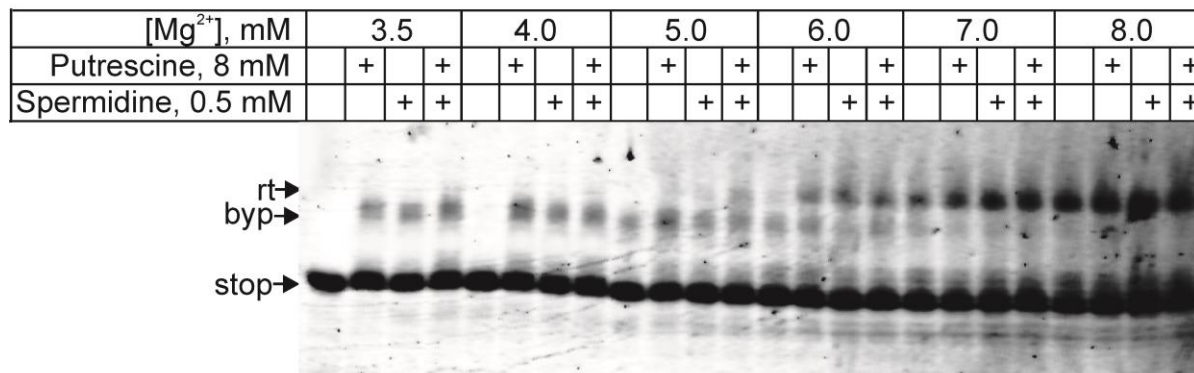
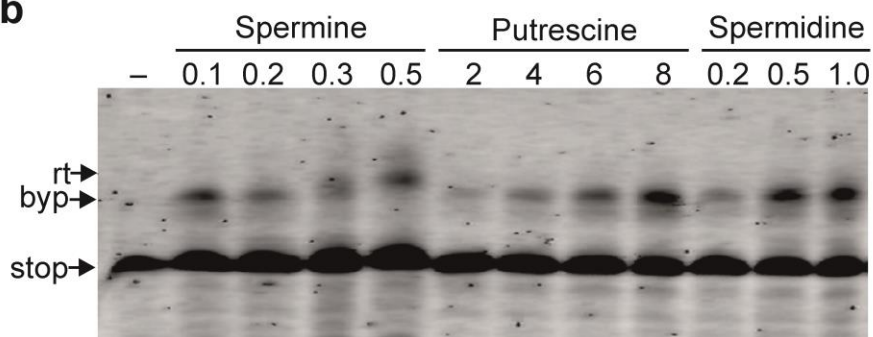


