The molecular basis of tRNA selectivity by human pseudouridine synthase 3 (PUS3)

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Figure 1A. Uncropped SDS-PAGE of purified $PUS3_{WT}$ and PUS_{D118A}.

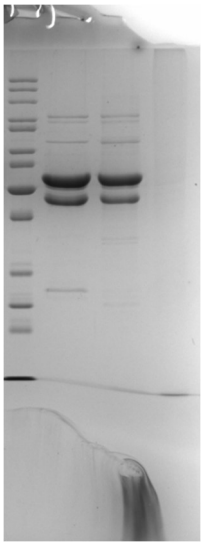


Figure 1B. Uncropped urea gel for detecting Ψ_{39} formation in tRNAs by $PUS3_{WT}$ and PUS_{D118A}.

Figure 2E. Uncropped urea gel for detecting Ψ_{39} formation in tRNAs by PUS3 wild-type and mutants.

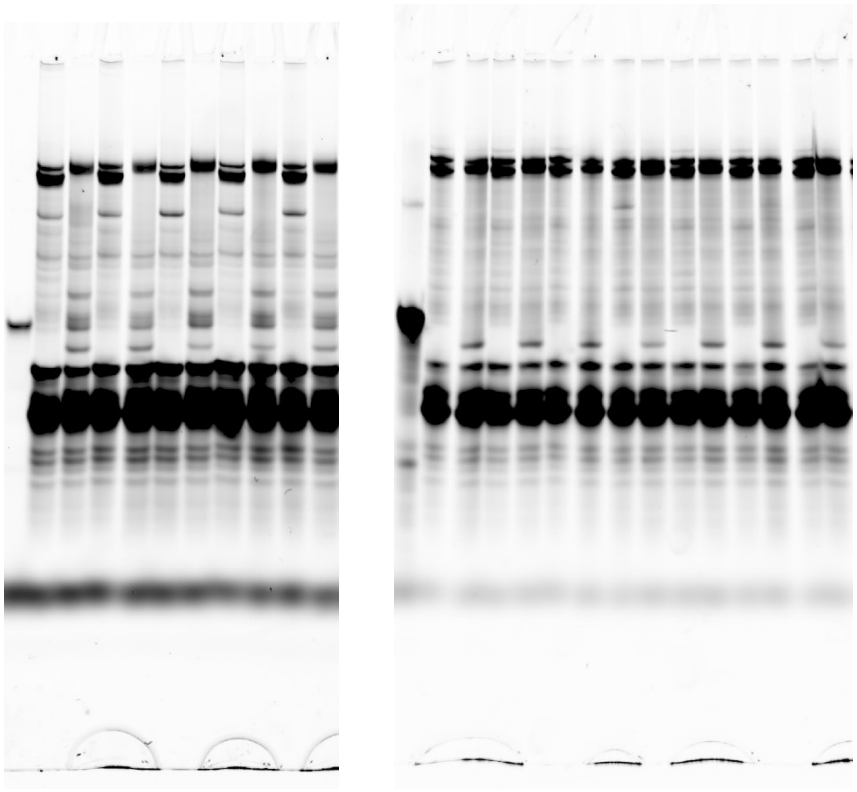


Figure 3B. Uncropped urea gel for detecting Ψ_{39} formation in tRNAs (in mature $tRNA^{Arg}_{UCU}$ and pre-$tRNA^{Arg}_{UCU}$) by $PUS3_{WT}$.

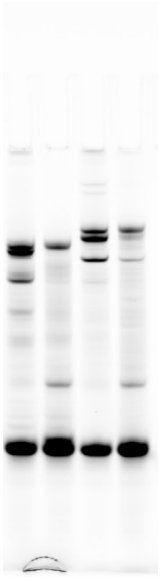


Figure 5B. Uncropped Western blots of detecting endogenous PUS1 and PUS3 proteins and GAPDH in 293 cells (Left: anti-PUS1; Middle: anti-PUS3; Right: anti-GAPDH).

