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Supplemental information

Dasatinib is a potent enhancer for CAR T cell generation by CD3-targeted lentiviral vectors

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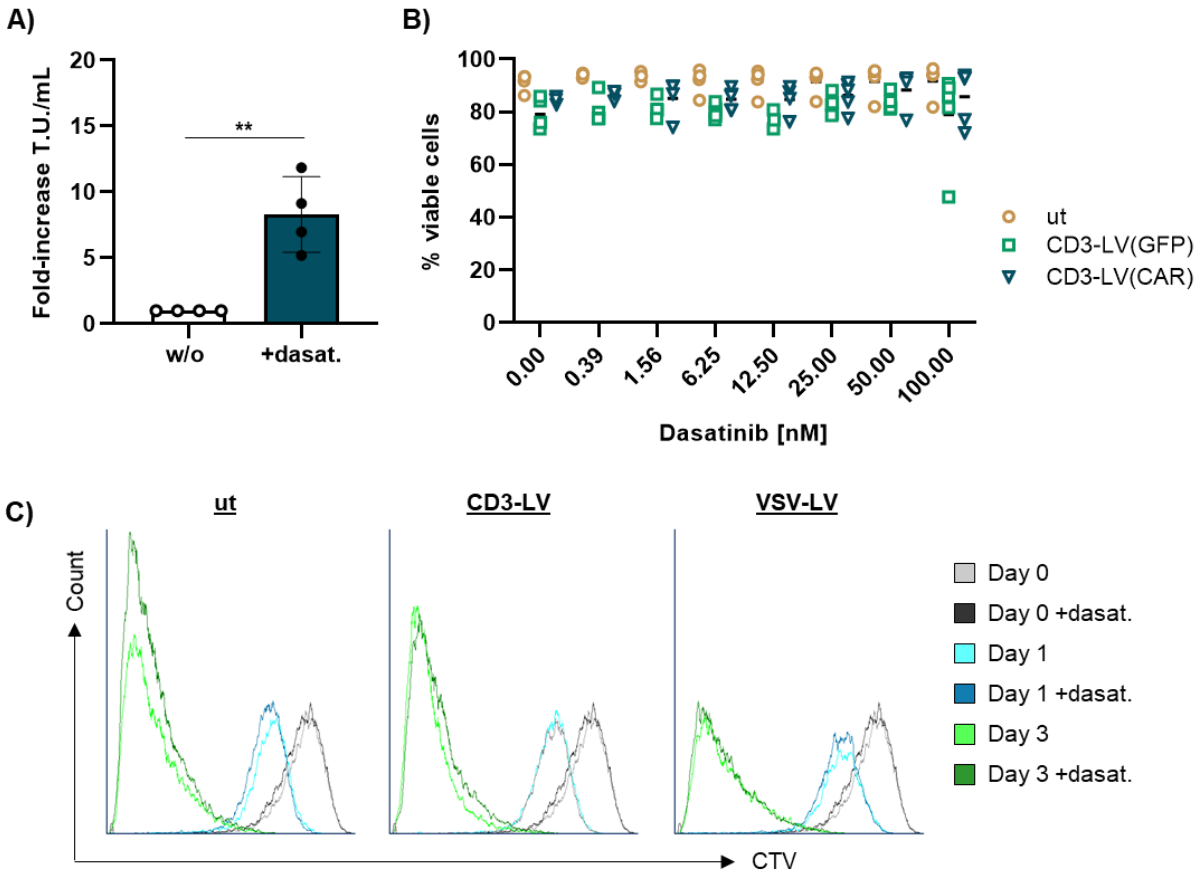

Figure S1: Dasatinib does not interfere with viability or proliferation of activated PBMC.

(**A**) Activated PBMC were incubated in 50 nM dasatinib-containing (+dasat.) medium or without (w/o) dasatinib for 5 h during transduction. Scatter bar diagrams summarize fold-increase in transducing units/mL (T.U./mL) three days post transduction, comparing transduction in presence of dasatinib to transduction without dasatinib with CD3-LV(GFP). Each data point is an average value referring to a particular vector stock tested on one to four donors. Values are shown as mean ± standard deviation (SD). $^{**}p < .01$, by ratio paired t-test. (**B**) Cells were incubated in 0.39-100 nM dasatinib-containing media for 7 h during transduction with CD3-LV delivering GFP or a CD19-CAR. Viability was determined three days post transduction and each value shown refers to the mean determined from four to five donors. (**C**) Activated PBMC were labeled with CellTraceViolet (CTV) prior to transduction with CD3-LV, VSV-LV or remained untransduced. Histograms show fluorescence intensity of CTV from day zero to day three after transduction in presence (+dasat.) or absence of dasatinib.

