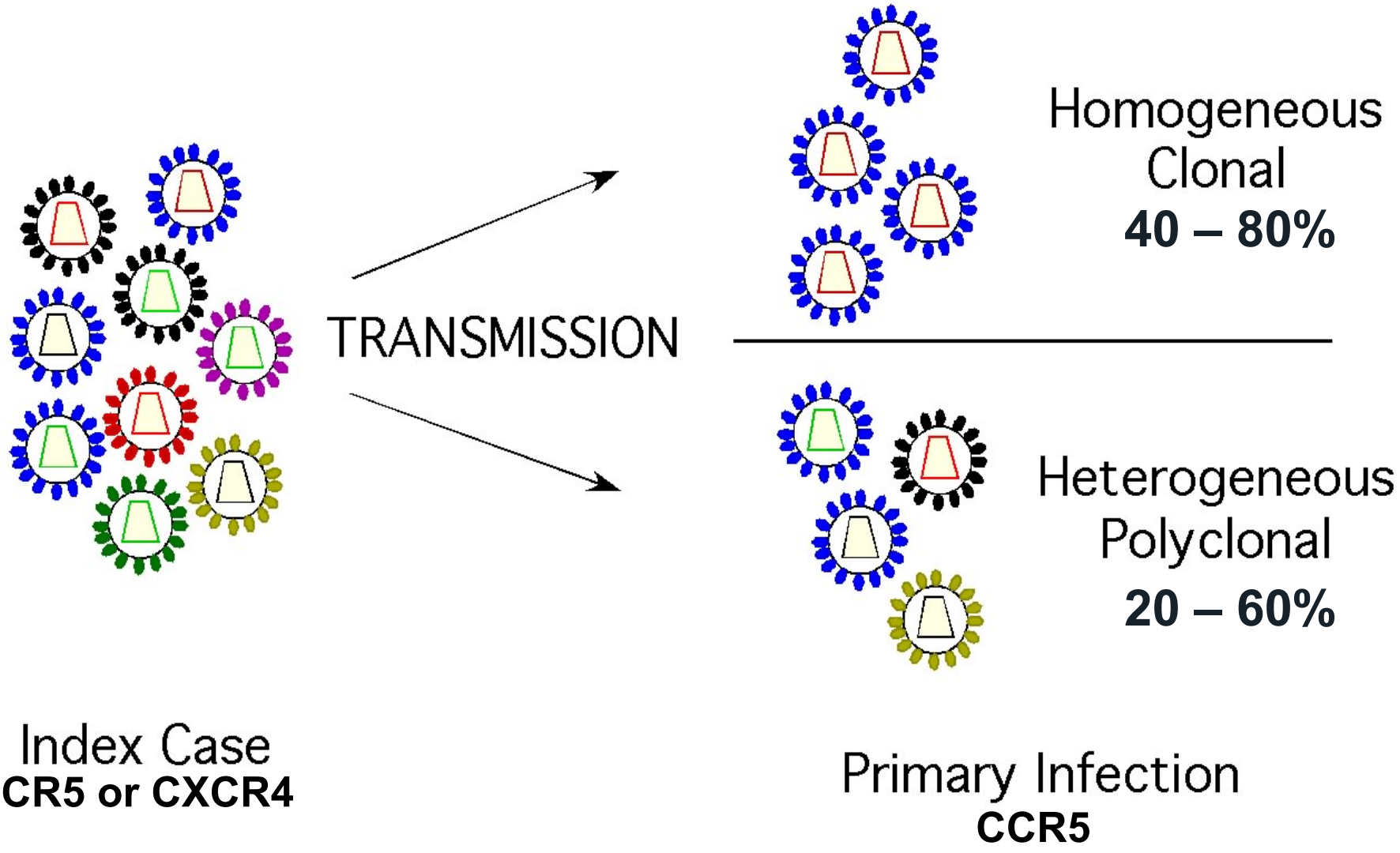
Cell Associated Transmission

Manish Sagar

HIV-1 TRANSMISSION



HIV acquisition versus exposure

Exposure	Relative Risk per 10,000 Exposures
Blood Transfusion	9,000
Needle-sharing	67
Receptive penile-anal intercourse	50
Percutaneous needle stick	30
Receptive penile vaginal intercourse	10
Insertive penile anal intercourse	6.5
Insertive penile vaginal intercourse	5
Receptive penile oral intercourse	1
Insertive penile oral intercourse	0.5

Powers et al., Lancet Infect. Dis. 2008

Ancestral strains are preferentially transmitted

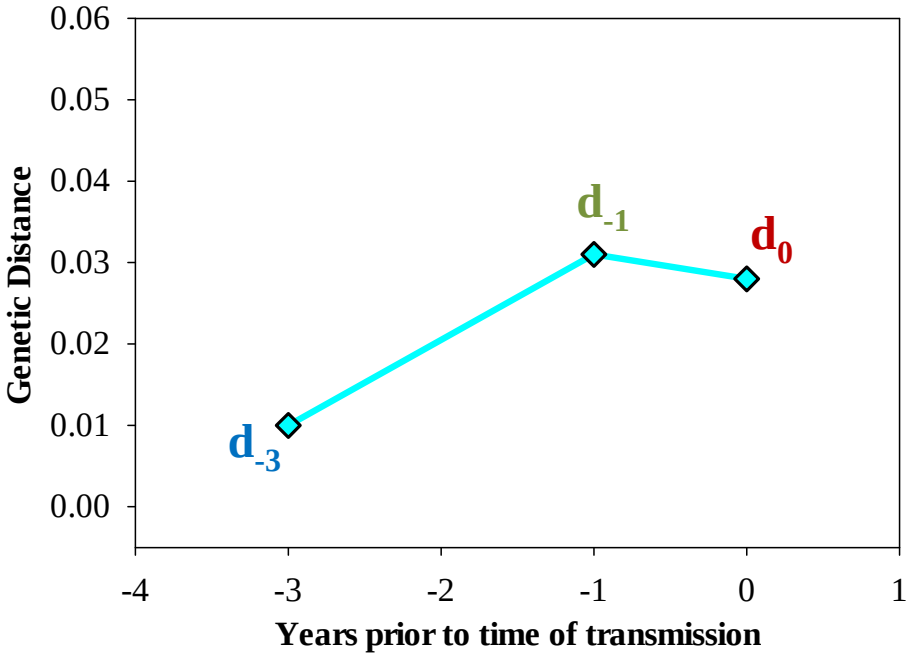
A)

HIV Status by Year

	1994	1995	1996	1997	1998	1999	2000	2001	2002
Male Partner	+	n/a	+	+	+	n/a	n/a	n/a	n/a
Female Partner	n/a	n/a	-	+	n/a	n/a	n/a	n/a	n/a

d_{-3} d_{-1} d_0

B)



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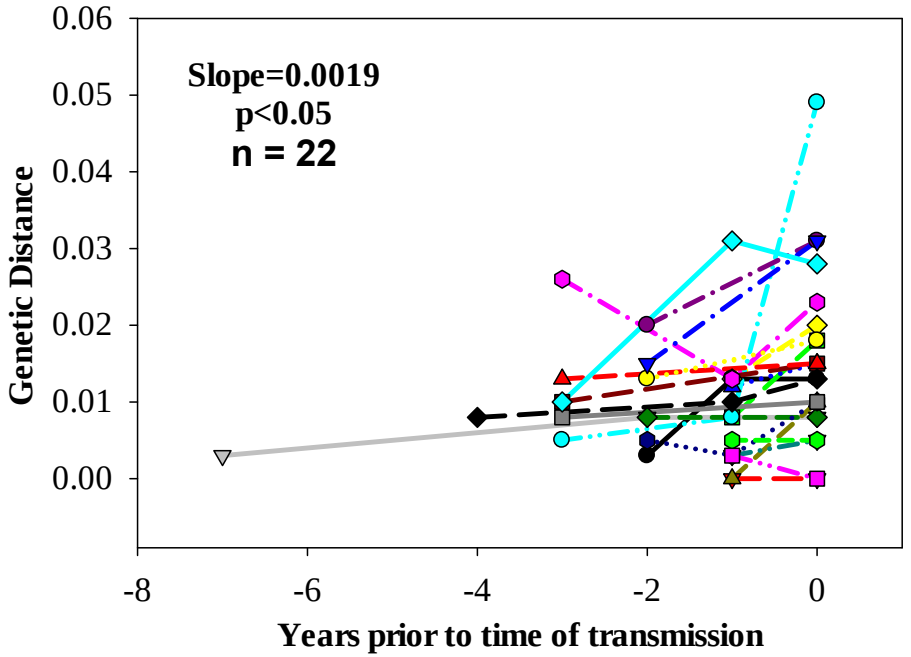
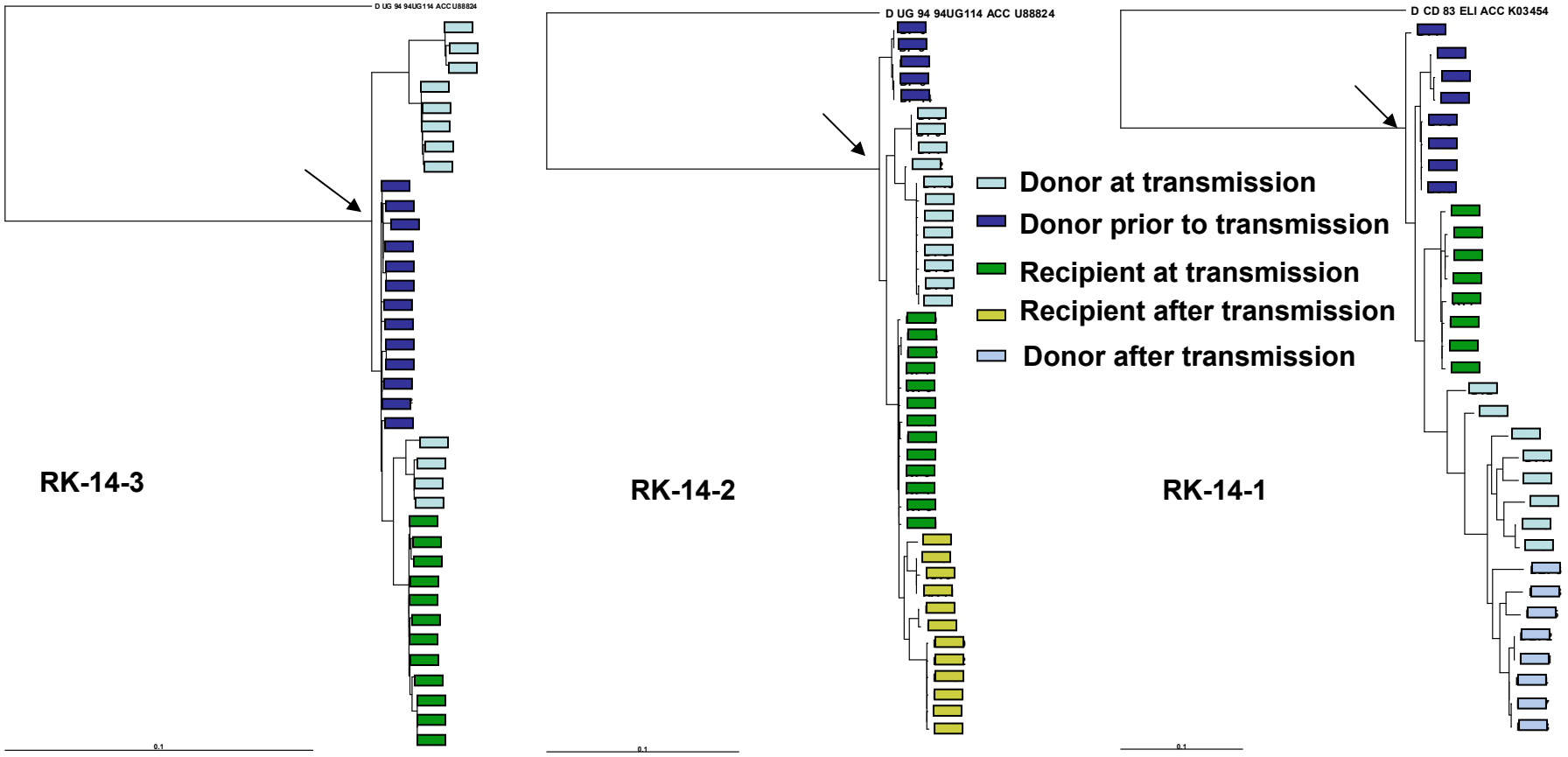


