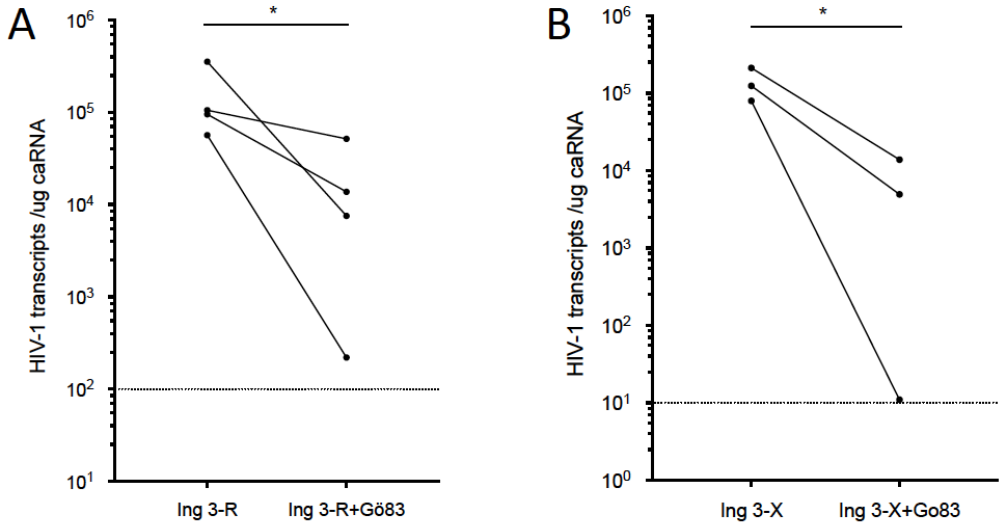
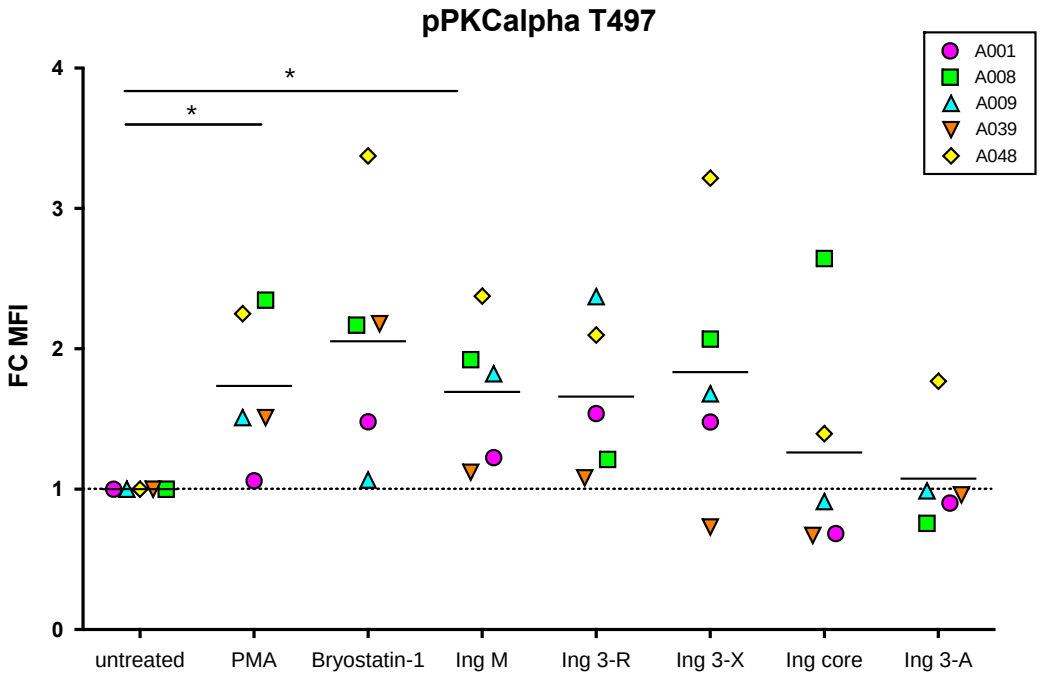
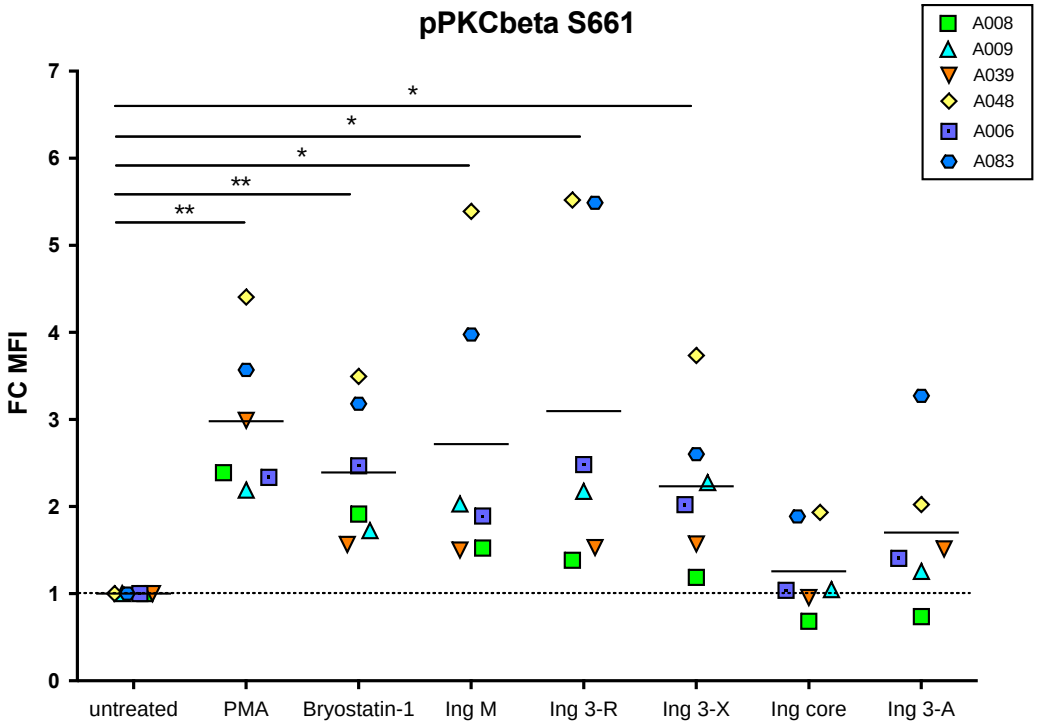
Supplemental Figure 1: Protein Kinase C inhibitor Gö6983 block ingenol-induced latency reversal in J-Lat 10.6

