

SUPPLEMENTARY DATA

Supplementary Figure 1. Multiple doses of Bortezomib regulates CTLA-4.

Transcript levels of *CTLA-4* are increased with bortezomib, from 0.05 to 10 μ M. Normal primary CD4⁺ T cells were purified as described in Materials and Methods followed by treatment with bortezomib (0.05 μ M, dotted line with diamond; 0.1 μ M, dash line with square, and 10 μ M, dash line with triangle) or untreated (solid line with circle) and concomitant stimulation with PMA/A23187 over a 12 h time course. Total RNA was isolated for qRT-PCR analysis as previously described. Results are the averages of 4 individual normal donors analyzed by qPCR normalized to *B2M* and presented as the fold increase over unstimulated normal cells.

Supplementary Figure 2. *NFAT1* and *FOXP3* expression levels are not augmented

by bortezomib. Normal primary CD4⁺ T cells were purified as described in Materials Methods followed by treatment with 0 μ M (solid line with circle), 0.1 μ M (dashed line with square) or 10 μ M (dotted line with triangle) bortezomib and stimulation with PMA/A23187 over a 12 h time course with 3 h intervals. Total RNA was isolated for qPCR analysis for **(A)** *NFAT1* and **(B)** *FOXP3* expression levels as previously described. Results are the averages of 4 individual normal donors analyzed by qPCR normalized to *B2M* and presented as the fold increase over unstimulated normal cells \pm SEM * p <0.05, ** p <0.005.

Supplementary Figure 3. *GATA3* knockdown by siRNA leads to decreased *IL-4* transcription but not *GAPDH* with bortezomib. Samples were prepared as in Figure 6. Transcript levels of **(A) *IL-4*** and **(B) *GAPDH*** were measured by qPCR for samples treated with siCTRL (black bars) or si*GATA3* (white bars) as previously described. Results are presented as averages of three independent experiments \pm SEM (* $p < 0.05$).

