

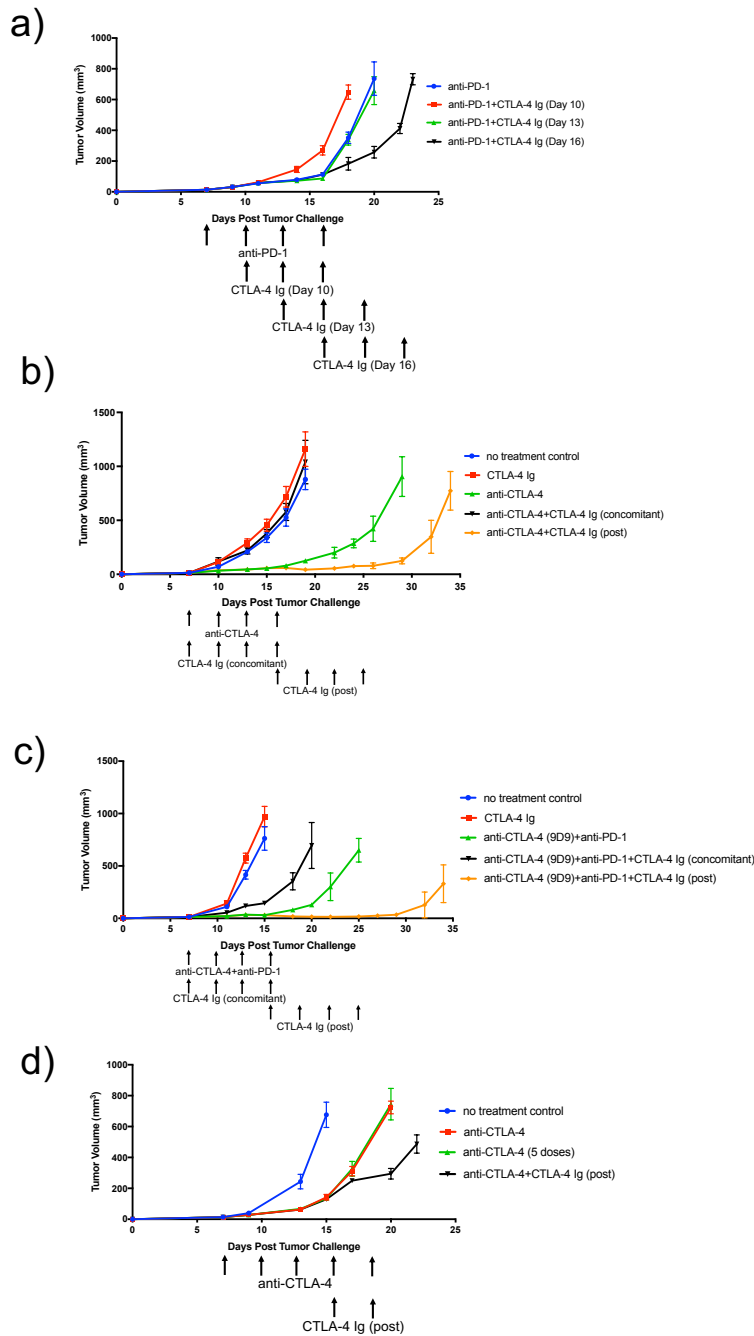
## **Supporting Information for Post-immunotherapy CTLA-4 Ig treatment improves antitumor efficacy.**

