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Supplemental Material to:

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Small RNAs derived from the 5′ end of tRNA can inhibit protein translation in human cells

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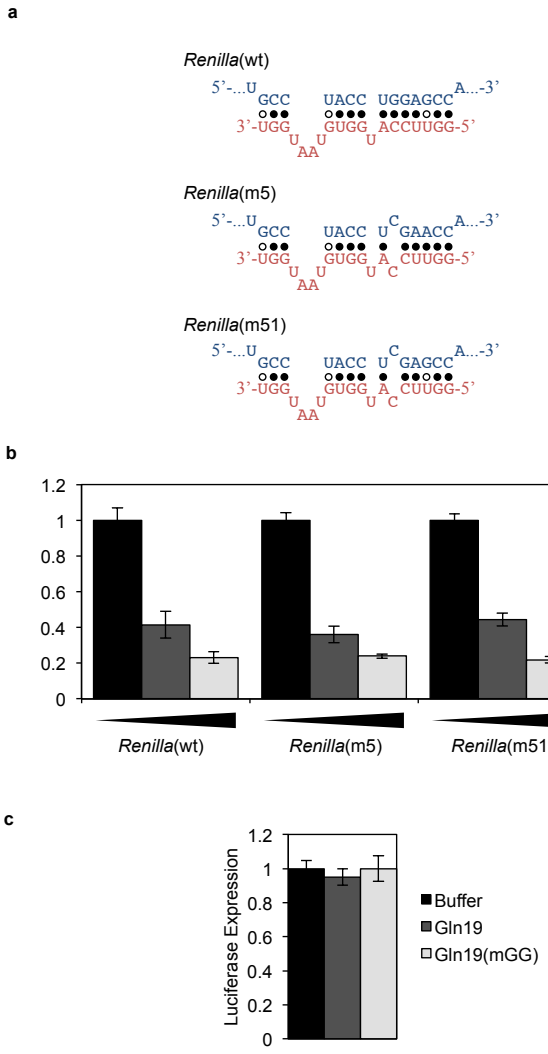
Supplemental Figure 1

Diagrams of small RNA target sites in reporter constructs used for *in vivo* assays. psiCheck-2-derived plasmids contain the indicated number of repeats of predicted small RNA target sites [complementary to either *let-7* or tRF(Gln)]. "Perfect" constructs have no mismatched basepairs, "mismatch" constructs have mismatches to small RNA bases 9–12 and "control" constructs additionally have mismatches to small RNA bases 3–5 and base 12 (if applicable). Target sites are shown in blue, and the tRF(Gln) or *let-7* sequences are shown in red. Basepairs are indicated with filled circles.

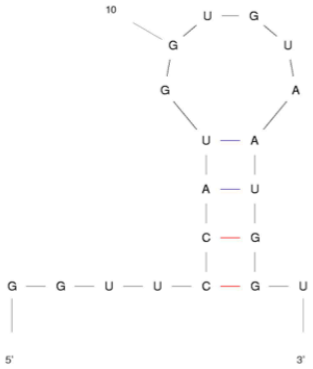
Supplemental Figure 2



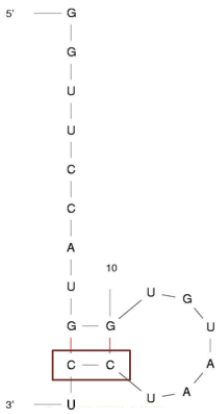
5′ tRFs do not affect luciferase enzyme activity, and repression of *Renilla* luciferase translation does not require a miRNA-like target site in the coding sequence. **a**. A potential Gln19 target site in the *Renilla* luciferase coding sequence, and the two mutations made in order to disrupt this site. These mutations are silent and do not affect the *Renilla* amino acid sequence. Gln19 is shown in red and the target site is shown in blue. Watson–Crick basepairs are indicated with filled circles and G:U wobble basepairs are indicated with non-filled circles. **b**. The mRNAs shown in (**a**) were used in an *in vitro* translation reaction containing increasing concentrations of Gln19, and *Renilla* activity subsequently measured. The concentrations of oligo used were 0, 5 and 10 μM. **c**. tRFs do not affect *Renilla* luciferase enzyme activity. *Renilla* luciferase was translated *in vitro*. 10 μM Gln19 or Gln19(mGG), or buffer alone, was subsequently added and luciferase activity measured.

