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Supplemental information

Efficient lentiviral transduction method to gene modify cord blood $CD8^+$ T cells for cancer therapy applications

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Supplemental Material

Figure S1

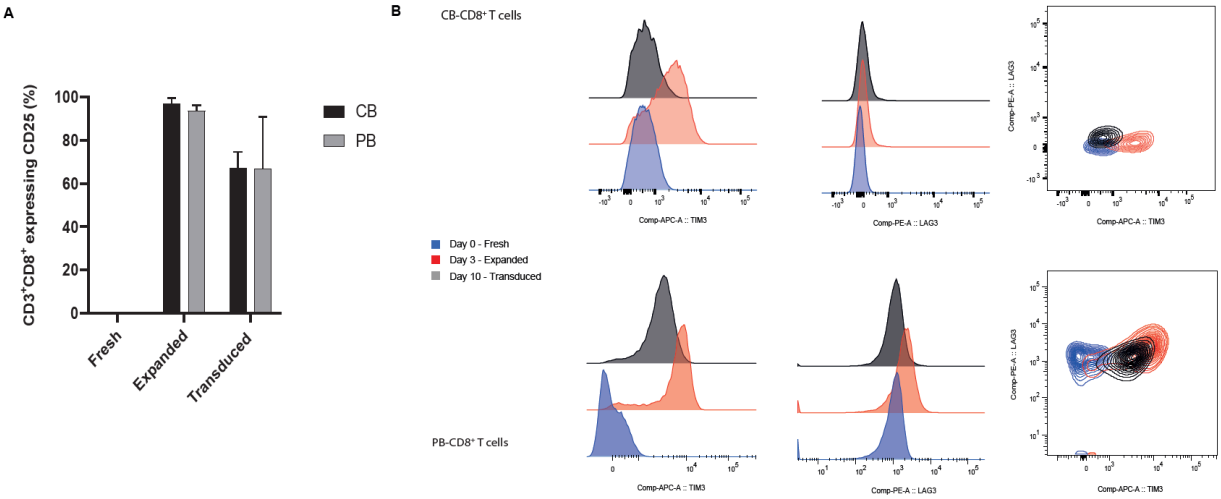

Figure S1. *Expression of CD25, TIM3 and LAG3 in CB and PB CD8+T cells*

(A) CD25 expression in CB (black) and PB (gray). **(B)** TIM3 and LAG3 expression histograms in both CB (top) and PB (bottom) $CD3^+CD8^+$ T cells, in the three different time points: Day 0 – Fresh (blue), Day 3 - Expanded (red), Day 10 – Transduced (black). **(C)** Co-staining of TIM-3 and LAG-3 representative plots for both CB and PB $CD3^+CD8^+$ T cells. The data corresponds to ≥ 3 independent experiments and are shown as average ± standard deviation.

