

Supplementary Materials

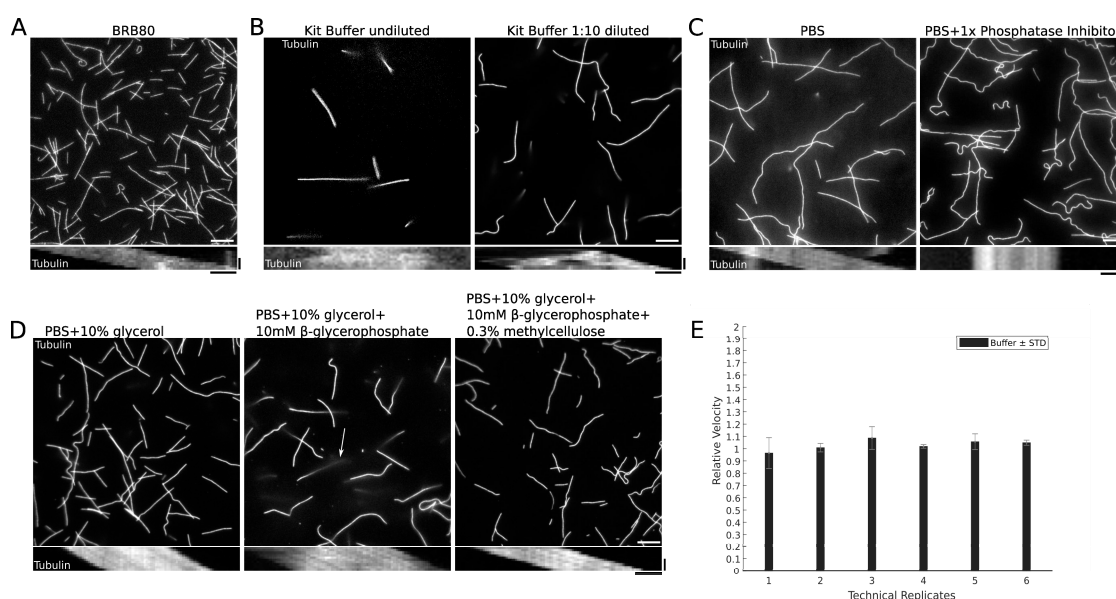


Figure S1. Optimal buffer conditions for protein-enriched, kinesin-1-dependent microtubule gliding motility assays consist of PBS supplemented with 10% glycerol, 10 mM β -glycerophosphate, and 0.3% methylcellulose. Top of each panel: Kinesin-1-dependent gliding of rhodamine-labeled microtubules: (A) in presence of BRB80 alone, (B) at indicated dilutions of whole cell lysis buffer utilized in a Nuclear Extract Kit for whole cell lysates (Active Motif, Waterloo, Belgium), (C) in presence of PBS alone or supplemented with 1x phosphatase inhibitor cocktail (HaltTM, Thermo Fisher Scientific, Waltham, MA, USA), (D) in presence of PBS supplemented with 10% glycerol, additional 10 mM β -glycerophosphate (white arrow marks a detaching microtubule), and in combination with 0.3% methylcellulose. Scale bar = 10 μ m. Bottom of each panel: Representative kymographs showing the kinesin-1-dependent movement of microtubules in the respective conditions. Horizontal scale bar = 2 μ m, vertical = 5 s. (E) Relative kinesin-1-dependent microtubule gliding velocities (see Materials and Methods) of six technical replicates in standard motility buffer.

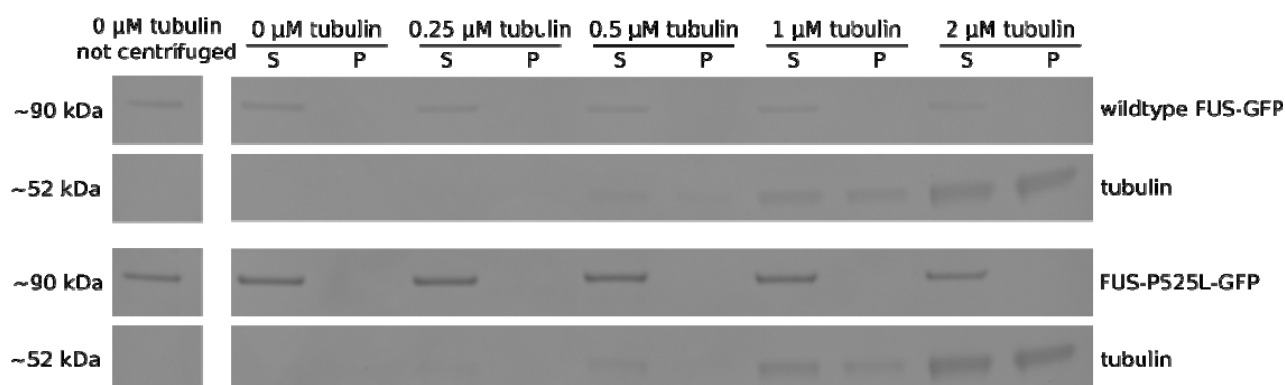


Figure S2. Recombinant wildtype FUS-GFP and FUS-P525L-GFP does not bind to microtubules. Pull-down assay of microtubules at indicated concentrations of tubulin incubated with 500 nM of either recombinant wildtype FUS-GFP or FUS-P525L-GFP.

