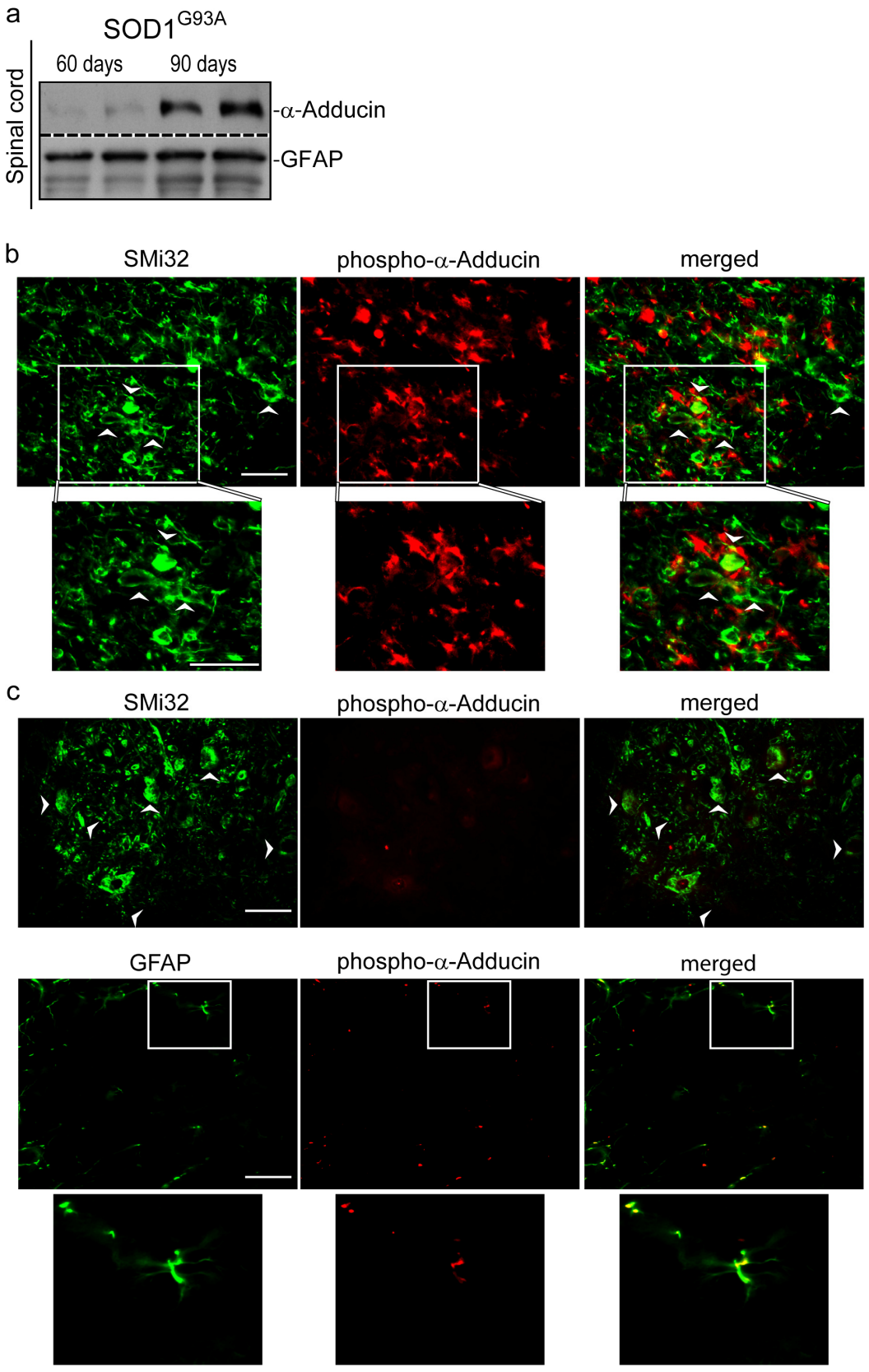
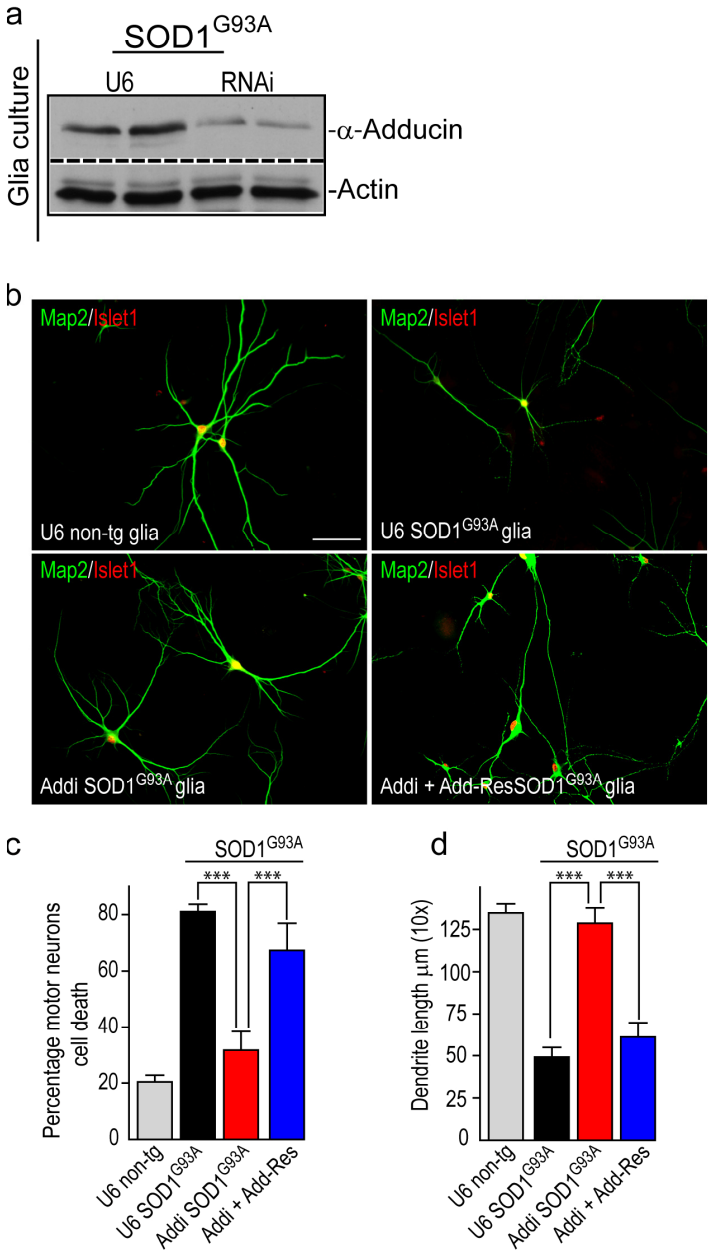