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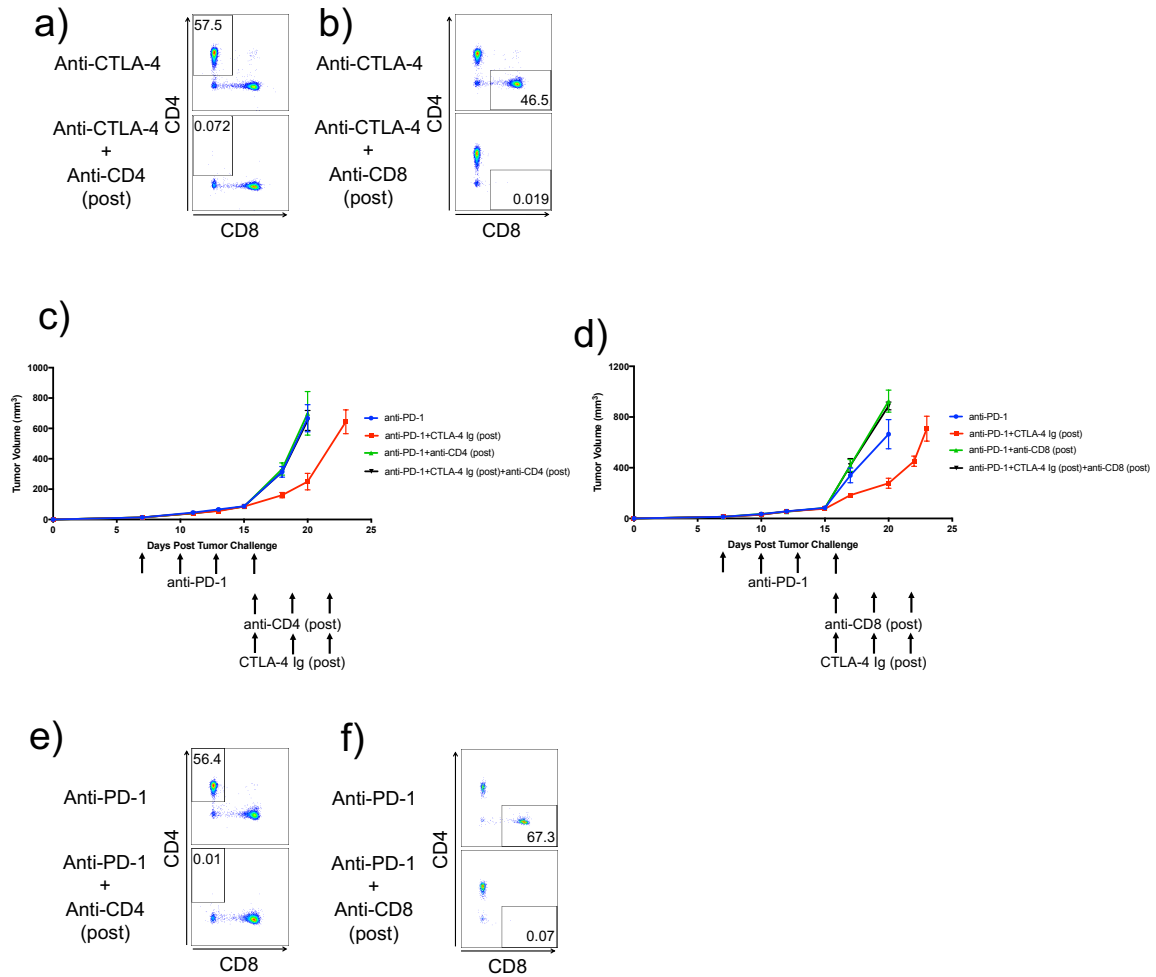
### **This PDF file includes:**

Figures S1 to S4



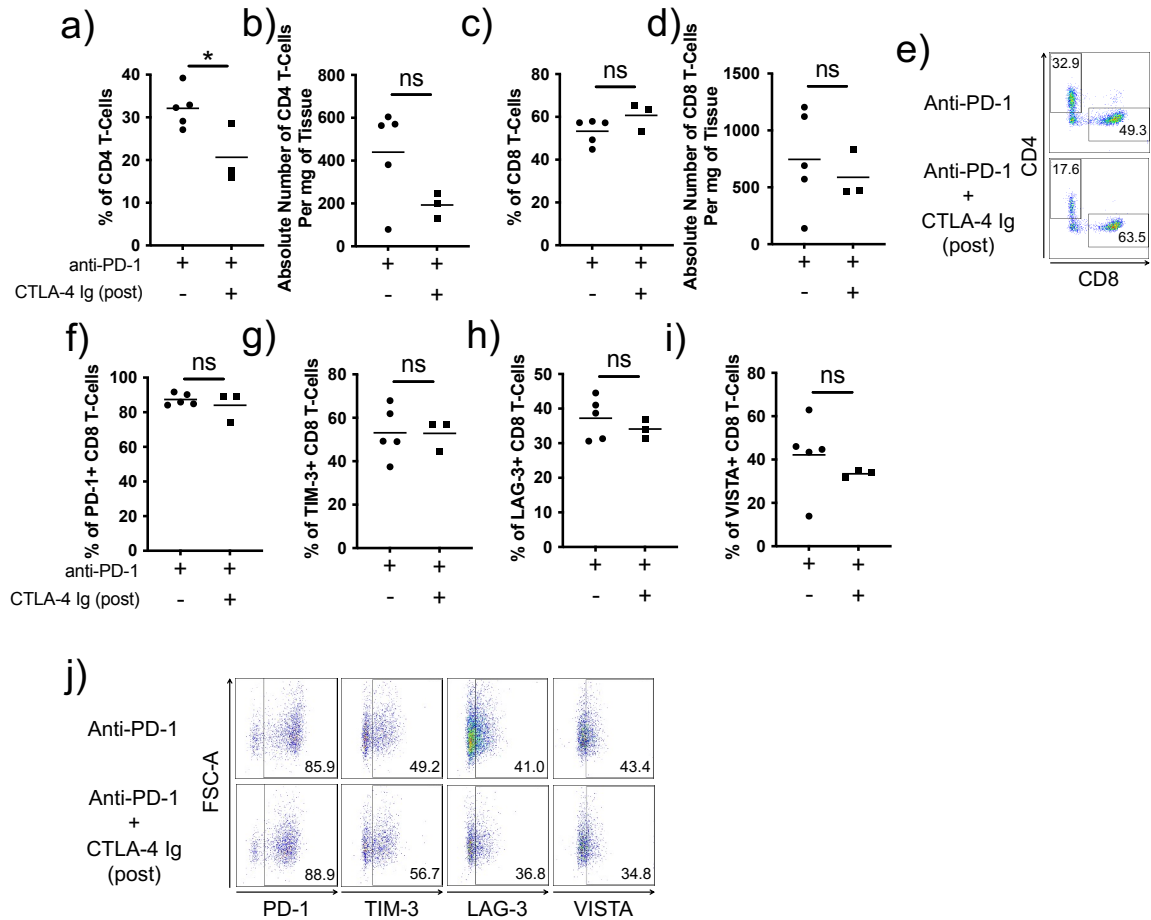
**Fig. S1. CTLA-4 Ig (post) improves antitumor efficacy of ICT. A)** Tumor growth curves of C57BL/6J mice harboring B16F10 tumors were treated with anti-PD-1 on day 7, 10, 13, and 16. Mice were then treated with CTLA-4 Ig which began on day 10, 13, or 16. **B)** Tumor growth curves of C57BL/6J mice harboring MC38

