Cell Reports, Volume *43*

Supplemental information

Retinoic acid enhances HIV-1 reverse transcription and transcription in macrophages via mTOR-modulated mechanisms

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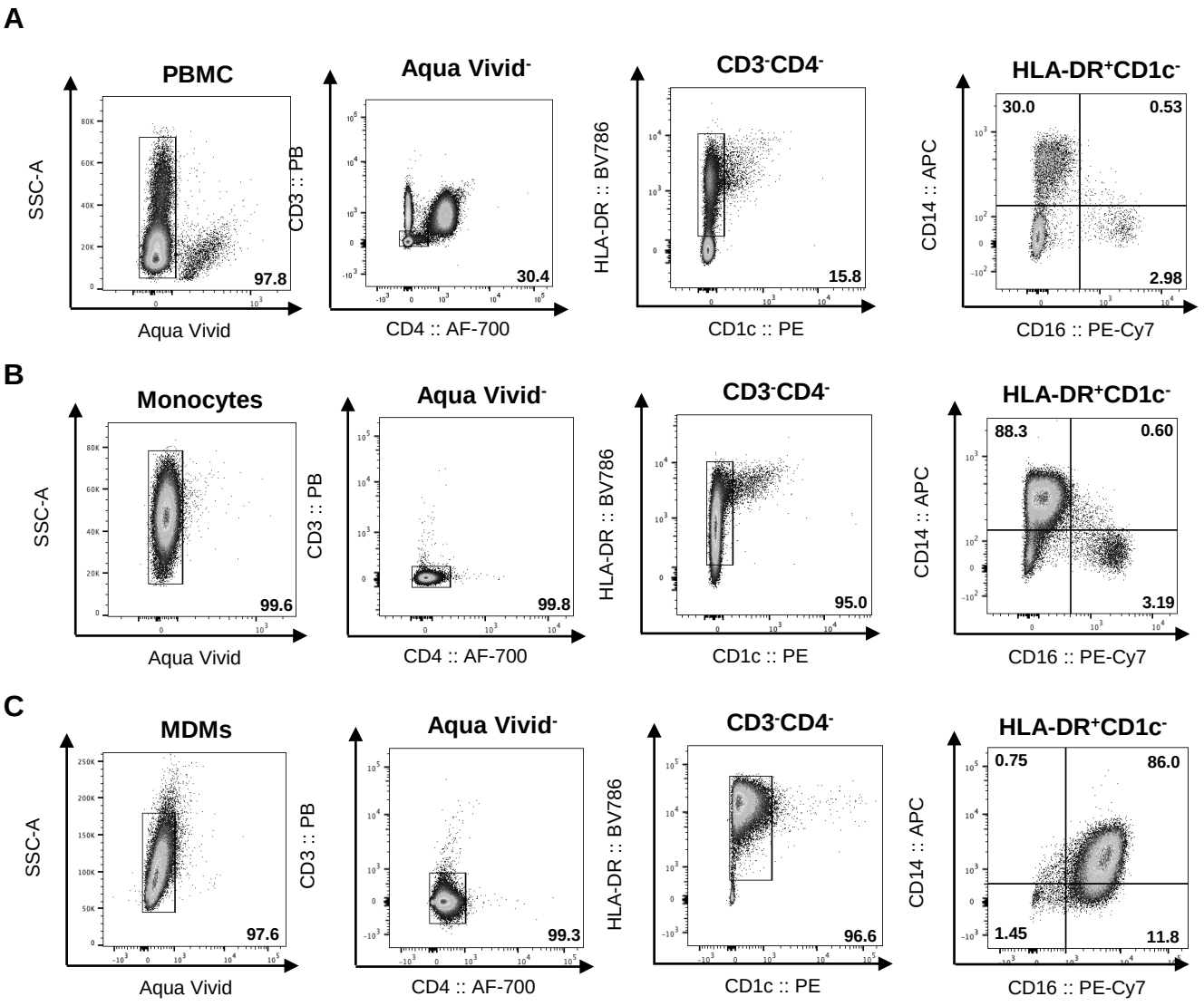
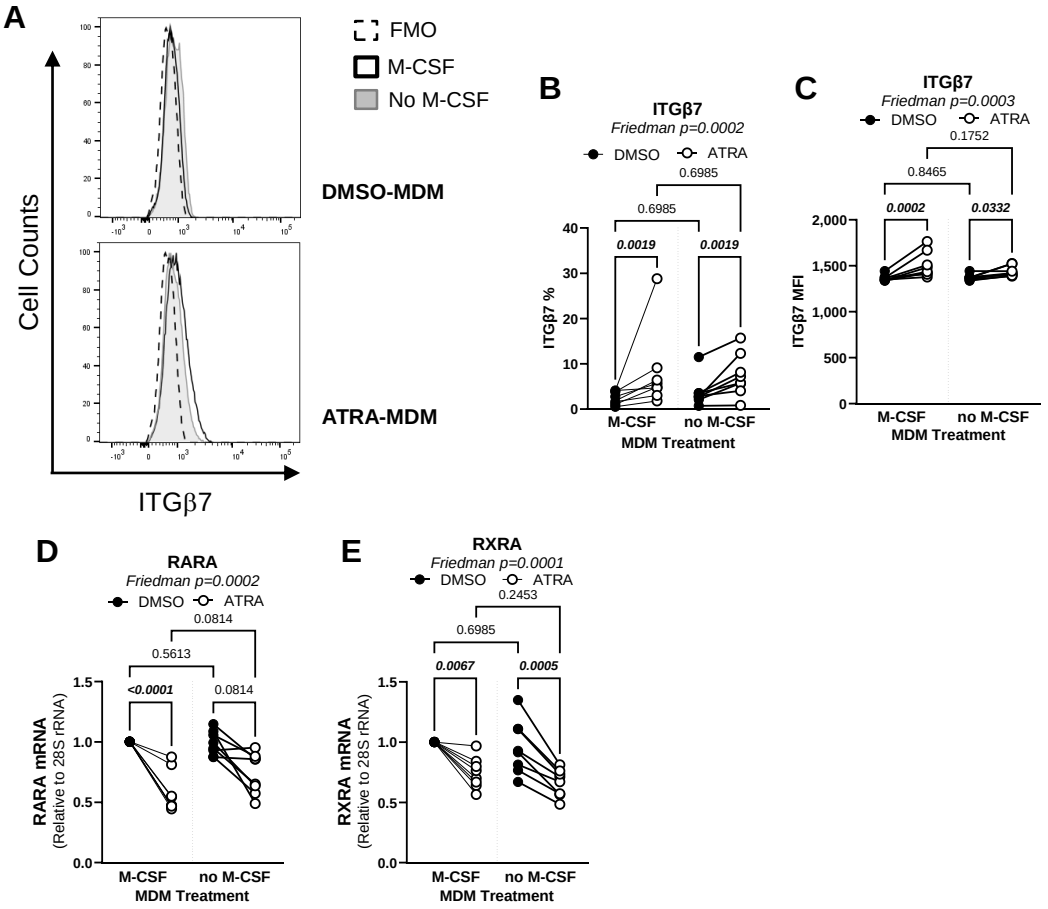
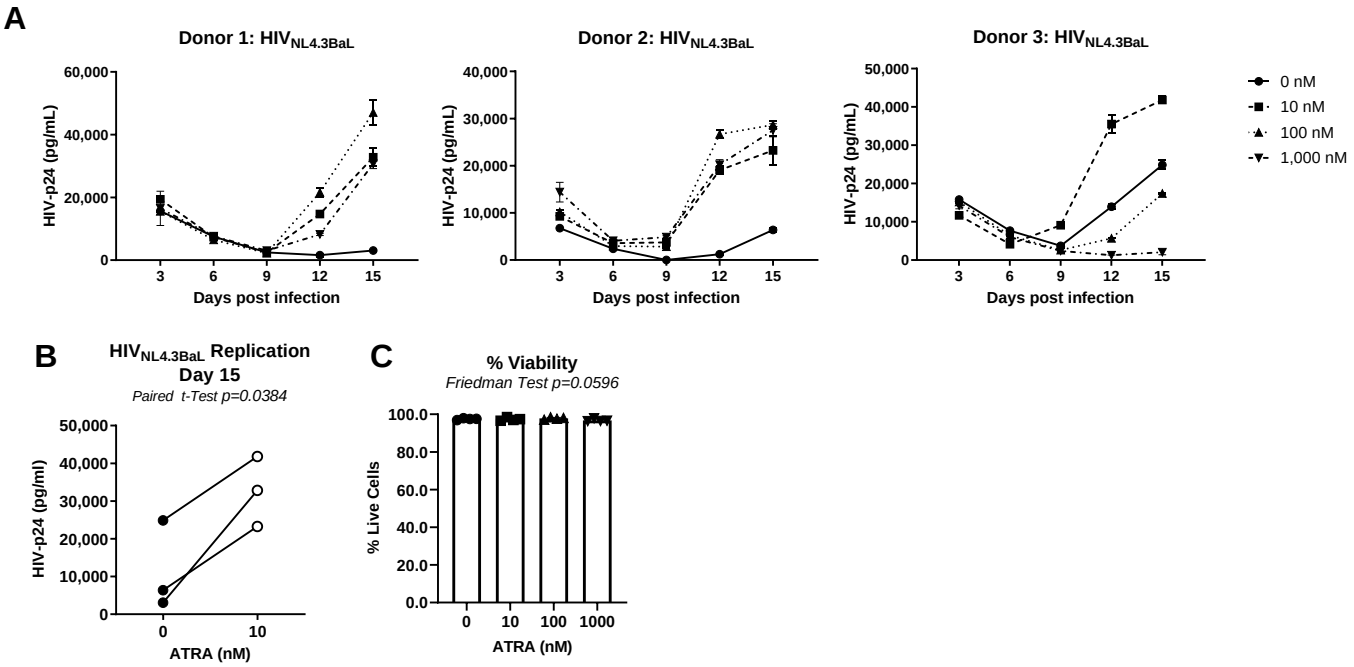


