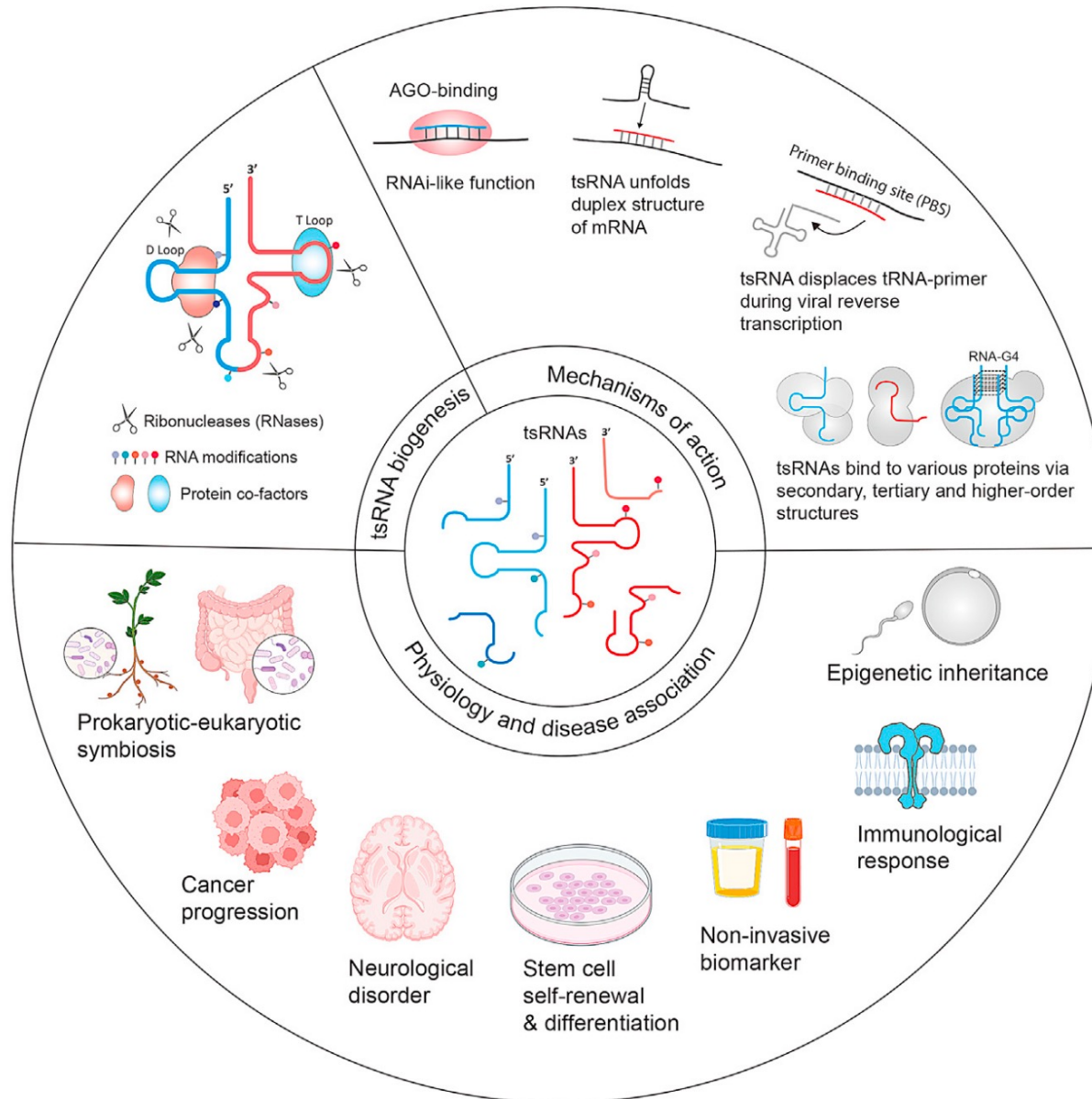


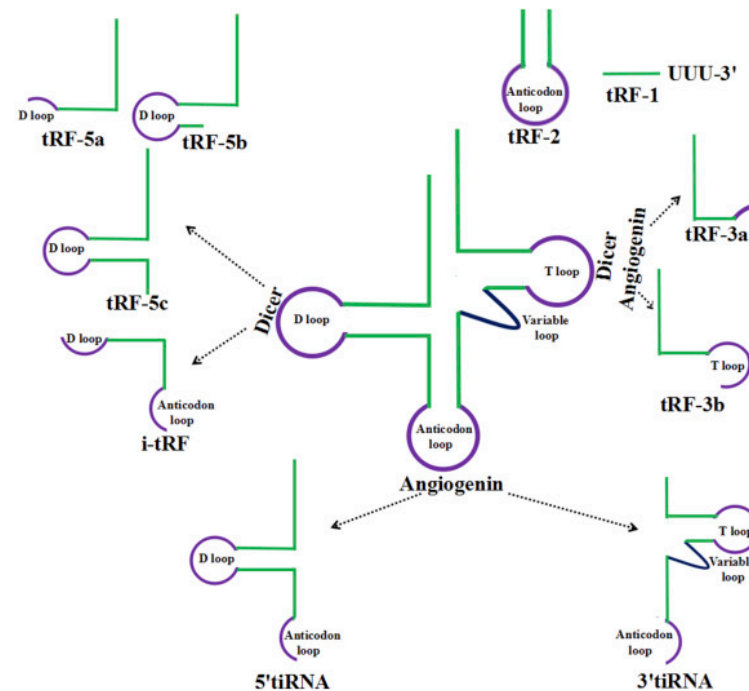
**tRNA fragments**

# tRNA fragments



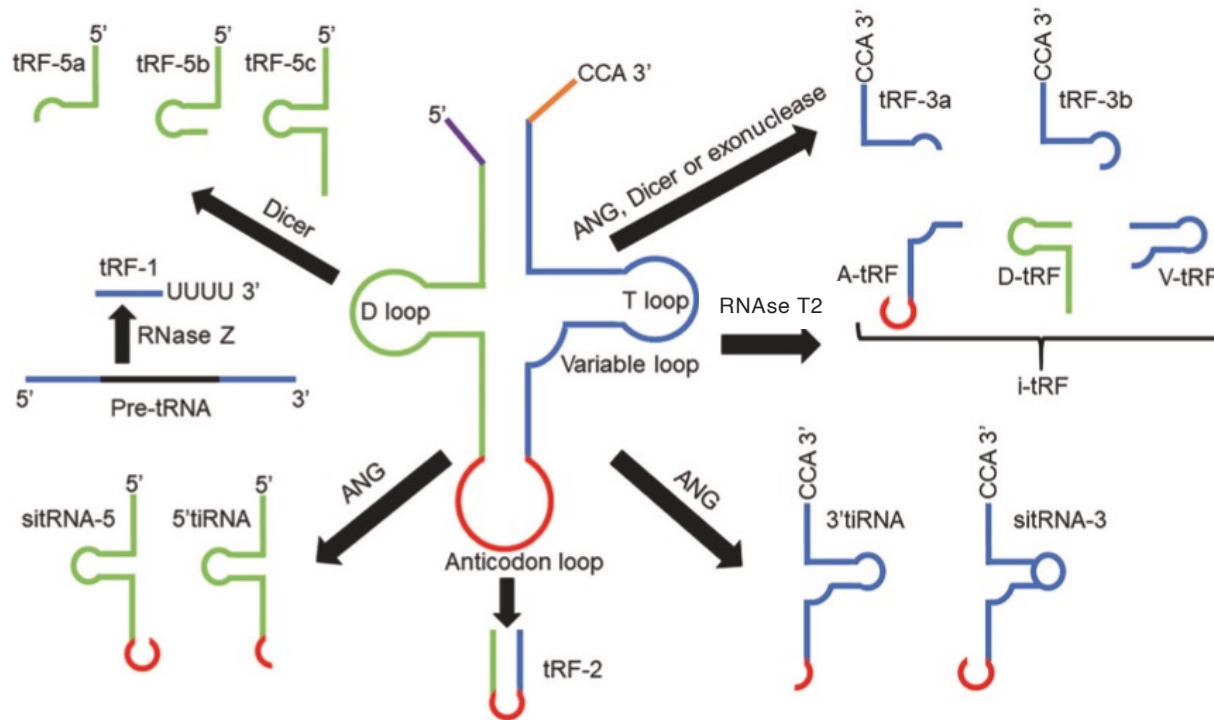
# Fragmentation expands the function of tRNAs

Fragmentation of tRNA unmasks sequence elements that are normally covered in the folded structure, providing sites for binding regulatory proteins. tRNA fragments (tRFs) range in length from 16 to 35 nucleotides (nts) and are produced through cleavage of