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Supplemental information

Identification of aryl hydrocarbon receptor as a barrier to HIV-1 infection and outgrowth in $CD4^+$ T cells

Debashree Chatterjee, Yuwei Zhang, Christ-Dominique Ngassaki-Yoka, Antoine Dutilleul, Soumia Khalfi, Olivier Hernalsteens, Tomas Raul Wiche Salinas, Jonathan Dias, Huicheng Chen, Yasmine Smail, Jean-Philippe Goulet, Brendan Bell, Jean-Pierre Routy, Carine Van Lint, and Petronela Ancuta

Supplemental Table 1: Clinical parameters of HIV-infected study participants receiving viral suppressive antiretroviral therapy (ART).

Participant ID	Sex	Age *	Ethnicity	CD4 #	CD8 #	CMV	PVL &	Time since infection**	ART	Time of aviremia**
ART #1	M	36	C	398	775	-	<40	154	Complera	22
ART #2	M	49	C	542	803	N/A	<40	13	Stribild	N/A
ART #3	M	51	C	458	899	+	<40	227	Truvada Viramune	N/A
ART #4	M	30	C	841	1322	+	<40	149	Sustiva Truvada	N/A
ART #5	M	47	C	598	605	+	<40	80	Stribild	N/A
ART #6	M	33	C	425	1156	+	<40	182	Atripla	N/A
ART #7	M	54	C	908	854	N-A	<40	89	Stribild	70

M, male; *, years; C, Caucasian; #, cells/µl; CMV, cytomegalovirus status; (-), negative; (+), positive; PVL, plasma viral load; &, HIV-RNA copies per ml plasma; **, months; ART, antiretroviral therapy; N/A, information not available

Supplemental Table 2: Clinical parameters of HIV-uninfected study participants.

Participant ID	Sex	Age*	Ethnicity	CD4 counts#	CD8 counts#	CMV
HIV- #1	M	60	C	710	370	+
HIV- #2	M	63	C	675	205	+
HIV- #3	M	71	C	576	176	+
HIV- #4	M	56	C	507	269	+
HIV- #5	F	40	C	N/A	N/A	+
HIV- #6	M	69	C	634	346	+
HIV- #7	M	41	C	1,144	826	+
HIV- #8	M	35	C	854	492	+
HIV- #9	F	57	C	624	333	+
HIV- #10	M	47	C	604	147	+
HIV- #11	M	66	C	1,115	545	+
HIV- #12	M	60	C	704	487	+
HIV- #13	M	44	C	845	333	+
HIV- #14	M	68	C	865	589	+
HIV- #15	M	59	C	821	197	-
HIV-#16	N/A	N/A	N/A	N/A	N/A	N/A
HIV-#17	N/A	N/A	N/A	N/A	N/A	N/A

M, male; F, female; *, years; C, Caucasian; #, cells/µl; CMV, cytomegalovirus status; (-), negative; (+), positive;

N/A, information not available

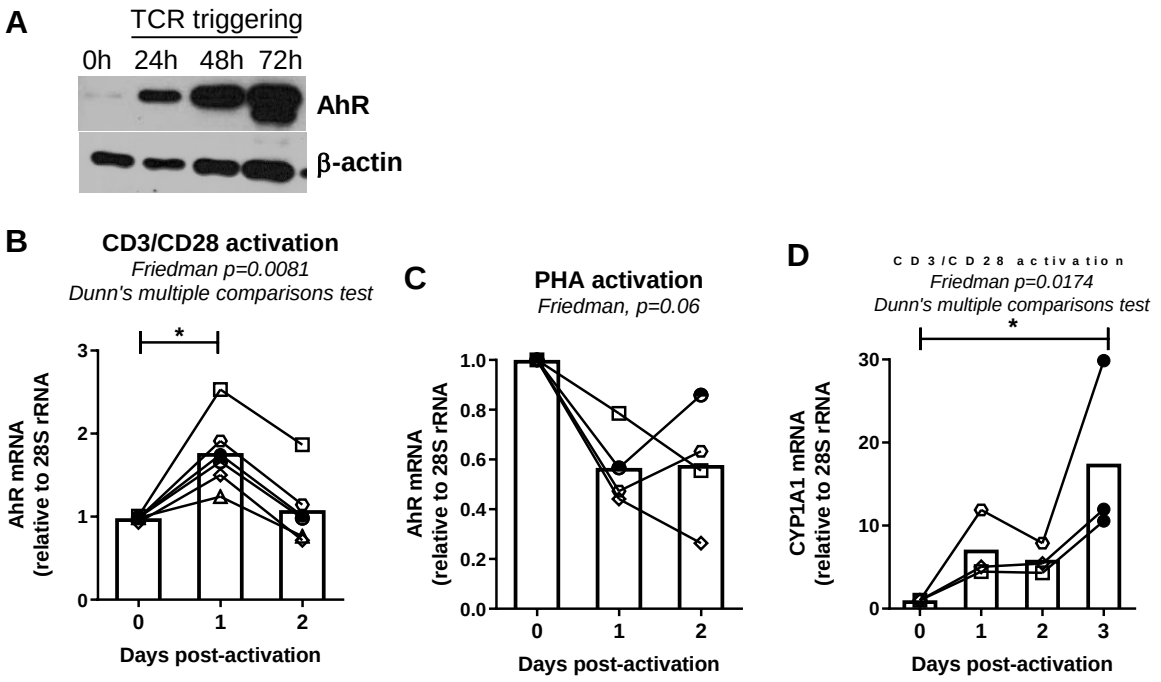
Supplemental Table 3: Primers and probes for PCR/RT-PCR. Related to STAR Methods.

Identification	Sequence	Provider	Identifier
Primer AA55	5'-CGT CTA GAG ATT TTC CAC AC-3'	IDT	N.A.
Primer M667	5'-CTA ACT AGG GAA CCC ACT G-3'	IDT	N.A.
CD3 external primer 1	5'-ACT GAC ATG GAA CAG GGG AAG-3'	IDT	N.A.
CD3 external primer 2	5'- CCA GCT CTG AAG TAG GGA ACA TAT-3'	IDT	N.A.
Primer GagR	5'- AGC TCC CTG CTT GCC CAT A-3'	IDT	N.A.
Primer Alu1	5'- TCC CAG CTA CTG GGG AGG CTG AGG-3'	IDT	N.A.
Primer Alu2	5'- GCC TCC CAA AGT GCT GGG ATT ACA G-3'	IDT	N.A.
Primer LM667	5'- ATG CCA CGT AAG CGA AAC TCT GGC TAA CTA GGG AAC CCA CTG-3'	IDT	N.A.
Primer LambdaT	5'- ATG CCA CGT AAG CGA AAC T-3'	IDT	N.A.
Primer AA55M	5'- GCT AGA GAT TTT CCA CAC TGA CTA A-3'	IDT	N.A.
Primer SK30	5'- GGT CTG AGG GAT CTC TAG-3'	IDT	N.A.
Primer SK29	5'- ACT AGG GAA CCC ACT GCT-3'	IDT	N.A.
CD3 internal primer 1	5'- CCT CTC TTC AGC CAT TTA AGT A-3'	IDT	N.A.
CD3 internal primer 2	5'- GGC TAT CAT TCT TCT TCA AGG T-3'	IDT	N.A.
Probe LTR-LC	5'-LC640-CACTCAAggCAAgCTTTATTgAggC-3'-phosphate	TIB MolBiol	N.A.
Probe LTR-FL	5'- CACAACAgACgggCACACACTACTTgA-3'-Flurescein	TIB MolBiol	N.A.
Probe P1	5'-ggCTgAAggTTAgggATACCAATATTCCTgTCTC-3'-Flurescein	TIB MolBiol	N.A.
Probe P2	5'-LC640-CTAgTgATgggCTCTTCCCTTgAgCCCTTC-3'-phosphate	TIB MolBiol	N.A.