a
SOD1G93A
60 days
90 days
Spinal cord
-α-Adducin
-GFAP
b
SMi32
phospho-α-Adducin
merged
c
SMi32
phospho-α-Adducin
merged
GFAP
phospho-α-Adducin
merged

Supplementary Figure 1

α-Adducin in spinal cord is upregulated in symptomatic SOD1^{G93A} spinal cord within astrocytes.

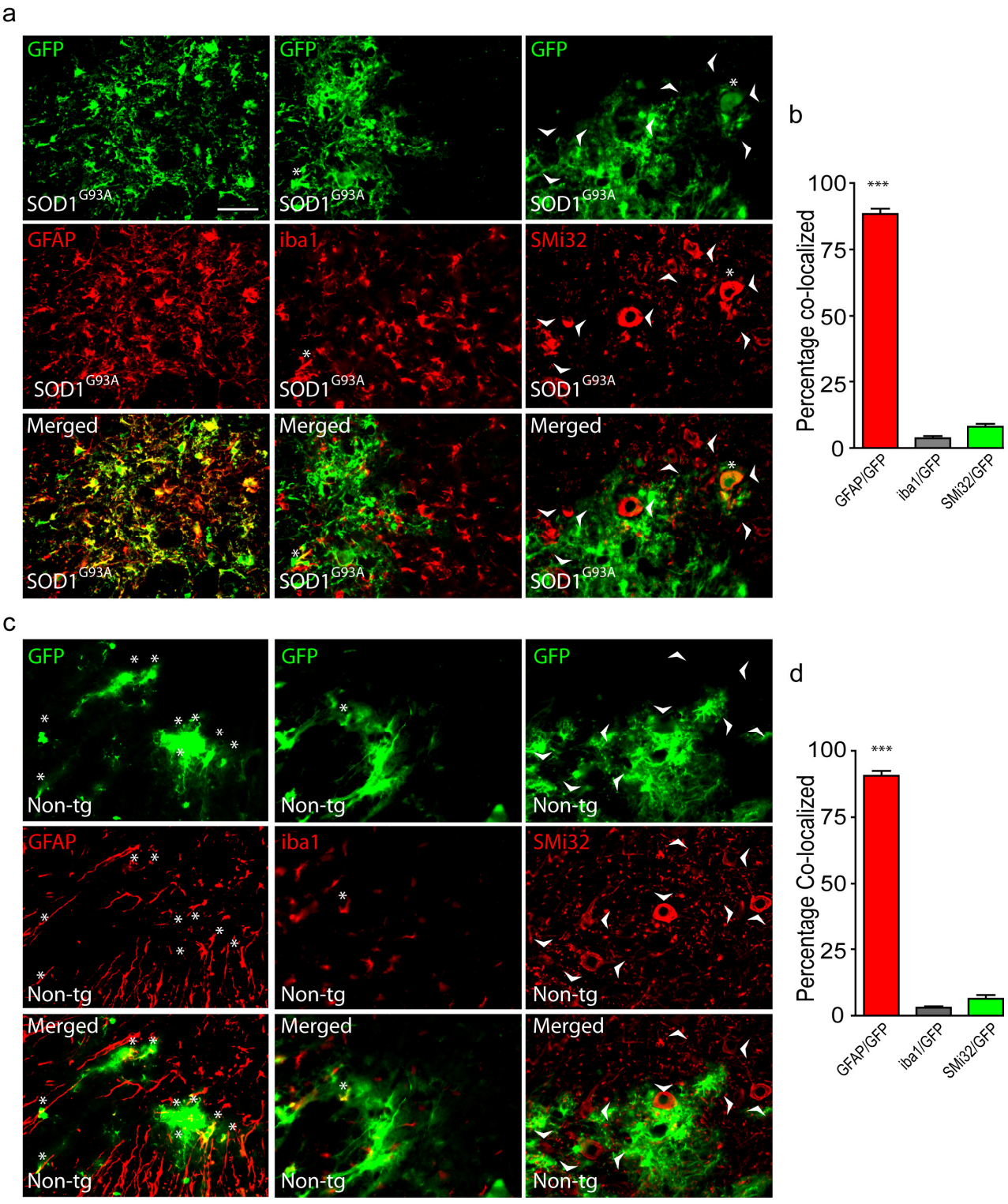
(**a**) Immunoblots for α-Adducin protein at 60 and 90 day old SOD1^{G93A} mice show α-Adducin is upregulated at 90 days. (**b**) Immunohistochemistry with in sections of the lumbar spinal cord from symptomatic SOD1^{G93A} mice displays Ser436-phosphorylated α-Adducin does not co-localize with the motor neuron marker (SMi32). Arrowheads indicate motor neurons; scale bar 50μm. (**c**) Immunohistochemistry from sections of control wild type lumbar spinal cord displays Ser436-phosphorylated α-Adducin does not co-localize with the motor neuron marker (SMi32); *upper panels*. Arrowheads indicate motor neurons. Immunohistochemistry from sections of control wild type lumbar spinal cord displays Ser436-phosphorylated α-Adducin co-localize with the astrocyte marker (GFAP) lower panel*s*. Scale bar 50μm. (**a**) are cropped; full length images are presented in **Supplementary Figure 11**.


