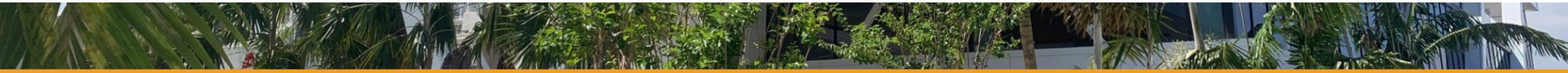
Suppression of viral rebound by a Rev-dependent lentiviral particle in SIV-infected rhesus macaques

Yuntao Wu

Center for Infectious Disease Research, George Mason University

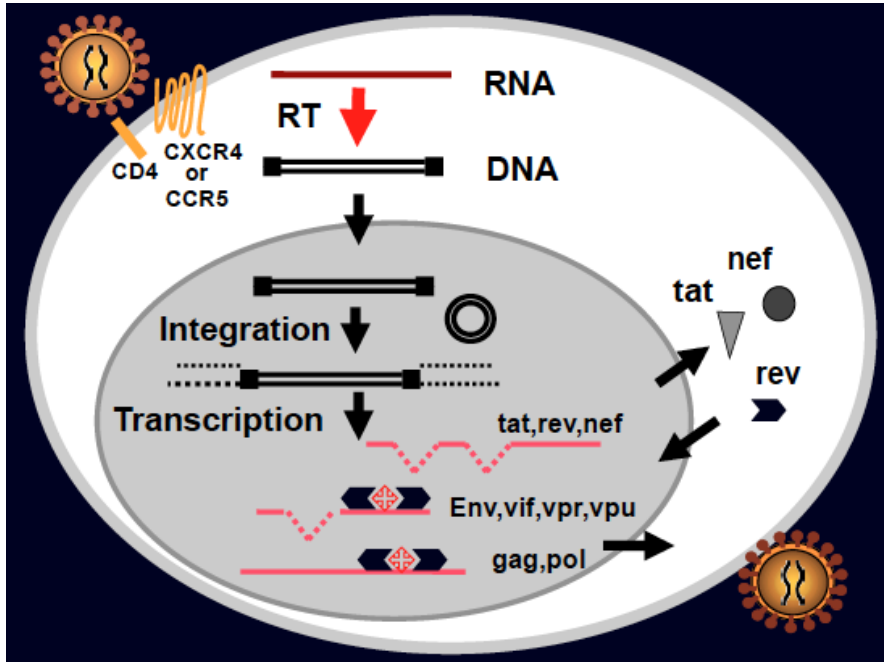
ywu8@gmu.edu

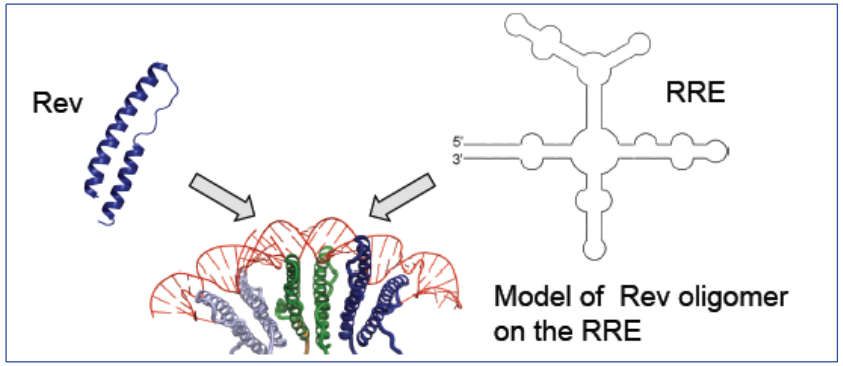


CONFLICTS OF INTEREST

B. Hetrick and Y. Wu are co-founders of Viropeutics Inc., a George Mason University spin-out company

Rev-dependent selective targeting of HIV reservoirs (2002 – 2024)





(Daugherty, Liu, and Frankel, 2010)

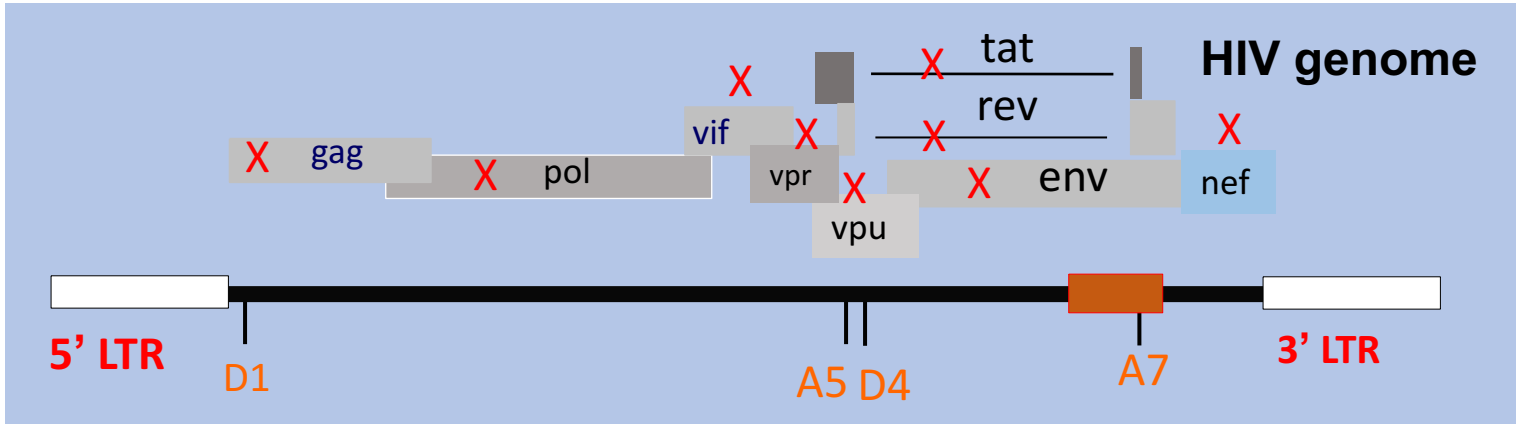
HIV "Rev" as the trigger

1) **Rev is expressed only in HIV$^+$ cells**
2) **Rev is essential for HIV replication**
3) **Rev interacts with RRE** (Maliam, 1989)
4) **Rev/RRE regulates HIV mRNA splicing and the nuclear export of unspliced mRNA**

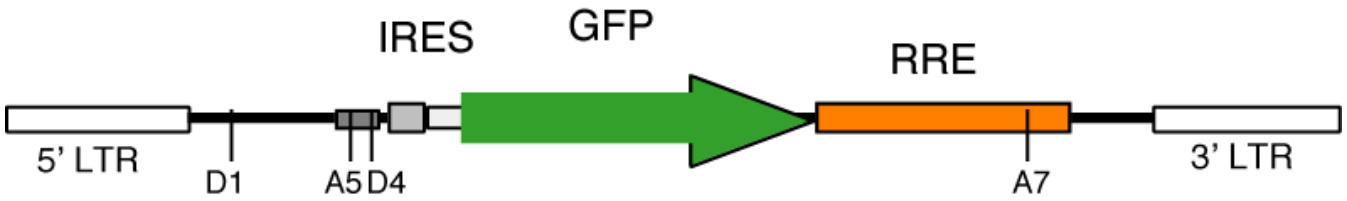
Other functions of Rev/RRE interaction:

- Genome dimerization and packaging (Anson, 2003)
- HIV structural protein translation (Arrigo, 1991)

Development of the HIV Rev-dependent lentiviral vector



- Delete all HIV genes
- Use REV/RRE to regulate Rev-dependent gene expression



pNL-GFP-RRE(SA)

(Available from NIH AIDS Reagent Program – Cat# ARP-11466)

