# Understanding PUS7 and its impact in disease


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## 1 Introduction.

Pseudouridine (ψ) is the C5-glycosidic isomer of uridine (**Figure 1**), formed by pseudouridine synthase (PUS) enzymes. This modification influences the structure and function of multiple RNA species [1]. One RNA modifying enzyme catalyzes ψ incorporation at uridine 13 in tRNA. In disease, there is an increase in pseudouridylation at uridine 50, mediated by this RNA modifying enzyme [2].

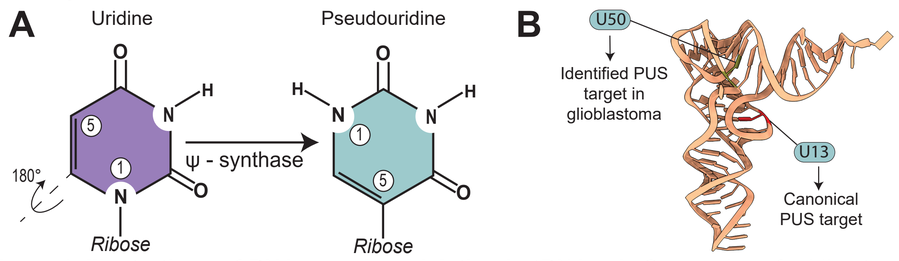



**Figure 1. Illustrations of RNA pseudouridylation in tRNAs. A.** Conversion of uridine to pseudouridine. **B**. 3D conformation of a tRNA with PUS-dependent pseudouridine sites.

## 2 Aim and approach.

Our aims is to understand underlying mechanism of RNA modifying enzymes in fundamental RNA regulation and impacts in human health. We elucidated the activity of one RNA modifying enzyme in vitro and in vivo. To do so, we took a combined approach using biochemical (**Figure 2**), structural, and genetic characterisations.

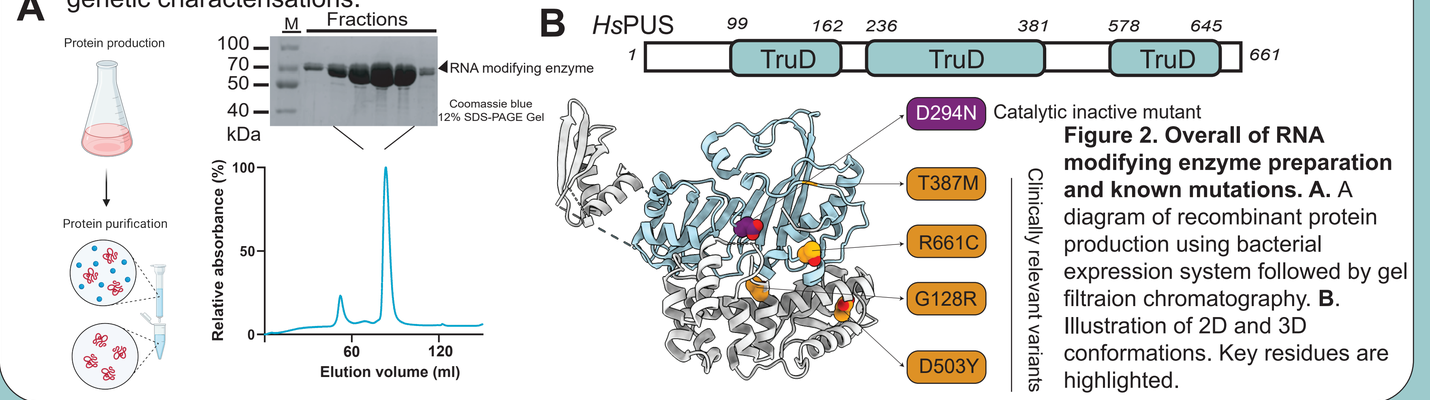



**Figure 2. Overall of RNA modifying enzyme preparation and known mutations. A.** A diagram of recombinant protein production using bacterial expression system followed by gel filtraion chromatography. **B.** Illustration of 2D and 3D conformations. Key residues are highlighted.

