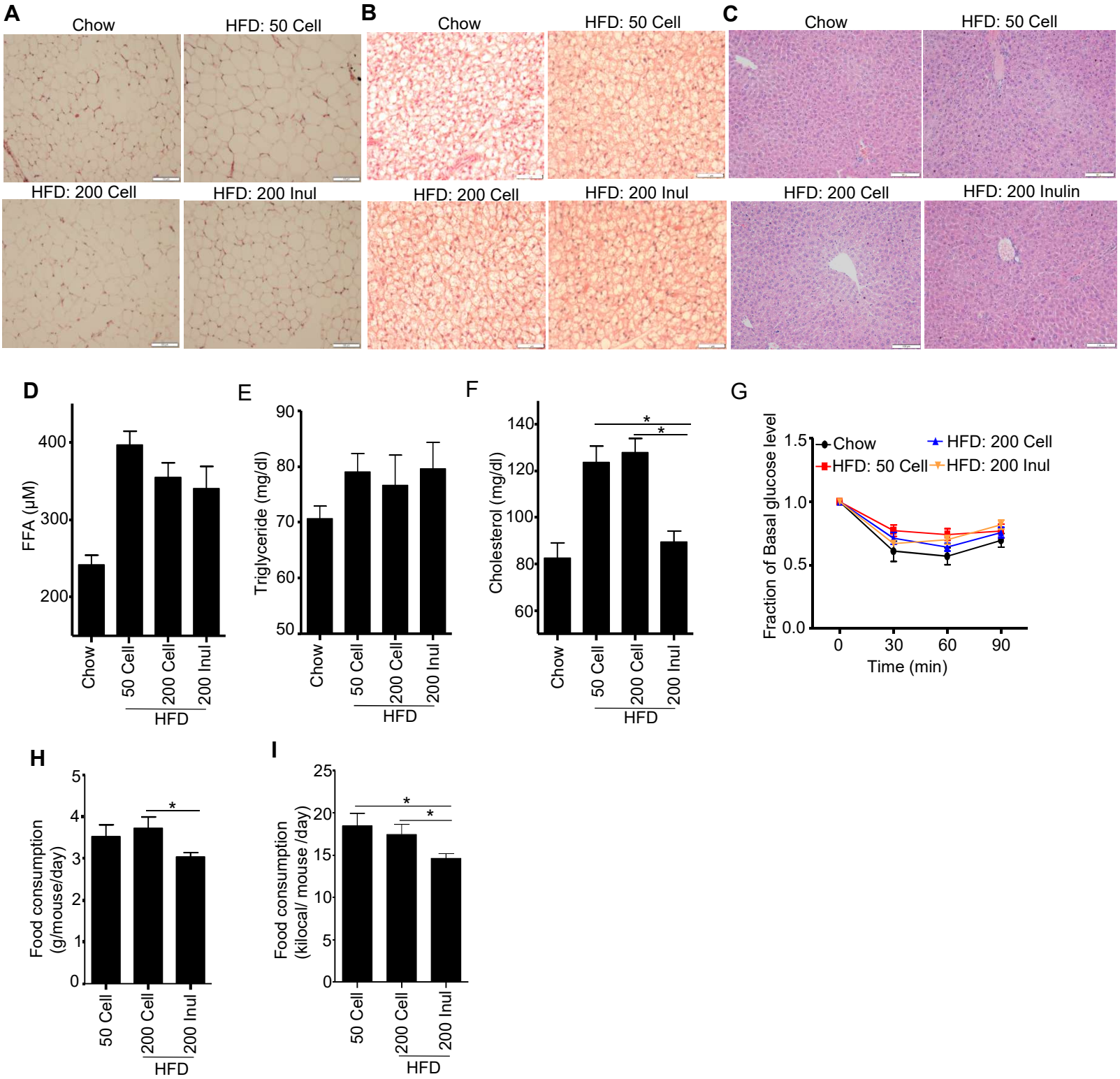

Figure S1, related to figure 1. Effect of inulin on HFD induced metabolic disease. C57BL/6 male mice were fed chow, HFD, or HFD supplemented with cellulose or inulin for 4 weeks (n=5). A-C) Epididymal adipose tissue (A), Brown adipose tissue (B) and liver (C) were stained with H&E, and representative images were showed. Scale bars, 100 µm (A), 50µm (B), 100 µm (C). D-F) Free fatty acid (FFA), triglycerides and cholesterol were measured in serum of mice at day 28 post diets treatment. G) The decrease in glucose level relative to basal glucose level after insulin injection was calculated. H-I) the food intake was measured. Data were expressed as mean ± SEM. Statistical significance was assessed by unpaired Student t test. *p<0.05; **p<0.01.

