ONLINE METHODS

Mice. Wild-type C57BL/6 female mice were purchased from Harlan Bioscience. Mice with conditional deletion of the gene encoding PU.1 (Sfpi1<sup>Lck<sup>-/-</sup></sup>) on the C57BL/6 background were previously described<sup>42</sup> and mated to mice carrying a Cre transgene under control of an Lck promoter (B6(CBA)-Tg(Lck-cre)1540Jxm/J). Mice were maintained in pathogen-free conditions and all studies were approved by the Indiana University School of Medicine Animal Care and Use Committee.

Murine T helper cell differentiation. Naïve CD4<sup>+</sup>CD62L<sup>+</sup> T cells were purified from spleens and lymph nodes using magnetic isolation (Miltenyi Biotec). Naïve CD4<sup>+</sup> T cells (1 x 10<sup>6</sup> cells/ml of complete RPMI-1640 medium) were cultured with plate-bound anti-CD3 (145-2C11, 2 μg/ml, BD Biosciences) and soluble anti-CD28 (37.51, 0.5 μg/ml, BD Biosciences) under T<sub>H2</sub> (IL-4 at 10 ng/ml (PeproTech) and anti-IFNγ (XMG) at 10 μg/ml) or T<sub>H9</sub> (IL-4 at 10 ng/ml (R&D Systems), TGF-β at 2 ng/ml (R&D Systems) and anti-IFNγ at 10 μg/ml) conditions. T<sub>H1</sub> and T<sub>H17</sub> conditions were previously described<sup>43</sup>. After 3 days, cultures were supplemented with complete RPMI-1640 medium. After 5-6 days of culture, differentiated cells were restimulated with plate bound anti-CD3 at 1 μg/ml for 1, 2, or 3 days, and the cell-free supernatant was collected after centrifugation and stored at −20°C until use. The amounts of IL-9 produced were determined using ELISA with capture anti-IL-9 (D8402E8, BD Biosciences) and biotin-labeled secondary anti-IL-9 (D9302C12, BioLegend). As noted, cultures were supplemented with 10 μg/ml anti-IL-10 during the differentiation or 2 ng/ml TGF-β1 during the anti-CD3 restimulation. In some experiments the cell pellets collected after one day of restimulation were used for RNA extraction. Chromatin immunoprecipitation and DNA affinity precipitation assay (DAPA) were performed as previously described<sup>7-9</sup>. Briefly, biotinylated oligonucleotides are incubated with cell extracts and bound proteins are precipitated with streptavidin-agarose before immunoblot. Competitions are performed with non-biotinylated oligonucleotides. VISTA analysis was performed with human and mouse genomic sequences. Primers for ChIP assay were Il9CNS1a (CAGTCTACCAGCATCTTCCAGTCTAGC and GTGGGCACCTGGGTATCGTCTTGATGTC), and Il9CNS1b (GTCACTTGACAAAGGCTGTCTTATGCCC and CAGAACCAGACTATTGGAAGAGCATC)
and II9CNS2 (AATTACAGAATTTCGCCCTG and GTTAATGCACAATTGTGCCAATCC).

**Retroviral transduction.** Bicistronic retroviral vectors encoding mouse PU.1 and EGFP or IRF4 and hCD4 were described previously\(^8,9\). After 2 days of differentiation, T helper cells were transduced with retroviral supernatant containing polybrene following the protocol described\(^8\). In transduced cells, GFP positive cells were sorted by flow cytometry before cytokine production and gene expression analyses.

**Quantitative RT-PCR.** Total RNA was isolated from either unstimulated or anti-CD3 (2\(\mu\)g/ml) restimulated cells using Trizol and reverse transcribed according to manufacturer’s instructions (Invitrogen Life Technologies). Quantitative PCR was performed with Taqman Fast Universal PCR Master Mix and commercially available primers for human and mouse genes using the 7500 Fast Real-Time PCR system (Applied Biosystems). RNA was normalized to expression levels of \(\beta\)2-microglobulin and relative expression was calculated using the \(-\Delta\Delta\text{Ct}\) method.

**Intracellular Cytokine Staining.** After differentiation as indicated, cells were incubated in monensin for the last 2 h of a 5 h anti-CD3 or PMA-ionomycin restimulation as indicated. Intracellular cytokine staining was performed by standard protocol using fluorochrome conjugated IL-9 (RM9A4, mouse, BioLegend; MH9A4, human, BD Pharmingen) and IL-10 (JES5-16E3), IL-4 (11B11), IL-13 (JES10-5A2), (BD Pharmingen), IL-17 (eBio17B7) or Foxp3 (FJK-16s, eBioscience) specific antibodies to stain the cells and evaluated by flow cytometry using FACScalibur instrument (Beckton Dickinson). Results were analyzed by WinMDI.

