

Supplemental Table S1. DNA oligonucleotide sequences used in this study.

DNA sequence	Description
<p>GCGAGTGAATTCTAATACGACTCACTATAGGAA <u>ACGACTCGAGTAGAGTCGAAAATTAGCTCGCCA</u> GTTAGCGAGGTCTGTCCCCACACGACAGATAAT CGGGTGCAACTCCCGCCCCTCTTCCGAGGGTC ATCGGAACCAGTTGGAGTCGAGTAGACTCCAAC AAAAGAAACAACAACAACGGATCCGCGAT</p>	<p>Gene block (dsDNA) fragment including the TLS representative from turnip yellow mosaic virus and flanking normalization hairpins (underlined) for chemical probing. Used as a template for all chemical probing and aminoacylation experiments.</p>
<p>GCGAGTGAATTCTAATACGACTCACTATAGGAA <u>ACGACTCGAGTAGAGTCGAAAAAGGAGCGACT</u> AGGGGTCGCGGTTGCTAAGACACAGCAATCAA GAGGGGTGCAAATCCCCCCTGAACCGGAGGG TTATCCGGCCAGTTGGAGTCGAGTAGACTCCA ACAAAGAAACAACAACAACGGATCCGCGA T</p>	<p>Gene block (dsDNA) fragment including the TLS representative from Japanese soil-borne wheat mosaic virus and flanking normalization hairpins (underlined) for chemical probing. Used as a template for all chemical probing and aminoacylation experiments.</p>
<p>GCGAGTGAATTCTAATACGACTCACTATAGGAA <u>ACGACTCGAGTAGAGTCGAAAATGTGCCGACAA</u> GTTGTCGGGAGTGCAAGACACGACTTAGTG GCTGGCGTAGACTGCTGGTGCGCAAACCTATA GAGTTTGCGTAGGTGCAAATCCCCCGTCCATAC GGAGCGATATCCGTCCAGTTGGAGTCGAGTA <u>GACTCCAACAAAAGAAACAACAACAACGGA</u> TCCGCGAT</p>	<p>Gene block (dsDNA) fragment including the TLS representative from peanut clump virus RNA 2 and flanking normalization hairpins (underlined) for chemical probing. Used as a template for all chemical probing and aminoacylation experiments.</p>
<p>GCGAGTGAATTCTAATACGACTCACTATAGGAA <u>ACGACTCGAGTAGAGTCGAAAAAGGGTAGCC</u> AGTGCTACCGCGATCTATAAACGATCGTTCAAG AGGGTGCAACTCCCCCCCCTTGGAGGGTATC CAAGCCCAGTTGGAGTCGAGTAGACTCCAACAA AAGAAACAACAACAACAACGGATCCGCGAT</p>	<p>Gene block (dsDNA) fragment including the WT TLS representative from Colombian potato soil-borne virus RNA 3 and flanking normalization hairpins (underlined) for chemical probing. Used as a template for all chemical probing and aminoacylation experiments.</p>
<p>GCGAGTGAATTCTAATACGACTCACTATAGGAA <u>ACGACTCGAGTAGAGTCGAAAAAGGGTAGCC</u> AGTGCTACCGCGATCTACACACGATCGTTCAAG AGGGTGCAACTCCCCCCCCTTGGAGGGTATC CAAGCCCAGTTGGAGTCGAGTAGACTCCAACAA AAGAAACAACAACAACAACGGATCCGCGAT</p>	<p>Gene block (dsDNA) fragment including the UAA → CAC anticodon mutation TLS representative from Colombian potato soil-borne virus RNA 3 and flanking normalization hairpins (underlined) for chemical probing. Used as a template for all chemical probing and aminoacylation experiments.</p>
<p>GCGAGTGAATTCTAATACGACTCACTATAGGAA <u>ACGACTCGAGTAGAGTCGAAAAGGACAAATACT</u> GACTTCTAGCCGTTTTTGGAGATAATTA ACTCATTATCCGGCTTATGGAAGGGTAGCCAGT GCTACCGCGATCTATAAACGATCGTTCAAGAGG GTGCAACTCCCCCCCCTTGGAGGGTATCCAA</p>	<p>Gene block (dsDNA) fragment including the 5'-extended TLS representative from Colombian potato soil-borne virus RNA 3 and flanking normalization hairpins (underlined) for chemical probing. Used as a template for all chemical probing and aminoacylation experiments.</p>

GCCCAGTTGGAGTCGAGTAGACTCCAACAAAA GAAACAACAACAACGGATCCGCGAT	
GCGAGTGAATTCTAATACGACTCACTATAGGAA ACGACTCGAGTAGAGTCGAAAATCGGACGAGT GATACAATTGACCCAGGGTCATCCTGCAAAACA CGCAGGTTTCCGATAGTGGTGCAAATCCACCC GCCAGTCGTCGGTGGTCCCTTGCGGGACCTAT ACGGTACCAGTTGGAGTCGAGTAGACTCCAACA AAAGAAACAACAACAACGGATCCGCGAT	Gene block (dsDNA) fragment including the TLS representative from Nudaurelia capensis beta virus and flanking normalization hairpins (underlined) for chemical probing. Used as a template for all chemical probing and aminoacylation experiments.
