Cell Reports, Volume *42*

Supplemental information

Intravenous heterologous prime-boost vaccination activates innate and adaptive immunity to promote tumor regression

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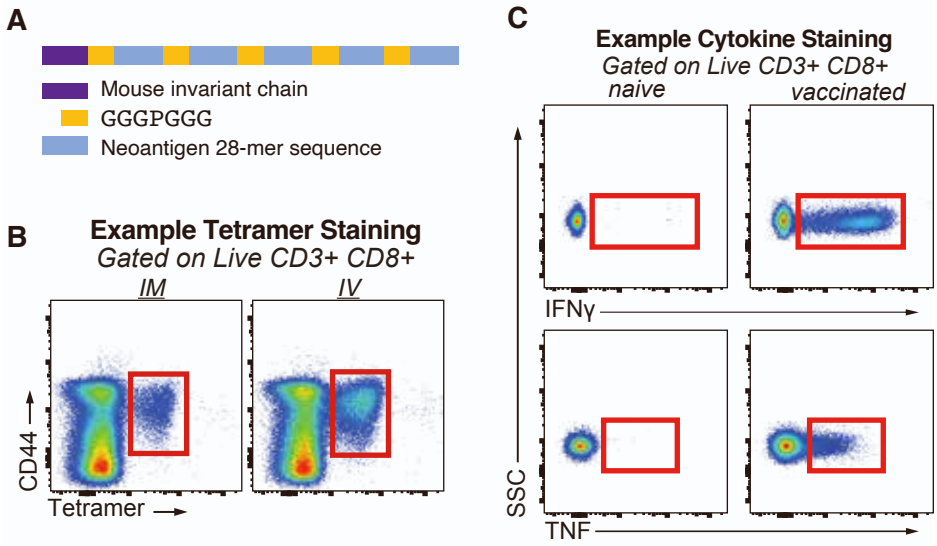

Figure S1. Neoantigen cassette construct and example staining of peptide restimulated T cell responses.

(A) Neoantigen cassette structure encoded in ChAdOx1 vectors. Mouse invariant chain at the N-terminus followed by a spacer sequence – to prevent formation of chimeric epitopes, and a pentamer repeat of the neoantigen-spacer.

(B) Example staining of Reps1-tetramer+ CD8 T cells in IM and IV vaccinated mice 2 weeks post vaccination.

(C) Example IFNγ and TNF staining following peptide restimulation in both vaccinated and unvaccinated mice

