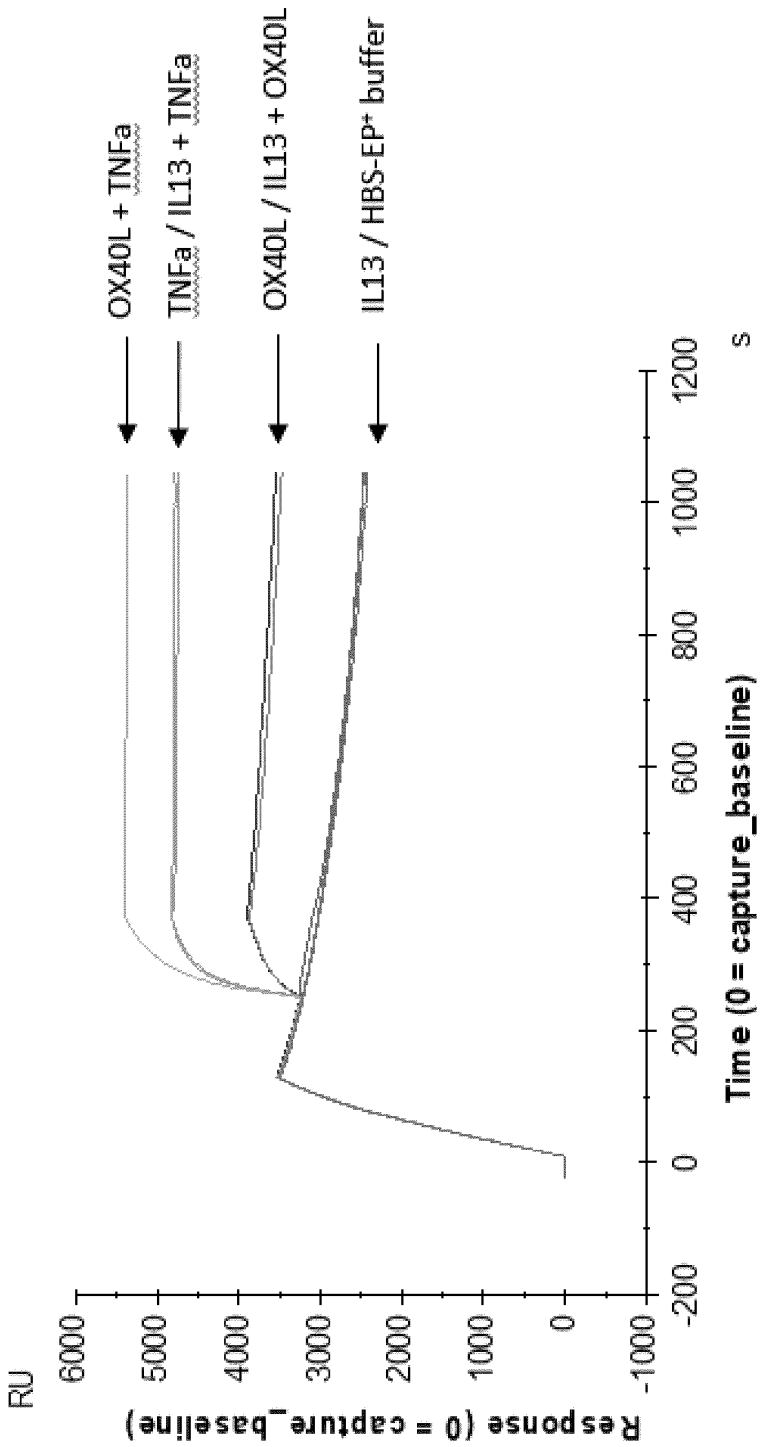

OX40L + TNFa
TNFa / IL13 + TNFa
OX40L / IL13 + OX40L
IL13 / HBS-EP+ buffer
RU
6000
5000
4000
3000
2000
1000
0
-1000
Response (0 = capture_baseline)
-200
0
200
400
600
800
1000
1200
Time (0 = capture_baseline)
s

Figure 1

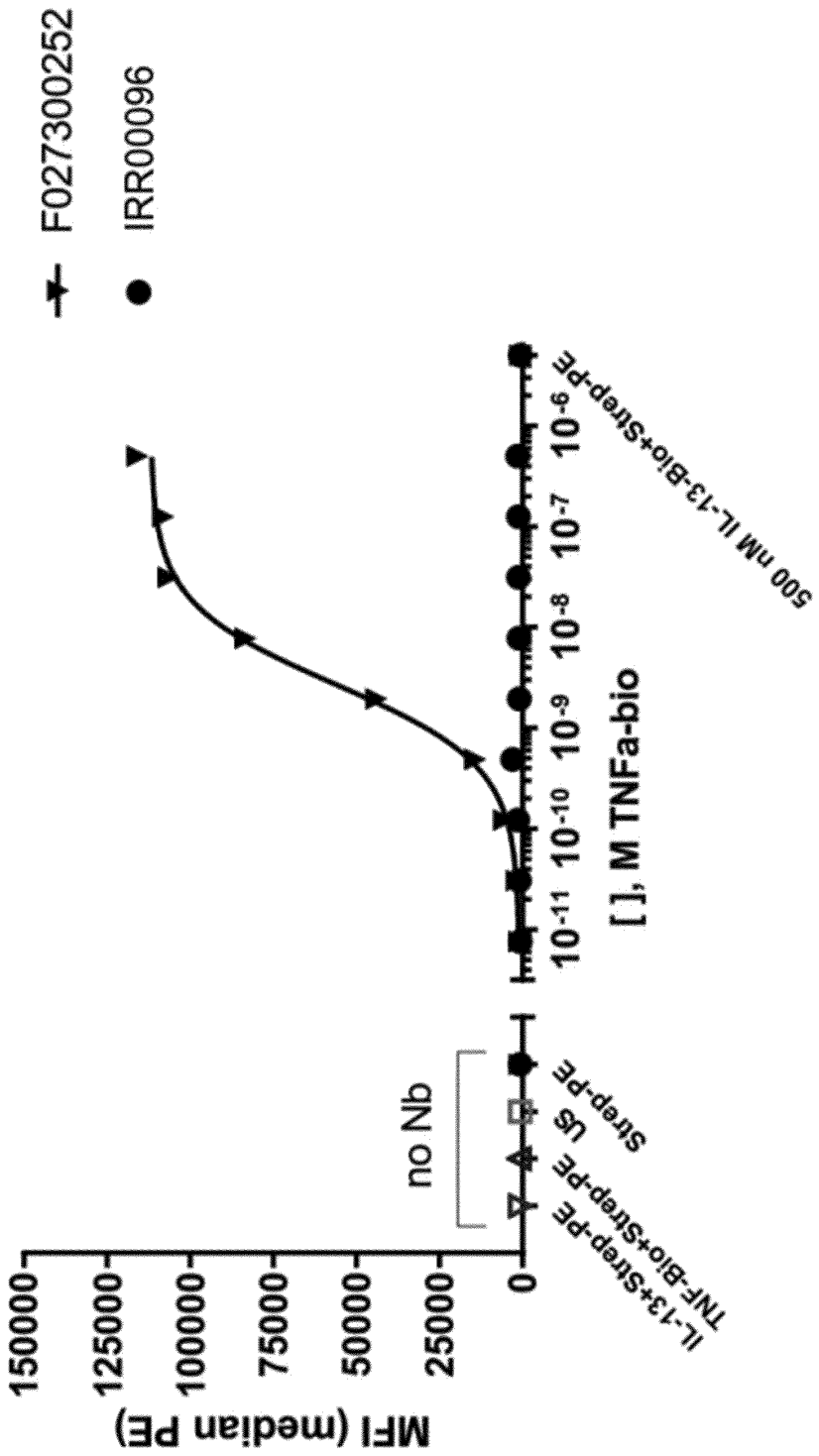

F027300252
IRR00096
MFI (median PE)
150000
125000
100000
75000
50000
25000
0
no Nb
IL-13+Strep-PE
TNF-Bio+Strep-PE
Strep-PE
[ ], M TNFa-bio
10-11
10-10
10-9
10-8
10-7
10-6
500 nM IL-13-Bio+Strep-PE

Figure 2

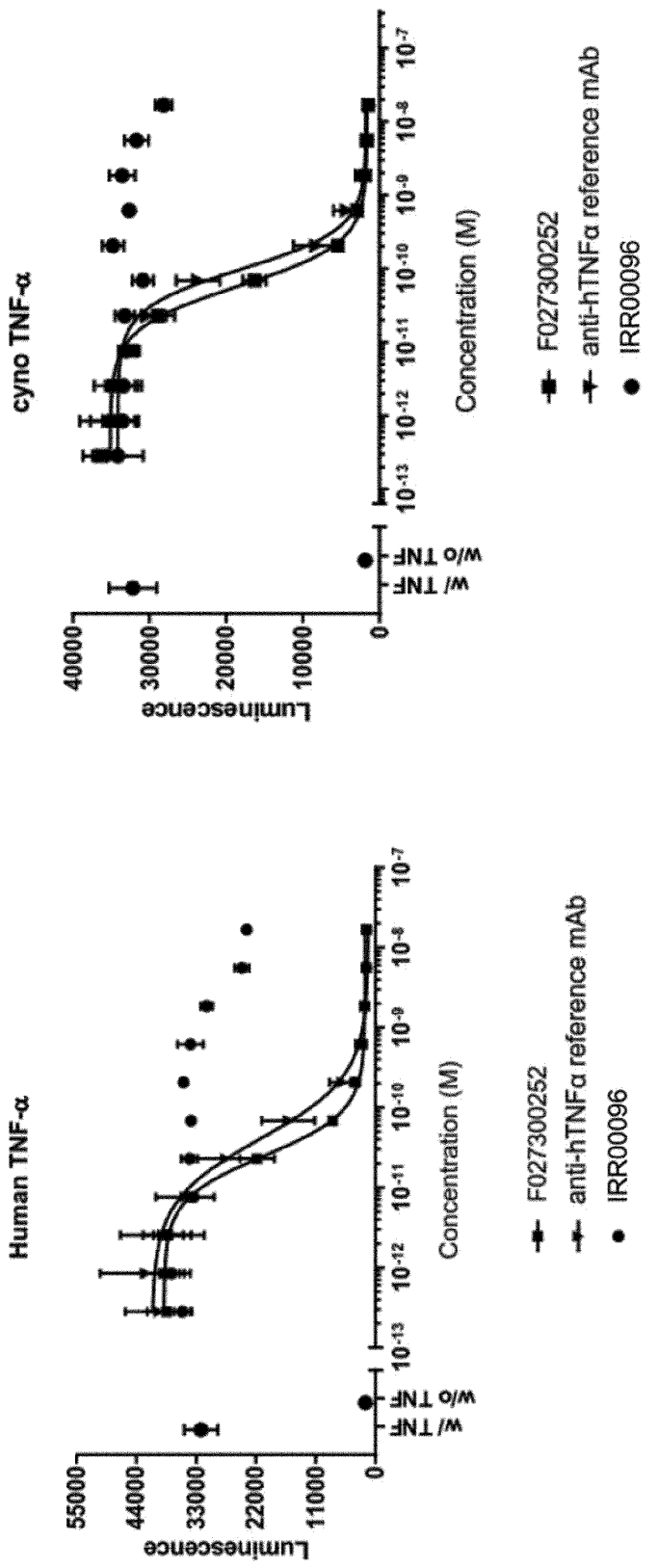

cyno TNF-α
Luminescence
40000
30000
20000
10000
0
w/ TNF
w/o TNF
10^-13
10^-12
10^-11
10^-10
10^-9
10^-8
10^-7
Concentration (M)
F027300252
anti-hTNFα reference mAb
IRR00096
Human TNF-α
Luminescence
55000
44000
33000
22000
11000
0
w/ TNF
w/o TNF
10^-13
10^-12
10^-11
10^-10
10^-9
10^-8
10^-7
Concentration (M)
F027300252
anti-hTNFα reference mAb
IRR00096

