S. Figure 1. Characterization and detection of functional BiTE protein from transfected 293Ts. (A) Diagram representing the BiTE lentiviral construct with EF1α promoter, the truncated CD19 (CD19t) marker of transduction, T2A self-cleaving peptide, EGFRvIII-targeting bi-specific T cell engager (BiTE), and Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element (WPRE). Details of the BiTE protein structure are shown below. A longer linker (sequence: GCC GGC TCC ACC TCT GGG AGT GGA AAA CCT GGC TCC GGG GAA GGA TCT ACA AAG GGA) is between variable heavy (VH) and variable light (VL) domains for both the 806 (EGFRvIII binding portion) and the CD3 (OKT3) binding scFv. A short linker (sequence: ACG GGG AGG CGG CGG CAG T) was encoded between the scFVs. The C terminus of the BiTE protein includes a 6 X His tag (His) for purification. (B, C) Supernatant of transfected 293Ts was harvested three days post-transfection with BiTE-encoding plasmid and incubated for three days with CD3 T cells isolated from human donor PBMCs and EGFRvIII-K562 target cells. CD25 (B) and CD69 (C) were measured on CD4 and CD8 T cells following incubation. Untransfected 293T supernatant and samples without target cells added are shown as controls. n = 2 independent experiments. (D) Supernatants from transfected 293T or transduced macrophages were concentrated and purified using either nickel column (Ni+) or protein L magnetic beads (L+) and run on an SDS-Page gel. Proteins loaded on the gel were blotted onto a nitrocellulose membrane and probed with an anti-His antibody to detect BiTE protein. CD19t GEM or untransfected 293T supernatants are shown as a negative controls.

S. Figure 2. Amount of BiTE protein secreted by transduced macrophages accumulates over time but does not correlate with integration events. (A) Representative dot plots of both wild-type, untransduced macrophages (WT) and macrophages transduced at 750 LP/cell with the BiTE lentivirus (BiTE GEMs) stained for CD19 truncated marker encoded in the lentiviral construct are shown. Quantification of CD19 staining from nine independent donors is shown in the graph to the right. (B) Graph shows the concentration of BiTE protein in supernatant from 5 x 10^5 BiTE or CD19t control GEMs, with accumulation over seven days following lentiviral transduction (Acc) or without, as determined by a competitive inhibition His ELISA. (C) Supernatant accumulated for 14 days following transduction of 3 x 10^6 BiTE or CD19t control GEMs at 750 LP/cell was concentrated and BiTE protein was purified with protein L magnetic beads. Concentration of BiTE in the purified supernatant was determined using a competitive inhibition ELISA and is shown for nine independent donors. 5 x 10^5 GEMs from the same nine donors were transduced with BiTE lentivirus at 750 LP/cell and genomic DNA was isolated on day 7 post-transduction for a WPRE assay. Graph shows BiTE protein concentration with corresponding WPRE copy number. Line depicts regression line with best fit.

S. Figure 3. BiTE GEMs encoding the H7 secretion sequence secrete more functional BiTE protein than the GMCSF secretion sequence. (A) Supernatant from transfected 293T cells (encoding the human heavy chain (H7) or GMCSF secretion sequence) was collected on day 3 post-transfection and incubated with EGFRvIII-expressing K562 target cells. Representative dot plots show percentage of EGFRvIII-K562 cells bound by BiTE protein with C terminal His tag as detected with a PE-labeled anti-His antibody. Supernatant from untransfected 293T cells is shown as a negative control. (B, C) Representative dot plots showing the T cell activation markers CD25 (B) and CD69 (C) on CD4 and CD8 T cells following incubation with macrophages transduced with BiTE lentivirus encoding either the GMCSF or H7 secretion sequence and EGFRvIII-K562 target cells in the presence of EGFRvIII-K562 target cells for three days, n = 2 independent donors. Untransduced macrophages are shown as a negative control.

S. Figure 4. TNFα, IFNγ, and PD-1 are upregulated by T cells in degranulation and activation assays in the presence of BiTE GEMs and EGFRvIII U87 target cells. (A, B) Autologous T cells and GEMs were incubated for 48 hours prior to addition of EGFRvIII U87 (U87+) or control U87 (U87-) target cells for 5 hours in the presence of brefeldin A. Percentage of T cells expressing TNFα (A) or IFNγ (B) following

incubation is shown. (C) Representative dot plots of PD-1 activation on CD4 or CD8 T cells following a three day incubation with BiTE or CD19t control GEMs and U87+ or U87- control cells. (A, B, C) Quantification of the percentage of T cells positive for each of the markers is shown for three independent donors, run in duplicate. Donors not run in duplicate are shown with grey symbols. Error bars depict mean and standard deviation. T cells, GEMs, and target cells were incubated at a ratio of 25: 3: 1, respectively. P values shown were determined by a two-tailed, ratio paired t test. (D) Activated cellular processes as determined by Panther analysis and gene set from Nanostring data in Figure 2F comparing T cell responses to purified BiTE protein or BiTE GEMs in the presence of target cells relative to no Mac controls.

