Supplementary Data (Vance *et al*)

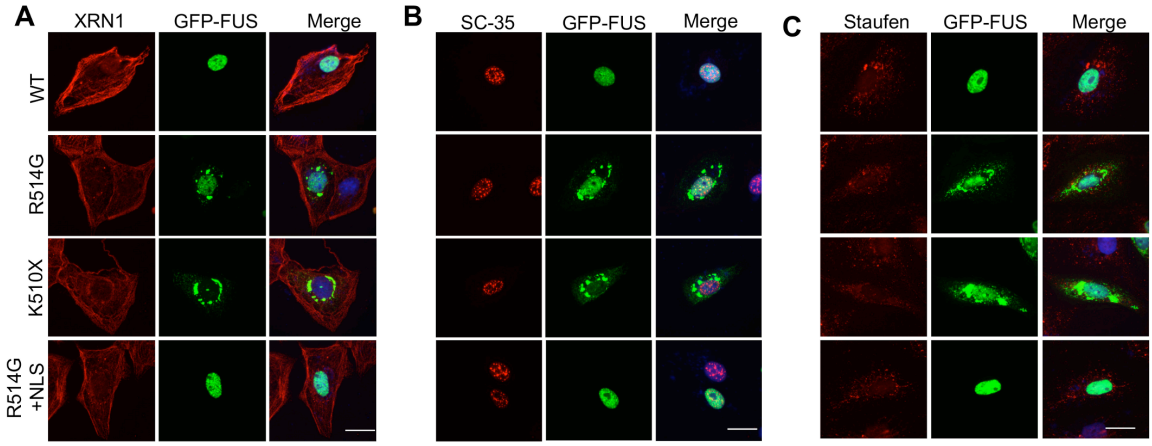

Figure S1: GFP-FUS does not co-localise with markers for p-bodies ,nuclear speckles or RNA transport granules.

Immunofluorescent staining of CV-1 cells transfected with GFP-FUS^{WT}, GFP-FUS^{R514G}, GFP-FUS^{K510X} and GFP-FUS$^{R514G+NLS}$ (green) and immunostained for the marker of p-bodies XRN1 (red, A), the nuclear speckle marker SC-35 (red, B) and a marker for RNA transport granules (red, C). There was no co-localisation between the cytoplasmic mutant GFP-FUS and any of these markers. Nuclei are stained blue with DAPI in the merge. Scale bar 25 µm.

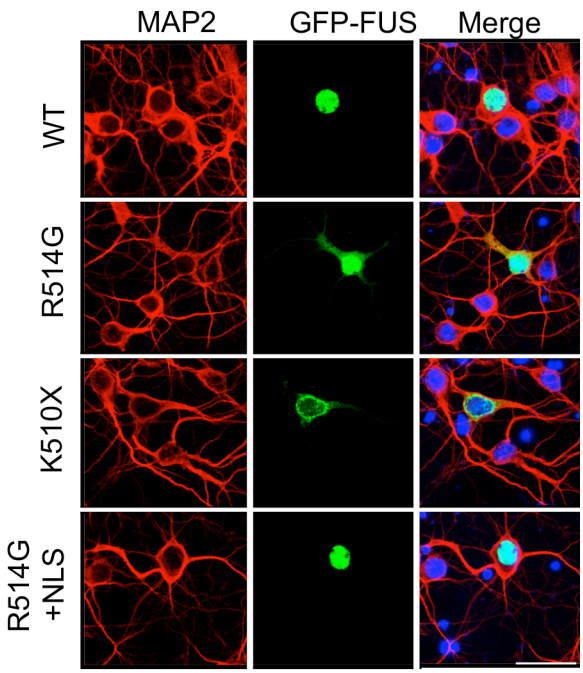

Figure S2: Mutant GFP-FUS is mislocalised in cortical Neurons

Immunostaining for MAP2 (red) in rat primary cortical neurons confirms that GFP-FUS^{R514G} and GFP-FUS^{K510X} are mislocalised whilst GFP-FUS^{WT} and GFP-FUS$^{R514G+NLS}$ (green) remain nuclear in neuronal cells. Scale bar 50 µm.

