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C9orf72 interacts with coilin and influences Cajal body dynamics and splicing

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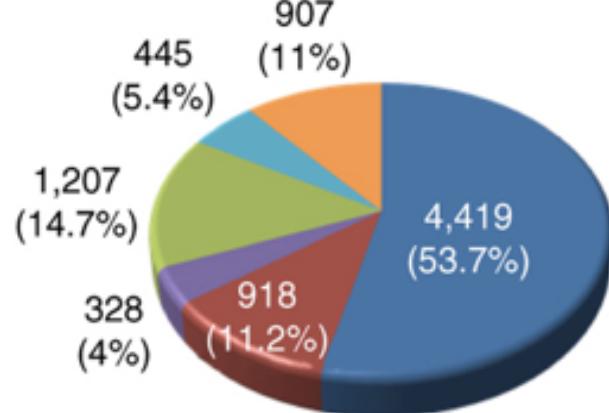


Splicing is defective in C9ALS

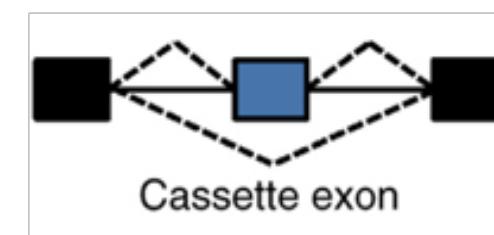
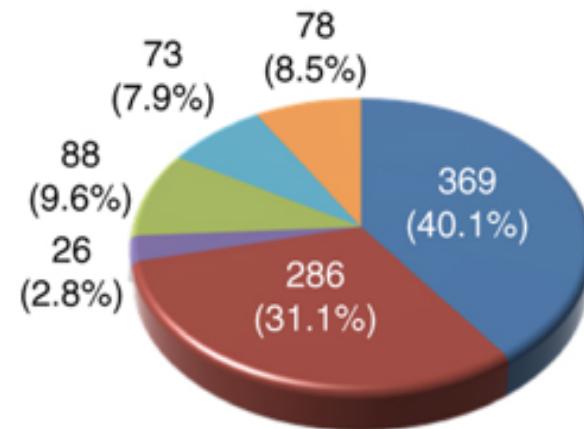
- C9ALS patients have splicing defects that correlate with disease severity

Splicing defects unique to C9ALS

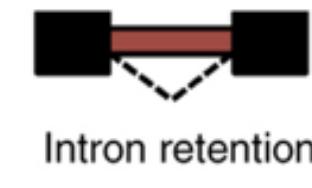
C9ALS Cerebellum



C9ALS Cortex



Cassette exon

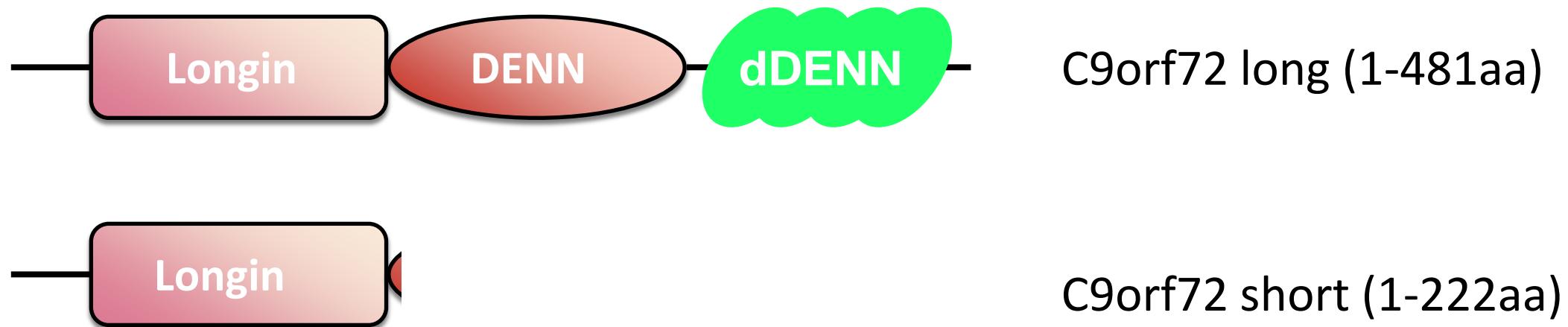


Intron retention



C9orf72 protein function

- Two protein isoforms:

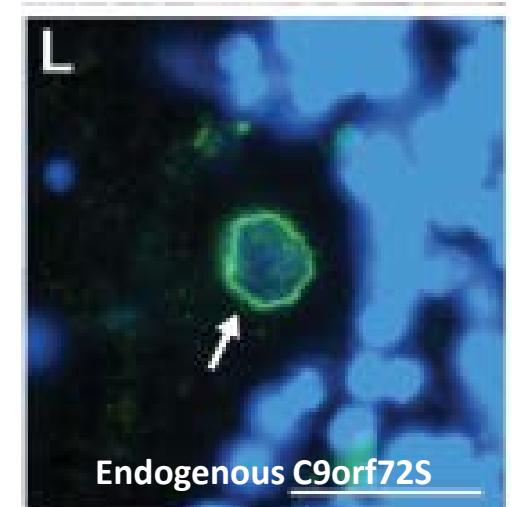
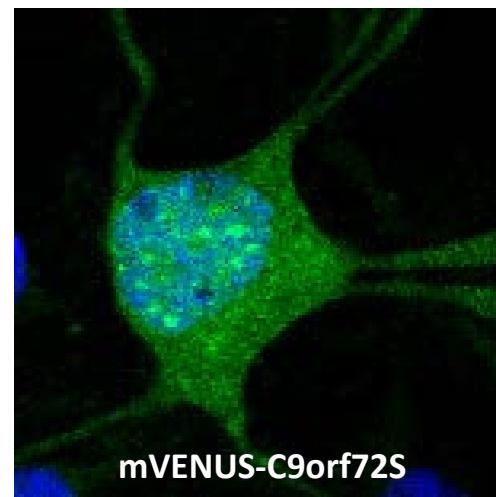


