Supplementary Figures

Novel lectin-based chimeric antigen receptors target Gb3-positive tumour cells

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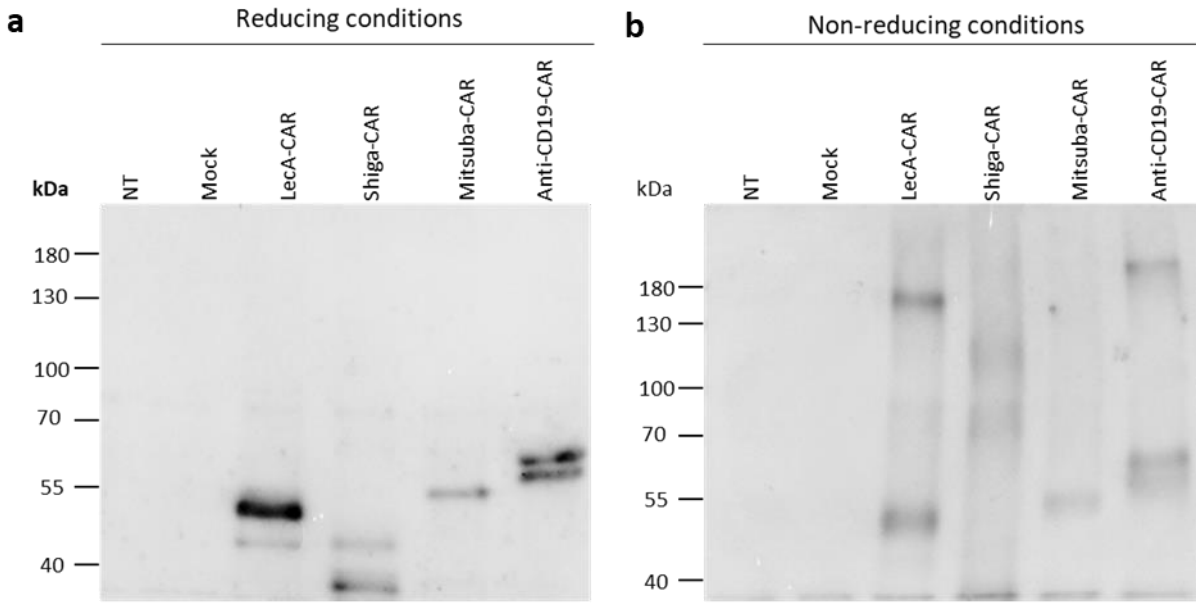


Fig. S1 Incorporation of the selected CARs in PBMCs. For the analysis, non-transduced (NT) and transduced (with empty vector) mock cells were included as controls, in addition to the panel of the lectin-CARs: LecA-CAR, Shiga-CAR, Mitsuba-CAR and anti-CD19-CAR. Primary T cells were lysed either with reducing SDS buffer (**a**)or non-reducing buffer (**b**)plus 5 μl of urea. Under reducing conditions, all CARs exhibited the expected sizes. Meanwhile, higher molecular weight complexes were observed in non-reducing gels. Despite the presence of identical cysteines in the hinge and transmembrane regions of all CARs, each CAR construct displayed a distinct pattern of oligomerization.