mature and premature tRNAs at various positions. These cleavages can occur essentially anywhere along the length of the tRNA and are context-dependent. Notably, the majority of tRFs overlap one another and are not adjacent, which suggests complex biogenesis processes.



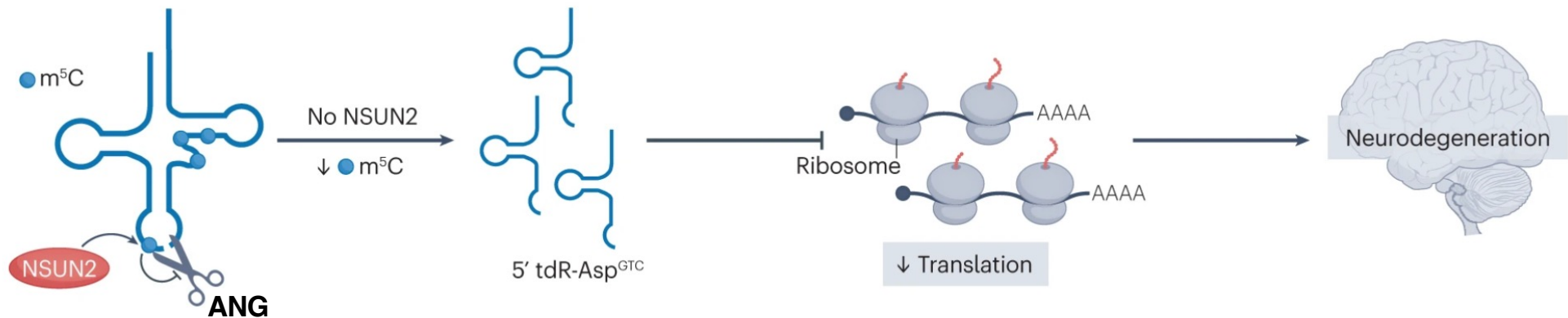
# Biogenesis of tRFs

At present, tRF production is known to depend on several enzymes including **Angiogenin**, **RNase T2**, **Dicer** and **RNase Z/ELAC2**.

