Supplemental Materials

Molecular Biology of the Cell

Zorbas et al.

FIGURE S1

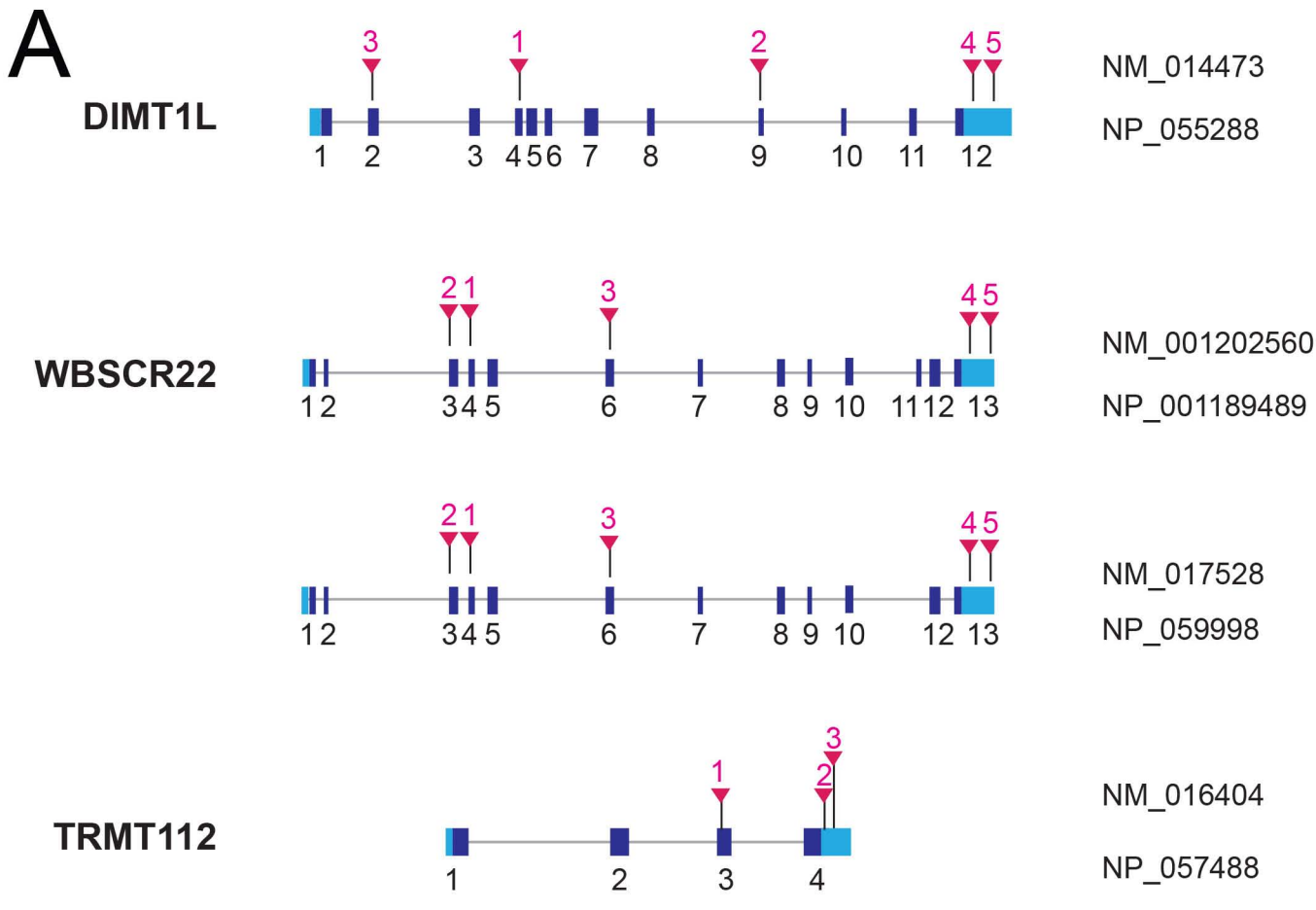

A
DIMT1L
NM_014473
NP_055288
WBSCR22
NM_001202560
NP_001189489
NM_017528
NP_059998
TRMT112
NM_016404
NP_057488
B
Remaining mRNA level (relative to Scr)
120
100
80
60
40
20
0
1
3
2
2
2
5
1
3
8
11
25
16
23
Scr #1 #2 #3 #4 #5
Scr #1 #2 #3
Scr #1 #2 #3 #4 #5
WBSCR22
TRMT112
DIMT1L

