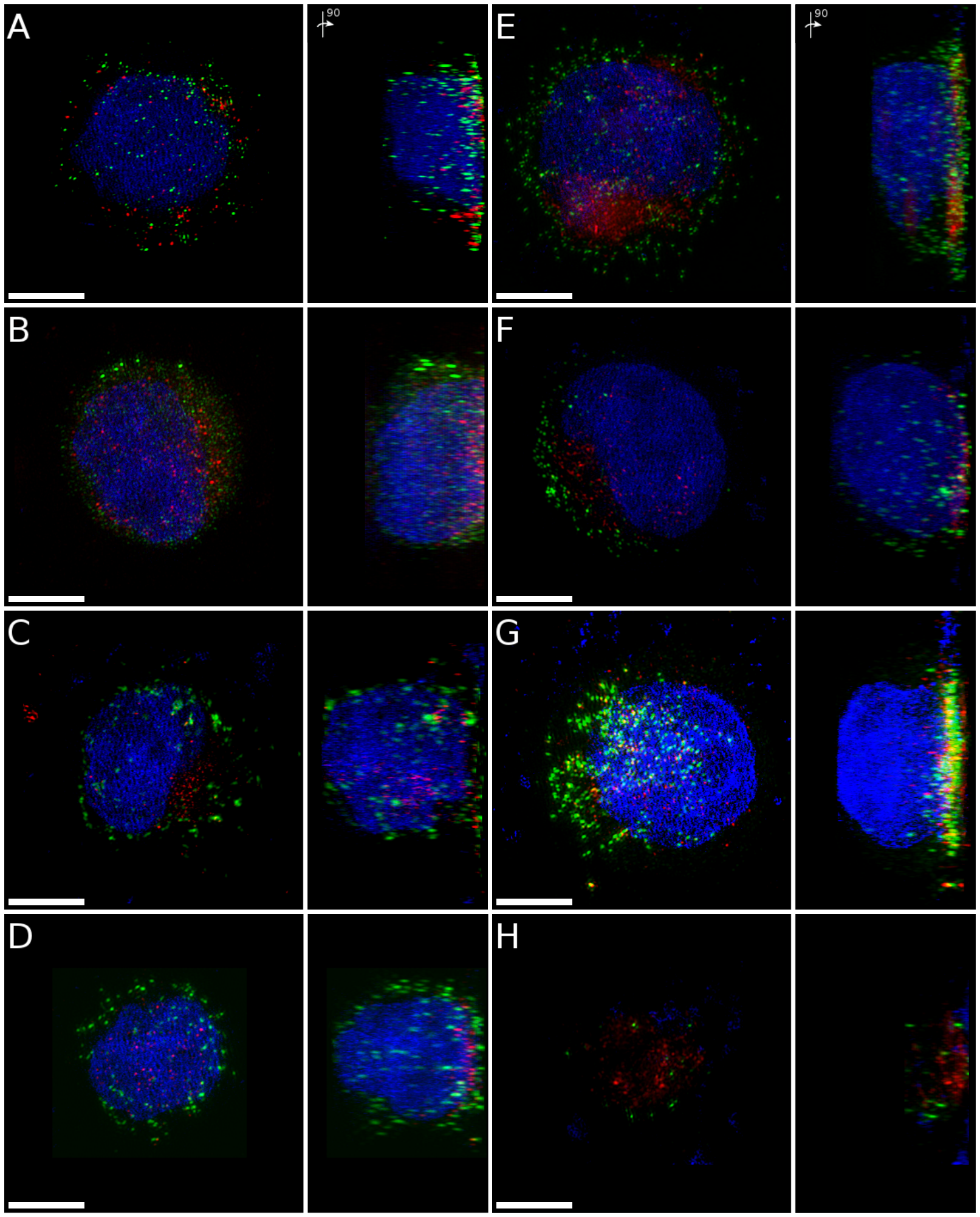
