Supplemental Material

A high-throughput screening assay for silencing established HIV-1 macrophage infection identifies nucleoside analogues that perturb H3K9me3 on proviral genomes

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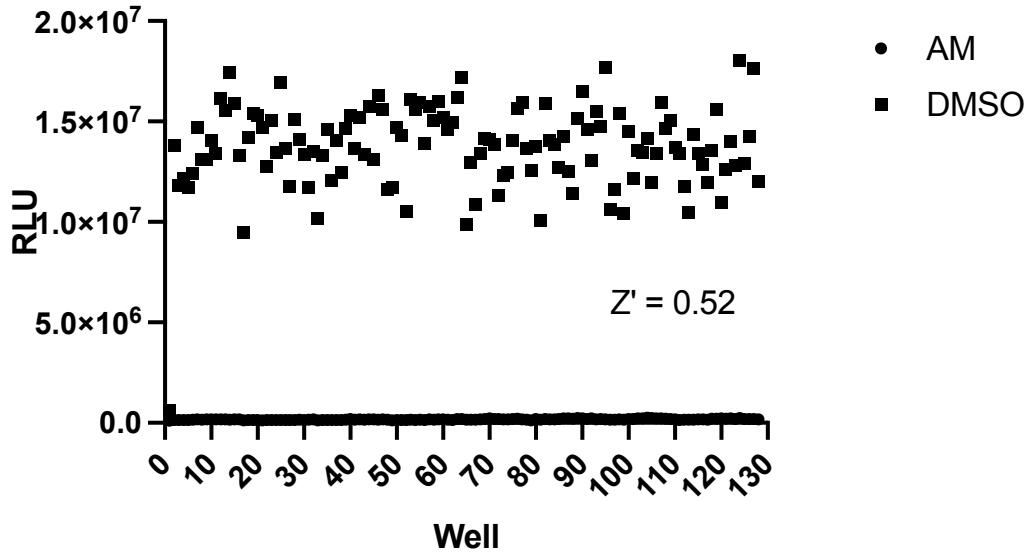

Figure S1. Z'-score for HTS assay.

