

# MG53 preserves neuromuscular junction integrity and alleviates ALS disease progression

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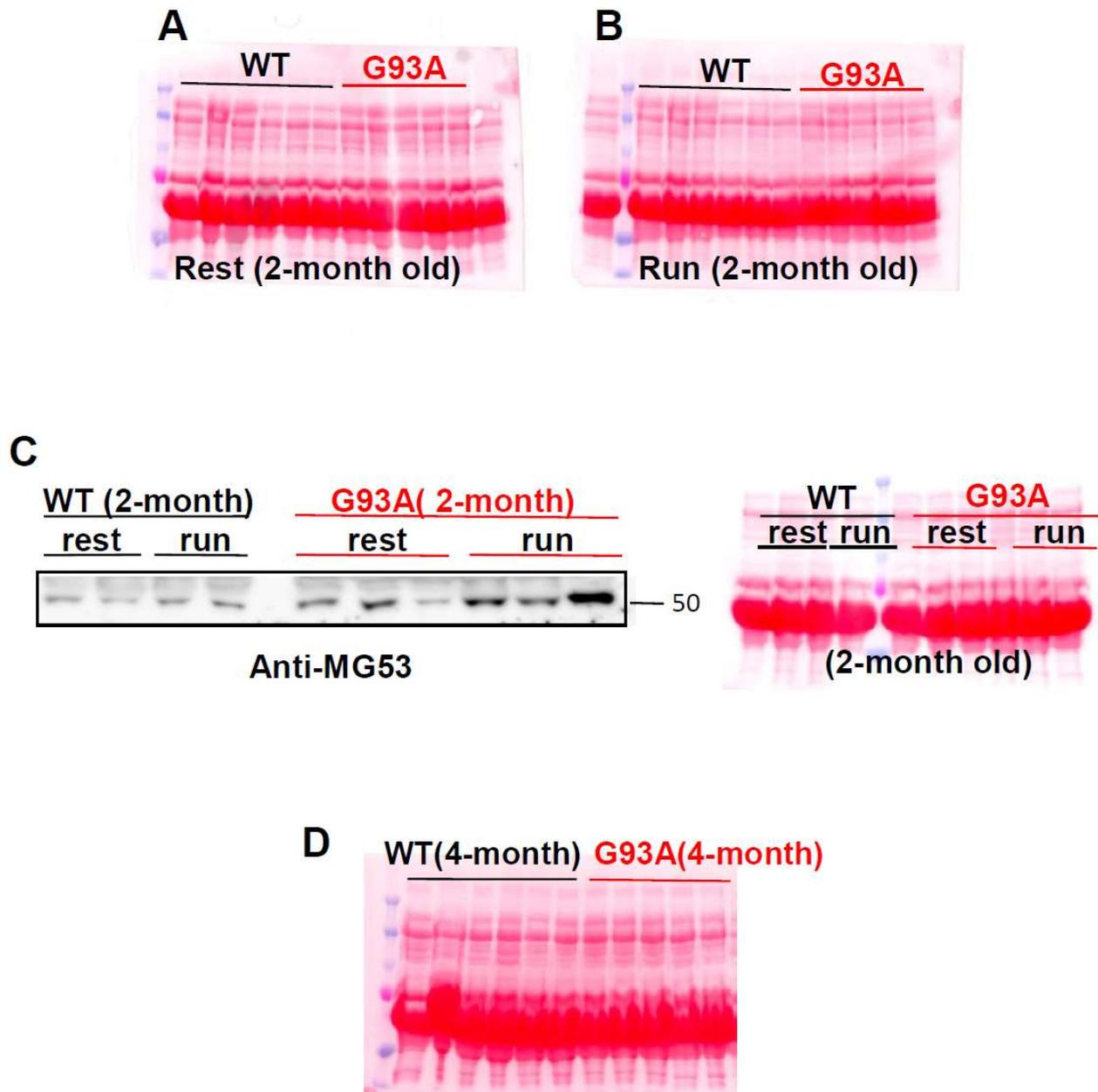
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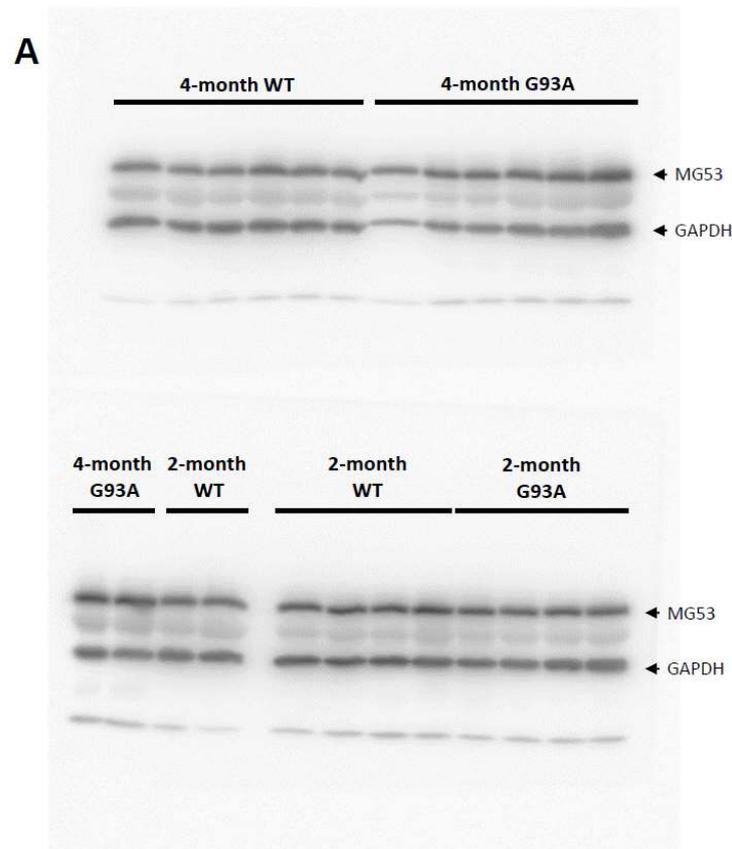
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## Supplementary Figure S1



