

ALS Associated Mutations in Matrin 3 Alter Protein-Protein Interactions and Impede mRNA Nuclear Export

Authors: Ashley Boehringer^{1,2}, Krystine Garcia-Mansfield³, Gurkaran Singh^{1,2}, Nadine Bakkar¹, Patrick Pirrotte^{3,4}, Robert Bowser^{1,4*}

Affiliations:

¹Department of Neurobiology, Barrow Neurological Institute, Phoenix, AZ, USA.

²School of Life Sciences, Arizona State University, Phoenix, AZ, USA.

³Collaborative Center for Translational Mass Spectrometry, Translational Genomics Research Institute, Phoenix, AZ, USA

⁴These authors jointly directed this work

*To whom correspondence should be addressed: Robert Bowser, Department of Neurobiology, Barrow Neurological Institute, 350 W Thomas Road, Phoenix, AZ 85213.

Email: Robert.bowser@dignityhealth.org.

Supplemental Table 1:

Accession	Gene	Wild Type			Ser85Cys Mutant			Phe115Cys Mutant			Pro154Ser Mutant			Thr622Ala Mutant		
		AvgP	SAINT	Manual	AvgP	SAINT	Manual	AvgP	SAINT	Manual	AvgP	SAINT	Manual	AvgP	SAINT	Manual
Q60668	Hnrnpd		+++	+++	1	+++	+++	1	+++	+++	1	+++	+++	1	+++	+++
Q61029-3	Tmpo	1	++	+++	1	+++	+++				0.96	++	+++			
P62270	Rps18	1	+++	+++				1	+++	+++	1	++	+++	1	++	+++
P62301	Rps13	1	+++	+++					++	+++	0.98	++	+++	0.99	++	+++
P84228	Hist1h3b	0.99	++	+++				0.96	++	+++						
O08583	Alyref	0.98	+	+												
Q9D1J3	Samp	0.97	++	+++		+	+++		++	+++	1	++	+++	0.98	++	+++
Q9QXS1-7	Plec	0.97	++	+++		++	+++									+++
Q9D554	Sf3a3	0.87	+	+								+				
P57784	Snrpa1	0.85	+	+					+	+		+				
Q8CH18	Ccar1	0.84	+	+	0.74	+	+	0.88	+	+		+			+	
P62281	Rps11	0.73	+	+	0.96	++	+	0.9	++	+		+	+	0.87	+	+
P08775	Polr2a	0.71	+	+					+	+	0.74	+	+		+	+
Q8BG05	Hnrnpa3			+	1	+	+	1	+	+			+			+
P05213	Tuba1b				1	+++	+++								++	+++
P99024	Tubb5				1	+++	+++									
Q91YR7	Prpf6				0.96	+	+		+		0.87	+	+			
Q9Z1N5	Ddx39b			+	0.95	+	+		+	+		+	+			+
O35691	Pnn				0.9	+	+									
Q9D0E1	Hnrmpm				0.88	+	+									+
Q99M28	Rnps1		+	+	0.85	+	+		+	+		+	+	0.82	+	+
P14873	Map1b				0.73	+	+		+	+	0.72	+	+	0.98	+	+
Q6NZF1	Zc3h11a		++	+++				1	++	+++	1	+++	+++	1	+++	+++
P61358	Rpl27		+	+++				0.98	++	+++		++	+++	0.96	++	+++
P68134	Acta1							0.98	++	+++						
A2BDX3	Mocs3					++	+++	0.98	++	+++		++	+++			
P62751	Rpl23a					++	+++	0.95	++	+++				0.96	++	+++
Q9JHJ0	Tmod3		++	+++				0.95	++	+++						
Q9DBG3	Ap2b1		+					0.9	+	+	0.86	++	+		+	
Q8VHZ7	Imp4		+					0.7	+					0.86	+	+
Q8BG81	Poldip3		+	+		+	+		+	+	1	+	+	0.71	+	+
Q9Z1X4-2	Ilf3										1	++	+++	1	+++	+++
Q9DB96	Ngdn								++	+++	0.98	++	+++	0.98	++	+++
P62137	Ppp1ca		++	+++					+	+++	0.98	++	+++		+	+++
Q8K4P0	Wdr33										0.98	++	+++		+	+++
P62911	Rpl32										0.96	++	+++	1	++	+++
P62267	Rps23								++	+++	0.96	++	+++	0.96	++	+++
A2AIV2	Kiaa1429										0.96	++	+++			
Q8BL97-4	Srsf7													1	+++	+++
P20152	Vim					+++	+++							1	++	+++
Q6P5B0	Rrp12		+	+++					++	+++				0.98	++	+++
Q8CGP2	Hist1h2bp													0.98	++	+++
P62754	Rps6					+	+++							0.98	++	+++
Q9CZU3	Skiv2l2		+	+++					++	+++		++	+++	0.96	++	+++
Q8BMK4	Ckap4								+	+++				0.96	++	+++
Q8K4L0	Ddx54								++	+++		+	+++	0.96	++	+++
Q07646	Mest													0.96	++	+++
P62849	Rps24								++	+++		+	+++	0.96	++	+++
Q8C4J7	Tbl3								++	+++				0.96	++	+++
Q501J6	Ddx17								+			+		0.94	+	+
Q8CH25	Sltm								+	+		+	+	0.86	+	+
Q921Y2	Imp3													0.75	+	
P14869	Rplp0		+						+			+	+	0.7	+	+
P02535	Krt10		+						+	+						
P56959	Fus		+													
Q8CFQ3	Aqr		+													
Q35326	Srsf5		+	+		+	+		+	+		+	+		+	+
Q9WV55	Vapa		+												+	+
Q91VJ5	Pqbp1		+						+							
O70503	Hsd17b12		+			+	+		+						+	+
Q9DAW6	Prpf4		+			+										
Q8QZY9	Sf3b4		+	+		+										
Q8BTI8	Srrm2		+			+						+				
Q6NV83	U2surp		+	+		+			+	+		+	+++			
Q9D287	Bcas2		+	+++												
P43276	Hist1h1b		+	+++		+	+++									
P04104	Krt1		+	+++		++	+++									
Q99PL5	Rrbp1		+	+++		+	+++								++	+++
Q9CQI7	Snrpb2		+	+++		+	+++		+	+++						
Q9QX47	Son		+	+++												
Q91VR2	Atp5c1		+	+++											+	+++
Q8BMS1	Hadha		+	+++												
Q91YE7	Rbm5		+	+++		+	+++		++	+++		++	+++			

