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☐ Initial submission ☐ Revised version ☒ Final submission

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size

Describe how sample size was determined.

All available specimens were analyzed from all available time points prior to HIV infection. The original cohort was powered to detect the effectiveness of TFV gel, as previously described (Abdool Karim et al Science 2010).

2. Data exclusions

Describe any data exclusions.

Participant specimens for the original trial were not available for 13% of participants. We were not able to carry out analyses in instances where storage consent was not provided, no specimens were available, or when participants acquired HIV before samples could be obtained.

3. Replication

Describe whether the experimental findings were reliably reproduced.

Clinical specimens were analyzed. A portion were analyzed in duplicate to calculate intra-plate variability and these data are available if required.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

Samples were analyzed randomly, with plate design done without any knowledge of clinical or outcome variables.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

Laboratory personnel were blinded to all clinical and epidemiological data, and were only given access (in some cases) once the final cytokine data was locked.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ A statement indicating how many times each experiment was replicated
☐	☒ The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☐	☒ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals noted
☐	☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒ Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.	Bio-Plex Manager software version 6, SPSS version 24, SAS version 9.3, and Microsoft Excel 14.7.2

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.	No unique materials were used.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	All antibodies used in Multiplex ELISA assays were validated by Biorad.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.	No eukaryotic cell lines were used.
b. Describe the method of cell line authentication used.	No eukaryotic cell lines were used.
c. Report whether the cell lines were tested for mycoplasma contamination.	No eukaryotic cell lines were used.
d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.	No eukaryotic cell lines were used.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.	No animals were used in this study.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.	All participants were women enrolled in the CAPRISA 004 clinical trial that tested the safety and efficacy of TFV 1% Gel (Abdool Karim et al Science 2010). We have included analysis of study arm at randomization and HIV acquisition, stratified by mucosal cytokine defintions of inflammation and study product adherence. A number of co-variates collected during the clinical trial have been used to adjust multi-variable analyses; most of these relate to risk factors for HIV acquisition and/or potential correlates of mucosal cytokine levels.