
Daily treatment with hBD2
2% DSS daily administration by the drinking water
Days
1
4
8
10
Anti-TNF antibody
Sacrifice and colon removed for histology

Fig. 1

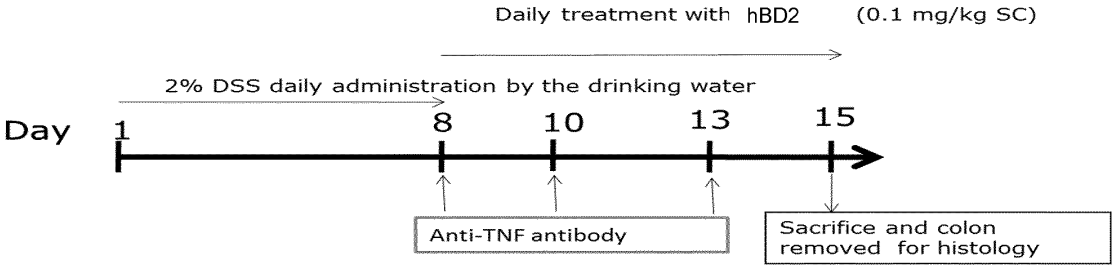

Daily treatment with hBD2 (0.1 mg/kg SC)
2% DSS daily administration by the drinking water
Day
1
8
10
13
15
Anti-TNF antibody
Sacrifice and colon removed for histology

Fig. 2

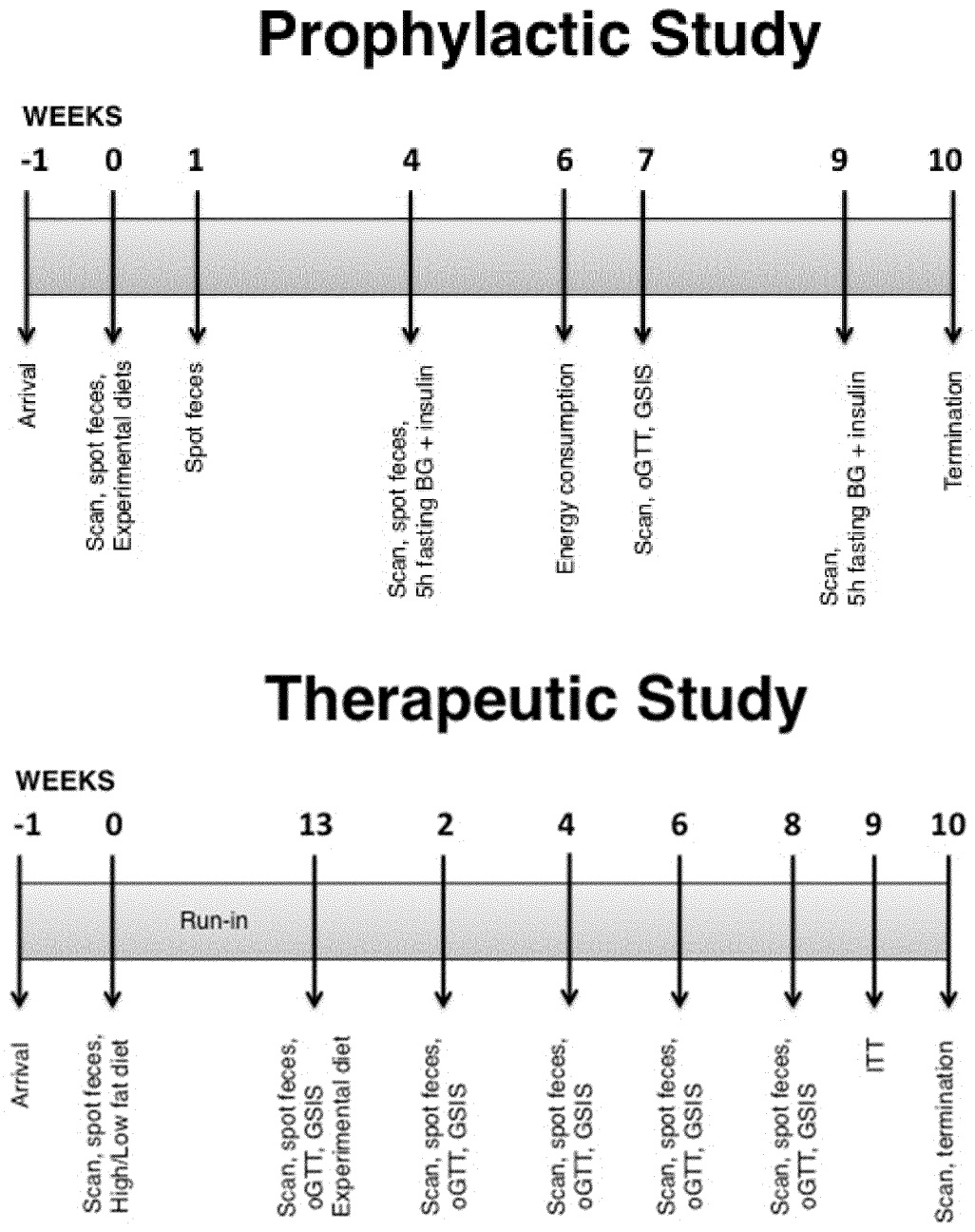

Prophylactic Study
WEEKS
-1
0
1
4
6
7
9
10
Arrival
Scan, spot feces, Experimental diets
Spot feces
Scan, spot feces, 5h fasting BG + insulin
Energy consumption
Scan, oGTT, GSIS
Scan, 5h fasting BG + insulin
Termination
Therapeutic Study
WEEKS
-1
0
13
2
4
6
8
9
10
Run-in
Arrival
Scan, spot feces, High/Low fat diet
Scan, spot feces, oGTT, GSIS Experimental diet
Scan, spot feces, oGTT, GSIS
Scan, spot feces, oGTT, GSIS
Scan, spot feces, oGTT, GSIS
Scan, spot feces, oGTT, GSIS
ITT
Scan, termination

Fig. 3

Group #	N	Sensitization *s.c.* (day 0)	Challenge *i.n.*/mouse Day 14	Treatment Days 11, 12 and 13	Measurements and sampling 48hrs following challenge (day 16)
1	12	100 µL of CFA dissolved in saline	50 µL saline	-	• AHR (resistance and compliance; Buxco) N=6/group • BALF (total and differential cell counts) N=12/group • Lungs (frozen for cytokine concentration) N=12/group
2	12	100 µg HDM/0.2 mL saline+CFA/ mouse	25µg HDM/50µL saline	Vehicle *i.n.*	
3	12			Vehicle *p.o.*	
4	12			hbD2 *i.n.* 1.2 mg/kg/day (0.4 mg/kg TID)	
5	12			hbD2 *p.o.* 1.2 mg/kg/day (0.4 mg/kg TID)	

Fig. 4

Group #	N	Sensitization *s.c.* (day 0)	Challenge *i.n.*/mouse Day 14	Treatment Day 14 Dosing regimen and route to be agreed	Measurements and sampling 48hrs following challenge (day 16)
1	14	100 µL of CFA in saline	50 µL saline	-	• Blood for plasma (N=14) • Lungs for histopathology (N=8) • Lungs frozen for cytokine determination (N=6)
2	14	100 µg HDM/0.2 mL saline+CFA/ mouse	25µg HDM/50µL saline	Vehicle	
3	14			hbD2 *p.o.* 1.2 mg/kg/day (0.4 mg/kg TID)	
4	14			hbD2 *i.n.* 1.2 mg/kg/day (0.4 mg/kg TID)	

Fig. 5

```
HBD1            ------DHYNCVSSGGQCLYSACPIFTKIQGTCYRGKAKCCK---
HBD2            ---GIGDPVTCLKSGAICHPVFCPRRYKQIGTCGLPGTKCCKKP-
HBD3            GIINTLQKYYCRVRGGRCAVLSCLPKEEQIGKCSTRGRKCCRRKK
HBD4            -----ELDRICGYGTARCR-KKCRSQEYRIGRCPN-TYACCLRK-
                          *    . *    *       * *      **
```

Fig. 6a

```
HD5             -ATCYCRTGRCATRESLSGVCEISGRLYRLCCR
HD6             AFTCHCRR-SCYSTEYSYGTCTVMGINHRFCCL
                  **:**   * : *   *.* : *  :*:**
```

Fig. 6b

```
HD6       -------AFTCH-----CR-RSCYSTEYSYGTCTVMGINHRFCCL---
HD5       --------ATCY-----CRTGRCATRESLSGVCEISGRLYRLCCR---
HBD1      ------DHYNCVSSGGQCLYSACPIFTKIQGTCYRGK---AKCCK---
HBD2      ---GIGDPVTCLKSGAICHPVFCPRRYKQIGTCGLPG---TKCCKKP-
HBD3      GIINTLQKYYCRVRGGRCAVLSCLPKEEQIGKCSTRG---RKCCRRKK
HBD4      -----ELDRICGYGTARCR-KKCRSQEYRIGRCPN-T---YACCLRK-
                    *      *    *       * *         **
```

Fig. 6c

Description	Score
Change in Body Weight:	
<1%	0
1-5%	1
>5-10%	2
>10-15%	3
>15%	4
Rectal Bleeding:	
Negative	0
Positive	4
Stool Consistency:	
Normal	0
Loose Stools	2
Diarrhoea	4

Fig. 7

Description	Score
No changes observed	0
Minimal scattered mucosal inflammatory cell infiltrates, with or without minimal epithelial hyperplasia.	1
Mild scattered to diffuse inflammatory cell infiltrates, sometimes extending into the submucosa and associated with erosions, with minimal to mild epithelial hyperplasia and minimal to mild mucin depletion from goblet cells.	2
Mild to moderate inflammatory cell infiltrates that were sometimes transmural, often associated with ulceration, with moderate epithelial hyperplasia and mucin depletion.	3
Marked inflammatory cell infiltrates that were often transmural and associated with ulceration, with marked epithelial hyperplasia and mucin depletion.	4
Marked transmural inflammation with severe ulceration and loss of intestinal glands.	5

Fig. 8

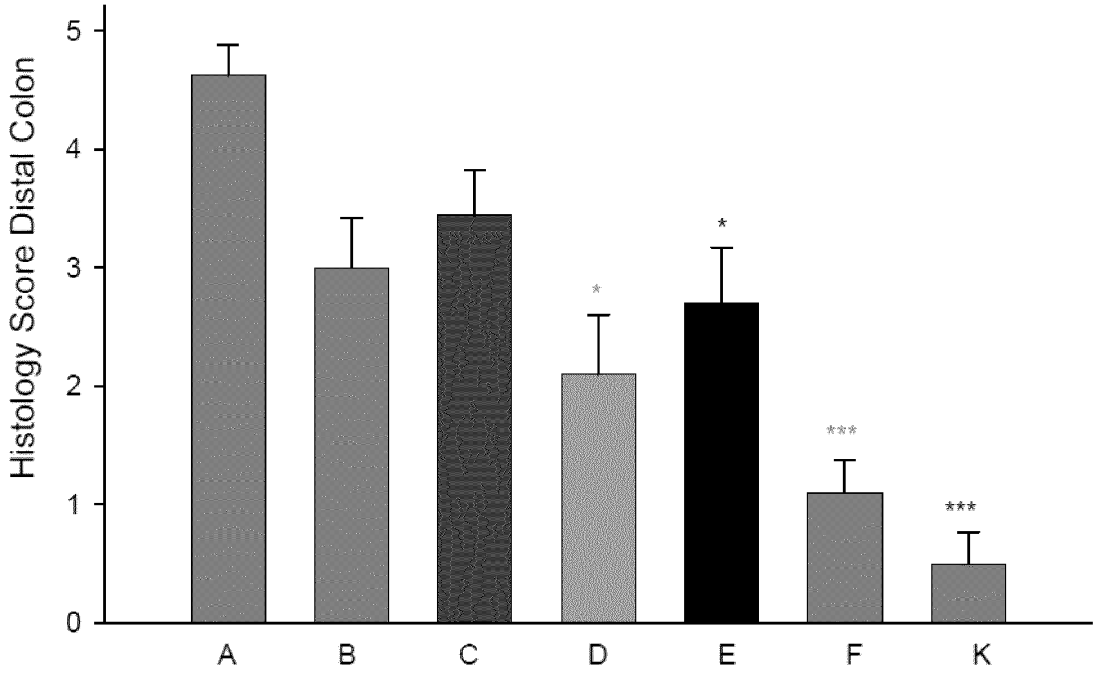

Histology Score Distal Colon
5
4
3
2
1
0
A
B
C
D
E
F
K
*
*
***
***

Fig. 9

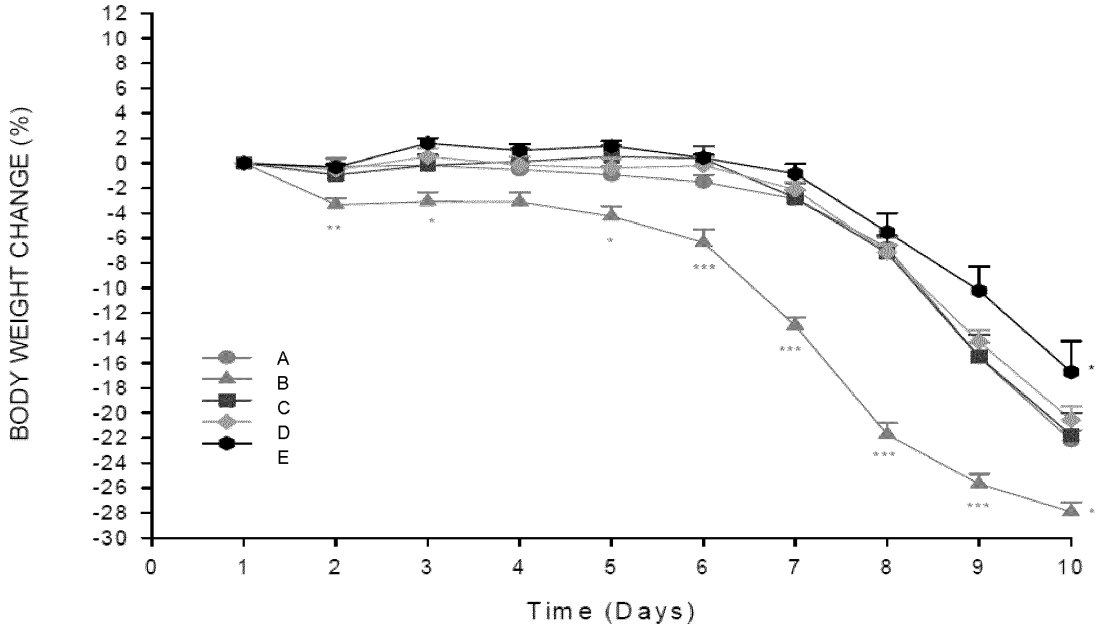

BODY WEIGHT CHANGE (%)
12
10
8
6
4
2
0
-2
-4
-6
-8
-10
-12
-14
-16
-18
-20
-22
-24
-26
-28
-30
0
1
2
3
4
5
6
7
8
9
10
Time (Days)
A
B
C
D
E