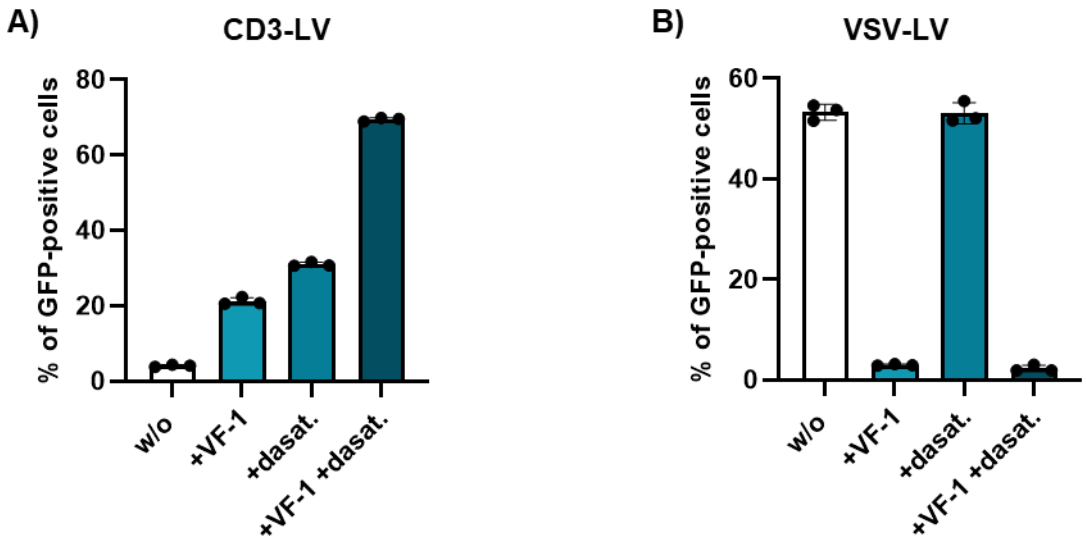

Figure S2: Dasatinib and Vectofusin-1 (VF-1) mediate a synergistic effect on gene delivery.

Activated PBMC were transduced with $2x10^{10}$ particles CD3-LV (**A**) or $8x10^{8}$ particles VSV-LV (**B**). Transduction was either performed without transduction enhancers, with VF-1, with 100 nM dasatinib or with both VF-1 and dasatinib. GFP-expression of the cells was determined three days post transduction by flow cytometry and is shown as scatter bar diagrams with the mean ± SD determined from technical triplicates of one donor.