Figure 3

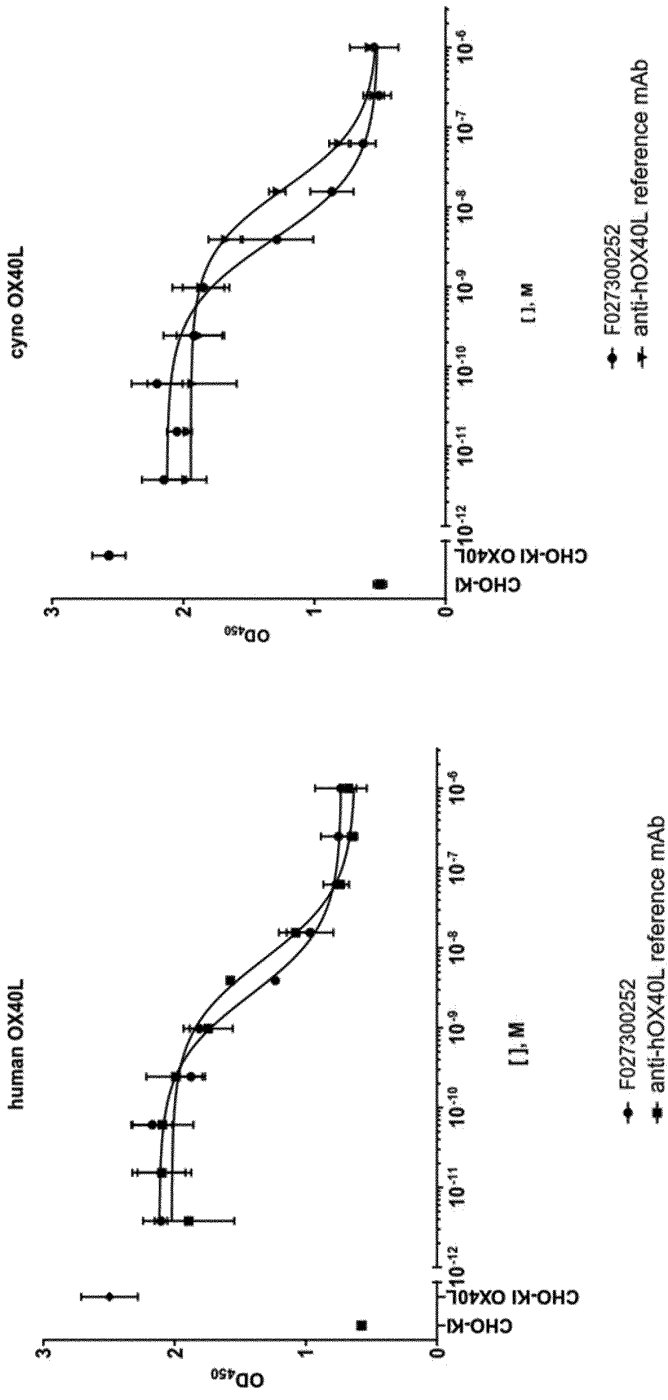

cyno OX40L
human OX40L
OD450
CHO-KI
CHO-KI OX40L
[ ], M
F027300252
anti-hOX40L reference mAb

Figure 4

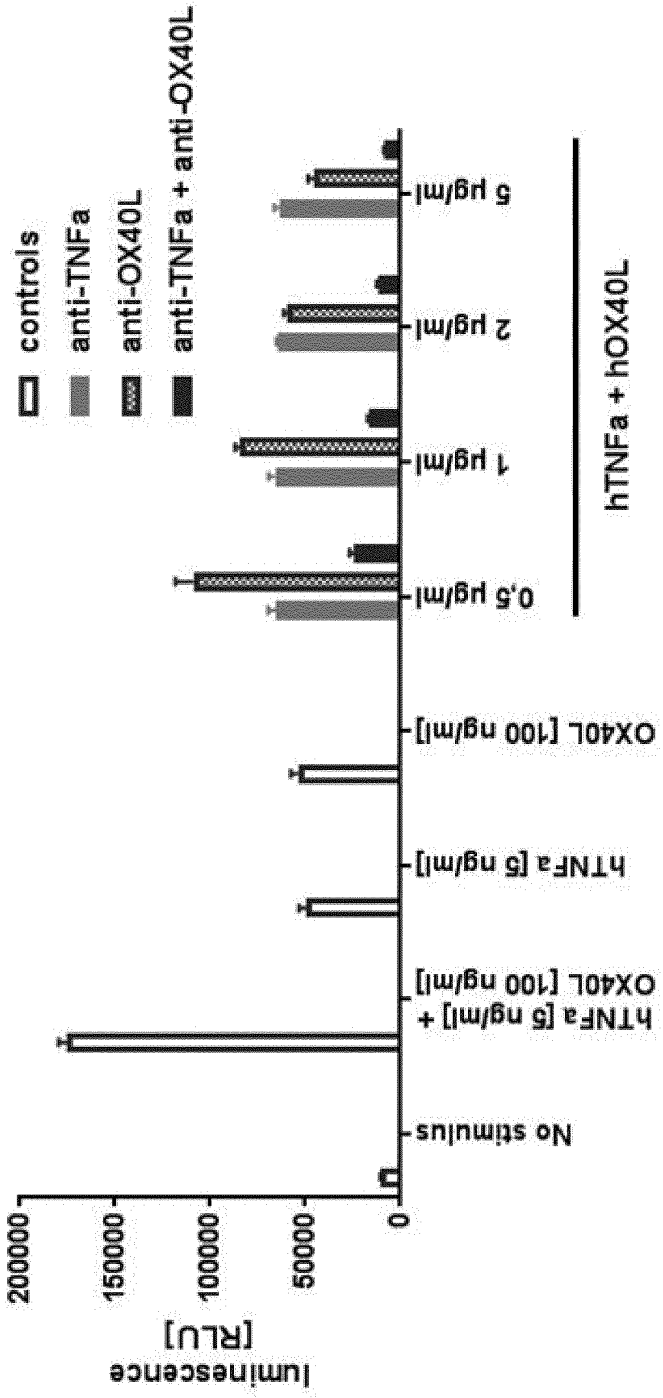

luminescence [RLU]
200000
150000
100000
50000
0
No stimulus
hTNFa [5 ng/ml] + OX40L [100 ng/ml]
hTNFa [5 ng/ml]
OX40L [100 ng/ml]
0,5 µg/ml
1 µg/ml
2 µg/ml
5 µg/ml
hTNFa + hOX40L
controls
anti-TNFa
anti-OX40L
anti-TNFa + anti-OX40L

Figure 5

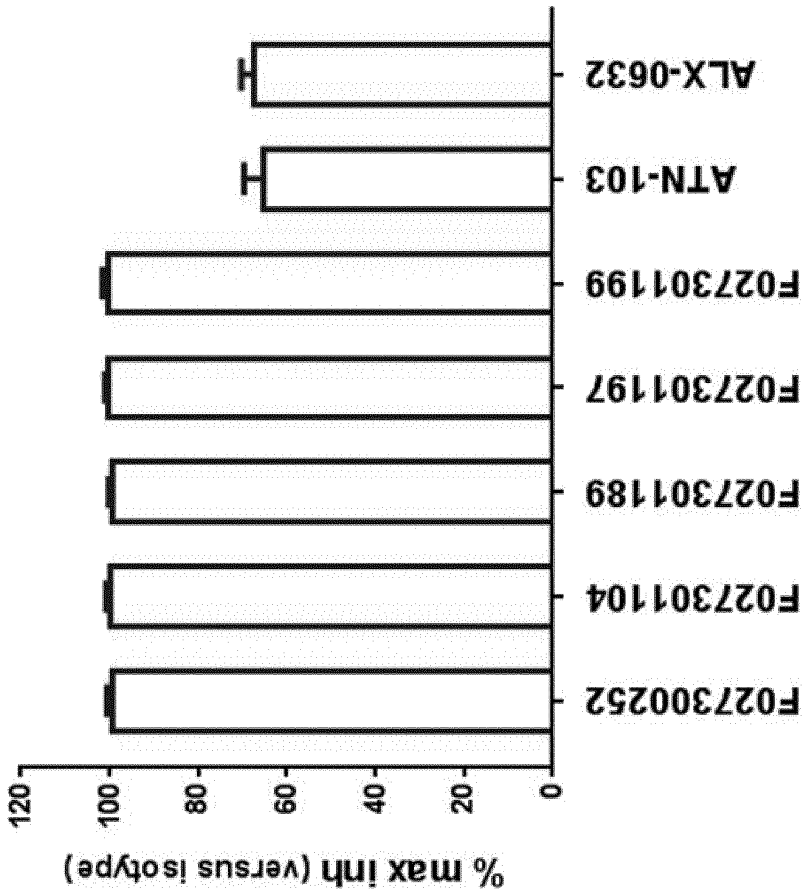

% max inh (versus isotype)
120
100
80
60
40
20
0
F027300252
F027301104
F027301189
F027301197
F027301199
ATN-103
ALX-0632

