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Histone deacetylase inhibition reduces deleterious cytokine release induced by ingenol stimulation

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Abstract

Introduction: Though antiretroviral therapy has led to viral suppression and increased quality of life for patients living with HIV-1, strategies to eliminate the HIV-1 latent reservoir are still necessary to eliminate HIV. Latency reversal with superior latency reversal agents (LRAs) such as protein kinase C (PKC) agonists is a promising strategy for unveiling and eliminating the latent HIV-1 reservoir. However, PKC agonists induce T cell activation and deleterious pro-inflammatory cytokine production. Secondary pharmacological agents combined with LRAs have been previously shown to reduce deleterious pro-inflammatory cytokine secretion without inhibiting HIV-1 viral reactivation. Histone deacetylase inhibitors (HDACi) are also known for inhibiting deleterious pro-inflammatory cytokines in the context of graft-versus-host disease and rheumatoid arthritis in addition to being known to synergize with PKC agonists. In this study we investigated whether HDACi and other epigenetic modifiers could decrease PKC-induced pro-inflammatory cytokines secretion while simultaneously synergizing with the PKC agonists Ingenol-3,20-dibenzoate, to enhance latency reversal.

Methods: We screened an epigenetic modifier library in health donor human peripheral blood mononuclear cells (PBMCs) to identify compounds ('hits') that reduced intracellular IL-6 pro-inflammatory cytokine production induced by PKC agonist Ingenol-3,20-dibenzoate. We

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then further tested reducers of intracellular IL-6 ('hits') for their ability to synergize with Ingenol-3,20-dibenzoate in the J-LAT 10.6 model of HIV-1 latency. The most promising epigenetic modifier from both screens, the HDACi Panobinostat, was then further tested for its ability to reduce pro-inflammatory cytokines and synergize with Ingenol-3,20-dibenzoate.

Results: We show that co-treatment with Ingenol-3,20-dibenzoate and Panobinostat reduces pro-inflammatory cytokines and enhances latency reversal *in vitro*. Panobinostat suppressed pro-inflammatory cytokine production when combined with Ingenol-3,20-dibenzoate *ex vivo* when using aviremic patient cells, but antagonized Ingenol-3,20-dibenzoate dependent latency reversal *ex vivo*.

Conclusion: The combination of Panobinostat and Ingenol-3,20-dibenzoate reduces deleterious cytokine production but is not a suitable latency reversal combination therapy.

Keywords: HIV-1; Latency Reversing Agent; Ingenol-3,20-dibenzoate; Panobinostat; Synergy; Antagonist; Cytokine

Introduction

Antiretroviral therapy (ART) has profoundly improved the lives of individuals living with HIV-1 by reducing plasma viremia levels below the limit of detection (~20-50 copies/ml) [1-3] and allowing for reconstitution of the adaptive immune system. However, ART is unable to eradicate HIV-1 due to the presence of a silent, but long-lived latent viral reservoir [4-6]. The presence of this latent reservoir prevents patients from being removed from ART, making ART a lifelong necessity [7]. Additionally, not all patients have access to ART or are compliant with ART regiments due to high costs [8], side effects [9], and social stigmas [10, 11]. Aviremic individuals taking ART are prone to immune dysfunction conditions [12] and neurologic complications associated with HIV-1 [13] underscoring the importance of strategies targeting the latent HIV-1 reservoir.

Many strategies have been proposed to eliminate the latent HIV-1 reservoir including the "shock and kill" strategy [14], in which latency reversing agents (LRAs) are used to transcriptionally reactivate the latent reservoir prior to targeting with immune-mediated

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mechanisms [14, 15]. Additional strategies include the use of broadly neutralizing antibodies (bNAbs), cytokines, vaccines or other immune-mediated mechanisms to enhance recognition of HIV-1 infected cells via the immune system [16, 17] and identification of cellular markers specific to cells harboring latent HIV-1 to target latently infected cells [12]. While all strategies hold merit, enhanced targeting of latently infected cells via immune-mediated mechanisms would still be dependent on unveiling the latent reservoir. Therefore, unveiling latent HIV-1 via the use of LRAs may be necessary before additional targeting of infected cells.

Several classes of compounds have emerged as potential LRAs including positive transcription elongation factor b (PTEFb) activators, cytokines, histone deacetylase inhibitors (HDACi), and protein kinase C (PKC) agonists [15, 18]. However, not all LRAs appear to be created equal. Spina *et al*. [18] demonstrated that PKC agonists are superior LRAs, shown to reactivate latent HIV-1 across multiple models of HIV-1 latency, whereas other well characterized LRAs such as HDACi were unable to reactivate to the same extent in some models of HIV-1 latency. Additionally, PKC agonists have been shown to reactivate latent provirus and lead to cell death of HIV-1 infected cells [19]. PKC agonists are thought to reactivate latent HIV-1 though activation of NF-κB and have been shown to additionally activate AP-1 and NFAT [20].

