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Supplemental Information

Nuclear Envelope Protein SUN2 Promotes Cyclophilin-A-Dependent Steps of HIV Replication

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FIGURE S1

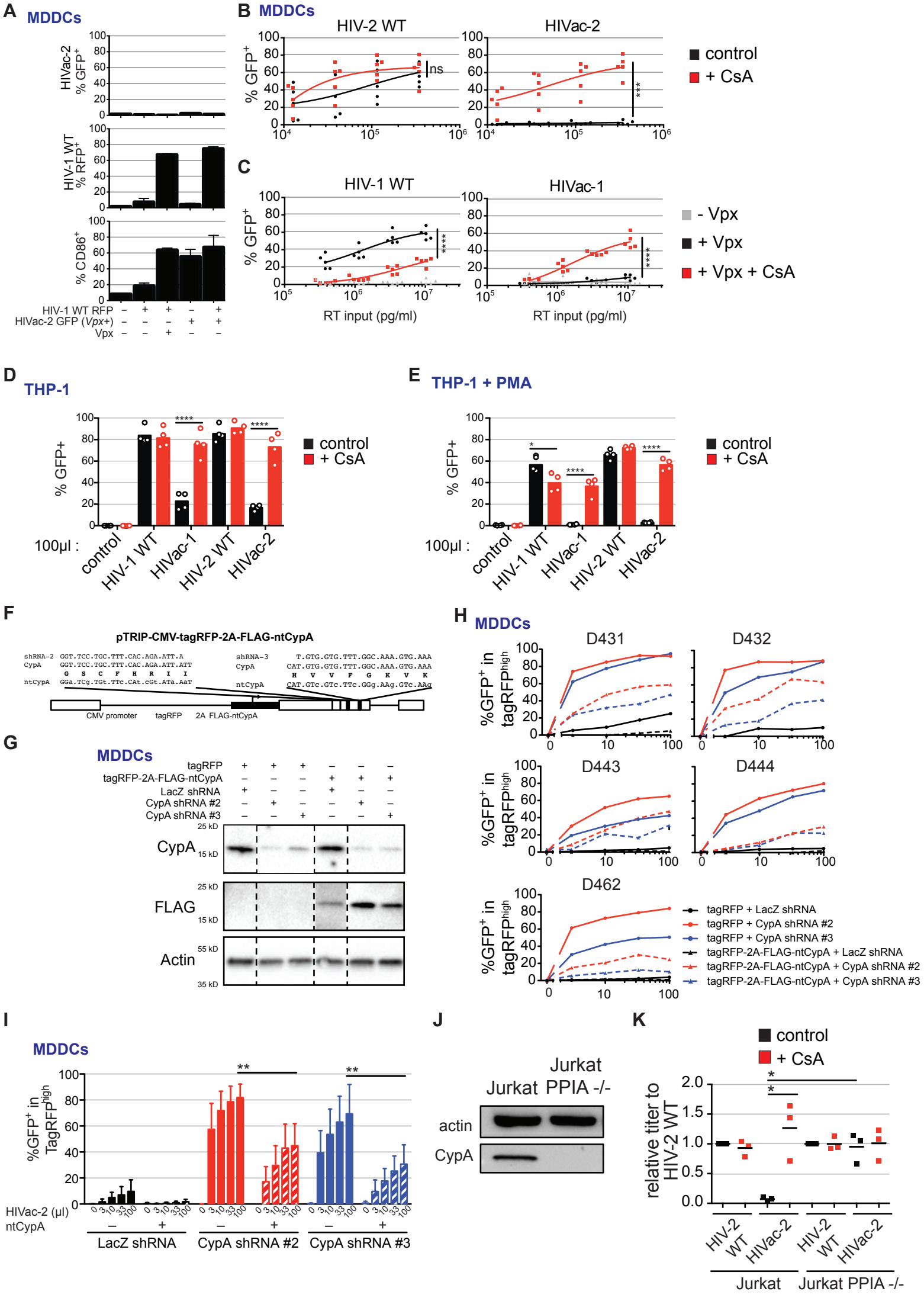
A
MDDCs
HIVac-2 % GFP+
HIV-1 WT % RFP+
% CD86+
HIV-1 WT RFP
HIVac-2 GFP (Vpx+)
Vpx
B
MDDCs
HIV-2 WT
HIVac-2
% GFP+
ns
***
control
+ CsA
C
HIV-1 WT
HIVac-1
% GFP+
****
RT input (pg/ml)
- Vpx
+ Vpx
+ Vpx + CsA
D
THP-1
% GFP+
control
+ CsA
100µl :
control
HIV-1 WT
HIVac-1
HIV-2 WT
HIVac-2
E
THP-1 + PMA
% GFP+
control
+ CsA
100µl :
control
HIV-1 WT
HIVac-1
HIV-2 WT
HIVac-2
F
pTRIP-CMV-tagRFP-2A-FLAG-ntCypA
shRNA-2 GGT.TCC.TGC.TTT.CAC.AGA.ATT.A
CypA GGT.TCC.TGC.TTT.CAC.AGA.ATT.ATT
G S C F H R I I
ntCypA GGa.TCg.TGt.TTc.CAt.cGt.ATa.AaT
shRNA-3 T.GTG.GTG.TTT.GGC.AAA.GTG.AAA
CypA CAT.GTG.GTG.TTT.GGC.AAA.GTG.AAA
H V V F G K V K
ntCypA CAT.GTc.GTc.TTc.GGg.AAg.GTc.AAg
CMV promoter
tagRFP
2A FLAG-ntCypA
G
MDDCs
tagRFP
tagRFP-2A-FLAG-ntCypA
LacZ shRNA
CypA shRNA #2
CypA shRNA #3
25 kD
CypA
15 kD
FLAG
Actin
55 kD
35 kD
H
MDDCs
D431
D432
D443
D444
D462
%GFP+ in tagRFPhigh
tagRFP + LacZ shRNA
tagRFP + CypA shRNA #2
tagRFP + CypA shRNA #3
tagRFP-2A-FLAG-ntCypA + LacZ shRNA
tagRFP-2A-FLAG-ntCypA + CypA shRNA #2
tagRFP-2A-FLAG-ntCypA + CypA shRNA #3
I
MDDCs
%GFP+ in TagRFPhigh
**
HIVac-2 (µl)
ntCypA
LacZ shRNA
CypA shRNA #2
CypA shRNA #3
J
Jurkat
Jurkat PPIA -/-
actin
CypA
K
control
+ CsA
relative titer to HIV-2 WT
HIV-2 WT
HIVac-2
Jurkat
Jurkat PPIA -/-

Figure S1 CypA is required for a restriction of HIV-1 and HIV-2 capsid mutants, related to Figure 1

(A) GFP, RFP and CD86 expression in MDDCs 48h after infection or co-infection with HIVac-2 (encoding GFP in Nef) and HIV-1 WT (encoding RFP in Nef) and pseudotyped with VSV-G in the presence or absence of SIVmac251 VLPs (n=2, bar indicates mean +/- SEM).

(B) Dose-response GFP expression for infections with HIV-2 and HIVac-2 as in 1A (n=5, paired t-test, ***p<0.001, "ns" stands for not statistically significant, line indicates mean).

(C) Dose-response GFP expression for infections with HIV-1 and HIVac-1 as in 1A (n=5, paired t-test, ****p<0.0001, line indicates mean).

