SUPPLEMENTARY FIGURES

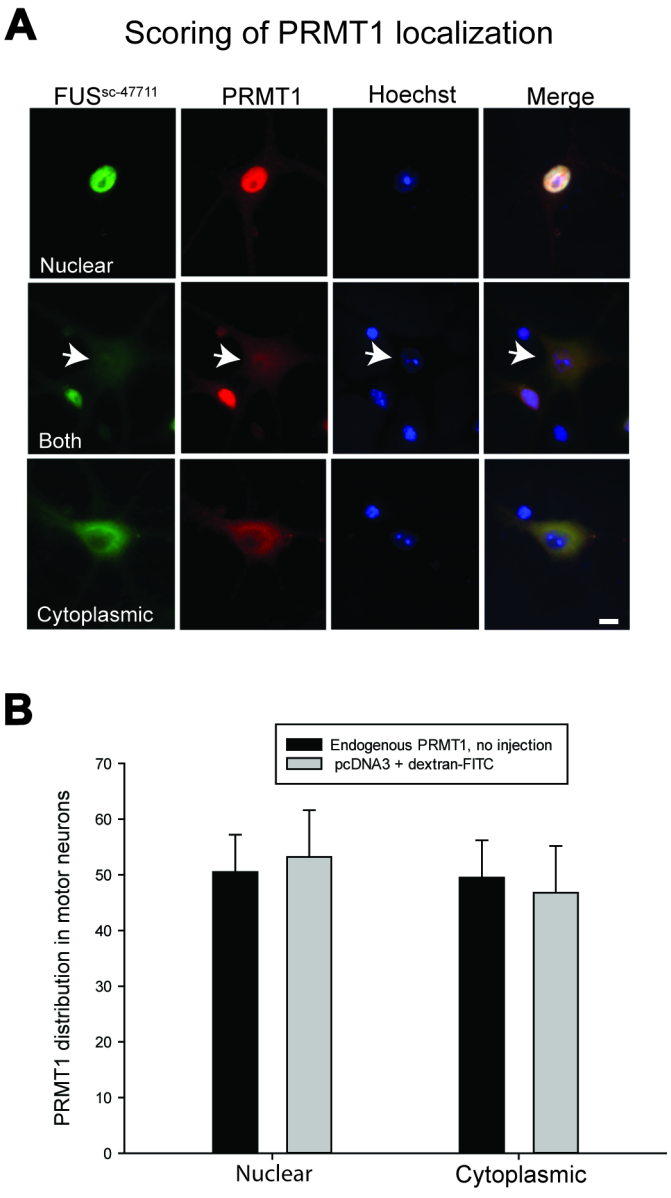
Cytoplasmic sequestration of FUS/TLS associated with ALS alters histone marks through loss of nuclear arginine protein methyltransferase 1

Michael Tibshirani, Miranda L. Tradewell, Katie R. Mattina, Sandra Minotti, Wencheng Yang, Hongru Zhou, Michael J. Strong, Lawrence J. Hayward, Heather D. Durham

Supplementary Material Fig. S1. Localization of PRMT1 in cultured motor neurons.

