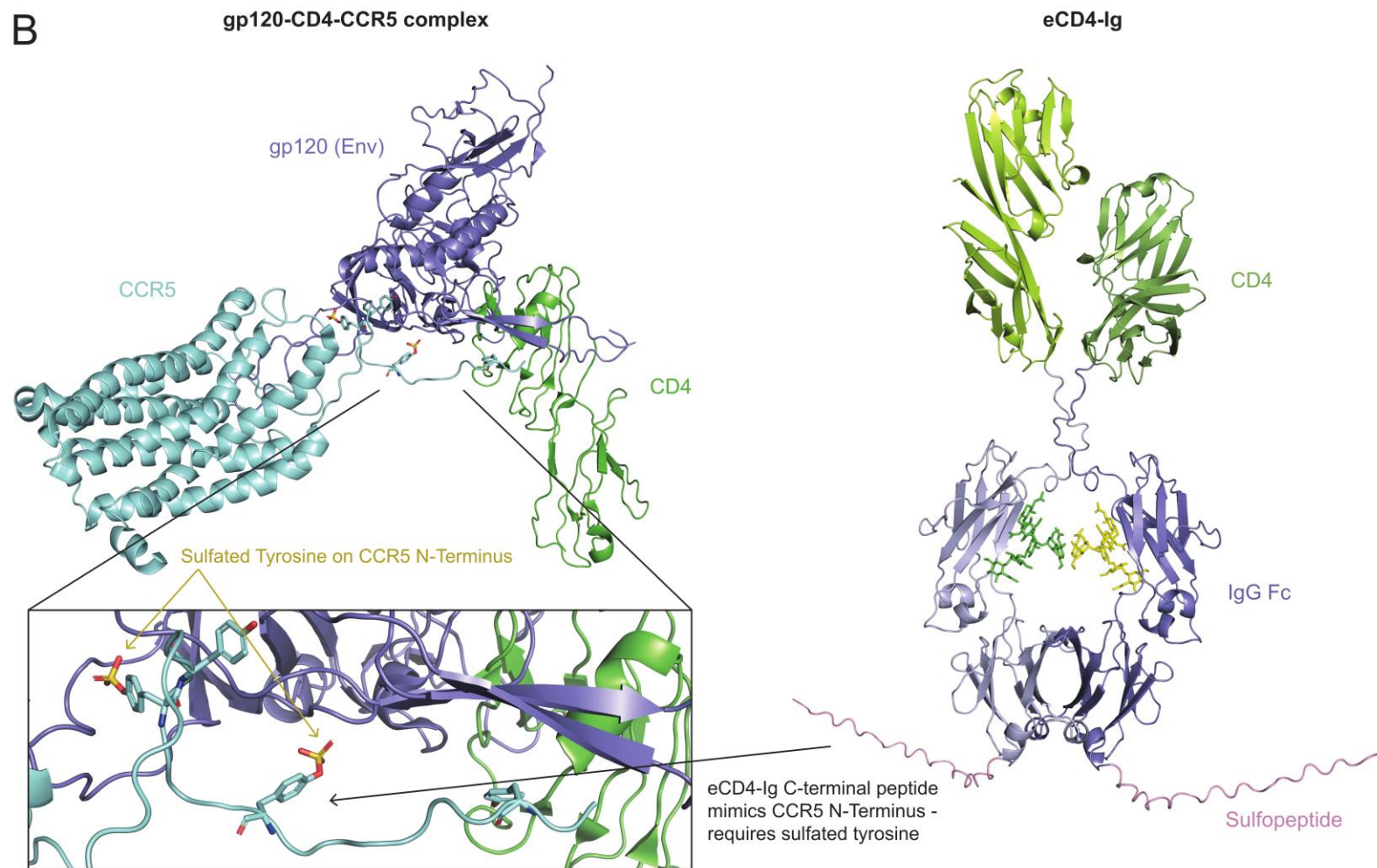
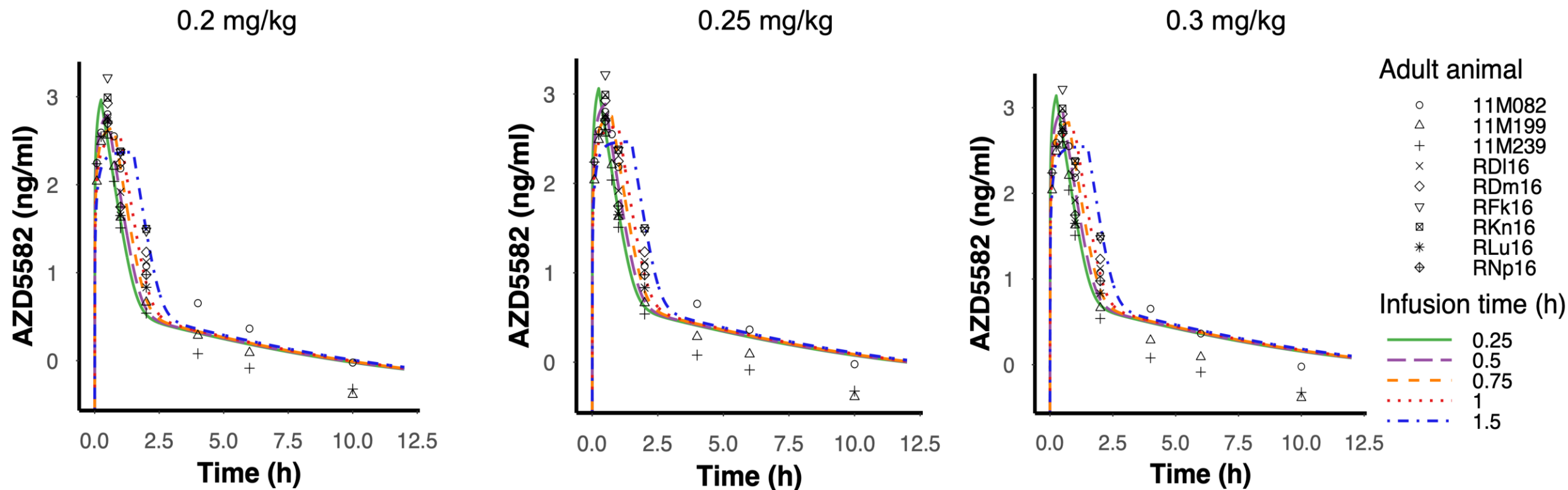


A ShortHinge Rhesus IgG1: DIVVLA-----GGGSKPPTCPPCPAPELLGGP
 Wild Type Rhesus IgG1: DIVVLA^{EIKT}CGGGSKPPTCPPCPAPELLGGP