FIGURE S2

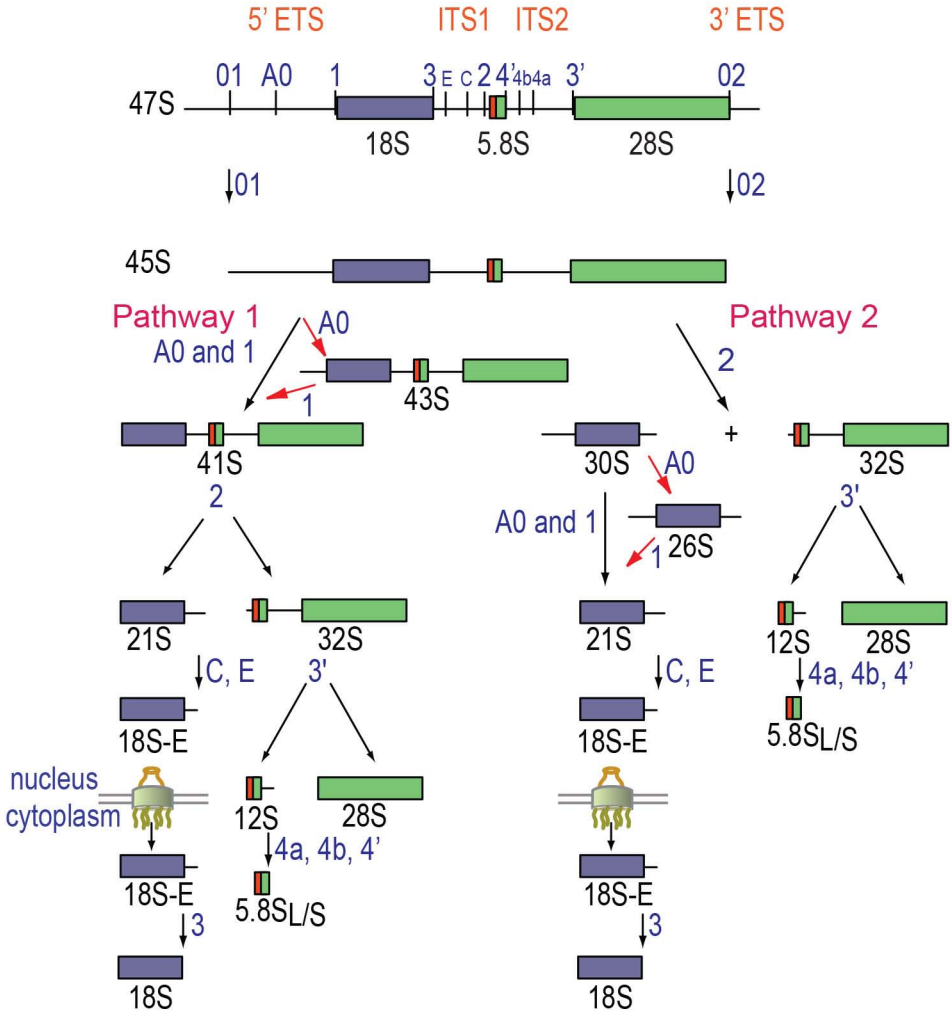

5' ETS
ITS1
ITS2
3' ETS
01
A0
1
3
E
C
2
4'
4b
4a
3'
02
47S
18S
5.8S
28S
01
02
45S
Pathway 1
A0
A0 and 1
1
43S
41S
2
Pathway 2
2
+
30S
A0
32S
A0 and 1
3'
1
26S
21S
32S
21S
12S
28S
C, E
3'
C, E
4a, 4b, 4'
18S-E
18S-E
5.8SL/S
nucleus
cytoplasm
12S
28S
4a, 4b, 4'
18S-E
18S-E
5.8SL/S
3
3
18S
18S

