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Understanding and exploiting CD8^{+} T cells in highly pathogenic SIV infection: Application to vaccine and immunotherapy development

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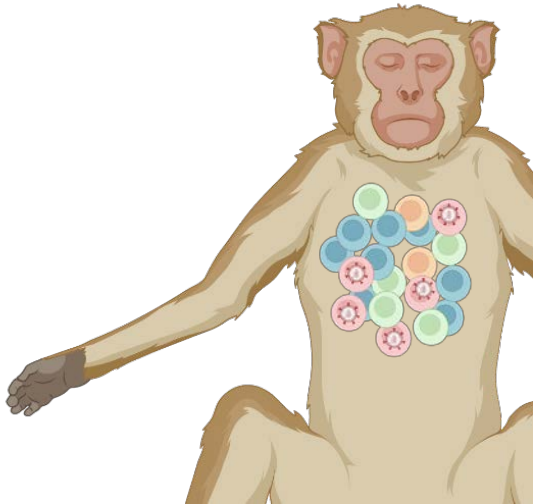
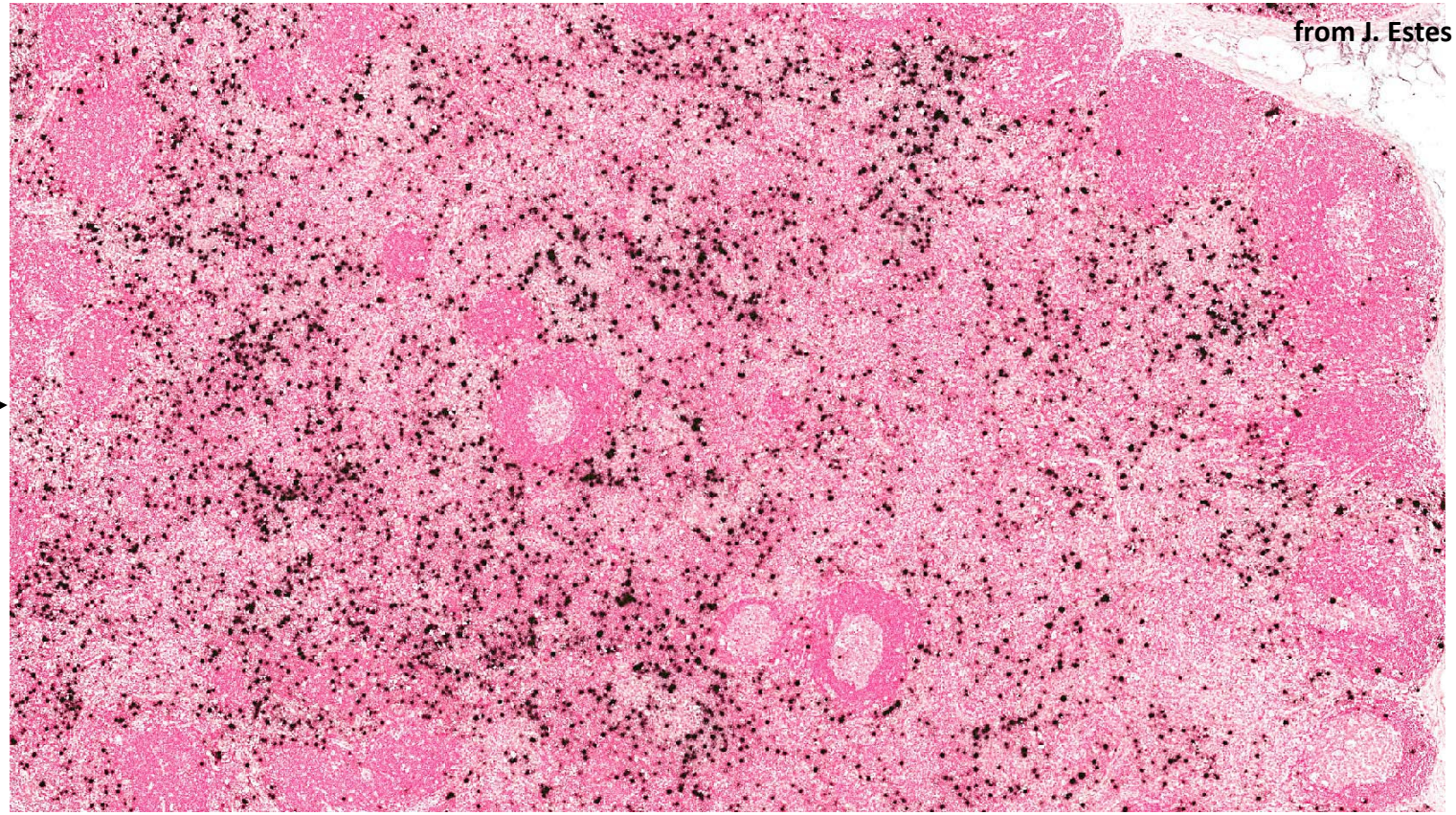


Intrinsic immune evasion mechanisms, rapid, massive viral replication, a high rate of mutation, and functional plasticity make HIV/SIV a formidable opponent for the adaptive immune system, mostly certainly including the usually potent anti-viral $CD8^+$ T cell response.

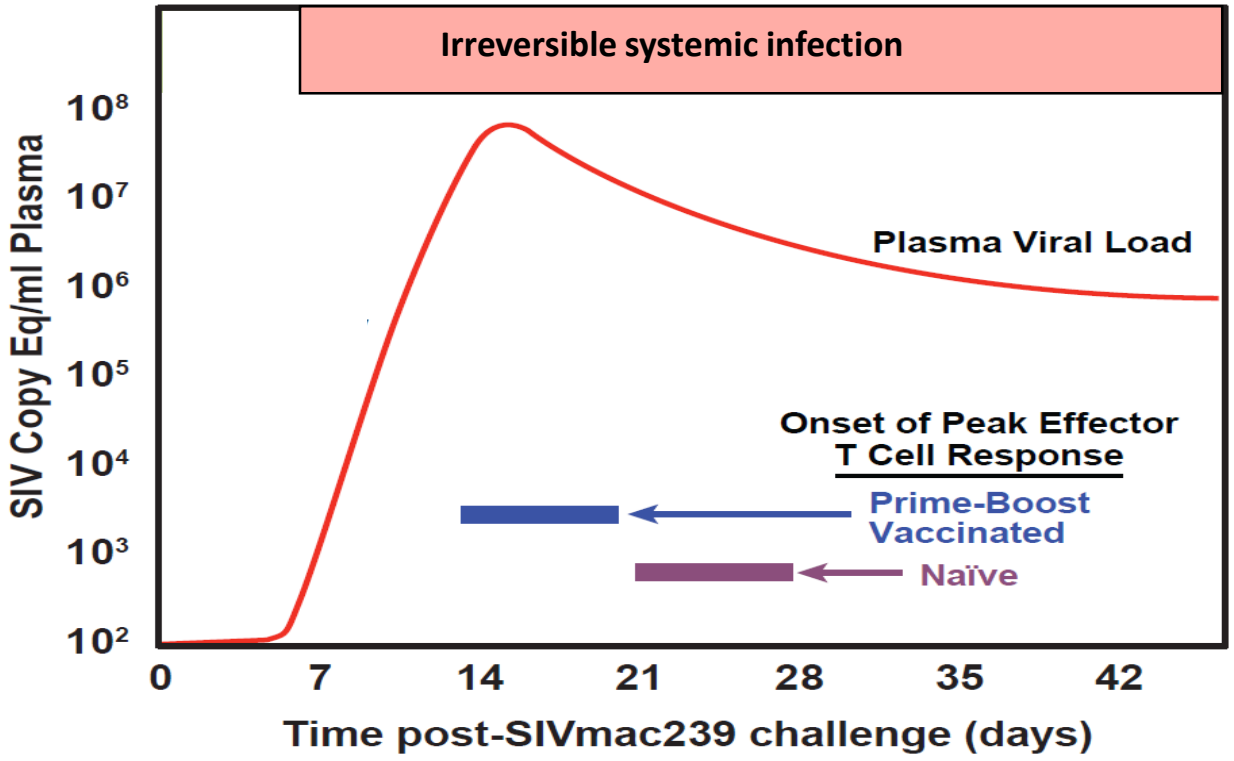
This is what the earliest immune effector response "sees" in primary SIV infection!

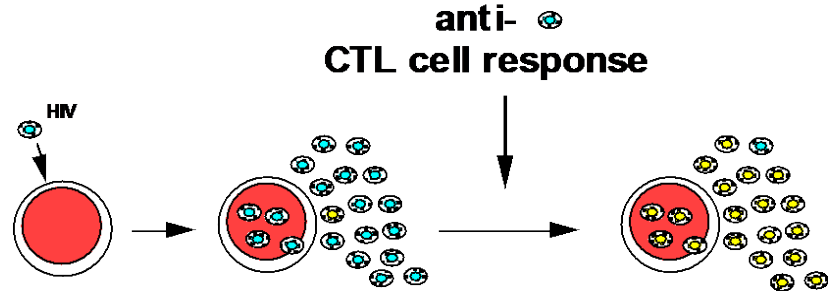


SIVmac239
(day 14 pi)

Weakly anti-viral responses are overwhelmed, and highly effective responses prompt rapid mutational escape, often with little consequence on the subsequent course of infection.

In most infections, adaptive immune responses simply come too little (insufficient effector level/efficiency) and/or too late to efficiently suppress viral replication, let alone clear infection.

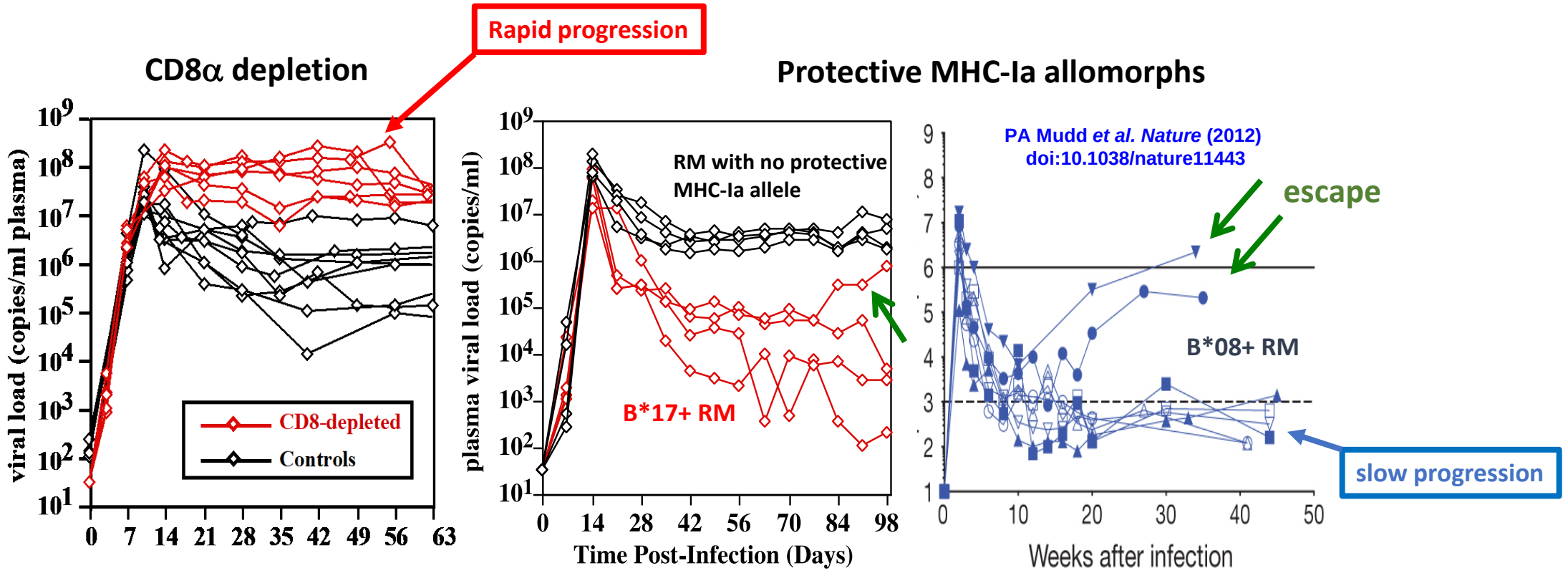




Mutational Immune Escape

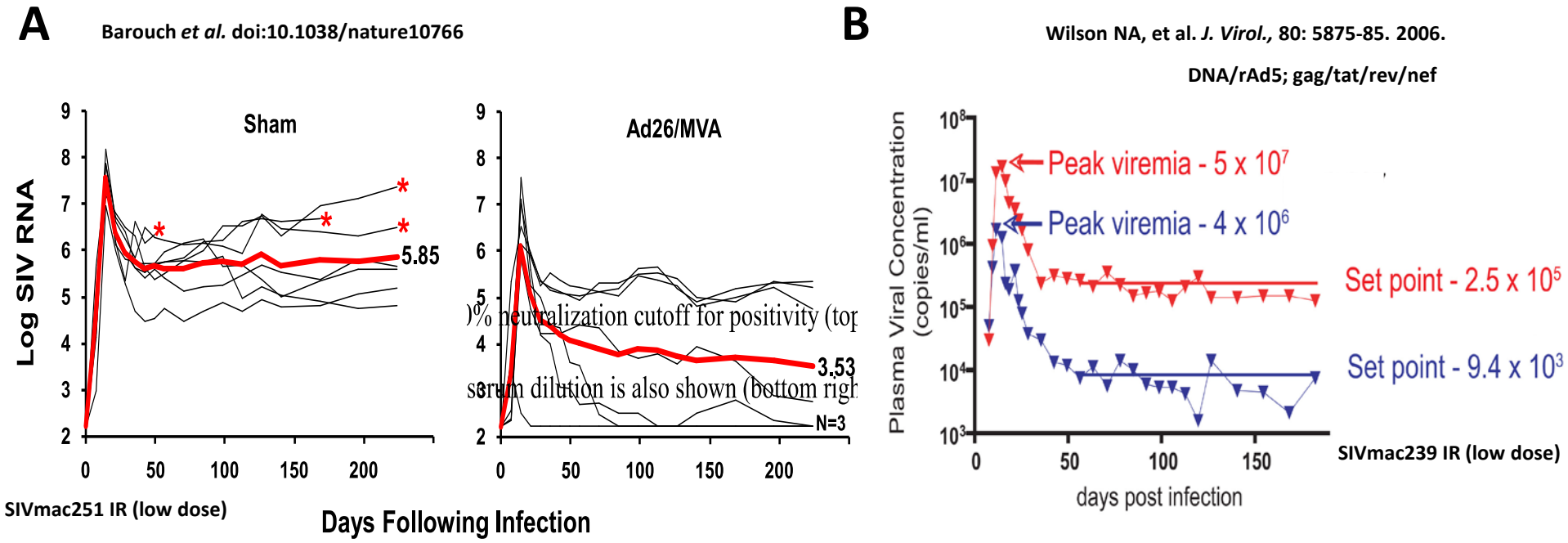
This is even before considering the deleterious effect of HIV/SIV immune pathogenesis ($CD4^+$ T cell destruction) on the virus-specific immune response, and the ability of HIV/SIV to established long-term latency.

However, failure to establish complete viral control or clearance does not mean these immune responses have no effect . . . $CD8^+$ T cell responses ameliorate the tempo of SIV pathogenesis in rhesus macaques and in the right circumstances* mediate substantial viral control:

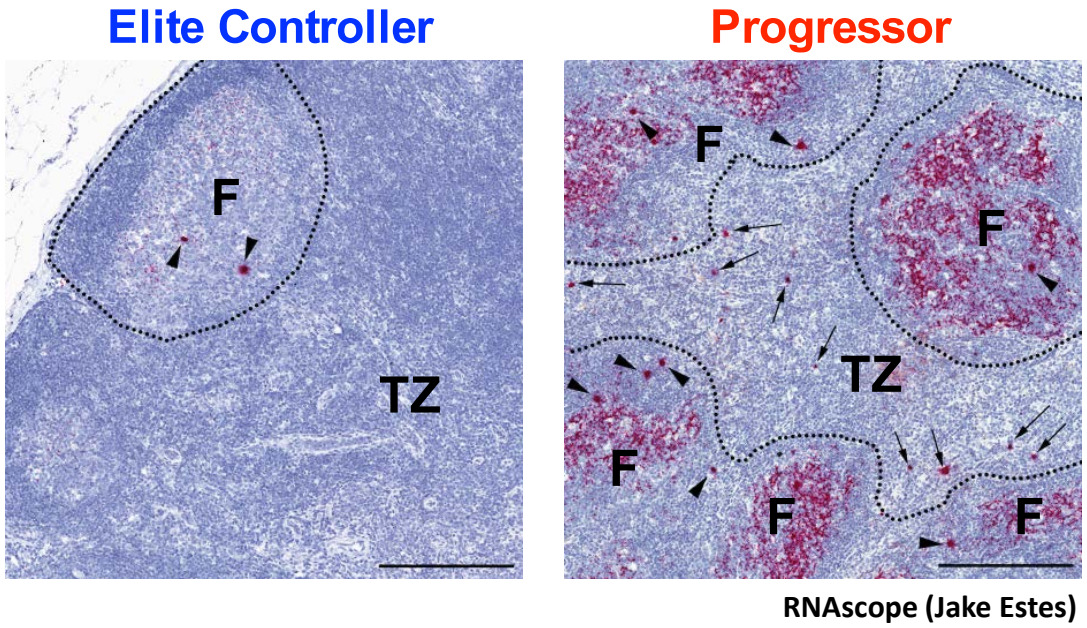


*usually monkeys with "protective" MHC-Ia alleles.

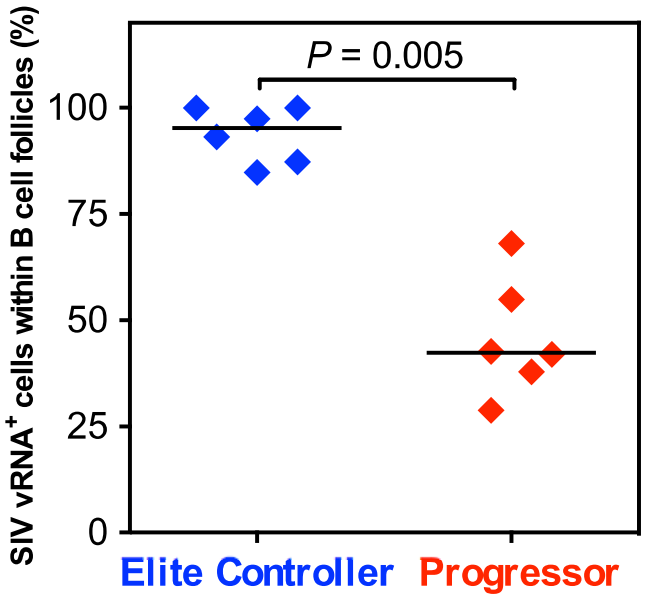
And . . . the best T cell-targeted vaccines can also manifest substantial SIV control, albeit often incomplete and transient:

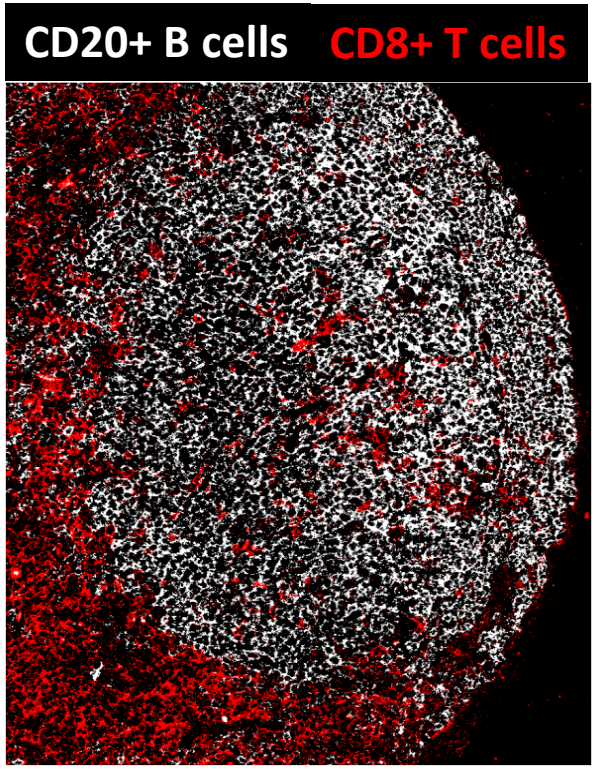


The natural or post-vaccination elite SIV control (pvl < 600 copies/ml) that occurs in a subset of monkeys is perhaps more impressive than one might initially think . . . As almost all the residual viral replication is restricted to B cell follicles, structures that largely exclude effector CD8+ T cells . . . Productive infection is largely cleared from extra follicular zones!



RNAscope (Jake Estes)

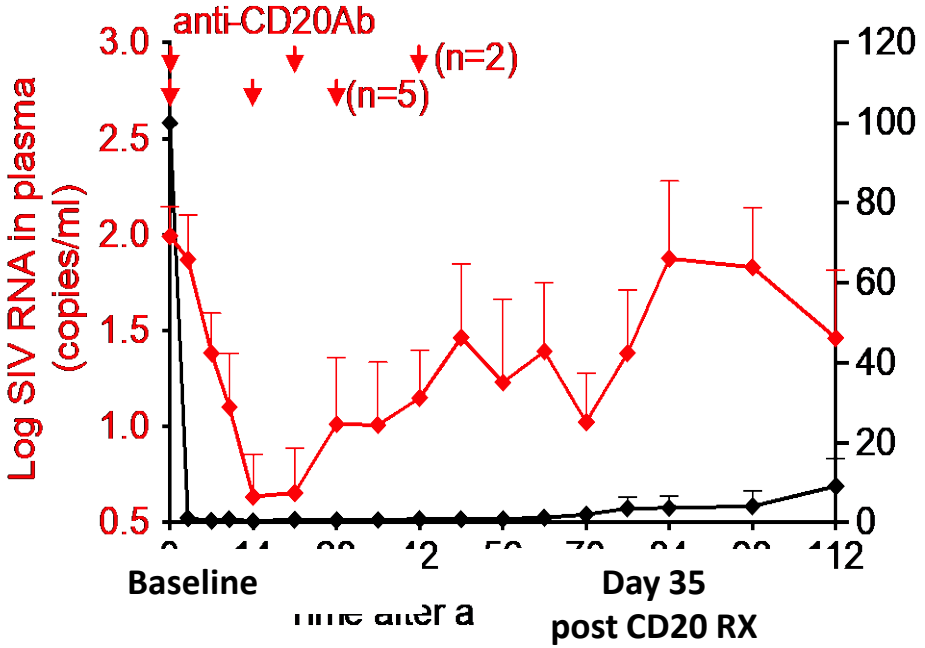


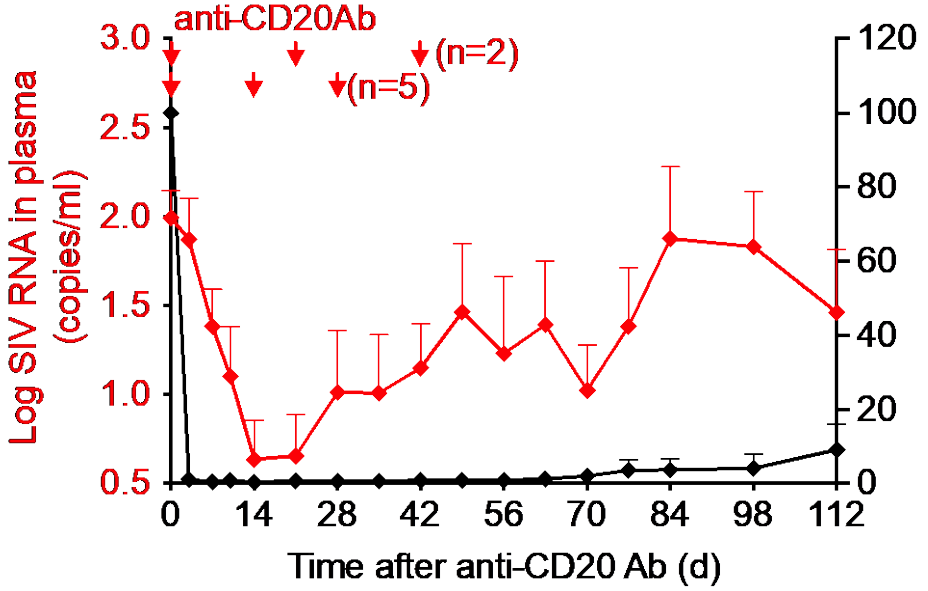


Fukazawa Y, et al. *Nature Medicine*, 21:132-9, 2015. doi:10.1038/nm.3781.

T cell depletion obviates this restriction, releasing productive infection outside of follicles.

And anti-CD20 treatment, which depletes B cells and disrupts B cell follicles . . .





. . . transiently (further) enhances viral control*.

(*monkeys must manifest initial control)

The benefit of natural CD8⁺ T cell responses (primed during primary infection) is even more apparent in monkeys that are virally suppressed during primary infection and later taken off anti-retroviral therapy (ART) - although not able to prevent SIV rebound post-ART, these responses are able to restrict post-ART viral replication setpoints by 1.5 – 2.0 logs relative to untreated primary infection . . .

The Journal of Clinical Investigation RESEARCH ARTICLE

CD8⁺ T cells fail to limit SIV reactivation following ART withdrawal until after viral amplification

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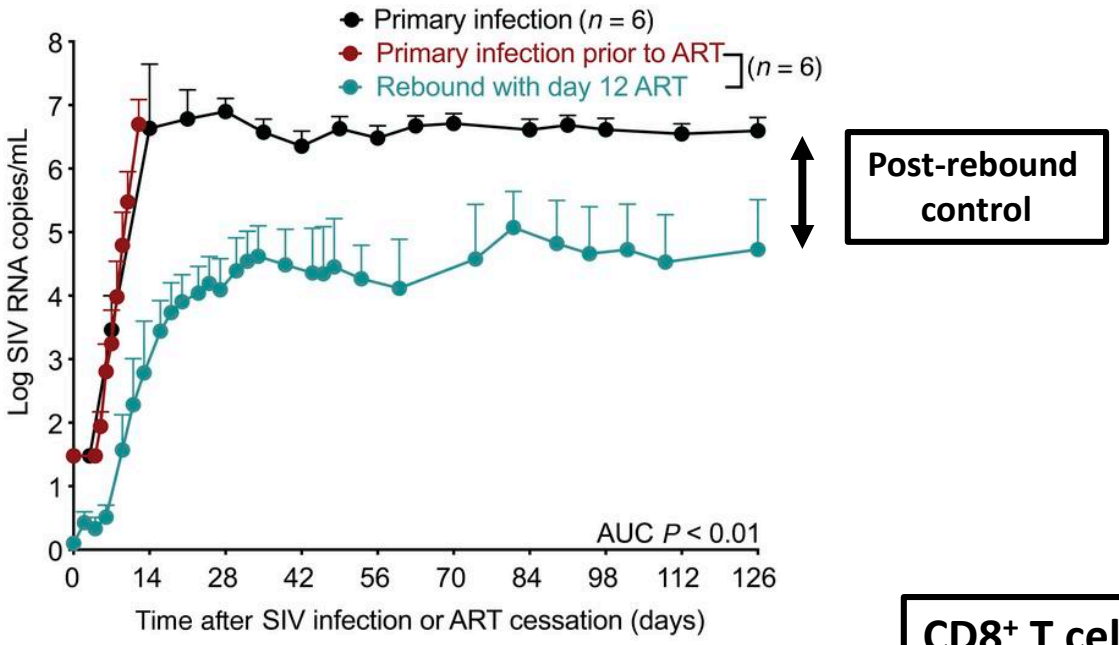
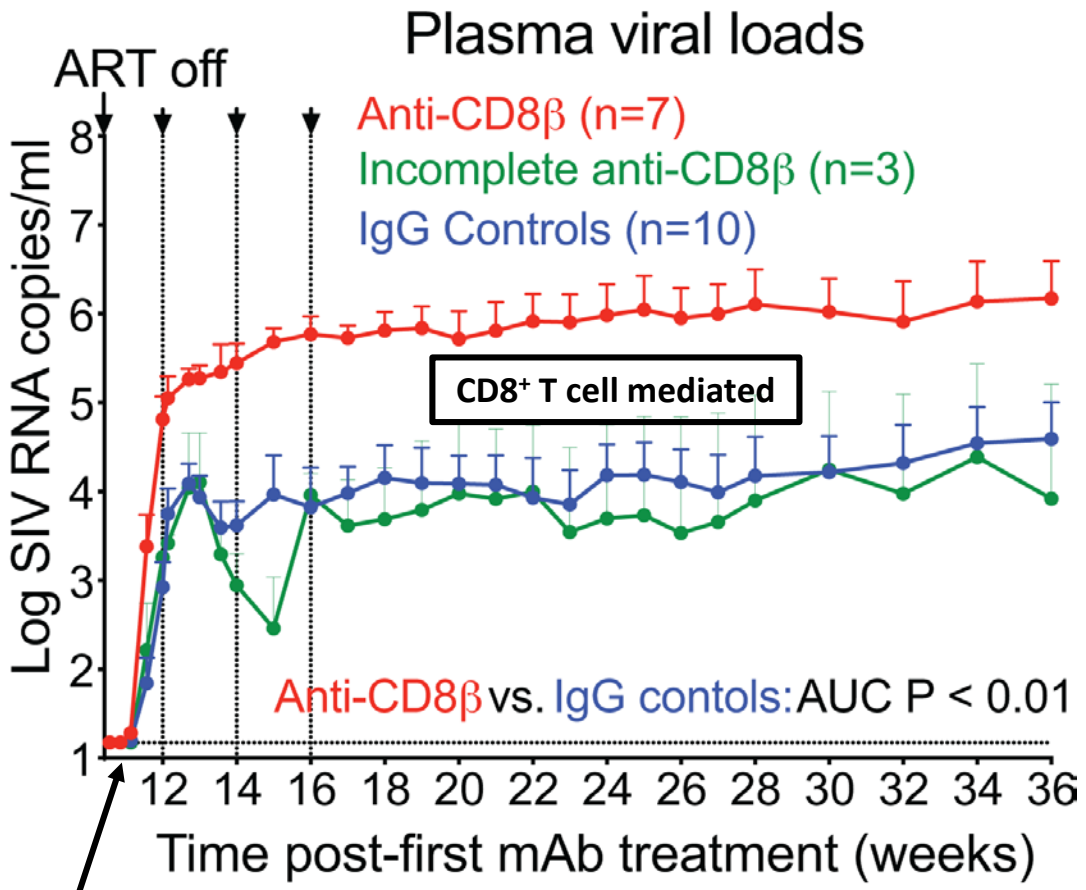




CD8⁺ T cell depletion has no significant effect on early rebound dynamics

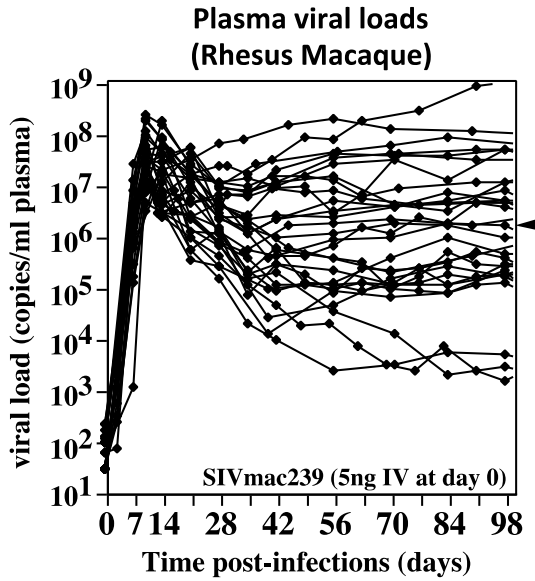
For more than 2 decades, my group has sought to use the Rhesus Macaque (RM) model to understand both the effectiveness and limitations of $CD8^+$ T cell responses against SIV and based on this knowledge, develop vaccine and immunotherapies that are more effective than natural responses.

<u>Today I will present 2 stories on this topic, one mature, and one more nascent</u>:

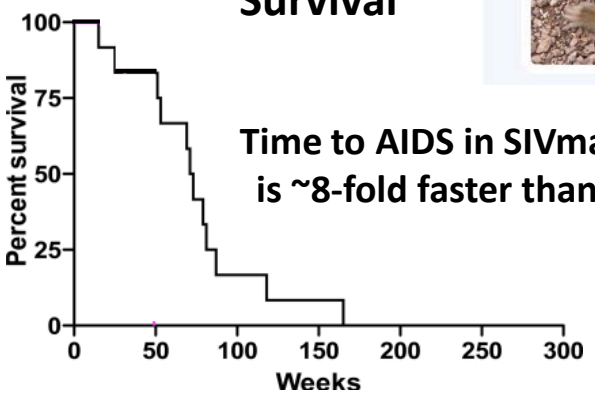
1. Exploitation of the unique immunobiology of Cytomegalovirus (CMV) to create a more effective HIV/SIV vaccine, and

2. Immunotherapeutic manipulation of the immune:viral intercept post-ART release to potentiate post-ART viral control.

<u>All studies use the SIVmac239 virus clone</u>



[Nearly 2 logs higher than typical HIV]



Time to AIDS in SIVmac239-infected RM is ~8-fold faster than for HIV infection!

- Highly replicative (fit)
- Highly pathogenic (chronic/progressive)
- Immune evasive
- Neutralization resistant

SIVmac239 is HIV on "speed" = high bar

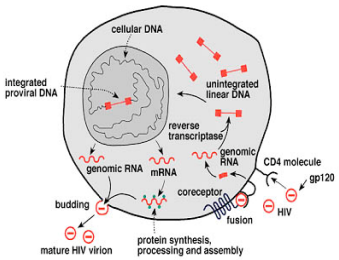
Once primary HIV/SIV infection attains both the replicating viral mass needed for mutational escape and establishes the long-term latent viral reservoir, the likelihood of full immune control becomes increasing remote, considerations that suggest that suggest and early effective immune intercept will be critical for general/complete viral control . . .

Thinking about a prophylactic HIV/SIV vaccine . . .

STAGES OF PRIMARY INFECTION

STEP 1
Mucosal Translocation and Initial Infection

Long-term latent reservoir

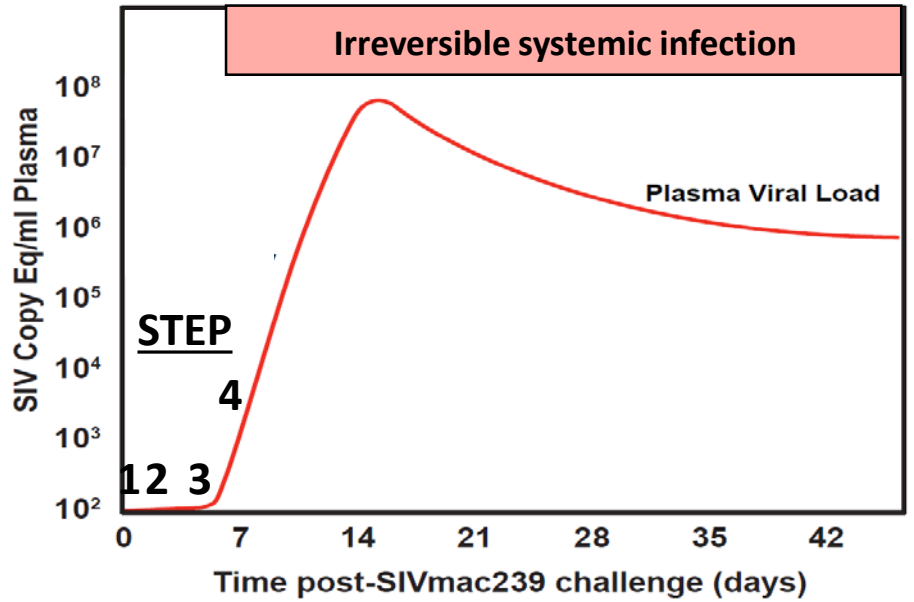


STEP 2
Local Replication at Portal of Entry

STEP 3
Early Spread
(Amplification and Broadcast of Infection)

LINE OF NO RETURN

STEP 4
Generalized Systemic Replication



. . . Which has implications for the type of vaccine-elicited immune response that has the highest likelihood of success

STAGES OF PRIMARY INFECTION

STEP 1
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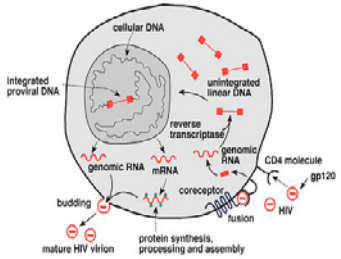


LINE OF NO RETURN

IMMUNE EFFECTOR RESPONSE

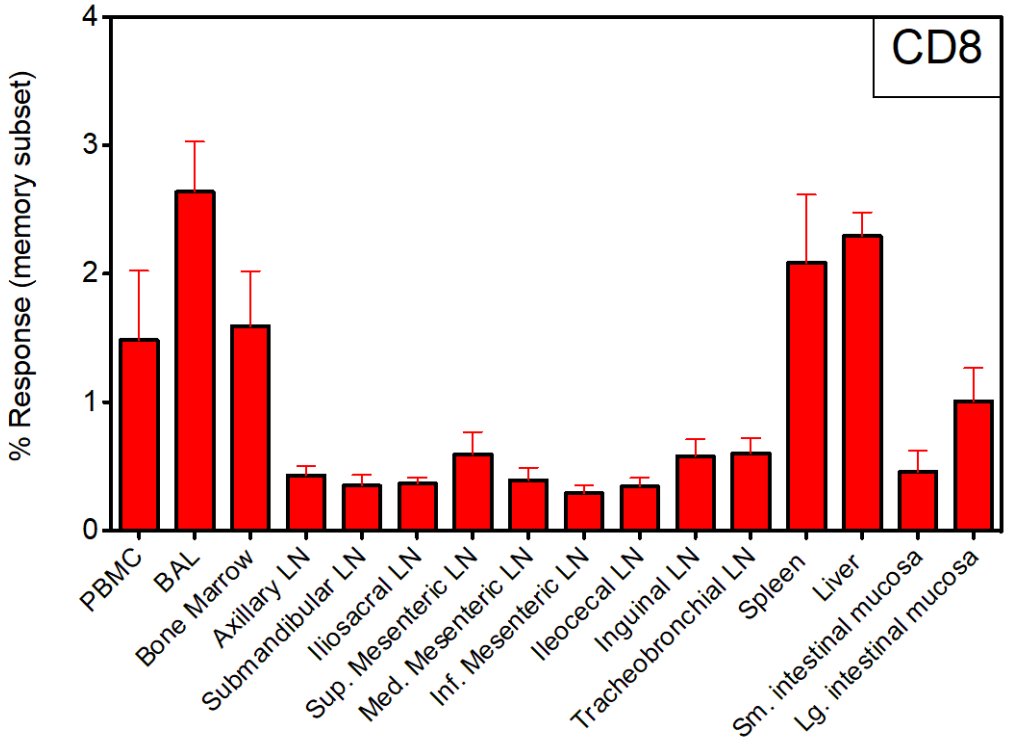
Pre-established antibodies

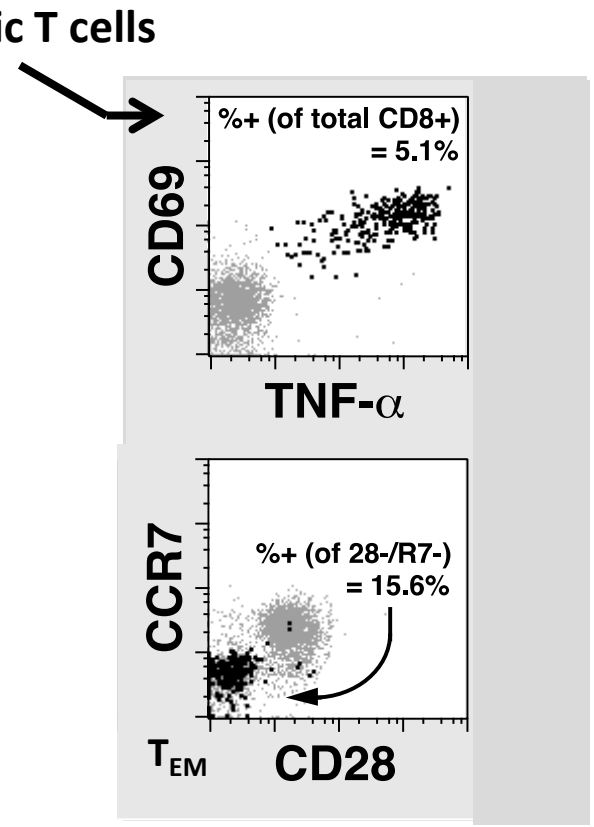
Pre-positioned effector differentiated responses (effector memory)

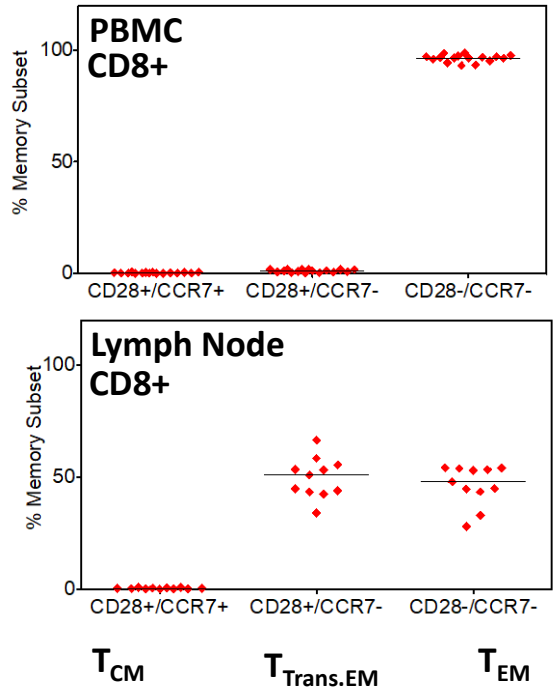
Central memory (requiring anamnestic response for effector generation/distribution)

Conclusion: need pre-positioned effector-differentiated memory $CD8^+$ T cell responses

Relative to all known primate infections, Cytomegalovirus is renown for its high magnitude effector-differentiated CD8+ memory T cell responses . . .

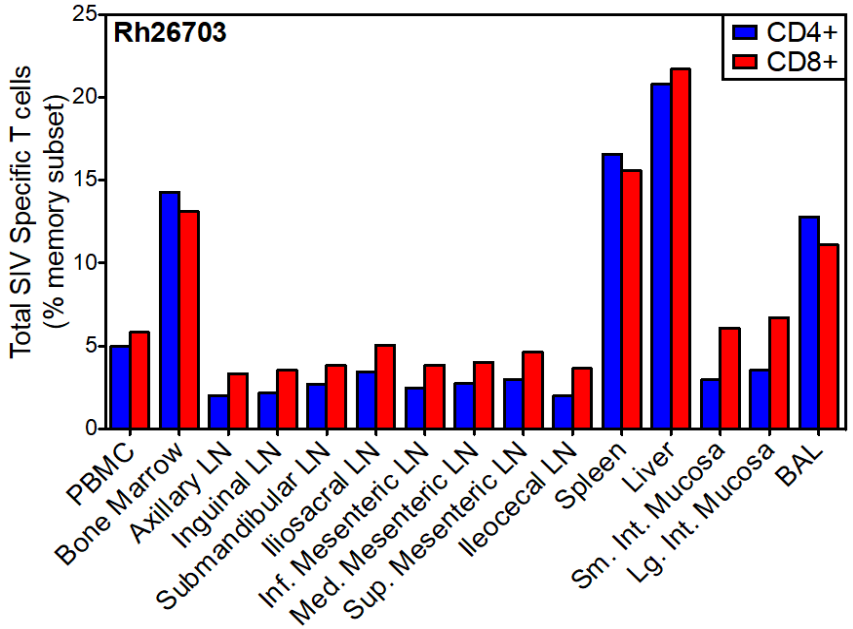




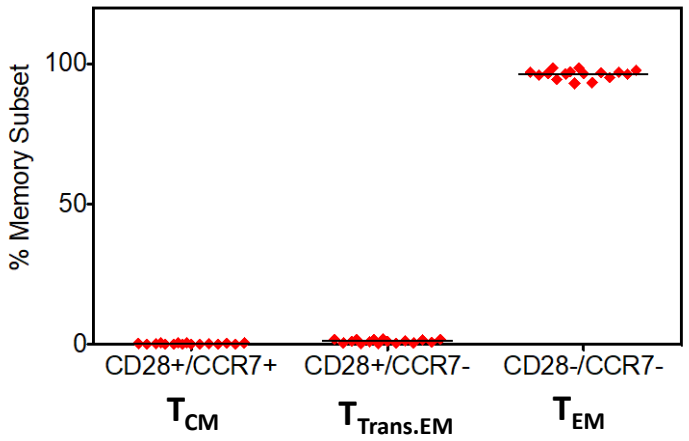


The original concept that guided the development of CMV vectors . . .

