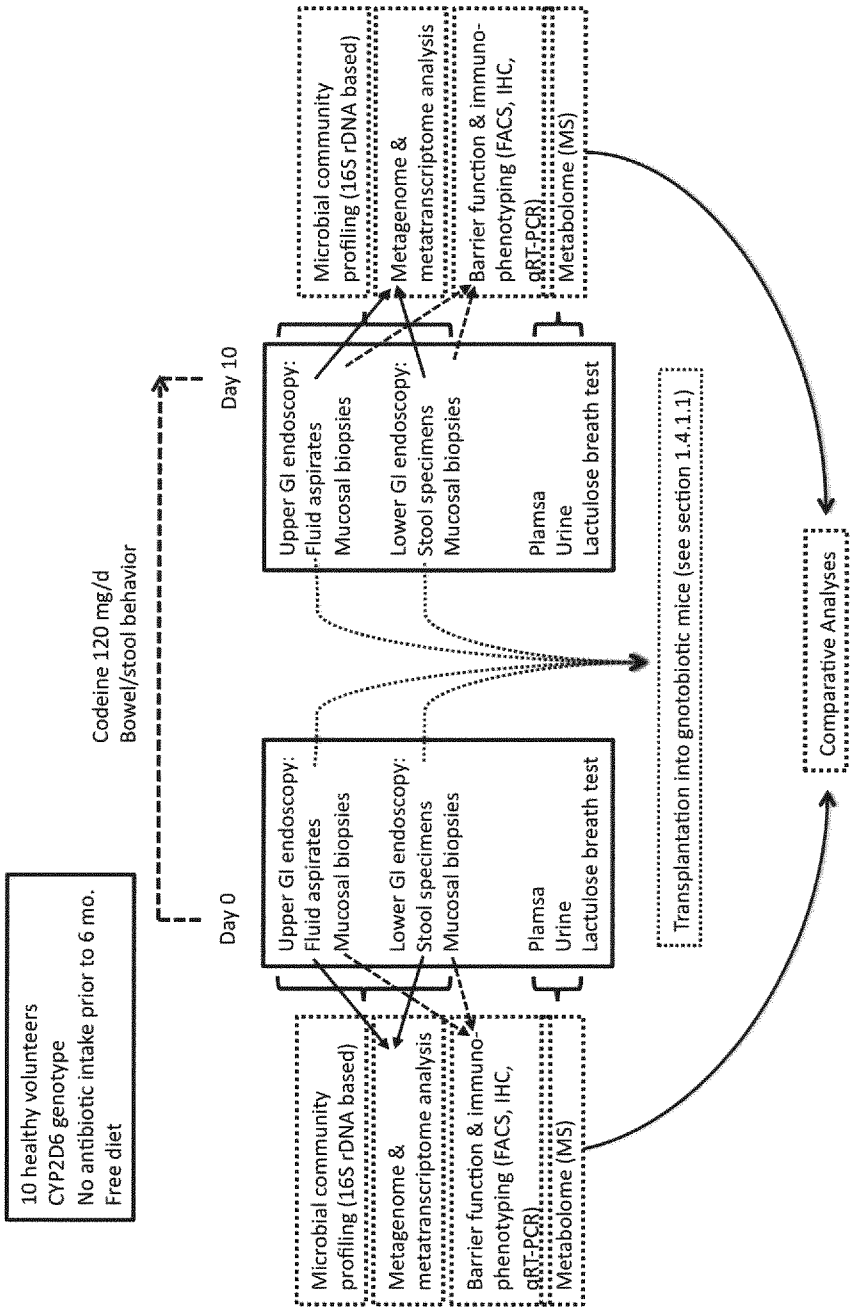

10 healthy volunteers
CYP2D6 genotype
No antibiotic intake prior to 6 mo.
Free diet
Codeine 120 mg/d
Bowel/stool behavior
Day 0
Day 10
Upper GI endoscopy:
Fluid aspirates
Mucosal biopsies
Lower GI endoscopy:
Stool specimens
Mucosal biopsies
Plamsa
Urine
Lactulose breath test
Microbial community profiling (16S rDNA based)
Metagenome & metatranscriptome analysis
Barrier function & immuno-phenotyping (FACS, IHC, qRT-PCR)
Metabolome (MS)
Transplantation into gnotobiotic mice (see section 1.4.1.1)
Comparative Analyses

