Internal Name	GI	Locus Tag	Length (AA)	Strain	SEQ ID NOS:	Cloned SEQ ID NOS:	primers SEQ ID NOS:
DIF44	126698424	CD0844	313	630	78 & 79	138 & 139	370 & 371
DIF51	126698583	CD0999	331	630	80 & 81	140 & 141 356 & 357	372 & 373
DIF130	126700259	CD2645	424	630	92 & 93	152 & 153 358 & 359	380 & 381
DIF153	126700446	CD2830	220	630	299 & 300	321 & 322	346 & 347
DIF183	126701297	CD3669	191	630	186 & 187	188 & 189 360 & 361	424 & 425
DIF192	126698619	CD1035	679	630	104 & 105	164 & 165	384 & 385
DIF208	126700447	CD2831	972	630	132 & 133	432 & 433	422 & 423
DIF208A	126700447	CD2831	448	630	110 & 111	170 & 171	414 & 415
DIF208B	126700447	CD2831	457	630	112 & 113	172 & 173	416 & 417
DIF232	126698615	CD1031	693	630	124 & 125	184 & 185	400 & 401

Fig. 1

Name	Protein	PSORTb	Secretome 630	Secretome 20291	NMR	Confocal microscopy
Dif44	cell surface protein cwp25	Cell wall		yes	folded	surface
Dif51	ABC transporter substratebinding protein lipoprotein	Unknown			folded	surface
Dif130	extracellular solute-binding protein	Unknown/ Multiple Localizatio n				
Dif153	Hypothetical protein CD2830	unknown	yes	yes		surface
Dif183	hypothetical protein CD3669	unknown	yes	yes	folded	surface
Dif192	Nacetylmuramoyl -L-alanine amidase cwp16	Cell wall	yes	yes	folded	surface
Dif208 Dif208 A, Dif208 B	collagen-binding protein sortase substrate	Cell wall		yes	Partially folded	surface
Dif232	Unknown	Unknown				surface

Fig. 2

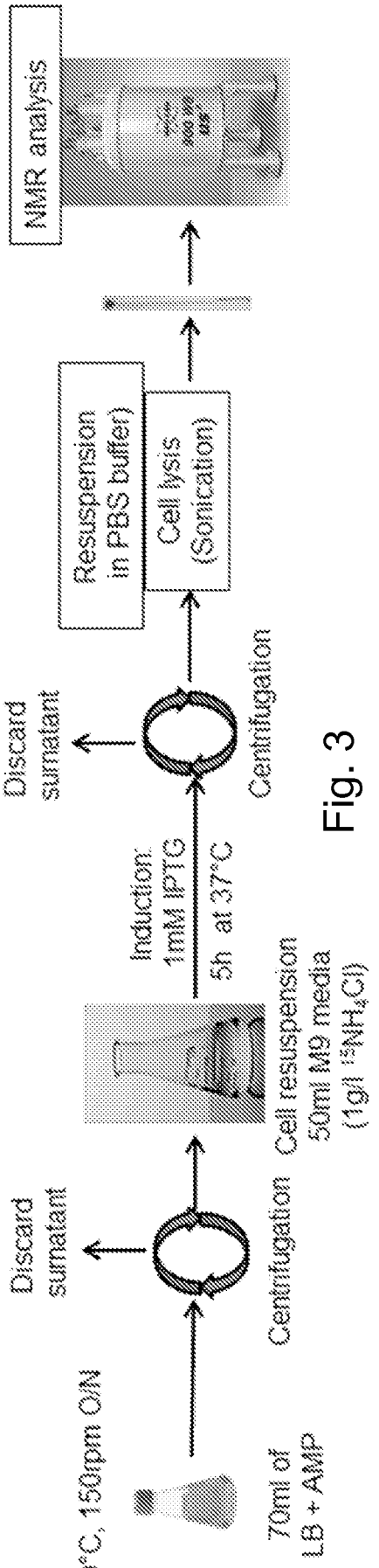

30°C, 150rpm O/N
70ml of
LB + AMP
Centrifugation
Discard
surnatant
Cell resuspension
50ml M9 media
(1g/l $^{15}NH_4Cl$)
5h at 37°C
Induction:
1mM IPTG
Centrifugation
Discard
surnatant
Cell lysis
(Sonication)
Resuspension
in PBS buffer)
NMR analysis

Fig. 3

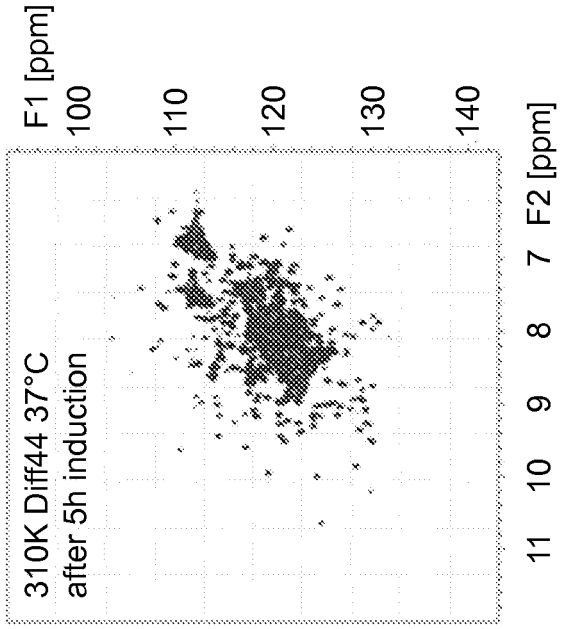

Dif44 Folded
310K Diff44 37°C
after 5h induction
F1 [ppm]
100
110
120
130
140
11
10
9
8
7
F2 [ppm]

Fig. 4

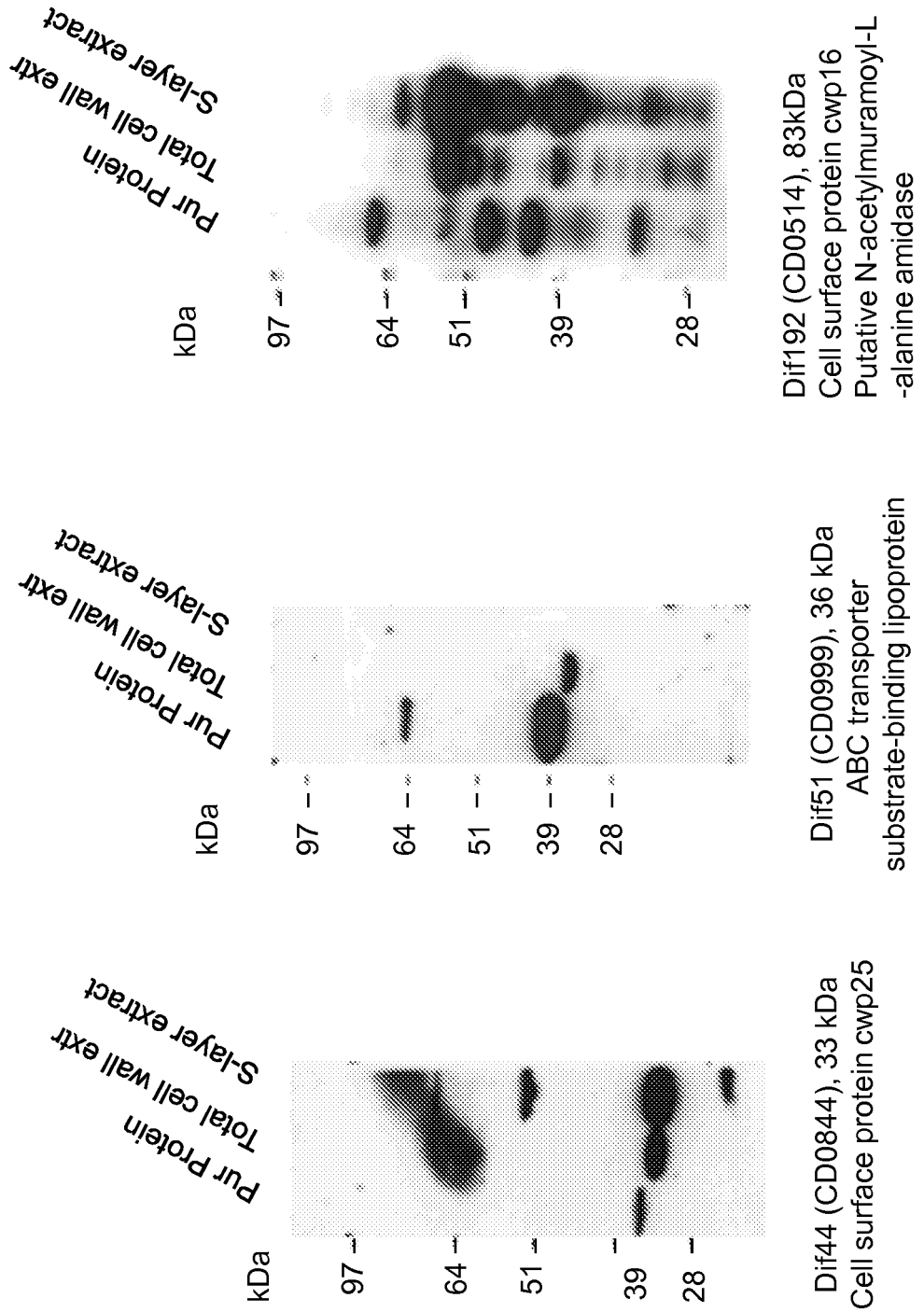
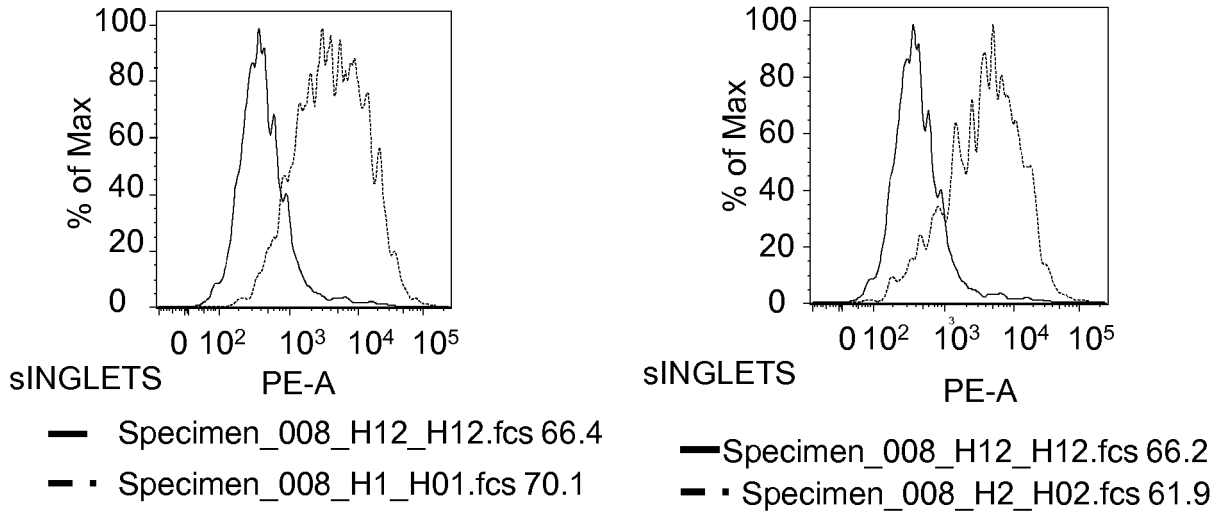

kDa
97
64
51
39
28
Pur Protein
Total cell wall extr
S-layer extract
Dif192 (CD0514), 83kDa
Cell surface protein cwp16
Putative N-acetylmuramoyl-L
-alanine amidase
Dif51 (CD0999), 36 kDa
ABC transporter
substrate-binding lipoprotein
Dif44 (CD0844), 33 kDa
Cell surface protein cwp25

