Figure 1

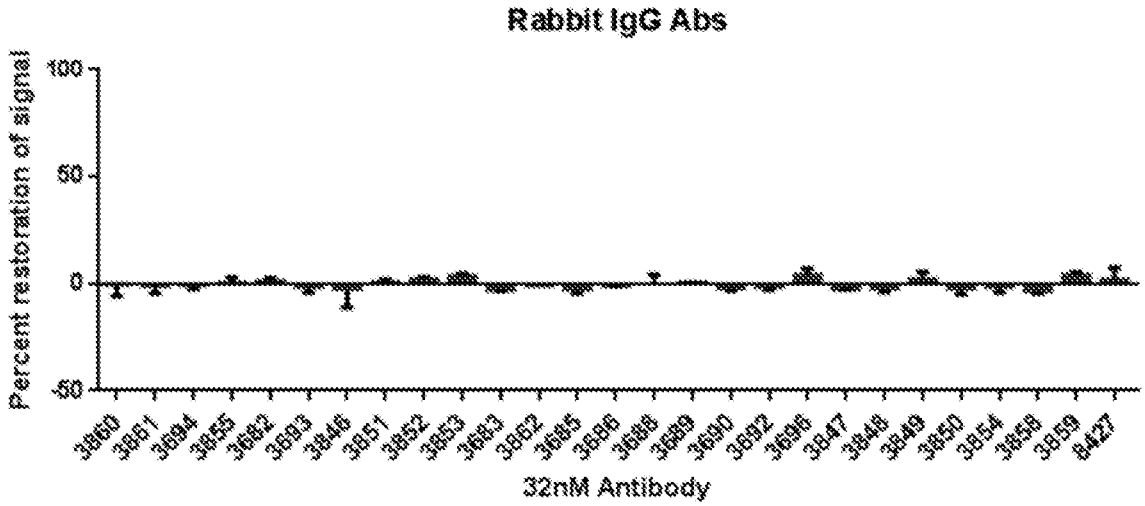


Figure 2

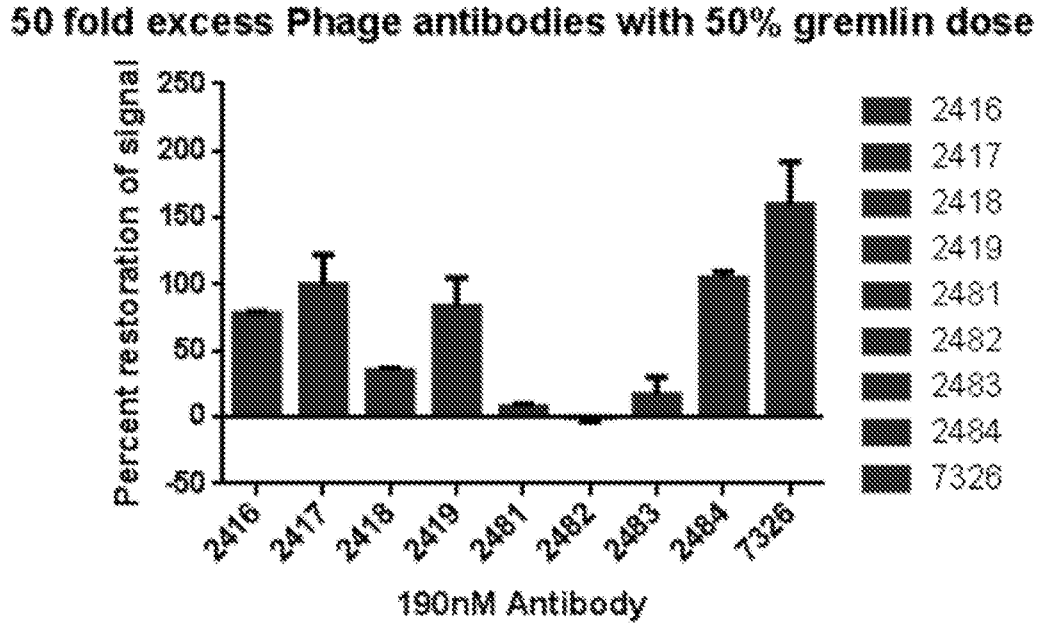
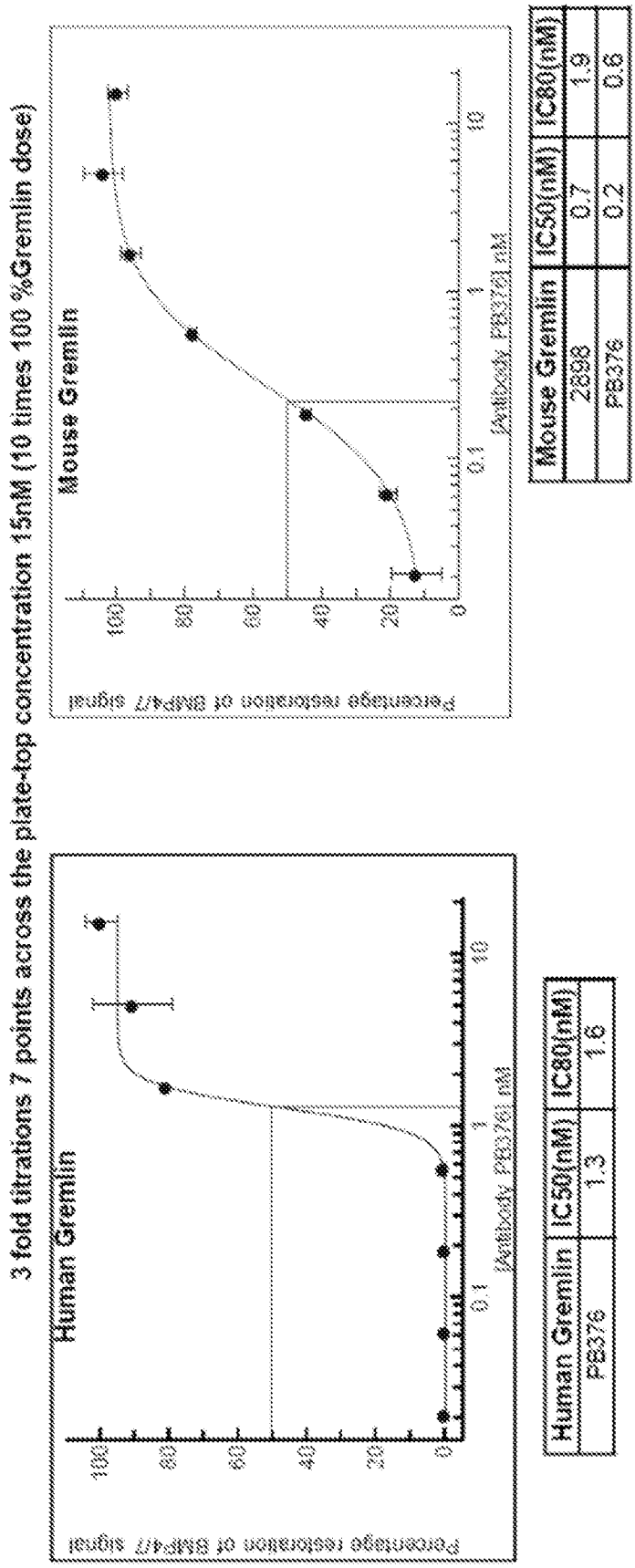


Figure 3



Human Gremlin	IC50(nM)	IC80(nM)
PB376	1.3	1.6

Mouse Gremlin	IC50(nM)	IC80(nM)
2898	0.7	1.9
PB376	0.2	0.6

Figure 4

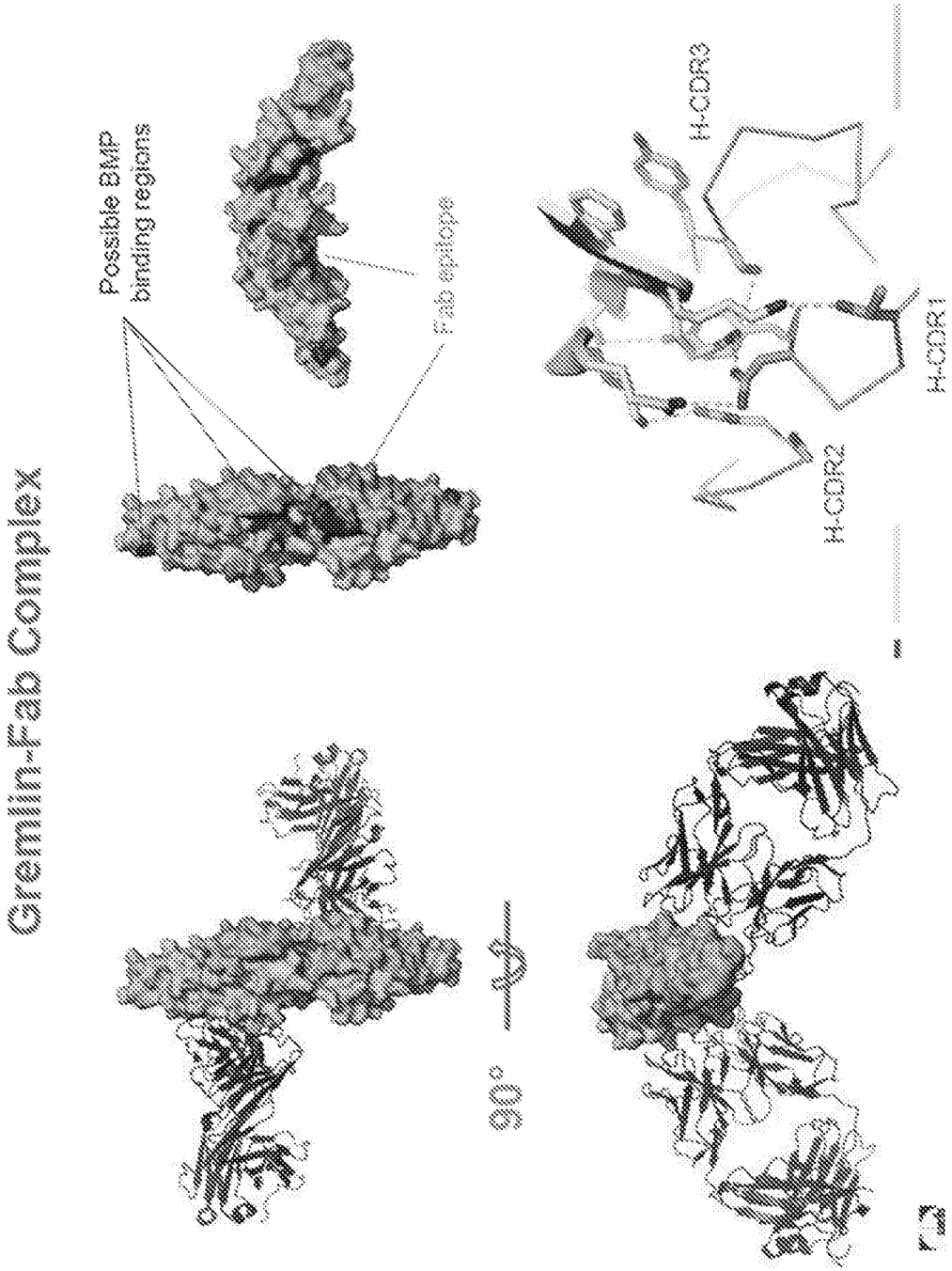

Gremlin-Fab Complex
Possible BMP binding regions
Fab epitope
H-CDR3
H-CDR2
H-CDR1
90°

Figure 5

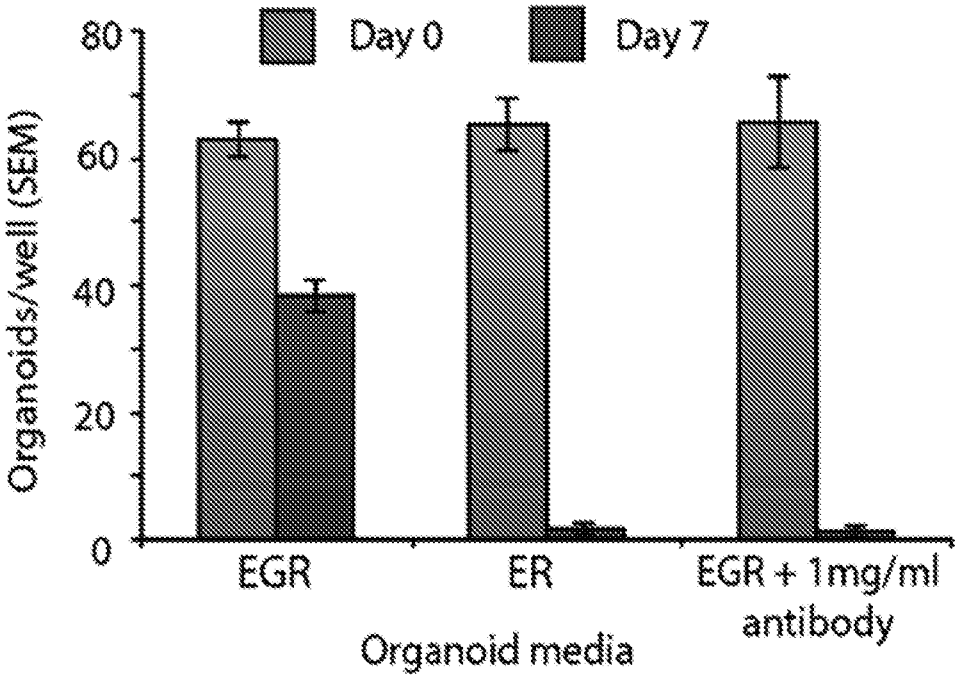


Figure 6

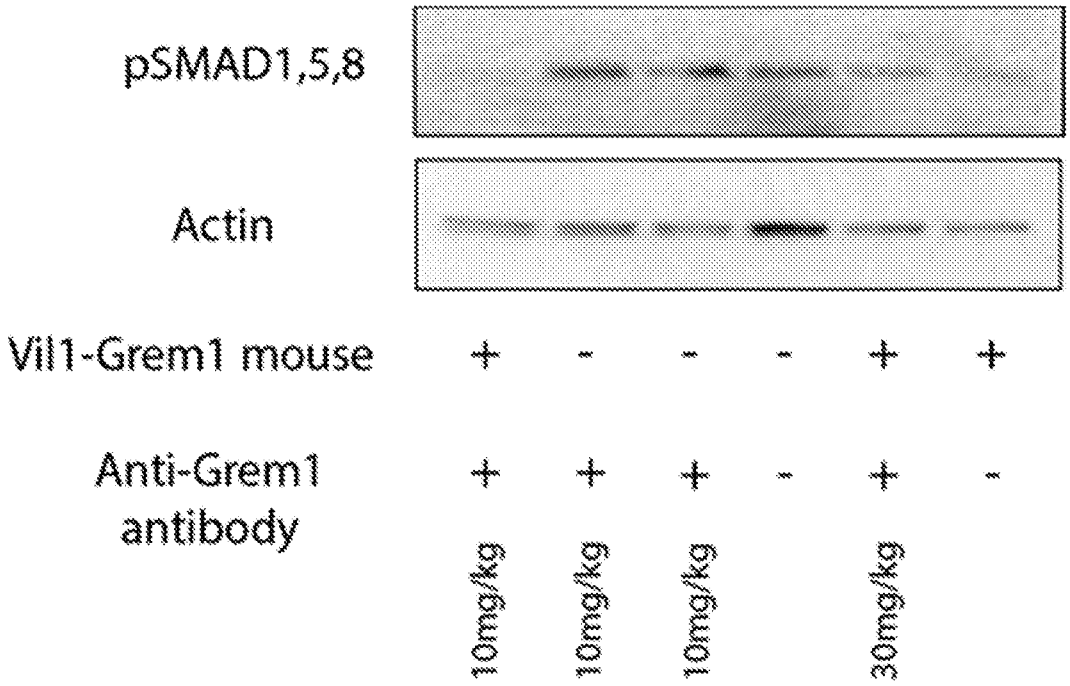


Figure 7

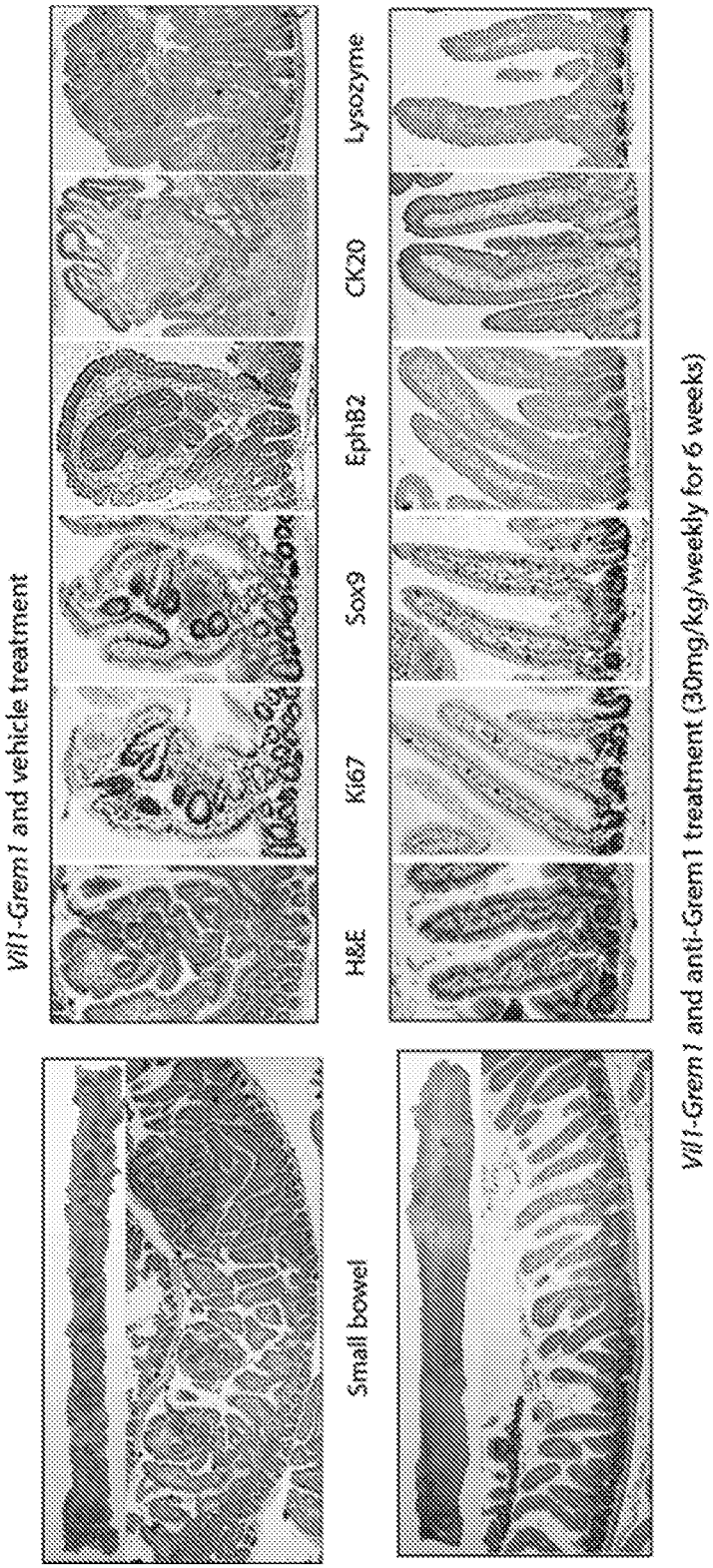

Vill-Grem1 and vehicle treatment
H&E
Ki67
Sox9
EphB2
CK20
Lysozyme
Small bowel
Vill-Grem1 and anti-Grem1 treatment (30mg/kg/weekly for 6 weeks)