Figure 1

Figure 2

Group	Route of Admin	Formulation	Study Design	# Mice/ Sex-Type	Conc. (mg/mL)	Dose Level		Day of Admin.	Tissue Collection Time Points Post-Dose
						mg/kg	mL/kg		
1	Not Applicable	NA	Sample prep. (Control)	6M C57BL/6 RAG1 -/-	NA	NA	NA	NA	**Day 8**[b]: Immediately post AM dose, tissues and blood collected per text description
2	Oral (Gavage)	Vehicle	Charcoal Transit	6M C57BL/6 RAG1 -/-	NA	NA	10	1-8[a]	**Day 8:** 45 min post AM dose, small intestine collected
3	Oral (Gavage)	Vehicle	Sample Preparation	6M C57BL/6 RAG1 -/-	NA	NA	10	1-8[a]	**Day 8:** Immediately post AM dose, tissues and blood collected per text description
4	Oral (Gavage)	Oxycodone	Charcoal Transit	6 M C57BL/6 RAG1 -/-	1	10	10	1-8[a]	**Day 8:** 45 min post AM dose, small intestine collected
5	Oral (Gavage)	Oxycodone/ Naloxone 2:1 (w/w)	Charcoal Transit	6 M C57BL/6 RAG1 -/-	1/0.5	10/5	10	1-8[a]	**Day 8:** 45 min post AM dose, small intestine collected
6	Oral (Gavage)	Oxycodone	Sample Preparation	8 M C57BL/6 RAG1 -/-	1	10	10	1-8[a]	**Day 8:** Immediately post AM dose, tissues and blood collected per text description
7	Oral (Gavage)	Oxycodone/ Naloxone 2:1 (w/w)	Sample Preparation	8 M C57BL/6 RAG1 -/-	1/0.5	10/5	10	1-8[a]	**Day 8:** Immediately post AM dose, tissues and blood collected per text description

a. Animals in Groups 2-7 were orally administered the dose three times a day for seven days and once for the eighth day. For Groups 2, 4 and 5 fifteen minutes after the day 8 morning dosing, each mouse was fed a charcoal meal and 30 min following the charcoal meal each mouse was sacrificed and GI tract was collected for charcoal movement measurement.
b. Animals in Group 1 did not receive any treatment or vehicle and were removed from cage and weighed so they get same handling as dosed group
c. For Groups 1, 3, 6 and 7, animals were sacrificed after the first dose on day 8 and blood and tissue samples were collected and measured as described in example 2.

