Supplementary Materials

ARCHAEAL FIBRILLARIN-NOP5 HETERODIMER 2'-O-METHYLATES RNA INDEPENDENTLY OF THE C/D GUIDE RNP PARTICLE

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Supplemental Figures

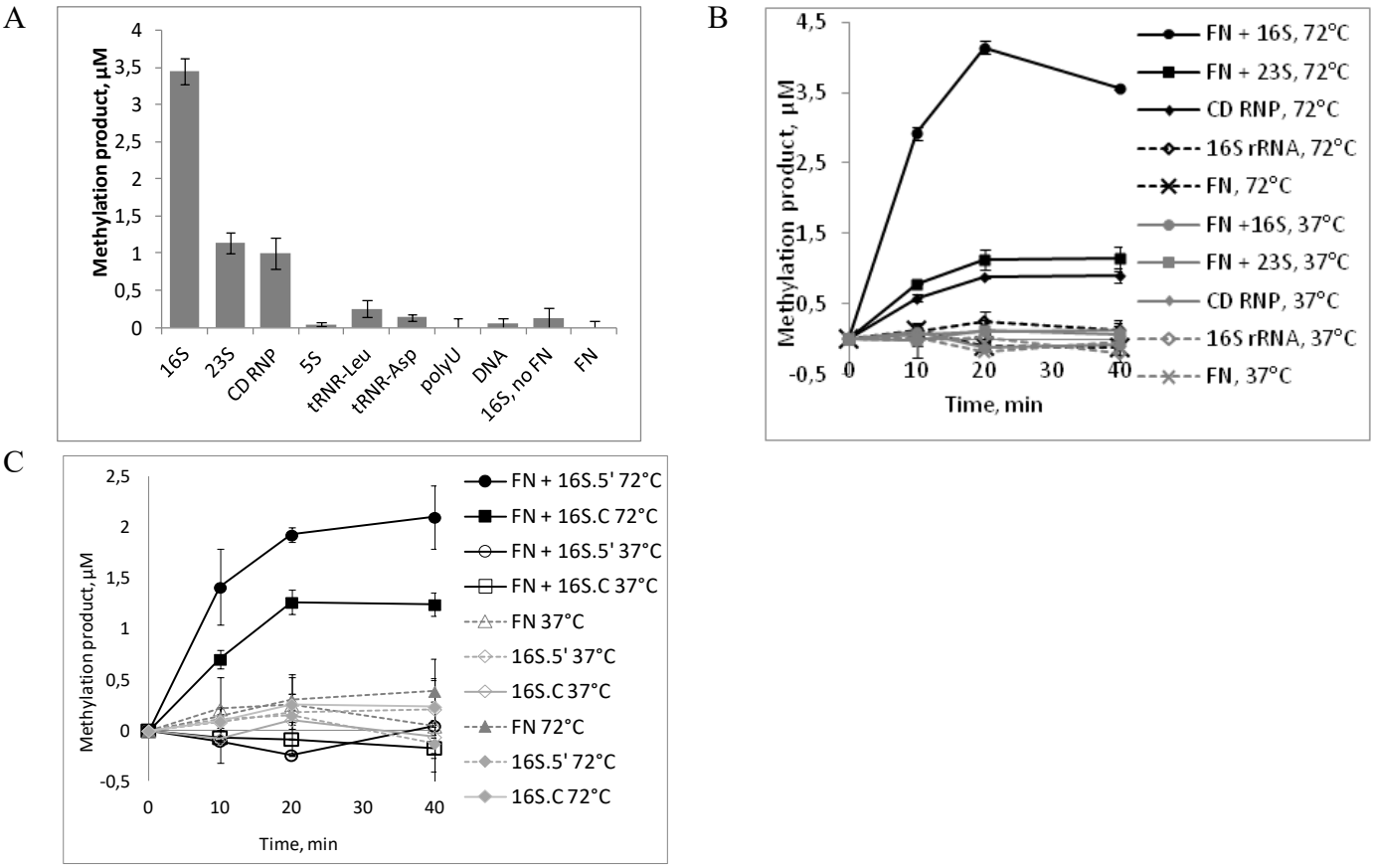
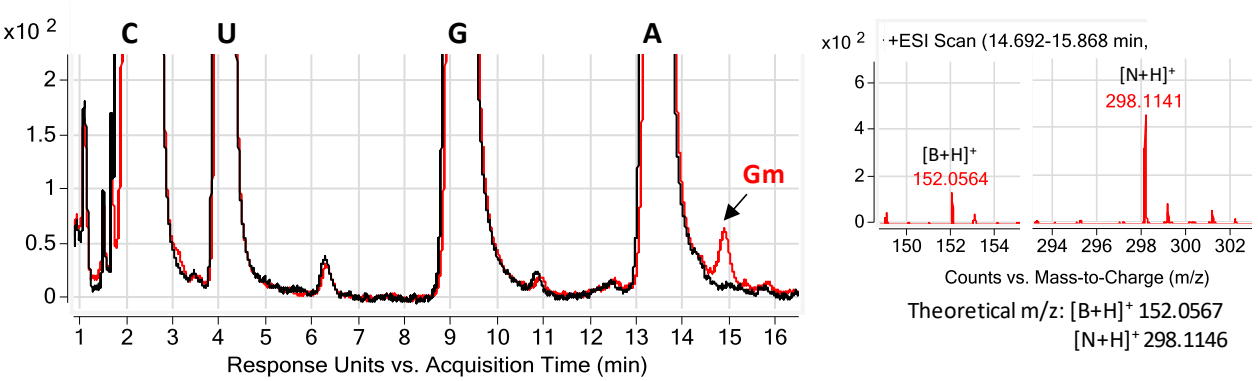


Figure S1. *Pyrococcus abyssi* stand-alone aFib-Nop5 (FN) heterodimer methylates *P.abyssi* 16S and 23S rRNA. (A) 1 µM *in vitro* transcribed *P.abyssi* 5S, 16S and 23S rRNAs, tRNA-Leu(CAA), *M.musculus* tRNA-Asp, as well as polyU, or Herring sperm sonicated DNA were exposed to 1 µM aFib-Nop5 at 72 ºC. For comparison, 1 µM C/D sR47 RNP was incubated with 1 µM tRNA-Leu(CAA). (B) Temperature dependence of the aFib-Nop5 heterodimer activity. The methylation reactions of aFib-Nop5 on 16S or 23S rRNA, and the C/D sR47 RNP on tRNA-Leu(CAA), were incubated either at 72 ºC or 37 ºC. (C) The activity of aFib-Nop5 on 16S.5' and 16S.C also requires high temperature. The reaction mixtures containing 1 µM aFib-Nop5 and 1 µM either 16S.5' or 16S.C were incubated either at 72 ºC or 37 ºC.

