Dissecting HIV cell-to-cell infection with flow cytometry

Metroflow October 18, 2016

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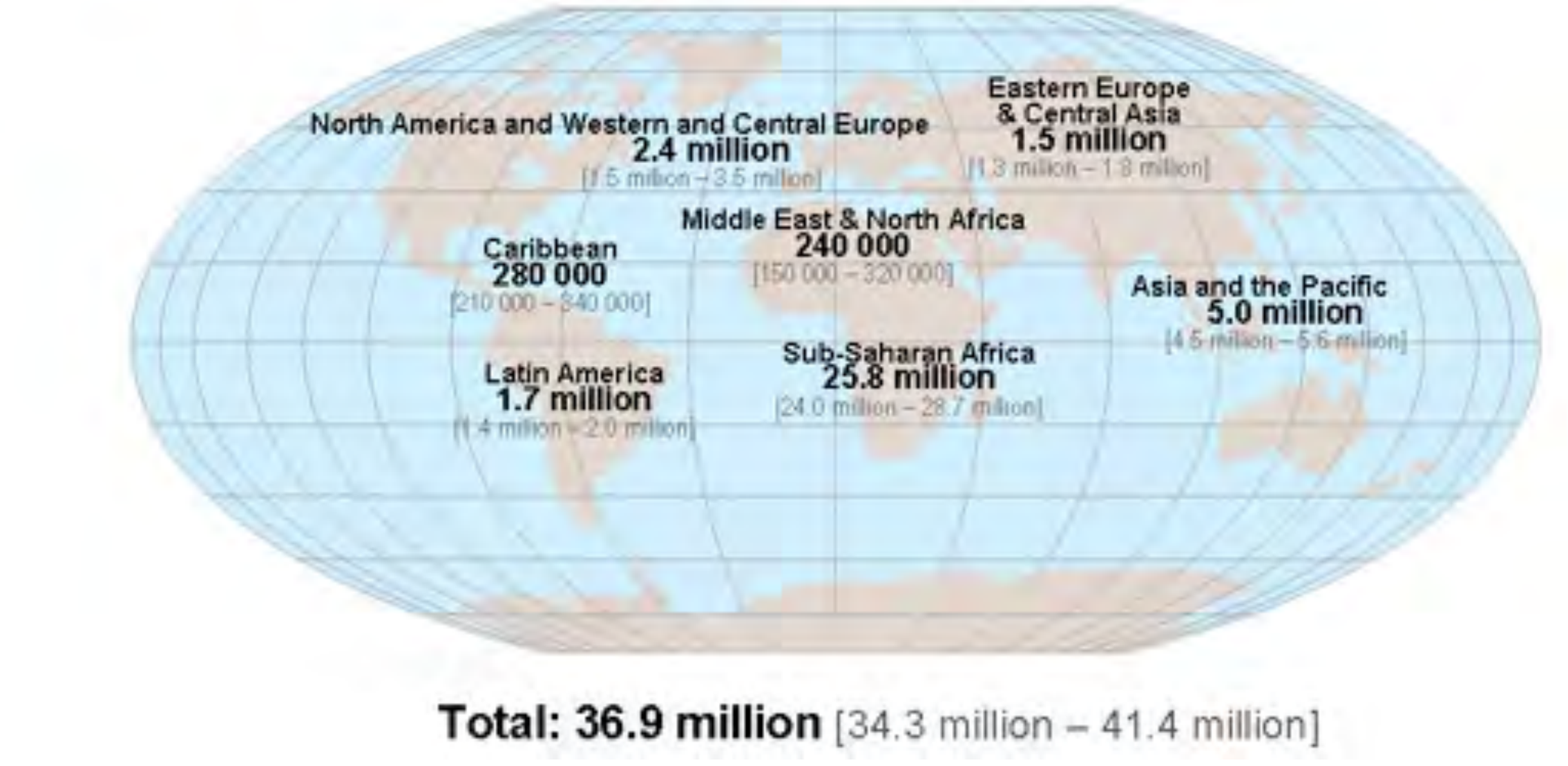
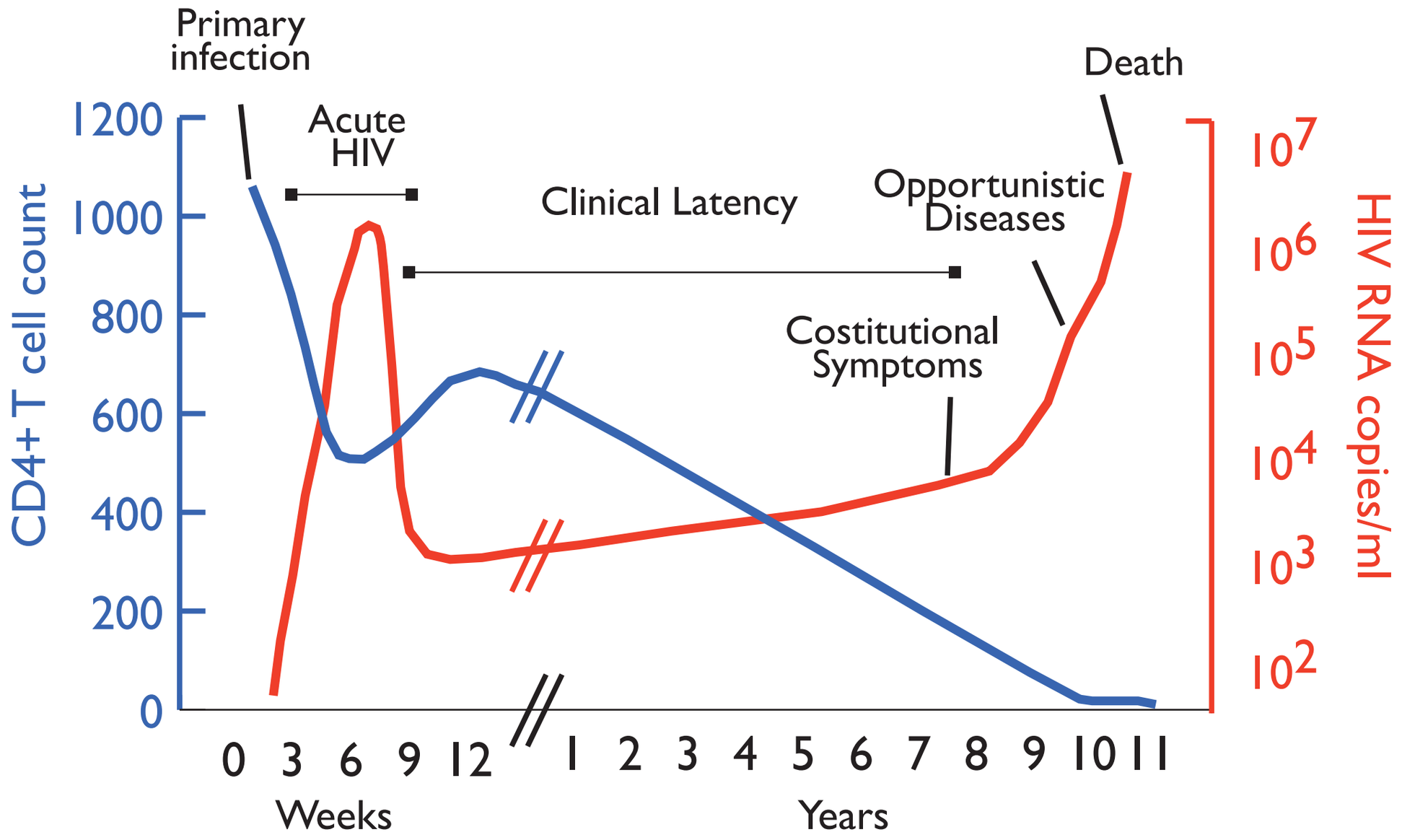
Global HIV 2014



