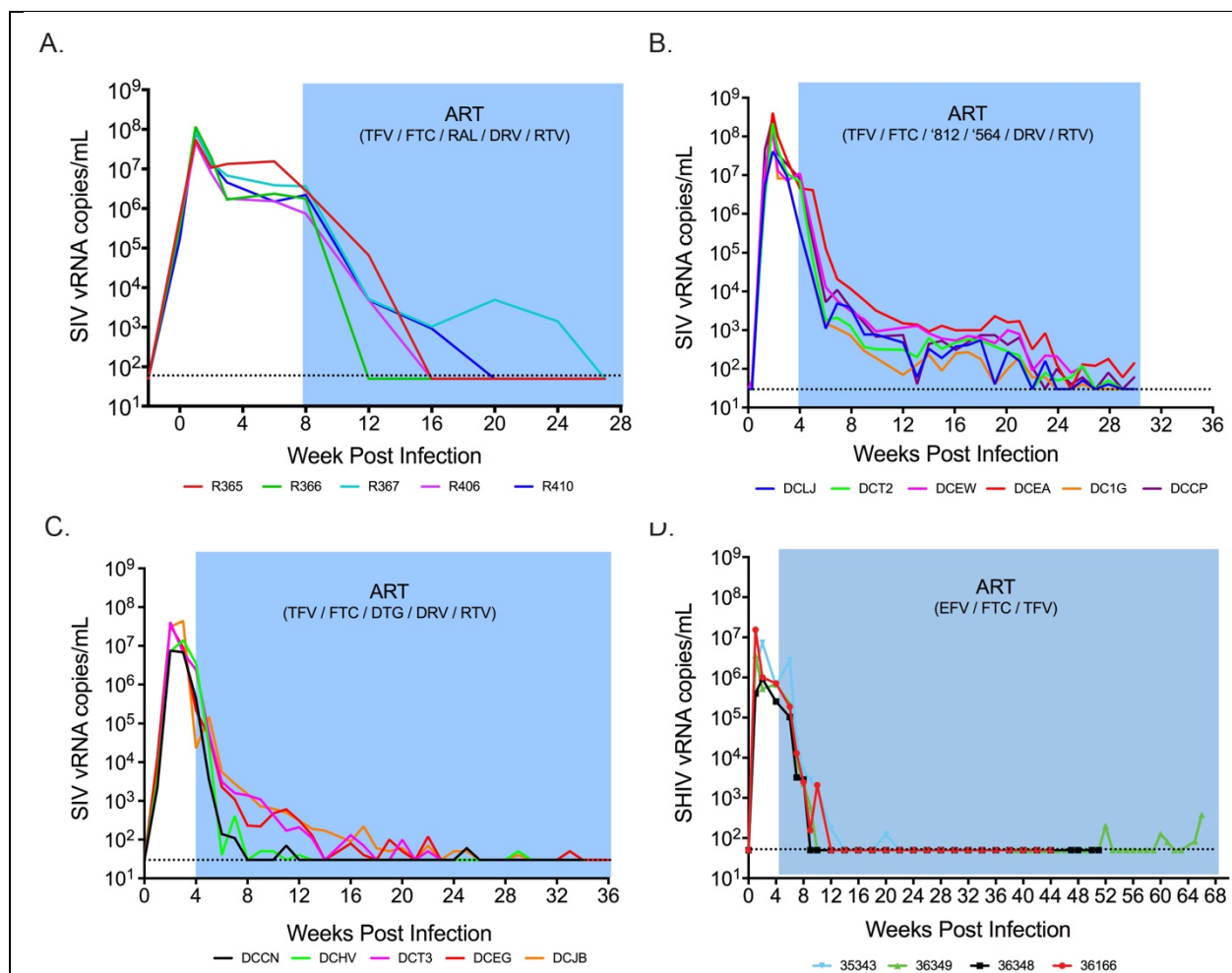
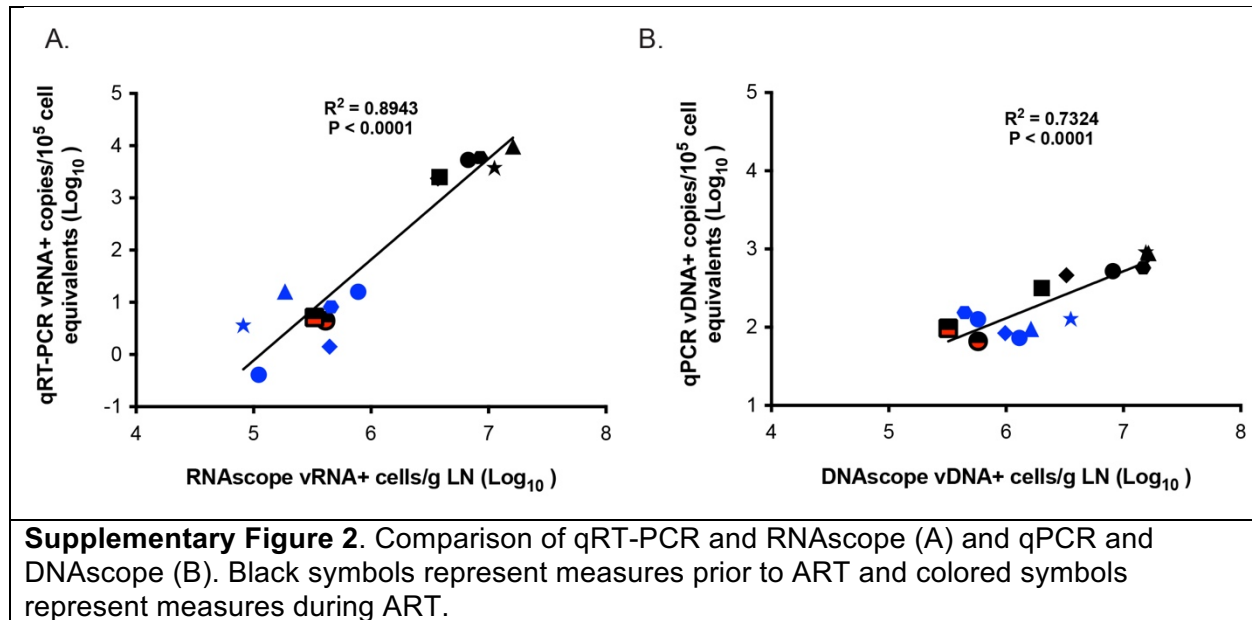
