Supplementary Information to

Schäck et al.: **Eukaryotic life without tQ^{CUG}**: the role of Elongator-dependent tRNA modifications in *Dictyostelium discoideum*

This file contains

Table S1. Oligonucleotides used in this study.

Gene	Application[a]	Name[b]	Primer	Sequence
elp3	Gene deletion	left arm fw	#2043	AGCGCGTCTCCAATGACTAGTGCAGGCGCTAAACCAGTTTGTAAG
		left arm rev	#2044	AGCGCGTCTCCGTTGTTGCTAAATGTGGGCAACGATGTGG
		right arm fw	#2045	AGCGCGTCTCCCTTCGGTTTACTTCGTCTTAGAAAATGTTCAG
		right arm rev	#2046	AGCGCGTCTCCTCCCACTAGTAAGGAAACCTCAAATCATATCTTACTTGG
	Wt detection	outer fw	#2047	TGAATGACCCAAGACCTGTTTC
		inner fw	#2048	GCACGTGATACAAATCGTGGTCAT
		outer fw	#2049	ACCTGGACAATATACACAAATATTACC
		outer rev	#2050	GCCTTCAACAAGCCATGCTCC
	Over expression	N-term fw	#2059	AGAAGATCTATGTCATTAATGAATGACCCAAGACC
		N-term rev	#2106	AGAACTAGTTTAACCAAGATATTTTGAAACGTAAACACC
		C-term fw	#2061	AGAAGATCTTCATTAATGAATGACCCAAGACCTGTT
		C-term rev	#2062	AGAACTAGTTTAACCAAGATATTTTGAAACGTAAACACC
	HAT mutant		p1	AGCGGCTCTTCAATGATGTCATTAATGAATGACCCAAG
			p2	AGCGGCTCTTCGATAAAATTGATGTTGAAATTTAGTTGGATC
			p3	AGCGGCTCTTCGTATTTTACTTTATTAATGGAAGAAGCTG
			p4	AGCGGCTCTTCTCCCACCAAGATATTTTGAAACGTAAAC
	SAM mutant	outer fw	#2205	AGCGGCTCTTCAATGATGTCATTAATGAATGACCCAAG
		rev 1	#2205	AGCGGCTCTTCGTATACTGAAATATTACCAGTCATTGCTAAAT
		fwd 2	#2205	AGCGGCTCTTCGATATTCACCAGGTGGACCAGATTCAG
		outer rev	#2206	AGCGGCTCTTCTCCCACCAAGATATTTTGAAACGTAAAC
ctu1	Gene deletion	left arm fw	#2334	AGCGCGTCTCCAATGACTAGTGACAGGGGGGCGTCATTAACAA
		left arm rev	#2335	AGCGCGTCTCCGTTGTTTGTTGCTGTATTTTCCATTTTATTTATTTATTTGTTTG
		right arm fw	#2336	AGCGCGTCTCCCTTCAGTACTTCAGCAGCAACAACAACAAC
		right arm rev	#2337	AGCGCGTCTCCTCCCACTAGTTCAGATTTACTTGTTTTTGGATCAACTTTAATAATC
	Wt detection	outer fw	#2338	GATGAATTTTAAACAAATATTCAAAGGGGGAG
		inner fw	#2339	TATCCTTACTTTTCTTTCCAACCTAAGTAA
		outer fw	#2340	AGAGGAAAAGATAATAATAATAATAGTAATAATAATGAT
		outer rev	#2341	CTTGATACATTACAAACTTCACATAATTTTGATTTC
trm9	Gene deletion	left arm fw	#2537	AGCGCGTCTCCAATGACTAGTCATGACCATTGATTATCATAGATCTTTTGA
		left arm rev	#2538	AGCGCGTCTCCGTTGTCATCACTAATCTCTGGTACTCCT
		right arm fw	#2538	AGCGCGTCTCCCTTCAGAATGTGAAATGGTTGAAAATAATTTAGATCA
		right arm rev	#2539	AGCGCGTCTCCTCCCACTAGTTCTAGTGAAGTTTGGATTGGTACTTC
	Wt detection	outer fw	#2540	CCAGAAGGTACAGTAGATACAAC
		inner fw	#2541	CAAGATGATACTAATGGTGATGGC
		outer fw	#2542	GAAGAAGATAAAATTATTGAAAAATCAGGTTC
		outer rev	#2543	GGTATTTACCATTACCACAACCAAC
tQ^{CUG}	Gene deletion	left arm fw	#2583	AGCGCGTCTCCAATGACTAGTCTCCTTCATATGCTAATTCTCCACTAC
		left arm rev	#2584	AGCGCGTCTCCGTTGCCTCACATTATCATTTGGACAGATTCCA
		right arm fw	#2585	AGCGCGTCTCCCTTCCAACGAAAAATGCGCAATTTGGATACGT
		right arm rev	#2586	AGCGCGTCTCCTCCCACTAGTCCTGAAAGTTTTTCAATAACAATTTCACTAATTTCC
	Over expression	gen fw hindIII	#2526	AGAAAGCTTGGAATCTGTCCAAATGATAATGTGAG
		gen rev hindIII	#2527	TCTAAGCTTGATTAACATTCTTATTTTAATTTGTGGAGATTAG
	Northern Blot	5' probe		GTCCTACTGAGATTCGAACTC
tQ^{UUG}		gen fw xhoI	#2530	AGACTCGAGCAAATCTAGGTATTGGGCAAAAATAG
		gen rev xhoI	#2529	TCTCTCGAGGTTTTGTTTTATTTTGTTTTTATTTTGGTTTTGTG
	Northern Blot	5' probe		GGTGCTAACCAATTACACTATAAAA
tK^{UUU}	Northern Blot	5' probe		TGCGCTCTACCGACTGAGCT
tE^{UUC}	Northern Blot	5' probe		TTACCGACTACACCAATGAGGA
tG^{GCC}	Northern Blot	5' probe		GGCAGGGATGCATCATACCA