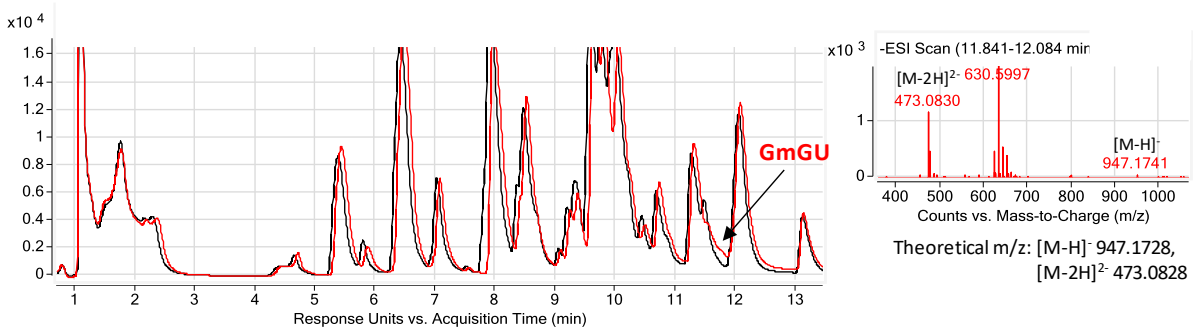
A

16S.5' P1



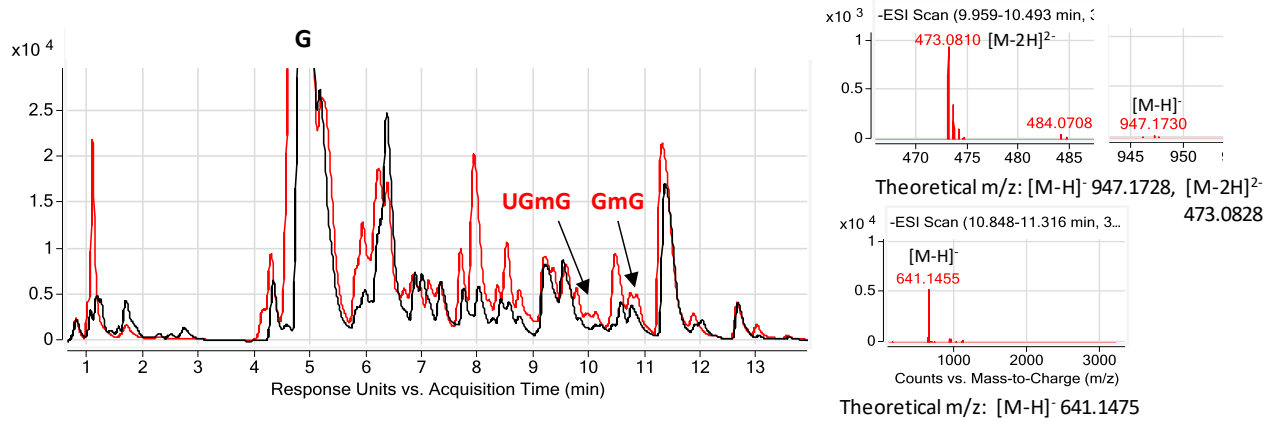
Theoretical m/z: $[B+H]^+$ 152.0567
$[N+H]^+$ 298.1146