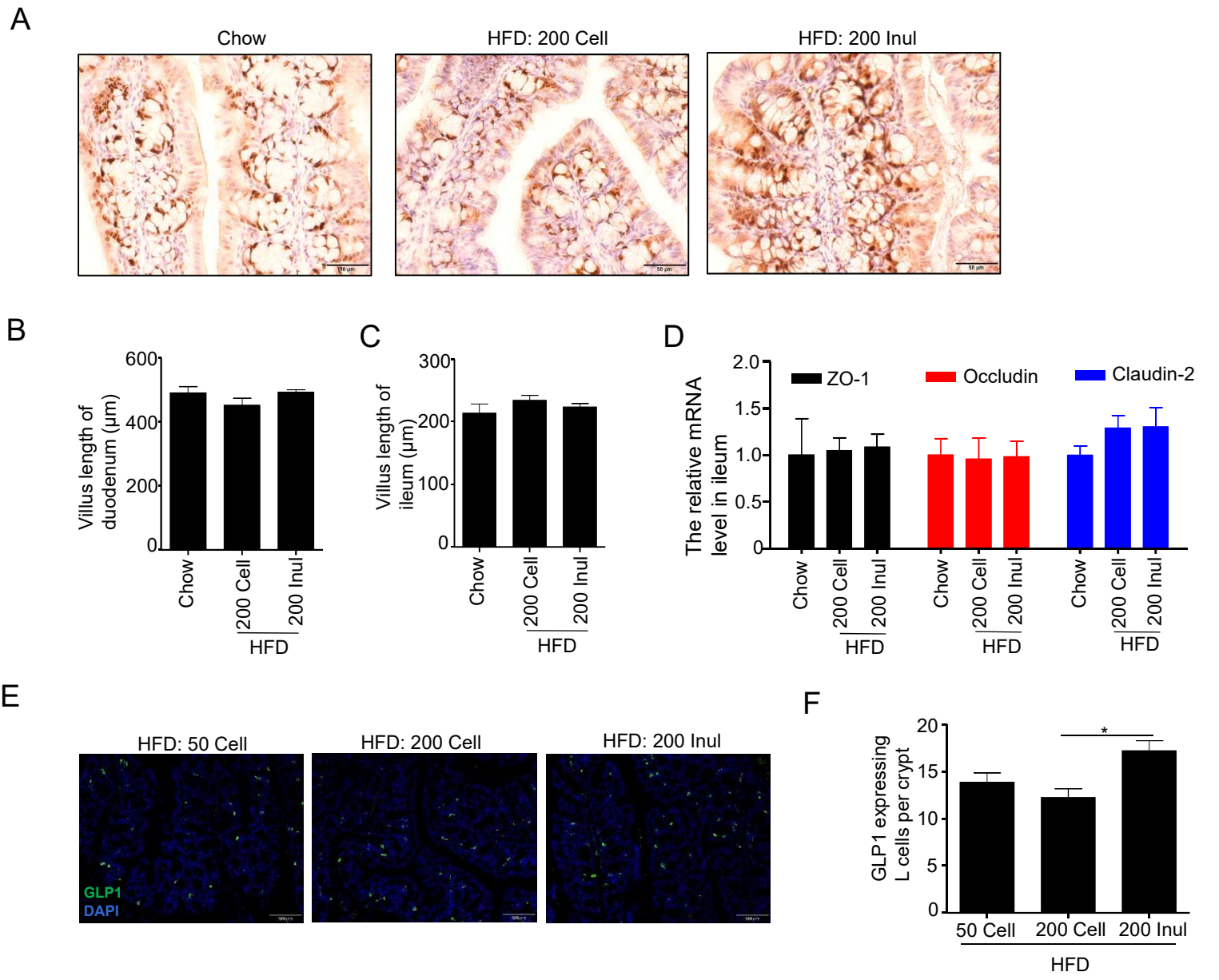

Figure S2, related to figure 2. Extended analysis of the effect of HFD supplemented with cellulose or inulin on intestine.

A) The colon was stained with PCNA. Representative images are shown. Scale bars, 50 μm. B-C. The small intestine including duodenum and ileum was stained by HE, the villus length in duodenum (B) and ileum (C) was measured. D) The mRNA was extracted from ileum, and the expression of ZO-1, Occludin and Claudin-2 was analyzed by RT-PCR. E-F) The proximal colon was stained with GLP1 (E), and GLP1 positive cells were counted (F). Scale bars, 50 μm. Data were expressed as mean ± SEM. Statistical significance was assessed by unpaired Student t test. *$p<0.05$.