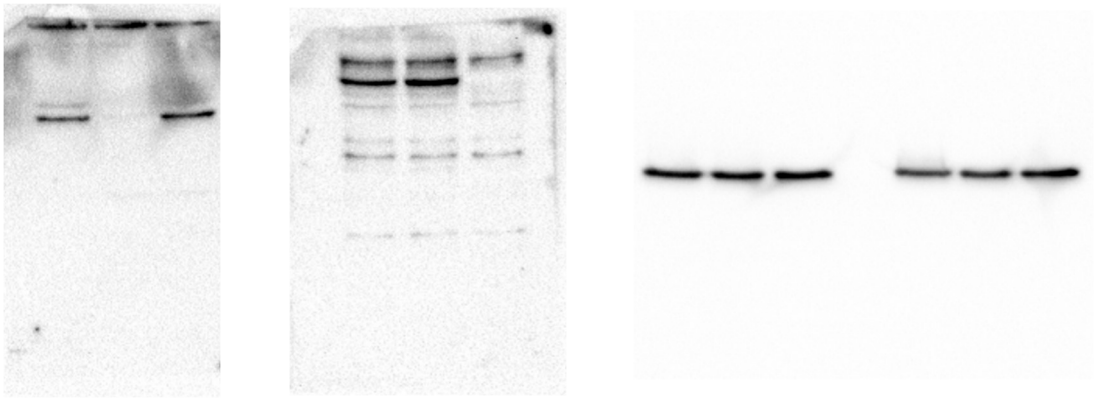


Figure 5B. Uncropped urea gel for detecting Ψ_{28} and Ψ_{39} in tRNAs in WT, PUS1$^{-/-}$ and PUS3$^{-/-}$ cells (left: detection of Ψ_{28}; Right: detection of Ψ_{39}).

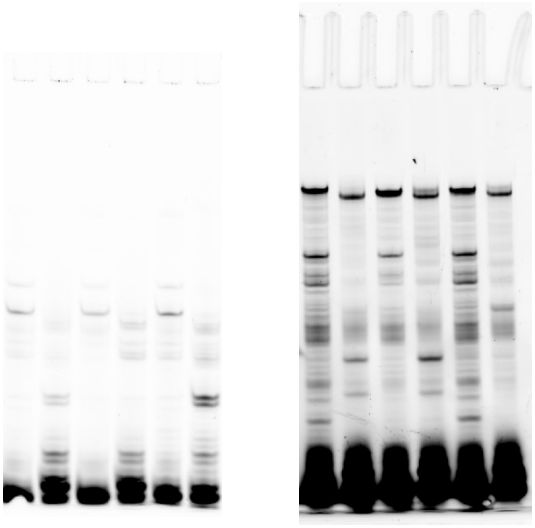


Figure 6A. Uncropped urea gel for detecting PUS1- or PUS3-dependent Ψ formation in *MT-ND4* and $tRNA^{Gln}_{UUG}$ *in vitro* (Left: CMC on MT-ND4 with PUS1; Right: CMC on tRNA with PUS1 and PUS3 and MT-ND4 with PUS3).

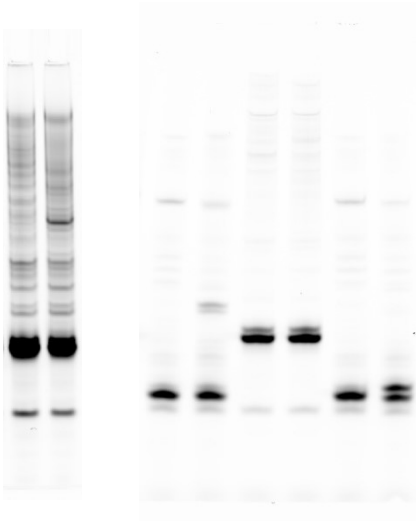


Figure 7B. Uncropped SDS-PAGE of purified $PUS3_{WT}$ and mutants.