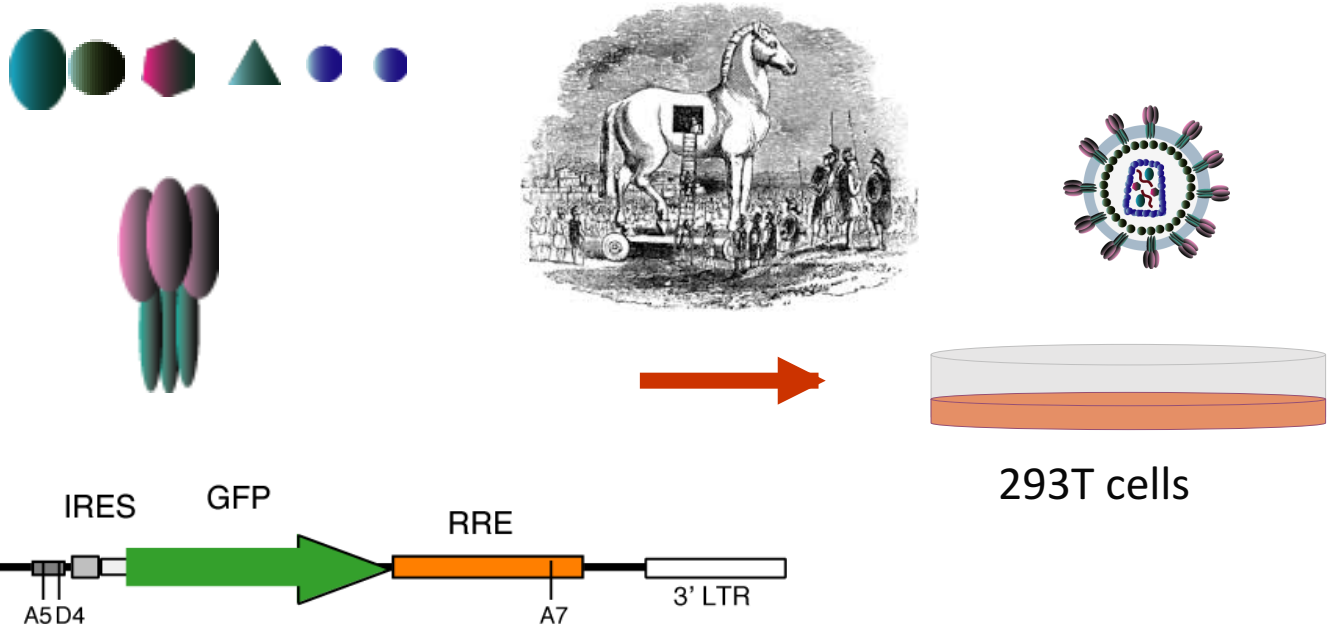
Wu *et al.* 2007, *Retrovirology* 4:12

Wu *et al.* 2007, *Current HIV Research 5: 395*

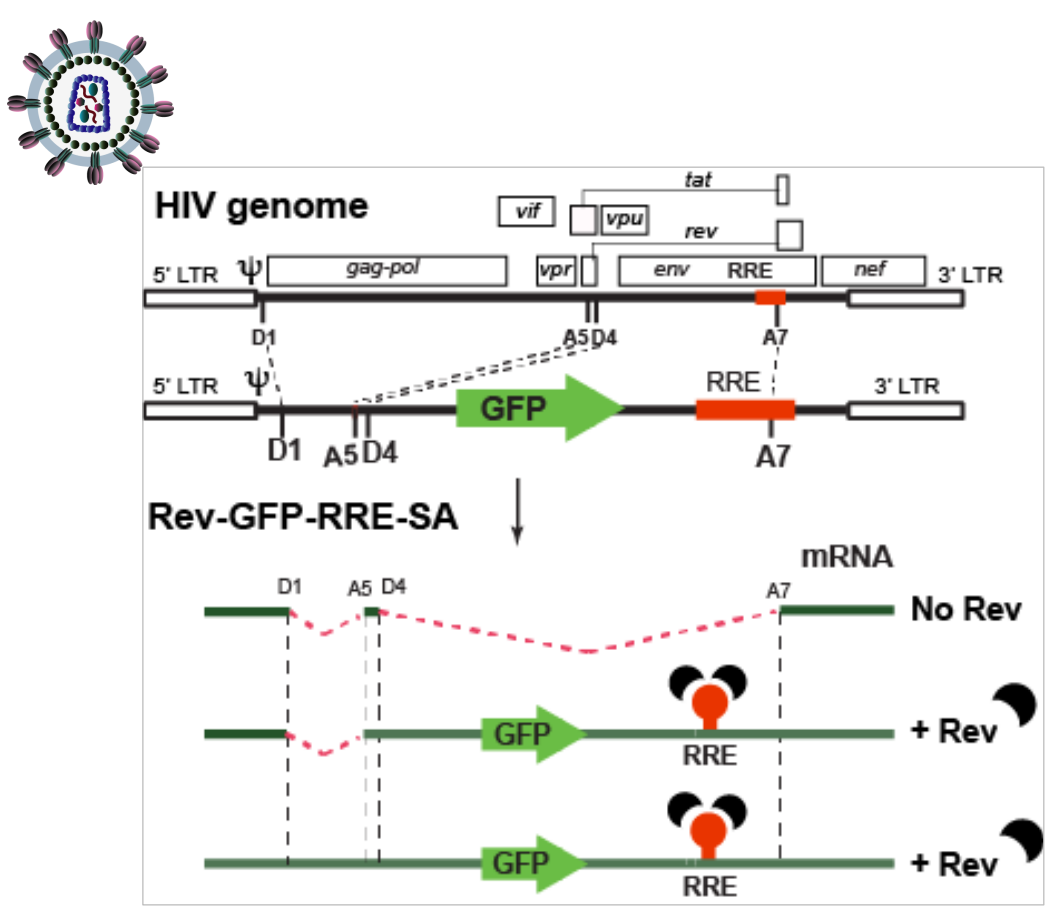
Young *et al.* 2008, *Retrovirology* 5:36

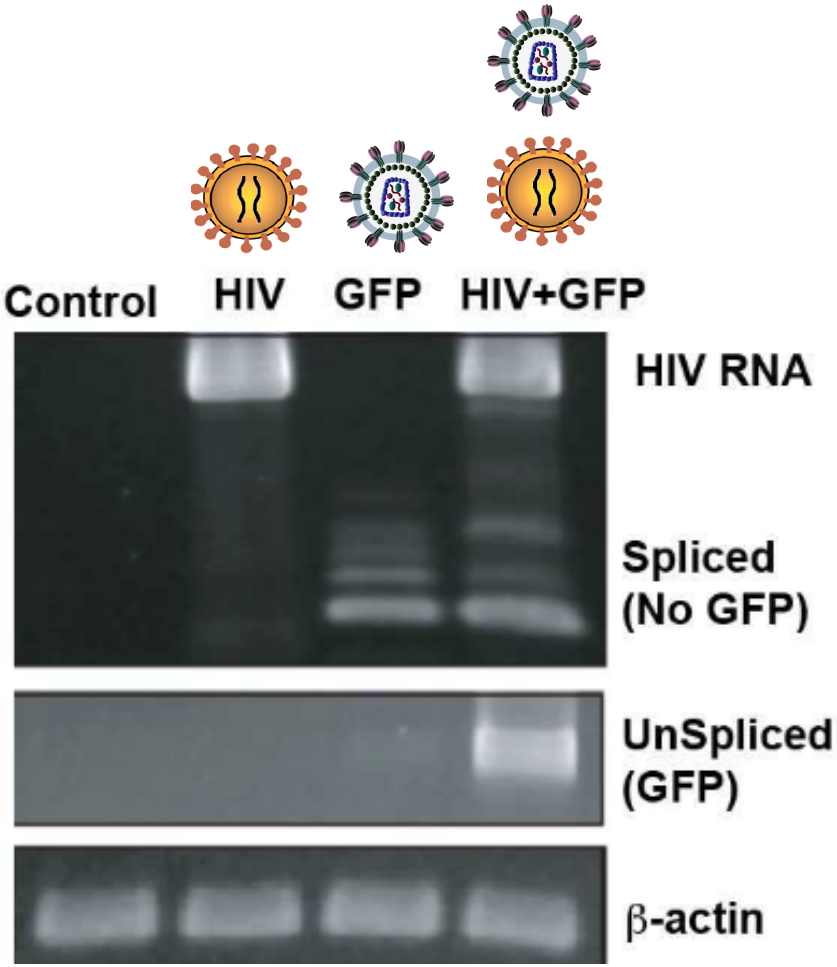
Wang *et al.* 2010, *Gene Therapy* 17:1063

Assembly of the HIV Rev-dependent lentiviral particles



Rev-dependent, selective expression of GFP only in HIV+ cells



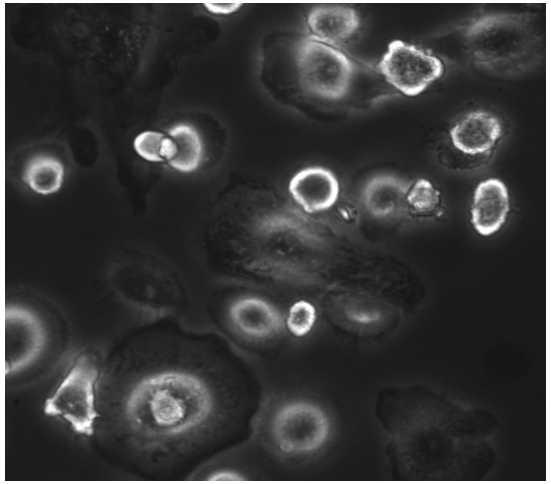


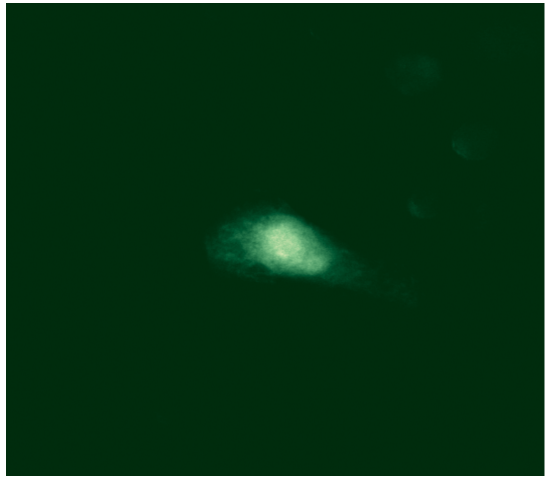
Wu *et al.* 2007, *Retrovirology* 4:12

Specific marking of HIV+ macrophages by the Rev-dependent particles

HIV-1(AD8)