Redd et al., JID 2012

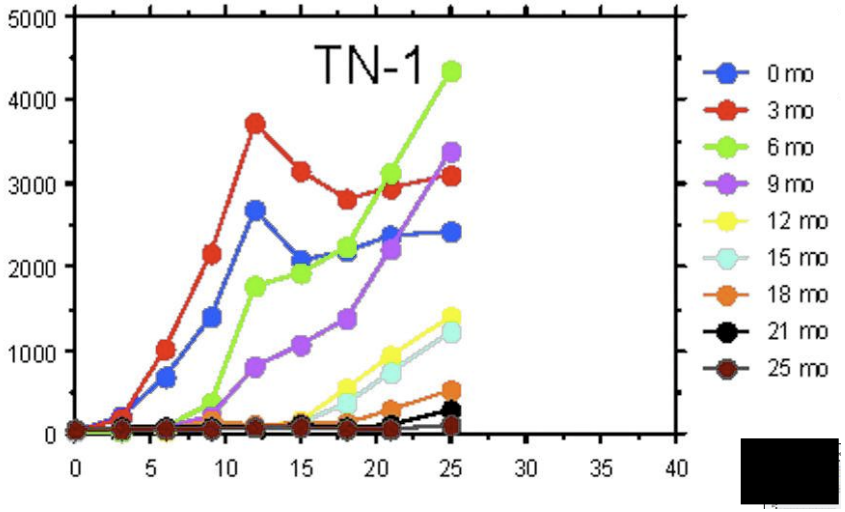
Transmitted viruses are more closely related to previously circulating strains

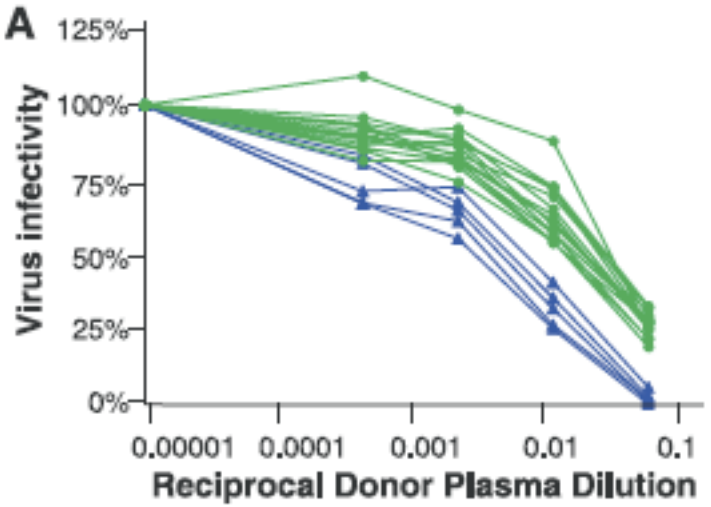


Redd et. al. JID 2012

Previously circulating strains are sensitive to contemporaneous autologous sera

Viruses present in newly infected subjects are highly sensitive to the transmitting partner antibodies



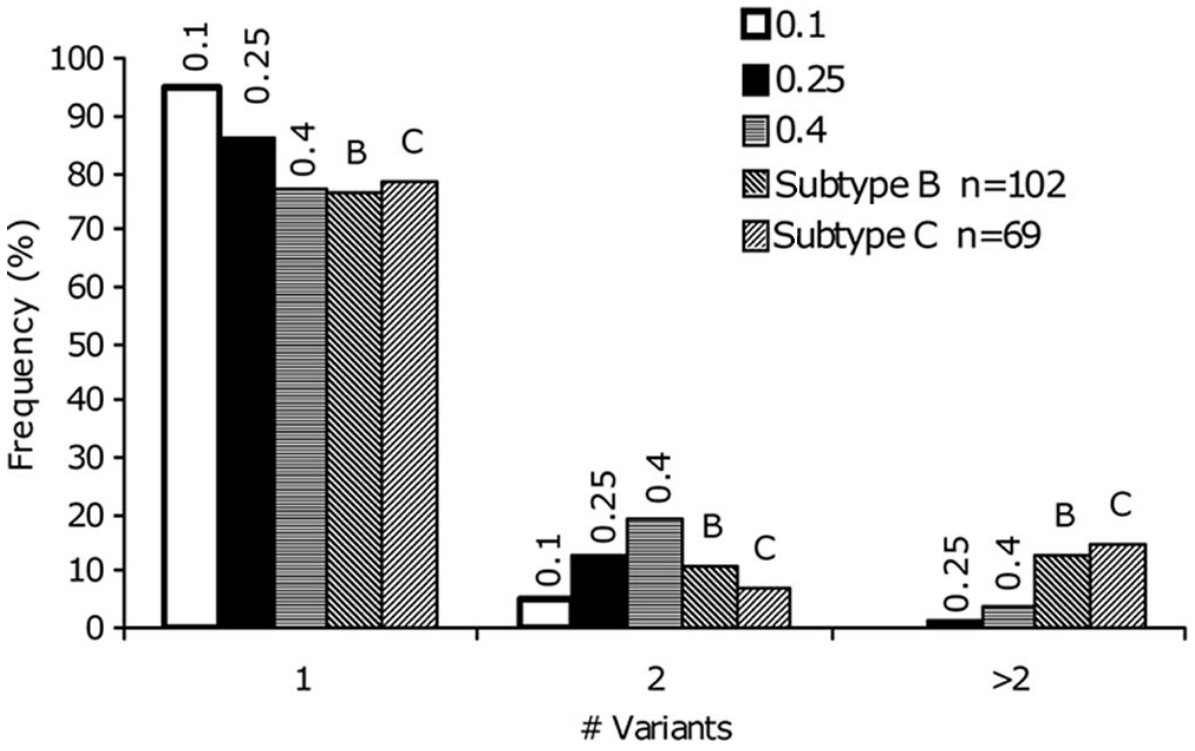


Richman et al., PNAS 2003
Derdeyn et al., Science 2004

How do newly infected individuals acquire strains that are both more closely related to previously circulating donor strains and likely more sensitive to antibodies present in the donor at the time of transmission?

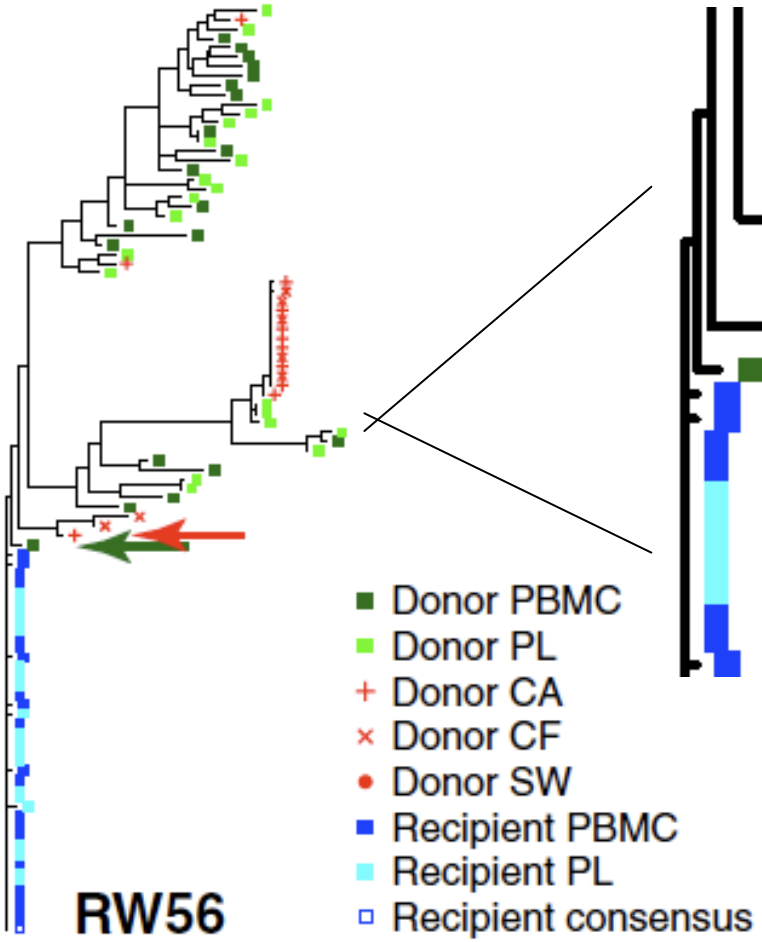
- **Hypothesis: Newly infected individuals may acquire HIV-1 from cell associated as opposed to cell-free virus.**

