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Supplemental information

In vivo generation of CAR T cells in the presence of human myeloid cells

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Supplemental Information

Table S1: Particle concentration and transducing units of applied LV stocks.

Vector	Particle/mL	[a]T.u./mL
[b]CD4-LV	1.3×10^{12}	[d]1.4×10^{7}
[b]CD8-LV	1×10^{12}	[e]1.2×10^{7}
[b]CD4-LV	6×10^{11}	[d]3×10^{6}
[b]CD8-LV	5.7×10^{11}	[f]5.5×10^{6}
[c]CD4-LV	4.3×10^{11}	[f]1.7×10^{7}
[c]CD4-LV^{sh}	9.9×10^{11}	[f]6.5×10^{7}
[c]CD8-LV	7.3×10^{11}	[f]2.6×10^{6}
[c]CD8-LV^{sh}	1.8×10^{12}	[f]3.7×10^{6}

[a](T.u.) Transducing units.

[b]stock used in Figure 1.

[c]stock used in Figure 5.

[d]t.u. determined on A301 cells.

[e]t.u. determined on J76S8ab cells.

[f]t.u. determined on PBMCs.

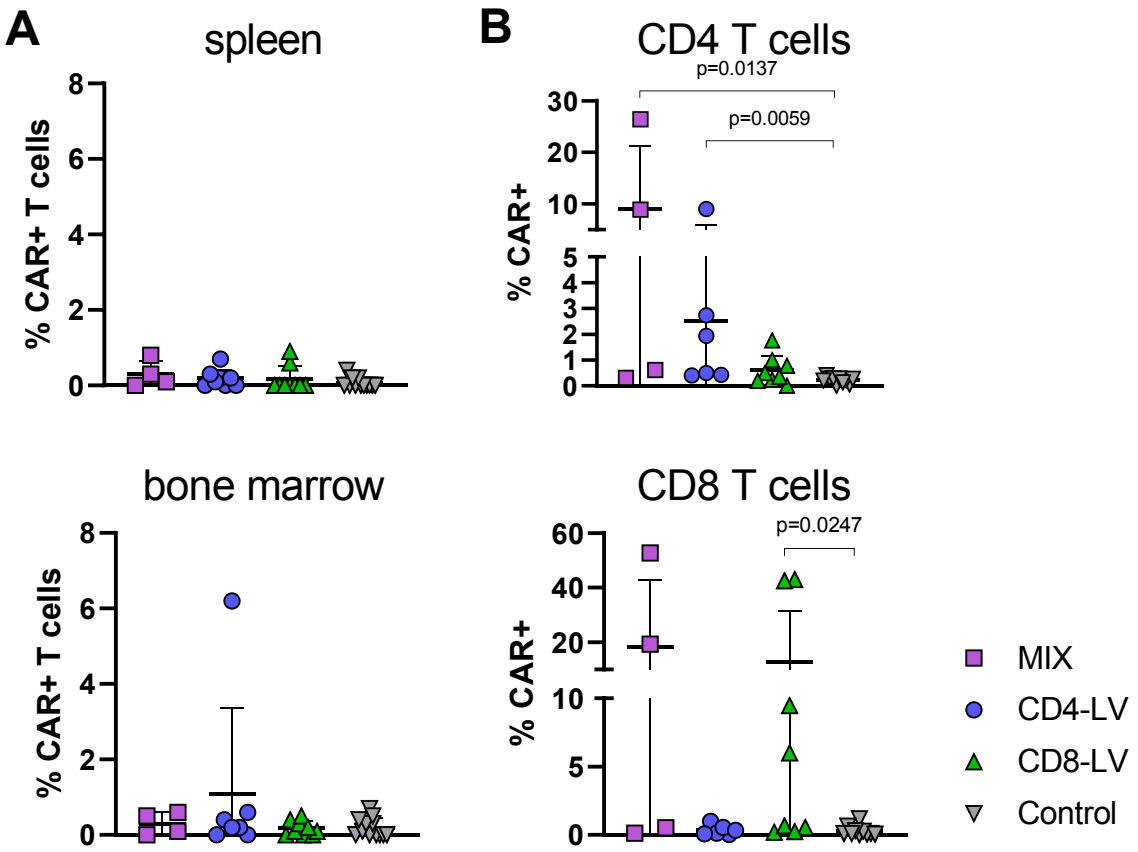

Figure S1: CAR T cells in organs and co-culture.