N/A, information not available; N.A., Not applicable

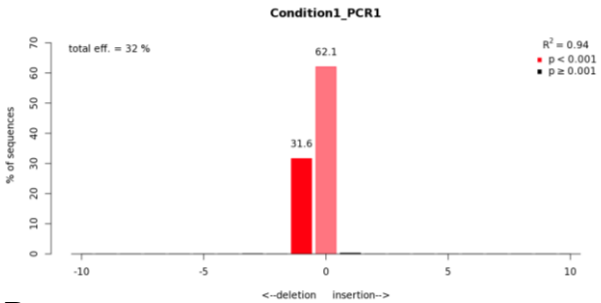
28S Forward Primer	5′-CGAGATTCCTGTCCCCACTA-3′	IDT	N.A.
28S Reverse Primer	5′ GGGGCCACCTCCTTATTCTA-3′	IDT	N.A.
Primer AhR	N/A	Qiagen	GeneGlobe ID: QT02422938
Primer CYP1A1	N/A	Qiagen	GeneGlobe ID: QT00012341
Primer BACH2	N/A	Qiagen	GeneGlobe ID: QT00038304
Primer IL-22	N/A	Qiagen	GeneGlobe ID: QT00034853
Primer HIC1	N/A	Qiagen	GeneGlobe ID: QT00203175
Primer CEBPB	N/A	Qiagen	GeneGlobe ID: QT00237580
Primer SIRT1	N/A	Qiagen	GeneGlobe ID: QT00051261
AhR crRNA sequence #1	5'- AAGTCGGTCTCTATGCCGCT -3'	IDT	Genscript
AhR crRNA sequence #2	5'- TTGCTGCTCTACAGTTATCC -3'	IDT	Genscript
Primer for AhR KO genotyping Forward	5'-GGGAATCACTGTGCTACAAATGC-3'	IDT	N.A.
Primer for AhR KO genotyping Reverse	5'-CAGAAAATCCAGCAAGATGGTGT -3'.	IDT	N.A.
Primer for CHIP assay AhR#1 Forward	5'-CACCCACACAAACCTGACCT-3'	IDT	N.A.
Primer for CHIP assay AhR#1 Reverse	5'-GGCCCTAGAATCCCCCTGTA-3'	IDT	N.A.
Primer for CHIP assay AhR#2 Forward	5'-TTTTCCGAACTGGGGCTGTG-3'	IDT	N.A.
Primer for CHIP assay AhR#2 Reverse	5'-TTCGCCCTCCCACCTGTA-3'	IDT	N.A.
Primer for CHIP assay AhR#1+2 Forward	5'-AGTTCCGGGGGAGAAGGG-3'	IDT	N.A.
Primer for CHIP assay AhR#1+2 Reverse	5'-GCGGGGAGGGCGTTATATC-3'	IDT	N.A.
Primer for CHIP assay MMP9 promoter Forward	5'-TCTCATGCTGGTGCTGCC-3'	IDT	N.A.
Primer for CHIP assay MMP9 promoter Reverse	5'-CTTTAAGGAGGCGCTCCTGTG-3'	IDT	N.A.
Primer for TIDE assay AhR#1crRNA	5'- TTCGGAAGAATTTAACCCATTC-3'	IDT	N.A.
Primer for TIDE assay AhR#2 crRNA	5'-AACTGACGCTGAGCCTAAGAAC-3'	IDT	N.A.

N/A, information not available; N.A., Not applicable

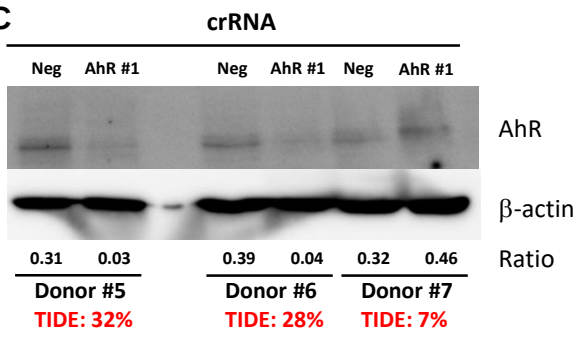


Supplemental Figure 1 (related to Figure 1): AhR expression and activation is induced by TCR triggering but not PHA stimulation in memory CD4⁺ T-cells. Memory CD4⁺ T-cells isolated from PBMCs of HIV-uninfected individuals were stimulated with CD3/CD28 Abs for 1-3 days or PHA for 2 days. AhR protein expression was measured by western blotting *ex vivo* and at days 1, 2, and 3 post-TCR triggering **(A)**. In parallel, total RNA was extracted from cells activated with CD3/CD28 Abs for 1-3 days (**B)** or PHA (10 µg/ml) for 2 days (**C)** and used for the quantification of AhR mRNA **(B-C)** and CyP1A1 mRNA **(D)** by real-time RT-PCR (n=4-6). Friedman test p-values, with Dunn's multiple comparisons indicated on the graphs.

