EVALUATION OF TEST ARTICLES IN AN *IN VITRO* T CELL ACTIVATION ASSAY.

FINAL REPORT

STUDY NUMBER

MD-3-3-093-1052

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REPORT DATE

April 30, 2008

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In vitro phase initiation:	March 17, 2008
Completion of *in vitro* phase:	March 19, 2008
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1. SUMMARY

11 Test Articles were evaluated in an *in vitro* T cell activation assay using human peripheral blood mononuclear cells (PBMC). Human PBMC's were incubated with immobilized anti-CD3 antibody in the presence and absence of 11 Test Articles, each at 3 concentrations. T cell activation was assessed by cell proliferation and cytokine production. Co-stimulation with anti-CD28 antibody was also performed.

1.1. *Conclusions*

- At high concentrations (50 nM), triptolide and triptonide inhibited human PBMC cell proliferation.
- Triptonide (10 nM) and CAY10470 (100 nM, 1000 nM) inhibited anti-CD3 activated cell proliferation.
- Triptolide (10 nM), triptonide (10 nM) and CAY10470 (100 nM, 1000 nM) inhibited anti-CD3 activated IL-2 production.
- Triptolide (10 nM) and triptonide (10 nM) inhibited anti-CD3/anti-CD28 activated IL-2 production.
- Triptolide (10 nM) and triptonide (10 nM) inhibited anti-CD3 activated TNF-α production.
- Triptonide (10 nM) inhibited anti-CD3/anti-CD28 activated TNF-α production.

2. OBJECTIVE

The objective of this study was to evaluate 11 Test Articles in an *in vitro* T cell activation assay.

3. REGULATORY GUIDELINES

This study does not follow any specific regulatory guidelines. This study follows standard operating procedures in place at MD Biosciences, Inc., St. Paul, Minnesota.

4. ARCHIVING

The following records are stored in the archives of MD Biosciences, Inc. in St. Paul, Minnesota for 2 years:

A copy of the final report, the study protocol, documentation of all raw data and specimens generated during the conduct of the study.

5. TEST MATERIALS

5.1. *Test Articles*

TA-ID	Sponsor ID	Batch/Lot No.	Physical State	Storage Cond.	Exp. Date
TA-070124	Triptolide	20480-107	white solid	4˚C	31-Jan-09
TA-070125	Triptonide	20482-202	white solid	4˚C	31-Jan-09
TA-070126	(QNZ) CAY10470	NA	crystalline solid	-30˚C	31-Jan-10
TA-070127	GB67A	NA	white solid	4˚C	31-Jan-09
TA-070128	GB67B	NA	white solid	4˚C	31-Jan-09
TA-070129	GB615	NA	white solid	4˚C	31-Jan-09
TA-070130	GB616	NA	white solid	4˚C	31-Jan-09
TA-070131	GB594	NA	white solid	4˚C	31-Jan-09
TA-070132	GB595	NA	white solid	4˚C	31-Jan-09
TA-070133	GB65B	NA	white solid	4˚C	31-Jan-09
TA-070134	GB117	NA	white solid	4˚C	31-Jan-09

5.2. *Experimental/Reference Articles*

Name	Vendor	Cat. No.	Lot No.	Exp. Date	Storage	Use
RPMI-1640	Invitrogen	61870-036	383190	30-Nov-08	4˚C	PBMC culture
Heat inactivated fetal bovine serum (FBS)	Invitrogen	10082-147	291539	30-Jun-12	-80˚C	PBMC culture
Penicillin/streptomycin stock solution	Cambrex	17-602E	1106064	27-Nov-08	-30˚C	PBMC culture
T-cell activation control plate	BD Biosciences	354730	65897	NA	4˚C	Activation
T-cell activation plate, anti-CD3	BD Biosciences	354725	88430	21-May-08	4˚C	Activation
Anti-CD28 antibody	R&D Systems	MAB342	AEG0407041	03-Dec-08	-30˚C	Activation
Cyclosporin A	Sigma	C3662	126K4051	06-Dec-09	4˚C	Reference Article
IL-2 Ab Bead Kit, Human	Invitrogen	LHC0021	1383782	31-Jul-08	4˚C	Analyte Assay
TNF-α Bead Kit, Human	Invitrogen	LHC3011	370059B	30-Apr-09	4˚C	Analyte Assay
Multiplex Buffer Kit	Invitrogen	LHB0001	299782D	31-Jul-09	4˚C	Analyte Assay
CellTiter-Glo Cell Viability Assay	Promega	G7571	252036	31-Oct-09	-30˚C	Proliferation Assay
DMSO	Sigma	D2650	107K2315	31-Oct-09	RT	Solvent
Phosphate Buffered Saline (PBS)	Invitrogen	14190-144	1403804	31-Mar-10	RT	Solvent
Ethanol	Sigma	362808	00333HH	NA	RT	Solvent

5.3. *Peripheral blood mononuclear cells*

Donor	Vendor	Catalog Number	Lot Number	Storage
1	SeraCare	72001	042805	Liquid N_2

5.4. *Culture media*

Complete Culture Media (CM): RPMI-1640 + 10% FBS + 100 U/ml penicillin + 100 µg/ml streptomycin.

5.5. *Preparation of Test Articles*

20 mM Test Article stock solutions were prepared in DMSO:

20 mM Stock Solutions				
Test Article	**MW**	**mg**	**DMSO (ml)**	**mg/ml**
TA-070124	360.4	2.8	0.388	7.208
TA-070125	358.4	3.6	0.502	7.168
TA-070126	356.4	5	0.701	7.128
TA-070127	208.3	8.4	2.016	4.166
TA-070128	208.3	11.2	2.688	4.166
TA-070129	224.3	10.5	2.341	4.486
TA-070130	224.3	5.4	1.204	4.486
TA-070131	242.3	9.3	1.919	4.846
TA-070132	222.3	7.4	1.664	4.446
TA-070133	194.2	5.1	1.313	3.884
TA-070134	210.2	4.5	1.070	4.204

1000 X Test Article stock solutions were prepared in DMSO:

1000 X Stock Solutions				
	Volume	**Source**	**Diluent**	**Total Volume**
4000 µM	50 µl	20 mM	200 µl DMSO	250 µl
400 µM	25 µl	4000 µM	225 µl DMSO	250 µl
200 µM[1]	125 µl	400 µM	125 µl DMSO	250 µl
40 µM[1]	25 µl	400 µM	225 µl DMSO	250 µl
4 µM	2.5 µl	400 µM	247.5 µl DMSO	250 µl
[1]Prepare only for triptolide and triptonide.				

4 X Test Article working solutions were prepared in CM:

4 X Working Solutions				
	Volume	**Source**	**Diluent**	**Total Volume**
4000 nM	5 µl	4000 µM	5 ml CM	5 ml
400 nM	5 µl	400 µM	5 ml CM	5 ml
200 nM[1]	5 µl	200 µM	5 ml CM	5 ml
40 nM[1]	5 µl	40 µM	5 ml CM	5 ml
4 nM	5 µl	4 µM	5 ml CM	5 ml
[1]Prepare only for triptolide and triptonide.				

2 X Test Article working solutions were prepared in CM:

2 X Working Solutions				
	Volume	**Source**	**Diluent**	**Total Volume**
2000 nM	0.5 ml	4000 nM	0.5 ml	1 ml
200 nM	0.5 ml	400 nM	0.5 ml	1 ml
100 nM[1]	0.5 ml	200 nM	0.5 ml	1 ml
20 nM[1]	0.5 ml	40 nM	0.5 ml	1 ml
2 nM	0.5 ml	4 nM	0.5 ml	1 ml
[1]Prepare only for triptolide and triptonide.				

5.6. *Preparation of cyclosporin A*

A cyclosporin A stock solution of 10 mg/ml was prepared in ethanol. Cyclosporin A working solutions of 400 ng/ml (4 X) and 200 ng/ml (2 X) were prepared by diluting the stock solution in CM.

5.7. *Preparation of vehicle control*

Vehicle control working solutions were prepared by diluting DMSO to a final concentration of 0.1% (4 X) and 0.05% (2 X) in CM.

5.8. *Preparation of anti-CD28*

An anti-CD28 antibody stock solution of 500 µg/ml was prepared in PBS. An anti-CD28 antibody working solution of 4 µg/ml (4 X) was prepared by diluting the stock solution in CM.

6. TEST PROCEDURE

6.1. *Culture setup*

Cryopreserved PBMC's were thawed, washed with CM and tested for viability using Trypan blue (PBMC viability = 92%). Cells were resuspended to 4 x 10^6 cells/ml in CM.

6.2. *T cell activation: IL-2/TNF-α production assay*

50 µl of 4 X vehicle, cyclosporin A or Test Article working solutions was added to control and anti-CD3 coated plates. 50 µl of CM was added to the – anti-CD28 plates and 50 µl of 4 X anti-CD28 was added to + anti-CD28 plates. 100 µl of 4 x 10^6 cells/ml was added to each well (400,000 cells/well). Plates were incubated at 37˚C with 5% CO_2 for 48 hours.

Cell culture supernatants were collected after 48 hours of T cell activation and stored at -30˚C until assayed. IL-2 and TNF-α levels were determined using a Luminex-based assay according the manufacturer's instructions. Data were collected using a Luminex 100 (Luminex Corporation, Austin, TX). Standard curves were generated using a 5-parameter logistic curve fitting equation weighted by 1/y (StarStation V 2.0; Applied Cytometry Systems, Sacramento, CA). Each sample reading was interpolated from the appropriate standard curve. Calculated concentrations were multiplied by the appropriate dilution factor when necessary.

6.3. *T cell activation: cell proliferation assay*

50 µl of 2 X vehicle, cyclosporin A or Test Article working solutions was added to control and anti-CD3 coated plates. 50 µl of 4 x 10^6 cells/ml was added to each well (200,000 cells/well). Plates were incubated at 37˚C with 5% CO_2 for 48 hours.

After 48 hours of T cell activation, cell proliferation was determined using the CellTiter-Glo Luminescent Cell Viability kit (ATP assay) according to the manufacturer's instructions. Measurements were made using a FLUOstar Omega microplate reader (BMG Labtech, Durham, NC). Relative luminescent units (RLU) are reported.

7. DATA EVALUATION

Values were analyzed using one-way ANOVA followed by Tukey's post test comparing sample values to the appropriate vehicle value (Prism V 4.0, GraphPad Software, San Diego, CA).

8. RESULTS

8.1. *Cell proliferation*

50 nM triptolide and triptonide significantly reduced cell proliferation when grown in the absence of anti-CD3 antibody (39% of vehicle, $p < 0.001$ and 28% of vehicle, $p < 0.001$, respectively). Triptolide at 1 nM and 10 nM and triptonide at 1 nM did not significantly affect cell proliferation (Table 1, Figure 1). 10 nM triptonide (80% of vehicle, $p < 0.001$), 1 nM CAY10470 (86% of vehicle, $p < 0.05$), 100 nM CAY10470 (72% of vehicle, $p < 0.001$), 1000 nM CAY10470 (68% of vehicle, $p < 0.001$), 1 nM GB117 (82% of vehicle, $p < 0.001$) and 100 nM GB117 (85% of vehicle, $p < 0.01$) showed an intermediate level of reduced cell proliferation. Therefore, care should be taken when evaluating the effect of these compounds on T cell activation. The remaining Test Articles did not reduce cell proliferation in the absence of anti-CD3 antibody.

8.2. *T cell activation*

The Test Articles did not significantly induce IL-2 or TNF-α production from PBMC's not treated with anti-CD3 antibody (Tables 2 and 3). Cell proliferation, IL-2 production and TNF-α production increased upon incubation with anti-CD3 antibody (Tables 1 – 3, Figures 2, 3 and 5). Co-stimulation with anti-CD3 and CD28 increased the mean production of IL-2 and TNF-α (Tables 2 and 3, Figures 4 and 6).

8.3. *Effect of cyclosporin A on inflammatory mediator production*

The Reference Article, 100 ng/ml cyclosporin A, significantly decreased anti-CD3 stimulated cell proliferation (63% of vehicle, $p < 0.001$), IL-2 production (1% of vehicle, $p < 0.001$) and TNF-α production (9% of vehicle, $p < 0.05$; Tables 1 – 3, Figures 2, 3 and 5). Cyclsporin A also decreased the anti-CD3/anti-CD28 co-stimulation of IL-2 (3% of vehicle, $p < 0.001$) and TNF-α production (34% of vehicle, $p > 0.05$; Tables 2 and 3, Figures 4 and 6). However, the latter was not statistically significant.

