

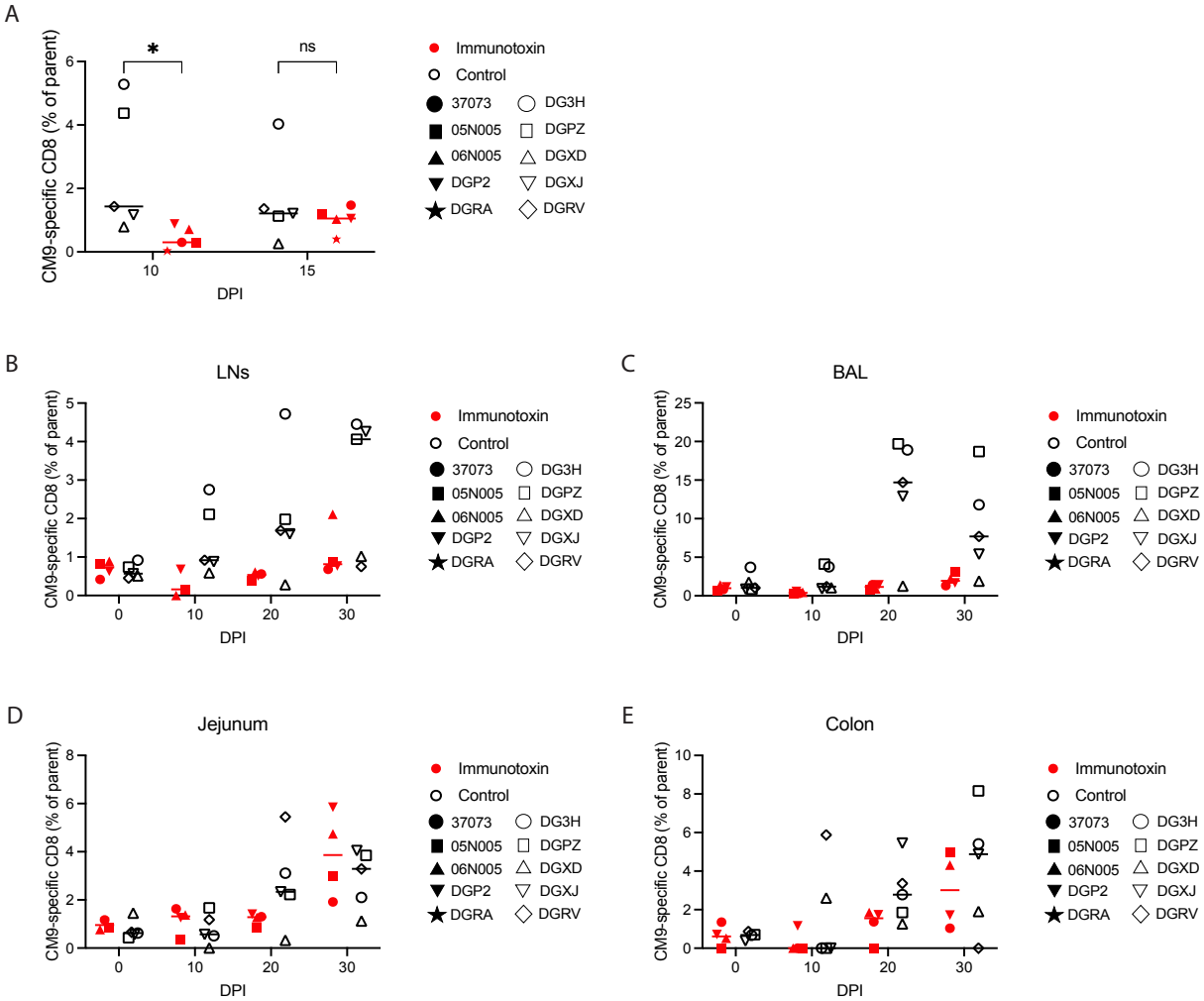
SUPPLEMENTAL MATERIAL

Immunotoxin-mediated depletion of Gag-specific CD8⁺ T cells undermines natural control of SIV

