Fig. S1

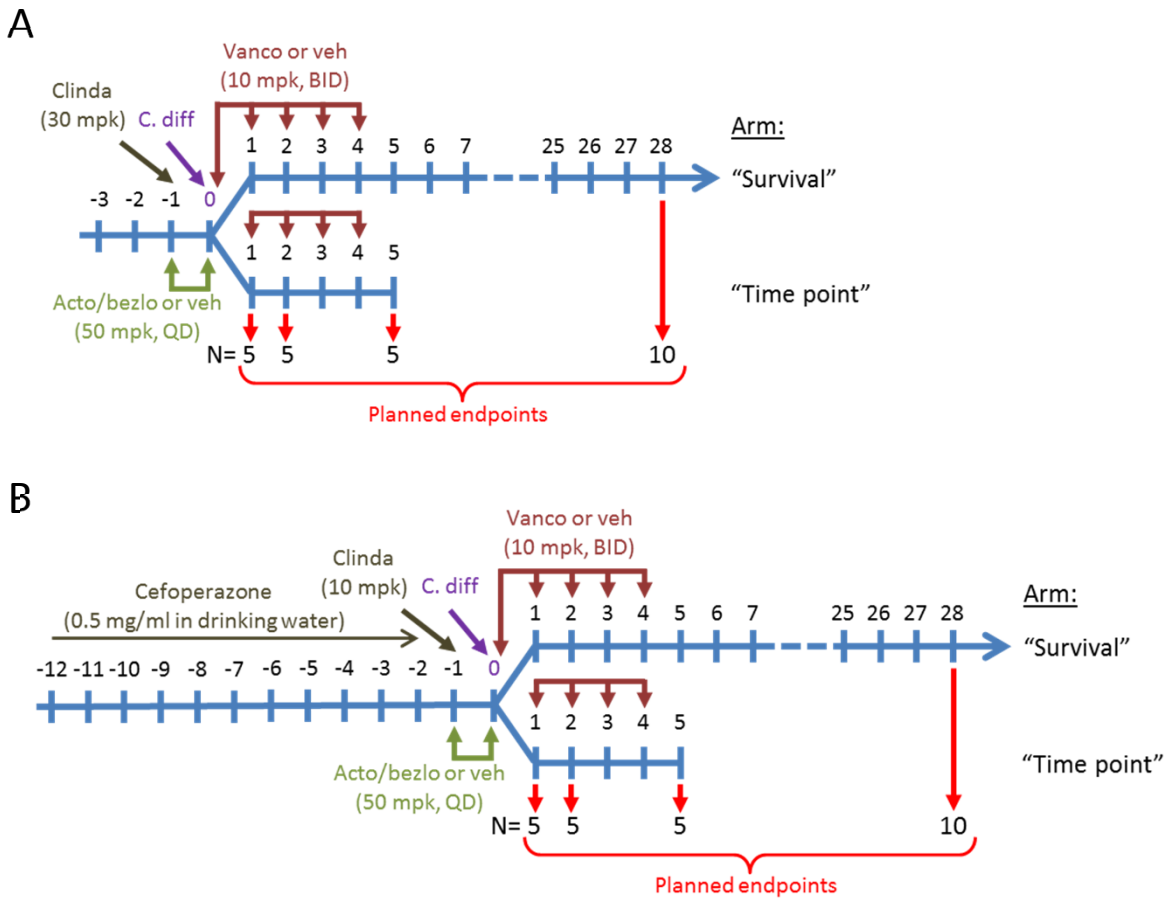

Fig. S1 *Schematic representations of timelines for rodent CDI models used in this study.* A) Hamster model. B) Mouse model. Times of administration of cefoperazone, clindamycin ("Clinda"), vancomycin ("Vanco"), actoxumab/bezlotoxumab ("Acto/bezlo") or vehicle ("veh") , and of *C. difficile* challenge ("C diff"), are indicated. Numbers of animals intended to be euthanized ("N") are as indicated at Days 1, 2, 5 and 28. Actual numbers euthanized were affected by animal survival. "mpk" = milligrams per kilogram; "QD" = "quaque die" (once daily); "BID" = "bis in die" (twice a day)