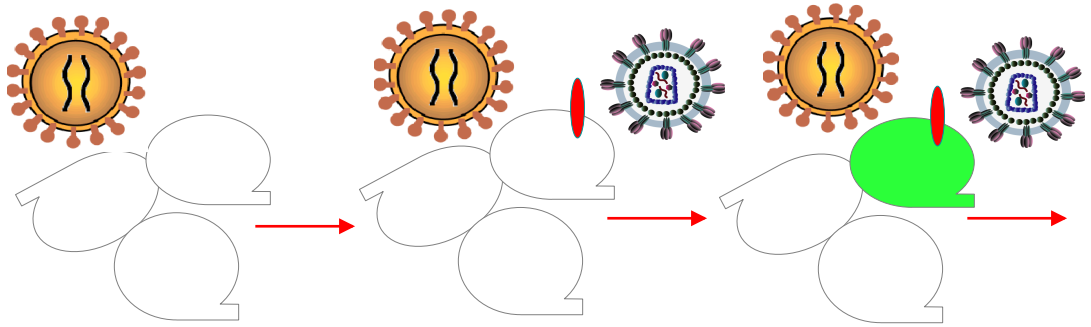
HIV-1(AD8) + NL-GFP-RRE



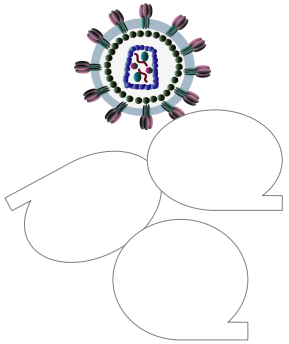


Specific marking of HIV+ T cells by the Rev-dependent particles

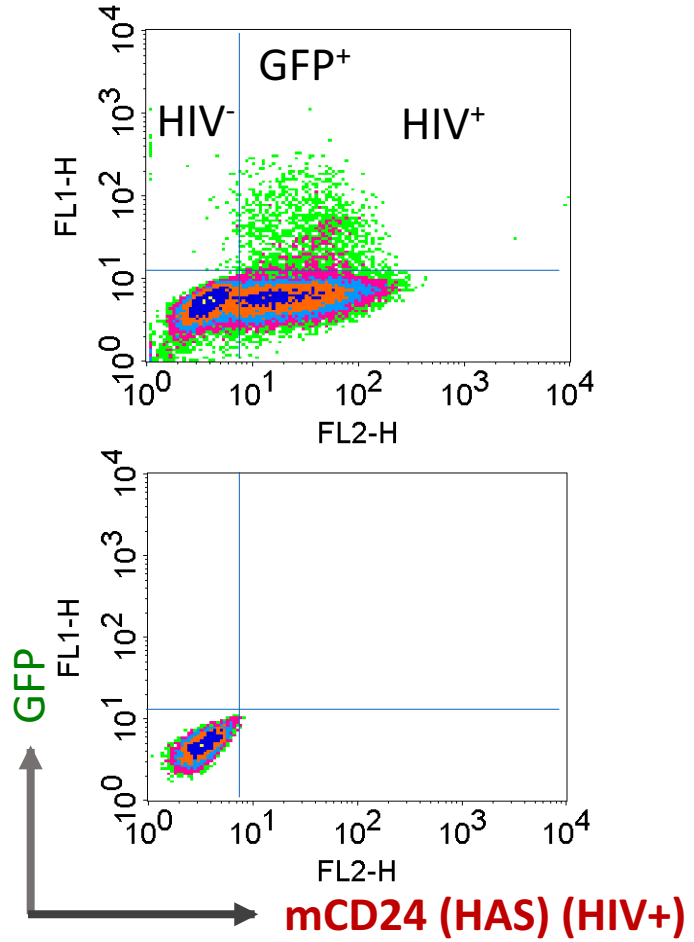
HIV(HAS) + **NL-GFP-RRE**



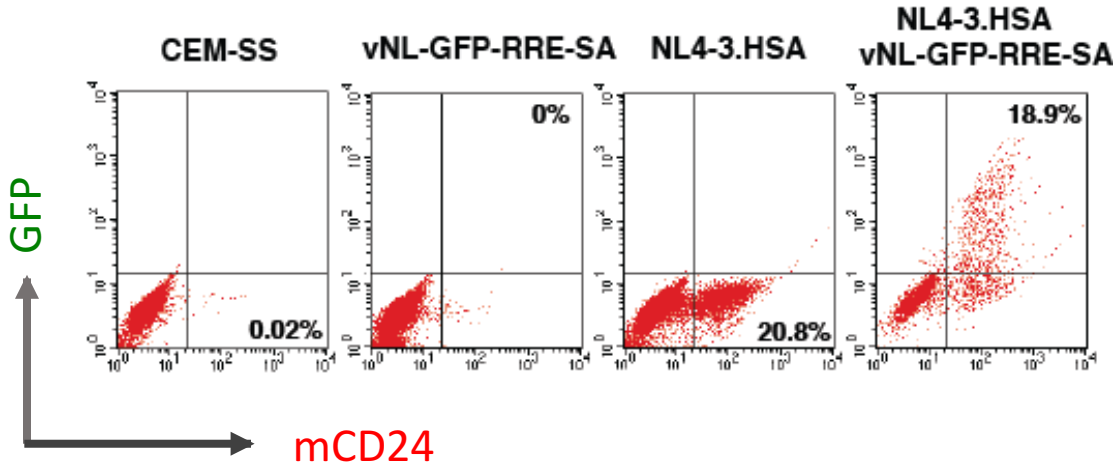
NL-GFP-RRE only

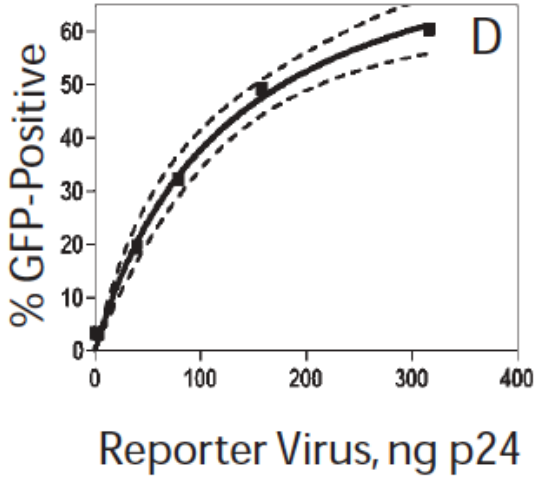


Wu *et al.* 2007, *Retrovirology 4*:12



Specific marking of 90% HIV$^+$ T cells by the Rev-dependent lentiviral particles



