A FUS-DEPENDENT GENE NETWORK UNDERLIES COGNITIVE AND BEHAVIORAL IMPAIRMENT IN ALS

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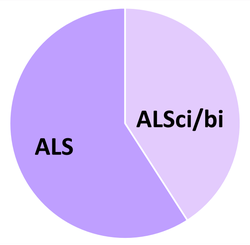
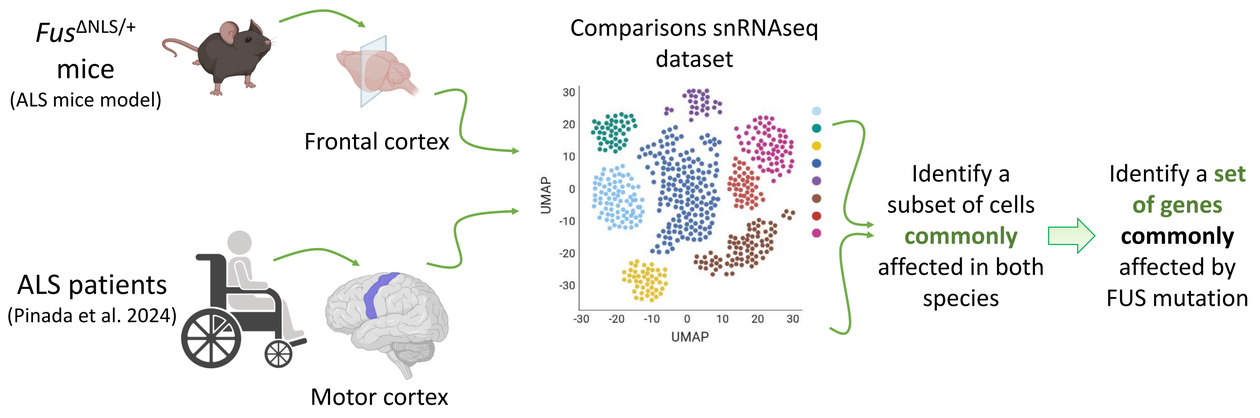
1 Context and Aims

ALS is the most frequent adult-onset disease of motor neurons. **Additional extra-motor deficits** (in up to 50% ALS patients), unrelated to the motor system, and leading to several forms of ALS are observed: ALS with cognitive impairment (ALS-ci), and ALS with behavioral impairment (ALS-bi). Some patients even present frontotemporal dementia symptoms (ALS-FTD). **Familial ALS-FUS** are associated with mutations in FUS-NLS domain, leading to FUS cytoplasmic mislocalization. In **sporadic forms of ALS**, FUS mislocalization is also observed (i.e. without mutation). FUS is an RNA-binding protein that is involved in multiple steps of RNA metabolism. FUS is mislocalized in sporadic ALS motor neurons and neurons in cortex. (Tyzack et al. 2019, Rifai et al. 2022).

Could cytoplasmic delocalization of FUS alter genes expression, leading to cognitive and behavioral impairments in ALS?

