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Supplemental Information

Regulation of HIV-1 Gag-Pol Expression

by Shiftless, an Inhibitor of

Programmed -1 Ribosomal Frameshifting

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Table S1. ISGs screened for activity to inhibit HIV-1 -1PRF. Related to Figure 1.

Gene Name	Relative -1PRF efficiency	Gene Name	Relative -1PR efficiency	Gene Name	Relative -1PRF efficiency	Gene Name	Relative -1PRF efficiency
Empty Vector	100.0	XRN2	88.3	CREM	99.9	APOL6	108.8
C19orf66	53.5	TEAD4	88.4	B3GNT2	100.5	IFI44	109.1
FAM122C	70.3	FTSJD2	89.0	NAPSA	100.8	GBP4	109.6
ASPHD2	70.4	PLAC8	89.2	RUNX3	100.9	LGALS3BP	109.8
IER3	73.2	CASP4	90.6	LMO2	101.5	FNDCC5	110.5
AGPAT4	74.3	C14orf149	90.9	RNF213	101.8	RTP4	111.6
MC3R	74.7	ARHGAP25	91.2	CKB	102.2	NPC2	111.7
CASP10	76.0	RNASET2	91.3	IFI27	102.5	TMEM140	111.8
LHFPL1	77.2	MSMB	91.7	CCDC146	102.5	SP140	111.8
MYO5C	77.8	GSDMD	92.5	EPSTI1	102.8	GSN	112.7
FRMD3	79.0	SLAMF8	94.3	ST3GAL	103.0	TRIM31	113.8
GALM	80.0	BOC	94.9	NPHP1	103.0	GLYATL1	115.4
ETV4	81.2	TNFRSF1A	95.0	MFN1	103.0	IFI44L	116.0
BRWD1	81.4	RNF149	95.0	MR1	103.0	SELL	116.9
TMEM229B	81.7	PDE6H	96.0	REC8	104.4	CD69	118.5
C1orf38	81.8	XRN1	96.2	TMEM45B	104.6	IFIT5	120.2
ENDOD1	81.9	SLC38A5	96.2	IDH1	104.6	INPP1	120.8
CD164	82.4	C20orf103	96.4	PDS5A	104.7	IFI6	123.2
PHLDA2	82.9	MOXD1	97.1	MGC87042	105.4	DDAH2	123.5
TOR1B	83.9	MS4A12	97.4	DCLRE1C	105.5	MX2	126.4
TMEM106A	84.2	BCL2A1	97.8	FAM109B	105.7	DDX60L	127.3
GPR84	85.7	NEXN	97.9	EGR1	106.7	CXorf21	129.0
PARP3	87.4	AZU1	98.0	USP18	107.2	XAF1	132.1
TPPP2	88.1	SNX9	98.3	ENTPD1	107.2	FCGR2C	134.4
FGD2	88.3	RASGRP3	99.1	TIMD4	107.6	PIM1	140.4

The dual-luciferase reporter pDual-HIV(-1) was transfected into 293T cells together with an empty vector or a plasmid expressing an ISG. At 36 h posttransfection, luciferase activities were measured. The Fluc/Rluc ratio reflects the -1PRF efficiency. The relative -1PRF efficiency in the cells transfected with the empty vector was set as 100.

Table S2. Raw data of Figure 2A. Related to Figure 2.

EXP1	EV			Flag-SFL		
	Fluc	Rluc	Fluc/Rluc*100	Fluc	Rluc	Fluc/Rluc*100
HIV-1(0)	1600	4344	36.8	852.7	2674	31.9
HIV-1(-1)	105.2	5080	2.07	38.94	3915	0.99
RSV	28.08	4336	0.65	8.743	4034	0.22
HTLV	171	8814	1.94	47.11	6251	0.75
MMTV	450.2	8872	5.07	95.90	4655	2.06
HIV-2	99.71	6893	1.45	23.54	6184	0.38
SIV	108.1	6333	1.71	48.65	8007	0.61
SINV	59.82	1641	3.65	18.73	1941	0.96
PEG10	486.4	6454	7.54	106.7	3625	2.94
CCR5	4.934	134.0	3.68	4.713	304.6	1.55

