HIV-1 transcription is regulated by splicing factor SRSF1

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SUPPLEMENTAL MATERIALS AND METHODS

CD4+ T cells isolation and activation.

CD4+ cells were isolated by negative selection from leukapheresis-enriched peripheral blood mononuclear cells (PBMC) obtained from unidentified healthy donors (OneBlood, Palm Beach, FL) using the EasySep Human CD4+ T Cell Enrichment Kit (StemCell Technologies) as recommended by the manufacturer. Cells were activated for 48 hours in RPMI, 10% FBS, IL2 20U/mL (Peprotech, Cat# AF-200-02), anti-CD28 antibody (BD bioscience Cat# 340975) at a final concentration of 1ug/mL on 24 well plates coated with anti-CD3 antibody (BioXCell, Cat# BE0001-2). 48 hours after activation total RNA was isolated utilizing the RNAqueous-Micro Total RNA Isolation Kit (LifeTechnologies).

SUPPLEMENTAL REFERENCES

1. Cartegni, L., Wang, J., Zhu, Z., Zhang, M.Q. and Krainer, A.R. (2003) ESEfinder: A web resource to identify exonic splicing enhancers. *Nucleic acids research*, **31**, 3568-3571.
2. Ledderose, C., Heyn, J., Limbeck, E. and Kreth, S. (2011) Selection of reliable reference genes for quantitative real-time PCR in human T cells and neutrophils. *BMC research notes*, **4**, 427.

SUPPLEMENTAL FIGURE LEGENDS.

Figure S1. Cell viability assay. Cell viability was measured by quantifying cellular ATP production. Viability of the cells transfected with the SRSF1 expression clones is relative to the pEGFP transfected control and was assayed 6 days after transfection to account for longer term effects of SRSF1 overexpression.

Figure S2. Sequences predicted to function as splicing enhancers when bound by SRSF1 and other SR proteins. The TAR (A) and the 5′ hairpin of the 7SK RNA (B) sequences

were analyzed utilizing the ESEfinder 3.0 (1). Consensus binding sequences and matrix score for a number of SR proteins are indicated.

Figure S3. SRSF1 mRNA expression in CD4+ T cells following activation and HEK293 cells after transfection of the SRSF1 coding plasmid. (A) CD4+ T cells were isolated from 6 healthy donors. Following activation with anti-CD3/CD28 antibodies and IL2 SRSF1 for 48 hours gene expression was assayed by qPCR utilizing the housekeeping genes RPL13A and IPO8, which expression has been shown to remain constant after activation (2), as normalizing controls. Unstimulated resting CD4+ T cells were utilized as relative expression controls and assigned the arbitrary value 1. (B) HEK293 cells were transfected with the T7-tagged SRSF1 and a control EGFP-coding plasmids. RNA was extracted and analyzed by qPCR 48 hours after transfection. (C) SRSF1 expression was compared in HEK293 cells and activated CD4+ T cells utilizing the housekeeping genes GAPDH, RPL13A and IPO8 as normalizing controls. The value of 1 was arbitrarily assigned to the SRSF1 expression level in HEK293 cells.

Table S1. Gene name, original clone name and source (academic and commercial) for the RBP expression clones.

Fig. S1

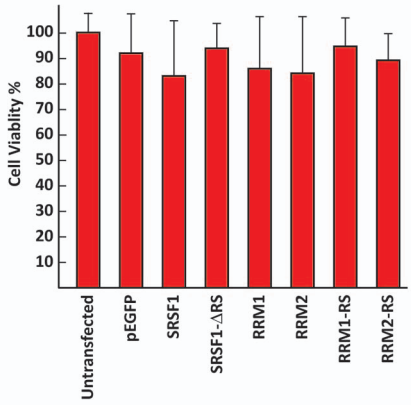
Cell Viability %
100
90
80
70
60
50
40
30
20
10
Untransfected
pEGFP
SRSF1
SRSF1-ΔRS
RRM1
RRM2
RRM1-RS
RRM2-RS

