Supplemental Material

EFFICIENT FRACTIONATION AND ANALYSIS OF RIBOSOME ASSEMBLY INTERMEDIATES IN HUMAN CELLS

Figure S1

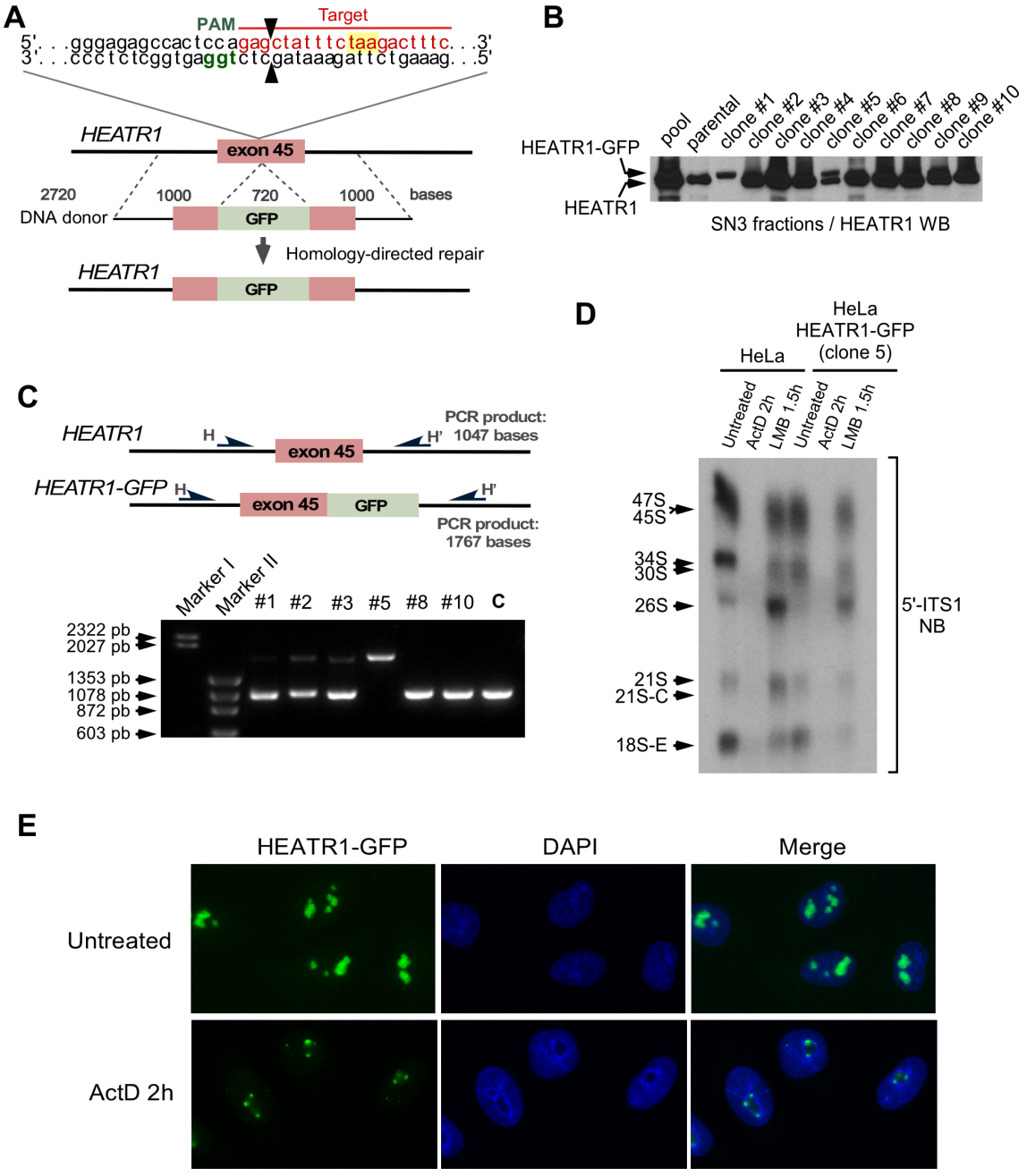
A
PAM
Target
5'. . .gggagagccactccagagctatttctaagactttc. . .3'
3'. . .ccctctcggtgaggtctcgataaagattctgaaag. . .5'
HEATR1
exon 45
2720
1000
720
1000
bases
DNA donor
GFP
Homology-directed repair
HEATR1
GFP
B
HEATR1-GFP
HEATR1
pool
parental
clone #1
clone #2
clone #3
clone #4
clone #5
clone #6
clone #7
clone #8
clone #9
clone #10
SN3 fractions / HEATR1 WB
C
HEATR1
H
exon 45
H'
PCR product:
1047 bases
HEATR1-GFP
H
exon 45
GFP
H'
PCR product:
1767 bases
Marker I
Marker II
#1
#2
#3
#5
#8
#10
C
2322 pb
2027 pb
1353 pb
1078 pb
872 pb
603 pb
D
HeLa
HeLa
HEATR1-GFP
(clone 5)
Untreated
ActD 2h
LMB 1.5h
Untreated
ActD 2h
LMB 1.5h
47S
45S
34S
30S
26S
21S
21S-C
18S-E
5'-ITS1
NB
E
HEATR1-GFP
DAPI
Merge
Untreated
ActD 2h

