

SUPPLEMENTARY TABLES AND FIGURES

Table S1. Sequence of primers used for point mutagenesis

Primers with restriction sites:

eRFNde 5'-GAGATATACATATGGCGGACGACCC-3'
eRFBst(rev) 5'-CCATTCTTAAGCGGGCAAACGCAAGG-3'

Mutant primers:

E25A (rev) 5'-GGGCCGCCACCAAGCTC-3'
E25Q (rev) 5'-GGGCCGCCTGCAAGCTC-3'
R28A 5'-ATTGCCAGCGGCCGCTCCAAGCTC-3'
R28K 5'-CCATTGCCCTTGCCGCTCCAAGCTC-3'
G29V (rev) 5'-GCCATTGACGCGGGCC-3'
N30A (rev) 5'-GCTGGTGCCAGCGCCGCGGGC-3'
N30D (rev) 5'-GCTGGTGCCATCGCCGCGGGC-3'
G31Y 5'-ATGCTGGTGTAATTGCCGCG-3'
G31V 5'-ATGCTGGTGACATTGCCGCG-3'
T32A 5'-ATATCATGCTGGCGCCATTGCC-3'
T32S 5'-ATATCATGCTGCTGCCATTGCCG-3'
S33A (rev) 5'-CAATGATATCATGGCGGTGCC-3'
S33T (rev) 5'-CAATGATATCATGGTGGTGCCATT-3'
S36I (rev) 5'-GGAATGATCAATATTATCATGCTGGTGC-3'
S36T(rev) 5'-GGAATGATCAATGTTATCATGCTGGTG-3'
M51L 5'-CATCCGCTAACAGTTTTGCCACTC-3'
M51R 5'-CATCCGCTAAGCGTTTTGCCACTC-3'
E55A 5'-GCAGTTCCAAACGCATCCGCTAAC-3'
E55Q 5'-GCAGTTCCAAACTGATCCGCTAAC-3'
E55R 5'-GCAGTTCCAAACCGATCCGCTAAC-3'
E55D 5'-GCAGTTCCAAAGTCATCCGCTAAC-3'
A59V(rev) 5'-GACTTAATGTTAGATACAGTTCCAAACTCATC-3'
A59S(rev) 5'-GACTTAATGTTAGATGAAGTTCCAAACTCATC-3'
S60V 5'-ACTGCAGTTAACATTAAGTCACGAGTAAACCG-3'
N61A 5'-CTGCATCTGCCATTAAGTCACGAGTAAACCG-3'
N61S 5'-CTGCATCTTCCATTAAGTCACGAGTAAACCG-3'
N61D 5'-CTGCATCTGACATTAAGTCACGAGTAAACCG-3'
I62A 5'-CTGCATCTAACGCGAAGTCACGAGTAAACCG-3'
I62V 5'-CTGCATCTAACGTGAAGTCACGAGTAAACCG-3'
K63E (rev) 5'-GGTTTACTCGTGACTCAATGTTAGATGC-3'
K63A 5'-CTGCATCTAACATTGCCTCACGAGTAAACCG-3'
K63Q 5'-CTGCATCTAACATTCAGTCACGAGTAAACCG-3'
K63R 5'-CTGCATCTAACATTCGCTCACGAGTAAACCG-3'
S64A 5'-CTGCATCTAACATTAAGGCCCGAGTAAACCG-3'
S64D 5'-CTGCATCTAACATTAAGGACCGAGTAAACCG-3'
R65A 5'-GTTTACTGCTGACTTAATGTTAGATGC-3'
N67D (rev) 5'-CTGAAAGGCGGTCTACTCGTGAC-3'
R68A (rev) 5'-GTAAACGCCCTTTCAGTCCTGGG-3'
S70A (rev) 5'-CAGGACTGCAAGGCGGTTTACT-3'
S70T (rev) 5'-CCAGGACTGTAAGGCGGTTTACTC-3'
V71F 5'-TGGCTCCCAGGAATGAAAGGCG-3'
V71A 5'-CTCCAGGGCTGAAAGGCGGTT-3'
G73S (rev) 5'-ACAGATGTAATGGCTGACAGGACTGAAAGG-3'
I75A (rev) 5'-TGTACAGATGTAGCGGCTCCCA-3'
T76A(rev) 5'-GTTGTACAGATGCAATGGCTCCC-3'
V78A (rev) 5'-GTTTGAGTCTTTGTTGTGCAGATGTAATG-3'
Q79E (rev) 5'-AGTTTGAGTCTTTGTTCTACAGATGTAATGG-3'
Q79K (rev) 5'-AGTTTGAGTCTTTGTTTTACAGATGTAATGG-3'
Q80E (rev) 5'-AGTTTGAGTCTTTCTTGTACAGATGTAATGG-3'
R81E (rev) 5'-AGTTTGAGTCTTTGTTGTACAGATGTAATGG-3'
Y96F (rev) 5'-CAA TTG TTC CAC AGA ATA CAA CCA GAC-3'
Y96A (rev) 5'-CAATTGTTCCACAGGCTACAACCA-3'
V101A 5'-GTGGAACAATTGCAACAGAAGAAGG-3'