EXP2	EV			Flag-SFL		
	Fluc	Rluc	Fluc/Rluc*100	Fluc	Rluc	Fluc/Rluc*100
HIV-1(0)	1191	3089	38.56	633.6	1841	34.4
HIV-1(-1)	100.8	4361	2.31	35.67	3671	0.97
RSV	13.34	1424	0.94	4.704	1477	0.32
HTLV	157.6	7896	2.00	42.18	6438	0.66
MMTV	487.8	9256	5.27	158.4	7528	2.10
HIV-2	65.88	4436	1.49	24.67	6648	0.37
SIV	101.9	5659	1.80	56.70	9564	0.59
SINV	46.94	1112	4.22	15.72	1947	0.81
PEG10	575.8	8214	7.01	140.0	5759	2.43
CCR5	4.242	106.0	4.00	3.666	187.0	1.96

EXP3	EV			Flag-SFL		
	Fluc	Rluc	Fluc/Rluc*100	Fluc	Rluc	Fluc/Rluc*100
HIV-1(0)	343.5	910.2	37.74	151.4	463.0	32.70
HIV-1(-1)	18.22	929.0	1.96	5.656	651.4	0.87
RSV	6.818	766.0	0.89	1.663	731.8	0.23
HTLV	32.41	1773	1.83	7.368	1041	0.71
MMTV	134.1	2664	5.03	33.45	1588	2.11
HIV-2	15.24	1107	1.38	3.702	1057	0.35
SIV	18.61	1181	1.58	7.65	1360	0.56
SINV	12.97	368.4	3.52	3.622	405.6	0.89
PEG10	98.22	1408	6.98	16.95	557.1	3.04
CCR5	1.514	38.31	3.95	1.324	85.61	1.55

Luciferase activities were measured with the Dual-Luciferase Reporter Assay System. Fluc: Firefly luciferase; Rluc: Renilla luciferase.

Table S3. Sequences of DNA primers. Related to STAR methods

oligos for:	oligo name	target	Sequence (5'-3')
qPCR	qFirefly luciferase-F	Firefly luciferase	CCAGGGATTTCAGTCGATGT
	qFirefly luciferase-R		AATCTCACGCAGGCAGTTCT
	qGag-Pol-F	HIV-1	AAAGGCACAGCAAGCAGCAG
	qGag-Pol-R	Gag-Pol	ACCATTTGCCCTGGAGGTT
	qGAPDH-F	GAPDH	TCGGAGTCAACGGATTTG
	qGAPDH-R		GCATCGCCCCACTTGATT
	qPelo-F	Pelo	GACCGACAACAACTGCTCCTG
	qPelo-R		AGCCACAGTAGGGTCACAAAGG
	qHBS1L-F	HBS1L	CGAGGATCCACTGTGTAAGAAG
qHBS1L-R	AGGGTAGGAAATCAGAGCATAAC		
pDual-HIV2(-1) construction	HIV-2-F	HIV-2	CGGGGGTACCTGGTAAGCCAGGACACATC
	HIV-2-R	-1PRF	CGCGGATCCTGCTGTTGGTGTGACCCCC
pDual-SIV(-1) construction	SIV-F	SIVmac	CGGGGGTACCTGGAAAATGGACCATGTT
	SIV-R	-1PRF	CGCGGATCCAGCAGTTGGCATCAGCCCC
pDual-SINV(-1) construction	SVNI-F	SVNI	CGGGGGTACCGCGCTGCTGCTCCTGCT
	SVNI-R	-1PRF	CGCGGATCCATACGGTATCTGTGGCAC
pDual-PEG10(-1) construction	PEG10-F	PEG10	GTTGGTACCAGGTCCTACGCTGACA
	PEG10-R	-1PRF	TTGGATCCAGGCATCATCTTGTGGGGA
pDual-CCR5(-1) construction	CCR5-F	CCR5	GGGGTACCCCTCCTGACAATCGATA
	CCR5-R	-1PRF	ATGGATCCTGGAAAATGAGAGCTGCA
pCMV-PMT construction	PMT-F	Truncated HIV-1 Gag	AGGATCCGCCACCATGGGTGCGAGAGCGT CG
	PMT-R		AGAATTCTCAAAAATTAGCCTGTCTCTCAG
pMD19-T7-PRF construction	PRF-F	HIV-1 -1PRF	<u>TAATACGACTCACTATAGGGGACACCAAAT</u> GAAAGATT
	PRF-R		<u>GAATTC</u> CACGCGTGAAGAAAATTC
pSuper-retro-MMT V(-1) construction	MMTV-F	MMTV	GGAAGATCTGGTACCTCCTCCCTGG
	MMTV-R	-1PRF	CCCAAGCTTAAAATAGGATCTCTG