Human MX2 is an interferon–induced post–entry inhibitor of HIV–1 infection

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Animal cells harbour multiple innate effector mechanisms that inhibit virus replication. For the pathogenic retrovirus human immunodeficiency virus type 1 (HIV–1), these include widely expressed restriction factors1, such as APOBEC3 proteins2, TRIM5–α, BST2 (refs 4, 5) and SAMHD1 (refs 6, 7), as well as additional factors that are stimulated by type 1 interferon (IFN–α)–induced myxovirus resistance 2 (MX2, also known as MXB) protein as a potent inhibitor of HIV–1 infection and as a key effector of IFN–α–mediated resistance to HIV–1 infection. MX2 suppresses infection by all HIV–1 strains tested, has equivalent or reduced effects on divergent simian immunodeficiency viruses, and does not inhibit other retroviruses such as murine leukemia virus. The Capsid region of the viral Gag protein dictates susceptibility to MX2, and the block to infection occurs at a late post–entry step, with both the nuclear accumulation and chromosomal integration of nascent viral complementary DNA suppressed. Finally, human MX1 (also known as MXA), a closely related protein that has long been recognized as a broadly acting inhibitor of RNA and DNA viruses, including the orthomyxovirus influenza A virus8–12, does not affect HIV–1, whereas MX2 is ineffective against influenza virus. MX2 is therefore a cell–autonomous, anti–HIV–1 resistance factor whose purposeful mobilization may represent a new therapeutic approach for the treatment of HIV/AIDS.

We reported previously that IFN–α pre–treatment of cultured human cells and cell lines establishes patterns of HIV–1 infection ranging from severe (monocyte–derived macrophages (MDMs), the monocytic line THP–1 and the glioblastoma line U87–MG), to intermediate (primary CD4+ T cells), to minimal (lines such as CEM, HUT78 or Jurkat)10,17. We therefore used transcriptional profiling to define the human dynamin–like, IFN–induced myxovirus resistance 2 (MX2, also known as MXB) protein as a potent inhibitor of HIV–1 infection and as a key effector of IFN–α–mediated resistance to HIV–1 infection. MX2 suppresses infection by all HIV–1 strains tested, has equivalent or reduced effects on divergent simian immunodeficiency viruses, and does not inhibit other retroviruses such as murine leukemia virus. The Capsid region of the viral Gag protein dictates susceptibility to MX2, and the block to infection occurs at a late post–entry step, with both the nuclear accumulation and chromosomal integration of nascent viral complementary DNA suppressed. Finally, human MX1 (also known as MXA), a closely related protein that has long been recognized as a broadly acting inhibitor of RNA and DNA viruses, including the orthomyxovirus influenza A virus8–12, does not affect HIV–1, whereas MX2 is ineffective against influenza virus. MX2 is therefore a cell–autonomous, anti–HIV–1 resistance factor whose purposeful mobilization may represent a new therapeutic approach for the treatment of HIV/AIDS.

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As an initial screen for individual anti–viral capability, parental U87–MG CD4+ CXCR4+ cultures were untreated or treated with IFN–α, or transduced with high–titre stocks of each vector, as well as with negative control vectors expressing green fluorescent protein (GFP) or CD8, or a positive control expressing the TRIM5–cytoplphilin A (TRIMCyp) fusion protein of owl monkeys, a well–established post–entry inhibitor of HIV–1 (ref. 18). The cultures were induced with doxycycline and >85% of the cells in each culture were confirmed as E2–Crimson–positive (not shown). Five separate wells of each culture were then challenged with one of five escalating doses of HIV–1/ Nef–internal ribosome entry signal (IRES)–Renilla, a modified replication–competent virus, and productive infection quantified by monitoring activity of the Renilla luciferase reporter at 48 h (Fig. 1b). Only MX2 exhibited a clear anti–viral phenotype, with the levels of inhibition typically exceeding 90% and approaching those achieved with TRIMCyp or treatment with IFN–α. Similar results were obtained using vesicular stomatitis virus G–glycoprotein (VSV–G)–pseudotyped challenge virus, demonstrating that MX2–mediated inhibition occurs independently of the route of virus entry (Extended Data Fig. 1), as well as with CEM–SS and 293T target cells (Extended Data Fig. 2). The expression profile of MX2 in MDMs, primary T cells and cell lines was assessed by immunoblot (Fig. 1c) and quantitative PCR with reverse transcription (qRT–PCR) (Extended Data Fig. 3), confirming both IFN–α inducibility as well as preferential expression in cells displaying IFN–α–induced resistance to infection10,17.