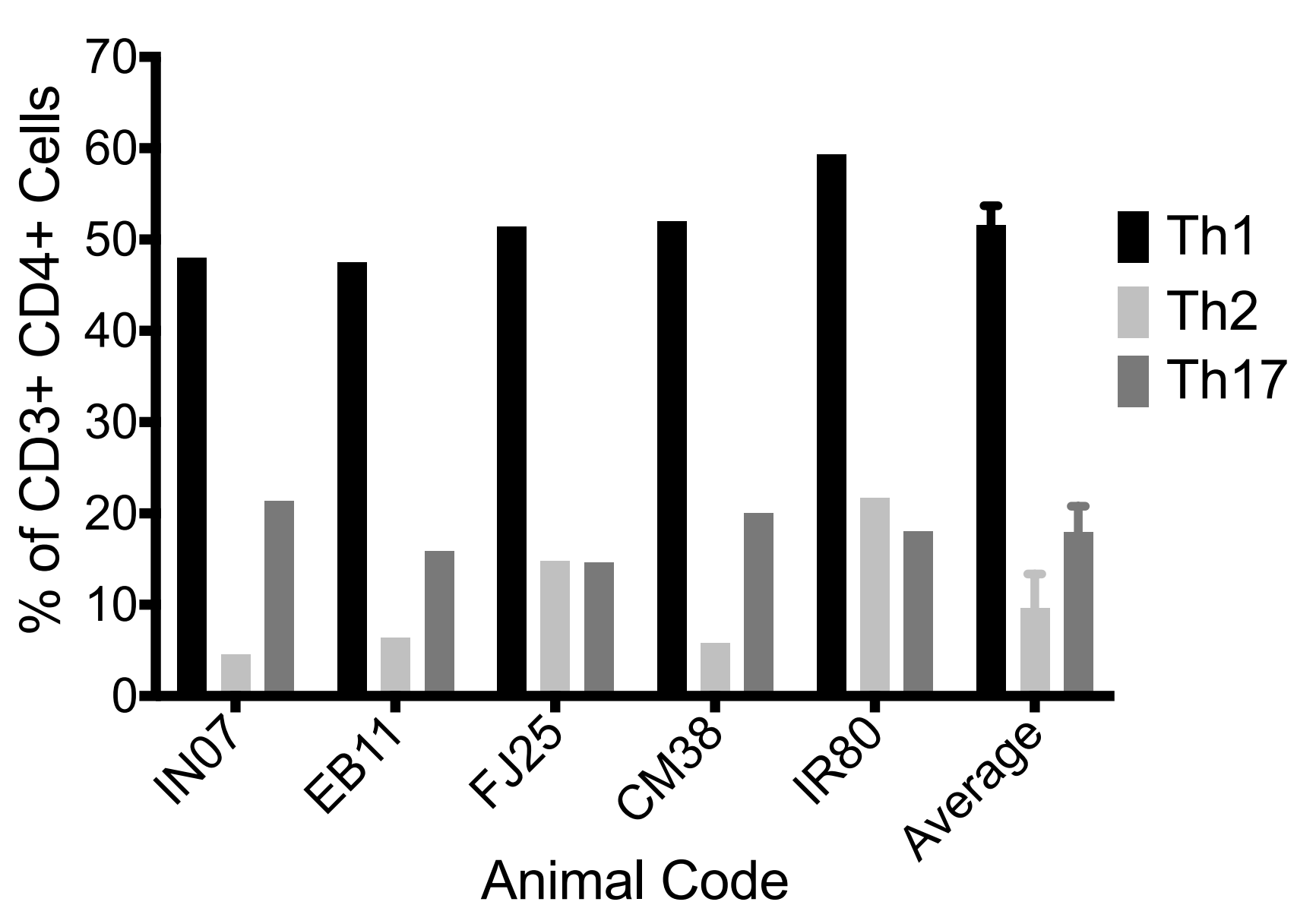