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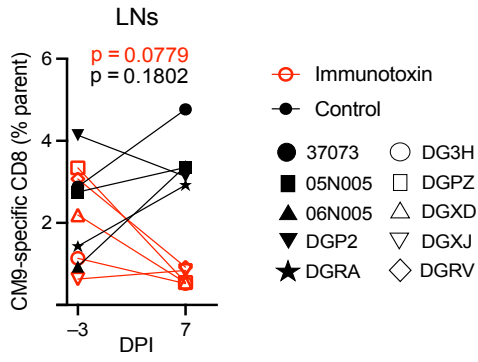
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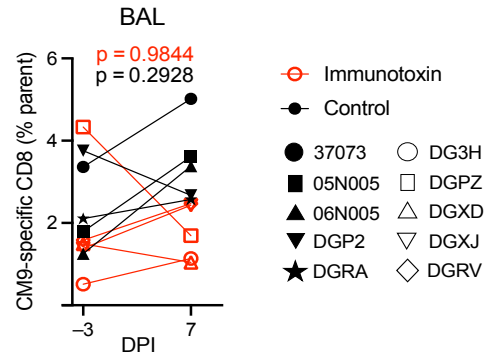
Supplemental Figure 1. Immunotoxin administration does not alter CM9-specific CD8⁺ T cell frequencies in tissues during acute infection with SIV. Experimental details as in **Figure 2**. **(A)** CM9-specific CD8⁺ T cell frequencies among PBMCs (parent = CD8⁺ T cells). **(B)** CM9-specific CD8⁺ T cell frequencies in LNs (parent = CD8⁺ T cells). **(C)** CM9-specific CD8⁺ T cell frequencies in BAL (parent = CD8⁺ T cells). **(D)** CM9-specific CD8⁺ T cell frequencies in jejunum (parent = CD8⁺ T cells). **(E)** CM9-specific CD8⁺ T cells in colon (parent = CD8⁺ T cells). Each symbol represents one macaque (A–E). Horizontal bars indicate median values (A–E). Significance was determined using a two-way ANOVA with Šídák correction. DPI, days post-infection; ns, not significant.

Supplemental Figure 2. Immunotoxin administration does not modulate germline contributions to the clonotypic repertoire of CM9-specific CD8⁺ T cells during acute infection with SIV. Experimental details as in **Figure 2.** **(A)** Heatmap analysis of TRBV segment use among CM9-specific CD8⁺ T cell repertoires from control and immunotoxin-treated macaques. **(B)** Heatmap analysis of TRBJ segment use among CM9-specific CD8⁺ T cell repertoires from control and immunotoxin-treated macaques. DPI, days post-infection.