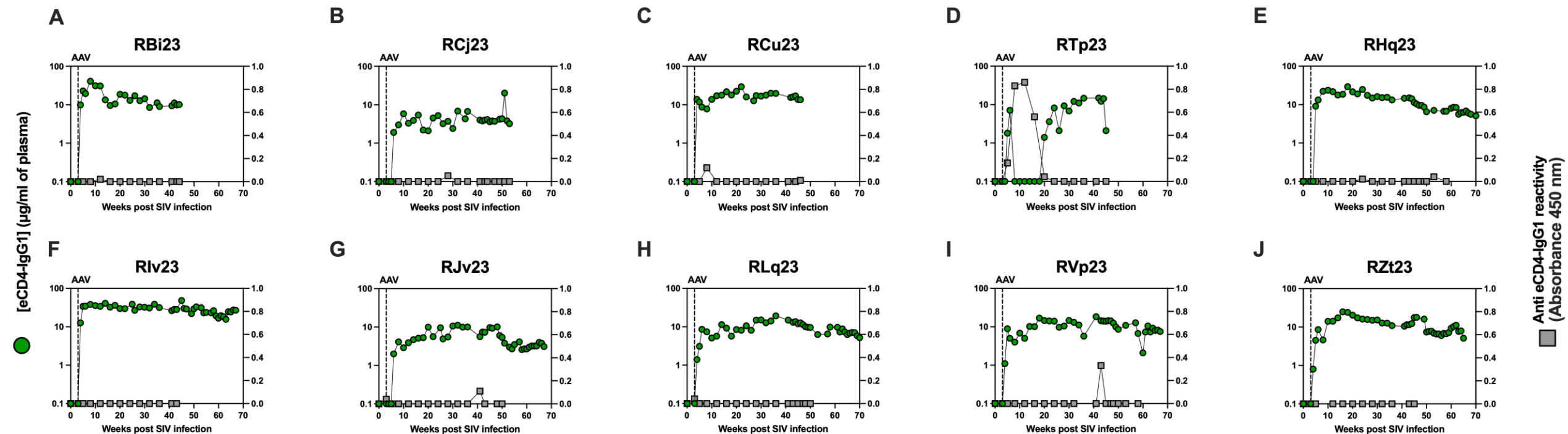
CD4 Hinge CH2



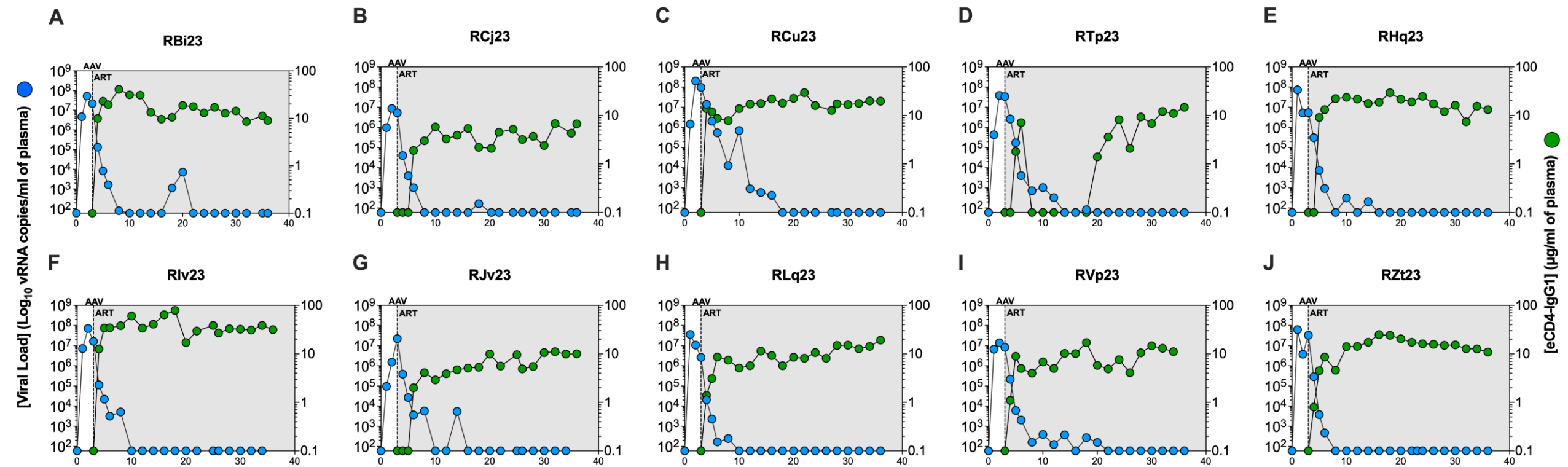
Supplemental Figure 1. Structural features of eCD4-Ig. (A) Sequence alignment of wild-type and short-hinge rhesus IgG1 (Variant Emm058). The indicated cysteine normally pairs with the immunoglobulin light chain; its deletion prevents misfolding in the absence of a light chain. **(B)** Structural basis for sulfotyrosine dependence. *Left*, cryo-EM structure of HIV-1 Env gp120 (slate) bound to CD4 (green) and CCR5 (cyan; PDB 6MEO), highlighting sulfated CCR5 N-terminal tyrosines contacting the CD4-induced gp120 surface. *Right*, AlphaFold model of eCD4-IgG1 showing CD4 D1–D2 (green), IgG1 Fc (slate), and C-terminal CCR5-mimetic sulfopeptides (pink). The sulfopeptide occupies the functional position of the CCR5 N-terminus and requires tyrosine sulfation for high-affinity Env binding. Because TPST2 catalyzes tyrosine sulfation in the trans-Golgi during protein secretion, AAV9-eCD4-IgG1 and AAV9-TPST2 were co-formulated and co-administered.



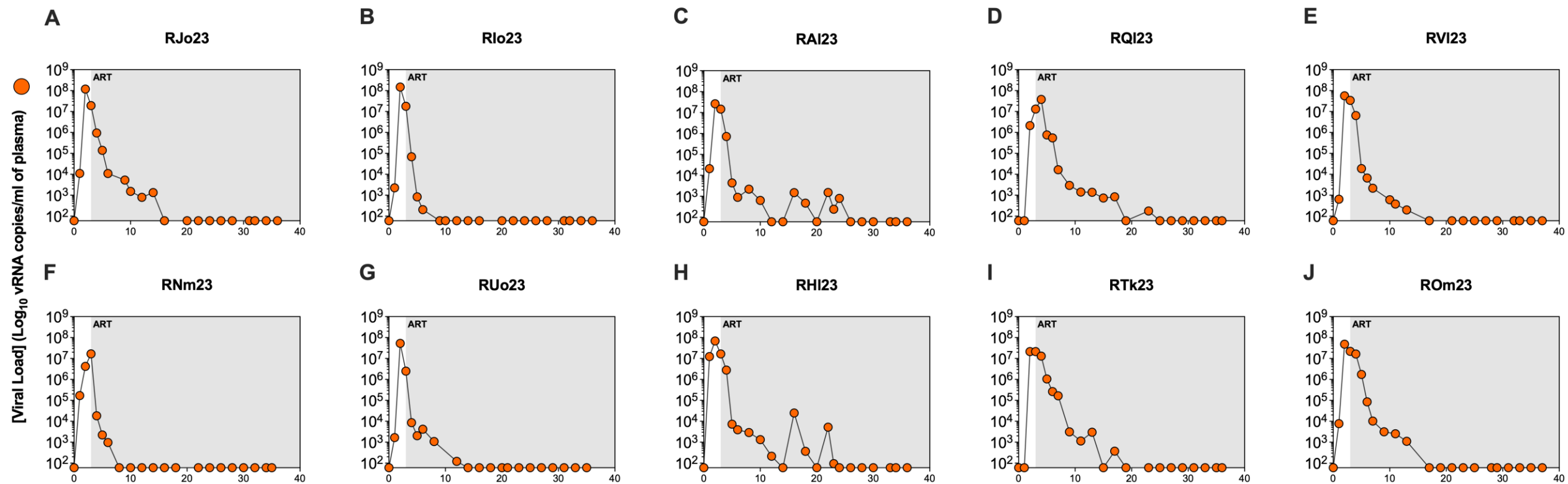
Supplemental Figure 2. Pharmacokinetic modeling of AZD5582 in infant rhesus macaques. Simulated plasma concentration–time profiles of AZD5582 in infant rhesus macaques at doses of 0.2 mg/kg (left), 0.25 mg/kg (middle), and 0.3 mg/kg (right), generated from a three-compartment population PK model fit in NONMEM to infant plasma concentrations following 0.1 mg/kg IV AZD5582. Within each panel, curves correspond to infusion durations of 0.25, 0.5, 0.75, 1, and 1.5 hours. Open symbols denote observed plasma concentrations from adult rhesus macaques at 1, 2, 4, 6, and 10 hours following a 0.1 mg/kg AZD5582 infusion.