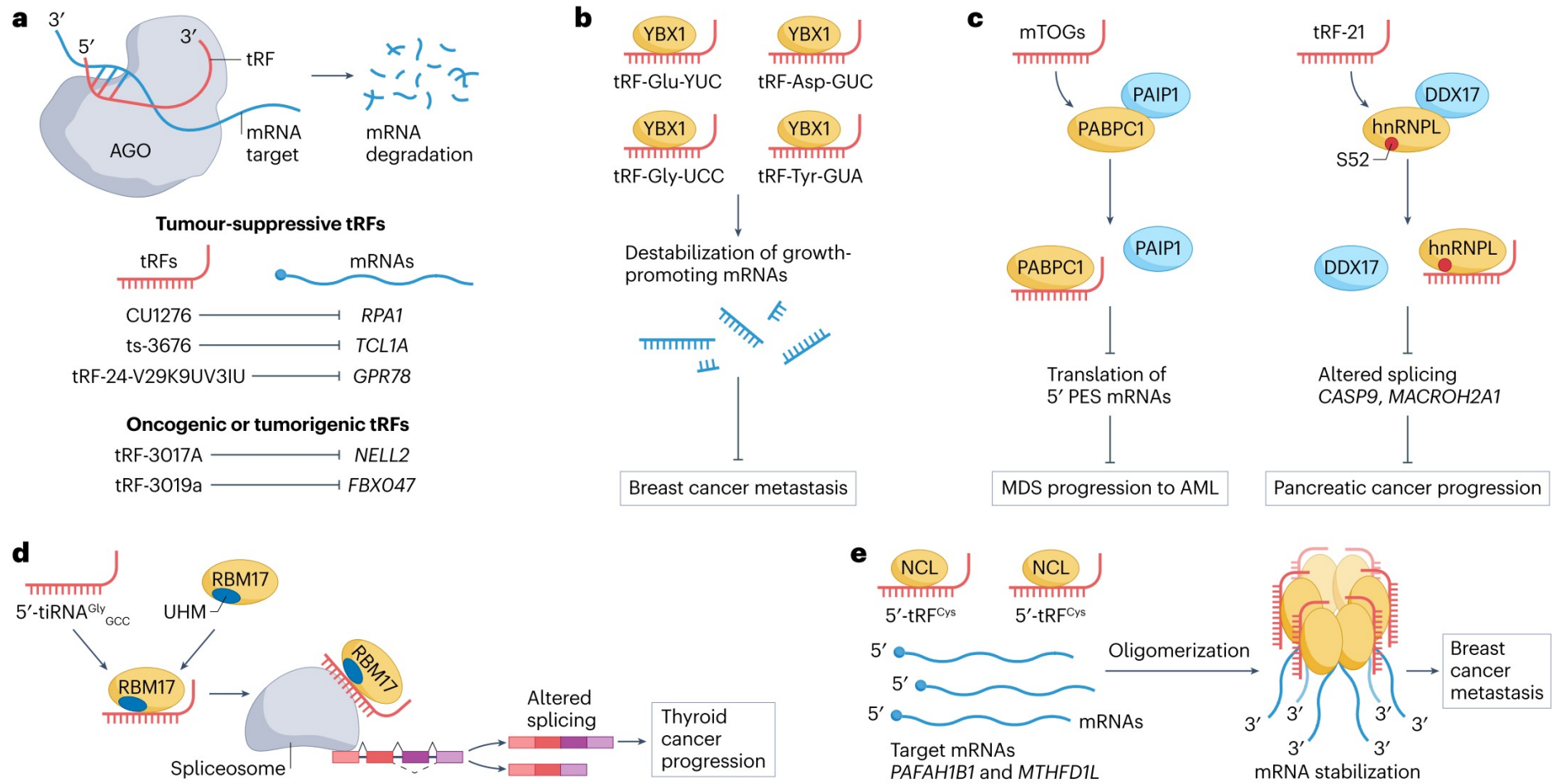


# Aberrant methylation of tRNAs links cellular stress to neuro-developmental disorders

Loss of m<sup>5</sup>C, installed by the methyltransferase NSUN2, increases the **angiogenin (ANG)** mediated endonucleolytic cleavage of tRNA leading to an accumulation of 5'-tRNA-derived small RNA fragments. Accumulation of 5'-tiRNA fragments in the absence of NSUN2 reduces protein translation rates and activates stress pathways leading to neurodegeneration.

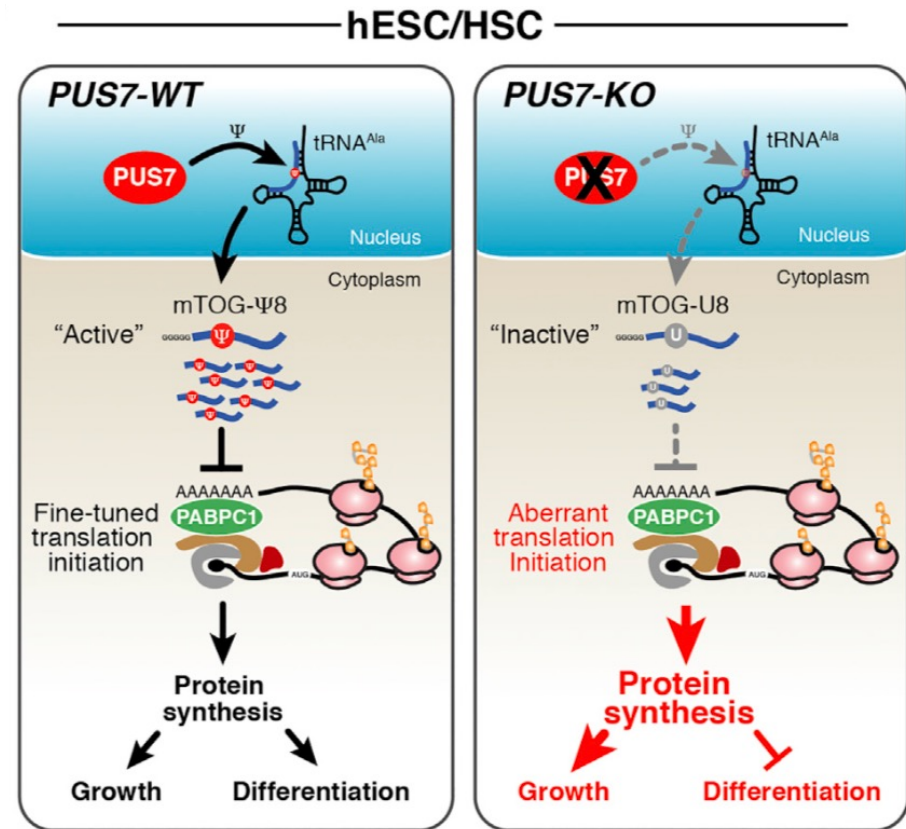


# Mechanisms of tRFs in cancer



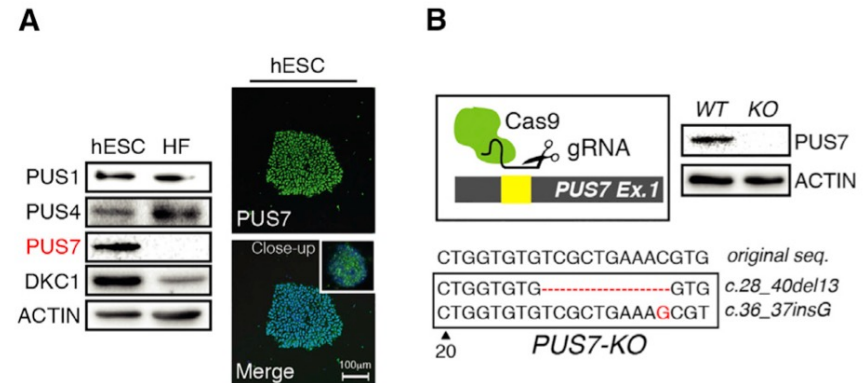
# Pseudouridylation of tRNA-Derived Fragments Steers Translational Control in Stem Cells