Fig. 10

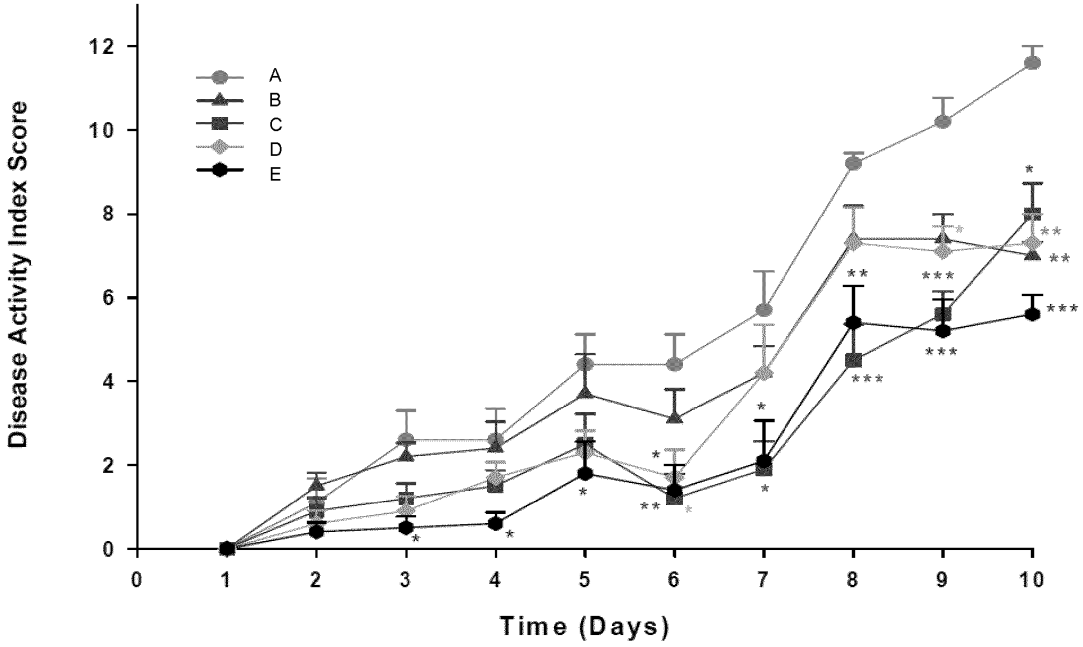

Disease Activity Index Score
Time (Days)
A
B
C
D
E
0
2
4
6
8
10
12
0
1
2
3
4
5
6
7
8
9
10

Fig. 11

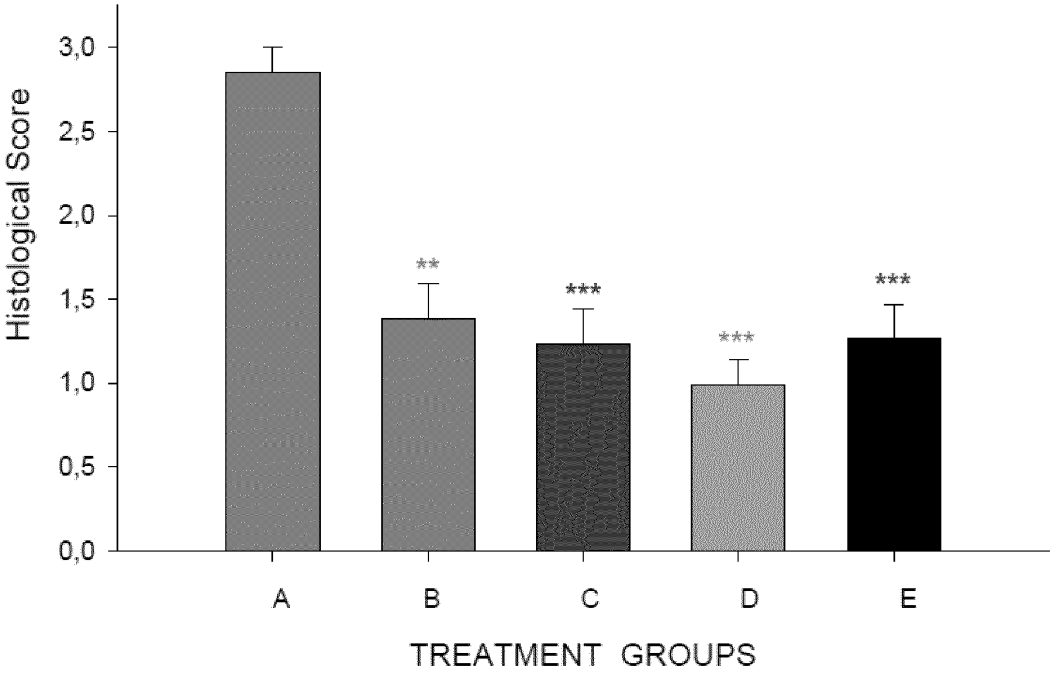

Histological Score
TREATMENT GROUPS
A
B
C
D
E
0,0
0,5
1,0
1,5
2,0
2,5
3,0

Fig. 12

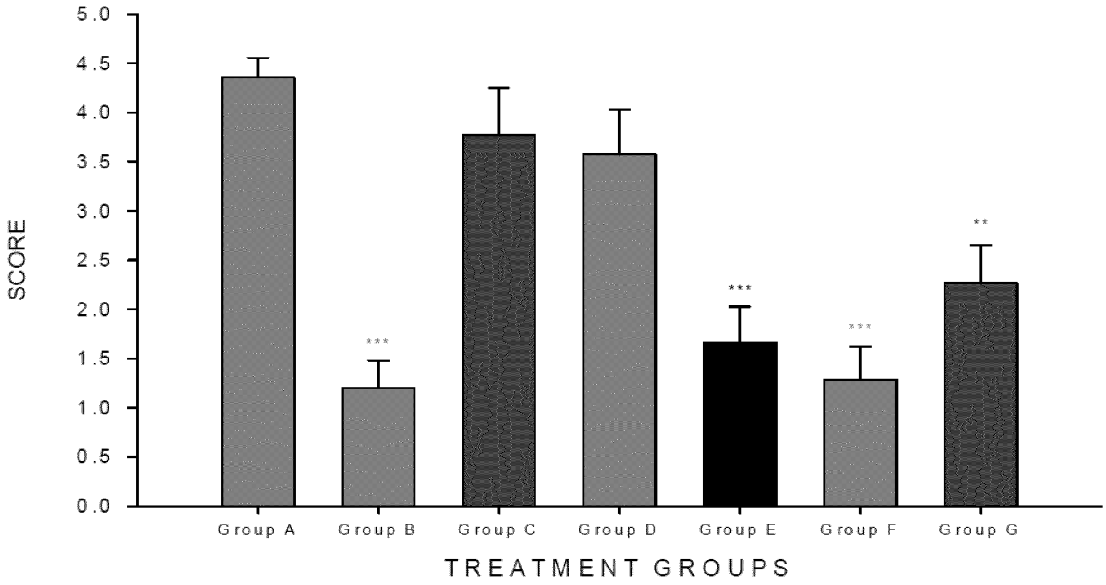

SCORE
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
***
***
***
**
Group A
Group B
Group C
Group D
Group E
Group F
Group G
TREATMENT GROUPS

Fig. 13

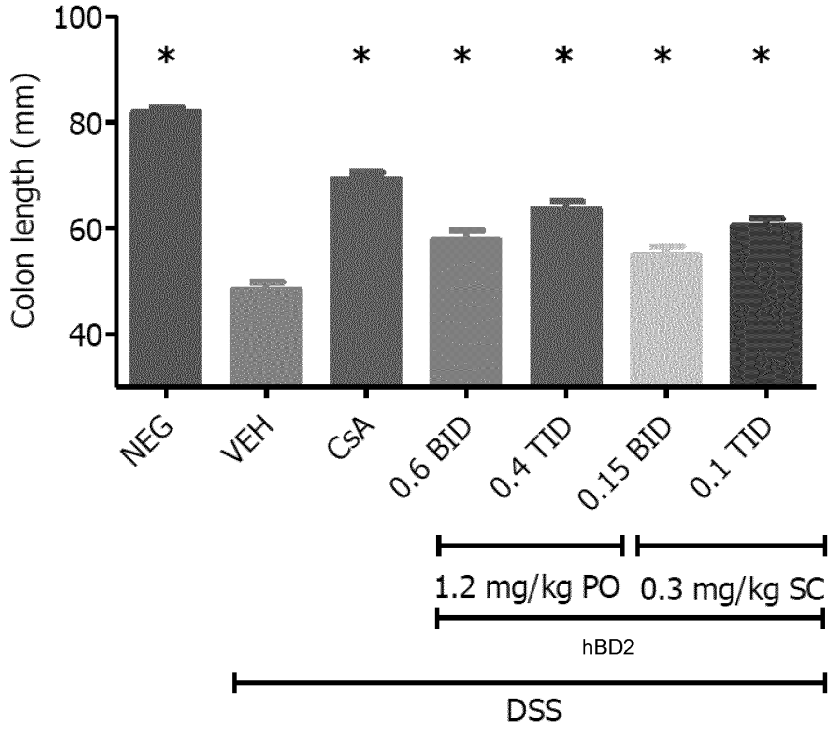

Colon length (mm)
100
80
60
40
*
*
*
*
*
*
NEG
VEH
CsA
0.6 BID
0.4 TID
0.15 BID
0.1 TID
1.2 mg/kg PO
0.3 mg/kg SC
hBD2
DSS

Fig. 14

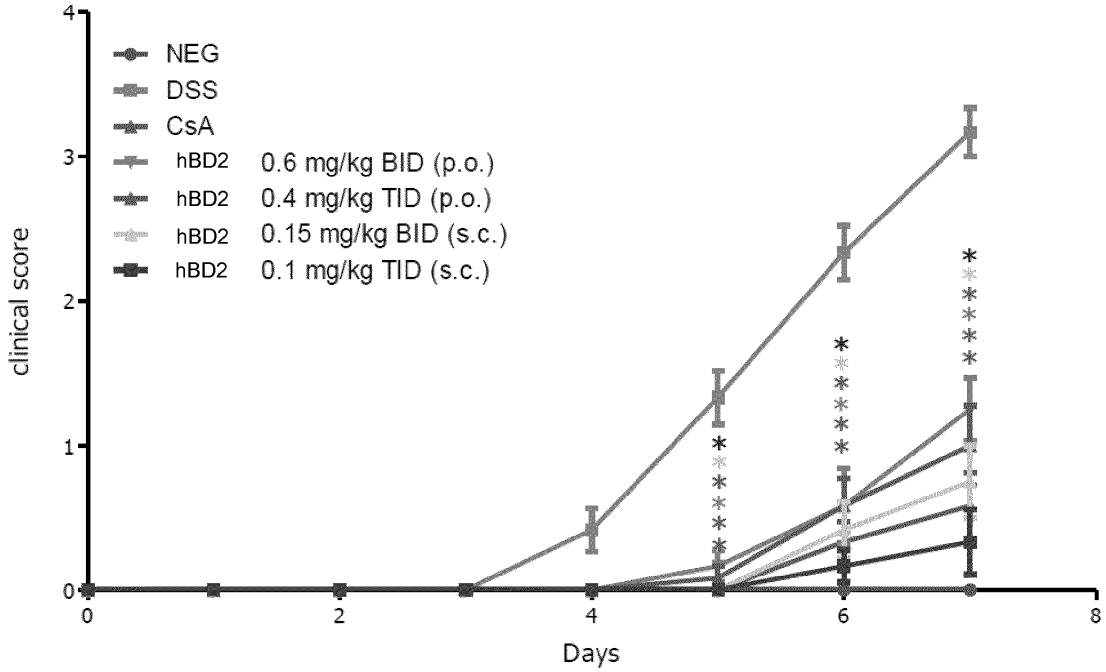

NEG
DSS
CsA
hBD2 0.6 mg/kg BID (p.o.)
hBD2 0.4 mg/kg TID (p.o.)
hBD2 0.15 mg/kg BID (s.c.)
hBD2 0.1 mg/kg TID (s.c.)
clinical score
Days
0
1
2
3
4
0
2
4
6
8

Fig. 15

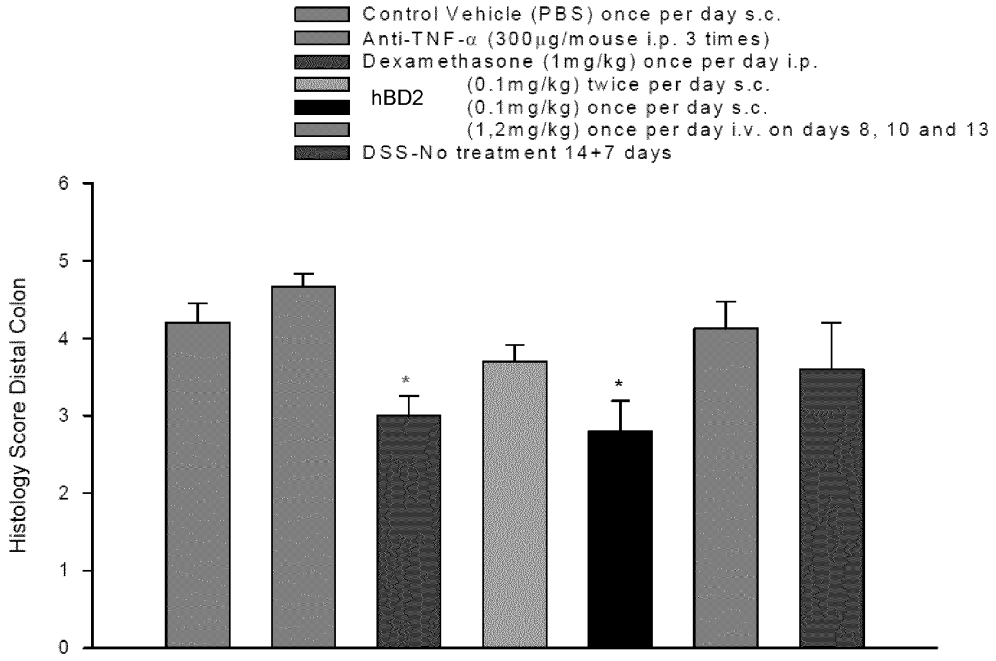

Control Vehicle (PBS) once per day s.c.
Anti-TNF-α (300µg/mouse i.p. 3 times)
Dexamethasone (1mg/kg) once per day i.p.
hBD2 (0.1mg/kg) twice per day s.c.
(0.1mg/kg) once per day s.c.
(1,2mg/kg) once per day i.v. on days 8, 10 and 13
DSS-No treatment 14+7 days
Histology Score Distal Colon
0
1
2
3
4
5
6
*
*

Fig. 16

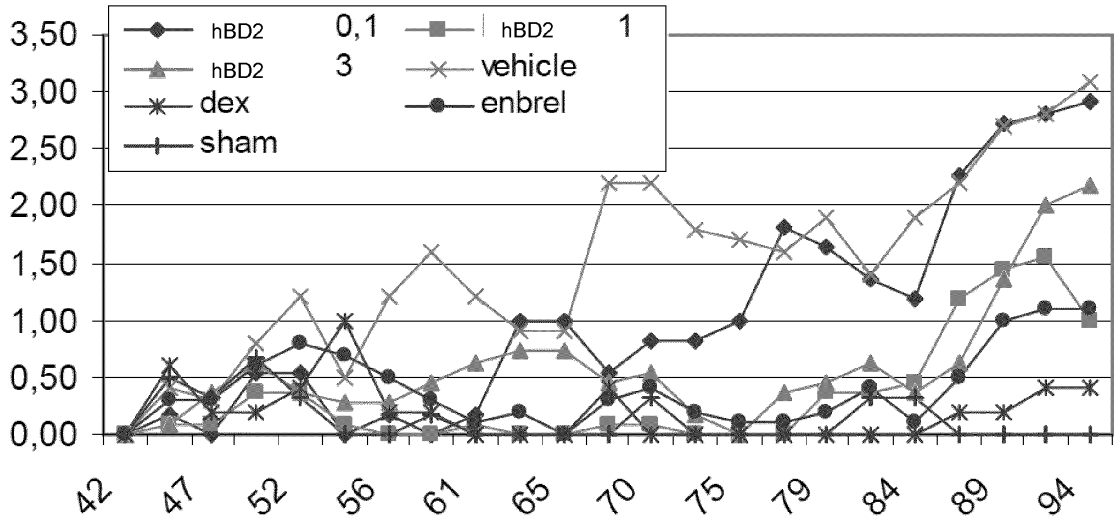

hBD2 0,1
hBD2 1
hBD2 3
vehicle
dex
enbrel
sham
3,50
3,00
2,50
2,00
1,50
1,00
0,50
0,00
42
47
52
56
61
65
70
75
79
84
89
94

