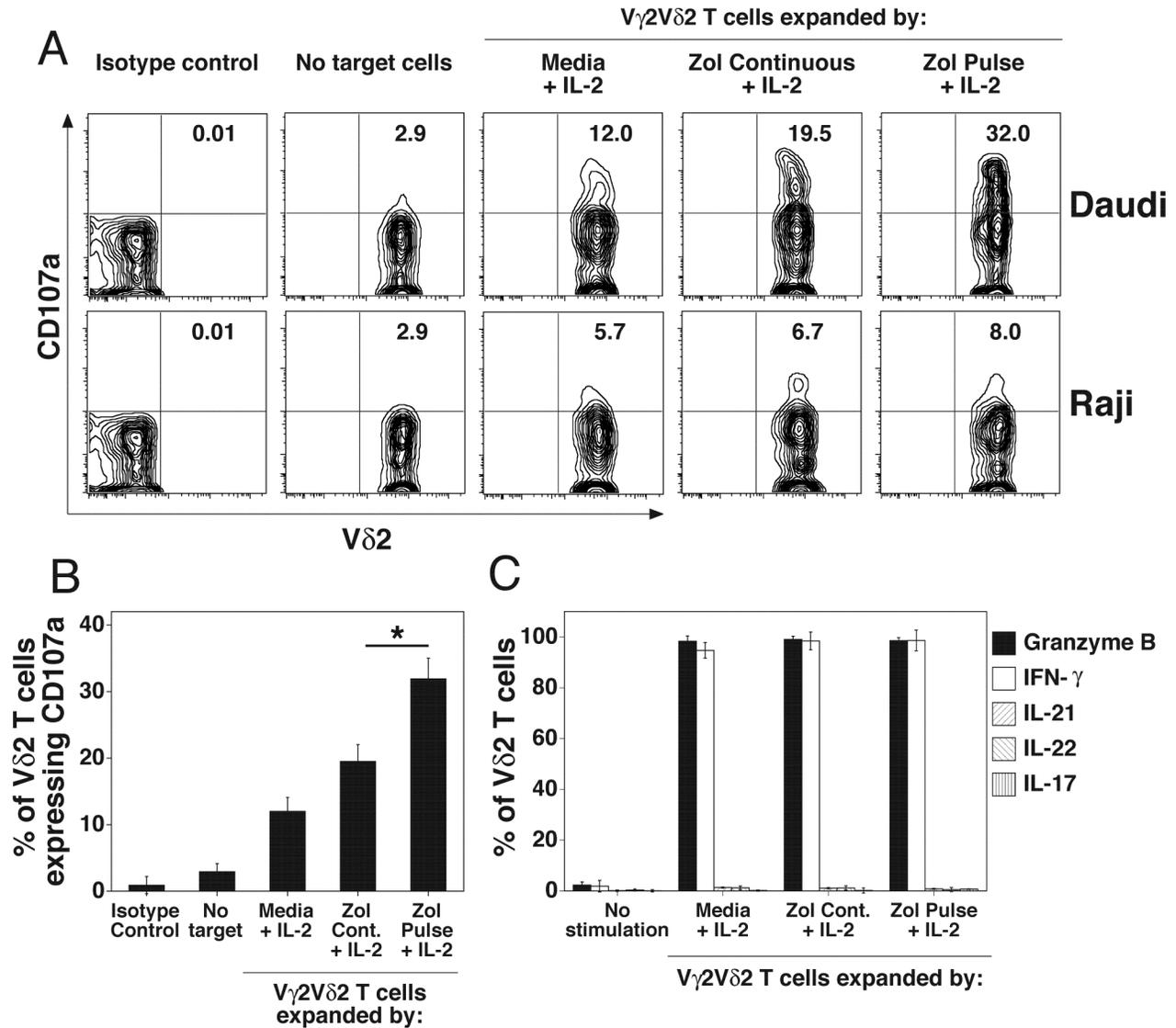
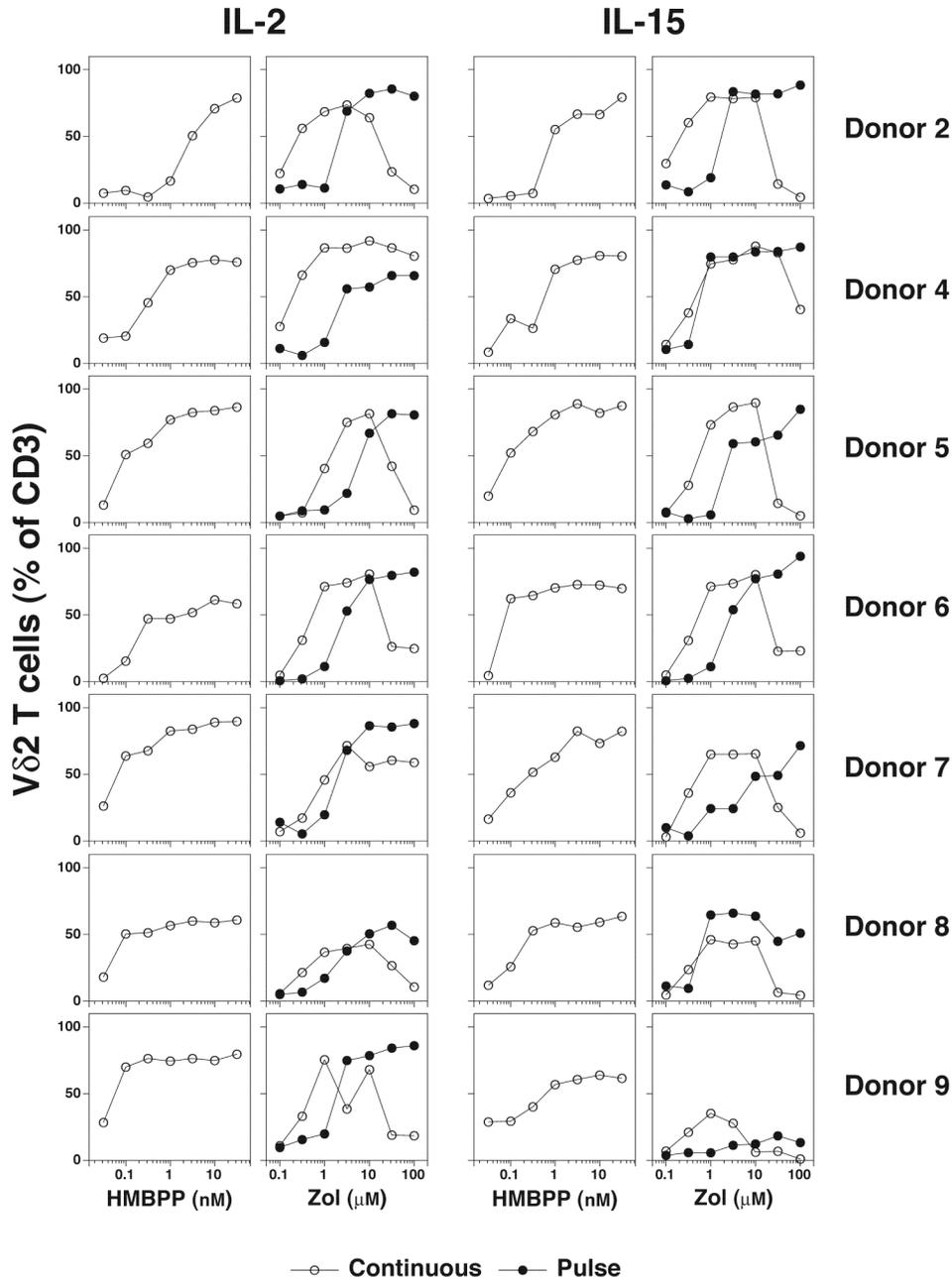


**Figure S1.** Purity of V $\gamma$ 2V $\delta$ 2 T cells after positive magnetic bead separation. V $\gamma$ 2V $\delta$ 2 T cells expanded by either continuous or pulse zoledronate stimulation for 14 d and then purified by magnetic bead separation. Expanded V $\gamma$ 2V $\delta$ 2 T cells (*top panels*) were washed, resuspended to  $1 \times 10^7$  cell/ml, and reacted with APC-conjugated anti-human V $\delta$ 2 mAb (clone B6) for 20 min. Cells were then washed twice with purification buffer and anti-APC magnetic beads were added. After incubation on ice for 15 min, the cells were washed twice, resuspended in 500  $\mu$ l of purification buffer, and loaded onto LS columns for positive selection. Purified V $\delta$ 2 T cells were removed from the magnet, eluted, washed, and then counted for use for adoptive transfer or other functional assays. Cell purity was evaluated by flow cytometry (*bottom panels*) and was at least  $> 95\%$  for use.