Figure 3

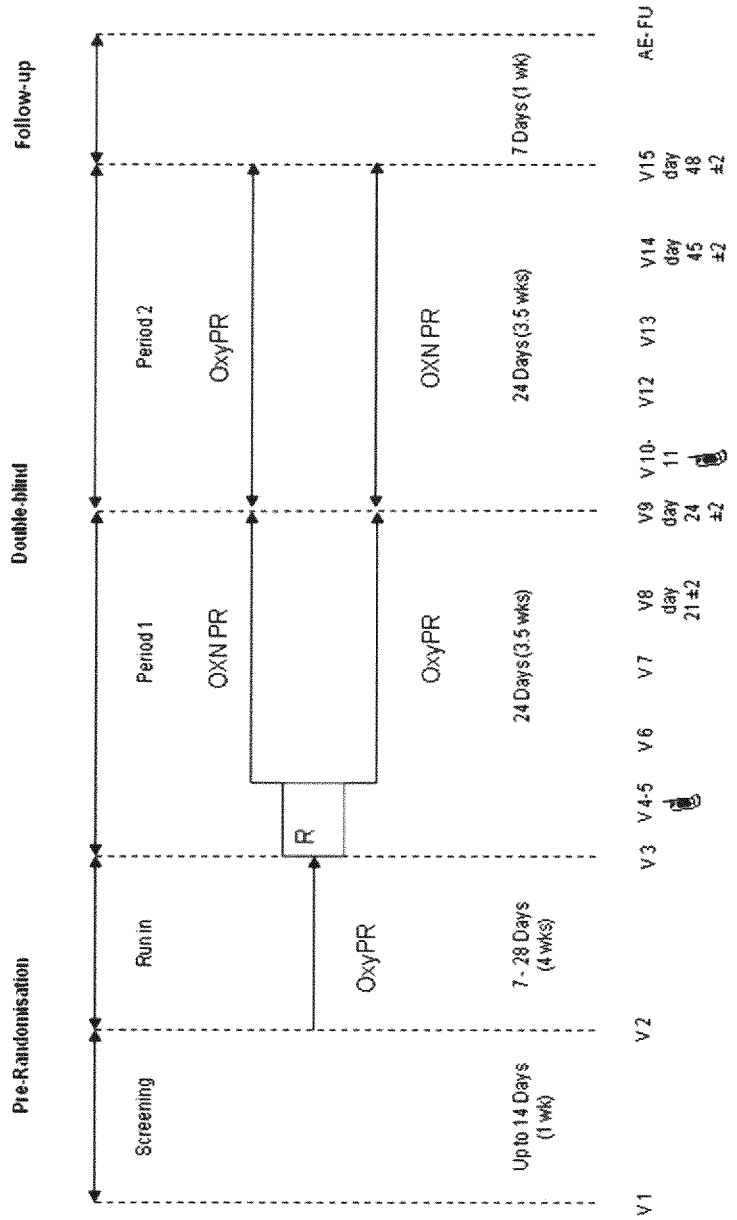

Phase
Pre-Randomisation
Double-blind
Follow-up
Period
Screening
Run in
Period 1
Period 2
Up to 14 Days (1 wk)
7 - 28 Days (4 wks)
24 Days (3.5 wks)
24 Days (3.5 wks)
7 Days (1 wk)
OxyPR
OXN PR
OxyPR
OXN PR
R
V1
V2
V3
V4-5
V6
V7
V8 day 21 ±2
V9 day 24 ±2
V10-11
V12
V13
V14 day 45 ±2
V15 day 48 ±2
AE-FU
R = randomisation

Figure 4

Phase	Pre-Randomisation			Double-Blind										
Period	Screening	Run-in[1]		Cross-over Period 1[1]					Cross-over Period 2[1]					
Study Visit	Visit 1	Visit 2	Visit 3	Visit 4, 5	Visit 6	Visit 7	Visit 8	Visit 9	Visits 10, 11	Visit 12	Visit 13	Visit 14	Visit 15[2]	AE FU[6]
Study Day[3]			0	2-6	7	14	21	24	26-30	31	38	45	48	V15+7
Duration	Up to 14 days	7 to 28 days		3.5 weeks					3.5 weeks					7 days
Telephone visit				X					X					
Investigational site visit at pain management clinic	X	X	X		X	X		X		X	X		X	X
Investigational site visit at gastroenterologist's clinic							X					X		
Informed Consent (IC)	X													
IC for Pharmacogenetic Sampling	X													
Assess Inclusion/Exclusion Criteria	X	X	X											
Demography	X													
Physical Exam	X		X					X					X	
Vital Signs Measurements	X		X					X					X	
Medical History and Current Medical Conditions	X													
Assess Prior and Current Medication Use	X													

Figure 4 cont.

Phase	Pre-Randomisation			Double-Blind										
Period	Screening	Run-in[1]		Cross-over Period 1[1]					Cross-over Period 2[1]					
Study Visit	Visit 1	Visit 2	Visit 3	Visit 4, 5	Visit 6	Visit 7	Visit 8	Visit 9	Visits 10, 11	Visit 12	Visit 13	Visit 14	Visit 15[2]	AE FU[6]
Study Day[3]			0	2-6	7	14	21	24	26-30	31	38	45	48	V15+7
Duration	Up to 14 days	7 to 28 days		3.5 weeks					3.5 weeks					7 days
Telephone visit				x					x					
Investigational site visit at pain management clinic	x	x	x		x	x		x		x	x		x	x
Investigational site visit at gastroenterologist's clinic							x					x		
Clinical Laboratory Tests (hematology, chemistry, urinalysis)	x							x					x	
Blood Sampling for Pharmacogenetic Analysis			x											
Pregnancy Test[4]	x (urine)							x (urine)					x (urine)	
12-lead ECG	x							x					x	
Site Study Staff to Phone Subject		Approx. every 2 - 4 days		x					x					
DoloTest®	x		x		x			x		x			x	
Pain Intensity Scale- "Average Pain over last 24 Hours" (subject)		Daily in diary												
Bowel Function Measures (subject)[5]		Daily in diary												
OxyIR Pain Rescue Medication Use (subject)[7]		Daily on medication wallet		Daily on medication wallet										
Bisacodyl Suppository Use (subject)[7]		Daily on medication wallet		Daily on medication wallet										