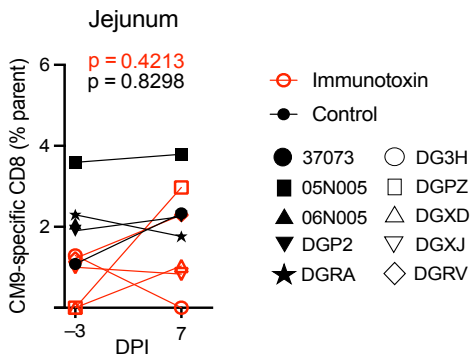
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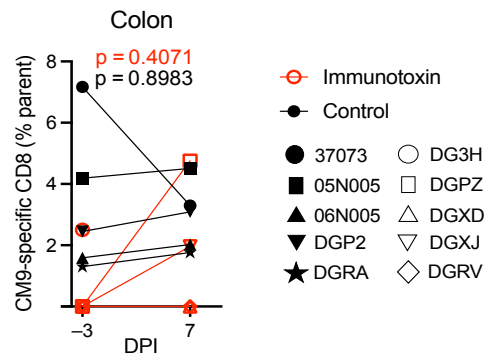
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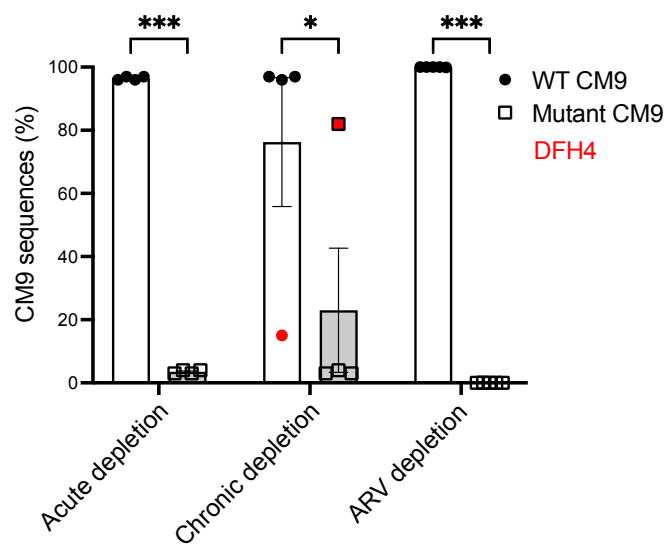