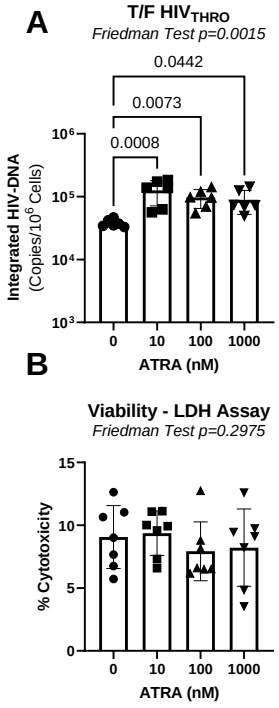
Supplemental Figure 1 (relative to Figure 1): Phenotyping analysis of monocytes and MDMs. Monocytes were isolated from PBMCs by negative selection using magnetic beads (Miltenyi). Monocyte-derived macrophages (MDMs) were generated by culturing monocytes in the presence of M-CSF (20 ng/mL) for six days, as depicted in Figure 1A. In each experiment, the cell purity and phenotype were determined by flow cytometry analysis of matched PBMCs **(A)**, monocytes **(B)**, and MDMs **(C)** upon staining with CD3, CD4, HLA-DR, CD1c, CD14 and CD16 Abs. The viability dye Aqua Vivid was used to exclude dead cells from the analysis. Monocytes and macrophages were identified as cells with a $CD3^{-}CD4^{-}HLA\text{-}DR^{+}CD1c^{-}$ and $CD3^{-}CD4^{-}HLA\text{-}DR^{+}CD1c^{-}CD14^{+}CD16^{+}$ phenotype, respectively. Shown are results generated with cells from one donor representative of results generated with MDMs from 10 HIV-uninfected donors.



