Exploring the impact of NUP50 loss on nuclear pore complex integrity and the structural consequences of ALS-linked variants

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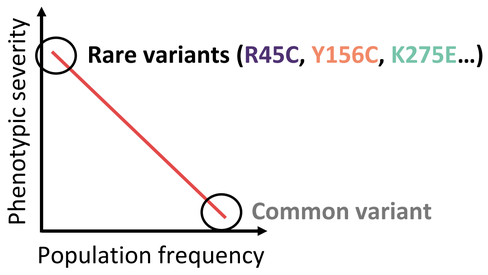
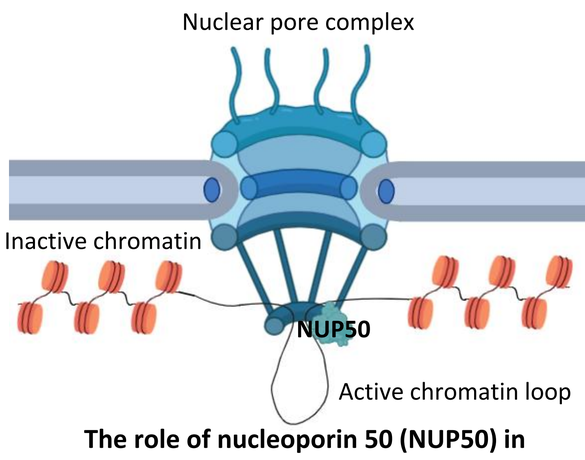
Introduction

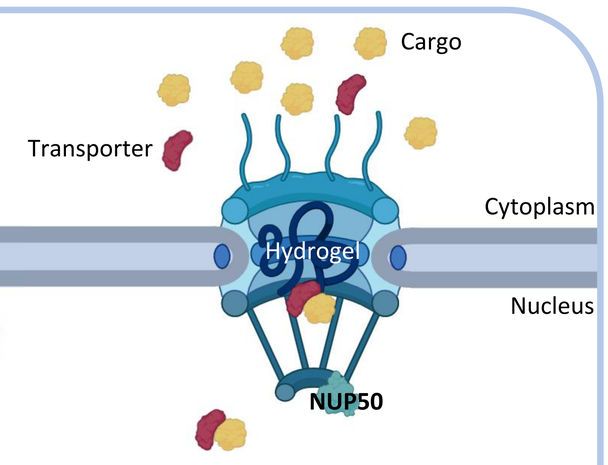
In our laboratory, we identified a **common NUP50 variant** in ALS patients associated with **reduced protein levels and increased ALS risk**, as well as several **rare variants (R45C, Y156C, K275E…) of unknown significance** [1].



Population frequency and phenotypic severity of ALS-associated NUP50 variants.



The role of nucleoporin 50 (NUP50) in chromatin organisation.



The role of NUP50 in nucleocytoplasmic transport.