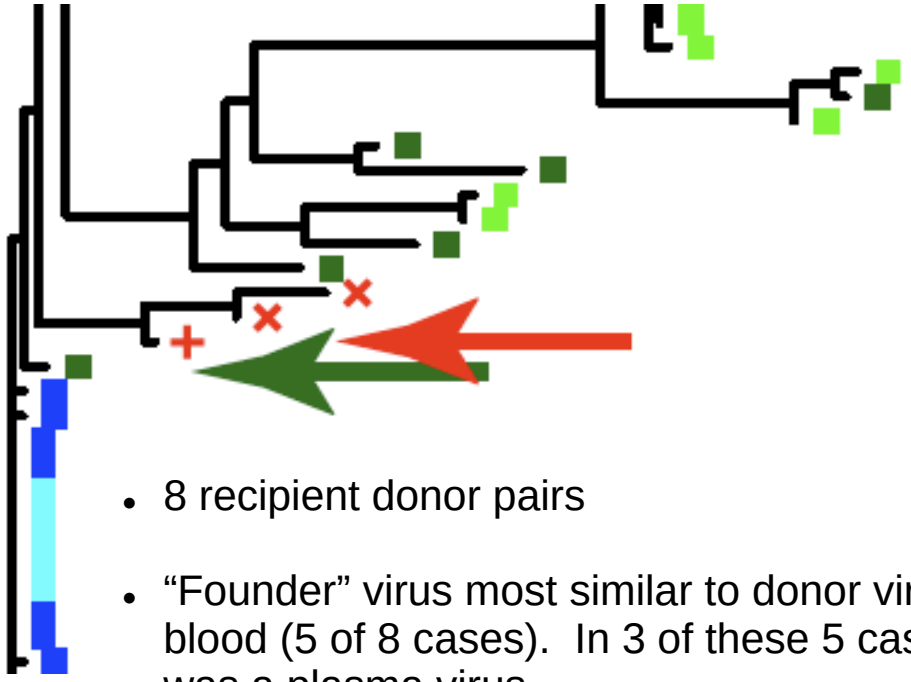
Acquisition of multiple variants are likely linked events



Abrahams et al., JVI 2009

Transmitted viruses

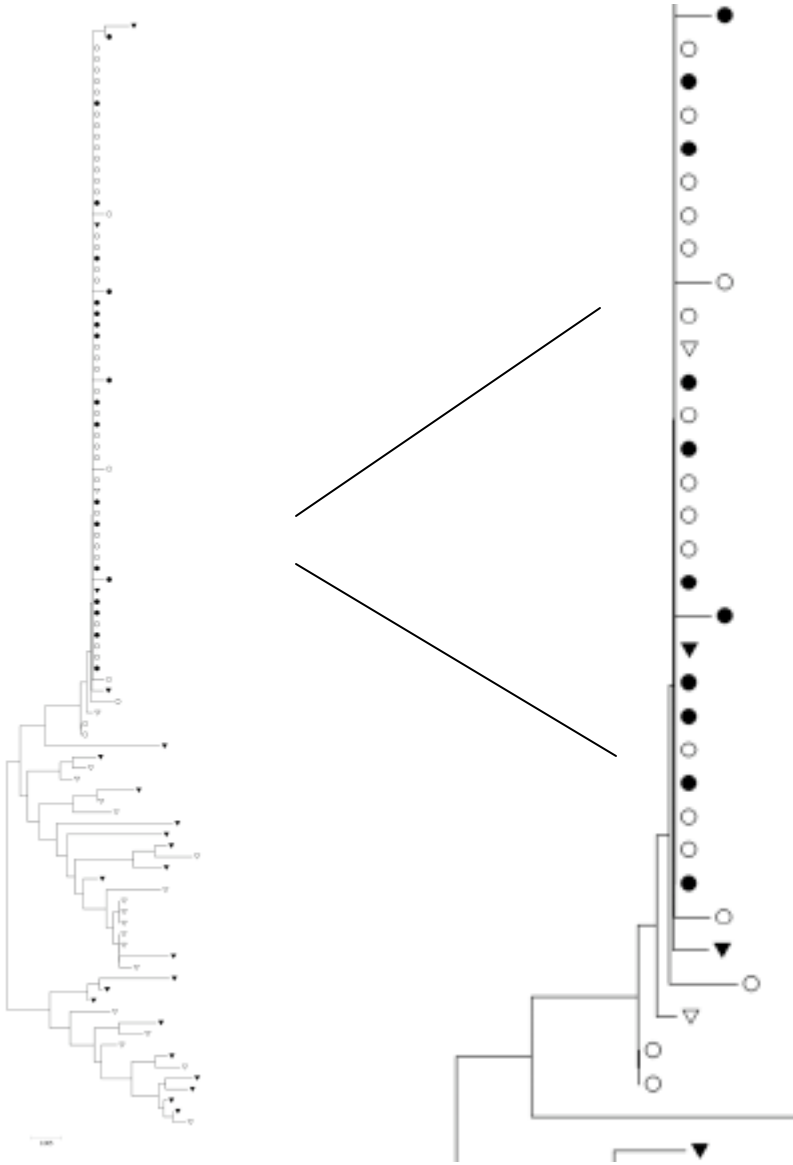




- 8 recipient donor pairs
- "Founder" virus most similar to donor virus found in blood (5 of 8 cases). In 3 of these 5 cases, source was a plasma virus.
- In the remaining cases, "founder" virus most closely related to a genital swab isolate (RNA) and in 1 case cell associated.

Boeras et. al., PNAS 2011

PBMC or plasma derived virus?



● Recipient PBMC

○ Recipient Plasma

▼ Donor PBMC

▽ Donor Plasma

- Sampled 8 recipient donor pairs.
- All newly infected subjects were sampled during acute infections.
- Donor variant most closely related to recipient sequence from plasma in 3/8 cases and/or in PBMC samples in 6/8 cases.

Frange et. al., Plos One 2013

Sequencing Studies

- Analysis of recipient donor pairs shows that plasma or PBMC donor virus is often most closely related to the founder virus in the newly infected partner.
- Lack of compartmentalization between plasma and PBMC donor virus make it difficult to conclusively demonstrate if acquired virus is from the plasma or PBMC.

Phenotypic studies

- Recipient viruses have a unique phenotype that confers fitness for transmission, potentially enhanced cell to cell transmission.

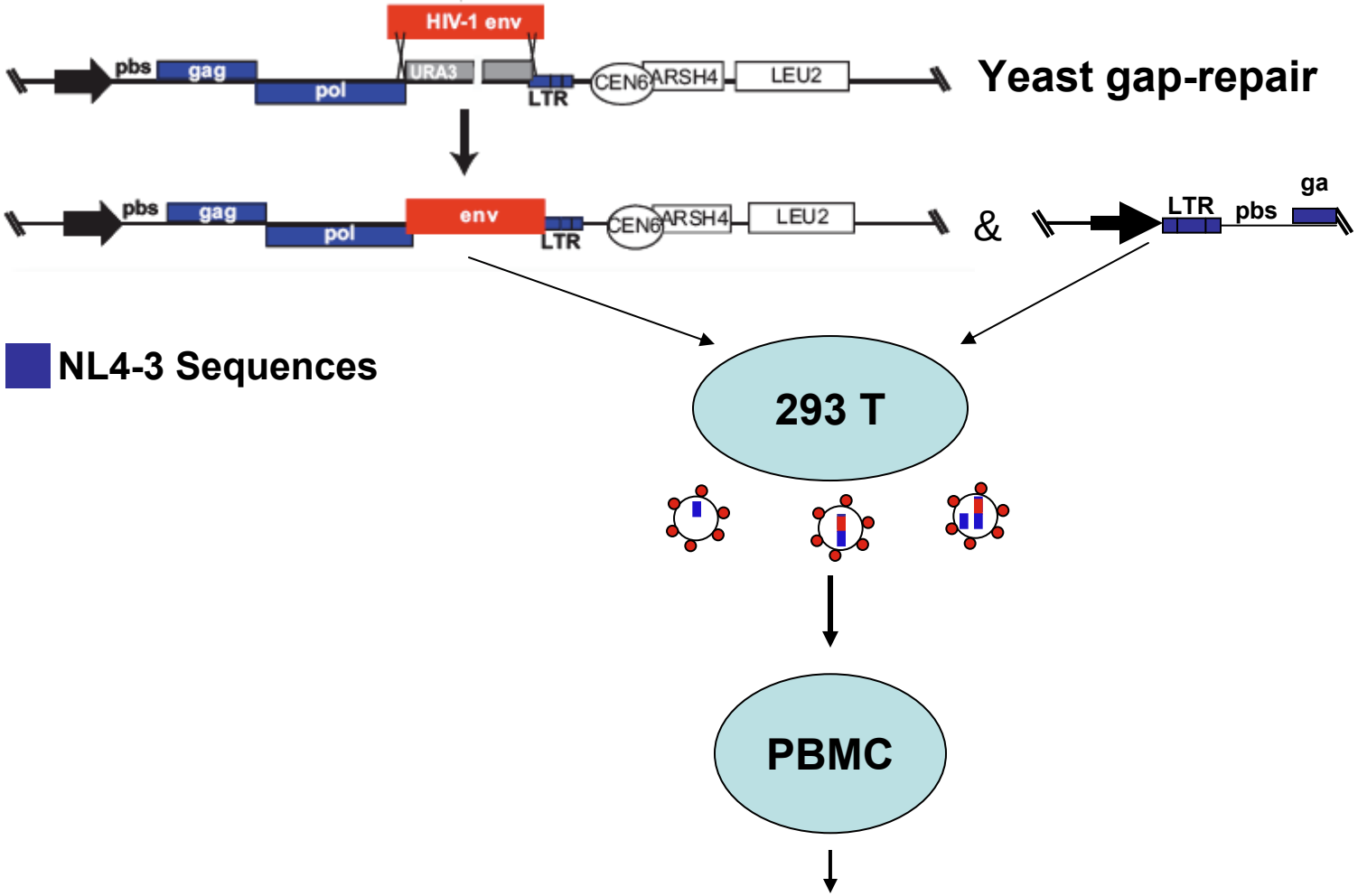
- Compare recipient and transmitter virus phenotypic properties.