FIGURE S3

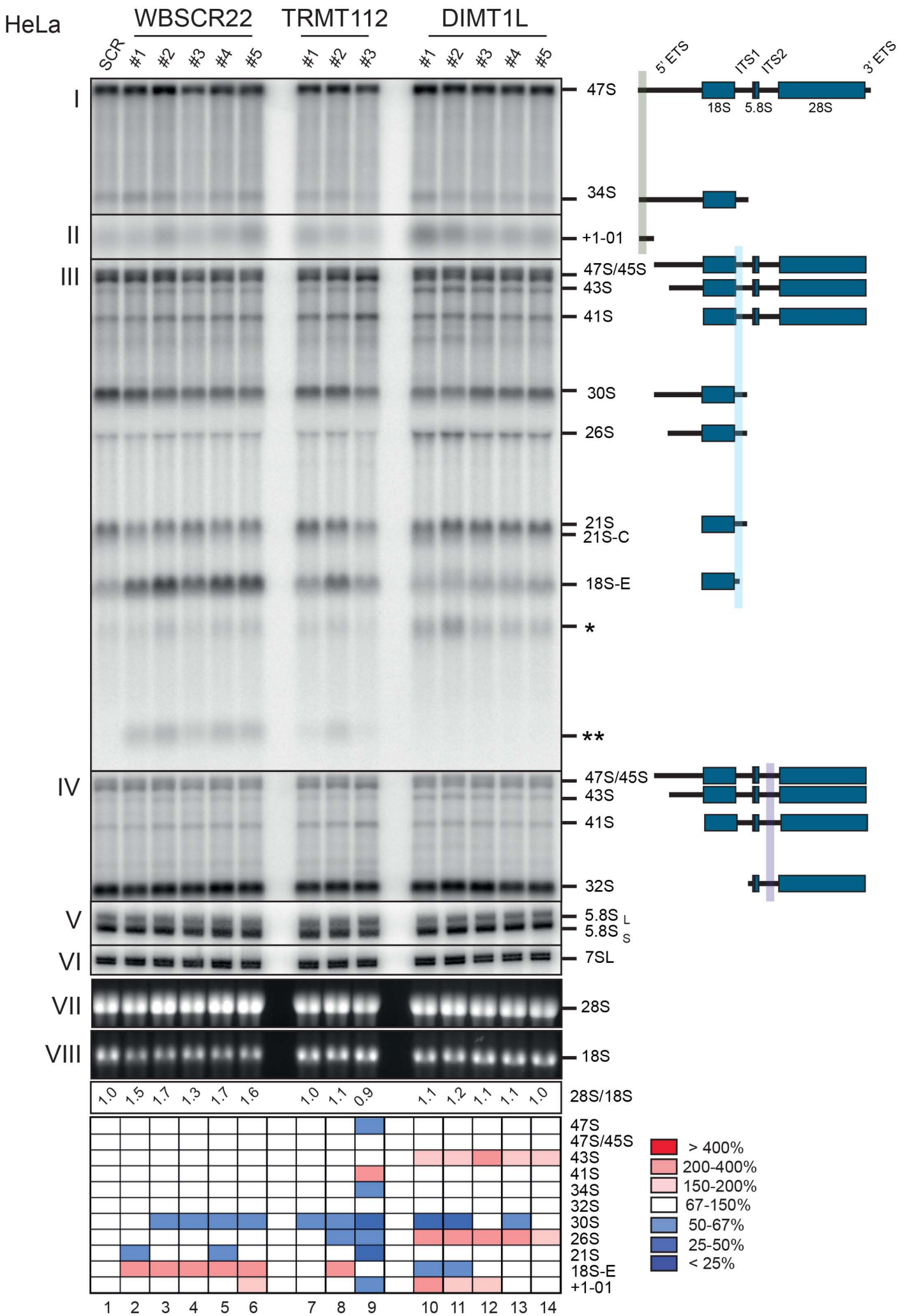