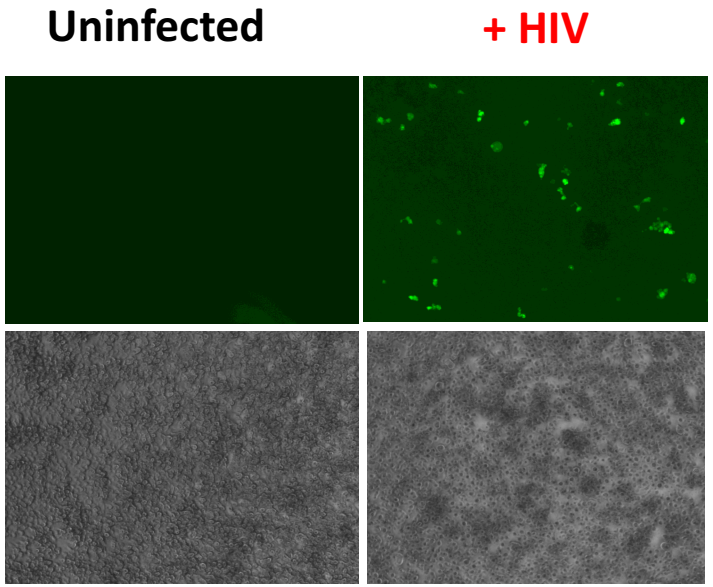


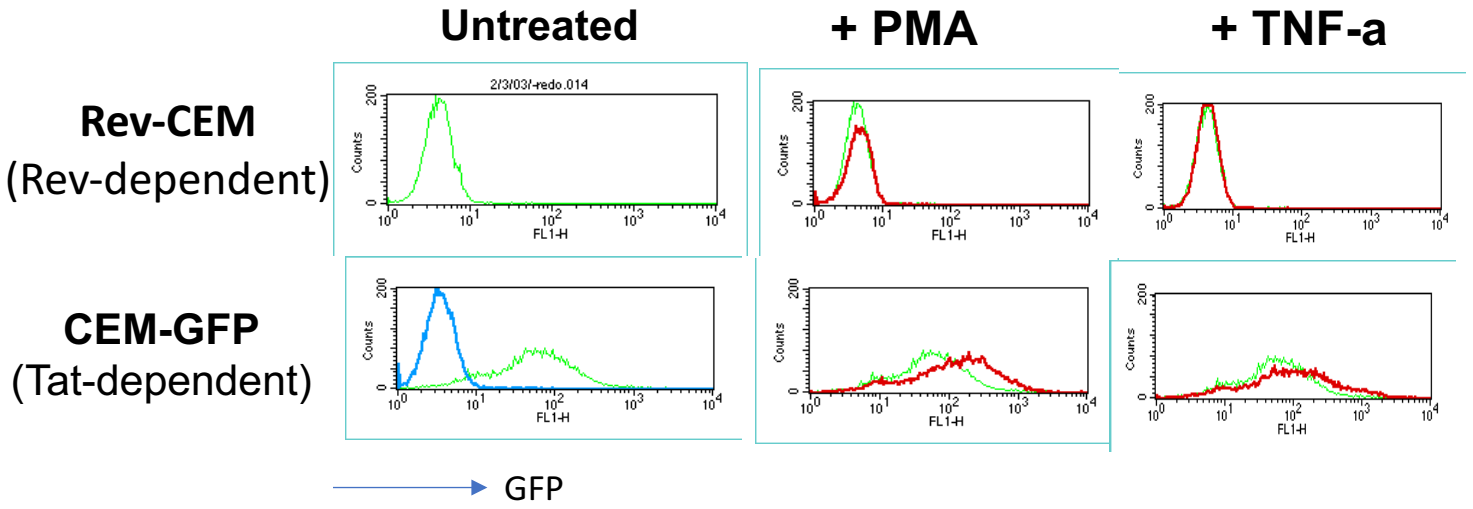
Wu *et al.* 2007, *Retrovirology* 4:12

Young *et al.* 2008, *Retrovirology* 5:36

High Specificity of the HIV Rev-dependent indicator cells

Rev-CEM GFP indicator cell *(available from NIH AIDS Reagent Program – Cat# ARP-11447)*





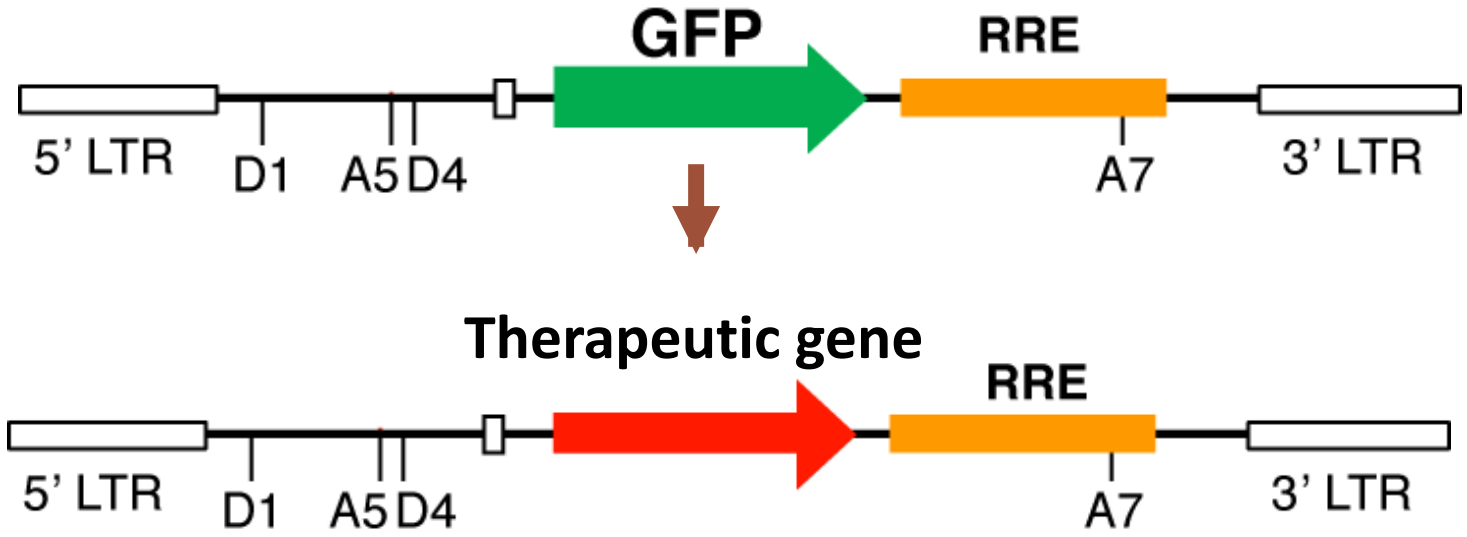
Wu *et al.* 2007, *Current HIV Research 5: 395*

Wu *et al.* 2007, *Retrovirology 4*:12

pNL-GFP-RRE(SA)-based Rev-dependent cells by others:

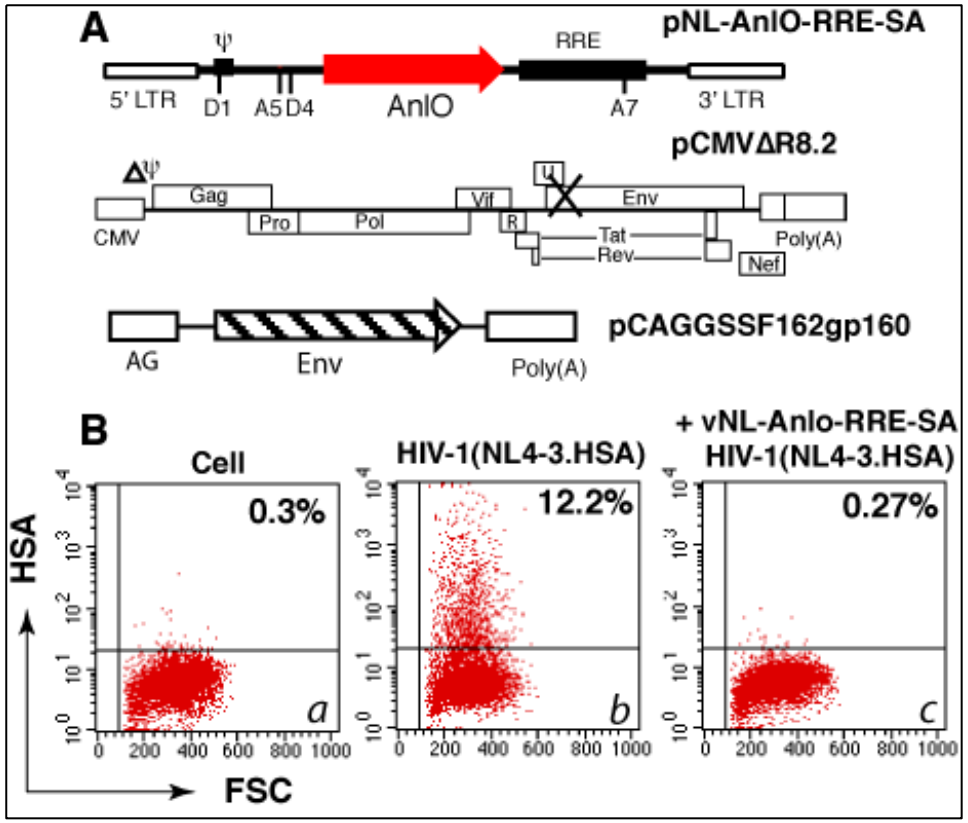
Sup-GGP (Salasc et al, 2019, Scientific Reports, 19325)
TZM-GFP (Gludish et al. 2020, Scientific Reports, 19900)
Rev-GGR (Benhur Lee et al. 2017, US Patent- US9,719,127 B2)
Rev-CEM-D4/-E7 (Alex Sigal, Jackson et al. 2018, eLife, 30134)

