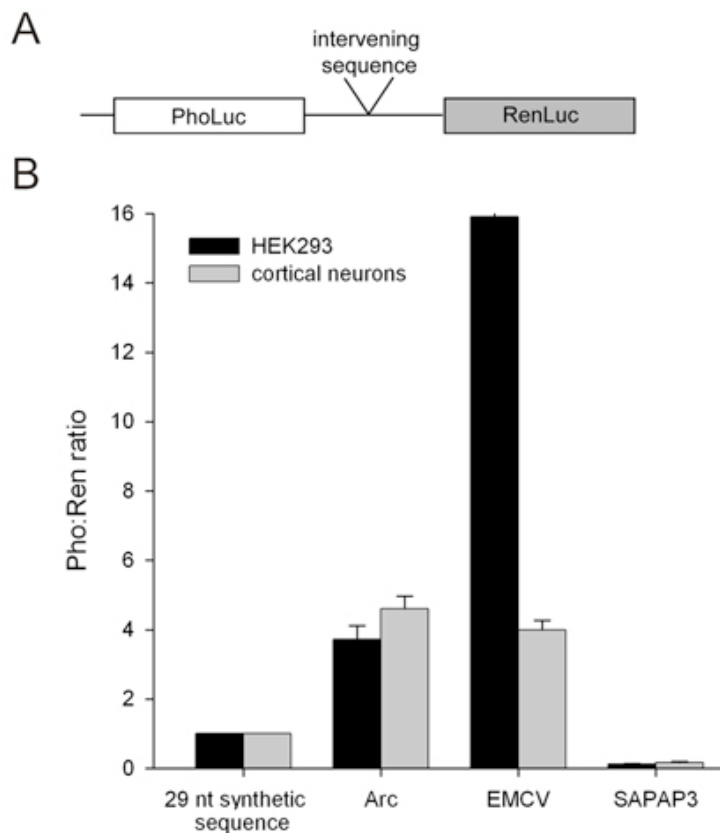


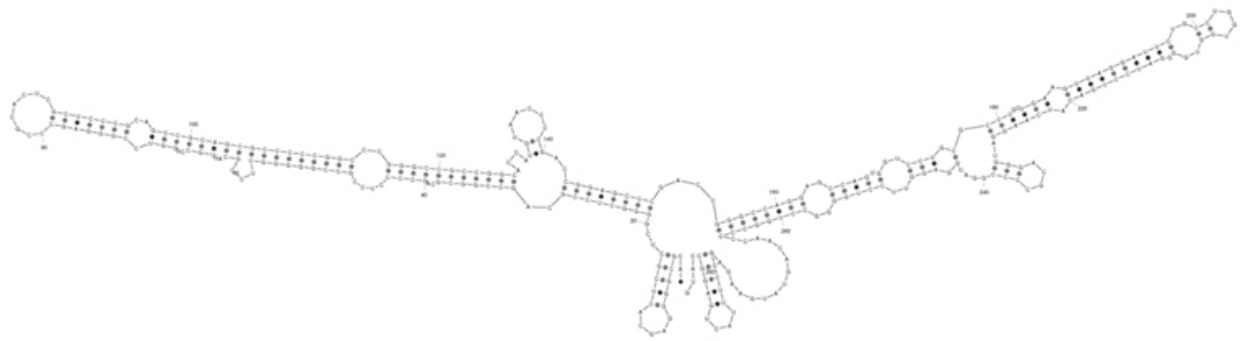
Synthesis of two SAPAP3 isoforms from a single mRNA is mediated via alternative translational initiation

