Table 1. Nucleotide sequences of 5' leaders used in shunting experiments

5' Leader	Description
p1-uAUG	Five repeats of the 9-nt poly(A) spacer (SIII) interspersed with a 9-nt spacer based on a segment of the β-globin 5' UTR [nt 9-17 (SI)]. The sequence between the 4th and 5th (SI/SIII) repeat has been mutated at various nucleotides to A and is spaced 25-nt upstream of the *Photinus* initiator AUG with β-globin 5' UTR sequence. Cloned into the mammalian expression vector pGL3c. *TTCTGACAT* AAAAAAAAA *TTCTGACAT* AAAAAAAAA *TTCTGACAT* AAAAAAAAA *TTCTGACAT* AAAAAAAAA *TTCTGACAT* C**A***CC***AAA***GA *TTCTGACAT* AAAAAAAAA *GACTCACAACCCCAGAAACAGACAT*
p1+uAUG	Five repeats of the 9-nt poly(A) spacer (SIII) interspersed with a 9-nt spacer based on a segment of the β-globin 5' UTR [nt 9-17(SI)]. The sequence between the 4th and 5th (SI/SIII) repeat has been mutated at various nucleotides to introduce an upstream AUG in optimal context and is spaced 25-nt upstream of the *Photinus* initiator AUG with β-globin 5' UTR sequence. Cloned into the mammalian expression vector pGL3c. *TTCTGACAT* AAAAAAAAA *TTCTGACAT* AAAAAAAAA *TTCTGACAT* AAAAAAAAA *TTCTGACAT* AAAAAAAAA *TTCTGACAT* C**A***CC***ATG***GA *TTCTGACAT* AAAAAAAAA *GACTCACAACCCCAGAAACAGACAT*
p2-uAUG	Four repeats of the 8-nt *Gtx* TEE and a 9-nt poly(A) spacer (SIII) interspersed with a 9-nt spacer based on a segment of the β-globin 5' UTR [nt 9-17 (SI)]. The spacer sequence between the 4th *Gtx* TEE and the (SI/III) spacer has been mutated at various nucleotides to A and is spaced 25-nt upstream of the *Photinus* initiator AUG with β-globin 5' UTR sequence. Cloned into the mammalian expression vector pGL3c. *TTCTGACAT* **CCGGCGGGA** *TTCTGACAT* **CCGGCGGGA** *TTCTGACAT* **CCGGCGGGA** *TTCTGACAT* **CCGGCGGGA** *TTCTGACAT* C***A****CC****AAA****GA *TTCTGACAT* AAAAAAAAA *GACTCACAACCCCAGAAACAGACAT*
p3-uAUG	Five repeats of the 8-nt *Gtx* TEE interspersed with a 9-nt spacer based on a segment of the β-globin 5' UTR [nt 9-17 (SI)]. The spacer sequence between 4th and 5th *Gtx* TEE has been mutated at various nucleotides to A and is spaced 25-nt upstream of the *Photinus* initiator AUG with β-