a
SOD1G93A
U6
RNAi
Glia culture
-α-Adducin
-Actin
b
Map2/Islet1
U6 non-tg glia
U6 SOD1G93A glia
Addi SOD1G93A glia
Addi + Add-ResSOD1G93A glia
c
SOD1G93A
***
***
Percentage motor neurons cell death
0
20
40
60
80
U6 non-tg
U6 SOD1G93A
Addi SOD1G93A
Addi + Add-Res
d
SOD1G93A
***
***
Dendrite length µm (10x)
0
25
50
75
100
125
U6 non-tg
U6 SOD1G93A
Addi SOD1G93A
Addi + Add-Res

Supplementary Figure 2

Expression of a RNAi-resistant form of α-Adducin in $SOD1^{G93A}$ astrocytes restores the ability of $SOD1^{G93A}$ astrocytes to induce non-cell autonomous motor neuron cell death.

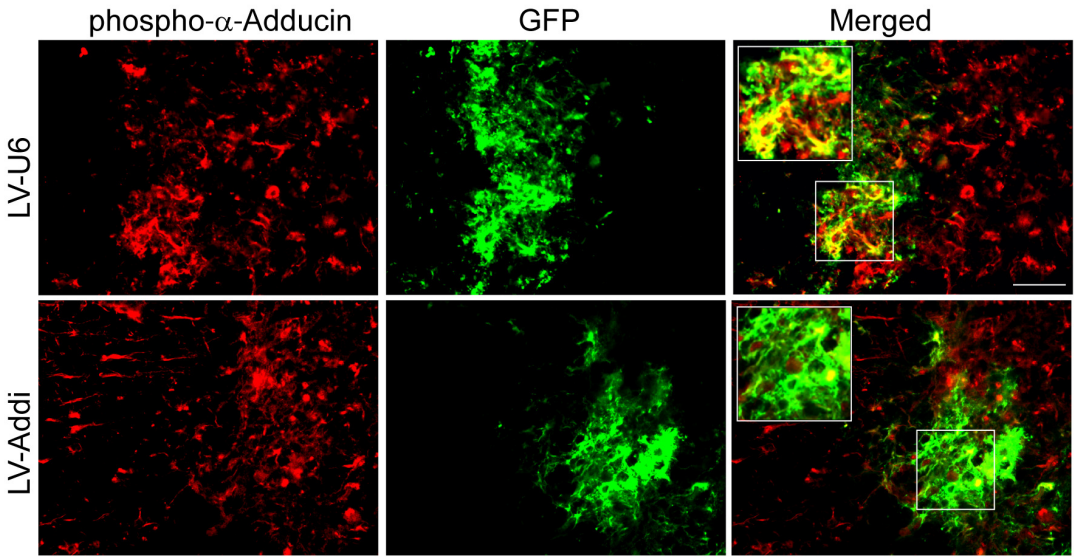
(**a**) Knockdown of α-Adducin relative to control U6 in astrocytes (**b**) Co-cultured astrocytes and motor neurons were subjected to immunocytochemistry with the motor neuron nuclear protein Islet1 (red) and the dendrite protein MAP2 (green); scale bar 50μm. Wild type astrocytes transfected with the control U6 or α-Adducin RNAi plasmid had little or no effect on motor primary motor neurons cell death or dendrite abnormalities (*upper left panel)*; quantified (**c** and **d**). Control U6 $SOD1^{G93A}$ astrocytes induced non-cell autonomous motor neuron cell death and dendrite abnormalities (*upper right panel*); quantified (**c** and **d**). Knockdown of α-Adducin in $SOD1^{G93A}$ astrocytes protected motor neurons against the non-cell autonomous cell death and dendrite abnormalities (*lower left panel*); quantified (**c and d**). Expressions of an RNAi-resistant form of α-Adducin (Add-Res) in the background of α-Adducin RNAi in $SOD1^{G93A}$ astrocytes restored the ability of the $SOD1^{G93A}$ astrocytes to induce non-cell autonomous cell death and dendrite abnormalities in motor neurons (*lower right panel*); quantified (**d** and **e**). All data in bar charts show mean ± s.e.m (***$p<0.001$; unpaired t-test). (**a)** are cropped; full length images are presented in **Supplementary Figure 11**.


a
GFP
SOD1G93A
GFAP
iba1
SMi32
Merged
b
Percentage co-localized
100
75
50
25
0
***
GFAP/GFP
iba1/GFP
SMi32/GFP
c
Non-tg
d
Percentage Co-localized

Supplementary Figure 3

Lentivirial mediated knockdown *in vivo* predominately target astrocytes.