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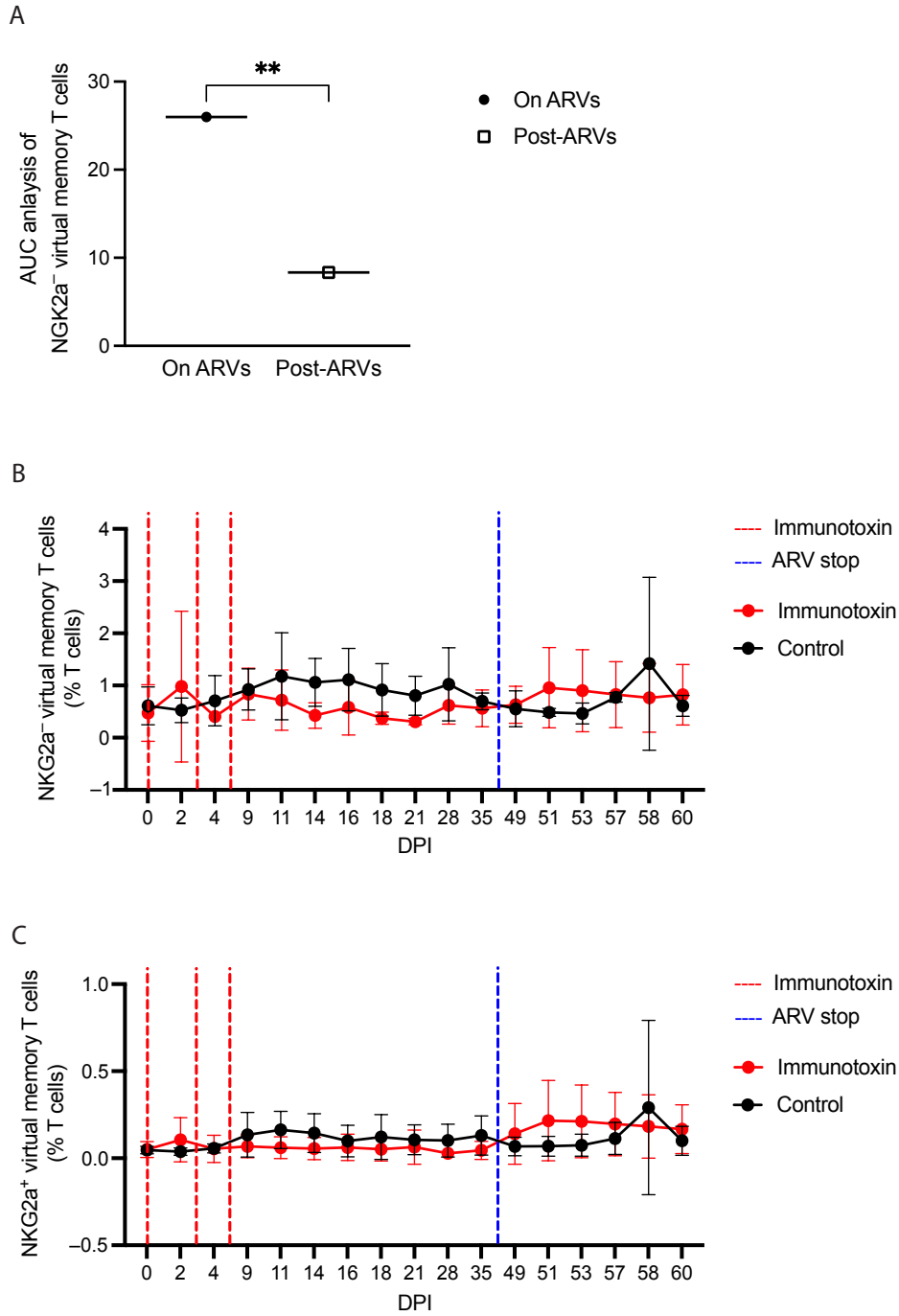
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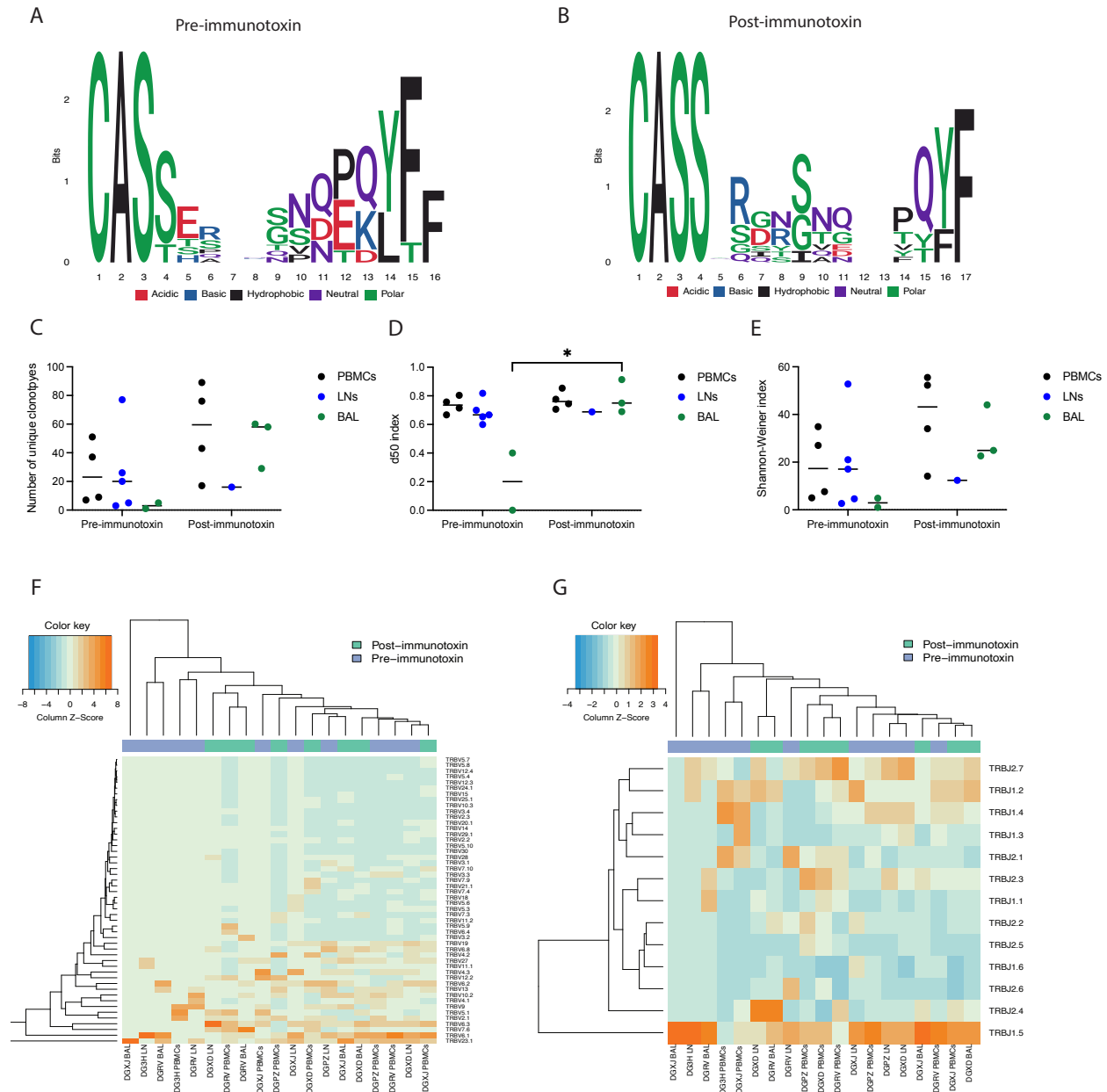