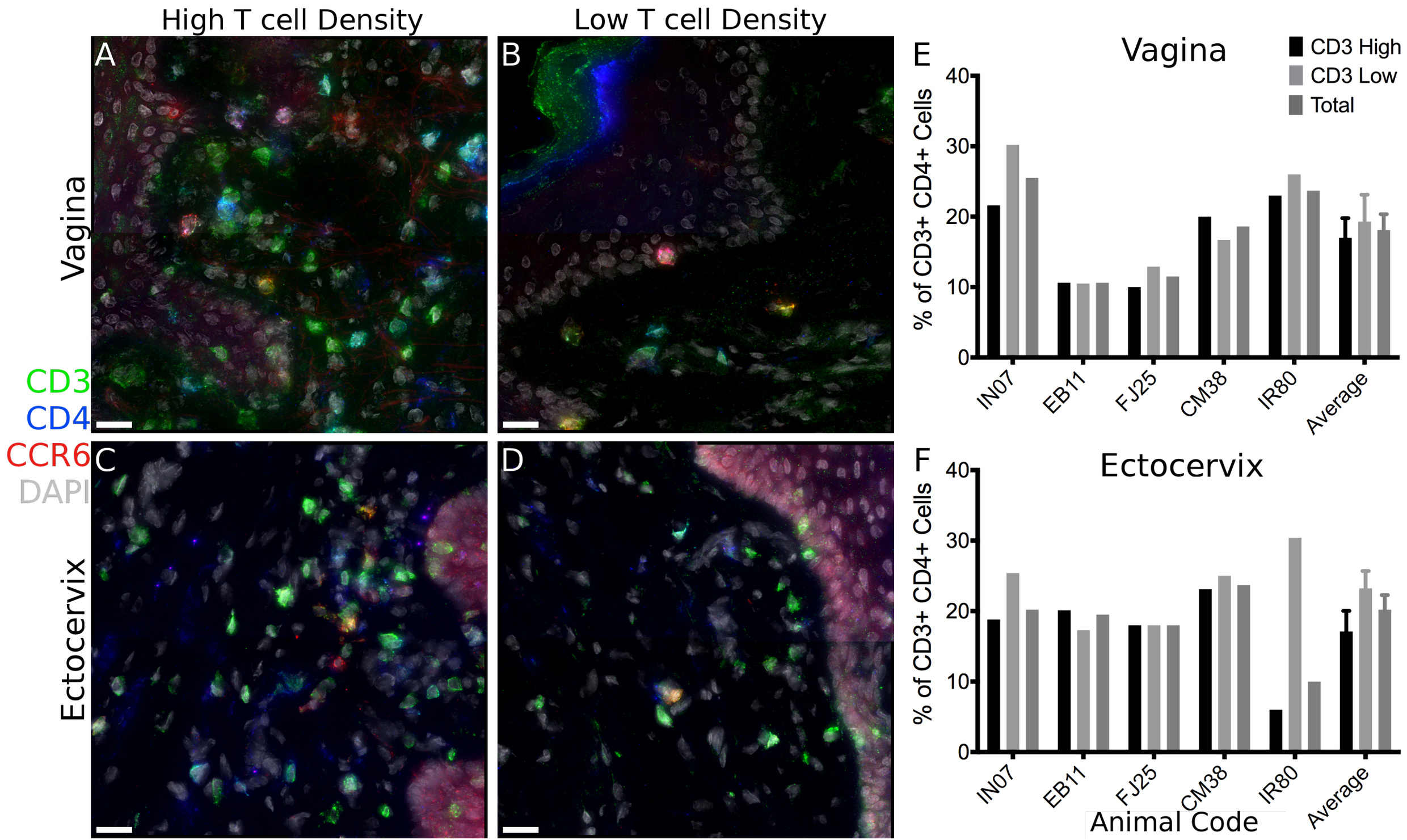

