

Figure S1

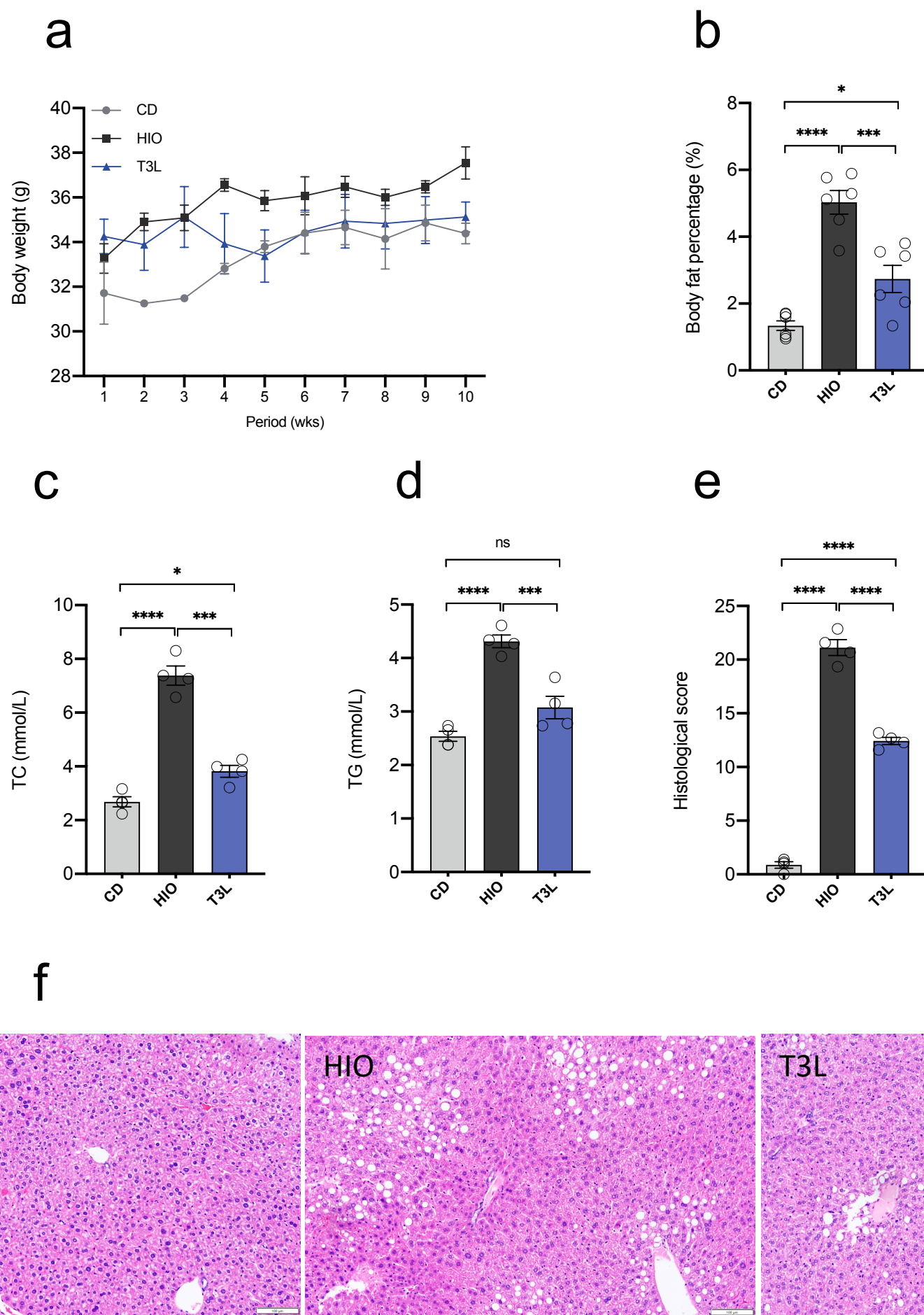


Figure S2

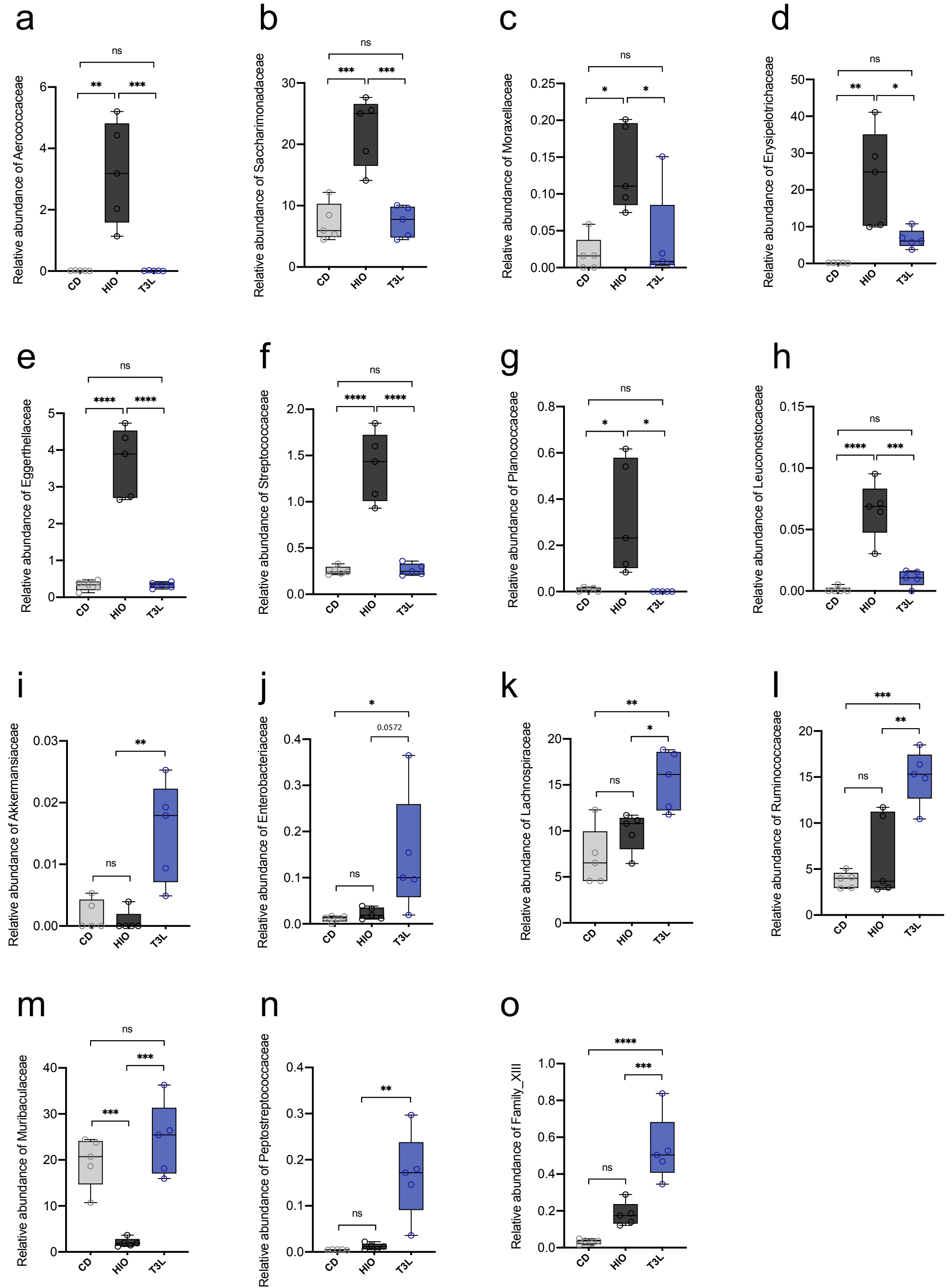


Figure S3

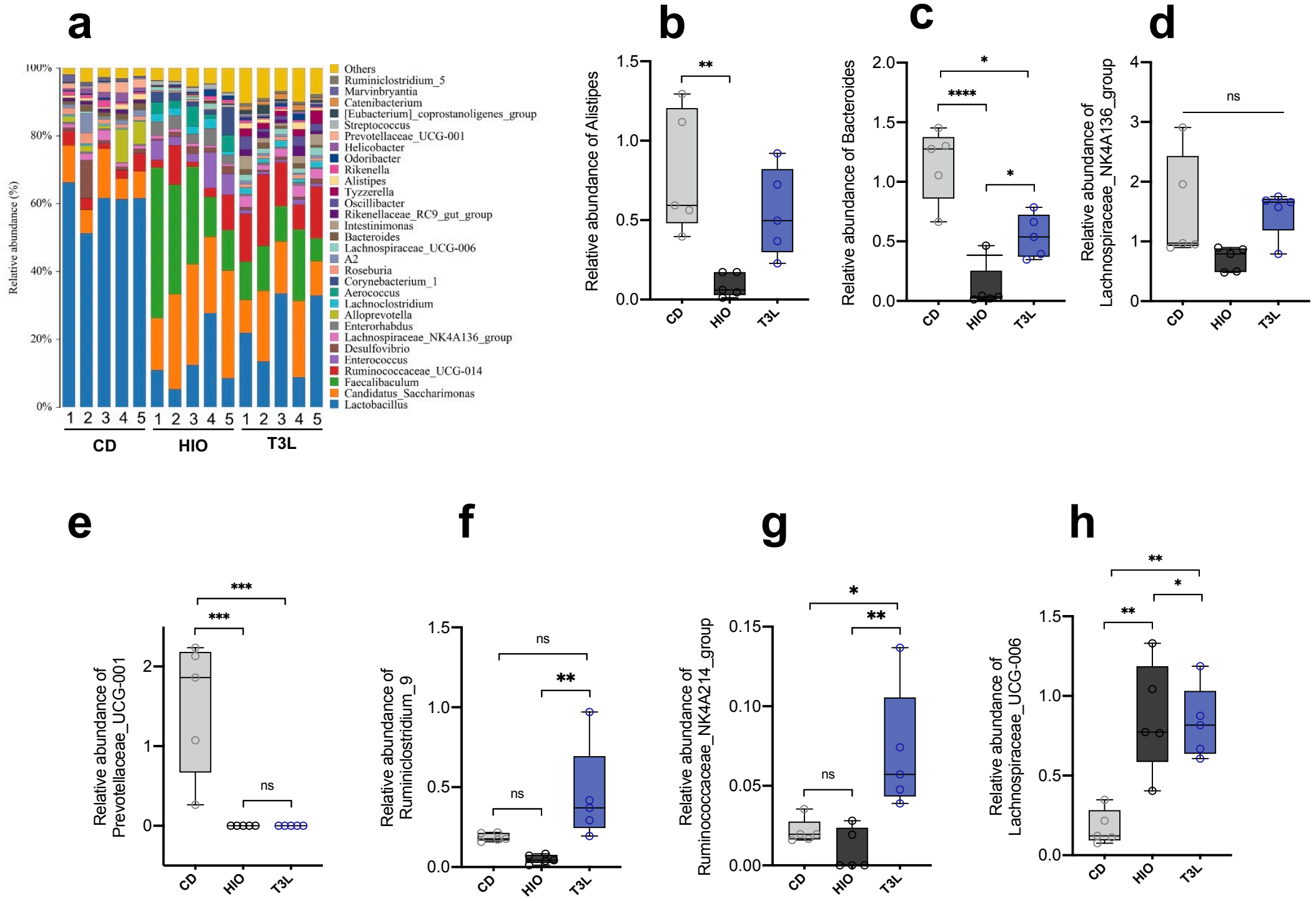


Figure S4

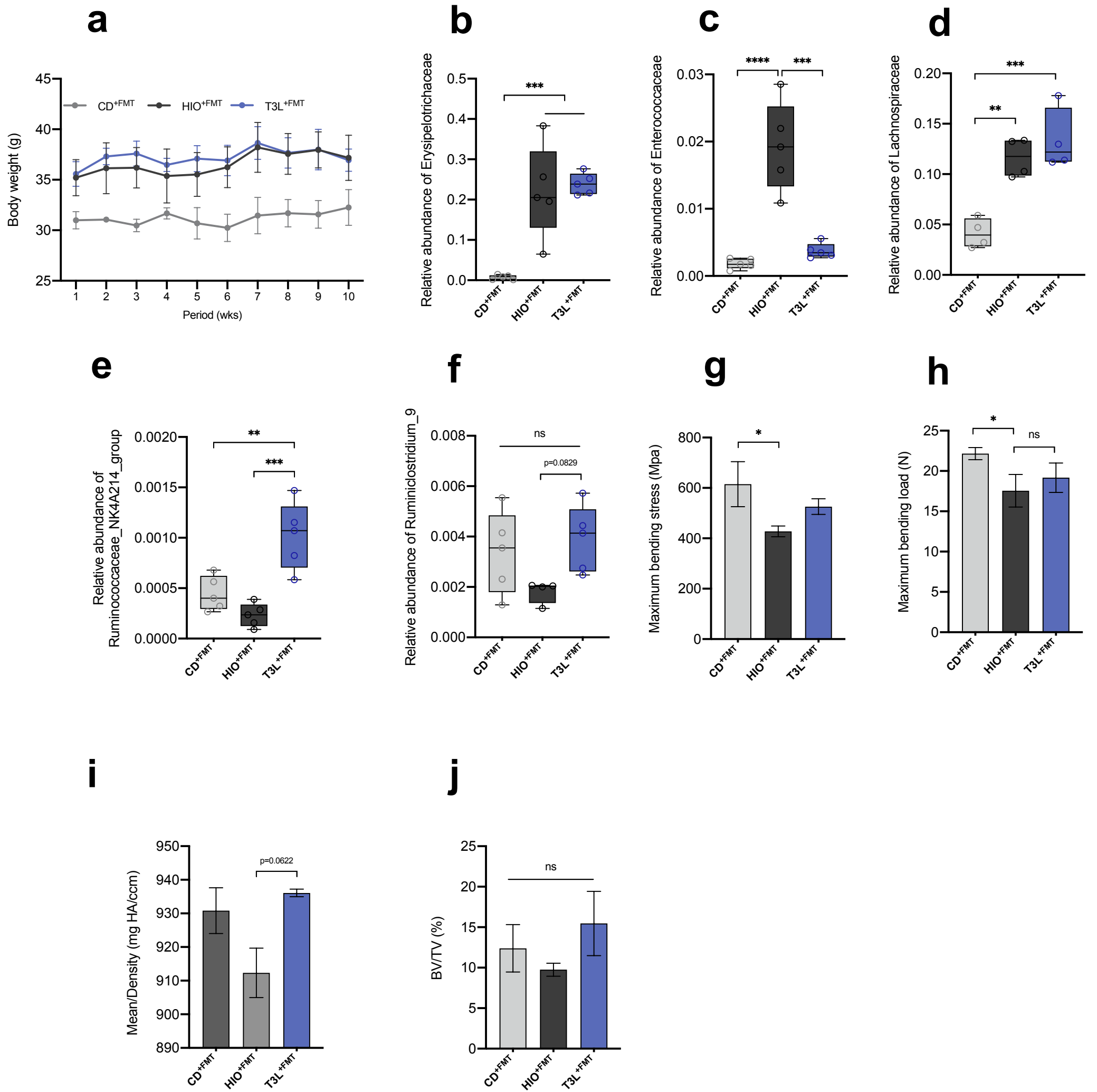


Figure S5

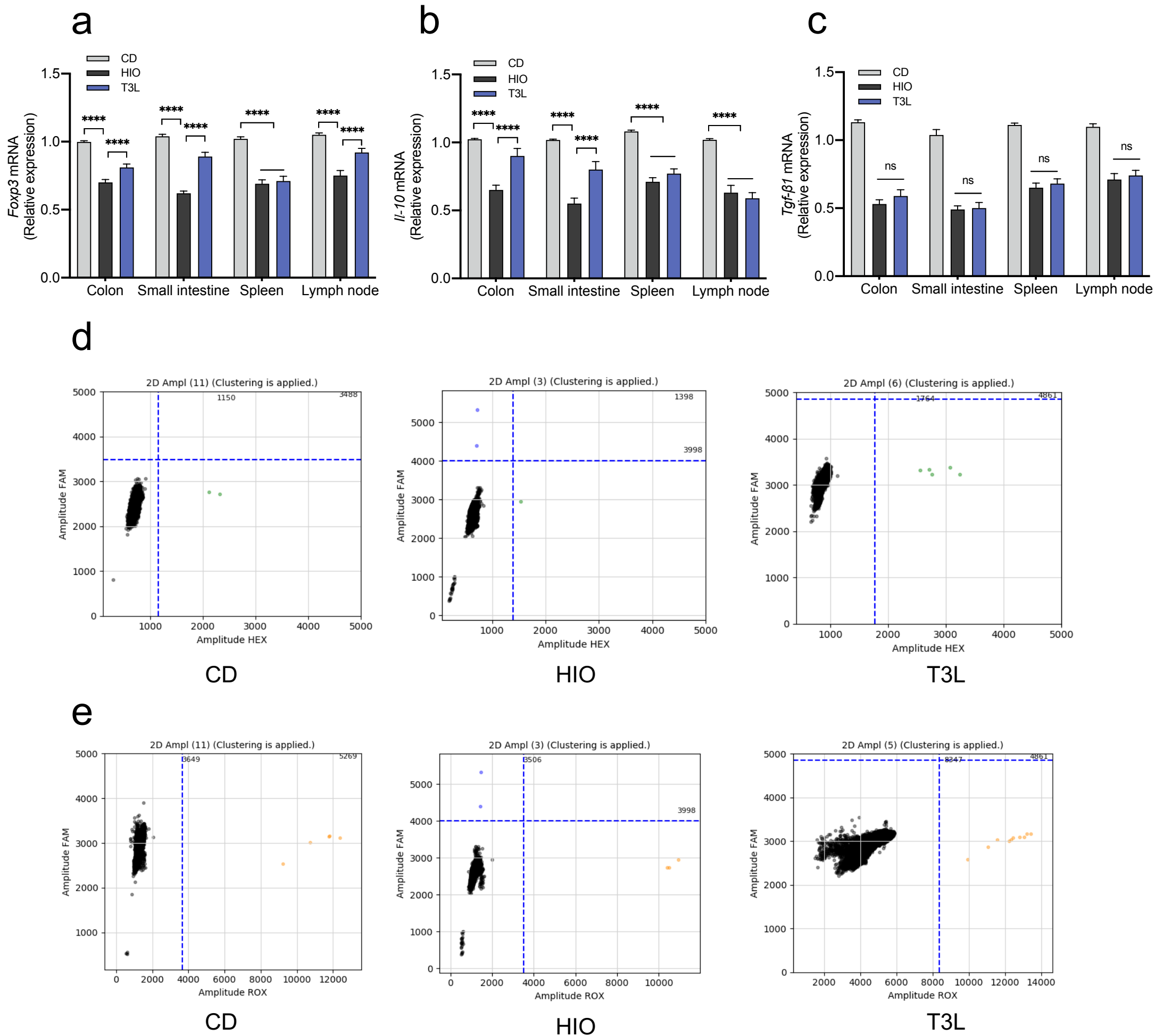


Figure S1 T3L reduces fat accumulation in HFD-fed mice. Related to Figure 1. (a) Effect of T3L treatment on body weight. (b) body weight percentage. (c) TC levels. (d) TG levels. (e) histologic score. (f) liver tissue. CD, chow diet; HFD, high-fat diet; TC, cholesterol; TG, triglyceride. Results determined by one-way ANOVA. Data are