Figure 6

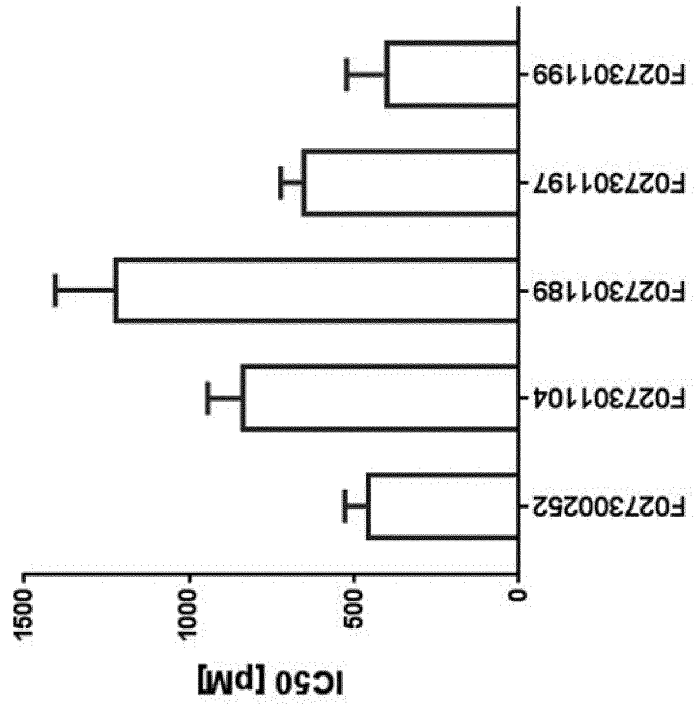

IC50 [pM]
1500
1000
500
0
F027300252
F027301104
F027301189
F027301197
F027301199

Figure 7

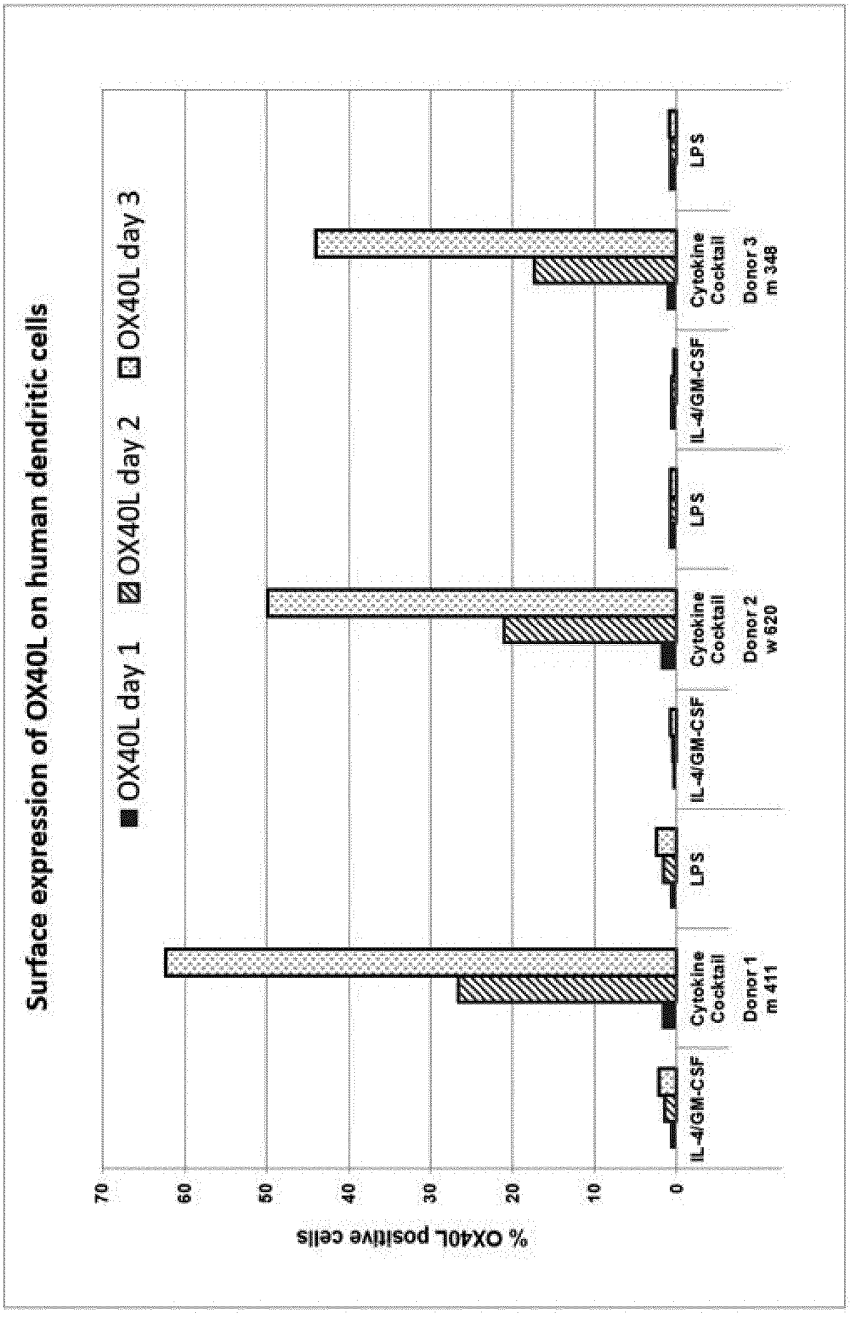

Surface expression of OX40L on human dendritic cells
OX40L day 1
OX40L day 2
OX40L day 3
% OX40L positive cells
70
60
50
40
30
20
10
0
IL-4/GM-CSF
Cytokine Cocktail
LPS
Donor 1 m 411
IL-4/GM-CSF
Cytokine Cocktail
LPS
Donor 2 w 620
IL-4/GM-CSF
Cytokine Cocktail
LPS
Donor 3 m 348

Figure 8

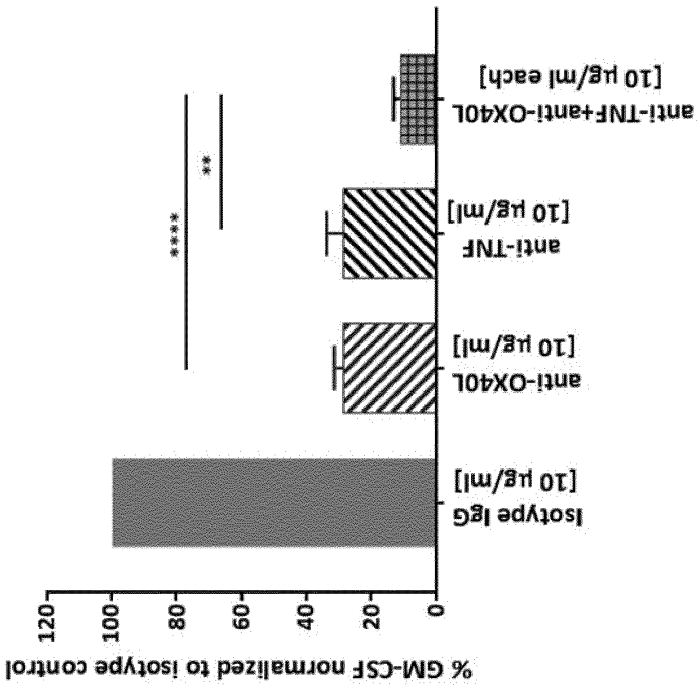

% GM-CSF normalized to isotype control
0
20
40
60
80
100
120
Isotype IgG [10 µg/ml]
anti-OX40L [10 µg/ml]
anti-TNF [10 µg/ml]
anti-TNF+anti-OX40L [10 µg/ml each]
****
**

Figure 9

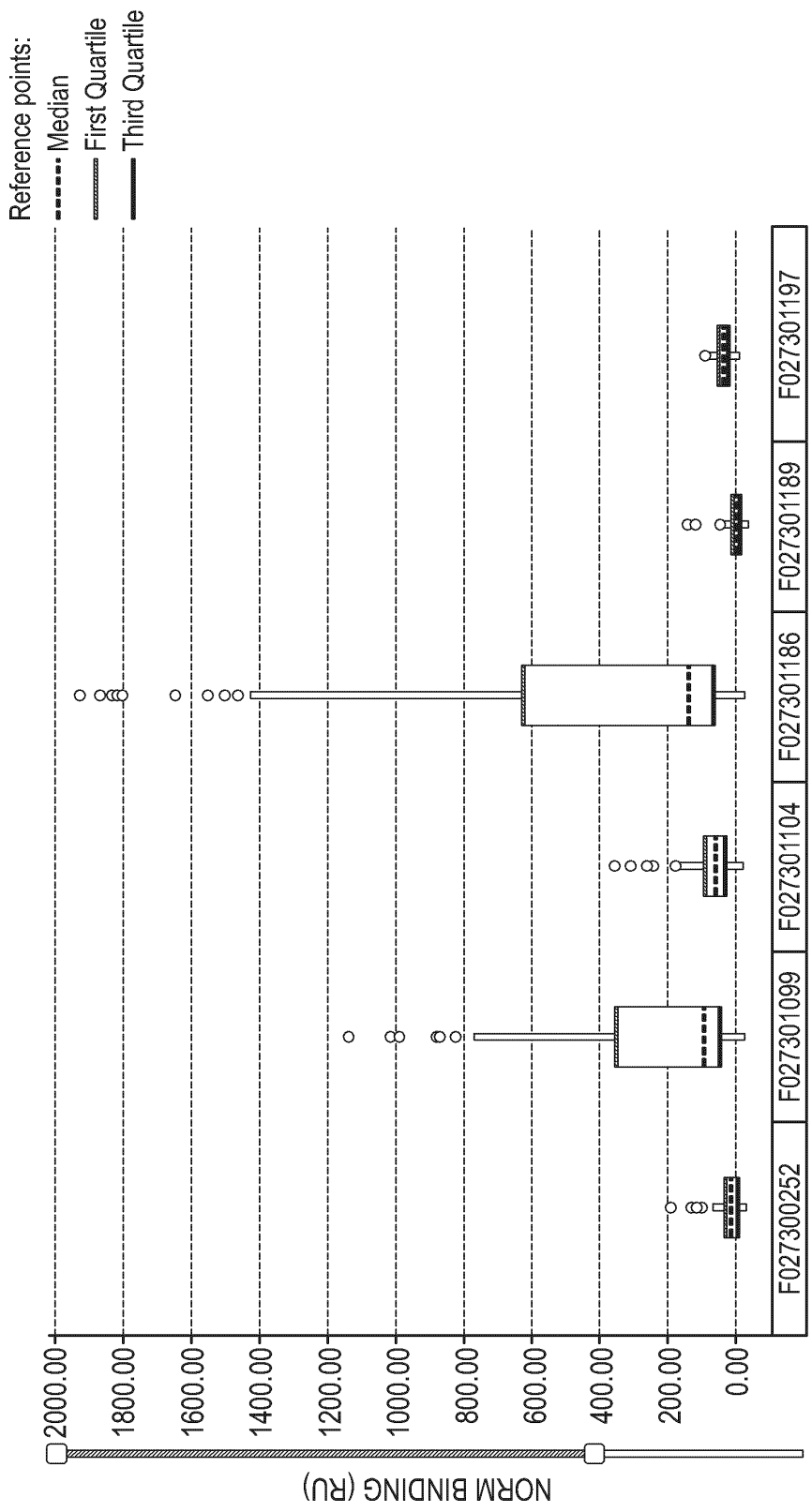

Reference points:
Median
First Quartile
Third Quartile
NORM BINDING (RU)
2000.00
1800.00
1600.00
1400.00
1200.00
1000.00
800.00
600.00
400.00
200.00
0.00
F027300252
F027301099
F027301104
F027301186
F027301189
F027301197

