

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Cell culture

Ghost(3) cells (AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH) were cultured in DMEM (Invitrogen) supplemented with 10% heat-inactivated fetal bovine serum (FBS, Biocrom), 0.2mM GlutaMAX™ (Life Tech), 500µg/mL G418 (Sigma), 100µg/mL Hygromycin (Sigma) and 1µg/mL Puromycin (Sigma). HEK293T cells (AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH) were cultured in DMEM (Invitrogen) supplemented with 10% heat-inactivated FBS. PBMCs from healthy anonymous donors were isolated from buffy coats processed by the Western Province Blood Transfusion Service (University of Cape Town HREC ref 317/2016). PBMCs were separated by density centrifugation over Lymphoprep (Alere). Monocytes were isolated using Ficoll (GE Healthcare) followed by CD14+ positive selection (Miltenyi microbeads), cultured and differentiated into macrophages for 7 days in RPMI (Invitrogen) supplemented with 10% heat-inactivated Human AB serum (IPLA), sodium pyruvate (Invitrogen), L-Glutamine (Invitrogen) and 20ng/mL M-CSF (Miltenyi).

Viral plasmids, virus stocks, infections and drugs

Viral stocks were generated by co-transfecting HEK293T cells with either HIV-1 clones BaL.01 and pSG3Δenv, or NL4-3 and AD8env (AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH) using Fugene6 (Roche). Supernatants were collected 48 hours post-transfection, supplemented with FBS to a final concentration of 20% and stored in aliquots at -80°C. HEK293T cell viral stocks were used to infect Ghost(3) cells at an MOI = 1.0 for up to 72 hours. For human macrophage infections, HIV-1 strain BaL (National Institute for Biological Standards and Controls) was propagated in PBMCs in RPMI-supplemented with 50 mM glutamine, 20% FBS, 100 IU/mL penicillin, 100 µg/mL streptomycin, and 20 IU/mL IL-2 (Sigma). Supernatants were filtered through a 0.22-µm PVDF membrane (Millipore), purified by ultracentrifugation through a 20% sucrose buffer and resuspended in RPMI medium supplemented with 5% human AB serum. Macrophages were infected to a final rate of ~ 4,5% for up to 9 days. Virus input was washed out 24 hours post-infection of Ghost(3) cells and three days

post-infection for macrophages. Where indicated, cells were treated with 10 μ M of either Doxorubicin (Sigma-Aldrich), Nutlin3a (Sigma-Aldrich), MAP2K1 inhibitor (U-0126, Sigma-Aldrich) or ERK2 inhibitor (FR180204, Sigma-Aldrich) for up to 48 hours.

Cloning and transfections

Ghost(3) cells were transfected 48 hours prior to infection with 25nM (final concentration) of ON-TARGETplus human HuR/ELAV1 siRNA SMARTpool (5' GAC AAA AUC UUA CAG GUU U 3', 5' GAC AUG UUC UCU CGG UUU G 3', 5' ACA AAU AAC UCG CUC AUG C 3', 5' GCU CAG AGG UGA UCA AAG A 3'; ThermoScientific) using RNAiMax (Invitrogen). Mouse full-length lincRNA-p21 (3073 bp) and truncated lincRNA-p21 (1889 bp) sequences (Huarte et al, 2010) were synthesised (GeneArt, Life Technologies) and sub-cloned via 5' SacI and 3' EcoRI into pCi-Neo (Promega). Ghost(3) cells were transfected for 21 hours (prior to infection) with either construct using Lipofectamine2000 (Invitrogen).

Immunofluorescence

For each experiment, cells were infected or treated with drugs on coverslips, fixed for 10 mins in fresh 4% paraformaldehyde at room temperature, then washed 3 times in PBS and permeabilised for 10 mins in ice-cold methanol at -20°C. Coverslips were washed once in PBS and incubated in blocking buffer (5% goat serum, 0.3% Triton-X100 in PBS) for 60 mins at room temperature. Cells were incubated in primary antibody solution (1% BSA, 0.3% Triton X-100 in PBS) overnight at 4°C using the following antibodies rabbit polyclonal anti-phospho-histone H2A.X Ser139 (Cell Signaling), rabbit monoclonal anti-phospho-ATM Ser1981 (Cell Signaling), rabbit polyclonal anti-phospho-p53 Ser46 (Cell Signaling), mouse monoclonal anti-HIV-1 p24 (AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH), mouse monoclonal anti-HuR 3A2 (Santa Cruz Biotechnology), goat polyclonal anti-hnRNP-K P-20 (Santa Cruz Biotechnology), and rabbit polyclonal anti-phospho-MAP2K1/MAP2K2 Ser218/Ser222 (Elabscience Biotechnology). Coverslips were washed 3 times (5 mins each on an orbital shaker) with wash buffer (0.05% Tween-20 in PBS), followed by incubation with secondary antibodies conjugated to either Atto-550 or Atto-565 or Atto-647 for 60 mins at room temperature. Coverslips were washed 3 times