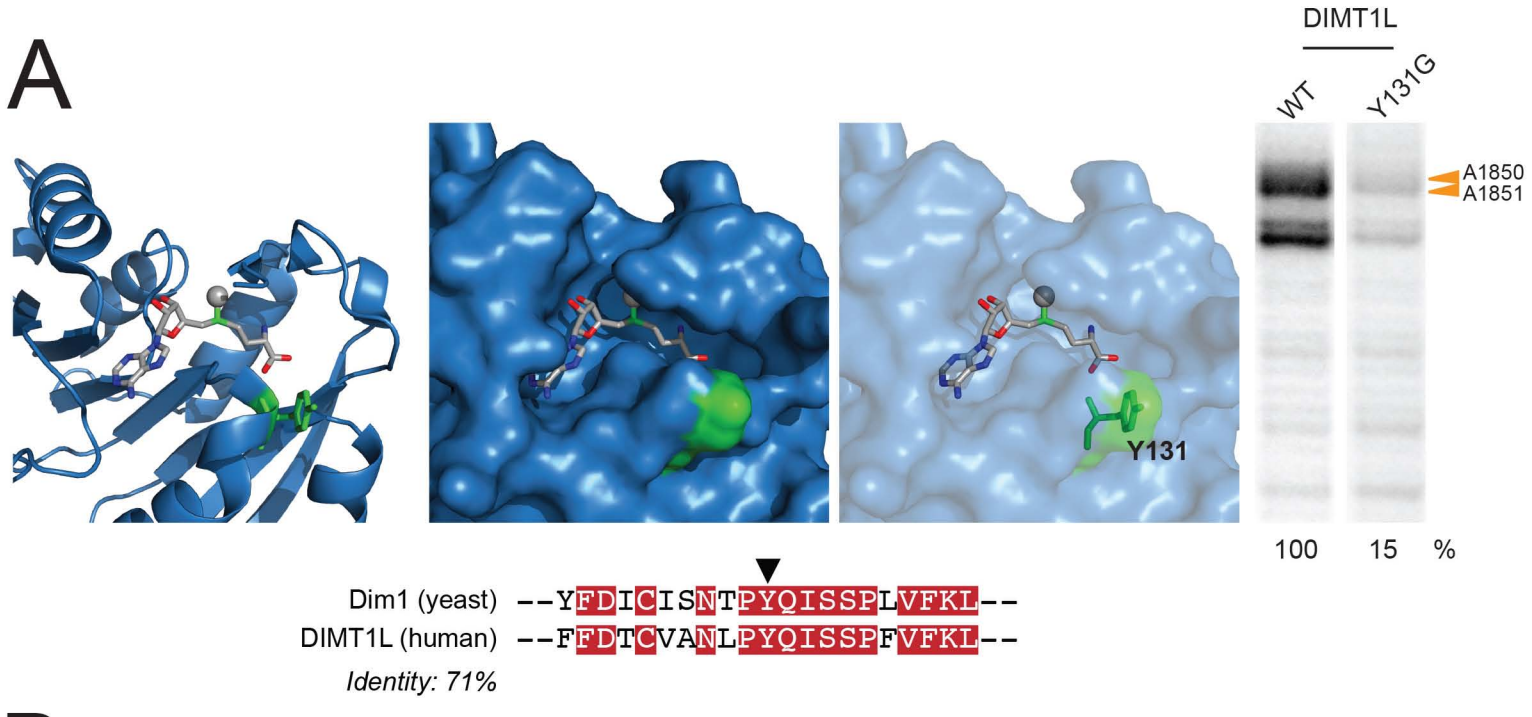