Figure 8

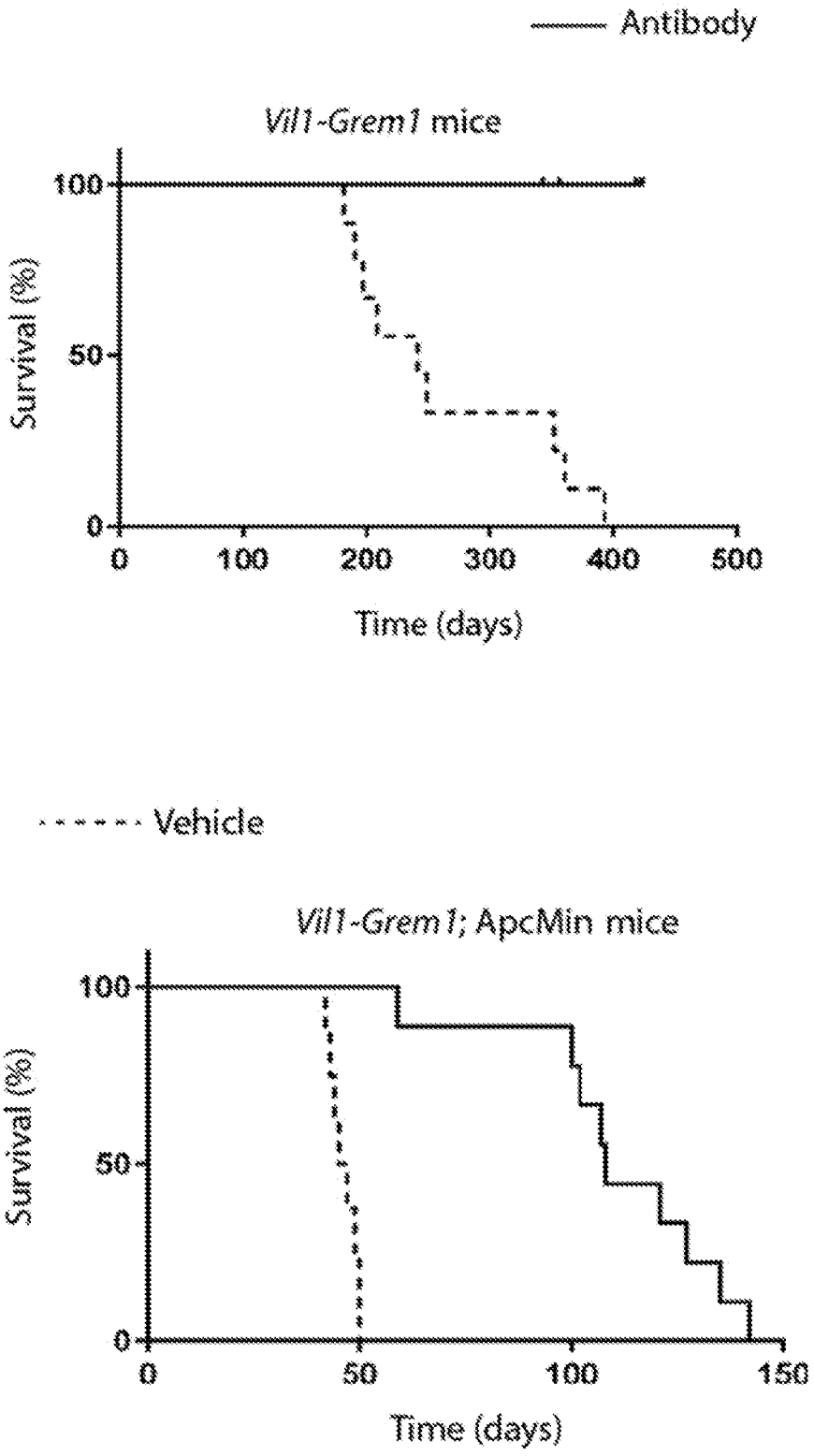

Antibody
Vil1-Grem1 mice
Survival (%)
100
50
0
0
100
200
300
400
500
Time (days)
Vehicle
Vil1-Grem1; ApcMin mice
Survival (%)
100
50
0
0
50
100
150
Time (days)

Figure 9

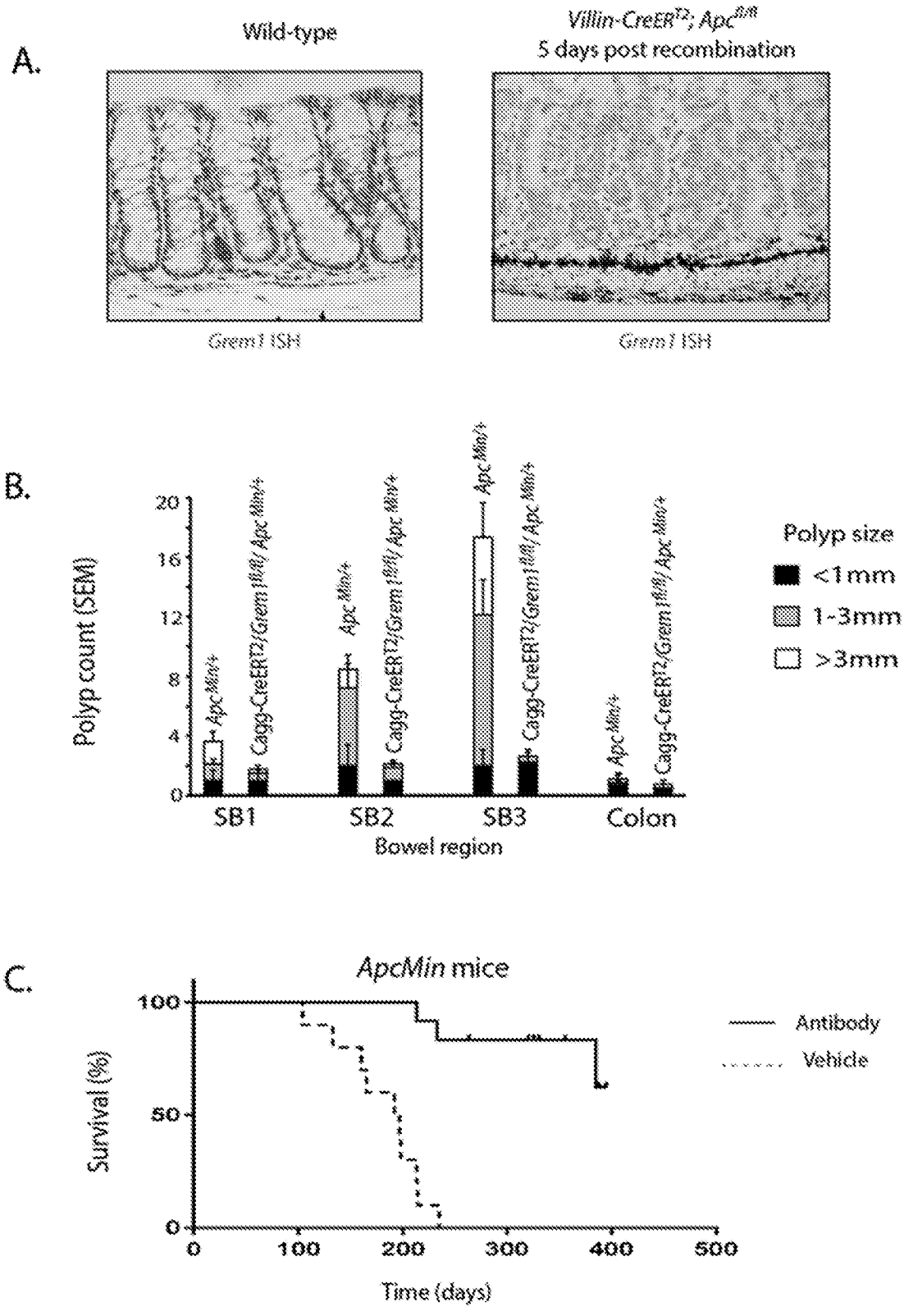

A.
Wild-type
Villin-CreER^{T2}; Apc$^{fl/fl}$
5 days post recombination
Grem1 ISH
Grem1 ISH
B.
Polyp count (SEM)
20
16
12
8
4
0
Apc$^{Min/+}$
Cagg-CreER^{T2}/Grem1$^{fl/fl}$/ Apc$^{Min/+}$
Apc$^{Min/+}$
Cagg-CreER^{T2}/Grem1$^{fl/fl}$/ Apc$^{Min/+}$
Apc$^{Min/+}$
Cagg-CreER^{T2}/Grem1$^{fl/fl}$/ Apc$^{Min/+}$
Apc$^{Min/+}$
Cagg-CreER^{T2}/Grem1$^{fl/fl}$/ Apc$^{Min/+}$
SB1
SB2
SB3
Colon
Bowel region
Polyp size
<1mm
1-3mm
>3mm
C.
ApcMin mice
Survival (%)
100
50
0
0
100
200
300
400
500
Time (days)
Antibody
Vehicle

Figure 10

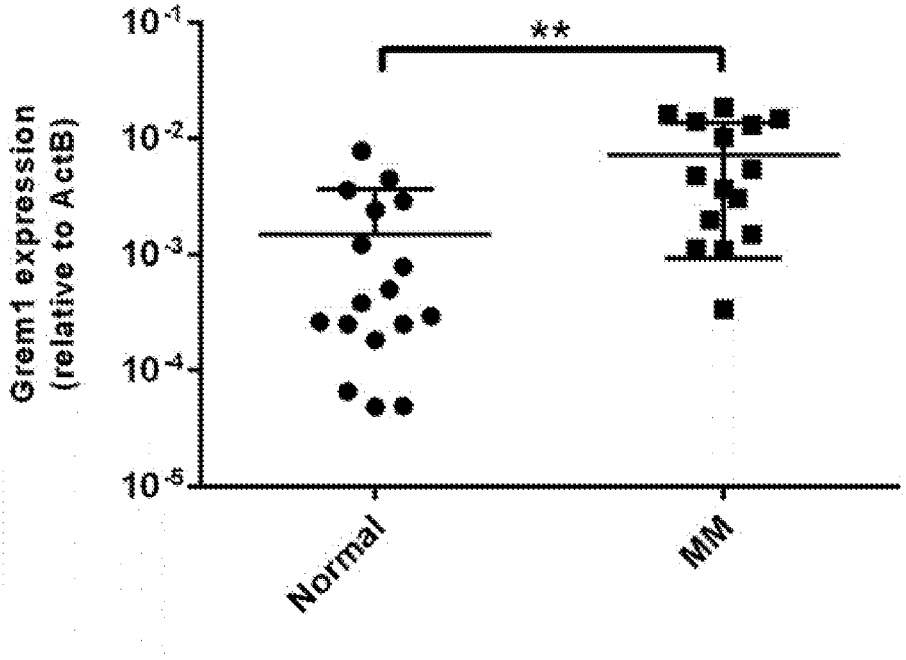
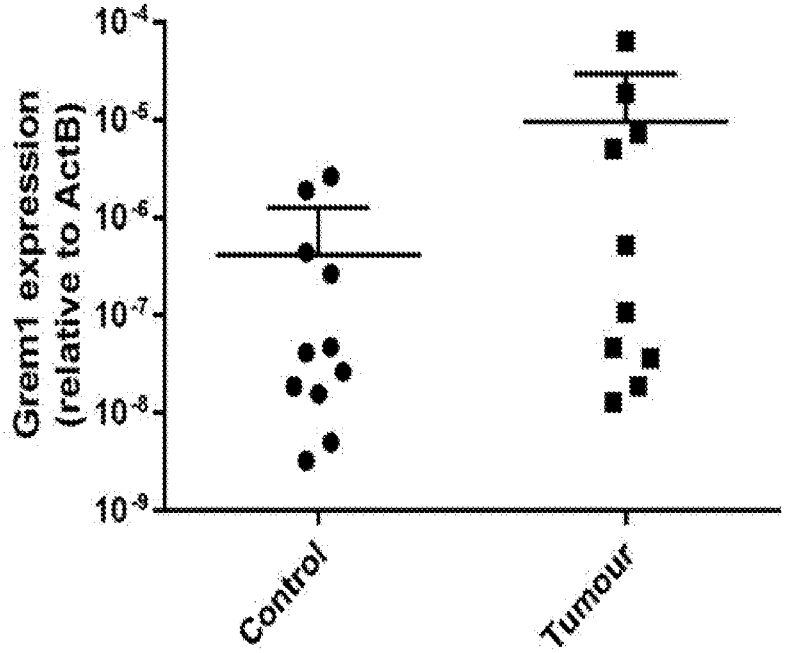

Grem1 expression
(relative to ActB)
10-1
10-2
10-3
10-4
10-5
**
Normal
MM

Figure 11

A


Grem1 expression
(relative to ActB)
10-4
10-5
10-6
10-7
10-8
10-9
Control
Tumour

B

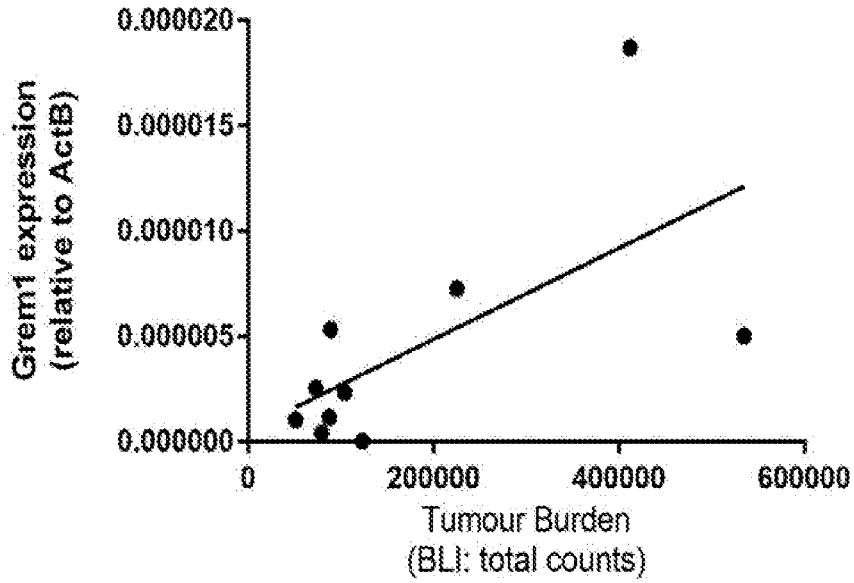
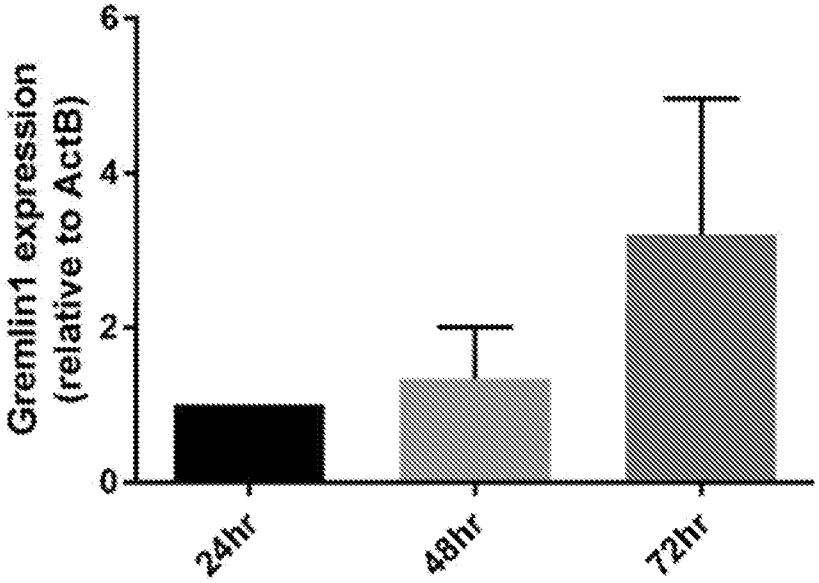

