Comparison of latent HIV-1 reactivation in multiple cell model systems

Celsa A. Spina, Jenny Anderson, Nancie M. Archin, Alberto Bosque, Jonathan Chan, Marylinda Famiglietti, Warner C. Greene, Angela Kashuba, Sharon R. Lewin, David M. Margolis, Matthew Mau, Debbie Ruelas, Suha Saleh, Kotaro Shirakawa, Robert F. Siliciano, Akul Singhania, Paula C. Soto, Valeri H. Terry, Eric Verdin, Christopher Woelk, Stacey Wooden, Sifei Xing, and Vicente Planelles

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Johns Hopkins University School of Medic

		TCR		PKC				Cytok		HDACi			PTEFb	Ca++
		Maximal Level of HIV Response with Different Stimuli												
T Cell Model	**Group**	aCD3/28	PHA-M	PMA	PMA+Io	Prostratin	Bryostatin	TNFα	IL-7 + IL-2	SAHA	MRK-1	MRK-11	HMBA	Ionomycin
Patient CD4 Cells	Margolis													
Quiescent CD4	Greene													
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J-Lat 5A8	Verdin													
J-Lat 6.3	"													
J-Lat 8.4	"													
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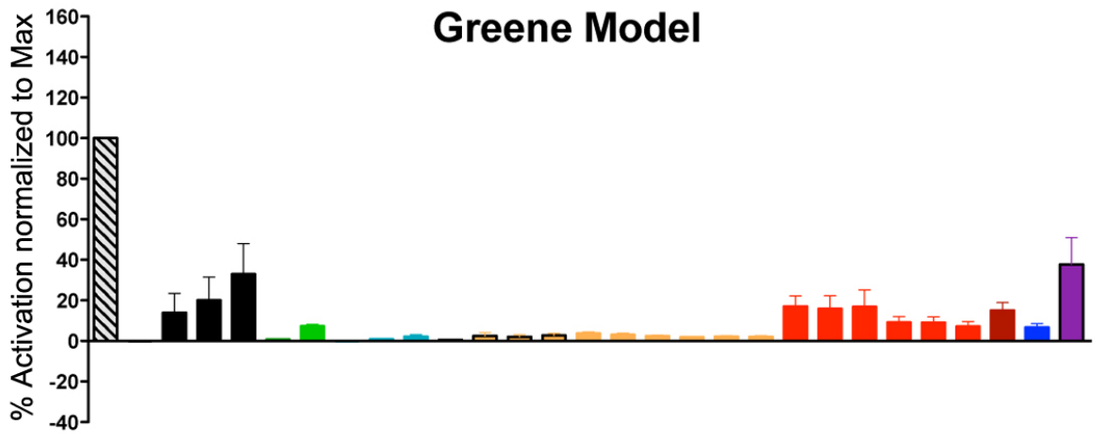