Having found that ectopic expression of MX2 is sufficient to confer resistance to HIV–1 infection, we used gene silencing to address the contribution of MX2 to the IFN–α–induced anti–viral state. U87–MG CD4+ CXCR4+ cells were transduced three to four times with either of two lentiviral vectors expressing MX2–specific short hairpin RNAs (shRNAs sh1 and sh2) or a non–targeting shRNA control vector. After at least 8 days, the cultures were incubated with or without IFN–α, challenged with HIV–1/Nef–IRES–Renilla, and infection monitored as Renilla luciferase activity (Fig. 2a). In cultures treated with IFN–α, MX2 silencing stimulated infection by five–to tenfold relative to the control, whereas no effect was noted in the absence of IFN–α, demonstrating that MX2 has a substantial role in the restriction of HIV–1 by IFN–α. Immunoblot analyses confirmed the efficiency of MX2 silencing (Fig. 2b, lanes 4 and 6), and similar results were obtained in a second cell line, THP–1 (Extended Data Fig. 4).

Human MX2 is a member of the IFN–inducible guanosine triphosphatase (GTPase) superfamily that includes proteins involved in cellular processes requiring membrane remodelling, such as vesicular transport and cytokinesis, as well as in resistance to intracellular pathogens14. The most closely related family member is human MX1 (63% amino acid sequence identity), which inhibits a variety of RNA and DNA viruses, including influenza A virus, La Crosse encephalitis virus and hepatitis B virus, and is thought to form an oligomeric ring that engages and disrupts viral nucleoprotein/repliaion complexes15,20. Conversely, relatively little information concerning MX2 function is available: it is nuclear as well as cytoplasmic and accumulates at the cytoplasmic face of nuclear pores15. MX2 may have a role in cell cycle progression, but has been discounted from further study (the latter being cytotoxic).

To define more closely how MX2 inhibits HIV–1 replication, we challenged parental U87–MG CD4+ CXCR4+ cells, cultured with or
Human MX2 is a potent inhibitor of HIV-1 infection. a, Schematic representation of the EasiLV (E2-Crimson antisense inducible lentiviral vector) system. pEasiLV-MCS contains an internal antisense and Tet-inducible expression cassette driving expression of a tricistronic RNA encoding the cDNA of interest, the reverse responsive tetracycline transactivator variant 3 (rtTA3) and the E2-Crimson indicator gene (TetOCMV, tetracycline operator-minimal cytomegalovirus promoter; PA, polyadenylation signal) (see Methods for details). b, Candidate cDNA screen in U87-MG CD4+ CXCR4+ cells. U87-MG CD4+ CXCR4+ cells were transduced with EasiLV expressing the different candidate cDNAs, CD8 (negative control), GFP (negative control) or TRIMCyp (positive control) cDNAs and either treated with doxycycline for 48 h, left untransduced (Ctrl) or treated with 1,000 U ml−1 IFN-α for 24 h before HIV-1 infection. The cells were infected with increasing viral inputs of NL4-3/Nef-IRES-Renilla (0.04–25 ng p24Gag) and infection efficiency was monitored 48 h later by measuring Renilla activity. Mean relative infection efficiencies with standard deviations from four independent experiments are shown. c, Immunoblot analysis of MX2 protein levels in control and IFN-α-treated Jurkat, HUT78, CEM-SS, primary CD4+ T cells, U87-MG, THP-1 and MDMs; HSP90 served as a loading control. The IFN-α-induced resistance phenotype of each cell type is shown underneath (−, no resistance; +, resistance).

Figure 2 MX2 is required for effective IFN-α-induced suppression of HIV-1. a, U87-MG CD4+ CXCR4+ cells expressing a control shRNA or two different shRNAs targeting MX2 were cultured with or without IFN-α (500 U ml−1) for 24 h. Cells were infected with five different doses of NL4-3/ Nef-IRES-Renilla (0.04–25 ng p24Gag) for 48 h, and Renilla activity was measured. Mean relative infection efficiencies from two independent experiments are shown. b, Immunoblot analysis of parallel samples from a. Protein levels of MX2 and SAMHD1 (positive control for IFN-α induction) were determined and HSP90 served as a loading control.

without IFN-α, and cells transduced with CD8- or MX2-expressing vectors, with wild-type HIV-1 and then collected total DNA at 2, 6, 24 and 48 h. The 48-h cultures were also analysed for p24 expression using flow cytometry, confirming MX2-mediated inhibition of viral gene expression (Extended Data Fig. 5). qPCR was then used to measure viral reverse transcripts representing three phases of replication: extended minus (first)-strand cDNA, 2-long terminal repeat (LTR) circular DNA (a marker for viral cDNA nuclear localization) and integrated (provirus) DNA (Fig. 3). As reported previously, IFN-α treatment severely blocked the accumulation of all HIV-1 cDNAs. By contrast, MX2 did not measurably affect the synthesis or accumulation of minus-strand cDNA, but reduced the levels of 2-LTR circles and proviruses by ~90%, possibly indicating a blockade to the nuclear uptake of viral replication complexes or a decrease in their stability.