Fig. 17

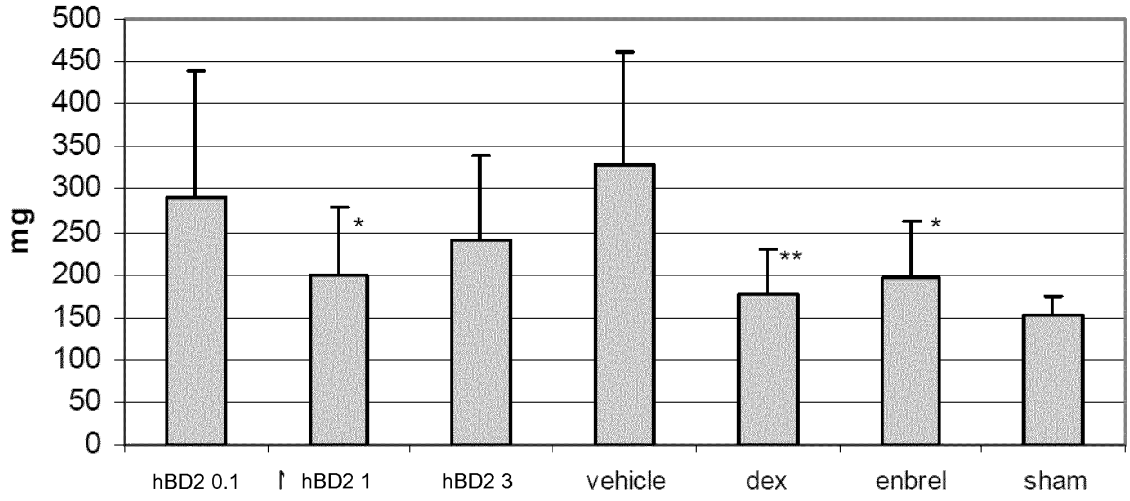

mg
500
450
400
350
300
250
200
150
100
50
0
*
**
*
hBD2 0.1
hBD2 1
hBD2 3
vehicle
dex
enbrel
sham

Fig. 18

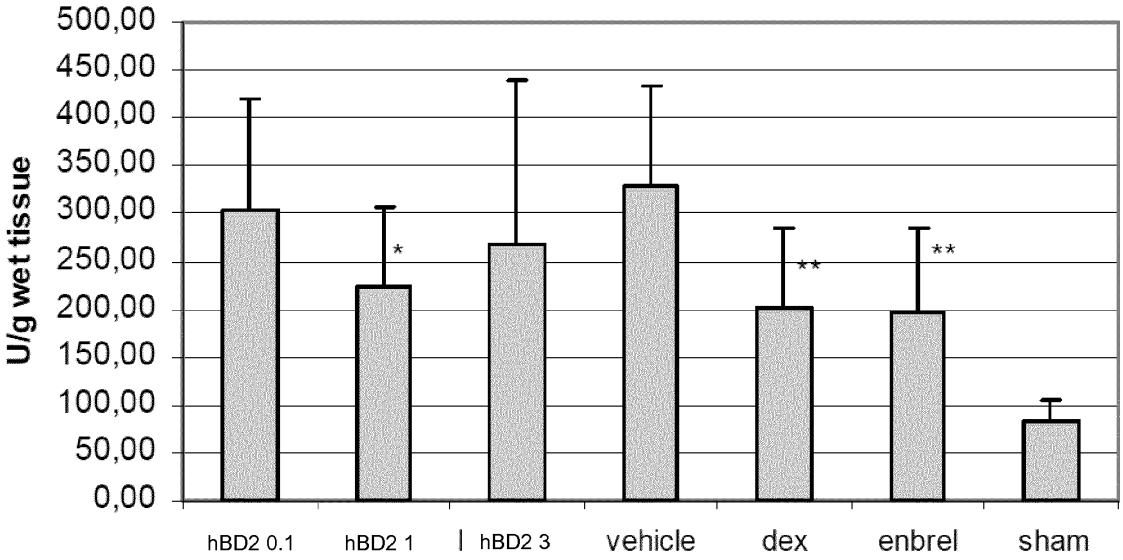

500,00
450,00
400,00
350,00
300,00
250,00
200,00
150,00
100,00
50,00
0,00
U/g wet tissue
*
**
**
hBD2 0.1
hBD2 1
hBD2 3
vehicle
dex
enbrel
sham

Fig. 19

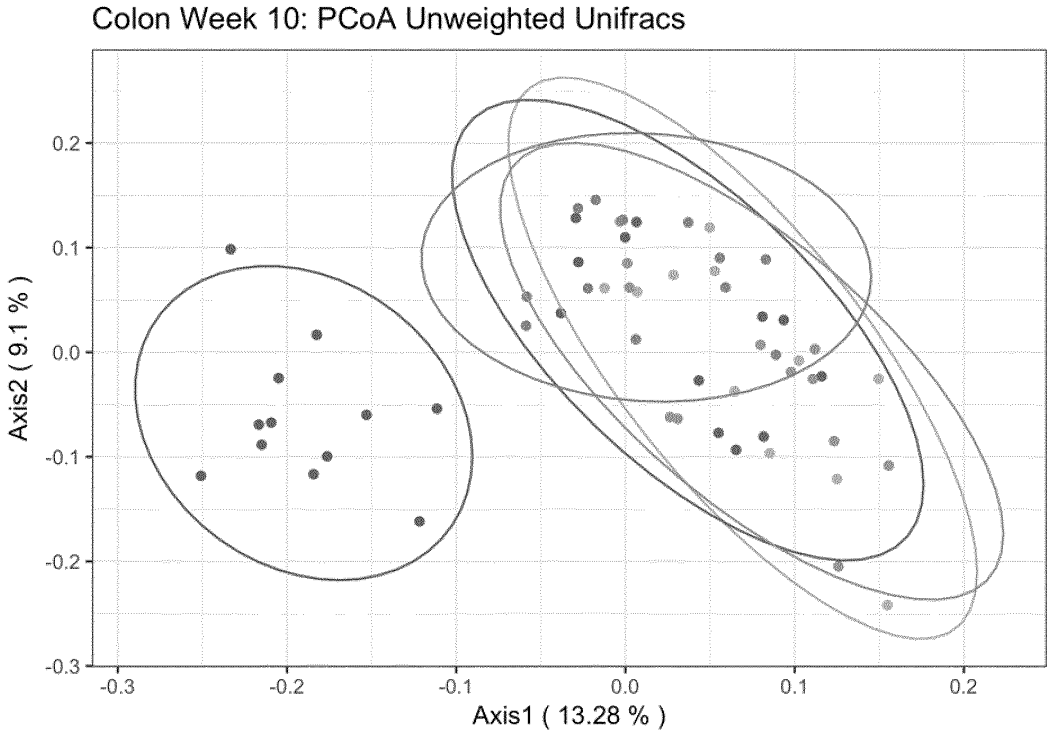

Colon Week 10: PCoA Unweighted Unifracs
0.2
0.1
0.0
-0.1
-0.2
-0.3
Axis2 (9.1 %)
-0.3
-0.2
-0.1
0.0
0.1
0.2
Axis1 (13.28 %)

Fig. 20

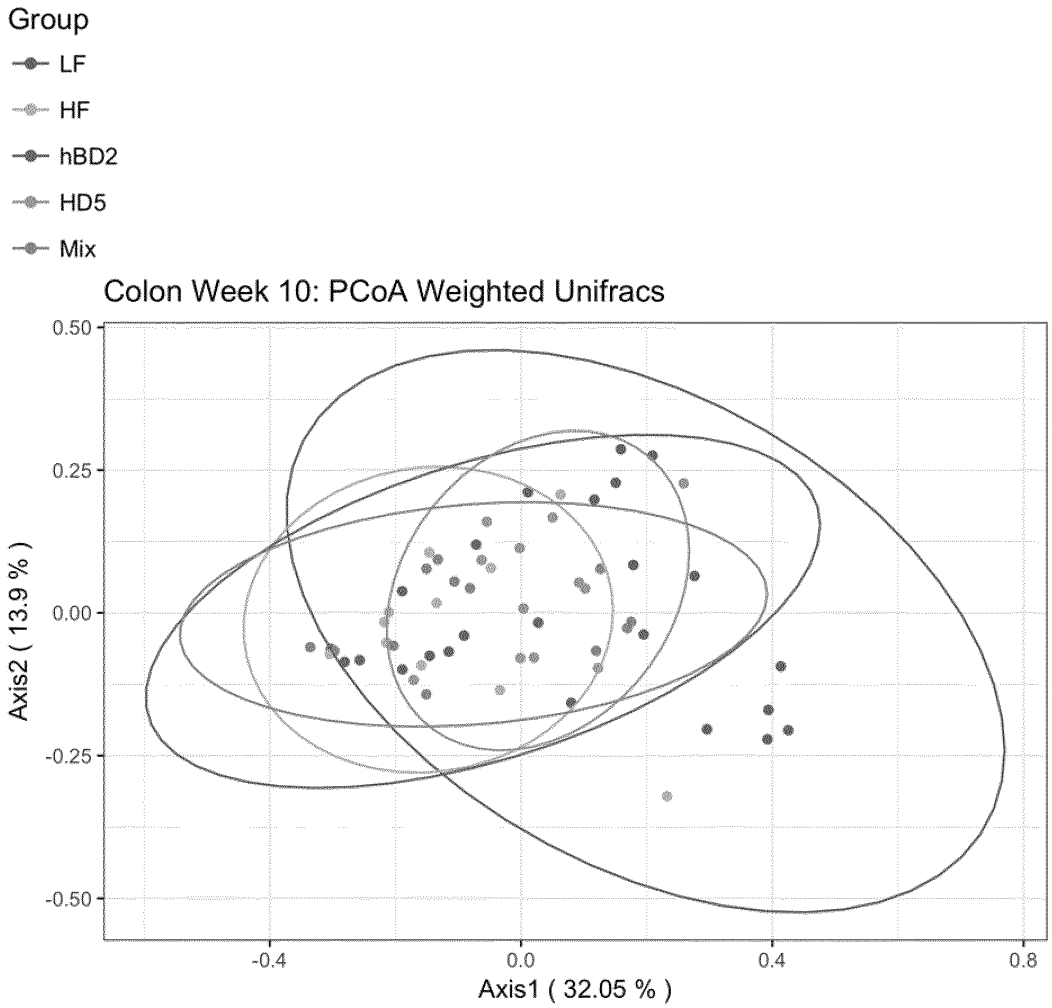

Group
LF
HF
hBD2
HD5
Mix
Colon Week 10: PCoA Weighted Unifracs
0.50
0.25
0.00
-0.25
-0.50
Axis2 (13.9 %)
-0.4
0.0
0.4
0.8
Axis1 (32.05 %)

FIG. 20 contd.

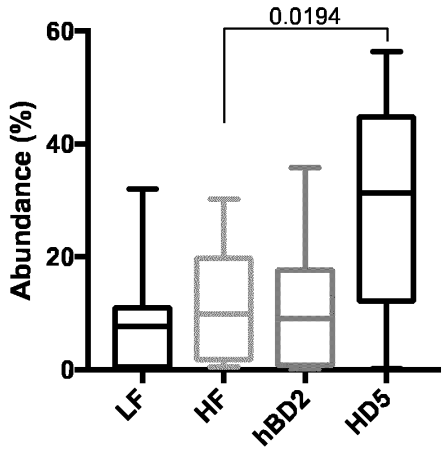

0.0194
60
40
20
0
Abundance (%)
LF
HF
hBD2
HD5

Fig. 21

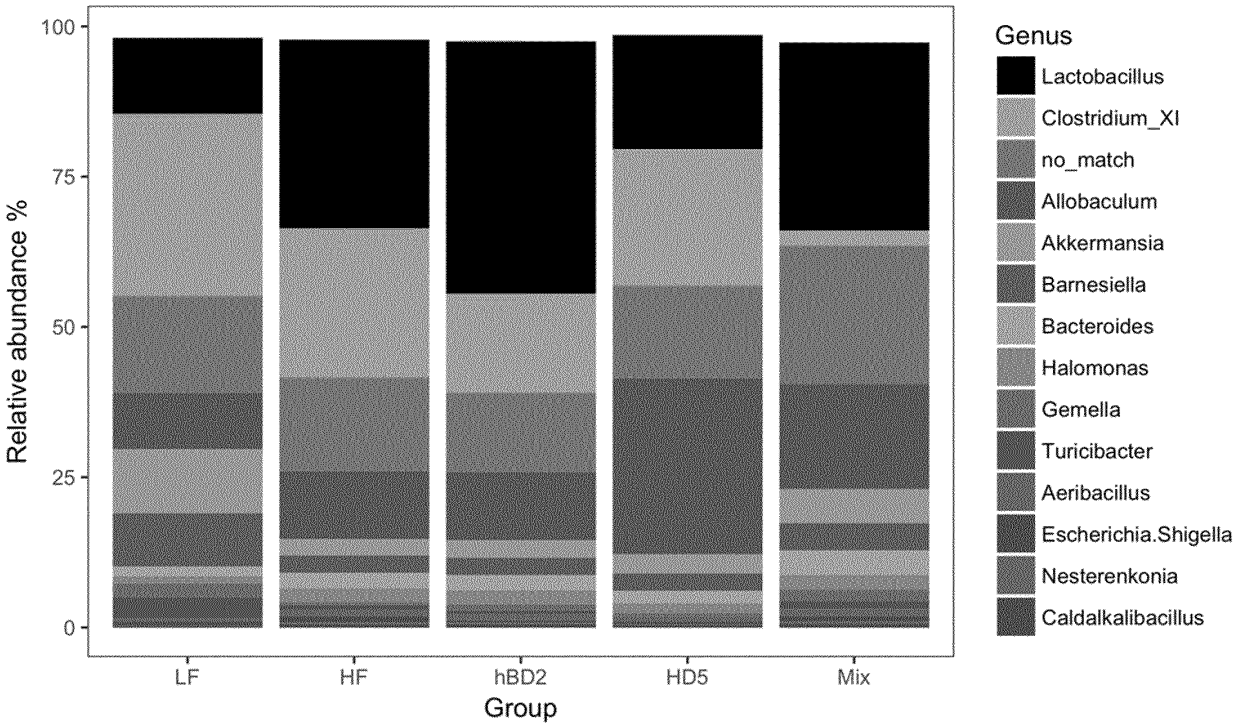

100
75
50
25
0
Relative abundance %
LF
HF
hBD2
HD5
Mix
Group
Genus
Lactobacillus
Clostridium_XI
no_match
Allobaculum
Akkermansia
Barnesiella
Bacteroides
Halomonas
Gemella
Turicibacter
Aeribacillus
Escherichia.Shigella
Nesterenkonia
Caldalkalibacillus

Fig. 22

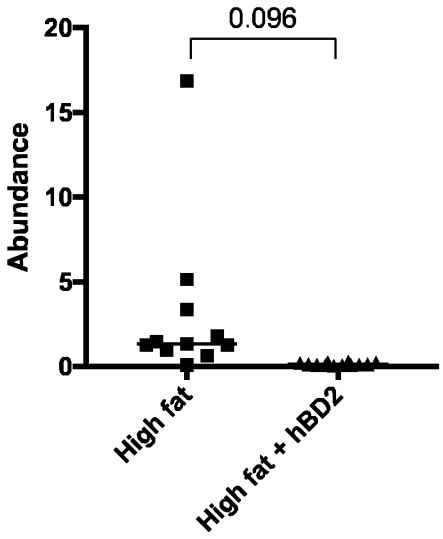

0.096
Abundance
20
15
10
5
0
High fat
High fat + hBD2

Fig 23.

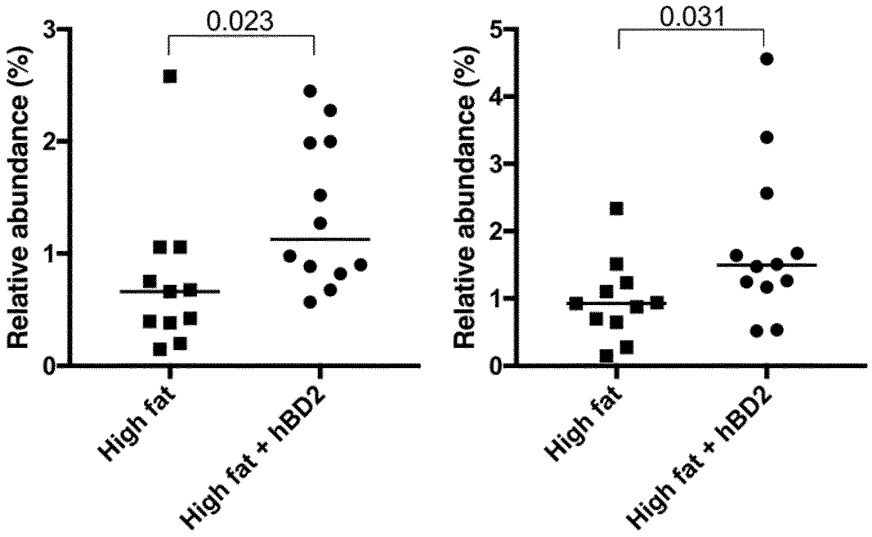

0.023
Relative abundance (%)
3
2
1
0
High fat
High fat + hBD2
0.031
Relative abundance (%)
5
4
3
2
1
0
High fat
High fat + hBD2

Fig 24.

