Supplementary Data

Supplementary Fig. 1. *Loa* mutation in dynein alters the sedimentation of mutant SOD1 in the spinal cord of $SOD1^{G93A}$/*Loa* mice. Spinal cord homogenates of equal protein contents prepared from $SOD1^{G93A}$ and SOD1G93A/*Loa* littermates at P120 (late stage of disease) were sedimented in 5-40% continuous sucrose gradient by centrifugation at 237,000 x *g* for 4 hrs at 4°C, then 16 equal-volume fractions were collected from top to bottom and analyzed by SDS-PAGE and western blotting detecting for SOD1. The bands representing the endogenous and transgenic $SOD1^{G93A}$ are indicated. We did not observe any significant alteration in the sedimentation of the endogenous mouse SOD1 in SDG centrifugations.

Supplementary Fig. 2. *Loa* mutation in dynein does not affect the level of $SOD1^{G93A}$ protein in $SOD1^{G93A}$/*Loa* mice. Three samples containing 1, 2 or 5 μg protein, of the brain homogenates used in sucrose sedimentation assays and prepared from $SOD1^{G93A}$ and $SOD1^{G93A}$/*Loa* littermates at P120 (late stage of disease) were analyzed by SDS-PAGE and western blotting detecting for SOD1 and α-tubulin as a loading control.

Supplementary Fig. 3. *Loa* mutation reduces the amount of mutant SOD1 in sub-cellular fractions containing mitochondria and ER microsomes. Brain homogenates of equal protein contents prepared from *A*, $SOD1^{G93A}$ and *B*, $SOD1^{G93A}$/*Loa* littermates at P120 were sedimented in 5-40% continuous sucrose gradient (as described in Figure 2) and analyzed by SDS-PAGE and western blotting detecting for SOD1and Cox4, calnexin and TGN38 as markers for mitochondria, ER and trans-Golgi network, respectively.

Supplementary Fig. 4. Organelle-associated $SOD1^{G93A}$ is largely protease- and detergent-resistant. *A*, Samples containing equal protein amounts of the BDG fraction 1 prepared from brain cortex of $SOD1^{G93A}$ and $SOD1^{G93A}$/*Loa* littermates at late-stage disease were incubated with 100 μg/ml proteinase K (PK) with or without 0.5% Triton X-100 for 15 min at room temperature then PK was inactivated by incubating the reactions with 10 mM PMSF for 10 min on ice. Digestions were analyzed by SDS-PAGE and immunoblotting detecting for calnexin, TGN38, Cox4 and SOD1. *B*, Samples containing equal protein amounts of the BDG fractions 1 and 3 prepared from spinal cords of SOD1G93A andSOD1G93A/*Loa* littermates at the late-stage of disease were incubated with 100 μg/ml PK with or without1% Triton X-100 and 1% SDS for 15 min at room temperature then PK was inactivated as in (A). Digestions were analyzed by SDS-PAGE and immunoblotting detecting for SOD1.

Supplementary Fig.5. Immunoprecipitation of the dynein/dynactin complex and mutant SOD1 from brains of four end-stage SOD1G93A mice. Brain homogenates from four different transgenic mice were subjected to IP using antibodies against dynein, dynactin and SOD1 linked with different solid supports. Samples of homogenate input (IN), supernatant after precipitation (S), last wash (W) and the IP product were analyzed by SDS-PAGE and western blotting. *A,* Brain homogenate from an end-stage SOD1transgenic mouse (125 days old) was incubated with protein A sepharose beads only (IP^-) or beads linked with polyclonal anti-SOD1, mouse monoclonal anti-p150 or mouse monoclonal anti-DIC antibodies. *B,* Brain homogenate from a second end-stage transgenic mouse was incubated with beads only or beads linked with mouse monoclonal anti-SOD1, polyclonal anti-SOD1 or mouse monoclonal anti-p150. *C,* Brain homogenate from a third end-stage transgenic mouse was incubated with beads only or beads linked with anti-DIC or anti-SOD1 antibodies. *D*, Brain of a fourth end-stage transgenic mouse was divided into three parts and homogenized in three different homogenization buffers (*a*, PBS; *b*, PBS without Ca2+ and Mg2+; or *c*, 50mM Tris, pH 8.1, 150mM NaCl, 5mM EDTA, 1% Triton X-100). The brain homogenates were incubated with anti-DIC antibody. Some of the brain homogenate prepared in buffer *b* was also incubated with excess of anti-DIC antibody (*b*+) to IP all dynein present in the sample.