S. Figure 5. BiTE GEMs did not significantly increase survival in subcutaneous or intracranial glioblastoma models. (A, B) Kaplan-Meier curves depicting the survival outcomes for each mouse that receive BiTE or CD19t control GEMs throughout the duration of the subcutaneous (A) or intracranial mouse (B) experiments as diagramed in Figure 5. CD19t GEM results are shown in a dotted line. BiTE GEM are shown in a solid black line. (C) Luminescent images of individual mice at the specified time points that received intracranial injection of EGFRvIII eGFP-ffluc U87s and T cells only in the absence of GEMs. The grey scale for luminescent imaging intensity is shown to the right of images.

S. Figure 6. Characterization of T cell responses to BiTE and IL-12 dual transduced GEMs. (A) Representative dot plots of wildtype (WT), untransduced macrophages, BiTE GEMs, or BiTE and IL-12 dual transduced GEMs stained for the CD19 marker on day 7 post-transduction. Percentage CD19 positive cells for four independent donors is shown in the graph to the right. (B) Supernatant from BiTE and IL-12 dual transduced GEMs harvested from day 7 post-transduction cells and used in an EGFRvIII-K562 binding assay as in Figure 1. Representative dot plots depict the percentage of EGFRvIII-K562 cells bound by BiTE protein in the dual transduced cells as detected by an anti-His PE-labeled antibody. Graph shows fold change in His stained positive cells following incubation with dual transduced GEM supernatant relative to BiTE GEM supernatant. (C) Supernatant was harvested on day 7 post-transduction for the comparison of IL-12 secreted by BiTE/IL-12 dual transduced and IL-12 GEMs. Graph shows the concentration in ng/mL of IL-12 detected in each GEM condition supernatant. (A-C) Bars on graphs depict mean for each condition with standard deviation. (D, E) 3×10^6 T cells were cultured with 5×10^5 BiTE/IL-12 dual transduced, BiTE, or IL-12 GEMs and 2×10^5 EGFRvIII U87 target cells. On day 3 of culture, T cells were harvested, lysed, and RNA was isolated and ran on a Nanostring human immunology v2 panel. Graphs show fold changes in normalized counts for the top up- and down-regulated genes for BiTE and IL-12 dual transduced GEMs, BiTE GEMs, and IL-12 GEMs in comparison to co-cultures with no Macs (D) or untransduced Macs (E). (F) Activated cellular processes as determined by Panther analysis and gene set from Nanostring data in Figure 6 comparing T cell responses in the presence of BiTE/IL-12 dual transduced GEMs, BiTE GEMs, or IL-12 GEMs and EGFRvIII-U87 target cells relative to CD19t control macrophages.

S. Figure 7. Recombinant IL-12 enhances BiTE-specific responses. (A) Diagram depicting *in vivo* subcutaneous experiment. Mice were injected subcutaneously with 1×10^6 EGFRvIII eGFP-ffluc U87s. On day 18 mice received a single intravenous injection of 1×10^7 activated T cells and starting on day 18 mice received IT injection of 1 μg purified BiTE protein and 12 ng recombinant human IL-12 daily for five days. (B) Luminescent images of individual mice from groups that received subcutaneous EGFRvIII eGFP-ffluc U87 injections only (tumor only) or in combination with T cells (no protein, T cells only), or injections of purified protein (Purified BiTE plus IL-12) is shown (Day 17-31). (C) Graph displays the percent change in each analyte detected in the supernatant of three day co-culture of BiTE/IL-12 dual transduced GEMs cultured with autologous T cells and EGFRvIII target cells relative to the same cultures with BiTE GEMs for three independent donors, each sample was run in duplicate. (D) Chemokines and

proteins detected from the co-culture of 3 x 10^6 autologous T cells added on day 7 post-transduction to 5 x 10^5 BiTE, BiTE/IL-12 dual transduced, or CD19t control GEMs and 2 x 10^5 EGFRvIII target cells. Graphs show concentration of cytokine detected for each analyte in pg/mL for CD19t GEMs (white), BiTE GEMs (black), or BiTE/IL-12 GEMs (grey), n = 3 independent donors run in duplicate.