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## **Supplemental Data**

## Structural Studies of V $\gamma$ 2V $\delta$ 2 T Cell Phosphoantigens

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Figure S1. <sup>1</sup>H and <sup>31</sup>P NMR Spectra of: A, 2 (<sup>1</sup>H); B, 2 (<sup>31</sup>P); C, 12 (<sup>1</sup>H); D, 12 (<sup>31</sup>P); E, 15 (<sup>1</sup>H); F, 15 (<sup>31</sup>P).

\* Denotes ethanol peaks in the <sup>1</sup>H spectra and + denotes inorganic diphosphate (PPi) in the <sup>31</sup>P spectra.



Figure S2. Structures of 6-Phosphogluconate (16), Ribulose-5-Phosphate (17), and 2-Dehydro-3-Deoxy-6-Phosphogluconate (18)

Conversion of **16** to **17** is carried out by 6-phosphogluconate dehydrogenase, decarboxylating, while conversion of **16** to **18** is carried out by 6-phosphogluconate dehydratase .



Figure S3. ESI MS/MS Parent/Daughter Spectra of: (Upper Panel). m/e = 275 Ion Species from *M. smegmatis* Extract (see Ref. 1 for More Details); (Lower Panel). 6-Phosphogluconate (**16**)



Figure S4. 3-Formyl-1-Butyl Diphosphate (2) and the Hyrate (14) Compound Do Not Antagonize  $V\gamma 2V\delta 2$  T Cell Responses to HMBPP (6)

Since it seemed possible that one of the two compounds could function as an antagonist for the other, we directly tested for inhibitory activity by stimulating  $V\gamma 2V\delta 2$  T cells with HMBPP in the presence of **2 (14)**. A. **2 (14)** does not inhibit HMBPP responses. Varying concentrations of HMBPP and **2 (14)** were mixed and used to stimulate the 12G12  $V\gamma 2V\delta 2$  T cell clone with fixed Va2 cells as APC. Responses to **2 (14) (B)** and to HMBPP (C) alone. Note that addition of **2 (14)** only slightly inhibits the peak response to HMBPP and does not alter the EC<sub>50</sub>.



Figure S5. Vy2V82 T Cells Expand in Response to 2 (14) but Not 6-Phosphogluconate (16)

**A**, Flow cytometric analysis of V $\gamma$ 2V $\delta$ 2 T cell expansion on day 7. **B**, Expansion of V $\gamma$ 2V $\delta$ 2 T cells at day 7 and day 14. PBMC from 2 normal donors were cultured with either medium, 0.316  $\mu$ M HMBPP, 50  $\mu$ M IPP, 50  $\mu$ M ethyl pyrophosphate (EPP), 50  $\mu$ M **2 (14)** (3-FBPP), and 0.316, 1, 3.16, and 10 mM 6-phosphogluconate (6-PG). On day 3, IL-2 was added to 1 nM. On day 7 and day 14, PBMC were harvested and analyzed by two-color flow cytometry using anti-Va2 and anti-CD3 specific mAbs. The results of the 10 mM 6-phosphogluconate stimulation are shown.