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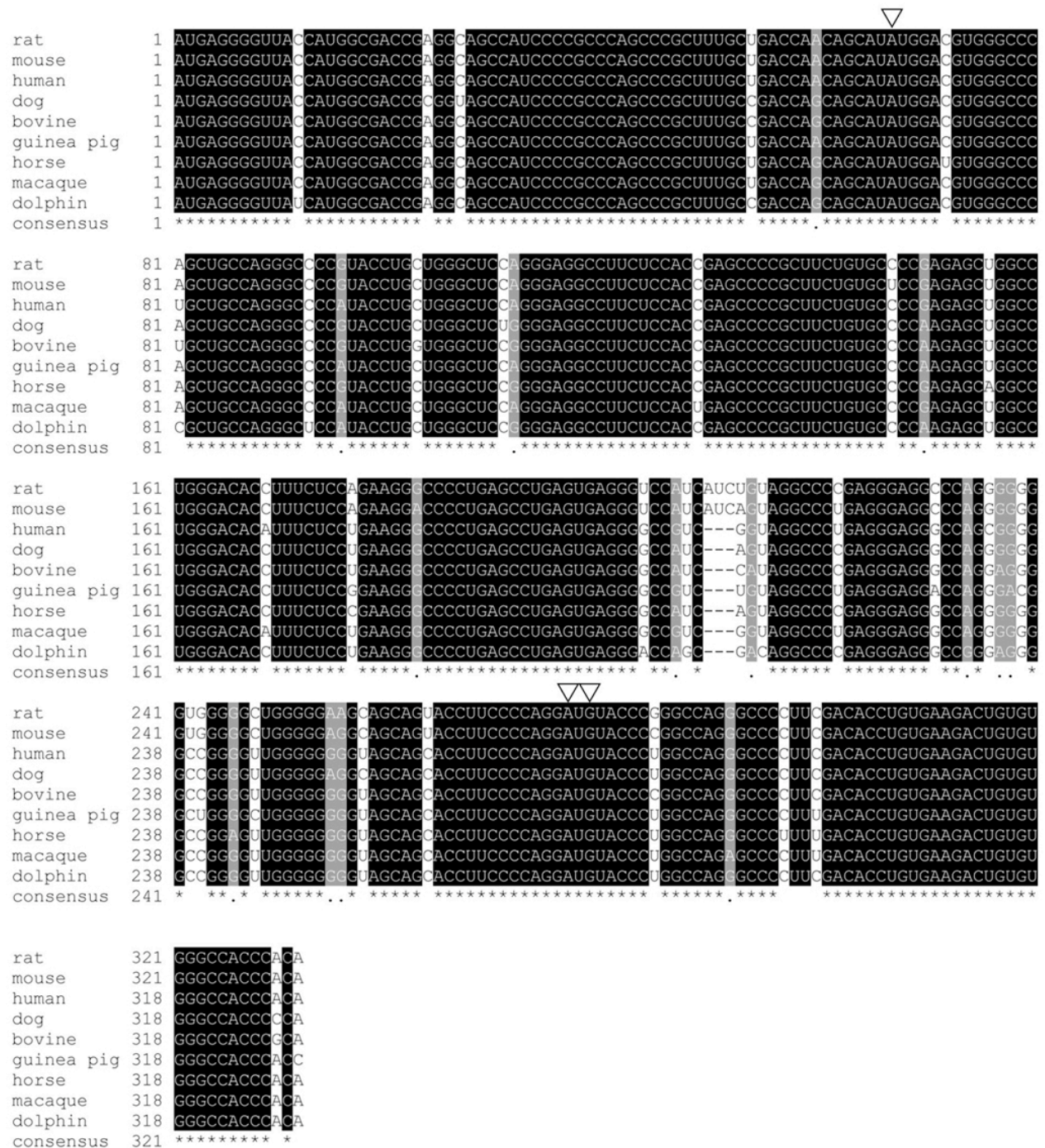
Supplementary information



Supplementary Figure S1 | The S3-5'UTR does not act as an IRES. (A) Schematic representation of bicistronic mRNAs used to test for IRES activity. (B) The S3-5'UTR exhibits no IRES activity. Extracts from transfected HEK293 cells and cortical neurons were assayed for luciferase activities.



Supplementary Figure S2 | Secondary structure of the S3-5'UTR predicted by MFOLD.



Supplementary Figure S3 | The 5' sequences of the SAPAP3 ORF including AUG⁺⁶⁷ and AUG⁺²⁷⁷ are conserved in transcripts from different mammalian species. Alignment of the 5' coding sequence of SAPAP3 mRNAs from rat (GenBank accession number AY530298), mouse (AY243848), human (NM_001080418) and dog (XM_532568). Nucleotides conserved amongst all species are highlighted by a black background. Positions of AUG⁺⁶⁷ (▽) and AUG⁺²⁷⁷ (▽▽) are indicated.

Supplementary Table S1 | 5'UTR sequences of transcripts encoded by different expression vectors.