Cohort demographics

Couple	Type[1]	Int. Days[2]	Partner Interval[3]	Recipient CCR5[4]	Recipient CXCR4[5]	Recipient Tropism[6]	Transmitter CCR5	Transmitter CXCR4	Transmitter Tropism
HF	FTM	17	3	7.24	<0.1	R5	8.27	<0.1	R5
888	MTF	74	19	10.39	<0.1	R5	11.91	<0.1	R5
890	MTF	138	12	3.79	<0.1	R5	2.27	<0.1	R5
394	MTF	93	2	7.79	<0.1	R5	10.85	<0.1	R5
927	MTF	324	46	12.55	<0.1	R5	13.37	<0.1	R5
2769	MTF	149	46	5.69	<0.1	R5	5.34	0.65	R5/X4
2810	MTF	161	23	5.49	<0.1	R5	6.12	<0.1	R5
SR-5	MTF	17	0	12.62	<0.1	R5	9.72	<0.1	R5
SR-20	MTF	91	34	6.70	<0.1	R5	7.24	<0.1	R5

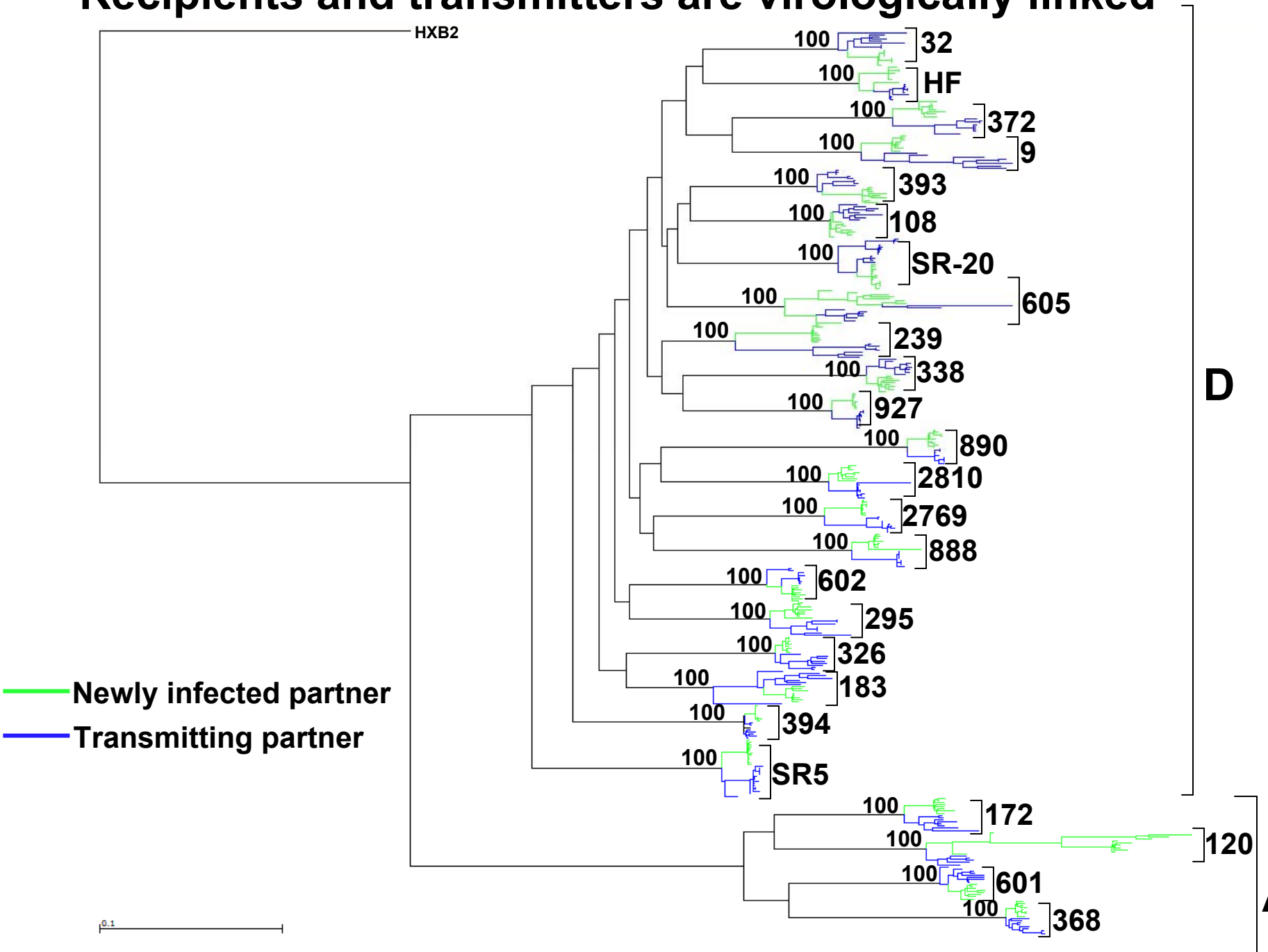
Replication Competent Recombinant Viruses

- **Full length envelope sequences amplified from each sample.**
  Median 4 (range 4 – 8) independent PCRs

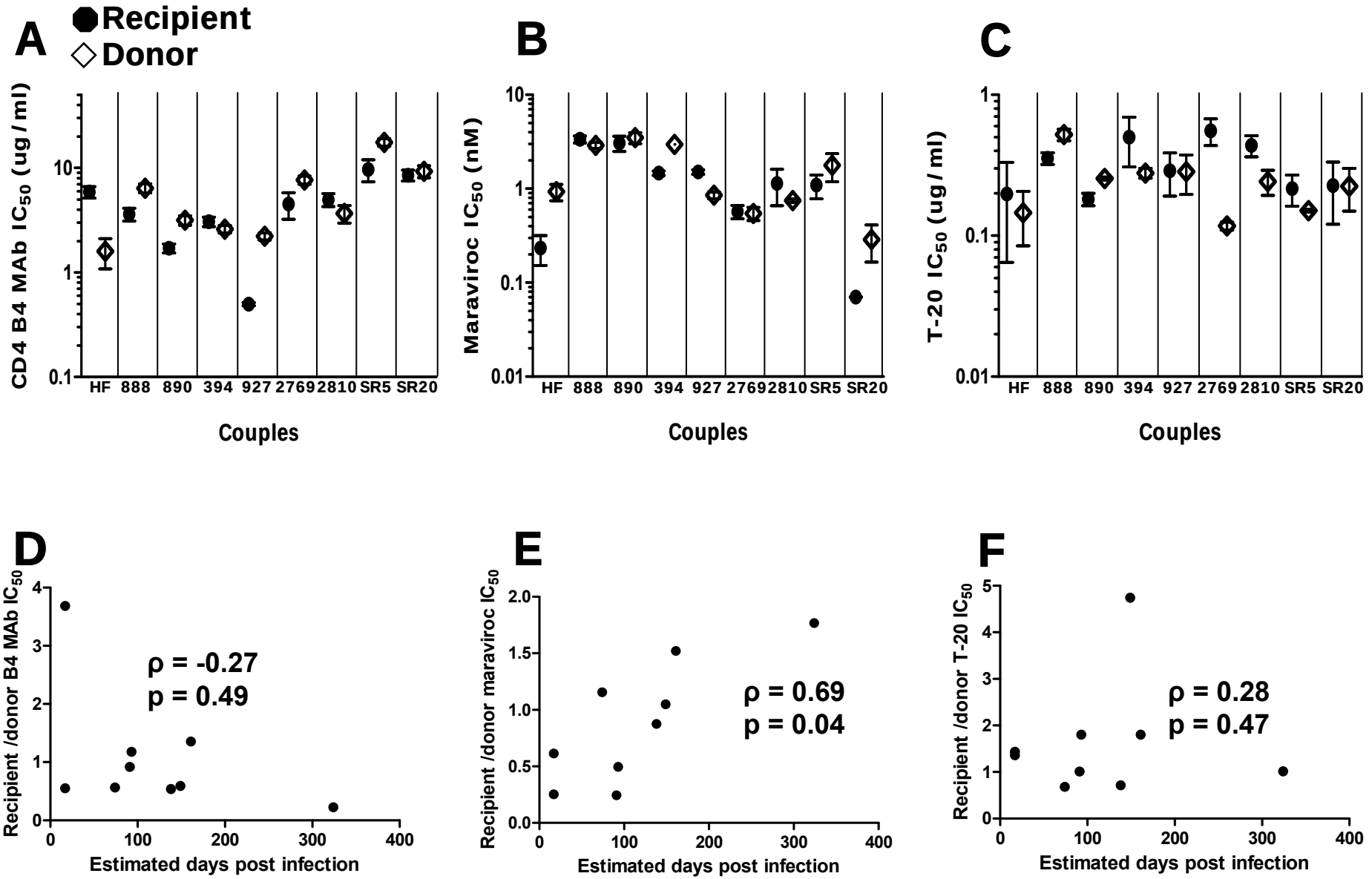


Etemad et al., JVI 2009
Dudley et al., Biotechniques 2009

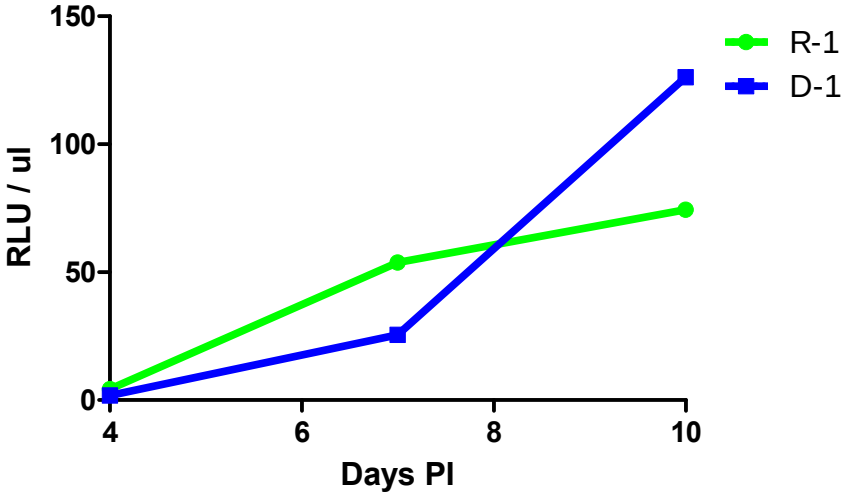
Recipients and transmitters are virologically linked