Figure 10

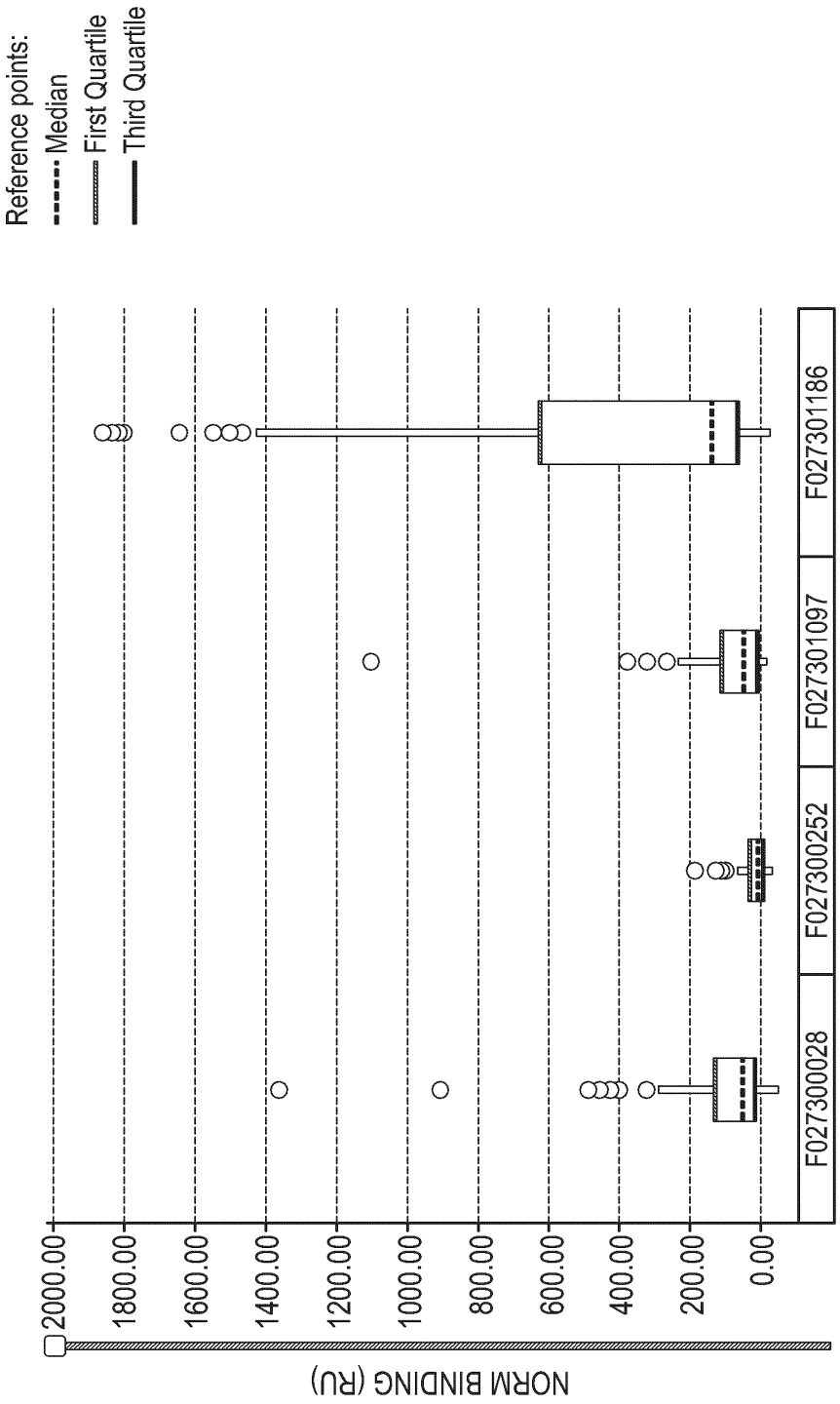
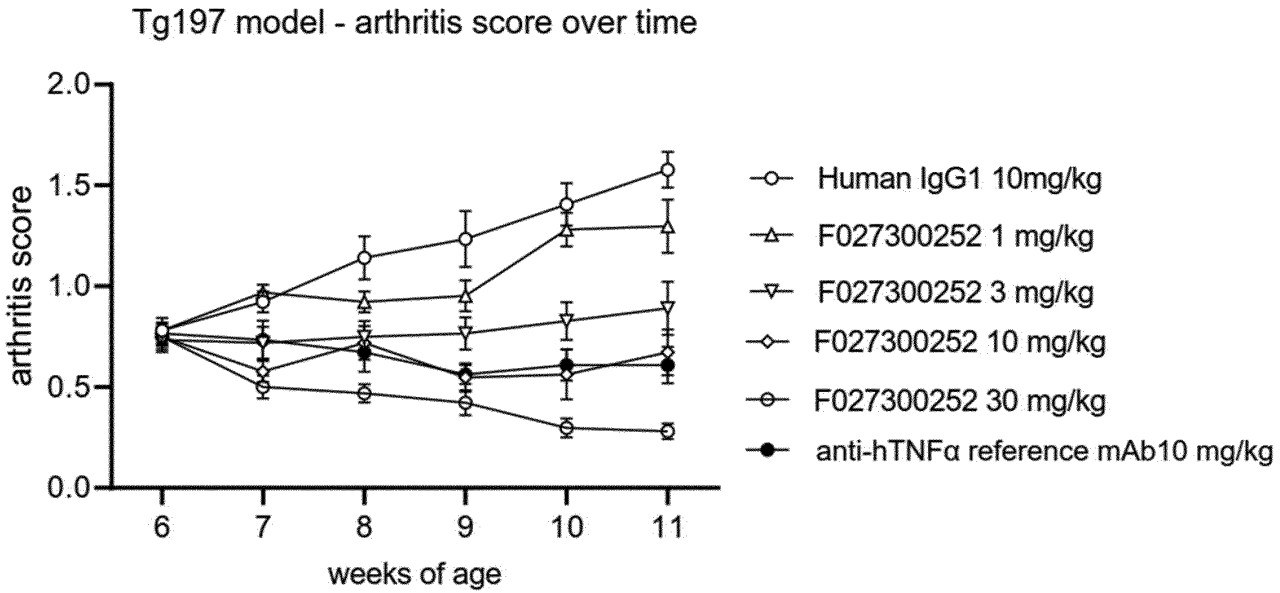
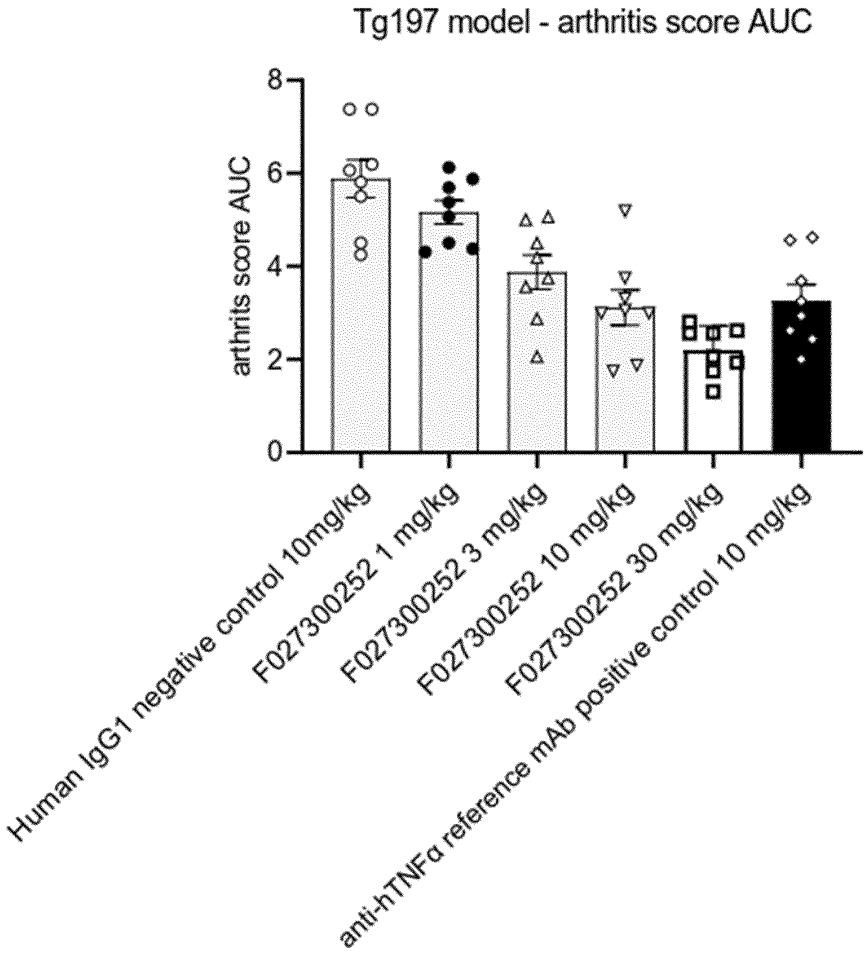

Reference points:
Median
First Quartile
Third Quartile
NORM BINDING (RU)
2000.00
1800.00
1600.00
1400.00
1200.00
1000.00
800.00
600.00
400.00
200.00
0.00
F027300028
F027300252
F027301097
F027301186

Figure 11



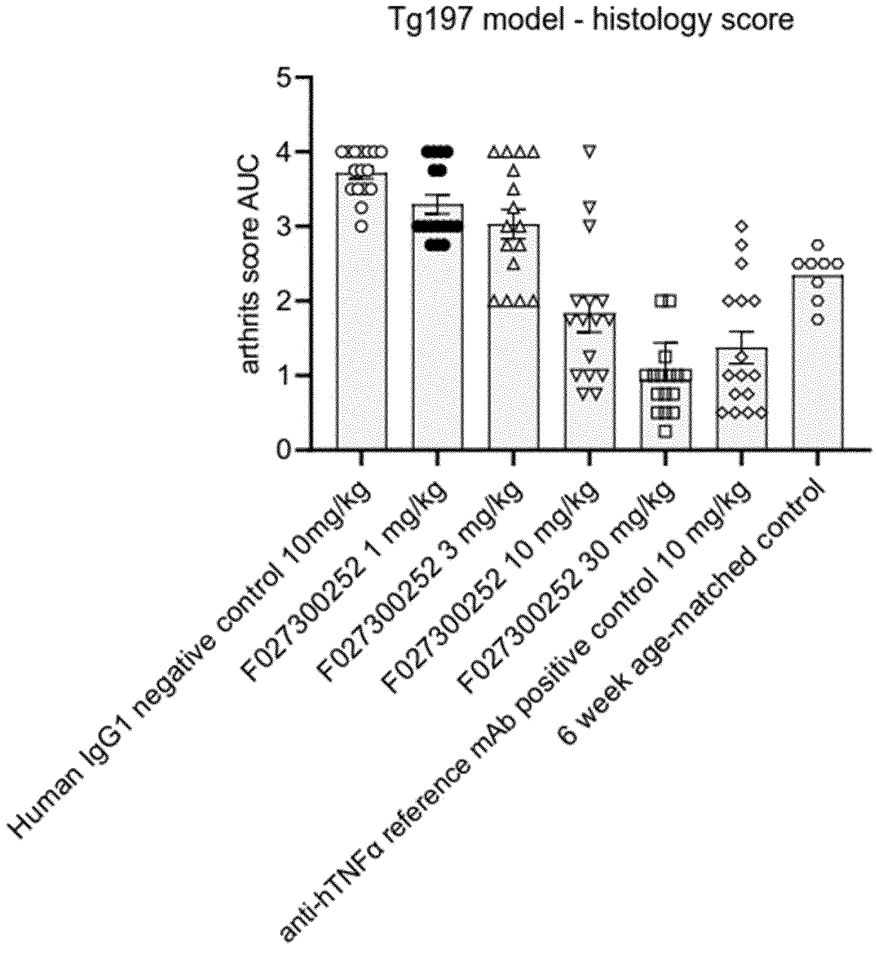
Bonferroni multiple comparisons test	week 7		week 8		week 9		week 10		week 11	
Human IgG1 vs F027300252 1 mg/kg	ns	>0.99	ns	0.48	ns	0.52	ns	>0.99	ns	0.503
Human IgG1 vs F027300252 3 mg/kg	ns	0.29	ns	0.06	ns	0.07	**	0.006	**	0.005
Human IgG1 vs F027300252 10 mg/kg	**	0.002	*	0.04	**	0.006	***	0.0007	***	0.0001
Human IgG1 vs F027300252 30 mg/kg	***	0.0005	**	0.001	**	0.002	****	<0.0001	****	<0.0001
Human IgG1 vs anti-hTNFα reference mAb 10 mg/kg	ns	0.572	*	0.03	**	0.007	***	0.0002	****	<0.0001

