Role of mutants FUS in nucleolar reorganization following DNA damage

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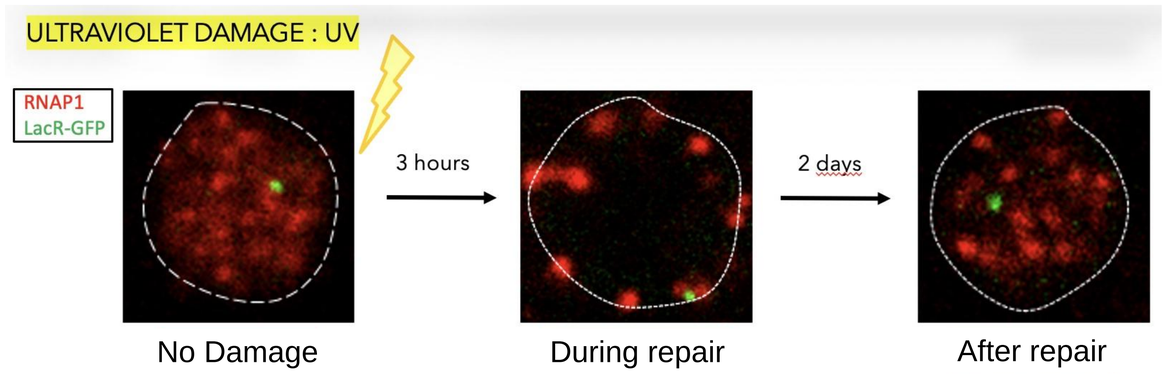
Introduction

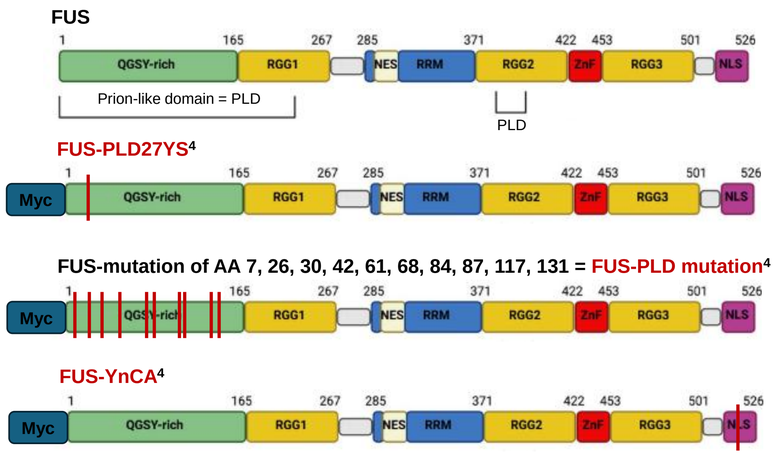
- FUS is an RNA / DNA binding protein, localized in the **nucleoplasm**[1]
- FUS is found to be mutated in **ALS**[2], a neurodegenerative disease
- FUS mutations cause **cytoplasmic mislocalization** and aggregation[2].

- Under **genotoxic stress**, **nucleolus** undergoes **reorganization,** RNA Polymerase I (**RNAP1**) and **rDNA** are relocalized to the **periphery of nucleolus**[3].
- Once repair is completed, nucleolar proteins **return** to their original position[3].
- This is an **active** process.

