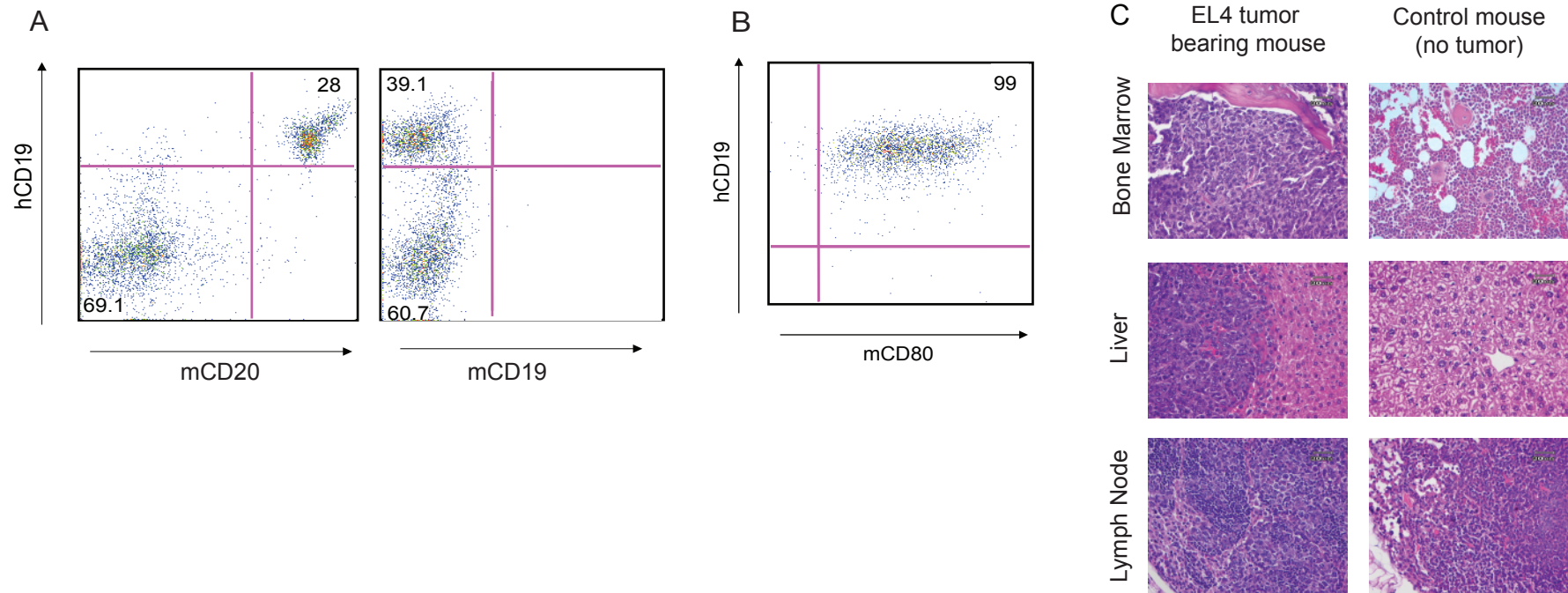


Supplementary Figure 1

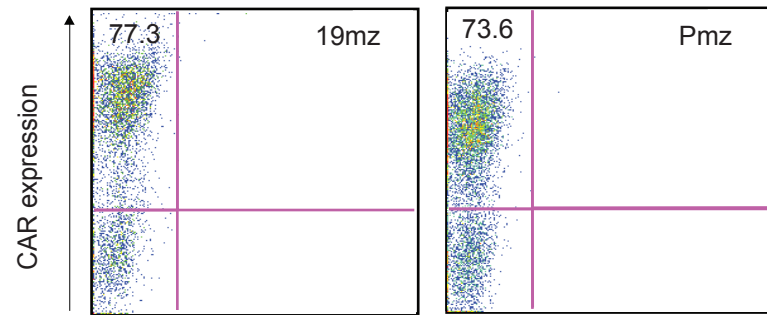


Supplementary Figure 1. Characterization of syngeneic murine model of disease.