Figure 4 cont.

Phase	Pre-Randomisation			Double-Blind										
Period	Screening	Run-in[1]		Cross-over Period 1[1]					Cross-over Period 2[1]					
Study Visit	Visit 1	Visit 2	Visit 3	Visit 4, 5	Visit 6	Visit 7	Visit 8	Visit 9	Visits 10, 11	Visit 12	Visit 13	Visit 14	Visit 15[2]	AE FU[6]
Study Day[3]			0	2-6	7	14	21	24	26-30	31	38	45	48	V15+7
Duration	Up to 14 days	7 to 28 days		3.5 weeks					3.5 weeks					7 days
Telephone visit				x					x					
Investigational site visit at pain management clinic	x	x	x		x	x		x		x	x		x	x
Investigational site visit at gastroenterologist's clinic							x					x		
Bowel Function Index (BFI) (interviewer)	x	x	x		x	x		x		x	x		x	
Bristol Stool Form Scale (BSFS)			x					x					x	
Stool sample (microbiota analysis & metabolomics)[8]			x				x					x		
Assess Concomitant Therapy Use		x	x		x	x		x		x	x		x	x[9]
H_2 Breath Test							x					x		
CH_4 Breath Test							x					x		
Gastric emptying (^{13}C-acetate Breath Test)							x					x		
Blood sample (immune, inflammatory response parameters, and serum metabolome)	x							x					x	
Abdominal girth							x					x		

Figure 4 cont.

Phase	Pre-Randomisation			Double-Blind										
Period	Screening	Run-in[1]		Cross-over Period 1[1]					Cross-over Period 2[1]					
Study Visit	Visit 1	Visit 2	Visit 3	Visit 4, 5	Visit 6	Visit 7	Visit 8	Visit 9	Visits 10, 11	Visit 12	Visit 13	Visit 14	Visit 15[2]	AE FU[6]
Study Day[3]			0	2-6	7	14	21	24	26-30	31	38	45	48	V15+7
Duration	Up to 14 days	7 to 28 days		3.5 weeks					3.5 weeks					7 days
Telephone visit				x					x					
Investigational site visit at pain management clinic	x	x	x		x	x		x		x	x		x	x
Investigational site visit at gastroenterologist's clinic							x					x		
Adverse Events (Non-elicited Reporting)		x	x	x	x	x		x	x	x	x		x	x[8]
Record subsequent analgesic therapy													x	x
Randomisation			x											
Call IRT to update subject status information	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Study Medication Dispensed		x	x		x	x		x		x	x			
Drug Accountability			x		x	x		x		x	x		x	
Subject Diary Dispensed		x												
Subject Diary Collection			x											
Discontinuation / End of Study													x	