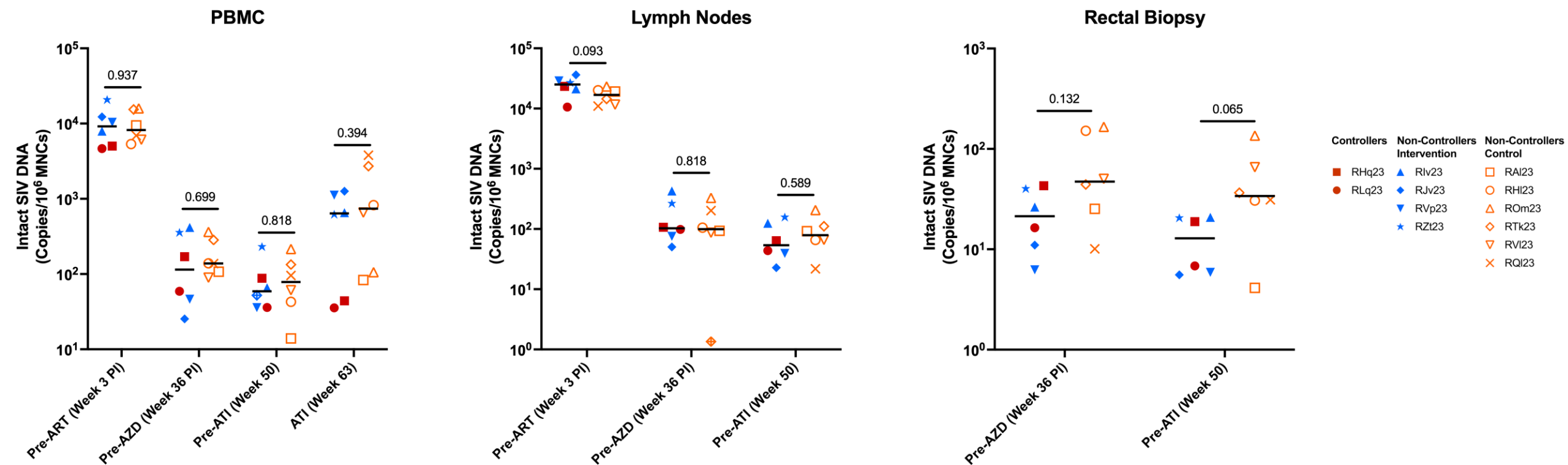
Supplemental Figure 3. Individual serum eCD4-IgG1 concentration and anti-drug antibody kinetics in intervention animals. (A–J) Longitudinal serum eCD4-IgG1 concentration (µg/mL; green, left y-axis) and anti-drug antibody (ADA) responses (anti-eCD4-IgG1 reactivity, OD at 450 nm; right y-axis) for each individual animal receiving AAV9-eCD4-IgG1, plotted as a function of weeks post-SIV infection. The dashed vertical line indicates the time of AAV9-eCD4-IgG1 administration. Each panel represents a single animal. ADA, anti-drug antibody; OD, optical density.



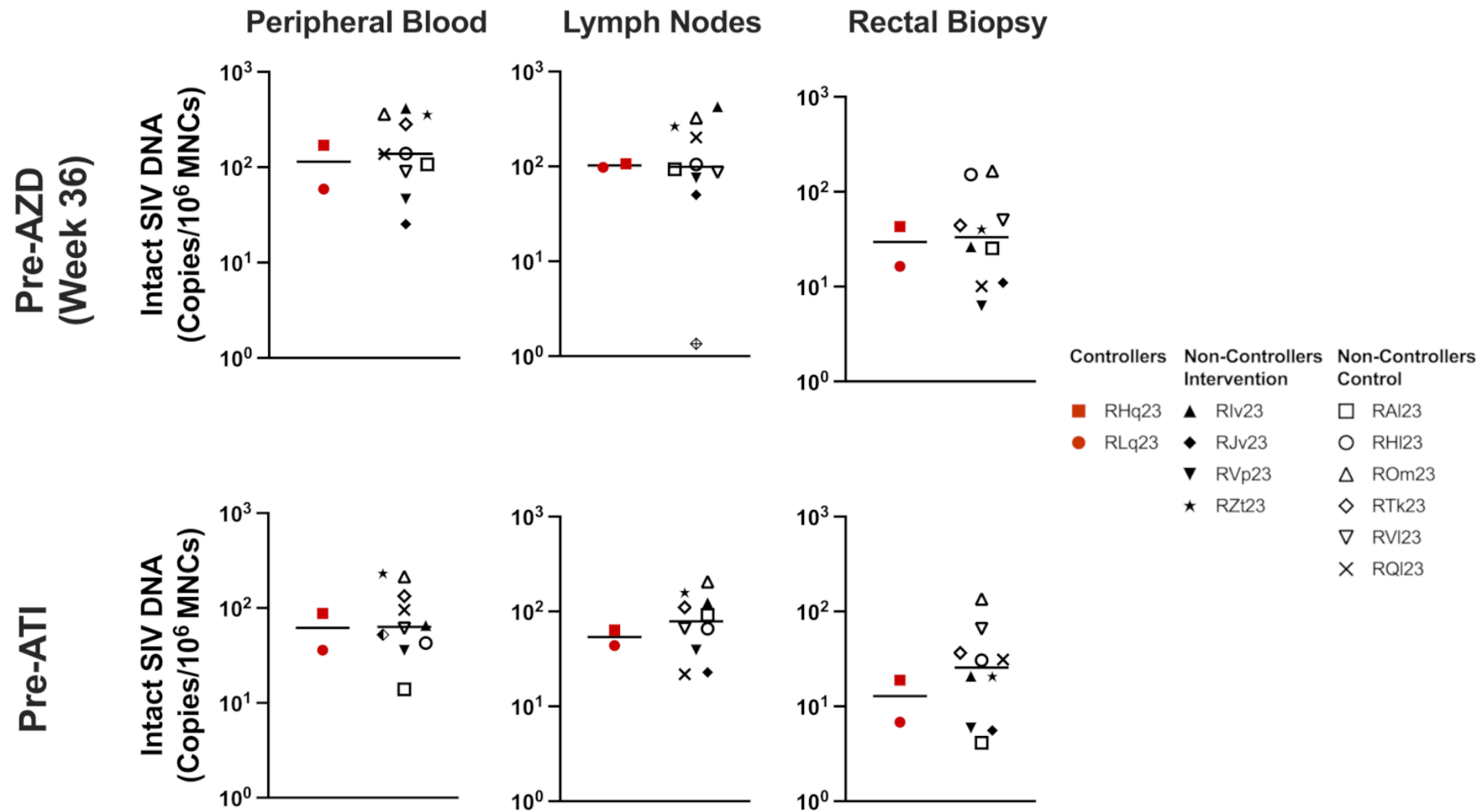
Supplemental Figure 4. Individual plasma viral load and serum eCD4-IgG1 concentration kinetics in intervention animals. (A–J) Longitudinal plasma SIV RNA levels (copies/mL, log_{10} scale; blue, left y-axis) and serum eCD4-IgG1 concentration ($\mu\text{g/mL}$; green, right y-axis) for each individual animal receiving AAV9-eCD4-IgG1, plotted as a function of weeks post-SIV infection. The dashed vertical line indicates the time of AAV9-eCD4-IgG1 administration. Gray shading denotes the period of ART administration. The dotted horizontal line indicates the assay limit of detection (60 copies/mL). Each panel represents a single animal. ART, antiretroviral therapy.