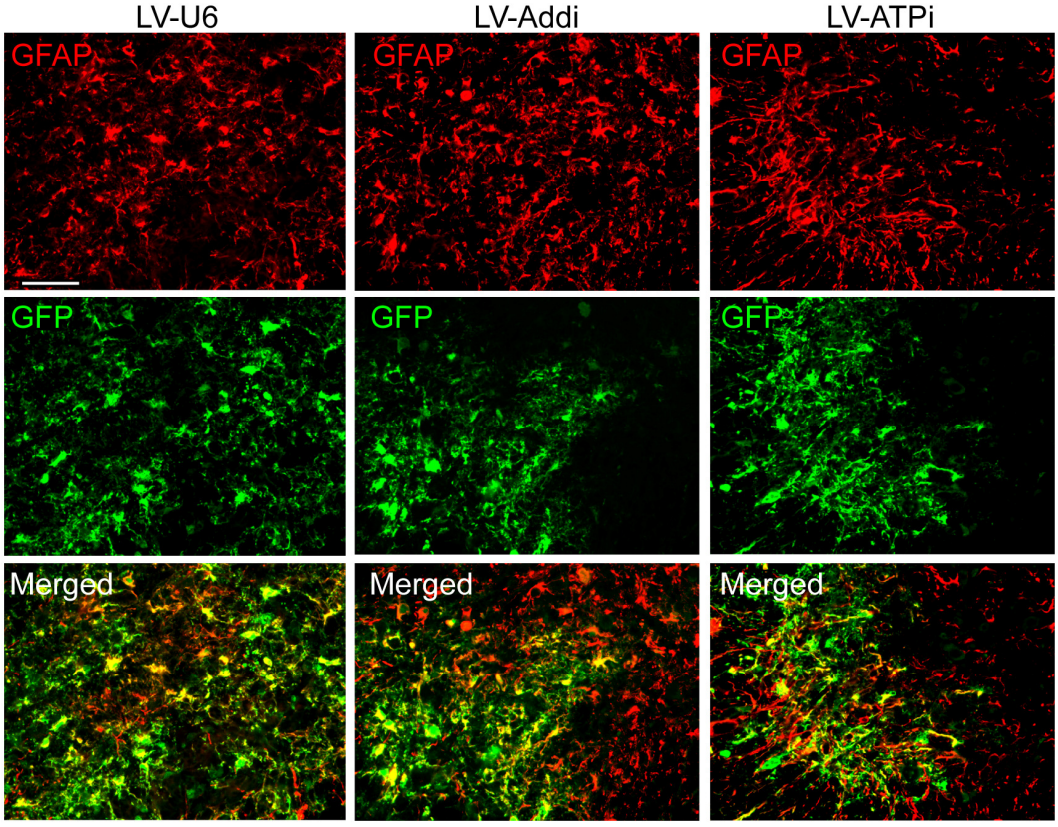
(**a**) Immunohistochemistry with GFP in sections of the lumbar spinal cord from $SOD1^{G93A}$ mice displaying percent GFP positive astrocytes (GFAP), microglia (Iba1) and motor neurons (SMi32) 30 days post-injection. Arrowheads indicate motor neurons; scale bar 50μm. (**b**) Quantifications of percent GFP positive cells revealed lentivirus predominately target astrocytes; n=~300 per cell type/three. All data in bar charts show ± s.e.m (***$p<0.001$; ANOVA). (**c**) Immunohistochemistry with GFP in sections of the lumbar spinal cord from wild type mice displaying percent GFP positive astrocytes (GFAP), microglia (Iba1) and motor neurons (SMi32) 30 days post-injection. Arrowheads indicate motor neurons; scale bar 50μm. (**d**) Quantifications of percent GFP positive cells revealed lentivirus predominately target astrocytes; n=~300 per cell type/three. All data in bar charts show ± s.e.m (***$p<0.001$; unpaired t-test).


phospho-α-Adducin
GFP
Merged
LV-U6
LV-Addi

Supplementary Figure 4

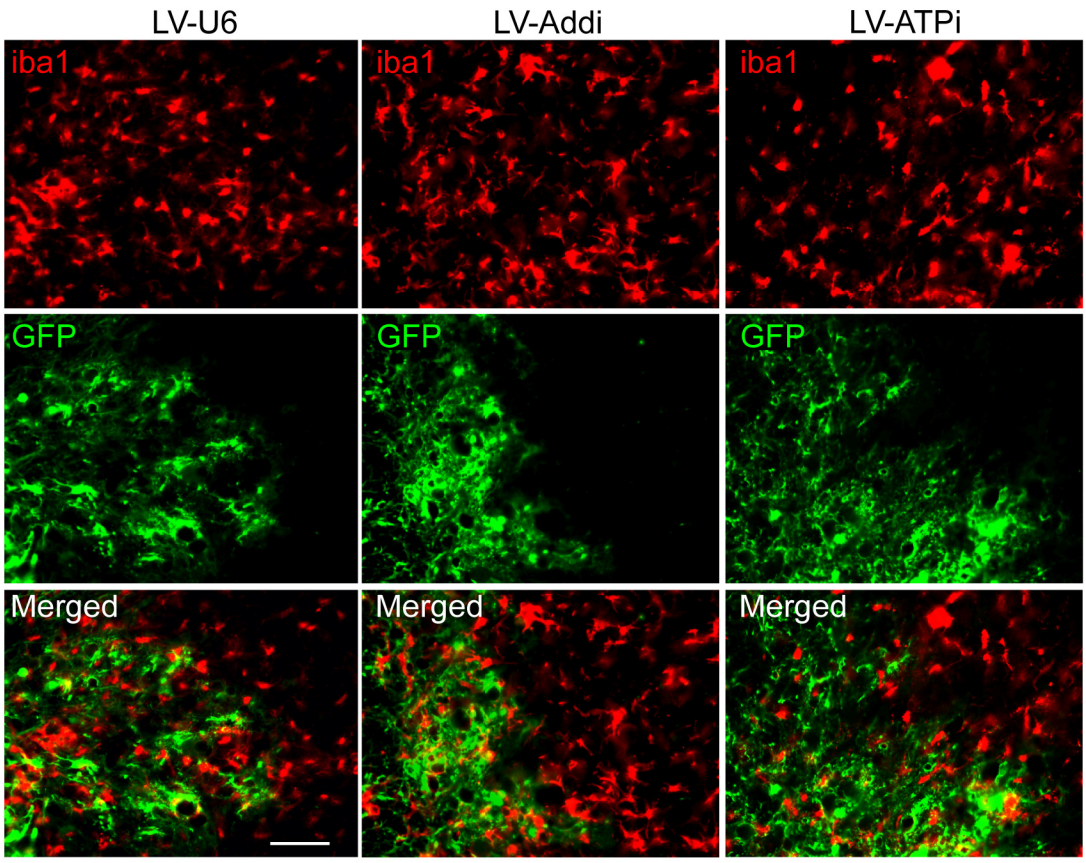
Knockdown of α-Adducin in SOD1^{G93A} mice decreases immunoreactivity for phosphorylated Ser436-α-Adducin.