We next examined the ability of MX2 to suppress infection by a range of primate lentiviruses including laboratory-adapted strains of HIV-1, HIV-1–transmitted founder strains, HIV-2 and simian immunodeficiency viruses (SIVs) derived from the rhesus macaque (SIVMAC), mandrill (SIVMND) or African green monkey (SIVAGM). This was quantified by using the virus-encoded Tat proteins that are expressed after infection to trans-activate an HIV-LTR/luciferase reporter cassette that was resident in target U87-MG cells. These reporter cells were transduced with either CD8- or MX2-expressing vectors and subsequently challenged with two doses of VSV-G-pseudotyped stocks of HIVs or SIVs. Measurement of luciferase levels at 48 h showed that all HIV-1s and SIVMND were susceptible to potent repression by MX2, whereas HIV-2, SIVMAC and SIVAGM were somewhat less sensitive (Fig. 4a). The analysis was then extended to three non-primate viruses, the lentiviruses equine infectious anemia virus (EIAV) and feline immunodeficiency virus (FIV), and the gammaretrovirus murine leukemia virus (MLV). Here, we used retroviral vectors encoding GFP and monitored single-cycle infectivity at 48 h by flow cytometry (Fig. 4b). Interestingly, whereas MX2 suppressed infection by the HIV-1–based vector by ~80%, no inhibition of the three non-primate viruses was observed, demonstrating that the human MX2 protein exhibits substrate selectivity, albeit to differing extents, for primate lentiviruses.

Current views on the post-entry progression of HIV-1 infection invoke the sustained presence of the viral Capsid (CA) protein within reverse transcription complexes, as well as a central role for CA in mediating interactions with host proteins such as cyclophilin A, TNPO3, NUP358 (also known as RANBP2), NUP153 or TRIM5-α that influence the fate of infection. To address whether CA
were either not infected (NI) or challenged with 10 ng p24Gag HIV-1IIIB and productive infection (Extended Data Fig. 5).

expression was also determined at 48 h in parallel samples to monitor

Figure 3 | MX2 inhibits the nuclear accumulation and integration of HIV-1 reverse transcripts. a–c, U87-MG CD4+ CXCR4+ cells were transduced with EasiLV expressing CD8 or MX2 and treated with doxycycline for 48 h, left untransduced (Ctrl) or treated with IFN-α for 24 h before infection. The cells were either not infected (NI) or challenged with 10 ng p24Gag HIV-1IIIB and collected at 2, 6, 24 or 48 h after infection for DNA extraction and qPCR analysis of minus-strand DNA (a), 2-LTR circle DNA (b) and integrated proviral DNA (c). Mean values of relative amounts of DNA (normalized to control at 48 h) from three independent experiments are shown. The detection limit for 2-LTR circle qPCR was ten copies per reaction, which corresponds to ~6% relative copies as indicated on the graph by a dashed grey line. p24Gag expression was also determined at 48 h in parallel samples to monitor productive infection (Extended Data Fig. 5).

determines the sensitivity of HIV-1 to MX2, we measured the effects of MX2 using GFP-encoding vectors carrying the P90A or N74D mutations in CA that inhibit/prevent interactions with CypA, TNPO3, NUP358 or NUP153, or that had the CA region of Gag replaced with SIVMAC CA (Fig. 4c). In contrast to the ~80% inhibition of wild-type Gag, the P90A and N74D CA variants were insensitive or only mildly sensitive to inhibition by MX2, respectively, and the SIV-CA-containing chimaera displayed modest inhibition, reflecting closely that of the parental SIVMAC protein. The observation that modifying HIV-1 CA can control MX2 susceptibility or escape suggests that CA is a specific target of MX2.