Supplementary Fig. 6. Sample preparation and buffers used for immunoprecipitation do not affect the dynein complex or the heterogeneity of mutant SOD1 protein in brains and spinal cords of $SOD1^{G93A}$ mice. Samples of the brain and spinal cord homogenates of three end-stage $SOD1^{G93A}$ mice prepared either in PBS or RIPA buffer for immunoprecipitation were analyzed on gradient (4-15%) non-denaturing and non-reducing acrylamide gels followed by immunodetection of dynein and SOD1, using anti-DIC and anti-SOD1 antibodies, respectively.

Supplementary Fig. 7. Immunoprecipitation of the dynein/dynactin complex and mutant SOD1 from spinal cords of non-transgenic and asymptomatic (118 days old) and symptomatic (131 days old) $SOD1^{G93A}$ mice. The IP procedure was conducted as described previously (19) in which the homogenates of spinal cords from non-transgenic (Non-Tg) and asymptomatic and symptomatic $SOD1^{G93A}$ mice were prepared in RIPA buffer then incubated with anti-DIC antibody or with the irrelevant anti-HA antibody as a negative control (IP^-). The IP products along with 1% or 0.2% of the input from non-transgenic and transgenic homogenates, respectively, were analyzed by SDS-PAGE and western blotting.

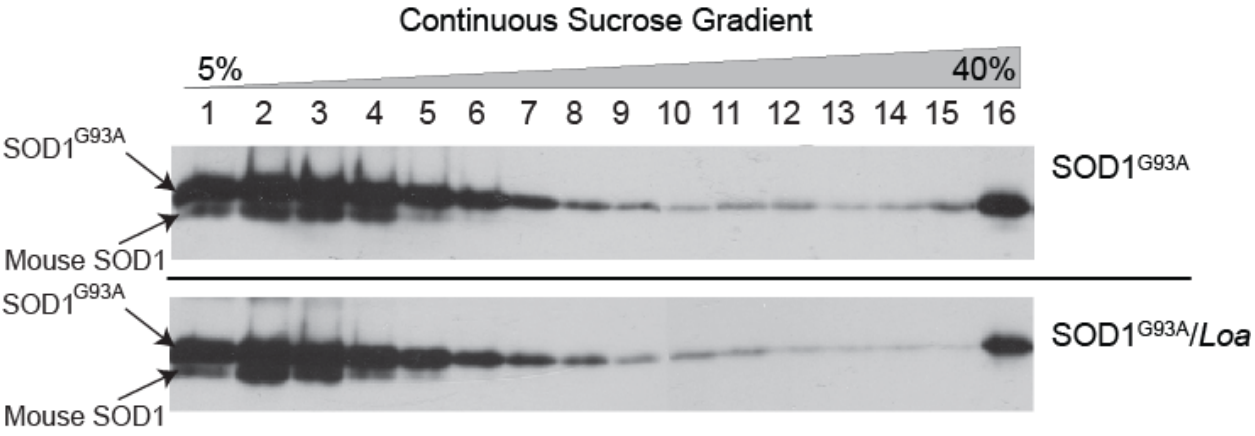
Supplementary Fig. 8. Effect of cross-linking on the immunoprecipitation of the dynein complex and mutant SOD1 from spinal cords of non-transgenic and $SOD1^{G93A}$ mice. The cross-linking procedure was carried out as recommended by the manufacturer and the IP procedure was conducted as described in reference (19). *A*, The homogenates of spinal cords from 100-day old non-transgenic (Non-Tg) and $SOD1^{G93A}$ mice were prepared in the cross-linking buffer (20mM HEPES, pH 7.4) and cleared by centrifugation. Equal amount of homogenate proteins were incubated on ice with or without 4 mM BS^3 for 2 hrs then Tris-HCl, pH 7.5 to a final concentration of 20 mM was added to both samples. After incubation on ice for further 30 min 10X RIPA buffer was added to a final concentration equivalent to 1X and the samples were incubated with beads linked with anti-DIC antibody. Some of the cross-linked homogenates were also incubated with the irrelevant anti-HA antibody as a negative control (IP^-). The beads were then washed three times with RIPA buffer. The IP products along with 1% of the input from non-transgenic and transgenic homogenates were analyzed by SDS-PAGE and western blotting. *B*, The experiment was repeated on a spinal cord from another 100-day old $SOD1^{G93A}$ mouse but without adding the cross-linker BS^3 to the samples and the immunoprecipitated complexes on beads were washed either three or four times with RIPA buffer as indicated in the figure. The IP products along with 0.2% of the input were analyzed by SDS-PAGE and western blotting.

