Supplemental Methods

Generation of noncovalent and covalent BiTEs

For glycolipid loading, glycolipids dissolved in aqueous buffer were added to fusion proteins at a molar ratio of 3:1 in PBS plus 0.05% Triton X-100 (for *in vitro* applications) or PBS + 0.05% Tween-20 (for *in vivo* experiments), and incubated for 16 hours at 22º C. For conjugate and conjugated BiTE formation, loaded complexes were transferred to ultra-low binding microtiter plate wells at 100 µl per well and cooled on ice. A fixed wavelength UV lamp (Schleicher & Schuell Rad-Free long wave UV lamp, λ = 365 nm) was placed directly over wells (mean distance to sample ~5 mm) containing complexes for 1 hour on ice to deliver a total dose of 600 $mJoules/cm^2$. Resulting conjugates and conjugated BiTEs were recovered from the wells, and excess glycolipid and detergent was removed using detergent-removal columns (Pierce) followed by buffer exchange into PBS using a 10 kDa MW cutoff centrifugal filtration unit (Amicon). Size exclusion chromatography using a S200 26/60 FPLC column (GE Healthcare) was performed to confirm the predominantly monomeric status of the conjugated BiTEs and remove trace amounts of multimers. All preparations of complexes and conjugates were confirmed to be free of significant levels of endotoxin (< 0.1 EU/mg) by Kinetic-QCL limulus amebocyte lysate test (Lonza).

Preparation of mouse splenocyte suspensions and TILs

Mouse spleens and tumors were harvested aseptically for preparation of single cell suspensions. Spleens were cut into fragments and gently ground between glass slides, and resulting suspensions were filtered through 40 µm nylon mesh, followed by

treatment with RBC lysis buffer (Sigma) for 3 minutes. The buffer was neutralized by adding 10 volumes of RPMI-1640 containing 10% FBS, centrifuged and resuspended in medium. Liver mononuclear cells were isolated from organs following treatment with Liberase (0.3 Wunsch units/ml) and 200 Units/ml DNase I (Roche) and passage through 40 μm nylon mesh, followed by Percoll gradient centrifugation as previously described (*45*). For isolation of tumor infiltrating lymphocytes (TILs), tumors were harvested from euthanized mice, cut into ~1 mm^3 pieces and processed using the enzyme solution provided in the Miltenyi mouse Tumor Dissociation Kit according to the supplier's instructions (Miltenyi Biotec). Cells were passed through a 40 μm nylon mesh, collected by centrifugation and resuspended 40% Percoll in RPMI-1640, and overlaid on 70% Percoll in RPMI-1640. This was centrifuged at 220 x g for 25 minutes at 15° C. The layer of lymphocytes from the interface of 40% and 70% Percoll was collected, treated with RBC lysis buffer, washed and resuspended in PBS with 5% FCS for antibody staining. For adoptive transfer studies and *in vitro* bioassays, iNKT cells were isolated from mouse splenocytes by immunomagnetic purification using the Miltenyi mouse $NK1.1^+$ iNKT purification kit according to the manufacturer's instructions (Miltenyi Biotec). Total T cells were isolated from spleens using Miltenyi mouse Pan-T cell isolation kit, and CD8 depletion was done using anti-CD8 beads (Miltenyi) for immunomagnetic negative selection. All magnetic bead purification steps were performed at 4° C.