Figure S2

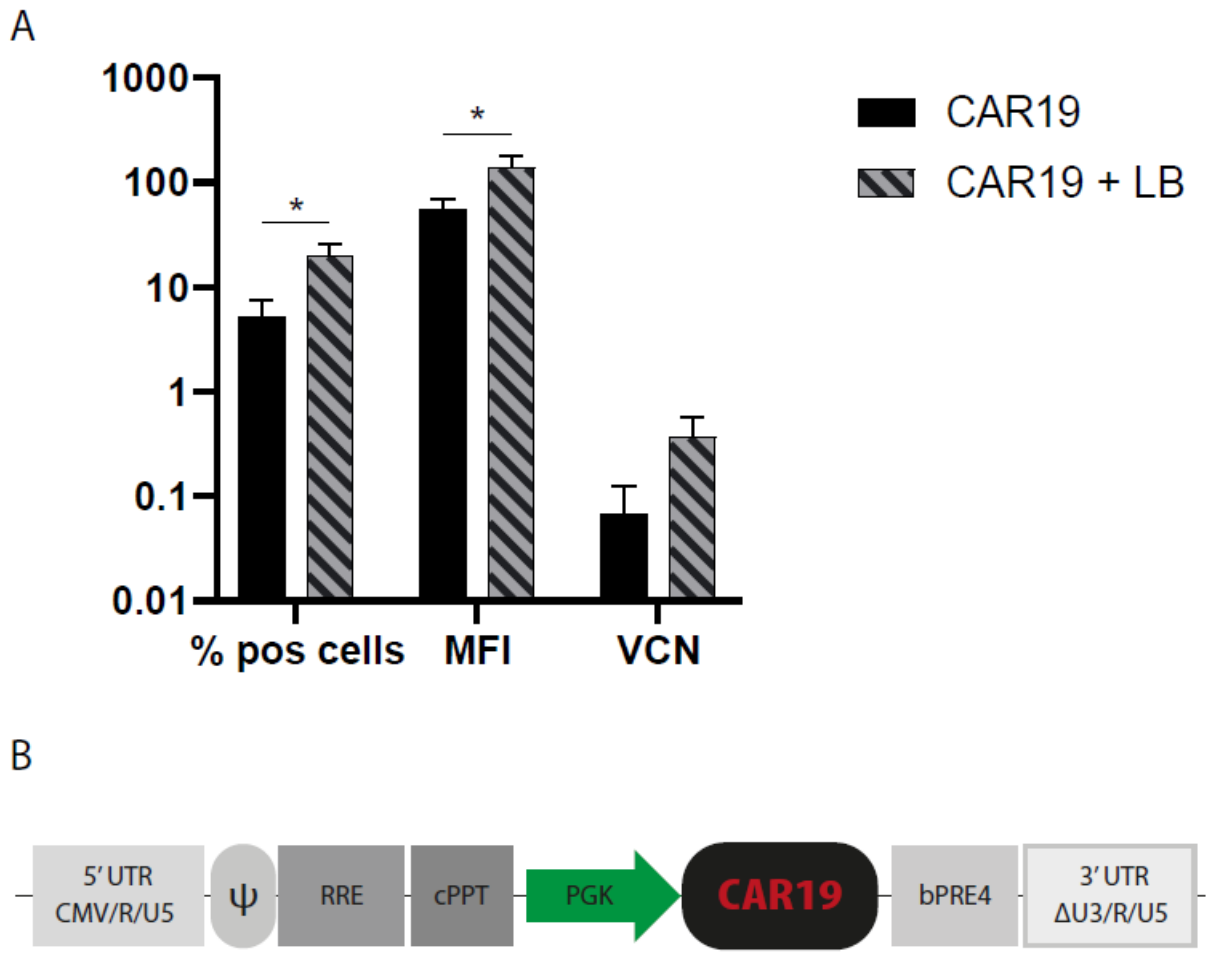

Figure S2. *LB increase transduction efficiency also with LV.CAR19 construct*

(A) CB $CD8^+$ T cells transduced with LV.CAR19 (MOI1) showed 5.2% ± 2.5 transduction efficiency (black), while the use of LB increased the % of transduced cells to 19.9 ± 5.9 (black stripes). The same trend is also visible for the MFI and the VCN. (B) Schematic representation of plasmid LV.CAR19 vector design. The data corresponds to ≥ 3 independent experiments and are shown as average ± standard deviation; $p \leq 0.05$ (*); $p \leq 0.01$ (**) ; $p \leq 0.001$ (***); $p \leq 0.0001$ (****).

Figure S3

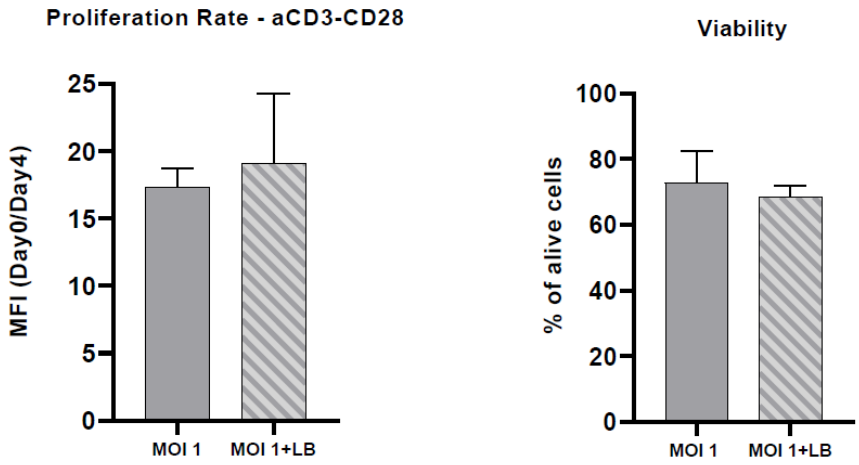

Figure S3. *LB effect on proliferation and cell viability on CB CD8$^+$ T cells transduced with an MOI 1*

(A) Proliferation rate after 4 days of aCD3/CD28 beads stimulation in CB CD8$^+$ T cells transduced with and MOI 1 in the presence of LB (gray stripes) compared to STND method (gray). **(B)** Cell viability analysis, 4 days after aCD3/CD28 beads stimulation, of CB CD8$^+$ T cells transduced with and MOI 1 in the presence of LB (gray stripes) compared to STND method (gray). The data corresponds to ≥ 3 independent experiments and are shown as average ± standard deviation; $p \leq 0.05$ (*); $p \leq 0.01$ (**) ; $p \leq 0.001$ (***); $p \leq 0.0001$ (****).

Figure S4

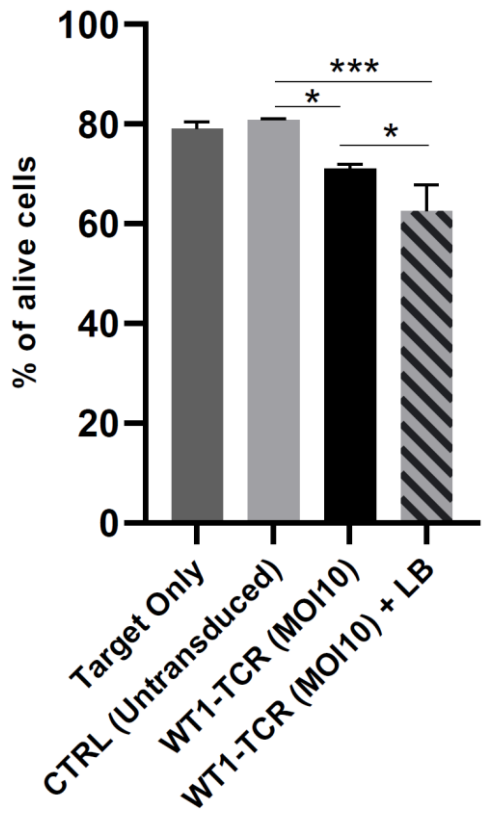

Figure S4

Killing capacity of WT1-TCR CB $CD8^+$ T cells transduced, in the presence or absence of LB, depicted as % of alive cells in a co-culture experiment with target cells loaded with $pWT1_{126}$. Co-culture experiments were performed in a ratio of 10:1 (Target:Effector). The data corresponds to ≥ 3 independent experiments and are shown as average ± standard deviation; $p \leq 0.05$ (*); $p \leq 0.01$ (**) ; $p \leq 0.001$ (***); $p \leq 0.0001$ (****).