Fig. S2

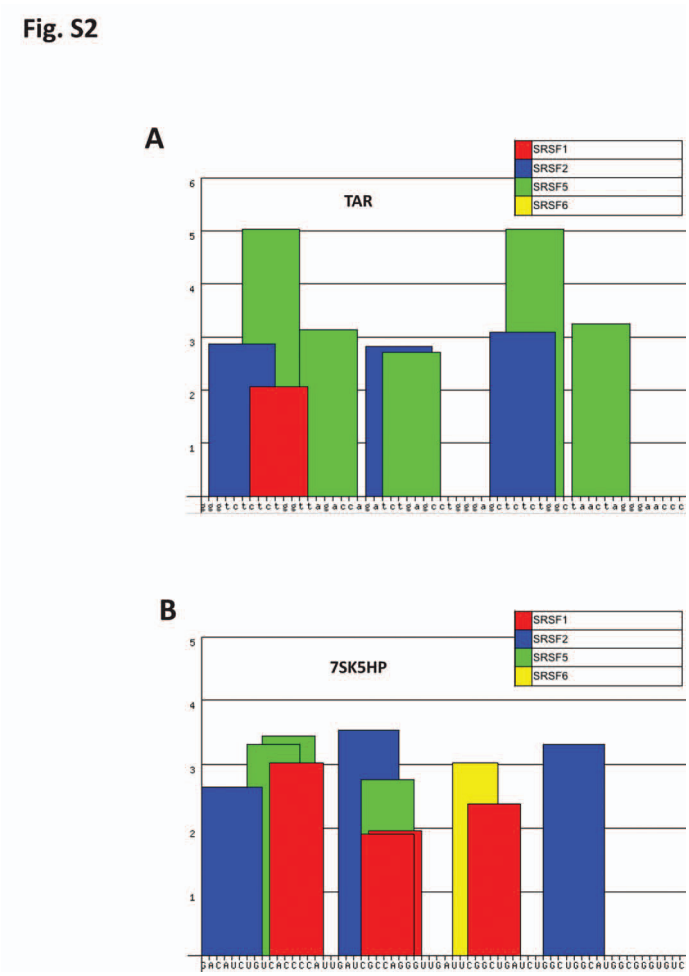
A
SRSF1
SRSF2
SRSF5
SRSF6
TAR
6
5
4
3
2
1
B
SRSF1
SRSF2
SRSF5
SRSF6
7SK5HP
5
4
3
2
1

Fig. S3

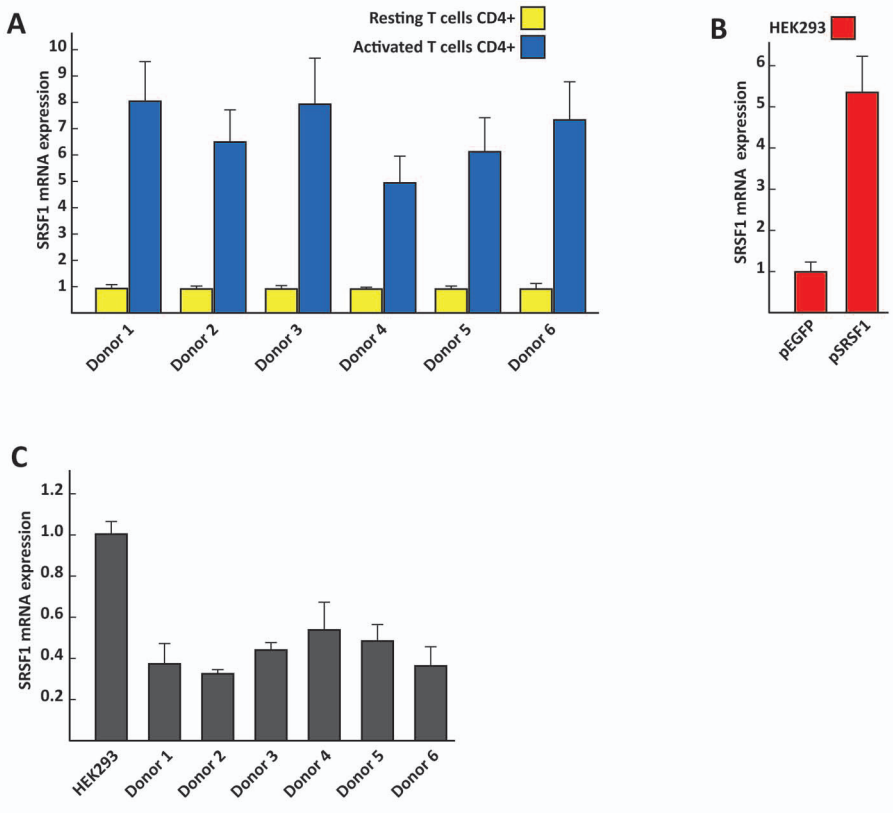
A
Resting T cells CD4+
Activated T cells CD4+
SRSF1 mRNA expression
10
9
8
7
6
5
4
3
2
1
Donor 1
Donor 2
Donor 3
Donor 4
Donor 5
Donor 6
B
HEK293
SRSF1 mRNA expression
6
5
4
3
2
1
pEGFP
pSRSF1
C
SRSF1 mRNA expression
1.2
1.0
0.8
0.6
0.4
0.2
HEK293
Donor 1
Donor 2
Donor 3
Donor 4
Donor 5
Donor 6