V101F 5'-TGTGGAACAATTTTCACAGAAGAAGG-3'
E107K 5'-GAAGAAGGAAAAGAAAAAGAAAGTCAACA-3'
E107Q 5'-GAAGAAGGAAAAGCAAAAAGAAAGTCAAC-3'
E107A 5'-GAAGAAGGAAAAGCAAAAAGAAAGTCAAC-3'
V110Y 5'-GAAAGGAAAAGAAATACAACATTGACTTTG-3'
V110I 5'-AGGAAAAGAAAATCAACATTGACTTTGA-3'
V110A 5'-GAAAGGAAAAGAAAAGCCAACATTGA-3'
T122Q 5'-CCAATTAATCAGTCATTGTATTTGTGTGAC-3'
S123F 5'-CCAATTAATACGTTCTTGTATTTGTGTGAC-3'
Y125F 5'-AATACGTCATTGTTTTTGTGTGACAAC-3'
Y125A 5'-AATACGTCATTGGCTTTGTGTGACAAC-3'
L126F 5'-CTGCATTGTATTTCTGTGACAACAAATTCC-3'
C127S 5'-GTATTTGTCTGACAACAAATTCC-3'
C127A 5'-TCATTGTATTTGGCTGACAACAAATTCC-3'
D128R 5'-GTGACAACAAATTCGCTACAGAGG-3'
D128G 5'-GTGTGGCAACAAATTCATACAGAGG-3'
N129A 5'-GTGTGACGCAAAATTCATACAGAGG-3'
N129P 5'-GTGTGACCCTAAATTCATACAGAGG-3'
N129D 5'-CATTGTGTGACGACAATTCATACAG-3'
F131A 5'-GACAACAAAGCCCATACAGAGGCTC-3'
F131Y 5'-GACAACAAATATCATACAGAGGCTC-3'
E134S 5'-ACAAATTCATACATCGGCTCTTACA-3'
E134A 5'-ACAAATTCATACAGCGGCTCTTAC-3'
E134Q 5'-ACAAATTCATACACAGGCTCTTACAG-3'

Table S2. The RF activity of the human eRF1 mutants

Position	Replacement	RF activity, %		
		UAA	UAG	UGA
E25	E25A E25Q	109	73	93
		116	130	109
R28	R28A R28K	73	57	85
		79	58	71
G29	G29V	107	78	102
N30	N30A N30D	94	94	104
		67	59	76
G31	G31Y G31V	52	53	56
		46	43	29
T32	T32A T32S	32	30	75
		75	93	112
S33	S33A S33T	100	90	63
		94	71	46
S36	S36I S36T	0	5	24
		120	86	97
M51	M51L M51R	65	100	85
		120	110	100
E55	E55A E55Q E55R E55D	117	48	90
		127	53	89
		80	20	120
		76	53	76
A59	A59S A59V	68	70	73
		46	36	41
S60	S60V	94	92	72
N61	N61A N61S N61D	64	68	75
		16	20	13
		16	20	6
I62	I62A I62V	3	5	8
		53	48	50
K63	K63A K63E K63Q K63R	22	20	24
		18	13	13
		5	7	13
		97	108	111
S64	S64A	110	92	90
R65	R65A	26	21	32
N67	N67D	2	2	6
R68	R68A	0	5	2
S70	S70A S70T	78	92	42
		60	55	30
V71	V71F V71A	33	77	18
		4	55	28
G73	G73S	83	77	84
I75	I75A	18	14	13
T76	T76A	71	90	61
V78	V78A	100	100	80
Q79	Q79K Q79E	210	220	200
		76	39	57
Q80	Q80E	14	10	15

