

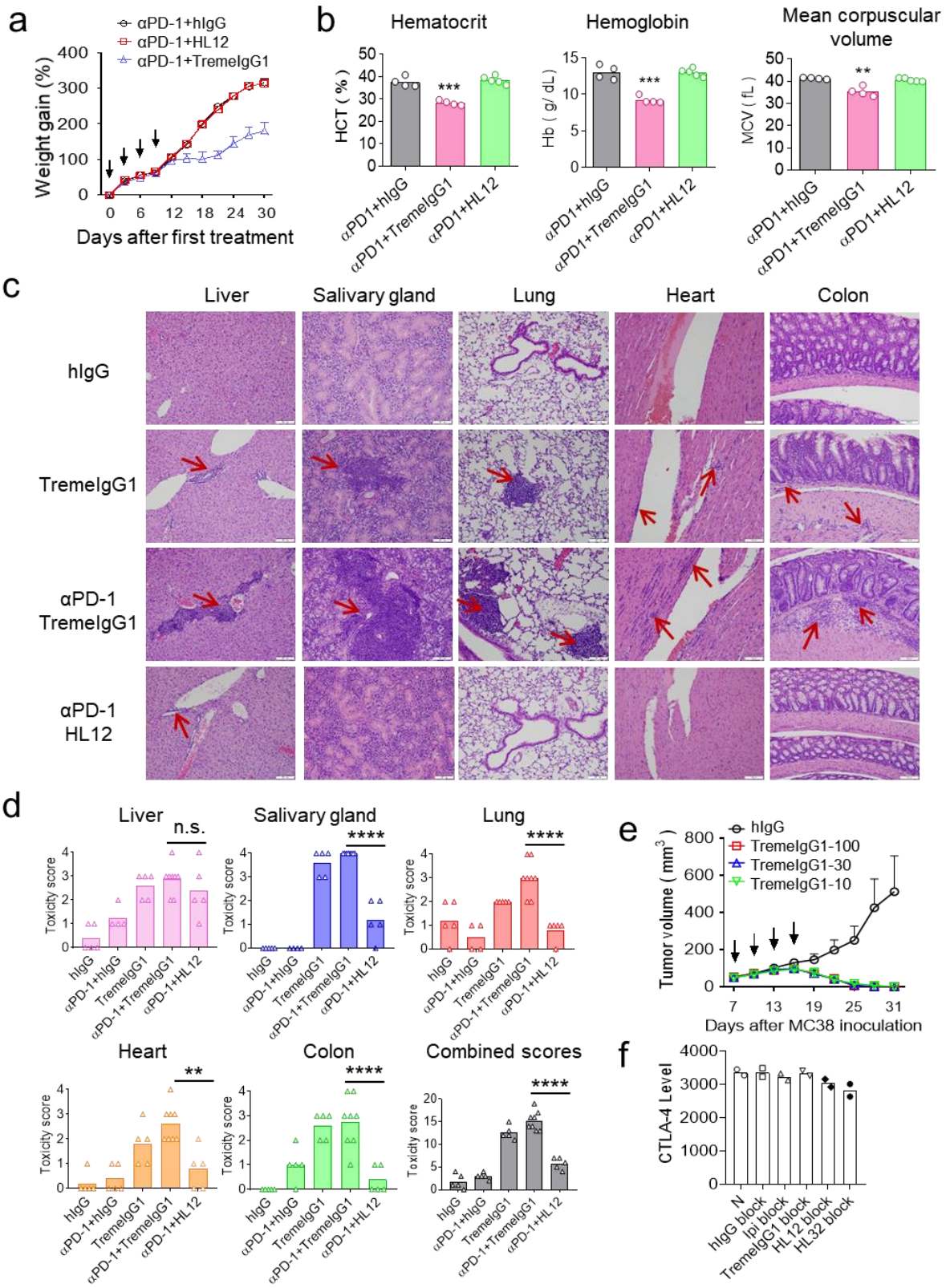


Figure S2. Down-regulation of surface CTLA-4 by anti-CTLA-4 mAbs correlates with their irAE, Related to Figure 2

(a) C57BL/6 *CTLA4^{h/h}* male mice were treated with control hIgG Fc plus anti-PD-1, TremelgG1 plus anti-PD-1, or HL12 plus anti-PD-1 respectively, at a dose of 100 µg/mouse/injection on days 10, 13, 16 and 19 after birth. Means and SEM of the weight gains of mice receiving different treatments are shown. n=5. One mouse in the TremelgG1 treated group died at day 31. (b) The CBC analysis of mice blood from (A) was performed on day 41 after birth. Data of blood hematocrit (HCT), total hemoglobin (Hb) and Mean Corpuscular Volume (MCV) are shown. (c) Representative images of H&E stained paraffin sections from different organs of mice with the indicated treatments. Representative inflammatory foci are marked with arrows. Scale bar, 200 µm. (d) Toxicity scores of internal organs and glands in (c). Data are mean ± S.E.M., n = 5–8 mice per group. Data were analyzed by one-way ANOVA with Bonferroni's multiple comparison tests. (e) MC38 bearing-*Ctla4^{h/h}* female mice (n=5) were i.p. treated with either control hIgG Fc (100 µg) or TremelgG1 (10 µg, 30 µg or 100 µg) on day 7, 10, 13 and 16 after tumor inoculation. (f) Antibody competition was analysis by adding saturated doses of anti-CTLA-4 mAbs to the cells before CTLA-4 staining with BNI3 when comparing with the hIgG group. Splenocytes of *Ctla4^{h/h}* mice were used for this test. Data are mean ± SEM. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. Representative data of three independent experiments in (a), (b) and (e) were shown. The samples in (c) and (d) were collected from three independent experiments and have been scored double-blind. Representative data of two independent experiments were shown in (f).