Supplementary Fig. 9. GST-SOD1 Pull down. The open reading frames of human wild-type SOD1,$SOD1^{A4V}$, $SOD1^{G37R}$, $SOD1^{G85R}$ and $SOD1^{G93A}$ were amplified by PCR using forward and reverse primers containing restriction sites for *Bam*H1 and *Xho*1, respectively, and subcloned into the glutathione *S* transferase(GST) bacterial expression vector pGEX-4T-1 (Amersham). *E.coli* BL21 (DE3) bacteria were transfected with the GST-tagged SOD1 constructs or pGEX-4T-1 vector and the proteins were expressed by IPTG induction. After lysing the cells the expressed GST and GST-tagged SOD1 proteins were purified using S-linked glutathione agarose beads. The captured GST and GST-SOD1 proteins on beads were then incubated with brain homogenate prepared from a non-transgenic C57BL/6 mouse. Beads were then washed four times with homogenization buffer then heated in 1X SDS sample loading buffer at 95°C for 5 min. The pull-downs (lanes 4-8) were then analysed by SDS-PAGE and western blotting detecting for SOD1 and DIC. Some of the $SOD1^{G93A}$-containing bacterial lysate (lane 1) and brain homogenate (Br H.) from an $SOD1^{G93A}$ mouse (lane 2) and the non-transgenic mouse brain homogenate (lane 3) were also analyzed to serve as positive controls for GST-SOD1, $SOD1^{G93A}$ and DIC.

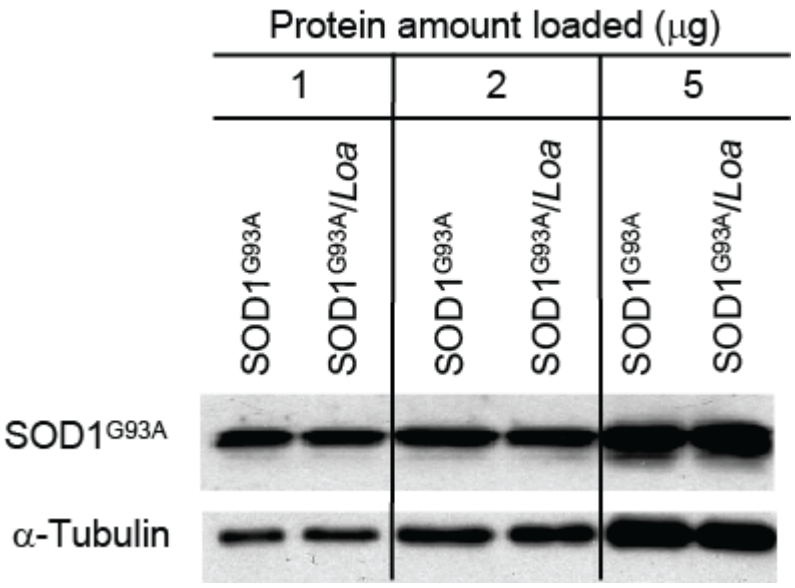
Supplementary Fig. 10. Immunoprecipitation of mutant SOD1 from brains of mouse embryos. Brain homogenates from E16.5 embryos of the four different genotypes (*Loa*/+, +/+, $SOD1^{G93A}$, and $SOD1^{G93A}$/*Loa*) produced by crossing $SOD1^{G93A}$ and *Loa*/+ mice were immunoprecipitated with anti-

SOD1antibody. Samples of homogenate input (IN), supernatant after precipitation (S), last wash (W) and the IP products were analyzed by SDS-PAGE and western blotting.

