SUPPLEMENTARY FIGUTES AND TABLES

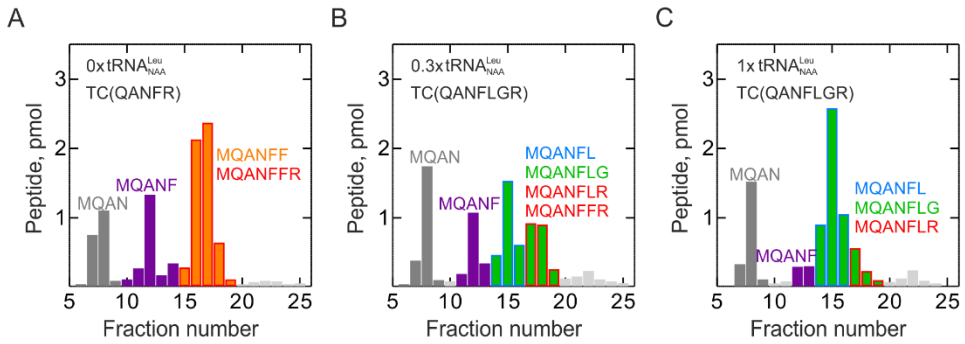

Figure S1. Analysis of the translation products by HPLC.

(A) Separation of the translation products in the absence of Leu-$tRNA_{NAA}^{Leu}$ specific for the UAA codon. tRNAs used for translation are Gln, Ala, Asn, Phe, and Arg (TC(QANFR)). Peptides are indicated: MQAN (grey), MQANF (purple), MQANFF (orange), and MQANFFR (red outline). Peptides were assigned based on different elution positions and confirmed using radioactively labeled amino acids: ^{14}C-labeled Gln, Ala and ^{3}H-labeled Asn to identify MQAN; [^{14}C]Phe/f[^{3}H]Met ratio to distinguish between MQANF and MQANFF; [^{14}C]Arg to assign MQANFFR.

(B) Separation of the reaction products in the presence of limiting $tRNA_{NAA}^{Leu}$ (0.3 per ribosome). tRNAs used for translation were Gln, Ala, Asn, Phe, Leu, Gly and Arg, (TC(QANFLGR)). Resulting peptides are MQAN (grey), MQANF (purple), MQANFL (blue outline), MQANFLG (green), and MQANFFR/MQANFLR (red outline). Position of each peptide was determined using radioactive labels, as in (A), and in addition [^{14}C]Leu, [^{14}C]Arg and [^{3}H]Gly were used for identification of MQANFL, MQANFLR and MQANFLG, respectively.

(C) Same as **(B)**, but with higher excess of $tRNA_{NAA}^{Leu}$ (1.0 per ribosome). TCs are the same as in **(B)**. Peptide assignment was done as in **(A)** and **(B)**.

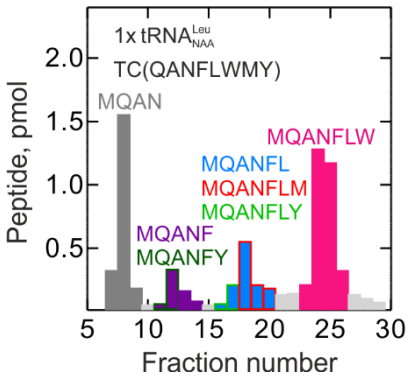

Figure S2. HPLC separation of products formed upon translation of –2 / +1 mRNA. Translation was performed at 1-fold $tRNA_{NAA}^{Leu}$ over the 70S. Resulting peptides are MQAN (grey), MQANF (purple), MQANFY (dark green outline), MQANFL (blue), MQANFLM (red outline), MQANFLY (light green outline) and MQANFLW (magenta). In the absence of $tRNA_{NAA}^{Leu}$ MQANFFM and MQANFFY peptides will be formed, which elute in the third peak on the chromatogram. To distinguish co-eluting peaks, radioactively labelled Phe, Leu, Met, Tyr and Trp amino acids were used.