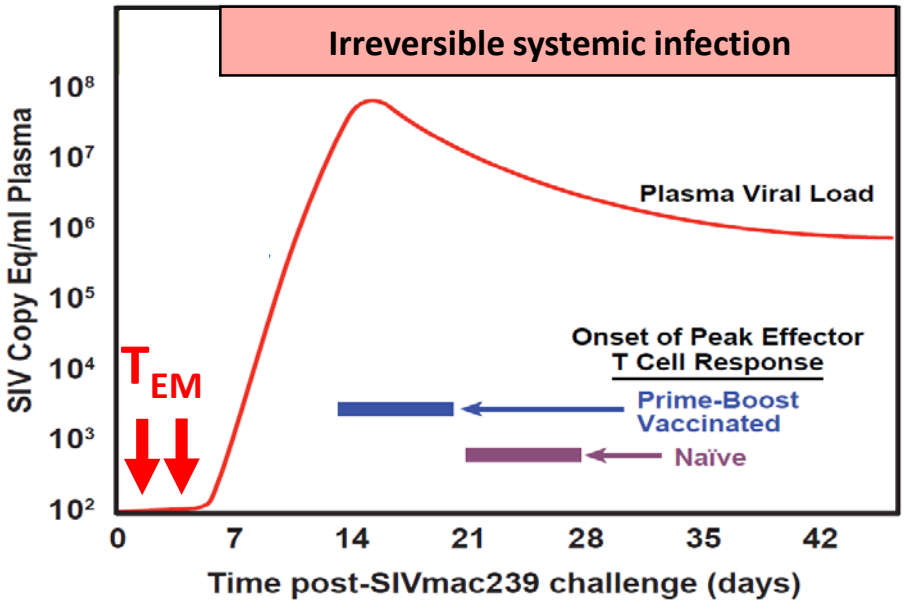
Necropsy analysis 700 days post RhCMV/SIV* vector vaccination



Differentiation phenotype of SIVgag-specific CD8+ T cells in blood



Whac-A-Pathogen



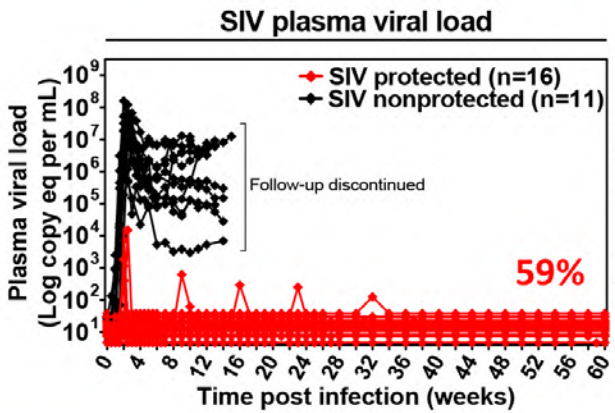
Whac-A-Pathogen

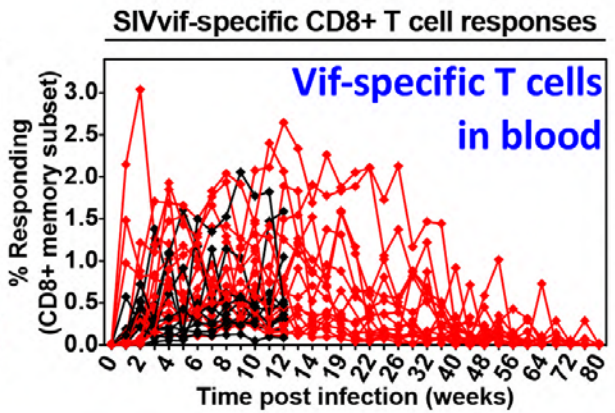
So, would a RhCMV-based SIV ("T_{EM}") vaccine prove efficacious against mucosal challenge with highly pathogenic SIV?

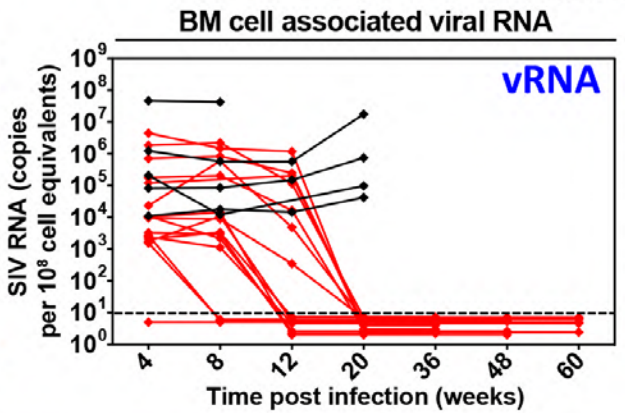
YES – a "T_{EM}" vaccine based on strain 68-1-based RhCMV/SIV vectors has manifested reproducible, stringent protection against highly pathogenic SIVmac239 infection in RM:

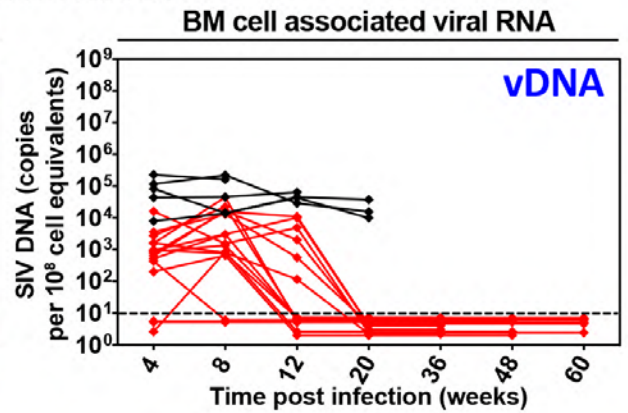
Repeated, limiting dose SIVmac239 challenge

BM = bone marrow

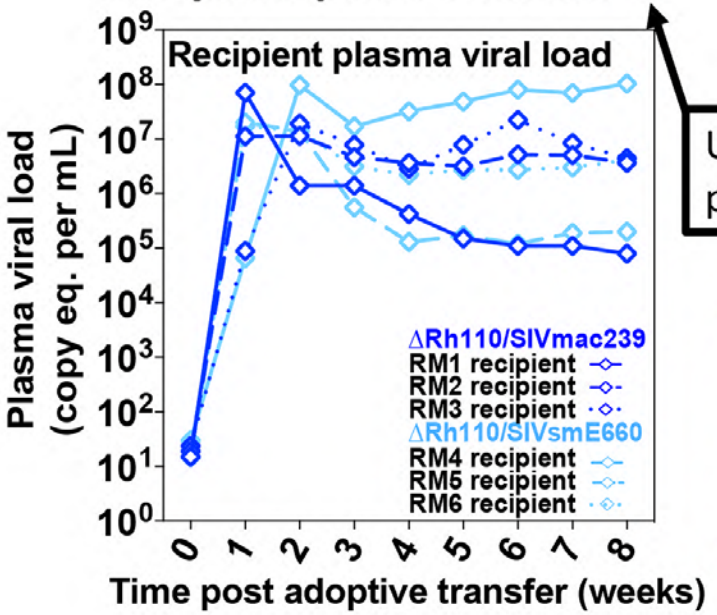








Early Adoptive Transfer +

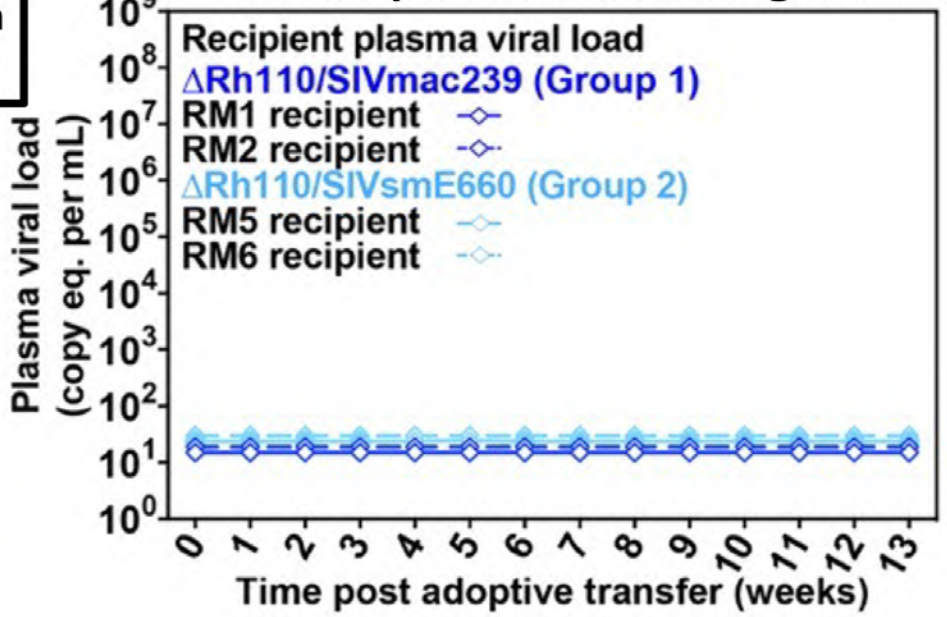


Up to 10^8 BM + PBMC + LN cells taken from same protected RM 420 to 728 days pi

Up to 10^8 BM + PBMC taken from protected RM prior to day 84 pi

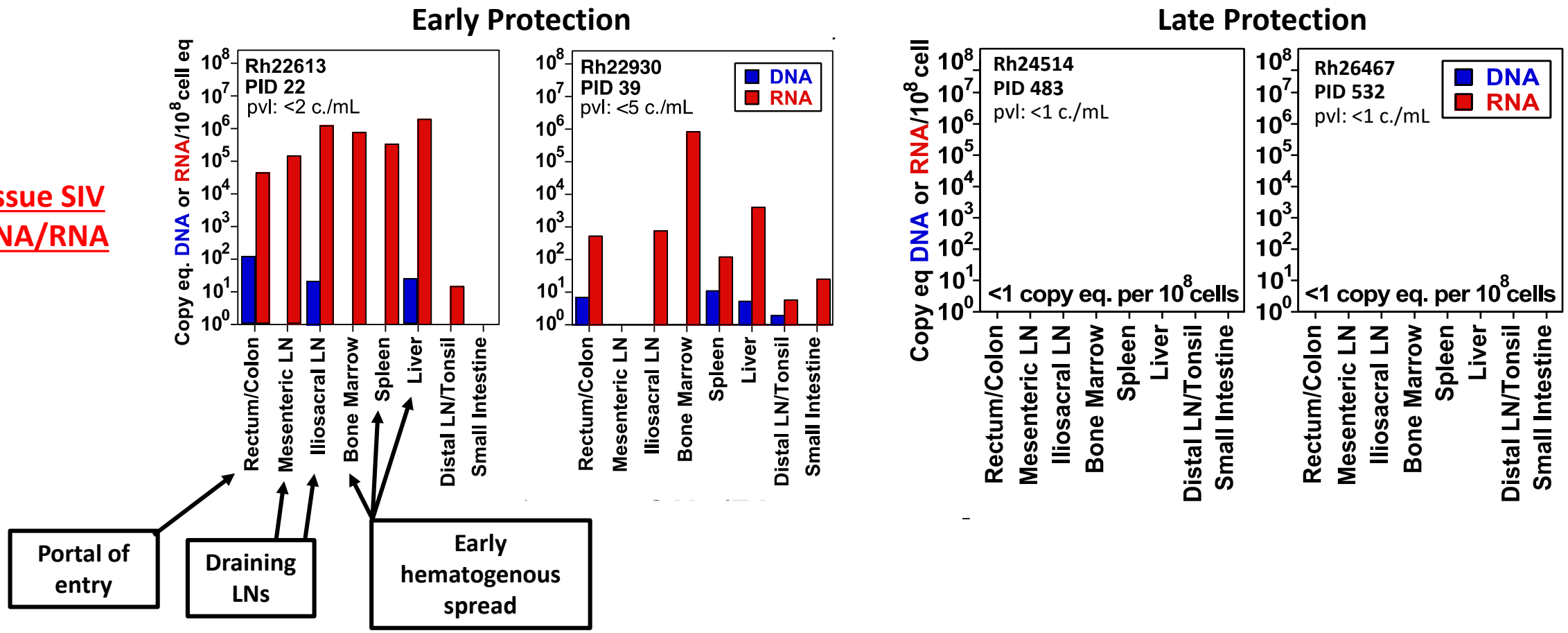
Cells from protected RM given IV to naïve RM, who are then followed for infection. Viral outgrowth in recipient RM indicates replication competent SIV.

Late Adoptive Transfer Negative



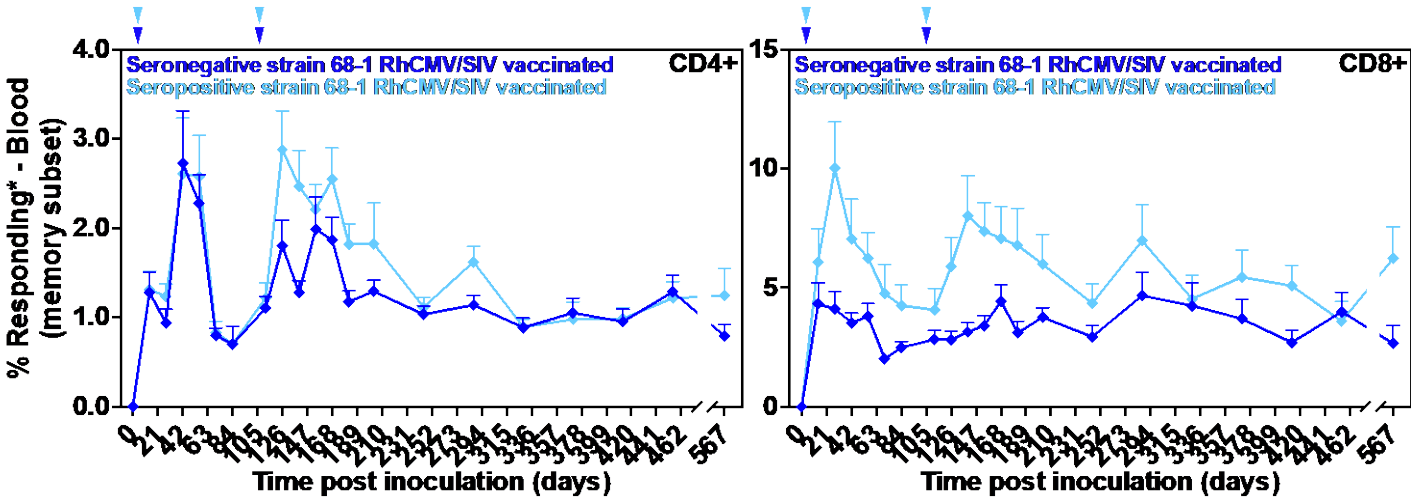
Characteristics of 68-1 RhCMV/SIV vector-induced SIV "replication arrest" efficacy: Infection followed by replication arrest followed by clearance!

Tissue SIV DNA/RNA

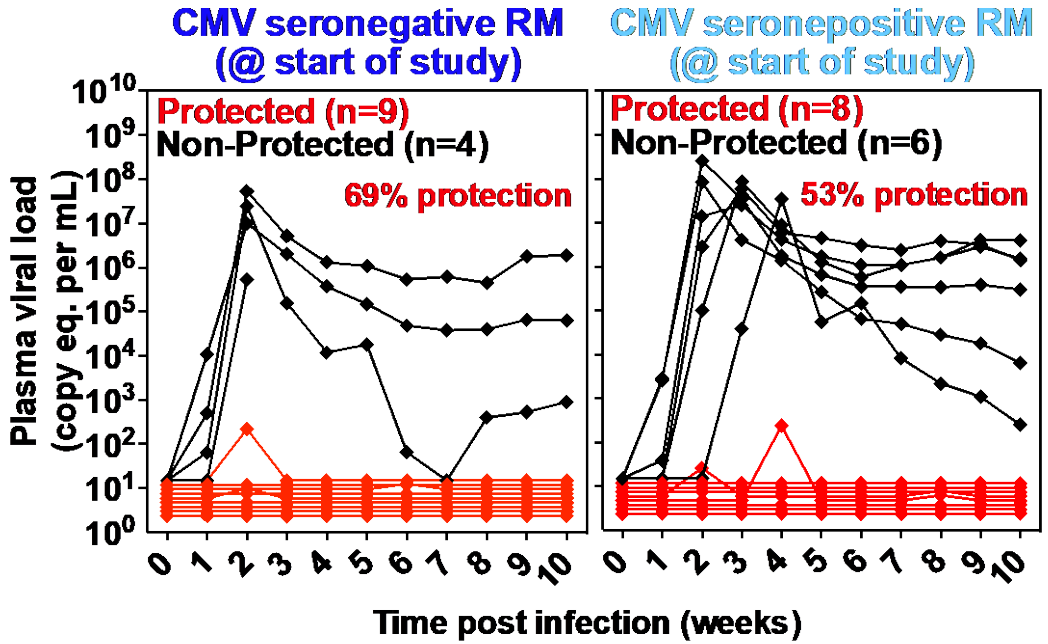


Both CMV^{+} and CMV^{-} RM can be protected by 68-1 RhCMV/SIV vaccination:

Vaccine Phase: CMV negative vs. positive RM (total SIV-specific* response)

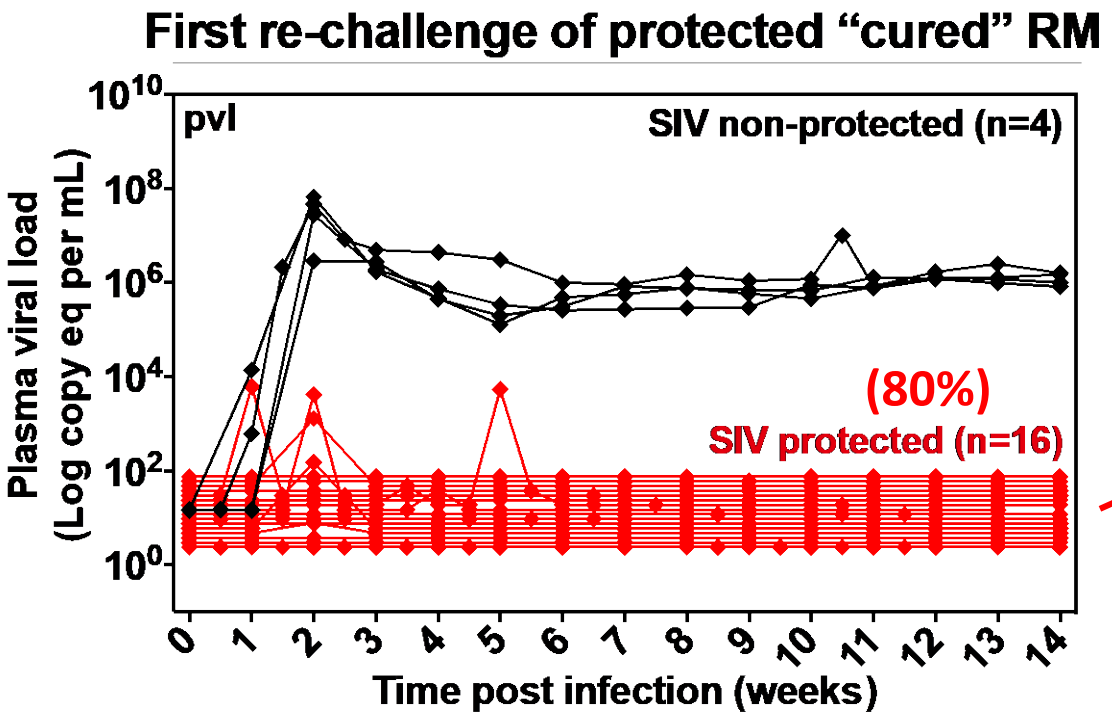


Challenge Phase:

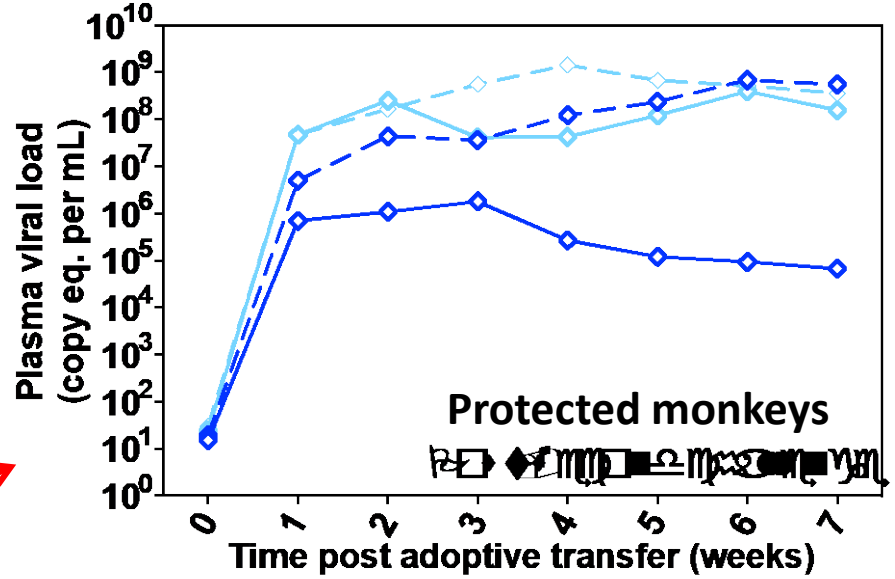


(CMV-negative RM are also negative for RRV, Herpes B, SRV, SFV, STLV)

68-1 RhCMV/SIV vaccine-protected “cured” RM can stringently control a second challenge . . . re-initiating replication arrest:



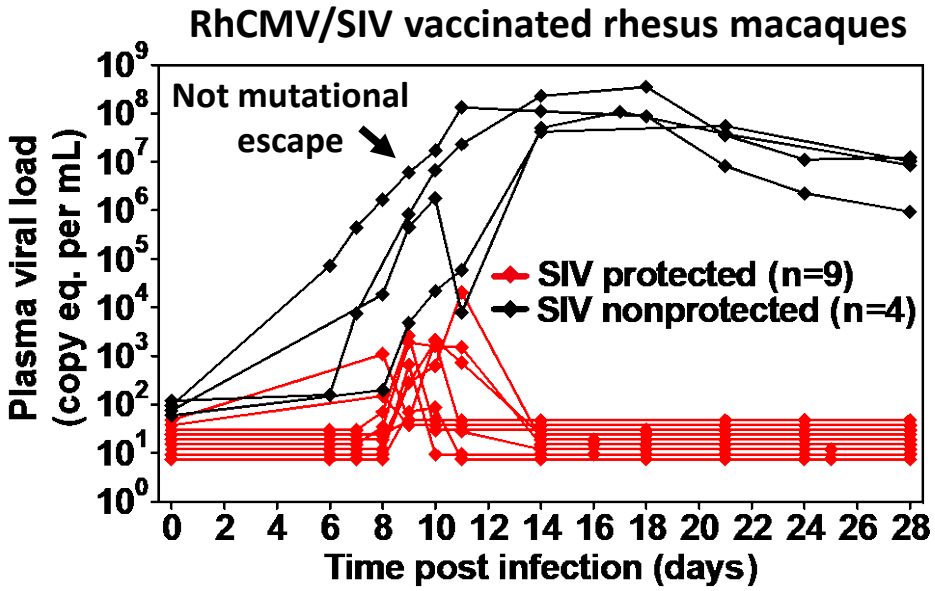
(female monkeys: IVag challenge)



	Tissue	PID	Number of cells (per tissue)	Total number of cells transferred
RM1	Bone marrow PBMC	14-84	5.0×10^6 7.7×10^7	8.2×10^7
RM2	Bone marrow PBMC	7-77	1.5×10^7 1.9×10^7	3.4×10^7
RM3	PBMC	7-84	5.2×10^7	5.2×10^7
RM4	Bone marrow PBMC	7-45	2.0×10^6 1.5×10^7	1.7×10^7

68-1 RhCMV/SIV vector-induced SIV replication arrest is an entirely different phenomenon than conventional (spontaneous or vaccine-elicited) elite SIV control:

Viral Replication Arrest



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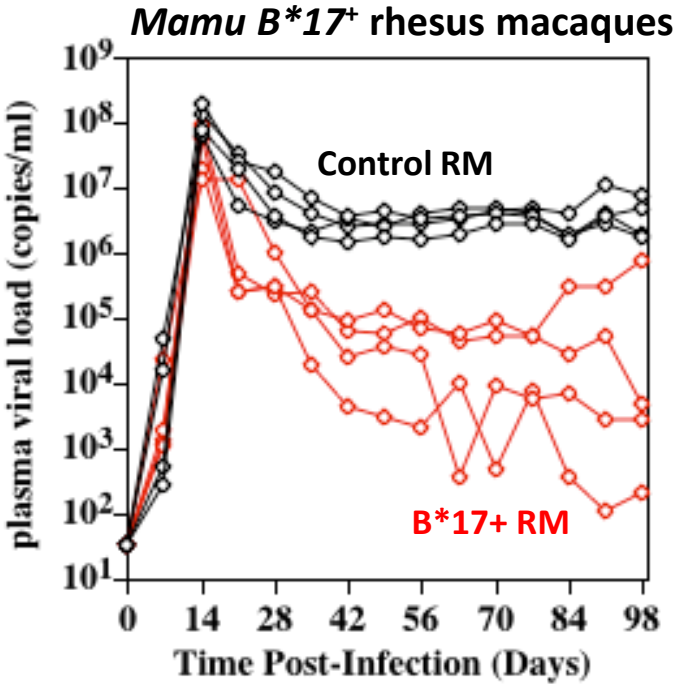
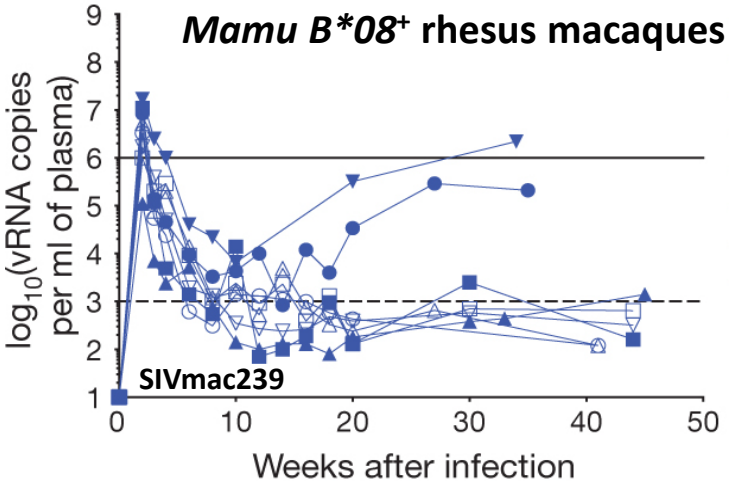
Viral Replication Suppression (elite control)



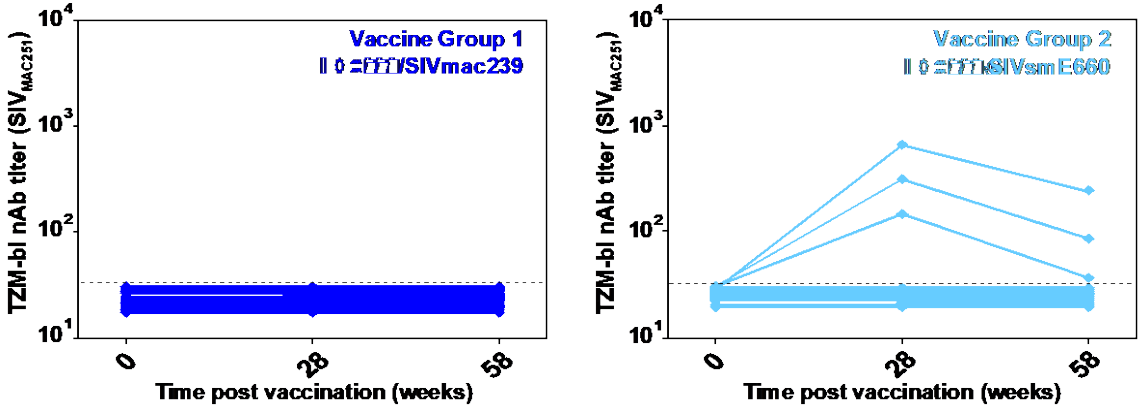


PA Mudd *et al. Nature* (2012) doi:10.1038/nature11443

So, what is the mechanism of this unique efficacy . . . Just robust anti-viral T_{EM}?

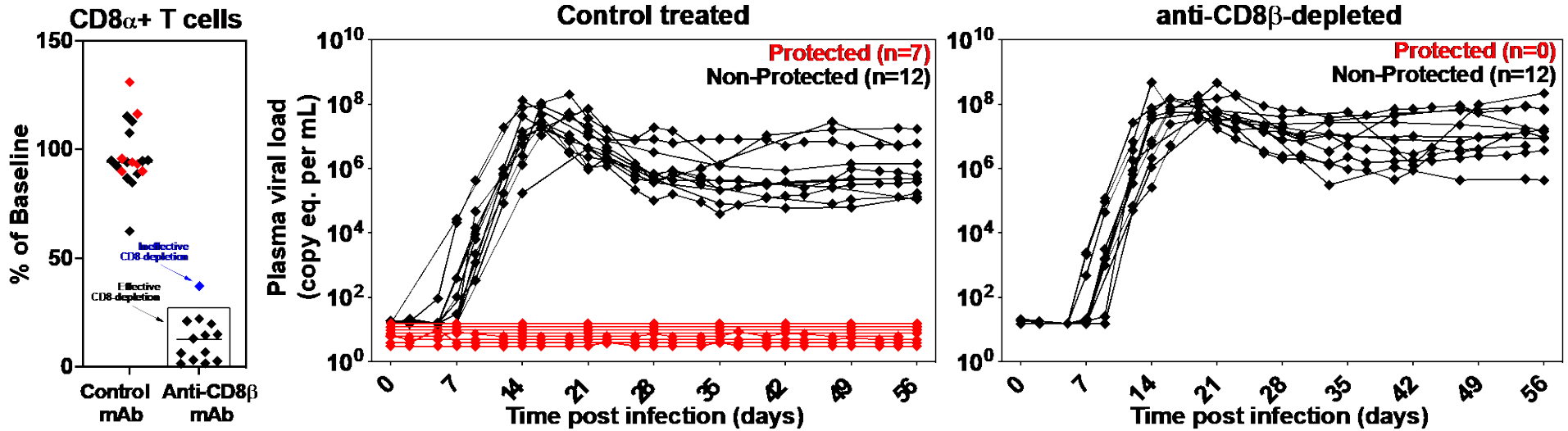
Antibodies vs. CD8$^+$ T cells?

Point #1



RhCMV/SIV vectors, including env-expressing vectors, have little to no ability to elicit Abs, and Env inserts are not required for efficacy = not antibodies.

Point #2

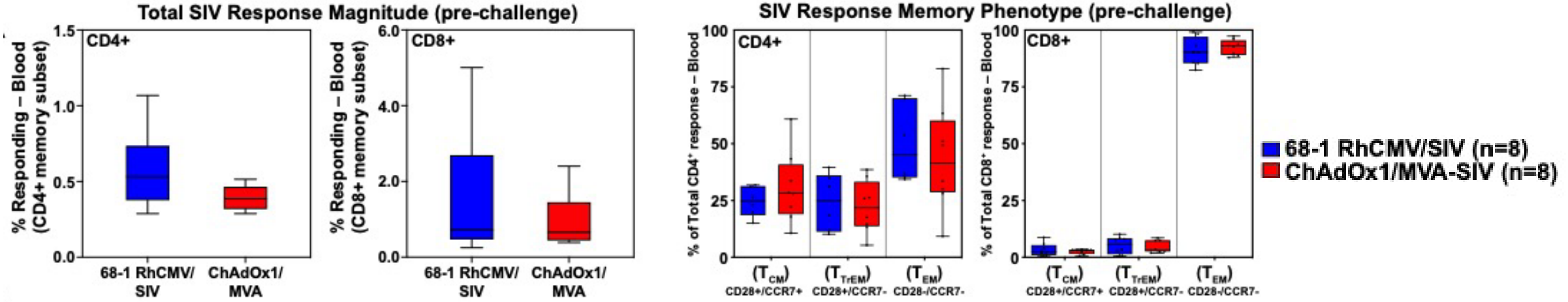


CD8β$^+$ T cell depletion prior to and during challenge abrogates protection, implicating CD8$^+$ T cell responses as crucial for establishing efficacy.

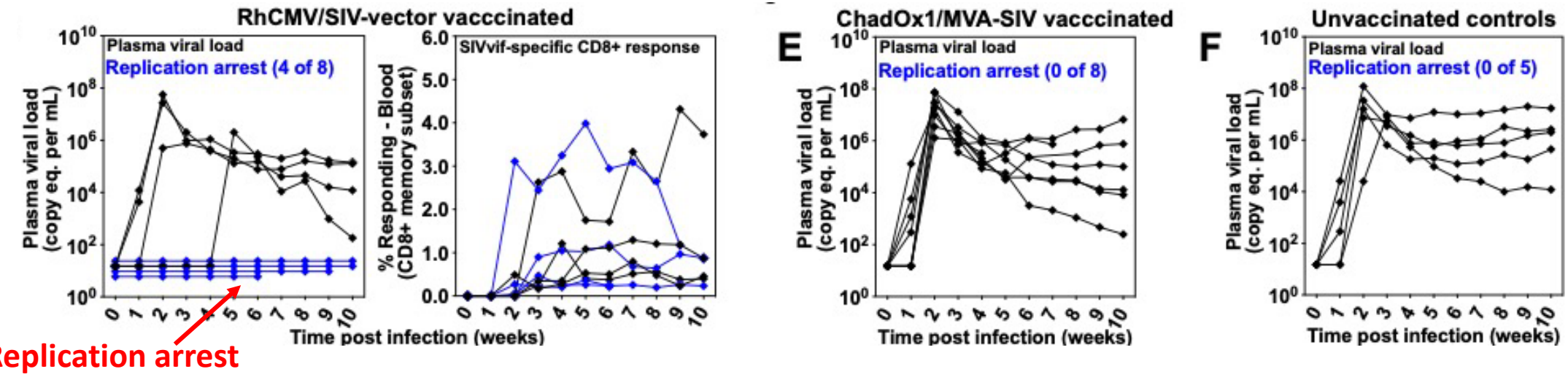
Just robust T_{EM}? . . . Effector memory-biased $CD8^+$ T cell responses elicited by other (non-CMV-based) vaccines fail to achieve replication arrest efficacy.

Immunogenicity

ChAd-OX/MVA prime boost (same inserts)* *with Tom Hanke

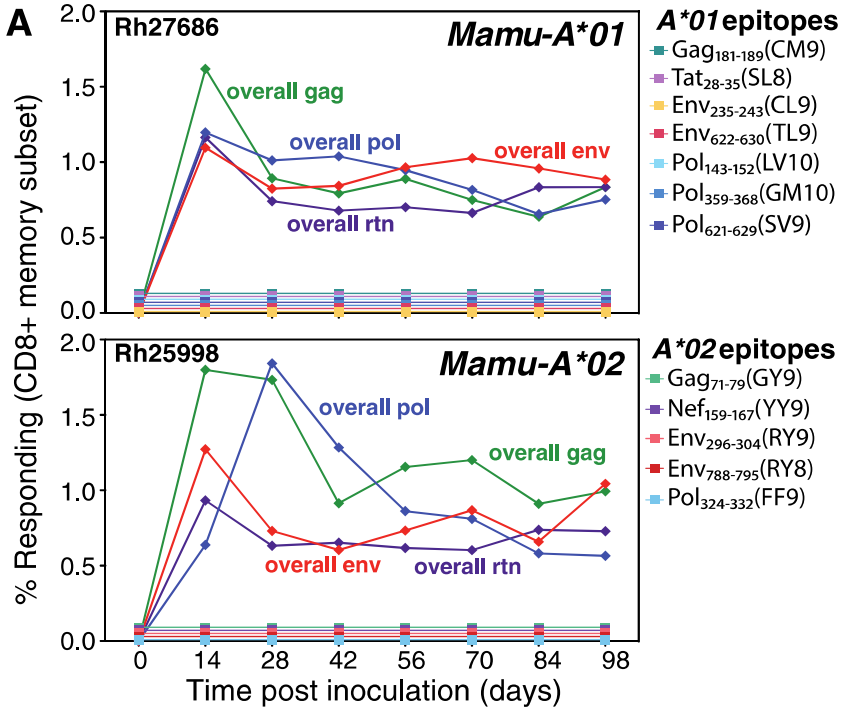


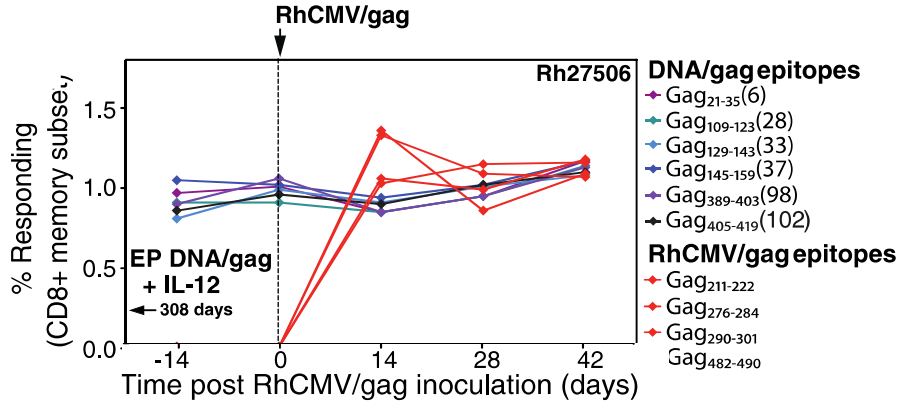
Challenge Outcome

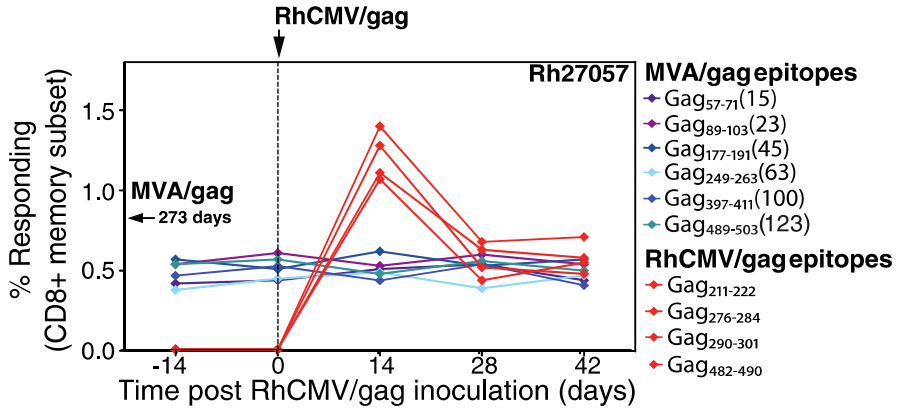


CMV vectors had other immunologic surprises in store . . .

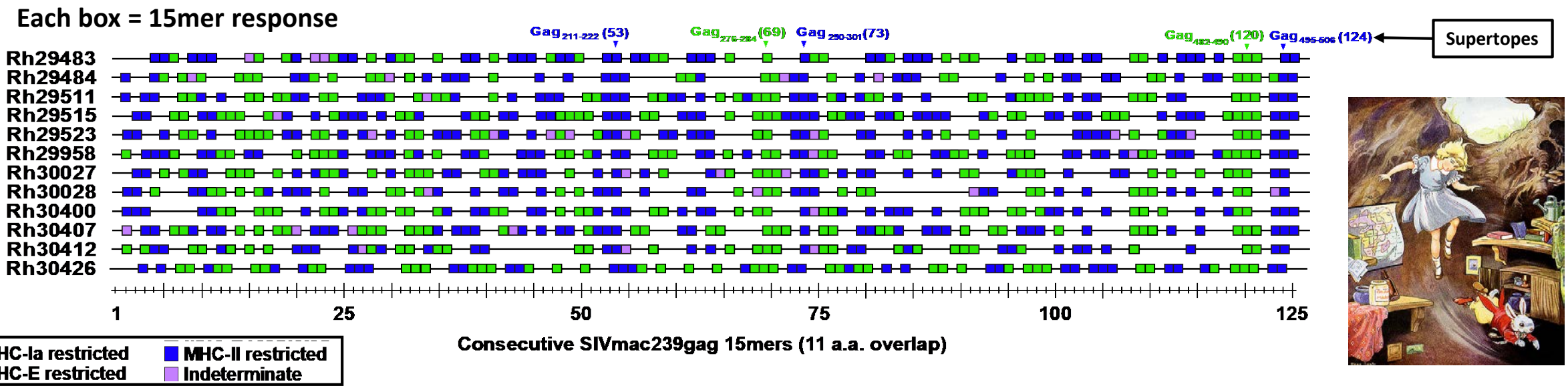
During our analysis of RhCMV vector immunogenicity, we noticed that the SIV-specific CD8+ T cells elicited by strain 68-1 RhCMV/SIV vectors did not recognize "canonical" MHC-Ia-restricted epitopes or overlap at all with MHC-Ia-restricted epitopes targeted by CD8+ T cells elicited by conventional vaccines or SIV itself:



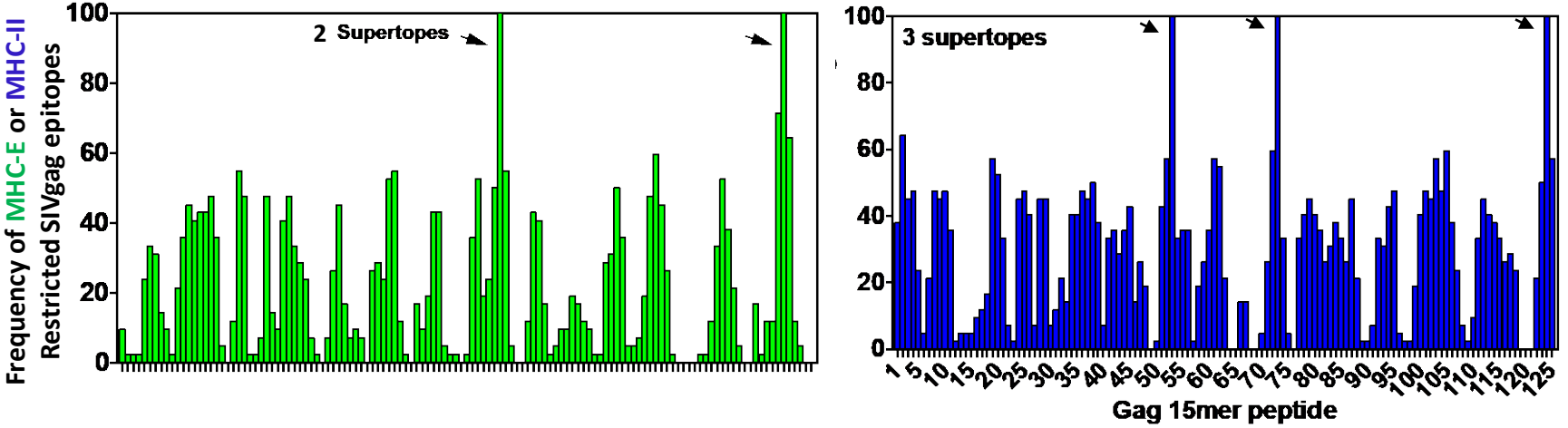




Indeed, further study reveal that the efficacious 68-1 RhCMV/SIV vaccines elicit CD8+ T cell responses entirely restricted by MHC-II or MHC-E and include universal epitopes (supertopes):

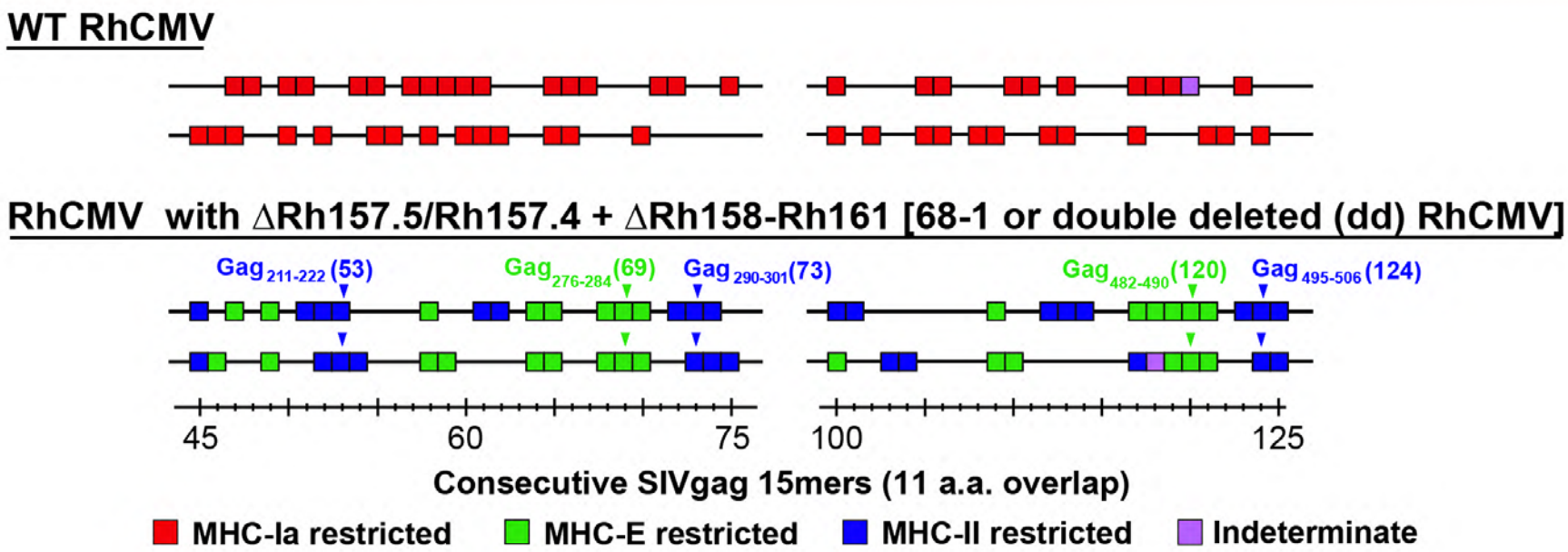


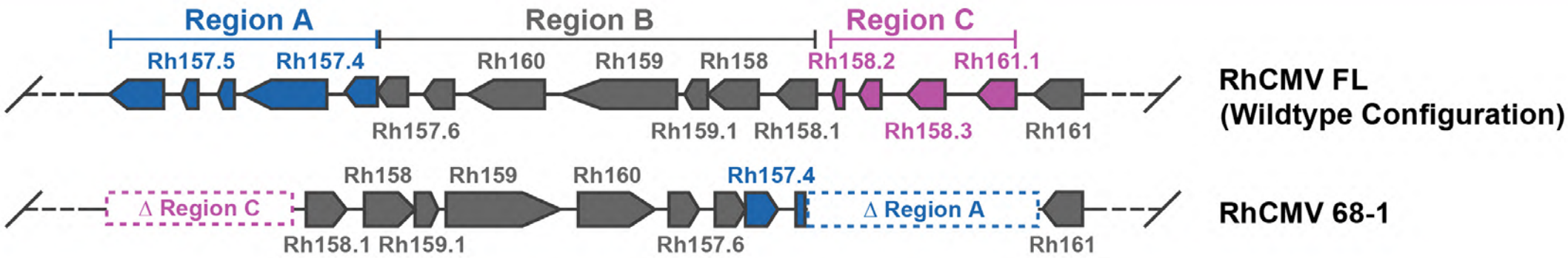
n = 42 68-1 RhCMV/SIVgag vaccinated RM



Originally, we didn't know the biologic basis of this unconventional (non-wildtype) response, or whether it had anything to do with efficacy . . .