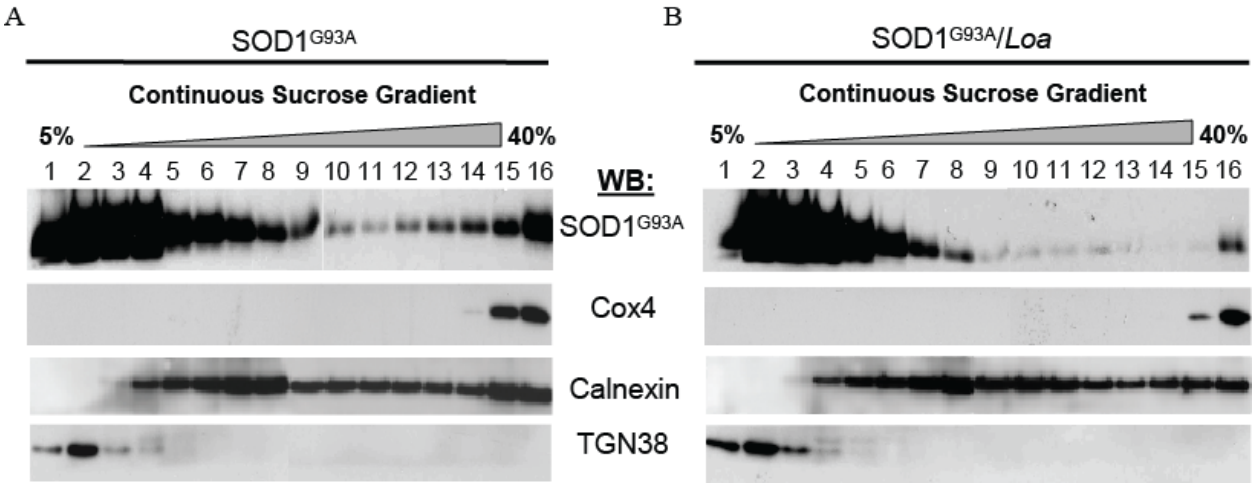
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