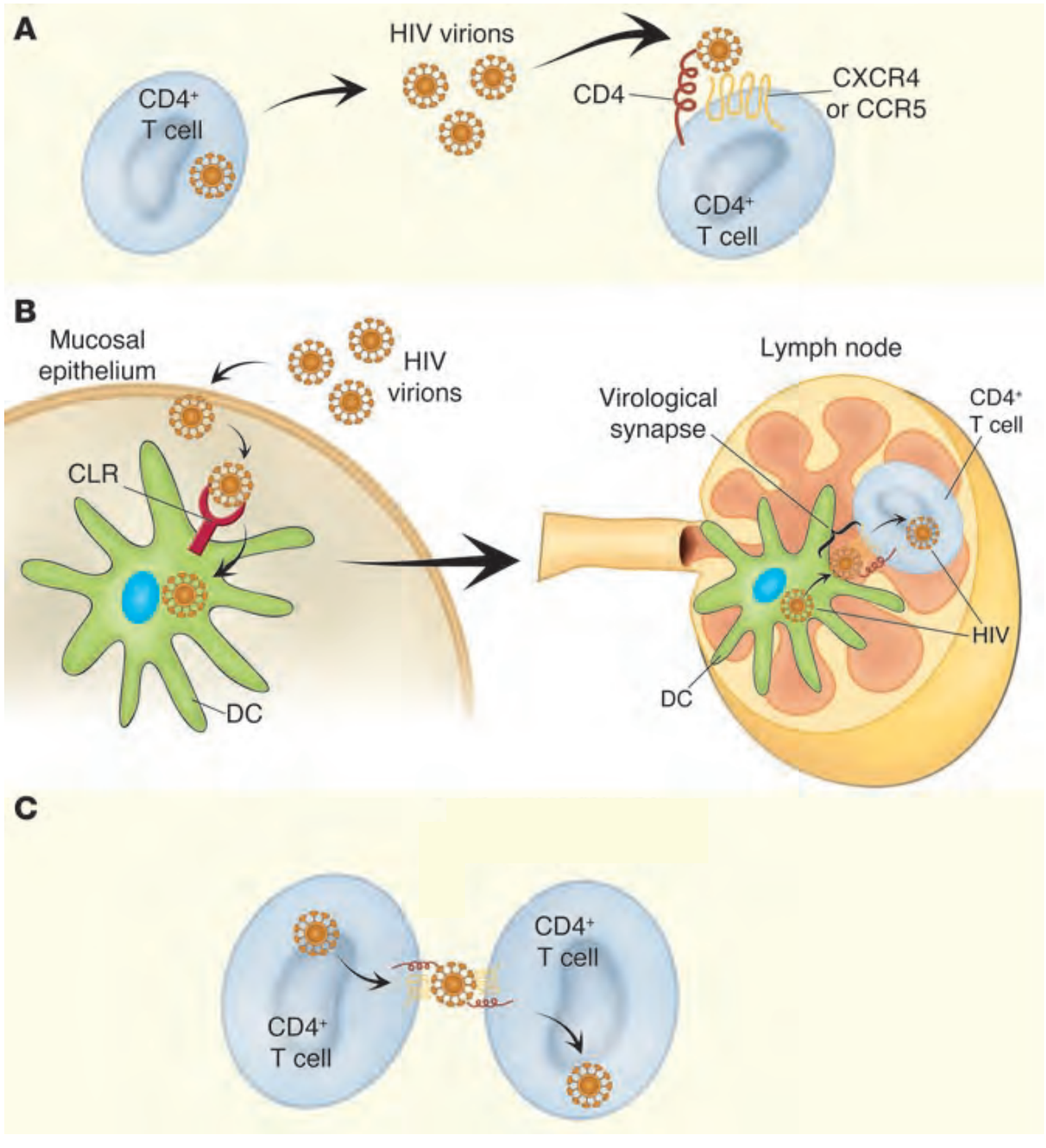
UNAIDS/WHO

Natural history of HIV infection



Adapted from Fauci, Ann Int Med 1996

Mechanisms of HIV spread



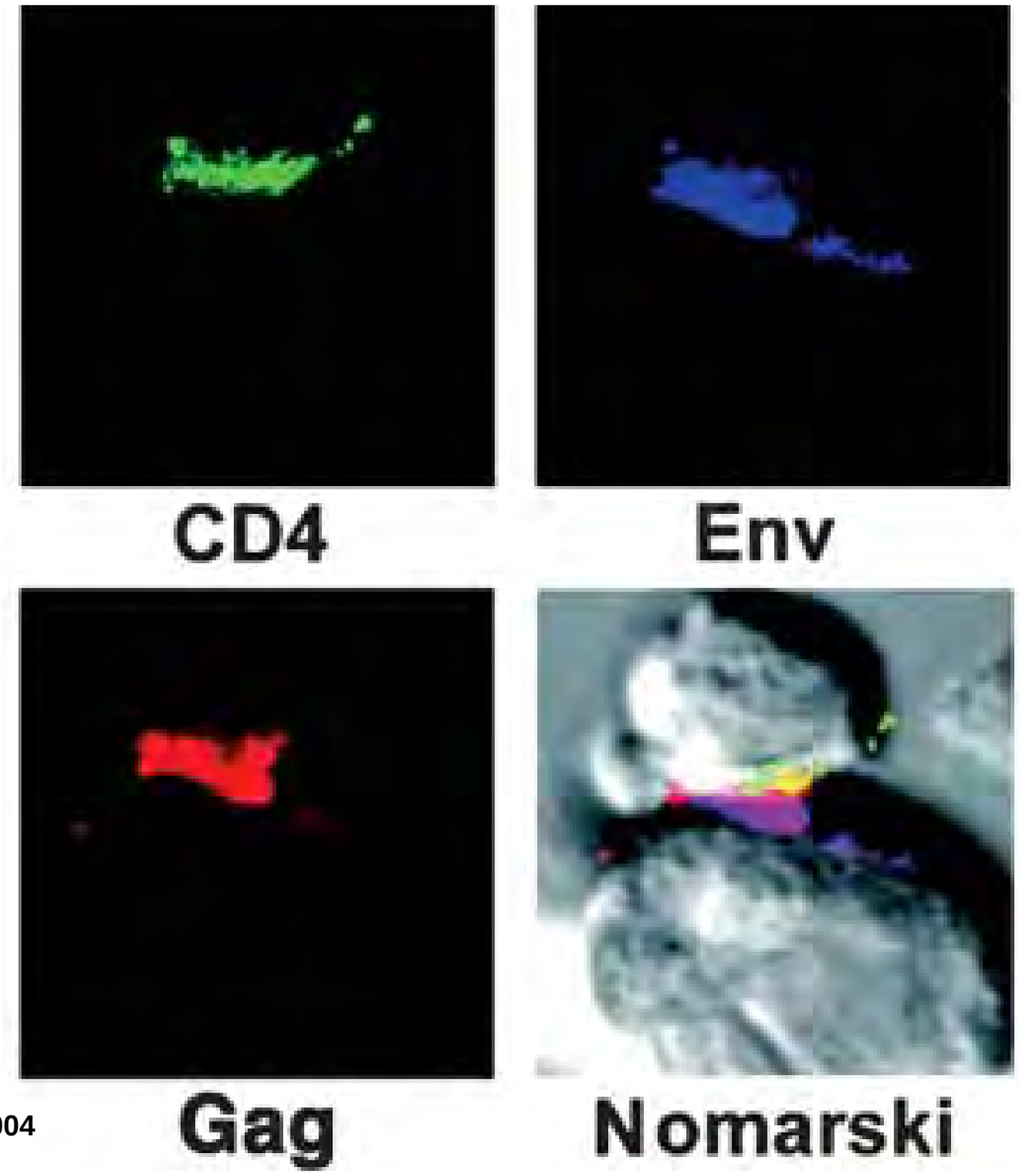
Cell free virus

Dendritic Cell to T cell

T cell to T cell

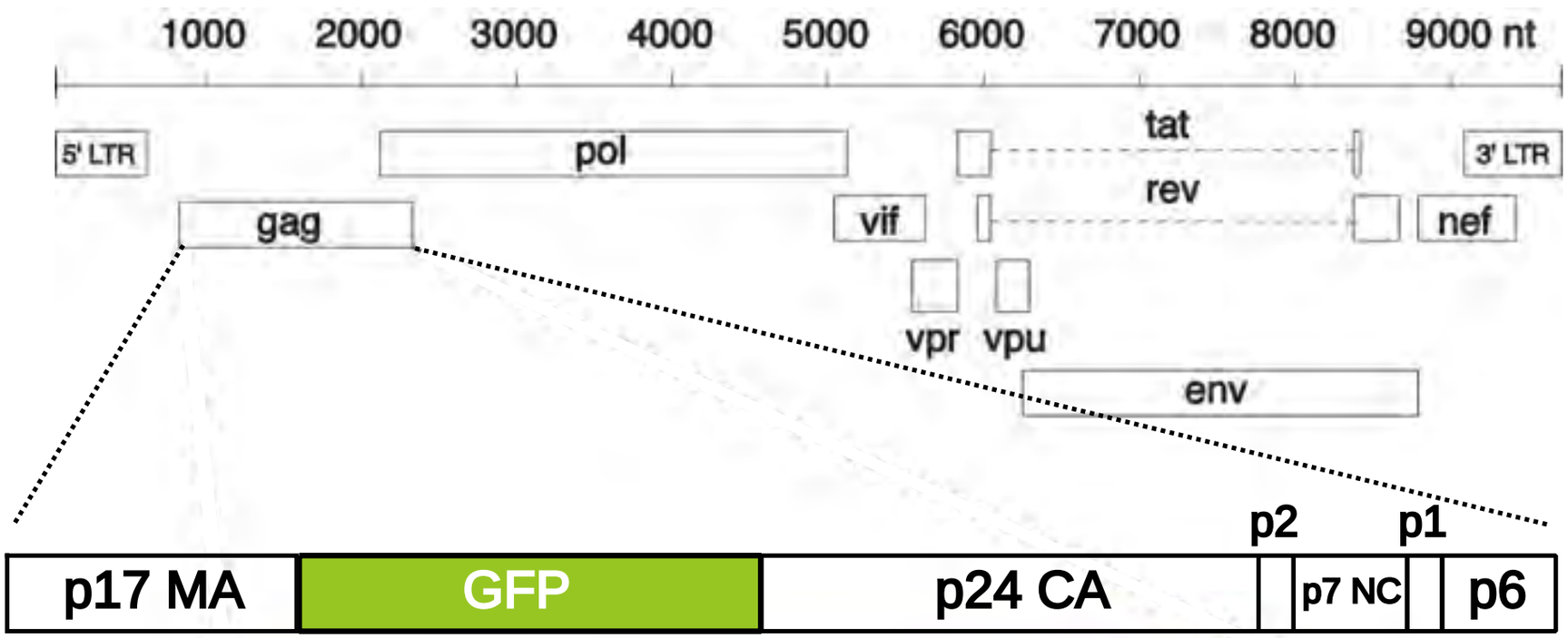
Piguet, et al. JCI 2004

T cell virological synapse:
CD4, Env and Gag colocalized at contact sites



Jolly et al J Exp Med, 2004

HIV-1 Gag-iGFP

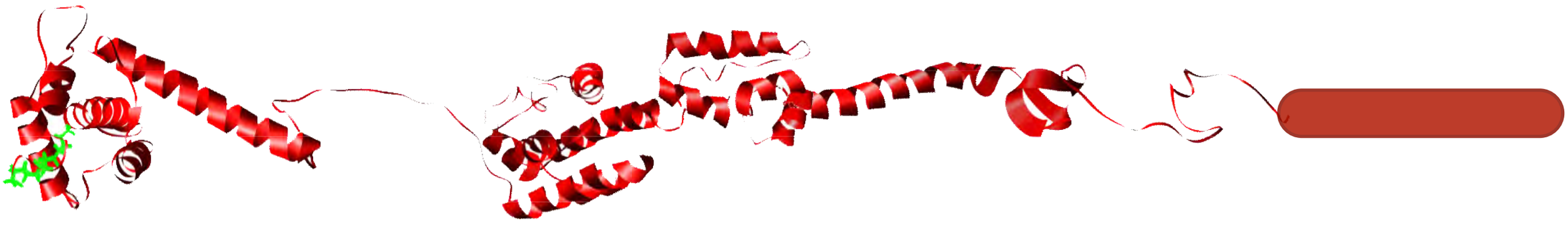


GFP internal interdomain insertion into core structural protein Gag

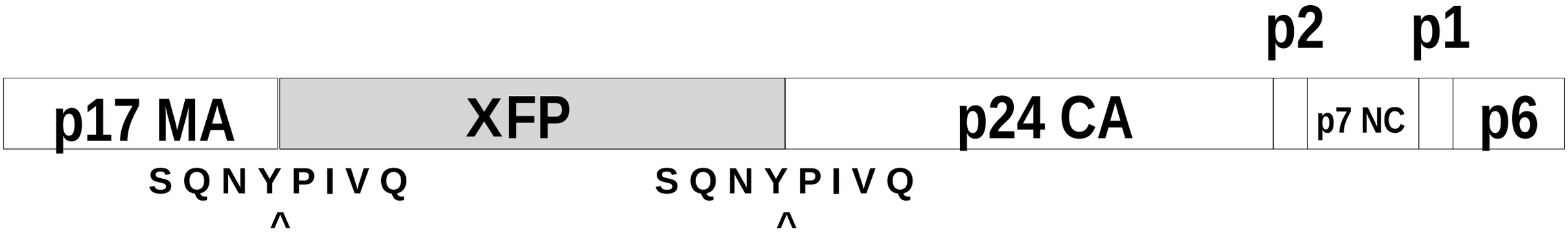
Maintains full genome & viral proteins

Hübner et al., 2007

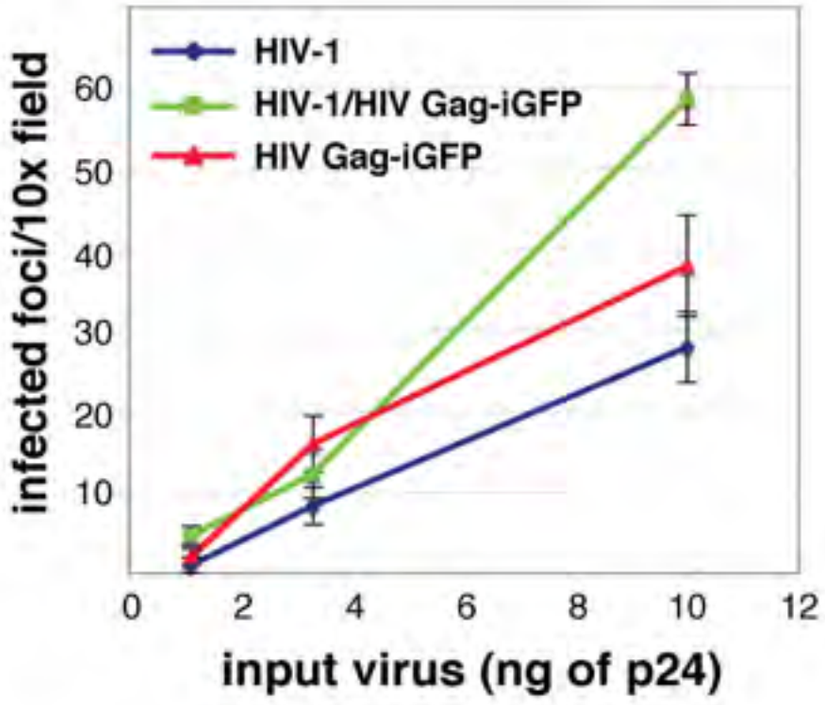
Internal fluorescently labeled Gag

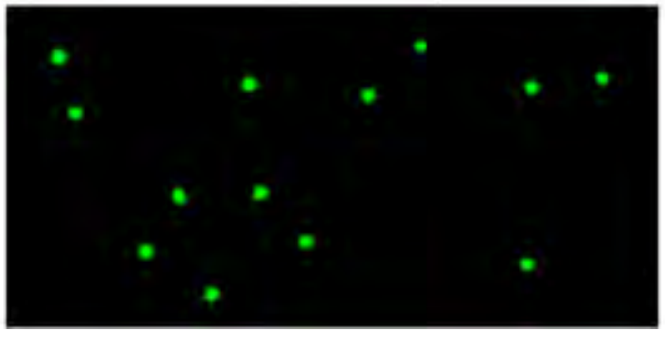


Internal fluorescently labeled Gag

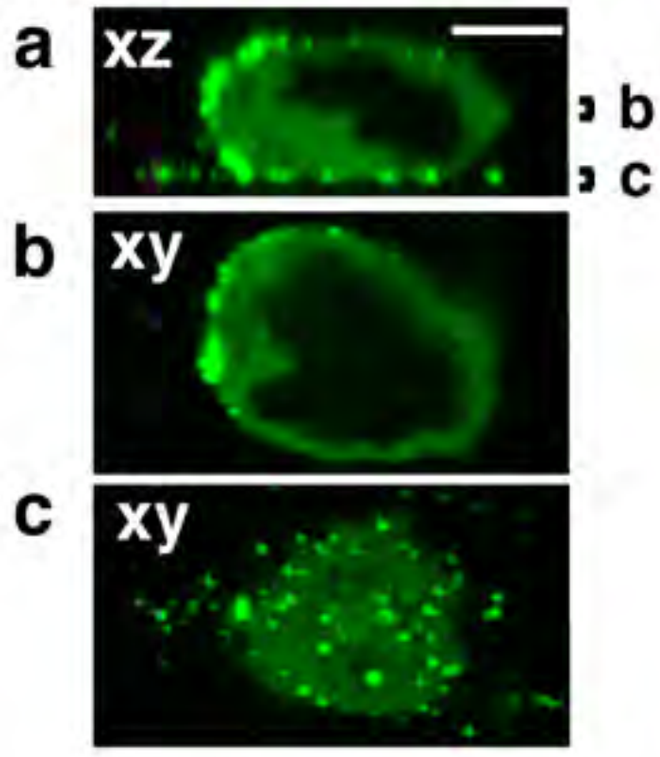


Infectious fluorescent virus particles



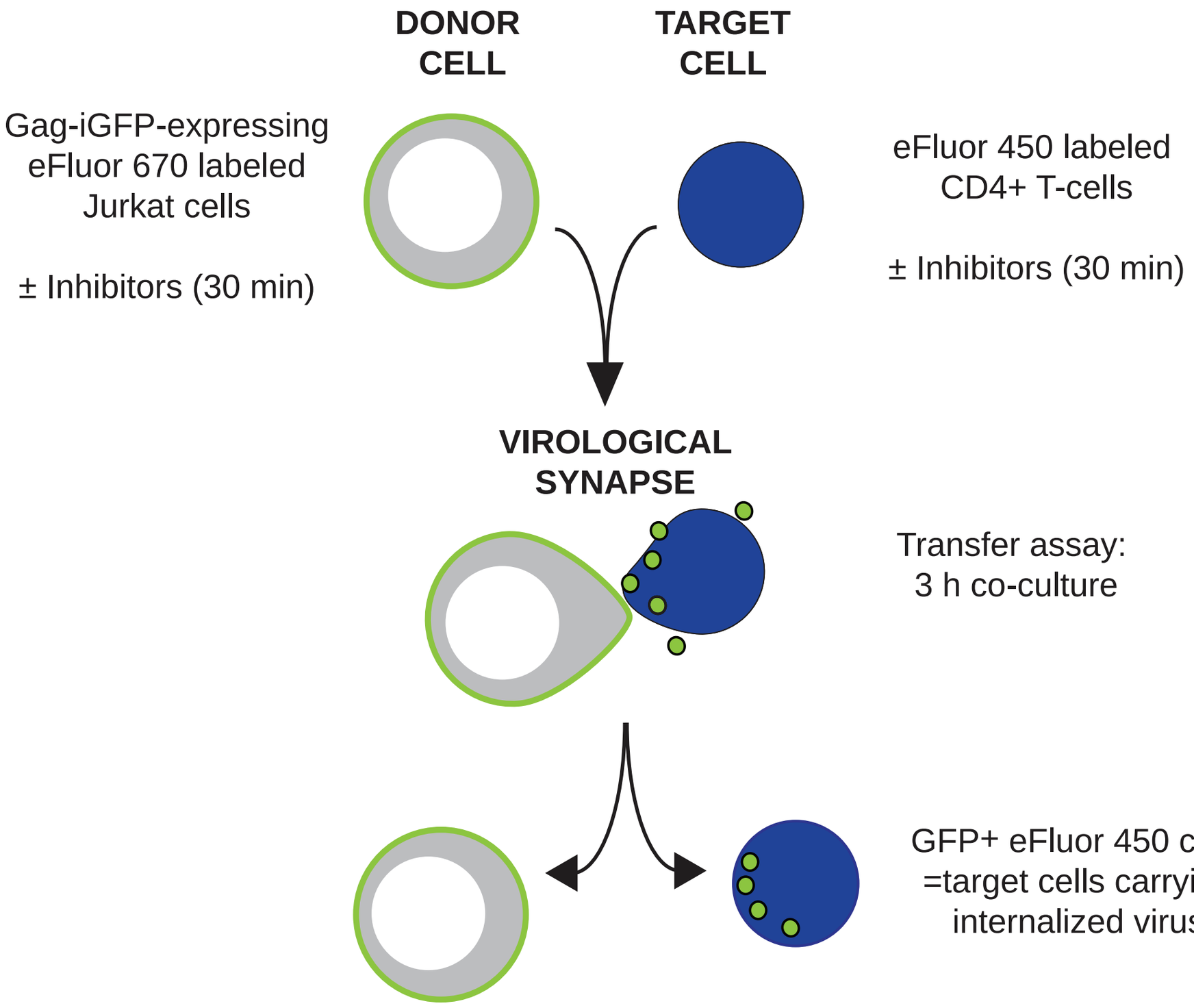


Cell-free Virus particles



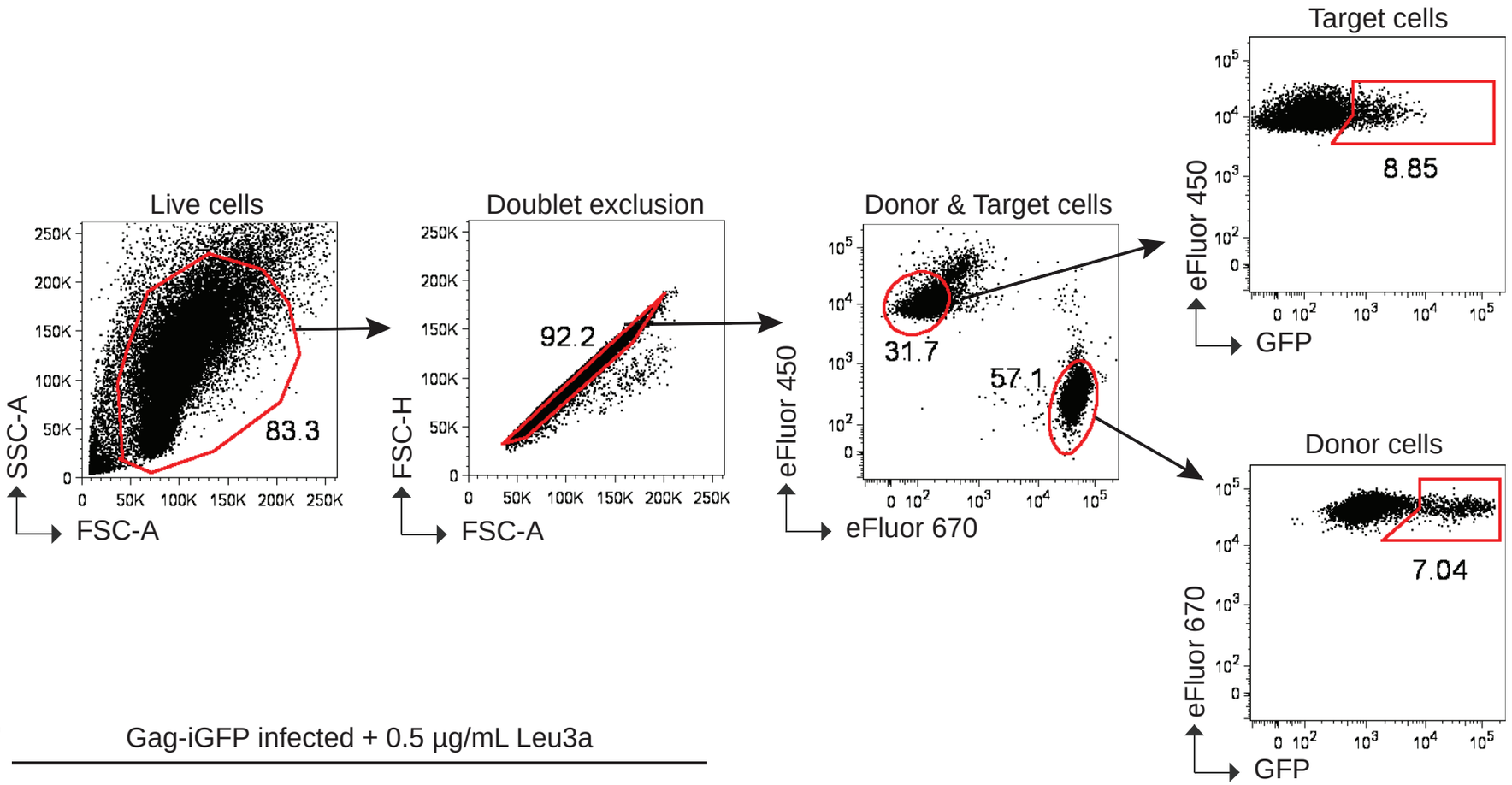
Cell-associated virus particles

Cell-to-cell HIV transfer



Donor Target Discrimination

A Gag-iGFP infected + no inhibitor



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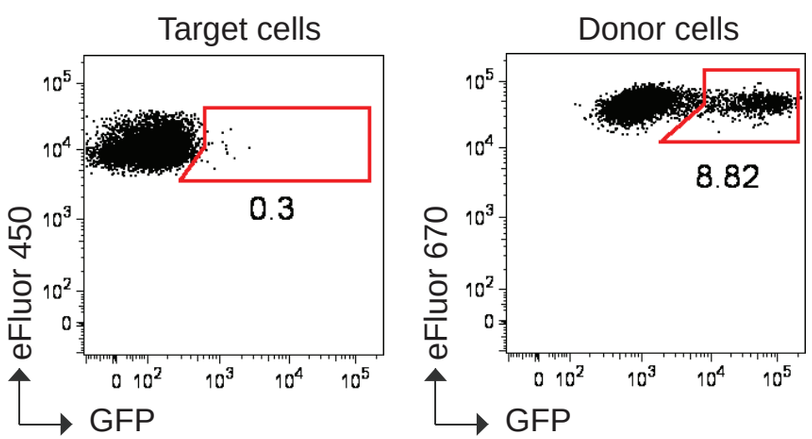
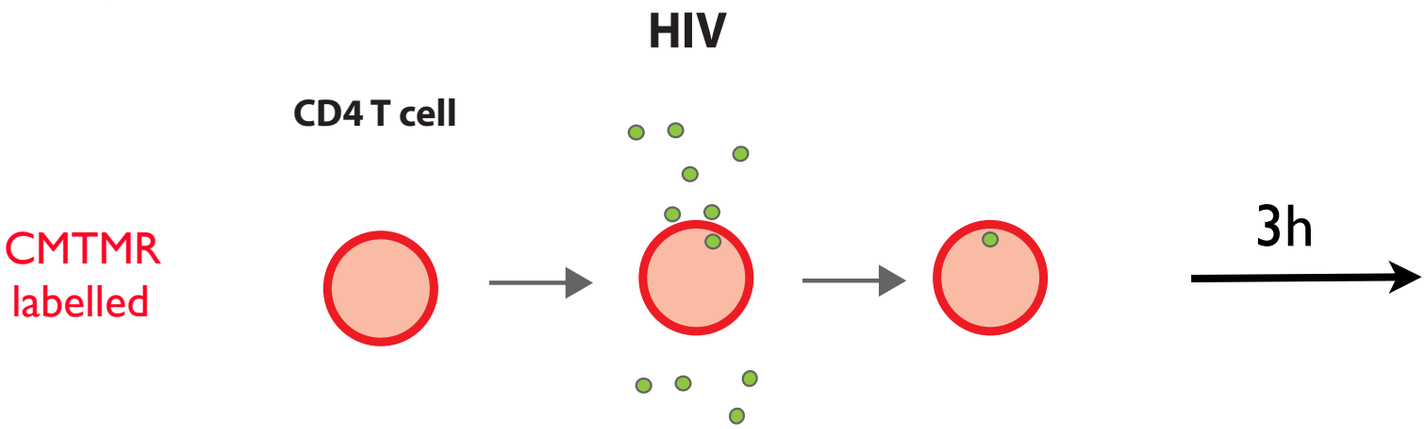
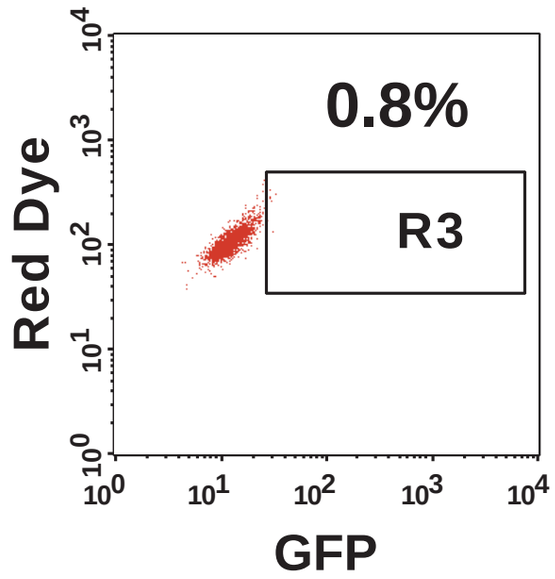


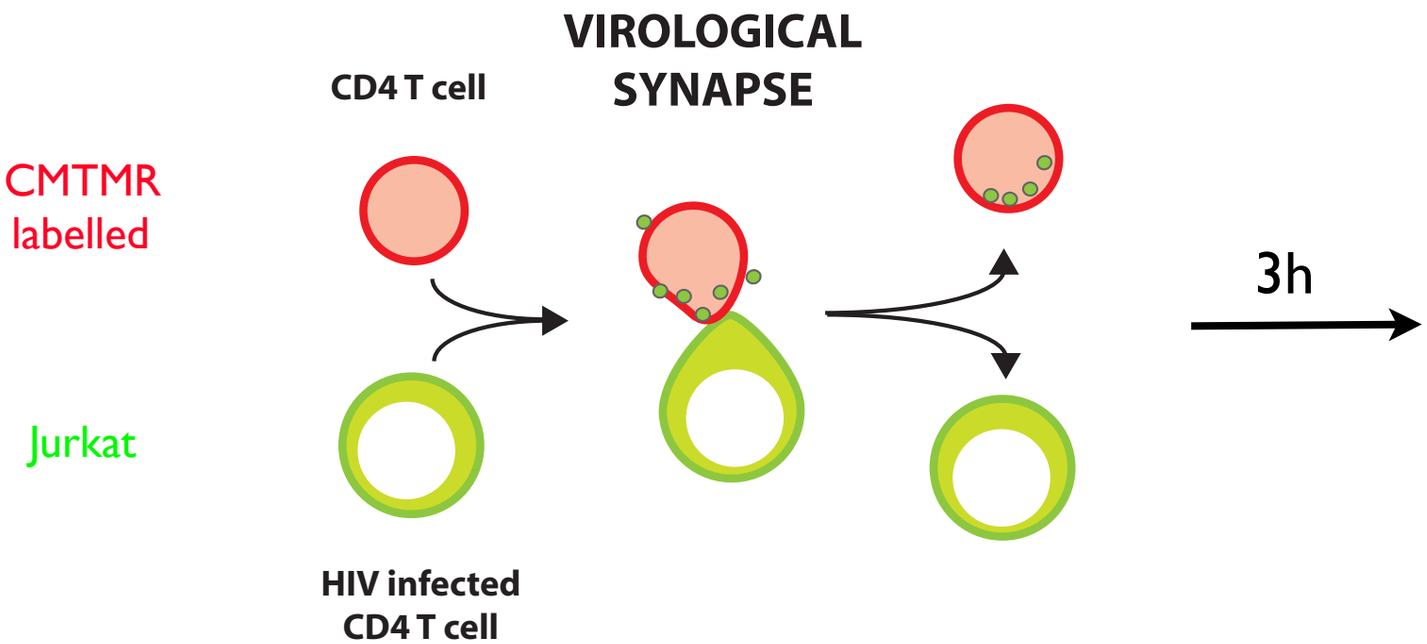
Efficiency of cell-free vs cell-associated viral uptake

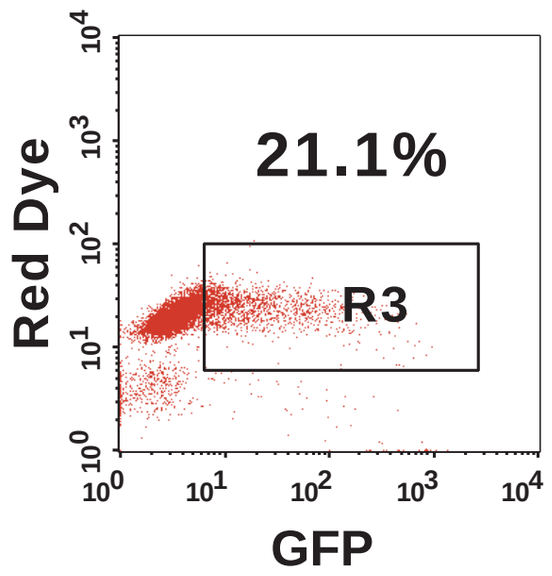
Cell-free





Cell-to-cell



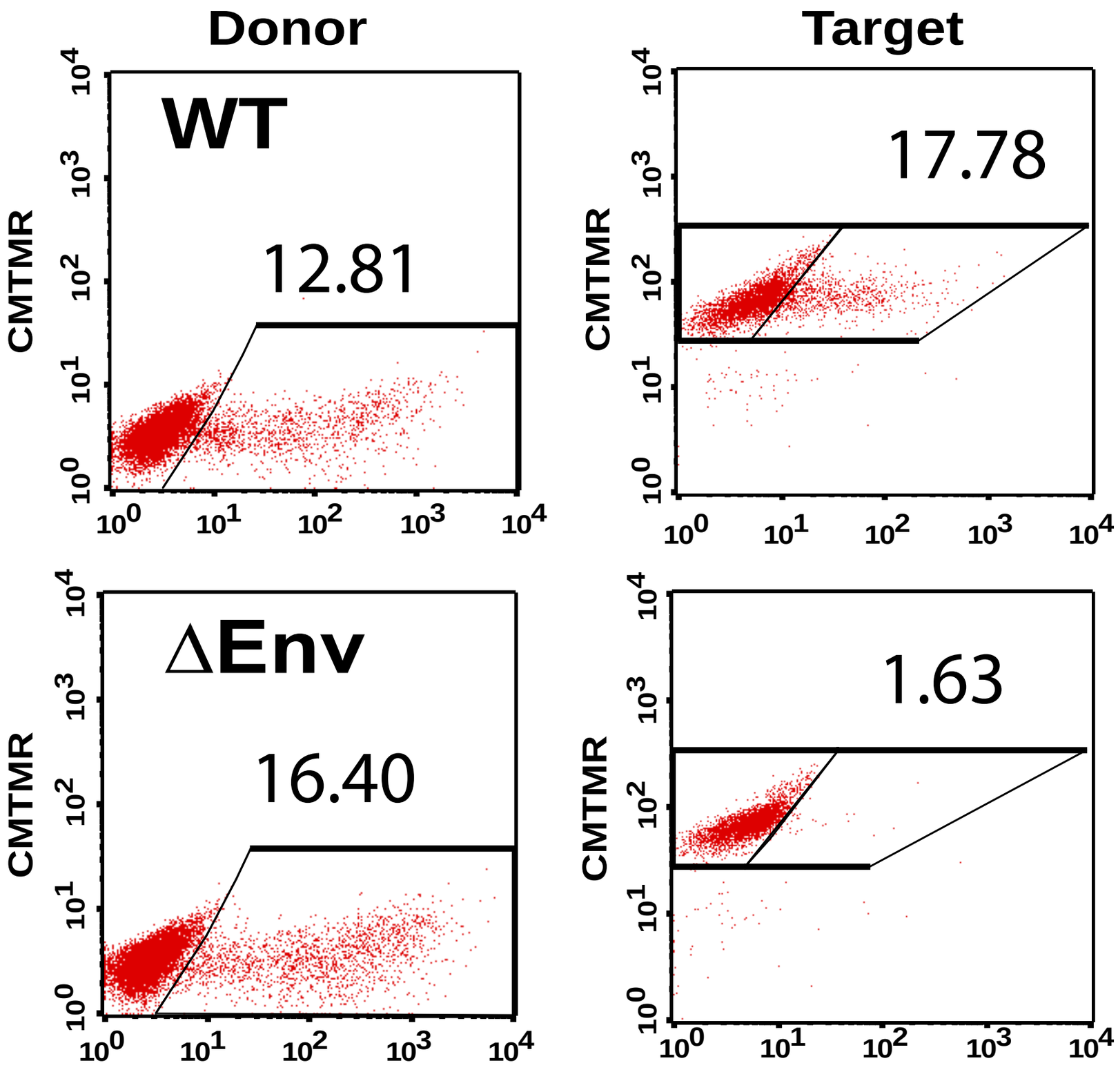


Chen P et al., J Virol 2007

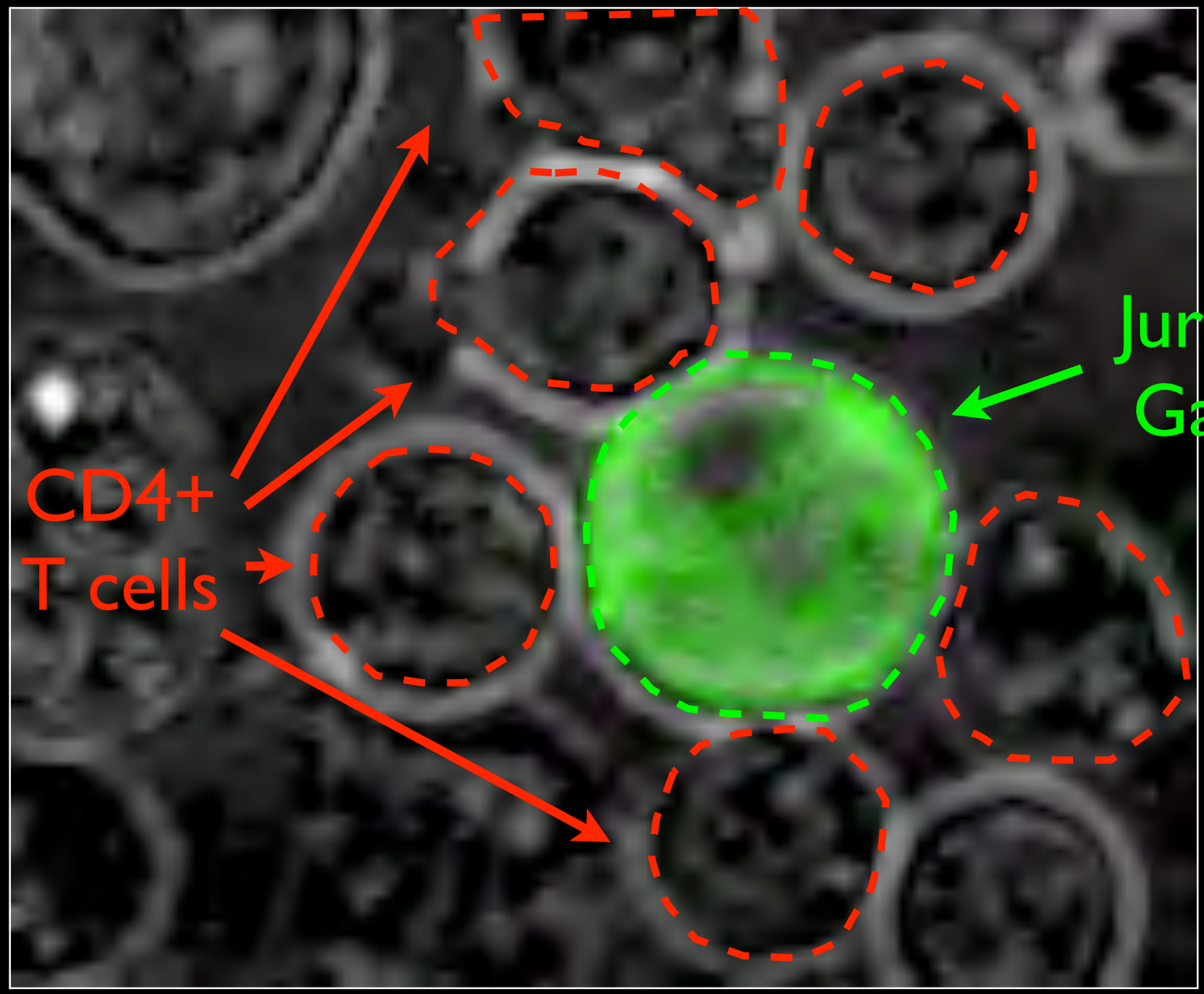
Gag synaptic button

Jurkat HIV Gag-iGFP &
CD4+ (CMRA)

