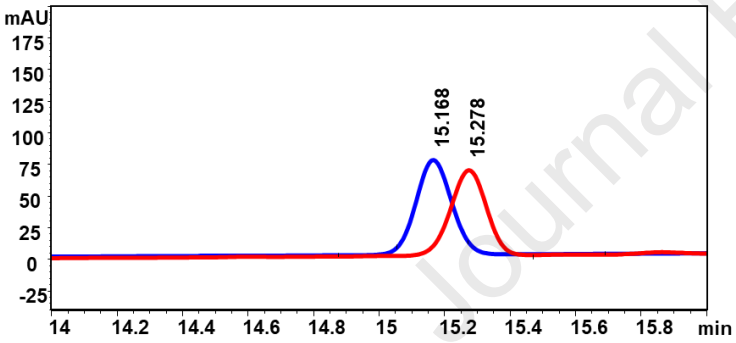
Supplementary Information

A



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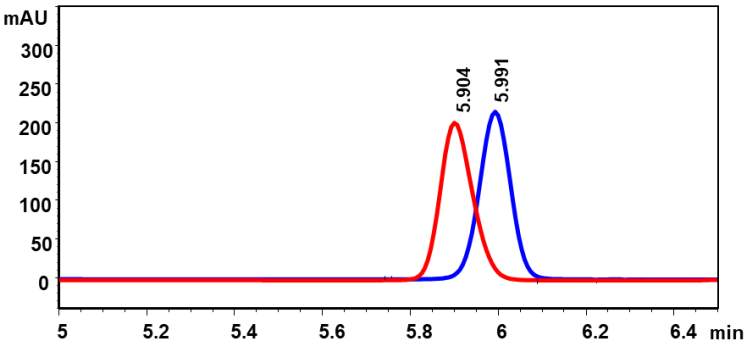

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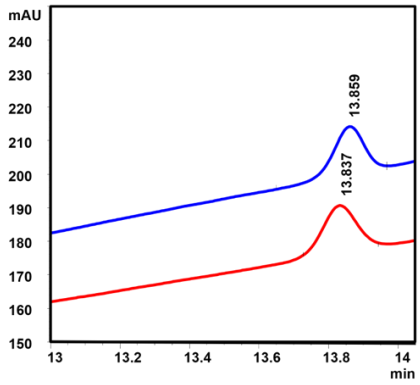


D

Drug	Before storage (mg)	After 90-day storage (mg)
ENG	14.2±0.95	13.76±0.89
EE	3.4±0.21	3.4±0.14
ISL	306±3.65	294.4±4.64

E

Metric	Before storage	After 90-day storage
Mass (mg)	7369±33.2	7560.3±36.4
Outer diameter (mm)	53.36±0.33	53.96±0.21
Cross-sectional diameter (mm)	7.64±0.08	7.51±0.19

Figure S1. Post-storage metrics and drug content. HPLC chromatograms of (A) ENG, (B) ISL, and (C) EE before and after 90 days storage in 40°C/75% RH. Red curves indicate HPLC readings before storage and blue curves indicate HPLC readings after storage. Chromatograms represent MPT IVR extraction samples before and after storage. (D) Drug content before and after storage. (E) IVR mass and dimensions before and after storage.

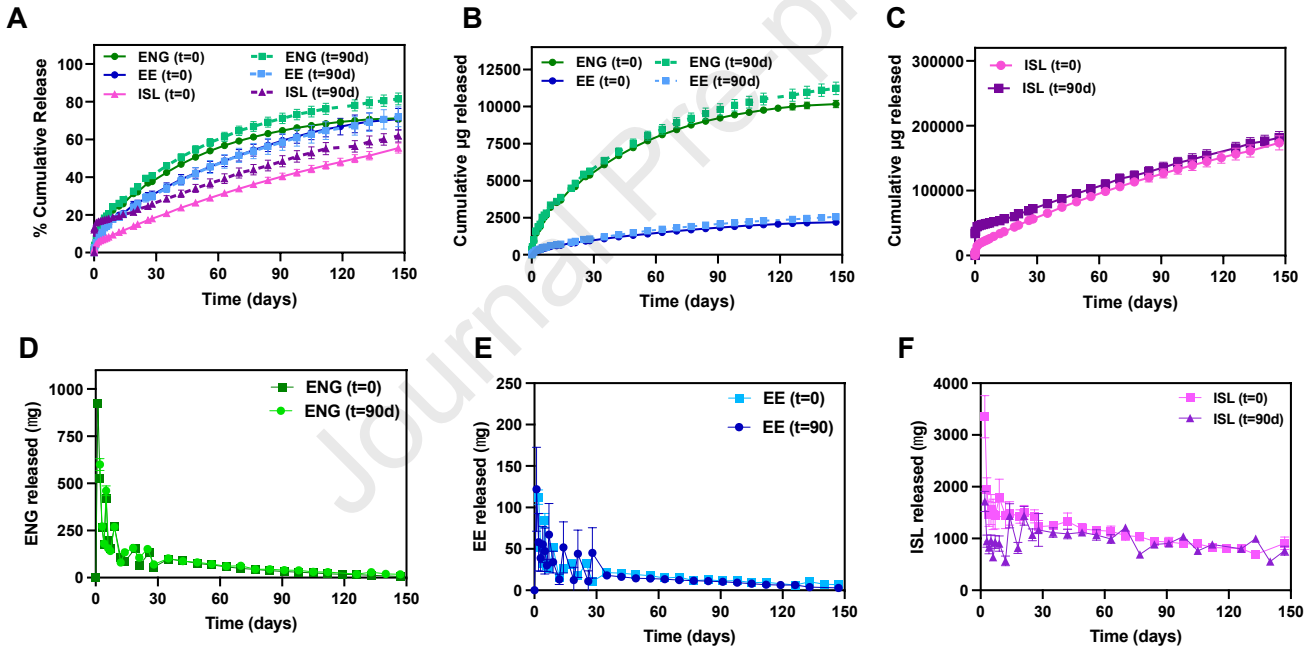


Figure S2. Post-storage in vitro release of MPT IVR. (A) Precent (%) cumulative in vitro release of MPT IVR post-storage (t=Day 90) compared to baseline (t=Day 0). (B) Cumulative release (µg) of ENG/EE post-storage (t=Day 90) compared to baseline (t=Day 0). (C) Cumulative release (µg) of ISL post-storage (t=Day 90) compared to baseline (t=Day 0). (D) Daily release (µg) of ENG before and after storage. (E) Daily release (µg) of EE before and after storage. (F) Daily release (µg) of ISL before and after storage. ISL release shown in (F) begins after the burst release (day

2) due to the extremely high burst release of ISL post-storage (44304±2070 μg ISL at 24 hours after storage compared to 14633±919 μg ISL at 24 hours at baseline). All experiments were done in triplicate in SVF pH 7 and error bars indicate standard deviation.