- The stem cell-enriched  $\Psi$  synthase PUS7 governs protein synthesis and cell growth.
- PUS7 binds distinct tRNAs and controls biogenesis of tRNA-derived fragments (tRFs) containing a 5' terminal oligoguanine (TOG).
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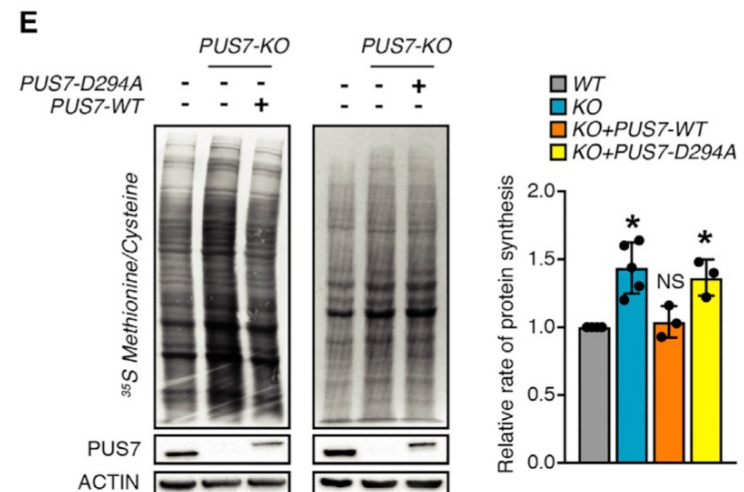


# PUS7 Governs Stem Cell Growth and Protein Synthesis Rates

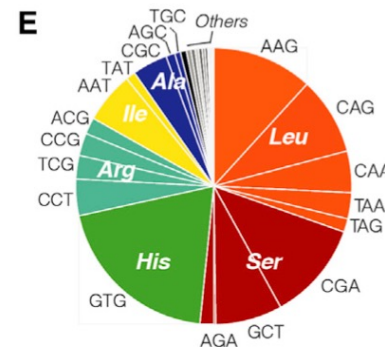
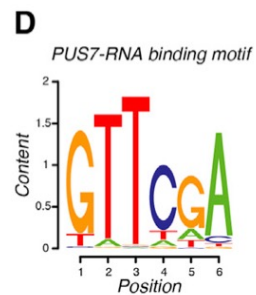
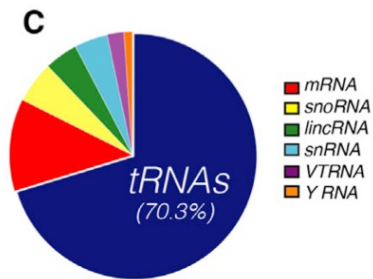
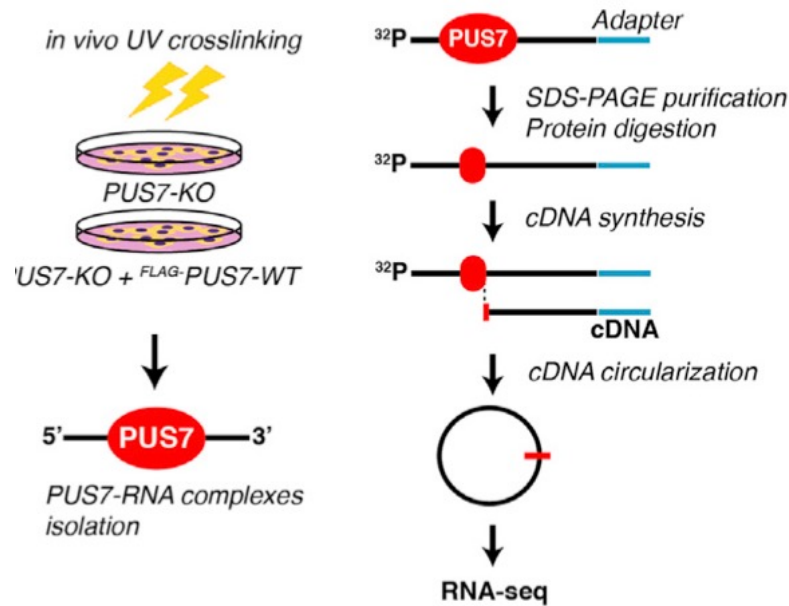
PUS7 is a stem cell-enriched  $\Psi$  synthase. A) Expression of PUS enzymes in human embryonic stem cells (hESCs) and fibroblasts (HFs). B) Schematic of CRISPR/Cas9-based mutagenesis of PUS7.



Translation is increased in *PUS7-KO* hESC. E) *De novo* protein synthesis measured by  $^{35}\text{S}$ -radioactive methionine/cysteine incorporation in WT and *PUS7-KO* cells  $\pm$  wild-type (*PUS7-WT*) or  $\Psi$  catalytically inactive PUS7 allele (*PUS7-D294A*).



# PUS7 Binds Specific tRNA Isoacceptors in hESCs



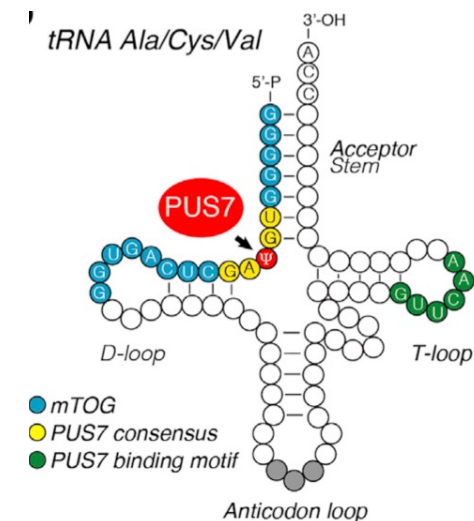
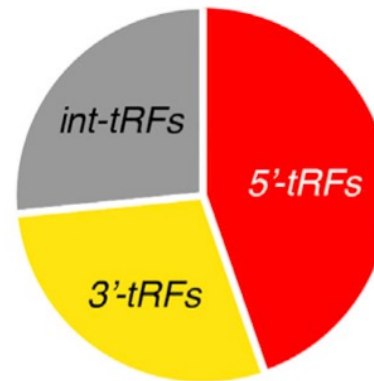
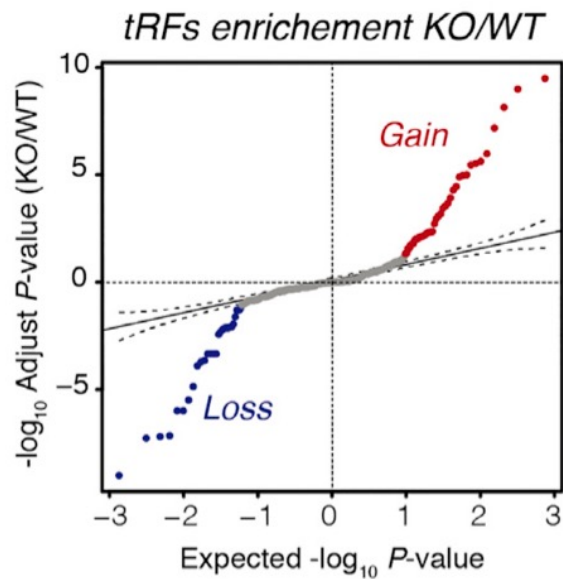
C) Pie chart shows distinct categories of RNA species bound to PUS7 in hESCs identified by iCLIP-seq.