These unconventionally restricted CD8+ T cell responses were observed with strain 68-1 RhCMV, but not wildtype RhCMV . . .





During multiple passages prior to BAC cloning, strain 68-1 had developed a "double deletion" of 2 gene clusters: Rh157.5/.4 (UL128/130) and Rh158-161 (UL147/147) . . .

We have elucidated the virologic determinants underlying these unconventionally restricted CD8s, resolving th viral genes and viral characteristics responsible for their elicitation and demonstrating that modulation of thes determinants differentially programs CD8$^+$ T cell response priming:

SCIENCE IMMUNOLOGY | RESEARCH ARTICLE

HIV

2021

Cytomegaloviral determinants of CD8$^+$ T cell programming and RhCMV/SIV vaccine efficacy

Daniel Malouli[1]*, Scott G. Hansen[1]*, Meaghan H. Hancock[1], Colette M. Hughes[1], Julia C. Ford[1], Roxanne M. Gilbride[1], Abigail B. Ventura[1], David Morrow[1], Kurt T. Randall[1], Husam Taher[1], Luke S. Uebelhoer[1], Matthew R. McArdle[1], Courtney R. Papen[1], Renee Espinosa Trethewy[1], Kelli Oswald[2], Rebecca Shoemaker[2], Brian Berkemeier[2], William J. Bosche[2], Michael Hull[2], Justin M. Greene[1], Michael K. Axthelm[1], Jason Shao[3], Paul T. Edlefsen[3], Finn Grey[4], Jay A. Nelson[1], Jeffrey D. Lifson[2], Daniel Streblow[1], Jonah B. Sacha[1], Klaus Früh[1†], Louis J. Picker[1†]

RESEARCH ARTICLE

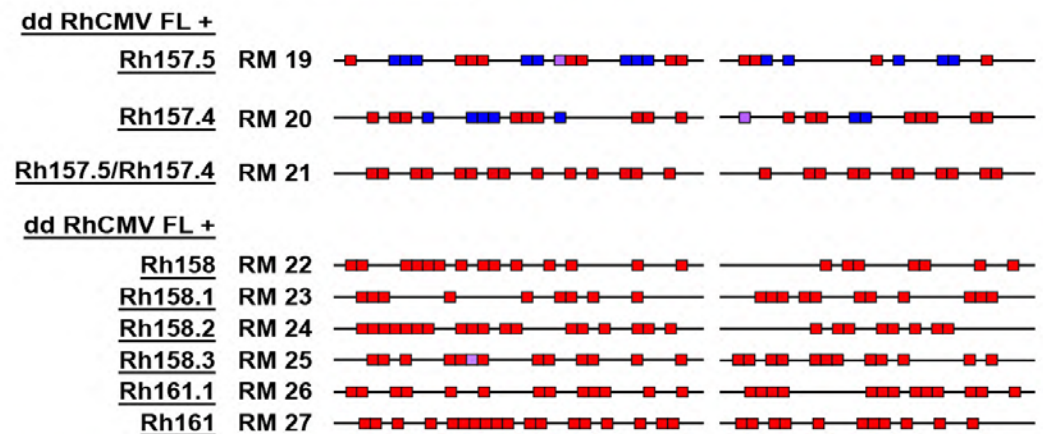
2021

VACCINES

Modulation of MHC-E transport by viral decoy ligands is required for RhCMV/SIV vaccine efficacy

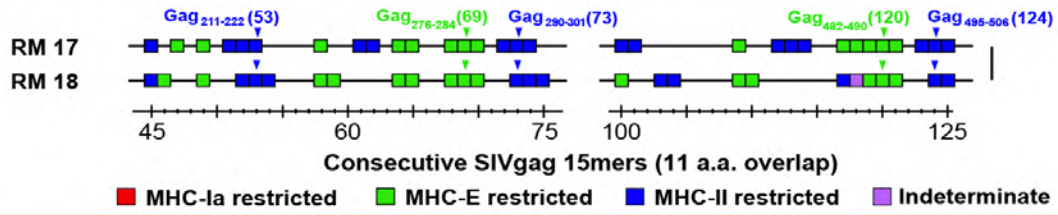
Marieke C. Verweij[1]†, Scott G. Hansen[1]†, Ravi Iyer[1]†, Nessy John[1]†, Daniel Malouli[1], David Morrow[1], Isabel Scholz[1], Jennie Womack[1], Shaheed Abdulhaqq[1], Roxanne M. Gilbride[1], Colette M. Hughes[1], Abigail B. Ventura[1], Julia C. Ford[1], Andrea N. Selseth[1], Kelli Oswald[2], Rebecca Shoemaker[2], Brian Berkemeier[2], William J. Bosche[2], Michael Hull[2], Jason Shao[3], Jonah B. Sacha[1], Michael K. Axthelm[1], Paul T. Edlefsen[3], Jeffrey D. Lifson[2], Louis J. Picker[1]*, Klaus Früh[1]*

Gene Manipulation:



MHC-Ia + -II

MHC-Ia only

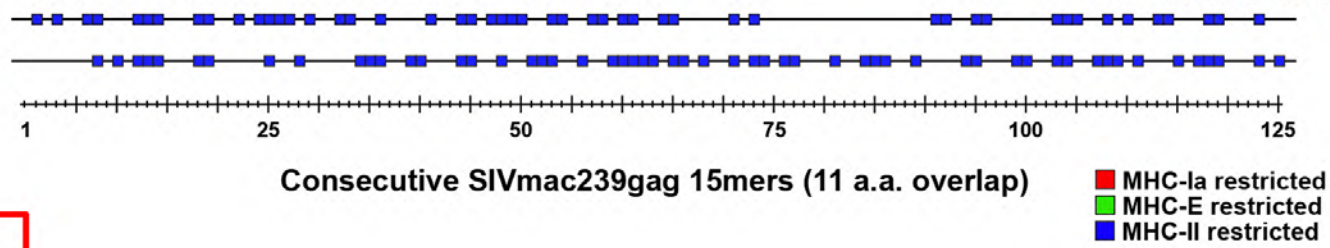


Starting vector*

MHC-E + -II

Rh67-deleted 68-1 RhCMV/SIV

MHC-II only



* dd = double deleted for Rh157.5/.4 + Rh158-161 = 68-1 genotype

RhCMV encodes both negative and positive regulators of CD8$^+$ T cell priming to itself!

RhCMV ORF	HCMV ortholog#	Protein Type	Known Function	Effect on CD8$^+$ T cell epitope targeting	Refs.
Rh67	UL40	ER[1] glycoprotein	MHC-E upregulation via viral VL9	Required for MHC-E	
Rh157.5	UL128	CC-chemokine	Tropism, PC[3] subunit	Inhibits MHC-E and MHC-II ST*	
Rh157.4	UL130	CXC-chemokine	Tropism, PC[3] subunit	Inhibits MHC-E and MHC-II ST*	
Rh158	UL147	CXC-chemokine	Unknown (UL147)	Inhibits MHC-E and MHC-II	
Rh158.1	UL146	CXC-chemokine	Neutrophil chemotaxis (UL146)	Inhibits MHC-E and MHC-II	
Rh158.2	UL146	CXC-chemokine	Neutrophil chemotaxis (UL146)	Inhibits MHC-E and MHC-II	
Rh158.3	UL146	CXC-chemokine	Neutrophil chemotaxis (UL146)	Inhibits MHC-E and MHC-II	
Rh161.1	UL146	CXC-chemokine	Neutrophil chemotaxis (UL146)	Inhibits MHC-E and MHC-II	
Rh161	UL146	CXC-chemokine	Neutrophil chemotaxis (UL146)	Inhibits MHC-E and MHC-II	
Rh189	US11	ER[1] glycoprotein	MHC-I degradation	Inhibits canonical MHC-Ia	
Rh214	US28	GPCR[2]	Latency and reactivation (US28)	Required for MHC-E**	Unpubl.
Rh220	US28	GPCR[2]	Latency and reactivation (US28	Required for MHC-E**	Unpubl.
None	US18	MHC-I	LILRB1-binding	Inhibits MHC-E and MHC-II	Unpubl

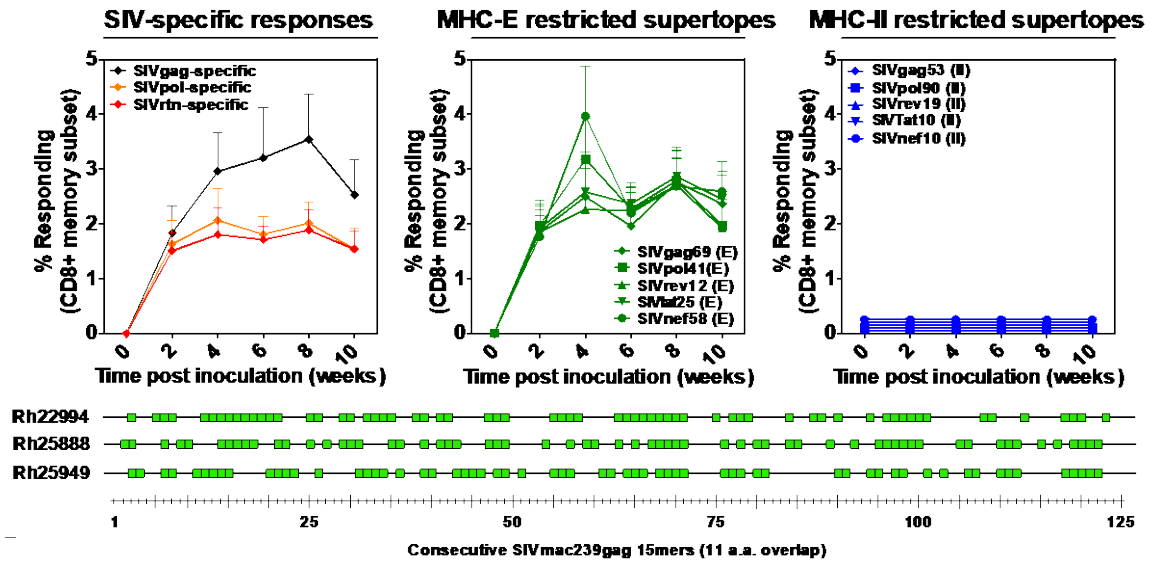
And HCMV encodes an additional unconventional response inhibitor that is not found in RhCMV . . .

Negative regulators (response inhibitors)

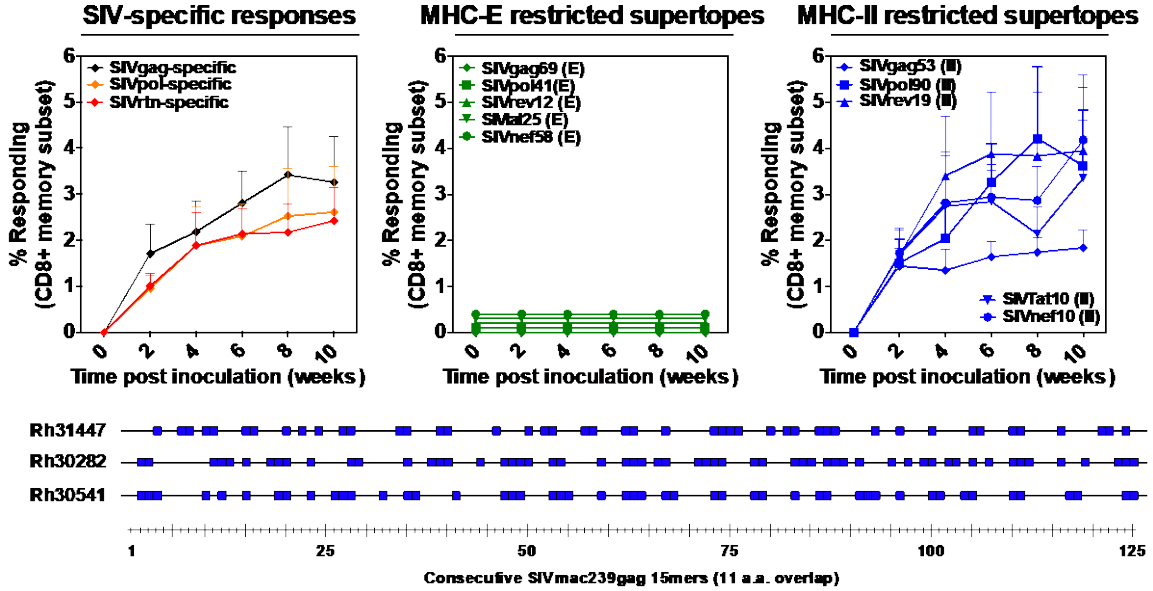
Positive regulators (response inducers)

Even better, tropism manipulation with cell-type specific microRNAs (miRs) programs for MHC-E-only (miR-126), MHC-II-only (miR-142), and MHC-Ia–only (miR-126 + miR-142) CD8$^+$ T cell responses:

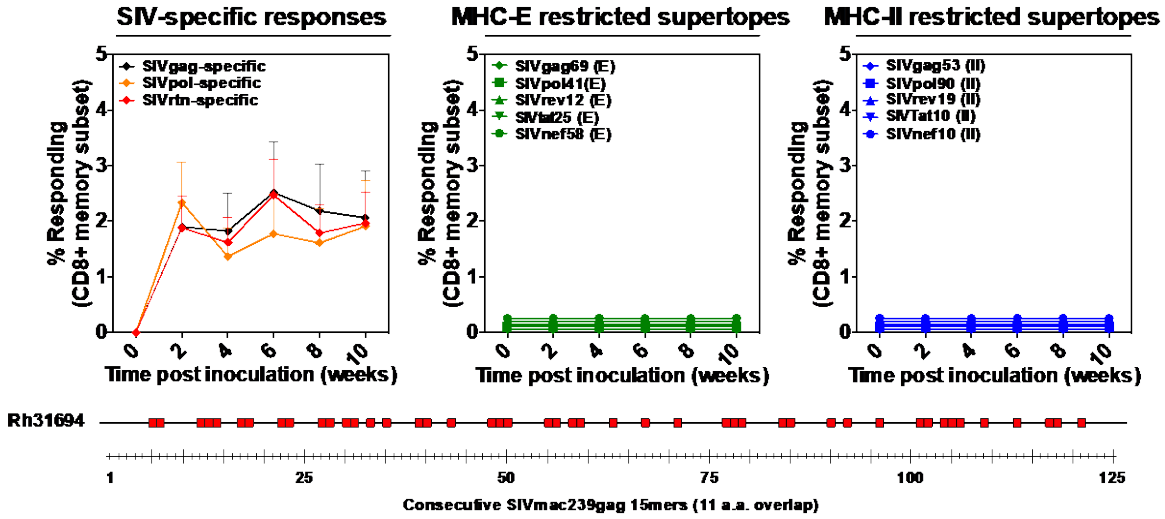
miR-126-restricted – blocks EC infection (MHC-E-only)



miR-142-restricted – blocks myeloid infection (MHC-II-only)



miR-126+miR-142-restricted (MHC-Ia-only)



SCIENCE IMMUNOLOGY | RESEARCH ARTICLE

VACCINES

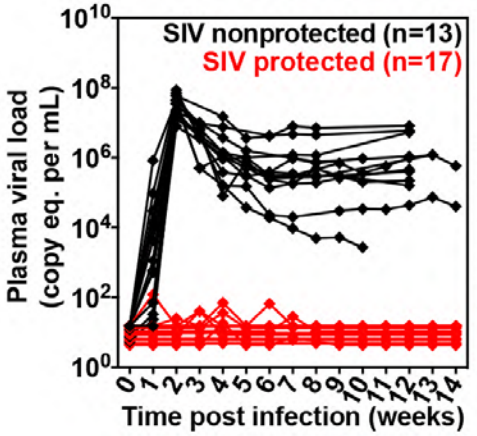
Myeloid cell tropism enables MHC-E–restricted CD8$^+$ T cell priming and vaccine efficacy by the RhCMV/SIV vaccine

Scott G. Hansen[1]†, Meaghan H. Hancock[1]†, Daniel Malouli[1], Emily E. Marshall[1], Colette M. Hughes[1], Kurt T. Randall[1], David Morrow[1], Julia C. Ford[1], Roxanne M. Gilbride[1], Andrea N. Selseth[1], Renee Espinosa Trethewy[1], Lindsey M. Bishop[1], Kelli Oswald[2], Rebecca Shoemaker[2], Brian Berkemeier[2], William J. Bosche[2], Michael Hull[2], Lorna Silipino[2], Michael Nekorchuk[1], Kathleen Busman-Sahay[1], Jacob D. Estes[1], Michael K. Axthelm[1], Jeremy Smedley[1], Danica Shao[3], Paul T. Edlefsen[3], Jeffrey D. Lifson[2], Klaus Früh[1], Jay A. Nelson[1], Louis J. Picker[1]*

Among differentially programmed 68-1 RhCMV/SIV vectors, only those with MHC-E-restricted CD8+ T cell responses manifest replication arrest efficacy.

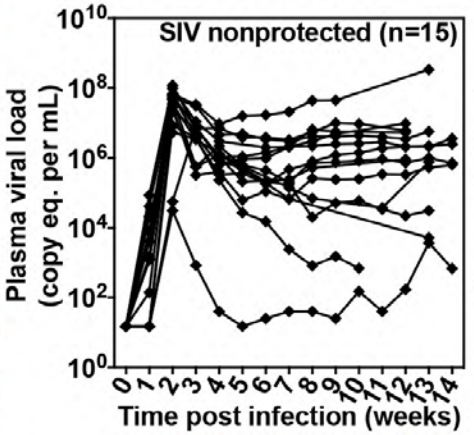
RhCMV/SIV vector-vaccinated

Strain 68-1 (UL128-130 deleted)



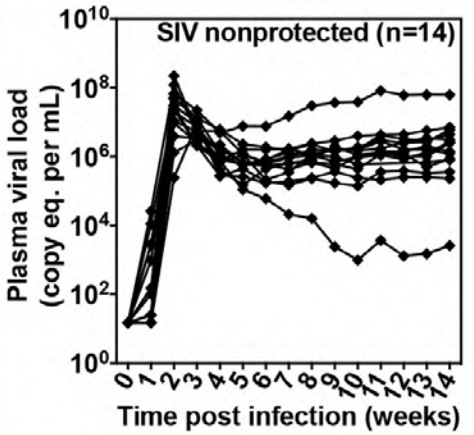
MHC-E and MHC-II

Strain 68-1.2 (UL128-130 repaired)



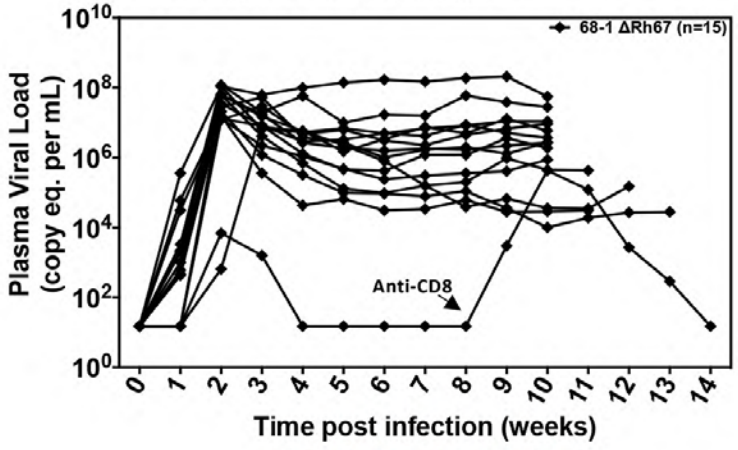
MHC-Ia-only

Strain 68-1.2 ΔUL128 (UL130 intact)



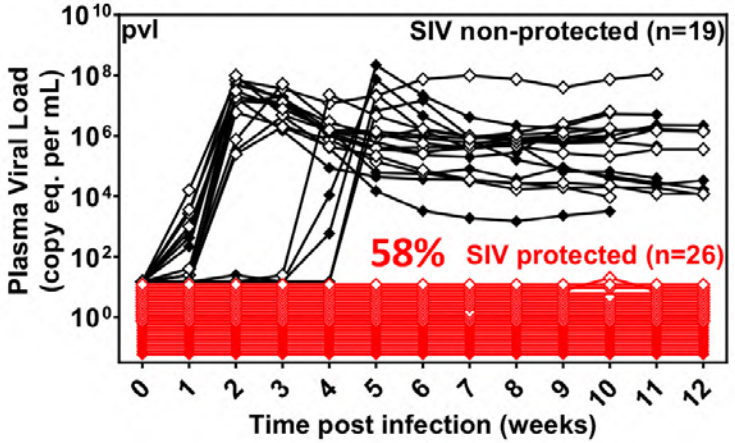
MHC-Ia and MHC-II

Rh67-deleted 68-1 RhCMV/SIV



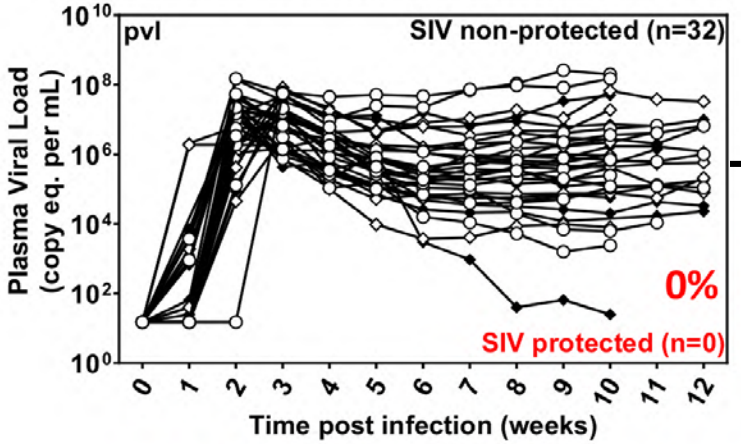
MHC-II-only

miR126-restricted
68-1 RhCMV/SIV vector-vaccinated



MHC-E-only

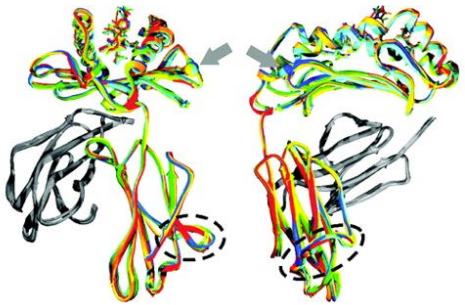
miR-142 and miR-142/126 restricted
68-1 RhCMV/SIV vector-vaccinated



MHC-Ia-only or MHC-II-only

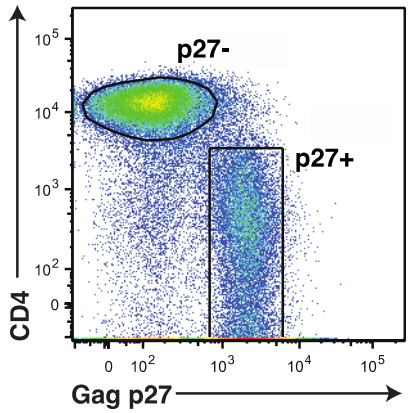
No difference in response magnitude and phenotype (T_{EM}-bias) relative to effective (MHC-E) vaccines.

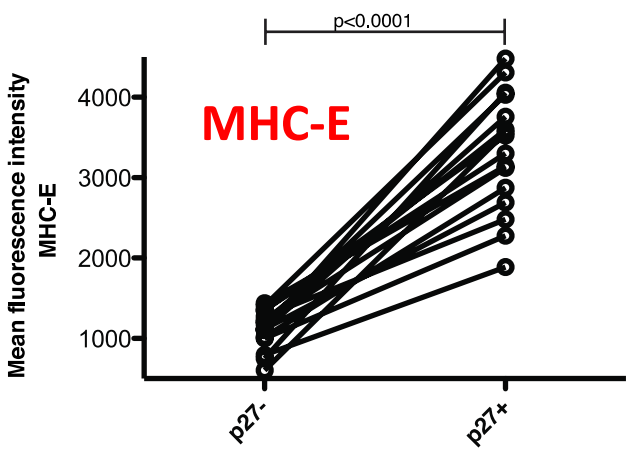
MHC-E!

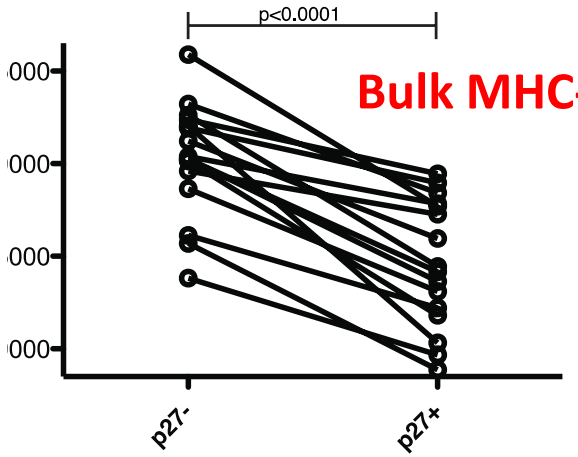


MHC E is the most conserved MHC I in primates and is relatively non-polymorphic. Its primary job is to present a 9-mer (VL9) from the leader sequence of MHC Ia alleles to NK cells, primarily to inhibitory CD94/NKG2A receptors, which allows NK cells to distinguish normal cells from those infected with viruses that down-regulate MHC Ia.

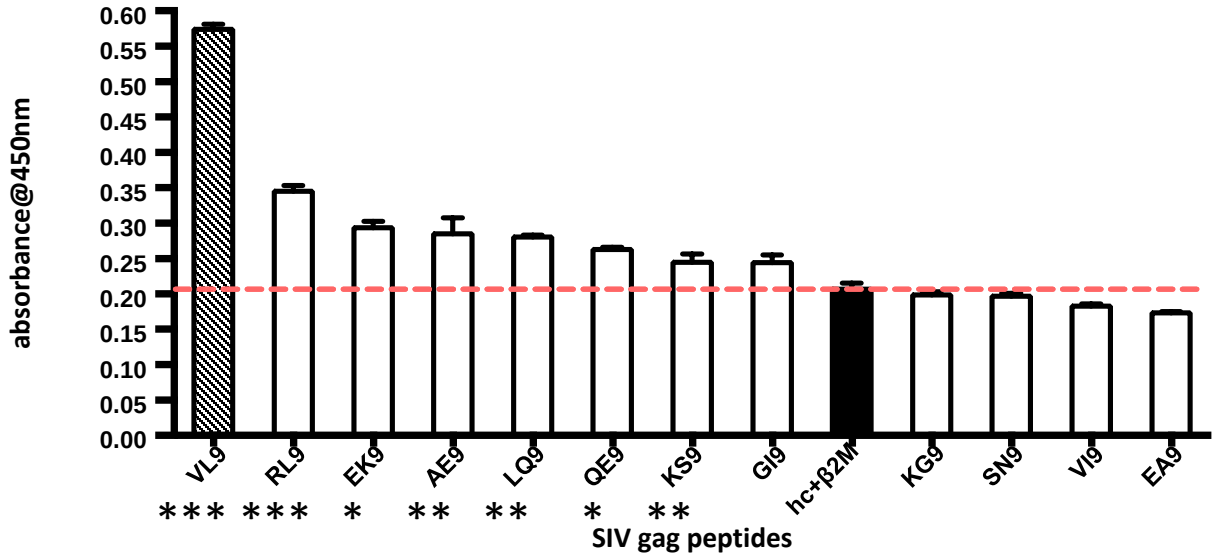
MHC E appears to be sufficiently important for regulating NK cell recognition that both CMV and HIV/SIV maintain specific mechanisms to up-regulate MHC-E expression on infected cells (while simultaneously down-regulating MHC-Ia) to avoid NK cell-mediated destruction:



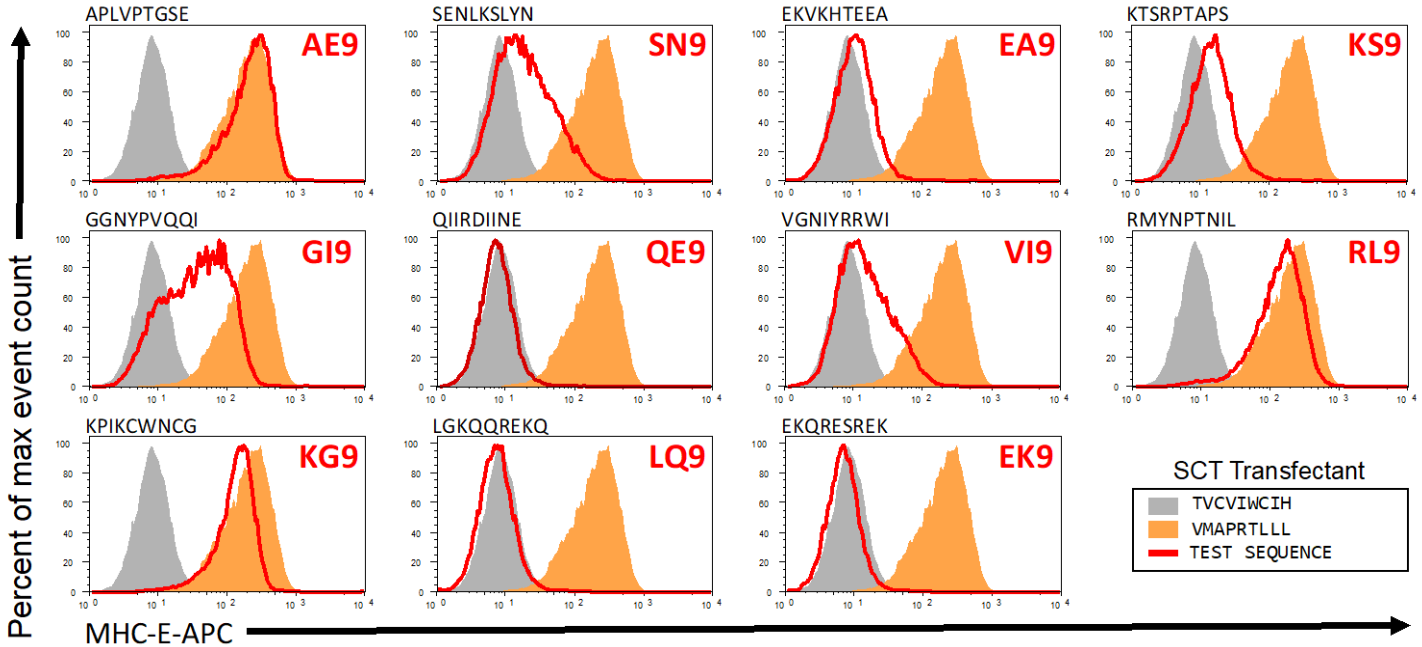




Other MHC-E points of interest: 1) binding affinity of non-VL9 peptides is low . . .



HLA-E/peptide "refold ELISA"



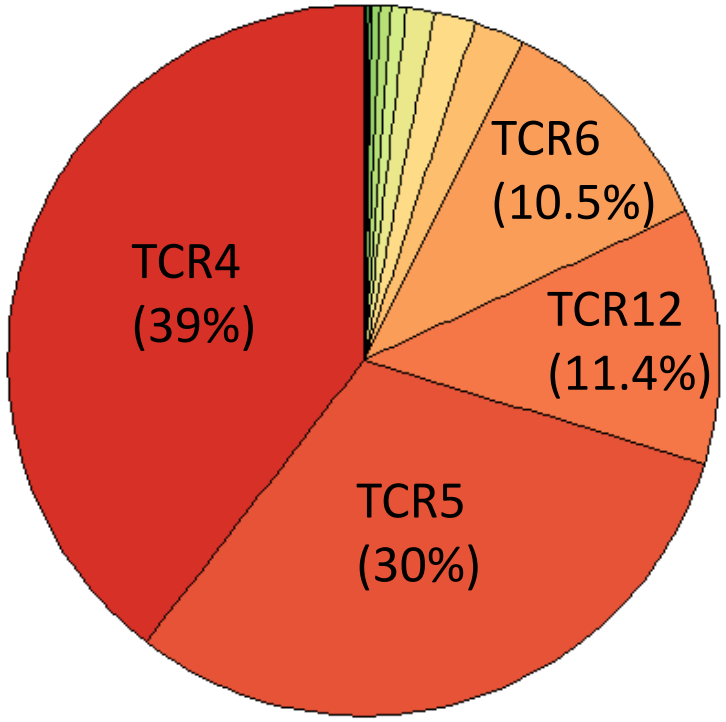
Single chain trimer (SCT) expression assay (Mamu-E)

Andrew McMichael (Oxford)

Point 2:

A relatively few supertope-reactive MHC-E-restricted TCR mediate SIV-infected cell recognition

SIV-infected cell recognition

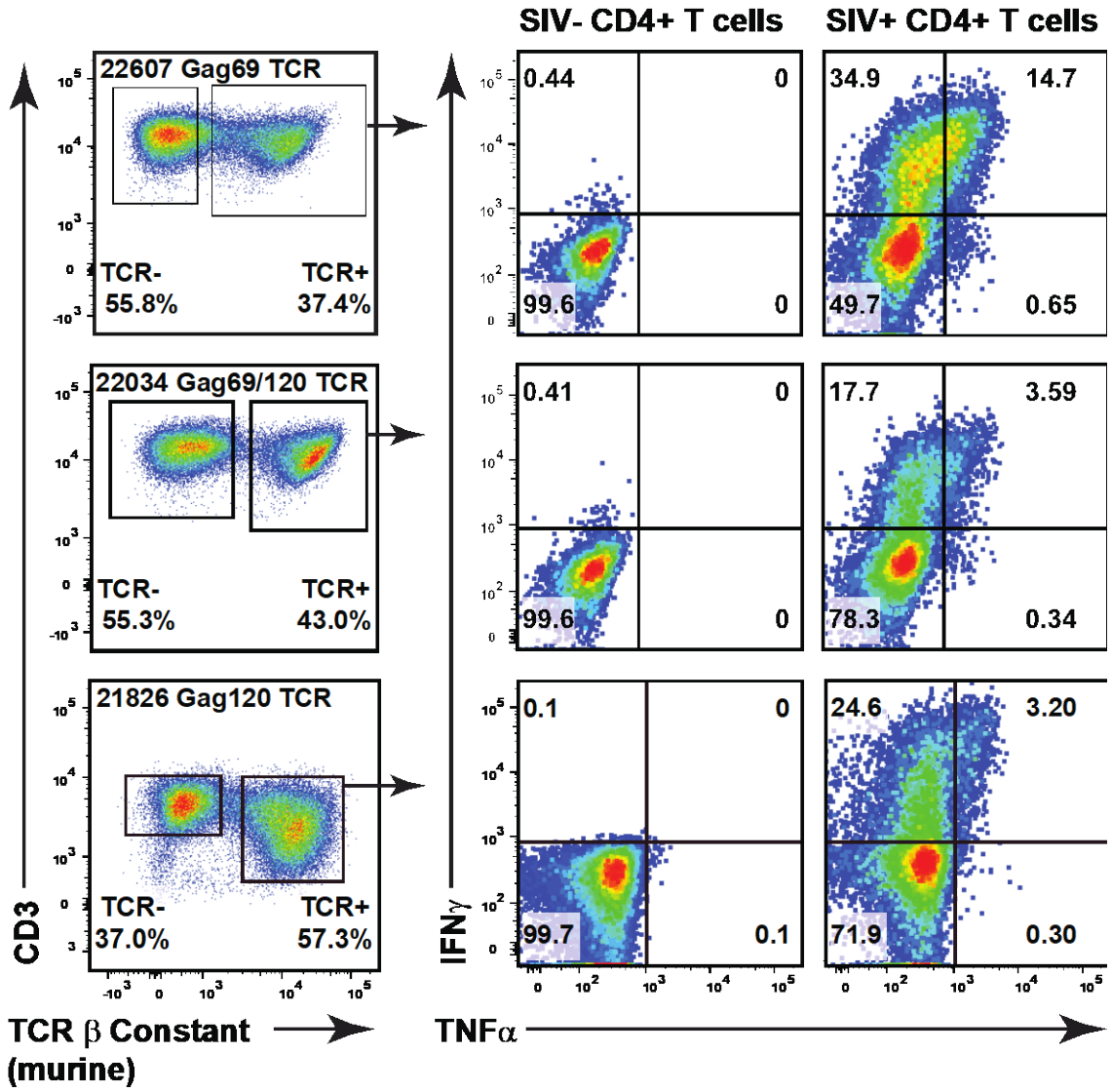


4 Clones Account for ~90% of SIV response!

22607 Gag69 TCR10

22034 Gag69/120 TCR5

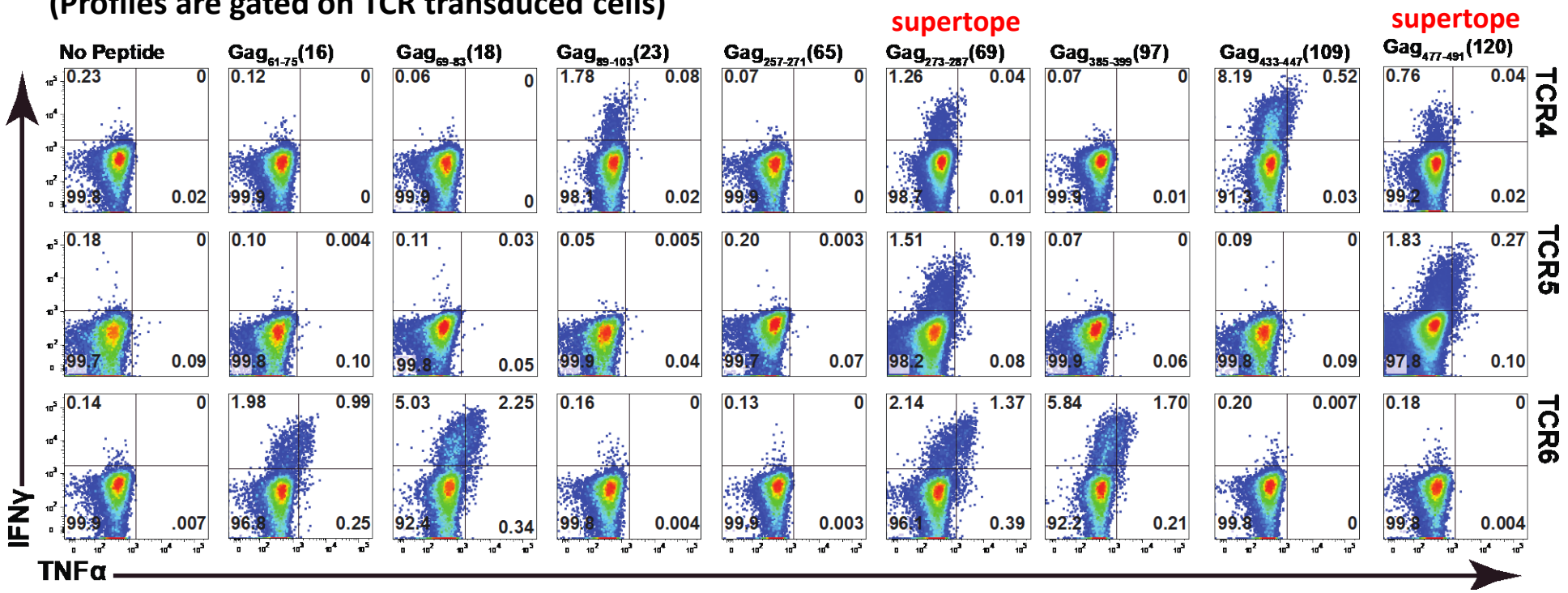
21826 Gag120 TCR2



Point 3: Almost universal cross-reactivity with structurally distinct peptides*

Analysis of TCR Transductants:

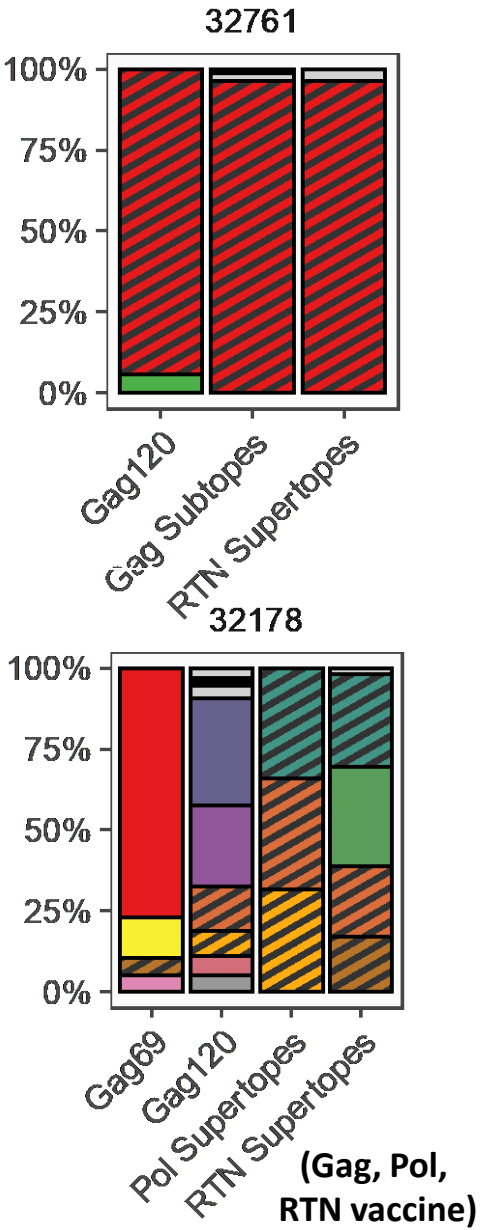
(Profiles are gated on TCR transduced cells)



TCR4 – Four Specificities
TCR5 – Two Specificities
TCR6 – Four Specificities

*one or more supertopes + variable subtopes

scRNAseq TCR analysis:

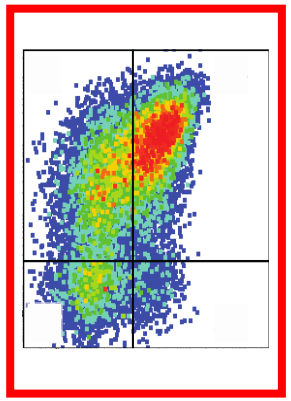


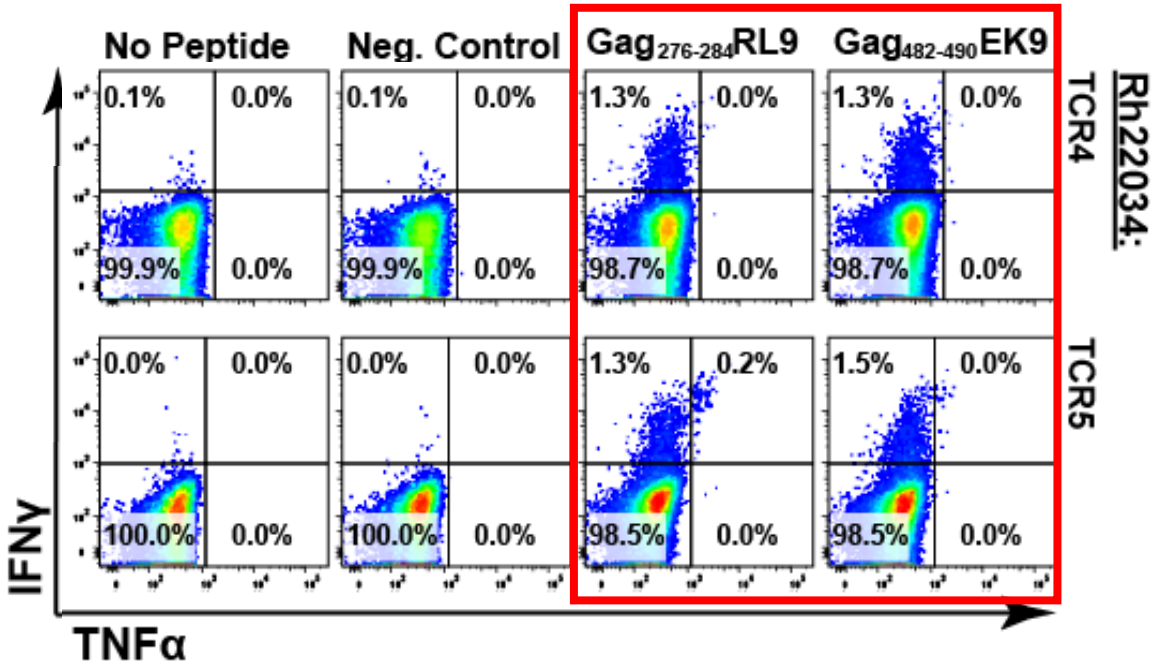
Thus, RhCMV vector-elicited CD8⁺ MHC-E-restricted T cells appear to achieve breadth by TCR cross-reactivity.