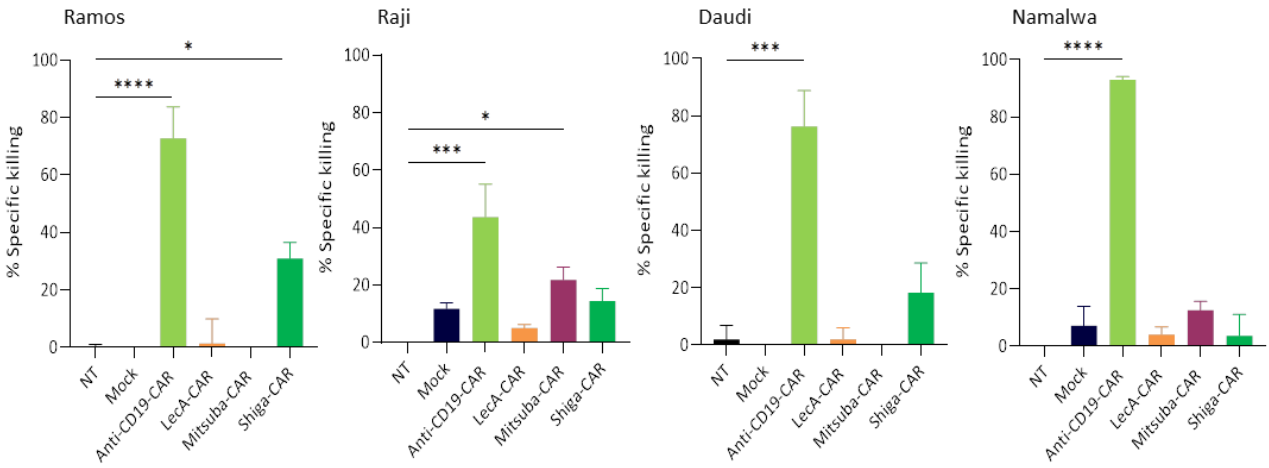


Fig S2 *In vitro* tumour cell killing by CAR T cells. Burkitt's lymphoma-derived cells lines. After 17 h, the anti-CD19 CAR showed the most prominent cytotoxic activity in all cell lines. The Shiga-CAR specific killing increased and showed 30% for Ramos cells, 14.4% for Raji cells and 18% for Daudi cells. n=3. One-way ANOVA followed by Dunnett's multiple comparisons test. Mean values ± s.e.m. are shown. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. Data represent at least three independent donors.

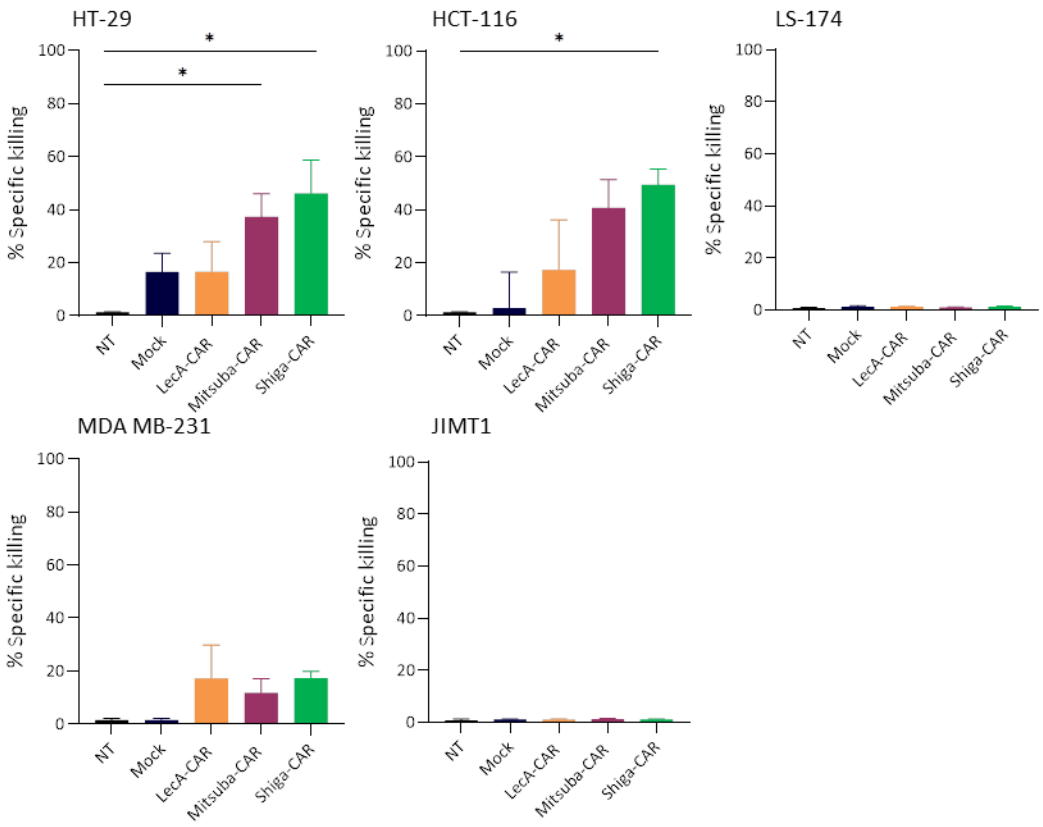


Fig. S4 In vitro solid tumour cell killing by CAR T cells. a Lectin-CARs specific killing against colorectal cancer cell lines (HT-29, HTC-116, LS-174) and breast cancer cell lines (MDA-MB-231 and JIMT1) was checked by bioluminescence assay. After 17 h, the Shiga-CAR and the Mitsuba-CAR appeared to be the best in killing colorectal cancer cells. One-way ANOVA followed by Dunnett's multiple comparisons test. Mean values ± s.e.m. are shown. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. Data represent at least three independent donors.

