

Supporting Information

Sub-chronic exposure of mice to cadmium perturbs their hepatic energy metabolism
and gut microbiome

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Table S1 Sequences of primer pairs used in the real-time quantitative PCR reactions

Gene name	Full name	Primer sequence
<i>Glut2</i>	Glucose transporter type 2	Forward 5'-ACCCTGTTCTTAACCGGG-3' Forward 5'-TGAACCAAGGGATTGGACC-3'
<i>Gk</i>	Glucokinase	Forward 5'-GGCCACCAAGAAGGAAAAGGT-3' Reverse 5'-CCTCTCCCACCTTTGACCAG CA-3'
<i>Pk</i>	Pyruvate kinase	Forward 5'-CAACAGGAAGGGTGTGAACTTG-3' Reverse 5'-ACAAAGGAGGCAAAGATGATGT-3'
<i>Chrebp</i>	Carbohydrate regulatory element binding protein	Forward 5'-CCCTCAGACACCCACATCTT-3' Reverse 5'-CAGAGCTCAGAAAGGGGTTG-3'
<i>Cs</i>	Citrate synthase	Forward 5'-AGCAAGGGAAGGCTAAGAAC-3' Reverse 5'-CCCGAGACACTCCAAACAG-3'
<i>Acl</i>	Atp citrate lyase	Forward 5'-AGGAAGTGCCACCTCCAACAGT-3' Reverse 5'-CGCTCATCACAGATGCTGGTCA-3'
<i>Acc</i>	Acetyl-coa carboxylase	Forward 5'-TAACAGAATCGACACTGGCTGGCT-3' Reverse 5'-ATGCTGTTCTCAGGCTCACATCT-3'
<i>Fas</i>	Fatty acid synthase	Forward 5'-GCAGCAAGTGTCACCAACAA-3' Reverse 5'-CTCATCGGAGCGCAGGATAGA-3'
<i>Scd1</i>	Stearoyl-coa desaturase-1	Forward 5'-TCCCTCCGGAAATGAACGAGAGAA-3' Reverse 5'-AGTGCAGCAGGACCATGAGAATGA-3'
<i>Fatp2</i>	Fatty acid transport protein 2	Forward 5'-GGAACCACAGGTCTTCCAAA -3' Reverse 5'-TAAAGTAGCCCCAACACGA -3'
<i>Fat</i>	Fatty acid transporter	Forward 5'-TTGCTGCCTTCTGAAATGTG-3' Reverse 5'-GCAGAATCAAGGGAGAGCAC-3'
<i>Fabp1</i>	Fatty acid-binding protein 1	Forward 5'-GTCAGCTGTGGAAAGGAAGC-3' Reverse 5'-GTCTCCAGTTCGCACTCCTC-3'
<i>Gpat</i>	Glyceraldehyde 3-phosphate acyltransferase	Forward 5'-CAACACCATCCCCGACATC-3' Reverse 5'-GTGACCT TCGATTATGCGATCA-3'
<i>Dgat1</i>	Diacylglycerol acyltransferase1	Forward 5'-GACGGCTACTGGGATCTGA-3' Reverse 5'-TCACCACACACCAATTCAGG-3'
<i>Dgat2</i>	Diacylglycerol acyltransferase2	Forward 5'-CGCAGCGAAAACAAGAATAA-3' Reverse 5'-GAAGATGTCTTGAGGGGCTG-3'
<i>Srebp1c</i>	Sterol-regulatory-element-binding protein-1c	Forward 5'-TATGGAGGGCATGAAACCCGAAGT-3' Reverse 5'-TTGACCTGGCTATCCTCAAAGGCT-3'
<i>Pparγ</i>	Peroxisome proliferator-activated receptor γ	Forward 5'-TTCGCTGATGCACTGCCTATG-3' Reverse 5'-CGAAGTTGGTGGGCCAGAA-3'
<i>Mtp</i>	Microsomal triglyceride transfer protein	Forward 5'-CACTCAGGCAATTCGAGACA-3' Reverse 5'-TATCGCTTTCTGGCTGAGGT-3'
<i>Mcad</i>	Medium chain acyl coA dehydrogenase	Forward 5'-GAGCCTGGGAACCTCGGCTTGA-3' Reverse 5'-GCCAAGGCCACCGCAACTTT-3'
<i>Cpt1</i>	Carnitine palmitoyltransferase-1	Forward 5'-CGCACGGAAGGAAAATGG-3' Reverse 5'-TGTGCCCAATATTCCTGG-3'