## 3 tRNA production and purification.

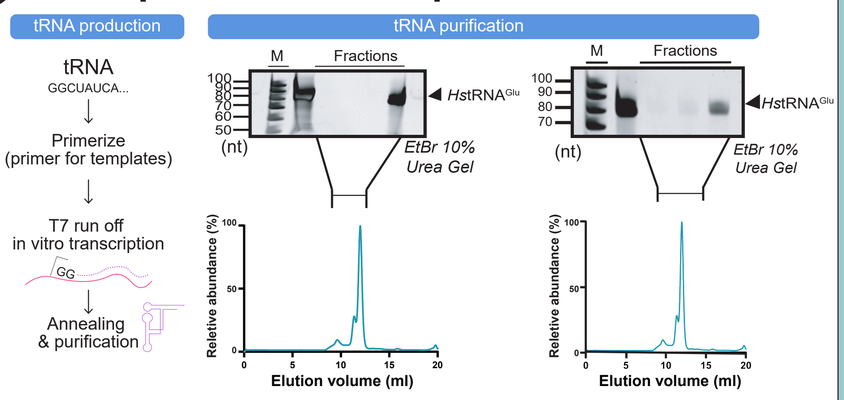



**Figure 3. Illustrations of tRNA production pipeline.** tRNAs were produced using in vitro transcription and purified via sequential steps of chromatography methods. This can be applied to produce internally fluorescent labeled RNA.

## 4 Biophysical characterisations of RNA modifying enzyme.

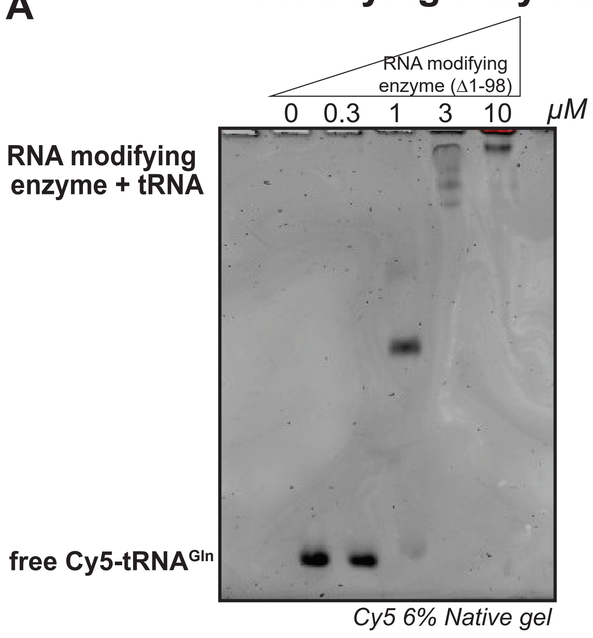



**Figure 4. RNA modifying enzyme interacts with tRNA at low nanomolar range. A.** Electrophoresis mobility shift assay presents RNA modifying enzyme interacts with Cy5-tRNA$^{Gln}$. The free tRNA and bound tRNA are indicated. **B**. Biophysical characterisations of the interaction using flow induced dispersion analysis. Left: hydrodynamic radius changes upon increasing RNA modifying enzyme concentration; Right: fluoresence ratio changes upon increasing RNA modifying enyme concentrations. The Kds are indicated in boxes.