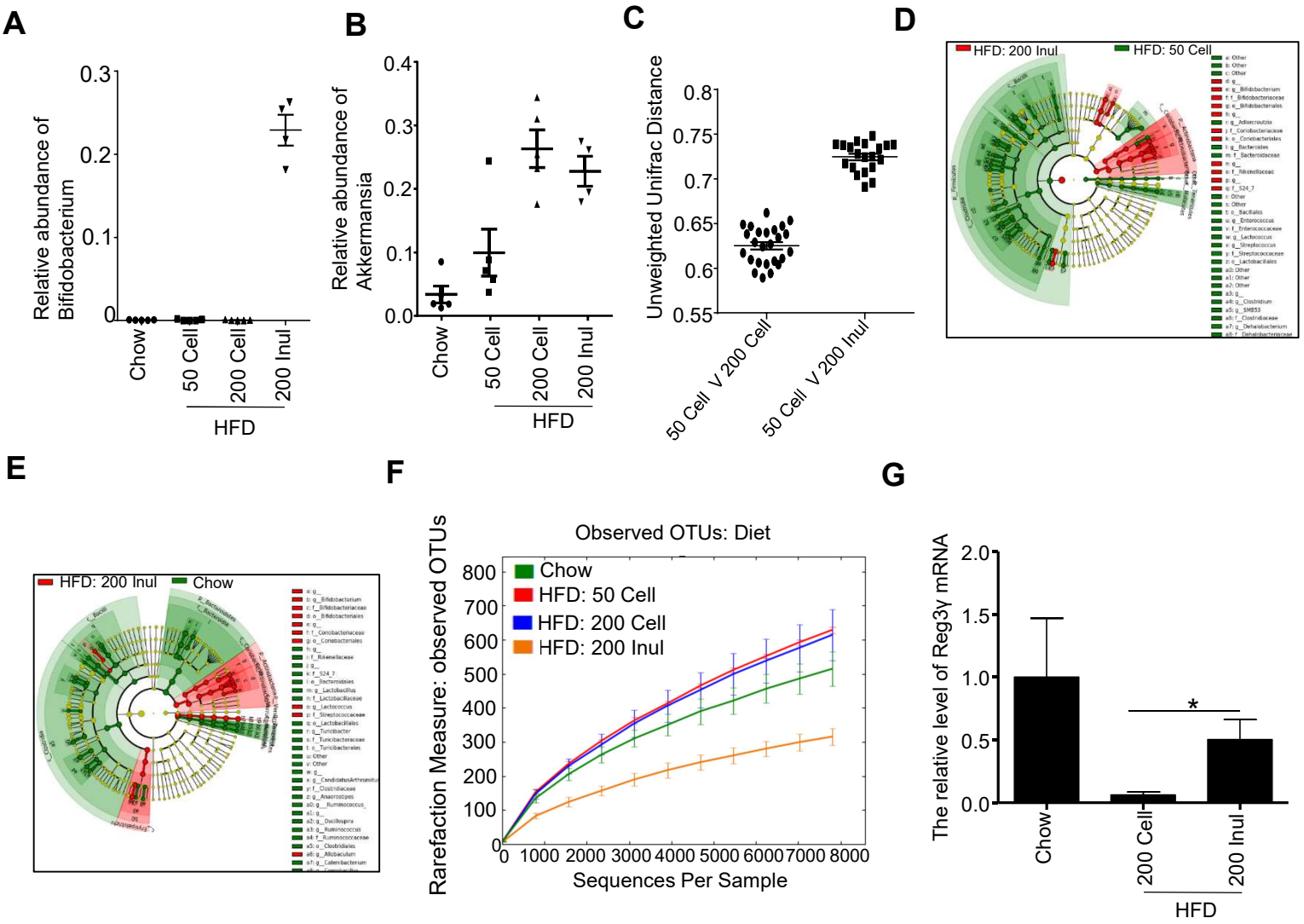

Figure S3, related to figure 3. Extended analysis of HFD and its enrichment with cellulose or inulin impact microbiota composition. A-B) The relative abundance of Bifidobacteria and Akkermansia in feces was analyzed by 16S rRNA gene sequence. C) Principal component analysis of the Unifrac distance. D-E) Taxonomic cladogram obtained from LEfSe analysis of 16S sequences by comparing HFD enrichment of Inulin with standard HFD (D) or chow (E). F) Rarefaction curves comparing the species richness (observed OTU number) during different diets treatment. G) Quantitation of Reg3γ in epithelial cells by RT-PCR. Data were expressed as mean ± SEM. Statistical significance was assessed by unpaired Student t test. *$p<0.05$.