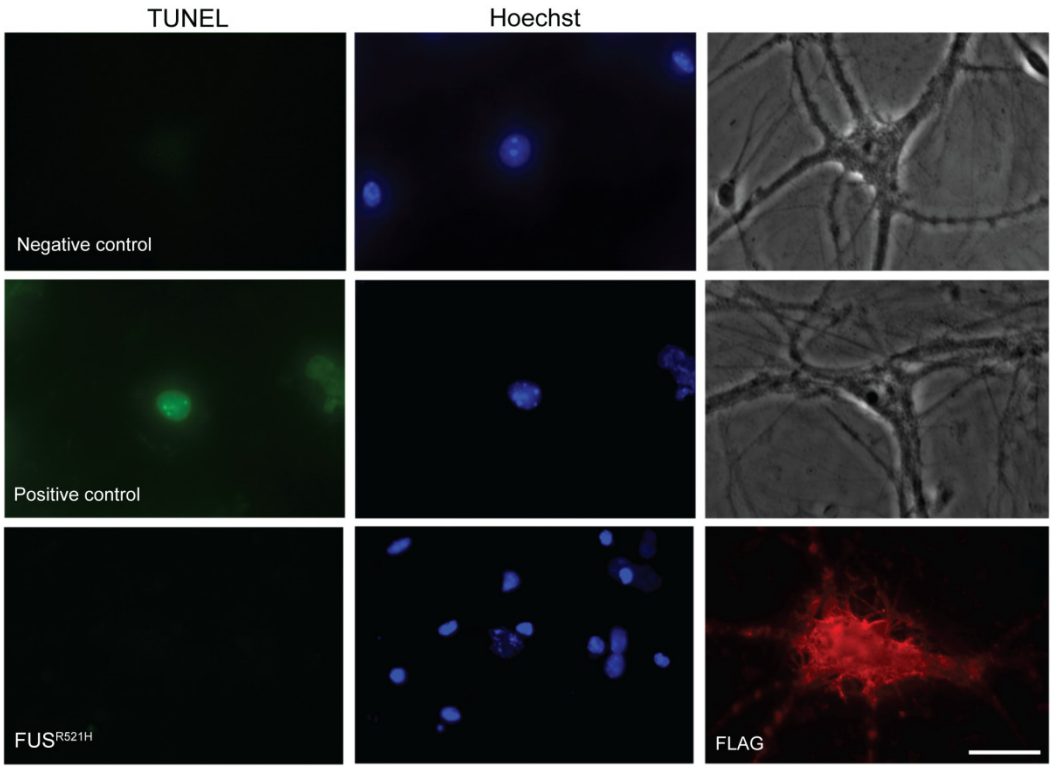
(**A)** Double labeling of cultured neurons with anti-FUS/anti-PRMT1 antibodies. PRMT1 localization was scored as nuclear, both nuclear and cytoplasmic, or cytoplasmic and quantified as presented in Fig. 2. Scale bar = 10 μm.

(**B)** Scoring of PRMT1 localization in uninjected neurons and neurons injected with FITC-Dextran, showing that the microinjection procedure has no effect on PRMT1 localization.



Supplementary Material Fig. S2. Neurons with cytoplasmic FUS and condensed chromatin are not apoptotic.

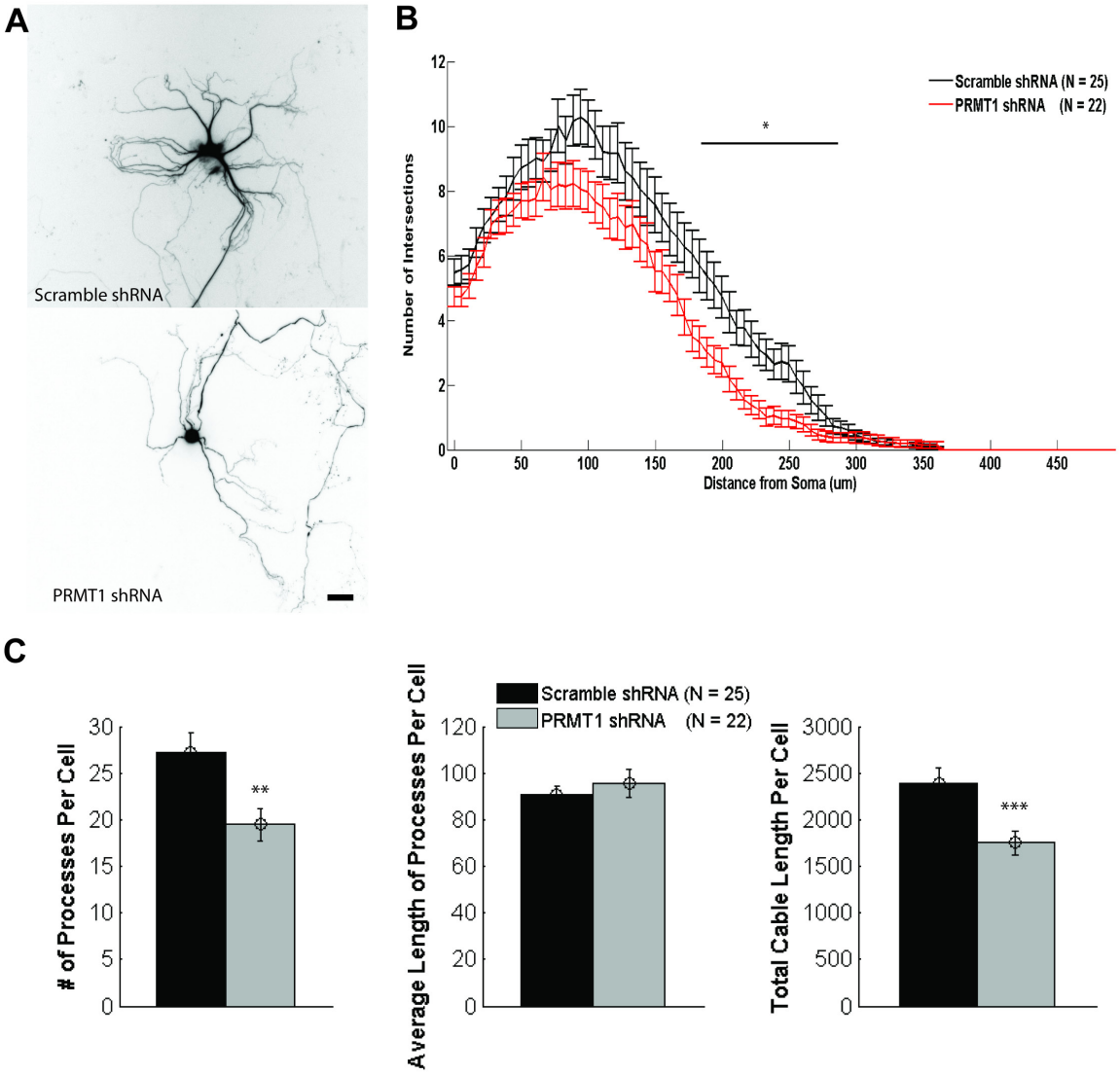
TUNEL assay was performed on neurons injected with FUS^{R521H} plasmid. Neurons with cytoplasmic FUS and condensed chromatin were not positive for TUNEL staining indicating intact DNA. Treatment of cultures with DNAse was used as a positive control; negative control was no treatment. Scale bar = 20 μm.