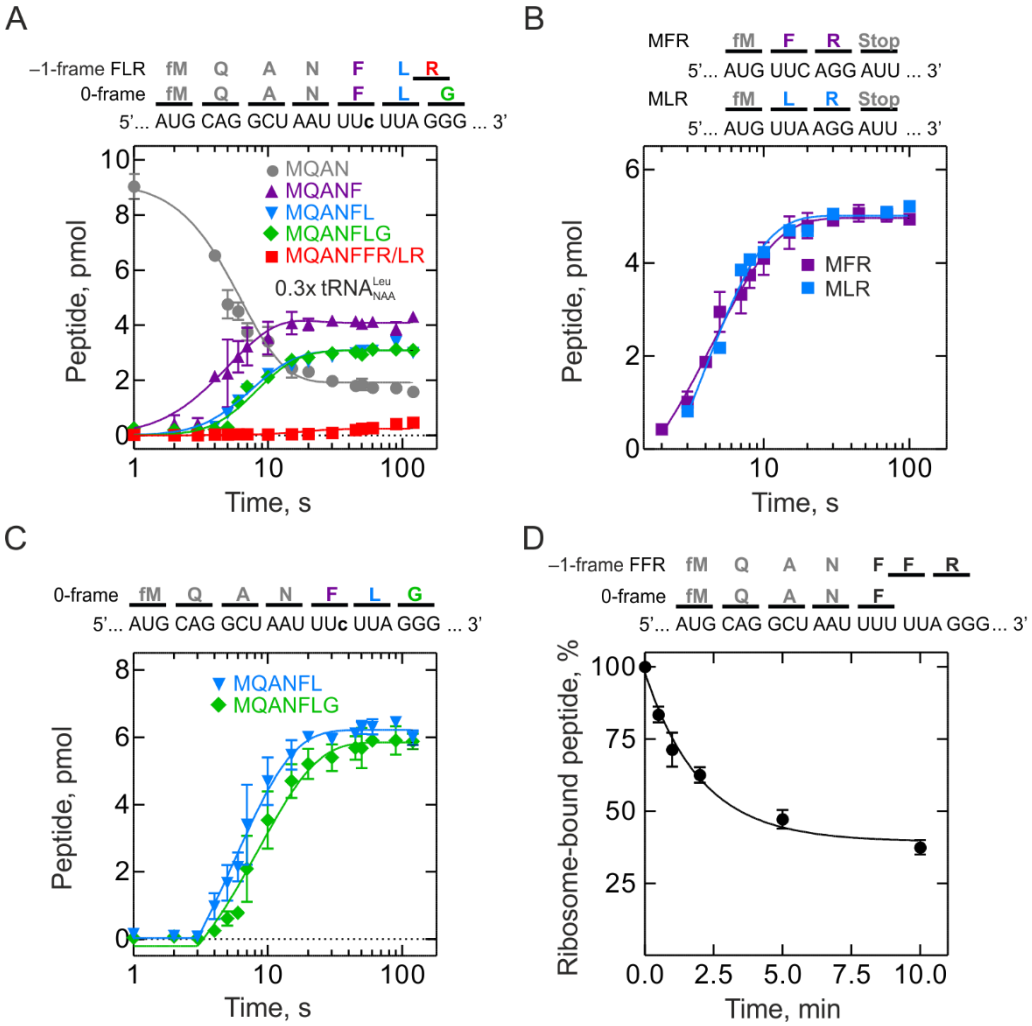

Figure S3. Supporting kinetic experiments.

(A) Translation time courses of the mRNA mutant with the disrupted SS1 (U_4C). Top panel: Sequences of mRNA and amino acids in –1- and 0-frames. Peptide products are MQAN (grey circles), MQANF (purple triangles), MQANFL (blue downward triangles hidden behind the green symbols), MQANFLG (green diamonds), and MQANFFR/MQANFLR (red squares). All tRNA concentrations were the same as in codon-walk experiments (Methods) and a 0.3-fold excess of $tRNA_{NAA}^{Leu}$ over ribosomes was used. Global fits are shown as continuous lines. The panel above shows amino acids incorporated into –1-frame FLR and 0-frame peptide.

