Supplementary Figure Legend:

Figure 1. High-fat diet increases protein level of Rspo3, LGR4/LGR5 and β-catenin in mouse colon. High-fat diet (HFD) increased protein levels of Rspo3 (**A**), LGR4 (**B**), LGR5 (**C**) and β-catenin (**D**) in mouse colon as determined by immunofluorescence. Images were analyzed with Image J and summarized with bar graphs in right panels. For statistical analysis, unpaired Student's *t*-test was used. *$p<0.05$, **$P<0.01$. In all plots, data are shown as the mean ± SEM. n=4.

Figure 2. Gene expression regulation of Rspo3. (**A**) High-fat diet (HFD) increased gene expression of Rspo3, which is reversed by concurrent administration of bile acid binder, cholestyramine (CHO, 6%) in rat ileum. Gene expression was normalized to the expression level of GAPDH. n=6. (**B**) Addition of pectin (30%), a fermentable fiber, or cellulose (30%), a non-fermentable fiber, showed no effect on HFD-induced upregulation of Rspo3 in mouse colon. (**C**) Oral administration of deoxycholic acid (DCA, 0.15% in drinking water) to HFD upregulated Rspo3 gene expression in the colon of germfree mice; HFD induced upregulation of Rspo3 in the colon of mice inoculated with both *Lactobacillus plantarum* (*L.P.*, 10^8 CFU, 0.1ml/mouse, oral gavage) and *Clostridium scindens* (*C. S.,* 10^8 CFU, 0.1ml/mouse oral gavage) , but not in mice inoculated with either *Lactobacillus plantarum* or *Clostridium scindens* alone. One-way ANOVA with Bonfferoni *post hoc* analysis was used. *P<0.05. n=4-9.

Figure 3. Antibiotic treatment prevented HFD-induced dysbiosis in rats. Additional OTUs (**A**, #41-60; **B**, #60-77) that were significantly enriched by HFD and reversed by antibiotic treatment. Linear Discriminant Analysis Effect Size (LEfSe) was used to determine which OTUs were differentially abundant. Antibiotic treatment decreased OTU richness (**C**) and Shannon diversity index (D). Within-community diversity (α-diversity) was calculated using Shannon diversity index (H') and OTU Richness. One-way ANOVA with Bonfferoni *post hoc* analysis *p<0.05, ** P<0.01. n=5.

Figure 4. Concentration of bile acids in mouse serum. (A) Concentration of unconjugated primary and secondary bile acids. (B) Taurine conjugated α- plus β- muricholic acids (Tauro- α- plus β- MCA). (C) Taurine conjugated cholic acid (Tauro-CA). One-way ANOVA with

Bonfferoni *post hoc* analysis. *p<0.05, **p<0.01. n=4-5. SPF: specific pathogen free. GF: Germ free. *L.P.: Lactobacillus plantarum. C.S.*: *Clostridium scindens*.

Figure 5. Detection of *Lactobacillus plantarum* and *Clostridium scindens* in mouse feces using qPCR. Two weeks after bacterial inoculation either specific primers and probe for *Lactobacillus plantarum* (**A**. top), or specific primers for *Clostridium scindens* were used for qPCR using mouse fecal DNA (**A**. bottom). **B.** Representative qPCR samples was resolved on agarose gel (1.2%), showing specific PCR products. ND: non-detectable. Ct: threshold cycle. n=6.

Figure 6. Effect of LCA and CDCA on Rspo3 gene expression in rat colon myofibroblasts. Double phased response to lithocholic acid (LCA) (**A**), and dose response to chenodeoxycholic acid (CDCA) (**B)** of Rspo3 gene expression in rat colon myofibroblasts. One-way ANOVA with Bonfferoni *post hoc* analysis *p<0.05, ** P<0.01. n=4-10.

Figure 7. Rspo3 mRNA level in the colon of wild type (wt) and Rspo3 knockout (Rspo3 KO) mice. One-way ANOVA with Bonfferoni *post hoc* analysis was used. *P<0.05, **p<0.01. n=3-6.

