SUPPLEMENTARY DATA

Figure S1

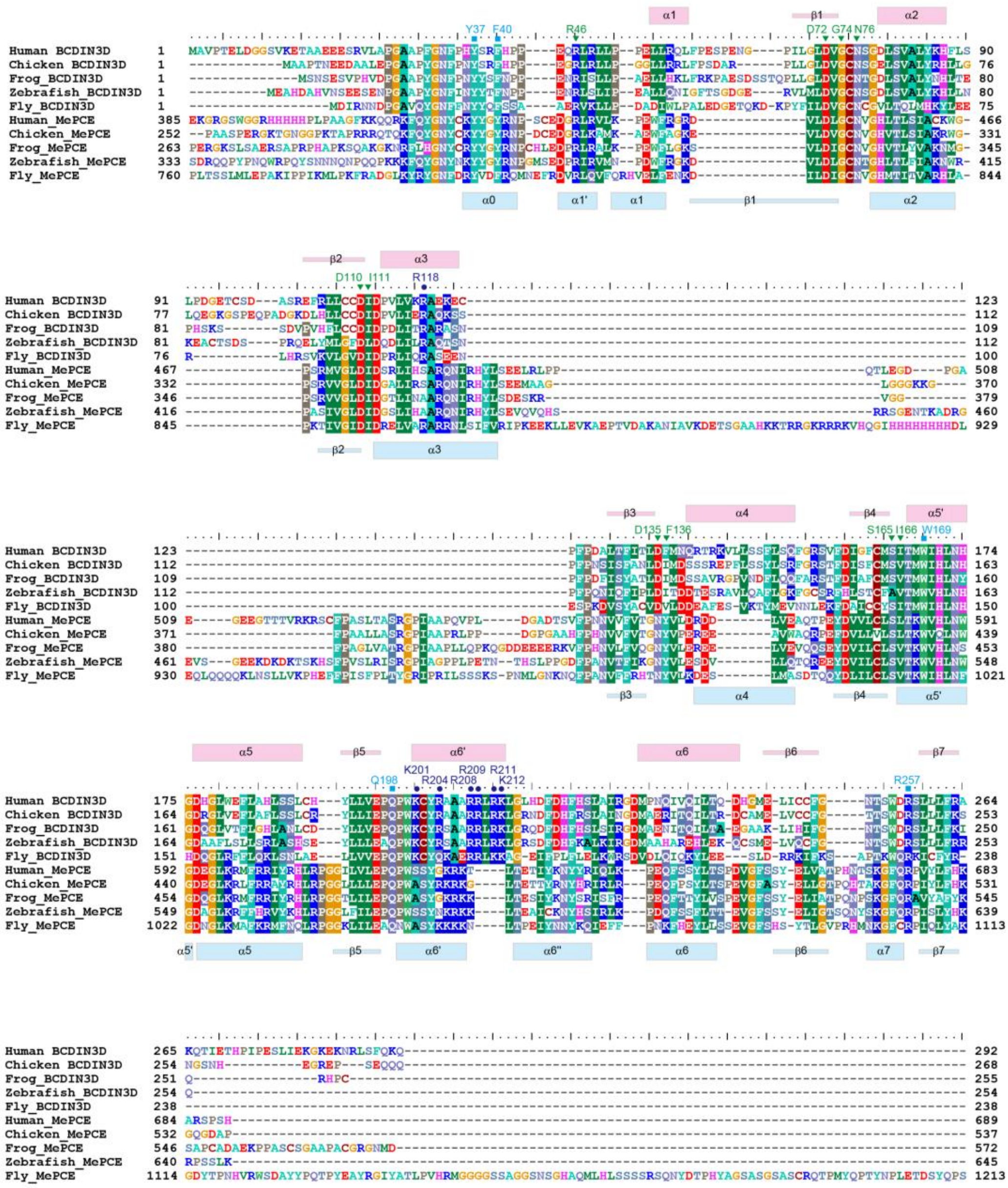