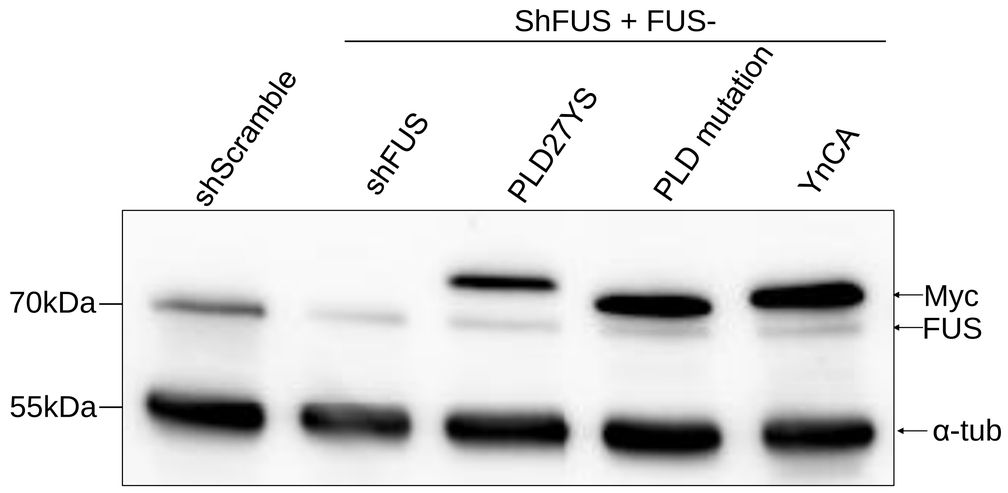


Methods

Genetic characterization of FUS mutants

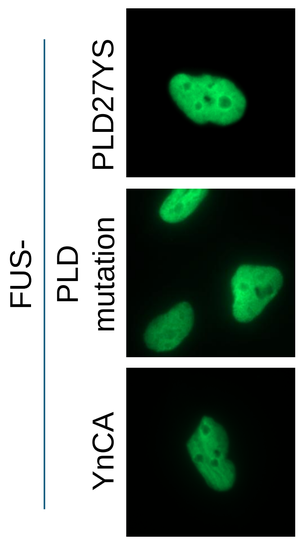