- Cytoplasm – Autophagy and trafficking via Rab protein interactions



C9orf72 protein localises to the nucleus

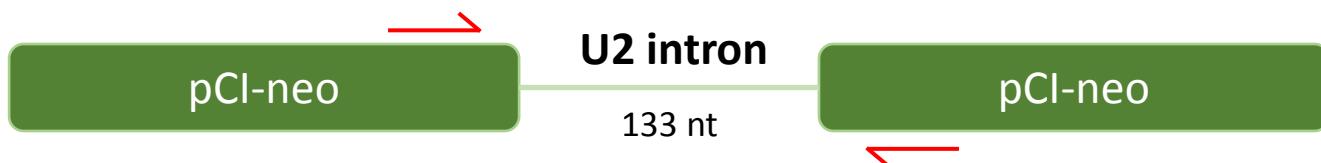
- C9orf72 proteins show nuclear localisation
- Proteomic analysis studies have identified nuclear proteins as C9orf72 interactors





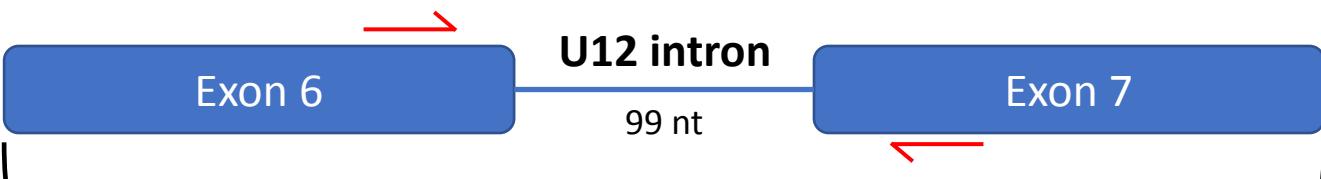
Using a reporter assay to investigate splicing

Major splicing



Unspliced = 388 nt
Spliced = 255 nt

Minor splicing



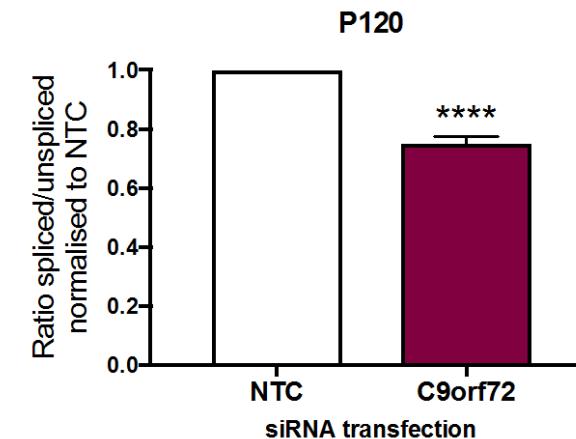
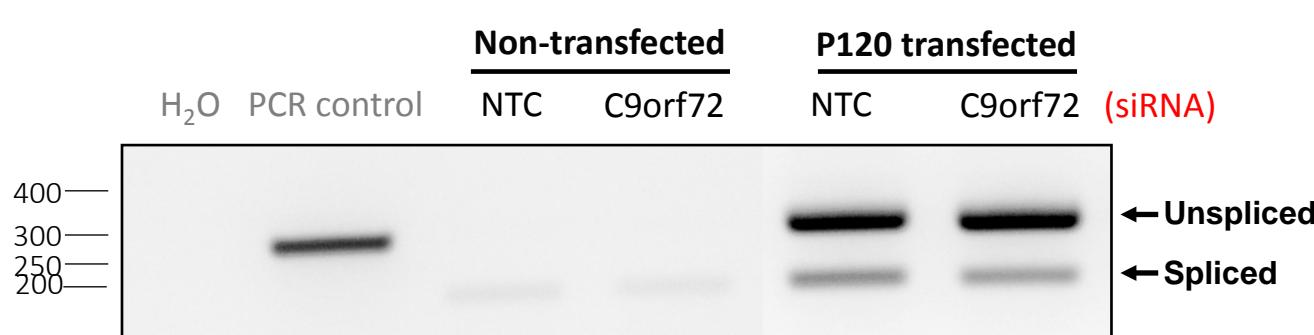
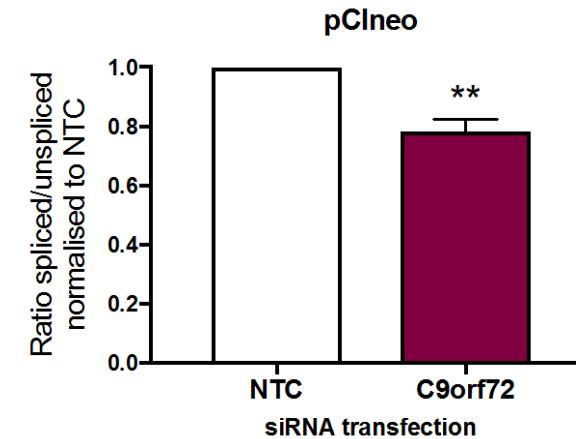
Unspliced = 250 nt
Spliced = 151 nt



