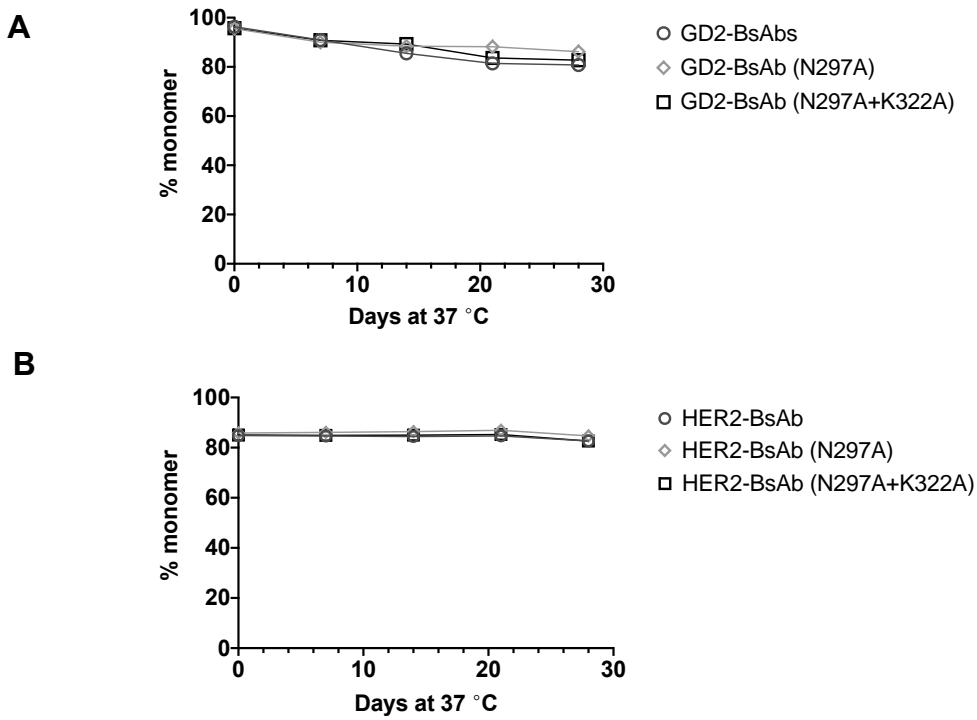


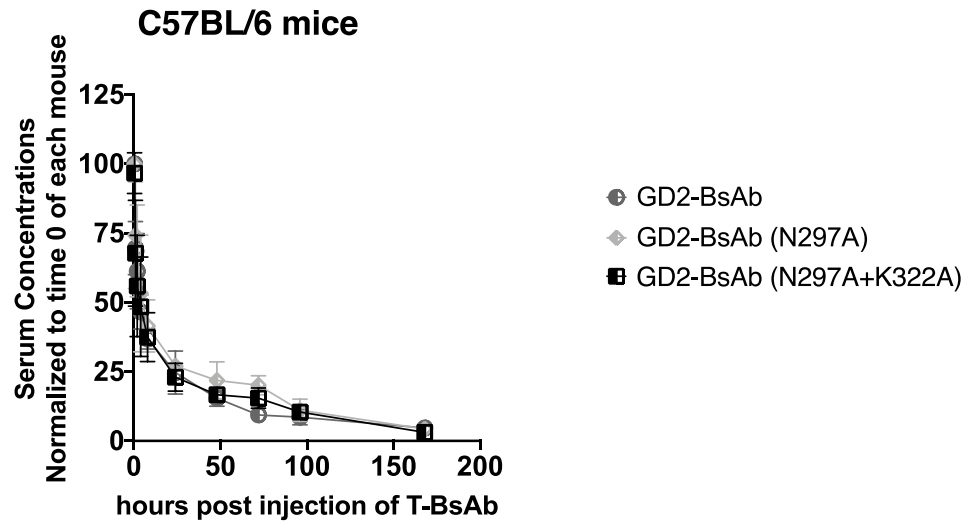
Figure S1



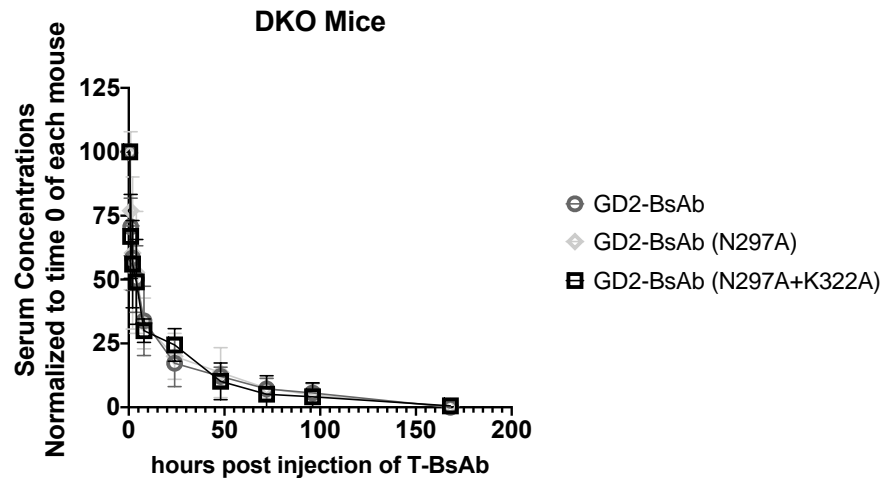
Supplementary Figure S1. Comparisons of BsAb purities and their stabilities at 37 °C over four weeks. A, GD2-BsAb and its Fc mutants. B, HER2-BsAb and its Fc mutants.

