## Materials and Methods

**Animals.** 24 outbred, Indian-origin female rhesus monkeys (*Macaca mulatta*) were genotyped and selected as negative for the protective MHC class I alleles *Mamu-A*01, Mamu-B*08,* and *Mamu-B*17*. TRIM5 polymorphisms were balanced equally among groups, and animals were otherwise randomly allocated. All monkeys were housed at Bioqual, Rockville, MD. Animals received 2 mg/kg PGT121 or an isotype matched sham control antibody against dengue virus serotype 3 by the intravenous route on day -1 and were infected with $5x10^4$ $TCID_{50}$ of our SHIV-SF162P3 stock (*8-10*) by the intravaginal route on day 0. Animals were necropsied on day 1, 3, 7, or 10 following infection, and multiple samples of 30 independent tissues were obtained for virologic, immunologic, and transcriptomic analyses. All assays were performed blinded. 12 additional rhesus monkeys were utilized for the initial PGT121 protection study, and 3 additional rhesus monkeys were utilized for the adoptive transfer study. All animal studies were approved by the appropriate Institutional Animal Care and Use Committee (IACUC).

**Viral RNA and DNA.** Plasma SHIV RNA levels were measured using a *gag* targeted quantitative real-time RT-PCR assay, and tissue levels of SHIV RNA and DNA were measured using *gag* targeted, nested quantitative hybrid real time/digital RT-PCR and PCR assays, essentially as described (*12*). Untouched CD4+ T lymphocytes were purified from lymph node mononuclear cells to >99% purity using a non-human primate CD4+ T Cell Isolation Kit (Miltenyi Biotec) prior to SHIV RNA and DNA assays.

**Cellular immune assays.** SIV Gag-specific cellular immune responses in blood and tissues were assessed by multiparameter ICS assays, essentially as described (*16, 17*). 9-color ICS assays were performed with predetermined titers of mAbs against CD3 (SP34; Alexa700), CD4 (L200; AmCyan), CD8 (SK1; allophycocyanin-cyanine 7 [APC-Cy7]), CD28 (L293; peridinin chlorophyll-A-cyanine 5.5 [PerCP-Cy5.5]), CD95 (DX2; phycoerythrin [PE]), CD69 (TP1.55.3; phycoerythrin-Texas Red [energy coupled dye; ECD]; Beckman Coulter), IFN-γ (B27; phycoerythrin-cyanine 7 [PE-Cy7]), IL-2 (MQ1-17H12; allophycocyanin [APC]) and TNF-α (Mab11; fluorescein isothiocya [FITC]). IFN-γ backgrounds were consistently <0.01% in PBMC and <0.05% in tissues.

**Microarray analyses.** Cells were isolated from tissues and lysed for RNA extraction and gene arrays were performed, essentially as described (*11, 12*). Reverse transcription reactions were performed to obtain cDNA, which was hybridized to the Illumina Human HT-12 version 4 Expression BeadChip according to the manufacturer's instructions and quantified using an Illumina iScan System. Data were collected with Illumina GenomeStudio software. Analysis of the genome array output data was conducted using the R statistical language and the Limma statistical package from Bioconductor. First, arrays displaying unusually low median intensity, low variability, or low correlation relative to the bulk of the arrays were tagged as outliers and were discarded from the rest of the analysis. Quantile normalization followed by a log2 transformation using the Bioconductor package LIMMA was applied to process microarrays. The Limma package was used to fit a linear model to each probe and to perform a moderated Student's t-test on various differences of interest between tissue types and time points. For data mining and functional analyses, genes that satisfied P <

0.05 were selected. Probes that did not map to annotated RefSeq genes and control probes were removed. When indicated, the expected proportions of false positives (FDR) were estimated from the unadjusted P-value. Transcriptomics data can be accessed at GEO (GSE83702) and NCBI (17948367).

**Gene set enrichment analysis (GSEA).** GSEA calculates an enrichment score that reflects the degree to which a set of genes is overrepresented among differently expressed genes (*19*). Leading edge analysis defines the particular genes contributing the most to the enrichment. The Hallmark v5 MSigDB database from the Broad Institute was used to mine canonical pathways. Supervised analysis was performed on contrasts of interest.

**Gene Mania network analysis.** Gene Mania networks were utilized to represent co-expression of genes associated with viral RNA positive tissues from PGT121 treated animals (*20*, *21*).

**Statistics.** To evaluate the differences in tissue viral RNA between the experimental and the naïve group, we applied Fisher's exact tests for equal medians. The differences in the proportion of positive viral RNA detection between experimental groups or between days were also evaluated using Fisher's exact tests. The differences in the number of anatomic sites with a positive viral RNA detection between groups were analyzed using Fisher's exact tests for equal medians and Poisson regression models for counts.

## Author Contributions

D.H.B., D.R.B., J.D.L, and R.P.S. designed the studies and interpreted the data. J.L., E.B., C.C., D.S., L.P., A.B., M.S., J.J., J.M., B.L., P.G., A.Ch., and D.H.B. led the conduct of the study and the immunologic assays. W.J.B., Y.L., E.C., B.B., K.O., P.A., and J.D.L. led the virologic assays. K.G. and R.P.S. led the transcriptomic assays. C.G., C.B., W.W., and M.G.L. led the clinical care of the rhesus monkeys. A.Co. and W.L. conducted the data and statistical analyses. D.H.B. wrote the paper with all co-authors.

