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Supplemental information

Accelerating clinical-scale production of BCMA CAR T cells with defined maturation stages

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Supplemental Information

Supplemental Table S1: Activity matrix used on the CliniMACS Prodigy

Timepoint	Activity	Timing	User operation
Day 0	Process setup	7-8 hrs	Required
Day 2	Waste bag exchange	Any time between day 2 and day 5	Required
	Volume reduction	15 min before transduction	No
	Transduction	46 hrs after start cultivation	Required
	Spin	15 min after transduction	No
Day 3	Culture wash	24 hrs after transduction	No
	Activate shaker	1 hr after culture wash	No
Day 5	Medium bag exchange	15 min before media exchange	Required
	Media exchange	48 hrs after culture wash	No
Day 6-8	Media exchange	Every 24 hrs after previous media exchange	No
	Activate shaker	1 hr after last media exchange	No
Day 9-11	Media exchange*	Every 12 hrs after previous media exchange	No
Day 12	End of culture	Any time	Yes

* If the process was terminated on day 10 or 11, media exchange was replaced by End of culture program step.

Supplemental Figures and Figure legends

Supplemental Figure 1

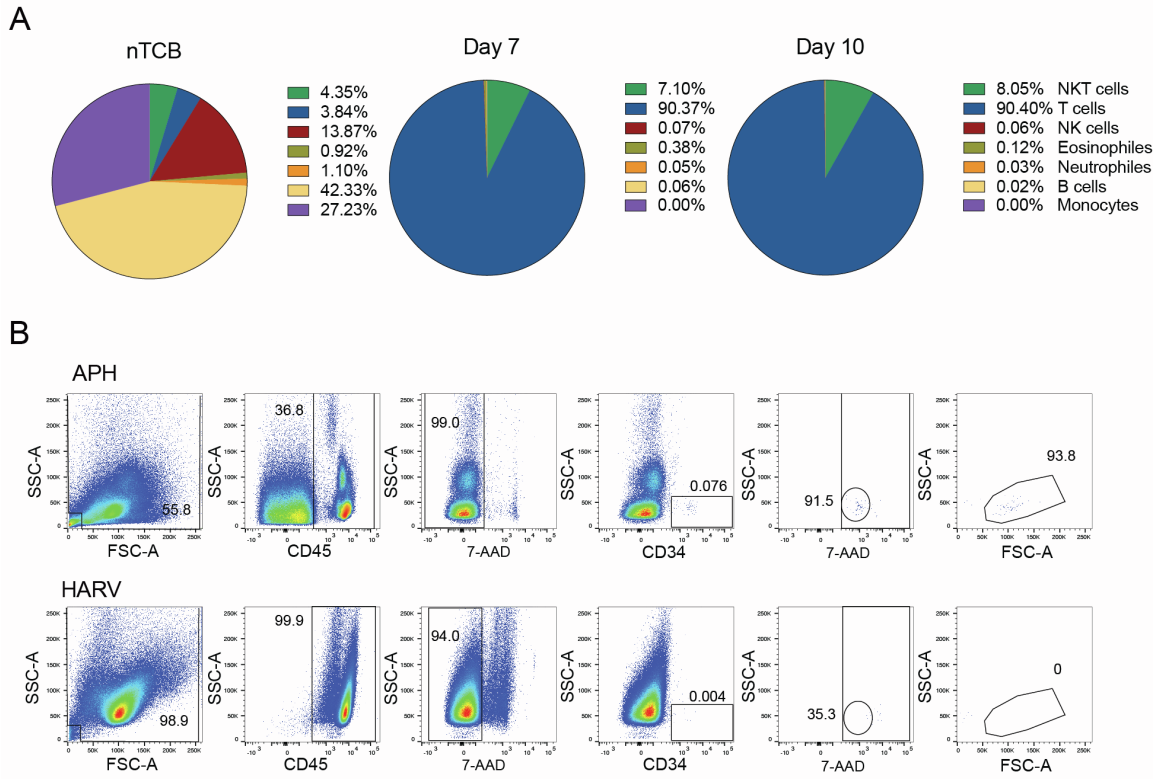

Figure S1. Flow cytometric analysis of leukocyte subpopulations at different process steps

