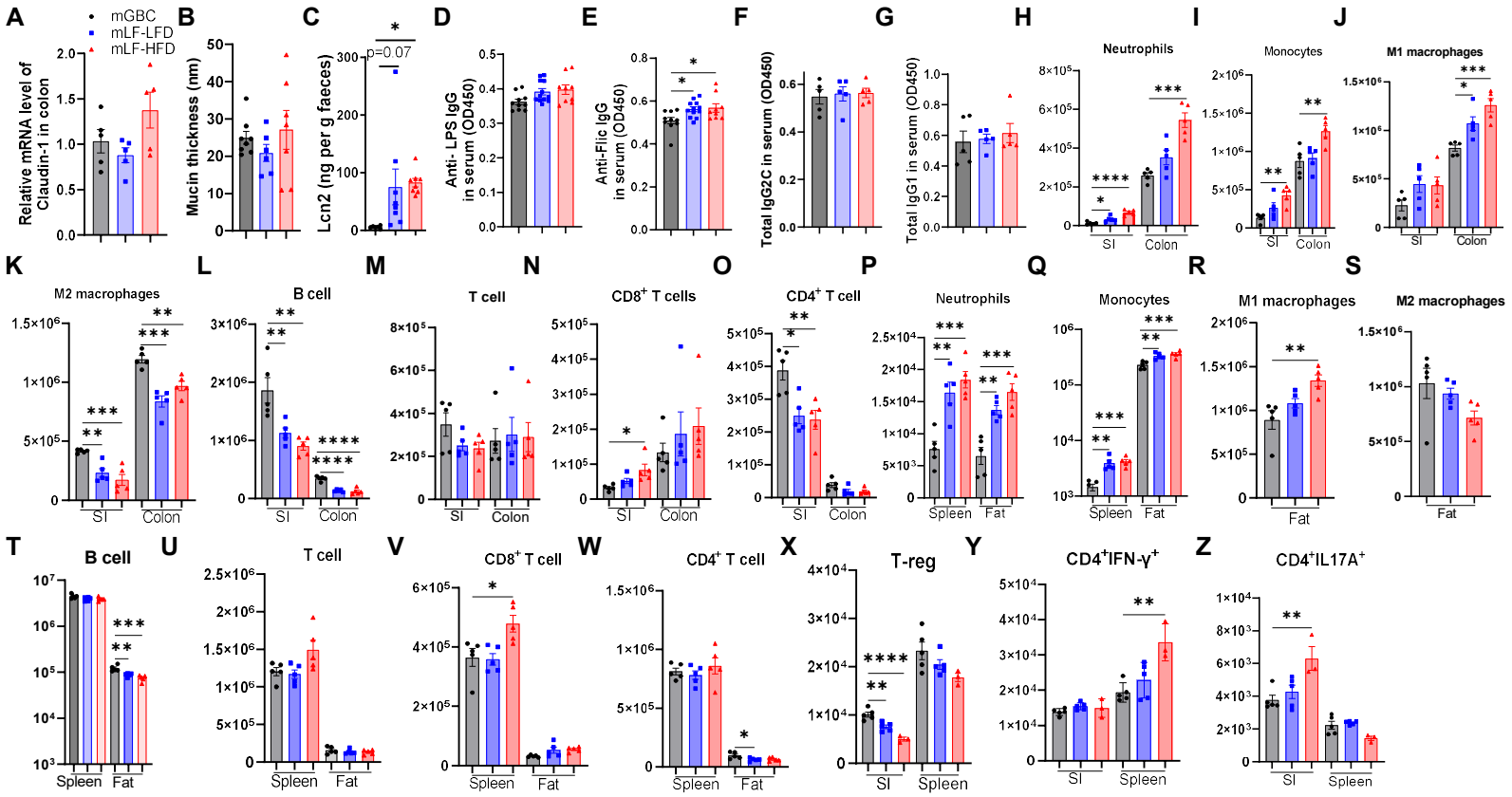
A
mGBC
mLF-LFD
mLF-HFD
Relative mRNA level of Claudin-1 in colon
B
Mucin thickness (nm)
C
Lcn2 (ng per g faeces)
p=0.07
D
Anti- LPS IgG in serum (OD450)
E
Anti-Flic IgG in serum (OD450)
F
Total IgG2C in serum (OD450)
G
Total IgG1 in serum (OD450)
H
Neutrophils
I
Monocytes
J
M1 macrophages
SI
Colon
K
M2 macrophages
L
B cell
M
T cell
N
CD8+ T cells
O
CD4+ T cell
P
Neutrophils
Q
Monocytes
R
M1 macrophages
S
M2 macrophages
Spleen
Fat
T
B cell
U
T cell
V
CD8+ T cell
W
CD4+ T cell
X
T-reg
Y
CD4+IFN-γ+
Z
CD4+IL17A+

Figure S3 (related to fig 5). Impact of maternal lactation diet on 12-week-old offspring.

