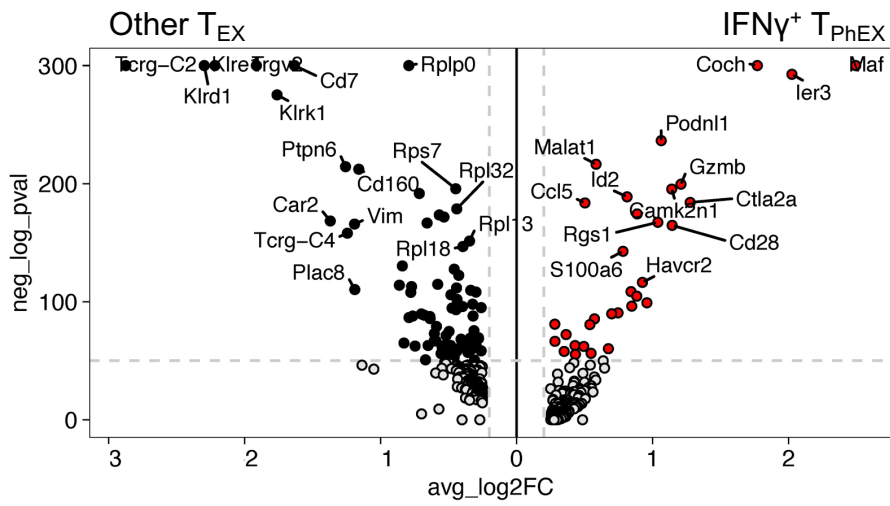
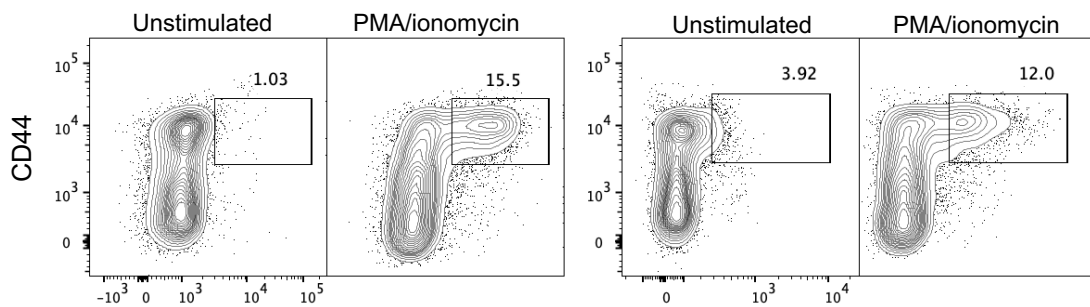
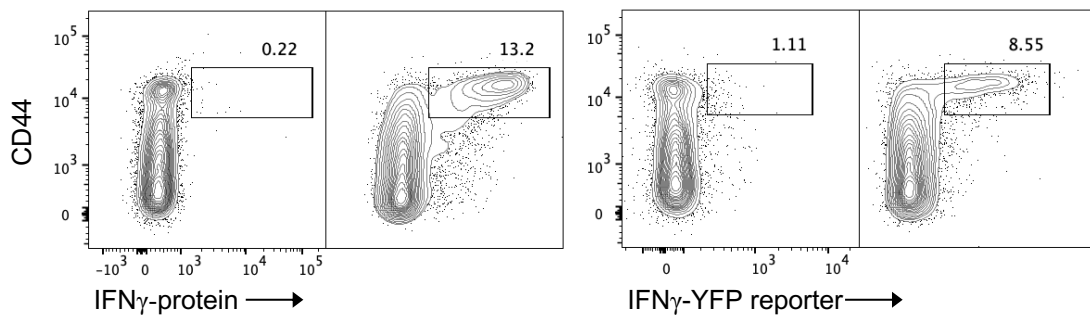
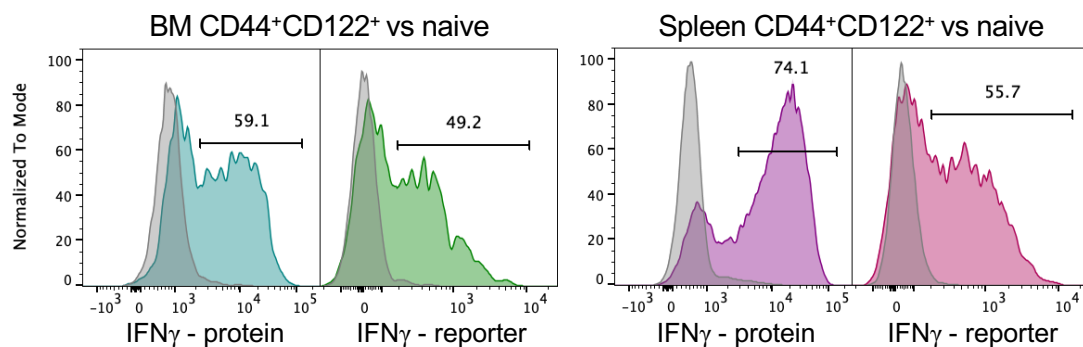