P51881	Slc25a5			+			+												
Q3UKJ7	Smu1					++	+++												
P27048	Snrpb						+++												
P62315	Snrpd1								+	+++									
P15508	Sptb								+	+++									
P47758	Srpb																++	+++	
Q9D0B0	Srsf9																	+	
Q08943	Ssrp1					++	+++		++	+++									
Q9WTS6	Tenm3					+	+++												
Q9ERA6	Tip11								+	+									
B1AZI6	Thoc2								+	+++							+	+++	
Q62318	Trim28																		
P26369	U2af2			+															
Q6EJB6	Utp14b					++	+++		++	+++			+	+++					
Q5SSI6	Utp18								+									+	+
Q9J13	Utp3								+										
Q9DCD2	Xab2																	+	
Q8BJ05	Zc3h14					+	+++												
O88532	Zfr																	+	+++

Supplemental Table 1: High and medium confidence protein interactors of Matrin-3 wild-type, Ser85Cys mutant, Phe115Cys mutant, Pro154Ser mutant and Thr622Ala mutant. Plus signs signify a fold change over empty vector of ≤ 10 (+), 10-50 (++) or ≥ 50 (+++). Medium confidence protein interactors were defined as those identified in two out of three replicates and with a fold change ≥ 2.5 over empty vector in the manual analysis, or a fold change ≥ 2.5 over empty vector and an avgP ≥ 0 in the SAINTexpress analysis. High confidence interactors were those with a fold change ≥ 2.5 over empty vector in both the SAINTexpress analysis and the manual analysis along with a SAINTexpress avgP value ≥ 0.7 .

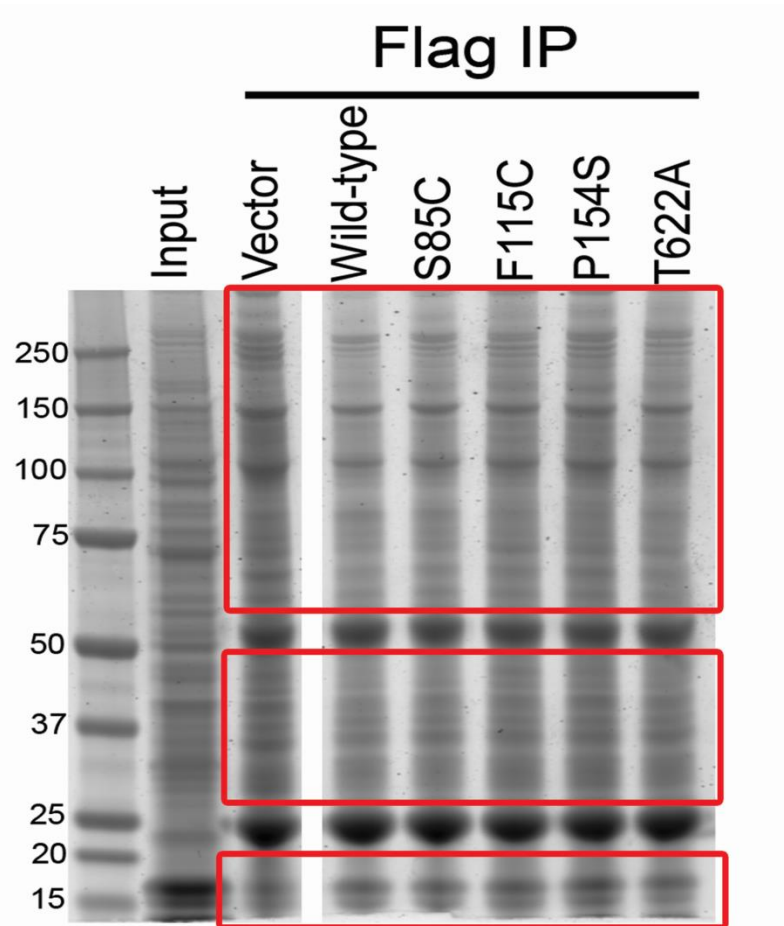
Supplemental Table 2:

Case ID	Gender	Age	PMI (hrs)
CON1	F	74	3
CON2	M	81	17
CON3	F	57	32
ALS1	M	72	3
ALS2	M	39	5.5
ALS3	M	83	21

Supplemental Table 2: Patient Demographics of lumbar spinal cord tissues used in the study..

PMI = post-mortem interval;. All diagnoses were performed by licensed neuropathologists.