A. **TIDE analysis– Editing efficacy - Donor 5**

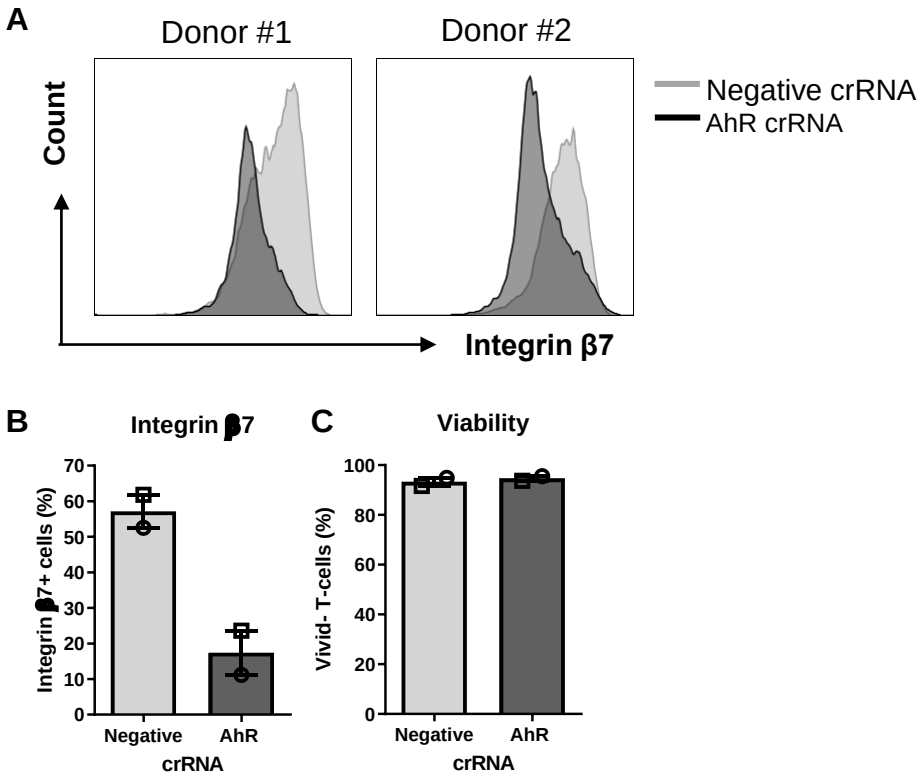


C

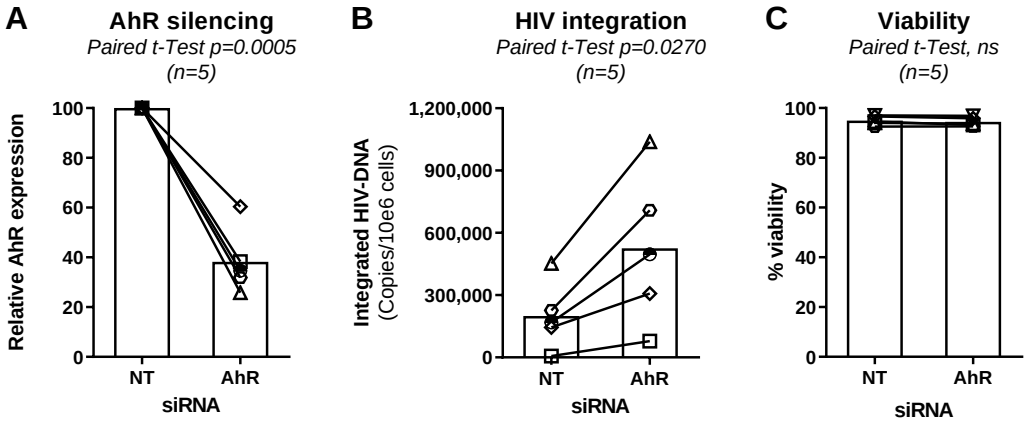


B. **TIDE Assay – Sequencing – Donor 5**

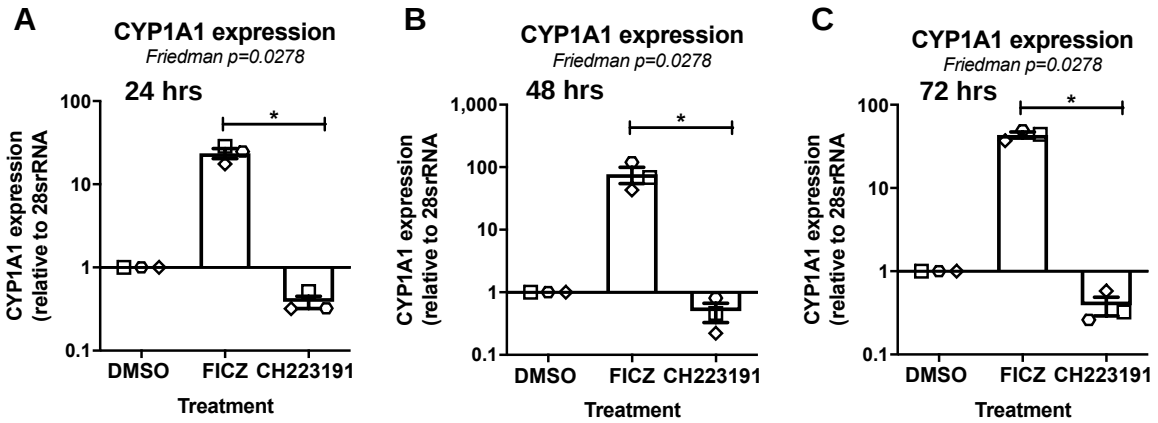
Supplemental Figure 2 (related to Figure 1): TIDE analysis of CRISPR/Cas9-mediated AhR gene editing efficacy in memory CD4⁺ T-cells. Memory CD4⁺ T-cells isolated from PBMCs of HIV-uninfected individuals were stimulated with CD3/CD28 Abs for three days and nucleofected with negative crRNA or AhR crRNA, as illustrated in Figure 1A. CRISPR/Cas9 experiments were performed with cells from three independent HIV-uninfected donors (Donors 5-7). At day 3 post-nucleofection, CRISPR/Cas9-mediated AhR gene editing efficiency was evaluated using the Tracking of Indels by DEcomposition (TIDE) analysis. Shown are results performed with cells from Donor 5 highlighting gene editing distribution plot obtained by TIDE analysis **(A)** and chromatogram sequence alignment results in comparison to cells nucleofected with the negative crRNA **(B)**. Red arrow highlights a potential C > T SNP in this participant. Results in panel 2B demonstrate a potential C > T SNP induced by AhR #1 crRNA. In parallel, western blotting was performed to visualize changes in AhR protein expression compared to β-actin level **(C)**. The AhR gene editing efficacy varied between donors (32% for Donor 5; 28% for Donor 6; and 7% for Donor 7), and coincided with changes in AhR protein expression. The ratio indicated below the gel figure was calculated as the ration between the density of AhR and the corresponding β-actin bands, as measured using the Image Lab software on the ChemiDoc Imaging System (BioRad).



