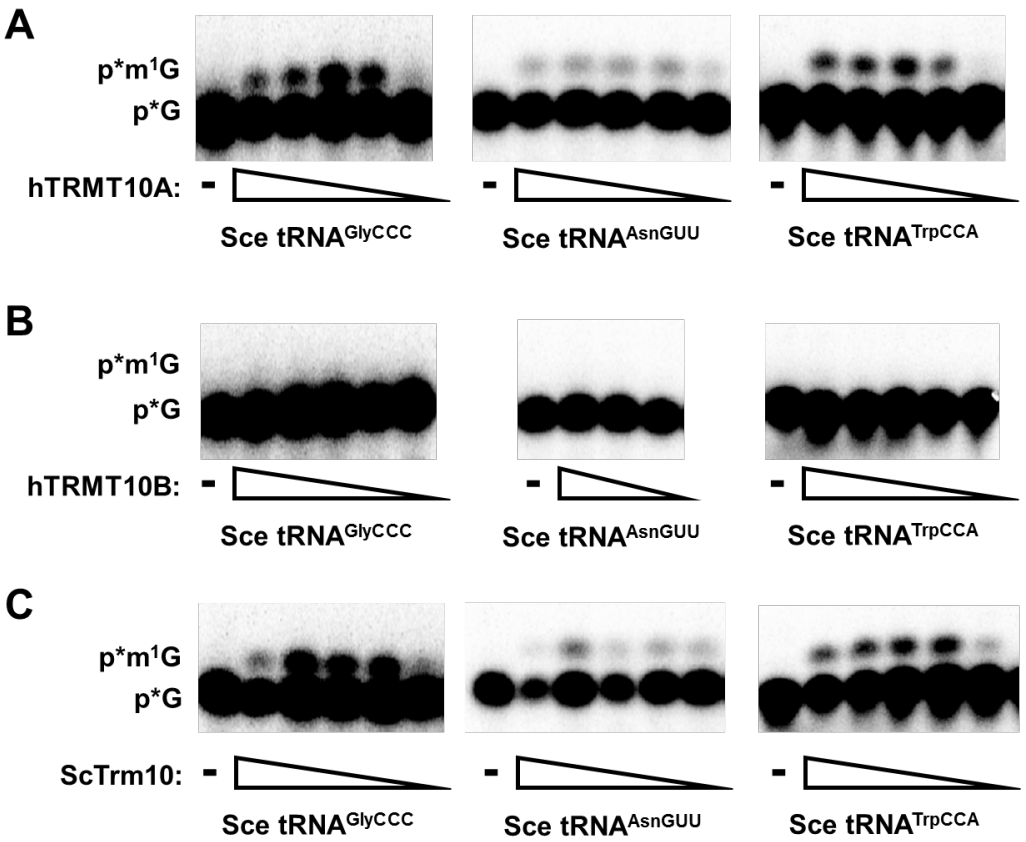
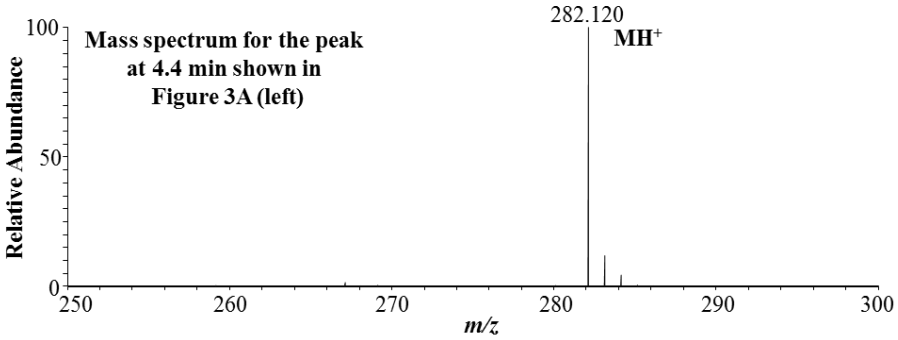
Supplementary Material for Howell et al., *Distinct substrate specificities of the human tRNA methyltransferases TRMT10A and TRMT10B*