A) Percentage of CAR T cells in the spleen and bone marrow is shown for the different vector groups of the experiment in Fig. 1. **B**) *In vitro* expanded CAR+ T cells after further cultivation of isolated splenocytes with irradiated Raji cells. Organ data are shown for each mouse with mean and standard deviation (SD) of the group. n= 4 (MIX), 8 (CD4-LV), 9 (CD8-LV) and 12 (Control) of two experiments. Co-culture data are shown for each mouse, performed in technical triplicates, with mean and SD of the group. n= 4 (MIX), 6 (CD4-LV), 8 (CD8-LV) and 10 (Control) of two experiments. Statistics were determined by non-parametric Kruskal-Wallis ANOVA with Dunn's multiple comparisons test and significant p-values are indicated.

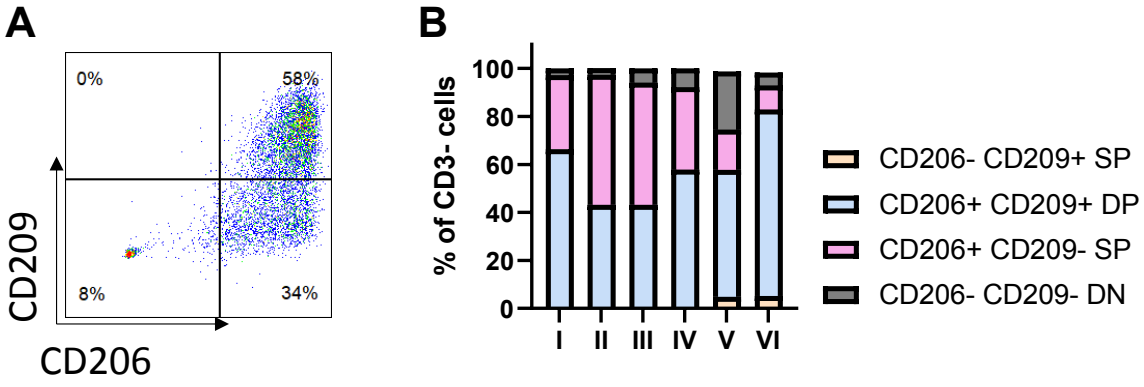

Figure S2: Characterization of polarized macrophages.

Macrophages, polarized from monocytes with GM-CSF, were characterized for surface expression of CD206 and CD209 by FACS. **A**) A representative FACS plot for macrophage polarization. **B**) Marker expression on polarized macrophages of five different donors used in four independent experiments. (SP) single positive, (DP) double positive, (DN) double negative.