D) Sequence analysis of PUS7-specific iCLIP products identifies a new putative PUS7-RNA-binding motif.

E) Pie chart shows enrichment of individual PUS7-bound tRNAs identified by iCLIP-seq normalized to their abundance in hESCs determined by RNA-seq.

# PUS7-Mediated $\Psi$ Directs tRNA-Derived Small RNAs

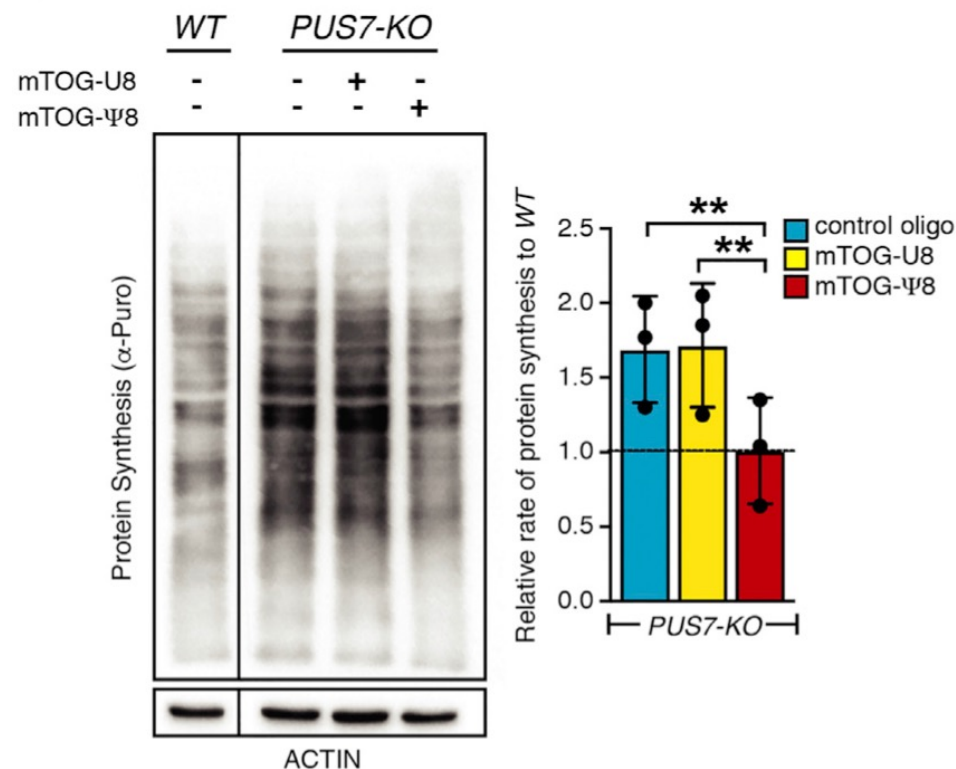
They identified specific tRF subsets significantly altered in *PUS7*-KO cells, which were primarily processed from the 5' end of specific cognate tRNAs. 5'-tRFs derived from tRNA containing a 5' terminal oligoguanine (TOG), such as tRNA-Ala, tRNA-Cys, and tRNA-Val, were significantly depleted in *PUS7*-KO cells.



Small RNA-seq

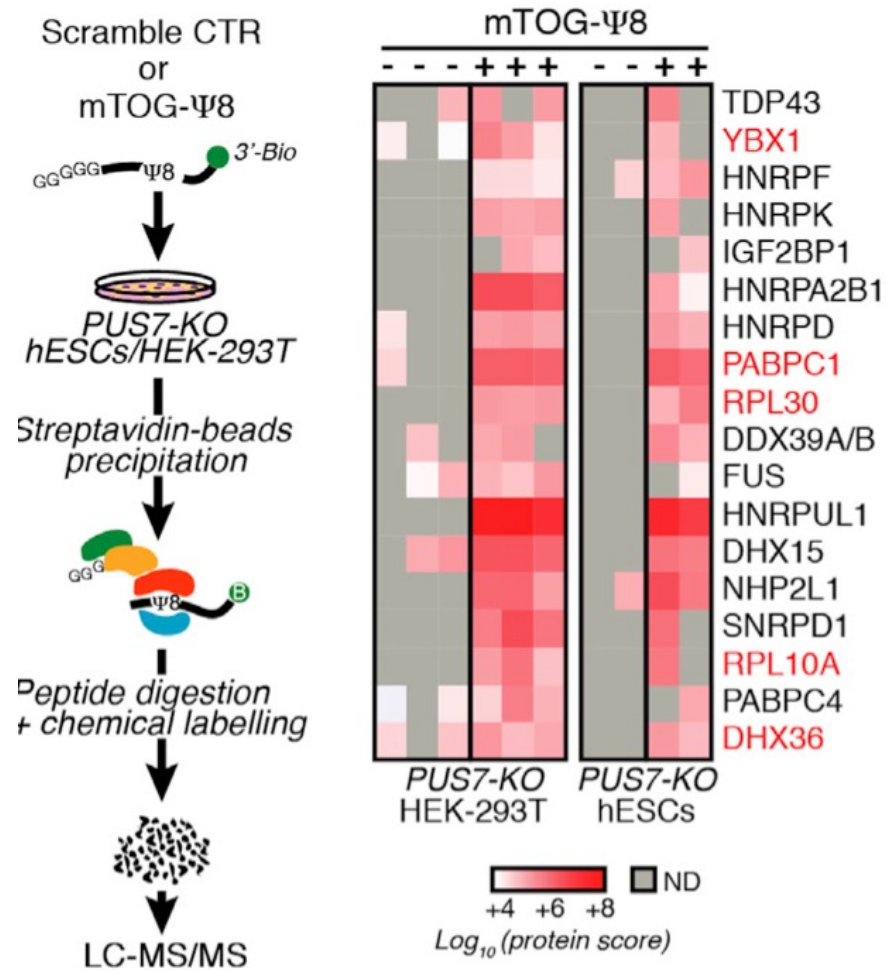
# Pseudouridylation of Specific 5-tRFs Fine Tunes Translation in hESCs

They measured protein synthesis in *PUS7-KO* hESCs transduced with differentially  $\Psi$  synthetic mTOG oligos. Strikingly, we uncovered that only mTOG- $\Psi$ 8 restored protein synthesis to control levels in these cells. This effect was not due to differences in mTOG levels, stability, or a specific response induced by transduction of  $\Psi$ -modified oligonucleotides. These results suggest that  $\Psi$  is critically required for 5'tRF-dependent translational repression *in vivo*.



