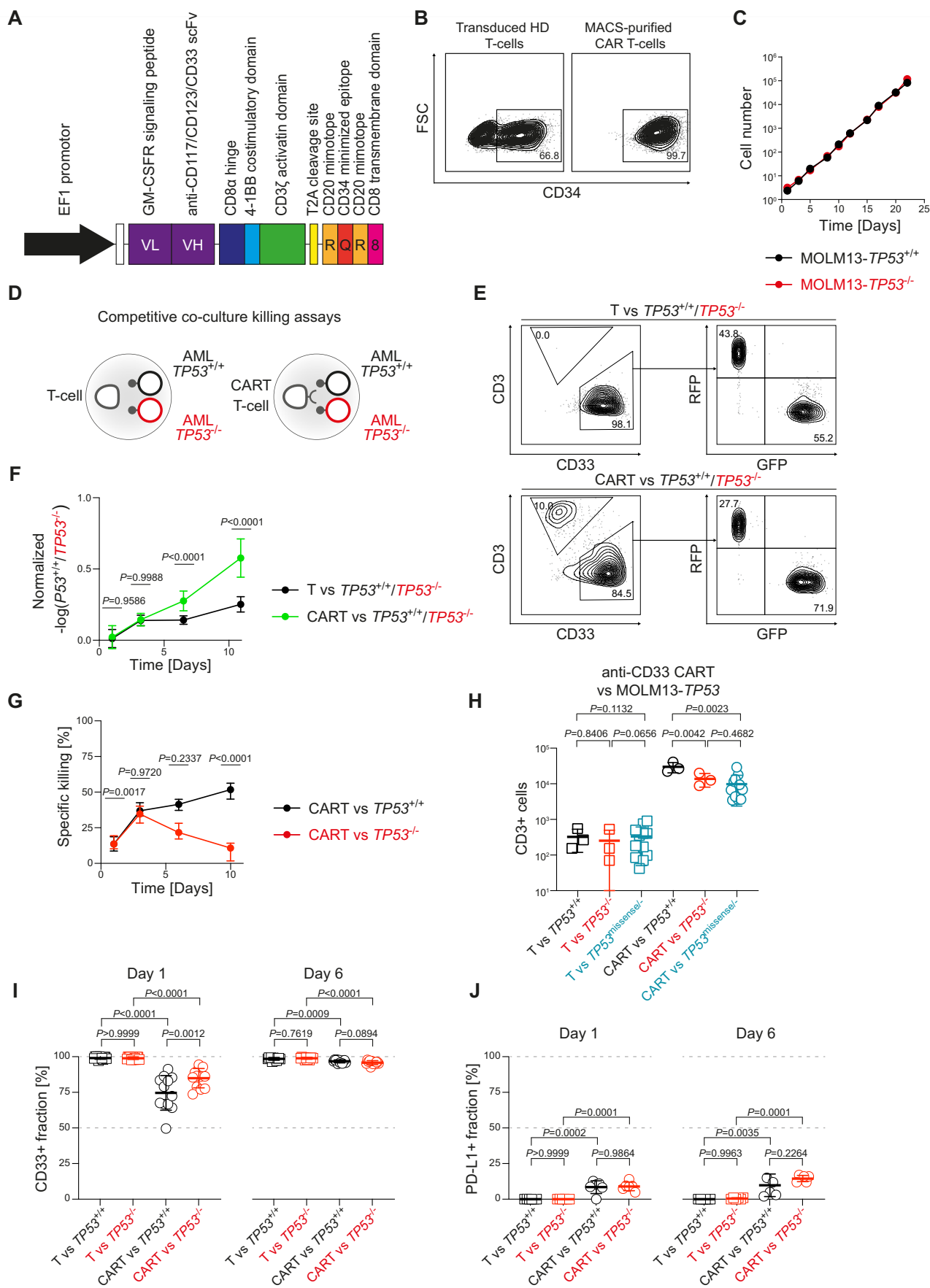
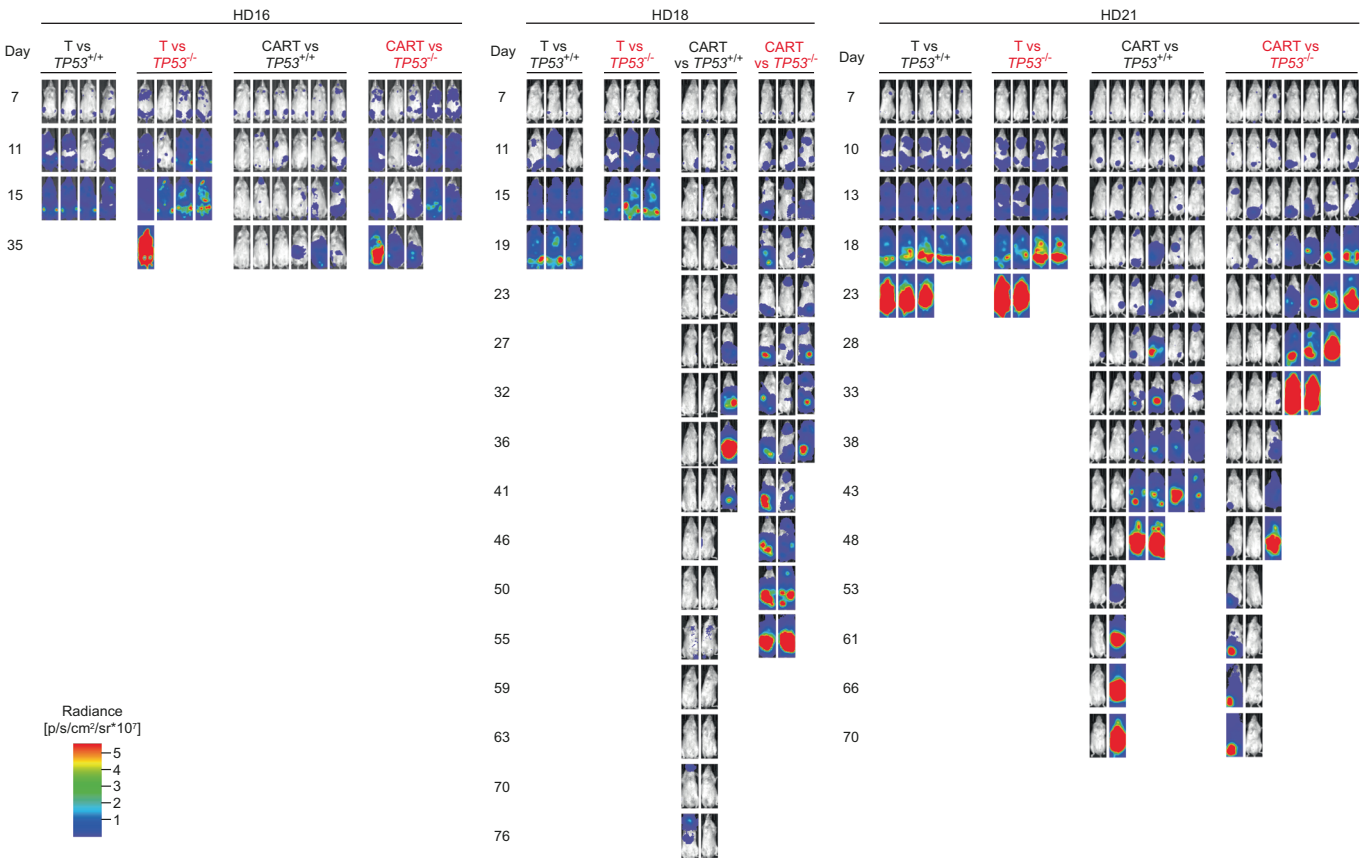


## Expanded View Figures

**Figure EV1. Details of TP53-associated resistance to in vitro CAR T-cell killing, relates to Figs. 1 and 2.**

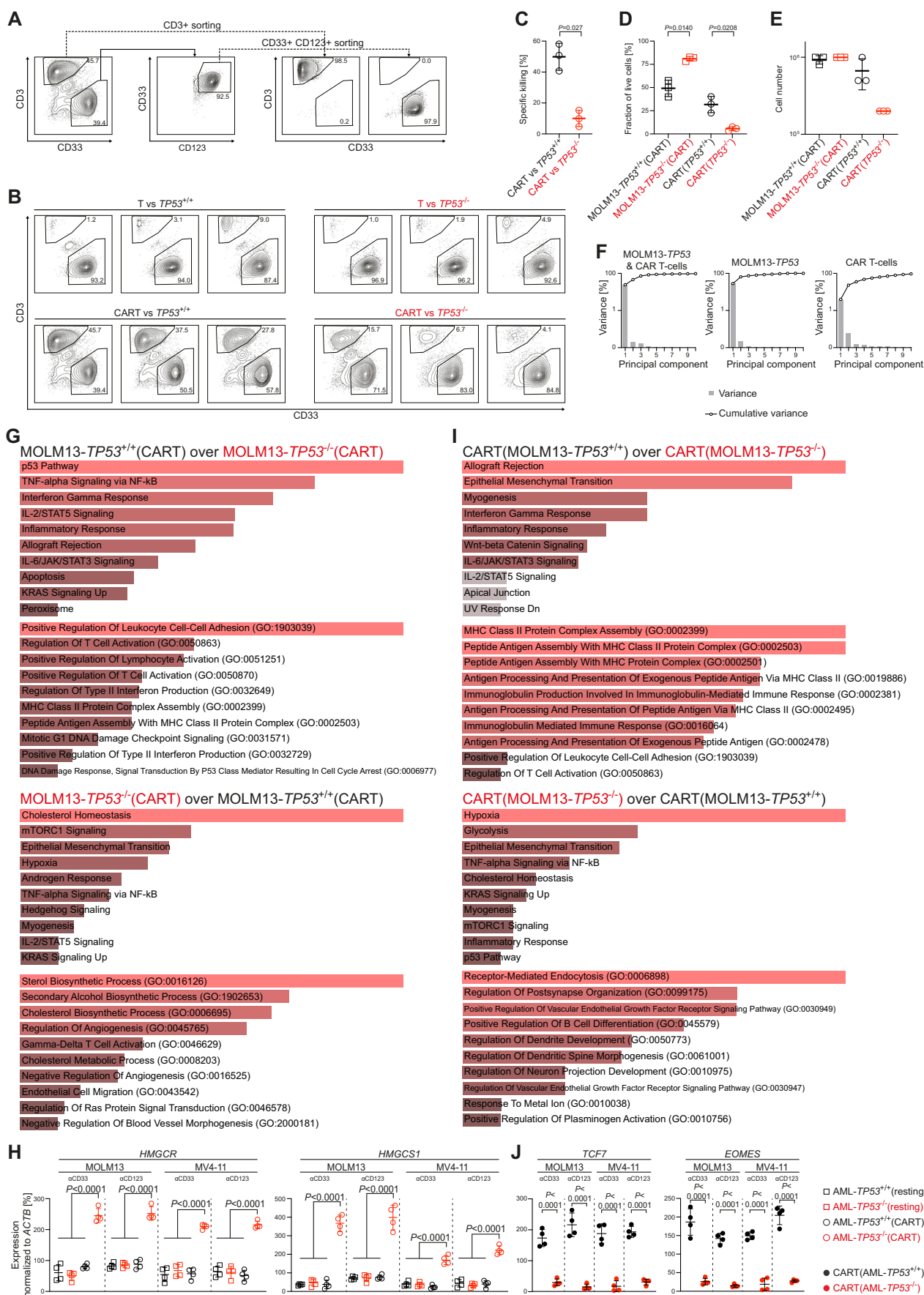
(A) Vector design for second-generation CAR expression under an EF1 promotor with a CD8 $\alpha$  hinge, a 4-1BB costimulatory domain, a CD3 $\zeta$  activating domain and an RQR8 identification and selection peptide. (B) Lentiviral T-cell transduction and MACS purification yielded a >95% pure CAR T-cell population for further experiments. (C) In vitro growth kinetics of unperturbed MOLM13-TP53<sup>+/+</sup> and MOLM13-TP53<sup>-/-</sup> leukemia cells. (D) Graphical representation of in vitro competitive co-incubation assay. (E) Representative FACS plots of in vitro killing assays showing ratio of MOLM13-TP53<sup>-/-</sup>GFP<sup>+</sup> over MOLM13-TP53<sup>+/+</sup>RFP<sup>+</sup> upon 10 days of co-incubation with untransduced T-cell controls and or CAR T-cells. Percentages of parental populations are