## 5 RNA modifying enzyme catalyses pseudouridylation on several tRNA.

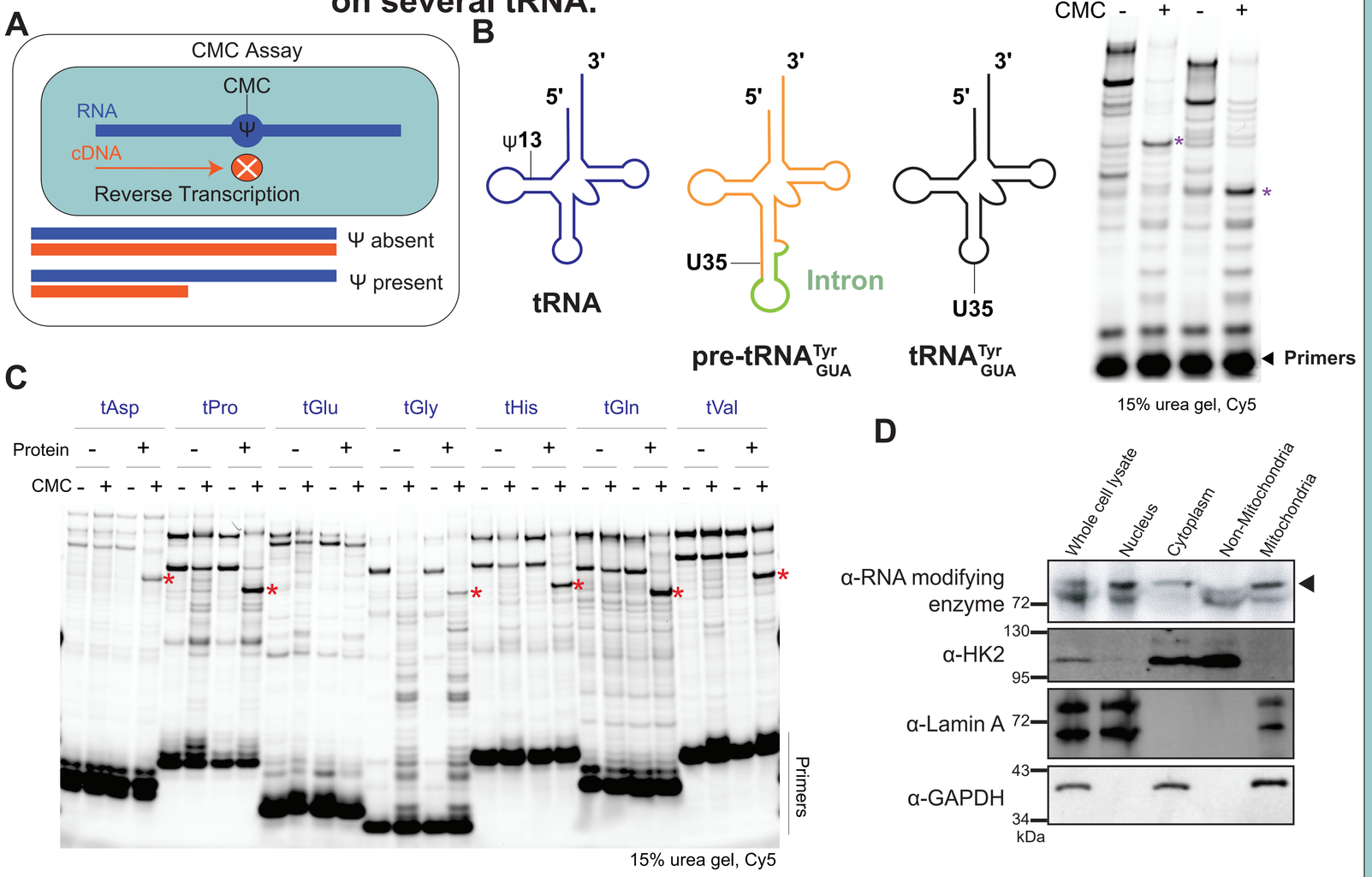



**Figure 5. RNA modifying enzyme - dependent pseudouridylation on mature and pre-mature tRNAs. A.** Illustration of a chemical coupled-primer extension assay to determine pseudouridine sites in RNA. **B-C.** Detection of pseudouridines in RNA. Cartoon illustration of different tRNAs and different RNA modifying enzyme-targeting sites (**B**). The truncated cDNA products are indicated in gels with * (**B and C**). **D**. Cellular localisation of RNA modifying enzyme in MDA-MB-231 cells.

## 6 PUS7 knockdown in the gut of *Drosophila Melanogaster*

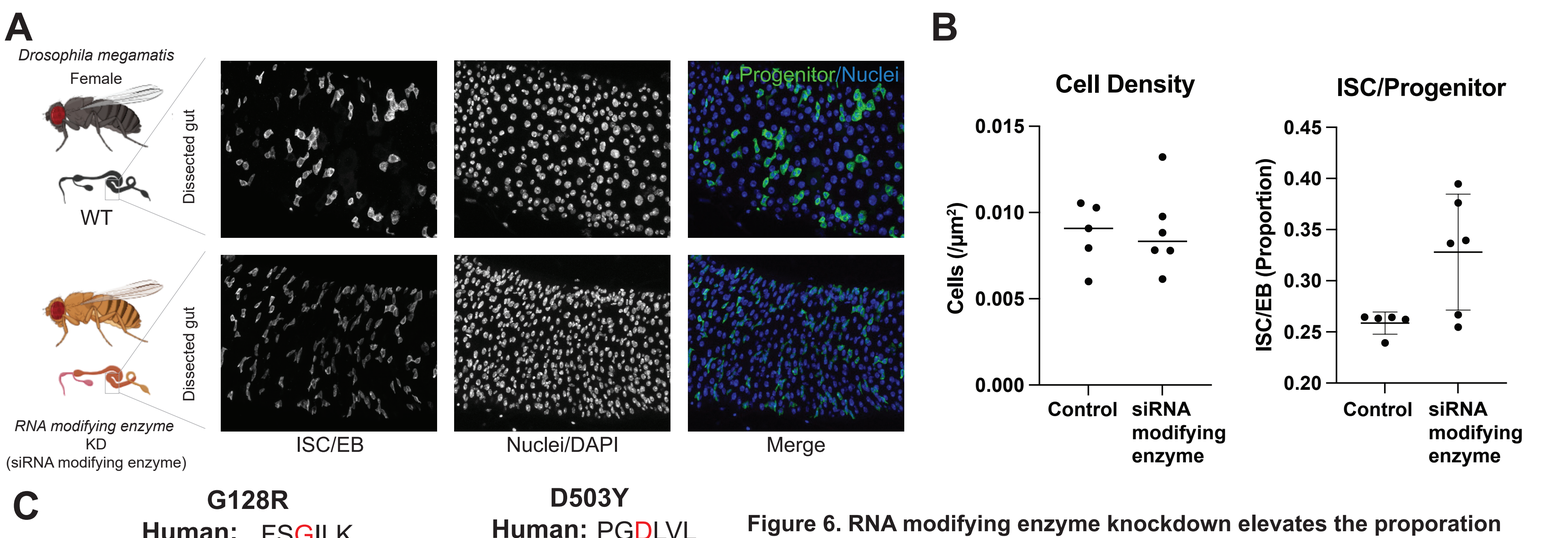
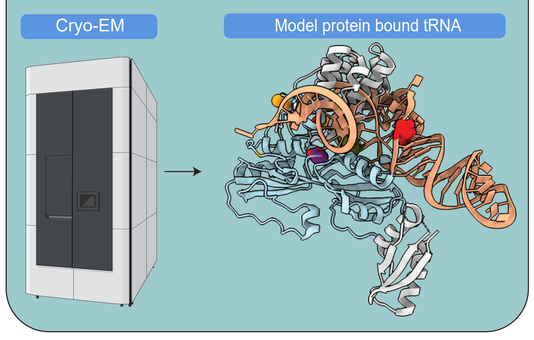