Figure 5

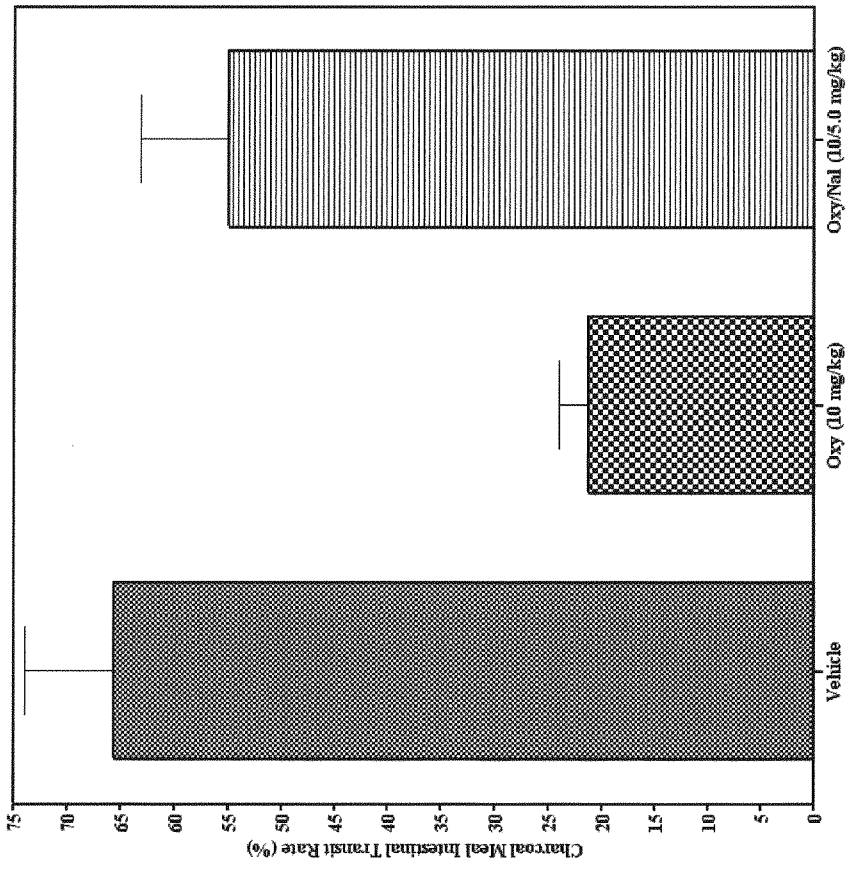

Charcoal Meal Intestinal Transit Rate (%)
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
Vehicle
Oxy (10 mg/kg)