shown. (F) -Log of MOLM13-TP53<sup>+/+</sup>/MOLM13-TP53<sup>-/-</sup> ratios normalized to the calculated initial MOLM13-TP53<sup>+/+</sup>/MOLM13-TP53<sup>-/-</sup> ratios plotted against time of co-incubation. Pooled results of all different E:T and KO:WT ratios are shown (biological replicates,  $n = 2$ ; three technical replicates per biological replicate; symbols represent means and error bars indicate SD; two-way ANOVA). (G) Calculated specific killing from in vitro competitive co-incubation assays of anti-CD33 CAR T-cells against a mixture of MOLM13-TP53<sup>+/+</sup> and MOLM13-TP53<sup>-/-</sup> leukemia cells. (H) Absolute CD3<sup>+</sup> cell numbers at an E:T ratio of 1:16 for untransduced T-cell controls and anti-CD33 CAR T-cells co-incubated with MOLM13-TP53<sup>+/+</sup> (black), MOLM13-TP53<sup>-/-</sup> (red) or MOLM13-TP53<sup>missense/-</sup> (blue) AML cells on day 6 (biological replicates,  $n = 2$ ; two technical replicates per biological replicate; symbols represent individual replicates; thickened lines indicate means and error bars indicate SD; two-way ANOVA). (I) CD33 target antigen density on MOLM13-TP53<sup>+/+</sup> and TP53<sup>-/-</sup> AML cells in co-incubation at an E:T of 1:16 with untransduced T-cell controls and anti-CD33-directed CAR T-cells on days 1 and 6 (biological replicates,  $n = 4$ ; three technical replicates for each biological replicates; symbols represent individual replicates; thickened lines indicate means and error bars indicate SD; two-way ANOVA). (J) PD-L1 surface expression on MOLM13-TP53<sup>+/+</sup> and TP53<sup>-/-</sup> AML cells in co-incubation with untransduced T-cell controls and anti-CD33-directed CAR T-cells on days 1 and 6 (biological replicates,  $n = 3$ ; three technical replicates for each biological replicate; symbols represent individual replicates; thickened lines indicate means and error bars indicate SD; two-way ANOVA).





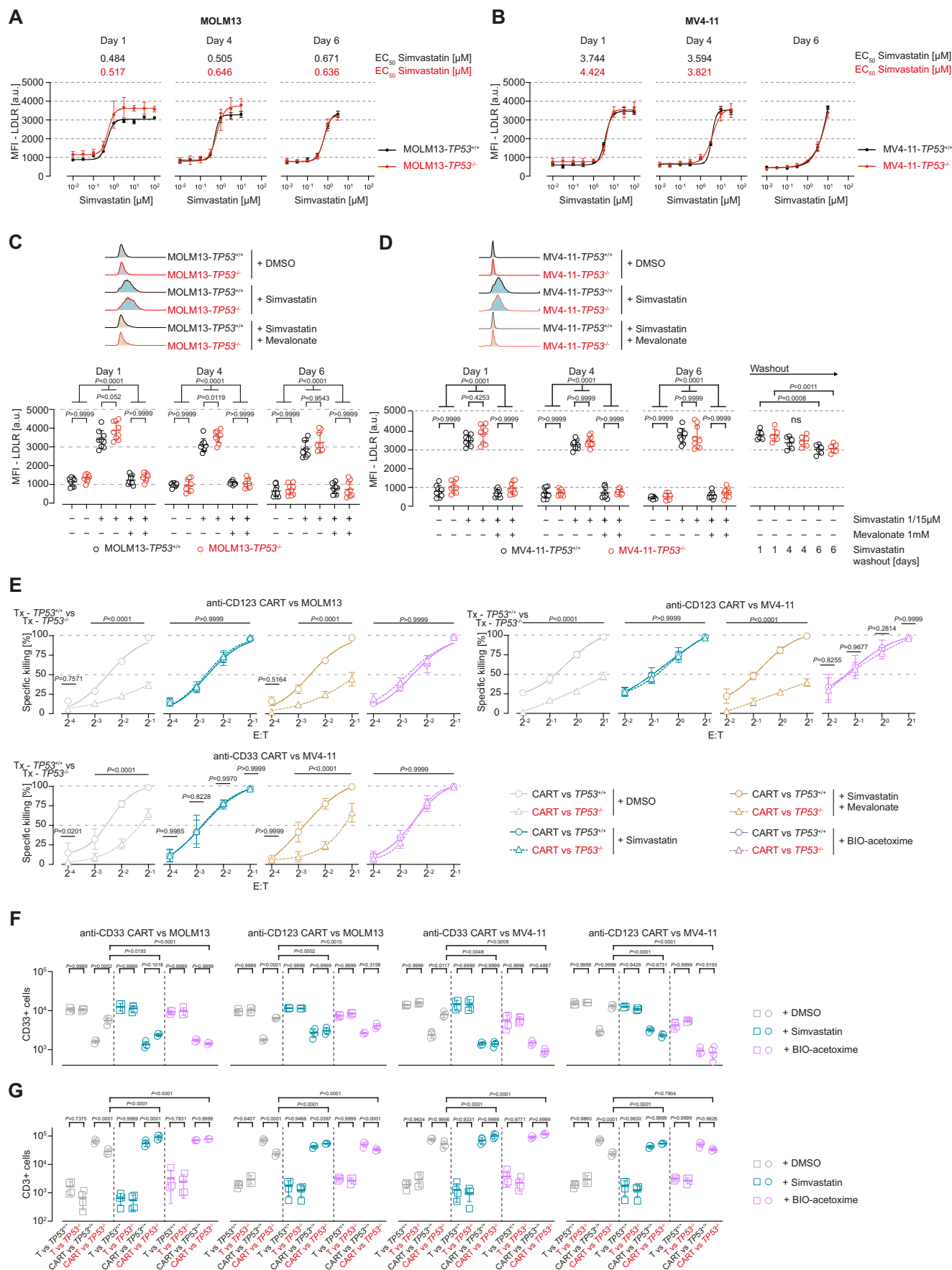
**Figure EV2. Details of therapeutic in vivo xenograft model, relates to Fig. 3.