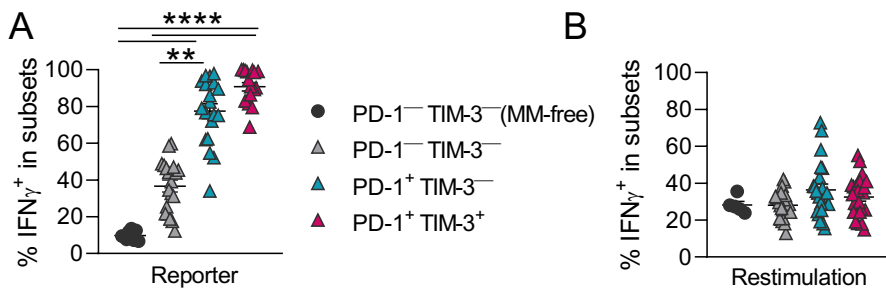
**Supplementary Figure 1: Myeloma generates a terminal  $T_{EX}$  subset with retained functional capacity in the bone marrow. (A)** Feature plots showing log gene expression of *Tox*, *Maf*, *Tcf7*, *Batf*, *Gzmb*, *Prf1* (perforin), *Gzma*, *Ifng*, *Cx3cr1*, *Havcr2* (TIM-3), *CD28*, *Ly6a* (Sca-1). **(B)** Co-embedding of single RNA sequencing data from Fig.1A and from LCMV infection from Giles et.al, *Nat Immunol*, 2022 colored by LCMV dataset. **(C)** Representative FACS plots showing TOX and TCF-1 expression on TpeX (maroon) identified by Ly108 and PD-1 expression at 7 weeks post-SCT. FMO controls for TOX and TCF-1 shown in grey.



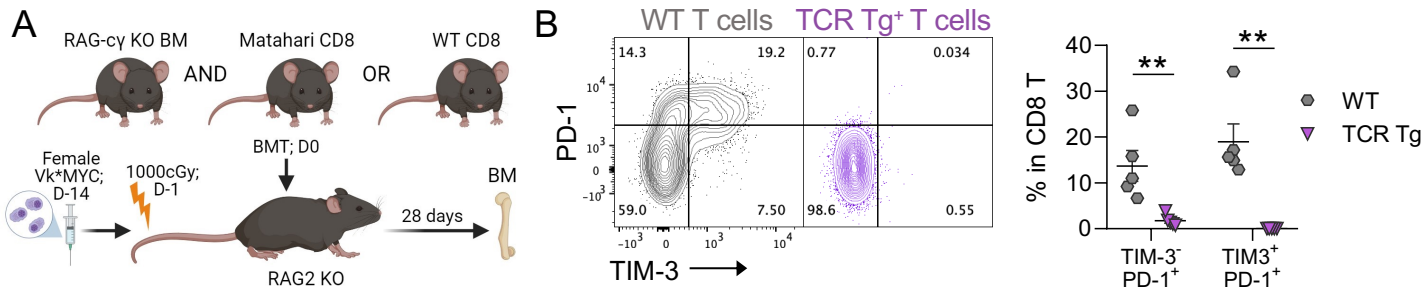
**Supplementary Figure 2: Differentially expressed genes between IFN $\gamma$ <sup>+</sup> T<sub>PHEX</sub> and other T<sub>EX</sub> clusters.** Volcano plot showing differentially expressed genes in the IFN $\gamma$ <sup>+</sup> T<sub>PHEX</sub> cluster vs other T<sub>EX</sub> clusters.

**A****BM****Spleen****B**

**Supplementary Figure 3: IFN $\gamma$  protein and YFP-reporter expression are both upregulated after PMA/ionomycin restimulation.** Spleen and bone marrow (BM) was harvested from IFN $\gamma$ -YFP reporter mice and cultured ex vivo for 4 hours in the presence of brefeldin A with or without PMA/ionomycin. Samples were split in half and IFN $\gamma$ -YFP was analyzed on unfixed cells and intracellular IFN $\gamma$ -protein was measured after fixation. **(A)** Contour flow cytometry plots of CD44 and IFN $\gamma$  expression within CD8 T cells from BM and spleen. **(B)** Histograms showing IFN $\gamma$  expression within memory (CD44<sup>+</sup>CD122<sup>+</sup>; colored) and naive (grey) CD8 T cells.