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C9orf72 knockdown decreases splicing

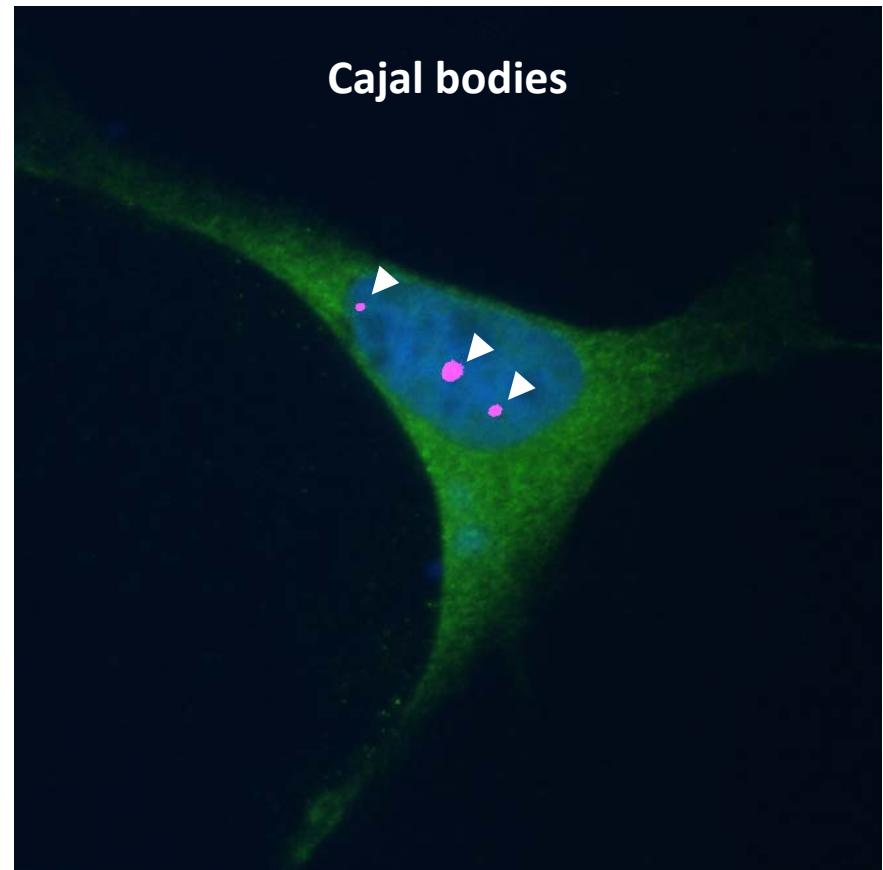
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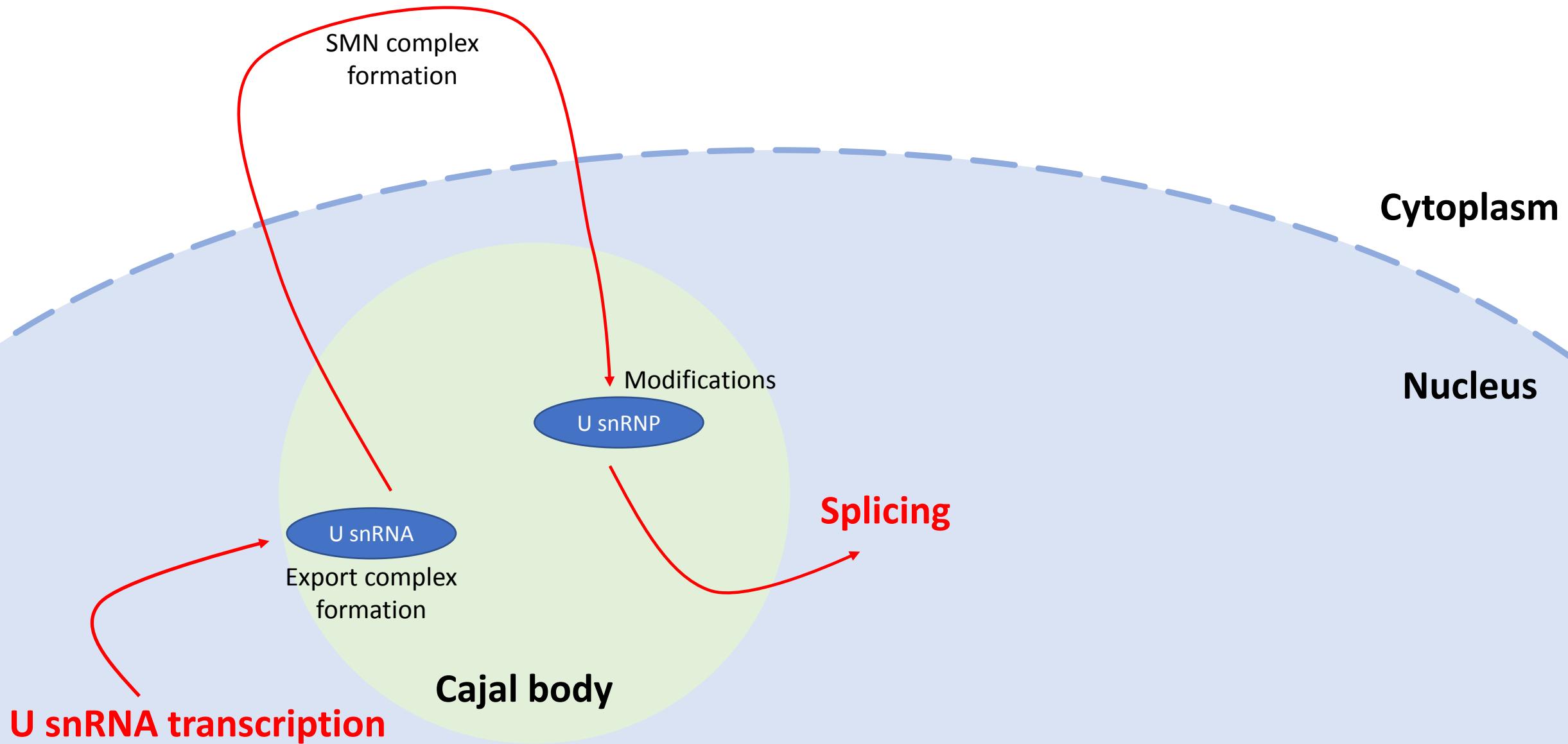
C9orf72 interacts with coilin