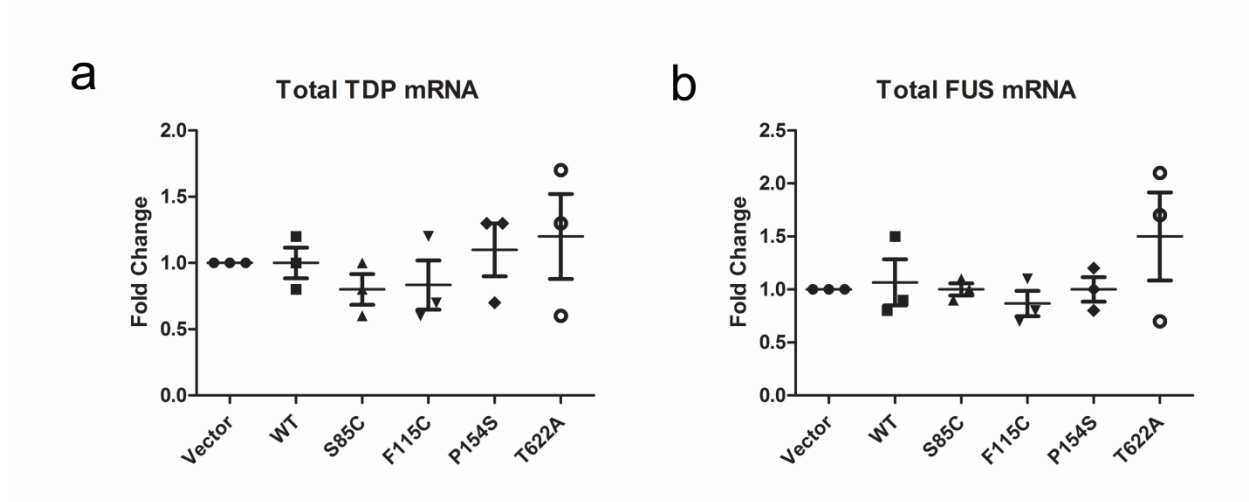
Supplemental Figure 1



SupplementalFigure 1: Representative image of Coomassie stained gel after IP pull-down.

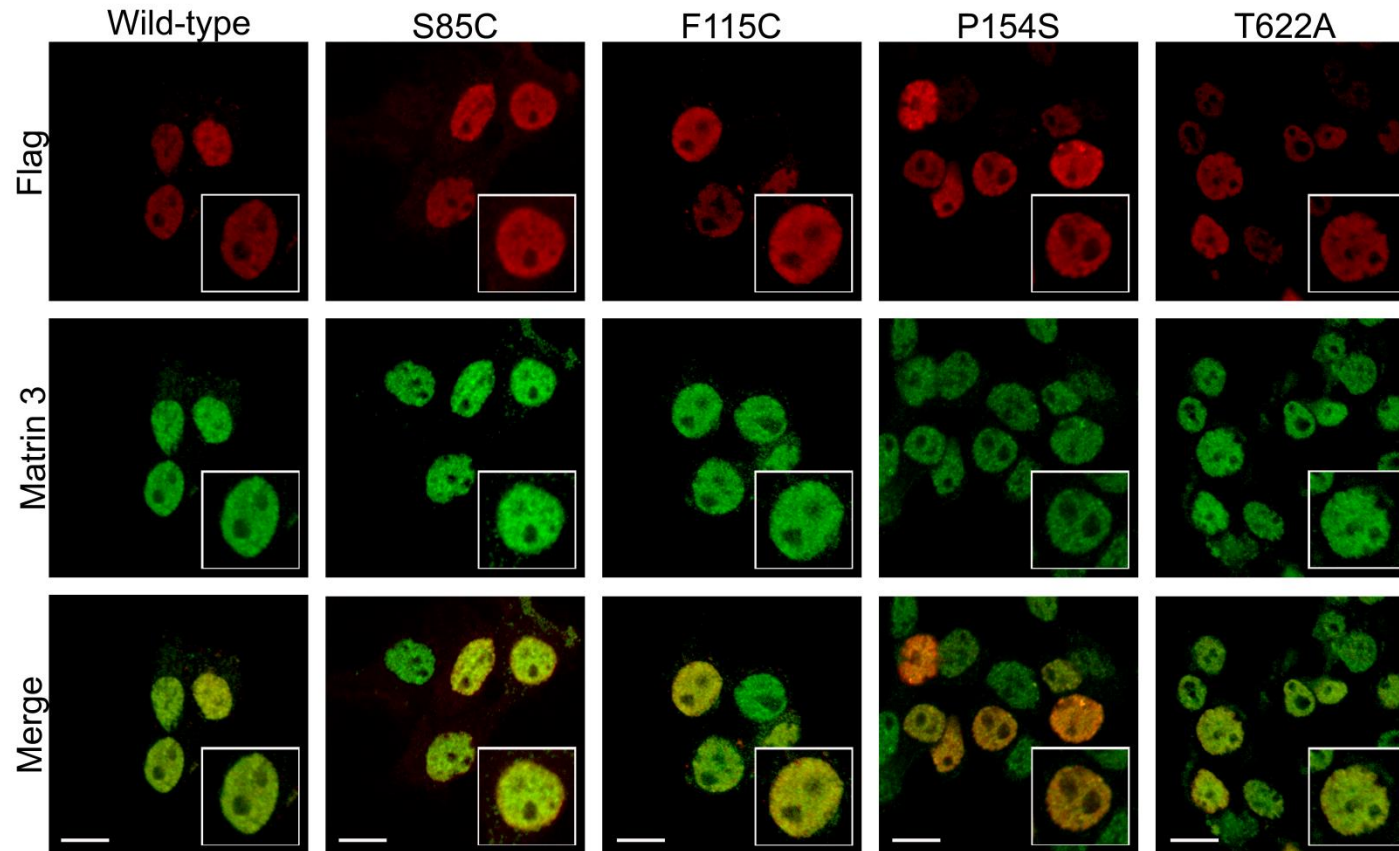
Input indicates total nuclear lysate before immunoprecipitation experiment (40 μ g of total protein) followed by immunoprecipitation using Flag agarose gel in NSC-34 cells stably expressing empty vector, wild-type Matrin 3 or one of the four mutations in Matrin 3. Red boxes indicate the area used for mass spectrometry experiments (IgG heavy and light chains were removed).