(D) GFP expression in THP-1 cells 48 hours after infection with HIV-1WT, HIVac-1, HIV-2 WT, and HIVac-2, in the presence of absence of CsA (n=4, paired t-test, ****p<0.0001, bar indicates mean). Virus inoculum volume is indicated.

(E) GFP expression in PMA-differentiated THP-1 cells 48 hours after infection with HIV-1WT, HIVac-1, HIV-2 WT, and HIVac-2, in the presence of absence of CsA (n=4, paired t-test, *p<0.05, ****p<0.0001, bar indicates mean). Virus inoculum volume is indicated.

(F) Design of the non-targetable CypA (ntCypA) open-reading frame for shRNA rescue used in 1D.

(G) Detection of endogenous CypA, FLAG-ntCypA and actin as in 1I. Dashes indicate image clipping of the same exposure. Not shown: FLAG-ntCypA is detected with anti-CypA at longer exposures.

(H) Dose-response of GFP expression in tagRFP^{high} cells for each individual donor. Virus inoculum volume is indicated.

(I) Averaged data on dose-response of GFP expression in tagRFP^{high} cells as in 1I (n=5, paired t-test, **p<0.01, bar indicates mean +/- SEM). Virus inoculum volume is indicated.

(J) Western blot analysis of CypA and actin expression on Jurkat and Jurkat *PPIA*$^{-/-}$ cell lines.

(K) Relative titer of HIV-2 WT and HIVac-2 on Jurkat and Jurkat *PPIA*$^{-/-}$ cell lines reported under HIV-2 WT infection, in the presence or absence of CsA (2μM) (n=3, paired t-test *p<0.05, line indicates mean).

FIGURE S2

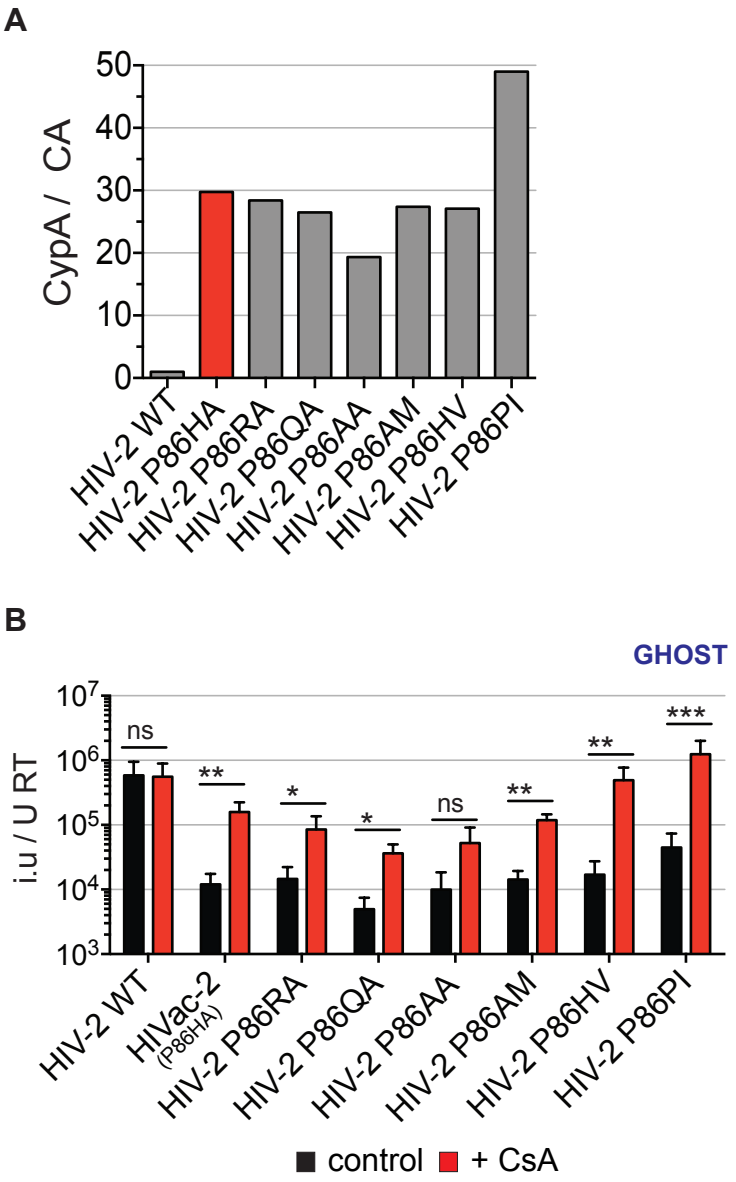
A
CypA / CA
50
40
30
20
10
0
HIV-2 WT
HIV-2 P86HA
HIV-2 P86RA
HIV-2 P86QA
HIV-2 P86AA
HIV-2 P86AM
HIV-2 P86HV
HIV-2 P86PI
B
GHOST
i.u / U RT
10^7
10^6
10^5
10^4
10^3
ns
**
*
*
ns
**
**
***
HIV-2 WT
HIVac-2 (P86HA)
HIV-2 P86RA
HIV-2 P86QA
HIV-2 P86AA
HIV-2 P86AM
HIV-2 P86HV
HIV-2 P86PI
control
+ CsA
C
Human MDDCs
10 µm
10 µm
10 µm
Macaque MDDCs
10 µm
10 µm
10 µm
10 µm

Figure S2 CypA induces a direct antiviral resistance against a family of capsid mutants in HIV and SIV that is conserved in human and macaque cells, related to Figure 2

(A) Ratios of CypA over capsid in viral particles, quantified from western blot analyses against Gag/Capsid and CypA on viral particles. Ratios were normalized to HIV-2 (n=1).

(B) Infectious titer of HIV-2 WT, HIVac-2 and additional HIV-2 mutants (as indicated) on GHOST cells normalized to reverse transcriptase activity in the viral inoculum, in the presence or absence of CsA (2μM) (n=3, paired t-test on log-transformed data, *p<0.05, **p<0.01, ***p<0.001, "ns" stands for not statistically significant, bar indicates mean +/- SEM).

(C) Human and Macaque MDDCs by Giemsa/May–Grünwald staining. The figure is a composite of different fields. Bar = 10 μm.