<i>Acox</i>	Acyl-Co A oxidase	Forward 5'-GCCCAACTGTGACTTCCATT-3' Reverse 5'-GGCATGTAACCCGTAGCACT-3'
<i>Ppar</i>	Peroxisome proliferator-activated receptor α	Forward 5'-CCTCAGGGTACCACTACGGAGT-3' Reverse 5'-GCCGAATAGTTCGCCGAA-3'
<i>I8s</i>		Forward 5'-CGAACGTCTGCCCTATCAACTT-3' Reverse 5'-CCGGAATCGAACCCCTGATT-3'
<i>LBP</i>	Lipopolysaccharide-binding protein	Forward 5'- GGCTCTGCAGAGAGAGCTGTACAA -3' Reverse 5'- TAGTTAAGGAATGCCTGGAACAGG -3'
<i>MD-2</i>		Forward 5'- ATGTTGCCATTTATTCTCTTTTCGACG-3' Reverse 5'- ATTGACATCACGGCGGTGAATGATG -3'
<i>TLR4</i>	Toll-like receptor 4	Forward 5'- GGTGGCACTCTCACTTCCT -3' Reverse 5'- ATCTGTAAGTGGTGGCAGCG -3'
<i>CD14</i>		Forward 5'- CCCAAGCACACTCACTCAAC -3' Reverse 5'- TCAGTCCTCTCTCGCCCAAT -3'
<i>g-proteobacteria</i>		Forward 5'- GAGTTTGATCATGGCTCA -3' Reverse 5'- GTATTACCGCGGCTGCTG -3'
<i>Firmicutes</i>		Forward 5'- GGAGYATGTGGTTTAATTCGAAGCA-3' Reverse 5'- AGCTGACGACAACCATGCAC -3'
<i>Bacteroidetes</i>		Forward 5'- GGARCATGTGGTTTAATTCGATGAT-3' Reverse 5'- AGCTGACGACAACCATGCAG -3'
<i>Bacteria</i>		Forward 5'- ACTCCTACGGGAGGCAGCAG -3' Reverse 5'- ATTACCGCGGCTGCTGG -3'

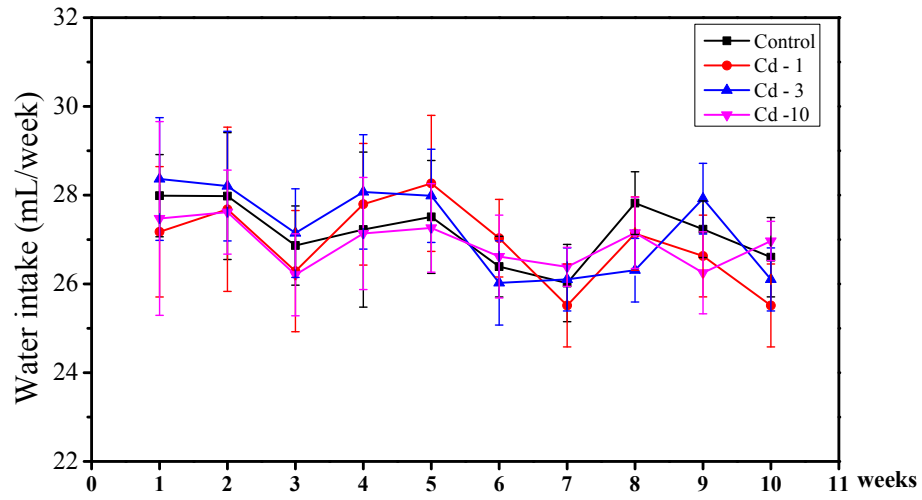
Table S2. Relative abundance of typical gut bacterial families in the measurements for control and Cd-10 mg/L exposed mice (n=4).