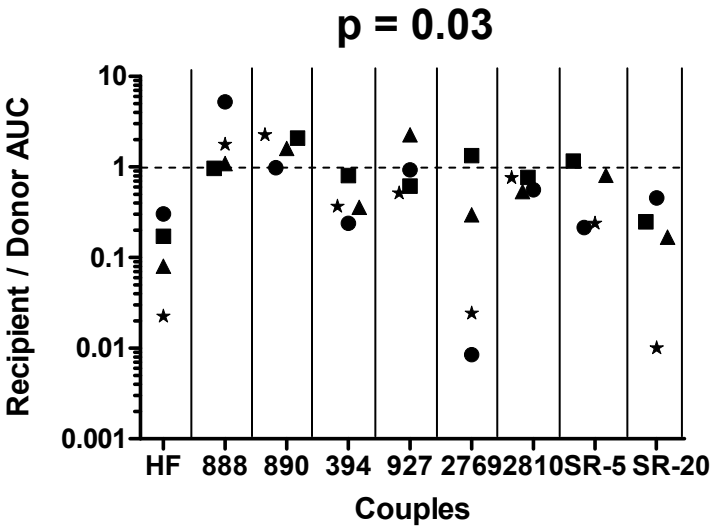


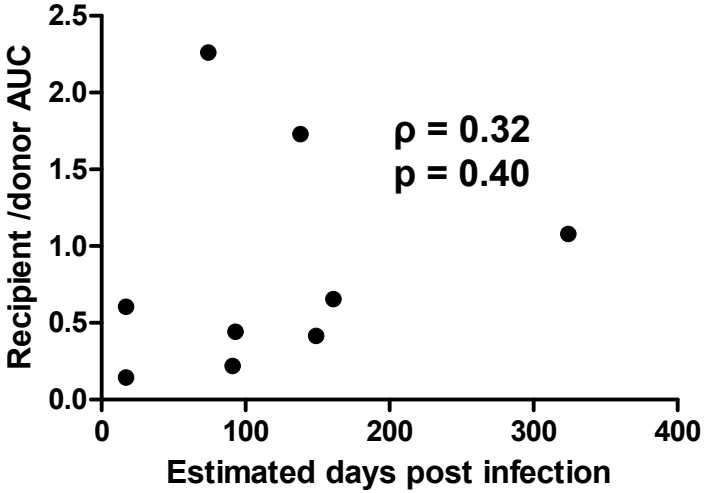
Sensitivity of receptor and fusion inhibitors