While PKC agonists are superior LRAs, they also induce T cell activation [21] and pro-inflammatory cytokine secretion. Spivak *et al.* [22] first demonstrated that latency reversal with PKC agonist Ingenol-3,20-dibenzoate used in combination with a second pharmacological agent, Ruxolitinib, an FDA approved Janus Kinase (JAK) inhibitor, to suppress deleterious pro-inflammatory cytokine secretion, led to potent reactivation of latent HIV-1 and suppression of pro-inflammatory cytokine secretion induced by Ingenol compounds. Additionally, Rapamycin, an inhibitor of mTOR, has been shown to reduce pro-inflammatory cytokine secretion induced upon reactivation of latent HIV-1 with αCD3/αCD28 [23]. These studies suggest that latency reversal and cytokine secretion can be uncoupled and that potent reactivation with PKC agonists may be achieved without deleterious side effects of cytokine release. Therefore, we were interested in determining if additional compounds could be used to inhibit deleterious cytokines upon reactivation of latent HIV-1 with PKC agonists.

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While Ruxolitinib and Rapamycin have been shown to reduce *in vivo* cytokine secretion in the context of HIV-1, HDACi have also been well characterized to inhibit pro-inflammatory cytokine secretion *in vivo* as a result of graft-versus-host disease [24] and rheumatoid arthritis [25] as well as *in vitro*, as a result of LPS stimulation of human peripheral blood mononuclear cells (PBMCs) [26]. Additionally, HDACi are well known LRAs and although they have been characterized as being less potent LRAs than PKC agonists, HDACi have been shown to synergize with PKC agonists when reactivating latent HIV-1 [27-29]. Therefore, we hypothesized that HDACi and other epigenetic modifiers could decrease PKC-induced pro-inflammatory cytokines secretion while simultaneously synergizing with PKC agonists to enhance latency reversal. In this study, we show that Panobinostat, a broad-spectrum HDAC inhibitor, decreases PKC-induced pro-inflammatory cytokines while simultaneously enhancing latency reversal *in vitro* through synergy with PKC agonist Ingenol-3,20-dibenzoate. While the cytokine suppression ability of Panobinostat was maintained *ex vivo* when using aviremic patient cells, we observed that Panobinostat failed to synergize with Ingenol-3,20-dibenzoate in this system and, instead, exerted antagonism.

Methods

Participants

Healthy donors and aviremic HIV-1 positive patients were recruited in accordance with University of Utah Institutional Review Board (IRB) protocols 67637 (approved May 31, 2017) and 58246 (approved Jan 4, 2017). Aviremic HIV-1 positive participants included in the study were aviremic (plasma viral loads less than 50 HIV-1 RNA copies/mL) for a minimum of 6 months and compliant with an ART regimen initiated during chronic HIV-1 infection for a minimum of 12 months.

In vitro epigenetic inhibitor screening

PMBCs were isolated from healthy donors using a Lymphoprep density gradient (Cat# 07861, StemCell Technologies) prior to being cultured in RPMI medium supplemented with 10% FBS and 5 U/mL penicillin/streptomycin overnight to remove monocytes via adherence. Non-

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adherent PBMCs were then cultured at a density of 1 x 10^5 cells/100µL in the presence of 100nM compounds from a Cayman Chemical Epigenetics Screening Library obtained from the University of Utah Drug Discovery Core Facility (Item No. 11076) or 100nM Ruxolitinib, a control for cytokine inhibition, for 1.5 h at 37°C. Cells were then incubated in media alone or the presence of 100nM Ingenol-3,20-dibenzoate for 40 hours post-stimulant exposure. At 40 hours post-stimulant exposure, 0.067 µL/100µL BD GolgiStop™ Protein Transport Inhibitor (Cat# 554724) was added to each sample to inhibit cytokine secretion. Cells were then fixed and stained at 48 hours prior to flow cytometry analysis.

Intracellular cytokine staining and flow cytometry

Cells were washed with 1x phosphate buffered saline (PBS) prior to staining the cells with 0.1µL/100 µL Fixable Viability Dye eFluor® 450 (Cat# 65-0863-14, Affymetrix eBioscience) for 30 min at 4°C. Cells were then washed with 1x PBS prior to fixing with 100 µL BD Cytofix/Cytoperm™ for 30 min at 4°C. Once cells were fixed they were washed with a perm/wash solution (1x PBS, 3% FBS, 0.1% Saponin, 0.05% Sodium Azide) prior to staining cells in 100 µL perm/wash with 0.5 µL APC anti-human IL-6 antibody (Cat# 501112, Biolegend®) overnight at 4°C. Cells were finally washed in 1x PBS and re-suspended in PBS prior to flow cytometry [BD FACSCanto™ flow cytometer with FACSDiva™ acquisition software (Becton-Dickinson, Mountain View, CA)] and analysis with FlowJo (TreeStar Inc, Ashland, OR).

Selection of compounds

The percentage of IL-6-positive cells in ingenol-3,20-dibenzoate-alone-treated cells was compared to cells treated with epigenetic modifiers and Ingenol-3,20-dibenzoate in order to calculate intracellular IL-6 fold change. Epigenetic modifiers that reduced intracellular IL-6 by fourfold or greater ('hits') were selected for a secondary screen to examine the ability of these compounds to synergize with Ingenol-3,20-dibenzoate.

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Synergy Screening

J-LAT 10.6 cells [30] were cultured in RPMI supplemented with 10% FBS and 5 U/mL penicillin/streptomycin. J-LAT 10.6 cells were plated in 96 well u-bottom plates at a cell density of 5 x 10^5/100µL. Cells were then treated with a concentration gradient (1µM, 500nM, 100nM, 50nM, 10nM) of top epigenetic modifier 'hits' for 1 hour prior to treatment with 45nM Ingenol-3,20-dibenzoate, a suboptimal concentration in order to visualize synergy, for 24 hours. JQ-1 (+) was used as a positive control for synergy as it has been previously described to synergize with PKC agonists [31]. At 24 hours cells were stained with Fixable Viability Dye eFluor® 450 and fixed as described above. Latency reversal was then measured by the percentage of GFP^+ cells by flow cytometry as described above.