Grem1 expression
(relative to ActB)
0.000020
0.000015
0.000010
0.000005
0.000000
0
200000
400000
600000
Tumour Burden
(BLI: total counts)

Figure 12

A **Non-contact**



B **Contact**

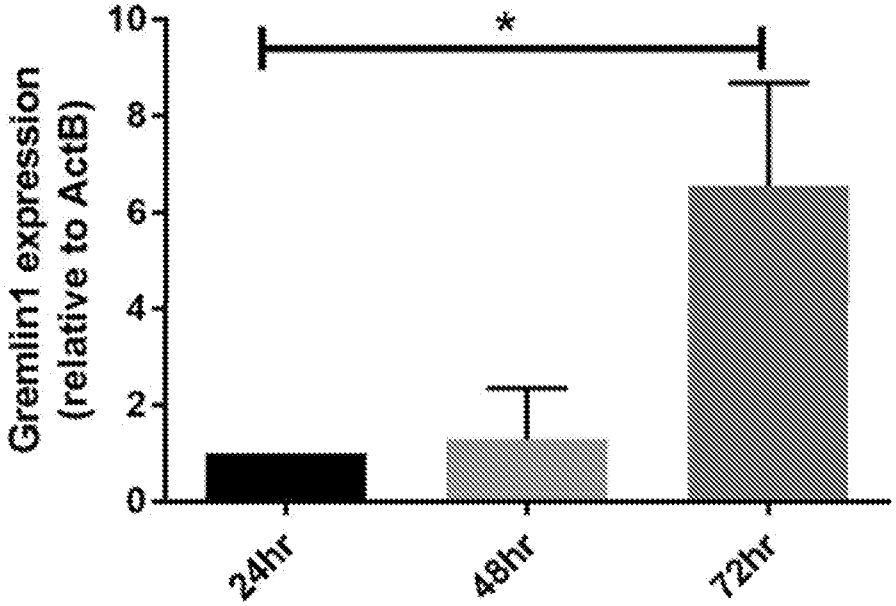


Figure 13

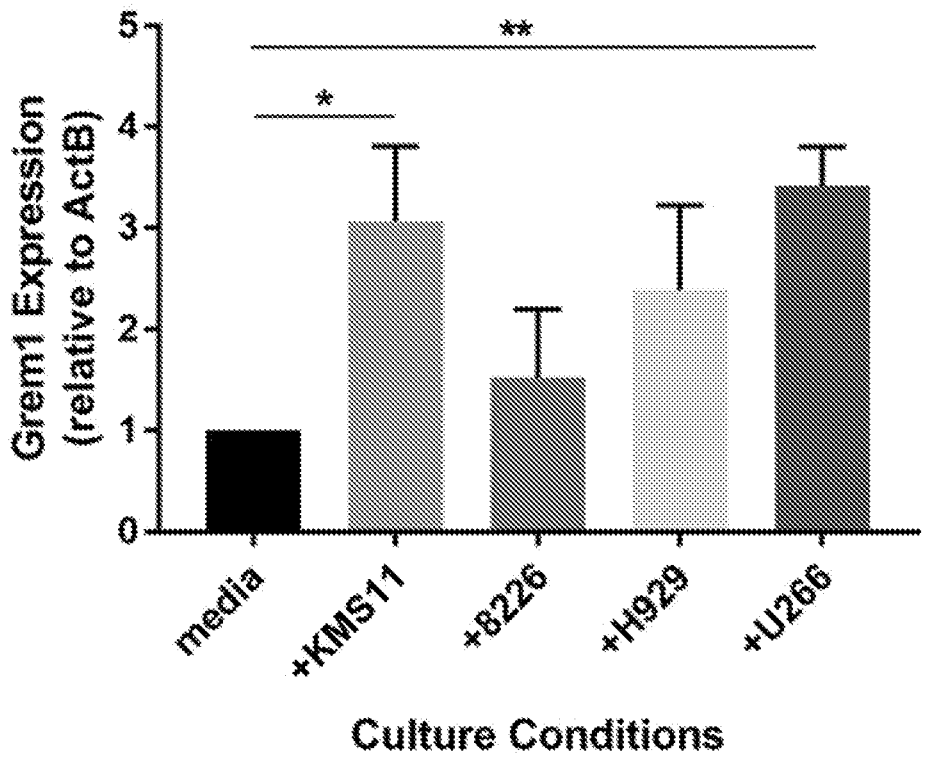

Grem1 Expression
(relative to ActB)
0
1
2
3
4
5
*
**
media
+KMS11
+8226
+H929
+U266
Culture Conditions

Figure 14

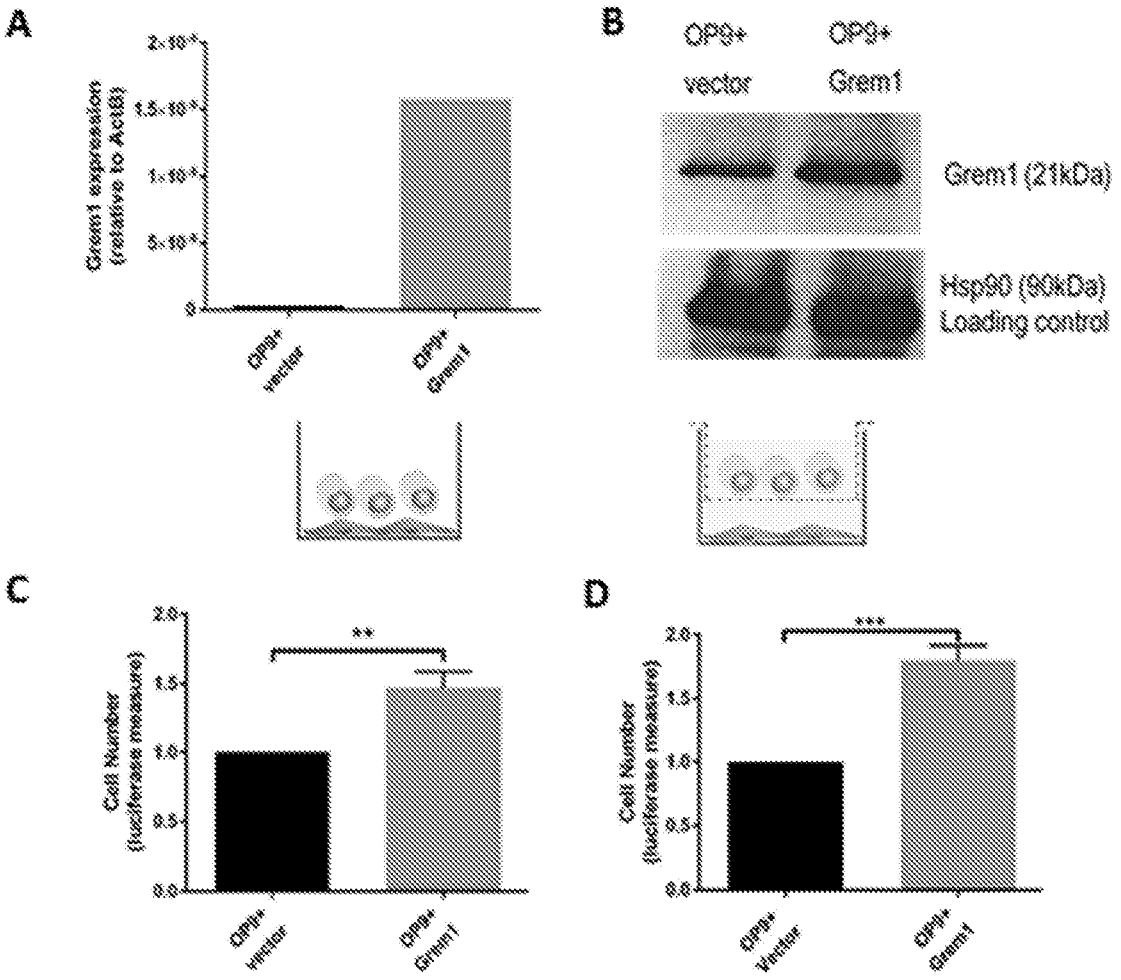

A
Grem1 expression (relative to Actb)
OP9+ vector
OP9+ Grem1
B
OP9+ vector
OP9+ Grem1
Grem1 (21kDa)
Hsp90 (90kDa) Loading control
C
Cell Number (luciferase measure)
**
OP9+ vector
OP9+ Grem1
D
Cell Number (luciferase measure)
***
OP9+ Vector
OP9+ Grem1

Figure 15

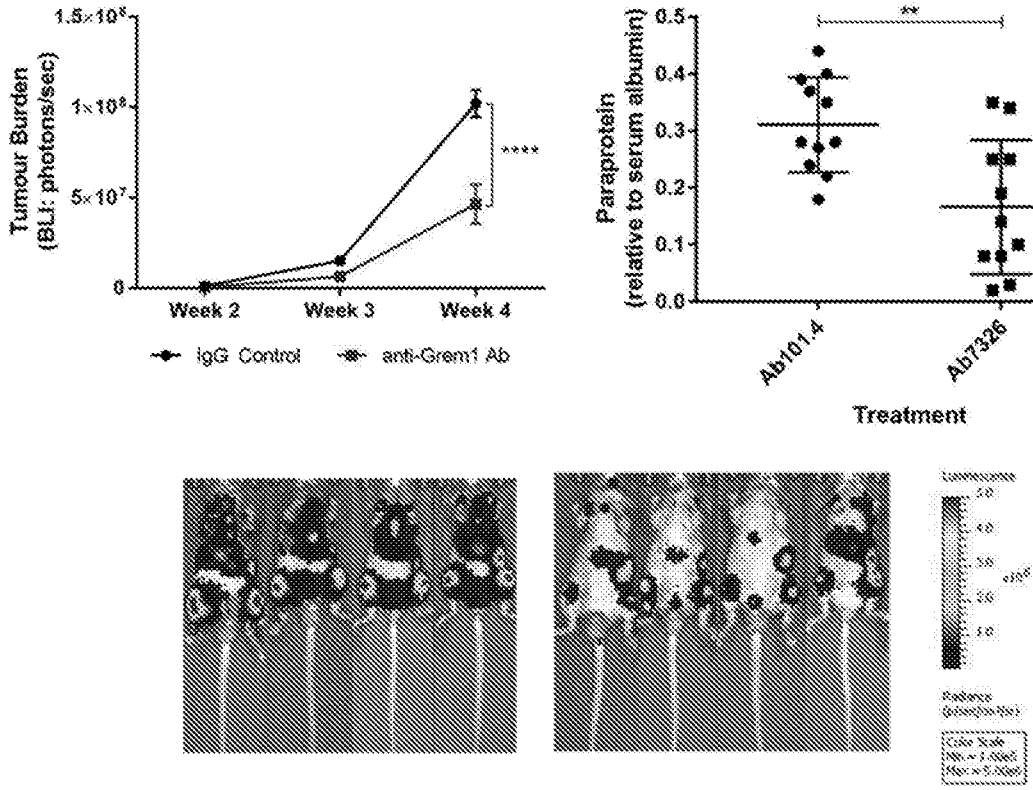

Tumour Burden
(BLI: photons/sec)
1.5×10⁸
1×10⁸
5×10⁷
0
Week 2
Week 3
Week 4
****
IgG Control
anti-Grem1 Ab
Paraprotein
(relative to serum albumin)
0.5
0.4
0.3
0.2
0.1
0.0
**
Ab101.4
Ab7326
Treatment

Figure 16

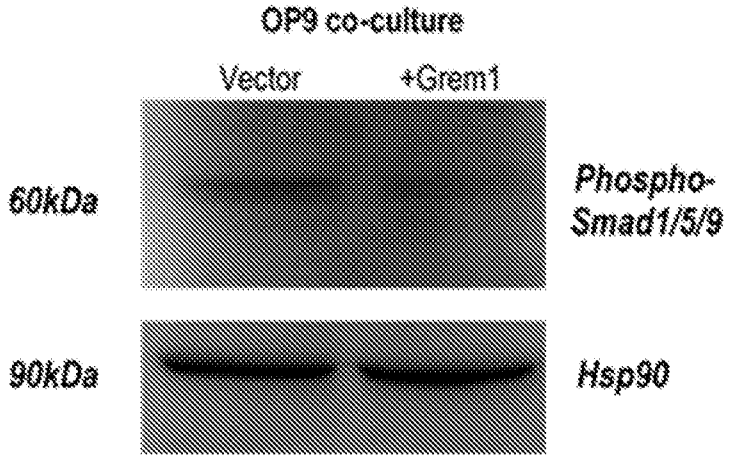


Figure 17

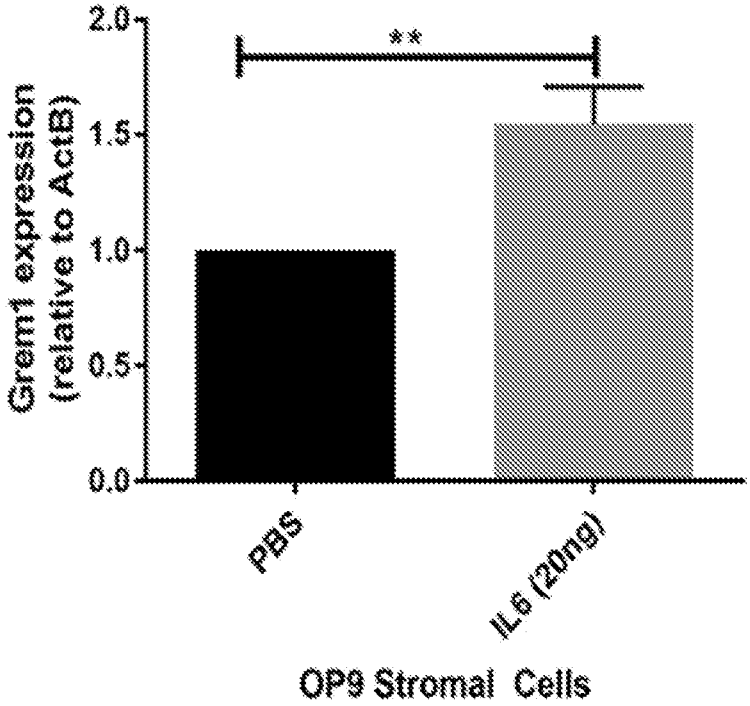


Figure 18

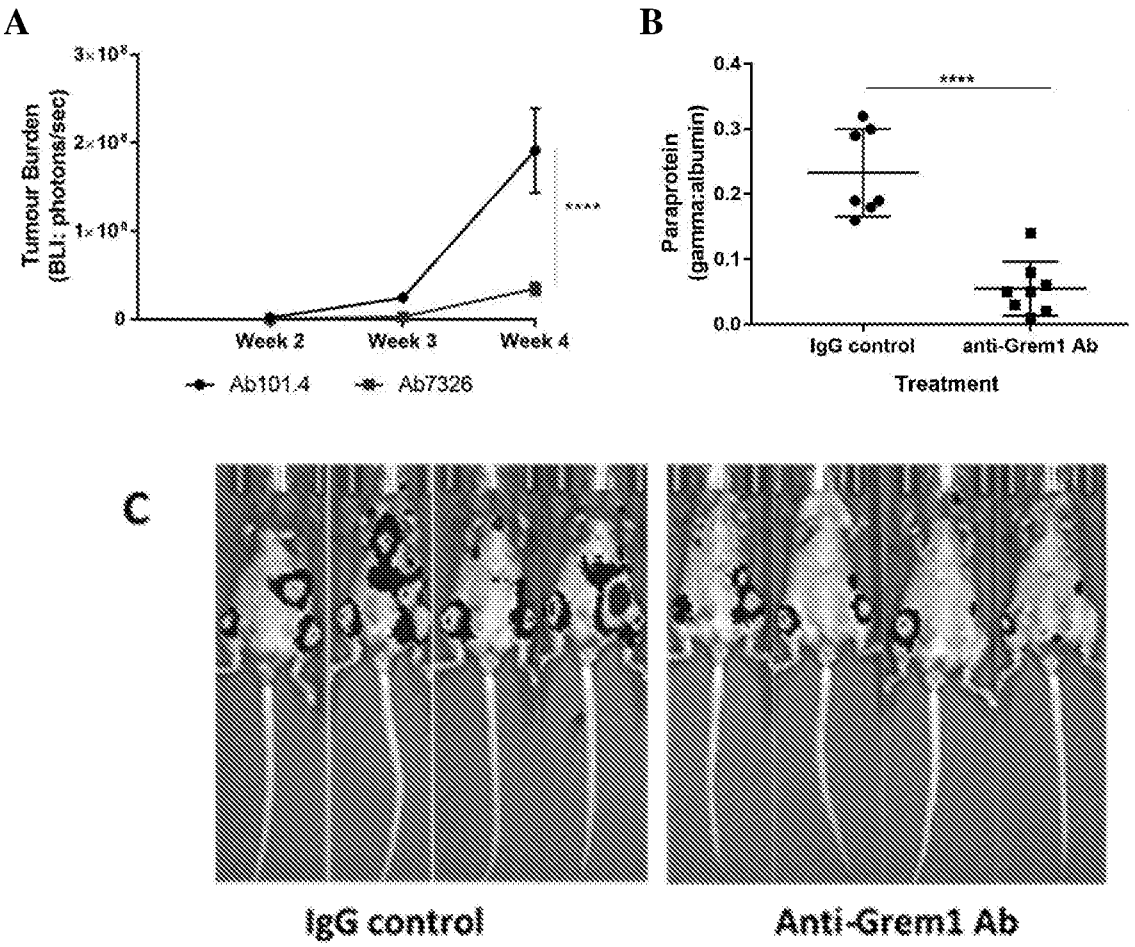

A
Tumour Burden (BLI: photons/sec)
3×10⁸
2×10⁸
1×10⁸
0
Week 2
Week 3
Week 4
****
Ab101.4
Ab7326
B
Paraprotein (gamma:albumin)
0.4
0.3
0.2
0.1
0.0
****
IgG control
anti-Grem1 Ab
Treatment
C
IgG control
Anti-Grem1 Ab