Supplementary Information

a

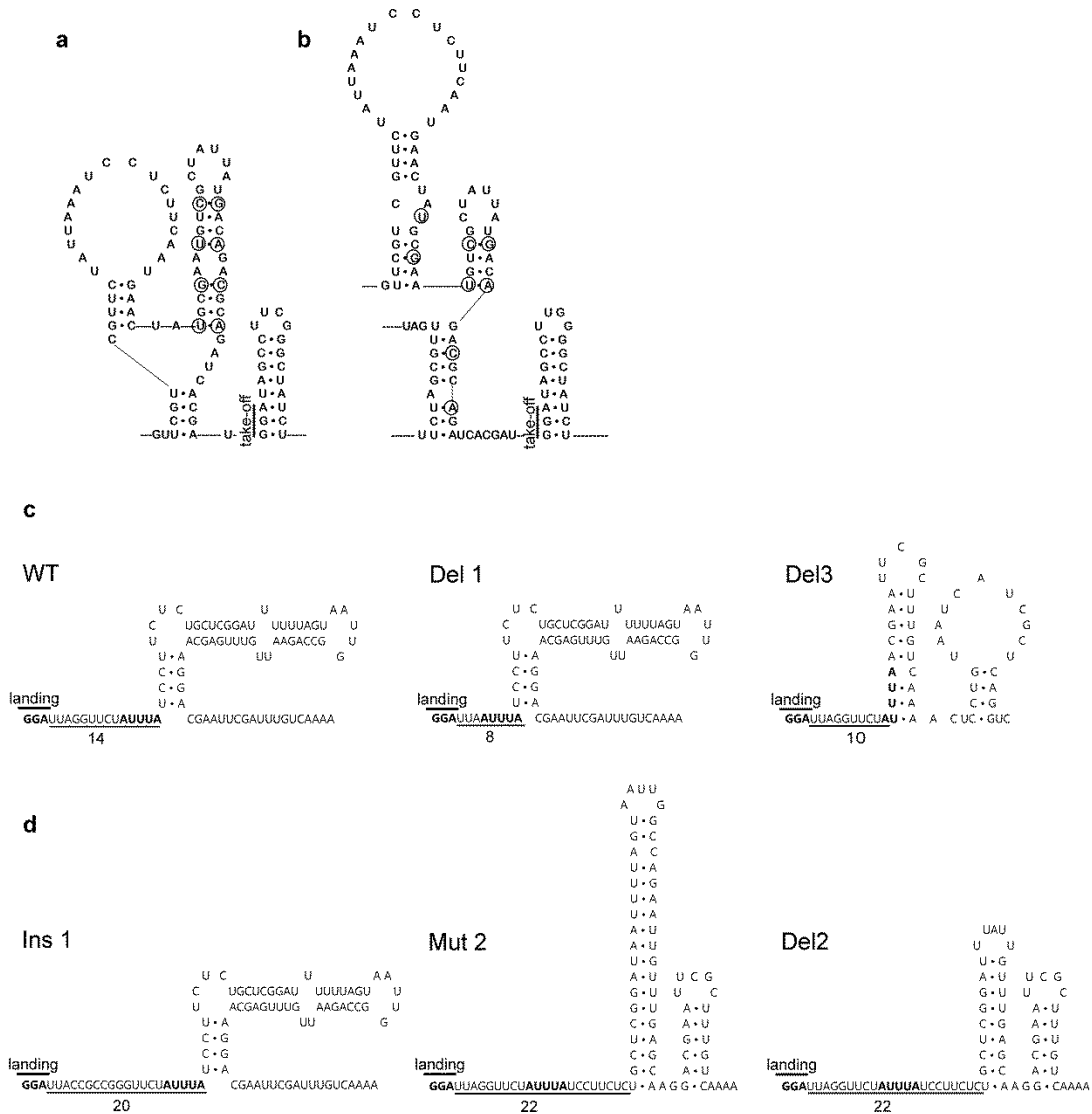


b



Supplementary Figure 1: Effect of polyamines on the efficiency and fidelity of bypassing.

(a) Addition of putrescine and spermidine at different concentrations of added Mg²⁺. Due to the presence of GTP (1 mM) and phosphoenolpyruvate (1 mM), the estimated concentration of free Mg²⁺ is lower than indicated by 1.5 mM¹⁶. (b) Different concentrations of polyamines at constant Mg²⁺ concentration (3.5 mM added, 2.0 mM free).



Supplementary Figure 2. Putative mRNA secondary structures of the 5'SL and 3'SL regions upstream and downstream the gap.

(a) 5'SL forms a long hairpin loop as predicted by mfold and chemical probing¹⁰. (b) Alternative structure of the 5'SL suggested for the Δ gap mRNA¹⁰. (c) mfold predictions for the 3'SL of the wt and various mRNA constructs that support efficient bypassing. (d) mfold predictions for the 3'SL of the mutants that abolish bypassing. Numbers indicate the distance between the landing site and the base of the putative hairpin. The AUUUA motif is shown bold for better comparison between the constructs.