Figure S2

A



B

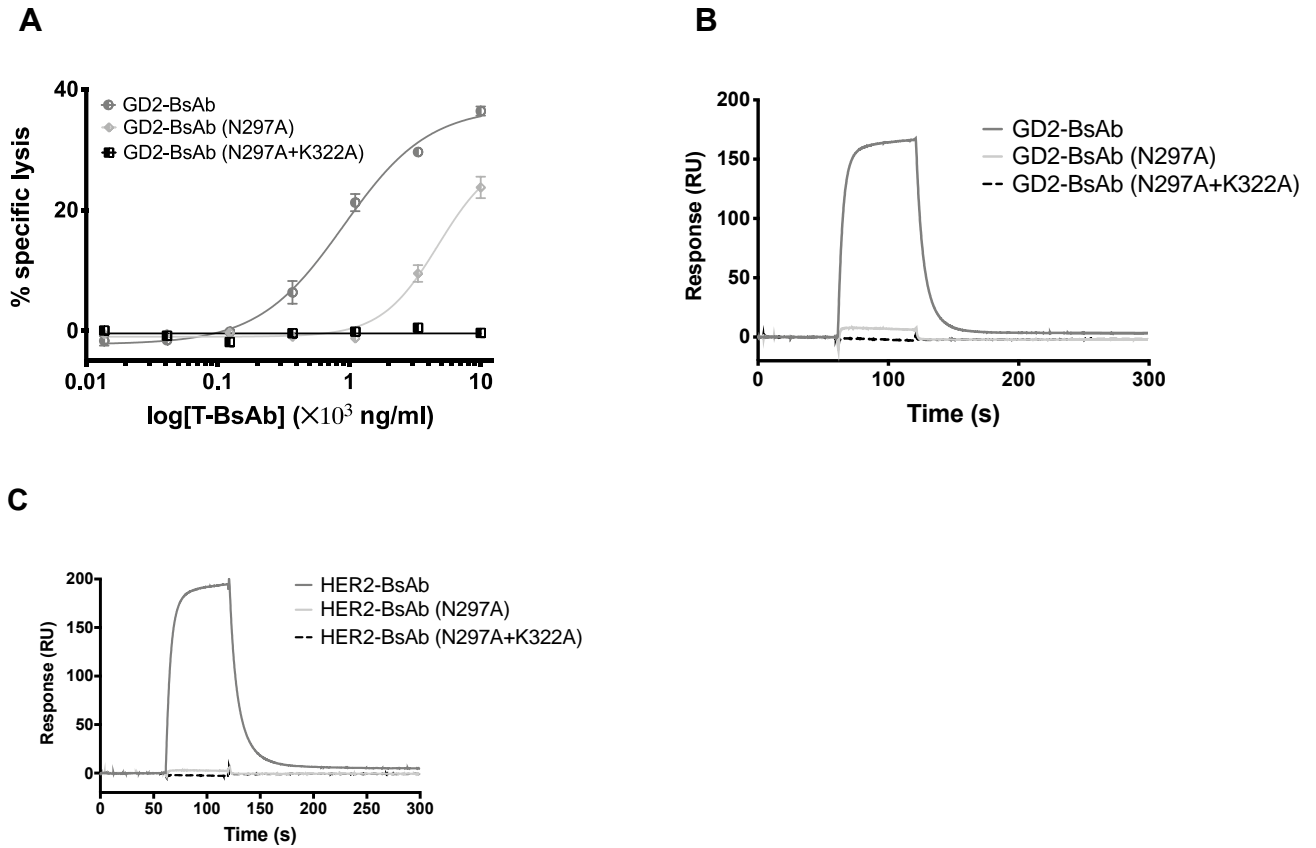


C

	$t_{1/2}$ (days)	
	C57BL/6	DKO
GD2-BsAb	2.19±0.51	2.40±0.36
GD2-BsAb (N297A)	2.04±0.52	2.82±0.42
GD2-BsAb (N297A+K322A)	1.93±0.21	2.32±0.39

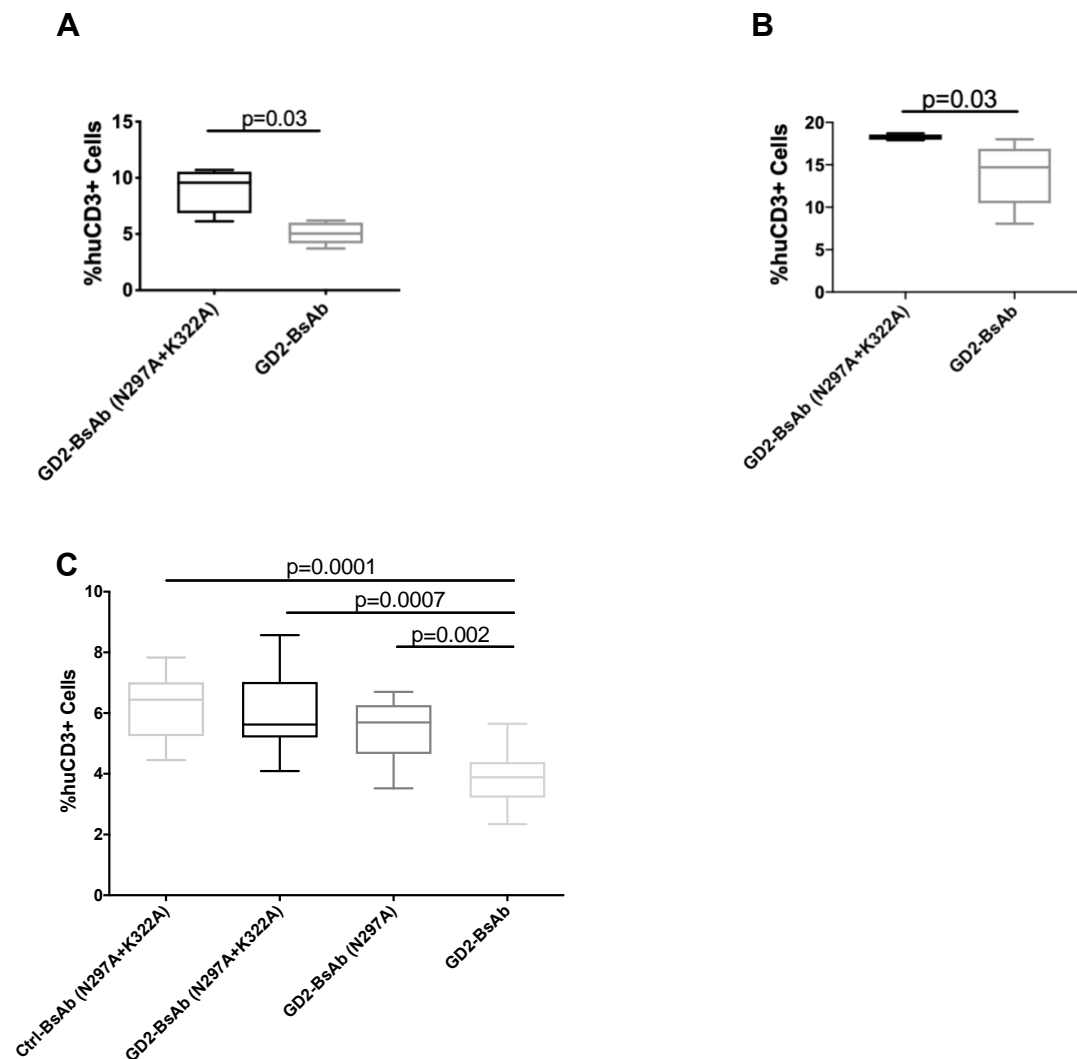
Supplementary Figure S2. **A**, Serum concentration (normalized to time zero for each individual mouse) of GD2-BsAb and Fc variants in C57BL/6 mice following one IV injection of 100 μ g BsAb, n=5. **B**, Serum concentration (normalized to time zero for each individual mouse) of GD2-BsAb and Fc variants in DKO mice following one IV injection of 100 μ g BsAb, n=5. **C**, Terminal half-lives of GD2-BsAb and its Fc mutants.