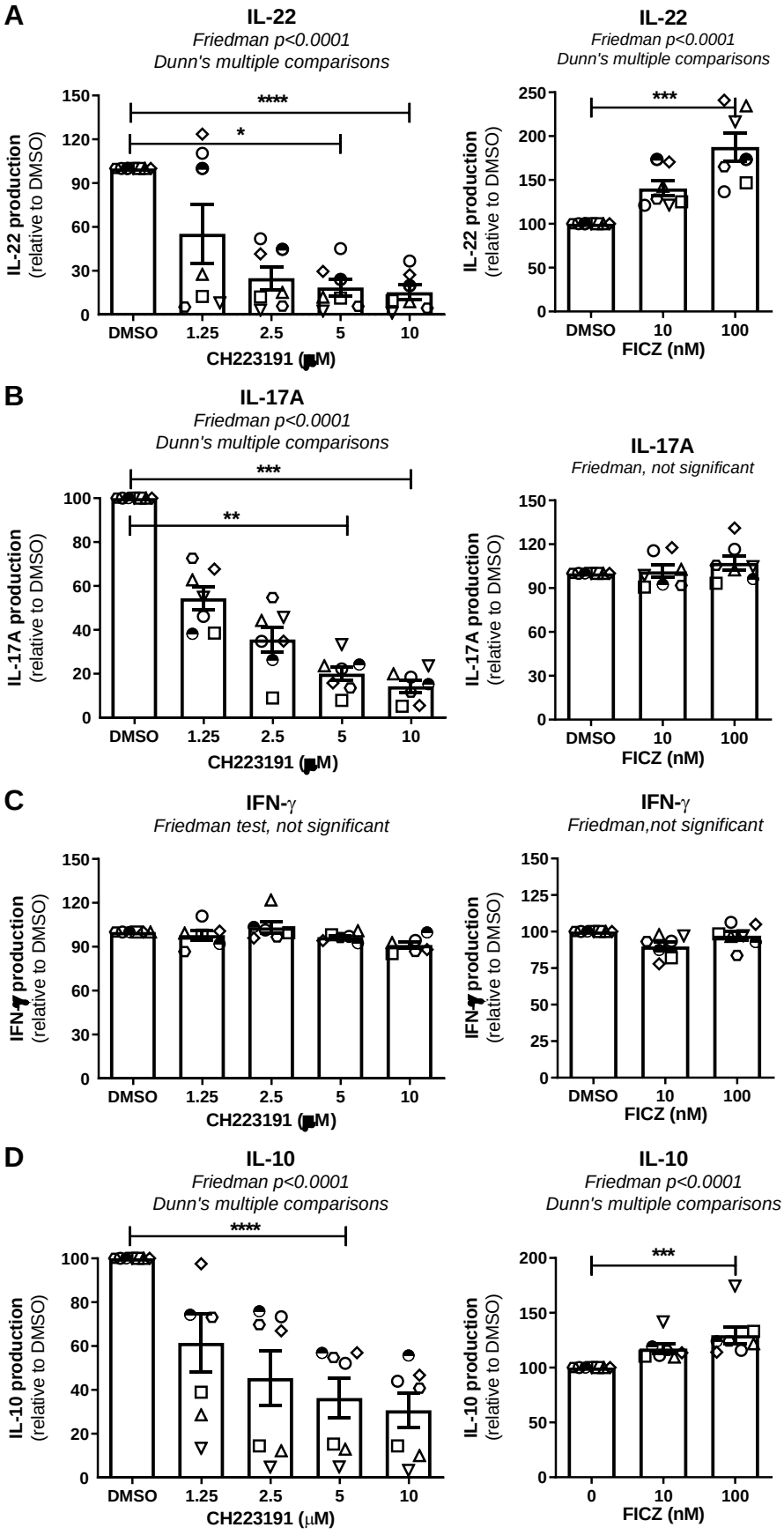
Supplemental Figure 3 (related to Figure 1): CRISPR/Cas9-mediated AhR KO downregulates integrin β7 expression in memory CD4⁺ T-cells. Memory CD4⁺ T-cells isolated from PBMCs of HIV-uninfected individuals were stimulated with CD3/CD28 Abs and nucleofected either with negative crRNA or with AhR crRNA, as in Figure 1. After four days, cells were analyzed by flow cytometry for the surface expression of integrin β7 (ITGB7) **(A-B)** and cell viability using the viability dye Aqua Vivid **(C)**. Shown are results from two different HIV-negative individuals, with each symbol representing a different donor.



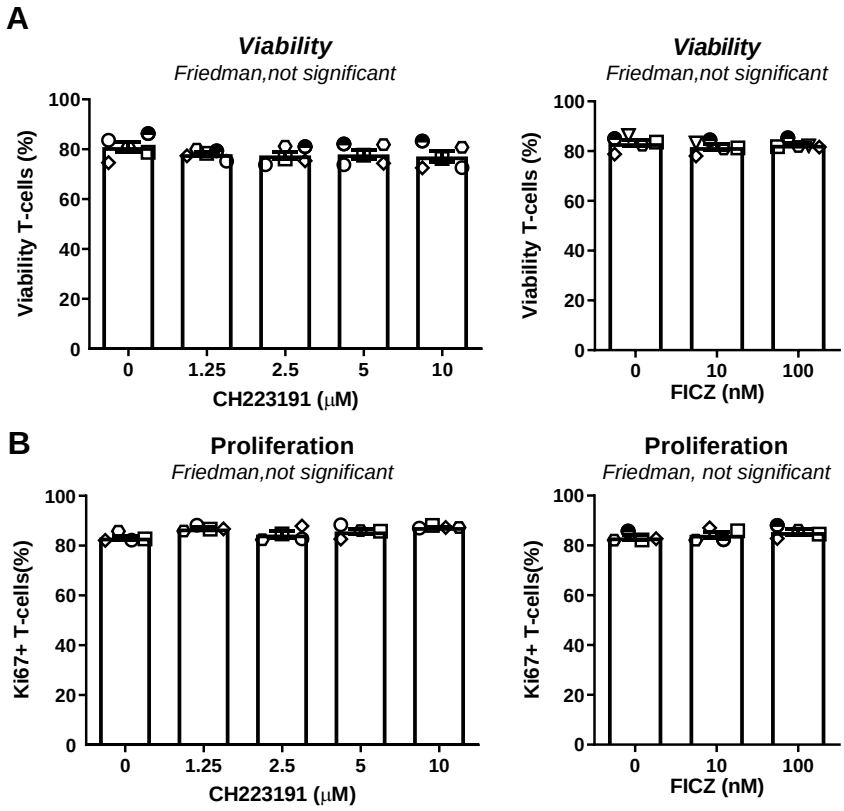
Supplemental Figure 4 (related to Figure 2): Small interfering RNA against AhR increases HIV-DNA integration in memory CD4⁺ T-cells. Memory CD4⁺ T-cells isolated from the PBMCs of HIV-uninfected individuals were stimulated *via* CD3/CD28 for 3 days. Then, cells were nucleofected with non-targeting (NT) and AhR-targetting small interfering RNA (siRNA). Nucleofected cells were cultured in the presence of IL-2 (5 ng/ml) for 24 hours and then exposed to replication-competent NL4.3BaL HIV-1 strain for 3 hours. Unbound virus was excluded by extensive washing. Cells were then cultured in the presence of IL-2 for 3 other days. Shown are AhR mRNA expression as measured by real-time RT-PCR **(A)**; levels of integrated HIV-DNA quantified by nested real-time PCR in cells **(B)**; and cell viability measured using the viability dye **(C)** upon siRNA silencing. Results were generated with cells from n=5 different HIV-uninfected individuals. Each symbol represents one different donor. Paired t-test p-values are indicated on the graphs.



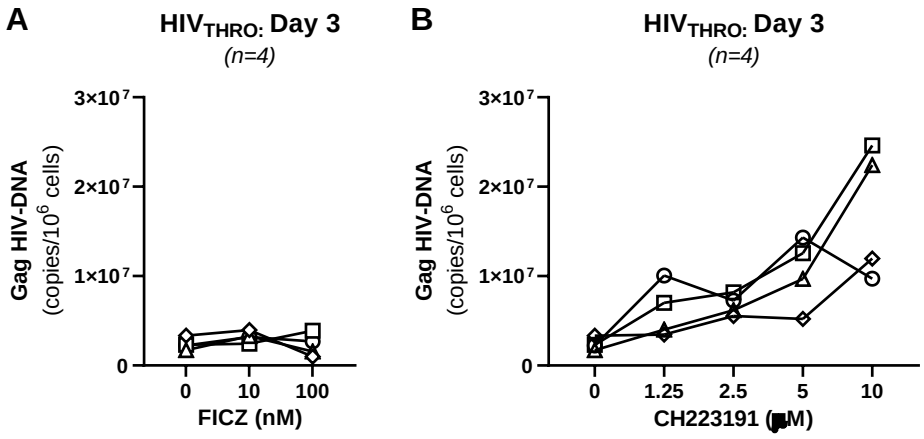
Supplemental Figure 5 (related to Figure 3): Pharmacological targeting of AhR pathway modulates CYP1A1 mRNA expression. Memory $CD4^+$ T-cells from the PBMCs of HIV-uninfected individuals were stimulated with CD3/CD28 Abs in the presence or the absence of FICZ (100 nM) or CH223191 (10 µM) for 24 (A), 48 (B) and 72 hours (C). CYP1A1 mRNA expression was measured by RT-PCR, with 28S rRNA used as control housekeeping gene. Shown are CYP1A1 mRNA expression relative to DMSO levels (DMSO considered 1). Results were generated with cells from n=3 different donors. Friedman test p-values, with Dunn's multiple comparisons indicated on the graphs.