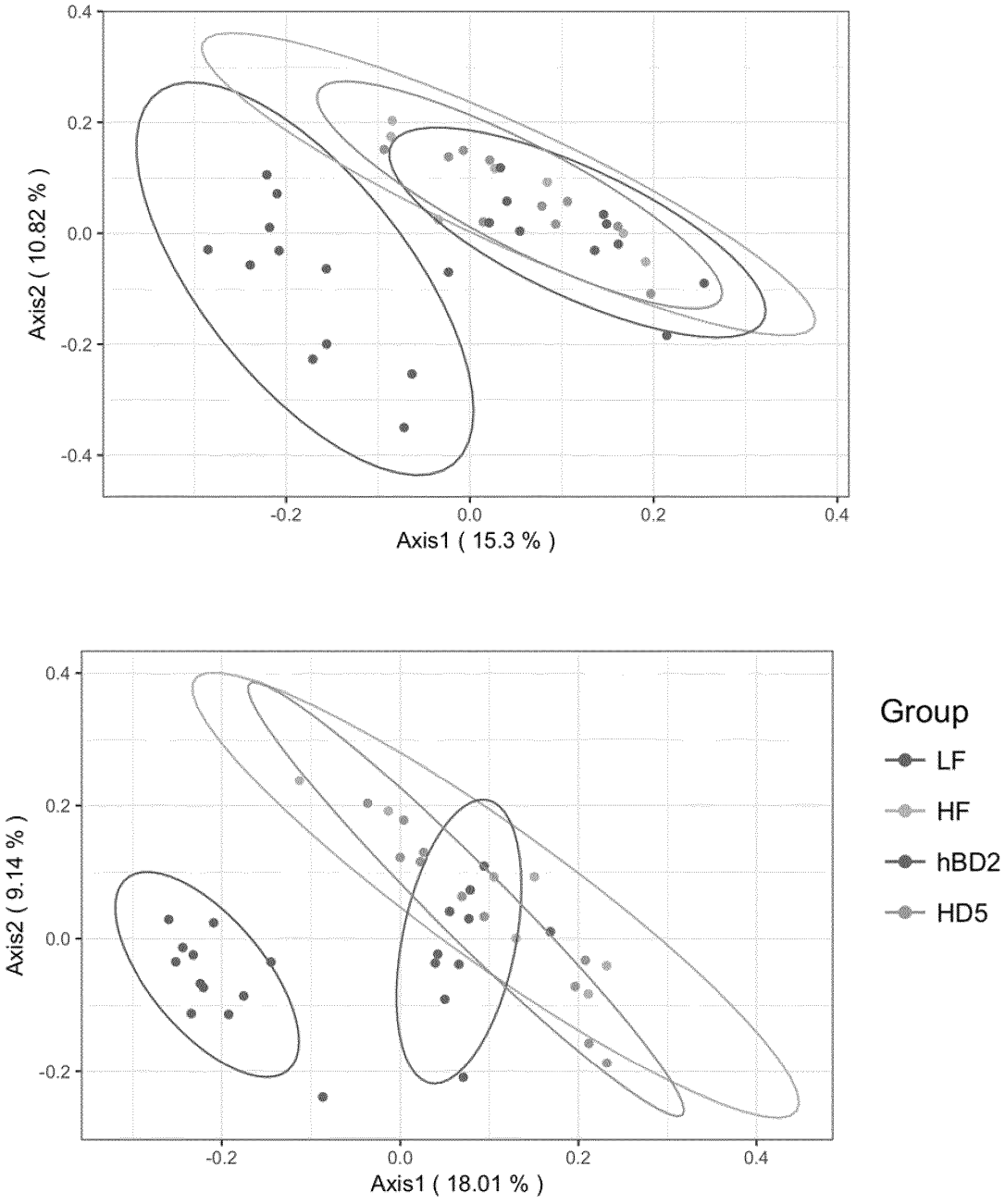

0.4
0.2
0.0
-0.2
-0.4
Axis2 (10.82 %)
-0.2
0.0
0.2
0.4
Axis1 (15.3 %)
0.4
0.2
0.0
-0.2
Axis2 (9.14 %)
-0.2
0.0
0.2
0.4
Axis1 (18.01 %)
Group
LF
HF
hBD2
HD5

Fig 25

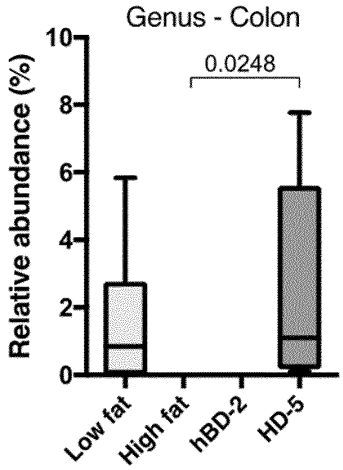

Genus - Colon
0.0248
Relative abundance (%)
10
8
6
4
2
0
Low fat
High fat
hBD-2
HD-5

Fig 26.

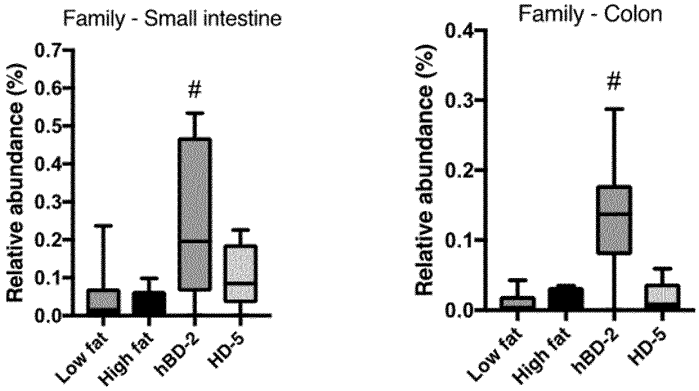

Family - Small intestine
Relative abundance (%)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
#
Low fat
High fat
hBD-2
HD-5
Family - Colon
Relative abundance (%)
0.4
0.3
0.2
0.1
0.0
#
Low fat
High fat
hBD-2
HD-5

Fig 27.

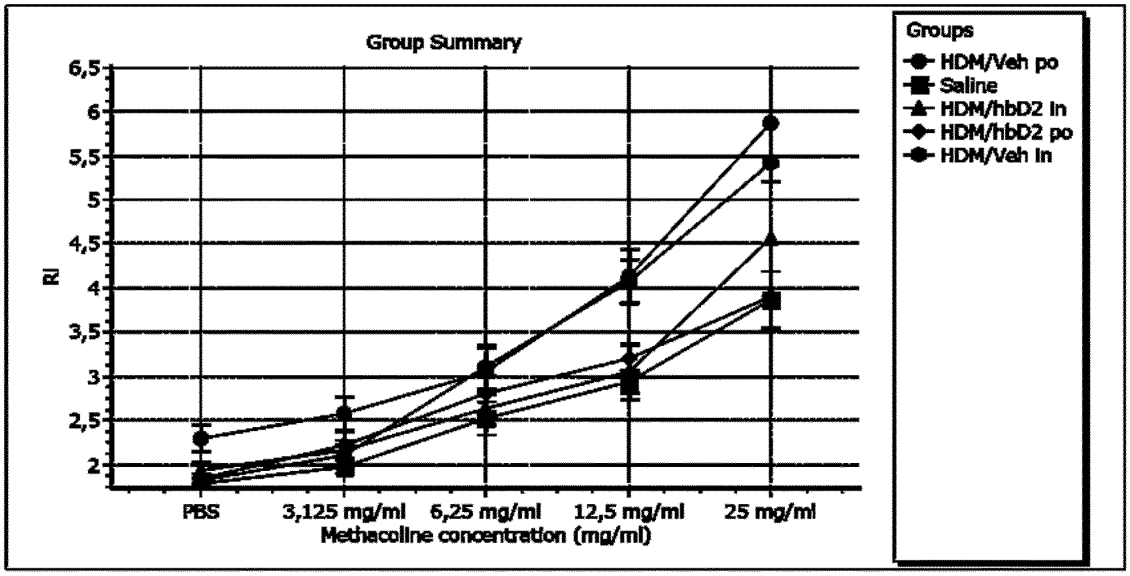

Group Summary
RI
6,5
6
5,5
5
4,5
4
3,5
3
2,5
2
PBS
3,125 mg/ml
6,25 mg/ml
12,5 mg/ml
25 mg/ml
Methacoline concentration (mg/ml)
Groups
HDM/Veh po
Saline
HDM/hbD2 in
HDM/hbD2 po
HDM/Veh in

Fig. 28

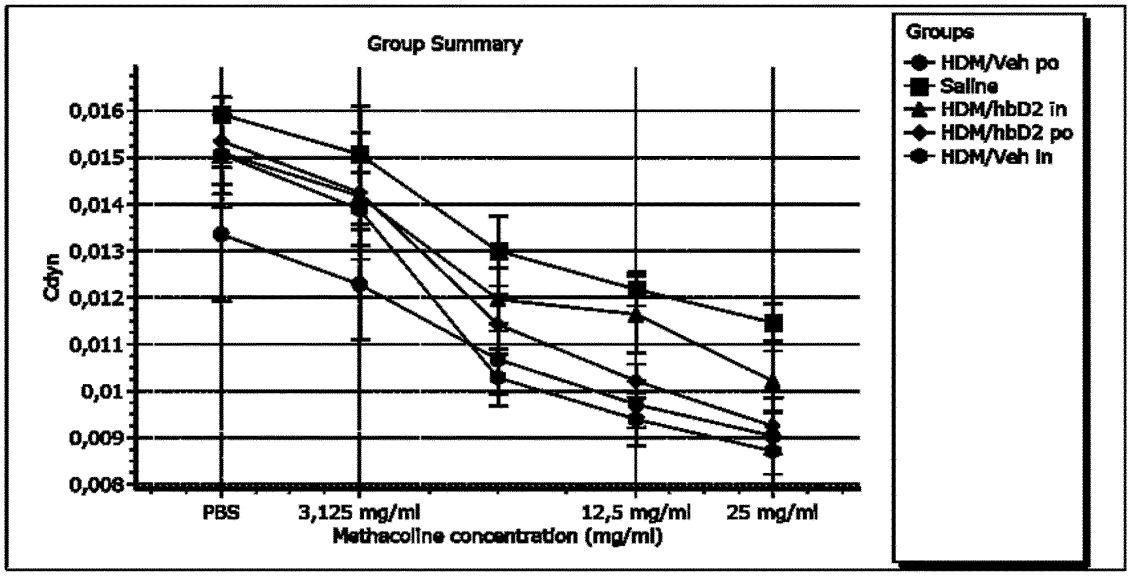

Group Summary
Cdyn
0,016
0,015
0,014
0,013
0,012
0,011
0,01
0,009
0,008
PBS
3,125 mg/ml
12,5 mg/ml
25 mg/ml
Methacoline concentration (mg/ml)
Groups
HDM/Veh po
Saline
HDM/hbD2 in
HDM/hbD2 po
HDM/Veh in

