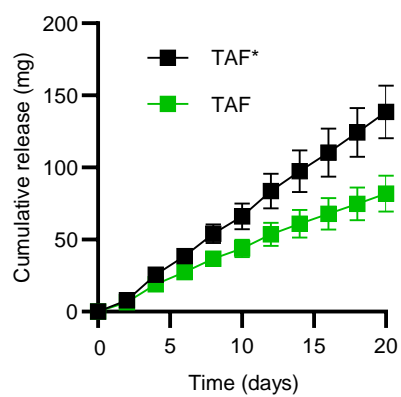


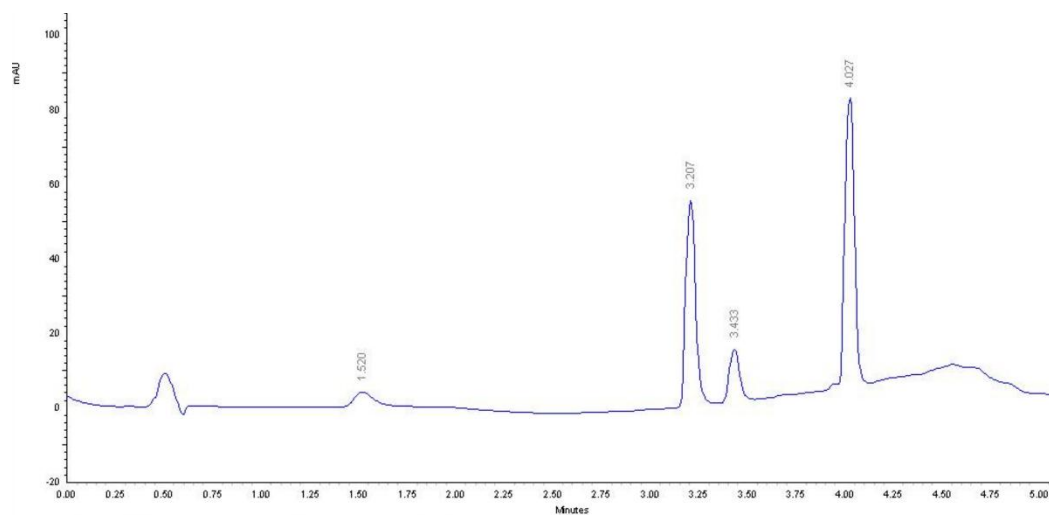
Supporting Information

Preventive efficacy of a tenofovir alafenamide fumarate nanofluidic implant in SHIV-challenged nonhuman primates

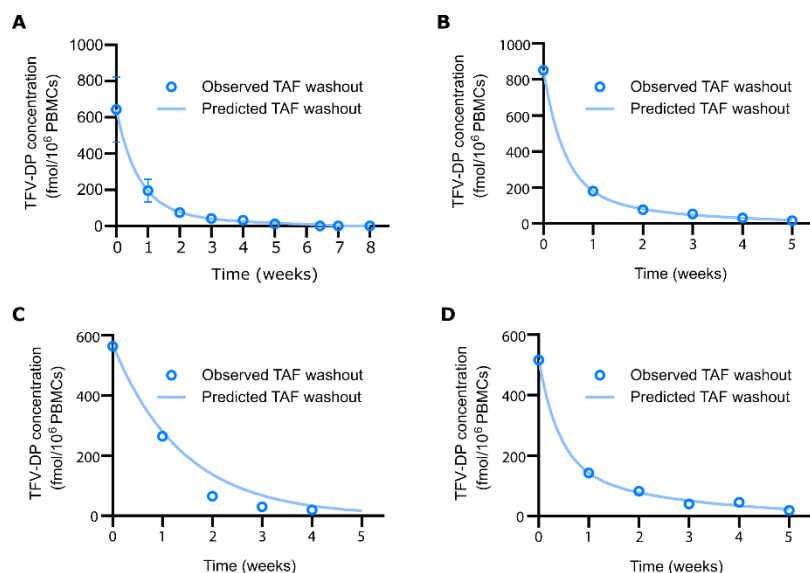
*Fernanda P. Pons-Faudoa, Antons Sizovs, Kathryn A. Shelton, Zoha Momin, Jean A. Niles, Lane R. Bushman, Jiaqiong Xu, Corrine Ying Xuan Chua, Joan E. Nichols, Sandra Demaria, Michael M. Ittmann, Trevor Hawkins, James F. Rooney, Mark A. Marzinke, Jason T. Kimata, Peter L. Anderson, Pramod N. Nehete, Roberto C. Arduino, Mauro Ferrari, K. Jagannadha Sastry, Alessandro Grattoni**