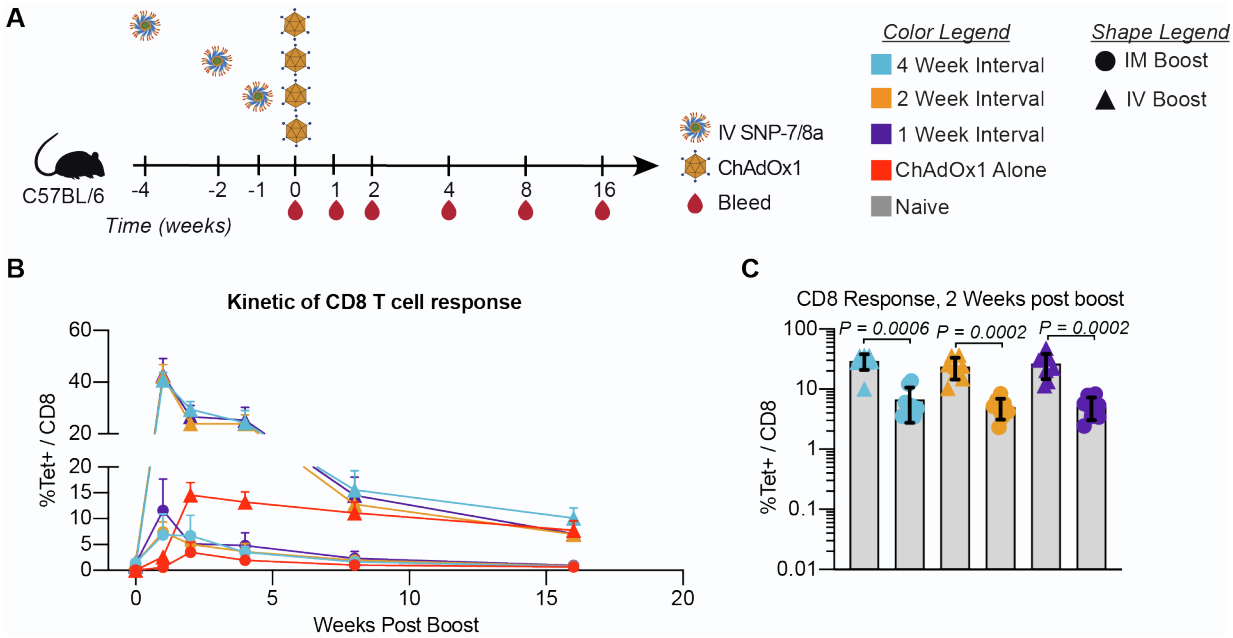

Figure S2. Interval between IV SNP prime and either IM or IV ChAdOx1 boost does not affect Reps1-specific CD8 T cell response magnitude.

(A) Interval vaccination study schematic. Mice are primed 4, 2, or 1 week prior to boosting with IV or IM ChAdOx1. Some mice receive only IV or IM ChAdOx1 and work as a benchmark for heterologous prime boost. Mice are bled to collect PBMCs for tetramer staining to quantify the Reps1-antigen specific response at the time of ChAdOx1 vaccination and then 1, 2, 4, 8, and 16 weeks post vaccination. The color legend corresponds to the interval, whereas the shape legend indicates the route of ChAdOx1 administration. All SNP vaccinations were given IV.

(B) Kinetic of Reps1-specific CD8 T cell responses over the course of the study.

(C) Comparison of the effect of route on magnitude of the Reps1-specific CD8 T cell response 2 weeks post boost, matched by interval.

(Stats) Data represented as mean ± SD (C) Mann-Whitney test, n = 8, data representative of 2 experimental repeats.

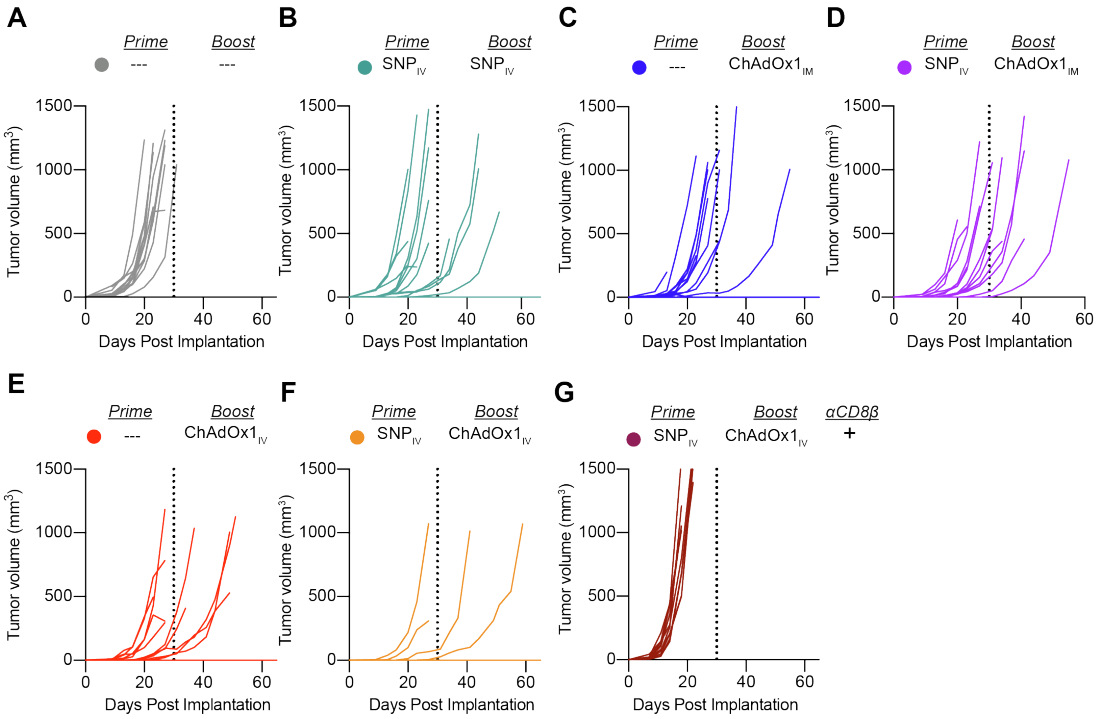

Figure S3. Individual tumor growth curves for prophylactic study groups.