Supplemental Figure 2



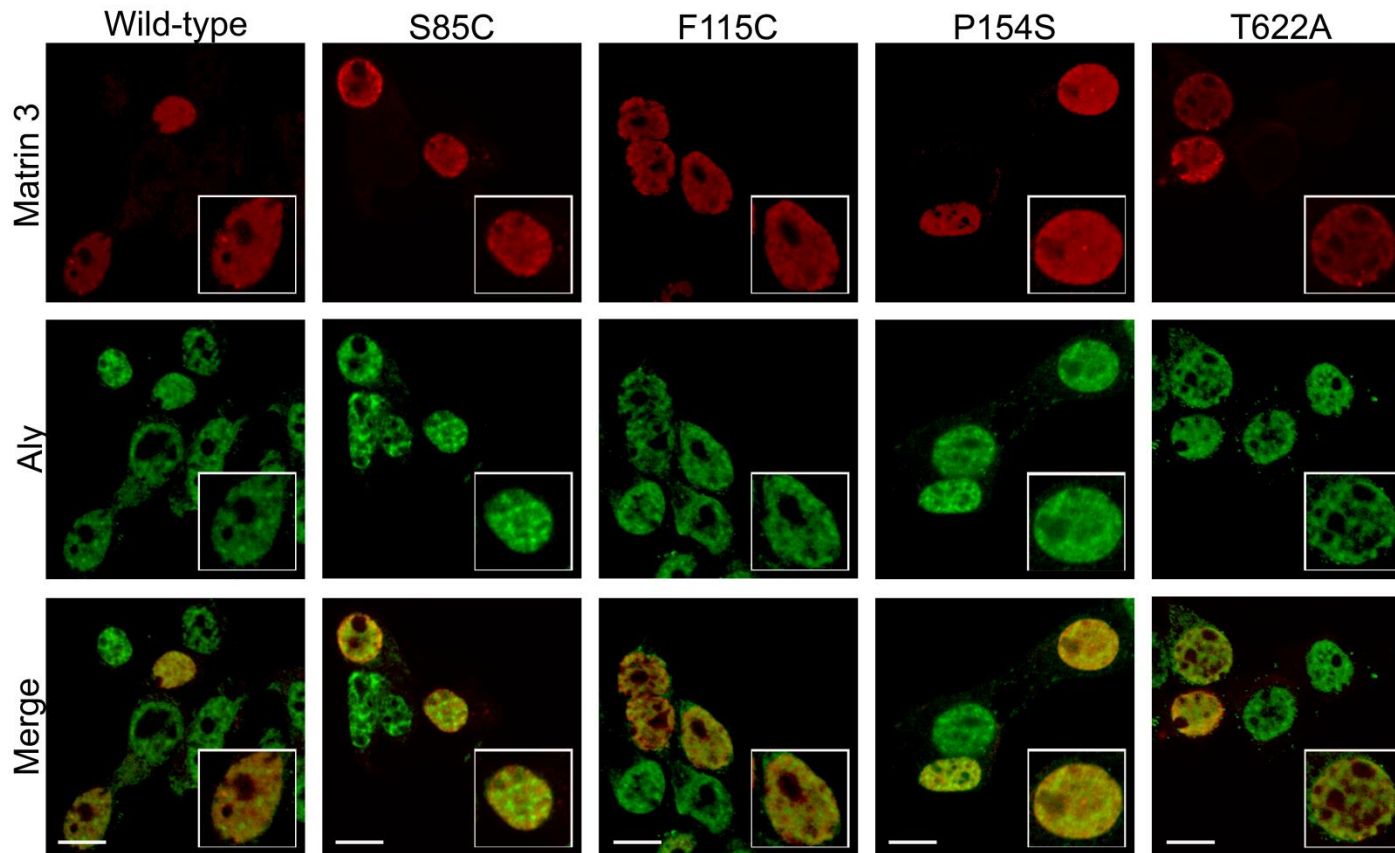
Supplemental Figure 2: Total TDP-43 and FUS mRNA levels by RT-PCR. Prior to fractionation experiments an aliquot of cells was separated and total RNA was extracted, followed by RT-PCR to determine the total levels of a) TDP-43 and b) FUS. mRNA levels were not altered by expression of wild-type or mutant Matrin 3. Error bars represent the mean \pm SEM of three independent experiments.