Supplementary Table 1 RT-PCR primers

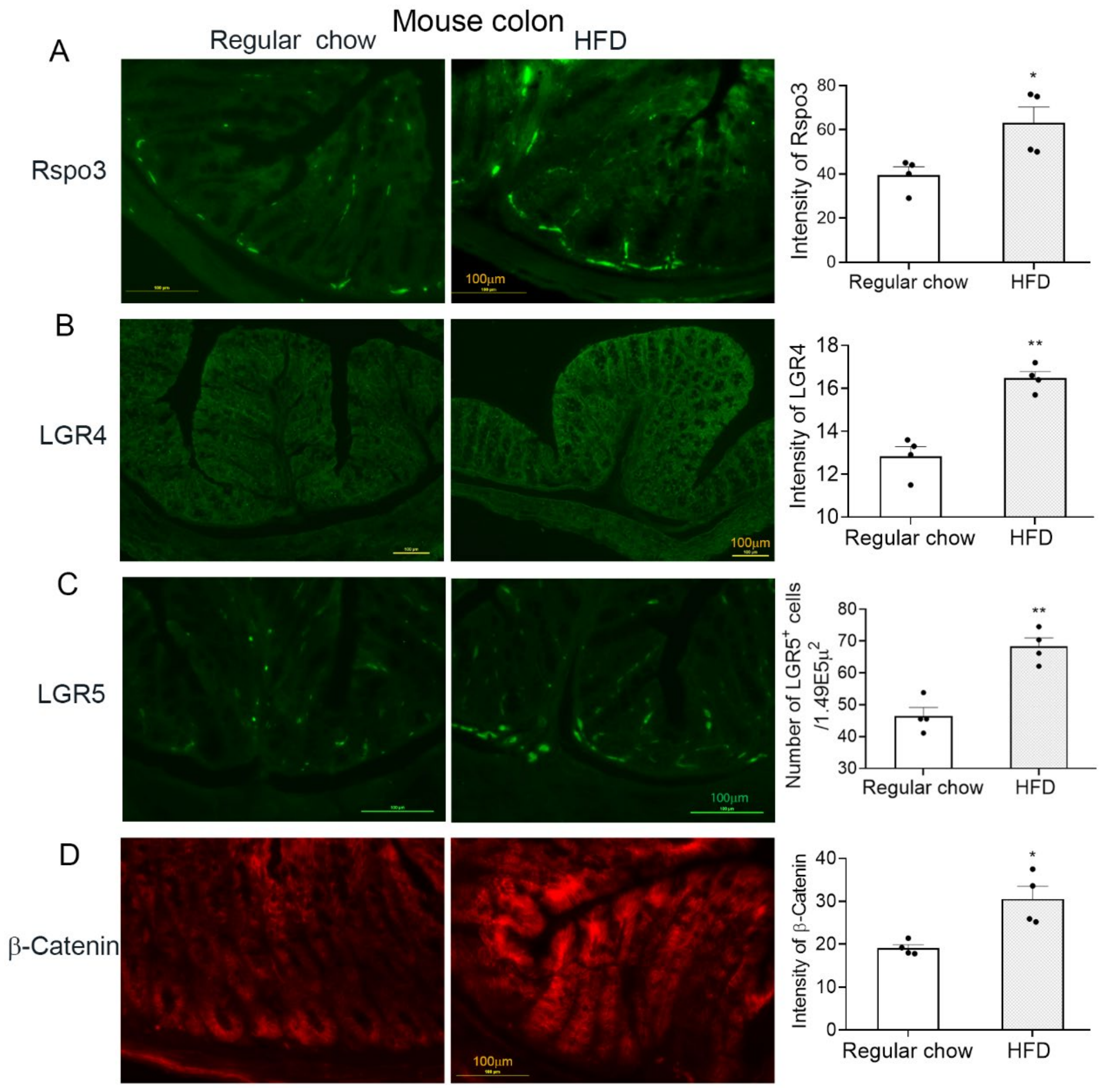
Genes	Forward(F), reverse (R) primers sequences, probe(P)
RT-PCR	
Rat Rspo1	F: 5’- CCTGCCCGCCTGGATACTTTGAT- 3’ R: 5’-TTCTTCCTGGTCGGGTGCCTATTG-3’
Rspo2	F: 5’-AAGGCAACCGATGGAGACGCAGTA-3’ R: 5’-TTTCGGAAGGCAGGCAGCATA-3’
Rspo3	F: 5’-TGCGCGACGTGTTCAGATTAC-3’ R: 5’-TGGTTGGGGGACACAGGTTC-3’
Rspo4	F: 5’-GTACCCGGGAGTGCCAGGAGGAGT-3’ R: 5’-GACCGGGCA-GATGGGAAAAGTAGC-3’
TGR5	F: 5’-CATGGCCCCAAGACCTACAAGAGTG-3’ R: 5’-TGGCAAGCAGGGAGAGGAAACAAA-3’
FXRα	F: 5'-GGGGCAACTGCGTGATGGAT-3' R: 5'-CGACTGCGGACCCTTTGAGC-3
PRX:	F : 5'-GCGGTCGGCTGGCTTACTGCT-3' R : 5' -GCCGTCCGTGCTGCTGAATA-3'
