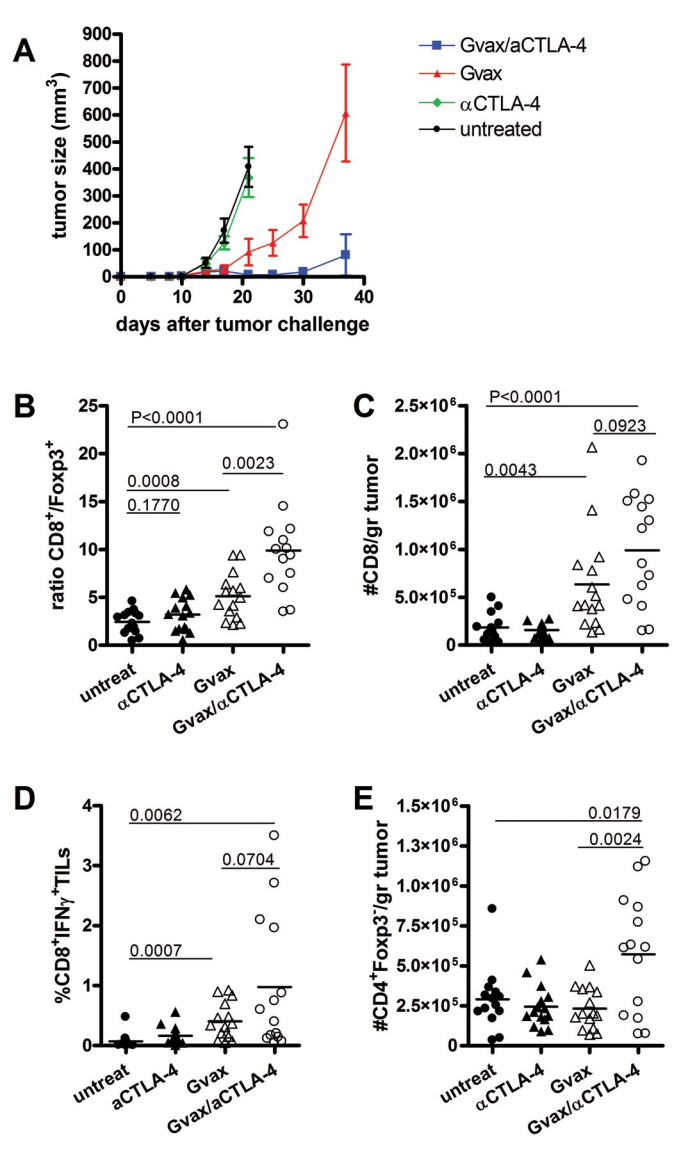
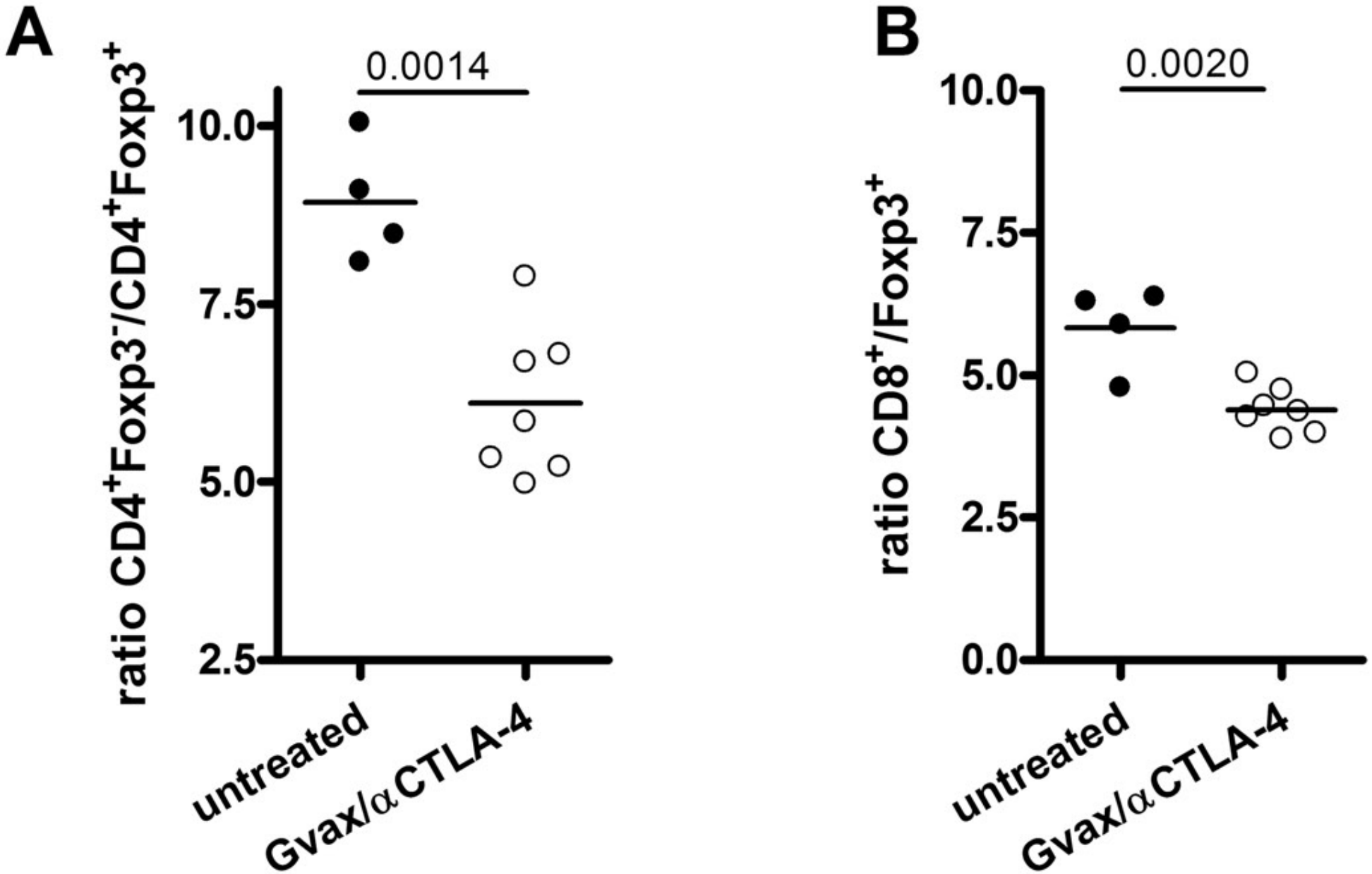
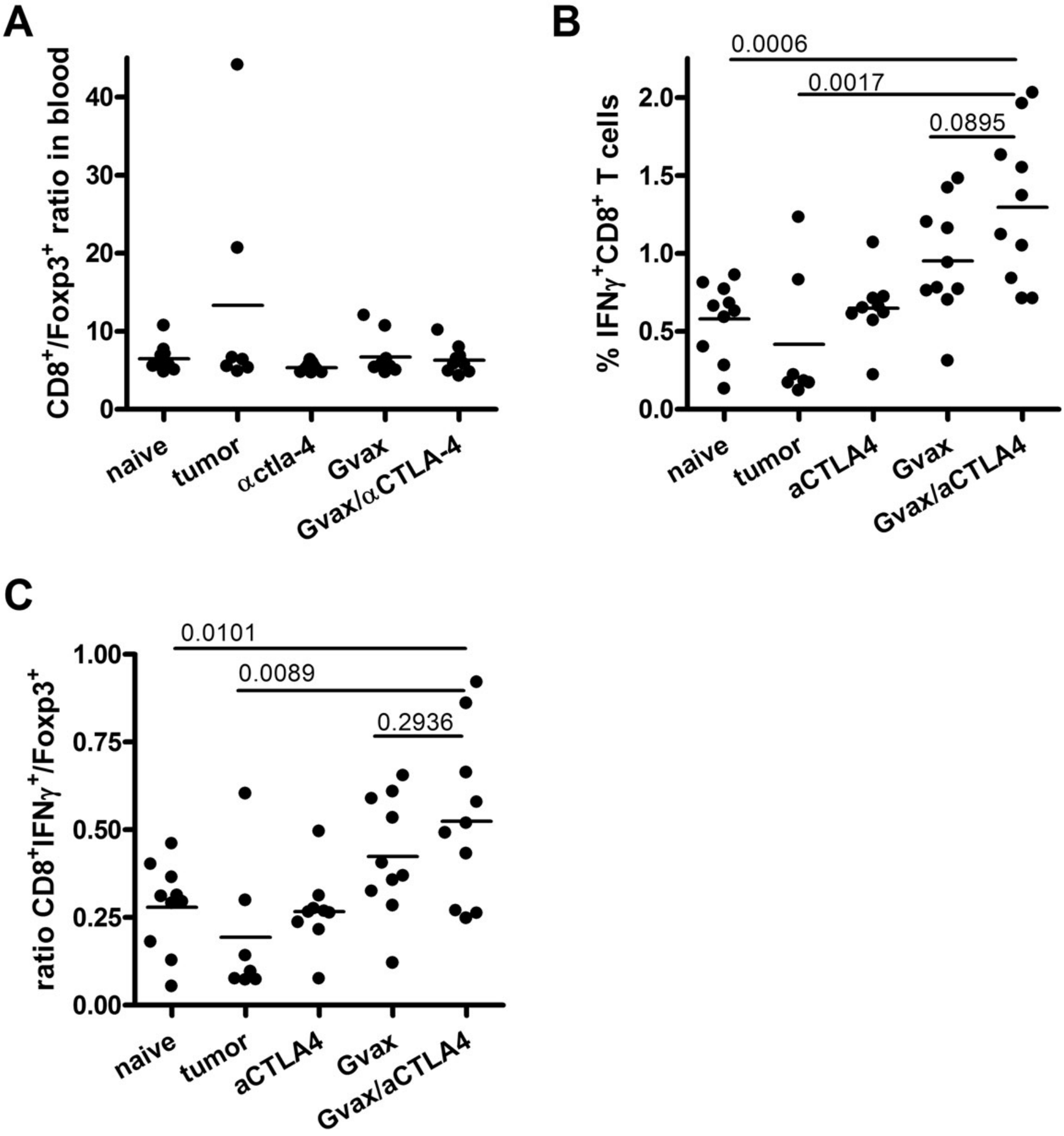

A
tumor size (mm3)
900
800
700
600
500
400
300
200
100
0
0
10
20
30
40
days after tumor challenge
Gvax/aCTLA-4
Gvax
αCTLA-4
untreated
B
ratio CD8+/Foxp3+
25
20
15
10
5
0
P<0.0001
0.0008
0.1770
0.0023
untreat
αCTLA-4
Gvax
Gvax/αCTLA-4
C
#CD8/gr tumor
2.5×10^6
2.0×10^6
1.5×10^6
1.0×10^6
5.0×10^5
0
P<0.0001
0.0923
0.0043
untreat
αCTLA-4
Gvax
Gvax/αCTLA-4
D
%CD8+IFNγ+TILs
4
3
2
1
0
0.0062
0.0704
0.0007
untreat
aCTLA-4
Gvax
Gvax/aCTLA-4
E
#CD4+Foxp3-/gr tumor
1.5×10^6
1.3×10^6
1.0×10^6
7.5×10^5
5.0×10^5
2.5×10^5
0
0.0179
0.0024
untreat
αCTLA-4
Gvax
Gvax/αCTLA-4


A
0.0014
ratio CD4+Foxp3-/CD4+Foxp3+
10.0
7.5
5.0
2.5
untreated
Gvax/αCTLA-4
B
0.0020
ratio CD8+/Foxp3+
10.0
7.5
5.0
2.5
0.0
untreated
Gvax/αCTLA-4


A
CD8+/Foxp3+ ratio in blood
0
10
20
30
40
naive
tumor
αctla-4
Gvax
Gvax/αCTLA-4
B
