

**Differential effects of cannabinoid receptor 2 agonists on HIV replication and inflammatory activation in monocyte-derived macrophages and induced pluripotent stem cell-derived microglia**

Alexander Starr<sup>1</sup> (ORCID: 0000-0002-0756-4082), Sara Rathore<sup>1</sup>, Marzieh Daniali<sup>2</sup> (ORCID: 0000-0001-7703-8777), Peter J. Gaskill<sup>2</sup> (ORCID: 0000-0003-0095-5424), Cagla Akay-Espinoza<sup>1</sup> (ORCID: 0000-0003-0199-3836), Kelly L. Jordan-Sciutto<sup>1</sup> (ORCID: 0000-0002-0827-1337)

<sup>1</sup> Department of Oral Medicine, School of Dental Medicine, University of Pennsylvania, Philadelphia, PA 19104

<sup>2</sup> Department of Pharmacology & Physiology, Drexel University College of Medicine, Philadelphia, PA 19102

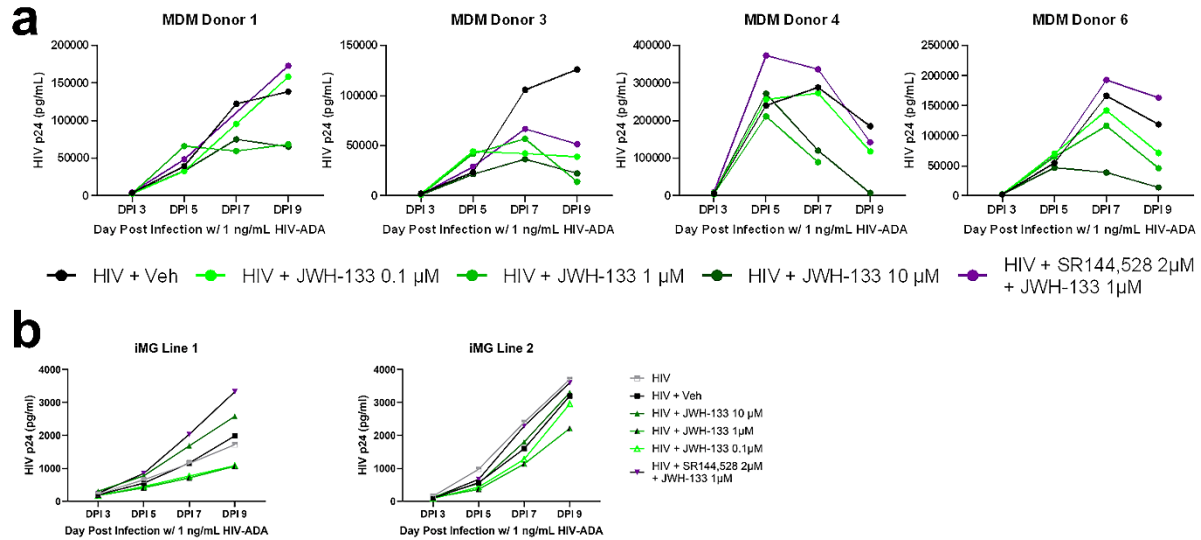
**Corresponding author:**

Kelly L. Jordan-Sciutto

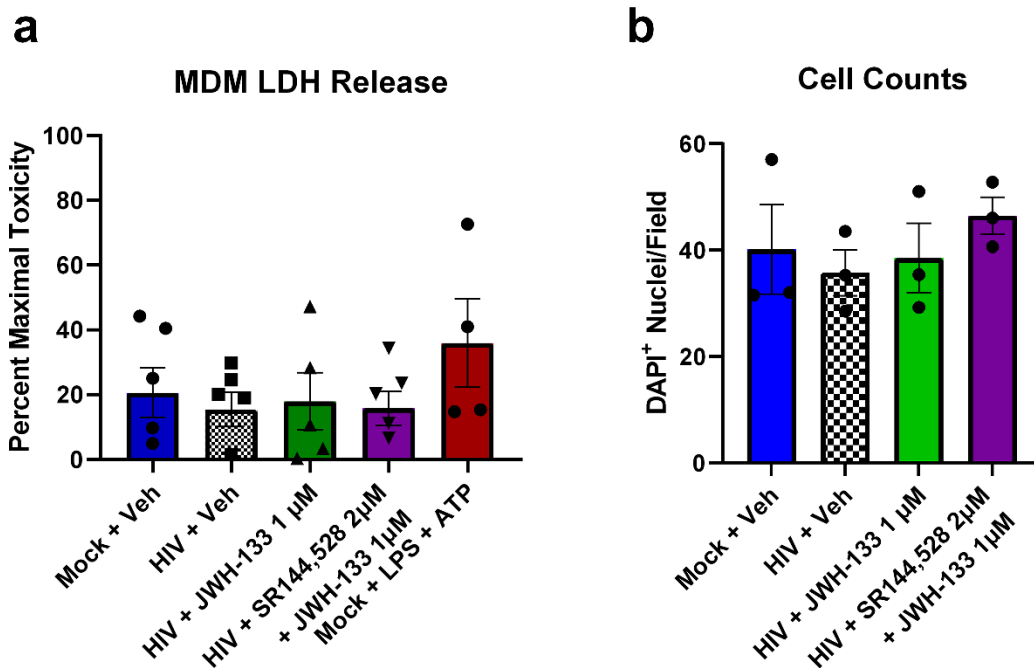
Department of Oral Medicine, School of Dental Medicine, University of Pennsylvania

240 S. 40th St, Rm 312 Levy, Philadelphia, PA 19104

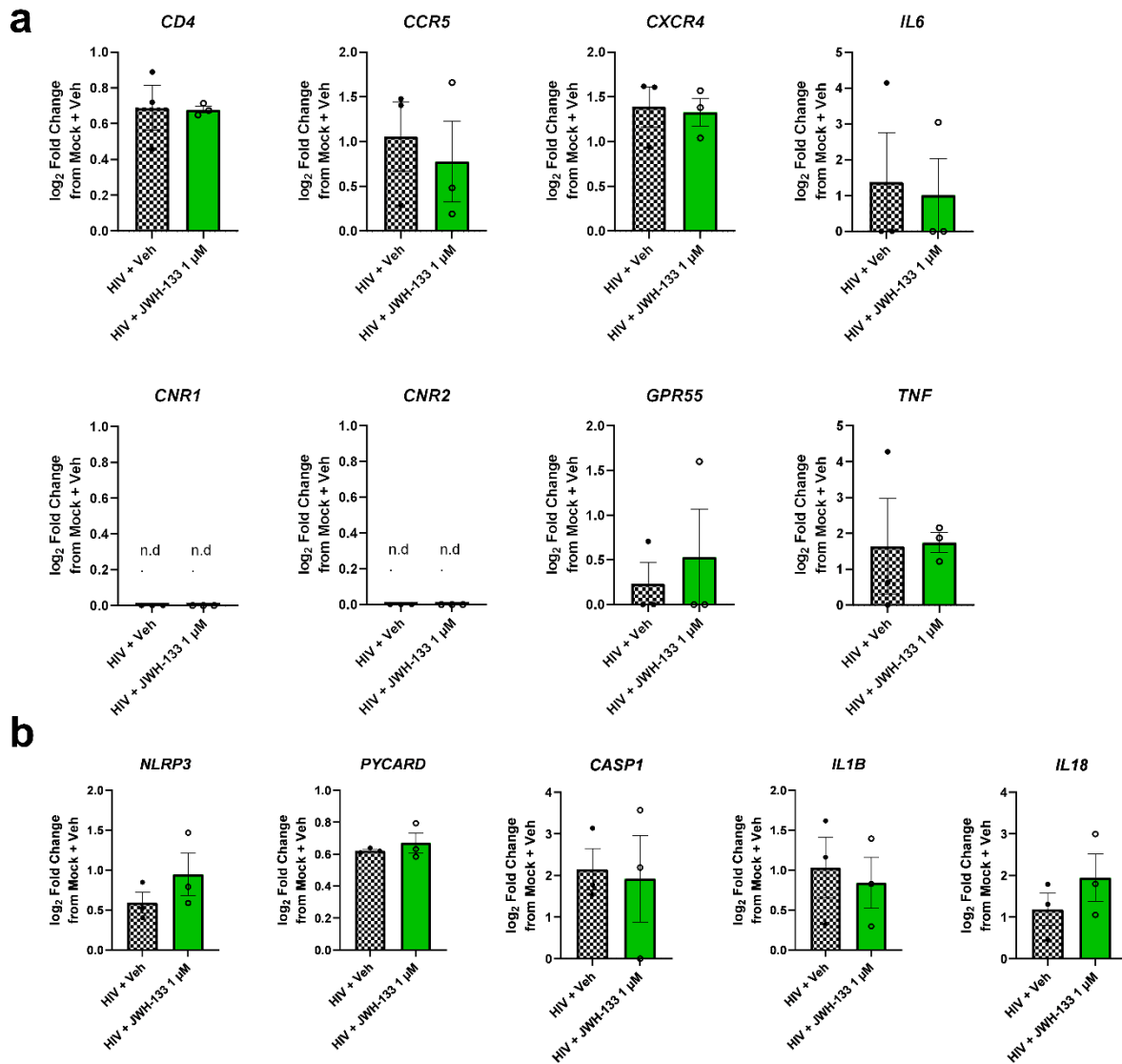
Phone: 215-898-4196; E-mail: jordank@upenn.edu



