

Figure S3. Evolutionary establishment and maintenance of miR-367 targeting of *SPAST* mRNA in primates. **A)** Base-pairing of the *Homo sapiens* (hsa) miR-367 miRNA with the *SPAST* 3'-UTR. The optimal seed motif (green text and yellow shade) for targeting by the miRNA is at position 1,594-1,600 of the *SPAST* 3'-UTR for human, and similarly the optimal seed motif is maintained in chimpanzee, orangutan, and gibbon (see **Fig. S3C**). In macaque and baboon, the *SPAST* target:miR-367 seed pairing includes a U:G pair (see **Fig. S3C**). Additional sequences that can contribute to the *SPAST* target:miR-367 pairing outside the seed are shown by gray shade. **B)** The miRNA seed motif for miR-367 is shared with six other miRNAs, any of which could target the *SPAST* 3'-UTR *in vivo*. At top, the human *SPAST* 3'-UTR sequence that is targeted by miR-367 is displayed, followed underneath by the 7 mature miRNA sequences (from miRBase) that would be capable of targeting the *SPAST* mRNA (yellow shade, miRNA seed and target sequences; gray shade, nucleotides that can contribute to auxiliary pairing, without gaps). **C)** Multisequence alignment in primate species for the boundaries of the Alu element insertion in the *SPAST* 3'-UTR, highlighting the 18-nt target site duplication (TSD; bold blue and underline) and the miR-367 target site (yellow shade, target sequence for optimal miR-367 seed; gray shade, auxiliary pairing). **D)** Multisequence alignment in mammals for the position of the Alu element insertion in the *SPAST* 3'-UTR, highlighting the 18-nt TSD present in catarrhine primates and single copy in the other mammalian species. In **C)** and **D)**: *, conserved in all species; ^, conserved in 13/14 species; +, presence of the Alu element insertion; - (in species name), absence of the Alu element insertion; ##Alu## represents the remaining Alu element sequences, not shown for illustrative purposes; T_n represents the number of T nucleotides at that position. **E)**

Primate phylogenetic tree showing the presence (+) or absence (-) of an Alu repetitive element insertion in the *SPASTIN* 3'-UTR, that includes a miR-367 optimal seed motif for targeting by the miRNA. An * indicates species in which the pairing of miR-367 with its target includes a G:U pair. The putative origin of the Alu element is shown by a red arrow.

A

human *SPAST* 3'-UTR 5'...GCUCUUGUUGCCAGGCUGGA**GUGCAAU**GGC...

|::| ||::| |||

hsa-miR-367 3'-AGUGGUAACGAUUU**CACGUUAA**-5'

macaque *SPAST* 3'-UTR 5'...GCUCUUGUUGCCAGGCUGGA**GUGAAAU**GGC...

|::| ||::| |||

mml-miR-367 3'-AGUGGUAACGAUUU**CACGUUAA**-5'

B

Human *SPAST* 3'-UTR 5'-UCUUGUUGCCAGGCUGGA**GUGCAAU**GGCGC-3'