- Yeast two-hybrid identified an interaction between C9orf72 and coilin
- Coilin is major protein component of Cajal bodies
- Membrane-less nuclear ‘suborganelles’ – concentrated in coilin, the SMN protein and spliceosomal snRNPs
- Sites of splicing machinery assembly





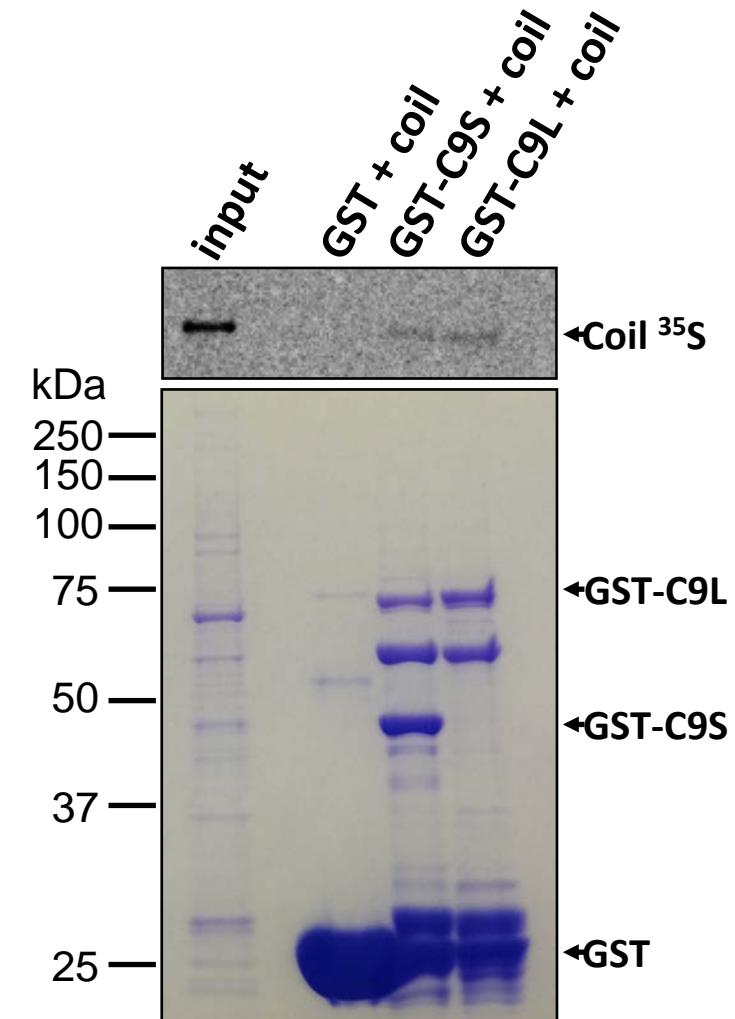
The role of Cajal bodies





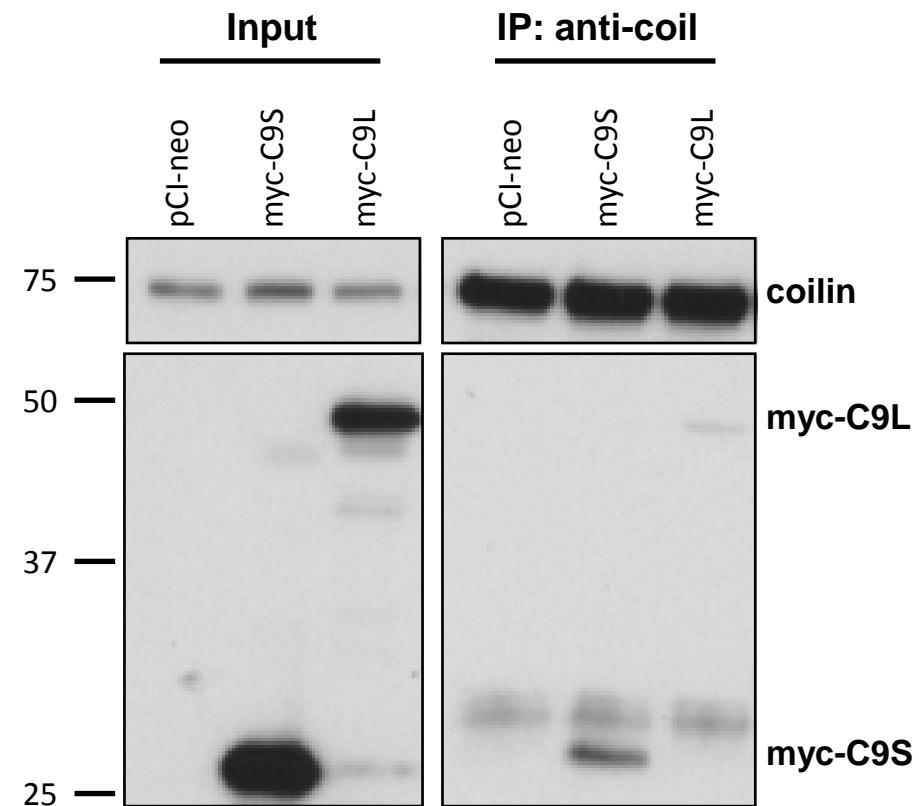
In Vitro GST-binding assay shows a direct interaction between C9orf72 and coillin