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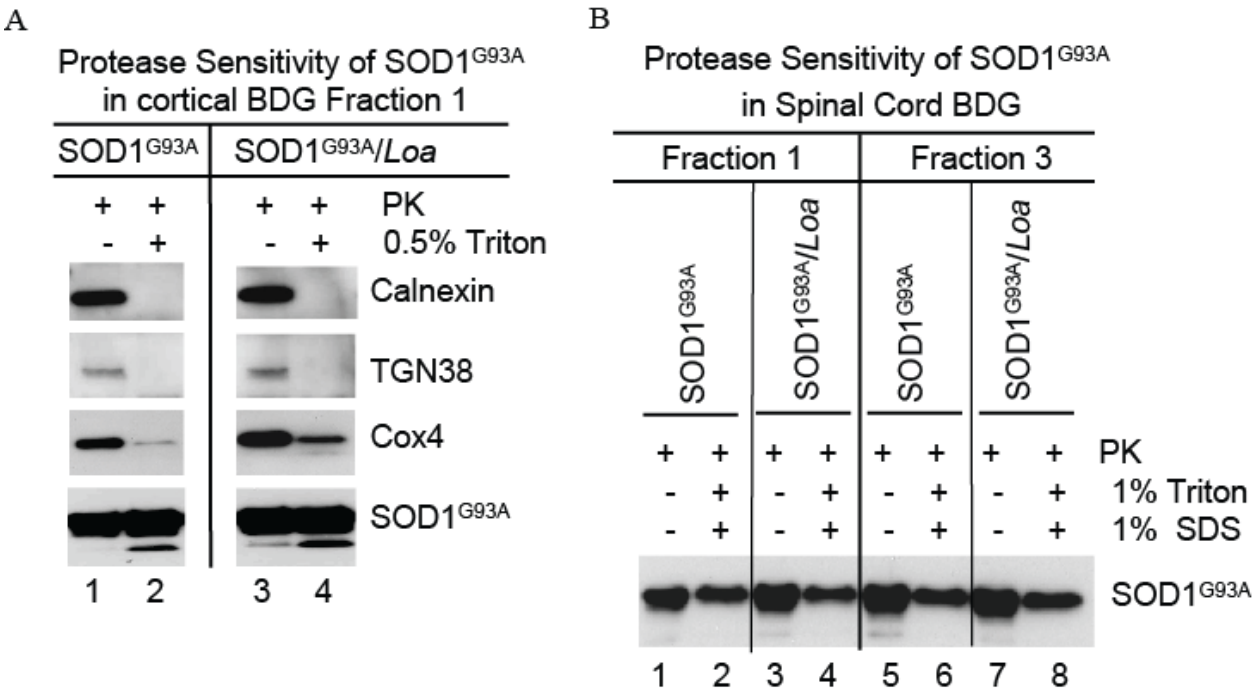
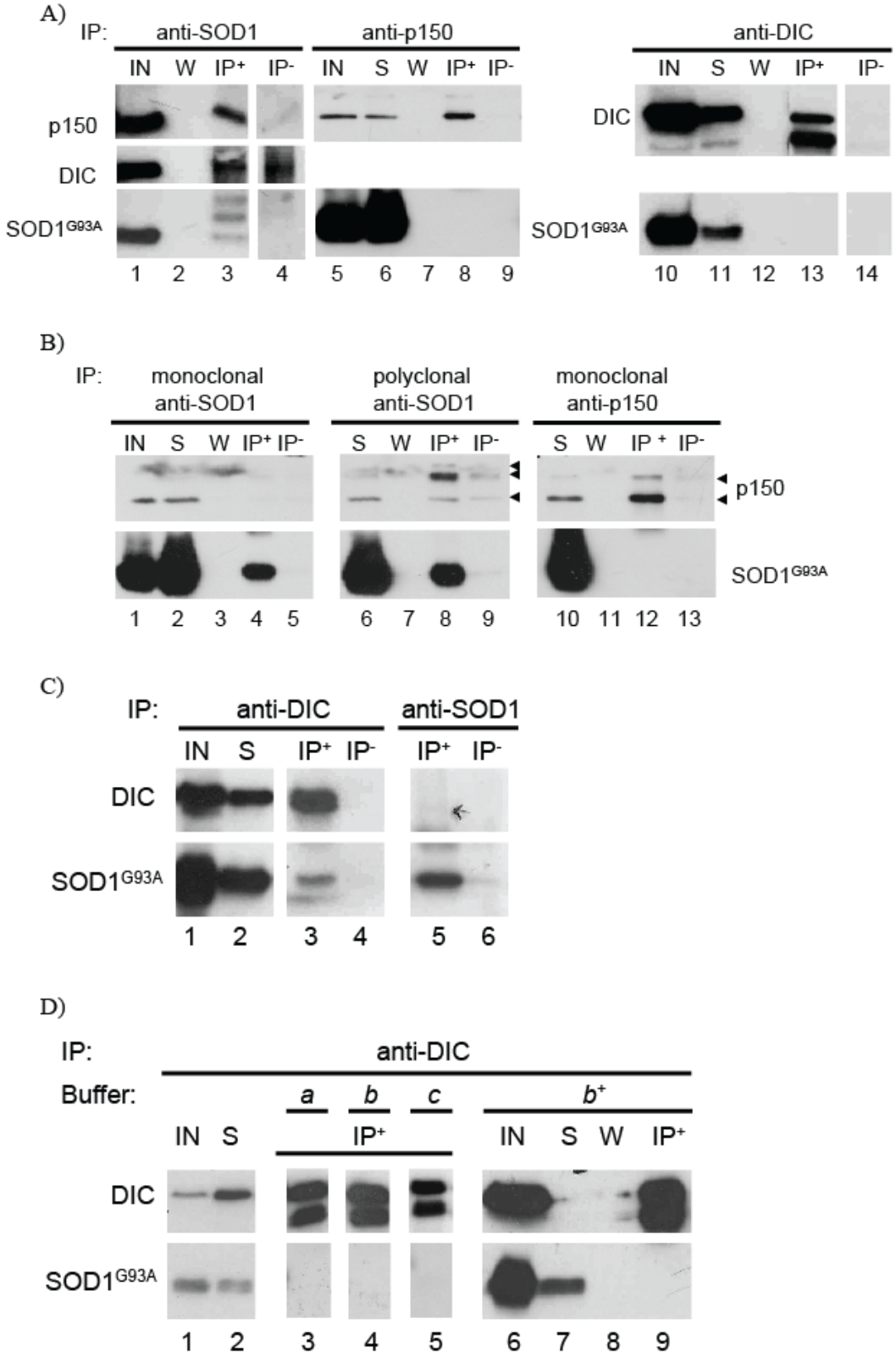