16S.5' RNase A



Theoretical m/z: $[M-H]^-$ 947.1728,
$[M-2H]^{2-}$ 473.0828

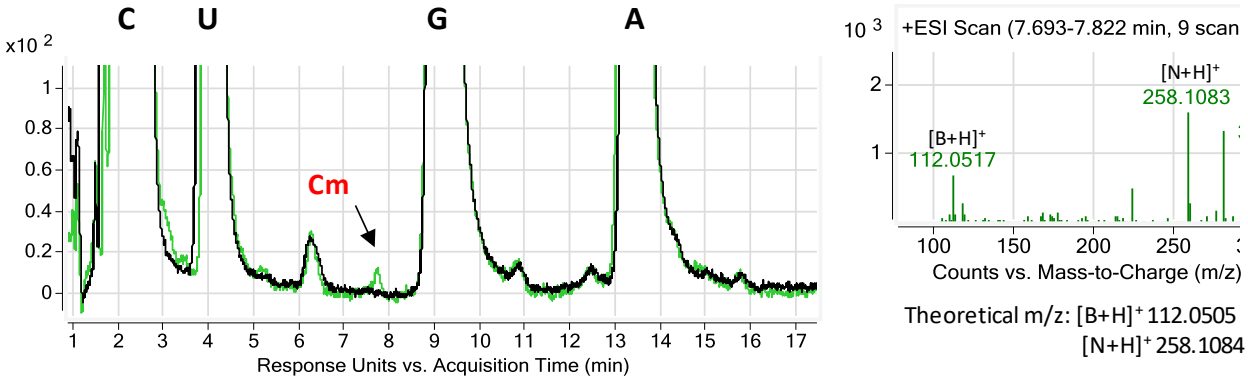
16S.5' RNase T1



Theoretical m/z: $[M-H]^-$ 947.1728, $[M-2H]^{2-}$ 473.0828

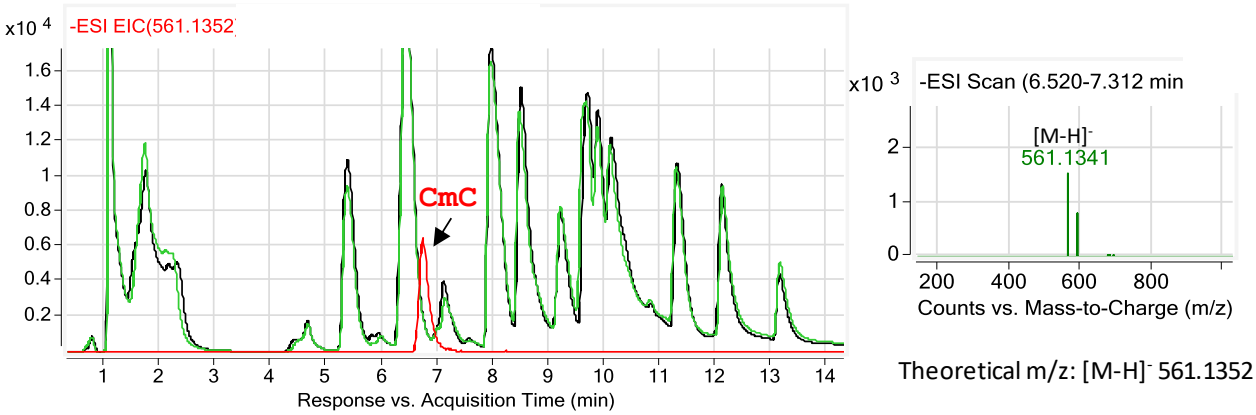
Theoretical m/z: $[M-H]^-$ 641.1475

16S.C P1



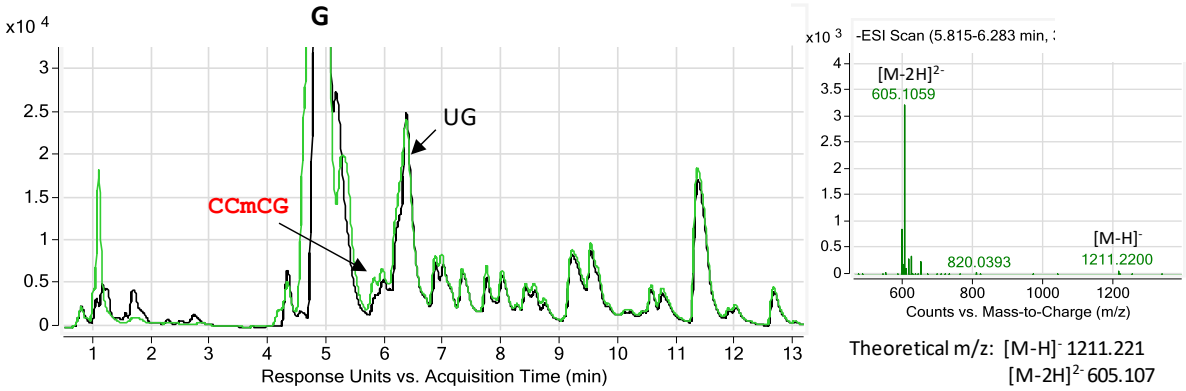
Theoretical m/z: $[B+H]^+$ 112.0505
$[N+H]^+$ 258.1084

16S.C RNase A



Theoretical m/z: $[M-H]^-$ 561.1352

16S.C RNase T1



Theoretical m/z: $[M-H]^-$ 1211.221
$[M-2H]^{2-}$ 605.107

B

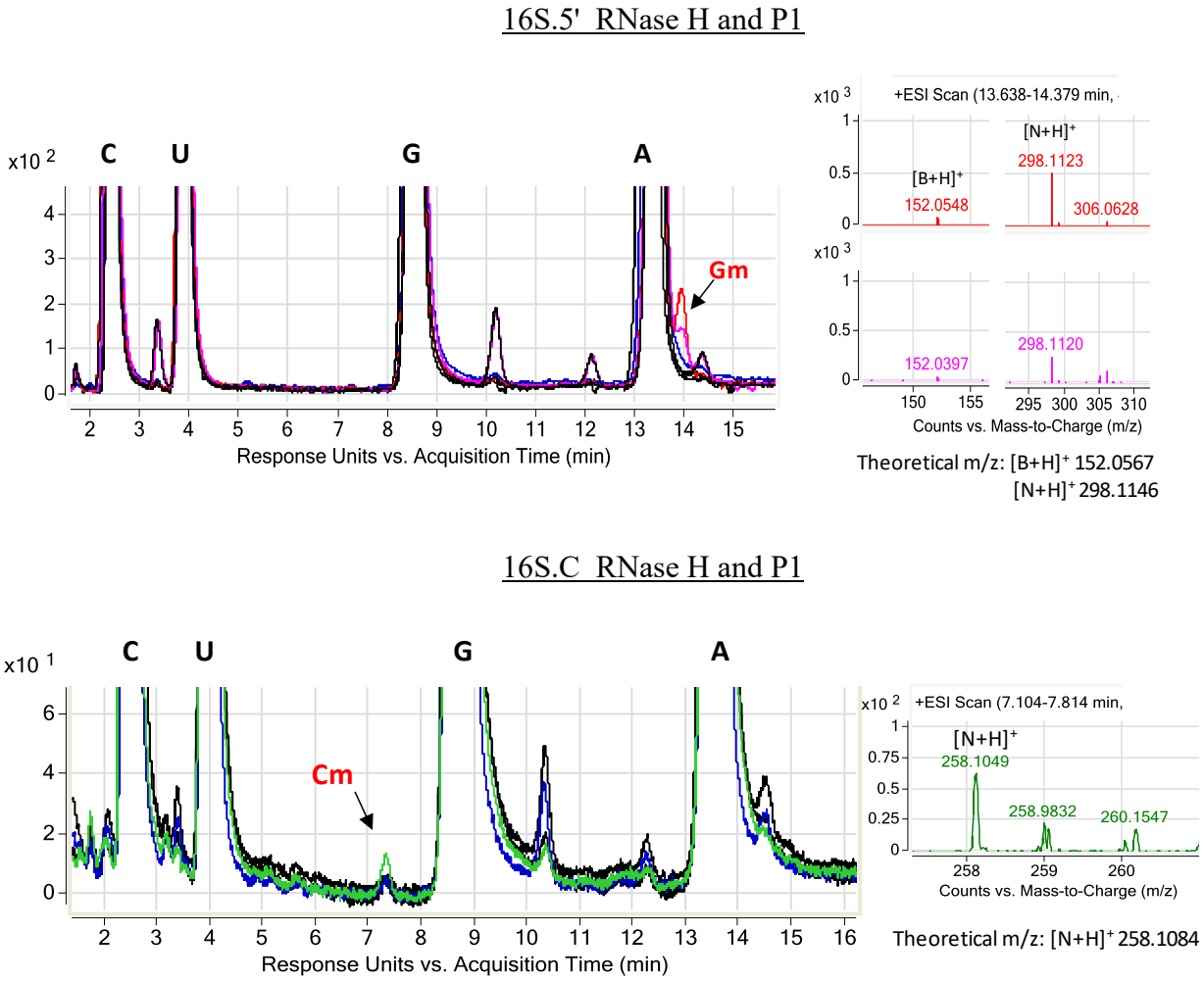


Figure S2. The specificity of the aFib-Nop5 stand-alone methylation revieled by HPLC/MS analysis. Overlaid UV chromatograms (left panel) are shown together with mass spectra of the indicated peaks (right panel). N denotes nucleoside; B – nucleobase; M – short oligonucleotide. (A) HPLC/MS analysis of 16S fragments after incubation with aFib-Nop5 and digestion with various nucleases (as indicated). Chromatograms are shown of methylation (red for 16S.5', green for 16S.C) and control (no aFib-Nop5, black) reactions. 16S.C RNase digestion product UV profile also has an overlayed CmC mass search profile (red) as the UV signal of this dinucleotide is obscured by other peaks. (B) P1 digestion and HPLC/MS analysis of 16S.5' and 16S.C after incubation with aFib-Nop5 and subsequent Rnase H treatment. 16S.5' was processed into fragments spanning 1-51th (UV absorption trace in magenta), 72-470th (blue) and 491-533th (red) nucleotides. 16S.C was processed into fragments spanning 431-826th (UV absorption trace in blue) and 845-962th (green) nucleotides. Control reactions contained no aFib-Nop5 (black). The resultant Gm nucleosides can only be attributed to G516 and the G47-49 triade, whereas Cm only to C847, based on previous experiments showing the sequence context of the methylated nucleosides (see Main Text Table 1).