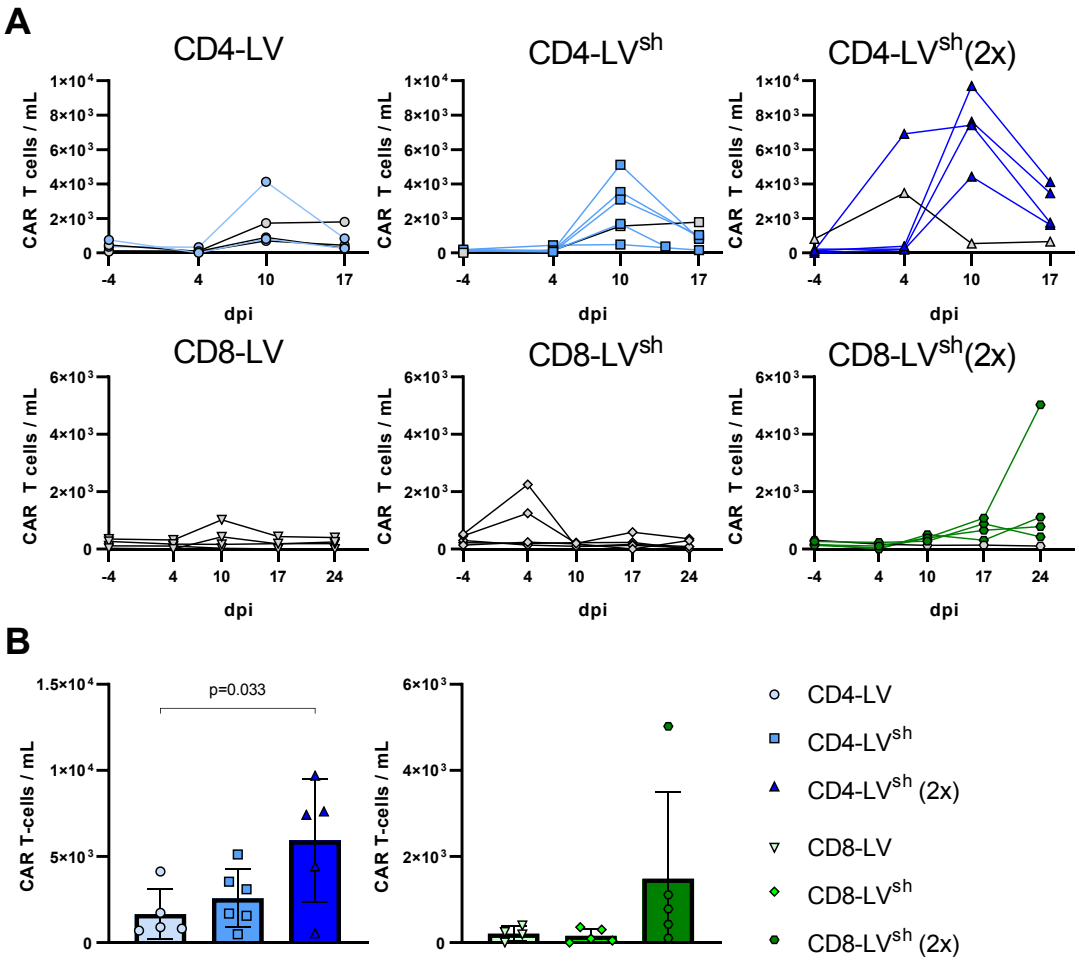

Figure S3: Absolute *in vivo* CAR T cell numbers in blood of huSGM3 mice.

See Figure 5 for experimental set up. **A**) Kinetic of CAR+ T cells in blood shown for each mouse. **B**) Side-by-side comparison of CAR T cell count for the CD4-LV group on day 10 and for the CD8-LV group on day 24. Each data point represents an individual mouse with mean and standard deviation of the group. Statistics for (**B**) were determined by one-way ANOVA with Turkey's multiple comparisons test and significant p-values indicated. n= 5 (CD4-LV), 6 (CD4-LV^{sh}), 5 (CD4-LV^{sh}(2x)), 4 (CD8-LV), 5 (CD8-LV^{sh}), 5 (CD8-LV^{sh}(2x)) in one experiment. dpi: days post injection.

