Persistent HIV reservoir suppression by (-)-hopeaphenol, a plant-derived stilbenoid

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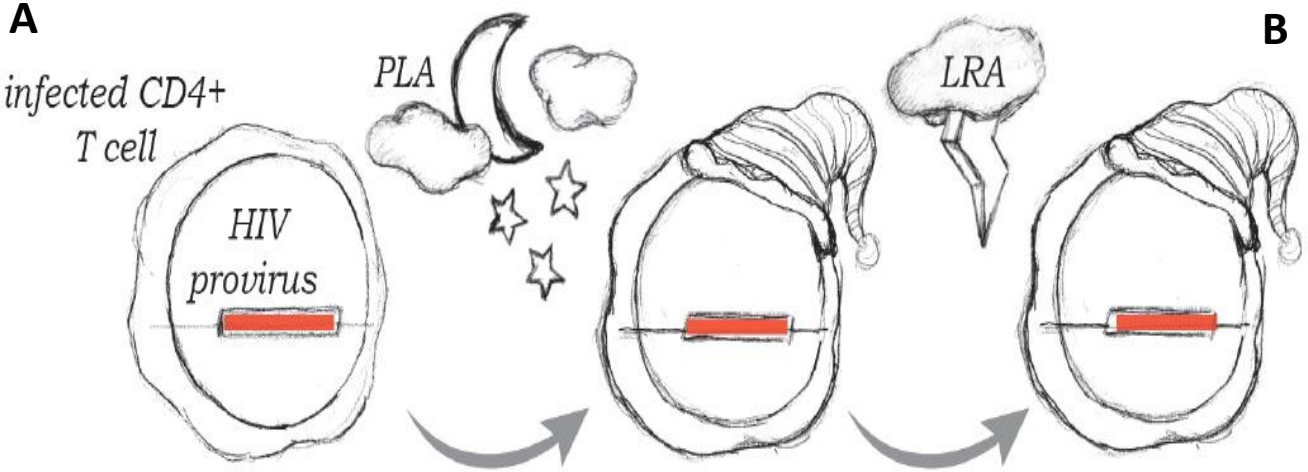
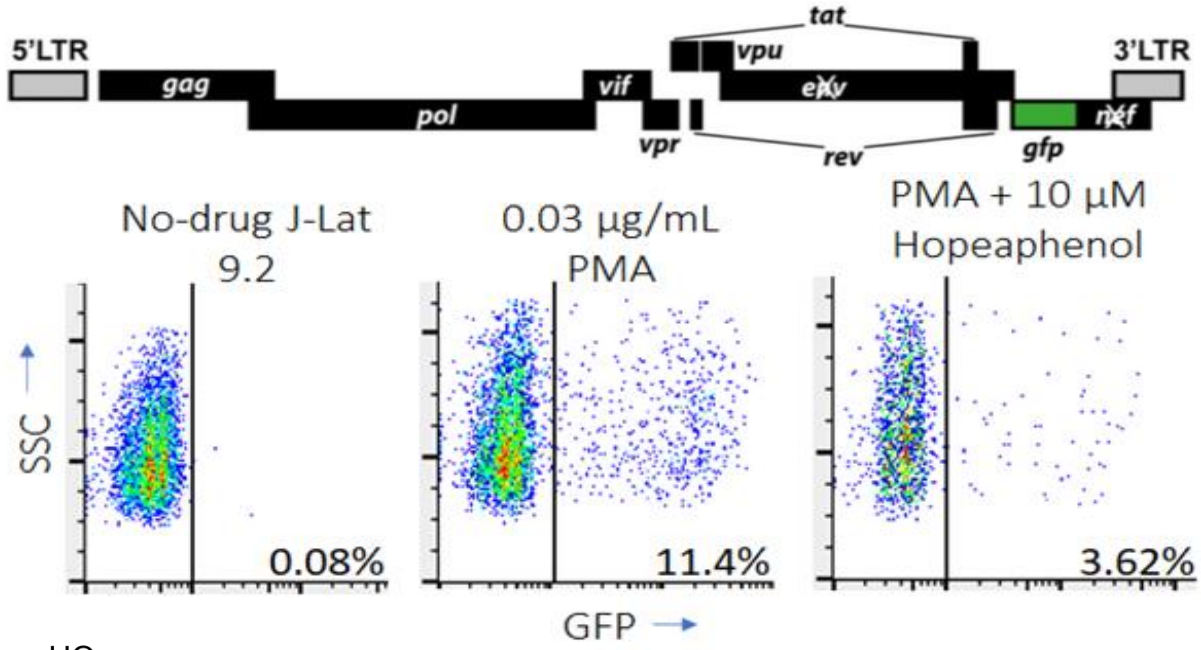
*The authors declare no conflicts of interest

