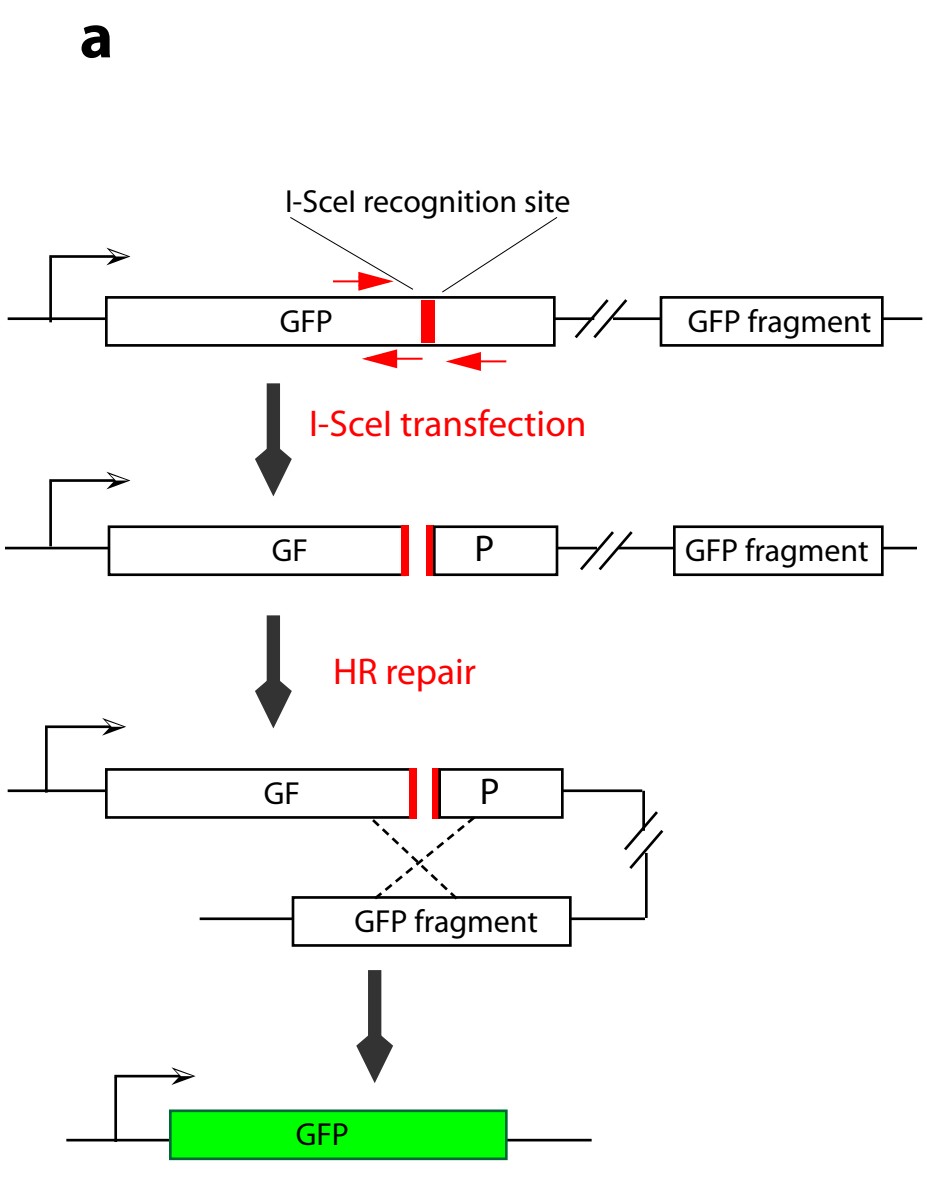
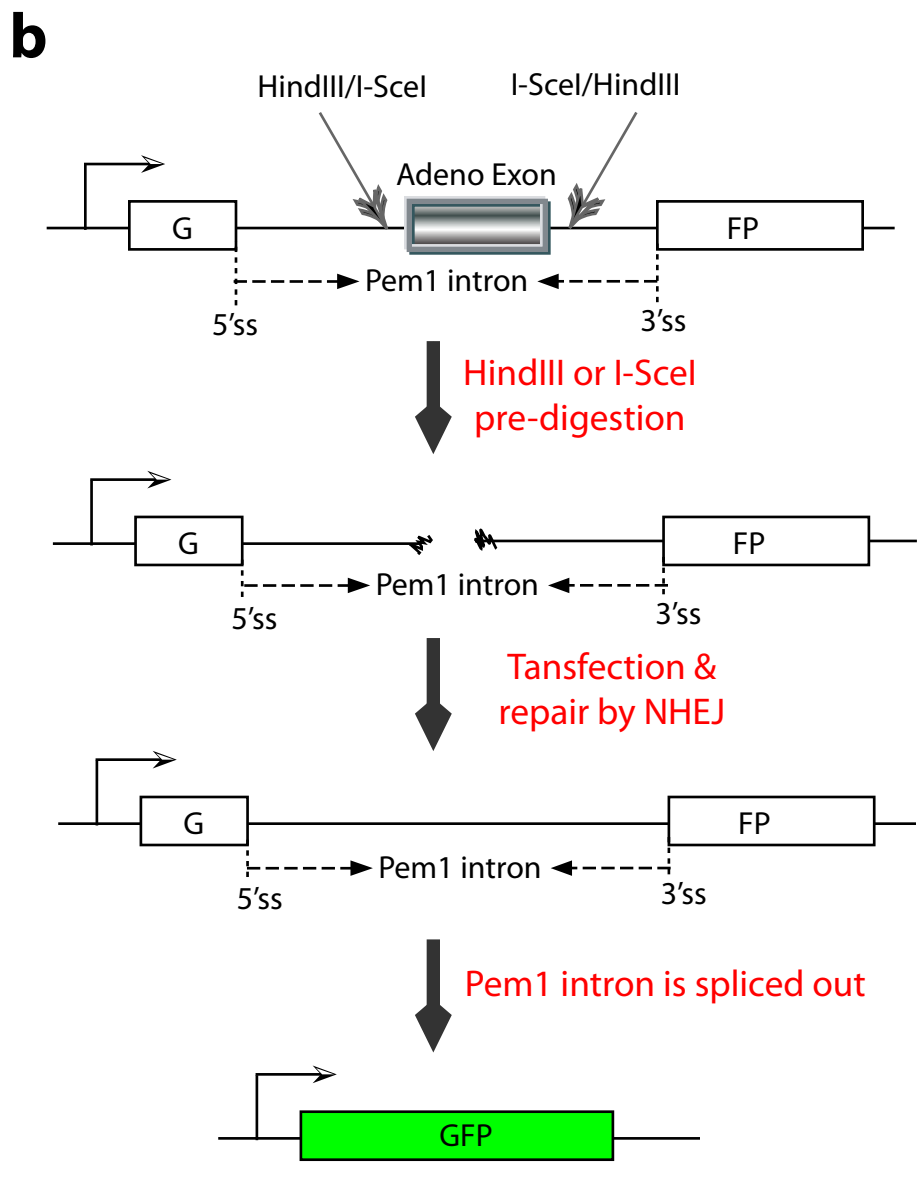
