SI Methods

Study population. Peripheral blood came from 63 healthy volunteers (31 male, 32 female, median age 33yr, range 3-69yr) and 10 osteoporotic patients (2 male, 8 female, median age 70yr, range 44-81yr) with informed consent and ethical approval (London - City and East Research Ethics Committee (REC) 13/LO/0548). Thymus samples were from neonatal (<24 months) cardiac surgery with informed consent and ethical approval (London - Queen Square REC 14/LO/2132).

Isolation of peripheral blood mononuclear cells (PBMC). PBMC were isolated from peripheral blood by Ficoll-Paque (GE Healthcare) gradient centrifugation at 400xg (brake off) for 35min at 20°C. Interface cells were harvested and washed in PBS supplemented with 10% foetal bovine serum (FBS) and 5mM ethylenediaminetetraacetic acid (EDTA) (both Gibco, Life Technologies).

Flow cytometry. Fluorochrome-conjugated antibodies were from eBioscience, Becton Dickinson or BioLegend: CD3ε (HIT3a), CD11a (HI111), CD16 (3G8), CD27 (O323), CD28 (CD28.2), CD45RA (HI100), CD56 (HCD56), CD57 (NK-1), CD62L (DREG-56), CD107a (H4A3), CD161 (HP-3G10), CCR2 (K036C2), CCR5 (HEK/1/85a), CCR6 (G034E3), CCR7 (G043H7), CX3CR1 (K0124E1), Vδ2 (B6), granzyme B (GB11), granzyme K (G3H69), granulysin (DH2), IFNγ (4S.B3), IL-17A (BL-168), IL18Rα (H44), PD-1 (EH12.2H7), perforin (dG9) and TNFα (MAb11). For chemokine receptors, cells were stained at 20°C with fluorochrome-conjugated antibodies diluted in FACS buffer (PBS, 2% FBS, 5 mM EDTA). For other surface markers PBMC were stained on ice, washed and re-suspended in FACS buffer containing 0.5μg/mL DAPI (Invitrogen) for dead cell exclusion, prior to analysis. For cytokines, cells were either stimulated with 50ng/mL phorbol 12-myristate 13- acetate (PMA; Sigma) and 1μg/mL ionomycin (Sigma), or 1nM HMB-PP (Sigma) and 100U/mL rhIL-2 (PeproTech), or 20μM zoledronate (Sigma) and 100U/mL rhIL-2 for 4h, 6h or 24h at 37°C; 10μg/mL Brefeldin A (eBioscience) and 2μM Monensin (eBioscience) were added for at least the last 2h, or 8h if stimulating cells for 24h. Cells were stained for surface markers, fixed with IC fixation buffer (eBioscience) for 15min on ice, permeabilised, and stained with intracellular cytokine-specific antibodies diluted in permeabilization buffer (eBioscience). Samples were acquired using a Canto-II, LSR-II or FACSAria-II flow cytometer (BD) and analysed using FlowJo software (Tree Star, Inc.).

Proliferation assays. First γδ T cells were enriched from PBMC by magnetic separation with a biotin-conjugated TCRδ antibody (Miltenyi Biotec). Subsequently, enriched γδ T cells were FAC-sorted into $V\delta2^{(+)}$ subsets on a FACSAria-II. Sorted cells were labelled with 5μM eFluor®670 (eBioscience) for 10min at 37°C with intermittent mixing and quenched with ice-cold RPMI-1640 (Life Technologies) containing 10% FBS. Labelled cells were cultured with Vδ2-depleted mononuclear accessory cells at a 1:1 ratio at 37°C in RPMI-1640 complete media (RPMI-1640 supplemented with 10% FBS and 1% penicillin/streptomycin) in 96-well round-bottom plates in the presence of 1μM zoledronate and 100U/mL rhIL-2. After 7-days, cells were analysed for eFluor®670 dilution. FAC-sorted $V\delta2^{(+)}$ subsets were also cultured

for 3 days with autologous CD14$^{(+)}$ monocytes, isolated by magnetic separation with a biotin-conjugated anti-CD14 antibody (BioLegend), at a 1:5 (Vδ2:monocyte) ratio with 1nM HMB-PP and 100U/mL rhIL-2, at 37°C in RPMI-1640 complete media.

Cytotoxicity assays. Vδ2$^{(+)}$ T cells were isolated to high purity with magnetic-activated cell sorting (MAC-sorted) using a biotin-conjugated anti-human Vδ2 antibody (Miltenyi Biotec) from either freshly isolated PBMC or Vδ2$^{(+)}$ T cell lines (from profile #1 and #6 donors) that had been expanded from PBMC over 12-days with 1nM HMB-PP and 100U/mL rhIL-2 with replenishment of rhIL-2 every 3-days. Sorted Vδ2$^{(+)}$ T cells were then rested for 16h in RPMI-1640 media supplemented with 100U/mL rhIL-2 +/- 100μM nafamostat mesylate (granzyme K inhibitor, Cayman Chemical)) before use. MOLT-4, Jurkat, HL-60, DOHH-2, Daudi, and HCT-116 cell lines were labelled with 1μM CellTrace Far Red DDAO-SE (Molecular Probes). Vδ2$^{(+)}$ T cells that were MAC-sorted from freshly isolated PBMC were pre-activated for 4h with 1nM HMB-PP and 100U/mL rhIL-2 before co-culture with target cell lines. All co-culture cytotoxicity assays were performed in 96-well round-bottom plates at a 5:1 effector:target ratio for 4h in RPMI-1640 supplemented with 100U/mL rhIL-2, +/-100μM Z-AAD-CMK (granzyme B inhibitor, Abcam). For ADCC assays, Daudi cells were rested or pre-treated with 1μg/mL anti-hCD20-hIgG1 (InvivoGen) for 30min at 37°C. Vδ2$^{(+)}$ T cells were then added and activated with 1nM HMB-PP and 100U/mL rhIL-2. Cells were stained with Annexin V-PE (BioLegend) and Zombie aqua (BioLegend) and analysed on a NovoCyte Flow Cytometer (ACEA Biosciences).

Degranulation assay. Vδ2$^{(+)}$ T cell degranulation was determined by CD107a surface expression. 1x10^6 PBMCs were cultured for 8h in 96-well round-bottom plates in RPMI-1640 complete media, with 20nM HMB-PP, 100U/mL of rhIL-2, 1μM Monensin and anti-human CD107a at 37°C. PBMC were then stained for cell surface markers as described.

Probability state modelling (PSM). GemStone™ version 1.0.69 (Verity Software House, Maine, USA) was used to model extracellular marker similarity of flow-cytometry data of Vδ2$^{(+)}$ T cells as per manufacturer's instructions. Modelling used simple rules/assumptions about directional changes in marker expression, with data from all 63 samples integrated into a final model.

Microarray analysis. Total RNA was extracted using RNeasy Micro Kit (Qiagen) according to manufacturer's instructions. After confirming concentration, purity, and integrity, total RNA was reverse-transcribed, then converted to cRNA using the Ambion® WT Expression Kit (Life Technologies). The HumanHT-12 v4 Expression BeadChip (Illumina) was used for the microarray. GEO database (http://www.ncbi.nlm.nih.gov/bioproject/); accession number GSE75519. Data were analysed using Genome Studio (Illumina) with a threshold for differential gene expression of ≥2-fold and *p*-value<0.05 (Benjamini and Hochberg False Discovery Rate).

Statistical analysis. Statistical analysis was performed by Prism 6.0 (GraphPad). Data are mean ±s.d.