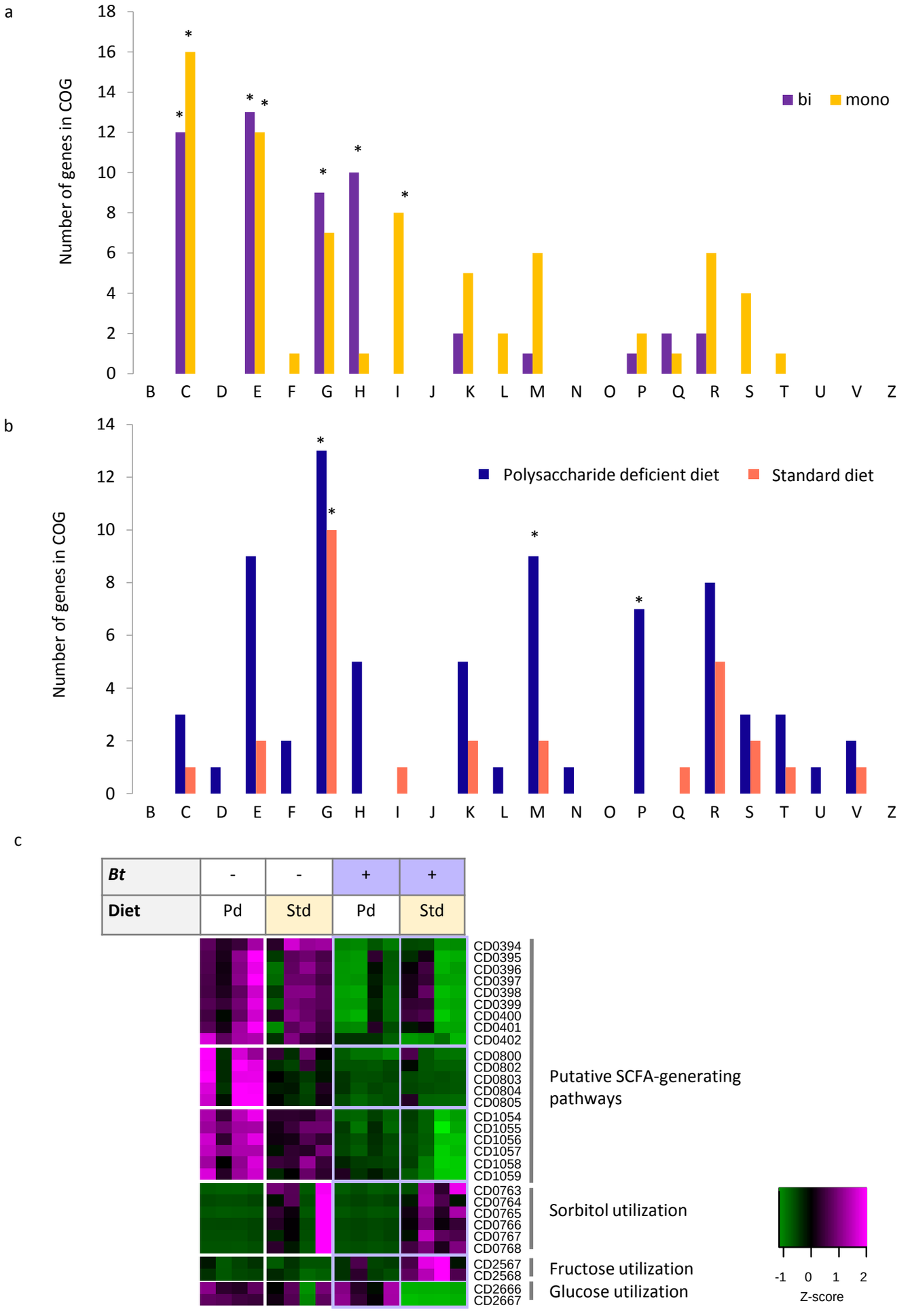
a
Number of genes in COG
bi
mono
B C D E F G H I J K L M N O P Q R S T U V Z
b
Number of genes in COG
Polysaccharide deficient diet
Standard diet
B C D E F G H I J K L M N O P Q R S T U V Z
c
Bt - - + +
Diet Pd Std Pd Std
CD0394 CD0395 CD0396 CD0397 CD0398 CD0399 CD0400 CD0401 CD0402
CD0800 CD0802 CD0803 CD0804 CD0805
CD1054 CD1055 CD1056 CD1057 CD1058 CD1059
Putative SCFA-generating pathways
CD0763 CD0764 CD0765 CD0766 CD0767 CD0768
Sorbitol utilization
CD2567 CD2568
Fructose utilization
CD2666 CD2667
Glucose utilization
-1 0 1 2
Z-score