TABLE S1

GENE	CONSTRUCT	SOURCE
ACIN	pcDNA3.1-Acinus S	Dr. Heiner Schaal, Universitatsklinikum Dusseldorf, DE
CELF1	tgcugbp	Dr. Thomas Cooper, Baylor College of Medicine, USA
CELF2	tgetr3	Dr. Thomas Cooper, Baylor College of Medicine, USA
CELF3	3.1CBP3R	Dr. Thomas Cooper, Baylor College of Medicine, USA
CELF4	NCELF4h	Dr. Thomas Cooper, Baylor College of Medicine, USA
CELF5	tgCELF5.4	Dr. Thomas Cooper, Baylor College of Medicine, USA
CELF6	3.1CELF6orf1	Dr. Thomas Cooper, Baylor College of Medicine, USA
EGFP	pEGFP-N1	Clontech Laboratories, USA
FUS	TLS 5:hTLS-CHOP	Dr. David Ron, University of Cambridge, UK
GRSF1	pCMV6-GRSF1	Origene Technologies, USA
HNRNPA1	pCG7-A1	Dr. Adrian R. Krainer Cold Spring Harbor Laboratory, USA
HNRNPA2B1	pCMV6-HNRNPA2B1	Origene Technologies, USA
HNRNPA3	pCMV6-HNRNPA3	Origene Technologies, USA
HNRNPC	pFlag-hnRNP C1/C2	Dr. Heiner Schaal, Universitatsklinikum Dusseldorf, DE
HNRNPD	pLJM60-Auf1	Dr. David Sabatini, Massachusetts Institute of Technology
HNRNPF	pCMV6-HNRNPF	Origene Technologies, USA
HNRNPH1	pCMV6-HNRNPH1	Origene Technologies, USA
HNRNPH3	pCMV6-HNRNPH3	Origene Technologies, USA
HNRNPL	pEFnFlag-hnRNP L	Dr. Kristen Lynch University of Pennsylvania, USA
HNRNPU	pCMV6-HNRNPU	Origene Technologies, USA
HTATSF1	pCMV6-HTATSF1	Origene Technologies, USA
MBNL1	FLAGMBNL1-4	Dr. Thomas Cooper, Baylor College of Medicine, USA
MBNL2	FLAGMBNL2var1	Dr. Thoma Cooper, Baylor College of Medicine, USA
MBNL3	FlagMBNL3	Dr. Thoma Cooper, Baylor College of Medicine, USA

NONO	pCMV6-NONO	Origene Technologies, USA
PCBP1	pENTR-PCBP1	Dr. Huda Zoghbi, Baylor College of Medicine, USA
PTBP1	Myc tag WT PTB	Dr. Douglas Black, UCLA, USA
RBFOX2	pFox2-alpha	Dr. Heiner Schaal, Universitatsklinikum Dusseldorf, DE
RNPS1	pFlag-RNPS1	Dr. Heiner Schaal, Universitatsklinikum Dusseldorf, DE
SAP18	pFlag-Sap18	Dr. Heiner Schaal, Universitatsklinikum Dusseldorf, DE
SFPQ	pEFnFlag-PSF	Dr. Kristen Lynch University of Pennsylvania, USA
SNRNP70	pFlag-U1-70K	Dr. Heiner Schaal, Universitatsklinikum Dusseldorf, DE
SNRPA	pFlag-U1-A	Dr. Heiner Schaal, Universitatsklinikum Dusseldorf, DE
SNRPC	pFlag-U1-C	Dr. Heiner Schaal, Universitatsklinikum Dusseldorf, DE
SNRPD3	pCMV6-SNRPD3	Origene Technologies, USA
SRSF1	pCGT7-SF2	Dr. Adrian R. Krainer Cold Spring Harbor Laboratory, USA
SRSF2	pGCT7-30	Dr. Adrian R. Krainer Cold Spring Harbor Laboratory, USA
SRSF3	pGCT7-20	Dr. Adrian R. Krainer Cold Spring Harbor Laboratory, USA
SRSF4	pGCT7-75	Dr. Adrian R. Krainer Cold Spring Harbor Laboratory, USA
SRSF5	pCMV6-SRSF5	Origene Technologies, USA
SRSF6	pCMV6-SRSF6	Origene Technologies, USA
SRSF7	pCMV6-SRSF7	Origene Technologies, USA
TARDBP	CMV-TDP43	Dr. Emanuele Buratti, ICGEB, Italy
TCERG1	pCMV6-TCERG1	Origene Technologies, USA
TIA1	eGFP-TIA-1	Dr. Juan Valcarcel, Centre de Regulació Genòmica, Spain
Tra2A	pCMV-myc Tra2alpha	Dr. Alan Cochrane, University of Toronto, Canada
Tra2B	pCMV-myc Tra2beta	Dr. Alan Cochrane, University of Toronto, Canada
U2AF1	U2AF65	Dr. Emanuele Buratti, ICGEB, Italy