8.4. *Effect of triptolide on T cell activation*

10 nM triptolide significantly reduced the anti-CD3 induction of IL-2 (1% of vehicle, $p < 0.001$) and TNF-α (5% of vehicle, $p < 0.05$; Tables 2 and 3, Figures 3 and 5). 10 nM triptolide also decreased the anti-CD3/anti-CD28 co-stimulated induction of IL-2 (4% of vehicle, $p < 0.001$) and TNF-α (13% of vehicle, $p > 0.05$; Tables 2 and 3, Figures 4 and 6). However, the latter was not statistically significant. Anti-CD3 induced cell proliferation was not significantly reduced by 10 nM triptolide (Table 1, Figure 2). While 1 nM triptolide reduced anti-CD3 induced IL-2 production to 48% of the vehicle control, the results were not statistically significant (Table 2, Figure 3).

The effects observed in the presence of 50 nM triptolide are likely due to a general inhibition of cell proliferation rather than a specific inhibition of T cell activation.

8.5. *Effect of triptonide on T cell activation*

10 nM triptonide significantly reduced the anti-CD3 induction of cell proliferation (73% of vehicle, $p < 0.001$), IL-2 production (1% of vehicle, $p < 0.001$) and TNF-α production (3% of vehicle, $p < 0.05$; Tables 1 – 3, Figures 2, 3 and 5). 10 nM triptonide also decreased the anti-CD3/anti-CD28 co-stimulated induction of IL-2 (3% of vehicle, $p < 0.001$) and TNF-α (7% of vehicle, $p < 0.05$; Tables 2 and 3, Figures 4 and 6). While 1 nM triptolide reduced anti-CD3 induced IL-2 production to 52% of the vehicle control, the results were not statistically significant (Table 2, Figure 3).

The results observed with 10 nM triptonide are significant enough that they are likely due to the inhibition of T cell activation rather than a general inhibition of cell proliferation. The effects observed in the presence of 50 nM triptonide are likely due to a general inhibition of cell proliferation rather than a specific inhibition of T cell activation.

8.6. *Effect of (QNZ) CAY10470 on T cell activation*

100 nM and 1000 nM (QNZ) CAY10470 reduced the anti-CD3 induction of cell proliferation (63% of vehicle, $p < 0.001$ and 64% of vehicle, $p < 0.001$, respectively), IL-2 production (27% of vehicle, $p < 0.001$ and 25% of vehicle, $p < 0.001$, respectively) and TNF-α production (27% of vehicle, $p > 0.05$ and 29% of vehicle, $p > 0.05$; Tables 1 – 3, Figures 2, 3 and 5). However, the latter was not statistically significant. These effects were less pronounced in anti-CD3/anti-CD28 co-stimulated cells (Tables 2 and 3, Figures 4 and 6). 1 nM (QNZ) CAY10470 increased IL-2 production (170% of vehicle, $p < 0.05$) in the presence of anti-CD3 and anti-CD28 (Table 2, Figure 4).

The results observed with (QNZ) CAY10470 are significant enough that they are likely due to the inhibition of T cell activation rather than a general inhibition of cell proliferation.

8.7. *Effect of GB67A on inflammatory mediator production*

GB67A did not affect anti-CD3 activated cell proliferation, IL-2 production or TNF-α production (Tables 1 – 3, Figures 2 – 6).

8.8. *Effect of GB67B on inflammatory mediator production*

GB67B did not affect anti-CD3 activated cell proliferation, IL-2 production or TNF-α production (Tables 1 – 3, Figures 2 – 6).

8.9. *Effect of GB615 on inflammatory mediator production*

GB615 did not affect anti-CD3 activated cell proliferation, IL-2 production or TNF-α production (Tables 1 – 3, Figures 2 – 6). 1000 nM GB615 increased IL-2 production (173% of vehicle, $p < 0.05$) in the presence of anti-CD3 and anti-CD28 (Table 2, Figure 4).

8.10. *Effect of GB616 on inflammatory mediator production*

GB616 did not affect anti-CD3 activated cell proliferation, IL-2 production or TNF-α production (Tables 1 – 3, Figures 2 – 6).

8.11. *Effect of GB594 on inflammatory mediator production*

GB594 did not affect anti-CD3 activated cell proliferation, IL-2 production or TNF-α production (Tables 1 – 3, Figures 2 – 6). 1 nM GB594 increased TNF-α production (196% of vehicle, $p < 0.05$) in the presence of anti-CD3 and anti-CD28 (Table 3, Figure 6).

8.12. *Effect of GB595 on inflammatory mediator production*

GB595 did not affect anti-CD3 activated cell proliferation, IL-2 production or TNF-α production (Tables 1 – 3, Figures 2 – 6).

8.13. *Effect of GB65B on inflammatory mediator production*

GB65B did not affect anti-CD3 activated cell proliferation, IL-2 production or TNF-α production (Tables 1 – 3, Figures 2 – 6).

8.14. *Effect of GB117 on inflammatory mediator production*

GB117 did not affect anti-CD3 activated cell proliferation, IL-2 production or TNF-α production (Tables 1 – 3, Figures 2 – 6).

9. CONCLUSIONS

Triptolide and triptonide reduced cell proliferation at 50 nM on control plates suggesting that these compounds are either cytoxic or cytostatic. This effect was less apparent at 1 nM and 10 nM. The reduction of anti-CD3 induced IL-2 and TNF-α production in the presence of 10 nM triptolide and triptonide suggests that the compounds are inhibiting T cell activation in human PBMC's. (QNZ) CAY10470 displayed a weaker, but noticeable inhibition of T cell activation. None of the other Test Articles analyzed in this study inhibited anti-CD3 induced cell proliferation, IL-2 production or TNF-α production.

Table 1. Cell proliferation assay.

Plate	Treatment	Concentration	Mean Luminescence (RLU)	Std.Dev.	Mean % Vehicle (Control or anti-CD3)[1]	P Value[2]
Control	Vehicle		19700	613	100%	
Control	Cyclosporin A	100 ng/ml	18286	545	93%	P > 0.05
Control	Triptolide	1 nM	18277	1003	93%	P > 0.05
Control	Triptolide	10 nM	17409	256	88%	P > 0.05
Control	Triptolide	50 nM	7608	872	39%	**P < 0.001**
Control	Triptonide	1 nM	17641	882	90%	P > 0.05
Control	Triptonide	10 nM	15737	725	80%	**P < 0.001**
Control	Triptonide	50 nM	5577	100	28%	**P < 0.001**
Control	(QNZ) CAY10470	1 nM	16882	372	86%	**P < 0.05**
Control	(QNZ) CAY10470	100 nM	14163	426	72%	**P < 0.001**
Control	(QNZ) CAY10470	1000 nM	13381	514	68%	**P < 0.001**
Control	GB67A	1 nM	18168	128	92%	P > 0.05
Control	GB67A	100 nM	17685	495	90%	P > 0.05
Control	GB67A	1000 nM	18902	694	96%	P > 0.05
Control	GB67B	1 nM	18422	1105	94%	P > 0.05
Control	GB67B	100 nM	18461	840	94%	P > 0.05
Control	GB67B	1000 nM	18403	957	93%	P > 0.05
Control	GB615	1 nM	19350	636	98%	P > 0.05
Control	GB615	100 nM	18549	218	94%	P > 0.05
Control	GB615	1000 nM	18703	131	95%	P > 0.05
Control	GB616	1 nM	17861	148	91%	P > 0.05
Control	GB616	100 nM	18245	1283	93%	P > 0.05
Control	GB616	1000 nM	18670	571	95%	P > 0.05
Control	GB594	1 nM	19032	1187	97%	P > 0.05
Control	GB594	100 nM	18651	372	95%	P > 0.05
Control	GB594	1000 nM	18377	582	93%	P > 0.05
Control	GB595	1 nM	18550	461	94%	P > 0.05
Control	GB595	100 nM	18511	924	94%	P > 0.05
Control	GB595	1000 nM	18200	344	92%	P > 0.05
Control	GB65B	1 nM	18809	647	95%	P > 0.05
Control	GB65B	100 nM	18753	236	95%	P > 0.05
Control	GB65B	1000 nM	18771	928	95%	P > 0.05
Control	GB117	1 nM	16241	1473	82%	**P < 0.001**
Control	GB117	100 nM	16676	1523	85%	**P < 0.01**
Control	GB117	1000 nM	17336	1186	88%	P > 0.05
anti-CD3	Vehicle		29132	717	100%	
anti-CD3	Cyclosporin A	100 ng/ml	18376	972	63%	**P < 0.001**
anti-CD3	Triptolide	1 nM	29325	1367	101%	P > 0.05
anti-CD3	Triptolide	10 nM	25055	1512	86%	P > 0.05
anti-CD3	Triptolide	50 nM	9184	1072	32%	**P < 0.001**
anti-CD3	Triptonide	1 nM	29341	346	101%	P > 0.05
anti-CD3	Triptonide	10 nM	21395	3541	73%	**P < 0.001**
anti-CD3	Triptonide	50 nM	6296	216	22%	**P < 0.001**
anti-CD3	(QNZ) CAY10470	1 nM	28814	337	99%	P > 0.05
anti-CD3	(QNZ) CAY10470	100 nM	18212	1553	63%	**P < 0.001**
anti-CD3	(QNZ) CAY10470	1000 nM	18599	767	64%	**P < 0.001**
anti-CD3	GB67A	1 nM	27352	2083	94%	P > 0.05
anti-CD3	GB67A	100 nM	29744	560	102%	P > 0.05
anti-CD3	GB67A	1000 nM	25807	674	89%	P > 0.05
anti-CD3	GB67B	1 nM	29208	687	100%	P > 0.05
anti-CD3	GB67B	100 nM	29891	1724	103%	P > 0.05
anti-CD3	GB67B	1000 nM	25370	897	87%	P > 0.05
anti-CD3	GB615	1 nM	29382	527	101%	P > 0.05
anti-CD3	GB615	100 nM	30640	2433	105%	P > 0.05
anti-CD3	GB615	1000 nM	30416	763	104%	P > 0.05
anti-CD3	GB616	1 nM	32317	999	111%	P > 0.05
anti-CD3	GB616	100 nM	29478	473	101%	P > 0.05
anti-CD3	GB616	1000 nM	30392	2263	104%	P > 0.05
anti-CD3	GB594	1 nM	28985	251	99%	P > 0.05
anti-CD3	GB594	100 nM	28116	1112	97%	P > 0.05
anti-CD3	GB594	1000 nM	29711	567	102%	P > 0.05
anti-CD3	GB595	1 nM	31052	2965	107%	P > 0.05
anti-CD3	GB595	100 nM	28677	963	98%	P > 0.05

Table 1. Cell proliferation assay.

Plate	Treatment	Concentration	Mean Luminescence (RLU)	Std.Dev.	Mean % Vehicle (Control or anti-CD3)[1]	P Value[2]
anti-CD3	GB595	1000 nM	29632	6228	102%	P > 0.05
anti-CD3	GB65B	1 nM	29777	1345	102%	P > 0.05
anti-CD3	GB65B	100 nM	27698	757	95%	P > 0.05
anti-CD3	GB65B	1000 nM	27278	2680	94%	P > 0.05
anti-CD3	GB117	1 nM	31213	845	107%	P > 0.05
anti-CD3	GB117	100 nM	27880	2103	96%	P > 0.05
anti-CD3	GB117	1000 nM	29428	1028	101%	P > 0.05

[1](Mean sample control plate/mean vehicle control plate) expressed as a percent; (mean sample anti-CD3 plate/mean vehicle anti-CD3 plate) expressed as a percent.
[2]P values from one-way ANOVA with Tukey's post test (sample vs. Vehicle or sample vs. Vehicle + anti-CD3). P values below 0.05 are bold.

Table 2. Mean IL-2 production.