Ex vivo cell culture, qPCR, and cytokine measurement

PBMCs were isolated from HIV-1 positive aviremic donors prior to the isolation of resting $CD4^+$ T cells (rCD4s) (EasySep™ Human Resting $CD4^+$ T Cell Isolation Kit, Cat # 17962, StemCell Technologies). rCD4s were cultured at a density of 5 x 10^6/mL in RPMI supplemented with 10% FBS and 5U/mL penicillin/streptomycin. As Panobinostat was seen to be the most potent epigenetic modifier in reducing intracellular IL-6 and synergizing with Ingenol-3,20-dibenzoate at multiple concentrations, it was added to rCD4s in the presence of medium or Ingenol-3,20-dibenzoate (100nM) for 72 hours. Medium or DMSO and Dynabead Human T-Activator αCD3/αCD28 (αCD3/αCD28) (Cat# 111.32D) stimulated cells were used as controls. At 72 hours post-stimulation, supernatant was collected and the rapid *ex vivo* evaluation of anti-latency assay (REVEAL) assay was performed as previously described [32] to quantify viral release. Statistical analysis was performed using software from GraphPad Prism Version 5.0f (GraphPad Software, Inc., San Diego CA). Statistical significance was calculated using a non-parametric Wilcoxon matched-pairs signed rank test. Additionally, supernatant was collected to measure *ex vivo* cytokine secretion. Supernatant was sent to ARUP Laboratories to measure *ex vivo* cytokine secretion via a commercially available quantitative multiplex bead assay to measure the following cytokines: interferon gamma (IFN-γ), tumor necrosis factor alpha (TNF-α), interleukin (IL) 1 beta (IL-1β), IL-2, soluble IL-2 receptor (IL-2r), IL-4, IL-5, IL-6, IL-8, IL-10, IL-

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12, and IL-13. Percent change in the secretion of pro-inflammatory cytokines compared to Ingenol-3,20-dibenzoate alone treated cells was calculated with GraphPad Prism Version 5.0f (GraphPad Software, Inc., San Diego CA).

Results

As HDACi have been previously described to inhibit cytokine secretion [24-26] and to synergize with PKC agonists [27-29], we investigated the ability of HDACi and other epigenetic modifiers to reduce pro-inflammatory cytokine secretion upon latency reversal with PKC agonists. A screen of 96 epigenetic modifiers was conducted to identify epigenetic modifier 'hits' that reduced the induction of pro-inflammatory cytokines during latency reversal with Ingenol 3,20-dibenzoate. Nine epigenetic modifiers from the epigenetic library were identified as reducing intracellular IL-6 by fourfold or greater when combined with Ingenol-3,20-dibenzoate (Figure 1). These 'hits' include six HDACi (Chemical Abstracts Service (CAS) # 743420-02-2 (Chidamide), CAS # 183506-66-3 (Apicidin), CAS # 209783-80-2 (Entinostat), CAS # 38937-66-5 (Suberhydroxamic Acid (SBHA)), CAS # 382180-17-8 (Pyroxamide), CAS # 404950-80-7 (Panobinostat)), one mixed lineage leukemia (MLL) inhibitor Cas # 890190-22-4 (WDR5-0103), and two compounds categorized as miscellaneous as they did not have the same function as other epigenetic modifiers in the screen. Out of the two 'hits' categorized as miscellaneous, the compound Cas # 160003-66-7 (Iniparib) has an unknown function and compound Cas # 1207113-88-9 (CCG-100602) is an inhibitor of Rho pathway mediated signaling [33]. The positive control Ruxolitinib also reduced intracellular IL-6 by greater than fourfold.

Compounds identified as 'hits' for their ability to suppress cytokine production were then tested for synergy with PKC agonist Ingenol-3,20-dibenzoate in J-LAT 10.6 cells [30], as calculated by the Bliss Independence model [34]. Upon visualization of latency reversal by the percentage of GFP^+ cells of Ingenol-3,20-dibenzoate (control) or Ingenol-3,20-dibenzoate plus epigenetic modifier treated cells, all nine epigenetic modifiers were identified to synergize with Ingenol-3,20-dibenzoate at one or more concentrations (Figure 2). Panobinostat (10nM, 50nM, 100nM, and 500nM) synergized with Ingenol-3-20-dibenzoate with the highest level of reactivation seen at 100nM. Panobinostat was not tested at 1µM due high cellular toxicity.

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Apicidin treatment led to the second highest level of reactivation but was only found to be synergistic at the highest concentration tested, 1uM. Following Apicidin, compounds Entinostat (10nM, 500nM, 1µM), Chidamide (1µM), Pyroxamide (1µM), CCG-100602 (50nM, 500nM, 1µM), SBHA (10nM), WDR5-0103 (100nM, 500nM, 1µM), and Iniparib (100nM) were found to be synergistic with Ingenol-3,20-dibenzoate but induced low levels of reactivation. JQ-1 (+) was used as a positive control for synergy as it has been previously described to synergize with PKC agonists [31] and was found to synergize with Ingenol-3,20-dibenzoate at all concentrations except 10nM. Ruxolitinib, the positive control for intracellular IL-6 reduction, as expected, did not synergize with Ingenol-3,20-dibenzoate.