FIGURE S3

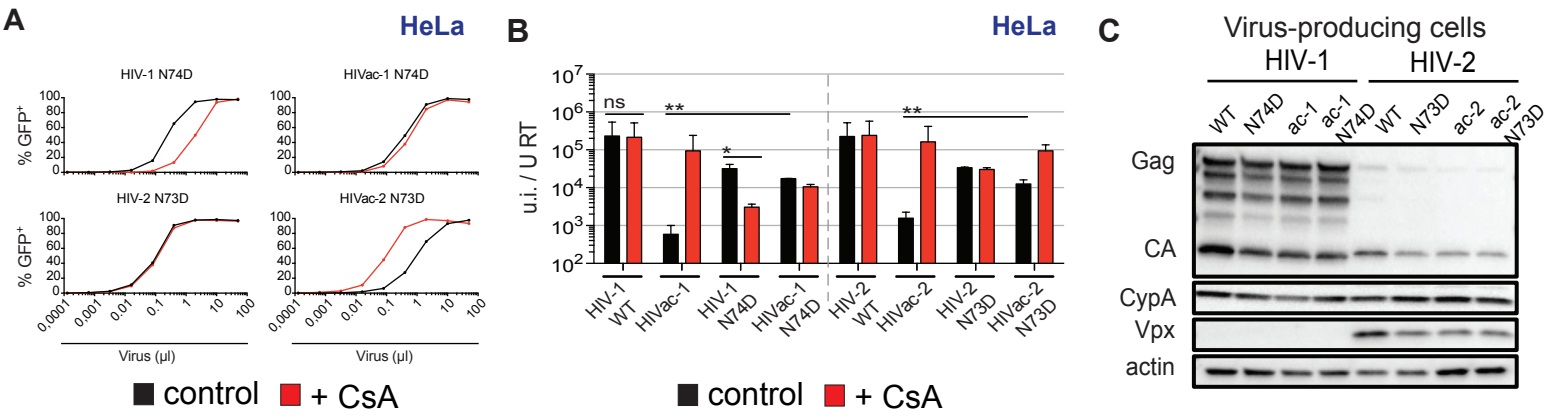
A
HeLa
HIV-1 N74D
HIVac-1 N74D
HIV-2 N73D
HIVac-2 N73D
% GFP+
Virus (µl)
control
+ CsA
B
HeLa
u.i. / U RT
ns
**
*
**
HIV-1 WT
HIVac-1
HIV-1 N74D
HIVac-1 N74D
HIV-2 WT
HIVac-2
HIV-2 N73D
HIVac-2 N73D
control
+ CsA
C
Virus-producing cells
HIV-1
HIV-2
WT
N74D
ac-1
ac-1 N74D
WT
N73D
ac-2
ac-2 N73D
Gag
CA
CypA
Vpx
actin

Figure S3 Second-site mutations in HIVac capsid rescue the CypA restriction, related to Figure 3

(A) Dose-response of GFP expression after infection of HeLa cells with the indicated viruses encoding, in the presence or absence of CsA (2μM) (n=3; representative data for 1 donor is shown). Virus inoculum volume is indicated.

(B) Infectious titer of indicated viruses on Hela cells, normalized to reverse transcriptase activity in the inoculum, in the presence or absence of CsA (2μM) (n=3, paired t-test on log-transformed data, *p<0.05, **p<0.01, "ns" stands for not statistically significant, bar indicates mean +/- SEM).

(C) Western blot analysis of Gag/Capsid, CypA,Vpx and actin in virus-producing cells as in 3E

FIGURE S4

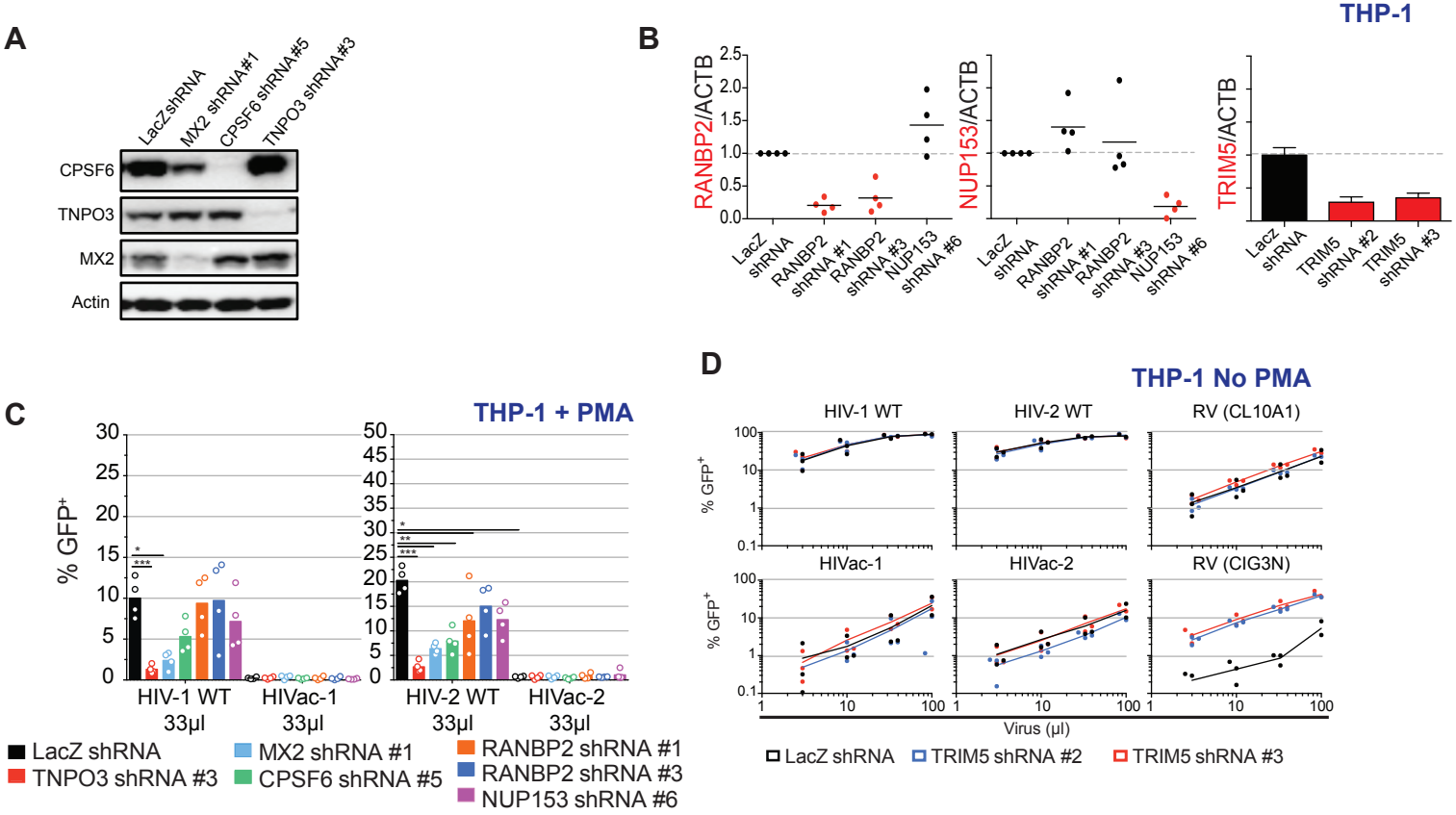
A
LacZ shRNA
MX2 shRNA#1
CPSF6 shRNA#5
TNPO3 shRNA#3
CPSF6
TNPO3
MX2
Actin
B
THP-1
RANBP2/ACTB
NUP153/ACTB
TRIM5/ACTB
LacZ shRNA
RANBP2 shRNA #1
RANBP2 shRNA #3
NUP153 shRNA #6
TRIM5 shRNA #2
TRIM5 shRNA #3
C
THP-1 + PMA
% GFP+
HIV-1 WT 33µl
HIVac-1 33µl
HIV-2 WT 33µl
HIVac-2 33µl
LacZ shRNA
TNPO3 shRNA #3
MX2 shRNA #1
CPSF6 shRNA #5
RANBP2 shRNA #1
RANBP2 shRNA #3
NUP153 shRNA #6
D
THP-1 No PMA
HIV-1 WT
HIV-2 WT
RV (CL10A1)
HIVac-1
HIVac-2
RV (CIG3N)
% GFP+
Virus (µl)
LacZ shRNA
TRIM5 shRNA #2
TRIM5 shRNA #3

Figure S4 Role of known capsid-associated factors in the restriction, related to Figure 4
(A) Western blot analysis of TNPO3, CPSF6, MX2 in THP-1 knock-down cell lines.
(B) Expression of NUP153, RANBP2 and TRIM5 measured by RT-qPCR in THP-1 knock-down cell lines (Line indicates mean, bar indicates mean +/- SEM).
(C) GFP expression as in 4A (n=4, paired t-test, *p<0.05, **p<0.01, ***p<0.001, bar indicates mean). Virus inoculum volume is indicated.
(D) Dose response of GFP expression as in 4B (n=4, line indicates mean). Virus inoculum volume is indicated.