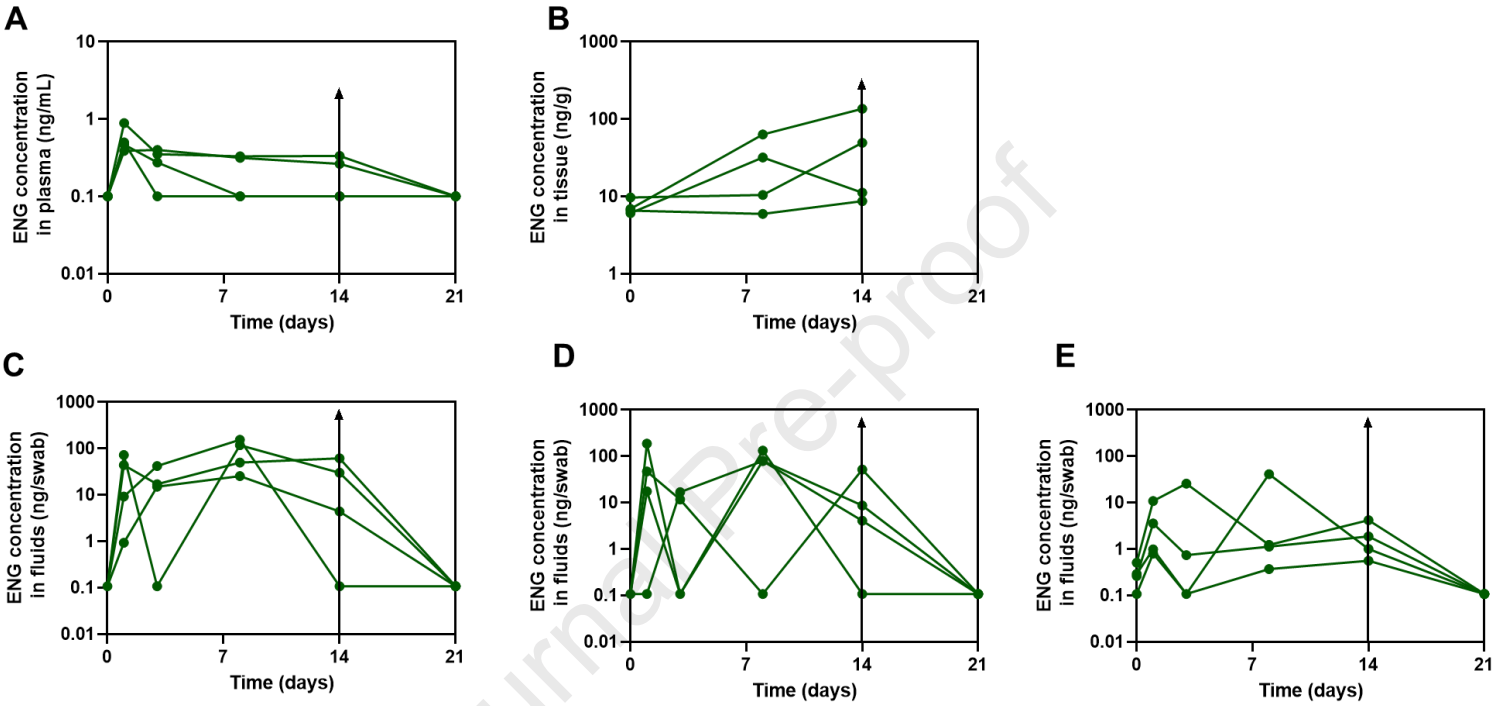


Figure S3. Individual replicates of ENG levels from MPT IVR in various sheep matrices. ENG levels in n=4 sheep in (A) plasma, (B) vaginal tissue, (C) proximal vaginal fluids, (D) distal vaginal fluids, and (E) rectal fluids. Black arrow indicates time when MPT IVR was removed. ENG lower limit of quantification (LLOQ) of 0.2 ng/mL in plasma, 17 ng/g in tissue, 0.215 ng/swab in fluids. Samples that were below the limit of quantification were represented as LLOQ/2.

