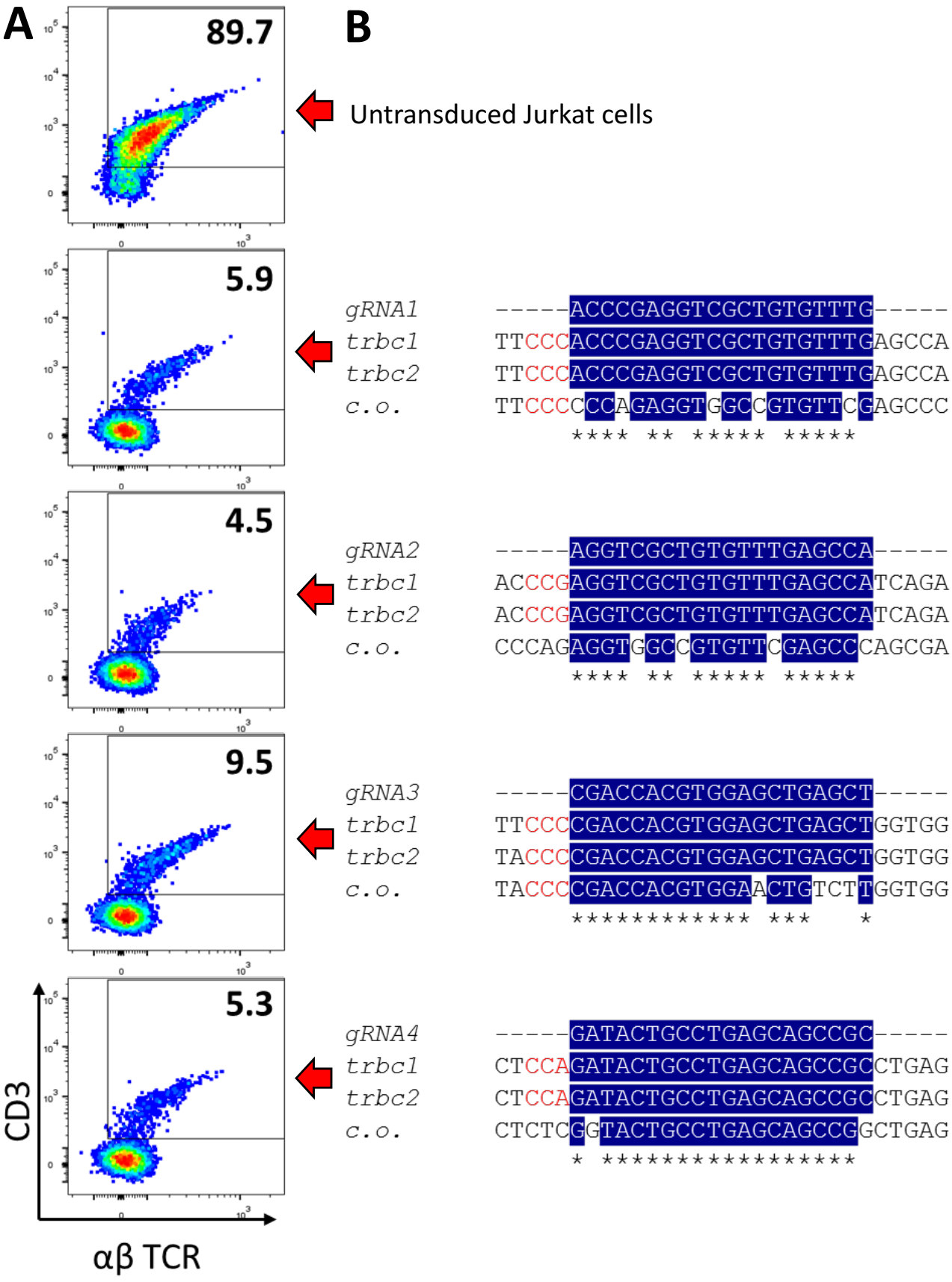
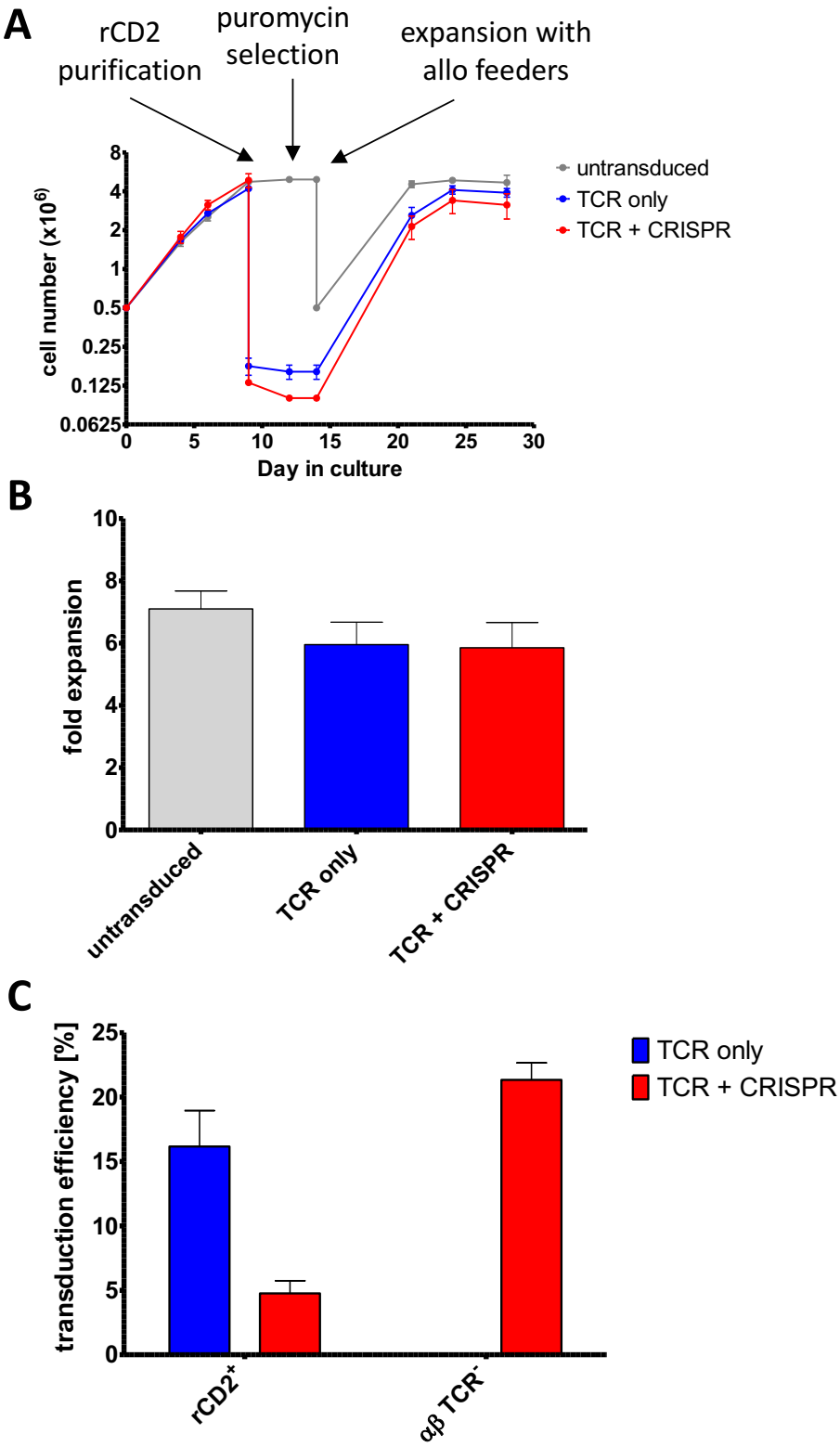