Fig. 5

Dif 232– 4ºC



Dif 232– 37ºC

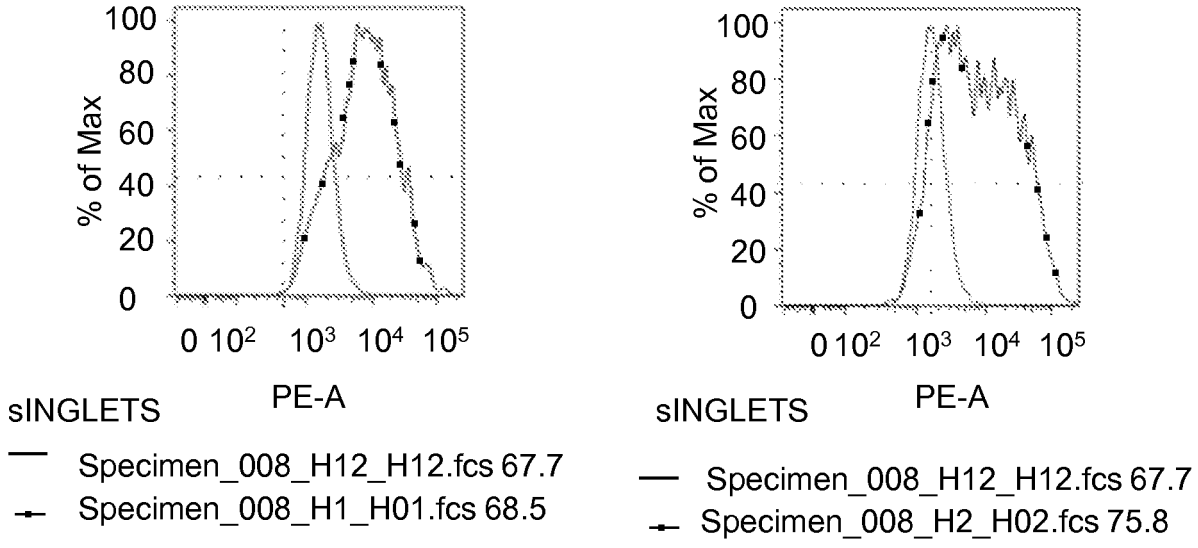


Fig. 6

Name	Annotation	Locus Tag	Strain	MW	Binding to the cells		
					FACS on Vero cells	Microscopy on Caco-2 cells	Microscopy on Vero cells
Dif44	cell surface protein cwp25	CD0844	630		-		
Dif208A	putative collagen-binding protein	CD2831	630	51	++	+/-	+/-
Dif232	putative cell wall anchored protein, cellulose synthase activity	CD1031	630	73	+ (37° C-4° C)	-	ND
Dif208B	putative collagen-binding protein	CD2831	630	52	++	++	
Dif51	ABC transporter, substrate-binding lipoprotein	CD0999	630	36	++ (37° C-4° C)	+	ND
Dif192	cell surface protein (putative N-acetylmuramoyl-L-alanine amidase) cwp16	CD1035	630	83	++ (37°C-4°C)	++	ND

Fig. 7

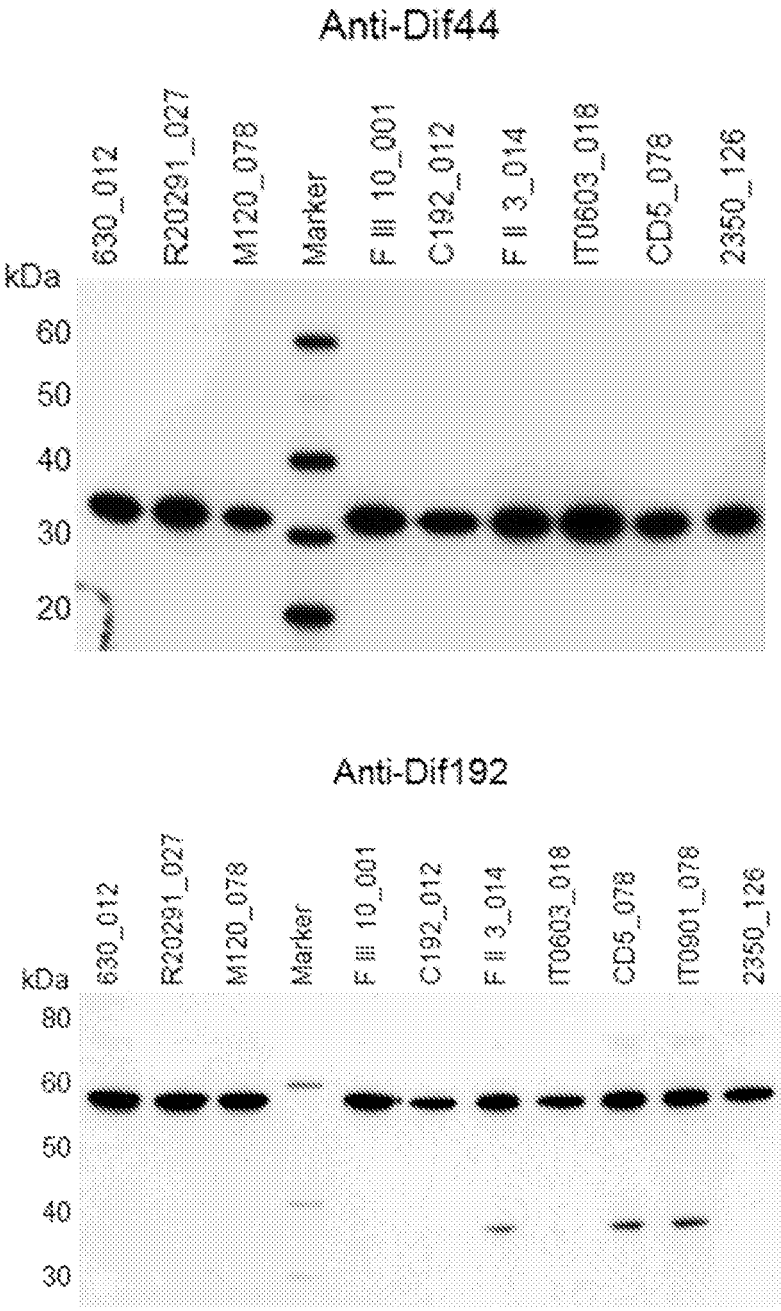
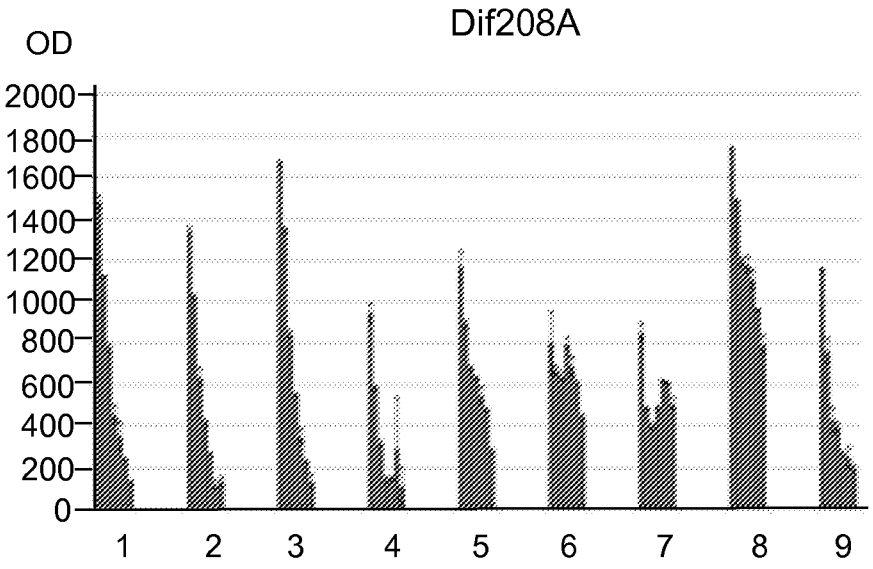
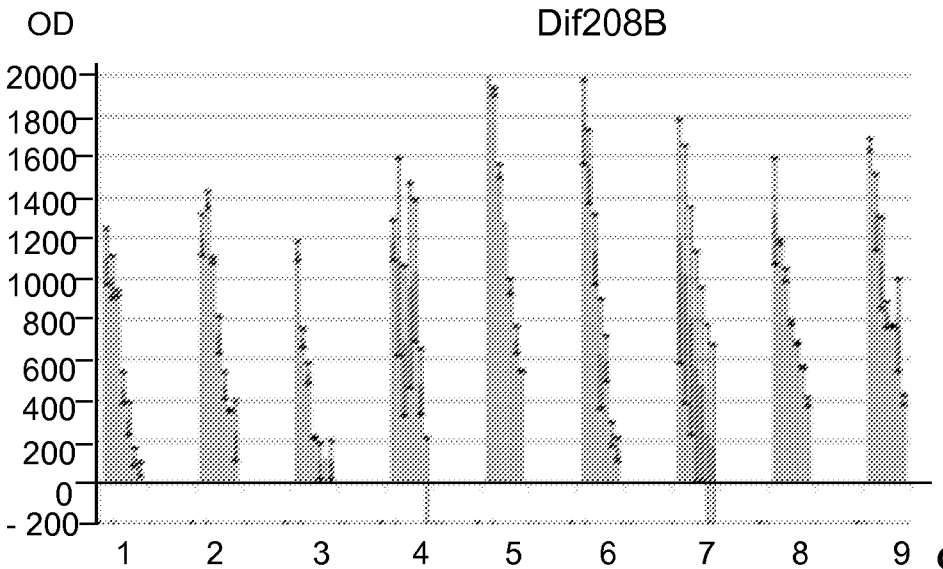

Anti-Dif44
630_012
R20291_027
M120_078
Marker
F III 10_001
C192_012
F II 3_014
IT0603_018
CD5_078
2350_126
kDa
60
50
40
30
20
Anti-Dif192
630_012
R20291_027
M120_078
Marker
F III 10_001
C192_012
F II 3_014
IT0603_018
CD5_078
IT0901_078
2350_126
kDa
80
60
50
40
30

Fig. 8



1: collagen I
2: collagen II
3: collagen III
4: collagen IV
5: Laminin
6: Fibronectin
7: Fibrinogen
8: Collagen V
9: collagen VI



1: collagen I
2: collagen II
3: collagen III
4: collagen IV
5: Collagen V
6: collagen VI
7: Laminin
8: Fibronectin
9: Fibrinogen

Summary of ELISA binding assays

	Collagen I	Collagen II	Collagen III	Collagen IV	Collagen V	Collagen VI	Laminin	Fibronectin	Fibrinogen
Dif208A	+	+	++	-	++	+	+	-	-
Dif208B	+	++	+	+	++	++	+	++	++

