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Supplemental Information

IL12 integrated into the CAR exodomain converts CD8$^+$ T cells to poly-functional NK-like cells with superior killing of antigen-loss tumors

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Supplementary Figures

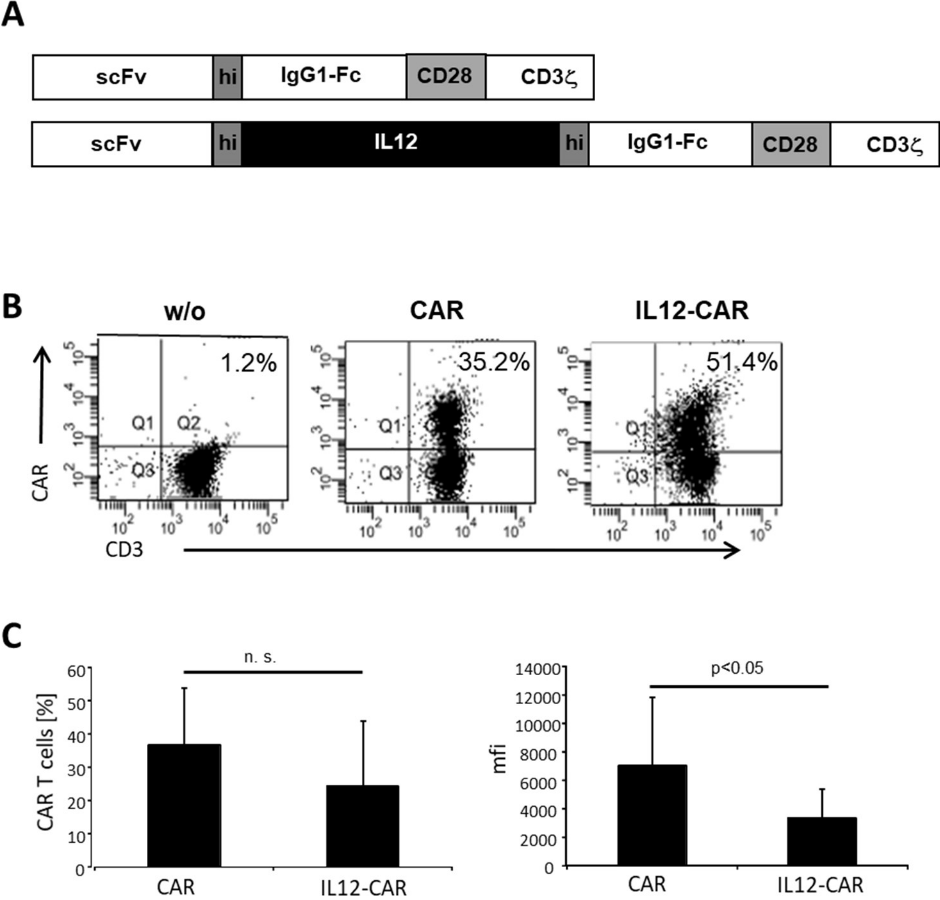

Figure S1

The IL12-CAR was expressed on the surface of peripheral blood T cells.

(A) Peripheral blood lymphocytes from healthy donors were retrovirally transduced for expression of the anti-CEA IL12-CAR or the homologous CAR without IL12. The CAR was detected by the anti-IgG antibody that binds to the extracellular spacer. A representative dot blot is shown.

(B) CAR expression by T cells. Figures represent mean values of the number of CAR T cells and mean fluorescence intensity (mfi) of the CAR in arbitrary units. The same numbers of T cells express the IL12-CAR, however, at lower levels per cell compared with the CAR without IL12. Data represent mean values of 10 healthy donors ± SD. Statistical analysis was performed by the Student´s T test; n.s., not significant.