represented as the mean \pm SEM, n=3-6. ns: not significant, * p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Figure S2 T3L ameliorated HFD-induced gut microbiota in family level. Related to Figure 2. (a) Related abundance of Aerococcaceae.(b) Related abundance of Saccharimonadaceae.(c) Related abundance of Moraxellaceae.(d) Related abundance of Erysipelotrichaceae.(e) Related abundance of Eggerthellaceae.(f) Related abundance of Streptococcaceae.(g) Related abundance of Planococcaceae.(h) Related abundance of Leuconostocaceae.(i) Related abundance of Akkermansiaceae.(j) Related abundance of Enterobacteriaceae.(k) Related abundance of Lachnospiraceae.(l) Related abundance of Ruminococcaceae.(m) Related abundance of Muribaculaceae.(n) Related abundance of Peptostreptococcaceae.(o) Related abundance of Family_XIII.

Figure S3 Relative abundance of genera regulated by T3L. Related to Figure 2. (a)Heatmap displaying genera in CD, HIO and T3L groups. (b) Relative abundance of *Alistipes*. (c) Relative abundance of *Bacteroides*. (d) Relative abundance of *Lachnospiraceae_NK4A136_group*. (e) Relative abundance of *Prevotellaceae_UCG-001*. (f) Relative abundance of *Ruminiclostridium_9*. (g) Relative abundance