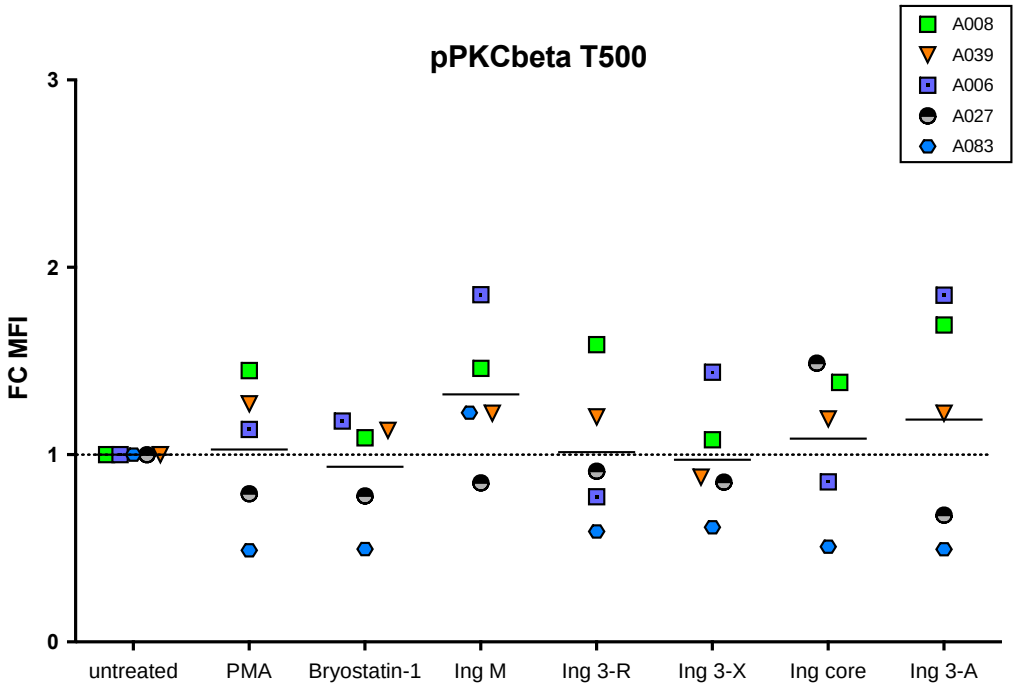


Resting CD4$^+$ T cells from aviremic HIV-1-infected participants were exposed to Ing 3-R (**panel A**) or Ing 3-X **(panel B**) at 100nM with or without four-hour pre-exposure to the pan-PKC inhibitor Gö6983 at 300nM. PKC inhibition resulted in a statistically significant average decrease in latency reversal of five logs (quantified as cell-associated HIV-1 RNA transcripts per ug of total cell-associated RNA) across a minimum of three independent experiments (Mann-Whitney test).