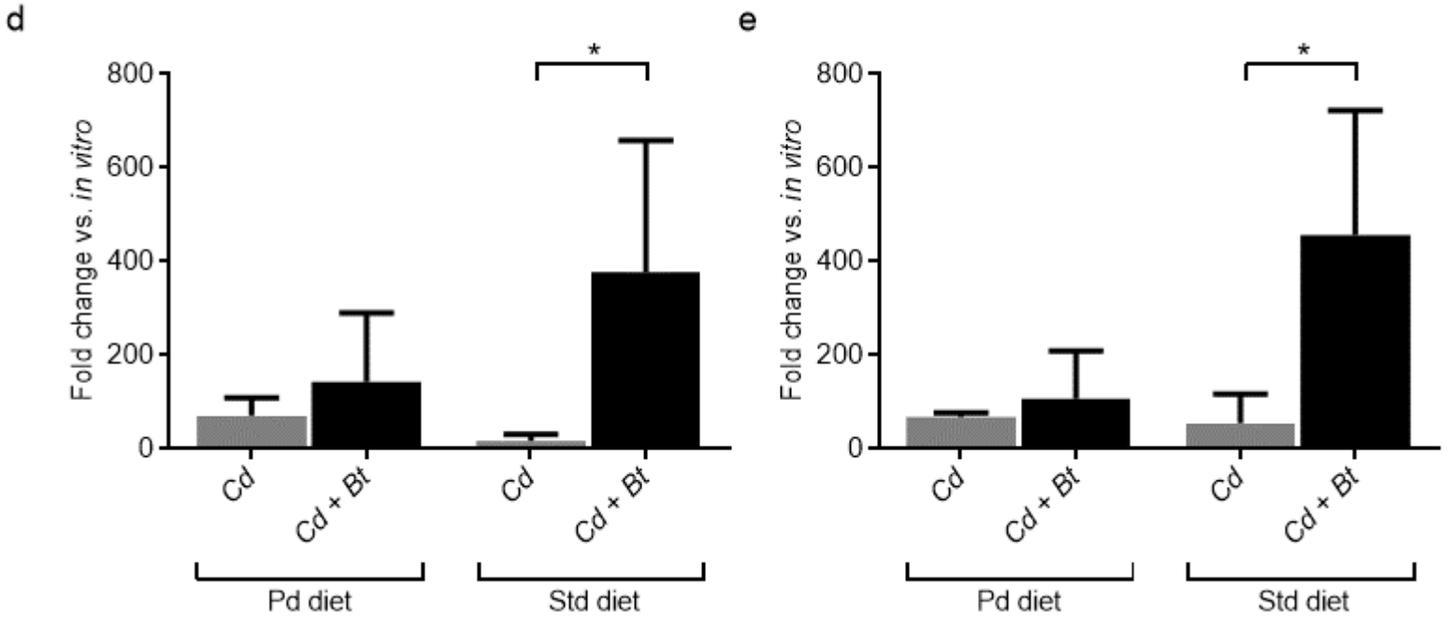

Figure S1, Related to Figure 1. *C. difficile* genes with altered expression in response to colonization state or diet.