**Supplementary Figure S1. CB<sub>2</sub> agonists dose-dependently reduce HIV replication in macrophages and microglia.** Quantification of HIV p24 levels in supernatants of human monocyte-derived macrophages (MDMs) (a) and human induced pluripotent stem cell-derived microglia (iMg) (b), measured by AlphaLISA



**Supplementary Figure S2. HIV<sub>ADA</sub> and CB<sub>2</sub>-specific cannabinoids are not cytotoxic in MDMs and iMg at DPI 9.** **a.** LDH release in MDM supernatants was quantified using the Cytotoxicity Detection kit. Data are presented as percentage of maximal cell toxicity in positive controls. n = 5 MDM donors. **b.** DAPI<sup>+</sup> iMg nuclei are averaged from 5 wells/condition across 3 differentiations.

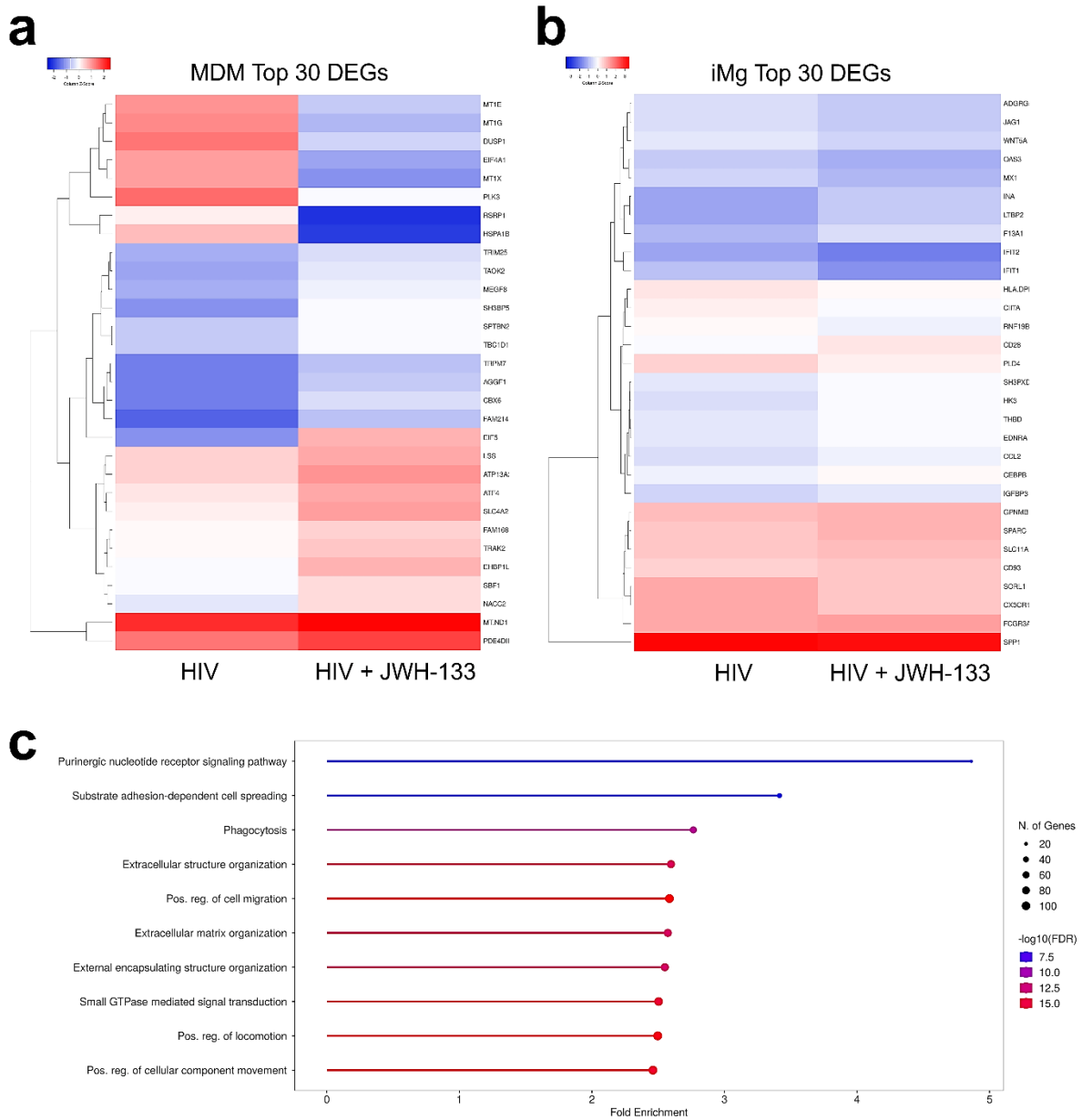


**Supplementary Figure S3. RNA-seq transcript counts are validated by qRT-PCR. a.**

Expression of key HIV entry, endocannabinoid system, and cytokine transcripts in MDMs, measured by qRT-PCR, represented as fold change from Mock + Veh, n = 3 MDM donors.

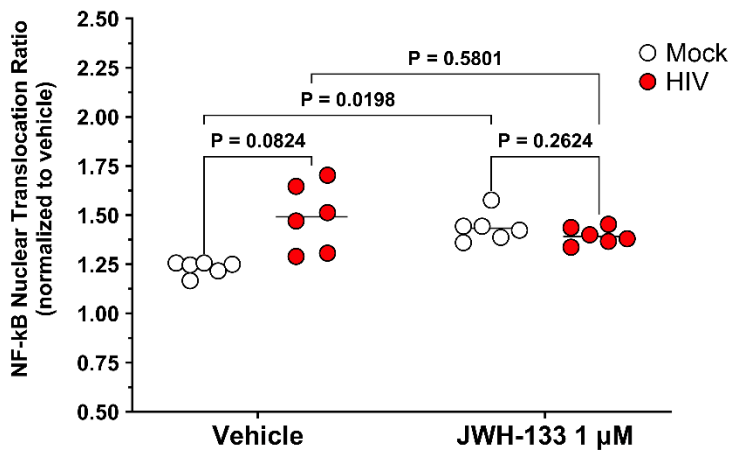
**b.** Expression of NLRP3 inflammasome transcripts in MDMs, measured by qRT-PCR, represented as fold change from Mock + Veh, n = 3 MDM donors

n.d., not detected



**Supplementary Figure S4. RNA-seq identifies divergent responses to CB<sub>2</sub> signaling across HIV-infected myeloid cell cultures. a.** Heatmap showing Manhattan plot clustering of log<sub>2</sub> normalized read counts per million of the top 30 significant differentially expressed genes (DEGs) in HIV + DMSO vs HIV + JWH-133 MDM, **b.** HIV + DMSO vs HIV + JWH-133 iMg. **c.** Top 10 GO Biological Process gene sets that are enriched in the

significant DEGs of HIV + JWH-133 MDM vs HIV + JWH-133 iMg. n = 2 MDM donors, n = 2 iMg lines



**Supplementary Figure S5. CB<sub>2</sub>-specific agonists do not suppress NLRP3 inflammasome priming in HIV-infected iMg.** Data showing the ratio of iMg with NF-κB nuclear translocation determined using immunofluorescence-based NF-κB translocation assay. Average of 3 wells/conditions from 2 differentiations in 3 iMg lines, two-way ANOVA with Tukey's correction for multiple comparisons