++ : OD > 800 with at least 3 protein concentrartions

+: OD > 400 with at least 3 protein concentrartions

Fig. 9

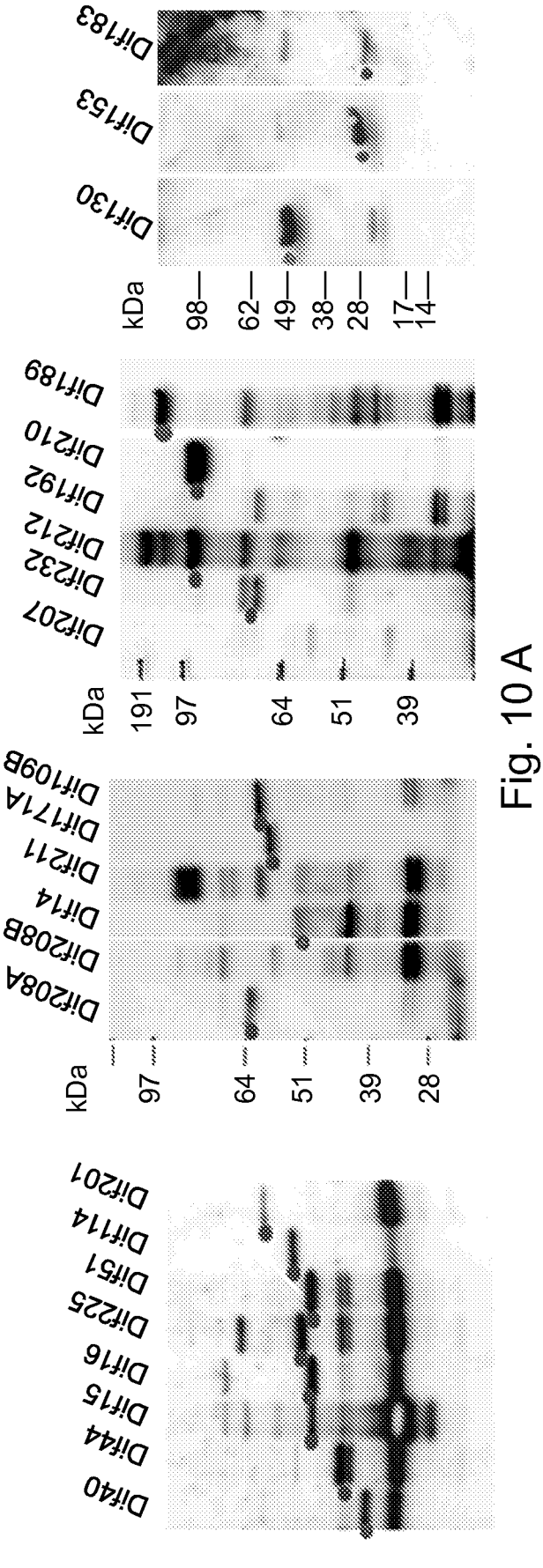


Fig. 10 A

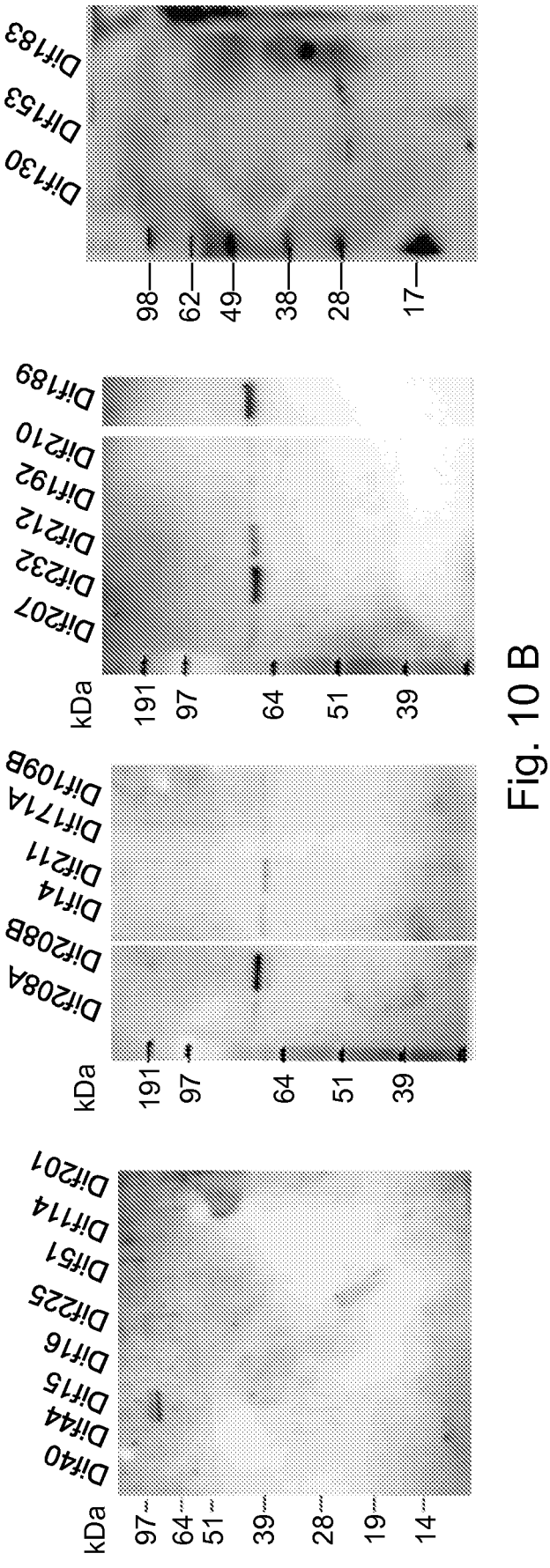


Fig. 10 B

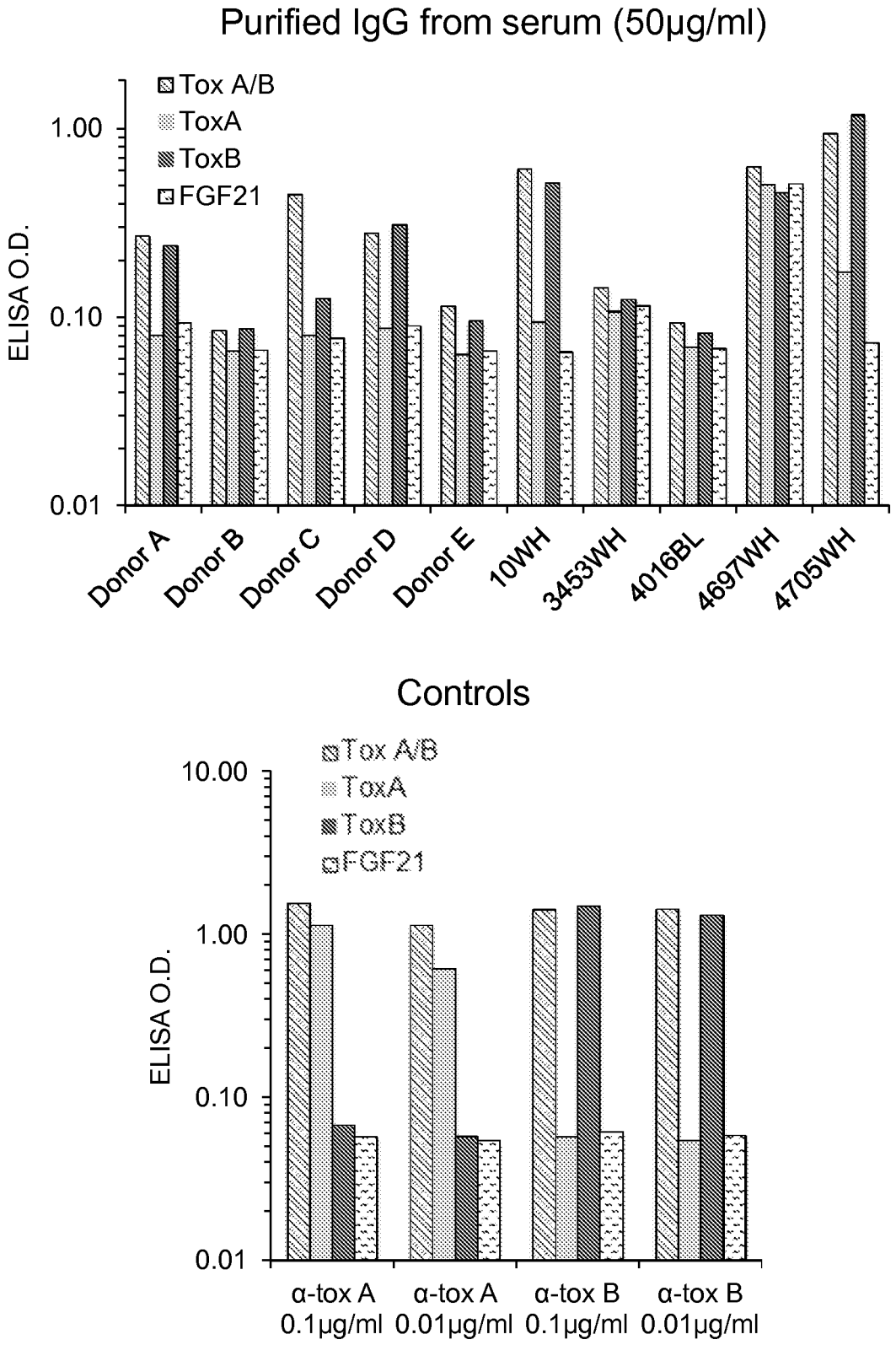
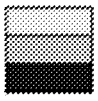

Purified IgG from serum (50µg/ml)
Tox A/B
ToxA
ToxB
FGF21
ELISA O.D.
1.00
0.10
0.01
Donor A
Donor B
Donor C
Donor D
Donor E
10WH
3453WH
4016BL
4697WH
4705WH
Controls
Tox A/B
ToxA
ToxB
FGF21
ELISA O.D.
10.00
1.00
0.10
0.01
α-tox A 0.1µg/ml
α-tox A 0.01µg/ml
α-tox B 0.1µg/ml
α-tox B 0.01µg/ml

Fig. 11

Method	PROTEIN CHIP											WESTERN BLOT
name of serum	No serum	DonorA 1:200	DonorB 1:200	DonorC 1:200	DonorD 1:200	DonorE 1:200	10WH 1:200	3453WH 1:200	4016BL 1:200	4697WH 1:500	4705WH 1:200	4705WH 1:500
Proteins	MFI	MFI	MFI	MFI	MFI	MFI	MFI	MFI	MFI	MFI	MFI	
Dif61 His	153	1050	2087	2041	1798	1305	966	2201	1125	2860	1189	NO
Dif65 His	218	1088	1911	1159	2745	1602	979	2065	8095	1555	486	NO
Dif130 His	853	1028	6154	933	2858	2859	737	9333	1142	6315	628	NO
Dif1836 His	73	1860	2071	7692	3430	2822	1798	2061	1138	775	1578	NO
Dif194 His	103	888	1049	1332	1101	956	738	1508	1121	1082	16976	YES
Dif210 His	138	1784	2871	6369	3012	2731	2249	2045	1987	1414	1092	NO
Dif211 His	934	2644	2985	7157	4564	2741	1607	5115	1625	2714	2385	NO
Dif231 His	195	1202	2683	1790	2862	1518	774	2750	1258	3503	5357	YES
Dif232 His	285	2016	1141	2113	3539	1218	614	2873	637	1196	7286	YES
Controls:												
NN-His	41	192	444	988	495	357	327	431	241	232	188	
PBS_Gly	19	28	32	62	25	30	33	26	29	25	47	

MFI: mean fluorescence intensity



MFI:> 2000
MFI:> 5000
MFI:> 10000

Fig. 12A

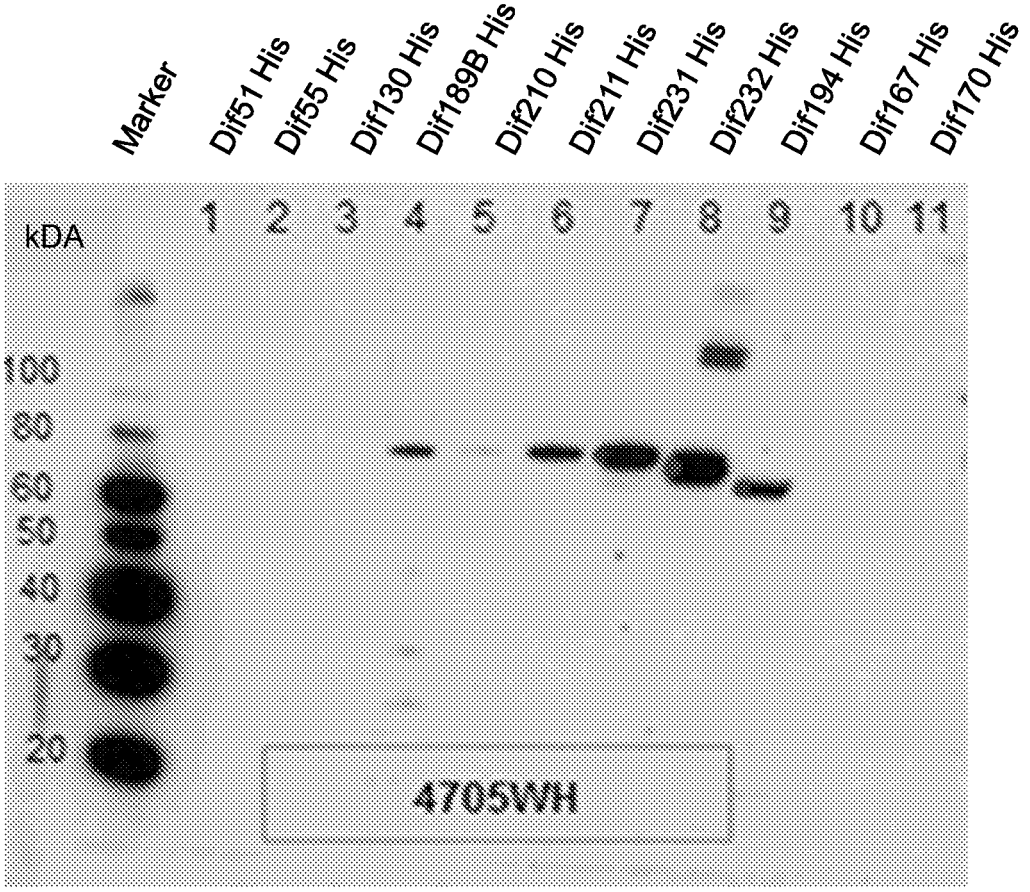

Marker
Dif51 His
Dif55 His
Dif130 His
Dif189B His
Dif210 His
Dif211 His
Dif231 His
Dif232 His
Dif194 His
Dif167 His
Dif170 His
kDA
1 2 3 4 5 6 7 8 9 10 11
100
80
60
50
40
30
20
4705WH