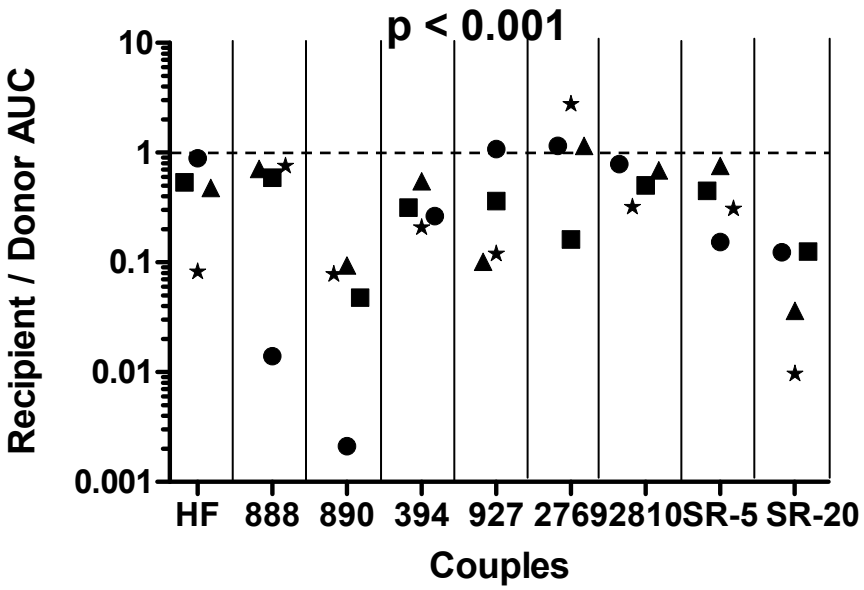
Replication in CD4+ T cells

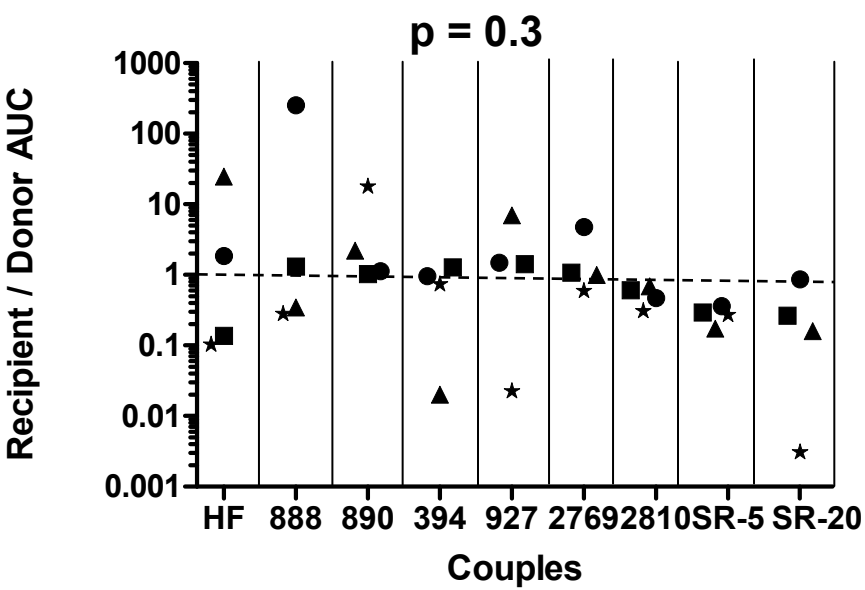


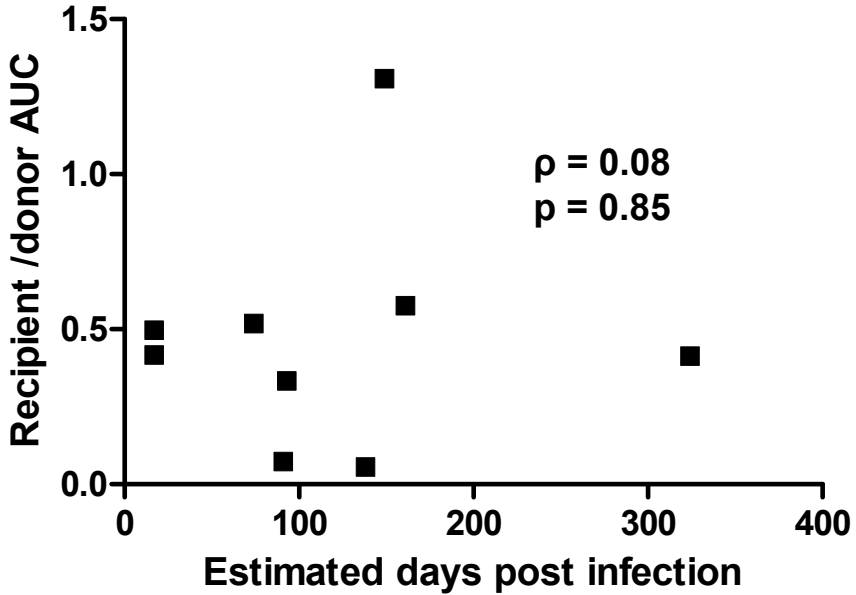


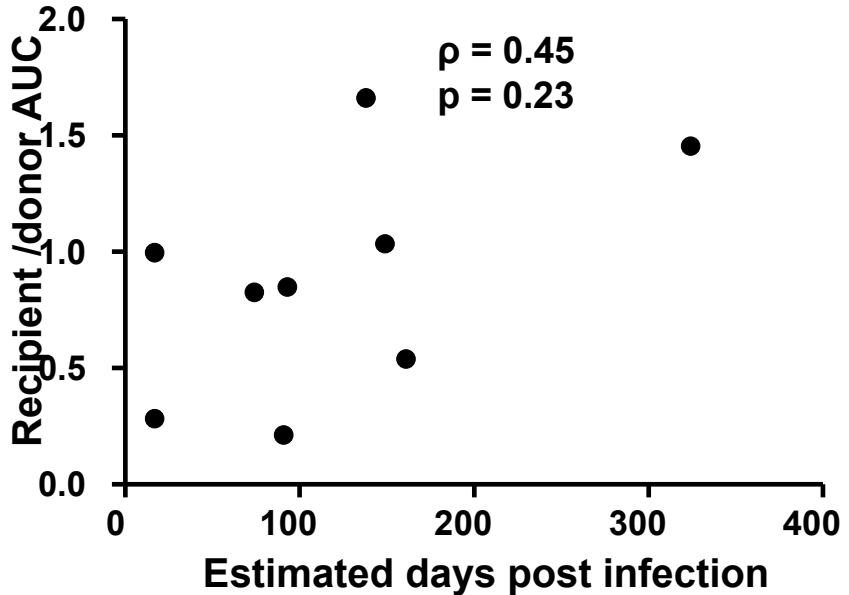


Replication in MDDC-CD4+ T cell cocultures

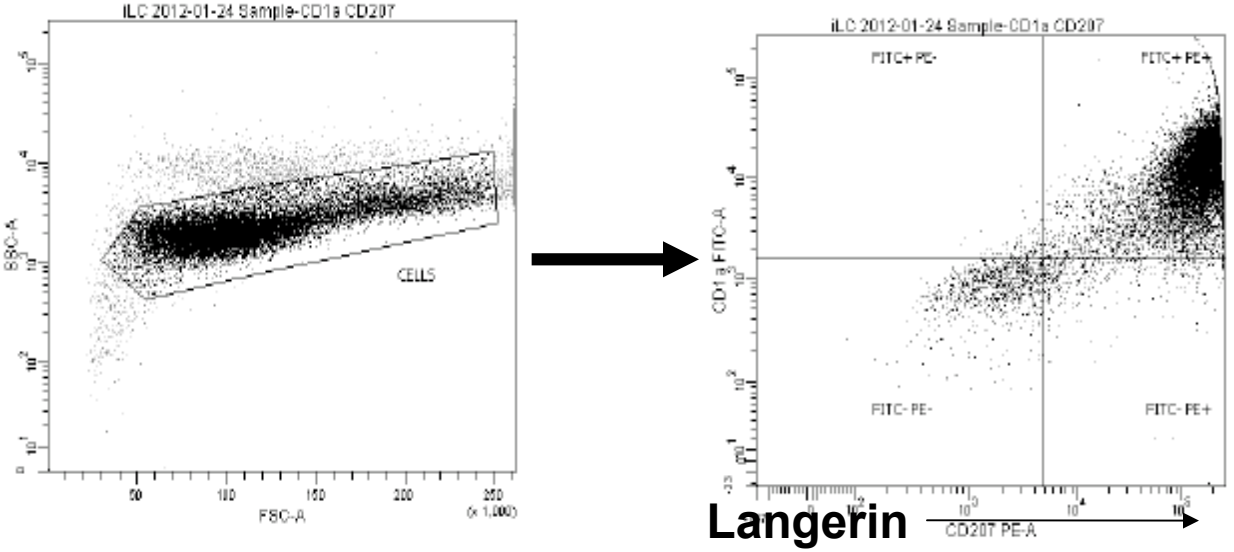


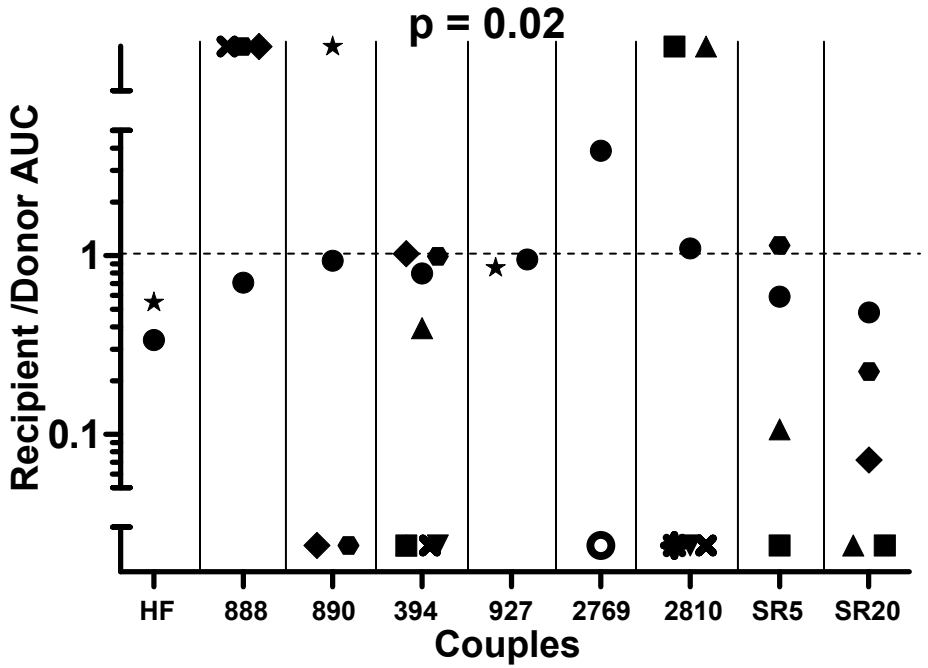


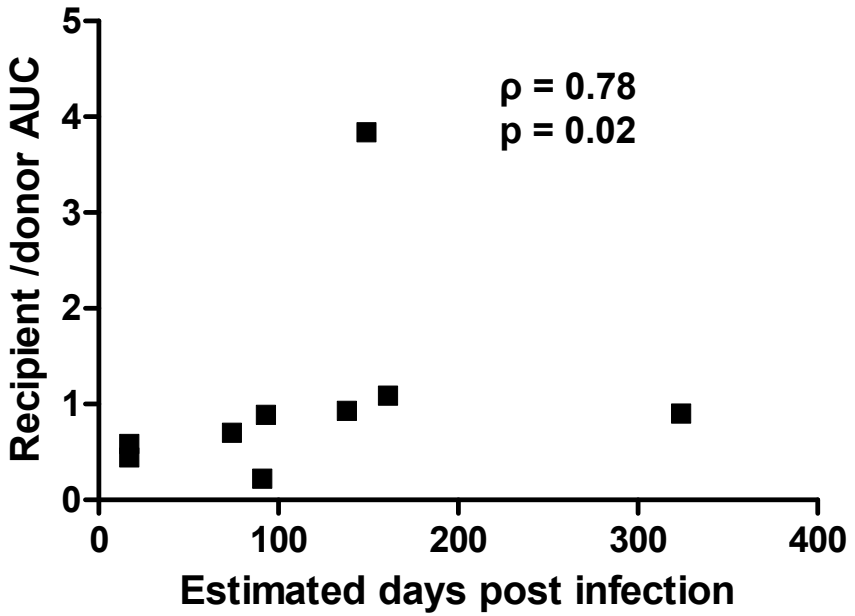


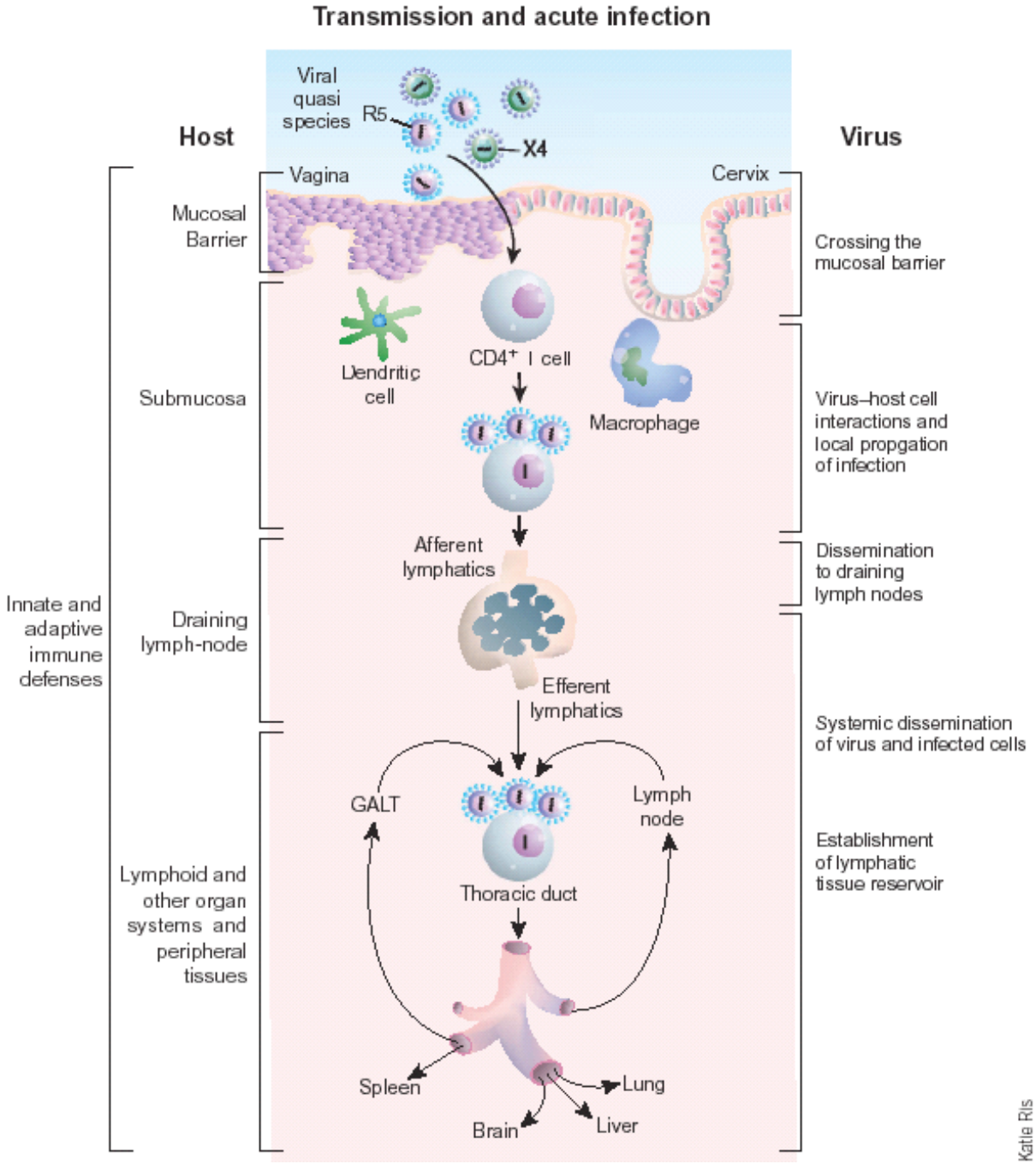


Replication in LC-CD4+ T cell cocultures



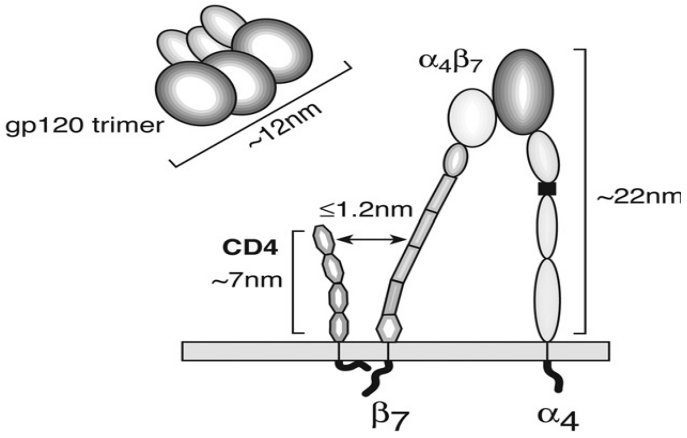






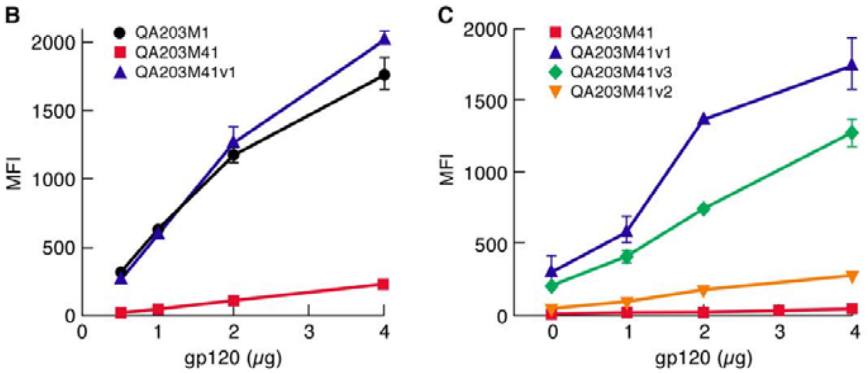
Pope et al., Nature Medicine 2003