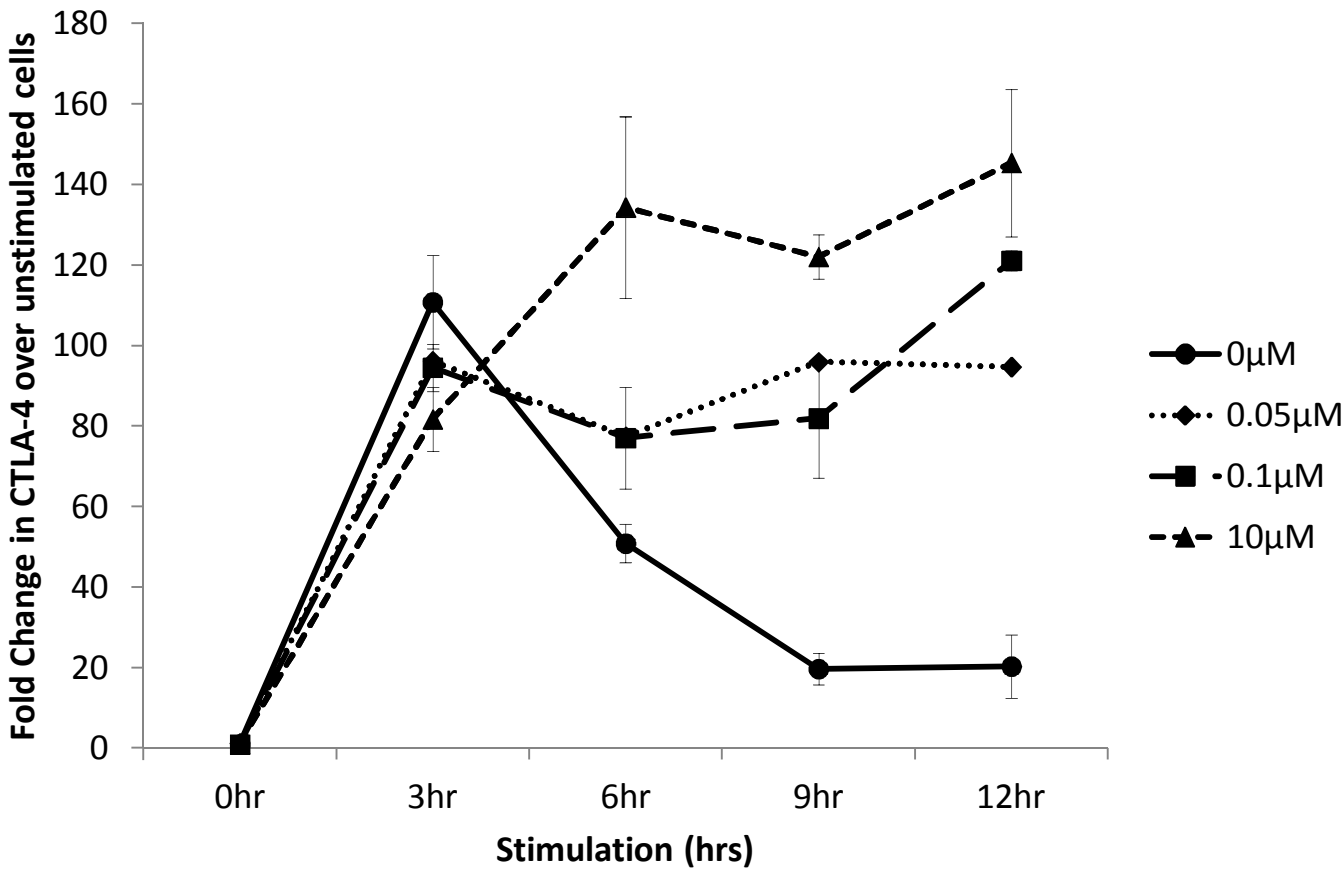
Supplementary Figure 4. Bortezomib treatment and apoptosis in primary CD4+ T cells. Cells were treated with the bortezomib concentration as indicated (green equals 0.1 μ M, blue equals 10 μ M) and stimulated with PMA/A23187 as described in the Materials and Methods. The cells were stained with Annexin V and analyzed by flow cytometry. Results are representative of three experiments.

Supplementary Table I. PCR Primer Sets

Gene	Forward	Reverse
B2M	TCTACTTTGAGTGCTGTCTCCATGT	AAGTTGCCAGCCCTCCTAGAG
CTLA-4	CTACCTGGGCATAGGCAACG	CCCCGAACTAACTGCTGCAA
FoxP3	ATCCGCCACAACCTGAGTCT	GTCCACACAGCCCCCTTCT
GAPDH	CCCACTCCTCCACCTTTGAC	CATACCAGGAAATGAGCTTGACAA
GATA3	TCTGGAGGAGGAATGCCAAT	CCGGGTAAACGAGCTGTTC

IL-4	CACAGGCACAAGCAGCTGAT	CTCTGGTTGGCTTCCTTCACA
NFAT1	TCCTGGAGATACCCTTGGAGC	AGTCGATGGTTGCCCTCATG
ChIP CTLA-4	GAGGACCCTTGTA CTCCAGGAA	CGAAAAGACAACCTCAAGCACTC