5' Leader	Description
	globin 5' UTR sequence. Cloned into the mammalian expression vector pGL3c. *TTCTGACAT* **CCGGCGGGA** *TTCTGACAT* **CCGGCGGGA** *TTCTGACAT* **CCGGCGGGA** *TTCTGACAT* **CCGGCGGGA** *TTCTGACAT C***A***CC***AAA***GA TTCTGACAT* **CCGGCGGGA** *GACTCACAACCCCAGAAACAGACAT*
p3+uAUG	Five repeats of the 8-nt *Gtx* TEE interspersed with a 9-nt spacer based on a segment of the β-globin 5' UTR [nt 9-17 (SI)]. The spacer sequence between 4th and 5th *Gtx* TEE has been mutated at various nucleotides to introduce an upstream AUG in optimal context and is spaced 25-nt upstream of the *Photinus* initiator AUG with β-globin 5' UTR sequence. Cloned into the mammalian expression vector pGL3c. *TTCTGACAT* **CCGGCGGGA** *TTCTGACAT* **CCGGCGGGA** *TTCTGACAT* **CCGGCGGGA** *TTCTGACAT* **CCGGCGGGA** *TTCTGACAT C***A***CC***ATG***GA TTCTGACAT* **CCGGCGGGA** *GACTCACAACCCCAGAAACAGACAT*
p4+uAUG	Four repeats of the 9-nt poly(A) spacer (SIII) and an 8-nt *Gtx* TEE interspersed with a 9-nt spacer based on a segment of the β-globin 5' UTR [nt 9-17 (SI)]. The spacer sequence between the 4th (SIII) and the 8-nt *Gtx* TEE has been mutated at various nucleotides to introduce an upstream AUG in optimal context and is spaced 25-nt upstream of the *Photinus* initiator AUG with β-globin 5' UTR sequence. Cloned into the mammalian expression vector pGL3c. *TTCTGACAT* AAAAAAAAA *TTCTGACAT* AAAAAAAAA *TTCTGACAT* AAAAAAAAA *TTCTGACAT* AAAAAAAAA *TTCTGACAT C***A***CC***ATG***GA TTCTGACAT* **CCGGCGGGA** *GACTCACAACCCCAGAAACAGACAT*
p5+AUG	Five repeats of the 8-nt *Gtx* TEE interspersed with a 9-nt spacer based on a segment of the β-globin 5' UTR [nt 9-17 (SI)]. The spacer sequence between 4th and 5th *Gtx* TEE has been mutated at various nucleotides to introduce an upstream AUG in optimal context and is in frame with the main *Photinus* luciferase reading frame by the introduction of an A into a 25-nt spacer sequence comprised of β-globin 5' UTR sequence indicated by an *. Cloned into the mammalian expression vector pGL3c. *TTCTGACAT* **CCGGCGGGA** *TTCTGACAT* **CCGGCGGGA** *TTCTGACAT* **CCGGCGGGA**

5' Leader	Description
	TTCTGACAT **CCGGCGGGA** *TTCTGACAT C***A***CC***ATG***GA TTCTGACAT* **CCGGCGGGA** *GACTCACAACCCCA*A**GAAACAGACAT*
p1hp	Five repeats of the 9-nt poly(A) spacer (SIII) interspersed with a 9-nt spacer based on a segment of the β-globin 5' UTR [nt 9-17 (SI)] upstream of a 128-nt hairpin structure (underlined) with a predicted energy of –126.1 kcal/mol. An (SI/SIII) repeat is located downstream of the hairpin structure and is spaced 25-nt upstream of the *Photinus* initiator AUG with β-globin 5' UTR sequence. Cloning into the pGL3c mammalian expression vector generates an *Mfe*I/*Eco*RI junction (CAATTC) and a unique AatII restriction site in the loop of the hairpin. AAAAAAAAA *TTCTGACAT* AAAAAAAAA *TTCTGACAT* AAAAAAAAA *TTCTGACAT* AAAAAAAAA *TTCTGACAT* AAAAAAAAA *TTCTGACA* CAATTC CCAGCGTAATCGGG AACGTCGTAGGGGTAAGCCATTGTACGACCACCGGCTCGAGGGGCCC GACGTC GGGCCCCTCGAGCCGGTGGTCGTACAATGGCTTACCCCTACGACGTTCCCGATTACGC TGG *TTCTGACAT* AAAAAAAAA *GACTCACAACCCCAGAAACAGACAT*
p2hp	Five repeats of the 8-nt *Gtx* TEE interspersed with a 9-nt spacer based on a segment of the β-globin 5' UTR [nt 9-17 (SI)] upstream of a 128-nt hairpin structure (underlined) with a predicted energy of -126.1 kcal/mol. An (SI)/poly(A) repeat is located downstream of the hairpin structure and are spaced 25-nt upstream of the *Photinus* initiator AUG with β-globin 5' UTR sequence. Cloning into the pGL3c mammalian expression vector generates an MfeI/EcoRI junction (CAATTC) and a unique AatII restriction site in the loop of the hairpin. **CCGGCGGGA** *TTCTGACAT* **CCGGCGGGA** *TTCTGACAT* **CCGGCGGGA** *TTCTGACAT* **CCGGCGGGA** *TTCTGACAT* **CCGGCGGGA** *TTCTGACAT* CAATTC CCAGCGTAATCGG GAACGTCGTAGGGGTAAGCCATTGTACGACCACCGGCTCGAGGGGCCC GACGTC GGGCCCCTCGAGCCGGTGGTCGTACAATGGCTTACCCCTACGACGTTCCCGATTACGC TGG *TTCTGACAT* AAAAAAAAA *GACTCACAACCCCAGAAACAGACAT*
p3hp	Five repeats of the 9-nt poly(A) spacer (SIII) interspersed with a 9-nt spacer based on a segment of the β-globin 5' UTR [nt 9-17 (SI)] upstream of a 128-nt hairpin structure (underlined) with a predicted energy of –126.1 kcal/mol. An (SI) spacer and a single copy of the 8-nt *Gtx* TEE are located downstream of the hairpin structure and are spaced 25-nt upstream of the *Photinus* initiator AUG with β-globin 5' UTR sequence. Cloning into the pGL3c mammalian expression vector generates an MfeI/EcoRI junction (CAATTC) and a unique AatII restriction site in the