NUP50 deficiency alters chromatin organisation

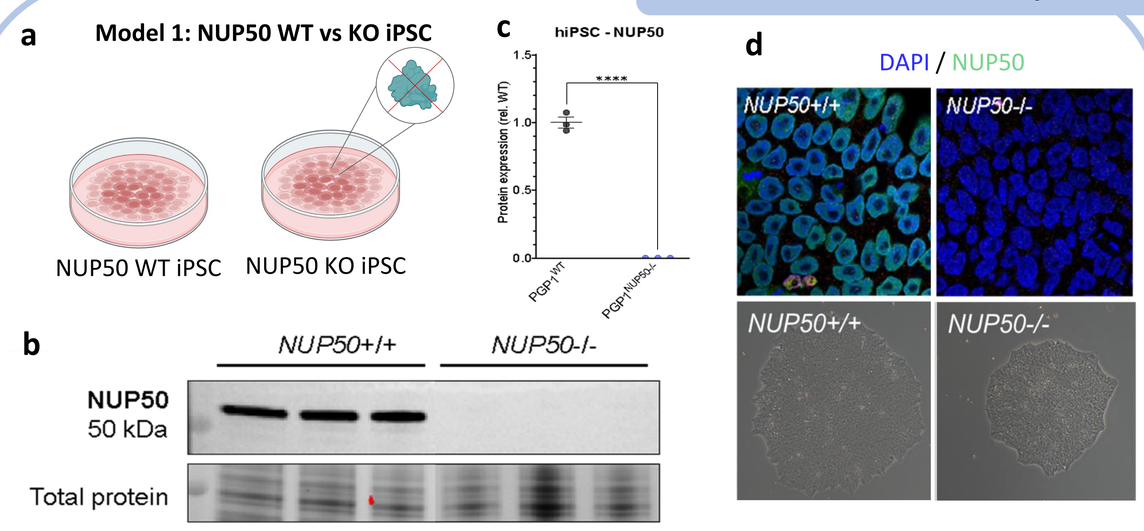


Figure 1: Characterisation of NUP50 WT and KO iPSC model. a) Schematic representation of NUP50 WT and KO iPSC. **b)** Western blot showing loss of NUP50 expression in KO iPSC. **c)** Quantification of NUP50 protein levels. **d)** Immunofluorescence staining confirming absence of NUP50 in KO iPSC colonies. ****p<0.0001, two-tailed unpaired t-test.

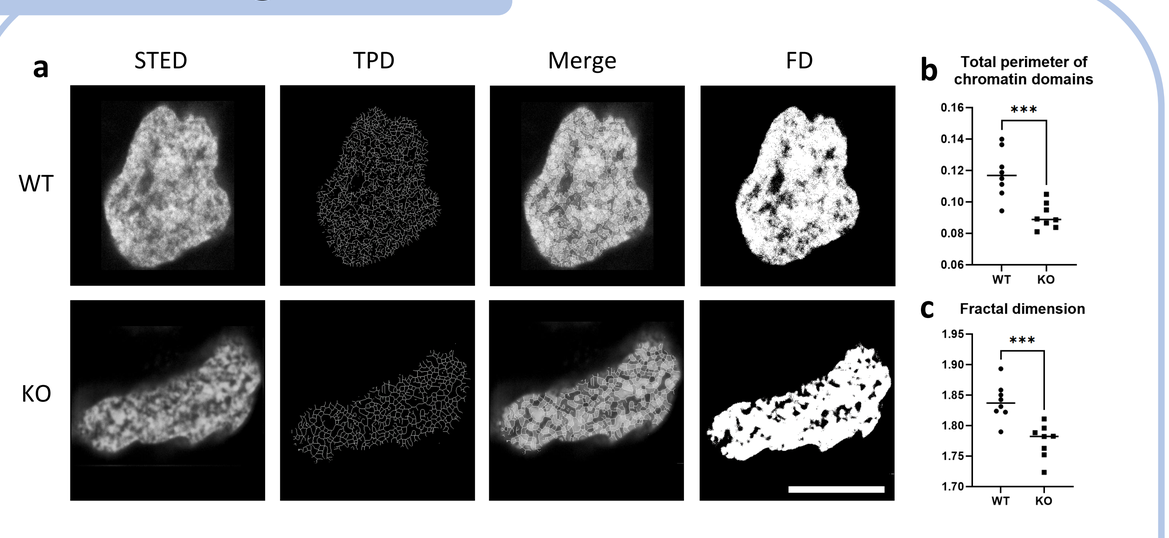


Figure 2: Chromatin organisation in NUP50 WT and KO iPSC. **a)** Representative stimulated-emission-depletion (STED) images of individual nuclei with corresponding masks used for total perimeter of chromatin domains (TPD) and fractal dimension (FD) quantification. Scale bar: 10 µm. **b)** Quantification of TPD shows increase in WT compared to KO. **c)** FD analysis reveals decreased values in KO compared to WT. ***p<0.001, two-tailed unpaired t-test.