Figure S1. **Generation of a HeLa-derived cell line that endogenously expresses HEATR1-GFP. (A)** Scheme showing the CRISPR strategy used to edit the *HEATR1* locus. The sequence targeted by the gRNA (in red), the protospacer adjacent motif (PAM) sequence (in green), the STOP codon (in yellow), the cleavage site (arrows) and the resulting knock-in product are shown. Sizes (bp) of the GFP sequence, left arm and right arm in the donor repair cassette are also indicated. **(B, C)** Western blot and PCR analyses of GFP-positive HeLa-derived clones selected after CRISPR-mediated gene editing. Cells of clone #5 express both GFP-fused and non-fused HEATR1. **(D)** Northern blot analyses showing the relative contents of 18S pre-rRNA species in the selected clone #5, in untreated and in ActD- and LMB- treated cells. The pre-rRNAs profiles of the gene-edited clones are similar to those observed in the parental HeLa line. **(E)** Subcellular distribution of HEATR1-GFP in gene-edited cells (clone #5) untreated and treated with ActD for 2 h. The protein is nucleolar and localizes to the nucleolar caps upon ActD treatment, which indicates that it is present in the dense fibrillar component (as expected for a component of the UTP-A subcomplex). The normal PSE fractionation profile and interaction with RBFs and the 30S pre-rRNA (Figs. 2B and 2D) also indicate that HEATR1-GFP is properly recruited to primary pre-40S ribosomes.

