

Figure 1

	1	2	3	4	5	6	7	8	9	10	11	12
A	E1-3A1	ET1-3B1	P3-2B8	P4-2F8	TT1-3C6	TT5-3E2	E1-3A1	ET1-3B1	P3-2B8	P4-2F8	TT1-3C6	TT5-3E2
B	E1-3B4	ET1-3D6	P3-2E11	T1-3D1	TT1-3C8	TT5-3E5	E1-3B4	ET1-3D6	P3-2E11	T1-3D1	TT1-3C8	TT5-3E5
C	E1-3E5	ET1-3E12	P3-2H7	T1-3G7	TT1-3D6	TT5-3E7	E1-3E5	ET1-3E12	P3-2H7	T1-3G7	TT1-3D6	TT5-3E7
D	E1-3E9	ET1-3F2	P4-2A6	T5-3A1	TT1-3E8	TT5-3G3	E1-3E9	ET1-3F2	P4-2A6	T5-3A1	TT1-3E8	TT5-3G3
E	E1-3F6	P1-2A11	P4-2B7	T5-3A7	TT1-3F5	TT5-3H7	E1-3F6	P1-2A11	P4-2B7	T5-3A7	TT1-3F5	TT5-3H7
F	E1-3H7	P2-2B5	P4-2C8	T5-3B12	TT1-3F6	2XYT	E1-3H7	P2-2B5	P4-2C8	T5-3B12	TT1-3F6	2XYT
G	E5-3B2	P2-2D12	P4-2F1	T5-3D4	TT1-3F9	2XYT	E5-3B2	P2-2D12	P4-2F1	T5-3D4	TT1-3F9	2XYT
H	2 X YT	P2-2E10	P4-2F2	T5-3H11	2 X YT	2 X YT	2 X YT	P2-2E10	P4-2F2	T5-3H11	2 X YT	2 X YT

Plate 1: Coating pattern

A. hGfTR-His (200ug/ml; 50ul + 4950ulPBS) 100ul/well						B. hGfTR-mIgG2a (1mg/ml; 10ul + 4990ul PBS) 100ul/well					
1	2	3	4	5	6	7	8	9	10	11	12

Plate 2: Coating pattern

ΑΜΟΔΕΛΟΤ						1	2	3	4	5	6	7	8	9	10	11	12
						3. mGfTR-His (400ug/ml; 25ul + 4975ulPBS) 100ul/well						D. mlgG2a (1mg/ml; 10ul + 4990ul PBS) 100ul/well					

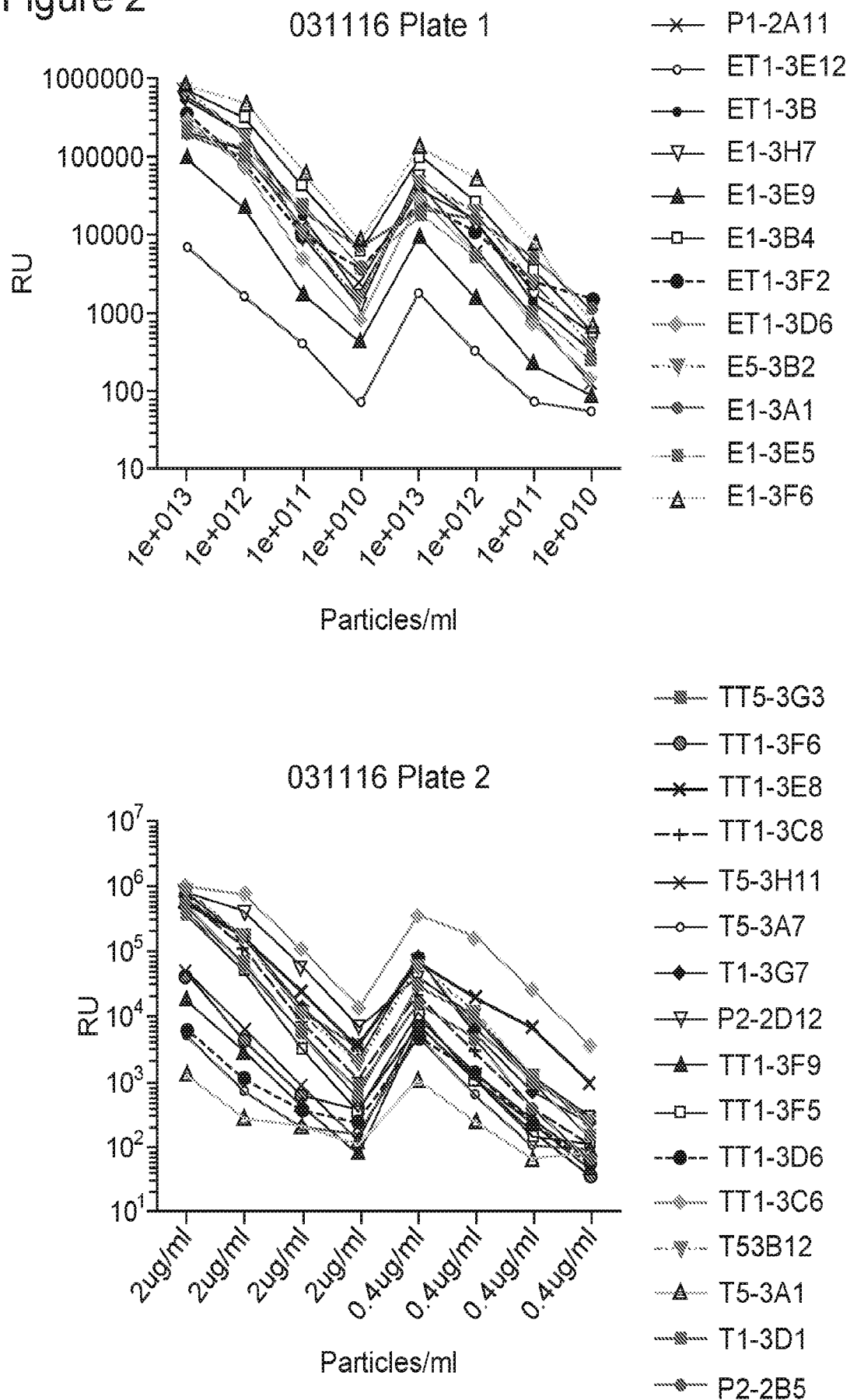
2/15

Figure 1 cont.