a., b., Each graph shows the number of genes in each COG category that were significantly upregulated in the comparison between (a.) germ-free mice mono-associated with *C. difficile* (mono) versus *Bt*-biassociated mice colonized with *C. difficile* (bi) and (b.) polysaccharide deficient diet versus standard diet. Letters represent the following COG categories: chromatin structure and dynamics (B); energy production and conversion (C); cell cycle, cell division and chromosome partitioning (D); amino acid transport and metabolism (E); nucleotide transport and metabolism (F); carbohydrate transport and metabolism (G); coenzyme transport and metabolism (H); lipid transport and metabolism (I); translation, ribosomal structure and biogenesis (J); transcription (K); replication, recombination and repair (L); cell wall, membrane and envelope biogenesis (M); cell motility (N); post-translational modification, protein turnover and chaperones (O); inorganic ion transport and metabolism (P); secondary metabolites biosynthesis, transport and catabolism (Q); general function prediction only (R); function unknown (S); signal transduction mechanisms (T); and intracellular trafficking, secretion and vesicular transport (U); and defense mechanisms (V). Bars labeled with * indicate a significant increase in gene abundance relative to representation in the genome (hypergeometric probability function $p < 0.01$).

c., Additional *C. difficile* operons with significant differences in expression between colonization state and diet. Colors indicate the deviation of each gene's signal above (green) and below (purple) its mean expression value across all sixteen *in vivo* samples (n=4/group) at 5 days post-infection in the cecal contents.
d., e., Induction of *C. difficile* genes from the succinate to butyrate pathway, including *cat1* (CD2343) (d) and *abfT* (CD2339) (e) in cecal contents at 5 days post-infection, normalized to growth in minimal media with glucose.