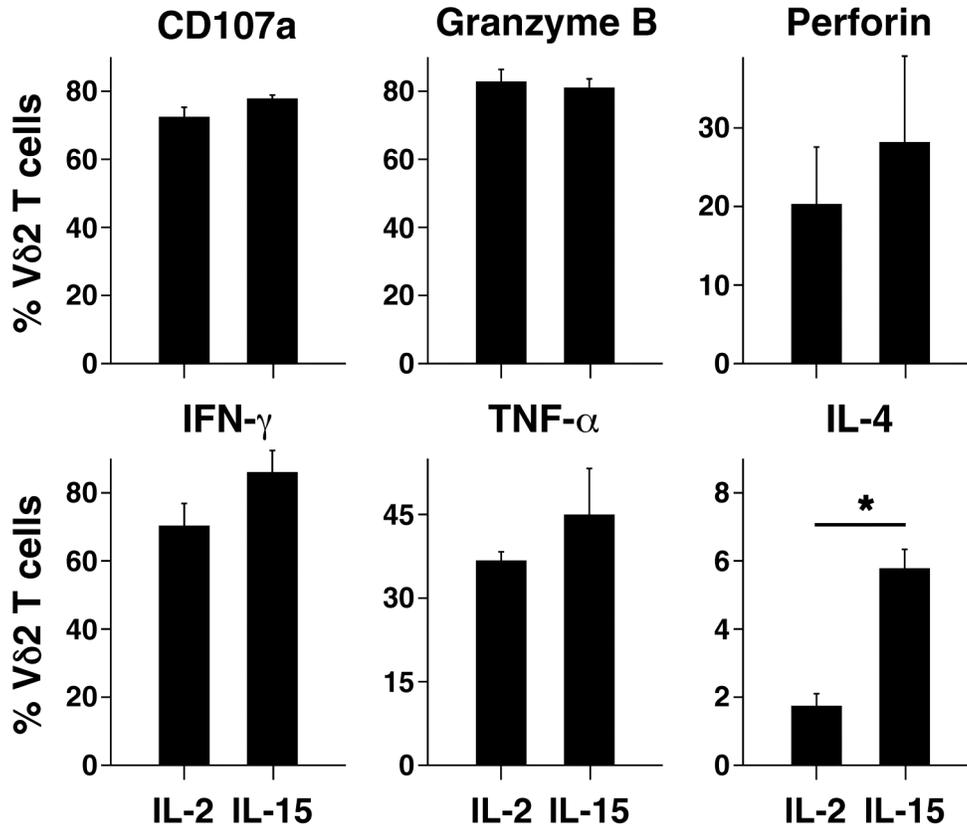


**Figure S2.** Expansion of  $V\gamma 2V\delta 2$  T cells by pulse zoledronate stimulation increased degranulation as measured by the expression of CD107a in response to the stimulatory Burkitt's lymphoma cell line, Daudi. **a** Increased expression of CD107a by  $V\gamma 2V\delta 2$  T cells expanded by pulse zoledronate stimulation when exposed to the stimulatory Daudi cell line.  $V\gamma 2V\delta 2$  T cells were expanded from PBMCs by either continuous or pulse zoledronate stimulation for 14 d.  $V\gamma 2V\delta 2$  T cell numbers were assessed by flow cytometric analysis. Unpurified  $V\gamma 2V\delta 2$  T cells were then mixed with Daudi or Raji Burkitt's lymphoma cells at 10:1 (E:T) ratio for 4 h in the presence of monensin and PE-Cy7-anti-CD107a. The cultures were then washed and stained with PE-anti-CD3 and FITC-anti-V $\delta 2$  mAbs and analyzed by flow cytometry. **b** Mean % of V $\delta 2$  T cells expressing CD107 after culturing with Daudi cells (n = 3 experiments). \*\* $p = 0.0058$  using the unpaired  $t$ -test. **c** Similar levels of IFN- $\gamma$  and granzyme B in  $V\gamma 2V\delta 2$  T cells expanded either by continuous or pulse zoledronate stimulation or by culturing in IL-2 after ionomycin/PMA stimulation for 4 h. Levels were determined by intracellular staining and flow cytometric analysis.