WB of cell lines construction



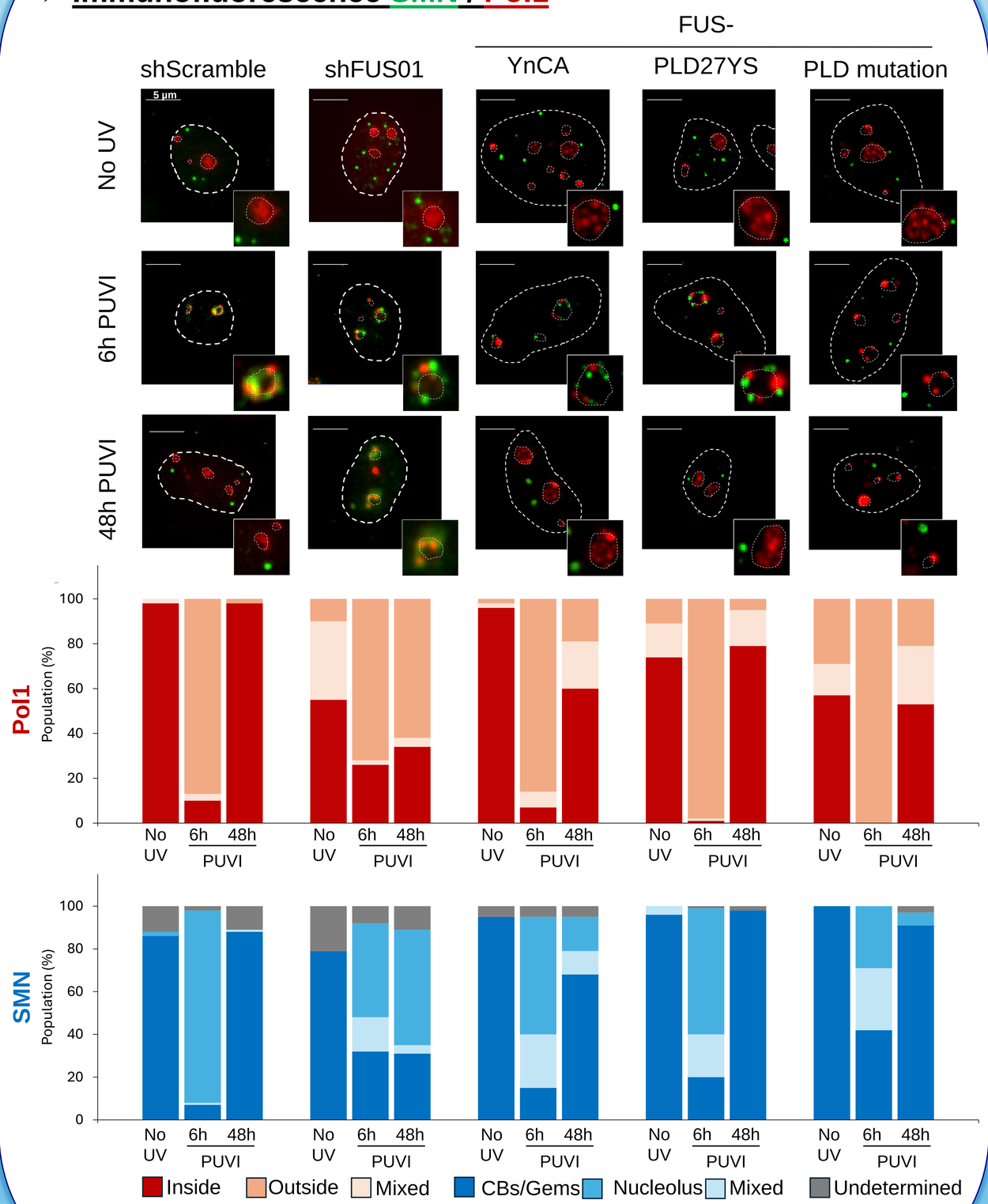
shRNA expression induced by Doxycycline (5 days)

