Vpr SHAPES THE PROVIRAL LANDSCAPE AND POLYCLONAL HIV-1 REACTIVATION PATTERNS IN VITRO

Edmond Atindaana[1], Jake Pitcher[1], Sarah Emery[2], Cleo Burnett[1], Jeffery Kidd[2], Alice Telesnitsky[1]

University of Michigan Medical School

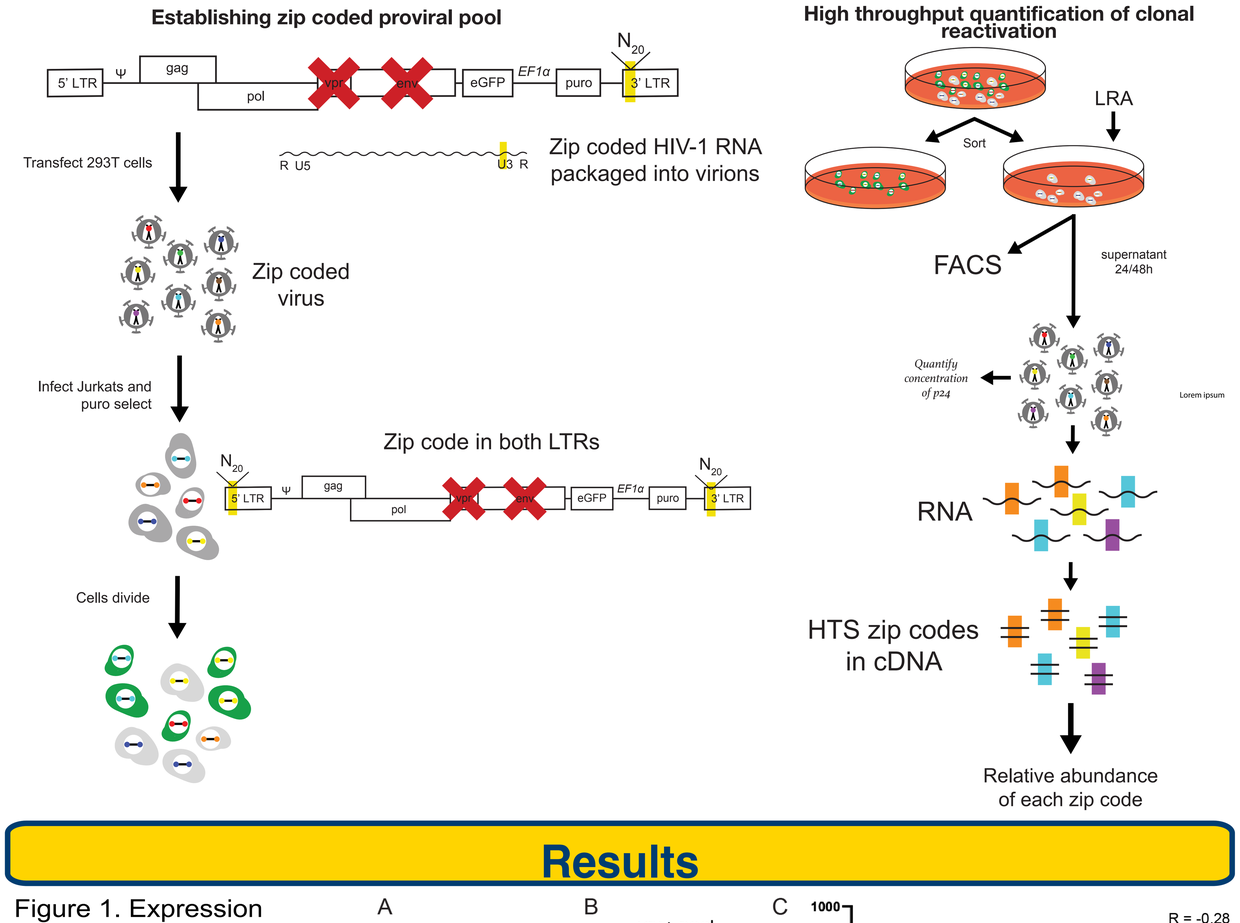
[1] Departments of Microbiology and Immunology, and [2] Human Genetics, University of Michigan, Ann Arbor, MI, USA.

