**Expanded View Figures**

**Figure EV1. Relevance of \textit{Fus}^{\text{NLS}} mice to human ALS.**

Upper panel: Scheme of the wild-type FUS protein. The NLS, encoded by exon 15, includes the C-terminal amino acids (aa 507–526, boundaries shown by the two dashed lines). Middle panels: 11 frameshift mutations (upper middle panel) and 2 truncating mutations (lower middle panel) in the \textit{FUS} gene have been identified in ALS families. The corresponding mutant FUS proteins are shown. Insertions of abnormal polypeptide sequences induced by frameshift mutations are shown as red boxes. Lower panel: structure of FUS\textsuperscript{ANLS} protein in \textit{Fus}\textsuperscript{NLS} mice.

<table>
<thead>
<tr>
<th>Frameshift mutations</th>
<th>Truncating mutations</th>
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<tbody>
<tr>
<td>G466VfsX14</td>
<td>R495X</td>
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<td>G472VfsX57</td>
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**Wild type**

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Wild type
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**Frameshift mutations**

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Frameshift mutations
G466VfsX14
G472VfsX57
G478LfsX23
Y485AfsX29
G492EfsX35
R495E/QfsX32
G497AfsX30
R502EfsX15
G504WfsX12
K510WfsX7
Q519lfsX9
```

**Truncating mutations**

```
Truncating mutations
R495X
Q519X
```

**\Delta NLS mice**

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\Delta NLS mice
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**Figure EV2.** Expression of the Fus gene in various tissues of Fus\(^{\Delta NLS}\) mice.

A. RT-PCR analysis of spinal cord and gastrocnemius muscle from 2 Fus\(^{+/+}\), 2 Fus\(^{\Delta NLS/+}\), and 2 Fus\(^{\Delta NLS/\Delta NLS}\) P0 mice using primers located in the STOP cassette, and thus specific to the Fus \(\Delta NLS\) mRNA (\(\Delta NLS\), upper panel), or primers located in exon 11 and 12, that is, upstream of the floxed cDNA insertion, and thus amplifying total Fus mRNA (Total, lower panel).

B. Immunoblot analysis of FUS protein in spinal cord and gastrocnemius of 2 Fus\(^{+/+}\), 2 Fus\(^{\Delta NLS/+}\), and 2 Fus\(^{\Delta NLS/\Delta NLS}\) mice using two different antibodies targeting the C-terminal (C-ter. 1 and C-ter. 2) or antibodies targeting the N-terminal (N-ter. 1) and internal parts (N-ter. 2) of FUS.

C. Representative confocal images for fluorescence immunocytochemical localization of FUS protein in mouse embryonic fibroblasts (MEFs).
Figure EV3. Genomewide expression changes identified by RNA-seq in Fus^{NLS/NLS} and Fus^{−/−} brains.

A. Quantification of Fus RNA levels by strand-specific RNA sequencing in brains from Fus^{NLS/NLS} (blue bars), Fus^{−/−} (red bars), and control littermates (Fus^{+/+}, black bars). RNA levels were determined by fragments per kilobase of transcript per million mapped reads (FPKM) value.

B. Unsupervised hierarchical cluster analysis using all RNAs expressed in brains of Fus^{NLS/NLS} mice (KI-1 to KI-5) and their control littermates (Ctrl-1 to Ctrl-4).

C. Unsupervised hierarchical cluster analysis using all RNAs expressed in brains of Fus^{−/−} mice (KO-1 to KO-5) and their control littermates (Ctrl-1 to Ctrl-5).
Figure EV4. FUS-dependent splicing alterations identified by RASL-seq.

A  Schematic representation of the RASL-seq strategy to measure ratios of alternative splicing isoforms from thousands of selected splicing events by high-throughput sequencing.

B  Unsupervised hierarchical cluster analysis using all splicing events sequenced in brains of Fus^{DNLS/DNLS} mice (KI-1 to KI-4), Fus^{DNLS/+} (HET-1 to HET-4), and their control littermates (Ctrl-1 to Ctrl-4).

C  Unsupervised hierarchical cluster analysis using all splicing events sequenced in brains of Fus^{+/C0/C0} mice (KO-1 to KO-5) and their control littermates (Ctrl-1 to Ctrl-5).

D  Venn diagram showing the number of overlapping splicing events that are misregulated in Fus^{DNLS/DNLS} and Fus^{+/C0/C0} mice similarly altered upon cytoplasmic mislocalization or complete loss of FUS.

E  Heat map using the fold changes of the 75 splicing events commonly regulated in Fus^{DNLS/DNLS} and Fus^{+/C0/C0} mice showing that 100% of the events were differentially included or excluded in the same direction.

F  Semi-quantitative RT–PCR analyses of selected targets shown in Fig 5C with alternatively spliced exons depicted in orange boxes with their flanking constitutive exons in blue boxes.
Figure EV5. The absence of protein aggregates and stress granules in Fus^{INLS/ANLS} mice.
A  Representative images of ubiquitin staining (green) in the ventral spinal cord.
B  Representative images of immunofluorescence staining of neurofilament heavy chain (green) and poly-ubiquitin (lysine 48, red). The neurofilament immunostaining shows normal filamentous staining, and the poly-ubiquitin staining is very weak and shows no positive aggregates.
C  Representative images of immunofluorescence staining of neurons labeled with NeuN (green) and TIAR (red), a stress granule marker.