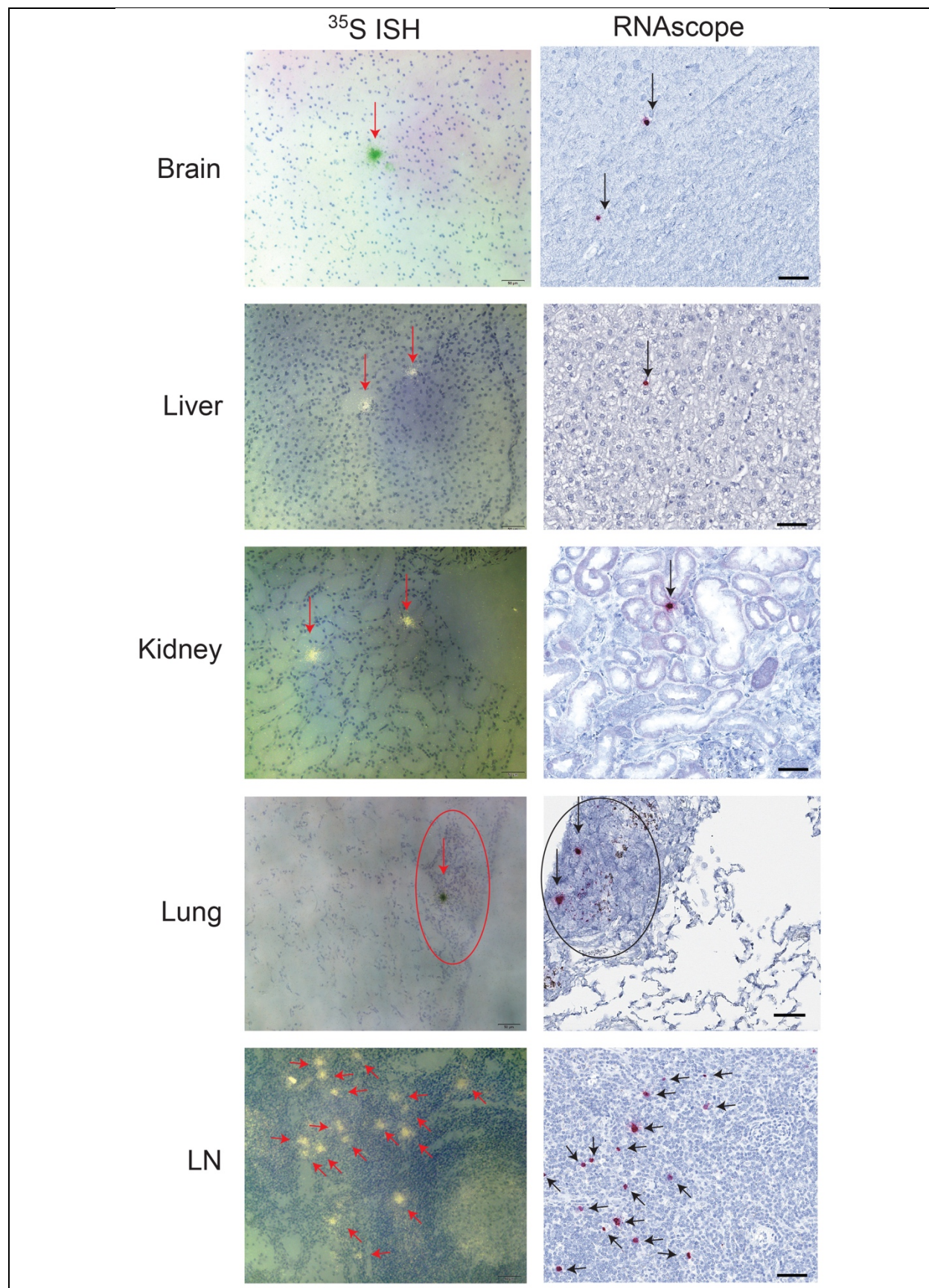