In a final series of experiments we assessed the effects of MX1 and MX2 on HIV-1 and influenza A virus replication (using analogous assays that measure the culmination of infection, viral RNA synthesis and protein expression). Influenza A virus genome segment replication was determined by co-transfecting 293T cells with a vector expressing a firefly luciferase-containing minigenome (as well as a vector expressing Renilla luciferase for normalization), together with vectors for wild-type MX1 or MX2 (Flag-tagged and -untagged), or the tagged GTPase-deficient MX1 derivatives K83A and T103A. The cells were infected with 25 ng p24Gag of NL4-3/Nef-IRES-Renilla and infection efficiencies from four independent experiments are shown. The percentage of GFP-expressing cells was evaluated by flow cytometry. Mean percentages of transduced cells from four independent experiments are shown. 

Figure 4 | Viral substrates for the human MX1 and MX2 proteins. a, U87-MG/LTR–Luc cells were transduced with EasiLV expressing CD8 or MX2. Cells were infected with two doses (1 and 10, corresponding to 50 and 500 pg RT) of VSV-G-pseudotyped HIV-1NL4-3, HIV-1YU2, HIV-1CH077.t, HIV-1CH106.c, HIV-1REJO.c, HIV-2ROD10, SIVMAC239, SIVAGM, SIVMND121. Luciferase activity was measured at 48 h. Mean values for three independent experiments are shown. b, CD8- or MX2-expressing U87-MG cells were challenged with HIV-1-, EIAV-, FIV- and MLV-based retroviral vectors expressing GFP at a multiplicity of infection (m.o.i.) of 0.25. The percentage of GFP-expressing cells was evaluated by flow cytometry. Mean percentages of transduced cells from four independent experiments are shown. c, CD8- or MX2-expressing U87-MG cells were challenged with GFP-encoding HIV-1-based vectors (containing wild-type (WT) CA, CAN74D,C, AP90A or CA from SIVMAC (CA31)), or an SIVMAC-based vector at a m.o.i. of 0.25 as in b. The percentage of GFP-expressing cells was evaluated, and mean percentages of transduced cells for four independent experiments (three for CASIV) are shown. d, 293T cells were co-transfected with expression plasmids for GFP (Neg Ctrl), IFITM3, untagged and Flag-tagged MX1 and MX2 (MX1–Fl and MX2–Fl), or the Flag-tagged MX1 GTPase-deficient mutants MX1(K83A) and MX1(T103A) along with an influenza A virus firefly luciferase minigenome plasmid and a Renilla luciferase expression plasmid. At 24 h, cells were infected with influenza A virus A/Victoria/3/75 (H3N2) at a m.o.i. of 2 and firefly and Renilla luciferase activities were measured 18 h after infection. Mean relative infection efficiencies for three independent experiments are shown. e, U87-MG CD4+ CXCR4+ cells were transduced with EasiLV expressing CD8, TRIMCyp, MX1, MX2 or the mutants MX1(K83A), MX1(T103A) and MX2(K131A). The cells were infected with 25 ng p24Gag of NL4-3/Nef-IRES-Renilla and infection efficiency was monitored at 48 h by measuring Renilla activity. Mean relative infection efficiencies from three independent experiments are shown.
cultures were infected with influenza A virus, and firefly luciferase expression measured 18 h later (Fig. 4d). As established previously, wild-type MX1, as well as IFITM3 (positive control)\textsuperscript{25}, suppressed replication by 75–80%, whereas the GTPase domain mutant proteins had lost anti-viral activity\textsuperscript{26,27}, consistent with previous studies, MX2 did not exert any inhibitory effect\textsuperscript{14}. The wild-type MX1 and MX2 proteins, and the K131A mutant of MX2 that does not bind GTP\textsuperscript{22}, were then examined for their HIV-1 inhibitory phenotypes in transduced U87-MG CD4\textsuperscript+ CCR5\textsuperscript+ cells as in Fig. 1b. In contrast to the results with influenza A virus, MX1 had no effect on HIV-1, and the mutated MX2(K131A) protein still retained a degree of anti-viral function (∼65% inhibition, Fig. 4e). Immunoblotting confirmed expression of the Flag-tagged proteins, although MX2(K131A) accumulated to a lower level than the wild-type protein (Extended Data Fig. 6).