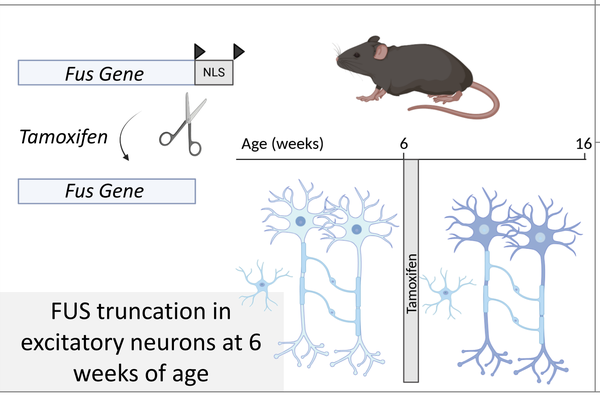


2 Global strategy



3 Material and methods

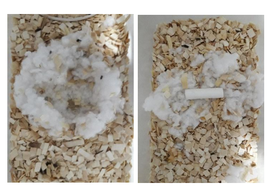
Mice model – Thy1-*Fus* exon15

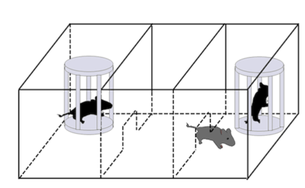


Behavioral analyses

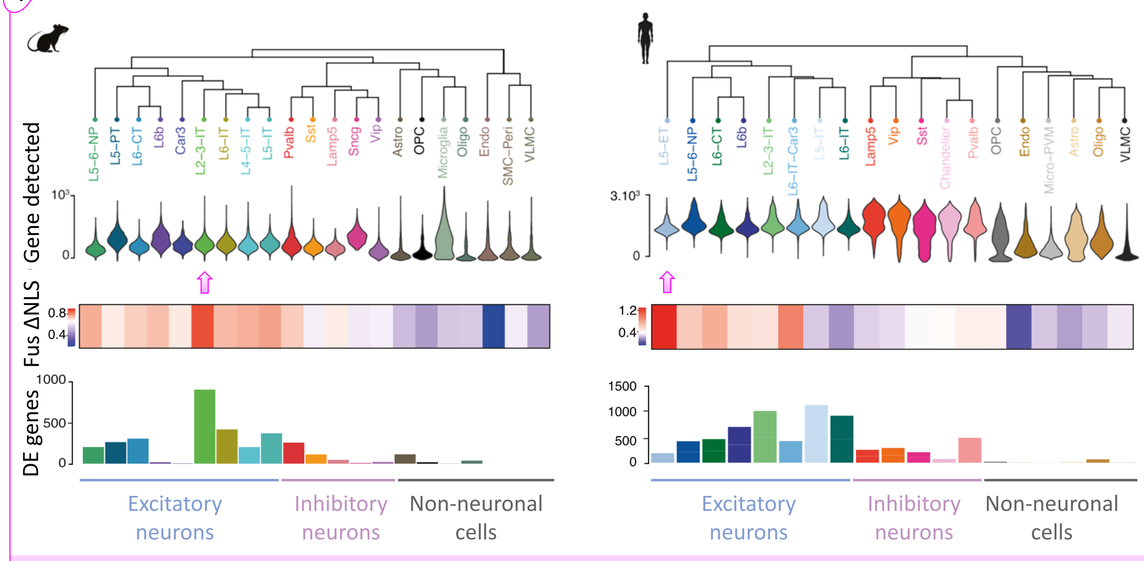
Motor test:
Grip test, Inverted grid test

Cognitive test: Nest building, 3-chamber test

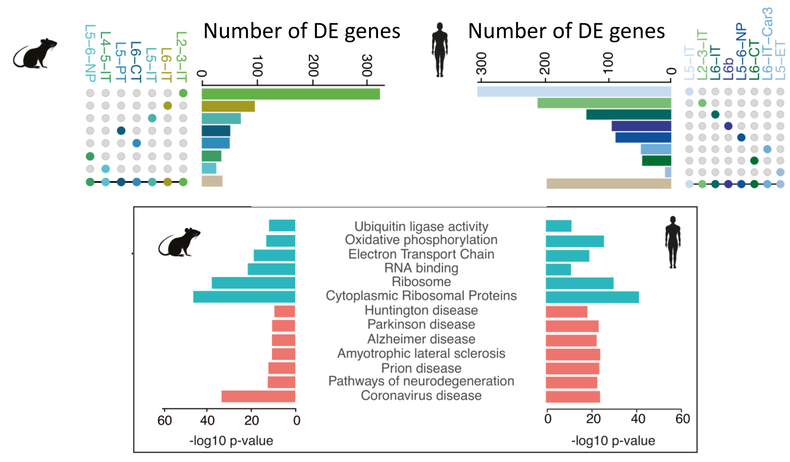




4 1. Mislocalization of FUS is sufficient to recapitulate transcriptomic alterations in ALS excitatory cortical projection neurons (CPN)

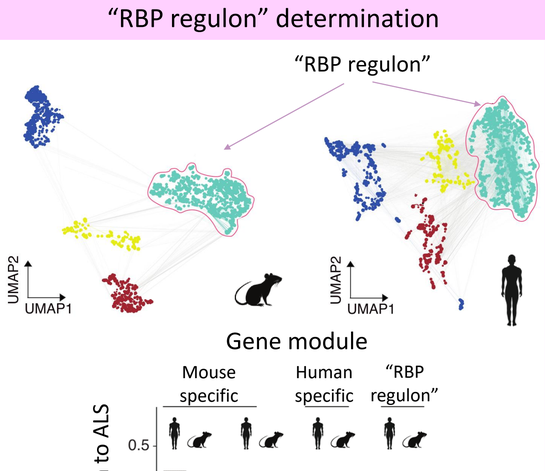


Cross-species snRNA seq shows similar transcriptomic alterations in excitatory CPN.