Taxon	Relative abundance (control)	Standard error (control)	Relative abundance (Cd)	Standard error (Cd)
Unassigned;Other;Other;Other;Other	0.021277217	0.001253929	0.032694413	0.003481444
k__Bacteria;Other;Other;Other;Other	5.83867E-06	5.83867E-06	4.47243E-06	4.47243E-06
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Dermacoccaceae	4.50566E-05	2.81986E-05	4.0388E-05	3.08101E-05
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micrococcaceae	5.83867E-06	5.83867E-06	1.09776E-05	6.55163E-06
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Nocardiaceae	0.005126736	0.001687476	0.002247767	0.001588292
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Pseudonocardiaceae	1.05755E-05	1.05755E-05	0	0
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Bifidobacteriales;f__Bifidobacteriaceae	0.000212334	7.5767E-05	0.00029376	0.0001228
k__Bacteria;p__Actinobacteria;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacteriaceae	0.000166111	3.76332E-05	7.95127E-05	4.51187E-05
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;Other	0.000116379	3.02942E-05	0.000312653	8.31558E-05
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__	0.000198588	3.6103E-05	0.00028114	9.66886E-05
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae	0.164943973	0.030222867	0.269951421	0.005899553
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Porphyromonadaceae	0.041319165	0.009707369	0.038692318	0.007339584
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Prevotellaceae	0.001799513	0.000374637	0.003581146	0.000533772
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae	0.04555692	0.002501438	0.033943709	0.007330979
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__S24-7	0.163291461	0.020085224	0.133989944	0.011471038
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__[Paraprevotellaceae]	0.074115052	0.017435739	0.180911985	0.029439998
k__Bacteria;p__Chloroflexi;c__Anaerolineae;o__SHA-20;f__	5.28776E-06	5.28776E-06	7.86213E-06	7.86213E-06
k__Bacteria;p__Cyanobacteria;c__4C0d-2;o__YS2;f__	1.11264E-05	6.43957E-06	0.000123025	9.85142E-05
k__Bacteria;p__Cyanobacteria;c__Chloroplast;o__Streptophyta;f__	3.39094E-05	1.37163E-05	5.65679E-05	2.45525E-05
k__Bacteria;p__Deferribacteres;c__Deferribacteres;o__Deferribacterales;f__Deferribacteraceae	0.000489498	0.00021984	3.29328E-05	1.96549E-05
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Bacillaceae	2.6479E-05	1.80416E-05	2.54477E-05	1.21E-05
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Planococcaceae	1.37415E-05	7.97586E-06	3.11743E-05	1.29934E-05
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Staphylococcaceae	6.58157E-05	4.41798E-05	4.47243E-06	4.47243E-06
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Lactobacillaceae	0.002765461	0.001719174	0.001291903	0.000505019
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Leuconostocaceae	0.000167471	8.43416E-05	0.000257794	8.86105E-05
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Streptococcaceae	0.002763553	0.000879357	0.000668498	0.000157533
k__Bacteria;p__Firmicutes;c__Bacilli;o__Turicibacterales;f__Turicibacteraceae	0.005392836	0.002682292	0.004747285	0.001966886
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;Other	0.000104517	6.8591E-05	4.69472E-05	1.41159E-05
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__	0.16209165	0.043207539	0.088665893	0.005725934
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Christensenellaceae	0.001728529	0.000340871	0.001251696	0.000366646
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae	0.013965475	0.010623378	0.004295597	0.001524887
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Dehalobacteriaceae	0.006479454	0.00045232	0.00700929	0.00081506
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae	0.065242525	0.017425199	0.034698936	0.005560401
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Peptococcaceae	0.000312133	9.6231E-05	0.000385217	0.000140037
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae	0.116624708	0.012788829	0.09363715	0.009802503