(B) Time courses of MFR (purple squares) and MLR (blue squares) formation on model fM-F-R(AGG)-Stop and fM-L-R(AGG)-Stop mRNAs, respectively. Phe-tRNA^{Phe}, Leu-tRNA$_{NAA}^{Leu}$ and Arg-tRNA^{Arg} were used at 1.6 μM (10-fold over 70S), 0.16 μM (1-fold over 70S) and 1.6 μM (10-fold over 70S) concentrations, respectively. Single-exponential fits are shown as continues lines. The rates of MFR and MLR formation are 0.22 ± 0.01 s^{-1} and 0.27 ± 0.03 s^{-1}, respectively. The panel above shows the sequence of the model mRNA with respective amino acids.

(C) Time courses of MQANFL (blue downward triangles) and MQANFLG (green diamonds) formation on U_4C mRNA at 1-fold excess of tRNA$_{NAA}^{Leu}$. Gly-tRNA^{Gly} was used at 1.5 μM (7.5-fold over 70S). The panel above shows amino acids incorporated into 0-frame peptide.

(D) Time courses of MQANF-tRNA^{Phe} drop-off from ribosomes upon translation of frameshifting wt mRNA. The complexes were prepared by mixing IC with TC(QANF) and incubating for 30 sec to 10 min at 37°C, which results in the formation of MQAN, MQANF and –1-frame MQANFFR peptides. Gln-tRNA^{Gln}, Ala-tRNA^{Ala}, Asn-tRNA^{Asn}, Phe-tRNA^{Phe} and Arg-tRNA^{Arg} were used at 0.5 μM each (10-times over 70S). The amount of total ribosome-bound peptide was calculated from the f[^{3}H]Met retained on a nitrocellulose membrane (0.45 μm, Sartorius) upon filtration and scintillation counting in Quickszint cocktail (Zinsser Analytic). MQANF and MQANFF peptides were distinguished based on the [^{14}C]Phe/f[^{3}H]Met ratio. The panel above shows amino acids incorporated into –1-frame FFR and 0-frame peptide.