VS-mediated viral transfer requires HIV Env

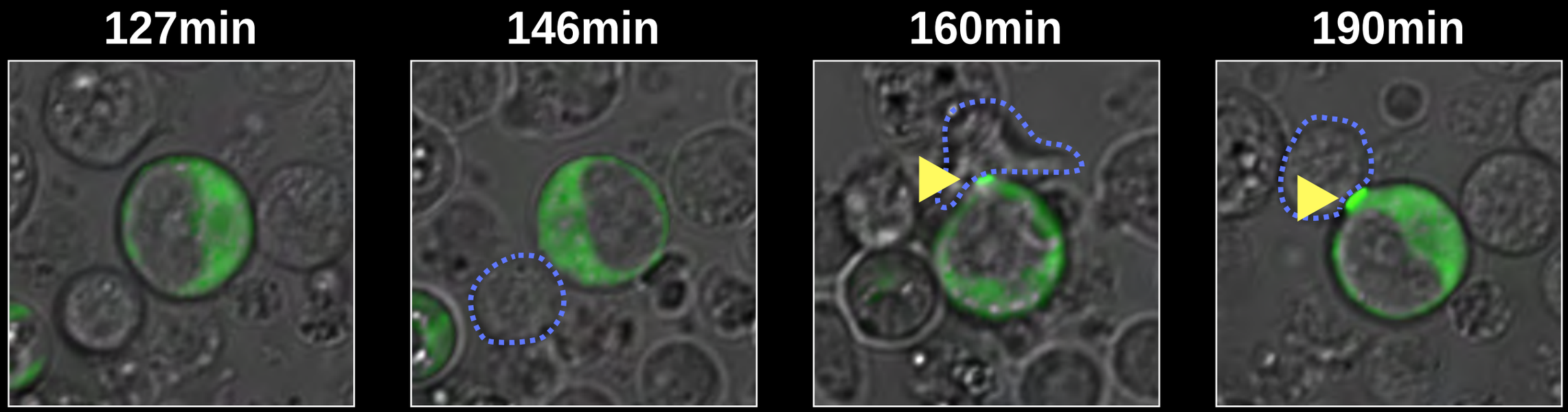


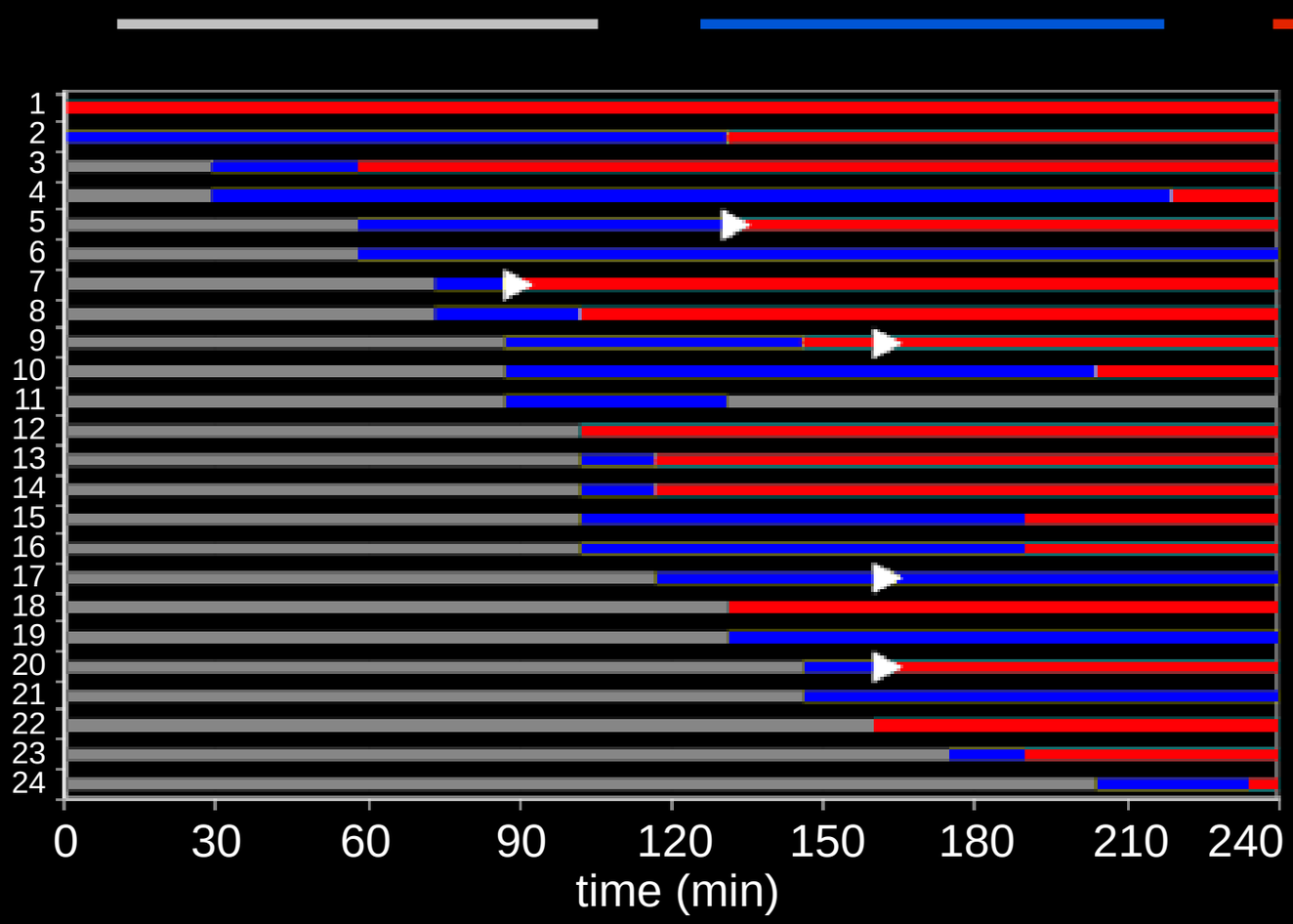
Virological Synapse formation



00:00 (min:sec)

Live tracking of HIV-infected cell conjugates over 4h

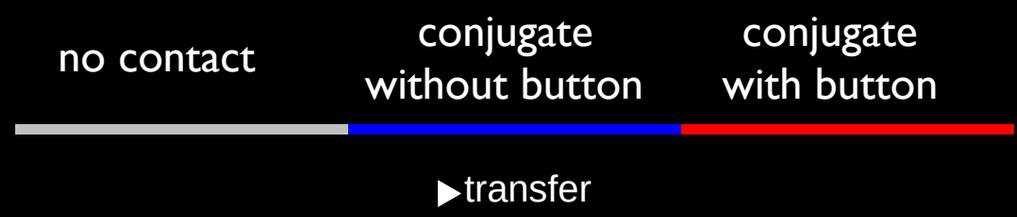




110 Jurkat HIV Gag-iGFP

22% form conjugates

83% Gag accumulates at synapse



Gag-iGFP recruitment to the VS and transfer

Jurkat HIV Gag-iGFP

CD4+ T cells

Gag-iGFP recruitment to the VS and transfer

02:35
min:sec

Hubner et al Science 2009

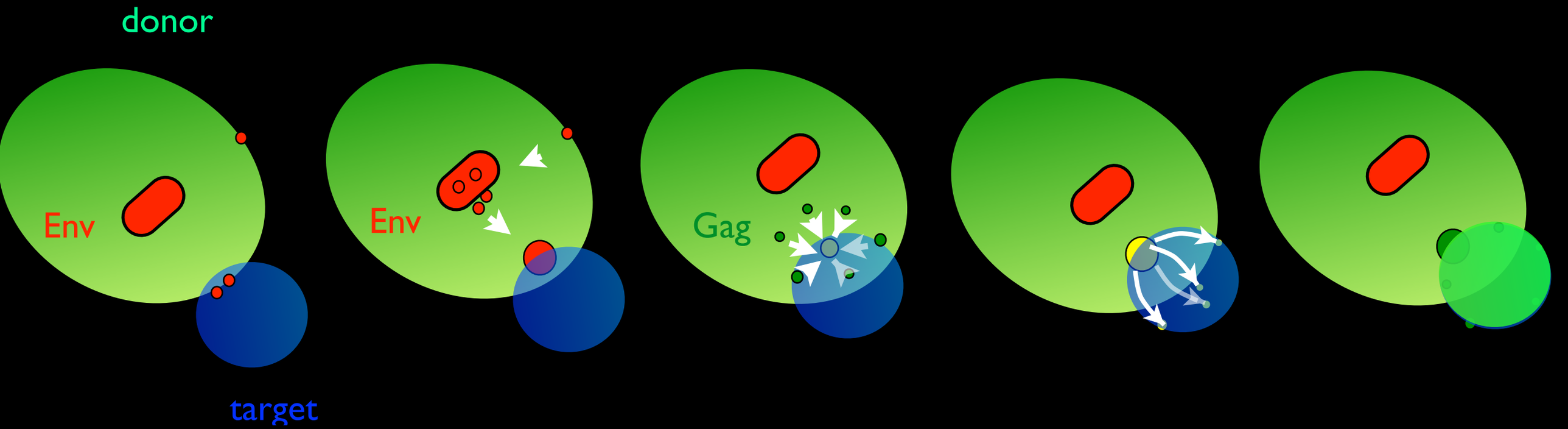
VS transfer leads to productive infection

00:00 (hours: min)

Gag-iGFP
+ GFP in place of *nef*

MT4

Stepwise model for VS formation and viral transfer



CD4:Env Adhesion

recycling Env targets VS

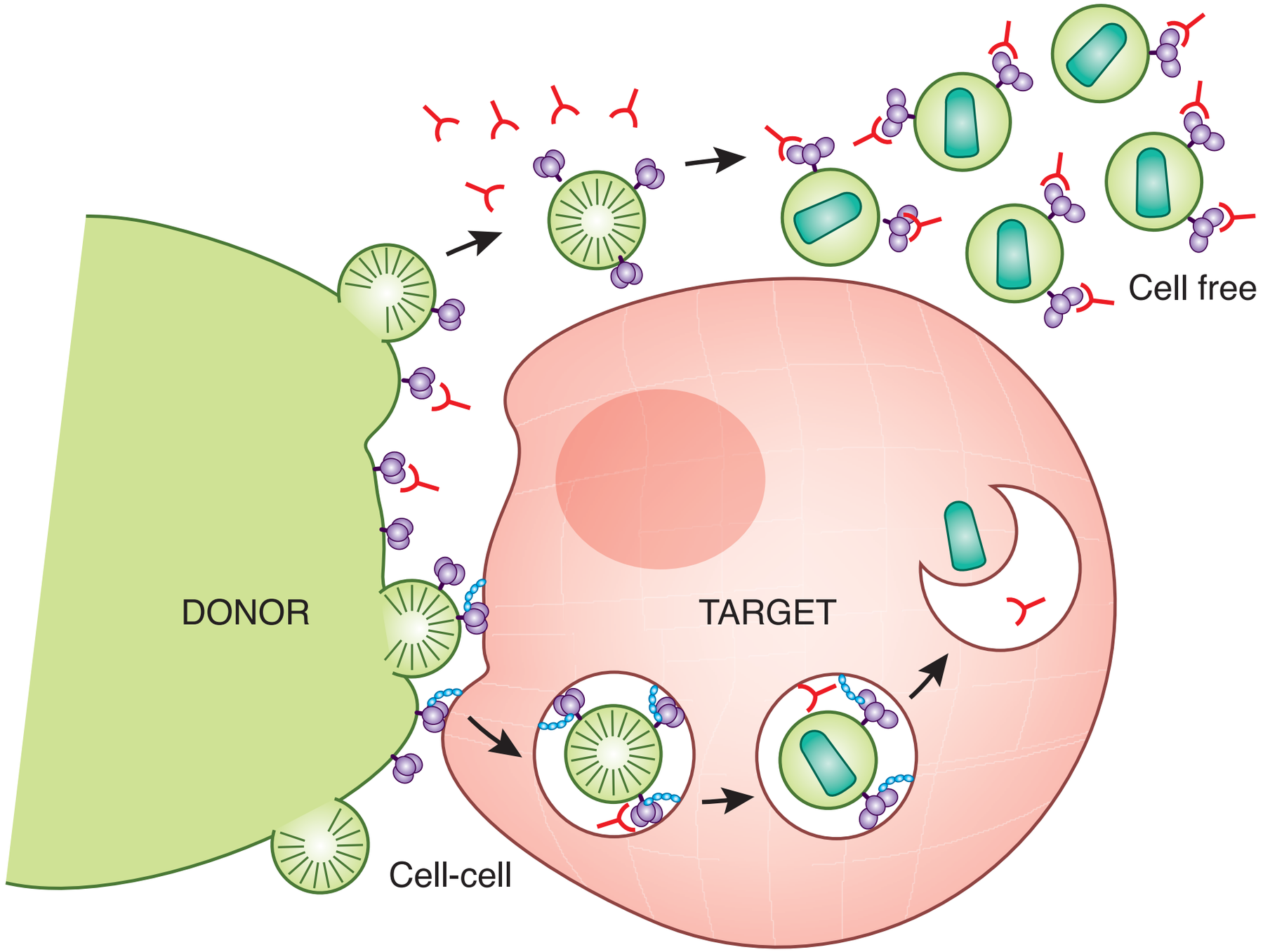
local Gag targets VS

endocytic viral uptake

viral fusion productive infection

Signaling in donor cell coordinates assembly: Signaling in target cell triggers endocytosis

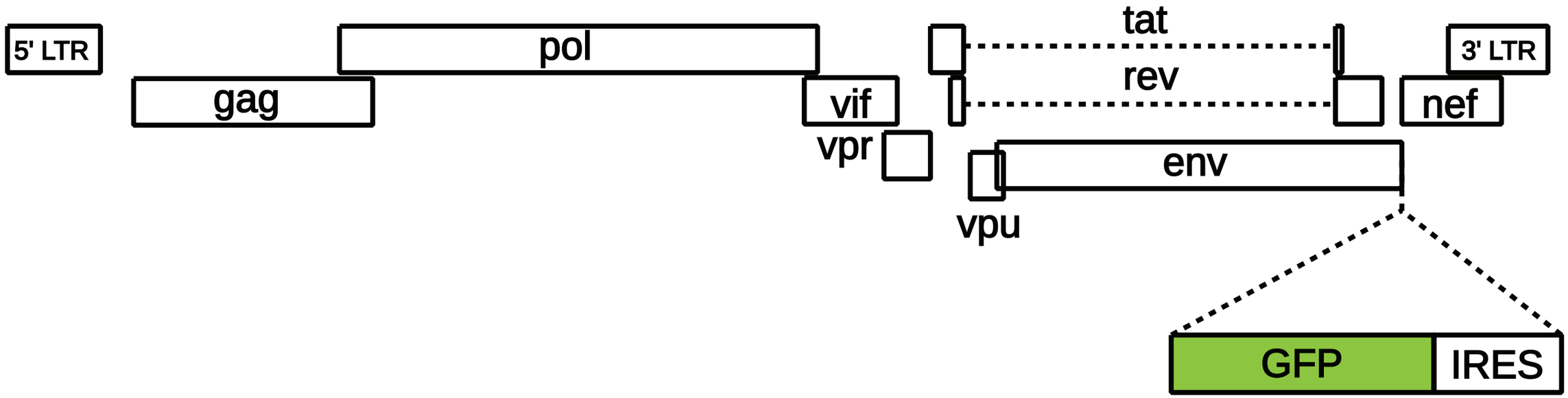
VS Neutralization Resistance



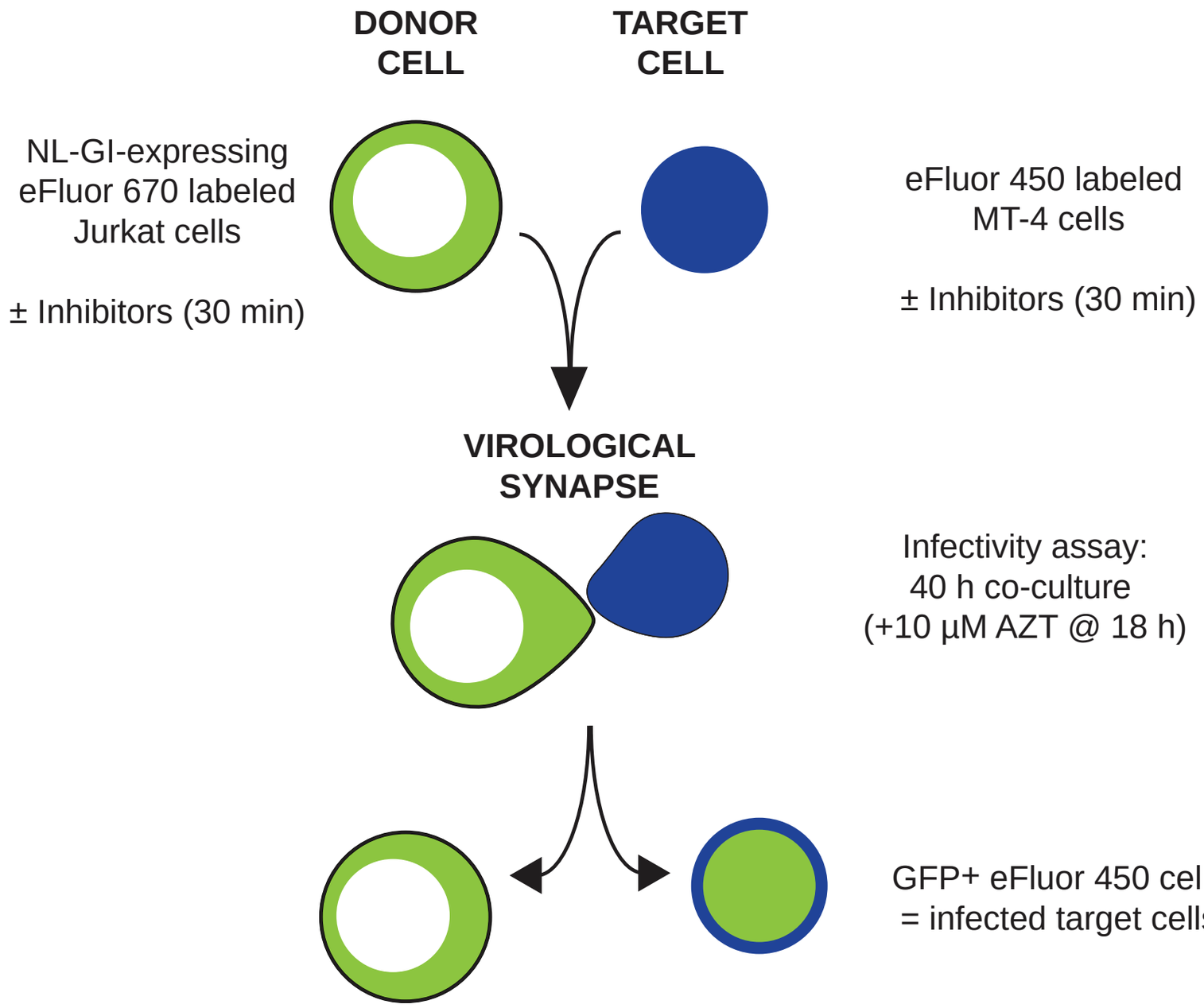
Abela, Plos Pathogen 2012, Durham et a J Virol 2012