Fig. S2

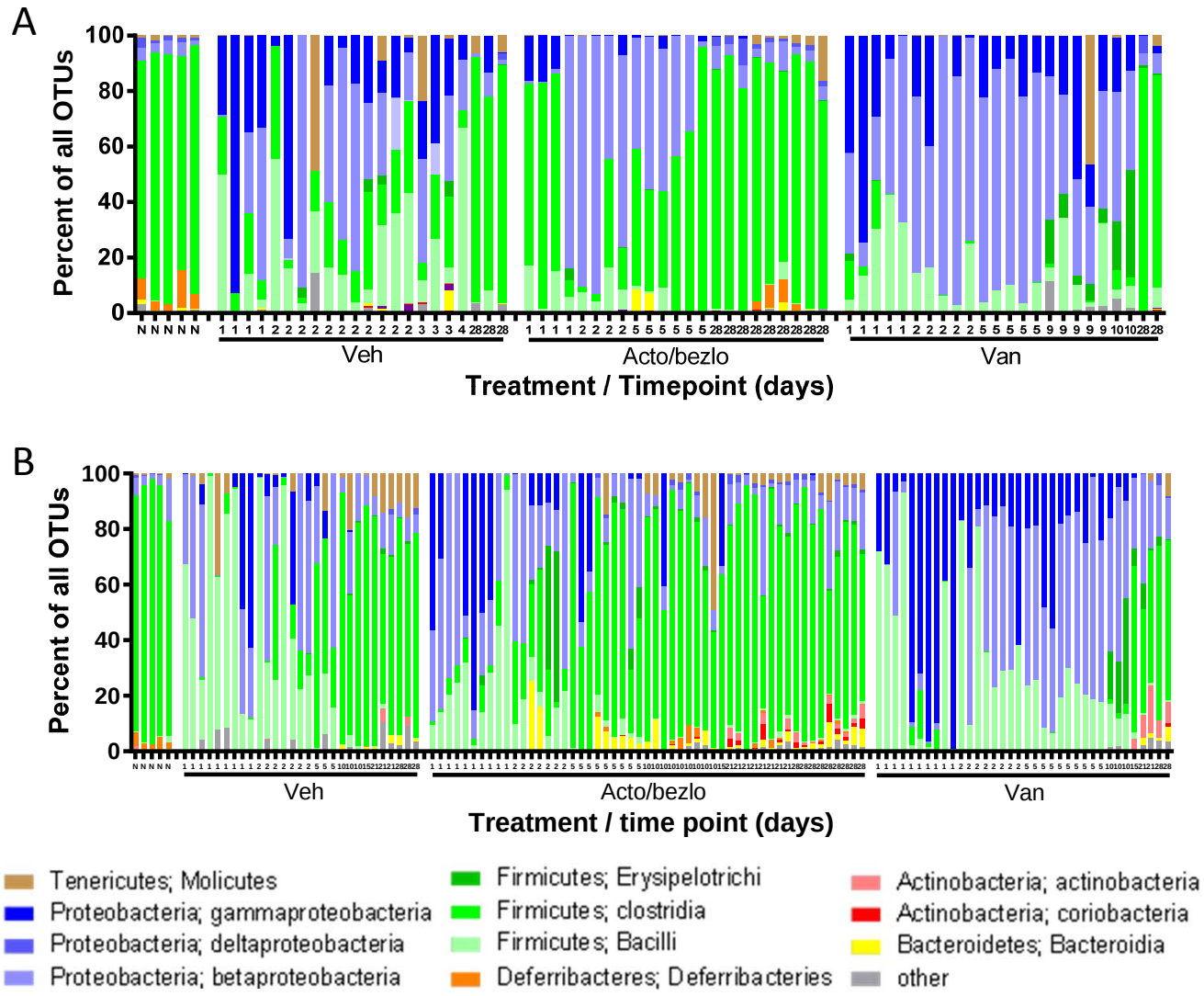

Fig. S2 *Effect of actoxumab/bezlotoxumab or vancomycin on microbiota composition of cecum content and feces in infected mice.* A) Relative microbiota composition (phylum and class, as indicated in legend) of cecum content samples from individual infected mice treated with vehicle (Veh), actoxumab/bezlotoxumab (Acto/bezlo) or vancomycin (Van), as indicated, at predefined time points (Days 1, 2, 5 and 28) or at time of death for animals that did not survive to predefined time points. Averages of animals within the same treatment group at the same timepoint are sown in Fig. 4C. B) As per panel A), but for fecal samples. Predefined time points were Days 1, 2, 5, 10, 15, 21 and 28 ("Survival" arm). Averages of animals within the same treatment group at the same timepoint are shown in Fig. 4F. "N" = naive.