Selective killing of HIV$^+$ cells by the Rev-dependent particles *in vitro*



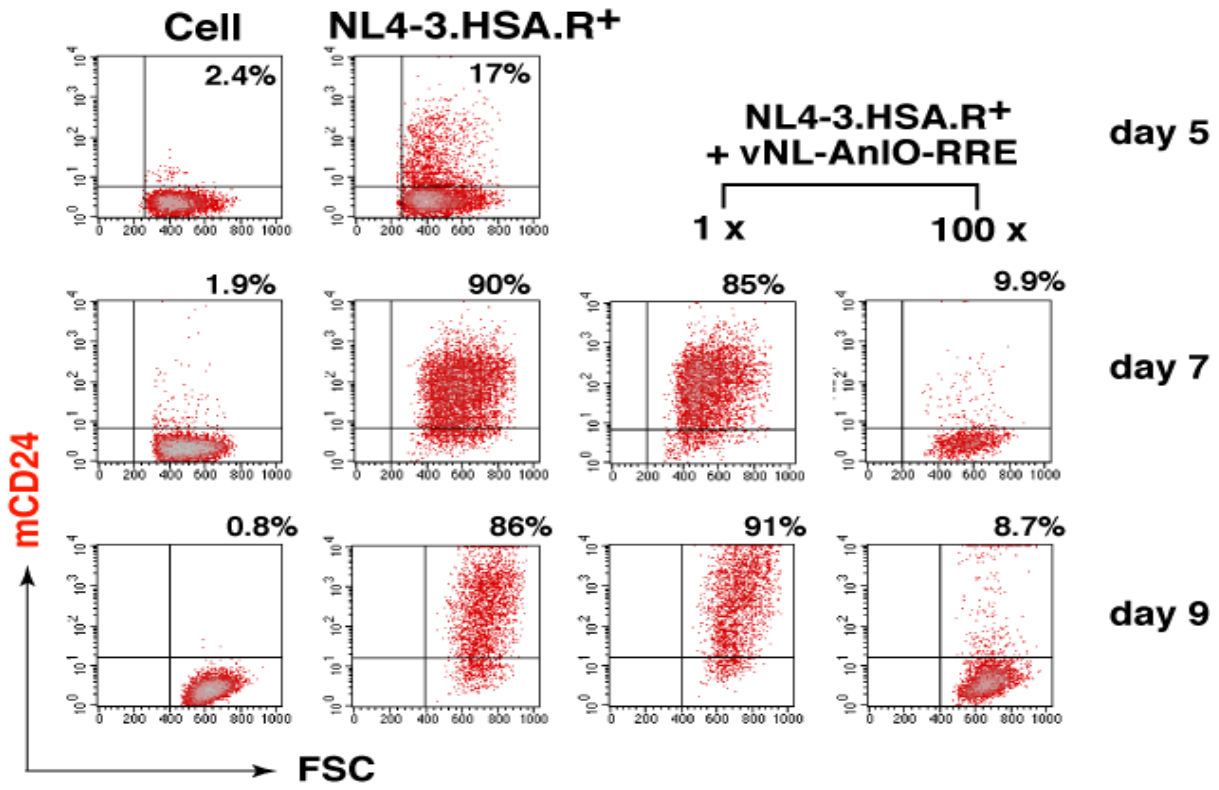
- **Anthrolysin O**
- **Diphtheria toxin A chain**

Proof-of-concept: killing of HIV+ macrophages *in vitro*



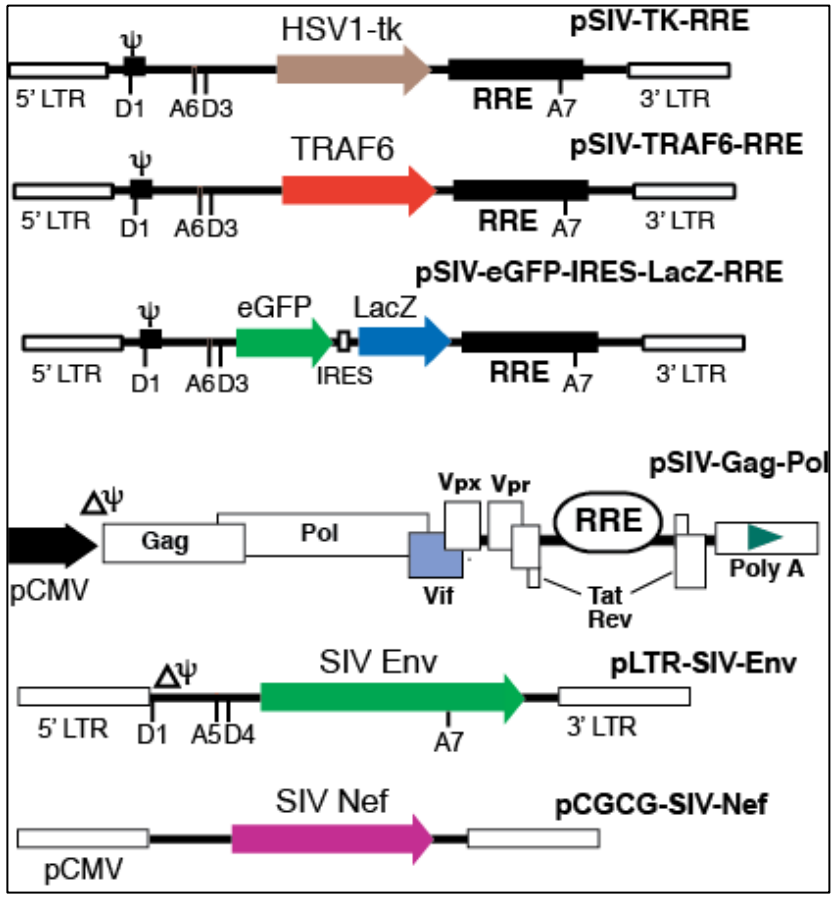
Young *et al.* 2008, *Retrovirology* 5:36

Proof-of-concept: killing of HIV+ T cells *in vitro*



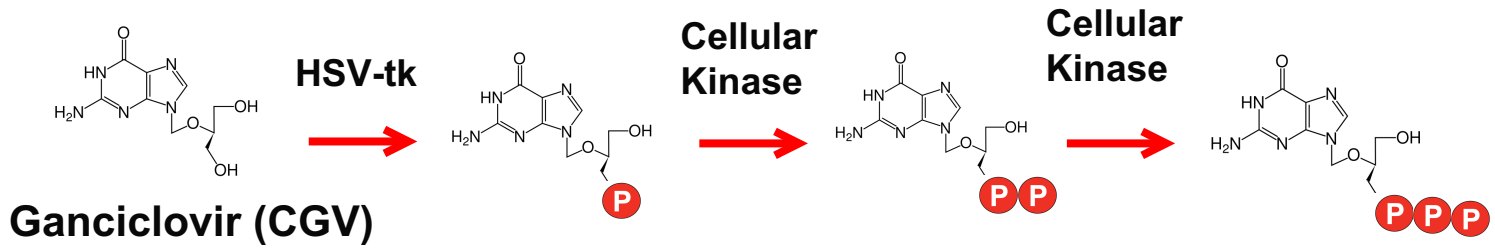
Young *et al.* 2008, *Retrovirology* 5:36

In vivo validation of the Rev-dependent vector in SIV/rhesus macaque



Helper vectors

Herpes simplex virus-1 thymidine kinase (HSV1-tk)



- Competitive inhibitor of dGTP
- Inhibit DNA polymerase chain elogation

- Superior safety profiles: over 30 years of research
- Capable of mobilization and amplification
- High titer particles can be assembled: > 10^{10}

Tumor necrosis factor-associated factor 6 (TRAF6)

THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 281, NO. 16, pp. 11235–11240, April 21, 2006
Printed in the U.S.A.

TRAF6 Regulates Cell Fate Decisions by Inducing Caspase 8-dependent Apoptosis and the Activation of NF-κB[*S]

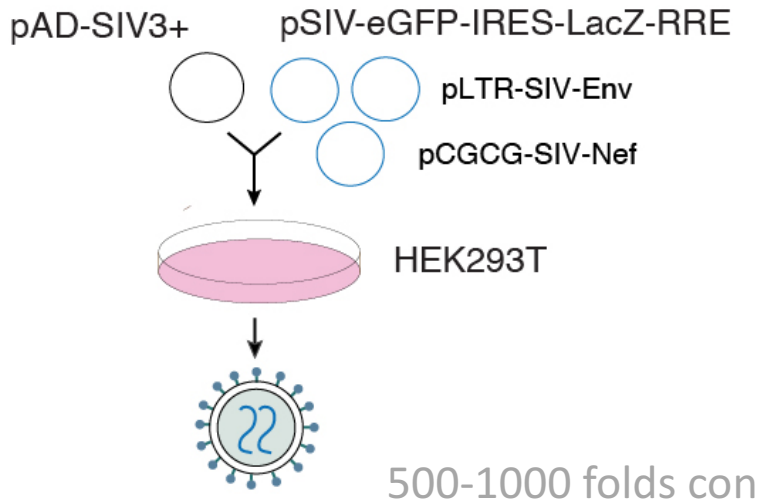
Received for publication, August 9, 2005, and in revised form, January 10, 2006 Published, JBC Papers in Press, January 25, 2006, DOI 10.1074/jbc.M508779200

Liusheng He[‡1], Xiaoli Wu[‡], Richard Siegel[§], and Peter E. Lipsky[§]