[a] Wt: wildtype

[b] fw: forward, rev: reverse, gen: genomic

Table S2: Elongator proteins in D. discoideum.

Name[a]	Gene ID[b]	ORF size	Protein size	Sequence Identity[c]
Elp1	DDB_G0284075	4286 bp	160 kDa	28%
Elp2	DDB_G0275651	2706 bp	100 kDa	27%
Elp3	DDB_G0290103	1680 bp	63 kDa	70%
Elp4	DDB_G0278783	1320 bp	48 kDa	20%
Elp5	DDB_G0276569	1270 bp	45 kDa	<20%
Elp6	DDB_G0268624	1130 bp	38 kDa	<20%

[a]Elp1-4 are annotated in dictybase.org, while Elp5 and Elp6 were identified by protein blast searches on the NCBI database (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins)

[b]Gene Identity at dictybase.org

[c]Protein sequence identity to the *S. cerevisae* homologue

Table S3: Mass spectrometric parameters for detection of nucleosides.

Name	RT[a] [min]	m/z Precursor	m/z Product	CE[b] [eV]
cm^5U*	1.6	303	171	10
C	1.8	244	112	40
ncm^5U	2.0	302	170	10
Psi	1.6	245	191	10
U	2.5	245	113	40
G	4.1	284	152	40
ncm^5s^2U	4.2	318	186	10
Q	4.4	410	295	10
s^2U	4.5	261	129	10
mcm^5U	5.0	317	185	10
A	5.3	268	136	40
^{15}N dA	5.6	257	141	10
mcm^5s^2U	6.4	333	201	10

[a]RT: retention time

[b]CE: collision energy

*Attention: Due to the similar RT and m/z of cm^5U and ncm^5U, special care is needed during data analysis to avoid integration of ncm^5U instead of cm^5U.

Table S4: Minimal detectable amount of modified nucleoside per tRNA [in %] in a 100 ng tRNA injection.

