Aging-induced pseudouridine synthase 10 impairs hematopoietic stem cells

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Supplemental Methods

Lentivirus Production and Transduction

The mouse cDNA (*Pus10* or *Pus10*^{D342A}) was cloned into the pRRL-PPT-SF-newMCS-IRES2-EGFP vector. The *Ddb1*-shRNA sequence was cloned into SF-LV-miRE-EGFP vector. Lentivirus was produced in HEK293T cells and concentrated by ultracentrifugation at 25000 rpm for 2.5 h. For lentiviral transduction, LSK (cKit$^+$ Sca1$^+$ Lineage$^-$) cells were sorted and cultured in 96-well plate (~1 × 10^5 cells per well) with 100ul SFEM medium (Stem Cell Technology, 09650) containing 20 ng/ml mSCF, 20 ng/ml mTPO and 1 % penicillin/streptomycin. Lentivirus was added to LSK cells. 72 h later, 2x10^4 GFP$^+$ cells were sorted and injected into lethally irradiated recipients.

Flow Cytometric Analysis and Cell Sorting

Bone marrow cells were harvested from femurs, tibias and pelvis. Viable cells were counted by Vi-CELL XR Cell Viability Analyzer (Beckman Coulter). Bone marrow cells were applied for hematopoietic cell and lineage cell analysis. Hematopoietic cells (antibodies containing CD117, Sca1, Lineage cocktail, CD34, CD150, CD127, CD135 and CD16/32) and lineage cells (antibodies containing CD3, B220 and CD11b) were stained with fluorochrome labeled antibodies and identified by BD LSRFortessa flow cytometer. For Chimerism analysis, red cells in peripheral blood were lysed by ACK buffer (150 mM NH_4Cl, 10 mM $KHCO_3$, 0.1 mM Na_2EDTA, pH7.2-7.4). Lineage cells stained with fluorochrome labeled antibodies (antibodies containing CD3, B220, CD11b, CD45.1 and CD45.2) and analyzed by flow cytometer. Data were analyzed using FlowJo software. For hematopoietic stem and progenitor cells sorting, cKit$^+$ cells were enriched, then stained with antibodies. LSK cells, HSPCs and HSCs were sorted by BD Influx. The antibodies were listed in Table S1.

Transplantations and peripheral blood analysis

2x10^4 GFP$^+$ LSK cells (CD45.2) and 2x10^5 competitor cells (CD45.1) were injected into lethally irradiated (10 Gy) recipient mice (CD45.2). 20 or 150 HSCs (CD45.2) and 3x10^5 competitor cells (CD45.1) were injected into lethally irradiated (10 Gy) recipient mice (CD45.1/2). Peripheral blood of recipients was collected to analyze donor-derived

chimerism (myeloid, B, and T cells) every 4 weeks until the 12^{th} or 16^{th} week.

HSPCs in vitro cultures

50 HSPCs ($CD48^-$ LSK) were sorted into 96-well plate by BD Influx and cultured in SFEM medium containing 20 ng/ml mSCF, 20 ng/ml mTPO and 1 % penicillin/streptomycin for 7 days. Then, the clones were photographed and the cell numbers of these clones were analyzed by BD LSRFortessa flow cytometer and FlowJo software.

Quantification of Ψ by liquid chromatography-tandem mass spectrometry

RNA was extracted and purified using miRNeasy Mini Kit (Qiagen). The RNA was digested into single nucleosides by Nucleoside Digestion Mix (NEB). These nucleosides were detected by a label-free quantitation method. Finally, the Ψ/U ratio was analyzed.

Real-time PCR

Total RNA was isolated using TRIzol reagent (Invitrogen) according to the manufacturer's instruction. Total RNA was reverse transcribed by PrimeScript RT reagent Kit (Takara), followed by RT-PCR using PowerUp™ SYBR™ Green mix (Applied Biosystems) with indicated primers on a QuantStudio-3 Real-time PCR System (Applied Biosystems). The primers were listed in Table S3.

Western Blot

Freshly isolated HSPCs or 32D cells were lysed in sodium dodecyl sulfate (SDS) loading buffer, sonicated for 5 cycles using Bioruptor (Diagenode) and denatured by boiling at 100℃ for 5 min. Samples were resolved on 10% SDS-PAGE, and the separated proteins were transferred onto a PVDF membrane. Membranes were blocked with 5% skimmed milk in Tris-buffered saline with Tween-20 (TBST) buffer for 1 h at room temperature and then probed with primary antibodies overnight at 4 °C.

32D cells were harvested and lysed with NETN buffer (20 mM Tris-HCl, pH 8.0, 100 mM NaCl, 1 mM EDTA, 0.5% Nonidet P-40, containing protease and phosphatase inhibitors cocktail) on ice for 30 minutes followed by centrifugation at 12000 rpm for 5 min. The supernatant was mixed with 2× protein loading buffer and denatured by boiling at 100 ℃ for 5 min. Samples were loaded onto 10% SDS-PAGE gel, and the

separated proteins were transferred onto a PVDF membrane. Membranes were blocked with 5% skimmed milk in Tris-buffered saline with Tween-20 (TBST) buffer for 1 h at room temperature and then probed with primary antibodies overnight at 4 °C.

Affinity Purification and Mass Spectrometry

HEK293T cells stably expressing Flag-tagged PUS10 were lysed in NETN buffer on ice for 30 min followed by centrifugation at 12000 rpm for 5 min. The supernatant was transferred to a fresh microcentrifuge tube and incubated with anti-Flag beads at 4 ℃ for 4 hr. Beads were washed with NTEN buffer 3 times and boiled in 30 μL 2 x protein loading buffer. Samples were loaded onto 10% SDS-PAGE gel and analyzed by mass spectrometry.

Co-Immunoprecipitations

Plasmids encoding SFB-tagged PUS10 or Myc-tagged DDB1, DCAF1, CUL4B were co-transfected into HEK293T cells. 24 hr later, the transfected HEK293T cells were harvested and lysed with NETN buffer on ice for 30 minutes followed by centrifugation at 12000 rpm for 5 min. The supernatant was transferred to a fresh microcentrifuge tube and incubated with 20 μL S-protein beads at 4 ℃ for 2 hr. Beads were washed with NETN buffer 3 times, mixed with 2× protein loading buffer and denatured by boiling. Samples were loaded onto 10% SDS-PAGE gel, and the separated proteins were transferred onto a PVDF membrane. Membranes were blocked and then probed with anti-Flag, anti-Myc antibodies.

Ubiquitination assay

Plasmids encoding SFB-tagged PUS10, Myc-tagged DDB1, DCAF1, CUL4B and HA-tagged Ub-WT, Ub-K48R were co-transfected into HEK293T cells. 24 hr later, the transfected HEK293T cells were harvested and lysed with 100 μL denaturing TS buffer (50 mM Tris-HCl, pH 7.5, 1% SDS, 5 mM N-ethymaleimide). The lysates were boiled at 100 ℃ for 10 min and sonicated for 5 cycles using Bioruptor (Diagenode). The lysates were diluted with 900 μL TNN buffer (0.5% Nonidet P-40, 50 mM Tris-HCl, pH 7.5, 250 mM NaCl, 5 mM N-ethymaleimide) followed by centrifugation at 12000 rpm for 10 min. The supernatant was transferred to a fresh microcentrifuge tube and incubated with 20 μL S-protein beads at 4 ℃ for 2 hr. Beads were washed with NETN

buffer 3 times, mixed with 2× protein loading buffer and denatured by boiling. Samples were loaded onto 10% SDS-PAGE gel, and the separated proteins were transferred onto a PVDF membrane. Membranes were blocked and then probed with anti-HA, anti-Flag, anti-Myc antibodies.

Blood Cell Counts

Peripheral blood was collected from mice and analyzed using an Auto Hematology Analyzer BC-5000 (MINDRAY).

Statistical analysis

Data are shown as mean ± SD. Student's t test (Two-tailed unpaired) was used for comparisons between the groups using GraphPad Prism 6.0 software.

Supporting Tables

Table S1 Key resources table

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Ter-119-Biotin	BioLegend	TER-119
Gr-1-Biotin	BioLegend	RB6-8C5
CD11b-Biotin	BioLegend	M1/70
CD11b-PerCP-Cy5.5	BioLegend	M1/70
CD3e-Biotin	BioLegend	145-2C11
CD3e-APC	BioLegend	145-2C11
CD4-Biotin	BioLegend	GK1.5
CD8a-Biotin	BioLegend	53-6.7
B220-Biotin	BioLegend	RA3-6B2
B220-V500	BioLegend	RA3-6B2
B220-Pacific Blue	BioLegend	RA3-6B2
Sca-1-PE-Cy7	BD Biosciences	D7
CD117-APC	BD Biosciences	2B8
CD150-PE	BioLegend	TC15-12F12.2
CD48-FITC	BioLegend	HM48-1
CD48-PerCP-Cy5.5	BioLegend	HM48-1
CD34-AlexaFlour700	eBioscience	RAM34
CD34-FITC	eBioscience	RAM34
CD135-CF594	BD Biosciences	A2F10.1
CD135-PE	BD Biosciences	A2F10.1
CD16/32-FITC	BD Biosciences	2.4G2
CD127-BV421	BD Biosciences	A7R34
CD45.1-FITC	BD Biosciences	A20
CD45.1-PE	BD Biosciences	A20
CD45.1-AlexaFluor700	BD Biosciences	A20
CD45.2-FITC	BD Biosciences	104
CD45.2-PE	BD Biosciences	104
CD45.2- PerCP-Cy5.5	BD Biosciences	104
Streptavidin-APC-Cy7	BioLegend	
PUS10	Abcam	ab185078
Myc	Biodragon	B1002
Flag	Cell Signaling Technology	2368S
HA	Cell Signaling Technology	3724S
CUL4B	Abclonal	A12696
DDB1	Abcam	Ab109027
H3	Cell Signaling Technology	4499
H4	Cell Signaling Technology	13919
GAPDH	Biodragon	B1034

Actin	Cell Signaling Technology	4970
Rabbit anti-mouse IgG (HRP conjugate)	Cell Signaling Technology	58802
Mouse anti-rabbit IgG (HRP conjugate)	Cell Signaling Technology	93702
Chemicals, Peptides, and Recombinant Proteins		
SCF	Peprotech	#250-03
TPO	Peprotech	#315-14
DMSO	Sigma-Aldrich	D2650
Fetal Bovine Serum	GEMINI	900-108
DMEM	Gibco	C11995500BT
StemSpan serum-free medium	Stem Cell Technologies	09650
DAPI	Sigma–Aldrich	D8417
Penicillin–streptomycin	Gibco	15140122
D-Hanks	Solarbio	H1045
Hepes	Solarbio	H1095
PBS	Solarbio	P1022
TRIzol	Invitrogen	15596018
Critical Commercial Assays		
PrimeScript RT Reagent Kit	Takara	RR047A
PowerUp SYBR Green mix	Applied Biosystems	A25780
miRNeasy Mini Kit	Qiagen	217004
RNeasy MinElute Cleanup Kit	Qiagen	74204
RNase-Free DNase Set	Sangon Biotech	B618253
Deposited Data		
Small RNA DM–Ψ-seq data		
Experimental Models: Cell Lines		
HEK293T	ATCC	
Experimental Models: Organisms/Strains		
Mouse: *Pus10*$^{-/-}$ mice	Mo Li Laboratory	N/A
Mouse: C57BL/6 (CD45.2)	Jackson Laboratory	N/A
Mouse: C57BL/6-SJL (CD45.1)	Jackson Laboratory	N/A
Oligonucleotides		
Primers for mouse genotyping and qRT-PCR, see Table S3	This paper	N/A
Recombinant DNA		
pRRL-PPT-SF-newMCS-IRES2-EGFP (Vector)	This paper	N/A
pRRL-*mPus10*	This paper	N/A
SF-LV-miRE-EGFP (Vector)	This paper	N/A
miRE-*Ddb1*-shRNA1	This paper	N/A
miRE-*Ddb1*-shRNA2	This paper	N/A

SFB-*hPus10*	This paper	N/A
HA-Ub-WT	Yuancai Liu Lab	N/A
HA-Ub-K48R	Yuancai Liu Lab	N/A
Myc-hDdb1	Yeguang Chen Lab	N/A
Myc-hDcaf1	This paper	N/A
Myc-hCul4b	Qiang Ding Lab	N/A
Software and Algorithms		
FlowJo Software	Becton, Dickinson and Company	N/A
GraphPad Prism 6	GraphPad Software	N/A
Adobe Illustrator CS6	Adobe	N/A
Adobe Photoshop CS5	Adobe	N/A

Table S2 The sequence of *Pus10*-gRNA and *Ddb1*-shRNA

Gene	Sequence	Application
Pus10-gRNA A1	TAGGTGCTTGTTCTCCTCAGTCAG	*Pus10*$^{-/-}$ mice
Pus10-gRNA A2	AAACCTGACTGAGGAGAACAAGCA	*Pus10*$^{-/-}$ mice
Pus10-gRNA B1	TAGGGCACAGCTGTTGTTCAGTTC	*Pus10*$^{-/-}$ mice
Pus10-gRNA B2	AAACGAACTGAACAACAGCTGTGC	*Pus10*$^{-/-}$ mice
Ddb1-shRNA1	TAGCATGAGAACTCTTGTCTGG	Knockdown DDB1
Ddb1-shRNA2	TAGGTCTCTAGTGAACTGGTTT	Knockdown DDB1

Table S3 Primers for genotyping and RT-PCR

Gene	Forward	Reverse	Application
Pus10	CAGCACGTAGCTGTAGAATACTG	GTTTGTAAGGTGCGGGAAGA	Genotyping
Pus10	TATTACGAAGGTGTGCCAAAAGG	GGACTACATCATTTCTTCCCAGG	RT-PCR
Actin	GTGACGTTGACATCCGTAAAGA	GCCGGACTCATCGTACTCC	RT-qPCR

Supplemental Figure Legends

Supplemental figure 1. Aging-activated PUS10 impairs the reconstitution capacity of HSPCs independently on its enzymatic activity.

(A) Representative western blot shows the efficient overexpression of PUS10 in lineage$^-$ cells with lentivirus carrying cDNA of *Pus10*. (B) This histogram depicts the protein level of PUS10 in lineage$^-$ cells with lentivirus carrying cDNA of *Pus10* from quantitative western blot data (n = 5). (C) The gating strategies for the frequency of indicated donor-derived GFP$^+$ cells. (D) The gating strategies for quantifying lineage distribution of the test donor-derived GFP$^+$ cells (B, T, myeloid cells).

Supplemental figure 2. No difference of pseudouridine modification profile between young and aged HSPCs.

Schematic of identified pseudouridine sites are marked in individual tRNA. Pseudouridine sites (red arrows) and levels of individual tRNA are identified in young and aged HSPCs. The x axis represents nucleotide position. The y axis represents pseudouridine levels.

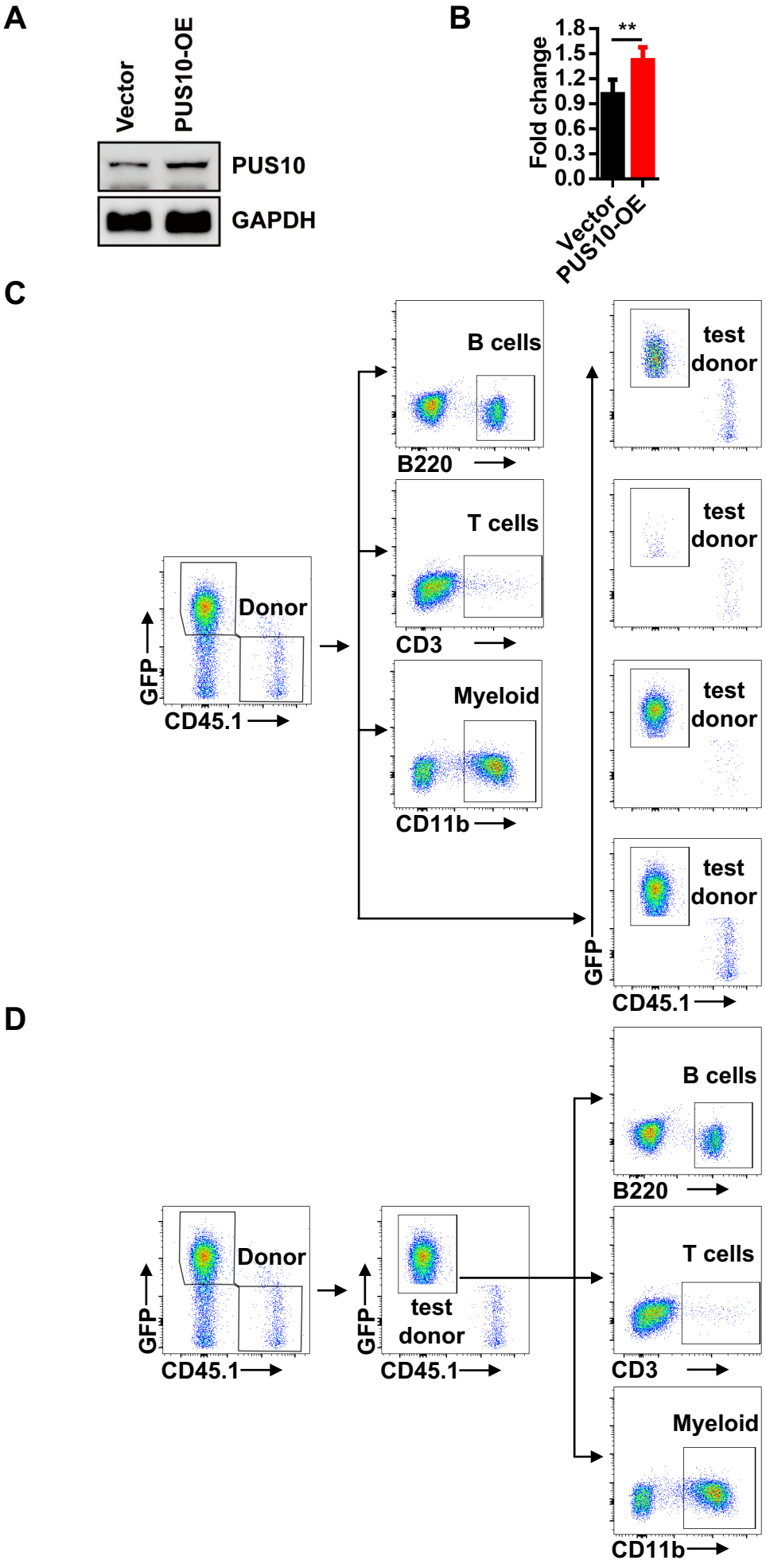
Supplemental figure 3. Aging-declined CRL4^{DCAF1}-mediated ubiquitination degradation signaling leads to the increase of PUS10.