Fig. 12B

Method	PROTEIN CHIP												WESTERN BLOT
name of serum	No serum	DonorA 1:500	DonorB 1:500	DonorC 1:500	DonorD 1:500	DonorE 1:500	10WH 1:500	2453WH 1:500	4016BL 1:500	4697WH 1:500	4705WH 1:200	4705WH 1:500
Proteins	MFI	MFI	MFI	MFI	MFI	MFI	MFI	MFI	MFI	MFI	MFI		
Dif51 His	209	697	\	**1384**	840	\	\	790	469	368	951	NO	
Dif104 His	148	624	\	**1631**	686	\	\	962	314	326	**1185**	**YES**	
Dif130 His	101	**1048**	\	**3466**	**1307**	\	\	**1897**	839	377	**2252**	**YES**	
Dif1698 His	72	166	\	384	178	\	\	270	90	124	180	NO	
Dif194 His	134	264	\	505	303	\	\	437	176	193	**1169**	**YES**	
Dif210 His	41	129	\	232	104	\	\	135	81	29	153	NO	
Dif211 His	564	**1098**	\	**2317**	**1133**	\	\	**1054**	612	704	**1287**	NO	
Dif231 His	52	487	\	**1215**	617	\	\	625	447	133	**1506**	**YES**	
Dif232 His	238	475	\	760	431	\	\	475	240	263	721	**YES**	
Controls:													
IRL-His	88	136	\	208	134	\	\	115	76	110	130		
PBS GW	22	35	\	32	22	\	\	36	26	24	26		

MFI: mean fluorescence intensity

MFI> 1000 and at least 5-fold higher than the negative control

Fig. 13A

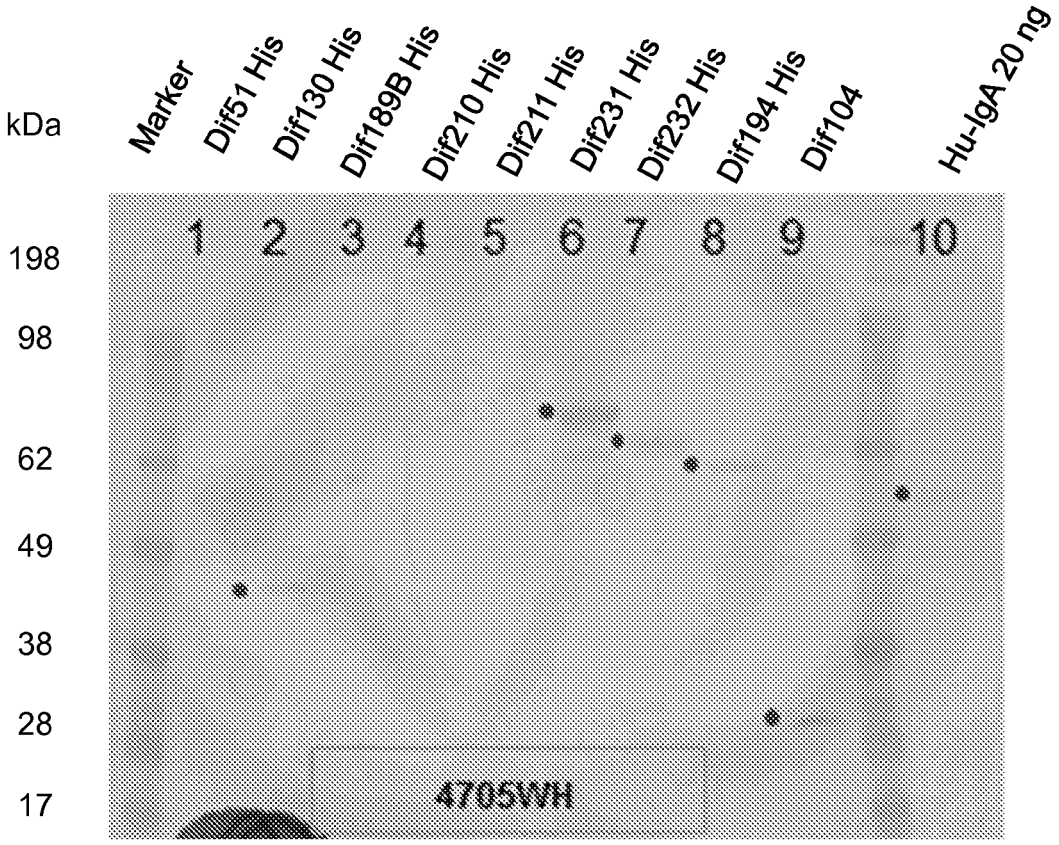

kDa
Marker
Dif51 His
Dif130 His
Dif189B His
Dif210 His
Dif211 His
Dif231 His
Dif232 His
Dif194 His
Dif104
Hu-IgA 20 ng
1 2 3 4 5 6 7 8 9 10
198
98
62
49
38
28
17
4705VH

Fig. 13B

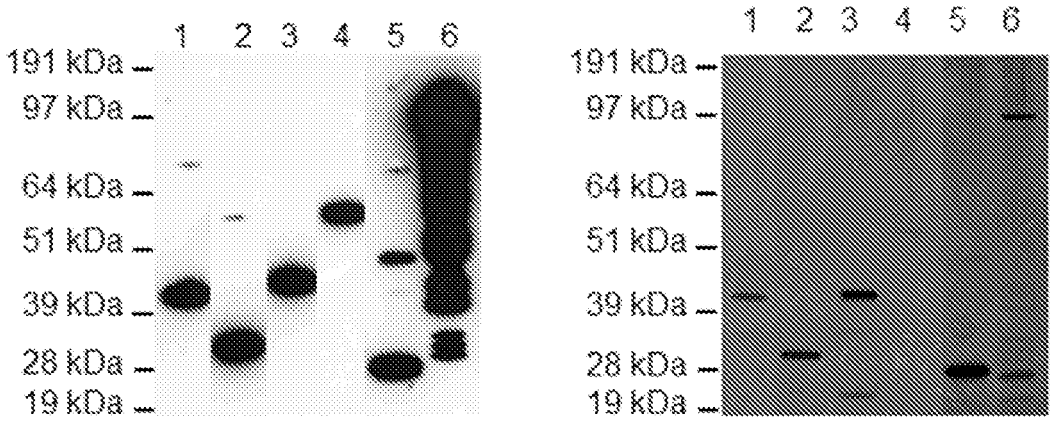


Fig. 14

```
LF          -DTKIQEAQLNINQEWNKALGLPKYTKLITFNVHNRYASNIVESAYLILNEWKNNIQSDL
DIF153      DSTTIQQNKDTLSQIVVFPTGN--YDKNEANAMVNRLAN--IDGKYLNALK-QNNLK---
             .*.**: : .:.*    . *   * *  :  : ** *.  ::. **   : :**::

LF          IKKVTNYLVDGNGRFVFTDITLPNIAEQYTHQDEIYEQVHSKGLYVPESRSILLHGPSKG
DIF153      IKLLSGKLTDEK-EYAYLKGVVP---KGWEGTGKTWDDVPGLGGSTVALR-IGFSNKGKG
            ** ::. *.* : .:.: . .:*   : :   .: :::* . *  .   * * : . .**

LF          VELRNDSEGFIHEFGHAVDDYAGYLLDKNQSDLVTNSKKFIDIFKEEGSNLT--SYGRTN
DIF153      HDAIN---LELHETAHAIDHIV--------LNDISKSAQFKQIFAKEGRSLGMVNYLGVY
             :  *     :** .**:*. .         : :::* :* :** :** .*   .*  .

LF          EAEFFAEAFRLMHSTDHAERLKVQKNAPKTFQFINDQIKFIINS
DIF153      PEEFFAESFAYYYLNQDTN-SKLKSACPQTYSFLQNLAK-----
              *****:*   : .:.::  *::. .*:*:.*:::  *
```

Fig. 15

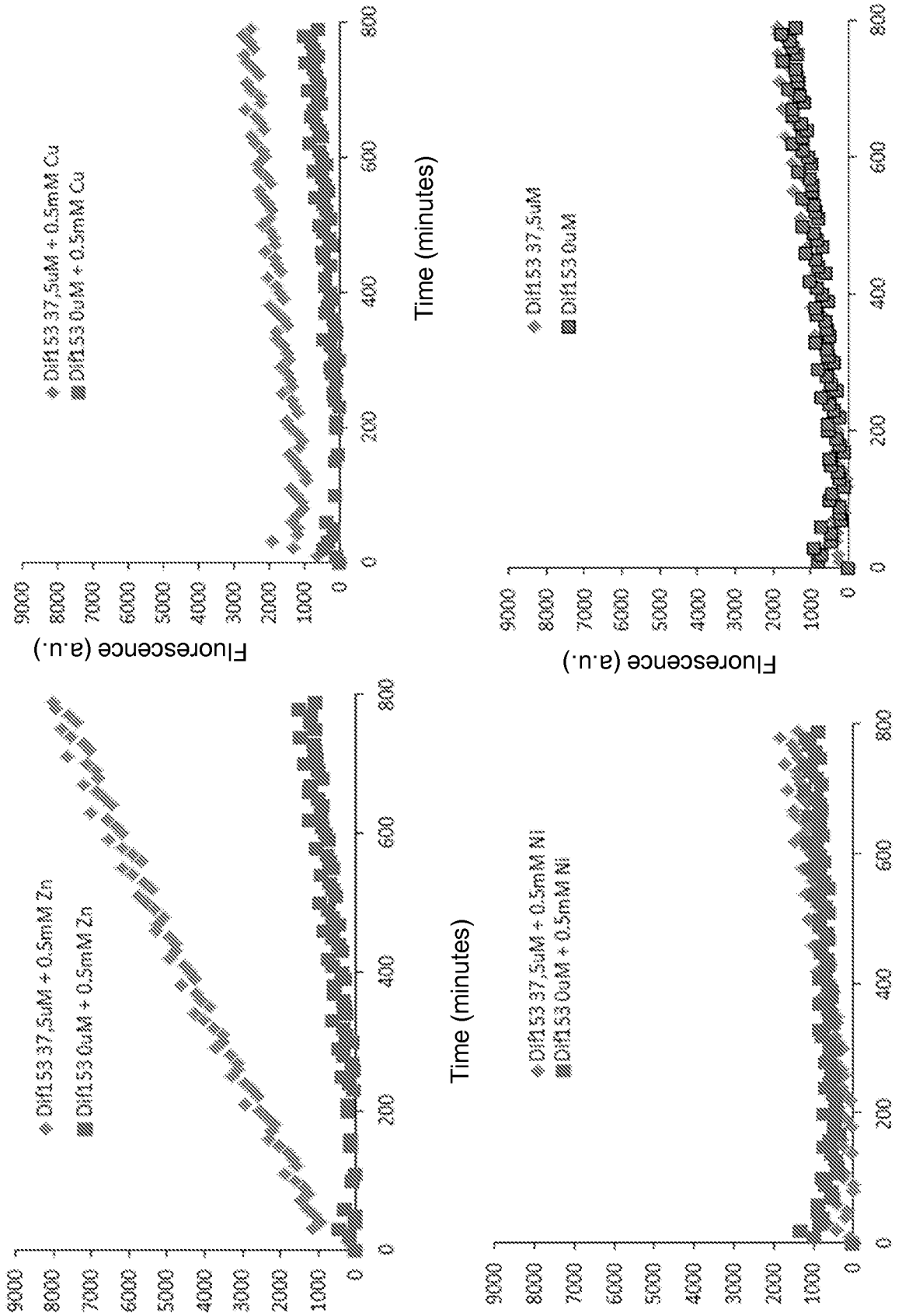

Fluorescence (a.u.)
Time (minutes)
Dif153 37,5uM + 0.5mM Cu
Dif153 0uM + 0.5mM Cu
Dif153 37,5uM + 0.5mM Zn
Dif153 0uM + 0.5mM Zn
Dif153 37,5uM + 0.5mM Ni
Dif153 0uM + 0.5mM Ni
Dif153 37,5uM
Dif153 0uM