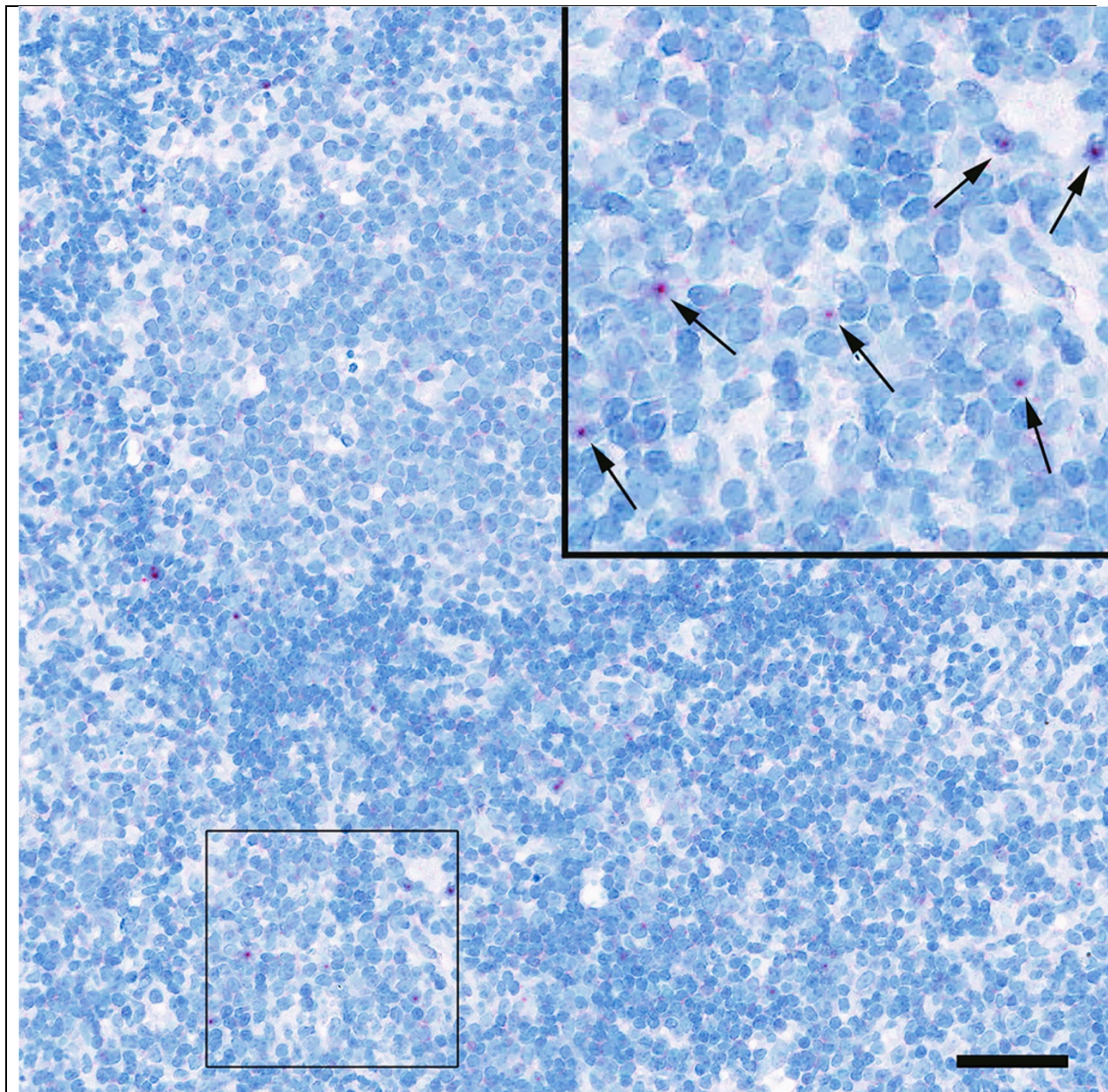


Supplementary Figure 1. Plasma viral load decay characteristics in each of 4 monkey cohorts studied after prolonged ART. A. Decay in 5 SIV infected animals treated ART for 20-22 weeks. B, C. Decay in additional SIV infected animals treated with the ART drugs listed. D. Decay in 4 RT-SHIV+ animals treated with efavirenz (RFV), emtricitibine (FTC), and tenofovir (TFV).