tumors were treated with anti-CTLA-4 (clone 9H10) and CTLA-4 Ig (concomitant) on day 7. Then, CTLA-4 Ig (post) was administered on day 16. **C)** Tumor growth curves of C57BL/6J mice harboring B16F10 tumors were first treated with combination of anti-CTLA-4 (clone 9D9) and anti-PD-1 or CTLA-4 Ig (concomitant). Then, mice were further treated with CTLA-4 Ig (post) on day 16 when the initial treatments were completed. **D)** Tumor growth curves of C57BL/6J mice harboring B16F10 tumors treated with anti-CTLA-4, anti-CTLA-4 (5 doses). Then, mice were treated with CTLA-4 Ig (post) after treatment with anti-CTLA-4 was completed. All experiments began with 5 mice per group. Representative experiment from duplicated experiments is shown.



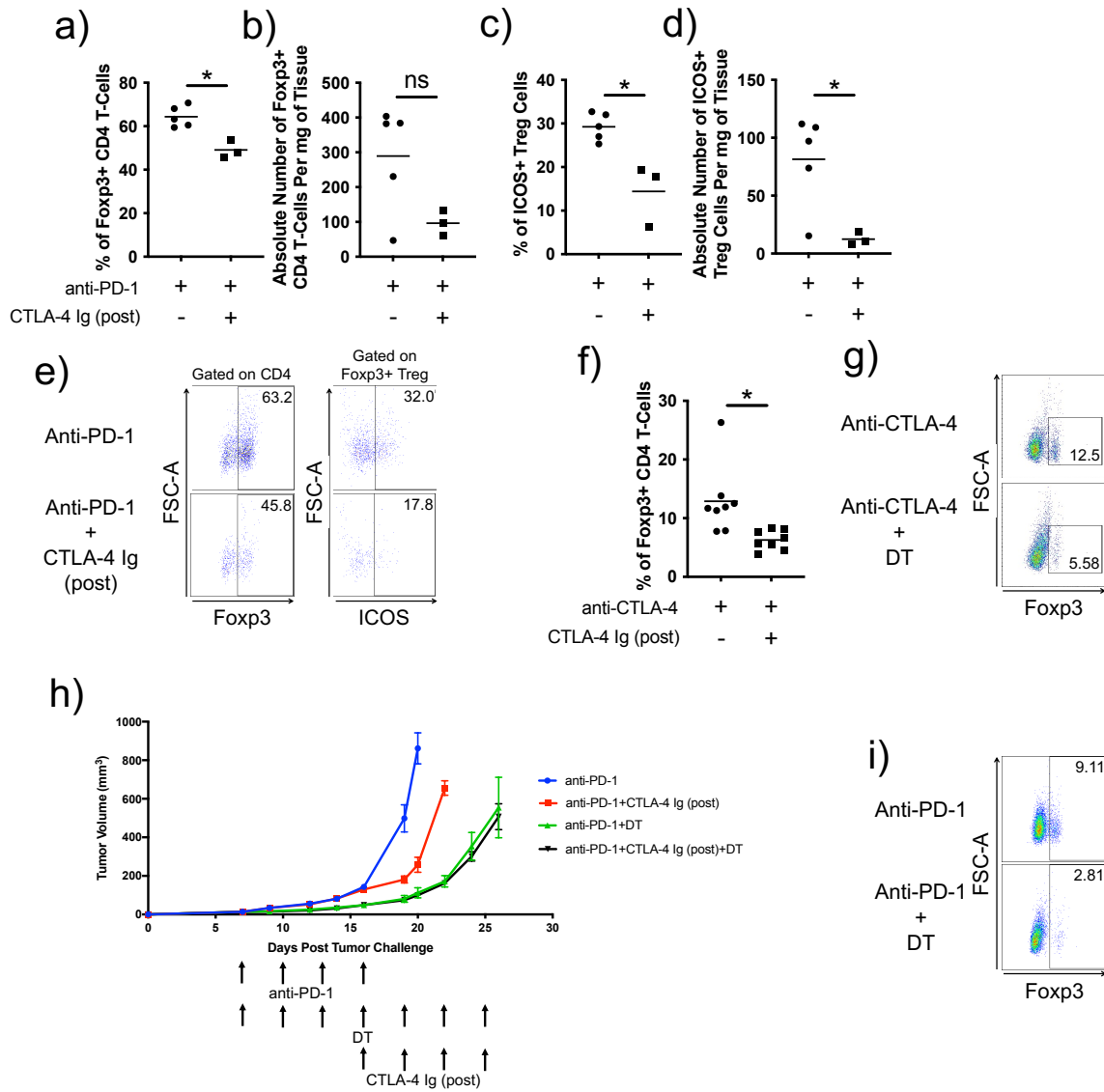
**Fig. S2. Antitumor response induced by CTLA-4 Ig (post) is T-cell-mediated.**