Fig. 16

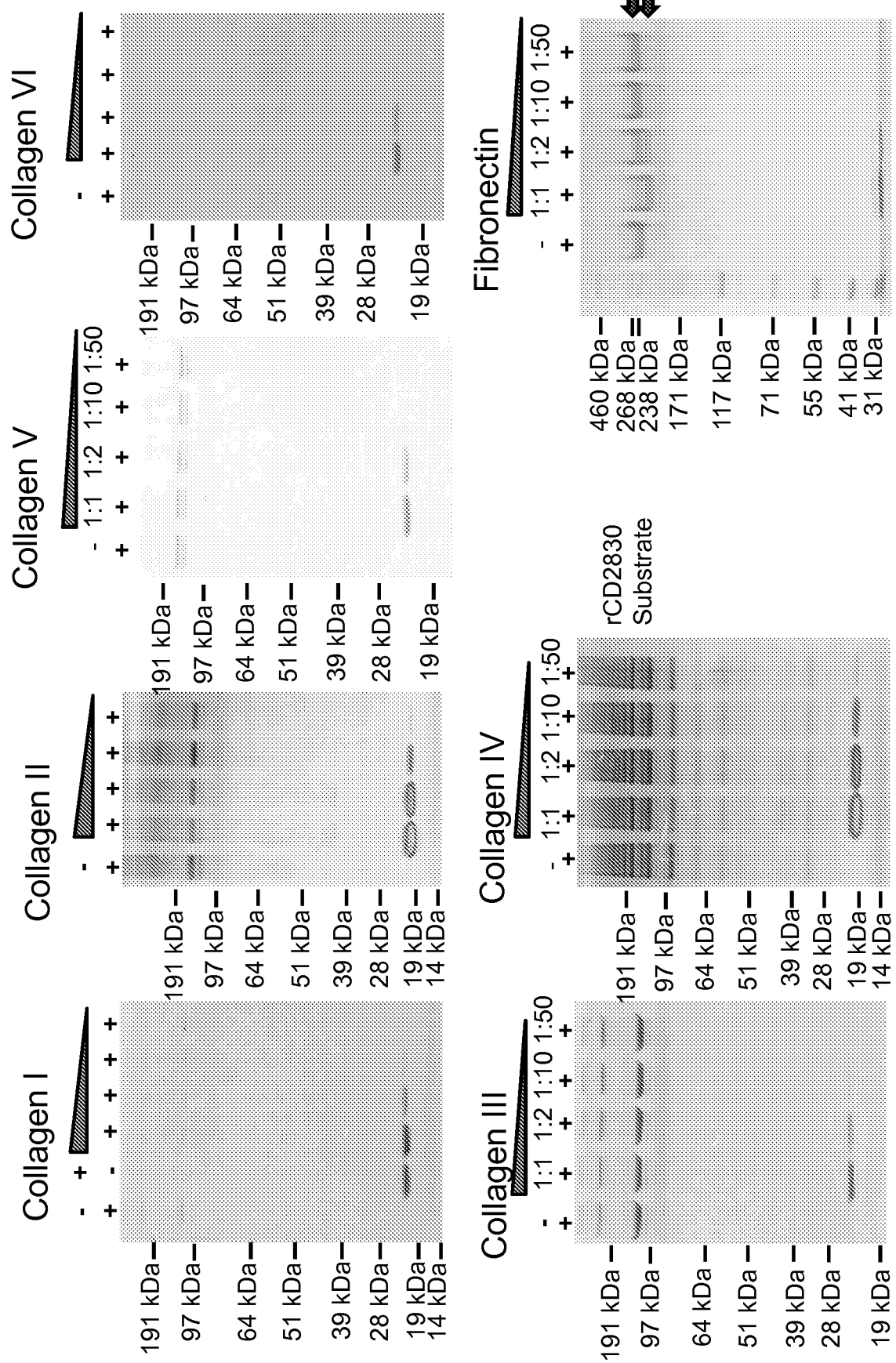

Collagen I
Collagen II
Collagen V
Collagen VI
Collagen III
Collagen IV
Fibronectin
rCD2830 Substrate
- + - + + + +
- 1:1 1:2 1:10 1:50
+ + + + + + +
191 kDa
97 kDa
64 kDa
51 kDa
39 kDa
28 kDa
19 kDa
14 kDa
460 kDa
268 kDa
238 kDa
171 kDa
117 kDa
71 kDa
55 kDa
41 kDa
31 kDa

Fig. 17

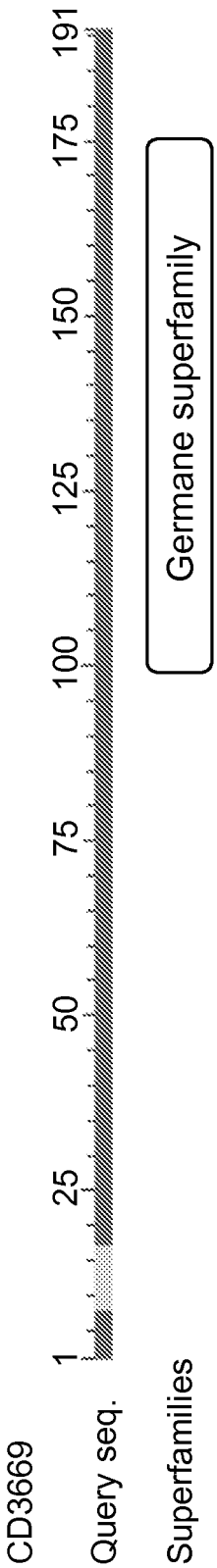

CD3669
Query seq.
1
25
50
75
100
125
150
175
191
Superfamilies
Germane superfamily

Fig. 18

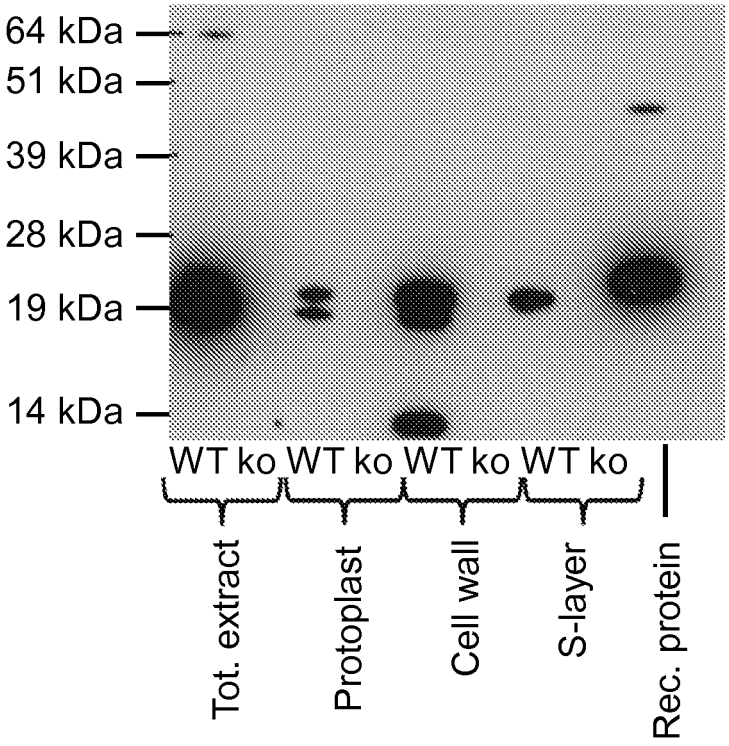


Fig. 19A

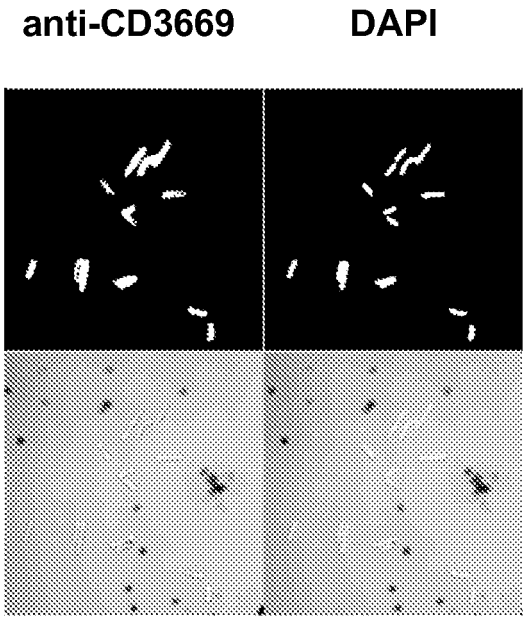


Fig. 19B

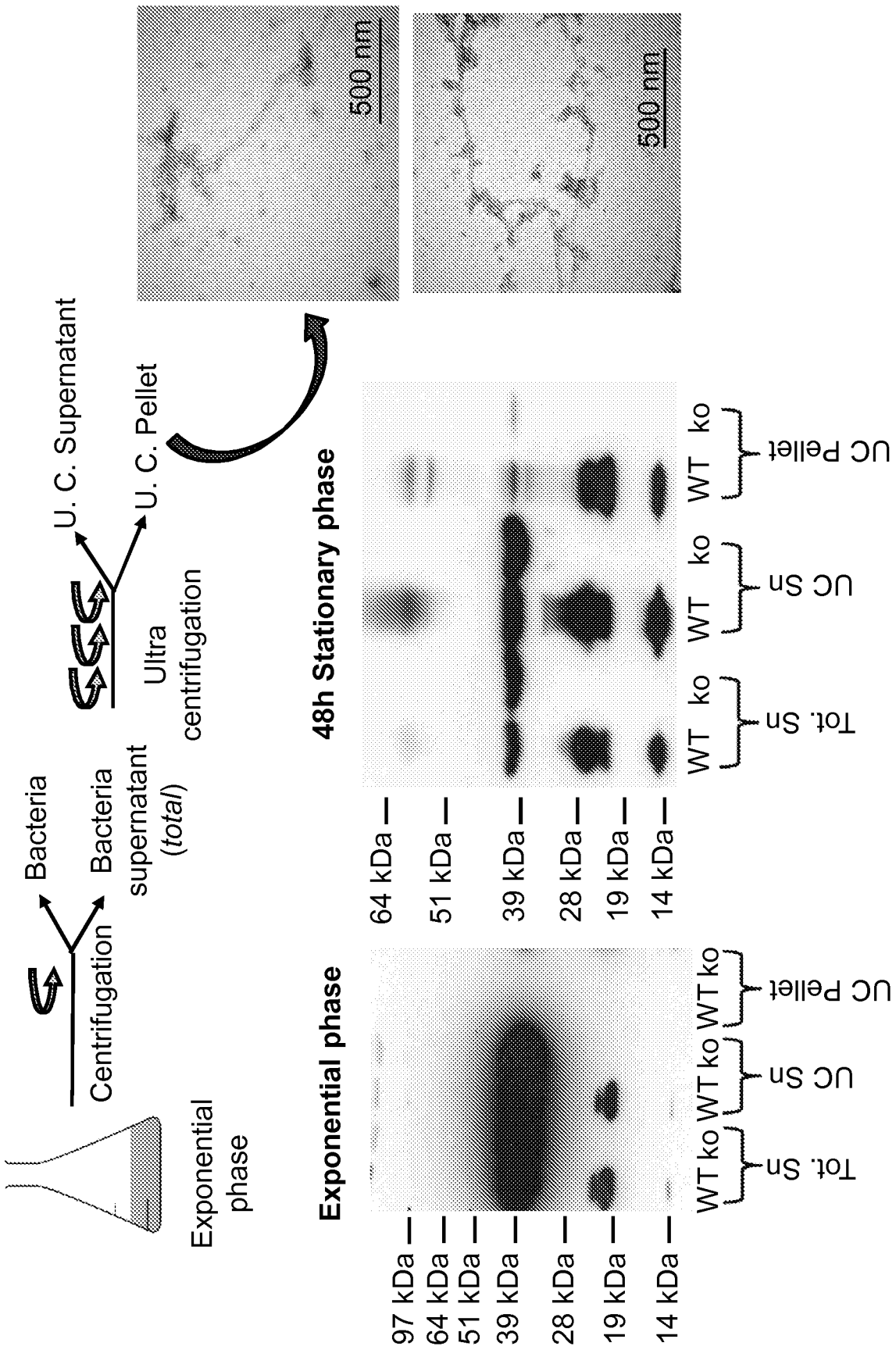

Exponential phase
Bacteria
Centrifugation
Bacteria supernatant (total)
Ultra centrifugation
U. C. Supernatant
U. C. Pellet
500 nm
500 nm
48h Stationary phase
Exponential phase
64 kDa
51 kDa
39 kDa
28 kDa
19 kDa
14 kDa
97 kDa
64 kDa
51 kDa
39 kDa
28 kDa
19 kDa
14 kDa
WT ko
Tot. Sn
WT ko
UC Sn
WT ko
UC Pellet