Here we describe the identification of human MX2 as an IFN-α/β-inducible anti-retroviral effector that, among primate immunodeficiency viruses, is most potent against HIV-1, but does not affect the non-primate viruses MLV, EIAV and FIV (Fig. 4a, b). Understanding the molecular details of MX2's recognition and inactivation of post-entry viral reverse transcription complexes, the interplay with other regulatory host proteins that interact with CA, and the basis for the dichotomy between MX2/HIV-1 inhibition and MX1/influenza virus inhibition with respect to GTPase function (Fig. 4d, e) will help to elucidate the mechanism of this new mode of cell-mediated resistance to retroviral infection. As viral inhibition occurs relatively late during infection and is manifested as the failure to accumulate viral cDNA in the nucleus (Fig. 3), the anti-viral action of MX2 is distinct from TRIM5-α or APOBEC3G-mediated inhibition of early reverse transcription or SAMHD1-mediated restriction through deoxynucleotidetriphosphate depletion\textsuperscript{14}.

Last, we note that although MX2 silencing substantially relieves IFN-α-induced resistance to HIV-1, measurable inhibition persists (Fig. 2 and Extended Data Fig. 4); taken together with the observation that IFN-α imposes an early block to HIV-1 reverse transcription (Fig. 3)\textsuperscript{10}, we speculate that additional IFN-stimulated factor(s) that interfere with the initial post-entry phases of HIV-1 infection remain to be discovered.

METHODS SUMMARY

Plasmids, cells, viral vectors and EasIvLV system. All reagents, including the novel inducible lentivirus vector pEasIvLV-MCS, are described in Methods. Candidate CDNAs were cloned into pEasIvLV-MCS for functional screening.

Virus infection. Lenti viral, retroviral and influenza A virus infections were monitored using standard reporter genes, and HIV-1 cDNA was measured by qPCR.

Microarray. Illumina HumanHT12v4 expression bead chips were probed with microarray.

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METHODS

HIV-1, HIV-2 and SIV molecular clones, lentiviral and retroviral vectors. HIV-1 proviral clones for the NL-3, YU-2 and IIB1 isolates have been described21-23. The NL-3/Nef-IREs-NeuIa reporter virus (which expresses NeuIa luciferase from an internal ribosome entry site) was provided by S. Chanda and K. Oliверi, and the NL-3/Nef-IREs-GFP reporter was obtained from the NIH AIDS Reagent and Reference Program (catalogue no. 11349, pBR43IeG-nef+, expresses GFP24). The transmitted founder HIV-1 molecular clones CH077,t CH106.c and REJO.c were provided by B. Hahn25. HIV-2 and SIV were amplified from pEASI-LV, p8.91, pptTRKrab49 and pMD.G at a ratio of 1:1:0.5:0.25, and cloning techniques. MX2(K131A)–Flag) were inserted into pEasiLV-MCS using standard mutagenesis and cloning techniques. The arrangement of this vector was amplified from pEF_Bos_2xFLAG_MDA555.cDNA's, as well as on the IFN-γ treatment due to cell type (relative to the CEM lymphoid cell line), and due to potential on-going transcription) and the BGH polyA (amplified from pcDNA3.1) downstream of the E2-Crimson coding sequence; (3) the entire inducible cassette was inserted into an HIV-1-based lentiviral vector (pRRL.sin.pCMV/ TCFW.PRE25) in place of the CMV-eGFP cassette in the antisense orientation, to create pEasiLV (E2-crimson antisense inducible lentiviral vector); and (4) pEasiLV-MCS was created by introducing a multiple cloning site (MCS) containing BamHI, AgeI, PacI, Pmel, Thh111, SnaBI, XhoI and NsiI restriction sites.

Cloning of candidate cDNAs. The candidate cDNAs (refer to Extended Data Table 1 for accession numbers), as well as IFITM3 cDNA (NM_021034), were amplified using the SuperScript III One-Step RT–PCR System with Platinum Taq (Invitrogen) from 20 to 100 ng RNA obtained from IFN-α-treated MDMs, except for IFIH1, which was amplified by PCR from pEF_Box2_xFLAG_MDA55, cDNAs, as well as CD8 and GFP (negative controls), were inserted into pEasiLV-MCS between the BamHI and XhoI sites (sites equivalent to pMD.G). The TRIMCyp cDNA (positive control) was amplified by PCR from pE鑫NATB-BcRcNod-luCyP4a and inserted into pEasiLV-MCS as an AgeI-XhoI fragment. cDNAs encoding MX1 and MX2 Flag-tagged at their carboxy termini, and the derivative GTPase domain mutants (MX1(K83A)–Flag, MX1(K131A)–Flag, MX2(K131A)–Flag) were inserted into pEasiLV-MCS between the BamHI and XhoI sites (sites equivalent to pMD.G). The TRIMCyp cDNA (positive control) was amplified by PCR from pE鑫NATB-BcRcNod-luCyP4a and inserted into pEasiLV-MCS as an AgeI-XhoI fragment.