A
90
E
90
B
F
C
G
D
H

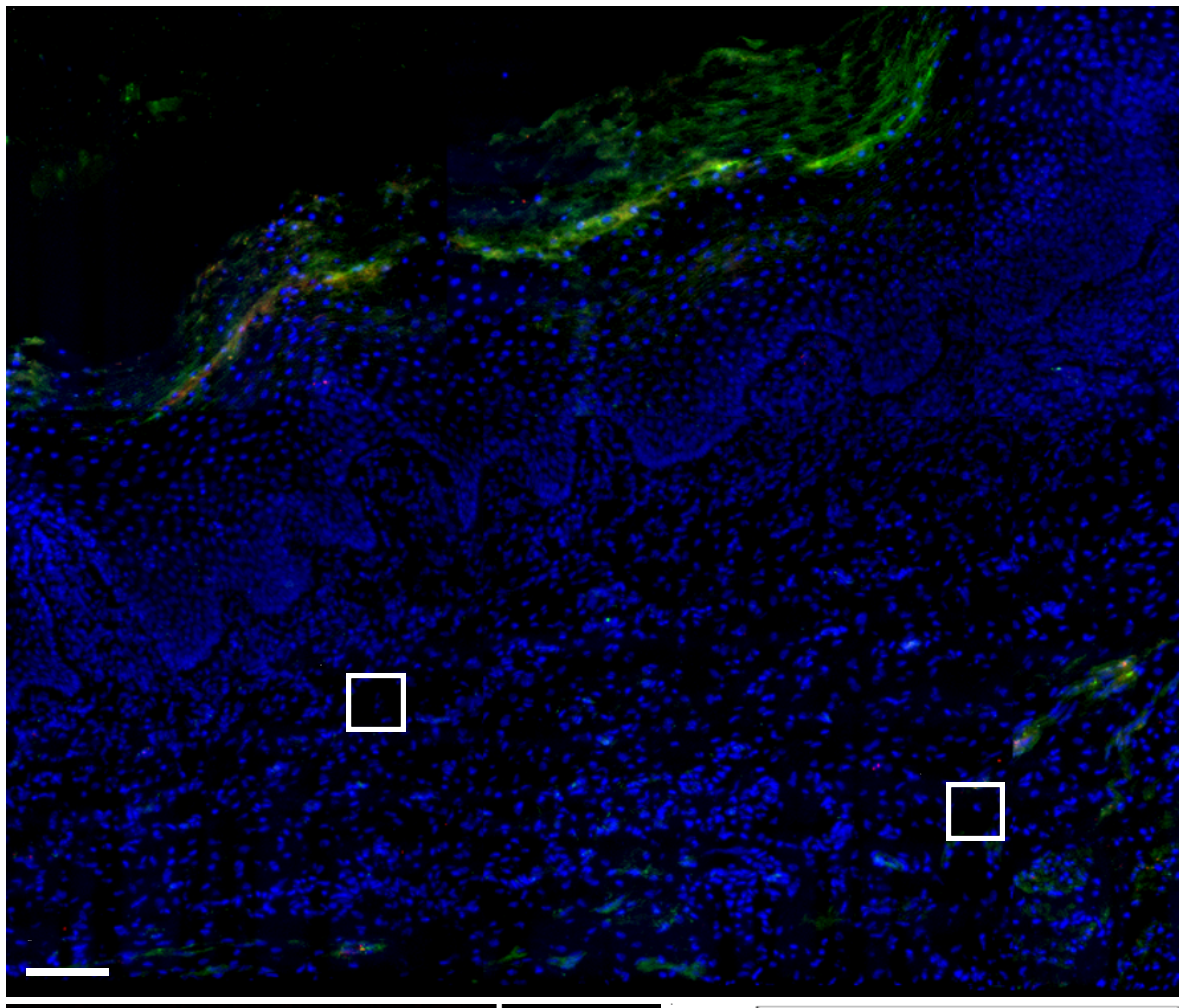
Supplemental Figure 1. Super-resolution imaging of SIV antigen expression in infected macaque PBMC, related to Figure 2. Cells from animal FJ25 were infected with SIVmac239 for 9 days then adhered to coverslips. Structured illumination super-resolution IF imaging of SIV Env (Red), Gag (Green), and nuclei (Blue) was used to detect **A-G**, infected and **H**, apoptotic cells. Gag accumulates primarily at the cell membrane while Env is found predominantly in the perinuclear space, consistent with translation in the trans-Golgi network. Scale bars measure 5μm.