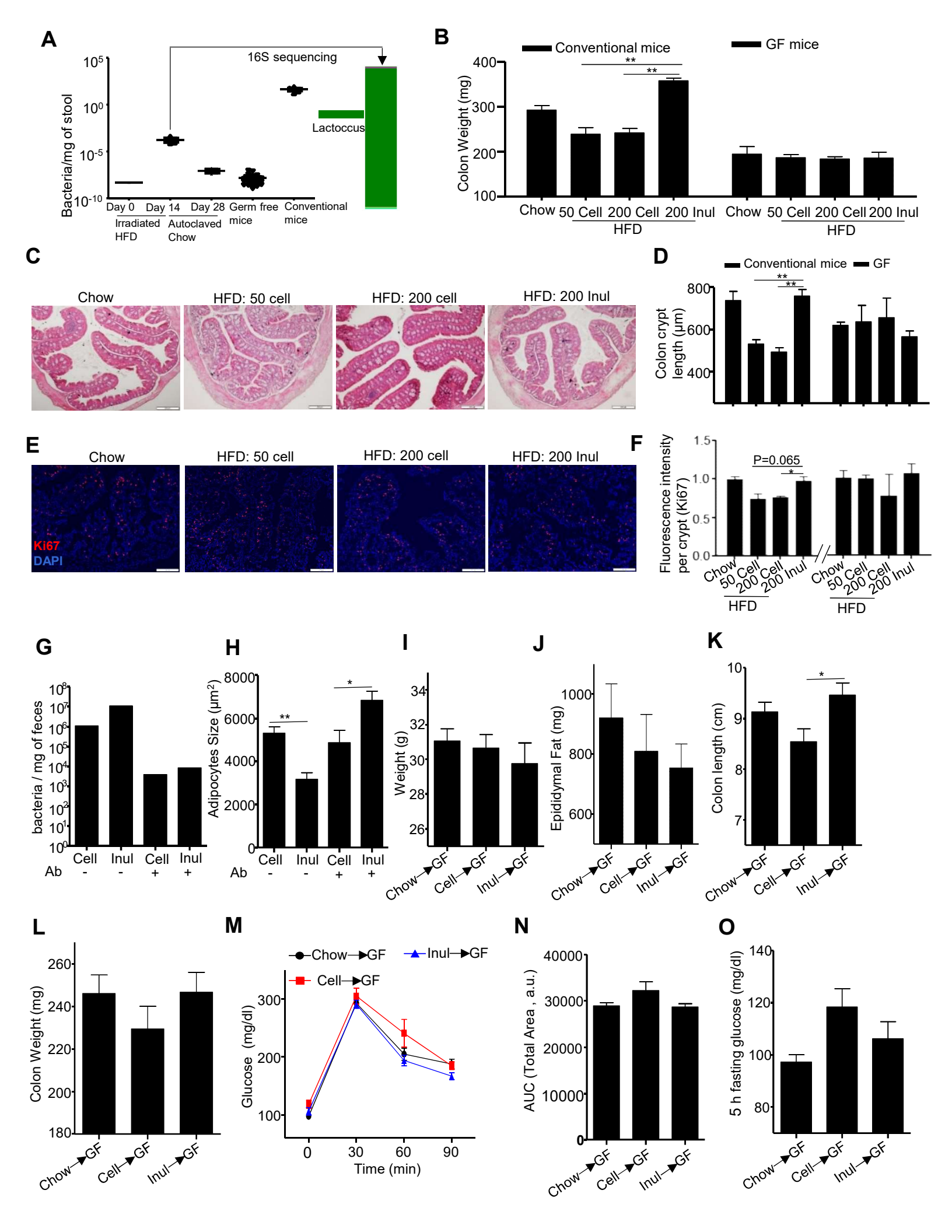
A
16S sequencing
Lactoccus
Bacteria/mg of stool
Day 0
Day 14
Day 28
Germ free mice
Conventional mice
Irradiated HFD
Autoclaved Chow
B
Conventional mice
GF mice
Colon Weight (mg)
Chow
50 Cell
200 Cell
200 Inul
HFD
C
Chow
HFD: 50 cell
HFD: 200 cell
HFD: 200 Inul
D
Conventional mice
GF
Colon crypt length (μm)
E
Ki67
DAPI
F
Fluorescence intensity per crypt (Ki67)
P=0.065
G
bacteria / mg of feces
Cell
Inul
Ab
H
Adipocytes Size (μm²)
I
Weight (g)
J
Epididymal Fat (mg)
K
Colon length (cm)
Chow→GF
Cell→GF
Inul→GF
L
Colon Weight (mg)
M
Glucose (mg/dl)
Time (min)
N
AUC (Total Area , a.u.)
O
5 h fasting glucose (mg/dl)

Figure S4, related to figure 4. Extended analysis of the role of microbiota in inulin induced beneficial effects. A) Swiss Webster GF mice were treated with irradiated HFD diet for 14 days, and were switched to autoclaved chow diet for 14 days, bacteria in the feces were measured by qPCR, and conventional mice were used as positive control. The DNA was extracted from feces of mice fed with irradiated HFD for 14 days, and was subjected to 16S rRNA gene sequences: Green = Firmicutes Bacilli, Lactobacillales Streptococcaceae Lactococcus. B-F) Conventional and germfree (GF) Swiss Webster mice (n=3-5) were treated with HFD, or HFD supplemented with cellulose or inulin. B) Colon mass. C) Representative colon histopathologic appearance by H&E staining in GF mice. Scale bars, 200 μm. D) Colon crypt length in conventional and GF mice. E) Representative images of colon stained by Ki67 in GF mice. F) Quantification of cell proliferation in colon assessed by Ki67 levels. G-H) C57BL/6 male mice were fed HFD supplemented with cellulose (HFD-200 Cell) or inulin (HFD-200 Inul) with or without antibiotic cocktail in drink water. The feces at day 6 post antibiotic treatment were collected to measure bacteria by qPCR (G), and the epididymal fat at the end of experiment was stained with H&E, adipocyte size was determined (H). I-O) Swiss Webster GF mice (n=3-5) were transplanted with microbiota from mice fed chow, or HFD enriched with cellulose or inulin. Transplanted mice were fed chow for 28 days. I) Body weight. J) Epididymal fat. K) Colon length. L) Colon weight. M-N) Glucose tolerance was measured (M) and area under curve (AUC) calculated (N). O) 5 h fasting glucose. Data were expressed as mean ± SEM. Statistical significance was assessed by unpaired Student t test. *$p<0.05$; **$p<0.01$.