Supplementary Figure 5



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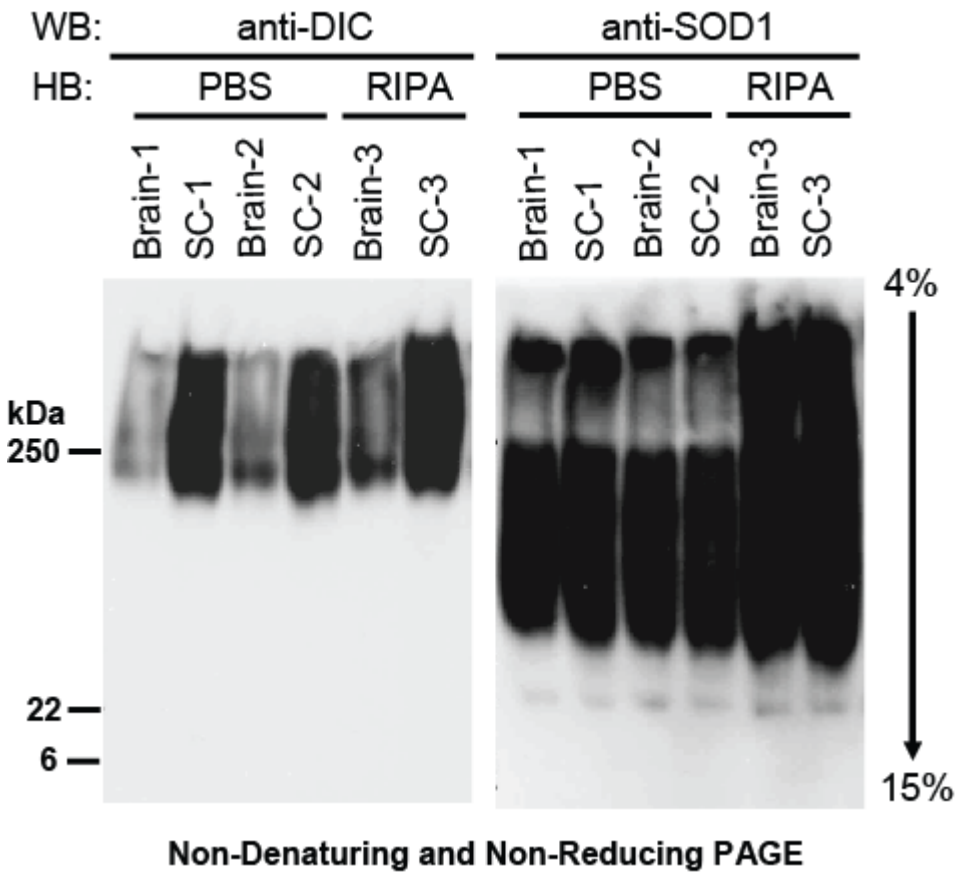