Fig. 20

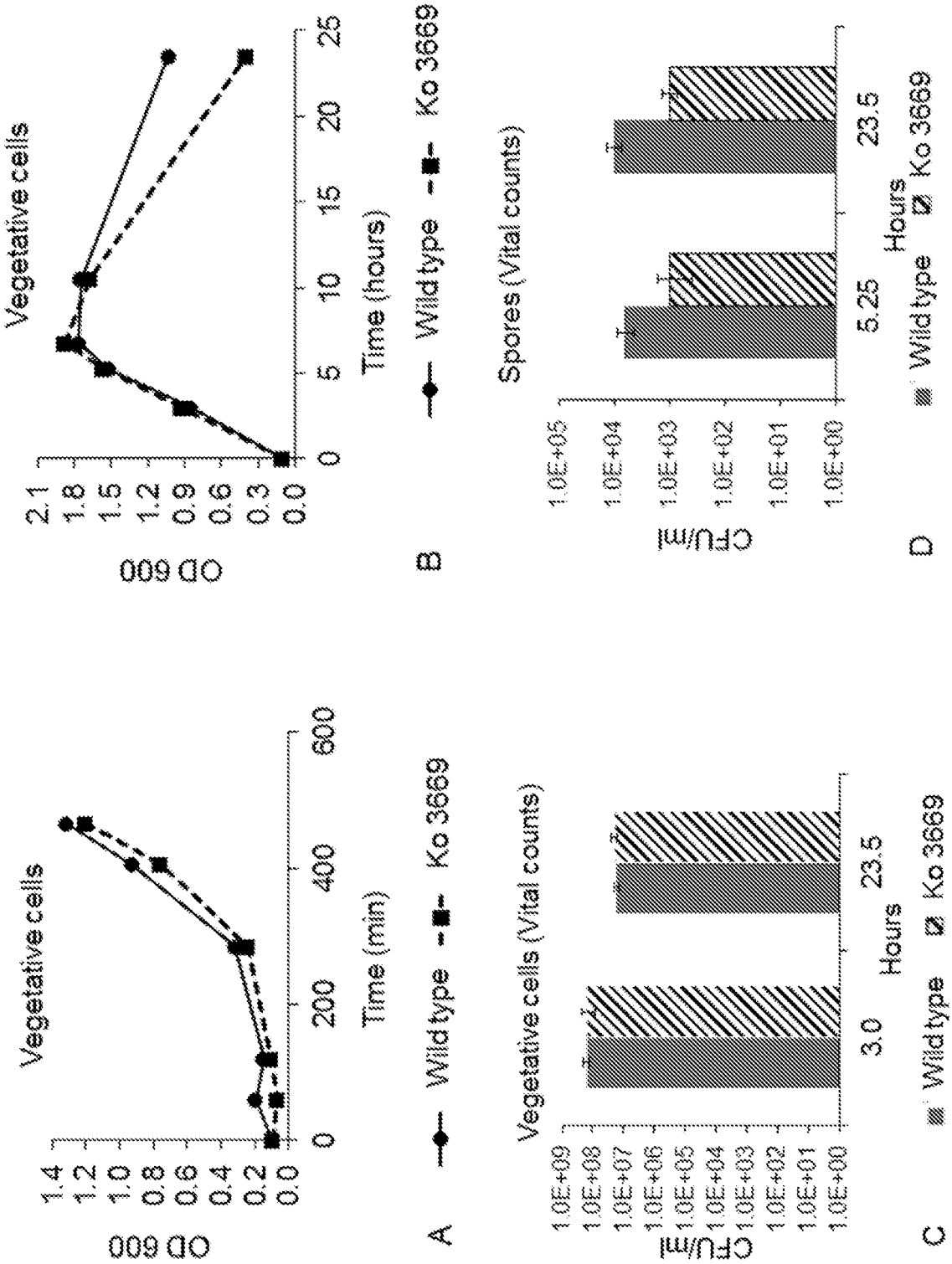

Vegetative cells
OD 600
Time (min)
Wild type
Ko 3669
A
Vegetative cells
OD 600
Time (hours)
Wild type
Ko 3669
B
Vegetative cells (Vital counts)
CFU/ml
Hours
3.0
23.5
Wild type
Ko 3669
C
Spores (Vital counts)
CFU/ml
Hours
5.25
23.5
Wild type
Ko 3669
D

Fig. 21

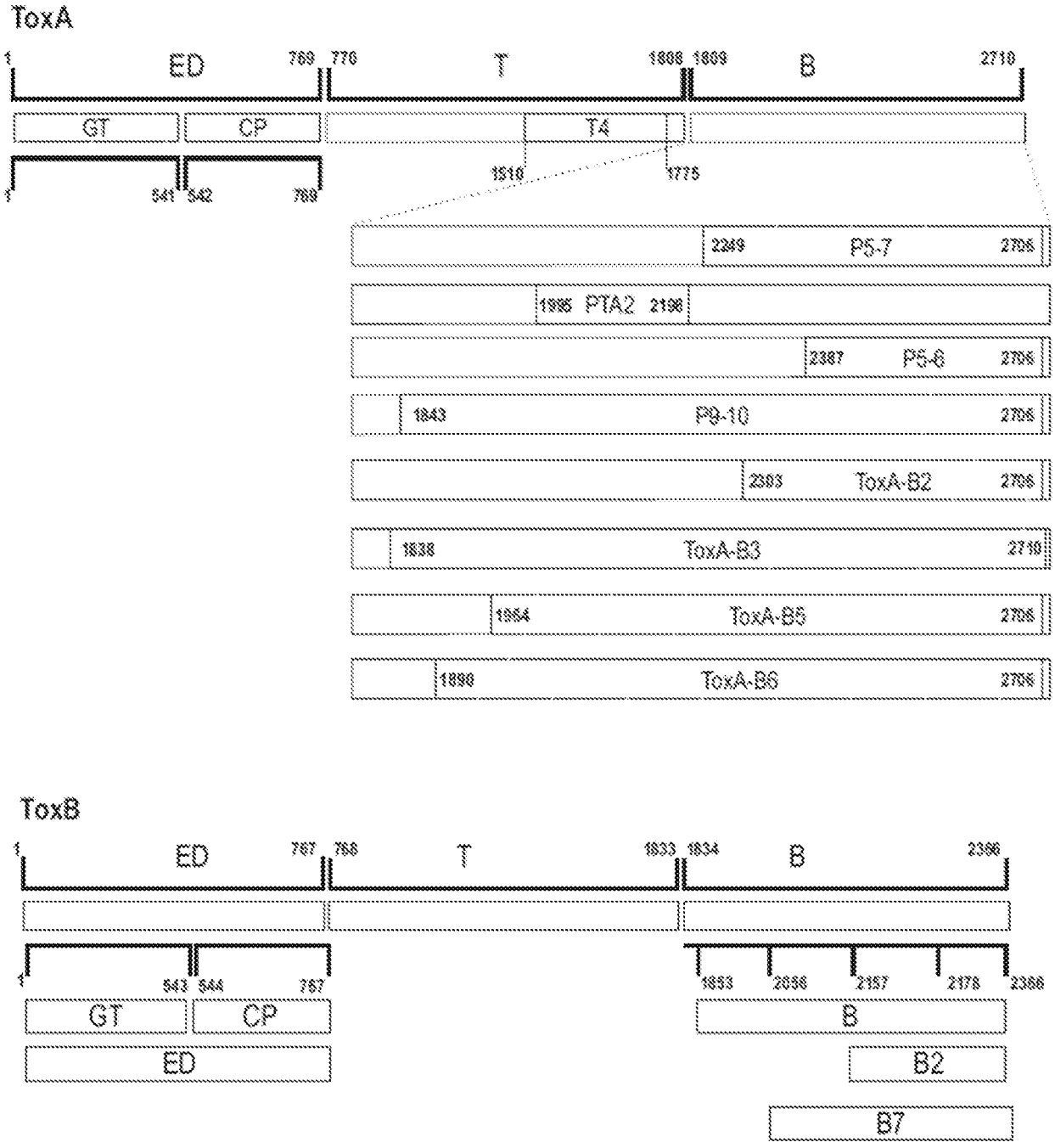

ToxA
ED
T
B
GT
CP
T4
P5-7
PTA2
P5-6
P9-10
ToxA-B2
ToxA-B3
ToxA-B5
ToxA-B6
ToxB
ED
T
B
GT
CP
ED
B
B2
B7

Fig. 22

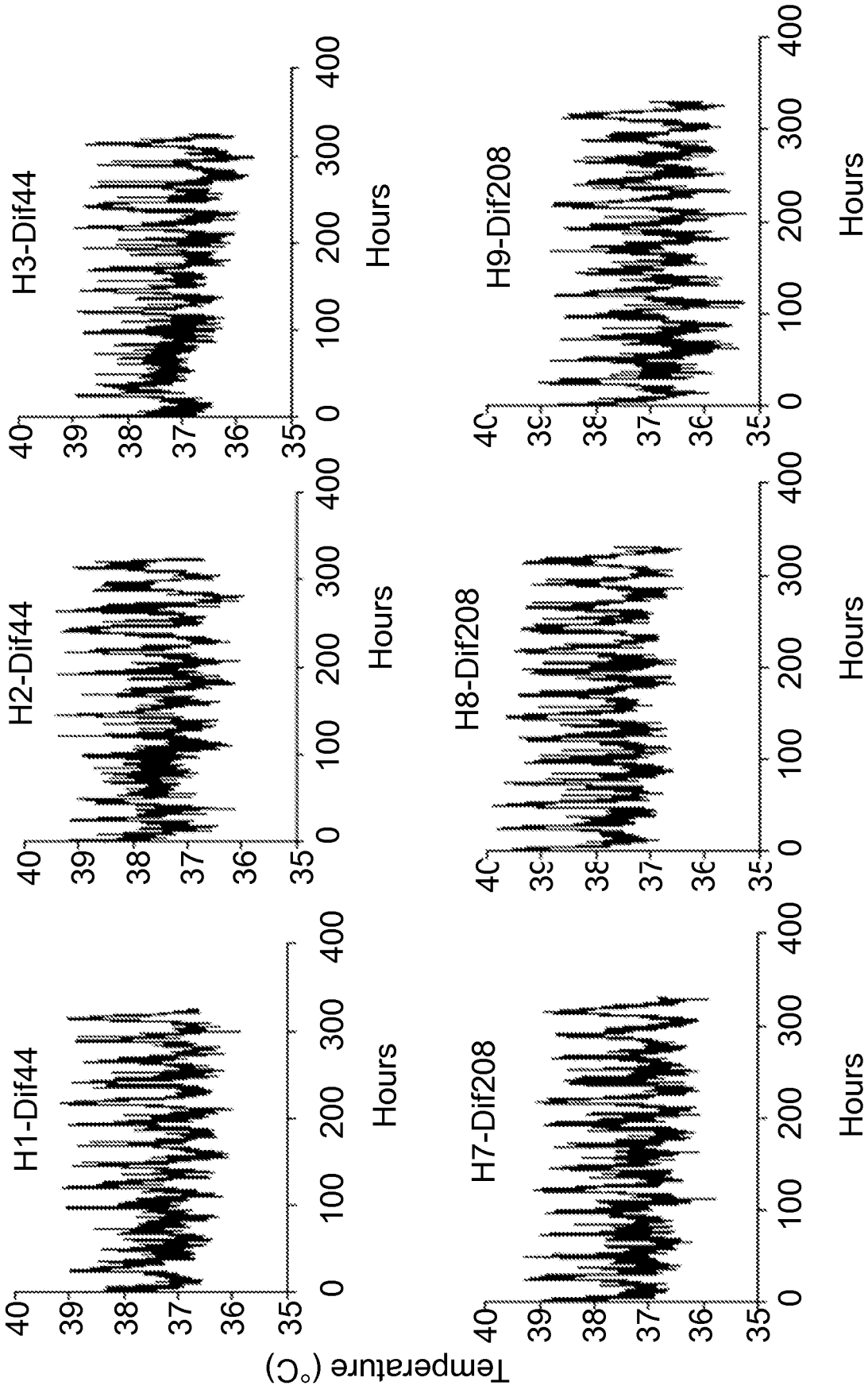

H1-Dif44
H2-Dif44
H3-Dif44
H7-Dif208
H8-Dif208
H9-Dif208
Temperature (°C)
Hours
40
39
38
37
36
35
0
100
200
300
400