Mouse Rspo1	F: 5’-CTCGCCCAAGCTCTTCATTCTGC-3’ R: 5’-GCCCGGCTCCTTGCTGTTCTTC-3’
Rspo2	F: 5’- CCCTGCGCTCGGCTGCTTCTA-3’ R: 5’-TGTTCATATCTGGGGCTCGGTGTC-3’
Rspo3	F: 5’-AGACTCGCCTAACACCTACAT -3’ R: 5’-TTGGCTTCTAACCCTTCTG-3’
Rspo4	F: 5’-CCCGGGAGTGCCAGGAAGAGTGTG -3’ R: 5’- GTGTGCGGGGTGGACGGTGAAGAT -3’
Human Rspo1	F: 5’- GACGCCCGCAACCCCGACAT -3’ R: 5’-GCTTTGCCCCCGACGTTCCAGTT -3’
Rspo2	F : 5’-AAGGCAACCGATGGAGACGCAGTA -3’ R : 5’- CCTTCGCCTTTGGTGTTCTCTTCC -3’
Rspo3	R: 5’-AACACGGGTCCGAGAAATAATACA -3’ F: 5’-CCCTTCTTTGATCTTTTGGCTCTT -3’
Rspo4	R: 5’- CCCTGGGGCGGCTGGAG-3’ F: 5’-GGGAAGGAGGGAGGGAGAAAGGAG-3’

