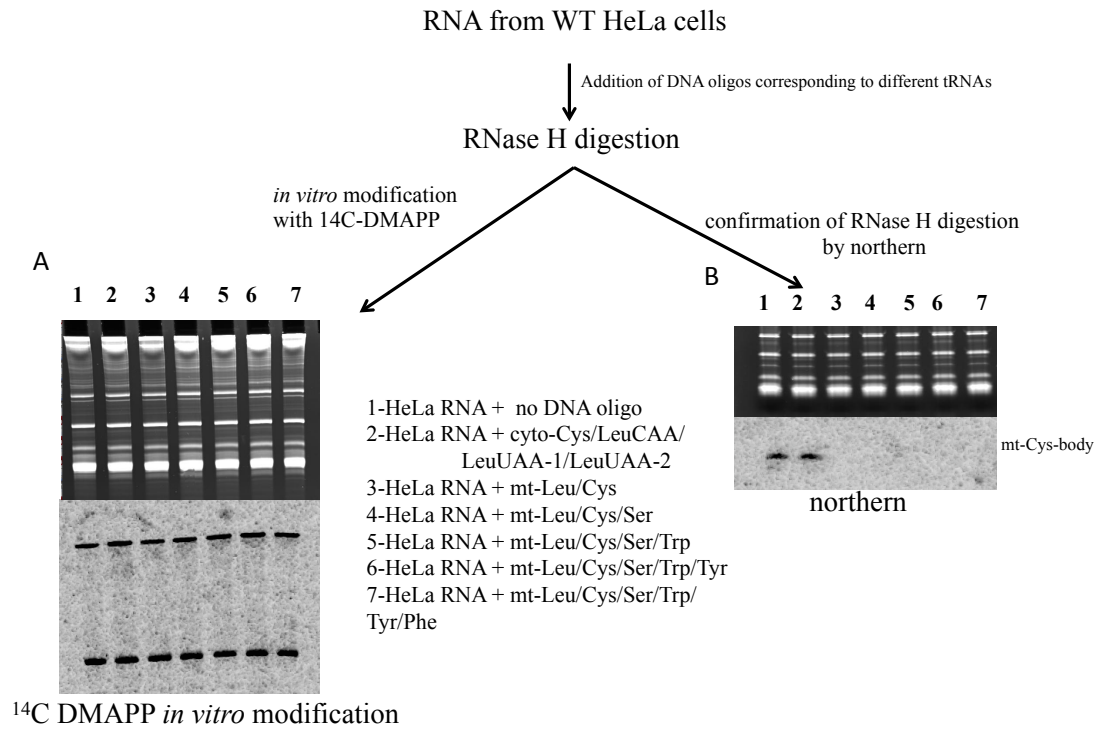


Lamichhane et al., Supplemental material

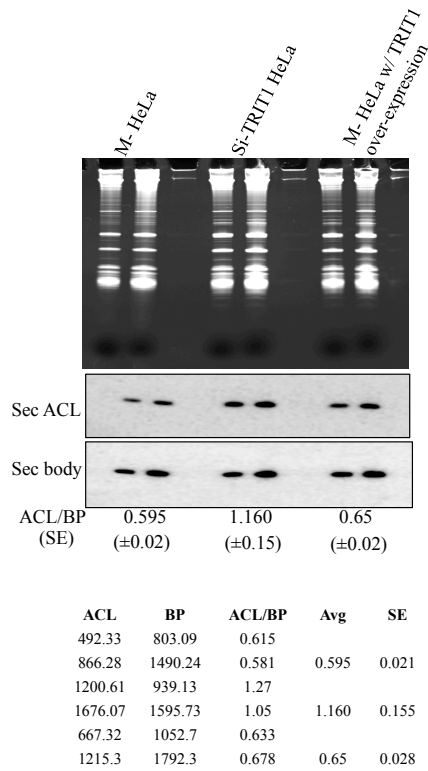
Supplemental Figure S1. The RNA modified *in vitro* by TRIT1, but not *in vivo*, is not tRNACys, tRNA^{Leu} or mt-tRNAs. As specified in the figure, the complementary DNA oligo of these selected tRNAs was added for elimination by RNase H. RNA was purified after RNase H digestion and split into two parts for further analysis: A) *in vitro* modification with ¹⁴C-DMAPP and His-TRIT1, and B) confirmation that elimination worked for mt-tRNACys.

Supplemental Figure S2: Overexpression of TRIT1 does not increase the i6A37 content of tRNA[Ser]Sec. Left: PHA6 assay. Right: Immunoblot; lanes 1 & 3 show independent control cell extracts, lanes 2 & 4 show independent TRIT1 over expression. Detection was with anti-TRIT1 Ab.

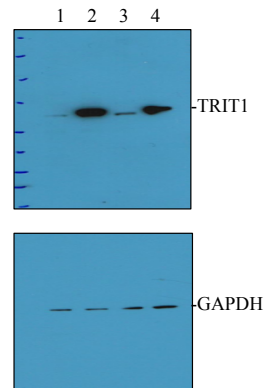
Supplemental Figure S1, Lamichhane et al.



Supplemental Figure S2, Lamichhane et al.



Measurement of i6A modification level of tRNA^{Sec} in TRIT1 over-expressed sample. Result shows that i6A modification level of tRNA^{Sec} is not increased in wild-type cells in response to TRIT1 over-expression



(Standard error is calculated using 1X and 2X samples)