Supplemental Figure 2: PKC Isoform Phosphorylation after Ingenol Exposure

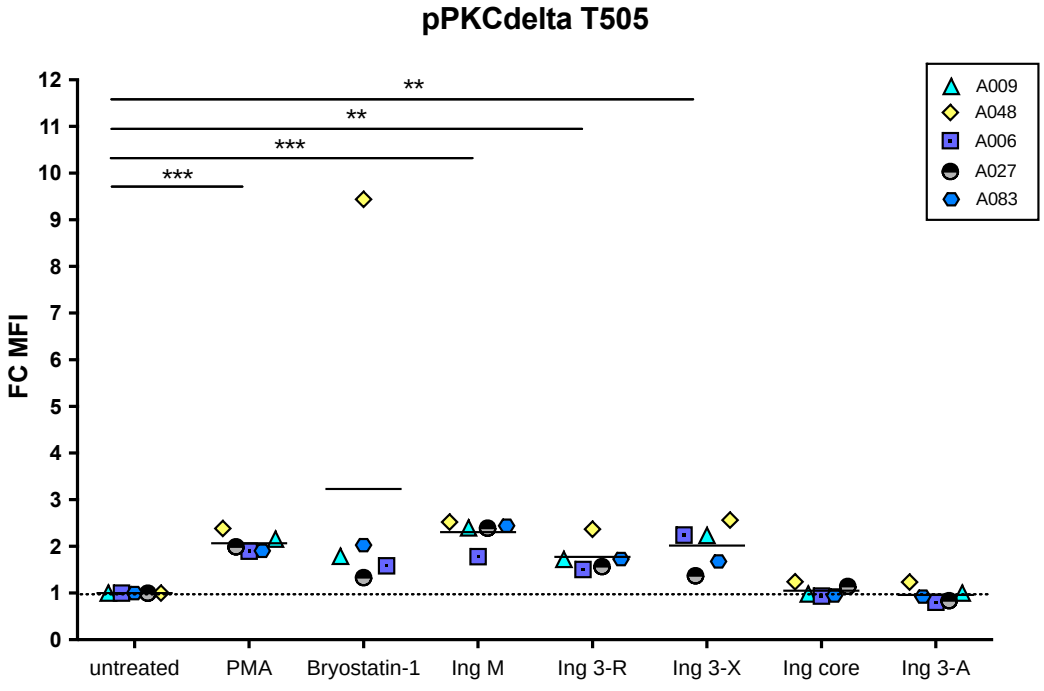





pPKCbeta T500
A008
A039
A006
A027
A083
FC MFI
0
1
2
3
untreated
PMA
Bryostatin-1
Ing M
Ing 3-R
Ing 3-X
Ing core
Ing 3-A

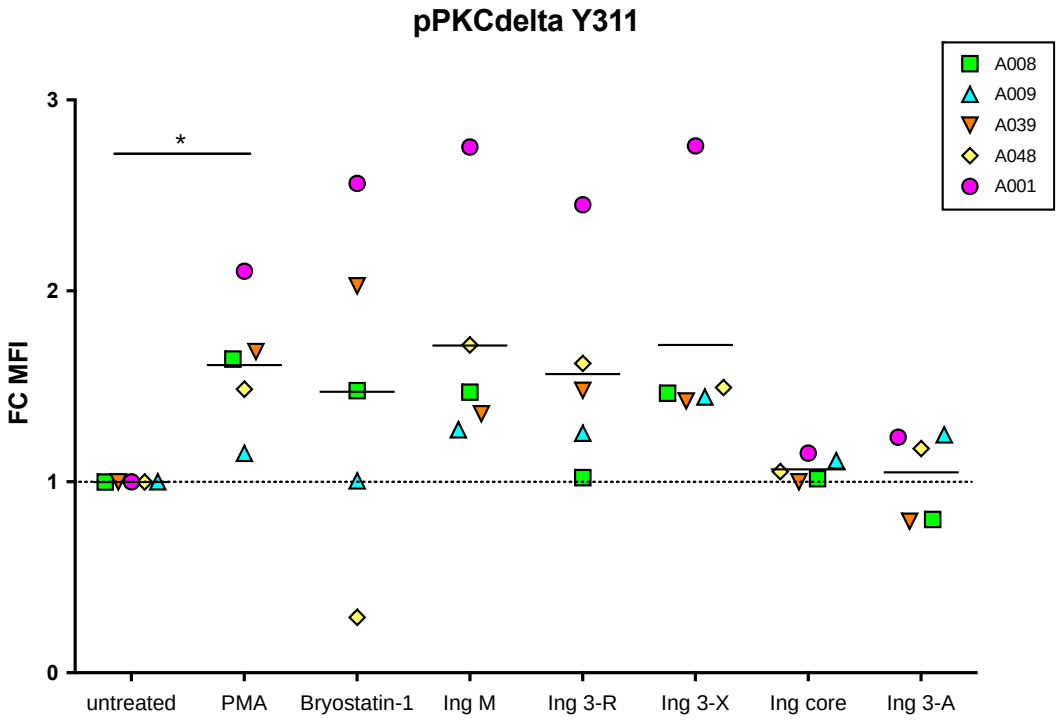
18

19


pPKCdelta T505
A009
A048
A006
A027
A083
**
**
***
***
FC MFI
0
1
2
3
4
5
6
7
8
9
10
11
12
untreated
PMA
Bryostatin-1
Ing M
Ing 3-R
Ing 3-X
Ing core
Ing 3-A