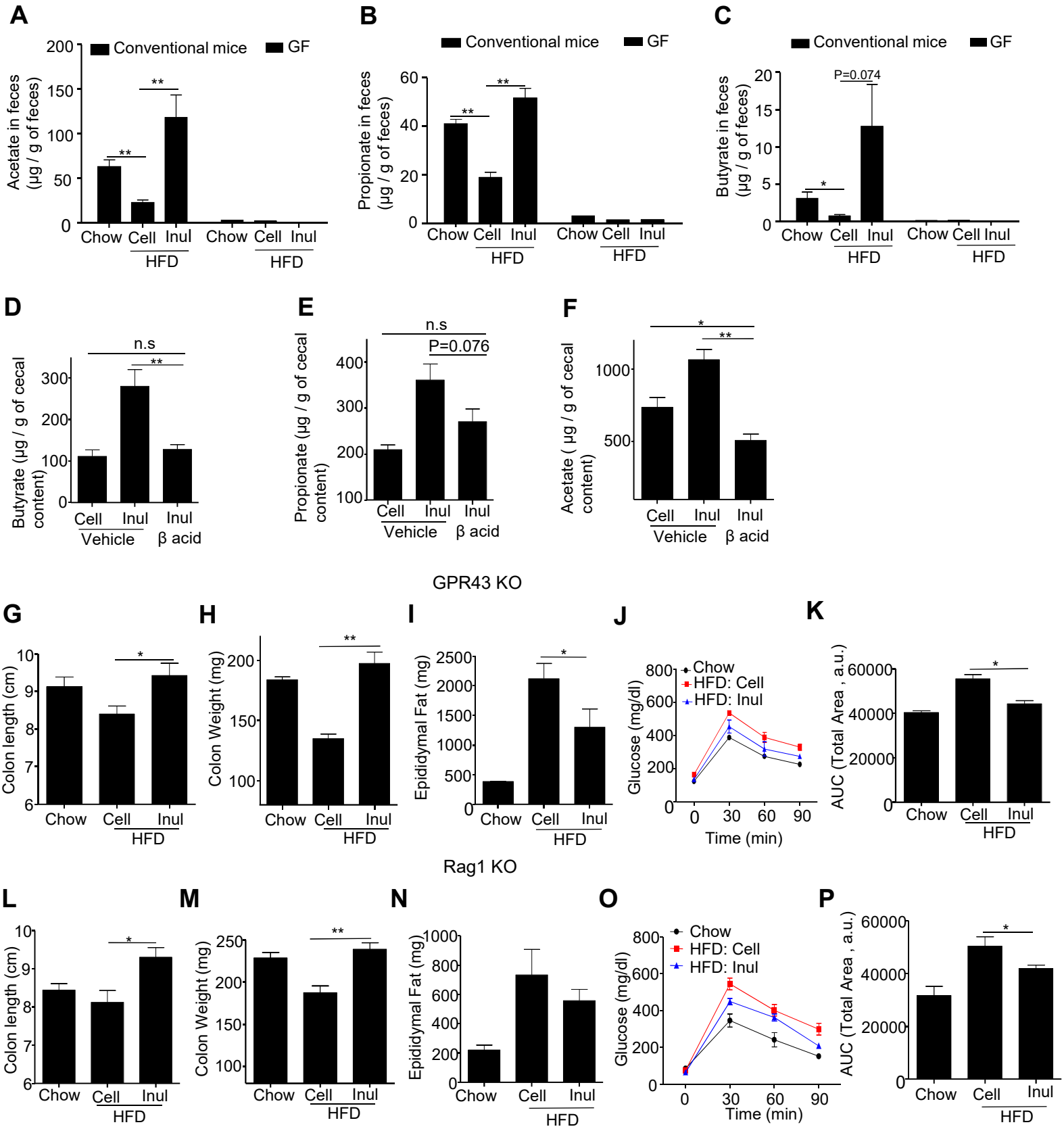
A
Conventional mice
GF
Acetate in feces (µg / g of feces)
Chow Cell Inul Chow Cell Inul
HFD
HFD
B
Propionate in feces (µg / g of feces)
C
Butyrate in feces (µg / g of feces)
P=0.074
D
Butyrate (µg / g of cecal content)
n.s
Cell Inul Inul
Vehicle β acid
E
Propionate (µg / g of cecal content)
P=0.076
F
Acetate (µg / g of cecal content)
GPR43 KO
G
Colon length (cm)
H
Colon Weight (mg)
I
Epididymal Fat (mg)
J
Glucose (mg/dl)
Chow
HFD: Cell
HFD: Inul
Time (min)
K
AUC (Total Area , a.u.)
Rag1 KO
L
M
N
O
P