of *Ruminococcaceae_NK4A214_group*. (h) Relative abundance of *Lachnospiraceae_UCG-006*.

Figure S4 T3L prevents HFD-induced bone loss through SCFAs mediated gut microbiota. (a) Body weight changes during 10 weeks fecal transplantation. (b) Relative abundance of Erysipelotrichaceae. (c) Relative abundance of Enterococcaceae. (d) Relative abundance of *Lachnospiraceae*. (e) Relative abundance of *Ruminococcaceae_NK4A214_group*. (f) Relative abundance of *Ruminiclostridium_9*. (g) Maximum bending stress. (h) Maximum bending load. (i) Mean/Density. (j) BV/TV.

Figure S5 Tregs relative cytokines expression in colon tissues and cells. (a) Relative mRNA expression of *Foxp3*. (b) Relative mRNA expression of *Il-10*. (c) Relative mRNA expression of *Tgf- β* . (d-e) number of gene copies of IL-10 and TGF- β .

1 **Table S1.** Gene primer sequences

Gene		Primer sequence (5'-3')
RANKL	F	5'- TCACTCTGTCCTCTTGGTA -3'
	R	5'- CGCTTCCCGATGTTTCA -3'
OPG	F	5'- GAGAGTGAGGCAGGCTAT -3'
	R	5'- TGTGAGGAGAGGAAGGAAG -3'
TRAP	F	5'- CACTCCCACCCTGAGATTTGT -3'
	R	5'- CATCGTCTGCACGGTTCTG -3'
Coll α 1	F	5'- ACGTCCTGGTGAAGTTGGTC -3'
	R	5'- TCCAGCAATACCCTGAGGTC -3'
CTSK	F	5'- TTGTGACCGTGATAATGTGA -3'
	R	5'- GCAGGCGTTGTTCTTATTC -3'