Supplemental Figure 3. Immunotoxin administration does not alter CM9-specific CD8⁺ T cell frequencies in tissues during treatment with ARVs. Experimental details as in **Figure 4**. **(A)** CM9-specific CD8⁺ T cell frequencies in LNs (parent = CD8⁺ T cells). **(B)** CM9-specific CD8⁺ T cell frequencies in BAL (parent = CD8⁺ T cells). **(C)** CM9-specific CD8⁺ T cell frequencies in jejunum (parent = CD8⁺ T cells). **(D)** CM9-specific CD8⁺ T cell frequencies in colon (parent = CD8⁺ T cells). **(E)** Proportions of wildtype (WT) and mutant CM9 epitope sequences identified in plasma samples. Each symbol represents one macaque (A–E). Significance was determined using a paired t test (A–D) or a mixed-effects ANOVA with Šídák correction (E). *p < 0.05, ***p < 0.001. DPI, days post-immunotoxin.



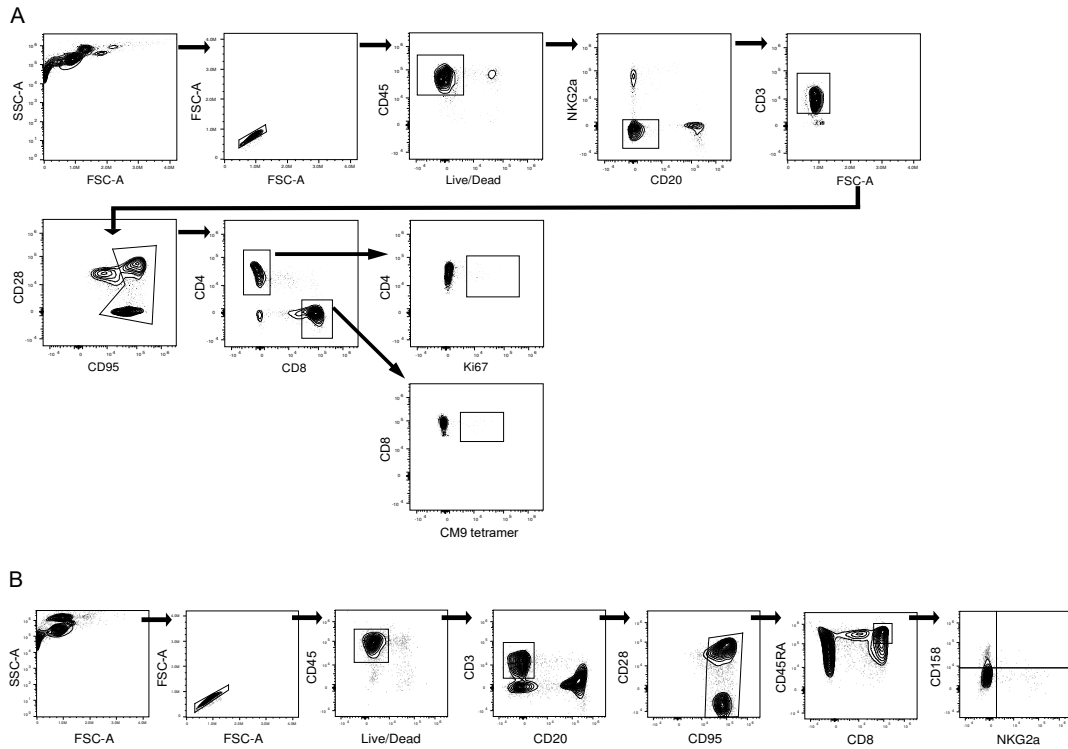
Supplemental Figure 4. T_{VM} cells are present in the circulation during treatment with ARVs. Experimental details as in **Figure 4**. **(A)** AUC analysis of NKG2a⁻ T_{VM} cells from all macaques across all time points during and after treatment with ARVs. **(B)** NKG2a⁻ T_{VM} cell frequencies among all circulating T cells in control and immunotoxin-treated macaques. **(C)** NKG2a⁺ T_{VM} cell