Gut homing receptor, α4β7



V1 stem

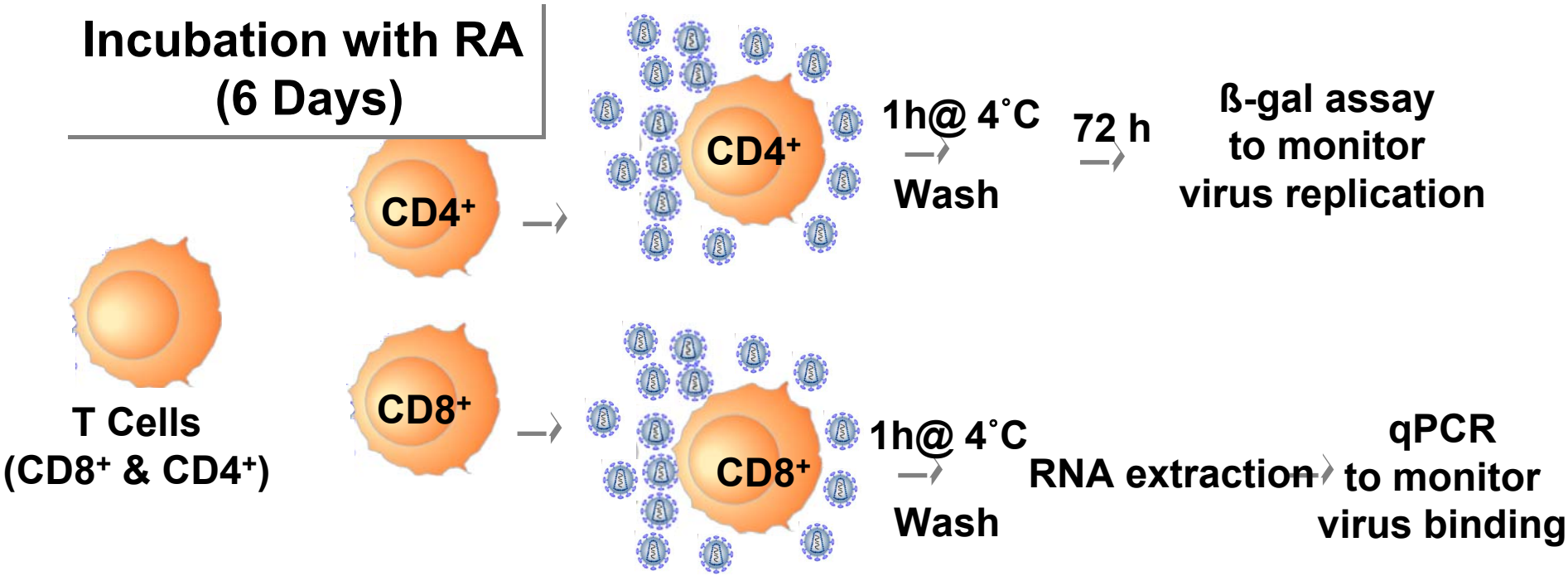
	isolate
CVTLECSNVNVTNNVT----------NDMGEEIKNCSFNMTTELRDKKQKTYSLFYKLDVVPFNNNRSQYRLINC	QA203M1 month 1
CVTLECSNVNVTNNVNVTNNVNVTNNVTNDMTGEIKNCSFNMTTELRDKKQKVYSLFYKLDVVPVNNNSSQYRLINC	QA203M41 month 41
CVTLECSNVNVTNNVNVTNNVQVTNQVTNDMTGEIKNCSFNMTTELRDKKQKVYSLFYKLDVVPVNNNSSQYRLINC	QA203M41 variant 1
CVTLECSNVNVTNNVNVTNNVNVTNNVTNDMTGEIKNCSFNMTTELRDKKQKVYSLFYKLDVVPVNQQSSQYRLINC	QA203M41 variant 2
CVTLECSNVNVTNNVNVTNNVQVTNQVTNDMTGEIKNCSFNMTTELRDKKQKVYSLFYKLDVVPVNQQSSQYRLINC	QA203M41 variant 3