- Incubate bacterially made GST-C9orf72 with recombinant ^{35}S -labelled coillin
- Pull down against GST
- Phosphor Screen detection of ^{35}S -coillin





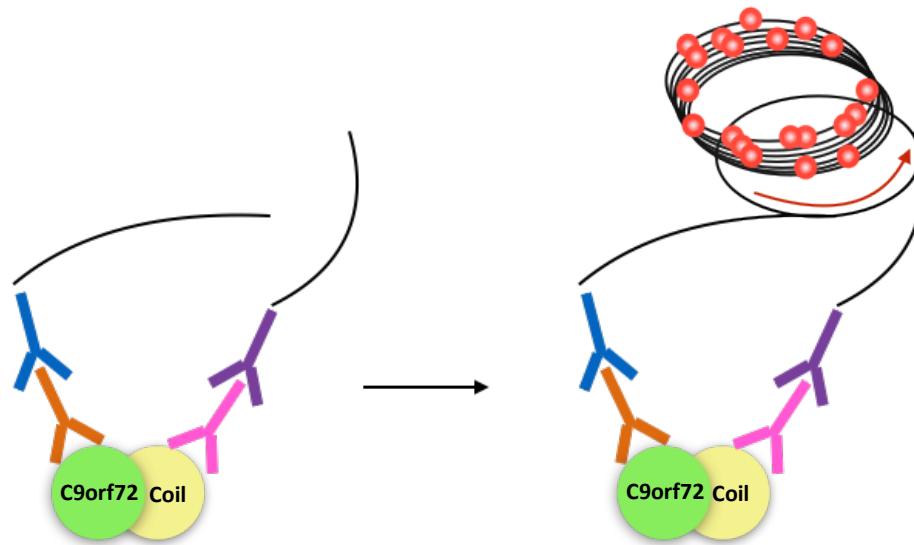
Co-IP confirms the C9orf72-coilin interaction in cells





Proximity Ligation Assay

- *In Situ* method of visualising protein-protein interactions in cells using fluorescence



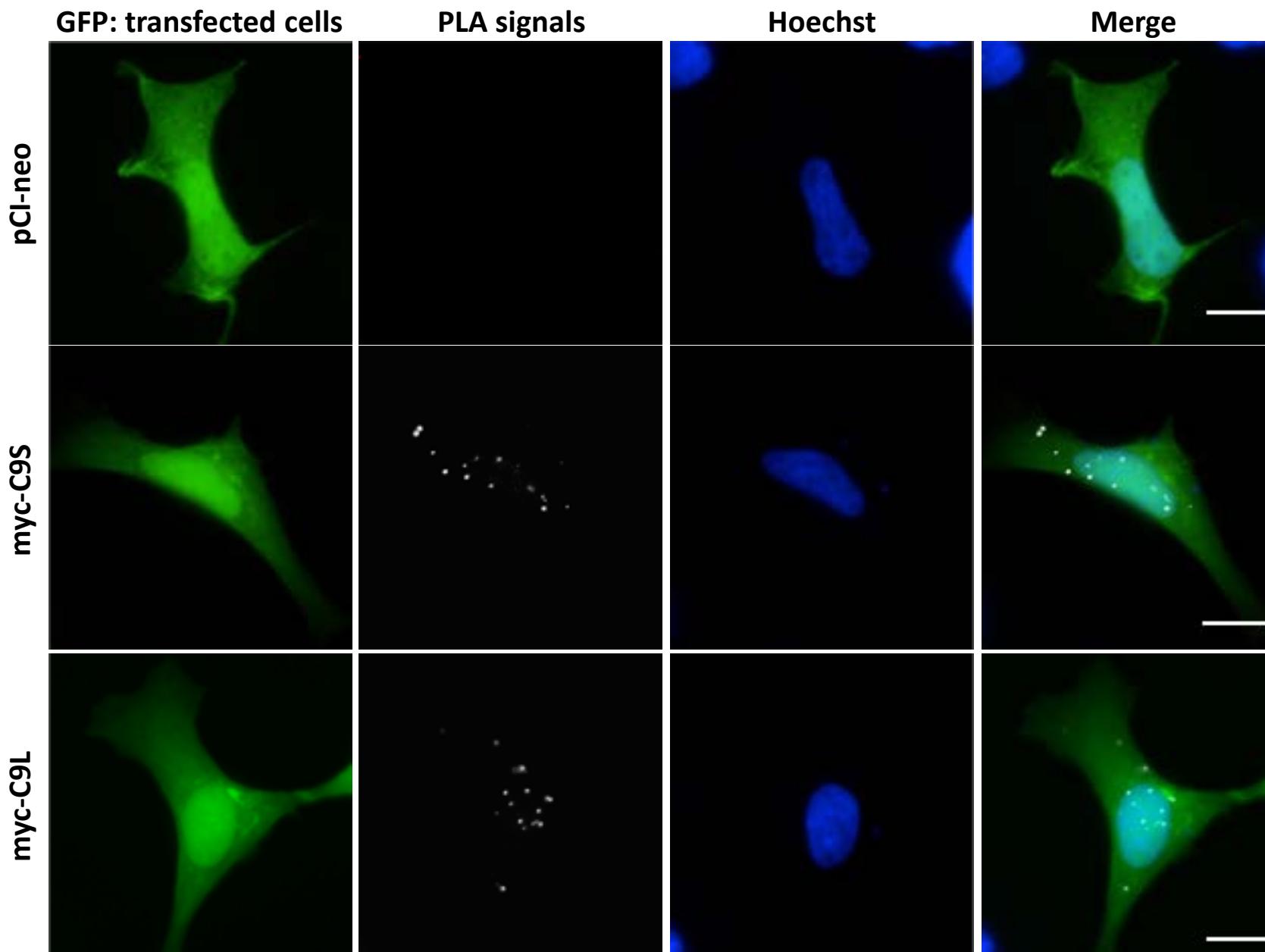
- Studied interaction between endogenous coillin and overexpressed myc-C9orf72