5' Leader	Description
	loop of the hairpin. **CCGGCGGGA** *TTCTGACAT* **CCGGCGGGA** *TTCTGACAT* **CCGGCGGGA** *TTCTGACAT* **CCGGCGGGA** *TTCTGACAT* CAATTC CCAGCGTAATCGG GAACGTCGTAGGGGTAAGCCATTGTACGACCACCGGCTCGAGGGGCCC GACGTC GGGCCCCTCGAGCCGGTGGTCGTACAATGGCTTACCCCTACGACGTTCCCGATTACGC TGG *TTCTGACAT* **CCGGCGGGA** *GACTCACAACCCCAGAAACAGACAT*
p4hp	Five repeats of the 9-nt poly(A) spacer(SIII) interspersed with a 9-nt spacer based on a segment of the β-globin 5' UTR [nt 9-17 (SI)] upstream of a 128-nt hairpin structure (underlined) with a predicted of –126.1 kcal/mol. An (SI) spacer and a single copy of the 8-nt *Gtx* TEE are located downstream of the hairpin structure and are spaced 25-nt upstream of the *Photinus* initiator AUG with β-globin 5' UTR sequence. Cloning into the pGL3c mammalian expression vector generates an MfeI/EcoRI junction (CAATTC) and a unique AatII restriction site in the loop of the hairpin. AAAAAAAAA *TTCTGACAT* AAAAAAAAA *TTCTGACAT* AAAAAAAAA *TTCTGACAT* AAAAAAAAA *TTCTGACAT* AAAAAAAAA *TTCTGACA* CAATTC CCAGCGTAATCGGG AACGTCGTAGGGGTAAGCCATTGTACGACCACCGGCTCGAGGGGCCC GACGTC GGGCCCCTCGAGCCGGTGGTCGTACAATGGCTTACCCCTACGACGTTCCCGATTACGC TGG *TTCTGACAT* **CCGGCGGGA** *GACTCACAACCCCAGAAACAGACAT*
p5hp	Five repeats of the 9-nt poly(A) spacer (SIII) interspersed with a 9-nt spacer based on a segment of the β-globin 5' UTR [nt 9-17 (SI)] upstream of a 128-nt hairpin structure (underlined) with a predicted energy of –126.1 kcal/mol. An (SI/SIII) spacer is located downstream of the hairpin structure and are spaced 25-nt upstream of the *Photinus* initiator AUG with β-globin 5' UTR sequence**.** Cloning into the pYESFFlucH yeast expression vector generates an MfeI/EcoRI junction (CAATTC) and a unique AatII restriction site in the loop of the hairpin. AAAAAAAAA *TTCTGACAT* AAAAAAAAA *TTCTGACAT* AAAAAAAAA *TTCTGACAT* AAAAAAAAA *TTCTGACAT* AAAAAAAAA *TTCTGACA* CAATTC CCAGCGTAATCGGG AACGTCGTAGGGGTAAGCCATTGTACGACCACCGGCTCGAGGGGCCC GACGTC GGGCCCCTCGAGCCGGTGGTCGTACAATGGCTTACCCCTACGACGTTCCCGATTACGC TGG *TTCTGACAT* AAAAAAAAA *GACTCACAACCCCAGAAACAGACAT*