Figure 12



Bonferroni multiple comparisons test	Adjusted P value	
Human IgG1 vs F027300252 1 mg/kg	ns	0.4126
Human IgG1 vs F027300252 3 mg/kg	***	0.0005
Human IgG1 vs F027300252 10 mg/kg	****	<0.0001
Human IgG1 vs F027300252 30 mg/kg	****	<0.0001
Human IgG1 vs anti-hTNFα reference mAb 10 mg/kg	****	<0.0001

Figure 13



Bonferroni multiple comparisons test	Adjusted P value	
Human IgG1 vs F027300252 1 mg/kg	ns	0.3023
Human IgG1 vs F027300252 3 mg/kg	*	0.0226
Human IgG1 vs F027300252 10 mg/kg	****	<0.0001
Human IgG1 vs F027300252 30 mg/kg	****	<0.0001
Human IgG1 vs anti-hTNFα reference mAb 10 mg/kg	****	<0.0001

Figure 14

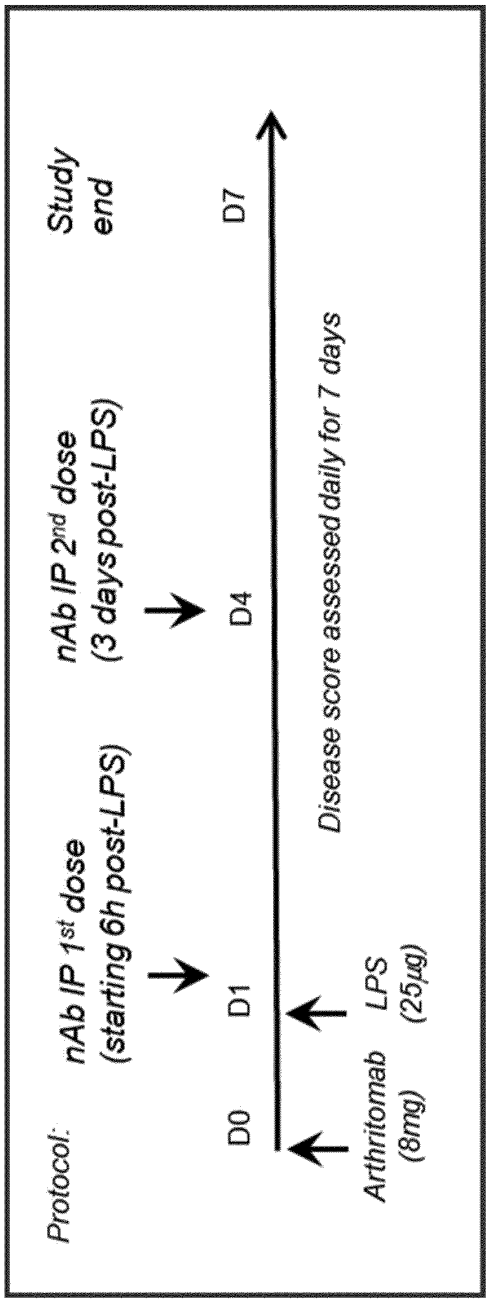
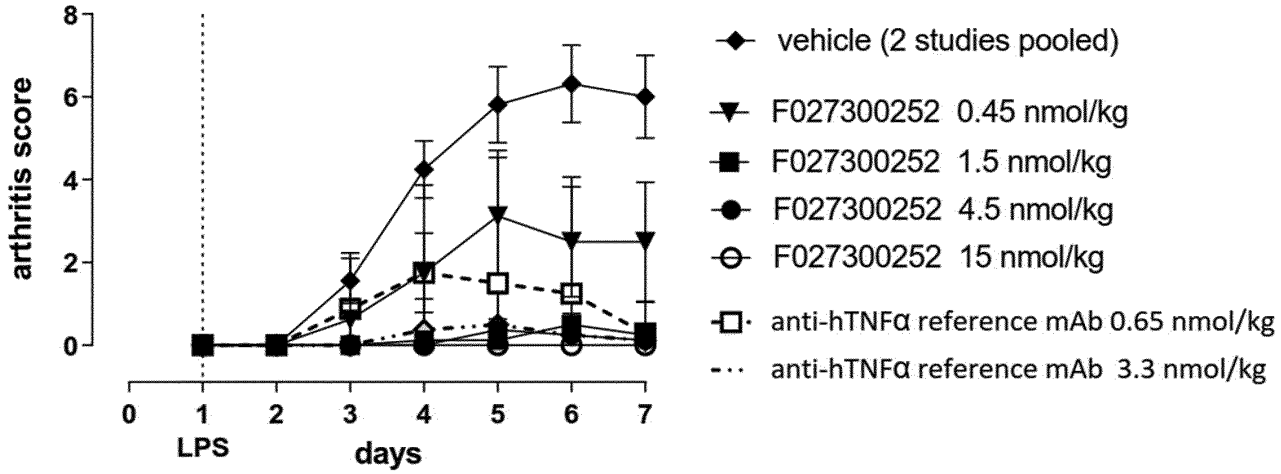
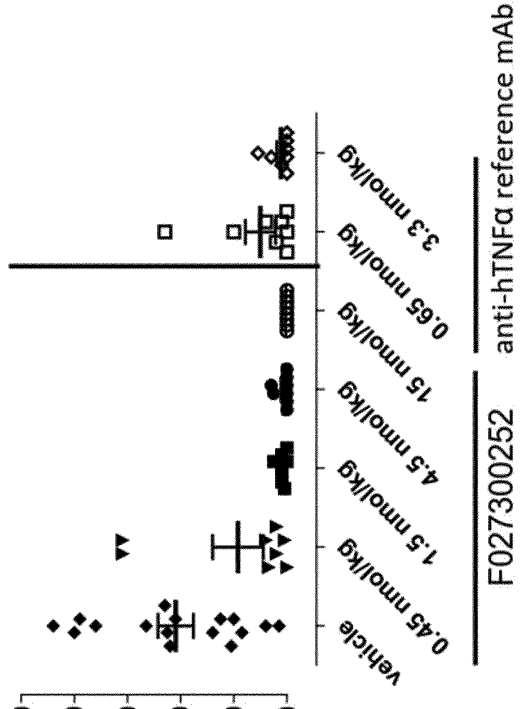

Protocol:
nAb IP 1st dose
(starting 6h post-LPS)
nAb IP 2nd dose
(3 days post-LPS)
Study
end
D0
D1
D4
D7
Arthritomab
(8mg)
LPS
(25µg)
Disease score assessed daily for 7 days

Figure 15



Bonferroni multiple comparisons test	day 4		day 5		day 6		day 7	
Human IgG1 vs F027300252 0.45 nmol/kg	ns	0.96	ns	0.92	*	0.02	ns	0.139
Human IgG1 vs F027300252 1.5 nmol/kg	*	0.04	****	<0.0001	****	<0.0001	****	<0.0001
Human IgG1 vs F027300252 4.5 nmol/kg	*	0.02	****	<0.0001	****	<0.0001	****	<0.0001
Human IgG1 vs F027300252 15 nmol/kg	*	0.02	****	<0.0001	****	<0.0001	****	<0.0001
Human IgG1 vs anti-hTNFα reference mAb 0.65 nmol/kg	ns	0.96	*	0.024	****	<0.0001	****	<0.0001
Human IgG1 vs anti-hTNFα reference mAb 3.3 nmol/kg	ns	0.09	***	0.00002	****	<0.0001	****	<0.0001

Figure 16



Bonferroni multiple comparisons test	Adjusted P value
Human IgG1 vs F027300252 0.45 nmol/kg	0.019
Human IgG1 vs F027300252 1.5 nmol/kg	<0.0001
Human IgG1 vs F027300252 4.5 nmol/kg	<0.0001
Human IgG1 vs F027300252 15 nmol/kg	<0.0001
Human IgG1 vs anti-hTNFα reference mAb 0.65 nmol/kg	0.0006
Human IgG1 vs anti-hTNFα reference mAb 3.3 nmol/kg	<0.0001

Figure 17

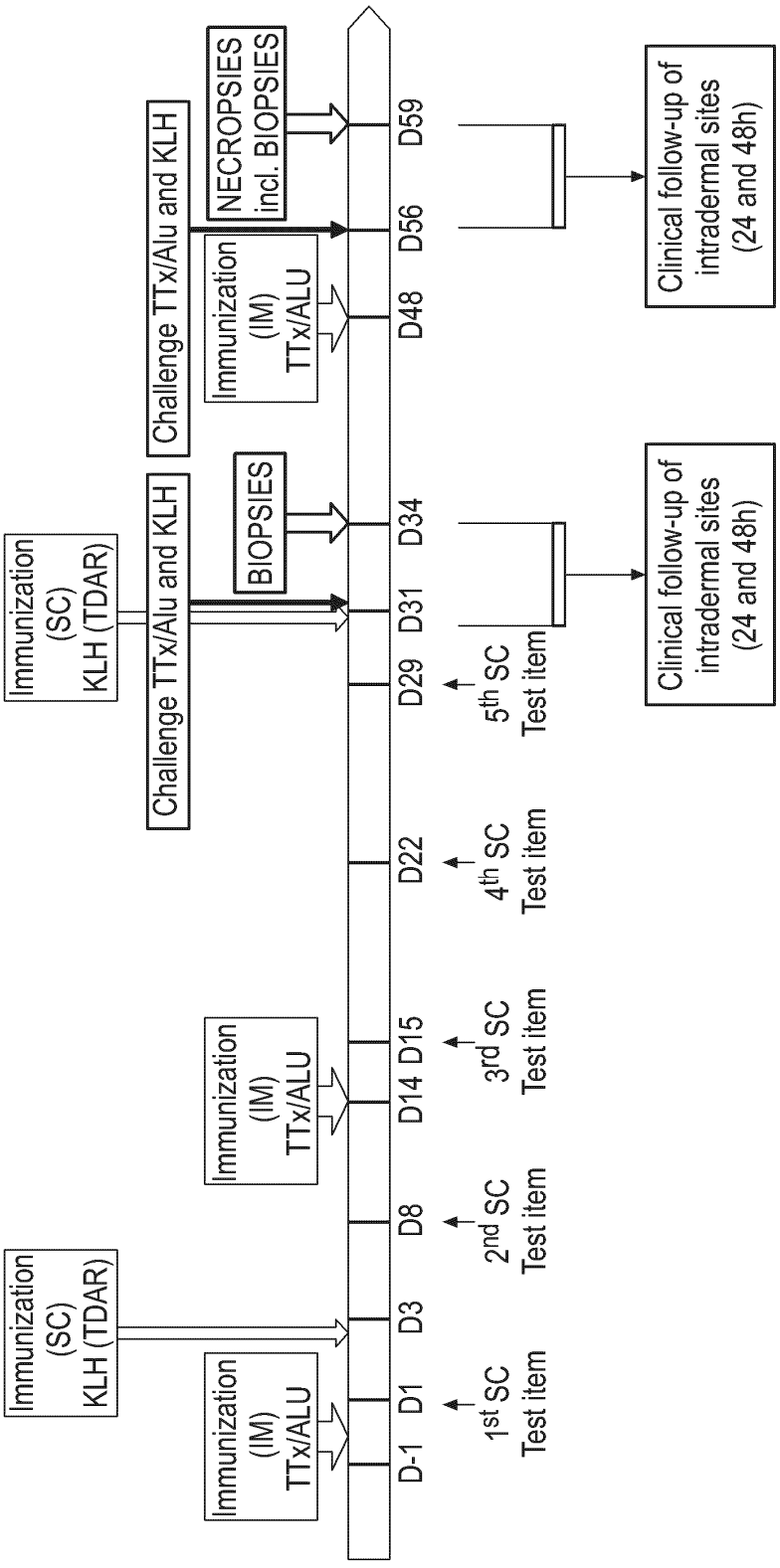

Immunization (SC) KLH (TDAR)
Immunization (IM) TTx/ALU
Immunization (SC) KLH (TDAR)
Challenge TTx/Alu and KLH
Immunization (IM) TTx/ALU
Challenge TTx/Alu and KLH
BIOPSIES
NECROPSIES incl. BIOPSIES
D-1
D1
D3
D8
D14
D15
D22
D29
D31
D34
D48
D56
D59
1st SC Test item
2nd SC Test item
3rd SC Test item
4th SC Test item
5th SC Test item
Clinical follow-up of intradermal sites (24 and 48h)
Clinical follow-up of intradermal sites (24 and 48h)