(A-G) Individual tumor growth curves for the groups receiving the indicated vaccinations. 30 days post tumor implantation marked with dotted line for comparison.

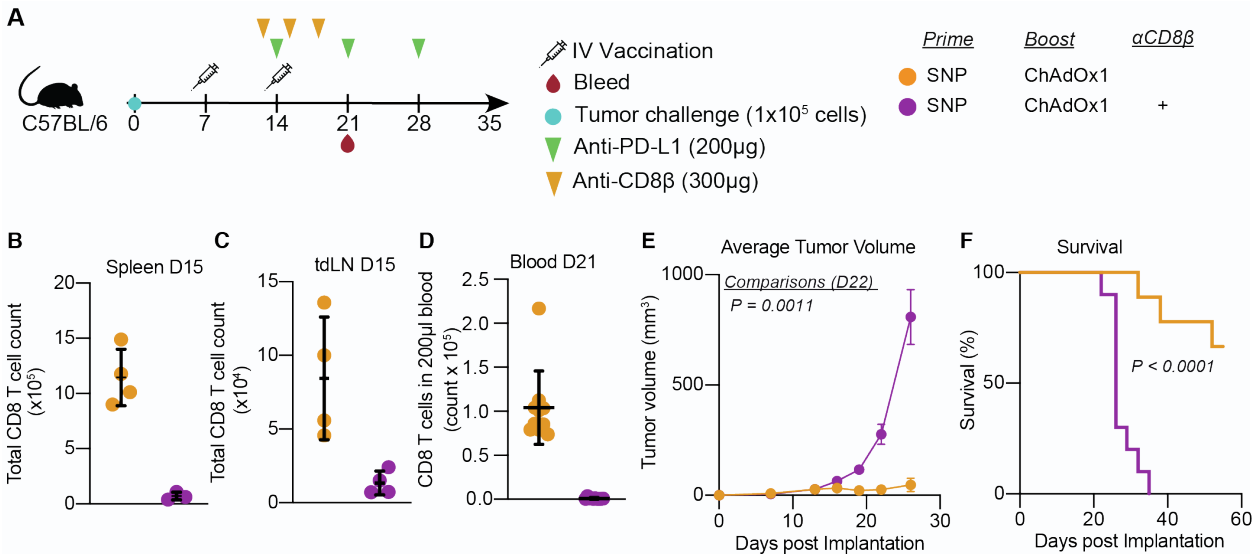

Figure S4. Tumor control in the therapeutic setting is dependent on CD8 T cells.

(A) Schematic of therapeutic study design. Mice were implanted with MC38 and vaccinated on day 7 and day 14 with the IV vaccinations indicated in the legend. Mice received 3 doses of αPD-L1 administered weekly beginning on day 14. Mice received 3 doses of a CD8 T cell depleting antibody before and after the boost vaccination. Spleens and tdLN were harvested on day 15 to assess effectiveness of CD8 depletion, and blood was collected on day 21 for the same purpose.

(B, C, D) CD8 T cell counts in the (B) spleen and (C) tdLN 1 day post-boost, and also in (D) blood 1 week post boost

(E) Average tumor growth curves for the IV heterologous prime boost group with and without CD8 T cell depletion.

(F) Survival curves for the IV heterologous prime boost group with and without CD8 T cell depletion.

(Stats) Data represented as mean ± SD (B, C, D) Mann-Whitney (need to add) (E) Two way ANOVA with Bonferroni correction for multiple comparisons, (F) Mantel-Cox Log-rank test. Panels B & C; n = 4, panels D, E, F; n = 9-10), data is from 1 experiment.