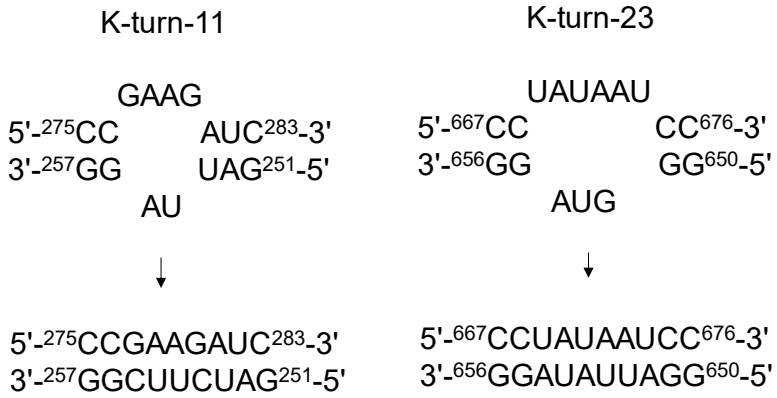
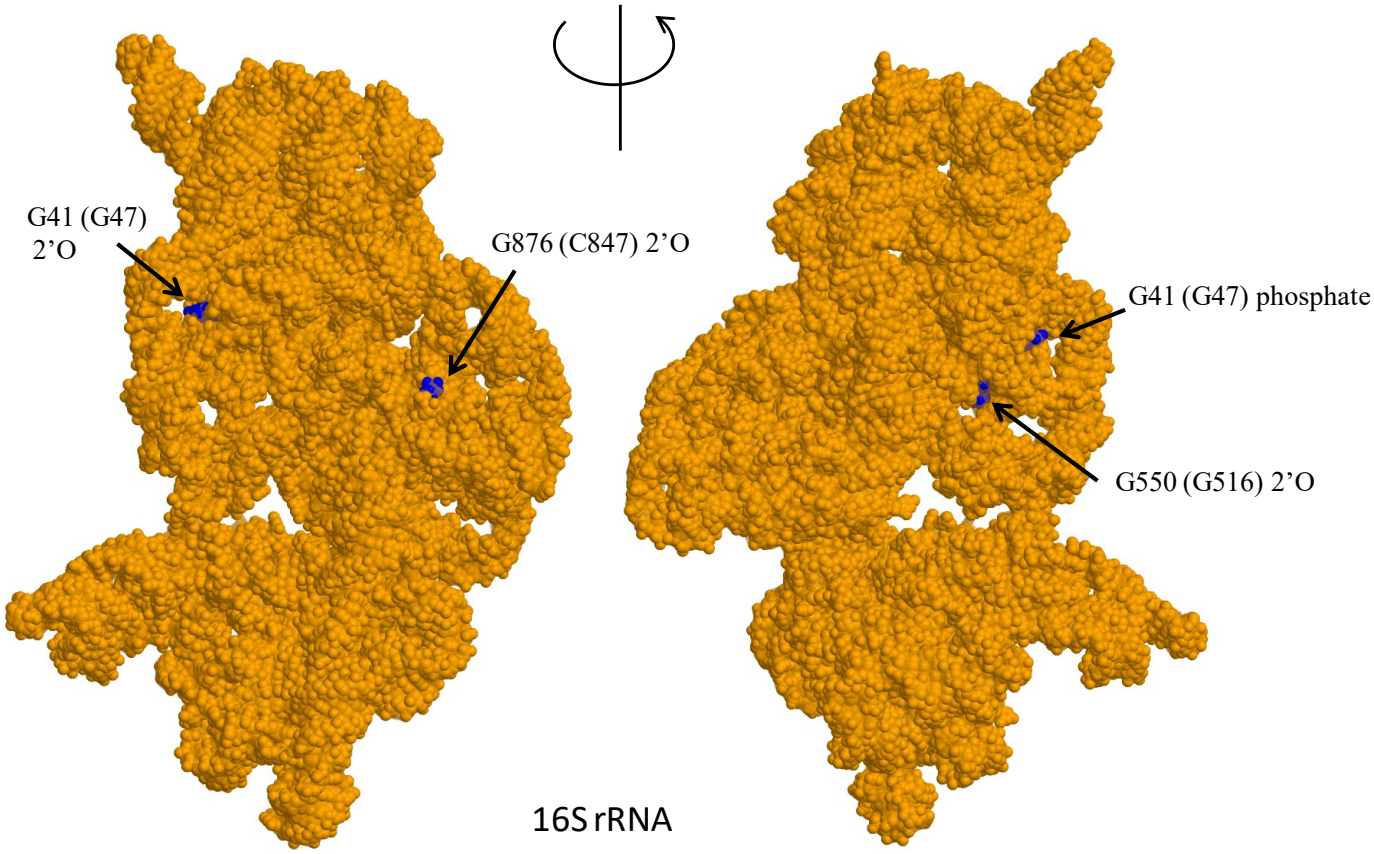


Figure S3. Schematic representation of K-turn-11 and K-turn-23 mutagenesis to Watson-Crick double-stranded regions in *P.abyssi* 16S.5' and 16S.C substrates, respectively.