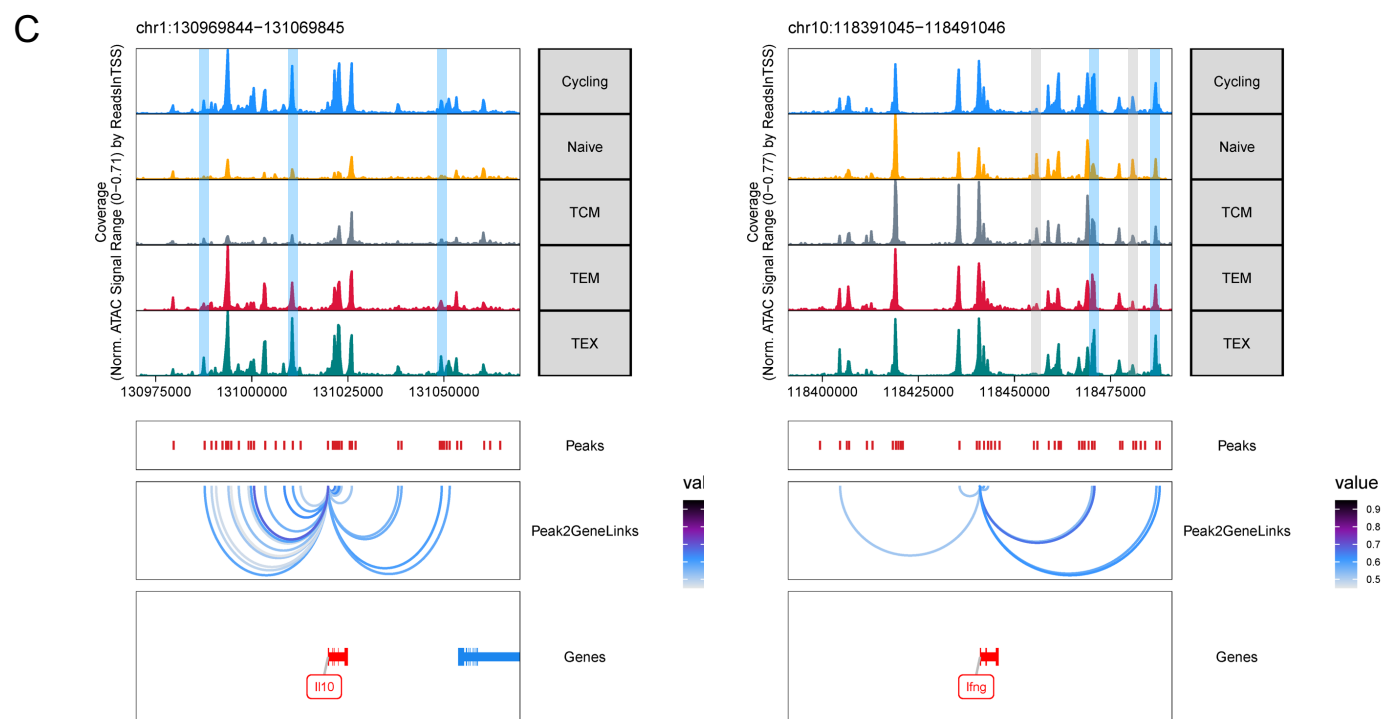
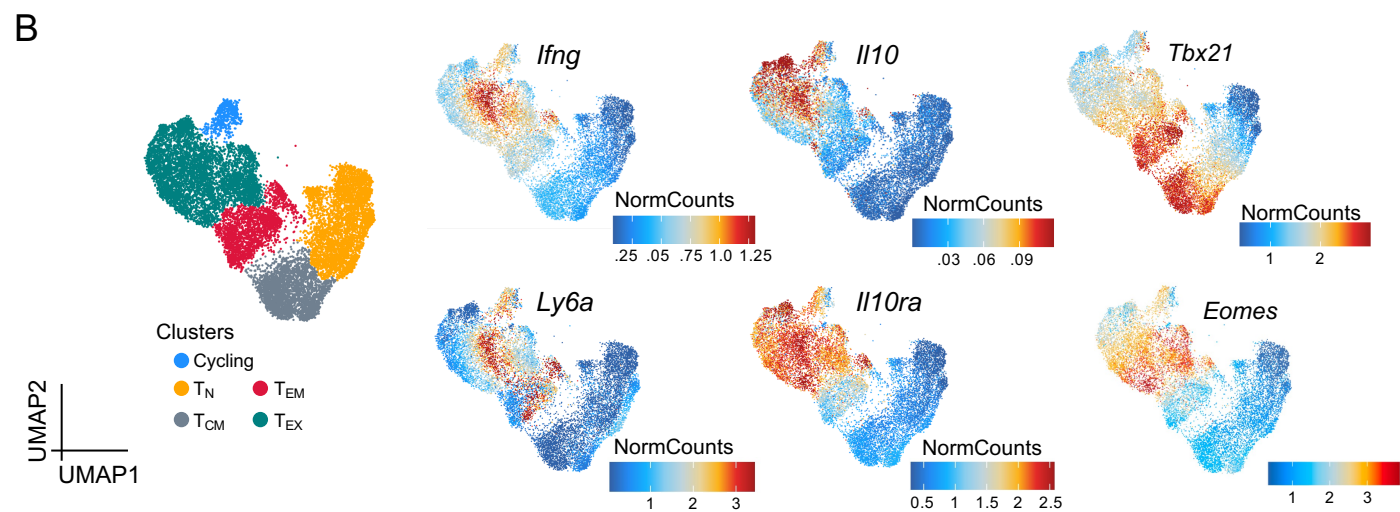
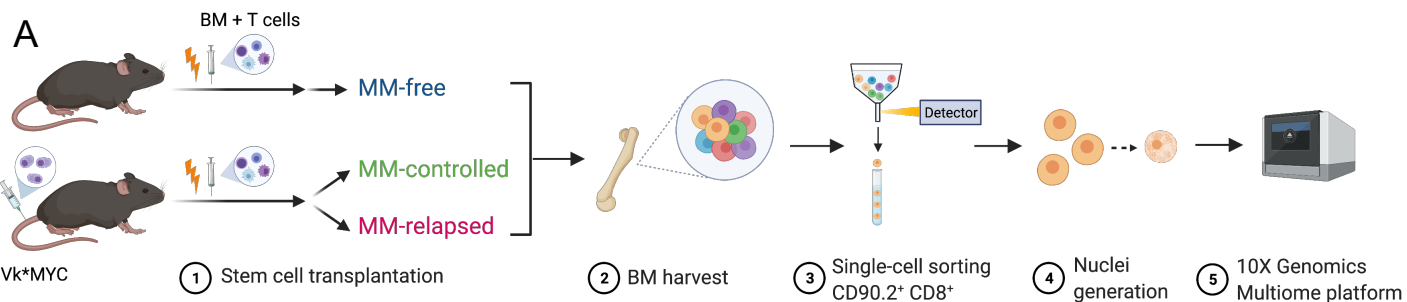


**Supplementary Figure 4: PD-1<sup>+</sup> T cells express more IFN $\gamma$  than PD-1<sup>-</sup> cells in vivo but not after ex vivo PMA/ionomycin restimulation.** Recipient C57Bl/6 x PTPRCA (CD45.1/CD45.2) mice were transplanted with HULK donor (IFN $\gamma$ -YFP x IL-10-GFP x FoxP3-RFP; CD45.2) grafts. Recipients were either never injected with tumor (MM-free) or progressive myeloma at 6-7 weeks post-transplant. BM was harvested for analysis of CD8 T cells using flow cytometry. **(A)** IFN $\gamma$ -YFP expression in unstimulated cells and **(B)** intracellular IFN $\gamma$ -protein expression after cells were cultured ex vivo for 4 hours in the presence of brefeldin A with PMA/ionomycin in subsets based on TIM-3 and PD-1 expression.