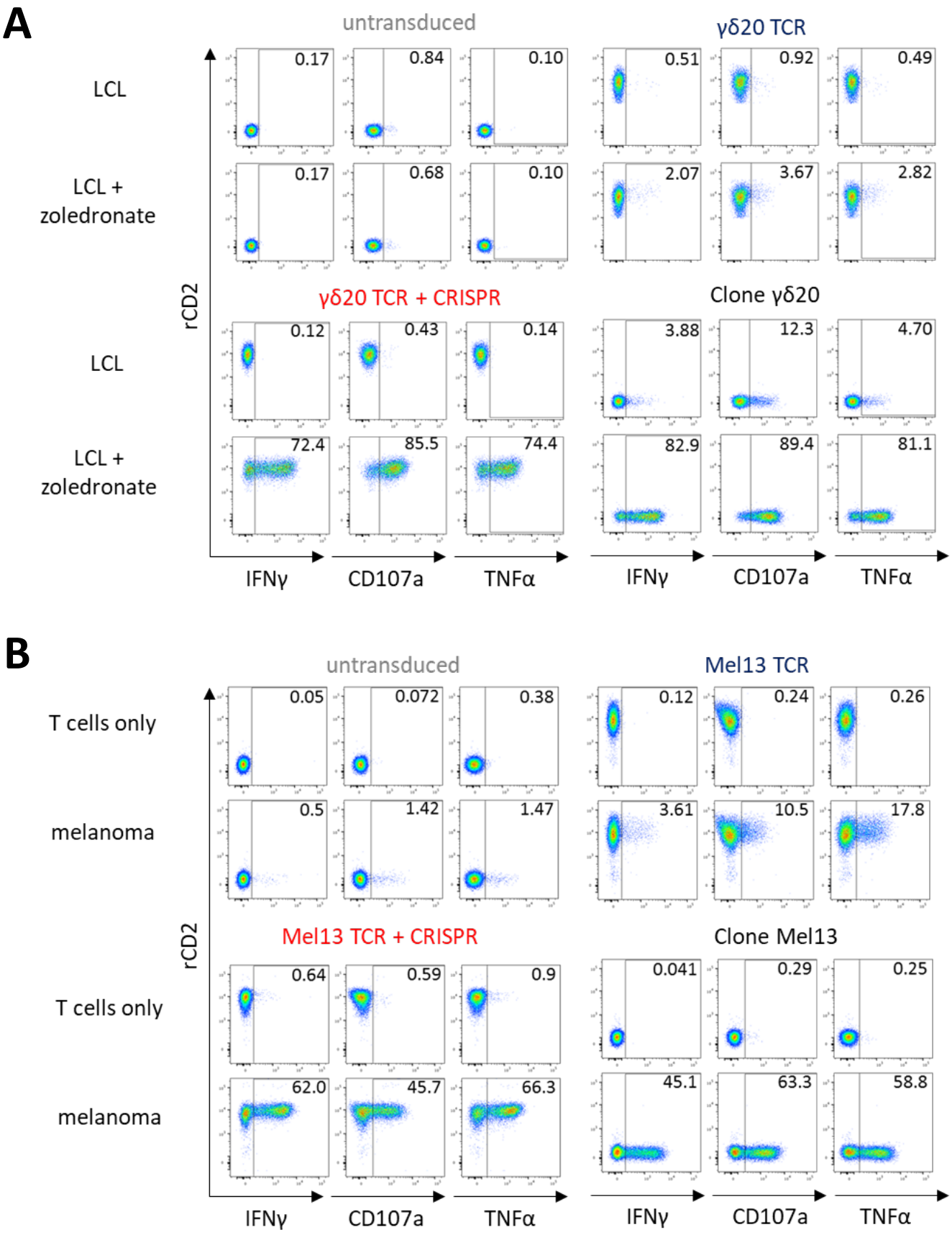