Plate	Treatment	Concentration	anti-CD28	Mean IL-2 (pg/ml)	Std. Dev.	Mean % Vehicle (anti-CD3)[1]	P Value[2]
Control	Vehicle		0	<LD	NA		
Control	Cyclosporin A	100 ng/ml	0	<LD	NA		
Control	Triptolide	1 nM	0	<LD	0.0		
Control	Triptolide	10 nM	0	<LD	NA		
Control	Triptolide	50 nM	0	<LD	NA		
Control	Triptonide	1 nM	0	<LD	0.0		
Control	Triptonide	10 nM	0	<LD	NA		
Control	Triptonide	50 nM	0	<LD	0.0		
Control	(QNZ) CAY10470	1 nM	0	<LD	NA		
Control	(QNZ) CAY10470	100 nM	0	<LD	NA		
Control	(QNZ) CAY10470	1000 nM	0	<LD	NA		
Control	GB67A	1 nM	0	<LD	NA		
Control	GB67A	100 nM	0	1.3	NA		
Control	GB67A	1000 nM	0	<LD	NA		
Control	GB67B	1 nM	0	<LD	0.0		
Control	GB67B	100 nM	0	<LD	NA		
Control	GB67B	1000 nM	0	<LD	NA		
Control	GB615	1 nM	0	<LD	0.0		
Control	GB615	100 nM	0	<LD	NA		
Control	GB615	1000 nM	0	<LD	NA		
Control	GB616	1 nM	0	<LD	0.0		
Control	GB616	100 nM	0	<LD	0.0		
Control	GB616	1000 nM	0	<LD	0.0		
Control	GB594	1 nM	0	<LD	NA		
Control	GB594	100 nM	0	<LD	NA		
Control	GB594	1000 nM	0	<LD	NA		
Control	GB595	1 nM	0	<LD	NA		
Control	GB595	100 nM	0	<LD	NA		
Control	GB595	1000 nM	0	<LD	NA		
Control	GB65B	1 nM	0	<LD	NA		
Control	GB65B	100 nM	0	<LD	NA		
Control	GB65B	1000 nM	0	<LD	NA		
Control	GB117	1 nM	0	<LD	NA		
Control	GB117	100 nM	0	<LD	NA		
Control	GB117	1000 nM	0	<LD	NA		
anti-CD3	Vehicle		0	1197.9	161.6	100%	
anti-CD3	Cyclosporin A	100 ng/ml	0	12.1	4.6	1%	**P < 0.001**
anti-CD3	Triptolide	1 nM	0	571.7	100.1	48%	P > 0.05
anti-CD3	Triptolide	10 nM	0	13.1	2.3	1%	**P < 0.001**
anti-CD3	Triptolide	50 nM	0	<LD	NA	0%	**P < 0.001**
anti-CD3	Triptonide	1 nM	0	620.1	67.2	52%	P > 0.05
anti-CD3	Triptonide	10 nM	0	13.6	3.2	1%	**P < 0.001**
anti-CD3	Triptonide	50 nM	0	<LD	NA	0%	**P < 0.001**
anti-CD3	(QNZ) CAY10470	1 nM	0	888.1	85.2	74%	P > 0.05
anti-CD3	(QNZ) CAY10470	100 nM	0	322.3	42.8	27%	**P < 0.001**
anti-CD3	(QNZ) CAY10470	1000 nM	0	302.3	46.7	25%	**P < 0.001**
anti-CD3	GB67A	1 nM	0	1056.6	338.4	88%	P > 0.05
anti-CD3	GB67A	100 nM	0	1287.1	504.2	107%	P > 0.05
anti-CD3	GB67A	1000 nM	0	930.9	300.3	78%	P > 0.05

Table 2. Mean IL-2 production.

Plate	Treatment	Concentration	anti-CD28	Mean IL-2 (pg/ml)	Std. Dev.	Mean % Vehicle (anti-CD3)[1]	P Value[2]
anti-CD3	GB67B	1 nM	0	1368.0	287.0	114%	P > 0.05
anti-CD3	GB67B	100 nM	0	879.3	238.5	73%	P > 0.05
anti-CD3	GB67B	1000 nM	0	1017.2	114.0	85%	P > 0.05
anti-CD3	GB615	1 nM	0	1122.7	246.6	94%	P > 0.05
anti-CD3	GB615	100 nM	0	1090.9	304.7	91%	P > 0.05
anti-CD3	GB615	1000 nM	0	1070.8	78.4	89%	P > 0.05
anti-CD3	GB616	1 nM	0	1194.2	59.6	100%	P > 0.05
anti-CD3	GB616	100 nM	0	1154.0	334.9	96%	P > 0.05
anti-CD3	GB616	1000 nM	0	1070.5	295.2	89%	P > 0.05
anti-CD3	GB594	1 nM	0	1399.6	94.8	117%	P > 0.05
anti-CD3	GB594	100 nM	0	1299.4	40.6	108%	P > 0.05
anti-CD3	GB594	1000 nM	0	1374.7	363.5	115%	P > 0.05
anti-CD3	GB595	1 nM	0	1322.0	193.7	110%	P > 0.05
anti-CD3	GB595	100 nM	0	1127.6	147.7	94%	P > 0.05
anti-CD3	GB595	1000 nM	0	1475.9	90.8	123%	P > 0.05
anti-CD3	GB65B	1 nM	0	1093.6	91.0	91%	P > 0.05
anti-CD3	GB65B	100 nM	0	973.1	170.5	81%	P > 0.05
anti-CD3	GB65B	1000 nM	0	1064.2	138.8	89%	P > 0.05
anti-CD3	GB117	1 nM	0	829.9	375.9	69%	P > 0.05
anti-CD3	GB117	100 nM	0	1243.5	74.2	104%	P > 0.05
anti-CD3	GB117	1000 nM	0	1008.9	143.0	84%	P > 0.05
anti-CD3	Vehicle		1 ug/ml	1935.8	707.9	100%	
anti-CD3	Cyclosporin A	100 ng/ml	1 ug/ml	50.4	5.0	3%	**P < 0.001**
anti-CD3	Triptolide	1 nM	1 ug/ml	1742.4	239.8	90%	P > 0.05
anti-CD3	Triptolide	10 nM	1 ug/ml	86.9	1.1	4%	**P < 0.001**
anti-CD3	Triptolide	50 nM	1 ug/ml	<LD	NA	0%	**P < 0.001**
anti-CD3	Triptonide	1 nM	1 ug/ml	1656.2	138.9	86%	P > 0.05
anti-CD3	Triptonide	10 nM	1 ug/ml	62.4	6.1	3%	**P < 0.001**
anti-CD3	Triptonide	50 nM	1 ug/ml	<LD	NA	0%	**P < 0.001**
anti-CD3	(QNZ) CAY10470	1 nM	1 ug/ml	3294.7	351.8	170%	**P < 0.05**
anti-CD3	(QNZ) CAY10470	100 nM	1 ug/ml	1117.2	140.3	58%	P > 0.05
anti-CD3	(QNZ) CAY10470	1000 nM	1 ug/ml	1116.8	96.9	58%	P > 0.05
anti-CD3	GB67A	1 nM	1 ug/ml	2674.1	277.5	138%	P > 0.05
anti-CD3	GB67A	100 nM	1 ug/ml	2578.2	371.2	133%	P > 0.05
anti-CD3	GB67A	1000 nM	1 ug/ml	2250.9	254.4	116%	P > 0.05
anti-CD3	GB67B	1 nM	1 ug/ml	3063.0	361.9	158%	P > 0.05
anti-CD3	GB67B	100 nM	1 ug/ml	2760.4	267.9	143%	P > 0.05
anti-CD3	GB67B	1000 nM	1 ug/ml	2223.6	231.5	115%	P > 0.05
anti-CD3	GB615	1 nM	1 ug/ml	2724.3	383.9	141%	P > 0.05
anti-CD3	GB615	100 nM	1 ug/ml	2462.3	1473.7	127%	P > 0.05
anti-CD3	GB615	1000 nM	1 ug/ml	3348.3	374.9	173%	**P < 0.05**
anti-CD3	GB616	1 nM	1 ug/ml	2840.3	538.6	147%	P > 0.05
anti-CD3	GB616	100 nM	1 ug/ml	2732.7	217.5	141%	P > 0.05
anti-CD3	GB616	1000 nM	1 ug/ml	3018.1	471.6	156%	P > 0.05
anti-CD3	GB594	1 nM	1 ug/ml	2972.1	416.7	154%	P > 0.05
anti-CD3	GB594	100 nM	1 ug/ml	2497.0	273.8	129%	P > 0.05
anti-CD3	GB594	1000 nM	1 ug/ml	2199.7	155.8	114%	P > 0.05
anti-CD3	GB595	1 nM	1 ug/ml	2362.1	305.9	122%	P > 0.05
anti-CD3	GB595	100 nM	1 ug/ml	2383.1	378.4	123%	P > 0.05
anti-CD3	GB595	1000 nM	1 ug/ml	2625.5	295.5	136%	P > 0.05
anti-CD3	GB65B	1 nM	1 ug/ml	3079.8	550.2	159%	P > 0.05
anti-CD3	GB65B	100 nM	1 ug/ml	2025.4	314.6	105%	P > 0.05
anti-CD3	GB65B	1000 nM	1 ug/ml	1774.9	181.8	92%	P > 0.05
anti-CD3	GB117	1 nM	1 ug/ml	2614.5	425.6	135%	P > 0.05
anti-CD3	GB117	100 nM	1 ug/ml	2736.2	483.1	141%	P > 0.05
anti-CD3	GB117	1000 nM	1 ug/ml	2752.9	576.9	142%	P > 0.05

[1](Mean sample control plate/mean vehicle control plate) expressed as a percent.
[2]P values from one-way ANOVA with Tukey's post test (sample vs. Vehicle or sample vs. Vehicle + anti-CD3). P values below 0.05 are bold.

Table 3. Mean TNF-α production.

Plate	Treatment	Concentration	anti-CD28	Mean TNF-α (pg/ml)	Std. Dev.	Mean % Vehicle (anti-CD3)[1]	P Value[2]
Control	Vehicle		0	<LD	NA		
Control	Cyclosporin A	100 ng/ml	0	<LD	NA		
Control	Triptolide	1 nM	0	<LD	NA		
Control	Triptolide	10 nM	0	<LD	NA		
Control	Triptolide	50 nM	0	<LD	NA		
Control	Triptonide	1 nM	0	<LD	NA		
Control	Triptonide	10 nM	0	<LD	NA		
Control	Triptonide	50 nM	0	<LD	NA		
Control	(QNZ) CAY10470	1 nM	0	<LD	NA		
Control	(QNZ) CAY10470	100 nM	0	31.4	NA		
Control	(QNZ) CAY10470	1000 nM	0	<LD	NA		
Control	GB67A	1 nM	0	<LD	NA		
Control	GB67A	100 nM	0	<LD	NA		
Control	GB67A	1000 nM	0	<LD	NA		
Control	GB67B	1 nM	0	<LD	NA		
Control	GB67B	100 nM	0	<LD	NA		
Control	GB67B	1000 nM	0	<LD	NA		
Control	GB615	1 nM	0	<LD	NA		
Control	GB615	100 nM	0	<LD	NA		
Control	GB615	1000 nM	0	<LD	NA		
Control	GB616	1 nM	0	<LD	NA		
Control	GB616	100 nM	0	<LD	NA		
Control	GB616	1000 nM	0	<LD	NA		
Control	GB594	1 nM	0	9.0	NA		
Control	GB594	100 nM	0	<LD	NA		
Control	GB594	1000 nM	0	<LD	NA		
Control	GB595	1 nM	0	<LD	NA		
Control	GB595	100 nM	0	<LD	NA		
Control	GB595	1000 nM	0	<LD	NA		
Control	GB65B	1 nM	0	<LD	NA		
Control	GB65B	100 nM	0	<LD	NA		
Control	GB65B	1000 nM	0	<LD	NA		
Control	GB117	1 nM	0	<LD	NA		
Control	GB117	100 nM	0	<LD	NA		
Control	GB117	1000 nM	0	<LD	NA		
anti-CD3	Vehicle		0	3380.4	307.8	100%	
anti-CD3	Cyclosporin A	100 ng/ml	0	306.0	306.5	9%	**P < 0.05**
anti-CD3	Triptolide	1 nM	0	2638.3	293.4	78%	P > 0.05
anti-CD3	Triptolide	10 nM	0	177.4	1.7	5%	**P < 0.05**
anti-CD3	Triptolide	50 nM	0	<LD	NA	0%	**P < 0.01**
anti-CD3	Triptonide	1 nM	0	2364.6	455.6	70%	P > 0.05
anti-CD3	Triptonide	10 nM	0	112.3	40.3	3%	**P < 0.05**
anti-CD3	Triptonide	50 nM	0	1.2	NA	0%	**P < 0.01**
anti-CD3	(QNZ) CAY10470	1 nM	0	2557.4	409.0	76%	P > 0.05
anti-CD3	(QNZ) CAY10470	100 nM	0	909.3	191.5	27%	P > 0.05
anti-CD3	(QNZ) CAY10470	1000 nM	0	986.1	34.8	29%	P > 0.05
anti-CD3	GB67A	1 nM	0	3315.9	1559.7	98%	P > 0.05
anti-CD3	GB67A	100 nM	0	4282.9	2725.2	127%	P > 0.05
anti-CD3	GB67A	1000 nM	0	2640.7	1008.8	78%	P > 0.05
anti-CD3	GB67B	1 nM	0	3334.2	864.1	99%	P > 0.05
anti-CD3	GB67B	100 nM	0	3702.0	1674.0	110%	P > 0.05
anti-CD3	GB67B	1000 nM	0	3230.3	565.6	96%	P > 0.05
anti-CD3	GB615	1 nM	0	2913.0	614.6	86%	P > 0.05
anti-CD3	GB615	100 nM	0	3421.1	1037.8	101%	P > 0.05
anti-CD3	GB615	1000 nM	0	2995.3	251.1	89%	P > 0.05
anti-CD3	GB616	1 nM	0	3978.8	561.9	118%	P > 0.05
anti-CD3	GB616	100 nM	0	2732.3	1225.5	81%	P > 0.05
anti-CD3	GB616	1000 nM	0	2538.6	716.8	75%	P > 0.05
anti-CD3	GB594	1 nM	0	4759.9	1272.2	141%	P > 0.05
anti-CD3	GB594	100 nM	0	3855.7	554.5	114%	P > 0.05
anti-CD3	GB594	1000 nM	0	4276.0	962.7	126%	P > 0.05

Table 3. Mean TNF-α production.