NUP50 variants alter NUP50 biochemical properties

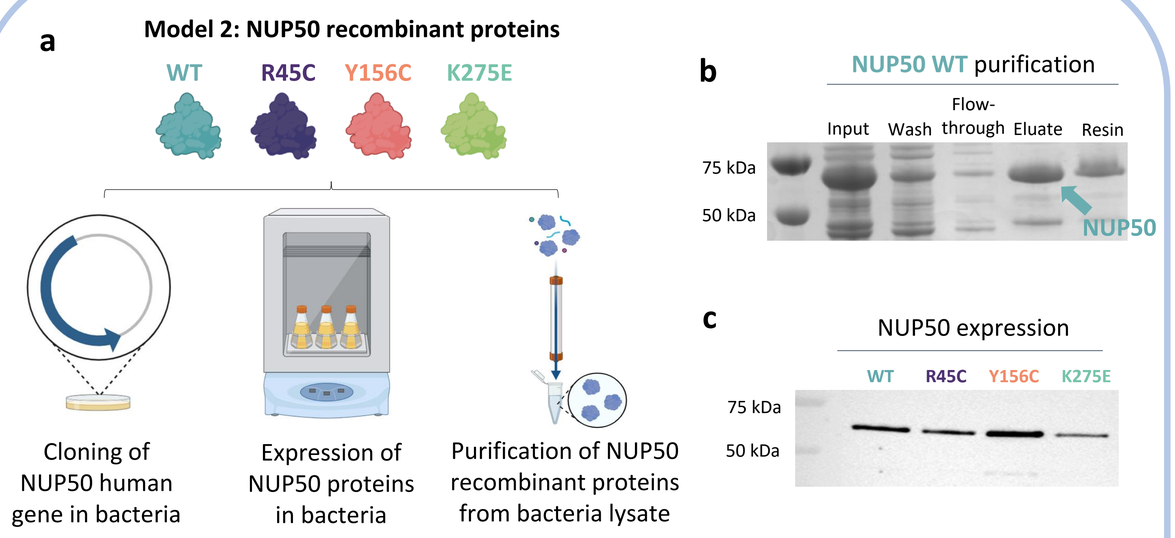


Figure 3: Production and validation of NUP50 WT and variants recombinant proteins. a) Workflow illustrating the production of NUP50 WT and variants recombinant proteins. **b)** SDS-PAGE showing successful purification of NUP50 WT. **c)** Western blot with anti-NUP50 antibody confirming the expression of NUP50 WT and variants.