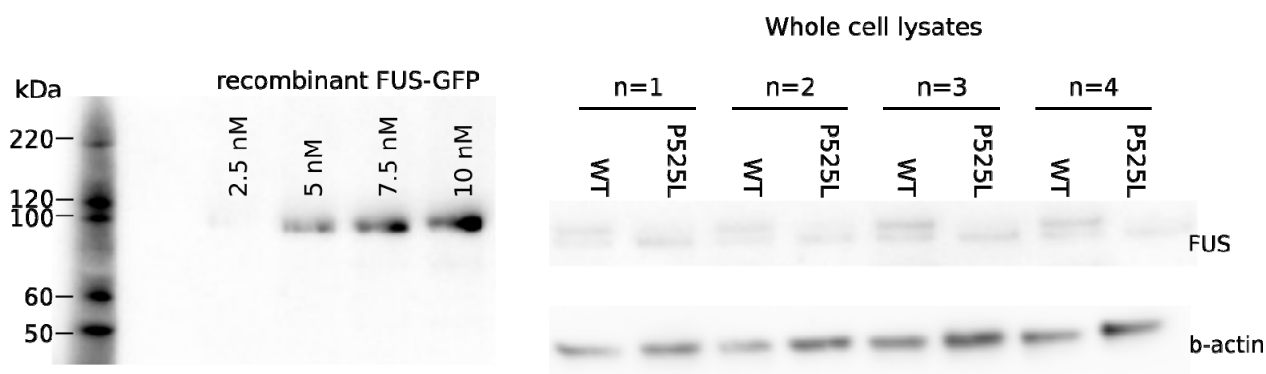


Figure S3. Western blot of FUS-GFP variants expressed in whole cell lysates of spinal motor neurons. Representative western blot of recombinant wildtype FUS-GFP at indicated concentrations and four biological replicates (n) of whole cell lysates obtained from spinal motor neurons expressing either wildtype FUS-GFP or FUS P525L GFP.