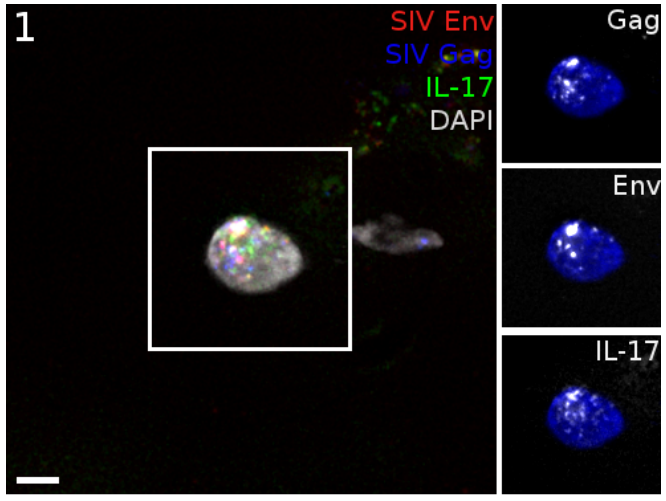

% of CD3+ CD4+ Cells
70
60
50
40
30
20
10
0
IN07
EB11
FJ25
CM38
IR80
Average
Animal Code
Th1
Th2
Th17

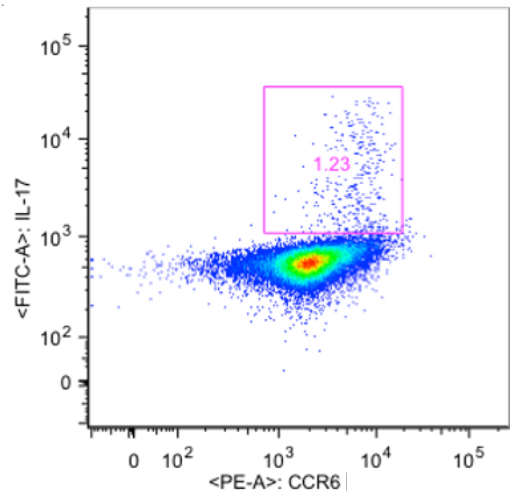
Supplemental Figure 2. SIV infected cells can produce IL-17 and these cells are CCR6+, related to Figure 3. **A**, Ectocervical tissue stained for SIV Env (Red), Gag (Blue), IL-17 (Green), and nuclei (Grey) demonstrates that SIV infected cells are IL-17 producing. Scale bars measure 5μm. Animal code: CM38. **B and C,** IL-17-producing CD4+ T cells were predominantly CCR6+ cell populations in vaginal tissues. Lymphocytes isolated from vaginal tissues were stimulated with PMA plus Ionomycin *in vitro* before staining.