Spinal cord from SOD1^{G93A} mice injected intraspinally with lentivirus expressing short hairpin RNAs targeting α-Adducin and encoding GFP (LV-Addi) or the corresponding control U6 (LV-U6) were subjected to immunohistochemistry using GFP and phospho-α-Adducin (red) antibodies. Knockdown of α-Adducin (LV-Addi) led to a decreased in immunoreactivity of phospho-α-Adducin within the GFP-labeled ventral horn as compared to control U6 (LV-U6) injected ventral horn; scale bar 50μm.


LV-U6
LV-Addi
LV-ATPi
GFAP
GFAP
GFAP
GFP
GFP
GFP
Merged
Merged
Merged

Supplementary Figure 5

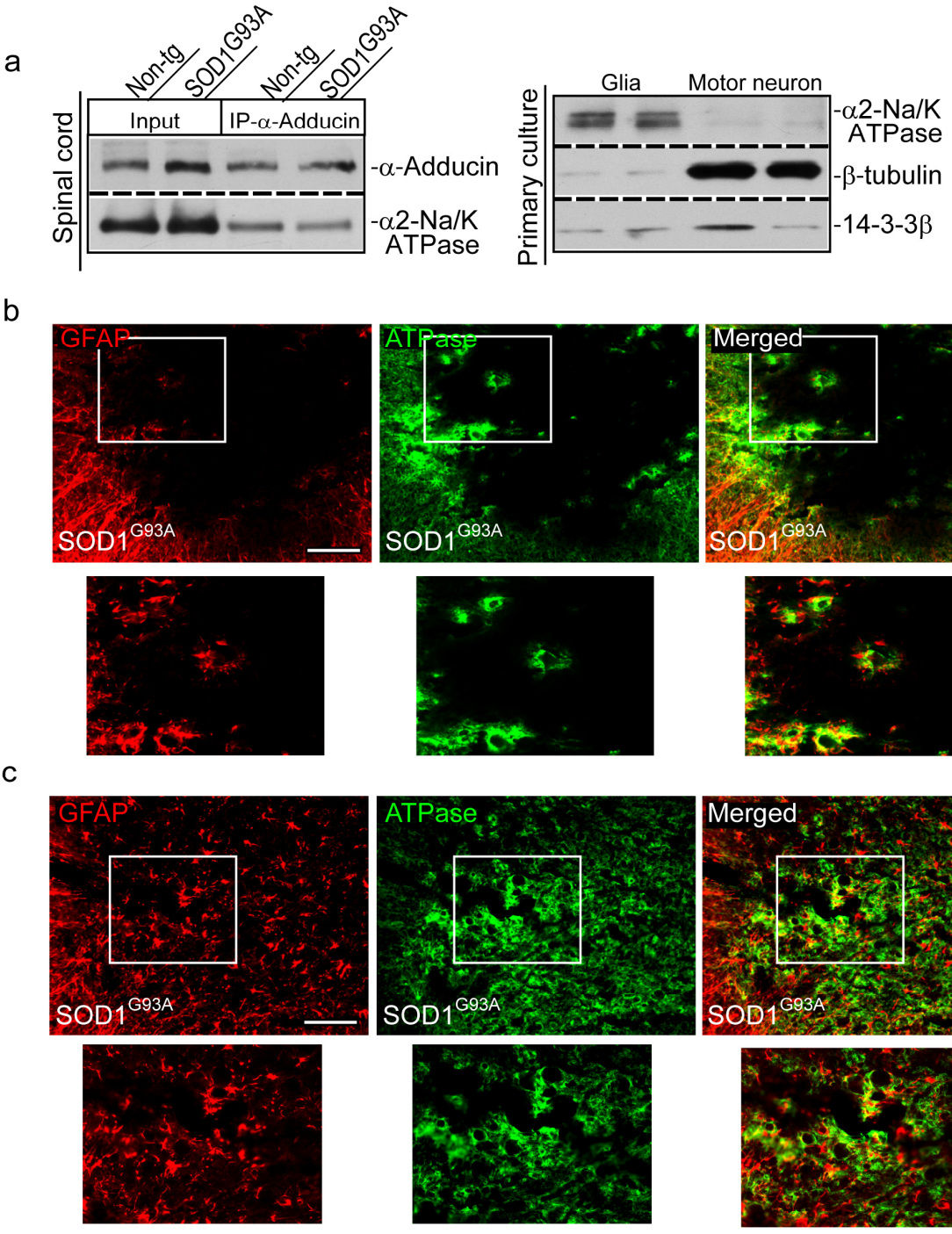
Knockdown of α-Adducin or α2-Na/K ATPase in SOD1^{G93A} mice do not alter gliosis in the spinal cord. Spinal cord from SOD1^{G93A} mice injected intraspinally with lentivirus expressing short hairpin RNAs targeting α-Adducin or α2-Na/K ATPase or the corresponding control U6 virus were subjected to immunohistochemistry using GFP and the GFAP (red) antibodies. Knockdown of α-Adducin (LV-Addi) or α2-Na/K ATPase (LV-ATPi) had little or no effect on the presence or abundance of astrocytes within the GFP-labeled ventral horn; scale bar 50μm.



