Supplemental Methods

Murine CD19 (mCD19) CAR stable producer cell line

The mCD19 CAR construct was kindly provided by Dr. Jim Kochenderfer[1]. Retroviral supernatant was produced with transient transfection of HEK 293GP cells with a packaging plasmid containing an anti-murine CD19 CAR and separate plasmid encoding a RD114-pseudotype envelope following a standard transfection protocol (Life Technologies). Supernatant was harvested at 48 and 72 hours and used for transduction of platinum-E (Plat-E) (ATCC), a high-titer retroviral production packaging cell line. Briefly, non-tissue culture treated 6-well plate were coated with 2 ml of diluted retronectin (12ug/ml) (Clontech, Mountain View, CA) for 2 to 3 hours at room temperature, then blocked with 2% BSA and rinsed with 1XPBS. Viral supernatant was added to each well and plates were spun at 32^0C, 2000g for 2 hours. Following centrifugation, Plat-E cells were suspended at a concentration of 5000 cells/ml, and 2 ml were added to the plates. The spin-inoculation was repeated once more 24 hours later. The transfected cells were analyzed for mCD19 CAR with Protein-L, sorted based on expression, and single cells were plated for further expansion and selection. The best clone was selected based on the surface expression intensity.

CAR T Cell Production

A single cell suspension was made from freshly harvested mouse spleen and T cells were enriched using mouse $CD3^+$ T-cell enrichment column (R&D, Minneapolis, MN) following the manufactures' protocol. T cells were activated for 24 hours with Mouse T-Activator CD3/CD28 Dynabeads (Life Technologies, Grand Island, NY) in the presence of IL-15 (10U/ml) and IL-7 (10ng/ml) in complete mouse media (CMM). Activated T cells were then transduced with the mCD19 retroviral supernatant harvested from the stable producer line. Dynabeads were removed 24 hours following the 2^{nd} spinoculation. The transduced T cells were harvested after 5 days and used either for *in vitro* analysis or for intravenous injections for *in vivo* experiments.

Adoptive T cell transfer and Vaccines

TCF3/PBX1.3 cells were lethally irradiated to150 Gy and administered intraperitoneally as vaccine for immunization in the vaccine protection model or to prime T cell donors in the adoptive T cell transfer model. Dendritic cells (DCs) used for vaccines were prepared from female B6 bone marrow as previously described.[2] Briefly, purified bone marrow cell were cultured *in vitro* for 6 days with GM-SCF (50ng/ml) and IL-4 (50ng/ml) to induce DC differentiation. For some groups, immature DCs were pulsed on day 6 with irradiated TCF3/PBX1 at 1:3 ratio. On day 7, immature DCs were activated with 1ug/ml of LPS for one day. On day 8, mature DCs were harvested and resuspended in serum-free RPMI and administered intraperitoneally as indicated.

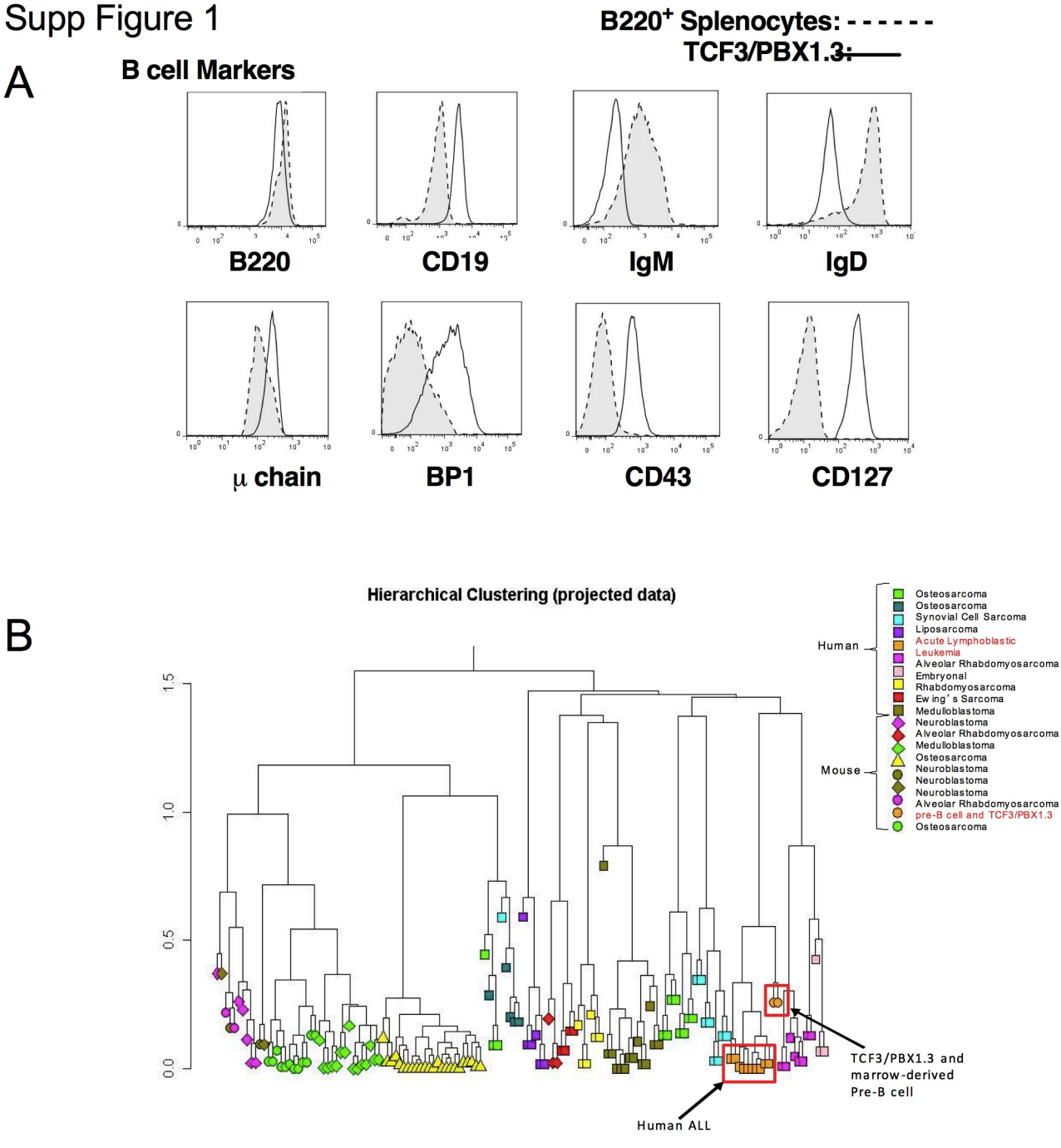
T cells were purified from single cell suspensions of lymph node or spleen from naïve or vaccinated donors using magnetic beads (Pan-T isolation kit, Miltenyi Biotec, Auburn, CA) following the manufacture's instruction. The isolated T cells were resuspended in serum-free RPMI media and administered intravenously through tail vein into the recipient mice.