Figure 18

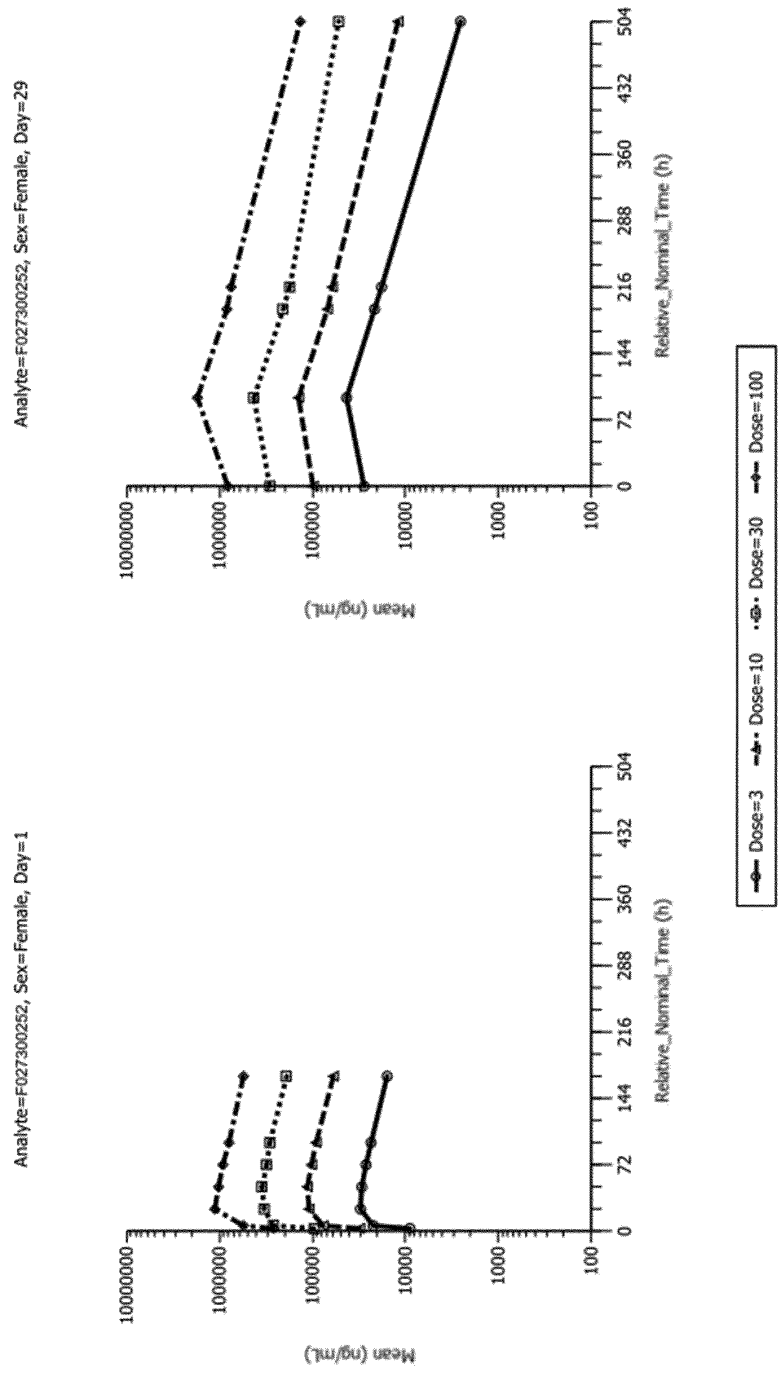
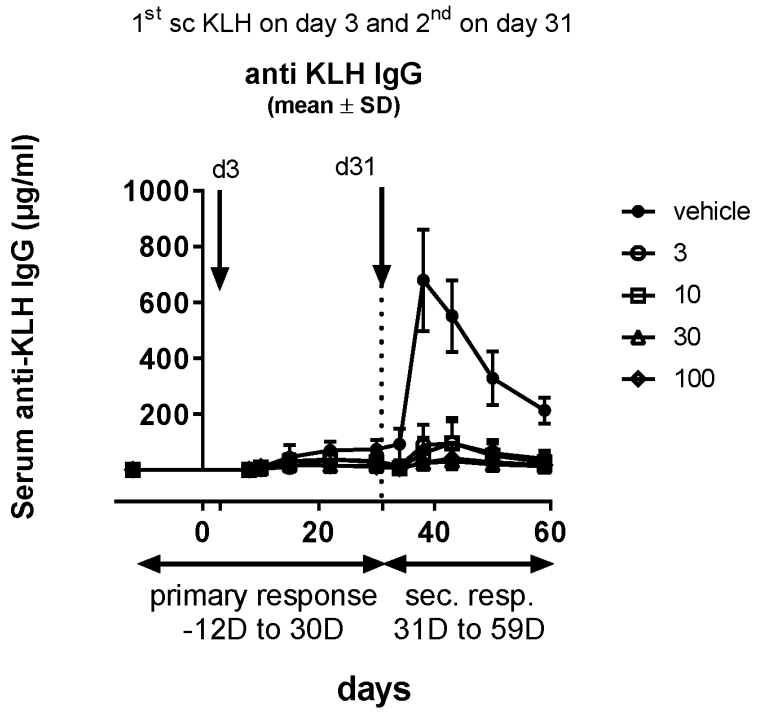

Analyte=F027300252, Sex=Female, Day=29
Analyte=F027300252, Sex=Female, Day=1
Mean (ng/mL)
Relative_Nominal_Time (h)
10000000
1000000
100000
10000
1000
100
0
72
144
216
288
360
432
504
Dose=3
Dose=10
Dose=30
Dose=100

Figure 19



Bonferroni multiple comp. test	Day 34		Day 38		Day 43		Day 50		Day 59	
Vehicle vs F027300252 3 mg/kg	*	0.0435	****	<0.0001	****	<0.0001	****	<0.0001	****	<0.0001
Vehicle vs F027300252 10 mg/kg	*	0.0285	****	<0.0001	****	<0.0001	****	<0.0001	****	<0.0001
Vehicle vs F027300252 30 mg/kg	*	0.0270	****	<0.0001	****	<0.0001	****	<0.0001	****	<0.0001
Vehicle vs F027300252 100 mg/kg	*	0.0231	****	<0.0001	****	<0.0001	****	<0.0001	****	<0.0001

Figure 20

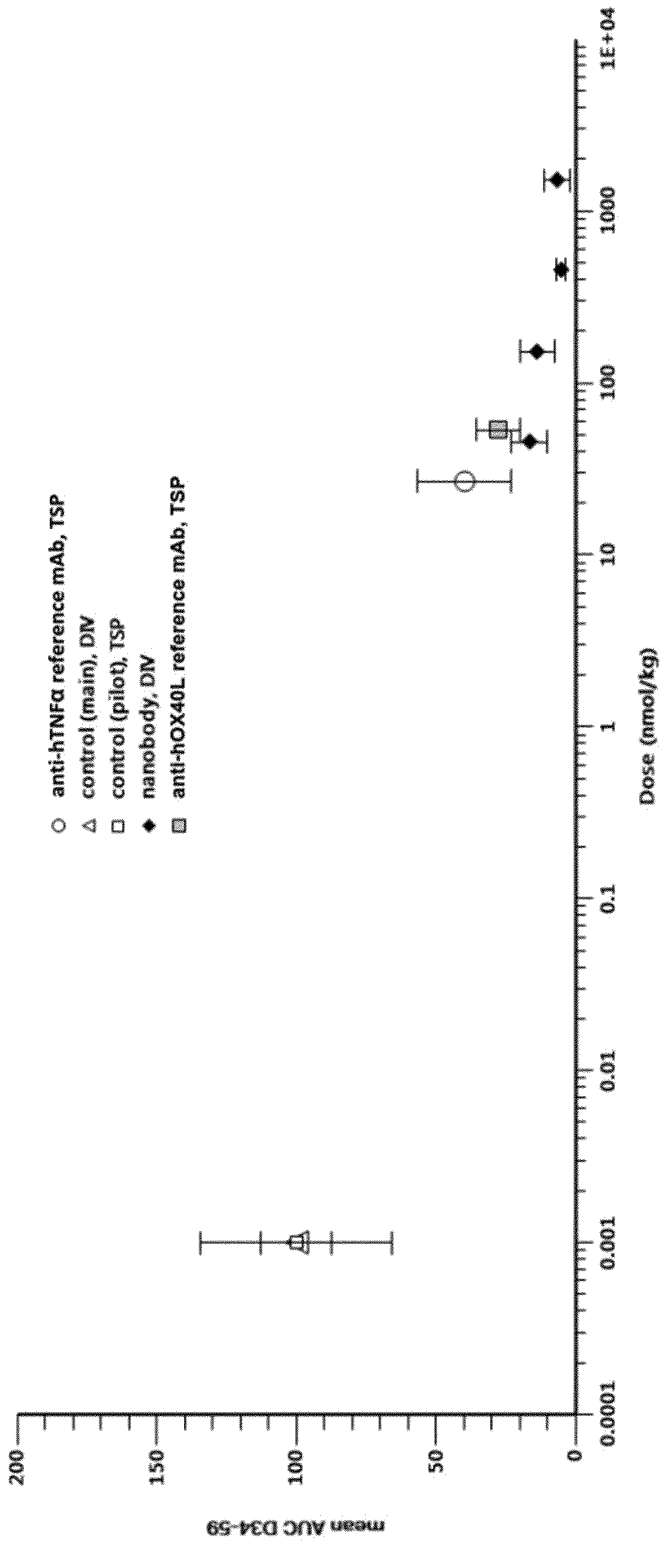
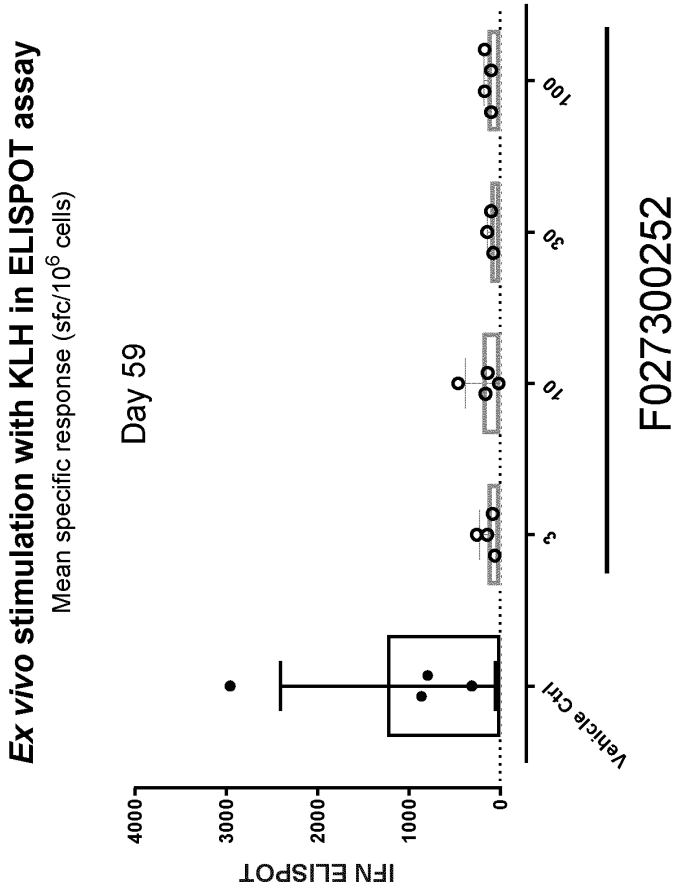
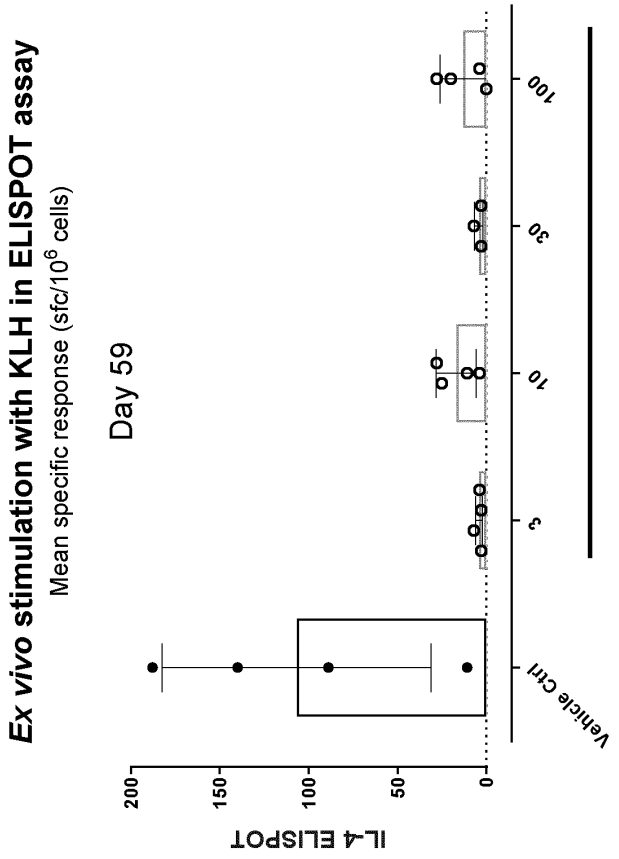