Supplementary Figure 6

Knockdown of α-Adducin or α2-Na/K ATPase in SOD1^{G93A} mice do not alter microgliosis in spinal cord. Spinal cord from SOD1^{G93A} mice injected intraspinally with lentivirus expressing short hairpin RNAs targeting

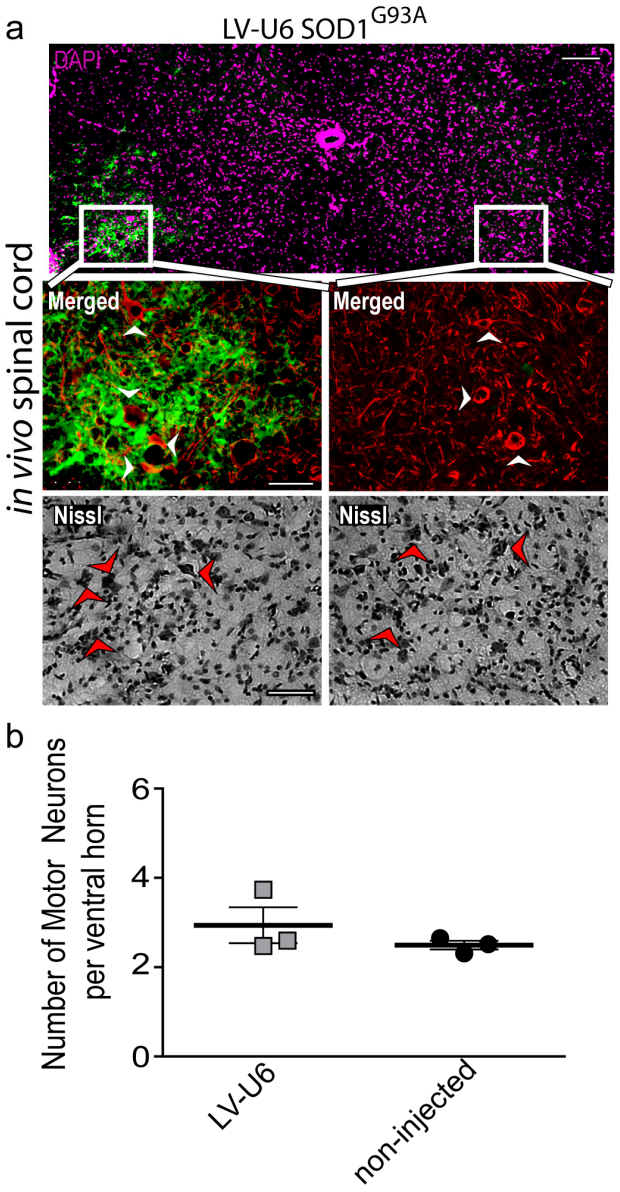
α-Adducin or α2-Na/K ATPase or the corresponding control U6 virus were subjected to immunohistochemistry using GFP and the Iba1 (red) antibodies. Knockdown of α-Adducin (LV-Addi) or α2-Na/K ATPase (LV-ATPi) had little or no effect on the presence or abundance of microglia within the GFP-labeled ventral horn; scale bar 50μm.


a
Non-tg
SOD1G93A
Non-tg
SOD1G93A
Input
IP-α-Adducin
Spinal cord
-α-Adducin
-α2-Na/K ATPase
Glia
Motor neuron
Primary culture
-α2-Na/K ATPase
-β-tubulin
-14-3-3β
b
GFAP
ATPase
Merged
SOD1G93A
SOD1G93A
SOD1G93A
c
GFAP
ATPase
Merged
SOD1G93A
SOD1G93A
SOD1G93A

Supplementary Figure 7

α2-Na/K ATPase co-immunoprecipitates with α-Adducin in spinal cord lysates and is specifically upregulated in astrocytes in symptomatic SOD1^{G93A} mice.

(**a**) Immunoblots show immunoprecipitated α-Adducin from SOD1^{G93A} and control wild type spinal cord lysates subjected to immunoblotting with the α-Adducin and α2-Na/K ATPase antibodies following glycine elution, confirming α2-Na/K ATPase as an interactor of α-Adducin (*left panels)*. Immunoblots show α2-Na/K ATPase is predominately expressed in primary glial cultures relative to primary motor neuron cultures enriched with the neuron marker β-tubulin. 14-3-3β is used as an internal control (*right panel*). (**b**) Immunohistochemistry with astrocyte marker GFAP and α2-Na/K ATPase antibody in sections of the lumbar spinal cord from SOD1^{G93A} mice at 60 days displays α2-Na/K ATPase expression within astrocytes; scale bar 50μm. (**c**) Immunohistochemistry with GFAP and α2-Na/K ATPase antibody in sections of the lumbar spinal cord from symptomatic SOD1^{G93A} mice at 120 days displays upregulation of α2-Na/K ATPase expression within astrocytes; scale bar 50μm. (**a)** are cropped; full length images are presented in **Supplementary Figure 11**.

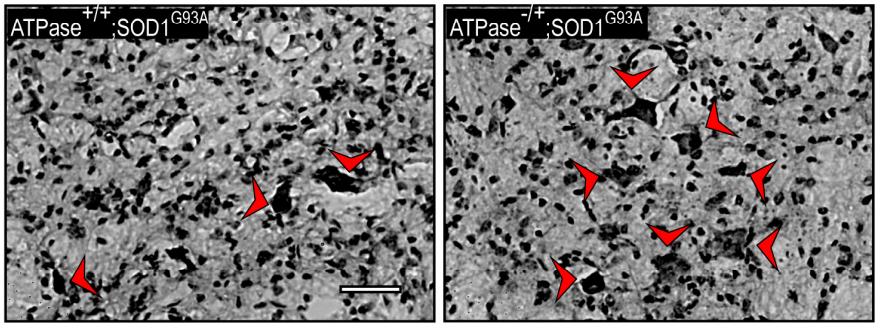

a
LV-U6 SOD1G93A
DAPI
in vivo spinal cord
Merged
Merged
Nissl
Nissl
b
Number of Motor Neurons per ventral horn
6
4
2
0
LV-U6
non-injected

Supplementary Figure 8

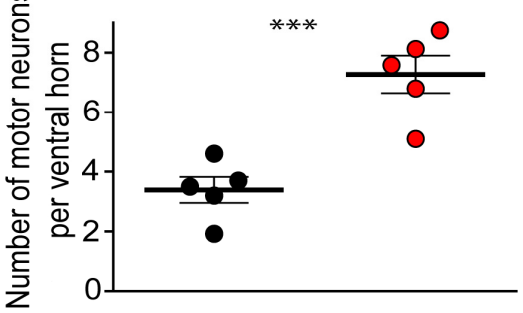
Intraspinally injection of control lentivirus in SOD1^{G93A} mice had no effect on motor neuron survival.

