

Figure S1. C5/C1' regions of  $^{13}\text{C}$ - $^1\text{H}$  HSQC spectra (bottom) of the anticodon stem-loop after ( $\psi_{32}\text{ACSL}^{\text{Phe}}$ ) and before ( $\text{ACSL}^{\text{Phe}}$ ) modification. Isomerization of uridine to form the C5-C1' glycosidic bond results in loss of the C5-H5 cross peak. One-dimensional slices (above) through the HSQC spectra at the chemical shift corresponding to  $\text{U}_{32}$  C5. No residual uridine could be detected in the sample after modification with RluA enzyme.