(-)-Hopeaphenol is a potential candidate for “Block-and-Lock”-based HIV remission

A



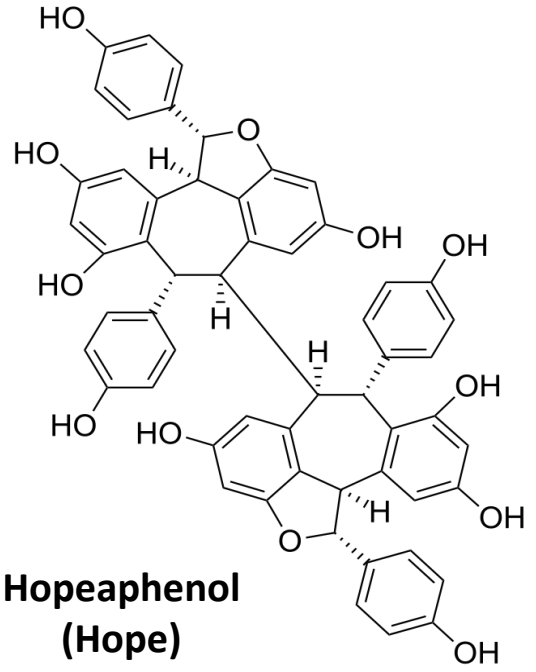
B



A, While ART durably suppresses HIV replication, virus persists within cellular reservoirs. One experimental approach to inactivate HIV reservoirs, frequently termed “Block-and-Lock,” involves use of pro-latency agents (PLAs) to reinforce long-term and durable proviral latency, even after agent discontinuation and/or subsequent proviral stimuli with Latency Reversing Agents (LRAs).

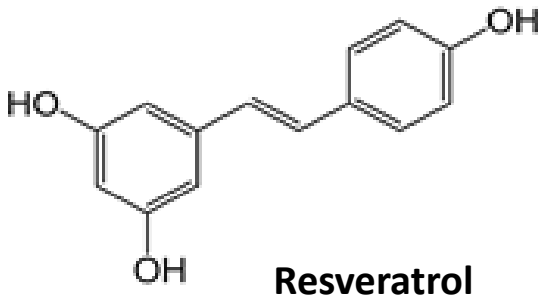
B, 527 pure compounds from Compounds Australia were screened for ability to inhibit PMA-induced latency reversal in J-Lat 9.2 cells (Jordan et al, 2003). The most active compound was (-)-hopeaphenol (**C**), a tetramer of resveratrol (**D**). Here we identify hopeaphenol as a novel candidate Block-and-Lock agent.