R81	R81E	7	0	6
Y96	Y96A Y96F	63 106	37 67	73 86
V101	V101A V101F	95 93	90 93	85 94
E107	E107Q E107K E107A	118 122 136	125 146 135	123 133 134
V110	V110I V110A V110Y	100 98 95	99 100 93	92 98 96
T122	T122Q T122Q/S123F/L124M/L126F (QFM_F)	38 18	29 13	47 106
S123	S123F	38	21	31
Y125	Y125F Y125A	92 0	78 3	92 2
L126	L126F	85	77	105
C127	C127S C127A	67 50	62 62	63 63
D128	D128R D128G	157 129	162 105	142 97
N129	N129A N129P N129D N129P/K130Q (DPQ)	32 112 55 21	40 77 45 33	28 142 70 63
F131	F131A F131Y	15 55	13 52	37 62
E134	E134S E134A E134Q	100 99 96	80 82 75	78 94 100

The RF activity of the wild-type eRF1 was considered 100%, error values varied from 5 to 10% for different eRF1 mutants.

The amino acid residues of the N domain important for stop codon decoding are marked in bold.

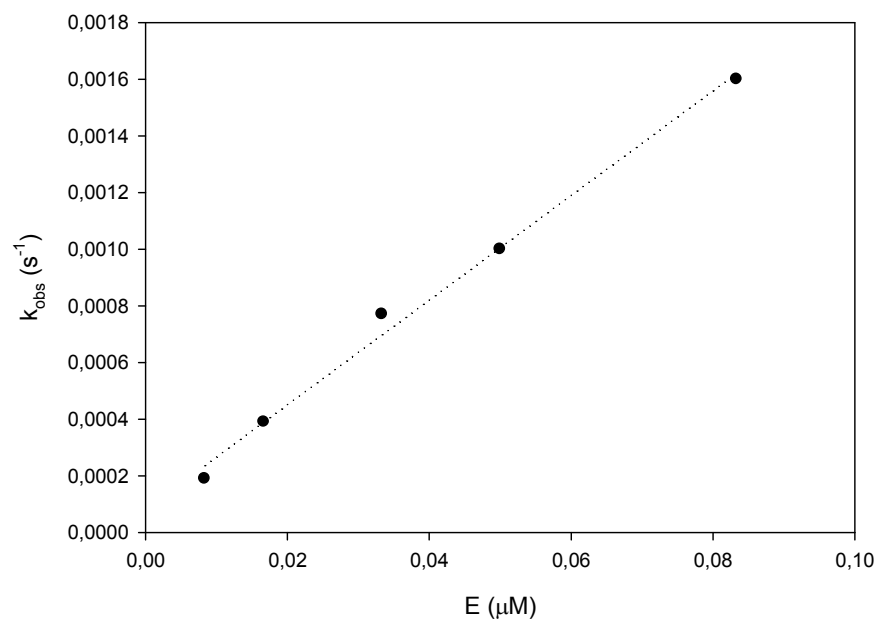


Figure S1. Graph of k_{obs} versus eRF1 concentration (E). k_{obs} was determined in 50 μl termination reaction containing 0.0125 pmol of the preTC and different amounts of eRF1 (0.25 – 4 pmol).

