Supplementary Materials: IL-12 Gene Electrotransfer Triggers a Change in Immune Response within Mouse Tumors

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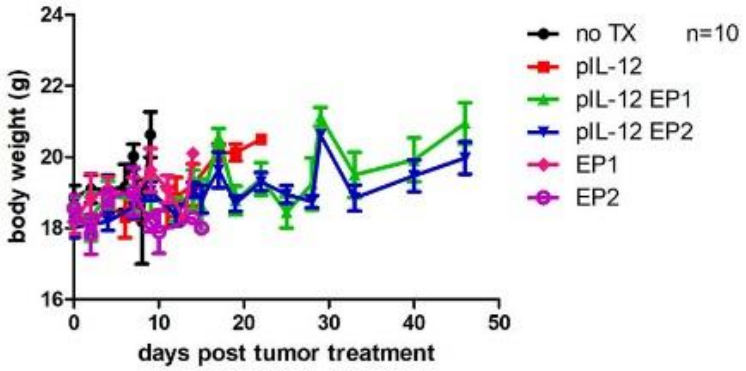

Figure S1. pIL-12 GET therapy associated with minimal systemic toxicity. On day 7, C57BL/6 mice were inoculated with B16F10 cells (1×10^6/50μL, s.c in the left flank.). Tumor-bearing C57BL/6 mice were treated with pIL-12 GET on day 0, 4 and 7. Animal weight data. Tumor-bearing mice did not show weight loss comparing the no TX group. The data presented are representative of two independent experiments. Each value represents the mean +/− SEM of the group (animals in each group, n = 8~13).

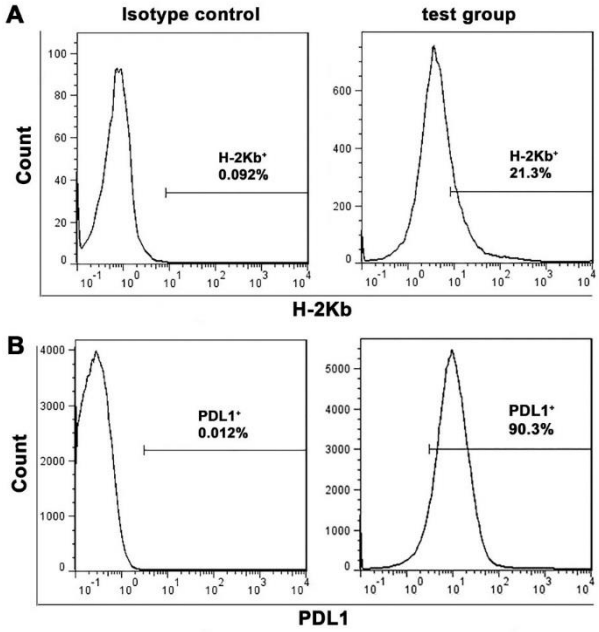

Figure S2. H-2Kb and PDL1 expression in B16F10 melanoma tumor cells. The H-2Kb and PDL1 expression in B16F10 tumor cells from tumor tissue (no TX group) were detected with flow cytometry at day 9. H-2Kb (**A**) and PDL1 (**B**) expression in B16F10 melanoma cells.