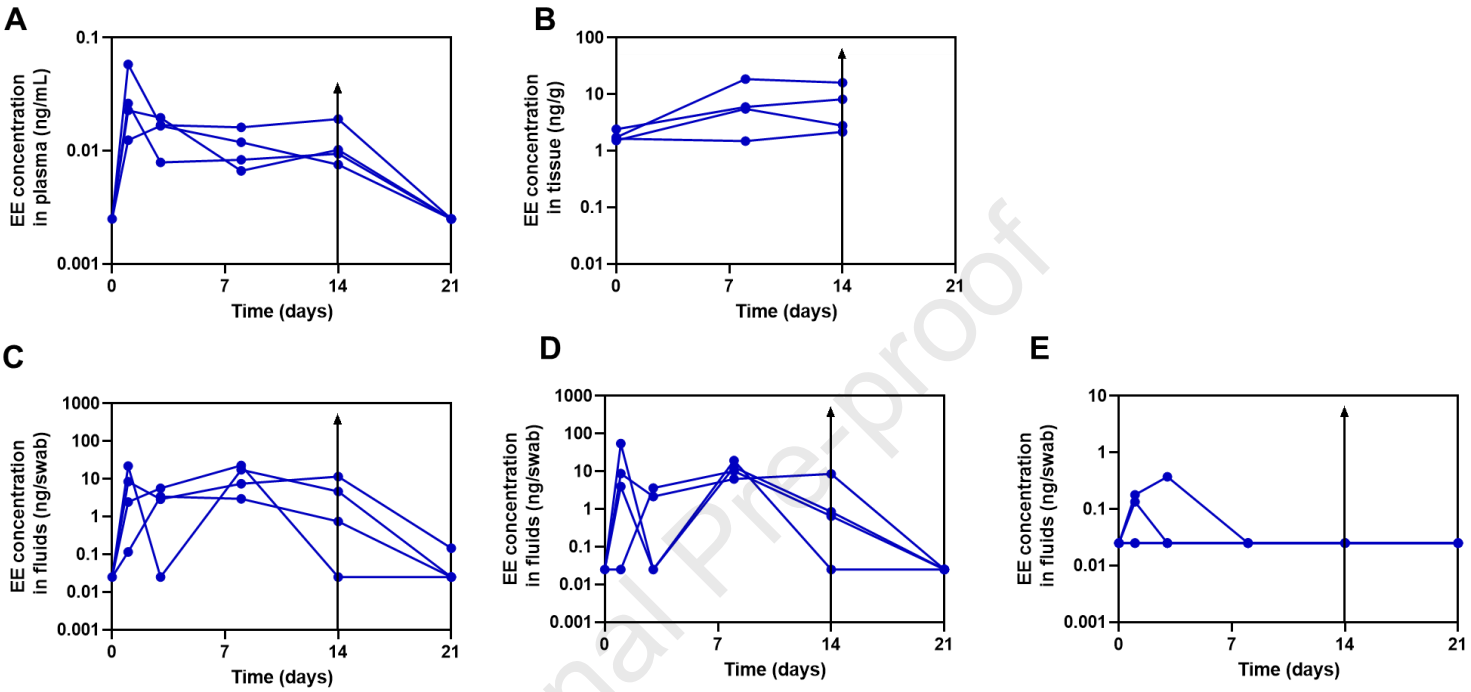


Figure S4. Individual replicates of EE levels from MPT IVR in various sheep matrices. EE levels in n=4 sheep in (A) plasma, (B) vaginal tissue, (C) proximal vaginal fluids, (D) distal vaginal fluids, and (E) rectal fluids. Black arrow indicates time when MPT IVR was removed. EE LLOQ of 0.005 ng/mL in plasma, 4.2 ng/swab in tissue, and 0.05 ng/swab in fluids. Samples that were below the limit of quantification were represented as LLOQ/2.

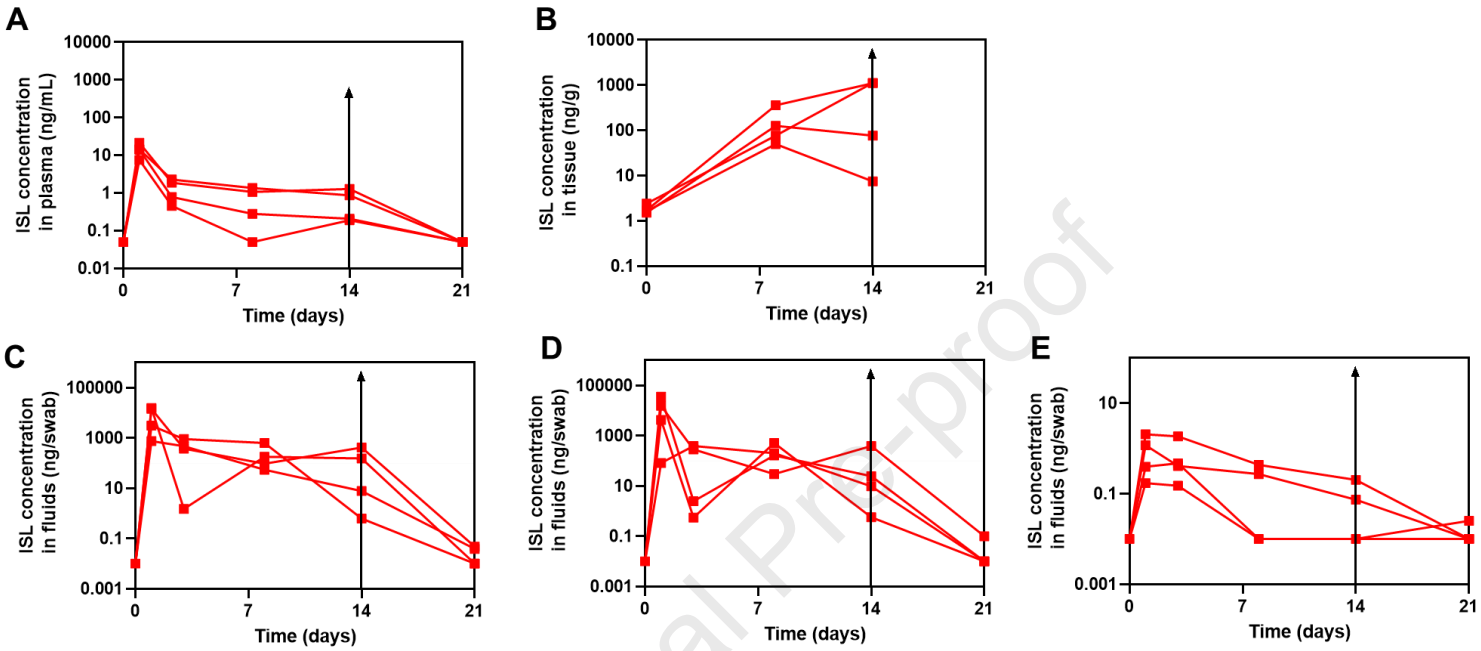


Figure S5. Individual replicates of ISL levels from MPT IVR in various sheep matrices. ISL levels in n=4 sheep in (A) plasma, (B) vaginal tissue, (C) proximal vaginal fluids, (D) distal vaginal fluids, and (E) rectal fluids. Black arrow indicates time when MPT IVR was removed. ISL LLOQ of 0.1 ng/mL in plasma, 4.24 ng/g in tissue, and 0.0215 ng/swab in fluids. Samples that were below the limit of quantification were represented as LLOQ/2.