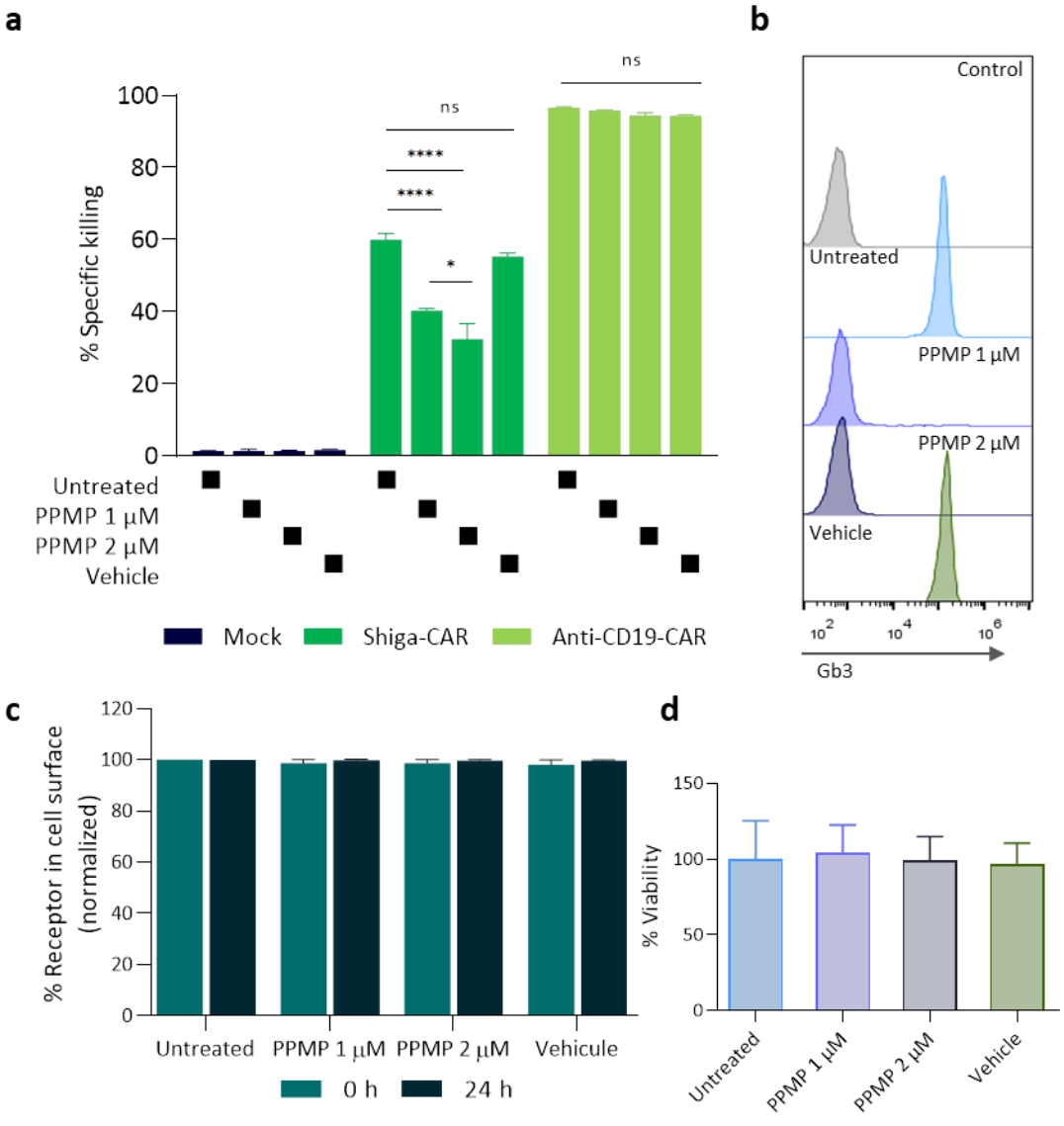


Fig. S3 Lectin-CARs specifically recognize Gb3. **a** Gb3 surface expression assayed by flow cytometry in Ramos cells treated with PPMP as indicated. Gb3 was detected by staining with labeled StxB at day 3. Data represent one experiment out of three independent experiments. **b** Ramos Gb3$^+$ cells were treated with PPMP for three days and then co-incubated for 24 h with the indicated effector cells: mock, Shiga-CAR and anti-CD19-CAR T cells. Two-way ANOVA followed by Dunnett's multiple comparisons test. Mean values ± s.e.m. are shown. n=3, simultaneously performed co-incubation. *$p < 0.05$, **$p < 0.01$ ***$p < 0.001$, ****$p < 0.0001$, ns, not