Supplementary Information

Supplementary Material and Methods

Plasmid construction The transposable element vectors for inducible expression of RFP-FUS\textsuperscript{wt} and EGFP-FUS\textsuperscript{R521C} and EGFP-FUS\textsuperscript{P525L} were derived from the enhanced piggyBac (ePiggyBac) vector epB-Bsd-TRE described in Rosa and Brivanlou, 2011. Briefly, a cassette encoding for the rtTA-Advanced protein (Clontech) was fused to the Puromycin or Blasticidin resistance coding sequences through a T2A self-cleavage peptide element, and put under the control of the ubiquitous pUbc promoter in the epB-Bsd-TRE vector. The resulting plasmids (epB-Puro-TT and epB-Bsd-TT) hold on the opposite direction the tetracycline-responsive promoter element (TRE), followed by a short multicloning site. Therefore both elements of the TET-ON system are present in the same vector. The RFP and EGFP coding sequences, devoid of the stop codon, were then inserted in the epB-Puro-TT and epB-Bsd-TT plasmids, respectively, generating the epB-Puro-TT-RFP and epB-Bsd-TT-EGFP. Finally, the coding sequences of FUS, wild type or mutated, were cloned in frame with the fluorescent proteins, generating the epB-Puro-TT-RFP-FUS\textsuperscript{wt}, epB-Bsd-TT-EGFP-FUS\textsuperscript{R521C} and epB-Bsd-TT-EGFP- FUS\textsuperscript{P525L}.

Drosha \textit{in vitro} processing. \textit{In vitro} processing assay was carried out as previously described (Lee and Kim, 2007). Pri-miRNA substrates were prepared by \textit{in vitro} transcription, using T7 RNA polymerase (Promega), from PCR amplified templates (oligonucleotides are listed in Table II), in the presence of [\textalpha-32P]UTP (Perkin-Elmer). 100'000 cpm of each pri-miRNA transcript were incubated with 15 mg of SK-N-BE nuclear extract cells at 37°C for 90 minutes.

Supplementary references


Supplementary Figure Legends

\textbf{Figure S1.} (A) miRNA and protein levels during SK-N-BE cells differentiation. SK-N-BE cells were induced to differentiate with retinoic acid (RA) and incubated for the indicated times (0, 1, 3, 6 and 10 days). miR-9, miR-125b and miR-132 were analyzed by Northern blot using corresponding specific oligonucleotides. 5.8S rRNA was used as internal control. The histogram indicates the relative levels normalized for the 5.8S signal. Lower panel: Western blot analysis of N-Myc and FUS proteins at the same time points. (B) miRNA profiling in SK-N-BE and HeLa cells treated with anti-FUS siRNA (siFUS) or with AllStars Negative Control siRNA (siScr). SK-N-BE cells were cultured in RA for 6 days. Pie charts and tables showing the percentage of miRNA deregulated more than 0.2 fold.

\textbf{Figure S2.} FUS interference. (A) Levels of neuronal-specific miRNAs in RA-treated SK-N-BE cells in two different sets of RNAi experiments where the residual FUS levels were 45% and 20% respectively. AllStars Negative Control siRNA (siScr) were utilized as control. Left panels: Western blot analysis of FUS and GAPDH proteins. Relative quantification (RQ) of FUS versus GAPDH is shown with respect to the siScr condition set to a value of 1. Right panels: histograms of miRNA levels analysed by qPCR normalized for the snoRNA-U25 internal control. The values are
the average from 3 independent experiments and are expressed with respect to the siScr condition set to a value of 1. (B) SK-N-BE cells were treated with siRNAs against the 3’UTR of FUS (siFUS-3’) or scrambled siRNA (siScr) and maintained in retinoic acid (RA) for 6 days. miRNA levels were analyzed by RT-qPCR. The histogram represents the average of 3 different measurements. miRNA levels were normalized for the snoRNA-U25 internal control.

**Figure S3.** Coomassie staining showing the quality of purified recombinant GST-FUS\(^{WT}\) and GST-FUS\(^{R521C}\). Different amounts of BSA protein are used as quantity control. Marker of molecular weight is also shown.