5' Leader	Description
p6hp	Five repeats of the 8-nt *Gtx* TEE interspersed with a 9-nt spacer based on a segment of the β-globin 5' UTR [nt 9-17 (SI)] upstream of a 128-nt hairpin structure (underlined) with **a** predicted energy of –126.1 kcal/mol. An (SI) poly(A) spacer is located downstream of the hairpin structure and are spaced 25-nt upstream of the *Photinus* initiator AUG with β-globin 5' UTR sequence. Cloning into the pYESFFlucH yeast expression vector generates a unique AatII restriction site in the loop of the hairpin and an EcoRI restriction site. **CCGGCGGGA** *TTCTGACAT* **CCGGCGGGA** *TTCTGACAT* **CCGGCGGGA** *TTCTGACAT* **CCGGCGGGA** *TTCTGACAT* **CCGGCGGGA** *TTCTGACAT* GAATTC CCAGCGTAATCGG GAACGTCGTAGGGGTAAGCCATTGTACGACCACCGGCTCGAGGGGCCC GACGTC *GGG*CCCCTCGAGCCGGTGGTCGTACAATGGCTTACCCCTACGACGTTCCCGATTACGC TGG *TTCTGACAT* AAAAAAAAA *GACTCACAACCCCAGAAACAGACAT*
p7hp	Five repeats of the 8-nt *Gtx*-TEE interspersed with a 9-nt spacer based on a segment of the β-globin 5' UTR [nt 9-17 (SI)] upstream of a 128-nt hairpin structure (underlined) with a predicted energy of –126.1 kcal/mol. An (SI) spacer and a single copy of the 8-nt *Gtx* TEE are located downstream of the hairpin structure and are spaced 25-nt upstream of the *Photinus* initiator AUG with β-globin 5' UTR sequence. Cloning into the pYESFFlucH yeast expression vector generates a unique AatII restriction site in the loop of the hairpin and an EcoRI restriction site. **CCGGCGGGA** *TTCTGACAT* **CCGGCGGGA** *TTCTGACAT* **CCGGCGGGA** *TTCTGACAT* **CCGGCGGGA** *TTCTGACAT* **CCGGCGGGA** *TTCTGACAT* GAATTC CCAGCGTAATCGG GAACGTCGTAGGGGTAAGCCATTGTACGACCACCGGCTCGAGGGGCCC GACGTC GGGCCCCTCGAGCCGGTGGTCGTACAATGGCTTACCCCTACGACGTTCCCGATTACGC TGG *TTCTGACAT* **CCGGCGGGA** *GACTCACAACCCCAGAAACAGACAT*
p8hp	Five repeats of the 9-nt poly(A) spacer (SIII) interspersed with a 9-nt spacer based on a segment of the β-globin 5' UTR [nt 9-17 (SI)] upstream of a 128-nt hairpin structure (underlined) with **a** predicted energy of –126.1 kcal/mol. An (SI) spacer and a single copy of the 8-nt *Gtx* TEE are located downstream of the hairpin structure and are spaced 25-nt upstream of the *Photinus* initiator AUG with β-globin 5' UTR sequence. Cloning into the pYESFFlucH yeast expression vector generates an MfeI/EcoRI junction (CAATTC) and a unique AatII restriction site in the loop of the hairpin.

