**Supplementary Methods**

**Ethics statement**

All animal procedures received prior approval from the Institutional Animal Care and Use Committee (IACUC) of the Centers for Disease Control and Prevention and were conducted in a United States Department of Agriculture (USDA)-registered, Office of Laboratory Animal Welfare (OLAW)-assured, and (AAALAC)-international-accredited animal facility following the Guide for the Care and Use of Laboratory Animals [1].

**Infection detection and characterization during efficacy study**

Blood was collected on the day of each challenge to measure viral RNA (vRNA). Animals were confirmed protected if tested vRNA- and seronegative during virus challenges and during the follow-up period of 6 weeks. Plasma SHIV RNA levels were assessed by qRT-PCR (Invitrogen SuperScript™ III Platinum™ One-Step qRT-PCR Kit, Fisher Scientific Company, Pittsburgh, PA), as described previously [2, 3]. Infected animals were tested for the emergence of K65R and integrase resistance mutations by Illumina MiSeq next-generation sequencing (NGS) with a cutoff for variant detection analysis at 1% of the total variant population [3].

**TAF/EVG terminal PK**

The terminal PK was conducted in SHIV-positive macaques and designed to define vaginal tissue drug levels one week after vaginal TAF/EVG insert application, corresponding to the time of SHIV exposure during efficacy studies (Figure 2A of the main manuscript). To assess accumulation after multiple dosings, the vaginal biopsies were collected one week after the first insert application from live animals and one week after the fourth weekly insert applications during the terminal procedure.

**Specimen processing and drug level detection**

Sample collection and drug analysis were performed as described previously [2, 3]. Whole blood samples were separated for plasma and PBMC. Vaginal pinch biopsies were collected with a pediatric speculum and biopsy forceps with a 3.3 mm jaw (Radial Jaw™ 3 Biopsy Forceps, Boston Scientific or equivalent). TFV-DP concentrations in PBMC and vaginal tissue were measured by high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) (Sciex, Foster City, CA, Shimadzu Scientific, Columbus, MD) [2, 3].

**Progesterone testing**

Progesterone levels in plasma were measured by liquid chromatography/mass spectrometry as described previously [4, 5]

1. National Research Council Committee for the Update of the Guide for the C, Use of Laboratory A. The National Academies Collection: Reports funded by National Institutes of Health. Guide for the Care and Use of Laboratory Animals. Washington (DC): National Academies Press (US), **2011**.

2. Dobard CW, Peet MM, Nishiura K, et al. Single dose topical inserts containing tenofovir alafenamide fumarate and elvitegravir provide pre- and post-exposure protection against vaginal SHIV infection in macaques. eBioMedicine **2022**; 86:104361.

3. Makarova N, Singletary T, Peet MM, et al. Pharmacokinetics and efficacy of topical inserts containing tenofovir alafenamide fumarate and elvitegravir administered rectally in macaques. eBioMedicine **2022**; 86:104338.

4. Saltzman W, Schultz-Darken NJ, Scheffler G, Wegner FH, Abbott DH. Social and reproductive influences on plasma cortisol in female marmoset monkeys. Physiology & behavior **1994**; 56:801-10.

5. Makarova N, Henning T, Taylor A, et al. Topical tenofovir protects against vaginal SHIV infection in macaques co-infected with chlamydia trachomatis and trichomonas vaginalis. Aids **2017**.

**Supplemental Table 1. Variability in TFV-DP detection in PBMCs during efficacy studies.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Macaque ID | Instances of no detectable TFV-DP | Total No. of observations | Percentage |
| 8h PEP | **A10W0421** | 4 | 14 | 29 |
|  | A11W008 | 5 | 14 | 36 |
|  | A11W056 | 2 | 14 | 14 |
|  | A11W066 | 2 | 14 | 14 |
|  | A12W033 | 10 | 14 | 71 |
|  | PDK2 | 13 | 14 | 93 |
| 24h PEP | **A6P02046** | 2 | 14 | 14 |
|  | A11192 | 4 | 14 | 29 |
|  | A12W021 | 3 | 14 | 21 |
|  | **A12W026** | 2 | 14 | 14 |
|  | **Z14276** | 3 | 14 | 21 |
|  | Z15004 | 6 | 14 | 43 |
| Medians |  | 3.5 | 14 | 25 |

**1**Infected animals are highlighted in bold

**Supplemental Figure 1.**



**Supplemental figure 1. Progesterone levels during efficacy study** Weekly levels in individual animals at the time of challenge 6 or 7 days after dosing during 8h (A) and 24h (B) PEP efficacy studies. Arrows indicate the time of infection.

**Supplemental Figure 2.**



**Supplemental Figure 2. Variations in TFV-DP levels in PBMCs between animals during efficacy studies.** Weekly levels of TFV-DP in PBMCs collected at the time of challenge starting 1 week after dosing during 8h and 24h PEP efficacy studies. The red dots represent TFV-DP levels at the estimated time of infection (i.e., 1 week prior to the detection of plasma viral RNA). The green dots represent values at the second challenge, 1 week after the first insert application. Values below LOQ (black dotted line) were given a value of ½ of the LOQ (LOQ was 100 fmol/sample corresponding to 10 fmol/106 cells).