**Figure S4.** Intracellular localization of wild type and mutated FUS proteins. (A) Schematic representation of the epB-Puro-TT-RFP-FUS\(^{wt}\), epB-Bsd-TT-EGFP-FUS\(^{R521C}\) and epB-Bsd-TT-EGFP-FUS\(^{P525L}\) constructs. Triangles indicate the 5’ and 3’ Terminal Repeats (TR) of the epiggyBac vector. (B) SK-N-BE cells were co-transfected with epB-Puro-TT-RFP-FUS\(^{wt}\) and epB-Bsd-TT-EGFP-FUS\(^{R521C}\) (top panels) or with epB-Puro-TT-RFP-FUS\(^{wt}\) and epB-Bsd-TT-EGFP-FUS\(^{P525L}\) (bottom panels), together with a plasmid encoding for the epiggyBac transposase. After selection with 1µg/ml Puromycin and 10 µg/ml Blasticidin, stably transfected cells were induced with 0,2µg/ml Doxycyclin for 3 days. Images were taken with a Zeiss Axio Observer A1 fluorescence microscope at 20X magnification. Scale bar=20 µm.

**Figure S5.** FUS depletion does not affect Drosha-mediated processing in vitro. In vitro processing with \(^{32}\)P-UTP labelled pri-miR-9-2, pri-miR-125-b and pri-miR-132 using nuclear extracts from SK-N-BE cells treated either with AllStars Negative Control siRNA (siScr) or siRNA against FUS (siFUS). The mock samples with no extract are used as controls.
Figure S1

A

![Graph showing expression levels of miR-9, miR-125b, and miR-132 over 10 days of RA treatment.]

B

- **SK-N-BE**
  - Downregulated: 149 (79.26%)
  - Unaffected: 22 (11.70%)
  - Upregulated: 17 (9.04%)
  - Total: 188 (100.00%)

- **HeLa**
  - Downregulated: 53 (30.46%)
  - Unaffected: 59 (33.91%)
  - Upregulated: 62 (35.63%)
  - Total: 174 (100.00%)
Figure S2

A

<table>
<thead>
<tr>
<th>siScr</th>
<th>siFUS</th>
</tr>
</thead>
<tbody>
<tr>
<td>FUS</td>
<td>[Image]</td>
</tr>
<tr>
<td>GAPDH</td>
<td>[Image]</td>
</tr>
</tbody>
</table>

RQ:

|  | siScr | siFUS |
|  | 1     | 0.45  |

|  | siScr | siFUS |
|  | 1     | 0.20  |

Graphs:

- siScr vs. siFUS for miR-9, miR-125b, and miR-132
- miR-9, miR-125b, miR-132 levels

B

Graphs:

- miR-9, miR-125b, miR-128, miR-132, miR-212, miR-134, miR-143, miR-192, miR-199a, miR-628-5p, miR-15a, miR-432
- siScr vs. siFUS-3'
Figure S3

**GST-FUS WT**

**GST-FUS R521C**

- 0,2 µg of BSA
- 0,5 µg of BSA

**4-12% gel**

- KD 115
- KD 80
- KD 65
- KD 50
- KD 40

**Coomassie**
Figure S4

A

epB-Puro-TT-RFP-FUS\textsuperscript{wt}

epB-Bsd-TT-EGFP- FUS\textsuperscript{R521C/P525L}

B

\textbf{EGFP-FUS}\textsuperscript{R521C} \hspace{1cm} \textbf{RFP-FUS}\textsuperscript{WT} \hspace{1cm} \textbf{merge}

\textbf{EGFP-FUS}\textsuperscript{P525L} \hspace{1cm} \textbf{RFP-FUS}\textsuperscript{WT} \hspace{1cm} \textbf{merge}
Figure S5

pri-miR-9-2 pri-miR-125b-2 pri-miR-132

mock siScr siFUS mock siScr siFUS mock siScr siFUS

pre-miRNA