In vivo cell depletion

Anti-CD4(GK1.5), Anti-CD8(2.43) were purchased from National Cell Culture Center. Anti-CD4 and/or anti-CD8 were given three times a week at a dose 200ug/mouse. All antibodies were administered intraperitoneally beginning three days prior to tumor challenge and continuing for three weeks.

1. Kochenderfer, J.N., Yu, Z., Frasheri, D., Restifo, N.P. & Rosenberg, S.A. Adoptive transfer of syngeneic T cells transduced with a chimeric antigen receptor that recognizes murine CD19 can eradicate lymphoma and normal B cells. *Blood* **116**, 3875-3886 (2010).
2. Capitini, C.M.*, et al.* Bone marrow deficient in IFN-{gamma} signaling selectively reverses GVHD-associated immunosuppression and enhances a tumor-specific GVT effect. *Blood* **113**, 5002-5009 (2009).

Supp Figure 1

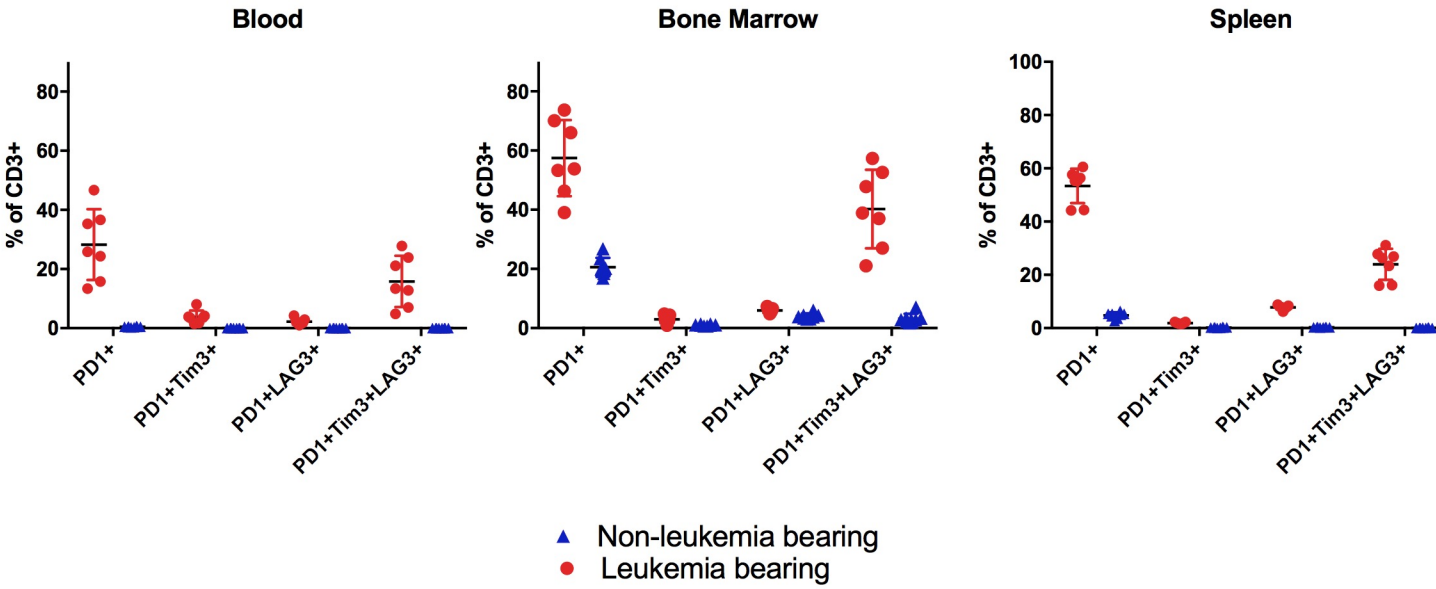


Experiment	Tumor Initiating Cells
#1	7%
#2	3%
#3	4%
Average	**4.7%**

Supp Figure 2

Primary ALL - TCF3/PBX1 Transgenic

A



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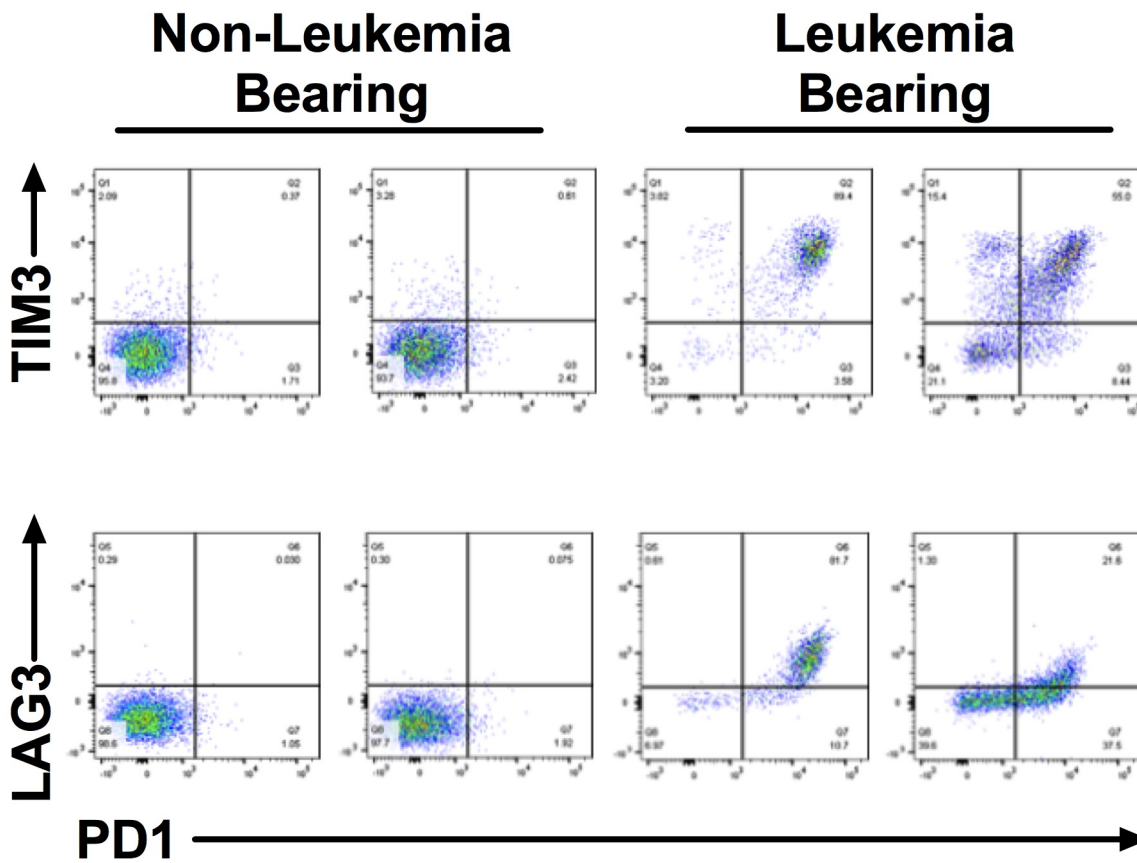
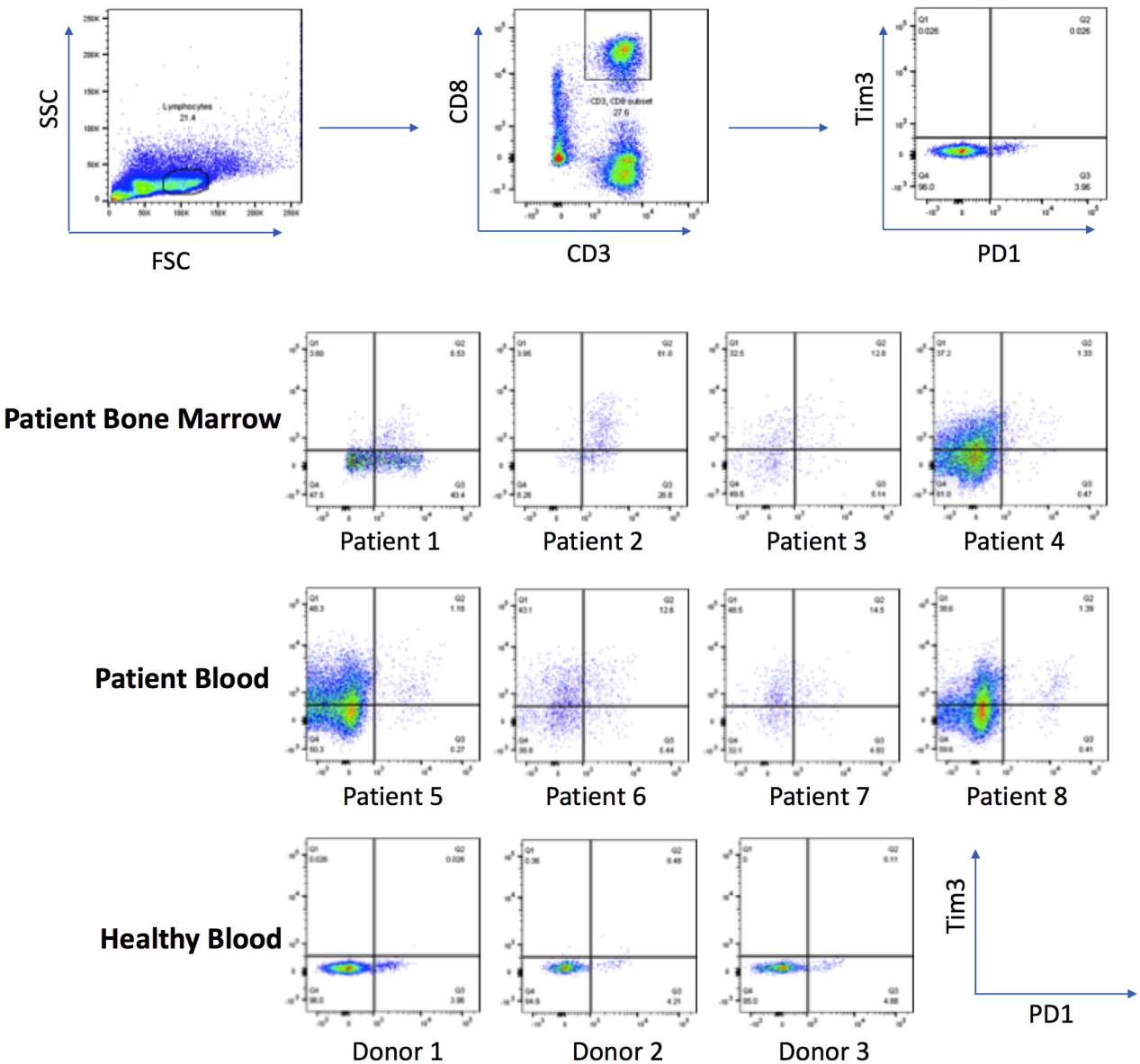


