cancers

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Article

Defucosylation of Tumor-Specific Humanized Anti-MUC1 Monoclonal Antibody Enhances NK Cell-Mediated Anti-Tumor Cell Cytotoxicity

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Supplementary Materials

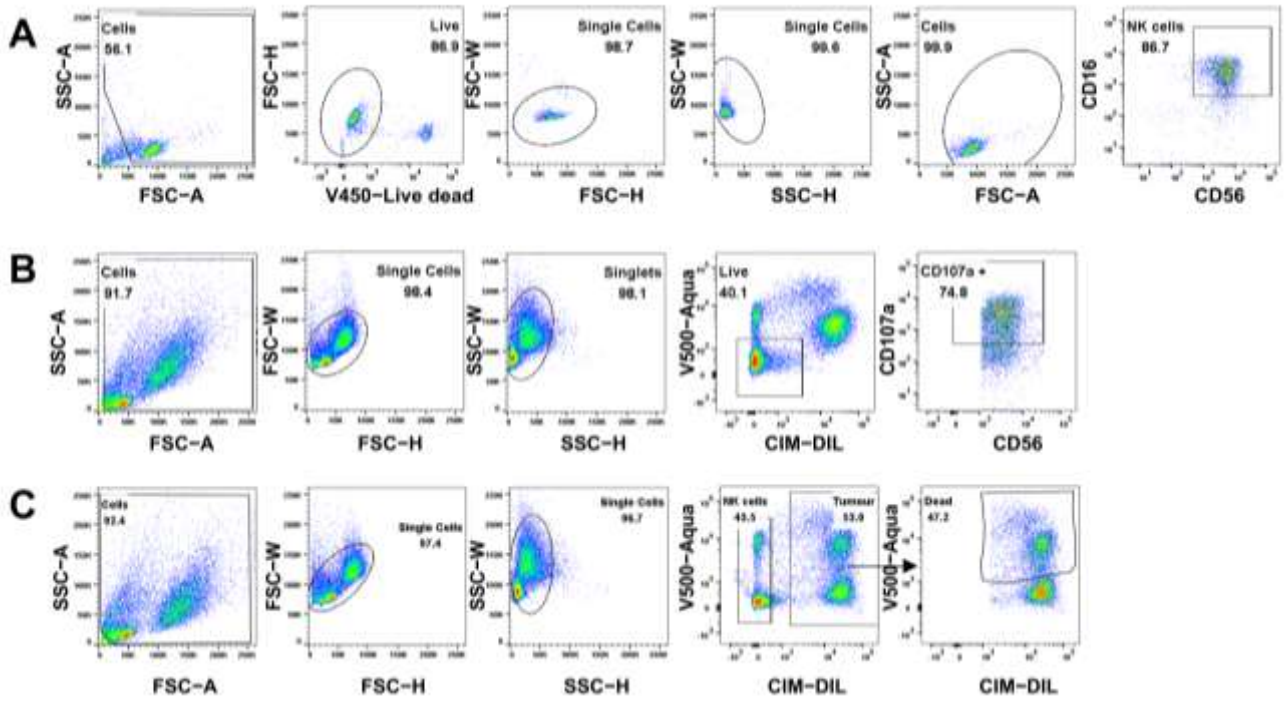

Figure S1. Flow cytometric analysis of NK cell purity, degranulation and cytotoxicity. (**A**) Representative sample of flow cytometric analysis of NK cell purity and CD16 expression after NK cell isolation using negative selection. (**B**) Example of flow cytometric analysis of NK cell degranulation, measured as CD107a expression. (**C**) Example of flow cytometric analysis of NK cell cytotoxicity, measured as the percentage of tumor cell death. Tumor cells were labeled with the dye CIM-DIL. Numbers in plots indicate gate frequencies.