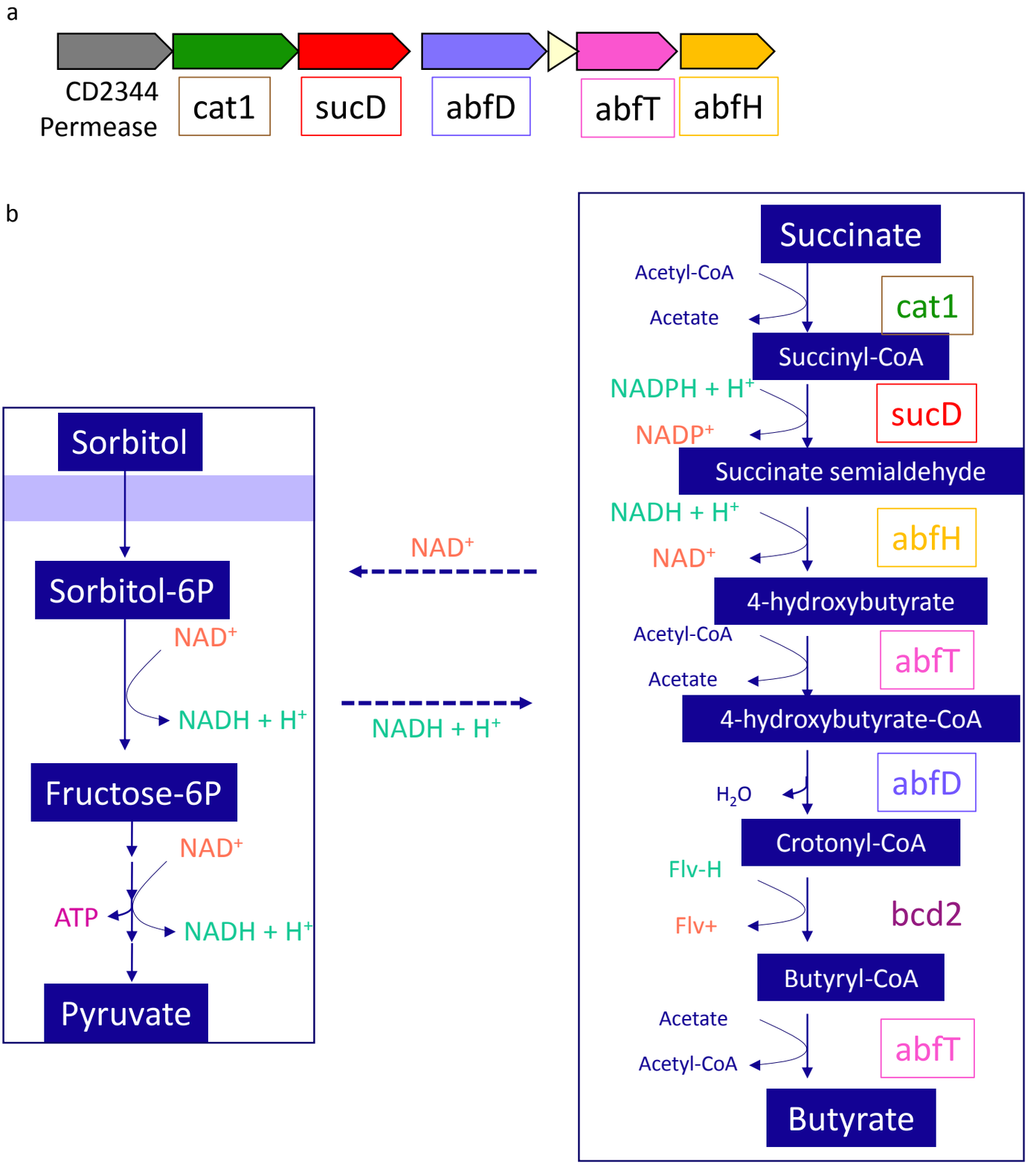

Figure S2, Related to Figure 2. Overview of the *C. difficile* succinate-to-butyrate operon and pathway.

a. Schematic of the gene cluster comprising the succinate-to-butyrate pathway in *Clostridium difficile strain 630.*

b. Schematic of the metabolic pathway for the conversion of succinate to butyrate and fermentation of sorbitol. Potential complementarity of electron flow is noted between pathways.