Modification	LOQ modification per tRNA [%]	LOQ[a] in fmol	LOD[b] in fmol
cm^5s^2U*	1,318	100	10
cm^5U	0,05	2	1
mcm^5s^2U	0,26	10	1
mcm^5U	0,26	10	1
ncm^5s^2U	0,26	10	10
s^2U	0,05	2	10

*not detectable in any of the analyzed tRNA samples.

[a]lower limit of quantification (LOQ) of modified nucleosides from calibration measurements.
[b]Limit of detection (LOD) of modified nucleosides from calibration measurements.

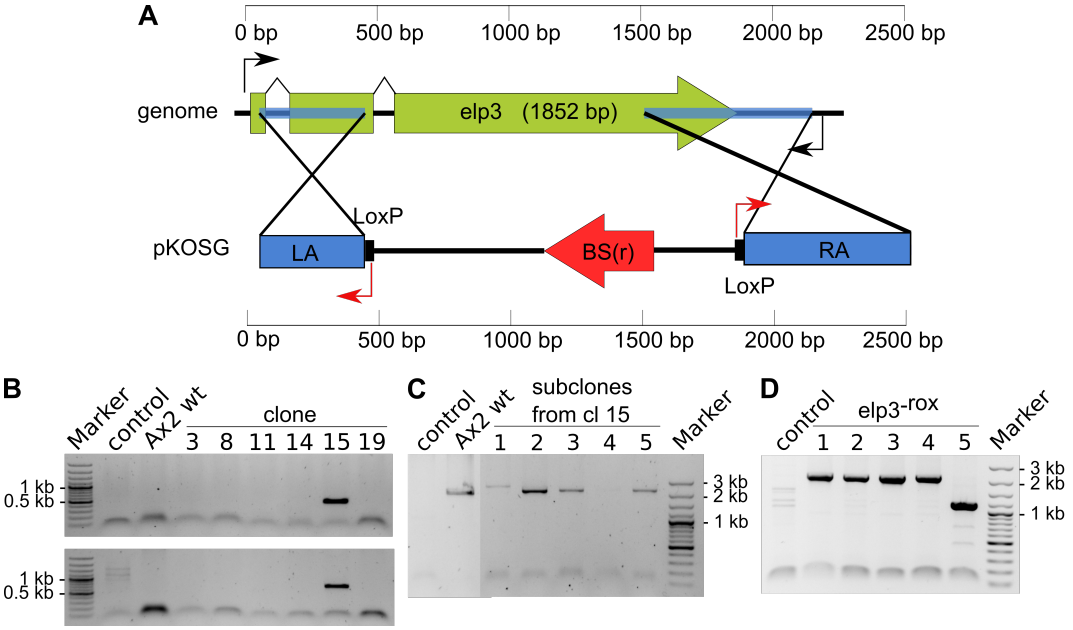

Figure S1. Deletion of the *D. discoideum elp3* gene.
(A) Top: Sketch of the *elp3* gene (green arrow) with two introns in its genomic context. Left (LA) and right arm (RA) for homologous recombination (crosses) are indicated in blue and have sizes of 400 bp and 637 bp, respectively. Bottom: pKOSG construct for homologous recombination featuring a blasticidin S resistance (BS(r)) cassette flanked by LA and RA. The thin black and red arrows represent the binding sites for outer wild type primers and LoxP primers, respectively. Except for primer binding sites, figure is drawn to scale. (B) PCR screening for LA (top) and RA (bottom) integration using primers specific for the genomic locus and the LoxP-sites in the construct. A signal is only expected when integration occurred at the designated genomic locus. (C) PCR Screening over the genomic area using outer wild type primers (black arrows). Upon integration of the pKOSG construct, the amplicon is expected to be 374 bp larger than that of the Ax2 wild type. The picture has been pasted together omitting irrelevant lanes. (D) PCR screening for removal of the BS(r) cassette upon transient expression of Cre recombinase. Successful recombination is expected to result in an amplicon with a size reduced by 1500 bp (clone 5 = elp3^{-rox}). (B-D) Control is a PCR reaction without added DNA template. Marker: GeneRuler 100bp DNA Ladder Plus. PCR products were separated electrophoretically on 1 % (B) or 0.8 % (C, D) agarose gels and visualized by Ethidium bromide staining.