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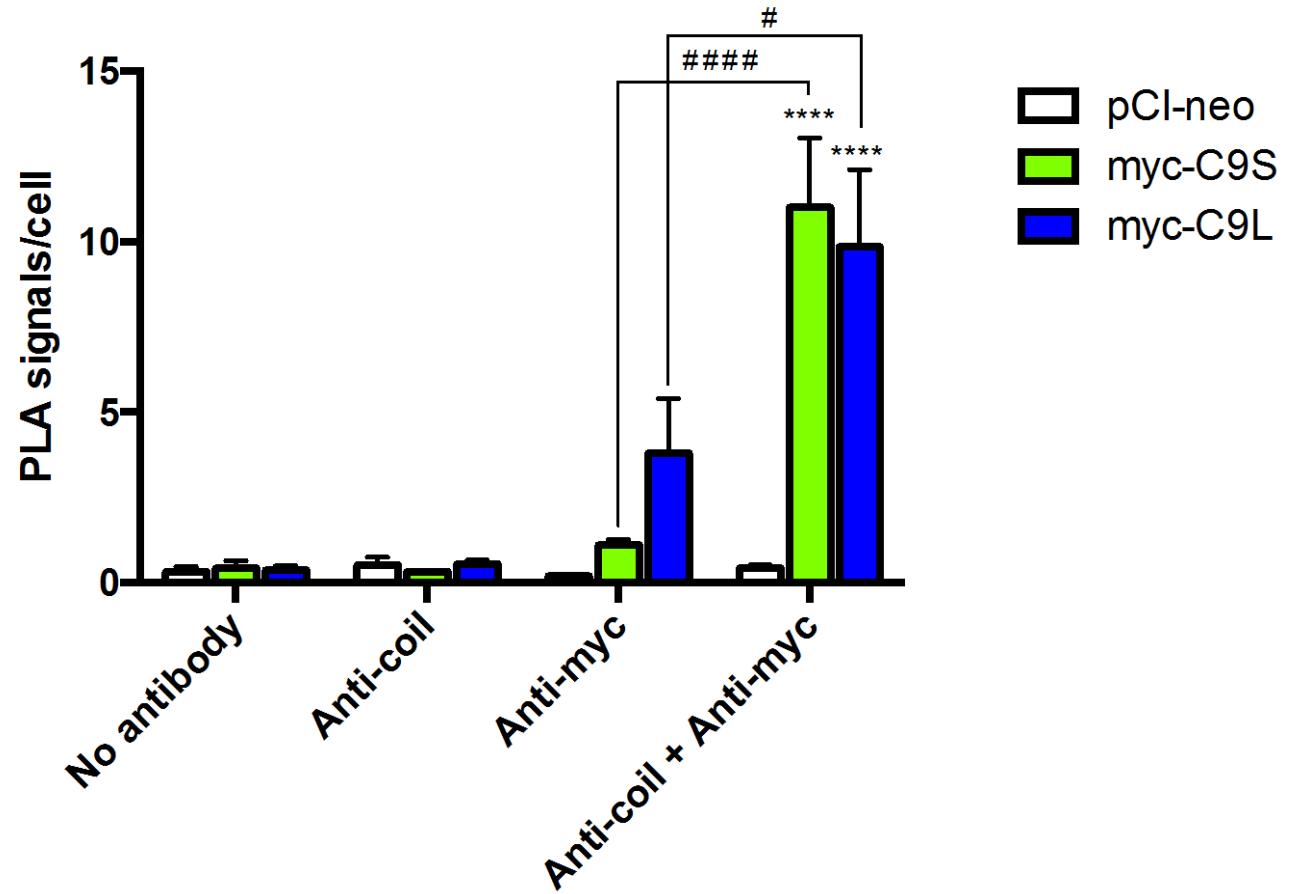
PLA confirmation of the C9orf72-coilin interaction

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PLA further confirms the interaction between C9orf72 and coilin