Supplementary Table 2 qPCR primers and probes

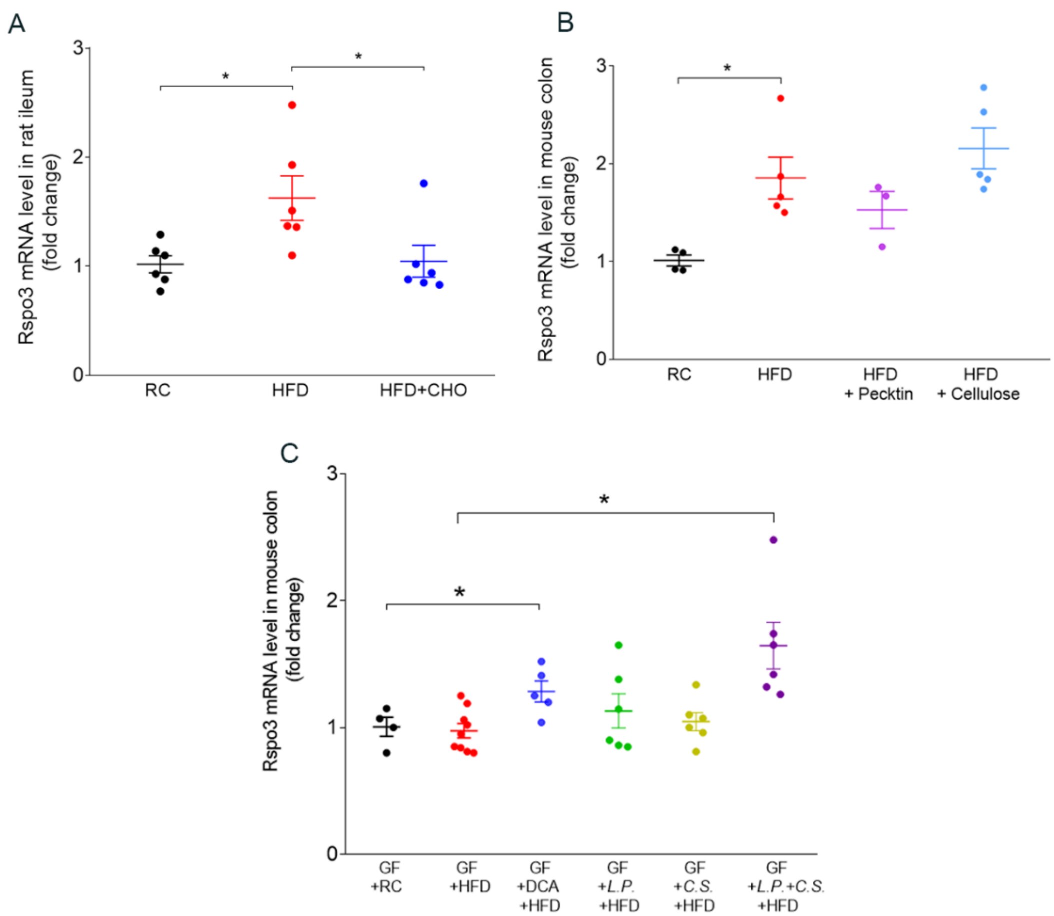
Genes	Forward(F), reverse (R) primers sequences, probe(P)
qPCR Rat Rspo3	F:5'-AACCAGCGAGACAAGAACG -3' P: 5'-/56FAM/AGTGTTCAAAGGGAGAGCGAGGG -3' R: 5'-TCCAAACCTTTGCTGTCAGAG-3'
LGR4	F:5'-AGCCAGAGACTTTGCTAATC-3' P:5'-/56FAM/AGTCACACCCCCAAAATGCACAAC-3' R:5'-CTGTAGCACCTTTCTCTTTTGTC-3'
LGR5	F:5'-CACAGCCTGGAGACTTTAGA-3' P:5'-/56FAM/TTGGAGAGTGTCTTGATTGCAGTGGG-3' R:5'-GAGAAGGGTTGCCTACGAA-3'
β-Catenin	F:5'-TGCCTTCAGATCTTAGCTTA-3' P:5'-/56FAM/TGGTGGACCCCAAGCCTTAGTAAAC-3' R:5'-AGACAGCACCTTCAGCAC-3'
TGR5	F: 5'TGCTGTGACTCTTTGATCCTC -3' P: 5'-/56FAM/TGACATCATGGGTCTTGGCGCA-3' R:5'-CTCTGGGAATGGCTGACAG-3'
Mouse Rspo3	F: 5'-CAACCAGCGAGACAAGAACT -3' P: 5'-/56FAM/AGAAGTGTTCAAAGGGAGAGCGAGG -3' R: 5'-TCCAAACCTTTGCTGTCAGAG -3
LGR4	F:5'- AGCCAGAGACTTTGCCAATC-3' Probe and Revere are the same as rat
LGR5	The same as rat
β-Catenin	The same as rat
Lactobacillus plantarum	F: 5'-TTACATTTGAGTGAGTGGCGAACT-3' P: 5'-/56FAM/GTGAGTAACACGTGGGWAACCTGCCC-3' R: 5'-AGGTGTTATCCCCCGCTTCT-3'
Clostridium scindens	F: 5'-GCATTTGGAACTGCGTGG-3' R: 5'-CGTTACGCGCTTTGGCATCG-3'