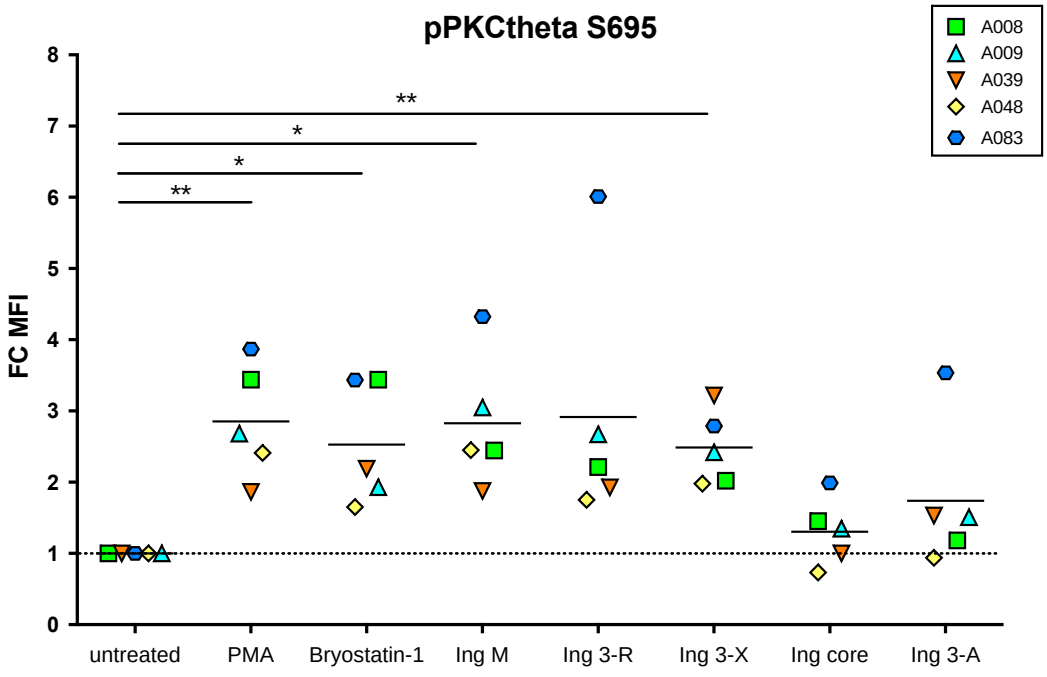
20

21


pPKCdelta Y311
A008
A009
A039
A048
A001
FC MFI
3
2
1
0
*
untreated
PMA
Bryostatin-1
Ing M
Ing 3-R
Ing 3-X
Ing core
Ing 3-A

22

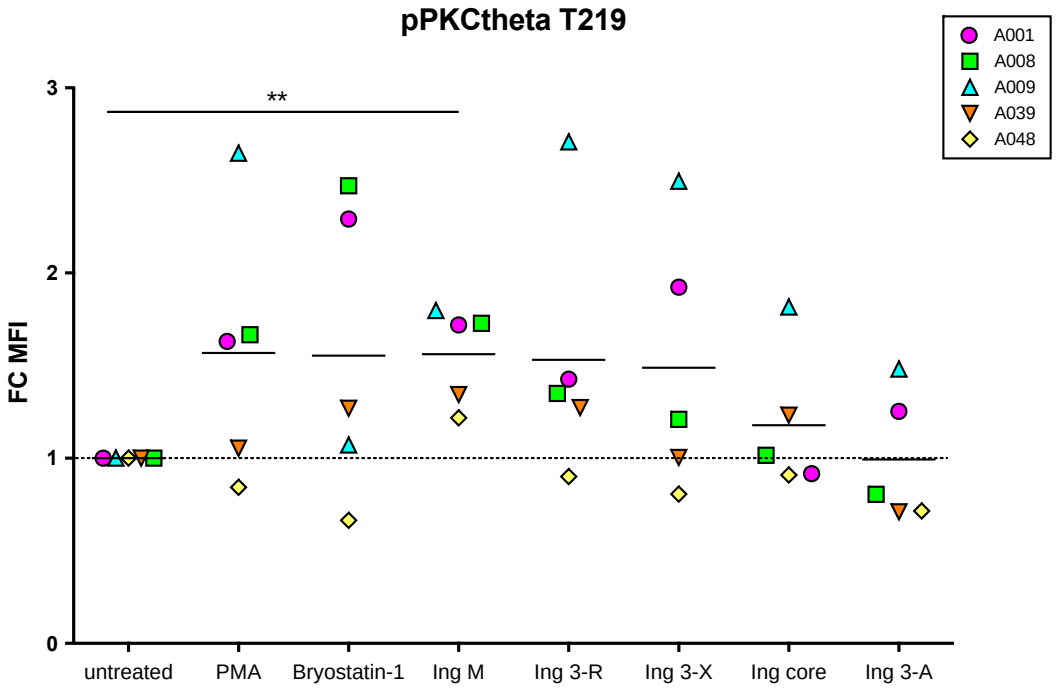
23


pPKCtheta S695
A008
A009
A039
A048
A083
FC MFI
8
7
6
5
4
3
2
1
0
**
*
*
**
untreated
PMA
Bryostatin-1
Ing M
Ing 3-R
Ing 3-X
Ing core
Ing 3-A