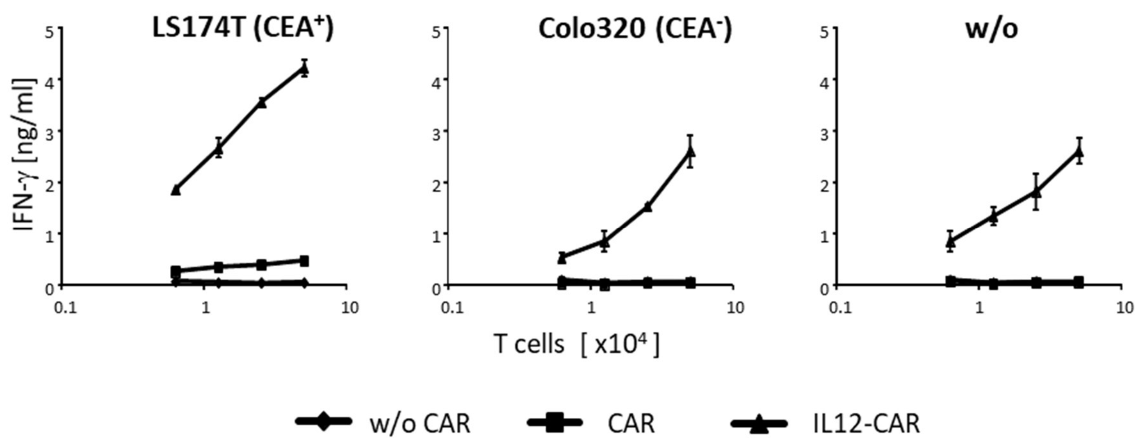

Figure S2

IL12-CAR induced IFN-γ secretion independently of CAR engagement of target.

T cells with and without engineered CAR (each 0.625-5x10^4 cells/well) were co-cultivated for 48 h in 96 micro-test plates with CEA^+ LS174T or CEA^- Colo320 tumor cells (each 2.5x10^4 cells/well) and without tumor cells (w/o), respectively. Supernatants were tested for IFN-γ by ELISA. Numbers represent mean values of technical triplicates ± SD. Data of a representative experiment out of 3 are shown.

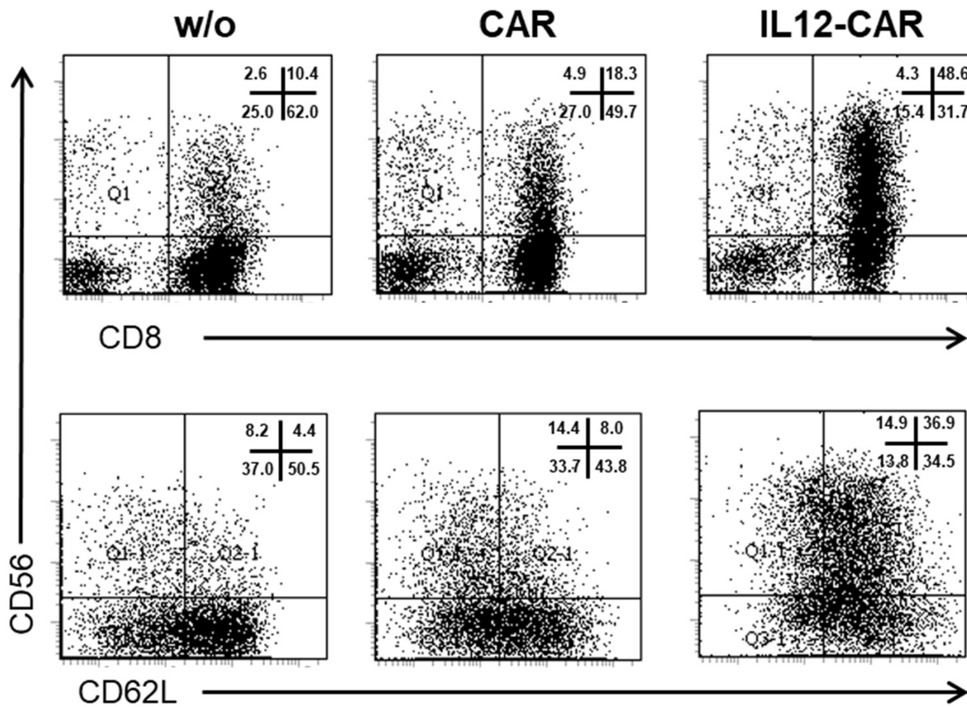

Figure S3

IL12-CAR T cells acquired a $CD8^{+}CD56^{+}CD62L^{high}$ phonotype.

Peripheral blood lymphocytes were engineered to express the conventional CAR or the IL12-CAR, both of same specificity for CEA, and tested 4-6 days after transduction for $CD8^{+}CD56^{+}CD62L^{high}$ T cells by flow cytometry. Non-transduced T cells (w/o CAR) served as control. Dot blots of a representative blood donor are shown.