**

Pseudo-colored bioluminescence measurements taken at the indicated days showing leukemic burden in the respective groups of treated and control mice. The three biological replicates with different HD untransduced T-cells and CAR T-cells are shown, ( $n = 53$  mice in total and  $n = 3$  biological replicates; T-cells vs. MOLM13-TP53<sup>+/+</sup>Luc<sup>+</sup>=12, T-cells vs. MOLM13-TP53<sup>-/-</sup>Luc<sup>+</sup>=11, CAR T-cells vs. MOLM13-TP53<sup>+/+</sup>Luc<sup>+</sup>=15, CAR T-cells vs. MOLM13-TP53<sup>-/-</sup>Luc<sup>+</sup>=15).



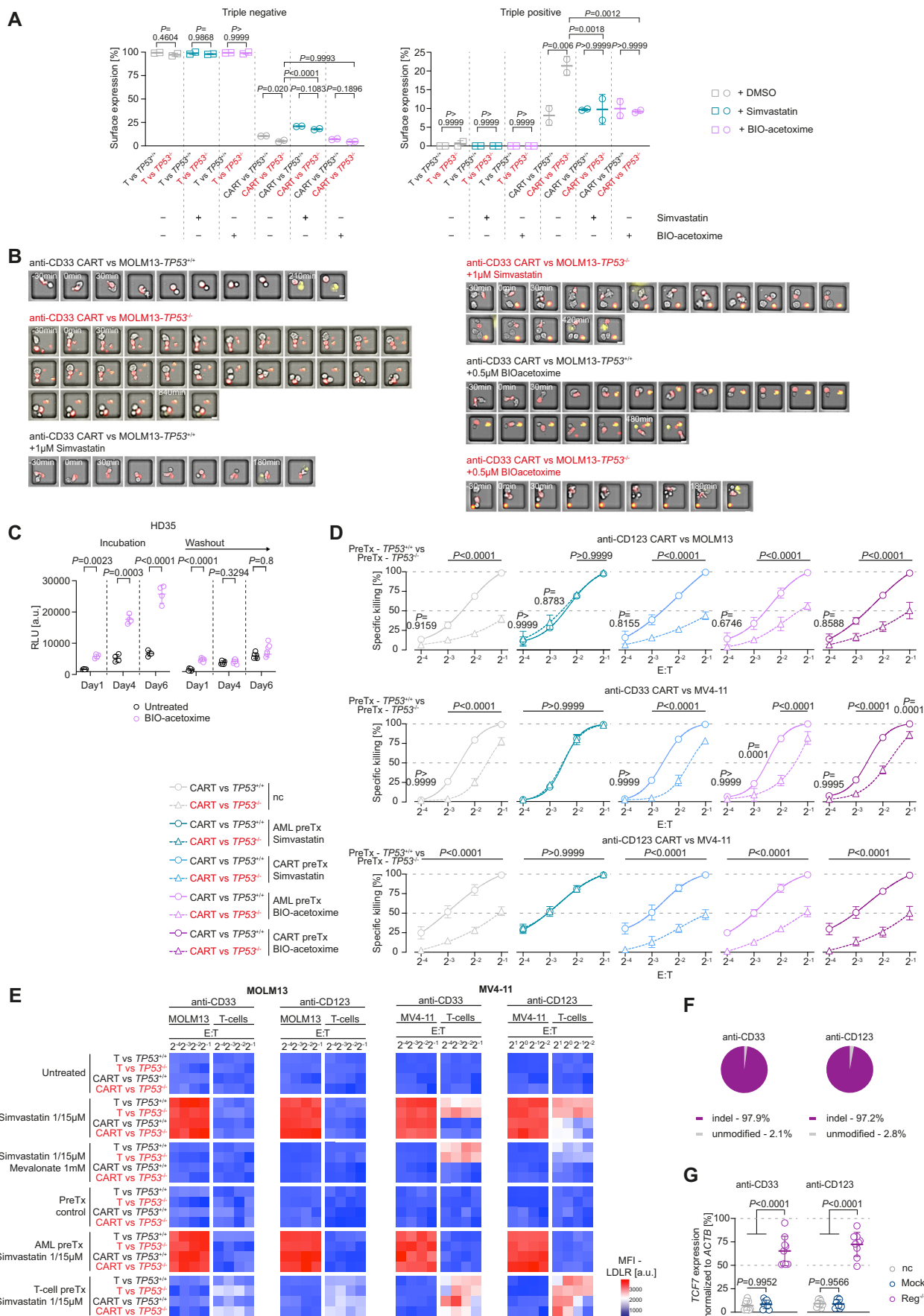
◀ **Figure EV3. Details of sorting procedure and gene expression profiling results, relates to Fig. 4.**

(A) Gating strategy for flow cytometry sorting as shown by an example (i.e., same data) from panel (B): CD3<sup>+</sup> T-cells were sorted as shown, CD3<sup>+</sup>CD33<sup>+</sup> events were further gated on CD33<sup>+</sup>CD123<sup>+</sup> to sort double-positive MOLM13-TP53 AML leukemia cells. The resulting cell populations of >97% purity are shown on the right two panels. (B) FACS plots of  $n = 3$  technical replicates of in vitro co-culture assays subjected to flow cytometry sorting and mRNA