Figure 19

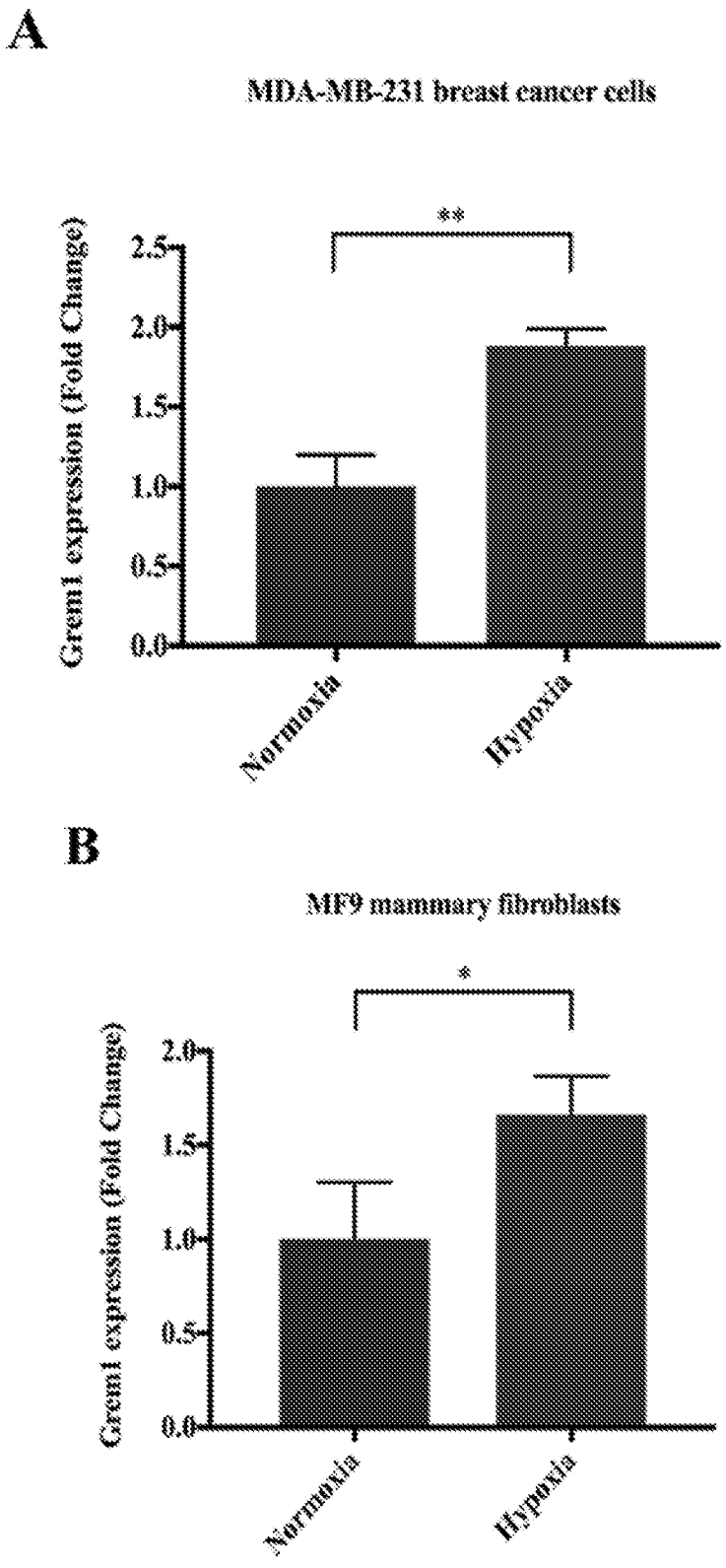

A
MDA-MB-231 breast cancer cells
**
Grem1 expression (Fold Change)
2.5
2.0
1.5
1.0
0.5
0.0
Normoxia
Hypoxia
B
MF9 mammary fibroblasts
*
Grem1 expression (Fold Change)
2.0
1.5
1.0
0.5
0.0
Normoxia
Hypoxia

Figure 20

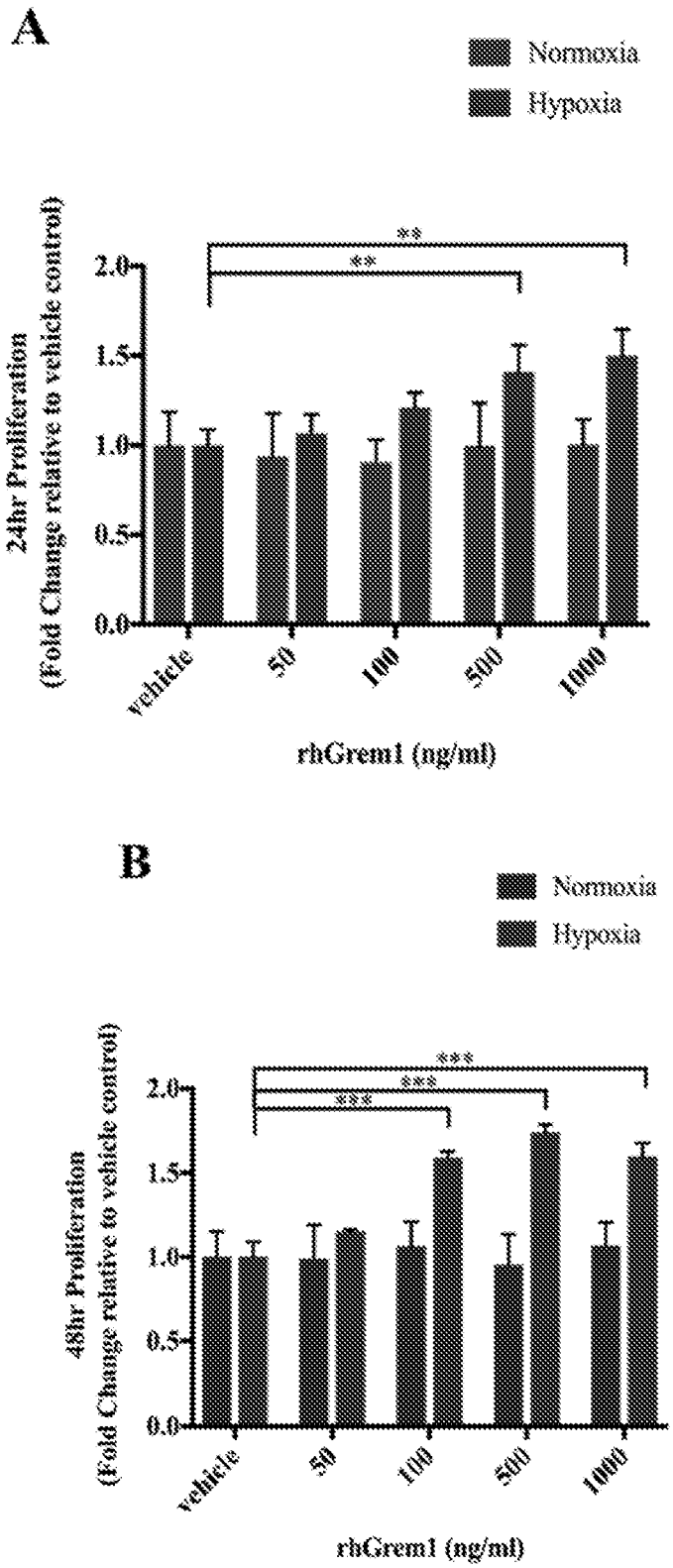

A
Normoxia
Hypoxia
24hr Proliferation
(Fold Change relative to vehicle control)
2.0
1.5
1.0
0.5
0.0
**
**
vehicle
50
100
500
1000
rhGrem1 (ng/ml)
B
Normoxia
Hypoxia
48hr Proliferation
(Fold Change relative to vehicle control)
2.0
1.5
1.0
0.5
0.0
***
***
***
vehicle
50
100
500
1000
rhGrem1 (ng/ml)

Figure 21

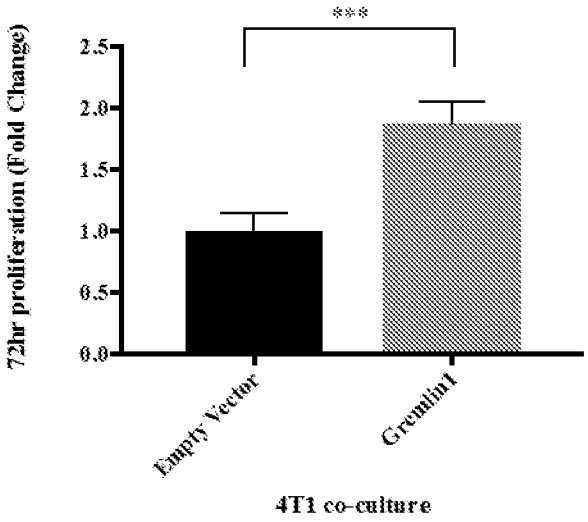

***
72hr proliferation (Fold Change)
2.5
2.0
1.5
1.0
0.5
0.0
Empty Vector
Gremlin1
4T1 co-culture

Figure 22

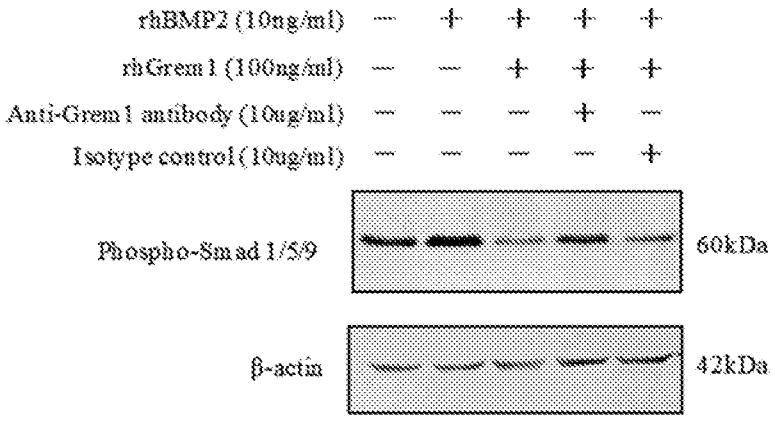


Figure 23

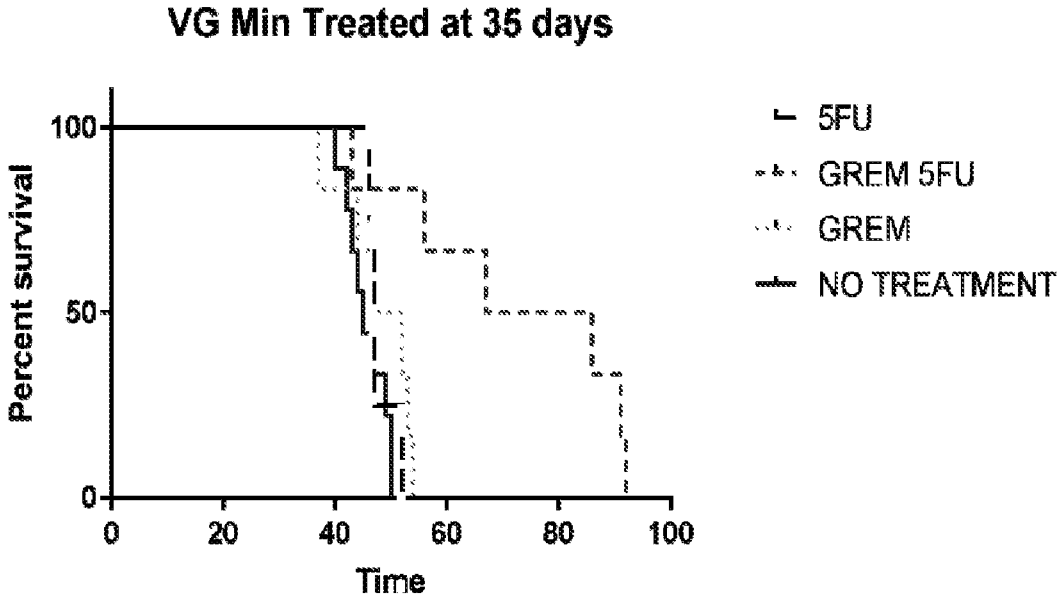


Figure 24

N = Normal tissue expression (where available)
T = Tumour expression

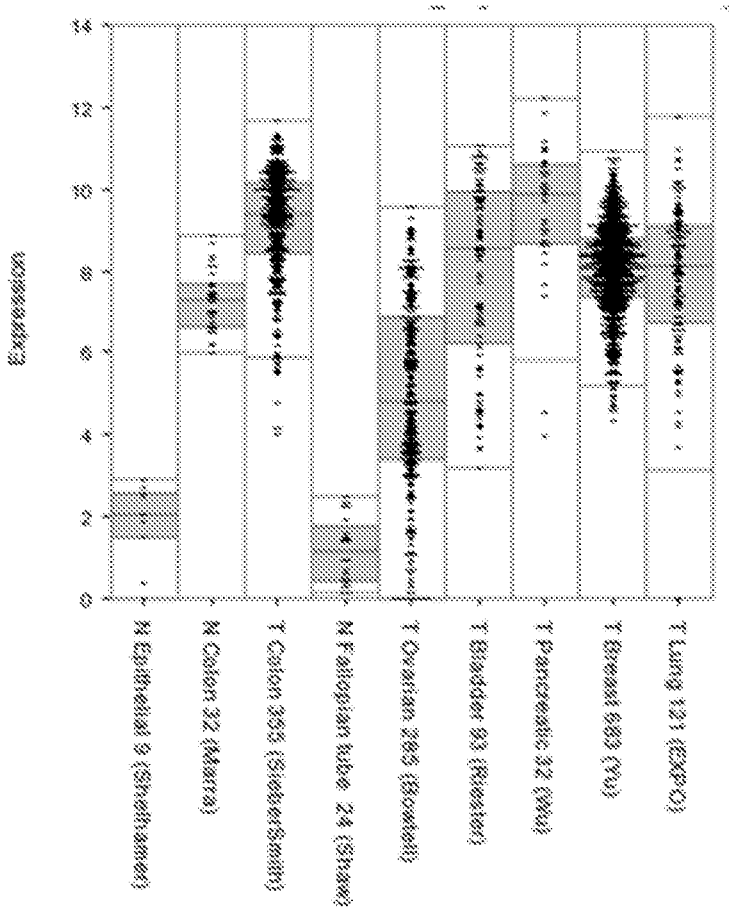


Figure 25

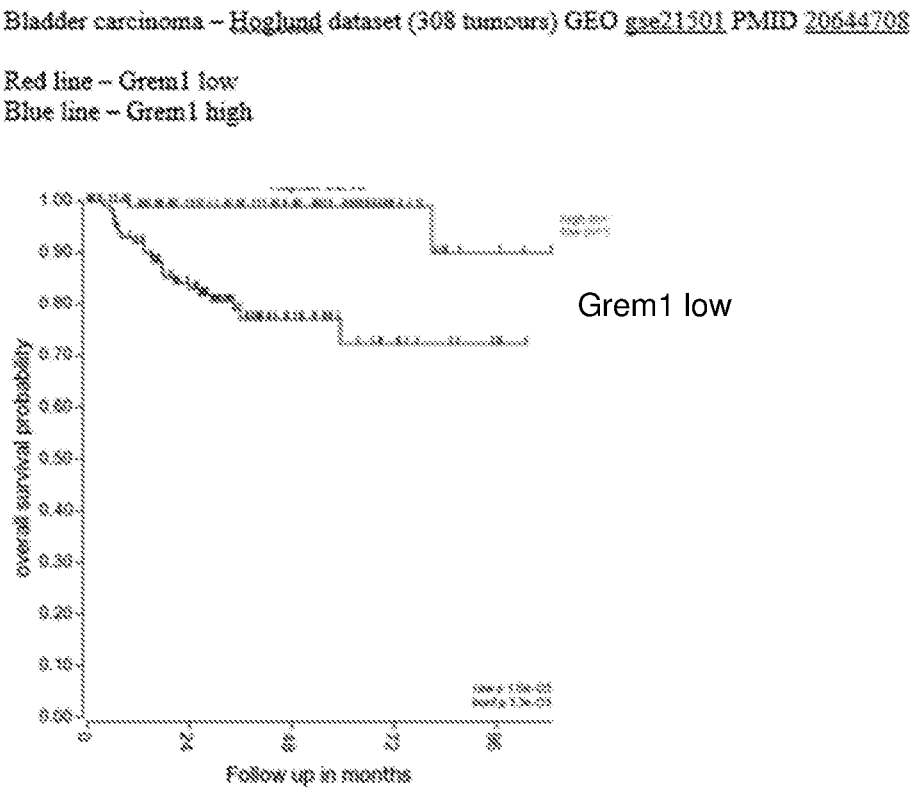

Bladder carcinoma – Hoglund dataset (308 tumours) GEO gse31301 PMID 20644708
Red line – Grem1 low
Blue line – Grem1 high
Grem1 low
overall survival probability
Follow up in months