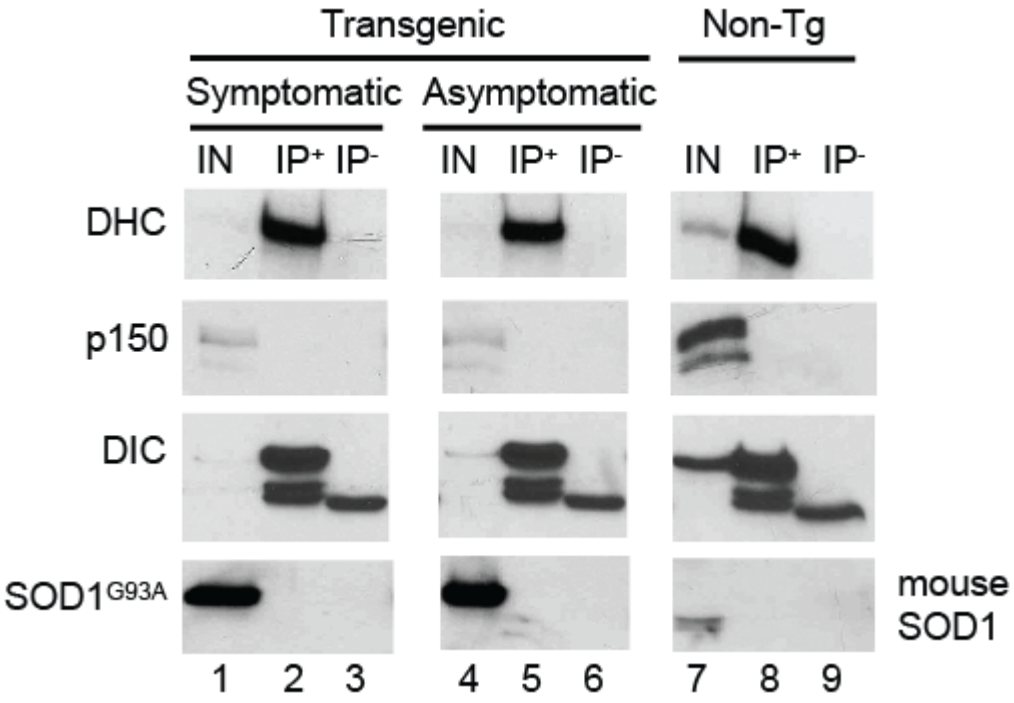
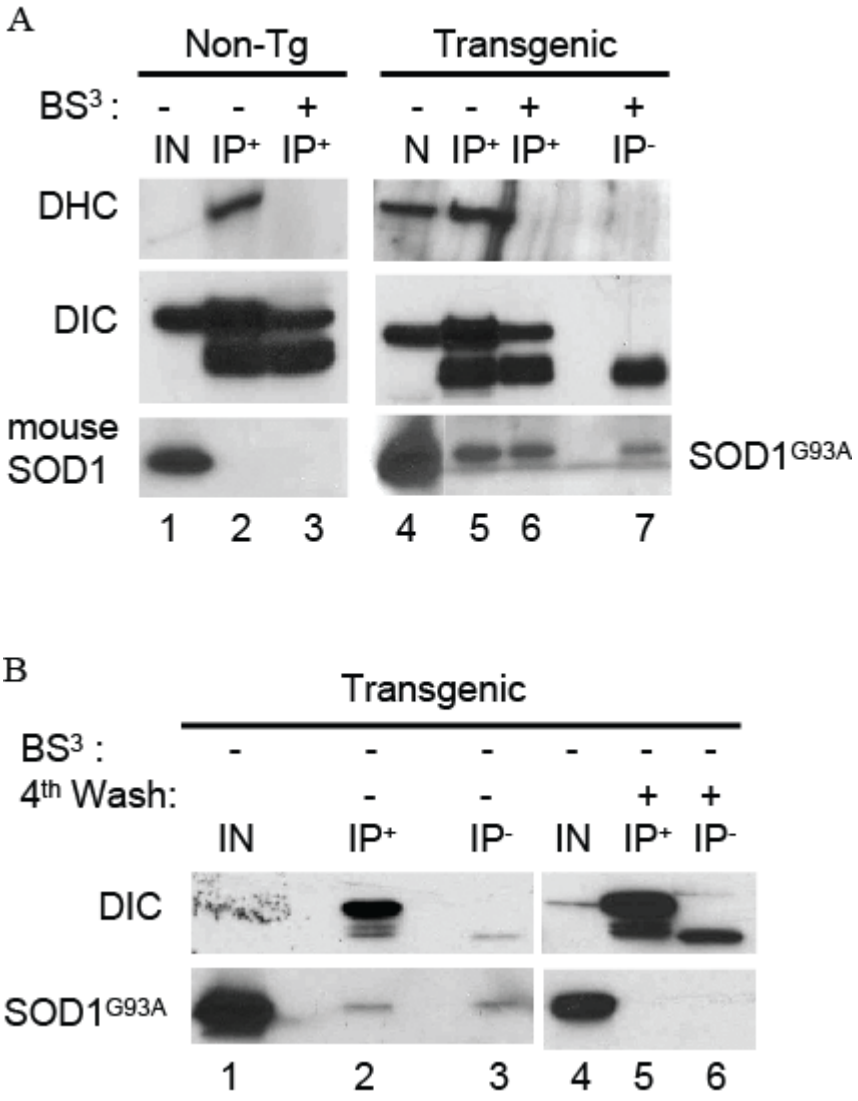


