Composite SMG5-SMG6 PIN domain formation is essential for nonsense-mediated mRNA decay

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Background: Nonsense-mediated mRNA decay (NMD)

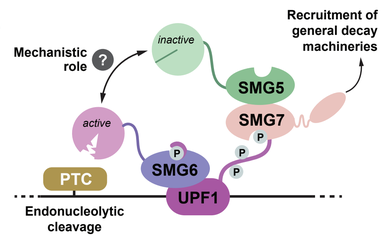
Nonsense-mediated mRNA decay: Co-translational mRNA surveillance pathway in eukaryotes, which mediates the rapid degradation of transcripts containing premature termination codons (PTCs)

- **Quality control** by preventing the accumulation of potentially deleterious truncated proteins
- **Regulation of gene expression** by controlling the steady-state levels of some physiological transcripts

Degradation of NMD substrates proceeds via two major branches:

- **SMG6** initiates an endonucleolytic pathway by cleaving NMD substrates in close proximity to the PTC
- **SMG5-SMG7** mediate a deadenylation/ decapping pathway by recruiting general decay machineries

- **How do cells avoid that SMG6 acts on random transcripts?**
- **Why does SMG6 only exhibit weak activity *in vitro*, but shows robust function in cellular asays?**
- **How is the SMG5 PIN domain involved in SMG6-mediated decay of NMD substrates?**



The SMG5 PIN domain is essential for endonucleolytic cleavage in human NMD

The catalytically inactive PIN domain of SMG5 is required for SMG6-mediated endonucleolytic cleavage of NMD substrates in human cells.

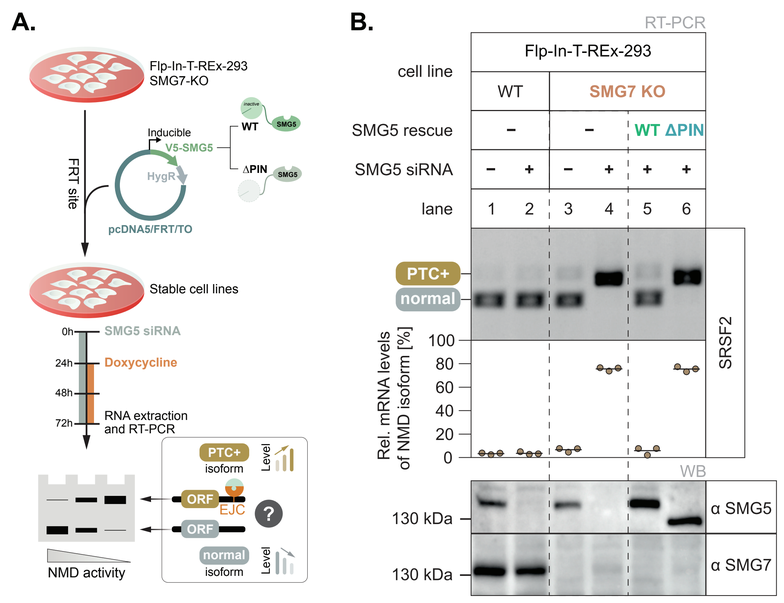


Figure 1: A. Schematic overview of NMD rescue assays in human cells. **B.** Rescue assays with SMG5 WT and SMG5 ΔPIN rescue constructs in WT or SMG7 KO cell lines treated with SMG5 siRNA.