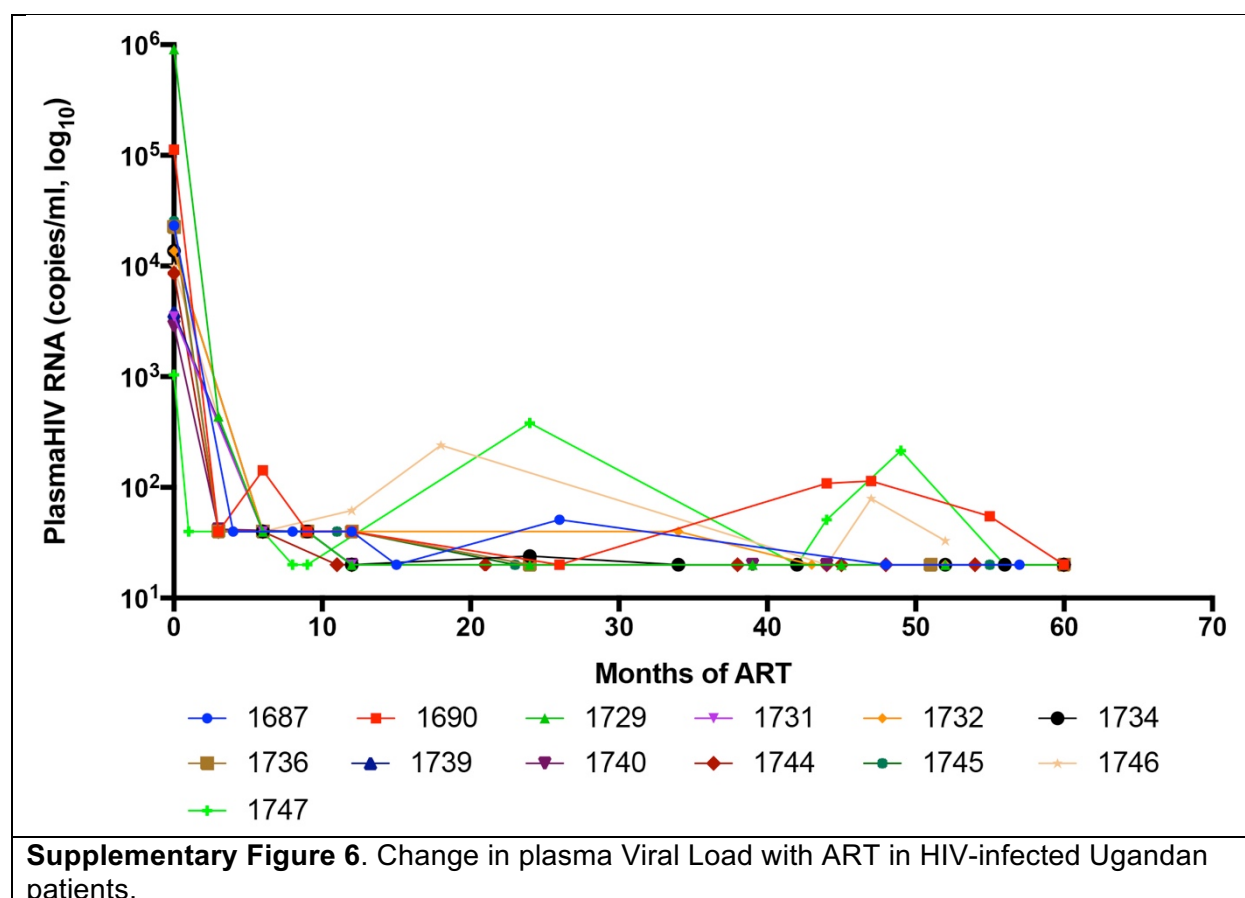