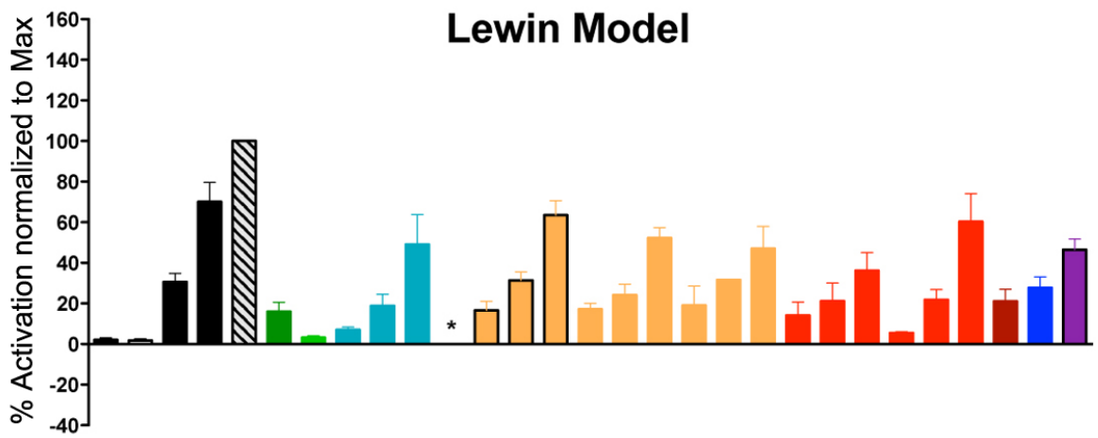

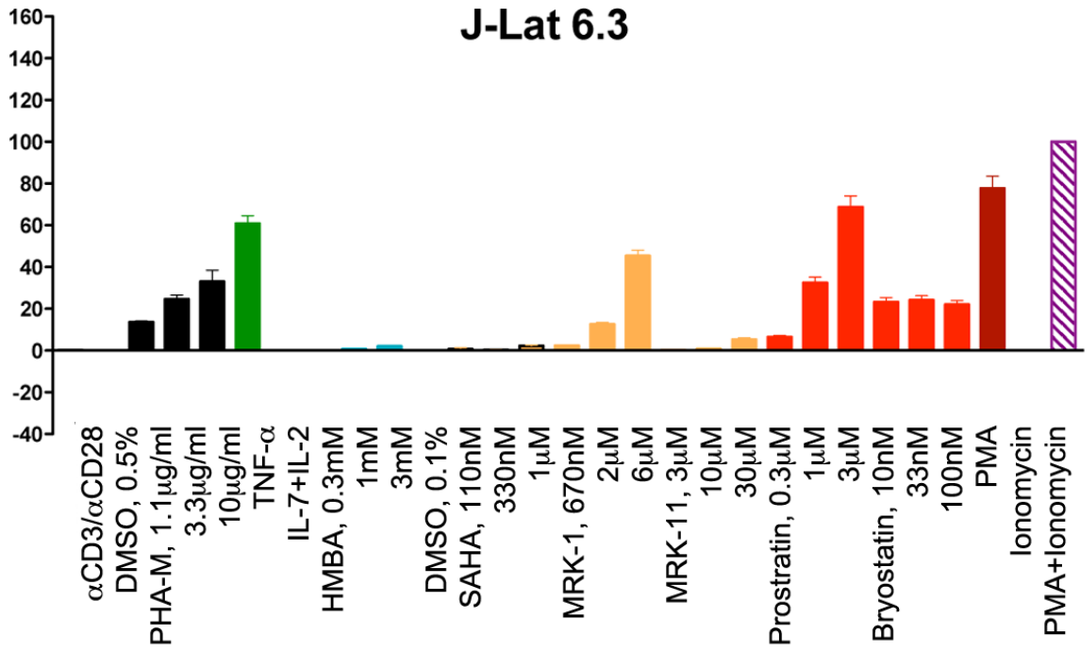

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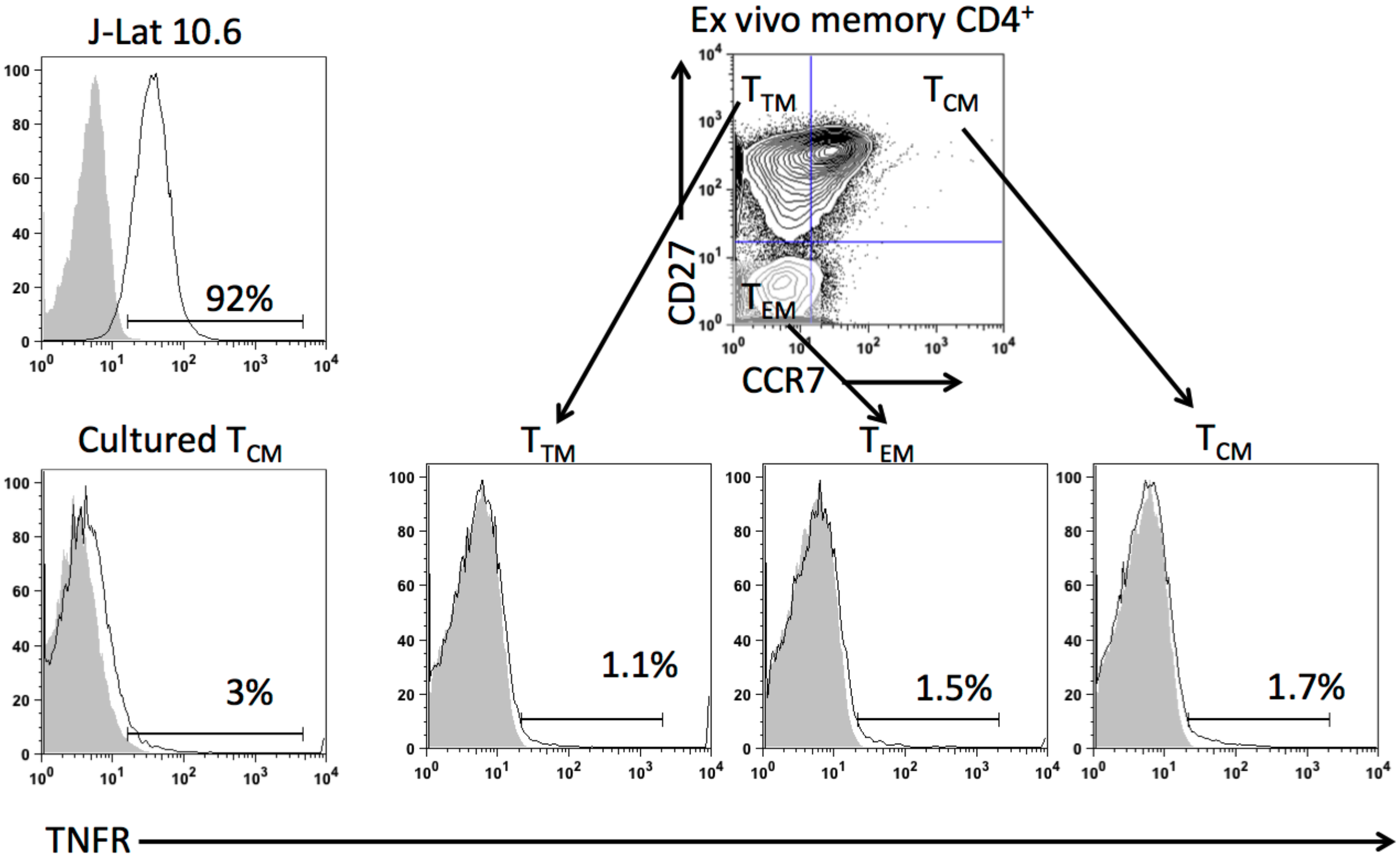
- The maximal response for most primary models was obtained through TCR stimulation. Some models were refractory toαCD3+αCD28
- Cells were universally responsive to PKC agonists, in particular phorbol esters PMA and prostratin
- TNFα was very variable and so was IL-2+IL-7 treatment

Figure S2


J-Lat 10.6
92%
Ex vivo memory CD4+
T TM
T CM
T EM
CD27
CCR7
Cultured T CM
T TM
T EM
T CM
3%
1.1%
1.5%
1.7%
TNFR

		TCR				PKC		Cytok	HDACi			PTEFb	Ca++
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- HDACi (and HMBA): Lewin model highly responsive to all. Some systems not at all responsive.

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Histone Deacetylase Inhibitors (HDACis) That Release the Positive Transcription Elongation Factor b (P-TEFb) from Its Inhibitory Complex Also Activate HIV Transcription*

Received for publication, February 25, 2013, and in revised form, March 27, 2013 Published, JBC Papers in Press, March 28, 2013, DOI 10.1074/jbc.M113.464834

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Background: HDACis activate HIV transcription.
Results: P-TEFb release from 7SK snRNP correlates better than histone H3 or tubulin acetylation with HIV reactivation by HDACis in cell lines.
Conclusion: Levels of P-TEFb must be increased before HDACis can reactivate HIV in resting primary $CD4^+$ T cells.
Significance: Levels and activity of P-TEFb are critical for HIV reactivation in all cells.