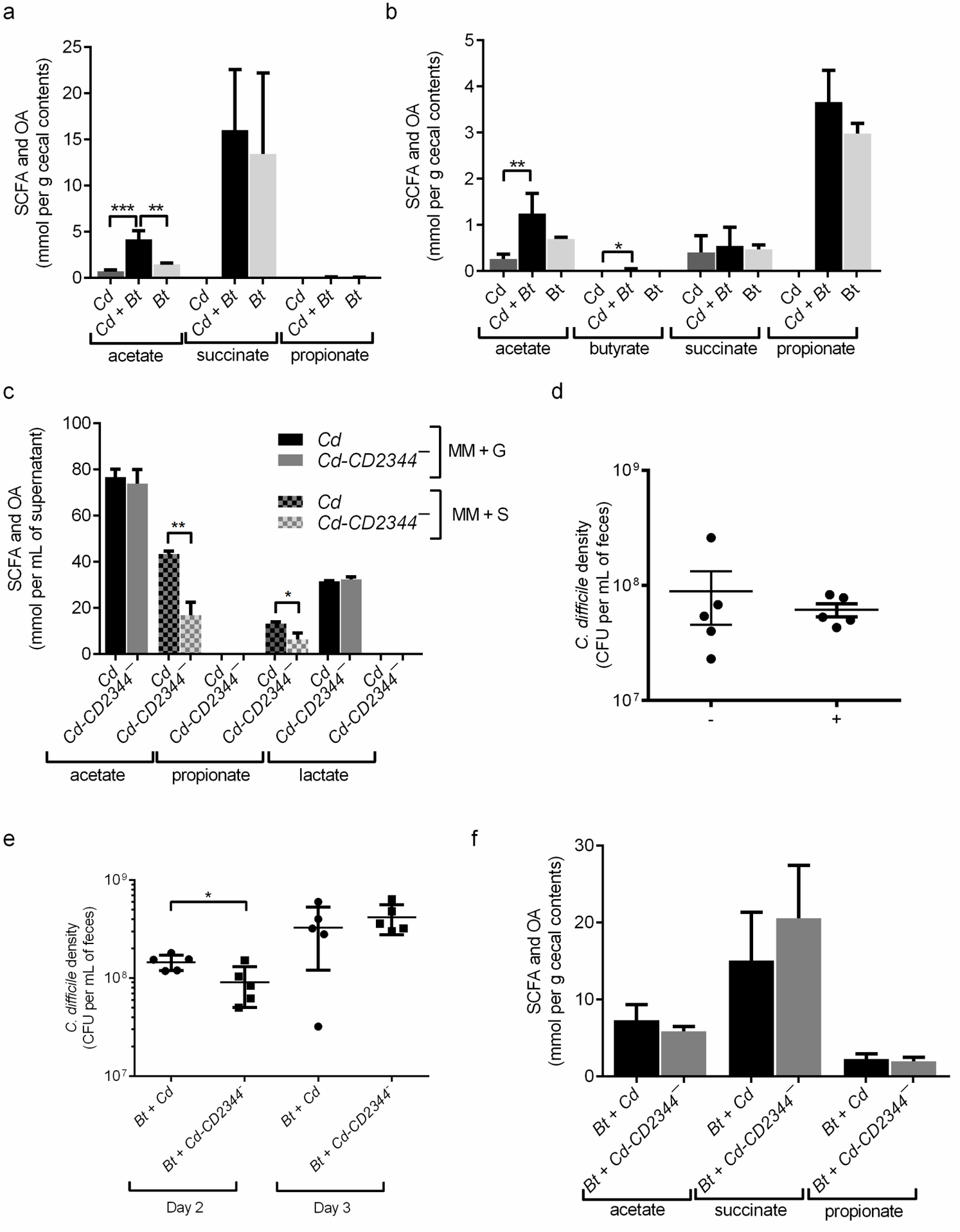
a
SCFA and OA
(mmol per g cecal contents)
25
20
15
10
5
0
***
**
Cd
Cd + Bt
Bt
acetate
succinate
propionate
b
SCFA and OA
(mmol per g cecal contents)
5
4
3
2
1
0
**
*
acetate
butyrate
succinate
propionate
c
SCFA and OA
(mmol per mL of supernatant)
100
80
60
40
20
0
Cd
Cd-CD2344⁻
MM + G
MM + S
acetate
propionate
lactate
d
C. difficile density
(CFU per mL of feces)
10⁹
10⁸
10⁷
-
+
e
C. difficile density
(CFU per mL of feces)
10⁹
10⁸
10⁷
*
Bt + Cd
Bt + Cd-CD2344⁻
Day 2
Day 3
f
SCFA and OA
(mmol per g cecal contents)
30
20
10
0
Bt + Cd
Bt + Cd-CD2344⁻
acetate
succinate
propionate

Figure S3, Related to Figure 3. *Cd* and *Cd-CD2344*$^{-}$ production of SCFAs and density in *Bt*-biassociation experiments and *in vitro.*

a. Concentration of short-chain fatty acids acetate, succinate and propionate in cecal contents of mice consuming standard diet and colonized with *C. difficile* (*Cd*), *Bt* (*Bt*) or *C. difficile* and *Bt* (*Cd* + *Bt*) at 5 days post-infection (n=4/group).

b. Concentration of short-chain fatty acids acetate, butyrate, succinate and propionate in cecal contents of mice consuming polysaccharide-deficient diet and colonized with *C. difficile* (*Cd*), *Bt* (*Bt*) or *C. difficile* and *Bt* (*Cd* + *Bt*) and at 5 days post-infection (n=4/group).