Table S1. MPT IVR median (range) sheep plasma levels

Drug	Day 1 (ng/mL)	Day 3 (ng/mL)	Day 8 (ng/mL)	Day 14 (ng/mL)	Day 21 (ng/mL)

ISL	14.3 (7.54-21.6)	1.33 (0.46-2.28)	0.67 (0.05-1.35)	0.54 (0.19-1.28)	0.05 (0.05-0.05)
ENG	0.48 (0.39-0.89)	0.31 (0.1-0.4)	0.21 (0.1-0.33)	0.18 (0.1-0.33)	0.1 (0.1-0.1)
EE	0.025 (0.012-0.058)	0.017 (0.008-0.019)	0.01 (0.007-0.016)	0.01 (0.008-0.019)	0.0025 (0.0025-0.0025)

Table S2. MPT IVR median (range) sheep vaginal tissue levels

Drug	Day 8 (ng/g)	Day 14 (ng/g)
ISL	101.5 (50-361)	593 (7.44-1124)
ENG	21.2 (5.96-63.2)	30.3 (8.65-136)
EE	5.75 (1.48-18.6)	5.48 (2.17-16)

Table S3. MPT IVR median (range) sheep vaginal and rectal fluid levels

Drug	Day 1 (ng/swab)			Day 3 (ng/swab)			Day 8 (ng/swab)		
	Vaginal (proximal)	Vaginal (distal)	Rectal	Vaginal (proximal)	Vaginal (distal)	Rectal	Vaginal (proximal)	Vaginal (distal)	Rectal
ISL	8719 (753-15803)	9879 (83-35905)	0.8 (0.17-2.1)	423 (1.5-905)	144 (0.6-387)	0.4 (0.15-1.89)	137 (55-632)	184 (30-510)	0.14 (0.01-0.44)
ENG	26.6 (0.93-72.2)	32.4 (0.1-188)	2.3 (0.8-10.8)	16.1 (0.1-41.9)	5.9 (0.10-16.8)	0.42 (0.10-25.8)	83.9 (25.6-155)	79.7 (0.1-133)	1.18 (0.37-41.1)
EE	5.45 (0.12-21.9)	6.3 (0.03-54.8)	0.08 (0.03-0.18)	3.15 (0.03-5.65)	1.09 (0.03-3.63)	0.03 (0.03-0.37)	12.47 (2.97-22.8)	11.6 (6.2-19.4)	0.03 (0.03-0.03)

Table S3-continued

Drug	Day 14 (ng/swab)			Day 21 (ng/swab)		
	Vaginal (proximal)	Vaginal (distal)	Rectal	Vaginal (proximal)	Vaginal (distal)	Rectal
ISL	81 (0.62-415)	17 (0.6-391)	0.04 (0.01-0.2)	0.02 (0.01-0.05)	0.01 (0.01-0.1)	0.01 (0.01-0.03)
ENG	17.2 (0.1-61.5)	6.4 (0.1-51.6)	1.4 (0.6-4.19)	0.1 (0.1-0.1)	0.1 (0.1-0.1)	0.1 (0.1-0.1)
EE	2.69 (0.03-11.5)	0.75 (0.03-8.58)	0.03 (0.03-0.03)	0.03 (0.03-0.14)	0.03 (0.03-0.03)	0.03 (0.03-0.03)

A

Control sheep (no MPT IVR treatment)	Days		
Sheep ID P1062	1	8	15
Epithelium	Score		
Hyperplasia, epithelial	0	0	0
Inflammatory cells, intraepithelial	2	2	2
Globular cells (Mott Cells)	1	0	0
Edema, intraepithelial	1	0	0
Edema, interepithelial	2	1	1
Apoptosis, individual cells	1	1	0
Parakeratosis	0	0	1
Submucosa	Score		
Infiltrates, PMNs	1	1	1
Infiltrates, eosinophils	0	0	0
Infiltrates, mononuclear	2	2	2
Sheep ID P1129	1	8	15
Epithelium	Score		
Hyperplasia, epithelial	1	1	0
Inflammatory cells, intraepithelial	2	2	1
Globular cells (Mott Cells)	1	0	0
Edema, intraepithelial	1	2	1
Edema, interepithelial	1	2	1
Apoptosis, individual cells	1	0	0
Parakeratosis	1	1	1
Submucosa	Score		
Infiltrates, PMNs	0	0	0
Infiltrates, eosinophils	0	0	0
Infiltrates, mononuclear	1	1	1

B

Sheep received MPT IVR	Days post-IVR insertion		
Sheep ID P1063	0	8	14
Epithelium	Score		
Hyperplasia, epithelial	0	1	1
Inflammatory cells, intraepithelial	1	1	2
Eosinophils, intraepithelial	0	0	1
Globular cells (Mott Cells)	0	0	0
Edema, intraepithelial	2	1	0
Edema, interepithelial	1	1	1
Apoptosis, individual cells	0	0	1
Parakeratosis	0	0	2
Submucosa	Score		
Infiltrates, PMNs	0	2*	1
Infiltrates, eosinophils	0	1	2
Inflitrates, mononuclear	1	1	1
Sheep ID P1100	1	8	15
Epithelium	Score		
Hyperplasia, epithelial	0	1	2
Inflammatory cells, intraepithelial	1	1	2
Eosinophils, intraepithelial	0	0	1
Globular cells (Mott Cells)	0	0	0
Edema, intraepithelial	2	1	1
Edema, interepithelial	1	1	1
Apoptosis, individual cells	0	0	0
Parakeratosis	1	2	1
Submucosa	Score		
Infiltrates, PMNs	1	1*	1
Infiltrates, eosinophils	0	1	1
Infiltrates, mononuclear	0	1	2