Figure S3



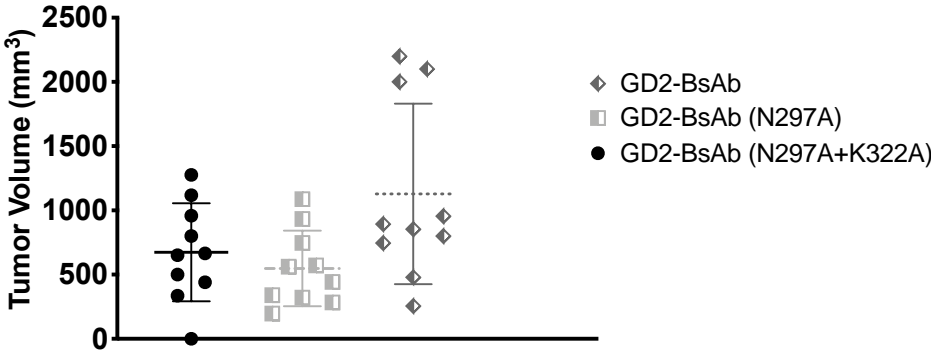
Supplementary Figure S3. A, Comparison of complement-dependent cytotoxicities between GD2-BsAb and its Fc mutants against GD2(+) IMR-32 cells. Exogenous complement source was human serum diluted at 1:80. **B**, Binding kinetics (by SPR) of GD2-BsAb and its two mutants (25 nM) on human C1q and **C**, Binding kinetics (by SPR) of HER2-BsAb and its two mutants (25 nM) on human C1q.

Figure S4



Supplementary Figure S4. A, Comparison of T cell counts in blood 24 hours after one dose of GD2-specific BsAbs in huCD3ε transgenic tumor-free mice, measured by flow cytometry (n=5 each group). **B**, Comparison of T cell counts in spleen 72 hours after one dose of GD2-specific BsAbs in huCD3ε transgenic tumor-free mice, measured by flow cytometry (n=5 each group). **C**, Comparison of T cell counts in blood after four doses of GD2-specific BsAbs in huCD3ε transgenic tumor (EL4)-bearing mice, measured by flow cytometry (n=10 each group).