(A) Colon claudin-1 expression via qRT-PCR.

(B) Colon mucus thickness.

(C) Fecal Lcn2.

(D&E) Serum anti-LPS (D) and anti-flagellin (E).

(F&G) Total serum IgG2C (F) and IgG1 (G).

(H-Z) Quantitation of neutrophils (Neu), monocytes, M1/M2 macrophage, B and T lymphocytes including T-cell subset in small intestine (SI), colon, spleen or epididymal fat by FACS analysis. The number of different immune cells population was showed. One-way ANOVA: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$.

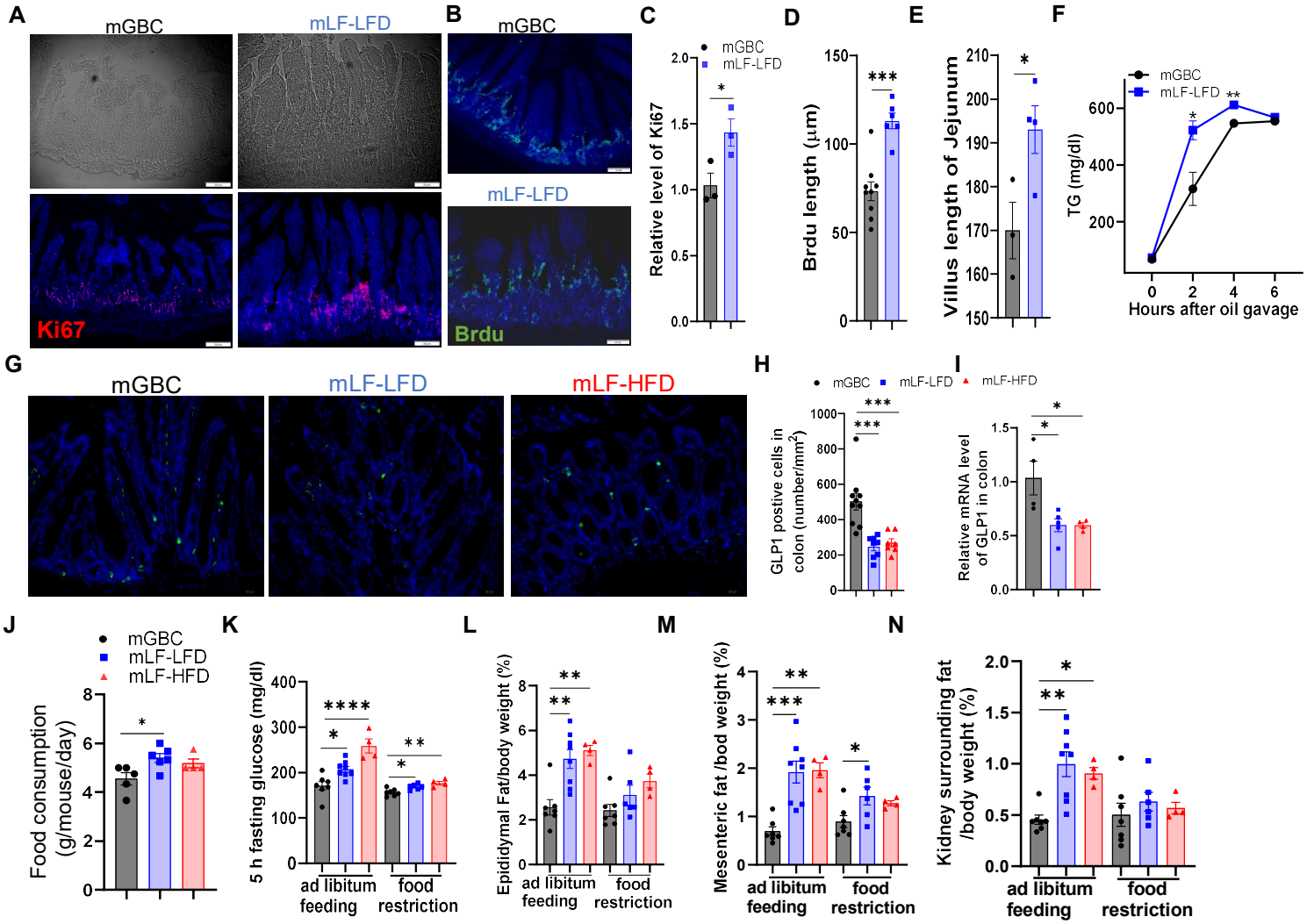
A
mGBC
mLF-LFD
Ki67
B
mGBC
mLF-LFD
Brdu
C
mGBC
mLF-LFD
Relative level of Ki67
D
Brdu length (μm)
E
Villus length of Jejunum
F
mGBC
mLF-LFD
TG (mg/dl)
Hours after oil gavage
G
mGBC
mLF-LFD
mLF-HFD
H
mGBC
mLF-LFD
mLF-HFD
GLP1 postive cells in colon (number/mm2)
I
Relative mRNA level of GLP1 in colon
J
mGBC
mLF-LFD
mLF-HFD
Food consumption (g/mouse/day)
K
5 h fasting glucose (mg/dl)
ad libitum feeding
food restriction
L
Epididymal Fat/body weight (%)
ad libitum feeding
food restriction
M
Mesenteric fat /bod weight (%)
ad libitum feeding
food restriction
N
Kidney surrounding fat /body weight (%)
ad libitum feeding
food restriction