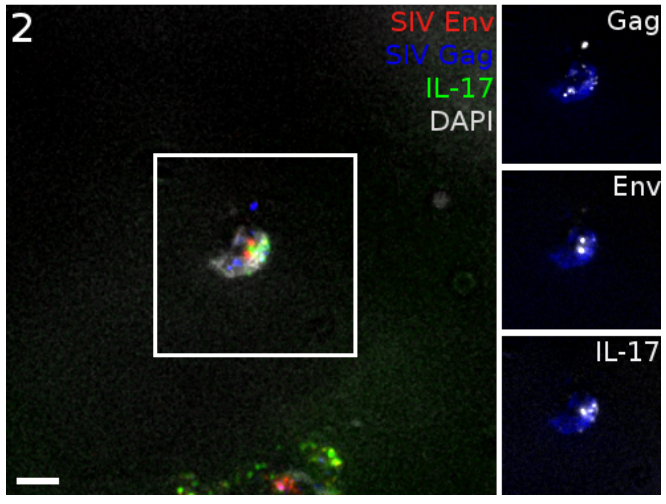

High T cell Density
Low T cell Density
A
B
C
D
Vagina
Ectocervix
CD3
CD4
CCR6
DAPI
E
Vagina
CD3 High
CD3 Low
Total
% of CD3+ CD4+ Cells
0
10
20
30
40
IN07
EB11
FJ25
CM38
IR80
Average
F
Ectocervix
% of CD3+ CD4+ Cells
0
10
20
30
40
IN07
EB11
FJ25
CM38
IR80
Average
Animal Code

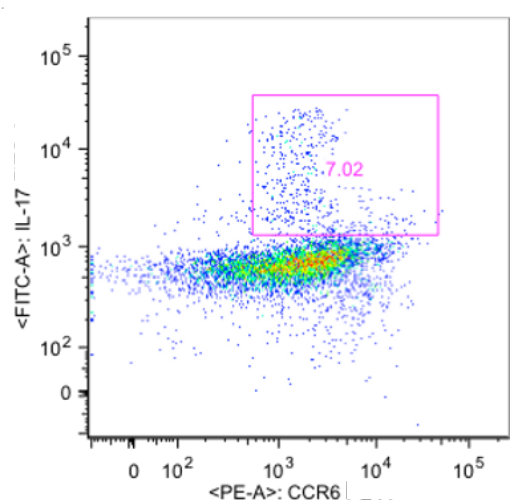
Supplemental Figure 3. Phenotype of T helper subsets in the vagina and ectocervix of 5 rhesus macaques was determined by chemokine receptor expression, related to Figure 3. The proportion of CD3 and CD4 expressing cells that were positive for CXCR3, CCR4 or CCR6 determined the Th1, Th2 or Th17-like classification, respectively. An average of 400 T helper cells per animal were phenotyped. Error bars indicate SEM.