Fig. S3

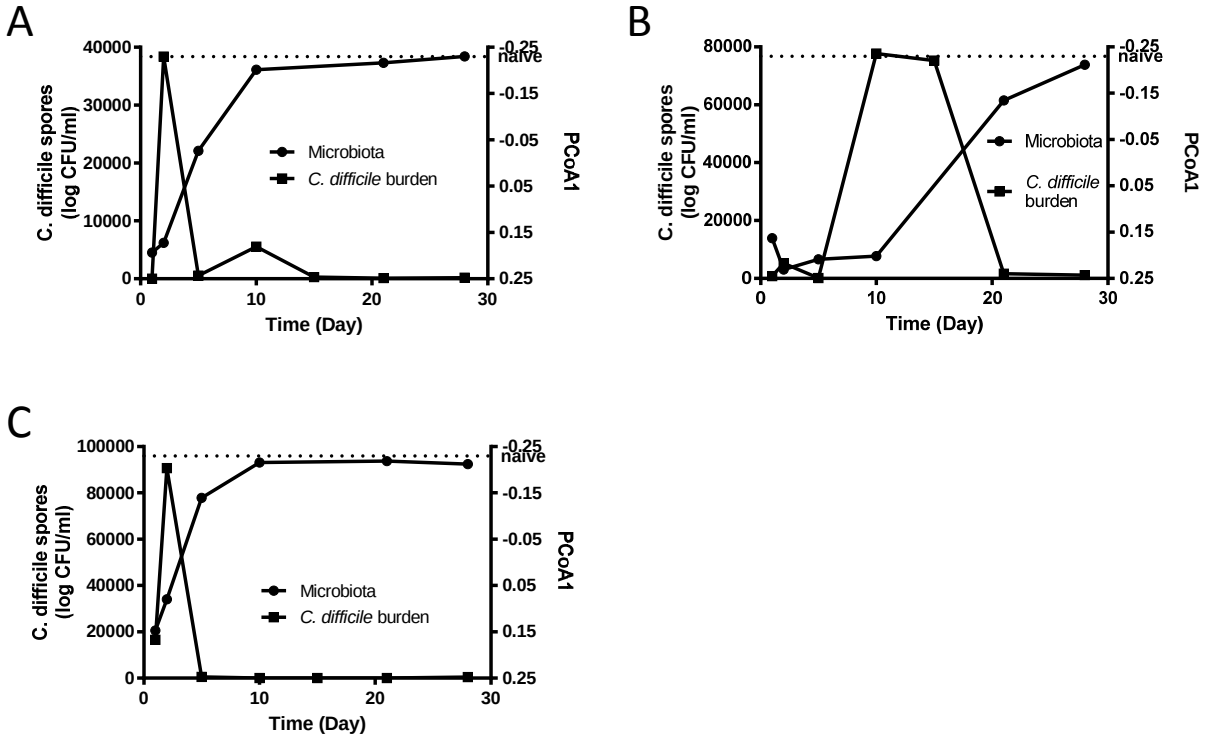

Fig. S3 *Inverse relationship between C. difficile burden and gut microbiota composition in fecal samples from infected mice.* A) *C. difficile* levels (CFU/mL) and microbiota composition (PCoA1) in fecal samples of vehicle-treated mice at predefined time points (Days 1, 2, 5, 10, 15, 21, 28; "Survival" arm). *C. difficile* levels are averages of individual data points shown in Fig. 3E, with data averaged and displayed in linear scale rather than log scale. PCoA1 values are taken from Fig. 4E, with Day 15 values omitted because a single animal was analyzed per group at that time point. B) As per A), but for vancomycin-treated animals. C) As per A), but for actoxumab/bezlotoxumab-treated animals.

Table S1 – Scoring system for gross intestinal score

Scoring Scheme Table:	**Score**	**Additional Comments**
Normal	0	No difference from wild type
Normal - loose content	0.5	No difference from wild type but lacking formed faeces in the lower 1/3 of the colon
Normal - empty	1	No difference from wild type but a total lack of contents, colon is flat
Slightly inflamed	1.5	Sections of the GI tract are enlarged compared to wild type
Slightly inflamed & empty	2	Multiple sections of the GI tract are enlarged compared to wild type. No faecal content
Inflamed	2.5	Multiple sections of the GI tract are enlarged compared to wild type or smaller sections are grossly enlarged
Inflamed & Red	3	Multiple sections of the GI tract are enlarged compared to wild type or smaller sections are grossly enlarged. Redness and haemorrhages on the outer surface of the GI tract.
Enlarged	4	Large sections of the GI tract are grossly enlarged compared to wild type
Inflamed & Enlarged	5	Mega colon or mega ileum with redness and haemorrhages