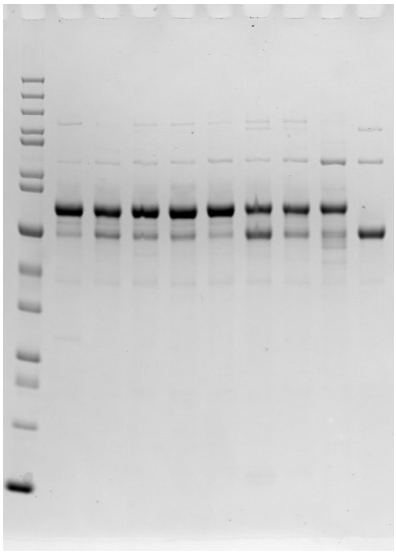


Figure 7E. Uncropped urea gel for detecting Ψ_{39} formation in tRNAs by PUS3 wild-type and clinically-relevant mutants.

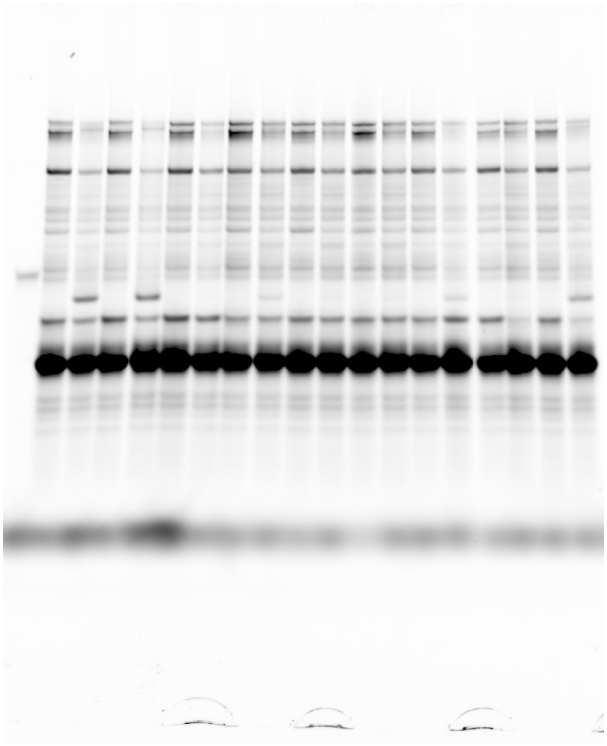
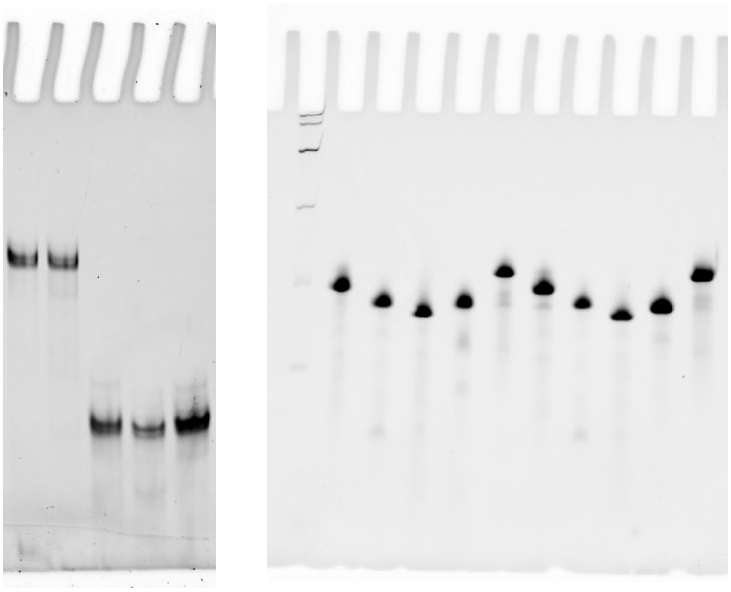
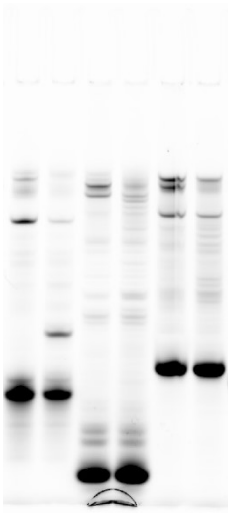


Figure 10. Uncropped Western blots of detecting exogenous PUS3 proteins, EGFP and Lamin in 293T cells (Top: anti-PUS3; Middle: anti-EGFP; Bottom: anti-Lamin). Of note; gels are reversed.