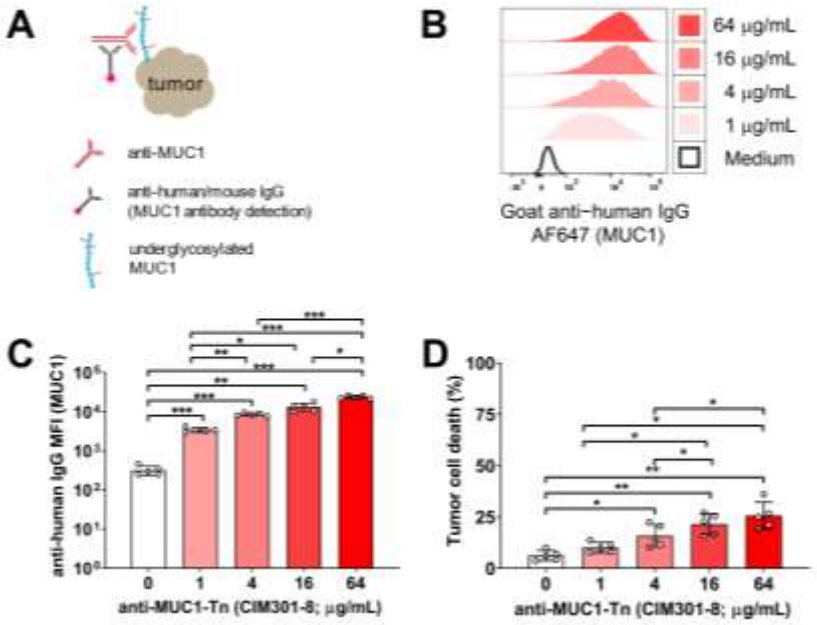

Figure S2. Higher concentrations of humanized anti-MUC1 antibodies induce Jurkat cell death. (**A**) Schematic overview of the analysis of the effects of increasing concentration of defucosylated CIM301-8 anti-MUC1-Tn/STn antibodies on Jurkat tumor cells. (**B**) Overlay histogram showing binding of anti-MUC1 antibodies (CIM301-8; defucosylated Fc-tail) to Jurkat cells, detected using a labeled anti-human IgG antibody. One representative sample is shown. (**C**) Quantification of anti-MUC1 CIM301-8 binding to Jurkat cells as described in B, expressed as median fluorescence index. (**D**) Quantification of anti-MUC1-induced cell death of Jurkat cells after incubation with increasing concentrations of CIM301-8 antibodies (without NK cells). Differences between groups were calculated using one-way ANOVA with Tukey's multiple comparisons test. Not significant (n.s.); $p < 0.05$ (*); $p < 0.01$ (**); $p < 0.0001$ (***).