(**a**) Spinal cord from end stage SOD1^{G93A} mice injected at age 90 days intraspinally with the control lentivirus encoding GFP (LV-U6 SOD1^{G93A}) was subjected to immunohistochemistry at end stage. End stage was defined as a time point at which the animal was unable to upright itself within 30s of placement on its side. Immunohistochemistry with GFP in SOD1^{G93A} lumbar sections revealed delivery of control injected virus (LV-U6) into the ventral horn; scale bar 100μm. Alternating GFP positive sections were subjected to immunohistochemistry using the GFP antibody and the neurofilment-SMi32 antibody (red), a motor neuron marker, or Nissl stained (*lower panels*) for quantification of surviving motor neurons within GFP-labeled injected ventral horn and contralateral non-injected ventral horn (n≥20 sections per animal); scale bar 50μm. Control LV-U6 SOD1^{G93A} mice (n=3) displayed equivalent degeneration of motor neurons within injected GFP-labeled ventral horn and non-injected contralateral ventral horn. Arrowheads indicate surviving motor neurons; quantification shown in (**b**).

a


ATPase+/+;SOD1G93A
ATPase-/+;SOD1G93A

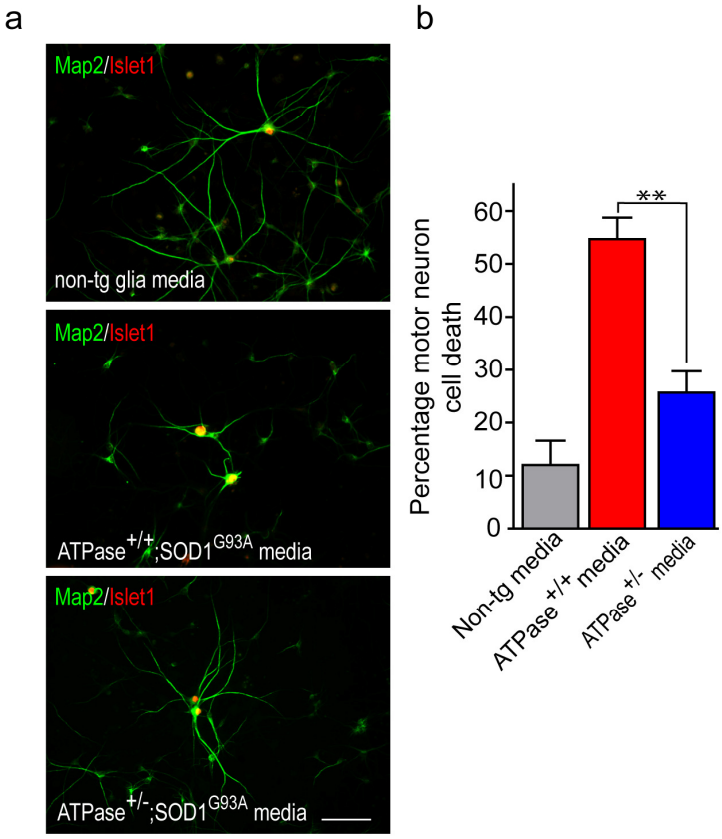
b


Number of motor neurons per ventral horn
0
2
4
6
8
***

Supplementary Figure 9

Heterozygous disruption of the α2-Na/K ATPase gene in $SOD1^{G93A}$ mice delays motor neuron degeneration.

(**a**) Nissl stained sections from endstage control $SOD1^{G93A}$ mice (n=5) and aged-matched $SOD1^{G93A}$ littermates heterozygous-null for the α2-Na/K ATPase allele (n=5) displayed more than twice the number of motor neurons in $ATPase^{+/-}$;$SOD1^{G93A}$ than control $SOD1^{G93A}$ mice. Arrow heads indicate surviving motor neurons; quantification shown in (**b**); scale bar 50μm (***$p<0.001$; unpaired t-test).


a
b
Map2/Islet1
non-tg glia media
Map2/Islet1
ATPase+/+;SOD1G93A media
Map2/Islet1
ATPase+/-;SOD1G93A media
Percentage motor neuron cell death
0
10
20
30
40
50
60
**
Non-tg media
ATPase +/+ media
ATPase +/- media

Supplementary Figure 10

Condition media from heterozygous-null from α2-Na/K ATPase SOD1^{G93A} astrocytes is neuroprotective.

(**a**) Precondition media from wild type, SOD1^{G93A}, and heterozygous-null α2-Na/K ATPase; SOD1^{G93A} astrocytes were exposed to motor neurons and subjected to immunocytochemistry with antibodies recognizing the motor neuron nuclear protein Islet1 (red) and the dendrite protein MAP2 (green); scale bar 50μm. Precondition media from wild type astrocytes had little or no effect on motor neuron survival (*upper panels*); quantified (**b**). Preconditioned medium from SOD1^{G93A} astrocytes induced non-cell autonomous motor neuron cell death (*middle*); quantified (**b**). Preconditioned medium from heterozygous-null α2-Na/K ATPase; SOD1^{G93A} astrocytes protected motor neurons against the non-cell autonomous cell death (*lower panel*); All data in bar charts show mean ± s.e.m (***$p<0.001$; unpaired t-test).