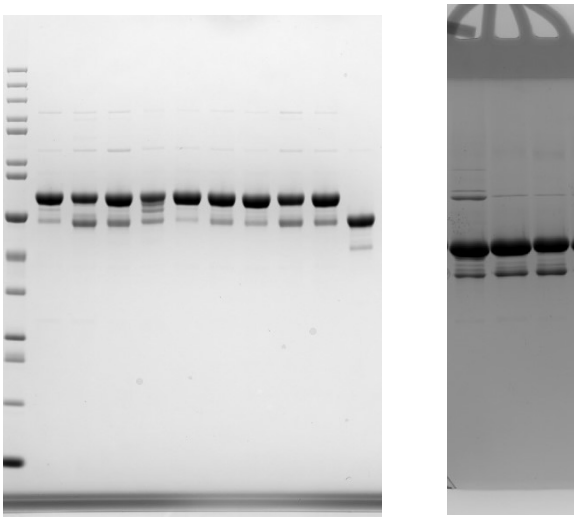
Supplementary Figure 1B. Uncropped urea gels for *in vitro* produced RNAs.



Supplementary Figure 1D. Uncropped urea gel for detecting Ψ_{39} formation in tRNAs by PUS3 wild-type.

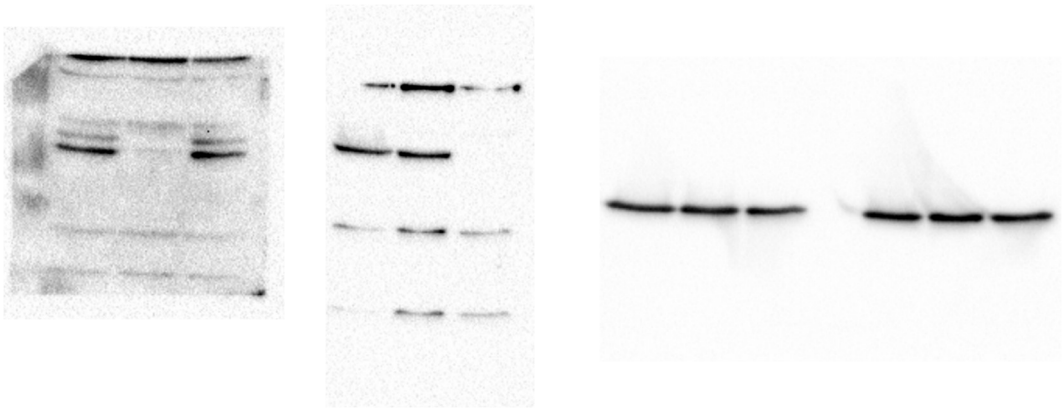


Supplementary Figure 4A. Uncropped SDS-PAGE of purified $PUS3_{WT}$ and mutants.

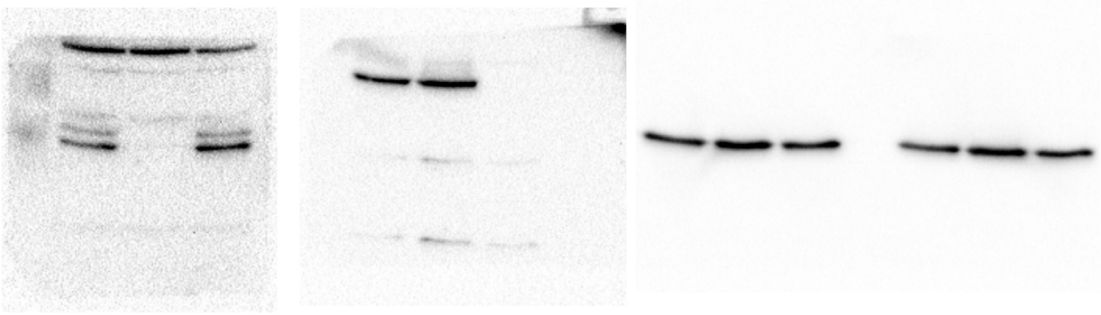


Supplementary Figure 8A. Uncropped Western blots of detecting endogenous PUS1 and PUS3 proteins and GAPDH in 293 cells (Left: anti-PUS1; Middle: anti-PUS3; Right: anti-GAPDH).

