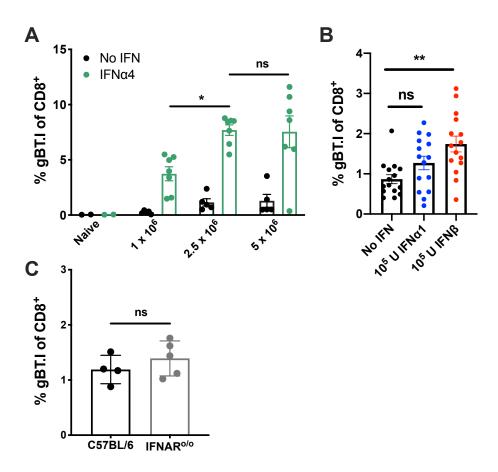
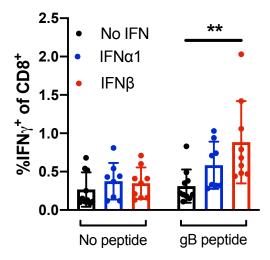


Supplementary Material

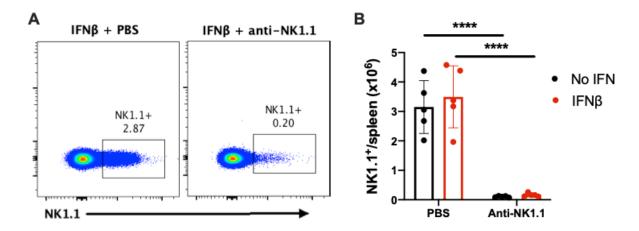
1 Supplementary Figures



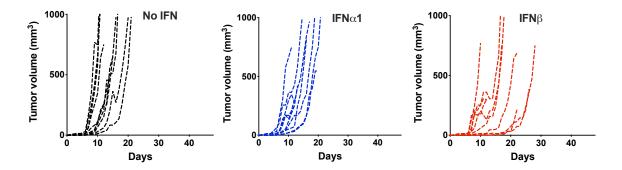
Supplementary Figure 1. Optimisation and validation of vaccination strategy. A,B C57BL/6 mice received 5 x 10^4 naïve gBT.I cells one day prior to vaccination with irradiated B16.Kbloss.gB±IFN cells. Mice received titrated amounts of B16.Kbloss.gB or B16.Kbloss.gB_IFN α 4 (n = 2-7 per group, from 1-2 independent repeats) (A) or 2.5 x 10^6 B16.Kbloss.gB cells with or without 10^5 U recombinant IFN α 1 or IFN β (n = 15 per group from 4 independent repeats) (B). Seven days post-vaccination, spleens were harvested and expansion of gBT.I cells was measured. C 2.5 x 10^6 naïve gBT.I cells were passively transferred into C57BL/6 and IFNARolo mice (n = 4-5 per group). Splenocytes were analysed thiry days post-transfer for the presence of gBT.I cells. Data is analysed by two-way ANOVA (A), one-way ANOVA (B) or unpaired t-test (C), **p < 0.01, ns = not significant (p > 0.05). Bars represent mean ± SEM.



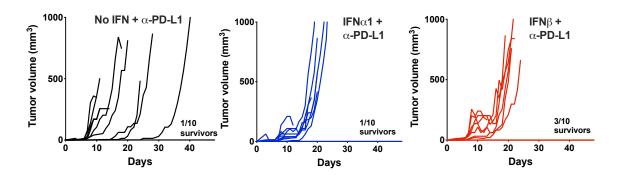
Supplementary Figure 2. Increase in tumor-specific CD8⁺ T cells mediated by IFN β is present at 60 days post-vaccination. C57BL/6 mice were harvested sixty days post-vaccination with 2.5 x10⁶ irradiated B16.Kb^{loss}.gB±IFN cells (n = 8-9 per group) and IFN γ -producing gB-specific CD8⁺ T cells were measured. Data is pooled from 2 independent repeats and analysed by two-way ANOVA, **p < 0.01. Bars represent mean ± SEM.



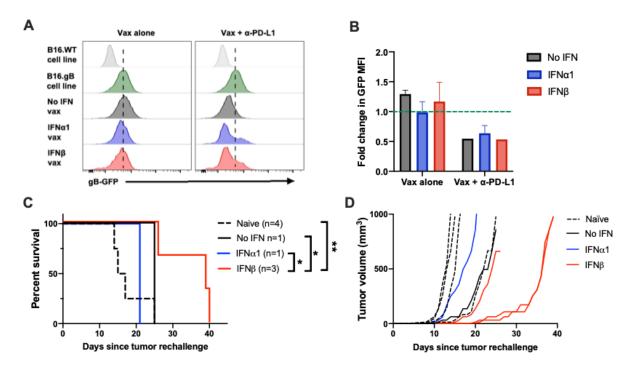
Supplementary Figure 3. Anti-NK1.1 treatment results in successful depletion of NK cells. A,B Representative flow plots of NK1.1 expression (A) and enumeration of NK1.1 cells (B) in the spleen of mice vaccinated seven days prior with 2.5 x 10^6 irradiated B16.Kbloss.gB±IFN β cells. Mice either received control PBS or 200 μ g anti-NK1.1 one day prior and post vaccination (n = 5 per group). Asterisks indicate significant difference by two-way ANOVA, ****p < 0.001. Bars represent mean ± SEM.



Supplementary Figure 4. Vaccination with IFN β delays tumor progression. Individual tumor growth of individual C57BL/6 mice vaccinated with 2.5 x 10⁶ irradiated B16.Kb^{loss}.gB±IFN cells three days post-B16.gB tumor inoculation (n = 10 per group from two independent experiments).



Supplementary Figure 5. Vaccination with IFN β synergises with anti-PD-L1 checkpoint blockade therapy to increase overall survival. Individual tumor growth of C57BL/6 mice vaccinated with 2.5 x 10^6 irradiated B16.Kbloss.gB±IFN cells three days post-B16.gB tumor inoculation. Mice recieved α -PD-L1 on days six, nine and twelve post-challenge (n = 10 per group from two independent experiments).



Supplementary Figure 6. Anti-PDL1 may promote tumor antigen downregulation compared to vaccination alone, and increased epitope spreading when combined with vaccination plus IFN β . Mice were vaccinated with 2.5 x 10⁶ irradiated B16.Kb^{loss}.gB±IFN cells three days post-B16.gB tumor inoculation, with some mice receiving three doses of anti-PD-L1 on days six, nine and twelve post-challenge. (A) Representative histograms of gB-GFP expression and (B) fold decrease in GFP MFI of tumors harvested at endpoint (n = 1-4 per group). (C) Survival and (D) individual growth curves of surviving mice rechallenged with B16 wildtype tumors sixty days post-initial tumor inoculation (n = 1-4 per group). Significance was assessed by Log-rank Mantel-Cox test, **p < 0.05, *p < 0.1. Bars represent mean ± SEM.