



Supplementary Information for

Differential control of human Treg and effector T cells in tumor immunity by Fc-engineered anti-CTLA-4 antibody

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Figs. S1 to S6

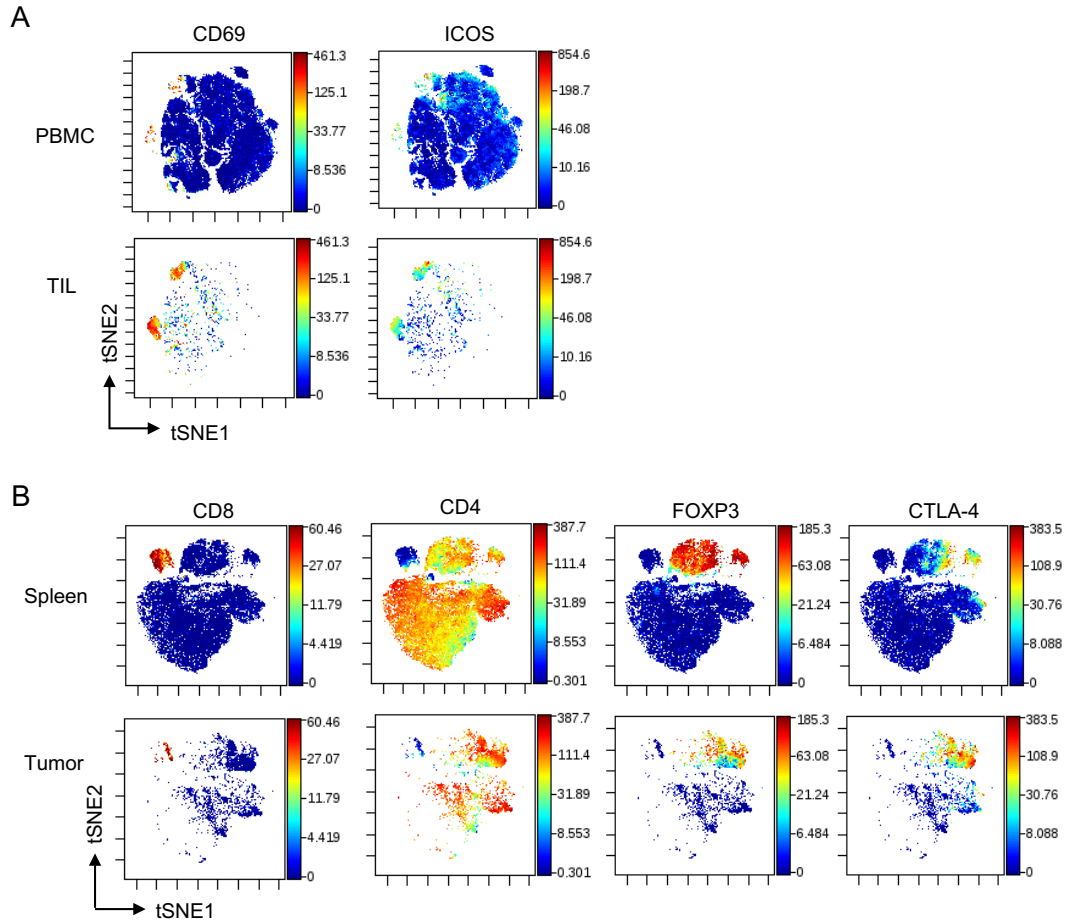


Fig. S1. CTLA-4 expression by Treg and CD8⁺ T cells.

(A) PBMCs and TILs from human melanoma patients by CyTOF analysis as in Fig. 1E. viSNE plots of CD4⁺ and CD8⁺ T cells with heat maps for indicated marker expression levels are shown (n=2). (B) CyTOF analysis of CTLA-4 expressing clusters in spleen and tumor from CMS5a fibrosarcoma-bearing BALB/c mice (n=2). viSNE plots of selected markers on CD4⁺ and CD8⁺ T cells are shown.

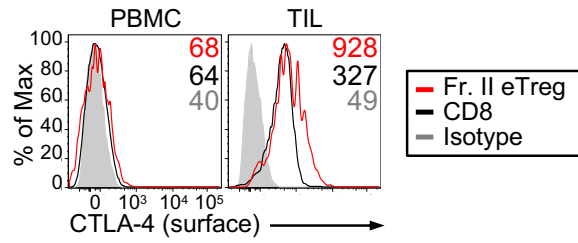


Fig. S2. CTLA-4 expression by Treg and CD8⁺ T cells in humans. Representative histogram for the surface CTLA-4 staining of tumor-infiltrating (n=11) and circulating (n=14) human Fr. II eTreg and CD8⁺ T cells from melanoma patients.