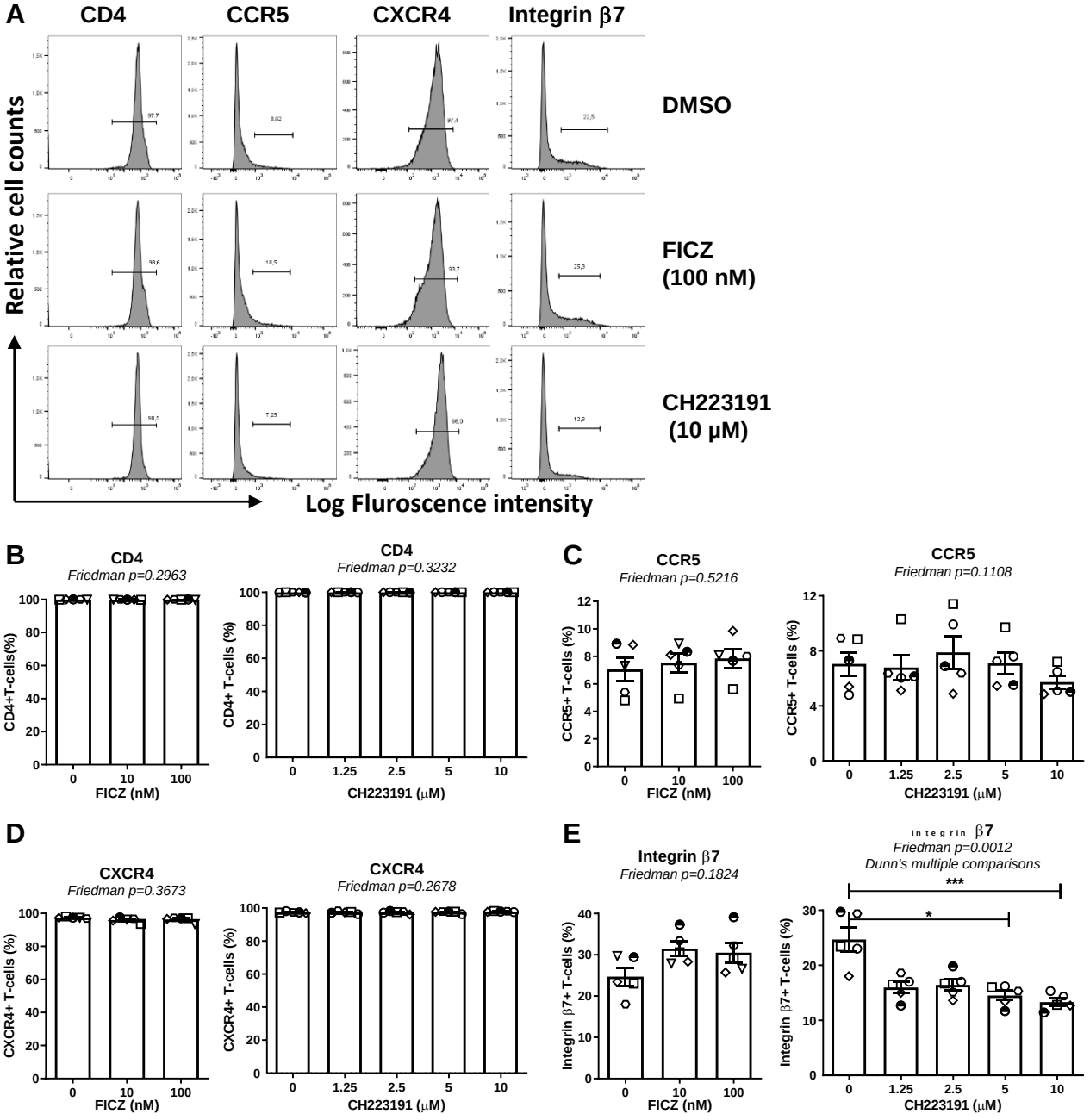
Supplemental Figure 6 (related to Figure 3): Pharmacological targeting of AhR modulates IL-22, IL-17A and IL-10 but not IFN-γ expression in primary CD4⁺ T-cells. Memory CD4⁺ T-cells isolated as described in Figure 1 were stimulated with CD3/CD28 Abs in the presence or the absence of different concentrations of the AhR antagonist CH223191 (1.25, 2.5, 5 or 10 µM) or agonist FICZ (10 and 100 nM). Shown are levels of IL-22 **(A)**, IL-17A **(B)**, IFN-γ **(C)**, and IL-10 **(D)** measured by ELISA in cell culture supernatants collected from cells exposed to CH223191 (**left panels**) and FICZ (**right panels**) at day 3 post-TCR triggering. Results were generated with cells from n=7 HIV-uninfected individuals. Each symbol represents one donor. Shown are the Friedman test p-values, and the significance of Dunn's multiple comparisons (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).



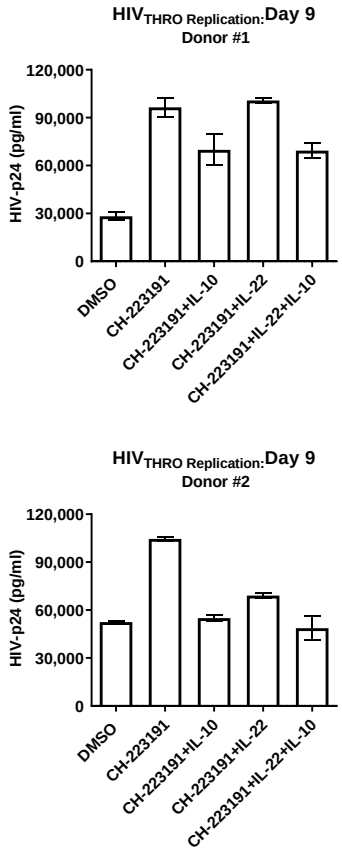
Supplemental Figure 7 (related to Figure 3): Pharmacological targeting of AhR pathway does not affect cell viability and proliferation: Memory CD4$^+$ T-cell were isolated from HIV-uninfected individuals and stimulated with CD3/CD28 Abs in the presence or the absence of CH223191 (1.25, 2.5, 5 or 10 μM) or FICZ (10 and100 nM) for 3 days, as in Supplemental Figure 7. Shown are cell viability was measured by staining using the viability dye aqua vivid **(A)**, as well as cell cycle progression measured by intranuclear staining with Ki-67 Abs (a surrogate marker of cell proliferation) **(B)**. Results were generated with cells from n=4-5 HIV-uninfected individuals. Friedman test p-values, with Dunn's multiple comparisons indicated on the graphs.