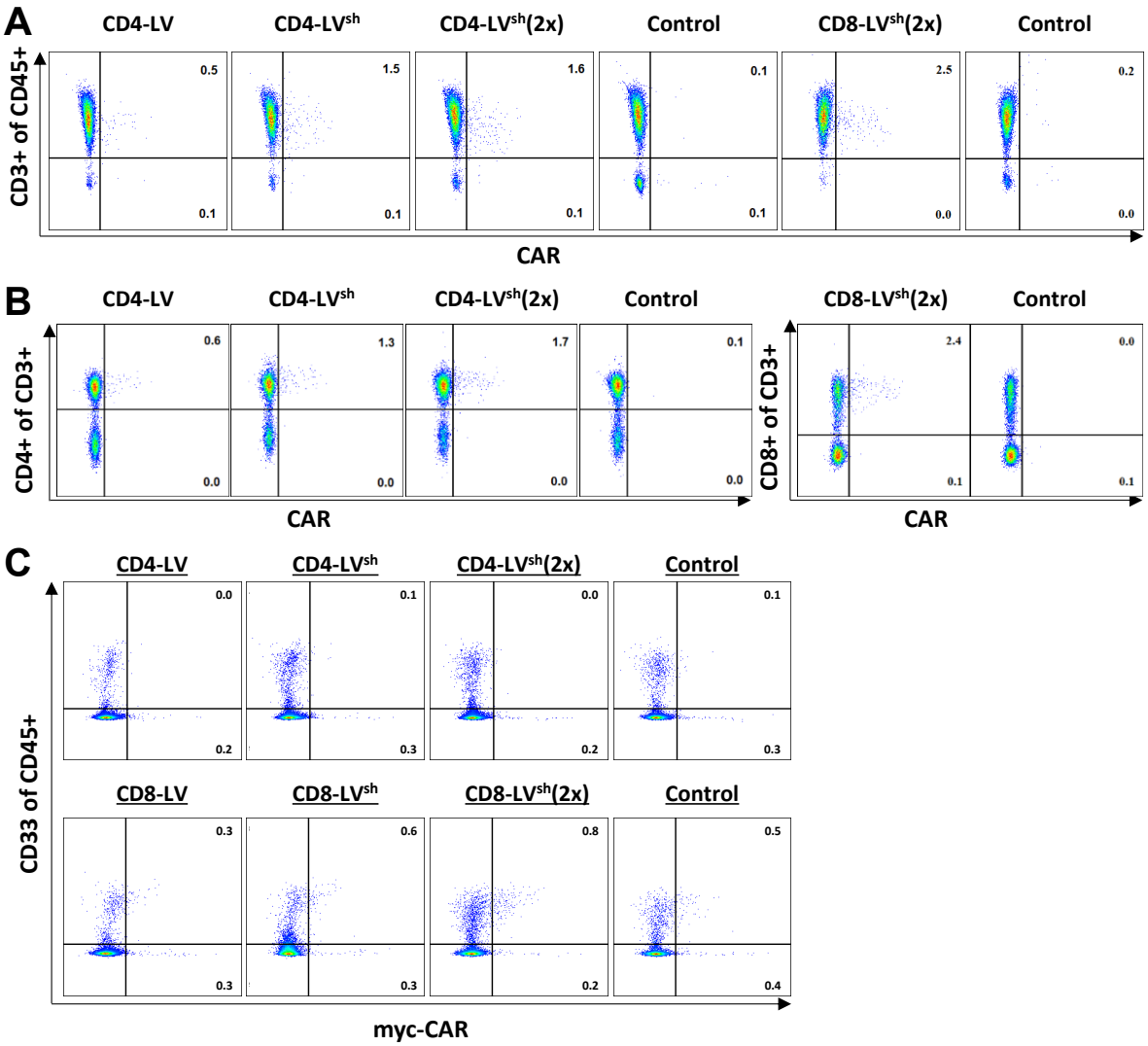

Figure S4: FACS plots of CAR signal in blood and spleen.

See Figure 5 for experimental set up. **A**) Representative FACS plots of *in vivo* generated CAR T cells in blood for human T cell specific transduction are presented as CD3+ of human CD45+ cells positive for CAR, detected via its myc-tag. **B**) Representative FACS plots from blood for specific transduction in T cell subtype are shown as CD4+ or CD8+ of CD3+ cells positive for CAR. **C**) Representative FACS plots from spleen for background signal in CD33+ of human CD45+ cells. Frequency of CAR signal as percent is indicated in the plots.