Figure S4 (related to fig 5). Food restriction largely normalized DIO severity in offspring of fiber deprived dams.

A-J, 12-week-old GBC-fed offspring of dams fed indicating lactating diet(m) were assayed.

(**A-C**) Measure of enterocyte proliferation/migration in 12-week-old offspring, Ki67 staining (A&C), Brdu labeling (B&D) and jejunal villus length (E).

(F) Fat absorption as indicated by levels of serum TG following injection of tyloxapol and orally gavaged with olive oil

(**G-H**) Colonic GLP^+ L cells quantitated by immunofluorescence microscopy.

(**I**) Colonic epithelial GLP1 expression by qRT-PCR.

(J) Offspring mice fed with GBC at 12 weeks of age were measured for food consumption.

(**K-N)** 12-week-old offspring of mice fed indicating lactating diet(m) were administer LF-HFD fed ad libitum or restricted (2.8g/per day) for 10 days at which time indicated parameters were measured. Student's t test (C, D, E, F). One-way ANOVA (H, I, J, K, L, M, N). *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$.

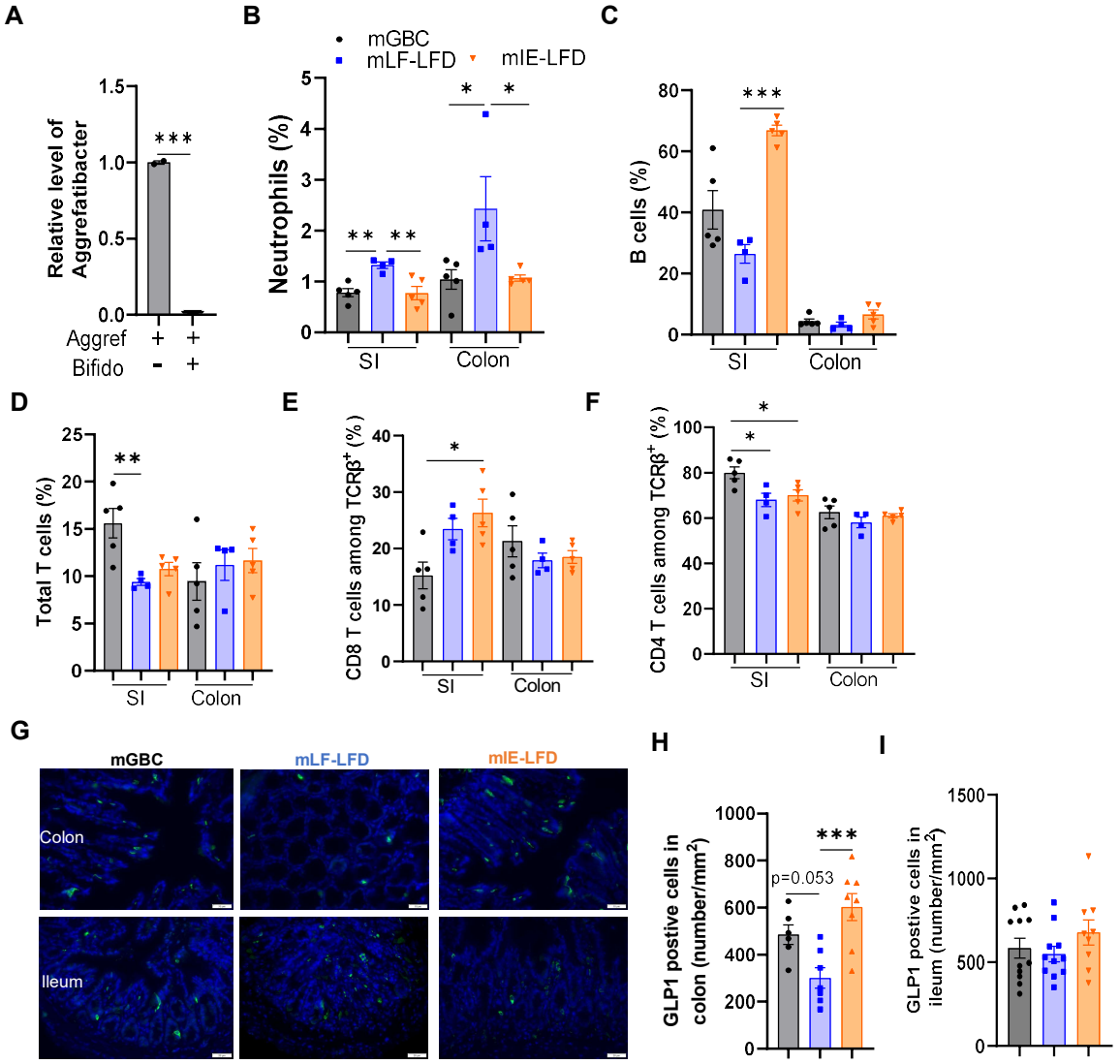
A
Relative level of Aggrefatibacter