Supplemental Figure 3



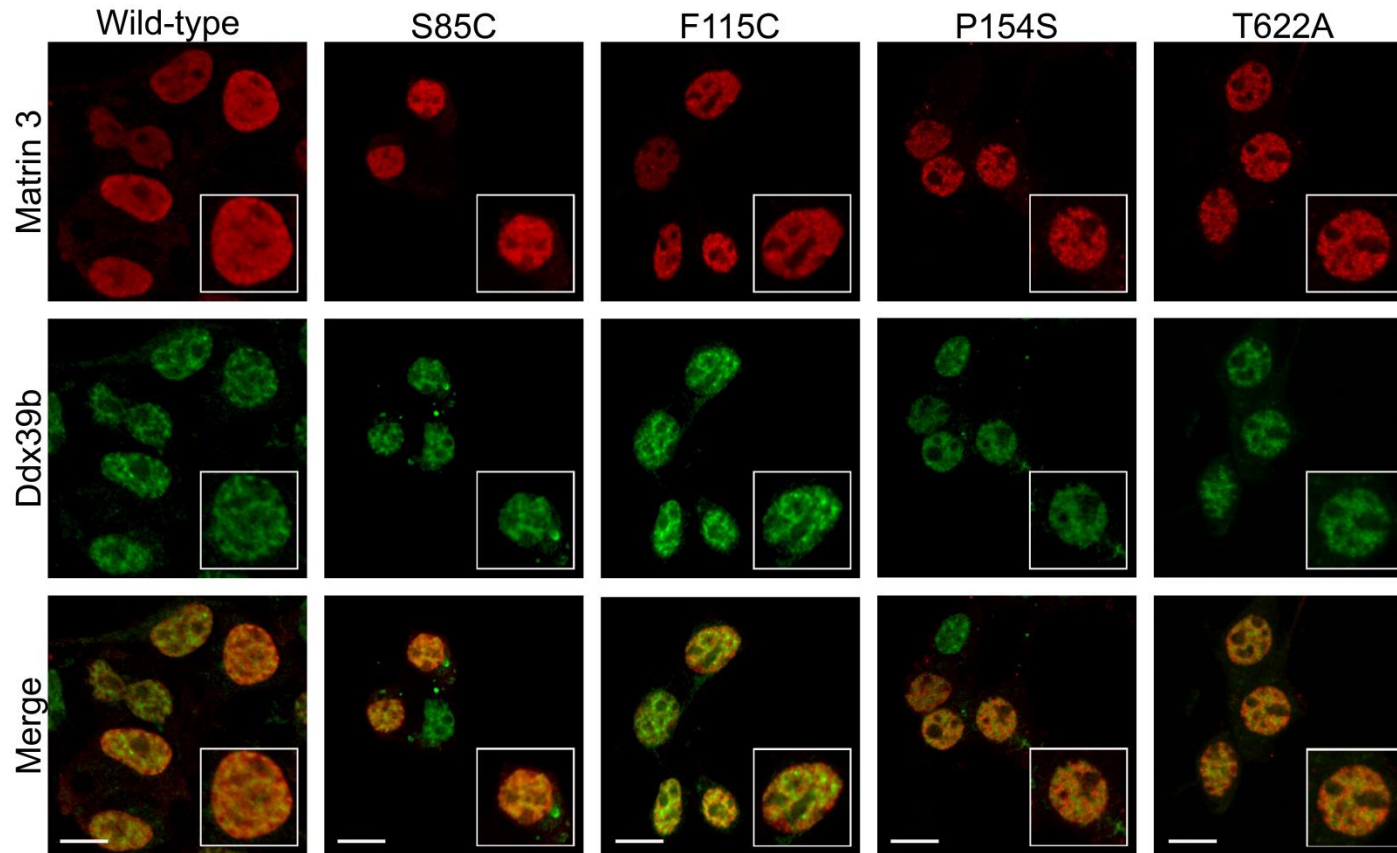
Supplemental Figure 3: Immunofluorescence images of NSC-34 cells transiently transfected with wild-type or mutant Matrin 3 then subjected to co-localization analysis. Representative images from immunofluorescence staining., flag is shown in red marking exogenous Matrin 3 and endogenous Matrin 3 is shown in green, merged image of two signals below. Insets indicate higher magnification images. Scale bar indicates 10 μ m.

Supplemental Figure 4



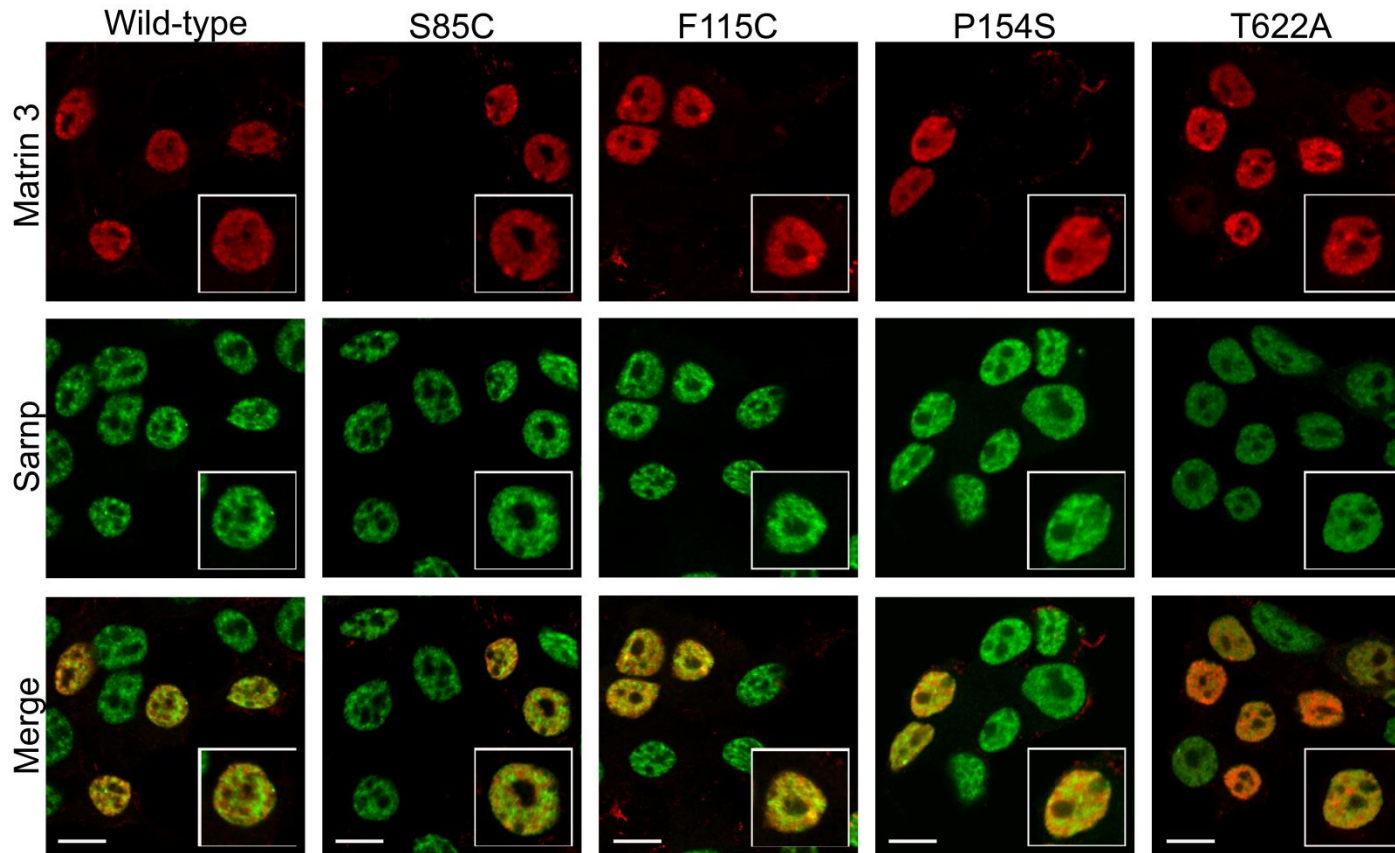
Supplemental Figure 4: Immunofluorescence images of NSC-34 cells transiently transfected with wild-type or mutant Matrin 3 then subjected to co-localization analysis. Representative images from immunofluorescence staining, flag is shown in red marking exogenous Matrin 3 and Aly is shown in green, merged image of two signals below. Insets indicate higher magnification images. Scale bar indicates 10 μ m.