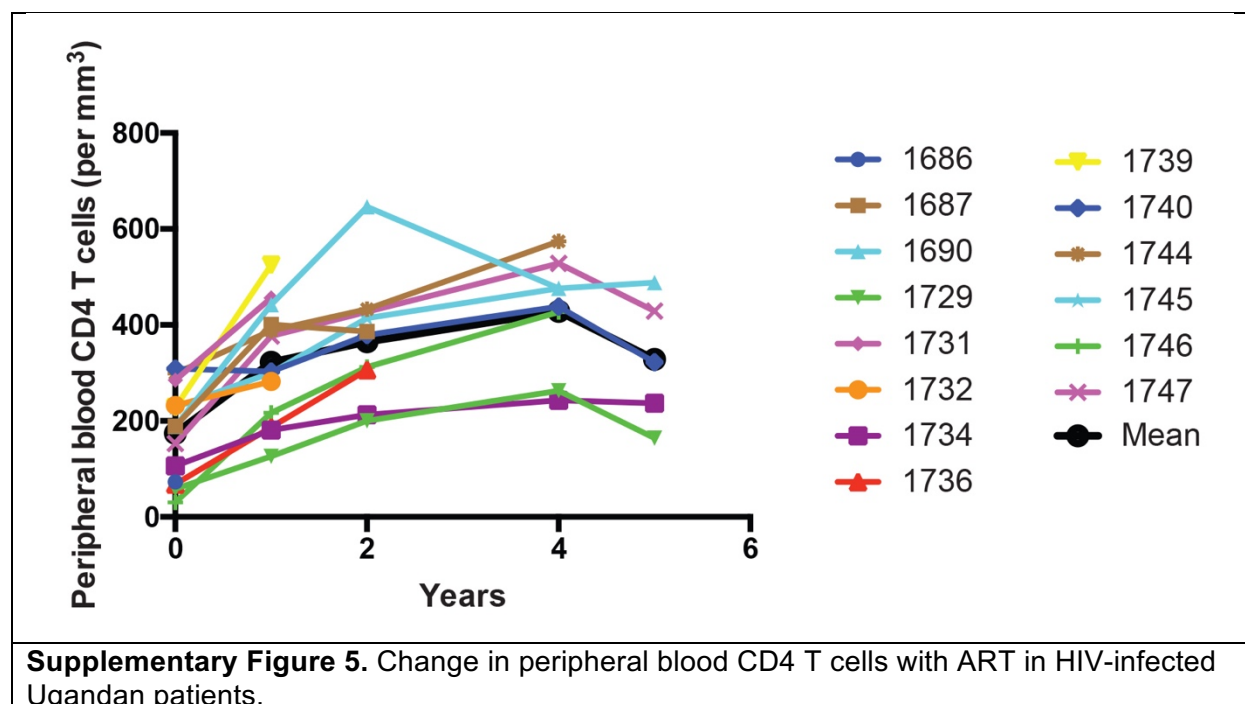