**Supplementary Figure S1 (A,B)** Ponceau S staining of the immunoblotting assay in **Figure 2D** verifies equal loading amount of the serum samples of 2-month old mice. **(C)** Due to limited numbers of lanes in the acrylamide gel, the additional blotting assay of 2 WT and 3 G93A serum samples before and after running were evaluated in a separate gel. Data from this blot were also included in **Figure 2D** quantification results. **(D)** Ponceau S staining of the immunoblotting assay in **Figure 2E** verifies equal loading amount of the serum samples of 4-month old mice.

## Supplementary Figure S2



**B**

4 month		WT vs G93A: 4M		2 month		WT vs G93A: 2M	
WT	MG53/GAPDH	mean	p value	WT	MG53/GAPDH	mean	p value
1	0.879	0.867	0.000210	1	0.958	1.003	0.650967
2	0.809			2	0.884		
3	0.778			3	0.940		
4	0.912			4	1.101		
5	0.860			5	0.991		
6	0.962			6	1.142		
G93A				G93A			
1	2.011	1.449		1	1.017	0.976	
2	1.334			2	1.047		
3	1.367			3	0.924		
4	1.157			4	0.915		
5	1.395						
6	1.329						
7	1.354						
8	1.647						

**Supplementary Figure S2.** (A) The original blots for MG53 protein level in TA muscles derived from G93A and WT mice at the age of 2-month and 4-month. Anti-MG53 and anti-GAPDH were applied together during blotting. (B) Quantification of the MG53 expression level normalized to the GAPDH expression. Note, there is no significant change in MG53 expression level at the age of 2 months,  $p > 0.5$ ,  $n = 4-6$  mice; while the MG53 expression level is significantly higher in G93A muscle at the age of 4 months,  $p = 0.0002$ ,  $n = 6-8$  mice.