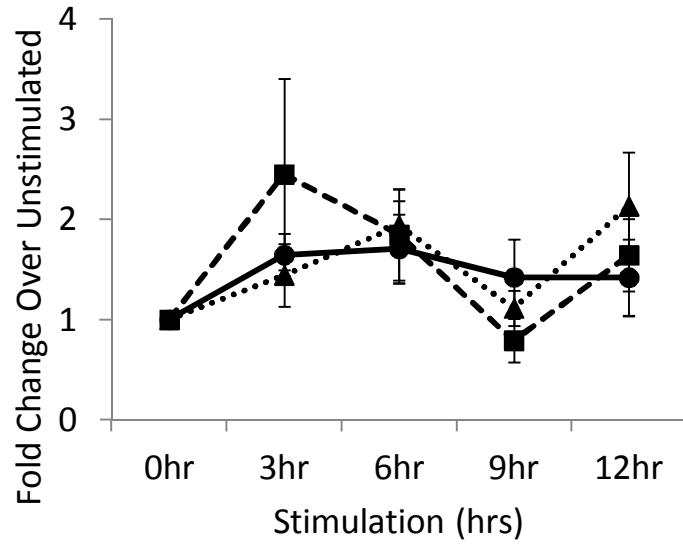
Supplementary Figure 1



Supplementary Figure 2

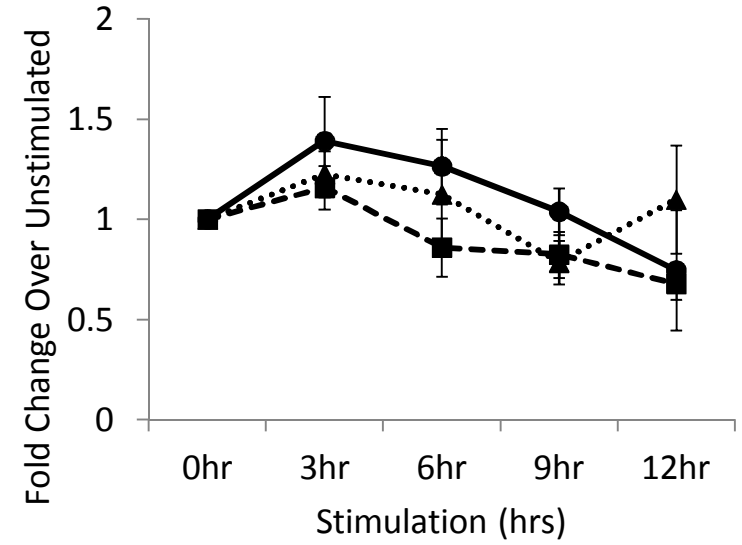
(A)

NFAT1



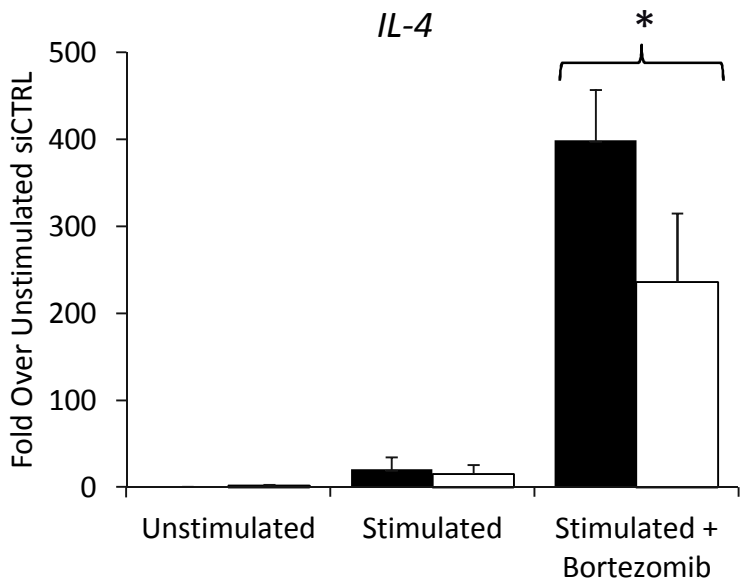
(B)