FIGURE S5

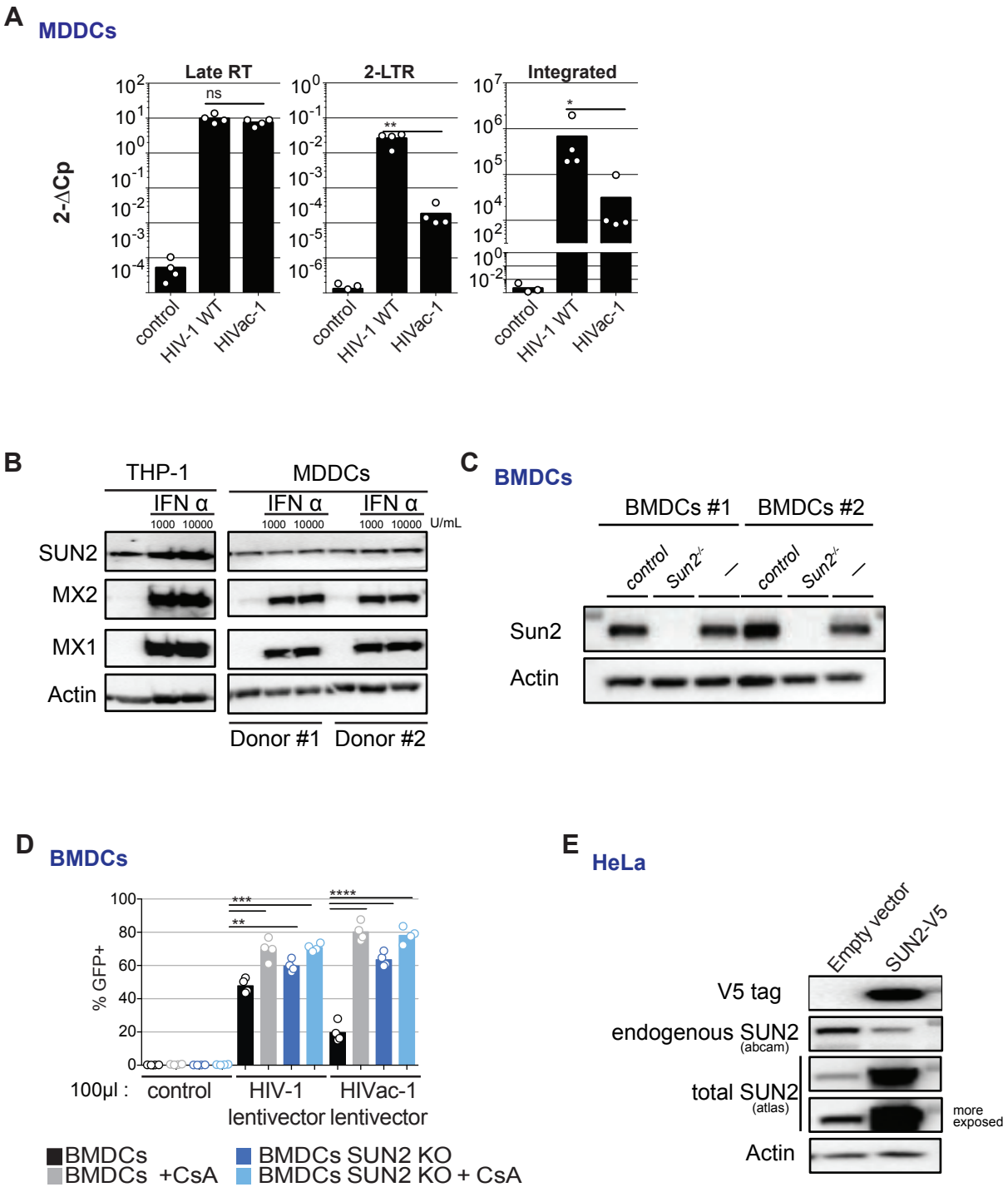
A
MDDCs
Late RT
2-LTR
Integrated
ns
**
*
2-ΔCp
control
HIV-1 WT
HIVac-1
B
THP-1
MDDCs
IFN α
1000 10000
U/mL
SUN2
MX2
MX1
Actin
Donor #1
Donor #2
C
BMDCs
BMDCs #1
BMDCs #2
control
Sun2-/-
Sun2
Actin
D
BMDCs
% GFP+
***
****
100µl :
control
HIV-1 lentivector
HIVac-1 lentivector
BMDCs
BMDCs +CsA
BMDCs SUN2 KO
BMDCs SUN2 KO + CsA
E
HeLa
Empty vector
SUN2-V5
V5 tag
endogenous SUN2 (abcam)
total SUN2 (atlas)
more exposed
Actin

Figure S5 SUN2 is essential for the CypA restriction of HIVac capsid, related to Figure 5

(A) Quantification of late RT, 2LTR circles and integrated viral cDNA products 18 hours after infection of DCs with HIV-1 WT or HIVac-1 in the presence of SIVmac251 VLPs. (n=4; paired t-test on log-transformed data, ***p<0.001, "ns" stands for not statistically significant, bar indicates mean).

(B) Western blot analysis of SUN2 expression in THP-1 cell lines and MDDCs after 24h of IFNα treatment (1000 U/ml and 10000 U/ml).

(C) Western blot analysis of Sun2 protein expression in BMDCs (WT control or *Sun2-/-*) after 7 days of differentiation (dash indicates an unrelated sample).

(D) GFP expression in BMDCs as in 5B and 5C (n=4, paired RM ANOVA one-way test, ****p<0.0001, "ns" stands for not statistically significant, bar indicates mean). Virus inoculum volume is indicated.

(E) Western blot analysis of SUN2 expression in control HeLa cells (Empty vector) or HeLa overexpressing SUN2 (SUN2-V5). Atlas anti-SUN2 detects endogenous and V5-tagged SUN2, while Abcam anti-SUN2 detects only endogenous SUN2.