## Supplementary Figure Legends

**Figure S1. Protective efficacy of PGT121 in rhesus monkeys.** Rhesus monkeys received 2 mg/kg PGT121 (N=6) or sham control (N=6) by the intravenous route on day -1 and were challenged intravaginally with $5x10^4$ $TCID_{50}$ SHIV-SF162P3 on day 0. Plasma viral RNA copies/ml are shown for 6 months following challenge.

**Figure S2. Summary of viral RNA in distal tissues following SHIV-SF162P3 challenge.** Box-and-whisker plots depict the number of viral RNA positive tissues distal to the female reproductive tract per monkey in PGT121 treated animals and sham controls on day 1-3, day 7, and day 10 following SHIV-SF162P3 challenge. P-values reflect Fisher's exact tests.

**Figure S3. SIV Gag-specific T lymphocyte responses in tissues following SHIV-SF162P3 challenge on days 1-3.** SIV Gag-specific, IFN-γ-positive CD8+ and CD4+ T lymphocyte responses were analyzed by intracellular cytokine staining assays across multiple tissues at necropsy in animals on days 1-3 following SHIV-SF162P3 challenge. Values to the right of the vertical line indicate samples distal to the female reproductive tract.

**Figure S4. SIV Gag-specific T lymphocyte responses in tissues following SHIV-SF162P3 challenge on day 7.** SIV Gag-specific, IFN-γ-positive CD8+ and CD4+ T lymphocyte responses were analyzed by intracellular cytokine staining assays across

multiple tissues at necropsy in animals on day 7 following SHIV-SF162P3 challenge. Values to the right of the vertical line indicate samples distal to the female reproductive tract.

**Figure S5. SIV Gag-specific T lymphocyte responses in tissues following SHIV-SF162P3 challenge on day 10.** SIV Gag-specific, IFN-γ-positive CD8+ and CD4+ T lymphocyte responses were analyzed by intracellular cytokine staining assays across multiple tissues at necropsy in animals on day 10 following SHIV-SF162P3 challenge. Values to the right of the vertical line indicate samples distal to the female reproductive tract.

**Figure S6. Multidimensional scaling plot reveals patterns of gene expression.** The largest transcriptomic signal (33% of the variation in the transcriptome) reflected differences observed in the different tissue groups, including female reproductive tract tissues (FRT), gastrointestinal tissues (GI), and lymph nodes (LN). Individual tissues were grouped based on Euclidean distance for dimension reduction. The second largest transcriptomic signal (16% of the variation in the transcriptome) distinguished PGT121 treated animals compared with sham controls.

**Figure S7. Samples for transcriptomic analysis in viral RNA positive tissues from PGT121 treated animals.** Sample details for the data shown in Figure 3A.

**Figure S8. Checkerboard plots showing upregulated and downregulated pathways in viral RNA positive compared with viral RNA negative tissues from PGT121 treated animals.** Pathways are shown on the y-axis and the respective leading edge genes (gene members contributing the most to enrichment of the pathways) are depicted on the x-axis on day 1 following SHIV-SF162P3 challenge. The color gradient represents the difference in expression between the two groups (log2FC). The leading edge genes presented on this plot are significantly differentially expressed between the two groups (LIMMA t-test with $P < 0.05$). Red and blue boxes correspond to upregulation and downregulation of specific genes in each respective pathway.

**Figure S9. Network of genes that positively correlated with levels of viral RNA in tissues from PGT121 treated animals.** Top upregulated pathways are shown with false discovery rates (FDR) for the data shown in Figure 3C.

**Figure S10. Temporal progression of transcriptomic changes in viral RNA positive tissues from PGT121 treated animals.** (A) Number of differentially expressed interferon-stimulated genes (ISGs) and total genes over time in the peripheral blood, female reproductive tract tissues (FRT), lymph nodes (LN), and gastrointestinal tissues (GI) ($P<0.05$, $|FC|>1.3$). (B) Heatmap depicts differentially expressed ISGs in viral RNA positive tissues in PGT121 treated animals on day 7 versus day 1 following SHIV-SF162P3 challenge, highlighting the emergence of antiviral restriction factors. Gene expression is represented as a gene-wise standardized expression (Z-score) with $P < 0.05$. Red and blue correspond to upregulated and downregulated genes, respectively.

**Figure S11. Overlap of transcriptomic signature of viral RNA positive tissues from PGT121 treated animals with ISGs associated with viral replication.** Venn diagrams represent overlap of gene signatures of viral RNA positive tissues from PGT121 treated animals with an interferon-stimulated gene (ISG) signature that reflects replicating viruses from Schoggins et al (*22*). A 31-gene overlap signature is shown.

**Figure S12. Summary of viral RNA and viral DNA data from PGT121 treated animals following SHIV-SF162P3 challenge.**