Fig. 29

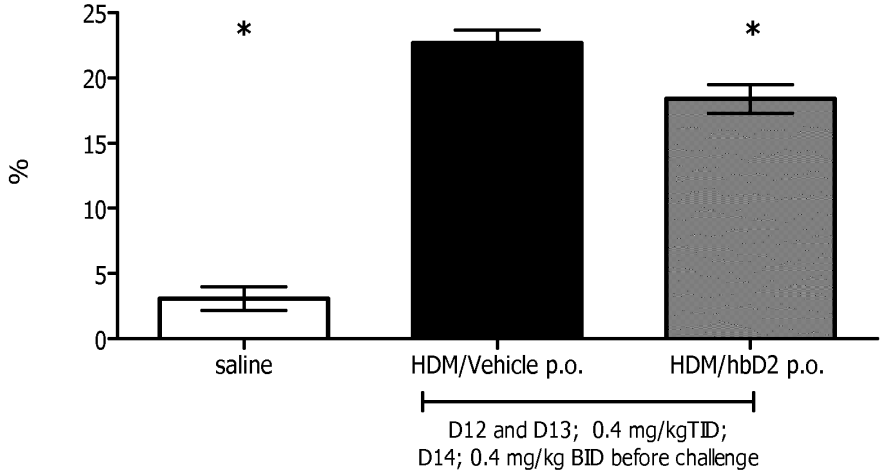


Fig. 30

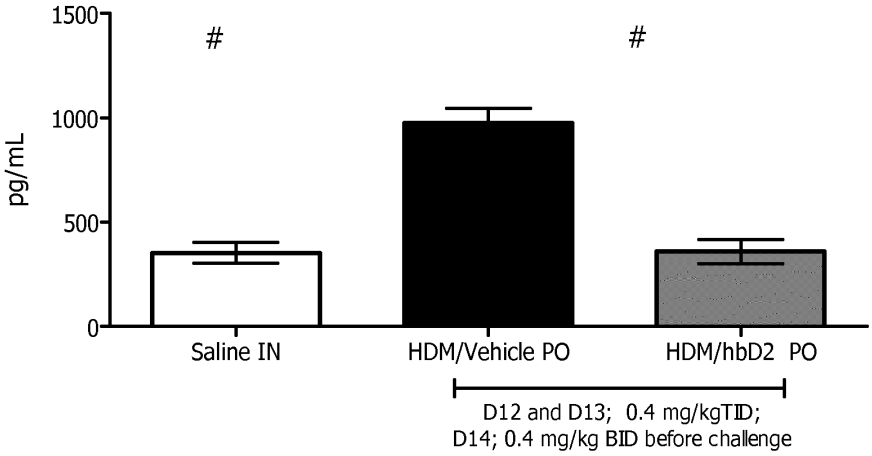


Fig. 31a

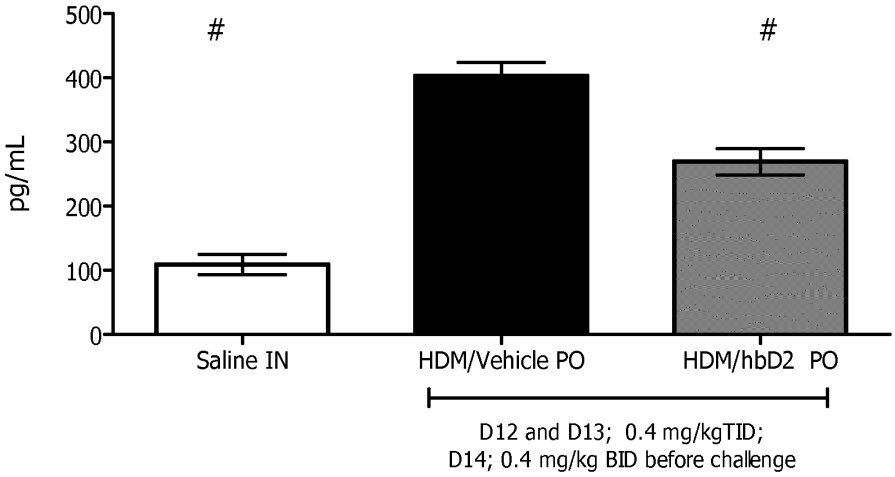


Fig. 31b

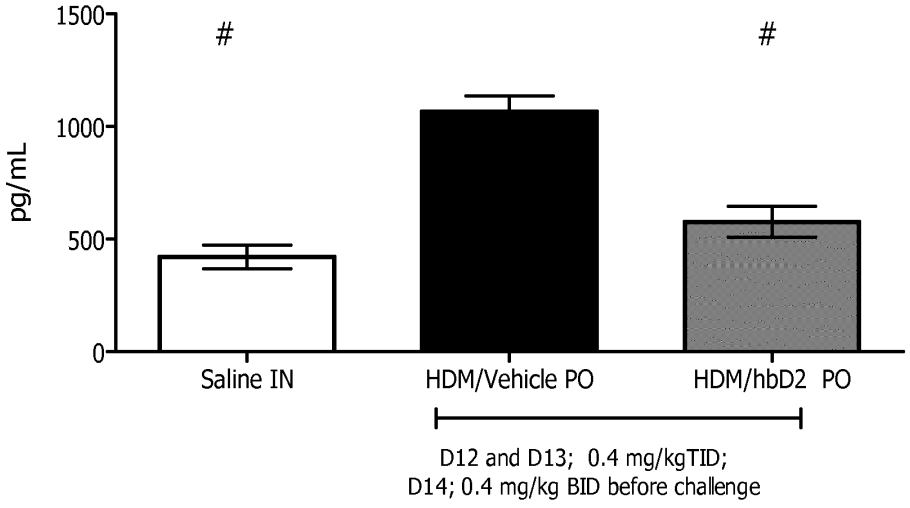


Fig. 31c

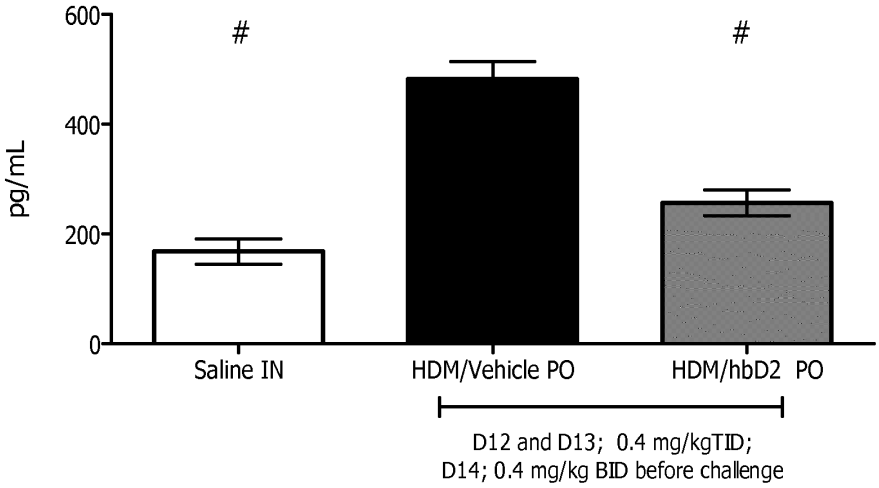


Fig. 31d

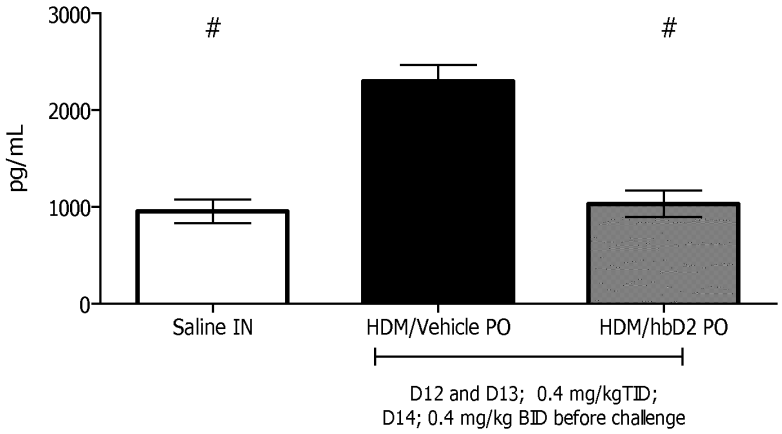


Fig. 31e

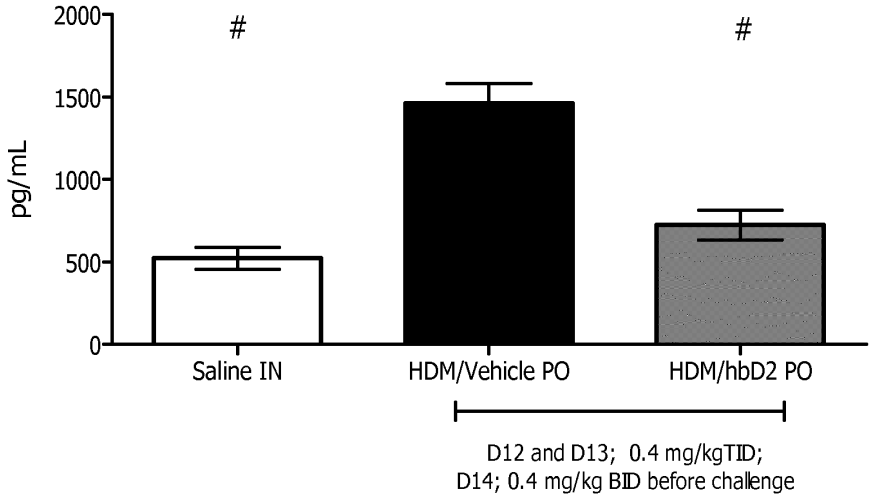

2000
1500
1000
500
0
pg/mL
#
#
Saline IN
HDM/Vehicle PO
HDM/hbD2 PO
D12 and D13; 0.4 mg/kgTID;
D14; 0.4 mg/kg BID before challenge

Fig. 31f

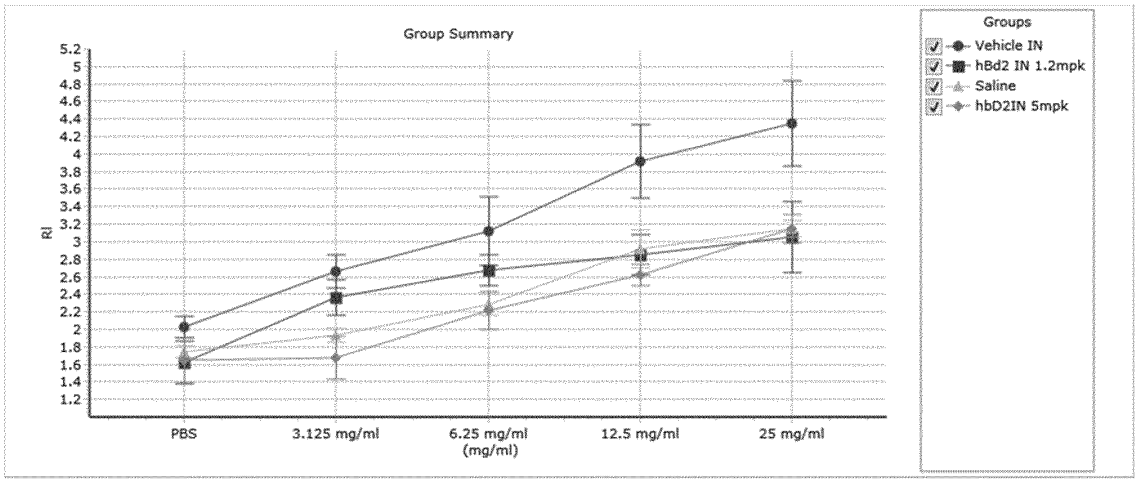


Fig. 32a.

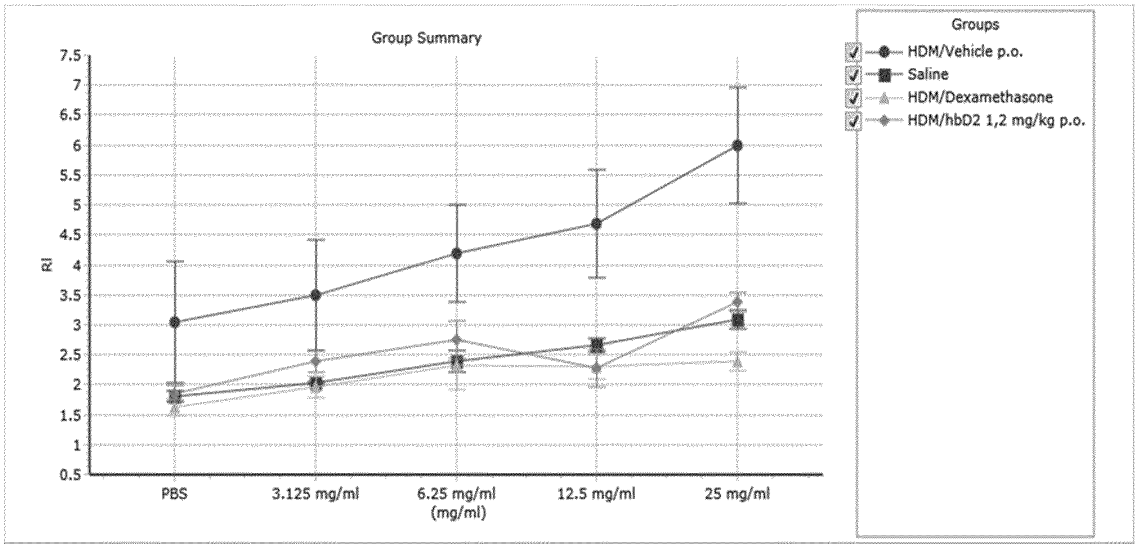


Fig. 32b.

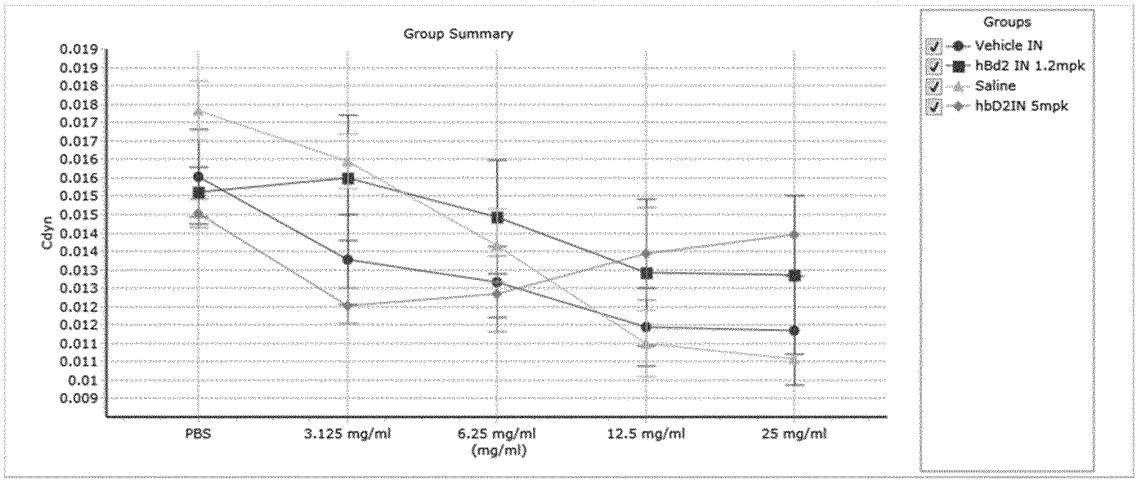


Fig. 33a

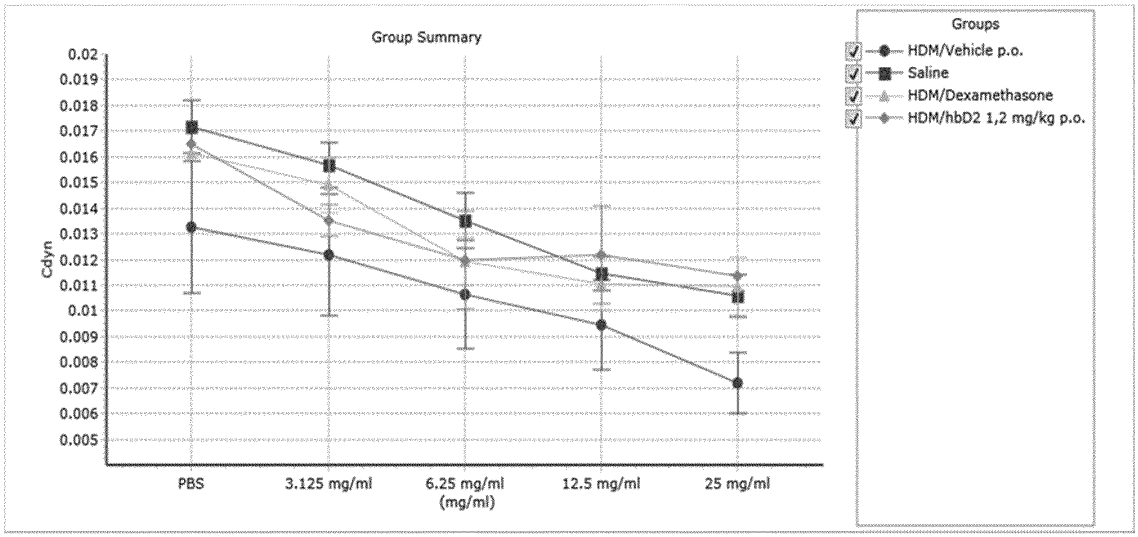


Fig. 33b

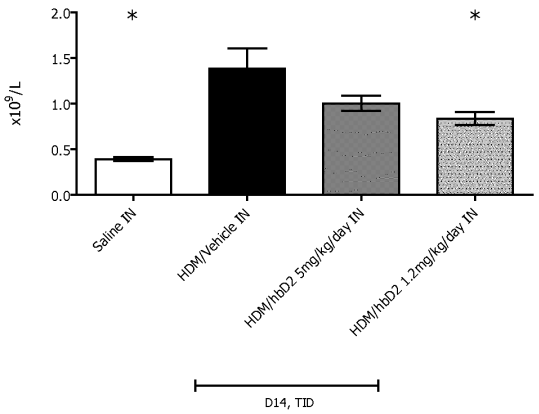


Fig 34a

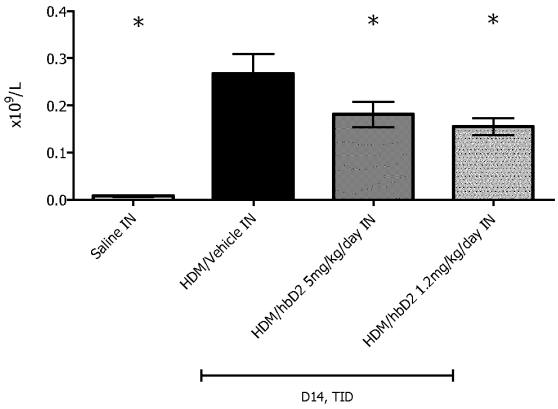


Fig 34b

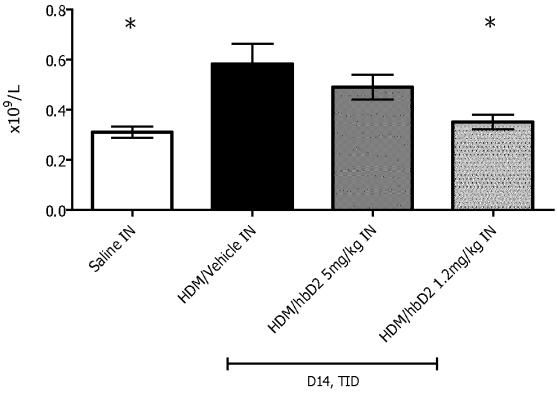


Fig. 34c

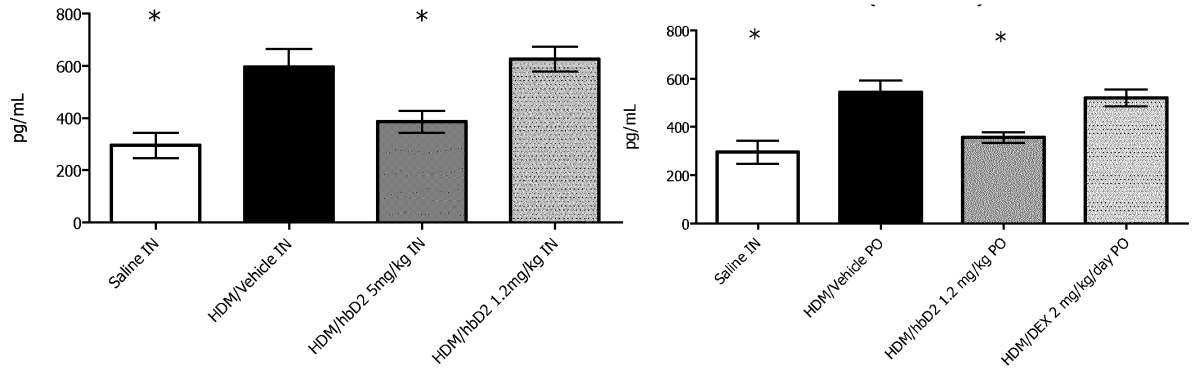

pg/mL
800
600
400
200
0
*
*
Saline IN
HDM/Vehicle IN
HDM/hbD2 5mg/kg IN
HDM/hbD2 1.2mg/kg IN
pg/mL
800
600
400
200
0
*
*
Saline IN
HDM/Vehicle PO
HDM/hbD2 1.2 mg/kg PO
HDM/DEX 2 mg/kg/day PO