**Figure 6. RNA modifying enzyme knockdown elevates the proporation of intestinal stem cells within** *Drosophila Melanogaster* **A.** Representative immunoflourescence images of intestinal stem cells (ISCs) and enteroblasts (EBs) within the midgut of Drosophila. **B.** Quantification of cell density and proportion of ISCs/EBs in WT and RNA modifying enzyme KD cells. **C.** Sequence alignment of 2 clinically relevant RNA modifying enzyme variants, providing a baseline to investigate their function in vivo.

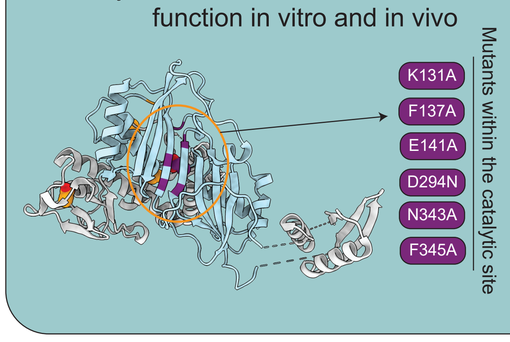
## 7 Conclusion and future perspectives

Biophysical data indicates that RNA modifying enzyme binds tRNA via a 1:1 interaction with cellular localisation analysis suggesting that enzyme activity occurs throughout the cell. Through the CMC assay we have identifed several tRNA that undergo pseudouridylation mediated by RNA modiffying enzyme. Kockdown of RNA modifying enzyme eleavting the proportion of ISCs within the midgut of *Drosophila Melanogaster* highlights the role of the RNA modifying enzyme in cellular homeostasis.

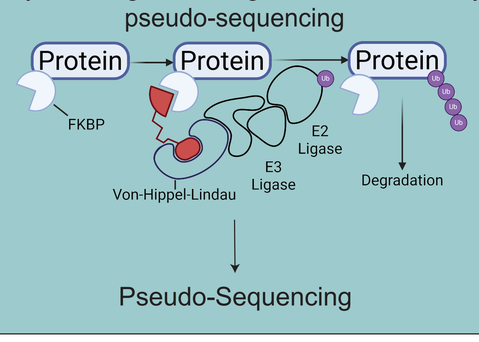
Resolve a strcuture of the protein - tRNA complex binding through Cryo-EM




Characterise various RNA modifying enzyme mutants to better understand its function in vitro and in vivo




Characterise ψ sites dependent on RNA modifying enzyme using dTAG degradation followed by pseudo-sequencing





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**References**

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2) Cui Q, Yin K, Zhang X, Ye P, Chen X, Chao J, Meng H, Wei J, Roeth D, Li L, Qin Y, Sun G, Zhang M, Klein J, Huynhle M, Wang C, Zhang L, Badie B, Kalkum M, He C, Yi C, Shi Y. Targeting PUS7 suppresses tRNA pseudouridylation and glioblastoma tumorigenesis. Nat Cancer. 2021 Sep;2(9):932-949. doi: 10.1038/s43018-021-00238-0. Epub 2021 Aug 16. PMID: 35121864; PMCID: PMC8809511.