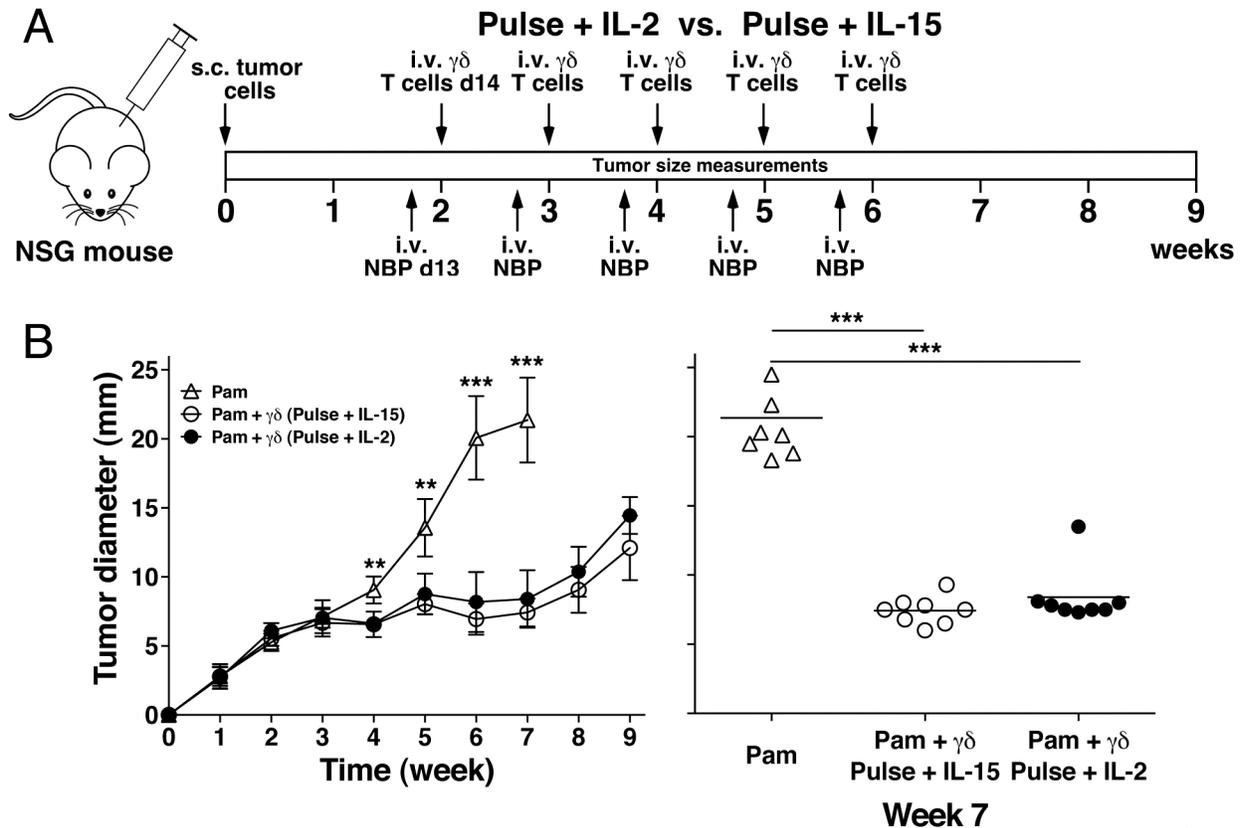




**Figure S4.** Comparison of the expansion of V $\gamma$ 2V $\delta$ 2 T cells in response to varying doses of HMBPP and zoledronate with IL-2 or IL-15. Data are from Fig. 1 and Fig. 5. Human V $\gamma$ 2V $\delta$ 2 T cells were expanded ex vivo from PBMC from each donor by exposure to HMBPP or zoledronate in parallel. Human PBMC were cultured in 96-well plates either continuously with varying starting concentrations of HMBPP or zoledronate (open circles) or pulsed with zoledronate for 4 h (closed circles) followed by washing twice. IL-2 or IL-15 was added to 1000 IU for IL-2 or 100 ng/ml for IL-15 on day 3. Thereafter, media with the respective cytokine was changed every 2-3 d depending on cell growth. On day 14, V $\gamma$ 2V $\delta$ 2 T cell numbers were determined by flow cytometric analysis.