Table S2 – *C. difficile* burdens (log CFU/ml) in content of intestinal segments in individual mice and hamsters (data used to generate averages in Fig. 3A and B)

Hamsters

Treatment	Timepoint (hours)	Small Intestine	Cecum	Colon
Acto/bezlo	21	<1.23	<1.23	<1.23
	21	<1.23	<1.23	<1.23
	21	<1.23	<1.23	<1.23
	21	<1.23	<1.23	<1.23
	21	<1.23	<1.23	<1.23
	43	2.44	3.82	3.64
	43	3.25	3.5	4.07
	43	2.7	3.99	4.78
	43	<1.23	3.99	5.03
	43	3.58	3.6	5.22
	114	<1.23	2.99	2.96
	114	<1.23	3.05	3.58
	114	<1.23	2.91	3.1
	114	<1.23	4.79	3.56
	114	<1.23	2.93	4.67
	138	1.92	3.58	2.75
	138	1.52	3	<1.23
	138	<1.23	<1.23	2.97
	138	<1.23	3.82	3.12
	138	<1.23	3.92	3.48
	259	2.07	3.43	2.93
	666	<1.23	<1.23	<1.23
	666	<1.23	<1.23	<1.23
	666	<1.23	<1.23	<1.23
	666	<1.23	<1.23	<1.23
Vancomycin	22	<1.23	<1.23	<1.23
	22	<1.23	<1.23	<1.23
	22	<1.23	<1.23	<1.23
	22	<1.23	<1.23	<1.23
	22	<1.23	<1.23	<1.23
	43	<1.23	<1.23	<1.23
	43	<1.23	<1.23	<1.23
	44	<1.23	<1.23	<1.23
	44	<1.23	<1.23	<1.23
	44	<1.23	<1.23	<1.23
	115	<1.23	<1.23	<1.23
	115	<1.23	<1.23	<1.23
	115	<1.23	<1.23	<1.23
	115	<1.23	<1.23	<1.23
	115	<1.23	<1.23	<1.23
	238	2.99	4.83	4.7
	274	3.1	3.1	4.9
	260	<1.23	<1.23	3.99
	259	<1.23	<1.23	3.64
	281	2	2.48	3.45
	318	1.7	2.18	3.48
	298	2.81	2.07	<1.23
	308	4.45	2.18	3.3
	308	1.92	3.07	3.3
	322	3.48	3.37	3.4
Vehicle	24	<1.23	<1.23	<1.23
	21	<1.23	<1.23	<1.23
	21	<1.23	<1.23	<1.23
	21	<1.23	<1.23	<1.23
	21	<1.23	<1.23	<1.23
	32	2.68	3.5	3.58
	32	<1.23	2.43	4.74
	32	<1.23	2.58	2.48
	30	<1.23	2.75	2.95
	36	<1.23	2.68	3.65
	39	2.99	2.94	3.83
	39	3.35	2.56	2
	35	<1.23	2.88	3.8
	40	3.3	2	5.21
	40	3.58	2.52	5.15
	38	2.18	2.86	3.6
	37	<1.23	3.86	3.73
	36	<1.23	2.99	3.56
	36	<1.23	2.89	3.37
	35	<1.23	2.91	2.37
	39	<1.23	3.04	3.99
	41	<1.23	3.04	4.16
	35	<1.23	2.45	2.82
	36	<1.23	2.12	2.9
	39	1.7	3.7	4.05