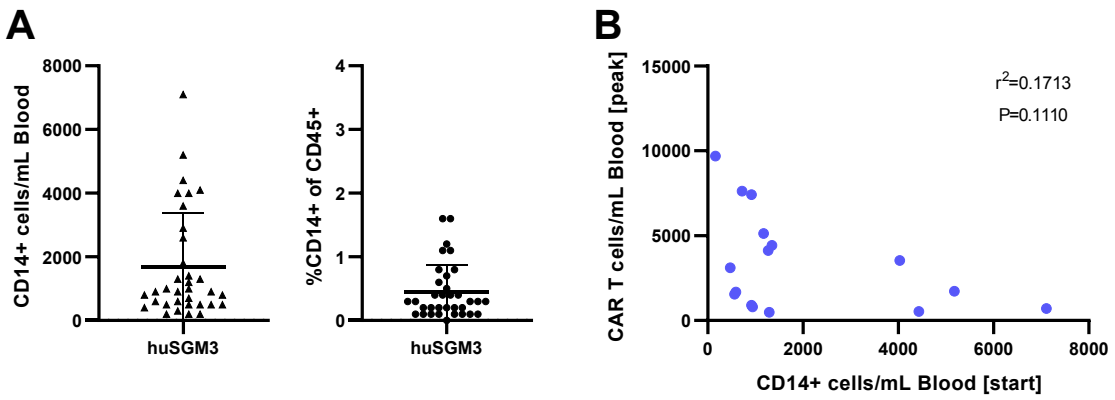

Figure S5: Correlation of monocyte level with *in vivo* CAR T cell generation by CD4-LV.

A) Blood of all huSGM3 mice from Figure 5 were analyzed for human CD14+ cells before experiment start (day -4) by flow-cytometry analysis. CD14+ Cells were pre-gated for human CD45+, human CD3- and human CD19- cells. Individual mice are shown as data point with mean and standard deviation for n=34. **B)** Correlation of CD14+ cell number in the blood before experiment start (day -4) with *in vivo* generated CAR T cell numbers in the blood on day 10 for CD4 targeted LVs. Individual mice are shown as data point for n=16. Correlation in **(B)** was determined by Pearson correlation analysis with indicated r^2 and P value.

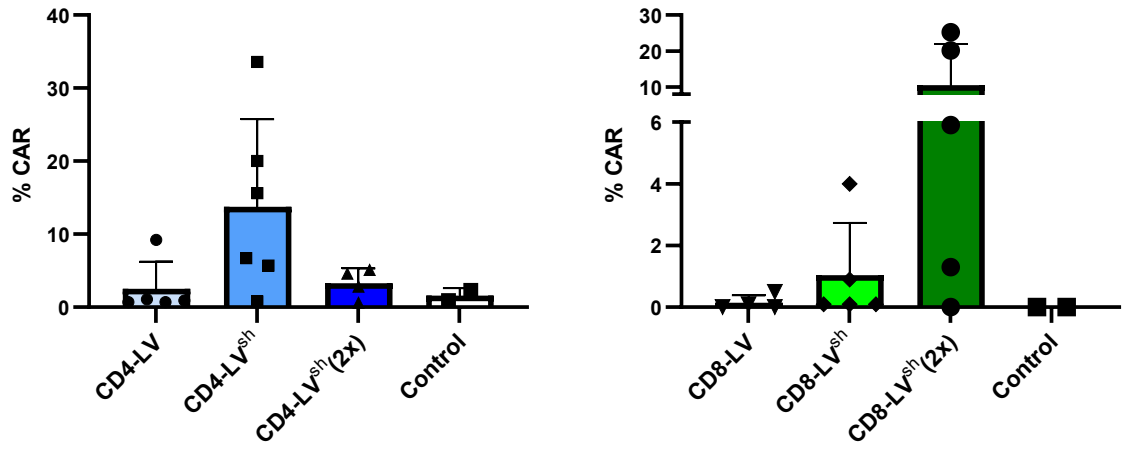

Figure S6: *In vitro* CAR T cell expansion from splenocytes.

See Figure 5 for experimental set up. Expansion of CAR T cells from splenocytes of LV injected huSGM3 mice upon co-culture with irradiated Nalm6 tumor cells for seven days. Percentage of expanded CAR+ T cells of the respective subpopulation is shown for each mouse as data point with mean and standard deviation of the group. Statistics were determined by one-way ANOVA with Dunnett's multiple comparisons test.