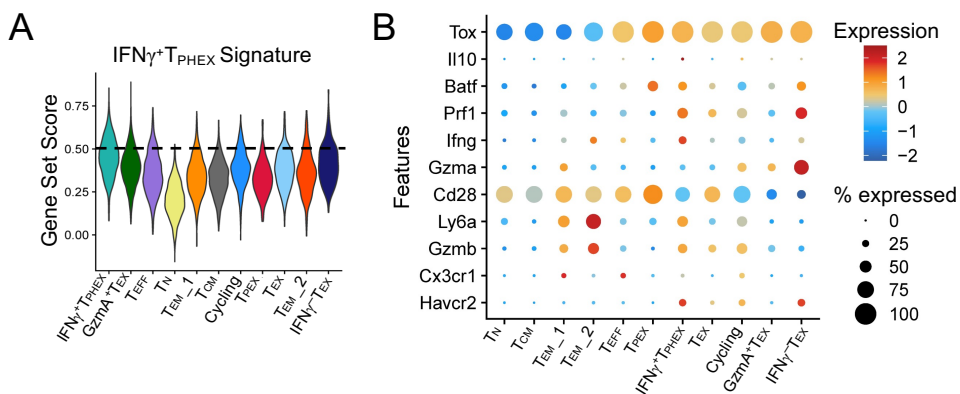
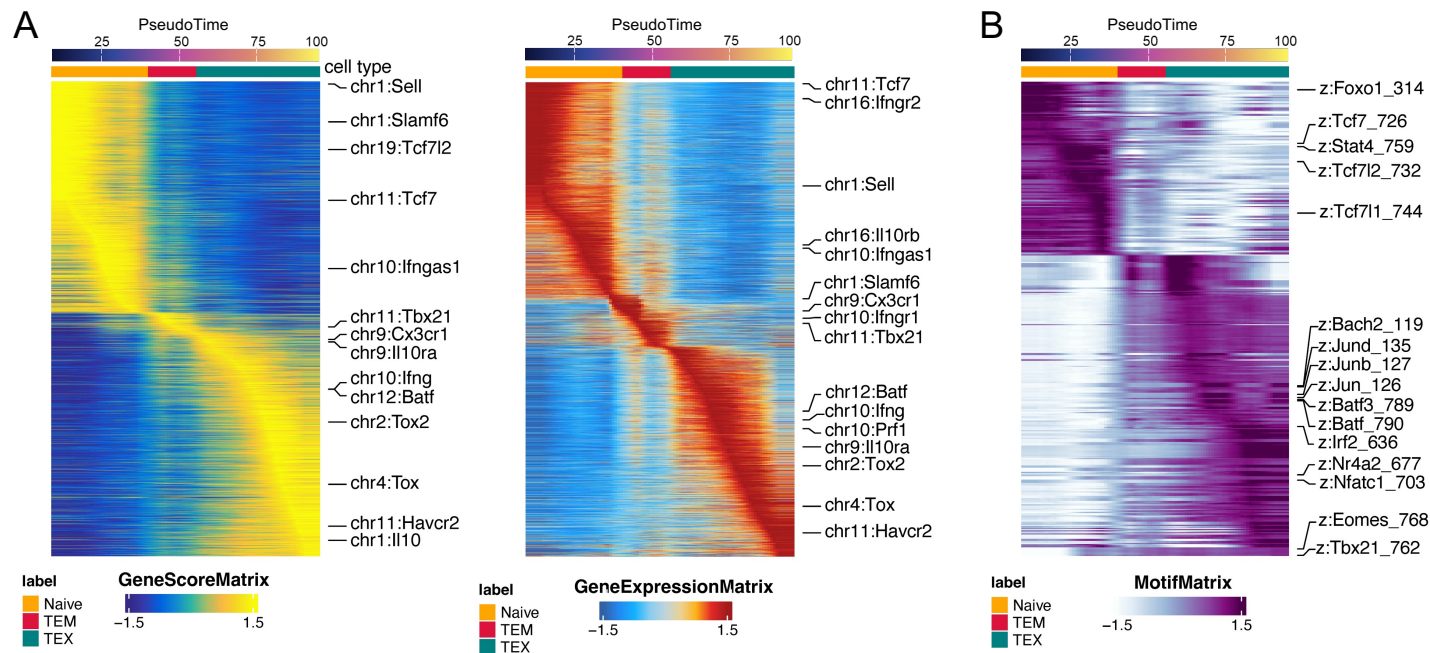