Infectious GFP-expressing HIV

NL-GI *(for infectivity assay)*

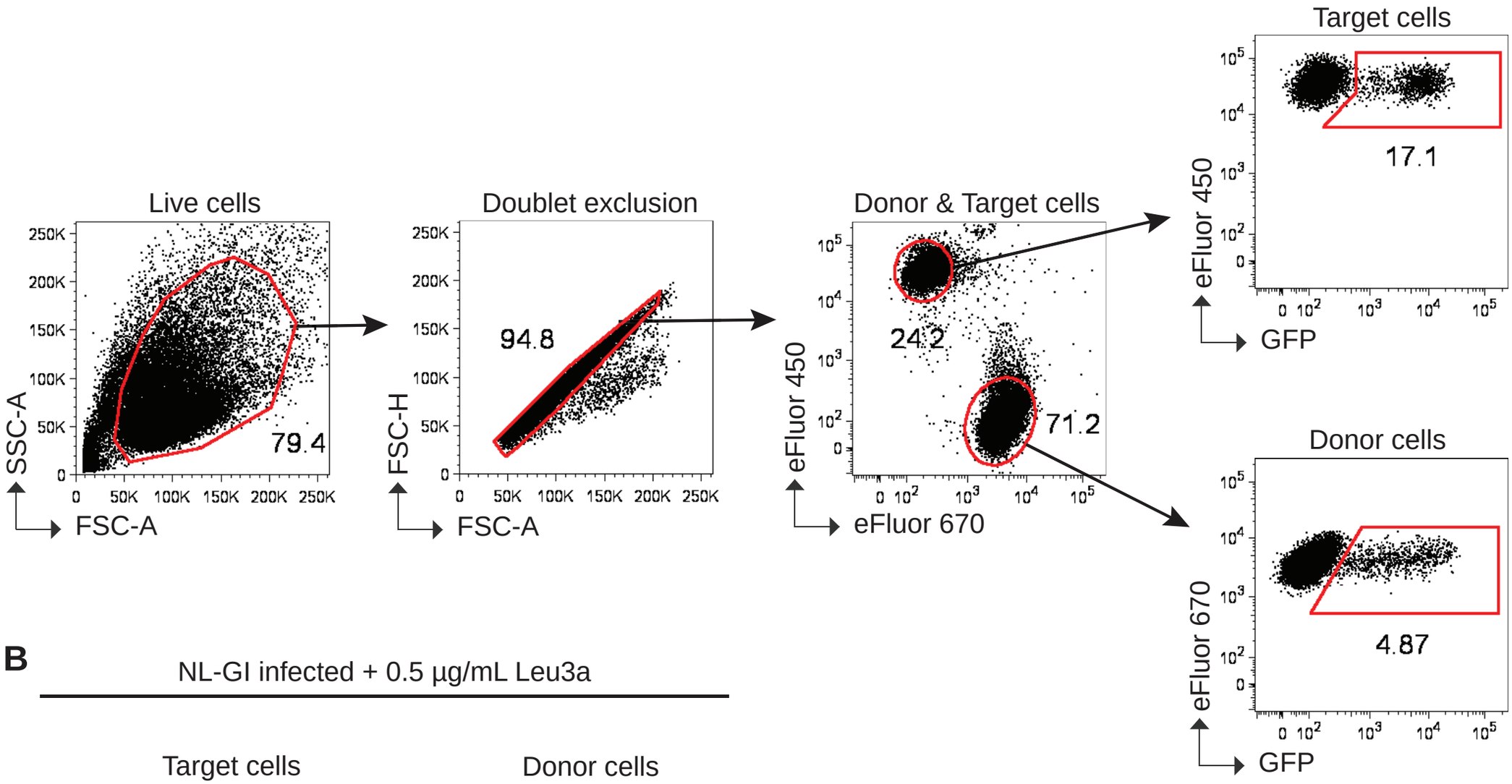


Cell-to-cell HIV infection



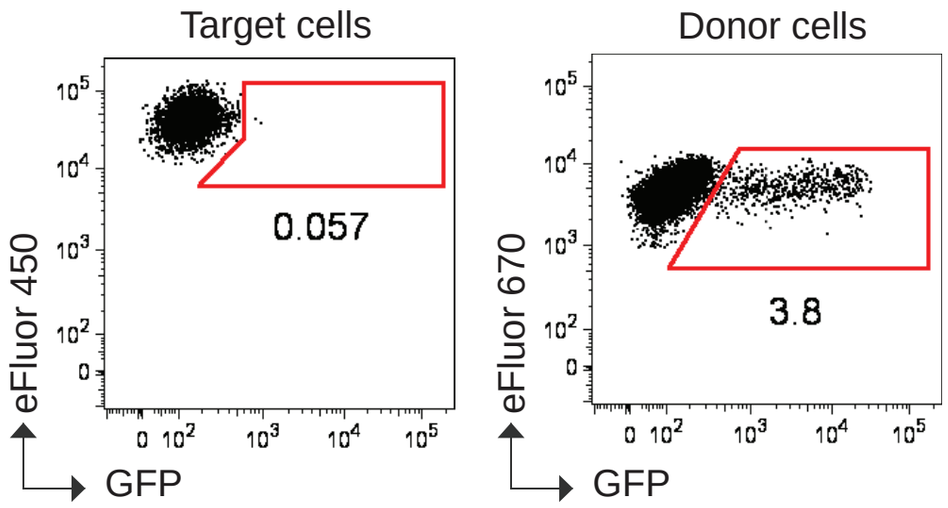
Donor Target Discrimination

A NL-GI infected + no inhibitor

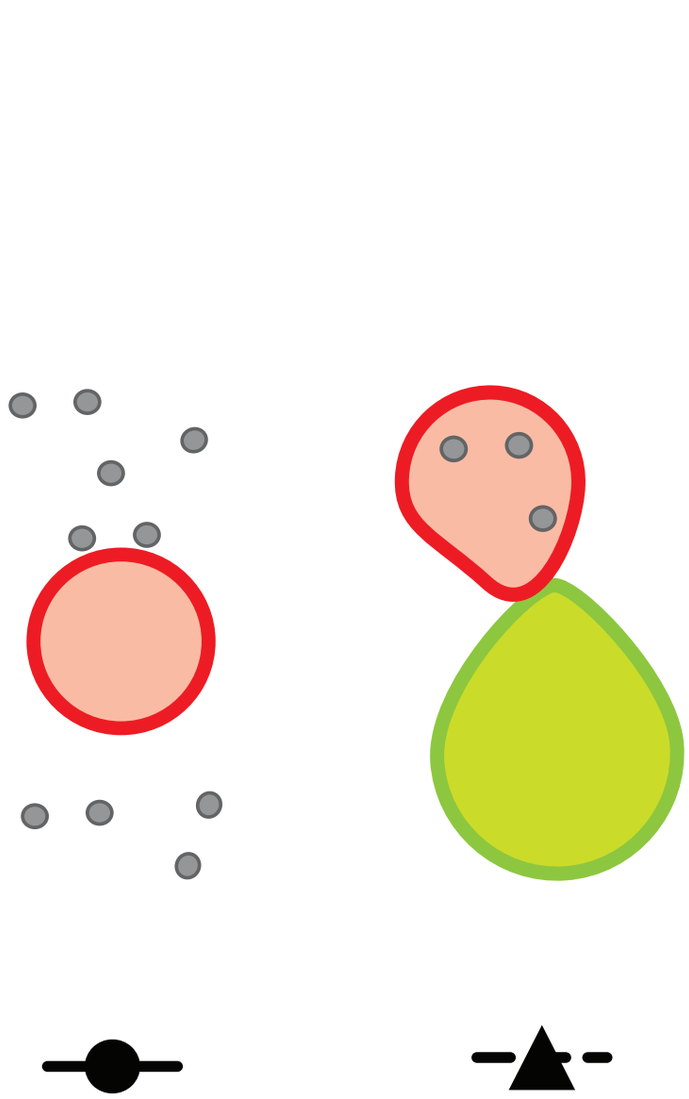


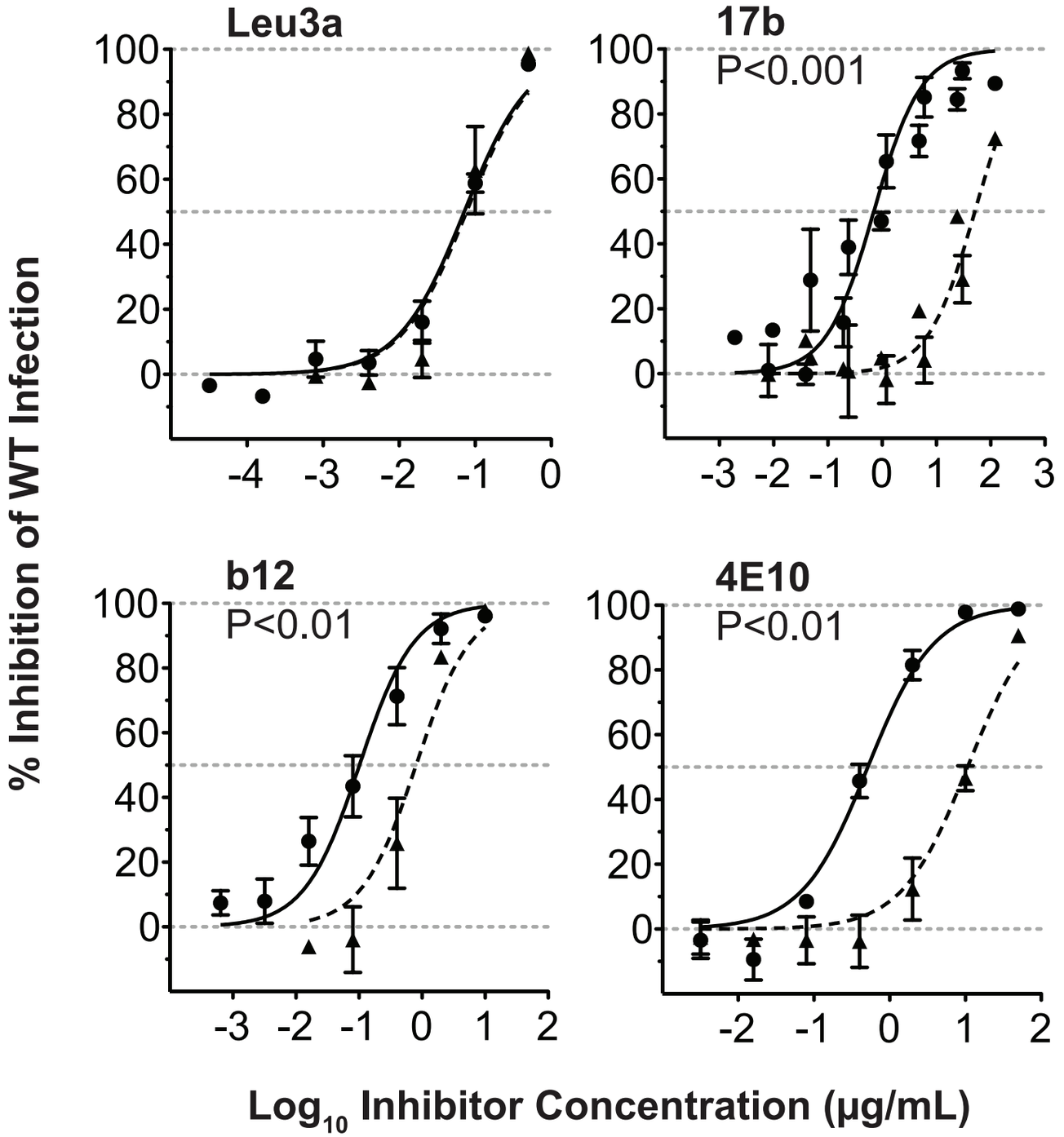
B

NL-GI infected + 0.5 µg/mL Leu3a



Resistance of VS infection to neutralizing antibodies





Durham et a J Virol 2012

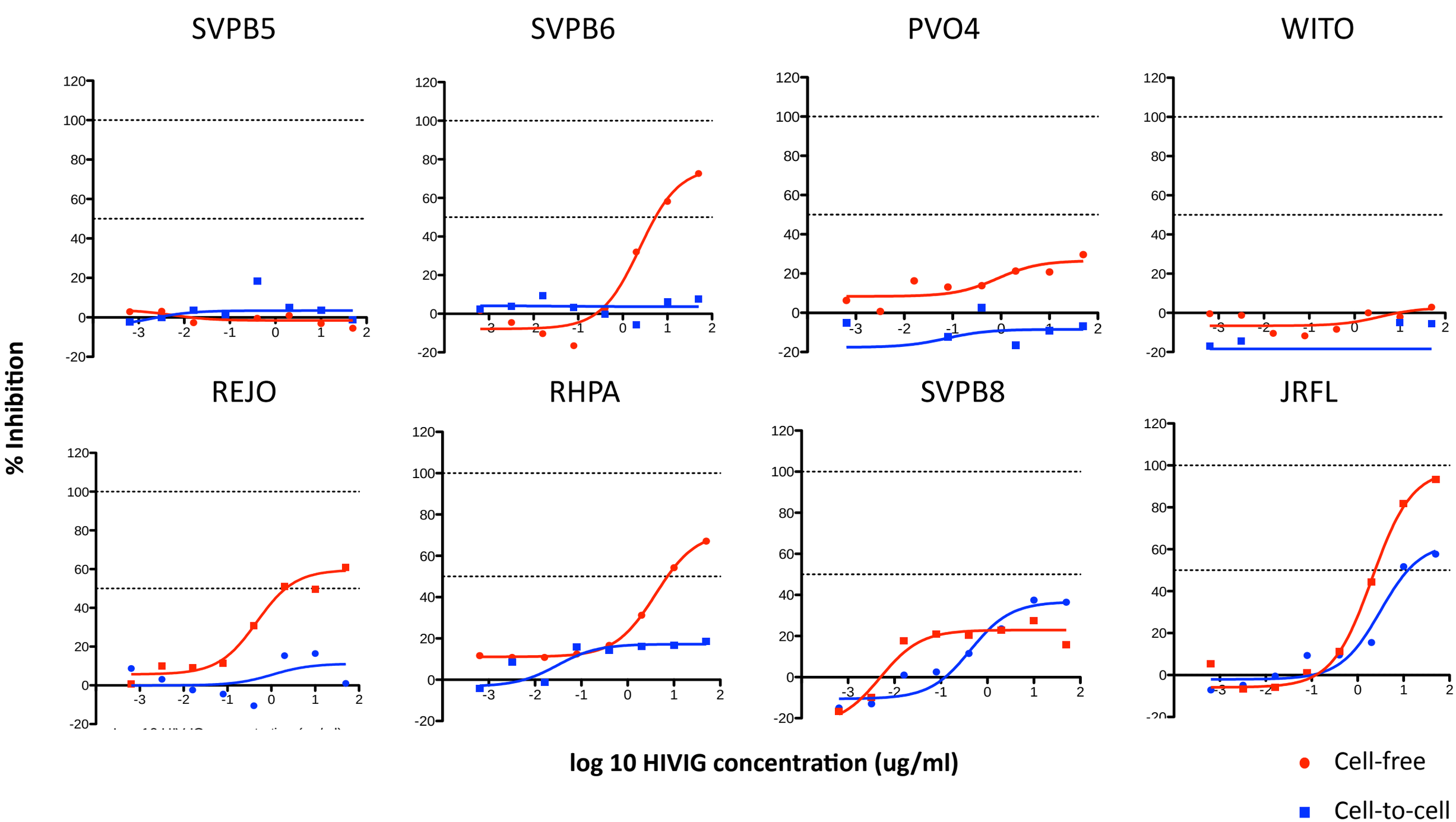
Primary isolate transmitted founder HIV Env clones

Env clone name	Origin	Tier	Subtype	Fiebig
SVPB5	Washington DC	1B	B	V
SVPB6	Trinidad	2	B	V
PVO, clone 4	Italy	3	B	III
WITO 4160	Alabama	2	B	II
REJO 4541	Alabama	2	B	II
RHPA 4259	Tennessee	2	B	<V
SVPB 8	Trinidad	2	B	IV