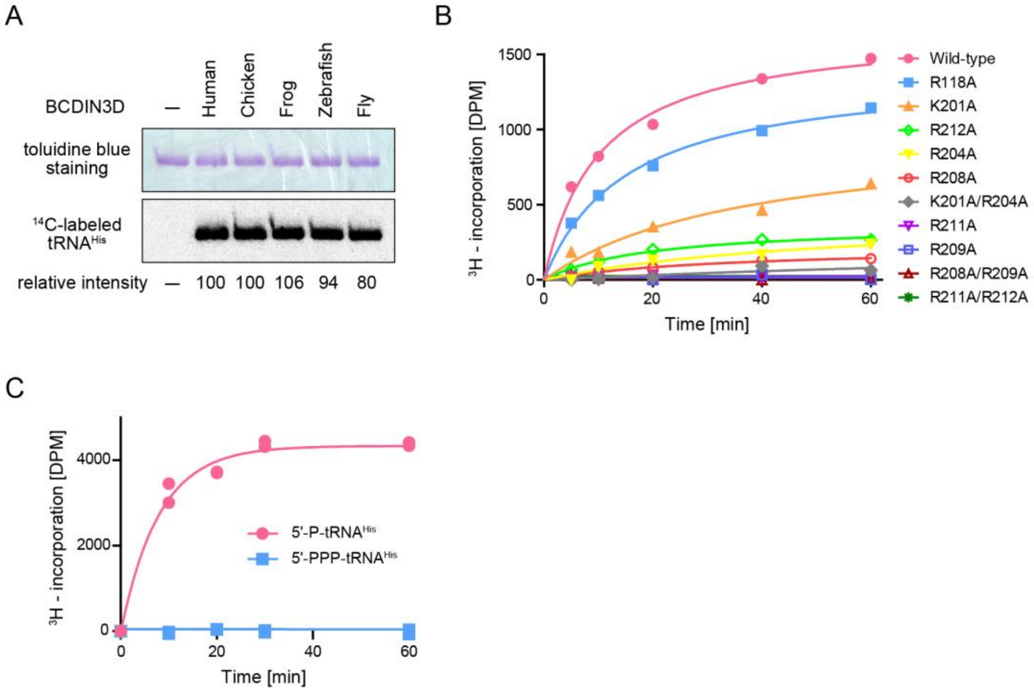