Fig. 35

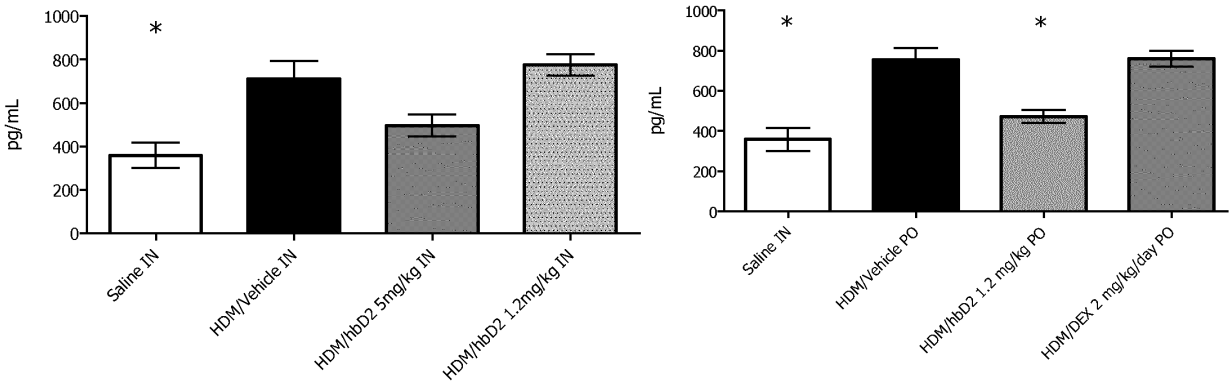

pg/mL
1000
800
600
400
200
0
*
Saline IN
HDM/Vehicle IN
HDM/hbD2 5mg/kg IN
HDM/hbD2 1.2mg/kg IN
pg/mL
1000
800
600
400
200
0
*
*
Saline IN
HDM/Vehicle PO
HDM/hbD2 1.2 mg/kg PO
HDM/DEX 2 mg/kg/day PO

Fig. 36

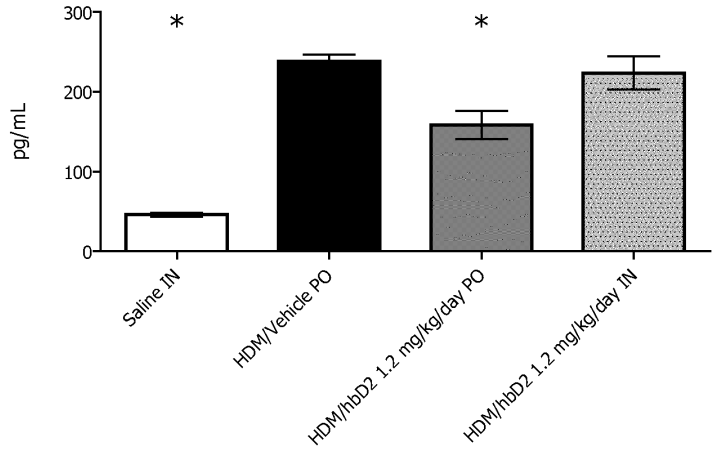

300
200
100
0
pg/mL
*
*
Saline IN
HDM/Vehicle PO
HDM/hbD2 1.2 mg/kg/day PO
HDM/hbD2 1.2 mg/kg/day IN

Fig. 37

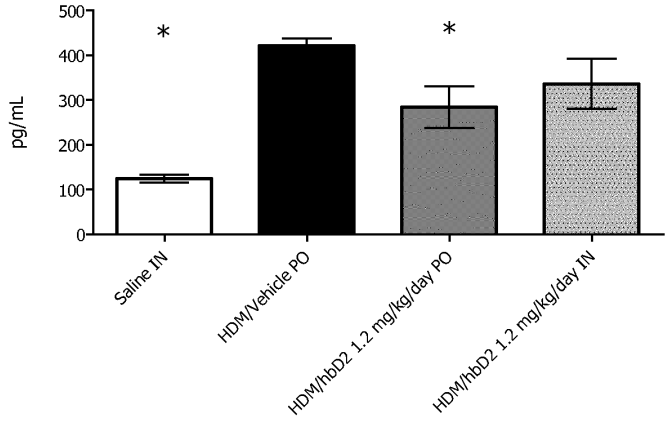

500
400
300
200
100
0
pg/mL
*
*
Saline IN
HDM/Vehicle PO
HDM/hbD2 1.2 mg/kg/day PO
HDM/hbD2 1.2 mg/kg/day IN

Fig. 38

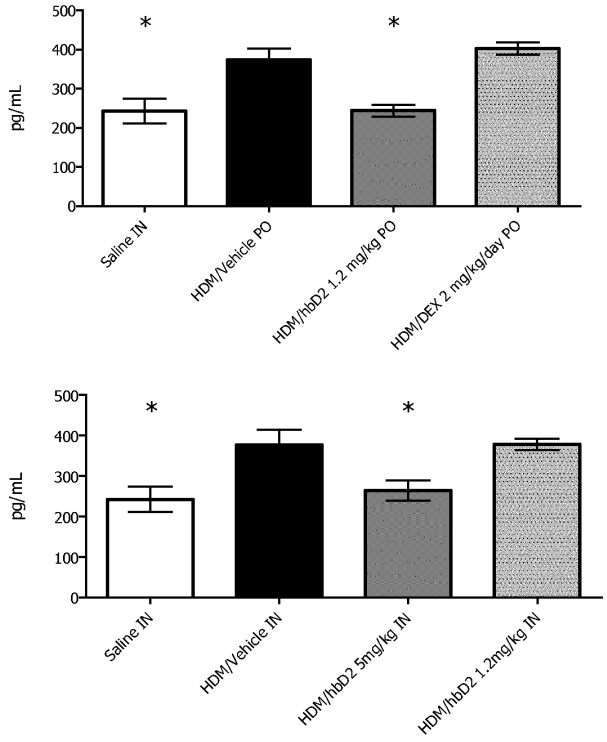

pg/mL
500
400
300
200
100
0
*
*
Saline IN
HDM/Vehicle PO
HDM/hbD2 1.2 mg/kg PO
HDM/DEX 2 mg/kg/day PO
pg/mL
500
400
300
200
100
0
*
*
Saline IN
HDM/Vehicle IN
HDM/hbD2 .5mg/kg IN
HDM/hbD2 1.2mg/kg IN

Fig. 39

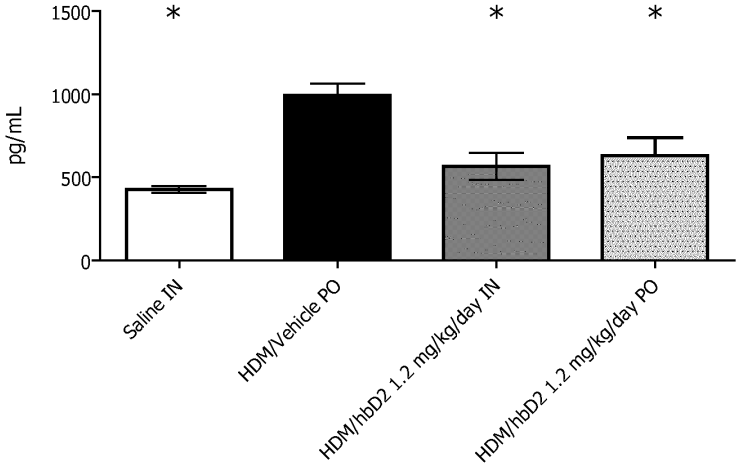

pg/mL
1500
1000
500
0
*
*
*
Saline IN
HDM/Vehicle PO
HDM/hbD2 1.2 mg/kg/day IN
HDM/hbD2 1.2 mg/kg/day PO

Fig. 40

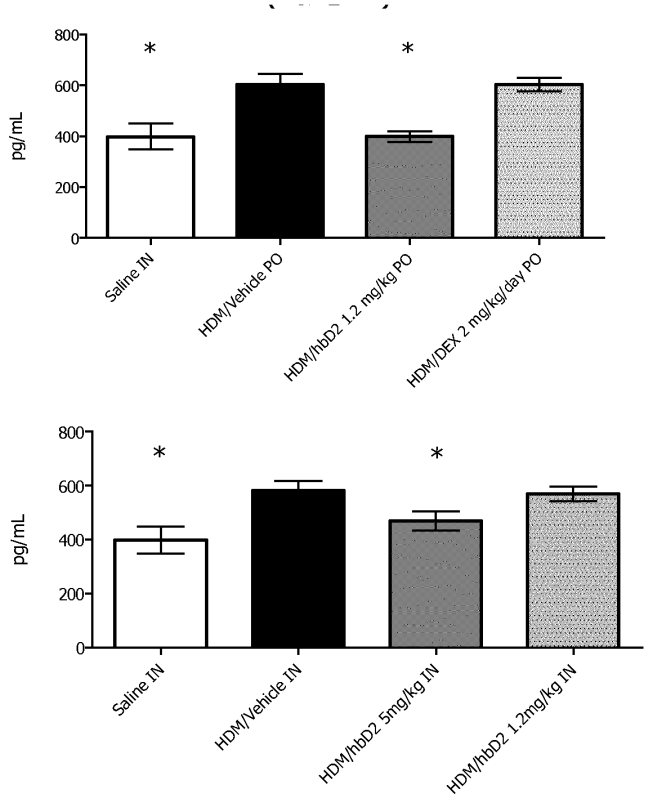

800
600
400
200
0
pg/mL
*
*
Saline IN
HDM/Vehicle PO
HDM/hbD2 1.2 mg/kg PO
HDM/DEX 2 mg/kg/day PO
800
600
400
200
0
pg/mL
*
*
Saline IN
HDM/Vehicle IN
HDM/hbD2 5mg/kg IN
HDM/hbD2 1.2mg/kg IN

Fig. 41

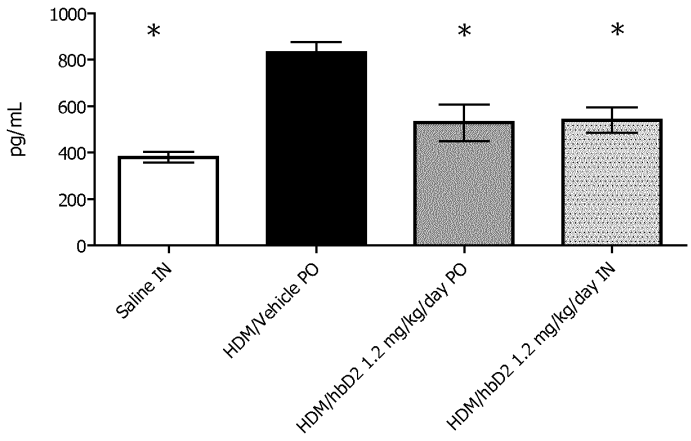

1000
800
600
400
200
0
pg/mL
*
*
*
Saline IN
HDM/Vehicle PO
HDM/hbD2 1.2 mg/kg/day PO
HDM/hbD2 1.2 mg/kg/day IN

Fig. 42

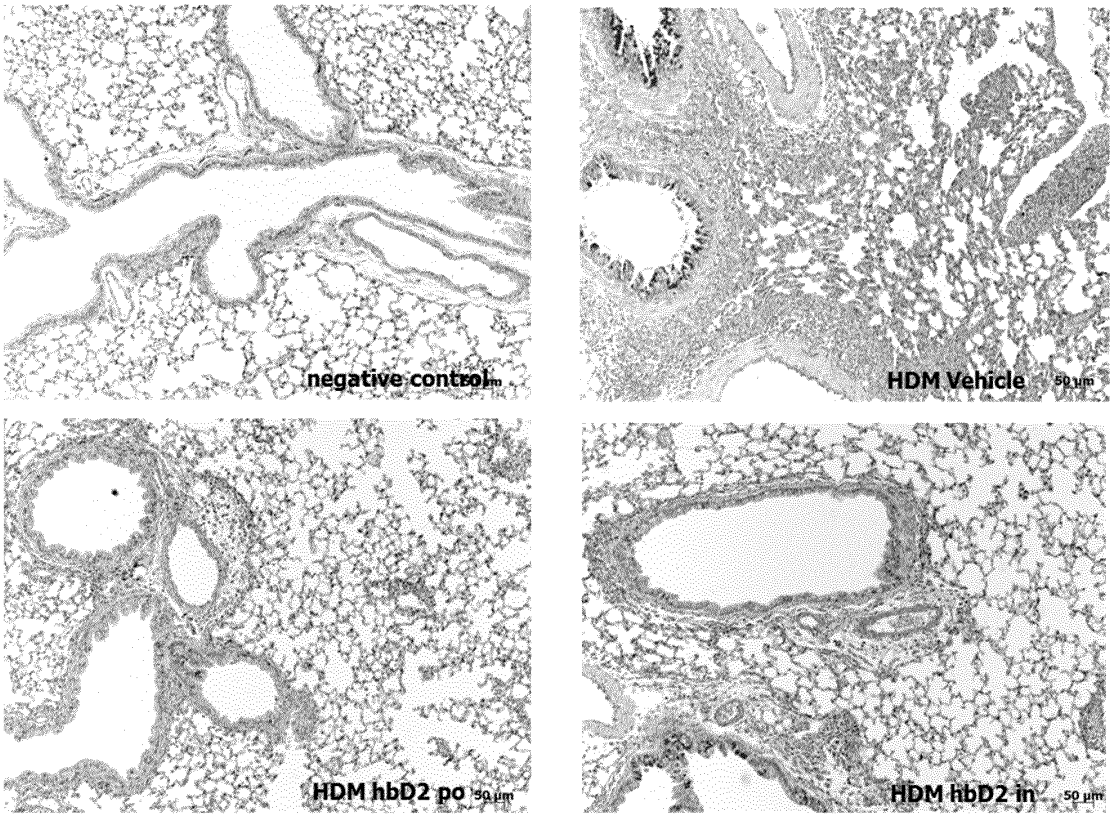

negative control
50 µm
HDM Vehicle
50 µm
HDM hbD2 po
50 µm
HDM hbD2 in
50 µm

Fig. 43

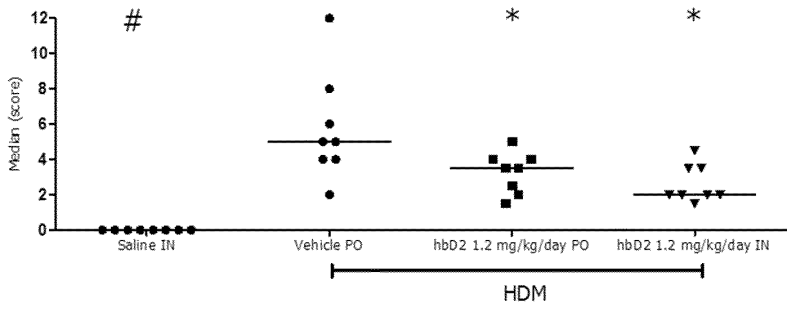

#
*
*
Median (score)
12
10
8
6
4
2
0
Saline IN
Vehicle PO
hbD2 1.2 mg/kg/day PO
hbD2 1.2 mg/kg/day IN
HDM

Fig. 44

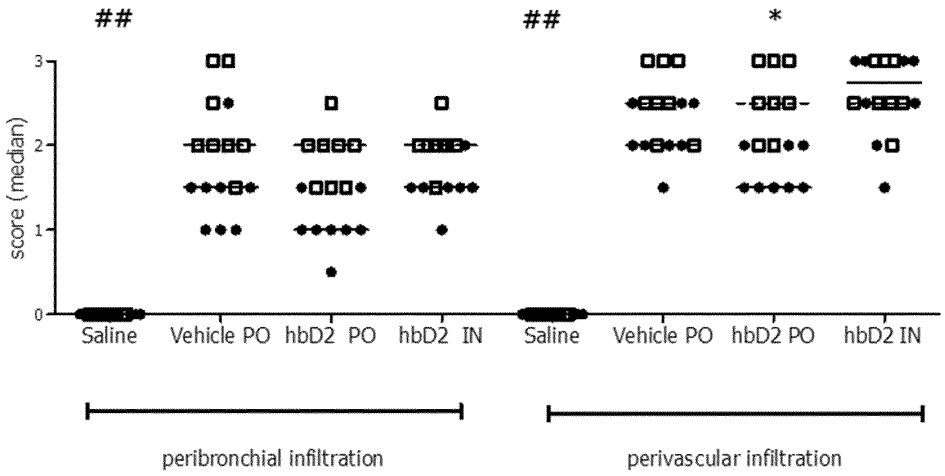

##
##
*
score (median)
3
2
1
0
Saline
Vehicle PO
hbD2 PO
hbD2 IN
Saline
Vehicle PO
hbD2 PO
hbD2 IN
peribronchial infiltration
perivascular infiltration