Montefiori, Gao, and LI Hahn, Salazar-Gonzalez

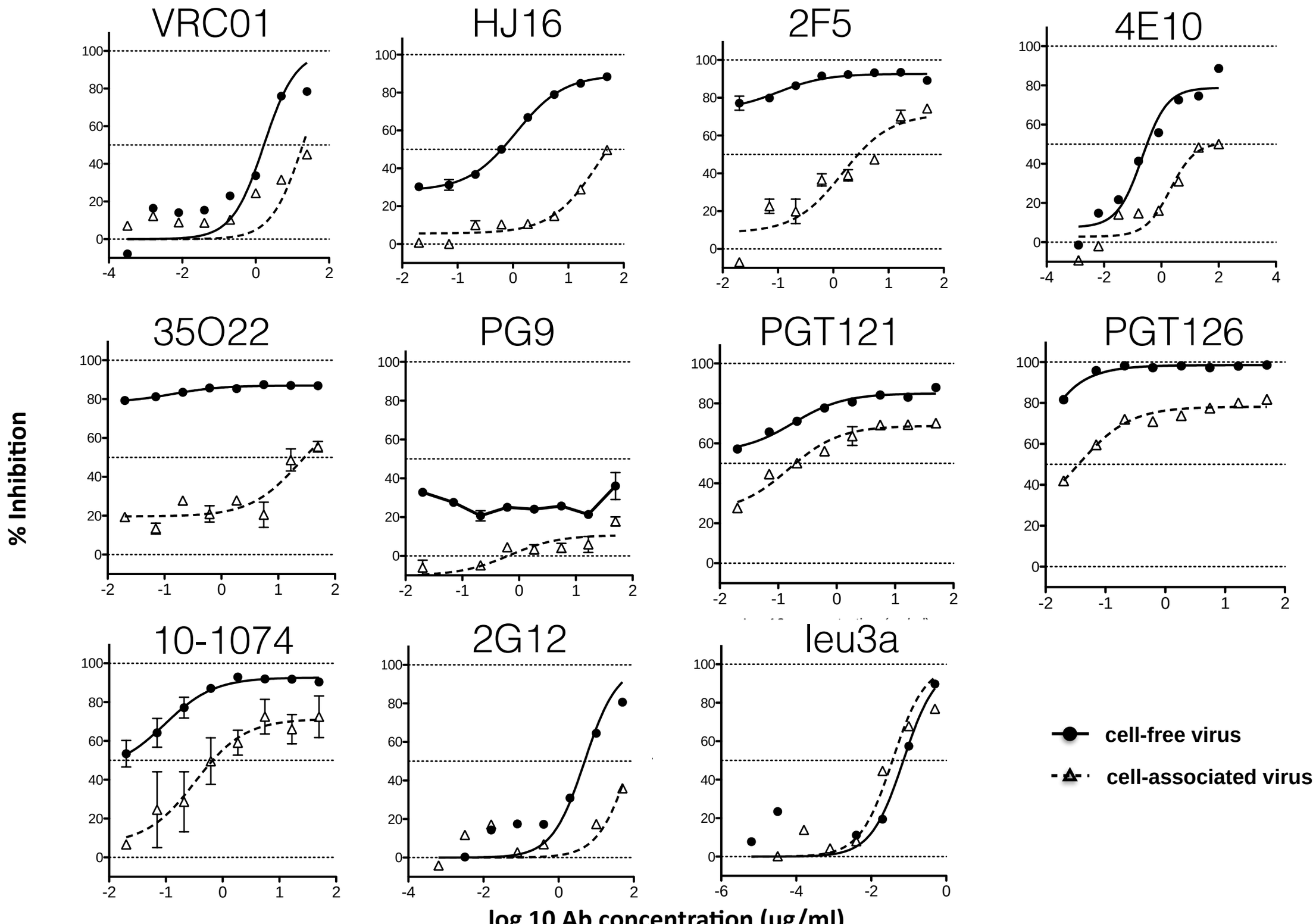
Li et al, unpublished

Clade B primary isolates cell-free vs cell-to-cell neutralization by HIVIG

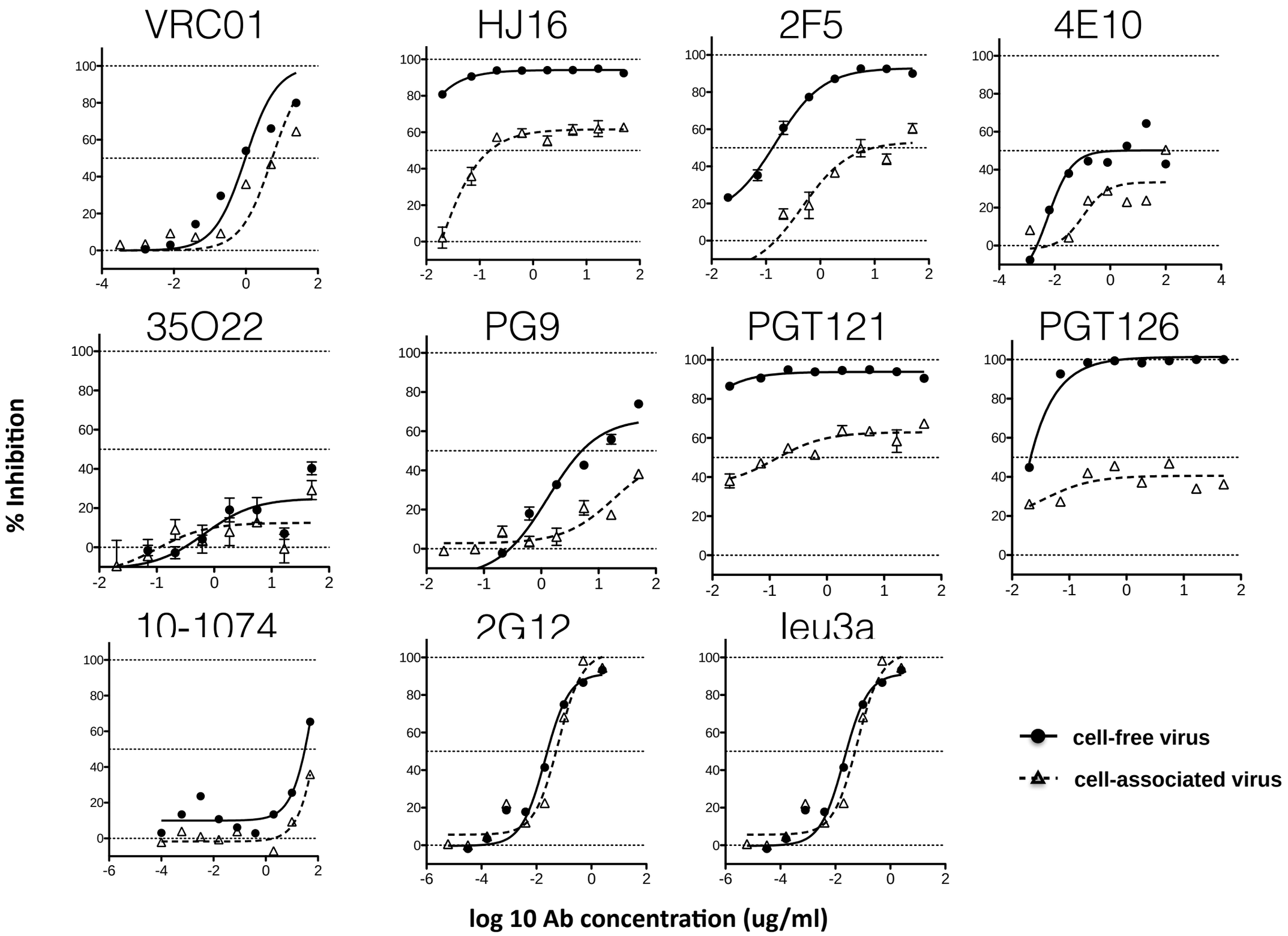


Li et al, unpublished

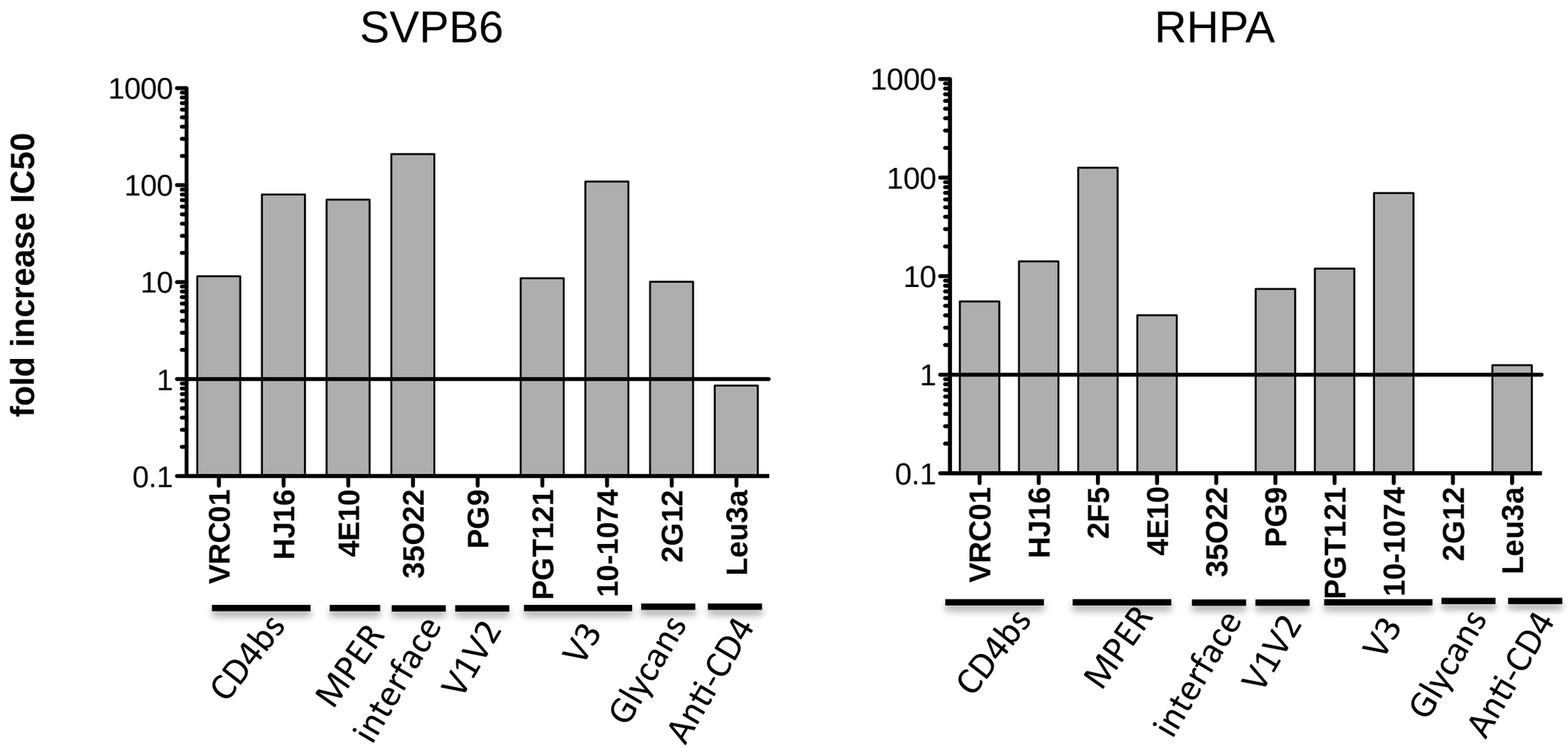
Neutralization of T/F HIV SVPB6



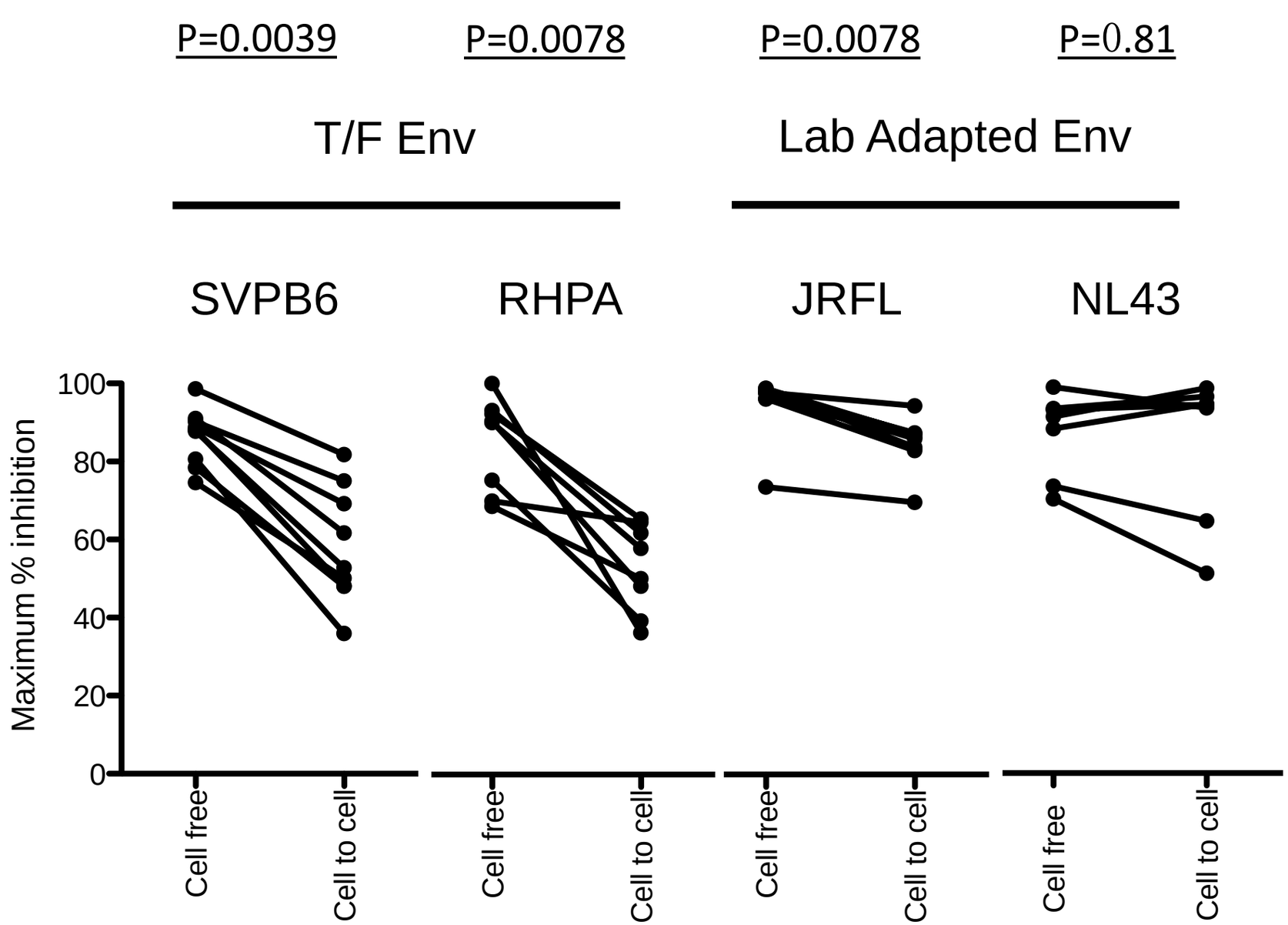
Neutralization of T/F HIV RHPA



Fold Increase in IC50 in cell-to-cell infection over cell-free infection



Incomplete neutralization of T/F Env by potent bnAb



Wilcoxon matched-pairs signed rank test

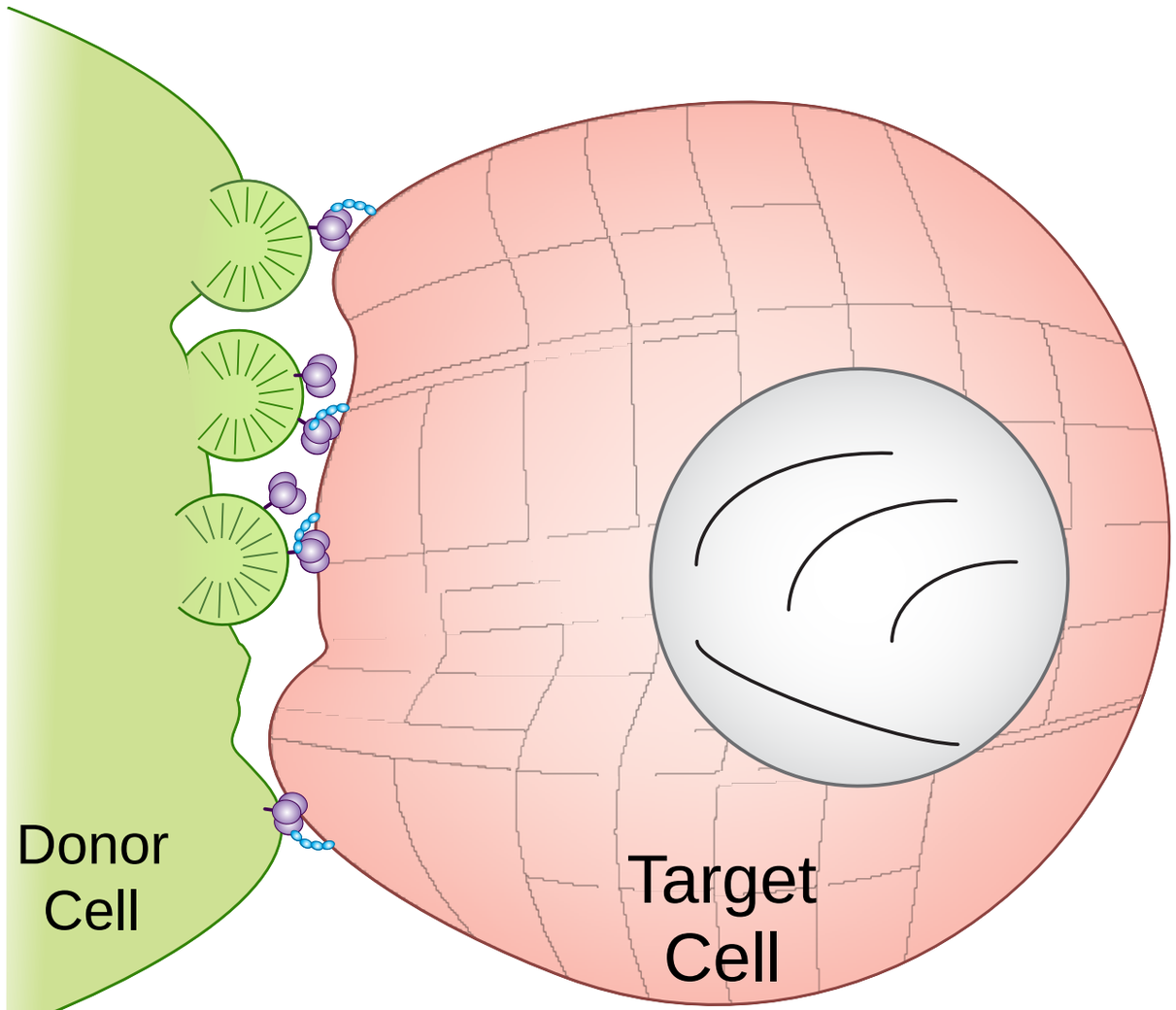
Neutralization of TF Env viruses engaged in VS

- TF Env -- more difficult to neutralize than lab strains -- especially by cell-cell infection
- Increases in IC50 and decreases in Max neutralizing activity are observed
- Low background of the flow based assay is critical for accurately assessing the maximum neutralizing capacity

Does cell-to-cell infection occur during infection in vivo?

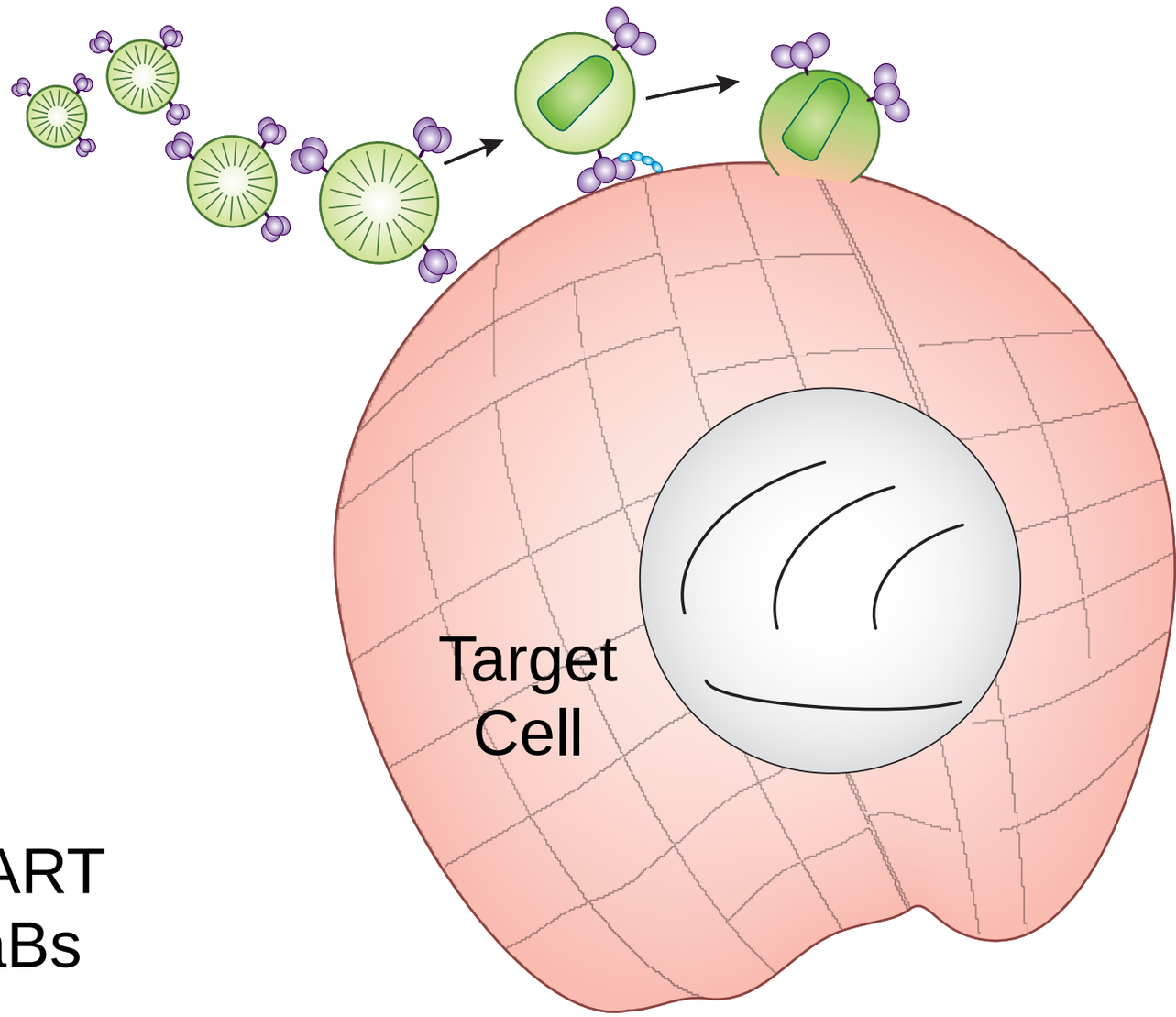
Multicopy HIV infection during cell-cell infection

Cell-to-cell Transmission



- Reduced sensitivity to certain ART
- Reduced neutralization by bNaBs

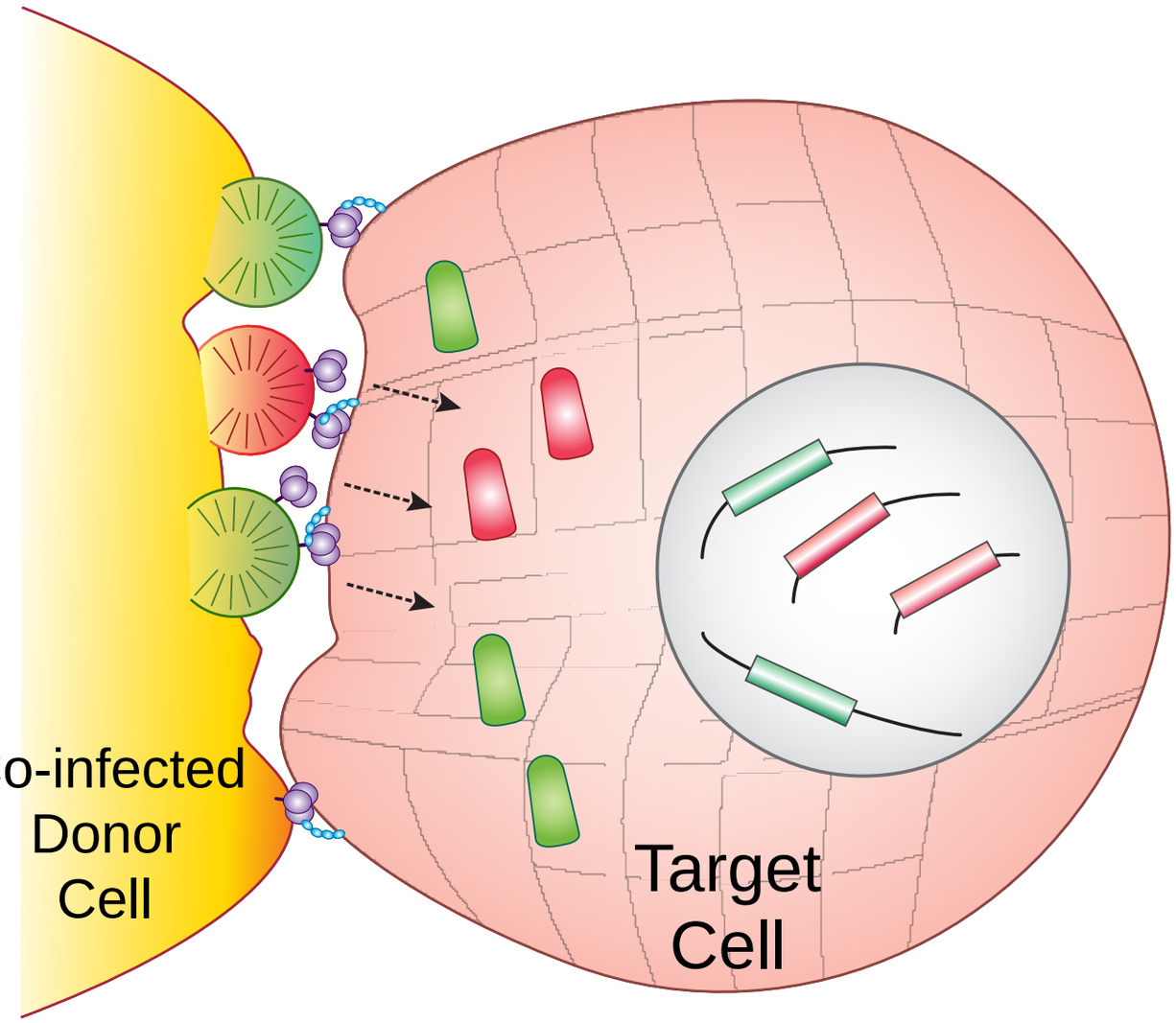
Cell-Free Transmission



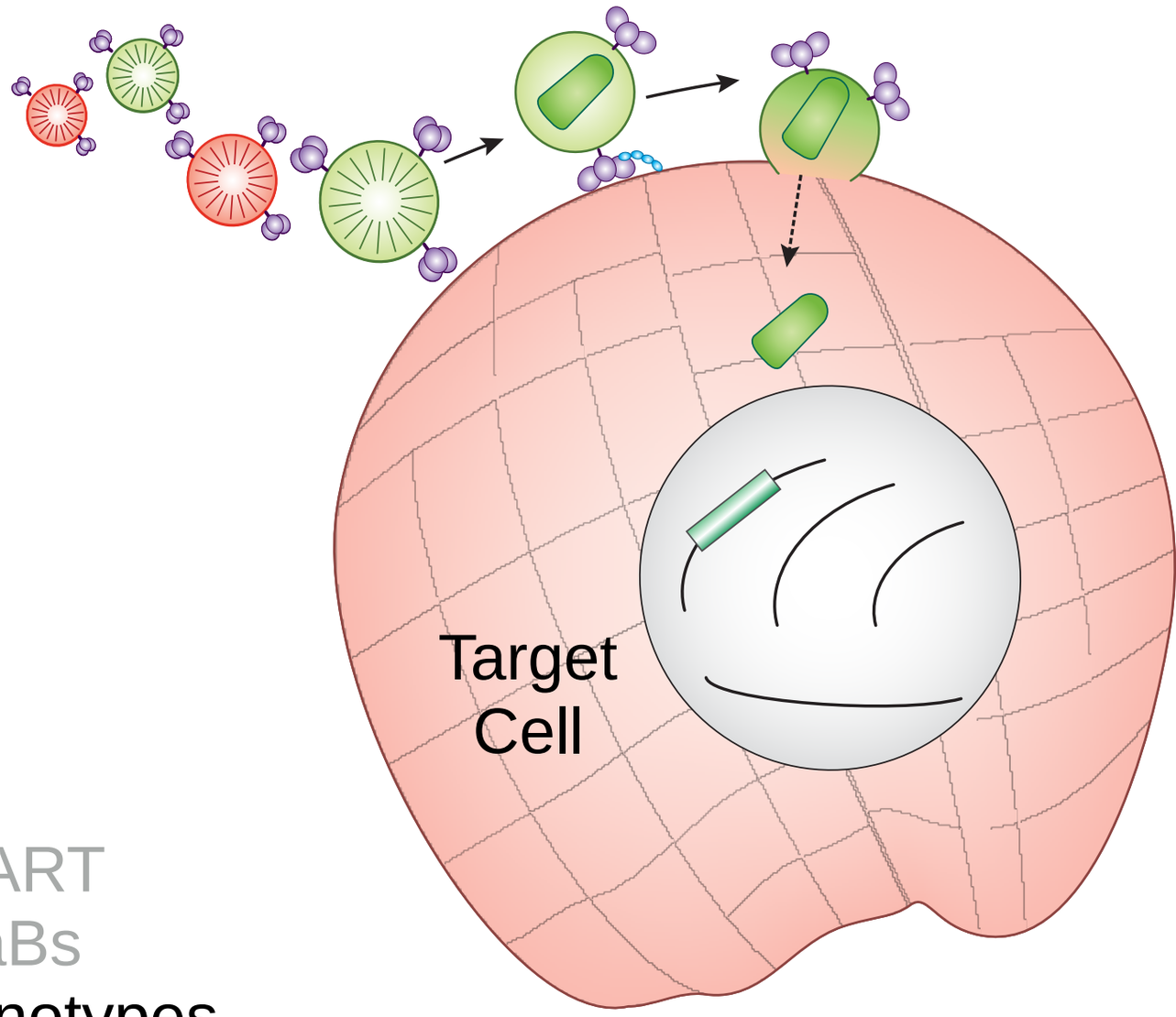
Del Portillo et al. J Virol 2011
Sigal et al. Nature 2011
Agosto et al. PPath 2014

Multicopy HIV infection during cell-cell infection

Cell-to-cell Transmission



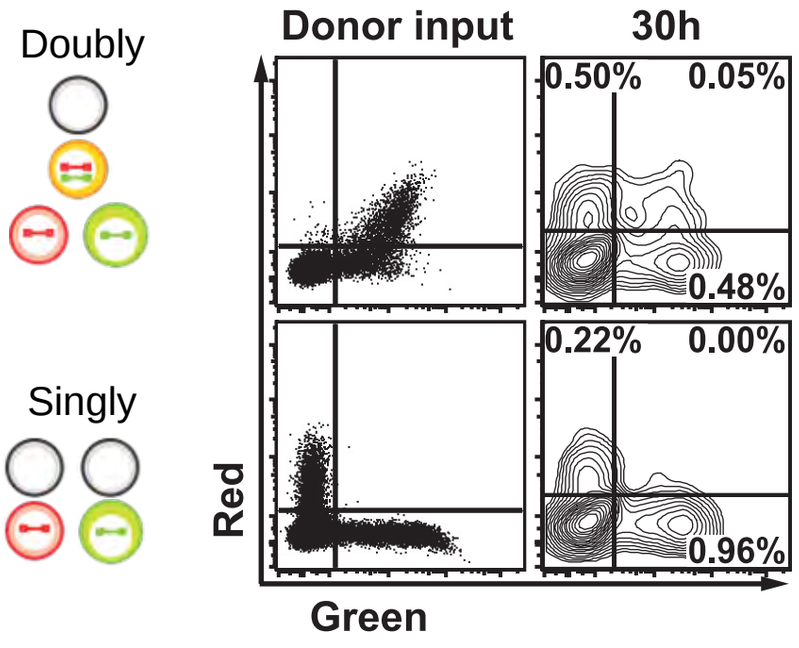
Cell-Free Transmission



- Reduced sensitivity to certain ART
- Reduced neutralization by bNaBs
- Co-transmission of multiple genotypes

Del Portillo et al. J Virol 2011
Sigal et al. Nature 2011
Agosto et al. PPath 2014

VS promote the co-transmission of multiple HIV-1 copies

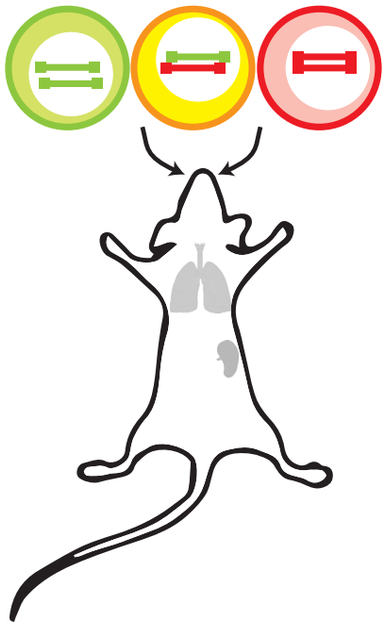


Del Portillo, A et al. 2011. JVI

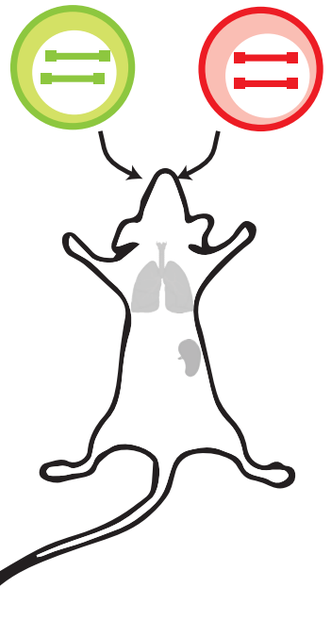
Experimental design to detect multi copy infection in vivo