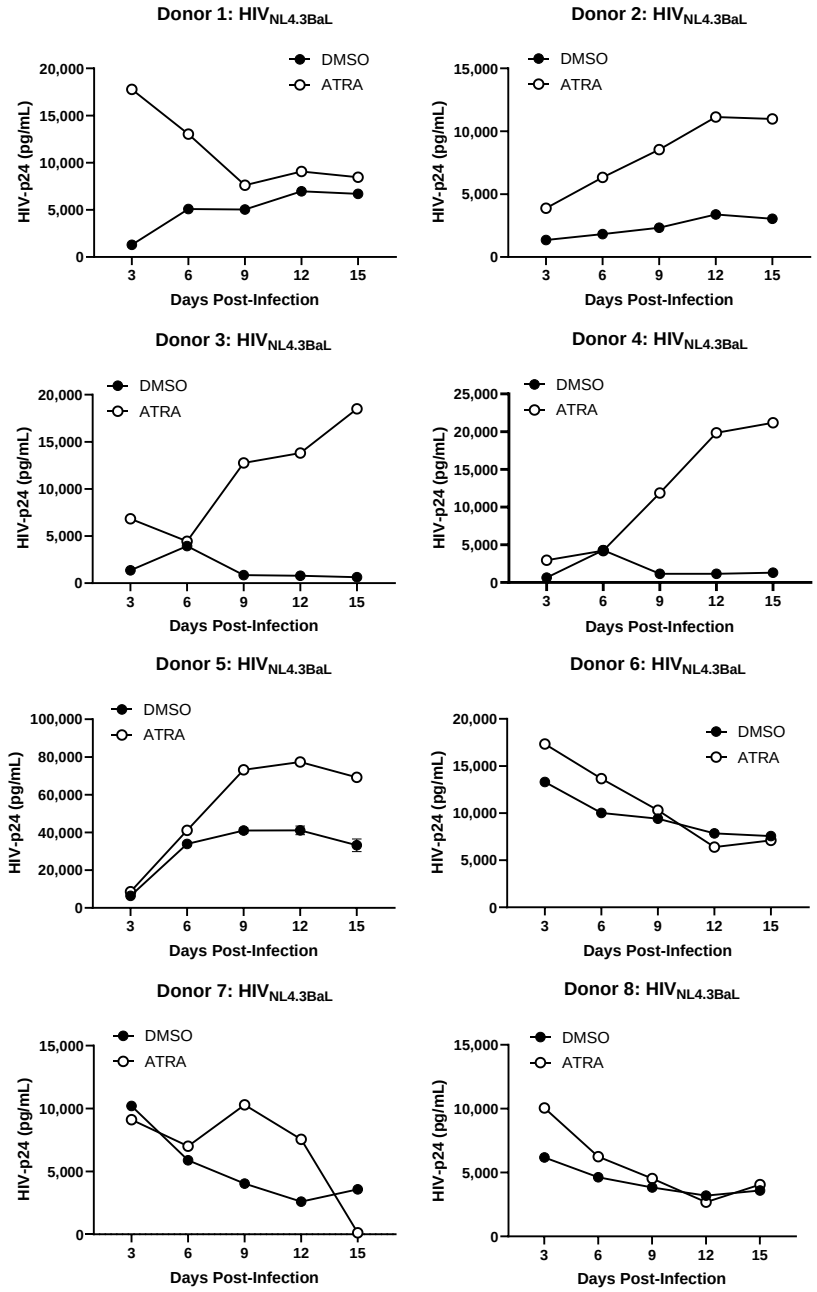
Supplemental Figure 2 (relative to Figure 1): Effect of ATRA on the expression of ITGB7 protein, and RARA and RXRA mRNA in MDMs treated with ATRA. Shown are histograms for ITGB7 expression on MDMs pretreated with ATRA and/or M-CSF from one representative participants **(A)** as well as statistical analysis of the frequency of ITGß7^{+} MDM **(B),** and ITGß7 MFI **(C)** in ATRA-treated MDM from n=8 participants. Total RNA extracted from MDMs of n=8 HIV-uninfected participants generated in the presence or the absence of M-CSF and or ATRA, as in Figure 3. Shown are levels of RARA **(D)** and RXRA mRNA expression **(E)**, as measured by SYBR-Green real-time RT-PCR. Friedman and uncorrected Dunn's Test are indicated on the graphs.