		TCR			PKC			Cytok		HDACi			PTEFb	Ca++
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- TNF**α** was very variable and so was IL-2+IL-7 treatment
- HDACi (and HMBA): Lewin model highly responsive to all. Some systems not at all responsive.
- Ca^{++} moderate responses but only in primary models

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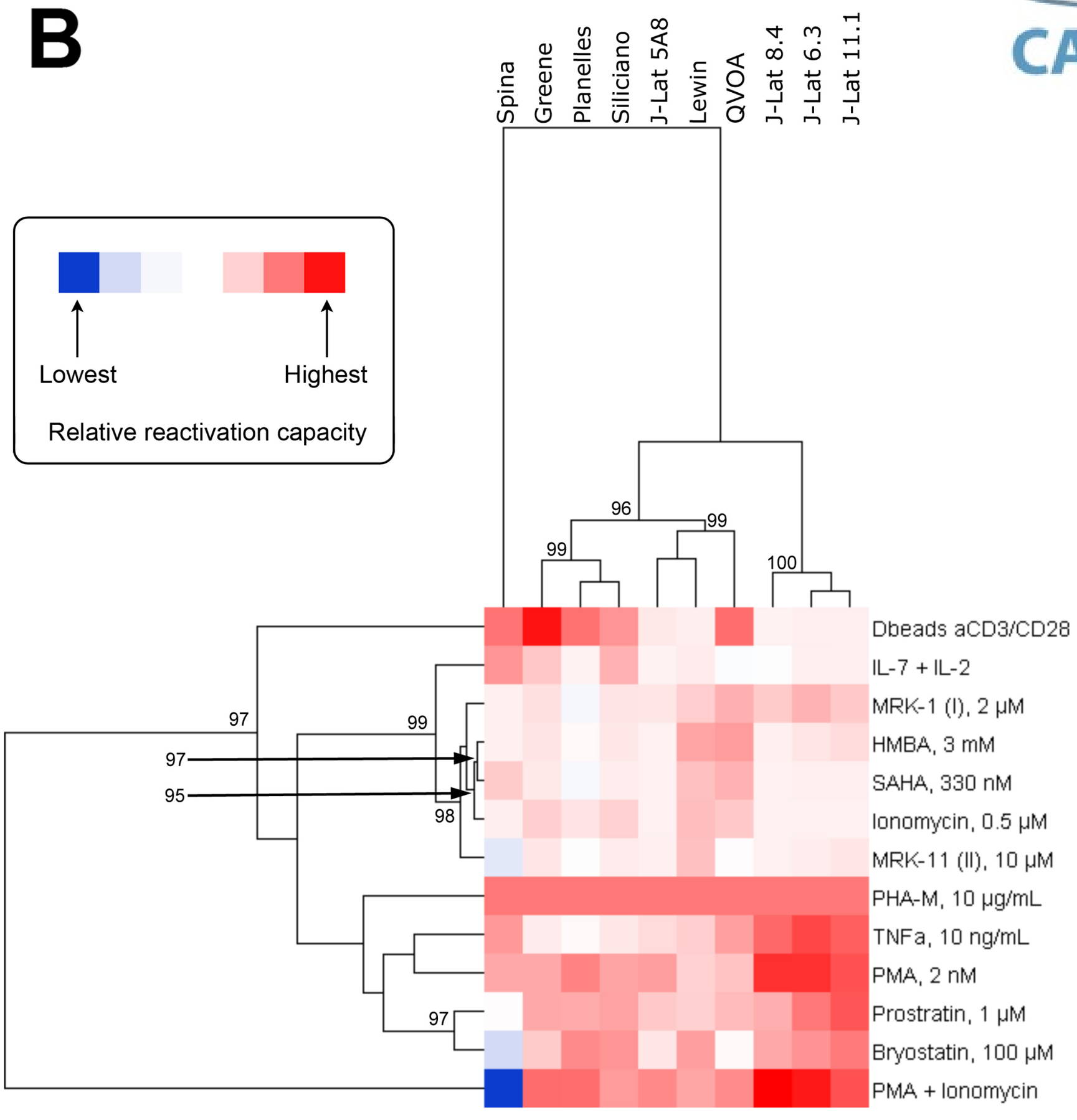
B