Plate	Treatment	Concentration	anti-CD28	Mean TNF-α (pg/ml)	Std. Dev.	Mean % Vehicle (anti-CD3)[1]	P Value[2]
anti-CD3	GB595	1 nM	0	4718.9	1456.3	140%	P > 0.05
anti-CD3	GB595	100 nM	0	3557.0	158.5	105%	P > 0.05
anti-CD3	GB595	1000 nM	0	3907.9	203.5	116%	P > 0.05
anti-CD3	GB65B	1 nM	0	3393.8	652.3	100%	P > 0.05
anti-CD3	GB65B	100 nM	0	2773.6	1250.8	82%	P > 0.05
anti-CD3	GB65B	1000 nM	0	3277.8	347.6	97%	P > 0.05
anti-CD3	GB117	1 nM	0	2703.8	311.5	80%	P > 0.05
anti-CD3	GB117	100 nM	0	3507.9	738.8	104%	P > 0.05
anti-CD3	GB117	1000 nM	0	2701.3	1123.1	80%	P > 0.05
anti-CD3	Vehicle		1 ug/ml	3751.5	1133.7	100%	
anti-CD3	Cyclosporin A	100 ng/ml	1 ug/ml	1269.7	585.6	34%	P > 0.05
anti-CD3	Triptolide	1 nM	1 ug/ml	4428.0	643.7	118%	P > 0.05
anti-CD3	Triptolide	10 nM	1 ug/ml	500.1	94.7	13%	P > 0.05
anti-CD3	Triptolide	50 nM	1 ug/ml	3.3	0.8	0%	**P < 0.05**
anti-CD3	Triptonide	1 nM	1 ug/ml	4729.2	2082.0	126%	P > 0.05
anti-CD3	Triptonide	10 nM	1 ug/ml	268.5	76.8	7%	**P < 0.05**
anti-CD3	Triptonide	50 nM	1 ug/ml	5.7	2.1	0%	**P < 0.05**
anti-CD3	(QNZ) CAY10470	1 nM	1 ug/ml	5646.6	1746.8	151%	P > 0.05
anti-CD3	(QNZ) CAY10470	100 nM	1 ug/ml	2183.8	592.8	58%	P > 0.05
anti-CD3	(QNZ) CAY10470	1000 nM	1 ug/ml	1342.1	264.7	36%	P > 0.05
anti-CD3	GB67A	1 nM	1 ug/ml	4033.8	362.1	108%	P > 0.05
anti-CD3	GB67A	100 nM	1 ug/ml	4251.7	623.8	113%	P > 0.05
anti-CD3	GB67A	1000 nM	1 ug/ml	3609.3	1515.7	96%	P > 0.05
anti-CD3	GB67B	1 nM	1 ug/ml	5519.8	308.8	147%	P > 0.05
anti-CD3	GB67B	100 nM	1 ug/ml	4962.1	473.0	132%	P > 0.05
anti-CD3	GB67B	1000 nM	1 ug/ml	4142.2	1027.0	110%	P > 0.05
anti-CD3	GB615	1 nM	1 ug/ml	5009.1	585.0	134%	P > 0.05
anti-CD3	GB615	100 nM	1 ug/ml	4085.0	2641.1	109%	P > 0.05
anti-CD3	GB615	1000 nM	1 ug/ml	6218.4	1805.2	166%	P > 0.05
anti-CD3	GB616	1 nM	1 ug/ml	4732.3	1355.1	126%	P > 0.05
anti-CD3	GB616	100 nM	1 ug/ml	4867.6	893.3	130%	P > 0.05
anti-CD3	GB616	1000 nM	1 ug/ml	4263.3	930.8	114%	P > 0.05
anti-CD3	GB594	1 nM	1 ug/ml	7339.1	1429.9	196%	**P < 0.05**
anti-CD3	GB594	100 nM	1 ug/ml	3976.9	392.9	106%	P > 0.05
anti-CD3	GB594	1000 nM	1 ug/ml	4200.5	635.0	112%	P > 0.05
anti-CD3	GB595	1 nM	1 ug/ml	4430.8	491.5	118%	P > 0.05
anti-CD3	GB595	100 nM	1 ug/ml	4317.8	1216.9	115%	P > 0.05
anti-CD3	GB595	1000 nM	1 ug/ml	5748.4	393.4	153%	P > 0.05
anti-CD3	GB65B	1 nM	1 ug/ml	5751.5	1573.3	153%	P > 0.05
anti-CD3	GB65B	100 nM	1 ug/ml	3654.8	930.2	97%	P > 0.05
anti-CD3	GB65B	1000 nM	1 ug/ml	4086.8	1156.7	109%	P > 0.05
anti-CD3	GB117	1 nM	1 ug/ml	3951.8	582.1	105%	P > 0.05
anti-CD3	GB117	100 nM	1 ug/ml	2646.0	524.7	71%	P > 0.05
anti-CD3	GB117	1000 nM	1 ug/ml	3069.7	1228.4	82%	P > 0.05

[1](Mean sample control plate/mean vehicle control plate) expressed as a percent.
[2]P values from one-way ANOVA with Tukey's post test (sample vs. Vehicle or sample vs. Vehicle + anti-CD3). P values below 0.05 are bold.

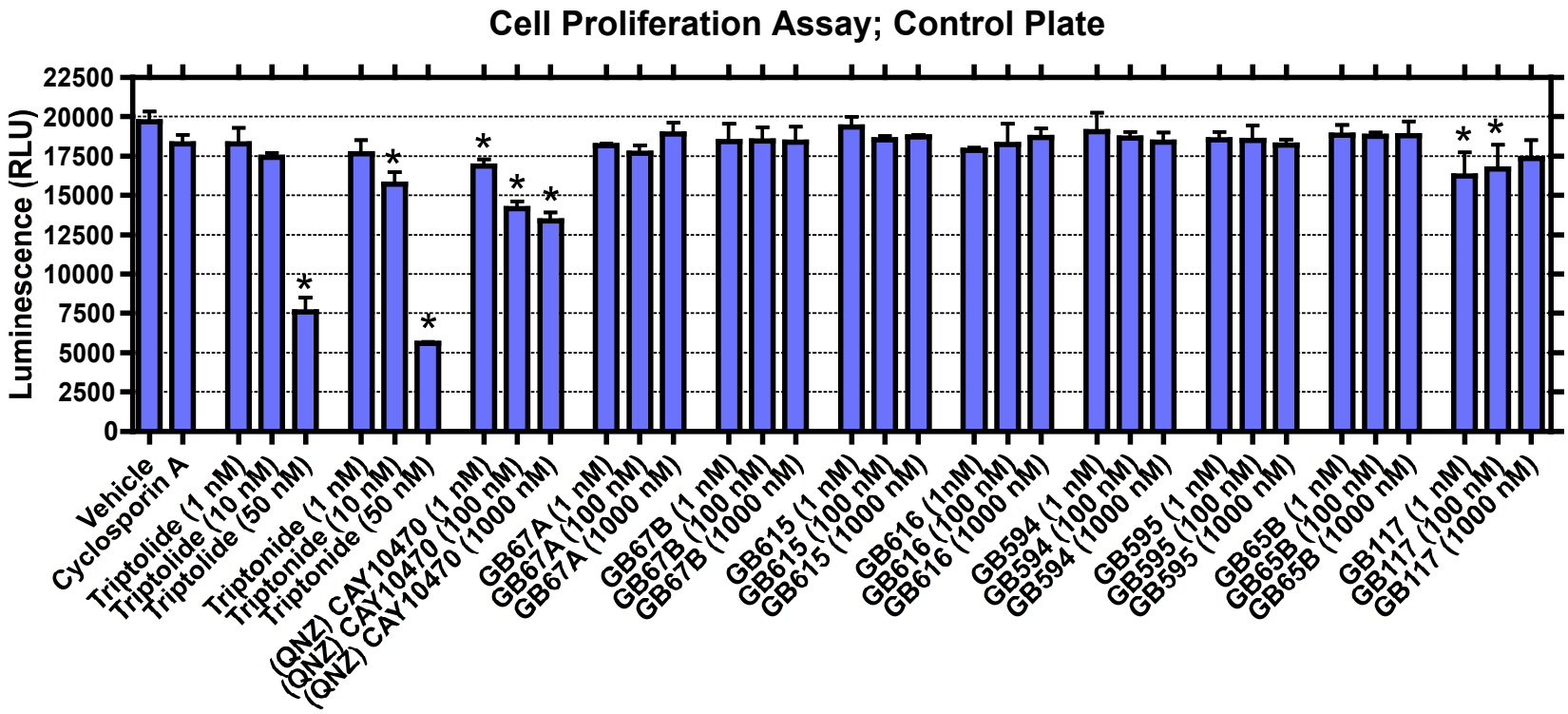

Figure 1. Cell proliferation assay, control plate. PBMC's were incubated on uncoated control plates for 48 hours. Cell proliferation was determined using a luminescent ATP cell viability assay. Mean values are shown. Error bars represent standard deviations. Values were analyzed by one-way ANOVA with Tukey's post-test comparing sample values to the vehicle value. *P < 0.05.