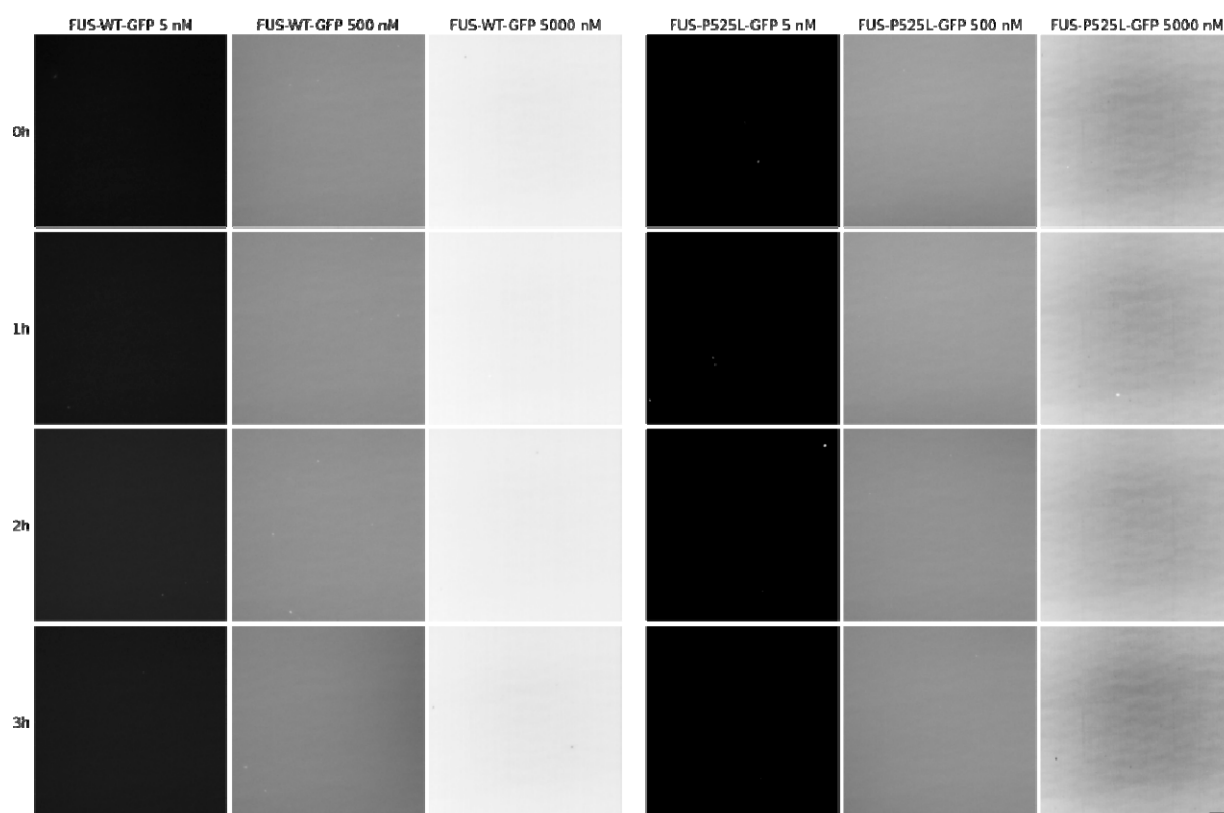


Figure S4. Recombinant wildtype FUS-GFP and FUS-P525L-GFP do not aggregate over the course of three hours in our assay conditions. Free recombinant wildtype FUS-GFP (left three columns) and ALS-associated FUS-P525L-GFP (right three columns) in standard motility buffer at indicated concentrations (left to right) and time points (top to bottom). Scale bar = 10 μ m.

Table S1. Significance values (*p*-values) for kinesin-1-dependent microtubule gliding in the presence of recombinant human wildtype FUS-GFP or FUS-P525L-GFP, standard motility buffer or protein control (BSA) over the course of three hours at indicated concentrations and time points (referring to Figures 3).

5 nM	FUS-WT-GFP	FUS-P525L-GFP	BSA	Time
Buffer	0.9991	0.9885	0.9502	0 h
FUS-WT-GFP	-	0.9977	0.9079	
FUS-P525L-GFP	-	-	0.8285	
Buffer	>0.9999	>0.9999	>0.9999	1 h
FUS-WT-GFP	-	>0.9999	>0.9999	
FUS-P525L-GFP	-	-	>0.9999	
Buffer	>0.9999	0.9985	0.9976	2 h
FUS-WT-GFP	-	0.9131	0.9024	
FUS-P525L-GFP	-	-	>0.9999	
Buffer	>0.9999	0.9975	0.9933	3 h
FUS-WT-GFP	-	0.9968	0.9928	
FUS-P525L-GFP	-	-	>0.9999	
500 nM	FUS-WT-GFP	FUS-P525L-GFP	BSA	Time
Buffer	0.3289	0.0135	>0.9999	0 h
FUS-WT-GFP	-	0.4325	0.2927	
FUS-P525L-GFP	-	-	0.0111	
Buffer	0.1702	0.0543	>0.9999	1 h
FUS-WT-GFP	-	>0.9999	>0.9999	
FUS-P525L-GFP	-	-	0.5244	
Buffer	0.2112	0.1338	0.8306	2 h
FUS-WT-GFP	-	0.9952	0.7023	
FUS-P525L-GFP	-	-	0.5587	
Buffer	0.1695	0.3686	0.871	3 h
FUS-WT-GFP	-	0.9684	0.469	
FUS-P525L-GFP	-	-	0.7612	
5000 nM	FUS-WT-GFP	FUS-P525L-GFP	BSA	Time
Buffer	0.1765	0.5127	0.9899	0 h
FUS-WT-GFP	-	0.8327	0.0971	
FUS-P525L-GFP	-	-	0.3302	
Buffer	0.0162	>0.9999	>0.9999	1 h
FUS-WT-GFP	-	0.3011	0.016	
FUS-P525L-GFP	-	-	0.4378	
Buffer	0.0465	0.3882	0.9997	2 h
FUS-WT-GFP	-	0.6555	0.0375	
FUS-P525L-GFP	-	-	0.3377	
Buffer	0.0449	0.3301	0.9964	3 h
FUS-WT-GFP	-	0.6869	0.0691	
FUS-P525L-GFP	-	-	0.4408	

Table S2. Significance values (*p*-values) for kinesin-1-dependent microtubule gliding in the presence of recombinant human 2N4R tau-GFP at indicated concentrations (referring to Figure 2).