Microarray analysis. In total, 1–2 × 10⁶ primary CD4⁺ T cells (previously activated with IL-2 and PHA), MDMs, CEM, CEM-SS, Jurkat, U87-MG, HT1080, U937, PMA-U937, THP-1 and PMA-THP-1 cells were collected 24 h after IFN-γ treatment, or no treatment. RNA was isolated using the miRNeasy kit with on-column DNase treatment (Qiagen). A total of 500 ng RNA was used for complementary RNA probe preparation using the Illumina TotalPrep RNA Amplification Kit (Ambion), according to the manufacturer’s instructions. The probes were hybridized on Illumina HT12v4 bead arrays following the manufacturer’s standard hybridization and scanning protocols.

Raw signals, detection P values, bead numbers and bead-level standard errors were exported for regular and control probes from GenomeStudio. Data were imported into R using the Biocductor beadarray and illuminaHumanv4.db packages. Microarray probes annotated by the latter as not or badly mapping to the reference genome were excluded. Data then were background-corrected, quantile-normalized and log₂-transformed using the limma implementation of the negc method26. Using limma, a factorial linear model (cell type × treatment) was fitted to data, and relative array quality weights were computed. The range of fold change was small (a few fives), the median range of fold change per array was 2 (±4-fold upregulation) on the comparison between MDMs and the CEM cells as well as on the IFN-γ treatment versus no treatment control, implying an additive effect size of >4 (±16-fold upregulation) on gene expression.

EasiLV inducible lentiviral vector system. The arrangement of this vector is diagrammed in Fig. 1a. pEasiLV-MCS (pBRIL.sin.cPPT(Tet2/OcmV/MCS, IRES.rtTA3-2A-E2-Crimson) antisense.WPRE) was constructed using the following steps: (1) the Tet2OcmV/d2GFP-IREs-tTA3 tetracycline (Tet)-inducible expression cassette from TRTraitor3 (provided by J. Seppey)27 was modified by overlapping PCR to remove the stop codon from the Tet3 coding sequence and to add, in frame, both the sequence coding the 2A-like peptide from Thogoto asigna virus (hereafter called the 2A peptide (NH2-GSGEGRGSLLTCGDVEENPGP), which allows the generation of two independent proteins by a co-translational cleavage process28,29) and the E2-Crimson fluorescent reporter gene (2); the inducible cassette was further modified to add a synthetic polyadenylation signal (polyA, amplified from pGL4, Promega) upstream of the Tet inducible promoter (to stop any potential on-going transcription) and the BGH polyA (amplified from pCDNA3.1) downstream of the E2-Crimson coding sequence; (3) the entire inducible cassette was inserted into an HIV-1-based lentiviral vector (pRRL.sin.pCMV/TGF.PRE25) in place of the CMV-eGFP cassette in the antisense orientation, to create pEasiLV (E2-crimson antisense inducible lentiviral vector); and (4) pEasiLV-MCS was created by introducing a multiple cloning site (MCS) containing BamHI, AgeI, PacI, Pmel, Thh111, SnaBI, XhoI and NsiI restriction sites.

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cDNAs encoding MX1 and MX2 Flag-tagged at their carboxy termini, and the derivative GTPase domain mutants (MX1(K83A)–Flag, MX1(K131A)–Flag, MX2(K131A)–Flag) were inserted into pEasiLV-MCS using standard mutagenesis and cloning techniques.

Viral production. To produce EasiLV particles, 293T-TetR-KRAB cells were co-transfected with pEasiLV, p8.91, pPTiKRAB40 and pMD.G at a ratio of 1:1:0.5:0.25, respectively, with Fugene 6 reagent (Promega). The medium was replaced after overnight incubation and viral particles collected at 24 h, filtered and used directly to transduce target cells. After a few hours, the media was replaced and dexamethasone added (0.5 µg ml⁻¹, Sigma–Aldrich) to induce transgene expression. The percentage of E2-Crimson-positive cells was scored by flow cytometry (FACSCalibur, BD Biosciences) after 48 h and was typically >85%.

HIV-1 particles were produced by standard polyethylenimine (PEI) transfection of 293T monolayers. VSVG-pseudotyped HIV and SIV stocks were prepared by co-transfection using a 3:1 ratio of provirus to pMD.G41, the culture medium was changed at ~6 h, and virus containing supernatant collected at ~36 h.