GCGAGTGAATTCTAATACGACTCACTATAGG	Forward ssDNA primer to amplify all templates for chemical probing experiments.
GTTGTTGTTGTTGTTTCTTTTGTGGAGTCTACT C	Reverse ssDNA primer to amplify all templates for chemical probing experiments.
TAATACGACTCACTATAGGTTAGCTCGCCAGTT AGCGAGG	Forward ssDNA primer to amplify the TYMV TLS template for <i>in vitro</i> aminoacylation experiments.
mUmGGTTCCGATGACCCTCGGA	Reverse ssDNA (with two 2' O-methyl bases at the 5' end) primer to amplify the TYMV TLS template for <i>in vitro</i> aminoacylation experiments.
TAATACGACTCACTATAGGAGGAGCGACTAGGG GTCG	Forward ssDNA primer to amplify the JSWMV TLS template for <i>in vitro</i> aminoacylation experiments.
mUmGGGCCGGATAACCCTCCG	Reverse ssDNA (with two 2' O-methyl bases at the 5' end) primer to amplify the JSWMV TLS template for <i>in vitro</i> aminoacylation experiments.
TAATACGACTCACTATAGGTGTGCCGACAAGTT GTCGGG	Forward ssDNA primer to amplify the PCV RNA 2 TLS template for <i>in vitro</i> aminoacylation experiments.
mUmGGGACGGATATCGCTCCG	Reverse ssDNA (with two 2' O-methyl bases at the 5' end) primer to amplify the PCV RNA 2 TLS template for <i>in vitro</i> aminoacylation experiments.
TAATACGACTCACTATAGGAAGGGTAGCCAGTG CTACCG	Forward ssDNA primer to amplify the CPSbV RNA 3 TLS template for <i>in vitro</i> aminoacylation experiments.
mUmGGGCTTGGATACCCTCCAAGG	Reverse ssDNA (with two 2' O-methyl bases at the 5' end) primer to amplify the CPSbV RNA 3 TLS template for <i>in vitro</i> aminoacylation experiments.
TAATACGACTCACTATAGGTCCGACGAGTGATA CAATTGACCCAG	Forward ssDNA primer to amplify the NCBV TLS template for <i>in vitro</i> aminoacylation experiments.

mUmGGTACCGTATAGGTCCCGC	Reverse ssDNA (with two 2' O-methyl bases at the 5' end) primer to amplify the NCBV TLS template for <i>in vitro</i> aminoacylation experiments.
TAATACGACTCACTATAGGCAATTGACCCAGGG TCATCCTGC	Forward ssDNA primer to amplify the 5'-truncated NCBV TLS template for <i>in vitro</i> aminoacylation experiments.
TAATACGACTCACTATAGGAGGGTTGGCCGAGT GGTCTAAGGCGGCAGACTTAAGATCTG	Forward overlapping primer to create a template for the yeast tRNA ^{Leu} <i>in vitro</i> aminoacylation assay.
mUmGGTGAAGGATGCGAGGTTCGAACTCGCGC GGACAACCGTCCAACAGATCTTAAGTCTGCCGC C	Reverse overlapping (with two 2' O-methyl bases at the 5' end) primer to create a template for the yeast tRNA ^{Leu} <i>in vitro</i> aminoacylation assay.
TAATACGACTCACTATAGGTGGTTTGCCCGAGT GGTTAAGGGGGAAGACTTAAGATCTTC	Forward overlapping primer to create a template for the potato tRNA ^{Leu} <i>in vitro</i> aminoacylation assay.
mUmGGTGGTGGCTGTGGGGTTCGAACCCACG CGCACTTATGTGCAGAAGATCTTAAGTCTTCCC CC	Reverse overlapping (with two 2' O-methyl bases at the 5' end) primer to create a template for the potato tRNA ^{Leu} <i>in vitro</i> aminoacylation assay.