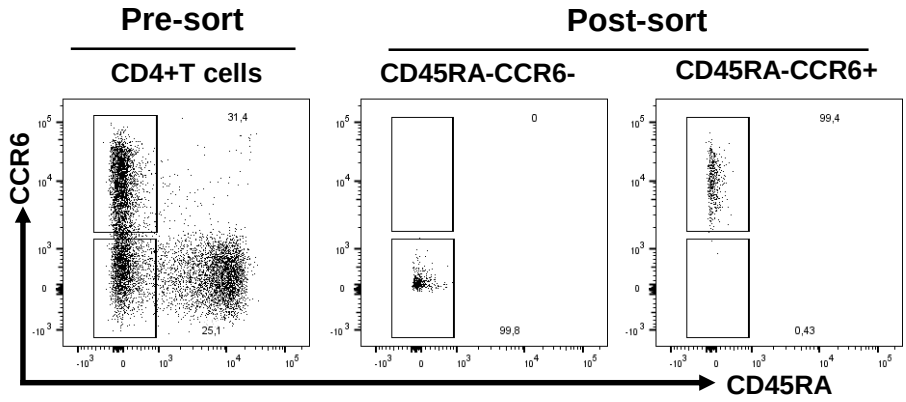
Supplemental Figure 8 (related to Figure 3): Dose-response effect of AhR drugs on HIV infection. Memory CD4$^+$ T-cells were activated *via* CD3/CD28 and cultured in the presence or the absence of different concentrations of FICZ (0, 10, 100 nM) or CH223191 (0, 1.25, 2.5, 5, 10 µM), as in Supplemental Figure 7, for 3 days. Cells were exposed to HIV$_{THRO}$ for 3 hours. The unbound virus was removed by extensive washing and cells were cultured in the presence of recombinant human IL-2 (5 ng/ml) and in the presence or the absence of FICZ **(A)** or CH223191 **(B)** . At day 3 post-infection cells were harvested and Gag HIV-DNA was measured by real time PCR. Shown are results from experiments performed with cells from n=4 different donors, each symbol indicates one donor.



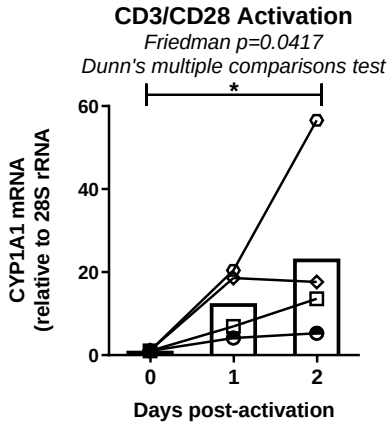
Supplemental Figure 9 (related to Figure 3): AhR antagonism downregulates integrin β7 expression without modulating the expression of the HIV-1 receptor CD4 or co-receptors CCR5 and CXCR4. Memory CD4⁺ T-cells were activated *via* CD3/CD28 and cultured in the presence or the absence of different concentrations of CH223191 **(left panels)** or FICZ **(right panels)**, as in Supplemental Figure 7. Cells were then analyzed by flow cytometry for the surface expression of CD4 **(A),** CXCR4 **(B)**, CCR5 **(C),** and integrin β7 **(D)**. Shown are results generated with cells from one representative donor **(A)** and statistical analysis of results were generated with cells from n=7 HIV-uninfected individuals **(B-E; FICZ, left panels; CH223191, right panels).** Friedman test p-values, with Dunn's multiple comparisons indicated on the graphs.



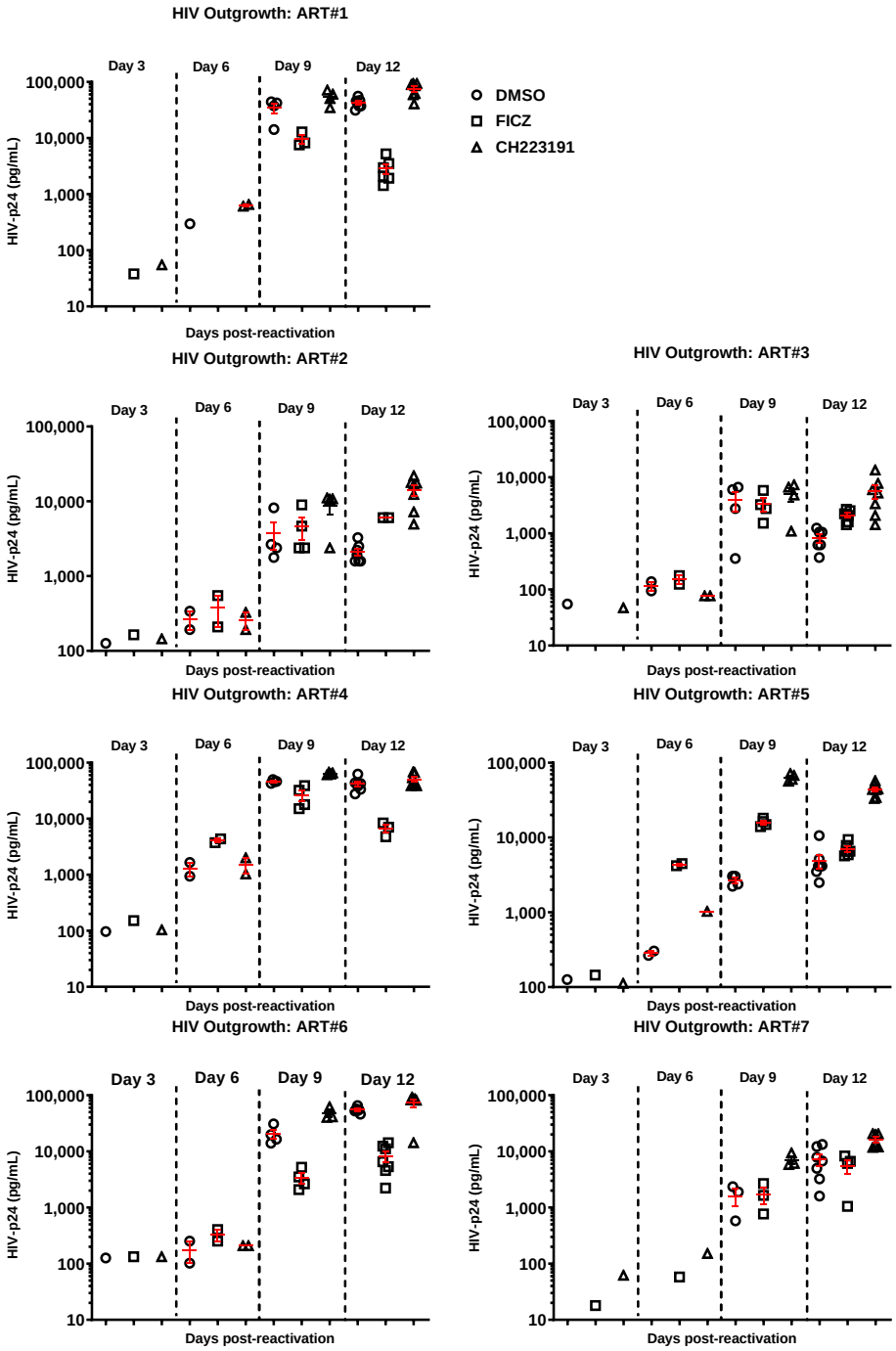
Supplemental Figure 10 (related to Figure 3): IL-10 and IL-22 supplementation counteracts the proviral effects of CH223191: Memory CD4$^+$ T-cell were isolated from HIV-uninfected individuals, stimulated with CD3/CD28 Abs and cultured in the presence or the absence of CH223191, as well as human recombinant IL-10 (10 ng/ml) and/or IL-22 (25 ng/ml) for 3 days. Cells were exposed to the replication competent T/F HIV$_{THRO}$ for 3 hours and cultured for additional 9 days. Media containing CH223191 and cytokines was refreshed every 3 days. Shown are HIV-p24 levels measured by ELISA in cell-culture supernatants at day 9 post-infection. Experiments were performed with cells from two different donors. Results represent mean±SD of ELISA triplicate values.