extraction. (C) Calculated specific killing from co-incubation assay of MOLM13-TP53<sup>+/+</sup> (black) or MOLM13-TP53<sup>-/-</sup> (red) with anti-CD33 CAR T-cells used for RNA-sequencing (biological replicate,  $n = 1$ ; three technical replicates; symbols indicate individual technical replicates; thickened line represents mean and error bars indicate SD; unpaired Student's  $t$  test). (D) Fraction of live cells from co-incubation assay used for RNA-sequencing. (biological replicates,  $n = 1$ ; 3 technical replicates; symbols represent individual replicates; thickened lines indicate mean and error bars represent SD; unpaired Student's  $t$  test). (E) Total sorted cell numbers for the respective conditions used for mRNA sequencing. (F) Proportion of variance plot. Bars represent the specific proportion of total variance explained by the principal component (PC) and curve represents the cumulative variance explained by PC and all PCs before it. (G) Pathways and gene ontology (GO) terms related to biological processes (BPs) enriched in MOLM13-TP53 under CAR T-cell attack. (H) RT-qPCR measuring expression of *HMGCR* and *HMGCS1* transcripts normalized to *ACTB* in MOLM13-TP53 and MV4-11-TP53 cells sorted from co-incubation assays with either untransduced T-cells [AML-TP53(resting)] or CD33-/CD123-directed CAR T-cells [AML-TP53(CART)] (biological replicates,  $n = 2$ ; 2 technical replicates per biological replicate; symbols indicate individual replicates; thickened lines indicate mean and error bars indicate SD; one-way ANOVA). (I) Pathways and gene ontology (GO) terms related to biological processes (BPs) enriched in CAR T-cells co-incubated with MOLM13-TP53. (J) RT-qPCR measuring expression of *TCF7* and *EOMES* transcripts normalized to *ACTB* in CAR T-cells sorted from co-incubation assays with TP53<sup>+/+</sup> [CART(AML-TP53<sup>+/+</sup>)] or TP53<sup>-/-</sup> [CART(AML-TP53<sup>-/-</sup>)] target cells lines MOLM13 and MV4-11 (biological replicates,  $n = 2$ ; 2 technical replicates per biological replicate; symbols indicate individual replicates; thickened lines indicate mean and error bars indicate SD; unpaired Student's  $t$  test).