Global protein synthesis measured by puromycin incorporation in WT and *PUS7-KO* hESCs transduced with scramble control and mTOG- $\Psi$ 8 or mTOG-U8 oligos.

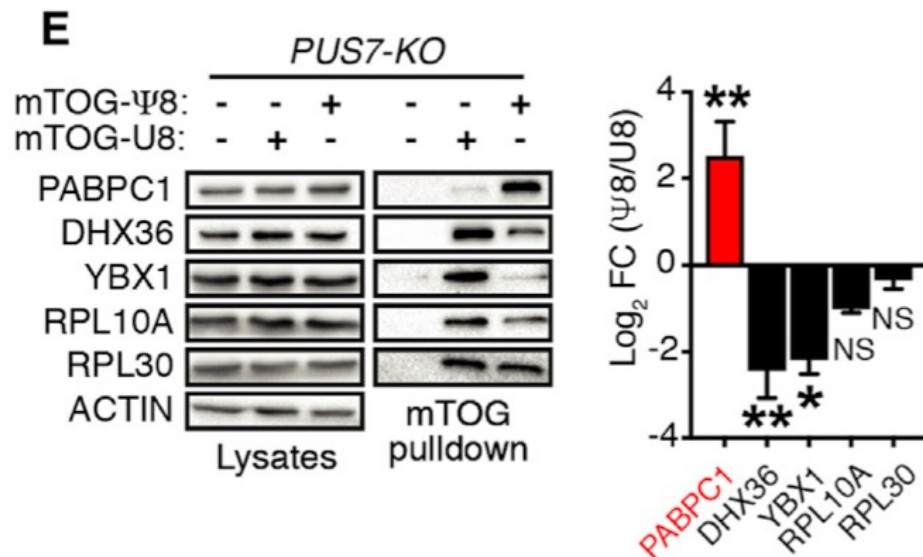
# Pseudouridylated mTOG binds PABPC1



Oligo-specific protein complexes are immunoprecipitated using streptavidin beads and subjected to MS-based quantitative proteomics. Heatmap shows proteins enriched in mTOG-Ψ8 immunoprecipitates in both *PUS7-KO* HEK293T and hESCs.

# $\Psi$ promotes mTOG-PABPC1 complex formation

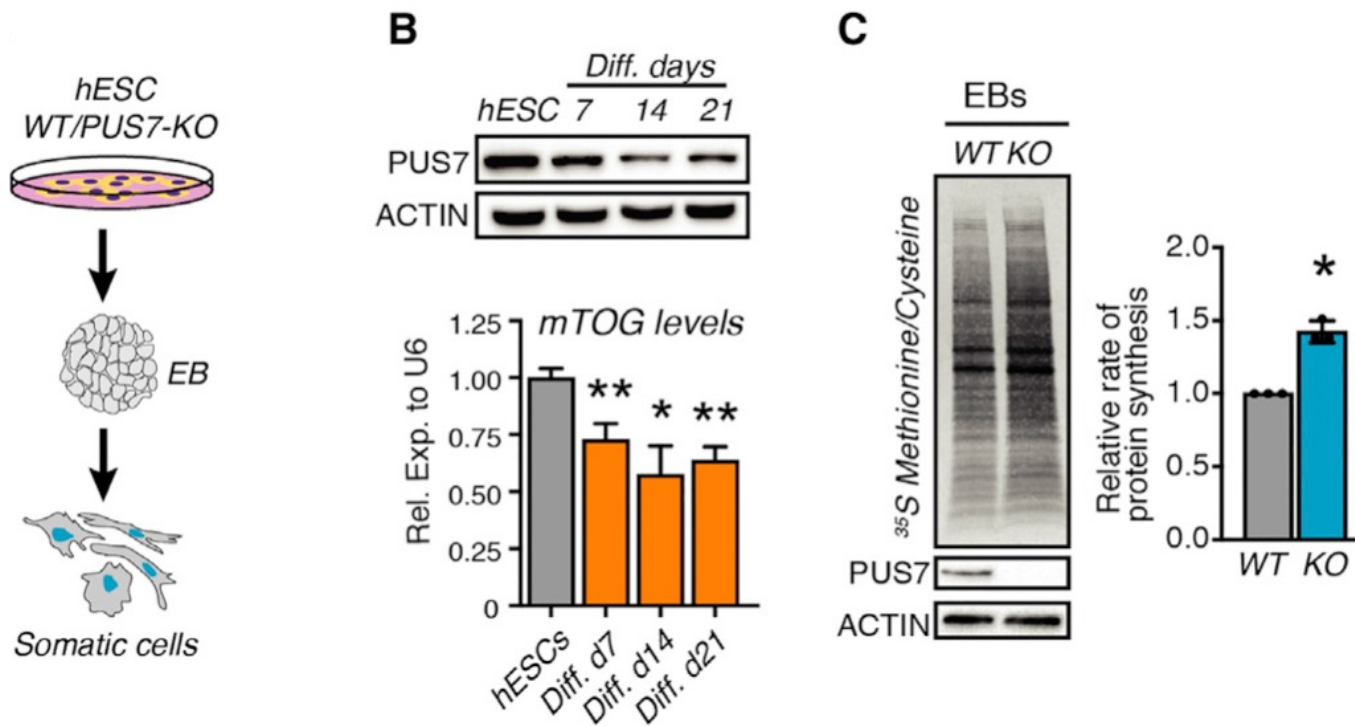
$\Psi$  strongly and selectively increased the association between mTOGs and polyadenylate-binding protein 1 (PABPC1), a central initiation factor highly enriched in mTOG- $\Psi$ 8 precipitates.



mTOG-binding proteins are co-precipitated using 3'-biotynilated control and mTOG-Y8 or mTOG-U8 in *PUS7-KO* hESCs.