C



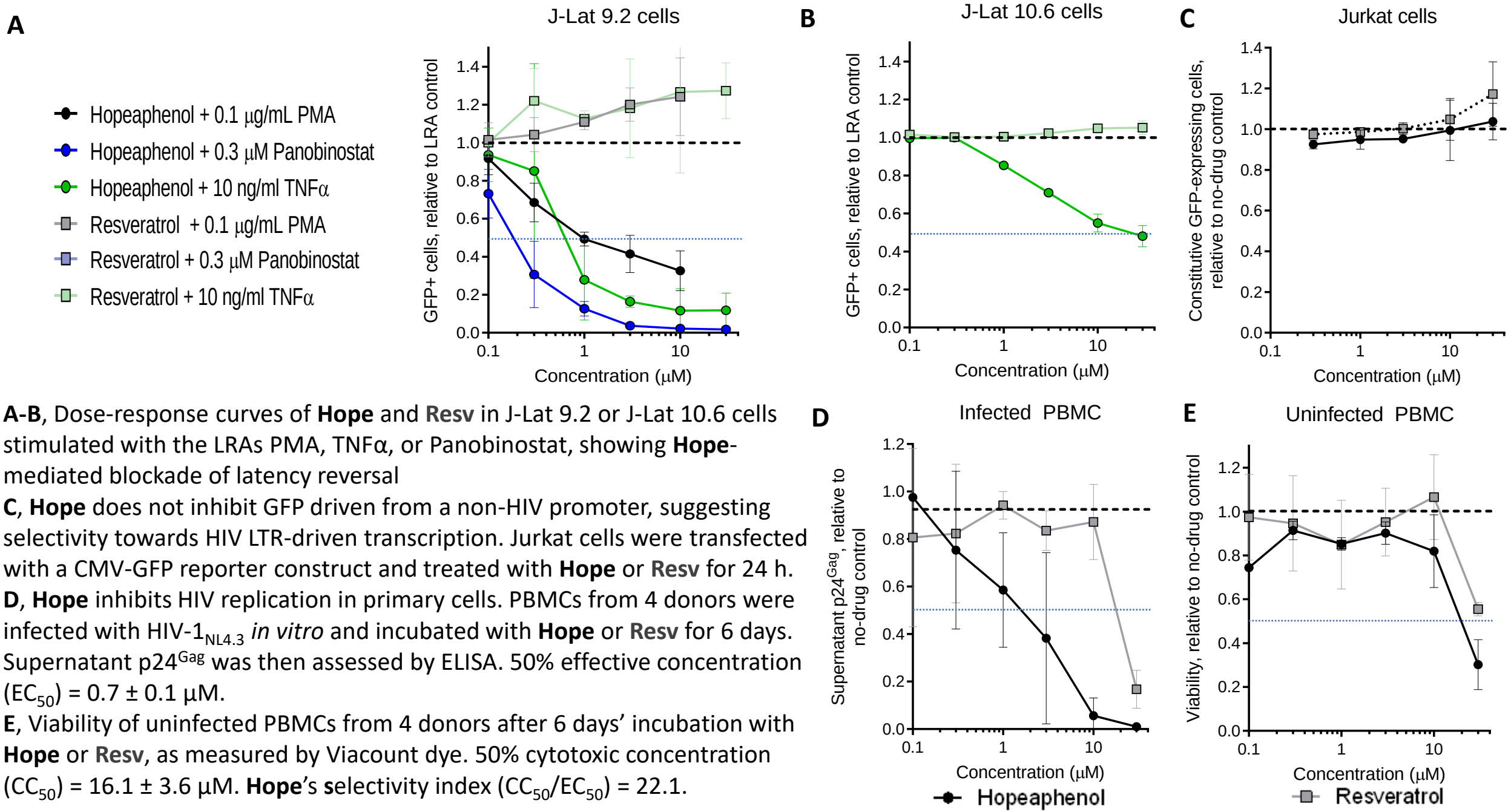
Hopeaphenol (Hope)

D



Resveratrol (Resv)

Hopeaphenol selectively reinforces HIV-1 latency *in vitro* and inhibits viral replication in primary PBMCs



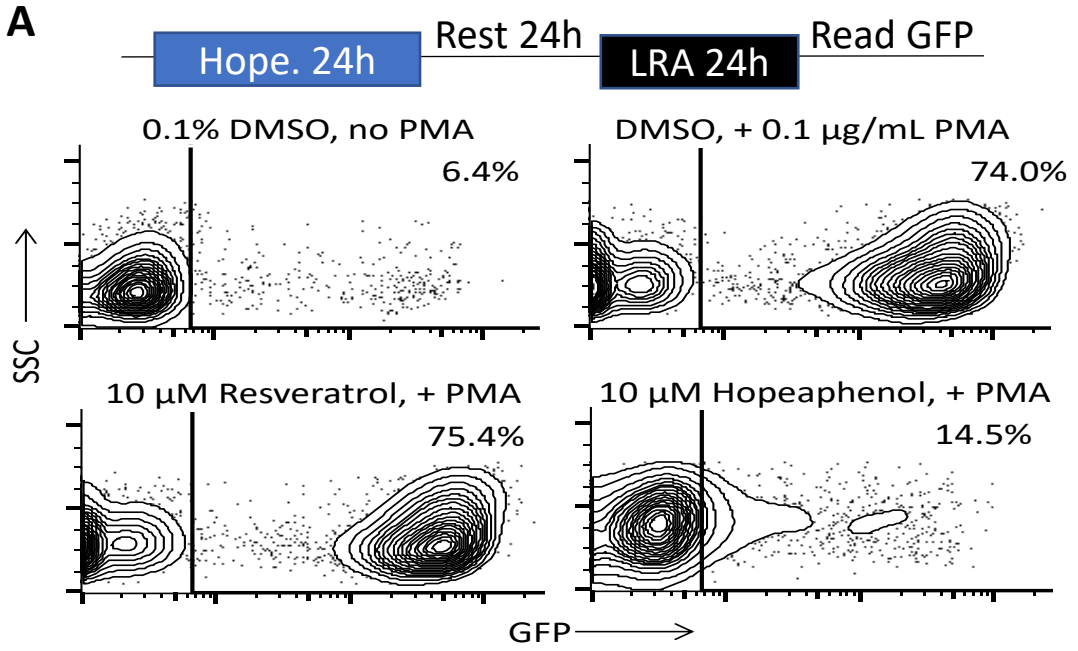
A-B, Dose-response curves of **Hope** and **Resv** in J-Lat 9.2 or J-Lat 10.6 cells stimulated with the LRAs PMA, TNFα, or Panobinostat, showing **Hope**-mediated blockade of latency reversal

