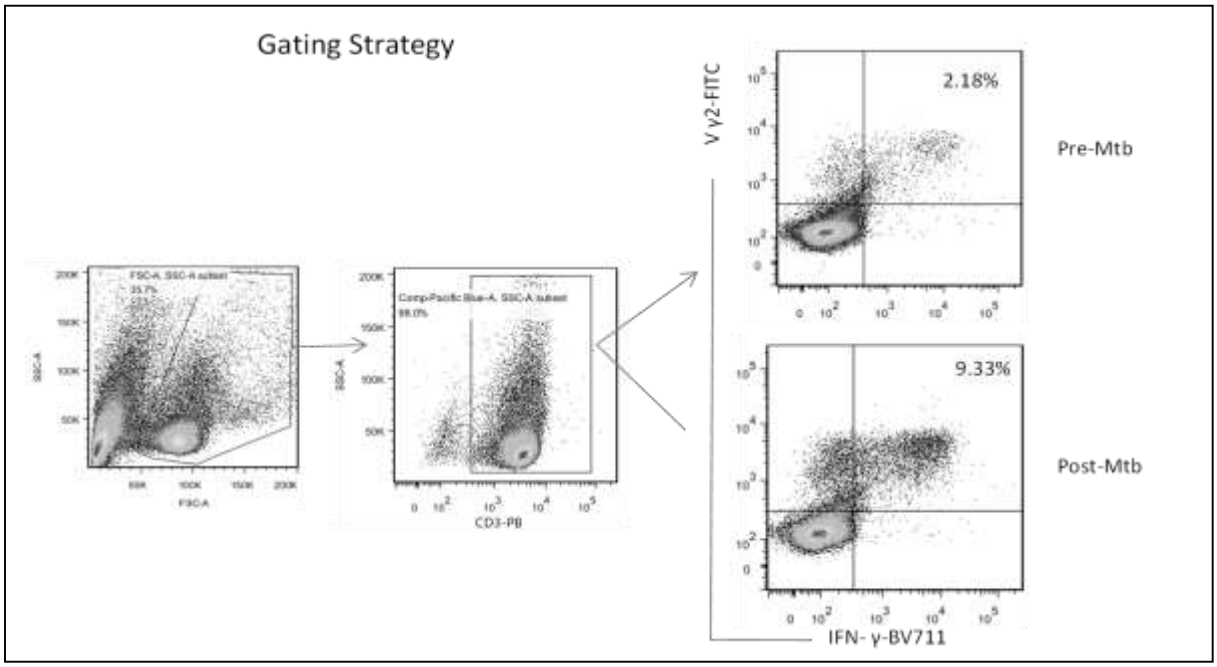
Supporting (Sup.) information for expression, purification and characterization of recombinant macaque IL-17A, IL-17F and IL-22 For isolation of macaque IL-17/IL-22 mRNA, PBMC were separated from heparinized blood of the monkeys by Ficoll-Paque plus (Amersham, Piscataway, NJ) density gradient centrifugation. Cells were activated for 48 hr with 200 ng/ml phorbol 12-myristate 13-acetate (PMA) and 1 μg/ml ionomycin (Sigma-Aldrich, St. Louis, MO). Total RNA was extracted from activated cells using TRIzol (Invitrogen, Carlsbad, CA) method and then converted to cDNA by using SMART cDNA synthesis kit (Clonetech, Mountain View, CA). Each cDNA sample was PCR-amplified by high-fidelity polymerase using the specific primers designed based on macaque IL-17A, IL-17F and IL-22 cDNA sequences (PubMed), respectively.

The coding sequence of macaque IL-17A, IL-17F or IL-22 cDNA was cloned into pXmh vector to express a protein tagged with C-terminal 6-his, and electroporated into Lactococcus lactis stain PA1001 for secretion of each of these cytokines as we previously described (Zeng G et al., 2009). Vector pXmh is a derivative of L. lactis expression vector pPA3, by which expressed protein contains a c-myc tag for western blotting analysis with anti c-myc antibody (clone 9E10, Sigma-Aldrich) and a 6-his tag for purification(Yuan Z et al., 2009). The sequences of forward and reverse primers for cytokine expression are as follows: IL-17A, 5-CATGCCATGGGAGGAATAGCAATCCCACGAAAT/ 5-ACATGCATGCGGCTACATGGTGGACAAT; IL-17F, 5-CATGCCATGGGAAGGAAAATCCCCAAAGTAG

/5- ACATGCATGCCTGCACATGGTGGACGAC; IL-22, 5-CATGCCATGGGAGCGCCCGTCAGCTCCCAC /5-ACATGCATGCAATGCAGGCATTTCTCAGAG. The host cells were incubated overnight at 30℃ in GM17 media containing chloramphenicol (5 μg/ml), with two addition of nisin A to induce the expression and secretion of target proteins. The secreted proteins were concentrated and subjected to affinity purification by Ni-NTA purification system (Invitrogen). Western blotting and SDS-PAGE were performed to examine the expression level and purity of these proteins. The concentration of purified proteins was determined by BCA protein assay kit (Pierce.). IL-23 is heterodimer consisting of two units, p19 and p40, and requires post-translational modifications and glycosylation for its bio-activity. Due to these requirements, macaque IL-23 could not be expressed in *L. lactis* prokaryotic expression system, and therefore recombinant human IL-23 (R&D system) was purchased for the experiments in the current study.