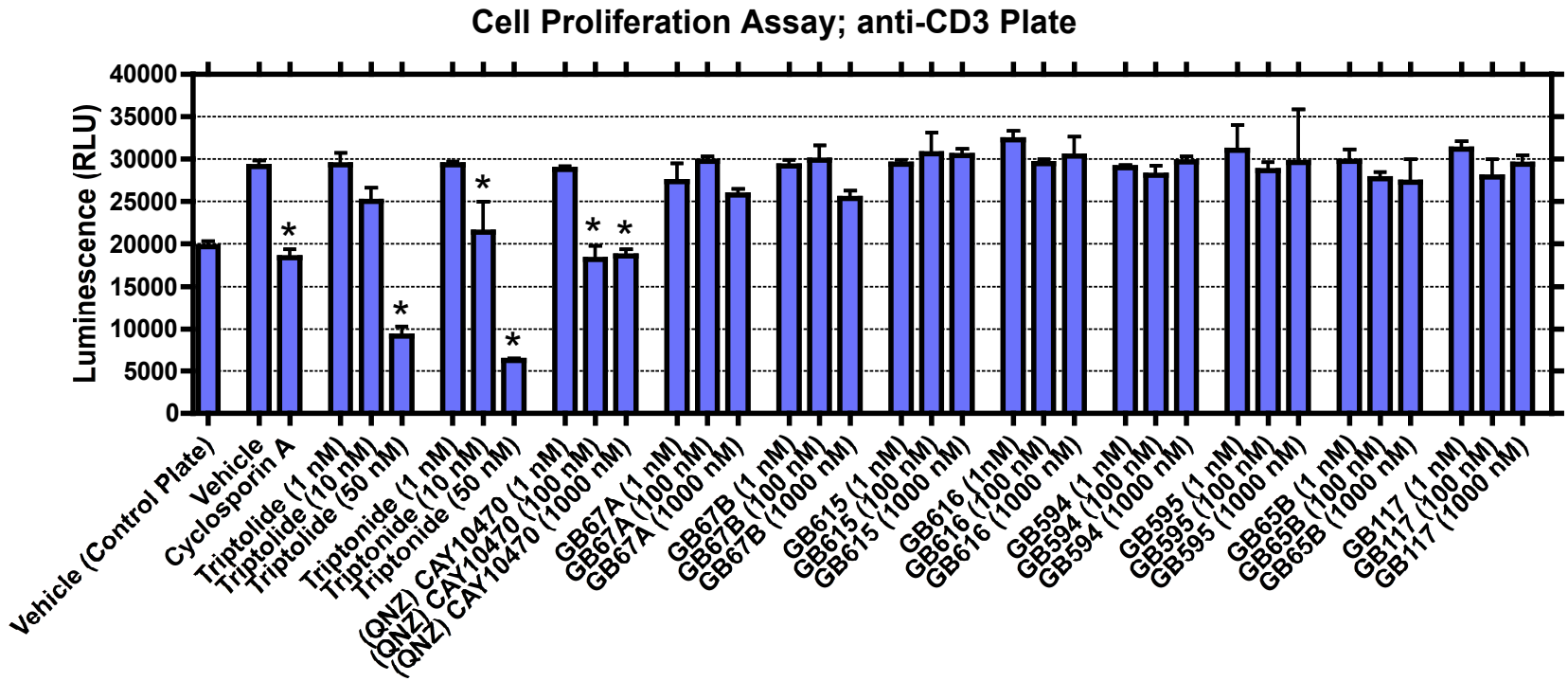

Figure 2. Cell proliferation assay, anti-CD3 plate. PBMC's were incubated on anti-CD3 coated plates for 48 hours. Cell proliferation was determined using a luminescent ATP cell viability assay. Mean values are shown. Error bars represent standard deviations. The vehicle value from the control plate is shown as a reference. Values were analyzed by one-way ANOVA with Tukey's post-test comparing sample values to the vehicle + anti-CD3 value. *$P < 0.05$.

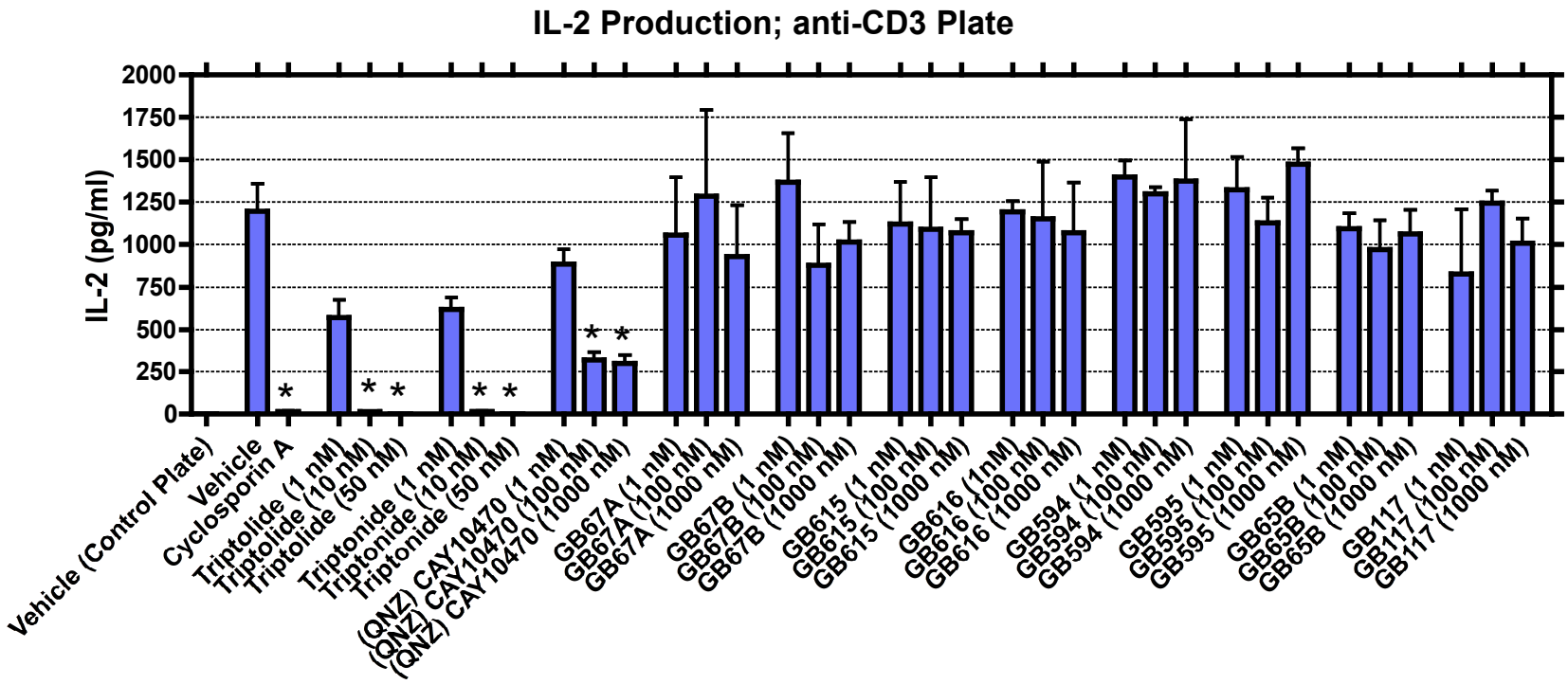

Figure 3. Mean IL-2 production, anti-CD3 plate. PBMC's were incubated on uncoated control plates and anti-CD3 plates for 48 hours. Mean values from PBMC's grown on anti-CD3 coated plates are shown. The vehicle control sample grown on the uncoated plate is shown as a reference. Error bars represent standard deviations. Values were analyzed by one-way ANOVA with Tukey's post-test comparing sample values to the vehicle + anti-CD3 value. *$P < 0.05$.

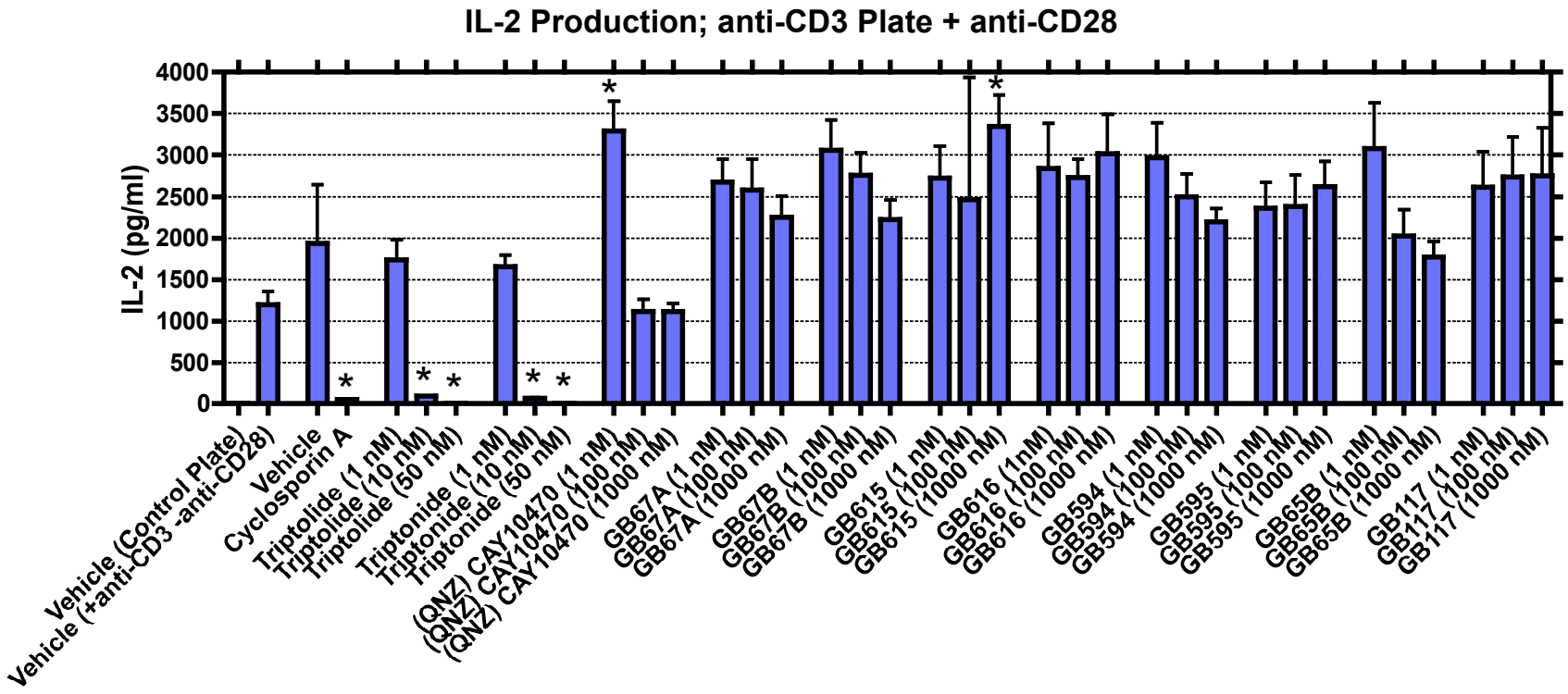

Figure 4. Mean IL-2 production, anti-CD3 plate + anti-CD28. PBMC's were incubated on anti-CD3 coated plates in the presence of anti-CD28 antibody plates for 48 hours. Mean values from PBMC's grown on anti-CD3 coated plates in the presence of anti-CD28 are shown. The vehicle control samples grown on uncoated plates and on anti-CD3 coated plates in the absence of anti-CD28 are shown for reference. Error bars represent standard deviations. Values were analyzed by one-way ANOVA with Tukey's post-test comparing sample values to the vehicle + anti-CD3 + anti-CD28 value. *P < 0.05.

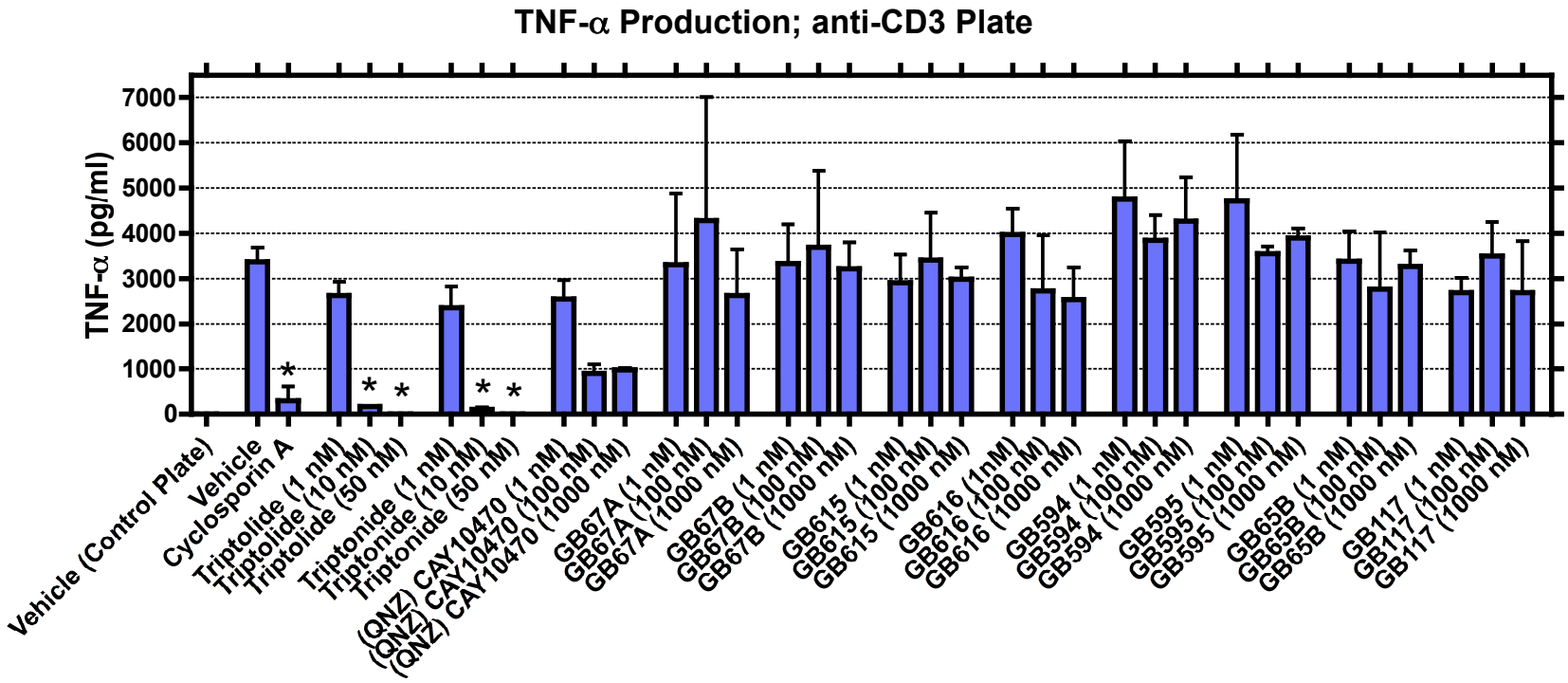

Figure 5. Mean TNF-α production, anti-CD3 plate. PBMC's were incubated on uncoated control plates and anti-CD3 plates for 48 hours. Mean values from PBMC's grown on anti-CD3 coated plates are shown. The vehicle control sample grown on the uncoated plate is shown for reference. Error bars represent standard deviations. Values were analyzed by one-way ANOVA with Tukey's post-test comparing sample values to the vehicle + anti-CD3 value. *$P < 0.05$.