The expression of *Pus10* between young and old HSCs in the GSE27686, GSE39553, GSE4332 and GSE6503 datasets.

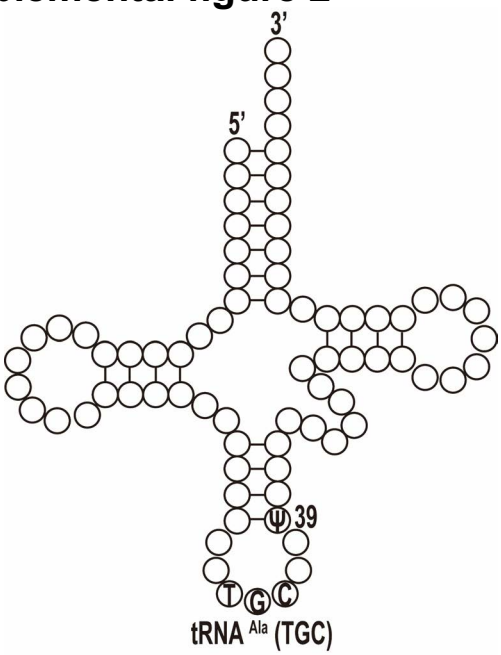
Supplemental figure 4. Young *Pus10*$^{-/-}$ mice exhibit no influence on hematopoietic homeostasis and HSC function.

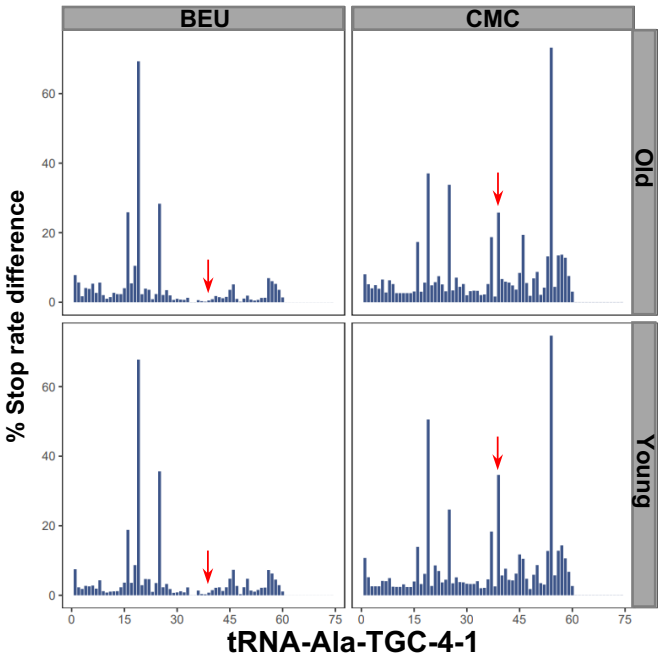
(A) The gating strategies for the frequency of B, T, myeloid cells in PB and BM of WT and *Pus10*$^{-/-}$ mice. (B) The gating strategies for quantifying the progenitors and HSCs in WT and *Pus10*$^{-/-}$ mice. (C) The gating strategies for the frequency of indicated donor-derived CD45.2$^+$ cells. (D) The gating strategies for quantifying lineage distribution of the test donor-derived CD45.2$^+$ cells (B, T, myeloid cells).