Supplemental Figure 5



Supplemental Figure 5: Immunofluorescence images of NSC-34 cells transiently transfected with wild-type or mutant Matrins 3 then subjected to co-localization analysis. Representative images from immunofluorescence staining, flag is shown in red marking exogenous Matrins 3 and Ddx39b is shown in green, merged image of two signals below. Insets indicate higher magnification images. Scale bar indicates 10 μ m.

Supplemental Figure 6



Supplemental Figure 6: Immunofluorescence images of NSC-34 cells transiently transfected with wild-type or mutant Matrin 3 then subjected to co-localization analysis. Representative images from immunofluorescence staining, flag is shown in red marking exogenous Matrin 3 and Sarnp is shown in green, merged image of two signals below. Insets indicate higher magnification images. Scale bar indicates 10 μ m.

Supplementary Figure 7

Figure 1c:

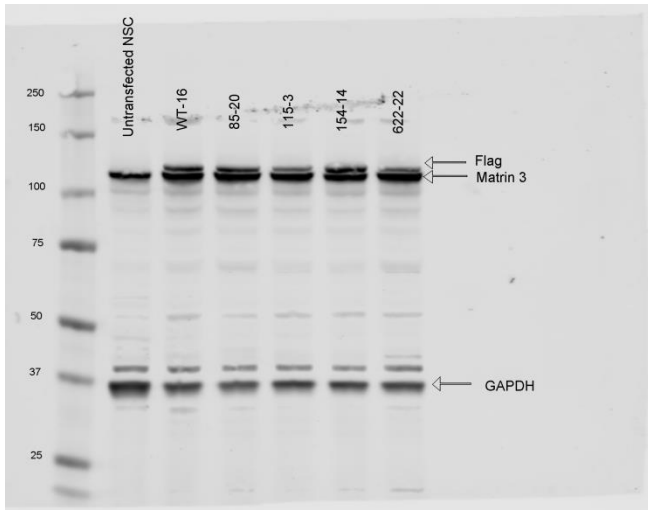
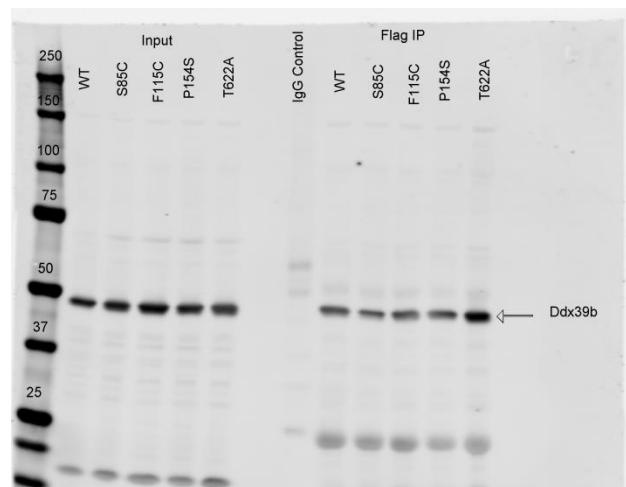
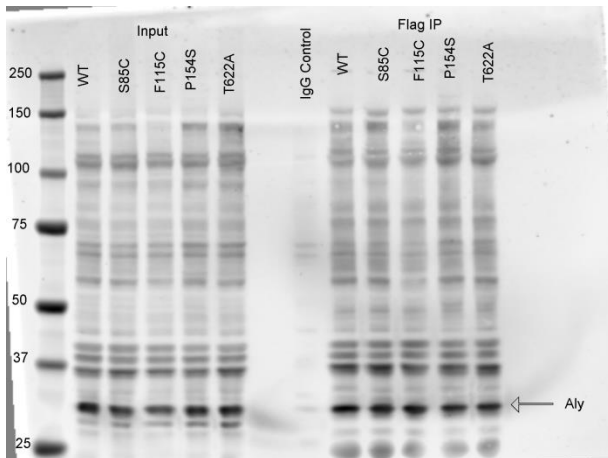
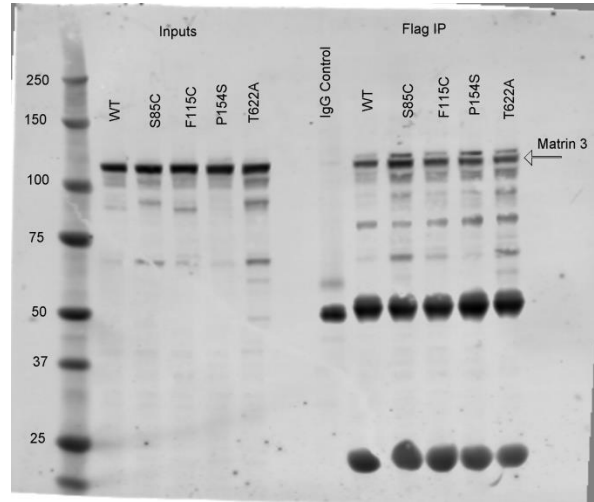
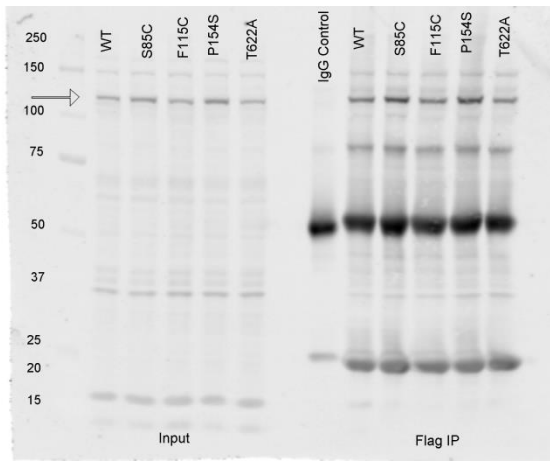


