TITLE: Antitumor effects of the GM3(Neu5Gc) ganglioside-specific humanized antibody 14F7hT against *Cmah*-transfected cancer cells.

Running title: Targeting of GM3(Neu5Gc)-expressing *Cmah*-transfected cells by a humanized antibody.

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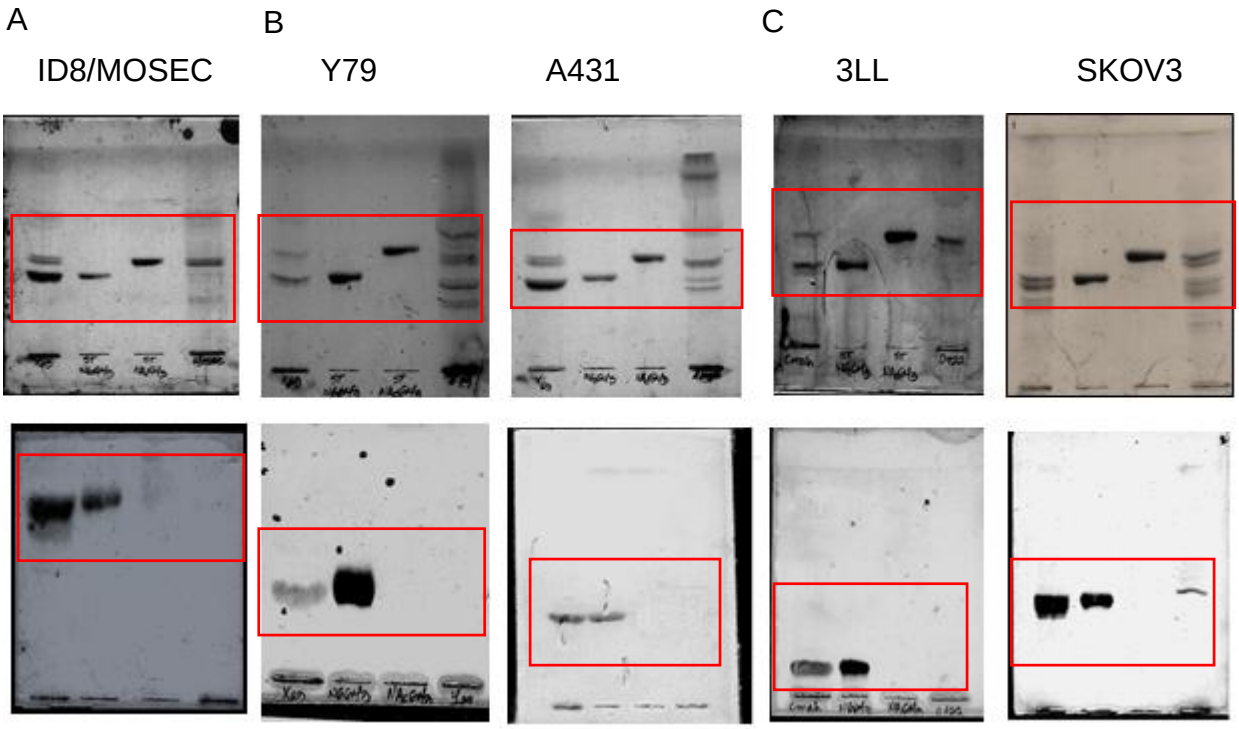
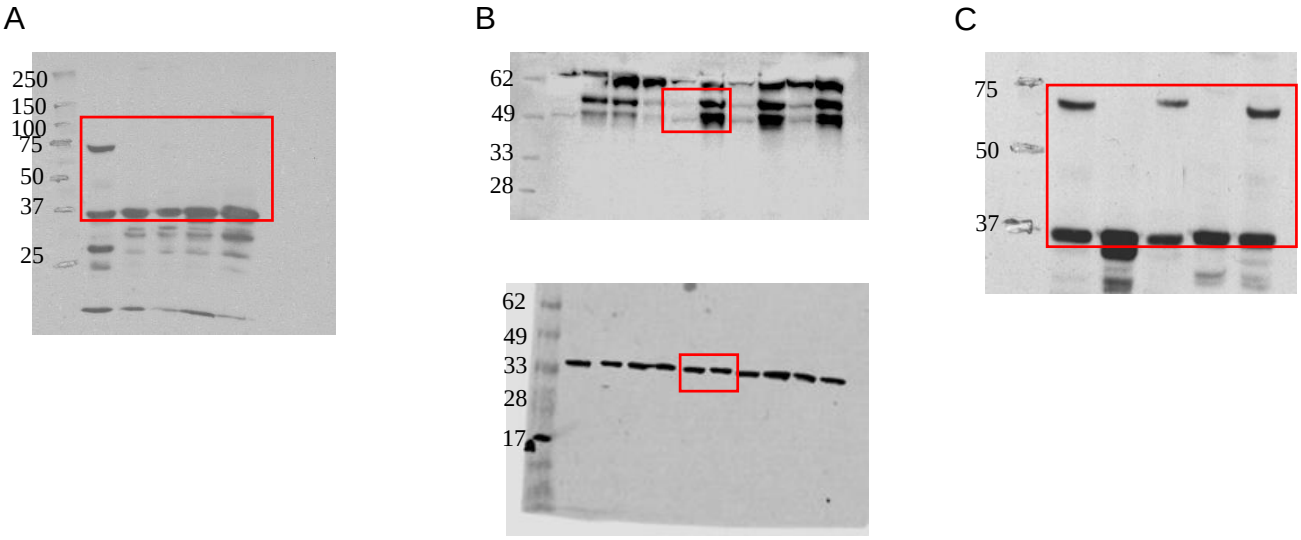
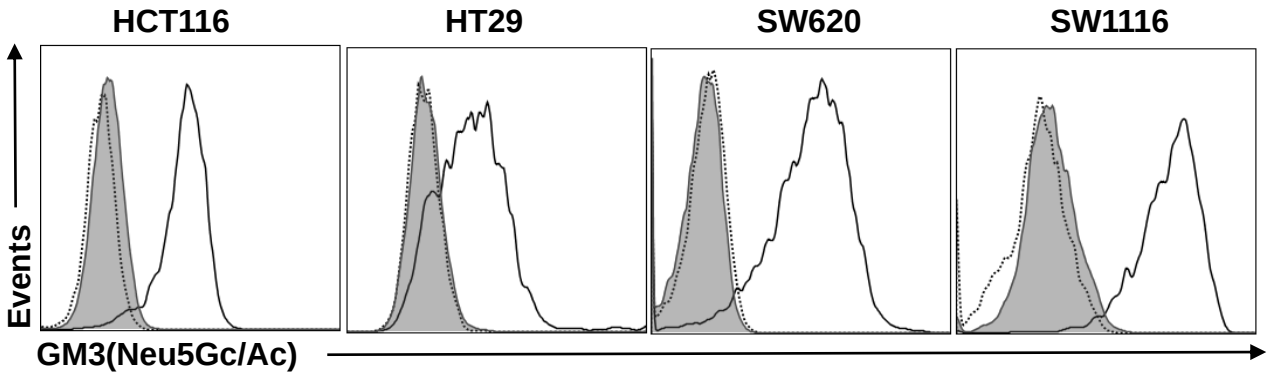