Foxp3	F	5'- GCAGAGAGGTATTGAGGGTGG -3'
	R	5'- CCACAGCATGGGTCTGTCTTC -3'
IL-10	F	5'- CCAAGCCTTATCGGAAATGA -3'
	R	5'- TCCTGAGGGTCTTCAGCTTC -3'
TGF- β	F	5'- CAATTCCTGGCGTTACCTTG -3'
	R	5'- AGCCCTGTATTCCGTCTCCT -3'
Ffar2	F	5'- GGCTTCTACAGCAGCATCTA -3'
	R	5'- AAGCACACCAGGAAATTAAG -3'
HDAC2	F	5'-CTATCCCGCTCTGTGCCCTA-3'
	R	5'-CCTCCTTGACTGTACGCCAT-3'
HDAC7	F	5'-CTTTCTCAGGCTGCTCTCCC -3'
	R	5'-AGGGCTATGGAGGTGCATCA -3'
HDAC9	F	5'- GCGGTCCAGGTAAAAACAGAA-3'
	R	5'- GCCACCTCAAACACTCGCTT-3'
P300	F	5'- ACCACCACCAGCAACA -3'
	R	5'- AAGGAGCAGCAGGAAGT -3'
CREBBP	F	5'- CTGAGATGATGGAAGAGGATT -3'
	R	5'- GAAGGAGATGTTGATTGTGAG -3'