C

Sheep received MPT IVR	Days post-IVR insertion		
Sheep ID P1107	0	8	14
Epithelium	Score		
Hyperplasia, epithelial	0	2	1
Inflammatory cells, intraepithelial	1	2	2
Eosinophils, intraepithelial	0	0	0
Globular cells (Mott Cells)	0	0	0
Edema, intraepithelial	1	1	1
Edema, interepithelial	1	1	2
Apoptosis, individual cells	1	0	0
Parakeratosis	0	2	1
Submucosa	Score		
Infiltrates, PMNs	0	1	2*
Infiltrates, eosinophils	0	0	1
Inflitrates, mononuclear	1	1	1
Sheep ID P1108	1	8	15
Epithelium	Score		
Hyperplasia, epithelial	0	1	1
Inflammatory cells, intraepithelial	1	1	1
Eosinophils, intraepithelial	0	0	0
Globular cells (Mott Cells)	0	0	0
Edema, intraepithelial	0	1	1
Edema, interepithelial	1	1	1
Apoptosis, individual cells	0	0	0
Parakeratosis	1	1	2
Submucosa	Score		
Infiltrates, PMNs	0	2*	0
Infiltrates, eosinophils	0	1	0
Infiltrates, mononuclear	1	1	1

Figure S6. Semiquantitative inflammation and immune cell infiltrate scores of (A) control sheep (no IVR treatment) and (B and C) sheep that received IVR for 14 days. Score of 0 indicates no finding, 1 indicates minimal finding, 2 indicates mild finding, 3 indicates moderate finding, 4 indicates marked finding, and 5 indicates severe finding. Asterisk (*) denotes neutrophils in close association with superficial submucosal vessels.

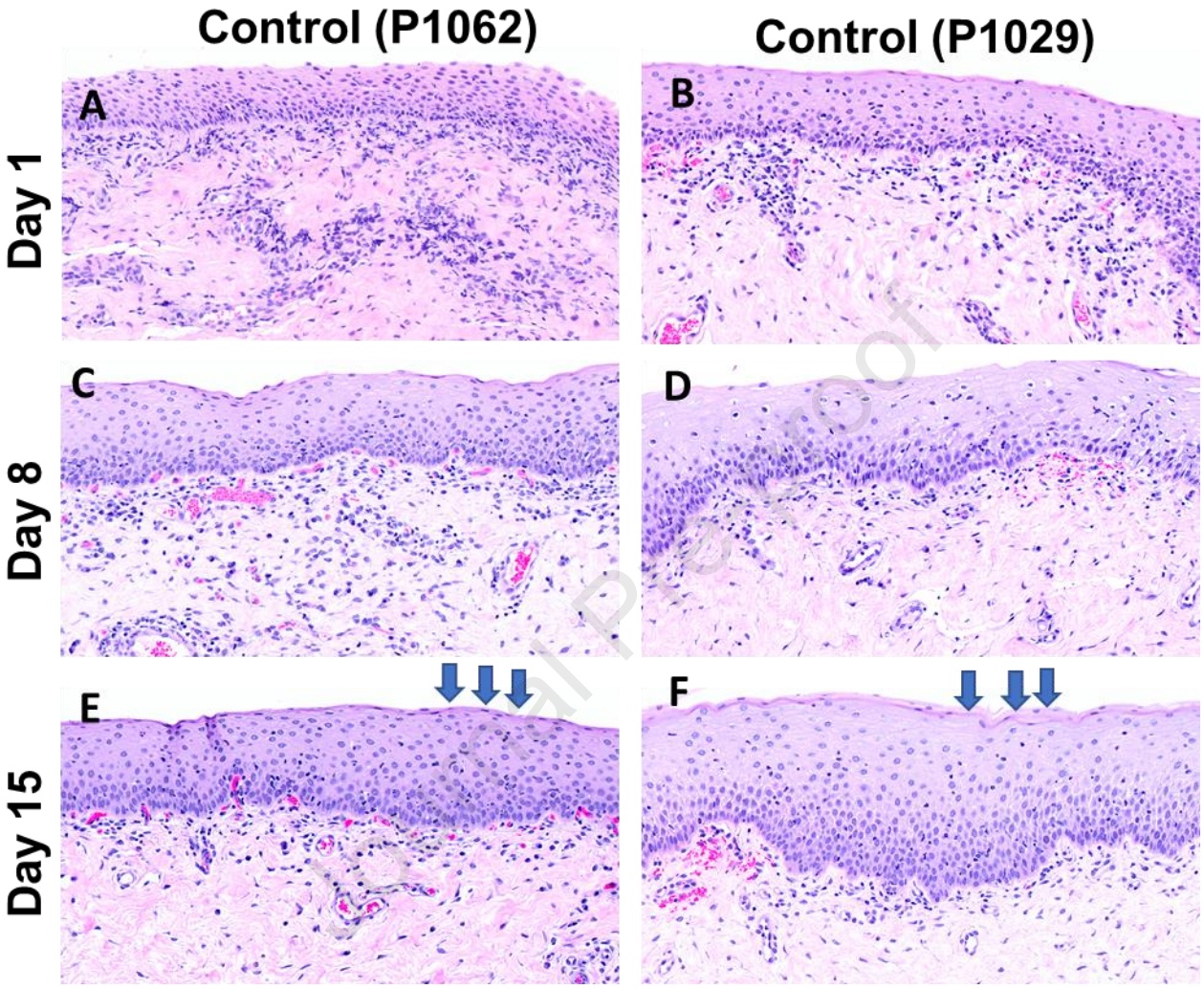


Figure S7. Histological images of control sheep (no IVR insertion) vaginal tissue. Day 1 images of (A) sheep ID P1062 and (B) sheep ID P1029. Day 8 images of (C) sheep ID P1062 and (D) sheep ID P1029. Day 15 images of (E) sheep ID P1062 and (F) sheep ID P1029. These sheep were used as the no treatment controls to assess histology and safety data compared to sheep that were administered the MPT IVR. Blue arrows represent parakeratosis.