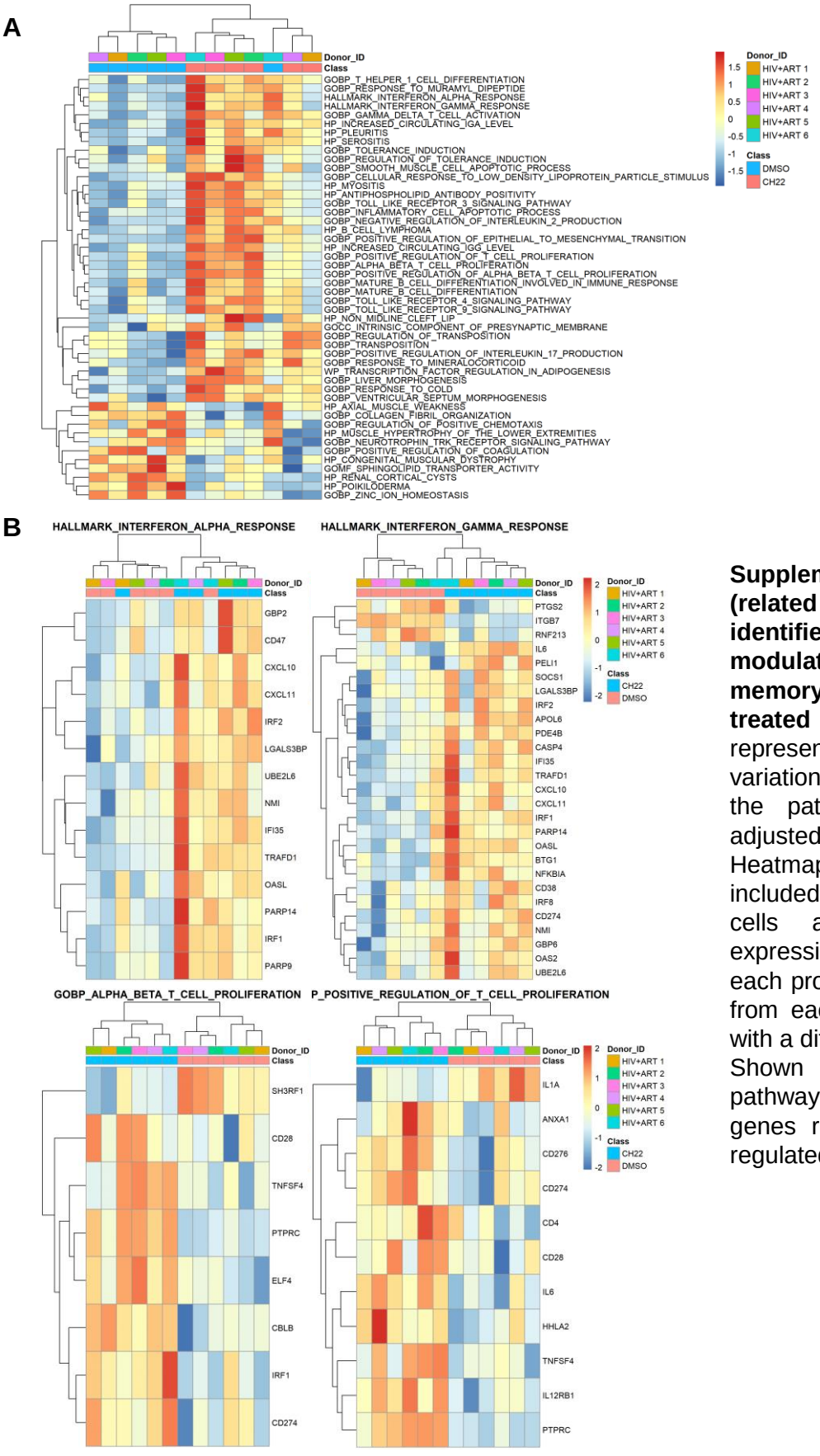
Supplemental Figure 11 (related to Figure 4): Flow cytometry sorting on memory CCR6$^+$ and CCR6$^-$ CD4$^+$ T-cells. Total CD4$^+$ T-cells were isolated by negative selection using magnetic beads and stained with CD45RA, and CCR6 Abs thus allowing the sorting of memory (CD45RA$^+$) CCR6$^+$ and CCR6$^-$ T-cells. Shown is the cell phenotype pre and post-sort, with a purity >99%, from experiments performed with cells from one donor representative of results generated with cells from n>4 different donors.