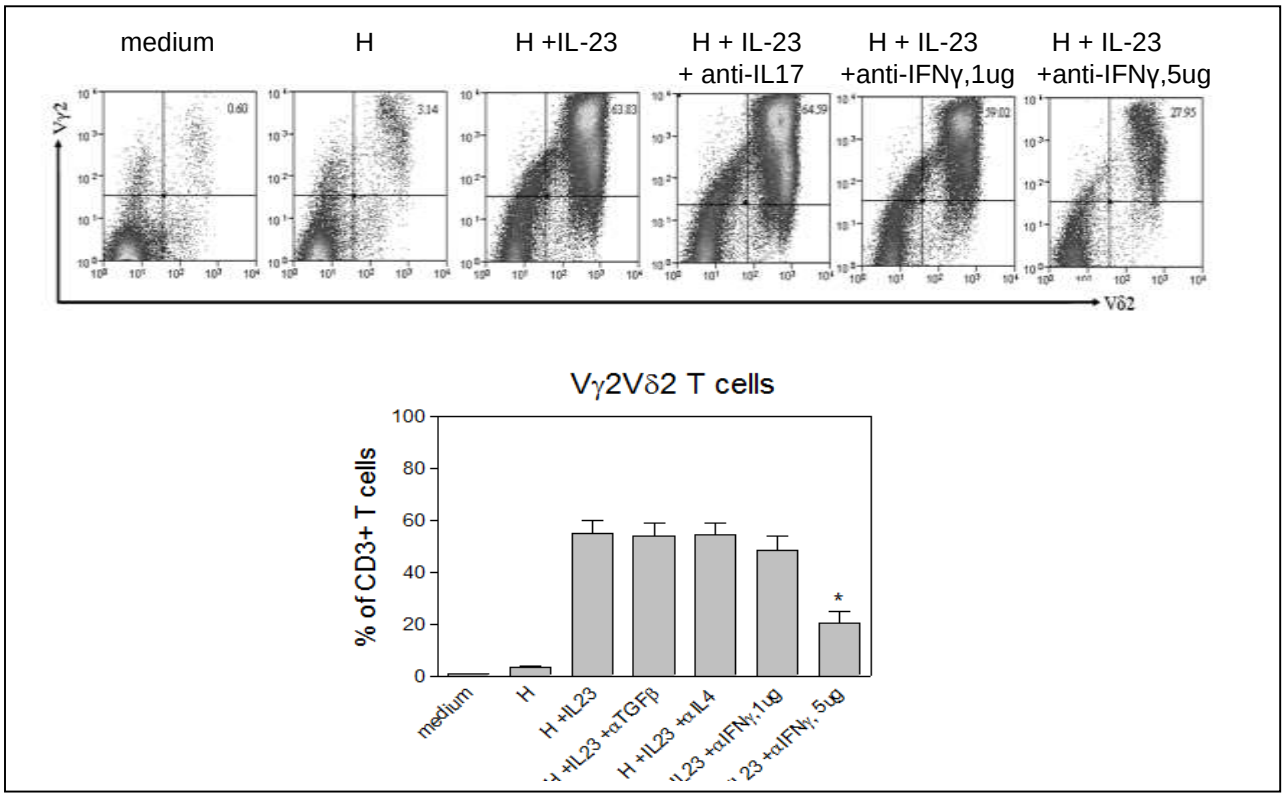
Supporting Fig. 1 Flow cytometry gating strategy for analysis of Vγ2+ T effector cells capable of producing cytokines. Shown is an example for IFN-γ+Vγ2+ T effector cells.



Supporting Fig. 2. Anti-IFN-γ Ab reduces the ability of HMBPP/IL-23 co-stimulation to expand Vγ2Vδ2 T cells.

Sup. Fig. 2a shows representative flow histograms from one representative monkey (7742) indicating that HMBPP/IL-23-induced expansion of Vγ2Vδ2 T cells was inhibited by anti-IFN-γ mAb (5 μg/ml), but not by anti-IL-17.

Sup. Fig. 2b shows pooled graph data indicating percentage numbers of Vγ2Vδ2 T cells in PBMC cultures treated with media alone, HMBPP, HMBPP+IL-23, HMBPP+IL-23+ anti-TGF-β, or HMBPP+IL-23 + anti-IFN-γ mAb. Data were mean values with SEM error bars from PBMC of 3 monkeys in two independent experiments. Mouse IgG does not have detectable effect(Fig.2).



Supporting Fig.3. HMBPP/IL-23-induced proliferation of Vγ2Vδ2 T cells required APC contact and involved the conventional and novel protein kinase C(PKC) signaling pathways.

Sup. Fig.3a shows representative flow cytometry histograms indicating CFSE-based proliferation of un-separated Vγ2Vδ2 T cells in PBMC (upper panels) and purified Vγ2 T cells in the trans-wells non-contacting with monocytes (lower panels) using cells derived from the macaque 7741. PBMC and Vγ2+ T cells were labeled with CSFE, and placed in the upper cell compartment, and cultured for 7 days in the presence of medium, HMBPP, IL-23(not shown), HMBPP +IL-23, or HMBPP +IL-2. Purified Vγ2+ T cells proliferate vigorously only in the presence of HMBPP +IL-2 but not HMBPP +IL-23. Similar results were seen in an additional experiment using samples from two macaques.

Sup. Fig.3b shows representative flow histograms indicating that the ability of Vγ2+ T cells to proliferate in response to HMBPP + IL-23 co-stimulation was recovered once mixing or

contacting with monocytes in culture. Similar data were seen from two other experiments using samples from macaques 7741 and 7742.

Sup. Fig. 3c, HMBPP/IL-23-induced proliferation of Vγ2Vδ2 T cells involved the conventional and novel protein kinase C(PKC) signaling pathways. Shown are representative flow cytometry histograms from above two macaques. Data demonstrate that both PKC α/β inhibitor GO6976 and PKC θ inhibitor rottlerin inhibit HMBPP/IL-23-induced proliferation of Vγ2Vδ2 T cells, respectively. Data were derived from day 7 culture.