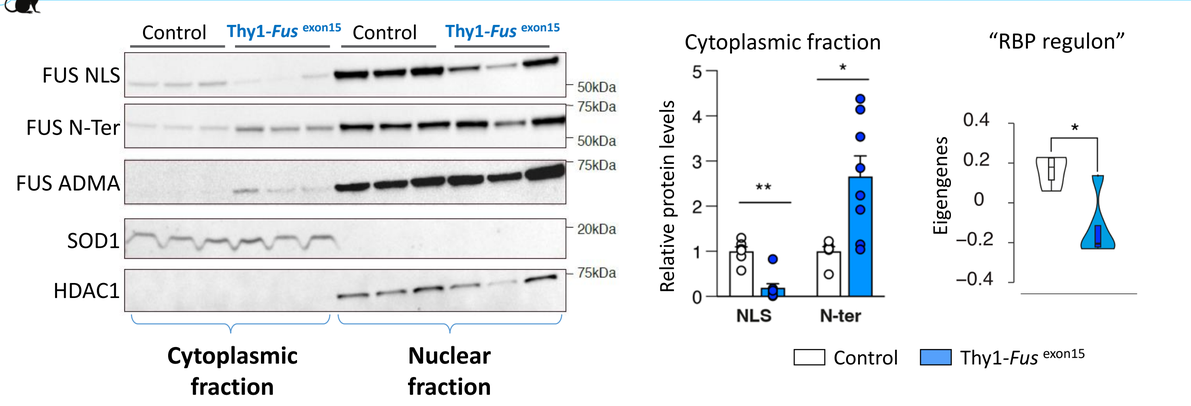


Higher number of differentially expressed genes in excitatory neurons (*vs.* inhibitory neurons and non-neuronal cells) and more specifically in CPN (Layer 5 extra telencephalic – L5-ET in human and their equivalents in mice – L2-3 IT)

"RBP regulon" determination

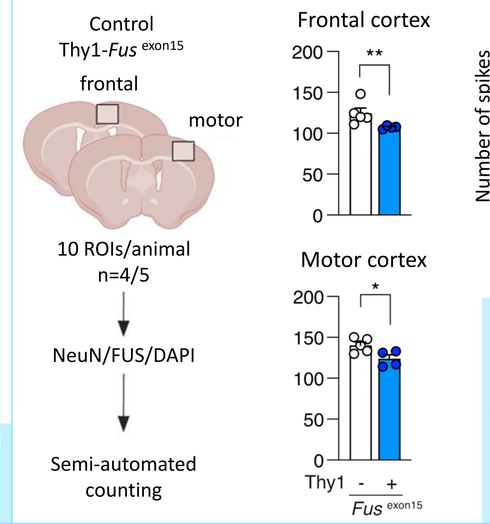


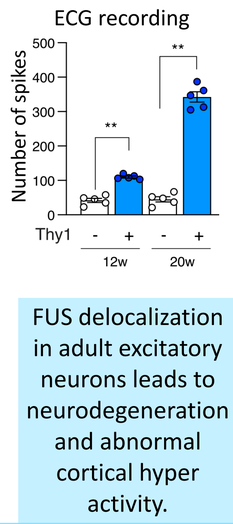
5 2. Adult-onset FUS mislocalization in excitatory projection neurons