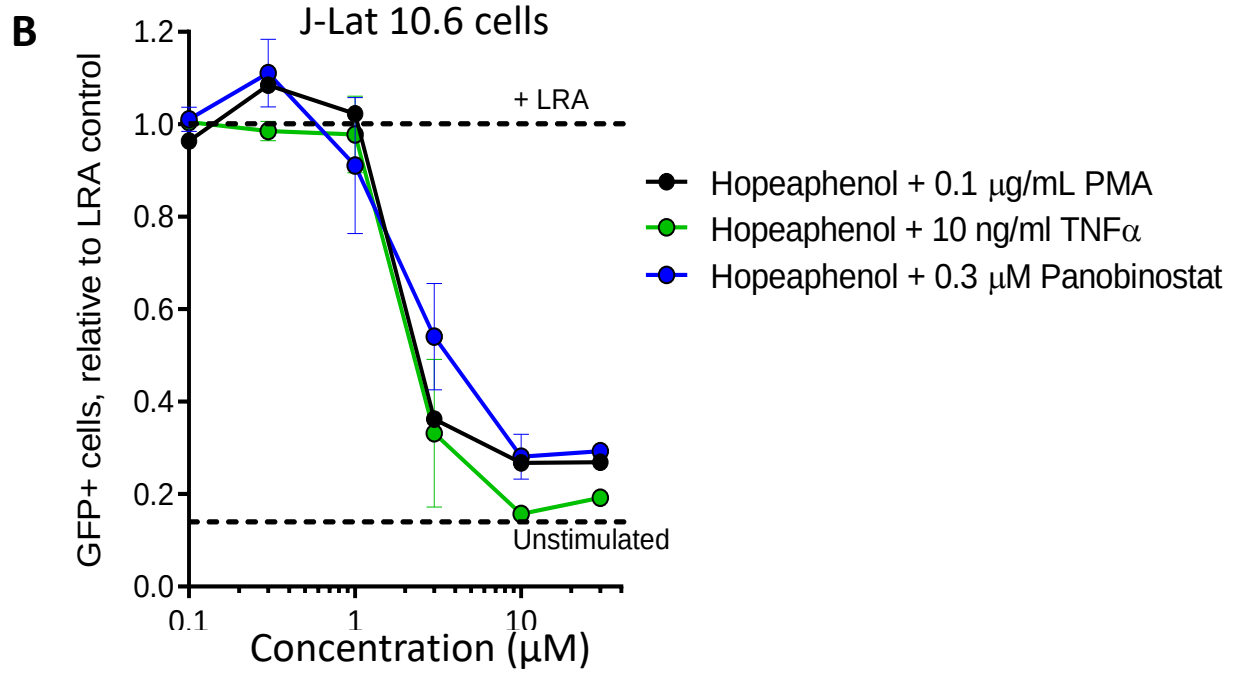
C, **Hope** does not inhibit GFP driven from a non-HIV promoter, suggesting selectivity towards HIV LTR-driven transcription. Jurkat cells were transfected with a CMV-GFP reporter construct and treated with **Hope** or **Resv** for 24 h.