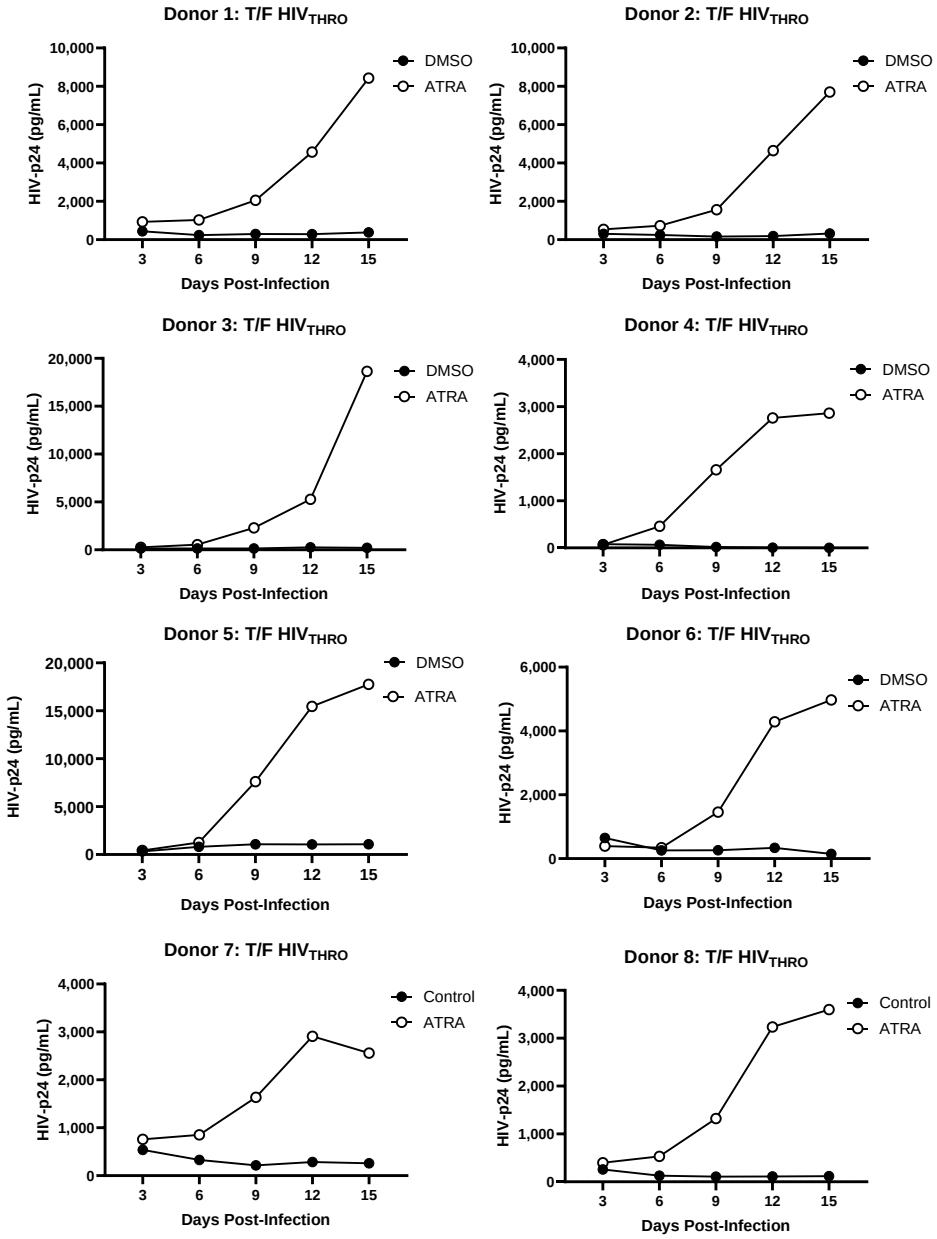
Supplemental Figure 3 (relative to Figure 1): The dose-effect of ATRA on $HIV_{NL3.3BaL}$ replication and viability in MDMs. MDMs were generated in the presence or the absence of different concentrations of ATRA (10, 100 and 1,000 nM), as depicted in Figure 1A, and were exposed to $HIV_{NL4.3BaL}$ (30 ng HIV-p24/well per 10^6 MDMs in 300 µl media) for 3 hours. Unbound virions were removed by extensive washing. Infected MDMs were cultured in media containing M-CSF (20 ng/mL), in the presence/absence of ATRA at the indicated concentrations. Cell-culture supernatants were collected every 3 days for HIV-p24 ELISA quantification, and fresh media containing M-CSF and/or ATRA was added every 3 days. **(A)** Shown are the kinetics of HIV-1 replication *per* donor at days 3, 6, 9, 12 and 15 post-infection and **(B)** statistical analysis performed at days 15 post-infection with MDMs from n=3 HIV-uninfected donors. **(C)** Cell viability was measured by flow cytometry using the Aqua Vivid viability dye (n=4) prior infection. Paired t-Test **(B)** and Friedman and uncorrected Dunn`s multiple comparison p-values **(C)** are indicated on the graphs.



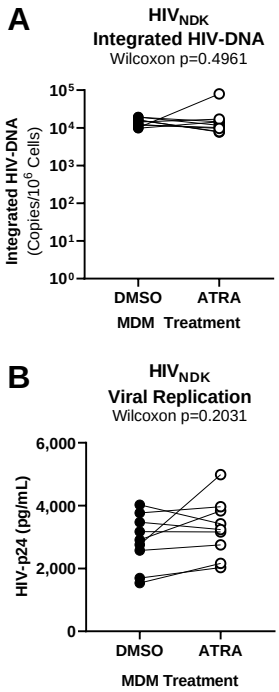
Supplemental Figure 4 (relative to Figure 1): The dose-effect of ATRA on HIV$_{THRO}$ integration and viability in MDMs. MDMs were generated in the presence or the absence of different concentrations of ATRA (10, 100 and 1,000 nM), as depicted in Figure 1A, and were exposed to HIV$_{THRO}$ (5 ng HIV-p24/well per 10^6 MDMs in 300 µl media) for 3 hours. Unbound virions were removed by extensive washing. Infected MDMs were cultured in media containing M-CSF (20 ng/mL), in the presence/absence of ATRA at the indicated concentrations for 3 days. Shown are levels of integrated HIV-DNA measured by nested real-time PCR at day 3 post-infection **(A)** and cell viability measured by lactate dehydrogenase (LDH) activity assay in cell culture supernatants prior infection **(B)** in cells from n=7 study participants. Friedman and uncorrected Dunn`s multiple comparison p-values are indicated on the graphs.