Colposcopy Findings					
Sheep	**Day 0**	**Day 1**	**Day 3**	**Day 8**	**Day 14**
P1063	Pink, Normal, petechiae	Pink, Erythema – mild, petechiae	Pink, Erythema – mild	Pink, Erythema – mild	Pink, Normal
P1100	Pink, Normal	Pink, Erythema – mild, petechiae,	Pink, Erythema – mild	Pink, Erythema – mild	Pink, Normal
P1107	Pink, Normal	Pink, Erythema – mild	Pink, Erythema – mild	Pink, Erythema – mild, Petechiae	Pink, Normal
P1108	Pink, Normal	Pink, Erythema – mild	Pink, Erythema – mild	Mottled, Erythema – mild	Pink, Normal

Table S4. Colposcopy findings in sheep. Normal findings of either normal tissue, mild erythema, or petechiae were found throughout the study in sheep treated with MPT IVR.

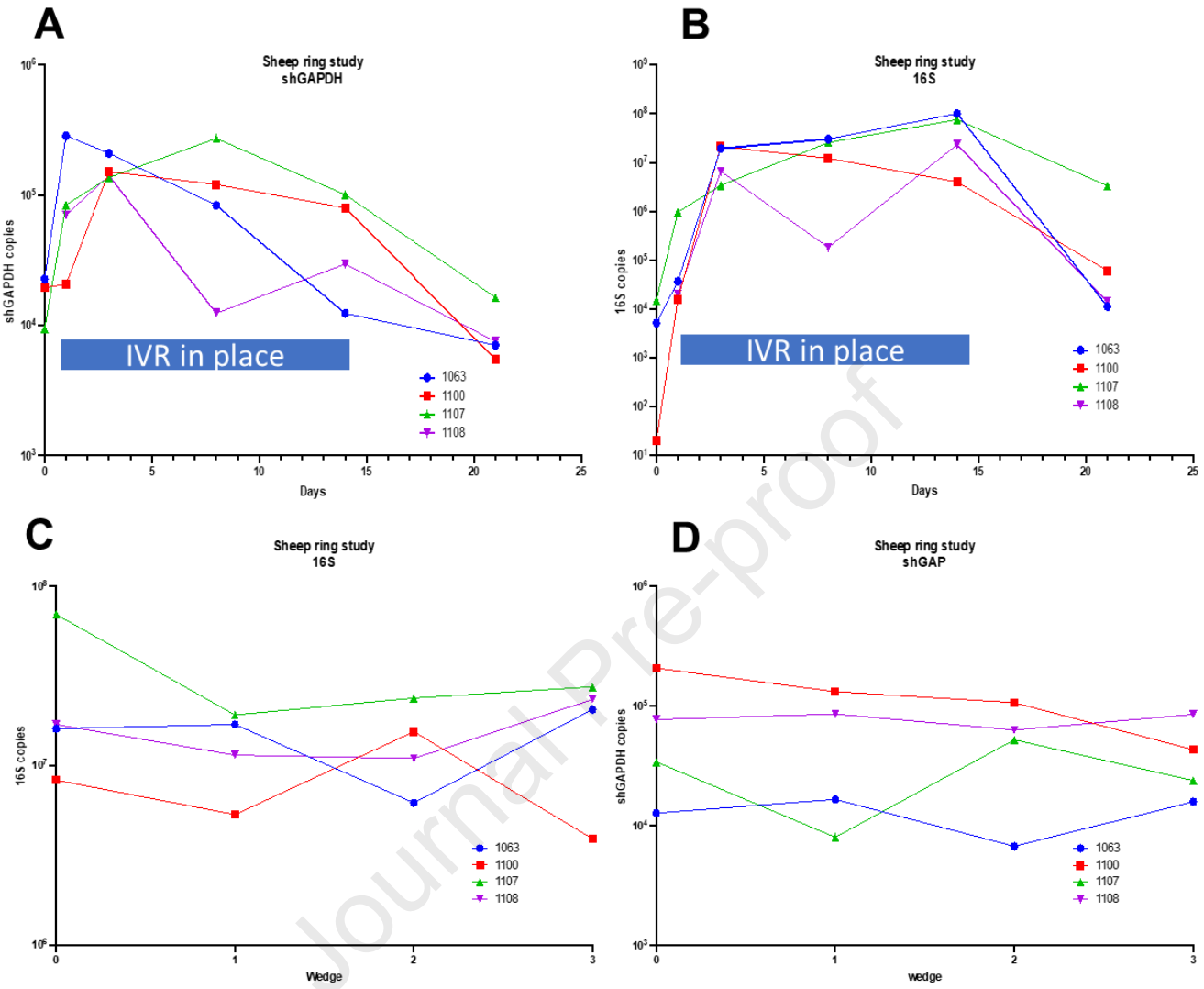
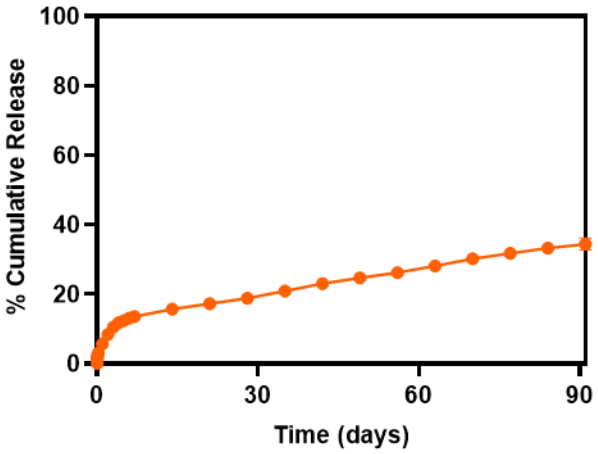


Figure S8. Individual replicates of sheep GAPDH and bacterial 16S rDNA from vaginal microbiota analyses. Individual sheep replicates of the number of copies of the **(A)** sheep GAPDH or **(B)** bacterial 16S rDNA from vaginal swabs from the 4 animals were quantified by PCR (qPCR) are shown at baseline (day 0) and then 24 hours after IVR placement through day 14 and then again on day 21, 7 days after the rings were removed. **(C & D)** The 4 removed MPT IVRs were processed sterilely allowing analysis of both **(C)** sheep GAPDH and **(D)** bacterial 16S copies using

the same qPCR analyses. The fluid carried by the ring (wedge 0) was compared to the material adhered to each of 3 equidistant wedges (wedges 1-3) from each of the 4 MPT IVRs.