Fig. 45

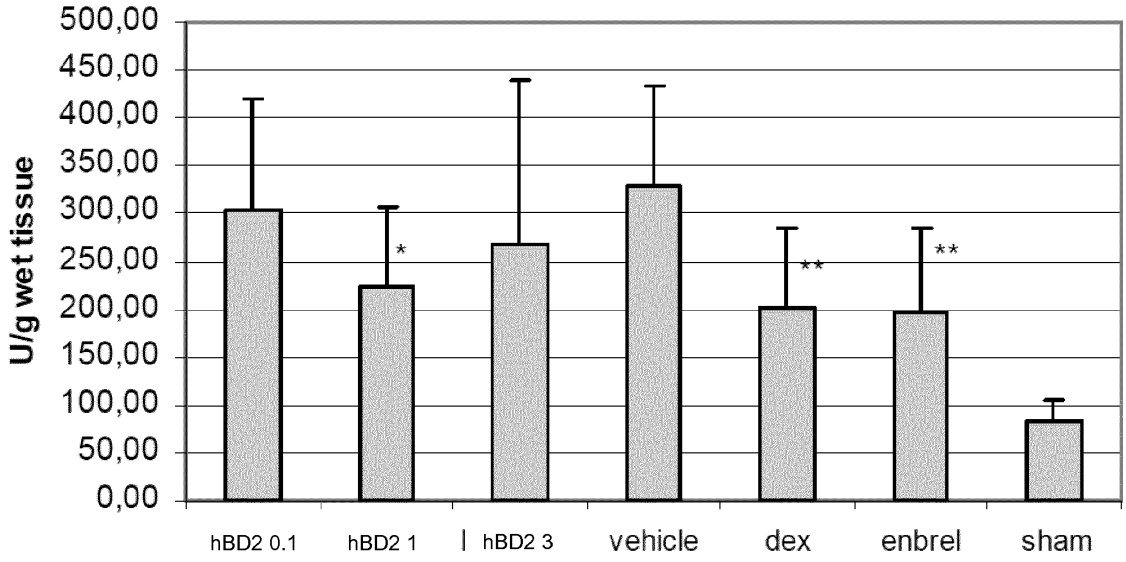

500,00
450,00
400,00
350,00
300,00
250,00
200,00
150,00
100,00
50,00
0,00
U/g wet tissue
*
**
**
hBD2 0.1
hBD2 1
hBD2 3
vehicle
dex
enbrel
sham

Fig. 46

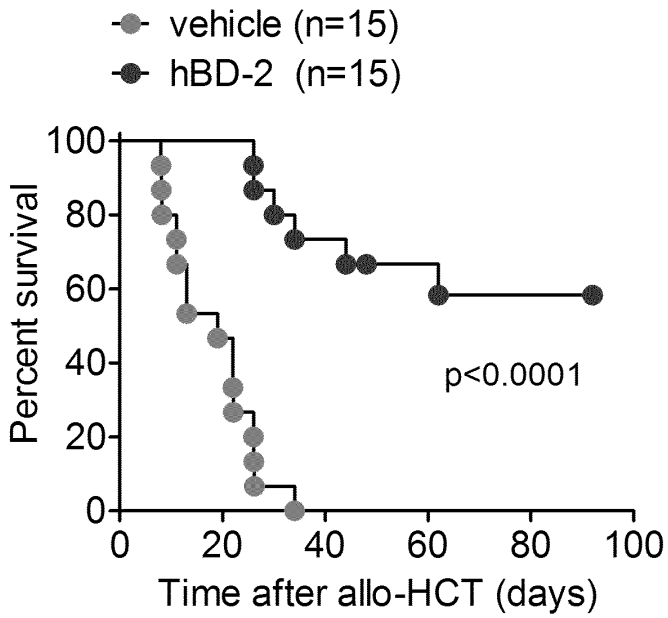

vehicle (n=15)
hBD-2 (n=15)
100
80
60
40
20
0
Percent survival
p<0.0001
0
20
40
60
80
100
Time after allo-HCT (days)

Fig. 47

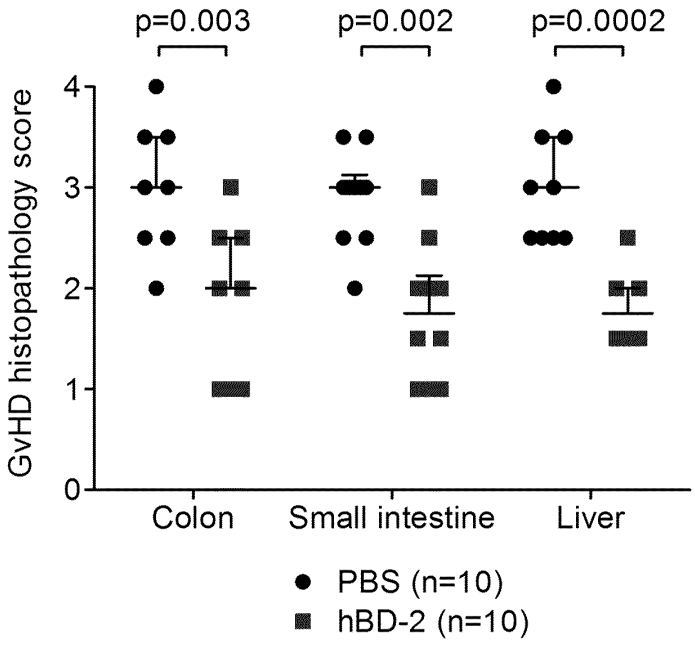

p=0.003
p=0.002
p=0.0002
GvHD histopathology score
4
3
2
1
0
Colon
Small intestine
Liver
PBS (n=10)
hBD-2 (n=10)

Fig 48

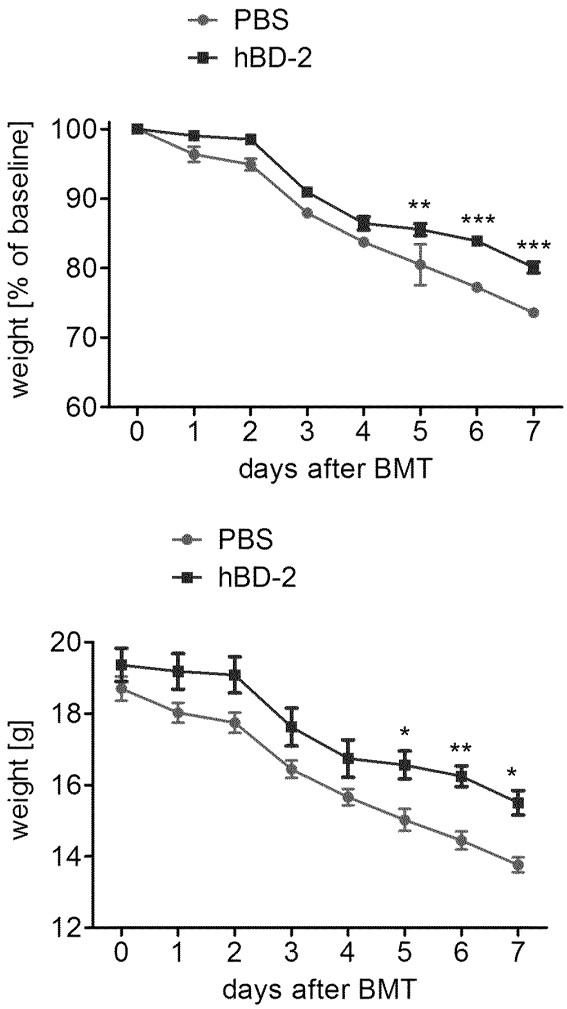

PBS
hBD-2
weight [% of baseline]
100
90
80
70
60
0 1 2 3 4 5 6 7
days after BMT
**
***
***
PBS
hBD-2
weight [g]
20
18
16
14
12
0 1 2 3 4 5 6 7
days after BMT
*
**
*