Clone2:

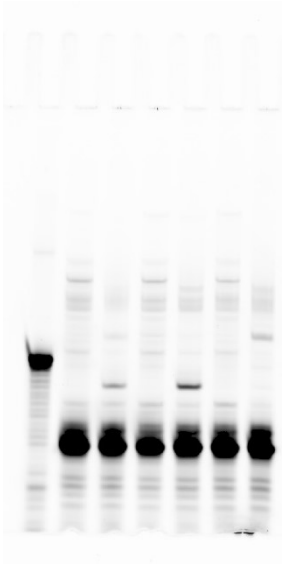


Clone3:

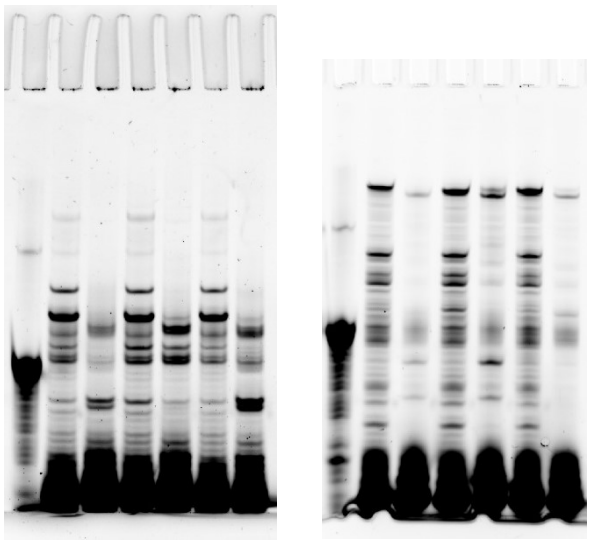


Supplementary Figure 8B. Uncropped urea gels for detecting Ψ_{28} and Ψ_{39} formation in tRNAs in 293 cells.

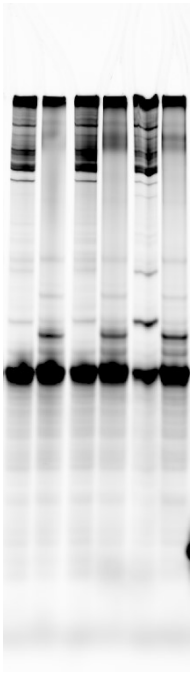
Clone2:



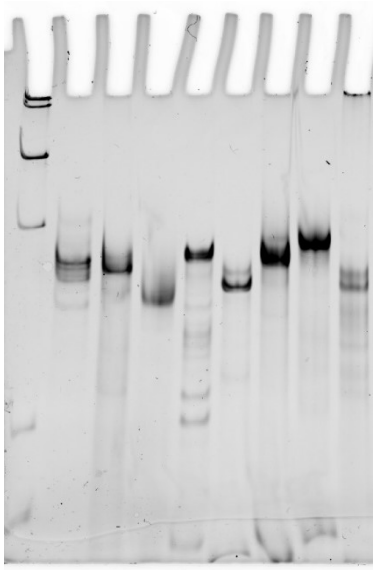
Clone3 (Left: Ψ_{28} ;Right: Ψ_{39}):



Supplementary Figure 8G. Uncropped urea gels for detecting Ψ in 18s rRNA in 293 cells.



Supplementary Figure 10A. Uncropped urea gel for *in vitro* transcribed RNAs.



Supplementary Figure 10B. Uncropped urea gel for detecting PUS3-dependent Ψ formation in mRNAs and $tRNA^{Gln}_{UUG}$ *in vitro*.