Supplementary Table 1. List of primers used to introduce mutations in mRNA.

	mRNA	Reverse primer	Forward primer
1.	Mut1	GACAAAGCGAATTCGTGCTTGCTCAAACAATTCTGGCC	GGCCAGAATTGTTTGAGCAAGCACGAATTCGCTTTGTC
2.	Del1	TAATCCAATAATCTCTTAATTATGAGG	ATTTATCCTTCTCTGCTCGGATTTTTAGTAATTGGCC
3.	Ins1	GGCCTAATCCAATAATCTCTTAATTATGAGG	GCCGTTCTATTTATCCTTCTCTGCTCGGATTTTTAG
4.	Del2	AAATCCGAGCAGAGAAGGATAAATAGAACC	ATTGTTTGAGCAAGGACGAATTCGC
5.	Mut2	CTGGCCAATTACTAAAATTATCCGAGCAGAGAAGGATAAATAGAACC	GGTTCTATTTATCCTTCTCTGCTCGGATAATTTAGTAATTGGC CAG
6.	Rev1	CATTAACCGGTCTTAACAAACTCGTTCCTTAATAGAACCTAATCCAATA ATCTC	ATTTTTAGGCTCGTCTCTTCTCCTCGAATTCGCTTTGTCAA ACT CCTGTAATCATCGC
7.	Del3	GACAAAGCGAATTCGATAAATAGAACCTAATCC	GGATTAGTTCATTTATCGAATTCGCTTTGTC
8.	(UUC) ₁₇	GAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAATCC AATAATCTCTTAATTATGAGG	TTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCCCT GTAATCATCGCTCAGGTC
9.	2M	CCATCGTGATCCGCATCCGTTATAATAGCGACATTCGC	GCGAATGTCGCTATTATAACGGATGCGGATCACGATGG
10.	2MC	GCCCGAAGGCTATCCATCGTGATCCGCATCCGTTATAATAGCAACGTTT GCGTAGTTCATTGAAGAGG	CCTCTTCAATGAACTACGCAAACGTTGCTATTATAACGGATGC GGATCAGCATGGATAGCCTTCGGGC
11.	4M	GCCCGAAGGCTATCCATCGTGATCCGCATCCGTTATAATAGCGACATTC GCATAGTTCATTG	CAATGAACTATGCGAATGTCGCTATTATAACGGATGCGGATCA CGATGGATAGCCTTCGGGC
12.	4MC	CGTGATCCGCATCCGTTATAATAGCAACGTTGCGTAGTTCATTGAAGA GGATTTAATAGAACG	CGTTCATTAATCCTCTTCAATGAACTACGCAAACGTTGCTAT TATAACGGATGCGGATCAGC
13.	K14E	GCAATTTATATTTTTCCATATCAACGCTAGAAGAATC	GATTCTTCTAGCGTTGATATGGAAAAATATAAATTGC
14.	K15E	GTTCTGCAATTTATATTCTTTCATATCAACGC	GCGTTGATATGAAAGAATATAAATTGCAGAAC
15.	Y16H	CATTGTTCTGCAATTTATGTTTTTTCATATCAACGC	GCGTTGATATGAAAAACATAAATTGCAGAACAATG