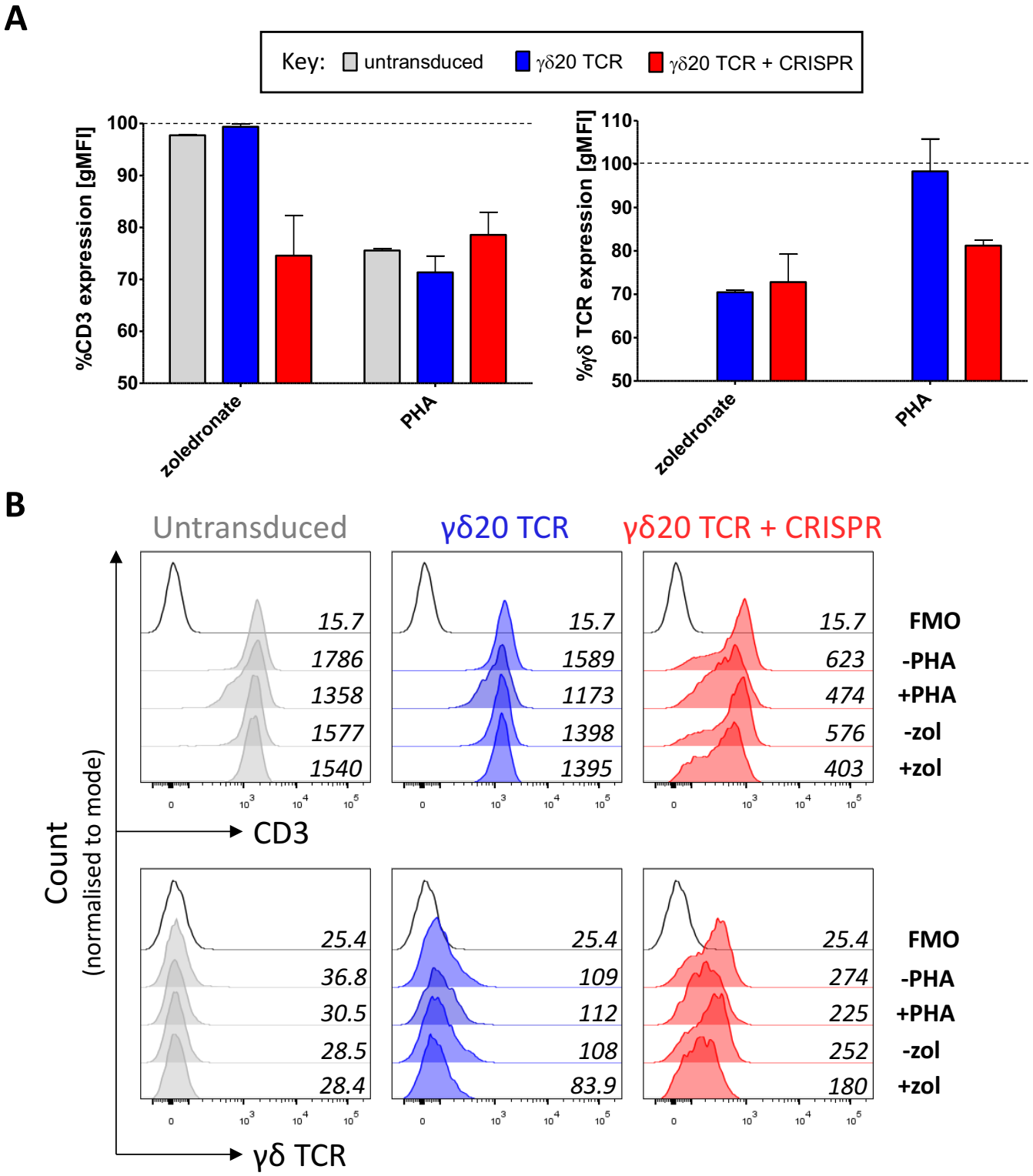
Supplementary Figure 1. Design and validation of gRNAs targeting *trbc* loci. (**A**) Expression of the endogenous αβ TCR-CD3 complex on T-cell leukemia line Jurkat E6.1, untransduced (wt) and transduced with 4 gRNAs targeting *trbc* gene segments (gRNA1-4). Numbers on dot plots indicate percentage of cells expressing αβ TCR-CD3 complex. (**B**) Alignment of tested gRNAs to *trbc1*, *trbc2* and codon-optimized (c.o.) *trbc* sequence used in transgenic αβ TCRs. Protospacer adjacent motif (PAM) is shown in red while blue highlight indicates nucleotide match between gRNA and *trbc*. gRNA sequence is shown as reverse complement in all four cases.