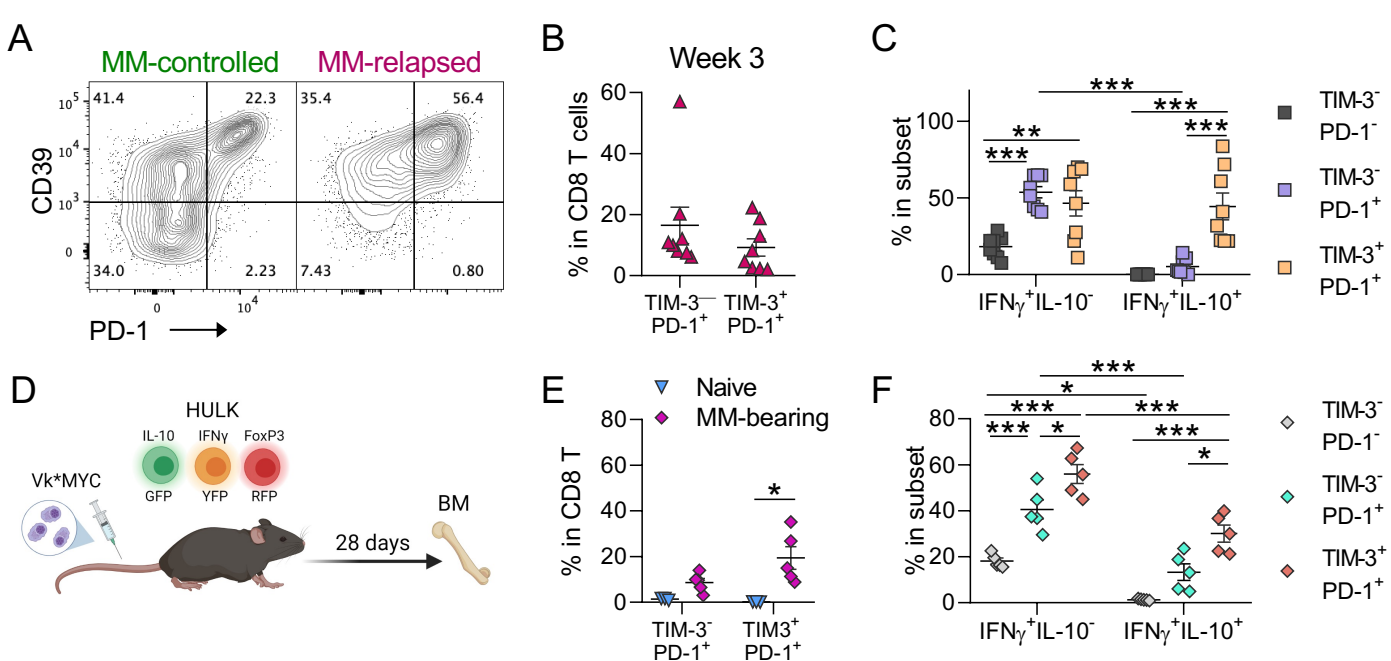
**Supplementary Figure 5: TIM-3 is not expressed on bystander CD8 T cells in mice with relapsed myeloma.** **(A)** Experimental design schematic. RAG2KO mice were injected with Vk12653 and, after 14 days, were transplanted with Rag2/Il2rg KO BM with either Matahari or WT CD8 T cells. **(B)** Representative flow cytometry plots and quantification of PD-1 and TIM-3 expression on WT and Matahari CD8 T cells in MM-bearing mice at 4 weeks post-transplant (n = 5). Mann-Whitney test. All data is mean  $\pm$  SEM. Each symbol represents an individual mouse. \*\* p<0.01.





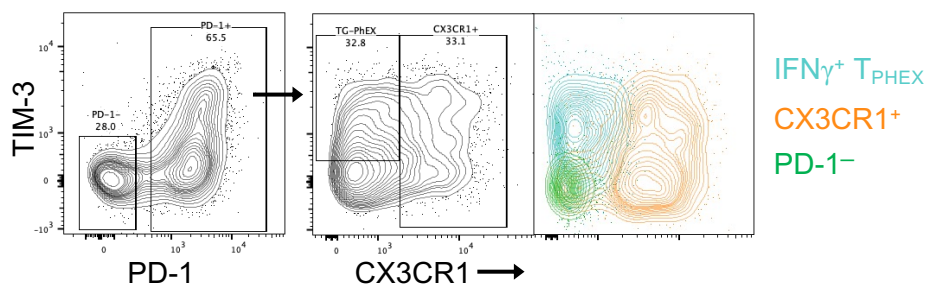
**Supplementary Figure 6: T<sub>EX</sub> cells have increased chromatin accessibility and gene expression in *Ifng* and *Il10* genes.** (A) Experimental design. BM was harvested at 6 weeks post-transplant from mice transplanted in the absence of tumor (MM-free) and from mice with controlled (MM-controlled) or progressive (MM-relapsed) myeloma. (B) WNN embedding with clusters from Figure 3A colored by clusters (left) and single cell gene expression of *Ifng*, *Il10*, *Tbx1*, *Ly6a*, *Il10ra* and *Eomes*. *Ifng* gene expression is also depicted in Fig.3E. (C) ATAC-seq coverage plot at the *Il10* locus (left) and *Ifng* locus (right). Peaks that are linked to gene expression uniquely gained or with increased accessibility in TEX are highlighted in blue. Peaks that are lost in TEX and are not correlated with gene expression are in grey.