Figure S5, related to figure 5. Prevention of metabolic syndrome by inulin does not require GPR43 or T and B lymphocytes. A-C) Feces samples from GF and conventional mice, which were fed chow, HFD supplemented with cellulose (HFD-200 Cell) or inulin (HFD-200 Inul), were used to analyze SCFA including acetate, propionate, and butyrate. D-F) C57BL/6 male mice (n=5) were fed HFD supplemented with cellulose (HFD-200 Cell) or inulin (HFD-200 Inul) with drink water containing β acid or vehicle for 4 weeks. The cecal content (n=5) was extract to measure the concentration of SCFA including butyrate, propionate and acetate. G-K) GPR43 KO (n=4) mice were treated with chow, HFD supplemented with cellulose (HFD-200 Cell) or inulin (HFD-200 Inul) for 4 weeks. G) Colon length. H) Colon weight. I) Epididymal fat pad. J-K). Glucose tolerance was measured (J) and area under curve (AUC) calculated (K). L-P) Rag1 KO mice were fed chow, HFD supplemented with cellulose (HFD-200 Cell) or inulin (HFD-200 Inul) for 4 weeks. L) The colon length. M) Colon weight. N) Epididymal fat pad. O-P) Glucose tolerance test. Data were expressed as mean ± SEM. Statistical significance was assessed by unpaired Student t test. *$p<0.05$; **$p<0.01$; n.s, not significance.