SMG5 and SMG6 directly interact to form a composite PIN domain

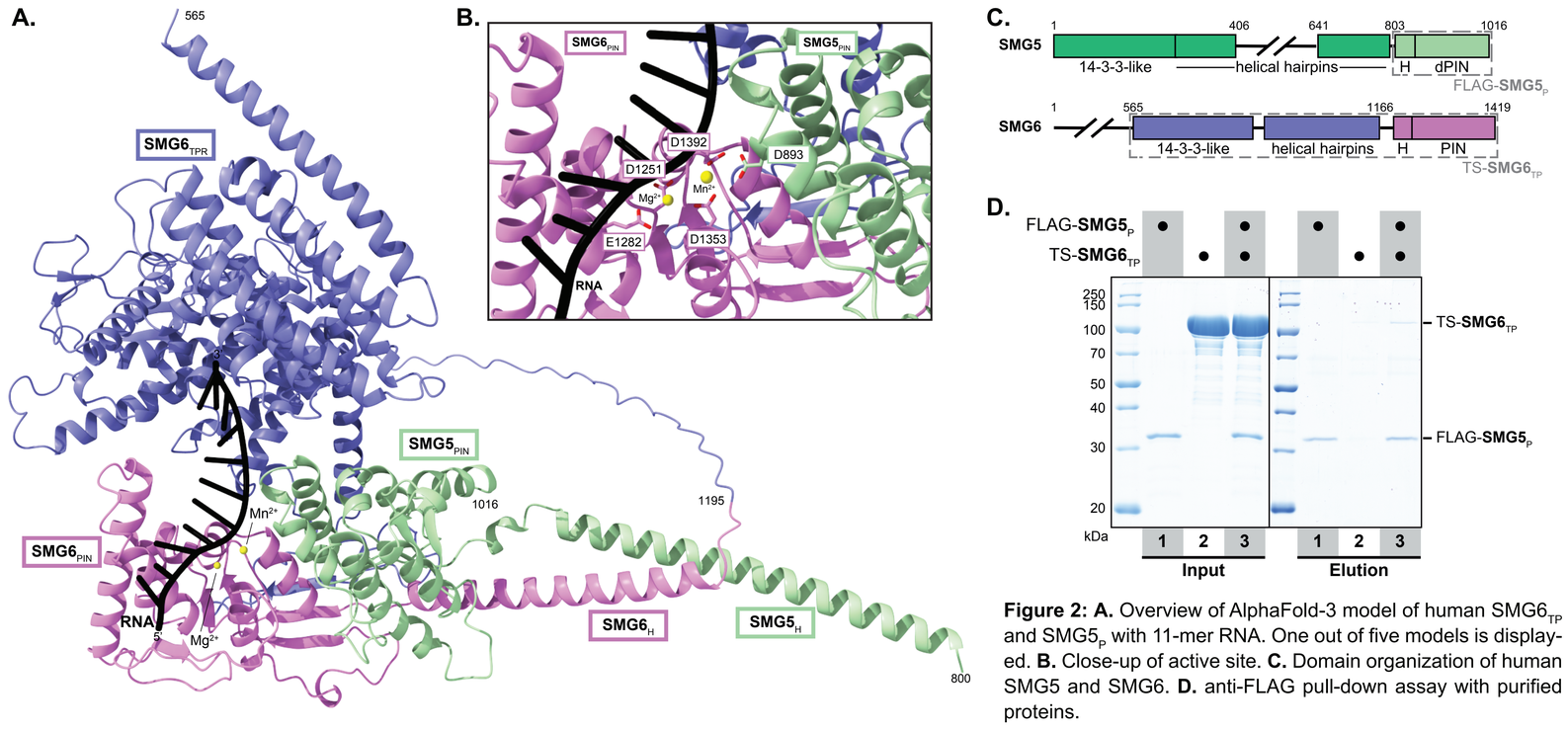


Figure 2: A. Overview of AlphaFold-3 model of human $SMG6_{TP}$ and $SMG5_P$ with 11-mer RNA. One out of five models is displayed. **B.** Close-up of active site. **C.** Domain organization of human SMG5 and SMG6. **D.** anti-FLAG pull-down assay with purified proteins.

SMG5 and SMG6 are consistently predicted to interact via their PIN domains by AlphaFold-3.

SMG6 is co-precipitated in sub-stoichiometric amounts by SMG5, indicating the presence of a direct yet weak interaction.

The SMG5 PIN domain stimulates the endonucleolytic activity of SMG6

The PIN domain of SMG5 strongly stimulates the catalytic activity of SMG6 *in vitro*.