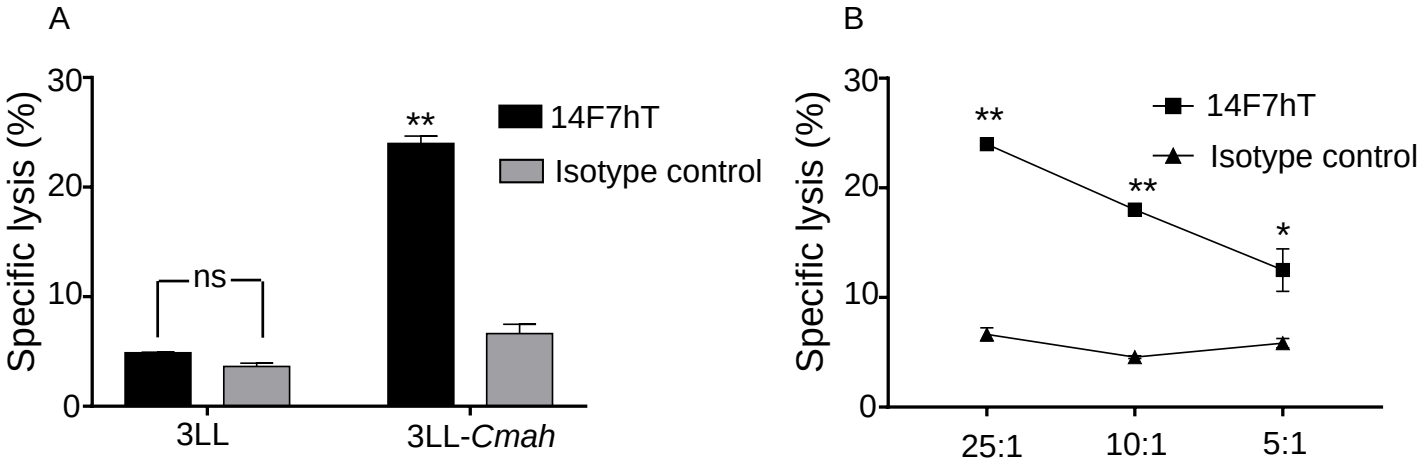
Supplementary figure 1. Original scans of HPTLC plates. Uncropped scans corresponding to images shown in Figure 1B (A), Figure 2B (B) and Figure 4B (C). Upper panels: orcinol staining; bottom panels: 14F7 immunostaining.