Plate 1	hGITR-His	hGITR-His	hGITR-His	hGITR-His	hGITR-His	hGITR-miG	hGITR-miG	hGITR-miG	hGITR-miG	hGITR-miG	hGITR-miG	hGITR-miG
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.052	1.828	0.616	0.048	1.996	0.254	1.895	1.936	0.062	0.054	2.156	0.973
B	1.087	1.097	0.051	0.209	1.405	0.046	2.171	1.974	0.044	1.533	1.845	1.534
C	0.763	0.091	0.047	0.412	1.983	0.045	1.992	1.789	0.053	2.086	2.146	2.157
D	0.821	0.052	1.982	0.047	0.054	0.048	1.949	1.908	0.046	0.052	2.481	1.563
E	1.855	2.085	0.488	0.047	1.131	0.049	2.197	2.473	0.492	0.045	1.452	1.478
F	1.957	1.261	0.054	0.217	0.048	0.094	2.165	1.698	0.046	1.763	1.253	0.043
G	1.950	0.671	0.067	0.049	0.046	0.044	2.071	2.014	0.047	0.045	0.425	0.044
H	0.057	0.374	1.407	0.060	0.054	0.045	0.056	0.462	1.282	1.264	0.054	0.053
Plate 2	mGITR-His	mGITR-His	mGITR-His	mGITR-His	mGITR-His	mGITR-His	mlgG2a	mlgG2a	mlgG2a	mlgG2a	mlgG2a	mlgG2a
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.071	0.053	1.450	0.423	0.052	0.047	0.066	0.058	0.116	0.048	0.106	0.072
B	0.050	0.047	0.044	0.046	0.043	0.042	0.044	0.608	0.056	0.069	0.061	1.861
C	0.069	0.047	1.854	0.044	0.046	0.044	0.048	0.077	0.188	0.050	0.041	0.054
D	0.048	0.058	2.175	0.041	0.045	0.047	0.078	0.046	0.053	0.043	0.048	0.455
E	0.063	0.049	0.366	0.042	0.045	0.044	0.140	0.064	0.601	0.088	0.067	0.050
F	0.055	0.050	1.985	0.049	0.047	0.043	0.044	0.124	0.049	0.046	0.519	0.054
G	0.057	0.055	0.938	0.061	0.043	0.045	0.047	0.045	0.217	0.064	0.046	0.051
H	0.051	0.112	0.160	0.054	0.051	0.053	0.047	1.083	0.395	0.433	0.049	0.050