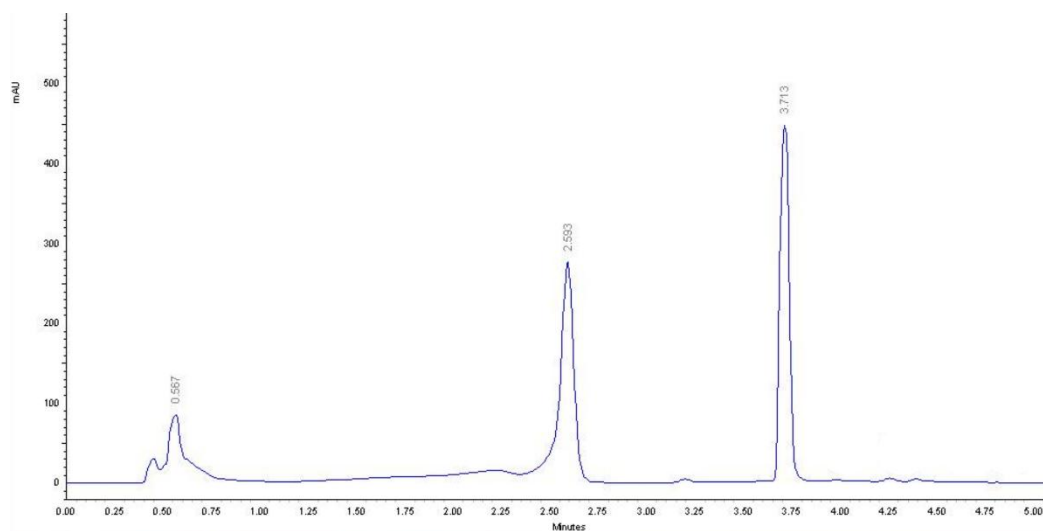
Supporting Figure S1. Cumulative in vitro release of TAF from nTAF (n=5). Sum of TAF* shown in black. Data presented as mean \pm SEM.