16.	K17E	CGAACATTGTTCTGCAATTCATATTTTTTCATATC	GATATGAAAAAATATGAATTGCAGAACAATGTTTCG
17.	L18S	GAACGACGAACATTGTTCTGCGATTATATTTTTTC	GAAAAAATATAAATCGCAGAACAATGTTCTGTCGTTTC
18.	Q19E	GAACGACGAACATTGTTCTCCAATTTATATTTTTTC	GAAAAAATATAAATGGAGAACAATGTTCTGTCGTTTC
19.	N20K	GAACGACGAACATTCTTCTGCAATTTATATTTTTTC	GAAAAAATATAAATGCAGAAGAATGTTCTGTCGTTTC
20.	N21K	GAACGACGAACCTTGTTCTGCAATTTATATTTTTTC	GAAAAAATATAAATGCAGAACAAGTTCTGTCGTTTC
21.	V22D	GAACGACGATCATTGTTCTGCAATTTATATTTTTTC	GAAAAAATATAAATGCAGAACAATGATCGTCGTTTC
22.	R23L	GAAGAGGATTTAATAGAACGAAGAACATTGTTCTGTC	GCAGAACAATGTTCTTCTGTTCTATTAATCCTCTTC
23.	R24L	GAAGAGGATTTAATAGAAAGACGAACATTGTTCTGTC	GCAGAACAATGTTCTGTTCTTCTATTAATCCTCTTC
24.	S25F	GAAGAGGATTTAATAAAGACGACGAACATTGTTCTGTC	GCAGAACAATGTTCTGTTCTTCTATTAATCCTCTTC
25.	I26F	GTTTCATTGAAGAGGATTTAAAAGAACGACGAACATTG	CAATGTTCTGTCGTTCTTTTAAATCCTCTTCAATGAAC
26.	K27I	CGCATAGTTCATTGAAGAGGATATAATAGAACGACG	CGTCTGTTCTATTATATCCTCTTCAATGAACTATGCG
27.	S28F	CATTCGCATAGTTCATTGAAGAGAATTTAATAGAACG	CGTTCATTAATCTCTTCAATGAACTATGCGAATG
28.	S29F	CATTCGCATAGTTCATTGAAAAGGATTTAATAGAAC	GTTCTATTAATCCTTTTCAATGAACTATGCGAATG
29.	S30L	CGACATTCGCATAGTTCATTAAGAGGATTTAATAG	CTATTAATCCTCTTTAATGAACTATGCGAATGTCG
30.	NP4	GAAGAGGATTTAATAGAACGACGAACATCGTTCCTTCAATTCATATCTTT CATATCAACGCTAGAAGAATC	GAAAAAATATAAATGCAGAACAATGTTAGTCGTA CTATTGAAT ATGTTCTGTCGTTCTATTAATCCTCTTC
31.	NP5	GCGACATTCGCATAGTTCATTGAAGTGGATTCAATAGTACGACTAACAT TGTTCTGCAATTTATATTTTTTC	GAAAAAATATAAATGCAGAACAATGTTAGTCGTA CTATTGAAT CCACTTCAATGAACTATGCGAATGTCGC
32.	NP6	GAAGAGGATTTAATAGAACGACGAACGTTATTTGTAACTTGACTTCTT CATATCAACGCTAGAAGAATC	GATTCTTCTAGCGTTGATATGAAGAAGTACAAGTTACAAAATA ACGTTCTGTCGTTCTATTAATCCTCTTC