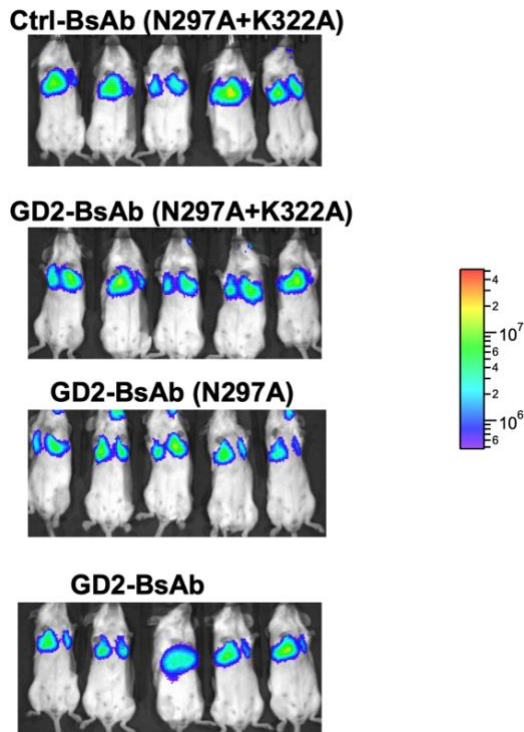
Figure S5



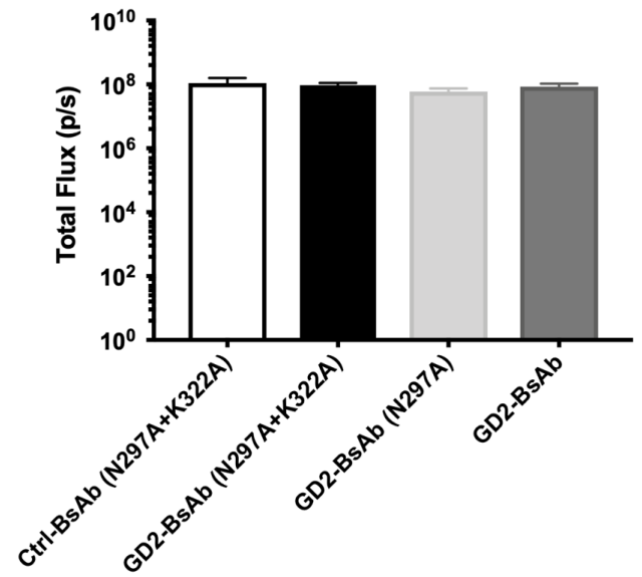
Supplementary Figure S5. Individual EL4 tumor volumes in huCD3ε-tg mice following treatment with different BsAbs on day 31 post treatment (n=10 per group).