**Human T helper cell differentiation.** Use of human cells was approved by the Indiana University IRB. Naive CD4\(^+\) T cells were isolated from PBMC using magnetic sorting (Miltenyi Biotec). Th2 and Th9 primed cultures were activated with CD3/CD28 Dynabeads (Invitrogen) supplemented with IL-4 (20 ng/ml; R&D Systems) and anti-IFN-\(\gamma\) (25718, 2.5 \(\mu\)g/ml; R&D Systems), or IL-4 (20 ng/ml) and human TGF-\(\beta\)1 (5 ng/ml), respectively. The cells were cultured at 37 °C in a humidified 5% CO\(_2\) incubator. Cells were expanded on day 3 by transferring to a 6-well plate with 5 ml culture media. Cells on day 5 culture were replated (0.5x10\(^6\) cells/ml) and
stimulated with plate-bound anti-CD3 (HIT3a, 2 μg/ml; BD Pharmingen). After culturing for 24 h, both supernatant and cells were collected for ELISA and qPCR, respectively. IL-9 ELISA was performed using reagents from BioLegend (MH9A4, MH9D1).

**siRNA transfection.** T\textsubscript{H}2 or T\textsubscript{H}9 cells were transfected with control or SPI1-specific siRNA (Santa Cruz Biotechnology) using the human T cell nucleofector kit (Amaxa Biosystems) as per manufacturer’s instructions. T cells (3 x 10\textsuperscript{6}) were suspended in human T cell Nucleofector Solution and transfected with 1 μg siRNA by using the T-23 transfection program. Transfected cells were plated in pre-warmed culture media supplemented with human IL-2 (50 U/ml; R&D Systems) and cultured at 37°C in a humidified 5% CO\textsubscript{2} incubator. After culturing for 24 h, both supernatant and RNA from cells were collected for ELISA and qPCR.

**Multiplex analysis of atopic patient PBMC cytokine production.** Patient sample collection and analysis was approved by the Indiana University IRB and required parental consent for samples from infants. Five ml of venous blood was collected by venipuncture. PBMCs were separated on a gradient of Ficoll-paque\textsuperscript{TM} PLUS (GE Healthcare). Cells were counted with trypan blue and stored in DMSO-freezing medium (Bioveris). Patients were defined as atopic or non-atopic based on positive allergen-specific serum IgE\textsuperscript{31}. Following thaw, isolated PBMCs from non-atopic vs atopic patients were activated with plate-bound anti-CD3 (HIT3a, 2 μg/ml; BD Pharmingen) and cultured for 3 days at 37 °C in a humidified 5% CO\textsubscript{2} incubator. Supernatant IL-9 concentration was measured using Multiplex Bead Immunoassays as per manufacturer’s protocol (Millipore). Samples and standards were loaded to a filter plate (Millipore) with multiplex beads following vortexing and sonication. The plate was incubated on a shaker (Lab-Line Instruments, Inc) in the dark at 24°C for 1 hour. Plate was washed twice with wash buffer before biotinylated detection antibody was added to each well and the plate was incubated for 30 min at 24°C. Streptavidin-phycoerythrin was added to each well and incubated for 30 min at 24°C. After washing twice with wash buffer, sheath fluid was added to each well and shaken for 5 min. The assay plate was then transferred to the Luminex 200 instrument (Luminex Corporation) for acquisition and analysis. Cytokine concentrations were calculated using Bio-Plex Manager 2.3 software with a five parameter curve-fitting algorithm applied for standard curve calculations.
**Induction of Allergic Airway Inflammation.** Mice were sensitized by intraperitoneal injections of Ova (Sigma) adsorbed with alum (Sigma) at a dose of 20 µg Ova / 2 mg alum on days 0 and 7. On day 14, mice were exposed to intranasal Ova (100 µg) per day for 5-6 consecutive days. In specified experiments, mice were given 20 µg control or anti-IL-9 Ab intravenously 30 minutes before the first, third and fifth challenges. Mice were euthanized by intraperitoneal injection of pentobarbital (5 mg/mouse), 48 h after the last intranasal challenge. The trachea was cannulated and lungs lavaged 3 times with 1 ml of PBS. The cells recovered in BAL fluid were counted with a hemocytometer. Eosinophils, neutrophils, T cells, B cells, and mononuclear cells in the BAL were distinguished by cell size and the expression of CD3, B220, CCR3, CD11c, and MHC class II, as described by a flow cytometric method\(^4^4\). Cytokine concentrations in cell-free BAL fluid were measured using multiplex reagents (Millipore). For qPCR measurements, lung tissues were homogenized in a tissue lyser (Qiagen) and RNA isolated with RNeasy kit (Qiagen) was used to synthesize cDNA for subsequent analysis. Peripheral immune responses in sensitized and challenged wild type or \(Sfpi1^{lck−/−}\) mice were assessed by culturing splenocytes with anti-CD3 (4 µg/ml) or OVA (100 µg/ml) for 72 h before cell free supernatants were tested for cytokines using ELISA. Paraffin-embedded sections were stained with hematoxylin & eosin (H&E) in order to evaluate the infiltration of inflammatory cells using light microscopy.

**Airway hyperreactivity.** Airway hyperreactivity to methacholine challenge was determined 24 h after the last intranasal challenge. Non-invasive unrestrained whole body plethysmography (Buxco System) was used to record airway responsiveness via a dimensionless parameter termed enhanced pause (Penh) to estimate the total pulmonary resistance, an indicator of bronchoconstriction. Mice were placed into whole body plethysmographs and baseline measurements recorded. Saline was administered by nebulization for 2 min followed by increasing doses of methacholine, and Penh was recorded over 5 min.