Supp Figure 3

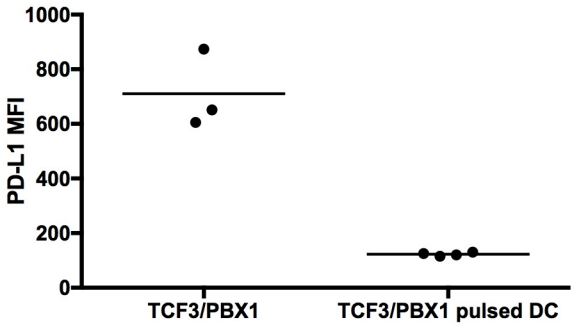
Gating Strategy for Human Samples:



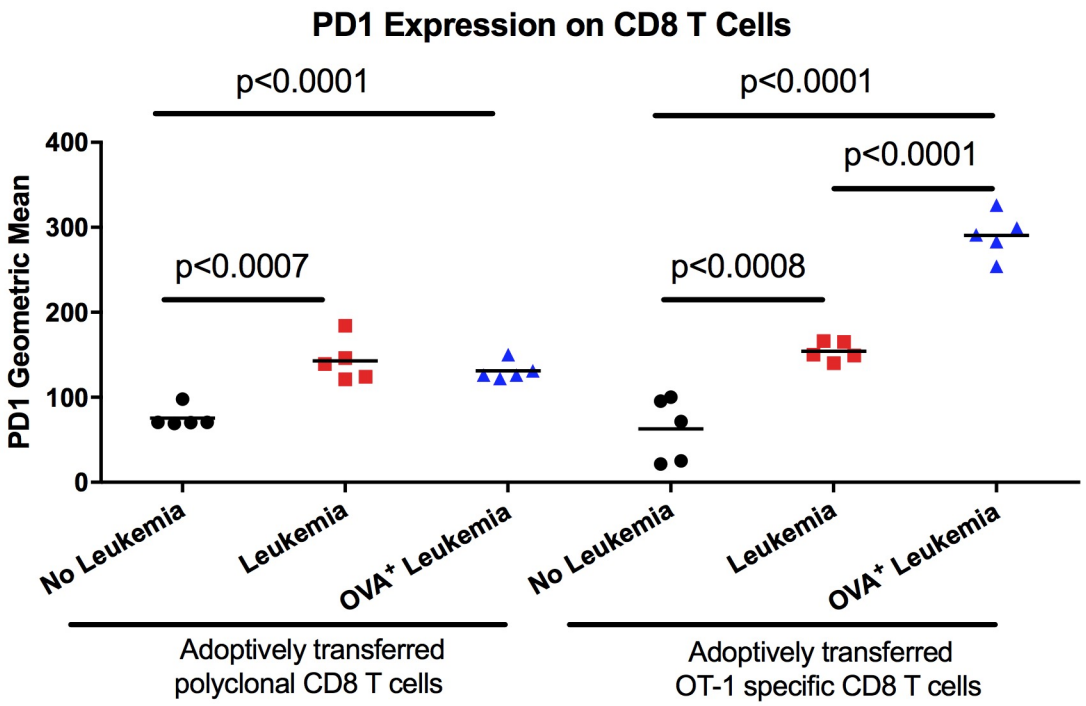
Patient 1: 13 year old male with multiply relapsed/refractory ALL and two prior allogeneic hematopoietic stem cell transplants (HSCT)
Patient 2: 7 year old male with relapsed/refractory ALL and no prior HSCT
Patient 3: 11 year old female with primary refractory leukemia and high burden disease after 3 failed induction attempts
Patient 4: 23 year old male with relapsed/refractory ALL and no prior HSCT
Patient 5: 16 year old male with relapsed/refractory ALL and no prior HSCT
Patient 6: 22 year old male with relapsed/refractory ALL and no prior HSCT
Patient 7: 2 ½ year old female with biphenotypic leukemia who presented with ALL following HSCT
Patient 8: 19 year old male with relapsed/refractory ALL and no prior HSCT