c. Concentrations of acetate, propionate or lactate produced by *Cd* or *Cd-CD2344*$^{-}$ in minimal media containing 0.5% glucose (filled bars, MM + G) or 0.1% succinate (checkered bars, MM + S) (n=3/group).

d. *Cd-CD2344*$^{-}$ mutant *C. difficile* (*Cd-CD2344*$^{-}$) density in feces of germ-free mice consuming polysaccharide-deficient diet and administered water (-) or water containing 1% sorbitol and 1% succinate (+) at 1 day post-infection (n=5/group).

e. *C. difficile* density in feces of *Bt*-biassociated mice infected with wild-type *C. difficile* (*Bt* + *Cd*) or *Cd-CD2344*$^{-}$ mutant *C. difficile* (*Bt* + *Cd-CD2344*$^{-}$) at day 2 and 3 post-infection (n=5/group).

f. Levels of acetate, succinate and propionate detected in cecal contents of *Bt*-biassociated mice infected with wild-type *C. difficile* (*Cd*) or *Cd-CD2344*$^{-}$ mutant *C. difficile* (*Cd-CD2344*$^{-}$) at day 3 post-infection (n=5/group).

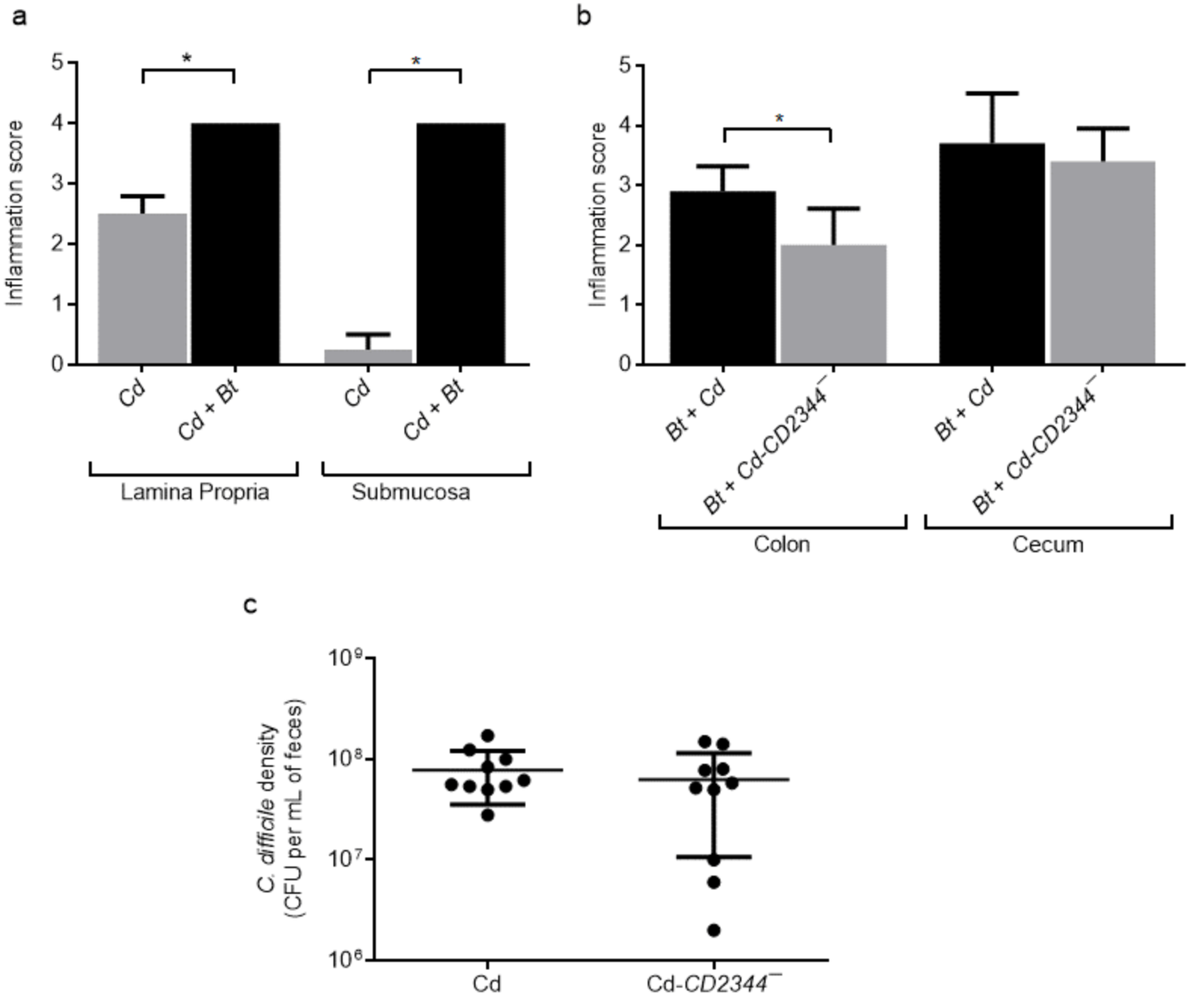

Figure S4, Related to Figure 4. *C. difficile*-induced inflammation is greater in *Bt*-biassociation.