A) Flow cytometric analysis of the CliniMACS Prodigy products of bulk runs P1, P2 and P3. Depicted is the leukocyte composition in the non-target cell bag (nTCB) obtained as a negative selection product of MACS purification, and intermediate products on day 7 and day 10. Antibody panels defining the indicated leukocyte subpopulations were used for NKT cells: $7AAD^{-}CD45^{+}CD3^{+}CD16^{+}/CD56^{+}$; T cells: $7AAD^{-}CD45^{+}CD3^{+}CD16^{-}/CD56^{-}$, additionally $CD4^{+}CD8^{-}$ T cells and $CD8^{+}CD4^{-}$ T cells; NK cells: $7AAD^{-}CD45^{+}CD3^{-}CD14^{-}CD19^{-}SSC\text{-}A^{low}CD16^{+}/CD56^{+}$; Eosinophils: $7AAD^{-}CD45^{+}CD3^{-}CD14^{-}CD19^{-}SSC\text{-}A^{high}CD16^{-}/CD56^{-}$; Neutrophils: $7AAD^{-}CD45^{+}CD3^{-}CD14^{-}CD19^{-}SSC\text{-}$

$A^{high}CD16^{+}/CD56^{+}$; B cells: $7AAD^{-}CD45^{+}CD3^{-}CD14^{-}CD19^{+}$; Monocytes: $7AAD^{-}CD45^{+}CD3^{-}CD14^{+}CD19^{-}$; CD16 and CD56 were stained in the same channel. Samples were run in technical duplicates and results are displayed as mean. The pie charts represent the mean of n=3 Prodigy runs.

B) Representative gating scheme for the identification of $CD45^{+}CD34^{+}$ hematopoietic stem cells ($Debri^{-}CD45^{+}7AAD^{-}SSC\text{-}A^{low}CD34^{+}$).