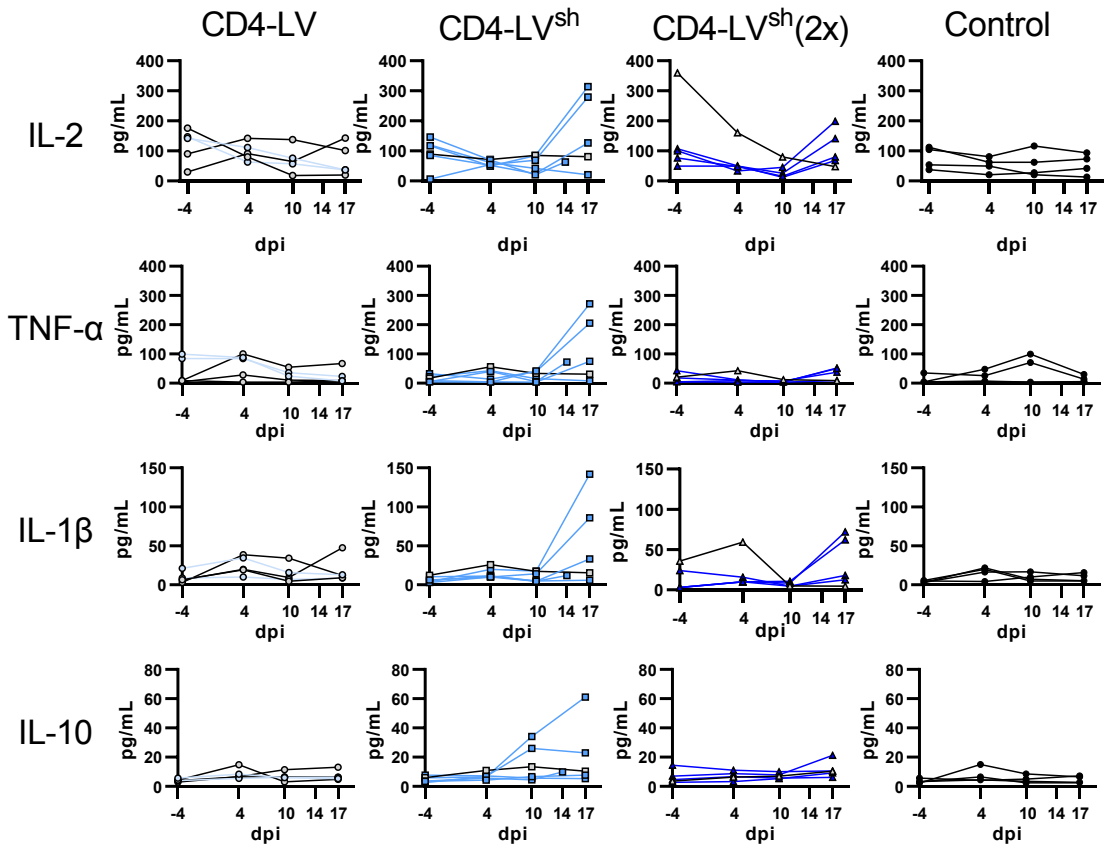

Figure S7: Kinetic of further plasma cytokines in CD4-LV injected huSGM3 mice.

See Figure 5 for experimental set up. Plasma cytokines of huSGM3 mice injected with the indicated LVs were determined by bead-based multi-analysis kit. The concentrations for each cytokine over time are shown for each mouse. Mice determined as CAR negative in blood are depicted in grey with black connecting lines. n= 5 (CD4-LV), 6 (CD4-LV^{sh}), 5 (CD4-LV^{sh} (2x)) and 4 (Control) from one experiment. dpi: days post injection.