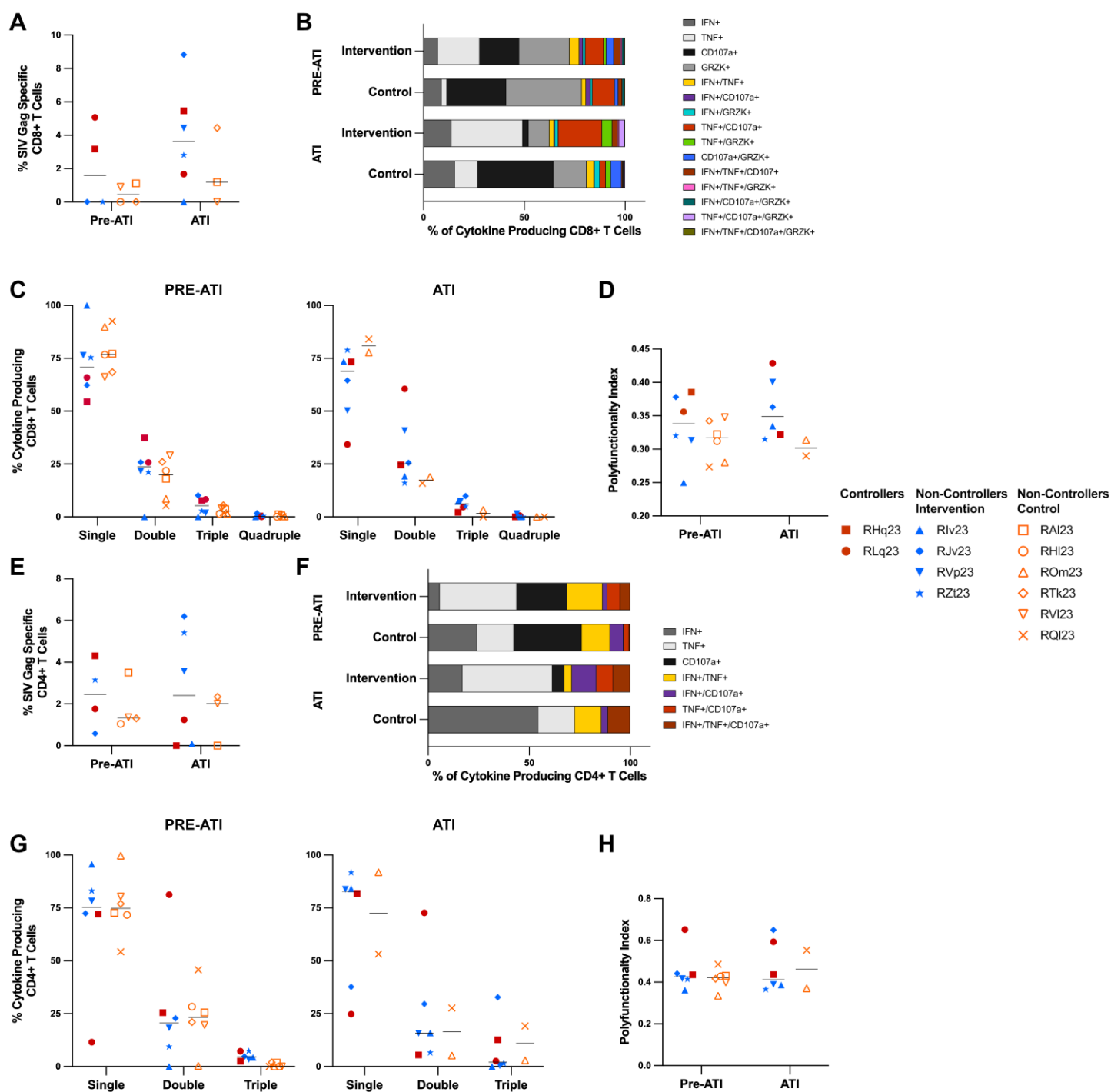
Supplemental Figure 5. Individual plasma viral load kinetics in control animals. (A–J) Longitudinal plasma SIV RNA levels (copies/mL, log₁₀ scale; orange) for each individual control animal, plotted as a function of weeks post-SIV infection. Gray shading denotes the period of ART administration. The dotted horizontal line indicates the assay limit of detection (60 copies/mL). Each panel represents a single animal. ART, antiretroviral therapy.