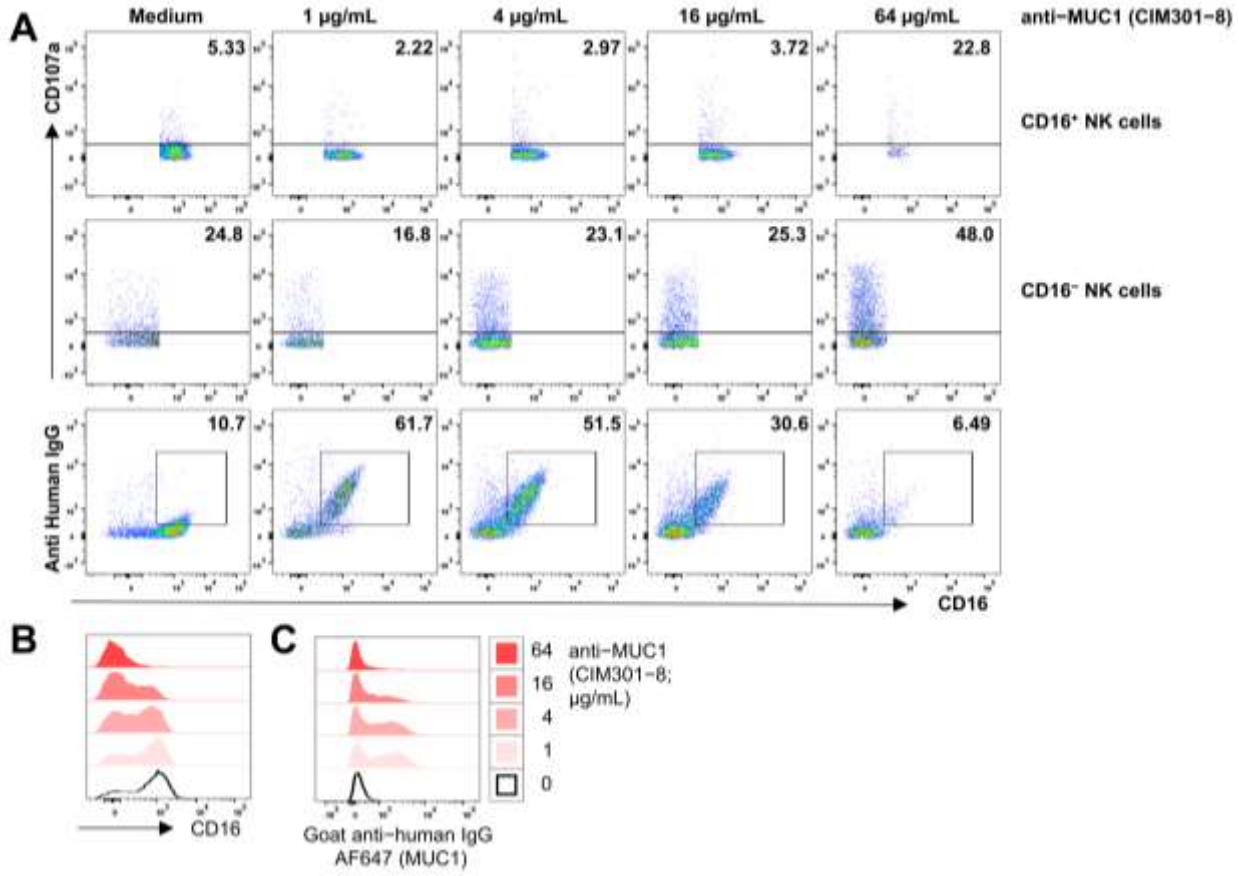

Figure S3. Increasing concentrations of humanized anti-MUC1 antibodies activate NK cells and induce down-regulation of CD16 expression. (**A**) Representative flow cytometry dot plots of CD107a expression on CD16 $^{+}$ (top row) and CD16^{-} (middle row) NK cells and the CIM301-8 binding (bottom row). Human NK cells were incubated with increasing concentrations of anti-MUC1 (CIM301-8; defucosylated Fc-tail). Increasing concentrations of defucosylated CIM301-8 anti-MUC1 antibody incubated with primary human NK cells only for 4h. Binding of the MUC1 antibody was determined using flow cytometric analysis labeled anti-human IgG antibodies. (**B**) Overlay histogram of CIM301-8 binding via the Fc tail on NK cells with increasing CIM301-8 concentrations. (**C**) Representative overlay histogram of CD16 expression on NK cells after incubation with CIM301-8 antibody. In all panels, one representative sample is shown.

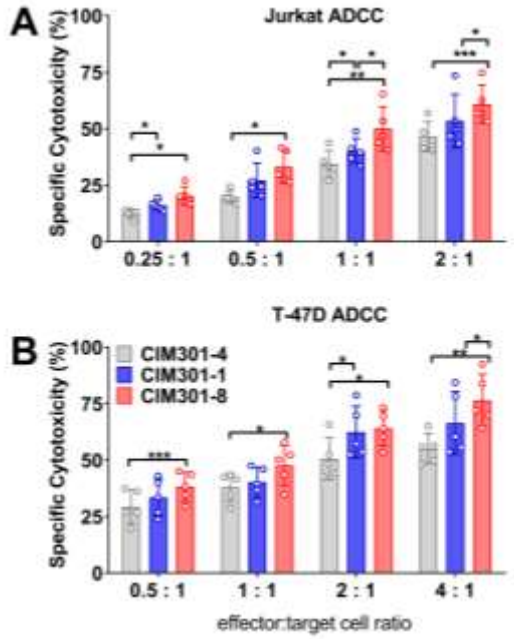

Figure S4. Humanized anti-MUC1 antibody enhances NK cell cytotoxicity via ADCC. (**A**) Quantification of antibody-dependent cellular cytotoxicity against Jurkat cells at different E:T ratios and in the presence of the indicated antibody at 1µg/mL after 4h of co-culture. Bars indicate mean ± SD, dots are 5 individual NK cell donors from independent experiments. (**B**) As A, with T-47D as tumor cells. Statistical analysis using two-way ANOVA plus Tukey's multiple comparisons test. Not significant (n.s.); $p < 0.05$ (*); $p < 0.01$ (**); $p < 0.001$ (***).