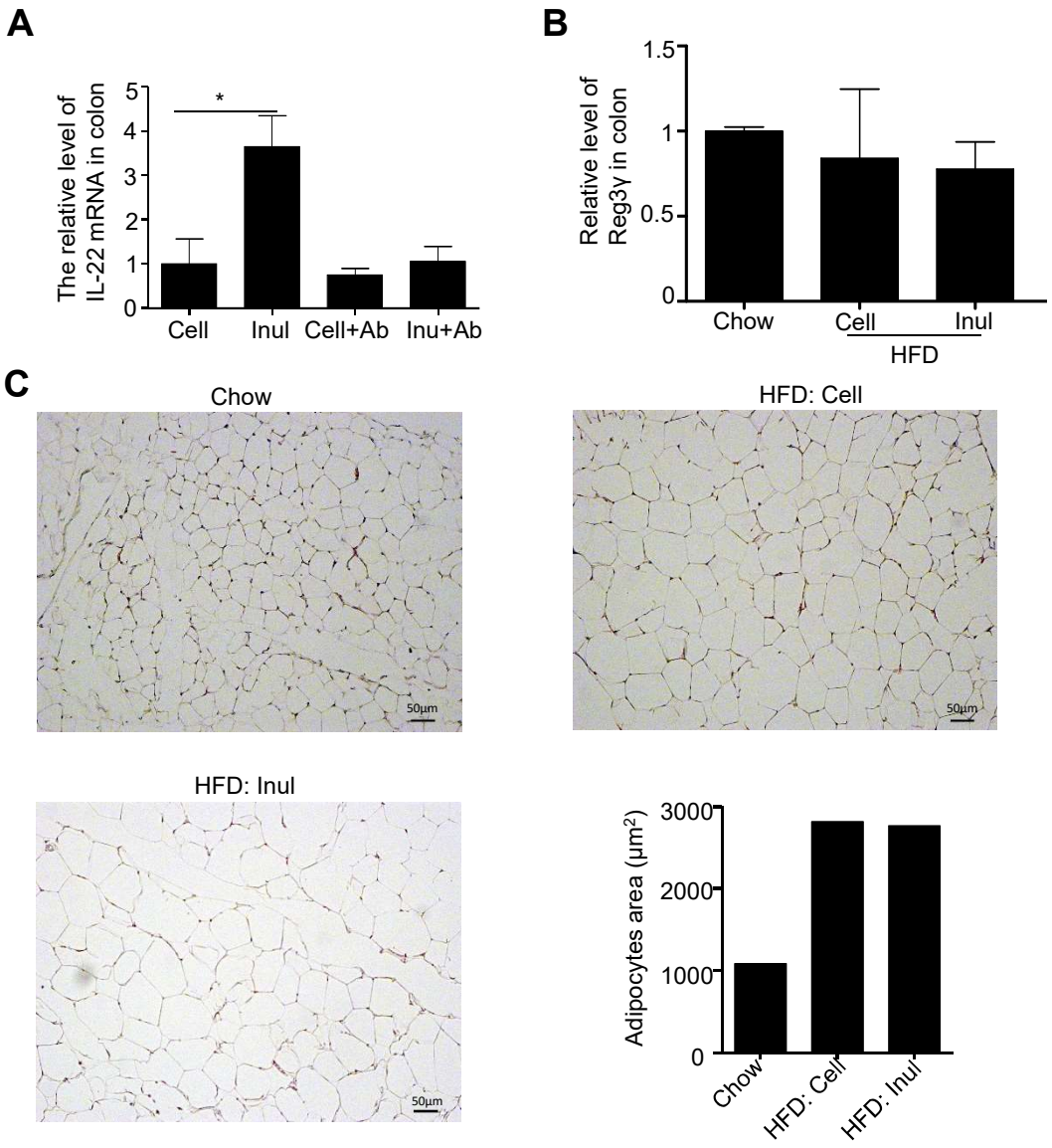

Figure S6, related to figure 6. Extended analysis of the role of IL-22 during inulin treatment in metabolic syndrome induced by HFD A) mRNA was extracted from transverse colons of mice fed HFD supplemented with cellulose or inulin wherein drinking with water containing, or lacking, antibiotics (Ab). Expression level of IL-22 was analyzed by qRT-PCR. B) Quantitation of Reg3γ in colon of GF mice by qRT-PCR. C) Epididymal adipose of IL-22KO mice was stained with hematoxylin-eosin, the cell size of adipose tissue was calculated by using image J. Data were expressed as mean ± SEM. Statistical significance was assessed by unpaired Student t test. *$p<0.05$.

