Homo sapiens FUS RNA binding protein (FUS), NM\_004960.4, mRNA sequence

1191 TGGAGGTGGTGGCAGTGGTGGTGGTGGCCGAGGAGGATTTCCCAGTGGAGGTGGTGGCGGTGGAGGACAG

1261 CAGCGAGCTGGTGACTGGAAGTGTCCTAATCCCACCTGTGAGAATATGAACTTCTCTTGGAGGAATGAAT

FUS-splicing-F

1331 GCAACCAGTGTAAGGCCCCTAAACCAGATGGCCCAGGAGGGGGACCAGGTGGCTCTCACATGGGGGGTAA

1401 CTACGGGGATGATCGTCGTGGTGGCAGAGGAGGCTATGATCGAGGCGGCTACCGGGGCCGCGGCGGGGAC

1471 CGTGGAGGCTTCCGAGGGGGCCGGGGTGGTGGGGACAGAGGTGGCTTTGGCCCTGGCAAGATGGATTCCA

1541 GGGGTGAGCACAGACAGGATCGCAGGGAGAGGCCGTATTAATTAGCCTGGCTCCCCAGGTTCTGGAACAG

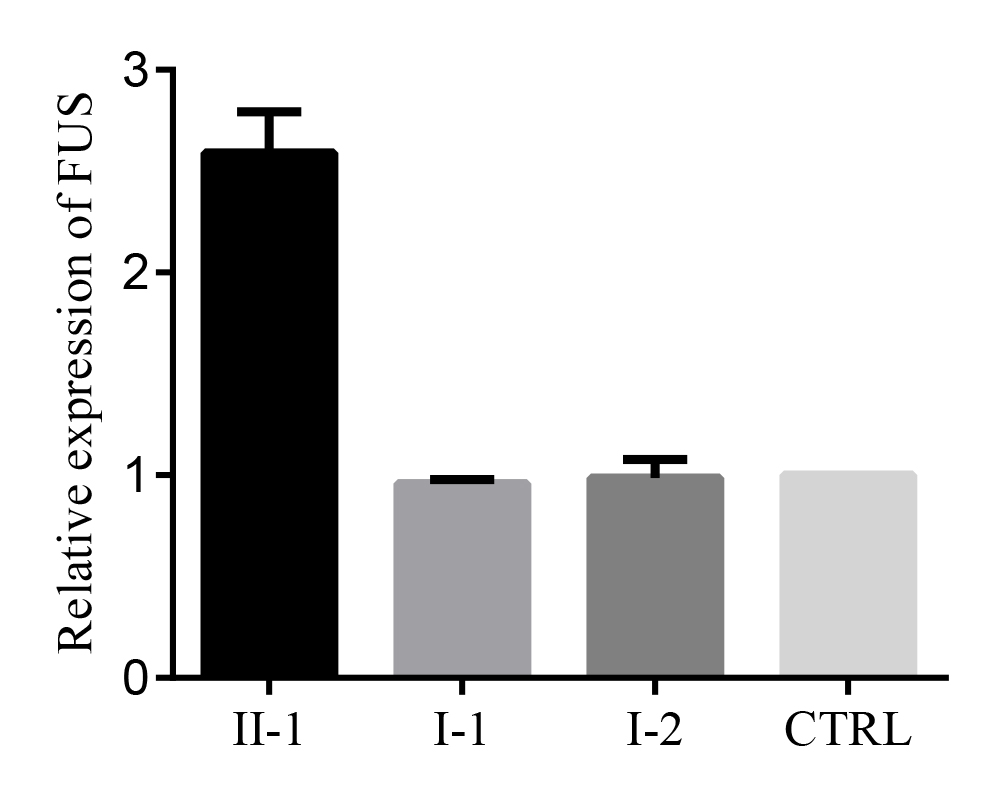
G V S T D R I A G R G R I N \*

1611 CTTTTTGTCCTGTACCCAGTGTTACCCTCGTTATTTTGTAACCTTCCAATTCCTGATCACCCAAGGGTTT

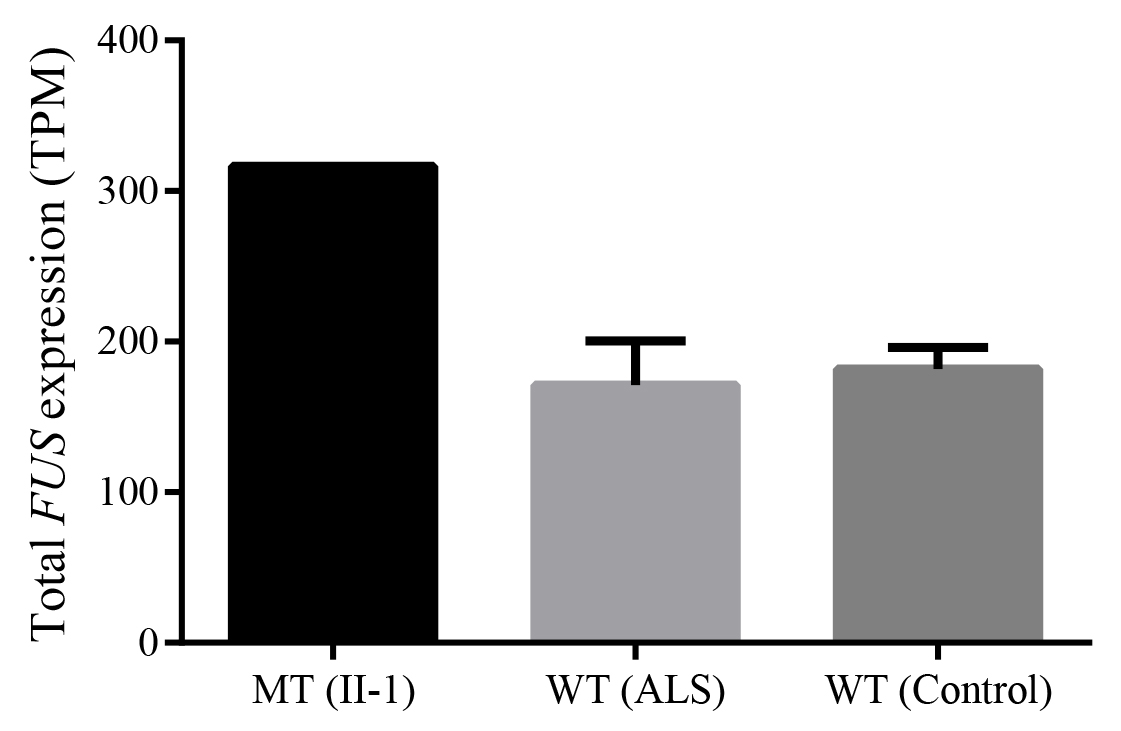
FUS-splicing-R

1681 TTTTGTGTCGGACTATGTAATTGTAACTATACCTCTGGTTCCCATTAAAAGTGACCATTTTAGTTAAATT

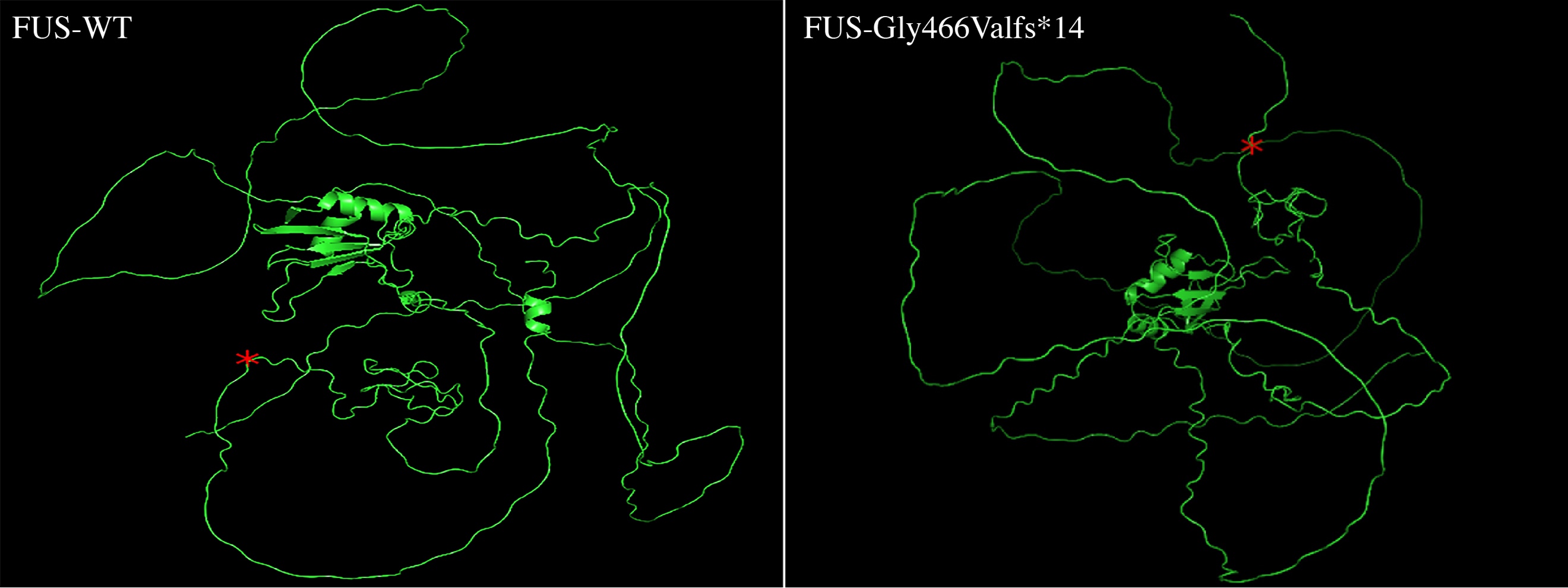
**Supplementary Figure 1**. The translation product of *FUS* with the c.1394-2delA mutation. The sequence of WT *FUS* exon14 was highlighted in blue; The bases with yellow background indicated the newly translated sequences. The underlined sequences represent the primer targeting region for RT-PCR.