A



B

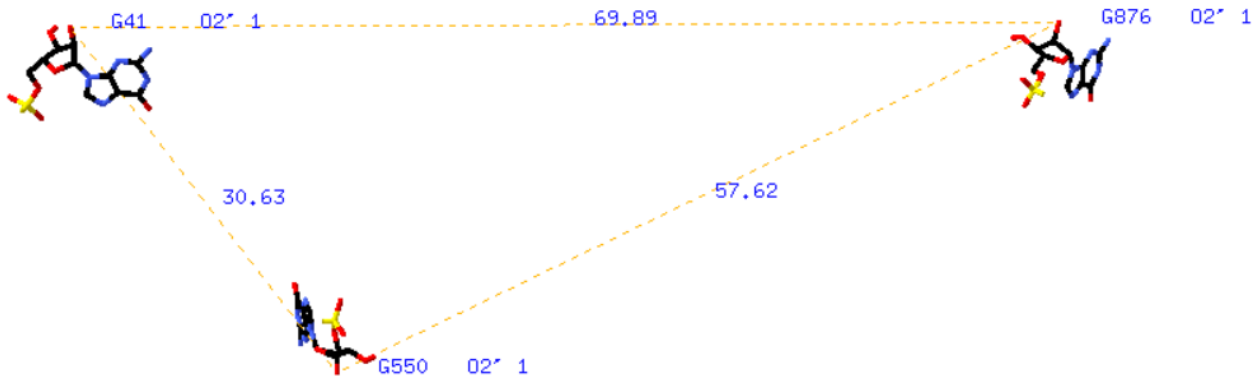


Figure S4. The aFib-Nop5 target site positions in a tertiary structure of 16S rRNA. Equivalent site nucleotides (G41, G550 and C876, respectively) are shown in the tertiary structure of a bacterial ribosome small subunit (Wimberly et al. 2000), PDB id 1j5e. (A) Target site accessibility from the two sides of the structure. (B) Distances between the target sites' 2'-O moieties.

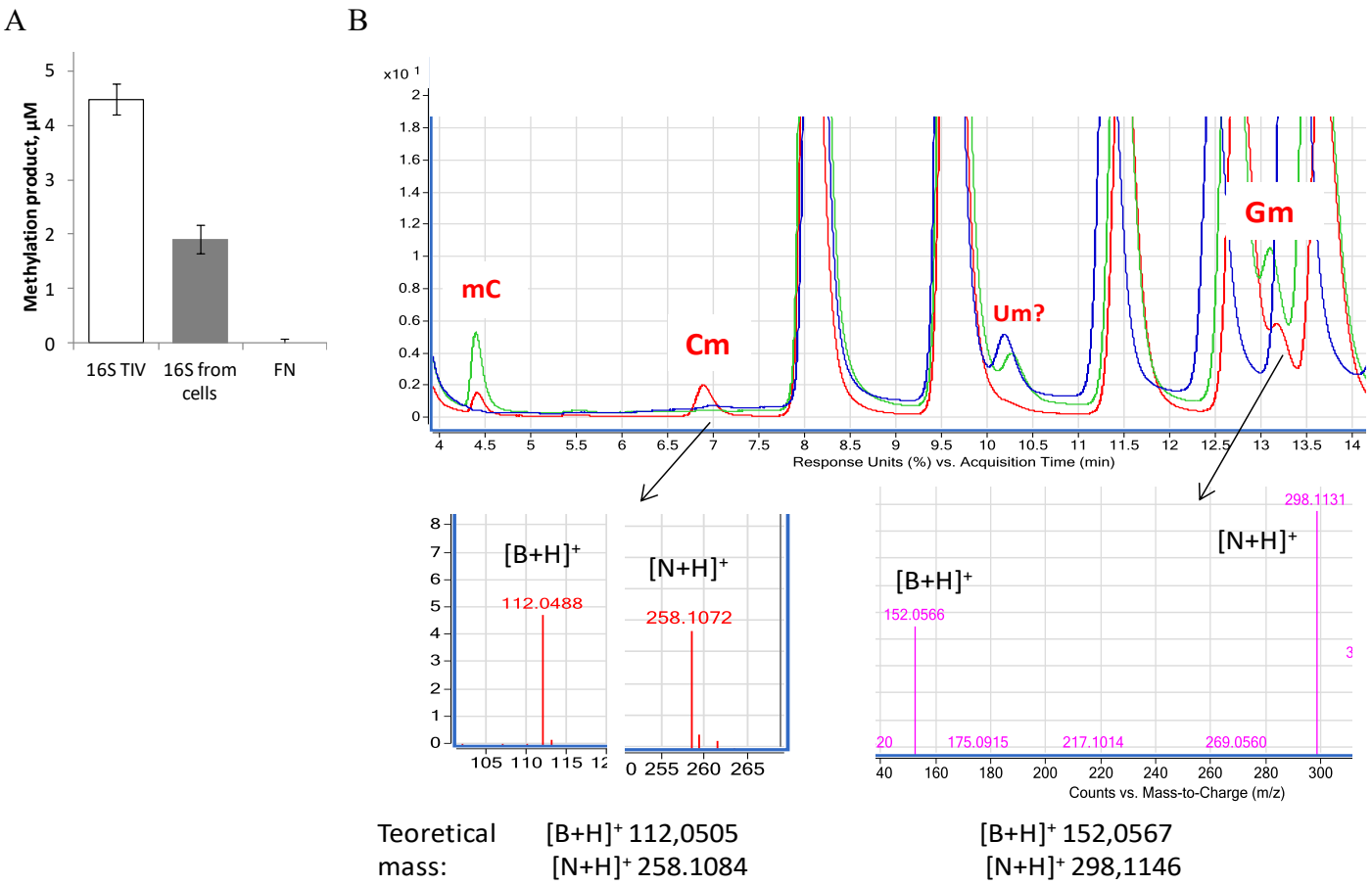


Figure S5. Analysis of 2'-O-methylation status at the aFib-Nop5 heterodimer target sites in 16S rRNA extracted from *P.abyssi* cells (see Supplemental methods). (A) 1 µM 16S rRNA, transcribed *in vitro* (TIV) or extracted from *P.abyssi* cells, was incubated with 1 µM aFib-Nop5. The extent of tritium incorporation was significantly lower compared to the *in vitro* transcribed substrate, suggesting that either of the targets might be methylated *in vivo*, or the reduced activity might be a consequence of a more stringent secondary and tertiary structure of the highly overall modified rRNA extracted from the cells. Fractions of middle-sized and small RNAs extracted from the cells were also tested and showed no apparent aFib-Nop5 activity *in vitro* (not shown). (B) P1 nuclease digestion and HPLC-MS analysis of RNA fragments of roughly 60 nt long spanning the aFib-Nop5 target sites (UV absorption trace: G47 region – blue, G516 – green and C847 – red line) in 16S rRNA extracted from *P.abyssi* cells. Chromatogram peaks corresponding to methylated nucleoside species are shown. The entity of the methylated nucleoside species was confirmed by a subsequent mass spectrometry analysis (lower panel). There was no Gm present in the G47 region. RNase A and T1 digestion product analysis did not attribute the found 2'-O-methylated nucleosides to G516 or C847, respectively (not shown).