Point 4: Relatively low triggering efficiency of most MHC-E-restricted TCR

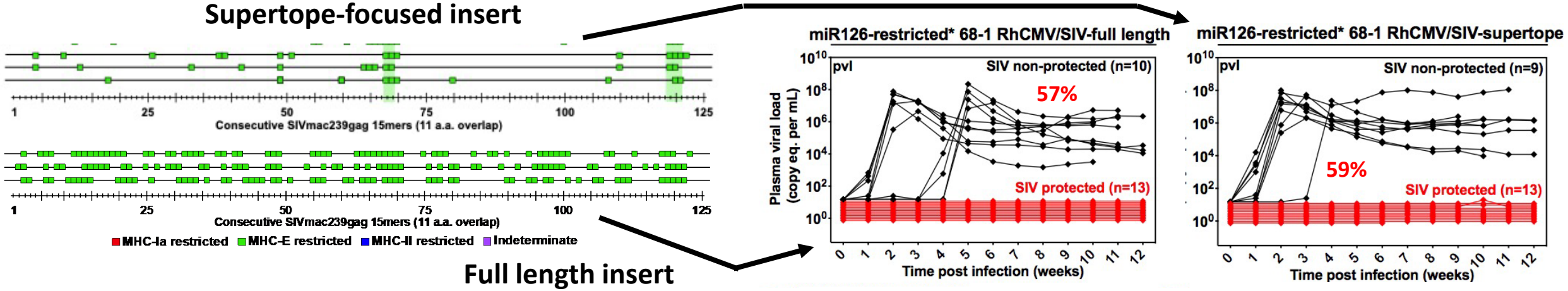
Conventional MHC-Ia-restricted TCR

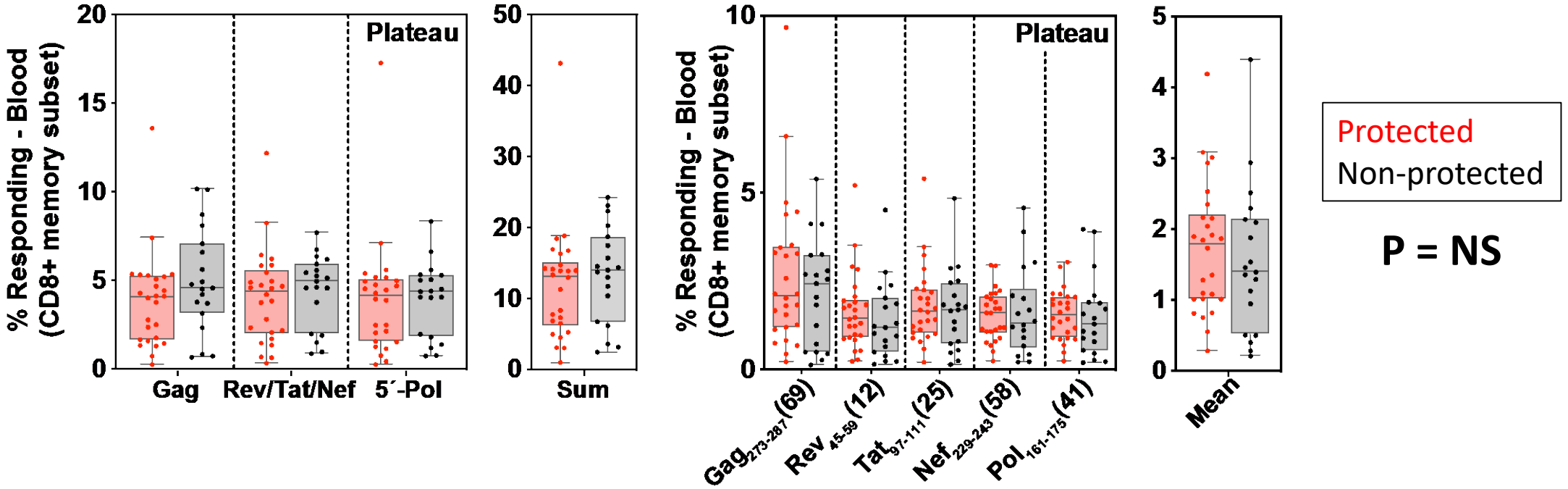
2 BLCL: GY9





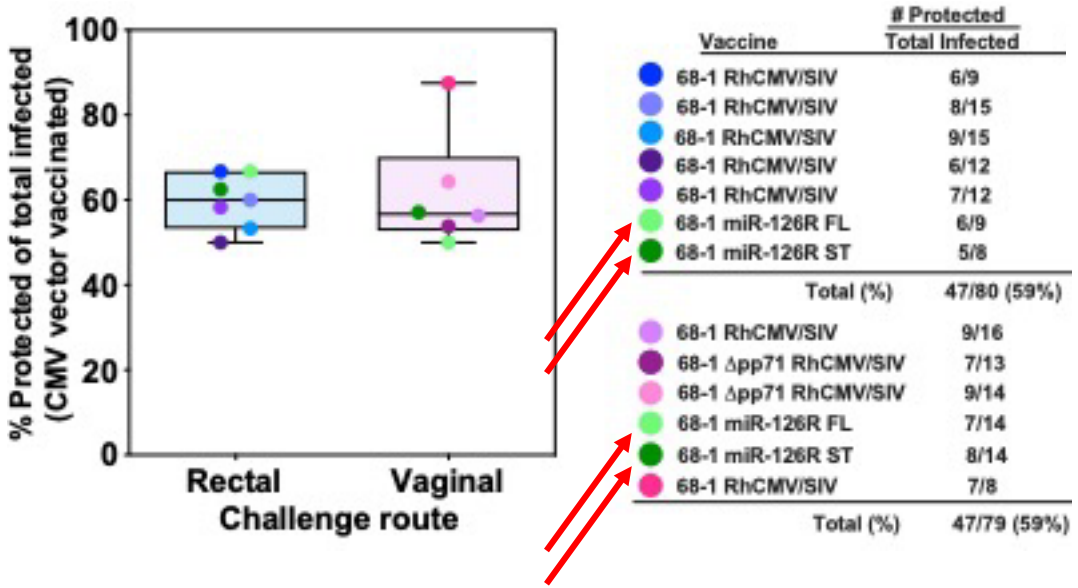
. . . Of note, there was no correlation between the breadth or magnitude of these MHC-E-restricted CD8+ T cell responses and outcome:





And, even when only MHC-E-restricted CD8s are induced, the level of efficacy was not increased over the original 68-1 vaccine . . .

Published cohorts:



Vaccine	# Protected / Total Infected
68-1 RhCMV/SIV	6/9
68-1 RhCMV/SIV	8/15
68-1 RhCMV/SIV	9/15
68-1 RhCMV/SIV	6/12
68-1 RhCMV/SIV	7/12
68-1 miR-126R FL	6/9
68-1 miR-126R ST	5/8
Total (%)	47/80 (59%)
68-1 RhCMV/SIV	9/16
68-1 Δpp71 RhCMV/SIV	7/13
68-1 Δpp71 RhCMV/SIV	9/14
68-1 miR-126R FL	7/14
68-1 miR-126R ST	8/14
68-1 RhCMV/SIV	7/8
Total (%)	47/79 (59%)

Intrarectal challenge (59% median overall efficacy)

Intravaginal challenge (59% median overall efficacy)

These observations suggest that while MHC-E-restricted CD8+ T cell responses are required for efficacy, additional immunologic determinants are also necessary . . .

Indeed, the Gale Lab has demonstrated that efficacy is significantly correlated with vaccine modulated immune-related genes in whole blood:

PLOS PATHOGENS

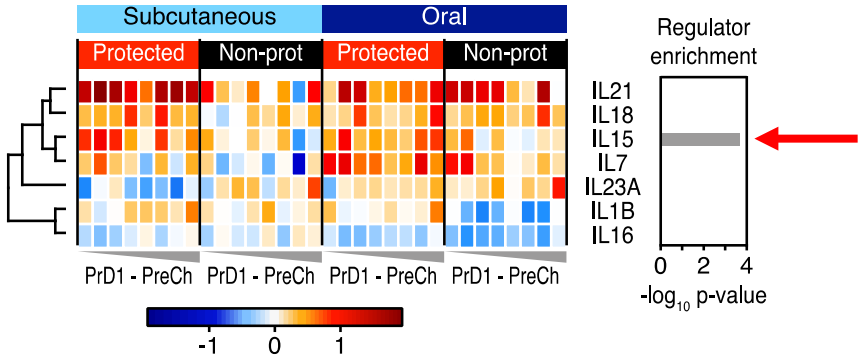
RESEARCH ARTICLE

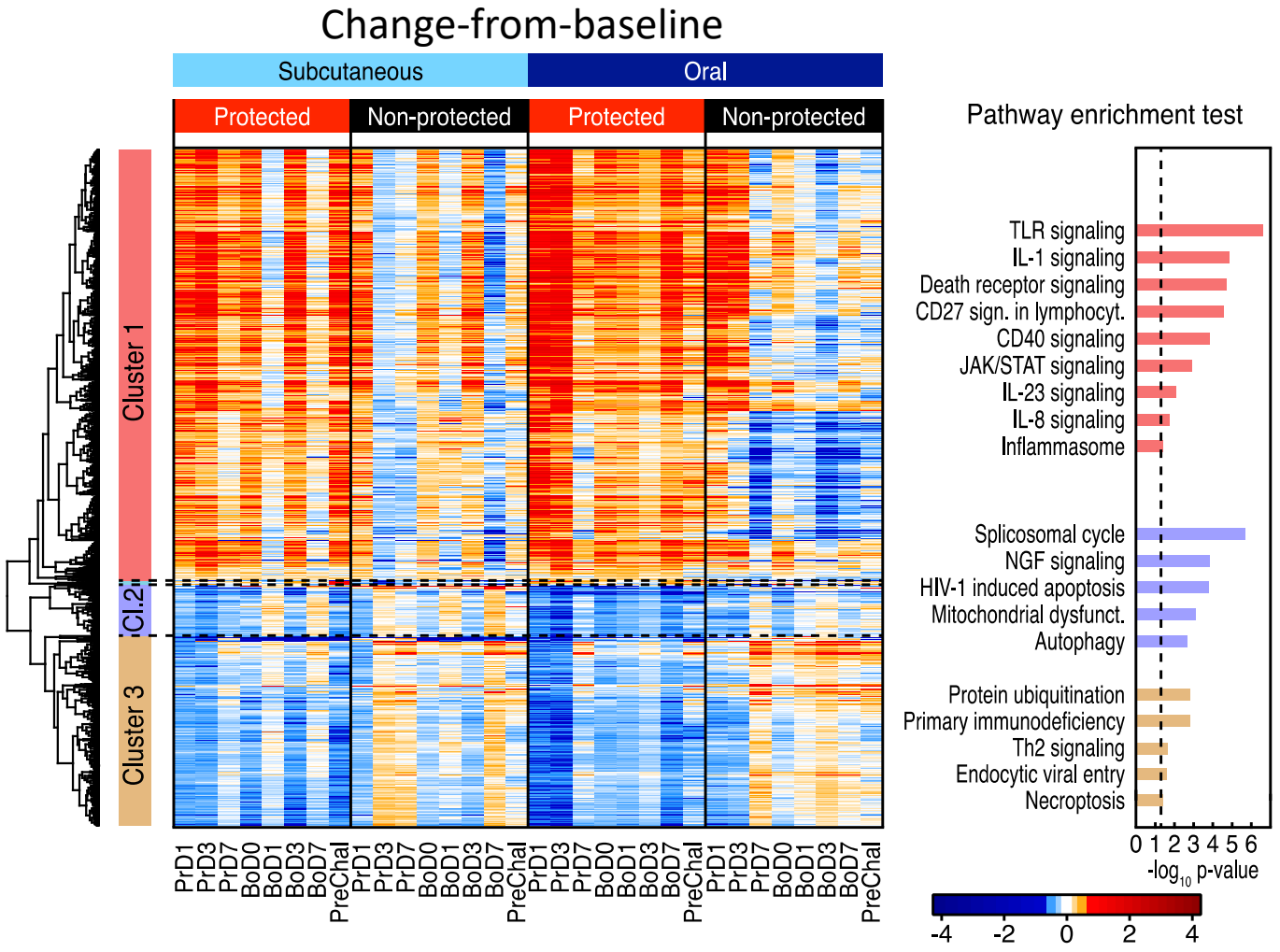
Interleukin-15 response signature predicts RhCMV/SIV vaccine efficacy

Fredrik Barrenäs[1], Scott G. Hansen[2], Lynn Law[3], Connor Driscoll[3], Richard R. Green[3], Elise Smith[3], Jean Chang[3], Inah Golez[3], Taryn Urion[3], Xinxia Peng[4], Leanne Whitmore[3], Daniel Newhouse[3], Colette M. Hughes[2], David Morrow[2], Kurt T. Randall[2], Andrea N. Selseth[2], Julia C. Ford[2], Roxanne M. Gilbride[2], Bryan E. Randall[2], Emily Ainslie[2], Kelli Oswald[5], Rebecca Shoemaker[5], Randy Fast[5], William J. Bosche[5], Michael K. Axthelm[2], Yoshinori Fukazawa[2], George N. Pavlakis[6], Barbara K. Felber[7], Slim Fourati[8], Rafick-Pierre Sekaly[8], Jeffrey D. Lifson[5], Jan Komorowski[1], Ewelina Kosmider[9], Danica Shao[9], Wenjun Song[9], Paul T. Edlefsen[9], Louis J. Picker[2]*, Michael Gale, Jr[3]*

1 Department of Cell and Molecular Biology, Uppsala University, Uppsala, Sweden, **2** Vaccine and Gene Therapy Institute and Oregon National Primate Research Center, Oregon Health & Science University, Beaverton, Oregon, United States of America, **3** Center for Innate Immunity and Immune Disease, Department of Immunology, University of Washington, Seattle, Washington, United States of America, **4** Department of Molecular Biomedical Sciences and Bioinformatics Research Center, North Carolina State University, Raleigh, North Carolina, United States of America, **5** AIDS and Cancer Virus Program, SAIC Frederick, Inc., Frederick National Laboratory, Leidos Biomedical Research, Inc., Frederick, Maryland, United States of America, **6** Human Retrovirus Section, Vaccine Branch, National Cancer Institute at Frederick, Frederick, Maryland, United States of America, **7** Human Retrovirus Pathogenesis Section, Vaccine Branch, National Cancer Institute at Frederick, Frederick, Maryland, United States of America, **8** Department of Pathology, Case Western Reserve University, Cleveland, Ohio, United States of America, **9** Statistical Center for HIV/AIDS Research and Prevention, Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center, Seattle, Washington, United States of America

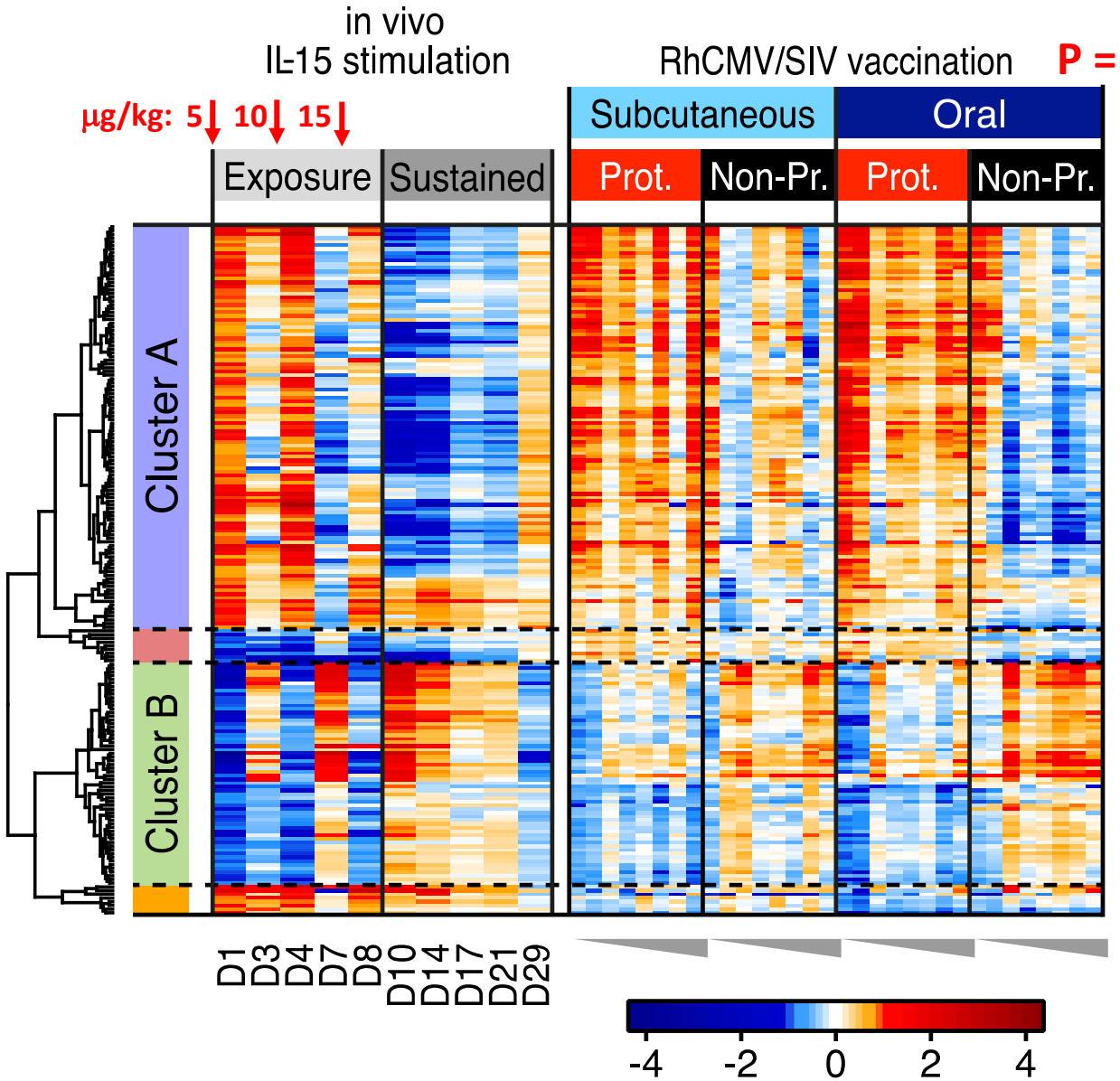
* pickerl@ohsu.edu (LP); mgale@uw.edu (MG)



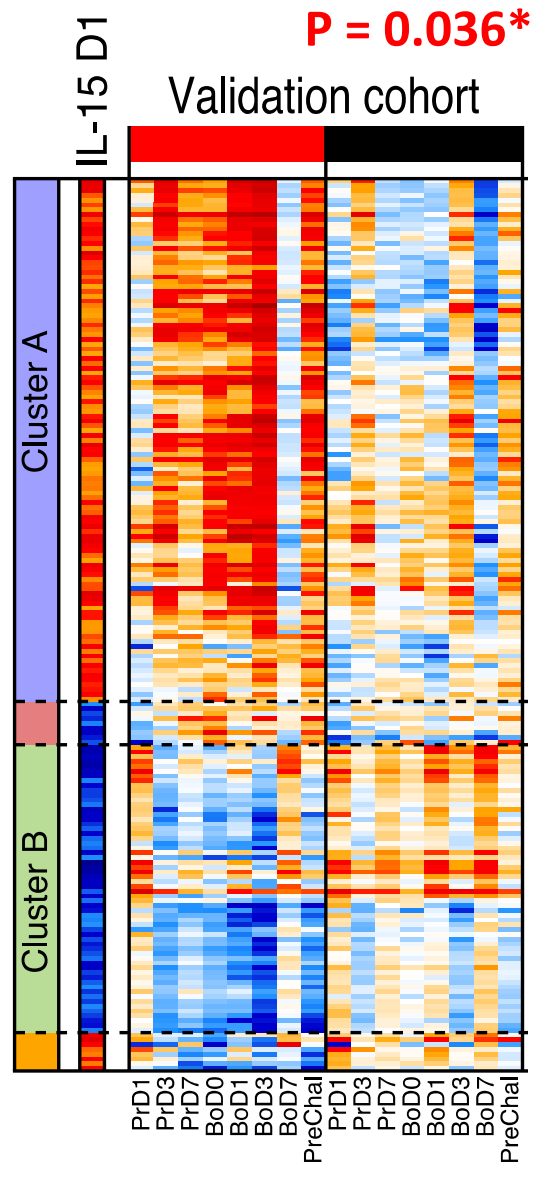


Protective signature is established early but must be maintained for protection to occur.

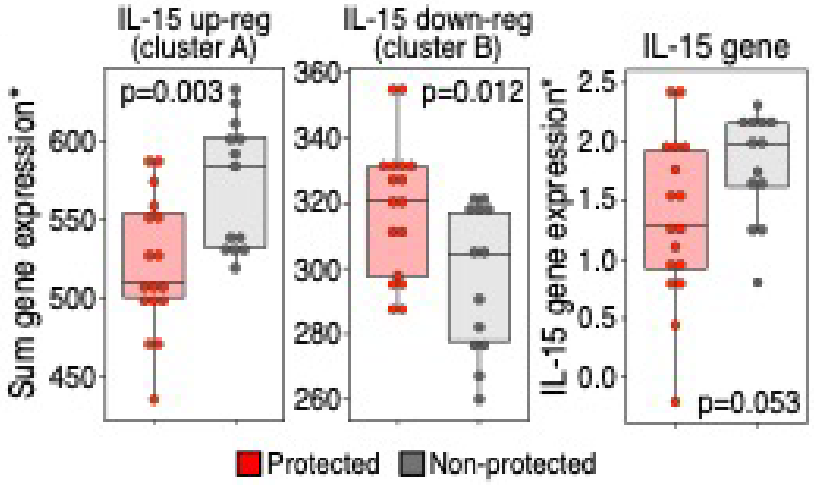
IL-15 signaling is a central component (node) of the protection-associated transcriptional signature, which is relevant since IL-15 is a primary regulator of effector memory T cell differentiation, homing and TCR triggering thresholds.



*permutation analysis
(P. Edlefsen/E. Kosminder)

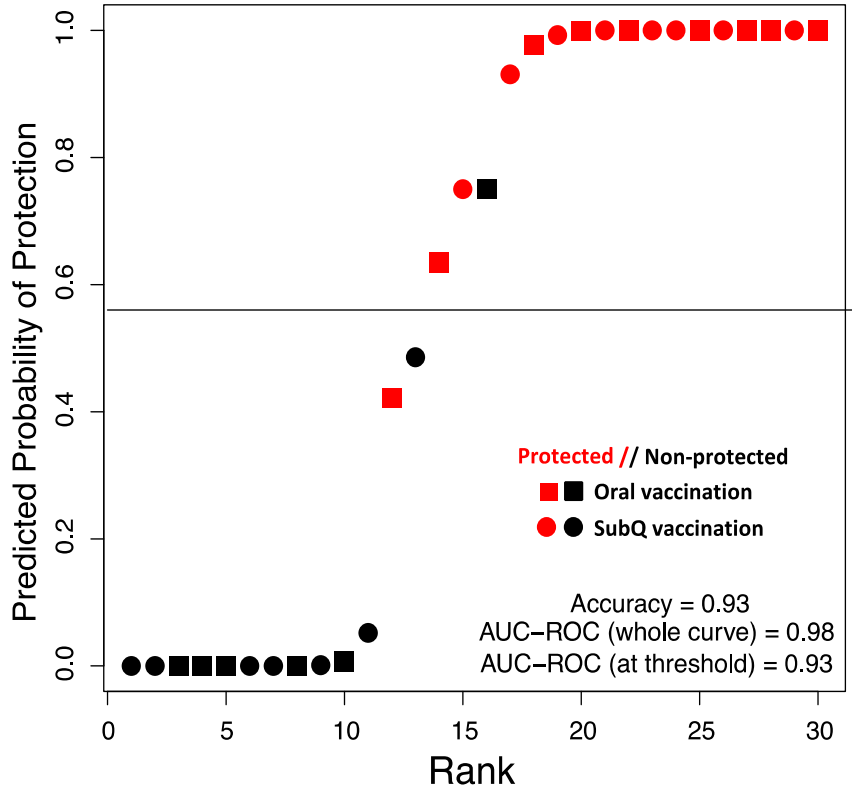


Of note, examining the IL-15 regulated gene clusters at baseline (pre-vaccination) revealed a "reversed" protection-associated expression signature . . .



. . . RM with lower pre-vaccination levels of IL-15 expression and IL-15 signaling tend to have higher induction of IL-15 signaling after vaccination and are more likely to be protected!

Indeed, a score based on baseline IL-15 signaling activity and absolute T + NK cell counts in blood predicts RhCMV/SIV vaccine efficacy (93%):



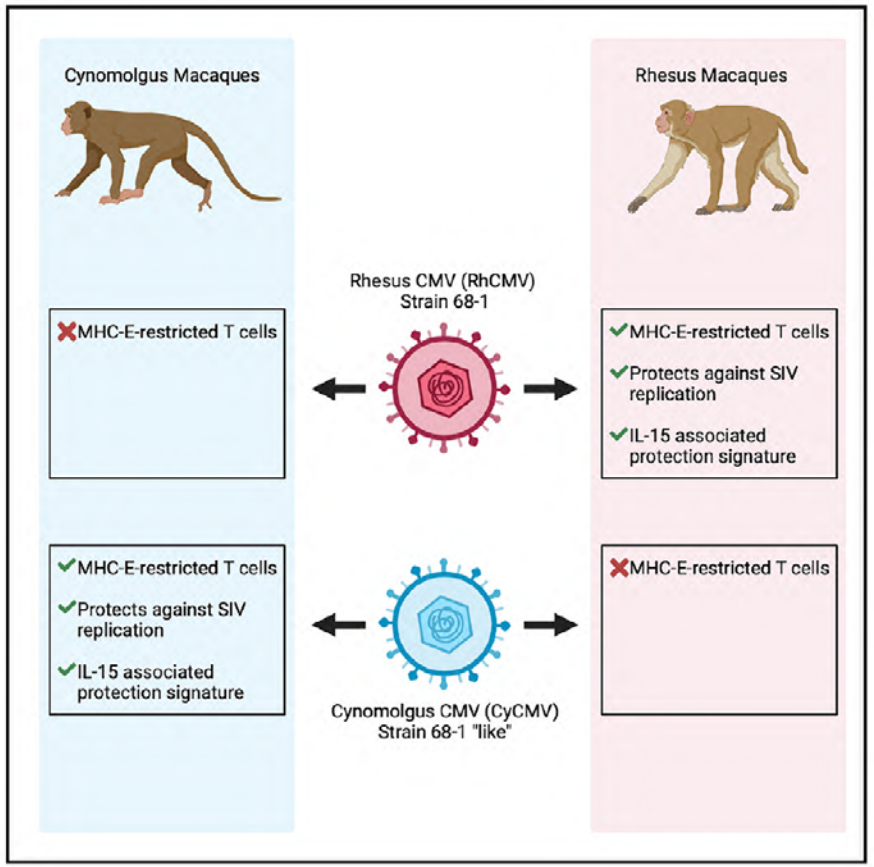
Thus, pre-vaccination IL-15 quiescence correlates with robust post-vaccination IL-15 signaling with both correlating with efficacy . . .

Translation to a second money species

Cell Host & Microbe

Cytomegalovirus-vaccine-induced unconventional T cell priming and control of SIV replication is conserved between primate species

Graphical abstract



Authors

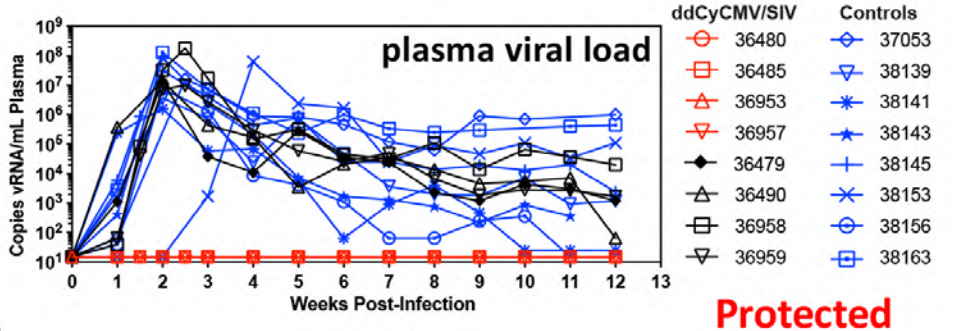
Daniel Malouli, Roxanne M. Gilbride, Helen L. Wu, ..., Louis J. Picker, Scott G. Hansen, Jonah B. Sacha

Correspondence

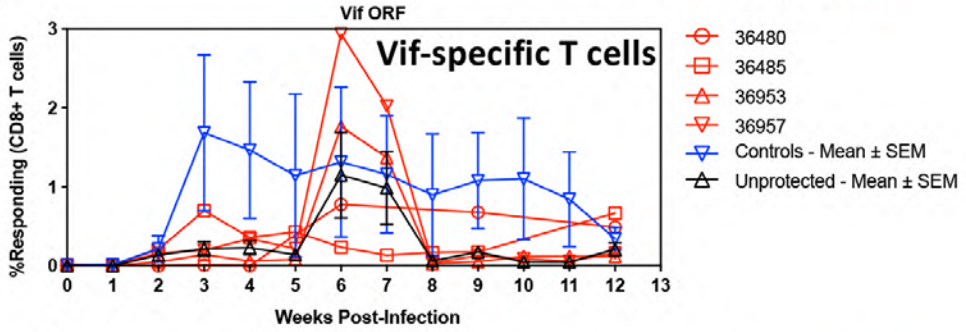
hansensc@ohsu.edu (S.G.H.), sacha@ohsu.edu (J.B.S.)

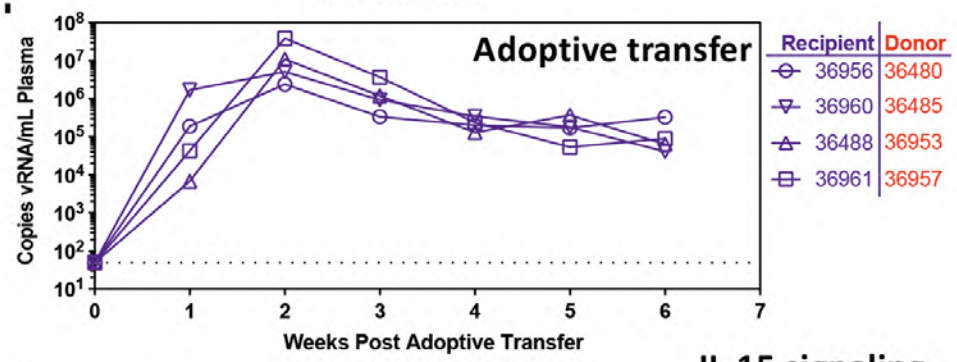
In brief

Malouli et al. show that the unusual MHC-E-restricted CD8+ T cells that are required for protection against simian immunodeficiency virus (SIV) observed in rhesus macaques vaccinated with rhesus CMV/SIV vectors is a fundamental property of primate CMVs. A protection-associated IL-15 signature is also conserved, and this finding highlights the promise of this vector for prophylactic HIV vaccine development.

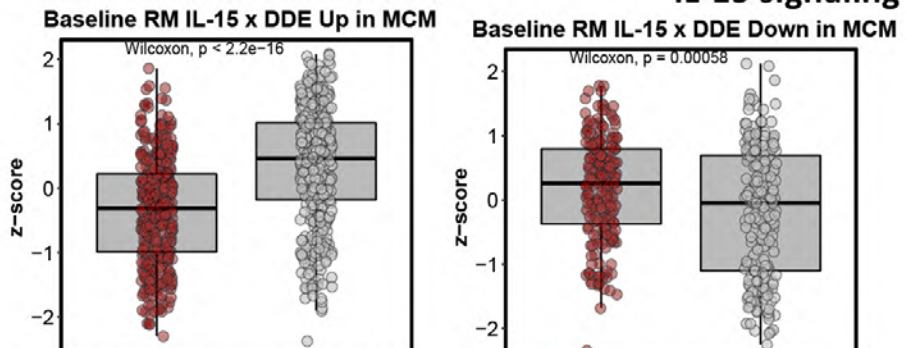


Protected





IL-15 signaling



Replication arrest efficacy therefore appears to require the following:

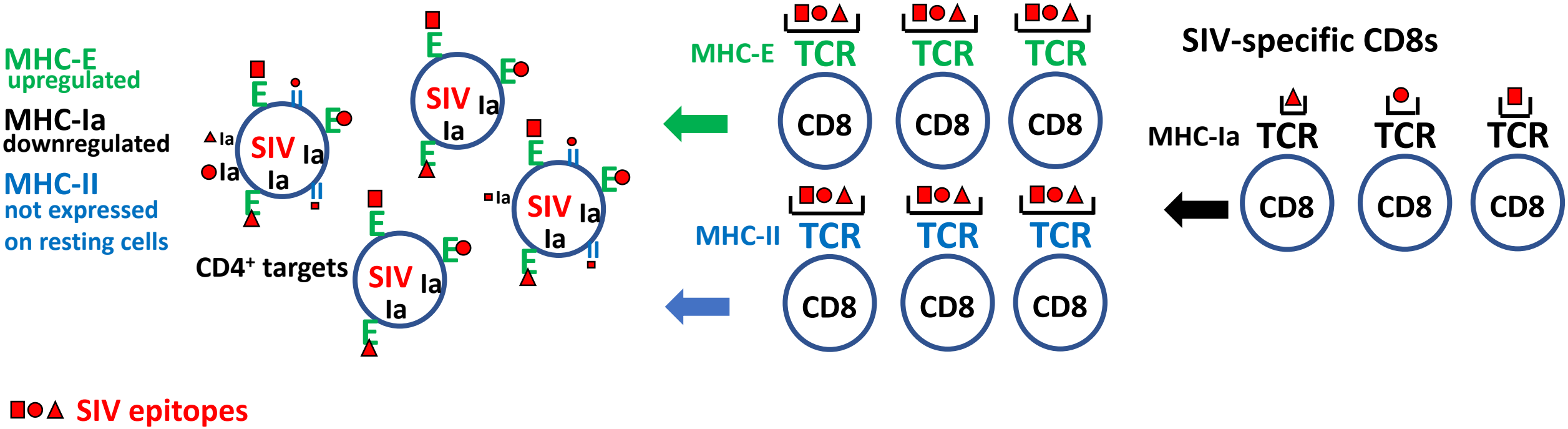
1. **MHC-E-restricted CD8$^+$ T cells that have the following characteristics:**
   - **Recognition of supertope(s) ± variable other epitopes that are "loosely" MHC-E-bound**
   - **Relatively few, low avidity, highly cross-reactive SIV-specific TCR**
   - **High magnitude in intercept tissues; effector differentiated (T_{EM})**
2. **Sustained innate immune response to vaccination that features IL-15 signaling.**

These features suggest that RhCMV/SIV-stimulated induction and maintenance of "excess" IL-15 signaling might be necessary for functional "competency" of MHC-E-restricted CD8$^+$ T cells, possibly in terms of the TCR triggering thresholds, tissue migration and anti-viral effector programs needed for efficacy.

But why are MHC-E-restricted CD8$^+$ T cells, with their relatively weak response characteristics, uniquely capable of SIV replication arrest?

Speculative (but Testable) Possibilities:

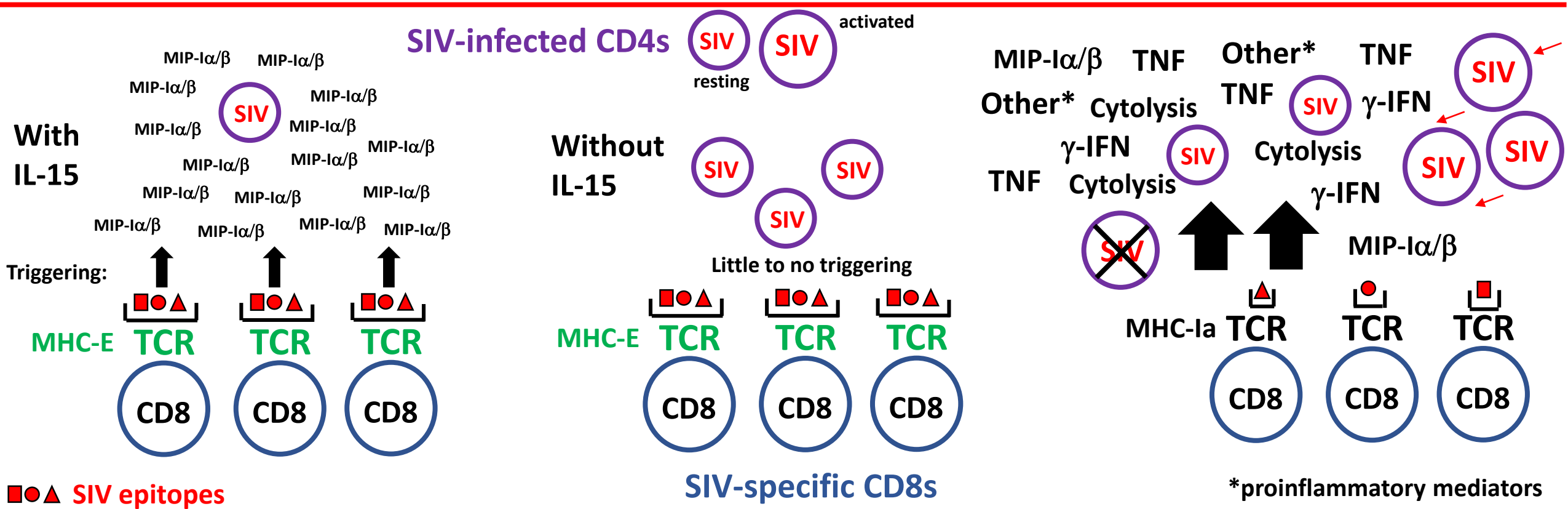
1. **Better SIV-infected cell recognition efficiency in primary infection?**
   - Perhaps related to MHC-E upregulation by infected cells to escape NK cells vs. MHC-Ia-downregulation and inconsistent MHC-II expression on infected cells? or to,
   - TCR cross-reactivity



Speculative (but Testable) Possibilities:

2. A more effective functional profile due to characteristics of TCR recognition of MHC-E presented epitopes (low avidity with abundant cross-reactivity)

- Differential CD8⁺ T cell differentiation (epigenetic modulation?) during vaccine phase and/or
- Low avidity recognition leading to induction of infection spread-suppressing mechanisms without the "excess" immune activation that would facilitate SIV replication and spread efficiency.
- IL-15 signaling might be required to enable the proper responses, perhaps by enabling the low avidity TCR to "fire" *in vivo* to otherwise tune homing/migration and/or the effector response



Trends in
Immunology

CellPress
OPEN ACCESS

Feature Review

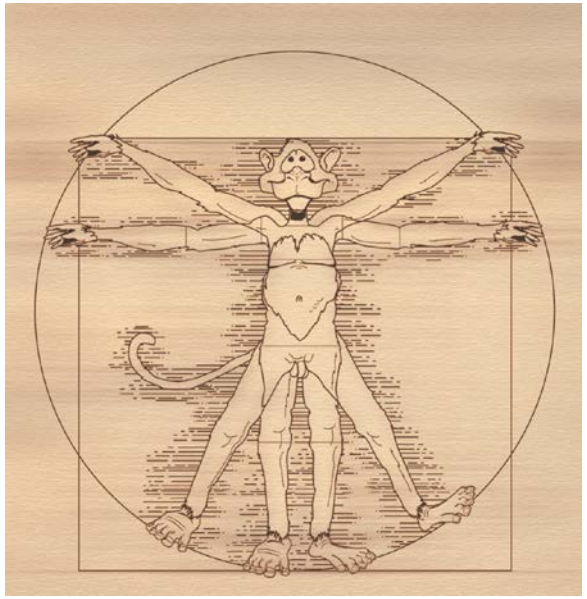
Programming cytomegalovirus as an HIV vaccine

Louis J. Picker [1,*] Jeffrey D. Lifson,[2] Michael Gale, Jr,[3] Scott G. Hansen,[1] and Klaus Früh[1]

Up-to-date review of the biology of CMV vectored HIV/SIV vaccines.

Will a properly engineered HCMV vector recapitulate the unique biology and immunogenicity of RhCMV vectors in humans?

RhCMV/SIV$_{cons.}$ Vir-1388 ortholog



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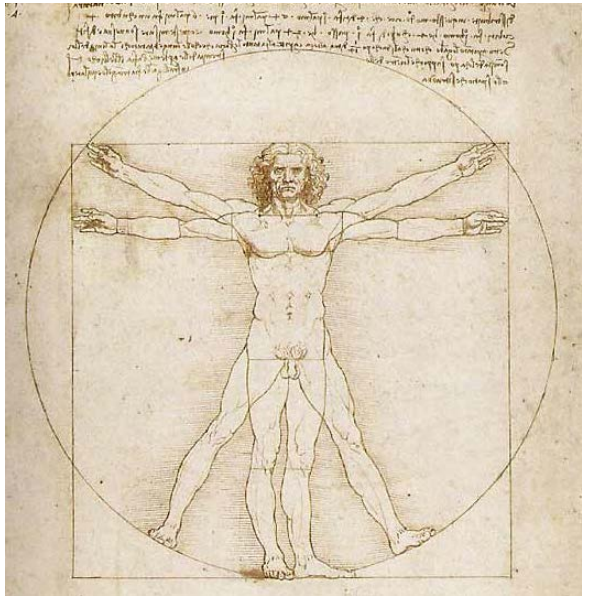
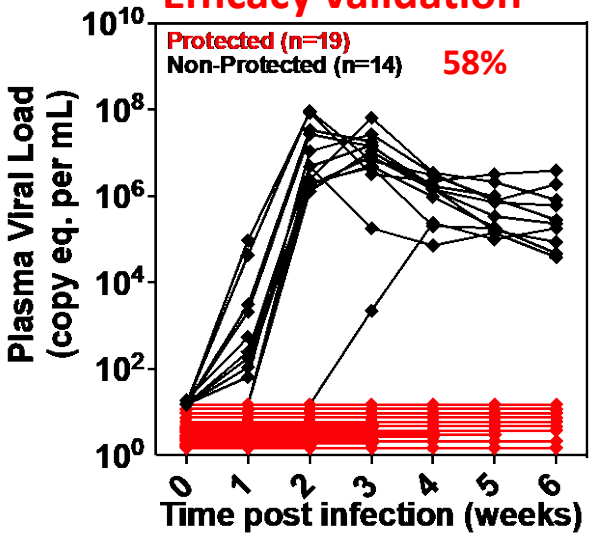


Vir-1388

ΔUL128/130; ΔUL146/147; ΔUL18 HCMV expressing conserved region HIVgag/pol/nef global episensus insert

Efficacy validation



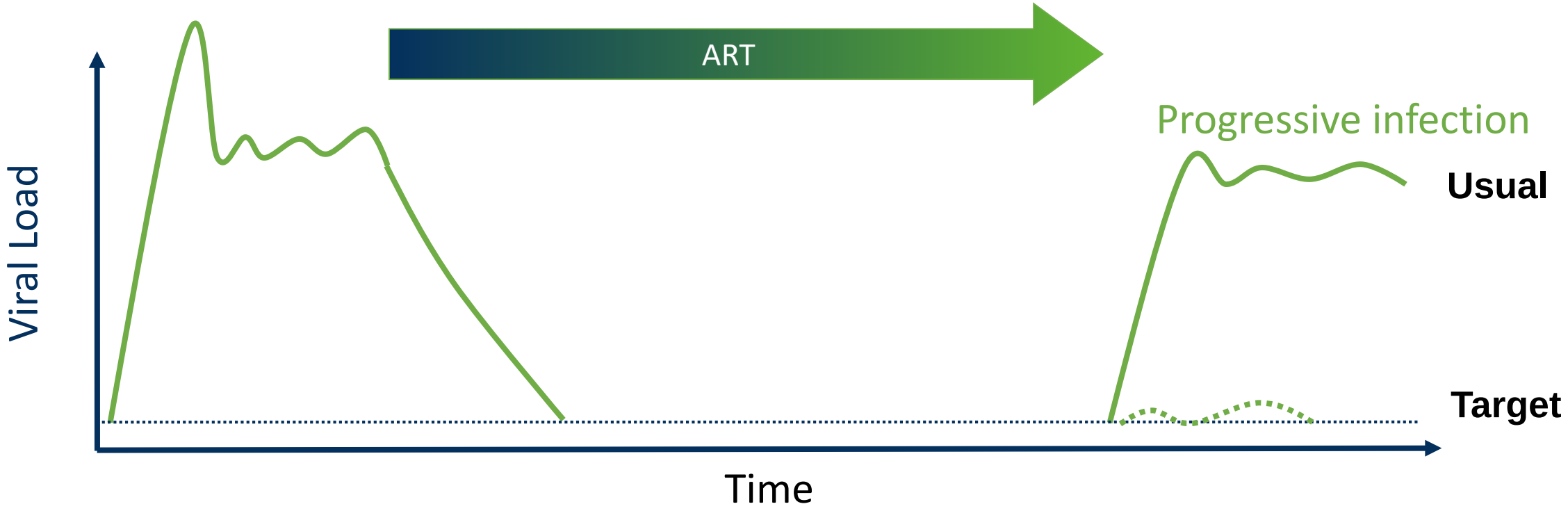
HVTN-142

Opened 9/20/23

To Investigate Safety, Reactogenicity and Immunogenicity of VIR-1388 Compared With Placebo in Participants Without HIV

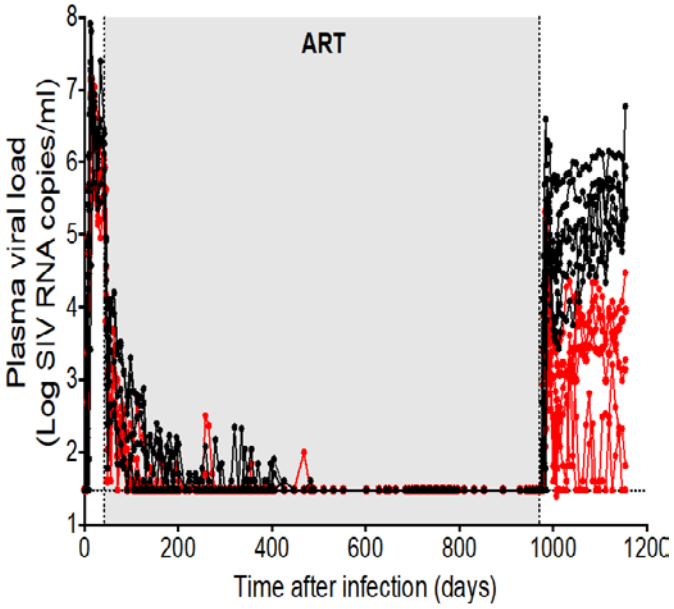
ClinicalTrials.gov Identifier: NCT05854381

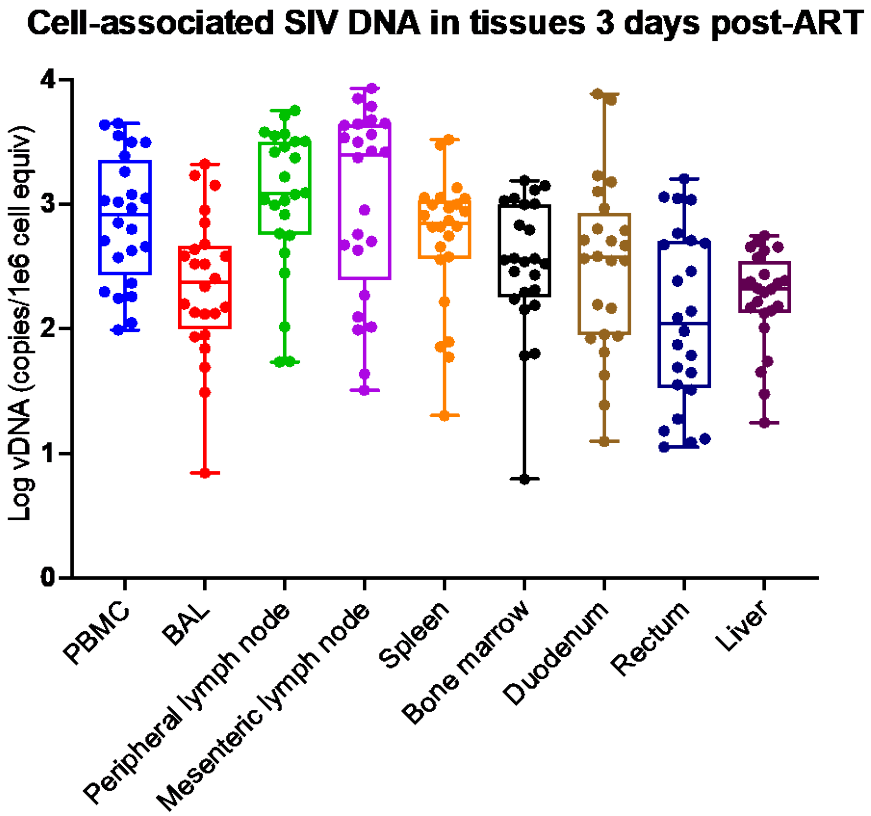
Switching gears . . . From prevention to therapy

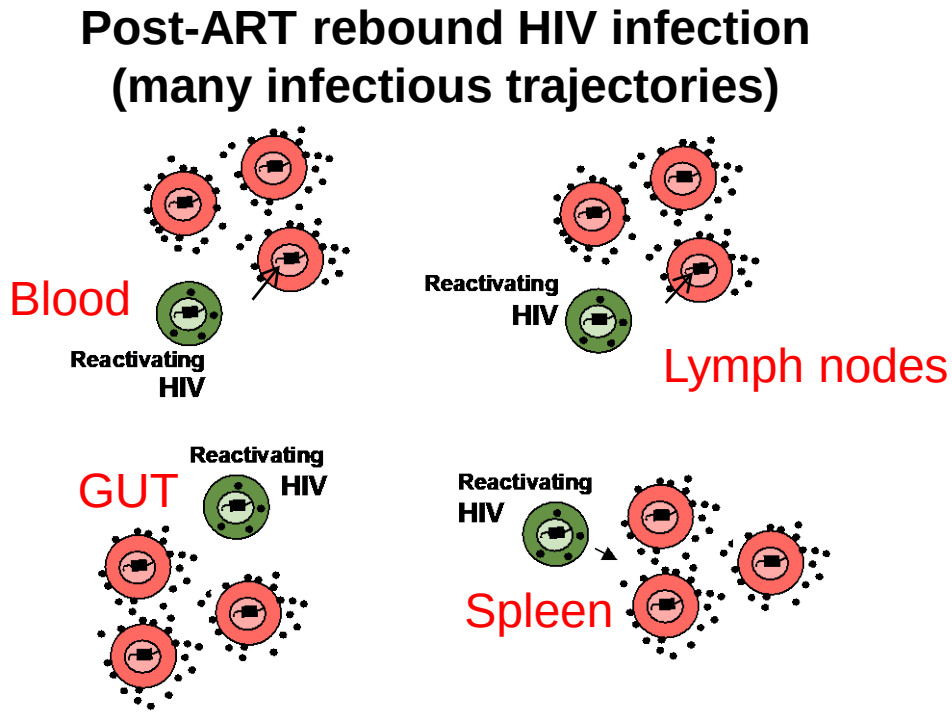


Can we abrogate (or stringently control) HIV/SIV rebound after ART discontinuation?