significant. Representative data of one out of two independent donors **c** CAR expression on the cell surface of T cells was assayed by FLAG-tag staining and flow cytometry analysis upon PPMP treatment for 24 h. Mean values ± s.e.m. are shown. Representative data of one out of two independent donors. **d** PPMP cytotoxicity was assessed using a viability assay in Ramos cells at day 3. Mean values ± s.e.m. are shown. One-way ANOVA followed by Dunnett's multiple comparisons test. *$p < 0.05$, **$p < 0.01$ ***$p < 0.001$, ****$p < 0.0001$. Data represent at least three independent experiments.

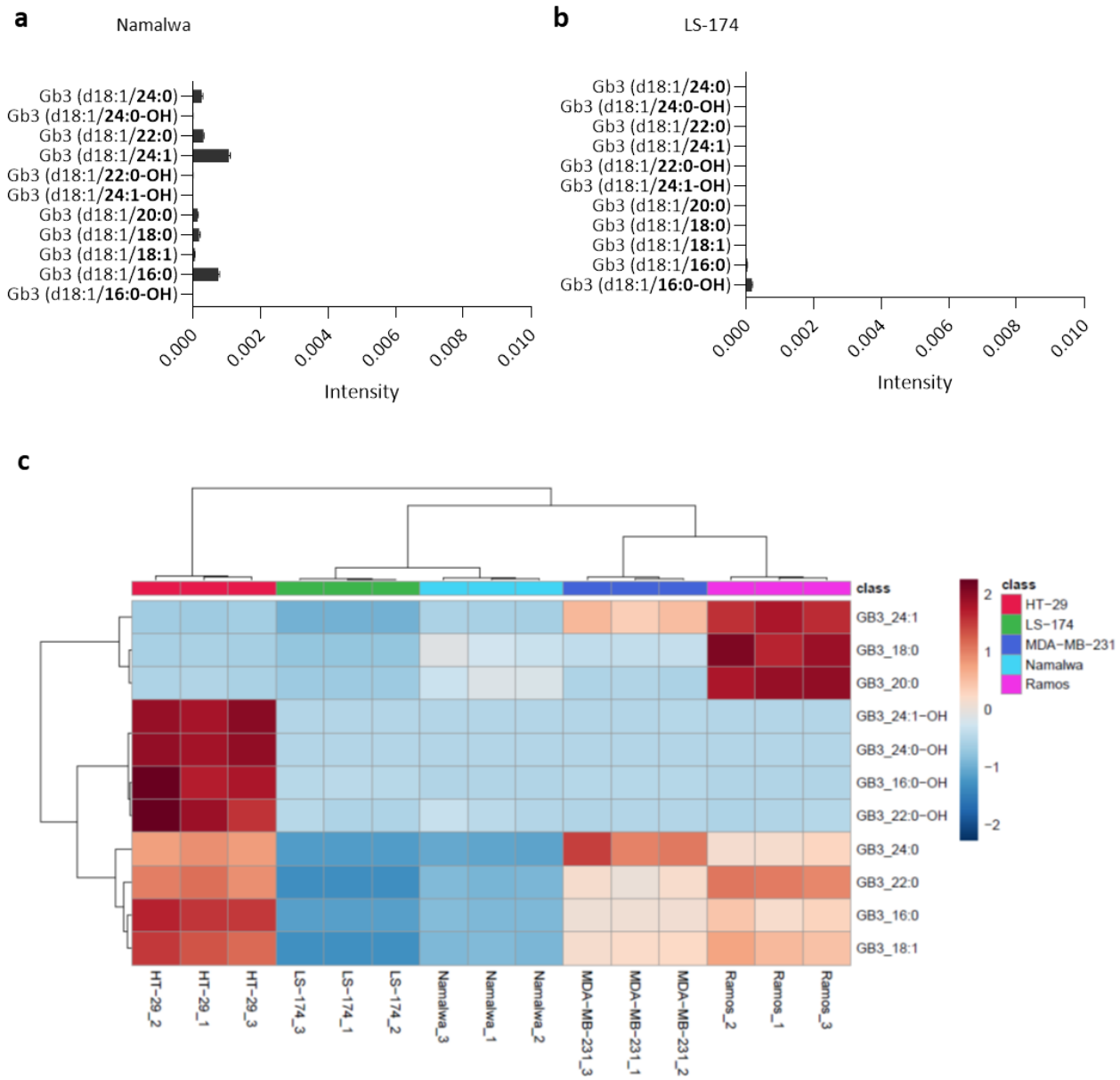
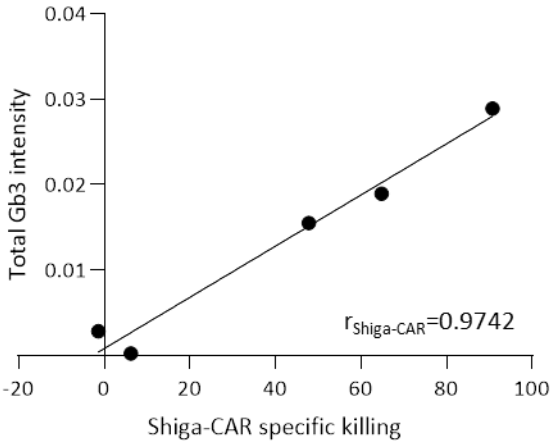
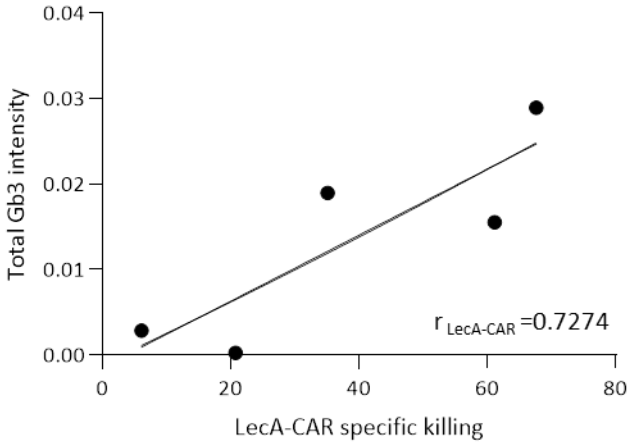