5'-/5- 6FAM/AAAAAAAAAAAAAAAAAATGGTTCCGA TGACCCTCGGAAGAGG	FAM-labeled RT primer.
CATCATCATCACAGCAGCGGCCTGGTGCCGCG CGGCAGCCATAtgATGAGCAGCGGTCTGGTATT AGAGAACACGGCACGCCGTGACGCTCTGATCG CCATTGAAAAGAAATACCAGAAAATCTGGGCCG AGGAACATCAATTTGAGATCGATGCACCTTCAA TCGAGGATGAGCCTATTACCATGGACTCGGAAG AACTTCATCGCACTTATCCAAAGTTTATGAGCTC CATGGCTTATCCGTATATGAACGGTGTATTGCA CGCGGGGCACTGTTTTACGCTTCAAAGGTGGA GTTTAGCATTGGGTTTGAGCGTATGAACGGGAA GCGTGCCCTTTTTCCCTGGGTTTCCACTGTAC AGGCATGCCTATTTTGGCCTGCGCCGATAAACT TAAACGTGAAGCTGAATTGTTTGGTAAGAACTT CGACAACGTGCCAGCGGAAGAGGAAGAAATTA AAGAAGAAACCCCTGCGGAGAAAGATCACGAG GATGTAACCAAATTCAAAGCTAAGAAAAGTAAA GCTGCGGCCAAAAGGGGCGCGGCAAGTACCA GTTCGAAATCATGTTGCAATTAGGTATCCCGCG CGAAGAAATTATTAAGTTTTCGGACGCGAAGTA CTGGCTGACCTACTTTCCGCCGCTGTGCGAGT CCGATTGCACCTCGTTGGGTGCTCGTATCGACT GGCGCCGTTTCATTCGTAACGACCGACGCTAATC CATATTATGACGCGTTTATCCGCTGGCAAATGA	Gene block fragment (dsDNA) #1 for the N-terminal region of the LeuRS protein from <i>S. cerevisiae</i> . Used in conjunction with gene block fragment #2 (see below) for Gibson assembly and a pET15b vector for protein expression in <i>E. coli</i> .

ATAAACTTAAGGCTGCCGGAAGATCAAGTTCG
GGGAACGTTATACGATTTATAGTGAAAAGGACG
GCCAAGCGTGCATGGACCATGATCGCCAATCT
GGCGAGGGGGTTACACCCCAGGAGTACATTGG
CGTAAAAATCGAGGCTTTGGAATTCGCTGACGA
TGCAGCTAAAATCATCGACTCCTCTTCAGATCT
GGATAAGTCCAAAAAGTTTTATTTTGTGCGCAGCT
ACCCTTCGCCCGGAGACTATGTATGGACAAACA
TGCTGCTTCGTTTCGCCGACGATCGAATACGGG
ATCTTCGACGCGGGAGACAGTTATTTTATTA
CAGAGCGCGCATTCAAAAACATGAGCTACCAGA
AACTTACTCCTAAGCGCGGTTTTTACAAACCTAT
TGTAACCGTTCCTGGCAAGGCATTTATTGGAAC
GAAAATCCACGCACCTCAGTCGGTGTATCCCGA
ACTTCGTATTCTGCCGATGGAGACGGTTATTGC
AACTAAAGGTACGGGGGTTGTTACGTGTGTGCC
TTCAAACCTACCAGACGATTACATCACCACAAA
AGACTTATTACACAAGCCAGAATATTACGGTATT
AAGCCTGAATGGATCGATCATGAGATTGTTCCG
ATTATGCATACTGAGAAGTACGGTGACCTTACA
GCGAAGGCGATCGTAGAAGAGAAAAAATTCAG
AGCCCTAAAGATAAGAACTTGTTGGCCGAGGCT
AAAAAAATTGCTTATAAAGAGGATTATTACACGG
GCACCATGATTTATGGTCCATATAAGGGCGAGA
AAGTTGAGCAAGCCAAAAATAAGGTAAAGGCGG
ATATGATCGCTGCGGGTGAGGCCTTCGTGTATA
ATGAACCCGAGTCACAAGTTATGTCACGTTCCG
GAGACGACTGCATTGTCAGTTTGGAGGACCAAT