**Supplementary Table S1: Demographics of humandecedents**

ID	Race/ Gender	Age at onset	Age at death	Site of onset	Diagnosis	C9ORF72 expansion	SOD1 mutation
38	WF	33	34	Upper + lower extremities	fALS	Yes	No
88	WM	57	59	Bulbar	fALS + FTD	Yes	No
90	WF	57	59	Bulbar + Respiratory	sALS	No	No
93	WM	65	68	left hand	sALS	No	No
102	BF	70	72	Bulbar	sALS	No	No
104	WF	69	71	Bulbar	sALS	No	No
105	WM	61	69	left leg	sALS	No	No
107	WM	67	69	left hand	sALS	No	No
112	WM	67	69	right hand	sALS	No	No
116	WF	64	67	Left foot	sALS	No	No

ID	Race/ Gender	Age at death	Diagnosis	Cause of Death
103	WM	22	Non-Neurological Control	Metastatic synovial sarcoma (no CNS mets)
123	BM	52	Non-Neurological Control	Pancreatic Cancer (no CNS mets)
129	WF	63	Non-Neurological Control	Colon cancer with liver & lymph node mets (no CNS mets)

**Supplementary Table S2: Biospecimen information for humansamples**

ID	Biospecimen type	Anatomic site	Vital state of patient	Collection	Type of stabilization	Type of long term preservation	Constitution of preservative	Storage duration	Shipping temperature	Composition Assessment & selection
38	Skeletal muscle	Diaphragm	Postmortem	Autopsy	Formalin fixed	Paraffin embedded	10% neutral- buffered formalin	21 years	25°C	Clinical, genetic & neuropathological
88	Skeletal muscle	Diaphragm	Postmortem	Autopsy	Formalin fixed	Paraffin embedded	10% neutral- buffered formalin	1-7 years	25°C	Clinical, genetic & neuropathological
90	Skeletal muscle	Psoas	Postmortem	Autopsy	Formalin fixed	Paraffin embedded	10% neutral- buffered formalin	1-7 years	25°C	Clinical, genetic & neuropathological
93	Skeletal muscle	Psoas	Postmortem	Autopsy	Formalin fixed	Paraffin embedded	10% neutral- buffered formalin	1-7 years	25°C	Clinical, genetic & neuropathological
102	Skeletal muscle	Diaphragm	Postmortem	Autopsy	Formalin fixed	Paraffin embedded	10% neutral- buffered formalin	1-7 years	25°C	Clinical, genetic & neuropathological
104	Skeletal muscle	Diaphragm	Postmortem	Autopsy	Formalin fixed	Paraffin embedded	10% neutral- buffered formalin	1-7 years	25°C	Clinical, genetic & neuropathological
105	Skeletal muscle	Psoas	Postmortem	Autopsy	Formalin fixed	Paraffin embedded	10% neutral- buffered formalin	1-7 years	25°C	Clinical, genetic & neuropathological
107	Skeletal muscle	Psoas	Postmortem	Autopsy	Formalin fixed	Paraffin embedded	10% neutral- buffered formalin	1-7 years	25°C	Clinical, genetic & neuropathological
112	Skeletal muscle	Psoas	Postmortem	Autopsy	Formalin fixed	Paraffin embedded	10% neutral- buffered formalin	1-7 years	25°C	Clinical, genetic & neuropathological
116	Skeletal muscle	Diaphragm/ psoas	Postmortem	Autopsy	Formalin fixed	Paraffin embedded	10% neutral- buffered formalin	1-7 years	25°C	Clinical, genetic & neuropathological
103	Skeletal muscle	Diaphragm/ psoas	Postmortem	Autopsy	Formalin fixed	Paraffin embedded	10% neutral- buffered formalin	1-7 years	25°C	Clinical, genetic & neuropathological
123	Skeletal muscle	Psoas	Postmortem	Autopsy	Formalin fixed	Paraffin embedded	10% neutral- buffered formalin	1-7 years	25°C	Clinical, genetic & neuropathological
129	Skeletal muscle	Psoas	Postmortem	Autopsy	Formalin fixed	Paraffin embedded	10% neutral- buffered formalin	1-7 years	25°C	Clinical, genetic & neuropathological