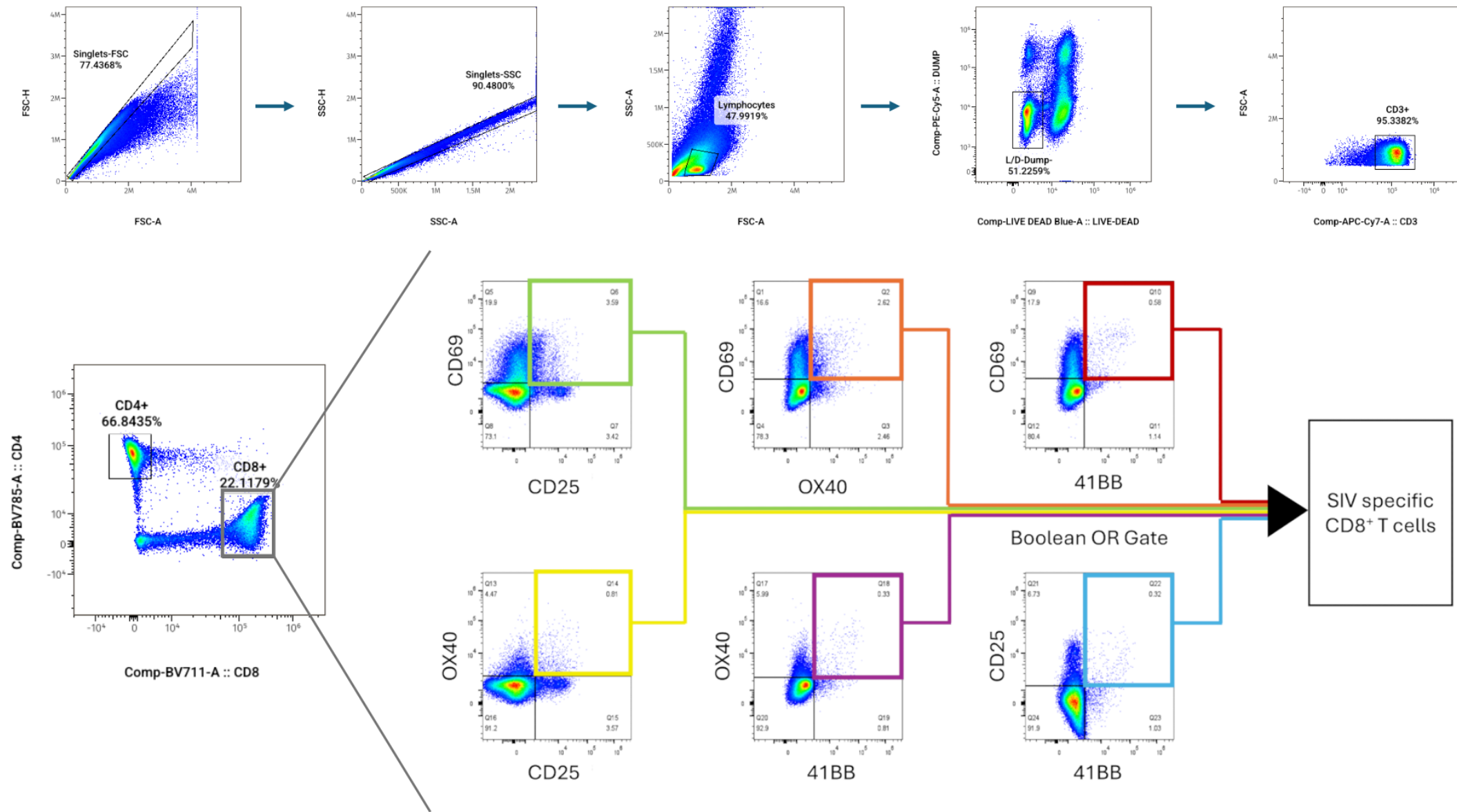
Supplemental Figure 6. Intact proviral DNA levels in intervention and control animals across study timepoints. (A–C) Quantification of intact SIV proviral DNA by IPDA, expressed as copies per 10^6 mononuclear cells, in PBMCs (A), LNMCs (B), and RBMCs (C) from ATI cohort animals ($n = 12$) at pre-ART (week 3 post-infection), pre-AZD5582 (week 36 post-infection), pre-ATI (week 50 post-infection), and during ATI (week 13 post-ATI). Group comparisons between intervention ($n=6$) and control ($n=6$) animals at each timepoint were performed using two-tailed Mann-Whitney U tests; exact P values are shown. Controllers are shown as red symbols, intervention non-controllers as filled blue symbols, and control non-controllers as open orange symbols. Each symbol represents an individual animal and symbol shapes are identified in the figure key. Symbol colors denote subsequent controller classification and were not used for grouping. ATI, analytical treatment interruption; ART, antiretroviral therapy; IPDA, intact proviral DNA assay; LNMC, lymph node mononuclear cells; PBMC, peripheral blood mononuclear cells; RBMC, rectal biopsy mononuclear cells.



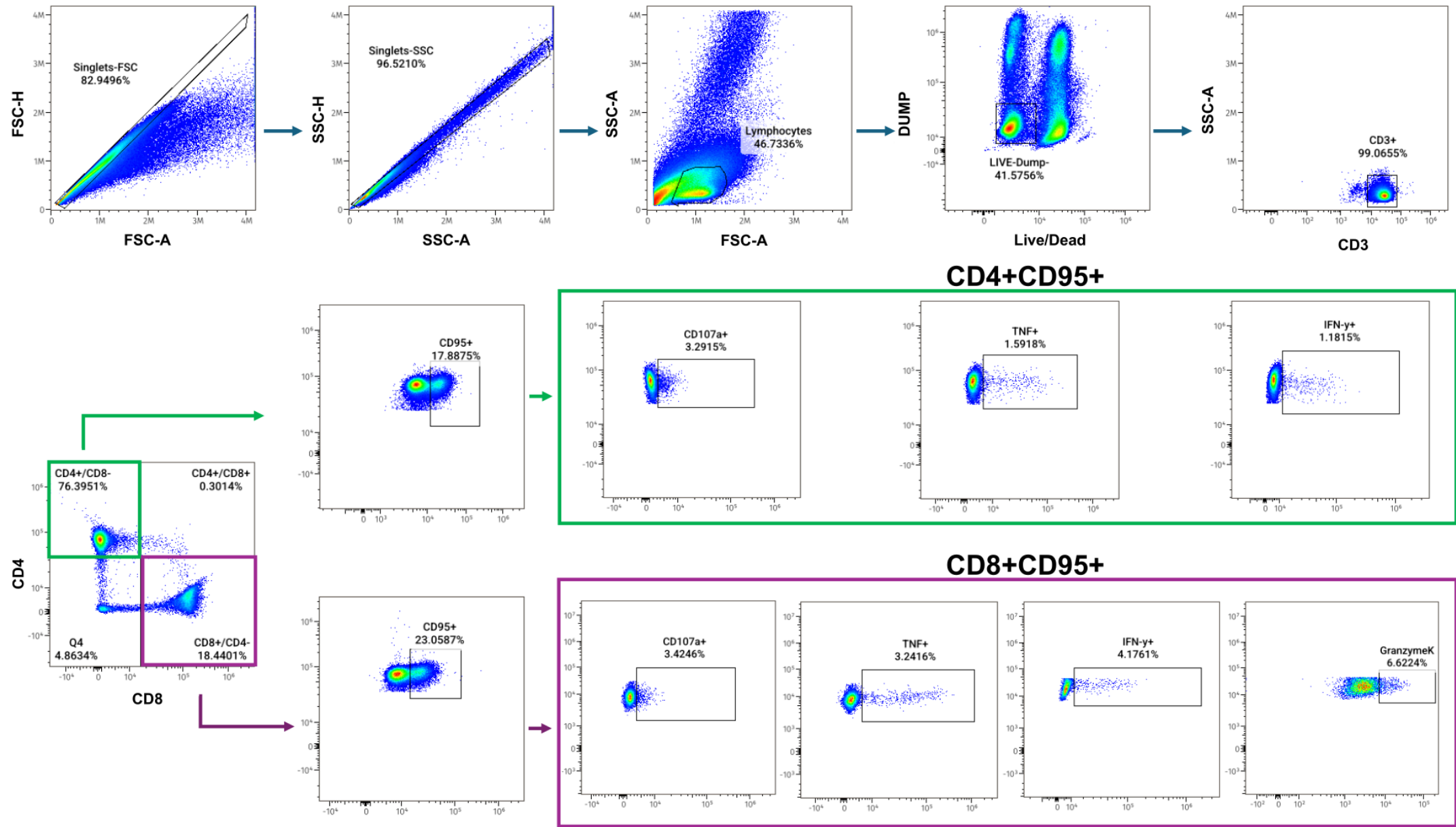
Supplemental Figure 7. Intact proviral DNA levels in controllers and non-controllers at pre-AZD5582 and pre-ATI timepoints. Quantification of intact SIV proviral DNA by IPDA, expressed as copies per 10^6 mononuclear cells, in PBMCs (left), LNCs (center), and RBMCs (right) at pre-AZD5582 (week 36 post-infection; top) and pre-ATI (week 50 post-infection; bottom) timepoints. Data are shown for ATI cohort animals only (controllers, $n = 2$; non-controllers, $n = 10$). Given the small number of controllers ($n = 2$), results should be interpreted descriptively. Controllers are shown as red symbols; non-controllers from the intervention group are shown as filled black symbols and those from the control group as open black symbols. Each symbol represents an individual animal and symbol shapes are identified in the figure key. ATI, analytical treatment interruption; IPDA, intact proviral DNA assay; LNC, lymph node mononuclear cells; PBMC, peripheral blood mononuclear cells; RBMC, rectal biopsy mononuclear cells.