vector	5'UTR sequence
pEGFP-N3	TCAGATCCGCTAGCGCTACCGGACTCAGATCTCGAGCTCAAGCTTCGAATTCTGCAGTCGACGGTACCGCGGGCCCGGGATCCATCGCCACC
p5'S3-EGFP	TCAGATCCGCTAGCGCTACCGGACTCAGATCTCGAGCTCAAGCTTCGAATTCACTAGTGATTGCGGCCGCTCAGCACTCGCTCGGGGCTCCGCAGCCGGCGCAGCCCCCTCCGCCGGGGGCTCCGCAGTGCCGGGTCCGGGAGCCCGCACTCGCTCCCGCAGCCGAGCGCCCCCGGGCCGCCGGCACCATGTAACCCCGACCCGAGCCCGACCGGCCAGGAGCCAGTGGTCTGCGCCTGCTGAAGTTAGGATTCTGCCTGGTGGGGATCCTGACATCAAGGATGGGACGCCCCGGATGGAGGTTCTGGGGCTGGCCCAACAGTATGAAGAGCCTTTACTGAGGCC
pFS3	TCAGATCCGCTAGCCCTTTACTGGCACC
pS1-FS3	TCAGATCCGCTAGCGAATTCGAGTGACATCTGGTTAACGCCATCAAGGACAGCAACAATCTGAAGTCGGGGTCTCCTGTGTTGGAAGCTCGGAAAAATAAAAACCCAGAGAGTGAGCGTCAAAGGTTACATCTGCTCTGGGGTTTGCTGAGTGTCACCTGAAGGCCCTGTGTCTGCTTCAGAGTGCTGCTGTTGAATGACAAAGACGCTAAGAACAGTCTTGCCCAAGACGGGTCTGGAACCTAACCGGGTTCCCATGACCCCTGTGATTGCTTCCAGTCAGCAGCTAGCCCTTTACTGGCACC
pS3-FS3	TCAGATCCGCTAGCGCGGCCGCTCAGCACTCGCTCGGGGCTCCGCAGCCGGCGCAGCCCCCGGGGGCTCCGCAGTGCCGGGTCCGGGAGCCCGCACTCGCTCCCGCAGCCGCAGCGCCCCCGGCCCGGGCCGCCGGCACCATGTAACCCCGACCCGGAGCCGACCCGGCCAGGAGCCAGTGGTCTGCGCCTGCTGAAGTTAGGATTCTGCCTGTGGTGGGGATCCTGACATCAAGGATGGGACGCCCCGGATGGAGGTTCTGGGGCTGGCCCCAACAGTATGAAGAGGCTAGCCCTTTACTGGCACC
pS3Δ53-124-FS3	TCAGATCCGCTAGCGCGGCCGCTCAGCACTCGCTCGGGGCTCCGCAGCCGGCGCAGCCCCCGGGCCACCATGTAACCCCGACCCGAGCCCGACCCGGCCAGGAGCCAGTGGTCTGCGCCTGCTGAAGTTAGGATTCTGCCTGGTGGTGGGGATCCTGACATCAAGGATGGGACGCCCCGGATGGAGGTTCTGGGGCTGGCCCCAACAGTATGAAGAGGCTAGCCCTTTACTGGCACC
pS3Δ150-FS3	TCAGATCCGCTAGAGACCCGGGCCAGGAGCCAGTGGTCTGCGCCTGCTGAAGTTAGGATTCTGCCTGGTGGTGGGGATCCTGACATCAAGGATGGGACGCCCCGGATGGAGGTTCTGGGGCTGGCCCCAACAGTATGAAGAGGCTAGCCCTTTACTGGCAC
pS3Δ203-FS3	TCAGATCCGCTAGAGTGGTGGGGATCCTGACATCAAGGATGGGACGCCCCGGATGGAGGTTCTGGGGCTGGCCCCAACAGTATGAAGAGGCTAGCCCTTTACTGGCAC
puORF2+3AAG-Δ150-FS3	TCAGATCCGCTAGAGACCCGGGCCAGGAGCCAGTGGTCTGCGCCTGCTGAAGTTAGGATTCTGCCTGGTGGTGGGGATCCTGACATCAAGGAAGGGACGCCCCGGAAGGAGGTTCTGGGGCTGGCCCCAACAGTATGAAGAGGCTAGCCCTTTACTGGCAC
puORF2AAG-S3-FS3	TCAGATCCGCTAGCGCGGCCGCTCAGCACTCGCTCGGGGCTCCGCAGCCGGCGCAGCCCCCGGGGGCTCCGCAGTGCCGGGTCCGGGAGCCCGCACTCGCTCCCGCAGCCGCAGCGCCCCCGGCCCGGGCCGCCGGCACCATGTAACCCCGACCCGGAGCCGACCCGGCCAGGAGCCAGTGGTCTGCGCCTGCTGAAGTTAGGATTCTGCCTGGTGGGGATCCTGACATCAAGGAAGGGACGCCCCGGATGGAGGTTCTGGGGCTGGCCCCAACAGTATGAAGAGGCTAGCCCTTTACTGGCAC
puORF3AAG-S3-FS3	TCAGATCCGCTAGCGCGGCCGCTCAGCACTCGCTCGGGGCTCCGCAGCCGGCGCAGCCCCCGGGGGCTCCGCAGTGCCGGGTCCGGGAGCCCGCACTCGCTCCCGCAGCCGCAGCGCCCCCGGCCCGGGCCGCCGGCACCATGTAACCCCGACCCGGAGCCGACCCGGCCAGGAGCCAGTGGTCTGCGCCTGCTGAAGTTAGGATTCTGCCTGGTGGGGATCCTGACATCAAGGATGGGACGCCCCGGAAGGAGGTTCTGGGGCTGGCCCCAACAGTATGAAGAGGCTAGCCCTTTACTGGCAC