Representative FACS plots demonstrating that **A)** CD4 or **B)** CD8 T-cells were depleted after anti-CD4 or anti-CD8 treatment. Tumor growth curves of C57BL/6J mice harboring B16F10 tumors were first treated with combination of anti-PD-1. Then, mice were further treated with CTLA-4 Ig (post) in combination with **C)** anti-CD4 or **D)** anti-CD8 when the initial treatments were completed. Representative FACS plots demonstrating that **E)** CD4 or **F)** CD8 T-cells were depleted. All experiments began with 5 mice per group. Representative experiment from duplicated experiments is shown.



**Fig. S3. CTLA-4 Ig (post) treatment does not affect CD8 T-cells.** C57BL/6J mice harboring B16F10 tumors were initially treated with anti-PD-1. Once the initial treatments were completed, mice were further treated with CTLA-4 Ig (post). Dot-plot representation of **A)** frequency and **B)** absolute number of CD4 T-cells in B16F10 tumor on day 24. Dot-plot representation of **C)** frequency and **D)** absolute number of CD8 T-cells in B16F10 tumor on day 24. **E)** Representative FACS plots demonstrating percentages of CD4 and CD8 T-cells. Dot-plot representation of frequency of **F)** PD-1+, **G)** TIM-3+, **H)** LAG-3+, and **I)** VISTA+ CD8 T-cells. **J)** Representative FACS plots demonstrating percentages of PD-1+, TIM-3+, LAG-

3+, and VISTA+ CD8 T-cells. All experiments began with 5 mice per group. Representative experiment from duplicated experiments is shown.



**Fig. S4. Antitumor response induced by CTLA-4 (post) is Treg-dependent.**

Mice were first treated with anti-PD-1 and then with CTLA-4 lg (post). Dot-plot representation of **A)** frequency and **B)** absolute number of Foxp3+ CD4 T-cells on day 24. Dot-plot representation of **C)** frequency and **D)** absolute number of ICOS+ Tregs on day 24. **E)** Representative FACS plots demonstrating percentages of Foxp3+ CD4 and ICOS+ Tregs. **F)** Dot-plot representation of frequency of Foxp3+ CD4 T-cells. **G)** Representative FACS plots demonstrating percentages of CD4 T-

cells that express Foxp3. **H)** Tumor growth curves of C57BL/6J mice harboring B16F10 tumors were first treated with combination of anti-PD-1 and DT. Then, mice were further treated with CTLA-4 Ig (post) when the initial treatments were completed. **I)** Representative FACS plots demonstrating percentages of CD4 T-cells that express Foxp3. All experiments began with 5 mice per group. Representative experiment from duplicated experiments is shown.