Spina
Greene
Planelles
Siliciano
J-Lat 5A8
Lewin
QVOA
J-Lat 8.4
J-Lat 6.3
J-Lat 11.1
96
99
99
100
97
99
97
95
98
97
Dbeads aCD3/CD28
IL-7 + IL-2
MRK-1 (I), 2 µM
HMBA, 3 mM
SAHA, 330 nM
Ionomycin, 0.5 µM
MRK-11 (II), 10 µM
PHA-M, 10 µg/mL
TNFa, 10 ng/mL
PMA, 2 nM
Prostratin, 1 µM
Bryostatin, 100 µM
PMA + Ionomycin
Lowest
Highest
Relative reactivation capacity

Sherrill-Mix *et al. Retrovirology* 2013, **10**:90
http://www.retrovirology.com/content/10/1/90

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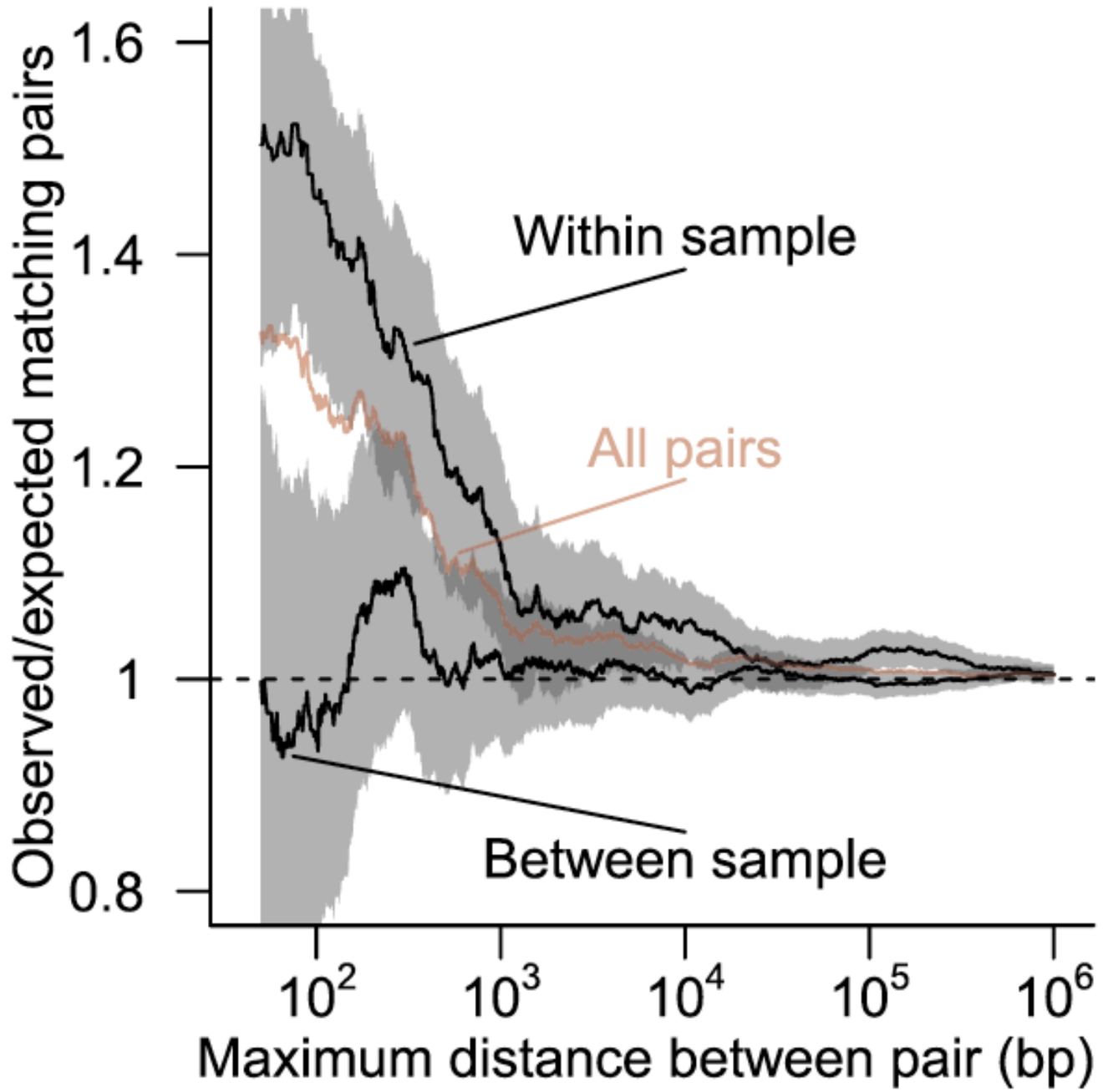
RESEARCH Open Access