3/15

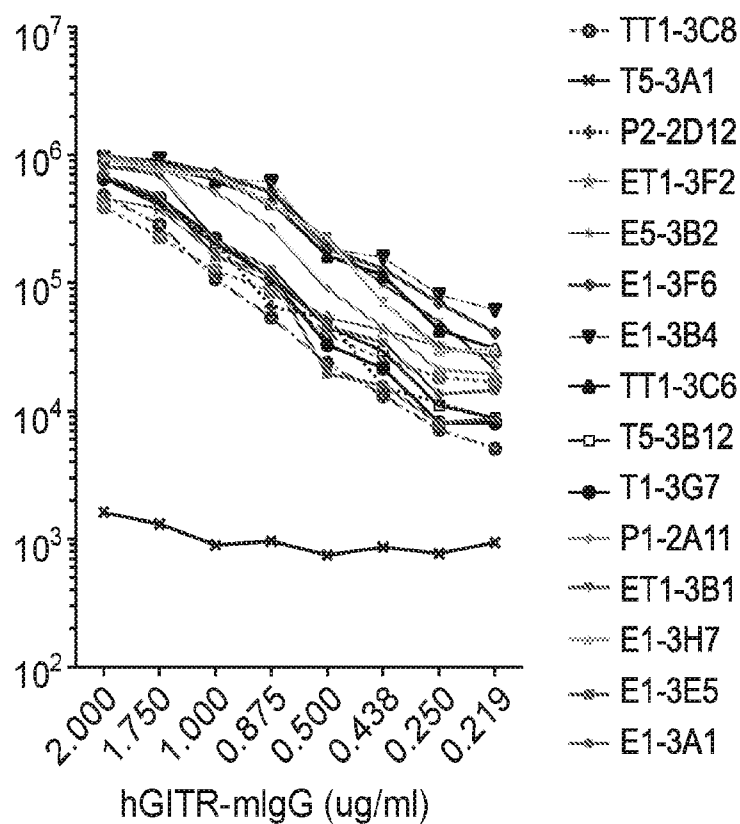
Figure 2



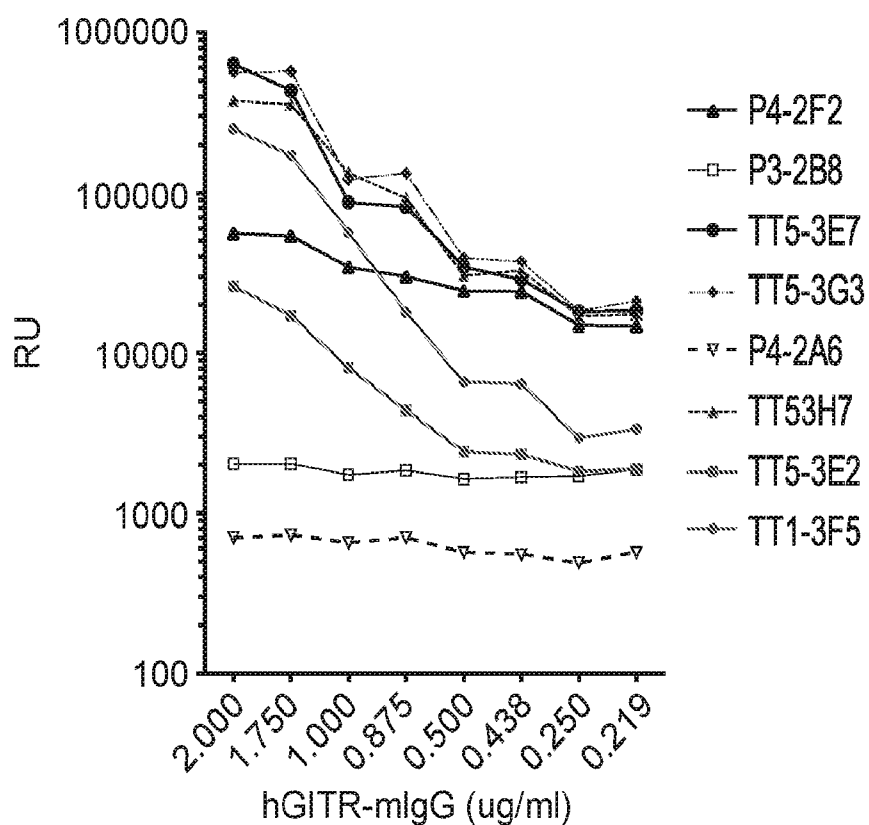
4/15

Figure 3

031816 Plate 1



031816 Plate 2



5/15

Figure 4

CL-354 (G1TR stable CHO cell pool)										CL-355 (CA9 stable CHO cell pool)		
1	2	3	4	5	6	7	8	9	10	11	12	
A	E1-3B4	4.00E+12	4.00E+11	4.00E+10	P2-2D12	4.00E+12	4.00E+11	4.00E+10	TT5-3E7	E1-3B4	P2-2D12	TT5-3E7
B	E1-3E5	4.00E+12	4.00E+11	4.00E+10	TT1-3C6	4.00E+12	4.00E+11	4.00E+10	TT5-3G3	E1-3E5	TT1-3C6	TT5-3G3
C	E1-3E9	4.00E+12	4.00E+11	4.00E+10	TT1-3C8	4.00E+12	4.00E+11	4.00E+10	TT5-3H7	E1-3E9	TT1-3C8	TT5-3H7
D	E1-3F6	4.00E+12	4.00E+11	4.00E+10	TT1-3F5	4.00E+12	4.00E+11	4.00E+10	ET1-3F2	E1-3F6	TT1-3F5	ET1-3F2
E	E1-3H7	4.00E+12	4.00E+11	4.00E+10	P1-2A11	4.00E+12	4.00E+11	4.00E+10	P4-2F2	E1-3H7	P1-2A11	P4-2F2
F	E5-3B2	4.00E+12	4.00E+11	4.00E+10	P2-2B5	4.00E+12	4.00E+11	4.00E+10	anti-G1TR	E5-3B2	P2-2B5	anti-G1TR
G	ET1-3B1	4.00E+12	4.00E+11	4.00E+10	TT5-3E2	4.00E+12	4.00E+11	4.00E+10	anti-CA9	ET1-3B1	TT5-3E2	anti-CA9
H	ET1-3E12	4.00E+12	4.00E+11	4.00E+10	T5-3A1*	4.00E+12	4.00E+11	4.00E+10	anti-M13	ET1-3E12	T5-3A1*	anti-M13

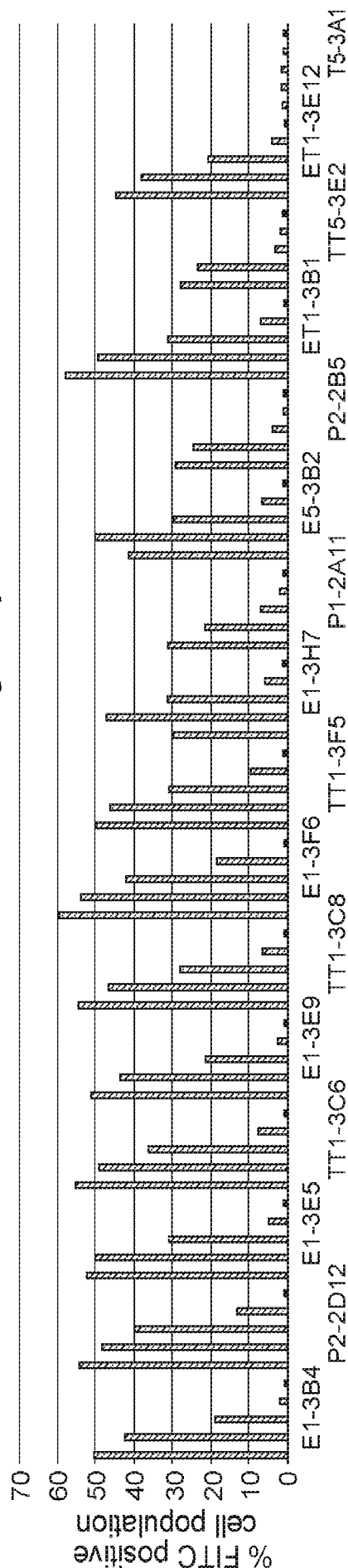
The 2nd FACS - G1TR binding assay 04/07/2016

Figure 5

The 3rd FACS - GTR binding assay 04/14/2016
Single concentration with phage supernatant from single colonies