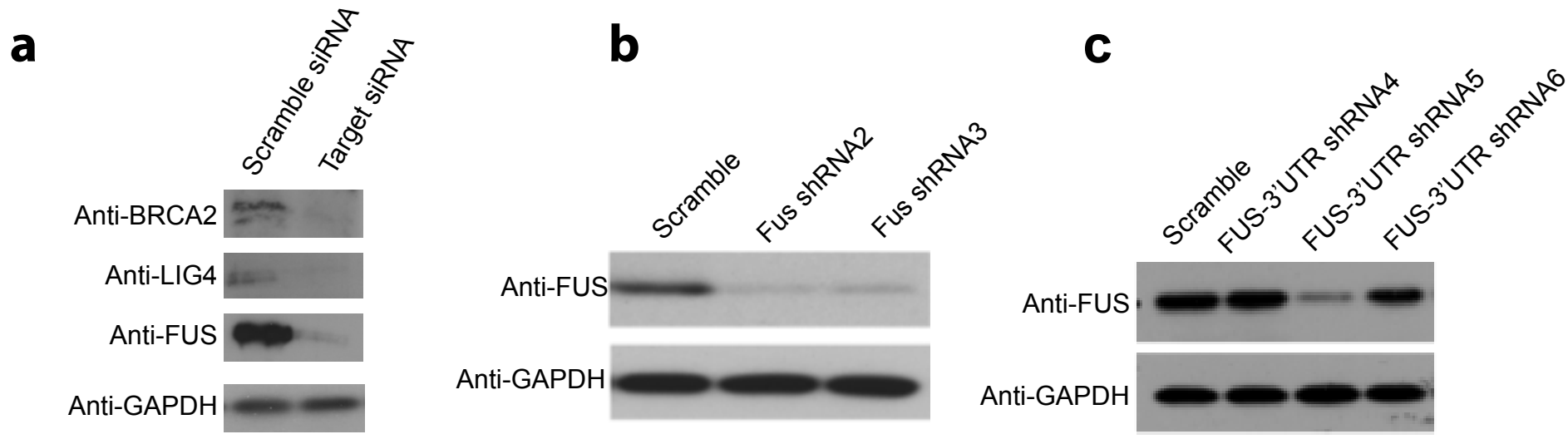
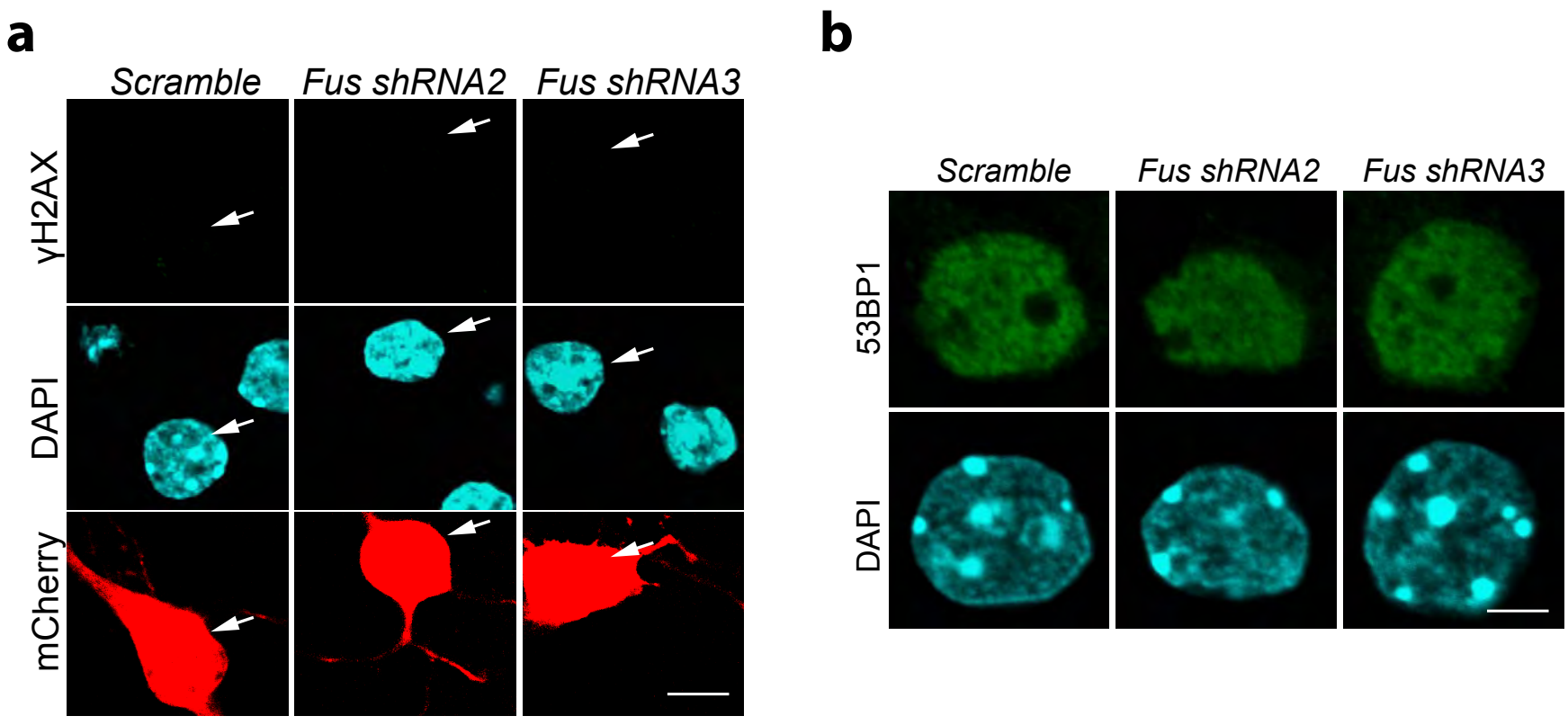