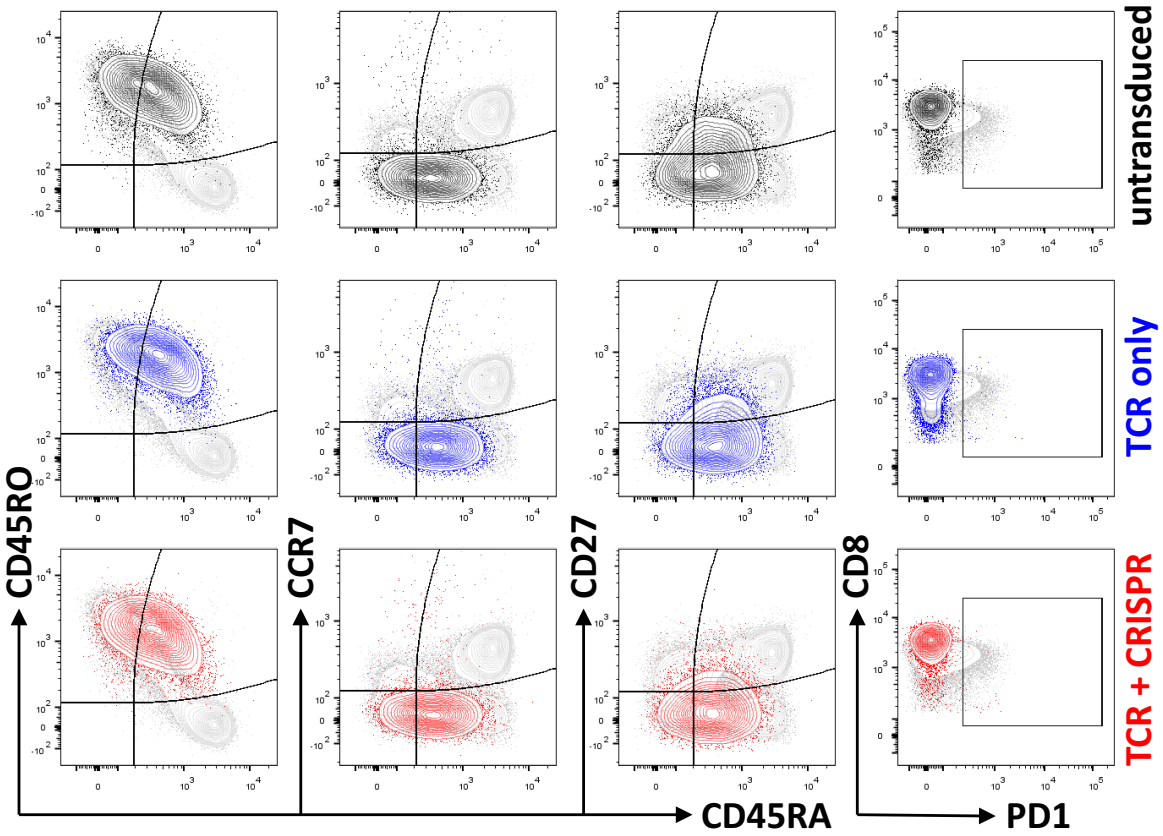
Supplementary Figure 2. The kinetics and efficacy of lentiviral transduction of primary T-cells. (**A**) T-cells (5×10^5 cells per condition) were isolated on day 0 and plated with CD3/CD28 beads. The cells were transduced with TCR only or TCR and CRISPR lentiviruses on day 1, and cultured until day 9, followed by magnetic pullout of $rCD2^+$ cells which were then plated with 2 µg/ml puromycin (TCR+CRISPR only). Puromycin selection was carried out until day 14 when the transduced cells (or a 5×10^5 untransduced cells) were expanded in presence of allogeneic irradiated feeders and PHA. The cells were counted every 2-4 days by trypan blue exclusion. (**B**) Following the initial selection, transduced or untransduced T-cells were expanded with allogeneic feeders and PHA every 14-28 days. The viable cells were counted after the expansion. (**C**) γδ20 transduced T-cells (with or without TCR-β CRISPR) were stained for rCD2 and αβ TCR on day 9 after isolation from peripheral blood (prior to any form of selection). Mean and standard deviation from three donors are shown.