k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Veillonellaceae	0.000138217	5.66031E-05	0.000248297	7.71923E-05
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__[Mogibacteriaceae]	0.000621138	6.56863E-05	0.000522851	0.000129474
k__Bacteria;p__Firmicutes;c__Erysipelotrichi;o__Erysipelotrichales;f__Erysipelotrichaceae	0.004984959	0.000906066	0.002053294	0.001227285
k__Bacteria;p__Proteobacteria;Other;Other;Other	1.16565E-05	6.78754E-06	4.47243E-06	4.47243E-06
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__RF32;f__	2.38848E-05	1.65227E-05	8.94486E-06	8.94486E-06
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Methylobacteriaceae	5.83867E-06	5.83867E-06	7.23505E-06	7.23505E-06
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria;o__Rhizobiales;f__Rhizobiaceae	0	0	1.44701E-05	1.44701E-05
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Alcaligenaceae	1.32113E-05	7.72972E-06	2.91416E-05	1.693E-05
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae	5.28776E-06	5.28776E-06	4.47243E-06	4.47243E-06
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfovibrionales;f__Desulfovibrionaceae	0.037748033	0.005006804	0.034205647	0.006100142
k__Bacteria;p__Proteobacteria;c__Epsilonproteobacteria;o__Campylobacteriales;f__Helicobacteraceae	0.05939575	0.009026323	0.026749619	0.013126674
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Alteromonadales;f__Shewanellaceae	1.80253E-05	1.20542E-05	2.33122E-05	8.02302E-06
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae	2.4317E-05	8.8642E-06	3.065E-05	2.05351E-05
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Oceanospirillales;f__Halomonadaceae	0.000136547	1.05898E-05	7.83571E-05	2.44242E-05
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Moraxellaceae	9.51797E-05	9.51797E-05	0	0
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Pseudomonadaceae	1.58633E-05	1.58633E-05	3.54454E-05	2.74654E-05
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Vibrionales;f__Pseudoalteromonadaceae	3.70143E-05	3.70143E-05	3.14485E-05	3.14485E-05
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Vibrionales;f__Vibrionaceae	3.82127E-05	3.82127E-05	0.000105994	5.44483E-05
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Xanthomonadales;f__Xanthomonadaceae	3.59943E-05	5.06253E-06	0.000113263	4.37809E-05
k__Bacteria;p__Spirochaetes;c__Spirochaetes;o__Spirochaetales;f__Spirochaetaceae	5.83867E-06	5.83867E-06	7.86213E-06	7.86213E-06
k__Bacteria;p__TM7;c__TM7-3;o__CW040;f__F16	0.000115058	5.3999E-05	0.000444417	0.000223415
k__Bacteria;p__Tenericutes;c__Mollicutes;o__Anaeroplasmatales;f__Anaeroplasmataceae	1.7516E-05	1.7516E-05	0	0
k__Bacteria;p__Tenericutes;c__Mollicutes;o__RF39;f__	1.37415E-05	7.97586E-06	0	0
k__Bacteria;p__Verrucomicrobia;c__Verrucomicrobiae;o__Verrucomicrobiales;f__Verrucomicrobiaceae	1.32113E-05	7.72972E-06	0.001007512	0.000790857
k__Bacteria;p__[Thermi];c__Deinococci;o__Deinococcales;f__Trueperaceae	1.05755E-05	1.05755E-05	0	0

The "Other" assignments are typically due to ambiguity when the RDP classifier tries to assign at the desired level. The “f_” or “f_unassigned” means that it matches a reference sequence, but that reference sequence is not well defined.

A



B

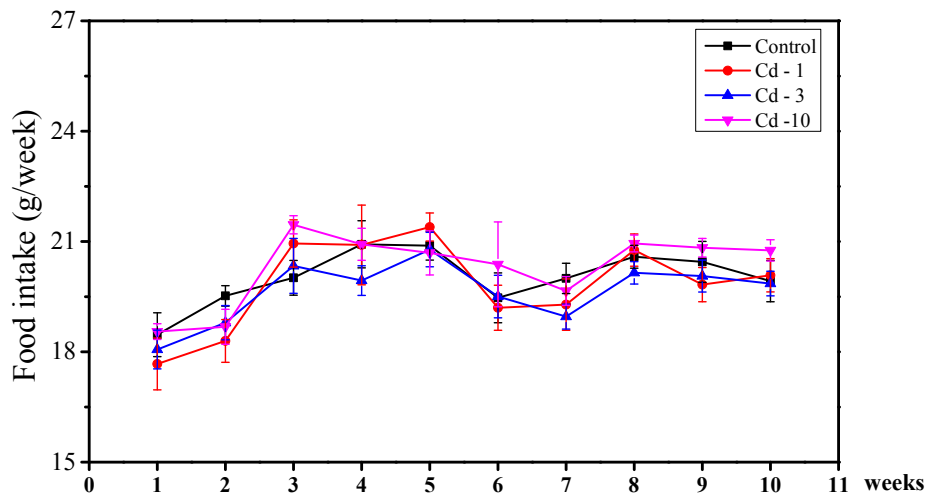


Figure S1. Water intake and food consumption across treatment groups (n=8). Intakes of water (A), food (B) every week by control mice or mice exposed to 1, 3 or 10 mg/L Cd.