Supplemental Figure 8. SIV-specific T-cell frequency and functional quality in intervention and control animals. (A) Frequency of SIVmac239 Gag-specific CD8⁺ T-cells measured by activation-induced marker (AIM) assay at pre-ATI and ATI timepoints in intervention ($n = 6$) and control ($n = 6$) animals. **(B)** Mean distribution of 15 Boolean combinations of IFN- γ , TNF- α , CD107a, and granzyme K among function-positive Gag-specific CD8⁺ T-cells by group and timepoint. Values are means of individual-animal proportions and sum to 100%. **(C)** Proportions of single-, double-, triple-, and quadruple-cytokine-producing SIVmac239 Gag-specific CD8⁺ T-cells compared between intervention and control groups at pre-ATI (left) and ATI (right). The y-axis indicates the proportion of cytokine-producing cells and the x-axis indicates the functional combination. **(D)** Polyfunctionality index of SIVmac239 Gag-specific CD8⁺ T-cells compared between intervention and control groups at pre-ATI and ATI. **(E)** Frequency of SIVmac239 Gag-specific CD4⁺ T-cells measured by AIM assay compared between intervention and control groups at pre-ATI and ATI timepoints. **(F)** Mean distribution of 7 Boolean combinations of IFN- γ , TNF- α , and CD107a among function-positive Gag-specific CD4⁺ T-cells by group and timepoint. Values are means of individual-animal proportions and sum to 100%. **(G)** Proportions of single-, double-, triple-, and quadruple-cytokine-producing SIVmac239 Gag-specific CD4⁺ T-cells compared between intervention and control groups at pre-ATI (left) and ATI (right). **(H)** Polyfunctionality index of SIVmac239 Gag-specific CD4⁺ T-cells compared between intervention and control groups at pre-ATI and ATI. Horizontal lines indicate the median. Sample size varies by panel due to cell loss during post-cryopreservation stimulation; available samples are shown. Controllers are shown as red symbols, intervention non-controllers as filled blue symbols, and control non-controllers as open orange symbols. Each symbol represents an individual animal and symbol shapes are identified in the figure key. ATI, analytical treatment interruption; AIM, activation-induced marker.



Supplemental Figure 9. Gating strategy for activation-induced marker (AIM) assay. Rhesus macaque PBMC were stimulated for 24 hours with an SIVmac239 Gag peptide pool. Singlets were identified by FSC-A/FSC-H and SSC-A/SSC-H, followed by lymphocyte gating on FSC-A/SSC-A. Viable cells negative for a dump channel (CD14, CD16, CD20) were selected, and CD3⁺ T-cells were divided into CD4⁺ and CD8⁺ subsets. Within CD8⁺ T-cells, AIM⁺ cells were defined as the boolean union of all six pairwise double-positive combinations of CD25, CD69, OX40, and 41BB, corresponding to cells expressing at least two of the four markers; cells in multiple combinations are counted once. Gates were set on paired unstimulated samples. Plots show raw frequencies from a representative stimulated sample; reported AIM⁺ values are background-subtracted. The same strategy was applied to CD4⁺ T-cells.



Supplemental Figure 10. Gating strategy for intracellular cytokine staining. Rhesus macaque PBMC were stimulated for 6 hours with an SIVmac239 Gag peptide pool. Singlets were identified by FSC-A/FSC-H and SSC-A/SSC-H, followed by lymphocyte gating on FSC-A/SSC-A. Viable cells negative for a dump channel (CD14, CD16, CD20) were selected, and CD3⁺ T-cells were divided into CD4⁺ and CD8⁺ subsets. Antigen-experienced cells were defined as CD95⁺. Responses were assessed as CD107a⁺, TNF⁺, and IFN- γ ⁺ within CD4⁺CD95⁺ cells, and CD107a⁺, TNF⁺, IFN- γ ⁺, and granzyme K⁺ within CD8⁺CD95⁺ cells. Gates were set on paired unstimulated samples. Values indicate frequency of the parent population

Supplemental Table 1. Individual Animal Pre-Intervention Characteristics

Animal	Cohort	Group	Sex	Age of Infection (Weeks)	Number of PO Challenges Needed for Infection	MAMU A*01 Allele	Peak Plasma Viral Load (Log₁₀)	Pre-ART Plasma Viral Load (Log₁₀)
RBi23	Dose Optimization	Intervention	F	5.3	1	Positive	7.72	7.34
RCj23	Dose Optimization	Intervention	M	4.4	1	Negative	6.93	6.72
RCu23	Dose Optimization	Intervention	M	3.4	1	Negative	8.30	7.98
RTp23	Dose Optimization	Intervention	F	5.0	1	Negative	7.59	7.53
Rlo23	Dose Optimization	Control	F	4.9	1	Positive	8.17	7.25
RJo23	Dose Optimization	Control	M	5.0	1	Negative	8.07	7.28
RNm23	Dose Optimization	Control	M	4.9	1	Positive	7.22	7.22
RUo23	Dose Optimization	Control	M	12.4	6	Negative	7.72	6.40
RHq23	Analytical Treatment Interruption	Intervention	M	4.6	1	Negative	7.86	6.73
Rlv23	Analytical Treatment Interruption	Intervention	M	4.3	1	Positive	7.85	7.21
RJv23	Analytical Treatment Interruption	Intervention	F	4.3	1	Negative	7.35	7.35
RLq23	Analytical Treatment Interruption	Intervention	F	4.4	1	Positive	7.55	6.42
RVp23	Analytical Treatment Interruption	Intervention	M	4.9	1	Negative	7.14	6.92
RZt23	Analytical Treatment Interruption	Intervention	F	12.9	7	Negative	7.51	7.51
RAI23	Analytical Treatment Interruption	Control	M	4.6	1	Positive	7.42	7.16
RHI23	Analytical Treatment Interruption	Control	F	4.4	1	Negative	7.84	7.22
ROm23	Analytical Treatment Interruption	Control	F	9.1	4	Positive	7.68	7.34
RQI23	Analytical Treatment Interruption	Control	M	5.4	2	Positive	7.58	7.58
RTk23	Analytical Treatment Interruption	Control	F	5.9	2	Positive	7.33	7.11
RVI23	Analytical Treatment Interruption	Control	F	6.7	1	Negative	7.75	7.53

Supplemental Table 2. Adverse Events in Dose Optimization Cohort

Animal	Dose (mg/kg)	Number of Doses Received	Clinical Findings	Laboratory Findings	Disposition	Pathology Findings
RBi23	0.3	5	Respiratory distress, hyperthermia, hypotension during administration of doses 3-5, managed with steroids and supplemental oxygen. Post-treatment emesis and diarrhea after dose 4. Unresponsive respiratory failure during last dose leading to euthanasia	AST elevation, lymphopenia, CK and Creatinine elevations	Euthanasia	Inflammation, necrosis and apoptosis of the liver, lungs and colon.
RCu23	0.25	5	Respiratory distress, hyperthermia, hypotension during administration of last dose that did not respond to management with steroids or supplemental oxygen.	AST elevation, lymphopenia, CK and Creatinine elevations	Euthanasia	Intestinal hemorrhages with associated necrosis, and multifocal interstitial pneumonia.
RTp23	0.25	5	Respiratory distress, hypotension during administration of last dose, responsive to steroids and supplemental oxygen. Hematochezia post treatment leading to euthanasia.	AST elevation, lymphopenia, CK and Creatinine elevations	Euthanasia	Intestinal hemorrhages with associated necrosis, and multifocal interstitial pneumonia. Bacterial dissemination, secondary to translocation
RCj23	0.3	7	Respiratory distress, hyperthermia, hypotension during administration of doses 3-5, managed with steroids. Severe respiratory distress on dose 7 leading to treatment discontinuation.	AST elevation, lymphopenia, CK and Creatinine elevations	Treatment Stopped	N/A

AST, aspartate aminotransferase; CK, creatine kinase; N/A, Non-Applicable.