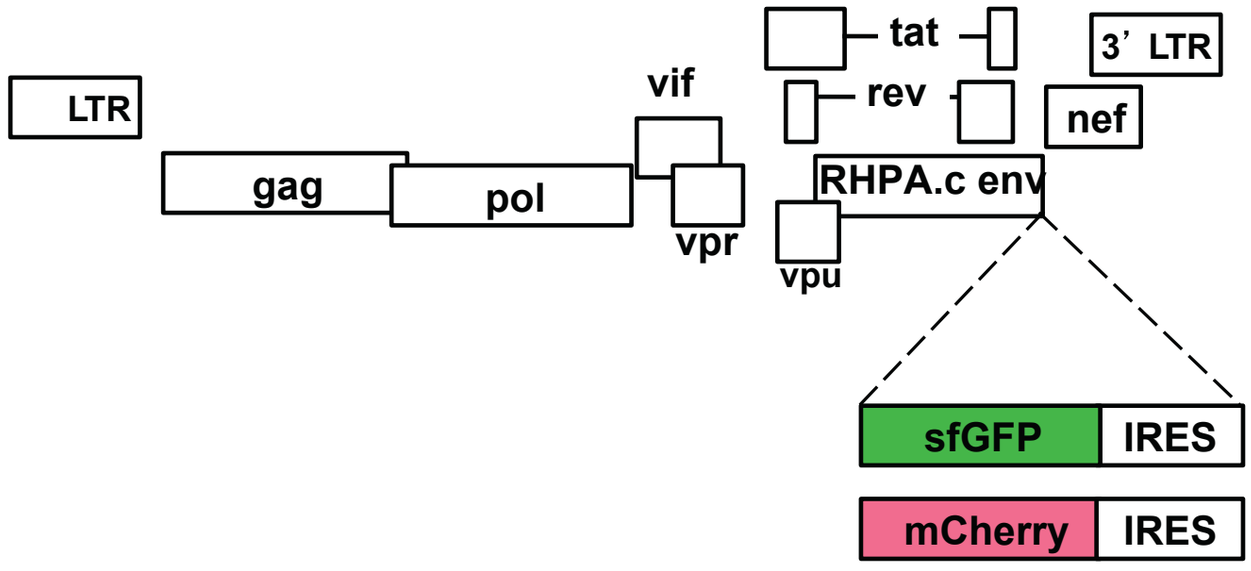
Cell-associated inocula

Doubly infected

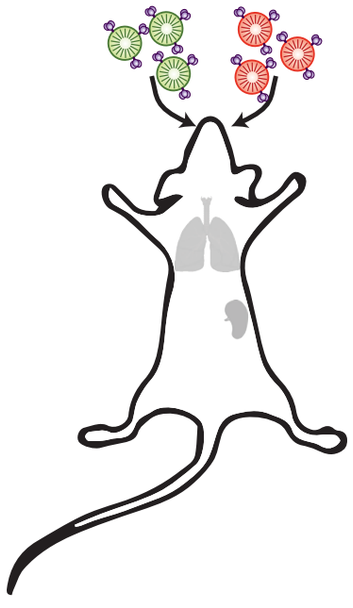


Singly infected





Cell-free inocula



Experimental design to detect multi copy infection in vivo

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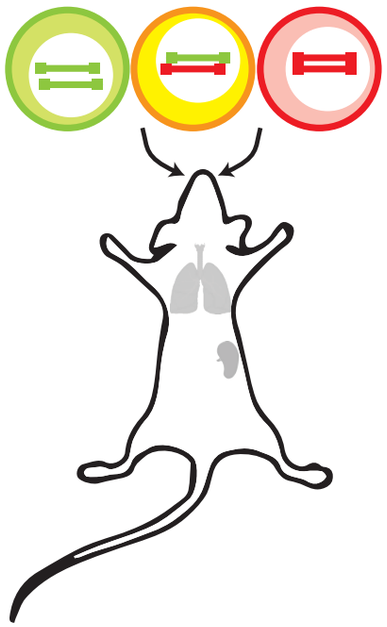
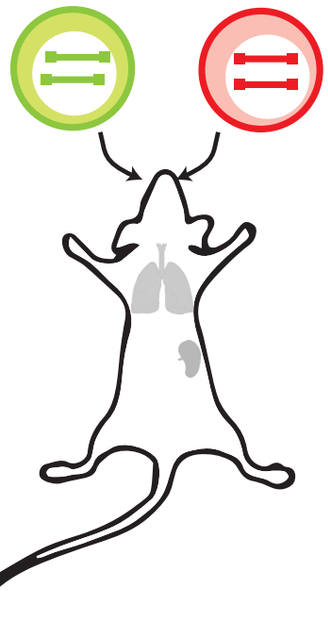


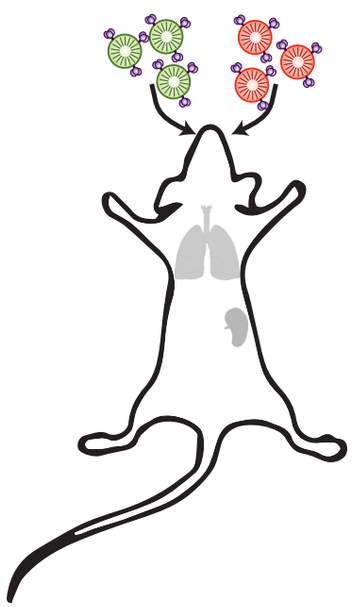
Singly infected



Inject Intravenously

Analyze 3 days post infection

Cell-free inocula

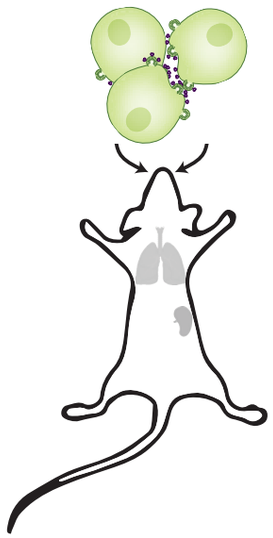


Inject Intravenously

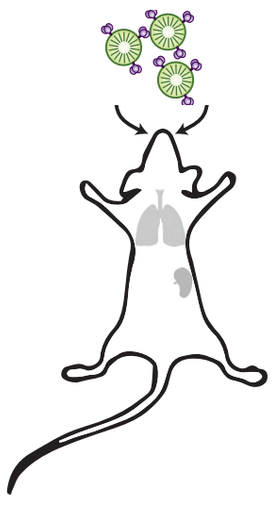
Analyze 3 days post infection

Robust acute infection in humanized mice, with fluorescent HIV using T/F Env

Cell-cell infection



Cell free infection



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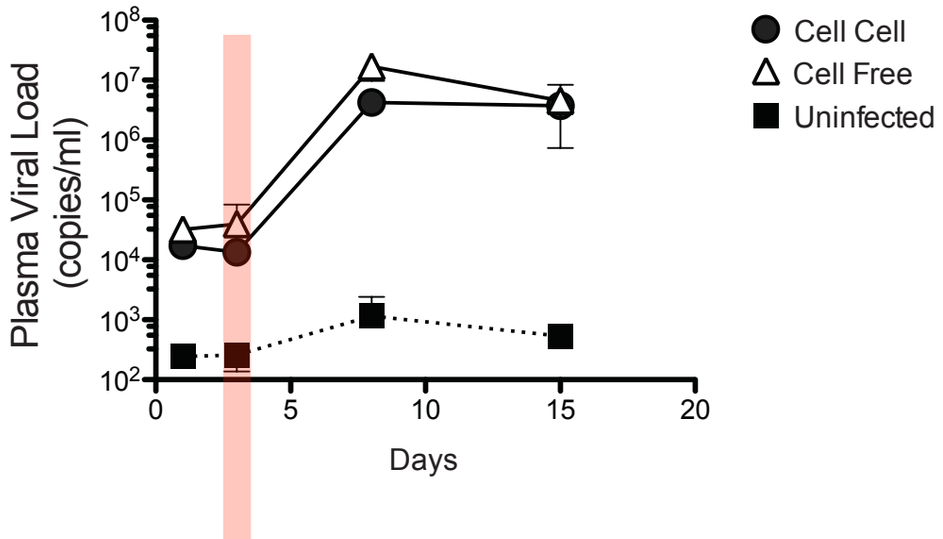
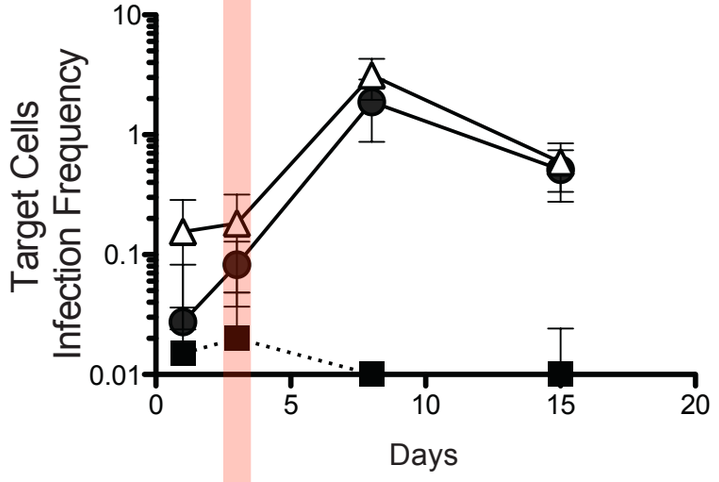
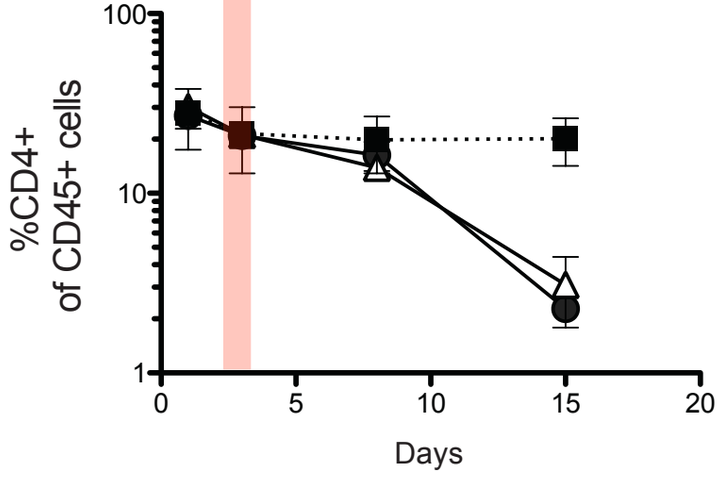
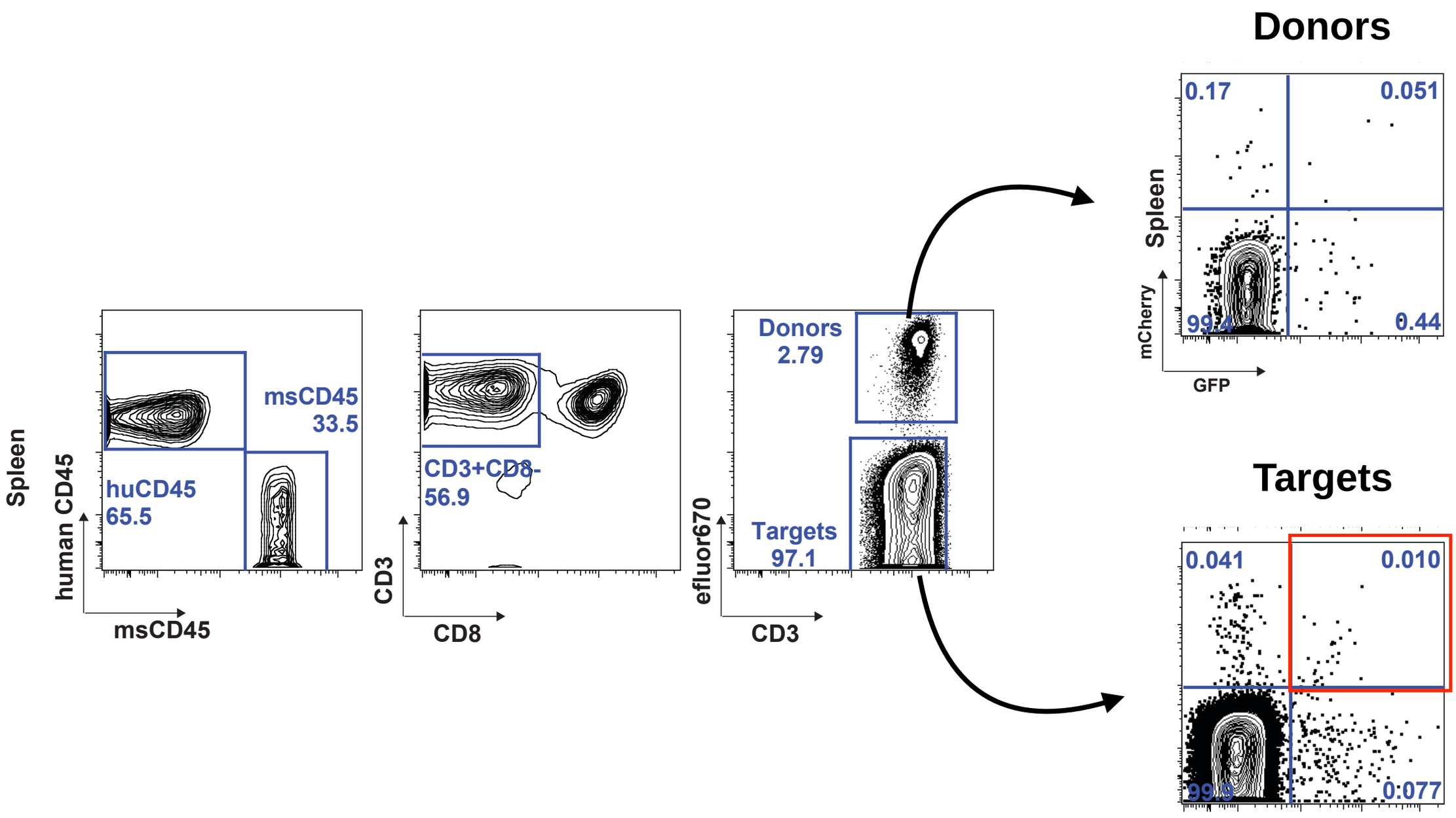






Examining multicopy infection *in vivo* mediated by cell-associated virus

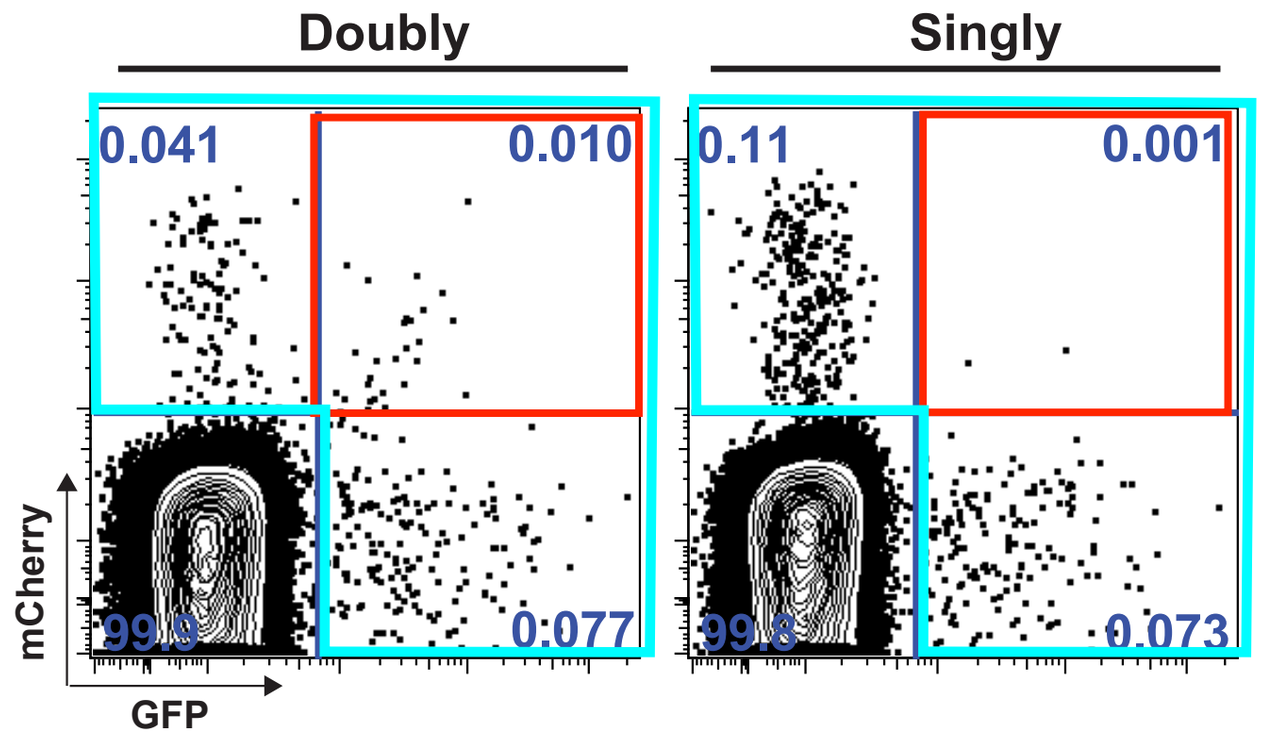


Experimental design to detect multiply infected cells

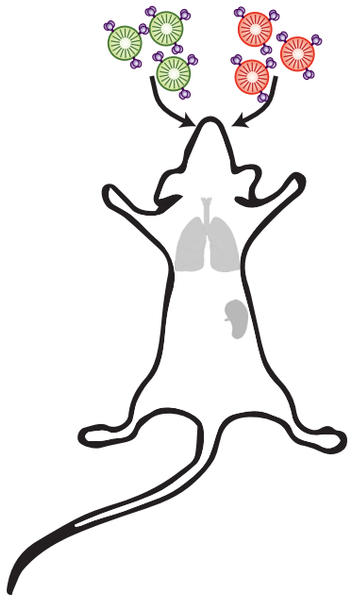
Cell-associated inocula

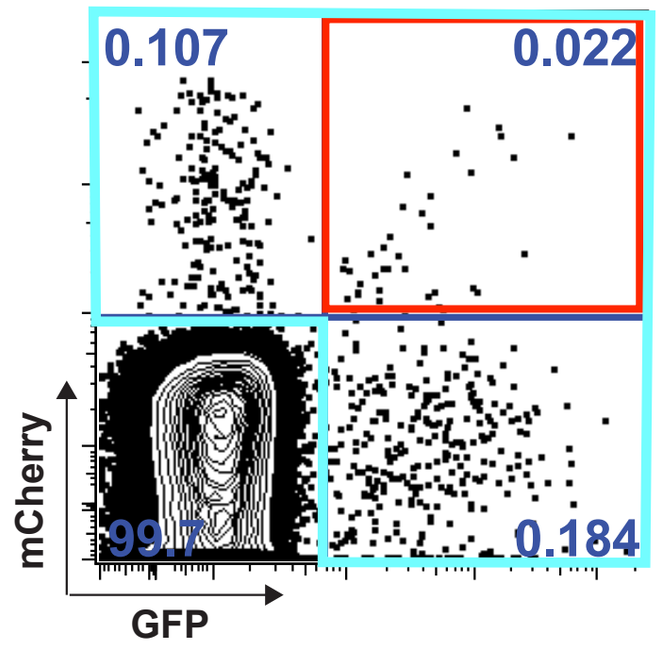
Doubly infected

Singly infected



Cell-free inocula





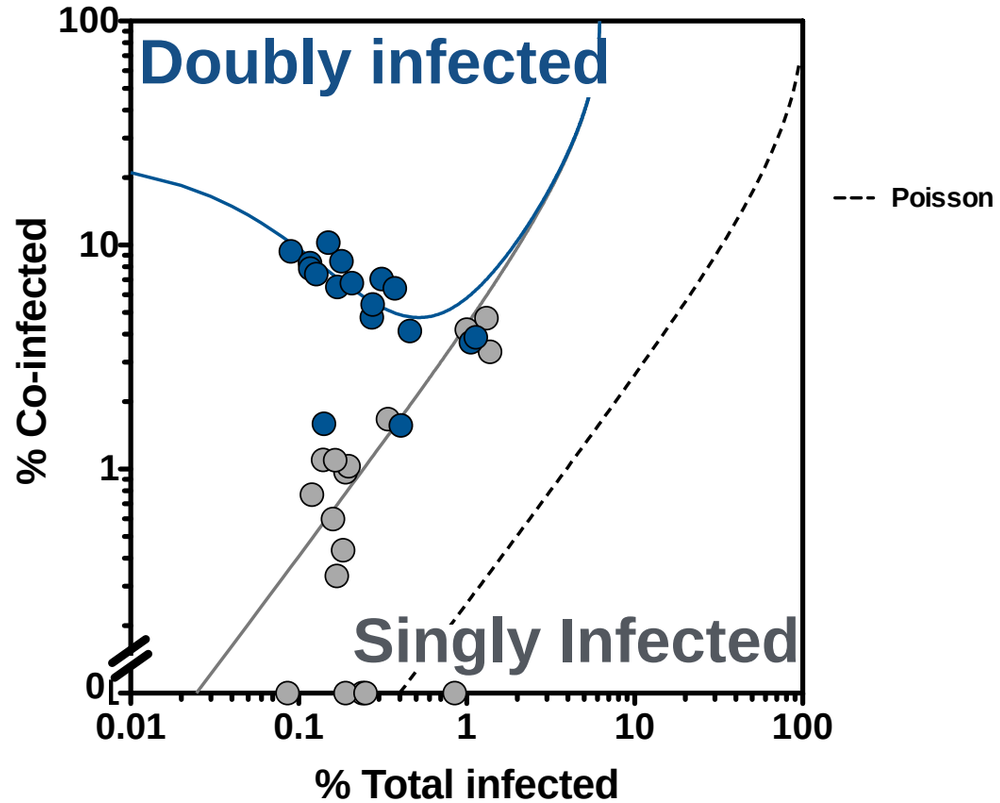
Cell-to-cell HIV-1 transmission promotes multicopy infection *in vivo*