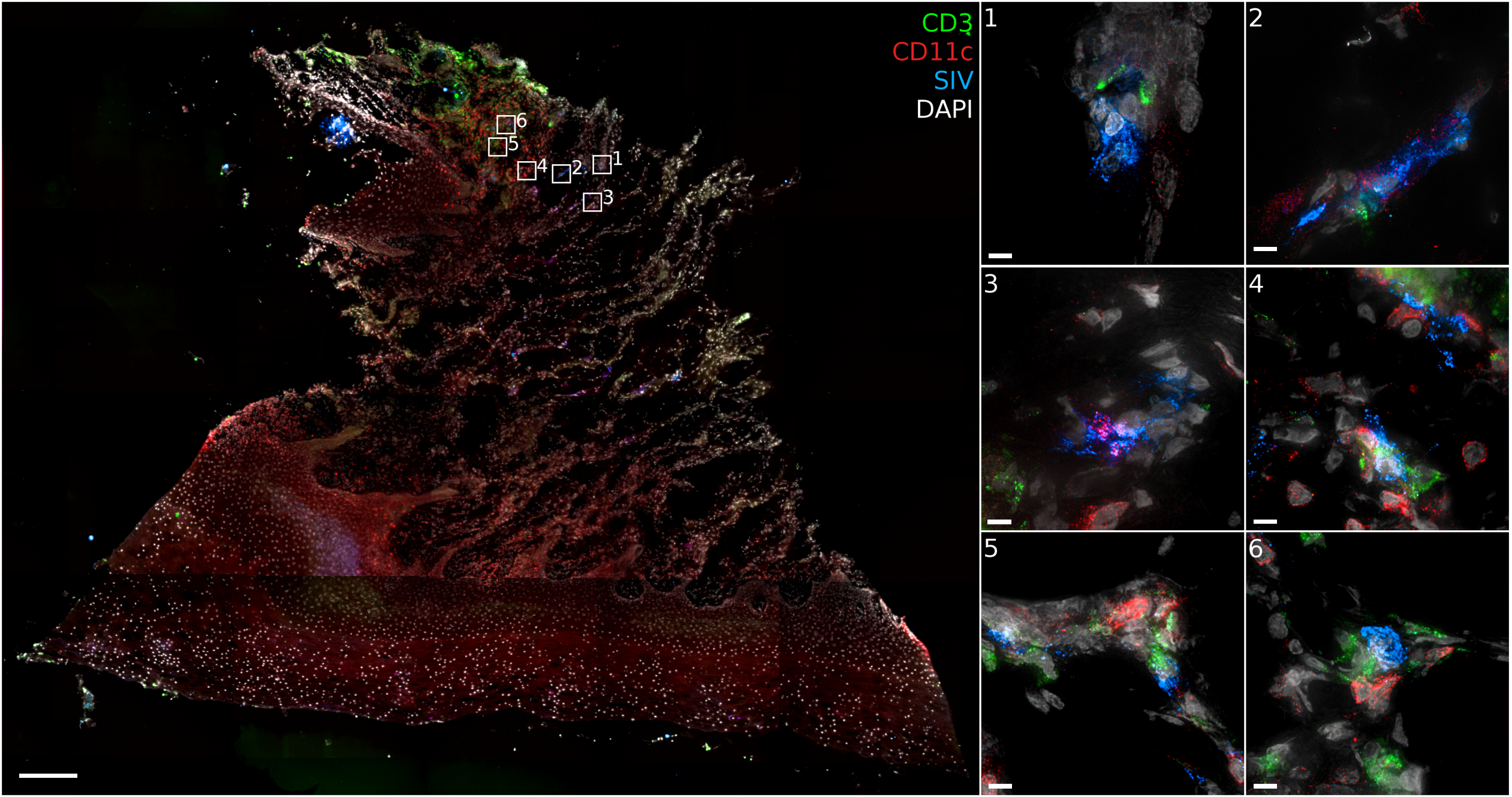

1
SIV Env
SIV Gag
IL-17
DAPI
Gag
Env
IL-17


1.23
<FITC-A>: IL-17
<PE-A>: CCR6


2
SIV Env
SIV Gag
IL-17
DAPI
Gag
Env
IL-17


7.02
<FITC-A>: IL-17
<PE-A>: CCR6

Supplemental Figure 4. Phenotyping Th17 cells in high and low lymphocyte density areas of the vagina and ectocervix by chemokine receptor expression, related to Figure 3. Areas of **A** and **C**, high and **B** and **D**, low densities of T cells were imaged in the **A** and **B**, vagina **C** and **D**, and ectocervix. CD3 (yellow), CD4 (red), CCR6 (green) and nuclei were visualized to define cell density and proportion of Th17 cells out of all T helper cells. Quantification of Th17 cells in **E**, vagina and **F**, ectocervix per animals and the overall average. In total, 7300 T cells were analyzed. Error bars indicate SEM. Animal code: FJ25, CM38.


CD3
CD11c
SIV
DAPI
1
2
3
4
5
6

Supplemental Figure 5. Spatial distribution of SIV infected cells in ectocervical tissue, related to Figure 5. Ectocervical tissue stained for CD3 (Green), CD11c (Red), SIV Env and Gag (Blue), and nuclei (Grey) show a clustering of SIV infected cells below the basement membrane of stratified squamous epithelium. Numbered boxes indicate the location of regions of interest shown in corresponding insets. Scale bars measure 5μm. Animal code: FJ25.

Supplemental Movie 1. Volume view of a lysed cell, related to Figure 5. Ectocervical tissue stained for SIV Gag (Green), Env (Red) and nuclei (Blue) shows viral antigens spread over a large area, not associated with a single cell and not colocalized into released viral particles. Scale bar measures 5μm.