Supplemental Table 3. eCD4-IgG1 Pharmacokinetic Properties

Animal ID	Cmax (µg/mL)	Time to Cmax (Weeks)	Concentration at Last Timepoint (µg/mL)	Time to First Detectable (Weeks)	Geometric Mean 3 WPI – Last Timepoint (µg/mL)	Geometric Mean 3 WPI – Plateau 17 WPI (µg/mL)	Geometric Plateau 17 WPI – Last Timepoint (µg/mL)
RBi23	40.80	8.00	10.10	4.00	14.35 (1.57)	19.64 (1.73)	12.00 (1.30)
RCj23	20.10	51.00	3.20	6.00	4.01(1.56)	3.63 (1.51)	4.19 (1.58)
RCu23	29.40	22.00	13.50	4.00	15.81 (1.34)	13.35 (1.42)	17.31 (1.24)
RHq23	29.30	18.00	5.10	5.00	11.38 (1.65)	17.22 (1.41)	10.39 (1.62)
Rlv23	48.30	45.00	27.00	4.00	27.86 (1.32)	31.20 (1.45)	27.11 (1.28)
RJv23	11.10	32.00	3.10	6.00	4.88 (1.64)	3.61 (1.42)	5.15 (1.65)
RLq23	19.20	36.00	5.20	4.00	8.43 (1.61)	5.50 (1.99)	9.38 (1.41)
RTp23	14.80	42.00	2.10	5.00	6.19 (2.34)	3.57 (2.64)	6.73 (2.31)
RVp23	18.40	41.00	7.50	4.00	9.05 (1.76)	5.36 (2.08)	10.40 (1.53)
RZt23	24.90	16.00	5.10	4.00	9.94 (1.82)	7.69 (3.00)	10.58 (1.50)
Mean	23.94	33.67	7.98	4.67	10.84	10.13	11.24
SD	11.67	14.33	7.47	0.84	6.99	9.21	6.69

Supplemental Table 4. Longitudinal Mixed-Effects Modeling of Plasma Viral Load, Weeks 3-36 post-infection

Effect/Component	Num df/Variance	Den df/SD	<i>F</i>	<i>P</i>
Fixed Effects (Type III tests)				
Group	1	99.67	0.82	0.369
Time (Natural cubic spline; df = 4)	4	352.10	534.28	<0.001
Group x Time	4	352.10	2.30	0.059
Random effects (Variance components)				
Animal (Random Intercept)	0.089	0.299		
Residual	0.371	0.609		

Summary of fixed and random effects for log₁₀ plasma viral load from 20 animals (*n* = 380 observations) between weeks 3 and 36 post-infection. Fixed effects were evaluated using Type III tests, with time modeled as a natural cubic spline (df = 4). The group effect assesses the overall difference in viral load between intervention and control animals across the post-ART period, while the group × time interaction evaluates differences in longitudinal trajectories. Random effects include a random intercept to account for between-animal variability and a residual term for within-animal variance. Denominator degrees of freedom were estimated using Satterthwaite’s approximation. Num df, numerator degrees of freedom; Den df, denominator degrees of freedom; SD, standard deviation.

Supplemental Table 5. Comparison of pre-ATI virological parameters between post-treatment controllers and non-controllers

Variable	Controllers (n=2) Median (IQR)	Non-controllers (n=10) median (IQR)	Median fold Difference (Controllers/non-controllers)	Overlap with non-controllers
Reservoir size				
Intact proviral DNA in PBMCs, Week 3 (pre-ART) <i>(IPDA copies per 10⁶ PBMC)</i>	4836.24 (4744.74 – 4927.74)	9936.81 (7184.11 – 14638.18)	2.05x lower	Below non-controller IQR
Intact proviral DNA in lymph nodes, Week 3 (pre-ART) <i>(IPDA copies per 10⁶ LNMC)</i>	16912 (10547 – 23276)	20607 (13758 – 27363)	1.22x lower	Within non-controller range
Intact proviral DNA in PBMCs, Week 36 (pre-AZD5582) <i>(IPDA copies per 10⁶ PBMC)</i>	114.50 (59.05 – 170.0)	138.40 (78.82 – 358.20)	1.21x lower	Within non-controller range
Intact proviral DNA in lymph nodes, Week 36 (pre-AZD5582) <i>(IPDA copies per 10⁶ LNMC)</i>	102.40 (98.18 – 106.7)	99.34 (69.18 – 280.8)	1.03x higher	Within non-controller range
Intact proviral DNA in rectal biopsies, Week 36 (pre-AZD5582) <i>(IPDA copies per 10⁶ RBMC)</i>	26.64 (16.37 – 42.91)	33.27 (10.78 – 165.6)	1.25x lower	Within non-controller range
Intact proviral DNA in PBMCs, pre-ATI <i>(IPDA copies per 10⁶ PBMC)</i>	62.02 (48.99 – 75.04)	63.31 (45.17 – 124.12)	1.02x lower	Within non-controller range
Intact proviral DNA in lymph nodes, pre-ATI <i>(IPDA copies per 10⁶ LNMC)</i>	53.80 (43.76 – 63.84)	78.97 (35.15 – 132.7)	1.47x lower	Within non-controller range
Intact proviral DNA in rectal biopsies, pre-ATI <i>(IPDA copies per 10⁶ RBMC)</i>	12.86 (6.83 – 18.88)	25.69 (5.81 – 43.92)	2.75x lower	Within non-controller range
Plasma viremia burden				
Geometric mean plasma viremia, Weeks 1–3 <i>(log₁₀ copies/mL)</i>	5.73 (5.69 – 5.78)	5.17 (5.00–5.86)	3.63x higher ^B	Within non-controller range
Peak plasma viremia, Weeks 1–3 <i>(log₁₀ copies/mL)</i>	7.73 (7.65 – 7.80)	7.63 (7.37–7.78)	1.26x higher ^B	Within non-controller range
Geometric mean plasma viremia, Weeks 4–36 <i>(log₁₀ copies/mL)</i>	2.18 (2.14 – 2.23)	2.62 (2.42–2.91)	2.75x lower ^B	Below non-controller IQR
Peak plasma viremia, Weeks 4–36 <i>(log₁₀ copies/mL)</i>	5.22 (4.97 – 5.38)	6.24 (5.49–7.05)	10.47x lower ^B	Below non-controller IQR
Interventions				
Geometric mean plasma eCD4-IgG concentration ^A <i>(μg/mL)</i>	7.47 (7.41 – 7.52)	8.49 (6.97–12.87)	1.14x lower	Within non-controller range
Geometric mean AZD5582-phase plasma viremia <i>(log₁₀ copies/mL)</i>	1.64 (1.42 – 1.78)	1.22 (1.03–1.80)	2.63x higher ^B	Within non-controller range
Peak AZD5582-phase plasma viremia <i>(log₁₀ copies/mL)</i>	6.52 (6.45 – 6.57)	6.14 (5.96–6.36)	2.40x higher ^B	Above non-controller IQR

Data for controllers (n=2) are shown as individual animal values; non-controller data are median (IQR). Because only two animals met the controller definition, statistical testing was not performed. The median fold difference is calculated on the linear scale (non-controller median relative to controller median); the final column describes the position of the two controller values relative to the non-controller interquartile range. ^AeCD4-IgG concentrations were compared between controllers (n=2) and intervention non-controllers (n=4); control animals did not receive eCD4-IgG. ^BAbsolute viremia comparison. ATI, analytical treatment interruption; ART, antiretroviral therapy; AZD5582, latency-reversing agent; IPDA, intact proviral DNA assay; PBMC, LNMC, and RBMC, peripheral blood, lymph node, and rectal biopsy mononuclear cells.