Figure S2



A
BCDIN3D
—
Human
Chicken
Frog
Zebrafish
Fly
toluidine blue staining
14C-labeled tRNAHis
relative intensity — 100 100 106 94 80
B
3H - incorporation [DPM]
1500
1000
500
0
0
20
40
60
Time [min]
Wild-type
R118A
K201A
R212A
R204A
R208A
K201A/R204A
R211A
R209A
R208A/R209A
R211A/R212A
C
3H - incorporation [DPM]
4000
2000
0
0
20
40
60
Time [min]
5'-P-tRNAHis
5'-PPP-tRNAHis

Figure S3

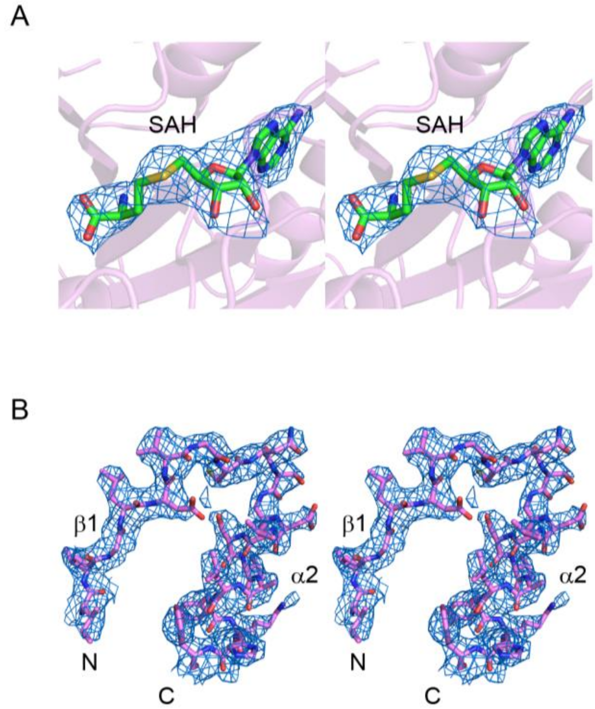
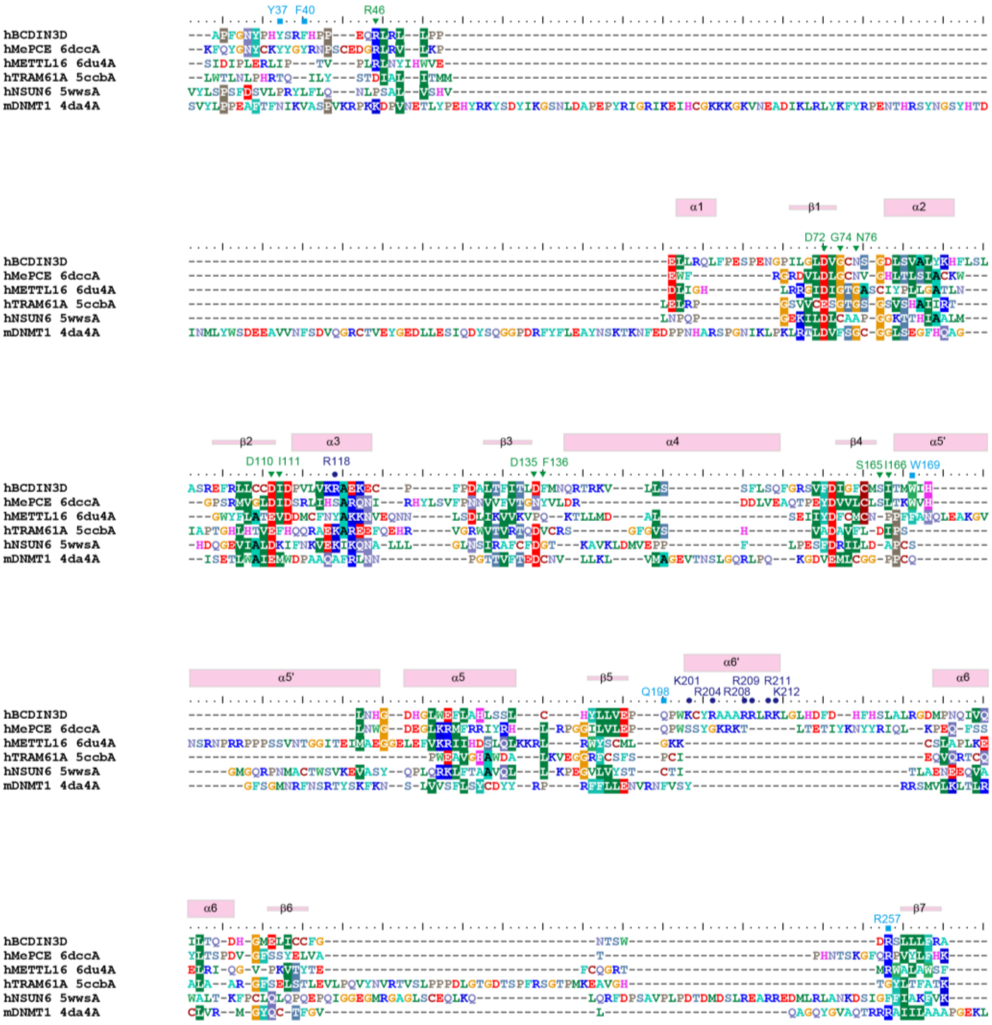