As Panobinostat was observed to be the most potent reducer of intracellular IL-6 and led to the highest level of reactivation by synergizing with Ingenol-3,20-dibenzoate at multiple concentrations, Panobinostat was then tested for the same activities *ex vivo* in $CD4^+$ T cells isolated from HIV-1 positive aviremic patients. Panobinostat in combination with Ingenol-3,20-dibenzoate was found to substantially reduce secreted levels of TNF-α (91.5% ± standard deviation (SD) 10.4), IFN-γ (98.3% ± SD 1.0), IL-1β (54.8% ± SD 44.2), and IL-6 (83.8% ± SD 8.4) (n=2) (donors H012 and H035) (Figure 3A) consistent with our results from our *in vitro* intracellular IL-6 screen. The combination of Ingenol-3,20-dibenzoate and Panobinostat also diminished the induction of IL-5, IL-10, IL-13, and IL-2r, with IL-12 levels remaining unchanged in both donors and IL-8 and IL-2 levels showing inconsistent trends between donors (data not shown). However, when examining the ability of Panobinostat and Ingenol-3,20-dibenzoate to synergize *ex vivo*, our encouraging initial screening results in J-Lat 10.6 cells failed to reproduce in patient cells. Ingenol-3,20-dibenzoate alone and αCD3/αCD28 alone significantly induced reactivation of the latent reservoir (mean = 16096 mRNA copies/mL, p value = 0.0313 and mean = 15286 mRNA copies/mL, p value = 0.0078 respectively, n=8) (Figure 3B). Panobinostat alone resulted in potent viral reactivation in rCD4s from three donors (H012, H026, and H035) out of 8 (mean = 6959 mRNA copies/mL, n = 8). The addition of Panobinostat to Ingenol-3,20-dibenzoate (mean = 7535 mRNA copies/mL, n = 8) reduced the overall response, when compared to Ingenol alone (mean = 16096 mRNA copies/mL, p value = 0.0313, n=8). Panobinostat was determined to be antagonistic to Ingenol-3,20-dibenzoate by the Bliss

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Independence model [34]. In three out of six donors the addition of Panobinostat to Ingenol-3,20-dibenzoate to rCD4s ablated reactivation completely (donors H010, H020, and H033) with reactivation only seen in the donors whose cells reactivated with Panobinostat alone (H012, H026, H035). A possible interpretation for these results is that Panobinostat has a potent suppressive activity over Ingenol-3,20-dibenzoate, which appears to be dominant. Reactivation in cells from two donors, H013 and H018, was only observed upon treatment with αCD3/αCD28.

Discussion

In order to eliminate the latent HIV-1 reservoir, LRAs must be evaluated based on their potency and deleterious side effects. The most potent LRAs that have emerged across multiple models of HIV-1 latency are PKC agonists [18]. However, PKC agonists are known to induce T cell activation [21] and deleterious cytokines [22, 35]. Previous reports have demonstrated that LRAs can be combined with a second pharmacological agent to suppress deleterious pro-inflammatory cytokines induced upon treatment with PKC agonists [22] and TCR stimulation [23]. HDACi such as SAHA [24, 26], Trichostatin A [25], and Nicotinamide [25] have been shown to reduce pro-inflammatory cytokines in the context of graft-versus-host disease [24], rheumatoid arthritis [25], and LPS-stimulated human PBMCs [26]. Additionally, HDACi have been shown to synergize with PKC agonists when reactivating latent HIV-1 [27-29]. Therefore, we sought to evaluate the ability of HDACi and other epigenetic modifiers for their ability to suppress pro-inflammatory cytokines and to synergize with the PKC agonist Ingenol-3,20-dibenzoate. We found that Panobinostat, a broad-spectrum inhibitor of HDACs, reduced pro-inflammatory cytokines in healthy donor PBMCs and synergized with Ingenol-3,20-dibenzoate in J-LAT 10.6 cells *in vitro.* Furthermore, we observed that the addition of Panobinostat to Ingenol-3,20-dibenzoate substantially reduced TNF-α, IFN-γ, IL-1β, and IL-6 in cells from HIV-1 positive aviremic individuals *ex vivo*. However, Panobinostat appeared to act in an antagonist manner to Ingenol-3,20-dibenzoate by diminishing or inhibiting reactivation in rCD4s isolated from aviremic patients. As we demonstrated a synergistic relationship between Panobinostat and Ingenol-3,20-dibenzoate in the J-LAT 10.6 *in vitro* HIV-1 latency model but saw an

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antagonist relationship between the two compounds in regards to latency reversal *ex vivo* we turned to the literature for possible explanations.