Supplementary Figure 3. Functional response of TCR-transduced CD8⁺ T-cells shown as individual functions (IFNγ, CD107a, TNFα). The response of (**A**) γδ20 TCR-transduced CD8⁺ T-cells to LCL or LCL pre-incubated with zoledronate or (**B**) Mel13 TCR-transduced T-cells to a HLA-A2⁺ melanoma. Only viable CD3⁺ cells were included in the analysis while the gates for cells positive for a given function were set based on appropriate fluorescence minus one and biological controls. Numbers on dot plots refer to percentage of cells positive for a given function. Representative data from three donors and two independent experiments are shown.

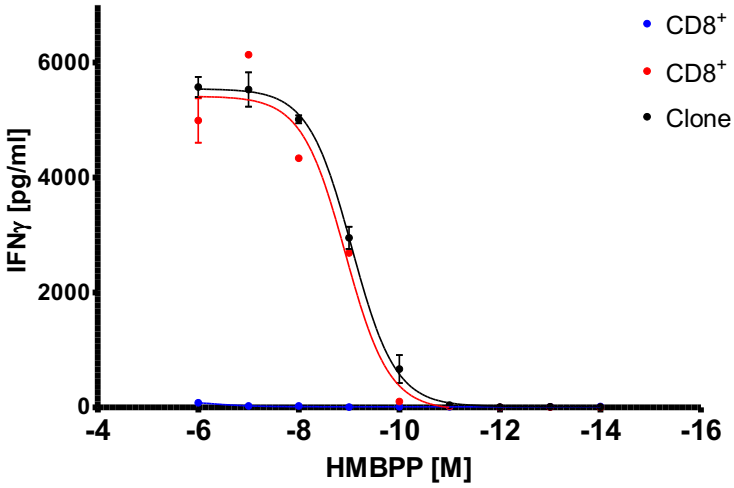


Supplementary Figure 4. TCR-CD3 complex undergoes downregulation upon antigen stimulation. γδ20 TCR transduced CD8⁺ T-cells and untransduced control cells were activated for 5 h with pan T-cell stimulus PHA or γδ20 TCR specific stimulus zoledronate (zol; in presence of an LCL cell line). Following incubation, cells were stained for CD3 and γδ TCR expression. Gating was performed to include only the viable T-cells. At least 10,000 viable events were acquired. (**A**) CD3 and TCR downregulation was calculated by dividing the geometric MFI of staining of stimulated by unstimulated sample, after subtracting FMO values. Mean and standard deviation is shown. (**B**) Representative staining for CD3 and γδ TCR. Numbers on histograms correspond to geometric MFI of staining. FMO, fluorescence minus one.



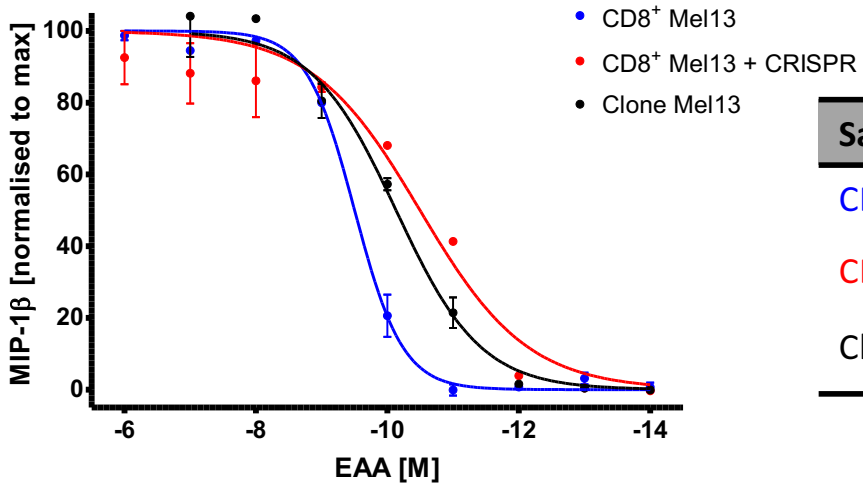
Supplementary Figure 5. Phenotypic profile of untransduced and transduced T-cells. Untransduced (top row), single transduced (TCR only; middle row) or double transduced (TCR+CRISPR; bottom row) T-cells were stained for phenotypic markers CD45RO, CD45RA, CCR7, CD27 and PD-1. Freshly isolated PBMC were stained in parallel as a control (shown in grey). Lymphocytes were gated based on scatter properties, followed by exclusion of doublets and dead cells. Only $CD3^{+}CD8^{+}$ cells were taken for further analysis.