Supplemental tables

Table S1. SnoScan search* of 16S-targeting C/D guide RNAs in Halobacteriacea.

*Every output was sorted manually. Here are shown the selected hits that resembled the thermoarchaeal C/D RNAs at most. The conserved boxes assigned by SnoScan are highlighted in yellow, and the base-paired region in green. The putative boxes manually assigned by M.T. are highlighted in grey. M.T. comments in cyan.
The species are listed in the order of increasing probability of the hits to be biologically relevant.

```
Target: 16S rRNA
Query: Complete genome or plasmid
==============================
Cutoffs (bits)
C Box: 4.33   D'Box: -14.43      Compl score: 8.0      Final score: 14.0
Min Match: 9 bp                  Max mismatch: 2 bp
Max C-D Box Dist: 60 bp          Min D box-Match Dist w/D' Box present: 10 bp
--------------------------------------------------------------------------------
```

Haloarcula marismortui

No hits

Halanaeroarchaeum sulfurireducens

```
>> gi|933677909|gb|CP011564.1|  15.78  (837715-837640)  Cmpl: 2651605543-Am1194 (-)
11/2 bp  Gs-DpBox: 837689 (27)  Len: 76

C Box:  AUGAUGA   Sc: 12.73    (837711-837705)                C-D box dist: 59 bp
D Box:  CUGA      Sc: 8.05
D'Box:  CUGA      Sc: 7.34              D'box Guide Transit Sc: -0.67

No known meth site found       Guide Seq Sc: -5.85  (14.71 -1.47 -18.10 -1.00)

Poor target complementarity.
                                    *
Db seq:  5'-                   CCGAGACAAUGGG -3'  2651605543   (1191-1203)
                               |||| ||| :|||
Qry seq: 3'-             AGUCUGGCUGUGUAGCCC -5'  gi|933677909|gb|CP011564.1|
          (837677-837689)


C Box-> Guide Seq Gap Sc: -2.16 (15 bp)            Guide Seq-> D Box Gap Sc: -2.76
(26 bp)

No terminal stem:               +[C Box] -N- UGGACCGU - 5'             Stem Sc: -
1.71 (1 bp)
                                |              |
                                +--[D Box] - UCGAUCAC - 3'             Stem
Transit Sc: -0.90


>Summary      [ C Box ] --         -- [ Cmpl/ Mism ]  X [D'Bx] --       -- [D Bx]
Length
>Meth Am1194  [AUGAUGA] --  15 bp  -- [  11 / 2    ]  1 [CUGA] -- 26 bp -- [CUGA]
76 bp
>Sc    15.78  [ 12.73 ] --  -2.16  -- [ 14.71 bits ]    [7.34]    -2.76    [8.05]

Seq: >gi|933677909|gb|CP011564.1|  15.78  (837715-837640)  Cmpl: 2651605543-Am1194
Len: 76
```

```
Seq: GGUGAUGAUGAGCGGUCAGAAGACGACCCGAUGUGUCGGUCUGAUAGAUGUCCUCGAGUU
Seq: CAUCGACGACCUGAUC
```

Halomicrobium mukohataei

```
>> gi|257167632|gb|CP001689.1|  15.05  (65405-65471)  Cmpl:
gi|444303794|ref|NR_074216.1|-Cm475 (-)  12/1 bp  Gs-D box: 65452 (48)  Len: 67  TS

C Box:  AUGAUGU   Sc: 7.48    (65409-65415)            C-D box dist: 50 bp
D Box:  CUGA      Sc: 8.05
D'Box:  None                                       D box Guide Transit Sc: -1.44

No known meth site found        Guide Seq Sc: 2.56  (23.13 -1.47 -18.10 -1.00)

                                 *
Db seq:  5'-                  UACCGGCAGCACG -3'  gi|444303794|ref|NR_074216.1|
           (472-485)
                              |||||||||||  |
Qry seq: 3'-             AGUCAAUGGCCGUCGUCC -5'  gi|257167632|gb|CP001689.1|
           (65464-65452)


C Box-> Guide Seq Gap Sc: -4.08 (36 bp)

Terminal stem:                    +[C Box] -N-  CGACCCAA - 5'              Stem Sc:
3.58 (5 bp)
                                  |              :||||
                                  +--[D Box] - GAUUGGGG - 3'               Stem
Transit Sc: -1.11


>Summary      [ C Box ]        --          --           [ Cmpl/ Mism ]  X [D Bx]
Length
>Meth Cm 475  [AUGAUGU]        --  36 bp   --           [  12 / 1    ]  1 [CUGA]   67
bp
>Sc    15.05  [  7.48 ]        --  -4.08   --           [ 23.13 bits ]    [8.05]

Seq: >gi|257167632|gb|CP001689.1|  15.05  (65405-65471)  Cmpl:
gi|444303794|ref|NR_074216.1|-Cm475  Len: 67
Seq: AGCUAUGAUGUAGUAGUCGUCACGGGCCUCCCAGAGUGGUUCGUGGGCCUGCUGCCGGUA
Seq: ACUGAGA
```

Halorhabdus utahensis

```
>> gi|257051090|ref|NC_013158.1|  15.17  (1044291-1044366)  Cmpl:
gi|444303784|ref|NR_074206.1|-Cm1001 (-)  12/0 bp  Gs-DpBox: 1044306 (16)  Len: 76  TS

C Box:  AUGGUGA   Sc: 8.15    (1044295-1044301)                C-D box dist: 59 bp
D Box:  CUGA      Sc: 8.05
D'Box:  UUGA      Sc: 3.82              D'box Guide Transit Sc: -0.67

No known meth site found        Guide Seq Sc: 0.96  (21.89 -1.12 -18.10 -1.72)

                                   *
Db seq:  5'-                    GCCGCCGUCAGC -3'  gi|444303784|ref|NR_074206.1|
           (997-1006)
                                |||:||||||||
Qry seq: 3'-                AGUUCGGUGGCAGUCG -5'  gi|257051090|ref|NC_013158.1|
           (1044317-1044306)


C Box-> Guide Seq Gap Sc: -4.76 (4 bp)      Guide Seq-> D Box Gap Sc: -1.59 (39 bp)
```

```
Terminal stem:                    +[C Box] -N-  CCGCCGUG - 5'              Stem Sc:
2.31 (4 bp)
                                  |              ||:| ::
                                  +--[D Box] - UGGUGAUG - 3'               Stem
Transit Sc: -1.11


>Summary      [ C Box ] --         -- [ Cmpl/ Mism ]  X [D'Bx] --       -- [D Bx]
Length
>Meth Cm1001  [AUGGUGA] --   4 bp  -- [  12 / 0    ]  0 [UUGA] -- 39 bp -- [CUGA]   76
bp
>Sc    15.17  [  8.15 ] --  -4.76  -- [ 21.89 bits ]    [3.82]    -1.59    [8.05]

Seq: >gi|257051090|ref|NC_013158.1|  15.17  (1044291-1044366)  Cmpl:
gi|444303784|ref|NR_074206.1|-Cm1001  Len: 76
Seq: GCCGAUGGUGAUGUCGCUGACGGUGGCUUGAUCUGGUUCGCCGUCGAUGACCGAAACCUG
Seq: UAGCGUCUUGCUGAUG
```