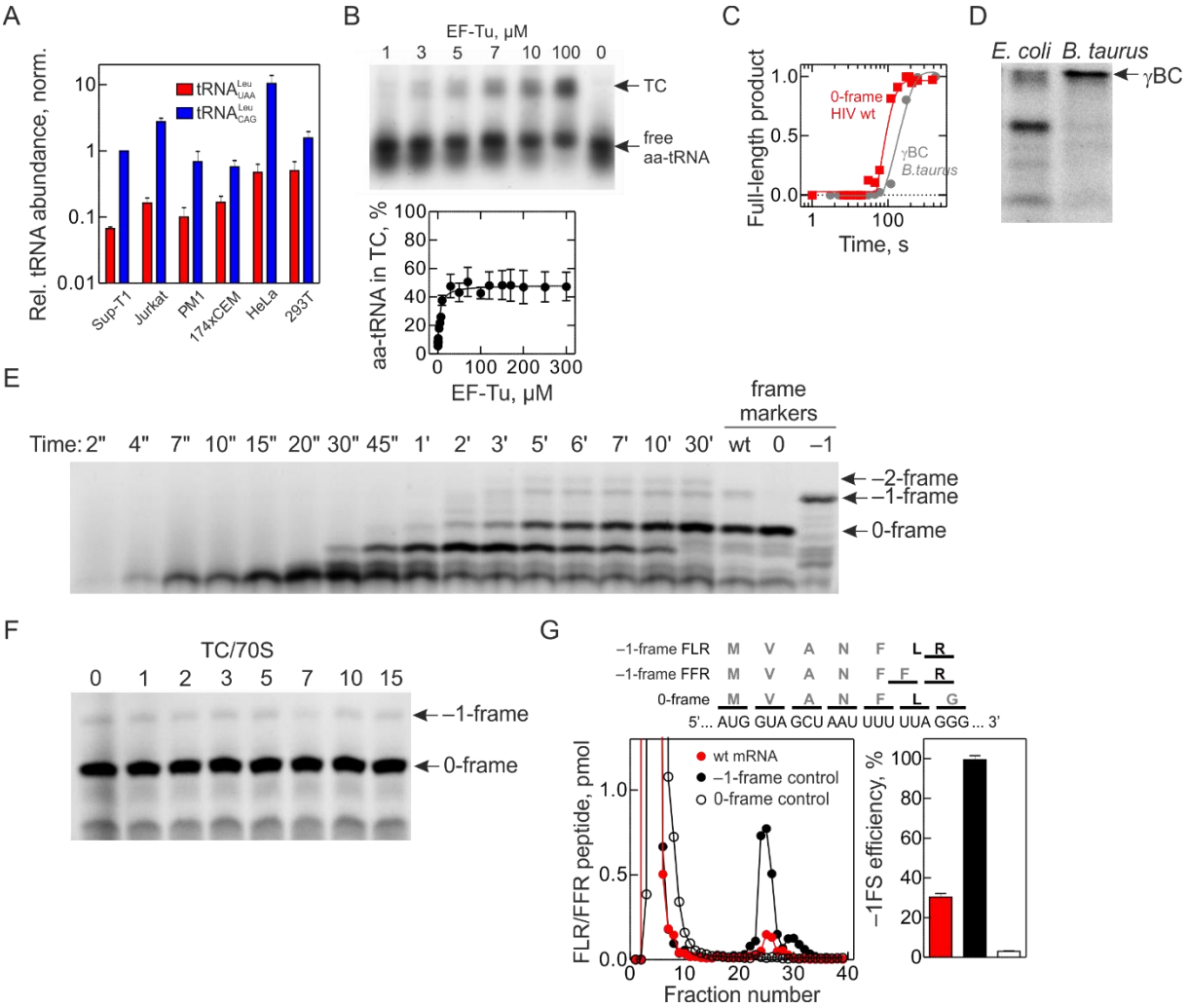

Figure S4. Characterization of the heterologous and homologous eukaryotic *in vitro* translation systems.

(A) Relative abundance of Leu-tRNA$_{UAA}^{Leu}$ (red) and Leu-tRNA$_{CAG}^{Leu}$ (blue) normalized to 18S rRNA in different cell lines. For better comparison, the ratio tRNA$_{CAG}^{Leu}$/18S rRNA in SupT1 cells was arbitrarily set to 1 and the values obtained for other cell types were normalized to this value. Error bars represent s.e.m of three independent experiments.

(B) Top panel. Formation of TC between *E. coli* EF-Tu and human native aa-tRNA (1.4 μM) monitored at varying concentrations of EF-Tu by electrophoretic mobility shift assay. TC formation was analyzed by native gel-electrophoresis using 5% PAGE supplemented with DTT (125 μM) and GTP (10 μM). The gels were run at 4°C in electrophoresis buffer (50 mM

Tris-HCl, pH 7.5, 10 mM magnesium acetate, 75 mM ammonium acetate, 1 mM EDTA, 10 μM GTP), stained with Gel Red (Biotium) and scanned using a UV transilluminator (Amersham™ imager 600). Band intensities were evaluated using the MultiGauge software. Bottom panel: TC formation as a function of EF-Tu concentration. The amount of the formed TC was calculated as a ratio between the EF-Tu-bound (TC) and free aa-tRNA multiplied by 100%.

(C) Time courses of synthesis of full-length γB-crystallin on the native mRNA from *B. taurus* (grey circles) and 0-frame peptide on wt HIV-1 mRNA (red squares). The fraction of 0-frame was calculated as a ratio between 0-frame product and all products of translation.

(D) Translation of γB-crystallin (γBC) in a heterologous system with human aa-tRNA using mRNAs with native (*B. taurus*) and harmonized (*E. coli)* codon usage. The full-length γB-crystallin is marked with an arrow.

(E) Time courses of 0, –1, and –2-frame peptides synthesized on no-stop mRNA. Times of translation and frame markers are shown above the gel. Frames are indicated with arrows.

(F) Titration of *E. coli* TC(Leu_{NAA}) on wt mRNA. Excesses of TC(Leu_{NAA}) are shown above the gel.