Supplementary Figure 3. Representative images from brain, kidney, liver, and lymph node using ^{35}S SIV RNA ISH (red arrows) and SIV RNAscope (black arrows) to identify vRNA+ cells.



Supplementary Figure 4. Representative image of lymph node, DNA ISH using DNAscope. Red signal located over the nucleus of the cell identifies those cells that are vDNA+ (black arrows).



Organ	Average SIV RNA+ Cells/Gram	Organ weight as a percentage of total weight (%)	Total Cells/Organ
Heart	7.54×10^3	0.44%	2.89×10^5
Brain	2.16×10^2	2.93%	5.58×10^4
Kidney (x2)	1.42×10^4	0.52%	1.29×10^6
Liver	1.88×10^3	3.17%	5.24×10^5
Gut	5.57×10^5	3.78%	1.86×10^8
Lungs (x2)	3.10×10^4	0.96%	5.26×10^6
LN	8.73×10^5	1.00%	7.68×10^7
Spleen	1.53×10^5	0.09%	1.19×10^6

Supplementary Table 1. Total estimated frequency of vRNA+ cells in each organ system. Estimated vRNA+ cells/gram of tissue from the animal sampled in acute infection were determined from a series of 5 sections from each organ and the average weight of each organ was estimated from the total weight of the animal using published necropsy literature to determine what average percent of the total body weight in a particular(1). Note the animal weight at necropsy was 8800 grams(1).

A.

	P568 (Peak Viremia)	R096 (Early Chronic)	P533 (Late Chronic)
Brain	2.16×10^2	7.67×10^2	4.51×10^4
Heart	ND	ND	1.95×10^3
Lung	1.88×10^3	3.07×10^4	7.75×10^5
Liver	6.99×10^5	1.91×10^3	4.69×10^4
Kidney	3.10×10^4	2.83×10^4	8.14×10^2
Spleen	1.76×10^4	1.58×10^5	1.69×10^5
Axillary LN	1.53×10^5	2.51×10^6	1.80×10^6
Mesenteric LN	3.42×10^5	1.95×10^6	1.90×10^6
Inguinal LN	1.11×10^6	6.11×10^6	1.36×10^6
Colonic LN	5.10×10^5	3.59×10^6	3.88×10^6
Jejunum	5.82×10^5	7.87×10^5	3.31×10^6
Duodenum	2.05×10^5	5.27×10^5	3.92×10^4
Ileum	7.54×10^3	2.56×10^5	1.24×10^5
Ascending Colon	1.09×10^6	1.09×10^5	5.51×10^5
Transverse Colon	2.13×10^5	2.09×10^5	9.61×10^4
Rectum	ND	9.17×10^5	7.59×10^6
Prostate	1.42×10^4	ND	ND
Seminal Vesicle	9.74×10^5	6.45×10^3	

B.