Oxy/Nal (10/5.0 mg/kg)

Figure 6

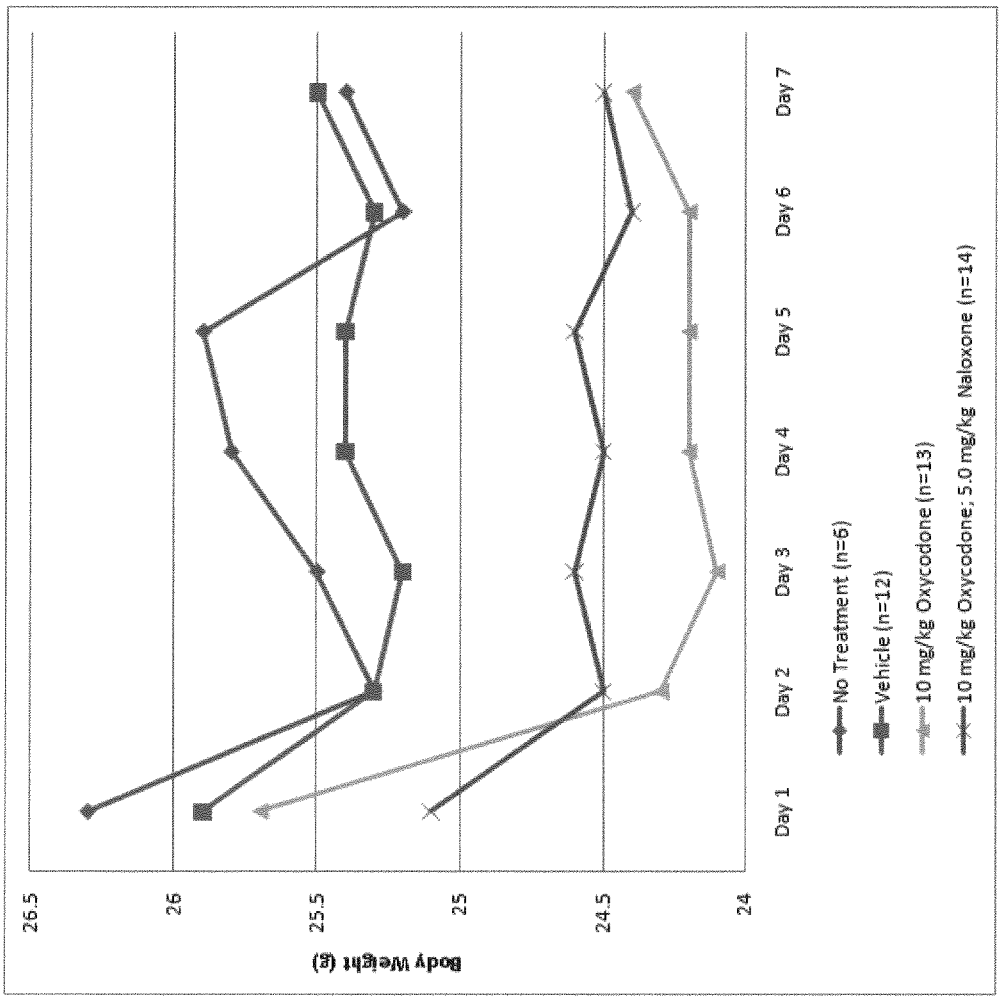
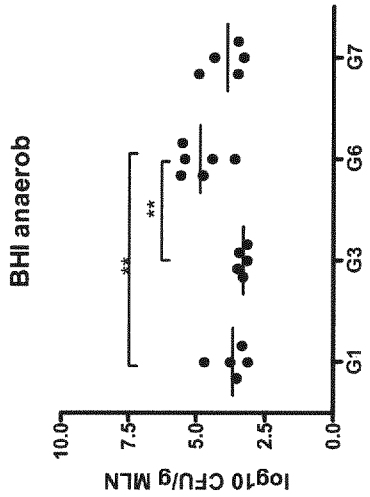
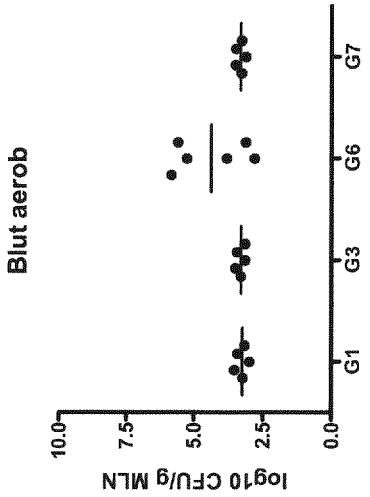