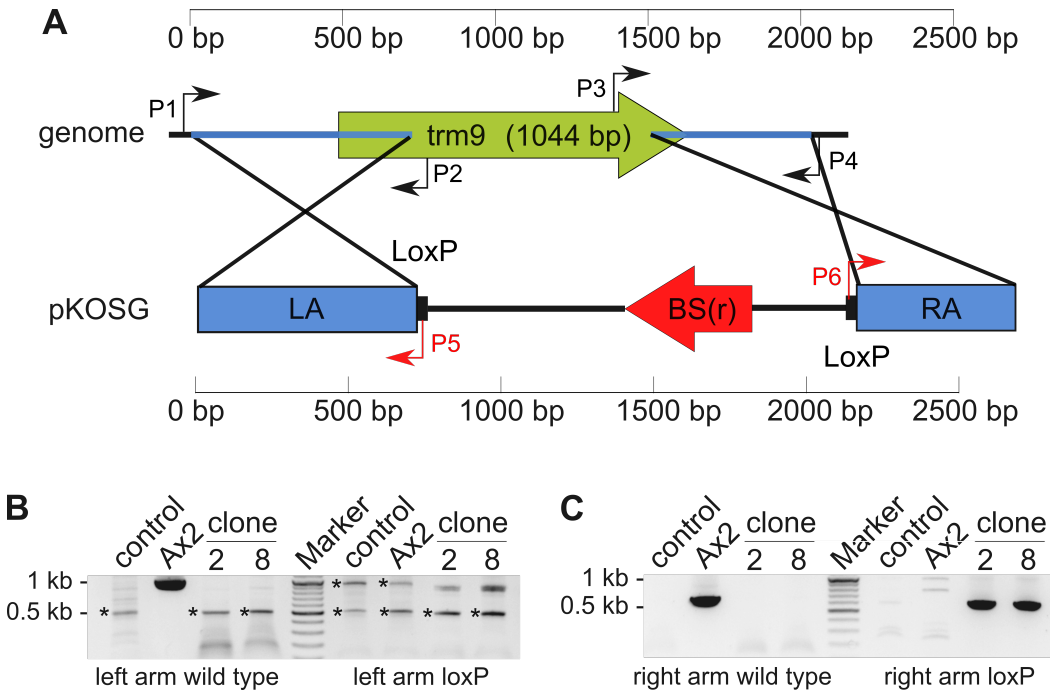

Figure S2. Deletion of the *D. discoideum trm9* gene.

(A). Top: Sketch of the *trm9* gene (green arrow) in its genomic context. Left (LA) and right arm (RA) for homologous recombination (crosses) are indicated in blue and have sizes of 720 bp and 523 bp, respectively. Bottom: pKOSG construct for homologous recombination featuring a blasticidin S resistance (BS(r)) cassette surrounded by LA and RA. P1-P4 (thin black arrows) represent the binding sites for wild type primers and P5 and P6 (red arrows) that for the LoxP primers. Except for primer binding sites, figure is drawn to scale. (B) PCR screening for LA wild type signals using P1 and P2 (left) and for LA pKOSG integration using P1 and P5 (right). (C) PCR screening for RA wild type signals using P3 and P4 (left) and for RA pKOSG integration using P6 and P4 (right). (B-C) Control is a PCR reaction without added DNA template and Ax2 is the wild type. Bands marked with * indicate unspecific PCR products that are also seen in the control without added DNA template. Marker = GeneRuler 100bp DNA Ladder Plus. PCR products were separated electrophoretically on 1 % agarose gels and visualized by Ethidium bromide staining.