Fig. 1a

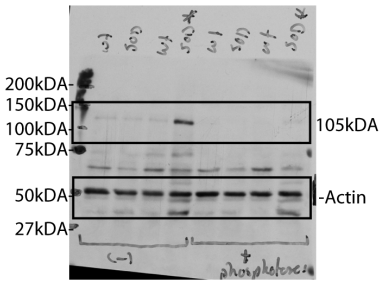


Fig. 1b

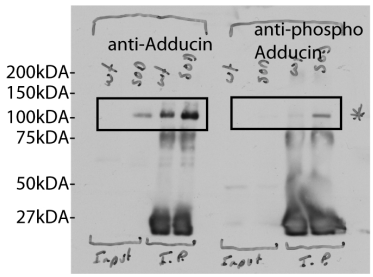


Fig. 1c

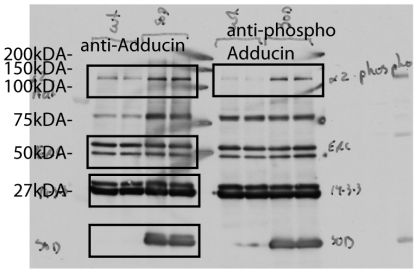


Fig. 1d

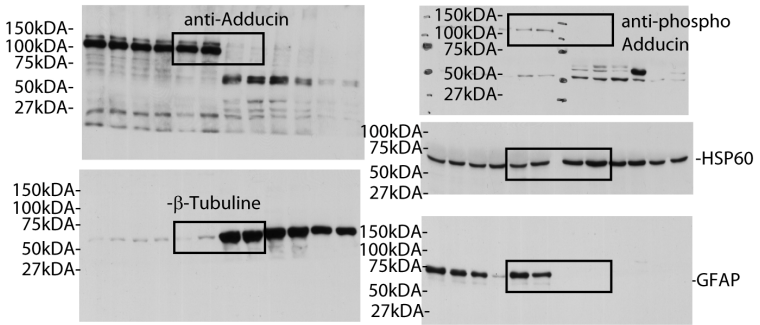


Fig. 3a

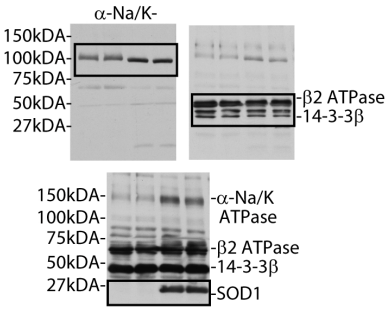


Fig. 3b

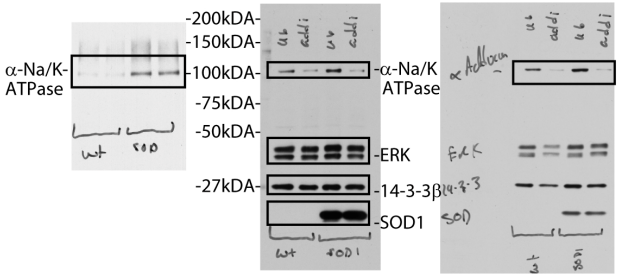


Fig. 6a

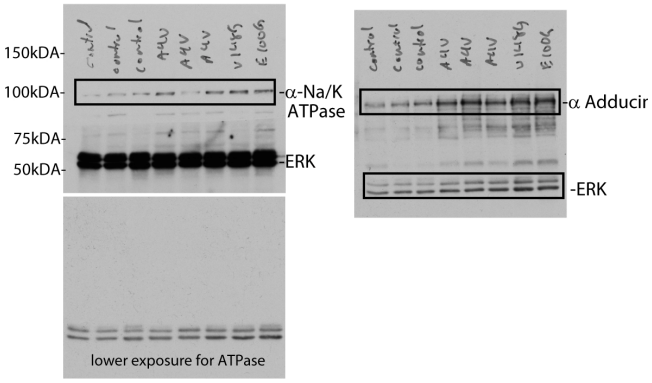
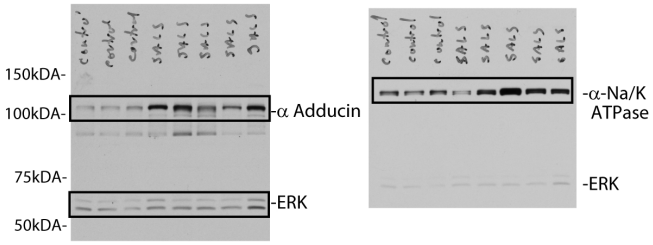
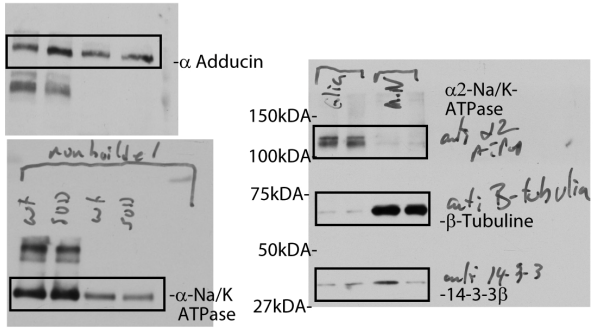


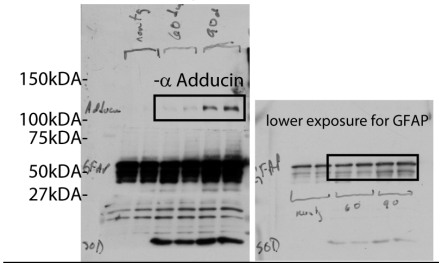
Fig. 6d



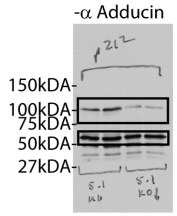
Supplementary Fig. 7a



Supplementary Fig. 1



Supplementary Fig. 2



Supplementary Figure 11

Full scans of key Western blot data. In many experiments, membranes were stripped and reblotted with a second antibody.