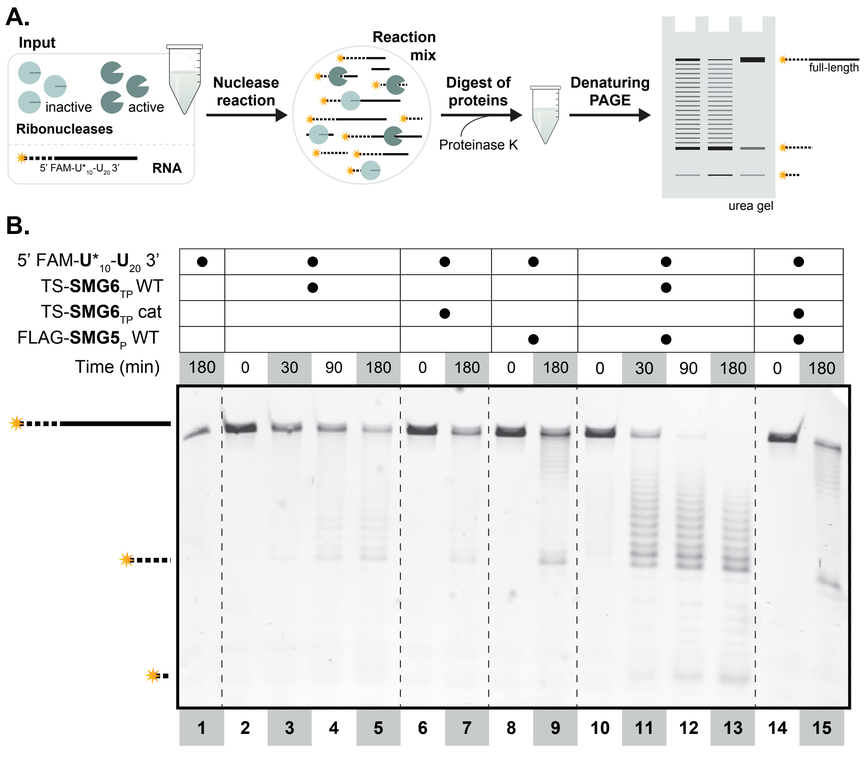


Figure 3: A. Schematic overview of *in vitro* nuclease assays with purified proteins. **B.** *In vitro* nuclease assay with time-course set-up for activity of SMG6 in absence and presence of the SMG5 PIN domain.

SMG5 completes the active site of SMG6 in the composite NMD endonuclease

Mutational analysis reveals key residues of SMG5 required for cPIN formation, catalytic activity and substrate binding.