A



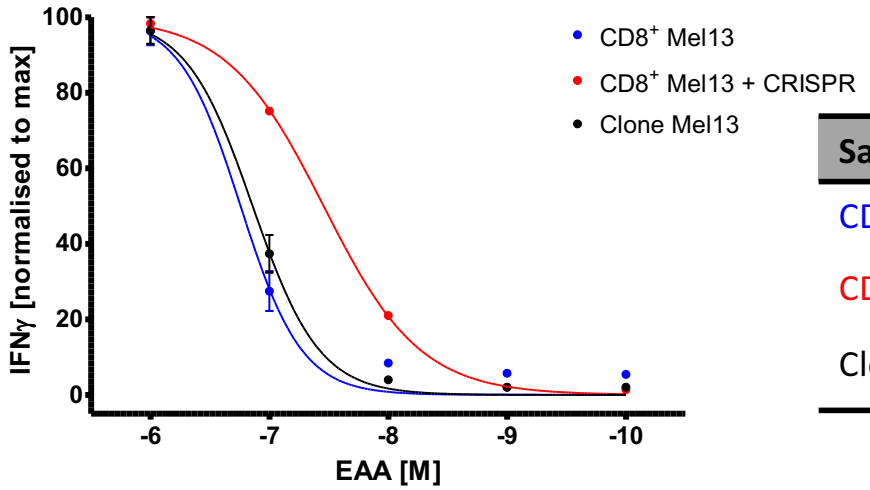
Sample	EC_{50} [M]
$CD8^+$ γδ20	ND
$CD8^+$ γδ20 + CRISPR	1.2×10^{-9}
Clone γδ20	8.8×10^{-10}

B



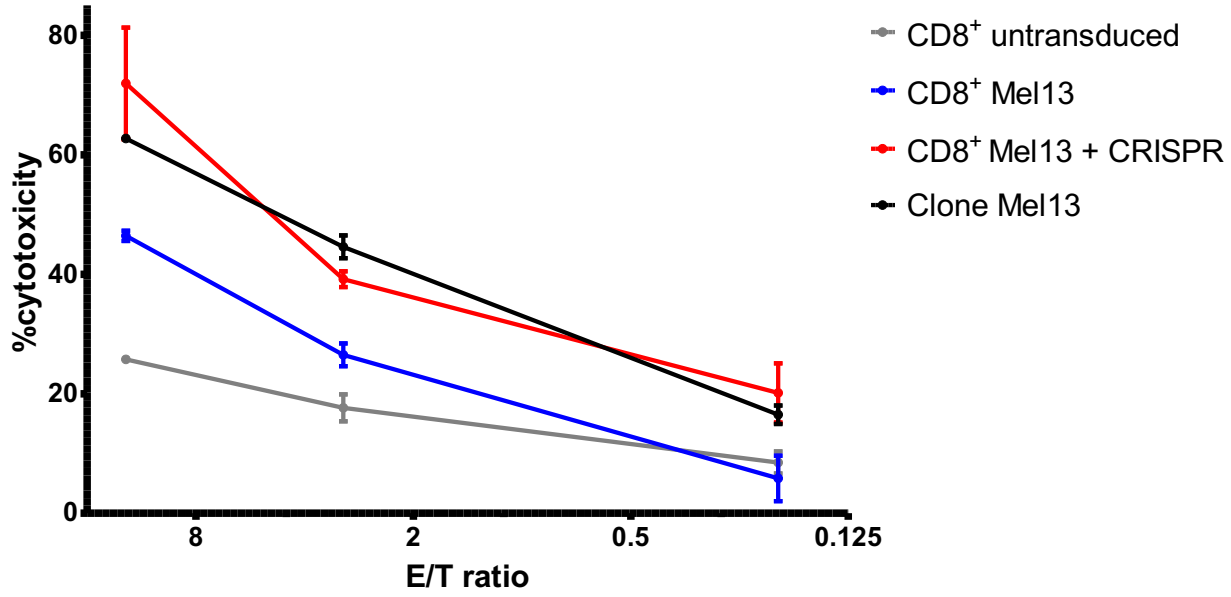
Sample	EC_{50} [M]
$CD8^+$ Mel13	3.1×10^{-10}
$CD8^+$ Mel13 + CRISPR	3.2×10^{-11}
Clone Mel13	7.3×10^{-10}