It has been well described that there are discrepancies between latently infected T cell lines and primary cell models of HIV-1 latency [18]. Spina et al. [18] demonstrated that compounds found to be strong LRAs in some cell models of latency did not induce reactivation in others which likely explains the discrepancies between our *in vitro* and *ex vivo* synergy/antagonism results. In regards to why Panobinostat would antagonize Ingenol-3,20-dibenzoate induced reactivation, upon examining the literature we found several potential explanations. We found that Panobinostat, as well as another HDACi, Dacinostat, are known to induce acetylation of the chaperone protein heat shock protein 90 (Hsp90) leading to reduced binding of ATP to Hsp90 [36]. This acetylation prevents Hsp90 from interacting with its client proteins Bcr-Abl and Akt leading to reduced levels of these proteins [36, 37] and reduced levels of pAkt in chronic myeloid leukemia blast crisis (CML-BC) K562 cells and acute leukemia MV4-11 (AML) cells [38]. Bcr-Abl and Akt have both been shown to modulate NF-κB [39, 40] indicating that diminished Bcr-Abl and Akt protein due to acetylation of Hsp90 would likely cause reduced levels of NF-kβ expression and in turn reduced reactivation.

Additionally, Hsp90 has been shown to directly control HIV-1 reactivation from latency [41, 42], a relationship that is significantly diminished upon inhibition of Hsp90 by 17-AAG [41], an Hsp90 inhibitor. Inhibition of Hsp90 by 17-AAG was seen to significantly reduce PKC induced reactivation by Prostratin, while it did not appear to significantly reduce latency reversal by the HDACi SAHA [41]. This observation suggests that HDACi induction of latency reversal is independent of Hsp90 activity while PKC agonist activity is tied to Hsp90 and could, in theory, explain why Panobinostat alone induced reactivation in some donors but led to reduced reactivation when combined with Ingenol-3,20-dibenzoate. However, further studies are necessary before we can propose a definitive mechanism for why Panobinostat acts antagonistically towards Ingenol-3,20-dibenzoate.

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Conclusions

Panobinostat substantially decreases Ingenol-3,20-dibenzoate-induced pro-inflammatory cytokine release but significantly inhibits latency reversal *ex vivo*. While a Panobinostat and Ingenol-3,20-dibenzoate combination does not appear to be a suitable latency reversal combination therapy, other epigenetic modifiers could potentially be used in a combination therapy with PKC agonists to reduce the induction of pro-inflammatory cytokines. However, further studies are necessary to determine the effects of these epigenetic modifiers on latency reversal when used in combination with PKC agonists.

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Competing interests

The authors declare no competing interests.

Ethics approval

Heathy donors and HIV-1 positive aviremic individuals on ART were recruited for phlebotomy according to Institutional Review Board (IRB) protocols 67637 (approved May 31, 2017) and 58246 (approved Jan 4, 2017) at the University of Utah.

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Figure Legends

Figure 1. Epigenetic modifiers screening identifying compounds that dampen cytokine production induced by Ingenol-3,20-dibenzoate. Screening of 96 epigenetic modifiers for compounds that reduced intracellular IL-6 induced by Ingenol-3,20-dibenzoate in healthy donor PBMCs (n=1) revealed nine epigenetic modifier 'hits' that reduce intracellular IL-6 by fourfold or greater (at or below dotted line) when compared to Ingenol treatment alone. Compounds are listed by CAS number and color coded according to categorized function. All nine 'hits' were selected for further testing.

Figure 2. Epigenetic modifiers screening identifying compounds that synergize with PKC agonist Ingenol-3,20-dibenzoate in J-LAT 10.6 cells. Screening of control Ruxolitinib and nine epigenetic modifier 'hits' identified from screening of epigenetic modifiers that reduced intracellular IL-6 cytokine production. JQ1 (+) was included as a positive control for synergy with PKC agonist Ingenol-3,20-dibenzoate. Epigenetic modifiers and controls were tested in a concentration gradient from 1µM to 10nM (green triangle) and viral reactivation was measured by the percentage of GFP^+ cells. All nine epigenetic modifier 'hits' from the previous screen were identified by Bliss Independence to synergize (asterisk) with Ingenol-3,20-dibenzoate (Ing DB) at one or more concentrations when combined with Ingenol-3,20-dibenzoate (solid bars) compared to epigenetic modifiers alone (empty bars), Media alone (dotted line), and 45nM Ingenol-3,20-dibenzoate alone (solid line). Bars are colored according to the category of compound: red (HDACi); yellow (MLL inhibitor); orange (BET inhibitor); sea green (JAK inhibitor).

Figure 3A. Panobinostat reduces pro-inflammatory cytokine release induced by Ingenol-3,20-dibenzoate *ex vivo*. Bars represent the mean change in pro-inflammatory cytokine concentrations in the supernatant of rCD4s isolated from HIV-1 positive aviremic individuals (n = 8) and treated with Panobinostat and Ingenol-3,20-dibenzoate for 72 hours compared to Ingenol-3,20-dibenzoate alone. Standard deviation is indicated by error bars.

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Figure 3B. Panobinostat antagonizes Ingenol-3,20-dibenzoate induced viral reactivation *ex vivo*. Treatment of rCD4 cells isolated from HIV-1 positive aviremic individuals (n = 8) with Panobinostat resulted in viral reactivation in three donors (H012, H026, and H035) out of eight. Ingenol-3,20-dibenzoate treatment significantly increased viral latency reactivation by inducing latency reversal in six out of eight donors. The combined treatment of Panobinostat and Ingenol-3,20-dibenzoate resulted in reactivation only in donors seen to reactivate with Panobinostat alone treatment (H012, H026, and H035). The positive control αCD3/αCD28 resulted in viral reactivation in all donors. Data from donors H013, H018, H020, and H026 was previously published [32], excluding the Panobinostat and Ingenol-3,20-dibenzoate combined data. *P value <0.05; ** P value <0.01.