**Figure S5.** Functional capabilities of  $V\gamma 2V\delta 2$  T cells expanded by pulse zoledronate stimulation with IL-15 are similar to those expanded with IL-2. Human PBMCs were pulsed with zoledronate (100  $\mu$ M) for 4 h and then washed twice before re-culture either with IL-15 (50 ng/ml) or IL-2 (1000 IU/ml) for 14 d. Expanded  $V\gamma 2V\delta 2$  T cells were purified by positive selection or left unpurified. PC-3 cells were treated overnight with pamidronate (200  $\mu$ M) and then washed. Pamidronate-treated PC-3 cells were incubated with unpurified or purified  $V\gamma 2V\delta 2$  T cells for 4 h in duplicate samples with monensin followed by surface and intracellular mAb staining. Staining was assessed by flow cytometric analysis. Mean  $\pm$  SD is shown. Representative of two experiments. \* $p < 0.05$  using the unpaired  $t$ -test.



**Figure S6.** Adoptive transfer of  $V\gamma 2V\delta 2$  T cells expanded by pulse zoledronate stimulation with IL-15 in combination with pamidronate controlled PC-3 prostate tumor growth in NSG mice similarly to  $V\gamma 2V\delta 2$  T cells expanded by pulse zoledronate stimulation with IL-2; tumor diameter data for Fig. 7. **a** Schema of treatment protocol used to evaluate the anti-tumor efficacy of  $V\gamma 2V\delta 2$  T cells. Immunodeficient NSG mice were s.c. inoculated with human PC-3 prostate cancer cells on day 0. On day 13, pamidronate (50  $\mu\text{g}/\text{kg}$ ) was given i.v. On day 14,  $1 \times 10^6$  purified  $V\gamma 2V\delta 2$  T cells expanded by pulse zoledronate stimulation with either IL-15 or IL-2 were inoculated i.v. Treatments were repeated weekly until week 6. Longitudinal and transverse diameters of the tumors were measured twice weekly until week 9. **b, left panel**  $V\gamma 2V\delta 2$  T cells stimulated by pulse zoledronate exposure with IL-15 showed similar anti-tumor immunity compared with those expanded with IL-2. Mean PC-3 tumor diameter  $\pm$  SD is shown for 7-8 mice per group treated with either pamidronate alone (open triangles), pamidronate with purified  $V\gamma 2V\delta 2$  T cells derived by pulse zoledronate stimulation with IL-15 (open circles), or pamidronate with purified  $V\gamma 2V\delta 2$  T cells derived by pulse zoledronate stimulation with IL-2 (closed circles).  $***p < 0.001$  compared with mean tumor volume of mice treated with  $V\gamma 2V\delta 2$  T cells derived by pulse zoledronate stimulation with IL-2 using the Mann-Whitney  $U$  test. **Right panel**, Tumor volume at week 7 of individual mice treated with pamidronate alone (open triangles), pamidronate with  $V\gamma 2V\delta 2$  T cells derived by pulse zoledronate stimulation with IL-15 (open circles), or pamidronate with  $V\gamma 2V\delta 2$  T cells derived by pulse zoledronate stimulation with IL-2 (closed circles). Bars represent mean values.  $***p < 0.001$  using the Mann-Whitney  $U$  test.