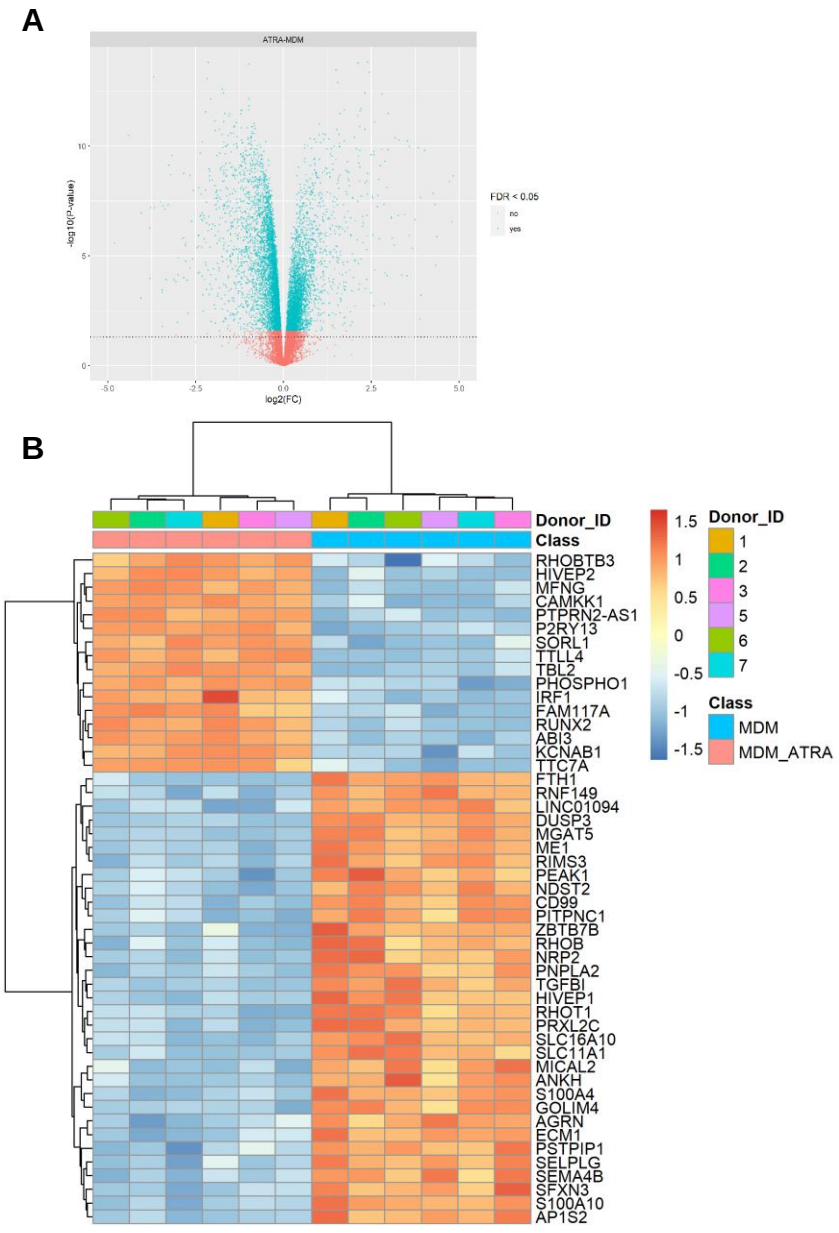
Supplemental Figure 5: Kinetics of HIV$_{NL4.3BaL}$ in ATRA-treated MDM (relative to Figure 1). MDMs treated or not to ATRA (10 nM) were exposed to HIV$_{NL4.3BaL}$ (30 ng HIV-p24/well per 10^6 MDMs in 300 µl media) as in Supplemental Figure 2, and cultured in the presence of M-CSF for up to 15 days. Cell-culture supernatants were collected every 3 days for HIV-p24 ELISA quantification, and fresh media containing M-CSF and/or ATRA was added every 3 days. Shown are the kinetics of HIV$_{NL4.3BaL}$ replication in MDMs from n=8 different study participants.



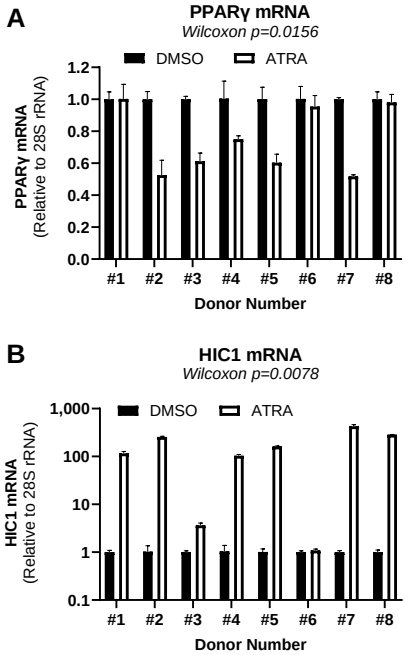
Supplemental Figure 6 (relative to Figure 1): Kinetics of HIV$_{THRO}$ in ATRA-treated MDM. MDMs treated or not to ATRA (10 nM) were exposed to HIV$_{THRO}$ (5 ng HIV-p24/well per 10^6 MDMs in 300 µl media) as in Supplemental Figure 2, and cultured in the presence of M-CSF (20 ng/ml) for up to 15 days. Cell-culture supernatants were collected every 3 days for HIV-p24 ELISA quantification, and fresh media containing M-CSF and/or ATRA was added every 3 days. Shown are the kinetics of HIV$_{THRO}$ replication in MDMs from n=8 different study participants.