	R365	R366	R367	R406	R410
Brain	ND	1.80×10^3	ND	7.7×10^3	
Heart	ND	ND	ND	3.93×10^2	
Lung	9.04×10^3	1.11×10^3	9.27×10^2	6.69×10^4	8.84×10^3
Liver	ND	6.55×10^2	ND	1.67×10^3	1.86×10^2
Kidney	ND	5.38×10^2	ND	5.48×10^2	8.77×10^2
Spleen	ND	4.76×10^4	1.08×10^5	1.14×10^5	
Axillary LN	ND	ND	ND	6.69×10^4	1.18×10^3
Mesenteric LN	1.14×10^4	ND	ND	7.64×10^4	1.87×10^4
Inguinal LN	ND	ND	ND	1.10×10^4	1.71×10^4
Colonic LN	1.46×10^4	ND	ND	ND	1.05×10^5
Jejunum	ND	ND	ND	2.93×10^6	1.76×10^5
Duodenum	2.98×10^3	2.52×10^5	4.78×10^4	1.11×10^6	4.05×10^4
Ileum	1.02×10^4	ND	1.11×10^6	4.87×10^6	2.92×10^4
Ascending Colon	2.47×10^4	6.54×10^4	1.01×10^6	1.09×10^5	7.16×10^5
Transverse Colon	2.11×10^4	2.15×10^5	4.08×10^5	3.01×10^6	5.45×10^5
Rectum	1.53×10^4	ND		3.42×10^5	
Prostate	*	*	*	*	*
Seminal Vesicle	*	*	*	*	*

C.

	35483	35609	36606	36650
Cerebrum	ND	1.1×10^3	ND	ND
Cerebellum	3.7×10^2	ND	ND	ND
Basal Ganglion			ND	ND
Bone Marrow	2.5×10^4	3.7×10^5	ND	7.9×10^4
Lungs	2.3×10^4	6.2×10^4	1.6×10^3	1.2×10^5

Liver	4.8×10^3	6.2×10^4	ND	3.1×10^4
Thymus	9.1×10^3	1.1×10^5	ND	4.4×10^4
Spleen	2.5×10^5	3.6×10^6	1.4×10^5	2.8×10^6
Axillary LN	4.0×10^5	ND	7.0×10^3	2.4×10^6
Iliac LN	5.3×10^5	2.4×10^6	ND	4.4×10^6
Mesenteric LN	2.9×10^5	8.5×10^6	1.4×10^4	5.9×10^6
Inguinal LN	6.3×10^4	4.1×10^6	5.8×10^3	3.3×10^6
Jejunum	5.9×10^4	1.8×10^6	2.2×10^2	3.1×10^6
Ileum	1.5×10^3	3.9×10^6	1.4×10^4	3.9×10^5
Colon	6.7×10^4	2.4×10^5	1.1×10^3	5.8×10^5

D.

	36348	35349	36166	35343
Cerebrum	1.1×10^2	2.4×10^2	1.2×10^2	1.1×10^2
Cerebellum	ND			
Spinal Cord				ND
Bone Marrow	ND	ND	ND	ND
Thymus	ND	ND	ND	ND
Heart				1.8×10^2
Lungs	1.2×10^2	6.8×10^2	1.9×10^2	ND
Liver	1.9×10^2	ND	ND	ND
Kidney				ND
Spleen	3.3×10^2	2.8×10^2	6.0×10^2	ND
Cervical LN				ND
Axillary LN	1.3×10^3	1.7×10^2	ND	
Iliac LN	3.5×10^3	1.4×10^3	7.8×10^2	ND
Inguinal LN	ND	3.2×10^2	1.1×10^3	ND
Mesenteric LN	1.1×10^3	4.2×10^2	7.9×10^3	ND
Jejunum	ND	ND	3.6×10^2	ND
Duodenum				ND
Ileum	8.8×10^2	ND	2.8×10^2	7.6×10^2
Colon	ND	ND		ND