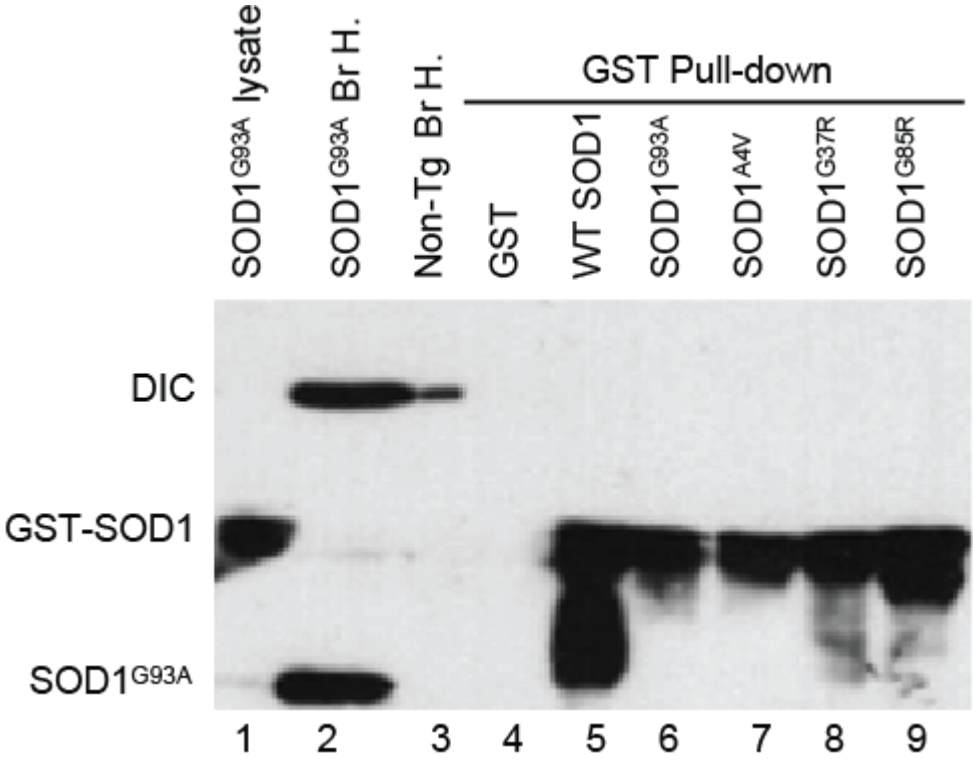
Supplementary Figure 7



Supplementary Figure 8



Supplementary Figure 9



Supplementary Figure 10