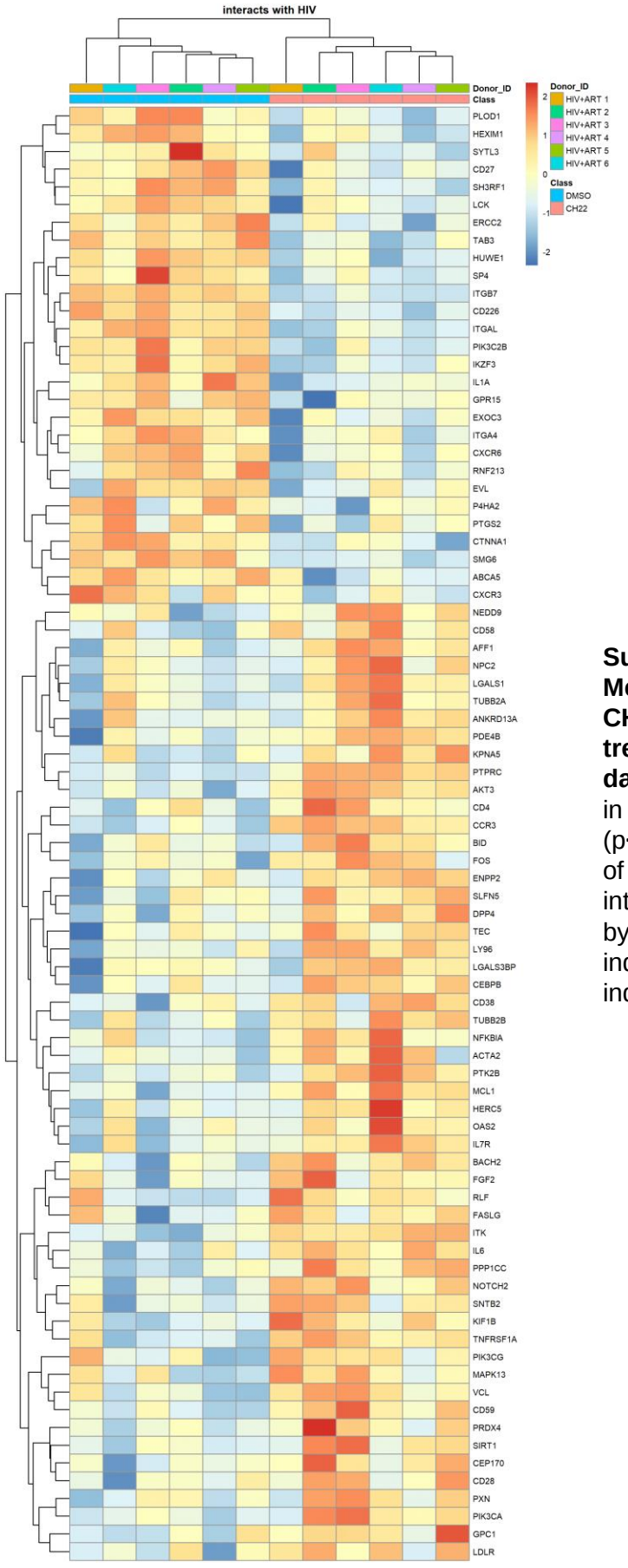
Supplemental Figure 12 (related to Figure 5): TCR triggering increases CYP1A1 expression in memory CD4⁺ T-cells of ART-treated PLWH. Memory CD4⁺ T-cell were isolated from ART-treated PLWH and stimulated with CD3/CD28 Abs for up to 2 days. CYP1A1 mRNA expression was measured by RT-PCR and normalized to 28S rRNA levels. Results were generated with cells from n=4 different donors. Friedman test p-values, with Dunn's multiple comparisons indicated on the graphs.



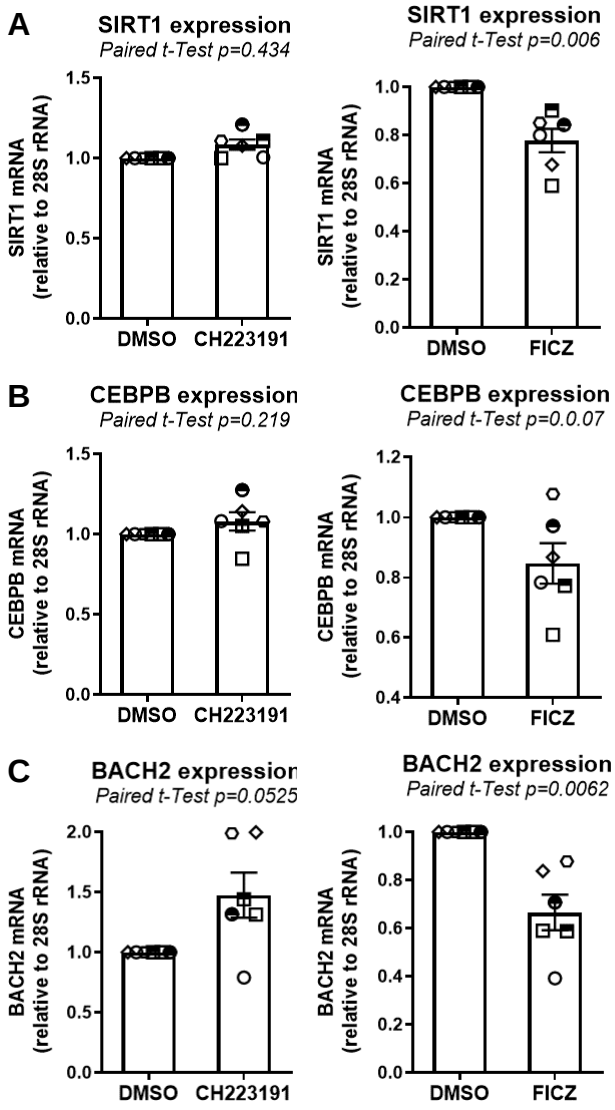
Supplemental Figure 13 (related to Figure 5): The AhR blockade boosts HIV-1 outgrowth in CD4⁺ T-cells of ART-treated PLWH. The viral outgrowth assay (VOA) was performed, as in Figure 6A. Briefly, memory CD4⁺ T-cells isolated from the PBMCs of ART-treated PLWH were activated with CD3/CD28 Abs and cultured in the presence of IL-2 and in the presence or the absence of FICZ (100 nM) or CH223191 (10 μM) for up to 12 days. Supernatants were collected and media containing IL-2 (5 ng/ml) and drugs refreshed every 3 days. HIV-p24 levels were measured in cell-culture supernatants by ELISA. Shown are HIV-p24 levels in cell-culture supernatants collected from replicate wells at days 3 (1 replicate), 6 (2 replicates), 9 (4 replicates) and 12 (8 replicates) post-activation in individual donors (n=7).



Supplementary Figure 14 (related to Figure 6): GSVA identifies pathways modulated by CH223191 in memory CD4⁺ T-cells of ART-treated PLWH: Heatmap representing the gene set variation analysis (GSVA) for all the pathways with a FDR-adjusted p-value <0.05. Heatmap represents pathways included in MSigDB: Heatmap cells are scaled by the expression level z-scores for each probe individually. Results from each donor are indicated with a different color code (n=6). Shown are top regulated pathways **(A)** and individual genes related to selected top regulated pathways **(B).**



Supplementary Figure 15 (related to Figure 6): Meta-analysis of genes modulated by CH223191 in memory CD4+ T-cells of ART-treated PLWH using the NCBI HIV interaction database. Transcripts modulated by CH223191 in memory $CD4^+$ T cells of ART-treated PLWH ($p<0.05$; FC cut-off 1.3) were matched to the lists of human genes included on the NCBI HIV interaction database. Heat- map cells are scaled by the expression level z-scores for each probe individually. Results from each donor are indicated with a different color code (n=6).



Supplementary Figure 16 (related to Figure 6): Pharmacological targeting of the AhR pathway modulates expression of SIRT1, CEBPB and BACH2 mRNA: Briefly, memory CD4$^+$ T cells isolated from the PBMCs of ART-treated PLWH (n=6) were stimulated by CD3/CD28 Abs and cultured in the presence or the absence of CH223191 (10 µM) and FICZ (100 nM) for 18 hours. Total RNA was extracted, and expression levels of SIRT1 **(A)**, CEBPB **(B)** and BACH2 **(C)** transcripts were quantified by qPCR. Each symbol represents one individual donor: bars represent median values. Wilcoxon matched pairs signed rank test are indicated on the graphs.