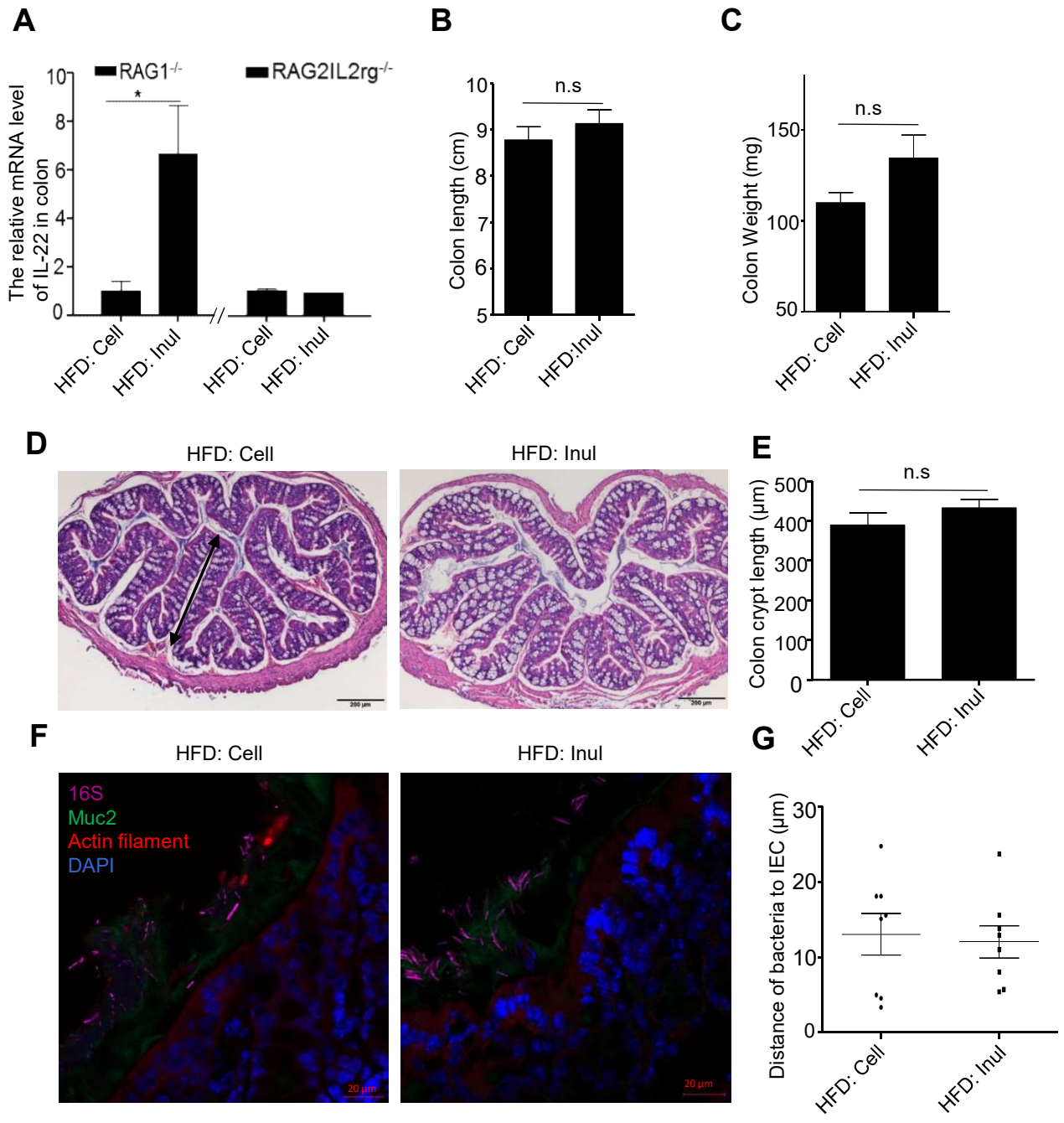

Figure S7, related to figure 6. Loss of inulin-induced IL-22 expression in colon and ablation of inulin's beneficial effects in RAG2IL2rg$^{-/-}$ mice. RAG2IL2rg$^{-/-}$ mice (n=5) were fed with HFD supplemented cellulose (HFD-200 Cell) or inulin (HFD-200 Inul) for 6 days. A) mRNA was extracted from transverse colon and used to measure IL-22 expression by qRT-PCR. B) Colon length. C) Colon weight. D-E) The proximal colon was stained by HE (D), and crypt length (shown as a double-headed arrow) was measured (E). F-G) The transverse colon was fixed in carnoy solution, and microbiota localization in colon was analyzed by immunohistochemical staining (F), Bacterial vs epithelial distance was quantified (G). Data were expressed as mean ± SEM. Statistical significance was assessed by unpaired Student t test. *$p<0.05$; n.s, not significance.

Table S1. The composition of the purified diets used in this study. Related to STAR Methods and Fig. 1

Product #	D12492		D13081106		D13081107	
	HFD-50 Cell		*HFD-200 Inul*		*HFD-200 Cell*	
	gm%	*kcal%*	gm%	*kcal%*	gm%	kcal%
Protein	26	*20*	23	*20*	22	20
Carbohydrate	26	*20*	40	*20*	22	*20*
Fat	35	*60*	31	*60*	29	*60*
Total		*100*		*100*		*100*
kcal/gm	5.2		4.6		4.4	
Ingredient	gm	*kcal*	gm	*kcal*	gm	*kcal*
Casein	200	*800*	200	*800*	200	*800*
L-Cystine	3	*12*	3	*12*	3	*12*
Corn Starch	0	*0*	0	*0*	0	*0*
Maltodextrin 10	125	*500*	75	*300*	125	*500*
Sucrose	68.8	*275*	68.8	*275*	68.8	*275*
Cellulose, BW200	50	*0*	0	*0*	200	*0*
Inulin, Orafti HP	0	*0*	200	*200*	0	*0*
Soybean Oil	25	*225*	25	*225*	25	*225*
Lard	245	*2205*	245	*2205*	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	*0*	0	*0*	0	*0*
FD&C Red Dye #40	0	*0*	0.05	*0*	0.025	*0*
FD&C Blue Dye #1	0.05	*0*	0	*0*	0.025	*0*
Total	773.85	*4057*	873.85	*4057*	923.85	*4057*

Table S2. Key Resource Table supplemental oligonucleotides. Related to Key Resource Table		
OLIGONUCLEOTIDES	SOURCE	IDENTIFIER
36B4: 5’ TCCAGGCTTTGGGCATCA-3’ and 5’CTTTATTCAGCTGCACATCACTCAGA-3’	Invitrogen	NA
IL-22: 5’-GTGCTCAACTTCACCCTGGA3’ and 5’-TGGATGTTCTGGTCGTCACC-3’	Invitrogen	NA
Reg3γ: 5’TTCCTGTCCTCCATGATCAAA-3’ and 5’CATCCACCTCTGTTGGGTTC-3’	Invitrogen	NA
CXCL1: 5’ TTGTGCGAAAAGAAGTGCAG-3’ and 5’TACAAACACAGCCTCCCACA-3’	Invitrogen	NA
IL-6: 5’ GTGGCTAAGGACCAAGACCA-3’ and 5’GGTTTGCCGAGTAGACCTCA-3’	Invitrogen	NA
TNF-α: 5’ CGAGTGACAAGCCTGTAGCC-3’ and 5’CATGCCGTTGGCCAGGA-3’	Invitrogen	NA
ZO-1: 5’ACCCGAAACTGATGCTGTGGATAG-3’ and 5’AAATGGCCGGGCAGAACTTGTGTA-3’	Invitrogen	NA
Occludin: 5’ATGTCCGGCCGATGCTCTC-3’ and 5’TTTGGCTGCTCTTGGGTCTGTAT-3’	Invitrogen	NA
Claudin-2: 5’ GTCATCGCCCATCAGAAGAT-3’ and 5’ACTGTTGGACAGGGAACCAG-3’	Invitrogen	NA
16S rRNA: 5′-AGAGTTTGATCCTGGCTCAG-3′ and 5′-CTGCTGCCTCCCGTAGGAGT-3′	Invitrogen	NA
16S rRNA sequence primers: 5′AATGATACGGCGACCACCGAGATCTACACTATGGTAATTGTGTGCCAGCMGCCGCGGTAA-3′; 5′ CAAGCAGAAGACGGCATACGAGATXXXXXXXXXXXX AGTCAGTCAG CC GGACTACHVGGGTWTCTAAT-3′	Invitrogen	NA
EUB338 probe: 5’-GCTGCCTCCCGTAGGAGT-3’, with a 5' labeling using Alexa 647	Invitrogen	NA