24

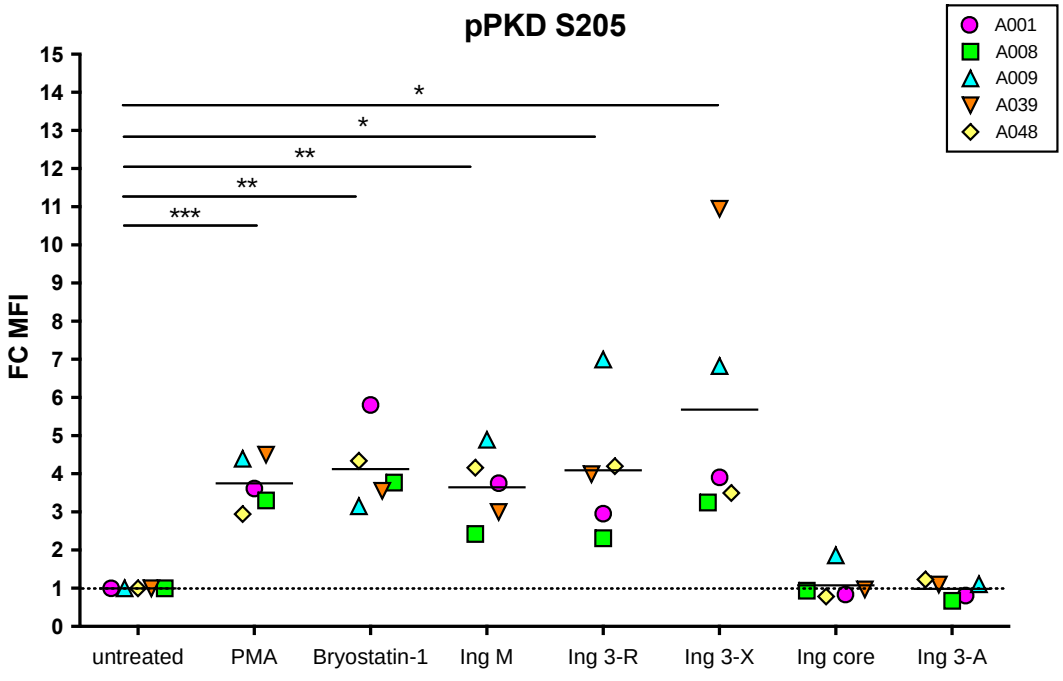
25

26


pPKCtheta T219
A001
A008
A009
A039
A048
FC MFI
3
2
1
0
**
untreated
PMA
Bryostatin-1
Ing M
Ing 3-R
Ing 3-X
Ing core
Ing 3-A

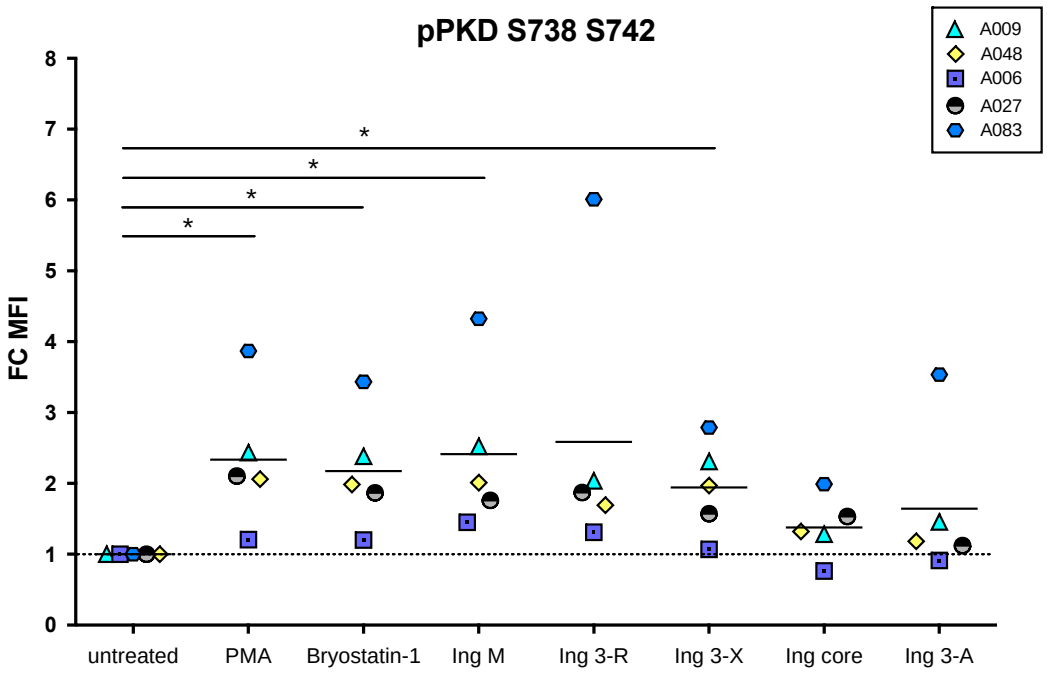
27

28


pPKD S205
A001
A008
A009
A039
A048
FC MFI
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
*
*
**
**
***
untreated
PMA
Bryostatin-1
Ing M
Ing 3-R
Ing 3-X
Ing core
Ing 3-A