Supplementary Tables 2 A-D: Tables providing raw data on frequency of vRNA+ cell/gram for each animal in each organ studied. Table 2A = 3 animals infected with SIV mac251 and sacrificed in acute, early and late chronic infection without ART, table 2B = macaques infected with SIV mac251 after 22 weeks of ART, Table 2C = 4 macaques infected with RT-SHIV and sacrificed after 26 weeks of infection without ART, and Table 1D = 5 macaques infected with RT-SHIV and sacrificed after 26 weeks of ART. ND, not detected. Cells in the table that are blank means the tissue was not available, * means the animal was female.

A. Tenofovir (TFV)

	TFV (ng/ml)	IC TFV-DP (foml/10 ⁶ cells)				Ratio to PBMC		
ID	Plasma	PBMC	LN	GALT	RALT	LN	GALT	RALT
DC1G	*	4783.33	3405	1812.5	4516	0.71	0.38	0.94
DCCP	83.06	3125	1530	10167	4153	0.49	3.25	1.33
DCT2	45.62	1532.89	1425	487.5	699	0.93	0.32	0.46
DCLI	41.14	1347.83	1635	587.5	785	1.21	0.44	0.58
DCEA	93.52	1652.34	2950	421.88	1213	1.79	0.26	0.73
DCEW	65.92	1925	1432.5	922.06	3545	0.74	0.48	1.84

B. Emtricitabine (FTC)

	FTC (ng/ml)	IC FTC-TP (fmol/10 ⁶ cells)				Ratio to PBMC		
ID	Plasma	PBMC	LN	GALT	RALT	LN	GALT	RALT
DC1G	*	4000	333.5	411	911	0.08	0.10	0.23
DCCP	61.0	2033	220	4861	724	0.11	2.39	0.36
DCT2	36.58	670.23	224.5	256.25	265	0.33	0.38	0.40
DCLI	31.23	457.61	270	364.42	126	0.59	0.80	0.28
DCEA	70.44	1304.69	346.25	178.91	354	0.27	0.14	0.27
DCEW	45.84	878.13	220.63	230.88	470	0.25	0.26	0.54

C. Darunavir (DRV)

	DRV (ng/ml)	IC DRV (fmol/10 ⁶ cells)				Ratio to PBMC		
ID	Plasma	PBMC	LN	GALT	RALT	LN	GALT	RALT
DC1G	*	BLQ	BLQ	BLQ	6531	NA	NA	NA
DCCP	59.6	172.5	BLQ	BLQ	68611	NA	NA	397.75
DCT2	188.7	225.33	BLQ	BLQ	1007	NA	NA	4.47
DCLI	275.9	114.13	BLQ	BLQ	1722	NA	NA	15.09
DCEA	43.7	41.02	BLQ	BLQ	406	NA	NA	9.89
DCEW	77.0	153.91	BLQ	BLQ	19773	NA	NA	128.50

Supplementary Table 3: Plasma and intracellular ARV drug concentrations. Samples where concentrations were below the limit of quantitation are noted as BLQ; ratios that could not be calculated are noted as NA, not available.

vRNA+ cells	Virus-producing cells
18	12
18	17
Supplementary Table 4: Direct comparison of frequency of vRNA+ cells in multiple sections from a LN of a suppressed macaque to the frequency of virus producing cells using ISH TSA/ELF.	

1. Fremming BD, Benson RE, Young RJ. Weights of organs in sixty-six male *Macaca mulatta* monkeys. *J Appl Physiol.* 1955;8(2):155-8. PubMed PMID: 13263256.