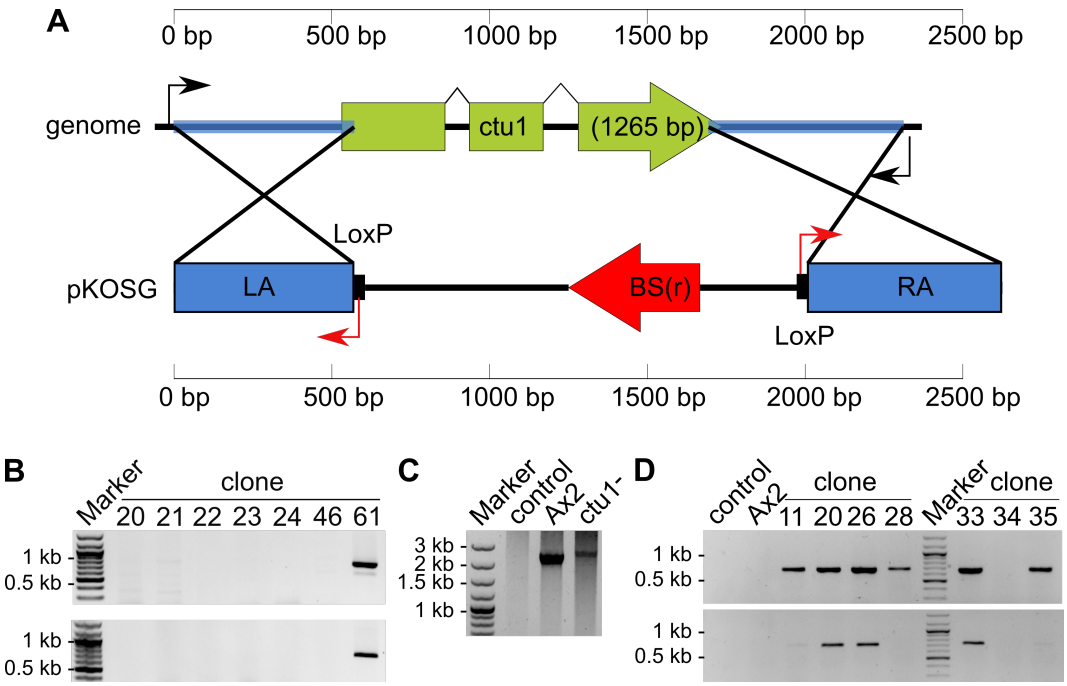

Figure S3. Deletion of the *D. discoideum ctu1* gene in the Ax2 and elp3⁻ strains.
(A) Top: Sketch of the *ctu1* gene (green arrow) in its genomic context. Left (LA) and right arm (RA) for homologous recombination (crosses) are indicated in blue and have sizes of 573 bp and 620 bp, respectively. Bottom: pKOSG construct for homologous recombination featuring a blasticidin S resistance (BS(r)) cassette surrounded by LA and RA. The thin black and red arrows represent the binding sites for outer wild type primers and LoxP primers, respectively. Except for primer binding sites, figure is drawn to scale. (B) PCR screening for LA (top) and RA (bottom) integration using primers specific for the genomic locus and the LoxP-sites in the construct. A signal is only expected when integration occurred at the designated genomic locus. (C) PCR Screening over the genomic area using outer wild type primers (black arrows). Upon integration of the pKOSG construct, the amplicon is expected to be 300 bp larger than that of the Ax2 wild type. (D) PCR screening for pKOSG-ctu1 integration in the elp3^{-rox} background for LA (top) and RA (bottom) integration using primers specific for the genomic locus and the LoxP-sites in the construct. A signal is only expected when integration occurred at the designated genomic locus. (B, D) Control is a PCR reaction without added DNA template. (B-D) Marker = GeneRuler 100bp DNA Ladder Plus. PCR products were separated electrophoretically on 1 % agarose gels and visualized by Ethidium bromide staining.