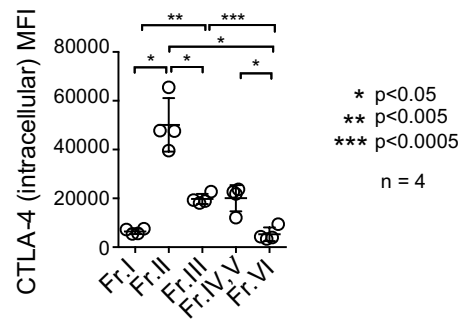


Fig. S3. CTLA-4 expression by Treg and CD4⁺ T cell fractions upon Melan-A stimulation. CTLA-4 expression by CD4⁺ fractions of PBMCs after 9 days of Melan-A stimulation alone, as in Fig. 2D (n=4).

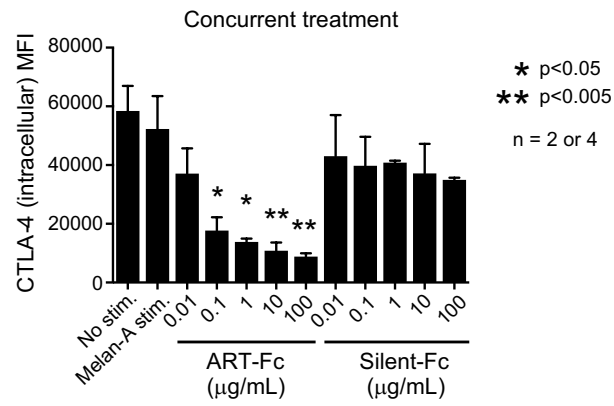


Fig. S4. CTLA-4 expression by eTreg cells treated with cell-depleting or non-depleting anti-CTLA-4 mAb in vitro. CTLA-4 expression levels of Fr. II eTreg cells treated with ART-Fc or silent-Fc anti-CTLA-4 mAb at indicated concentrations from day 0 concurrently with Melan-A stimulation in vitro (n=2 or 4). * $P \leq 0.05$, ** $P \leq 0.005$ by unpaired *t* tests against Melan-A stimulation alone.