A

Supp Figure 4

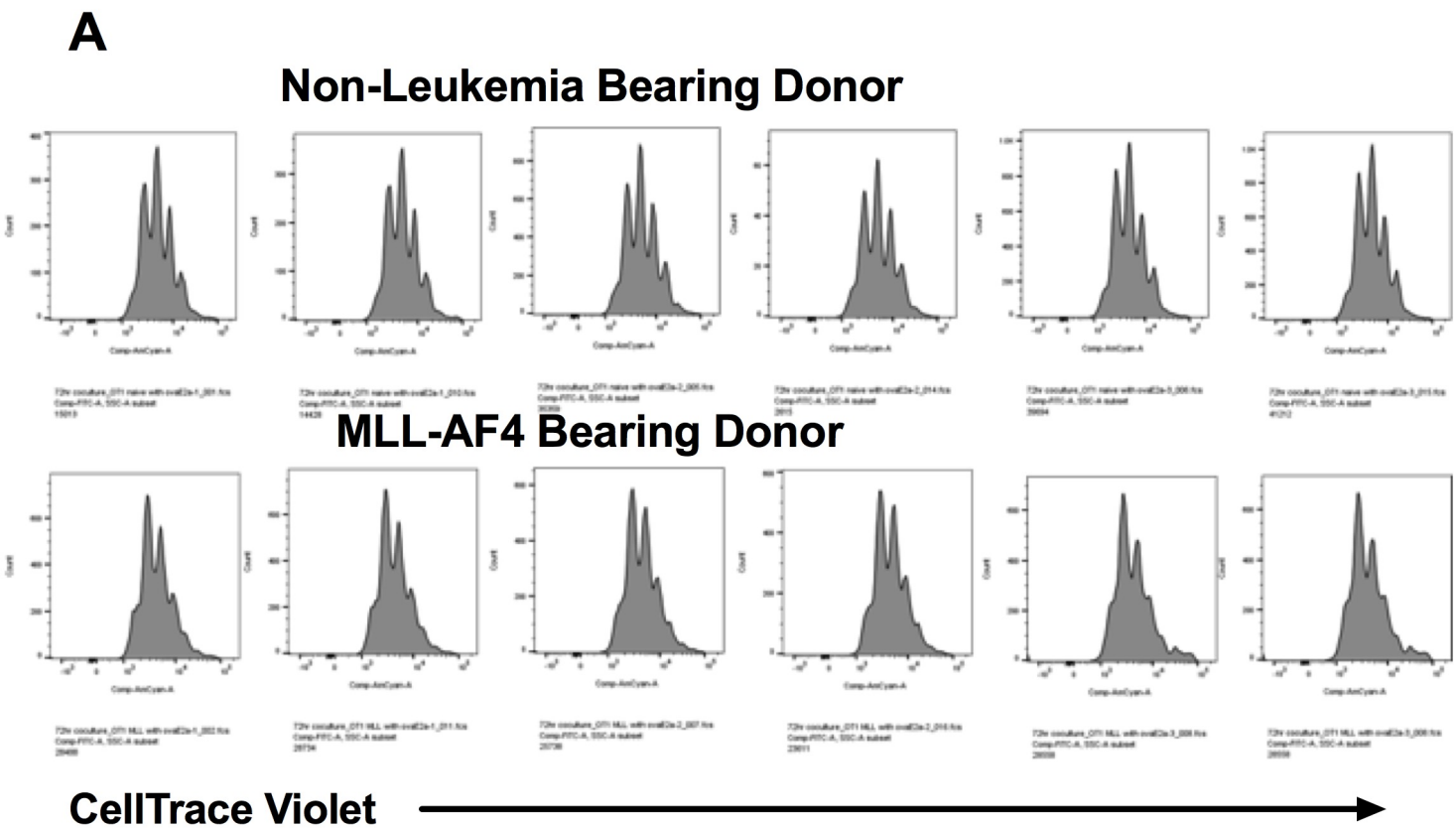


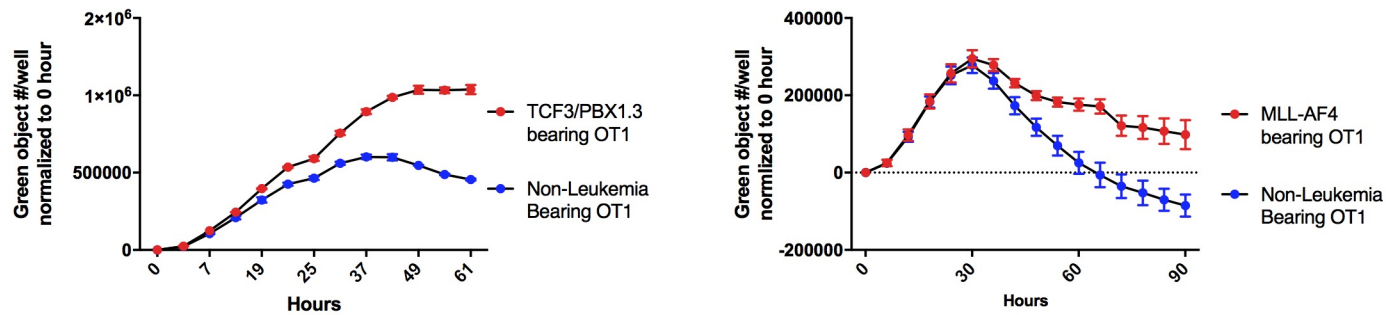
B



B) Congenic CD45.1 mice received adoptive transfer of dose $CD8^+$ T cells from healthy donors (CD45.2 or OT-1-TCR transgenic) one day prior to leukemia challenge, which consisted of either 10^5 TCF3/PBX1.3 or 10^5 TCF3/PBX1.3-OVA. 500 cGy irradiation on day *** One week after challenge, T cells were analyzed by flow cytometry for PD1 expression. Endogenous polyclonal T cells expressed increased PD1 compared to unexposed controls (TCF3/PBX1.3 $p<0.0007$, unpaired t test; TCF3/PBX1.3-OVA $p<0.0001$, unpaired t test), as did OVA-specific T cells (TCF3/PBX1.3 $p<0.0008$, unpaired t test; TCF3/PBX1.3-OVA $p<0.0001$, unpaired t test). Despite specificity for the OVA antigen, OT-1-TCR transgenic T cells had increased PD1 in the presence of TCF3/PBX1.3, suggesting a TCR-independent mechanism of PD1 upregulation.

Supp Figure 5

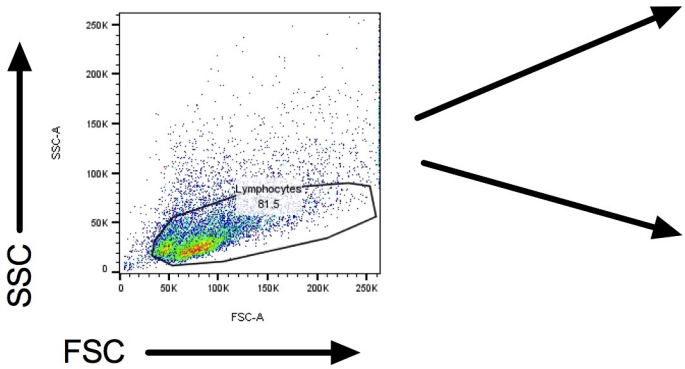




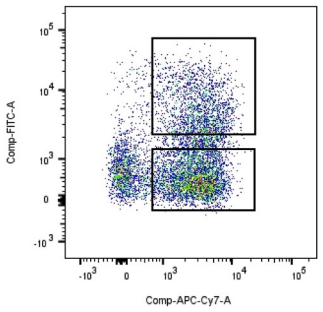