Figure S6

A

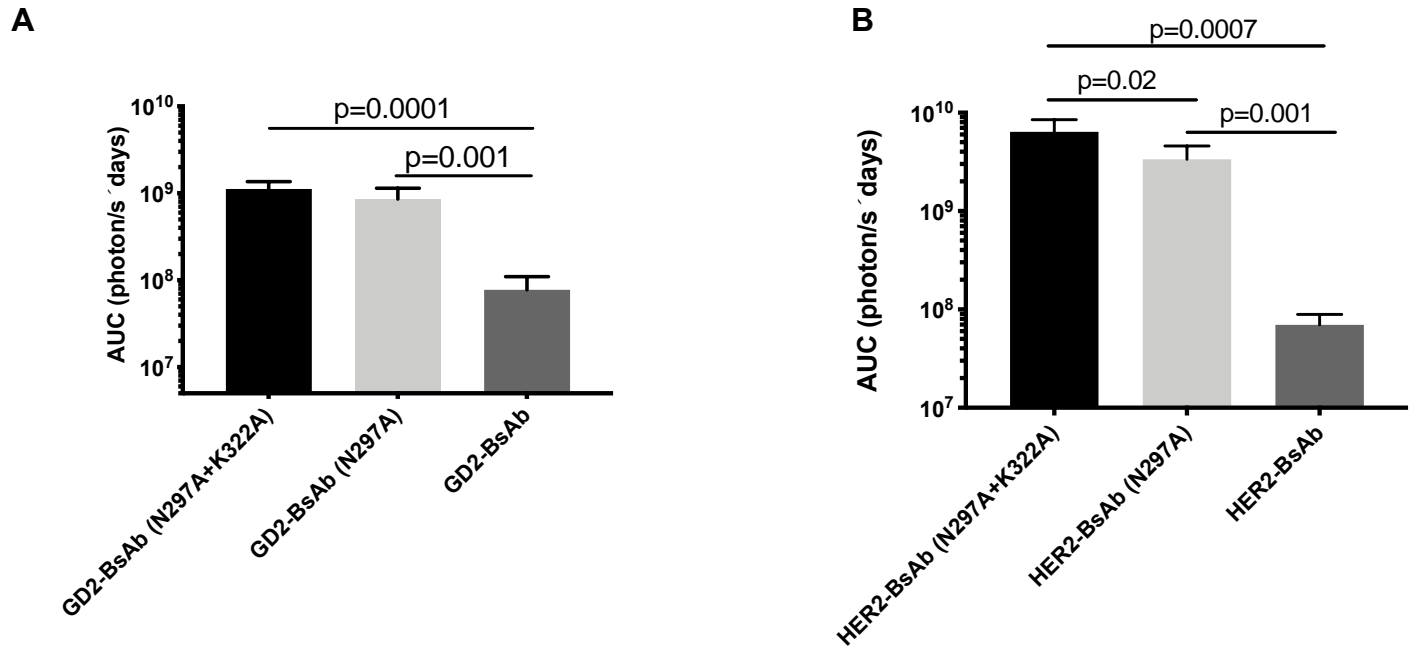


B



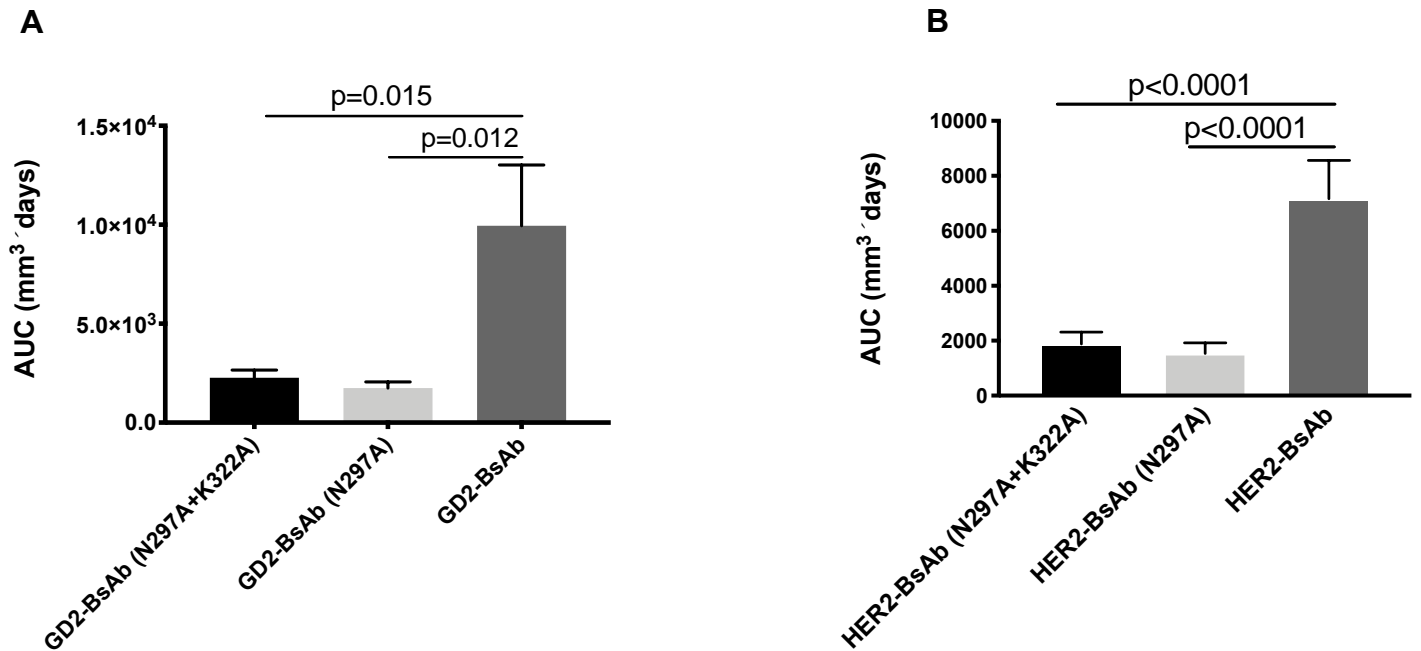
Supplementary Figure S6. A, T cell trafficking to mouse lungs imaged by bioluminescence 30 minutes after T cell injection: comparison between Ctrl-BsAb and GD2-binding BsAbs. B, Quantitation of T cell trafficking to mouse lungs measured by bioluminescence (total flux) expressed as photons per second (n=5 per group).