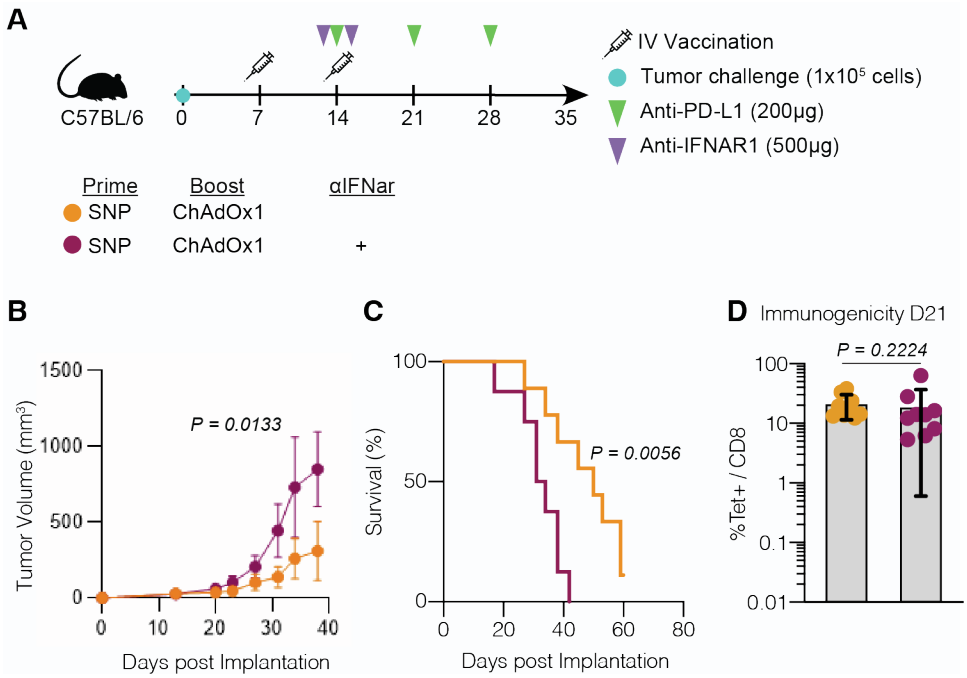

Figure S5. Type I IFN signaling is required for efficacy against Adpgk expressing B16F10 melanoma.

(A) Schematic of therapeutic study design. Mice were implanted with B16-F10 Adpgk and vaccinated on day 7 and day 14 with the IV vaccinations indicated in the legend. Mice received 3 doses of αPD-L1 administered weekly beginning on day 14. Some groups received saturating doses of IFNα receptor blocking antibody one day prior to and one day after the boost vaccination, as indicated in the legend.

(B) Average tumor growth curves for the IV heterologous prime boost group with and without IFNAR1 blockade

(C) Survival of the IV heterologous prime boost group with and without IFNAR1 blockade

(D) Adpgk-specific CD8 T cell response measured by tetramer staining one week post IV ChAdOx1 boost with or without IFNAR1 blockade.

(Stats) Panel B - Two-way ANOVA with Bonferroni correction for multiple comparisons. Panel C, Mantel-Cox Log-rank test. Panel D, Mann-Whitney test. For all panels, n = 9. Data from 1 experiment.

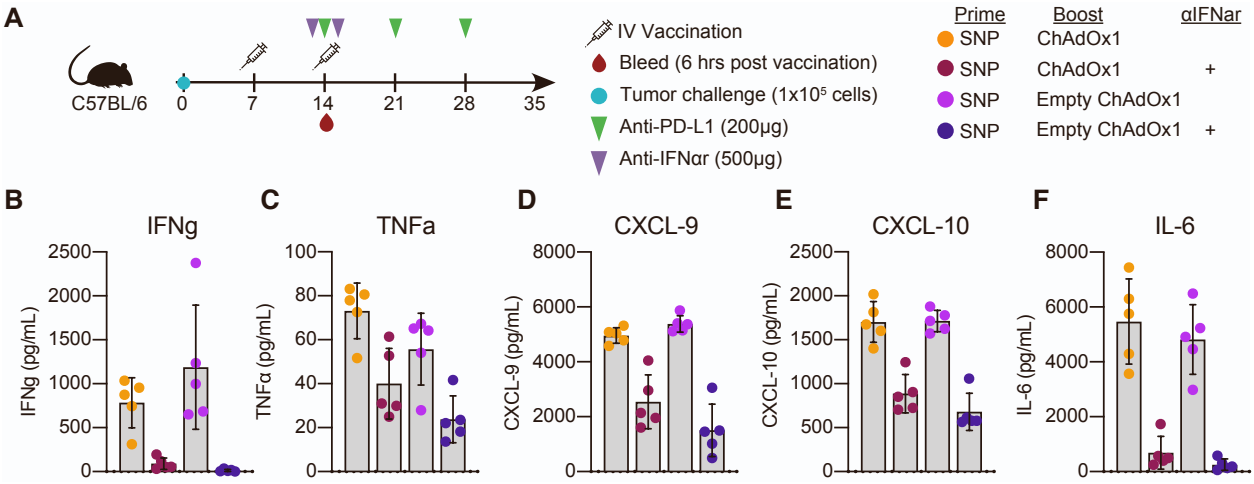

Figure S6. Production of pro-inflammatory systemic cytokines and activation of monocytes in the tumor is dependent on Type I IFNs.