FOXP3

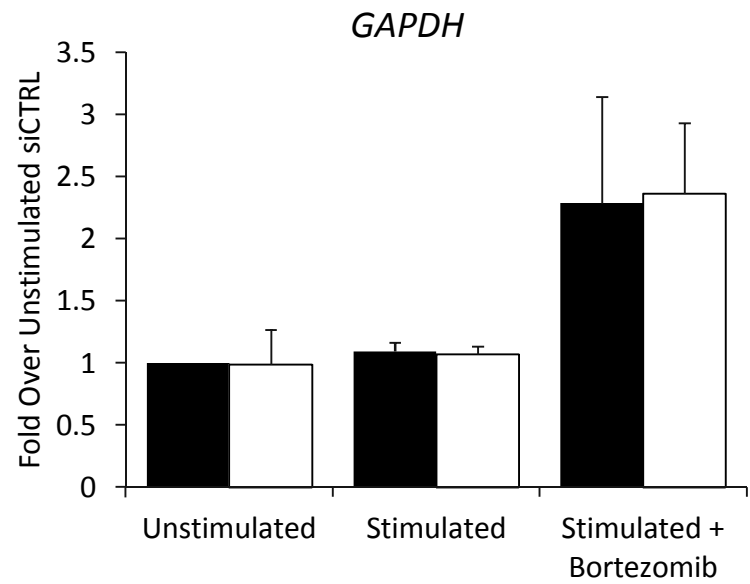


Supplementary Figure 3

(A)



(B)

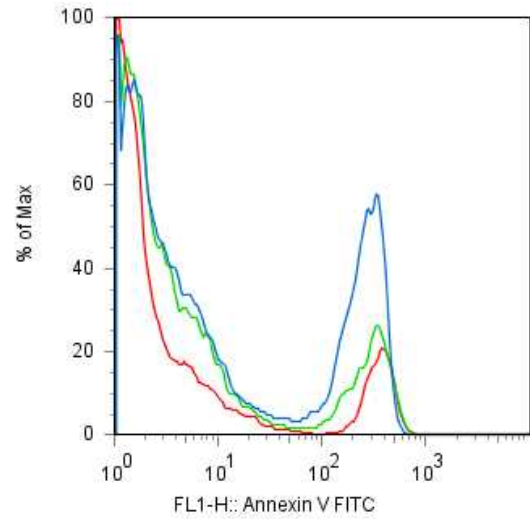
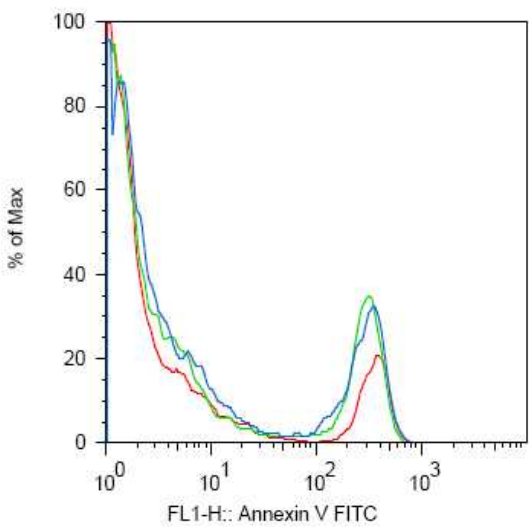
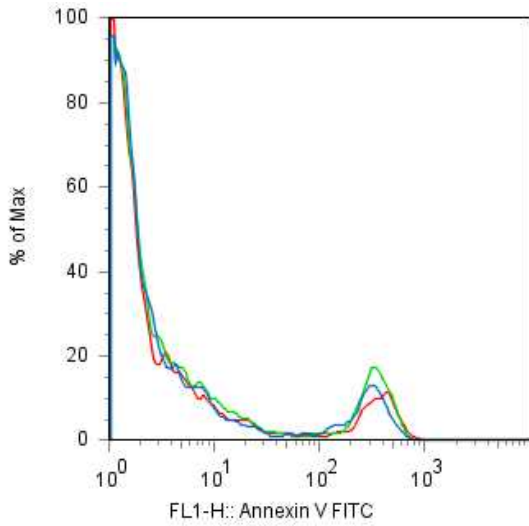


Supplementary Figure 4

6hr

9hr

12hr



- 0 μM
- 0.1 μM
- 10 μM