Supplemental Figure 2

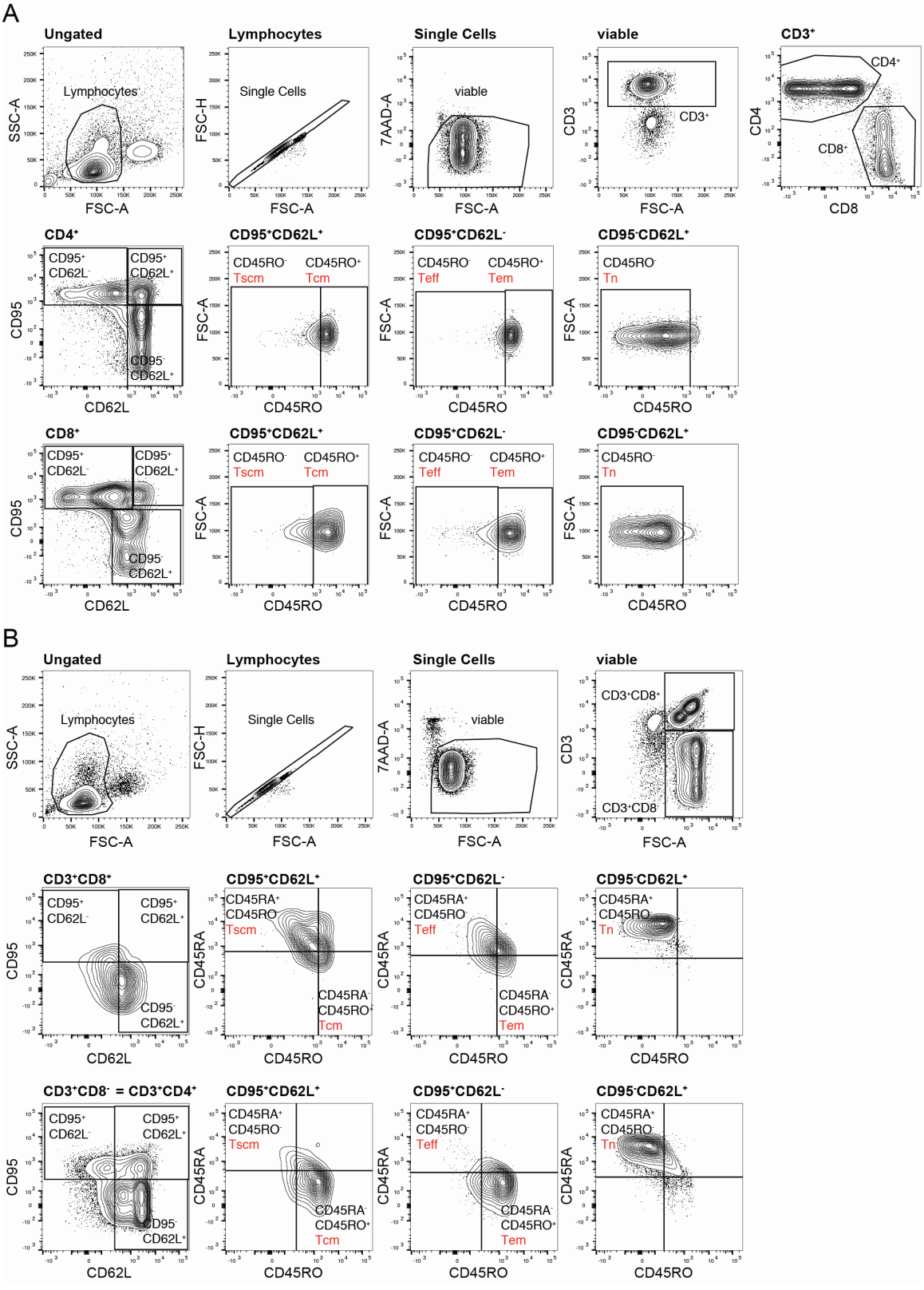

Figure S2. Gating strategy to define T cell subsets in flow cytometry

Flow cytometric analysis of different T cell subsets. **A**) The gating strategy for bulk runs P1, P2 and P3 (panel without CD45RA) is presented. T central memory (T_{CM}): $CD95^+CD62L^+CD45RO^+$; T stem cell memory (T_{SCM}): $CD95^+CD62L^+CD45RO^-$; T effector memory (T_{EM}): $CD95^+CD62L^-CD45RO^+$; T effector (T_{EFF}): $CD95^+CD62L^-CD45RO^-$; T naive (T_N): $CD95^-CD62L^+CD45RO^-$. **B**) The gates set in $T_{CM/SCM}$ runs P4, P5 and P6 (panel with CD45RA) are depicted. T_{CM}: $CD95^+CD62L^+CD45RO^+CD45RA^-$; T_{SCM}: $CD95^+CD62L^+CD45RO^-CD45RA^+$; T_{EM}: $CD95^+CD62L^-CD45RO^+CD45RA^-$; T_{EFF}: $CD95^+CD62L^-CD45RO^-CD45RA^+$; T_N: $CD95^-CD62L^+CD45RO^-CD45RA^+$. **A-B)** Representative dot plots for a MACS product are shown. Samples were analyzed after MACS separation (MACS), FACS sorting (Sort), on day 10 of culture (Day 10), and in the final formulation (HARV). For each marker, an isotype control or FMO was measured to determine the appropriate gate.