Lentiviral and retroviral vector stocks were obtained by PEI-mediated triple transfection of 293T cells with vectors expressing Gag-Pol (p8.91, pF9P3, pE53, pE55, pEF_Bos_2xFLAG_MDA55), vectors expressing miniviral genomes bearing a CMV-eGFP cassette (pRRL.sin.cPPT.CMV/GFP.WPRE, pGInvW, pSIN16.CeGFPW, pGAE1.0 and p13077), and pMD.G, at a ratio of 1:1:0:5.
Viral particles were filtered, and when required (that is, to enable NL4-3/Nef-IRES-Revil-nia or NL4-3/Nef-IRES-GFP to be used in 96-well format infections), virus containing supernatants were purified by ultracentrifugation through a sucrose cushion (20% w/v; 75 min; 4 °C, 145,370g using a Sorvall Surespin630 rotor), re-suspended in RPMI 1640 medium without serum and stored in aliquots at −80 °C.

Viral particles were normalized according to HIV-1 p24Gag ELISA (Perkin Elmer), by RT ELISA (Roche Applied Science) and/or by determining their infectious titres on U87-MG cells. The m.o.i. for lentiviral and retroviral vector stocks was determined by infecting a known number of U87-MG cells with standardized amounts of viral particles and evaluating by flow cytometry the percentage of infected cells 2–3 days later. For instance, an m.o.i. of 0.25 equates to the volume of virus necessary to obtain 25% of GFP-expressing U87-MG cells.

Lentiviral infection. All infections were initiated using standard conditions, often using a range of viral inocula. For infection with NL4-3/Nef-IRES-Revil-nia or GFP-expressing lentiviral and retroviral vector, U87-MG CD4+ CXCR4+, U87-MG/LTR-Luc or 293T cells were plated at 2.5–5 × 10⁶ per well in 96-well plates. CEM-SS cells were plated at 2 × 10⁶ per well in 96-well plates. When HIV-1 infection was followed by DNA extraction or p24Gag intracellular staining, U87-MG-CD4+ CXCR4+ cells were seeded at 2 × 10⁶ cells per well in 24-well plates. When required, cells were treated with IFN-α or reverse transcriptase inhibitors (AZT and 3TC, 10 μM each) for 24 h or at least 15 min, respectively, before viral challenge.

The efficiency of productive infection was analysed after −48 h by evaluating the percentage of GFP- or p24Gag-expressing cells using flow cytometry (FACSCalibur, BD Biosciences), or by measuring either firefly or Renilla luciferase activity (Promega). For p24Gag intracellular staining, the cells were washed in PBS, incubated for 10 min in trypsin to remove surface-associated virion particles, fixed and permeabilized (Intrastain kit, DAKO), and then stained with a p24Gag-specific antibody (KC57-RD1, Beckman Coulter)11.

Influenza A virus infection. Wild-type human MX1, MX2, or MX1 GTPase domain proteins were cloned into pEasiLV vector (Promega). For p24Gag intracellular staining, the cells were washed in PBS, incubated for 10 min in trypsin to remove surface-associated virion particles, fixed and permeabilized (Intrastain kit, DAKO), and then stained with a p24Gag-specific antibody (KC57-RD1, Beckman Coulter)11.