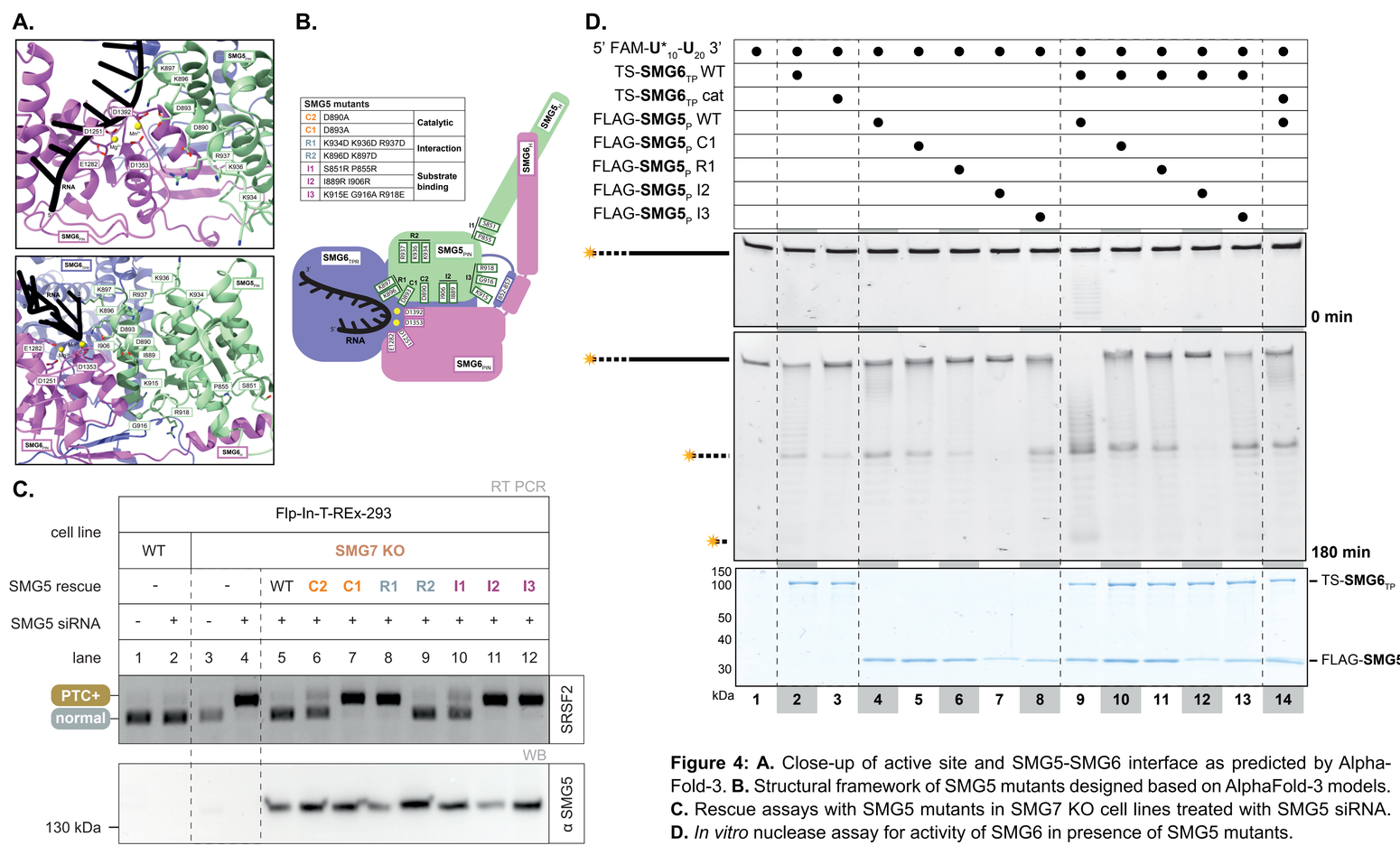


Figure 4: A. Close-up of active site and SMG5-SMG6 interface as predicted by AlphaFold-3. **B.** Structural framework of SMG5 mutants designed based on AlphaFold-3 models. **C.** Rescue assays with SMG5 mutants in SMG7 KO cell lines treated with SMG5 siRNA. **D.** *In vitro* nuclease assay for activity of SMG6 in presence of SMG5 mutants.

Conclusion

- SMG5 and SMG6 weakly but directly interact via their PIN domains (cPIN)
- Catalytic activity of SMG6 is stimulated by SMG5 *in vitro*
- SMG5 likely completes the SMG6 active site and contributes to positioning the RNA substrate

- Could the formation of composite PIN modules be a recurrent strategy in RNA surveillance?

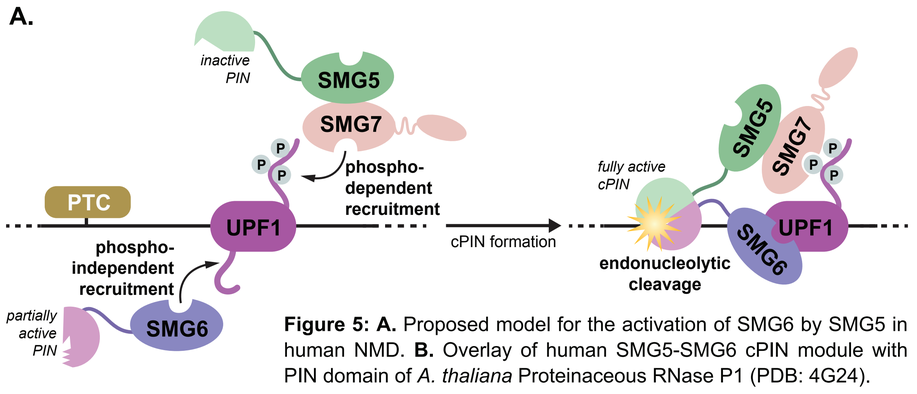


Figure 5: A. Proposed model for the activation of SMG6 by SMG5 in human NMD. **B.** Overlay of human SMG5-SMG6 cPIN module with PIN domain of *A. thaliana* Proteinaceous RNase P1 (PDB: 4G24).

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