**Figure EV4. Details of rescue co-incubation assays, relates to Figs. 5 and 6.**

LDLR mean fluorescence intensity and extrapolated EC50 of (A) MOLM13-TP53 and (B) MV4-11-TP53 AML cells incubated with increasing concentrations of simvastatin (biological replicates,  $n = 2$ ; symbols represent means; error bars indicate SD). (C) Upper panel: Representative FACS histograms of LDLR mean fluorescence intensity of isogenic MOLM13-TP53 AML cells with wild-type (MOLM13-TP53<sup>+/+</sup>) or null (MOLM13-TP53<sup>-/-</sup>) TP53 status in the presence of DMSO, simvastatin 1  $\mu$ M or simvastatin 1  $\mu$ M + mevalonate 1mM. Lower panel: LDLR mean fluorescence intensity of MOLM13-TP53 cells on days 1, 4 and 6 in the presence or absence of simvastatin 1  $\mu$ M and/or mevalonate 1mM (biological replicates,  $n = 3$ ; 2 technical replicates per biological replicate; symbols indicate individual replicates; thickened lines indicate means and error bars indicate SD; two-way ANOVA). (D) Upper panel: Representative FACS histograms of LDLR mean fluorescence intensity of isogenic MV4-11-TP53 AML cells with wild-type (MV4-11-TP53<sup>+/+</sup>) or null (MV4-11-TP53<sup>-/-</sup>) TP53 status in the presence of DMSO, simvastatin 15  $\mu$ M or simvastatin 15  $\mu$ M + mevalonate 1mM. Lower panel: LDLR mean fluorescence intensity of MV4-11-TP53 cells on days 1, 4 and 6 in the presence or absence of simvastatin 15  $\mu$ M and/or mevalonate 1mM as well as after washout of simvastatin (biological replicates,  $n = 3$ ; 2 technical replicates per biological replicate; symbols indicate individual replicates; thickened lines indicate means and error bars indicate SD; two-way ANOVA). (E) Summary data showing results from 3 different co-incubation assays of CAR T-cell-mediated killing of TP53<sup>+/+</sup> or TP53<sup>-/-</sup> AML cells over various E:T ratios in the presence of DMSO, simvastatin (1  $\mu$ M for MOLM13 and 15  $\mu$ M for MV4-11), simvastatin + mevalonate 1mM or BIO-acetoxime 0.5  $\mu$ M, respectively. Co-incubation assays include anti-CD123 CAR vs MOLM13-TP53, anti-CD33 CAR vs MV4-11-TP53 and anti-CD123 CAR vs MV4-11-TP53. (replicates,  $n = 2-3$ ; symbols represent mean; error bars indicate SD; two-way ANOVA). (F) Absolute CD33<sup>+</sup> target cell numbers from various co-incubation assays of untransduced T-cells or CAR T-cells with TP53<sup>+/+</sup> or TP53<sup>-/-</sup> AML cells at an E:T of 1:16 in the presence of DMSO, simvastatin (1  $\mu$ M for MOLM13 and 15  $\mu$ M for MV4-11) or BIO-acetoxime 0.5  $\mu$ M, respectively. Co-incubation assays include anti-CD33 CAR vs MOLM13-TP53, anti-CD123 CAR vs MOLM13-TP53, anti-CD33 CAR vs MV4-11-TP53 and anti-CD123 CAR vs MV4-11-TP53. (biological replicates,  $n = 2$ ; 2 technical replicates per biological replicate; symbols represent individual replicates; thickened lines indicate means and error bars indicate SD; ns, non-significant; two-way ANOVA). (G) Absolute CD3<sup>+</sup> effector cell numbers from various co-incubation assays of untransduced T-cells or CAR T-cells with TP53<sup>+/+</sup> or