Figure S2

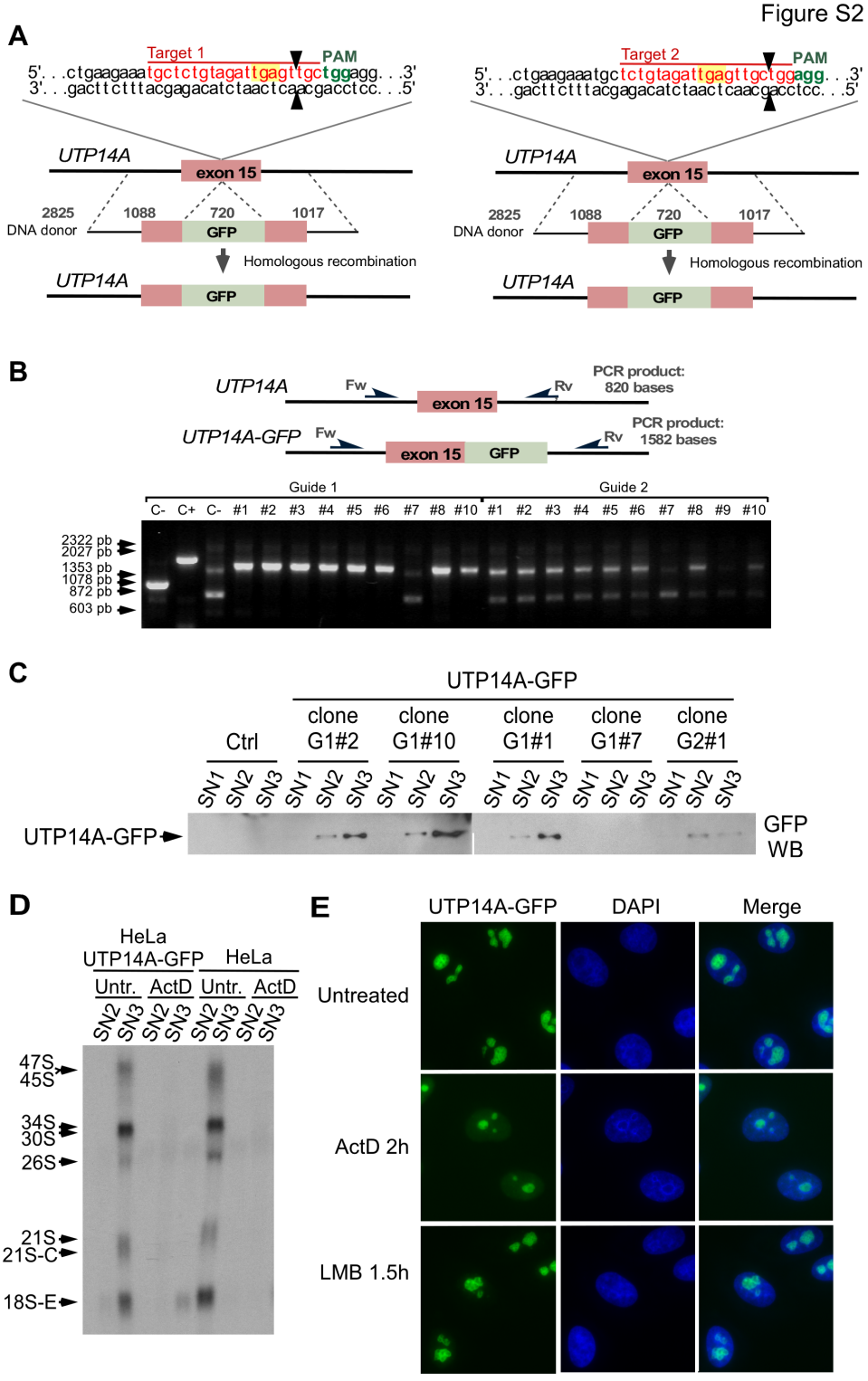
A
Target 1
PAM
5'. . .ctgaagaaatgctctgtagattgagttgctggagg. . .3'
3'. . .gacttctttacgagacatctaactcaacgacctcc. . .5'
UTP14A
exon 15
2825
DNA donor
1088
720
GFP
1017
Homologous recombination
UTP14A
GFP
Target 2
PAM
5'. . .ctgaagaaatgctctgtagattgagttgctggagg. . .3'
3'. . .gacttctttacgagacatctaactcaacgacctcc. . .5'
UTP14A
exon 15
2825
DNA donor
1088
720
GFP
1017
Homologous recombination
UTP14A
GFP
B
UTP14A
Fw
exon 15
Rv
PCR product:
820 bases
UTP14A-GFP
Fw
exon 15
GFP
Rv
PCR product:
1582 bases
Guide 1
Guide 2
C- C+ C- #1 #2 #3 #4 #5 #6 #7 #8 #10 #1 #2 #3 #4 #5 #6 #7 #8 #9 #10
2322 pb
2027 pb
1353 pb
1078 pb
872 pb
603 pb
C
UTP14A-GFP
Ctrl
clone G1#2
clone G1#10
clone G1#1
clone G1#7
clone G2#1
SN1 SN2 SN3 SN1 SN2 SN3 SN1 SN2 SN3 SN1 SN2 SN3 SN1 SN2 SN3 SN1 SN2 SN3
UTP14A-GFP
GFP WB
D
HeLa
UTP14A-GFP
HeLa
Untr. ActD Untr. ActD
SN2 SN3 SN2 SN3 SN2 SN3 SN2 SN3
47S
45S
34S
30S
26S
21S
21S-C
18S-E
E
UTP14A-GFP
DAPI
Merge
Untreated
ActD 2h
LMB 1.5h