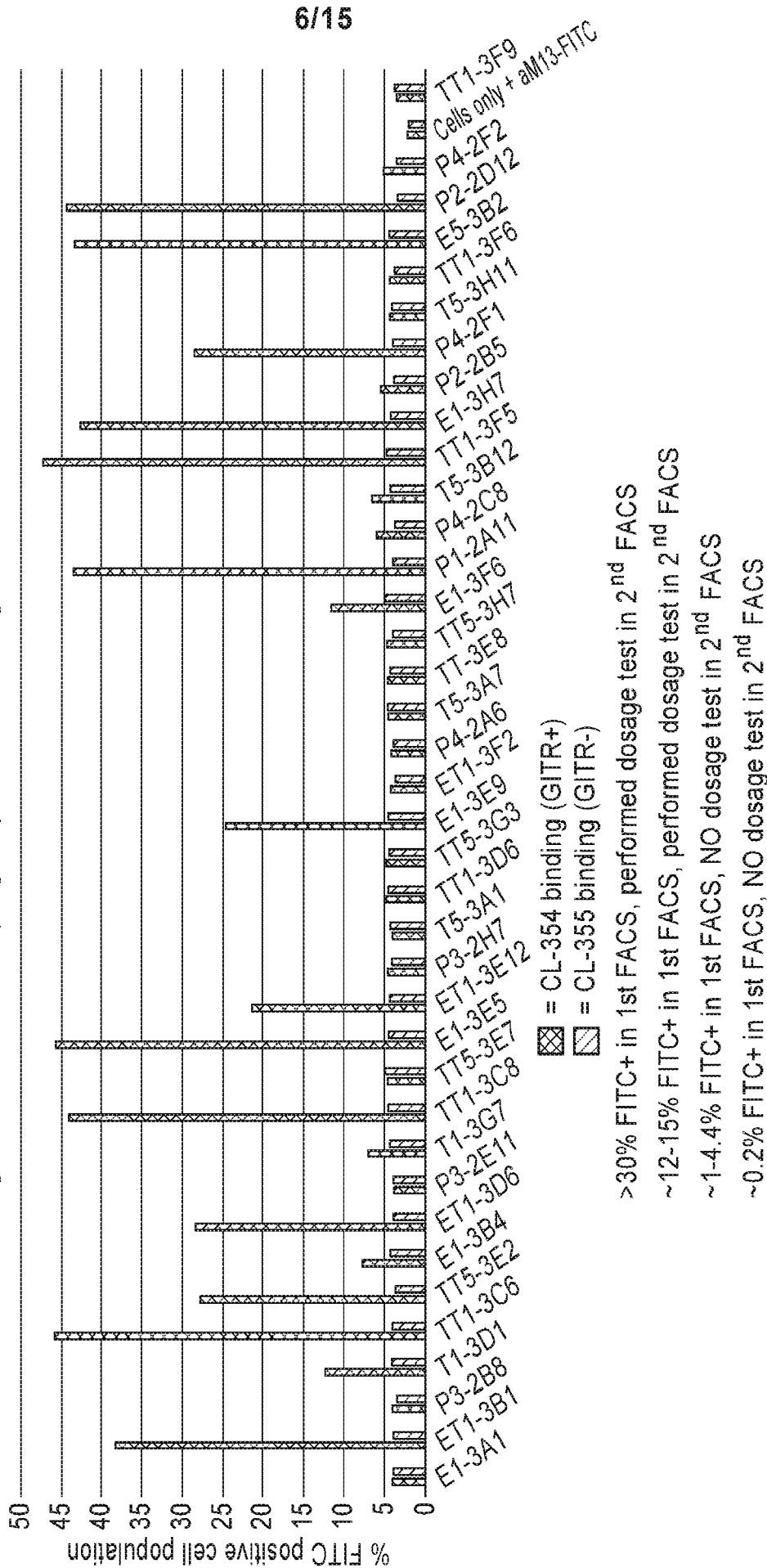


Figure 6

Plate 1 & 2 Clone map

	1	2	3	4	5	6	7	8	9	10	11	12
A	E1-3A1	ET1-3B1	P3-2B8	T1-3D1	TT1-3C6	TT5-3E2	E1-3A1	ET1-3B1	P3-2B8	T1-3D1	TT1-3C6	TT5-3E2
B	E1-3B4	ET1-3D6	P3-2E11	T1-3G7	TT1-3C8	TT5-3E7	E1-3B4	ET1-3D6	P3-2E11	T1-3G7	TT1-3C8	TT5-3E7
C	E1-3E5	ET1-3E12	P3-2H7	T5-3A1	TT1-3D6	TT5-3G3	E1-3E5	ET1-3E12	P3-2H7	T5-3A1	TT1-3D6	TT5-3G3
D	E1-3E9	ET1-3F2	P4-2A6	T5-3A7	TT1-3E8	TT5-3H7	E1-3E9	ET1-3F2	P4-2A6	T5-3A7	TT1-3E8	TT5-3H7
E	E1-3F6	P1-2A11	P4-2C8	T5-3B12	TT1-3F5		E1-3F6	P1-2A11	P4-2C8	T5-3B12	TT1-3F5	
F	E1-3H7	P2-2B5	P4-2F1	T5-3H11	TT1-3F6		E1-3H7	P2-2B5	P4-2F1	T5-3H11	TT1-3F6	
G	E5-3B2	P2-2D12	P4-2F2		TT1-3F9		E5-3B2	P2-2D12	P4-2F2		TT1-3F9	
H												

Plate 1: Coating pattern

1	2	3	4	5	6	7	8	9	10	11	12
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7/15

A	A. hG1TR-His (2100ug/ml: 50ul + 4950ulPBS) 100ul/well												B. hG1TR-mIgG2a (1mg/ml: 10ul + 4990ul PBS) 100ul/well											
B																								
C																								
D																								
E																								
F																								
G																								
H																								

Plate 2: Coating pattern

1	2	3	4	5	6	7	8	9	10	11	12
---	---	---	---	---	---	---	---	---	----	----	----

A	C. hG1TR-His (400ug/ml: 25ul + 4975ulPBS) 100ul/well												B. mlgG2a (1mg/ml: 10ul + 4990ul PBS) 100ul/well											
B																								
C																								
D																								
E																								
F																								
G																								
H																								

8/15

Figure 6 (cont.)

