

Supplemental Material to:

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Affinity-matured recombinant immunotoxin targeting gangliosides 3'-isoLM1 and 3'.6'-isoLD1 on malignant gliomas

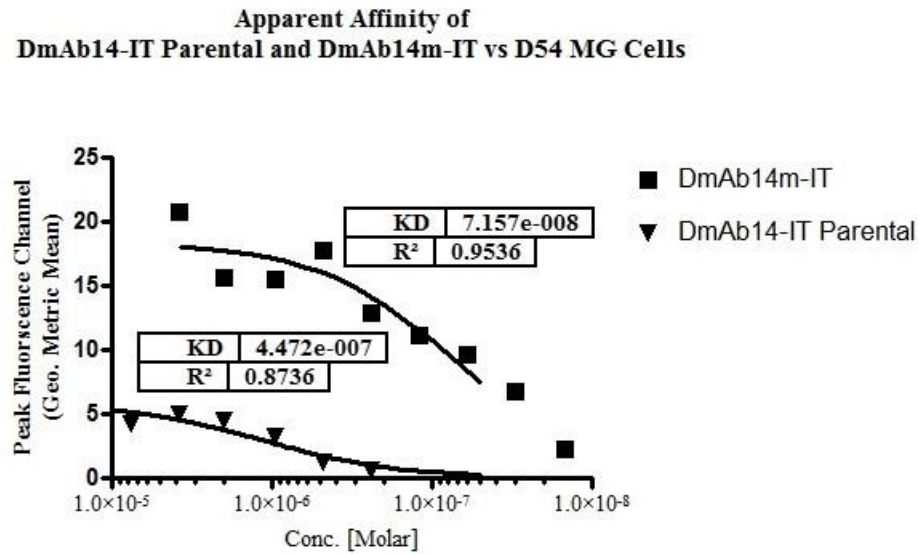
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Supplementary Figure 1.

Apparent Affinities of DmAb14-IT Parental and DmAb14m-IT on D54MG Cells

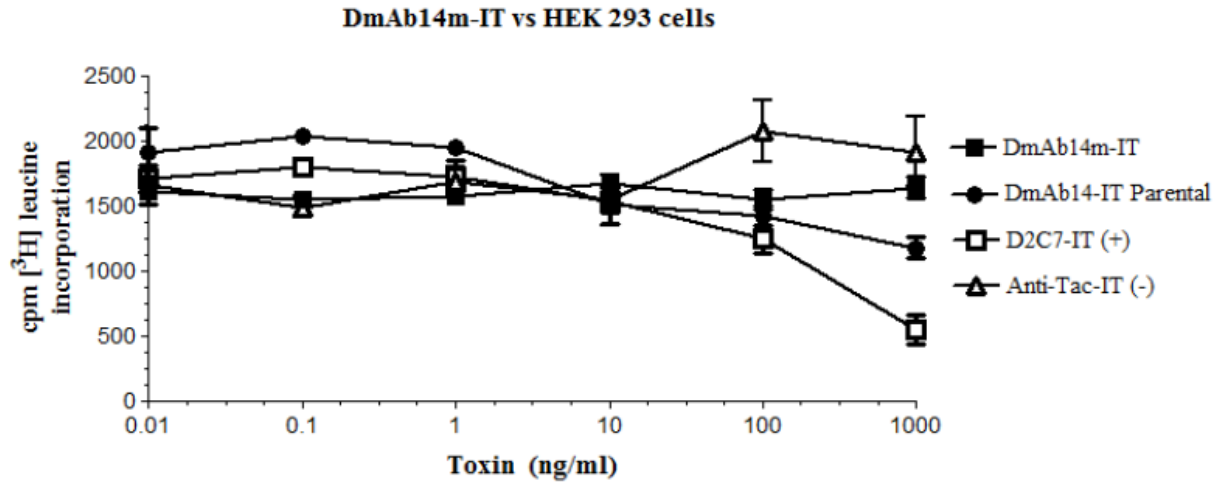


Supplementary Figure 1. Binding affinity (apparent affinity EC_{50}) of V_H CDR2 and V_L CDR1 mutant immunotoxins on D54MG cells.

Binding affinity K_d (apparent affinity EC_{50}) was measured by antibody dose dependent curve using flow cytometry to detect scFv immunotoxin binding against ganglioside-expressing D54MG cells. The K_d (apparent affinity EC_{50}) values were labeled beside each curve.

Supplementary Figure 2.

Cytotoxicity of DmAb14m-IT on negative control HEK293 cells



Supplementary Figure 2. Cytotoxicity of immunotoxins on HEK293 cells.

Inhibition of protein synthesis was determined as percentage of [^3H] leucine incorporation in HEK293 cells, after 20 hours of treatment with immunotoxins. The graph shows protein synthesis as measured by counts per minute of [^3H] leucine incorporated into protein. The dashed line indicates 50% inhibition of protein synthesis, the point at which half of the [^3H]-leucine has been incorporated in the absence of toxin. IC_{50} of the DmAb14m-IT on HEK293 cells is not applicable (Fig.2). The value shown is the mean \pm SD calculated from the means of three separate experiments.