frequencies among all circulating T cells in control and immunotoxin-treated macaques. Horizontal bars indicate mean values (A). Data are shown as mean \pm SD (B, C). Significance was determined using a paired t-test (A) or a two-way ANOVA with Dunnett correction (B, C). **p < 0.01. DPI, days post-immunotoxin.



Supplemental Figure 5. Immunotoxin administration does not modulate the clonotypic architecture of CM9-specific CD8⁺ T cell populations during treatment with ARVs. Experimental details as in **Figure 4**. **(A)** Logo plots and chemical classification of amino acids spanning the CDR3 β loops of the top 10 pooled clonotypes before immunotoxin administration. **(B)** Logo plots and chemical classification of amino acids spanning the CDR3 β loops of the top 10 pooled clonotypes after immunotoxin administration. **(C)** Repertoire diversity measured using

the number of unique clonotypes for CM9-specific CD8⁺ T cell populations isolated before and after immunotoxin administration. **(D)** Repertoire diversity measured using the d50 index for CM9-specific CD8⁺ T cell populations isolated before and after immunotoxin administration. **(E)** Repertoire diversity measured using the Shannon-Weiner index for CM9-specific CD8⁺ T cell populations isolated before and after immunotoxin administration. **(F)** Heatmap analysis of TRBV segment use among CM9-specific CD8⁺ T cell repertoires before and after immunotoxin administration. **(G)** Heatmap analysis of TRBJ segment use among CM9-specific CD8⁺ T cell repertoires before and after immunotoxin administration. Each symbol represents one macaque (C–E). Horizontal bars indicate median values (C–E). Significance was determined using a mixed-effects ANOVA with Šídák correction (C–E). * $p < 0.05$. Plots incorporate all sequences with a frequency of >2%. Post-immunotoxin = day 7 (B–G).



Supplemental Figure 6. Flow cytometric gating strategies. (A) Gating strategy for the identification of memory CD8⁺ T cells, CM9-specific CD8⁺ T cells, memory CD4⁺ T cells, and Ki67⁺ CD4⁺ T cells. **(B)** Gating strategy for the identification of NKG2a⁻ and NKG2a⁺ T_{VM} cells.






Supplemental Table 1. Participant macaques in the acute infection study.

Animal	Sex	Treatment	Symbol
37073	Female	3 × 350 pmol/kg of CM9 tetramer-immunotoxin	●
05N005	Female	3 × 350 pmol/kg of CM9 tetramer-immunotoxin	■
06N005	Female	3 × 350 pmol/kg of CM9 tetramer-immunotoxin	▲
DGP2	Male	3 × 350 pmol/kg of CM9 tetramer-immunotoxin	▼
DGRA	Male	3 × 350 pmol/kg of CM9 tetramer-immunotoxin	★
DG3H	Male	None	○
DGPZ	Male	None	□
DGXD	Male	None	△
DGXJ	Male	None	▽
DGRV	Male	None	◇

Supplemental Table 2. Participant macaques in the ARV study.