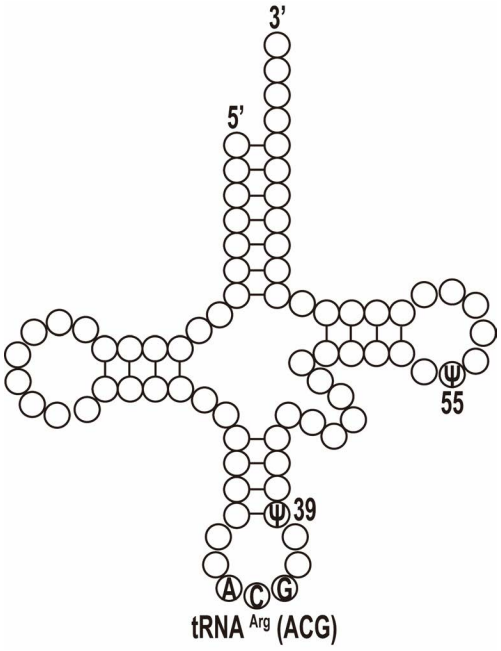
Supplemental figure 1

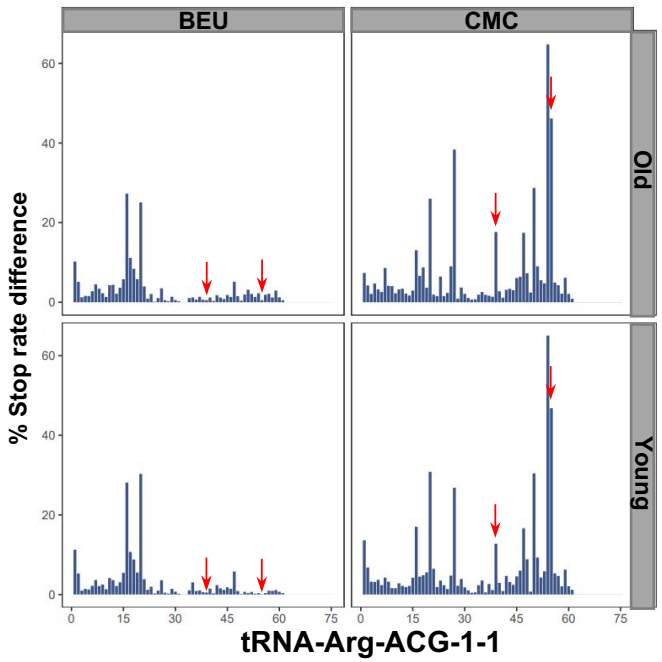


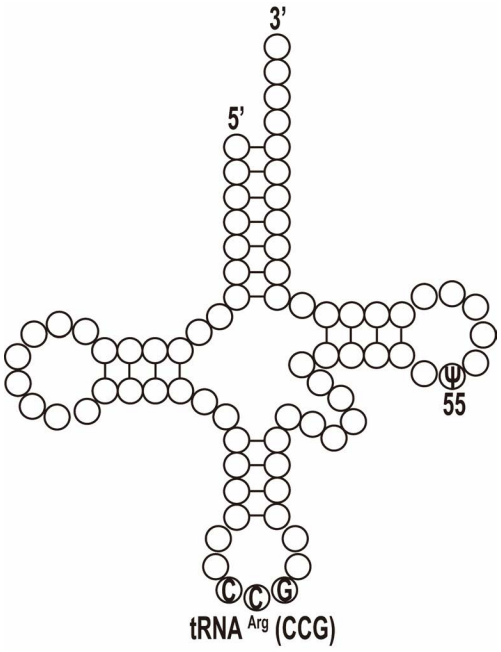
Supplemental figure 2

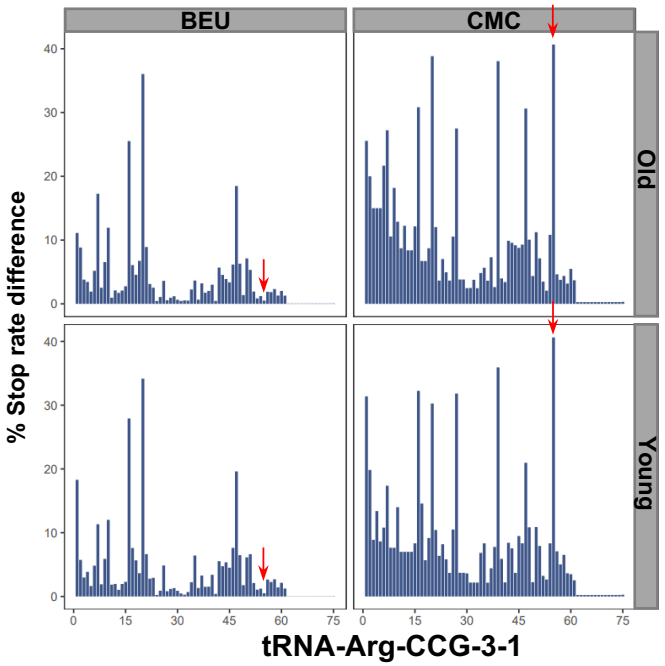


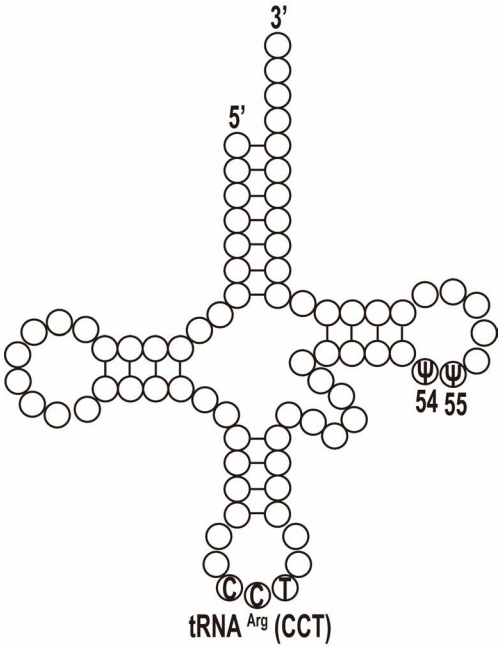








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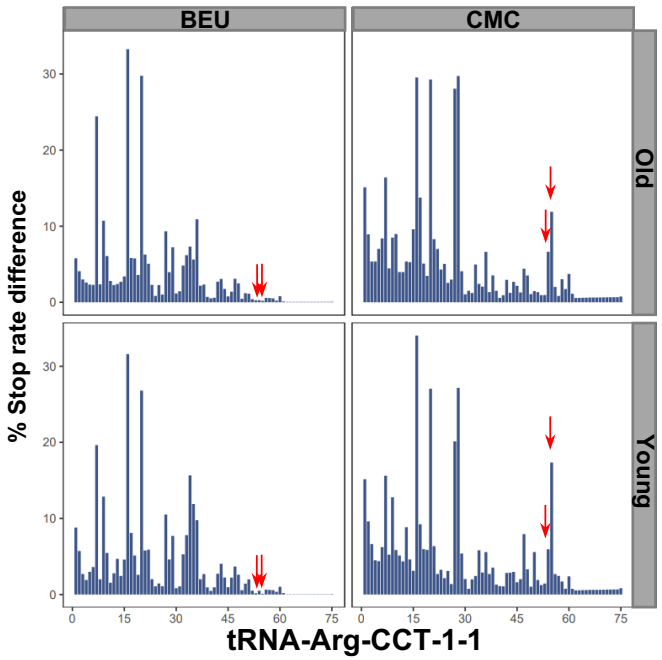
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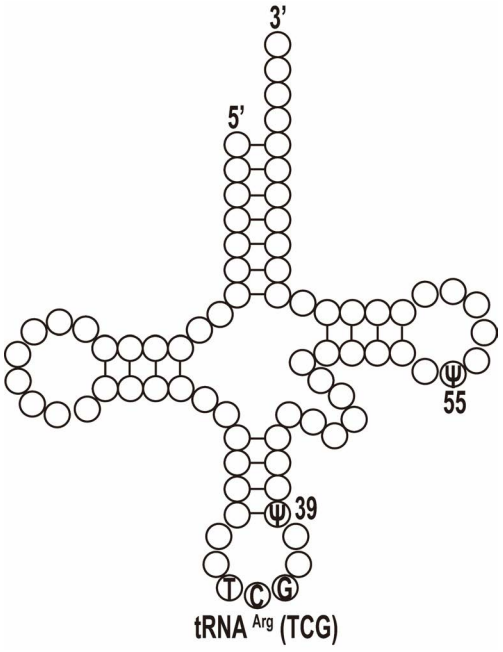
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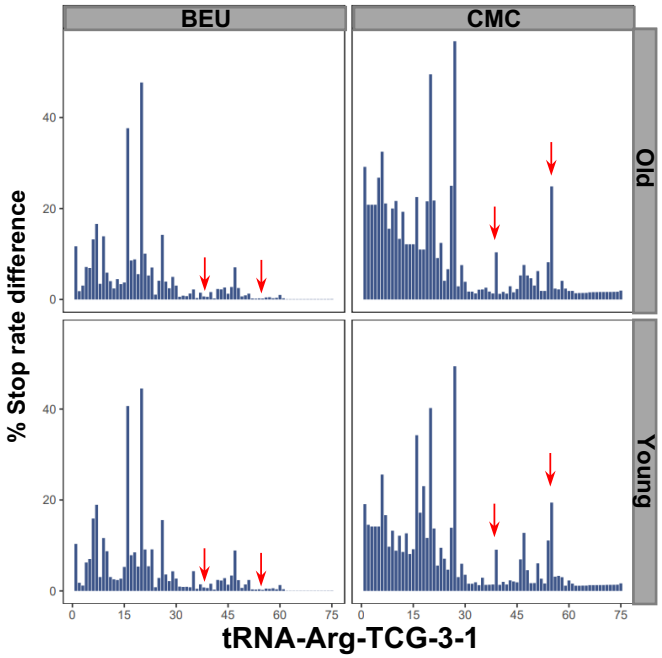
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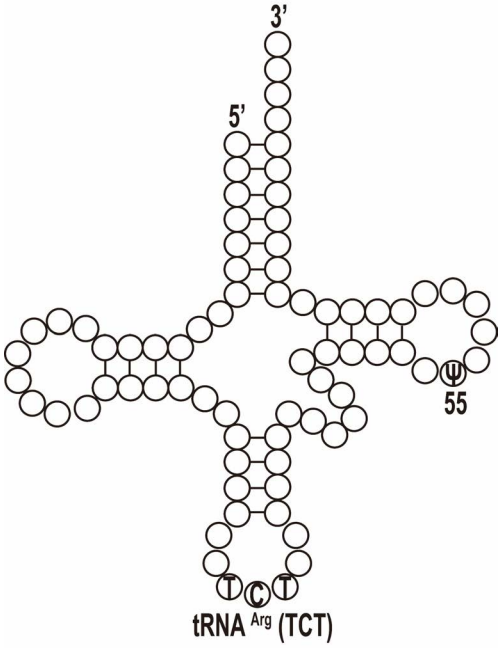
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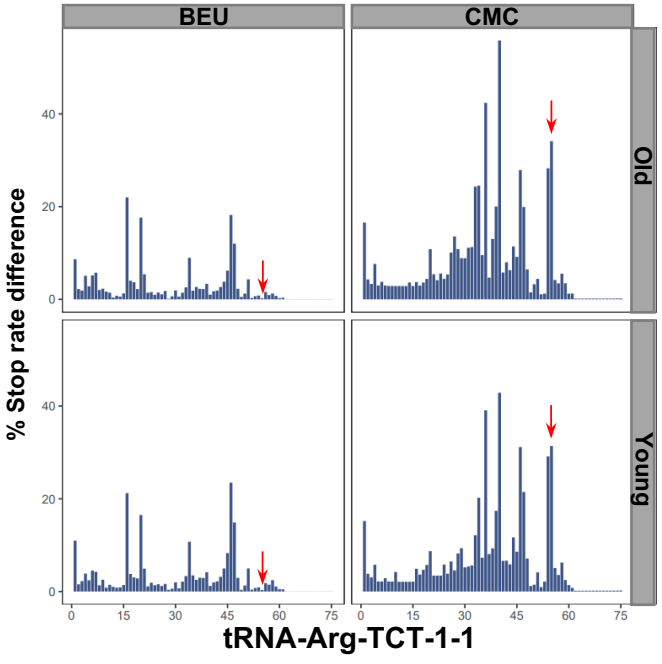
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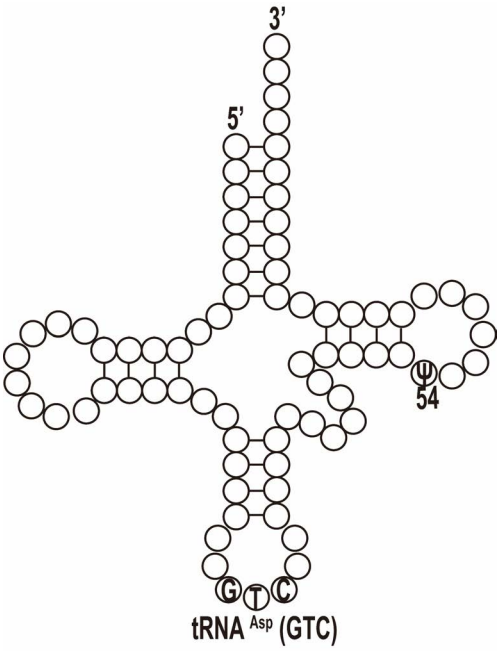
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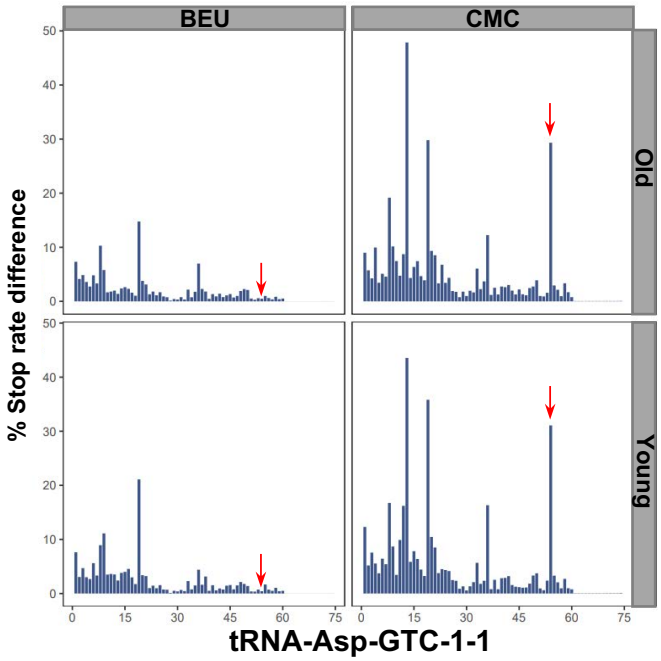
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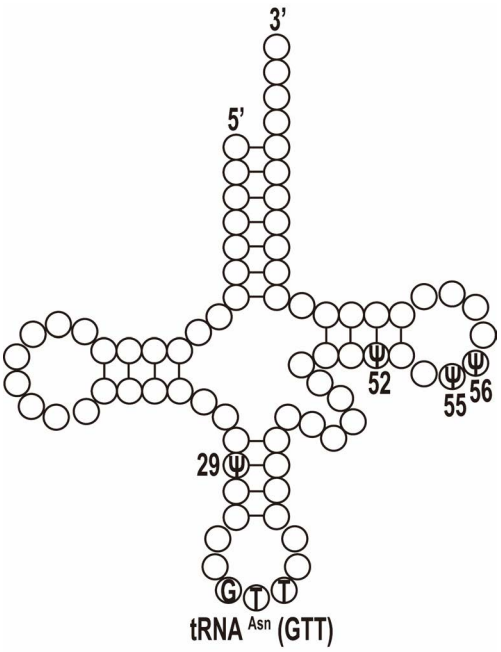
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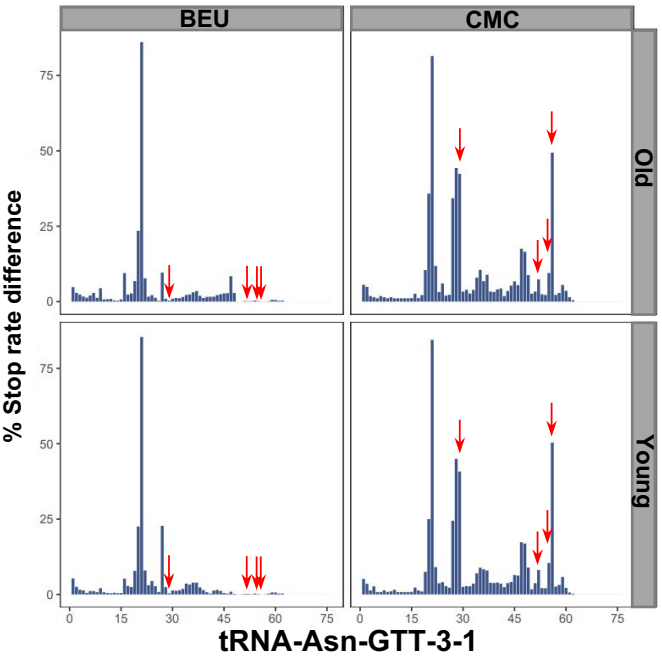
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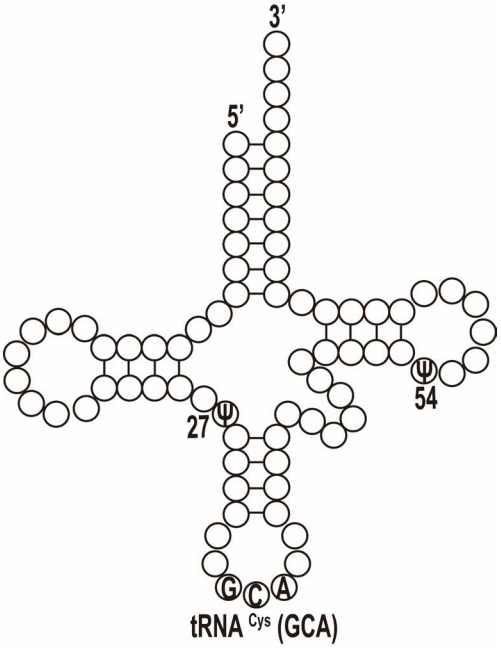
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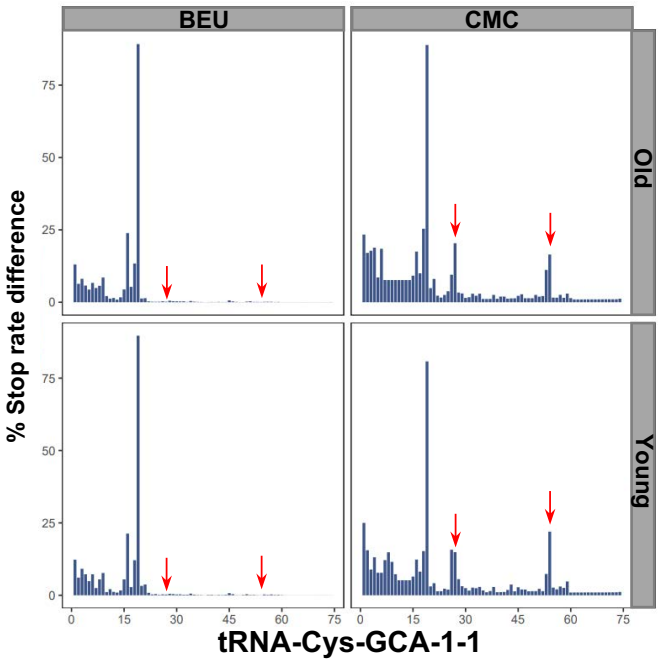
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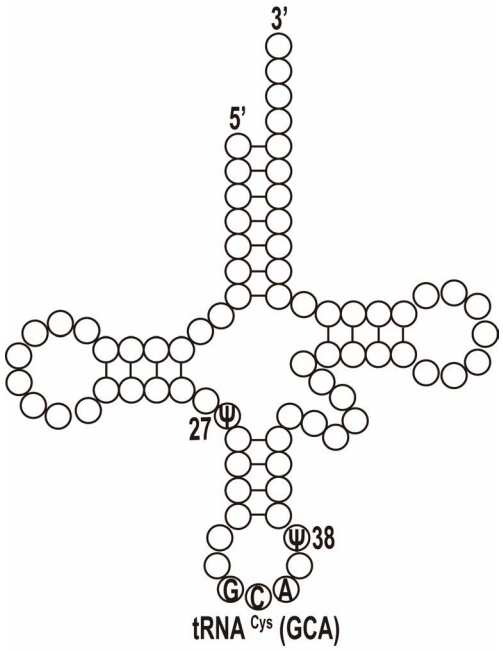
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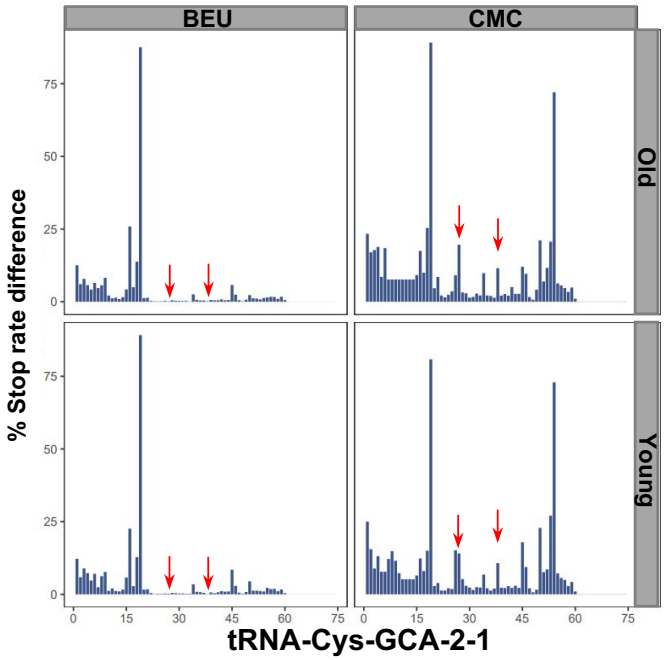
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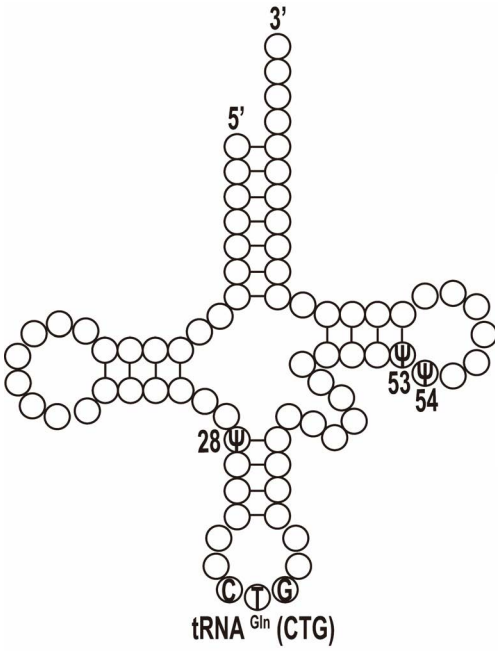
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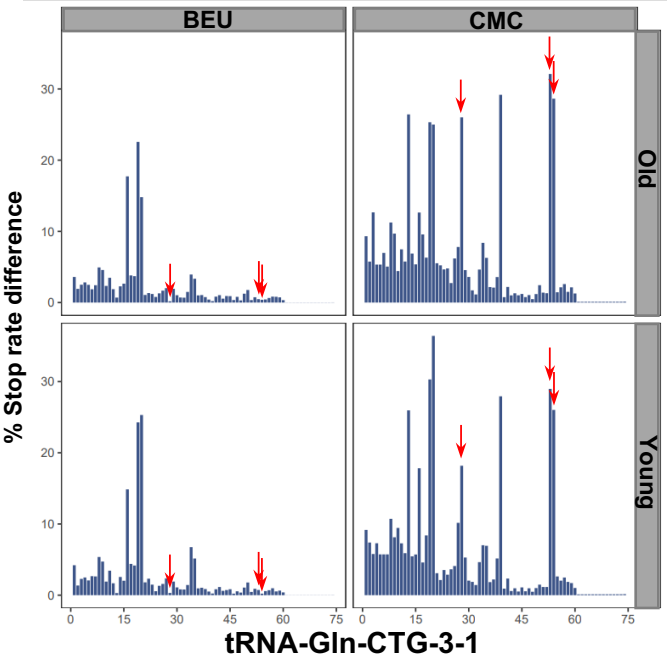
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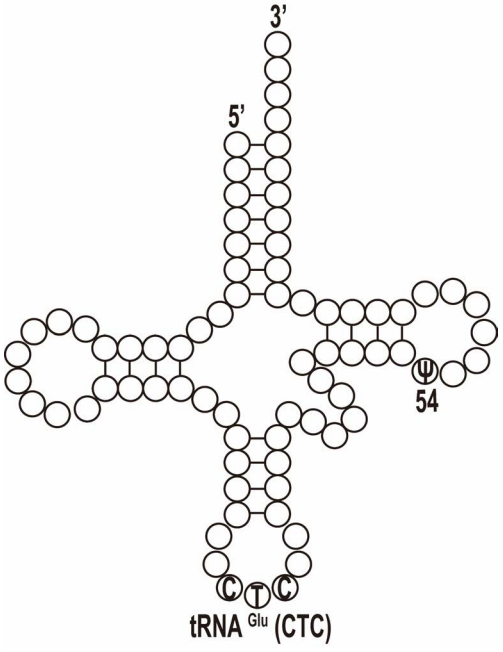
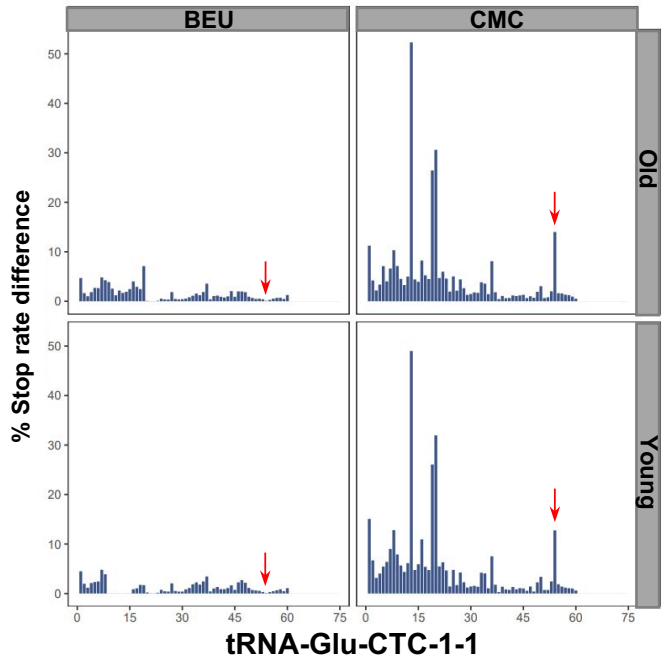
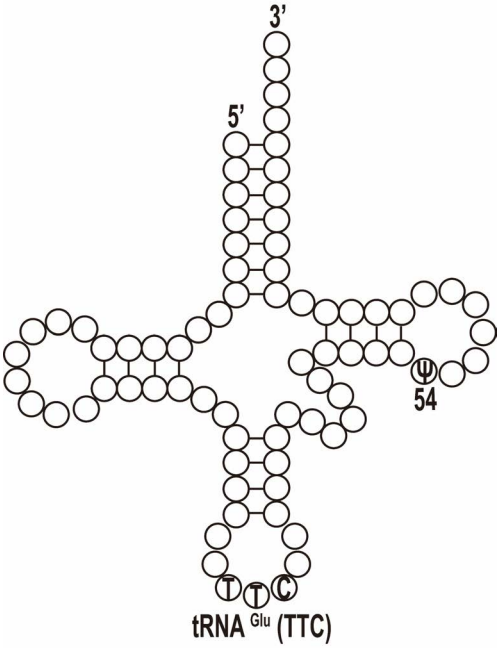
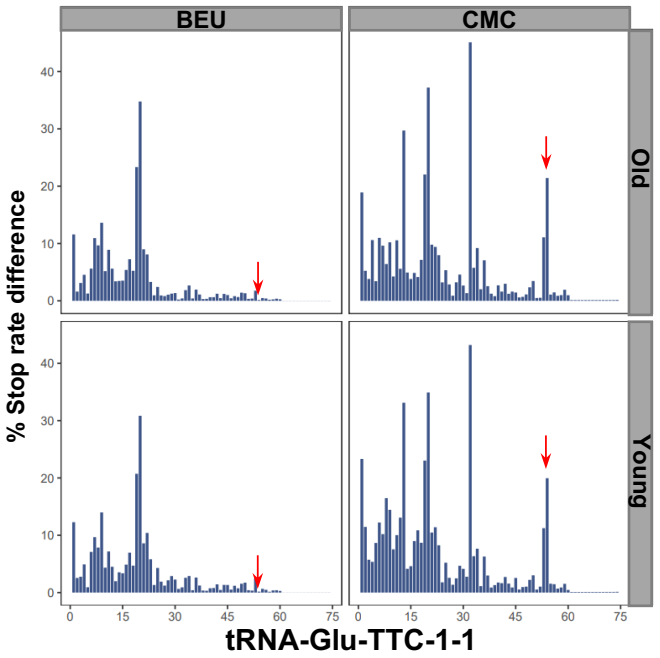
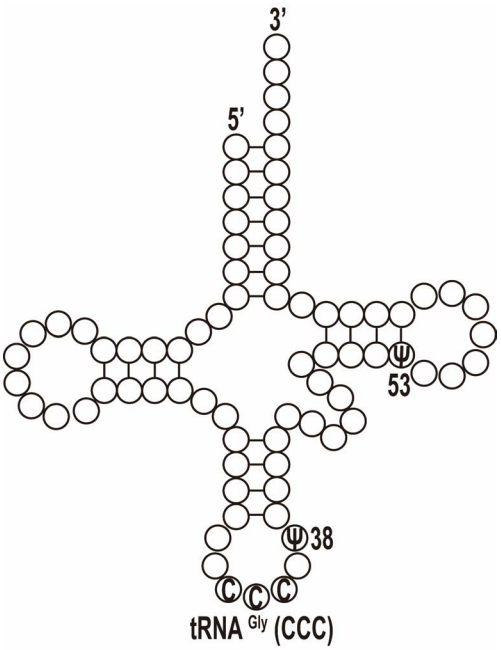
% Stop rate difference
tRNA-Gln-CTG-3-1