Mouse

Treatment	Timepoint (hours)	Small Intestine	Cecum	Colon
Acto/bezlo	20.5	<1.23	<1.23	2.92
	20.5	<1.23	<1.23	<1.23
	20.5	<1.23	<1.23	<1.23
	21.75	2.45	5.3	4.54
	22	2.34	6.22	4.07
	44	<1.23	3.87	2.52
	44	<1.23	4.79	3.07
	44.5	4.62	4.68	<1.23
	44.5	2.45	4.07	3.71
	113.75	3.7	5	3.88
	113.75	<1.23	<1.23	<1.23
	114.5	3.19	4.3	3.4
	114.5	2.7	5.5	2.81
	114.75	<1.23	2.6	<1.23
	147.35	<1.23	3.98	2.3
	667	<1.23	<1.23	<1.23
	667	<1.23	<1.23	<1.23
	667	<1.23	<1.23	<1.23
	667	<1.23	<1.23	<1.23
	667	<1.23	<1.23	<1.23
	667	<1.23	<1.23	<1.23
	667	<1.23	<1.23	<1.23
	667	<1.23	<1.23	<1.23
	667	<1.23	3.34	2.86
Vancomycin	20.45	5.34	4.56	3.07
	20.45	<1.23	4.5	<1.23
	21	4.12	5.07	4.43
	22.25	<1.23	<1.23	<1.23
	22.25	<1.23	<1.23	<1.23
	43	1.83	1.52	<1.23
	43	<1.23	2.12	<1.23
	43	<1.23	<1.23	<1.23
	43	<1.23	<1.23	<1.23
	43	<1.23	<1.23	<1.23
	114	<1.23	<1.23	<1.23
	114	<1.23	2	<1.23
	114.75	<1.23	<1.23	<1.23
	115	<1.23	<1.23	<1.23
	115	<1.23	<1.23	<1.23
	219	<1.23	2.71	<1.23
	215.2	<1.23	<1.23	<1.23
	219	<1.23	3	<1.23
	225.25	<1.23	4	1.52
	222.5	<1.23	<1.23	<1.23
	220	2.52	4.8	2.86
	233.5	<1.23	3.6	<1.23
	231.8	2.89	2.65	<1.23
	667	<1.23	<1.23	<1.23
	667	<1.23	<1.23	<1.23
Vehicle	20	<1.23	<1.23	<1.23
	20	2.71	2.9	2.9
	20	2.52	3.08	3.08
	21.5	2.3	3.43	<1.23
	21.5	<1.23	5.52	<1.23
	32.2	3.03	2.81	<1.23
	32.25	2.22	3.48	<1.23
	40.5	<1.23	2.12	<1.23
	40.5	2.56	2.62	<1.23
	45	<1.23	2.67	2.22
	45	1.83	3.74	2
	45	1.83	2.12	<1.23
	54.25	<1.23	3.79	1.7
	47.5	<1.23	2.48	<1.23
	54	1.52	2.95	<1.23
	40.5	2.22	3.8	<1.23
	56	<1.23	<1.23	<1.23
	43	<1.23	4	2.48
	62.5	<1.23	3.43	2.88
	61.75	<1.23	1.7	<1.23
	71	<1.23	2.34	2.12
	87	1.7	5.23	2.81
	667	<1.23	<1.23	<1.23
	667	<1.23	<1.23	<1.23
	667	<1.23	<1.23	<1.23

