

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

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

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18 **Supplementary figure 3. Variation in $\gamma\delta$ TCR repertoire before expansion**
19 **stimulus applied.**

20 Representative plots of three healthy adult donors. Cells are gated on CD3+/ $\gamma\delta$ TCR+
21 and represented in quadrants indicating V δ 1+, V δ 2+ or V δ 1^{neg}/V δ 2^{neg}/ $\gamma\delta$ TCR+

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23 **Supplementary figure 4. Killing of GD2+ Kelly by expanded $\gamma\delta$ T cells in the**
24 **presence of ch14.18 (black) or anti-CD20 control antibody (grey)** Black bars
25 represent killing of GD2+ Kelly cells opsonized with ch14.18, grey bars represent
26 killing in presence of control antibody, target is Kelly, at a range of E:T ratios (10:1-
27 1.25:1; 4hr ⁵¹Cr release assay).

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

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35 **Supplementary figure 6. (A) NKG2D expression of expanded**

36 **$\gamma\delta$ T cell subsets.** Comparison of NKG2D expression in V δ 1+, V δ 2+ and
37 V δ 1^{neg}/V δ 2^{neg} $\gamma\delta$ T cells following prolonged (21d) expansion with aAPC+B1,
38 determined using flow cytometry (NKG2D-APC), (n=3). (B) Production of interferon
39 gamma and Granzyme B by V δ 2+ $\gamma\delta$ T cells expanded with IPP+LCL and V δ 1+ or
40 V δ 1^{neg}/V δ 2^{neg} $\gamma\delta$ T cells expanded with aAPC+L, in the presence or absence
41 ofPMA/Ionomycin (representative FACS plots of 3 donors).
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