FIGURE S6

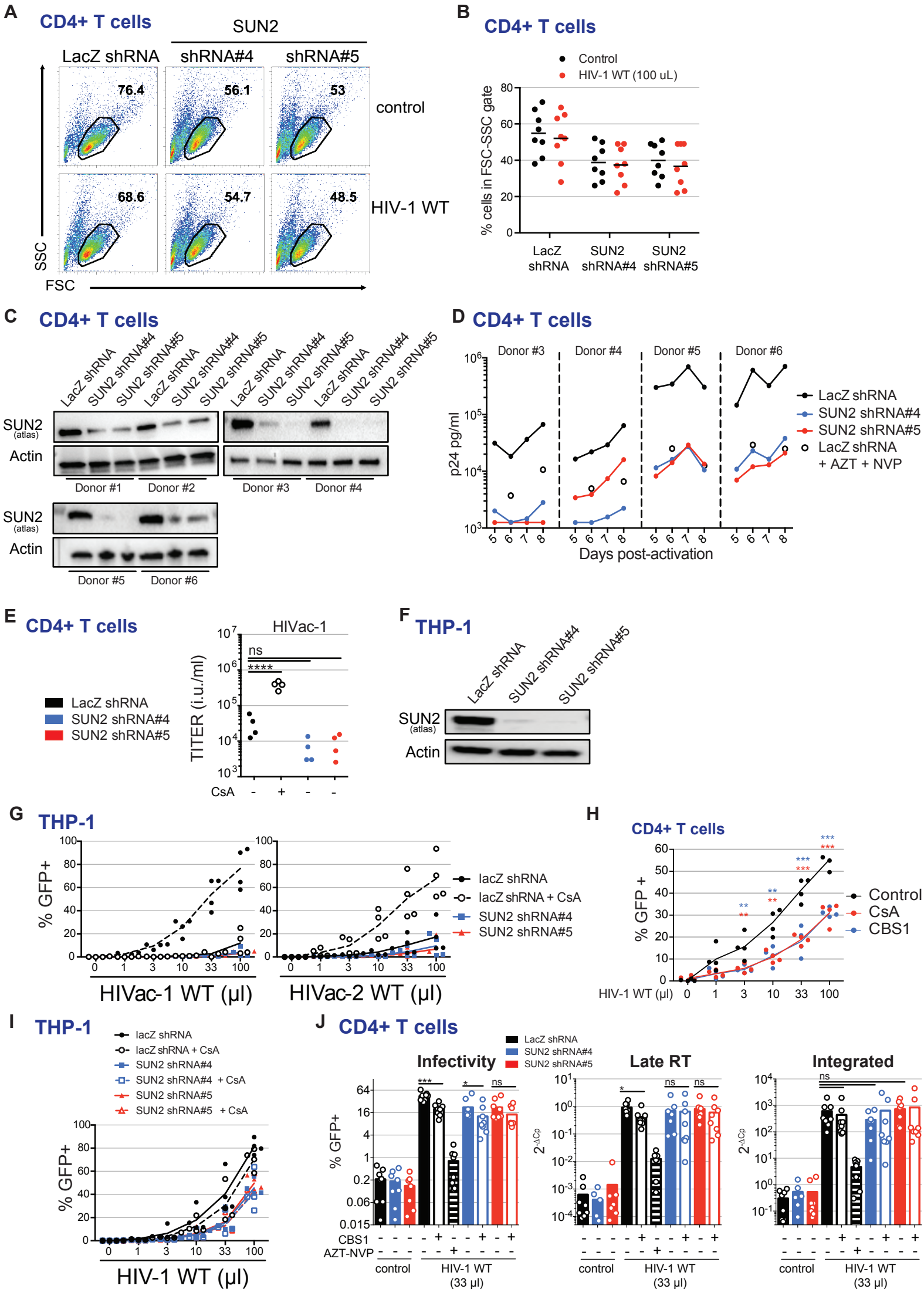
A
CD4+ T cells
SUN2
LacZ shRNA
shRNA#4
shRNA#5
76.4
56.1
53
control
68.6
54.7
48.5
HIV-1 WT
SSC
FSC
B
CD4+ T cells
Control
HIV-1 WT (100 uL)
% cells in FSC-SSC gate
LacZ shRNA
SUN2 shRNA#4
SUN2 shRNA#5
C
CD4+ T cells
SUN2 (atlas)
Actin
Donor #1
Donor #2
Donor #3
Donor #4
Donor #5
Donor #6
D
CD4+ T cells
Donor #3
Donor #4
Donor #5
Donor #6
p24 pg/ml
Days post-activation
LacZ shRNA
SUN2 shRNA#4
SUN2 shRNA#5
LacZ shRNA + AZT + NVP
E
CD4+ T cells
HIVac-1
TITER (i.u./ml)
ns
****
CsA
F
THP-1
G
THP-1
% GFP+
HIVac-1 WT (µl)
HIVac-2 WT (µl)
lacZ shRNA
lacZ shRNA + CsA
SUN2 shRNA#4
SUN2 shRNA#5
H
CD4+ T cells
% GFP +
HIV-1 WT (µl)
Control
CsA
CBS1
I
THP-1
lacZ shRNA
lacZ shRNA + CsA
SUN2 shRNA#4
SUN2 shRNA#4 + CsA
SUN2 shRNA#5
SUN2 shRNA#5 + CsA
% GFP+
HIV-1 WT (µl)
J
CD4+ T cells
LacZ shRNA
SUN2 shRNA#4
SUN2 shRNA#5
Infectivity
Late RT
Integrated
% GFP+
2-ΔCp
CBS1
AZT-NVP
control
HIV-1 WT (33 µl)

Figure S6 SUN2 is an essential host factor of HIV-1 and HIV-2 in primary CD4+ target cells, related to Figure 6

(A) FSC and SCC profiles of control and SUN2-depleted cells as in 6D (n=8).

(B) Proportion of cells in the FSC-SCC live cell gate as in S6A (n=8, line indicates mean).

(C) Western blot analysis of SUN2 and actin expression as in 6E (n=6).

(D) HIV-1 p24 concentration as in 6E, 6F (n=6; 4 additional donors shown).

(E) Titer of HIVac-1 calculated from infection as in 6D, in the presence or absence of CsA (2 μM) (n=4, paired RM ANOVA one-way test, ****p<0.0001, "ns" stands for not statistically significant).

(F) Western blot analysis of SUN2 and actin expression at the time of infection in THP-1 cells that were transduced with shRNA vectors against SUN2 (shRNA#4 or shRNA#5) or a shRNA control vector (LacZ shRNA).

(G) THP-1 as in S6F infected HIVac-1 or HIVac-2 encoding GFP in Nef and pseudotyped with VSV-G. Proportion of GFP-positive cells after 48 hours of infection (n=4, line indicates mean). Virus inoculum volume is indicated.

(H) Dose response of GFP expression in CD4+ T cells as in 6D, 48h after infection with HIV-1 WT, in the presence or absence of CsA (2 μM, red) or CBS1 (60 μM, line) (n=4, paired RM ANOVA one-way test, **p<0.01, ***p<0.001, line indicates mean). Virus inoculum volume is indicated.

(I) THP-1 as in S6F infected HIV-1 WT encoding GFP in Nef and pseudotyped with VSV-G. Proportion of GFP-positive cells after 48 hours of infection, in the presence or absence of CsA (2 μM) (n=4, line indicates mean). Virus inoculum volume is indicated.

(J) Proportion of GFP-positive cells and quantification of late RT and integrated viral cDNA products after 48 hours of infection as in 6D and treated or not with CBS1 (60μM) or 25 μM of AZT with 10 μM of NVP (n=8, paired RM ANOVA one-way test, ***p<0.001, *p<0.05, "ns" stands for not statistically significant). Virus inoculum volume is indicated.