Figure S4



Y37 F40
R46
hBCDIN3D
hMePCE 6dccA
hMETTL16 6du4A
hTRAM61A 5ccbA
hNSUN6 5wwsA
mDNMT1 4da4A
α1
β1
α2
D72 G74 N76
β2
α3
β3
α4
β4
α5'
D110 I111
R118
D135 F136
S165 I166 W169
α5'
α5
β5
α6'
α6
K201
R209 R211
Q198
R204 R208
K212
α6
β6
β7
R257

Figure S5

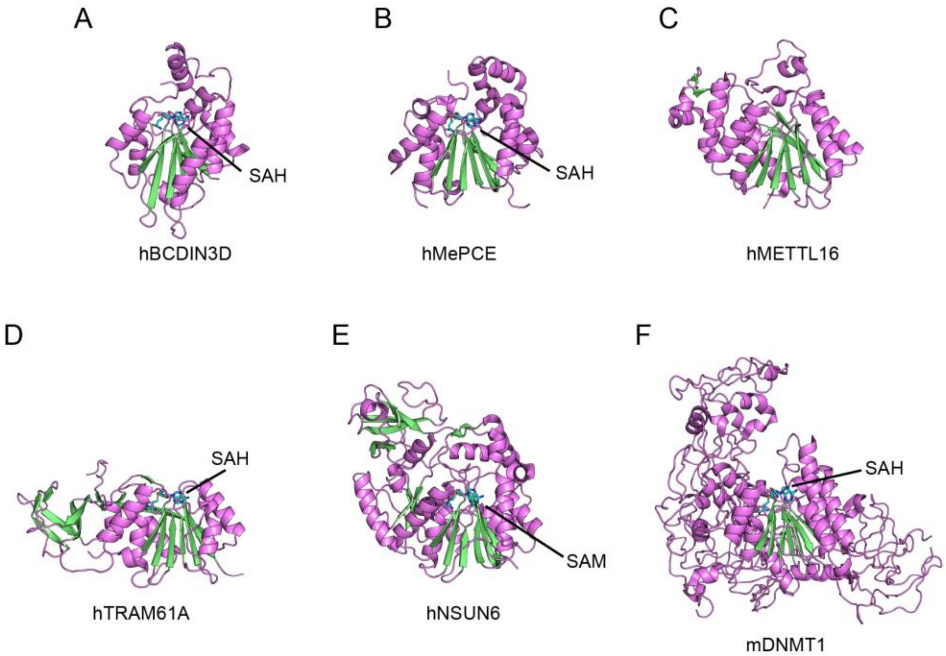

A
SAH
hBCDIN3D
B
SAH
hMePCE
C
hMETTL16
D
SAH
hTRAM61A
E
SAM
hNSUN6
F
SAH
mDNMT1

Table S1: Nucleotide sequences of the synthetic BCDIN3D genes

human BCDIN3D gene	ATGGCGGTTCCGACGGAACTGGATGGCGGCTCCGTCAAGGAAACCGCTGC GGAAGAAGAATCGCGCGTATTGGCCCCTGGTGCAGCACCATTTGGCAACTT TCCGCACTATTCGCGCTTCCATCCACCTGAACAGCGATTGCGCCTGTTACCG CCGGAATTGCTGCGTCAGTTGTTTCCGGAGAGTCCGGAGAATGGTCCGATT CTGGGGCTCGATGTGGGGTGTAACAGTGGAGACTTATCGGTGGCTCTGTAC AAACACTTTCTGTCACTGCCCGATGGTGAGACATGCAGTGACGCCTCTCGG GAATTTCGTCTGTTATGTTGCGATATCGATCCGGTTCTTGTTAAACGCGCGG AAAAAGAGTGTCCGTTCCCAGACGCGCTGACCTTTATCACTCTTGACTTCA TGAACCAACGCACCCGGAAAGTGCTGCTCTCCTCCTTTCTGTCTCAGTTCG GTCGTTCTGTGTTCGATATCGGTTTCTGTATGAGCATTACGATGTGGATTCAT CTGAATCATGGCGATCATGGTCTGTGGGAGTTTCTGGCCCATCTCAGCTCAC TTTGCCACTACCTGTTAGTCGAGCCACAACCGTGGAAATGCTATCGTGCTGC AGCGAGACGTCTACGCAAACTAGGCCTGCATGACTTTGACCACTTTCACTC ACTGGCGATTCGCGGAGATATGCCGAACCAGATCGTACAGATTCTGACCCA AGATCACGGCATGGAACTGATCTGCTGCTTTGGGAATACTAGCTGGGATCGT AGCTTATTGCTTTTCCGTGCCAAACAGACCATTGAAACGCATCCCATTCCTG AAAGCCTGATAGAAAAAGGCAAAGAAAAGAATCGCCTCAGCTTCCAAAAG CAGTAA