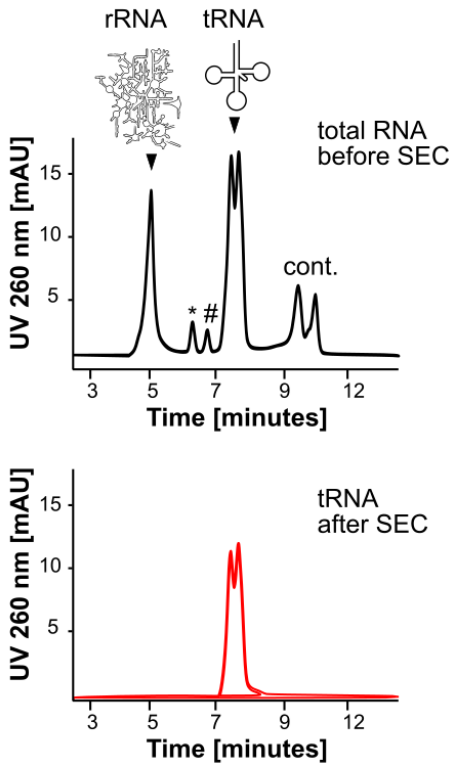

Figure S4. Size exclusion *chromatogram (SEC) of total RNA before SEC purification and tRNA fraction after SEC purification.* Top: Profile of total RNA isolated with TRI on a 300 Å size exclusion column. rRNA is separated from 5.8S rRNA (*), 5S rRNA (#) and tRNA. Later small contaminants like phenol elute from the column (cont.) between minute 10 and 12. Bottom: Profile of purified tRNA fraction after SEC purification as quality control.

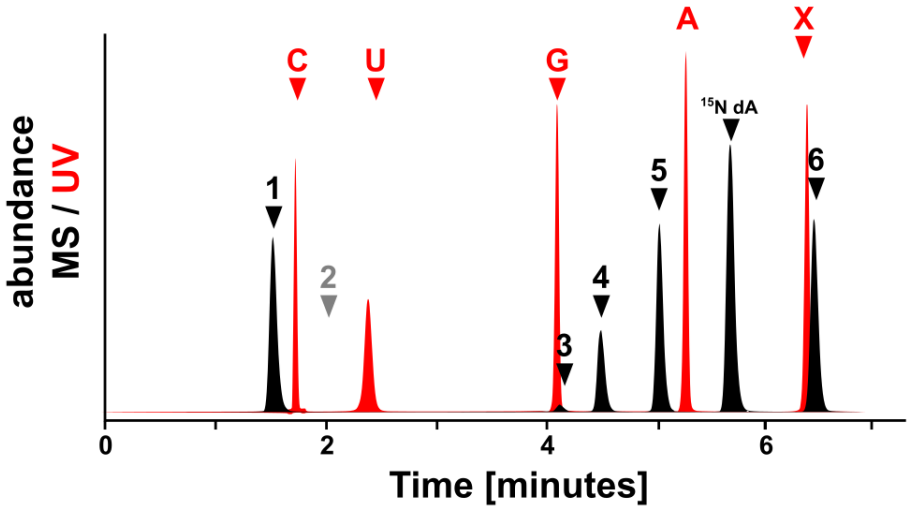

Figure S5. *LC-UV-MS/MS chromatogram of synthetic standards for analysis of modified nucleosides.* Red: UV signal at 260 nm for detection of canonical nucleosides cytidine (C), uridine (U), guanosine (G) and adenosine (A). The external standard theophylline is indicated as X. Black: MS/MS signal of mass transitions of modified uridine derivatives and internal standard ^{15}N-dA (Table S3). 1: cm^{5}U, 2: ncm^{5}U (not available as synthetic standard, position indicated by grey arrow only), 3: ncm^{5}s^{2}U, 4: s^{2}U, 5: mcm^{5}U and 6: mcm^{5}s^{2}U.