Figure S7



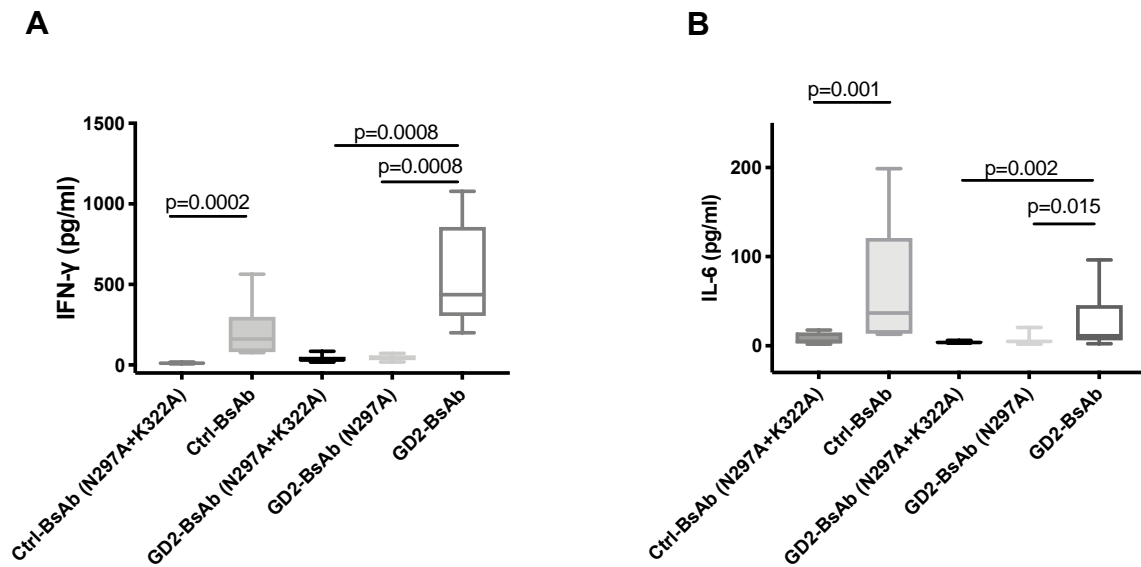
Supplementary Figure S7. **A**, AUC analysis of T cell trafficking into tumors measured by bioluminescence in mice treated with Ctrl-BsAb and GD2-binding BsAbs in Fig. 4B. **B**, AUC analysis of T cell trafficking into tumors measured by bioluminescence in mice treated with Ctrl-BsAb and HER2 series of BsAbs in Fig 5B.

Figure S8



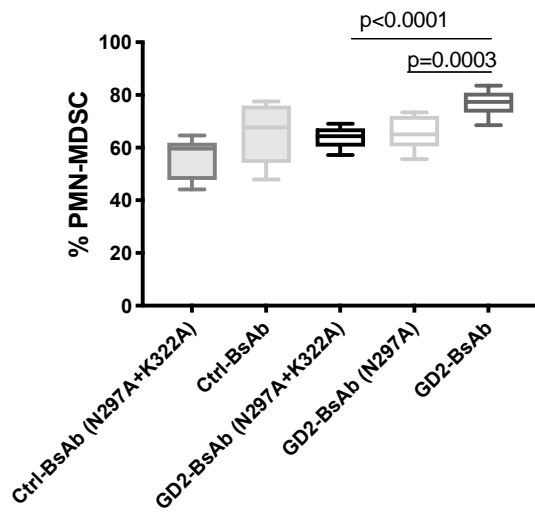
Supplementary Figure S8. **A**, AUC analysis of tumor growth in mice treated with Ctrl-BsAb and GD2-binding BsAbs in Fig. 4D. **B**, AUC analysis of tumor growth in mice treated with Ctrl-BsAb and HER2 series of BsAbs in Fig 5D.

Figure S9



Supplementary Figure S9. Serum cytokine levels in immunodeficient mice 24 hours after T cell injection. **A**, human IFN- γ . **B**, mouse IL-6 (n=5 per group).