anti-hTNFα reference mAb, TSP
control (main), DIV
control (pilot), TSP
nanobody, DIV
anti-hOX40L reference mAb, TSP
mean AUC D34-59
200
150
100
50
0
0.0001
0.001
0.01
0.1
1
10
100
1000
1E+04
Dose (nmol/kg)

Figure 21



Bonferroni multiple comp. test		Adjusted P Value
Vehicle vs F027300252 3 mg/kg	*	0.0461
Vehicle vs F027300252 10 mg/kg	ns	0.0609
Vehicle vs F027300252 30 mg/kg	ns	0.0594
Vehicle vs F027300252 100 mg/kg	*	0.0459

Figure 22



Bonferroni multiple comp. test		Adjusted P Value
Vehicle vs F027300252 3 mg/kg	**	0.0043
Vehicle vs F027300252 10 mg/kg	*	0.0112
Vehicle vs F027300252 30 mg/kg	**	0.0077
Vehicle vs F027300252 100 mg/kg	**	0.0083

Figure 23

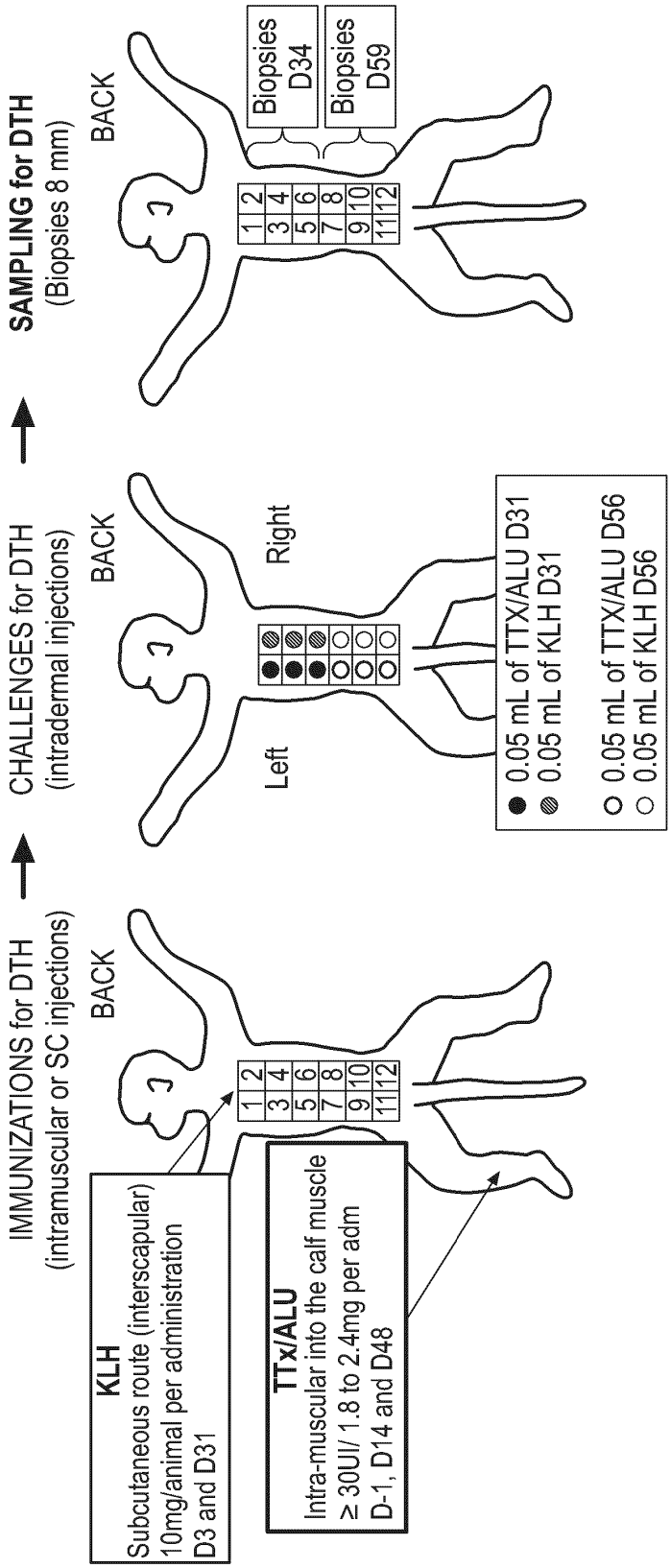
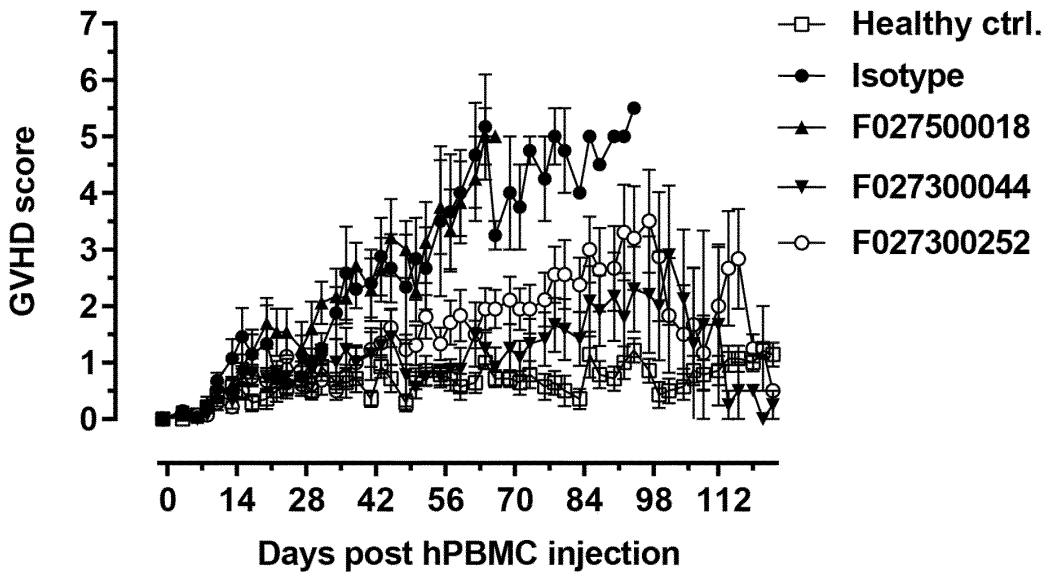
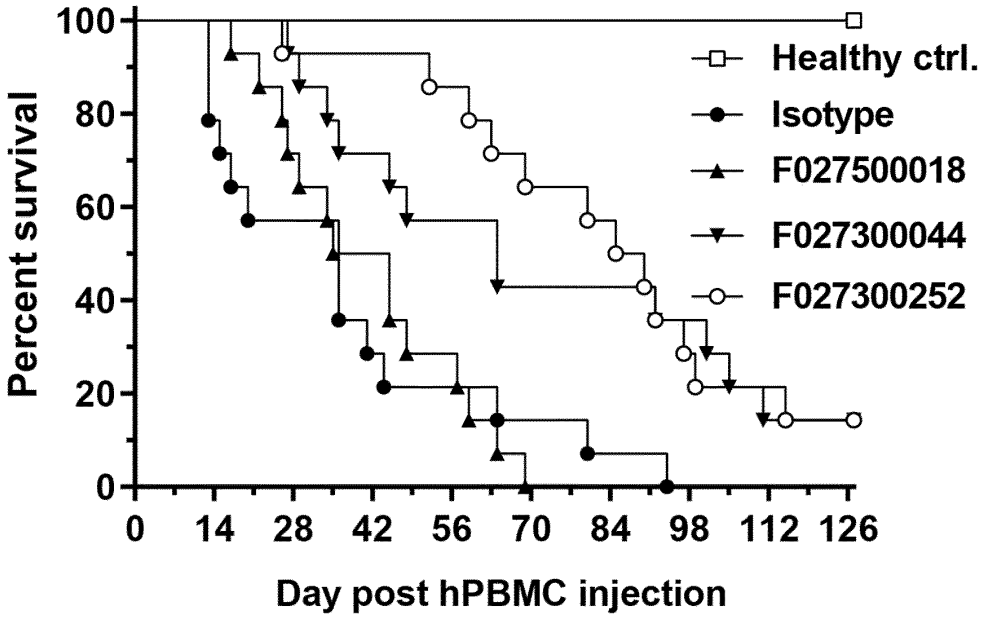

IMMUNIZATIONS for DTH
(intramuscular or SC injections)
KLH
Subcutaneous route (interscapular)
10mg/animal per administration
D3 and D31
TTx/ALU
Intra-muscular into the calf muscle
≥ 30UI/ 1.8 to 2.4mg per adm
D-1, D14 and D48
BACK
1 2
3 4
5 6
7 8
9 10
1112
CHALLENGES for DTH
(intradermal injections)
BACK
Left
Right
0.05 mL of TTX/ALU D31
0.05 mL of KLH D31
0.05 mL of TTX/ALU D56
0.05 mL of KLH D56
SAMPLING for DTH
(Biopsies 8 mm)
BACK
Biopsies D34
Biopsies D59