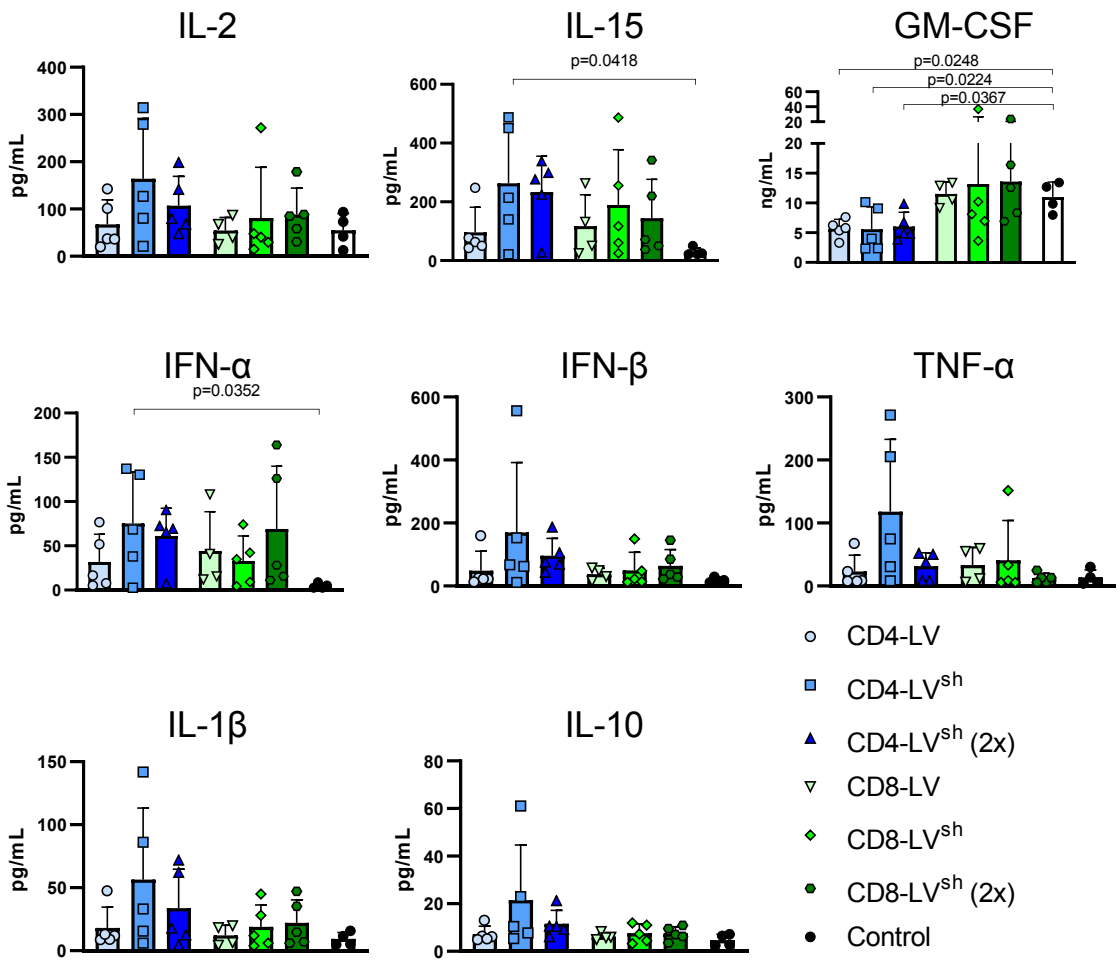

Figure S8: Plasma cytokines in LV injected huSGM3 mice on day 17.

See Figure 5 for experimental set up. Plasma cytokines of huSGM3 mice on day 17 after vector application were measured by bead-based multi-analysis kit. Individual mice are shown as data point with mean and standard deviation of the group. Statistics were determined by one-way ANOVA with Dunnett's multiple comparisons test with indicated significant p-values. n= 5 (CD4-LV), 6 (CD4-LV^{sh}), 5 (CD4-LV^{sh} (2x)), 4 (CD8-LV), 5 (CD8-LV^{sh}), 5 (CD8-LV^{sh}(2x)) and 4 (Control) from one experiment.