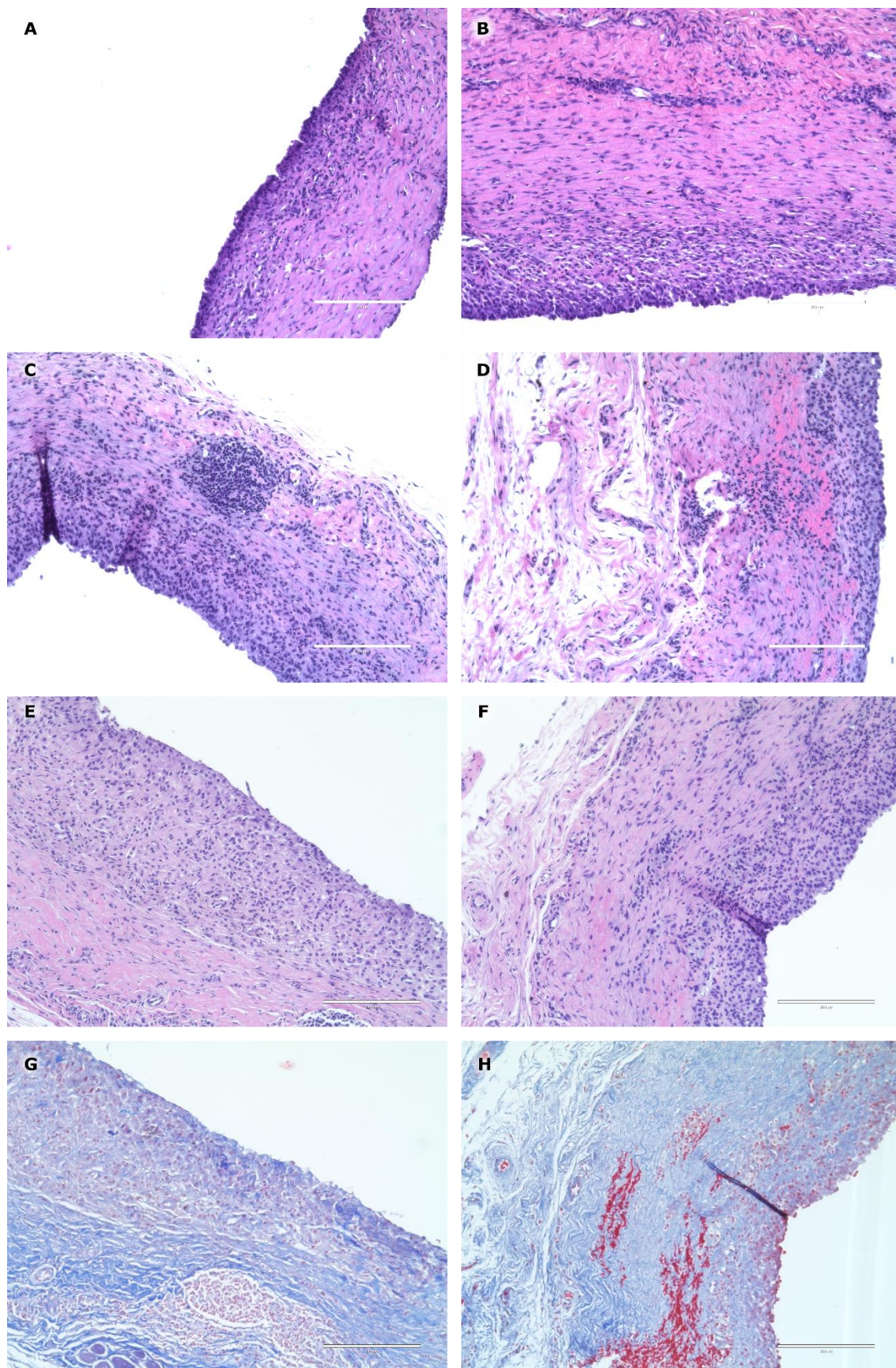
Supporting Figure S2. Representative HPLC chromatogram showing relative peak areas and retention times for TFV (RT= 1.52 min), TAF hydrolysis products (RT=3.207, 3.433 min), and TAF parent (RT=4.027 min) from nTAF implant on day of *in vitro* release.



Supporting Figure S3. (A) nTAF TFV-DP washout fitted to intravenous bolus injection two-compartment model to determine elimination rate constant. Data are presented as mean \pm SD. (B) PrEP 5 nTAF TFV-DP washout fitted to intravenous bolus injection two-compartment model to determine elimination rate constant. (C) PrEP 6 nTAF TFV-DP washout fitted to intravenous bolus injection two-compartment model to determine elimination rate constant. (D) PrEP 7 nTAF TFV-DP washout fitted to intravenous bolus injection two-compartment model to determine elimination rate constant.