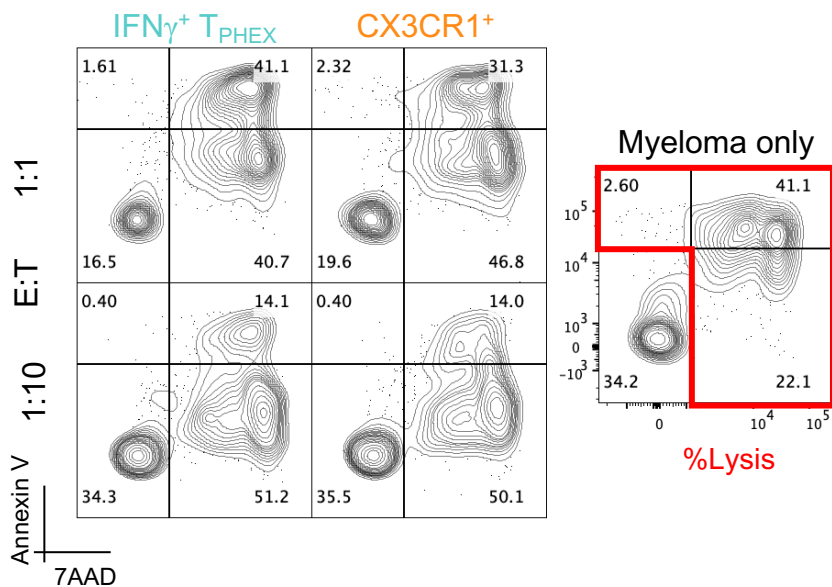


**Supplementary Figure 9: TIM-3<sup>+</sup> T cells are found in mice prior to frank relapse post-SCT and at relapse in the non-transplant setting.** **(A)** Representative FACS plots showing CD39 and PD-1 co-expression on CD8 T cells at 7 weeks post-SCT. **(B)** Frequency of TIM-3<sup>-</sup>PD-1<sup>+</sup> and TIM-3<sup>+</sup>PD-1<sup>+</sup> CD8 T-cells in bone marrow aspirates at 3 weeks post-transplant. Mann-Whitney test. **(C)** Frequency of IFN $\gamma$ <sup>+</sup>IL-10<sup>-</sup> and IFN $\gamma$ <sup>+</sup>IL-10<sup>+</sup> cells within subsets based on TIM-3 and PD-1 expression in MM-controlled mice ( $n = 8$ ). Two-Way ANOVA with Tukey's test. **(D)** Experimental design. **(E)** Frequency of TIM-3<sup>-</sup>PD-1<sup>+</sup> and TIM-3<sup>+</sup>PD-1<sup>+</sup> CD8 T-cells in naïve HULK mice ( $n = 3$ ) and HULK mice with high myeloma burden (MM-bearing;  $n = 5$ ). Two-Way ANOVA with Tukey's test. **(F)** Frequency of IFN $\gamma$ <sup>+</sup>IL-10<sup>-</sup> and IFN $\gamma$ <sup>+</sup>IL-10<sup>+</sup> cells within subsets based on TIM-3 and PD-1 expression in MM-bearing mice ( $n = 5$ ). Two-Way ANOVA with Tukey's test. Mann-Whitney test. All data is mean  $\pm$  SEM. Each symbol represents an individual mouse. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

