

Appendix

The tRNA pseudouridine synthase TruB1 regulates the maturation of let-7 miRNA

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1. Reagents and Tools Table

Reagents and Tools Table

Reagent/Resource	Reference or Source	Identifier or Catalog Number
	<p>Source (public): Stock center, company, other labs</p> <p>Reference: list relevant study (e.g. Smith et al, 2017) if referring to previously published work; use “this study” if new.</p> <p>If neither applies: briefly explain.</p>	Provide catalog numbers, stock numbers, database IDs or accession numbers, RRDs or other relevant identifiers.
<p>Experimental Models</p> <p>List cell lines, model organism strains, patient samples, isolated cell types etc. Indicate the species when appropriate.</p>		
Human cell line: HEK293FT	Thermo Fisher Scientific	Cat# R70007
Human cell line: A549	ATCC	Cat# ATCC® CCL-185
Human cell line: HeLa	ATCC	Cat# ATCC® CCL-2
<i>E. coli</i> BL21 (DE3)	Novagen	Cat# 69450
<i>E. coli</i> DH5α Competent Cells	Takara	Cat# 9057
<p>Recombinant DNA</p> <p>Indicate species for genes and proteins when appropriate</p>		
pcDNA3.1 (+)	Thermo Fisher Scientific	Cat# V79020
pcDNA3.1 containing GFP, TruB1 or its mutants sequences; see Materials and Methods and Appendix Table S4	This study	n/a
pLuc AKP	Our lab (Ito et al., 2017)	n/a
pLuc AKP containing antisense of let-7a or 3'UTR of KRAS sequences; see Materials and Methods and Appendix Table S4	This study	n/a
pRL-SV40	Promega	Cat# E2231
pCS2	Riken	Cat# RDB15116
pCLT; See Appendix Table S5	This study	n/a
pCLT containing GFP, TruB1 or its mutants sequences; see Materials and Methods and Appendix Table S4	This study	n/a
pCMV-VSVG-RSV-Rev	Addgene	Cat# #8454

pCAG-HIVgp	Addgene	Cat# #35616
pET22b	Merck Millipore	Cat# 69744
pET22b containing TruB1 or its mutants sequences; see Materials and Methods and Appendix Table S4	This study	n/a
Antibodies <i>Include the name of the antibody, the company (or lab) who supplied the antibody, the catalogue or clone number, the host species in which the antibody was raised and mention whether the antibody is monoclonal or polyclonal. Please indicate the concentrations used for different experimental procedures.</i>		
Anti-DDDDK-tag mAb (Flag, FLA-1)	MBL	Cat# M185-3L; RRID: AB_2687989
DGCR8 antibody	Abcam	Cat# Ab90579; RRID: AB_2049575
LIN28B (D4H1) antibody	Cell Signaling Technology	Cat# 11965; RRID: AB_2750978
Monoclonal Anti-beta-Actin antibody produced in mouse	Sigma-Aldrich	Cat# A5316; RRID: AB_476743
Anti-Mouse IgG, HRP-Linked Whole Ab Sheep	GE Healthcare	Cat# NA931
Anti-Rabbit IgG, HRP-Linked Whole Ab Donkey	GE Healthcare	Cat# NA934
Oligonucleotides and other sequence-based reagents <i>For long lists of oligos or other sequences please refer to the relevant Table(s) or EV Table(s)</i>		
See appendix Table S4 for all sequences of primers, probe and other nucleotides used in this paper.		
Chemicals, Enzymes and other reagents <i>(e.g. drugs, peptides, recombinant proteins, dyes etc.)</i>		
EcoR1	Takara	Cat# 1040A