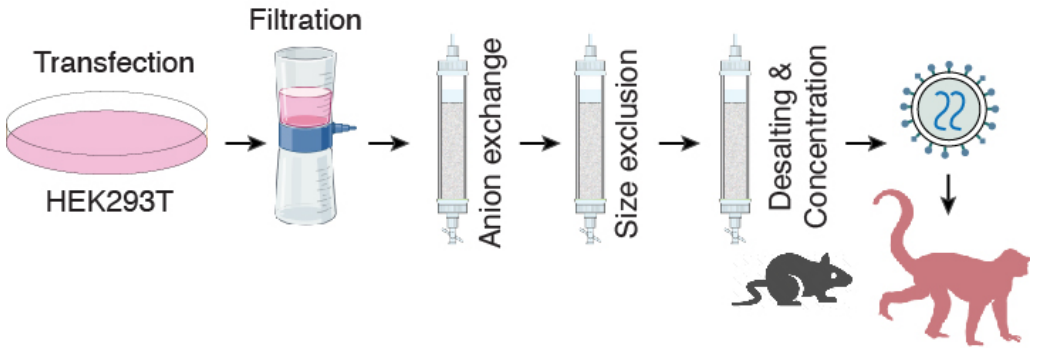
From the [‡]Flow Cytometry Section, Office of Science and Technology and the [§]Autoimmunity Branch, NIAMS, National Institutes of Health, Bethesda, Maryland 20892

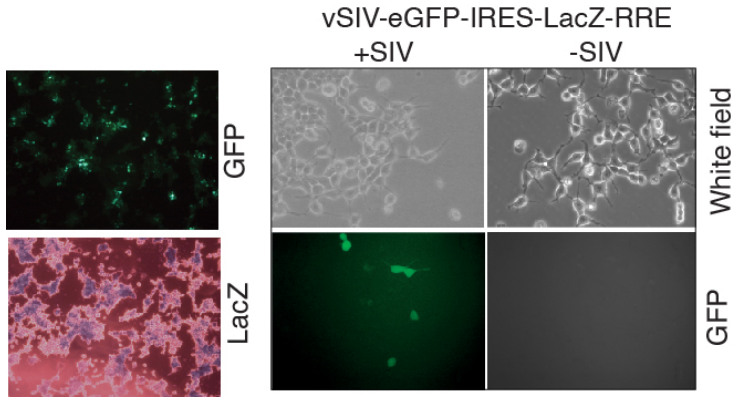
- endogenous human gene
- low level expression is tolerated
- over-expression triggers apoptosis

Assembly and concentration of the SIV Rev-dependent lentiviral particles



500-1000 folds concentration to 0.5 ml





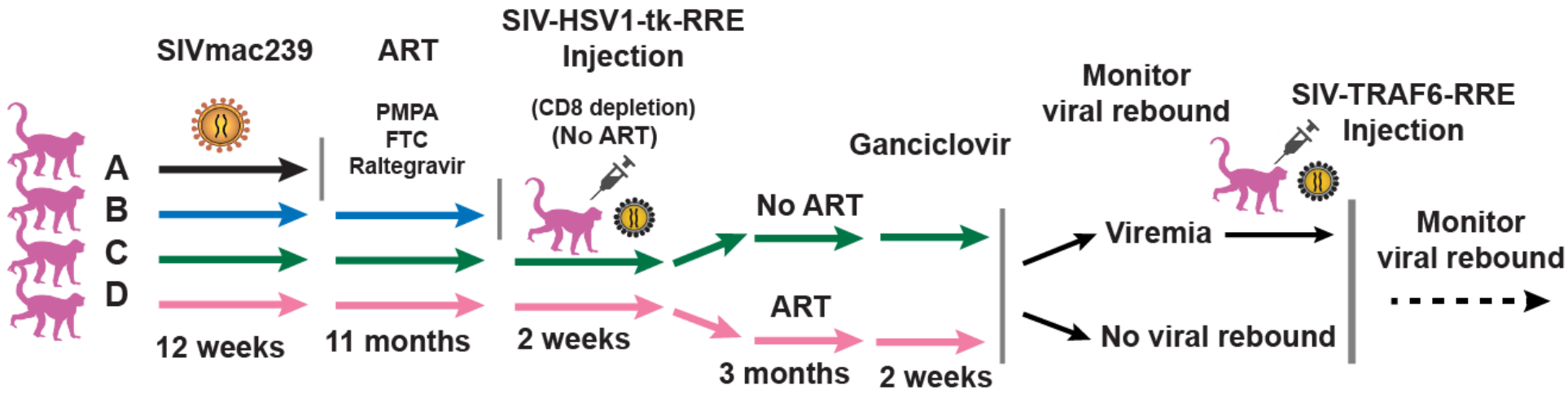
Safety test of SIV Rev-dependent vector particles in mice

Concentrated particles	i.v. injection (ml)	No. of mice	Days post injection	Observed results
vSIV-LacZ-GFP-RRE	0.1	5	14	No adverse effects
vSIV-LacZ-GFP-RRe	0.1(1:5)	5	14	No adverse effects
vSIV-HSV-tk-RRE	0.1	5	14	No adverse effects
vSIV-HSV-tk-RRE	0.1(1:5)	5	14	No adverse effects
vSIV-TRAF6(R)-RRE	0.1	5	14	No adverse effects
vSIV-TRAF6(R)-RRE	0.1(1:5)	5	14	No adverse effects

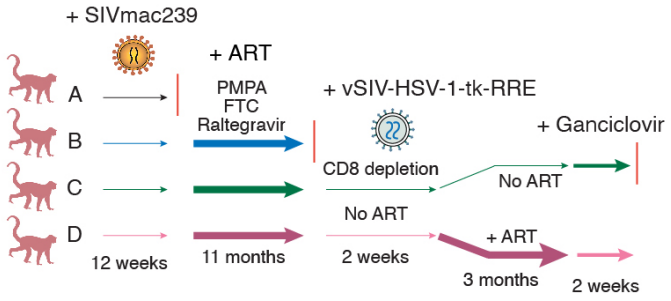
* Female BALB/cJ mice aged seven weeks were used for intravenous (i.v.) tail vein injection
All animals were reported healthy two weeks after particle injection.
1:5 is 1 to 5 dilution of the concentrated particles.

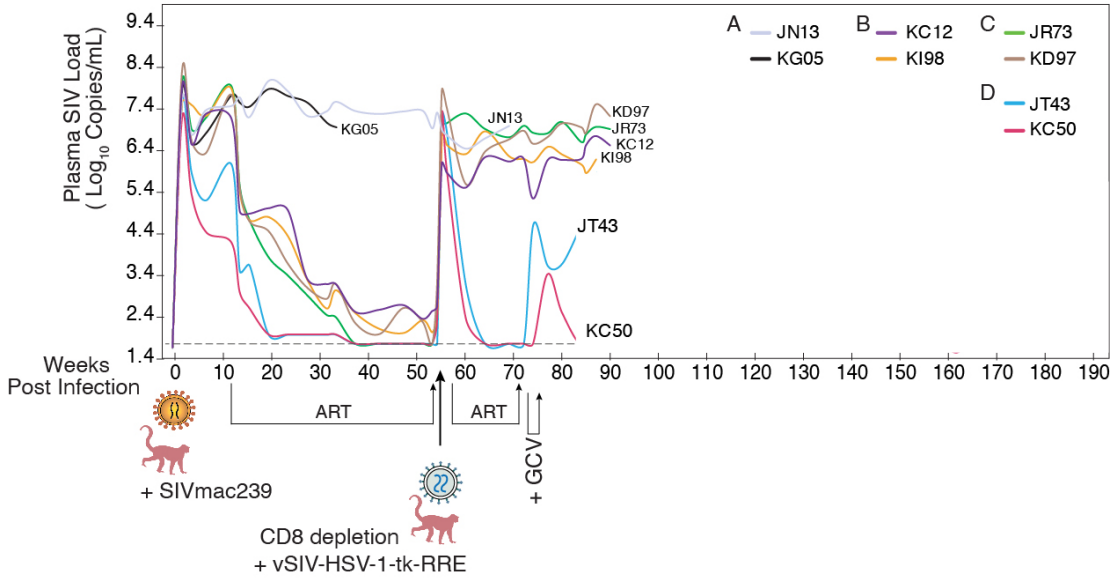
Hetrick et al. Gene Therapy, 2024, DOI: 10.1038/s41434-024-00467-9