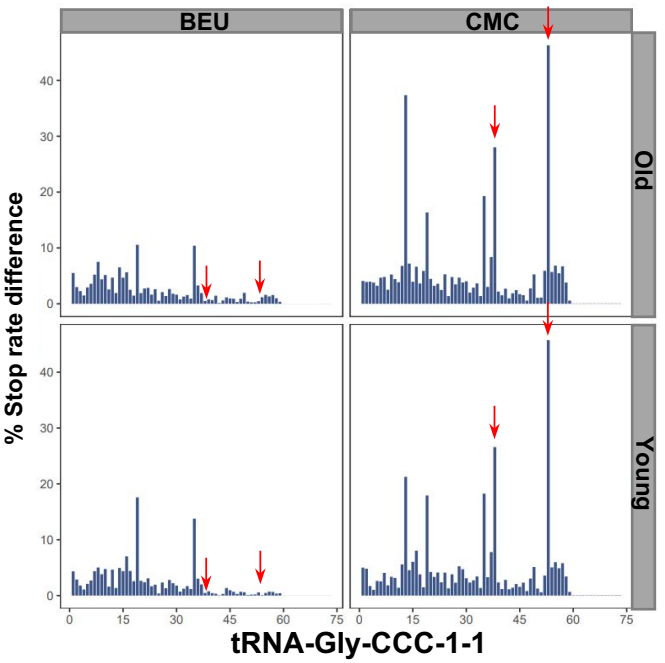

3'
5'
54
CTC
tRNA Glu (CTC)

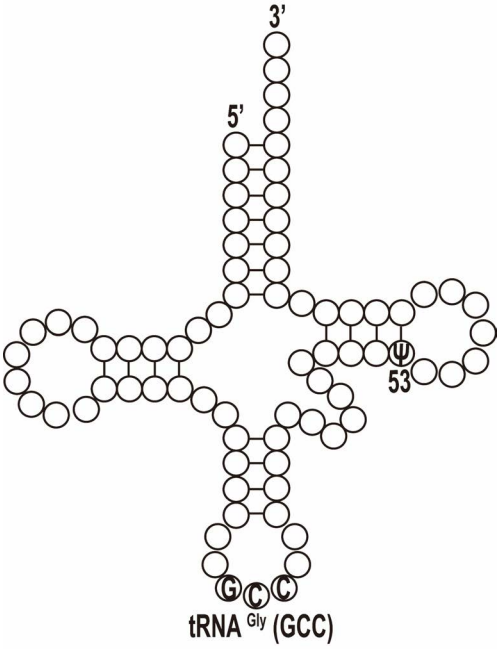

BEU
CMC
Old
Young
% Stop rate difference
tRNA-Glu-CTC-1-1

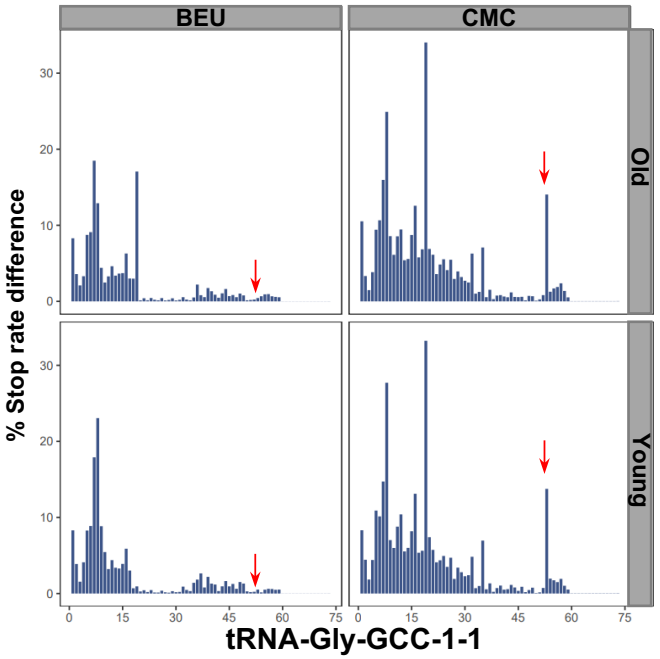

3'
5'
Ψ
54
T
T
C
tRNA Glu (TTC)

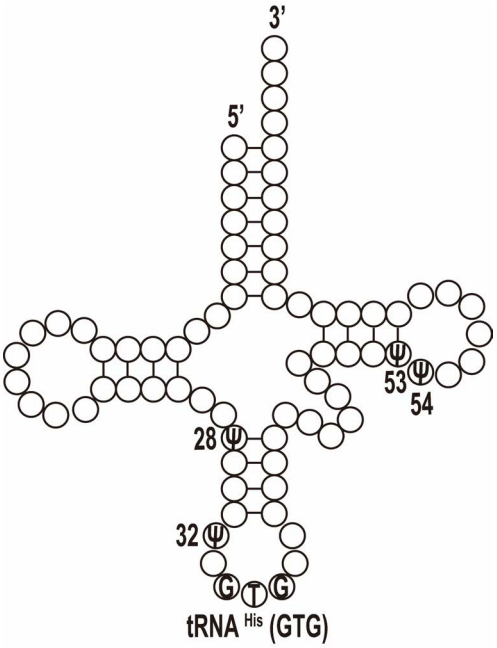

BEU
CMC
Old
Young
% Stop rate difference
0
10
20
30
40
0
15
30
45
60
75
tRNA-Glu-TTC-1-1

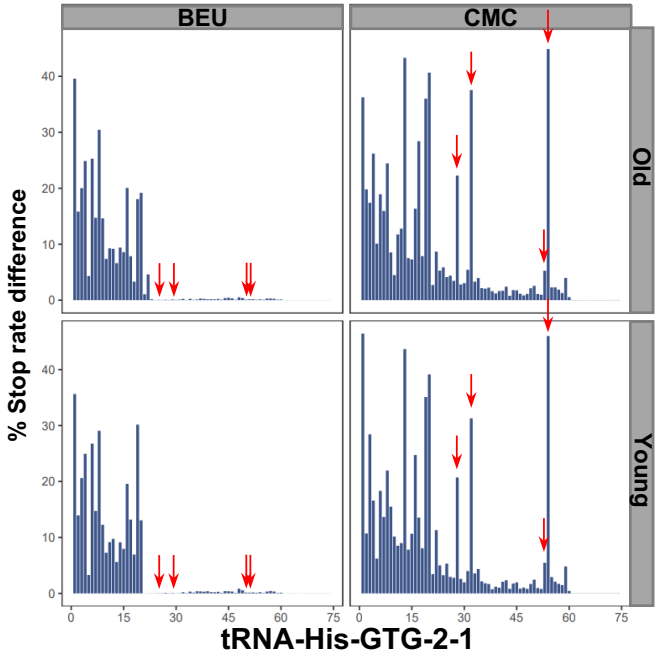

3'
5'
Ψ
53
Ψ
38
C
C
C
tRNA Gly (CCC)

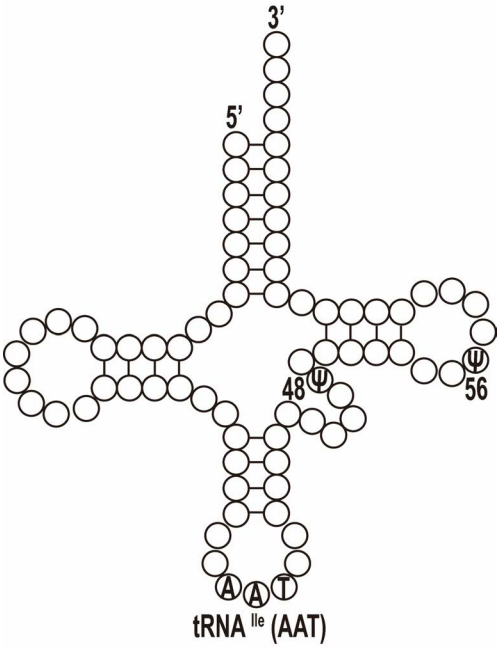

BEU
CMC
Old
Young
% Stop rate difference
0
10
20
30
40
0
15
30
45
60
75
tRNA-Gly-CCC-1-1

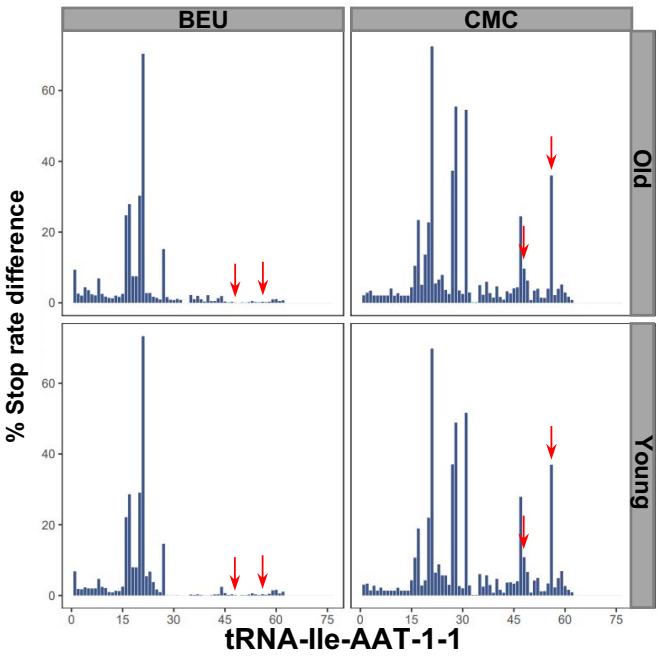

3'
5'
Ψ
53
G
C
C
tRNA Gly (GCC)

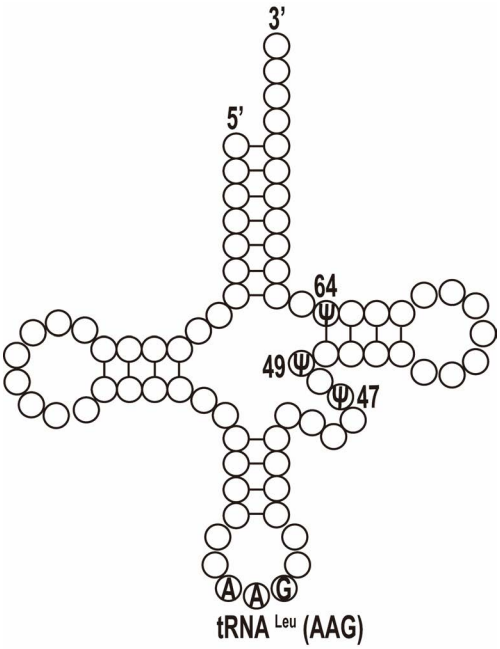

BEU
CMC
Old
Young
% Stop rate difference
0
10
20
30
0
15
30
45
60
75
tRNA-Gly-GCC-1-1

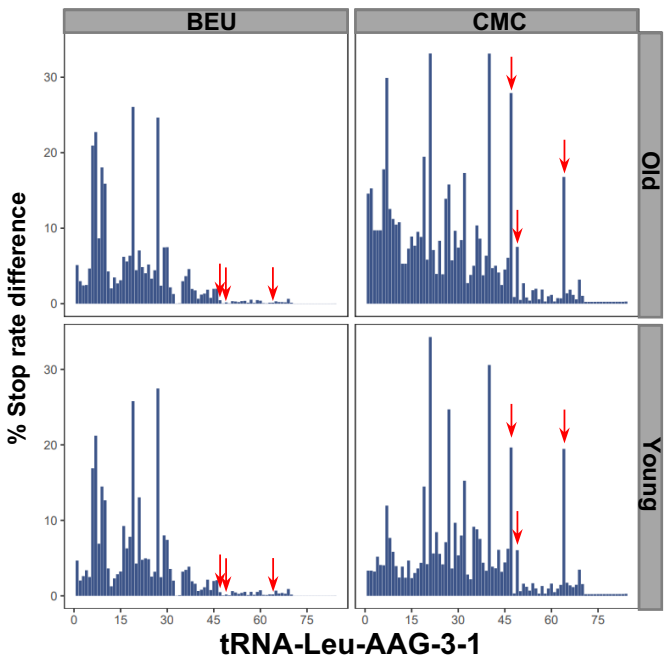

3'
5'
53
54
28
32
G T G
tRNA His (GTG)

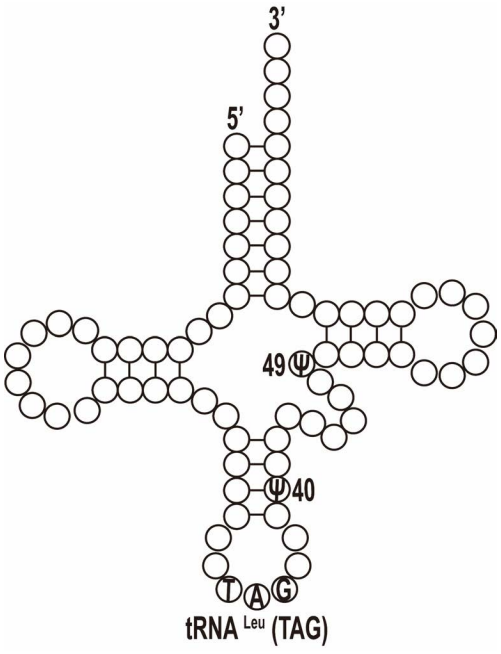

BEU
CMC
Old
Young
% Stop rate difference
tRNA-His-GTG-2-1

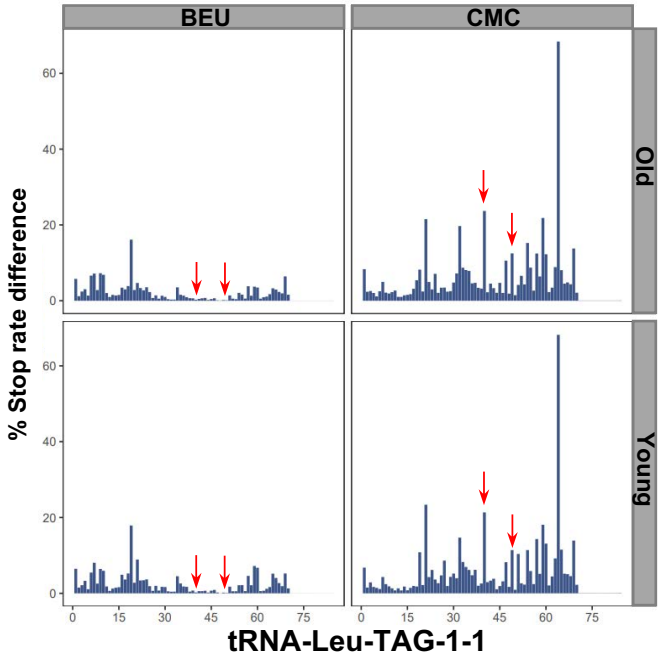

3'
5'
48
56
A A T
tRNA Ile (AAT)

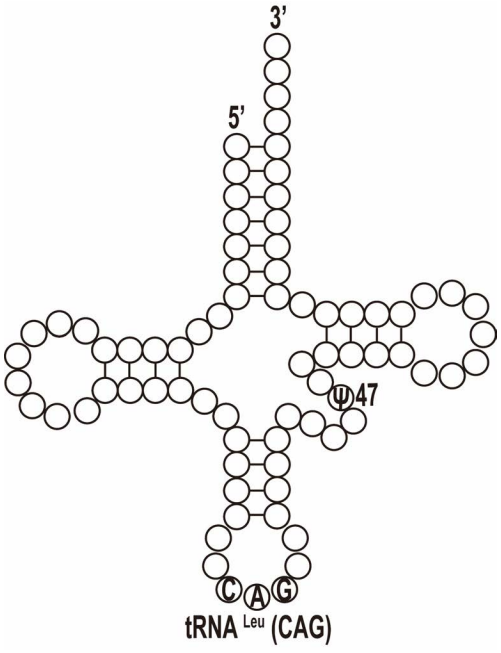

BEU
CMC
Old
Young
% Stop rate difference
tRNA-Ile-AAT-1-1

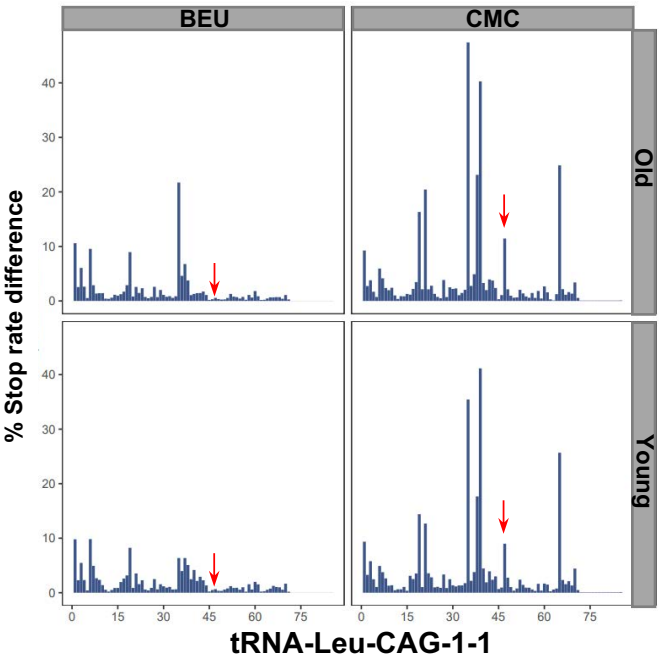

3'
5'
64
49
47
A A G
tRNA Leu (AAG)