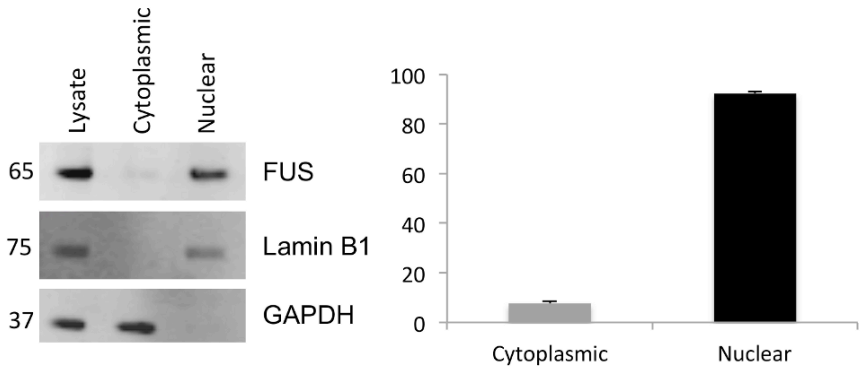

Figure S3: Endogenous FUS is localised mainly to the nucleus. Subcellular fractionation of HEK293T cells shows that the majority of FUS is localised to the nucleus.

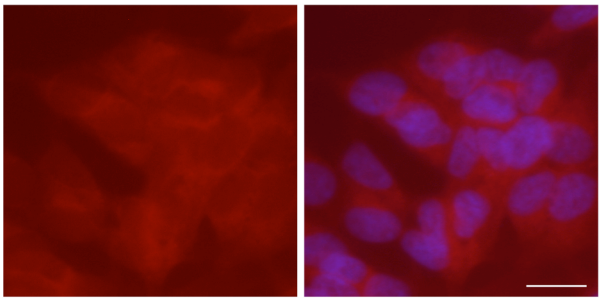

Figure S4: The absence of primary antibodies in PLA results in a weak diffuse cytoplasmic signal. PLA performed with no primary antibody does not result in formation of distinct puncta. Nuclei are stained blue with DAPI in the merge. Scale bar 25 µm.

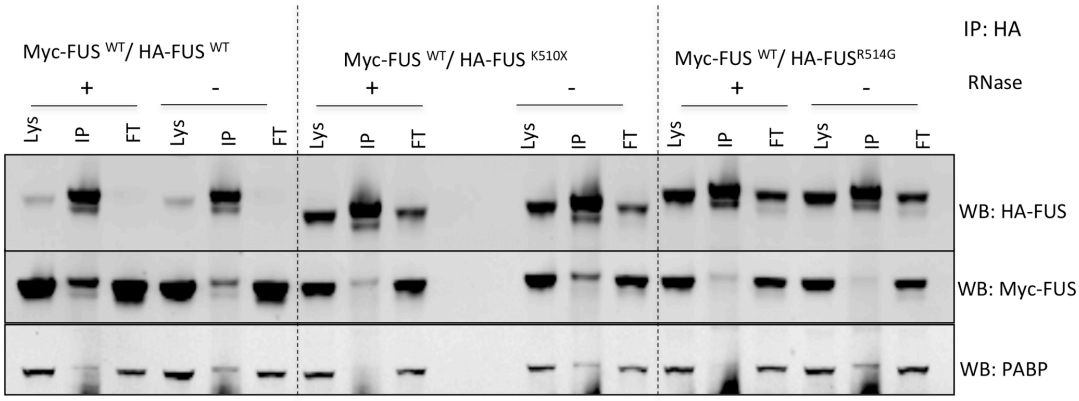

Figure S5: FUS forms a complex with itself, PABP and RNA.

Co-immunoprecipitation of HA-FUS, Myc-FUS and PABP from HEK293T cells. Immunoprecipitation with a HA antibody pulled down PABP along with Myc- FUS. (-) RNasin was added to block all RNase activity. (+) The interaction between HA-FUS and Myc-FUS was not altered by the addition of RNase whilst the co-immunopreciptation of PABP was completely abolished.