HeLa
WBSCR22
TRMT112
DIMT1L
SCR
#1
#2
#3
#4
#5
I
II
III
IV
V
VI
VII
VIII
47S
34S
+1-01
47S/45S
43S
41S
30S
26S
21S
21S-C
18S-E
*
**
32S
5.8S L
5.8S S
7SL
28S
18S
5' ETS
ITS1
ITS2
3' ETS
18S
5.8S
28S
1.0 1.5 1.7 1.3 1.7 1.6 1.0 1.1 0.9 1.1 1.2 1.1 1.1 1.0
28S/18S
47S
47S/45S
43S
41S
34S
32S
30S
26S
21S
18S-E
+1-01
> 400%
200-400%
150-200%
67-150%
50-67%
25-50%
< 25%
1 2 3 4 5 6 7 8 9 10 11 12 13 14

FIGURE S4


A
DIMT1L
WT
Y131G
A1850
A1851
Y131
100
15
%
Dim1 (yeast) --YFDICISNTPYQISSPLVFKL--
DIMT1L (human) --FFDTCVANLPYQISSPFVFKL--
Identity: 71%

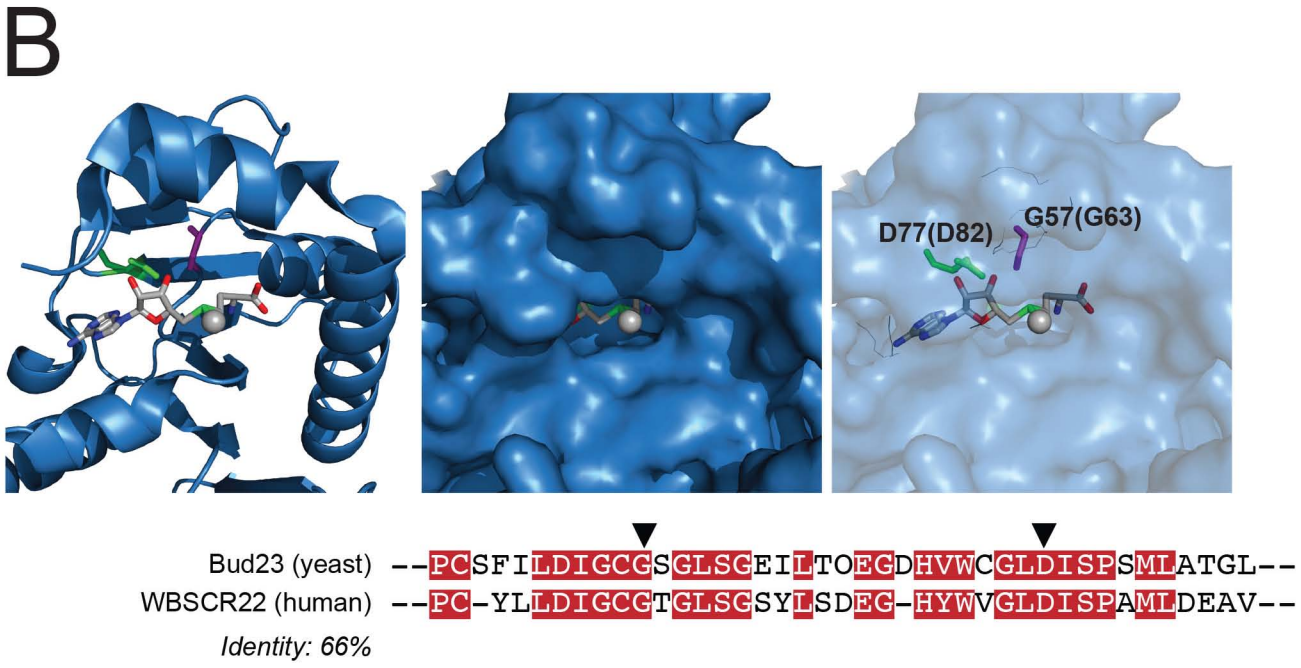

B
D77(D82)
G57(G63)
Bud23 (yeast) --PCSFILDIGCGSGLSGEILTQEGDHVWCGLDISPSMLATGL--
WBSCR22 (human) --PC-YLLDIGCGTGLSGSYLSDEG-HYWVGLDISPAMLDEAV--
Identity: 66%

Fig S1: Positions of siRNAs on transcripts targeted in this work, and siRNA-mediated depletion efficiency
A, The regions targeted by the siRNAs on mature mRNAs are shown on the structure of the pre-mRNAs. Exons are shown as blue boxes, introns as thin black lines. The 5' and 3' UTR elements are in cyan. There are two known isoforms of WBSCR22; both are targeted by the siRNAs used. siRNAs, depicted as red arrowheads, are numbered from 1 to 5.
B, The level of residual mRNAs was tested by qRT-PCR on total RNA extracted from HeLa cells incubated for 3 d with each siRNA. The signal was normalized to GAPDH, and expressed with respect to a nontargeting (Scr) control.