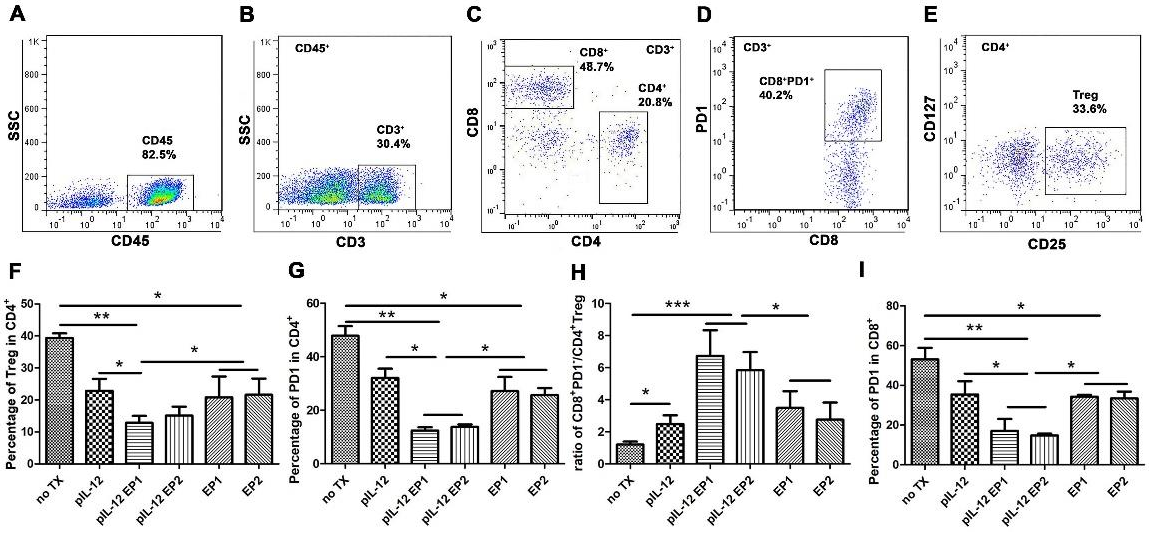

Figure S3. Exhausted $CD8^+PD1^-$, $CD4^+$ Treg in tumor-infiltrating lymphocytes (TILs). TILs from tumors tissue were collected at day 9 for flow cytometry assay. (**A**–**E**) Flow cytometry gating strategy used for defining immune cell subsets. (**F**–**I**) $CD4^+$ Treg, $CD4^+PD1^+$, ratio of $CD8^+PD1^-$/Treg, Exhausted $CD8^+PD1^+$ in TILs. One-way ANOVA, p * < 0.05, p ** < 0.01, p ***< 0.001.

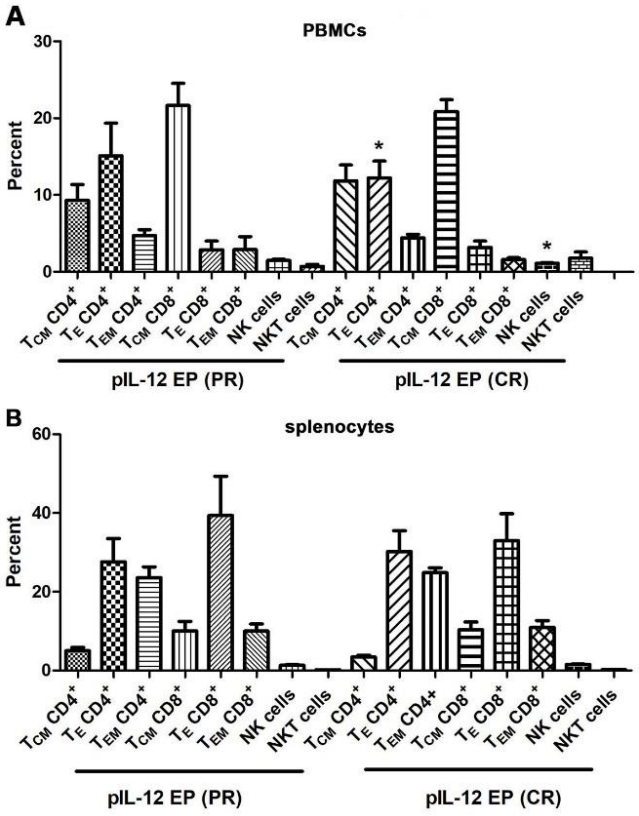

Figure S4. The changes of immune cells in pIL-12 GET induced prevention of new tumor formation following rechallenge. Peripheral blood mononuclear cells (PBMCs) (**A**) and splenocytes (**B**) were harvested at 20~30 days post rechallenge with 5 × 10^5 B16F10 cells from PR and CR mice for flow cytometry assay. Pooled data from two independent experiments are shown as mean +/− SEM. (animals in each group, n = 8~13). Independent t-test, p * < 0.05.