Supplementary figure 1. Removal of CD14+/CD11c+ cells prior to γδ TCR positive selection significantly increases sample purity. High levels of CD14+/CD11c+ contamination with apparent FITC positivity following isolation of γδ T cells from thawed aliquots of PBMC using 2 rounds of positive selection with Miltenyi anti-γδ TCR beads (A) is due to uptake of FITC conjugated anti-Hapten breads by CD14+/CD11c+ cells, suggested by clustering of FITC staining within these cells seen using fluorescence microscopy (B). Depleting CD14+ and CD11c+ cells removes these contaminants (C) and significantly improves purity of γδ T cell preparations from thawed PBMC aliquots (D).

Supplementary figure 2. Anti-γδ TCR clone B1 is a murine IgG1. To confirm that Leaf-purified clone B1 (anti-γδ TCR) would coat aAPC we used an APC conjugated antibody of the same isotype (murine IgG1 isotype control) as any interaction of this antibody must be via the Fc region. The APC conjugated IgG1 bound much better than murine IgG2, consistent with previous data (21), which demonstrated that aAPC could be coated in antibody of the IgG1 isotype.

Supplementary figure 3. Variation in γδ TCR repertoire before expansion stimulus applied. Representative plots of three healthy adult donors. Cells are gated on CD3+/γδ TCR+ and represented in quadrants indicating Vδ1+, Vδ2+ or Vδ1neg/Vδ2neg/γδ TCR+.

Supplementary figure 4. Killing of GD2+ Kelly by expanded γδ T cells in the presence of ch14.18 (black) or anti-CD20 control antibody (grey). Black bars represent killing of GD2+ Kelly cells opsonized with ch14.18, grey bars represent killing in presence of control antibody, target is Kelly, at a range of E:T ratios (10:1-1.25:1; 4hr ⁵¹Cr release assay).

Supplementary figure 5. GD2 staining of neuroblastoma cell lines. GD2 expression of five neuroblastoma cell lines used in killing assays in this study. The darker histogram represents an isotype staining control.

Supplementary figure 6. (A) NKG2D expression of expanded
γδT cell subsets. Comparison of NKG2D expression in Vδ1+, Vδ2+ and
Vδ1neg/Vδ2neg γδT cells following prolonged (21d) expansion with aAPC+B1,
determined using flow cytometry (NKG2D-APC), (n=3). (B) Production of interferon
gamma and Granzyme B by Vδ2+ γδT cells expanded with IPP+LCL and Vδ1+ or
Vδ1neg/Vδ2neg γδT cells expanded with aAPC+L, in the presence or absence
of PMA/Ionomycin (representative FACS plots of 3 donors).