Fig. 23

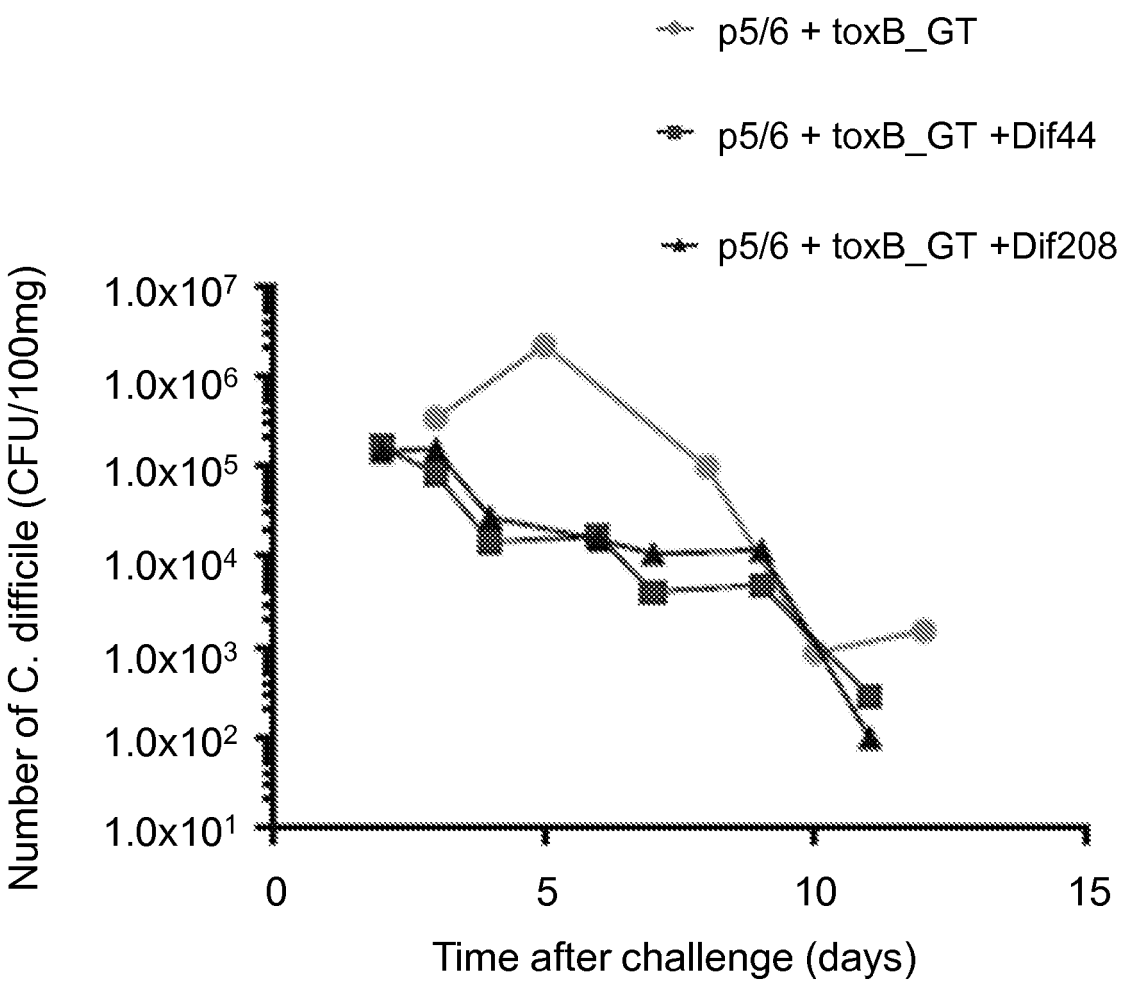

p5/6 + toxB_GT
p5/6 + toxB_GT +Dif44
p5/6 + toxB_GT +Dif208
Number of C. difficile (CFU/100mg)
1.0x10^7
1.0x10^6
1.0x10^5
1.0x10^4
1.0x10^3
1.0x10^2
1.0x10^1
0
5
10
15
Time after challenge (days)

Fig. 24

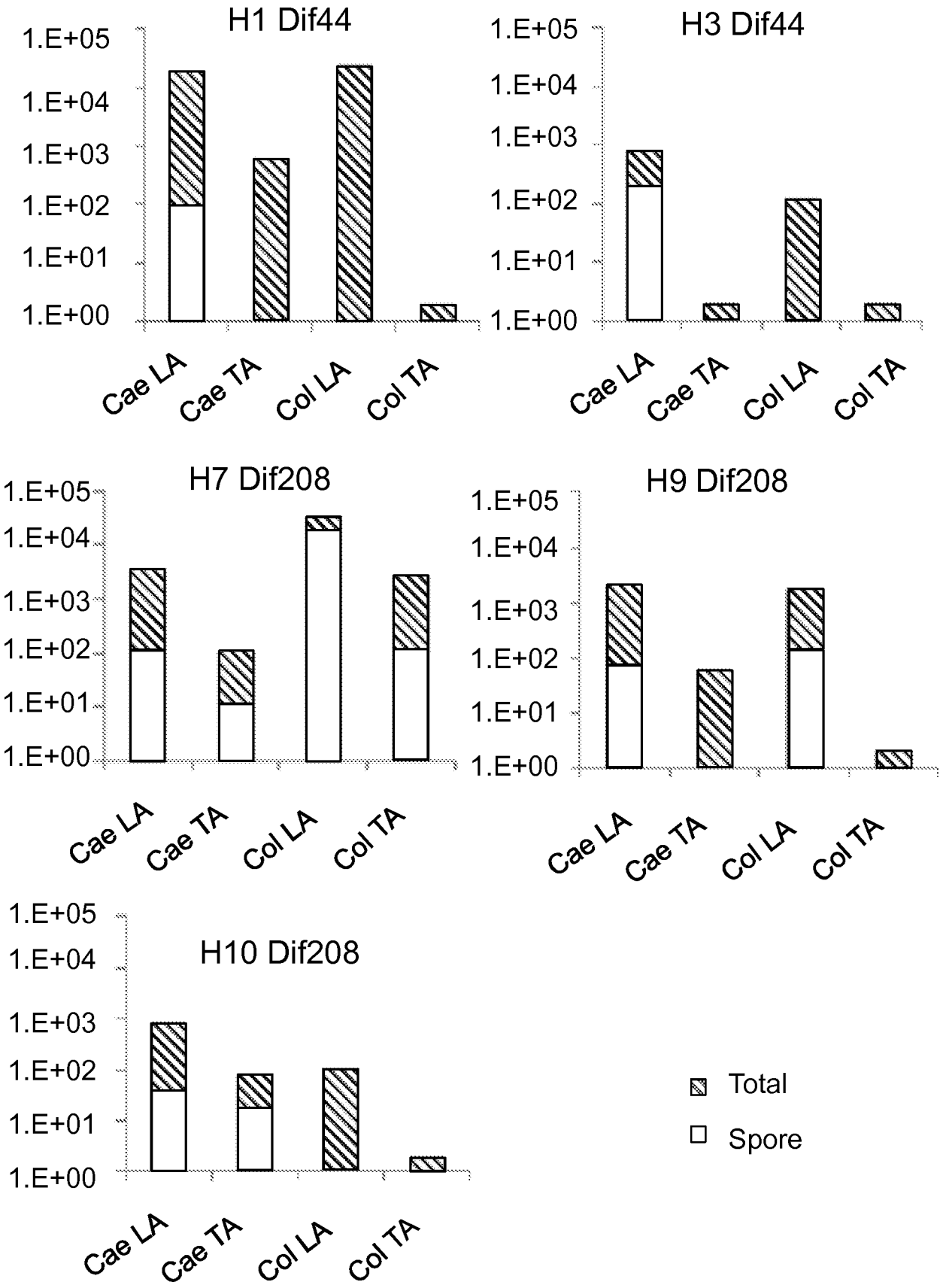

H1 Dif44
1.E+05
1.E+04
1.E+03
1.E+02
1.E+01
1.E+00
Cae LA
Cae TA
Col LA
Col TA
H3 Dif44
1.E+05
1.E+04
1.E+03
1.E+02
1.E+01
1.E+00
Cae LA
Cae TA
Col LA
Col TA
H7 Dif208
1.E+05
1.E+04
1.E+03
1.E+02
1.E+01
1.E+00
Cae LA
Cae TA
Col LA
Col TA
H9 Dif208
1.E+05
1.E+04
1.E+03
1.E+02
1.E+01
1.E+00
Cae LA
Cae TA
Col LA
Col TA
H10 Dif208
1.E+05
1.E+04
1.E+03
1.E+02
1.E+01
1.E+00
Cae LA
Cae TA
Col LA
Col TA
Total
Spore

Fig. 25

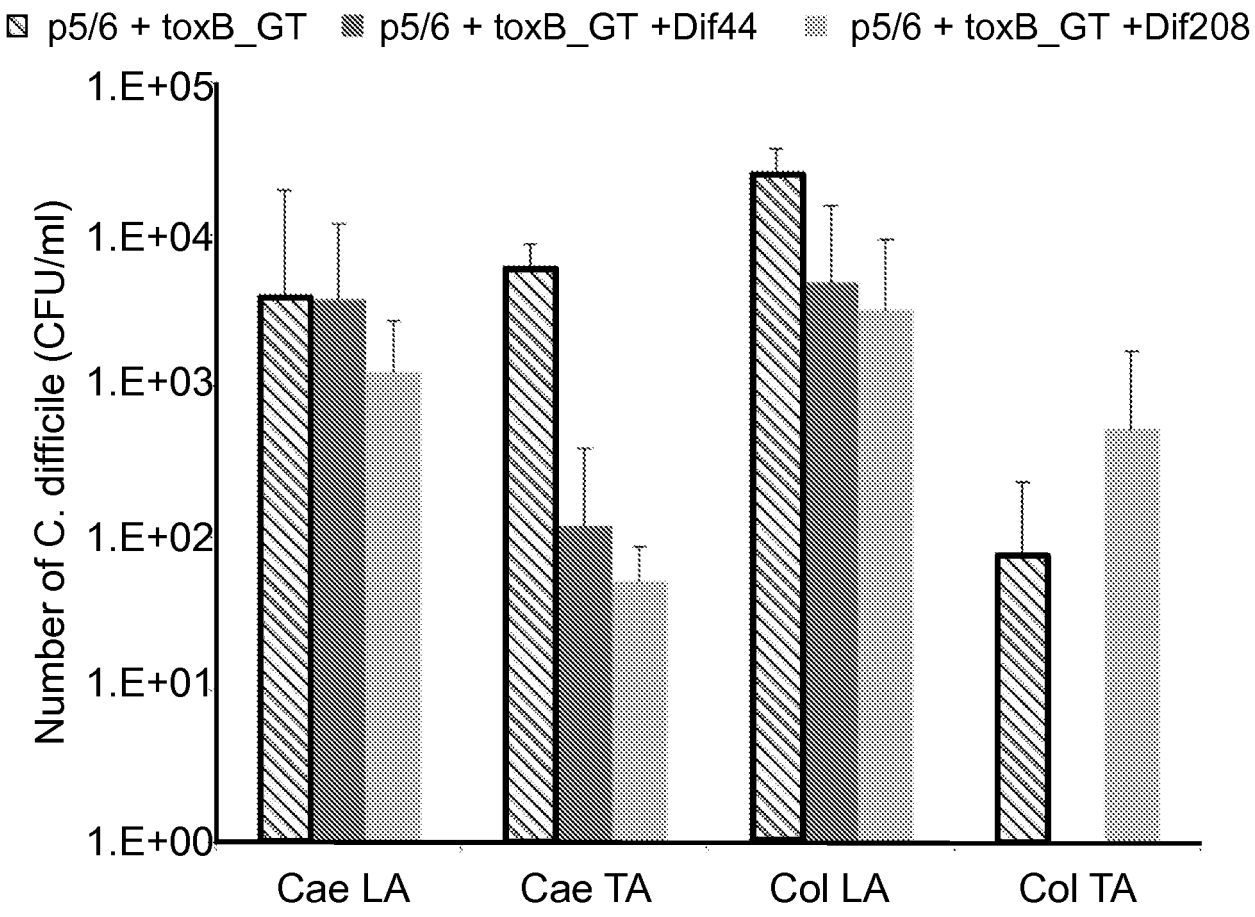

p5/6 + toxB_GT
p5/6 + toxB_GT +Dif44
p5/6 + toxB_GT +Dif208
Number of C. difficile (CFU/ml)
1.E+05
1.E+04
1.E+03
1.E+02
1.E+01
1.E+00
Cae LA
Cae TA
Col LA
Col TA