Supplemental Tables

Table S1 Mutagenized sequences used in the study, related to Experimental Procedures

Backbone	**Mutation**	**Original DNA sequence**	**Mutagenized sequence**
HIV-1	V86I-IAP91LPA-M96L (HIVac-1)	CAT CCA **GTG** CAT GCA GGG CCT **ATT GCA CCA** GGC CAG **ATG**	CAT CCA **ATC** CAT GCA GGG CCT **CTT CCA GCA** GGC CAG **CTG**
HIV-2	P86HA (HIVac-2)	CAT CCA ATA **CCA** GGC CCC TTA	CAT CCA ATA **CAT GCA** GGC CCC TTA
HIV-2	P86RA	CAT CCA ATA **CCA** GGC CCC TTA	CAT CCA ATA **AGG GCA** GGC CCC TTA
HIV-2	P86QA	CAT CCA ATA **CCA** GGC CCC TTA	CAT CCA ATA **CAG GCA** GGC CCC TTA
HIV-2	P86AA	CAT CCA ATA **CCA** GGC CCC TTA	CAT CCA ATA **GCT GCA** GGC CCC TTA
HIV-2	P86AM	CAT CCA ATA **CCA** GGC CCC TTA	CAT CCA ATA **GCT ATG** GGC CCC TTA
HIV-2	P86HV	CAT CCA ATA **CCA** GGC CCC TTA	CAT CCA ATA **CAT GTA** GGC CCC TTA
HIV-2	P86PI	CAT CCA ATA **CCA** GGC CCC TTA	CAT CCA ATA **CCA ATT** GGC CCC TTA
SIVmac239	SIVmac239 QPAPQQ85IHAGPLPA	CAG CAC CCA **CAA CCA GCT CCA CAA CAA** GGA CAA CTT	CAG CAC CCA **ATA CAT GCT GGC CCA TTA CCA GCG** GGA CAA CTT
HIV-1	N74D	GAG ACC ATC **AAT** GAG GAA GCT	GAG ACC ATC **GAT** GAG GAA GCT
HIV-2	N73D	GAG ATT ATC **AAT** GAG GAA GCA	GAG ATT ATC **GAT** GAG GAA GCA
psPAX2	psPAX2 variant	CAT CCA **GTG** CAT GCA GGG CCT **ATT GCA CCA** GGC CAG **ATG**	CAT CCA **ATC** CAT GCA GGG CCT **CTT CCA GCA** GGC CAG **CTG**

Table S2 shRNA used in the study, related to Experimental Procedures

Target	*shRNA identifier*	*Source or TRC identifier*	*Target sequence*
CypA (*PPIA*)	#1	custom	CTGGCATCTTGTCCATGGCAAA
CypA (*PPIA*)	#2	custom	GGTTCCTGCTTTCACAGAATTA
CypA (*PPIA*)	#3	custom	TGTGGTGTTTGGCAAAGTGAAA
TNPO3	#3	TRCN0000038331	CGGCGCACAGAAATTATAGAA
CPSF6	#5	TRCN00000000154	ACCATAGTAGATCACGAGAAA
NUP153	#6	(Matreyek and Engelman, 2011)	AGTGTTCAGTATGCTGTGTTTCT
RANBP2	#1	custom	AACTCAGTGCCTGATGGATATC
RANBP2	#3	custom	ACTTGTCAGTGTCCAAGTAAAC
MX2	#1	TRCN0000056713	GCCAACCAGATCCCATTTATA
TRIM5	#2	custom	CCCTGTGTGCCGGATCAGTTAC
TRIM5	#3	custom	TGGAGAGAAACTTCTACTCTTC
LacZ	/	custom	GCGATCGTAATCACCCGAGTG
SUN2	#4	TRCN0000143335	GCCTATTCAGACGTTTCACTT
SUN2	#5	TRCN0000143336	GAGGAAATCCAGCAACATGAA

Table S3 Antibodies used in the study, related to Experimental Procedures

Antigen	*Source*	*Clone or name*
CD86	eBioscience	IT2.2
CD14	eBioscience	61D3
CD11c (mouse)	BD Biosciences	HL3
CD4	BD Biosciences	RPA-T4
Gag/capsid	The following reagent was obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH: HIV-1 p24 Hybridoma (183-H12-5C) from Dr. Bruce Chesebro.	183-H12-5C
Vpx	The following reagent was obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH: HIV-2 Vpx Hybridoma (6D2.6) from Dr. John C. Kappes.	6D2.6
Actin	Millipore	C4
CypA (Rat)	R&D Systems	817815
CypA (Goat)	R&D Systems	AF3589
MX2	SIGMA	HPA030235
MX1	ABCAM	ab95926
CPSF6	Novus Biologicals	NBP1-85676
TNPO3	ABCAM	3152C2a
HA (tag)	Cell Signalling	C29F4
Flag (tag)	SIGMA	F3165
SUN2	Atlas	HPA001209
SUN2	ABCAM	EPR6557
V5 tag	eBioscience	TCM5

Table S4 Real-time quantitative PCR primers used in the study for RNA quantification, related to Experimental Procedures

Primer name	*Sequence*
Nup153-B-f	TTGAAGTATCTTTGTAGCCACCC
Nup153-B-r	GGGGCCAATTAAGCCTTACC
RANBP2-B-f	AGCAAGATCATATTCTTTAGCTTCA
RANBP2-B-r	CTGACGTGGAGCGGTACAT
TRIM5α-182-f	CTGTATTTACCTTGGGATCTGTG
TRIM5α-402-r	TCTAGCATGGACTTCTTGTGG
bactin737f	GGACTTCGAGCAAGAGATGG
bactin970r	AGCACTGTGTTGGCGTACAG

Table S5 Real-time quantitative PCR primers used in the study for DNA quantification, related to Experimental Procedures

Strain	*Apmlification*	*Primer Name*	*Sequence*	*Optimal annealing °C*
Human	beta-globin	bglobin-f	CCCTTGGACCCAGAGGTTCT	50
Human	beta-globin	bglobin-r	CGAGCACTTTCTTGCCATGA	50
HIV-2	Late RT	hiv2-3'U3-fwd	GAAGGGATGTTTTACCATTTAGTTA	50
	Late RT	hiv2-psi-rev	GTTCCAAGACTTCTCAGTCTTCTTC	50
HIV-2	2LTR	hiv2-3'U3-rev	TAACTAAATGGTAAAACATCCCTTC	50
	2LTR	hiv2-R-fwd	GTTCTCTCCAGCACTAGCAGGTA	50
HIV-2 First round	Integrated DNA	alu1	GCCTCCCAAAGTGCTGGGATTACAG	50
	Integrated DNA	hiv2-r	AAGGGTCCTAACAGACCAGGGTCT	50
HIV-2 Second round	Integrated DNA	hiv2-f2	GCAGGTAGAGCCTGGGTGTTC	50
	Integrated DNA	hiv2-r2	CAGGCGGCGACTAGGAGAGAT	50
HIV-1	Late RT	hiv1-3'U3-fwd	GCATGGAATGGATGACCCTGAGA	65
	Late RT	hiv1-psi-rev2	CGTCGAGAGATCTCCTCTGGCTTTA	65
HIV-1	2LTR JNCT	Junct4 -fwd	CAGTGTGGAAAATCTCTAGCAGTACTG	65
	2LTR JNCT	Junct2-rev	GCCGTGCGCGCTTCAGCAAGC	65
HIV-1 First round	Integrated DNA	alu1	GCCTCCCAAAGTGCTGGGATTACAG	65
	Integrated DNA	hiv1-psi-rev2	CGTCGAGAGATCTCCTCTGGCTTTA	65
HIV-1 Second round	Integrated DNA	hiv1-f2	CTGGGAGCTCTCTGGCTAACTA	65
	Integrated DNA	hiv1-r2	AACAGACGGGCACACACTACTT	65