TP53<sup>-/-</sup> AML cells at an E:T of 1:16 in the presence of DMSO, simvastatin (1  $\mu$ M for MOLM13 and 15  $\mu$ M for MV4-11) or BIO-acetoxime 0.5  $\mu$ M, respectively. Co-incubation assays include anti-CD33 CAR vs MOLM13-TP53, anti-CD123 CAR vs MOLM13-TP53, anti-CD33 CAR vs MV4-11-TP53 and anti-CD123 CAR vs MV4-11-TP53. (biological replicates,  $n = 2$ ; 2 technical replicates per biological replicate; symbols represent individual replicates; thickened lines indicate means and error bars indicate SD; two-way ANOVA).



**Figure EV5. Details of rescue and pretreatment co-incubation assays as well as Regnase-1-deficient CAR T-cells, relates to Figs. 5 and 6.**

(A) Fraction of T-cells negative for all three investigated exhaustion markers (right) and negative for all three markers (left) treated with the indicated compounds (one biological replicate,  $n = 2$  technical replicates; symbols represent means; error bars indicate SD; two-way ANOVA). (B) Representative stills of fluorescence live-cell time-lapse imaging data of anti-CD33 CAR T-cell engaging MOLM13-*TP53*<sup>+/+</sup> AML or MOLM13-*TP53*<sup>-/-</sup> AML cells in the presence of simvastatin 1  $\mu$ M or BIO-acetoxime 0.5  $\mu$ M (scale bars, 10  $\mu$ m). (C) Luminescence signal of T-cells transduced with a Wnt-responsive luciferase gene reporter system and incubated with or without BIO-acetoxime at 0.5  $\mu$ M for 1, 4 or 6 days as well as after washout of BIO-acetoxime. (Data shown from a second healthy T-cell donor than in Fig. 6C; biological replicates,  $n = 2$ ; 2 technical replicates per biological replicate; symbols indicate individual replicates; thickened lines denote means and error bars indicate SD; unpaired Student's *t* test). (D) Results from 3 different pretreatment co-incubation assays with anti-CD33 and anti-CD123 CAR T-cells against *TP53*<sup>+/+</sup> or *TP53*<sup>-/-</sup> AML (MOLM13 and MV4-11) cells at various E:T ratios. Co-incubations include: anti-CD123 CAR vs MOLM13-*TP53*, anti-CD33 CAR vs MV4-11-*TP53* and anti-CD33 CAR vs MV4-11-*TP53*. (biological replicates,  $n = 2$ -3; 2 technical replicates per biological replicate; symbols represent means; error bars indicate SD; two-way ANOVA). (E) Heatmaps depicting LDLR expression (mean fluorescence intensity) within treatment as well as pretreatment co-incubation assays with anti-CD33 and anti-CD123 CAR T-cells against MOLM13-*TP53* and MV4-11-*TP53* AML cells at various E:T ratios. (biological replicates,  $n = 3$ -4; 2 technical replicates per biological replicate; pseudocolors indicate signal of LDLR expression). (F) Sequencing results graphed as %indels of Regnase-1 CRISPRed anti-CD33 as well as anti-CD123 CAR T-cells from one healthy donor. (G) RT-qPCR measuring expression of *TCF7* transcripts normalized to *ACTB* in nc, mock and RegKO anti-CD33 and anti-CD123 CAR T-cells (biological replicates,  $n = 2$ ; 2 technical replicates per biological replicate; symbols indicate individual replicates; thickened lines indicate means and error bars indicate SD; paired Student's *t* test).