1.5
1.0
0.5
0.0
***
Aggref + +
Bifido - +
B
mGBC
mLF-LFD
mIE-LFD
Neutrophils (%)
5
4
3
2
1
0
* *
** **
SI
Colon
C
B cells (%)
80
60
40
20
0
***
SI
Colon
D
Total T cells (%)
25
20
15
10
5
0
**
SI
Colon
E
CD8 T cells among TCRβ+ (%)
40
30
20
10
0
*
SI
Colon
F
CD4 T cells among TCRβ+ (%)
100
80
60
40
20
0
* *
SI
Colon
G
mGBC
mLF-LFD
mIE-LFD
Colon
Ileum
H
GLP1 postive cells in colon (number/mm2)
1000
800
600
400
200
0
***
p=0.053
I
GLP1 postive cells in ileum (number/mm2)
1500
1000
500
0

Figure S5 (related to fig 6). Enrichment of maternal diet with inulin ameliorated low graded inflammation in offspring.

(A) qPCR-based measure Aggregatibacter (Aggref) growth in vitro with or without Bifidobacterium (Bifido).

(B-F) FACS analysis of immune cells in 12-week-old GBC-fed offspring of mice fed indicating lactating diet(m).

(G-I) GLP^+ L cells were assayed by immunofluorescence microscopy. Representative images (G) and quantitation of cells in colon (H) and small intestine (I). One-way ANOVA except A using student's t test: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