Figure 24



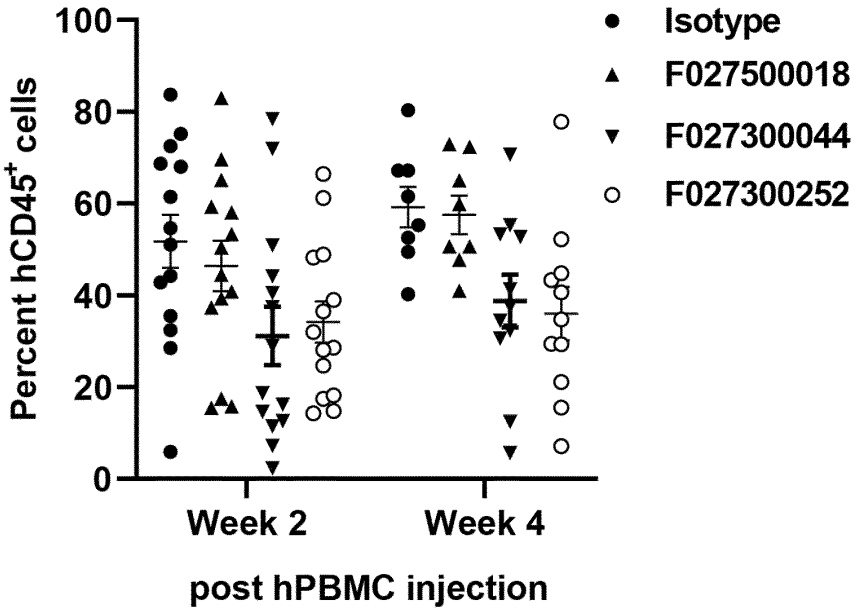
Bonferroni´s multiple comparisons test	Adjusted P values	
Healthy ctrl. vs. Isotype	***	<0.0001
Healthy ctrl. vs. F027500018	***	<0.0001
Healthy ctrl. vs. F027300044	ns	0.2113
Healthy ctrl. vs. F027300252	***	0.0004
Isotype vs. F027500018	ns	0.1184
Isotype vs. F027300044	***	<0.0001
Isotype vs. F027300252	***	<0.0001
F027500018 vs. F027300044	***	0.0003
F027500018 vs. F027300252	ns	0.0955
F027300044 vs. F027300252	ns	0.2349

Figure 25



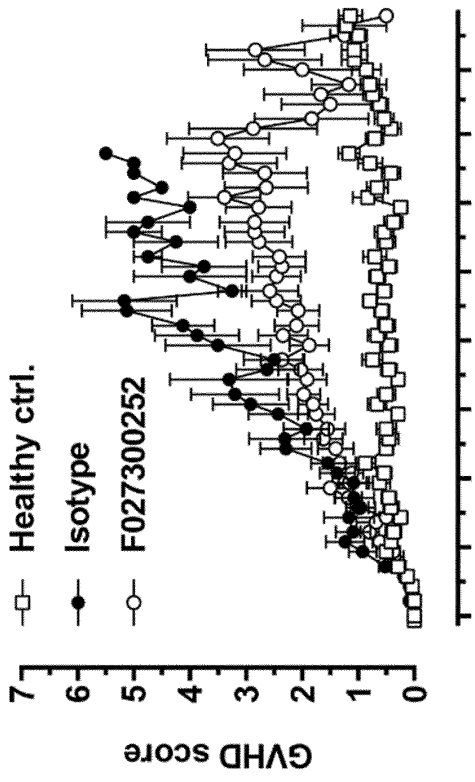
Log-rank (Mantel-Cox) test	Corrected P values	
Healthy ctrl. vs. Isotype	***	0.0003
Healthy ctrl. vs. F027500018	***	0.0003
Healthy ctrl. vs. F027300044	**	0.0069
Healthy ctrl. vs. F027300252	**	0.0070
Isotype vs. F027500018	ns	>0.9999
Isotype vs. F027300044	ns	0.0587
Isotype vs. F027300252	**	0.0035
F027500018 vs. F027300044	ns	0.0653
F027500018 vs. F027300252	***	0.0003
F027300044 vs. F027300252	ns	>0.9999

Figure 26



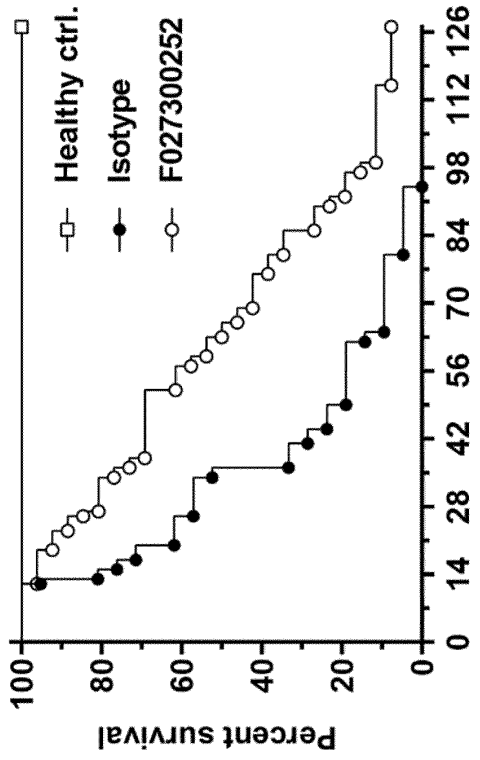
	Week 2		Week 4	
Bonferroni´s multiple comparisons test	**Adjusted P values**		**Adjusted P values**	
Isotype vs. F027500018	ns	>0.9999	ns	>0.9999
Isotype vs. F027300044	ns	0.0714	ns	0.2456
Isotype vs. F027300252	ns	0.2226	ns	0.0530
F027500018 vs. F027300044	ns	0.4781	ns	0.4395
F027500018 vs. F027300252	ns	>0.9999	ns	0.1043
F027300044 vs. F027300252	ns	>0.9999	ns	>0.9999

Figure 27



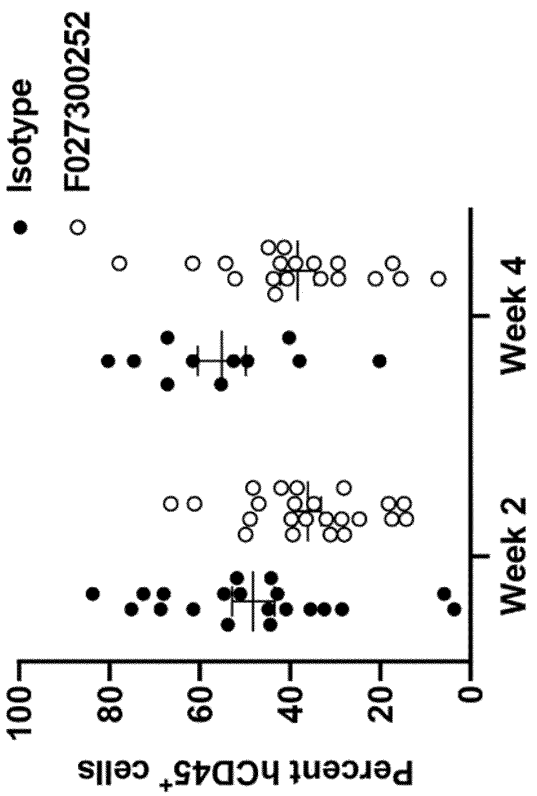
Bonferroni´s multiple comparisons test	Adjusted P values	
Healthy ctrl. vs. Isotype	***	<0.0001
Healthy ctrl. vs. F027300252	***	<0.0001
Isotype vs. F027300252	***	<0.0001

Figure 28



Log-rank (Mantel-Cox) test	Corrected P values	
Healthy ctrl. vs. Isotype	***	<0.0001
Healthy ctrl. vs. F027300252	***	0.0003
Isotype vs. F027300252	**	0.0030

Figure 29



	Week 2	Week 4
Bonferroni´s multiple comparisons test	Adjusted P values	Adjusted P values
Isotype vs. F027300252	ns	0.0503	**	0.0084

Figure 30

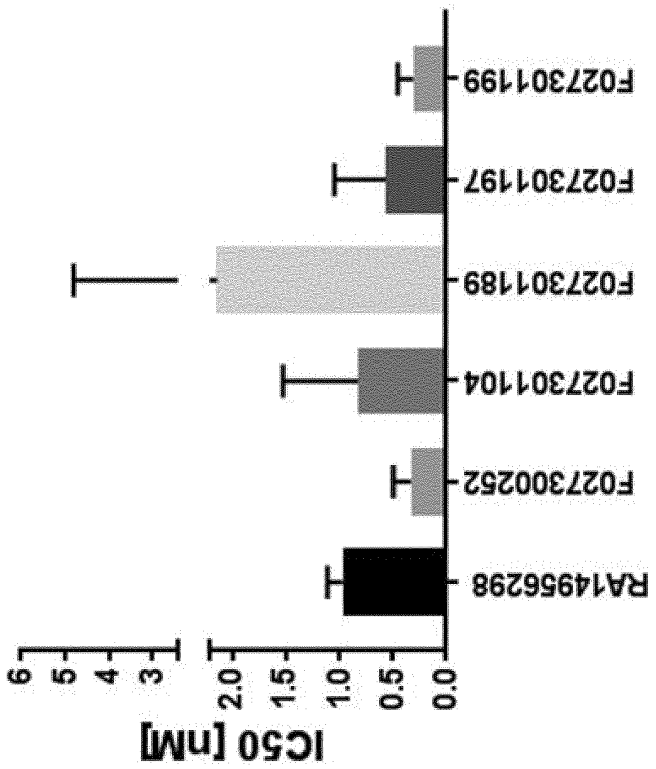

IC50 [nM]
6
5
4
3
2.0
1.5
1.0
0.5
0.0
RA14956298
F027300252
F027301104
F027301189
F027301197
F027301199

Figure 31

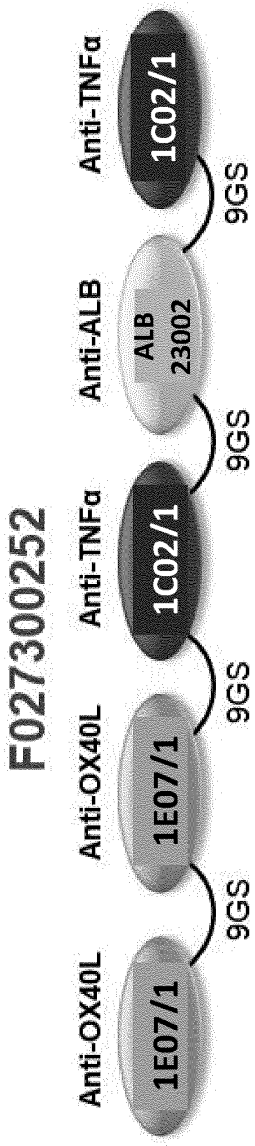

F027300252
Anti-OX40L
1E07/1
9GS
Anti-OX40L
1E07/1
9GS
Anti-TNFα
1C02/1
9GS
Anti-ALB
ALB 23002
9GS
Anti-TNFα
1C02/1

Figure 32