D, **Hope** inhibits HIV replication in primary cells. PBMCs from 4 donors were infected with HIV-$1_{NL4.3}$ *in vitro* and incubated with **Hope** or **Resv** for 6 days. Supernatant $p24^{Gag}$ was then assessed by ELISA. 50% effective concentration (EC_{50}) = 0.7 ± 0.1 μM.

E, Viability of uninfected PBMCs from 4 donors after 6 days' incubation with **Hope** or **Resv**, as measured by Viacount dye. 50% cytotoxic concentration (CC_{50}) = 16.1 ± 3.6 μM. **Hope**'s **s**electivity index (CC_{50}/EC_{50}) = 22.1.

Hopeaphenol-induced viral latency is maintained *in vitro* following compound withdrawal.

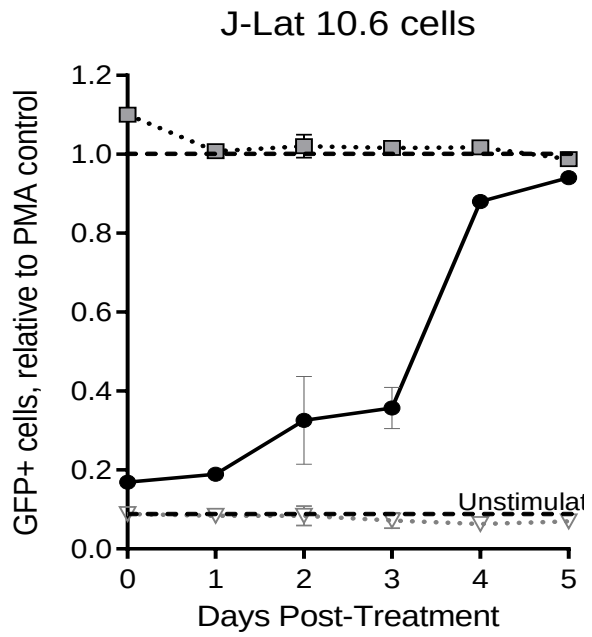


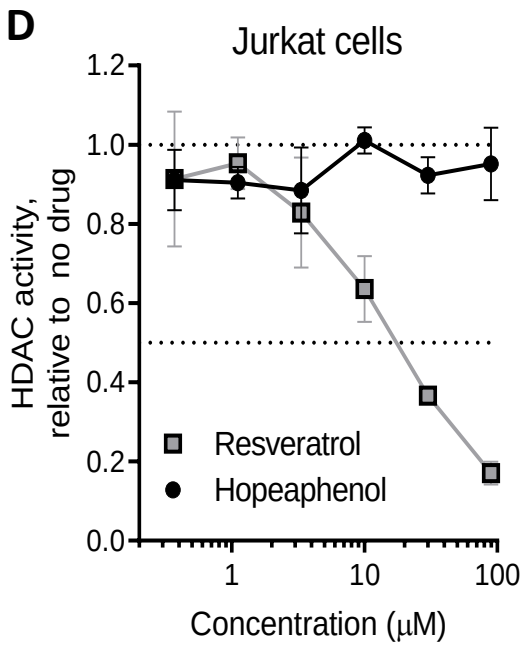


A-B, J-Lat 10.6 cells spontaneously press low levels of GFP (5-10%) and are highly sensitive to LRAs. Cells were treated with **Resv** or **Hope**, washed and rested for 24 h, then stimulated with LRAs. Cells pre-treated with **Hope** resisted LRA stimulation even after **Hope** washout, suggestive of block and lock, while those pre-treated with **Resv** did not.