Halobacterium sp. DL1

```
>> gi|573484043|gb|CP007060.1|  14.62  (2447649-2447721)  Cmpl: 2507054340-Cm508 (-)
11/0 bp  Gs-DpBox: 2447672 (24)  Len: 73

C Box:  CUGAUGA   Sc: 8.44    (2447653-2447659)                  C-D box dist: 56 bp
D Box:  CUGA      Sc: 8.05
D'Box:  ACGA      Sc: 1.72               D'box Guide Transit Sc: -0.67

No known meth site found        Guide Seq Sc: 2.90  (23.11 -1.12 -18.10 -1.00)

                                  *
Db seq:  5'-                    GGCCAGGCAAG -3'  2507054340      (505-519)
                                ||||||||||
Qry seq: 3'-               AGCACCCGGUCCGUUC -5'  gi|573484043|gb|CP007060.1|
          (2447682-2447672)


C Box-> Guide Seq Gap Sc: -2.16 (12 bp)    Guide Seq-> D Box Gap Sc: -2.76 (28 bp)

No terminal stem:                 +[C Box] -N- AGGAGCAC - 5'               Stem Sc: -
0.43 (2 bp)
                                  |             ||    |
                                  +--[D Box] - UCAAGAUC - 3'               Stem
Transit Sc: -0.90


>Summary      [ C Box ] --         -- [ Cmpl/ Mism ]  X [D'Bx] --       -- [D Bx]
Length
>Meth Cm 508  [CUGAUGA] --  12 bp  -- [  11 / 0    ]  1 [ACGA] -- 28 bp -- [CUGA]   73
bp
>Sc    14.62  [  8.44 ] --  -2.16  -- [ 23.11 bits ]    [1.72]    -2.76    [8.05]

Seq: >gi|573484043|gb|CP007060.1|  14.62  (2447649-2447721)  Cmpl: 2507054340-Cm508
Len: 73
Seq: GGAUCUGAUGAAGCAGGUCGACGCUUGCCUGGCCCACGAGCGCGAGGAGGCCGACGAGCA
Seq: GAGCGGCCUGAUC
```

```
>> gi|573484043|gb|CP007060.1|  15.92  (2386640-2386706)  Cmpl: 2507054340-Gm980 (-)
11/1 bp  Gs-DpBox: 2386668 (29)  Len: 67  TS

C Box:  AUGGUGA   Sc: 8.15    (2386644-2386650)                  C-D box dist: 50 bp
D Box:  CUGA      Sc: 8.05
D'Box:  CCGA      Sc: 5.24               D'box Guide Transit Sc: -0.67
```

```
No known meth site found        Guide Seq Sc: 2.26  (22.48 -1.12 -18.10 -1.00)

                                  *
Db seq:  5'-                    GCCGCCGUCAGC -3'  2507054340      (977-989)
                                |||||||||  ||
Qry seq: 3'-                AGCCGCGGCGGCAGCCG -5'  gi|573484043|gb|CP007060.1|
          (2386679-2386668)


C Box-> Guide Seq Gap Sc: -1.76 (17 bp)    Guide Seq-> D Box Gap Sc: -8.08 (16 bp)

Terminal stem:                    +[C Box] -N- AGUCGCGG - 5'                Stem Sc:
3.84 (6 bp)
                                  |             | |||| |
                                  +--[D Box] - UGAGCGAC - 3'                Stem
Transit Sc: -1.11


>Summary      [ C Box ] --         -- [ Cmpl/ Mism ]  X [D'Bx] --       -- [D Bx]
Length
>Meth Gm 980  [AUGGUGA] --  17 bp  -- [  11 / 1    ]  1 [CCGA] -- 16 bp -- [CUGA]   67
bp
>Sc    15.92  [  8.15 ] --  -1.76  -- [ 22.48 bits ]    [5.24]    -8.08    [8.05]

Seq: >gi|573484043|gb|CP007060.1|  15.92  (2386640-2386706)  Cmpl: 2507054340-Gm980
Len: 67
Seq: UGACAUGGUGAAGACGUCGACGGGGUUCGCCGACGGCGGCGCCGAGGUGCCGGACGUCGA
Seq: ACUGAUG
```

Halalcalicoccus jeotgali

```
>> gi|300709370|ref|NC_014297.1|  16.57  (618083-618011)  Cmpl:
gi|631252217|ref|NR_113415.1|-Um408 (-)  11/0 bp  Gs-DpBox: 618068 (16)  Len: 73  TS

C Box:  GUGAUGA   Sc: 10.76    (618079-618073)
C-D box dist: 56 bp
D Box:  CUGA      Sc: 8.05
D'Box:  CCGC      Sc: 1.30
D'box Guide Transit Sc: -0.67

No known meth site found        Guide Seq Sc: 0.81  (21.74 -1.12 -18.10 -1.72)

                                    *
Db seq:  5'-                    CGCUUUUCCCU -3'  gi|631252217|ref|NR_113415.1|
          (404-412)
                                |||||||||||
Qry seq: 3'-                CGCCGCGAAAAGGGA -5'  gi|300709370|ref|NC_014297.1|
          (618058-618068)


C Box-> Guide Seq Gap Sc: -4.76 (4 bp)     Guide Seq-> D Box Gap Sc: -1.59 (37 bp)

Terminal stem:                    +[C Box] -N-CCCUGCUA - 5'                 Stem Sc:
3.77 (5 bp)
                                  |              |||||
                                  +--[D Box] - CGACGAAA - 3'                Stem
Transit Sc: -1.11


>Summary      [ C Box ] --         -- [ Cmpl/ Mism ]  X [D'Bx] --       -- [D Bx]
Length
>Meth Um 408  [GUGAUGA] --   4 bp  -- [  11 / 0    ]  0 [CCGC] -- 37 bp -- [CUGA]   73
bp
>Sc    16.57  [ 10.76 ] --  -4.76  -- [ 21.74 bits ]    [1.30]    -1.59    [8.05]
```

```
Seq: >gi|300709370|ref|NC_014297.1|  16.57  (618083-618011)  Cmpl:
gi|631252217|ref|NR_113415.1|-Um408  Len: 73
Seq: CCCCGUGAUGAGUCGAGGGAAAAGCGCCGCUCCGGGAUCGCCGCCACGGCCGGCCGGGAA
Seq: GUCGGCGCUGACG
```