Supplemental Table 6. Linear Regression Models of ATI Outcomes

Outcome	Predictor	β	SE	CI 95%	R ²	F	P	n
Geometric mean plasma viremia, ATI	Geometric mean plasma viremia, Weeks 1–3	−0.012	0.034	−0.088 to −0.065	0.012	0.12	0.737	12
	Geometric mean plasma viremia, Weeks 4-36	20.121	26.603	−39.155 to 79.396	0.054	0.57	0.467	12
	Intact proviral DNA in PBMCs at ART initiation (Week 3)	1.931	2.237	−3.053 to 6.914	0.069	0.75	0.408	12
	Geometric mean AZD5582-phase plasma viremia	−64.235	263.964	−652.385 to 523.914	0.006	0.06	0.813	12
	Geometric mean plasma eCD4-IgG concentration	−1332.894	2531.444	−8361.310 to 5695.521	0.065	0.28	0.626	6 ^A
	Intact proviral DNA in PBMCs pre-ATI	−63.018	168.406	−438.251 to 312.214	0.014	0.14	0.716	12
Intact proviral DNA in PBMCs at ATI	Geometric mean plasma viremia, Weeks 1–3	−0.001	0.001	−0.004 to 0.001	0.183	2.25	0.165	12
	Geometric mean plasma viremia, Weeks 4-36	2.104	0.514	0.959 to 3.250	0.626	16.75	0.002^B	12
	Intact proviral DNA in PBMCs at ART initiation (Week 3)	0.018	0.071	−0.140 to 0.177	0.007	0.07	0.800	12
	Geometric mean AZD5582-phase plasma viremia	14.558	6.711	−0.395 to 29.512	0.320	4.71	0.055	12
	Geometric mean plasma eCD4-IgG concentration	−5.703	34.703	−102.055 to 90.649	0.007	0.03	0.877	6 ^A
	Intact proviral DNA in PBMCs pre-ATI	1.110	5.201	−10.479 to 12.699	0.005	0.05	0.835	12

Simple linear regression models examining pre-ATI predictors of virological outcomes following analytical treatment interruption. Each predictor was modeled independently. ^An = 6 for eCD4-IgG concentration comparisons; control group animals did not receive eCD4-IgG. ^BP < 0.05.
ATI, analytical treatment interruption; ART, antiretroviral therapy; β , regression coefficient; SE, standard error; CI, confidence interval; IPDA, intact proviral DNA assay; PBMC, peripheral blood mononuclear cells.

SUPPLEMENTAL METHODS

Animal Husbandry and clinical monitoring

Animals were socially housed indoors on a 12h:12h light-dark cycle, bottle-fed human infant formula (Similac Advance®, Abbott or equivalent), and transitioned at weaning to monkey chow (LabDiet 5038®; Purina) and fresh produce. Animals were monitored daily for clinical status, body weight, and overall health. Animals demonstrating progressive clinical decline, substantial weight loss, or laboratory evidence of organ toxicity were evaluated by veterinary staff and euthanized per institutional guidelines.

Preparation and administration of AZD5582

AZD5582–2HCl (ChemieTek) was dissolved at 0.4 mg/mL in sterile distilled water (Gibco Life Technologies) containing 10% Captisol (sulfobutyl ether β -cyclodextrin sodium salts; CyDex Pharmaceuticals), prepared fresh for each administration, and administered by slow intravenous infusion over approximately 45 minutes through an inline filter, as previously described (15).

Sample processing

Blood was collected into EDTA tubes; plasma was separated by centrifugation (800g, 15 minutes) and stored at -80°C . Lymph nodes were mechanically disrupted to remove connective tissue and passed through a 70 μm cell strainer. Mononuclear cells from blood and lymph node were isolated by density gradient centrifugation using Lymphocyte Separation Media (Corning). Rectal biopsies were enzymatically digested for 2 hours at 37°C in R10 medium containing collagenase I (0.75 mg/mL; Sigma-Aldrich) and DNase I (1.5 U/mL; Roche) supplemented with CaCl_2 , passed through a 70 μm cell strainer, and mononuclear cells isolated on a discontinuous Percoll gradient, with 30% Percoll in PBS layered over 60% Percoll in RPMI, centrifuged at 1,000g for 30 minutes at 4°C with low acceleration and no brake; cells at the interface were collected and washed in PBS. Mononuclear cells from all three compartments were counted, cryopreserved in fetal bovine serum containing 10% DMSO using a controlled-rate freezing container (Mr. Frosty, Thermo Fisher Scientific), and stored in liquid nitrogen.

Quantification of plasma eCD4-IgG1 and anti-drug antibodies

384-well high-binding plates (Corning #3700) were coated at 4°C overnight with 30 μL of anti-CD4 antibody (Millipore Sigma #SAB4700059) diluted to 1 $\mu\text{g/mL}$ in DPBS lacking Ca^{2+} and Mg^{2+} . The next day, wells were washed twice with PBS containing

0.05% Tween 20 (Teknova #B501-0500) and blocked with 5.0% (w/v) BSA and 0.05% Tween 20 in DPBS (pH 7.4) for 90 minutes at 37°C. Wells were aspirated and loaded with 50 µL of plasma samples serially diluted in blocking buffer alongside standard curves consisting of similarly diluted macaque serum spiked with recombinant eCD4-IgG1 and incubated for 1.5 hours. Wells were washed five times and incubated for 1 hour with 50 µL of HRP-conjugated anti-human Fcγ Fab fragment (Jackson Immuno #109-036-008) diluted 1:20,000 in DPBS lacking Ca²⁺ and Mg²⁺. Wells were washed 10 times, developed with 50 µL of TMB substrate (Thermo Fisher #34028), and neutralized with 50 µL of Liquid Stop Solution for TMB (Surmodics #LTSP-1000-01). Absorbance at 450 nm was recorded on a BioTek Synergy LX plate reader. Anti-eCD4-IgG1 antibodies were measured in the same format with plates coated with recombinant eCD4-IgG1 protein at 1 µg/mL.

Flow cytometry antibody panel

Viability: LIVE/DEAD Fixable Blue (Invitrogen). Dump channel (PE-Cy5): CD20 (2H7), CD16 (3G8), CD14 (M5E2), all BioLegend. Surface: CD3 (APC-Cy7, SP34-2; BD), CD4 (BV785, OKT4; BioLegend), CD8α (BV711, SK1; BioLegend), CD95 (BV605, DX2; BioLegend). AIM assay: CD25 (PE-Vio770, REA570; Miltenyi), CD69 (BV421, FN50; BioLegend), OX40 (PE, L106; BD), 4-1BB (APC, REA765; Miltenyi). ICS assay: CD107a (VioBlue, H4A3; Miltenyi), IFN-γ (PE, 4S.B3; BioLegend), TNF-α (BV650, MAb11; BD), granzyme K (eFluor 660, G3H69; Thermo Fisher).