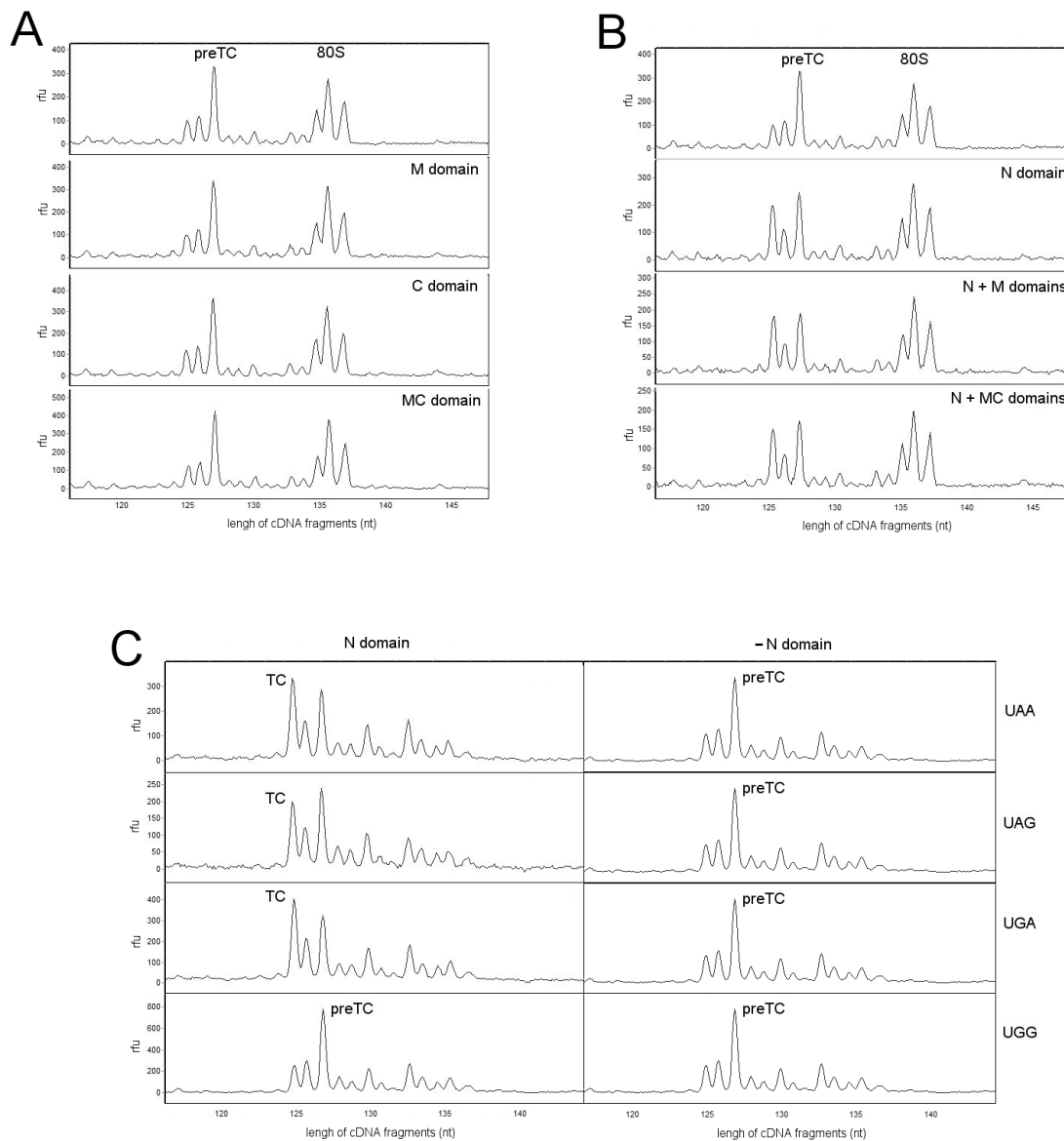


Figure S2. Conformational rearrangement of ribosomal complexes during termination of translation. (A) Toe-prints with M, C, and MC domains of human eRF1 on UAA codon, (B) toe-prints with N, N+M, and N+MC domains of human eRF1 on UAA codon (C) toe-prints with N domain of human eRF1 on different stop codons and UGG codon. cDNAs corresponding to pretermination complex and termination complex have the 127 and 125 nt length, respectively. RFU, relative fluorescence unit.