Cell-associated inocula

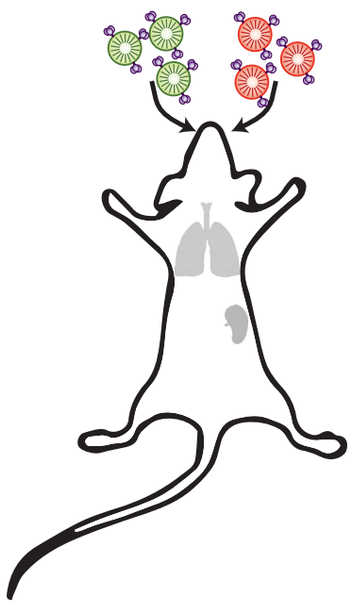
Doubly infected

Singly infected

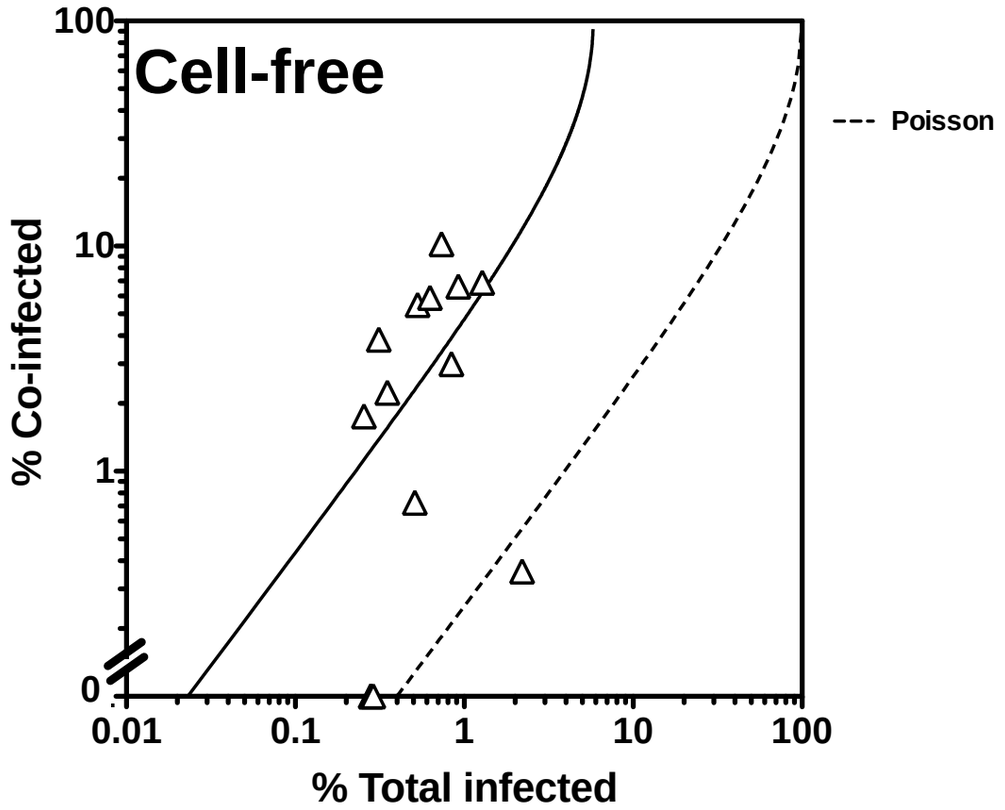
Cell-associated inocula



Cell-free inocula

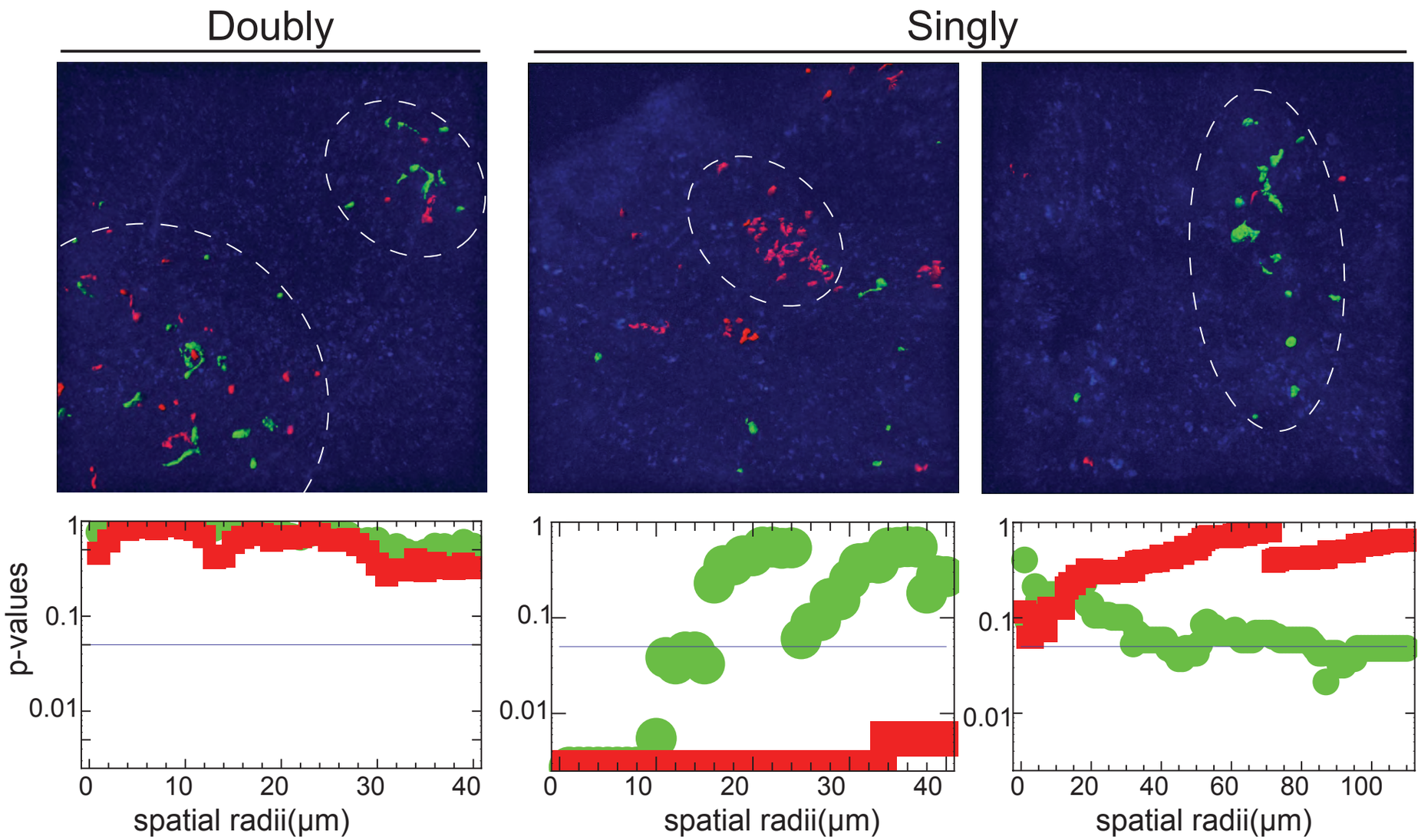


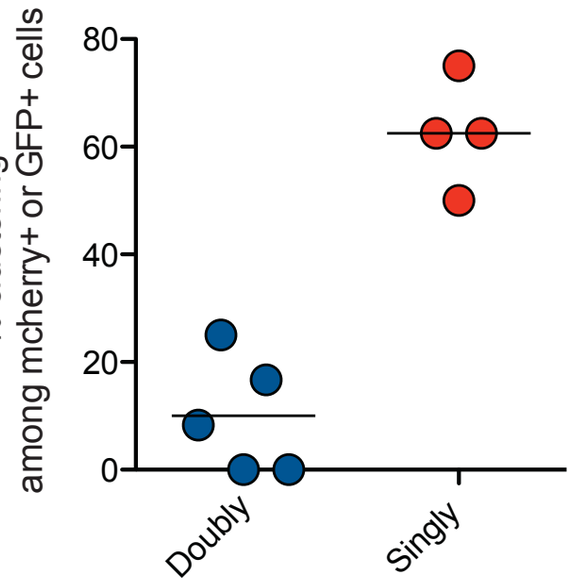
Cell-Free inocula



Law, K Cell Reports

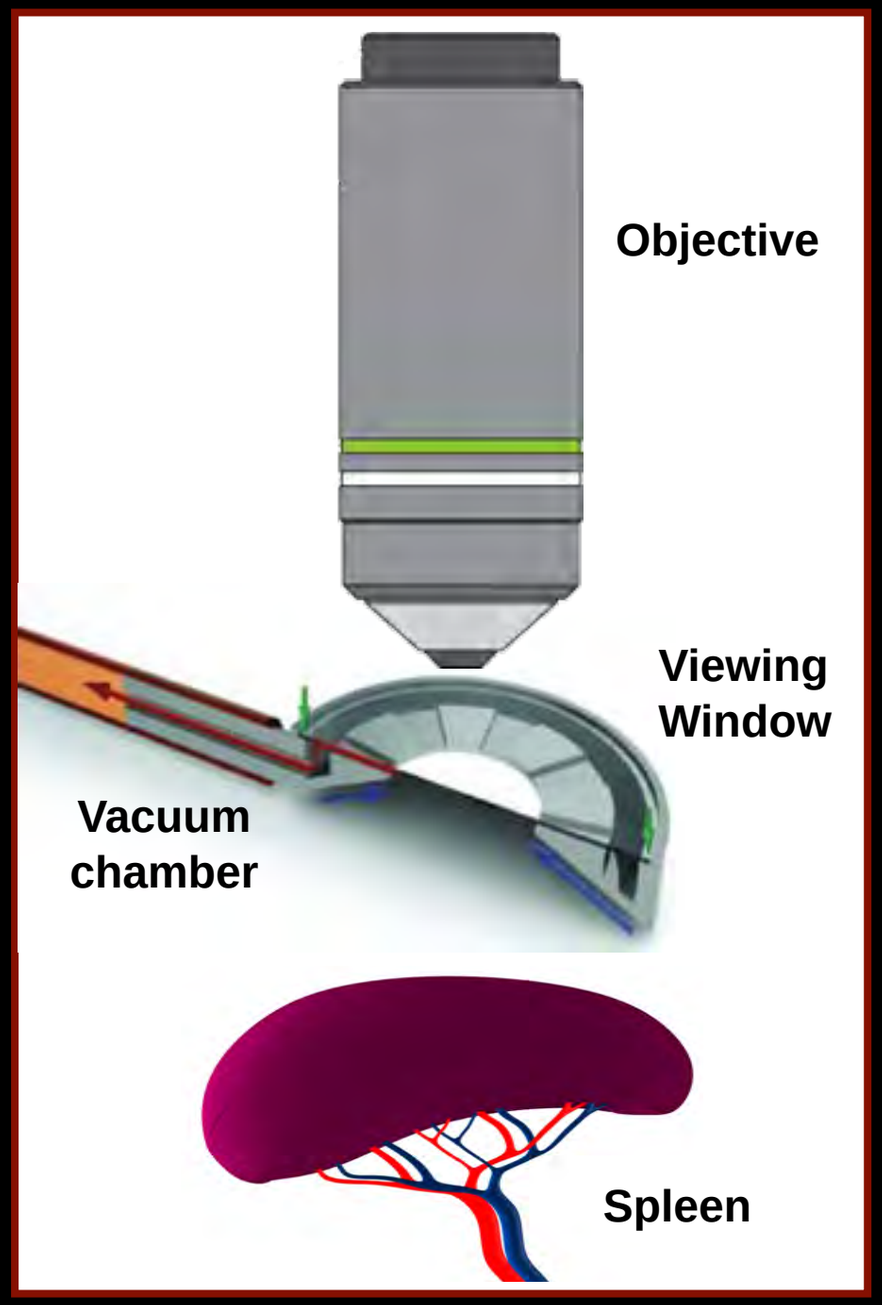
Singly infected mice show presence of clustering of single color populations

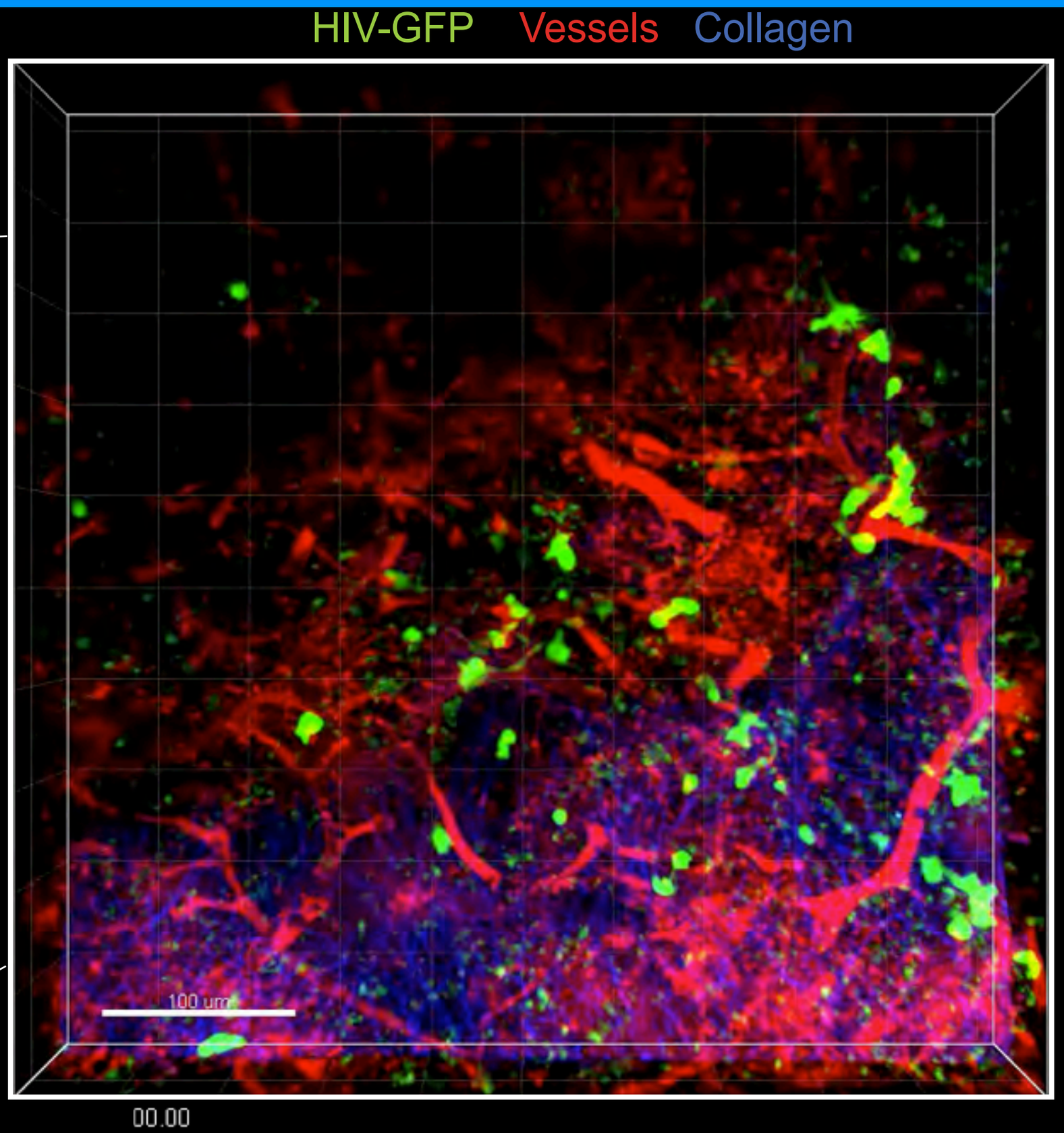




Wodarz D. & Komorova, N UC Irvine

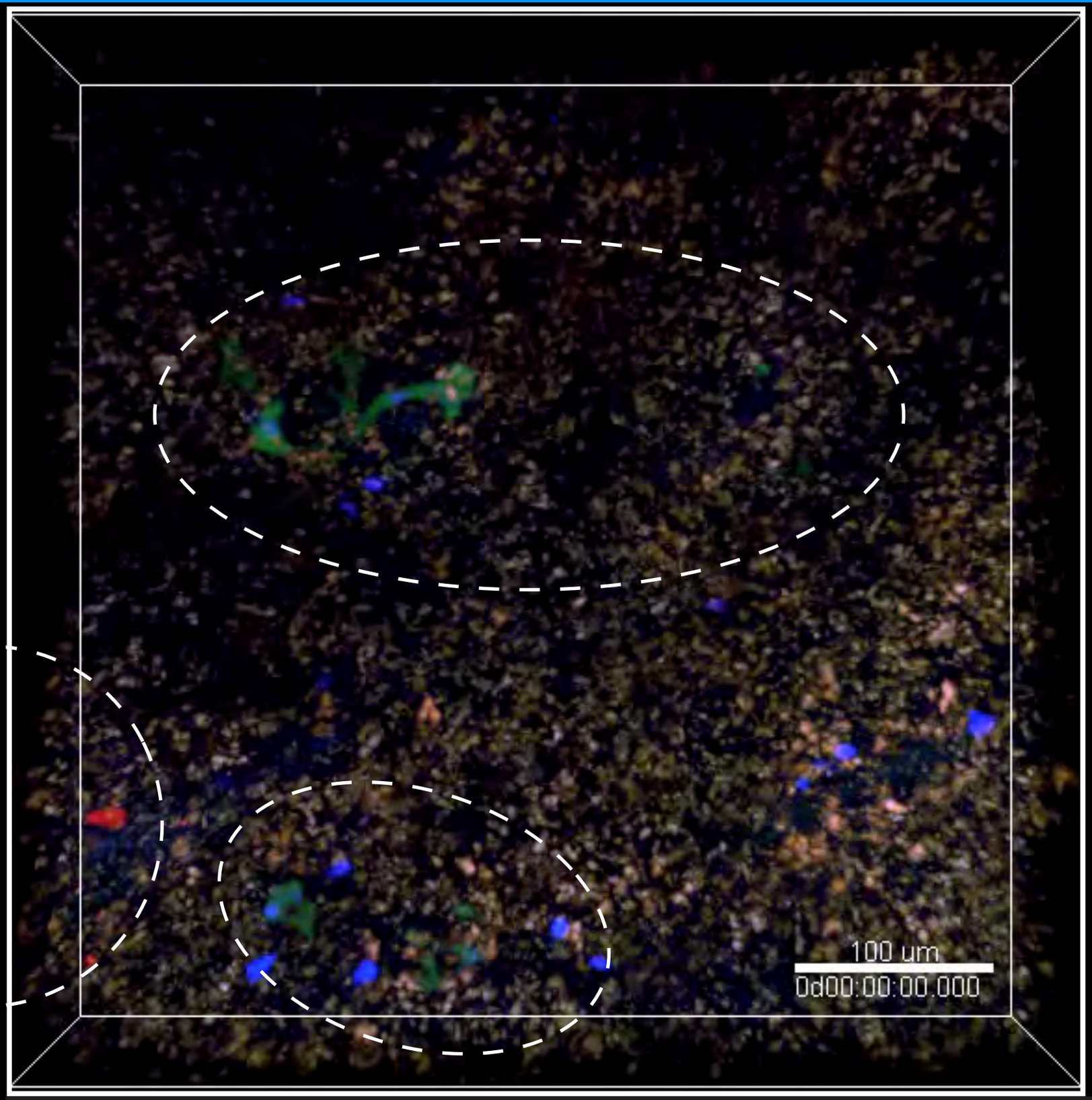
Intravital imaging: Imaging HIV-1 infected cells

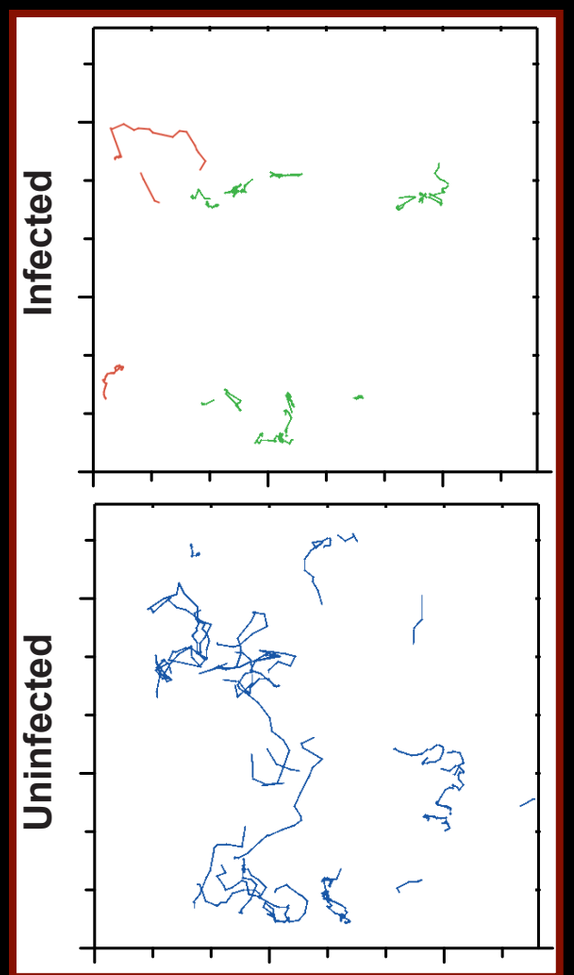


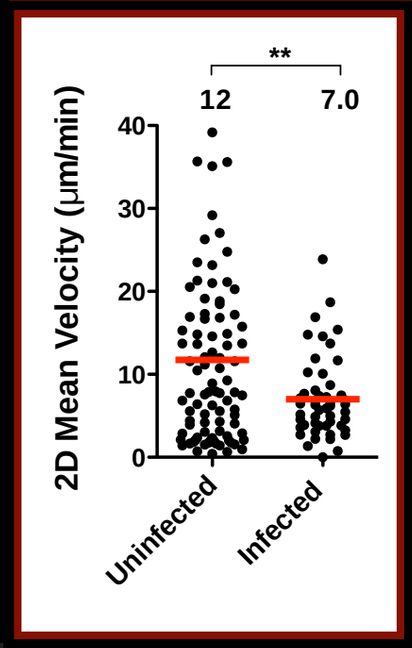


Law, K Submitted

Timelapse imaging show limited mixing of HIV variants in the the spleen





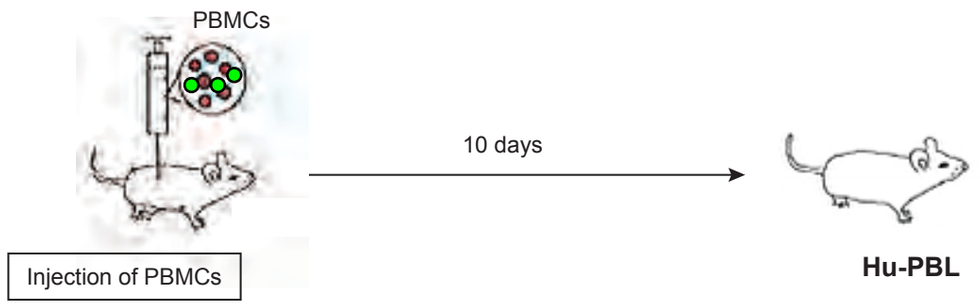


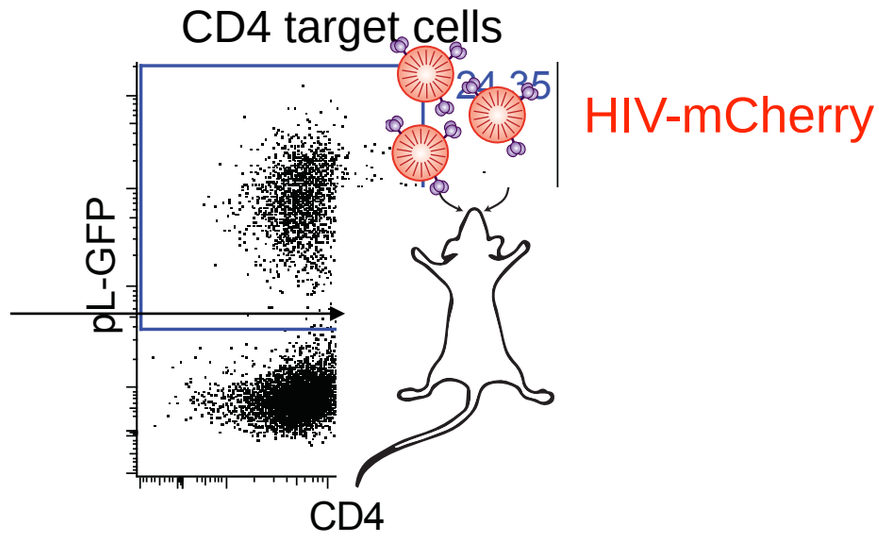
HIV-GFP HIV-mCherry Donor Cells Autofluorescence