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Figure 1

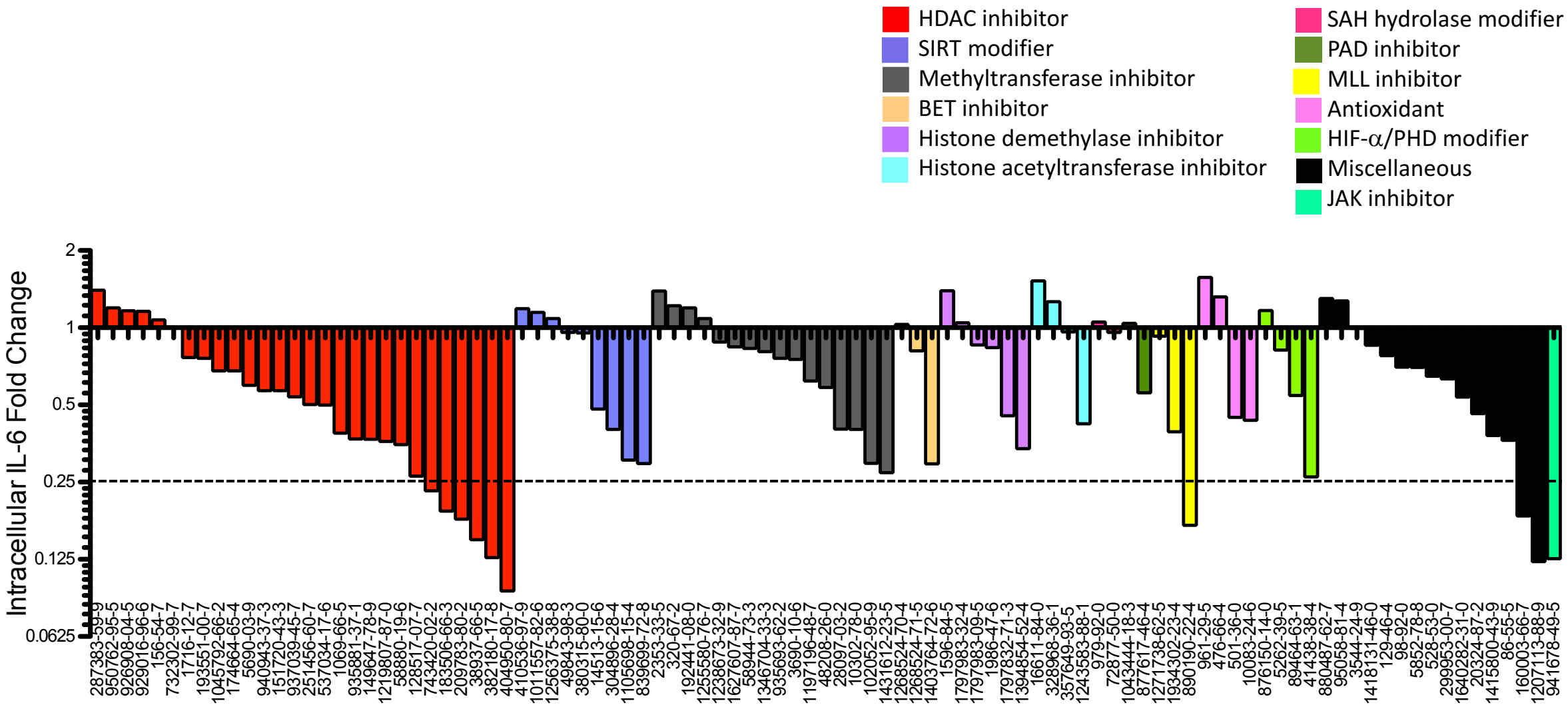
HDAC inhibitor
SIRT modifier
Methyltransferase inhibitor
BET inhibitor
Histone demethylase inhibitor
Histone acetyltransferase inhibitor
SAH hydrolase modifier
PAD inhibitor
MLL inhibitor
Antioxidant
HIF-α/PHD modifier
Miscellaneous
JAK inhibitor
Intracellular IL-6 Fold Change
2
1
0.5
0.25
0.125
0.0625
287383-59-9
950762-95-5
926908-04-5
929016-96-6
156-54-7