(G) Translation of the eukaryotic gag-pol wt mRNA using a fully reconstituted homologous mammalian *in vitro* translation system. Top panel: Sequence of gag-pol mRNA optimized for translation by eukaryotic translational machinery. Peptides produced in 0- and –1-frames are indicated above the sequence. Left panel: HPLC profile of –1 frameshifting peptides synthesized with wt (red circles), –1-frame control (closed circles) and 0-frame control (open circles) eukaryotic mRNAs. Peptides were monitored using [^{3}H]Arg. Right panel: –1FS efficiency measured with wt, –1-frame and 0-frame control mRNAs. Color code is as in the left panel. –1FS efficiency was calculated using [^{3}H]Arg on the frameshifting peptide MVANFLR or FFR and [^{14}C]Leu indicative of general translation efficiency.

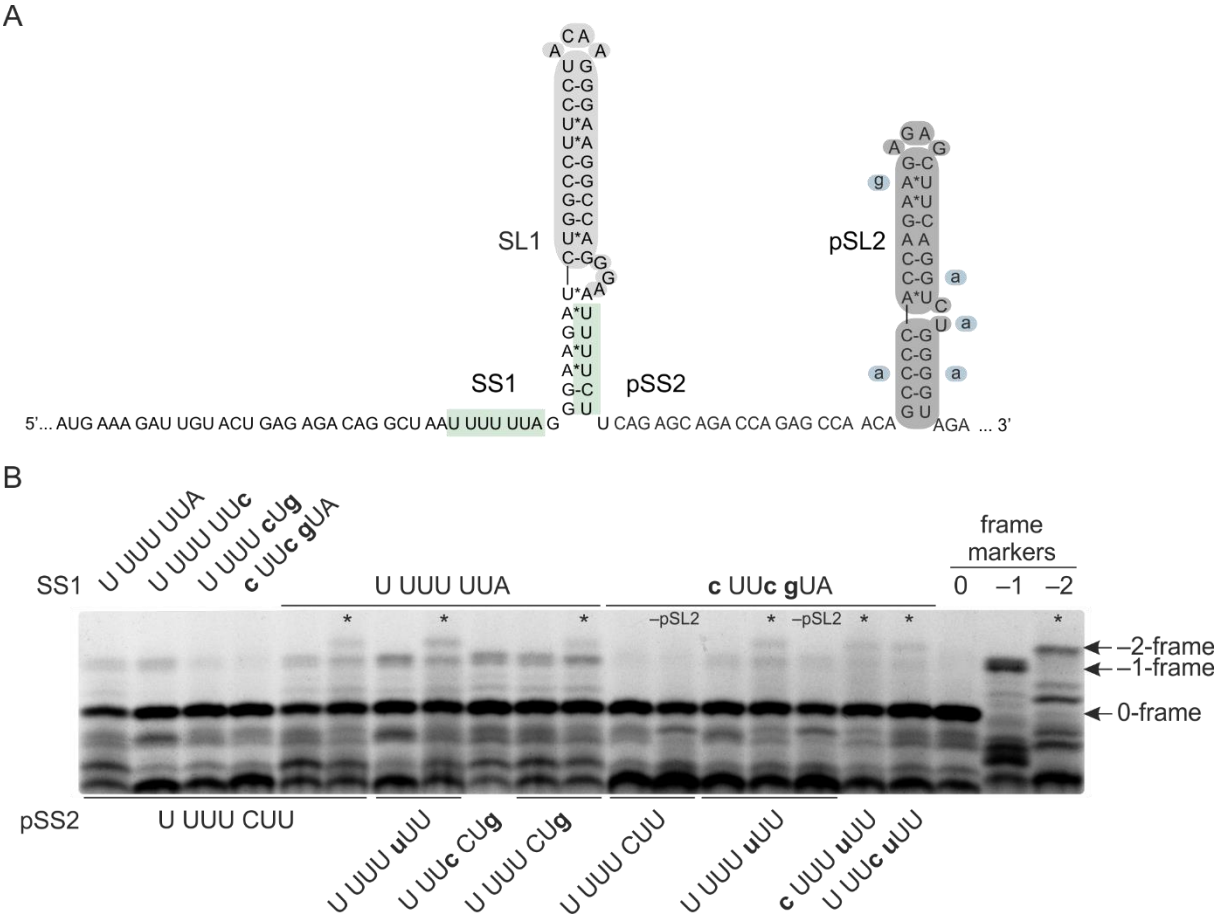

Figure S5. Translation experiments.