chicken BCDIN3D gene*	ATGGCAGCCCCGACCAACGAAGAAGATGCAGCACTGGAACCGGGTGCCGC ACCGTATGGCAACTTCCCGAACTATAGTCGTTTTCATCCGCCGGAGGGTCGC TTACGTTTACTGCCTGGCGGTCTGCTGCGCCGTTTATTCCCGAGCGATGCCC GCCCGTTACTGGGTCTGGATGTGGGTTGCAATAGCGGTGAGCTGAGCGTGG CCCTGTATCGTCACCTGTTAGGCCTGCAAGAAGGTAAAGGCAGTCCGGAAC AACCGGCCGACGGCAAAGATCTGCACCTGCTGTGCTGCGACATTGATCCGG TTCTGATTGAACGCGCCCAGAAAAGCAGCCCGTTTCCGAATAGCATTAGCT TCGCCAACCTGGACATCATGGATAGCAGCAGCCGCGAACCGTTCCTGAGCA GCTACTTAAGCCGTTTTGGCCGCAGCACCTTCGACATTAGCTTCTGCATGAG CGTGACCATGTGGATTCACCTGAATCATGGTGATCGCGGTCTGGTGGAATTT CTGGCCTTCCTGAGCAGTCTGTGTCGTTATCTGCTGATCGAGCCTCAACCGT GGAAGTGCTATCGCGCCGCAGCACGTCGTCTGCGTAAACTGGGCCGCAATG ATTTCGATCATTTCCGCAGCCTGGCCATCAATGGCGATATGGCAGAGCGCAT TACCCAGATTCTGACCCGTGACTGCGCTATGGAACTGGTGTGCTGCTTTGGT ACCACCAGTTGGGATCGCAGCCTGCTGCTGTTTAAGAGCAATGGCAGCAAT CATGAGGGCCGCGAACCGAGCGAACAGCAATAA
frog BCDIN3D gene	ATGGAAGCACATGACGCCCATGTGAACAGCGAAGAAAGCGAAAACCCGGG TGCCGCCCCGTATGGTAATTTTATCAACTACTATACTTTCAACCCGCCGGAA AATCGTCTGAGTCTGATCCCGGAAGCCCTGTTACAAAATATTGGCTTTACCA GCGGCGATGGCGAACGCGTGCTGATGCTGGACGTTGGCTGCAACAGCGGC GATCTGAGCGTTGCCCTGTATAAACACCTGCTGAACAAAGAGGCCTGCACC AGCGATAGTCCGCGCCAGGAACTGTATATGCTGGGCTTCGACCTGGATCAG GATTTAATCCTGCGTGCCCAGACCAGCAACCCGTTTCCGCAGAACATCCAG TTCATTCCGCTGGACATCACCGATGATACAGAGAGCCGCGCCGTTTTACAA GCCTTTCTGGGCAAATTCGGTTGTTCTCGCTTTCATCTGAGCACCTGCTTTG CCGTGACCATGTGGGTGCATCTGAATCATGGTGATGCCGCATTTCTGAGTCT GCTGAGCCGCCTGGCCAGCCATAGCGAATATCTGCTGCTGGAAGCACAGCC GTGGAAATGCTATCGCAGTGCAGCCCGTCGCTTACGTAAACTGGGCCGCAG CGACTTTGACCATTTCAAAGCACTGAAGATCCGCGGCGATATGGCCGCACA TGCACGCGAGCATCTGGAGAAGCAGTGTAGCATGGAACTGGTGCAGTGCT TTGGCAACACCAGTTGGGACCGCAGCCTGCTGCTGTTTCGCCGCCAGTAA