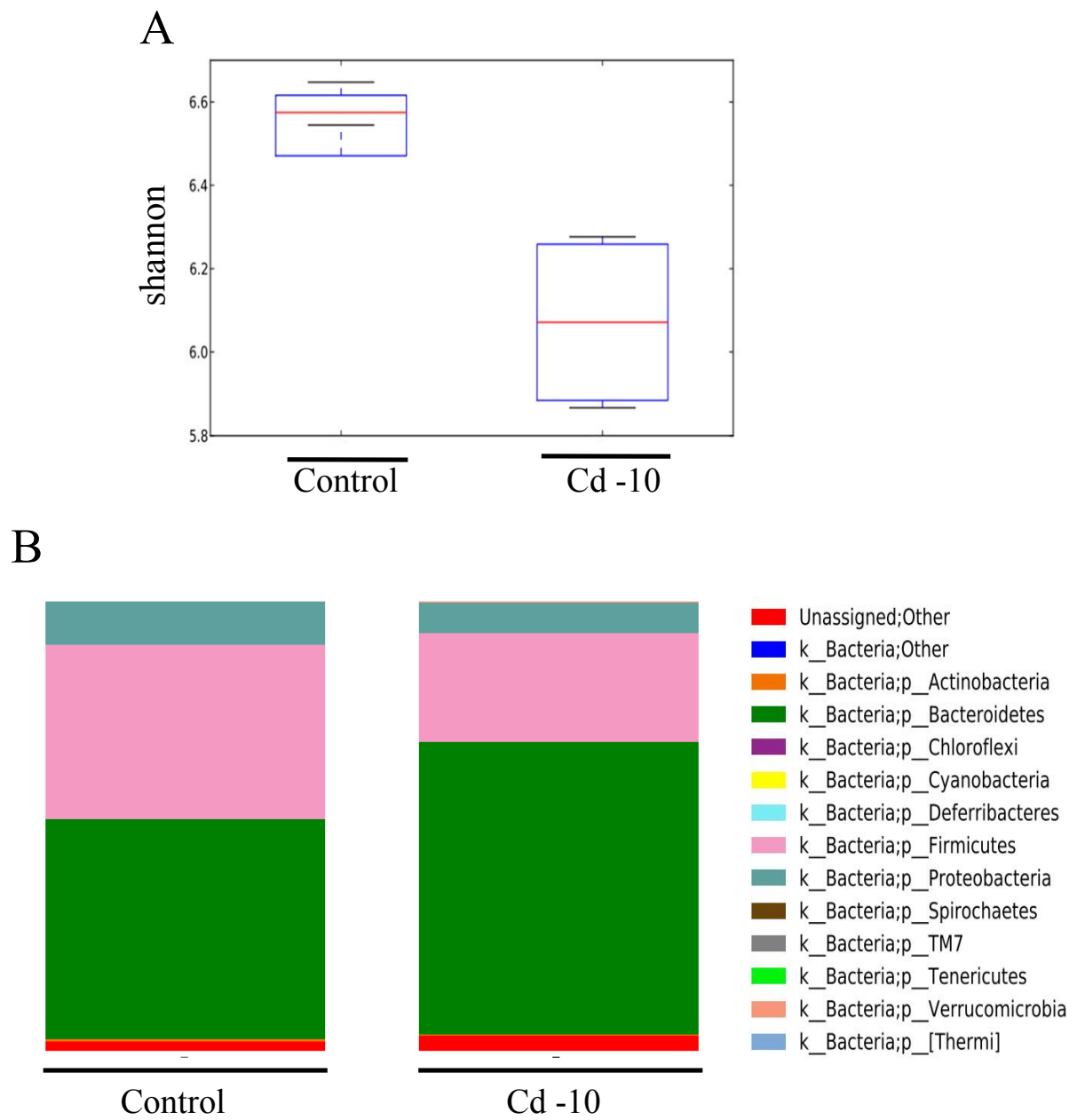


Figure S2. Alpha diversity boxplots and abundance of typical gut bacterial in the measurements across treatment groups (n=4). (A) Alpha diversity boxplots (Group, shannon); (B) Abundance of typical gut bacterial in the measurements for control and Cd exposed mice.