Gene	Primer sequence (5'-3')	
PCAF	F	5'- ATGAGTCCTGAAATGGCAGAGG -3'
	R	5'- CAAGTGAGAAACGTGAGCAGC -3'

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4 **Table S2.** Antibodies and buffers used in flow cytometry

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Item No.	Antibodies and buffers		Company
421002	Intracellular Staining Permeabilization Wash Buffer (10X)	100 mL	Biolegend
420801	Fixation Buffer	100 mL	Biolegend
424401	True-Nuclear™ Transcription Factor Buffer Set	120 tests	Biolegend
100510	FITC anti-mouse CD4	500 µg	Biolegend
101903	PE anti-mouse CD25	25 µg	Biolegend
126407	Pacific Blue anti-mouse FOXP3	25 µg	Biolegend
100306	FITC anti-mouse CD3	500 µg	Biolegend
100408	PE anti-mouse CD4	200 µg	Biolegend
100712	APC anti-mouse CD8a	100 µg	Biolegend
100510	FITC anti-mouse CD4	500 µg	Biolegend
101903	PE anti-mouse CD25	25 µg	Biolegend

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11 **Table S3.** Reference genomes for Mm Foxp3, Mm IL-10 and Mm TGF-beta

12 Mm Foxp3 Reference genome.

13 >NC_000086.8 Mus musculus strain C57BL/6J chromosome X, GRCm39

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MM Foxp3 Forward Primer F1	CGAAAGTGGCAGAGAGGTATT
MM Foxp3 Probe P1	CCCAGCCACTCCAGACAGAAGAAA
MM Foxp3 Reverse Primer R1	GCATGGGTCTGTCTTCTCTAAG
MM Foxp3 Forward Primer F2	CTATGCCACCCTTATCCGATG
MM Foxp3 Probe P2	TTTCATTGAGTGTCTCTGCCTCTCC
MM Foxp3 Reverse Primer R2	TGGTTTCTGAAGTAGGCGAAC

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16 **Mm IL10 reference genome**

17 >NC_000067.7 Mus musculus strain C57BL/6J chromosome 1, GRCm39

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MM IL10 Forward Primer F1	TTGAATTCCCTGGGTGAGAAG
MM IL10 Probe P1	TGAGGCGCTGTCATCGATTTCTCC
MM IL10 Reverse Primer R1	TCCACTGCCTTGCTCTTATTT
MM IL10 Forward Primer F2	AACATACTGCTAACCGACTCC
MM IL10 Probe P2	AACCCAAGTAACCCTTAAAGTCCTGCAT
MM IL10 Reverse Primer R2	CATTTCCGATAAGGCTTGGC

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20 **Mm TGF-beta reference genome**

21 >NC_000073.7 Mus musculus strain C57BL/6J chromosome 7, GRCm39

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MM TGFB Forward Primer F1	AGAGCCCTGGATACCAACTA
MM TGFB Probe P1	AGCTCCACAGAGAAGAAGACTGCTGT
MM TGFB Reverse Primer R1	CAACCCAGGTCCTTCCTAAAG
MM TGFB Forward Primer F2	CTATGCTAAAGAGGTCACCCG
MM TGFB Probe P2	TTCATAGATGGCGTTGTTGCGGT
MM TGFB Reverse Primer R2	TCCCGAATGTCTGACGTATTG

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