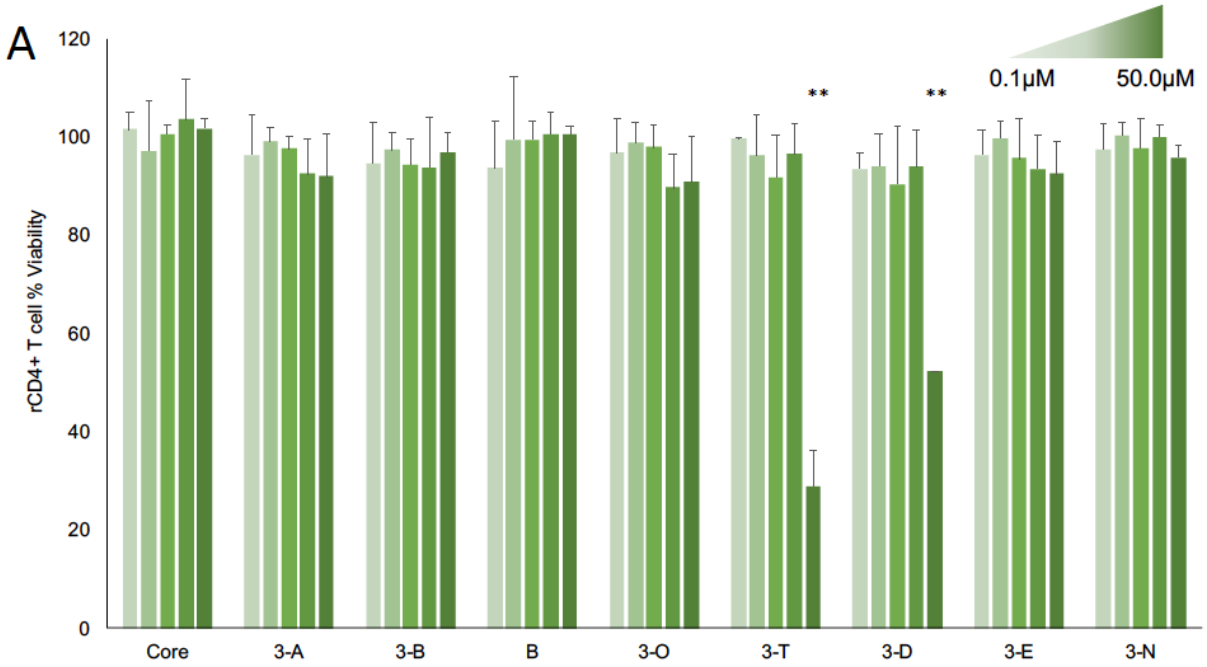
29

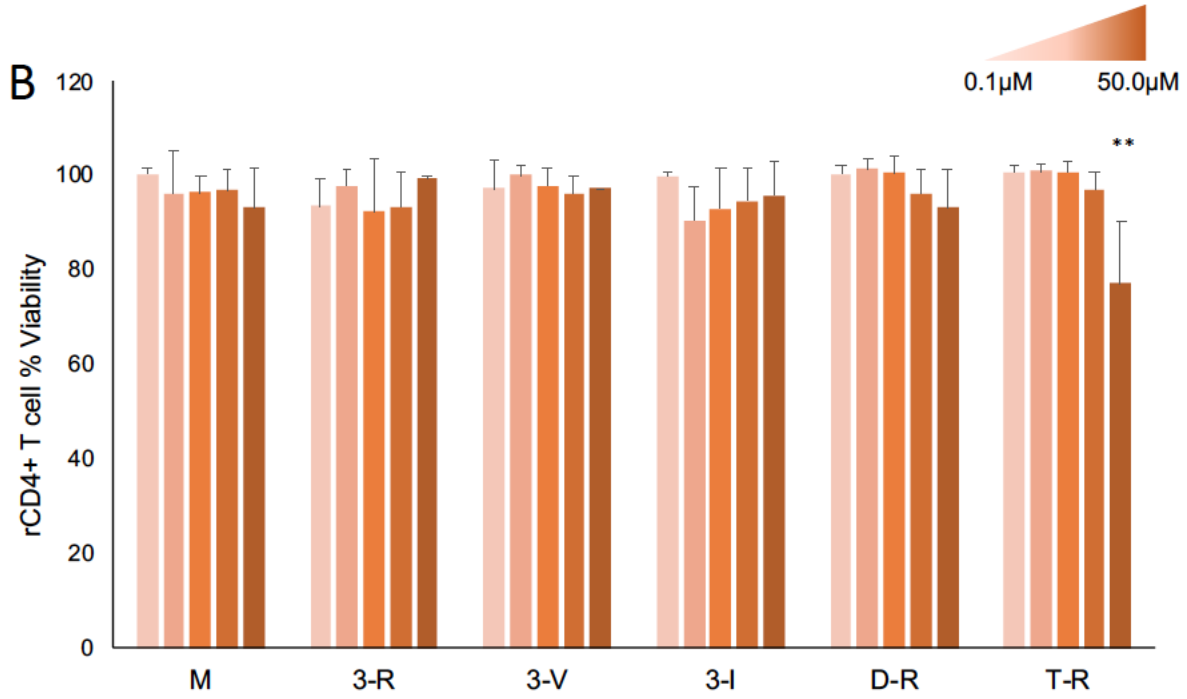
30

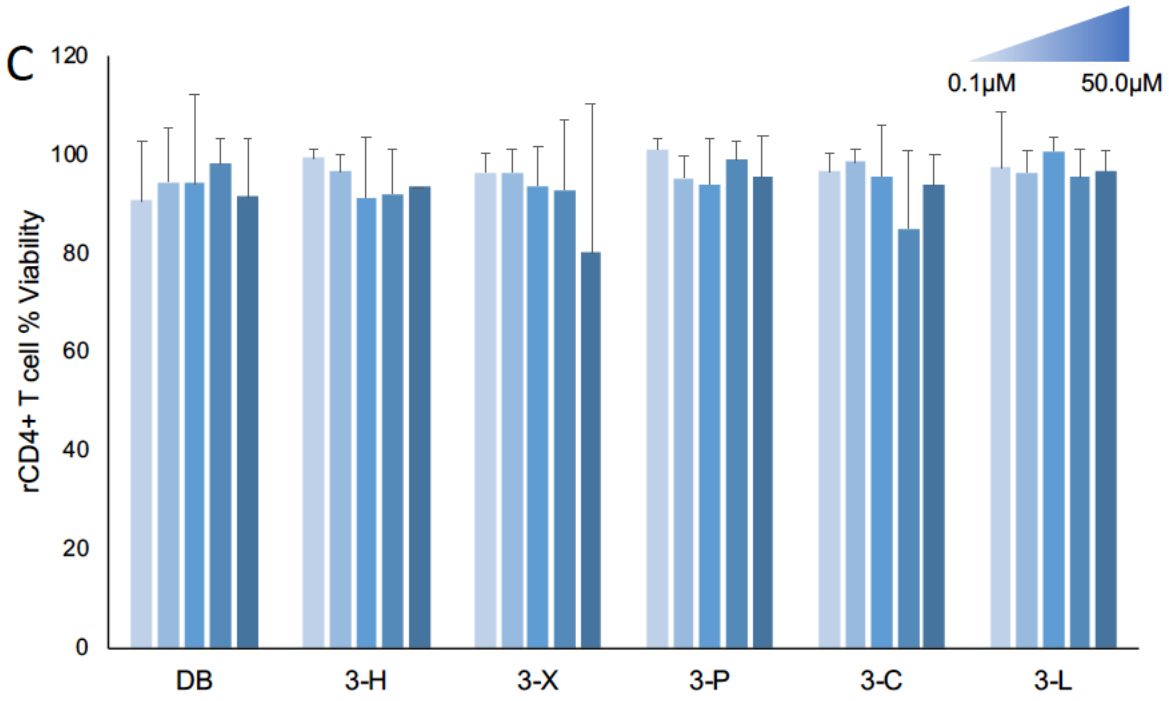


Resting primary CD4 cells isolated from HIV-1-uninfected participants (n=5) underwent 30-minute exposure to ingenol derivatives (100nM), Bryostatin-1 (100nM), or PMA (10 ng/mL). Cells underwent intracellular staining with anti-pPKC antibodies and were analyzed by flow cytometry. Changes in PKC isoform phosphorylation are represented as fold-change in mean fluorescence intensity (FC MFI) above untreated (negative) control cell cultures. Ingenol core and Ing 3-A, which have little to no latency reversal activity, did not induce any PKC isoform phosphorylation. Highly active ingenol derivatives Ing M, Ing 3-R, and Ing 3-X significantly induced phosphorylation of PKC isoforms PKCβ, PKCδ, PKCθ, and PKD protein. This PKC isoform phosphorylation pattern did not significantly differ among these ingenols or structurally distinct PKC agonists PMA or Bryostatin-1. Statistical significance was assessed using two-tailed paired student t-test with $p<0.05$ (*), $p<0.01$ (**), $p<0.001$(***).