732302-99-7
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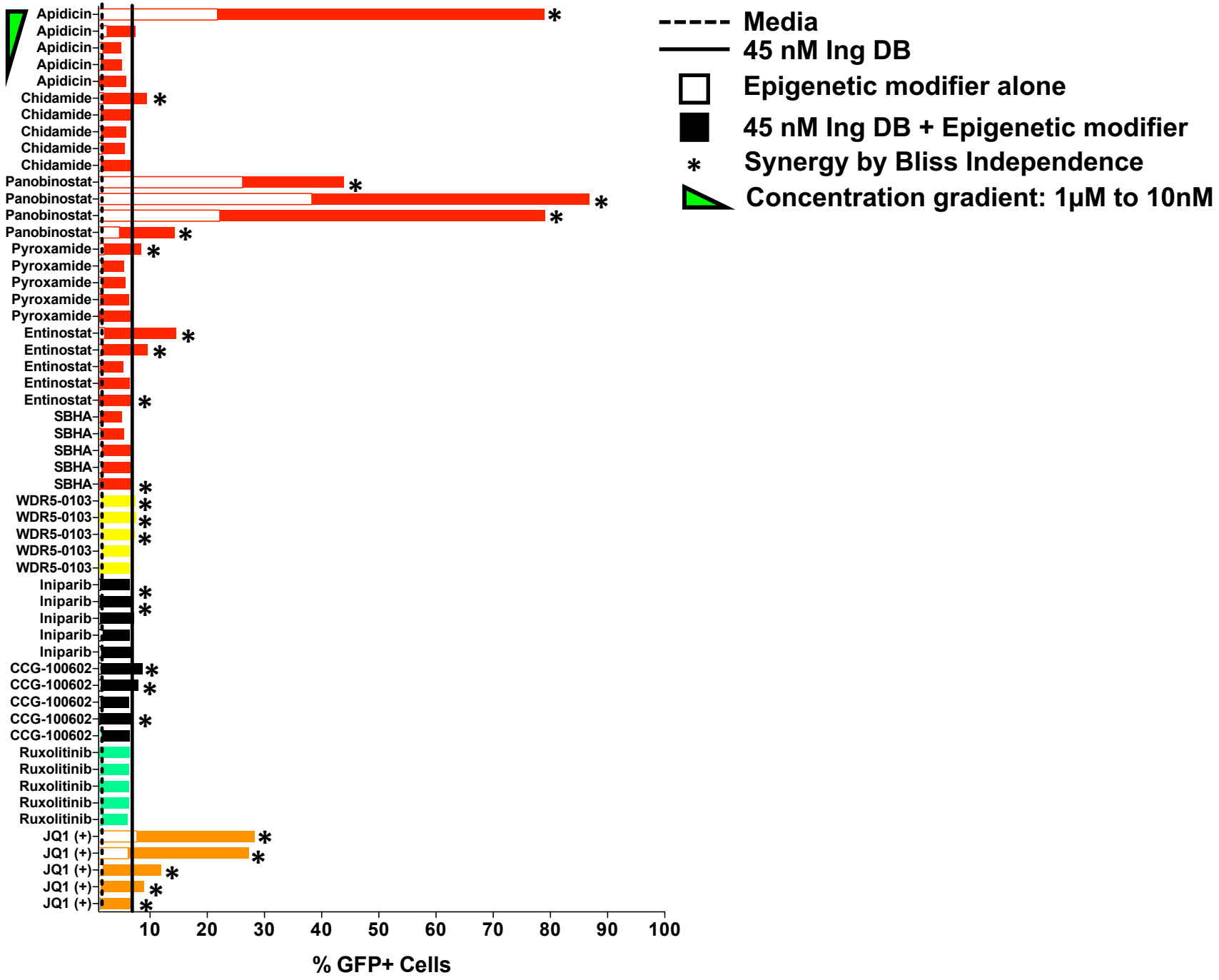
Figure 2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Chidamide
Panobinostat
Pyroxamide
Entinostat
SBHA
WDR5-0103
Iniparib
CCG-100602
Ruxolitinib
JQ1 (+)
10
20
30
40
50
60
70
80
90
100
% GFP+ Cells
Media
45 nM Ing DB
Epigenetic modifier alone
45 nM Ing DB + Epigenetic modifier
Synergy by Bliss Independence
Concentration gradient: 1μM to 10nM

