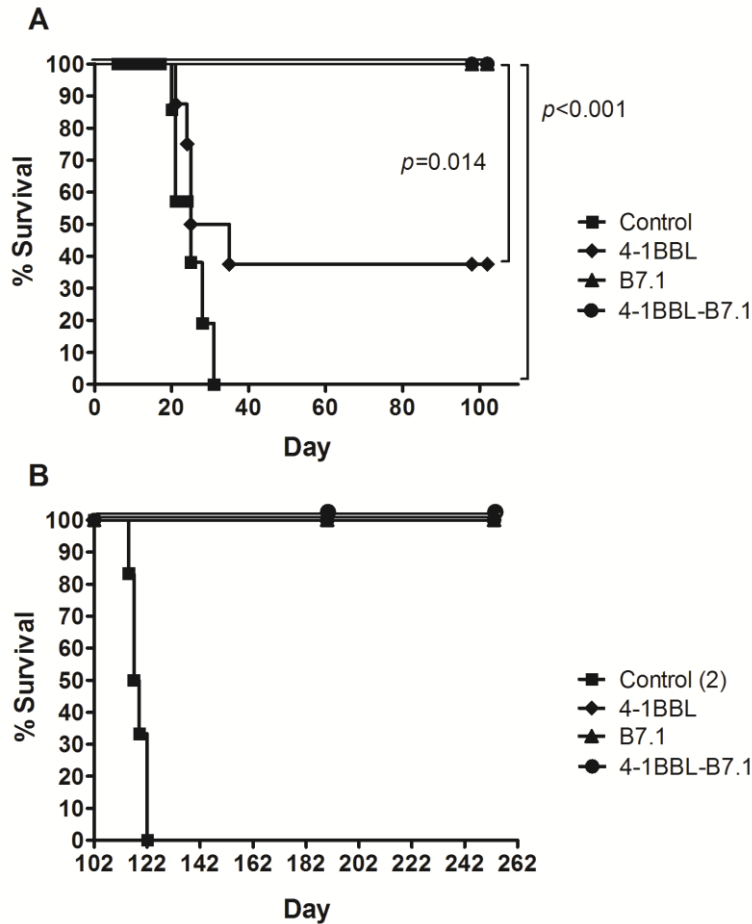


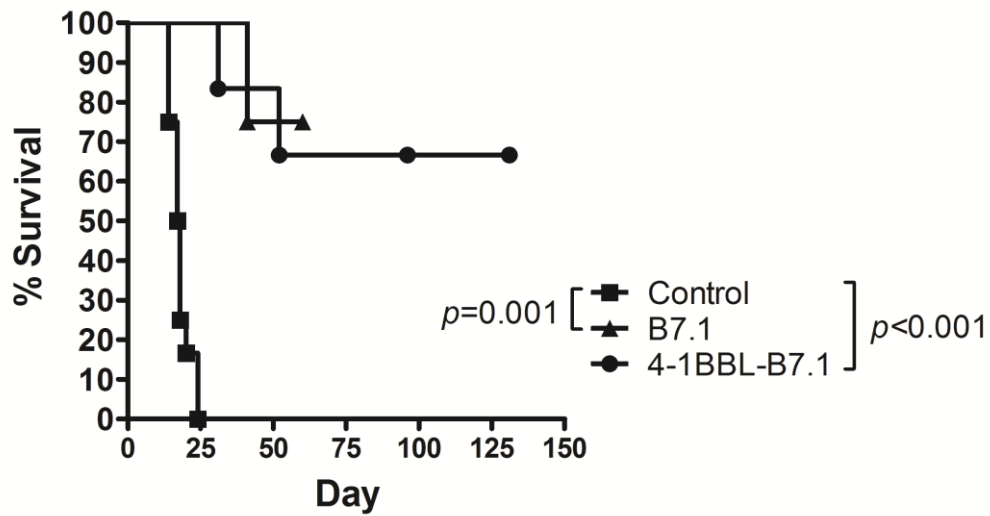
Supplementary Table 1. Comparison of B7.1 and 4-1BBL expression on B16-F10 wild-type cells with the levels expressed by the engineered vaccine cells.

Cell Line	Fold increase in expression*
B16-F10 MHC Class I	1
B7.1	1
4-1BBL	3
B16-F10-4-1BBL IFN γ / β : MHC Class I	24
4-1BBL	1104
B16-F10-B7.1 IFN γ / β : MHC Class I	18
B7.1	24
B16-F10-4-1BBL-B7.1 IFN γ / β : MHC Class I	24
B7.1	49
4-1BBL	465

* All fold increase values in co-stimulatory molecule expression are shown relative to the values produced by the isotype stained cells.



Supplementary Fig. 1 Vaccinating C57BL/6 mice with either the B16-F10-B7.1-IFN γ / β or B16-F10-4-1BBL-B7.1-IFN γ / β cells completely protects against tumour development when challenged with 5×10^5 live B16-F10-B7.1 cells. C57BL/6 mice remained unvaccinated as controls (n=6) or were treated with either B16-F10-4-1BBL-IFN γ / β (4-1BBL; n=6), B16-F10-B7.1-IFN γ / β (B7.1; n=7) or B16-F10-4-1BBL-B7.1-IFN γ / β cell vaccines (4-1BBL-B7.1; n=7). Mice were then challenged with 5×10^5 live B16-F10-B7.1 cells and survival assessed **A**. Mice that survived initial tumour challenge were further boosted with an additional two doses of vaccine. The vaccinated mice and a separate group of naïve mice were challenged with 5×10^5 live B16-F10-B7.1 cells and survival assessed **B**. Data for each panel are representative of two independent experiments and survival was analysed using the Log rank test.



Supplementary Fig. 2 Treating $LT\alpha^{-/-}$ mice with either the B16-F10-B7.1- $IFN\gamma/\beta$ or B16-F10-4-1BBL-B7.1- $IFN\gamma/\beta$ anticancer vaccine enhances survival after challenge with 5×10^5 live B16-F10-B7.1 cells. $LT\alpha^{-/-}$ mice were used as controls (n=12) or injected with either B16-F10-B7.1- $IFN\gamma/\beta$ (B7.1; n=4) or B16-F10-4-1BBL-B7.1- $IFN\gamma/\beta$ (4-1BBL-B7.1; n=6) vaccines. Four days following the final vaccination, all mice were challenged with 5×10^5 live B16-F10-B7.1 cells and survival assessed. Data are representative of two independent experiments and survival was analysed using the Log rank test.