Schematic of *in vivo* proof-of-concept animal study design



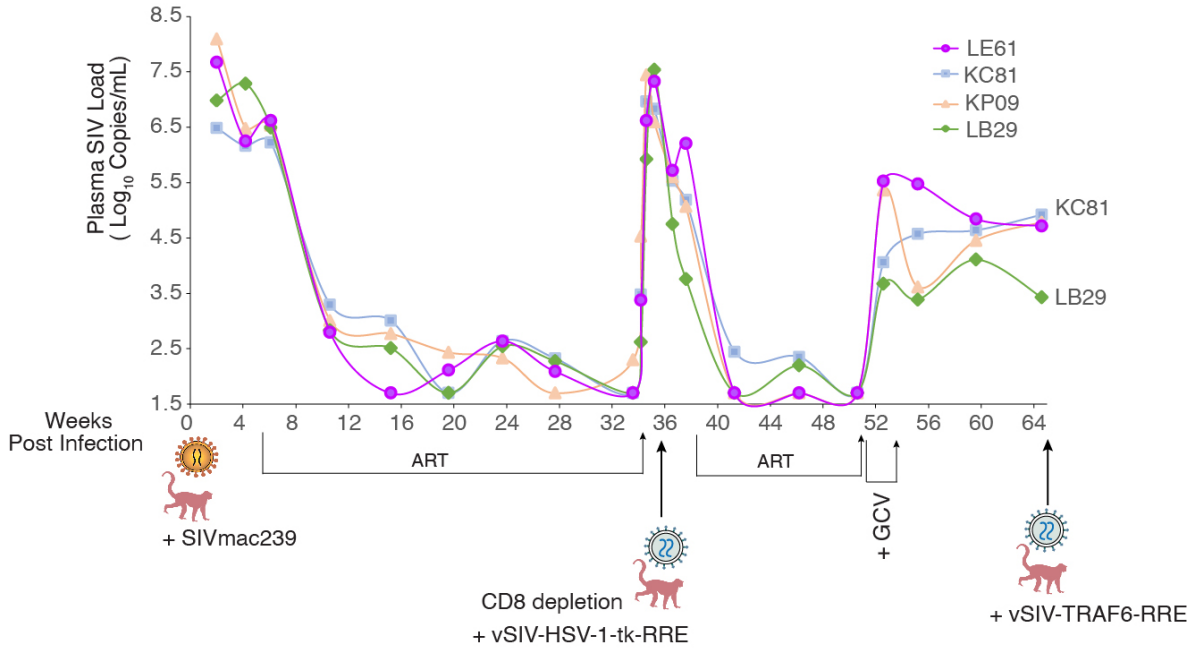
Partial inhibition of viral rebound by the Rev-HSV1-tk particles





A single Rev-HSV1-tk injection

3-4 log reduction



Rev-TRAF6-RRE adverse effects

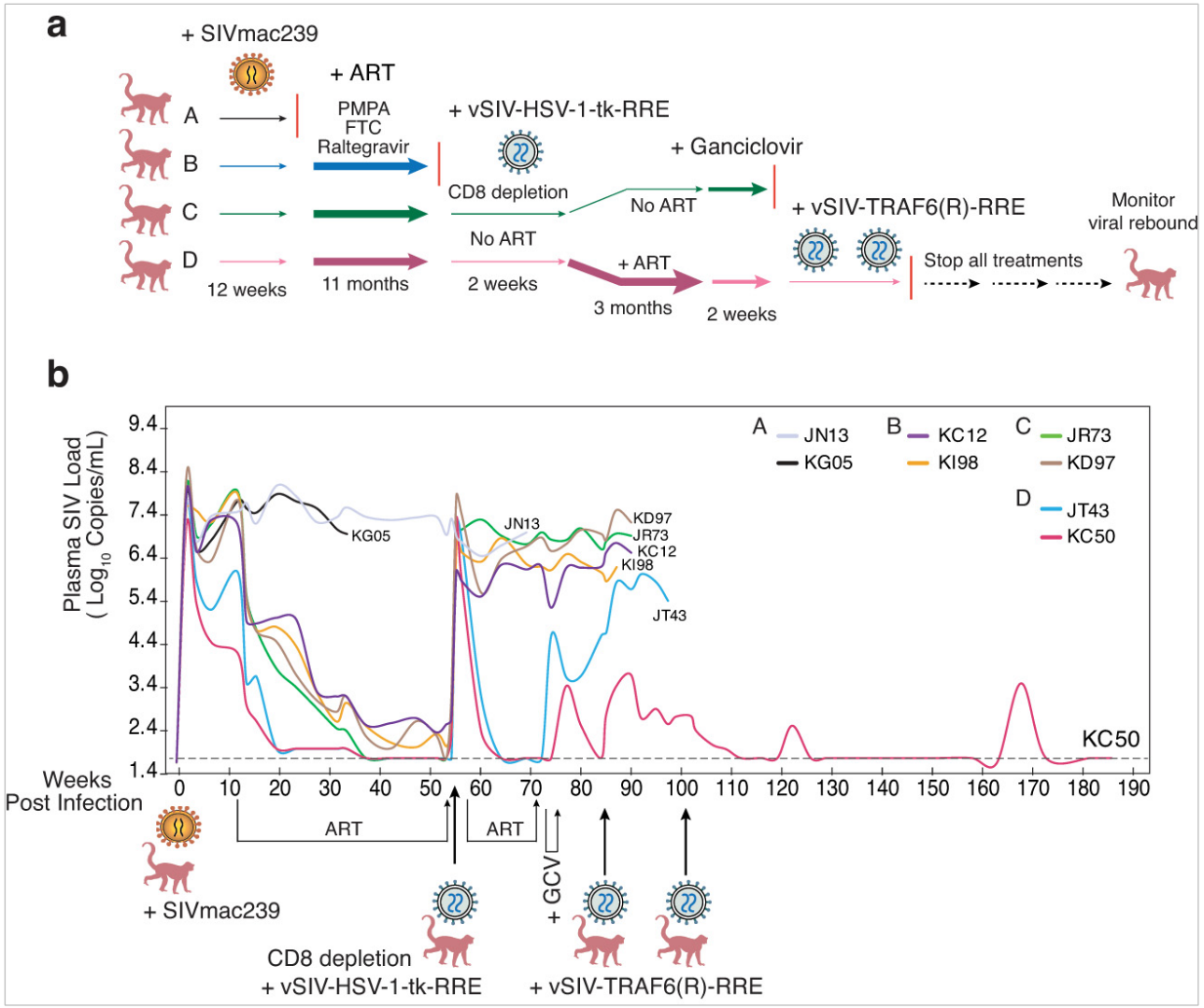
Hetrick et al. Gene Therapy, 2024, DOI: 10.1038/s41434-024-00467-9

Lessons learned:

- It is very difficulty, if not impossible, to kill all reservoir cells.

- Can we use Rev-targeting to partially modulate reservoir cells to stimulate immunity for immune control ?

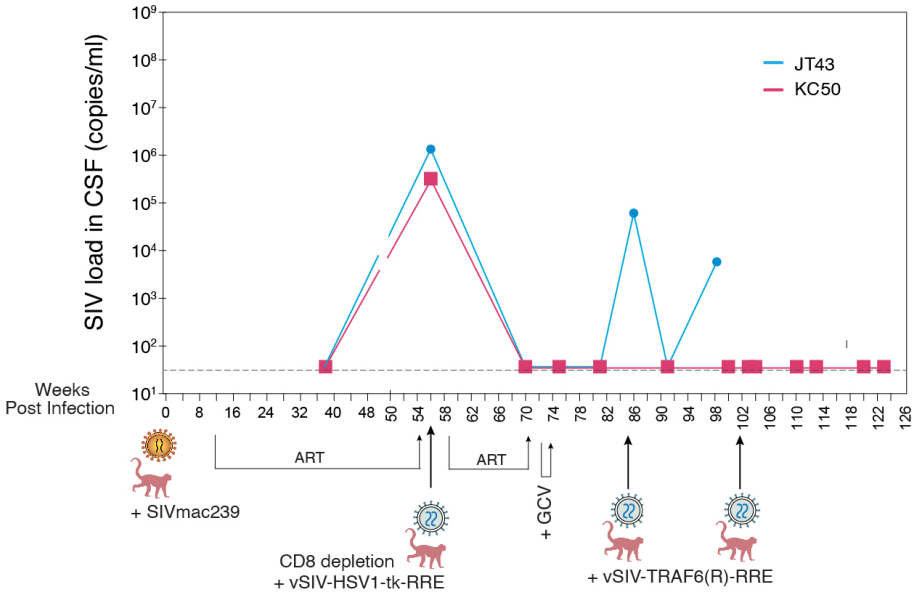
Proof-of-Concept: diminishing viral rebound with the Rev-dependent particles



KC50 self-controlled SIV to an undetectable level most of the time for 2 years after ART terminated

Hetrick et al. Gene Therapy, 2024, DOI: 10.1038/s41434-024-00467-9

KC50: undetectable viral load in cerebrospinal fluids & QVOA in peripheral CD4 T cells



QVOA of KC50 peripheral CD4 T cell reservoir at week 159 p.i.