GGTACGTAGATTATGGTGAAGAATCATGGAAGA
AGCAGGCCATCGAGTGTTTGAAGGGATGCAG
TTATTCGCGCCGGAGGTGAAAAATGCCTTTGAG
GGCGTTCTTGACTGGCTTAAGAACTGGGCCGTA
TGCCGCACGTACGGTCTGGGCACACGCCTTCC
ATGGGATGAAAAATATTTGGTGGAAAGTTTGAG
CGATTCAACCATCTACCAGTCCTTTTACACCATC
GCCACCTTCTTTTCAAGGACTACTATGGCAAT
GAGATTGGACCACTTGGAATTTCCGCTGACCAG
ATGACAGACGAGGTCTTTGACTACATCTTTCAG
CATCAAGATGATGTGAAGAATACGAATATTCCC
TTGCCAGCATTACAGAAATTACGCCGTGAATTC
GAATACTTTTACCCGCTTGACGTGTCAATTAGT
GGCAAAGACTTAATCCCTAATCATTTGACCTTCT
TCATTTATACCCATGTGGCGTTGTTCCCTAAAAA
GTTCTGGCCTAAAGGTATCCGTGCAAATGGTCA
CTTGATGCTTAATAATTCCAAAATGTCAAAGTCA
ACAGGAAATTTTATGACTCTTGAGCAAACGGTA
GAAAAGTTCGGTGCGGATGCTGCGCGCATCGC

<p> ATTTGCAGATGCCGCGATACAGTTGAGGACG CGAACTTTGACGAATCGAATGCTAATGCGGCAA TCCTTCGCCTGTTCAACTTGAAGGAGTGGGCG GAAGAAATTACCAAGGAAAGTAATCTTCGTACG GGGGAAATCACGGACTTCTTTGACATTGCATTG GAGCACGAGATGAACGCGCTTATTGAAAAAACT TATGAGCAGTATGCCTTGAATAACTATAAAAAAC GCCCTGAAGTACGGATTGTTGACTTCCAGGCC GCACGCGATTATTATCGTGAAGCATCTGGCGTG ATGCATAAGGATTTAATTGCCCGCTACATTGAG ACACAGGCATTGCTTTTGGCGCCTATCGCACCA CACTTTGCAGAGTACATTTACCGTGAGGTTCTT GGCAATCAGACAAGCGTTCAGAACGCCAAATTC CCCCGCGCGTCTAAGCCGGTAGACAAGGGTGT CTTGGCAGCGCTTGATTACCTGCGTAATCTTCA GCGCTCAATCCGCGAGGGTGAGGGGCAGGCC CTGAAGAAGAAAAAGGGGAAGTCAGCGGAAAT CGACGCATCAAAGCCCGTTAAGCTTACGTTATT GATCAGTGAATCTTTTCCTGAATGGCAATCTC </p>	
<p> GGGGAAGTCAGCGGAAATCGACGCATCAAAGC CCGTTAAGCTTACGTTATTGATCAGTGAATCTTT TCCTGAATGGCAATCTCAATGCGTTGAGATCGT CCGTAAGTATTTTCTGAGCAGACGCTTGATGA CAACAAAAAGGTGCGCGAGCACATCGAACCAA AAGAGATGAAACGTGCGATGCCATTTATCTCTC TGCTTAAACAGCGTCTGGCTAACGAAAAACCCG AGGACGTTTTTGAACGTGAGTTACAATTCAGTG AGATTGACACCGTTAAAGCAGCCGCACGTAACG TAAAGAAAGCCGCTCAGGCTCTTAAGATCGCAG AATTTTCGGCAATTAGCTTCCCGTACGGCGCTA AGACAGGCAAAGACATCTTCACGGGGGAAGAA GTTGAAATCCCCCAGTCACGAAGATCGTGGA GAACGCGGTCCCAGGAAATCCTGGGGTCGTCT TTCAAAACATCTAGGGATCCGGCTGCTAACAAA GCCCGAAAGGAAGCTGAGTTGGCTGC </p>	<p>Gene block fragment (dsDNA) #2 for the C-terminal region of the LeuRS protein from <i>S. cerevisiae</i>. Used in conjunction with gene block fragment #1 (see above) for Gibson assembly and a pET15b vector for protein expression in <i>E. coli</i>.</p>