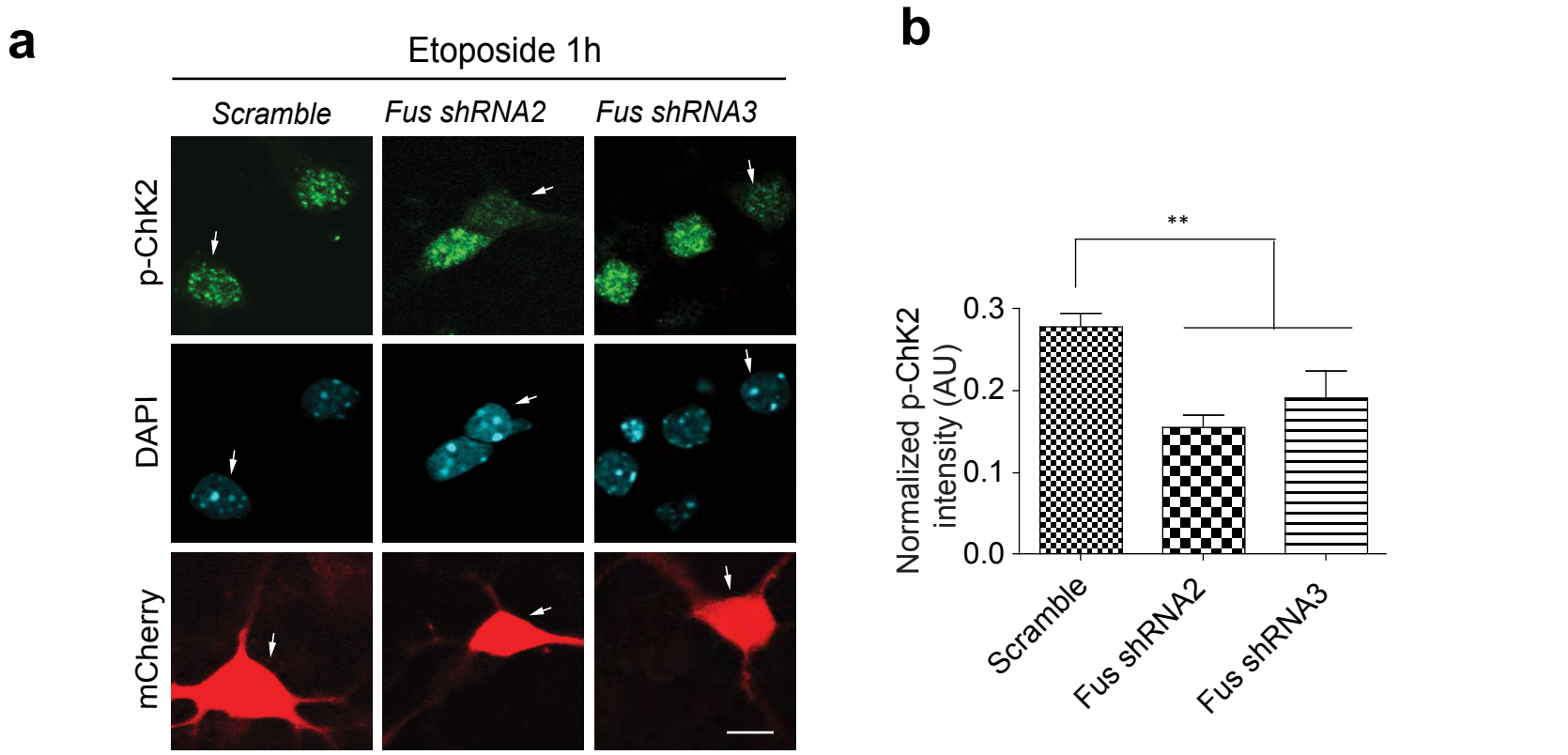
Supplemental figure 1. Schematic figures of HR and NHEJ reporter systems used in this study. (a). The I-SceI mediated DSB induction and repair by HR. A I-SceI endonuclease recognition site was inserted into the GFP coding sequence to create a nonfunctional GFP transgene, and a wild type GFP fragment was inserted downstream of the GFP tansgene. Transfection of I-SceI expressing construct will induce a DSB at the I-SecI site, and repair of this DSB by HR will successfully restore a functional GFP, which coule be detected by fluorescence microscope or by FACS. Arrows in up panel (red) represent the primers used for ChIP-qPCR analysis of the repair proteins accumulted at DSBs. (b). Reporter construct for NHEJ. The GFP coding sequence was interrupted by inserting an adenoviral exon flanked by artifical introns. The GFP is inactive due to the expression of the adeno exon in GFP gene; however, once digested with HindIII or I-SceI, the exon will be cutted out and a DSB will be created. Successful repair of this DSB using NHEJ will restore the expression of GFP. (Adapted from Oberdoerffer et al, cell, 2008; Seluanov et. al. PNAS. 2004).