(A) The wt mRNA used for translation experiments. In addition to SS1, pSS2, SL1 and pSL2, the schematic indicates which mutations were introduced to remove the structure at pSL2. The pSL2 structure was predicted by mFold software.

(B) Examples of translation reactions with HIV-1 mRNA variants containing mutations in SS1, pSS2, and pSL2. Sequences of SS1 and pSS2 variants are indicated above and below the gel, respectively. Mutated nucleotides are in small bold letters. –pSL2 indicates mutated pSL2. * represent mRNAs with removed stop codons in –2-frame.

Table S1. Rate constants of translation and frameshifting.

Step	**Product**	**Rate constant, s^{-1}**
–Leu-$tRNA_{NAA}^{Leu}$		
k1	M → M_n	0.13 ± 0.07
k2	MQAN	0.34 ± 0.17
k3	MQANF 0-frame	0.21 ± 0.06
k4	MQANF drop-off	0.007 ± 0.01
k5	**MQANFF**	**0.01 ± 0.009**
k6[a]	MQANFFR –1-frame	0.22 ± 0.01
+Leu-$tRNA_{NAA}^{Leu}$		
k1	M drop-off	0.13 ± 0.07
k2	MQAN	0.34 ± 0.17
k3	MQANF	0.24 ± 0.10
k4	MQANF drop-off	0.02 ± 0.01
k5	**MQANFF**	**0.03 ± 0.01**
k6[a]	MQANFFR –1-frame	0.22 ± 0.01
k7	**MQANFL**	**0.33 ± 0.06 / 0.9 ± 0.2***
k8[b]	**MQANFLG 0-frame**	**0.53 ± 0.22**
k9	**MQANFLR –1-frame**	**0.04 ± 0.02**
Arg-$tRNA^{Arg}$ incorporation in 0-frame[a]		
	MFR	0.22 ± 0.01 s^{-1}
	MLR	0.27 ± 0.03 s^{-1}

* The two rate constants correspond to 0.3- and 1.0-fold excess of $tRNA_{NAA}^{Leu}$ over ribosomes, respectively.

[a] The rate constant is from Supplementary Figure 3B.

[b] The rate constant is from Supplementary Figure 3C.

Table S2. Sequences of mRNAs used in translation with bacterial components.