EFdA in IVR (mg)	Burst at 24h (%)	Burst at 24h (µg)	Release rate (µg/day) Day 2-90
62	5.67±0.66	14633.9±919.5	169

Figure S9. In vitro release of ISL in macaque-sized IVR. % Cumulative in vitro release of ISL from macaque-sized IVRs with summary table of release kinetics. Release profile indicates average ± standard deviation of n=3 samples performed in SVF pH 8.

Table S4. ISL IVR median (range) macaque plasma levels

Drug	Day 3	Day 7	Day 14	Day 21	Day 28	Day 30
EFdA (ng/mL)	4.9 (0.34-7.86)	2.59 (0.19-5.11)	1.03 (0.05-1.54)	1.04 (0.05-1.42)	0.91 (0.1-0.99)	0.25 (0.05-0.29)

EFdA-TP (fmol/10^6 PBMCs)	251 (81.1-906)	338 (59.3-462)	191 (25.9-311)	120 (19.7-199)	91.7 (14.7-115)	41.8 (14-81.8)

Table S5. ISL IVR median (range) macaque vaginal tissues levels

Drug	**Day 7**		**Day 21**		**Day 30**	
	Vaginal (proximal)	Vaginal (distal)	Vaginal (proximal)	Vaginal (distal)	Vaginal (proximal)	Vaginal (distal)
EFdA (ng/g)	3200 (1289-12478)	4381 (2219-12571)	15125 (10452-22340)	3849 (728-62725)	85.4 (79.3-377)	98.5 (38.2-1194)
EFdA-TP (fmol/g)	155320 (153340-197841)	331975 (163059-2187119)	208149 (104306-591953)	66936 (22424-96050)	19633 (15469-243106)	28515 (8698-63955)

Table S6. ISL IVR median (range) macaque vaginal and rectal fluids levels

Drug	**Day 3**			**Day 7**			**Day 14**		
	Vaginal (proximal)	Vaginal (distal)	Rectal	Vaginal (proximal)	Vaginal (distal)	Rectal	Vaginal (proximal)	Vaginal (distal)	Rectal
EFdA (ng/swab)	16878 (3268-43215)	18211 (1034-21500)	121 (7.03-234)	1307 (237-1802)	525 (295-826)	12.1 (8.3-25.2)	503 (273-768)	217 (189-473)	6.86 (2.97-10.5)

Table S6 (continued)

Drug	Day 21			Day 28			Day 30		
	Vaginal (proximal)	Vaginal (distal)	Rectal	Vaginal (proximal)	Vaginal (distal)	Rectal	Vaginal (proximal)	Vaginal (distal)	Rectal
EFdA (ng/swab)	798 (194-2322)	473 (96.5-733)	4.99 (2.41-7.07)	1554 (639-2079)	372 (348-381)	13.1 (1.42-13.7)	10.75 (10.75-34.8)	10.75 (10.75-28.4)	1.27 (0.31-1.86)

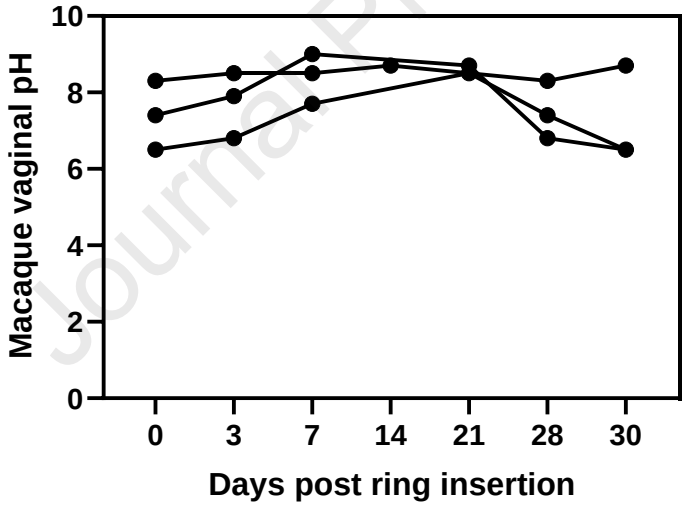


Figure S10. Longitudinal vaginal pH measurements in macaques that received ISL IVR. Vaginal pH measurements were obtained from the three pigtailed macaques before ring insertion (day 0), while the ring was present (days 3 to 28) and two days post ring removal (day 30). Each individual macaque's pH during the entire study is represented by a line. We were unable to obtain vaginal pH in two of the three macaques on day 14 as they were undergoing menses at that time.