Supplemental Experimental Procedures

Constructs
For PCR mutagenesis, corresponding sequences are listed in **Table S1**. CMV-VSVG, psPAX2, pLKO1puro and pSIV3+ were described elsewhere (Manel et al., 2010). Replication-competent X4-GFP is NL4-3 with GFP in Nef (Motsinger et al., 2002; Unutmaz et al., 1999). pTRIP-CMV-puro-2A and pTRIP-SFFV (coding for GFP) were generated from pTRIP (Zennou et al., 2000) and GAE-SFFV-GFP-WPRE obtained from Jean-Luc Battini (Mamede et al., 2013). pCIG3 N (a generous gift from Towers Greg (Bock et al., 2000)), pCL10A1 (Naviaux et al., 1996) and pRV.GFP were previously described. shRNA against LacZ, MX2, TNPO3, CPSF6, CypA(PPIA), RANBP2, NUP153, TRIM5 and SUN2 were in the pLKO1puro vector and are listed in **Table S2**.

Cells
Human peripheral blood mononuclear cells were isolated from buffy coats from normal human donors (approved by the Institut National de la Santé et de la Recherche Médicale ethics committee) using Ficoll-Paque PLUS (GE). Macaque peripheral blood mononuclear cells were isolated from macaques using 5% PBS 95% Ficoll-Paque PLUS. CD14+ cells were isolated by a positive selection with anti-human CD14 magnetic beads (Miltenyi). Purity was checked by flow cytometry using an anti-CD14 antibody (antibodies are listed in Table S3) and purity was superior to 99%. CD14+ cells were cultured in RPMI medium, 10% FBS (Biowest), Penicillin-Streptomicin, Gentamicin (50 µg/ml, Gibco) and HEPES (Gibco) in the presence of recombinant human GM-CSF (Miltenyi) at 10ng/ml and IL-4 (Miltenyi) at 50ng/ml. Fresh media was added at day 3, and cells were stimulated or infected at day 4. CD4+ T cells were isolated by a positive selection with anti-human CD4 magnetic beads (Miltenyi) from the CD14-negative fraction of PBMCs. Purity was checked by flow cytometry using an anti-CD4 antibody (antibodies are listed in Table S3) and purity was superior to 95-96%. CD4+ cells were cultured at 1 million per ml (200µl/well in round-bottomed 96-well plates) in RPMI medium, 10% FBS (Biowest), Penicillin-Streptomicin, Gentamicin (50µg/ml, Gibco) and 1000 U/ml human IL-2 and activated with the T Cell Activation/Expansion Kit (Miltenyi).
Bone marrow was taken from *Sun2*$^{-/-}$, *Sun1*$^{-/-}$ or WT littermate control mice, to differentiate mouse dendritic cells (BMDCs). Mouse bone-marrow progenitors were cultured for 10 days in DC medium (IMDM, FCS (10%), Glutamine (20mM), pen-strep (100U/mL) and 2-mercaptoethanol (50µM)), supplemented with granulocyte-macrophage colony stimulating factor (50ng/mL)-containing supernatant obtained from transfected J558 cells. After 4 days of differentiation, all cells were passed to a density of 10 millions per 165 cm^2.

Mice
All animal care and experimental procedures were conducted in accord with requirements approved by the Institutional Animal Care and Use Committee of Yale University. *Sun1*$^{-/-}$ (strain B6;129S6-*Sun1*^{tm1Mha}*n/J*), *Sun2*$^{-/-}$ (strain B6;129S6-*Sun2*^{tm1Mhan}/J) and C57BL/6 WT mice were obtained from Jackson Labs. *Sun1*$^{-/-}$ mice were previously generated through the replacement of exons 10-13 with a neomycin resistance cassette (Ding et al., 2007). *Sun2*$^{-/-}$ mice were previously generated through the replacement of exons 11-16 and part of exon 17 with a neomycin resistance cassette (Lei et al., 2009).

Virus production
For transfection of 3µg plasmid DNA per well, plasmid quantities were as follows: for VSV-G pseudotyped SIVmac VLPs, 0.4µg CMV-VSVG and 2.6µg pSIV3$^+$; for HIV-1, HIV-2 and SIVmac239 VSV-G pseudotyped viruses, 0.4µg CMV-VSVG and 2.6µg HIV or SIV DNA; for replication-competent HIV-1, 3µg X4-GFP; for MLV VSV-G pseudotyped viruses, 0.4µg CMV-VSVG, 1.6µg MLV DNA (pRV.GFP) and 1µg pCL10A1 (TRIM5α resistant) or pCIG3 N (TRIM5α sensitive); for shRNA vectors in MDDCs and THP-1 cells, 0.4µg CMV-VSV-G, 1µg psPAX2 and 1.6µg pLKO1puro-derived shRNA; for shRNA vectors in primary CD4+ T cells, 0.2µg HXB2 envelope expression plasmid (Boggiano et al., 2007), 0.2µg CMV-VSV-G, 1µg psPAX2 and 1.6 µg pLKO1puro-derived shRNA; for expression vectors 0.4 µg CMV-VSV-G, 1µg psPAX2 and 1.6µg pTRIP-CMV-tagRFP-2A, pLX304 (Empty vector) or pLX304-SUN2 (overexpression SUN2-V5, clone HsCD00436663 from DNASU; Genbank: AM392760). Empty pLX304 was generated by removing SUN2 from pLX304-SUN2 with BsrGI.

Infections.
For MDDCs infection, 50 µl of media or SIVmac VLPs were first added and 100 µl of media or dilutions of viral supernatants to 100µl of cells. Azidothymidine (AZT, SIGMA), Raltegravir (RAL, Euromedex) or Cyclosporin A (CypA, Selleckchem) were added respectively at 24µM, 20µM and 2µM. CBS1 is a small molecular inhibitor of CypA that binds the hydrophobic and the Abu pockets of CypA (Guichou et al., 2011) (Hakim Ahmed-Belkacem, Jean-François Guichou, Jean-Michel Pawlotsky, unpublished data) and was added at 2µM. For CD4+ T cells infection, one hundred microliter of media or dilutions of viral supernatants were added to 100µl of cells in presence or not of 2 µM of CsA, 60 µM of CBS1 or 25 µM of AZT plus 10 µM of

Nevirapine (NVP) (Sigma). For BMDCs infection, 100 µl of media or dilutions of viral supernatants were added in presence or not of 2 µM of CsA. 72h after infection, cells were stained with anti-mouse CD11c and analyzed.