Xho1	Takara	Cat# 1094A
Kpn1	Takara	Cat# 1068A
Nhe1	Takara	Cat# 1241A
Nde1	Takara	Cat# 1161A
BamH1	Takara	Cat# 1010A
Not1	Takara	Cat# 1166A
PrimeSTAR Max DNA Polymerase	Takara	Cat# R045A
DMEM medium	Sigma Aldrich	Cat# D5796
FBS, Qualified	Gibco	Cat# 10270106
OptiMEM	Gibco	Cat# 31985070
Penicillin-Streptomycin Solution	Wako	Cat# 16823191
Lipofectamine RNAiMAX	Thermo Fisher Scientific	Cat# 13778100
Fugene HD Transfection Reagent	Promega	Cat# E2311
Doxycycline	Clontech	Cat# 631311
PEI Max reagent	Polysciences	Cat# 24765-1
Polybrene	Merck Millipore	Cat# TR-1003
Puromycin	Thermo Fisher Scientific	Cat# A1113802
Alt-R® CRISPR-Cas9 crRNA; See Table S4	Integrated DNA Technologies	n/a
Alt-R® CRISPR-Cas9 tracrRNA	Integrated DNA Technologies	Cat# 1072532
Nuclease Free Duplex Buffer	Integrated DNA Technologies	Cat# 11010301
Alt-R® S.p. Cas9 Nuclease V3	Integrated DNA Technologies	Cat# 1081059
Trizma® base	Sigma Aldrich	Cat# T1503
Urea	Nacalai tesque	Cat# 35940-81
Chloroform	Wako	Cat# 038-02606
HCL	Wako	Cat# 086-03925
2-Propanol (isopropanol)	Wako	Cat# 166-04831
Glycerol	Wako	Cat# 075-00611
3M Sodium Acetate	NIPPON GENE	Cat# 316-90081
Polyoxyethylene(20)Sorbitan Monolaurate	Wako	Cat# 166-21115
ISOGEN	NIPPON GENE	Cat# 311-02501
THUNDERBIRD® SYBR qPCR Mix	TOYOBO	Cat# QPS-201
THUNDERBIRD® Probe qPCR Mix	TOYOBO	Cat# QPS-101
32P-γ-ATP	Perkin Elmer	Cat# NEG502Z
32P-α-UTP	Perkin Elmer	Cat# NEG507T
T4PNK	Takara	Cat# 2021S
Gel loading buffer II	Thermo Fisher Scientific	Cat# AM8547
Expresshyb	Clontech	Cat# 636832
EDTA	Wako	Cat# 345-01865
Boric Acid	Wako	Cat# 021-02195

Citric Acid (for SSC)	Wako	Cat# 038-06925
SDS	Wako	Cat# 191-07145
TaqMan™ Universal PCR Master Mix, No AmpErase UNG	Thermo Fisher Scientific	Cat# 4324018
DOC	Wako	Cat# 194-08311
BlockingOne	Nacalai tesque	Cat# 03953-95
RNase T1	Thermo Fisher Scientific	Cat# EN0541
TURBO™ DNase	Thermo Fisher Scientific	Cat# AM2238
Dynabeads™ Protein G	Thermo Fisher Scientific	Cat# 10004D
CIP	Takara	Cat# 2250A
T4 RNA ligase1	New England Biolabs	Cat# M0204S
NuPAGE® MOPS SDS Running Buffer	Thermo Fisher Scientific	Cat# NP0001
Proteinase K	Roche	Cat# 3115879001
Acid phenol :CHCL3	Thermo Fisher Scientific	Cat# AM9720
Pseudouridine	Carbosynth	Cat# NP09389
KCL	Wako	Cat# 163-03545
PMSF	Sigma Aldrich	Cat# P7626
DTT	Sigma Aldrich	Cat# D0632
MgCL2	Sigma Aldrich	Cat# 19-0220-5
isopropyl-β-D thiogalactopyranoside	Nacalai tesque	Cat# 19742-36
β-mercaptoethanol	Wako	Cat# 131-14572
imidazole	Wako	Cat# 093-00011
phenylmethylsulfonyl fluoride	Wako	Cat# 800263
NH4CL	Wako	Cat# 017-02995
Nuclease P1	New England Biolabs	Cat# M0660S
CMC	Tokyokasei	Cat# C0793
Bicine	DOJINDO	Cat# 150-25-4
Na2CO3	Wako	Cat# 197-01581
Dideoxynucleoside Triphosphate Set	Sigma Aldrich	Cat# 3732738001
phenol/chloroform/isoamyl alcohol	NIPPON GENE	Cat# 311-90151
RQ1 Dnase	Promega	Cat# M610A
Rnasin® Plus	Promega	Cat# N261B
Clean Blot IP Detection Reagent (HRP)	Thermo Fisher Scientific	Cat# 21230
Software <i>Include version where applicable</i>		
Bioconductor	https://www.bioconductor.org/	n/a
FastQC	https://github.com/s-andrews/FastQC	n/a
featureCounts	Liao et al., 2014	n/a