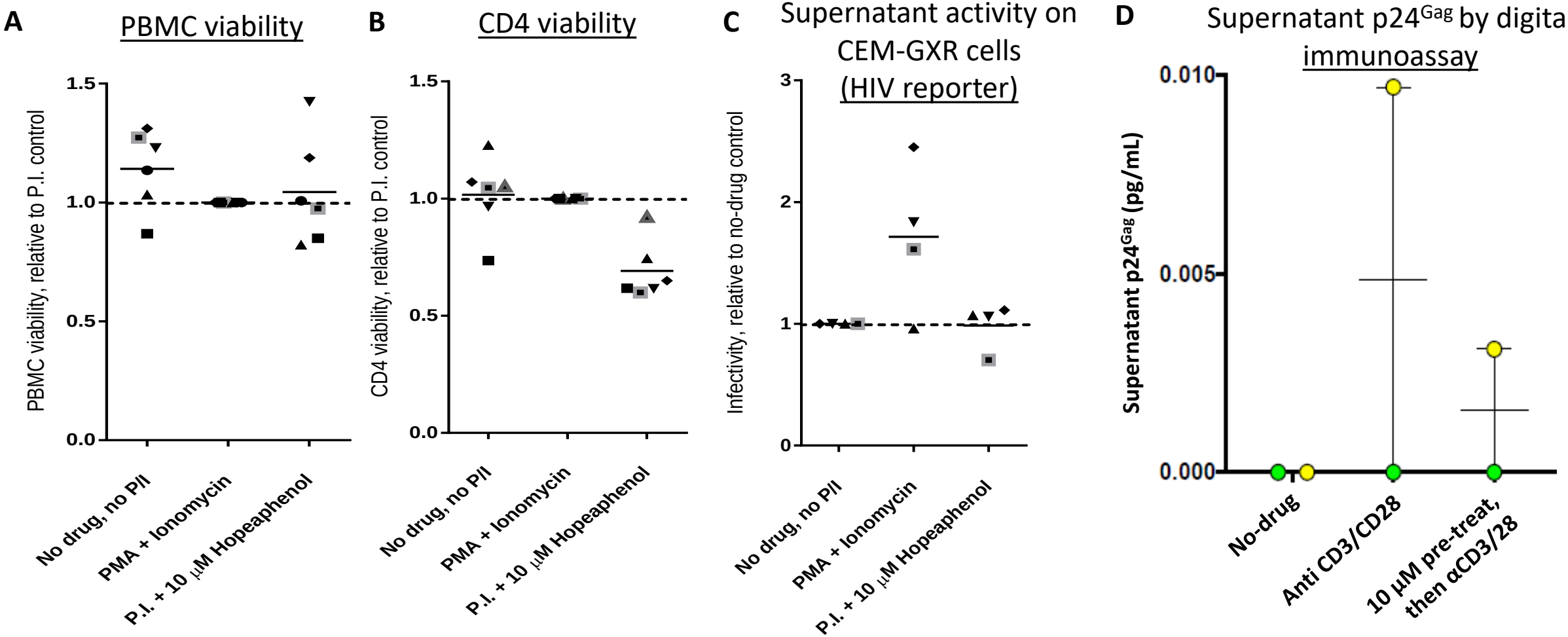
C, J-Lat 10.6 cells were pretreated with 10 µM **Hope** for 24 h then washed/rested for 0-5 days prior to PMA stimulation. **Hope**-mediated block and lock is maintained for up to 3 days post-wash.

D, Repressive epigenetics at the HIV LTR are likely required for durable block and lock. Cellular Histone Deacetylases (HDACs) are involved in maintaining HIV latency by compressing chromatin at the HIV LTR. The effects of **Hope** and **Resv** on HDAC activity in Jurkat cells was examined using the Promega HDAC-Glo assay. While **Resv** inhibited HDAC activity, **Hope** did not. Maintenance of HDAC activity is consistent with **Hope**'s ability to reinforce latency.





Hopeaphenol blocks viral reactivation *ex vivo* (?)