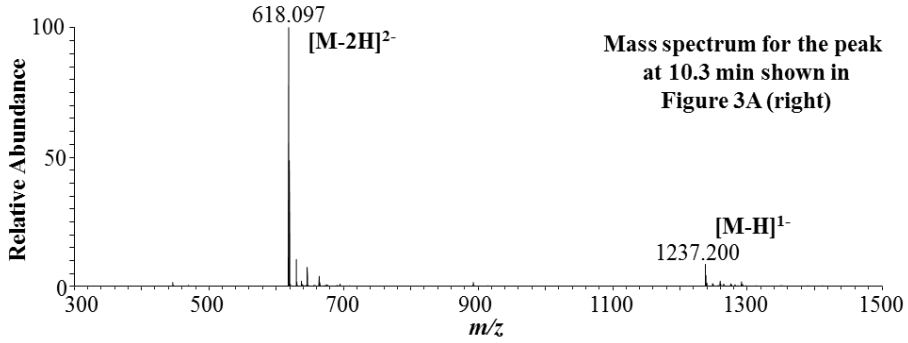
SUPPLEMENTAL FIGURE LEGENDS

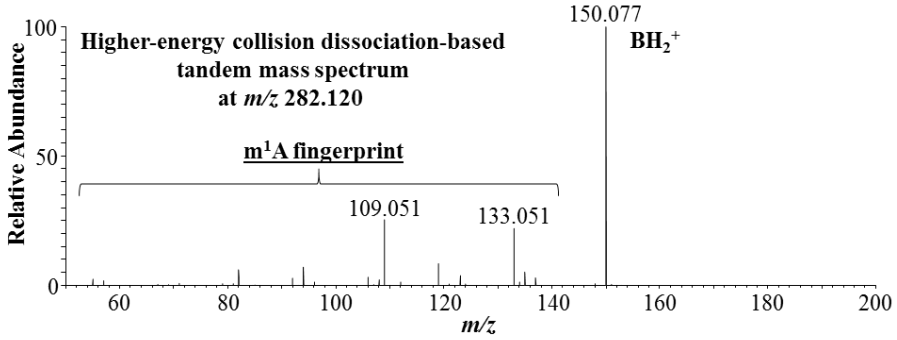
Figure S1. hTRMT10A, and not hTRMT10B, exhibit activity in vitro on *S. cerevisiae* tRNAs. Labeled tRNA were incubated with 5-fold dilutions of hTRMT10A (**A**, 15000 to 24 μg/mL), hTRMT10B (**B**, 3000 to 4.8 μg/mL), or ScTrm10 (**C**, 20000 to 32 μg/mL) or no enzyme (-) for 1 hour, followed by quenching, nuclease P1 digestion, and TLC separation of product (p*m^1G) from substrate (p*G) as in main text, Figure 2.

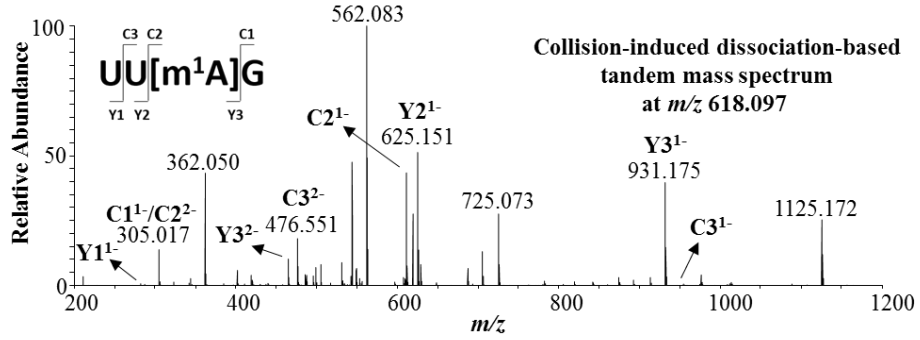
Figure S2. Mass spectra confirm ribonucleoside modification incorporated by hTRMT10B as m^1A at position 9 of $tRNA^{Asp}$. Mass spectrometric features of the nucleoside (left column) and oligonucleotide (right column) analyses of $tRNA^{Asp}$ transcript (with and without hTRMT10B incubation).


A
p*m1G
p*G
hTRMT10A:
-
Sce tRNAGlyCCC
Sce tRNAAsnGUU
Sce tRNATrpCCA
B
p*m1G
p*G
hTRMT10B:
-
Sce tRNAGlyCCC
Sce tRNAAsnGUU
Sce tRNATrpCCA
C
p*m1G
p*G
ScTrm10:
-
Sce tRNAGlyCCC
Sce tRNAAsnGUU
Sce tRNATrpCCA


Mass spectrum for the peak
at 4.4 min shown in
Figure 3A (left)
282.120
MH+
Relative Abundance
m/z


618.097
[M-2H]2-
Mass spectrum for the peak
at 10.3 min shown in
Figure 3A (right)
[M-H]1-
1237.200
Relative Abundance
m/z


Higher-energy collision dissociation-based
tandem mass spectrum
at m/z 282.120
m1A fingerprint
150.077
BH2+
109.051
133.051
Relative Abundance
m/z


UU[m1A]G
C3 C2 C1
Y1 Y2 Y3
Collision-induced dissociation-based
tandem mass spectrum
at m/z 618.097
562.083
C21-
Y21-
625.151
Y31-
931.175
362.050
C32-
476.551
C11-/C22-
305.017
Y32-
Y11-
725.073
C31-
1125.172
Relative Abundance
m/z