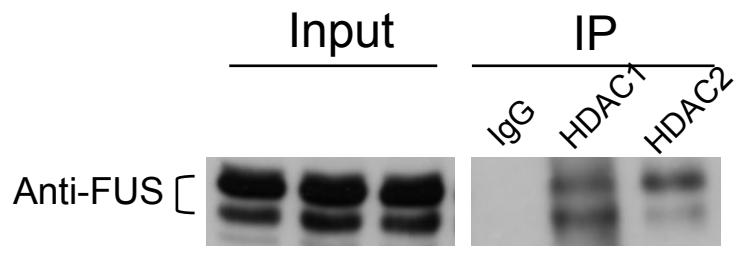
Supplemental Figure 2. Verification of the shRNAs and siRNAs used in this study. (a-c). Different siRNAs targeting human *BRCA2, LIG4, FUS* and shRNAs targeting mouse *Fus* (shRNA2 and 3) or human *FUS* (shRNA 2, 4-6) were transfected into 293T (a,c) cells and N2A cells (b) for 72 hours and cell lysate was collected for western blotting.



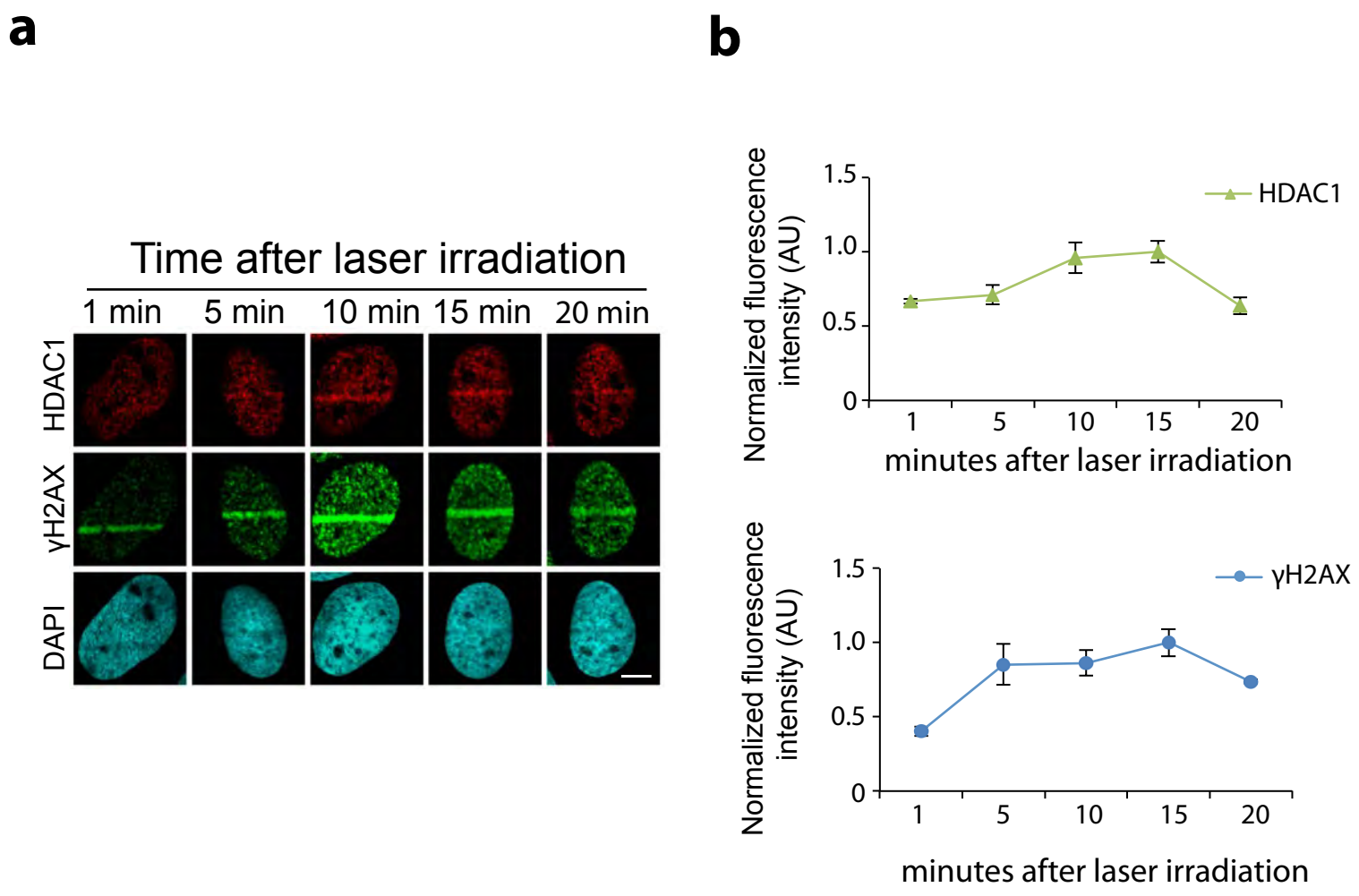
Supplemental Figure 3. Immunofluorescence staining of 53BP1 and γH2AX in vehicle treated neurons following *Fus* knockdown. (a). Primary cortical neurons were transfected with plasmids expressing *scrambled shRNA* or *Fus shRNAs* together with *mCherry*, treated with vechile for 1 hour, fixed and labeled for γH2AX. No γH2AX immunoreactivity was observed in both transfected (white arrows) and non-transfected neurons. scale bar: 8μm (b). Primary cortical neurons were transduced with lentivirus carrying shRNAs targeting *Fus*, or scrambled control shRNA. Neurons were treated with vehicle for 1 h and labeled with anti-53BP1 antibody. 53BP1 is uniformly distributed in the nuclei. Scale bar: 4μm