Figure 3A

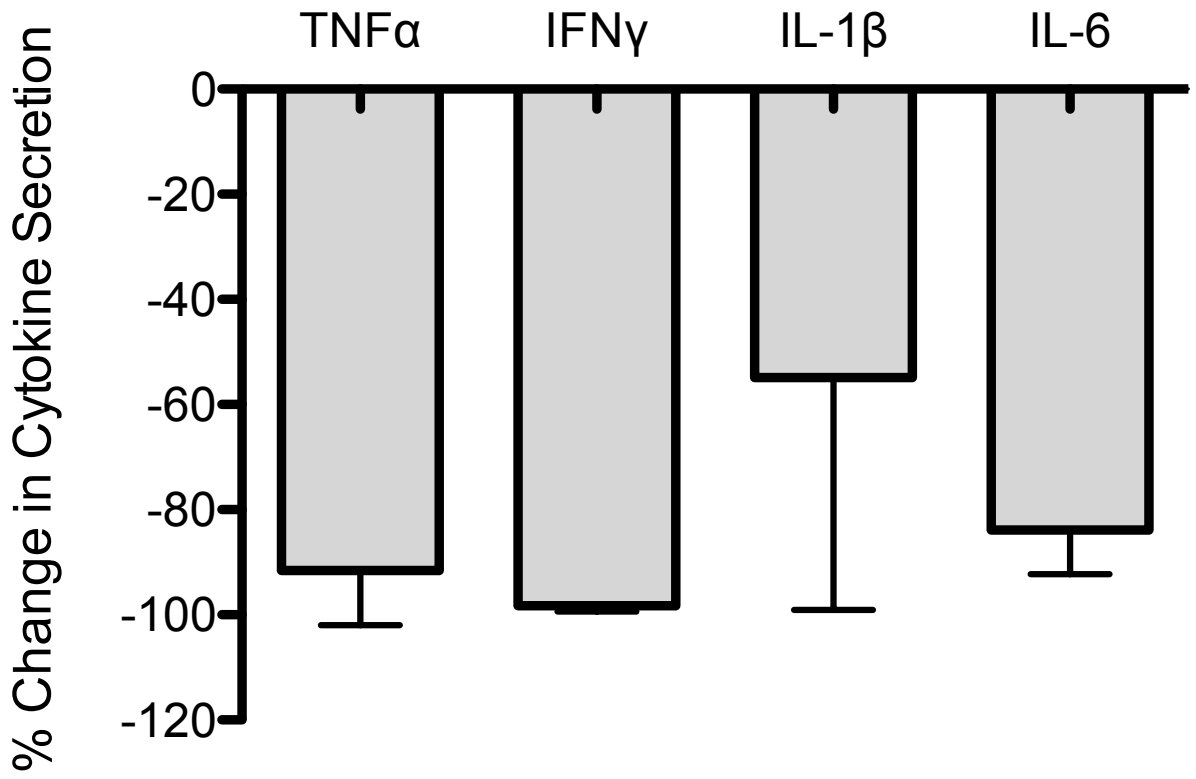
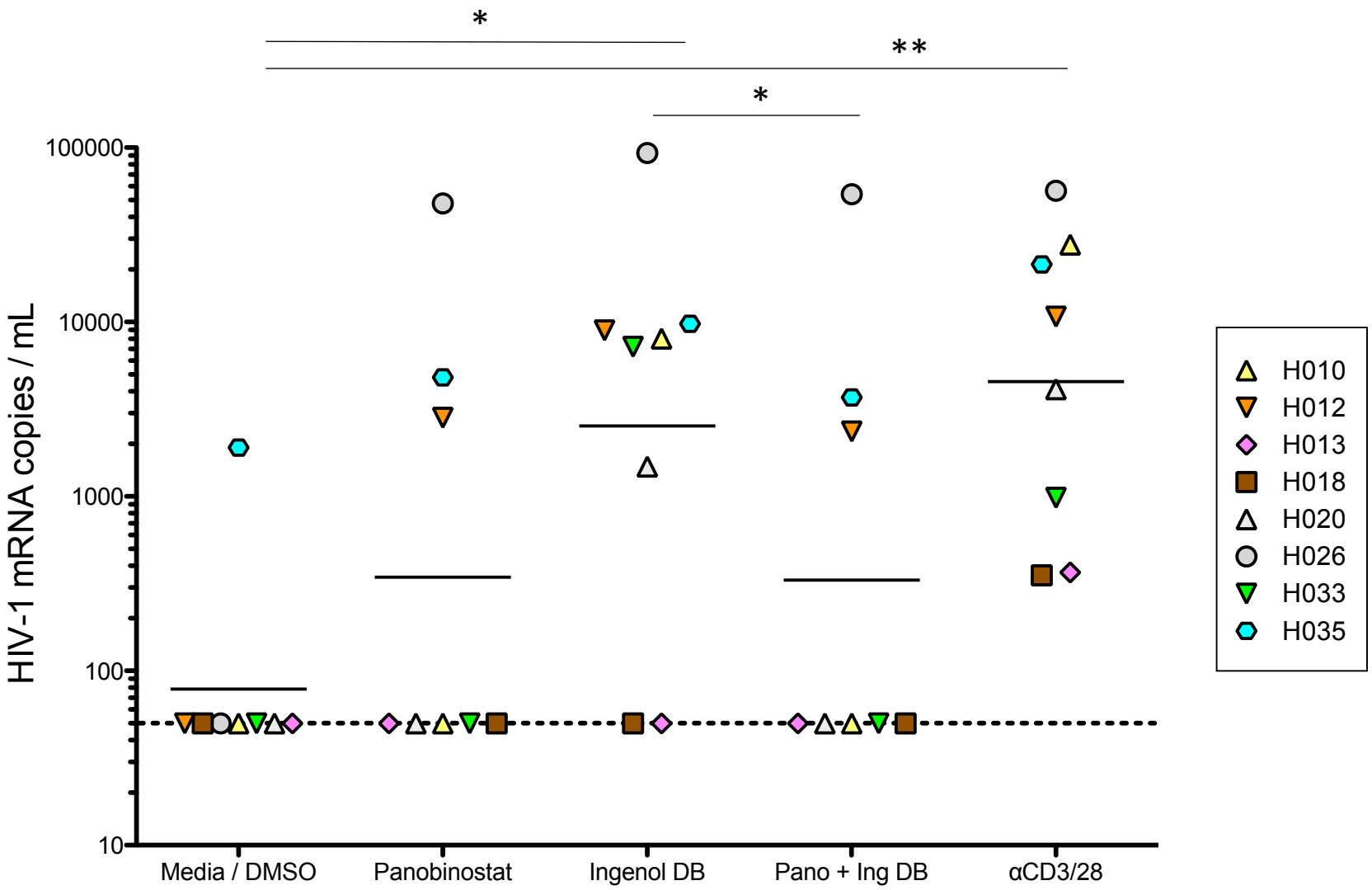

TNFα
IFNγ
IL-1β
IL-6
% Change in Cytokine Secretion
0
-20
-40
-60
-80
-100
-120

Figure 3B


*
**
*
100000
10000
1000
100
10
HIV-1 mRNA copies / mL
Media / DMSO
Panobinostat
Ingenol DB
Pano + Ing DB
αCD3/28
H010
H012
H013
H018
H020
H026
H033
H035