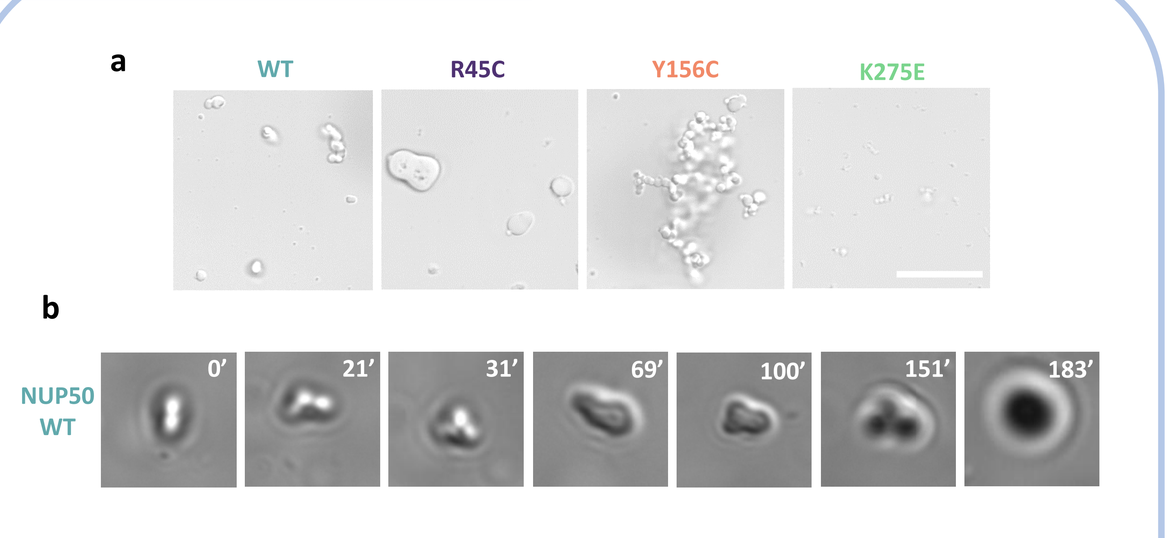
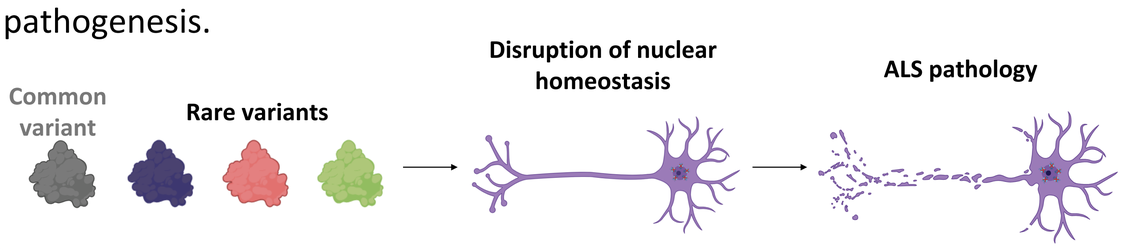
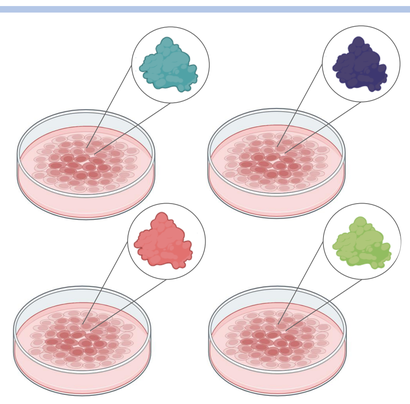


Figure 4: Liquid-liquid phase separation (LLPS) behavior of NUP50 WT and variants. **a)** Representative images showing droplet formations for NUP50 WT and variants under LLPS conditions. Variants R45C and Y156C form irregular aggregates, while K275E displays smaller droplets compared to WT. Scale bar: 20 µm. **b)** Time-lapse images illustrating droplet fusion events in NUP50 WT, confirming its liquid-like behavior.

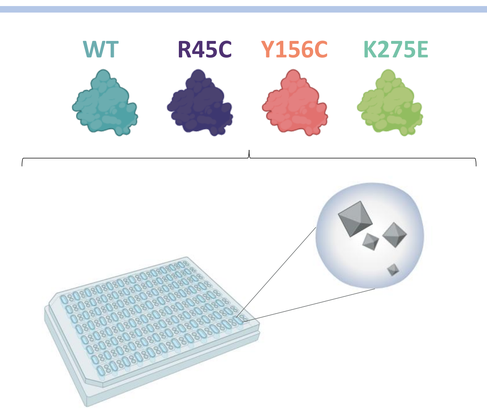
Conclusion & perspectives

Our results highlight the critical role of NUP50 in maintaining nuclear organisation and phase separation properties. Together, these findings suggest that NUP50 dysfunction contributes to altered nuclear homeostasis, providing new insights into its potential role in ALS pathogenesis.





Perspective 1: Nucleocytoplasmic transport defects and chromatin dysorganisation in NUP50 variants iPSC.



Perspective 2: Structural determination of NUP50 and ALS-linked variants by crystallisation.

[1] Megat S, Mora N, Sanogo J, et al. Integrative genetic analysis illuminates ALS heritability and identifies risk genes. Nat Commun. 2023; 14(1):342.