Table S3 – Statistical analysis of differences in microbiome composition in mouse cecum content

1st comparator	2nd comparator	Bacilli	Clostridia	Betaproteo-bacteria	Gammaproteo-bacteria
N	A/B1		***		
	A/B2		****	****	
	A/B5		****	****	
	A/B28				
	Va1	**	****	****	****
	Va2		****	****	
	Va5		****	****	
	Va9		****	****	**
	Va28				
	Ve1		****		****
	Ve2	**	****	****	
	Ve3-4	**	****		*
	Ve28				
A/B1	Va1		****		
	Ve1		****		
	A/B28		****		
A/B2	Va2			****	
	Ve2				
	A/B28		****	****	
A/B5	Va5		****	****	
	A/B28		****	****	
A/B28	Va28				
	Ve28				
Va1	Ve1				
	Va28		****	**	**
Va2	Ve2		**	****	
	Va28		****	****	
Va5	Va28		****	****	
Va9	Va28		****	***	
Va28	Ve28				
Ve1	Ve28		****		****
Ve2	Ve28		****	***	
Ve3-4	Ve28	*	****		

A/B = actoxumab/bezlotoxumab
Va = vancomycin
Ve = vehicle
Numbers indicate Day post-challenge
*=p<0.05; **=p<0.01; ***=p<0.001; ****=p<0.0001

Table S4 – Statistical analysis of differences in microbiome composition in mouse feces

1st comparator	2nd comparator	Bacilli	Clostridia	Betaproteo-bacteria	Gammaproteo-bacteria
N	A/B1	***	****	***	****
	A/B2		****	****	
	A/B5		**		
	A/B10				
	A/B15				
	A/B21				
	A/B28				
	Va1	****	****		****
	Va2	****	****	***	*
	Va5		****	****	**
	Va10		****	****	
	Va15				
	Va21		****		
	Va28				
	Ve1	****	****		
	Ve2	****	****		
	Ve5		****		
	Ve10				
	Ve15				
	Ve21				
	Ve28				
A/B1	A/B28	****	****	*	****
	Va1			****	***
	Ve1	****			*
A/B2	A/B28		****	**	
	Va2	****	*		
	Ve2	****			
A/B5	A/B28				
	Va5		****	****	
	Ve5				
A/B10	A/B28				
	Va10		****	****	
	Ve10				
A/B15	A/B28				
	Va15				
	Ve15				
A/B21	A/B28				
	Va21		****		
	Ve21				
A/B28	Va28				
	Ve28				

Table S4 (cont'd) – Statistical analysis of differences in microbiome composition in mouse feces

1st comparator	2nd comparator	Bacilli	Clostridia	Betaproteo-bacteria	Gammaproteo-bacteria
Va1	Ve1	*			****
	Va28	**	****		****
Va2	Ve2				
	Va28	***	****		
Va5	Ve5		****		
	Va28		****	***	
Va10	Ve10		****	*	
	Va28		****		
Va15	Ve15				
	Va28				
Va21	Ve21				
	Va28				
Va28	Ve28				
Ve1	Ve28	****	****		
Ve2	Ve28	****	****		
Ve5	Ve28				
Ve10	Ve28				
Ve15	Ve28				
Ve21	Ve28				
Ve28	Ve28				

A/B = actoxumab/bezlotoxumab
Va = vancomycin
Ve = vehicle
Numbers indicate Day post-challenge
*=p<0.05; **=p<0.01; ***=p<0.001; ****=p<0.0001