Supplemental Figure 3

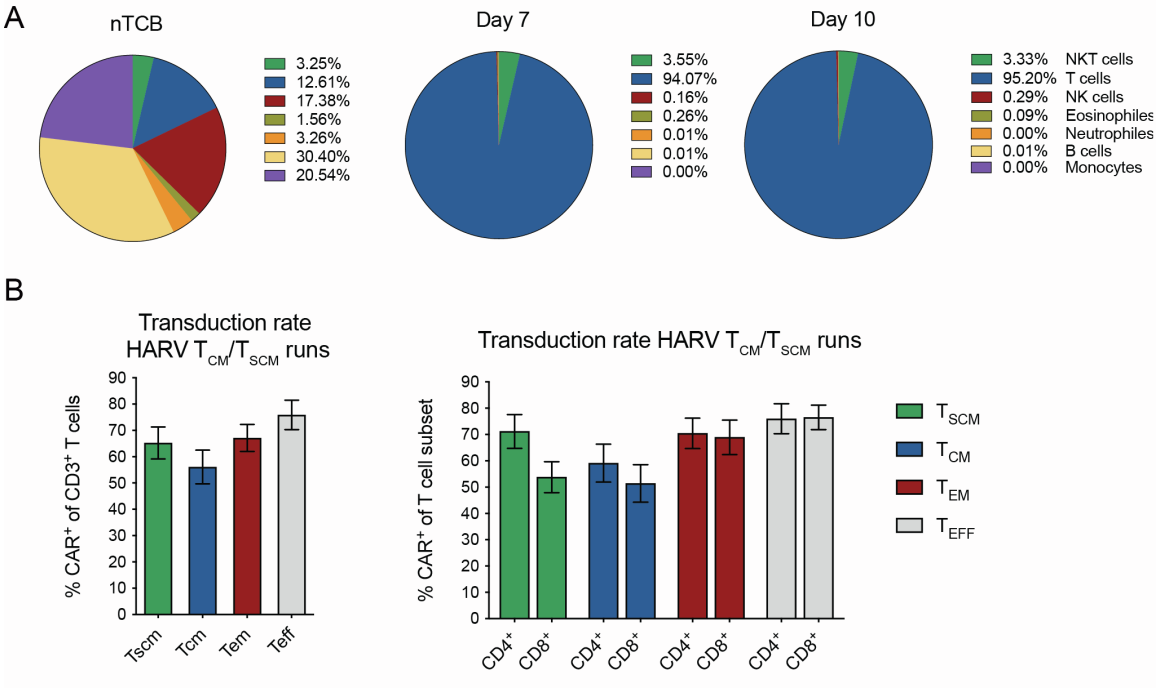

Figure S3. Phenotypic analysis of the cellular composition of T_{CM}/T_{SCM} BCMA CAR T cells

A) Flow cytometric analysis of leukocyte subpopulations obtained in $T_{CM/SCM}$ runs P4, P5 and P6 at different process steps was conducted (non-target cell bag (nTCB), an intermediate product on day 7 and on day 10. All leukocyte subpopulations were defined as in **Figure S1A**. Samples were run in technical duplicates and results are displayed as mean. **B)** Flow cytometric analysis of the transduction rate (CAR^+ T cells) of total $CD3^+$ T cells (left panel) or divided into $CD4^+$ and $CD8^+$ T cells (right panel) memory and effector subpopulations after harvest obtained in run $T_{CM/SCM}$ runs P4, P5 and P6. Subpopulations were gated as: T_{CM}: $CD95^+CD62L^+CD45RO^+CD45RA^-$; T_{SCM}: $CD95^+CD62L^+CD45RO^-CD45RA^+$; T_{EM}: $CD95^+CD62L^-CD45RO^+CD45RA^-$; T_{EFF}: $CD95^+CD62L^-CD45RO^-CD45RA^+$; T_N: $CD95^-CD62L^+CD45RO^-CD45RA^+$. The pie charts represent the mean of n=3 Prodigy runs.