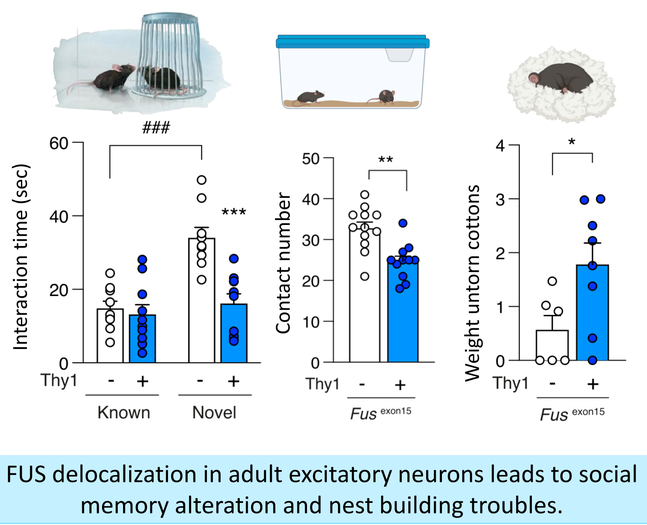
*, *vs.* control mice

FUS NLS domain truncation in excitatory projection neurons: **cytoplasmic delocalization** (increase of FUS N-Ter signal in cytoplasmic fraction) and increased FUS dimethylated form (ALS-FUS marker) in transgenic mice.
These mice suffer from "RBP regulon" dysregulation.





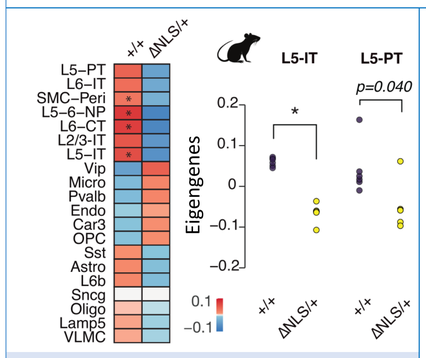
FUS delocalization in adult excitatory neurons leads to neurodegeneration and abnormal cortical hyper activity.



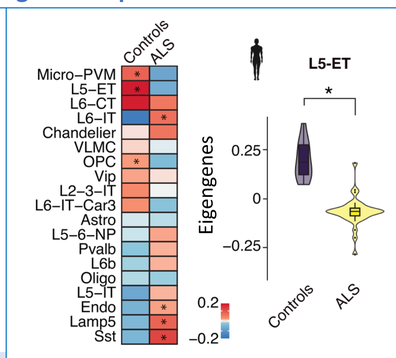
FUS delocalization in adult excitatory neurons leads to social memory alteration and nest building troubles.

6 3. FUS pathology and downregulation of the "RBP regulon" in patients with ALS-ci

Global measurement of "RBP regulon" expression across CPN

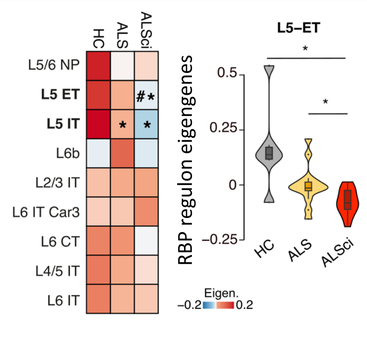


Dysregulation of the "RBP regulon" in L5-IT (intra-telencephalic) and L5-PT (extra-telencephalic).



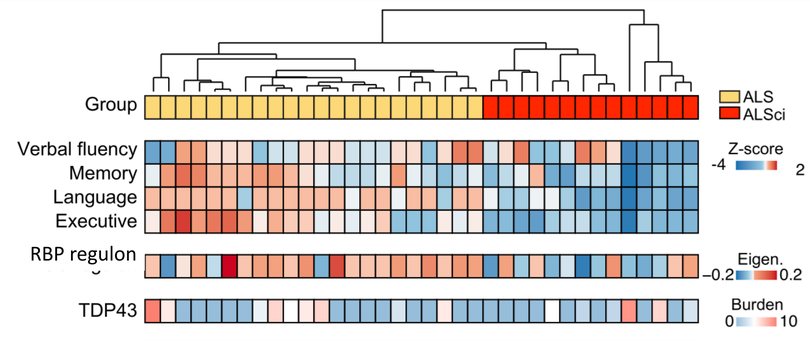
"RBP regulon" strongly downregulated in L5-ET in ALS-patient

Are CPNs more affected in ALS-ci patients?