Localization of FUS mutant (clonal selection)



Results 1

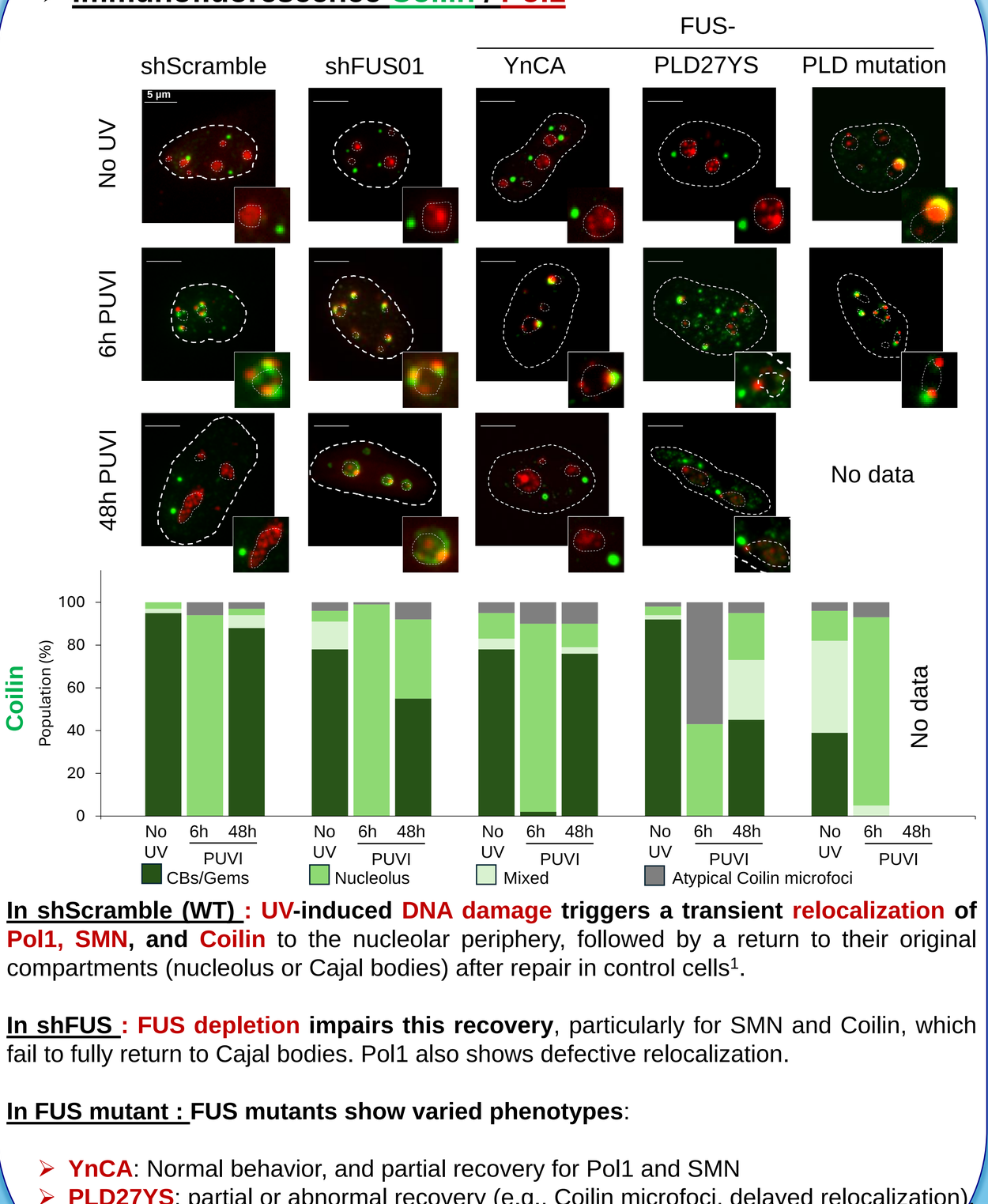
➢ **Immunofluorescence SMN / Pol1**



PRELIMINARY RESULTS

Results 2

➢ **Immunofluorescence Coilin / Pol1**



In shScramble (WT) : UV-induced DNA damage triggers a transient relocalization of Pol1, SMN, and Coilin to the nucleolar periphery, followed by a return to their original compartments (nucleolus or Cajal bodies) after repair in control cells[1].

In shFUS : FUS depletion impairs this recovery, particularly for SMN and Coilin, which fail to fully return to Cajal bodies. Pol1 also shows defective relocalization.

In FUS mutant : FUS mutants show varied phenotypes:

- **YnCA**: Normal behavior, and partial recovery for Pol1 and SMN
- **PLD27YS**: partial or abnormal recovery (e.g., Coilin microfoci, delayed relocalization).
- **PLD mutant**: persistent mislocalization even before UV, with no proper recovery.

PRELIMINARY RESULTS

Conclusion / Perspective

WE KNOW THAT FUS is essential for the correct dynamic relocalization of key nuclear components during the UV damage–repair cycle (RNAP1, SMN and Coilin)

WE WANT TO

- **Investigate the molecular mechanisms** regulating the relocalization of Pol1, SMN, and Coilin after stress, and FUS's direct role in these pathways.
- **Explore the functions of FUS's PLD domain**, particularly its involvement in nuclear compartment dynamics and protein/RNA interactions.
- **Establish a functional link with ALS**, by assessing whether similar nuclear relocalization defects occur in disease models.

References

1.Wang et al. 2015, **Nucleic acid-binding specificity of human FUS protein**

2.Yang et al. 2014, **Self-assembled FUS binds active chromatin and regulates gene transcription**

3.Musawi et al.2023, **Nucleolar reorganization after cellular stress is orchestrated by SMN shuttling between nuclear compartments.**

4.Scekic-Zahirovic et al, 2016, **Toxic gain of function from mutant FUS protein is crucial to trigger cell autonomous motor neuron loss**