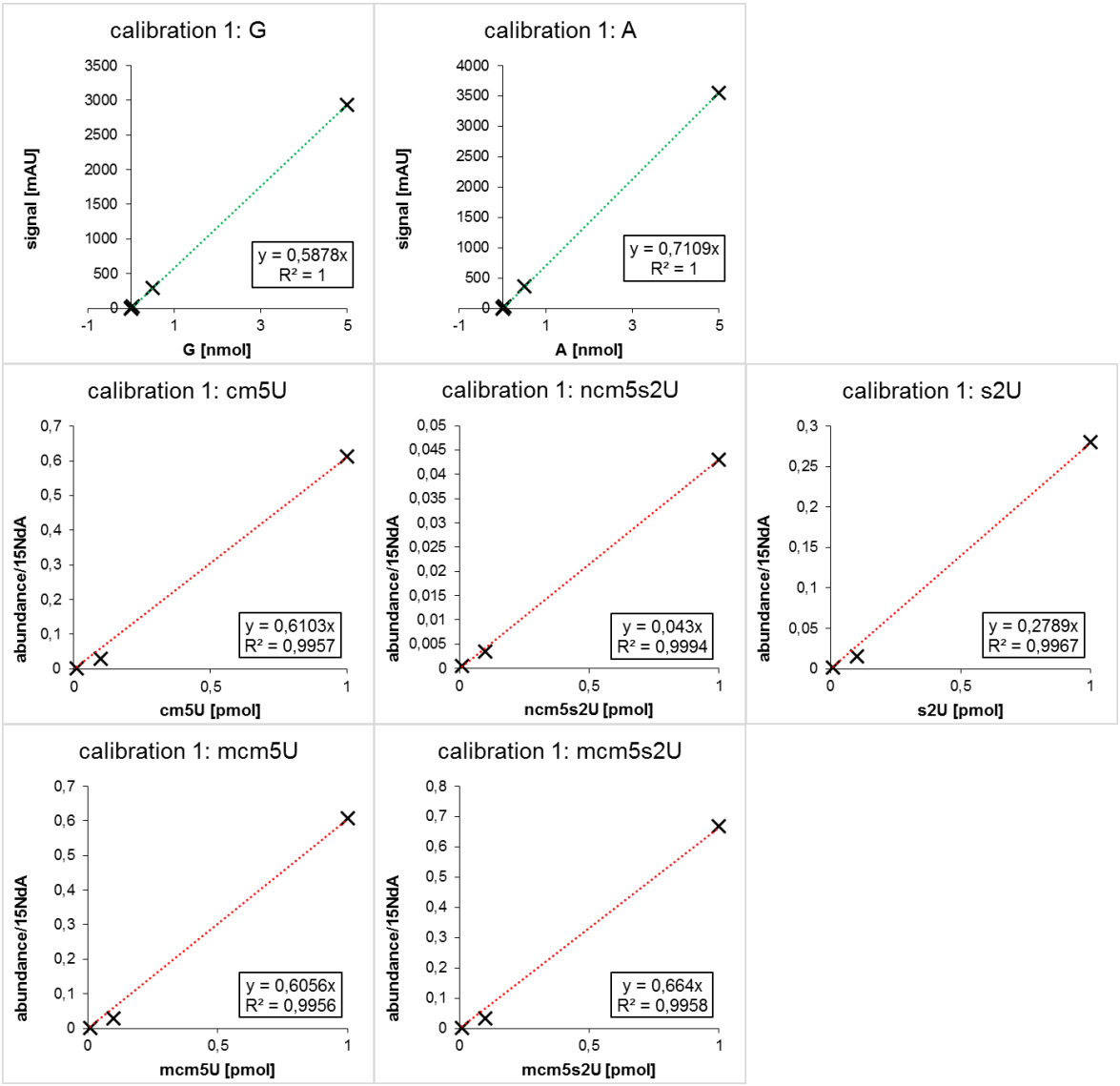

Figure S6. ***Calibration curves for quantification of nucleosides by photometric and mass spectrometric detection.*** Top: Exemplary calibration curve of guanosine (G) and adenosine (A) in the range of 0.5 to 5000 pmol (0.0005 to 5 nmol) at λ 260 nm. Middle: Exemplary calibration curve of cm^5U, ncm^5s^2U and s^2U in the range of 0.01 to 1 pmol at their respective mass transition (Supplemental Table S3). Bottom: Exemplary calibration curve of mcm^5U and mcm^5s^2U in the range of 0.01 to 1 pmol at their respective mass transition (Supplemental Table S3).