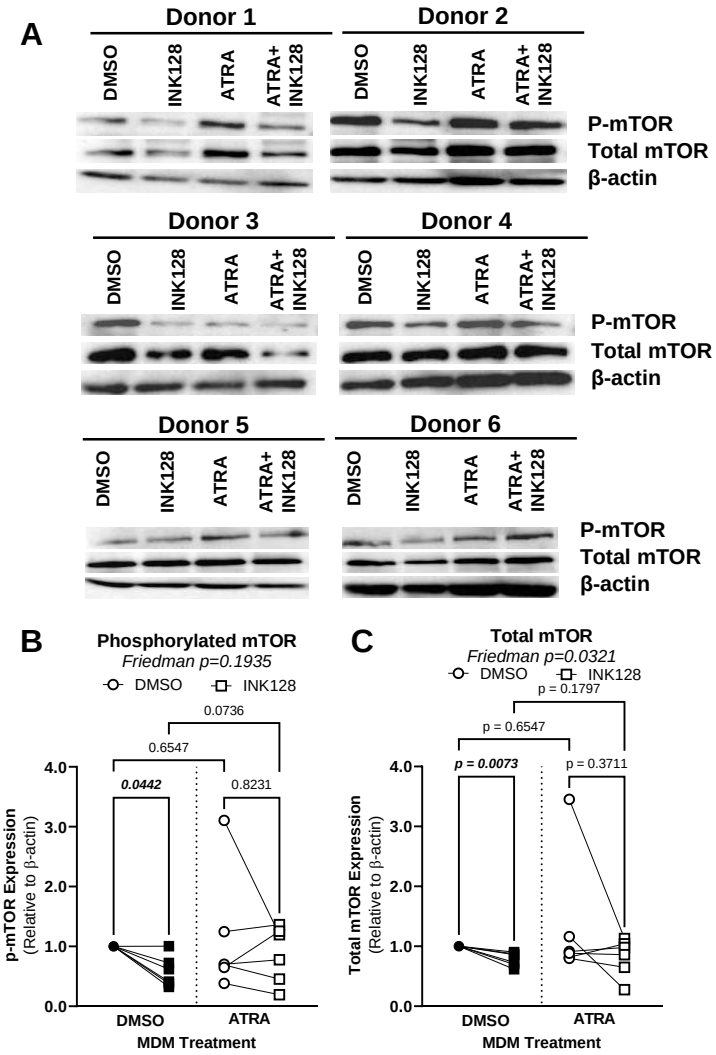
Supplemental Figure 7 (relative to Figure 1): ATRA does not impact on CXCR4-tropic HIV-1 integration and replication in MDMs. MDMs generated by culturing monocytes in media containing M-CSF (20 ng/mL), in presence or the absence of ATRA (10 nM), as in Figure 1A, were exposed to the CXCR4-troic (X4) HIV_{NDK} strain (50 ng HIV-p24/10^6 cells in 300 µl). Shown are levels of (**A)** integrated HIV-DNA measured by real-time nested PCR and **(B)** HIV-p24 measured by ELISA in cell culture supernatants at day 3 post-infection. Experiments were performed with MDMs from n=8 HIV-uninfected individuals. Wilcoxon test p-values are indicated on the graphs.



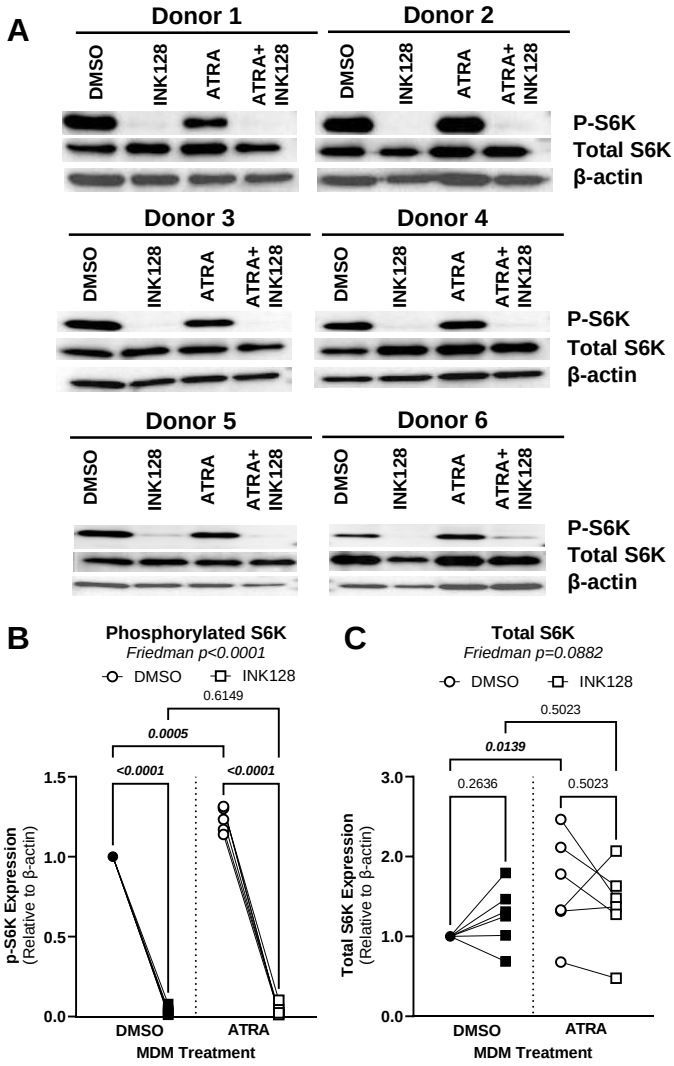
Supplemental Figure 8 (relative to Figure 4): Identification of genes modulated by ATRA in MDMs. Total RNA extracted from MDMs generated in the presence (ATRA-MDMs) or the absence (DMSO-MDMs) of ATRA (10 nM) were used for genome-wide RNA sequencing (Illumina technology), as in Figure 3A. **(A)** The volcano plot depicts the log2 fold change (FC) in gene expression levels (x axis; cut-off: 1.3) and the log10 p-values for differentially expressed genes (DEG; y axis). **(B)** The heatmap depicts the top 50 upregulated and downregulated DEG in MDMs generated in ATRA-MDMs *versus* DMSO-MDMs. Experiments were performed with MDMs from n=6 HIV-uninfected individuals.