Supplementary Table 3 Information on antibodies used in this study

Primary antibodies	**Host and clone**	**Species Specificity**	**Dilution**	**Catalog #**	**Sources**
BrdU	rat monoclonal	human Mouse	1:1200	OBT0030CX	Accurate Chemical and Scientific, Westbury, NY
β-Catenin	rabbit polyclonal	human mouse, rat,	1:100	9587	Cell Signaling, Danvers, MA
Cytokeratin 20	rabbit monoclonal	human mouse, rat,	1:100	Ab76126	abcam, Cambridge, UK
Desmin	rabbit polyclonal	human mouse, rat,	1:80	PA5-17182	Thermofisher Scientific, Waltham MA
Ki67	rabbit polyclonal	human mouse	1:100	PA1-38032	Thermofisher Scientific, Waltham MA
LGR4	rabbit polyclonal	human mouse, rat,	1:100	PA5-67868	Thermofisher Scientific, Waltham MA
LGR5	rat monoclonal	mouse	1:100	MAB8240	R&D Systems Minneapolis, MN
α-MSA	mouse monoclonal	human mouse, rat,	1:100	14-9760-80	Thermofisher Scientific, Waltham MA
R-spondin 3	rabbit polyclonal	mouse, rat, human	1:100	PA5-45136	Thermofisher Scientific, Waltham MA
Vimentin	Chiken polyclonal	human rat, mouse,	1:100	919101	Biolegend, San Diego, CA
Secondary antibodies	**Host**	**Species Specificity**	**Dilution**	**Catalog #**	**Sources**
Donkey anti-mouse IgG	Donkey, 488 or 594	mouse	1:200	715-485-150 715-515-150	Jackson ImmunoResearch, West Grove, PA
Donkey anti-rabbit IgG	Donkey, 488 or 594	rabbit	1:200	711-486-152 7110516-152	Jackson ImmunoResearch, West Grove, PA
Donkey anti-goat IgG	Donkey, 488 or 594	goat	1:200	705-485-147 705-515-147	Jackson ImmunoResearch, West Grove, PA
Donkey anti-chicken	Donkey, 594 or 350	chicken	1:200	703-585-155 703-155-155	Jackson ImmunoResearch, West Grove, PA
Goat anti-rat IgG	Goat, Cy3	rat	1:200	112-166-062	Jackson ImmunoResearch, West Grove, PA
Biotinylated Goat anti-rat IgG	Goat, Biotin	rat	1:200	BA-9400	Vector Laboratory, Burlingame, CA



Supplementary Figure 1.



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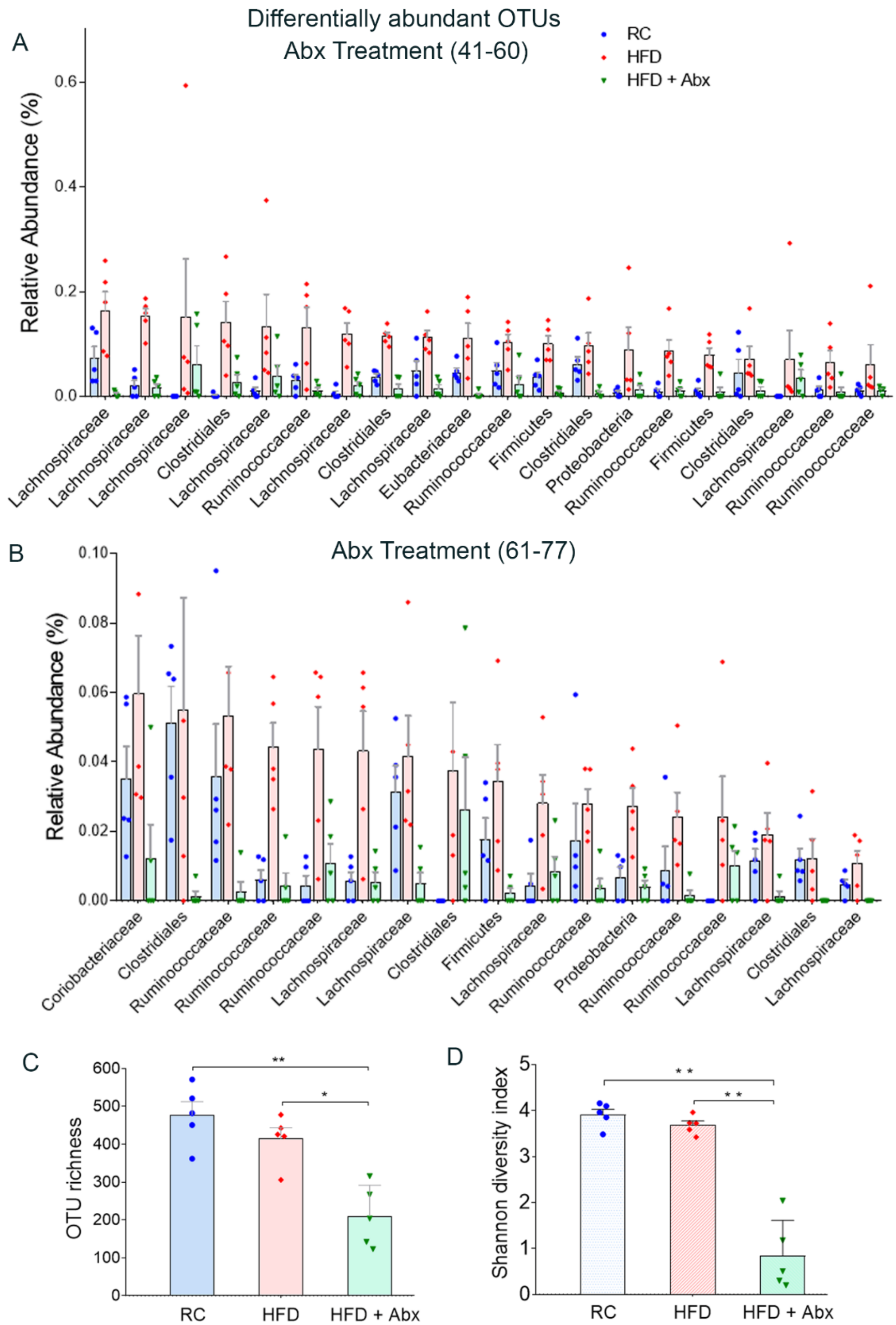