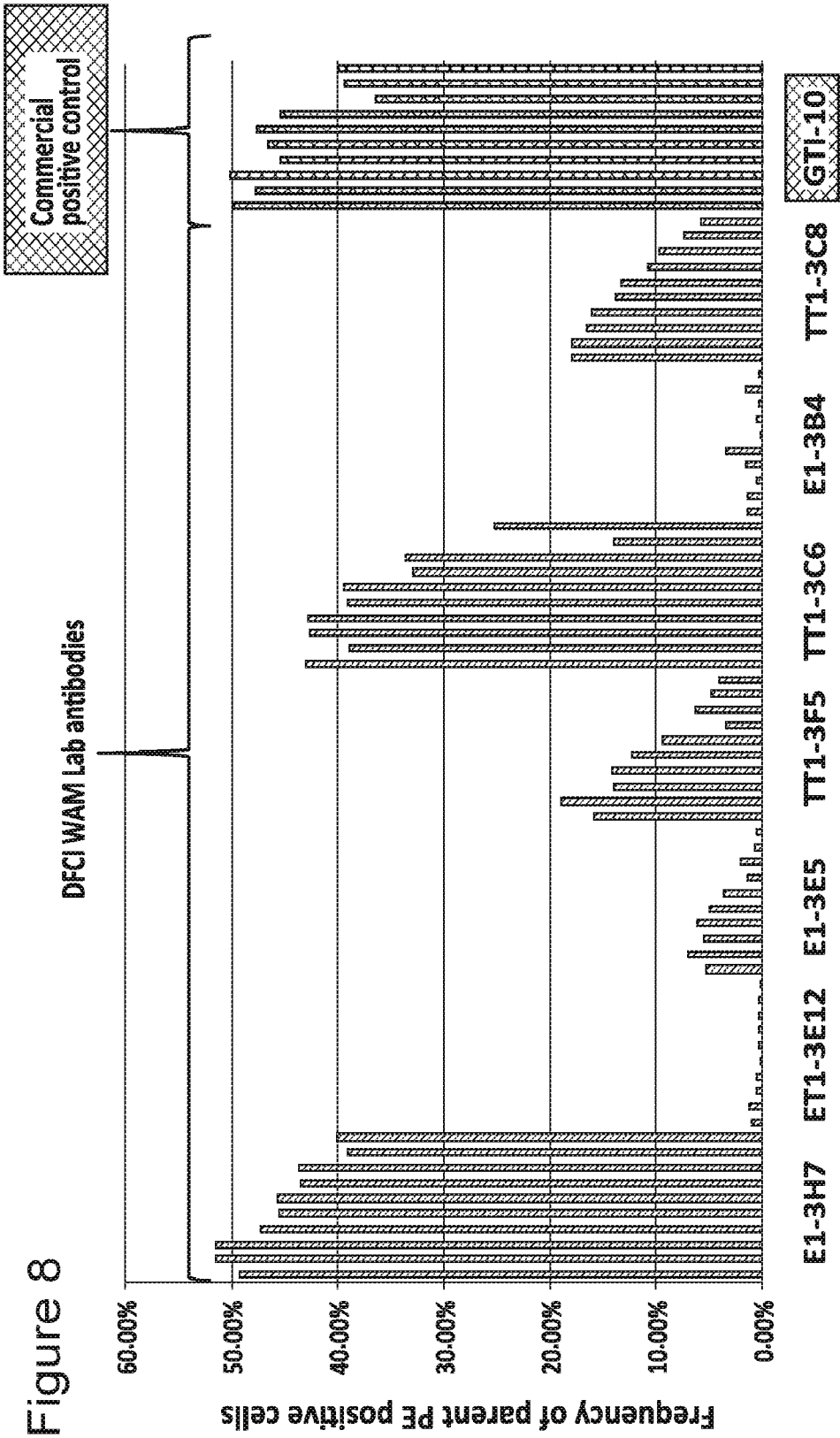
Plate 1	1	2	3	4	5	6	7	8	9	10	11	12
A	0.058	1.593	0.175	0.148	1.440	0.217	1.361	1.340	0.056	0.779	1.297	1.025
B	0.554	0.834	0.306	0.131	0.728	0.076	1.556	1.341	0.105	1.205	1.228	1.328
C	0.612	0.073	0.058	0.065	0.063	0.063	1.813	0.709	0.058	0.057	0.078	1.029
D	0.478	0.078	0.428	0.056	0.071	0.056	1.419	0.706	0.066	0.073	0.908	1.665
E	1.714	1.286	0.130	0.186	1.839	0.053	1.952	1.663	0.958	1.085	1.980	0.051
F	1.143	0.071	1.266	0.063	0.048	0.058	1.585	0.060	1.474	0.792	0.857	0.084
G	1.261	0.504	0.088	0.086	0.705	0.083	1.624	1.709	0.484	0.055	0.792	0.073
H	0.048	0.057	0.053	0.063	0.048	0.082	0.082	0.101	0.050	0.060	0.048	0.053

Plate 2	1	2	3	4	5	6	7	8	9	10	11	12
A	0.067	0.233	0.834	0.054	0.052	0.049	1.304	0.316	0.818	0.065	0.679	0.085
B	0.055	0.047	0.563	0.044	0.044	0.043	0.056	0.125	0.051	0.049	0.306	1.712
C	0.049	0.048	1.511	0.044	0.043	0.044	0.050	0.066	0.110	0.055	0.305	0.947
D	0.047	0.049	0.921	0.045	0.044	0.046	0.162	0.745	0.096	0.718	1.044	1.864
E	0.054	0.052	0.049	0.044	0.046	0.044	0.927	0.520	0.046	0.052	1.127	0.055
F	0.048	0.282	0.053	0.044	0.043	0.045	0.399	0.057	0.050	0.604	0.718	0.056
G	0.059	0.048	0.046	0.044	0.087	0.046	0.410	0.051	0.347	0.050	0.249	0.055
H	0.096	0.060	0.055	0.051	0.047	0.044	0.049	0.051	0.052	0.049	0.056	0.063