[hsa-miR-367-3p](#): 44-a**auugcac**uuuagcaauugguga-65

[hsa-miR-25-3p](#): 52-c**auugcac**uugucucggucuga-73

[hsa-miR-32-5p](#): 6-u**auugcac**auuacuaaguugca-27

[hsa-miR-92a-1-3p](#): 48-u**auugcac**uuguccggccugu-69

[hsa-miR-92a-2-3p](#): 48-u**auugcac**uuguccggccugu-69

[hsa-miR-92b-3p](#): 61-u**auugcac**ucguccggccucc-82

[hsa-miR-363-3p](#): 50-a**auugcac**gguaucacugua-71

C

human+ GAATATAGAAGATGCATGATTCTGGGT (T14) GAGACAGAGTTTCGCTCTTGTGGCCAGGCTGGAGTGCAATGGCGC##Alu##CCAGCCAGAAGATGCATGATTCTTAGGAT
 chimpanzee+ GAATATAGAAGATGCATGATTCTGTTT (T17) GAGACAGAGTTTCGCTCTTGTGGCCAGGCTGGAGTGCAATGGCGC##Alu##CCAGCCAGAAGATGCATGATTCTTAGGAT
 orangutan+ GACTATAGAAGATACATGATTCTGGGT (T20) GAGACAGAGTTTCGCTCTTGTGGCCAGGCTGGAGTGCAATGGCGC##Alu##CCAGCCAGAAGATGCATGATTCTTAGGAT
 gibbon+ GAATATGGAAGATGCATGATTCTGGGT (T15) GAGACAGAGTTTCGCTCTTGTGGCCAGGCTGGAGTGCAATGGCGC##Alu##CCGCCAGAAGATGCATGATTCTTAGGAT
 macaque+ GAATATAGAAGATGCATGAAT-CTGG (T5CT6) GAGACGGAGTTTGTCTCTTGTGGCCAGGCTGGAGTGTAATGGCGC##Alu##CCGCCAGAAGAGGCATGATTTTTTAGGAT
 baboon+ GAATATAGAAGATGCATGAAT-CTGG (T5CT6) GAGACGGAGTTTGTCTCTTGTGGCCAGACTGGAGTGTAATGGCAC##Alu##CCGCCAGAAGAGGCATGATTTTTTAGGAT
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D

human+ TTAAAGATTTG---TTTGAATATAGAAGATGCATGATTCT##Alu##AGAAGATGCATGATTCTT-----AGGATCATATGCTGTTTGTAGCCATAAGGTAAATCATGTCT
 chimpanzee+ TTAAAGATTTG---TTTGAATATAGAAGATGCATGATTCT##Alu##AGAAGATGCATGATTCTT-----AGGATCATATGCTGTTTGTAGCCATAAGGTAAATCATGTCT
 orangutan+ TTAAAGATTTG---TTTGACTATAGAAGATACATGATTCT##Alu##AGAAGATGCATGATTCTT-----AGGATCATATGCTGTTTGTAGCCATAAGGTAAATCATGTCT
 gibbon+ TTAAAGATTTG---TTTGAATATGGAAGATGCATGATTCT##Alu##AGAAGATGCATGATTCTT-----AGGATCATATGCTGTTTGTAGCCATAAGGTAAATCATGTCT
 macaque+ TTAAAGATTTG---TTTGAATATAGAAGATGCATGAAT-CT##Alu##AGAAGAGGCATGATTTTTT-----AGGATCATATGCTGCTCGTAGCCATAAAGTAAATCATGTCT
 baboon+ TTAAAGATTTG---TTTGAATATAGAAGATGCATGAAT-CT##Alu##AGAAGAGGCATGATTTTTT-----AGGATCATATGCTGTTCTGAGCCATAAAGTAAATCATGTCT
 marmoset- TTAAAGATTTG---TCTGAATAT-----AGAAGATGCATGATTCTT-----AGGATCATATGCTGTTTGTAGCCATAAGTTAAATCATGTCT
 pig- TTAAAGATTTA---TCTCAACAC-----AGATGTATGATCTCTT-----ACGGTCATATGCTATTTGTAGCCATAACTTAA-TCATGTCT
 cow- TTAAAGATTTA---TCTAAATGT-----AGAAGATGTATGATT-----ATATGCTGTTTGTAGCTATAACTTAAATCATGTCT
 rat- TTAAAGATTTAATACTTCAACAT-----AGAAGATGTATTATTCTT-----AGAATCATGCGCTGTTTTTAGCTGTAATTTAAATCACATCT
 mouse- TTAAAGATTTAATACTTAAATAT-----AGAAGATGGATTATTCTTTTCCTAGGATCATATGCTGTTTATAGCTGTAATTTAAATCATATCT
 sloth- TTAAAGATTTA---TCTAATTT-----AGAAGATGTATGATTCTT-----AGGATCAAGTCTGTTTGTAGCCATTACTTAAATCACATCT
 tarsier- GTAAAGATTTA---TCTAAATAT-----AGAAGATGTATGATTCTT-----AAGATCATATGCTGTTTATAGTCATAATTTAAATTATGTCT
 lemur- TTTACTCTTGC---TCT---TGT---AGAAGACATATAATTCTT-----AAGATCATACACTGTTTGTAGCCATAATTTGAATGATGTCT
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E