Fig S2: Simplified pre-rRNA processing pathway in human cells
Three of the four mature rRNAs, the 18S, 5.8S, and 28S rRNAs are produced from a single RNA Pol I transcript (47S). The 18S rRNA is the RNA component of the small subunit (40S); 5.8S and 28S are incorporated into the large subunit (60S). There is a third rRNA in the 60S subunit, 5S, which is independently produced by RNA Pol III (not shown). The mature sequences are embedded in noncoding 5' and 3' external transcribed spacers (ETS) and internal transcribed spacers (ITS1 and 2). Cleavage sites (in blue) and alternative pathways are indicated. For details, see www.ribosomesynthesis.com and ref. Mullineux and Lafontaine, 2012.

Fig S3: Pre-rRNA processing of HeLa cells depleted of WBSCR22, TRMT112, or DIMT1L
Total RNA extracted from HeLa cells treated for 3 d with an siRNA specific to the indicated target was resolved on a denaturing agarose gel and analyzed by northern blotting with specific oligonucleotide probes. Five distinct siRNAs (#1 to #5) were used for DIMT1L and WBSCR22 and three for TRMT112 (see Fig S1 and Table S3). A non-targeting control (SCR) was used as control. The positions of the probes used and the pre-rRNA intermediates detected are indicated to the right of the northern-blot panels. Detection of the RNA component of the signal recognition particle (7SL) was used as a loading control (panel VI). The probes used were as follows: panels I and II, LD1844; panel III, LD1827; panel IV, LD1828; panel V, probe LD2132; panel VI, probe LD2133. The mature 18S and 28S rRNAs were visualized by ethidium bromide staining of the agarose gel (panels VII and VIII). The mature 28S/18S ratio was calculated from Agilent Bioanalyzerelectropherograms. The major pre-rRNA intermediates detected were quantitated with a Phosphorimager and represented as a heatmap with a color code indicating the respective abundances normalized with respect to the SCR control. Note that the 12S, 7S, and 5.8S+40 RNAs were detected and quantified, but are not shown for the sake of simplicity.

Fig S4: 3-D representations of the SAM binding pocket area of human DIMT1L and yeast Bud23

The models illustrate the positions of the mutated residues in the catalytic pocket of each methyltransferase. From left to right: ribbon representation, surface rendering, and surface rendering with transparence. SAM and the residues mutated in this work are highlighted and shown as sticks. The carbon atoms of SAM are shown in light grey and the methyl group transferred during the methylation reaction as a grey sphere. Conservation between the yeast and human proteins in the area targeted by mutagenesis is illustrated by pairwise alignments underneath. The sequence identity percentages are indicated.
A, Human DIMT1L (model based on PDB entry 1ZQ9): residue Y131, in the immediate vicinity of SAM, is indicated in green. Right panel, the residual dimethylation activity in HCT116 cells expressing the Y131G catalytically deficient allele of DIMT1L was estimated to be of ~15%.
B, Yeast Bud23 (model based on PDB entry 4QTU, see Létoquart *et al.*, 2014): residues D77 and G57, lining the SAM binding site, are shown in green and purple, respectively. The equivalent residues in human are numbered in brackets.