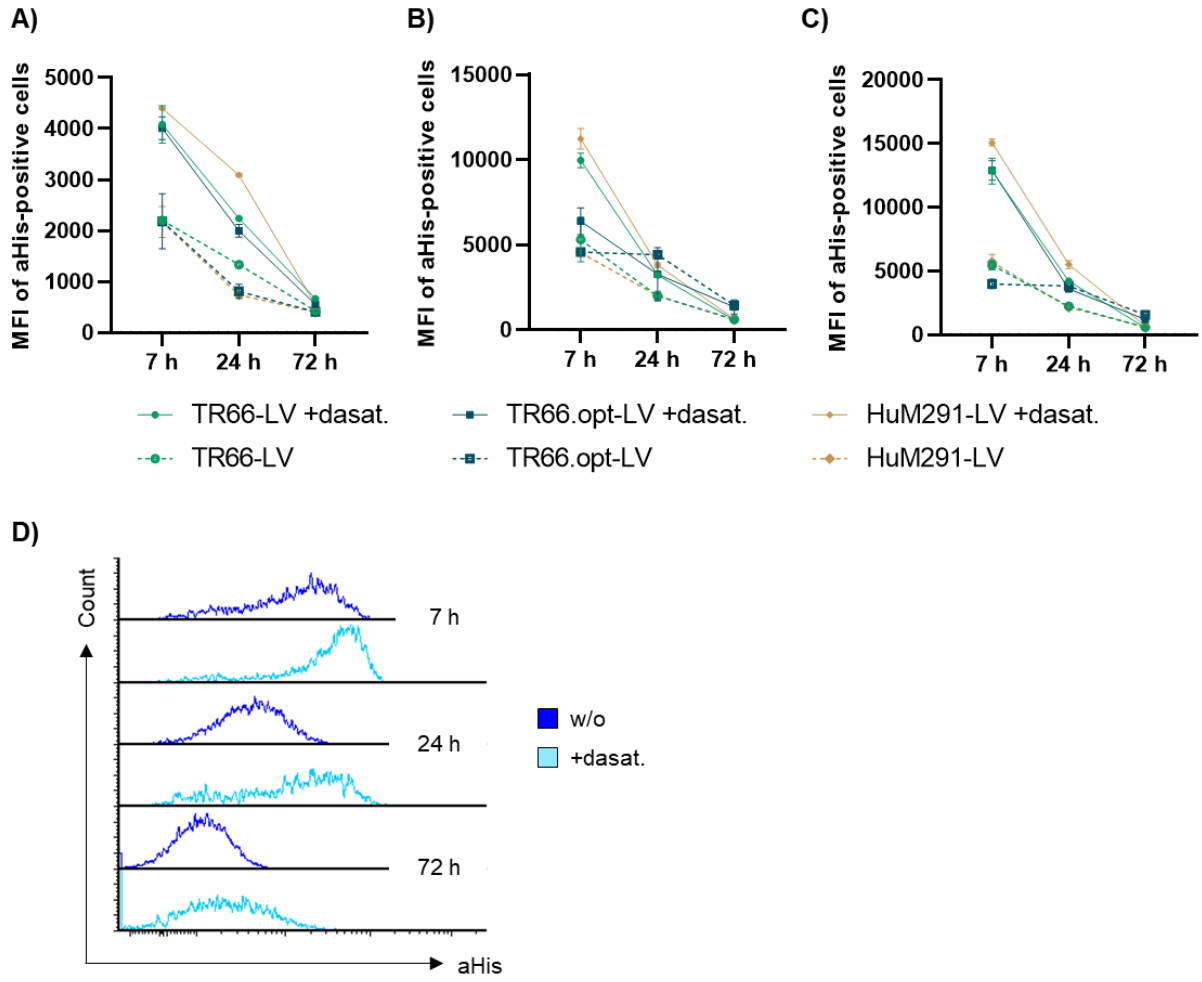

Figure S3: Increased binding of CD3-LV upon dasatinib treatment.

Activated PBMC were incubated in 100 nM dasatinib-containing medium or without dasatinib for 7 h during incubation with CD3-LVs. (A-C) Timelines show mean fluorescence intensity (MFI) of anti-His staining of three donors over 72 h post treatment with dasatinib, allowing detection of LVs bound to PBMC. Filled symbols and lines show the binding of LVs to cells treated with dasatinib (+dasat.), blank dots connected by dashed lines show binding of LVs to cells in absence of dasatinib. Mean ± SD of one donor measured in technical duplicates. (D) Representative histogram of fluorescence intensity of anti-His staining of one donor after 7 h, 24 h and 72 h.