Figure 4a:



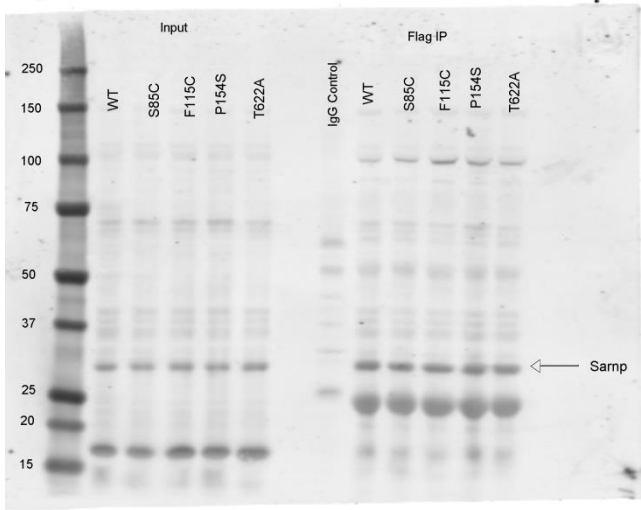


Figure 4b:

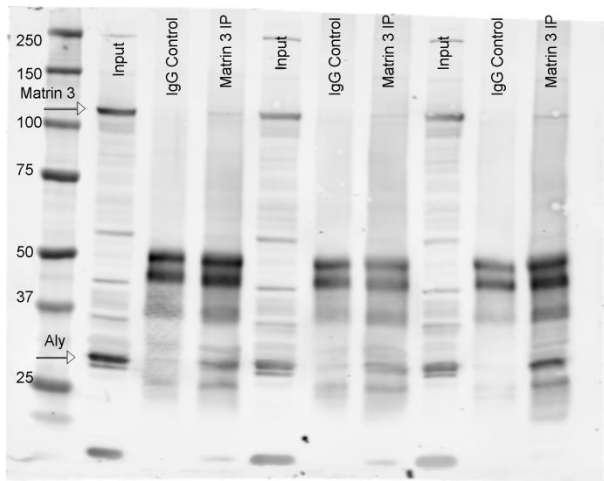


Figure 4c:

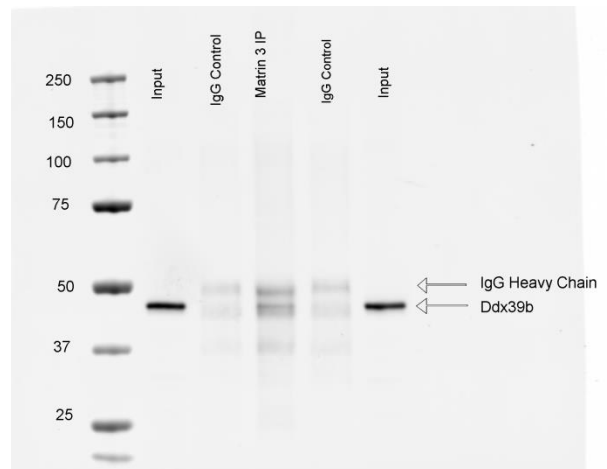
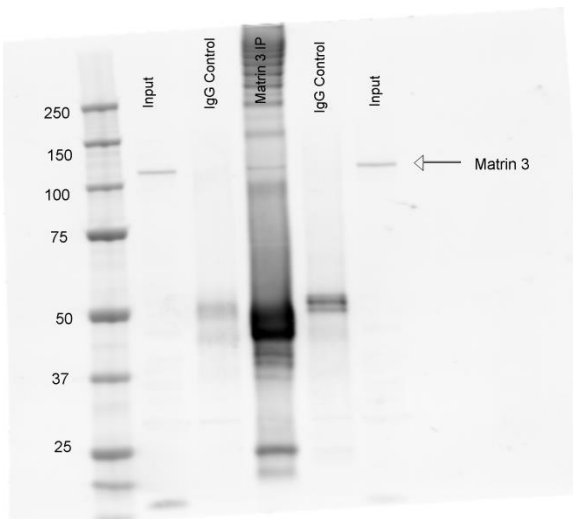