Figure S2. **Generation of a HeLa-derived cell line that endogenously expresses UTP14A-GFP. (A)** Scheme showing the CRISPR strategy used to edit the *UTP14A* locus. Two independent gRNAs were tried. The sequence targeted by the gRNA (in red), the protospacer adjacent motif (PAM) sequence (in green), the STOP codon (in yellow), the cleavage site (arrows) and the resulting knock-in product are shown. Sizes (bp) of the GFP sequence, left arm and right arm in the donor repair cassette are also indicated. **(B)** PCR analyses of GFP-positive HeLa-derived clones selected after CRISPR-mediated gene editing. **(C)** PSE fractionation profiles of three clones (G1#2, G1#10, G1#1) that, based on the PCR analyses, have the GFP knock-in introduced in all alleles of *UTP14A*. Two other clones with partial knock-ins were also analyzed. **(D)** Northern blot analyses showing the relative contents of 18S pre-rRNA species in the selected clone #10, in untreated and in ActD- and LMB- treated cells. The pre-rRNAs profiles of the gene-edited clone are similar to those observed in the parental HeLa line. **(E)** Subcellular distribution of UTP14A-GFP in the gene-edited selected cell line (clone #10) untreated and treated with ActD and LMB for the indicated times. The protein is nucleolar and remains in the nucleolar interior, excluded from the nucleolar caps, upon ActD treatment. This result is consistent with the protein's presence in the granular component of the nucleolus, which is the expected localization for UTP14A. The normal localization, PSE fractionation profile and growth of cells in the absence of the wild-type protein indicate that the UTP14-GFP fusion is fully functional.

Supplementary Table 1. Plasmids used in this study

Name	Description	Use	Reference
pX330	pX330-U6-Chimeric_BB-CBh-hSpCas9	Generation of Cas9/sg plasmids	Feng Zhang generous deposit at Addgene (plasmid # 42230)
pBN62	pX330-sg1CHEATR1	GFP knock-in at HEATR1 C-terminus (Cas9/sg plasmid)	This study
pHEATR1-HDR	pMA-gHEATR1(Chr.1: 236551904 - 236550905)-GFP-gHEATR1(Chr.1: 236550904 - 236549902)	GFP knock-in at HEATR1 C-terminus (HDR donor plasmid)	This study (GeneArt, Invitrogen)
pSG10	pX330-sg1CUTP14A	GFP knock-in at UTP14A C-terminus (Cas9/sg plasmid)	This study
pSG12	pBluescript-gUTP14A (Chr.X: 129928518 - 129929605)-GFP-gUTP14C (Chr.X: 129929606-129930626)	GFP knock-in at UTP14A C-terminus (HDR donor plasmid)	This study