mRNA	Sequence (5' to 3')
wt short HPLC	GGGAGACCGGAAUUCGAGCUCGCCCAAACGCGGUUGGAUUCCUGAUGAAAAGU UCUAUGAGGUGUAUA**AUG**CAGGCUAAUUUUUUUAGGGAAGAUCUGGCCUUCCU ACAAGGGAAGGCCAGGGAAUUUUCUUCAGAGCAGACC
U_4C HPLC	GGGAGACCGGAAUUCGAGCUCGCCCAAACGCGGUUGGAUUCCUGAUGAAAAGU UCUAUGAGGUGUAUA**AUG**CAGGCUAAUUU**c**UUAGGGAAGAUCUGGCCUUCCUA CAAGGGAAGGCCAGGGAAUUUUCUUCAGAGCAGACC
–2 / +1 HPLC	GGGAGACCGGAAUUCGAGCUCGCCCAAACGCGGUUGGAUUCCUGAUGAAAAGU UCUAUGAGGUGUAUA**AUG**CAGGCUAAUUUUUUUA**u**GGAAGAUCUGGCCUUCCUA CAAGGGAAGGCCAGGGAAUUUUCUUCAGAGCAGACC
wt long gel	GGGAGACCGGAAUUCGAGCUCGCCCAAACGCGGUUGGAUUCCUGAUGAAAAGU UCUAUGAGGUGUAUA**AUG**AAAGAUUGUACUGAGAGACAGGCUAAUUUUUUUAG GGAAGAUCUGGCCUUCCUACAAGGGAAGGCCAGGGAAUUUUCUUCAGAGCAGA CCAGAGCCAACAGCCCCACCAGAAGAGAGCUUCAGGUCUGGGGUAGAGACAAC AACUCCCCCUCAG*UAG*CAGGAGCCGAUAGACAAGGAACUGUAUCCUUUAACUU CCCUCAGGUCACUCUUUGGCAACGACCCCUCGUCACAAUAAAGAUAGGGGGGC AACUAAAGGAAGCUCUAUUAGAUACAGGAGCAGAUGAUACAGUAUUAGAAGA AAUGAGUUUGCCAGGAAGAUGGAAACCAAAAAUGAUAGGGGGAAUUGGAGGU UUUAUCA
0-frame control gel	GGGAGACCGGAAUUCGAGCUCGCCCAAACGCGGUUGGAUUCCUGAUGAAAAGU UCUAUGAGGUGUAUA**AUG**AAAGAUUGUACUGAGAGACAGGCUAA**c**UU**cg**UAGG GAAGAUCUGGCCUUCCUACAAGGGAAGGCCAGGGAAUUU**c**CUUCAGAGCAGAC CAGAGCCAACAGCCCCACCAGAAGAGAGCUUCAGGUCUGGGGUAGAGACAACA ACUCCCCCUCAG*UAG*CAGGAGCCGAUAGACAAGGAACUGUAUCCUUUAACUUC CCUCAGGUCACUCUUUGGCAACGACCCCUCGUCACAAUAAAGAUAGGGGGGCA ACUAAAGGAAGCUCUAUUAGAUACAGGAGCAGAUGAUACAGUAUUAGAAGAA AUGAGUUUGCCAGGAAGAUGGAAACCAAAAAUGAUAGGGGGAAUUGGAGGUU UUAUCA
–1-frame control gel	GGGAGACCGGAAUUCGAGCUCGCCCAAACGCGGUUGGAUUCCUGAUGAAAAGU UCUAUGAGGUGUAUA**AUG**AAAGAUUGUACUGAGAGACAGGCUAA**c**UU**cg**UA**a**G GGAAGAUCUGGCCUUCCUACAAGGGAAGGCCAGGGAAUUU**c**CUUCAGAGCAGA CCAGAGCCAACAGCCCCACCAGAAGAGAGCUUCAGGUCUGGGGUAGAGACAAC AACUCCCCCUCAG*UAG*CAGGAGCCGAUAGACAAGGAACUGUAUCCUUUAACUU CCCUCAGGUCACUCUUUGGCAACGACCCCUCGUCACAAUAAAGAUAGGGGGGC AACUAAAGGAAGCUCUAUUAGAUACAGGAGCAGAUGAUACAGUAUUAGAAGA AAUGAGUUUGCCAGGAAGAUGGAAACCAAAAAUGAUAGGGGGAAUUGGAGGU UUUAUCA
–2-frame	GGGAGACCGGAAUUCGAGCUCGCCCAAACGCGGUUGGAUUCCUGAUGAAAAGU

control gel	UCUAUGAGGUGUAUA**AUG**AAAGAUUGUACUGAGAGACAGGCUAA**c**UU**cg**UA**a**G GGAAGAUCUGGCCUUCCUACAAGGGAAGGCCAGGGAAUUU**c**CUU**u**CAGAGCAG ACCAGAGCCAACAGCCCCACCAGAAGAGAGCUUCAGGUCUGGGGU**c**GAGACAA CAACUCCCCCUCAG*UAG*CAGGAGCCGAU**c**GACAAGGAACUGUAUCCUUU**c**ACUU CCCUCAGGUCACUCUUUGGCAACGACCCCUCGUCACAAUAAAGAUAGGGGGGC AACUAAAGGAAGCUCUAUUAGAUACAGGAGCAGAU**c**AUACAGUAUUAGAAGAA AUGAGUUUGCCAGGAAGAUGGAAACCAAAAAUGAUAGGGGGAAUUGGAGGUU UUAUCA
fM-F-R(AGG)-Stop	GUUAACAGGUAUACAUACU**AUG**UUCAGGAUUAC
fM-L-R(AGG)-Stop	GUUAACAGGUAUACAUACU**AUG**UUAAGGAUUAC

Table S3. Long mRNAs with SS1 and pSS2 variants used to study –1FS in HIV-1.