Post-ART viral rebound is a very different scenario than primary infection.

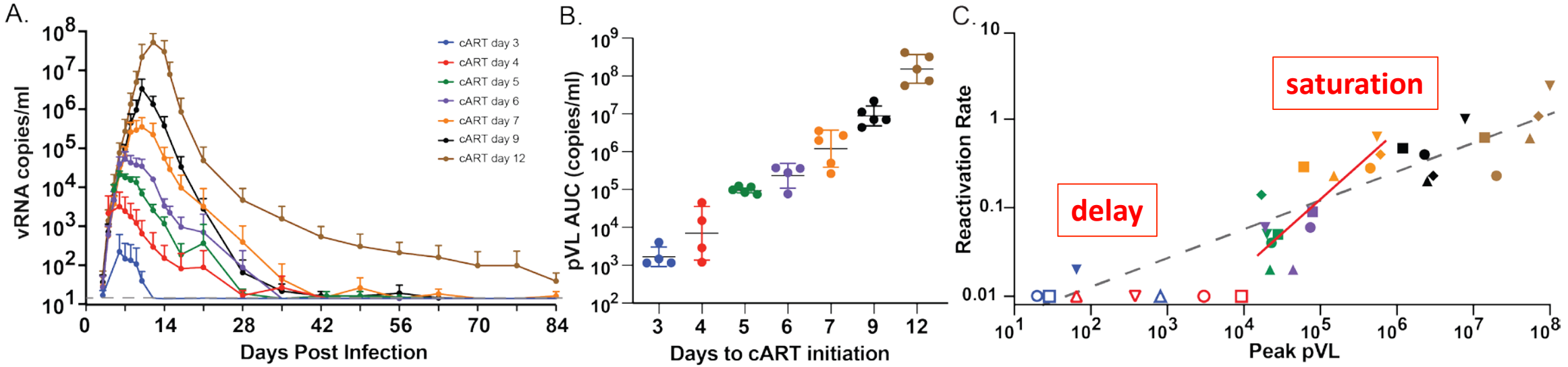


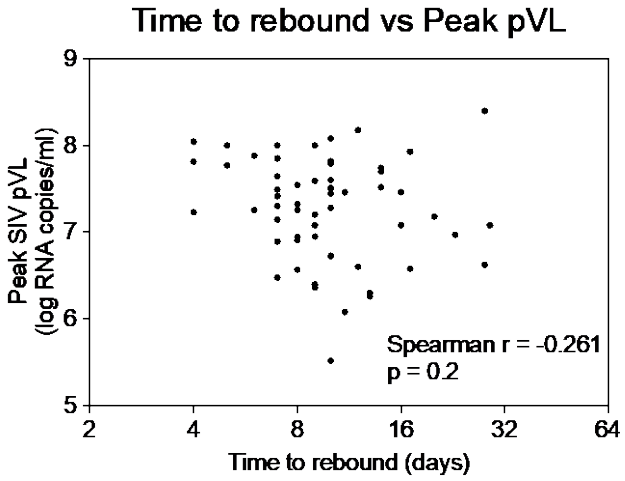


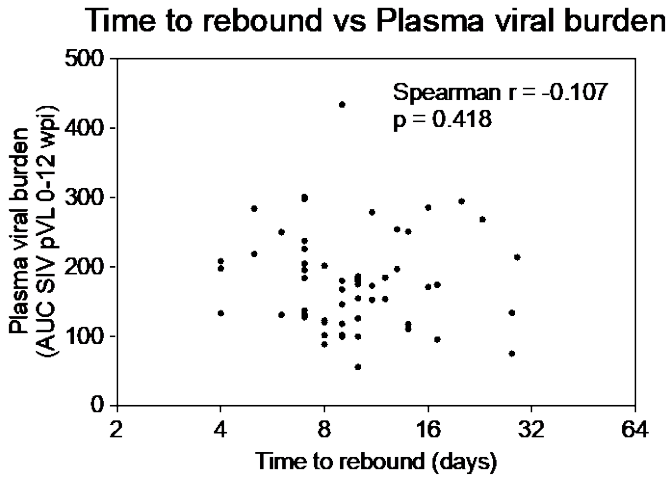


Systemic infection following ART release arises from a widely distributed, long-lived viral reservoir, resulting in multiple and continuous infectious trajectories.

Using barcoded SIV, we have also precisely defined development of the “rebound-competent” SIV reservoir and have defined rebound dynamics of “full” reservoir:

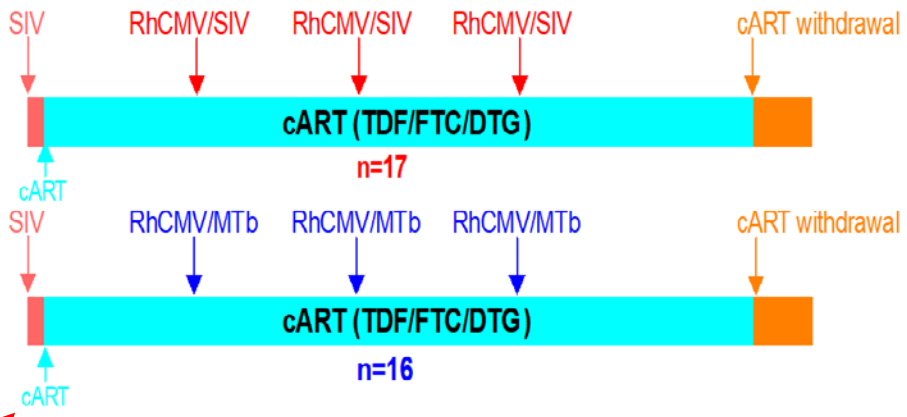




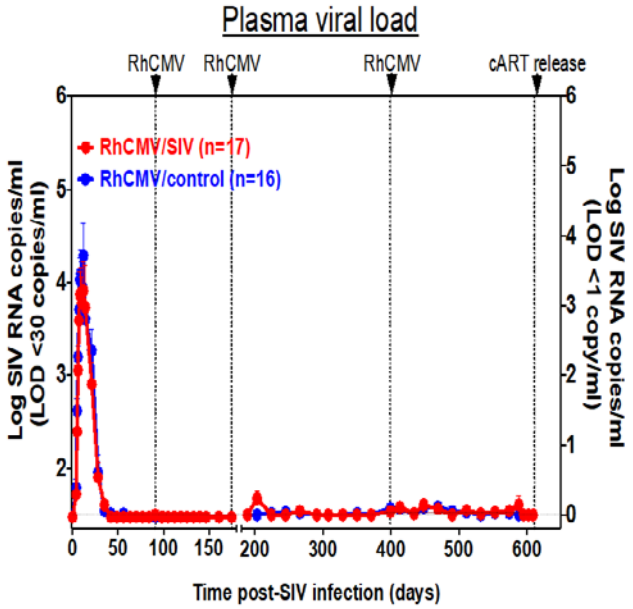


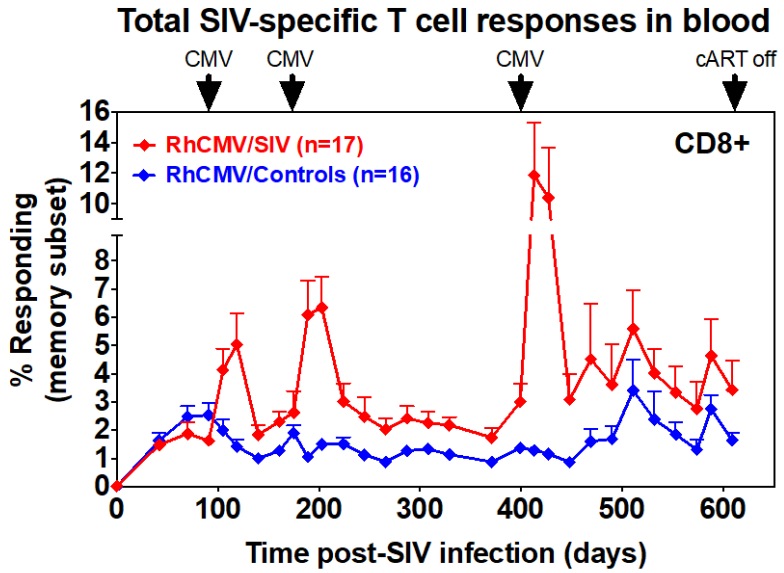
”Full” Reservoir

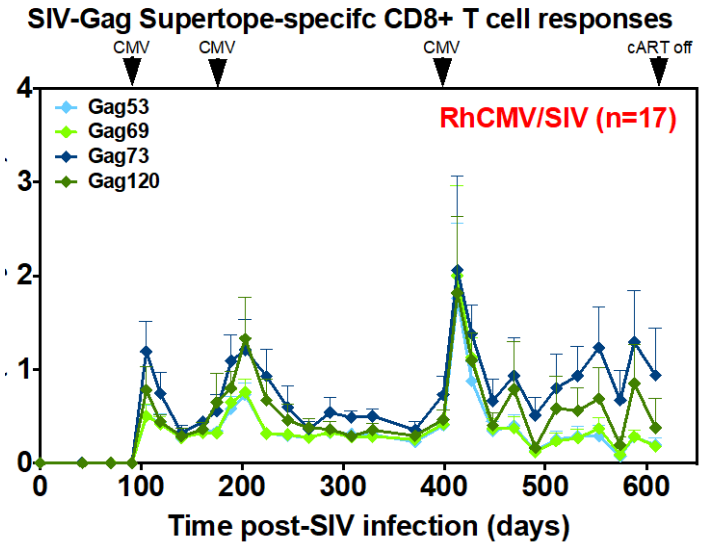
First Question: Would 68-1 RhCMV/SIV vaccination work as therapeutic HIV vaccine?



(Subgroups with ART initiation at days 4/5, 6, 7, 8/9)

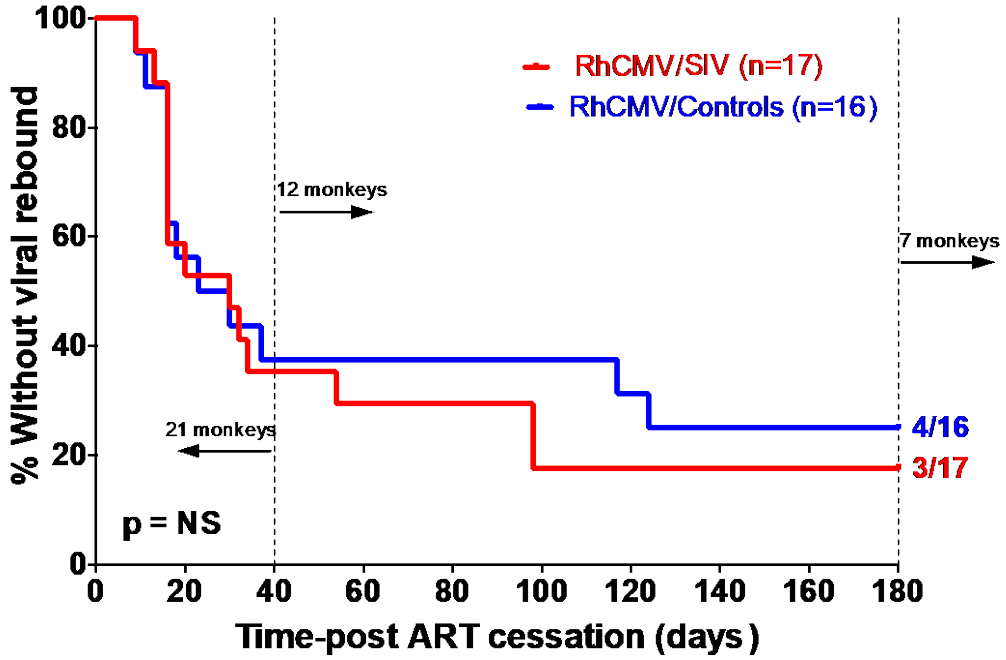


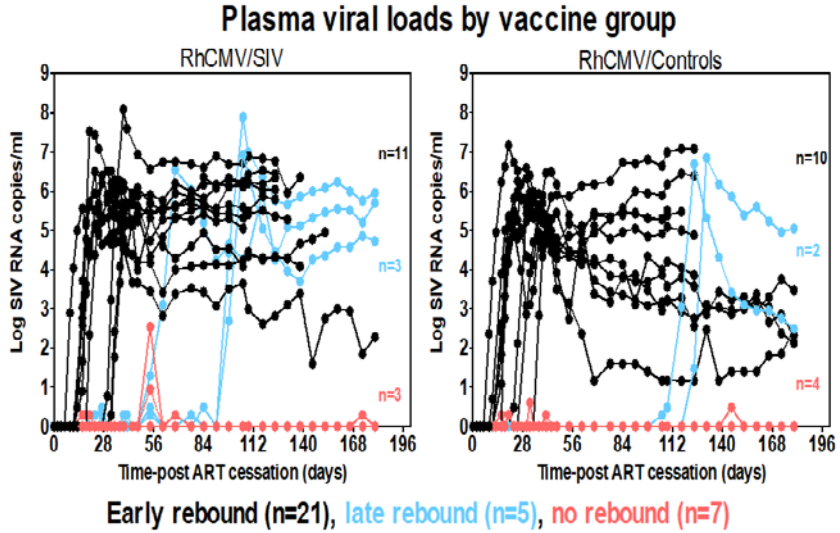




But, unfortunately, we saw no difference in time to viral rebound (or viral set point) post ART cessation in the SIV-specific vs. control vaccinated groups . . .

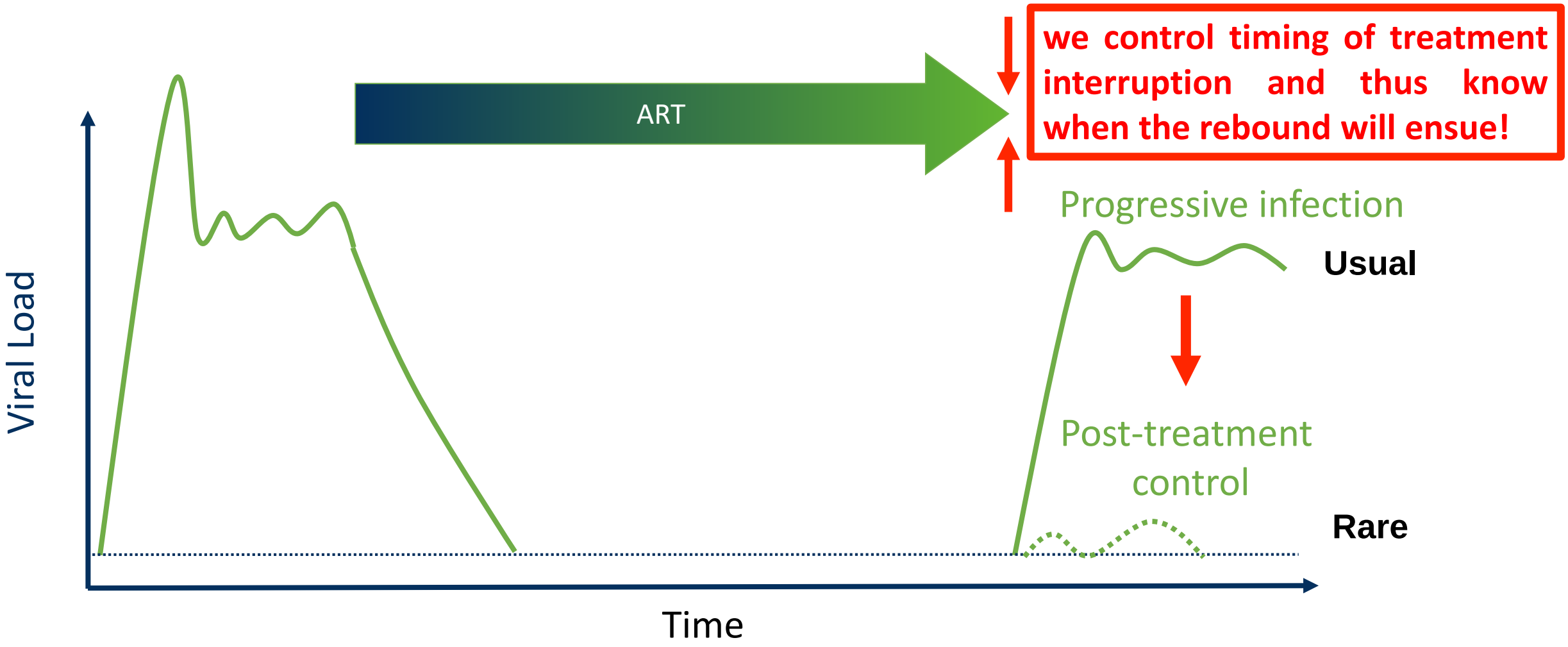
No evidence of replication arrest efficacy





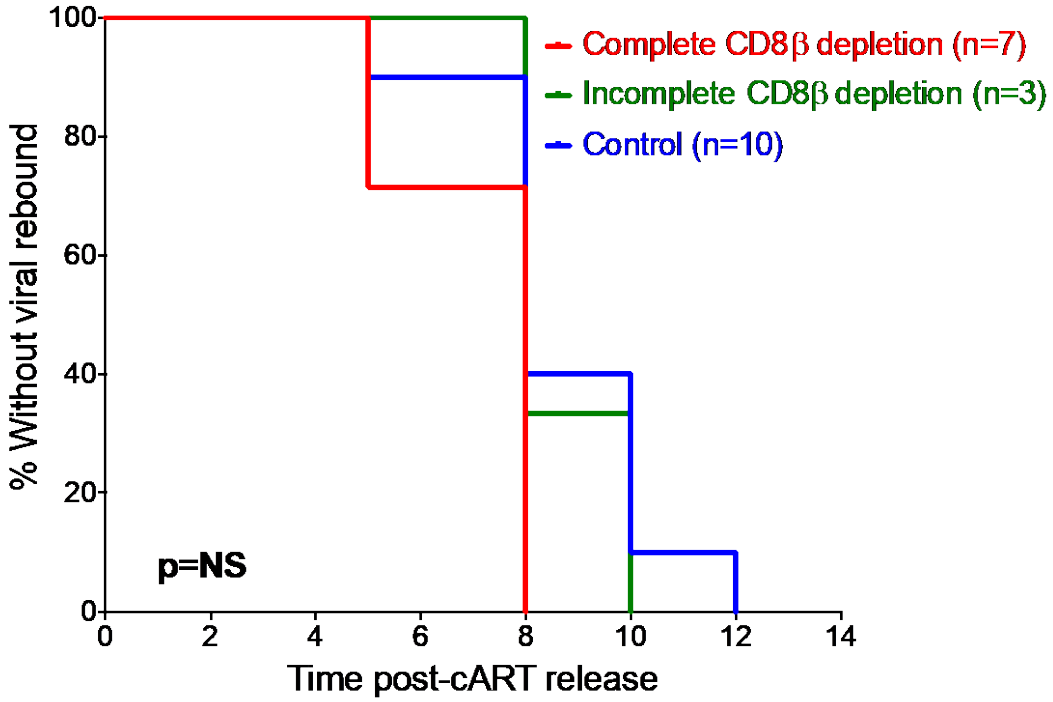
Whether this failure or RhCMV/SIV vectors is due to failure of more subtle aspect of immunogenicity (e.g. IL-15 programming) or simply to the number/diversity of infectious foci in post-ART rebound has not been determined, but as a practical matter, we are back to exploring optimization of conventional responses for immunotherapy of post-ART viral rebound.

Post-ART immune control has one big advantage over vaccine development for prevention or stringent suppression of new (primary) infection . . .

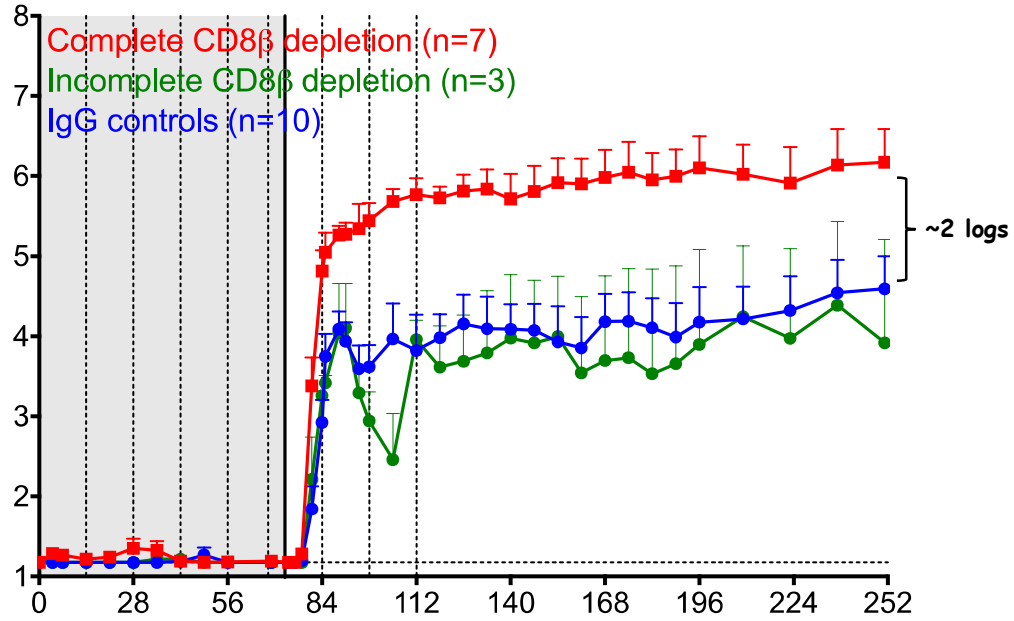


And, as previously mentioned, we have shown that the responses that develop during ART mediate a 2-log reduction in viral load setpoint, although have no effect on time-to-rebound.

Time-to-rebound



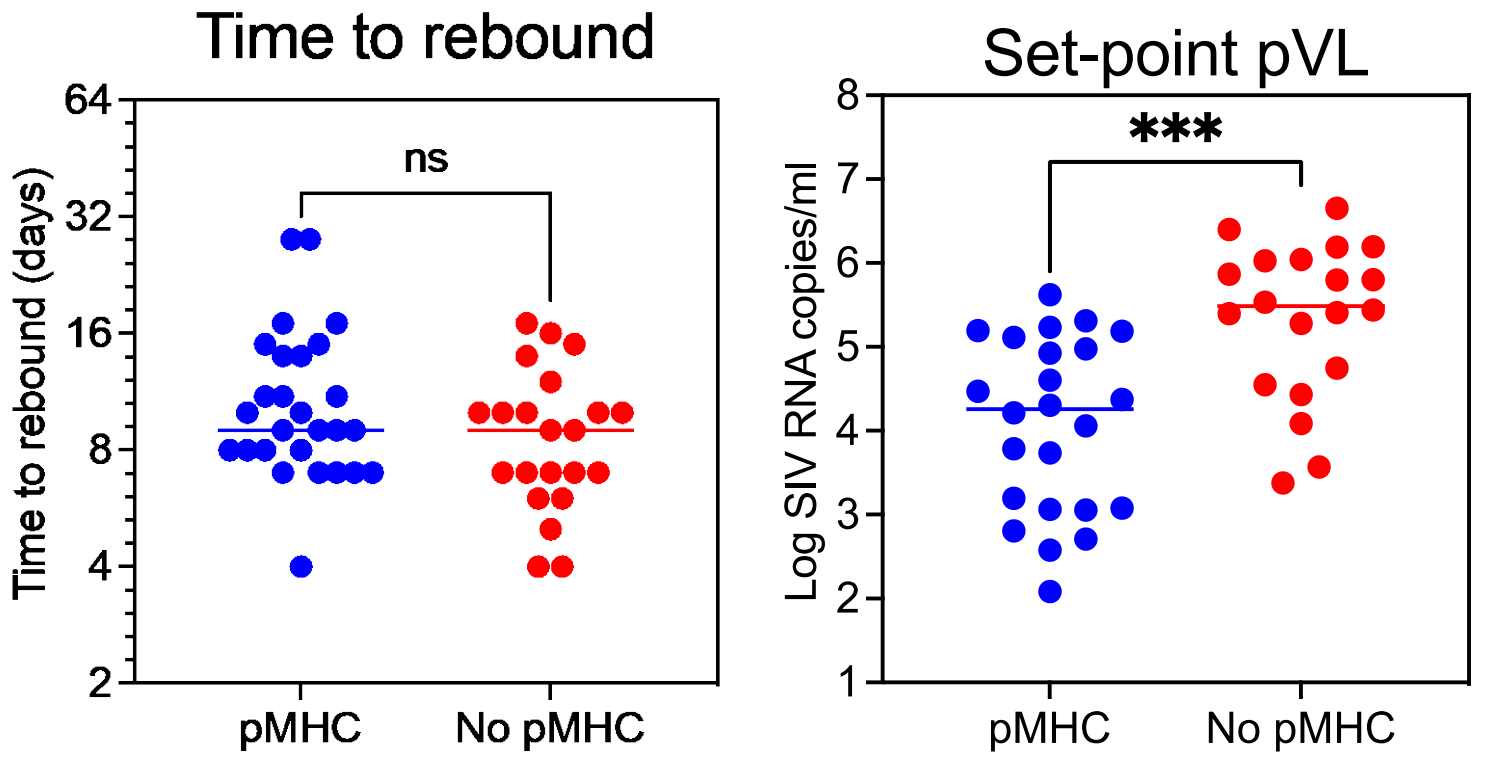
Plasma viral loads



No effect

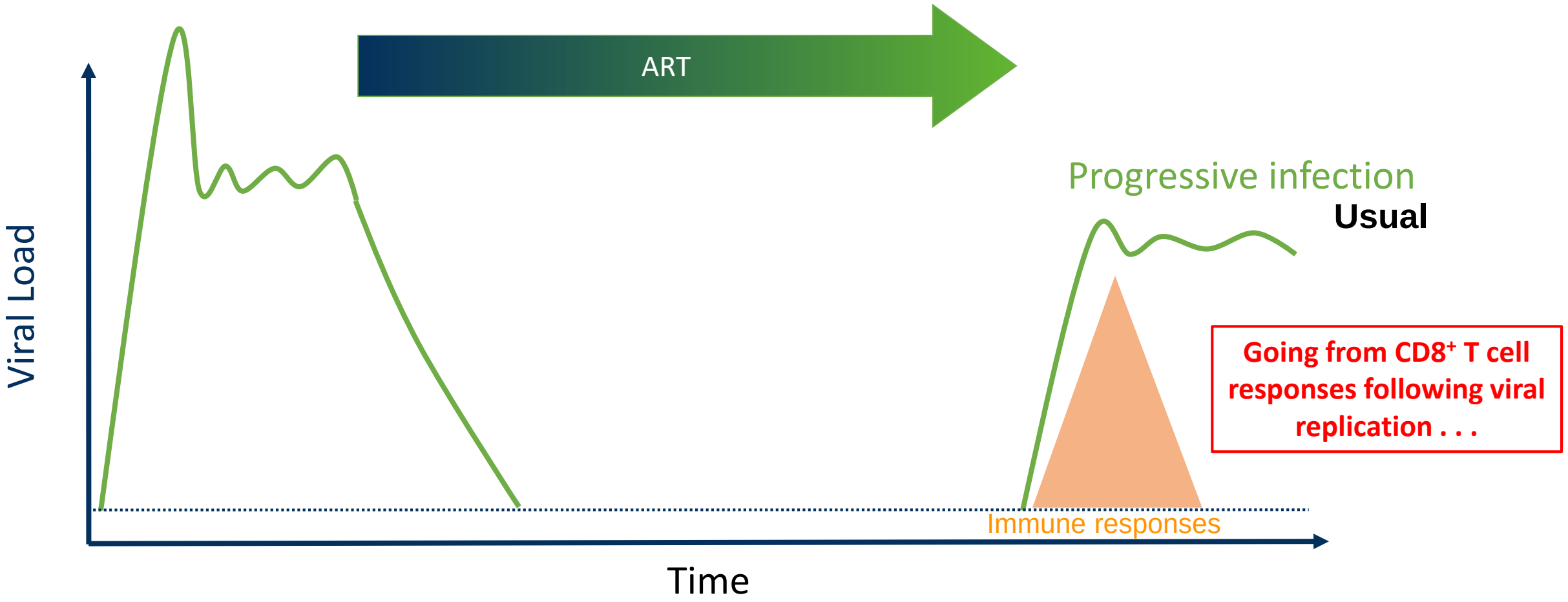
2 log reduction in plateau phase

In keeping with the CD8$^+$ T cell depletion data, protective MHC-Ia alleles are not correlated with time-to-rebound, but are correlated with better long-term post-ART viral control.



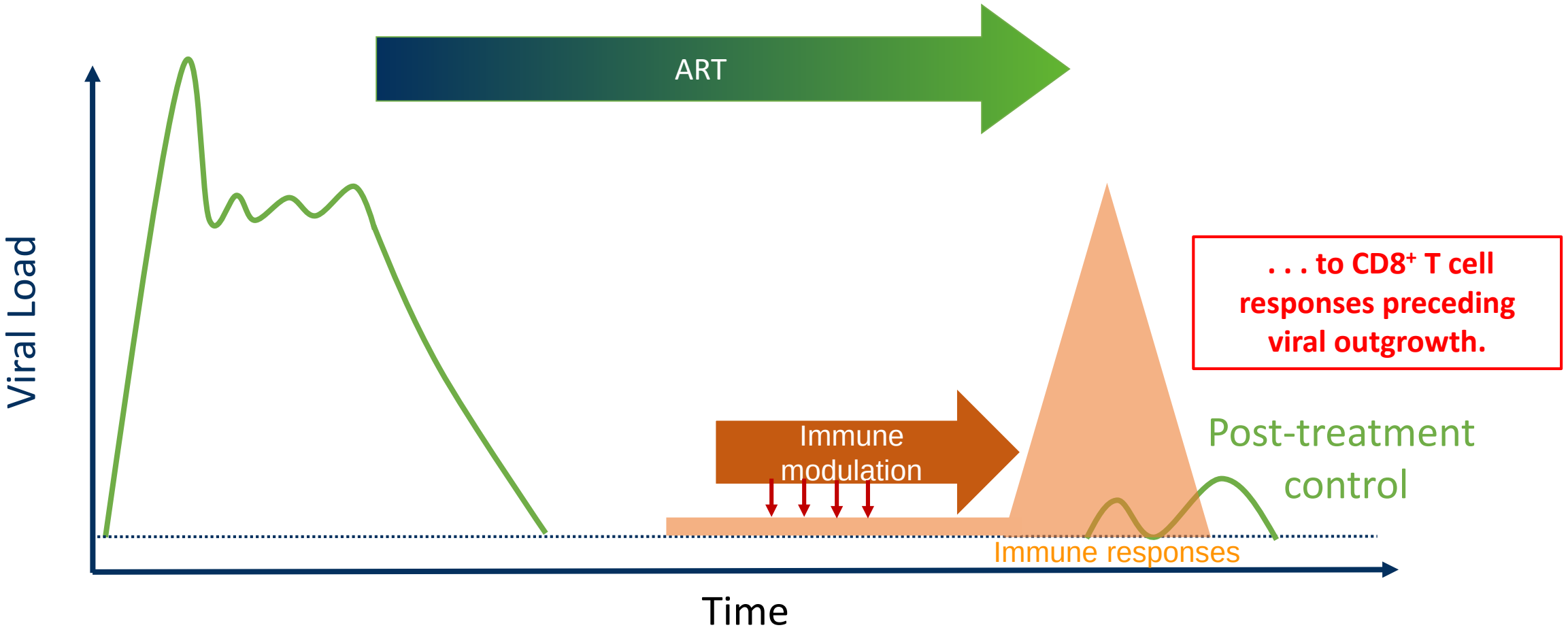
***p = 0.0001

The major goal of post-ART release immune therapeutics is to mobilize effective immune to prevent or stringently suppress post-ART viral rebound . . .



T cell anti-viral activity follows viral outgrowth: "too little, too late" with such an immune evasive virus.

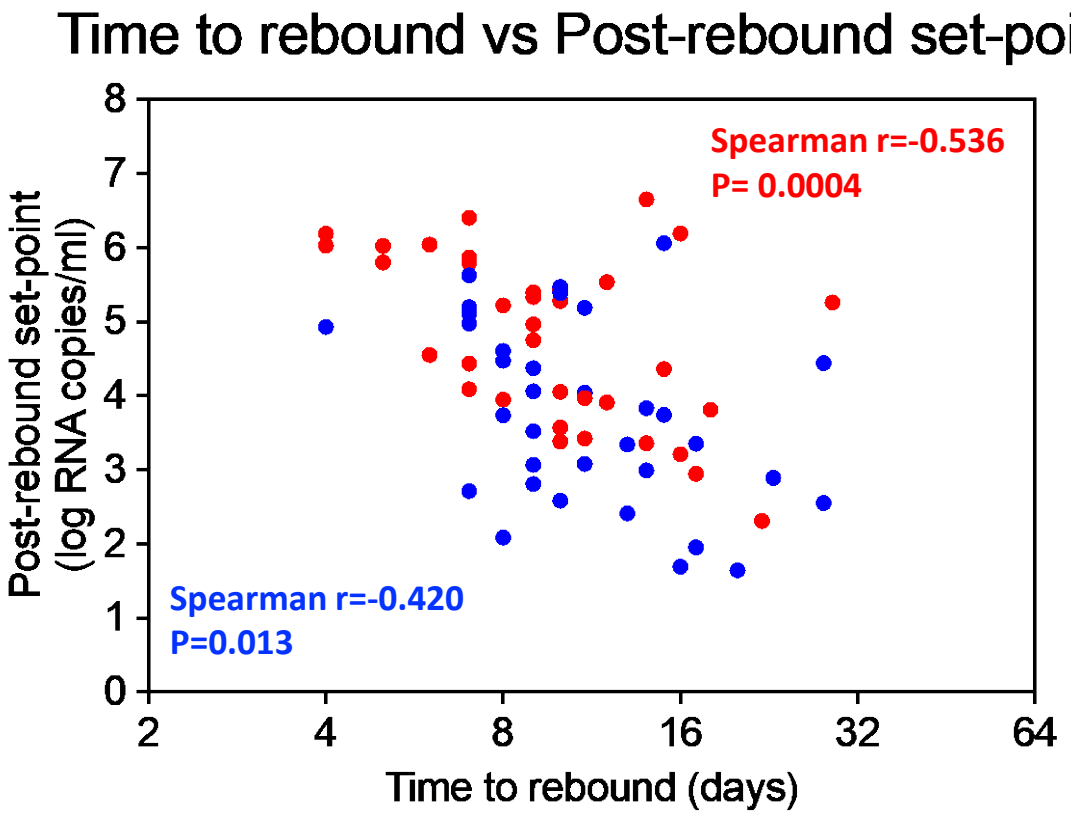
HIV cure interventions that accelerate and increase the anti-viral efficacy of the host immune "intercept" might reduce post-ART viral replication, potentially leading to long-term control off ART.



In this regard, we have noted a significant correlation between time-to-rebound* and post-ART viral setpoint. . .

(full reservoir model)

(*first above-threshold plasma viral load)



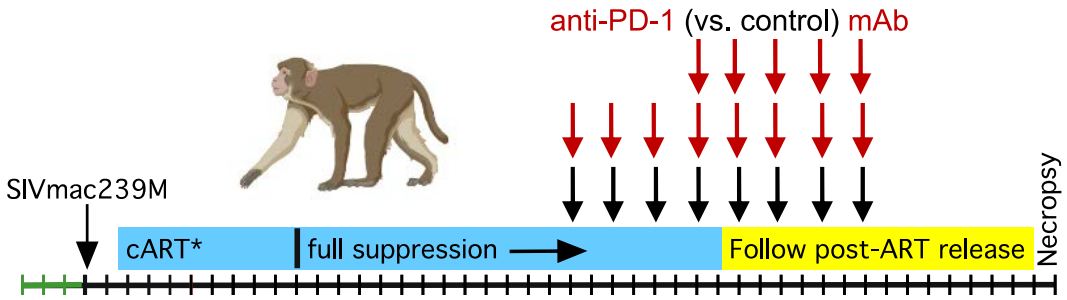
*Blue = protective MHC (n=34; A*01, B*08 or B*17)*
Red = no protective MHC (n=39)

Slower rebound is associated with better subsequent control

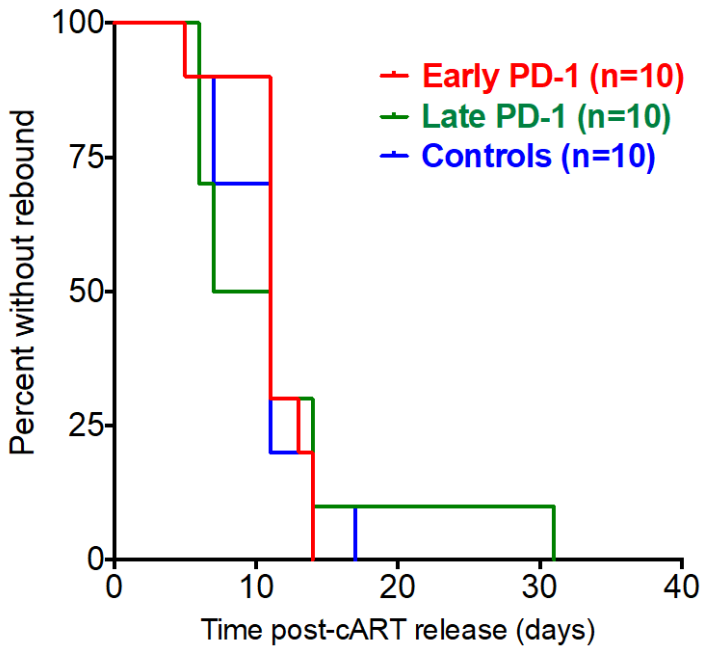
Overall Spearman r=-0.462
P<0.0001

. . . providing further evidence that immune intercept dynamics govern immune control, and that therapies proving early, stronger, properly focused immune responses relative to viral spread dynamics will result in better long-term control.

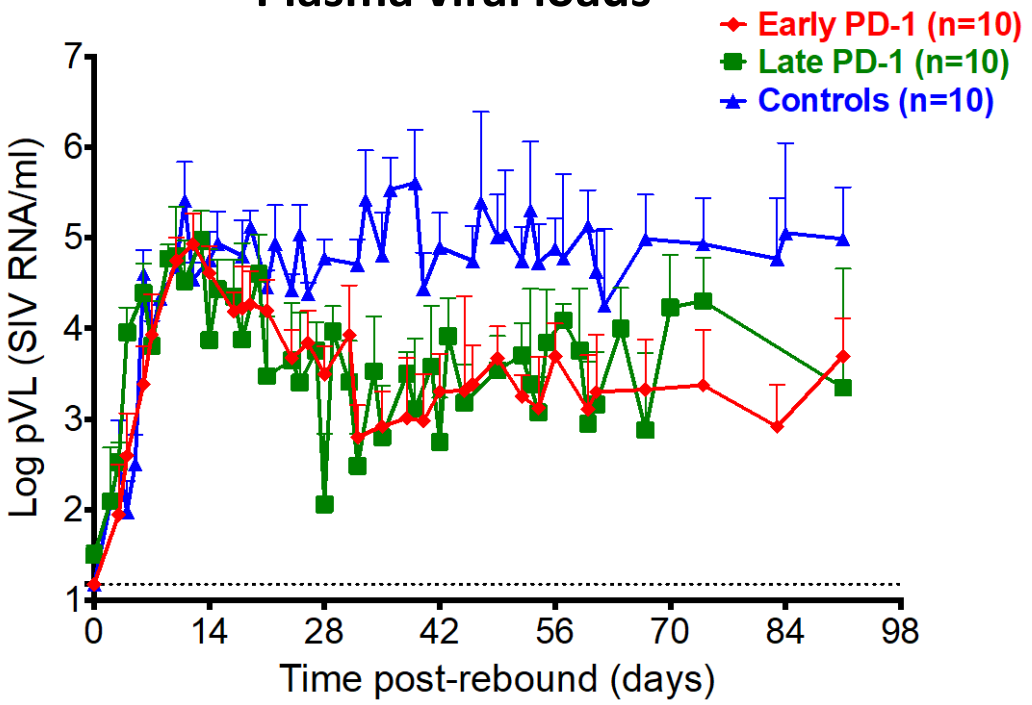
Checkpoint blockade (anti-PD-1) can increase effector efficiency of RM CD8$^+$ T cells . . . Can this approach potentiate post-ART viral control?



Time-to-rebound



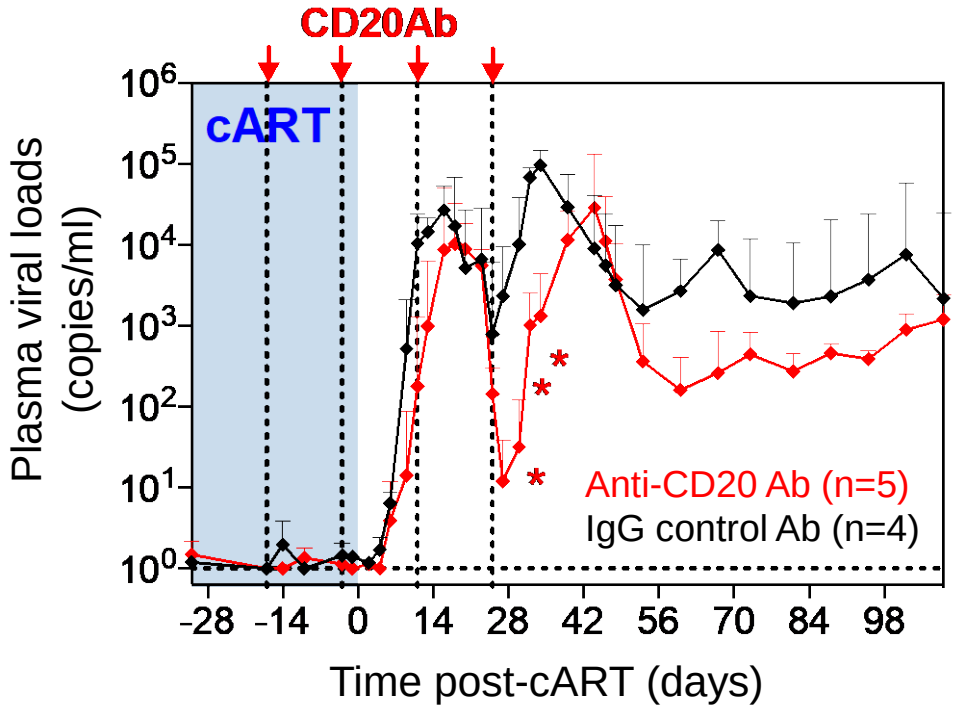
Plasma viral loads



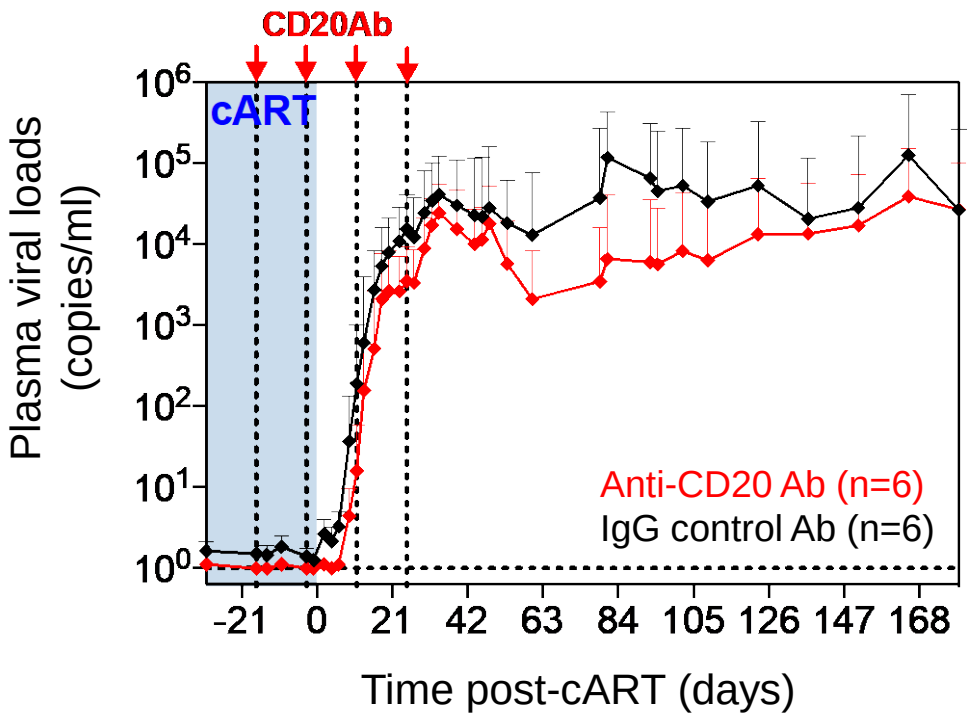
"more", but still "too little, and too late".