Figure 26

Pancreatic Ductal Adenocarcinoma – Yeh dataset (132 tumours) GEO gse21501, PMID 20644708

Red line – Grem1 low
Blue line – Grem1 high

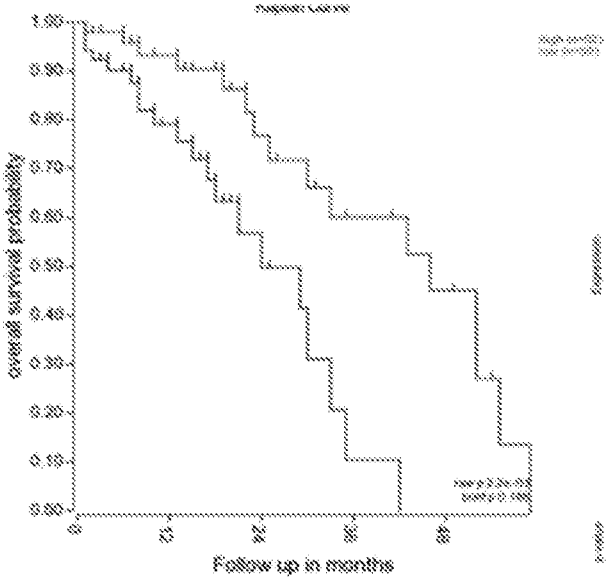


Figure 27

Ovarian adenocarcinoma – Mehta-Gringoriou dataset (107 tumours) GEO GSE26193, PMID 22101763

Red line – Grem1 low
Blue line – Grem1 high

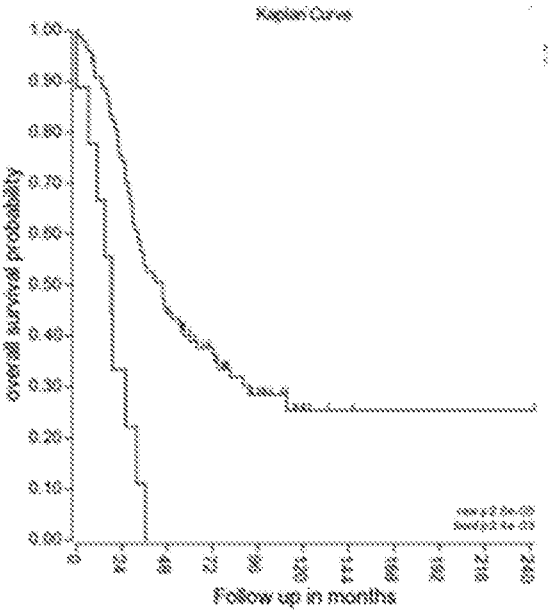


Figure 28

Basal breast carcinoma – Bertucci dataset (266 tumours). GEO gse21653. PMID 20490615

Red line – Grem1 low
Blue line – Grem1 high

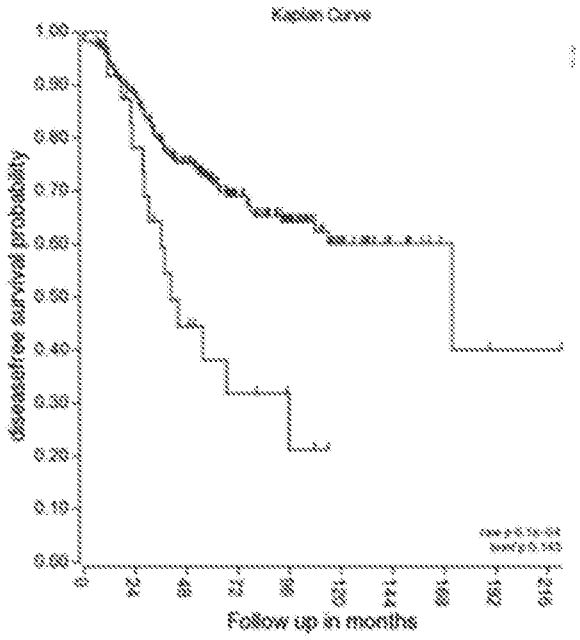


Figure 29

Lung carcinoma – Bild data set (114 tumours) GEO GSE3141 PMID 16273092

Red line – Grem1 low
Blue line – Grem1 high

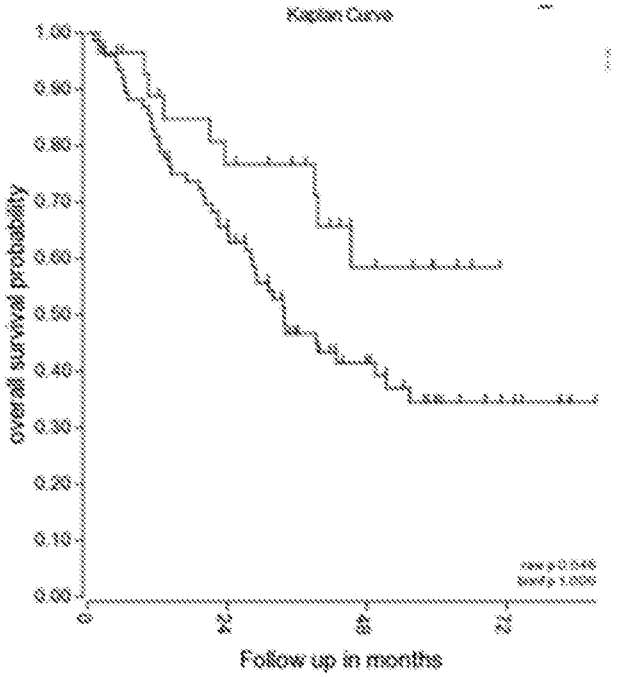


Figure 30

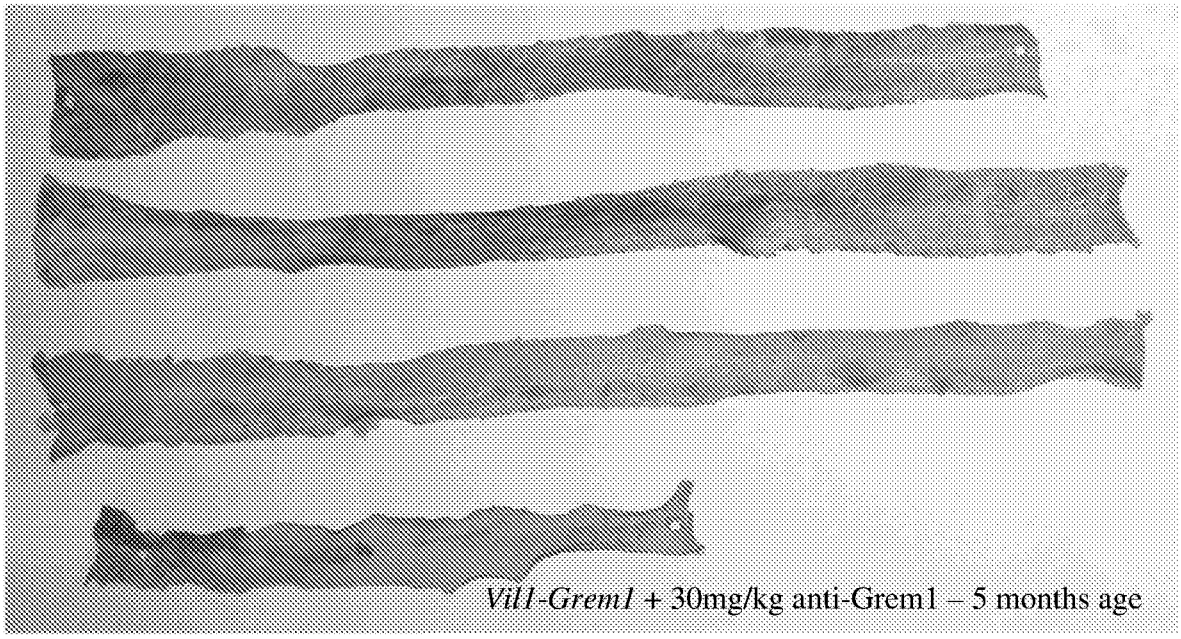
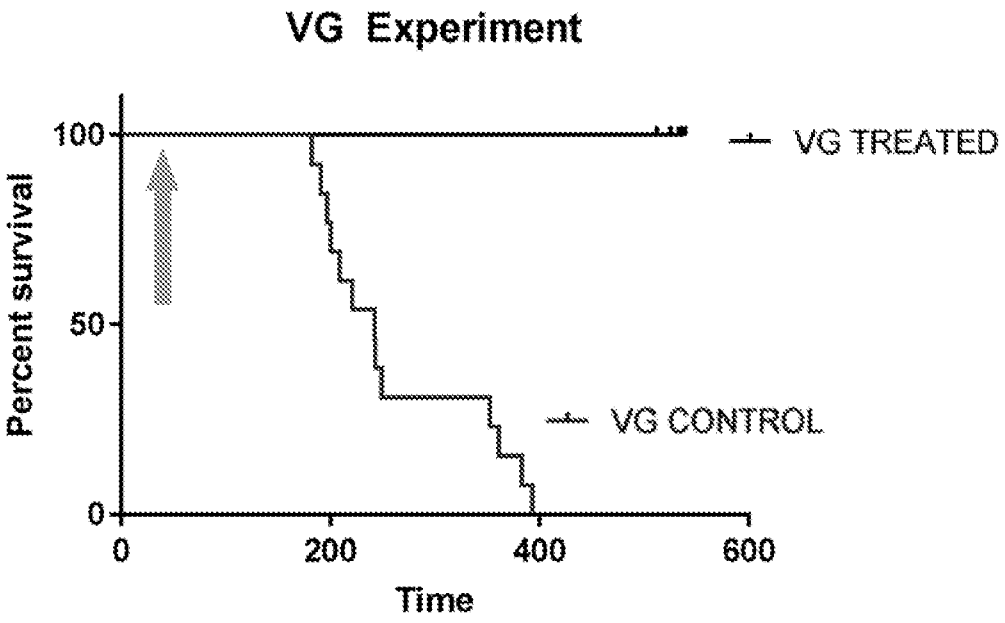


Figure 31

A


VG Experiment
Percent survival
100
50
0
0
200
400
600
Time
VG TREATED
VG CONTROL

B

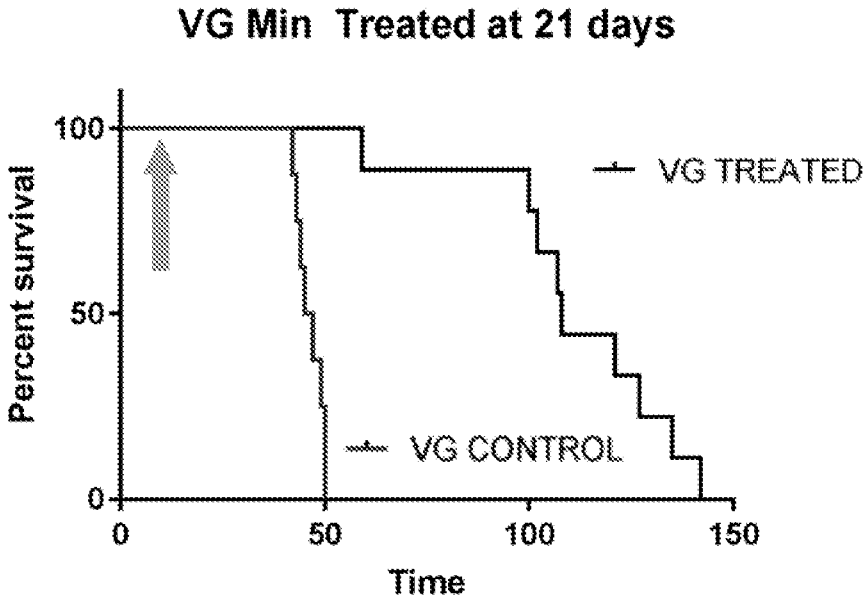
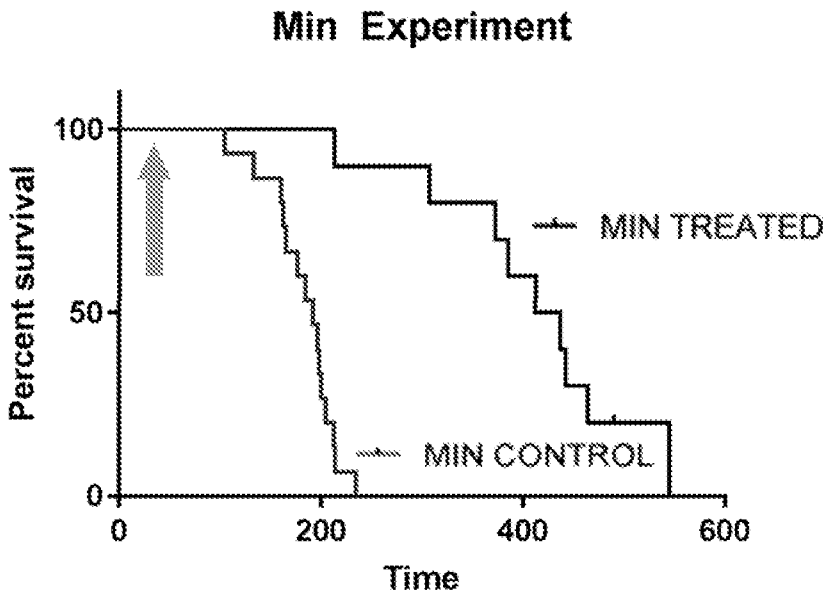

VG Min Treated at 21 days
Percent survival
100
50
0
0
50
100
150
Time
VG TREATED
VG CONTROL

Figure 32

A


Min Experiment
Percent survival
100
50
0
0
200
400
600
Time
MIN TREATED
MIN CONTROL