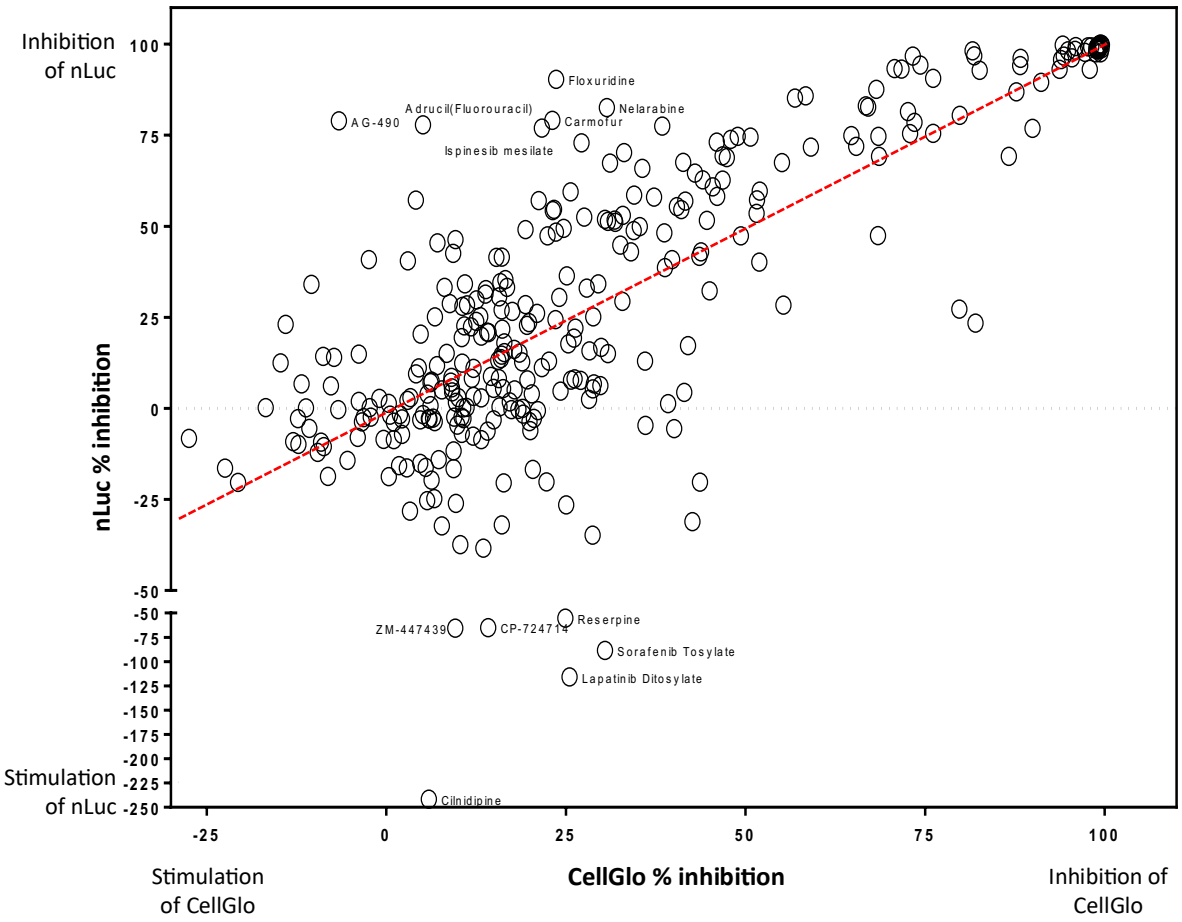

Figure S2. Scatter plot of primary HTS assay on Selleck Chem library. The red line indicates where equivalent effects on nLuc and CellTiter-Glo would lie, suggesting altered virus expression results from global cell effects. Compounds in the top left area show inhibition of virus expression disproportionate to cell viability effects, and compounds in the lower right area indicate stimulation of nLuc relative to cell viability effects.

Table S1. Libraries screened.

Library	Number of compounds
EPGN	80
SelleckChem	880
Anticancer	2500
Spectrum	2500
Total	5900

Table S2. Primers used for mutagenesis to generate reporter virus

Generate reporter cassette with 5' Not1 site, Kozak sequence, nLucPest gene, P2A sequence, 3' Not1 site:

Primer 1 (Not1-nLucP2A-forward) - Not1 site underlined, Kozak sequence bolded, nLuc 5' sequence italics:
5'-TCGGCTGCGG**CCGCC*ATG***GGTGGCAAGTGGTCAAAACG-3'

Primer 2 (nLucP2A-reverse) - nLucPest 3' sequence underlined, P2A 5' sequence bolded:
5'-**GCTGAAGTTAGTAGCTCCGCTTCC**GACGTTGATGCGAGC TGAAGCACAAGC-3'

Primer 3 (P2A oligo-reverse) – Not1 site underlined, P2A sequence bolded:
5'-
GCATTCGCGGCCGC**AGGTCCAGGGTTCTCCTCCACGTCTCCAGCCTGCTTCAGCAGGCTGAAGTTAGTAGCTCCGCTTCC**-3'

Primer 4 (P2A-Not1-reverse) - Not1 site underlined, P2A sequence bolded:
5'-GCATTCGCGGCCGC**AGGTCCAGGGTTCTCCTCCACG**-3'

Insert Not1 site between *env* and *nef* - Not1 site underlined:

primer-A: primer env/forward: 5'-GAGTTAGGCAGGGATACTCACC-3
primer-B: primer env/reverse: 5'-CCACC CAT G GCGGCCGC CTTATAGCAAAGCTCTTTCC-3
primer-C: primer nef/forward: 5'-GCTATAAG GCGGCCGC C ATGGGTGGCAAGTGGTC-3
primer-D: primer vector/reverse: 5'-CCGTACGTATAGGCTGCGC-3

Primers A and B amplified a fragment of YU2 *env* and introduce a Not1 site at the *env/nef* junction.
Primers C and D amplified a fragment of YU2 *nef*, LTR and vector and introduced a Not1 site at the *env/nef* junction.
Products were combined and amplified to generate a fragment containing env/Not1/nef/LTR/vector
Fragment was cloned back into the vector at Sal1 (5' in *env*) and Apa1 (3', in vector).

Amplify 5' env fragment to create 2bp insertion and stop codon – insertion bolded, Nde1 site underlined:

Env-stop-forward: 5'-TAGACAGGATGAGGATTAGAGCATGG-3'
Env-stop-reverse: 5'-CTGTATCATATG**TA**CTTTAGCATCTGATGCACAAAATAGAGTGGTGG-3'

Table S3. qPCR Primers

nef-forward:	5’-CGGCGACTGGAAGAAGCG-3’
nef-reverse:	5’-GTCTCTCTCTCCACCTTCTTCTTC-3’
tat-forward:	5’-CGGCGACTGAATTGGGTG-3’
tat-reverse:	5’-GTCTCTCTCTCCACCTTCTTCTTC-3’
gag-forward:	5’-CATGTTTTCAGCATTATCAGAAGGA-3’
gag-reverse:	5’-TGCTTGTTGTCCCCCCACT-3’
TAR short-forward:	5’-GGGTCTCTCTGGTTAGA-3’
TAR short- reverse:	5’-GGGTTTCCTAGCTAGCC-3’
TAR long-forward:	5’-GGGTCTCTCTGGTTAGA-3’
TAR long-reverse:	5’-CTGCTAGAGATTTTCCACACTGAC-3’
GUSB-forward:	5’-CGCCCTGCCTATCTGTATTC-3’
GUSB Reverse:	5’-TCCCCACAGGGAGTGTGTAG-3’