Natronomonas pharaonis

```
>> gi|76556520|emb|CR936257.1|  14.18  (278822-278888)  Cmpl:
gi|470467514|ref|NR_074179.1|-Gm457 (-)  10/0 bp  Gs-DpBox: 278841 (20)  Len: 67  TS

C Box:  GUGACGA   Sc: 6.25    (278826-278832)                    C-D box dist: 50 bp
D Box:  AUGA      Sc: 3.77
D'Box:  CCGA      Sc: 5.24                 D'box Guide Transit Sc: -0.67

No known meth site found        Guide Seq Sc: 1.32  (22.25 -1.12 -18.10 -1.72)

                                    *
Db seq:  5'-                      AGCCGCCGCG -3'  gi|470467514|ref|NR_074179.1|
          (453-464)
                                  ||||||||||
Qry seq: 3'-                 AGCCUCGGCGGCGC -5'  gi|76556520|emb|CR936257.1|
          (278850-278841)


C Box-> Guide Seq Gap Sc: -1.59 (8 bp)     Guide Seq-> D Box Gap Sc: -2.76 (28 bp)

Terminal stem:                     +[C Box] -N- AACGGCCG - 5'               Stem Sc:
3.72 (4 bp)
                                   |               |||| |
                                   +--[D Box] - ACGCCGAC - 3'               Stem
Transit Sc: -1.11


>Summary      [ C Box ] --         -- [ Cmpl/ Mism ]  X [D'Bx] --       -- [D Bx]
Length
>Meth Gm 457  [GUGACGA] --   8 bp  -- [  10 / 0    ]  0 [CCGA] -- 28 bp -- [AUGA]   67
bp
>Sc    14.18  [  6.25 ] --  -1.59  -- [ 22.25 bits ]    [5.24]    -2.76    [3.77]

Seq: >gi|76556520|emb|CR936257.1|  14.18  (278822-278888)  Cmpl:
gi|470467514|ref|NR_074179.1|-Gm457  Len: 67
Seq: CAACGUGACGACACUCGGUCGCGGCGGCUCCGAUACGACGGCCGUGAUGCUCGGCAACUA
Seq: CAUGAAC
```

```
>> gi|76556520|emb|CR936257.1|  15.59  (2094518-2094446)  Cmpl:
gi|470467514|ref|NR_074179.1|-Gm454 (-)  10/0 bp  Gs-DpBox: 2094498 (21)  Len: 73  TS

C Box:  GCGAUGA   Sc: 6.22    (2094514-2094508)                  C-D box dist: 56 bp
D Box:  CUGA      Sc: 8.05
D'Box:  CCGA      Sc: 5.24                 D'box Guide Transit Sc: -0.67

No known meth site found        Guide Seq Sc: 2.13  (22.34 -1.12 -18.10 -1.00)

                                   *
Db seq:  5'-                     CCAGCCGCCG -3'  gi|470467514|ref|NR_074179.1|
          (451-463)
                                 ||||||||||
Qry seq: 3'-                AGCCGGGUCGGCGGC -5'  gi|76556520|emb|CR936257.1|
          (2094489-2094498)


C Box-> Guide Seq Gap Sc: -1.59 (9 bp)     Guide Seq-> D Box Gap Sc: -2.76 (32 bp)
```

```
Terminal stem:                    +[C Box] -N- CCACGCGA - 5'              Stem Sc:
0.08 (2 bp)
                                  |              ||  |:
                                  +--[D Box] - AGUCAGUC - 3'              Stem
Transit Sc: -1.11


>Summary      [ C Box ] --         -- [ Cmpl/ Mism ]  X [D'Bx] --       -- [D Bx]
Length
>Meth Gm 454  [GCGAUGA] --   9 bp  -- [  10 / 0    ]  1 [CCGA] -- 32 bp -- [CUGA]   73
bp
>Sc    15.59  [  6.22 ] --  -1.59  -- [ 22.34 bits ]    [5.24]    -2.76    [8.05]

Seq: >gi|76556520|emb|CR936257.1|  15.59  (2094518-2094446)  Cmpl:
gi|470467514|ref|NR_074179.1|-Gm454  Len: 73
Seq: ACCGGCGAUGAUAGCGAGCACGGCGGCUGGGCCGAGAAUGAGGAACAGCGGCUCAGCAGC
Seq: AGCGAGGCUGAAG
```

Supplemental methods

Methylation status analysis of the target positions in vivo

Total RNA was prepared from *P. abyssi* cell paste by Trizol extraction followed by treatments with DNase RQ1 (Promega), proteinase K and phenol/chloroform extraction followed by ethanol precipitation. Size-selected RNA fractions were then PAGE-purified.

For the analysis of the *in vivo* 2'-O-methylation state of the aFib-Nop5 target sites in 16S rRNA, the RNA regions of interest were isolated by hybridization to the complementary deoxyoligonucleotides (Metabion) (adapted from (Auxilien et al. 2011):

G47 region TCGCATGGCTTAGTCGGACCCCCATAGCAGTGGCCTCCGGCAGGATCAACCGGAAT,

G519 - TAATAGTGGCCACCACTCGGGCCGCCGGTATTACCGCGGCGGCTGCCACCGGCCTTGCC,

C847 - TTTAAGTTTCAGCCTTGCGGCCGTACTCCCCAGGCGGCGGGCTTAACGGCTTCCCTA.

65 µg of total *P.abyssi* RNA was heated with 130 pmol of deoxyoligonucleotide at 85 °C for 1 min, followed by slow cooling to 45 °C over 2 h. Regions of RNA that were not protected by hybridization were digested away with RNase Cocktail Enzyme Mix (Thermo Fisher Scientific). The rRNA sequences paired to the deoxyoligonucleotide were resolved on a 6 % PAA denaturing gel and column-purified with accompanying DNase I treatment (Zymo Research). Each of the rRNA

sequences of ~60 nt was digested with either nuclease P1, RNase A or RNase T1 and further HPLC-MS analyzed as described in the main text.

Supplemental references

Auxilien S, Rasmussen A, Rose S, Brochier-Armanet C, Husson C, Fourmy D, Grosjean H, Douthwaite S. 2011. Specificity shifts in the rRNA and tRNA nucleotide targets of archaeal and bacterial m5U methyltransferases. *RNA* **17**: 45–53.