zebrafish BCDIN3D gene	ATGAGCAATAGCGAAAGCGTGCCGCACGTTGATCCTGGTGCAGCCCCGTAC GGCAACTTCCCGAACTACTATAGCTTCAACCCGCCGGAAAACCGCATTAGC CTGCTGCCGGCCGAACTGCTGCACAAGCTGTTCCGCAAACCGGCCGAGAG CGATAGTAGCACCCAGCCGCTGCTGGGTTTAGATGTGGGCTGCAATACCGG TGACCTGAGCGTTGCCCTGTACAACCATCTGACCGAACCTCACAGCAAGAG CAGCGATGTTCCGGTGCACTTTCTGTGCTGCGACATTGATCCGGACCTGATT ACCCGTGCACGTGCCAGCAACCCTTTTCCGGACTTCATCAGCTACGCAACC CTGGACATCATGGATAGCAGCGCCGTGCGTGGCCCGGTGAATGACTTCTTA CAACAGTTTGCCCGCAGCACCTTTGACATCGCCTTCTGCATGAGCGTGACC ATGTGGATTCATCTGAACTACGGCGATCAGGGCCTGGTGACCTTCCTGGGT CATCTGGCCAACCTGTGCGACTATCTGCTGGTGGAACCGCAACCGTGGAAA TGCTATCGTAGCGCAGCCCGTCGTCTGCGTAAACTGGGCCGTCAGGATTTC GATCACTTCCACAGCCTGAGCATTCGCGGCGACATGGCCGAGAACATCACC CAGATCTTAACAGCCGAAGGCGCCGCCAAACTGATCCACATCTTTGGCAAC ACCAGCTGGGACCGCAGCCTGCTGCTGTTTAAAATCCAGCGCCATCCGTGC TAA
fly BCDIN3D gene	ATGGATATTCGTAATAATGATCCGGGCGCCGTGCAATATGGCAATTTTTTCAA CTATTACCAGTTTAGCAGCGCAGCCGAGCGCGTGAAGTTACTGCCGGATGC CGATATTTGGCTGCCTGCCCTGGAGGACGGTGAGACCCAGAAAGATAAACC GTATTTTATTCTGGATGTGGGTTGCAACTGCGGCGTTCTGACCCAGCTGATG CACAAGTACCTGGAAGAACGTCTGCATCGCAGCGTGAAAGTGCTGGGCGT TGACATTGATCCGCGCCTGATCCAACGCGCCAGCGAGGAAAACGAGAGCC CGAAAGACGTGAGCTATGCCTGCGTGGACGTGCTGGATGATGAGGCCTTTG AAAGCGTGAAAACCTATATGGAAGTGAACAACCTGGAGAAGTTCGACGCC ATCTGCTGCTATAGCATCACCATGTGGATTCATCTGAATCATCATGATCAGGG CCTGCGCTTCTTCTTACAAAAACTGAGCAACCTGGCCGAACTGCTGGTGGT GGAACCGCAGCCGTGGAAATGCTATCAGAAAGCCGAGCGCCGCCTGAAAA AAGCCGGCGAAATCTTCCCGCTGTTTCTGGAACTGAAATGGCGCAGCGATG TGGACTTACAAATCCAGAAATACCTGGAGGAGAGCCTGGACCGCCGCAAA ATCTTTAAGAGCGCCCCGACCAAGTGGCAGCGTAAAATCTGCTTTTATCGCT AA

* The C-terminal residue of Chicken BCDIN3D (Q268) was omitted.