C

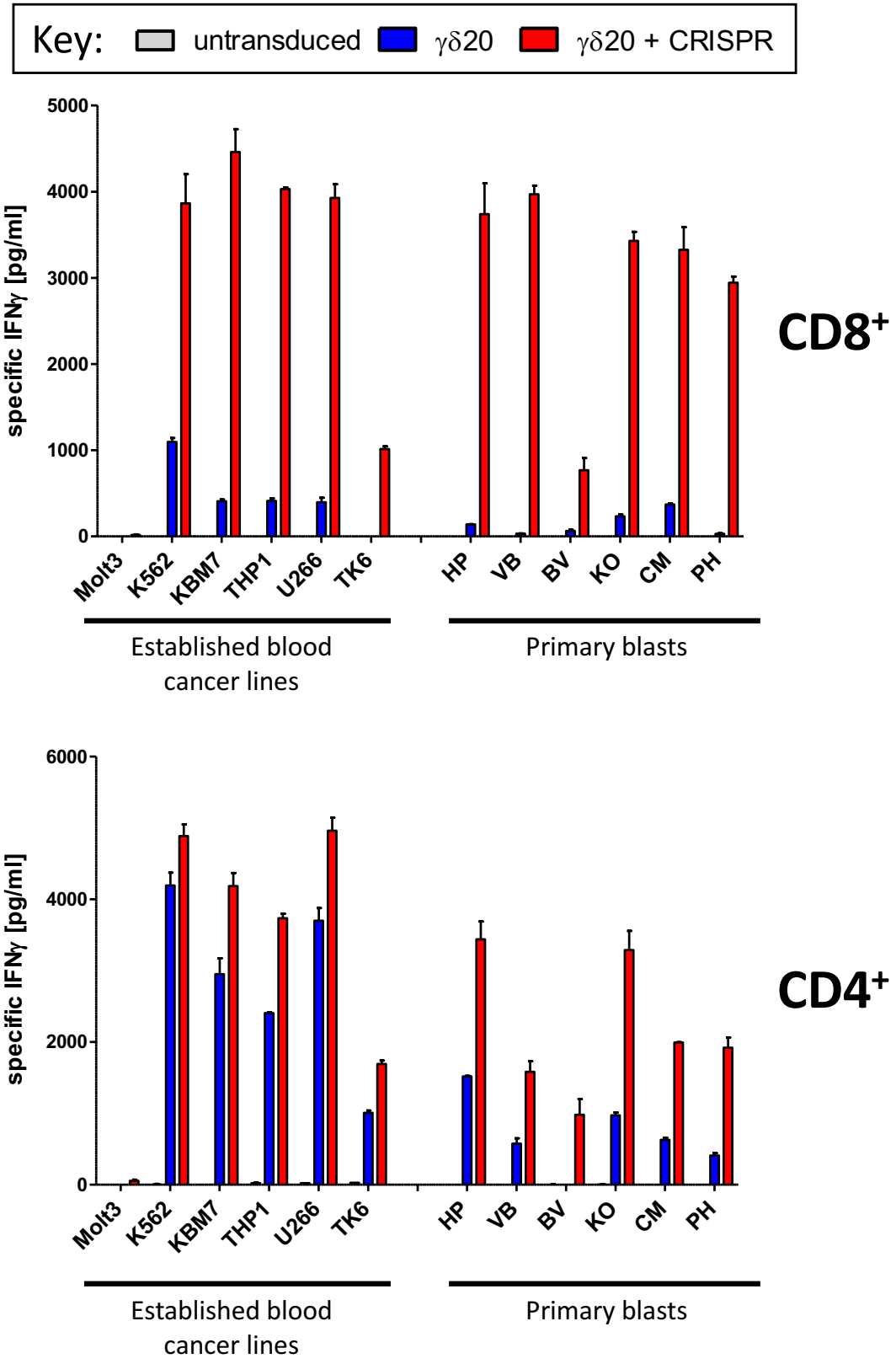


Sample	EC_{50} [M]
$CD8^+$ Mel13	1.7×10^{-7}
$CD8^+$ Mel13 + CRISPR	3.5×10^{-8}
Clone Mel13	1.4×10^{-7}