ALS-ci patients showed a decreased expression of the "RBP regulon" in L5-ET neurons.

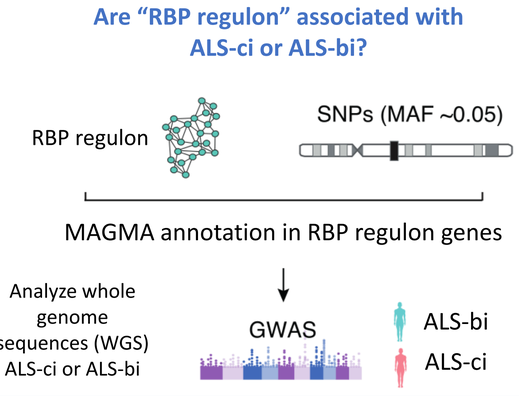
What about the link with cognitive impairment?

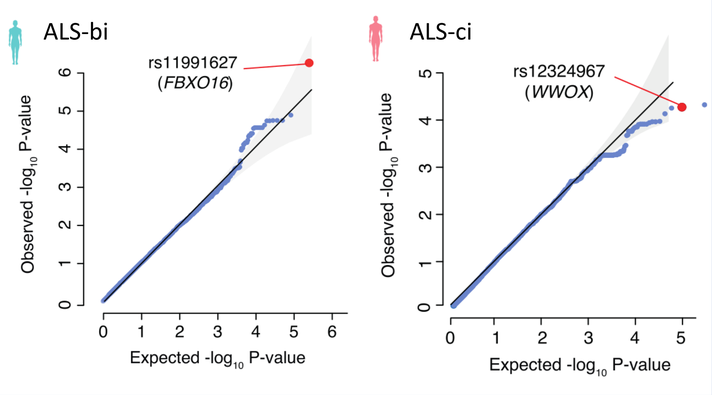


"RBP regulon" is specifically downregulated in ALS patients suffering from cognitive alterations.

7 4. The "RBP regulon" is highly enriched in ALS heritability and allows priorization of novel genes

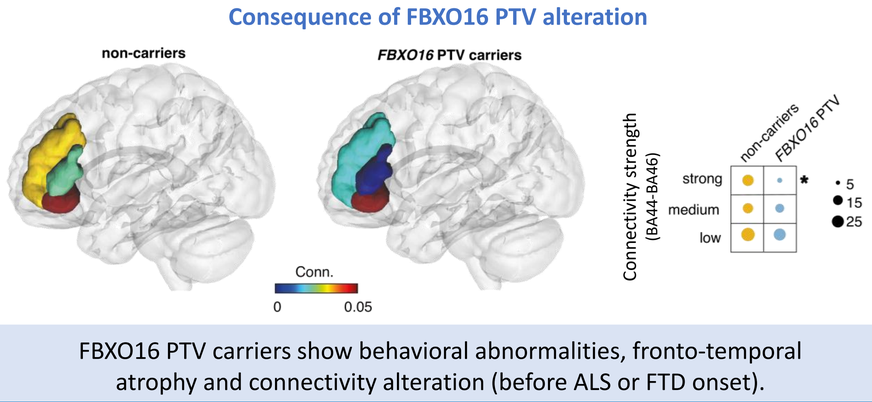
Are "RBP regulon" associated with ALS-ci or ALS-bi?





"RBP regulon" includes novel gene associated to cognitive impairment in ALS (***WWOX***) or to behavioral impairment in ALS (***FBXO16***).

Consequence of FBXO16 PTV alteration



FBXO16 PTV carriers show behavioral abnormalities, fronto-temporal atrophy and connectivity alteration (before ALS or FTD onset).

8 Conclusions and Perspectives

FUS mislocalization leads to altered transcriptomic in vulnerable cells in ALS and FTD.

- FUS mislocalization in adult cortical projection neurons is sufficient to compromise neuronal survival and alter behavior in mouse.
- A "RBP regulon" is downregulated in vulnerable neurons and highly enriched in ALS causing genes, that can be mined to identify novel ALS genes.

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Agence Nationale de la Recherche ANR
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