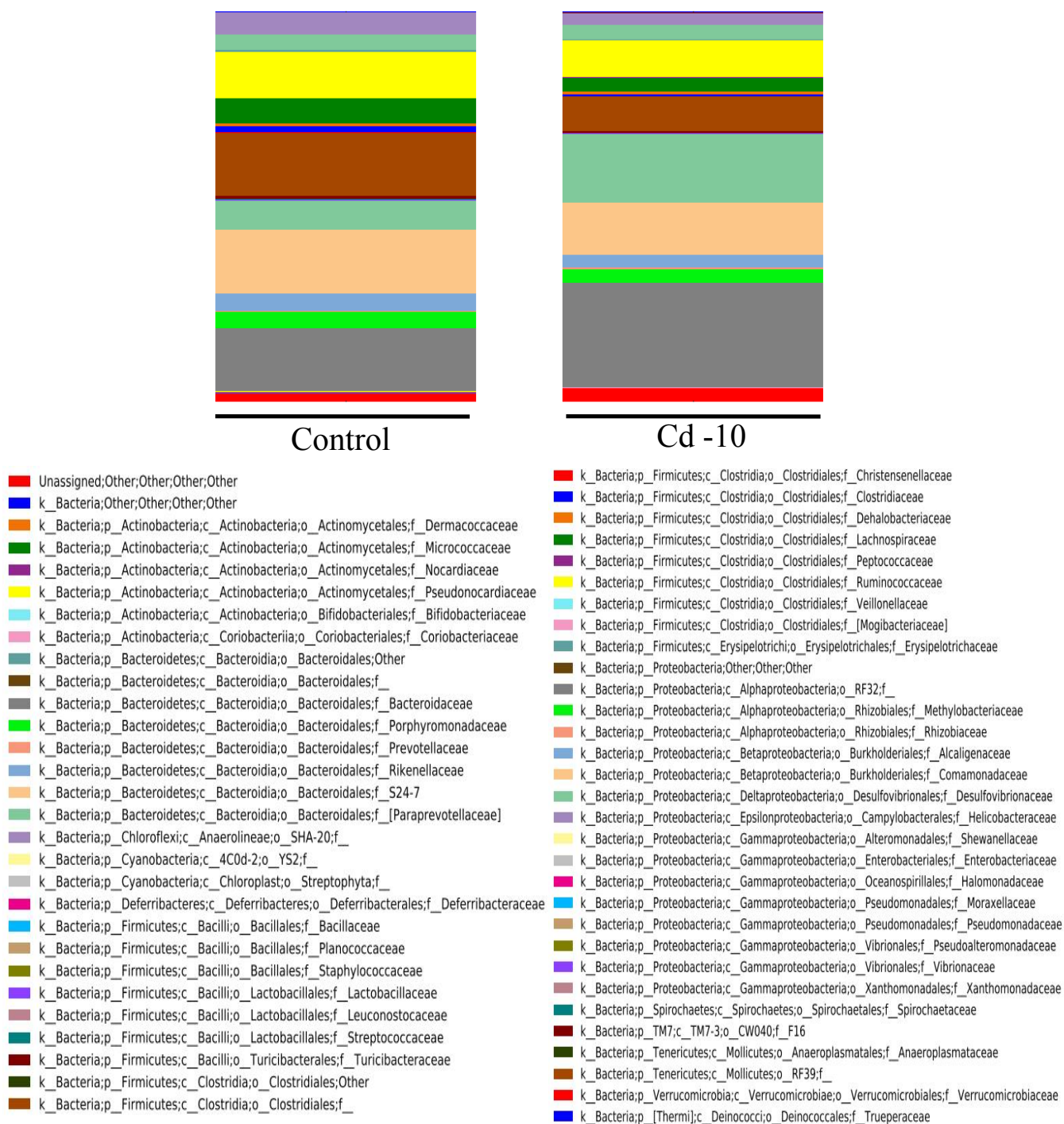


Figure S3. Abundance of typical gut bacterial families in the measurements for control and Cd exposed mice (n=4).