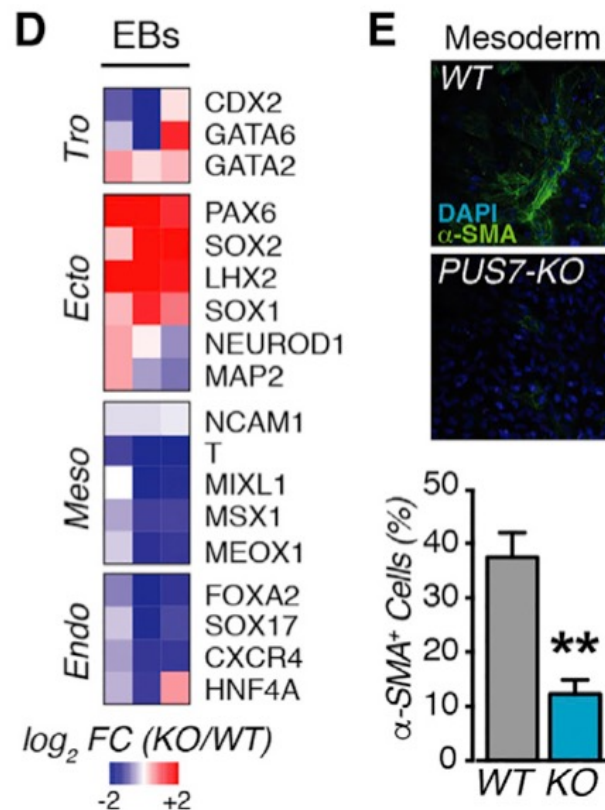
# PUS7 Is Required for Accurate Embryonic Differentiation

They observed a reduction in PUS7 (mRNA and protein) and mTOGs levels (B) accompanied by increased protein synthesis during hESC differentiation (C).



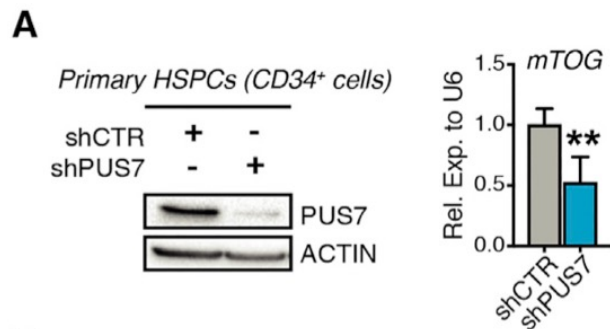
# PUS7 Is Required for Accurate Embryonic Differentiation

*PUS7*-KO EBs showed abnormal levels of multiple lineage-specific markers (D). Specifically, *PUS7* depletion greatly impaired mesodermal production as shown by dramatic reductions of terminally differentiated cells positive for the mesoderm marker  $\alpha$ -SMA/ACTA2 (E).

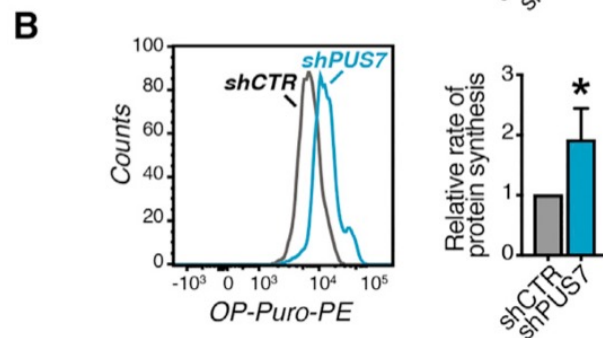


# PUS7-Mediated $\Psi$ Critically Governs Hematopoietic Stem Cell Function

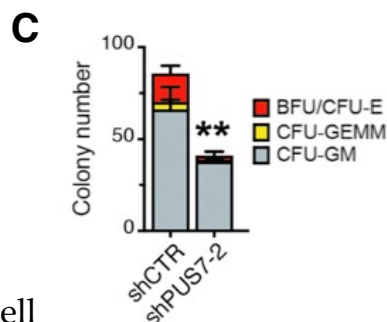
Lentiviral shRNA-mediated downregulation of PUS7 in CD34<sup>+</sup> HSPCs led to mTOGs reduction, increased protein synthesis, and severely blocked HSPCs differentiation



(A) Primary human HSPCs were transduced with lentiviruses expressing scramble control (shCTR) or PUS7 (shPUS7) shRNAs and the green fluorescent protein (GFP). Left: PUS7 protein levels in shCTR and shPUS7 in CD34<sup>+</sup>/GFP<sup>+</sup> HSPCs 3 days after lentiviral transduction. Right: Graph shows mean relative mTOG expression  $\pm$  SD in three independent experiments.