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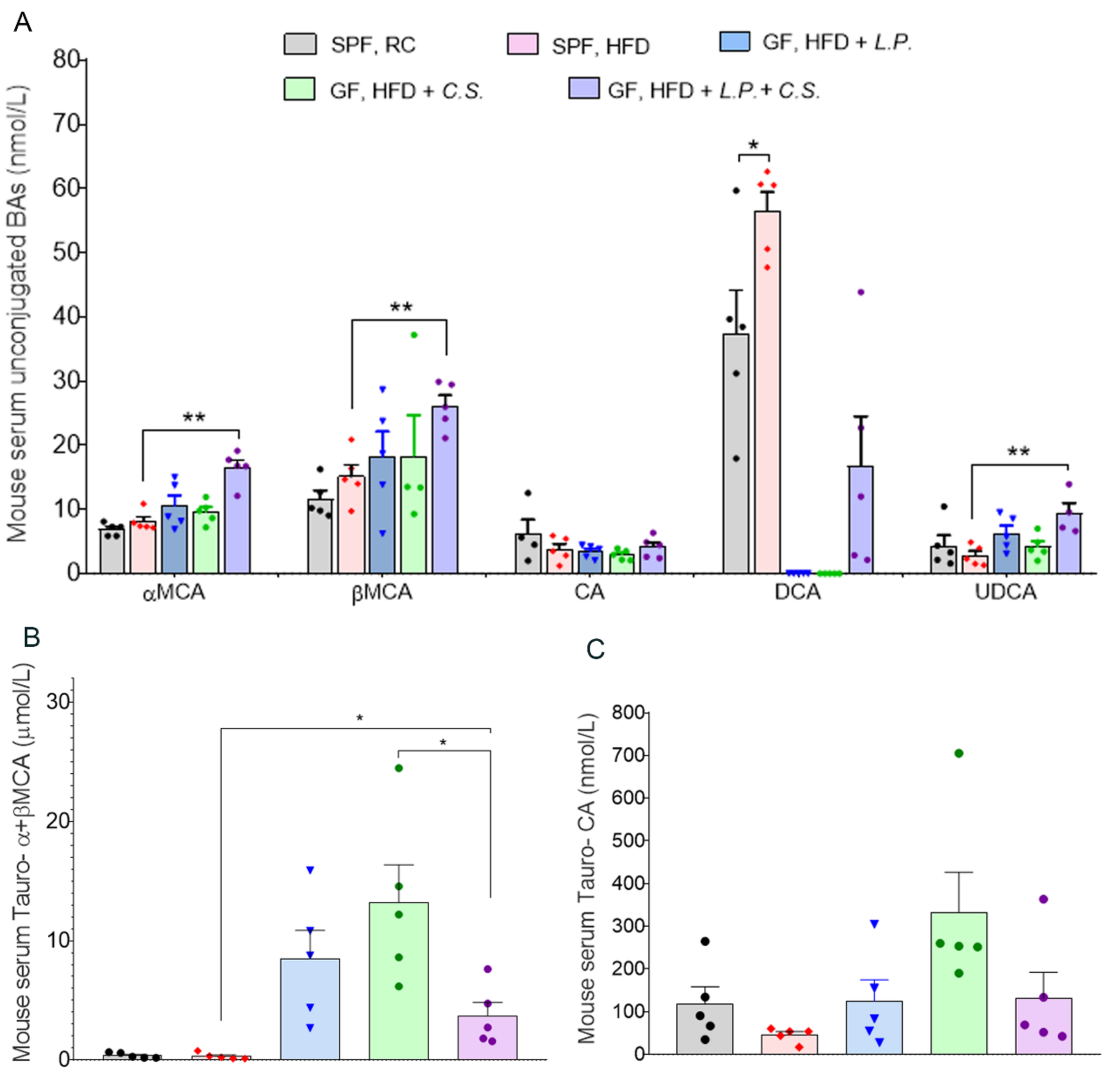