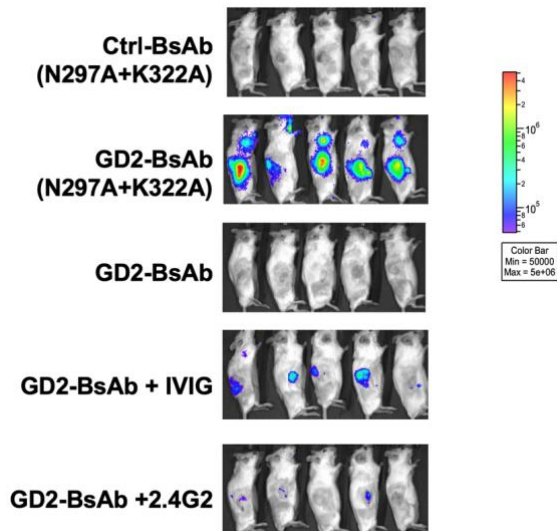
Figure S10



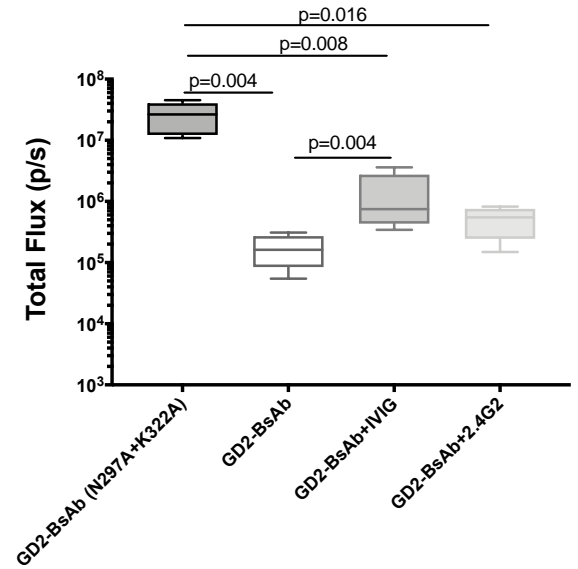
Supplementary Figure S10. Comparison of PMN-MDSC counts in blood 24 hours after ATC injection in DKO mice (n = 10), comparing treatments with Ctrl-BsAb with and without Fc mutations versus GD2-BsAb with and without Fc mutations.

Figure S11

A

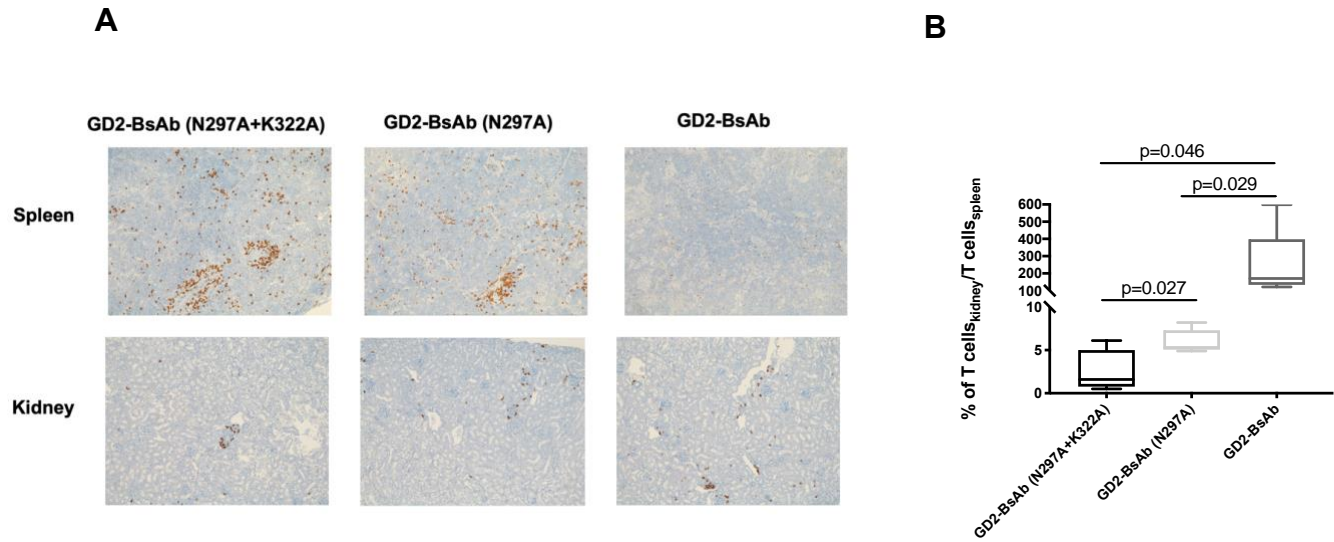


B



Supplementary Figure S11. Effects of Fc γ R blocking by IVIG and 2.4G2 on T cell trafficking into tumors. **A**, T cell trafficking imaged by bioluminescence on Day 5 post T cell injection. **B**, Quantitation of T cell trafficking into tumor sites measured by bioluminescence on Day 5 post T cell injection. N = 5 per group.

Figure S12



Supplementary Figure S12. Effects of complement-activating BsAb on T cell infiltration into kidneys **A**, Representative immunohistochemistry images of huCD45(+) T cells in spleen and kidney sections 72 hours after T cell injection (100X magnifications). **B**, Ratio of T cell numbers in kidney to spleen enumerated by IHC (n = 5 per group).