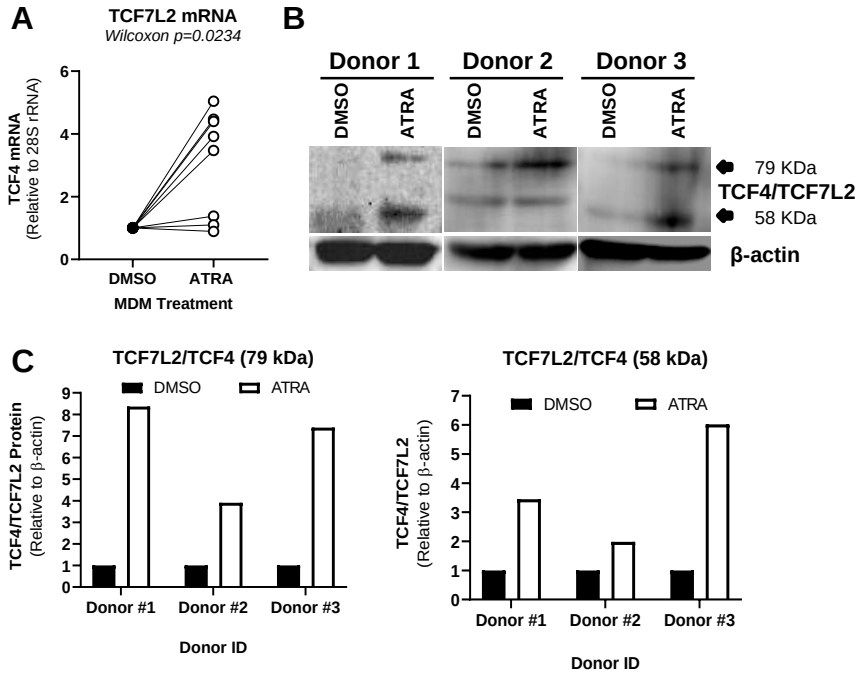
Supplemental Figure 9 (relative to Figure 4): RT-PCR validation of differential PPARγ and HIC1 mRNA expression in ATRA-MDMs versus DMSO-MDMs. Total RNA was extracted from MDMs generated in the presence or the absence of ATRA (10 nM), as in Figure 3. Shown are levels of PPARγ **(A)** and HIC1 mRNA expression **(B)**, as measured by SYBR-Green real-time RT-PCR. Wilcoxon p-values are indicated on the graphs. Experiments were performed with MDMs from n=8 HIV-uninfected individuals.



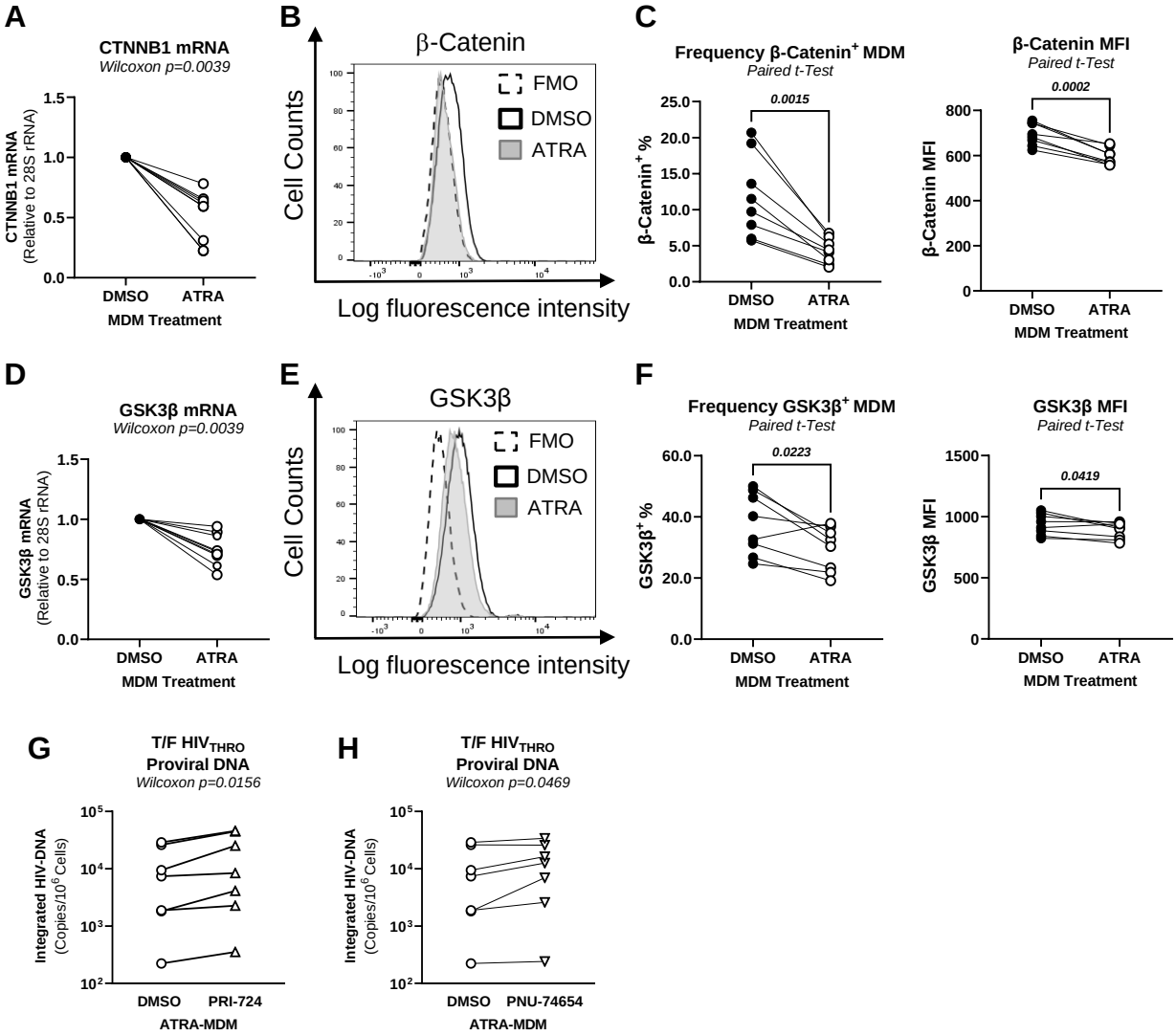
Supplemental Figure 10 (relative to Figure 5): Effect of ATRA on mTOR expression and phosphorylation. MDMs were generated in the presence or the absence of ATRA (10 nM; Day -2 until Day 0), as in Figure 1A. Cells harvested at Day 0 prior HIV exposure were used to generated cell lysates that were subject to SDS-gel electrophoresis for the visualisation/quantification of total and phosphorylated/total mTOR expression **(A)**, Shown is the visualisation of total mTOR (MW: 289 kDa), phosphorylated mTOR (MW: 289 kDa), and β-actin protein bands (MW: 42 kDa) **(A)** in MDMs from n=6 HIV-uninfected participants, upon incubation with specific Abs. Results in the graphs depict phosphorylated (**left panels**) and total (**right panels**) mTOR **(B)** normalized to ß-actin levels (n=6). Friedman and uncorrected Dunn's test are indicated on the graphs.