Fig. 26

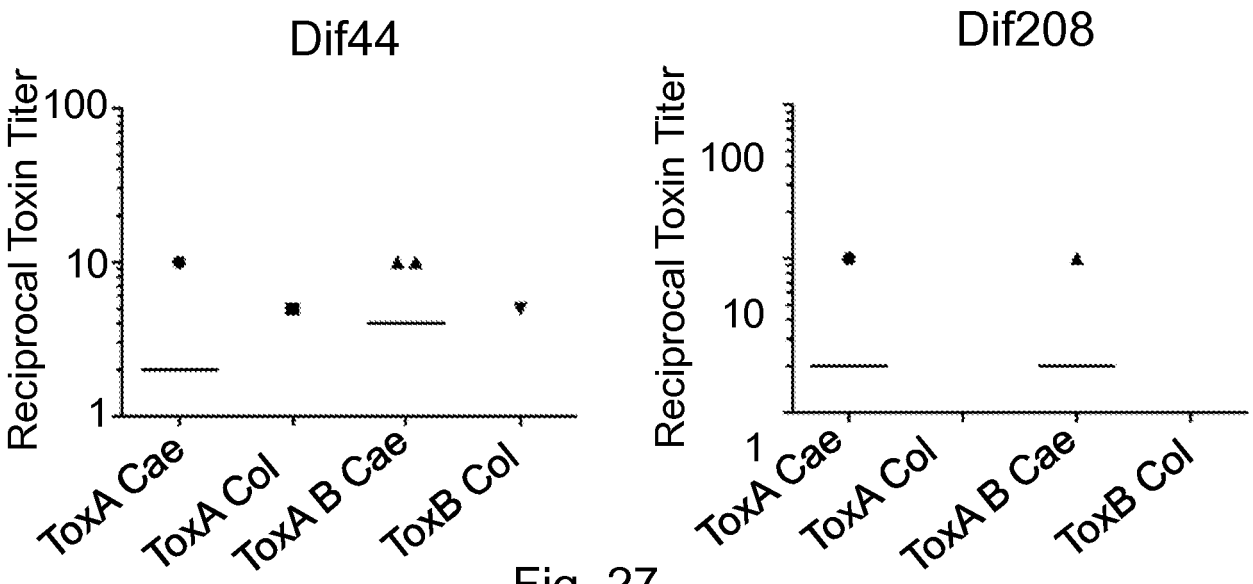

Dif44
Reciprocal Toxin Titer
100
10
1
ToxA Cae
ToxA Col
ToxA B Cae
ToxB Col
Dif208
Reciprocal Toxin Titer
100
10
1
ToxA Cae
ToxA Col
ToxA B Cae
ToxB Col

Fig. 27

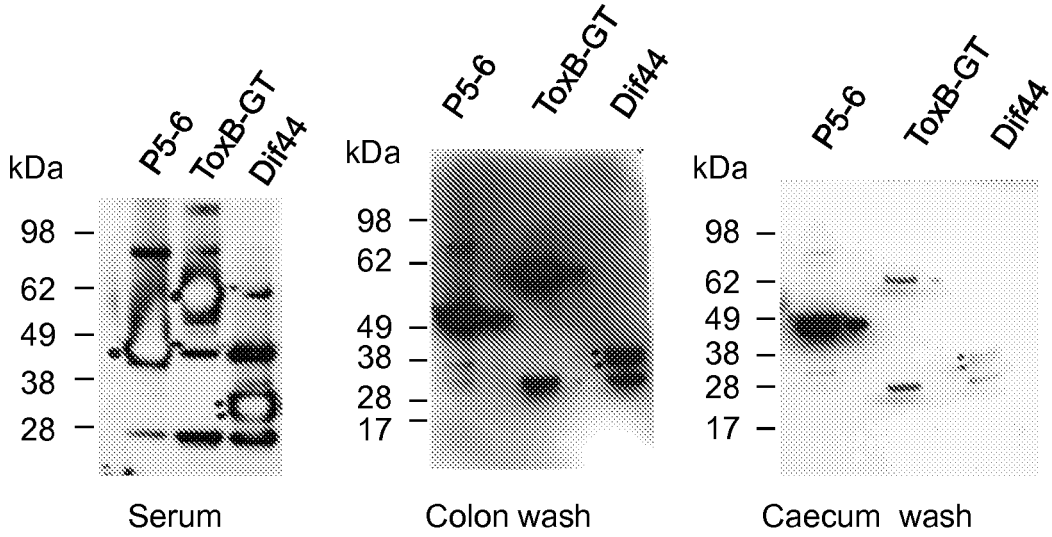

P5-6
ToxB-GT
Dif44
kDa
98
62
49
38
28
17
Serum
Colon wash
Caecum wash

Fig. 28

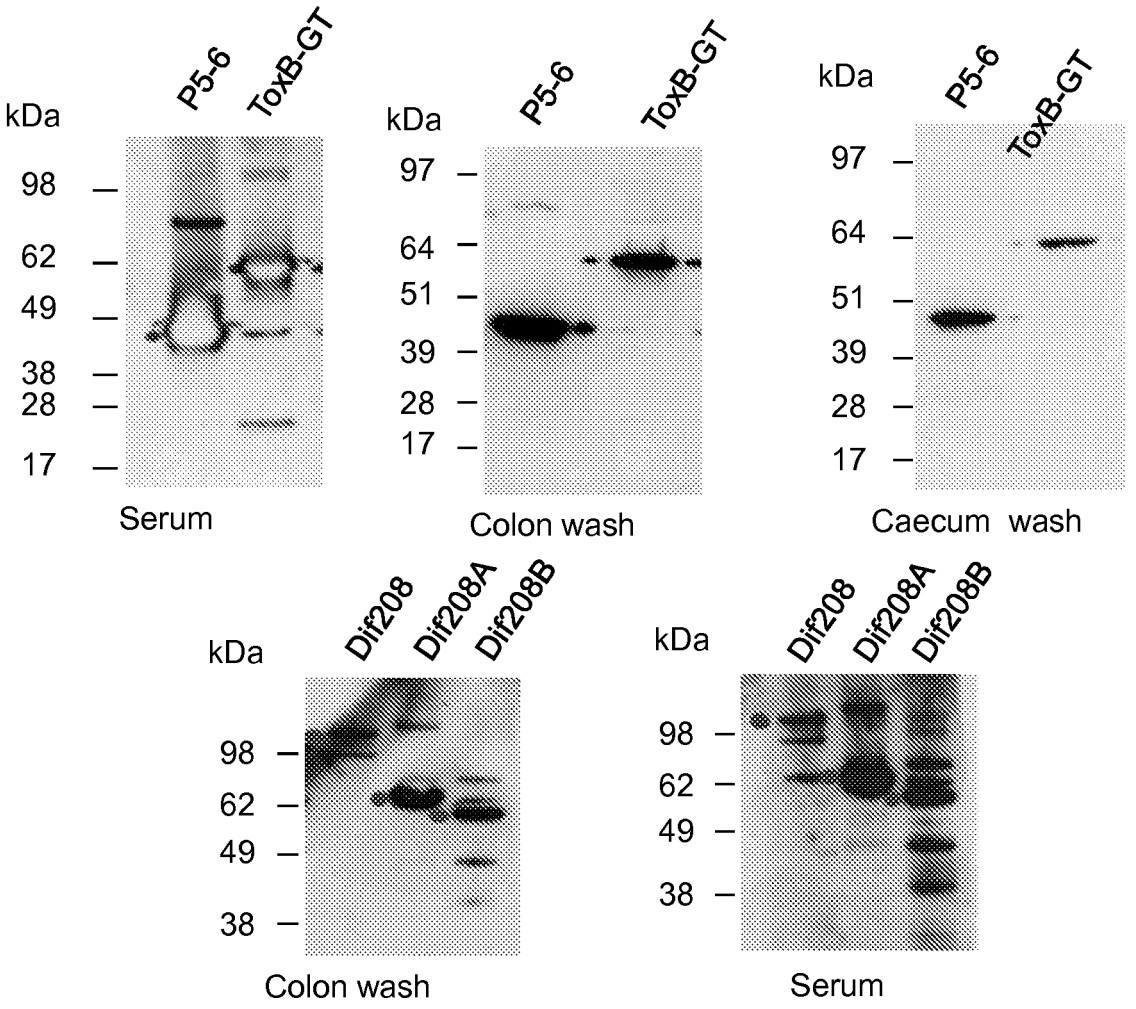

P5-6
ToxB-GT
Dif208
Dif208A
Dif208B
kDa
98
97
64
62
51
49
39
38
28
17
Serum
Colon wash
Caecum wash

Fig. 29

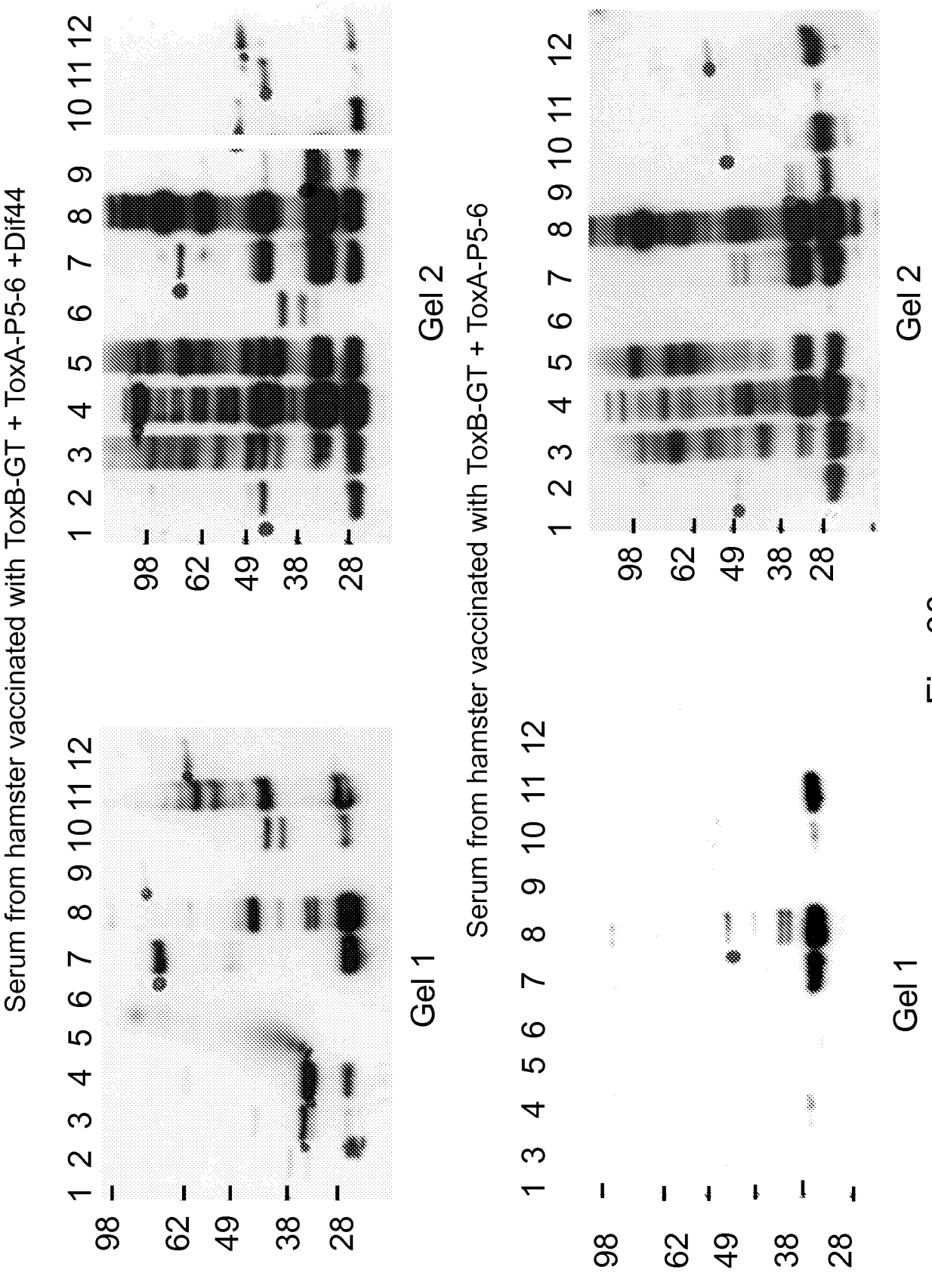

Serum from hamster vaccinated with ToxB-GT + ToxA-P5-6 +Dif44
1 2 3 4 5 6 7 8 9 10 11 12
98
62
49
38
28
Gel 1
Gel 2
Serum from hamster vaccinated with ToxB-GT + ToxA-P5-6

Fig. 30