A) OT1 T cells from leukemia-bearing or non-leukemia bearing donors were washed once in 1XPBS and label with 5mM CellTrace Violet (Thermo Fisher Scientific Catalog # C34557) in warm Complete Mouse Medium (CMM) at a final cell concentration 5E6 cells/ml. T cells were incubated for 20 min at room temperature in dark. Labeled T cells were washed with 5 volumes the original staining volume of CMM and incubate for another 5 min. The cells were washed 2 more times in PBS. T cells were resuspended at 3E6/ml, 100ul was added to a 96-well plate, and co-incubated with equal number and equal volume of PBS washed TCF3/ PBX1-OVA target cells for 3 days followed by flowcytometric analysis. Each plot is from a different donor mouse. B) 3E5 TCF3-PBX1-OVA-GFP in 500 ul of CMM media were loaded into a 24-well plate (Poly-D-Lysine coated 24-well plate, Corning, Catalog # 354414) 12 hours before adding equal volume and number of OT-1 T cells from donors as indicated into each well in triplicates. The plate was scanned for the GFP fluorescent expression to monitor the cell apoptosis using an IncuCyte ZOOM® system every 6 hours in a duration of 70 hours.

Supp Figure 6

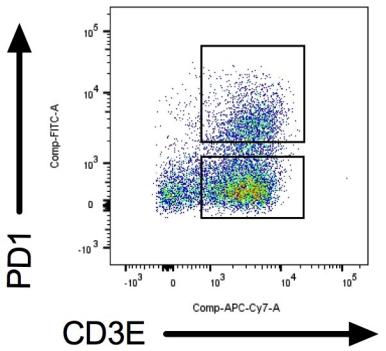
Sorting strategy:



Vaccinated Spleen



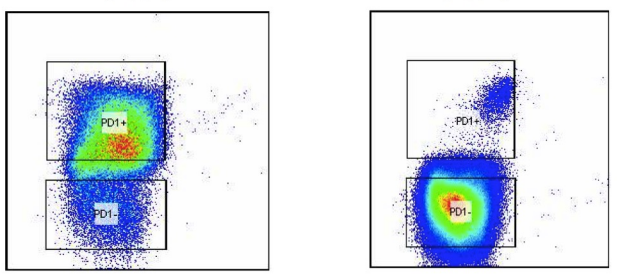
Leukemic Spleen

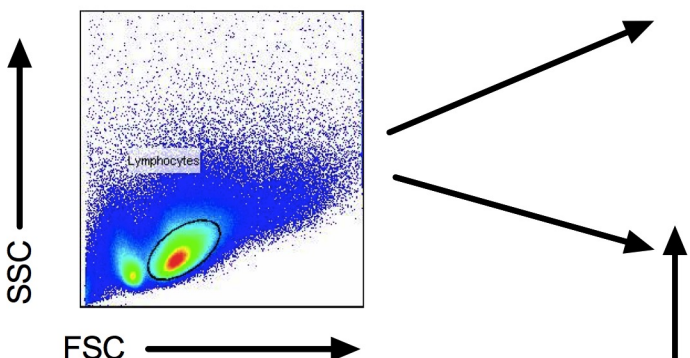


Post Sorting:

Vaccinated Spleen

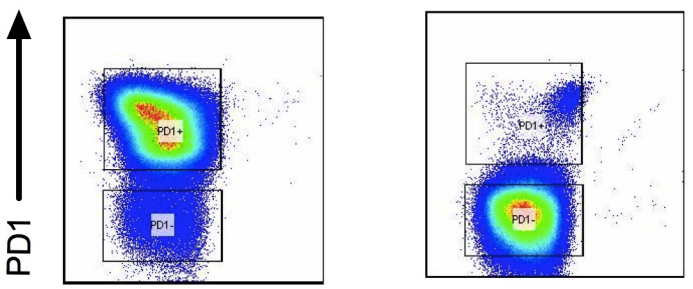
PD1⁺ **PD1-**





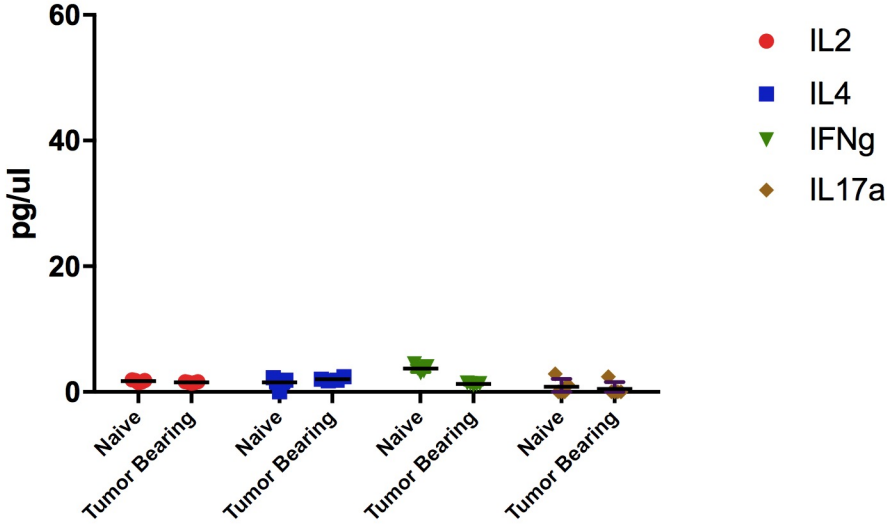
Leukemic Spleen

PD1⁺ **PD1-**

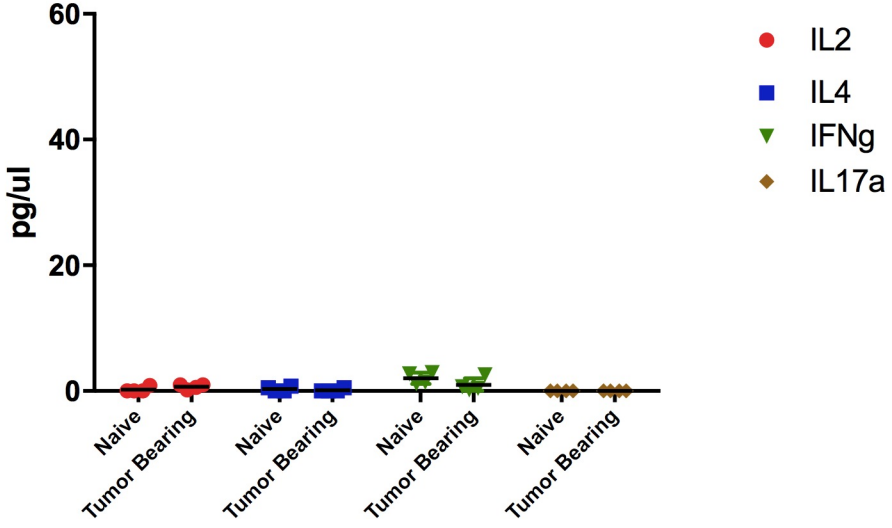


Supp Figure 7

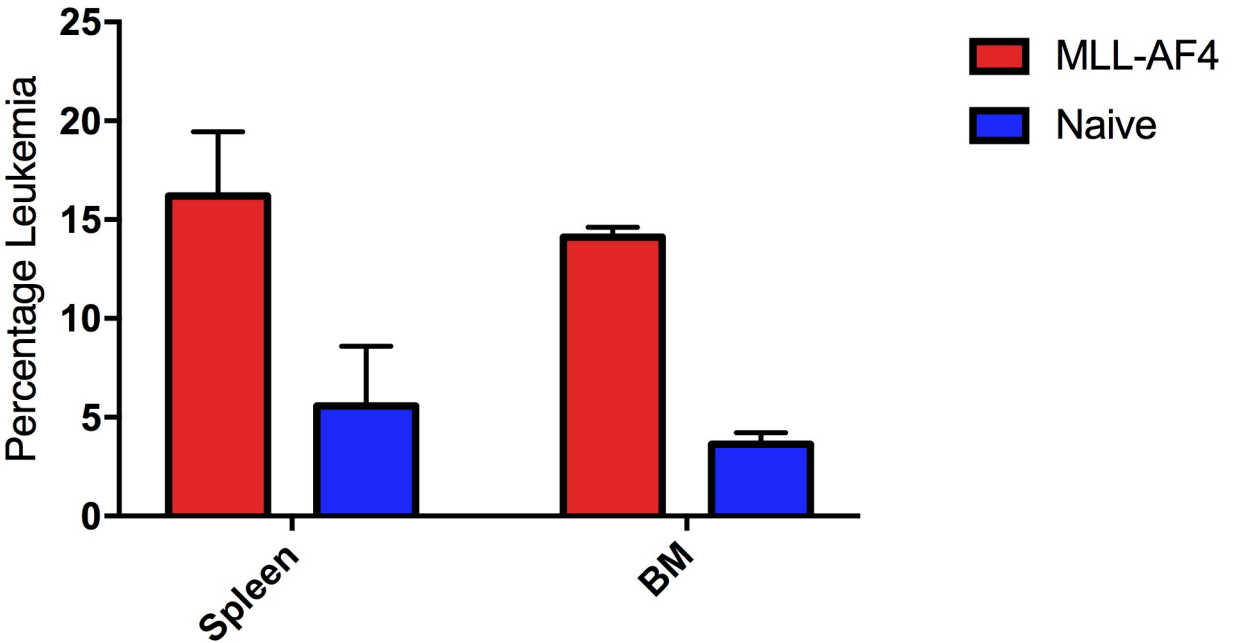
TCF3/PBX1



MLL-AF4

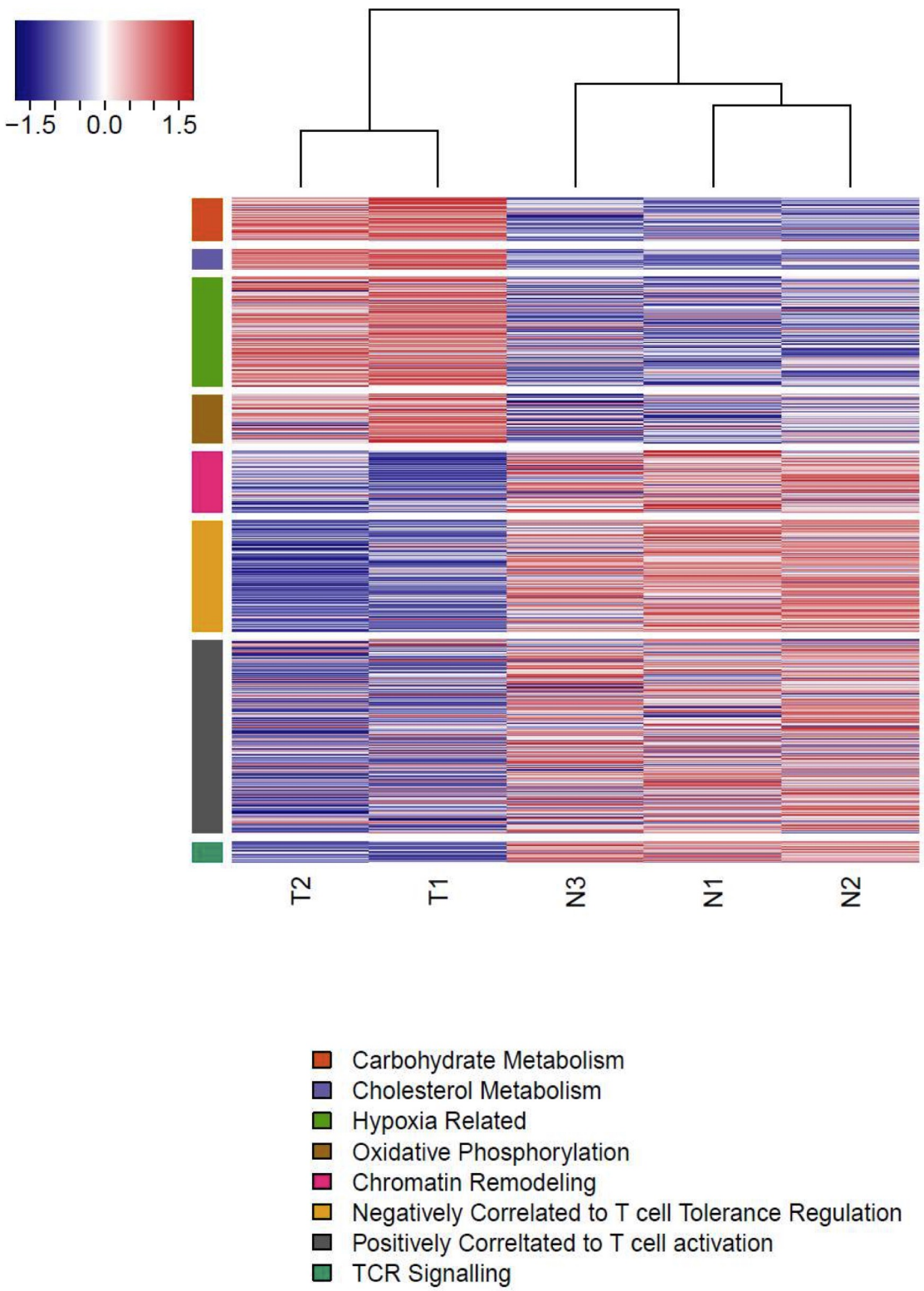


Supp Figure 8



Supplemental Figure 8: C57Bl/6 mice were irradiated (250cGy) and injected with $1x10^6$ MLL-AF4. Twenty one days later OT1 T cells were enriched from pooled splenocytes and bone marrow cells and used to generated CD19 CAR T cells. CART T cells were then injected into CD45.1 congenic mice bearing TCF3/PBX1.3. At day 14, bone marrow an spleen was analyzed for the presence of CD45.2+/CD19+ leukemia. $p<0.02$ by Mann-Whitney.

Supp Figure 9



Supp Figure 7