Supplemental Figure 11 (relative to Figure 5): ATRA increases the expression and the phosphorylation of S6K. MDMs were generated in the presence or the absence of ATRA (10 nM; Day -2 until Day 0), as in Figure 1A. Cells harvested at Day 0 prior HIV exposure were used to generated cell lysates that were subject to SDS-gel electrophoresis for the visualisation/quantification of total and phosphorylated/total S6K expression **(A)** Shown is the visualisation of total S6K (MW: 32 kDa) and phosphorylated S6K (MW: 32 kDa), and β-actin protein bands (MW: 42 kDa) **(A)** in MDMs from n=6 HIV-uninfected participants, upon incubation with specific Abs. Results in the graphs depict phosphorylated (**left panels**) and total (**right panels**) S6K expression levels **(B)** normalized to ß-actin levels (n=6). Friedman and uncorrected Dunn's test are indicated on the graphs.



Supplemental Figure 12 (relative to Figure 4): ATRA modulates the TCF4 mRNA and protein expression. ATRA-MDMs and DMSO-MDMs obtained as in Figure 3A, were used for the quantification of TCF4 mRNA expression by SYBR-Green RT-PCR **(A)**, as well as the visualisation/quantification of TCF4 protein expression by western blotting **(B-C).** Shown are statistical analysis of TCF4 mRNA expression in MDMs from n=8 HIV-uninfected donors **(A)**. Also, shown are TCF4 (MW: 79 KDa and 58 KDa) and β-actin (MW: 42 KDa) protein expression in MDMs **(B)** and relative expression of the two TCF4 bands (79 KDa, left panel; 58 KDa right panel) relative to β-actin **(C)** in MDMs from n=3 HIV-negative donors.



Supplemental Figure 13 (relative to Figure 4): ATRA modulates the expression of β-Catenin and GSK3β in MDMs. MDMs were generated in the presence/absence of ATRA as in Figure 1A and exposed to PRI-724 (0.1μM) or PNU-74654 (10 μM) two days before infection and the day of infection. Briefly, MDMs harvested prior HIV-1 infection were used for the analysis of β-Catenin **(A-C)** and GSK3β **(D-F)** mRNA expression by RT-PCR (**A** and **D**) and intranuclear protein expression by flow cytometry (**B-C** and **E-F**) (n=8) **(A** and **D)** Shown is the ATRA-mediated decrease in β-Catenin **(A)** and GSK3β **(D)** mRNA levels in MDMs from n=8 participants. (**B-C** and **E-F**) Shown are flow cytometry histograms for β-Catenin and GSK3β expression on MDM generated in the presence/absence of ATRA from one representative participants **(B, E)**, as well as statistical analysis of the β-Catenin **(C)** and GSK3β **(F)** expression, in terms of % **(left panels)** and MFI **(right panels)** in MDMs from n=8 participants. MDM generated in the presence of ATRA were exposed to PRI-724 **(G)** and PNU-74654 **(H)** T/F HIV_{THRO} to quantify HIV-DNA integration by nested real-time PCR at day 3 post-infection. Shown are statistical analysis performed with results generated from n=7 participants. Wilcoxon values are indicated on the graphs.

Supplemental Table 1: Primers and probes used for PCR and RT-PCR. Related to STAR

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
Primer 28S Forward	5′-CGAGATTCCTGTCCCCACTA-3′	IDT	N/A
Primer 28S Reverse	5′-GGGGCCACCTCCTTATTCTA-3′	IDT	N/A
Unspliced HIV-1 mRNA Forward	5'- TTCTTCAGAGCAGACCAGAGC-3'	IDT	N/A
Unspliced HIV-1 mRNA Reverse	5'- GCTGCCAAAGAGTGATCTGA-3'	IDT	N/A
GAPDH Forward	5'-GGACCTGACCTGCCGTCTAGAA-3'	IDT	N/A
GAPDH Reverse	5'- GGTGTCGCTGTTGAAGTCAGAG-3'	IDT	N/A
YWHAZ Forward	5'- ACTTTTGGTACATTGTGGCTTCAA-3'	IDT	N/A
YWHAZ Reverse	5'- CCGCCAGGACAAACCAGTAT-3'	IDT	N/A
5' LTR Nuc-1 region Forward	5'-TGTGTGCCCGTCTGTTGTGTGA-3'	IDT	N/A
5' LTR Nuc-1 region Reverse	5'-TCGGGCGCCACTGCTAGAGA-3'	IDT	N/A

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