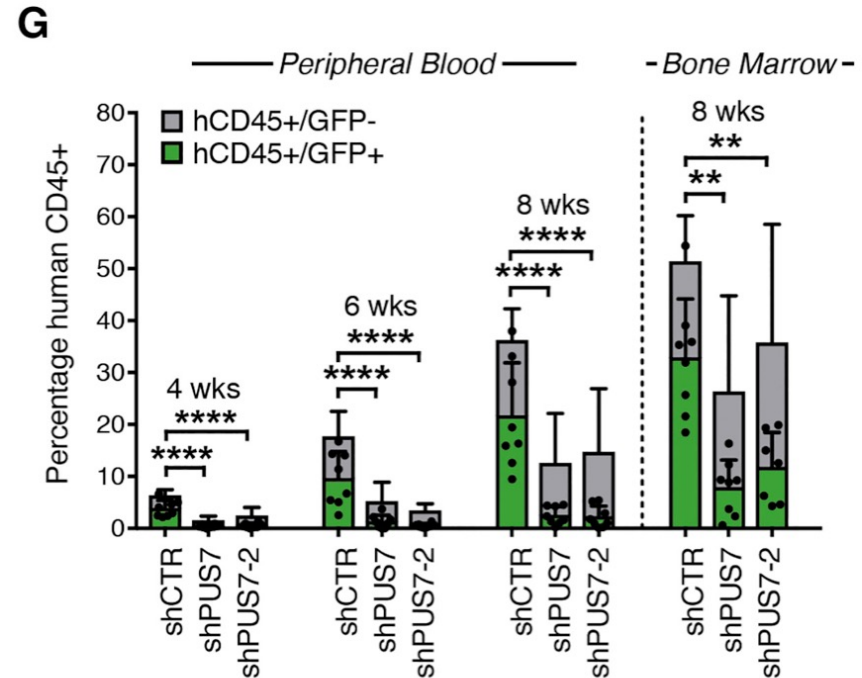
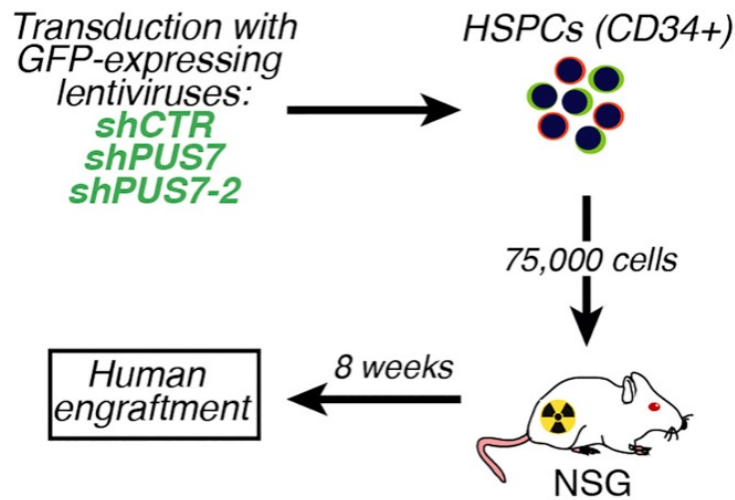
(B) Protein synthesis rates measured by Puro incorporation in GFP<sup>+</sup> shCTR and shPUS7 HSPCs.



(C) PUS7 is required for accurate differentiation of primary human HSPCs. Graph shows mean number of colony-forming unit granulocyte-erythrocyte-monocyte-megakaryocyte (CFU-GEMM) and granulocyte-monocyte (CFU-GM) and burst-forming unit-erythroid (BFU-E).

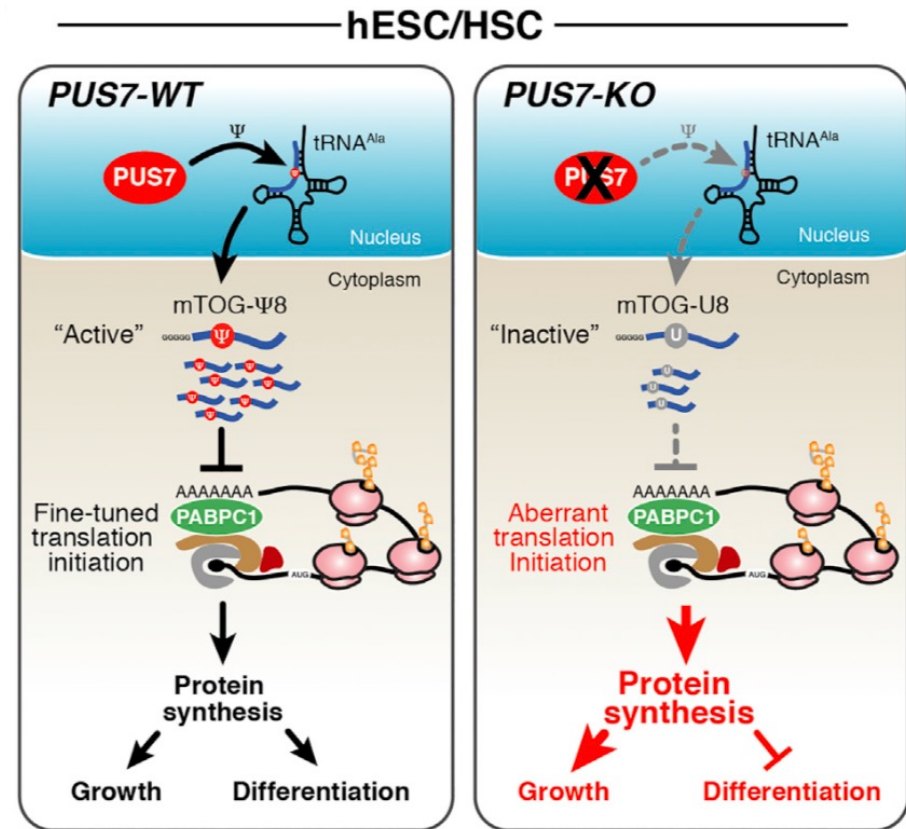
# PUS7 depletion impairs human engraftment in NSG mice

To investigate the *in vivo* effects of PUS7 loss, they performed xenotransplantation of primary human HSPCs infected with control or two distinct PUS7 shRNA-expressing lentiviruses into immunocompromised NOD/SCID/g (NSG) mice. Human multilineage engraftment within the peripheral blood (PB) of mice reconstituted with PUS7-depleted HSPCs was dramatically impaired (G). This defect was associated with a significant loss of PUS7-depleted hematopoietic cells (hCD45+/GFP+).



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# Pseudouridine-modified tRNA fragments repress aberrant protein synthesis and predict leukaemic progression in myelodysplastic syndrome

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Phuong Cao Thi Ngoc <sup>1</sup>, Magdalena Madej <sup>1</sup>, Roberto Munita<sup>1</sup>, Serena Fazio<sup>1</sup>, Simon Ekström <sup>4</sup>,  
Teresa Mortera-Blanco<sup>2</sup>, Monika Jansson<sup>2</sup>, Yasuhito Nannya<sup>5,6</sup>, Mario Cazzola <sup>3,7</sup>, Seishi Ogawa<sup>2,5,6</sup>,  
Luca Malcovati <sup>3,7</sup>, Eva Hellström-Lindberg <sup>2</sup>, Marios Dimitriou <sup>2</sup>✉ and Cristian Bellodi <sup>1</sup>✉

# 3'tRNA fragments (tFRs) limit the mobility of transposable elements in mammalian cells

22 nt tRFs post-transcriptionally silence coding-competent endogenous retroviruses (ERVs), while 18 nt tRFs specifically interfere with reverse transcription and retrotransposon mobility. The tRNA primer binding site (PBS) offers a unique target to specifically inhibit LTR-retrotransposons, and tRF-targeting is a potentially highly conserved mechanism of small RNA-mediated transposon control.