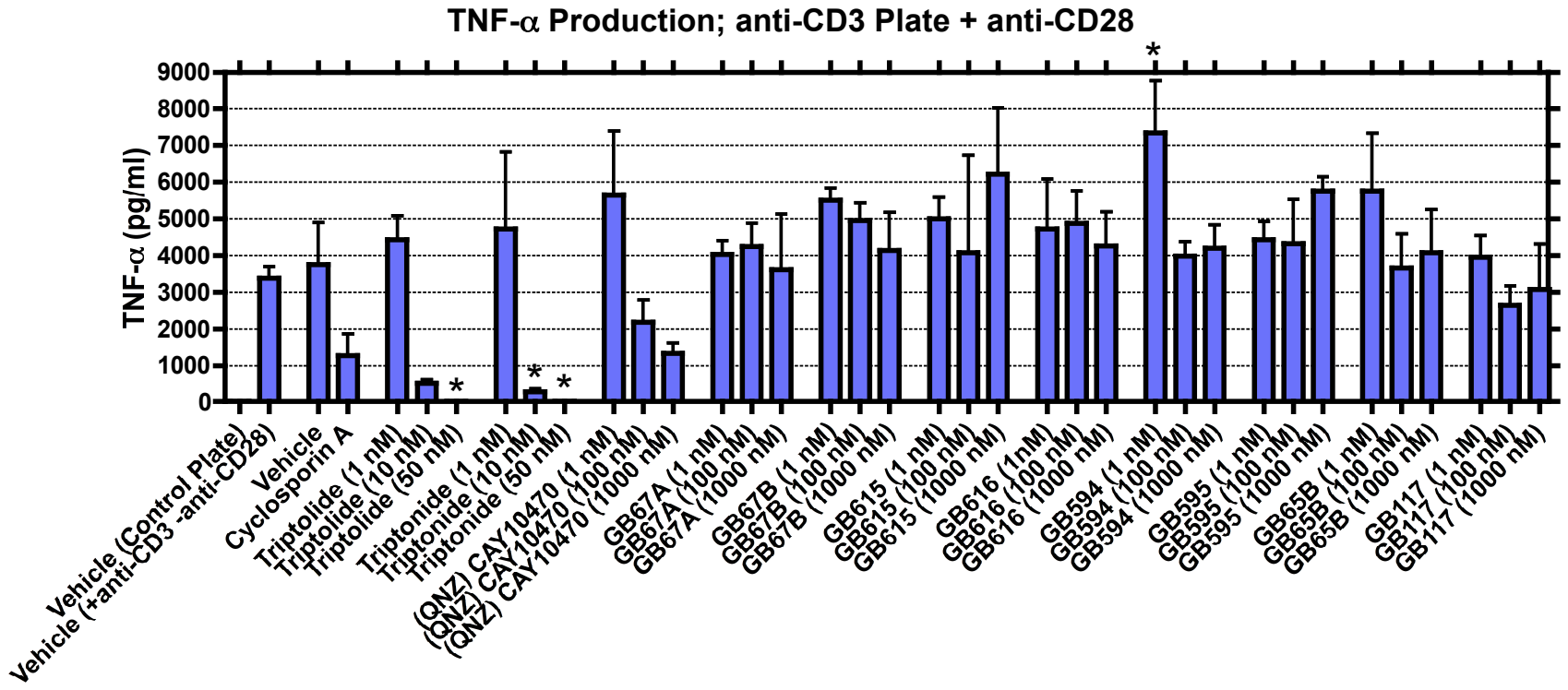

Figure 6. Mean TNF-α production, anti-CD3 plate + anti-CD28. PBMC's were incubated on anti-CD3 coated plates in the presence of anti-CD28 antibody plates for 48 hours. Mean values from PBMC's grown on anti-CD3 coated plates in the presence of anti-CD28 are shown. The vehicle control samples grown on uncoated plates and on anti-CD3 coated plates in the absence of anti-CD28 are shown for reference. Error bars represent standard deviations. Values were analyzed by one-way ANOVA with Tukey's post-test comparing sample values to the vehicle + anti-CD3 + anti-CD28 value. *P < 0.05.

Table 4. Cell proliferation assay raw data.

Plate	Treatment	Concentration	Luminescence (RLU)
Control	Vehicle		19915
Control	Vehicle		19008
Control	Vehicle		20176
Control	Cyclosporin A	100 ng/ml	18777
Control	Cyclosporin A	100 ng/ml	17699
Control	Cyclosporin A	100 ng/ml	18381
Control	Triptolide	1 nM	18686
Control	Triptolide	1 nM	19012
Control	Triptolide	1 nM	17134
Control	Triptolide	10 nM	17638
Control	Triptolide	10 nM	17132
Control	Triptolide	10 nM	17458
Control	Triptolide	50 nM	7601
Control	Triptolide	50 nM	8483
Control	Triptolide	50 nM	6740
Control	Triptonide	1 nM	18414
Control	Triptonide	1 nM	17829
Control	Triptonide	1 nM	16680
Control	Triptonide	10 nM	14967
Control	Triptonide	10 nM	16407
Control	Triptonide	10 nM	15838
Control	Triptonide	50 nM	5619
Control	Triptonide	50 nM	5649
Control	Triptonide	50 nM	5462
Control	(QNZ) CAY10470	1 nM	17179
Control	(QNZ) CAY10470	1 nM	17002
Control	(QNZ) CAY10470	1 nM	16464
Control	(QNZ) CAY10470	100 nM	14606
Control	(QNZ) CAY10470	100 nM	13756
Control	(QNZ) CAY10470	100 nM	14126
Control	(QNZ) CAY10470	1000 nM	13951
Control	(QNZ) CAY10470	1000 nM	13241
Control	(QNZ) CAY10470	1000 nM	12951
Control	GB67A	1 nM	18216
Control	GB67A	1 nM	18264
Control	GB67A	1 nM	18023
Control	GB67A	100 nM	18238
Control	GB67A	100 nM	17532
Control	GB67A	100 nM	17285
Control	GB67A	1000 nM	18578
Control	GB67A	1000 nM	19699
Control	GB67A	1000 nM	18430
Control	GB67B	1 nM	19341
Control	GB67B	1 nM	17196
Control	GB67B	1 nM	18729
Control	GB67B	100 nM	19039
Control	GB67B	100 nM	18847
Control	GB67B	100 nM	17498
Control	GB67B	1000 nM	17596
Control	GB67B	1000 nM	19460
Control	GB67B	1000 nM	18153
Control	GB615	1 nM	18941
Control	GB615	1 nM	19025
Control	GB615	1 nM	20083
Control	GB615	100 nM	18797
Control	GB615	100 nM	18387
Control	GB615	100 nM	18462
Control	GB615	1000 nM	18806
Control	GB615	1000 nM	18555
Control	GB615	1000 nM	18748
Control	GB616	1 nM	17879
Control	GB616	1 nM	17999
Control	GB616	1 nM	17704
Control	GB616	100 nM	17605

Table 4. Cell proliferation assay raw data.

Plate	Treatment	Concentration	Luminescence (RLU)
Control	GB616	100 nM	19722
Control	GB616	100 nM	17408
Control	GB616	1000 nM	19144
Control	GB616	1000 nM	18829
Control	GB616	1000 nM	18036
Control	GB594	1 nM	17902
Control	GB594	1 nM	18927
Control	GB594	1 nM	20268
Control	GB594	100 nM	18964
Control	GB594	100 nM	18240
Control	GB594	100 nM	18749
Control	GB594	1000 nM	18837
Control	GB594	1000 nM	18571
Control	GB594	1000 nM	17723
Control	GB595	1 nM	18875
Control	GB595	1 nM	18753
Control	GB595	1 nM	18022
Control	GB595	100 nM	19172
Control	GB595	100 nM	18906
Control	GB595	100 nM	17456
Control	GB595	1000 nM	18551
Control	GB595	1000 nM	18184
Control	GB595	1000 nM	17864
Control	GB65B	1 nM	18568
Control	GB65B	1 nM	19542
Control	GB65B	1 nM	18316
Control	GB65B	100 nM	18715
Control	GB65B	100 nM	19006
Control	GB65B	100 nM	18539
Control	GB65B	1000 nM	18340
Control	GB65B	1000 nM	19836
Control	GB65B	1000 nM	18138
Control	GB117	1 nM	17941
Control	GB117	1 nM	15386
Control	GB117	1 nM	15395
Control	GB117	100 nM	17552
Control	GB117	100 nM	17558
Control	GB117	100 nM	14917
Control	GB117	1000 nM	18031
Control	GB117	1000 nM	15966
Control	GB117	1000 nM	18010
anti-CD3	Vehicle		28308
anti-CD3	Vehicle		29476
anti-CD3	Vehicle		29613
anti-CD3	Cyclosporin A	100 ng/ml	19074
anti-CD3	Cyclosporin A	100 ng/ml	17266
anti-CD3	Cyclosporin A	100 ng/ml	18787
anti-CD3	Triptolide	1 nM	29682
anti-CD3	Triptolide	1 nM	27814
anti-CD3	Triptolide	1 nM	30478
anti-CD3	Triptolide	10 nM	25588
anti-CD3	Triptolide	10 nM	26228
anti-CD3	Triptolide	10 nM	23348
anti-CD3	Triptolide	50 nM	9179
anti-CD3	Triptolide	50 nM	8115
anti-CD3	Triptolide	50 nM	10259
anti-CD3	Triptonide	1 nM	29142
anti-CD3	Triptonide	1 nM	29741
anti-CD3	Triptonide	1 nM	29141
anti-CD3	Triptonide	10 nM	21326
anti-CD3	Triptonide	10 nM	17889
anti-CD3	Triptonide	10 nM	24970
anti-CD3	Triptonide	50 nM	6144
anti-CD3	Triptonide	50 nM	6543

Table 4. Cell proliferation assay raw data.