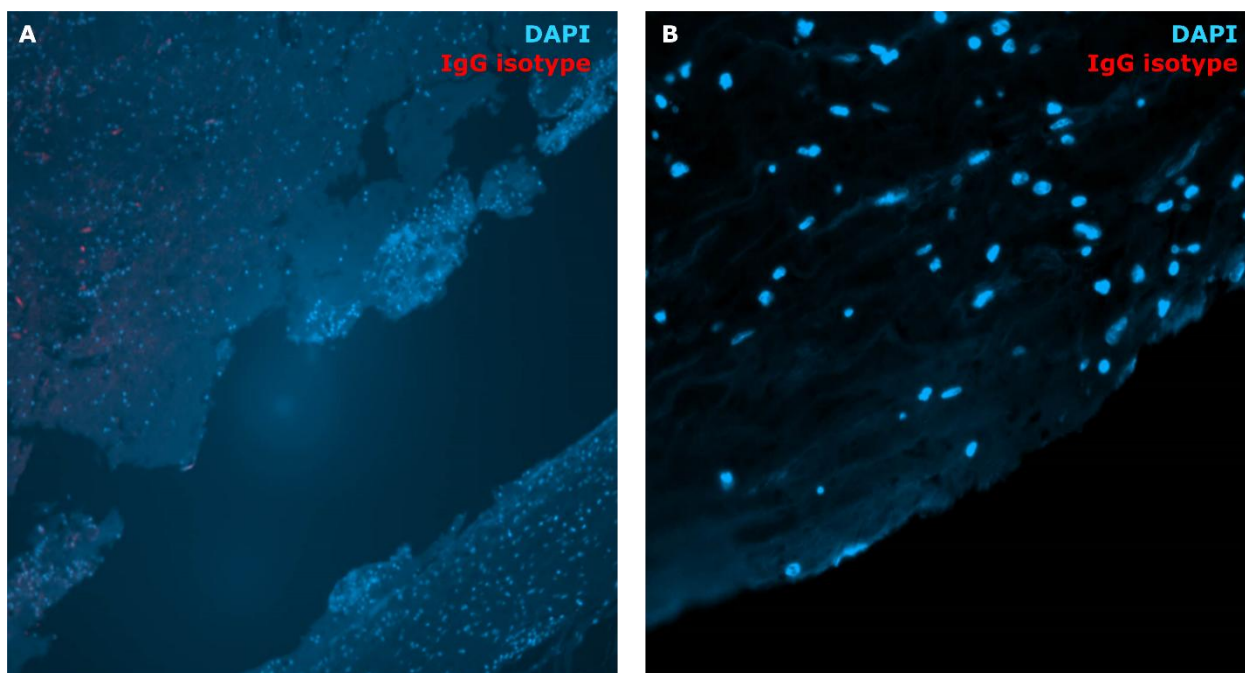


Supporting Figure S4. Representative HPLC chromatogram showing relative peak areas and retention times for TFV (RT=0.567 min), TAF hydrolysis products (RT=2.593 min), and TAF parent (RT= 3.713 min) from nTAF implant upon explantation on day 112.