Is the rebounding virus hiding in B cell follicles? Would follicular disruption with anti-CD20 potentiate post-ART control?

Mamu B*08+ RM
(protective allele)



Mamu A*01- B*08- B*17- RM
(no protective allele)

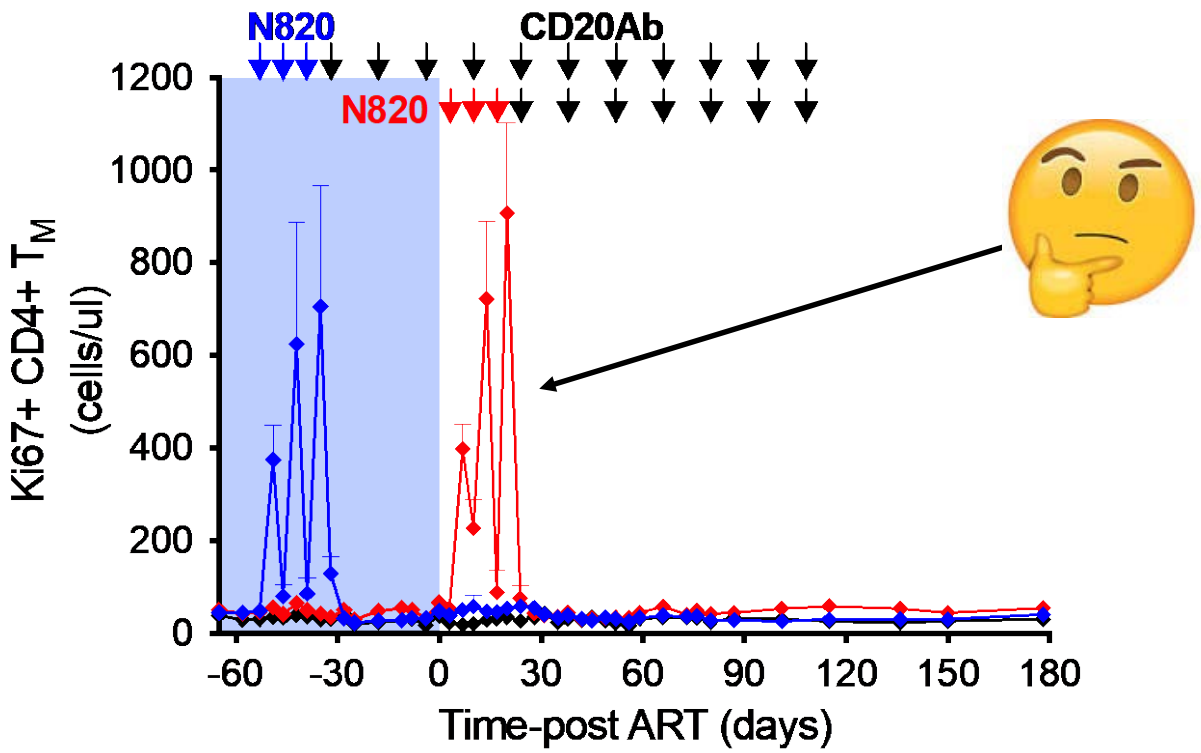


"A little, but not a lot"

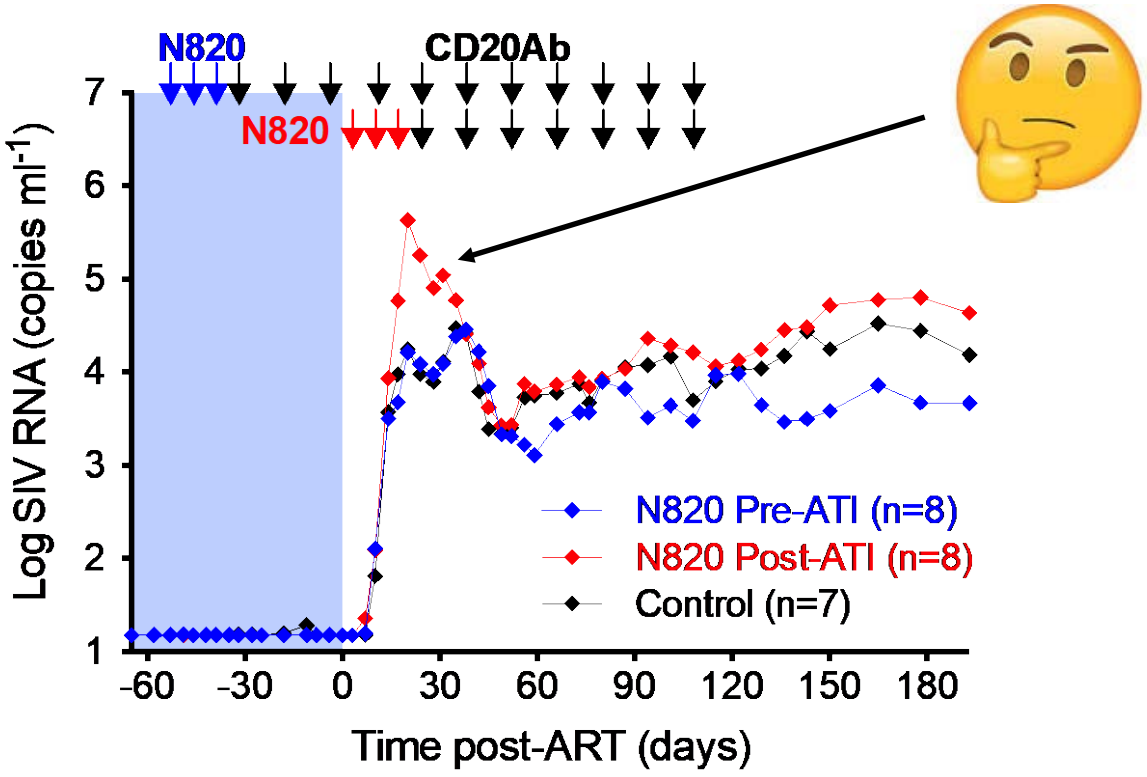
Moreover, adding IL-15 stimulation to anti-CD20 (N820) goes too far . . .

(all RM B*08+)

Proliferating CD4+ memory T cells



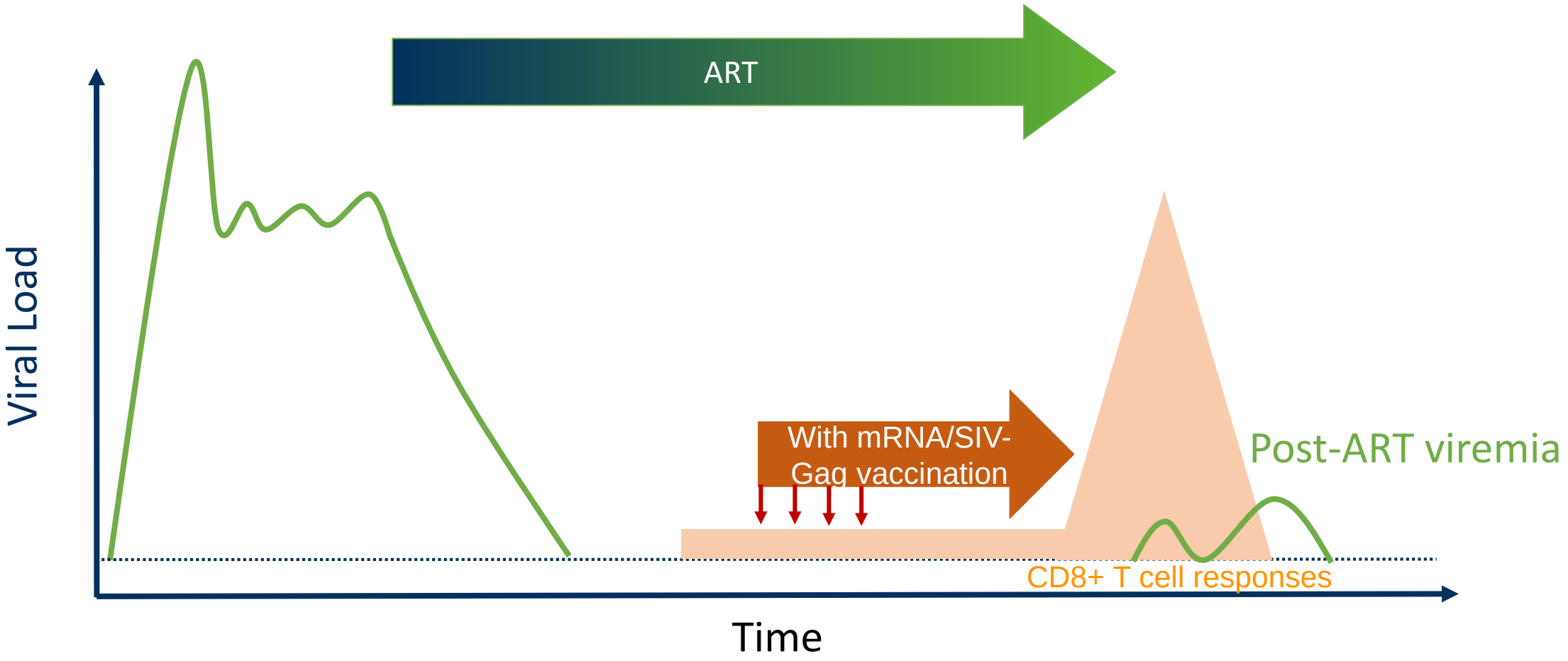
Plasma viral loads



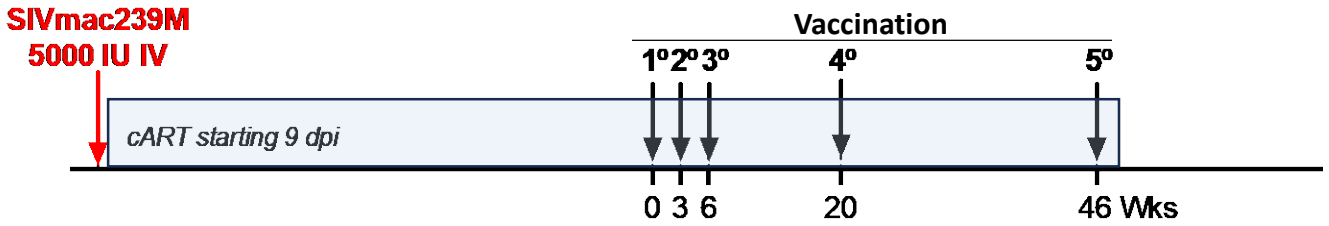
IL-15 (N820) at time of ART enhanced early, post-ART viral replication

. . . Increasing rather than decreasing post-ART viral replication.

Can therapeutic (T cell-targeted) vaccination during ART and a boost just prior to ART release delay rebound and facilitate viral control?

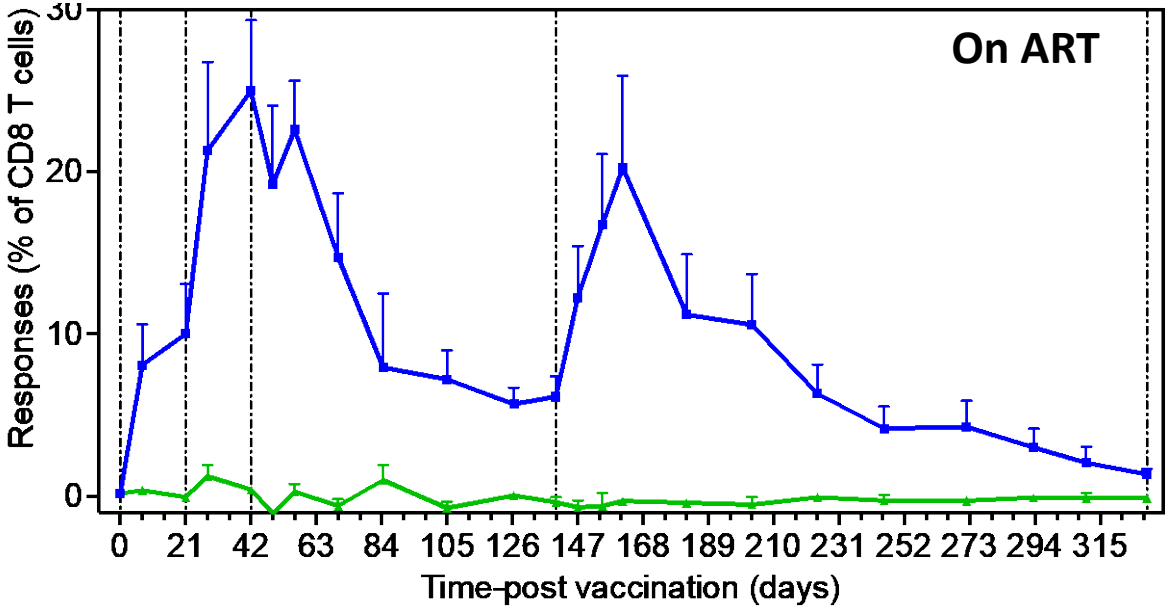


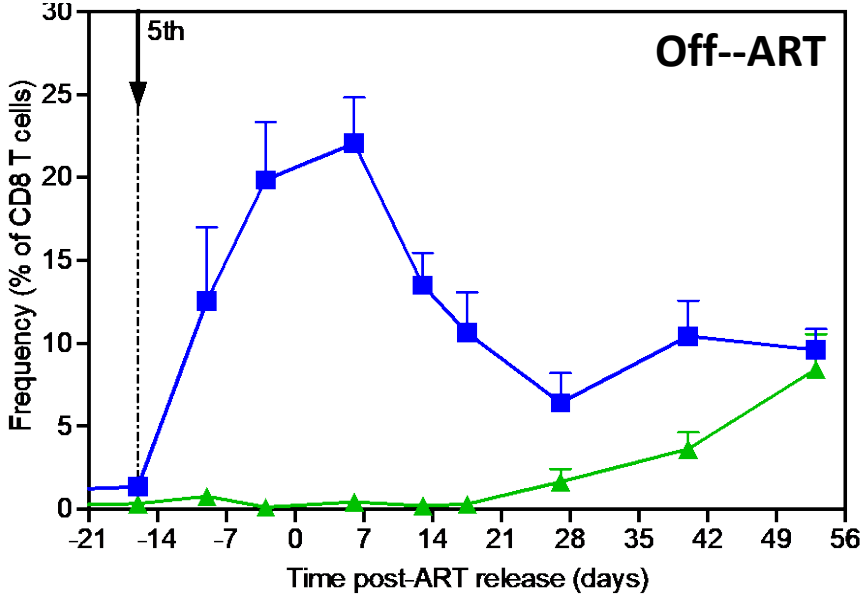
Assessment of an SIVgag-targeted mRNA T cell vaccine during ART, with boosting prior to ART release . . .



Final vaccine dose administered 2 weeks prior to ART interruption

SIVgag-specific CD8 T cell responses in lung wash (accessible effector site)

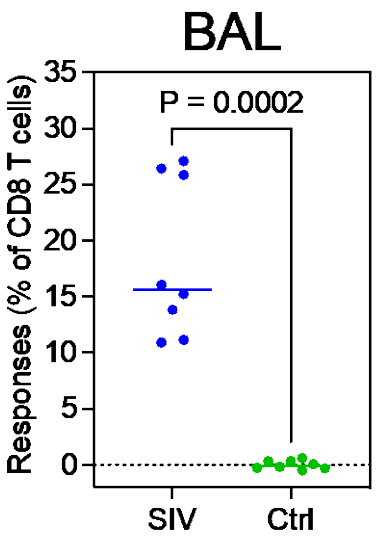


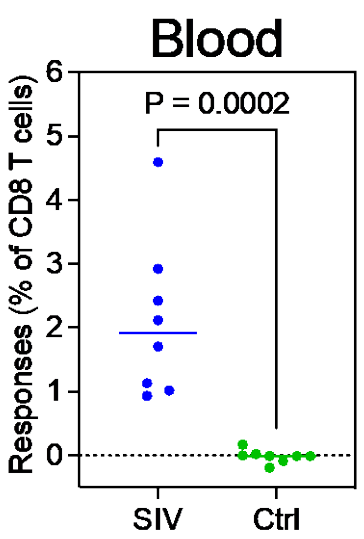


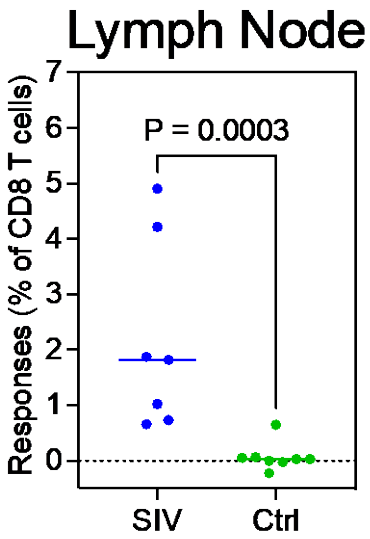
mRNA/SIV-Gag (n=8)

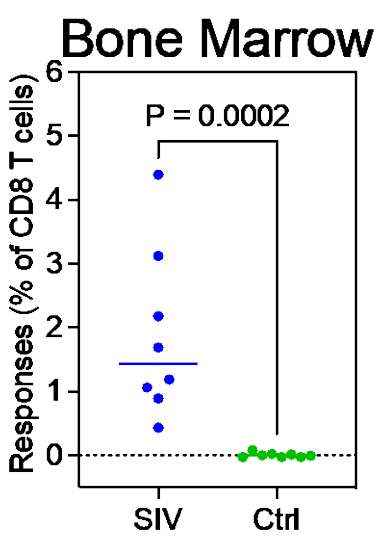
mRNA/Control (n=8)

mRNA/SIV-Gag LNP increased Gag-specific CD8+ T cell responses in most lymphoid tissues at the time of ART release

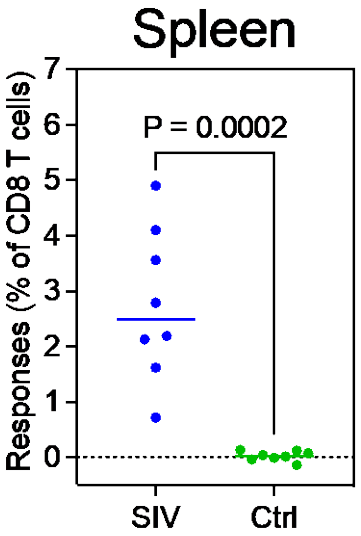


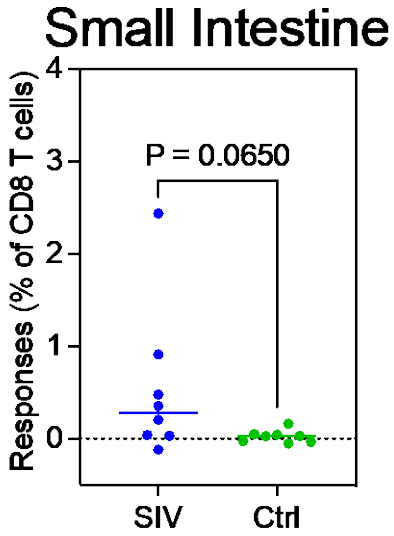


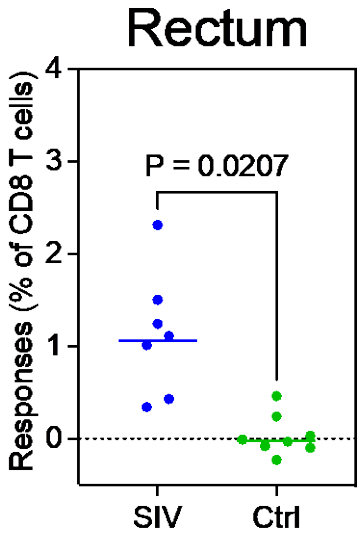


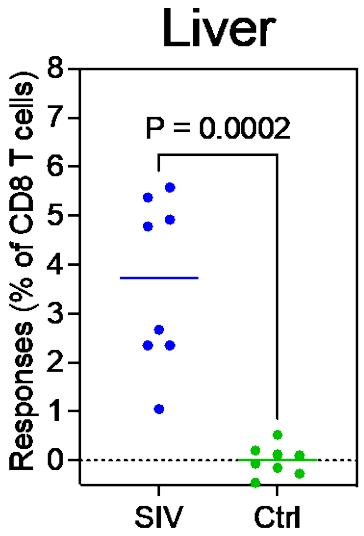


mRNA/SIV-Gag (n=8)
mRNA/Control (n=8)

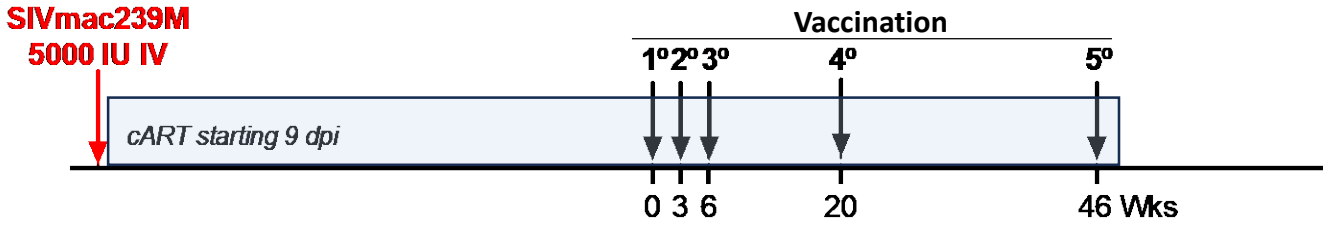




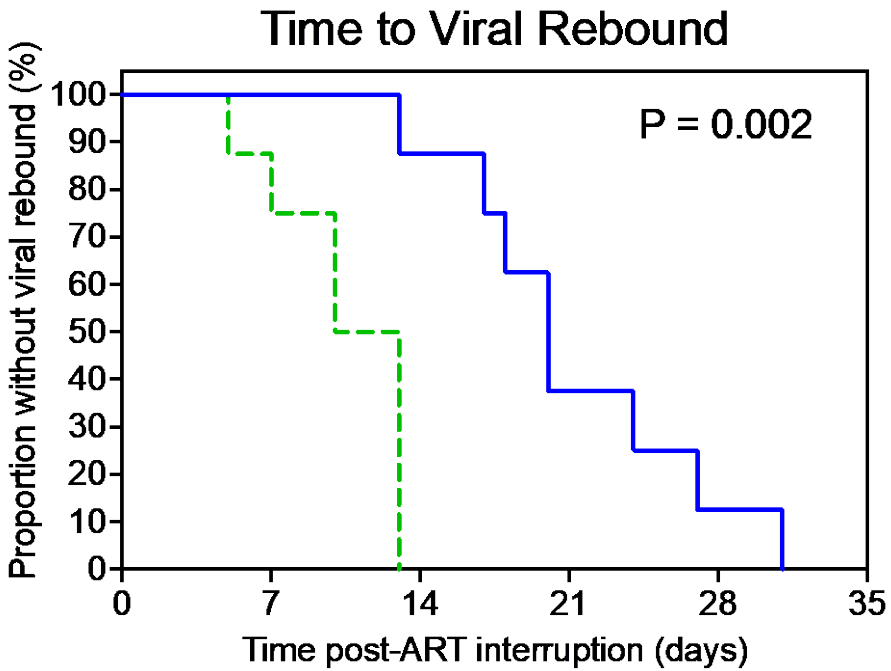


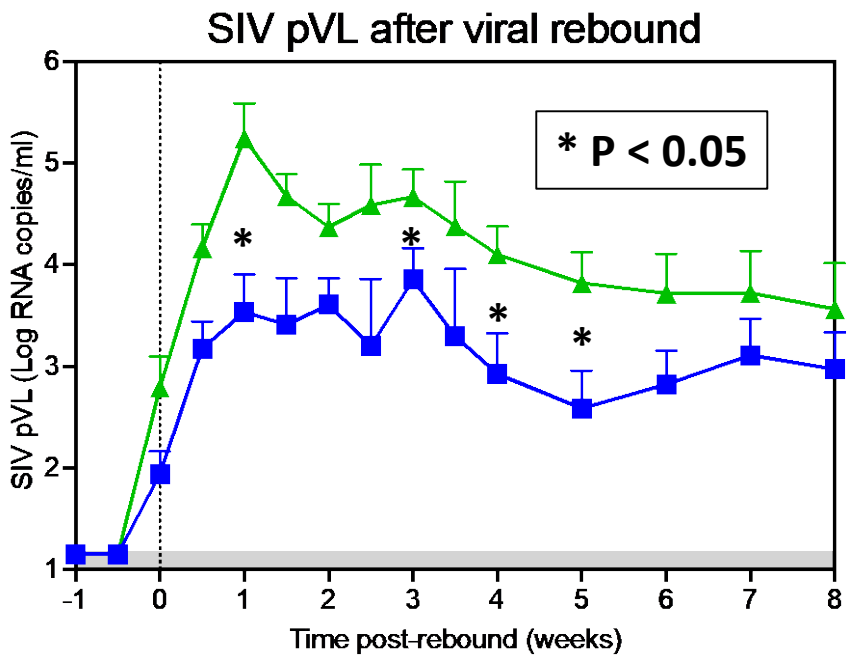


Importantly, this vaccine, timed to provide an early (pre-viremic) boost in CD8$^+$ T cell responses, both extended time-to-rebound and enhanced post-ART viral control . . .



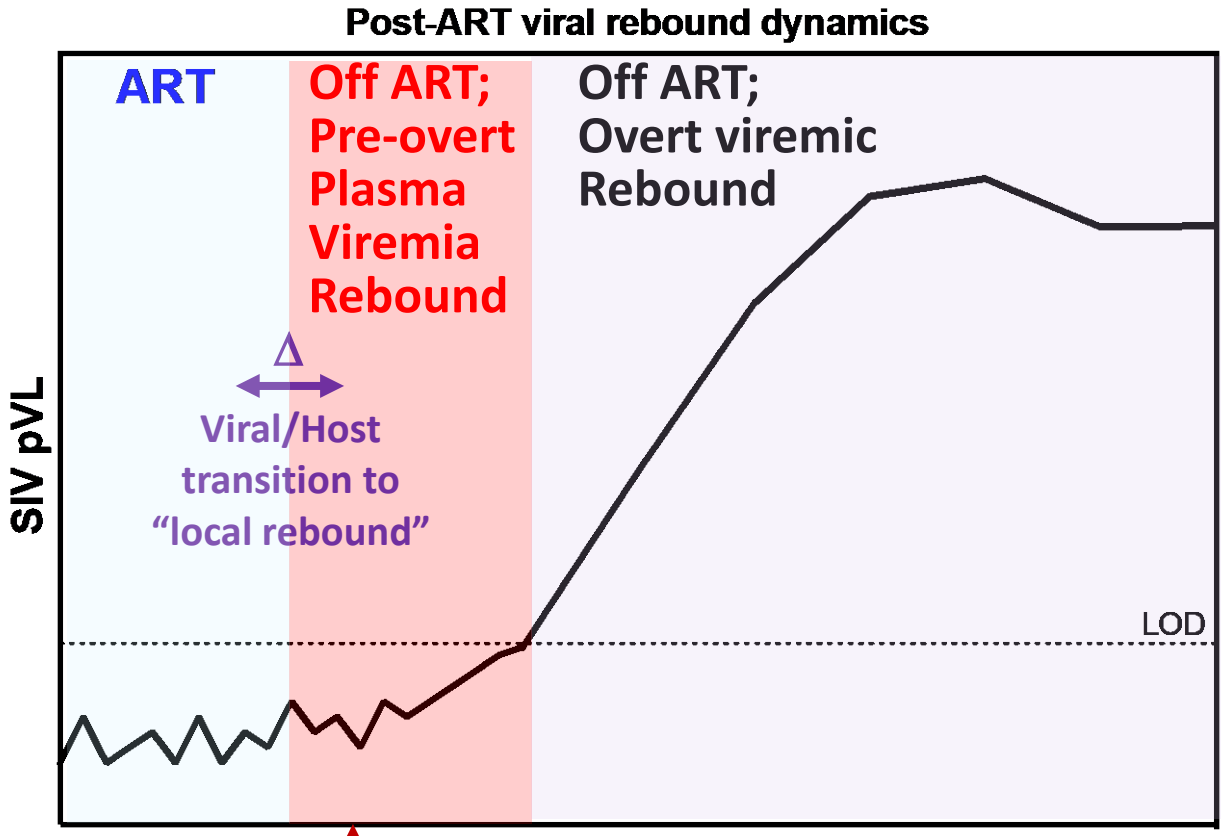
Final vaccine dose administered 2 weeks prior to ART interruption





While not a "home run" as the "additional" viral control mediated by the therapeutic vaccine was insufficient to completely abrogate rebound, these data do support the potential of therapies that provide an earlier, more potent immune "intercept" of rebound . . .

While we will continue to empirically build on these observations of partial therapeutic benefit, we felt these efforts would be substantially facilitated by a deeper understanding of the virology and immunology or early rebound . . .

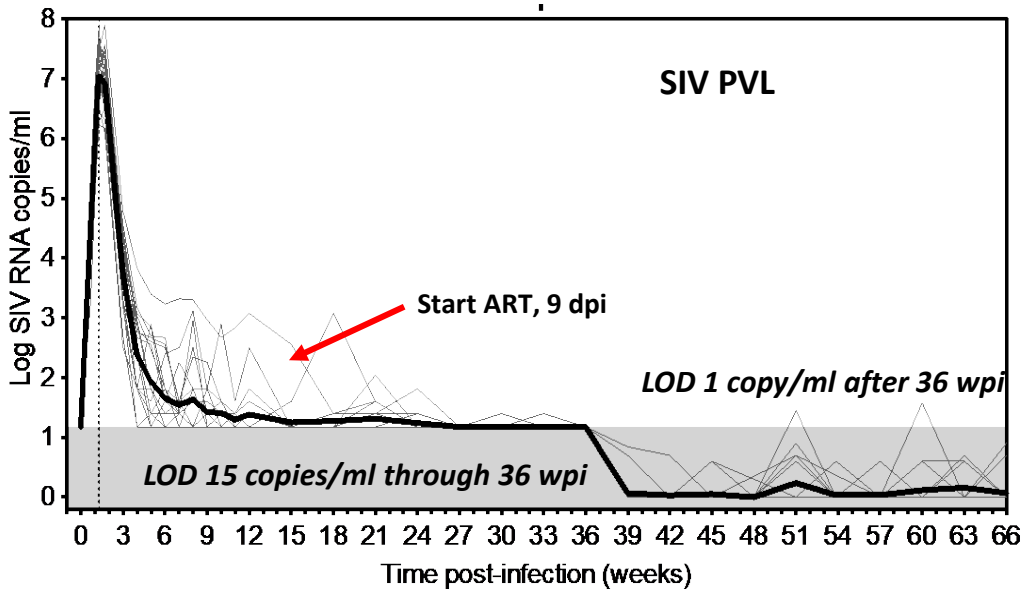


Unknown territory: "There be dragons here"

Key Questions

1. Following ART discontinuation, when and in which tissue sites does viral rebound initiate, through local SIV replication and within tissue spread?
2. What and where are the processes by which initial tissue local rebound is locally and ultimately, systemically, amplified?
3. What is the host response to local rebound and what role do local tissue "neighborhoods" and local innate/adaptive immune responses play in supporting or restricting viral rebound?

Can we capture early rebound in a serial necropsy study?

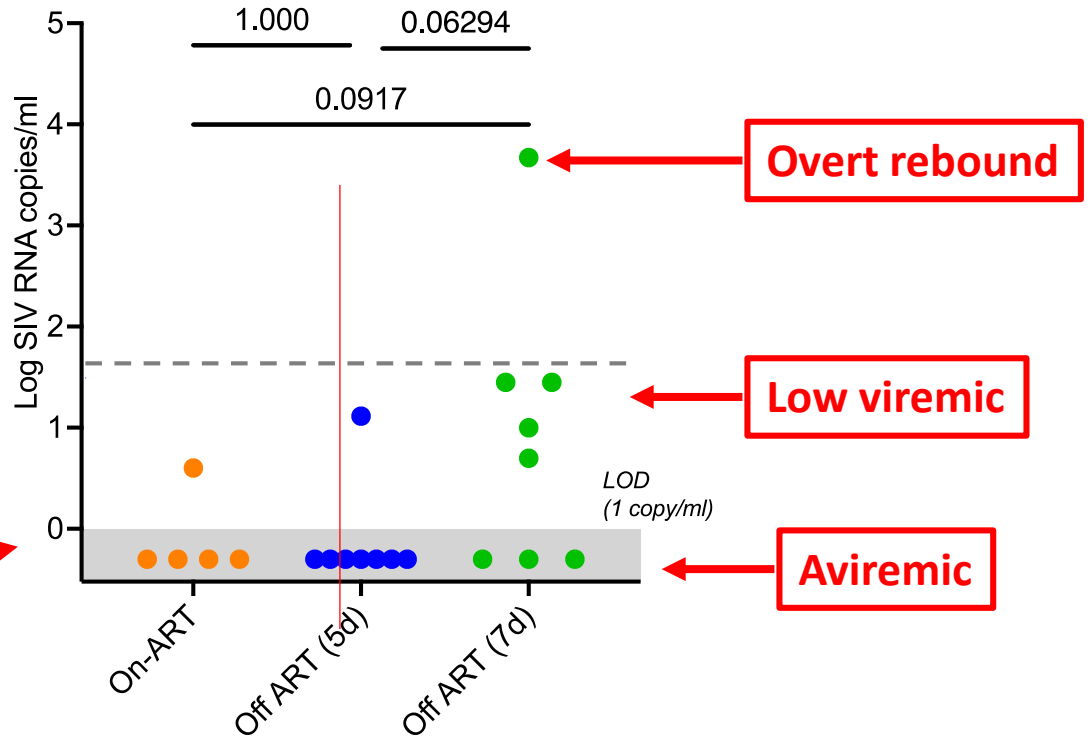


24 RM; i.v. barcoded SIVmac239M; ART started 9 dpi*; 70 weeks viral suppression

Necropsy Timing

1. Necropsy on ART (n = 6) (Cohort 1, comparator)
2. Necropsy on day 5 post-ART (n= 9) (Cohort 2a)
3. Necropsy on day 7 post-ART (n = 9) (Cohort 2b)

Design was "spot on" – except for one overt viremic rebounder at day 7, all other off-ART necropsies were performed with no detectable PVL (n = 11) or with PVL <60 (n = 6)



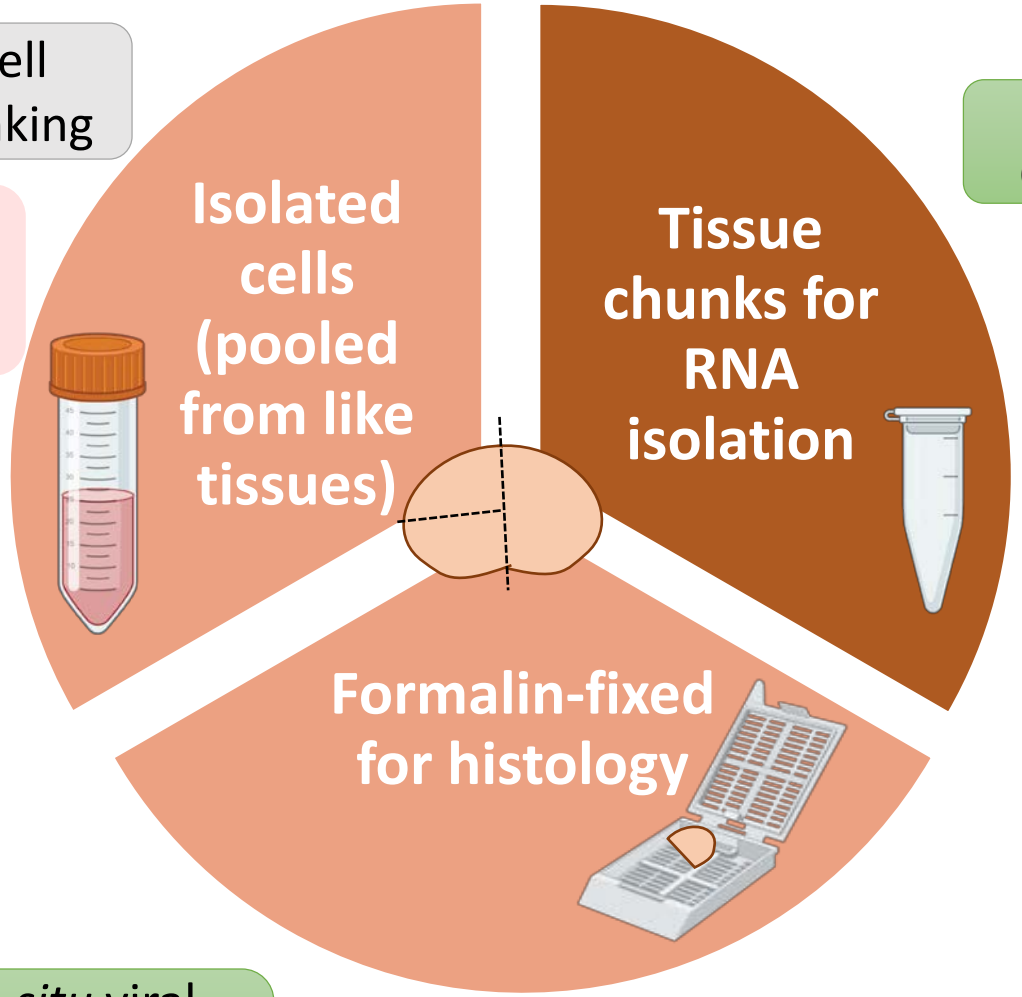
Over 400 samples collected per monkey, divided 3 ways:

vRNA/vDNA quant. (CAVL)

Cell banking

Immunophenotyping and ICS (flow cytometry)

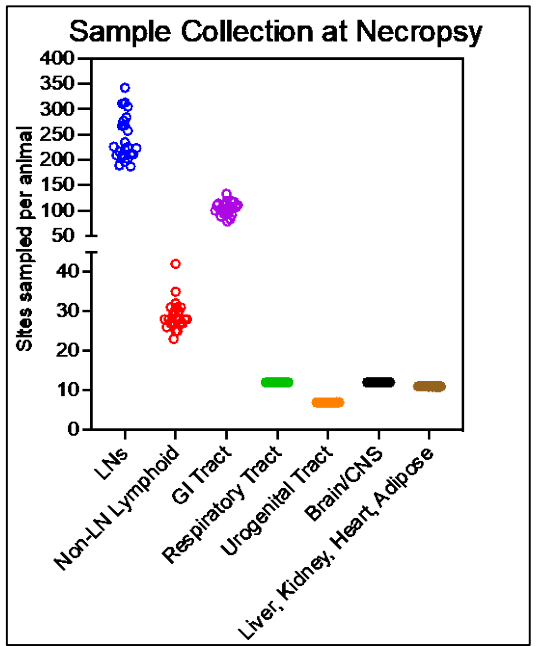
Single Cell Transcriptomics



vRNA/vDNA quantification (CAVL)

Barcode Sequencing

Bulk transcriptomics



In situ viral detection (RNAscope)

Spatial Transcriptomics

Spatial Proteomics

*** To provide detection of viral spread and the host response to such spread in same sample.**

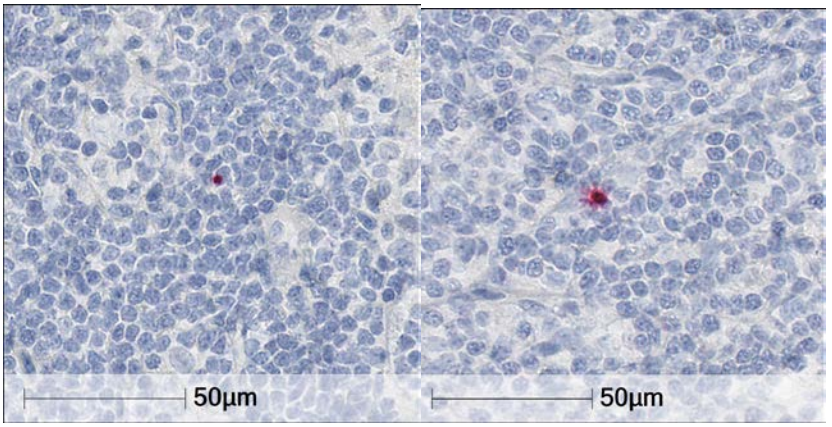
Criteria for local post-ART SIV replication and spread "local viral rebound":

Identify tissues with (and without) local rebound (SIV replication and spread) by comparision of **Cohort #2** (**off-ART**) **RM** to **Cohort #1 (on-ART) RM** for the following parameters:

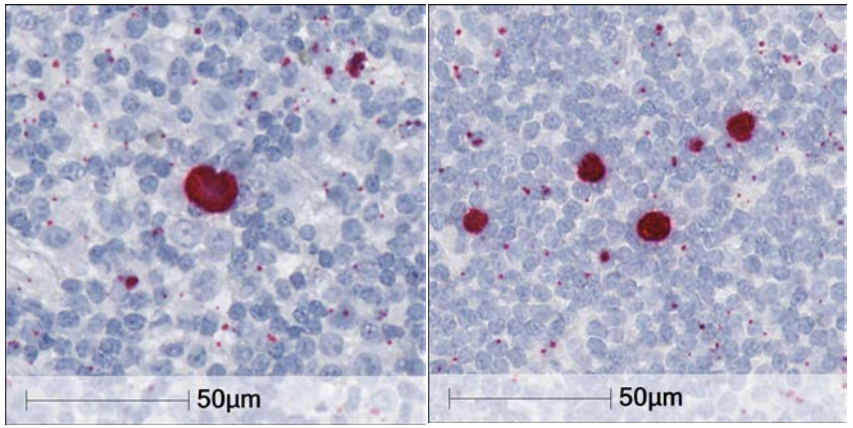
1. **Viral RNA/DNA ratio (CAVL)**
2. **Viral RNA content per infected cell (RNAscope)**
3. **Density/distribution of viral RNA$^+$ cells (RNAscope),**

Use barcode analysis to characterize these parameters at the level of viral clonotypes.