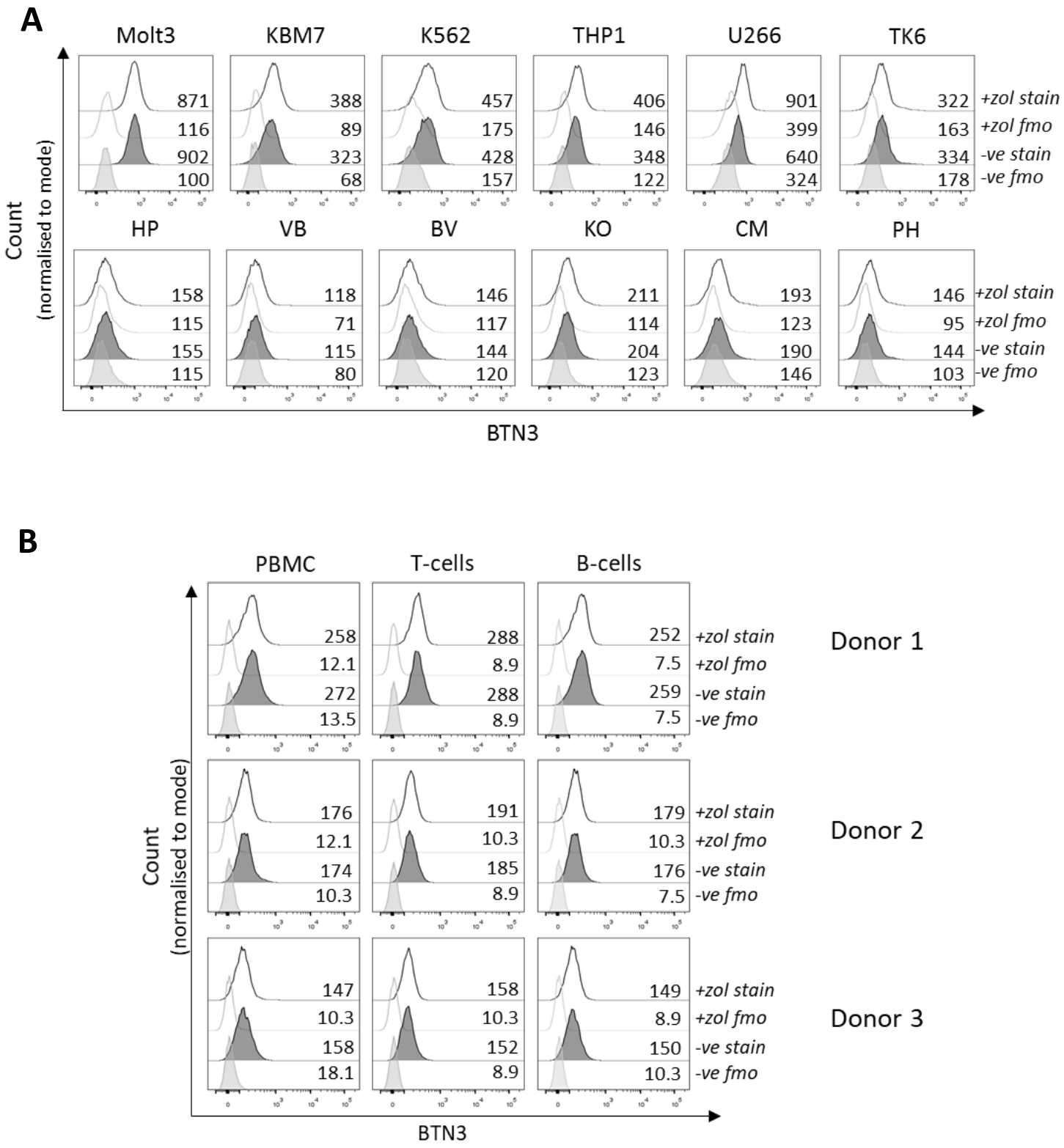
Supplementary Figure 6. Antigen sensitivity of γδ20 and Mel13 TCR transduced $CD8^+$ T-cells. (**A**) The sensitivity to the titrated antigen HMBPP was measured by IFNγ production after overnight incubation with the antigen and T2 cells used for antigen presentation. IFNγ concentration was normalized by subtracting the values of unstimulated cells. (**B**) The sensitivity to the titrated peptide EAAGIGILTV was measured by MIP-1β or (**C**) IFNγ production after overnight incubation with the antigen and T2 cells used for antigen presentation. The EC_{50} values were calculated in GraphPad Prism software by non-linear regression curve fitting.



Supplementary Figure 7. Mel13 TCR+CRISPR CD8+ T-cells show stronger cytotoxicity towards a melanoma cell line than Mel13 TCR-only transduced cells. 4 h cytotoxicity of transduced $CD8^+$ cells, as well as parental Mel13 T-cell clone, against a HLA-$A2^+$ melanoma cell line. Representative data are shown from two donors tested in two experiments carried out in duplicate.



Supplementary Figure 8. T-cells transduced with CRISPR replacement show a markedly stronger response to blood cancer lines than with standard transduction techniques. IFNγ secretion by transduced $CD8^+$ (top) or $CD4^+$ (bottom) T-cells after overnight co-incubation with a panel of established blood cancer lines of diverse lymphoid and myeloid origin, or patient-derived B ALL cells. Cancer cells were pre-incubated with zoledronate for 24 h before co-incubation with T-cells. IFNγ secretion was normalized by subtracting IFNγ produced by T-cells alone, and by cancer cells alone. No specific IFNγ secretion by T-cells was observed in absence of zoledronate pre-treatment. Representative data are shown from three donors and two experiments carried out in duplicate.



Supplementary Figure 9. Butyrophilin-3 expression on cancer cell lines and normal cells. (**A**) Cancer cell lines or (**B**) normal cells (untreated, -ve, or treated with 50 µM zoledronate, +zol) were stained with BTN3 antibody. The numbers on histograms refer to median fluorescence intensity of staining. Fmo, fluorescence minus one.