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Abstract

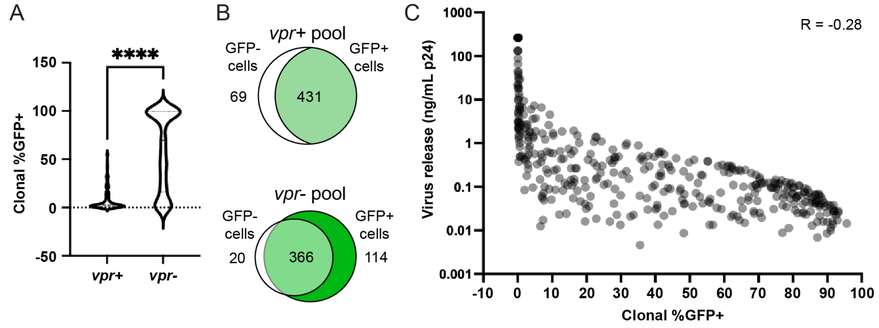
Integration site landscapes, clonal dynamics, and latency reversal with or without *vpr* were compared in HIV-1-infected Jurkat cell populations, and the properties of individual clones were defined. Clones differed in fractions of LTR-active daughter cells, with some clones containing few to no LTR-active cells while almost all cells were LTR-active for others. Clones varied over four orders of magnitude in virus release per active cell. Proviruses in largely LTR-active clones were closer to preexisting enhancers and promoters than low LTR-active clones. Unsurprisingly, major *vpr*+ clones contained fewer LTR-active cells than *vpr*- clones, and predominant *vpr*+ proviruses were farther from enhancers and promoters than those in *vpr*- pools. When the latency reactivation agents prostratin and JQ1 were applied separately or in combination, *vpr*+ and *vpr*- population-wide trends were similar, with dual treatment enhancement due in part to reactivated clones that did not respond to either drug applied separately. However, expression signatures of individual clones differed between populations. These observations highlight how Vpr, exerting selective pressure on proviral epigenetic variation, can shape integration site landscapes, proviral expression patterns, and reactivation properties.

Methods



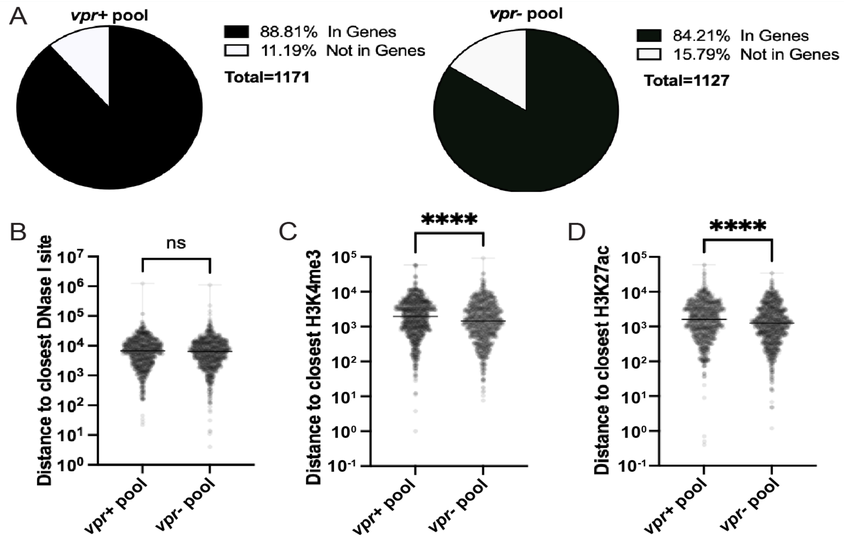
Results

Figure 1. Expression properties of individual clones within the vpr+ and vpr- pools.