Plate	Treatment	Concentration	Luminescence (RLU)
anti-CD3	Triptonide	50 nM	6200
anti-CD3	(QNZ) CAY10470	1 nM	28883
anti-CD3	(QNZ) CAY10470	1 nM	29112
anti-CD3	(QNZ) CAY10470	1 nM	28448
anti-CD3	(QNZ) CAY10470	100 nM	17765
anti-CD3	(QNZ) CAY10470	100 nM	16932
anti-CD3	(QNZ) CAY10470	100 nM	19940
anti-CD3	(QNZ) CAY10470	1000 nM	19481
anti-CD3	(QNZ) CAY10470	1000 nM	18218
anti-CD3	(QNZ) CAY10470	1000 nM	18097
anti-CD3	GB67A	1 nM	27141
anti-CD3	GB67A	1 nM	29532
anti-CD3	GB67A	1 nM	25383
anti-CD3	GB67A	100 nM	30223
anti-CD3	GB67A	100 nM	29128
anti-CD3	GB67A	100 nM	29881
anti-CD3	GB67A	1000 nM	26561
anti-CD3	GB67A	1000 nM	25597
anti-CD3	GB67A	1000 nM	25262
anti-CD3	GB67B	1 nM	28751
anti-CD3	GB67B	1 nM	29998
anti-CD3	GB67B	1 nM	28876
anti-CD3	GB67B	100 nM	29199
anti-CD3	GB67B	100 nM	31853
anti-CD3	GB67B	100 nM	28620
anti-CD3	GB67B	1000 nM	26082
anti-CD3	GB67B	1000 nM	24363
anti-CD3	GB67B	1000 nM	25665
anti-CD3	GB615	1 nM	28925
anti-CD3	GB615	1 nM	29958
anti-CD3	GB615	1 nM	29263
anti-CD3	GB615	100 nM	30934
anti-CD3	GB615	100 nM	32913
anti-CD3	GB615	100 nM	28073
anti-CD3	GB615	1000 nM	30160
anti-CD3	GB615	1000 nM	29813
anti-CD3	GB615	1000 nM	31274
anti-CD3	GB616	1 nM	31166
anti-CD3	GB616	1 nM	32820
anti-CD3	GB616	1 nM	32964
anti-CD3	GB616	100 nM	28953
anti-CD3	GB616	100 nM	29612
anti-CD3	GB616	100 nM	29870
anti-CD3	GB616	1000 nM	28653
anti-CD3	GB616	1000 nM	32951
anti-CD3	GB616	1000 nM	29571
anti-CD3	GB594	1 nM	29089
anti-CD3	GB594	1 nM	29167
anti-CD3	GB594	1 nM	28698
anti-CD3	GB594	100 nM	29397
anti-CD3	GB594	100 nM	27393
anti-CD3	GB594	100 nM	27558
anti-CD3	GB594	1000 nM	30193
anti-CD3	GB594	1000 nM	29854
anti-CD3	GB594	1000 nM	29086
anti-CD3	GB595	1 nM	30649
anti-CD3	GB595	1 nM	34197
anti-CD3	GB595	1 nM	28309
anti-CD3	GB595	100 nM	29274
anti-CD3	GB595	100 nM	27566
anti-CD3	GB595	100 nM	29192
anti-CD3	GB595	1000 nM	22463
anti-CD3	GB595	1000 nM	33711
anti-CD3	GB595	1000 nM	32723

Table 4. Cell proliferation assay raw data.

Plate	Treatment	Concentration	Luminescence (RLU)
anti-CD3	GB65B	1 nM	29822
anti-CD3	GB65B	1 nM	31099
anti-CD3	GB65B	1 nM	28410
anti-CD3	GB65B	100 nM	27483
anti-CD3	GB65B	100 nM	27071
anti-CD3	GB65B	100 nM	28539
anti-CD3	GB65B	1000 nM	28479
anti-CD3	GB65B	1000 nM	29148
anti-CD3	GB65B	1000 nM	24208
anti-CD3	GB117	1 nM	31860
anti-CD3	GB117	1 nM	31523
anti-CD3	GB117	1 nM	30257
anti-CD3	GB117	100 nM	26206
anti-CD3	GB117	100 nM	30241
anti-CD3	GB117	100 nM	27193
anti-CD3	GB117	1000 nM	28247
anti-CD3	GB117	1000 nM	30115
anti-CD3	GB117	1000 nM	29923

Table 5. Cytokine production raw data.

Plate	Treatment	Concentration	anti-CD28	IL-2 (pg/ml)	TNF-α (pg/ml)
Control	Vehicle		0	<LD	<LD
Control	Vehicle		0	0	<LD
Control	Vehicle		0	<LD	<LD
Control	Cyclosporin A	100 ng/ml	0	<LD	<LD
Control	Cyclosporin A	100 ng/ml	0	<LD	<LD
Control	Cyclosporin A	100 ng/ml	0	<LD	<LD
Control	Triptolide	1 nM	0	0	<LD
Control	Triptolide	1 nM	0	0	<LD
Control	Triptolide	1 nM	0	<LD	<LD
Control	Triptolide	10 nM	0	<LD	<LD
Control	Triptolide	10 nM	0	<LD	<LD
Control	Triptolide	10 nM	0	<LD	<LD
Control	Triptolide	50 nM	0	<LD	<LD
Control	Triptolide	50 nM	0	<LD	<LD
Control	Triptolide	50 nM	0	0	<LD
Control	Triptonide	1 nM	0	0	<LD
Control	Triptonide	1 nM	0	0	<LD
Control	Triptonide	1 nM	0	0	<LD
Control	Triptonide	10 nM	0	<LD	<LD
Control	Triptonide	10 nM	0	<LD	<LD
Control	Triptonide	10 nM	0	<LD	<LD
Control	Triptonide	50 nM	0	0	<LD
Control	Triptonide	50 nM	0	<LD	<LD
Control	Triptonide	50 nM	0	0	<LD
Control	(QNZ) CAY10470	1 nM	0	<LD	<LD
Control	(QNZ) CAY10470	1 nM	0	<LD	<LD
Control	(QNZ) CAY10470	1 nM	0	<LD	<LD
Control	(QNZ) CAY10470	100 nM	0	<LD	<LD
Control	(QNZ) CAY10470	100 nM	0	<LD	31
Control	(QNZ) CAY10470	100 nM	0	<LD	<LD
Control	(QNZ) CAY10470	1000 nM	0	<LD	<LD
Control	(QNZ) CAY10470	1000 nM	0	<LD	<LD
Control	(QNZ) CAY10470	1000 nM	0	<LD	<LD
Control	GB67A	1 nM	0	<LD	<LD
Control	GB67A	1 nM	0	<LD	<LD
Control	GB67A	1 nM	0	<LD	<LD
Control	GB67A	100 nM	0	<LD	<LD
Control	GB67A	100 nM	0	1	<LD
Control	GB67A	100 nM	0	<LD	<LD
Control	GB67A	1000 nM	0	0	<LD
Control	GB67A	1000 nM	0	<LD	<LD
Control	GB67A	1000 nM	0	<LD	<LD

Table 5. Cytokine production raw data.

Plate	Treatment	Concentration	anti-CD28	IL-2 (pg/ml)	TNF-α (pg/ml)
Control	GB67B	1 nM	0	<LD	<LD
Control	GB67B	1 nM	0	0	<LD
Control	GB67B	1 nM	0	0	<LD
Control	GB67B	100 nM	0	0	<LD
Control	GB67B	100 nM	0	<LD	<LD
Control	GB67B	100 nM	0	<LD	<LD
Control	GB67B	1000 nM	0	<LD	<LD
Control	GB67B	1000 nM	0	<LD	<LD
Control	GB67B	1000 nM	0	<LD	<LD
Control	GB615	1 nM	0	<LD	<LD
Control	GB615	1 nM	0	0	<LD
Control	GB615	1 nM	0	0	<LD
Control	GB615	100 nM	0	<LD	<LD
Control	GB615	100 nM	0	<LD	<LD
Control	GB615	100 nM	0	<LD	<LD
Control	GB615	1000 nM	0	<LD	<LD
Control	GB615	1000 nM	0	0	<LD
Control	GB615	1000 nM	0	<LD	<LD
Control	GB616	1 nM	0	0	<LD
Control	GB616	1 nM	0	0	<LD
Control	GB616	1 nM	0	<LD	<LD
Control	GB616	100 nM	0	0	<LD
Control	GB616	100 nM	0	0	<LD
Control	GB616	100 nM	0	0	<LD
Control	GB616	1000 nM	0	<LD	<LD
Control	GB616	1000 nM	0	0	<LD
Control	GB616	1000 nM	0	0	<LD
Control	GB594	1 nM	0	<LD	<LD
Control	GB594	1 nM	0	<LD	<LD
Control	GB594	1 nM	0	<LD	9
Control	GB594	100 nM	0	<LD	<LD
Control	GB594	100 nM	0	<LD	<LD
Control	GB594	100 nM	0	<LD	<LD
Control	GB594	1000 nM	0	<LD	<LD
Control	GB594	1000 nM	0	<LD	<LD
Control	GB594	1000 nM	0	<LD	<LD
Control	GB595	1 nM	0	<LD	<LD
Control	GB595	1 nM	0	0	<LD
Control	GB595	1 nM	0	<LD	<LD
Control	GB595	100 nM	0	<LD	<LD
Control	GB595	100 nM	0	<LD	<LD
Control	GB595	100 nM	0	<LD	<LD
Control	GB595	1000 nM	0	<LD	<LD
Control	GB595	1000 nM	0	<LD	<LD
Control	GB595	1000 nM	0	<LD	<LD
Control	GB65B	1 nM	0	<LD	<LD
Control	GB65B	1 nM	0	<LD	<LD
Control	GB65B	1 nM	0	<LD	<LD
Control	GB65B	100 nM	0	<LD	<LD
Control	GB65B	100 nM	0	<LD	<LD
Control	GB65B	100 nM	0	<LD	<LD
Control	GB65B	1000 nM	0	<LD	<LD
Control	GB65B	1000 nM	0	<LD	<LD
Control	GB65B	1000 nM	0	<LD	<LD
Control	GB117	1 nM	0	<LD	<LD
Control	GB117	1 nM	0	<LD	<LD
Control	GB117	1 nM	0	<LD	<LD
Control	GB117	100 nM	0	<LD	<LD
Control	GB117	100 nM	0	<LD	<LD
Control	GB117	100 nM	0	<LD	<LD
Control	GB117	1000 nM	0	<LD	<LD
Control	GB117	1000 nM	0	<LD	<LD
Control	GB117	1000 nM	0	<LD	<LD
anti-CD3	Vehicle		0	1071	3029

Table 5. Cytokine production raw data.

Plate	Treatment	Concentration	anti-CD28	IL-2 (pg/ml)	TNF-α (pg/ml)
anti-CD3	Vehicle		0	1143	3603
anti-CD3	Vehicle		0	1380	3509
anti-CD3	Cyclosporin A	100 ng/ml	0	11	70
anti-CD3	Cyclosporin A	100 ng/ml	0	8	652
anti-CD3	Cyclosporin A	100 ng/ml	0	17	195
anti-CD3	Triptolide	1 nM	0	508	2737
anti-CD3	Triptolide	1 nM	0	519	2870
anti-CD3	Triptolide	1 nM	0	687	2308
anti-CD3	Triptolide	10 nM	0	16	176
anti-CD3	Triptolide	10 nM	0	13	176
anti-CD3	Triptolide	10 nM	0	11	179
anti-CD3	Triptolide	50 nM	0	<LD	<LD
anti-CD3	Triptolide	50 nM	0	<LD	<LD
anti-CD3	Triptolide	50 nM	0	<LD	<LD
anti-CD3	Triptonide	1 nM	0	685	2691
anti-CD3	Triptonide	1 nM	0	551	1844
anti-CD3	Triptonide	1 nM	0	625	2559
anti-CD3	Triptonide	10 nM	0	11	91
anti-CD3	Triptonide	10 nM	0	17	159
anti-CD3	Triptonide	10 nM	0	13	87
anti-CD3	Triptonide	50 nM	0	<LD	<LD
anti-CD3	Triptonide	50 nM	0	<LD	1
anti-CD3	Triptonide	50 nM	0	<LD	<LD
anti-CD3	(QNZ) CAY10470	1 nM	0	826	2102
anti-CD3	(QNZ) CAY10470	1 nM	0	985	2894
anti-CD3	(QNZ) CAY10470	1 nM	0	853	2675
anti-CD3	(QNZ) CAY10470	100 nM	0	362	744
anti-CD3	(QNZ) CAY10470	100 nM	0	277	864
anti-CD3	(QNZ) CAY10470	100 nM	0	328	1119
anti-CD3	(QNZ) CAY10470	1000 nM	0	328	1014
anti-CD3	(QNZ) CAY10470	1000 nM	0	330	947
anti-CD3	(QNZ) CAY10470	1000 nM	0	248	998
anti-CD3	GB67A	1 nM	0	1304	5072
anti-CD3	GB67A	1 nM	0	671	2093
anti-CD3	GB67A	1 nM	0	1195	2782
anti-CD3	GB67A	100 nM	0	814	2139
anti-CD3	GB67A	100 nM	0	1229	3360
anti-CD3	GB67A	100 nM	0	1818	7350
anti-CD3	GB67A	1000 nM	0	584	1482
anti-CD3	GB67A	1000 nM	0	1097	3117
anti-CD3	GB67A	1000 nM	0	1112	3323
anti-CD3	GB67B	1 nM	0	1250	2455
anti-CD3	GB67B	1 nM	0	1158	3365
anti-CD3	GB67B	1 nM	0	1695	4182
anti-CD3	GB67B	100 nM	0	980	3974
anti-CD3	GB67B	100 nM	0	1051	5223
anti-CD3	GB67B	100 nM	0	607	1909
anti-CD3	GB67B	1000 nM	0	1138	3856
anti-CD3	GB67B	1000 nM	0	911	3079
anti-CD3	GB67B	1000 nM	0	1003	2756
anti-CD3	GB615	1 nM	0	983	2229
anti-CD3	GB615	1 nM	0	978	3090
anti-CD3	GB615	1 nM	0	1407	3420
anti-CD3	GB615	100 nM	0	1432	4413
anti-CD3	GB615	100 nM	0	994	3508
anti-CD3	GB615	100 nM	0	846	2343
anti-CD3	GB615	1000 nM	0	1082	2706
anti-CD3	GB615	1000 nM	0	987	3153
anti-CD3	GB615	1000 nM	0	1143	3127
anti-CD3	GB616	1 nM	0	1127	4627
anti-CD3	GB616	1 nM	0	1239	3638
anti-CD3	GB616	1 nM	0	1217	3671
anti-CD3	GB616	100 nM	0	1362	4117
anti-CD3	GB616	100 nM	0	1332	2293

Table 5. Cytokine production raw data.