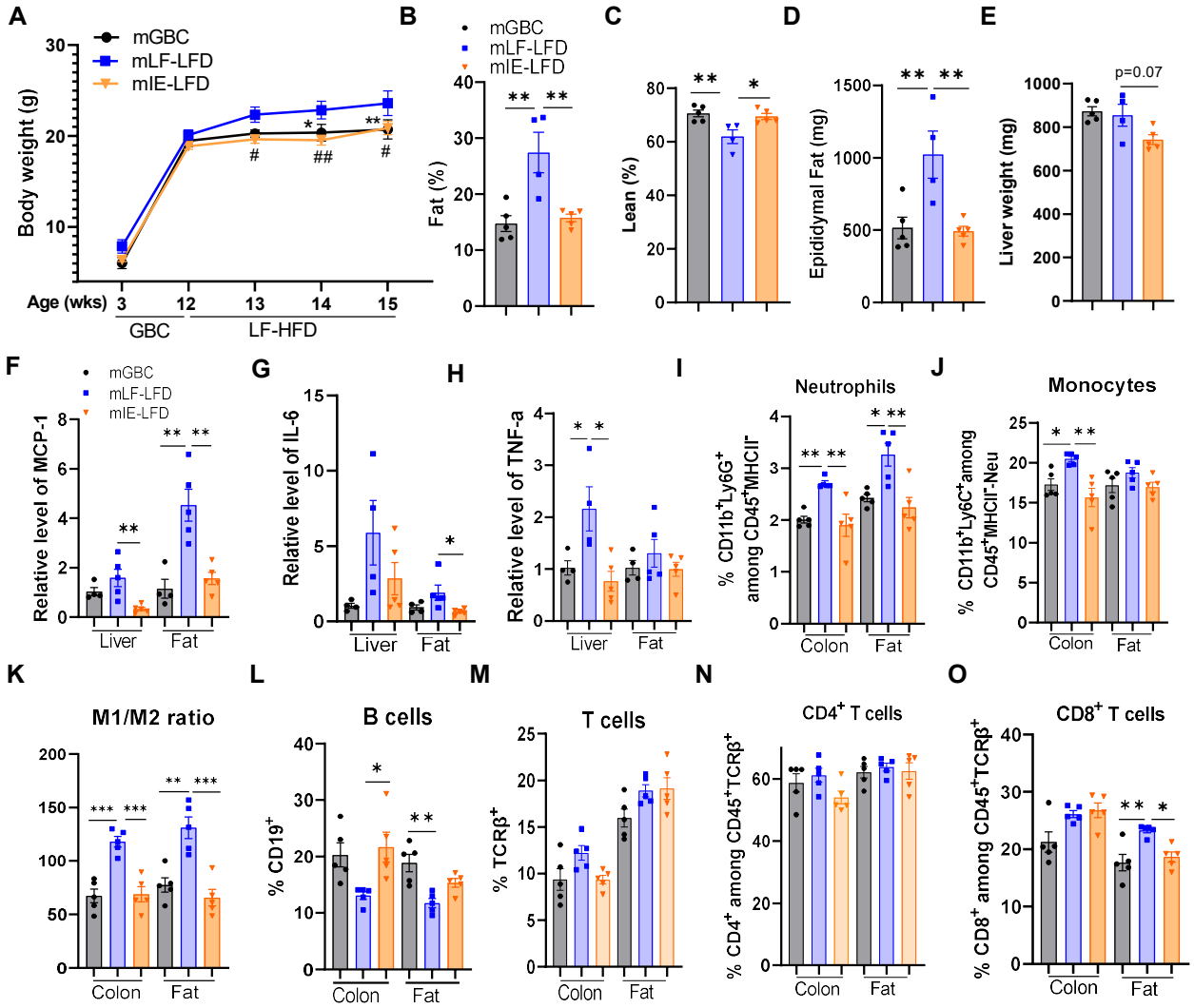
A
mGBC
mLF-LFD
mIE-LFD
Body weight (g)
Age (wks)
GBC
LF-HFD
B
Fat (%)
C
Lean (%)
D
Epididymal Fat (mg)
E
Liver weight (mg)
p=0.07
F
Relative level of MCP-1
G
Relative level of IL-6
H
Relative level of TNF-a
I
Neutrophils
% CD11b+Ly6G+ among CD45+MHCII-
J
Monocytes
% CD11b+Ly6C+among CD45+MHCII-Neu
K
M1/M2 ratio
L
B cells
% CD19+
M
T cells
% TCRβ+
N
CD4+ T cells
% CD4+ among CD45+TCRβ+
O
CD8+ T cells
% CD8+ among CD45+TCRβ+
Liver
Fat
Colon

Fig S6 (related to fig 7). Enrichment of maternal diet with inulin reduced offspring proneness to DIO.

(**A-E**) Female offspring of dams fed indicating lactating diet(m) were weaned onto GBC and, at 12 weeks of age, administered LF-HFD for 4 weeks. Body weight over time (A). Whole-body fat and lean mass at 15 weeks of age via MRI (B-C). Post-euthanasia measure of epididymal adipose (D) and liver weight (E).

(**F-O**) Male offspring with sequentially GBC and LF-HFD feeding as described above. Euthanized and tissues harvested for cytokine expression analysis via qRT-PCR (F-H), quantitation of neutrophils (Neu), monocytes, M1/M2 macrophage, B and T lymphocytes in colon and epididymal fat pad by FACS (I-O). One-way ANOVA: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. In A, $^{\#}$indicated mLF-LFD vs mIE-LFD.