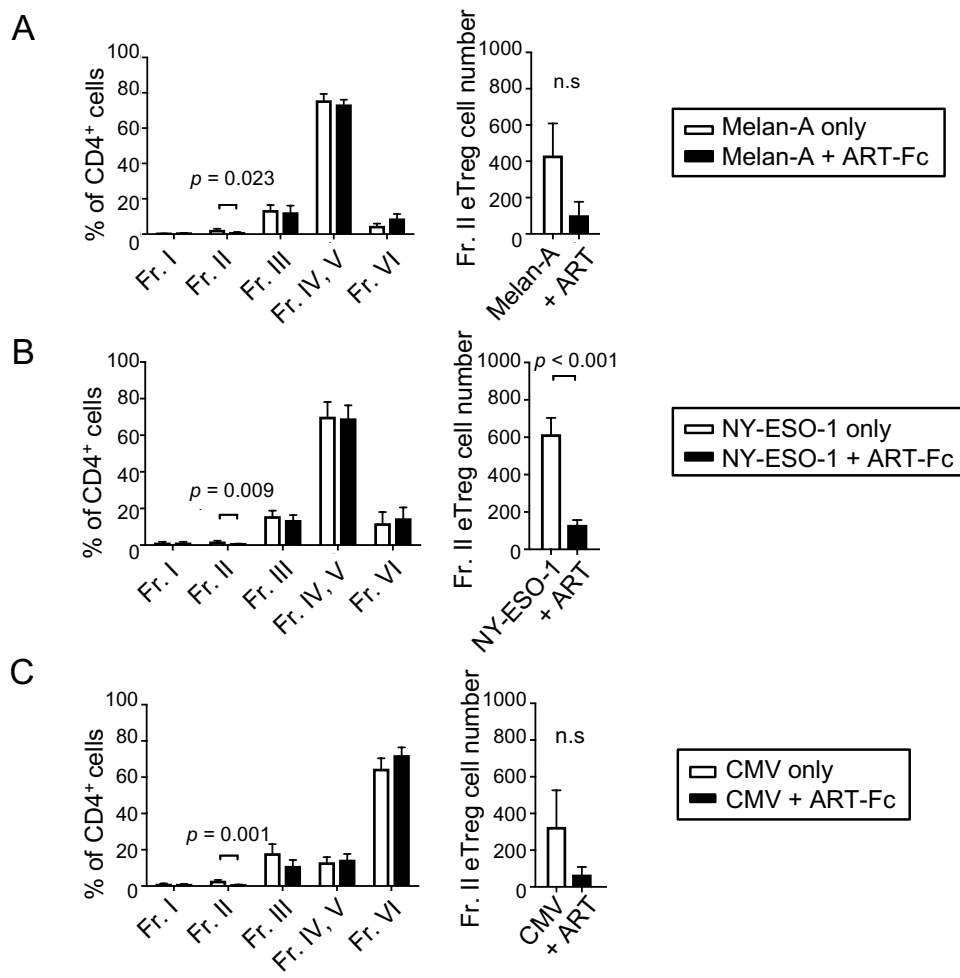


Fig. S5. Changes in other CD4⁺ T cell fractions with concurrent ART-Fc anti-CTLA-4 mAb treatment. (A to C) The percentage of CD4⁺ fractions (left) and the cell number of Fr. II eTreg cells (right) of PBMCs after 9 days of various antigen stimulation in the absence or the presence of ART-Fc, related to Figure 2G and H. Means ± SEM.

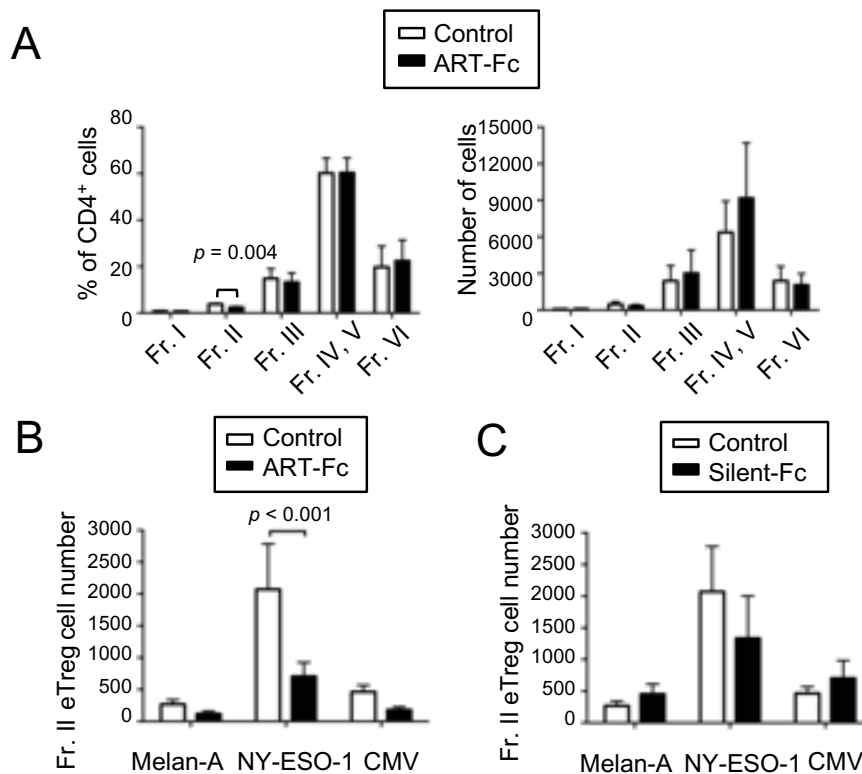


Fig. S6. Changes in other CD4⁺ T cell fractions and cell number of Fr. II eTreg cells.
(A) The percentage and absolute number of each CD4⁺ fraction after 5 days of ART-Fc treatment without antigen stimulation as in Figure 3A. **(B and C)** Changes in cell number of Fr. II eTreg cells after 8 days of various antigen stimulation, as shown in Figure 3B and D. PBMCs were pre-treated for 5 days with ART-Fc **(B)** or Silent-Fc **(C)**. Means \pm SEM. Depicted significances between two groups were calculated using t test for **(A)**, and Two-way ANOVA with Sidak's multiple comparison test for **(B)** and **(C)**.