

## Supplementary Data for

### **ALS mutations in FUS are clustered in the nuclear localization sequence and induce stress granules**

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Running title: NLS of FUS and stress granules induced by fALS mutants

**Supplementary Figure S1. Cytoplasmic mislocalization of the R521G familial ALS mutant FUS in NSC34, HEK293 and Drosophila S2 cells.** The NSC34 (A), HEK293 (B) and S2 (C) cells were transfected with GFP- (A and B) or Myc-tagged (C) WT or mutant FUS as described in the Materials and Methods. Confocal images of GFP-FUS or immunostaining of Myc\_FUS along with DAPI staining were acquired. The familial ALS mutant was localized in both the cytoplasm and nucleus while the cytoplasmic signal of WT FUS was undetectable. Protein inclusions were also observed in cells expressing mutant FUS. All scale bars are 10  $\mu$ m.

**Supplementary Figure S2. (A).** The NSC34 cells were transfected with GFP-tagged WT or C-17 deletion mutant FUS. Cytoplasmic localization of FUS- $\Delta$ 17 was observed while WT FUS was mostly localized in the nucleus. **(B).** Western blot of the cytoplasmic and nuclear fractions of NSC34 cells expressing WT FUS, R521G or  $\Delta$ 17 mutants. Increased levels of mutant FUS were found in the cytoplasmic fractions as compared to WT FUS. The quantification of the relative abundance of the cytoplasmic FUS normalized against the nuclear FUS was shown as C/N values at the bottom of the figure. Histone 4 (H4) and superoxide dismutase 1 (SOD1) were used as nuclear and cytoplasmic markers, respectively. The scale bar is 10  $\mu$ m.

**Supplementary Figure S3. Colocalization of the TIA-1 positive stress granules with mutant FUS inclusions.** HEK293 cells were transfected with GFP vector control, GFP-WT-FUS, or GFP-R521G-FUS. Confocal images of GFP, TIA-1 immunofluorescence and DAPI were obtained. TIA-1 positive cytoplasmic foci characteristic of stress granules were observed in cells expressing mutant FUS. The stress granules were also co-localized with the mutant FUS inclusions. All scale bars are 10  $\mu$ m.