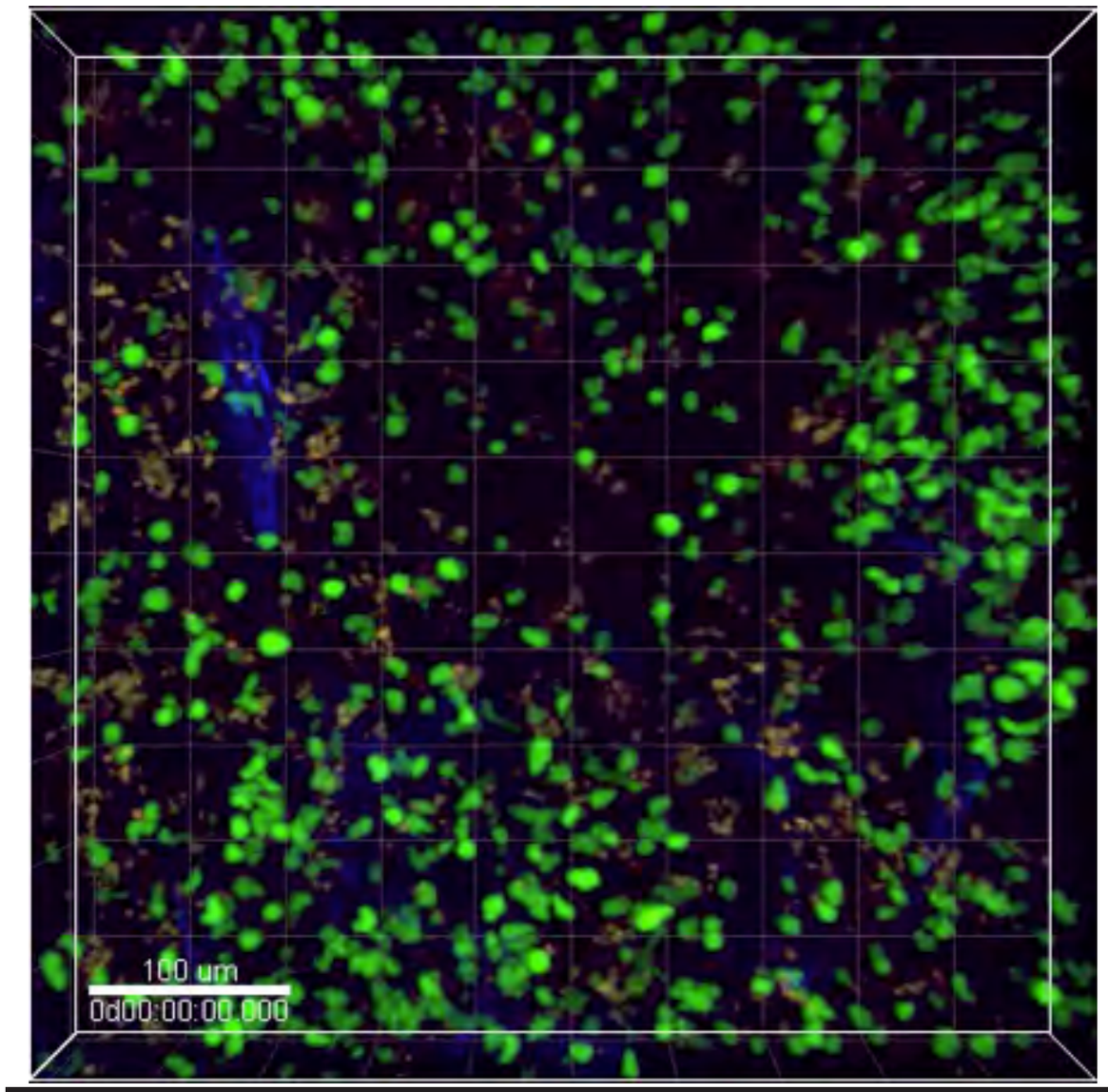
Law, K Submitted

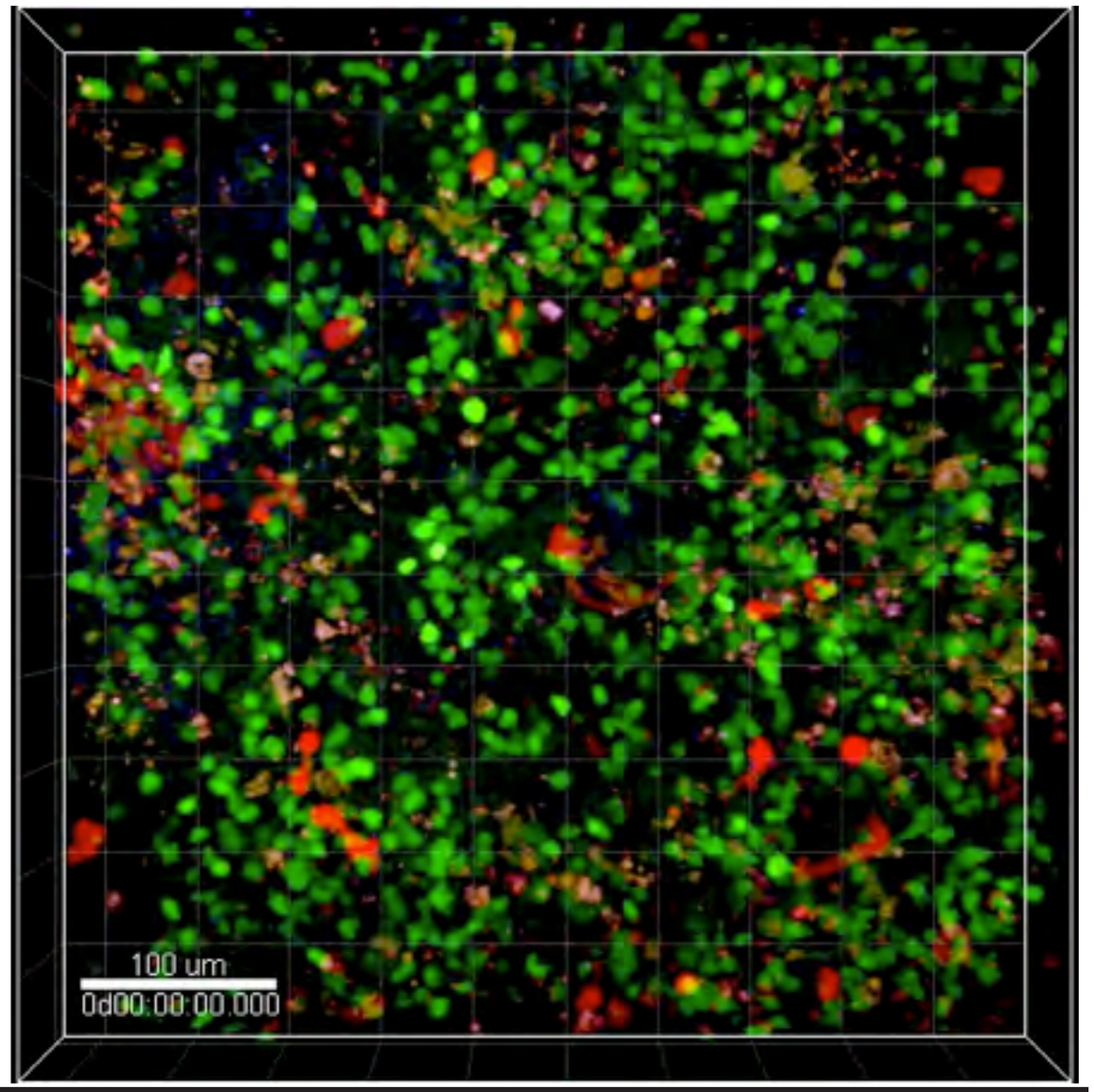
Tracking movement of uninfected target cells

GFP-transduced target cells



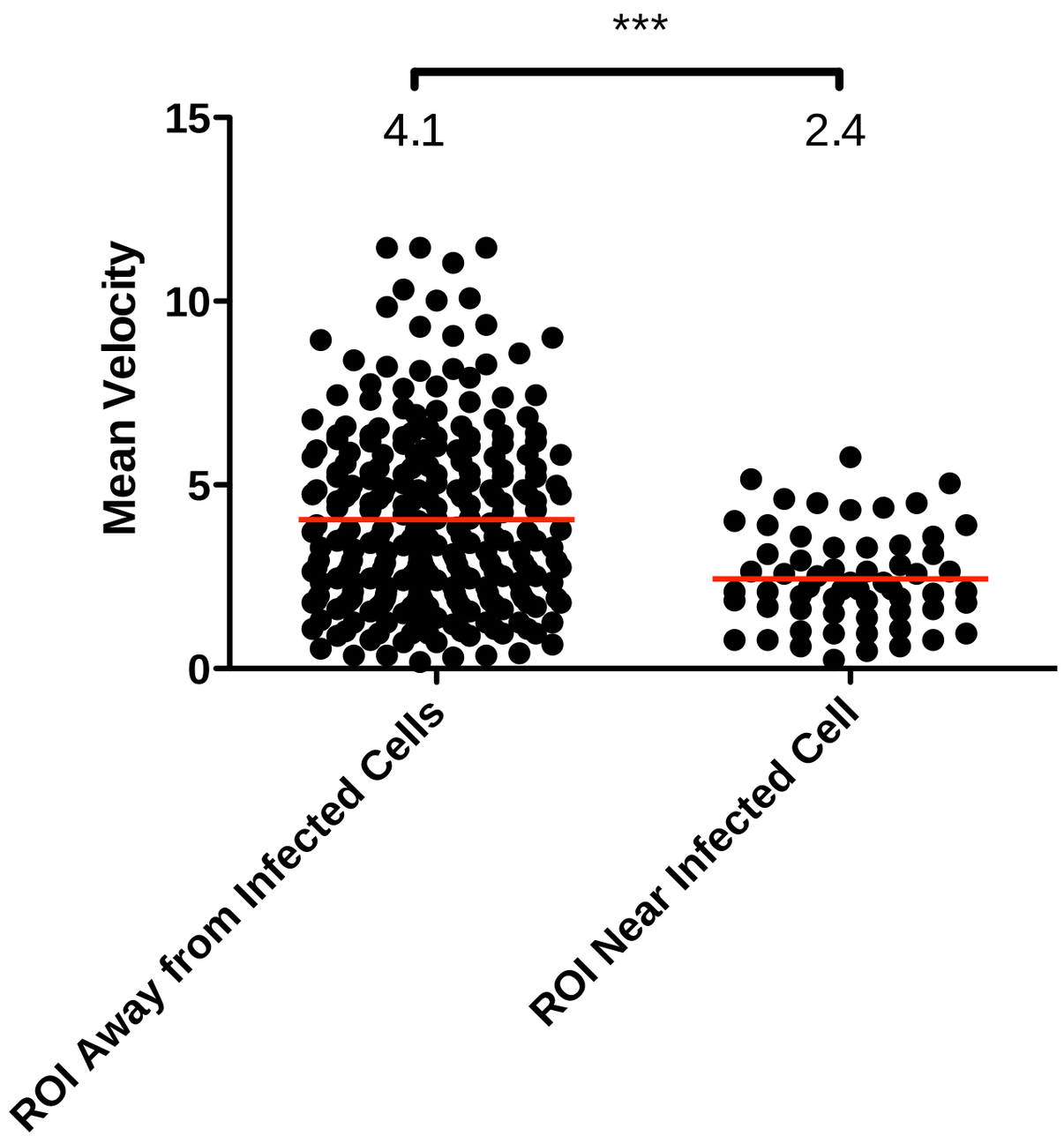






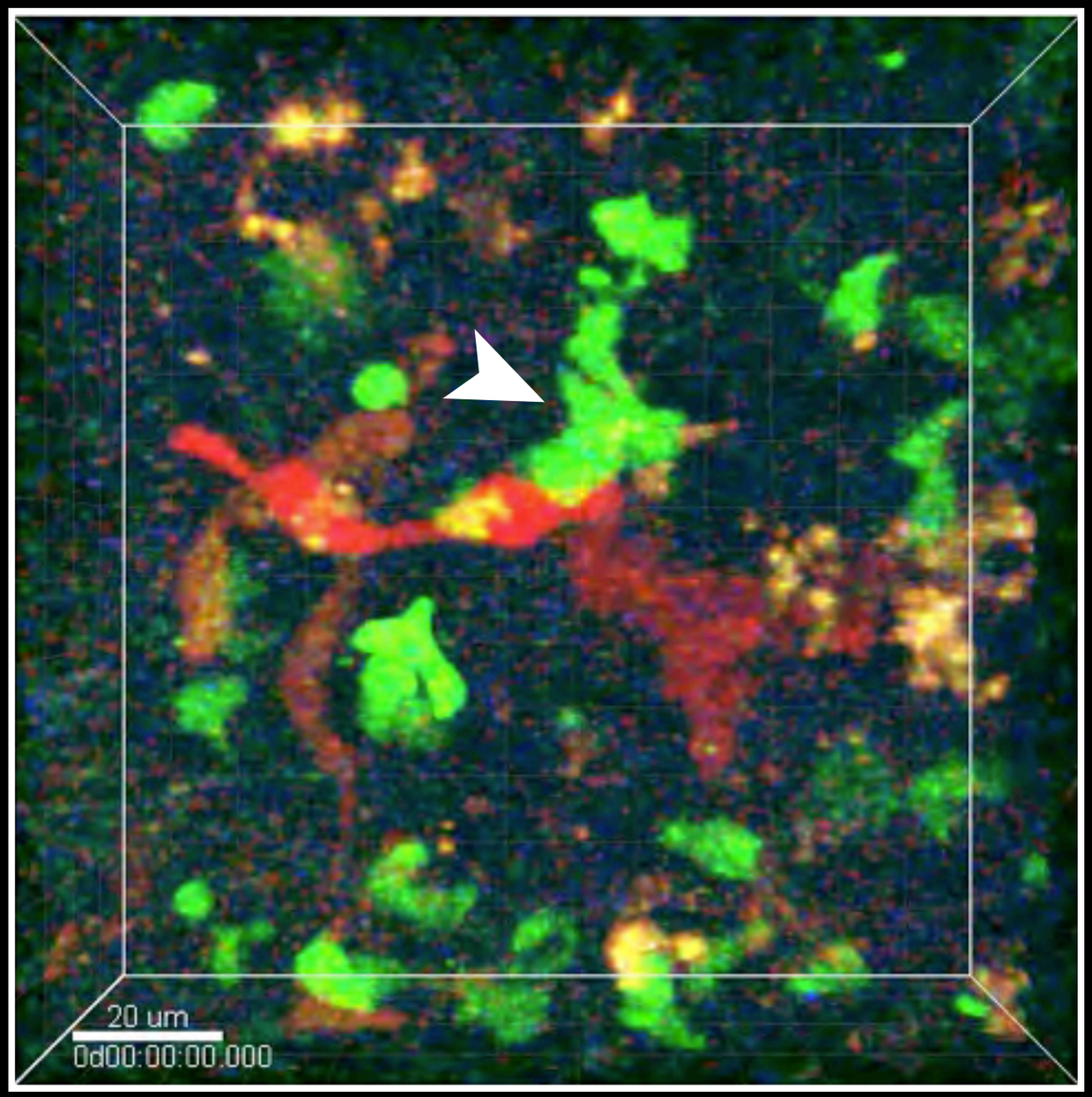
HIV-mCherry CD4 Target Cells SHM Autofluorescence

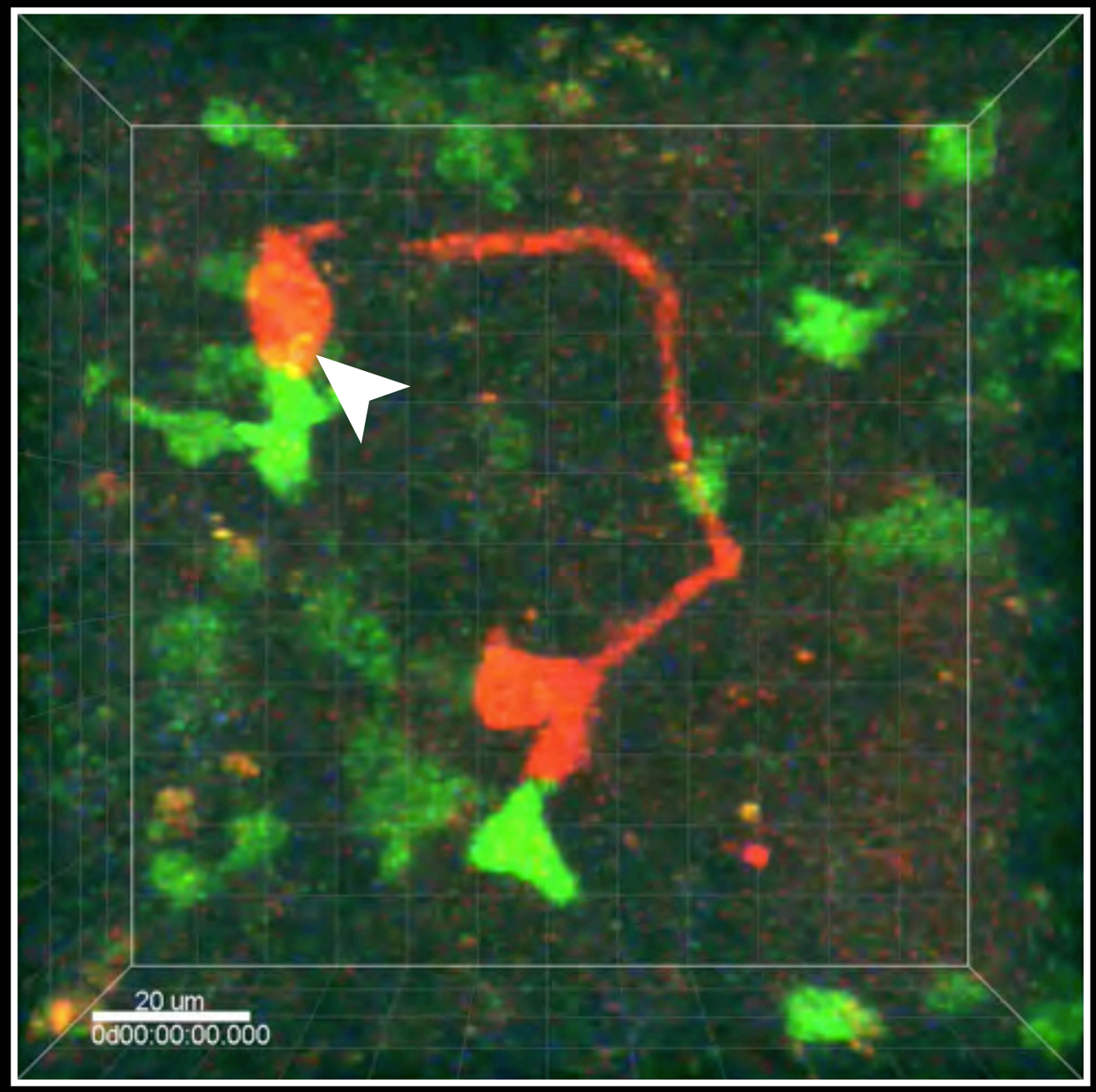
Target cells slow down in the vicinity of HIV infected cells



Elongated infected cells form long-lived contacts with target CD4+ T cells

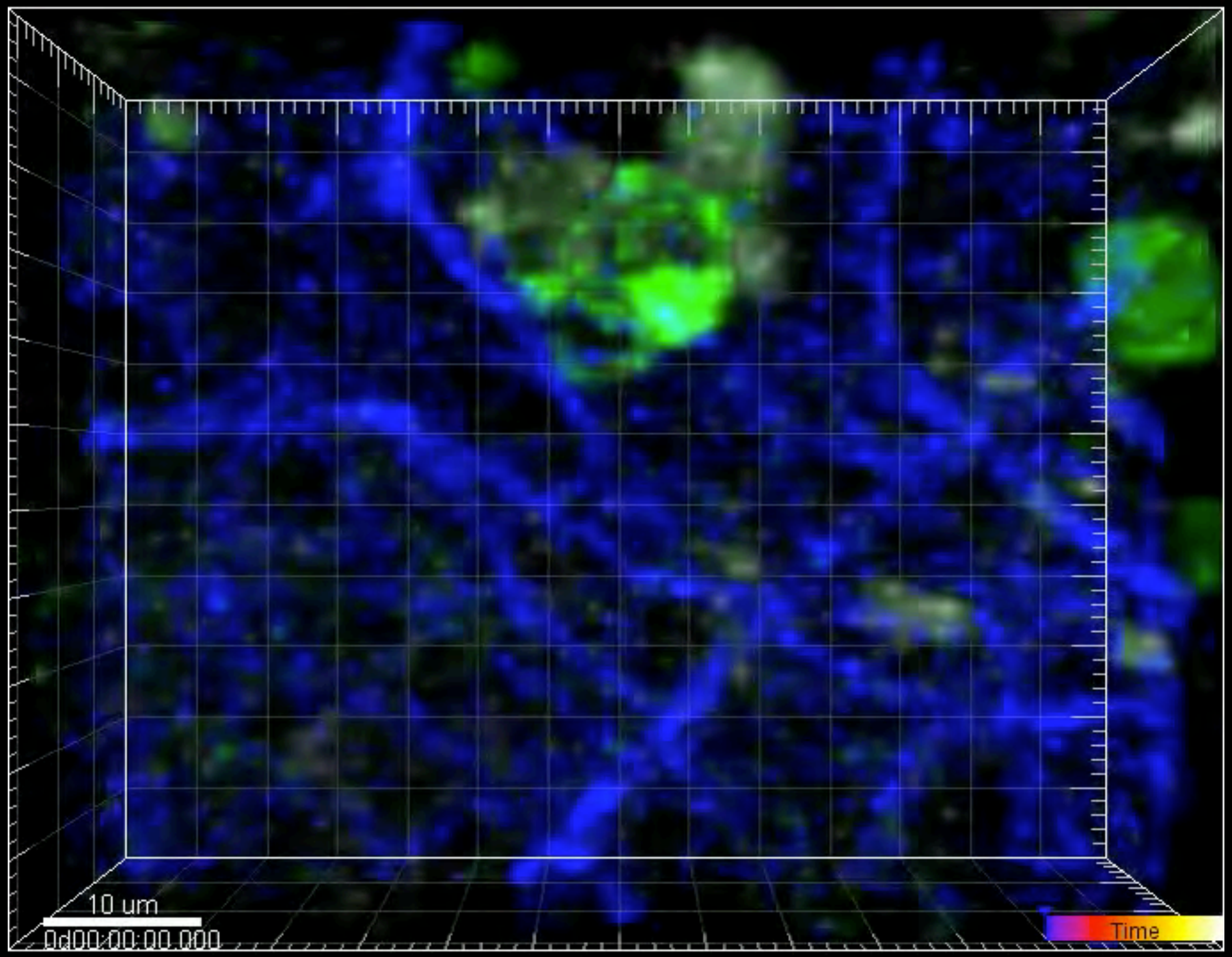
HIV-mCherry CD4 Target Cells SHM Autofluorescence





Law, K Submitted

Putative polarized HIV Gag-iGFP button in live humanized mouse in vivo



M4 Gag-iGFP RHPA 48 hpi

Evidence for cell-cell HIV infection in vivo

- Flow based inheritance assay shows infection of humanized mice with cell-associated HIV transmits multiple HIV copies
- At low infected cell density, genetic clustering is apparent--suggesting spread is local
- Genetic compartmentalization--tethered cells, diminished displacement of infected cells
- Target cells cluster around infected cells.

Cell cell spread and HIV pathogenesis

- Acute HIV spread in huMice maintains multicopy infection—Quasispecies
- Cell-cell interactions spread HIV
- Cell-cell spread promotes escape from antibodies —vaccines should target infected cells

Acknowledgments

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