Supplemental Figure 3: Primary rCD4^{+} T Cell Viability After Ingenol Exposure

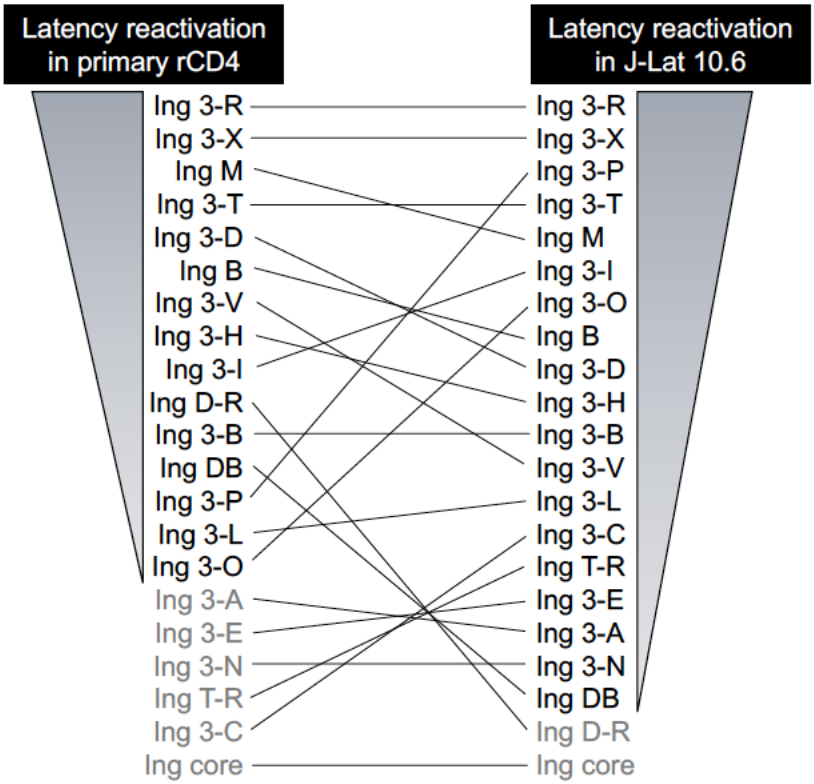






Resting CD4$^+$ T cells from aviremic HIV-1-positive participants (n=3) were exposed to linear, branched and cyclical ingenol-3-esters (**panels A, B** and **C** respectively) for 48 hours at concentrations ranging from 100nM to 50,000nM. Cell membrane permeability was determined by flow cytometry to quantify cytotoxicity induced by ingenol derivatives relative to medium-alone controls (set at 100% for each independent experiment). Color bars represent mean percentage of viable cells relative to this negative control, with standard deviation represented by error bars. No significant decrease in cellular viability occurred with any ingenol at concentrations between 100nM and 10,000nM. Ingenol derivatives 3-T, 3-D and T-R caused significant cell death at 50,000nM (unpaired T test, $P < 0.005$).

Supplemental Figure 4: Comparison of latency reversal efficacy between J-Lat 10.6 and primary T cells



Ingenol-3-esters are listed in order of decreasing latency reversing efficacy for primary resting $CD4^+$ T cells from aviremic participants (left) and J-Lat 10.6 cells (right). EC_{50} was used to define relative efficacy for J-Lats (**Figures 1-3**). For primary resting $CD4^+$ T cells, mean fold change in cell-associated HIV-1 mRNA transcript frequency in ingenol-exposed cultures (40nM for 48 hours in vitro) compared to medium-alone (negative) control cultures across a minimum of three independent experiments determined efficacy. Ingenols in grey showed no activity across any experiment. Four of the top five ingenols in J-Lats are represented among the top five ingenols in primary cells. Similarly, high EC_{50} values in J-Lats were predictive of minimal to no activity in primary cells.

Supplemental Table: Participant Characteristics

Participant	Age	Gender	Race / Ethnicity	$CD4^+$ T Cell Count[a]	Duration of Viral Suppression[b]	ART Regimen
H008	39	M	H	906	59	TAF/FTC/EVGc
H015	55	M	C	839	72	TAF/FTC/RAL
H016	46	M	C	1,959	56	TAF/FTC/RAL
H020	57	M	C	702	76	TAF/FTC/DRVr
H026	49	M	C	1,093	70	ABC/3TC/DTG
H031	58	M	C	677	38	TAF/FTC/RAL
H033	73	M	C	632	58	ABC/3TC/DTG
H043	49	M	C	464	47	ABC/3TC/DTG
H045	54	M	C	594	97	TAF/FTC/DTG
H052	54	M	C	281	13	TAF/FTC/RAL
H053	42	M	C	1047	30	TAF/FTC/DTG
H055	36	M	C	1,106	41	TAF/FTC/DTG

Abbreviations: 3TC, lamivudine; ABC, abacavir; C, non-Hispanic Caucasian; DRVr, darunavir boosted with ritonavir; DTG, dolutegravir; EVGc, elvitegravir boosted with cobicistat; FTC, emtricitabine; H, Hispanic; RAL, raltegravir; TAF, tenofovir alafenamide; TDF, tenofovir disoproxil fumarate

[a] Absolute CD4+ T cell count measured in cells/µL

[b] Consecutive months of documented viral load (plasma HIV-1 RNA) suppression below limit of clinical detection on ART