Plate	Treatment	Concentration	anti-CD28	IL-2 (pg/ml)	TNF-α (pg/ml)
anti-CD3	GB616	100 nM	0	768	1787
anti-CD3	GB616	1000 nM	0	755	1806
anti-CD3	GB616	1000 nM	0	1117	3239
anti-CD3	GB616	1000 nM	0	1340	2570
anti-CD3	GB594	1 nM	0	1290	3699
anti-CD3	GB594	1 nM	0	1453	4410
anti-CD3	GB594	1 nM	0	1456	6171
anti-CD3	GB594	100 nM	0	1340	3685
anti-CD3	GB594	100 nM	0	1300	3406
anti-CD3	GB594	100 nM	0	1258	4475
anti-CD3	GB594	1000 nM	0	1564	4453
anti-CD3	GB594	1000 nM	0	956	3237
anti-CD3	GB594	1000 nM	0	1604	5138
anti-CD3	GB595	1 nM	0	1164	4034
anti-CD3	GB595	1 nM	0	1538	3732
anti-CD3	GB595	1 nM	0	1263	6391
anti-CD3	GB595	100 nM	0	1227	3712
anti-CD3	GB595	100 nM	0	1198	3565
anti-CD3	GB595	100 nM	0	958	3395
anti-CD3	GB595	1000 nM	0	1580	4108
anti-CD3	GB595	1000 nM	0	1415	3701
anti-CD3	GB595	1000 nM	0	1433	3914
anti-CD3	GB65B	1 nM	0	1073	2777
anti-CD3	GB65B	1 nM	0	1015	4076
anti-CD3	GB65B	1 nM	0	1193	3329
anti-CD3	GB65B	100 nM	0	837	1551
anti-CD3	GB65B	100 nM	0	1164	4051
anti-CD3	GB65B	100 nM	0	917	2719
anti-CD3	GB65B	1000 nM	0	1217	3243
anti-CD3	GB65B	1000 nM	0	1029	3642
anti-CD3	GB65B	1000 nM	0	947	2949
anti-CD3	GB117	1 nM	0	434	2823
anti-CD3	GB117	1 nM	0	875	2350
anti-CD3	GB117	1 nM	0	1181	2939
anti-CD3	GB117	100 nM	0	1327	3693
anti-CD3	GB117	100 nM	0	1217	2694
anti-CD3	GB117	100 nM	0	1186	4136
anti-CD3	GB117	1000 nM	0	1115	3895
anti-CD3	GB117	1000 nM	0	1066	2543
anti-CD3	GB117	1000 nM	0	846	1666
anti-CD3	Vehicle		1 ug/ml	1155	2758
anti-CD3	Vehicle		1 ug/ml	2117	3510
anti-CD3	Vehicle		1 ug/ml	2536	4986
anti-CD3	Cyclosporin A	100 ng/ml	1 ug/ml	54	1278
anti-CD3	Cyclosporin A	100 ng/ml	1 ug/ml	52	1851
anti-CD3	Cyclosporin A	100 ng/ml	1 ug/ml	45	680
anti-CD3	Triptolide	1 nM	1 ug/ml	1596	4562
anti-CD3	Triptolide	1 nM	1 ug/ml	1612	3728
anti-CD3	Triptolide	1 nM	1 ug/ml	2019	4994
anti-CD3	Triptolide	10 nM	1 ug/ml	86	391
anti-CD3	Triptolide	10 nM	1 ug/ml	86	551
anti-CD3	Triptolide	10 nM	1 ug/ml	88	558
anti-CD3	Triptolide	50 nM	1 ug/ml	<LD	3
anti-CD3	Triptolide	50 nM	1 ug/ml	<LD	4
anti-CD3	Triptolide	50 nM	1 ug/ml	<LD	3
anti-CD3	Triptonide	1 nM	1 ug/ml	1499	6081
anti-CD3	Triptonide	1 nM	1 ug/ml	1763	5775
anti-CD3	Triptonide	1 nM	1 ug/ml	1706	2332
anti-CD3	Triptonide	10 nM	1 ug/ml	58	225
anti-CD3	Triptonide	10 nM	1 ug/ml	60	357
anti-CD3	Triptonide	10 nM	1 ug/ml	69	223
anti-CD3	Triptonide	50 nM	1 ug/ml	<LD	3
anti-CD3	Triptonide	50 nM	1 ug/ml	<LD	7
anti-CD3	Triptonide	50 nM	1 ug/ml	<LD	7

Table 5. Cytokine production raw data.

Plate	Treatment	Concentration	anti-CD28	IL-2 (pg/ml)	TNF-α (pg/ml)
anti-CD3	(QNZ) CAY10470	1 nM	1 ug/ml	3084	4972
anti-CD3	(QNZ) CAY10470	1 nM	1 ug/ml	3701	7630
anti-CD3	(QNZ) CAY10470	1 nM	1 ug/ml	3099	4338
anti-CD3	(QNZ) CAY10470	100 nM	1 ug/ml	1278	2078
anti-CD3	(QNZ) CAY10470	100 nM	1 ug/ml	1019	1651
anti-CD3	(QNZ) CAY10470	100 nM	1 ug/ml	1054	2823
anti-CD3	(QNZ) CAY10470	1000 nM	1 ug/ml	1095	1634
anti-CD3	(QNZ) CAY10470	1000 nM	1 ug/ml	1033	1274
anti-CD3	(QNZ) CAY10470	1000 nM	1 ug/ml	1223	1118
anti-CD3	GB67A	1 nM	1 ug/ml	2860	4337
anti-CD3	GB67A	1 nM	1 ug/ml	2807	4131
anti-CD3	GB67A	1 nM	1 ug/ml	2355	3633
anti-CD3	GB67A	100 nM	1 ug/ml	2157	4307
anti-CD3	GB67A	100 nM	1 ug/ml	2856	4846
anti-CD3	GB67A	100 nM	1 ug/ml	2722	3602
anti-CD3	GB67A	1000 nM	1 ug/ml	2328	2834
anti-CD3	GB67A	1000 nM	1 ug/ml	2458	5356
anti-CD3	GB67A	1000 nM	1 ug/ml	1967	2638
anti-CD3	GB67B	1 nM	1 ug/ml	2660	5463
anti-CD3	GB67B	1 nM	1 ug/ml	3360	5243
anti-CD3	GB67B	1 nM	1 ug/ml	3169	5853
anti-CD3	GB67B	100 nM	1 ug/ml	2458	5217
anti-CD3	GB67B	100 nM	1 ug/ml	2856	4416
anti-CD3	GB67B	100 nM	1 ug/ml	2967	5253
anti-CD3	GB67B	1000 nM	1 ug/ml	2064	5027
anti-CD3	GB67B	1000 nM	1 ug/ml	2118	3016
anti-CD3	GB67B	1000 nM	1 ug/ml	2489	4383
anti-CD3	GB615	1 nM	1 ug/ml	2318	4358
anti-CD3	GB615	1 nM	1 ug/ml	2774	5491
anti-CD3	GB615	1 nM	1 ug/ml	3081	5178
anti-CD3	GB615	100 nM	1 ug/ml	1189	1784
anti-CD3	GB615	100 nM	1 ug/ml	4077	6969
anti-CD3	GB615	100 nM	1 ug/ml	2121	3502
anti-CD3	GB615	1000 nM	1 ug/ml	3227	5304
anti-CD3	GB615	1000 nM	1 ug/ml	3049	5053
anti-CD3	GB615	1000 nM	1 ug/ml	3769	8298
anti-CD3	GB616	1 nM	1 ug/ml	3161	5624
anti-CD3	GB616	1 nM	1 ug/ml	3141	5400
anti-CD3	GB616	1 nM	1 ug/ml	2218	3173
anti-CD3	GB616	100 nM	1 ug/ml	2588	4362
anti-CD3	GB616	100 nM	1 ug/ml	2983	5899
anti-CD3	GB616	100 nM	1 ug/ml	2627	4342
anti-CD3	GB616	1000 nM	1 ug/ml	2875	3812
anti-CD3	GB616	1000 nM	1 ug/ml	3545	5334
anti-CD3	GB616	1000 nM	1 ug/ml	2635	3644
anti-CD3	GB594	1 nM	1 ug/ml	2570	5765
anti-CD3	GB594	1 nM	1 ug/ml	2944	8558
anti-CD3	GB594	1 nM	1 ug/ml	3402	7695
anti-CD3	GB594	100 nM	1 ug/ml	2308	3570
anti-CD3	GB594	100 nM	1 ug/ml	2372	4007
anti-CD3	GB594	100 nM	1 ug/ml	2811	4354
anti-CD3	GB594	1000 nM	1 ug/ml	2288	4735
anti-CD3	GB594	1000 nM	1 ug/ml	2291	3498
anti-CD3	GB594	1000 nM	1 ug/ml	2020	4368
anti-CD3	GB595	1 nM	1 ug/ml	2707	4632
anti-CD3	GB595	1 nM	1 ug/ml	2124	3871
anti-CD3	GB595	1 nM	1 ug/ml	2255	4790
anti-CD3	GB595	100 nM	1 ug/ml	2682	4364
anti-CD3	GB595	100 nM	1 ug/ml	2510	5511
anti-CD3	GB595	100 nM	1 ug/ml	1958	3078
anti-CD3	GB595	1000 nM	1 ug/ml	2785	5807
anti-CD3	GB595	1000 nM	1 ug/ml	2285	5329
anti-CD3	GB595	1000 nM	1 ug/ml	2807	6109
anti-CD3	GB65B	1 nM	1 ug/ml	3714	6120

Table 5. Cytokine production raw data.

Plate	Treatment	Concentration	anti-CD28	IL-2 (pg/ml)	TNF-α (pg/ml)
anti-CD3	GB65B	1 nM	1 ug/ml	2792	4027
anti-CD3	GB65B	1 nM	1 ug/ml	2733	7108
anti-CD3	GB65B	100 nM	1 ug/ml	2311	3213
anti-CD3	GB65B	100 nM	1 ug/ml	2077	4724
anti-CD3	GB65B	100 nM	1 ug/ml	1688	3028
anti-CD3	GB65B	1000 nM	1 ug/ml	1785	5186
anti-CD3	GB65B	1000 nM	1 ug/ml	1588	2880
anti-CD3	GB65B	1000 nM	1 ug/ml	1951	4195
anti-CD3	GB117	1 nM	1 ug/ml	2179	3281
anti-CD3	GB117	1 nM	1 ug/ml	2635	4323
anti-CD3	GB117	1 nM	1 ug/ml	3030	4251
anti-CD3	GB117	100 nM	1 ug/ml	2552	3168
anti-CD3	GB117	100 nM	1 ug/ml	2372	2118
anti-CD3	GB117	100 nM	1 ug/ml	3284	2652
anti-CD3	GB117	1000 nM	1 ug/ml	2413	4404
anti-CD3	GB117	1000 nM	1 ug/ml	2427	1985
anti-CD3	GB117	1000 nM	1 ug/ml	3419	2820

<LD: Below the level of the detection.
Values in grey were extrapolated below the lowest standard.