Results (con't)

Figure 2. Comparison of integration sites in vpr+ and vpr- pools. Integration sites for both vpr+ and vpr- pool cells harvested 10 days post-infection were determined and distances to the nearest indicated genome features were mapped and compared.



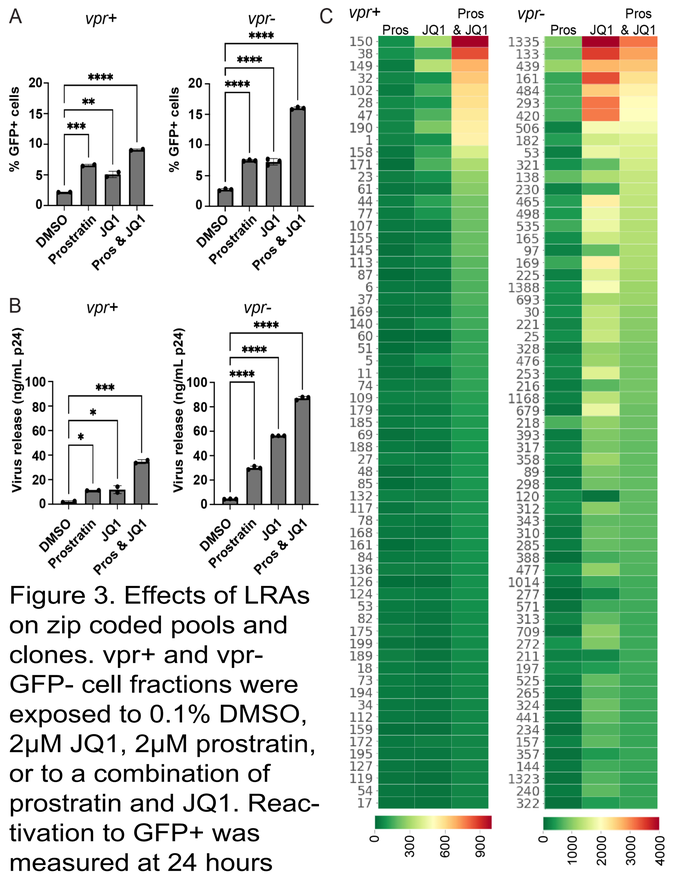
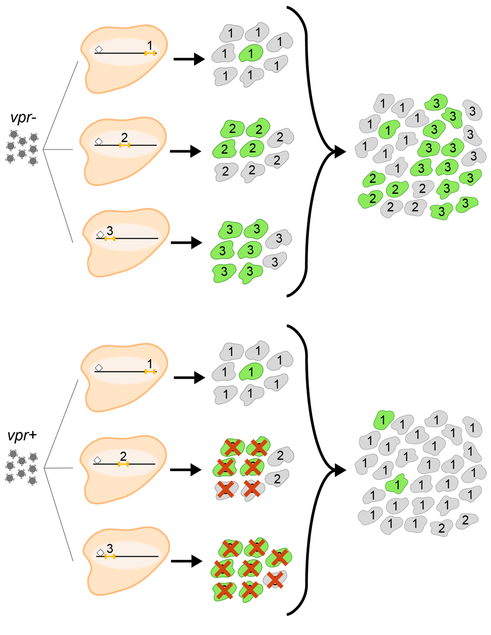


Figure 3. Effects of LRAs on zip coded pools and clones. vpr+ and vpr- GFP- cell fractions were exposed to 0.1% DMSO, 2μM JQ1, 2μM prostratin, or to a combination of prostratin and JQ1. Reactivation to GFP+ was measured at 24 hours post-infection by flow cytometry, and virus release

Figure 4. Schematic model of the effect of Vpr on integrant populations' proviral landscapes.



Conclusions

High LTR-active clones are depleted in the presence of Vpr.
vpr+ integration sites are farther away from H3K27ac and H3K4me3 marks than vpr- integration sites.
The clonal reactivation patterns differ between *vpr*+ and *vpr*- proviral populations.