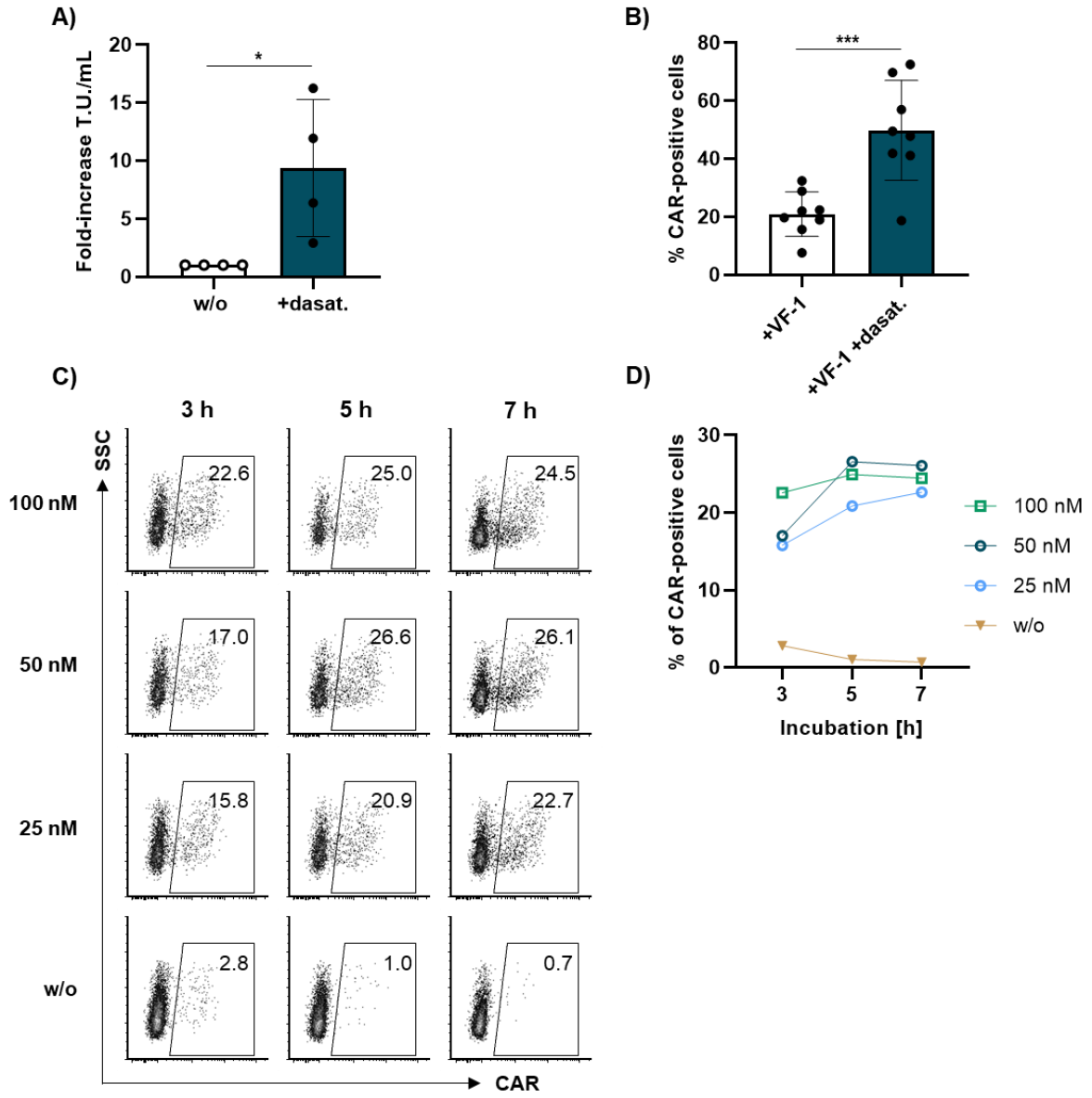

Figure S4: Dasatinib enhances CD3-targeted CD19-CAR gene delivery to activated PBMC in a time- and dose-dependent manner.

(**A**) Activated PBMC were incubated in 50 nM dasatinib-containing medium or without dasatinib for 5 h during transduction with CD3-LV. Scatter bar diagrams summarize fold-increase in T.U./mL three days post transduction, comparing transduction in presence of dasatinib to transduction without dasatinib with CD3-LV delivering a CD19-CAR. Each data point is an average value referring to a particular vector stock tested on one to four donors. Values are shown as mean ± SD. *$p < .05$, by ratio paired t-test. (**B**) Transduction of PBMC was performed in presence of VF-1 with or without dasatinib. CAR-expression of the cells was determined three days post transduction by flow cytometry and is shown as scatter bar diagrams as mean ± SD of eight donors. ***$p < .001$, by paired t-test. (**C-D**) Cells were incubated without,

25 nM, 50 nM or 100 nM dasatinib-containing media for 3 h, 5 h or 7 h during transduction. (**C**) Representative dot plots of CAR-expression on day three post transduction. (**D**) Superimposed symbols for different concentrations connected by line show CAR-expression three days post transduction of one donor.