B

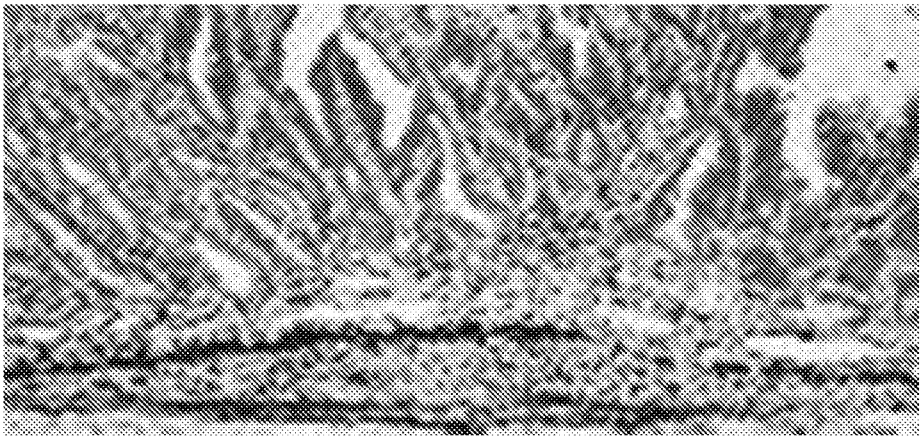


Figure 33

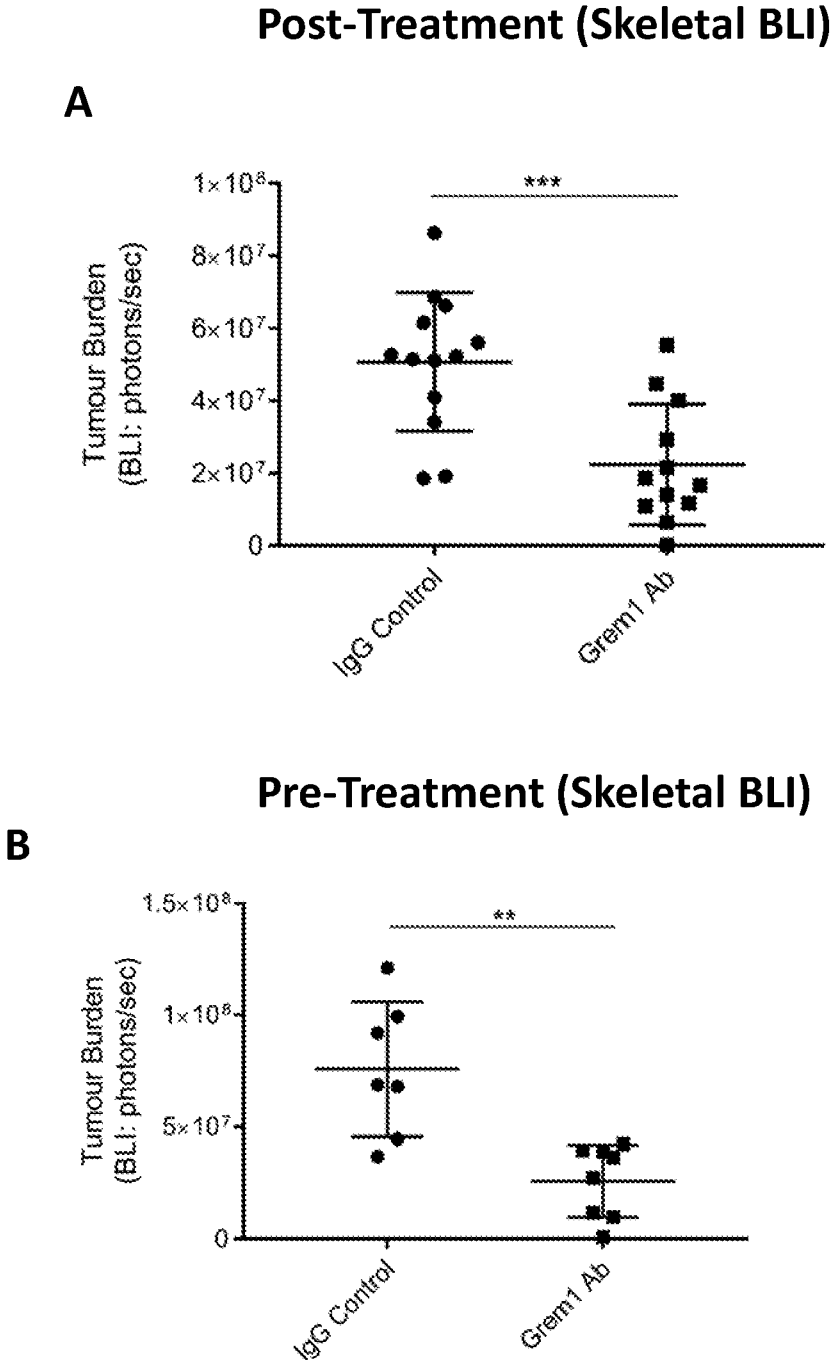
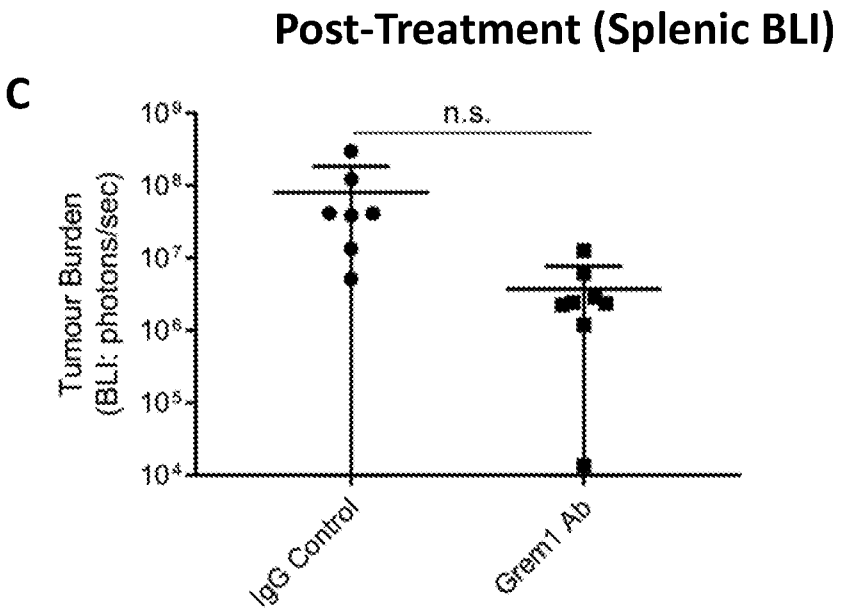

Post-Treatment (Skeletal BLI)
A
Tumour Burden (BLI: photons/sec)
1×10⁸
8×10⁷
6×10⁷
4×10⁷
2×10⁷
0
***
IgG Control
Grem1 Ab
Pre-Treatment (Skeletal BLI)
B
Tumour Burden (BLI: photons/sec)
1.5×10⁸
1×10⁸
5×10⁷
0
**
IgG Control
Grem1 Ab

Figure 33 cont'd



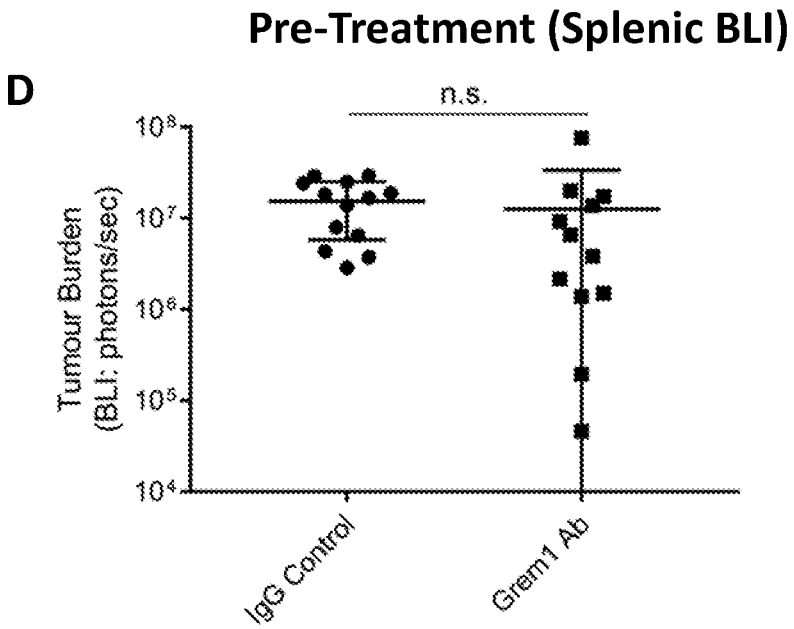
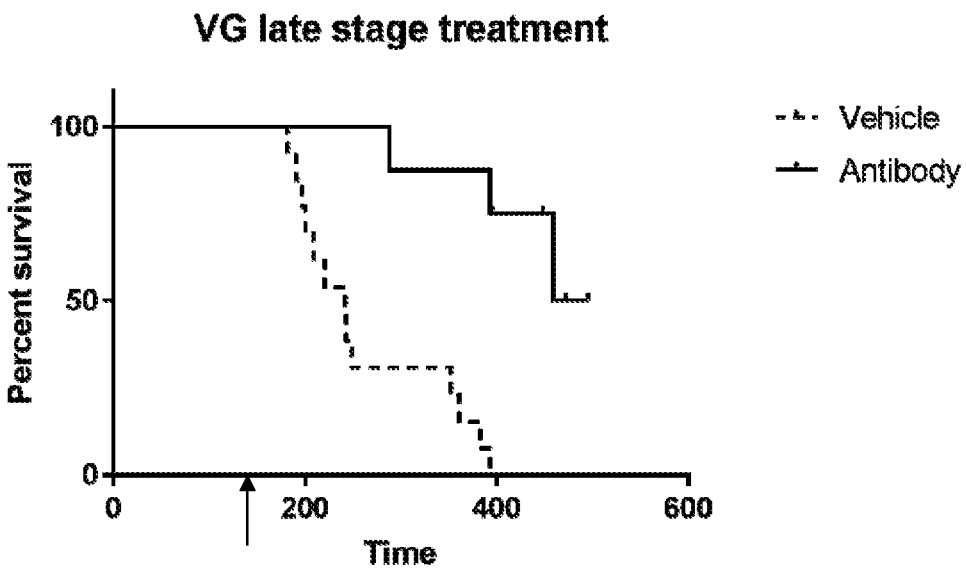


Figure 34

A


VG late stage treatment
Percent survival
100
50
0
0
200
400
600
Time
Vehicle
Antibody

B

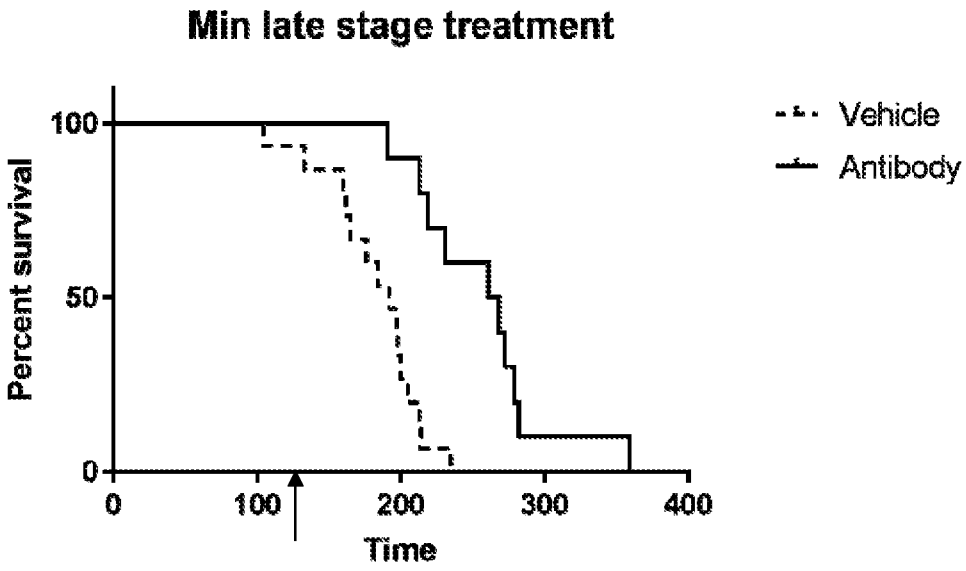
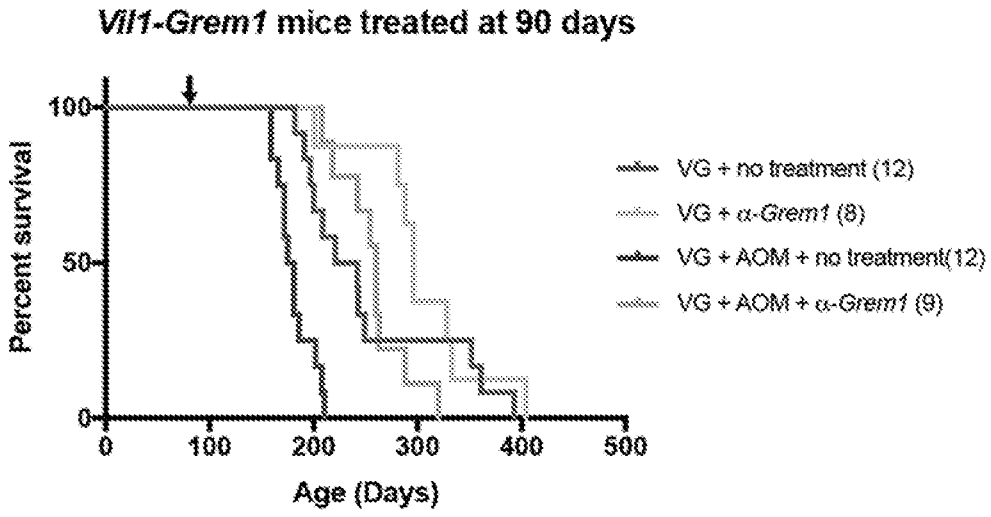
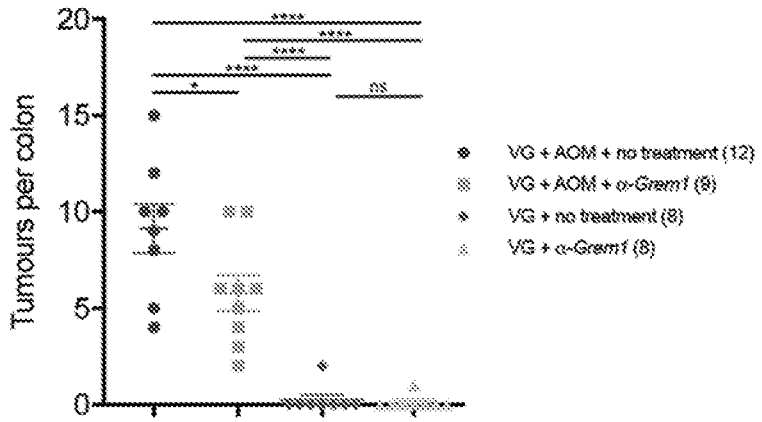

Min late stage treatment
Percent survival
100
50
0
0
100
200
300
400
Time
Vehicle
Antibody

Figure 35

A.



B.



C.

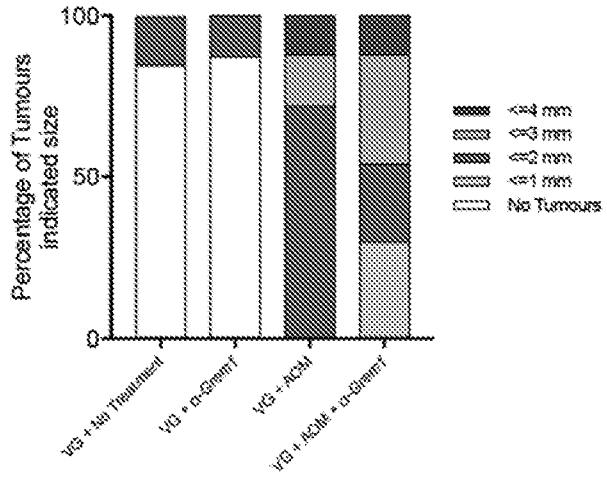


Figure 36

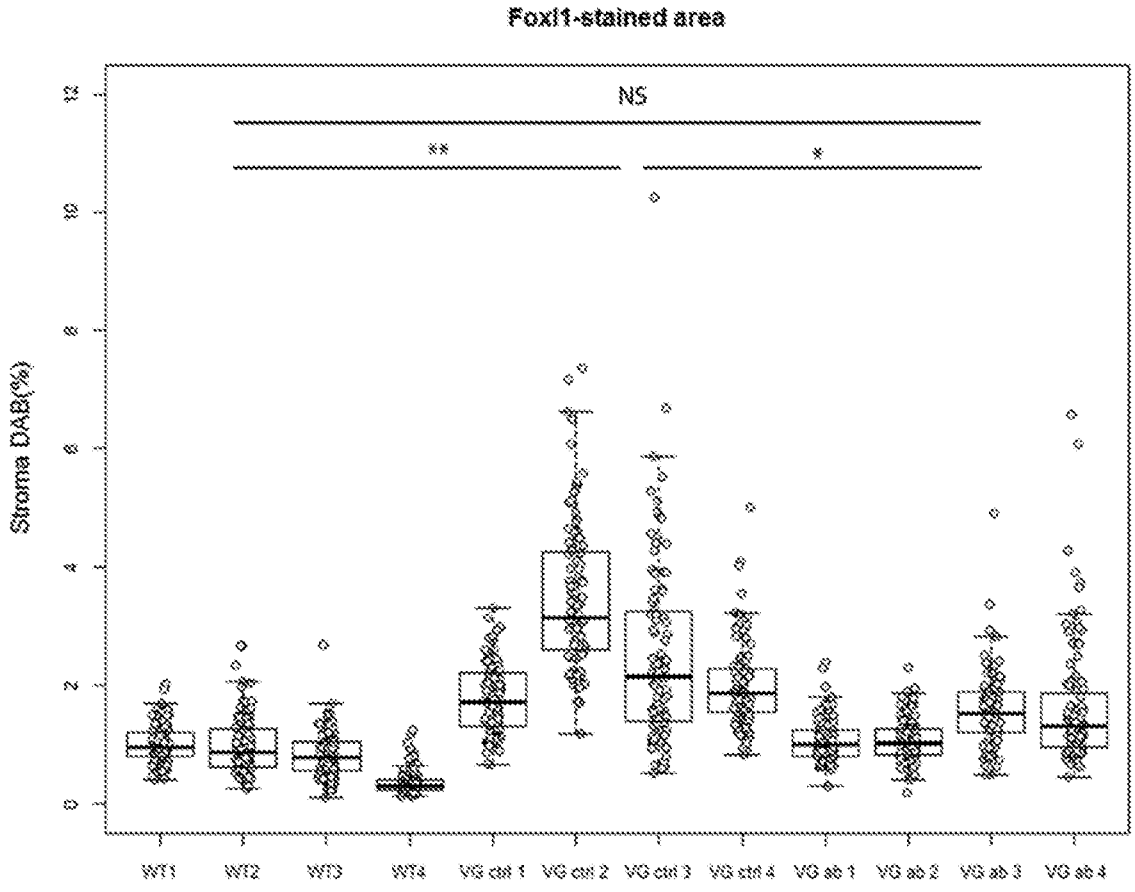

Foxl1-stained area
NS
**
*
Stroma DAB(%)
0
2
4
6
8
10
12
WT1
WT2
WT3
WT4
VG ctrl 1
VG ctrl 2
VG ctrl 3
VG ctrl 4
VG ab 1
VG ab 2
VG ab 3
VG ab 4