Animal	Sex	ARV regimen	Initial plasma VL (copies/mL)	Treatment	Symbol
37073	Female	1mL/kg/day sc [FTC (40 mg/kg) + TDF (5.1 mg/kg) + DTG (2.5 mg/kg)]	Below LOD	None	●
05N005	Female	1mL/kg/day sc [FTC (40 mg/kg) + TDF (5.1 mg/kg) + DTG (2.5 mg/kg)]	Below LOD	None	■
06N005	Female	1mL/kg/day sc [FTC (40 mg/kg) + TDF (5.1 mg/kg) + DTG (2.5 mg/kg)]	Below LOD	None	▲
DGP2	Male	1mL/kg/day sc [FTC (40 mg/kg) + TDF (5.1 mg/kg) + DTG (2.5 mg/kg)]	Below LOD	None	▼
DGRA	Male	1mL/kg/day sc [FTC (40 mg/kg) + TDF (5.1 mg/kg) + DTG (2.5 mg/kg)]	Below LOD	None	★
DG3H	Male	1mL/kg/day sc [FTC (40 mg/kg) + TDF (5.1 mg/kg) + DTG (2.5 mg/kg)]	Below LOD	3 × 350 pmol/kg of CM9 tetramer-immunotoxin	○
DGPZ	Male	1mL/kg/day sc [FTC (40 mg/kg) + TDF (5.1 mg/kg) + DTG (2.5 mg/kg)]	Below LOD	3 × 350 pmol/kg of CM9 tetramer-immunotoxin	□
DGXD	Male	1mL/kg/day sc [FTC (40 mg/kg) + TDF (5.1 mg/kg) + DTG (2.5 mg/kg)]	Below LOD	3 × 350 pmol/kg of CM9 tetramer-immunotoxin	△
DGXJ	Male	1mL/kg/day sc [FTC (40 mg/kg) + TDF (5.1 mg/kg) + DTG (2.5 mg/kg)]	65	3 × 350 pmol/kg of CM9 tetramer-immunotoxin	▽
DGRV	Male	1mL/kg/day sc [FTC (40 mg/kg) + TDF (5.1 mg/kg) + DTG (2.5 mg/kg)]	Below LOD	3 × 350 pmol/kg of CM9 tetramer-immunotoxin	◇

Supplemental Table 3. Participant macaques in the chronic infection study.

Animal	Sex	Initial plasma VL (copies/mL)	Treatment	Symbol
DFH4	Male	1,200,000	1 × 325 pmol/kg of CM9 tetramer-immunotoxin	
DGME	Male	4,400,000	1 × 650 pmol/kg of CM9 tetramer-immunotoxin	
DG2V	Male	9,000,000	1 × 1.3 nmol/kg of CM9 tetramer-immunotoxin	
08D030	Male	5,100	2 × 350 pmol/kg of CM9 tetramer-immunotoxin	
DGT4	Male	940	2 × 350 pmol/kg of CM9 tetramer-immunotoxin	

Supplemental Table 4. Antibodies used for flow cytometry and cell sorting.

Antigen	Fluorochrome	Clone	Supplier	Catalog ID
Ki67	FITC	35/Ki-67	BD Biosciences	556026
CD8	Pacific Blue	RPA-T8	BD Biosciences	558207
NKG2a	PE	Z199	Beckman Coulter	1M3291U
CD28	ECD	CD28.2	Beckman Coulter	6607111
CD45RA	BV750	5H9	BD Biosciences	747465
CD3	PerCP-Cy5.5	SP34-2	BD Biosciences	552852
CD4	BV650	OKT4	BioLegend	317436
CD45	BV786	D058-1283	BD Biosciences	563861
CD95	PE-Cy5	DX2	BioLegend	305610
CD20	APC-H7	2H7	BD Biosciences	560734
CD158	BV711	HP-MA4	BD Biosciences	752507
Live/Dead	Aqua Blue	N/A	Thermo Fisher Scientific	L34957