Tau-GFP	1 nM	5 nM	10 nM	15 nM	25 nM	50 nM	75 nM	100 nM
0 nM	>0.9999	>0.9999	>0.9999	0.8786	0.016	0.0005	0.0004	0.0004
1 nM	-	>0.9999	>0.9999	0.909	0.0189	0.0005	0.0005	0.0005
5 nM	-	-	>0.9999	0.9537	0.0258	0.0007	0.0007	0.0007
10 nM	-	-	-	0.9828	0.0366	0.001	0.001	0.001
15 nM	-	-	-	0.9828	0.2066	0.0067	0.0063	0.0062
25 nM	-	-	-	-	-	0.6482	0.6255	0.6216
50 nM	-	-	-	-	-	-	>0.9999	>0.9999
75 nM	-	-	-	-	-	-	-	>0.9999

Table S3. Significance values (*p*-values) for kinesin-1-dependent microtubule gliding in the presence of neural cell lysates of wildtype FUS-GFP or FUS-P525L-GFP cell lines, standard motility buffer or protein control (BSA) at indicated concentrations (referring to Figure 4B).

	FUS-WT-GFP	FUS-P525L-GFP	BSA	Concentration
Buffer	0.0691	0.0544	0.3116	50 ng/μL
FUS-WT-GFP	-	0.9992	0.8558	
FUS-P525L-GFP	-	-	0.7956	
Buffer	0.9973	0.3495	0.48	80 ng/μL
FUS-WT-GFP	-	0.2831	0.4451	
FUS-P525L-GFP	-	-	0.998	
Buffer	0.827	0.5112	0.1648	110 ng/μL
FUS-WT-GFP	-	0.9117	0.4083	
FUS-P525L-GFP	-	-	0.7726	
Buffer	0.9889	0.5873	0.5245	140 ng/μL
FUS-WT-GFP	-	0.6499	0.5884	
FUS-P525L-GFP	-	-	0.9891	

Table S4. Significance values (*p*-values) for kinesin-1-dependent microtubule gliding in the presence neural cell lysates of wildtype FUS-GFP or FUS-P525L-GFP cell lines, standard motility buffer or protein control (BSA) at a concentration of 80 ng/μL supplemented with 2N4R tau-GFP and the indicated concentrations (referring to Figure 4C).

	Tau-GFP	FUS-WT-GFP	FUS-P525L-GFP	BSA	Concentration of tau-GFP
Buffer	>0.9999	0.9876	0.9974	>0.9999	0 nM
Tau-GFP	-	0.9544	0.9895	>0.9999	
FUS-WT-GFP	-	-	0.9993	0.9279	
FUS-P525L-GFP	-	-	-	0.9786	
Buffer	>0.9999	0.967	0.9748	>0.9999	1 nM
Tau-GFP	-	0.9223	0.9416	0.9991	
FUS-WT-GFP	-	-	>0.9999	0.8187	
FUS-P525L-GFP	-	-	-	0.8505	
Buffer	0.9997	0.8977	0.9003	>0.9999	5 nM
Tau-GFP	-	0.858	0.8625	0.9989	
FUS-WT-GFP	-	-	>0.9999	0.719	
FUS-P525L-GFP	-	-	-	0.725	
Buffer	0.997	0.2203	0.3247	0.9987	10 nM
Tau-GFP	-	0.0966	0.1882	>0.9999	
FUS-WT-GFP	-	-	0.9978	0.0775	
FUS-P525L-GFP	-	-	-	0.1555	
Buffer	0.7365	0.1383	0.0462	0.977	15 nM
Tau-GFP	-	0.4992	0.1674	0.8932	

FUS-WT-GFP	-	-	0.9636	0.0992	
FUS-P525L-GFP	-	-	-	0.0182	
Buffer	0.0002	<0.0001	0.0029	0.0002	
Tau-GFP	-	0.9991	0.7958	>0.9999	25 nM
FUS-WT-GFP	-	-	0.6478	0.9995	
FUS-P525L-GFP	-	-	-	0.776	
Buffer	<0.0001	<0.0001	<0.0001	<0.0001	
Tau-GFP	-	>0.9999	>0.9999	>0.9999	50 nM
FUS-WT-GFP	-	-	>0.9999	>0.9999	
FUS-P525L-GFP	-	-	-	>0.9999	
Buffer	<0.0001	<0.0001	<0.0001	<0.0001	
Tau-GFP	-	>0.9999	>0.9999	>0.9999	75 nM
FUS-WT-GFP	-	-	>0.9999	>0.9999	
FUS-P525L-GFP	-	-	-	>0.9999	
Buffer	<0.0001	<0.0001	<0.0001	<0.0001	
Tau-GFP	-	>0.9999	>0.9999	>0.9999	100 nM
FUS-WT-GFP	-	-	>0.9999	>0.9999	
FUS-P525L-GFP	-	-	-	>0.9999	