Knock-down and overexpression

Table S2 lists the shRNA sequences used. THP-1 cells were transduced with a control empty vector pLKO1puro (shRNA vector targeting LacZ) or a single shRNA vectors in pLKO1puro. Puromycin was added at day 2 (2ng/ml). Knock-down efficiency was measured by western blotting or qPCR at day 10 (for THP-1 cells). Cells were seeded at 1 million per ml in 96-well flat bottom plate in 200 µl of final volume treated or not with PMA (30ng/ml) for 16 hours. Media was replaced with 100µl of fresh media and 100µl of media or dilutions of viral supernatants were added. Monocytes were transduced with shRNA vectors as previously described (Manel et al., 2010; Satoh and Manel, 2013). Knock-down efficiency was measured by western blotting or qPCR at day 4 or 6. DCs were harvested, counted, seeded at 0.5 million per ml in 96-well U bottom plate and infected or stimulated in 200 µl of final volume. For knock-down experiments in CD4+ T cells, after 24h of activation in 96-well plates, media was changed by 100µl of fresh media with 8µg/ml of protamine and cells were transduced with 100µl of shRNA lentivector supernatants. Cells were spinoculated at 1200g, 2h at 25°C. At day 3, media was changed in presence of puromycin (2ng/ml). For single-round infection experiments, cells were split at day 4 and day 6. At day 7, CD4+ T cells were harvested, counted, seeded and infected. For experiment with replication-competent virus X4-GFP, cells were harvested, counted, seeded and infected at day 4. 10h later, viral inoculum was washed. Accumulation of HIV-1 p24 was measured at days 5 to 8 by ELISA (Clontech Takara Lenti-X p24 Rapid Titer Kit). For overexpression in DCs, monocytes were transduced with vectors pTRIP-CMV-tagRFP-2A or pTRIP-CMV-tagRFP-2A-FLAG-ntCypA as previously described (Manel et al., 2010; Satoh and Manel, 2013). Overexpression efficiency was measured by western blotting or RFP expression at day 6. For overexpression in HeLa, cells were transduced with pLX304 or pLX304-SUN2 and selected with 15µg/mL of blasticidine. Overexpression efficiency was measured by western blotting at day 10.

HIV cDNA Real-time PCR

Total cellular DNA was harvested using a Nucleospin Tissue kit (Machery-Nagel). Real-time PCR analysis was adapted from (Brussel and Sonigo, 2003) and performed in a Roche LightCycler 480 using Roche 480 SYBR Green I master reagent in 20 µl final volume per well according to manufacturer specifications. Each sample was measured in triplicate for all primers (primer sequences are listed in **Table S5**). For beta-globin, primers were bglobin-f and bglobin-r. For HIV-2 late RT, primers were hiv2-3'U3-fwd and hiv2-psi-rev. For HIV-2 2LTR, primers were hiv2-3'U3-rev and hiv2-R-fwd. For HIV-2 integrated DNA two rounds of amplification were performed. For the first round, primers were alu1 and hiv2-r. For the second round, 1 µl of first-round reaction was used as template, and primers were hiv2-f2 and hiv2-r2. For HIV-1 late RT, primers were hiv1-3'U3-fwd and hiv1-psi-rev2. For HIV-1 2LTR JNCT, primers were Junct4 -fwd and Junct2-rev. For HIV-1 integrated DNA two rounds of amplification were performed. For the first round, primers were alu1 and hiv1-psi-rev2. For the second round, 1 µl of first-round reaction was used as template, and primers were hiv1-f2 and hiv1-r2. Cycling conditions were 1x 95°C 5'; 35x 95°C, 10"-50°C or 65°C, 20"-72°C 30". Relative concentrations of late RT, 2LTR and integrated viral DNA were calculated relative to beta-globin using the ΔCt method.

Gene expression quantification

Ten days after lentiviral vector transduction, total RNA was extracted from 1 million THP-1 cells using Nucleospin RNA II kit (Macherey-Nagel). cDNA was synthesized with random hexamer from 0.1µg total RNA using SuperScript III Reverse Transcriptase (Invitrogen). Real-time qPCR was performed using SYBR Green I Master (Roche). The relative quantities of target mRNAs were calculated between target RNA Cp and ACTB Cp ($2^{-\Delta Cp}$ method). The primer sequences are listed in **Table S4**.

Western Blotting

0.2 to 2 millions cells were lysed in 80 µL of Lysis buffer (50mM Tris HCl pH 8, 120mM NaCl, 4mM EDTA, 1% NP40 and 1x EDTA-free protease inhibitors cocktail (Roche)). Lysates were cleared by centrifugation at 6000g for 7 minutes at 4°C. Virus supernatants were filtered at 0.45µm, centrifugated at 16000g for 1 h 30 min at 4°C. Virus pellets were lysed in 15µl of Lysis buffer. Cellular and viral protein lysates were resolved on 4%-20% Biorad precast SDS-PAGE gels and transferred on nitrocellulose membrane. Proteins were blotted with antibodies as follow: mouse anti-Gag/capsid; mouse anti-Vpx; mouse anti-actin; rabbit anti-CypA (antibodies are listed in **Table S3**). ECL signal was recorded on the ChemiDoc XRS Biorad Imager. Data was analyzed and quantified with the Image Lab software (Biorad).

Morphological Analysis

Cells were subjected to cytospin and colored with May-Grunwald/Giemsa staining. Pictures were taken with a Nikon DS-FI1 High-definition color camera head on a Nikon Eclipse TS100 inverted microscope.

Immunofluorescence

HeLa cells monolayers grown on glass poly-Lysine coverslip were fixed with 4% paraformaldehyde in PBS at room temperature for 10 min and incubated in Glycine-PBS buffer (375 mg Glycine in 50ml of PBS) for 15min. Cells were permeabilized in 0.2% BSA, 0.1% Saponin in PBS for 30min and stained with SUN2 (Atlas Ig – 1/1000) and V5 (eBioscience Ig – 1/1000) in 0.2% BSA, 0.1% Saponin in PBS at room temperature for 5h. Followed this incubation, washes and secondary antibody staining were performed in the same buffers, with the corresponding immunoglobulin G (IgG) antibody conjugated to Alexa 488 (SUN2) or Alexa 647 (V5 tag) at the dilution of 1/500 at room temperature for 1h. The cells were mounted onto glass slides by using Dapi Fluoromont G (eBioscience) to stain nuclei and imaged on a Zeiss *LSM 780* Confocal Microscope. ImageJ (National Institutes of Health, Bethesda MD) software was used for image processing and for the Z-projection (base on the maximum of intensity for each z stack).

Ethic approval for macaque blood

Adult cynomolgus macaques (*Macaca fascicularis*) were used at CEA in accordance with French national regulation and under national veterinary inspectors (CEA permit no. A 92-032-02). CEA is in compliance with Standards for Human Care and Use of Laboratory of the Office for Laboratory Animal Welfare (OLAW; OLAW assurance no. A5826-01). The use of nonhuman primates at CEA is in accordance with recommendation with the European Directive (2010/63; recommendation no. 9). No suffering was specifically associated with the treatment and sample procedure to obtain nasal washes. The animals were used under the supervision of the veterinarians in charge of the animal facility. No suffering was specifically associated with the sampling procedure to obtain peripheral blood. Peripheral blood was obtained from healthy animals by venipuncture at the femoral vein after sedation with ketamine chrorhydrate (Rhone-Merieux, Lyon, France, 10 mg/kg) as previously described (Dioszeghy et al., 2006) and collected on heparinized CPT tubes (BD, Franklin Lakes, NJ).

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