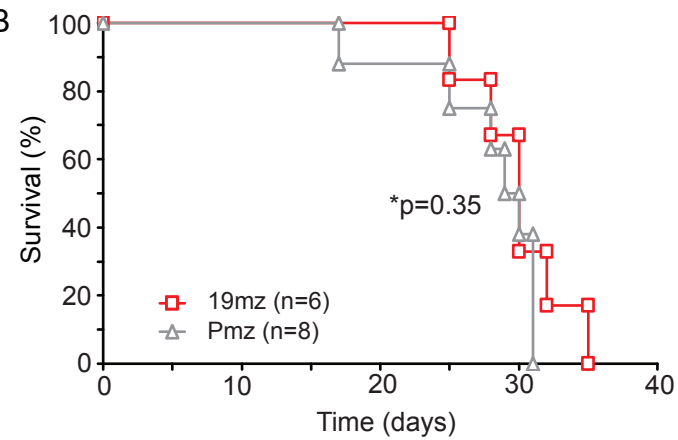
(A) Flow cytometry detecting human CD19 (hCD19) and mouse CD20 (mCD20) but not mouse CD19 (mCD19) on peripheral blood cells from C57BL6(mCD19^{-/-} hCD19^{+/-}) mice. (B) Flow cytometry demonstrates hCD19 and mCD80 expression on EL4(hCD19) tumor cells. (C) Hematoxylin eosin staining of bone marrow, liver and lymph node tissue in EL4(hCD19) tumor bearing mouse and control mouse (no tumor).

Supplementary Figure 2

A



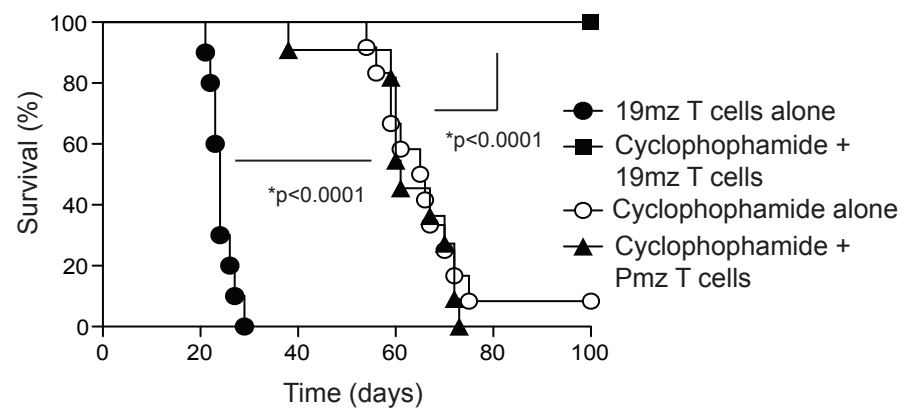
B



Supplementary Figure 2. hCD19 targeted T cells fail to eradicate systemic hCD19⁺ tumors in a syngeneic murine model.

(A) CAR expression on 19mz and Pmz T cells following retroviral gene transfer. Results shown are representative of multiple experiments. (B) Survival of systemic EL4 (hCD19) tumor bearing C57BL6(mCD19^{-/-} hCD19^{+/-}) mice treated with 19mz+ mouse T cells compared to control treated mice. Results shown represent combined data from at least two independent experiments.