Supplemental Figure 3

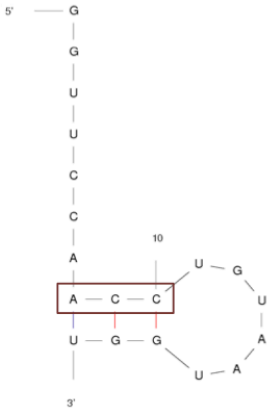
Gln19



Gln19(mGG)

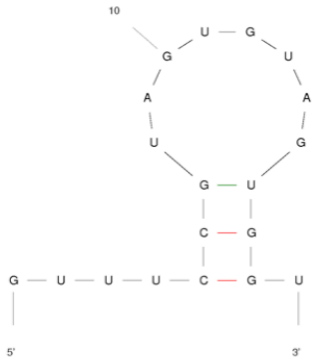


Gln19(mMid)

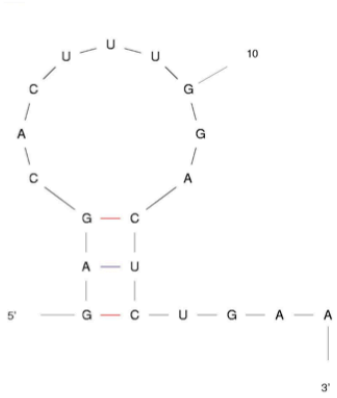


Glu19

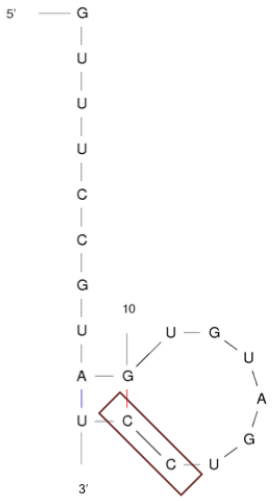
Val19



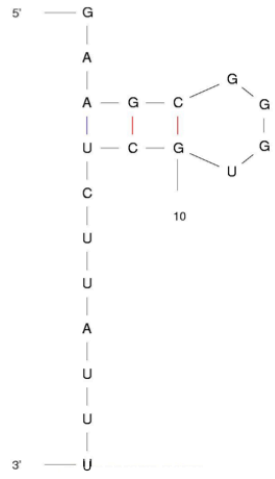
Glnmid



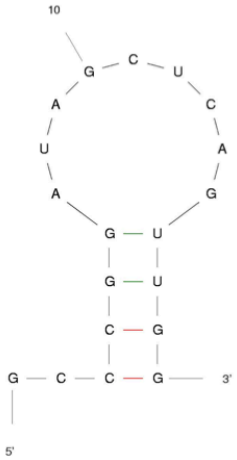
Val19(mGG)



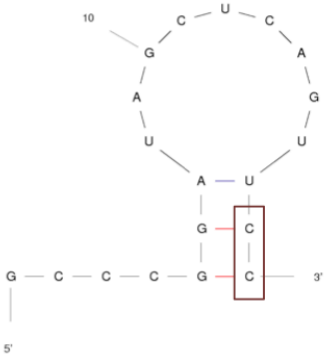
tRF-1001



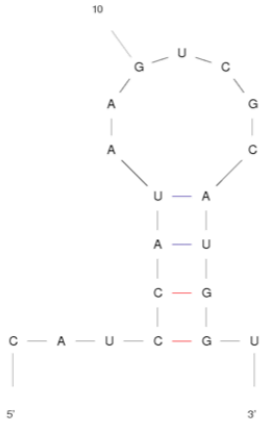
Lys19



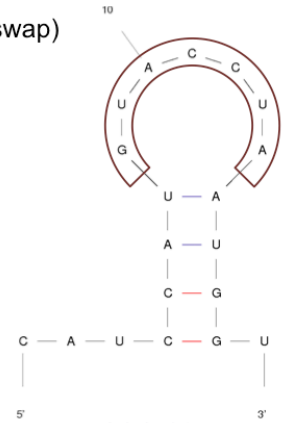
Lys19(mGG)