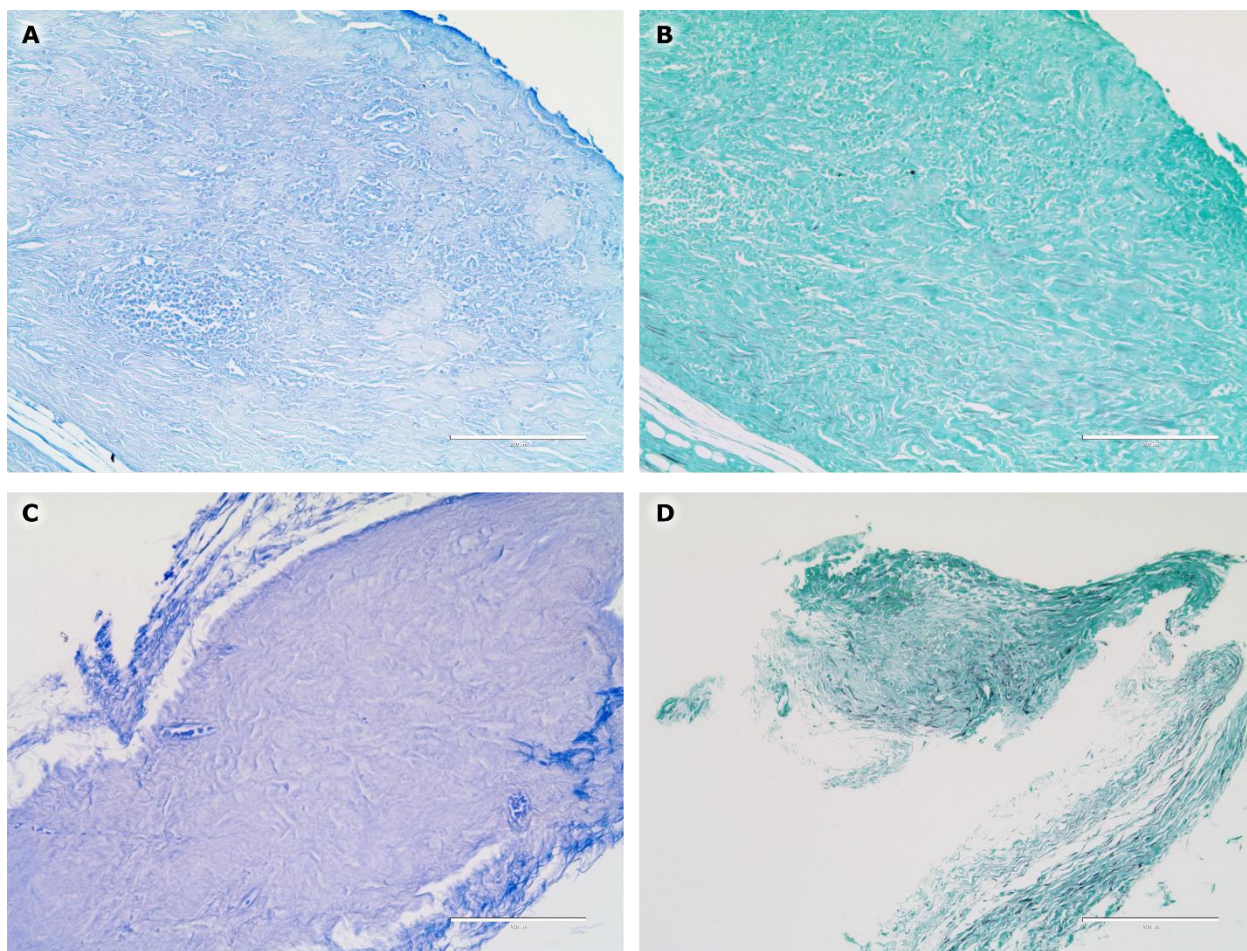


Supporting Figure S5. (A-F) Representative H&E NHP skin histology sections of fibrotic capsules in contact with TAF-releasing membrane. Inflammatory cells near peri-implant space, and collagen in the fibrotic capsule. (G-H) Masson Trichrome stains of (E-H),

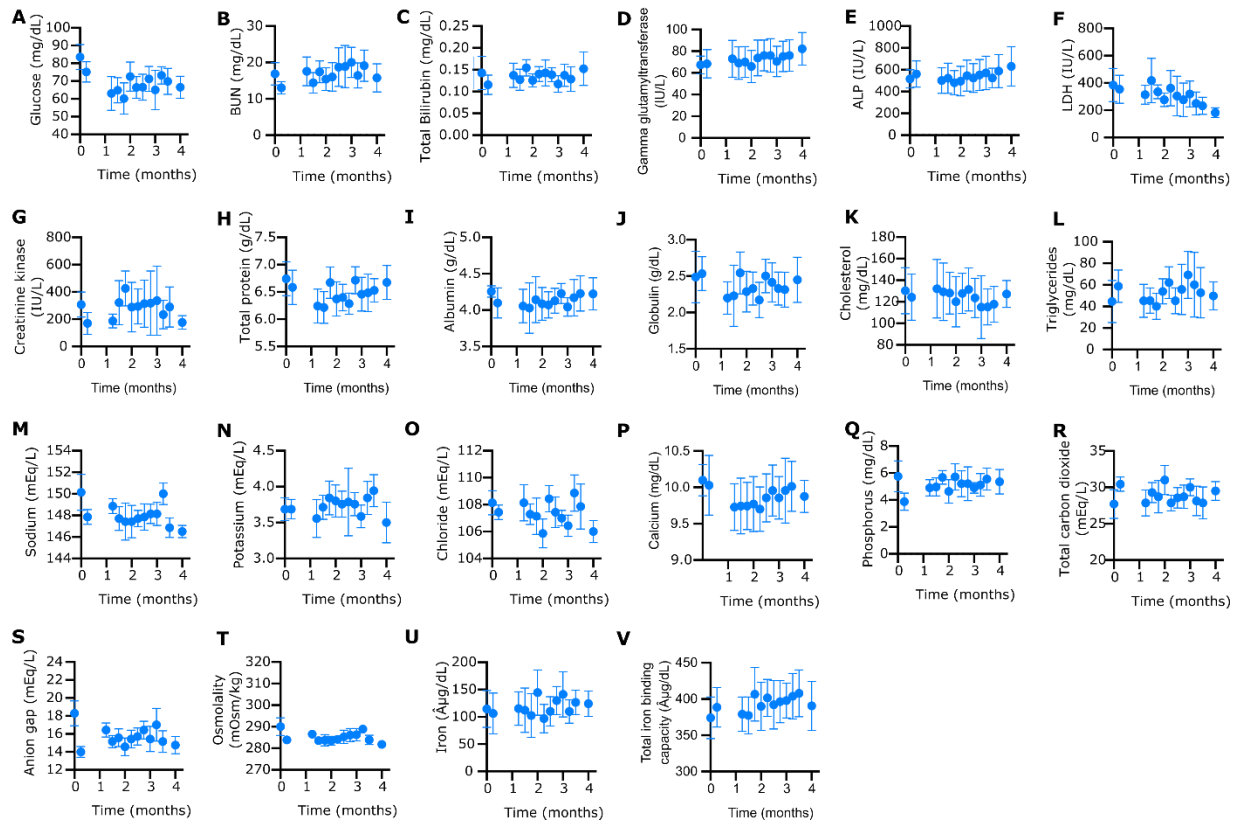
respectively, demonstrating collagen fibers in fibrotic capsule. All images taken at $20\times$ magnification with $200\ \mu\text{m}$ scale bar.