Supplementary Table 2. Oligonucleotides used in this study

No	Description	Sequence	Use
1	sgHEATR1 GUIDE1 F	CACCGGAAAGTCTTAGAAATAGCTC	Generation of Cas9/sg plasmid for *HEATR1* edition
2	sgHEATR1 GUIDE1 R	AAACGAGCTATTTCTAAGACTTTCC	Generation of Cas9/sg plasmid for *HEATR1* edition
3	gHEATR1 F2	GCCAAAAGACTATTCAGCAACTGG	Confirmation of GFP insertion at C-terminal end of HEATR1
4	gHEATR1 R2	GCCTTTTGCTTGCCACATTTCTGC	Confirmation of GFP insertion at C-terminal end of HEATR1
5	sgCUTP14A F1	CACCGTGCTCTGTAGATTGAGTTGC	Generation of Cas9/sg plasmid for *UTP14A* edition
6	sgCUTP14A R1	AAACGCAACTCAATCTACAGAGCAC	Generation of Cas9/sg plasmid for *UTP14A* edition
7	gUTP14A F1	CTGACATGCTAGCGGCTGAGGCGGGTGGATCACG AGGTCAGG	Generation of HDR donor for UTP14A edition
8	gUTP14A R1	CCAATGCCCGGGGCATCTACAGAGCATTTCTTCAG CTGTTTTTTGTGACG	Generation of HDR donor for UTP14A edition
9	gUTP14A F2	CTGACATAAGCTTGAGTTGCTGGAGGAGTGACAG CCAGGAGC	Generation of HDR donor for UTP14A edition
10	gUTP14A R2	CCAATGGGTACCGGTTCATAACGTTCAACATATTT TTAGAGG	Generation of HDR donor for UTP14A edition
11	gUTP14A F3	GCAGTGTCAGGGTGGTTTTTCAGACC	Confirmation of GFP insertion at C-terminal end of HEATR1
12	gUTP14A R3	CCTTGGTAAGAAAAGGAGGTGGCCC	Confirmation of GFP insertion at C-terminal end of HEATR1
13	5'-ITS1	CCTCGCCCTCCGGGCTCCGGGCTCCGTTAATGATC	Northern blot probe

Supplementary Table 3. siRNAs used in this study

siRNA	Gene Symbol	Gene name	Gene ID	siRNA ID	Sense siRNA Sequence	Source/Reference
si-ctrl		Silencer Select Negative Control No.1				Ambion / #4390843
UTP14A	*UTP14A*	U3 small nucleolar ribonucleoprotein, holomog A	10813	s21250	GGCCCAUGGAGUUUCUGAAtt	Ambion / #4392420
PWP2	*PWP2*	PWP2 periodic tryptophan protein homolog	5822	s11611	GAACCGAAGAAAAAUGACAtt	Ambion / #4392420
RPL5-1	*RPL5*	Ribosomal protein L5	6125	Custom (AD89JPV)	GGUUGGCCUGACAAAUUAUtt	Ambion / #4390827
RPL5-2	*RPL5*	Ribosomal protein L5	6125	Custom (ADAAYVO)	CUACCACUGGCAAUAAAGUtt	Ambion / #4390827
RPS6	*RPS6*	Ribosomal protein S6	6194	Custom (ADBJW1W)	GAAAGCCCUUAAAUAAAGAtt	Ambion / #4390827

Supplementary Table 4. Antibodies used in this study

Antibody	Raised in	Use	Source / Reference
ENP1	Rabbit	Western blot	Bethyl / A304-568A
Fibrillarin	Rabbit	Western blot	Santa Cruz (H-140) / sc-25397
GFP	Mouse	Western blot	Clontech / 632381
HEATR1	Mouse	Western blot	Santa Cruz (B-11) / sc-390445
Histone H3	Rabbit	Western blot	Abcam / ab1791
LTV1	Rabbit	Western blot	Sigma / HPA030161
PCNA	Mouse	Western blot	Abcam (PC10) / ab29
p53	Mouse	Western blot	Cell Signaling (1C12) / #2524
RIO2	Rabbit	Western blot	Novus Biologicals / NBP1-30098
RRP12	Mouse	Western blot	Santa Cruz (A-3) / sc-398593
RPL11	Rabbit	Western blot	Abcam / ab79352
RPL5	Rabbit	Western blot	Bethyl / A303-933A
RPS6	Rabbit	Western blot	Bethyl / A300-557A
TBL3	Rabbit	Western blot	Sigma / HPA042562
Tubulin	Mouse	Western blot	Calbiochem / CP06