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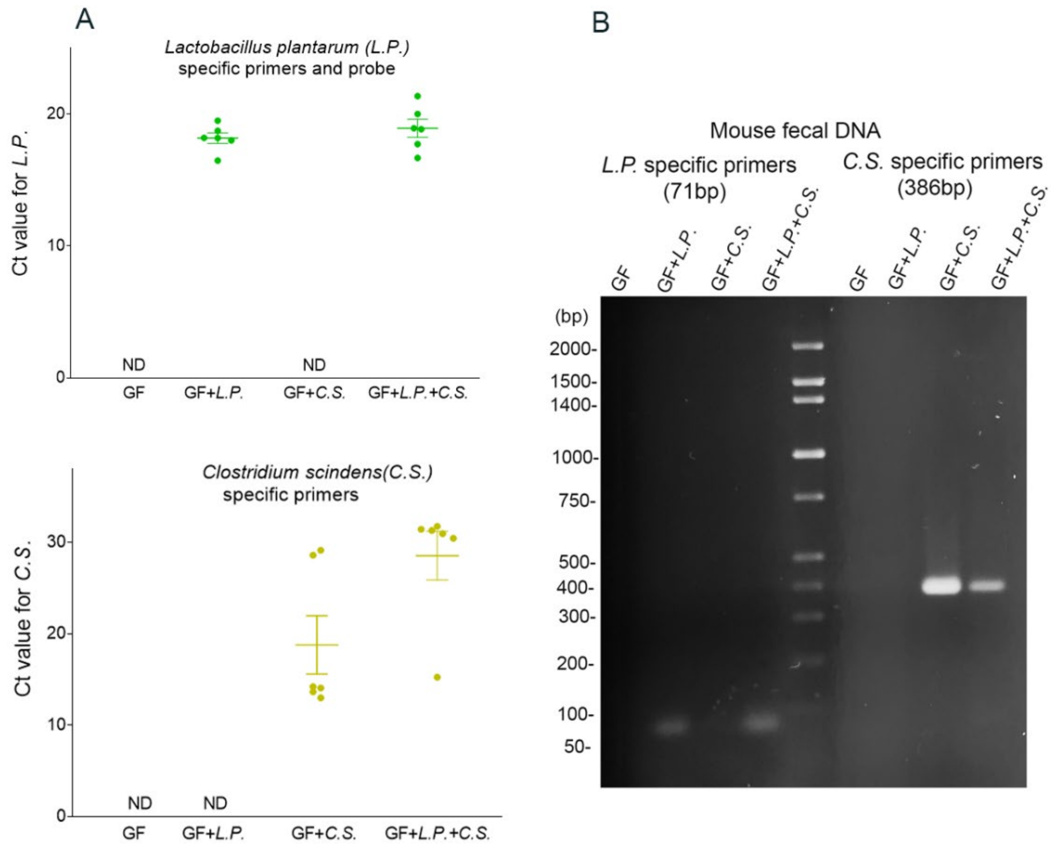