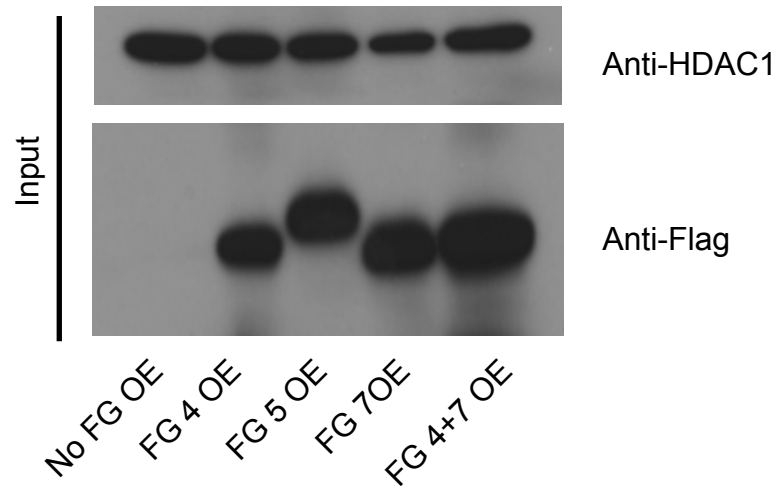
Supplemental Figure 4. Immunofluorescence staining of phospho-ChK2 (p-ChK2) in cultured primary neurons treated with ETO. (a) Primary cortical neurons were transfected with plasmids expressing *scrambled shRNA* or *Fus shRNAs* together with *mCherry*. 5 μM etoposide was added to induce DNA DSBs. Following 1 h etoposide treatment, neurons were fixed and labeled for p-ChK2 immunoreactivity. *Fus* knockdown neurons show reduced p-ChK2 immunoreactivity compared to *scrambled shRNA*-expressing neurons (white arrows). (b) p-ChK2 signal intensity in transfected neurons was measured by ImageJ (NIH). Scale bar: 8um. (** $p<0.01$, one-way ANOVA). (c). Verification of p-ChK2 antibody used for the immunofluorescnence staining.The p-ChK2 antibody (Cell Signaling #2661) detects a major band of approximately 62 kD. When the blot was stripped and re-blotted with ChK2 antibody (Cell Signaling #2662), the same 62 kD species was recognized. The p-ChK2 signal was minimal in vehicle-treated neurons and was enhanced with etoposide treatment, whereas phosphatase treatment completely abolished the signal. Note that the ChK2 antibody (Cell Signaling #2662) can recognize both human and mouse ChK2.



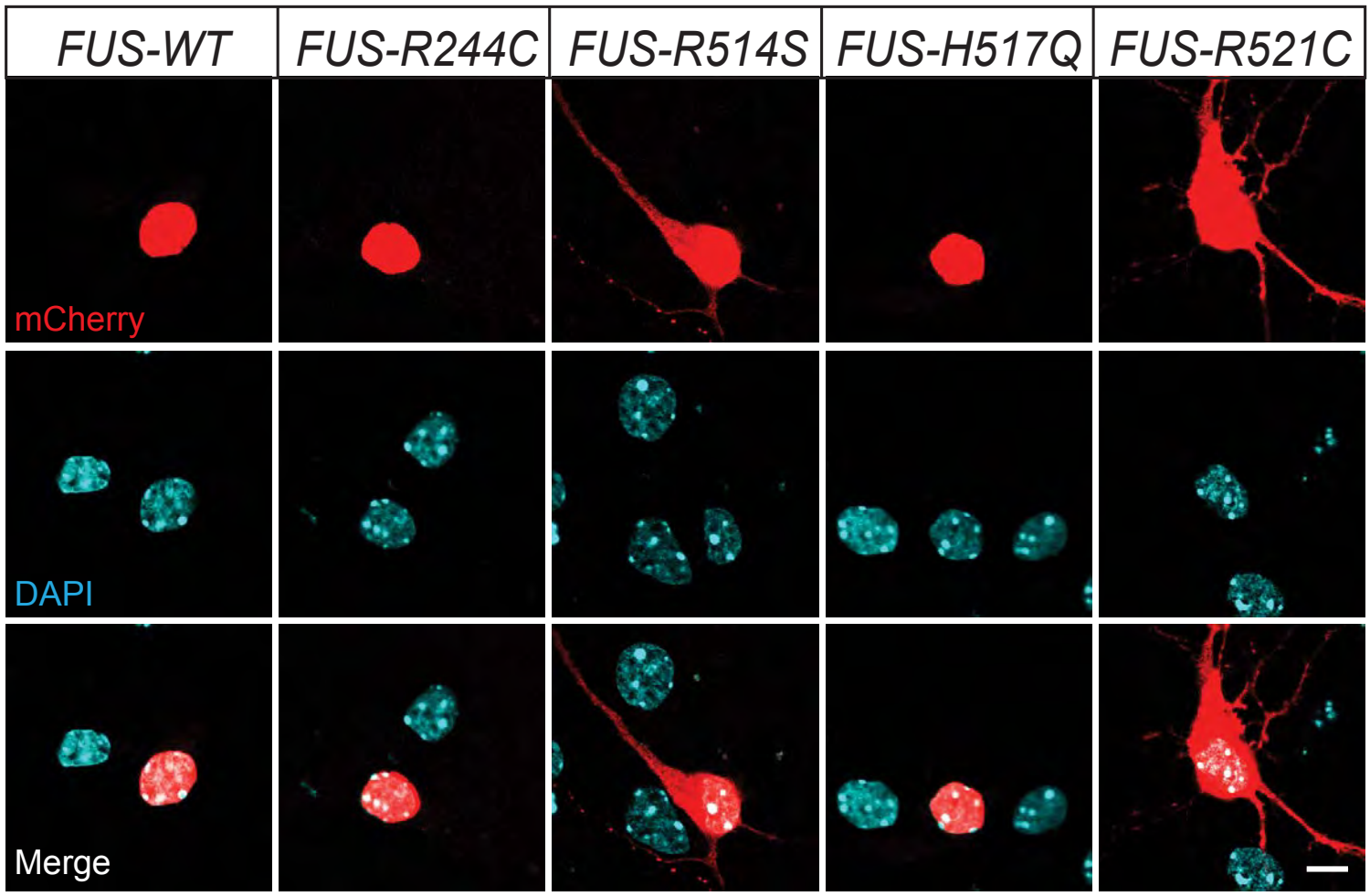
Supplemental Figure 5. *In vivo* interaction between HDAC and FUS. Nuclear extracts prepared from WT mouse hippocampus were immunoprecipitated with antibodies against HDAC1 and HDAC2 and blotted with anti-FUS antibody.