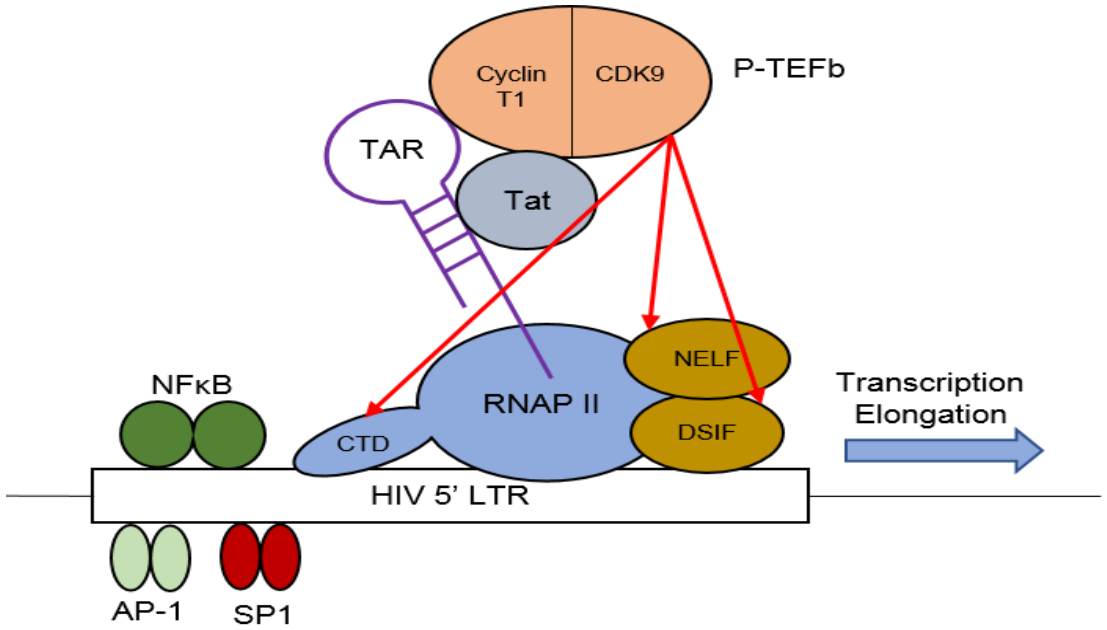


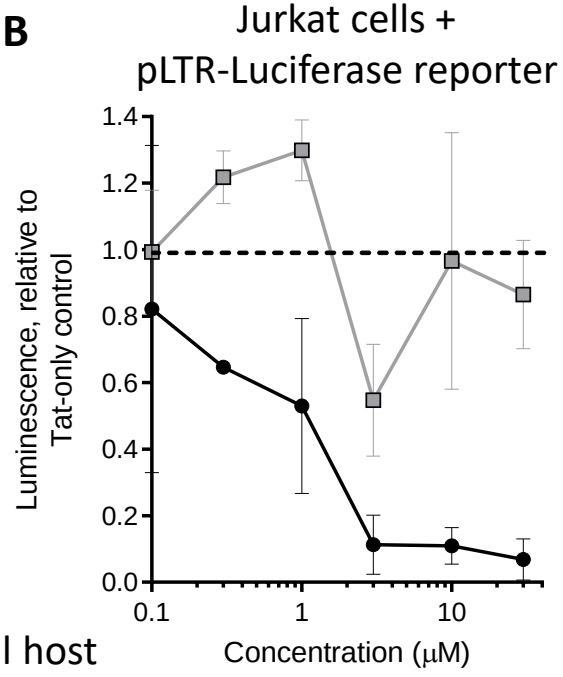
A-B, Cell viability, as measured by Guava Viacount, in PBMCs **(A)** and CD4+ T cells **(B)** from 6 HIV-infected donors on stably-suppressive ARVs. Cells were pre-treated with 10 µM **Hope**, washed and rested for 24 h, and stimulated with PMA + ionomycin (P.I).

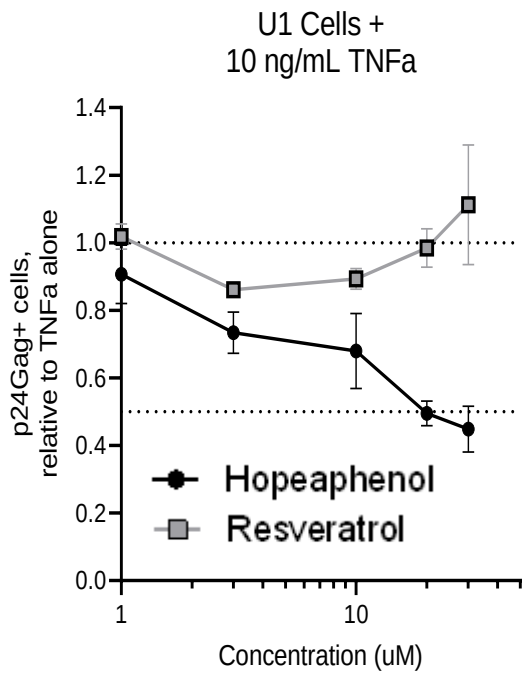
C, Effects of **Hope** pre-treatment (prior to washing and P/I treatment) on infectivity of supernatants from PBMC cultures from 4 HIV-infected donors. Supernatants were applied to CEM-GXR cells, which contain an HIV LTR-GFP reporter (PMID: 16182382).