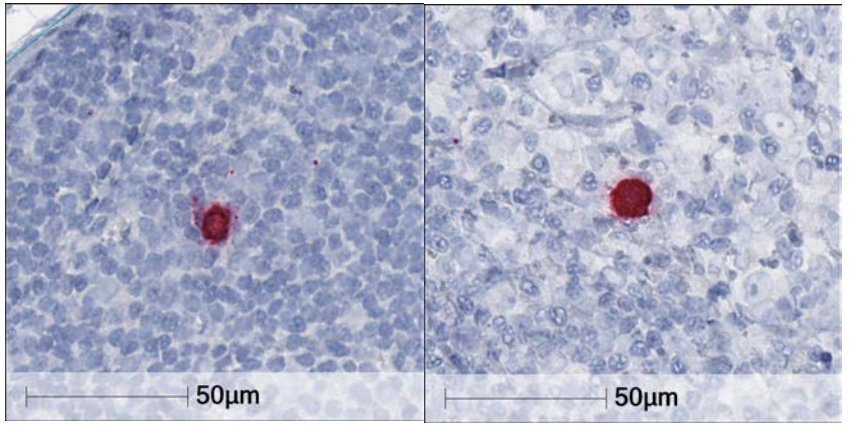
On ART (pVL <1)



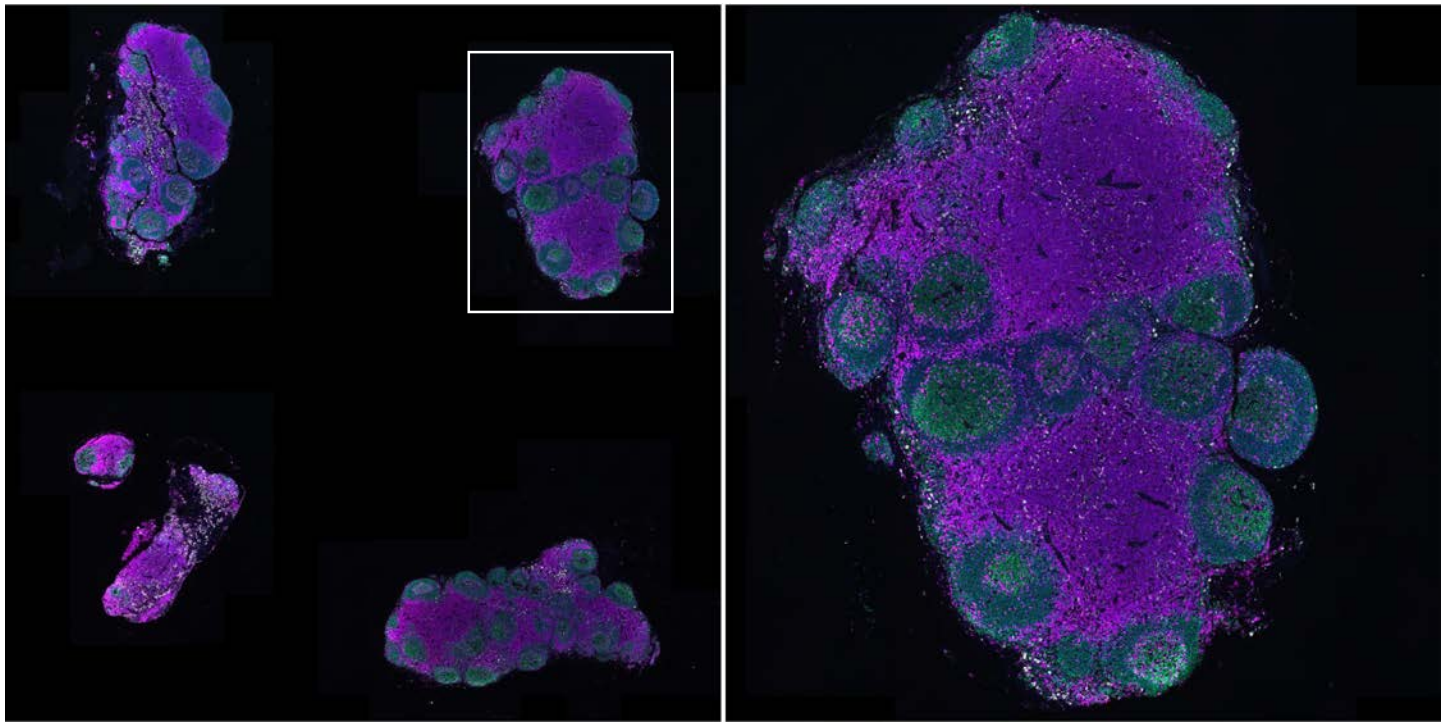
Peak Acute D12

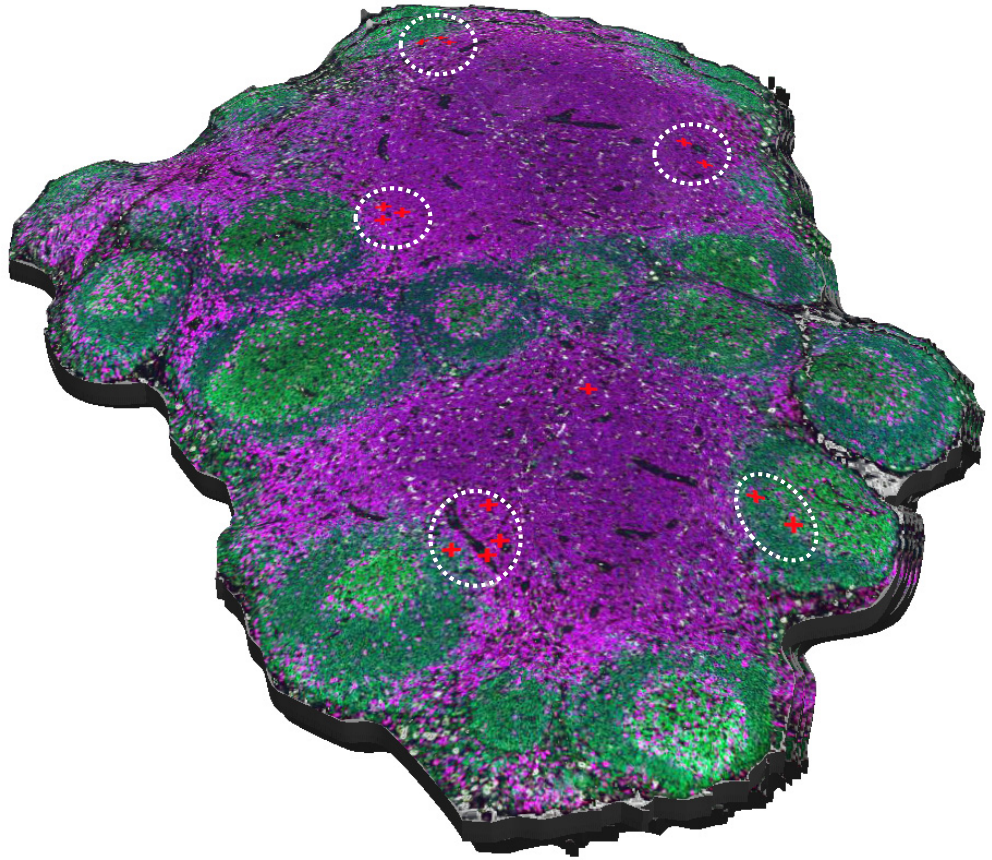


Off ART D12 (pVL 14,000)



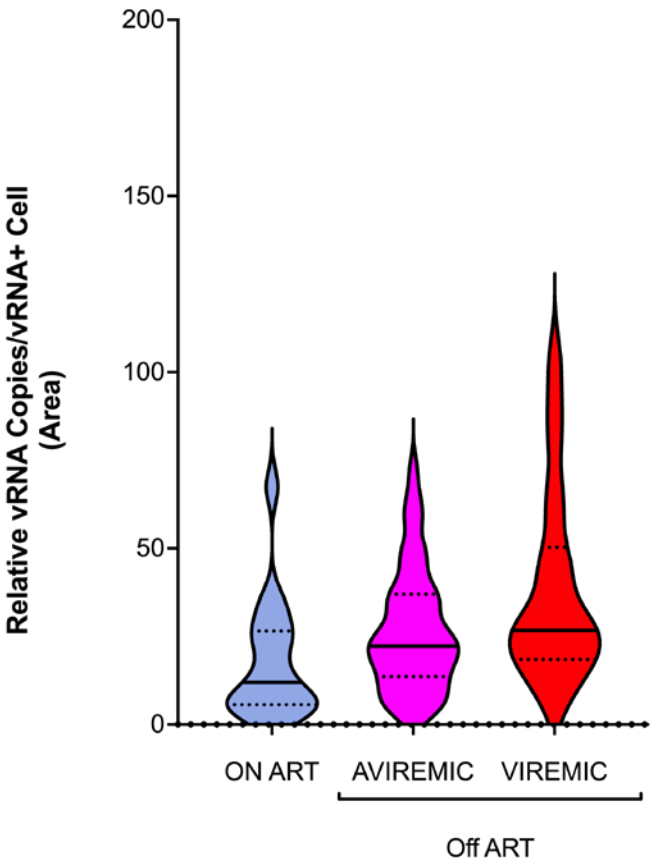
Local tissue rebound – Decreased distance between vRNA+ cells (increased focal density or clustering in 3-d) compared to on-ART:



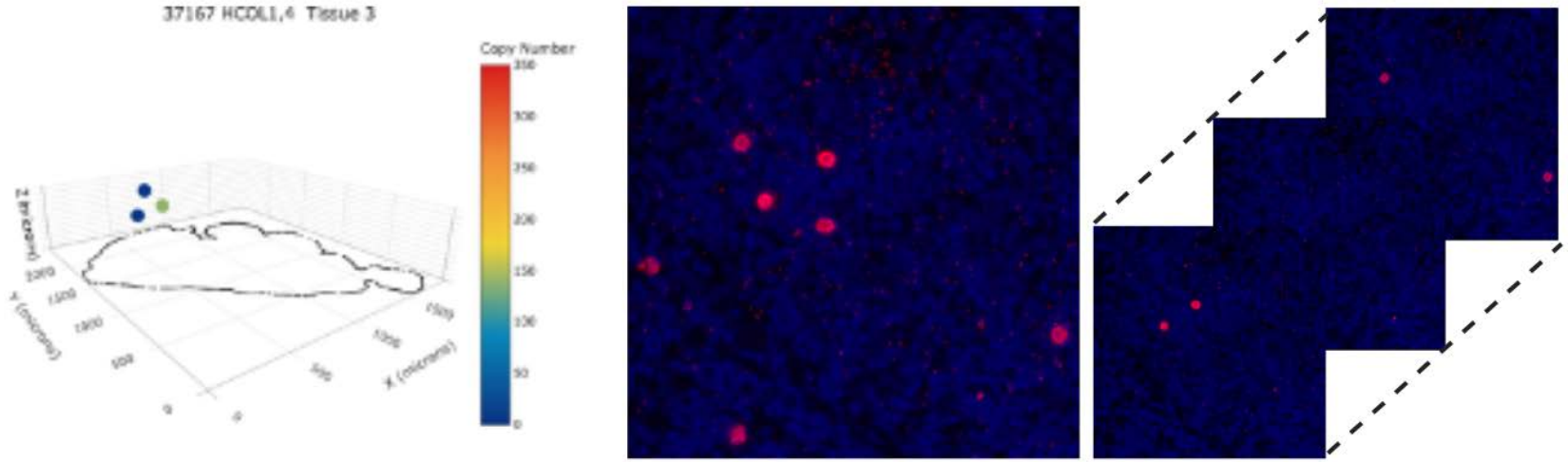


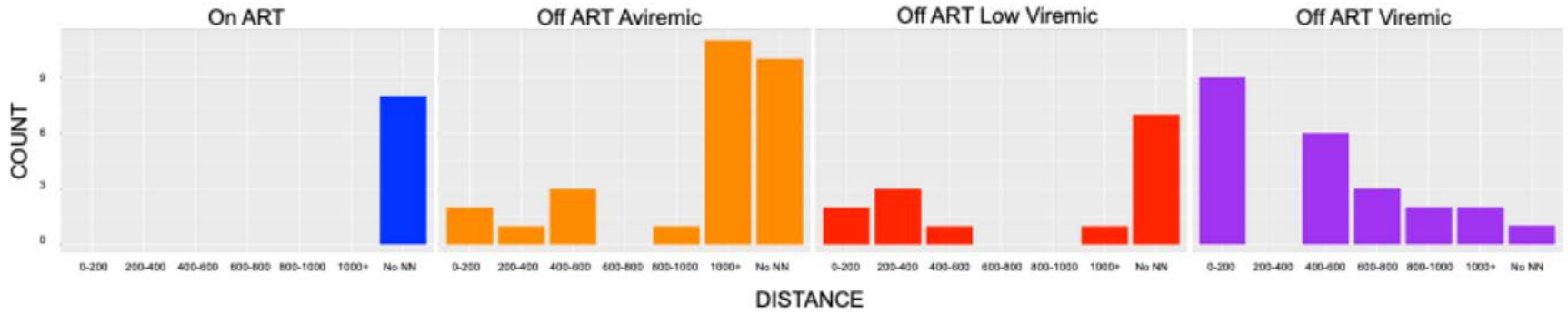
Spatial analysis confirms local rebound in the early Off-ART monkeys:

vRNA copies/vRNA+ cell



Clustering of vRNA+ cells

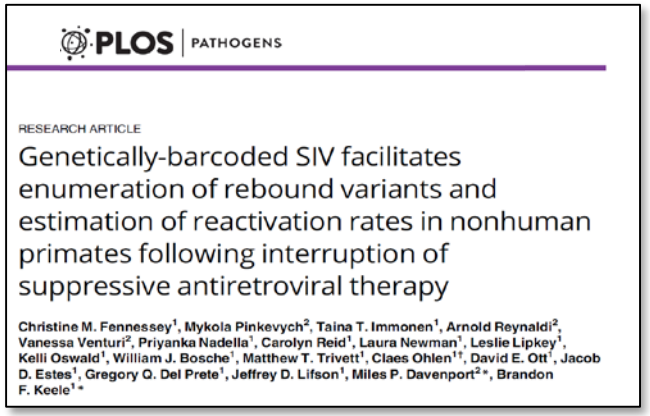




more clustering

Estes

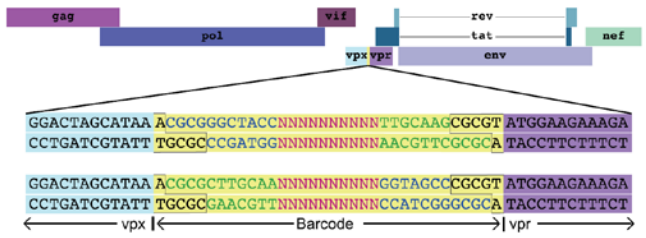
Viral barcoded-defined clonotypes contributing to off-ART rebound viremia. . .


PLOS | PATHOGENS

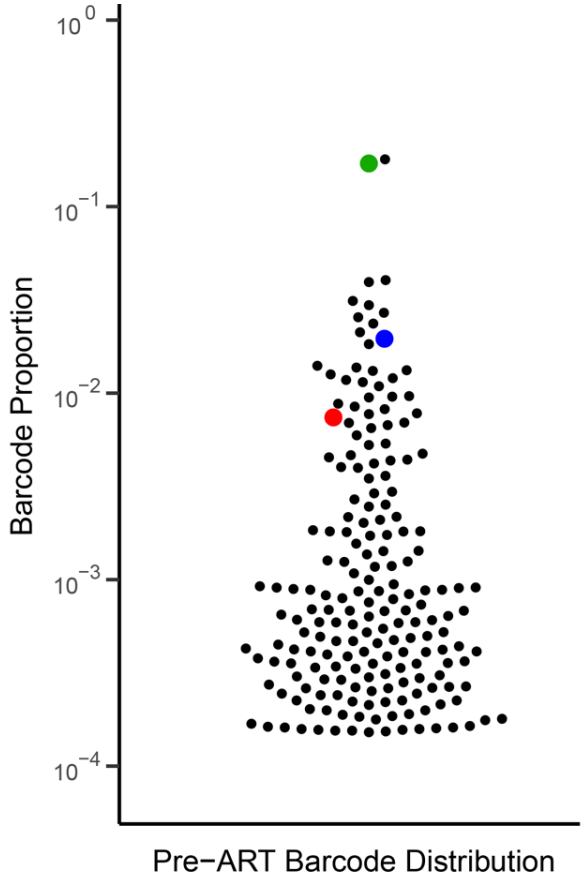
RESEARCH ARTICLE

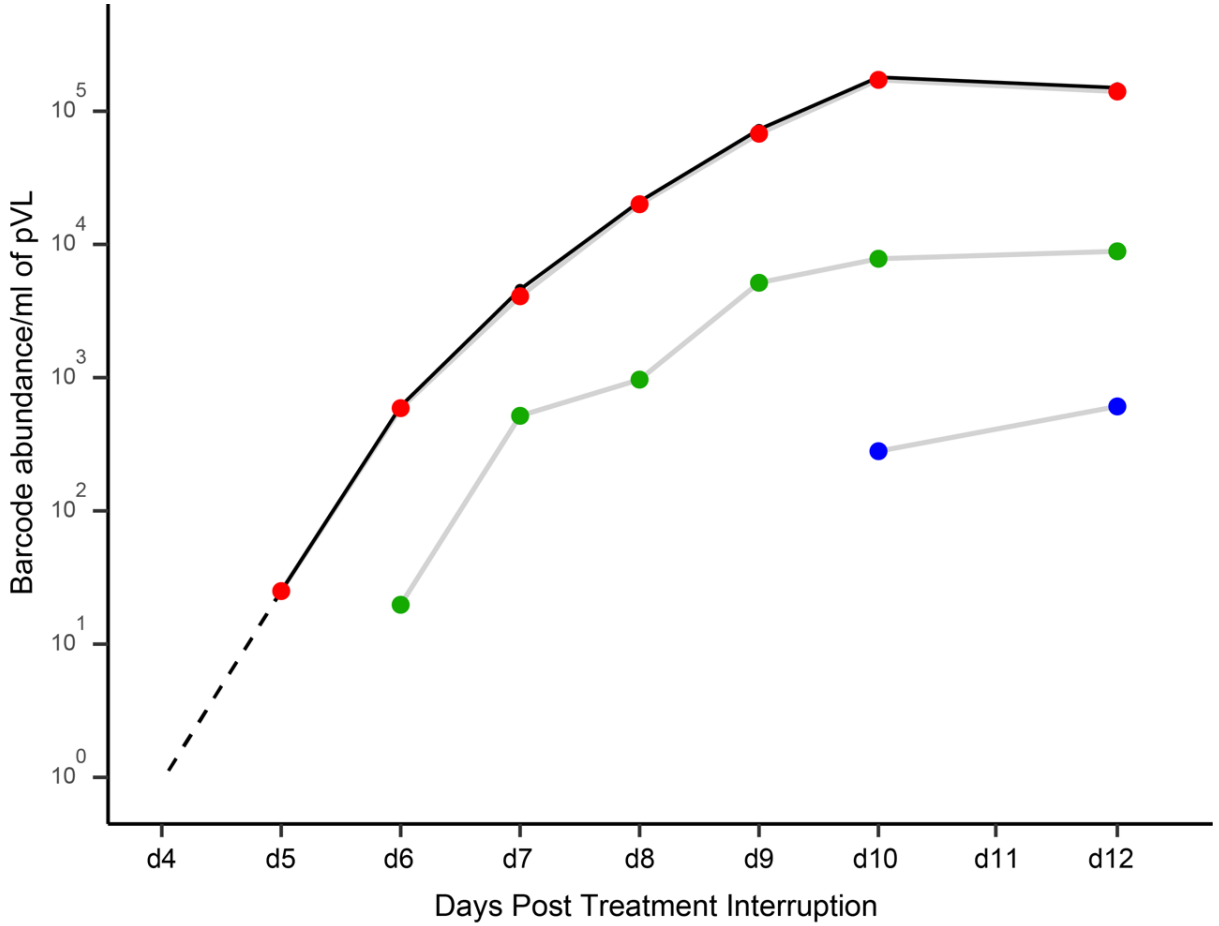
Genetically-barcoded SIV facilitates enumeration of rebound variants and estimation of reactivation rates in nonhuman primates following interruption of suppressive antiretroviral therapy

Christine M. Fennessey[1], Mykola Pinkevych[2], Taina T. Immonen[1], Arnold Reynaldi[2], Vanessa Venturi[2], Priyanka Nadella[1], Carolyn Reid[1], Laura Newman[1], Leslie Lipkey[1], Kelli Oswald[1], William J. Bosche[1], Matthew T. Trivett[1], Claes Ohlen[1†], David E. Ott[1], Jacob D. Estes[1], Gregory Q. Del Prete[1], Jeffrey D. Lifson[1], Miles P. Davenport[2]*, Brandon F. Keele[1]*



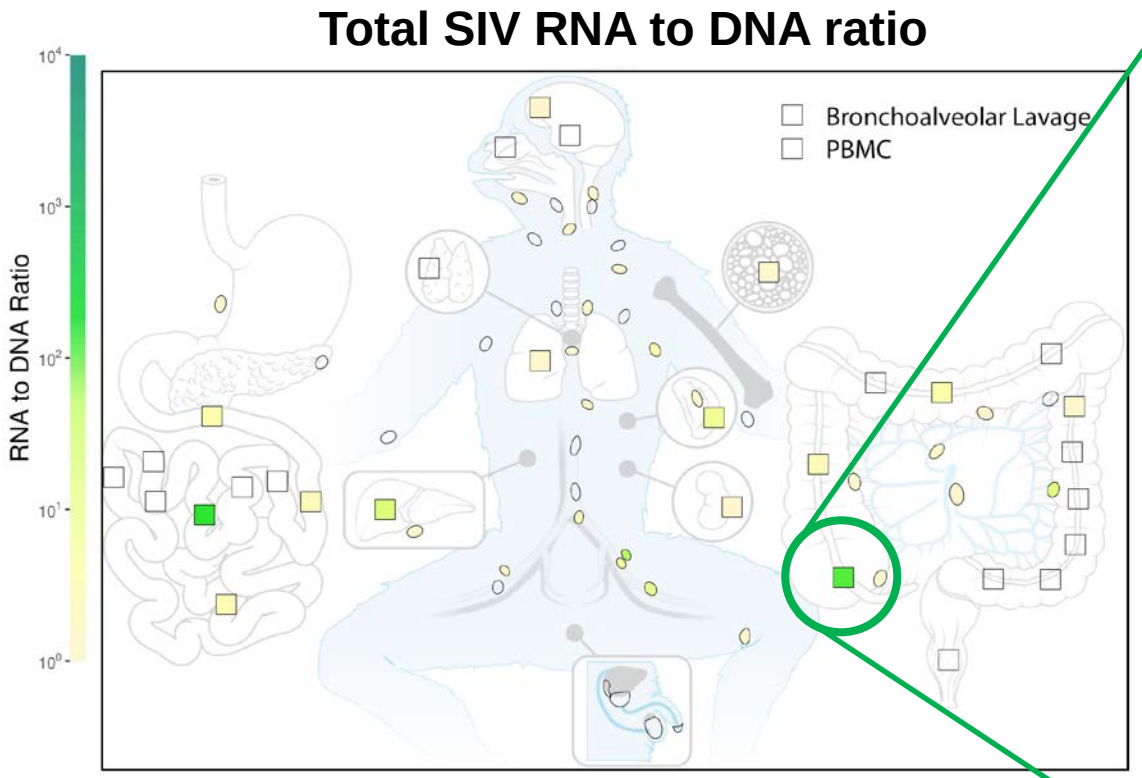
L604 ART Start 6 dpi
Nx Off ART d 12
pVL 150,000 c/ml

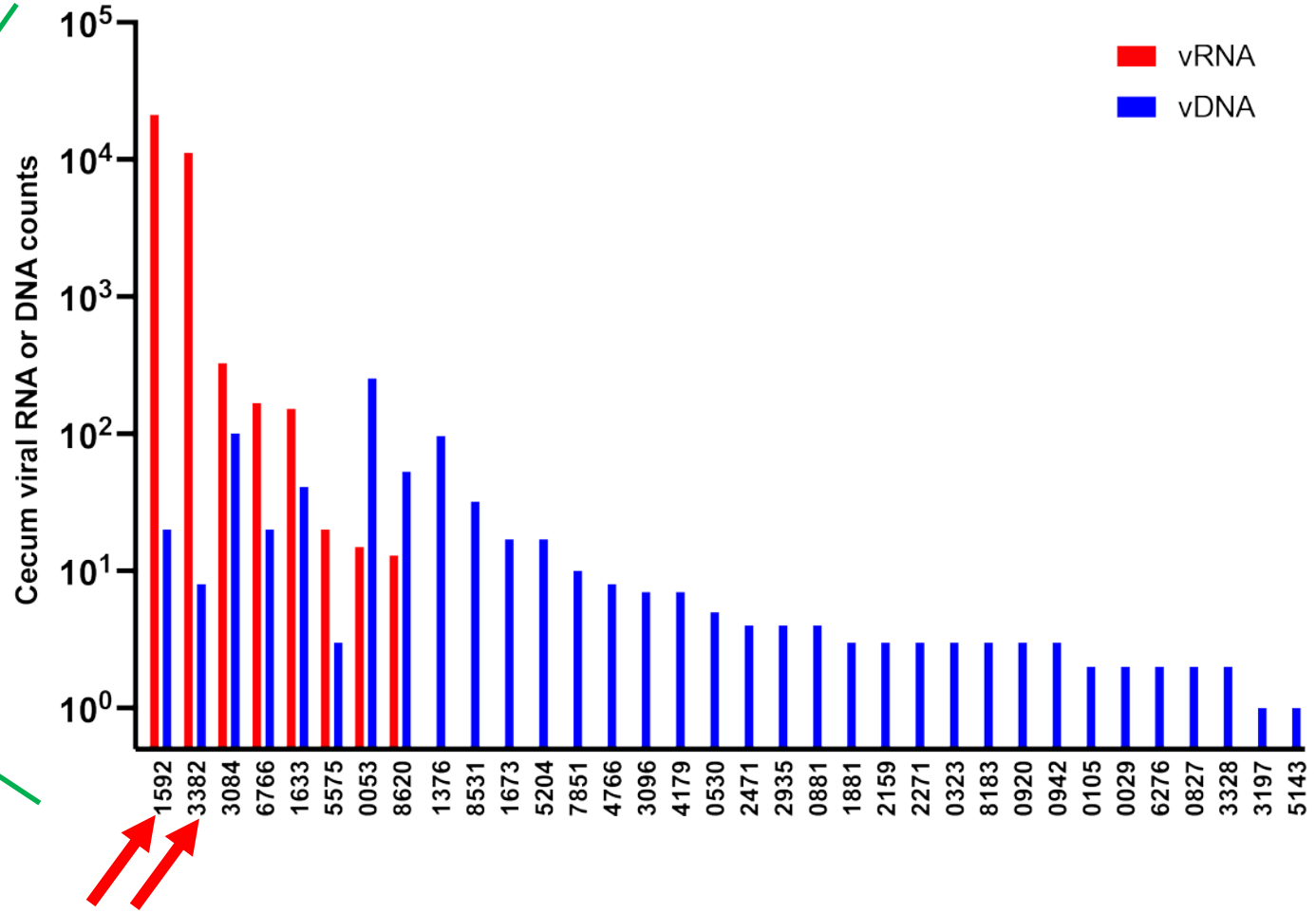




Tissue-localized barcode analysis can reveal the likely source of viral clonotypes contributing to rebound viremia.

DHGI ART Start 9dpi
Nx Off ART d 12
pVL 65 c/ml



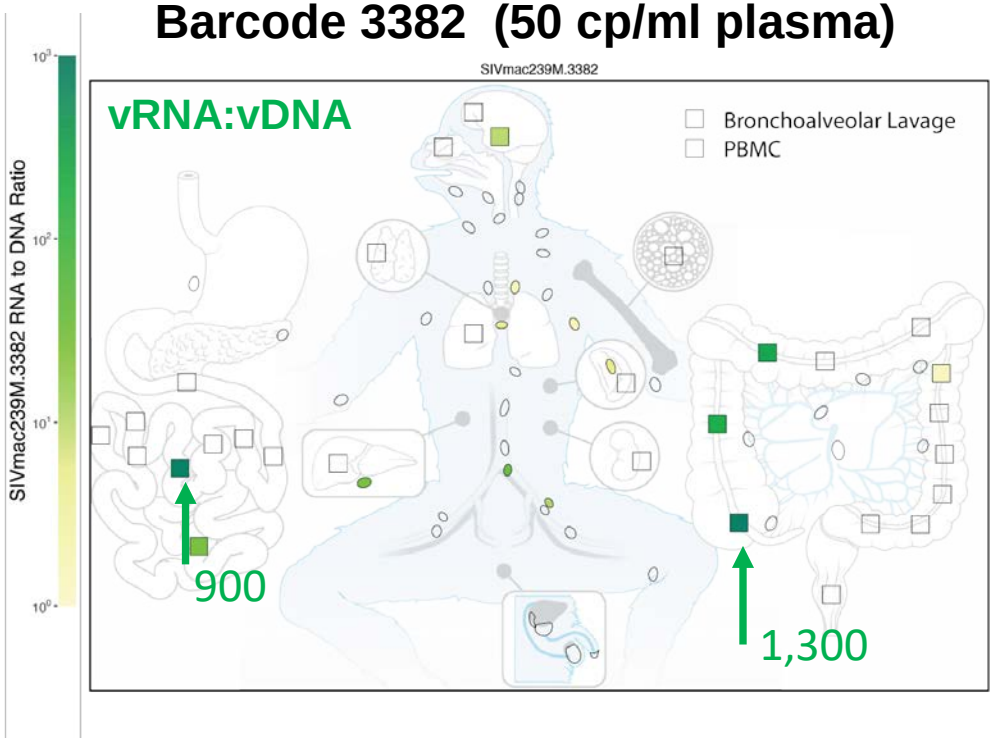


Lifson/Keele

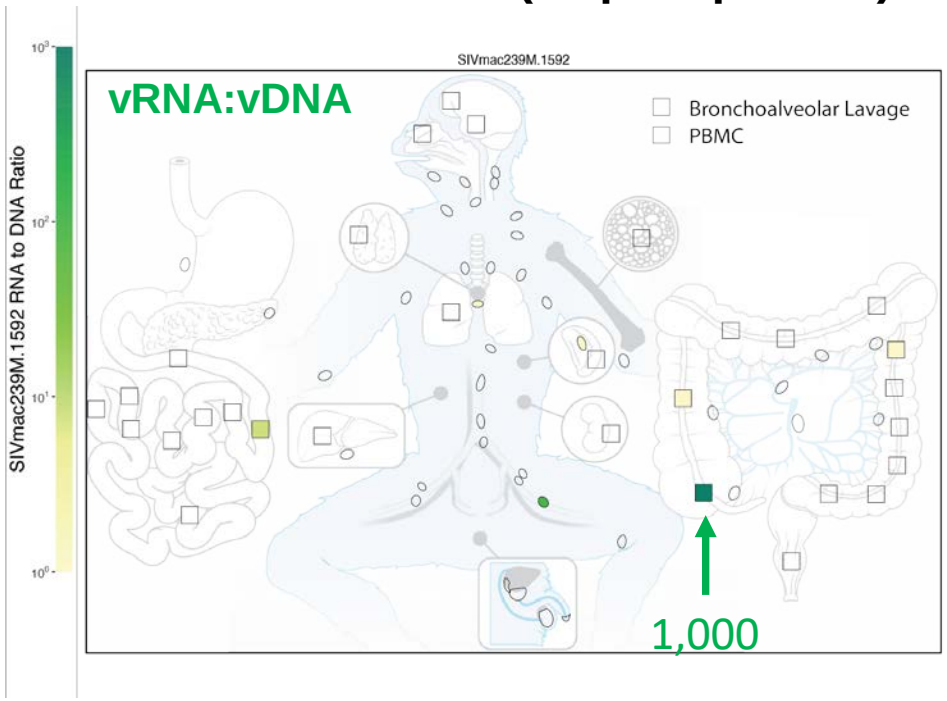
Tissue Localized Viral Barcodes Help Identify Potential Sites Contributing to Viral Rebound

DHGI ART Start 9dpi
Nx Off ART d 12
pVL 65 c/ml

Barcode 3382 (50 cp/ml plasma)



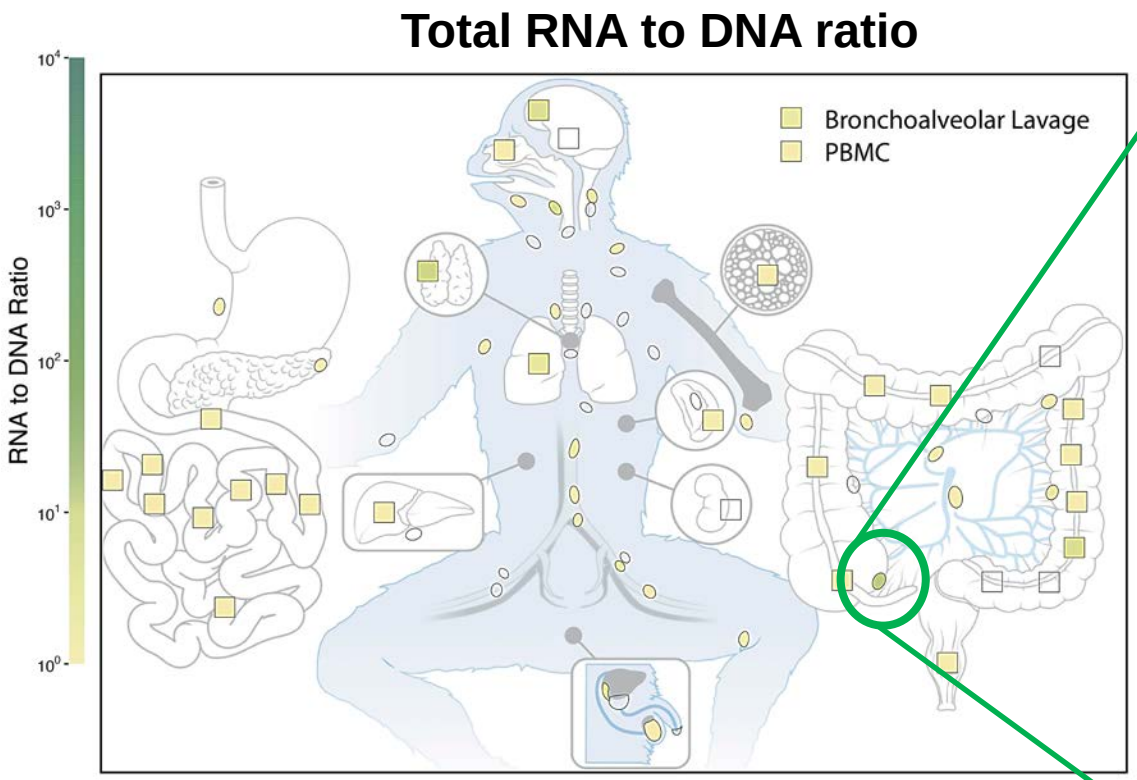
Barcode 1592 (2 cp/ml plasma)

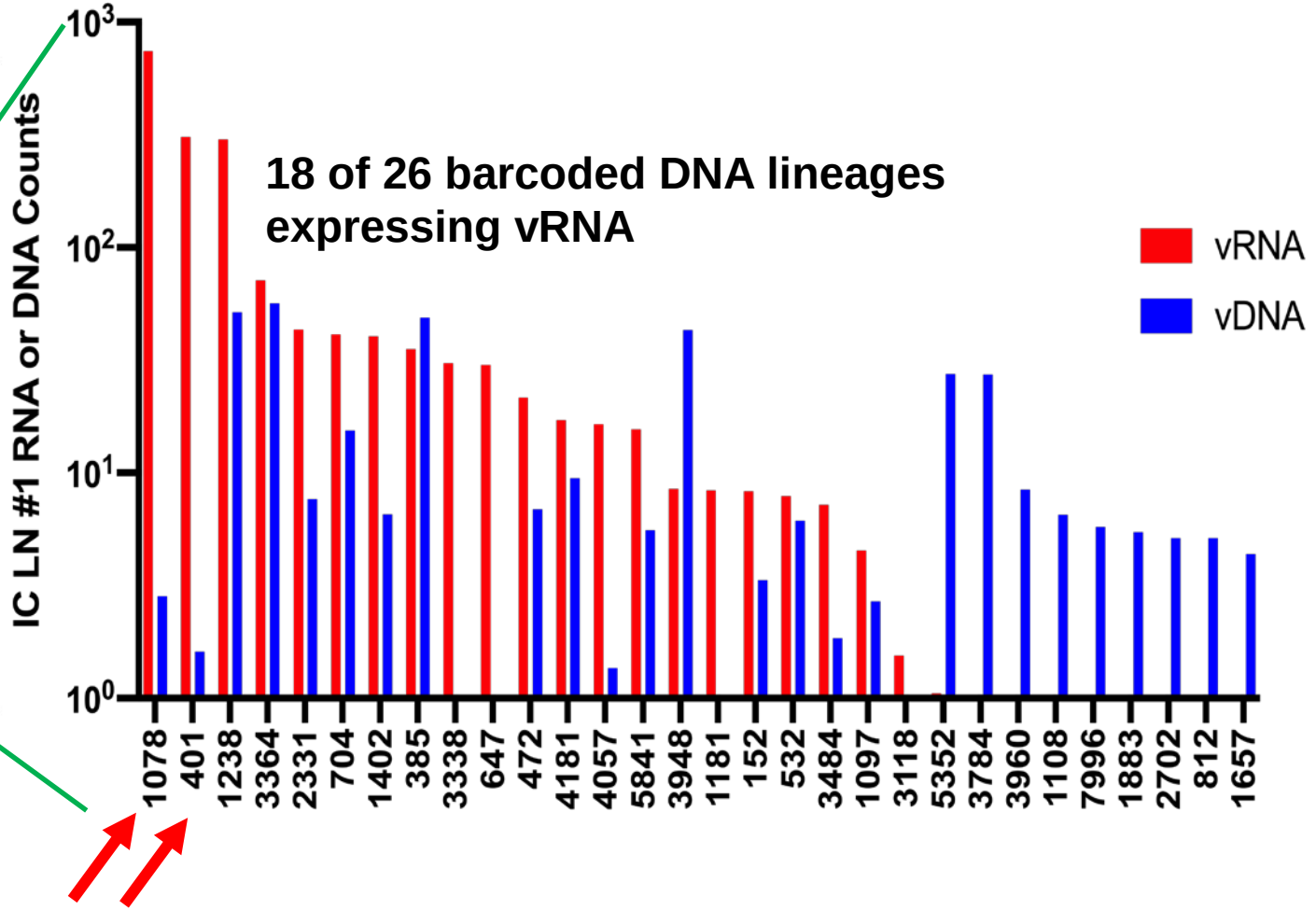


Tissue localized viral barcodes identify sites of local viral rebound

37167 ART Start 9dpi
Nx Off ART d 7
pVL 28 cp/ml

Ileocecal LN

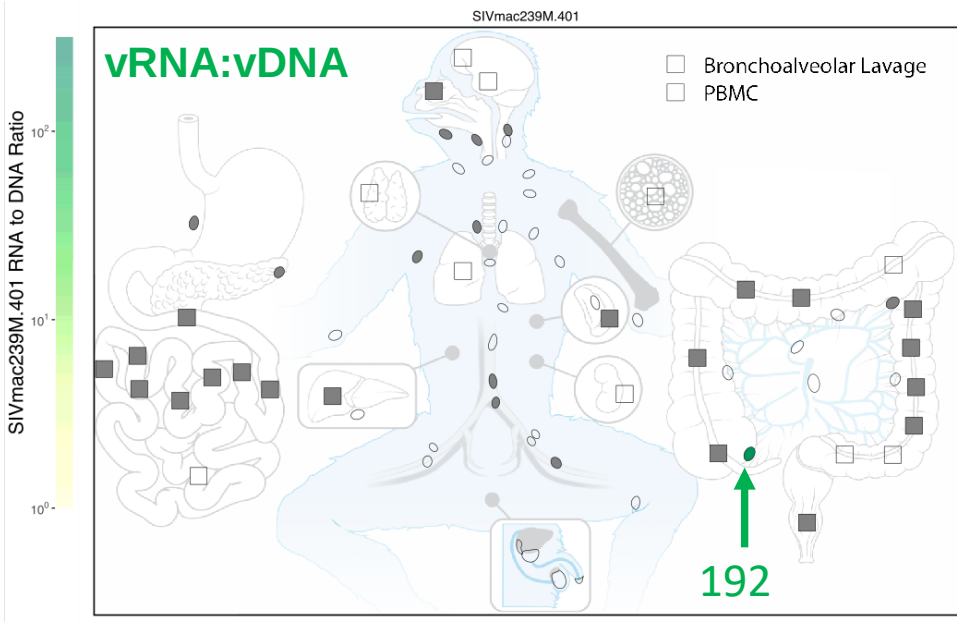




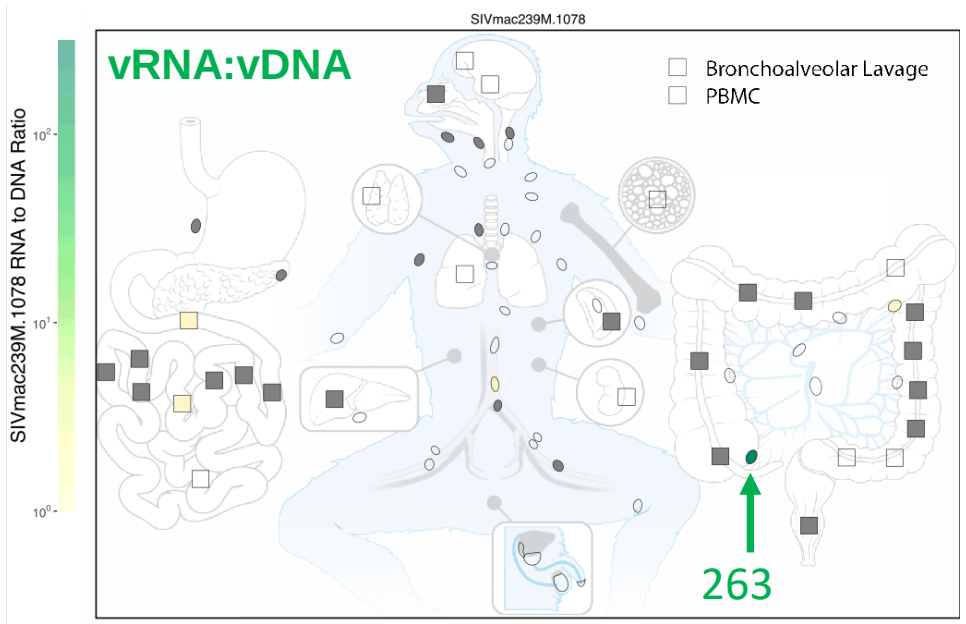
Tissue localized viral barcodes identify sites of local viral rebound

37167 ART Start 9dpi
Nx Off ART d 7
pVL 28 cp/ml

Barcode 401



Barcode 1078

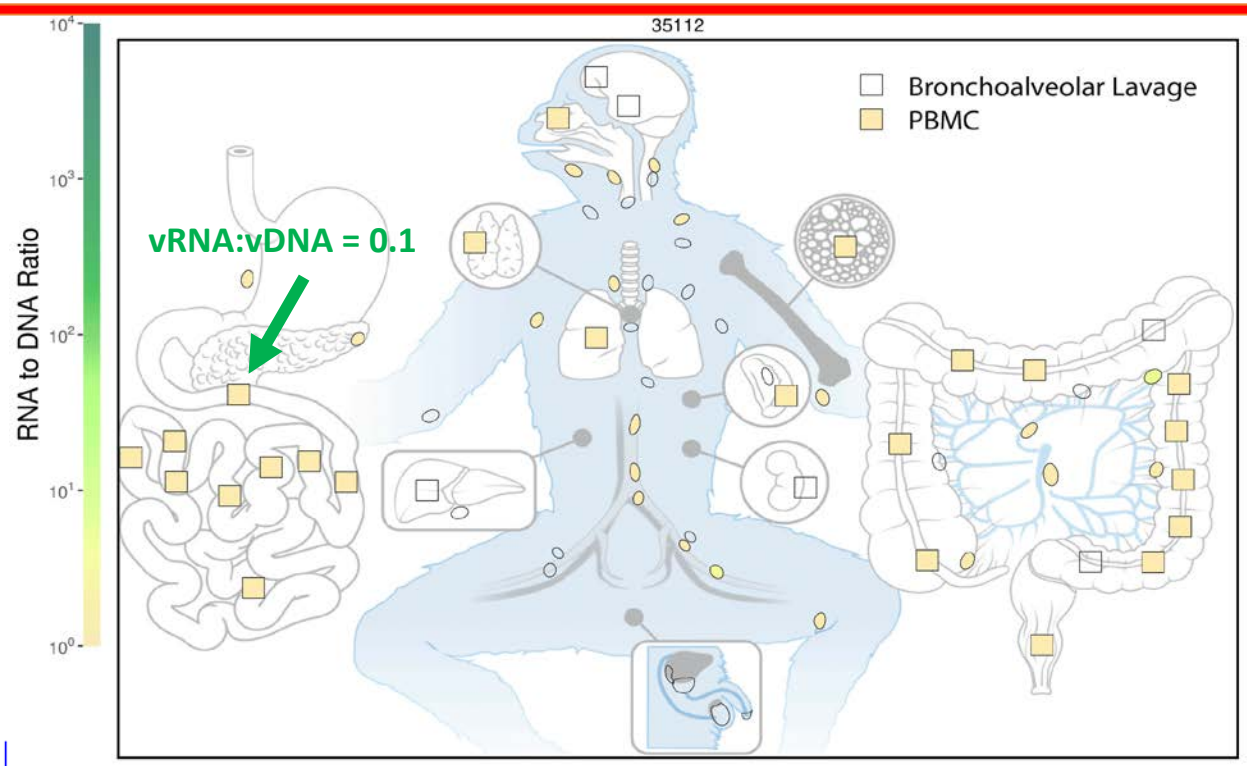


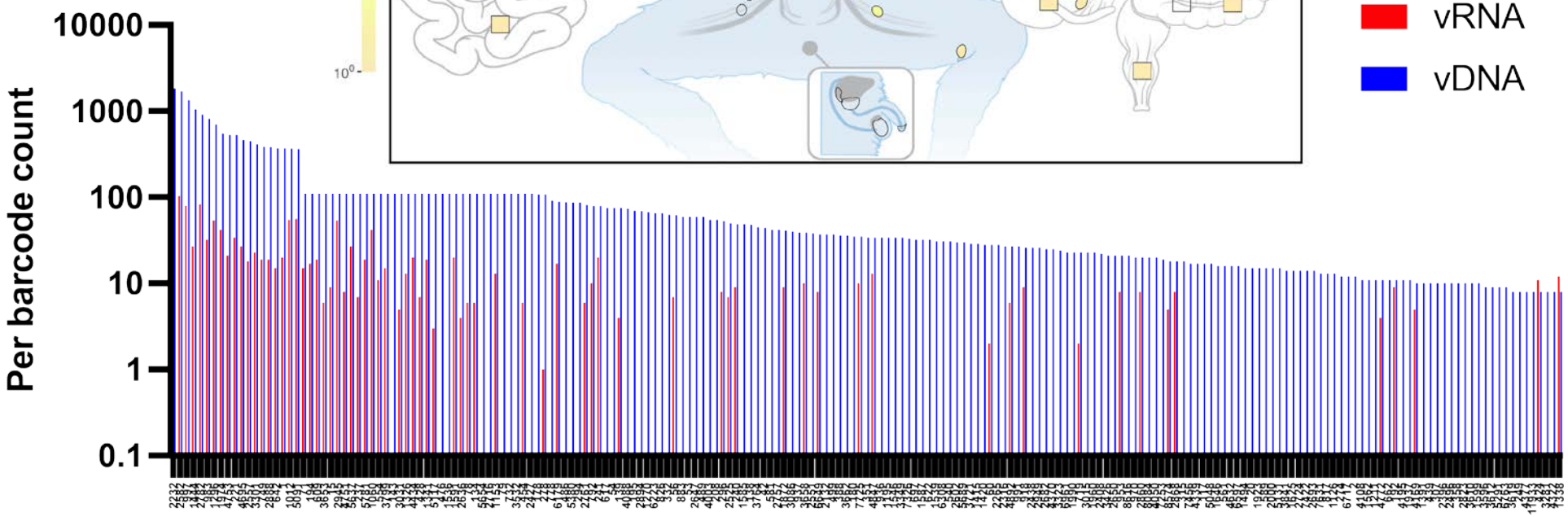
Ileocecal LN

Tissue localized viral barcodes identify sites that are likely not contributing to viral rebound