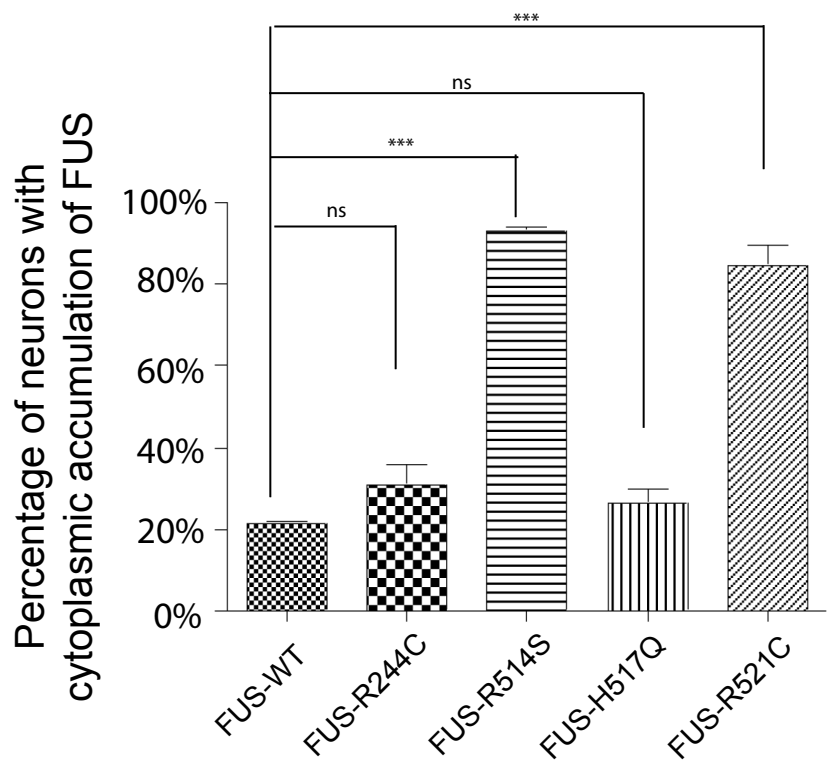


Supplemental Figure 6. Micro-irradiation assay for HDAC1 and γH2AX. (a). Immunolabeling for HDAC1 and γH2AX was conducted at the indicated times following the induction of DSBs via laser micro-irradiation of U2OS cells. Scale bar: 8μm. (b). Fluorescence intensity in the laser-irradiated area at each time points was first normalized to the signal of whole nucleus and then to the peak fluorescence intentsity accross time. (n=5-10)

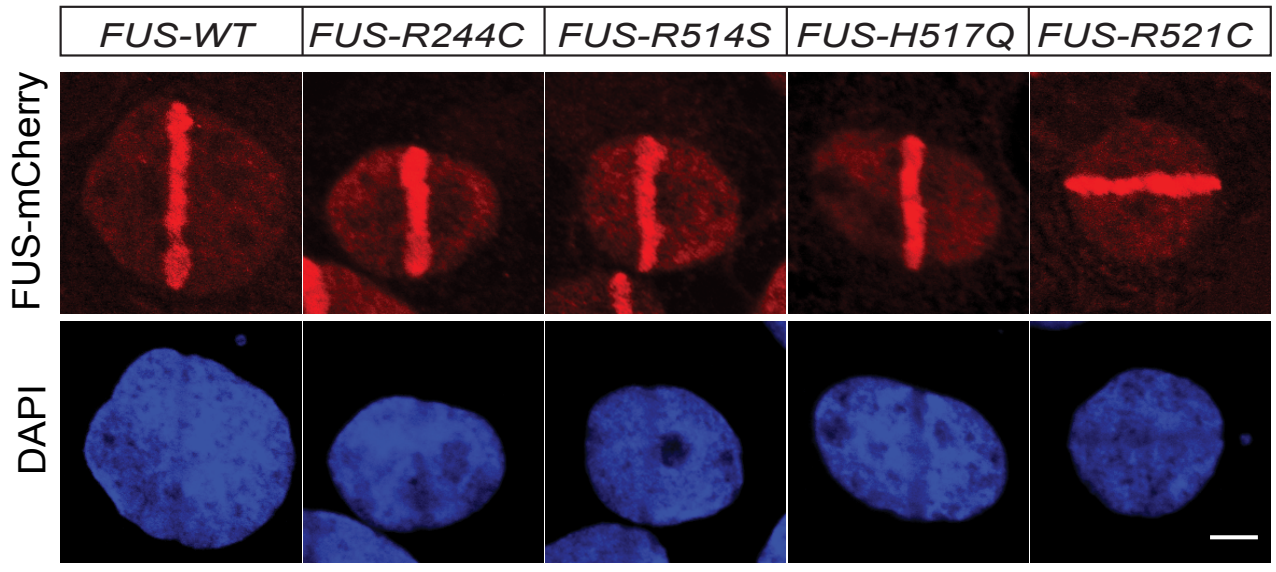


Supplemental Figure 7. Input controls for the experiment depicted in Figure 4b. Flag tagged FUS fragments (FG) 4, 5, 7, or 4+7 were transfected into 293T cells together with *FUS-mCherry* for 48 hr and processed for immunoprecipitation as shown in Figure 4b. This figure shows the input controls for these experiments.





Supplemental Figure 8. Cellular distribution of wild type and mutant FUS. Primary cultured neurons were transfected with *FUS-WT, FUS-R244C, FUS-R514S, FUS-H517Q,* or *FUS-R521C*, and the percentage of the cells showing a cytoplasmic accumulation of FUS was analyzed for each condition (n ≥ 50, ns: no significant difference. ***p<0.001, unpaired t-test). Scale bar: 6μm



Supplemental Figure 9. Laser micro-irradiation assay in U2OS cells expressing wild type FUS (FUS-WT) or fALS FUS mutants. Endogenous FUS was knocked down and replaced by mCherry tagged wild type or mutant FUS. Cells were fixed 10 minues after laser irradiation and processed for immunochemistry with anti-mCherry antibody. Scale bar: 4μm.