Fig 49 a and b

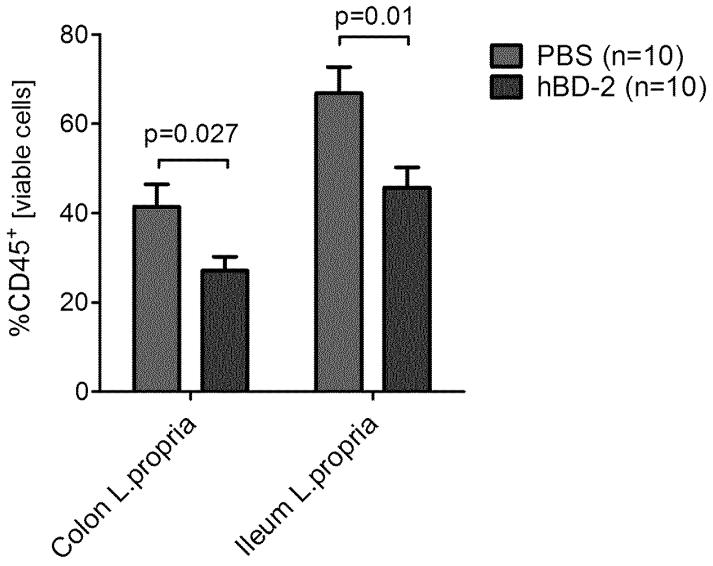


Fig 50

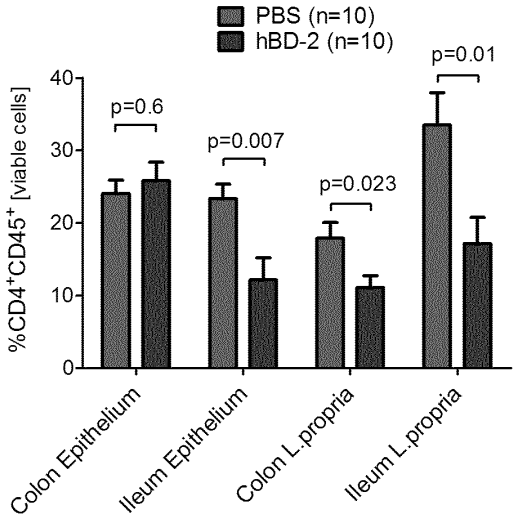


Fig 51a

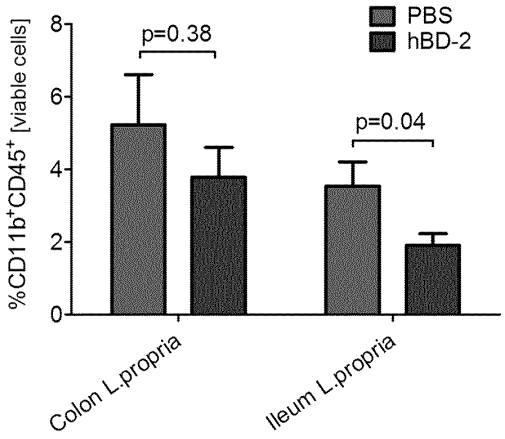


Fig 51b

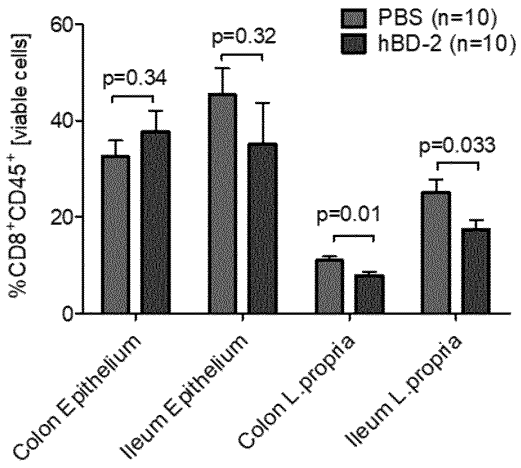


Fig 51c

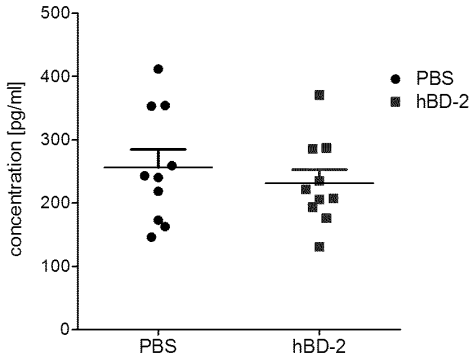


Fig 52a

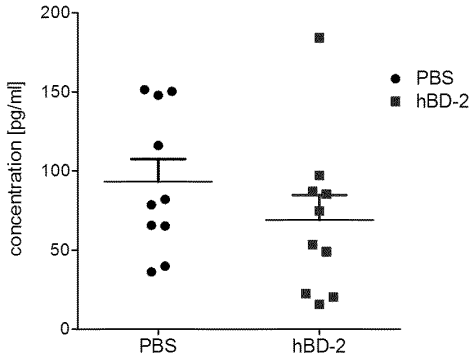


Fig 52b

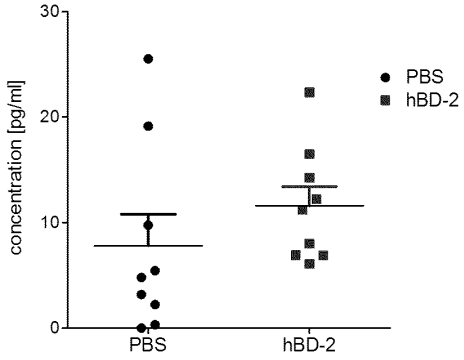


Fig 52c

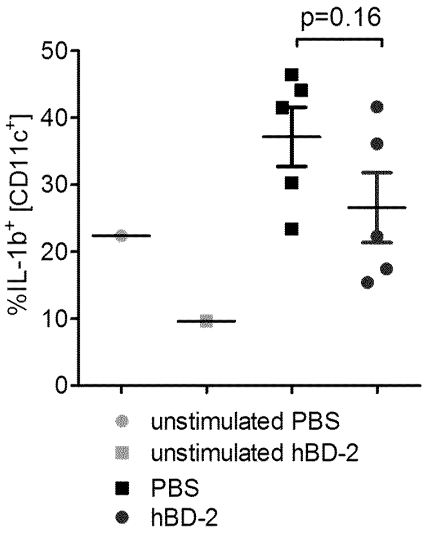


Fig 53a

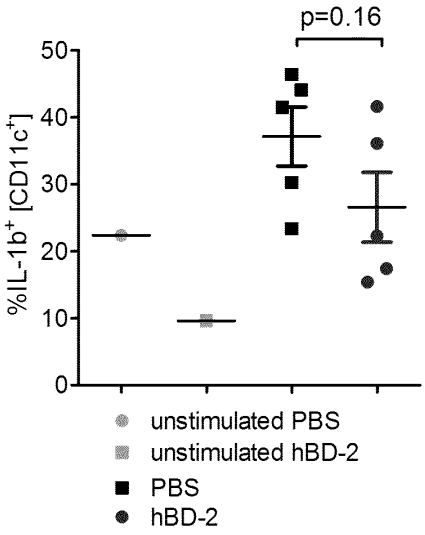


Fig 53b

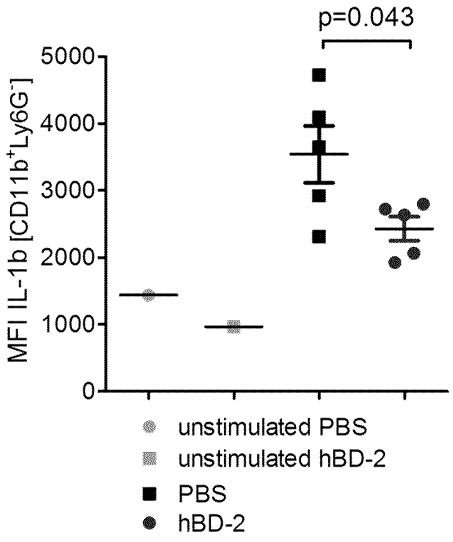


Fig 53c

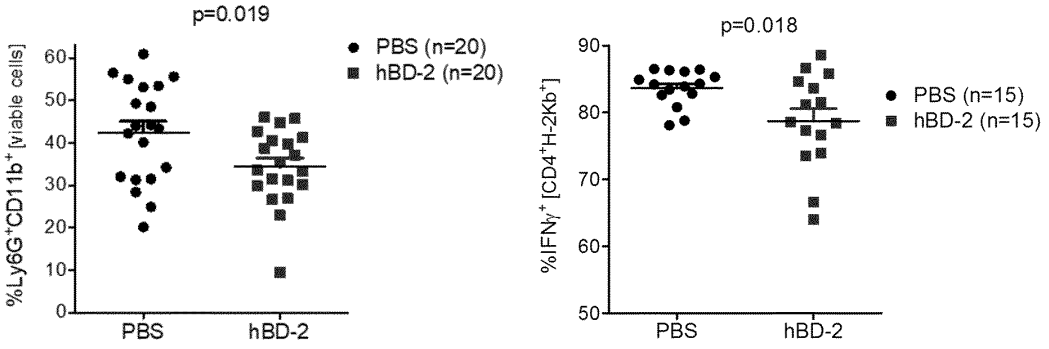


Fig 54 a and b

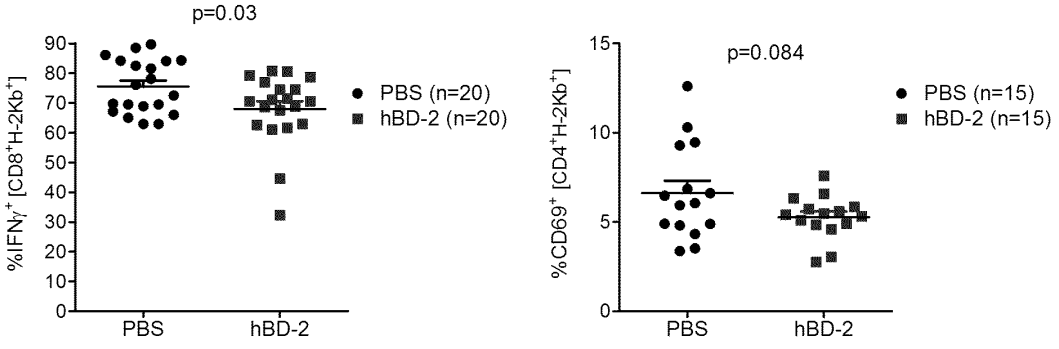


Fig 54 c and d

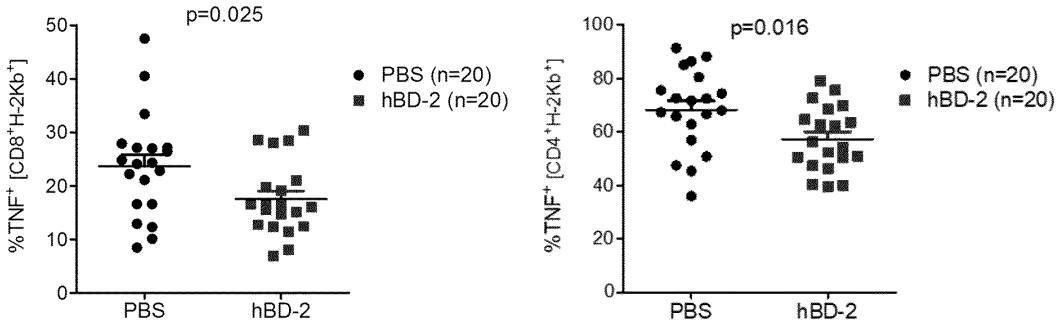


Fig 54 e and f

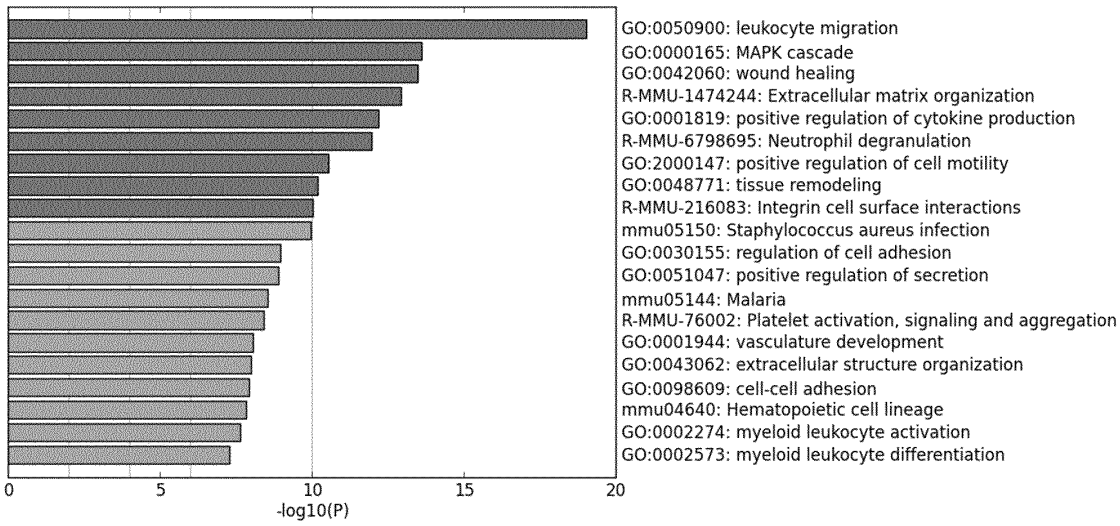


Fig 55

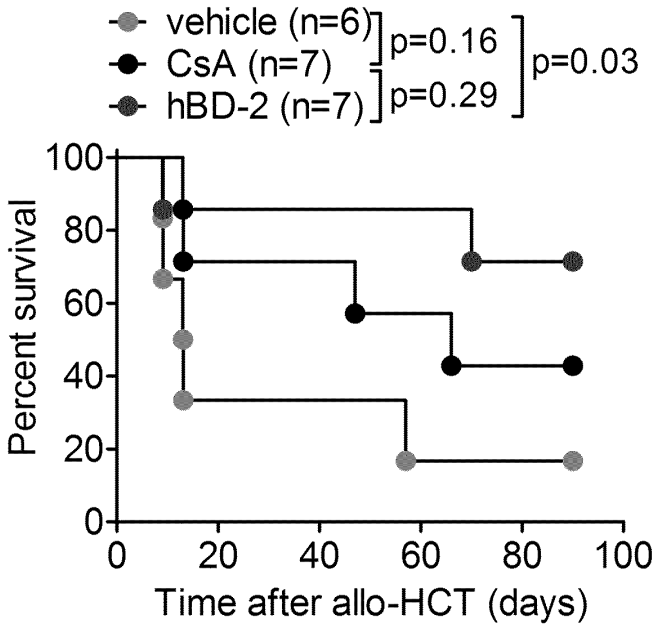

vehicle (n=6)
CsA (n=7)
hBD-2 (n=7)
p=0.16
p=0.29
p=0.03
Percent survival
100
80
60
40
20
0
0
20
40
60
80
100
Time after allo-HCT (days)

Fig 56

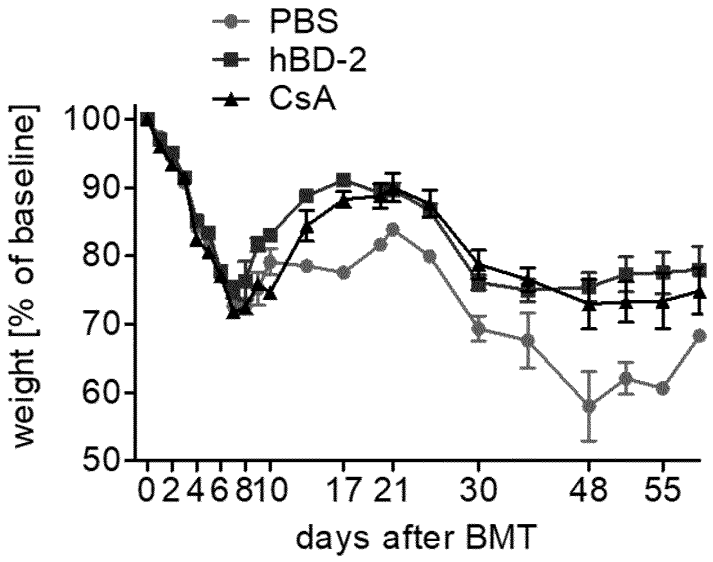

PBS
hBD-2
CsA
weight [% of baseline]
100
90
80
70
60
50
0 2 4 6 8 10
17 21
30
48
55
days after BMT

Fig 57

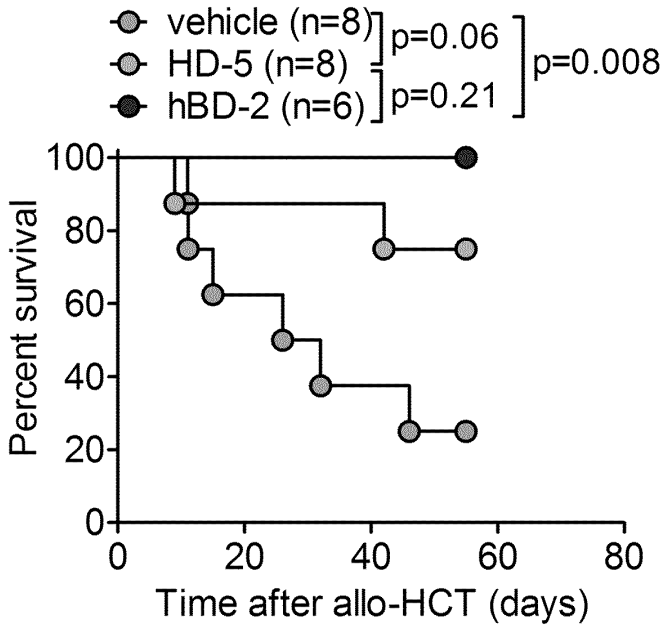

vehicle (n=8)
HD-5 (n=8)
hBD-2 (n=6)
p=0.06
p=0.21
p=0.008
Percent survival
100
80
60
40
20
0
0
20
40
60
80
Time after allo-HCT (days)

Fig 58

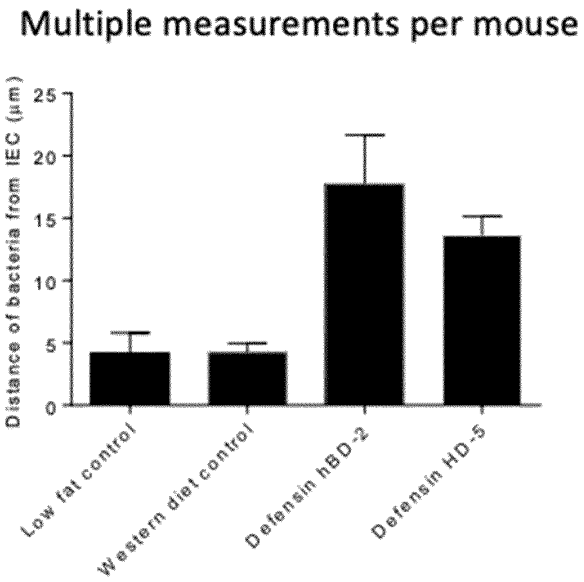

Multiple measurements per mouse
Distance of bacteria from IEC (µm)
25
20
15
10
5
0
Low fat control
Western diet control
Defensin hBD-2
Defensin HD-5

Fig 59

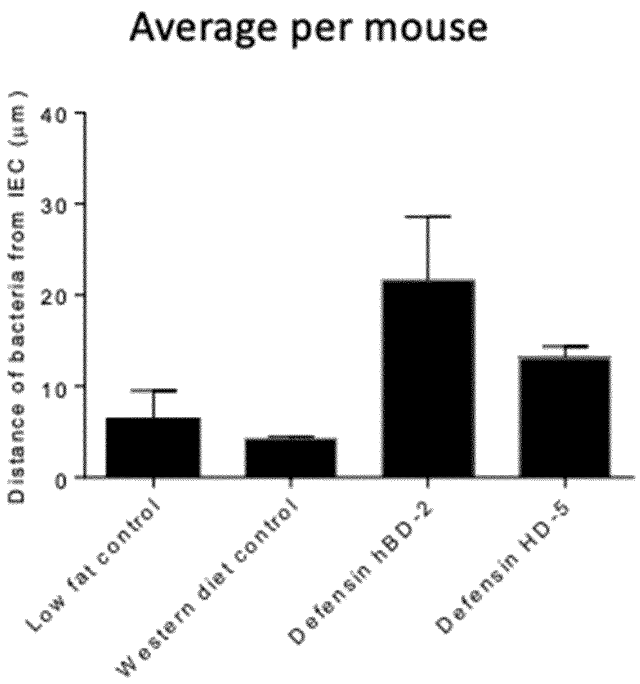


Fig 59 continued

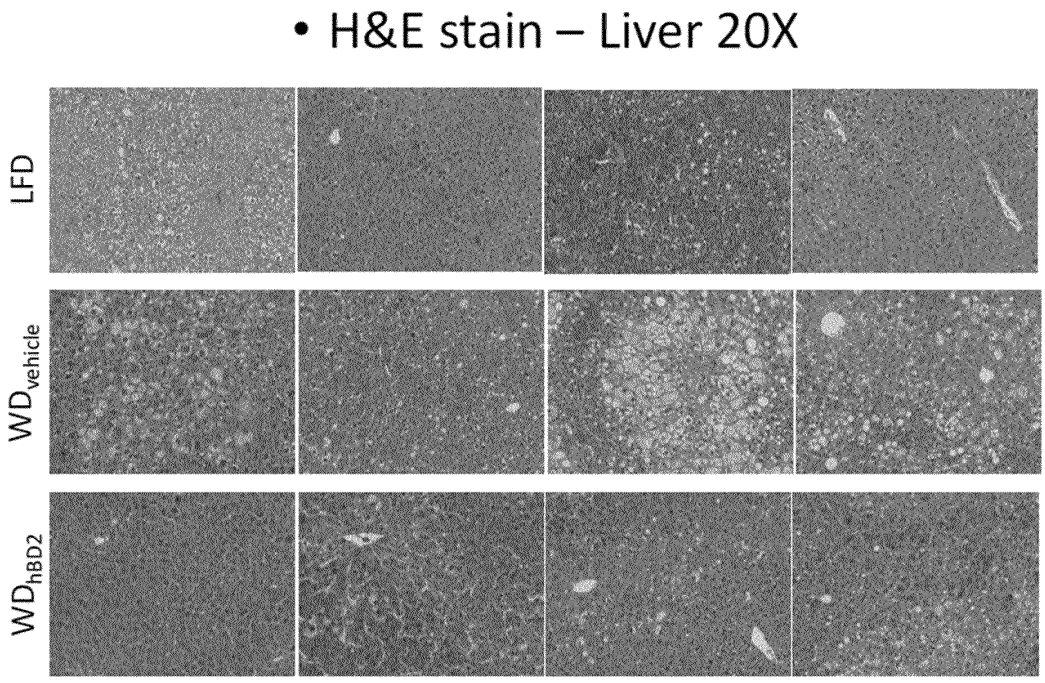


Fig 60