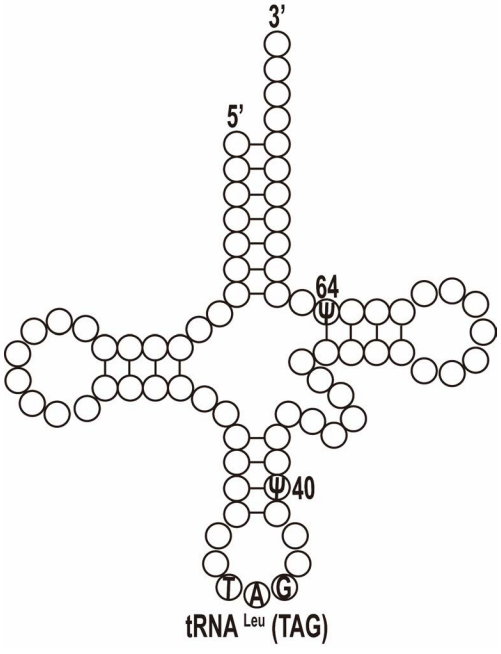

BEU
CMC
Old
Young
% Stop rate difference
tRNA-Leu-AAG-3-1

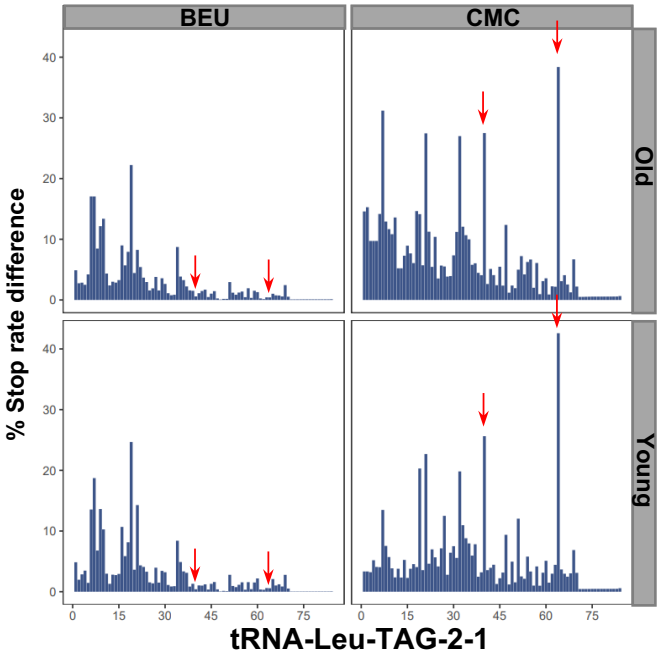

3'
5'
49Ψ
Ψ40
TAG
tRNA Leu (TAG)

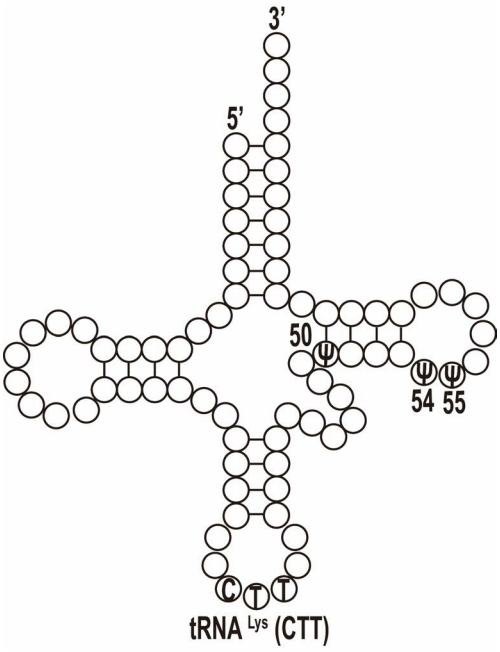

BEU
CMC
Old
Young
% Stop rate difference
tRNA-Leu-TAG-1-1

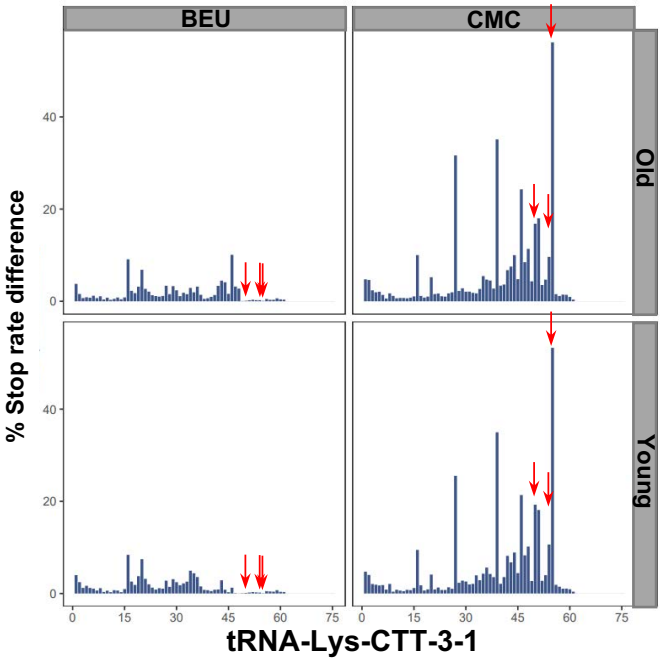

3'
5'
Ψ47
CAG
tRNA Leu (CAG)

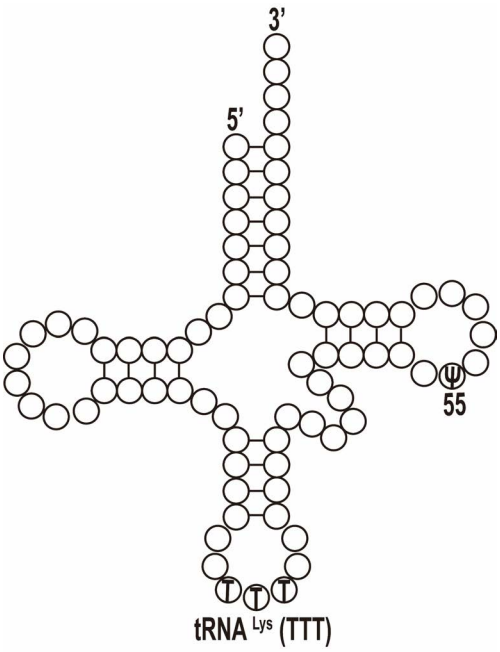

BEU
CMC
Old
Young
% Stop rate difference
tRNA-Leu-CAG-1-1

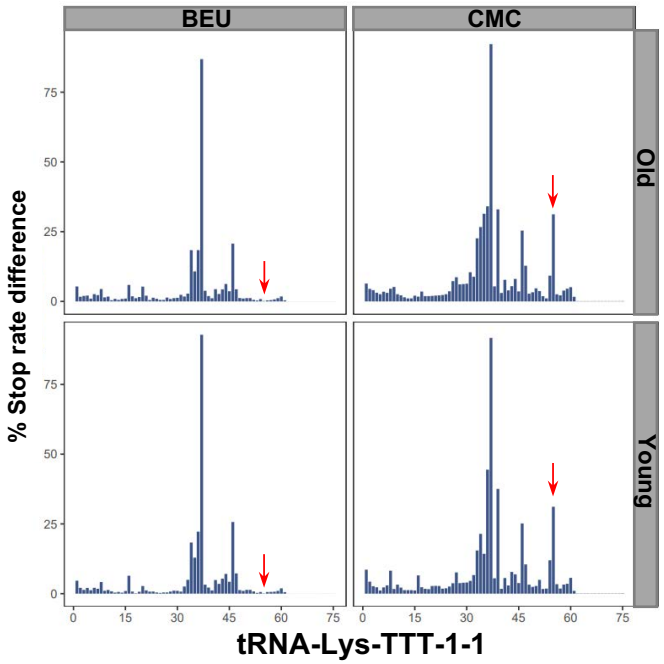

3'
5'
64Ψ
Ψ40
TAG
tRNA Leu (TAG)

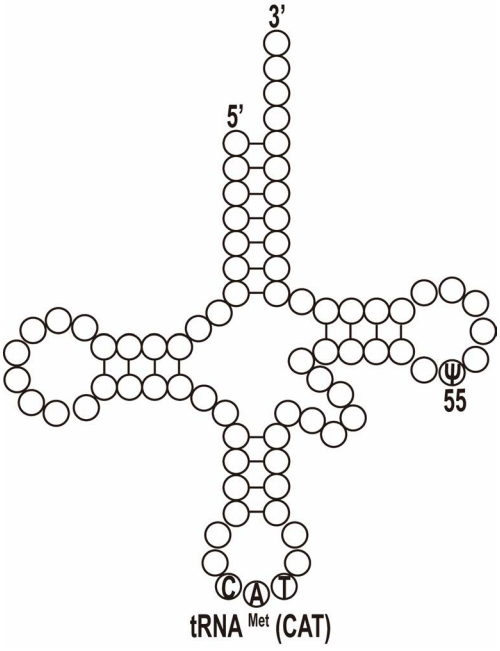

BEU
CMC
Old
Young
% Stop rate difference
tRNA-Leu-TAG-2-1

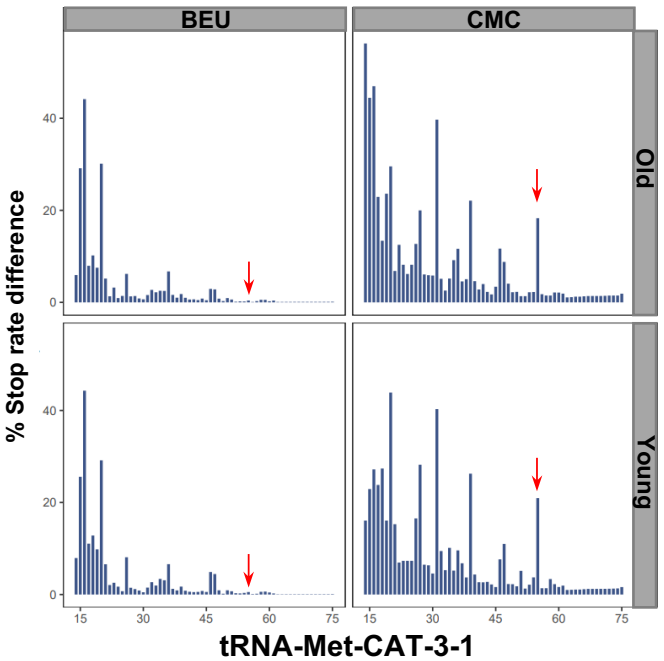

3'
5'
50
Ψ
54 55
C T T
tRNA Lys (CTT)

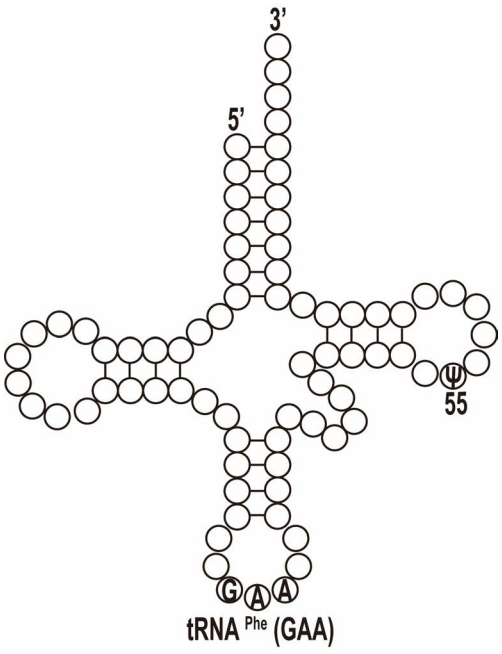

BEU
CMC
Old
Young
% Stop rate difference
0
20
40
0 15 30 45 60 75
tRNA-Lys-CTT-3-1

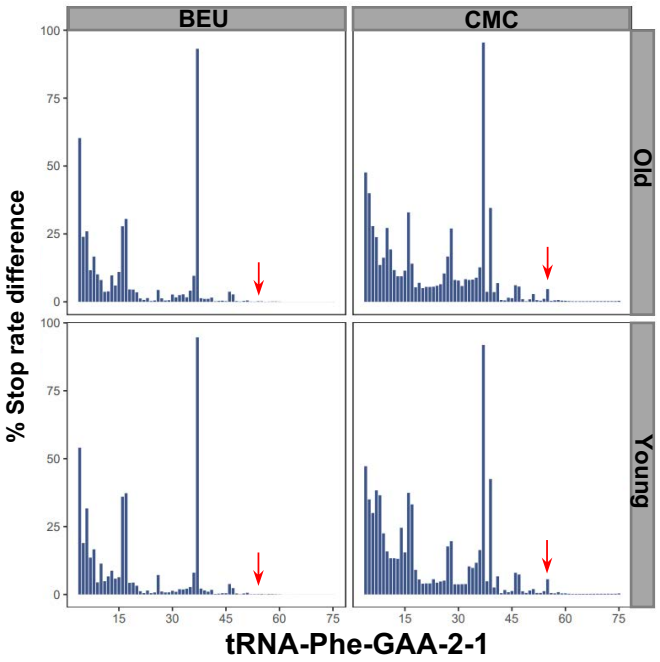

3'
5'
55
Ψ
T T T
tRNA Lys (TTT)

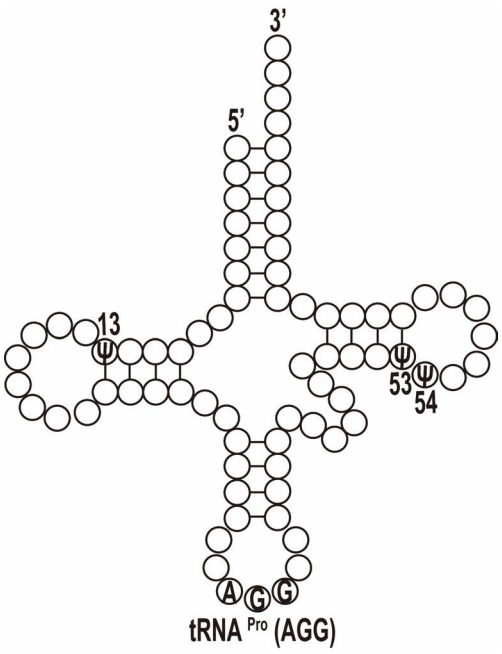

BEU
CMC
Old
Young
% Stop rate difference
0
25
50
75
0 15 30 45 60 75
tRNA-Lys-TTT-1-1

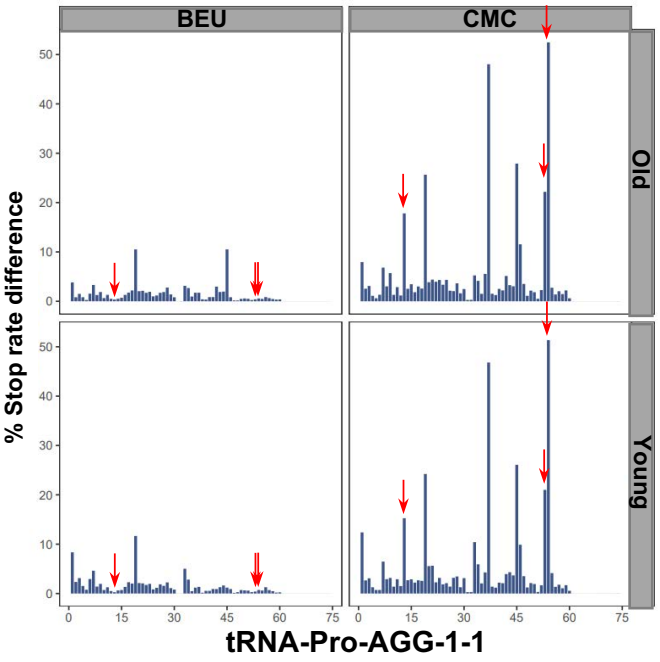

3'
5'
55
Ψ
C A T
tRNA Met (CAT)