Bowtie2	Langmead and Salzberg, 2012	http://bowtie-bio.sourceforge.net/bowtie2/index.shtml
SAMtools	Li et al., 2009	http://www.htslib.org
cutadapt (v.1.2.1)	Martin, 2011	http://cutadapt.readthedocs.io/en/stable/guide.html
FASTX-toolkit (v0.0.14)	Hannon Lab	http://hannonlab.cshl.edu/fastx_toolkit/
RSeQC (v2.6.4)	Wang et al., 2012	http://rseqc.sourceforge.net/
bedtools (v2.26.0)	Quinlan and Hall, 2010	http://bedtools.readthedocs.io/en/latest/
seqkit	Shen et al., 2016	https://bioinf.shenwei.me/seqkit/
MEME	Bailey et al., 2009	v.4.9.1. http://meme-suite.org
IGV	Robinson et al., 2011	https://software.broadinstitute.org/software/igv/
tRNAscan-SE 2.0	Low et al., 2016	http://lowelab.ucsc.edu/tRNAscan-SE/
R	https://www.r-project.org/	n/a
ImageJ	Schneider et al., 2012	https://imagej.nih.gov/ij/
Other <i>(Kits, instrumentation, laboratory equipment, lab ware etc. that are critical for the experimental procedure and do not fit in any of the above categories)</i>		
NEPA21 Super Electroporator	NEPAGENE	Cat# NEPA21
ECO DROPPER III	AS ONE	Cat# ICT-896III
ARVO X3	Perkin Elmer	Cat# 2030-0030A
MicroSpin G-25 Columns	GE Healthcare	Cat# 27532501
Hybond-N+	GE Healthcare	Cat# RPN203B
nitrocellulose membrane (Amersham Protran NC 0.2)	GE Healthcare	Cat# 10600001
7900HT Fast Real-Time PCR System	Thermo Fisher Scientific	Cat# 7900HT
PVDF (Immobilon® Membrane, 0.45um)	Merck Millipore	Cat# IPVH00010
CL-1000 Ultraviolet Crosslinker	UVP	Cat# 95-0174-03
Bioraptor® UCD-300	Cosmo BIO	Cat# UCD-300
NuPAGE™ 10% Bis-Tris gel	Thermo Fisher Scientific	Cat# NP0301BOX
Qubit3.0	Thermo Fisher Scientific	Cat# Q33216
TapeStation 2100 Bioanalyzer	Agilent technologies	n/a
Illumina MiSeq	Illumina	Cat# SY-410-1003
Ni-NTA agarose column	QIAGEN	Cat# 30210

HiTrap Heparin column	GE Healthcare	Cat# 17040601
HiLoad 16/60 Superdex 200 column	GE Healthcare	Cat# 28989335
TLC, Cellulose	Merck Millipore	Cat# 1.05716.0001
PrimeSTAR Mutagenesis Basal Kit	Takara	Cat# R046A
Nano Glo HiBiT Lytic Detection System	Promega	Cat# N2420
Dual-Glo Luciferase Assay System	Promega	Cat# E2920
SuperScript™ III First-Strand Synthesis System	Thermo Fisher Scientific	Cat# 18080051
TaqMan™ MicroRNA Reverse Transcription Kit	Thermo Fisher Scientific	Cat# 4366596
TaqMan™ MicroRNA Assays; See Table S4.	Thermo Fisher Scientific	n/a
mirVana™ miRNA Isolation kit	Thermo Fisher Scientific	Cat# AM1560
Megaplex™ RT Primers, Pool A (human)	Thermo Fisher Scientific	Cat# 4399966
TaqMan™ Array Human MicroRNA A Cards v2.0	Thermo Fisher Scientific	Cat# 4398965
ECL Select Western Blotting Detection Reagent	GE Healthcare	Cat# RPN2235
Phusion High Fidelity DNA Polymerase	New England Biolabs	Cat# M0530
NEB Next Library Quant Kit for Illumina	New England Biolabs	Cat# E7630S
MiSeq Reagent Kit v3	Illumina	Cat# 15043893
MEGA script T7 transcription Kit	Thermo Fisher Scientific	Cat# AM1333
RNeasy MinElute Cleanup kit	QIAGEN	Cat# 74204
RealTime-Glo MT Cell Viability Assay	Promega	Cat# G9712
High Sensitivity DNA Kit	Agilent technologies	Cat# 5067-4626
Decade Markers System (to make radiolabeled RNA markers)	Thermo Fisher Scientific	Cat# AM7778