9/15

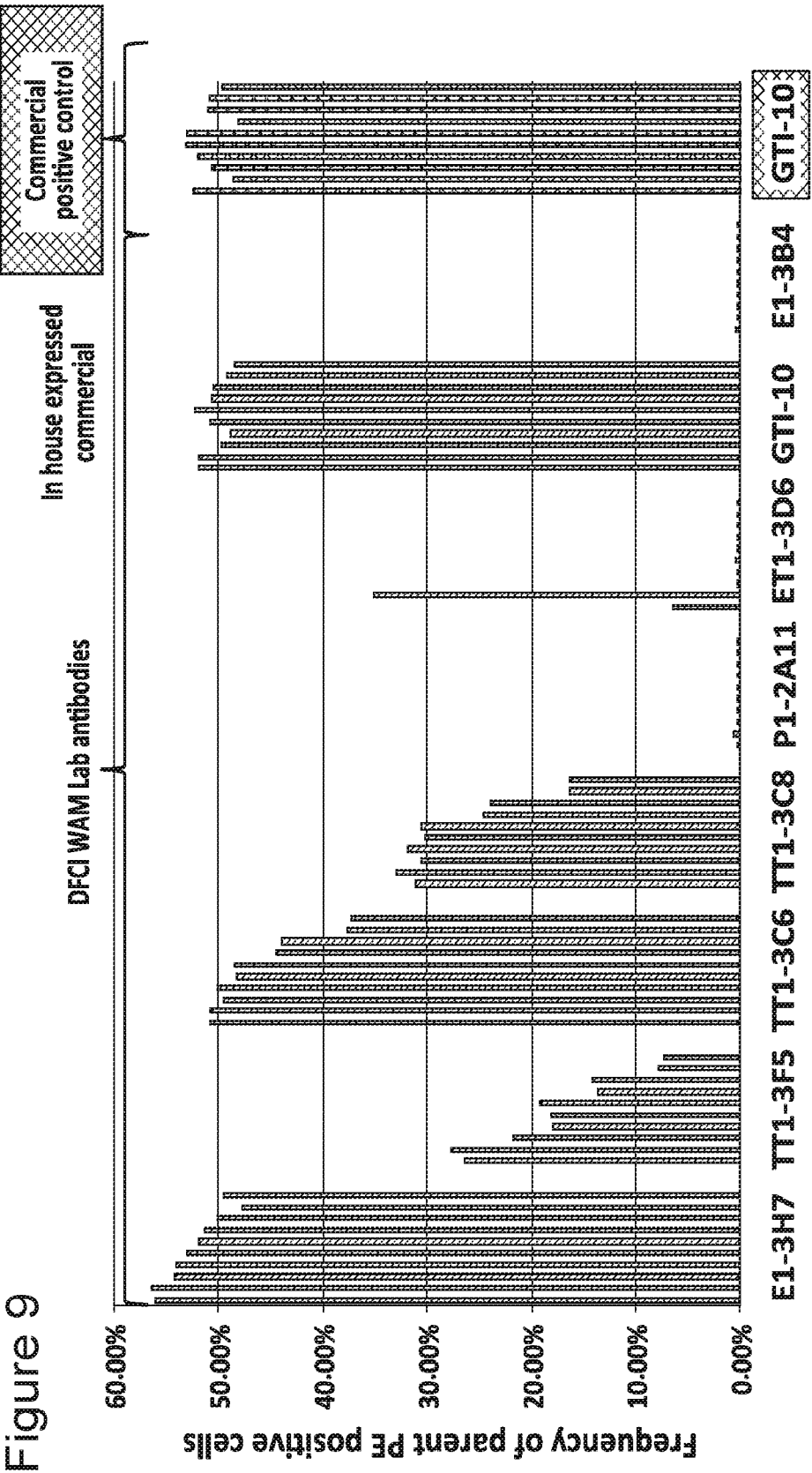
Figure 7

	4/20/16 ELISA	4/14/16 FACS	Previous dose response FACS
E1-3B4	++++	+	YES
E1-3E5	++++	++++	YES
E1-3E9	+++	++	YES
E1-3F6	++++/++	+	YES
E1-3H7	++++/+	++++	YES
E5-3B2	++++/+	++++	YES
ET1-3B1	+++/+	+++	YES
ET1-3D6	+++	++	
ET1-3E12	++	++	YES
P1-2A11	++++/++	++++	YES
P2-2D12	++++	++++	YES
P4-2F1	+++	++	
T1-3G7	+++	+	
TT1-3C6	+++/>++	++++	YES
TT1-3C8	+++/>+	++++	YES
TT1-3F5	++++/+++	++++	YES
TT5-3E2	+++	++	YES