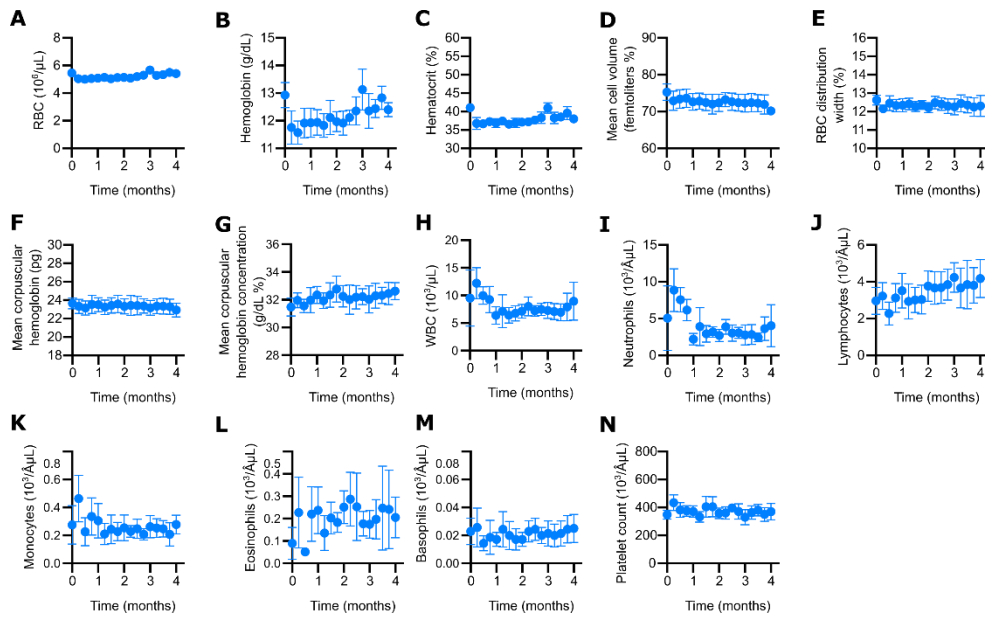
Supporting Figure S6. Negative control of representative NHP skin histology section of fibrotic capsule in contact with TAF-releasing membrane (A) DAPI nuclear stain (blue) at $10\times$ magnification show where cells are clustered, image is negative for IgG isotype (red). (B) DAPI nuclear stain (blue) shows healthy nuclei and at $20\times$ magnification IgG isotype (red) and DAPI noise disappears.



Supporting Figure S7. Representative NHP skin histology section of fibrotic capsule in contact with TAF-releasing membrane (A) AFB staining for presence of bacteria, (B) Grocott methenamine silver staining for presence of fungi. Representative NHP skin histology section in contact with control nPBS titanium implant (C) AFB staining for presence of bacteria, (D) Grocott methenamine silver staining for presence of fungi. All images stained negative and were taken at $20\times$ magnification with $200\ \mu\text{m}$ scale bar.



Supporting Figure S8. (A-V) Metabolic panel of rhesus macaques with nTAF. Baseline value for comparison is on day 0 pre-implantation. All data presented as mean \pm SD.



Supporting Figure S9. (A-N) CBC of rhesus macaques with nTAF. Baseline value for comparison is on day 0 pre-implantation. All data presented as mean \pm SD.

Supporting Table S1. Histological characteristics scoring system used to evaluate inflammation near the implants.