SUPPLEMENTAL TABLES

TABLE S1: plasmids

Plasmid name	insert	Marker, origin of replication	Reference
pET11a(His_6-WBSCR22) = **pVH475**	(His)6-WBSCR22 expressed from T7 promoter	Ap, Kn colE1 *E. coli* origin	This study
pACYC-Duet-1 (hTrm112) = **pFFh5**	TRMT112 expressed from T7 promoter	Ap, Kn, p15A *E. coli* origin	[1]
pDL0737	WBSCR22	in pDONR223	ORFeome, Prof Vidal (Harvard University)
pDL0811	pcDNA5-Flag	For tet-inducible Flag-Precision cleavage site-His-amino ter-tagged constructs	Gift from Prof Watkins (U. Newcastle)
pDL0846	DIMT1L	in pDONR223	ORFeome, Prof Vidal (Harvard University)
pDL0886	pcDNA5-Flag-DIMT1L-siRNAr-wt	A PCR fragment generated with oligonucleotides LD2819 and LD2820 on pDL0846 was cloned as a BamHI/XhoI fragment into pDL0811	This study
pDL0887	pcDNA5-Flag-DIMT1L-siRNAr-Y131G	Generated as pDL0886	This study
pDL0888	pcDNA5-Flag-WBSCR22-siRNAr-wt	A PCR fragment generated with oligonucleotides LD2817 and LD2818 on pDL0737 was cloned as a BamHI/XhoI fragment into pDL0811	This study
pDL0889	pcDNA5-Flag-WBSCR22-	Generated as pDL0888	This study

	siRNAr-G63E		
pDL0890	pcDNA5-Flag-WBSCR22-siRNAr-D82K	Generated as pDL0888	This study
pDL0891	pcDNA5-Flag-WBSCR22-siRNAr-G63E/D82K	Generated as pDL0888	This study

TABLE S2: oligonucleotides used for cloning purposes

Name	Sequence	Used for:
H6wbNdeI	GTACAAAAAAGTTCATATGCACCACCACCACCACCACGCGTCGCG CGGCCGGCGTCC	overexpress His6-WBSCR22 in bacteria
wbBglII	ACAAGAGATCTGATTAGAAGCGGGGCTTGC	overexpress His6-WBSCR22 in bacteria
LD2797	GATACTTGTGTGGCAAATTTGCCTGGACAGATCTCTTCGCCTTTTG TCTTC	DIMT1L Y131G FP
LD2798	GAAGACAAAAGGCGAAGAGATCTGTCCAGGCAAATTTGCCACACA AGTATC	DIMT1L Y131G RP
LD2161	GCTGGATATTGGCTGTGAGACTGGGCTGAGTGGAAG	WBSCR22 G63E FP
LD2162	CTTCCACTCAGCCCAGTCTCACAGCCAATATCCAGC	WBSCR22 G63E RP
LD2163	CTATTGGGTGGGCCTGAAAATCAGCCCTGCCATGC	WBSCR22 D82K FP
LD2164	GCATGGCAGGGCTGATTTTCAGGCCCACCCAATAG	WBSCR22 D82K RP
LD2665	ACTGGGCTGTCTGGTTCTTACTTATCAGATGAAGG	WBSCR22 siRNA s41529 silent mutation FP
LD2666	CCTTCATCTGATAAGTAAGAACCAGACAGCCCAGT	WBSCR22 siRNA s41529 silent mutation RP
LD2667	CACCCATCAATTTTCAGGAATGGGACGGCCTGGTGAGAATCACCT TTGTTAGGAAAAACAAGAC	DIMT1L siRNA s26097 silent mutation FP
LD2668	GTCTTGTTTTTCCTAACAAAGGTGATTCTCACCAGGCCGTCCCATT CCTGAAAATTGATGGGTG	DIMT1L siRNA s26097 silent mutation RP
LD2815	CTCTTTTTTCTGTTCTCGTCCGGGGTAGTCGAGCTGTCCTGCAGCT GTACCC	BamH1 cloning site removal from WBSCR22 ORF FP
LD2816	GGGTACAGCTGCAGGACAGCTCGACTACCCCGGACGAGAACAGA AAAAAGAG	BamH1 cloning site removal from WBSCR22 ORF RP
LD2817	CACCATCACCATGGATCCATGGCGTCGCGCGGCCGG	Cloning WBSCR22 into pCDNA5 FP
LD2818	GCTAGATCTAGACTCGAGCTAGAAGCGGGGCTTGCGC	Cloning WBSCR22 into pCDNA5 RP
LD2819	CACCATCACCATGGATCCATGCCGAAGGTCAAGTCG	Cloning DIMT1L into pCDNA5 FP
LD2820	GCTAGATCTAGACTCGAGCTAGGAAAAATGAATACCTTCTGC	Cloning DIMT1L into pCDNA5 RP