Figure 4d:

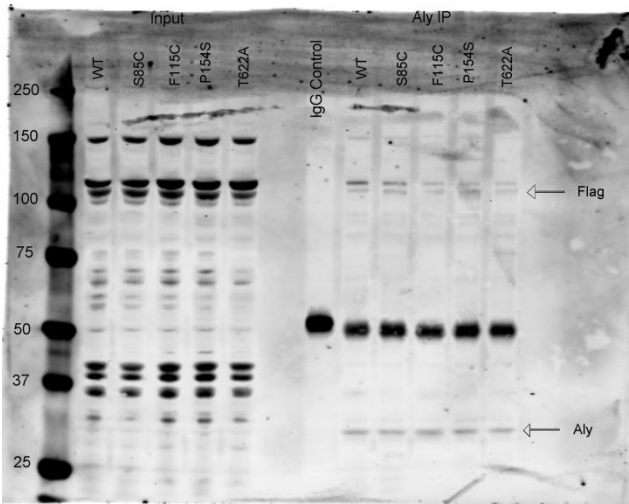
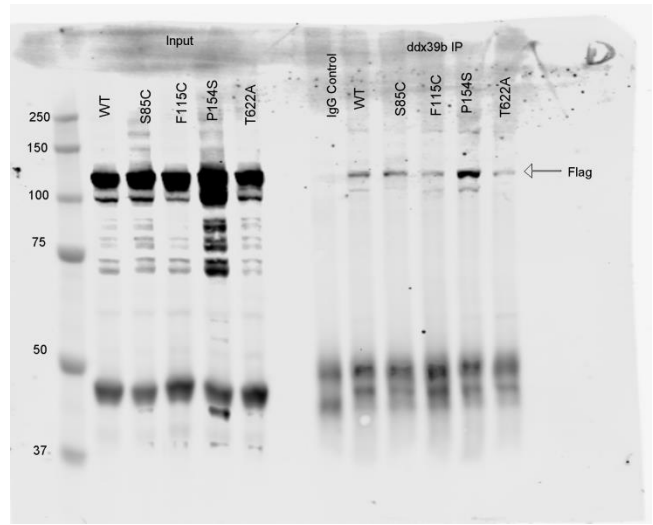
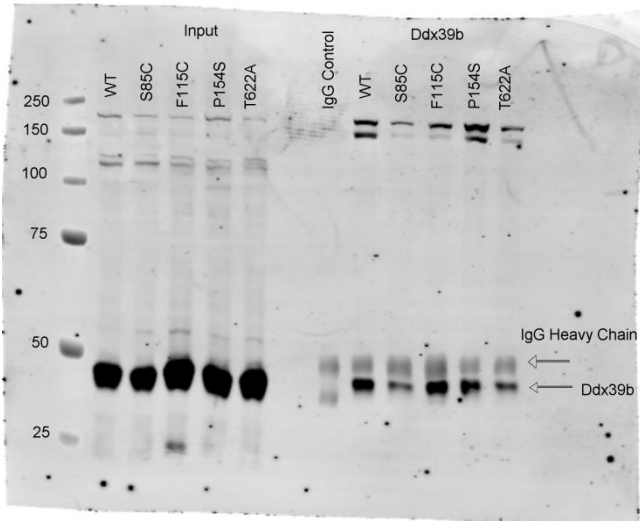
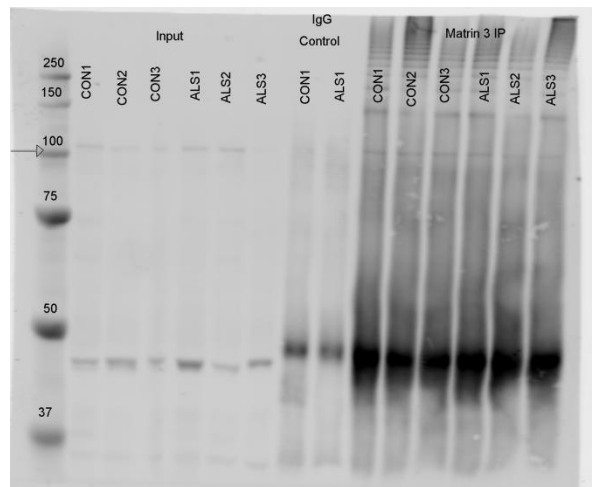
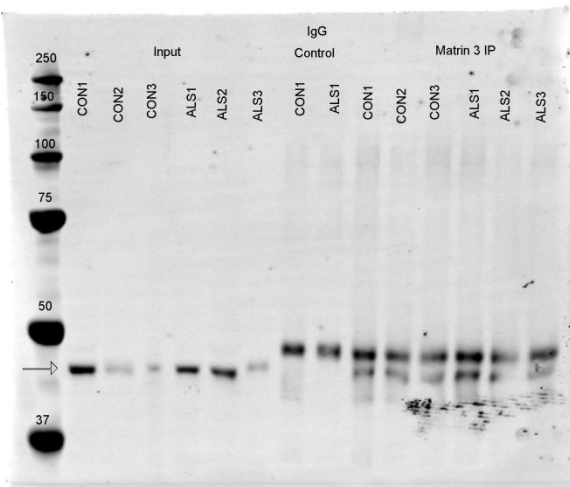


Figure 4g:



Supplementary Figure 7: Full length uncropped western blots shown in Figures 1c and 4a, 4b, 4c, 4d, 4g.