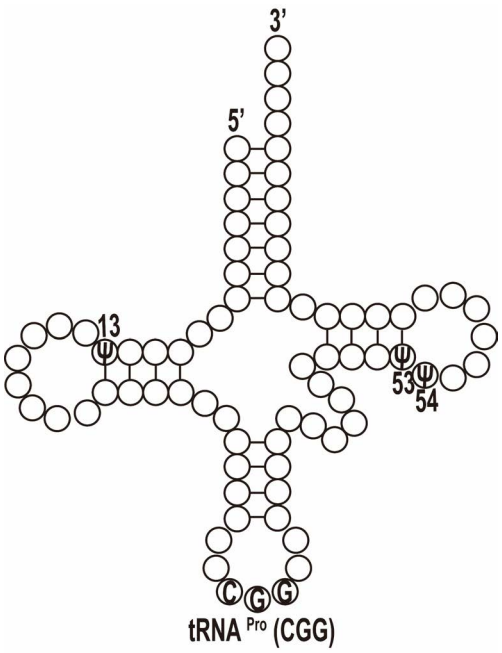

BEU
CMC
Old
Young
% Stop rate difference
0
20
40
15 30 45 60 75
tRNA-Met-CAT-3-1

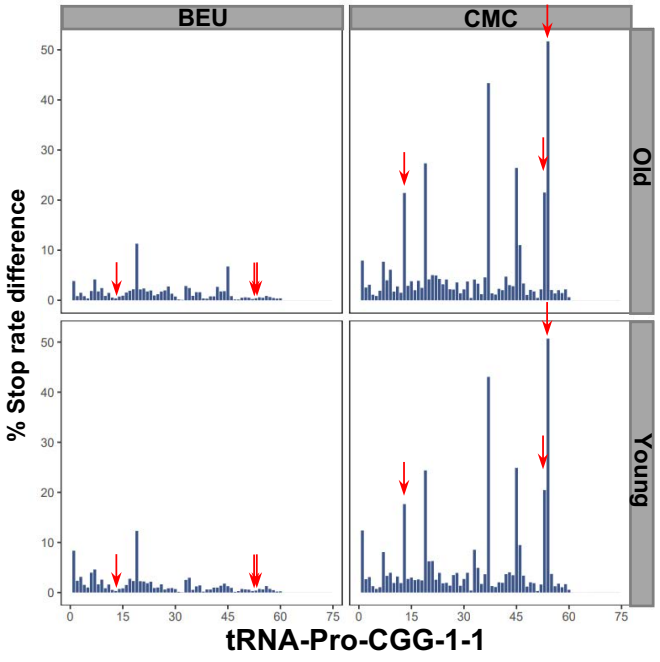

3'
5'
Ψ
55
G
A
A
tRNA Phe (GAA)

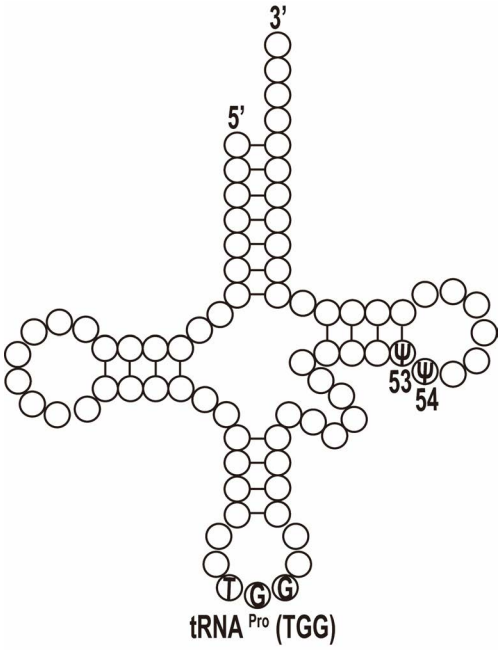

BEU
CMC
Old
Young
% Stop rate difference
tRNA-Phe-GAA-2-1

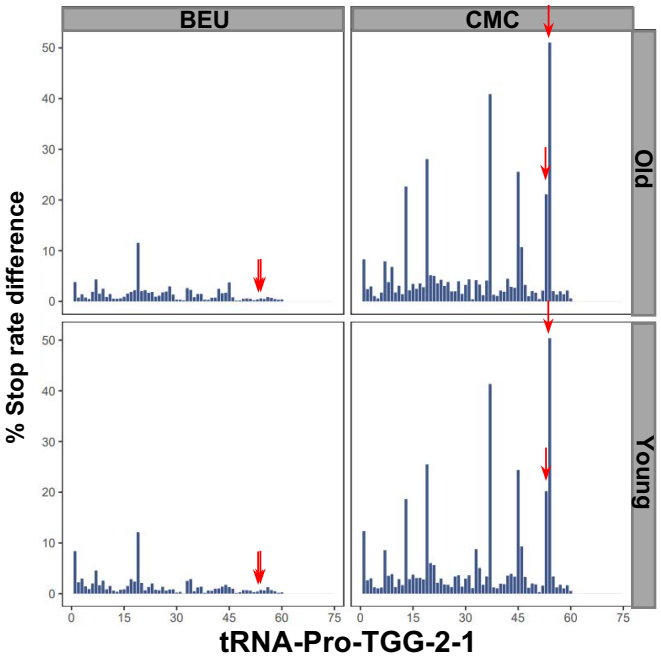

3'
5'
13
Ψ
53
Ψ
54
A
G
G
tRNA Pro (AGG)

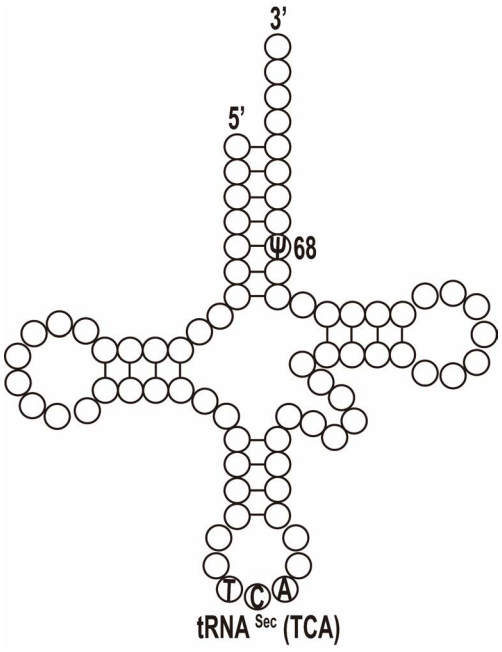

BEU
CMC
Old
Young
% Stop rate difference
tRNA-Pro-AGG-1-1

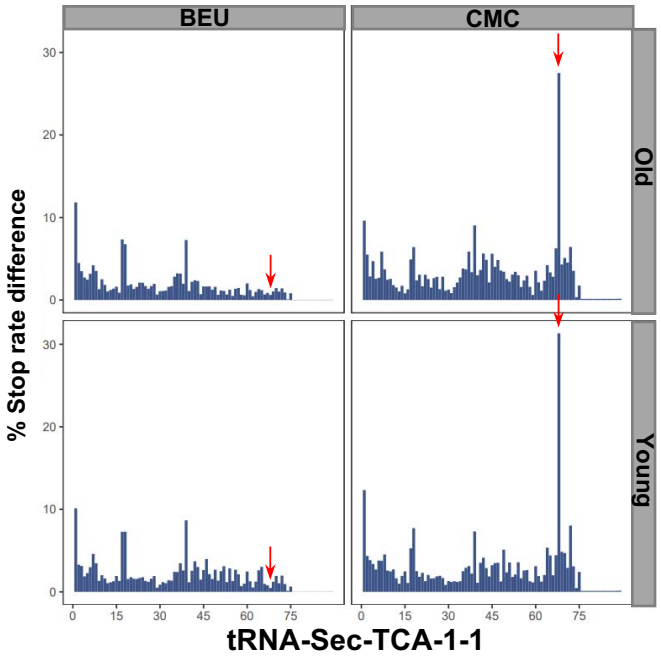

3'
5'
13
Ψ
53
Ψ
54
C
G
G
tRNA Pro (CGG)

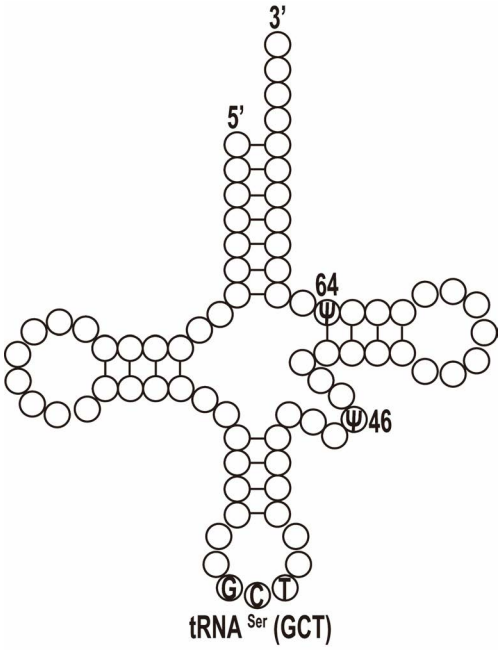

BEU
CMC
Old
Young
% Stop rate difference
tRNA-Pro-CGG-1-1

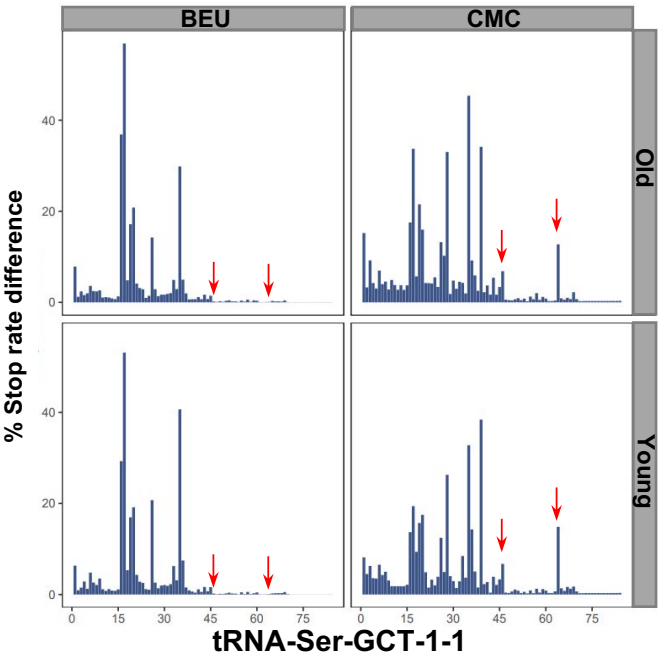

3'
5'
Ψ53
Ψ54
TGG
tRNA Pro (TGG)

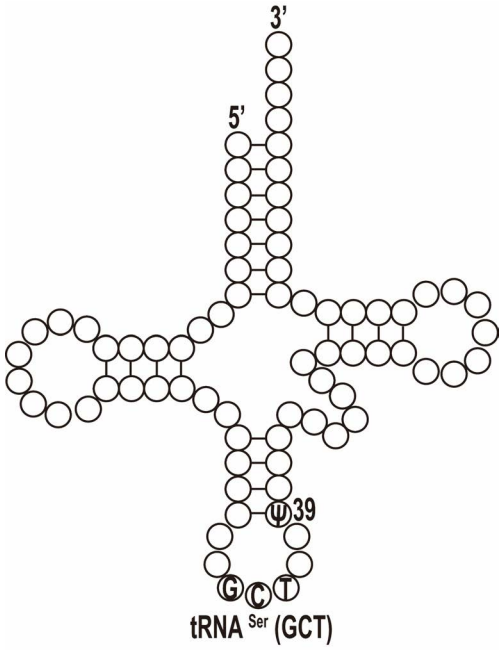

BEU
CMC
Old
Young
% Stop rate difference
tRNA-Pro-TGG-2-1

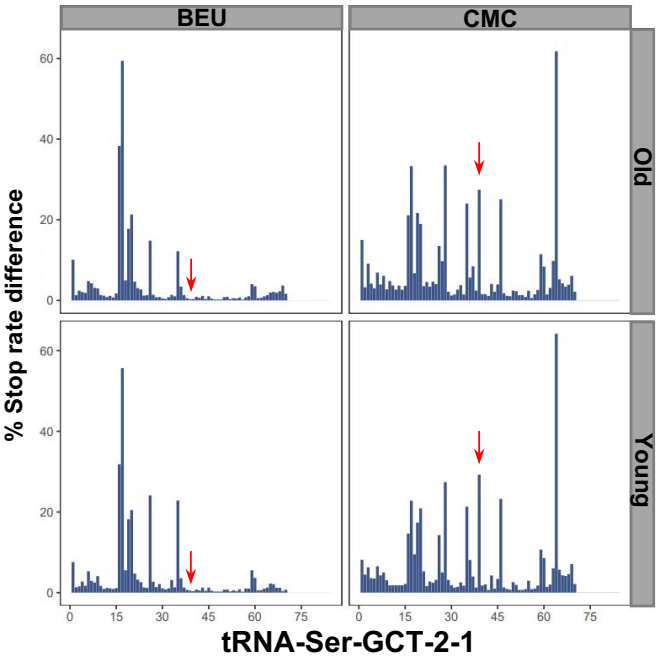

3'
5'
Ψ68
TCA
tRNA Sec (TCA)

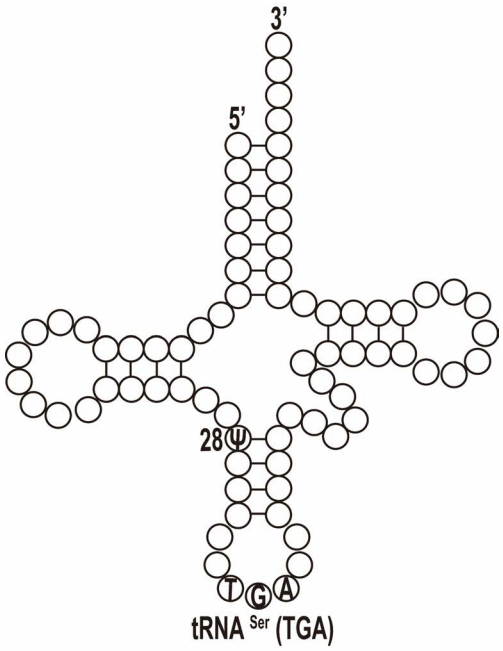

BEU
CMC
Old
Young
% Stop rate difference
tRNA-Sec-TCA-1-1

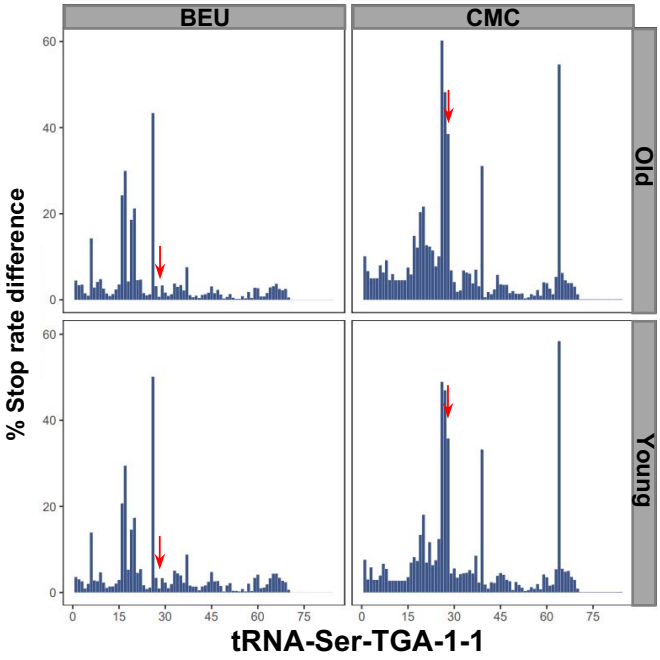

3'
5'
64
Ψ
Ψ46
GCT
tRNA Ser (GCT)

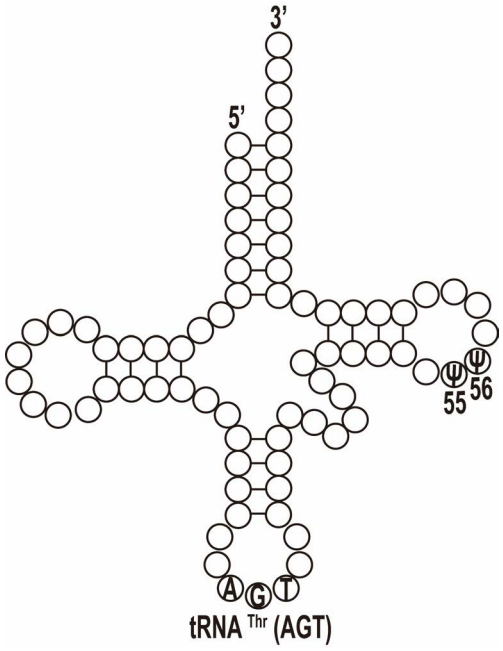

BEU
CMC
Old
Young
% Stop rate difference
tRNA-Ser-GCT-1-1

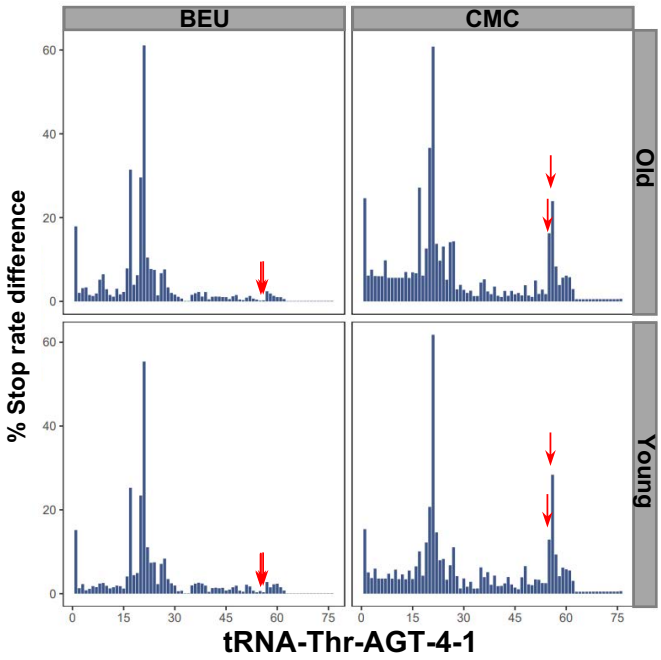

3'
5'
Ψ39
G C T
tRNA Ser (GCT)