	Score					Reactive inflammation multiplier ($m_{j,r}$)
	0	1	2	3	4	
Cell characteristic						
Polymorphonuclear cells	0/HPF	Rare, 1-5/HPF	5-10/HPF	Heavy infiltrate	Packed	2
Lymphocytes	0/HPF	Rare, 1-5/HPF	5-10/HPF	Heavy infiltrate	Packed	2
Plasma cells	0/HPF	Rare, 1-5/HPF	5-10/HPF	Heavy infiltrate	Packed	2
Macrophages	0/HPF	Rare, 1-5/HPF	5-10/HPF	Heavy infiltrate	Packed	2
Giant cells	0/HPF	Rare, 1-5/HPF	5-10/HPF	Heavy infiltrate	Packed	2
Tissue characteristic						
Necrosis	0	Minimal Narrow band	Mild	Moderate	Severe	2
Capsule thickness	0	Narrow band (<5 cells)	Moderate (5-10 cells)	Thick band (10-20 cells)	Extensive thick band	1
Tissue infiltrate	0	Minimal focal invasion of local tissue	Mild to multifocal inflammation in adjacent tissues	Moderate inflammation in adjacent tissues	Marked inflammation in adjacent tissues	1

Note: Reprinted from “A subcutaneous implant of tenofovir alafenamide fumarate causes local inflammation and tissue necrosis in rabbits and macaques”, by Su, J., 2020, *Antimicrobial Agents and Chemotherapy*, 64, e01893-19. Copyright © 2020 Su *et al.* Creative Commons Attribution 4.0 International license.

Supporting Table S2. Histopathological scores of nTAF (PrEP) and nPBS (Control) implants.

Animal 15-166 (PrEP 1)	Score			
	Pathologist 1	Pathologist 2	Pathologist 3	Average
Polymorphonuclear cells	2	2	3	2.3
Lymphocytes	3	2	3	2.7
Plasma cells	3	0	1	1.3
Macrophages	2	2	2	2.0
Giant cells	2	1	0	1.0
Necrosis	2	2	2	2.0
Capsule thickness	3	4	4	3.7
Tissue infiltrate	1	1	2	1.3
Overall total	18	14	17	16.3
Animal 17-087 (PrEP 2)	Score			
	Pathologist 1	Pathologist 2	Pathologist 3	Average
Polymorphonuclear cells	1	1	2	1.3
Lymphocytes	2	1	2	1.7
Plasma cells	2	0	1	1.0
Macrophages	3	3	2	2.7
Giant cells	2	1	0	1.0
Necrosis	0	0	1	0.3
Capsule thickness	3	4	3	3.3
Tissue infiltrate	1	1	1	1.0
Overall total	14	11	12	12.3
Animal 17-063 (PrEP 3)	Score			
	Pathologist 1	Pathologist 2	Pathologist 3	Average
Polymorphonuclear cells	1	2	3	2.0
Lymphocytes	2.5	2	3	2.5
Plasma cells	2	0	1	1.0
Macrophages	3	2	3	2.7
Giant cells	2	1	1	1.3
Necrosis	3	2	3	2.7
Capsule thickness	2	4	4	3.3
Tissue infiltrate	2	2	2	2.0
Overall total	17.5	15	20	17.5
Animal 15-112 (PrEP 4)	Score			
	Pathologist 1	Pathologist 2	Pathologist 3	Average
Polymorphonuclear cells	2	2	2	2.0
Lymphocytes	2	2	2	2.0
Plasma cells	1	0	0	0.3
Macrophages	3	1	2	2.0
Giant cells	1	0	0	0.3
Necrosis	2	3	1	2.0