Supplementary Material Fig. S3. Dendritic attrition is a consequence of PRMT1 knockdown.

Motor neurons were injected with Scramble shRNA or PRMT1 shRNA pool (1) along with mCherry to visualize cell morphology. Measurements of dendritic morphology were performed using Bonfire. Images taken after 3 days using a 10x objective were exported to 8-bit .tif images. Cell body and dendrites were traced using the semi-automated ImageJ tracing plugin, NeuronJ (http://www.imagescience.org/meijering/software/neuronj/). Branching points were designated using NeuroStudio and measurements of dendritic morphology were performed with Bonfire (2) using the resulting .swc file. Significance was determined using a Welch's t-test.

Neurons expressing PRMT1 shRNA showed decreased dendritic branching as indicated by (**A**) mCherry epifluorescence and (**B** and **C**) Sholl analysis compared to scramble shRNA. (B) Sholl curve shows fewer dendritic branches distant from the cell body with PRMT1 knockdown. (**C**) Knockdown of PRMT1 decreased the average number of processes per cell as well as total dendritic output. Asterisks indicate significant difference from scramble shRNA-injected neurons, *p<0.05, **p<0.01, ***p<0.001. Scale bar = 30 µm.



1. Tradewell, M.L., Yu, Z., Tibshirani, M., Boulanger, M.C., Durham, H.D. and Richard, S. (2012) Arginine methylation by PRMT1 regulates nuclear-cytoplasmic localization and toxicity of FUS/TLS harbouring ALS-linked mutations. *Hum. Mol. Genet.*, **21**, 136-149.

2. Langhammer, C.G., Previtera, M.L., Sweet, E.S., Sran, S.S., Chen, M. and Firestein, B.L. (2010) Automated Sholl analysis of digitized neuronal morphology at multiple scales: Whole cell Sholl analysis versus Sholl analysis of arbor subregions. *Cytometry. Part A : the journal of the International Society for Analytical Cytology*, **77**, 1160-1168.

Movie Legends

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Supplemental movies 1 & 2: Long term time-lapse imaging of neuronal cytoplasmic inclusion formation in cultured motor neurons expressing mutant human FUS. Neurons were microinjected with plasmid encoding eGFP-FUS^{R521H} and imaged in an Olympus VivaView incubator microscope on days 4-6 post-injection, every 15 min. Movie S1 shows FUS accumulating in the cytoplasm diffusely until inclusions form suddenly and coalesce into either globular or filamentous inclusions. Movie S2 shows formation of linear inclusions in motor neuron dendrites. Scale bar = 20 μm.