35112 ART Start 9dpi
Nx Off ART d 7
pVL 5 c/mL

Duodenum



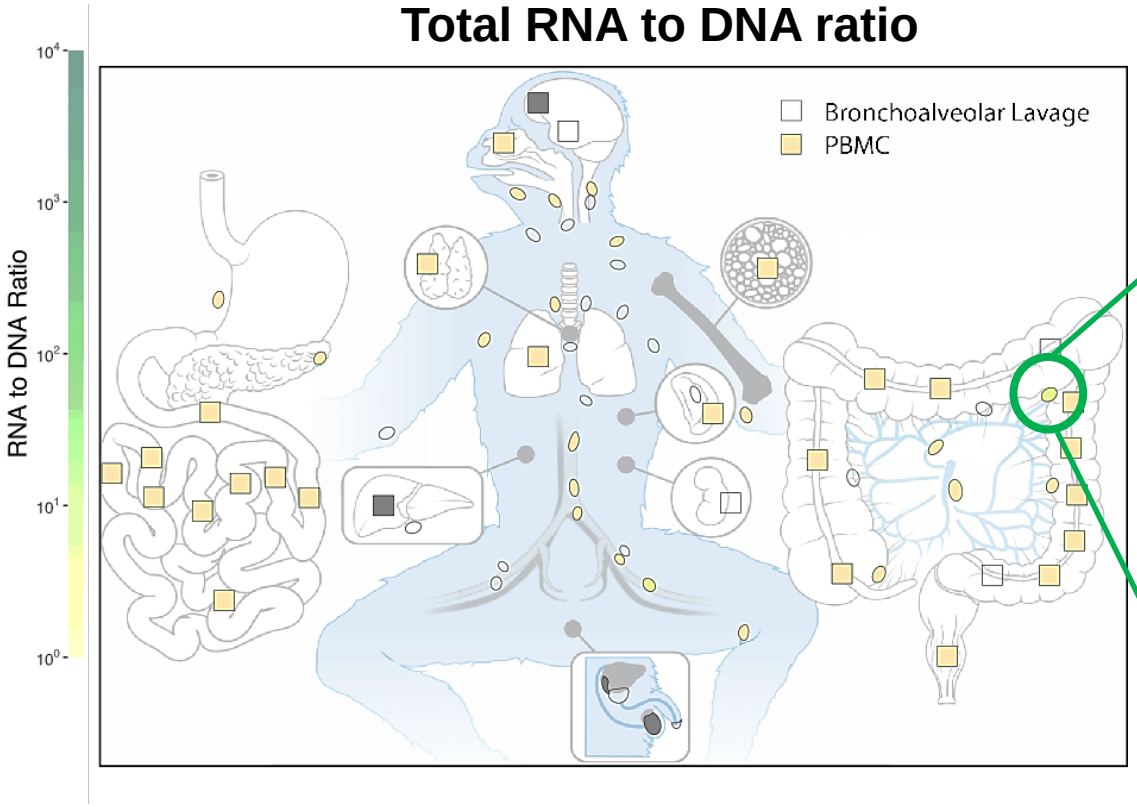


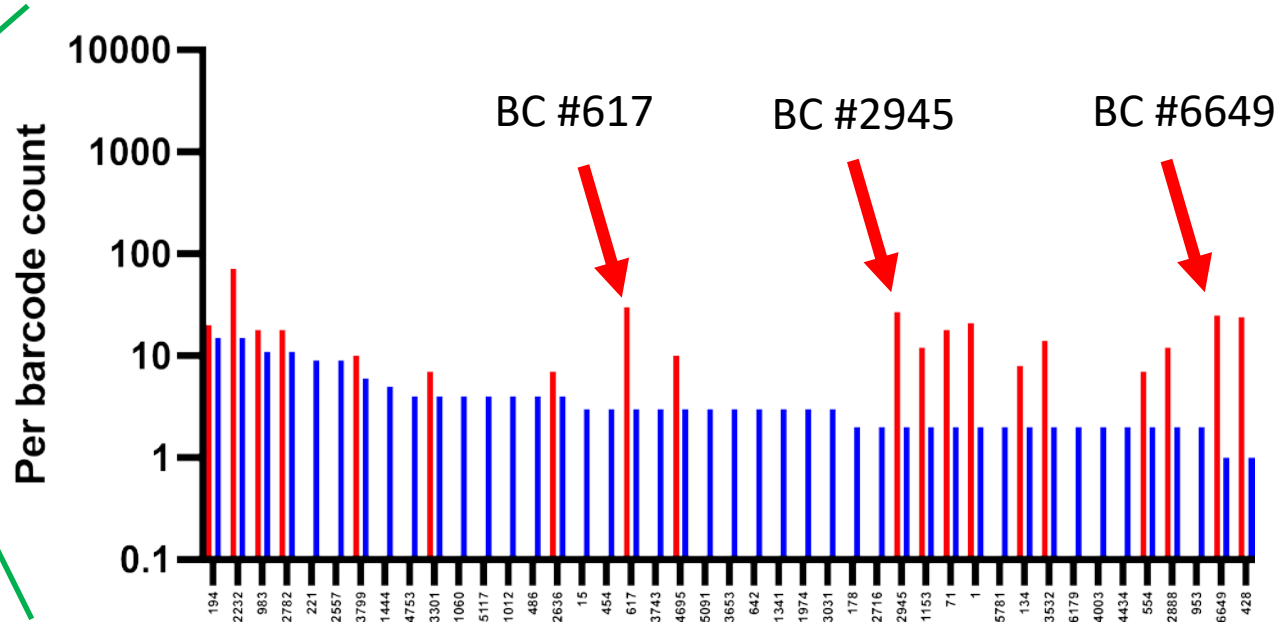
Tissue localized viral barcodes identify sites of local viral rebound

35112 ART Start 9dpi
Nx Off ART d 7
pVL 5 cp/ml

Colonic LN

Total RNA to DNA ratio



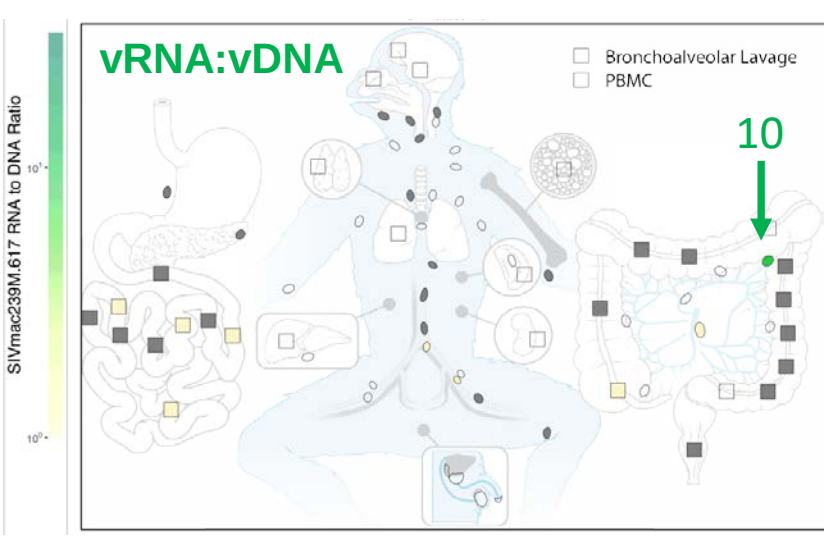


Tissue localized viral barcodes identify sites of local viral rebound

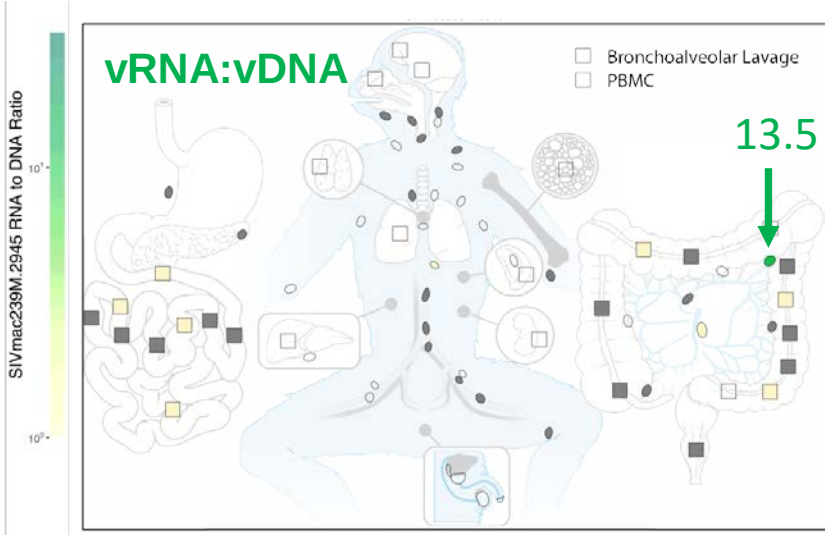
35112 ART Start 9dpi
Nx Off ART d 7
pVL 5 cp/ml

Colonic LN

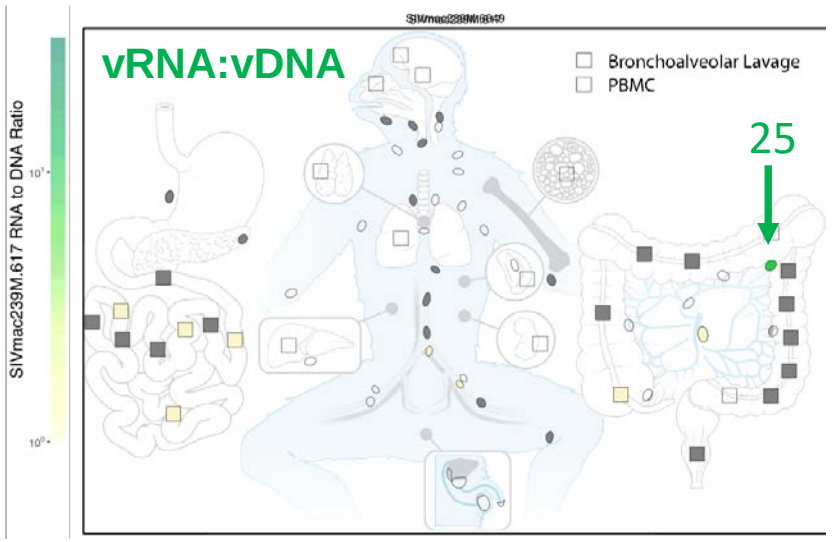
Barcode 617



Barcode 2945



Barcode 6649



Preliminary conclusions:

- **Early viral rebound is not a "needle-in-the-haystack" event that occurs in some special compartment that then spreads systemically in an explosive burst . . .**

- **Rather, it's a multi-, but generally pauci-focal process that occurs in various lymphoid tissues that differ in different individuals and appears (thus far) to stay "local" until relatively late (post overt viremia) in the process.**

- **The mechanisms that either restrict local rebound in sites that have SIV-infected cells, but lack such rebound or that promote such rebound in sites with rebound are unclear, but the comprehensive 'omics analysis planned in this study will reveal the immune pathways operating in these local neighborhoods, potentially shedding light on these mechanisms . . .**

- **. . . and providing targets for therapeutic interventions aimed at obviating rebound by enhancing restrictions and/or blocking inducers of rebound, or by facilitating effective immune intercept of rebound at the earliest stages.**

Final thoughts:

- **Completely successful immune responses teach us little about how the system works and its limitations; we learn more from failure, from trying to fix a broken system, which makes HIV/SIV an ideal model for immune exploration . . .**

- **In our work alone, we have discovered a completely novel immune response – MHC-E-restricted $CD8^+$ T cells – with unique properties potentially applicable not only to SIV/HIV, but many other pathogens and cancers . . .**

- **HIV vaccine and cure studies have demonstrated the importance of immune dynamics on $CD8^+$ T cell function, and tools we have developed will allow detailed investigation of the local interactions that mediate or fail to mediate, viral control.**

- **Monkeys are not humans, and will not model all aspects of human infection, but so far, the mechanisms revealed in detailed monkey studies have clear applicability to human infection, and in my view, will enable development of sophisticated new approaches to immunotherapy, with broad applicability.**

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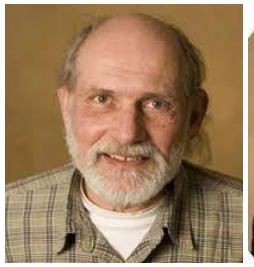
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David Morrow
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OHSU has licensed CMV technology, of which Dr. Picker is an inventor, to Vir Biotechnology, Inc., a company in which both OHSU and Dr. Picker have significant financial interest. Potential individual and institutional conflicts of interest have been reviewed and managed by OHSU.

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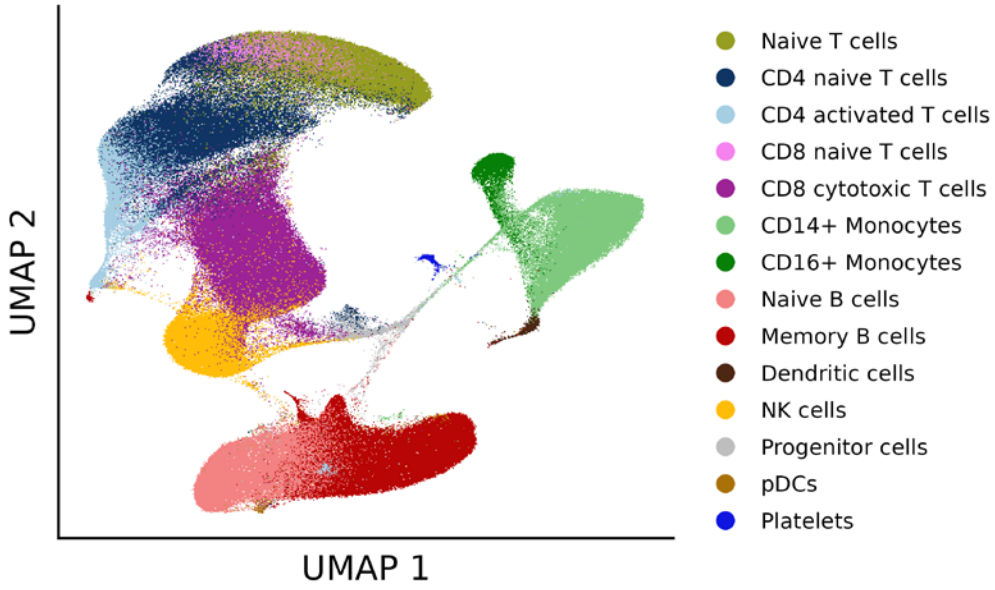
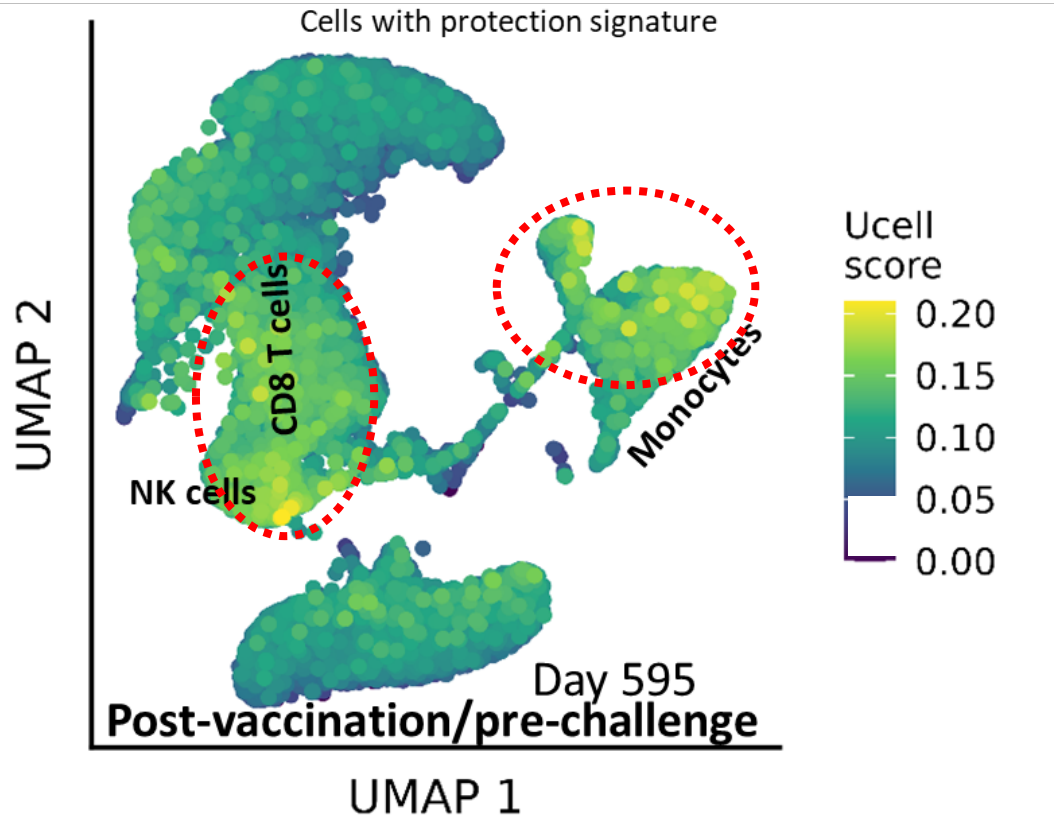
Single cell RNAseq of PBMC from vaccine study cohorts: Vaccine protection signature resides in monocytes, CD8⁺ T cells, and NK cells

Cell classification



Circulating cells with IL-15 protection signature



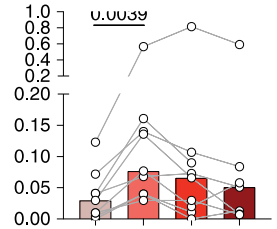
The "Vaccinal Effect" – the potential for neutralizing anti-HIV/SIV antibodies to from immune complexes that potentiate CD8$^+$ T cell responses . . .

LETTERS
https://doi.org/10.1038/s41591-019-0747-1
nature medicine

Combination anti-HIV-1 antibody therapy is associated with increased virus-specific T cell immunity

Julia Niessl[1,2,3], Amy E. Baxter[1,2,3,9], Pilar Mendoza[4], Mila Jankovic[4], Yehuda Z. Cohen[4], Allison L. Butler[4], Ching-Lan Lu[4,10], Mathieu Dubé[1], Irina Shimeliovich[4], Henning Gruell[5,6,7], Florian Klein[5,7,8], Marina Caskey[4], Michel C. Nussenzweig[4,11]* and Daniel E. Kaufmann[1,2,3,11]*

b



bNAb Rx during ART interruption

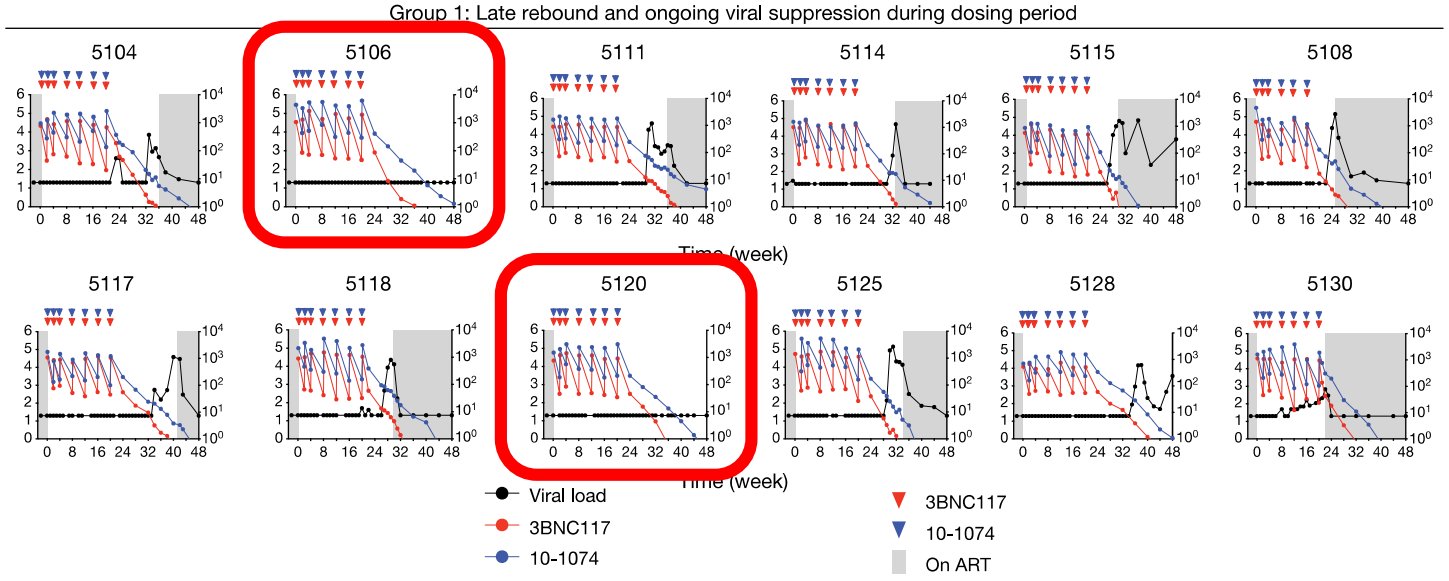
. . . Which might sustain viral control after decay of nAb levels

Article

Prolonged viral suppression with anti-HIV-1 antibody therapy

https://doi.org/10.1038/s41586-022-04597-1
Received: 14 November 2021
Accepted: 28 February 2022
Published online: 13 April 2022

Christian Gaebler[1], Lilian Nogueira[1], Elina Stoffel[1,2], Thiago Y. Oliveira[1], Gaëlle Breton[1], Katrina G. Millard[1], Martina Turroja[1], Allison Butler[1], Victor Ramos[1], Michael S. Seaman[3], Jacqueline D. Reeves[4], Christos J. Petropoulos[4], Irina Shimeliovich[1], Anna Gazumyan[1], Caroline S. Jiang[5], Nikolaus Jilg[6], Johannes F. Scheid[7], Rajesh Gandhi[6], Bruce D. Walker[8], Michael C. Sneller[9], Anthony Fauci[9], Tae-Wook Chun[9], Marina Caskey[1,11]✉ & Michel C. Nussenzweig[1,10,11]✉



K11 and ITS103.01 are novel neutralizing antibodies against SIVmac239

nature communications

Article

https://doi.org/10.1038/s41467-022-32783-2

Molecular insights into antibody-mediated protection against the prototypic simian immunodeficiency virus

Received: 11 February 2022

Accepted: 16 August 2022

Published online: 06 September 2022

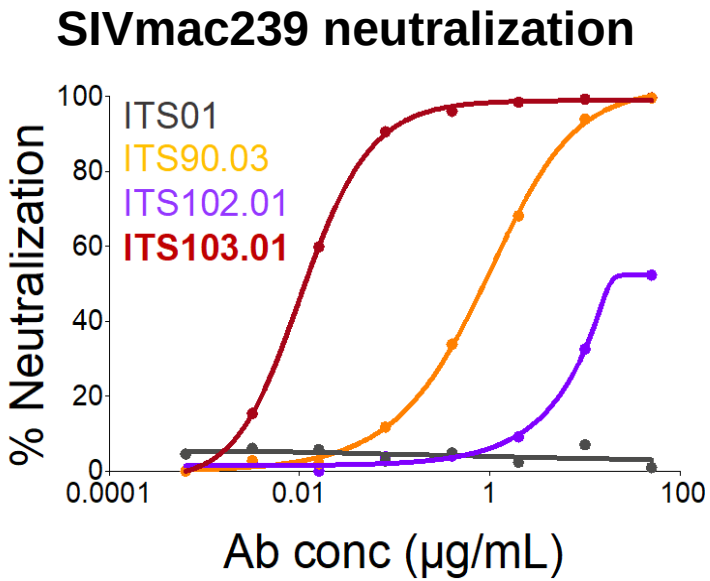
Check for updates

Fangzhu Zhao[1,2,3,13], Zachary T. Berndsen[2,3,4,13], Nuria Pedreño-Lopez[5,13], Alison Burns[1,2,3], Joel D. Allen[6], Shawn Barman[1,2,3], Wen-Hsin Lee[2,3,4], Srirupa Chakraborty[7], Sandrasegaram Gnanakaran[7], Leigh M. Sewall[2,3,4], Gabriel Ozorowski[2,3,4], Oliver Limbo[2,8], Ge Song[1,2,3], Peter Yong[1,2,3], Sean Callaghan[1,2,3], Jessica Coppola[1,2,3], Kim L. Weisgrau[9], Jeffrey D. Lifson[10], Rebecca Nedellec[1,2,3], Thomas B. Voigt[5], Fernanda Laurino[5], Johan Louw[5], Brandon C. Rosen[5,11], Michael Ricciardi[5], Max Crispin[6], Ronald C. Desrosiers[11], Eva G. Rakasz[9], David I. Watkins[5], Raiees Andrabi[1,2,3] ✉, Andrew B. Ward[2,3,4] ✉, Dennis R. Burton[1,2,3,12] ✉ & Devin Sok[1,2,3,8] ✉

K11 (Dennis Burton)

Glycan hole and V1/V4 loop

Documented ADCC activity



PLOS PATHOGENS

RESEARCH ARTICLE

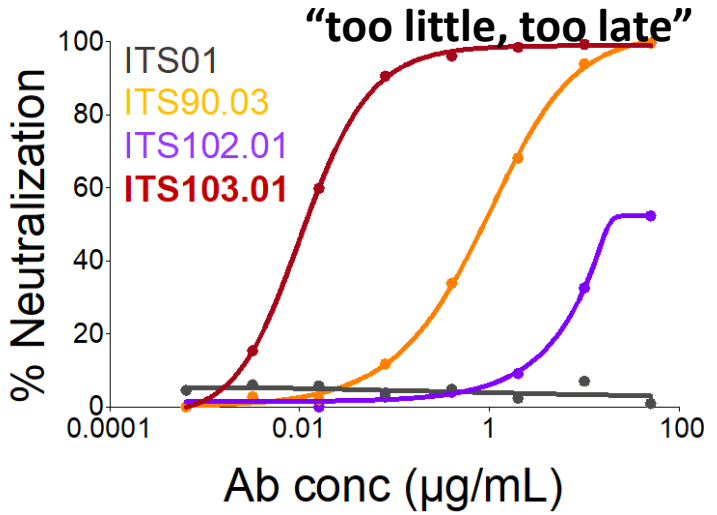
Broad coverage of neutralization-resistant SIV strains by second-generation SIV-specific antibodies targeting the region involved in binding CD4

Hugh C. Welles[1], Hannah A. D. King[1,2,3], Leonard Nettey[1], Nicole Cavett[1], Jason Gorman[1], Tongqing Zhou[1], Yaroslav Tsybovsky[4], Renguang Du[1], Kaimei Song[1], Richard Nguyen[1], David Ambrozak[1], Amy Ransier[1], Chaim A. Schramm[1], Nicole A. Doria-Rose[1], Adrienne E. Swanstrom[5], James A. Hoxie[6], Celia LaBranche[7], David C. Montefiori[7], Daniel C. Douek[1], Peter D. Kwong[1], John R. Mascola[1], Mario Roederer[1], Rosemarie D. Mason[1]*

ITS103.01 (Mario Roederer)

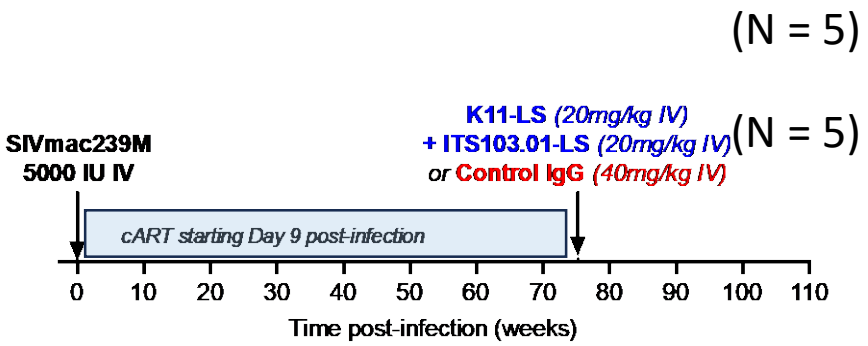
CD4 binding site

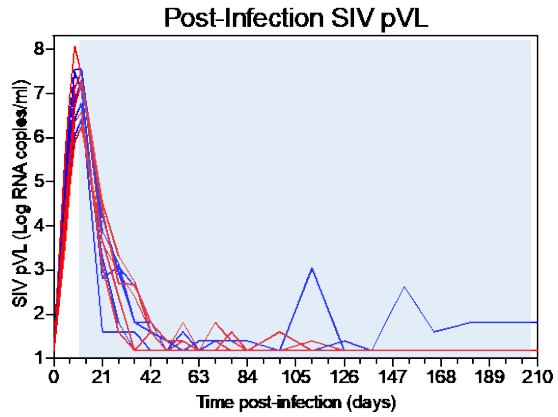
Documented ADCC activity

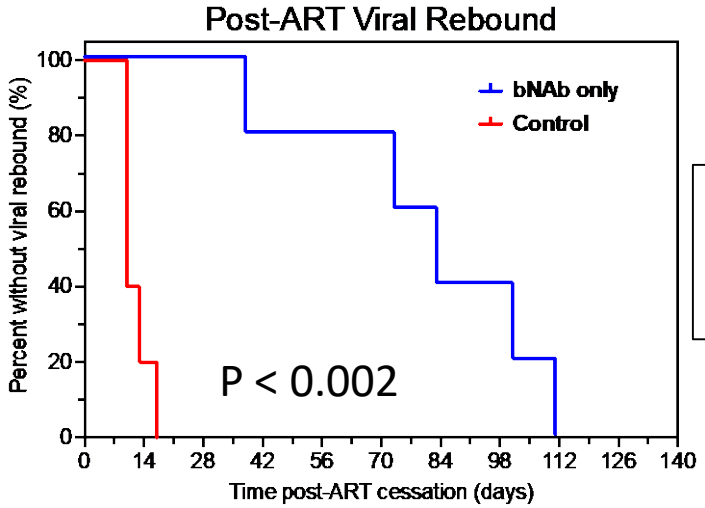


In pilot studies, the combination of these 2 mAbs was able to suppress *in vivo* viral replication at neutralizing titres above ~ 1/500.

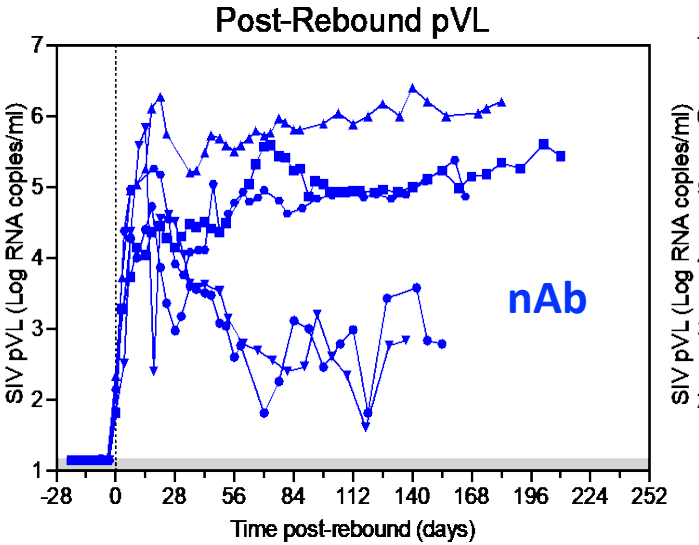
nAb Study 1: Day 9 ART; 72 weeks of viral suppression

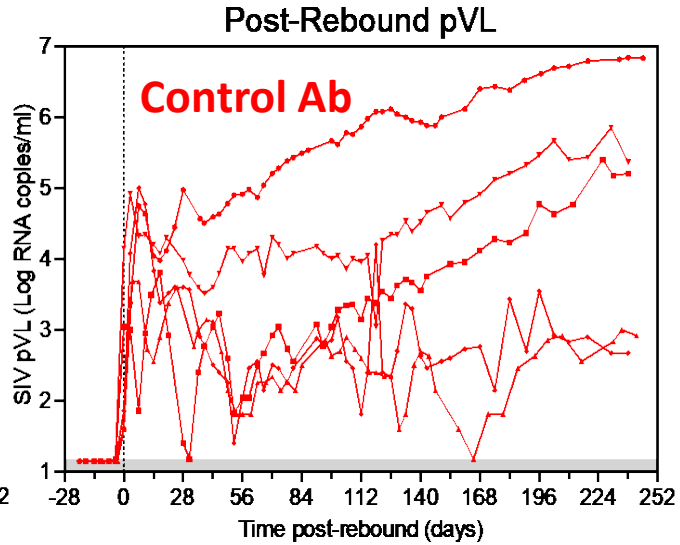




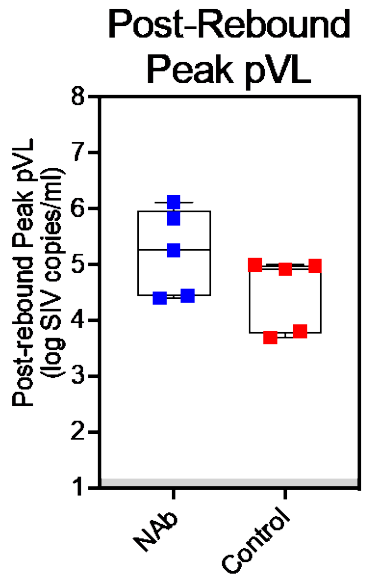


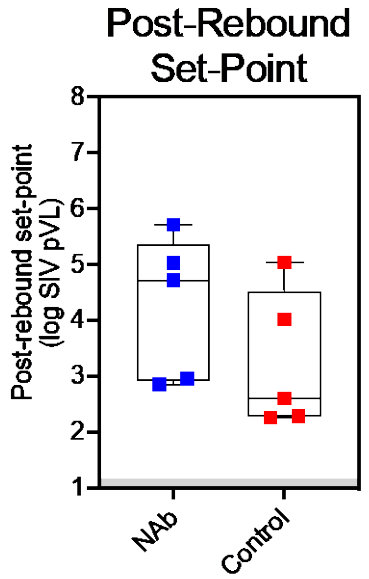
Median nAb titer prior to rebound: 1/330

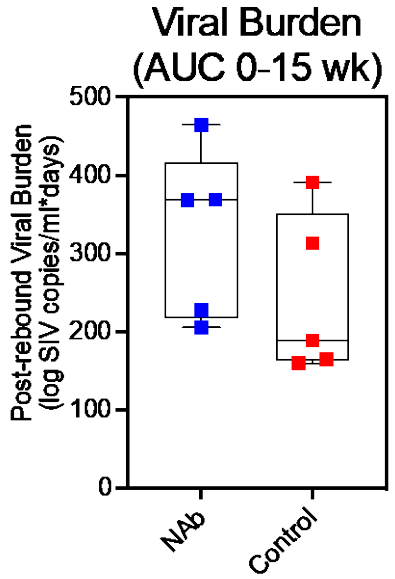




Normalized to rebound

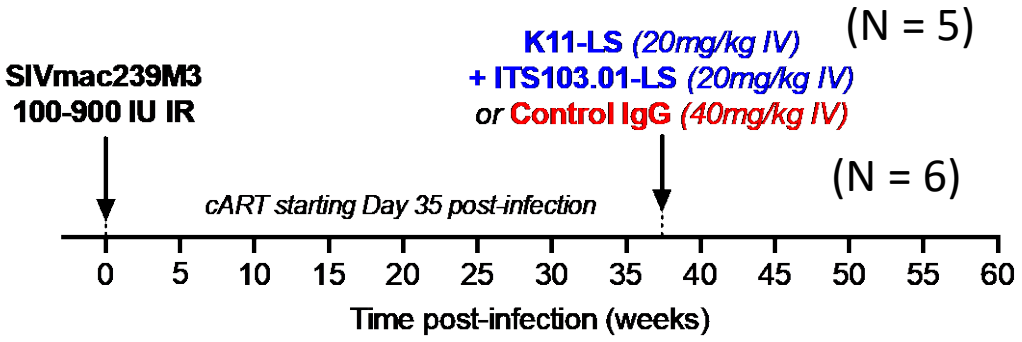


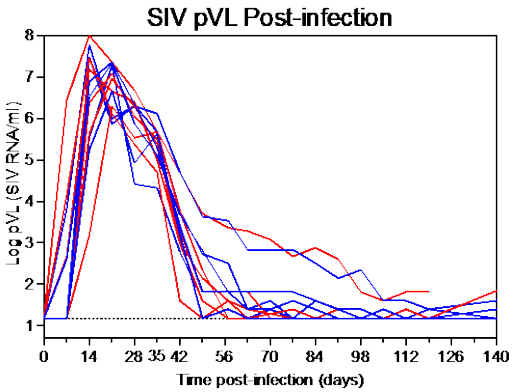




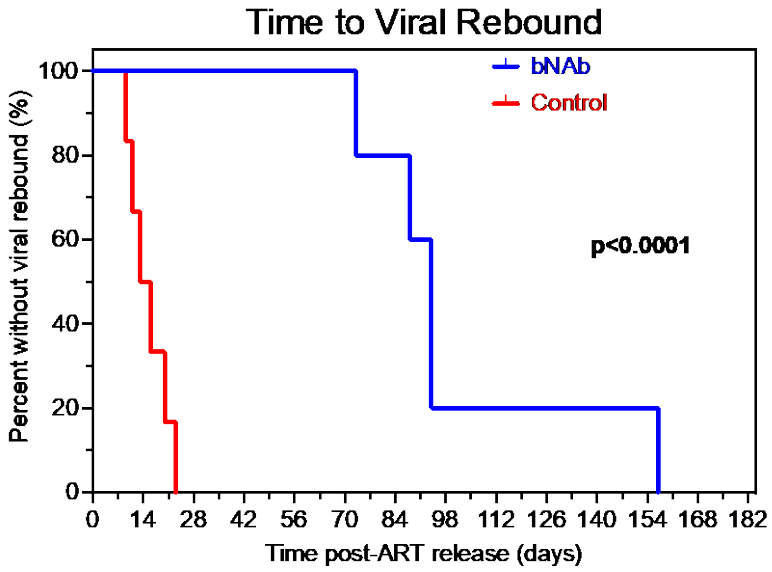
No difference in post-ART viral setpoint

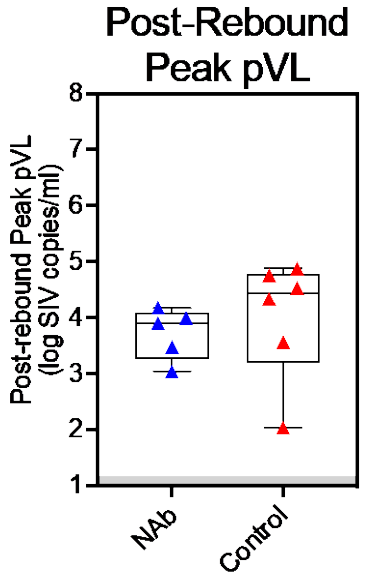
nAb study 2: Day 35 ART with protective MHC-Ia alleles

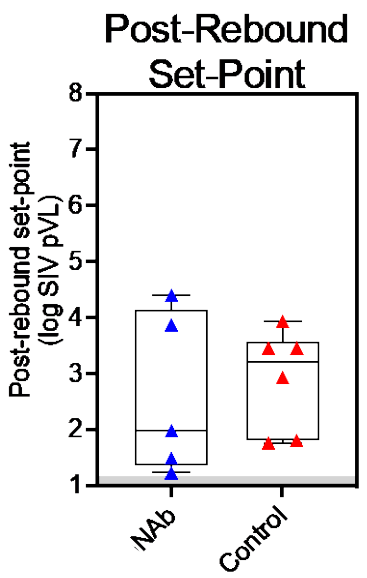


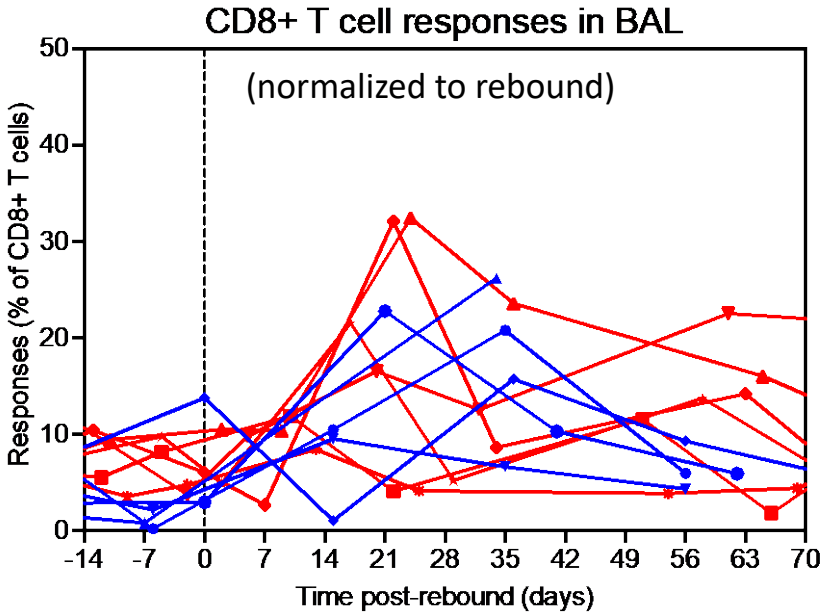


- *ART starting day 35 pi (response maturation)*
- *ART cessation at ~33 wpi (28-36)*
- *Groups balanced by*
  - *SIV pVL during acute infection*
  - *Sex (4 female, 2 male per group)*
  - *pMHC alleles (all)*



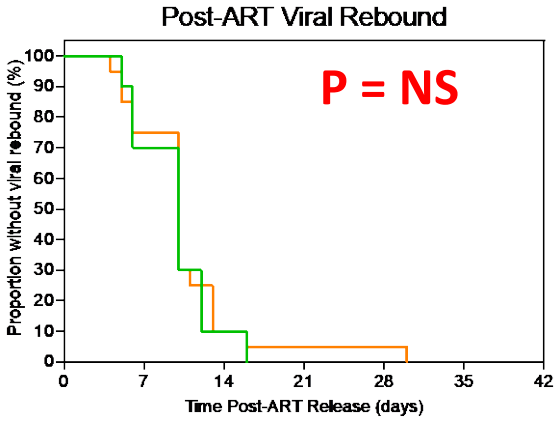


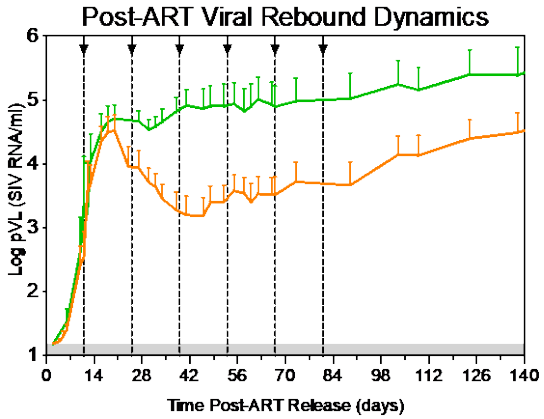




Finally, we tested the efficacy of PD1 blockade with and without nAb Rx . . .

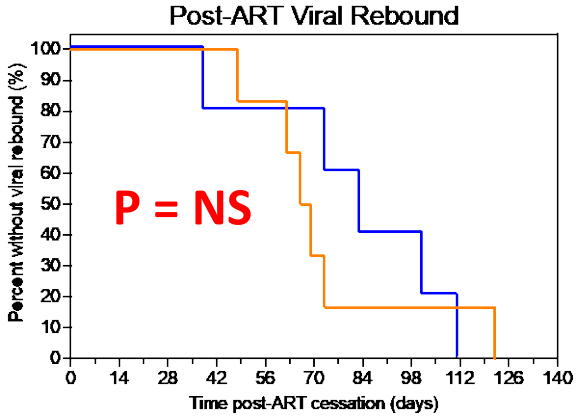
No nAb

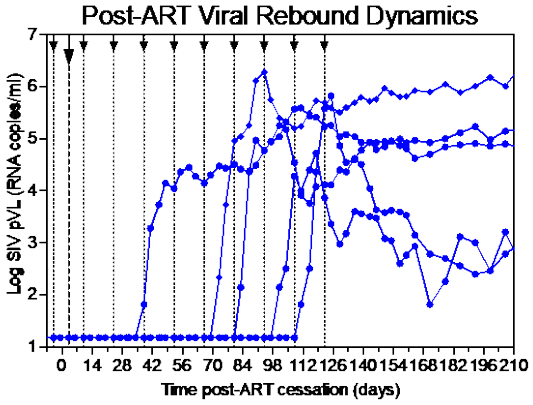


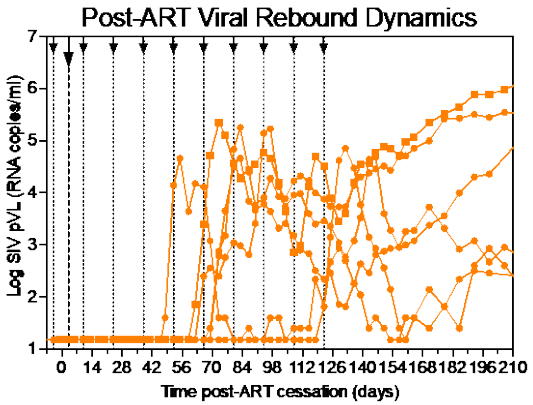


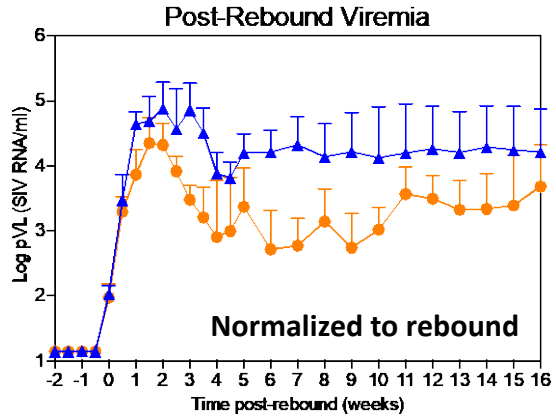
Green = Control (n=10)
Orange = PD1 blockade (n=20)

with nAb

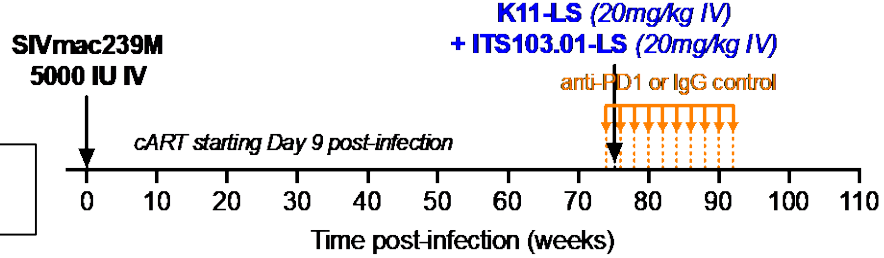








Blue = nAb (n=5)
Orange = nAb+PD1 blockade (n=6)



No apparent advantage with the nAb + anti-PD-1 combination