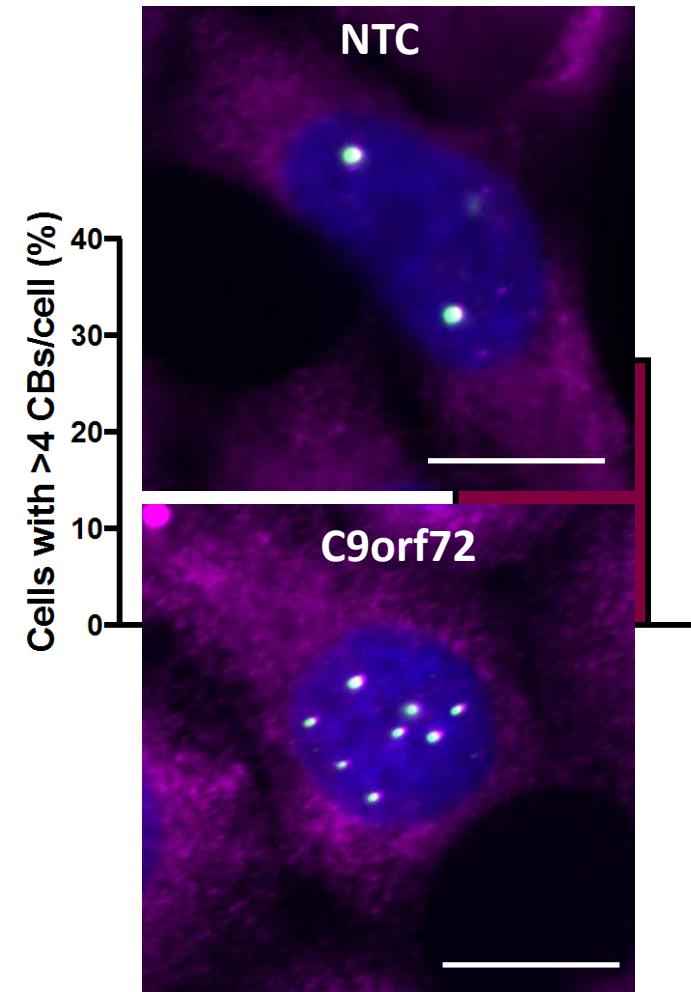
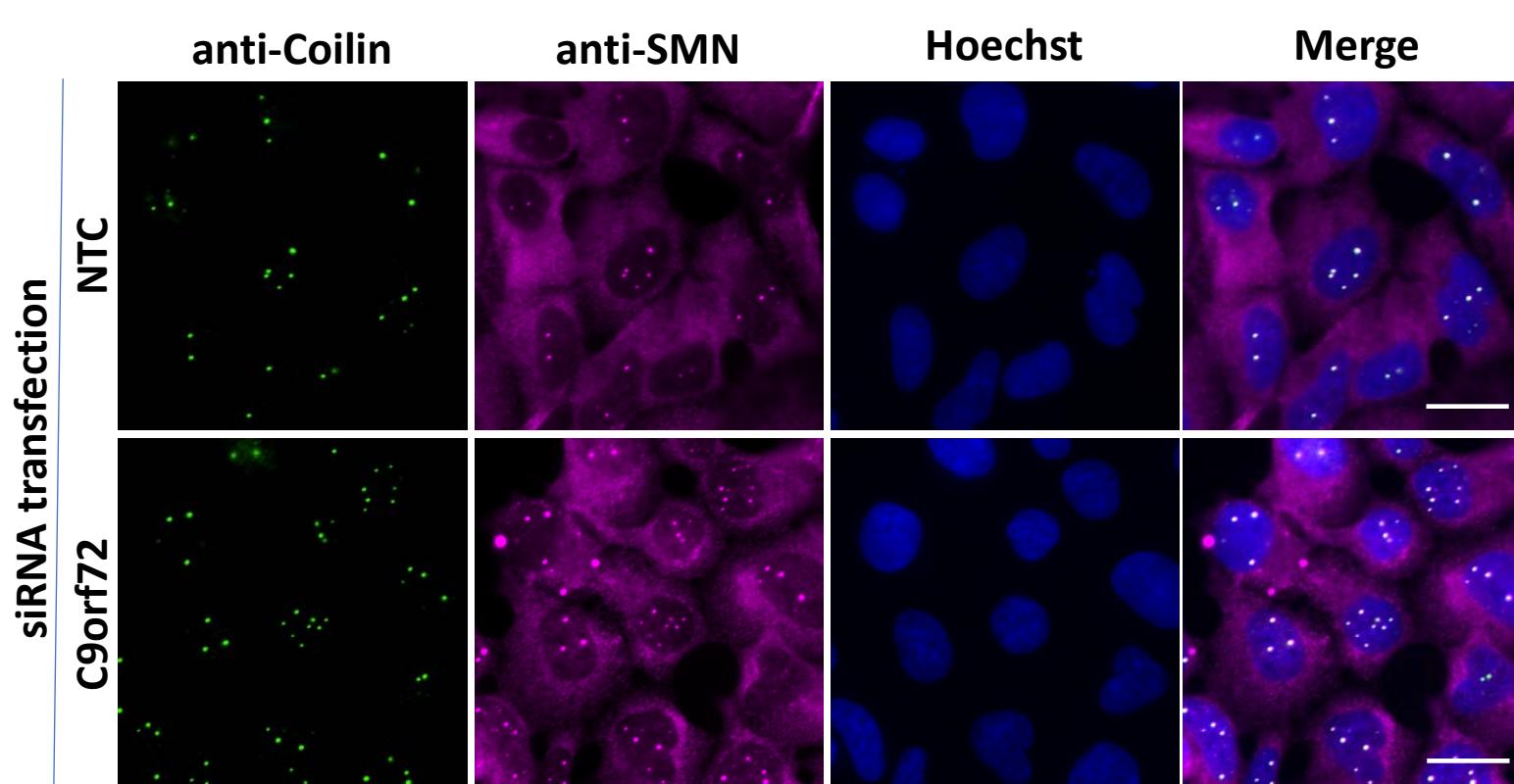




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C9orf72 knockdown increases Cajal bodies