D, Effects of **Hope** pre-treatment (prior to washing and Anti CD3/CD28 stimulation) on CD4+ T cells from 2 HIV-infected donors on supernatant p24Gag levels, as measured by digital immunoassay (PMID: 26369787).

Hopeaphenol blocks HIV-1 Tat-driven transcription through CDK9 inhibition





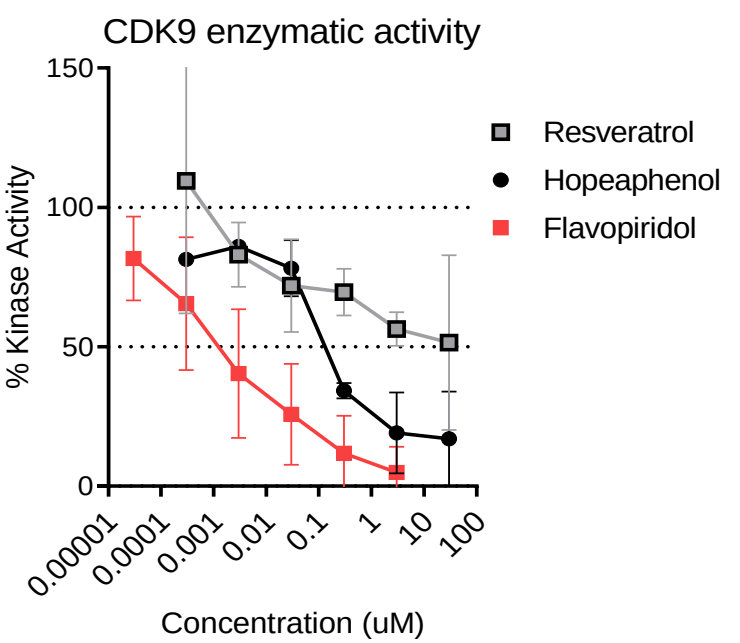


A, Productive HIV transcription is driven by the viral protein Tat and several host factors, including P-TEFb (made up of CDK9/Cyclin T1). P-TEFb is recruited by Tat to the LTR, where it phosphorylates RNAP II and several suppressive factors, stimulating productive transcription.

B, **Hope** inhibits Tat-driven transcription in Jurkat cells co-transfected with an HIV LTR-driven luciferase reporter and a Tat-expressing plasmid. Data was normalized to transfected, untreated cells. **Resv** did not inhibit Tat signalling, consistent with its inability to block latency reversal.

C, U1 cells are latently infected with a Tat-deficient provirus. **Hope** is able to block TNFα-mediated latency reversal in these cells, suggesting that it targets a factor involved in Tat signalling, but not Tat itself.

D, CDK9 is the kinase subunit of P-TEFb. **Hope** inhibits CDK9 kinase activity, as measured by the Promega ADP-Glo kinase assay and compared to the control inhibitor **flavopiridol**. **Hope** IC_{50} = 0.15 ± 0.06 μM



Conclusions

- Hopeaphenol inhibits HIV-1 replication and reinforces viral latency at low to sub-micromolar concentrations.
- Hopeaphenol's reinforced latency reversal persists even after its removal and/or subsequent stimulation by latency reversal agents in both cell lines and possibly *ex vivo.*
- It acts at least in part through silencing viral transcription, and blocks CDK9 activity.
- Block and Lock likely requires lasting, repressive epigenetic modifications at the HIV LTR. While Resv inhibits cellular HDAC activity, Hope does not, consistent with its ability to reinforce latency. Additional, potential epigenetic modifications are currently under investigation
- Taken together, hopeaphenol is a new candidate pro-latency agents which can be used to inform the emerging pharmacologically-based "Block-and-Lock" HIV-remission strategy.

Acknowledgements

CIHR IRSC
Canadian Institutes of Health Research
Instituts de recherche en santé du Canada

New Frontiers in Research Fund
Fonds Nouvelles frontières en recherche

SFU

Griffith UNIVERSITY
Griffith Institute for Drug Discovery
NatureBank

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