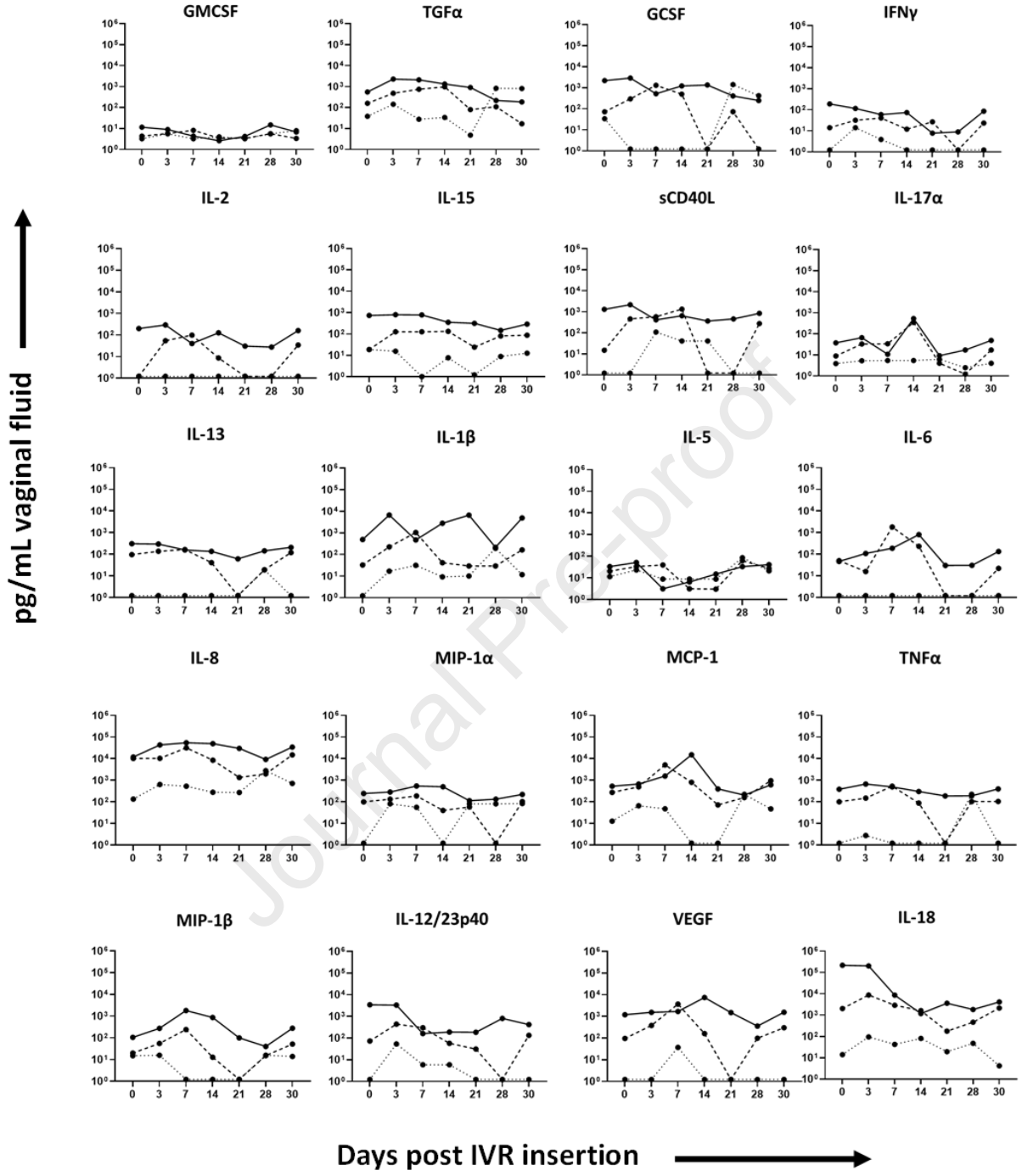


Figure S11. Vaginal fluid cytokine and chemokine concentrations (pg/mL) from macaques that received ISL IVR. Mucosal inflammation due to the presence of the ISL IVR was monitored by measuring 20 cytokines and chemokines in vaginal fluid collected throughout the study. The

concentrations obtained at baseline (day 0), while the ring was present (days 3-28), and post ring removal (day 30) are included. Each animal is represented independently (PRM2 dashed line, PFN2 dotted line, B0315 solid line).

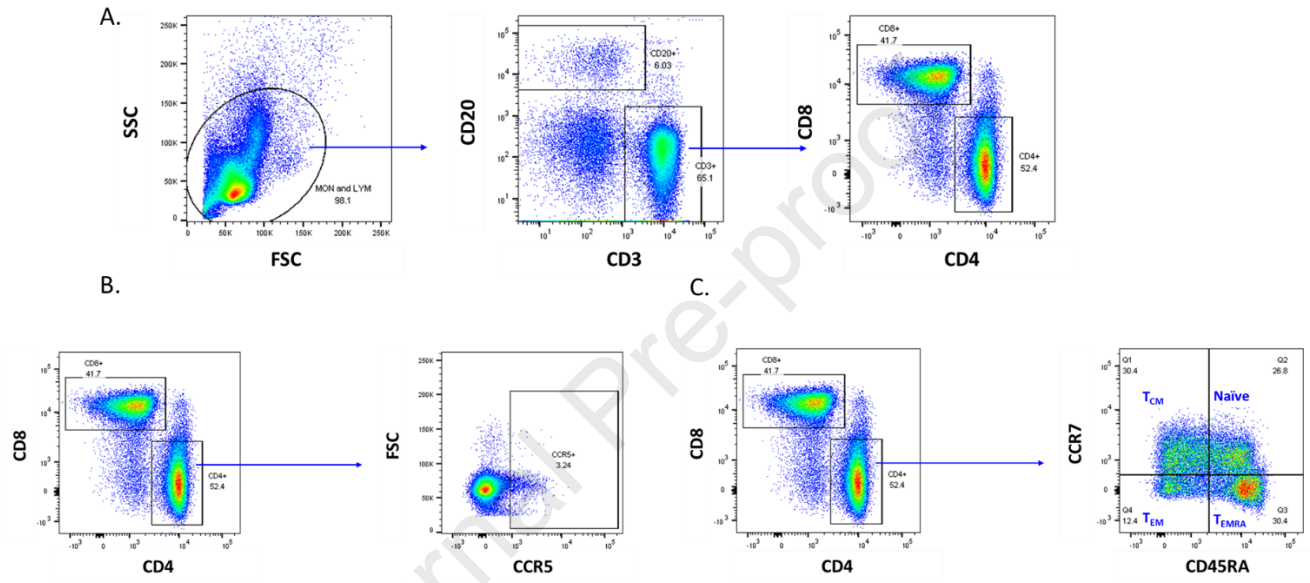


Figure S12. Representative FACS plots of CD4+ and CD8+ T cell and C4+ T cell subsets: A) CD4 and CD8 classification based on CD3 and CD20 expression B) CCR5 expression on CD3+ CD4+ T cells C) Maturational profile of CD3+ CD4+ T cells: CD4+ T_{CM} (central memory CCR7+/CD45RA-), CD4+ naïve (CCR7+/CD45RA+), CD4+T_{EMRA} (terminally differentiated effector memory, CCR7-/CD45RA+) and CD4+T_{EM} (effector memory, CCR7-/CD45RA- based on the expression profile of CD45RA and CCR7.