Capsule thickness	2	3	2	2.3
Tissue infiltrate	3	2	3	2.7
Overall total	16	13	12	13.7
Animal 16-133 (PrEP 5)	Score			
	Pathologist 1	Pathologist 2	Pathologist 3	Average
Polymorphonuclear cells	0	0	0	0.0
Lymphocytes	2	1	2	1.7
Plasma cells	1	1	0	0.7
Macrophages	1	0	1	0.7
Giant cells	0	0	0	0.0
Necrosis	0	0	0	0.0
Capsule thickness	1	3	2	2.0
Tissue infiltrate	1	1	0	0.7
Overall total	6	6	5	5.7
Animal 16-139 (PrEP 6)	Score			
	Pathologist 1	Pathologist 2	Pathologist 3	Average
Polymorphonuclear cells	0	0	NA	NA
Lymphocytes	0	0	NA	NA
Plasma cells	0	0	NA	NA
Macrophages	0	0	NA	NA
Giant cells	0	0	NA	NA
Necrosis	No capsule	1(fat)	NA	NA
Capsule thickness	No capsule	0	NA	NA
Tissue infiltrate	No capsule	0	0	NA
Overall total	0	0	0	0
Animal 17-010 (PrEP 7)	Score			
	Pathologist 1	Pathologist 2	Pathologist 3	Average
Polymorphonuclear cells	1	0	2	1.0
Lymphocytes	1	0	2	1.0
Plasma cells	1	0	1	0.7
Macrophages	1	0	2	1.0
Giant cells	0	0	0	0.0
Necrosis	0	1	0	0.3
Capsule thickness	1	0	3	1.3
Tissue infiltrate	1	0	0	0.3
Overall total	6	0	10	5.3
Animal 15-162 (Control 1)	Score			
	Pathologist 1	Pathologist 2	Pathologist 3	Average
Polymorphonuclear cells	0	1	2	1.0
Lymphocytes	1	1	1	1.0

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Plasma cells	1	0	0	0.3
Macrophages	2	1	2	1.7
Giant cells	0	0	0	0.0
Necrosis	0	0	0	0.0
Capsule thickness	2	4	3	3.0
Tissue infiltrate	0	0	0	0.0
Overall total	6	7	8	7.0
Animal 17-048 (Control 2)	Score			
	Pathologist 1	Pathologist 2	Pathologist 3	Average
Polymorphonuclear cells	0	0	NA	NA
Lymphocytes	0	0		NA
Plasma cells	0	0		NA
Macrophages	0	0		NA
Giant cells	0	0		NA
Necrosis	No capsule	1(fat)		NA
Capsule thickness	No capsule	0	NA	NA
Tissue infiltrate	No capsule	0	0	NA
Overall total	0	0	0	0
Animal 17-071 (Control 3)	Score			
	Pathologist 1	Pathologist 2	Pathologist 3	Average
Polymorphonuclear cells	0	0		NA
Lymphocytes	0	0		NA
Plasma cells	0	0		NA
Macrophages	0	0		NA
Giant cells	0	0		NA
Necrosis	No capsule	0		NA
Capsule thickness	No capsule	0	NA	NA
Tissue infiltrate	No capsule	0	0	NA
Overall total	0	0	0	0
Animal 16-131 (Control 4)	Score			

	Pathologist 1	Pathologist 2	Pathologist 3	Average
Polymorphonuclear cells	0	0	1	0.3
Lymphocytes	1.5	0	2	1.2
Plasma cells	1	0	0	0.3
Macrophages	2	0	2	1.3
Giant cells	1	0	0	0.3
Necrosis	0	0	0	0.0
Capsule thickness	3	3	2	2.7
Tissue infiltrate	1	0	1	0.7
Overall total	9.5	3	8	6.8
Animal 15-134 (Control 5)	Score			
	Pathologist 1	Pathologist 2	Pathologist 3	Average
Polymorphonuclear cells	1	2	1	1.3
Lymphocytes	1	2	2	1.7
Plasma cells	1	0	1	0.7
Macrophages	2	2	2	2.0
Giant cells	1	1	1	1.0
Necrosis	0	0	1	0.3
Capsule thickness	3	3	1	2.3
Tissue infiltrate	1	0	0	0.3
Overall total	10	10	9	9.7
Animal 17-004 (Control 6)	Score			
	Pathologist 1	Pathologist 2	Pathologist 3	Average
Polymorphonuclear cells	0	1	1	0.7
Lymphocytes	1.5	1	1	1.2
Plasma cells	1	0	0	0.3
Macrophages	2	2	2	2.0
Giant cells	1	1	0	0.7
Necrosis	0	0	0	0.0
Capsule thickness	3	4	3	3.3

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Tissue infiltrate	1	1	1	1.0
Overall total	9.5	10	8	9.2

All pathologists are board-certified. Pathologist 1, 2 and 3 are from Houston Methodist Hospital, Baylor College of Medicine and Weill Cornell Medicine, respectively. Animals PrEP 6, Control 2 and Control 3 were not used to calculate total histological score because the implant site was not easily identifiable.

Supporting Table S3. Urinalysis results in rhesus macaques with nTAF. Found in Excel File named: Supporting Table S3.