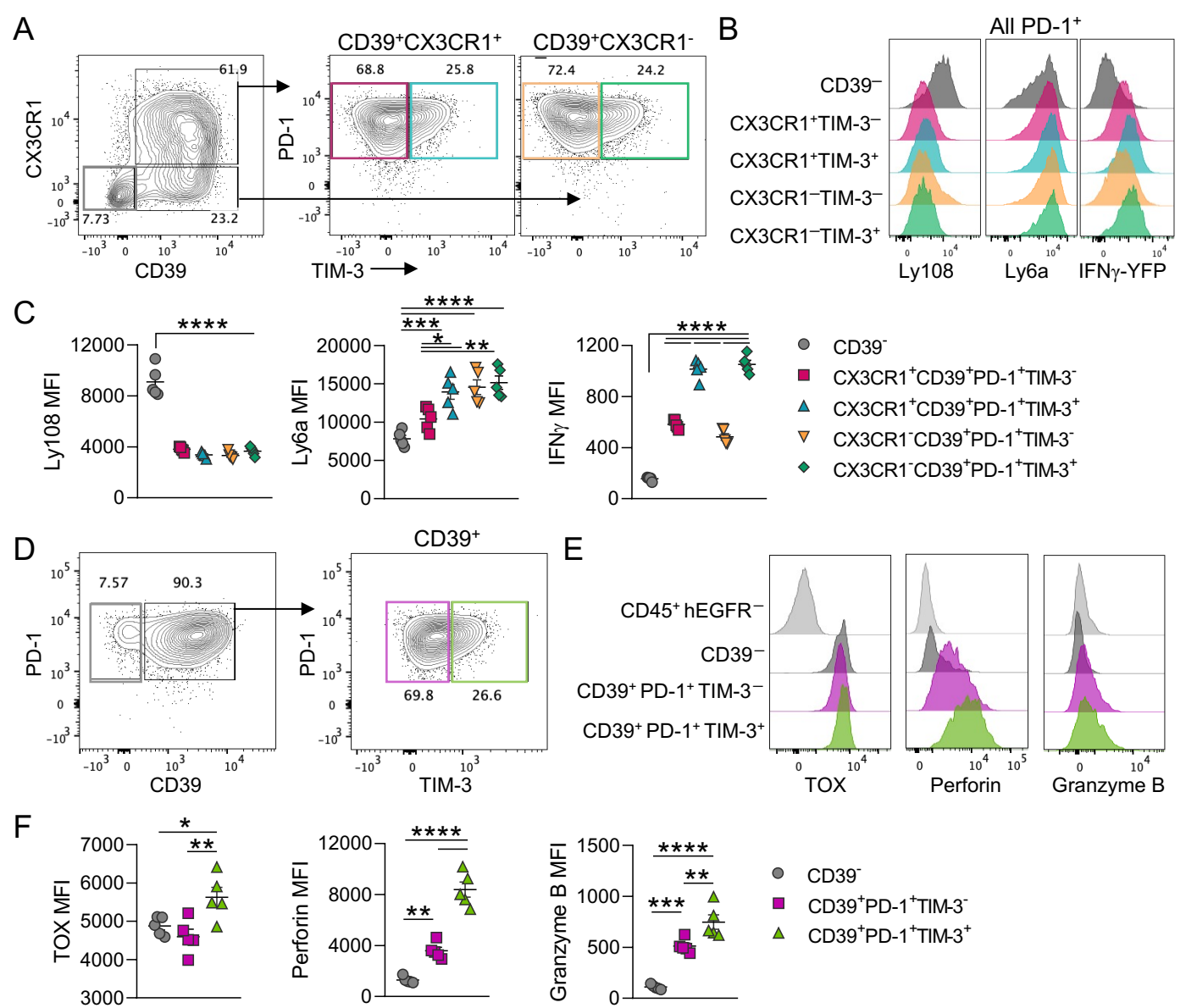
A



B

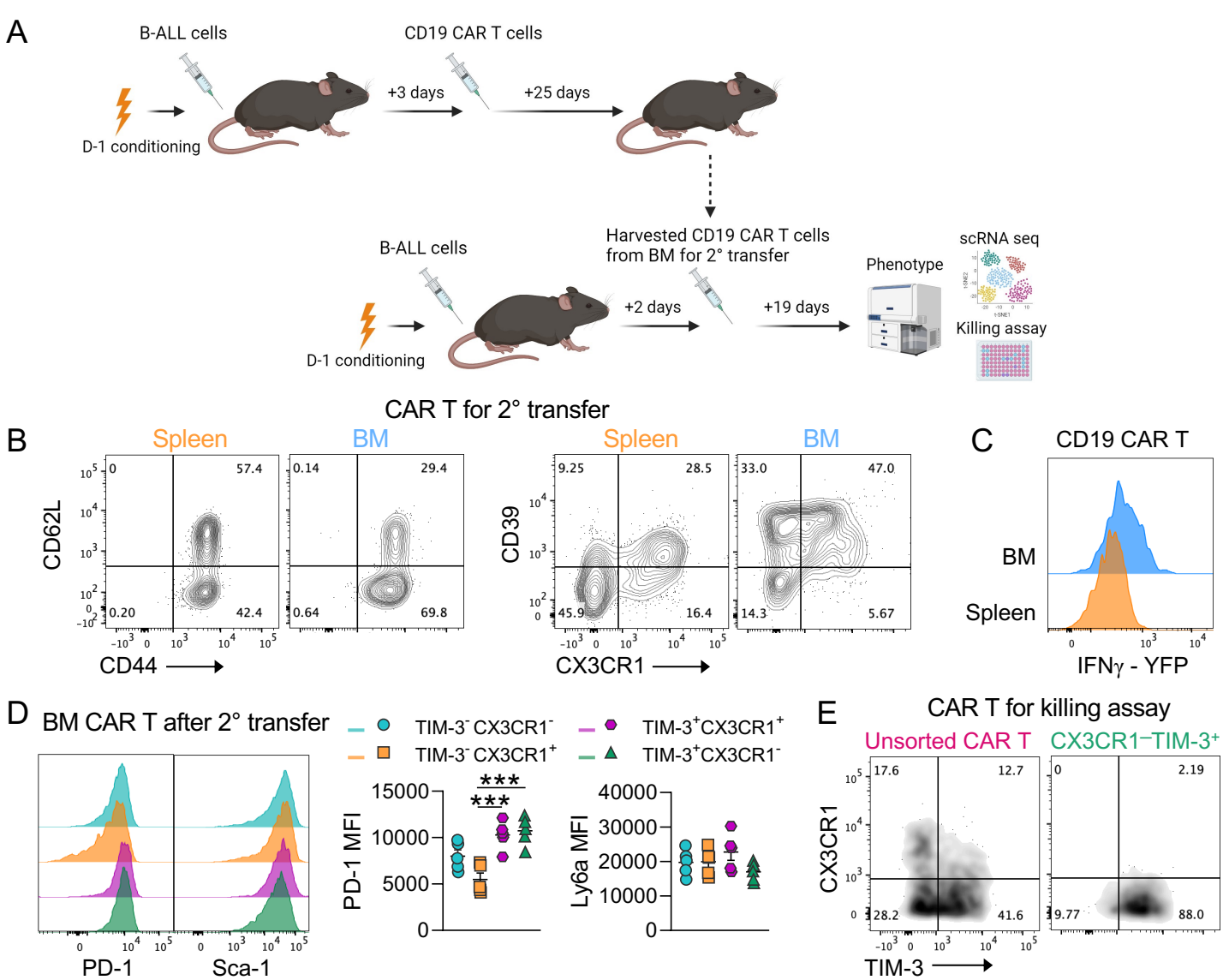


**Supplementary Figure 10: Ex vivo killing assay demonstrates potent anti-myeloma activity of IFN $\gamma$ <sup>+</sup> T<sub>PHEX</sub>.** (A) Contour plots depicting gating strategy for sort purification of PD-1<sup>+</sup>TIM-3<sup>+</sup>CX3CR1<sup>-</sup> (IFN $\gamma$ <sup>+</sup> T<sub>PHEX</sub>), CX3CR1<sup>+</sup>, and PD-1<sup>-</sup> T cells. (B) Representative flow cytometry plots showing Annexin V and 7AAD staining after T cell subsets were cultured with myeloma cells for 17 hours. % Killing (7AAD<sup>+</sup>) was calculated using a viability baseline from myeloma only wells.