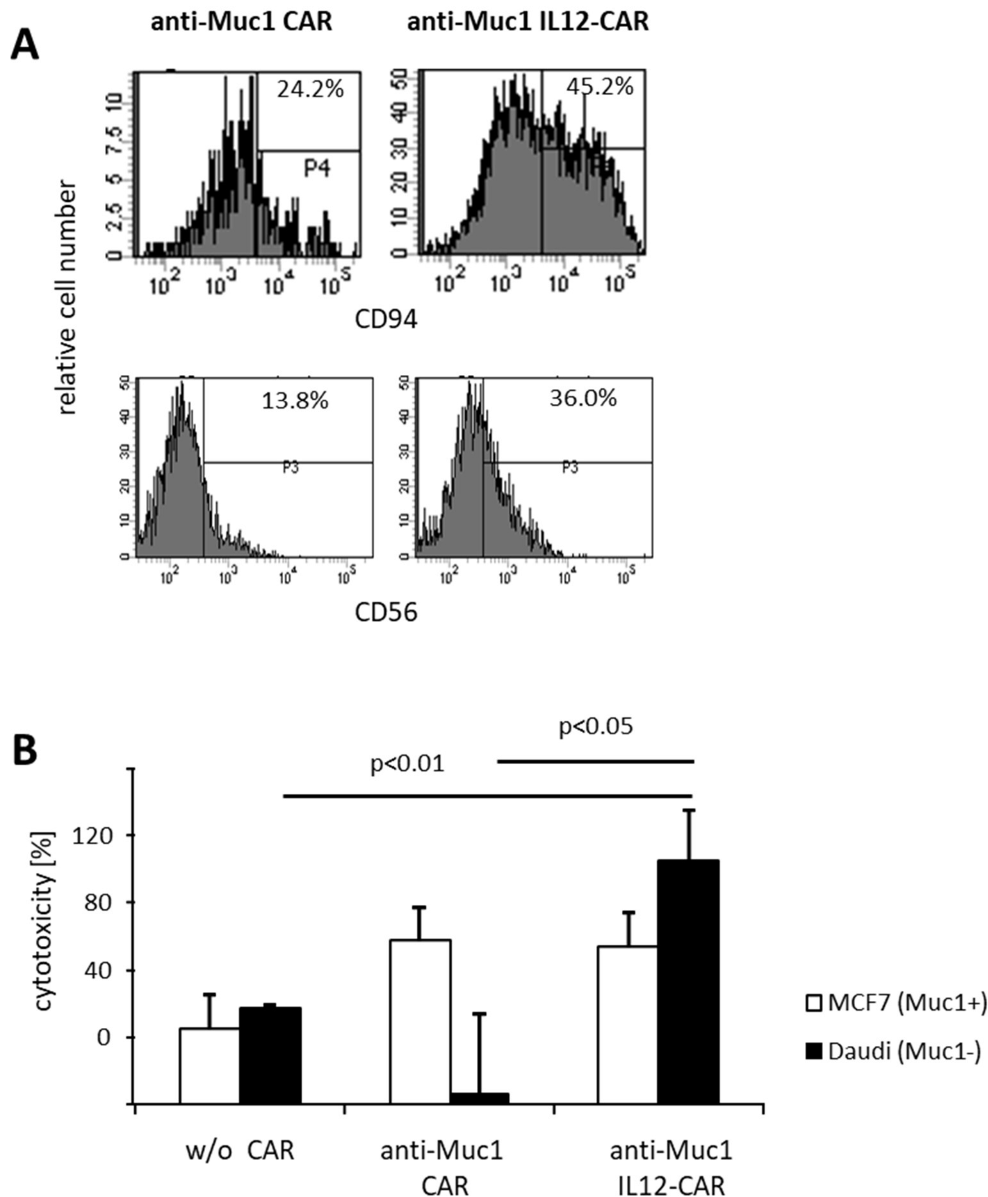

Figure S4

Acquisition of a CD94^{+}CD56^{+} phenotype and antigen-independent NK-like cytotoxicity by IL12-CAR T cells with specificity for Muc-1.

(A) T cells were engineered with a conventional CAR or IL12-CAR, each with specificity for Muc-1. CAR T cells were detected by the anti-IgG1 antibody that binds to the common extracellular spacer, gated and analyzed by flow cytometry 4 and 7 days post-transduction for CD94 and CD56 expression, respectively. Histograms of a typical experiment are shown.

(B) T cells with Muc1 specific conventional CAR or IL12-CAR were co-cultivated (2x10^{4} T cells/well) for 48 h with Muc1^{+} MCF7 or Muc1^{-} Daudi tumor cells (2x10^{4} T cells/well). Viability

of target cells was determined by the XTT assay and specific cytotoxicity was calculated. Numbers represent mean values of technical replicates ± SD. Representative results of two independent experiments are shown. Statistical differences were determined by Student´s T test.