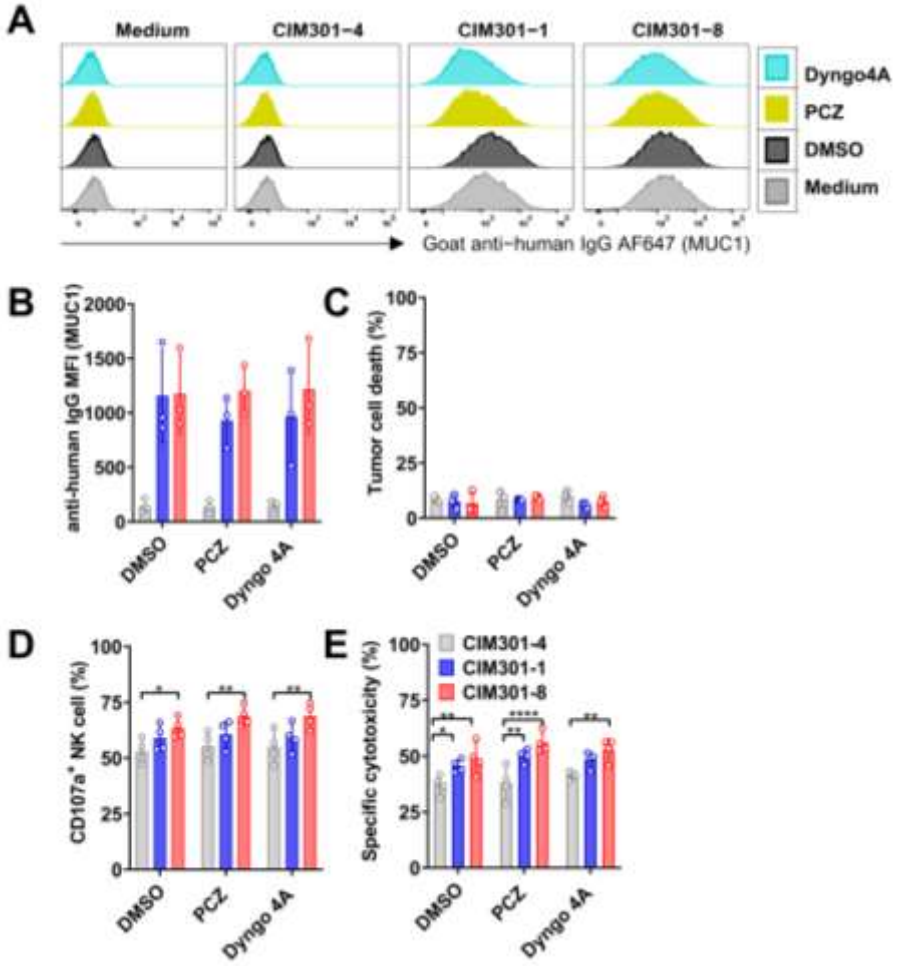

Figure S5. The endocytosis inhibitors PCZ and Dyngo4A neither upregulate the antigens on the tumor surface nor enhance the ADCC of Jurkat cells. (**A**) Overlay histograms of flow cytometric analysis of MUC1 epitope expression on Jurkat tumor cells in the presence of endocytosis inhibitors using regular (CIM301-1) or defucosylated (CIM301-8) anti-MUC1 antibodies or an irrelevant control antibody (CIM301-4). Anti-human IgG antibodies were used to detect antibody binding on tumor cells. Endocytosis inhibitors were dissolved in DMSO, here used as a negative control. One representative sample is shown. (**B**) Flow cytometric quantification of MFI of MUC1 expression levels on Jurkat cells after treatment with endocytosis inhibitors as described in (**A**). Differences between control antibody (CIM301-4; grey bars) and anti-MUC1 antibodies (regular CIM301-1 in blue and defucosylated CIM301-8 in red) were all statistically significant with $p < 0.05$ or smaller. (**C**) Viability of Jurkat tumor cells after incubation with anti-MUC1 antibodies with or without endocytosis inhibitors. Pooled data from 3 independent experiments performed at different timepoints. (**D**) Fraction of $CD107a^+$ degranulating human NK cells in co-cultures with Jurkat tumor cells at an effector/target ratio of 1:1 in the presence of anti-MUC1 antibodies and endocytosis inhibitors. NK cells and tumor cells were incubated for 4 h, with endocytosis inhibitors (5 μM PCZ, 30 μM Dyngo4A and 0.1% (*v*/*v*) DMSO) added during the last hour. (**E**) Antibody-dependent NK-cell mediated cytotoxicity against Jurkat tumor cells. Experimental setup as in (**D**). Panels (**D**) and (**E**) show pooled data from 4 independent experiments with different donors, performed at different time points. Differences between groups were determined using two-way ANOVA with Tukey's multiple comparisons test. Not significant (n.s.); $p < 0.05$ (*); $p < 0.01$ (**); $p < 0.0001$ (****).