pPhoLuc	TCTGGCTAACTAGAGAACCCACTGCTTACTGGCTTATCGAAATTAATACGACTCACTATAGGGAGACCCAAGCTGGCTAGCGTTTAAACTTAAGCTT
pS353-PhoLuc and pS35-PhoLuc	TAGAGAACCCACTGCTTACTGGCTTATCGAAATTAATACGACTCACTATAGGGAGACCCAAGCTGGCTAGAACGCGGGGAGCACTCGCTCGGGGCTCCGCAGCCGGCGCAGCCCCCTCGCCGGGGCTCCGCAGTGCCGGTCCGGGAGCCCGCACTCGCTCCCGCAGCCGCAGCGCCCCCGGCCGGCCGCCGGCACCATGTAACCCCGACCGGAGCCCGACCGGGCCAGGAGCCAGTGGTCTGCGCTGCTGAAGTTAGGATTCTGCCTGGTGGTGGGGATCCTGACATCAAGGATGGGACGCCCCGGATGGAGGTTCTGGGGCCTGGCCCCAACAGTATGAAGAGCCTTTACTGAGGCCAAGCTT
pS3	TCAGATCCGCTAGCCCTTTACTGAGGCC
pS3-S3	TCAGATCCGCTAGACGCGGGGAGCACTCGCTCGGGGCTCCGCAGCCGGCGCAGCCCTCCGCGGGGGCTCCGCAGTGCCGGTCCGGGAGCCCGCACTCGCTCCCGCAGCCGCAGCGCCCCCGGCCGGCCGCCGGCACCATGTAACCCCGACCGGAGCCCGACCGGGCCAGGAGCCAGTGGTCTGCGCTGCTGAAGTTAGGATTCTGCCTGGTGGTGGGGATCCTGACATCAAGGATGGGACGCCCCGGATGGGGGTTCTGGGGCCTGGCCCCAACAGTATGAAGAGCCTTTACTGAGGCC
pS3Δ150-S3	TCAGATCCGCTAGACCGGGCCAGGAGCCAGTGGTCTGCGCTGCTGAAGTTAGGATTCTGCCTGGTGGTGGGGATCCTGACATCAAGGATGGGACGCCCCGGATGGAGGTTCTGGGGCCTGGCCCCAACAGTATGAAGAGCCTTTACTGAGGCC
pS3Δ203-S3	TCAGATCCGCTAGAGTGGGGATCCTGACATCAAGGATGGGACGCCCCGGATGGGGTTCCTGGGGCCTGGCCCCAACAGTATGAAGAGCCTTTACTGAGGCC
puORF2+3 ^{AAG} -Δ203-S3	TCAGATCCGCTAGAGTGGGGATCCTGACATCAAGGAAGGGACGCCCCGGAAGGGGTTCTGGGGCCTGGCCCCAACAGTATGAAGAGCCTTTACTGAGGCC
puORF2 ^{AAG} -S3	TCAGATCCGCTAGACGCGGGGAGCACTCGCTCGGGGCTCCGCAGCCGGCGCAGCCCTCCGCGGGGGCTCCGCAGTGCCGGTCCGGGAGCCCGCACTCGCTCCCGCAGCCGCAGCGCCCCCGGCCGGCCGCCGGCACCATGTAACCCCGACCGGAGCCCGACCGGGCCAGGAGCCAGTGGTCTGCGCTGCTGAAGTTAGGATTCTGCCTGGTGTGGGGATCCTGACATCAAGGAAGGGACGCCCCGGATGGGGGTTCTGGGGCCTGGCCCCAACAGTATGAAGAGCCTTTACTGAGGCC
puORF3 ^{AAG} -S3	TCAGATCCGCTAGACGCGGGGAGCACTCGCTCGGGGCTCCGCAGCCGGCGCAGCCCTCCGCGGGGGCTCCGCAGTGCCGGTCCGGGAGCCCGCACTCGCTCCCGCAGCCGCAGCGCCCCCGGCCGGCCGCCGGCACCATGTAACCCCGACCGGAGCCCGACCGGGCCAGGAGCCAGTGGTCTGCGCTGCTGAAGTTAGGATTCTGCCTGGTGTGGGGATCCTGACATCAAGGATGGGACGCCCCGGAAGGGGTTCTGGGGCCTGGCCCCAACAGTATGAAGAGCCTTTACTGAGGCC
pΔAUG ⁺¹ -S3	TCAGATCCGCTAGCCAGCCCCGCTTTGCTGACCAACAGCAT
pΔAUG ⁺⁶⁷ -S3	TCAGATCCGCTAGCTACCTTCCCCAGG