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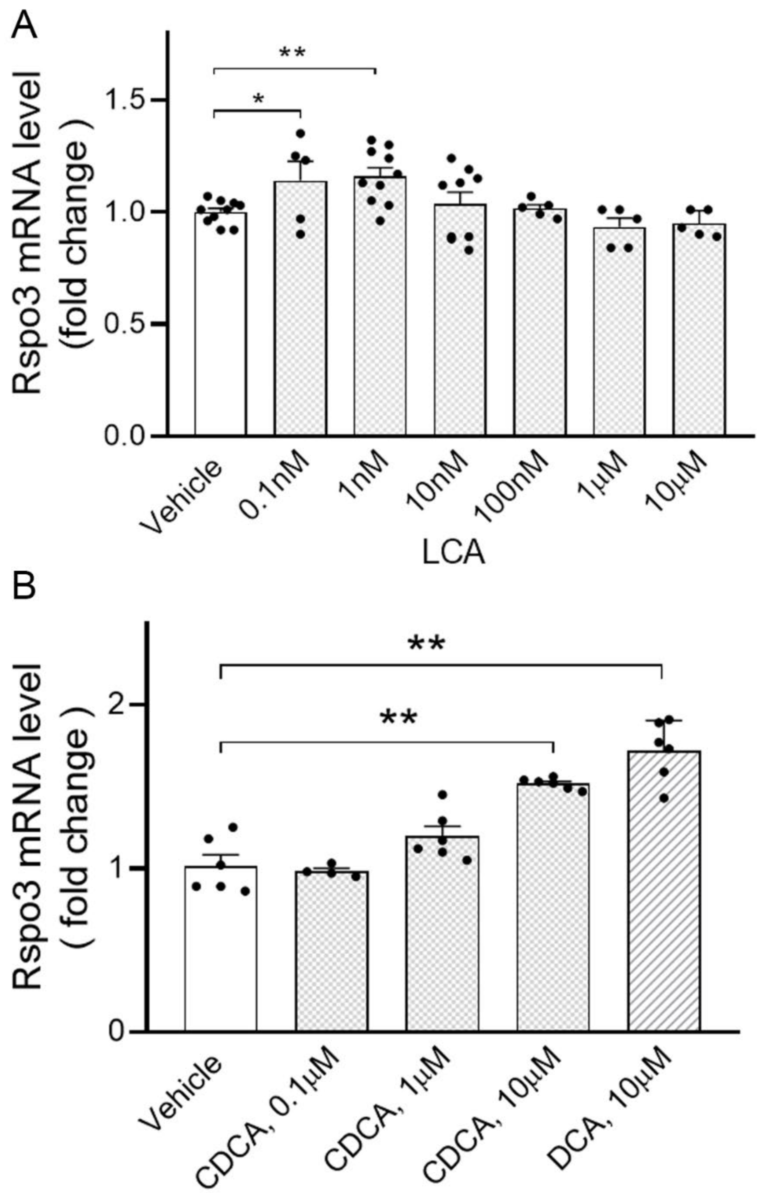

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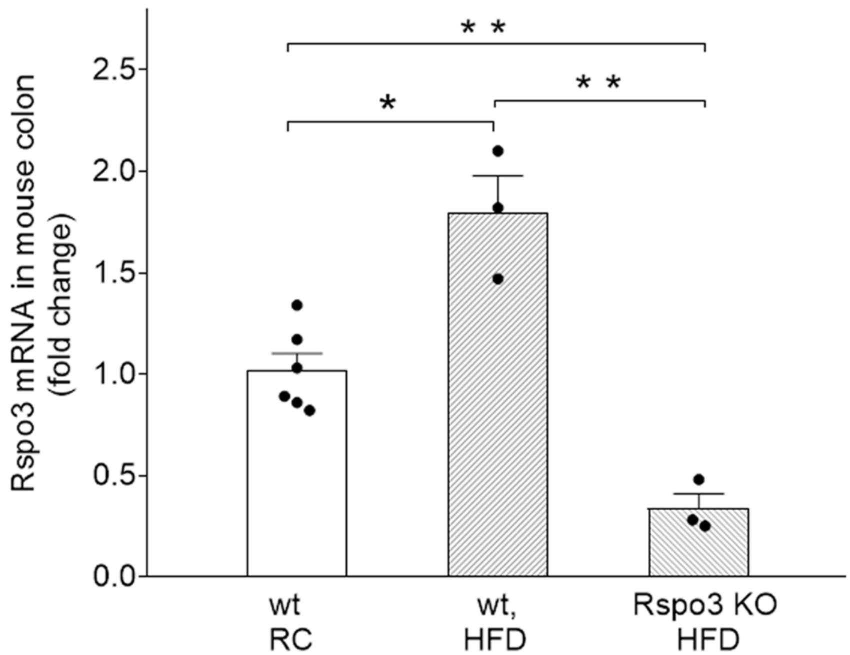

Supplementary Figure 7.