(5 mins each on an orbital shaker) with wash buffer (0.05% Tween-20 in PBS). Coverslips were incubated in equilibration buffer (0.4% glucose, 2X SSC) for 5 mins and counterstained with 1mg/ml DAPI (4',6-diamidino-2-phenylindole; Life Technologies). Coverslips were mounted in glox buffer (3.7mg/ml glucose oxidase, 1U catalase) and imaged. A range of 60 to 600 cells per treatment were imaged as described below.

Imaging and analysis

Cells were imaged on one of two microscopes: 1) a customised Nikon Ti Eclipse widefield fluorescent microscope using a 100x 1.49 N.A. Nikon Apochromat TIRF oil immersion objective, with mercury lamp illumination through the appropriate Semrock razor sharp filter sets at low camera gain in each of the fluorescent channels using an Andor iXion 897 EMCCD camera cooled to -80°C, and controlled using μ manager open source microscope management software (NIH and UCSF, USA). A 30ms exposure time was used for DAPI. Exposure times ranged from 200 to 500ms for other dyes. Each field of view was captured as a series of images acquired on multiple focal planes through the samples, across a range of 2-10mm in the axial plane. A 0.2mm piezo step-size was used for z-stacks. 2) An Andor Technology (Belfast, Northern Ireland) integrated Yokogawa CSU-W1 spinning disk confocal system and a Metamorph controlled Nikon TiE motorized microscope with a 60x, 1.4 NA phase oil immersion objective. Excitation sources were 405, 488 and 561 laser lines and emissions were detected through Semrock Brightline 465, 525 and 607 nm filters. Images were captured using an 888 EMCCD camera (Andor) and iQ2 software. Eight fields of view were captured per well (two wells per condition, from 3 donors), eight Z-sections per FOV with one phase and three fluorescent images. Signal intensities of all images were measured using Fiji (Schindelin et al, 2012). The contrast of images shown was adjusted to fit a 16 bit gray-scale.

Quantitative RT-PCR

PCR primer sets included:

P53 forward 5' TGTGACTTGACGTACTIONTCCC 3', reverse 5' ACCATCGCTATCTGAGCAGC 3'

CDKN1A/p21 forward 5' AGTCAGTTCCTTGTGGAGCC 3', reverse 5' GACATGGCGCCTCCTCTG 3'
HIV-1 Gag forward 5' ACTCTAAGAGCCGAGCAAGC 3', reverse 5' TGTAGCTGCTGGTCCCAATG 3'
lincRNA-p21 forward 5' GGGTGGCTCACTCTTCTGGC 3', reverse 5' TGGCCTTGCCCGGGCTTGTC 3'
HuR forward 5' AGAGCGATCAACACGCTGAA 3', reverse 5' TAAACGCAACCCCTCTGG AC 3'
MAP2K1 forward 5' ATGGATGGAGGTTCTCTGGA 3', reverse 5' TTTCTGGCGACATGTAGGACC 3'
HPRT forward 5' GCAGCCCTGGCGTCGTGATTA 3', reverse 5' CGTGGGGTCCTTTTCACCAGCA 3'

Apoptosis assay

Apoptosis was measured in Ghost(3) cells 30 hours post-infection or drug treatment using the NucView™ 488 Caspase-3 Assay Kit (Biotium) in a 96 well format. Notably, as the kit was only available with a 488nm dye, cells were analysed at the 30 hour time-point to minimise GFP input from the integrated Tat-driven reporter. An average of 3000 cells per condition were analysed with the exception of cells treated with Doxorubicin first followed by HIV-1 infection (Figure 1A) and cells transfected with lincRNA-p21 overexpression constructs followed by Doxorubicin treatment (Figure S2H). These conditions (⌘) yielded too few attached cells (<20) at 30 hours for similar analysis.