Figure 37

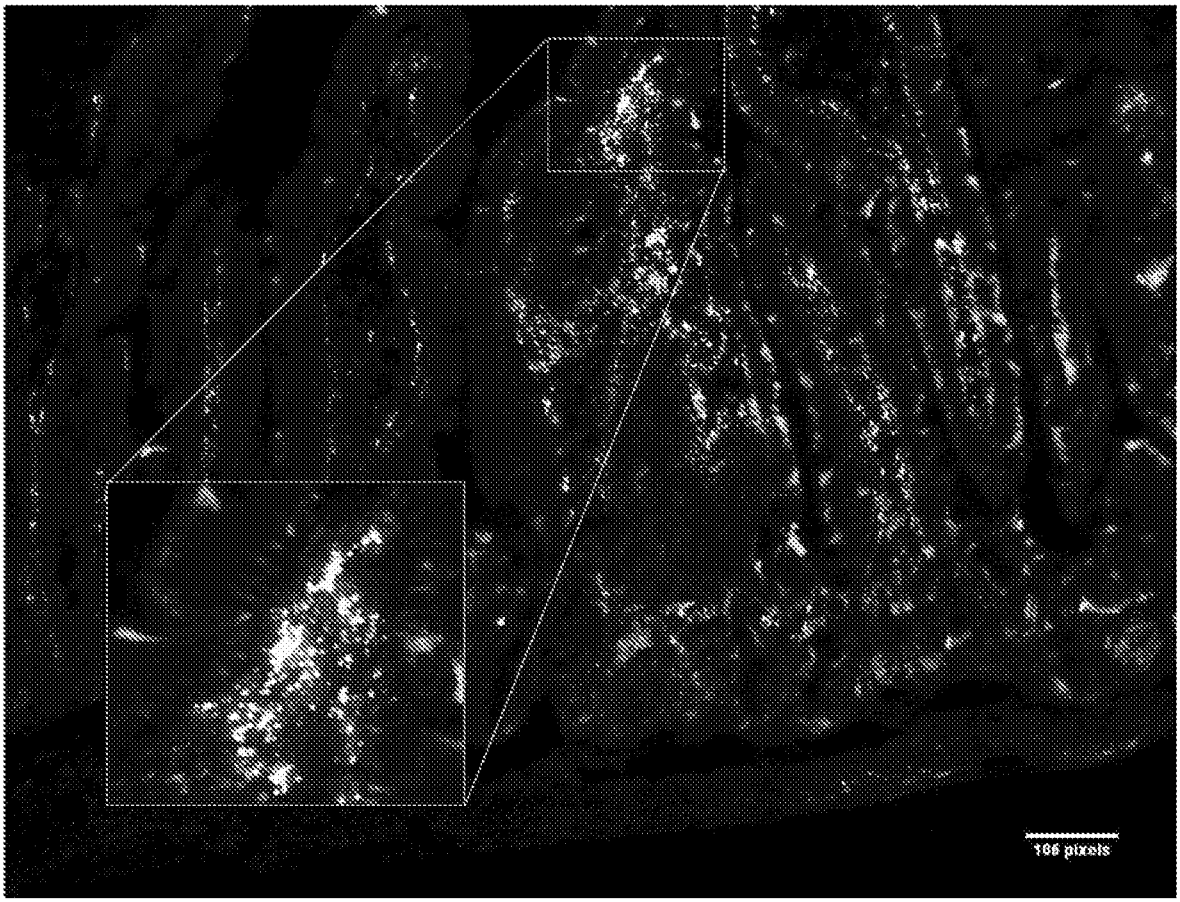


Figure 38

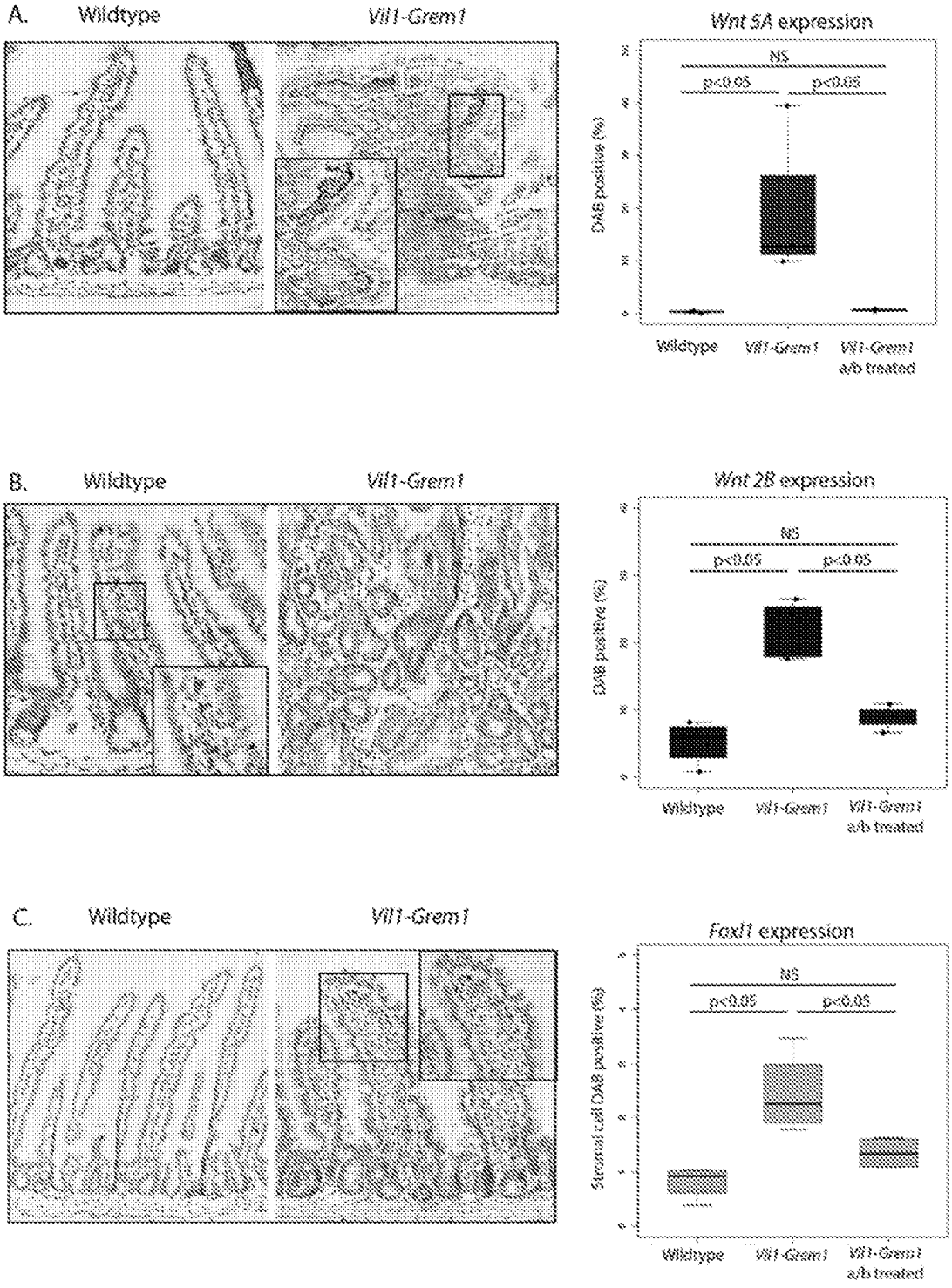

A.
Wildtype
Vil1-Grem1
Wnt 5A expression
NS
p<0.05
p<0.05
DAB positive (%)
Wildtype
Vil1-Grem1
Vil1-Grem1 a/b treated
B.
Wildtype
Vil1-Grem1
Wnt 2B expression
NS
p<0.05
p<0.05
DAB positive (%)
Wildtype
Vil1-Grem1
Vil1-Grem1 a/b treated
C.
Wildtype
Vil1-Grem1
Foxl1 expression
NS
p<0.05
p<0.05
Stromal cell DAB positive (%)
Wildtype
Vil1-Grem1
Vil1-Grem1 a/b treated

Figure 39

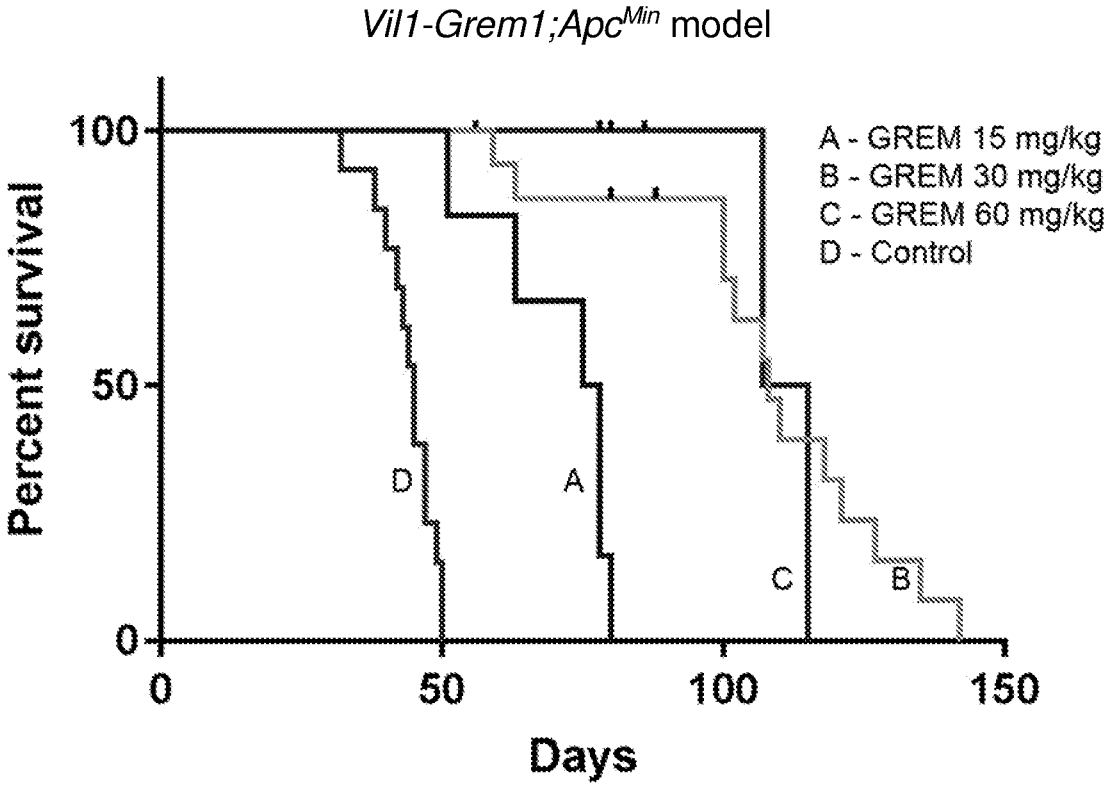

Vil1-Grem1;Apc^{Min} model
Percent survival
100
50
0
0
50
100
150
Days
A - GREM 15 mg/kg
B - GREM 30 mg/kg
C - GREM 60 mg/kg
D - Control
D
A
C
B

Figure 40

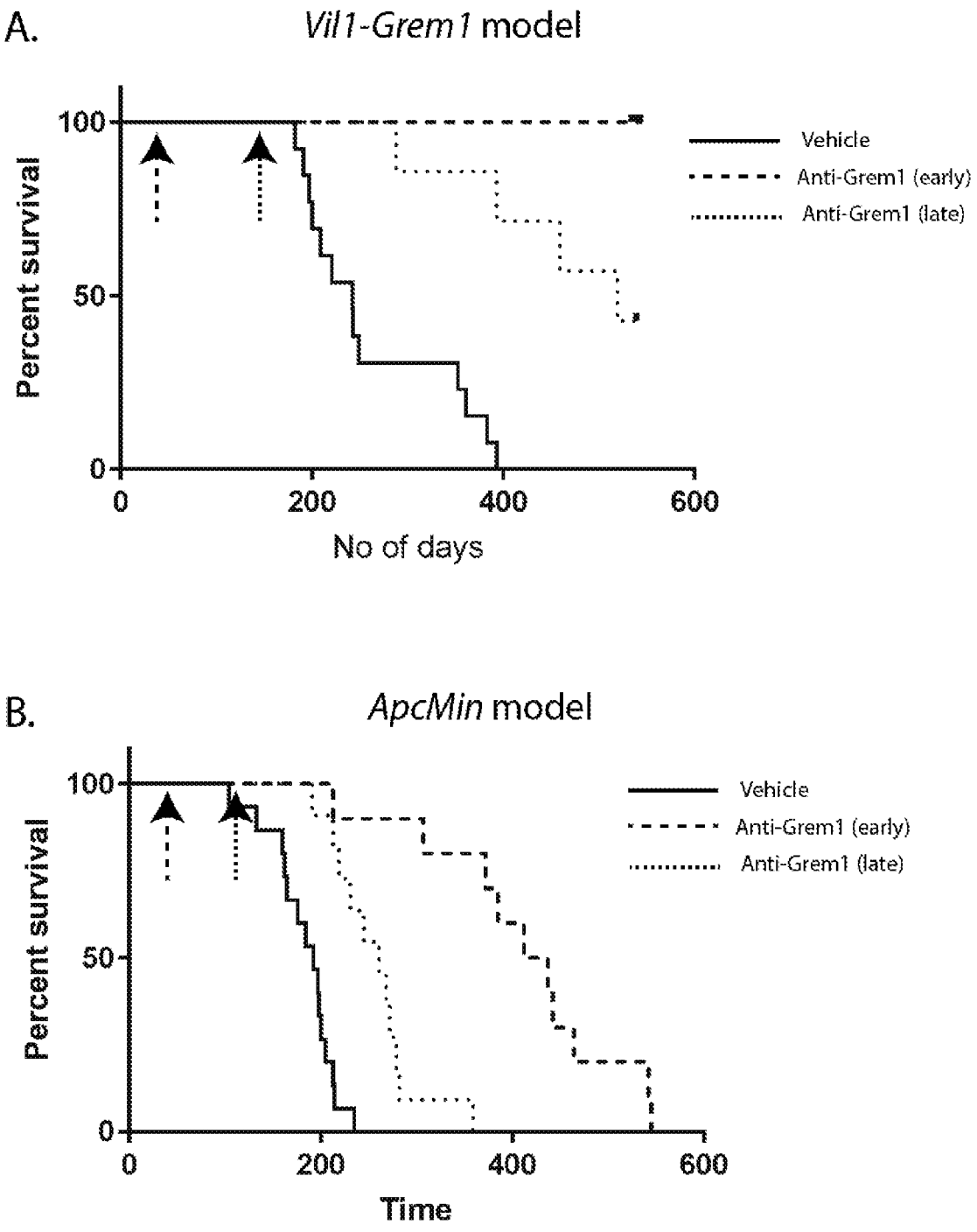
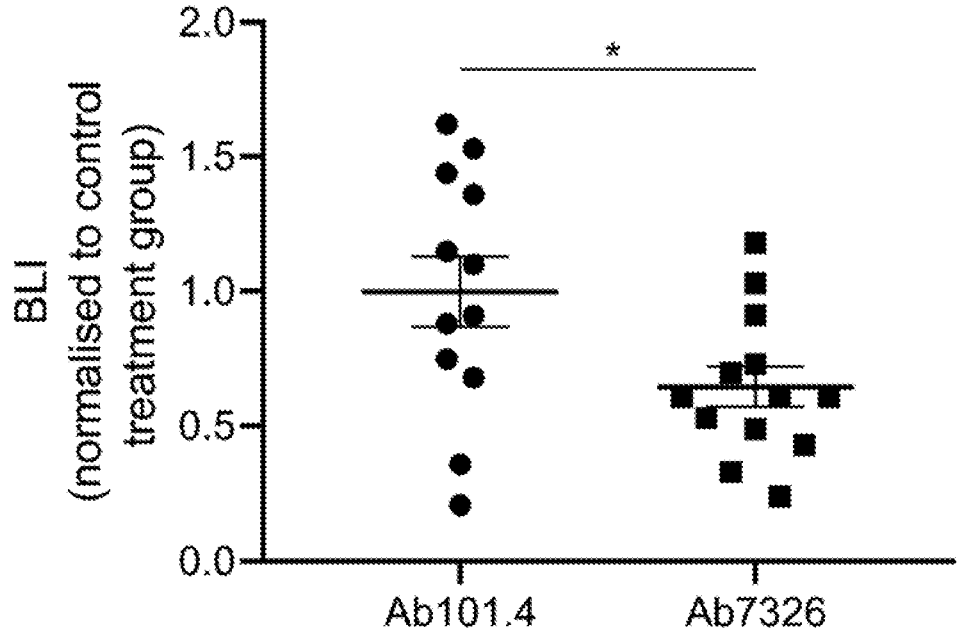

A.
Vil1-Grem1 model
Percent survival
100
50
0
0
200
400
600
No of days
Vehicle
Anti-Grem1 (early)
Anti-Grem1 (late)
B.
ApcMin model
Percent survival
100
50
0
0
200
400
600
Time
Vehicle
Anti-Grem1 (early)
Anti-Grem1 (late)