Supplemental Figure 4

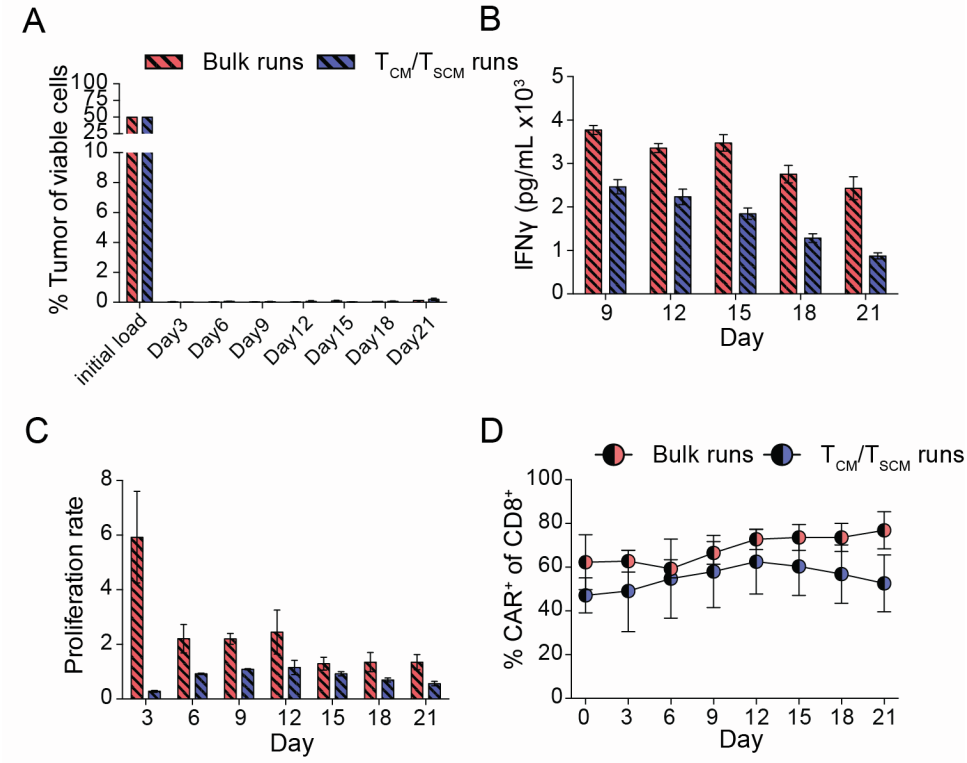

Figure S4. Repetitive antigen stimulation of isolated CD8$^+$ BCMA CAR T cells *in vitro* shows similar effector function, but low proliferative capacity of bulk- and T_{CM}/T_{SCM} -derived BCMA CAR T cells

BCMA CAR T cells were re-stimulated and then MACS bead-purified for CD8$^+$ T cells. CD8$^+$ BCMA CAR T cells were co-cultured in a 1:1 ratio with MM1.S$^{BCMA\text{-}high}$.eGFP target cells. After 72 hours, CAR T cells were retrieved and co-cultured with fresh MM1.S$^{BCMA\text{-}high}$.eGFP target cells for a total of 7 transfer rounds. Data represent the mean ± SEM of one experiment with bulk runs P1and P2, and $T_{CM/SCM}$ runs P4 and P5. **A**) Quantification of residual viable MM1.S$^{BCMA\text{-}high}$.eGFP target cells (7AAD$^-$CD3$^-$ GFP$^+$) by flow cytometry analysis after each round of co-culture. **B)** Cell-free supernatants were harvested at the indicated stimulation cycles and analyzed by ELISA

for IFNγ release; n = 3 replicates per run. **C)** After each round viable 7-AAD^{-}CD3^{+} T cells were counted and the proliferation rate was calculated as the ratio of input T cells versus T cell numbers after 72 hours. **D**) Frequency of transduced CAR^{+} T cells among viable CD3^{+}CD8^{+} T cells was measured.