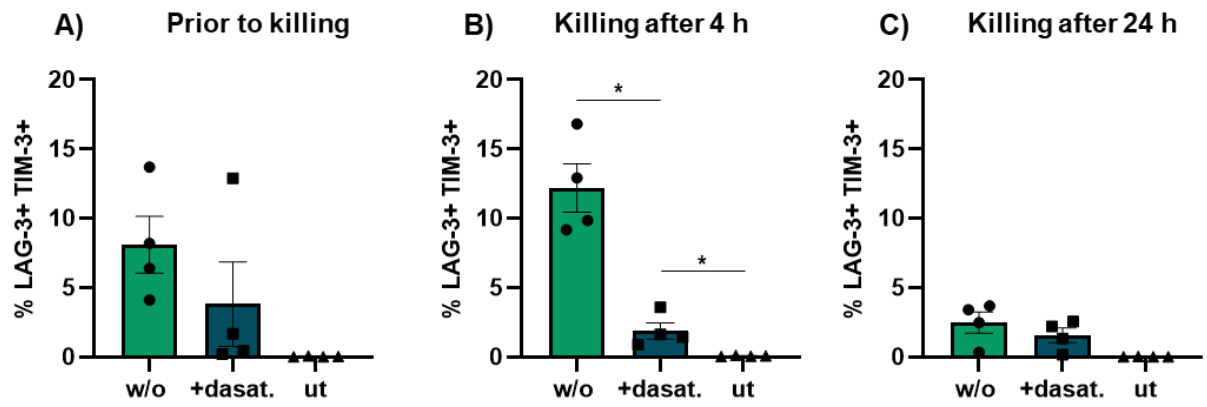

Figure S5: Coincident expression of LAG-3 and TIM-3.

Three days post transduction the level of LAG-3 TIM-3 double positive cells on T cells was determined prior to killing (= 0 h) (**A**), after 4 h of killing (**B**) and after 24 h of killing (**C**). Data is shown as mean ± SEM of four donors. *p < .05, by repeated measure one-way ANOVA followed by Tukey's multiple comparison test.

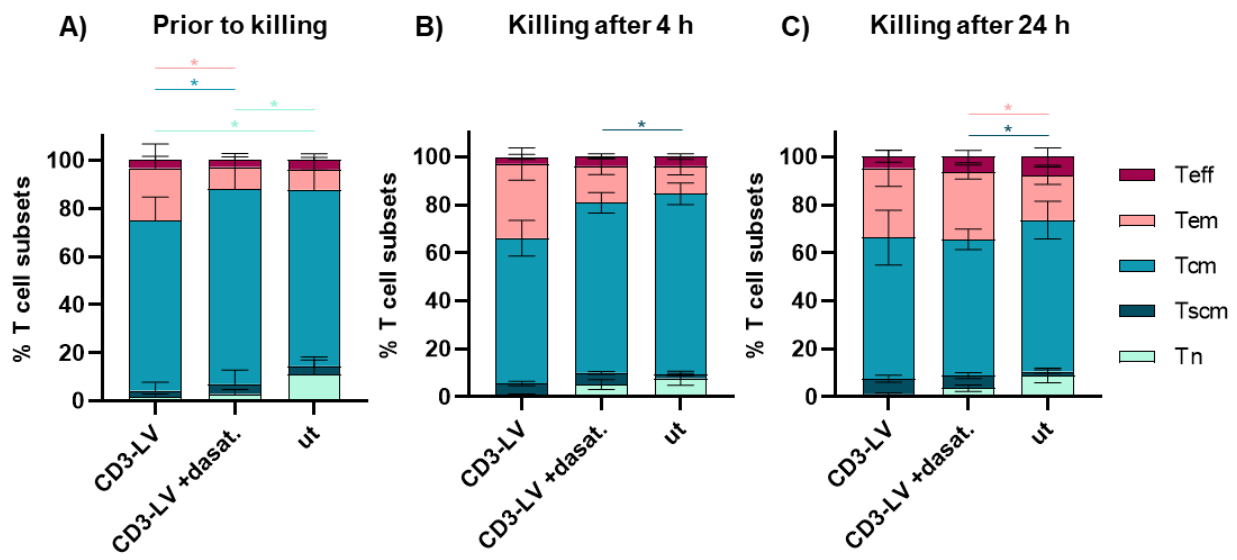

Figure S6: Dasatinib influences the T cell phenotype of transduced T cells.

Three days post transduction T cell phenotypes were determined prior to killing (**A**), after 4 h of killing (**B**) or after 24 h of killing (**C**). Data are shown as mean ± SEM of four to six donors. *$p < .05$, by repeated measure one-way ANOVA followed by Tukey's multiple comparison test.