SS1	pSS2	pSL2	No stop codons in –2-frame
U UUU UUA	U UUU CUU	+	
U UUU UU**c**	U UUU CUU	+	
U UUU **c**U**g**	U UUU CUU	+	
c UU**c** **g**UA	U UUU CUU	+	
U UUU UUA	U UUU CUU	+	*
U UUU UUA	U UUU **u**UU	+	
U UUU UUA	U UUU **u**UU	+	*
U UUU UUA	U UU**c** CU**g**	+	
U UUU UUA	U UUU CU**g**	+	
U UUU UUA	U UUU CU**g**	+	*
c UU**c** **g**UA	U UUU CUU	+	
c UU**c** **g**UA	U UUU CUU	–	
c UU**c** **g**UA	U UUU **u**UU	+	
c UU**c** **g**UA	U UUU **u**UU	+	*
c UU**c** **g**UA	U UUU **u**UU	–	
c UU**c** **g**UA	**c** UUU **u**UU	+	*
c UU**c** **g**UA	U UU**c** **u**UU	+	*

Table S4. Sequences of mRNAs used in eukaryotic homologous translation system.

mRNA	Sequence (5’ to 3’)
wt	GGGCAACAACAACAACAAGGAUCCAAAACAGACCACC**AUG**GUAGCUAAUUUUUUAGGGAAGAUCUGGCCUUCCUACAAGGGAAGGCCAGGGAAUUUUCUUCAGAGCAGACCAGAGUAAUAACCAACAGCCCCACCAGAAGAGAGCUUCAGGUCUGGGGUAGAGACUAAUAACUCCCCCUCAGAAGCAGGAGCCGAUACAGAGUGUGAGGGAAGGUCAAGCUU
–1-frame control	GGGCAACAACAACAACAAGGAUCCAAAACAGACCACC**AUG**GUAGCUAA**c**UU**cc**U**cc**GGGAAGAUCUGGCCUUCCUACAAGGGAAGGCCAGGGAAUUUUCUUCAGAGCAGACCAGAGUAAUAACCAACAGCCCCACCAGAAGAGAGCUUCAGGUCUGGGGUAGAGACUAAUAACUCCCCCUCAGAAGCAGGAGCCGAUACAGAGUGUGAGGGAAGGUCAAGCUU
0-frame control	GGGCAACAACAACAACAAGGAUCCAAAACAGACCACC**AUG**GUAGCUAA**c**UU**cc**U**c**GGGAAGAUCUGGCCUUCCUACAAGGGAAGGCCAGGGAAUUUUCUUCAGAGCAGACCAGAGUAAUAACCAACAGCCCCACCAGAAGAGAGCUUCAGGUCUGGGGUAGAGACUAAUAACUCCCCCUCAGAAGCAGGAGCCGAUACAGAGUGUGAGGGAAGGUCAAGCUU

Table S5. Primers used for qRT-PCR.

Primer	Sequence (5' to 3')
Primers for RT	
$tRNA_{UAA}^{Leu}$ and $tRNA_{CAG}^{Leu}$ –	GTCGTATCCAGAATTTGTTGCAACGAACAGGTCTGGATACGACT**AACCCACGC**
$tRNA_{UAC}^{Val}$	GTCGTATCCAGAATTTGTTGCAACGAACAGGTCTGGATACGACT**TTCCACTGG**
$tRNA_{CAC}^{Val}$	GTCGTATCCAGAATTTGTTGCAACGAACAGGTCTGGATACGACT**TTCYGCCCG**
18S rRNA	GTCGTATCCAGAATTTGTTGCAACGAACAGGTCTGGATACGA**CTAATGATC**
Forward primers for qPCR	
$tRNA_{UAA}^{Leu}$	GAGTGGATAAGGCGTTGGACT**TAA**
$tRNA_{CAG}^{Leu}$	GGTCTAAGGCGCTGCGTT**CAG**
$tRNA_{UAC}^{Val}$	TAGTGGTTATCACGTCTGCTT**TAC**
$tRNA_{CAC}^{Val}$	TAGTGGTTATCACGTTCGCCT**CAC**
18S rRNA	GTAACAAGGTTTCCGTAGGTGA
Reverse primer for qPCR	
universal	GTTGCAACGAACAGGTCTGGATACG

Sequences homologous to the tRNA or 18S rRNA genes as well as tRNA anticodons are in bold.