% IFNγ+CD8+ T cells
0.0
0.5
1.0
1.5
2.0
0.0006
0.0017
0.0895
naive
tumor
aCTLA4
Gvax
Gvax/aCTLA4
C
ratio CD8+IFNγ+/Foxp3+
0.00
0.25
0.50
0.75
1.00
0.0101
0.0089
0.2936
naive
tumor
aCTLA4
Gvax
Gvax/aCTLA4

Supplemental Figure 1: Independent contributions of Gvax and CTLA-4 blockade to intra-tumor CD8$^+$ T cell infiltration and differentiation. C57BL/6 mice were challenged with B16/BL6 tumor at day 0 and treated/not treated with Gvax or anti-CTLA-4 either alone or in combination at days 3, 6 and 9. A) Tumor size was monitored over time. Number of mice rejecting tumor is shown and is representative of 3 independent experiments. Untreated 0/10; anti-CTLA-4 0/10; Gvax 2/10 (p=0.4737); Gvax/anti-CTLA-4 8/10 (p=0.0007). In parallel experiments, 15 days after tumor challenge tumors were removed, weighed and analyzed by flow cytometry for the percentage of CD8$^+$, CD4$^+$ and Foxp3$^+$ T cells. b) ratio of CD8$^+$/Foxp3$^+$ T