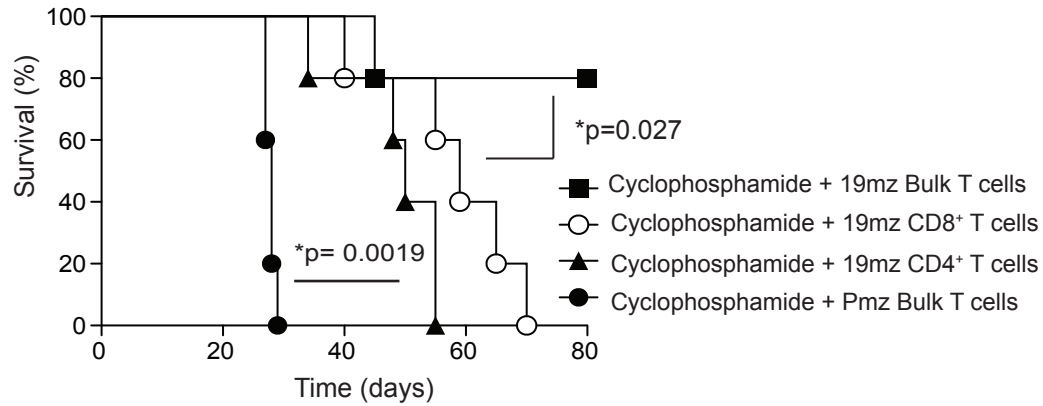
Supplementary Figure 3



Supplementary Figure 3. Cyclophosphamide pre-treatment combined with 19mz T cell infusion eradicated systemic EL4(hCD19) tumor.

C57BL6(mCD19^{-/-} hCD19^{+/-}) mice were inoculated with EL4(hCD19) tumor cells on day 0 followed by intraperitoneal injection of 250 mg/kg cyclophosphamide on day 2, then treated with either 19mz⁺ or Pmz⁺ T cells on day 3. Treatment with both cyclophosphamide and 19mz⁺ T cells resulted in long term survival compared to mice treated with 19mz⁺ T cells without cyclophosphamide. Additionally, mice treated with cyclophosphamide alone or additionally with Pmz⁺ T cells demonstrated significantly enhanced survival but all mice in these cohorts eventually succumbed to disease between 8 and 11 weeks post EL4(hCD19) tumor inoculation.

Supplementary Figure 4

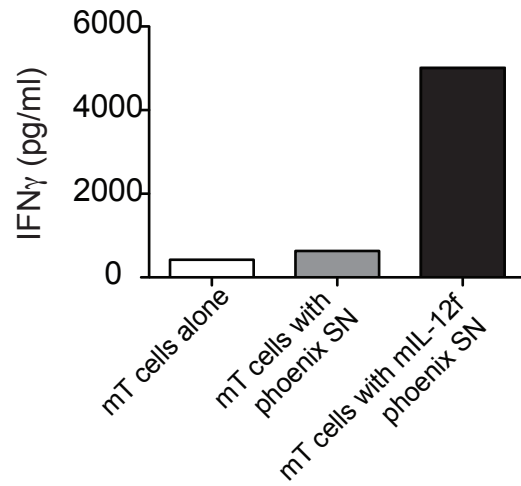


Supplementary Figure 4. CD4⁺ and CD8⁺ T cells are required for tumor eradication in mice treated with cyclophosphamide and hCD19 targeted T cells.

C57BL6(mCD19^{-/-}hCD19^{+/-}) mice were pre-treated with 250 mg/kg cyclophosphamide followed by iv inoculation with EL4(hCD19) tumor cells. Mice were subsequently treated with bulk 19mz⁺ T cells, sorted CD8⁺ 19mz⁺ T cells, sorted CD4⁺ 19mz⁺ T cells, or bulk Pmz⁺ T cells. Mice treated with cyclophosphamide followed by infusion of bulk 19mz⁺ T cells demonstrated long-term survival in contrast to cohorts treated with bulk Pmz⁺ T cells. While mice treated with either sorted CD4⁺ or CD8⁺ 19mz⁺ T cells alone had significantly increased survival when compared to control mice treated with bulk Pmz⁺ T cells, in contrast to mice treated with bulk 19mz⁺ T cells, the latter cohorts failed to eradicate systemic EL4(hCD19) tumors as evidenced by a failure to result in long term surviving mice.

Supplementary Figure 5

A



Supplementary Figure 5. Validation of the function of mIL-12f.

The function of mIL-12f was validated by culturing mouse T (mT) cells (activated with CD2/CD28 beads, Invitrogen) with supernatant (SN) from Phoenix ecotropic packaging cells retrovirally transduced with or without mIL-12f. Supernatants were collected after 24 hrs and tested for IFN γ using a multi-plex luminex assay.