Body Weight (g)
26.5
26
25.5
25
24.5
24
Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Day 7
No Treatment (n=6)
Vehicle (n=12)
10 mg/kg Oxycodone (n=13)
10 mg/kg Oxycodone; 5.0 mg/kg Naloxone (n=14)

Figure 7


BHI anaerob
log10 CFU/g MLN
10.0
7.5
5.0
2.5
0.0
G1
G3
G6
G7
**
**


Blut aerob
log10 CFU/g MLN
10.0
7.5
5.0
2.5
0.0
G1
G3
G6
G7

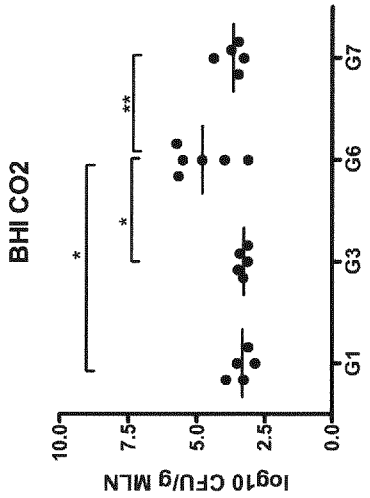
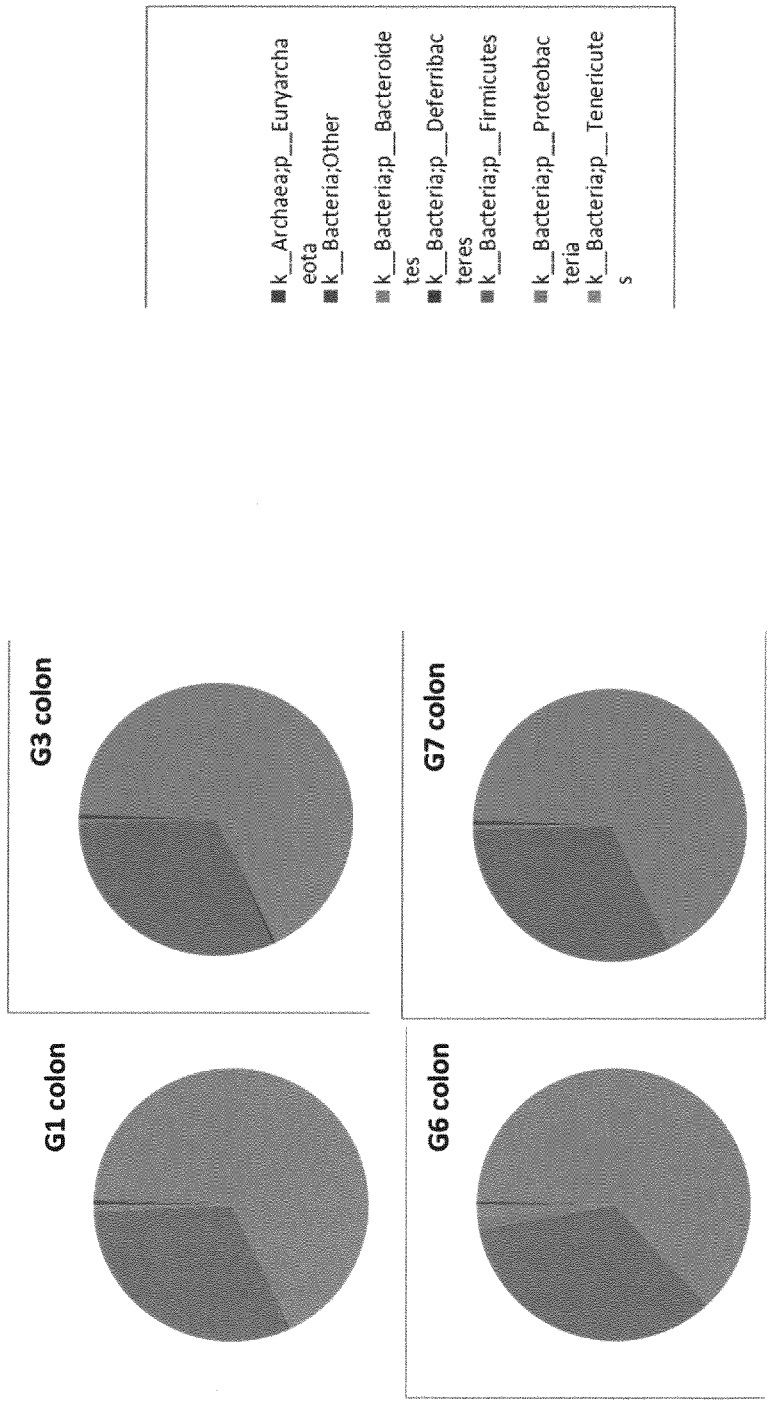

BHI CO2
log10 CFU/g MLN
10.0
7.5
5.0
2.5
0.0
G1
G3
G6
G7
*
*
**

Figure 8

G6 SI
G7 SI
k__Archaea;p__Euryarchaeota
k__Bacteria;Other
k__Bacteria;p__Bacteroidetes
k__Bacteria;p__Deferribacteres
k__Bacteria;p__Firmicutes
k__Bacteria;p__Proteobacteria
k__Bacteria;p__Tenericutes

Figure 9


G3 colon
G1 colon
G7 colon
G6 colon
k__Archaea;p__Euryarchaeota
k__Bacteria;Other
k__Bacteria;p__Bacteroidetes
k__Bacteria;p__Deferribacteres
k__Bacteria;p__Firmicutes
k__Bacteria;p__Proteobacteria
k__Bacteria;p__Tenericutes

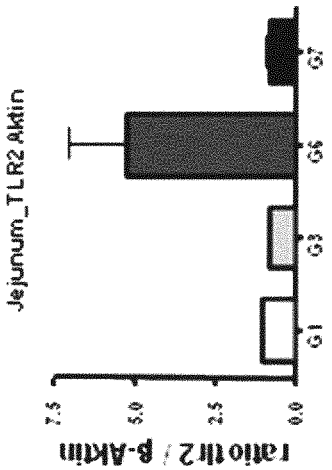


Figure 10