(A) Schematic of therapeutic study design carried out as described in figure 7.

(B-F) Cytokines detected in the serum 6 hours post boost vaccination (B) IFNγ (C) TNF (D) CXCL-9 (E) CXCL-10 (F) IL-6

(Stats) Data represented as mean ± standard deviation (B-F) Mann-Whitney test to compare within each vaccine strategy the effect of IFNα receptor blockade. For all panels, n = 5, data representative of 2 experimental replicates.

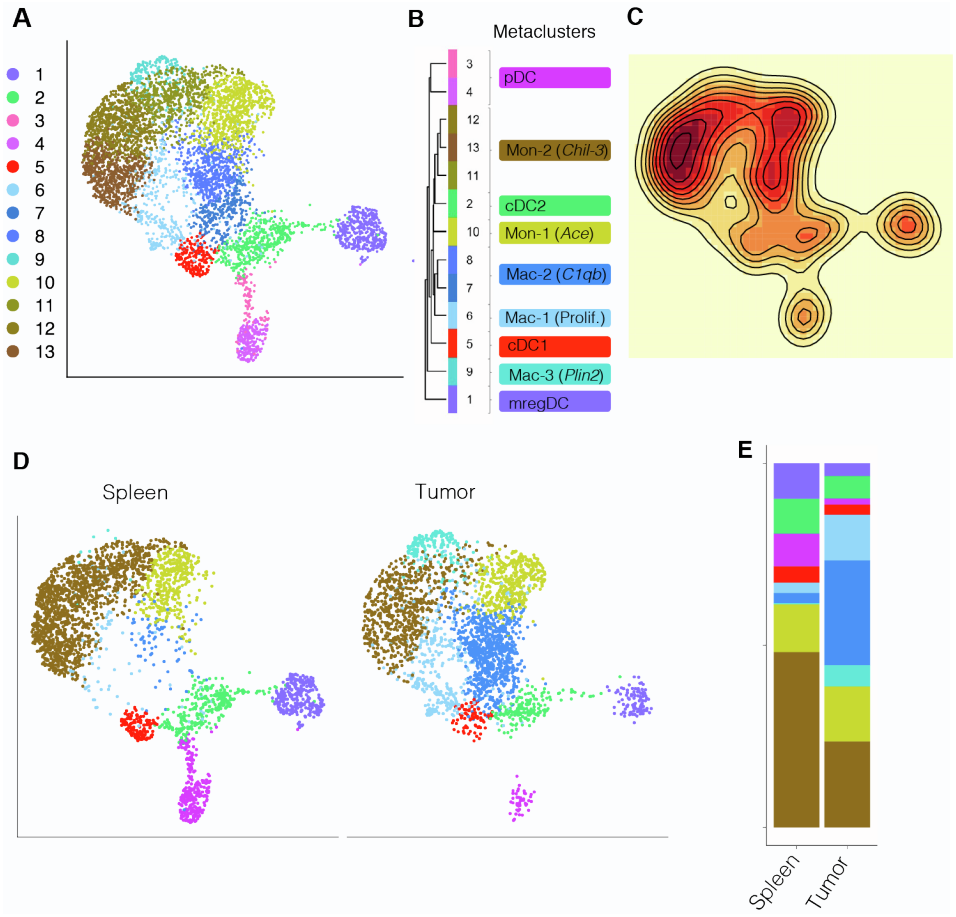

Figure S7. scRNA-seq of spleens and tumors reveals that all identified myeloid cell types are present in both tumor and spleens.

(A) Original clustering of scRNA-seq data visualized by UMAP dimensionality reduction

(B) Hierarchical clustering of original clusters into metaclusters

(C) Density plot to identify stable states in the visualized UMAP

(D) Metacluster UMAP segregated by tissue of origin of the cells (spleen and tumor)

(E) Bar graphs summarizes relative frequencies of myeloid metaclusters in spleen and tumor.