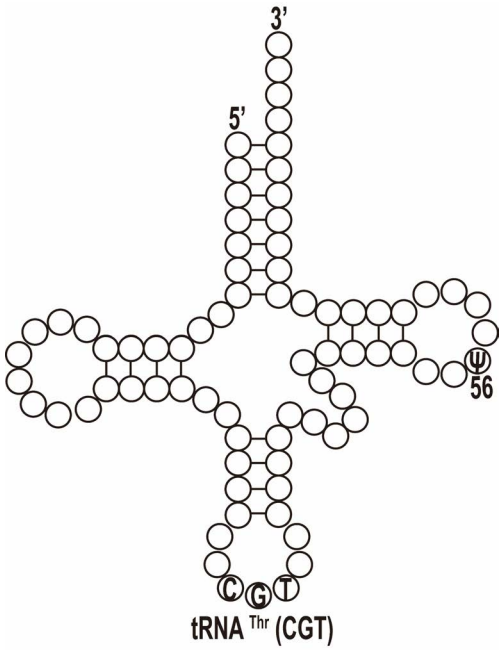

BEU
CMC
Old
Young
% Stop rate difference
tRNA-Ser-GCT-2-1

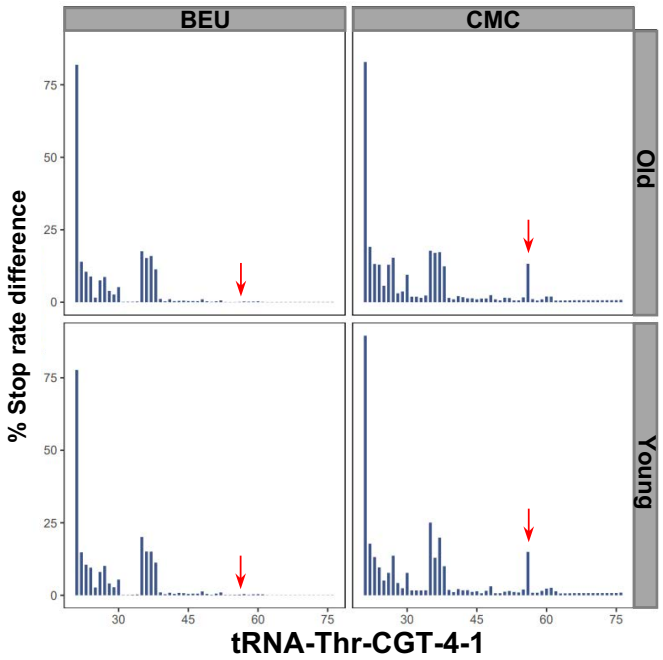

3'
5'
28Ψ
T G A
tRNA Ser (TGA)

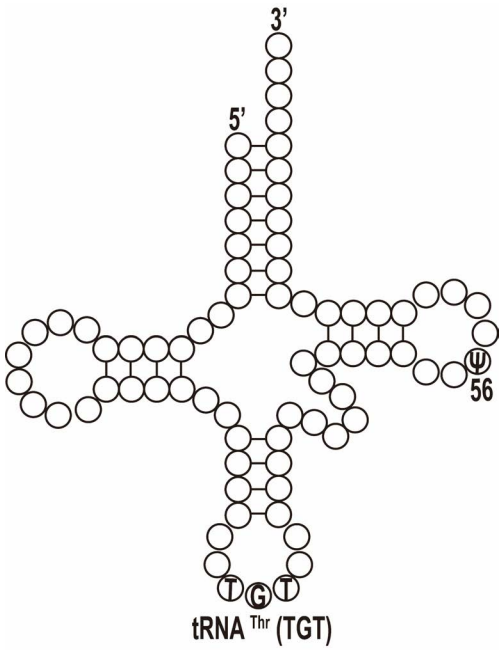

BEU
CMC
Old
Young
% Stop rate difference
tRNA-Ser-TGA-1-1

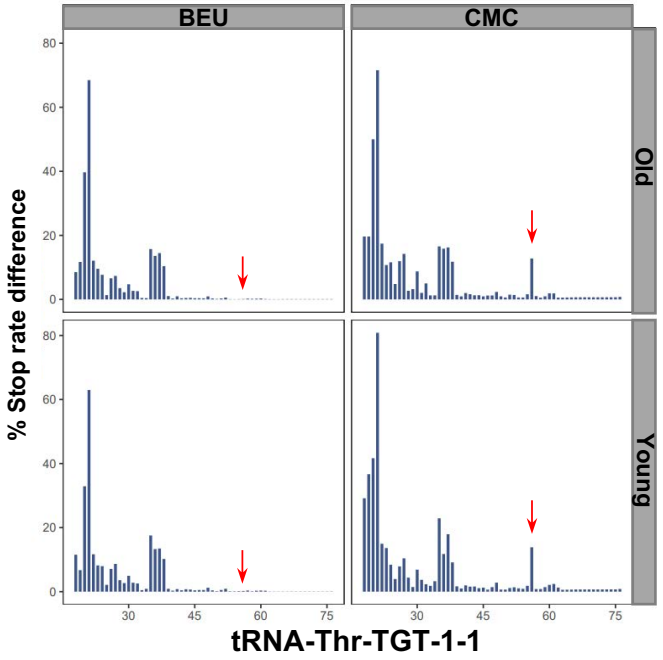

3'
5'
Ψ55 Ψ56
A G T
tRNA Thr (AGT)

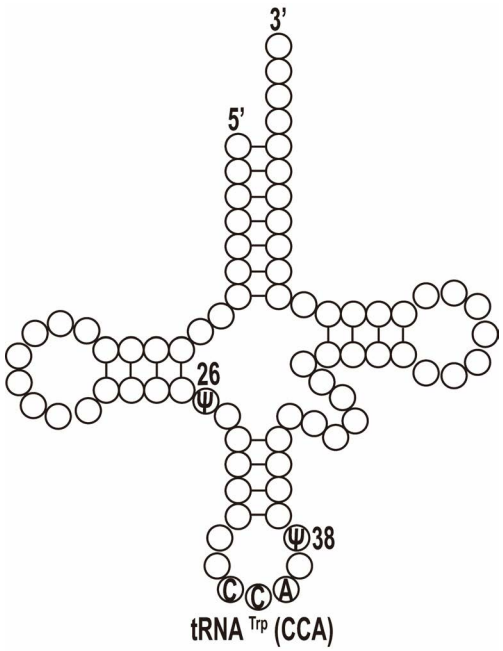

BEU
CMC
Old
Young
% Stop rate difference
tRNA-Thr-AGT-4-1

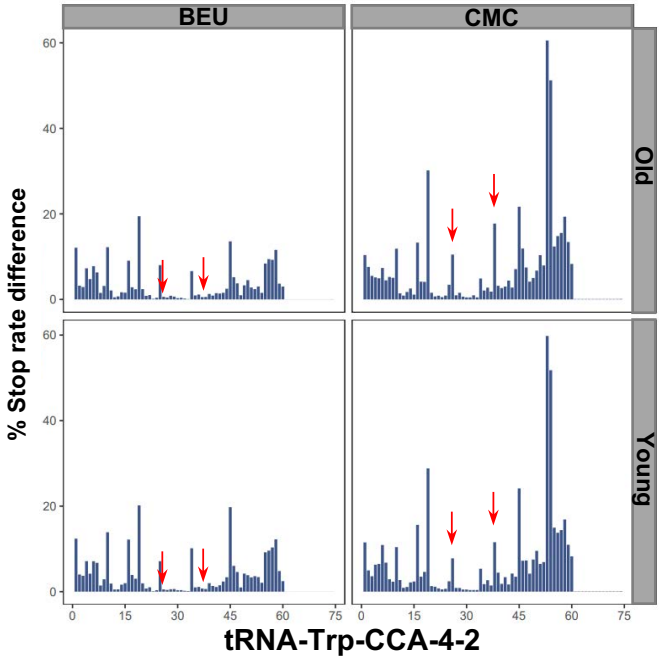

3'
5'
Ψ
56
C
G
T
tRNA Thr (CGT)

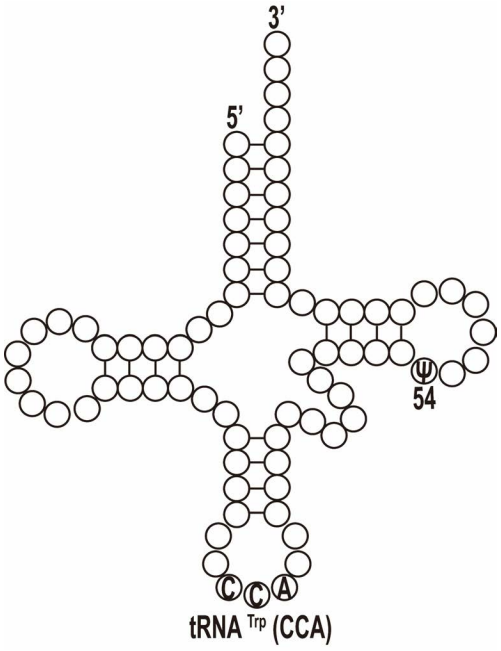

BEU
CMC
Old
Young
% Stop rate difference
tRNA-Thr-CGT-4-1

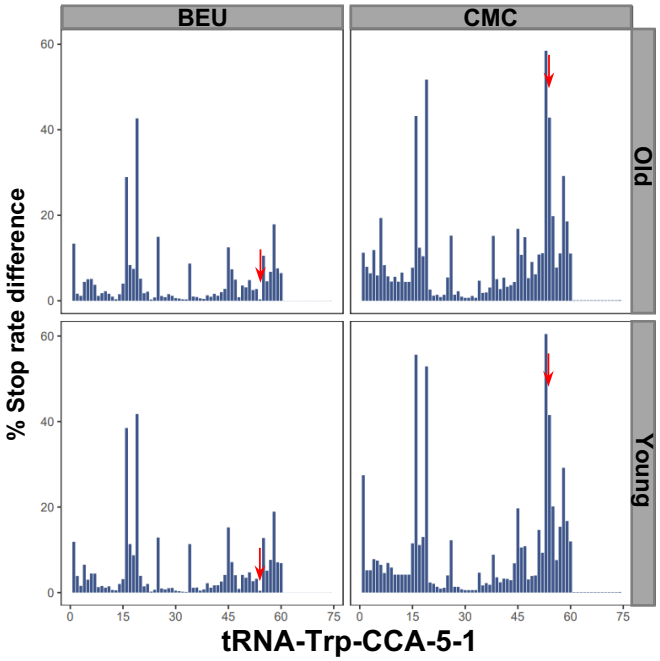

3'
5'
Ψ
56
T
G
T
tRNA Thr (TGT)

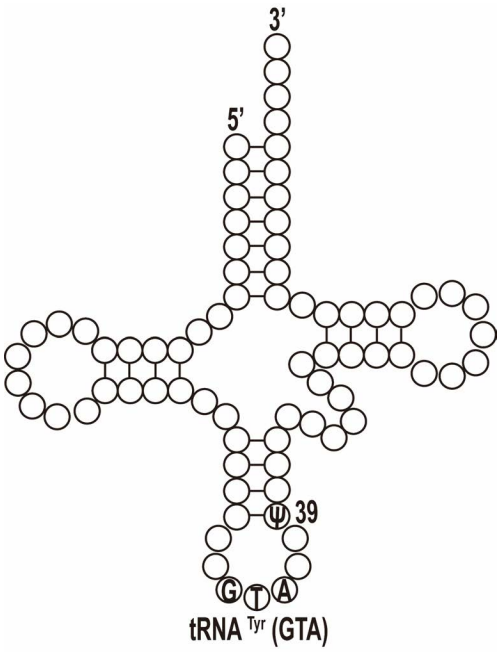

BEU
CMC
Old
Young
% Stop rate difference
tRNA-Thr-TGT-1-1

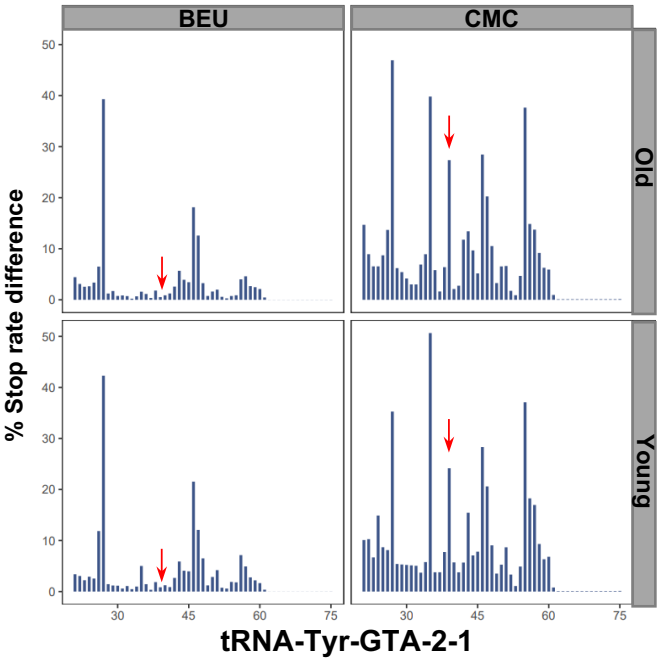

3'
5'
26
Ψ
Ψ38
C
C
A
tRNA Trp (CCA)

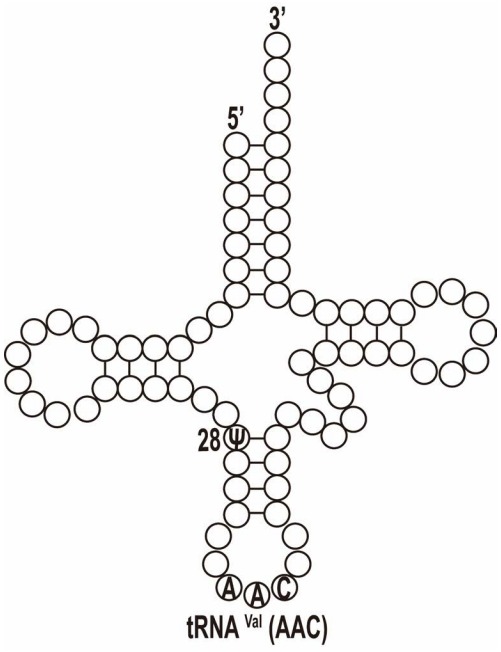

BEU
CMC
Old
Young
% Stop rate difference
tRNA-Trp-CCA-4-2

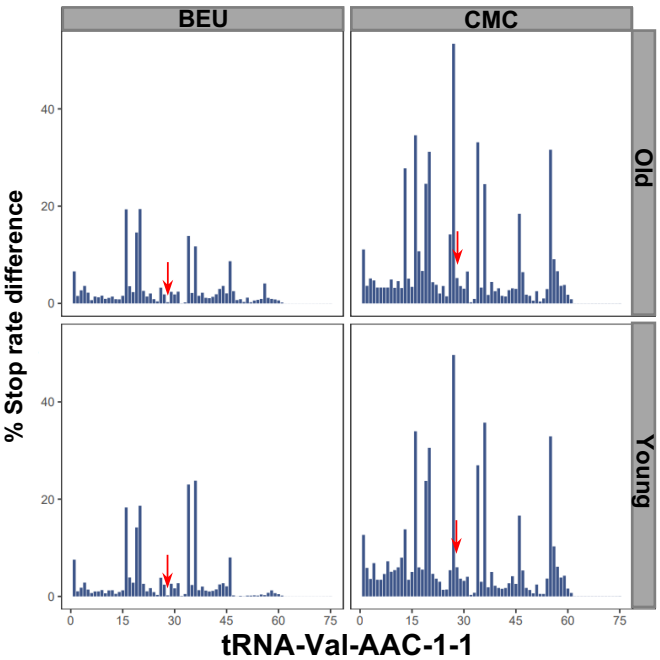

3'
5'
Ψ
54
C C A
tRNA Trp (CCA)