**Supplementary Figure 2.** Relative total *FUS* mRNA expression level in the mutation carrier (II-1), and three wild type carriers (I-1, I-2, and another independent healthy control [CTRL]). The expression of *FUS* was normalized to the expression housekeeping gene (*GAPDH*).



**Supplementary Figure 3.** RNA-seq revealed increased total *FUS* expression in splice site mutation carrier (II-1) when compared to non-carriers, including 3 ALS cases and 3 healthy controls. The error bar represents standard deviation. MT: mutant; WT: wild type; TPM: Transcript per million.



**Supplementary Figure 4**. Prediction of protein structures based on Alphafold. Left: FUS protein with wild type (WT) allele; Right: FUS protein with the *FUS* mutation (p.Gly466Valfs\*14). FUS protein with the mutation showed a truncated structure. The asterisk indicates the 465 locus of FUS protein.

**Supplementary Table 1** List of primers used for RT-qPCR

|  |  |
| --- | --- |
| Primer name | Sequence (5’ to 3’) |
| FUS-qPCR-F | ATGGCCTCAAACGATTATACCCA |
| FUS-qPCR-R | GTAACTCTGCTGTCCGTAGGG |
| GAPDH-F | GGAGCGAGATCCCTCCAAAAT |
| GAPDH-R | GGCTGTTGTCATACTTCTCATGG |