Fig. 3a

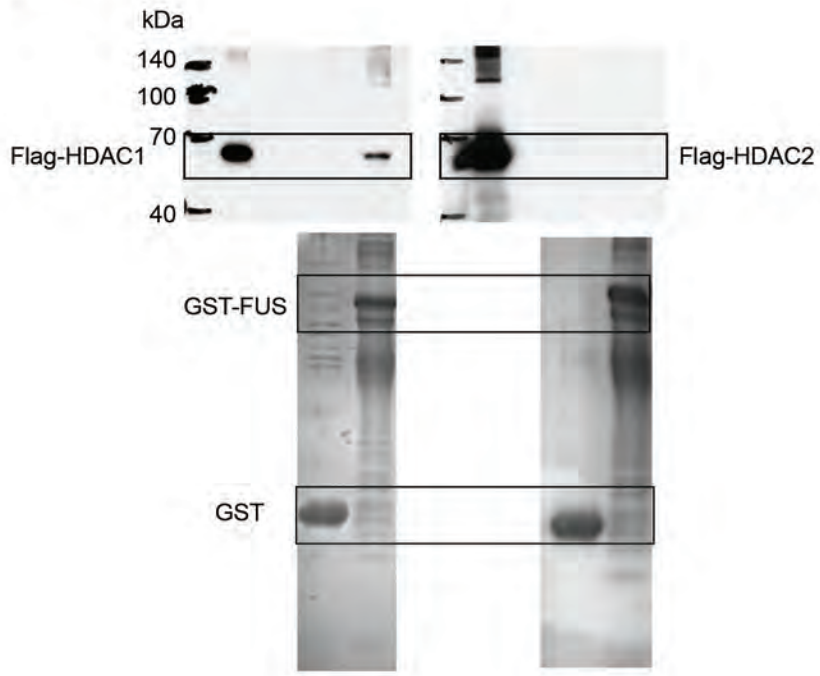


Fig. 3b

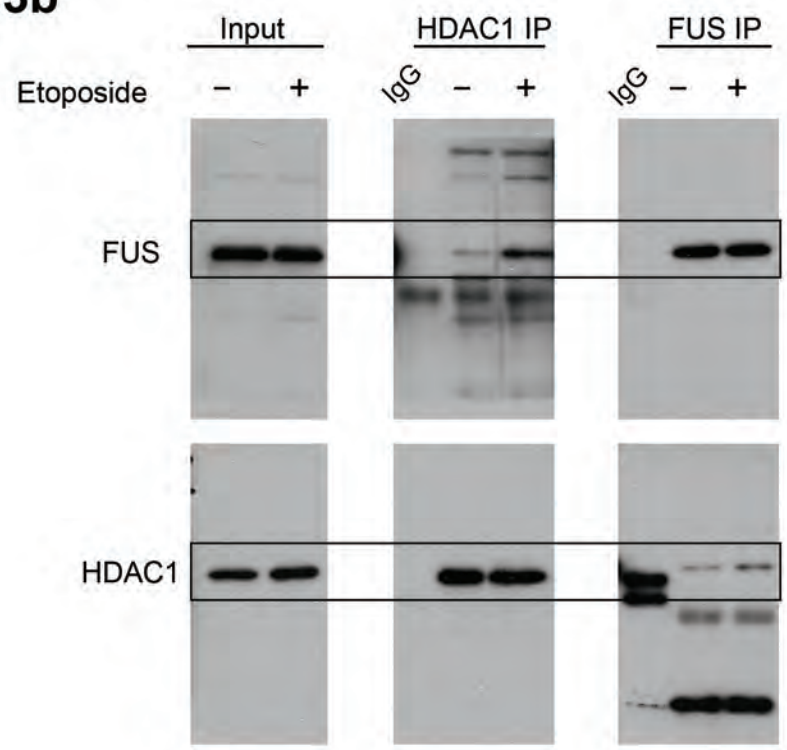


Fig. 4a

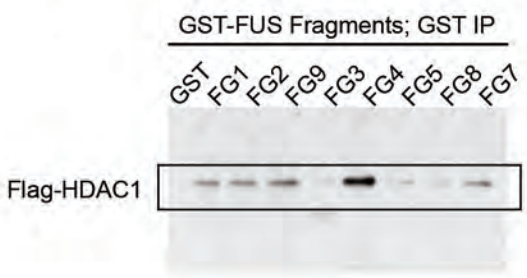


Fig. 4b

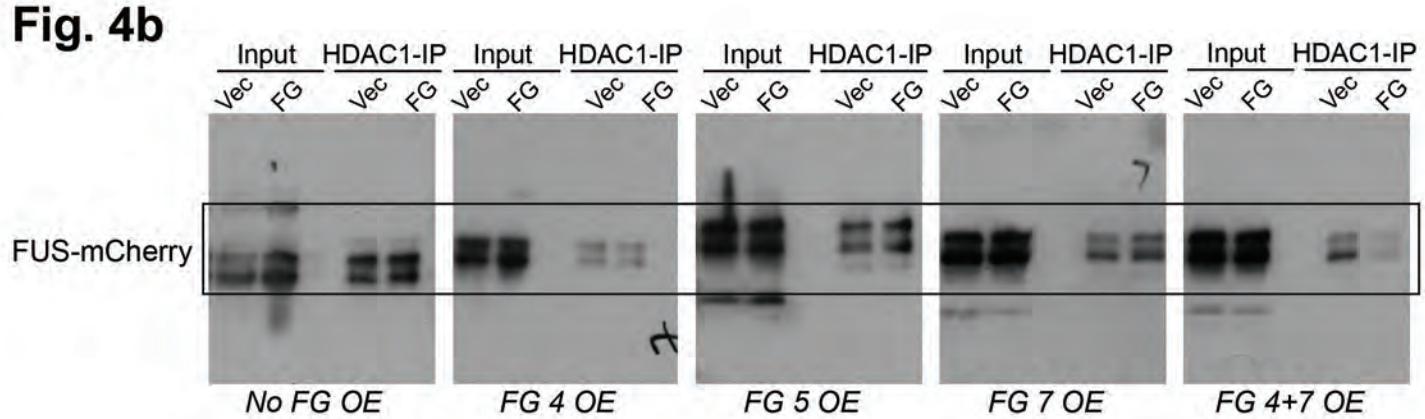


Fig. 5b

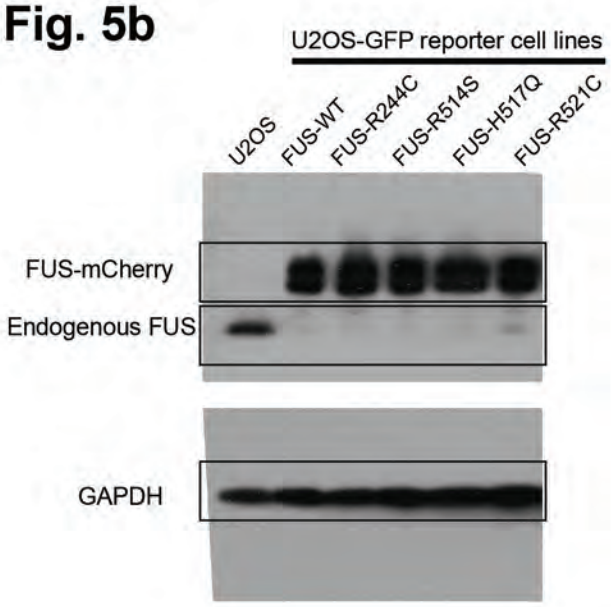


Fig. 5e

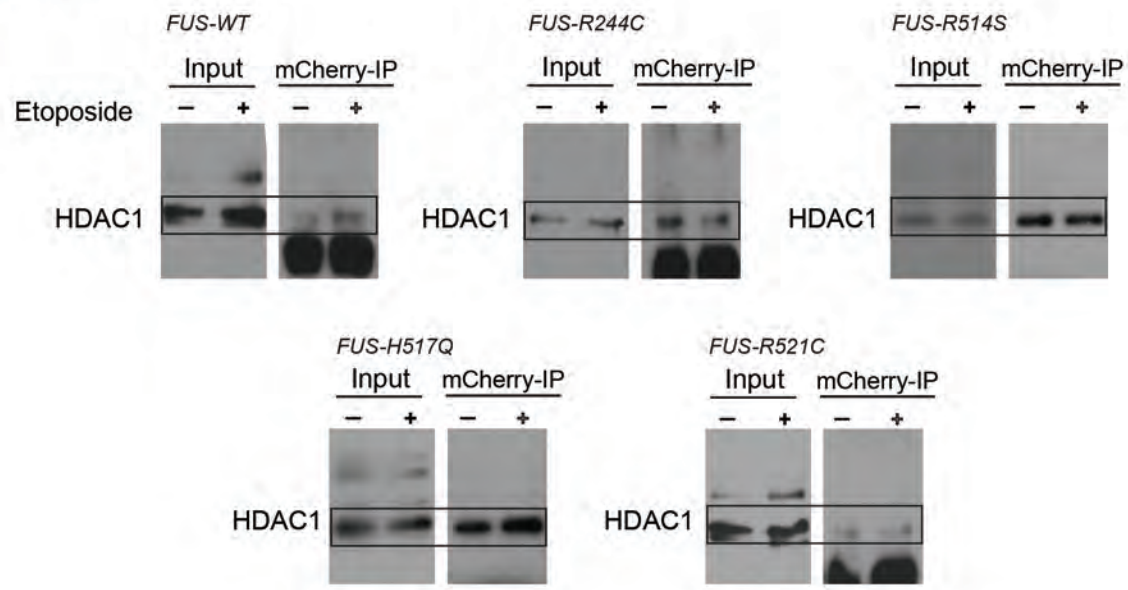
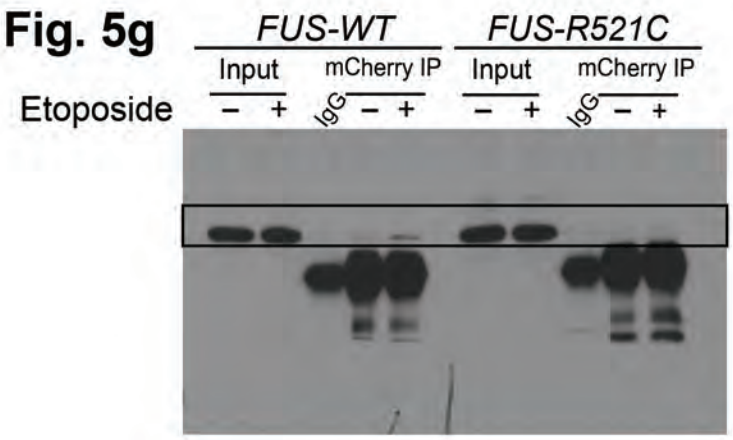


Fig. 5g



Supplemental Figure 10. Full-length pictures of the blots presented in the main figures.

Supplementary Table 1. Human tissue information.

Sample ID	Age	Gender	Diagnosis	FUS Mutation	Regions Examined
Control #1	57	Male	Hepatocellular carcinoma	--	Sensorimotor cortex, spinal cord
Control #2	88	Male	Poorly differentiated carcinoma	--	Sensorimotor cortex, spinal cord
Control #3	80	Male	Systemic amyloidosis	--	Sensorimotor cortex, spinal cord
ALS #1	15	Female	ALS-FUS	FUS-P525L	Senorimotor cortex, spinal cord
ALS #2	62	Female	ALS-FUS	FUS-R521C	Primary motor cortex, spinal cord