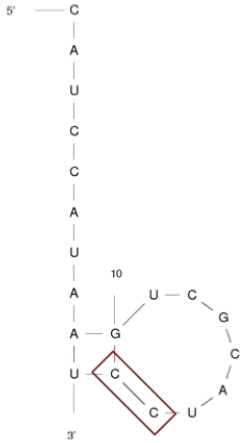
Artificial19



Artificial19(loopswap)



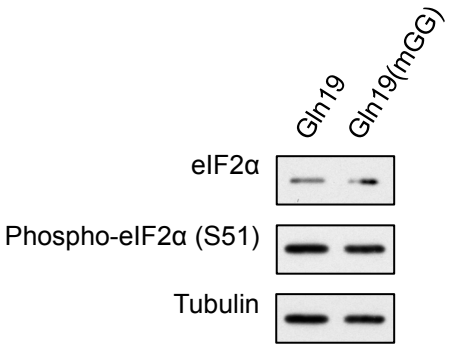
Artificial19(mGG)



Artificial19(unstruct)

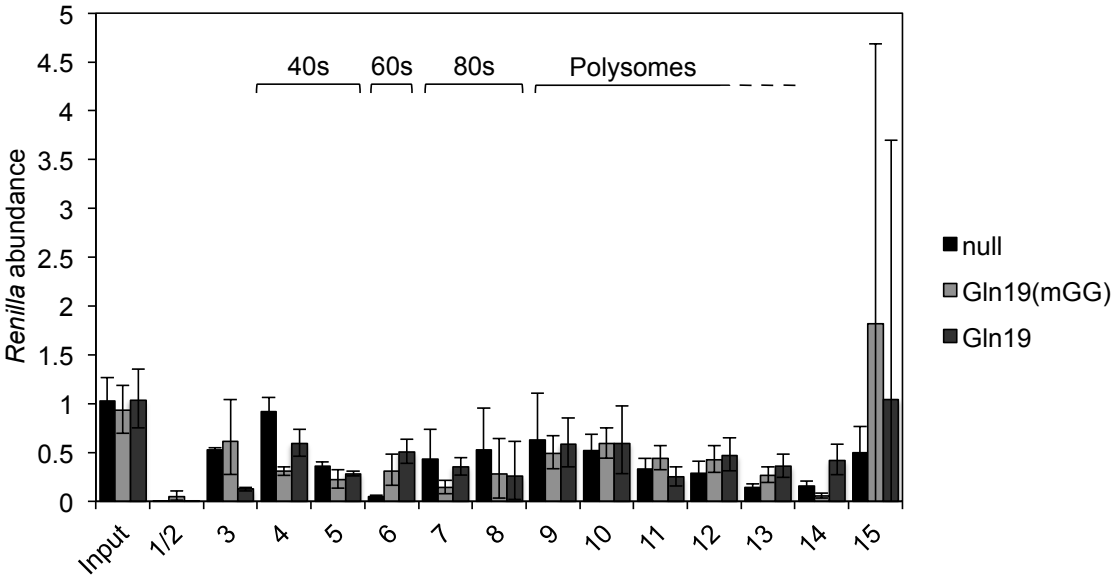
Oligonucleotide secondary structures as determined by mfold. The lowest free-energy structure is shown. For modified RNAs, the modified bases are outlined by a red box.

Supplemental Figure 4



5′ tRFs do not induce eIF2α phosphorylation. *In vitro* translation reactions were performed in the presence of 10 μM Gln19 or Gln19(mGG). The phosphorylation state of eIF2α was subsequently measured by western blotting.

Supplemental Figure 5



mRNA associated with polysomes does not change after Gln19 transfection. HeLa cells were transfected with either no small RNA (null), Gln19 or Gln19(mGG), plus the reporter psiCheck-2 to provide a known target mRNA (*Renilla*). The cell lysate was separated on a 10–50% (w/v) sucrose gradient to resolve polysomes, and *Renilla* abundance measured in each fraction by qPCR.