Table S2: List of synthetic oligonucleotides

hBCDIN3D_fromE14_Fw_NdeI	AGCTAGCTCATATGGAAACCGCTGCGGAAGAAG
hBCDIN3D_toK284_Rv_XhoI_nostop	AGCTCTCGAGCTTTTCTTTGCCTTTTTCTATC
bcdin3d-R118A-FW	GCCGCGGAAAAAGAGTGTCCGTTCCCAGAC
bcdin3d-R118A-RV	TTTAACAAGAACCGGATCGATATCGCAAC
bcdin3d-K201A-FW	GCATGCTATCGTGCTGCAGCGAGACGTC
bcdin3d-K201A-RV	CCACGGTTGTGGCTCGACTAACAGGTAG
bcdin3d-R204A-FW	GCACCTGCAGCGAGACGTCTACGCAAAC
bcdin3d-R204A-RV	ATAGCATTTCCACGGTTGTGGCTCGAC
Double201-204-RV	ATAGCATGCCCACGGTTGTGGCTCGAC
bcdin3d-R208A-FW	GCACGTCTACGCAAACTAGGCCTGCATGAC
bcdin3d-R208A-RV	CGCTGCAGCACGATAGCATTTCCACGG
bcdin3d-R209A-FW	GCACTACGCAAACTAGGCCTGCATGAC
bcdin3d-R209A-RV	TCTCGCTGCAGCACGATAGCATTTCCA
Double208-209-RV	TGCCGCTGCAGCACGATAGCATTTCCAC
bcdin3d-R211A-FW	GCCAAACTAGGCCTGCATGACTTTGACCAC
bcdin3d-R211A-RV	TAGACGTCTCGCTGCAGCACGATAGC
bcdin3d-K212A-FW	GCACTAGGCCTGCATGACTTTGACCAC
bcdin3d-K212A-RV	GCGTAGACGTCTCGCTGCAGCACGATAG
Double211-212-RV	GGCTAGACGTCTCGCTGCAGCACGATAG
bcdin3d-R257A-FW	GCAAGCTTATTGCTTTTCCGTGCCACC
bcdin3d-R257A-RV	ATCCCAGCTAGTATTCCCAAAGCAGCAG
46_Rev	CTGTTCAGGTGGATGGAAGCGCGAATAGTG
R46A_For	GCATTGCGCCTGTTACCGCCGGAATTGCTG
72-76 Rev	GAGCCCCAGAATCGGACCATTCTCCGG
D72A For	GCTGTGGGGTGTAACAGTGGAGACTTATCG
G74A_For	GATGTGGCGTGTAACAGTGGAGACTTATCG
72AG74A_For	GCTGTGGCGTGTAACAGTGGAGACTTATCG
N76A_For	GATGTGGGGTGTGCCAGTGGAGACTTATCGGTG
110-111_Rev	GCAACATAACAGACGAAATTCCCG
D110A For	GCTATCGATCCGGTTCTTGTTAAACGCGCG
I111G_For	GATGGCGATCCGGTTCTTGTTAAACGCGCG