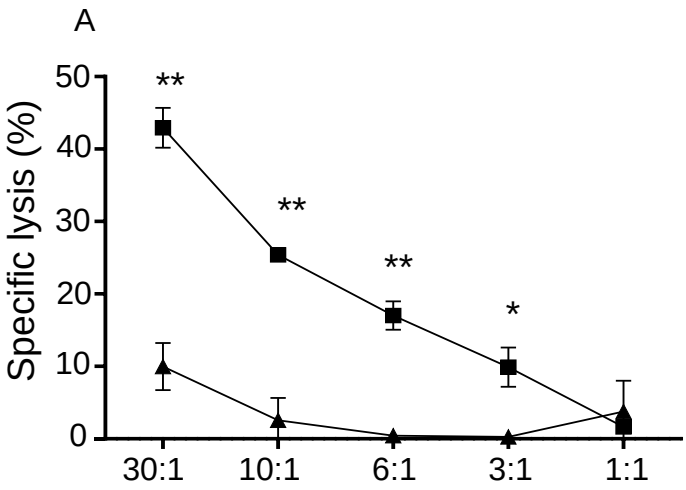
Supplementary figure 2. Original scans of Western blots. Uncropped scans corresponding to images shown in Figure 1C (A), Figure 3A (B) and Figure 4A (C).



Supplementary figure 3. GM3(Neu5Gc) expression in human colon cancer cell lines. Cells were stained with 10 µg/mL of 14F7hT (filled histogram), 7C1 (black line) or isotype-matched control antibody (itolizumab, dotted line) followed by a PE-conjugated anti-human IgG+IgM antiserum. Data are representative of two independent experiments.



Supplementary figure 4. 14F7hT-induced antibody-dependent cell-mediated cytotoxicity in recombinant CMAH-expressing cell lines. A) Induction of antibody-dependent cell-mediated cytotoxicity (ADCC) by 14F7hT with human PBMC in the mouse *Cmah*-transfected cell line 3LL-*Cmah*. Cells were stained with CFSE and cytotoxicity was measured using TO-PRO-3 dye by flow cytometry assay. PBMC from human healthy donors were used as effector cells. B) Cytotoxicity was evaluated at different effector:target cell ratios. Isotype-matched antibody (infliximab) was used as negative control. Statistical analysis was performed using Mann-Whitney *U* test. Data are representative of two independent experiments.



Supplementary figure 5. 14F7hT-opsonized 3LL-*Cmah* cells induce NK cells activation. A) Induction of antibody-dependent cell-mediated cytotoxicity (ADCC) by 14F7hT in the mouse *Cmah*-transfected cell line 3LL-*Cmah*. Cytotoxicity was measured by an LDH-release assay. NK cells from human healthy donors were used as effector cells. Cytotoxicity was evaluated at different effector:target cell ratios. Isotype-matched antibody (infliximab) was used as negative control. Statistical analysis was performed using Mann-Whitney *U* test. Data are representative of three independent experiments.

Supplementary table 1: Anti-ganglioside antibodies used in this study

Antibody	Isotype	Origin	Specificity	Antigen source	Assays	Affinity	Structural data	Selected references
14F7	mouse IgG1, κ	immunized BALB/c mouse	GM3(Neu5Gc)	mouse, horse, human[a], synthetic	ELISA, HPTLC, FACS, IHC, RIS	2-25 nM[b]	Fab, scFv[c]	1-9
14F7hT	human IgG1, κ	14F7 humanized by disruption of predicted T cell epitopes	GM3(Neu5Gc)	mouse, horse, human[a]	ELISA, HPTLC, FACS, IHC	ND	ND	10-12
7C1	human IgG1, κ	14F7hT binding site engineered for extended specificity	GM3(Neu5Gc)	mouse, horse	ELISA, HPTLC, FACS	ND	ND	11, 13
			GM3(Neu5Ac)	mouse, dog, human	ELISA, HPTLC, FACS			

[a]Tumor tissues.

[b]Measured by non-competitive ELISA.

[c]14F7 VH with alternative VL.

HPTLC: high-performance thin layer chromatography; IHC: immunohistochemistry; RIS: radioimmunoscintigraphy; ND: not determined.

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