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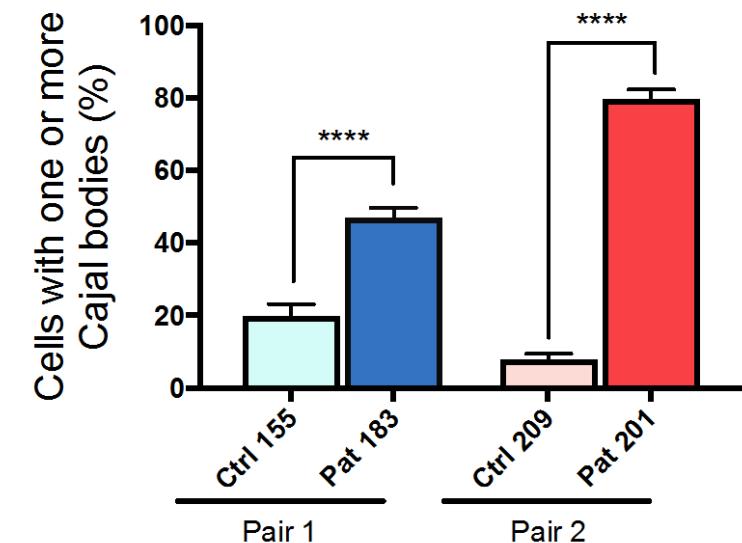
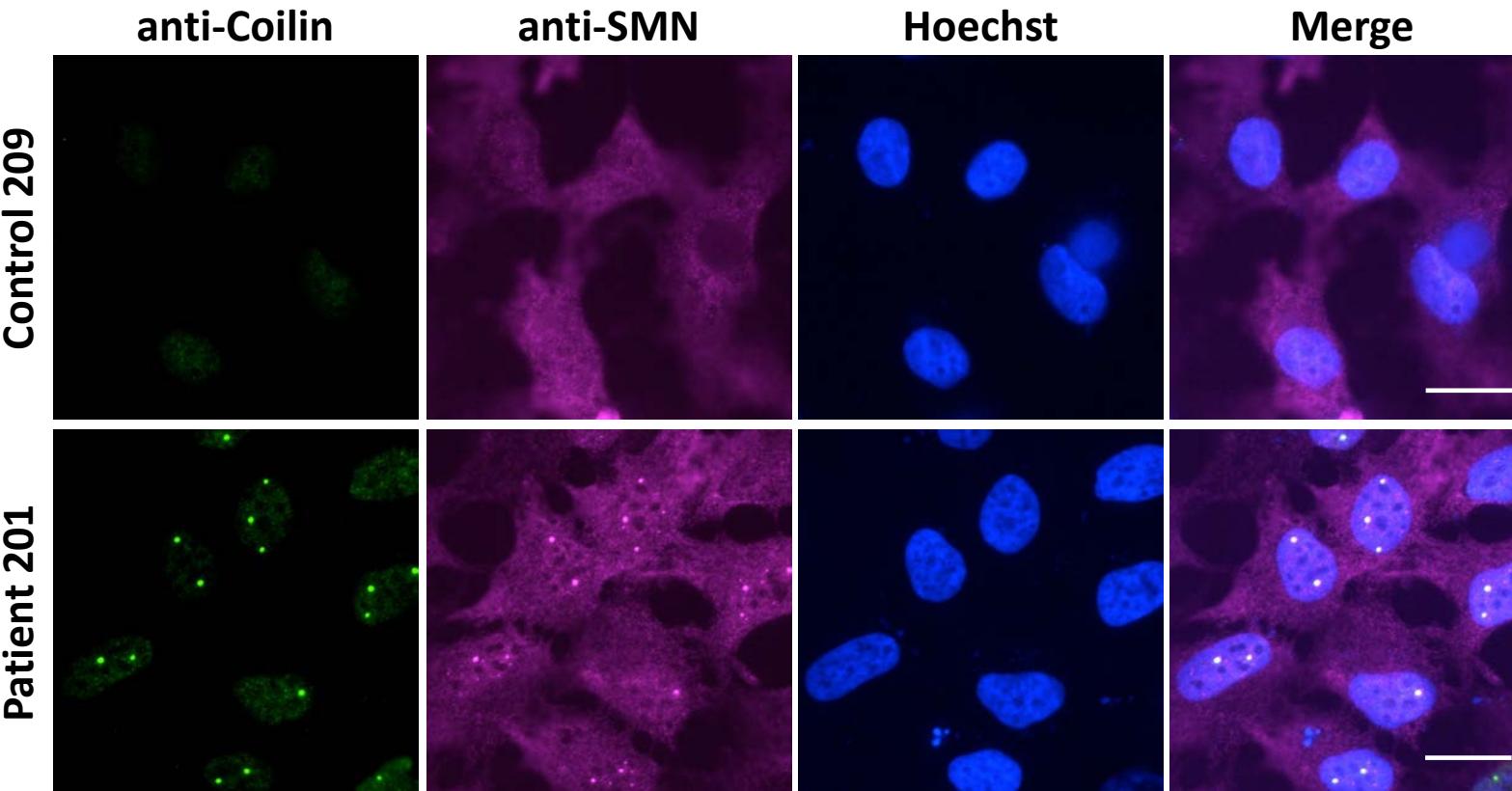




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Cajal bodies are increased in C9ALS patient iAstrocytes

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Conclusions

1. C9orf72 loss of function may contribute to defects in splicing
2. C9orf72 and colin interact *in vitro* and *in vivo*
3. C9orf72 knockdown increases Cajal bodies in cells
4. Patient iAstrocytes have increased Cajal bodies



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