cells in tumor infiltrates; c) number of CD8$^+$ T cells per gram of tumor; d) percentage of intra-tumor CD8$^+$ T cells that are IFNγ^+ upon re-stimulation with trp2 peptide; e) number of intra-tumor CD4$^+$Foxp3$^-$ T cells per gram of tumor. All graphs show cumulative data from 3 independent experiments with 4-5 mice/group.

Supplemental Figure 2: Gvax/αCTLA-4 treatment decreases the ratio of effector T cells to regulatory T cells in tumor draining lymph nodes. C57BL/6 mice were challenged with B16/BL6 tumor cells. Fifteen days after tumor challenge, tumor draining lymph nodes from untreated or Gvax/anti-CTLA-4-treated mice where analyzed by flow cytometry for their content of CD4$^+$, CD8$^+$ and Foxp3$^+$ positive T cells. A) Ratio of CD4$^+$Foxp3$^-$/CD4$^+$Foxp3$^+$ T cells. B) Ratio of CD8$^+$/Foxp3$^+$ T cells. Graphs show cumulative data from 2 independent experiments.

Supplemental Figure 3: Frequency of $CD8^+$ T cells producing IFN-γ is increased in peripheral blood upon Gvax/αCTLA-4 therapy. C57BL/6 mice were challenged with B16/BL6 tumor cells at day 0 and treated/not treated with Gvax or anti-CTLA-4 either alone or in combination at days 3, 6 and 9. Fifteen days after tumor challenge, peripheral blood was collected from all groups in heparinized tubes and was analyzed by flow cytometry for the expression of $CD8^+$ and $Foxp3^+$ positive T cells. A) Ratio of $CD8^+/Foxp3^+$ T cells. Additional blood samples were depleted of red blood cells and incubated overnight with 10μg/ml anti-CD3 mAb and monensin for the last four hours of culture. Samples were stained for CD8 and IFN-γ expression and data was plotted as b) percentage of $CD8^+IFN\text{-}\gamma^+$ T cells in blood, and c) ratio of $CD8^+IFN\text{-}\gamma^+/Foxp3^+$ T cells in peripheral blood. Graphs show cumulative data from 2 independent experiments.