Fig. S5 Lipid analysis by MS of model cell lines. The analysis revealed the total Gb3 abundance and identified the predominant isoforms for each cell line. a-b Gb3 isoforms present in Namalwa (a) and LS-174 cells (b). Mean values ± s.e.m. are shown. n=3, analyzed samples in one LC-MS sequence. c Heat map of Gb3 content normalized to the internal standard (IS) and the sum of all measured GSL. Range-scaled z scores are displayed.





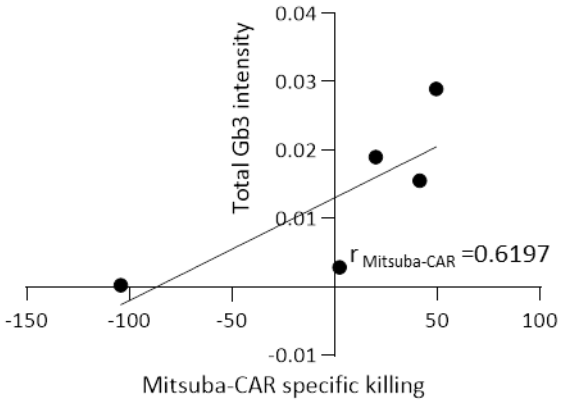


Fig. S6 Pearson correlation between the total Gb3 content of each cell line and the specific killing of the different lectin-CAR T cells. The total Gb3 content considers the sum of the intensities of all Gb3 isoforms detected on each cell line (HT-29, Ramos, MDA-MB-231, Namalwa and LS-174) by MS analysis. The correlation analysis revealed a strong Gb3 dependency for the Shiga-CAR T cell-induced cytotoxicity and moderate dependency for LecA-CAR T cell and Mitsuba-CAR T cells. The higher is the abundance of Gb3 on the target cells; the higher is the cytotoxicity.