Figure 41

A


BLI
(normalised to control
treatment group)
2.0
1.5
1.0
0.5
0.0
*
Ab101.4
Ab7326

B

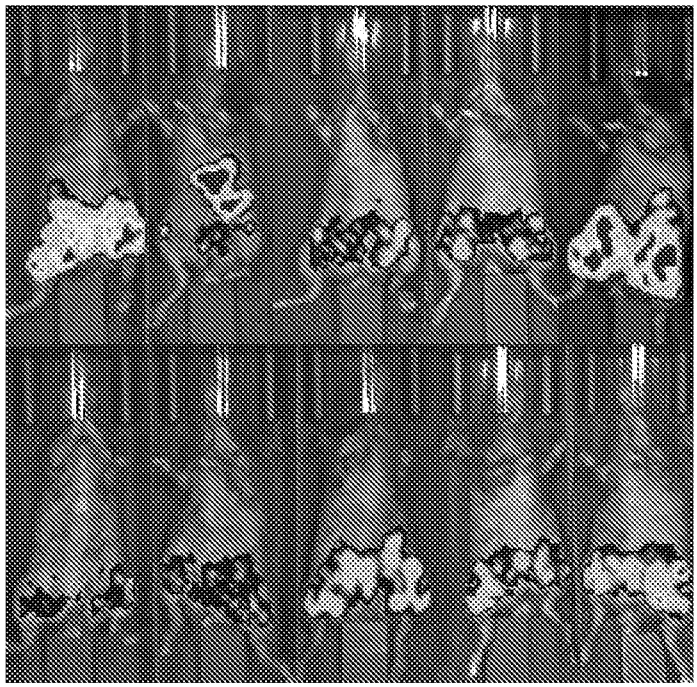

Ab101.4
Ab73263

Figure 42

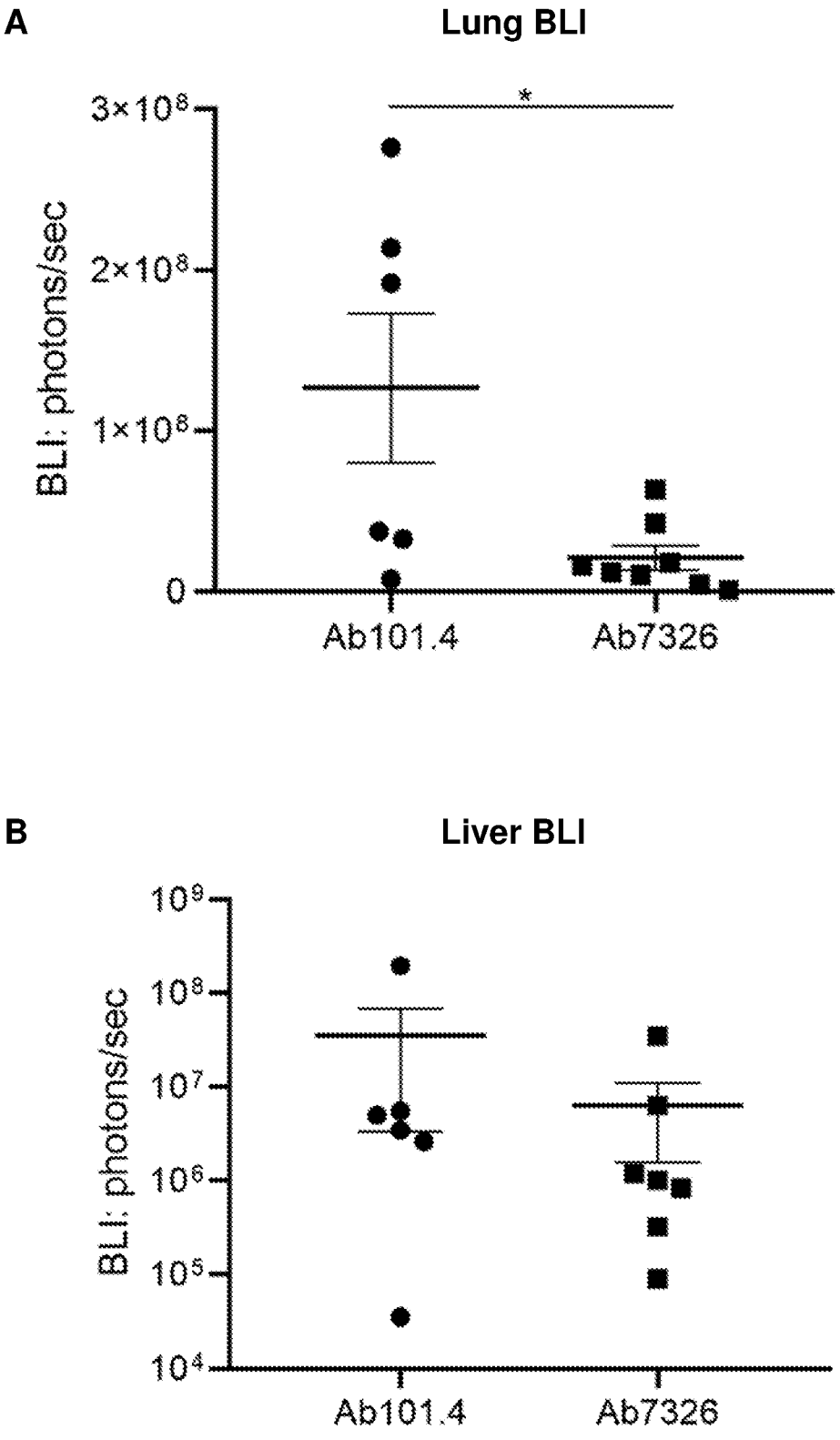

A
Lung BLI
BLI: photons/sec
3×10⁸
2×10⁸
1×10⁸
0
*
Ab101.4
Ab7326
B
Liver BLI
BLI: photons/sec
10⁹
10⁸
10⁷
10⁶
10⁵
10⁴
Ab101.4
Ab7326

Figure 43

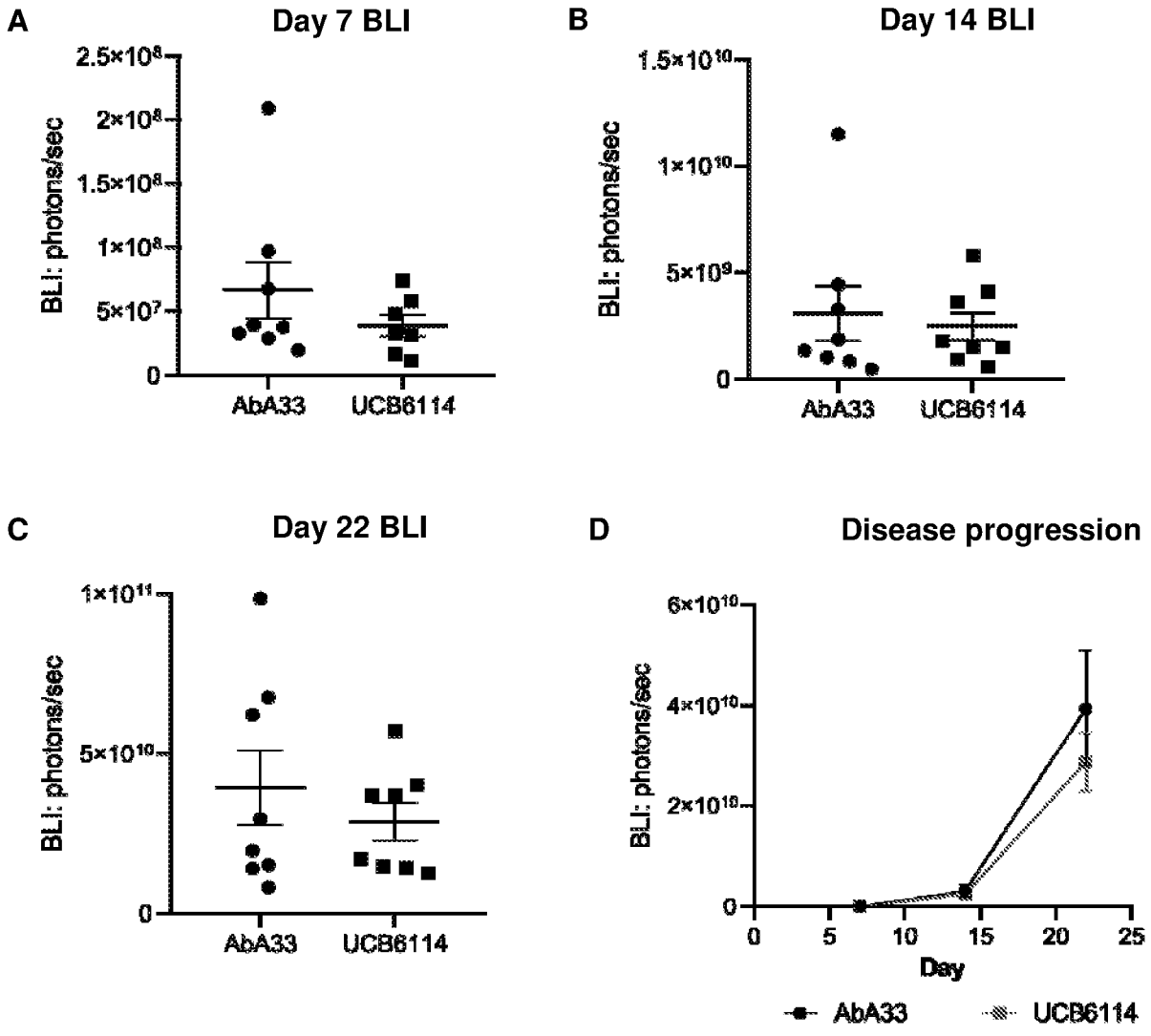


Figure 44

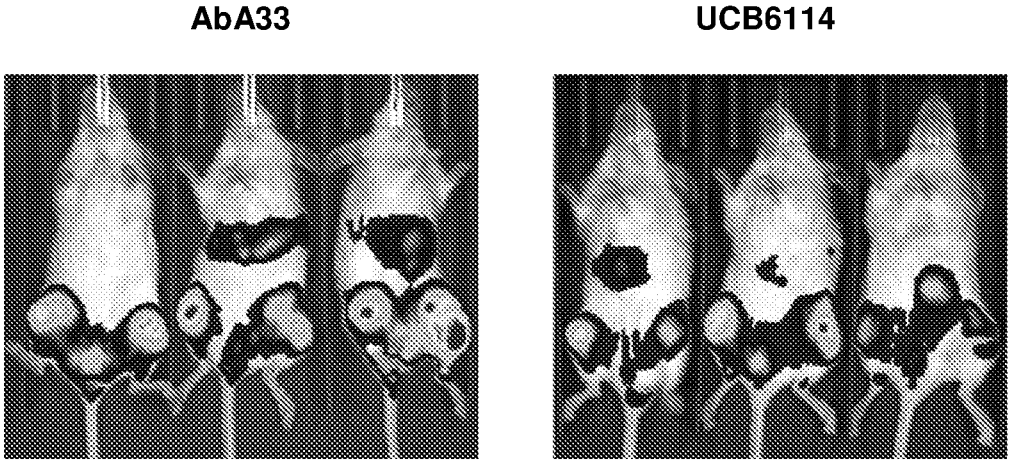


Figure 45

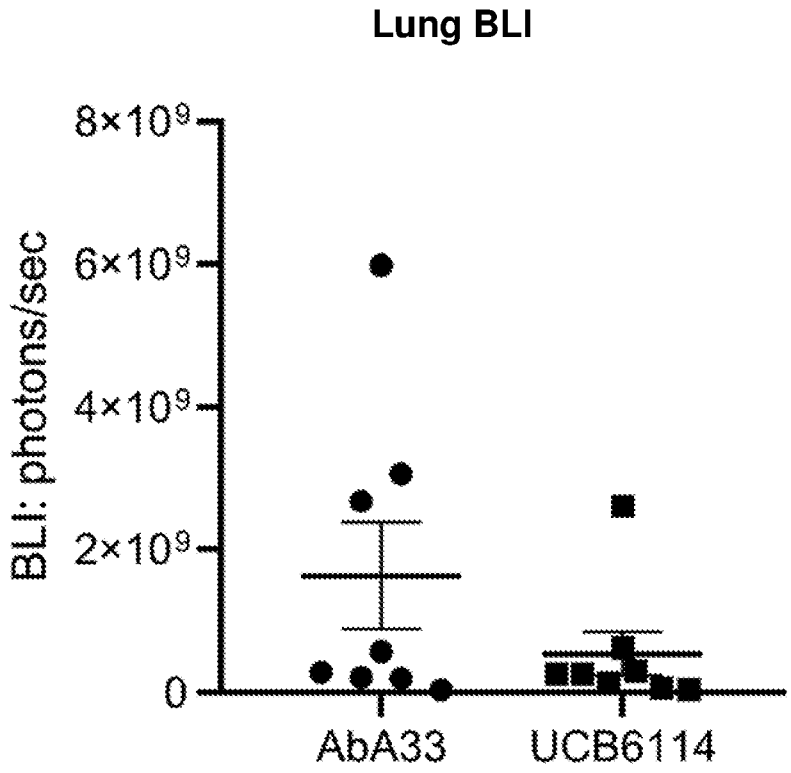


Figure 46

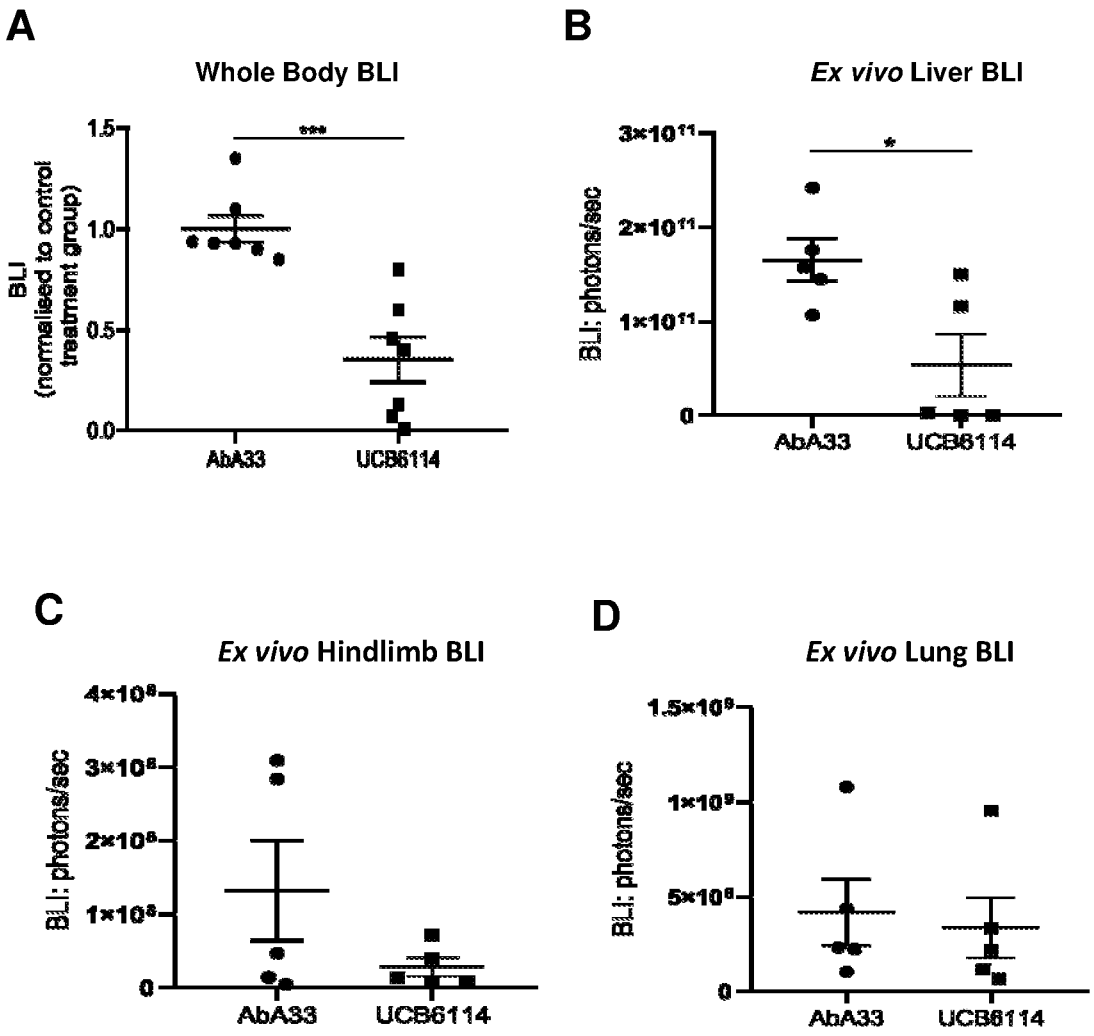

A
Whole Body BLI
BLI (normalised to control treatment group)
1.5
1.0
0.5
0.0
***
AbA33
UCB6114
B
Ex vivo Liver BLI
BLI: photons/sec
3×10^11
2×10^11
1×10^11
0
*
AbA33
UCB6114
C
Ex vivo Hindlimb BLI
BLI: photons/sec
4×10^8
3×10^8
2×10^8
1×10^8
0
AbA33
UCB6114
D
Ex vivo Lung BLI
BLI: photons/sec
1.5×10^9
1×10^9
5×10^8
0
AbA33
UCB6114

Figure 47