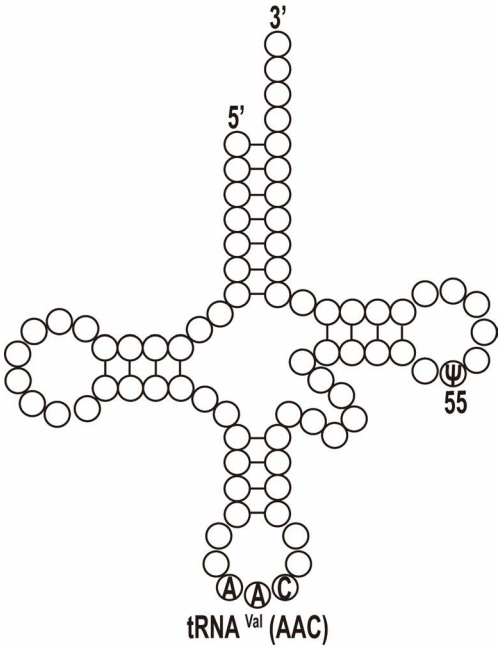

BEU
CMC
Old
Young
% Stop rate difference
tRNA-Trp-CCA-5-1

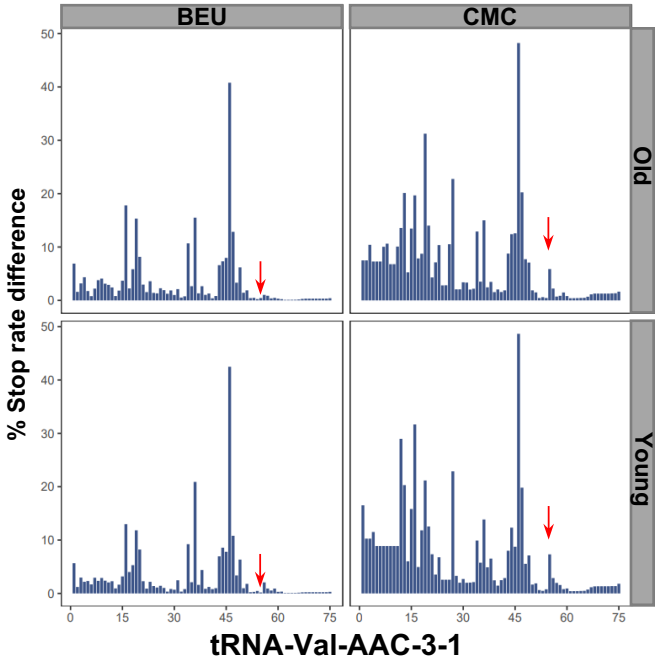

3'
5'
Ψ
39
G T A
tRNA Tyr (GTA)

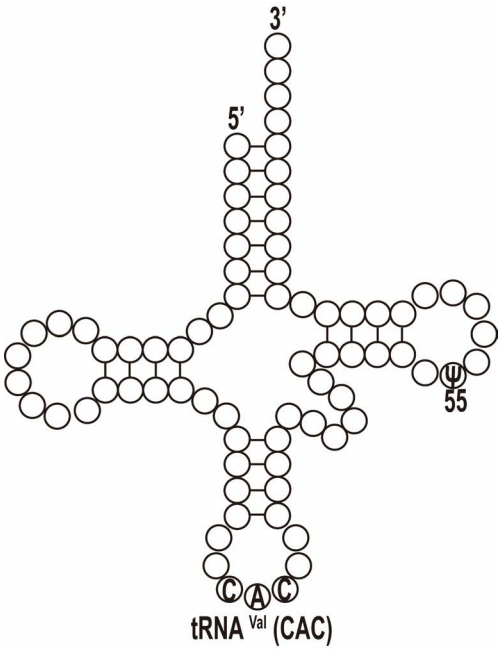

BEU
CMC
Old
Young
% Stop rate difference
tRNA-Tyr-GTA-2-1

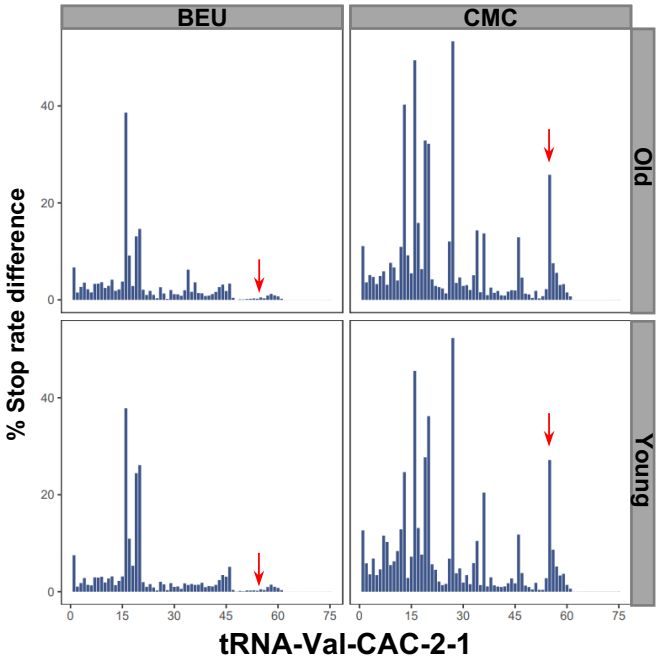

3'
5'
28
Ψ
A A C
tRNA Val (AAC)


BEU
CMC
Old
Young
% Stop rate difference
tRNA-Val-AAC-1-1

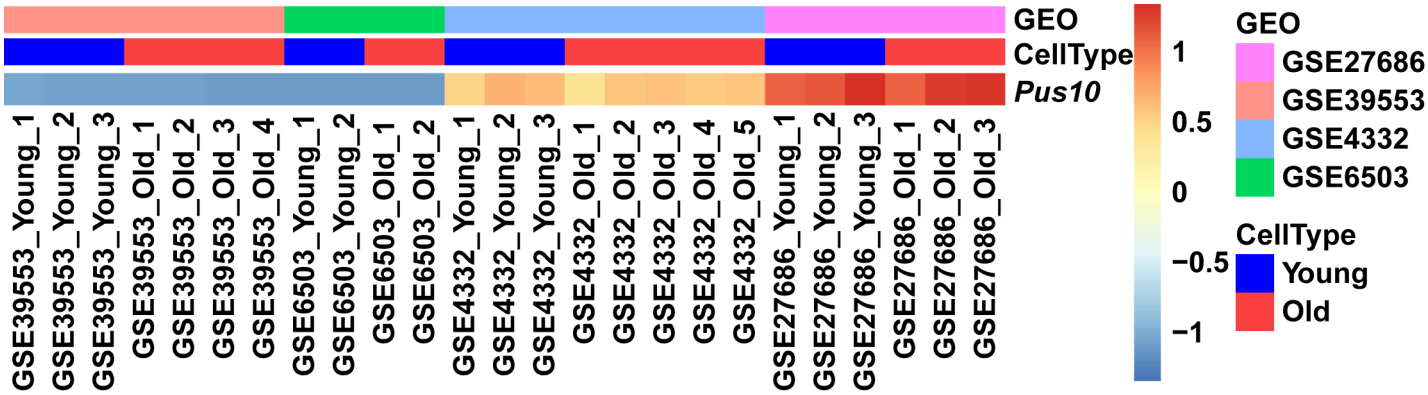

3'
5'
Ψ
55
A
A
C
tRNA Val (AAC)


BEU
CMC
Old
Young
% Stop rate difference
0
10
20
30
40
50
0
15
30
45
60
75
tRNA-Val-AAC-3-1

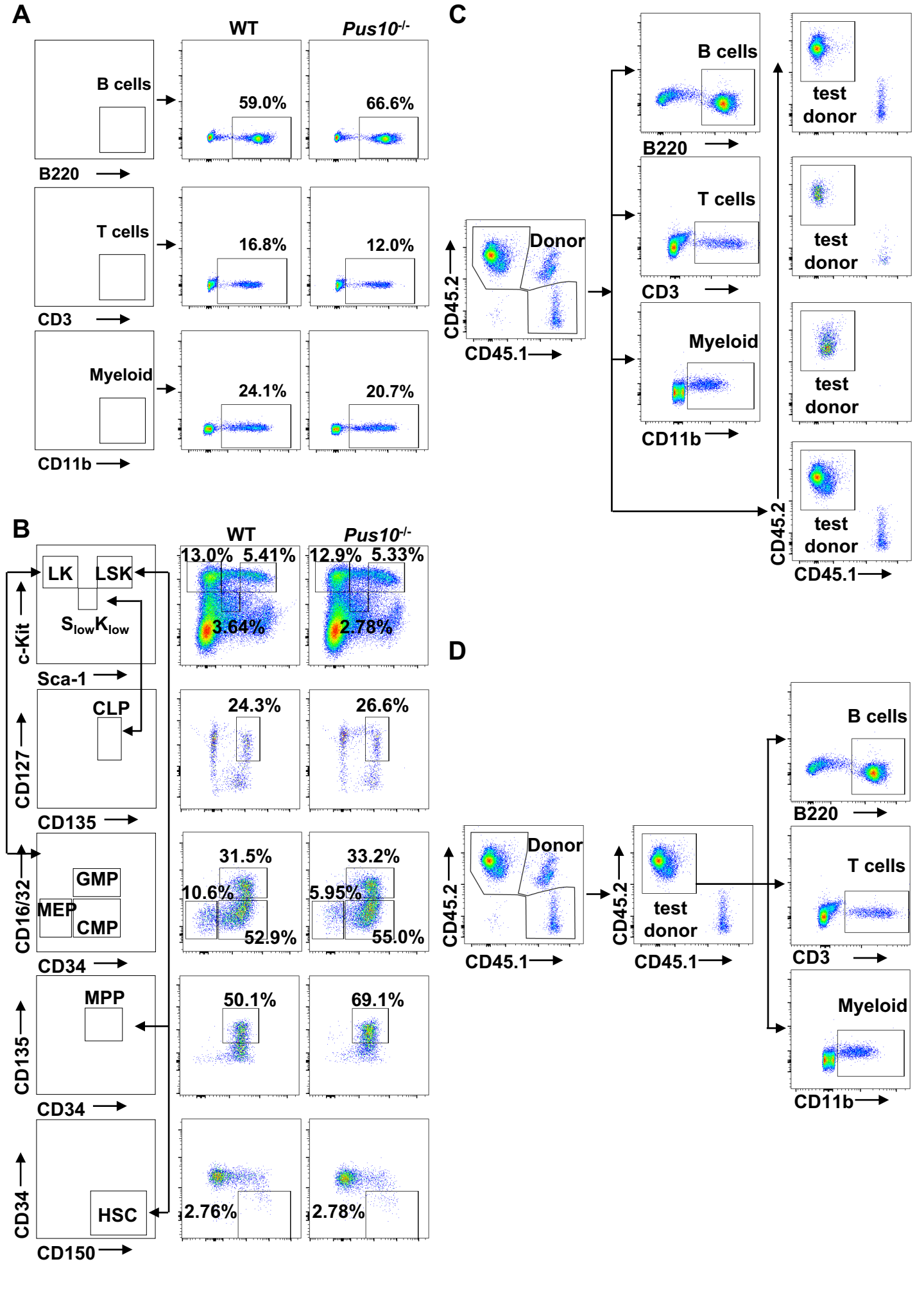

3'
5'
Ψ
55
C
A
C
tRNA Val (CAC)


BEU
CMC
Old
Young
% Stop rate difference
0
20
40
0
15
30
45
60
75
tRNA-Val-CAC-2-1

Supplemental figure 3

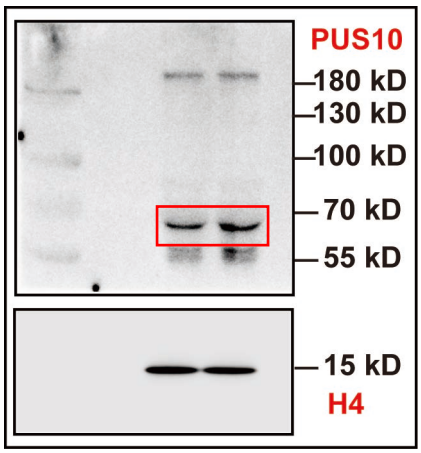


Supplemental figure 4

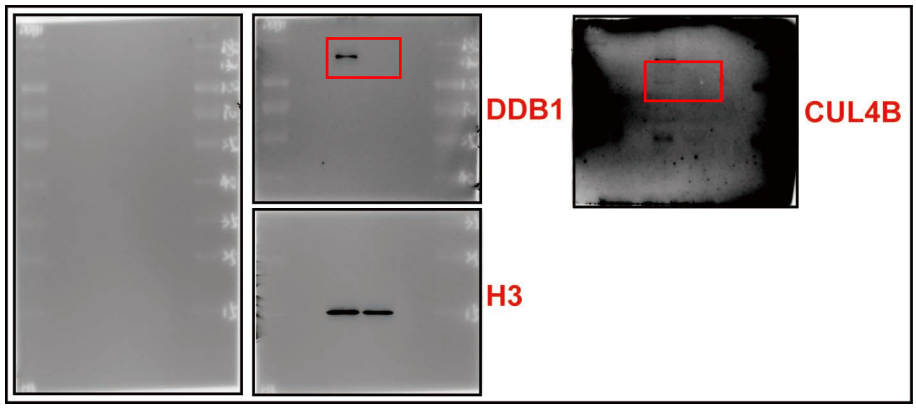


Uncropped blot Images for Fig.1A, Fig.3C,D,E,F and Fig.4B.

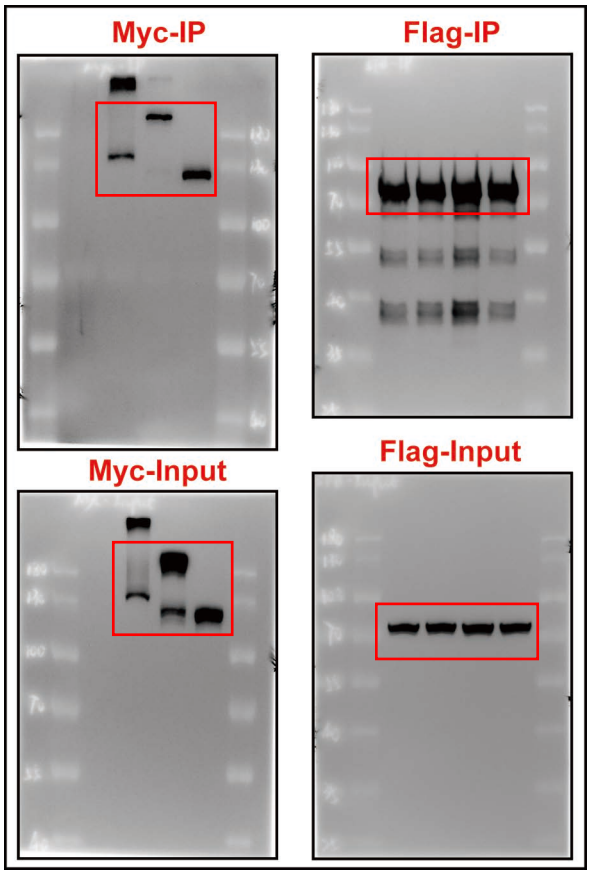
For Fig. 1A



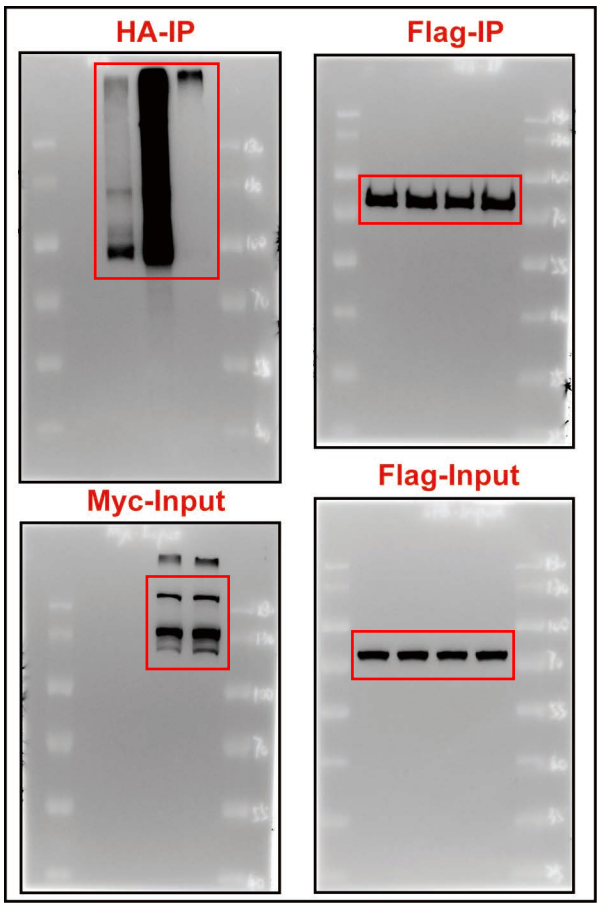
For Fig. 3E



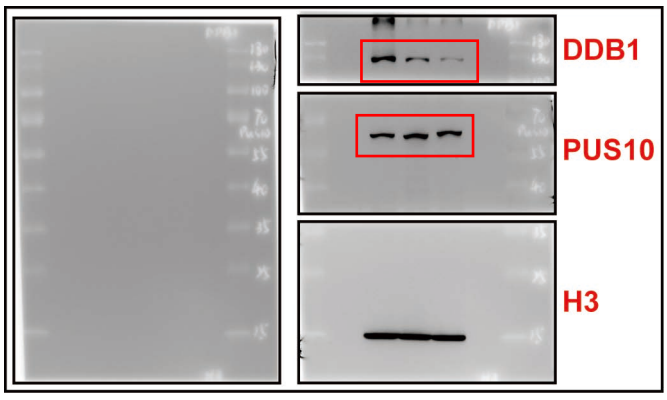
For Fig. 3C



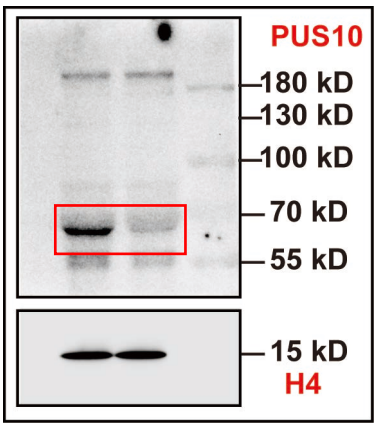
For Fig. 3D



For Fig. 3F



For Fig. 4B



Uncropped blot Images for Supplemental Fig.1A

For Supplemental. Fig. 1A