33.	NP7	GCGACATTTCGCATAGTTTCATAGAGGAAGACTTGATGGAGCGGCGGACA TTGTTCTGCAATTTATATTTTTTC	GAAAAAATATAAATTGCAGAACAATGTCCGCCGCTCCATCAAG TCTTCTCTATGAACTATGCCAATGTCCG
34.	gap -6	GTATTTCTATAGATAGCCCGAAGGCTATCC	ATTAAGAGATTATTGGATTAGGTTCTATTTATCCTTCTC
35.	gap -12	TTTCTATAGATAGCCCGAAGGCTATCC	AAGAGATTATTGGATTAGGTTCTATTTATCCTTCTCTGCTCGG
36.	gap +6 or (+6nt Ins1)or(+6nt unstr)	GGATAAATAGAACCTAATCCAATAATCTCTTAATTATGAGTATGAGGTAT TTCTATAGATAGCC	GGCTATCTATAGAAATACCTCATACTCATAATTAAGAGATTATT GGATTAGGTTCTATTTATCC
37.	gap +12 or (+12nt Ins1)or(+12nt unstr)	TATGAGTATGAGGTATTTCTATAGATAGCCCGAAGGCTATCC	CTCATAATTAAGAGATTATTGGATTAGGTTCTATTTATCC
38.	gap +18 or (+18nt unstr)	GAGTATGAGTATGAGGTATTTCTATAGATAGCCCGAAGGCTATCC	ATACTCATAATTAAGAGATTATTGGATTAGGTTCTATTTATCC
39.	gap +24	TATGAGTATGAGTATGAGGTATTTCTATAGATAGCCCGAAGGCTATCC	CTCATACTCATAATTAAGAGATTATTGGATTAGGTTCTATTTAT CC
40.	gap +30	GAGTATGAGTATGAGTATGAGGTATTTCTATAGATAGCCCGAAGGCTAT CC	ATACTCATACTCATAATTAAGAGATTATTGGATTAGGTTCTATT TATCC
41.	gap +36	TATGAGTATGAGTATGAGTATGAGGTATTTCTATAGATAGCCCGAAGGC TATCC	CTCATACTCATACTCATAATTAAGAGATTATTGGATTAGGTTCT ATTTATCC
42.	gap +42	GAGTATGAGTATGAGTATGAGTATGAGGTATTTCTATAGATAGCCCGAA GGCTATCC	ATACTCATACTCATACTCATAATTAAGAGATTATTGGATTAGGT TCTATTTATCC
43.	gap +6SL	CGAACGCTGTATTTCTATAGATAGCCCGAAGGCTATCC	CGCTATTAAGAGATTATTGGATTAGGTTCTATTTATCC
44.	gap +18SL	CGAACGCTTATGAGTATGAGGTATTTCTATAGATAGCCCGAAG	CGCTATTAAGAGATTATTGGATTAGGTTCTATTTATCCTTCTC
45.	gap +12SL	CGAACGCTTATGAGGTATTTCTATAGATAGCCCGAAGGCTATC	CGCTATTAAGAGATTATTGGATTAGGTTCTATTTATCCTTCTC
46.	Del12nt + 12nt Ins2	GAGAAGGATAAATAGAACCTAATCCAATAATAATAATAATCTCTTTTT CTATAGATAGC	GCTATCTATAGAAAAAGAGATTATTATTATTATTGGATTAG GTTCTATTTATCCTTCTC
47.	+6nt Ins2	GCAGAGAAGGATAAATAGAACCTAATCCAATAATAATAATCTCTTAATTA TGAGGTATTTCTATAG	CTATAGAAATACCTCATAATTAAGAGATTATTATTATTGGATTA GTTCTATTTATCCTTCTCTGC
48.	+12nt Ins2	GCAGAGAAGGATAAATAGAACCTAATCCAATAATAATAATAATAATCTCT TAATTATGAGGTATTTCTATAG	CTATAGAAATACCTCATAATTAAGAGATTATTATTATTATTATTG GATTAGGTTCTATTTATCCTTCTCTGC
49.	mut GAG to AUU	GGATAAATAGAACCTAATCCAATAAATTTAATTATGAGGTATTTCTATA G	CTATAGAAATACCTCATAATTAATTAATTATTATTGGATTAGGTTCT ATTTATCC
50.	mut GAG to CAC	GGATAAATAGAACCTAATCCAATAATGTGTTAATTATGAGGTATTTCTAT AG	CTATAGAAATACCTCATAATTAACACATTATTGGATTAGGTTCT ATTTATCC
51.	mut GAG to GGAGG	GGATAAATAGAACCTAATCCAATAACCTCCTAATTATGAGGTATTTCTAT AG	CTATAGAAATACCTCATAATTAGGAGGTTATTGGATTAGGTTCT ATTTATCC
52.	NP1	CATTATACACCTCATAGAACTTTTTCATCAGGAATCCAACCGCG	GAAAAAATATAAATTGCAGAACAATGTTTCGTCGTTCTATTAATC C
53.	NP2	GGATTTAATAGAACGACGAACATCGTTCTTCAATTCATATTTCTTTTATTA TACACCTCATAGAAC	GTTCTATGAGGTGTATAATGAAAGAATATGAATTGAAGAACGA TGTTTCGTCGTTCTATTTAAATCC
54.	NP3	CTGCAATTTATATTTTTTTCATATGAACGCAAGAAGCATCAACTTTTAGAA ATTTTATTATACACC	GGTGTATAATGAAATTTCTAAAAGTTGATGCTTCTTTCGCTTCAT ATGAAAAAATATAAATTGCAG
55.	T0	CTAAAAATCCGAGCAGAGATCCTTAAATAGAACCTAATCC	GGATTAGGTTCTATTTAAGGATCTCTGCTCGGATTTTTTAG