HIV latency and integration site placement in five cell-based models

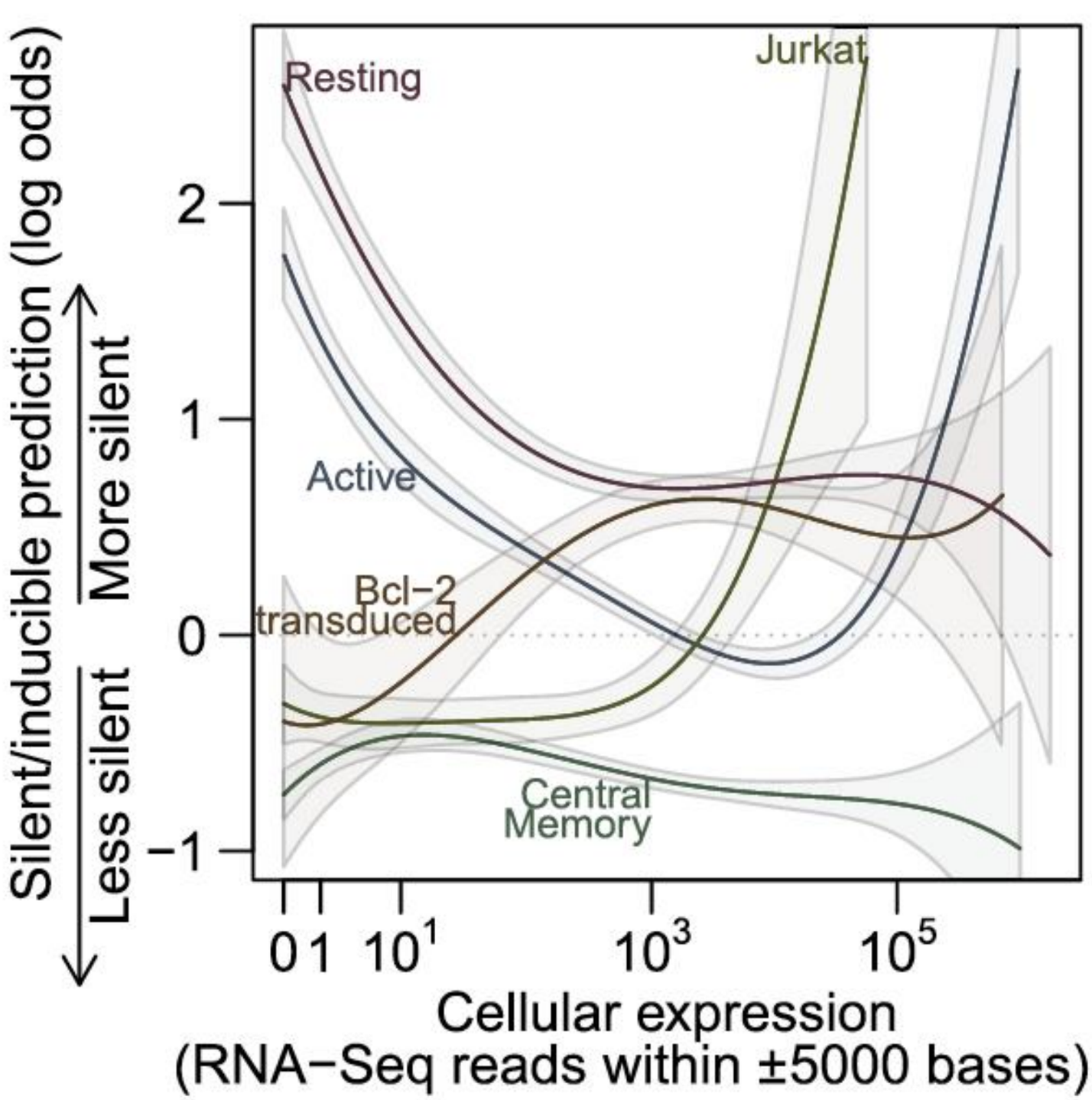
Scott Sherrill-Mix[1*], Mary K Lewinski[2], Marylinda Famiglietti[3], Alberto Bosque[4], Nirav Malani[1], Karen E Ocwieja[1], Charles C Berry[5], David Looney[2,6], Liang Shan[7], Luis M Agosto[9], Matthew J Pace[8], Robert F Siliciano[7], Una O'Doherty[8], John Guatelli[2,6], Vicente Planelles[4] and Frederic D Bushman[1]