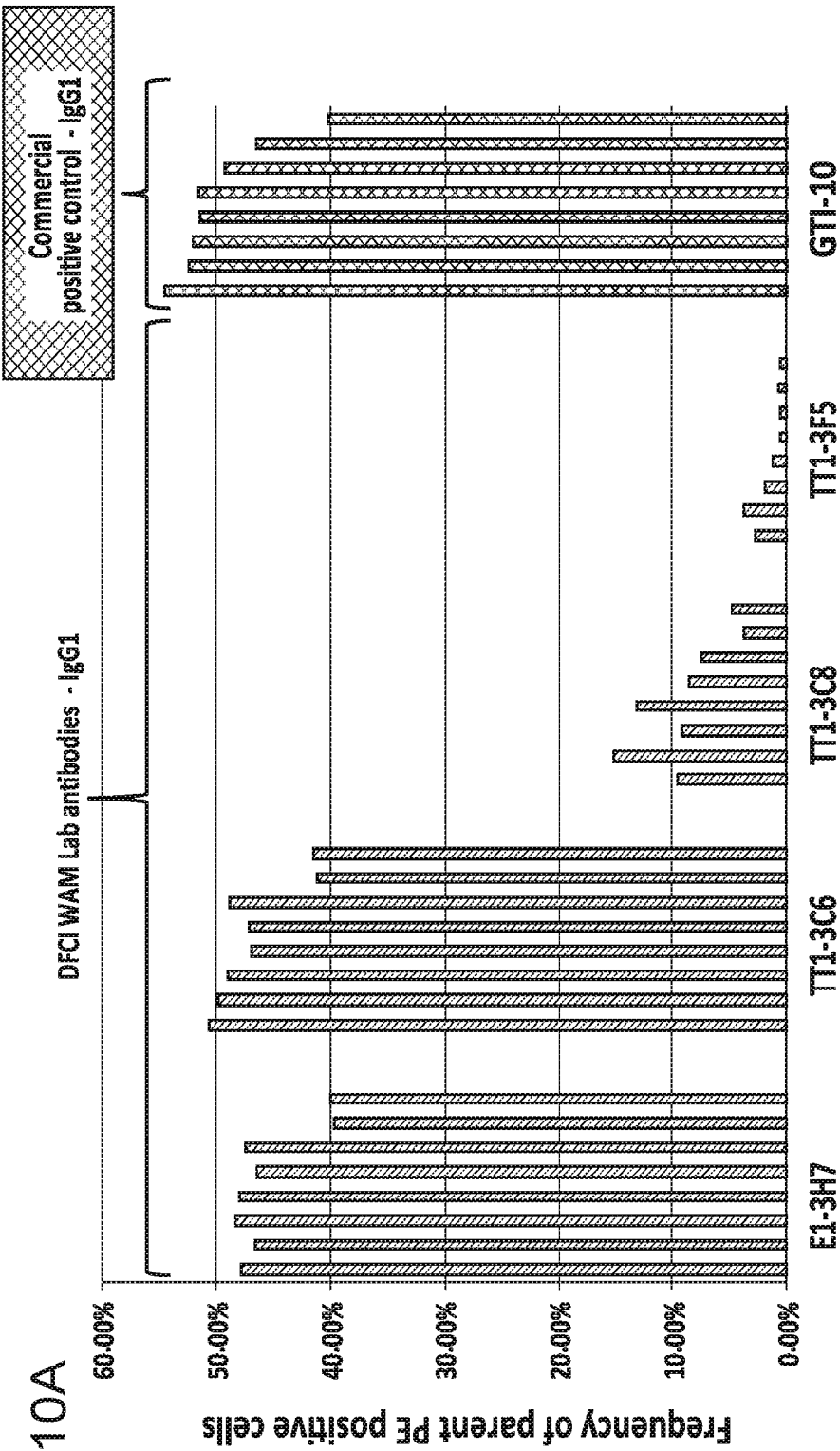


Anti-GITR mAb binding to the GITR(+) CHO cell lines were tested by 5-point 2X serial dilutions in duplicates. Each mAb starting concentration was 1.5ug/ml except that ET1-3F5 started 1 ug/ml & TT1-3C6 started at 0.75 ug/ml. Note that the highest concentration of each mAb showed no binding to GITR(-) control cells (data not shown).