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Extended Data Figure 1 | MX2 inhibits infection with VSV-G-pseudotyped HIV-1. U87-MG CD4+ CXCR4+ cells were transduced with EasiLV expressing the different candidate cDNAs, CD8, GFP or TRIMCyp cDNA controls and treated with doxycycline for 48 h, left untransduced (Ctrl) or treated with IFN-α for 24 h before infection. The cells were infected with increasing viral inputs of VSV-G-pseudotyped NL4-3/Nef-IRES-Renilla (0.04–5 ng p24Gag) and infection efficiency was monitored at 48 h by measuring Renilla activity. Mean relative infection efficiencies from four independent experiments are shown.
Extended Data Figure 2 | MX2 is active in other cell types. a, CEM-SS cells were transduced with EasILV expressing CD8 or MX2 and treated with doxycycline for 4–5 days and infected with increasing viral inputs of VSV-G-pseudotyped NL4-3/Nef-IRES-GFP (0.2–25 ng p24Gag). The efficiency of infection was measured at 72 h by scoring the percentage of GFP-expressing cells, among the E2-Crimson-expressing cells, by flow cytometry. Mean percentages of infected cells from independent duplicates are shown. b, 293T cells were transduced with lentiviral vectors containing a bicistronic expression cassette for either CD8 or MX2 with the puromycinR gene, and selected for puromycin resistance. Transduced cells were infected with increasing viral inputs of VSV-G-pseudotyped NL4-3/Nef-IRES-Renilla (0.2–25 ng p24Gag) and Renilla activity was measured at 48 h. Mean relative infection efficiencies from three independent experiments are shown.
Extended Data Figure 3 | MX2 relative levels of expression and IFN-α-inducibility. RNA was extracted from control (Ctrl) or IFN-α-treated (IFNα) PMA-treated or dividing THP-1 cells, MDMs, primary CD4⁺ T cells, U87-MG, HT1080, CEM, CEM-SS, HUT78 and Jurkat cells. 500-ng aliquots of RNA were reverse transcribed and the levels of MX2, as well as ISG15, and two endogenous controls, GAPDH and β-actin (ACTB), were analysed by qRT-PCR. The graph shows the mean of relative levels of expression (normalized to both endogenous controls and compared to THP-1 without IFN-α treatment) obtained in three independent experiments.
Extended Data Figure 4 | MX2 participates in IFN-α-induced resistance to HIV-1 in monocytic THP-1 cells. a, THP-1 cells expressing a control shRNA or two different shRNAs targeting MX2 were treated with or without IFN-α (500 U ml⁻¹) for 24 h. Cells were infected with five different doses of NL4-3/Nef-IRES-Renilla (0.04–25 ng p24Gag) for 48 h, and Renilla activity was measured. Mean relative infection efficiencies from two independent experiments are shown. b, Immunoblot analysis of parallel samples from a. Protein levels of MX2 and APOBEC3G (A3G, positive control for IFN-α treatment) were determined, and tubulin served as a loading control.
Extended Data Figure 5 | MX2 blocks productive HIV-1<sub>11B</sub> infection. U87-MG CD4<sup>+</sup> CXCR4<sup>+</sup> cells were transduced with EasiLV expressing CD8 or MX2 and treated with doxycycline for 48 h, left untransduced (Ctrl) or treated with IFN-α for 24 h before infection. Increasing viral inocula (indicated in ng p24<sup>Gag</sup>) were used to infect the cells with HIV-1<sub>11B</sub>. The levels of infection were analysed at 48 h by measuring the percentage of cells expressing p24<sup>Gag</sup> after intracellular staining. The means of three independent experiments are shown. This analysis accompanies the experiments shown in Fig. 3.
Extended Data Figure 6 | Expression of wild-type and GTPase domain mutants of MX1 and MX2. Immunoblot analysis of parallel samples from Fig. 4e. Protein levels of Flag-tagged MX1 and MX2 proteins were determined using a Flag-specific antibody and tubulin served as a loading control.
## Extended Data Table 1 | Candidate genes

| Probe     | Symbol | CEM EXP* | FC MDM / CEM† | FC IFN‡ | Accession  |
|-----------|--------|----------|---------------|---------|------------|
| ILMN_2058782 | IFI27  | 4.55     | 3.04          | 6.81    | NM_005532  |
| ILMN_1701789 | IFIT3  | 5.55     | 3.98          | 5.30    | NM_001549  |
| ILMN_2231928 | MX2    | 6.10     | 4.26          | 4.28    | NM_002463  |
| ILMN_1662358 | MX1    | 7.79     | 4.08          | 4.12    | NM_001144925 |
| ILMN_2410826 | OAS1   | 6.29     | 2.71          | 5.27    | NM_016816  |
| ILMN_1739428 | IFIT2  | 6.75     | 4.13          | 3.69    | NM_001547  |
| ILMN_1707695 | IFIT1  | 8.25     | 2.51          | 5.30    | NM_001548  |
| ILMN_1791759 | CXCL10 | 4.30     | 5.24          | 2.46    | NA         |
| ILMN_1657871 | RSAD2  | 7.02     | 2.46          | 4.26    | NM_080657  |
| ILMN_2347798 | IFI6   | 9.70     | 2.31          | 4.23    | NM_002038  |
| ILMN_1674811 | OASL   | 5.30     | 2.25          | 4.26    | NM_003733  |
| ILMN_1781373 | IFIH1  | 6.75     | 3.30          | 2.62    | NM_022168  |
| ILMN_1690105 | STAT1  | 9.05     | 2.61          | 2.75    | NA         |
| ILMN_2148785 | GBP1   | 9.06     | 2.60          | 2.01    | NM_002053  |

* Log₂ absolute expression in CEM.
† Log₂ fold-change in Macrophages versus CEM.
‡ Log₂ fold-change IFN treatment versus no treatment.