a. Mean pathology score of H&E stained formalin fixed sections from mouse cecum of mice in monoassociation with *C. difficile* (*Cd*) or *Bt*-biassociation with *C. difficile* (*Cd* + *Bt*) at day 5 post-infection (n=4/group). Statistical analysis conducted by Mann Whitney nonparametric test. * indicates $p<0.05$.

b. Mean pathology scores from the lamina propria of the colon or cecum of mice colonized with *Bt*-biassociated mice infected with wild-type *C. difficile* (*Bt* + *Cd*) or *Cd-CD2344¯* mutant *C. difficile* (*Bt* + *Cd-CD2344¯*) at day 3 post-infection (n=4/group). Statistical analysis conducted by Mann Whitney nonparametric test. * indicates $p<0.05$.

c. Density of C*. difficile* in feces of conventional, streptomycin-treated mice infected with wild-type *C. difficile* (*Cd*) or *Cd-CD2344¯* mutant *C. difficile* (*Cd-CD2344¯*) (n=10/group).

Supplemental Table S1, Related to Figure 1. Genes upregulated in *Bt*-biassociation vs. monoassociation

Fold change is provided when the gene's expression provided a false discovery rate of <1% for the indicated comparison. Blocks of genes highlighted in red, green, and yellow indicate the succinate-to-butyrate pathway, sialic acid utilization, and ethanolamine pathway, respectively.