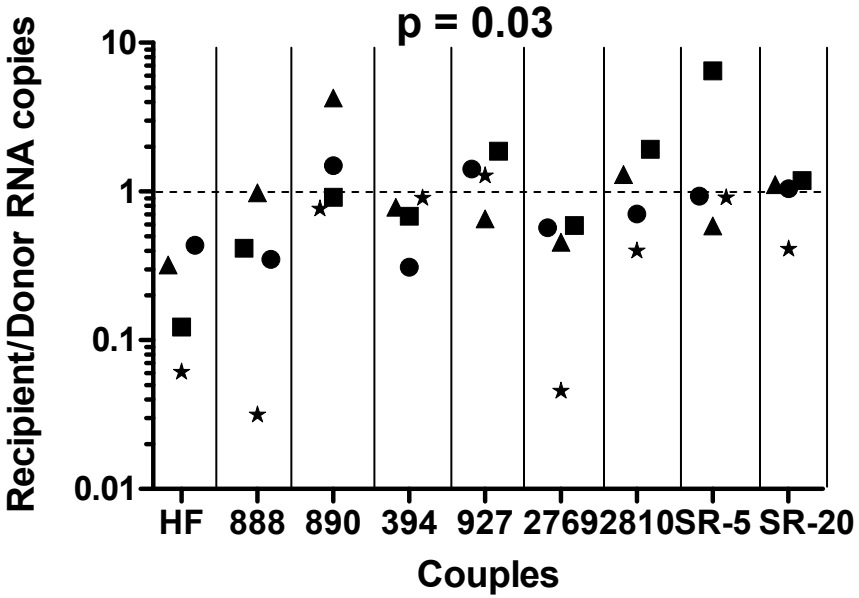
Cicala et al., PNAS 2009

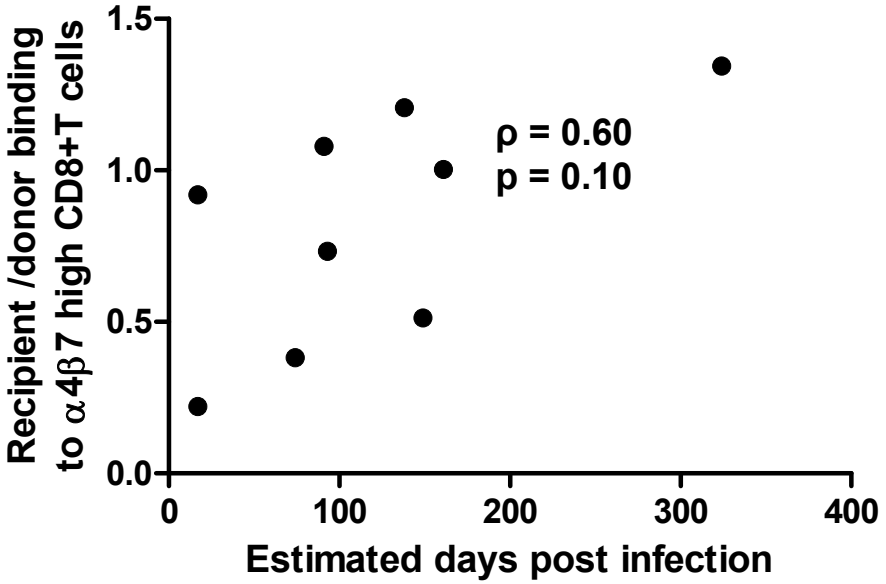
Nawaz et al., Plos Pathogens 2011

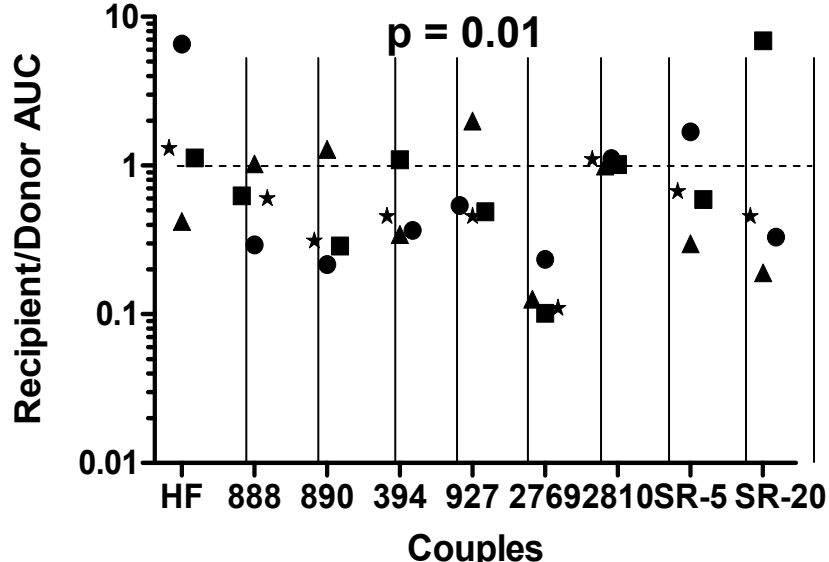
Gut homing receptor, α4β7

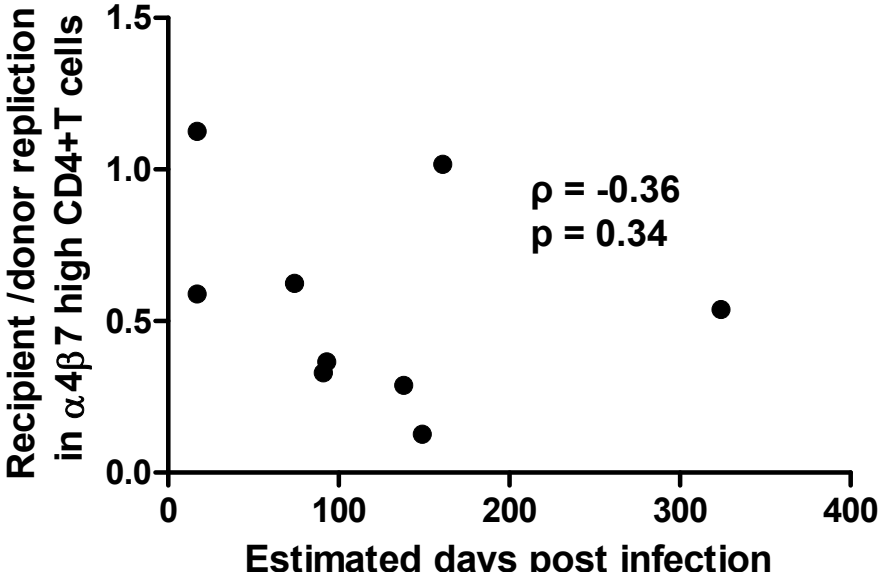


α4β7 usage


p = 0.03
Recipient/Donor RNA copies
10
1
0.1
0.01
HF
888
890
394
927
27692810
SR-5
SR-20
Couples


Recipient /donor binding to α4β7 high CD8+T cells
1.5
1.0
0.5
0.0
ρ = 0.60
p = 0.10
0
100
200
300
400
Estimated days post infection


p = 0.01
Recipient/Donor AUC
10
1
0.1
0.01
HF
888
890
394
927
27692810
SR-5
SR-20
Couples

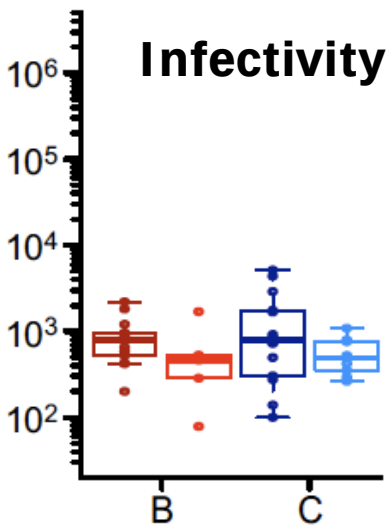

Recipient /donor repliction in α4β7 high CD4+T cells
1.5
1.0
0.5
0.0
ρ = -0.36
p = 0.34
0
100
200
300
400
Estimated days post infection

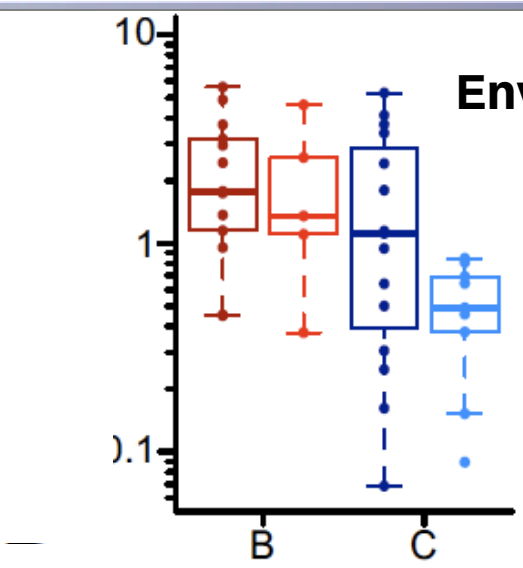
Transmitted founder versus chronic control viruses

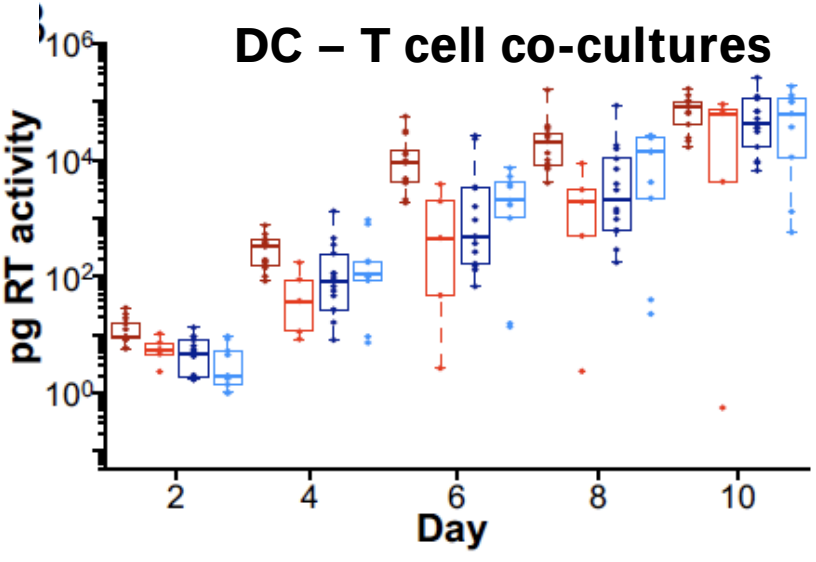
- Full length molecular clones of phylogenetically estimated T/F viruses
- 13 Subtype B T/F versus 5 CC from 4 subjects
- 14 Subtype C T/F versus 9 CC from different subjects
- All T/F were unrelated to CC

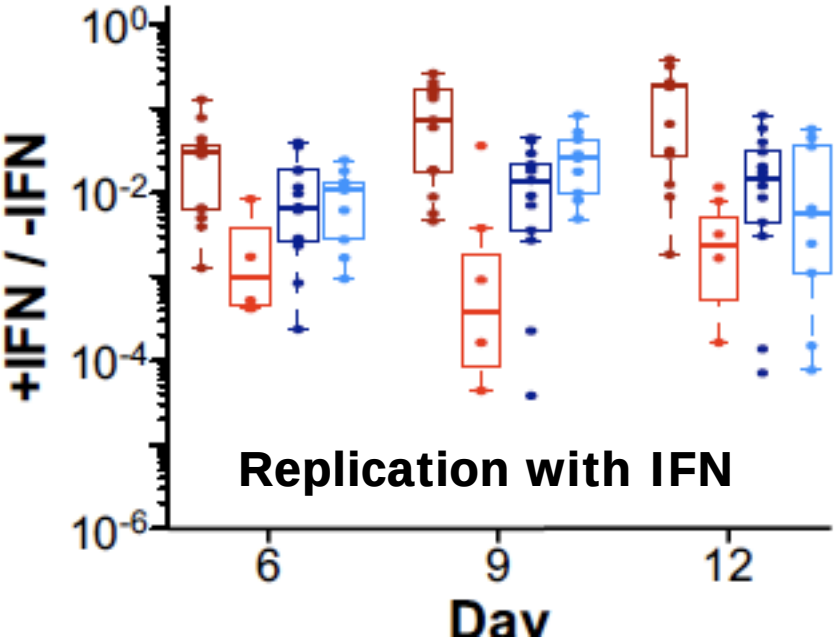
Parrish et al., PNAS 2013

T/F more infectious, higher env content, enhanced DC-T cell transmission and greater replication in presence of IFN-α









Early/Transmitted versus Chronic/Donor

Properties	Couples	T/F vs CC
Gene	Envelope	Full-length molecular clone
Sampling	Seronegative to 1 year	Phylogenetically estimated T/F
Comparison	Swarm present in transmitter	Unrelated chronic phase virus
Enhanced Infectivity	Transmitter	T/F
Higher replication in DC-T cells	Transmitter	T/F
α4β7 usage	Transmitter	No difference
CD4 usage	No significant difference	
CCR5 usage	No significant difference	
Fusion	No significant difference	

Conclusions

- Newly infected subjects may acquire cell associated virus.
- Cell associated and cell free virus are rarely compartmentalized making it difficult for sequence studies to determine origin of transmitted virus.
- Couple studies suggest that transmitter as compared to recipient swarm are more efficient in DC/LC T cell transfer.
- Because we did not examine T/F, it is possible that viruses with a transmission phenotype, such as cell associated replication, are selected against early after acquisition. In this case, variants with enhanced cell to cell replication must be enriched during the chronic phase of disease.
- Cell associated transmission may require different preventative strategies.

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