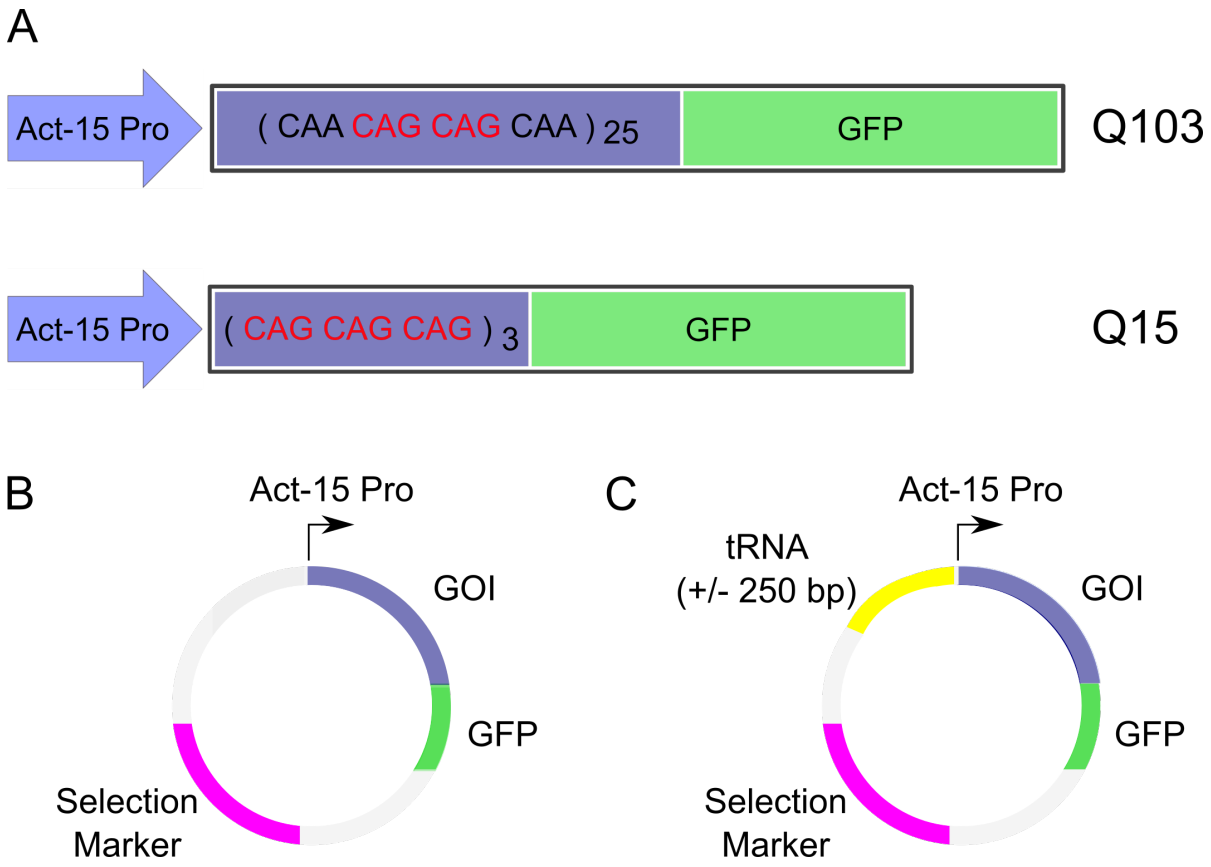

Figure S7. Sketch of poly glutamine leader constructs fused to GFP.
(A) Q103 is the extended human Huntington exon 1 and features a stretch of 103 glutamine codons, of which 50 % are CAA and CAG, respectively, upstream of GFP. Q15 is a synthetic leader construct consisting of 15 CAG codons. Both constructs are expressed from an Actin-15 promoter (Act-15 Pro). The leader constructs (GOI, gene of interest) were expressed from extrachromosomal pDM323 or pDM353 plasmids, either alone (B) or together with tRNA genes (C). For this, the sequence of the tRNA genes (+/- 250 bp genomic surrounding) were cloned in the same plasmid as the leader sequence (C). Please note that constructs are not drawn to scale.