5' Leader	Description
	AAAAAAAAA *TTCTGACAT* AAAAAAAAA *TTCTGACAT* AAAAAAAAA *TTCTGACAT* AAAAAAAAA *TTCTGACAT* AAAAAAAAA *TTCTGACAT* CAATTC CCAGCGTAATCGGG AACGTCGTAGGGGTAAGCCATTGTACGACCACCGGCTCGAGGGGCCC GACGTC GGGCCCCTCGAGCCGGTGGTCGTACAATGGCTTACCCCTACGACGTTCCCGATTACGC TGG *TTCTGACAT* **CCGGCGGGA** *GACTCACAACCCCAGAAACAGACAT*
p9hp	Five repeats of the 8-nt *Gtx* TEE containing a G*f*C mutation previously shown to eliminate its ability to enhance translation (1)[1] interspersed with a 9-nt spacer based on a segment of the β-globin 5' UTR [nt 9-17 (SI)] upstream of a 128-nt hairpin structure (underlined) with **a** predicted energy of –126.1 kcal/mol. An (SI) poly(A) spacer is located downstream of the hairpin structure and are spaced 25-nt upstream of the *Photinus* initiator AUG with β-globin 5' UTR sequence. Cloning into the pYESFFlucH yeast expression vector generates an MfeI/EcoRI junction (CAATTC) and a unique AatII restriction site in the loop of the hairpin. **CCC*GCGGGA** *TTCTGACAT* **CCC*GCGGGA** *TTCTGACAT* **CCC*GCGGGA** *TTCTGACAT* **CCC*GCGGGA** *TTCTGACAT* **CCC*GCGGGA** *TTCTGACAT* CAATTC CCAGCGTAAT CGGGAACGTCGTAGGGGTAAGCCATTGTACGACCACCGGCTCGAGGGGCCC GACGTC GGGCCCCTCGAGCCGGTGGTCGTACAATGGCTTACCCCTACGACGTTCCC GATTACGCTGG *TTCTGACAT* AAAAAAAAA *GACTCACAACCCCAGAAACAGACAT*
p10hp	Five repeats of the 8-nt *Gtx* TEE containing a G*f*C mutation previously shown to eliminate its ability to enhance translation (1) interspersed with a 9-nt spacer based on a segment of the β-globin 5' UTR [nt 9-17 (SI)] upstream of a 128-nt hairpin structure (underlined) with a predicted energy of -126.1 kcal/mol. An (SI) spacer and a single copy of the mutated 8-nt *Gtx* TEE are located downstream of the hairpin structure and are spaced 25-nt upstream of the *Photinus* initiator AUG with β-globin 5' UTR sequence. Cloning into the pYESFFlucH yeast expression vector generates an MfeI/EcoRI junction (CAATTC) and a unique AatII restriction site in the loop of the hairpin. **CCC*GCGGGA** *TTCTGACAT* **CCC*GCGGGA** *TTCTGACAT* **CCC*GCGGGA** *TTCTGACAT* **CCC*GCGGGA** *TTCTGACAT* **CCC*GCGGGA** *TTCTGACAT* CAATTC CCAGCGTAAT CGGGAACGTCGTAGGGGTAAGCCATTGTACGACCACCGGCTCGAGGGGCCC GACGTC GGGCCCCTCGAGCCGGTGGTCGTACAATGGCTTACCCCTACGACGTTCCC

[1] Please confirm or correct the addition of ref. 1.

5' Leader	Description
	GATTACGCTGG *TTCTGACAT* **CCC*GCGGGA** *GACTCACAACCCCAGAAACAGACAT*
p11hp	Five repeats of the 9-nt poly(A) spacer (SIII) interspersed with a 9-nt spacer based on a segment of the β-globin 5' UTR [nt 9-17 (SI)] upstream of a 128-nt hairpin structure (underlined) with **a** predicted energy of –126.1 kcal/mol. An (SI) spacer and a single copy of the mutated 8-nt *Gtx* TEE previously shown to eliminate its ability to enhance translation (1) are located downstream of the hairpin structure and are spaced 25-nt upstream of the *Photinus* initiator AUG with β-globin 5' UTR sequence. Cloning into the pYESFFlucH yeast expression vector generates an MfeI/EcoRI junction (CAATTC) and a unique AatII restriction site in the loop of the hairpin. AAAAAAAAA *TTCTGACAT* AAAAAAAAA *TTCTGACAT* AAAAAAAAA *TTCTGACAT* AAAAAAAAA *TTCTGACAT* AAAAAAAAA *TTCTGACAT* CAATTC CCAGCGTAATCGG GAACGTCGTAGGGGTAAGCCATTGTACGACCACCGGCTCGAGGGGCCC GACGTC GGGCCCCTCGAGCCGGTGGTCGTACAATGGCTTACCCCTACGACGTTCCCGATTA CGCTGG *TTCTGACAT* **CCC*GCGGGA** *GACTCACAACCCCAGAAACAGACAT*

Gtx sequences are in boldface; spacer sequences based on β-globin 5' UTR are in italics. β-globin 5' UTR numbering based on GeneBank accession no. J00413 for mouse β-globin gene.

1. Dresios, J., Chappell, S. A., Zhou, W. & Mauro, V. P. (2006) *Nat. Struct. Mol. Biol.* **13,** 30-34