		Both chows	Std	Pd
Gene ID	Gene Annotation	Fold Change	Fold Change	Fold Change
CD0135	PTS system, IIa component	10.2		15.9
CD0136	PTS system, IIb component	7.3		13.7
CD0137	PTS system, IIc component	10.8		18.3
CD0138	hypothetical protein	12.2		20.5
CD0139	glycosylasparaginase	7.3		14.3
CD0141	putative copper homeostasis protein	6.6		9.2
CD0324	putative cobalt transport protein			3.6
CD0325	cobalt transport protein			3.1
CD0334	aldehyde-alcohol dehydrogenase [includes: alcc	12.1		8.4
CD0448	putative amino acid racemase			4.6
CD0625	putative phage-related regulatory protein			1.7
CD0667	putative lantibiotic ABC transporter,ATP-binding			2.2
CD0678	putative transcriptional regulator			1.7
CD0990	3-isopropylmalate dehydratase large subunit			2.8
CD1009	GntR-family transcriptional regulator			6.4
CD1010	N-acetylglucosamine-6-phosphate deacetylase			8.2
CD1011	glucosamine-6-phosphate deaminase			8.2
CD1565	ketol-acid reductoisomerase	3.1		3.7
CD1594	putative O-acetylserine sulfhydrylase			5.0
CD1595	serine acetyltransferase			4.3
CD1599	putative phosphomethylpyrimidine kinase			11.3
CD1600	hydroxyethylthiazole kinase			10.0
CD1601	thiamine-phosphate pyrophosphorylase			9.0
CD1602	PTS system, IIb component			3.0
CD1702	thiamine biosynthesis protein			99.4
CD1702A	putative thiamine biosynthesis protein			58.9
CD1703	putative thiamine biosynthesis protein			90.1
CD1704	thiazole biosynthesis protein			134.7
CD1705	putative thiamine biosynthesis protein			134.9
CD1706	putative thiamine biosynthesis protein			34.6
CD1914	putative ethanolamine/propanediol ammonia-ly	3.0		
CD1918	putative ethanolamine/propanediol utilization p	2.5		
CD1919	putative ethanolamine/propanediol utilization c	2.8		

CD1978	ABC transporter, ATP-binding protein			3.0
CD2232	anaerobic sulfite reductase subunit B			2.1
CD2235	RpiR-family transcriptional regulator			9.0
CD2236	putative glucokinase	9.9		14.8
CD2237	hypothetical protein	10.1		24.1
CD2238	hypothetical protein	8.3		32.3
CD2239	putative sodium:solute symporter	15.9		57.4
CD2240	N-acetylneuraminate lyase	18.9		81.9
CD2241	putative N-acetylmannosamine-6-phosphate 2-	20.9		88.0
CD2338	NAD-dependent 4-hydroxybutyrate dehydrogen	5.3	4.9	
CD2339	4-hydroxybutyrate CoA transferase	7.1	6.9	
CD2340	hypothetical protein	5.0	5.1	3.3
CD2341	gamma-aminobutyrate metabolism dehydratase	4.9	4.9	3.9
CD2342	succinate-semialdehyde dehydrogenase (NAD(P	7.5	5.5	
CD2343	succinyl-CoA:coenzyme A transferase	8.2	5.3	
CD2344	hypothetical protein	7.7	4.3	
CD2370	nicotinate-nucleotide pyrophosphorylase (carbo	2.2	2.4	
CD2423	probable transporter	8.2		
CD2424	putative aminotransferase	8.3		
CD2425	phosphate butyryltransferase	10.2		
CD2568	PTS system, IIC component		2.4	
CD2702	putative branched-chain amino acid transport s	3.0		2.5
CD2938	hypothetical protein		1.7	

Supplemental Table S2, Related to Experimental Procedures. List of primers.

Name	Sequence	Notes
IBS	AAAAAAGCTTATAATTATCCTTAGTCATCGCATAAGTGC GCCCAGATAGGGTG	Primer used to make *Cd-CD2344*$^{-}$
EBS1d	CAGATTGTACAAATGTGGTGATAACAGATAAGTCGCAT AAGTTAACTTACCTTTCTTTGT	Primer used to make *Cd-CD2344*$^{-}$
EBS2	TGAACGCAAGTTTCTAATTTCGATTATGACTCGATAGAG GAAAGTGTCT	Primer used to make *Cd-CD2344*$^{-}$
EBS Universal	CGAAATTAGAAACTTGCGTTCAGTAAAC	Primer used to make *Cd-CD2344*$^{-}$
2344F	TGCAGCAGTTGGTCTATTGG	qPCR primer
2344R	AGCTAATCCAGGCGAACTTG	qPCR primer (For wild-type CD2344 amplification)
CDEP692	GTAAATTCAGATTCTCGGC	qPCR reverse primer (*Cd-CD2344*$^{-}$ mutant specific amplification)