135-136_Rev	AAGAGTGATAAAGGTCAGCGCGTC
D135A_For	GCCTTCATGAACCAACGCACCCGGAAAGTG
F136G_For	GACGGCATGAACCAACGCACCCGGAAAGTGCTG
165-166_Rev	CATACAGAAACCGATATCGAACAC
S165A_For	GCCATTACGATGTGGATTCATCTGAATCATGGC
I166G_For	AGCGGTACGATGTGGATTCATCTGAATCATGGC
hBCDIN3D_Y37A_m_Fw	GCGTCGCGCTTCCATCCACCTGAACAGCGATTGC
hBCDIN3D_Y37F_m_Fw	TTTTCGCGCTTCCATCCACCTGAACAGCGATTGC
hBCDIN3D_Y37F_Rv	GTGCGGAAAGTTGCCAAATGGTGCTGCACCAGGG
hBCDIN3D_R39F40A_m_Fw	CGCGCGCATCCACCTGAACAGCGATTGCGCCTG
hBCDIN3D_R39F40A_Rv	CGAATAGTGCGGAAAGTTGCCAAATGGTGCTGCAC
hBCDIN3D_W169F_m_Fw	TTTATTCATCTGAATCATGGCGATCATGGTCTG
hBCDIN3D_W169F_Rv	CATCGTAATGCTCATACAGAAACCGATATCGAAC
hBCDIN3D_Q198A_m_Fw	GCGCCGTGGAAATGCTATCGTGCTGCAGCGAGACG
hBCDIN3D_Q198A_Rv	TGGCTCGACTAACAGGTAGTGGCAAAGTGAGCTG

Supplementary Figure Legends

Supplementary Figure S1: Sequence alignments of BCDIN3Ds and MePCEs from various organisms.

Amino acid sequences of human BCDIN3D (HsBCDIN3D) and MePCE (HsMePCE) are aligned with those of BCDIN3D or MePCE from other organisms: *Gallus gallus*, *Xenopus laevis*, *Danio rerio*, and *Drosophila melanogaster*. Secondary structural elements of the human BCDIN3D (present study) and human MePCE (PDB ID: 6DCC) are depicted above and below the sequences, respectively. The α-helices and β-sheets are shown as bars. $tRNA^{His}$-interacting residues and SAM-interacting residues mutated in the biochemical assays are indicated.

Supplementary Fig. 2: $tRNA^{His}$ methylation by the BCDIN3Ds.

(A) *In vitro* methylations of the human $tRNA^{His}$ transcript by BCDIN3D proteins from various organisms. tRNAs were separated by gel electrophoresis under denaturing conditions, and stained with toluidine blue (upper). ^{14}C-methyated tRNAs were detected with a BAS-5000 imager (lower), and the relative ^{14}C-band intensities were calculated. The intensity of ^{14}C-methyated tRNA by human BCDIN3D was designated as 100. **(C)** *In vitro* methylation assay of the human $tRNA^{His}$ transcript with 5'-triphosphate (ppp-$tRNA^{His}$). ppp-$tRNA^{His}$ cannot be methylated by BCDIN3D.

Supplementary Fig. 3: Representative images of electron densities.

2*Fo*-*Fc* electron density contoured at 1.0 σ around **(A)** the SAH molecule and (**B**) residues corresponding to the β1-strand and α2-helix (residues I68-F88).

Supplementary Fig. 4: Sequence alignments of the Rossmann fold family methyltransferases.

The amino acid sequences of human BCDIN3D (hBCDIN3D) and human MePCE (hMePCE, PDB ID: 6DCC), human METTL16 (hMETTL16, PDB ID: 6DU4), human TRAM61A (hTRAM61A, PDB ID: 5CCB), human NSUN6 (hNSUN6, PDB ID: 5WWS), and mouse DNMT1 (mDNMT1, PDB ID: 4DA4) are aligned. Secondary structural elements of hBCDIN3D are depicted above the sequences. The α-helices and β-strands are shown as bars. $tRNA^{His}$-interacting residues and SAM-interacting residues mutated in the biochemical assays are indicated by blue circles and green triangles, respectively.

Supplementary Fig. 5: Structures of Rossmann fold family methyltransferases.

(**A**) - (**F**) Structures of (A) human BCDIN3D, (B) human MePCE (PDB ID: 6DCC), (C) human METTL16 (PDB ID: 6DU4), (D) human TRAM61A (PDB ID: 5CCB), (E) human NSUN6 (PDB ID: 5EES), and (F) mouse DNMT1 (PDB ID: 4DA4).

Supplementary Table S1: Nucleotide sequences of synthetic BCDIN3D genes.

Supplementary Table S2: List of synthetic oligonucleotides.