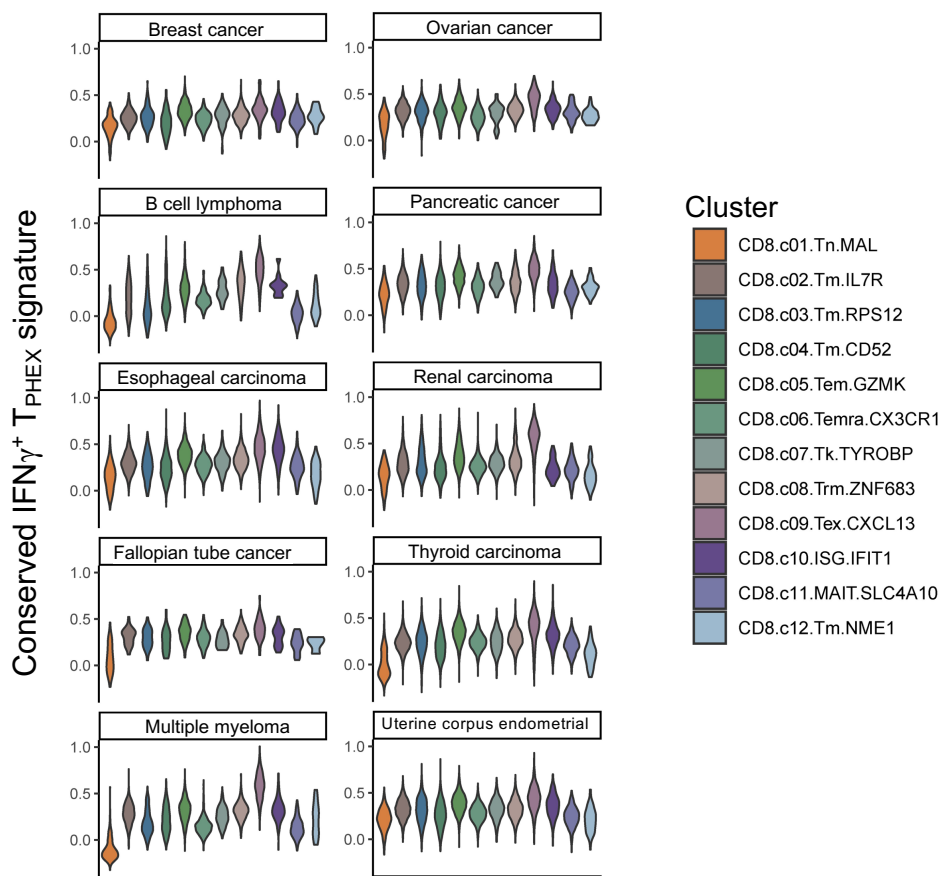


**Supplementary Figure 11: CD19 CAR T cells have an IFN $\gamma$ <sup>+</sup> T<sub>PHEx</sub> signature in the bone marrow.** C57Bl/6 mice bearing a CD19<sup>+</sup> B cell acute lymphoblastic leukemia were injected with murine CD19 CAR T cells. Mice that relapsed were harvested on D+9 after CAR T cell transfer and BM CAR T cells (human eGFR<sup>+</sup>) were analyzed. **(A)** Representative flow cytometry plot of T-cell subset gating strategy based on CX3CR1, CD39, TIM-3 and PD-1 expression. **(B)** Representative histograms showing expression of Ly108, Ly6a and IFN $\gamma$ -YFP MFI on T cell subsets. **(C)** Quantification of MFI of Ly108, Ly6a and IFN $\gamma$ -YFP on T cell subsets. **(D)** Representative FACS plots of gating strategy based on PD-1, CD39 and TIM-3 expression. **(E)** Histograms showing TOX, perforin and granzyme B expression in T cell subsets, including a CD45<sup>+</sup>hEGFR<sup>-</sup> control to account for background staining. **(F)** Quantification of MFI for TOX, perforin and granzyme B. All data is mean  $\pm$  SEM. Each symbol represents an individual mouse. One-way ANOVA with Tukey's test. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , \*\*\*\*  $p < 0.0001$ .





**Supplementary Data 12: CD19 CAR T cells develop an exhausted phenotype after chronic antigen exposure in vivo.** (A) Experimental design. B6.mice bearing a CD19<sup>+</sup> B cell acute lymphoblastic leukemia were injected with murine CD19 CAR T cells (HULK; B6.background). After 25 days, CAR T cells from BM were harvested and transferred to secondary B-ALL-bearing recipients (2° transfer). BM CAR T cells (human eGFR<sup>+</sup>) were harvested 19 days later and analyzed. (B) Representative flow cytometry plot of CAR T cell phenotype (left: CD44 and CD62L; right: CX3CR1 and CD39) in spleen and BM prior to 2° transfer. (C) Representative histograms showing expression IFN $\gamma$ -YFP in CAR T cells prior to 2° transfer. (D) Representative histograms and quantification of PD-1 and Ly6a MFI on CD19 CAR T cell subsets based on TIM-3 and CX3CR1 expression in 2° recipients ( $n = 5$ ). (E) Flow cytometry density plots depicting CX3CR1 and TIM-3 expression on CD8 CAR T cells from BM of 2° recipients before (left) and after (right) sorting of the CX3CR1-TIM-3<sup>+</sup> subset for an ex vivo killing assay. All data is mean  $\pm$  SEM. Each symbol represents an individual mouse. One-way ANOVA with Tukey's test. \*\*\*  $p < 0.001$ .



**Supplementary Figure 13: Expression of a conserved IFN $\gamma$ <sup>+</sup> T<sub>PHEX</sub> signature across CD8 T cell clusters in pan cancer dataset.** Violin plots showing gene scores for the conserved IFN $\gamma$ <sup>+</sup> T<sub>PHEX</sub> signature across CD8 T cell clusters, stratified by cancer type in the Zheng, Qin, Si et al. *Science*, 2021 dataset