TABLE S3: siRNAs used in this work

Lab ref	target	Lifetech Ref	Sense sequence
LD035	SCRAMBLED	s4390844	undisclosed

LD020	WBSCR22#1	s41529	GAGUGGAAGUUAUCUGUCAtt
LD021	WBSCR22#2	s41530	GCAACUCACGGAUGAUUGAtt
LD022	WBSCR22#3	s41531	CAAGAAGUCUGAAAACCCUtt
LD023	WBSCR22#4	s445233	CUGACAAAGUAGUAUUUUAtt
LD024	WBSCR22#5	s445234	AAAUGUUUUCUGCAGUAAAtt
LD068	TRMT112#1	s28228	GGCCGGUUGAGGGAUAUGAtt
LD027	TRMT112#2	s28229	GUAUUUUUGUUGAUCUAUAtt
LD026	TRMT112#3	s445236	CAAUGACACCAAACACAGUtt
LD069	DIMT1L#1	s26096	GGCUAGUAGCUGAACUUCAtt
LD070	DIMT1L#2	s26097	GGAUGGUCUAGUAAGGAUAtt
LD071	DIMT1L#3	s26098	GGAGGACUCAUGUUCAACAtt
LD072	DIMT1L#4	ACD007RR	AGCCGCAGAGACGCACAACtt
LD073	DIMT1L#5	ACD007RS	CCUUUAGCUCUGUUCCUCCtt
	PNO1#2	s32352	CCAAGGAUGUUAGUGCUCUtt

TABLE S4: oligonucleotides used for RNA Northern blotting, primer extension analysis, and qRT-PCR

Northern-blots:

LD1827	CCTCGCCCTCCGGGCTCCGTTAATGATC	5'ITS-1
LD1828	CTGCGAGGGAACCCCCAGCCGCGCA	ITS-2
LD1844	CGGAGGCCCAACCTCTCCGACGACAGGTCGCCAGAGGACAGCGTGTCAGC	5'ETS
LD2079	GGGGCGATTGATCGGCAAGCGACGCTC	5'ITS-2
LD2133	GCTCCGTTTCCGACCTGGGCC	7SL
LD2132	CAATGTGTCCTGCAATTCAC	5.8S

Primer extension:

LD2120	GTACAAAGGGCAGGGACTTAATC	map m^7G_{1639}
LD2122	GCCCTCCGGGCTCCGTTAATGATC	map m^7G_{1639}
LD2141	CGAGCGAGCGAACGAACGGGC	map $m^2_2A_{1850}m^2_2A_{1851}$
LD2165	GGCTTAATTTGACTCAACACGG	sequencing ladder
LD2166	GAACGAACGAGCGAGCGAAC	sequencing ladder

qRT-PCR:

LD2476	CACTGGGCTGAGTGGAAGTT	WBSCR22 FP
LD2477	TTACAGAGCCACTGCACAGC	WBSCR22 RP
LD2472	CTGTGGAATTCAACCCCAAC	TRMT112 FP
LD2473	GGTGCCCTCTATCACTTCCA	TRMT112 RP
LD1818	TGCACCACCAACTGCTTAGC	GAPDH FP
LD1819	GTTCAGCTCAGGGATGACC	GAPDH RP
LD2637	AGAGAATTTGCCCTCCGACT	DIMT1L FP
LD2638	CCTGAAAATTGATGGGTGGT	DIMT1L RP
LD2530	CACCATGCCTGGCTAATTTT	PNO1 FP
LD2531	GTATGGCCAAAACCACTGCT	PNO1 RP

REFERENCES

[1] Figaro S, Scrima N, Buckingham RH, Heurgue-Hamard V. HemK2 protein, encoded on human chromosome 21, methylates translation termination factor eRF1. FEBS letters. 2008;582:2352-6.