Supplemental Experimental Procedures

Cell culture and infection. 293T cells (American Type Culture Collection) and GHOST Bonzo+ cells (AIDS Reagent Repository) were cultured in Dulbecco's modified Eagle's medium (DMEM, HyClone) containing 10% fetal bovine serum (FBS). RM peripheral blood mononuclear cells (PBMC) were isolated from naive healthy animals, separated over Ficoll gradient and cultured at 2.5×10^6 cells mL^{-1}. Vaginal lymphocytes from RMs that were being necropsied as part of other studies were isolated by EDTA/Collagenase/Percoll treatment. PBMC and CEMx174 cells were maintained in RPMI containing 10% FBS. 48 hours prior to infection, PBMC were activated with $100IUml^{-1}$ IL-2 and $1\mu gmL^{-1}$ Phytohaemagglutinin (PHA). PBMCs (1×10^6 cells) were incubated with SIVmac251 at a multiplicity of 100 $TCID_{50}$ for 1h. Unattached virus was removed and cells were cultured at 2×10^5 cell per well. Cells were collected at day 3 and 5 post infection. To infect PBMC for microscopy studies, 100ng p27 SIVmac766 was added to activated cells. PMBC were maintained with IL-2 until 9 days post SIV infection.

Phenotyping. Flow cytometry for surface and intracellular staining was performed using standard protocols. Cells were stained with: CD3 (SP34), CD4 (SK3), CD8 (SK1), CCR5 (3A9), CCR6 (G034E3, BioLegend), Ki67 (B56), IL-17 (CZ8-23G1; Miltenyi Biotec) and LIVE/DEAD Fixable Aqua Dead Cell Stain Kit (Invitrogen, Grand Island, NY). Isotype-matched controls were included in experiments. All antibodies and reagents were purchased from BD Biosciences Pharmingen (San Diego, CA) unless noted. Samples were resuspended in BD Stabilizing Fixative and acquired on a FACS VERSE (Becton Dickinson, San Jose, CA). Data were analyzed with Flowjo software (Tree Star, Ashland, OR).

Nested PCR of SIVmac239 *gag* gene and LICh LTR. Genomic DNA was isolated from 3 to 5 mg of frozen tissue using the Qaigen DNeasy Blood & Tissue Kit (Qaigen N.V.). In each reaction, 250ng of DNA was utilized to detect either a 513-bp DNA sequence of the *gag* gene or a 468-bp sequence of the LICh LTR generated only after reverse transcription. Amplification procedure was based on previously described methodology (Milush et al., 2004). Primers used for *gag* detection were 5'-ATTAGCAGAAAGCCTGTTGGAG-3' (outer forward) and 5'- AGAGTGTCCTTCTTTCCCACAA -3' (outer reverse) followed by 5'- CATTCACGCAGAAGAGAAAGTG-3' (inner forward) and 5'-GGTATGGGGTTCTGTTGTCTGT-3' (inner reverse). LICh LTR detection primers were 5'-GCCTGTCAGAGGAAGAGGTTAG-3' (outer forward) and 5'- GCCTTCACTCAGCCGTACTC-3' (outer reverse) followed by 5'-TGGCTGACAAGAGGGAAACTC-3' (inner forward) and 5'-CTCCTTCAAGTCCCTGTTCG-3' (inner reverse). Amplification was performed using a Bio-Rad iCycler Thermal Cycler system (Bio-Rad Laboratories, Hercules, CA, USA). Each DNA sample was tested in 24 replicates. Negative controls were naïve RM tissue. Second round PCR products were separated on a 1.5% agarose gel and visualized by ethidium bromide staining. Sequences were confirmed by extracting DNA with Qaigen QIAquick Gel Extraction Kit and Sanger sequencing with second round primers.

Supplemental References

Milush, J.M., Kosub, D., Marthas, M., Schmidt, K., Scott, F., Wozniakowski, A., Brown, C., Westmoreland, S., and Sodora, D.L. (2004). Rapid dissemination of SIV following oral inoculation. AIDS *18*, 2371-2380.