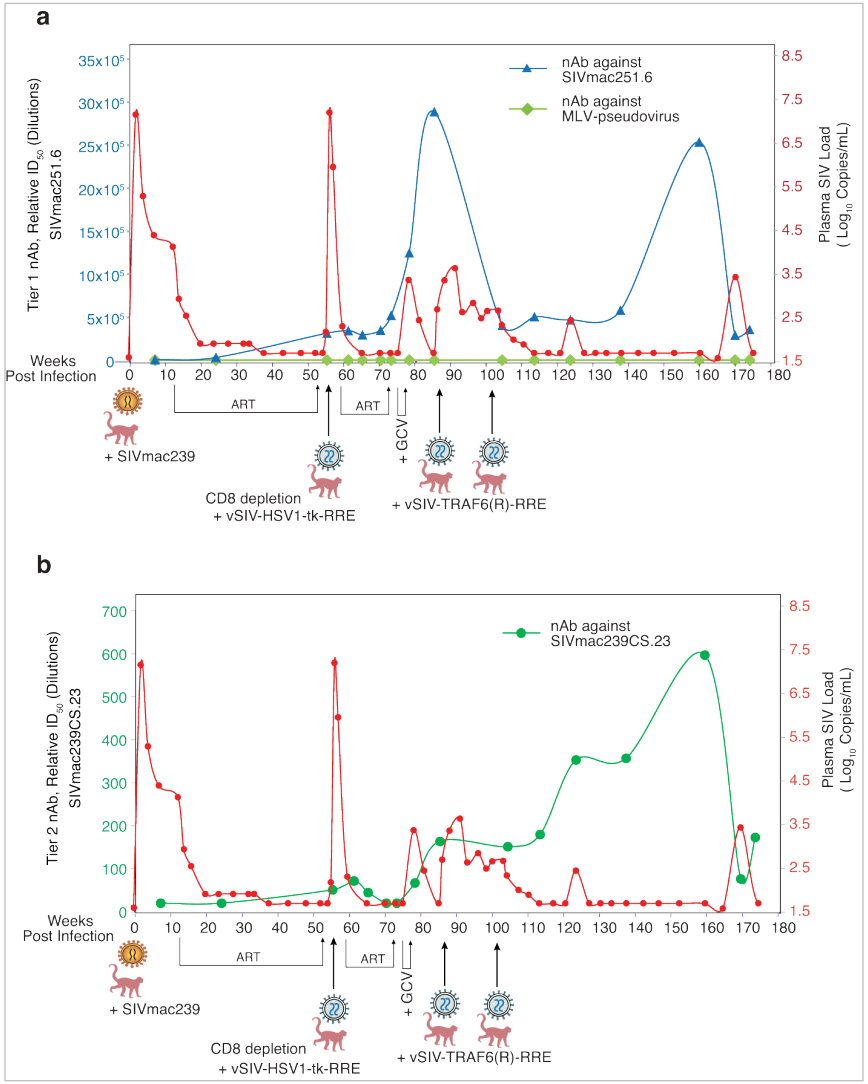
CD4 T cell number/well	Assay replicates	Co-culture days	Viral RNA copies/ml	Number SIV+ well
1,000,000	3	14	< 83	0
200,000	3	14	< 83	0
40,000	3	14	< 83	0
8,000	3	14	< 83	0
1,600	3	14	< 83	0
320	3	14	< 83	0

* The calculated IUMP is 0.18 IU/ml

Proof-of-concept:
Rev-dependent vector may also target microglia

- **Peripheral CD4 T cell reservoir undetectable**
- **Tissue reservoirs may still persist**

Induction of tier 2 neutralizing antibodies (nAb) by the Rev-dependent particles



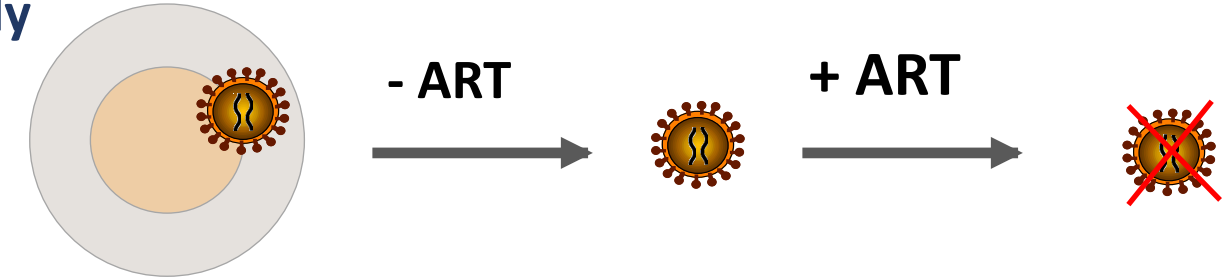
- **KC50 is negative for Mamu-A*01, B*08 and B*17 expression.**
- **The presence of high titers of tier 2 neutralizing antibodies was coincident with the period when plasma viremia remained undetectable.**

Hetrick et al. Gene Therapy, 2024, DOI: 10.1038/s41434-024-00467-9

Summary of Proof-of-concept Rev-targeting SIV/HIV immunotherapy *in vivo*

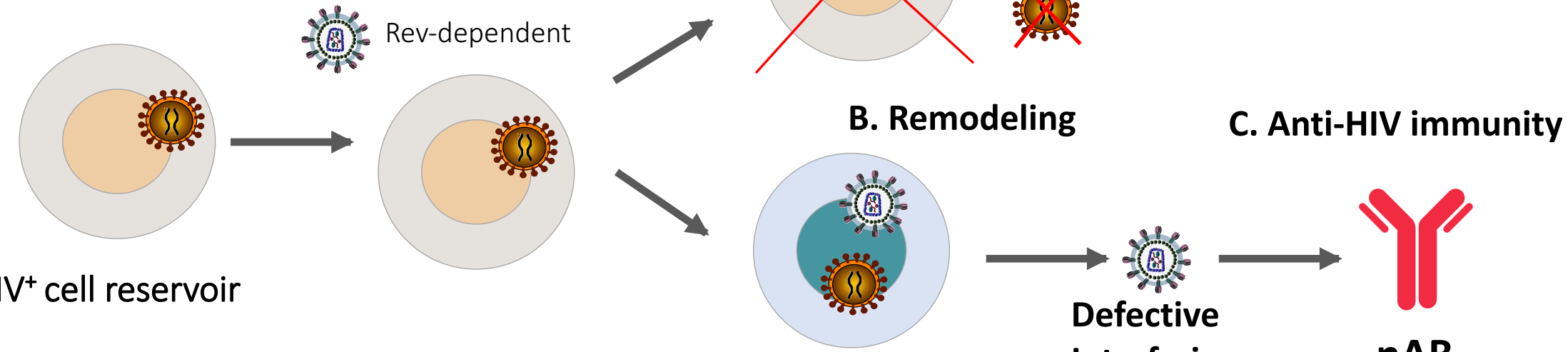
- Reduce viral reservoirs
- Inhibit viral rebound
- Stimulate neutralizing antibodies (nAB)

ART only



HIV+ cell reservoir

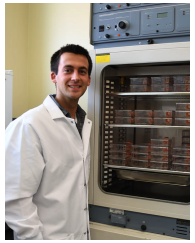
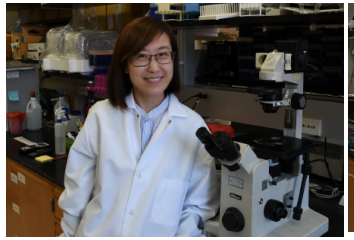
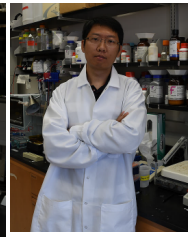
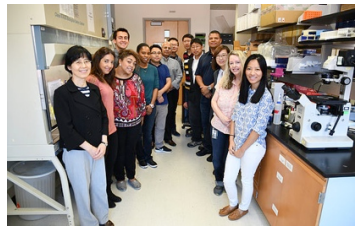
+ Rev-targeting HIV immunotherapy (RHIT)



HIV+ cell reservoir

Acknowledgements

- Brian Hetrick
- The Wu lab members


- Binghua Ling
- Summer Iqbal
- Ron Veazey
- TNPRC Core Facility

Tulane National Primate Research Center

Funding sources for this project:

05/01/2014 – 04/30/2019
R01 MH102144 , NIMH/NIH
Validation of the Rev-dependent vector for targeting SIV macrophage reservoirs
TNPRC base grant OD011104

2008 – 2012
New York City to Washington DC (NYCDC) AIDS Research Ride
(organizer: Marty Rosen)

04/01/2005 - 03/31/2007
R21 NS051130, NINDS
Targeting brain macrophages by a novel lentiviral vector

Duke Human Vaccine Institute
Duke University School of Medicine

David C. Montefiori
Celia C. LaBranche

NIH contract HHSN27220180

Welcome to the

Fourth International Workshop on HIV Persistence during Therapy

Radisson St. Martin Hotel
St. Martin, West Indies
December 8-11 2009