Table 1 HIV-1 integration datasets from *in vitro* models of latency

Title	Cell type	Virus	Time of harvest after infection	Sequencing	Generation of expressed vs. silent/inducible	Citation	Silent/inducible unique sites	Expressed unique sites
Jurkat	Jurkat cells	HIV vector pEV731 (LTR-Tat-IRES-GFP)	2 weeks	Sanger	TNFα, GFP expression	[19]	463 inducible	643
Bcl-2 transduced CD4$^+$	Primary CD4$^+$ T cells (Bcl-2 transduced)	HIV NL4-3-Δ6-drEGFP (inactivated *gag*, *vif*, *vpr*, *vpu*, *nef* and *env* replaced by GFP)	3 days + 3-4 weeks + 3 days	Sanger	Anti-CD3, anti-CD28 antibodies, GFP expression	[20]	446 inducible	273
Active CD4$^+$	Primary active CD4$^+$ T cells	HIV NL4-3	3 days	454	High vs. low Gag	[21]	1604 silent	1274
Resting CD4$^+$	Primary resting CD4$^+$ T cells	HIV NL4-3	3 days	454	High vs. low Gag	[21]	1942 silent	784
Central Memory CD4$^+$	Primary central memory CD4$^+$ T cells	HIV NL4-3 ΔNef GFP	2 days/9 days	IonTorrent	Anti-CD3, anti-CD28 antibodies, GFP expression	This paper	1729 inducible	3278



Shared expression status between near neighbors. The ratio of the number of pairs of proviruses with matching expression status to the number of matches expected by random pairings given the frequency of silent/inducible proviruses. All possible pairs of proviruses integrated within a given distance of each other on the same chromosome (red line) were separated into two sets; one with both proviruses from within the same cell culture model and one with proviruses paired between two different cell culture models (black lines). The shaded region shows the 95% Clopper-Pearson binomial confidence interval for within and between sample pairings. The dashed horizontal line shows the ratio of 1 expected if there is no association between the expression status of neighboring proviruses.



Cellular expression and latency. Predictions from a logistic regression of silent/inducible status on cellular RNA expression. High y-axis values are predicted to be silent/inducible. Dotted line shows where equal odds of silent/inducible and expressed are predicted. Solid lines show predictions from the regression for each sample and shaded regions indicate one standard error from the modeled predictions.

- Conclusions from integration site comparisons -

1. Certain chromosomal locations ARE associated with a tendency to harbor silent or active proviral integrations – but in a model-specific manner

2. No relationships between genomic features near the integration site and latency achieved significance in all models.

3. Integration into alphoid repeats frequently leads to a state of latency (significant in 3 models)

4. Proviruses from the same cellular model integrated in nearby positions did share the same latency status much more often than predicted by chance, indicating the presence of local features influencing latency