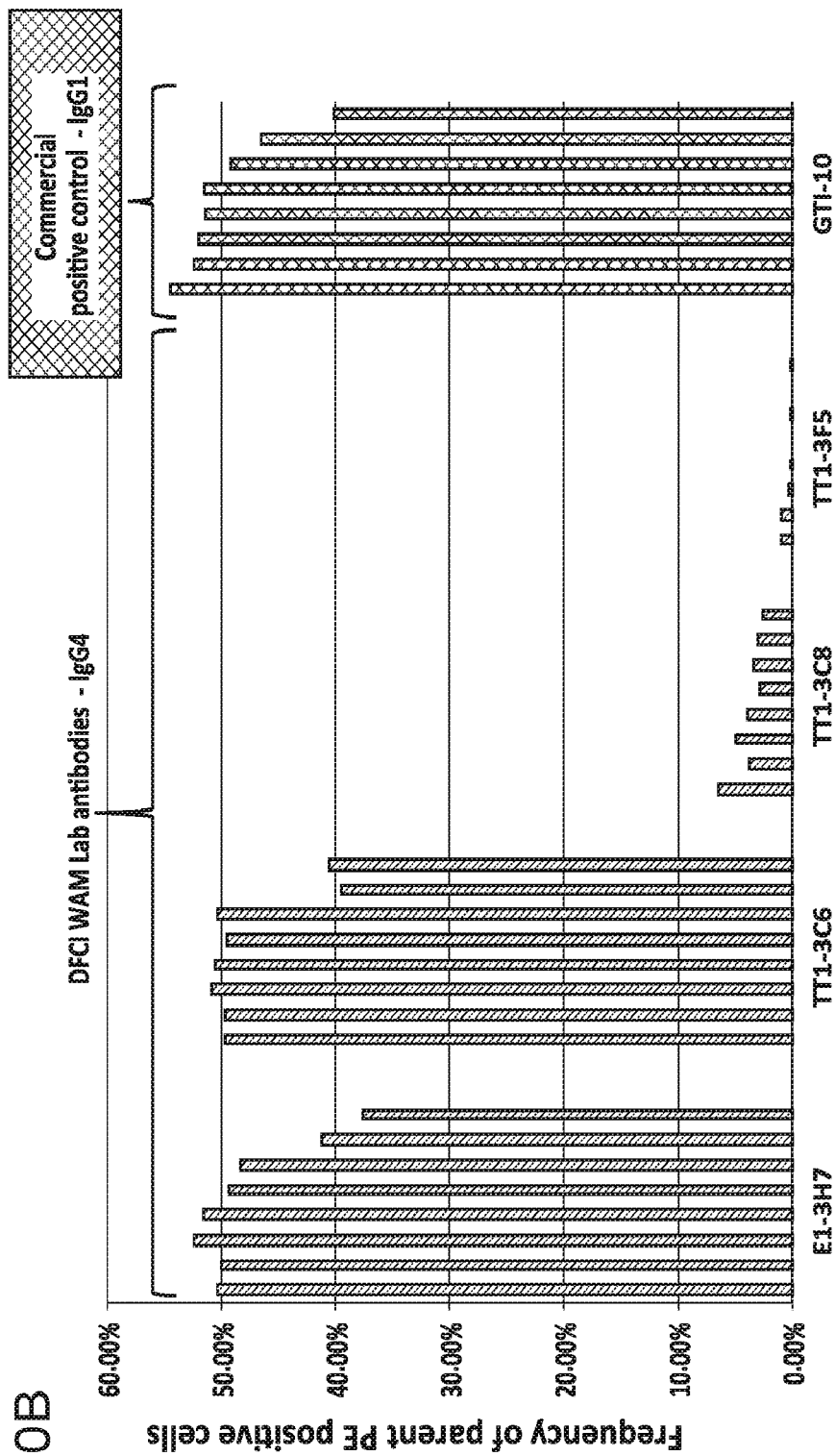
11/15



Anti-GITR mAb binding to the GITR(+) CHO cell lines were tested by 5-point 2X serial dilutions in duplicates. Each mAb starting concentration was 1.5ug/ml except that ET1-3F5 started 0.5 ug/ml, TT1-3C6 and TT1-3C8 started at 0.75ug/ml. Note that the highest concentration of each mAb showed no binding to GITR(-) control cells (data not shown).



Anti-GITR mAb binding to the GITR(+) CHO cell lines were tested by 4-point 2X serial dilutions in duplicates.
Final IgG1-FC(LALA) concentrations in Panel A are: 0.4ug/ml -> 0.2ug/ml -> 0.1ug/ml -> 0.05ug/ml
Except that ET1-3F5 started at 0.25ug/ml dilutions while E1-3H7 & GTI-10 started at 0.7ug/ml.
Final IgG4 concentrations in Panel B are: 0.5ug/ml -> 0.2ug/ml -> 0.1ug/ml -> 0.05ug/ml except that E1-3F5 started at 0.2ug/ml dilutions, GTI-10 IgG1 started at 0.7ug/ml
Note that the highest concentration of each mAb showed no binding to GITR(-) control cells (data not shown).



Anti-GTTR mAb binding to the GTTR(+) CHO cell lines were tested by 4-point 2X serial dilutions in duplicates.

Final IgG1-FC(LALA) concentrations in Panel A are: 0.4ug/ml -> 0.2ug/ml -> 0.1ug/ml -> 0.05ug/ml

Except that ET1-3F5 started at 0.25ug/ml dilutions while E1-3H7 & GTI-10 started at 0.7ug/ml.

Final IgG4 concentrations in Panel B are: 0.5ug/ml -> 0.2ug/ml -> 0.1ug/ml -> 0.05ug/ml except that ET1-3F5 started at 0.2ug/ml dilutions, GTI-10 IgG1 started at 0.7ug/ml

Note that the highest concentration of each mAb showed no binding to GTTR(-) control cells (data not shown).

Figure 11

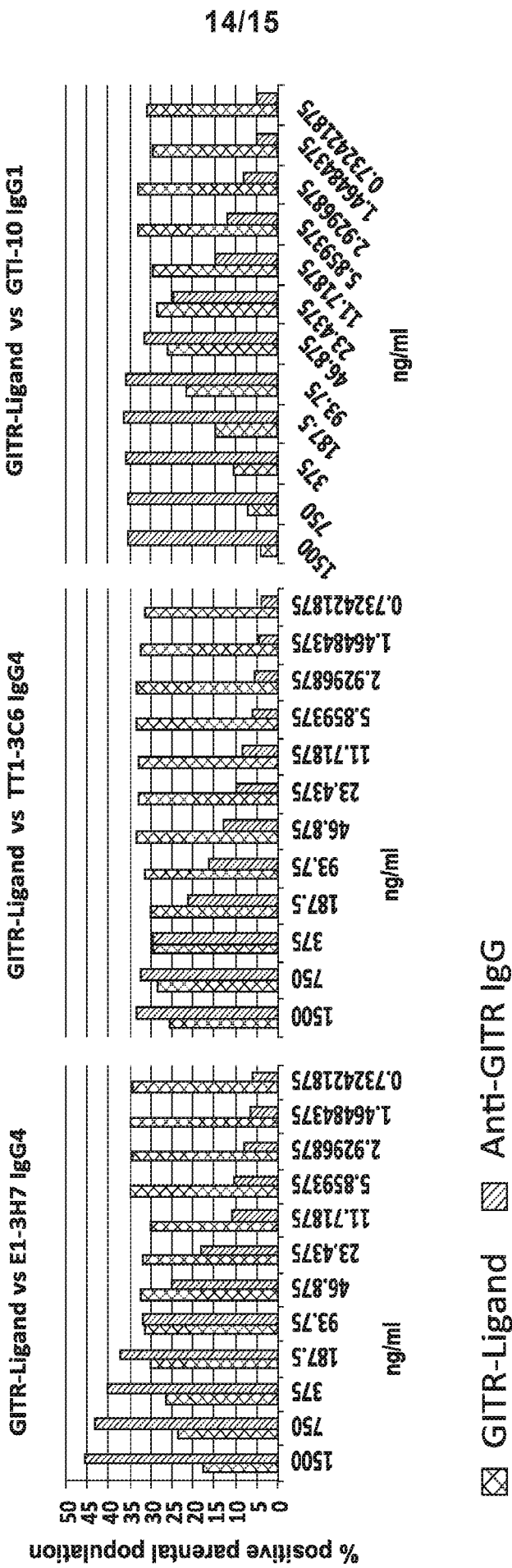


Figure 12

