Substantially elevating the levels of αB-crystallin in spinal motor neurons of mutant SOD1 mice does not significantly delay paralysis or attenuate mutant protein aggregation

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Figure S1. Amino acid sequence alignment of mouse and human αB-crys.

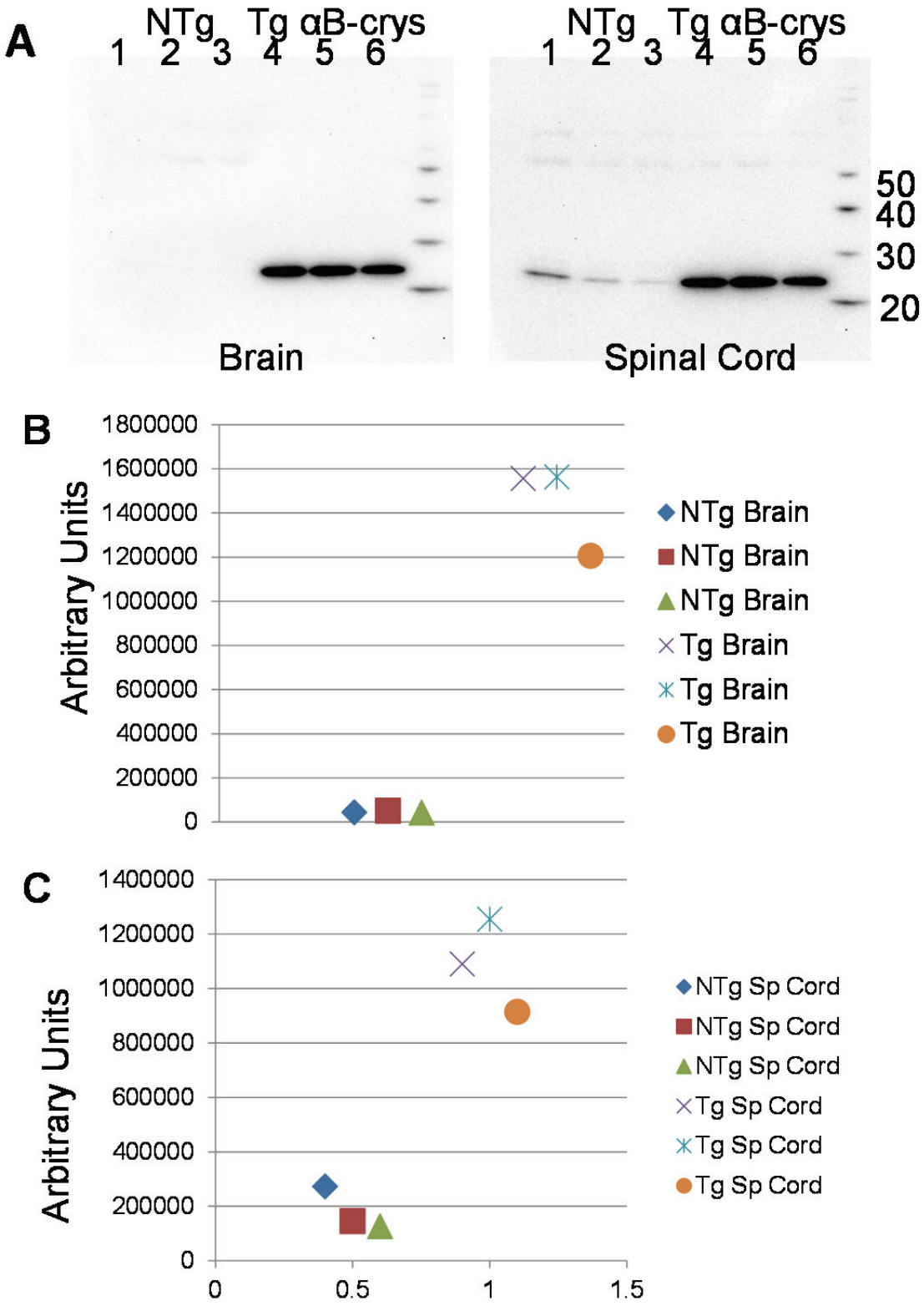

Figure S2. Quantification of αB-crys levels in αB-crys (Z104) transgenic mice. Immunoblots of αB-crys in the brain and spinal cord of Z104 transgenic mice (Panel A) were quantified using software provided with a FluorChem E imaging device (Protein Simple, Santa Clara, CA, USA). The intensity of each band was assigned an arbitrary unit by the software, indicative of the pixel values for each band. Homogenates from 3 animals were analyzed by SDS-PAGE and immunoblot before quantification. The increase in αB-crys levels in both brain and spinal cord were statistically significant ($p=0.00003$ and 0.001, respectively).

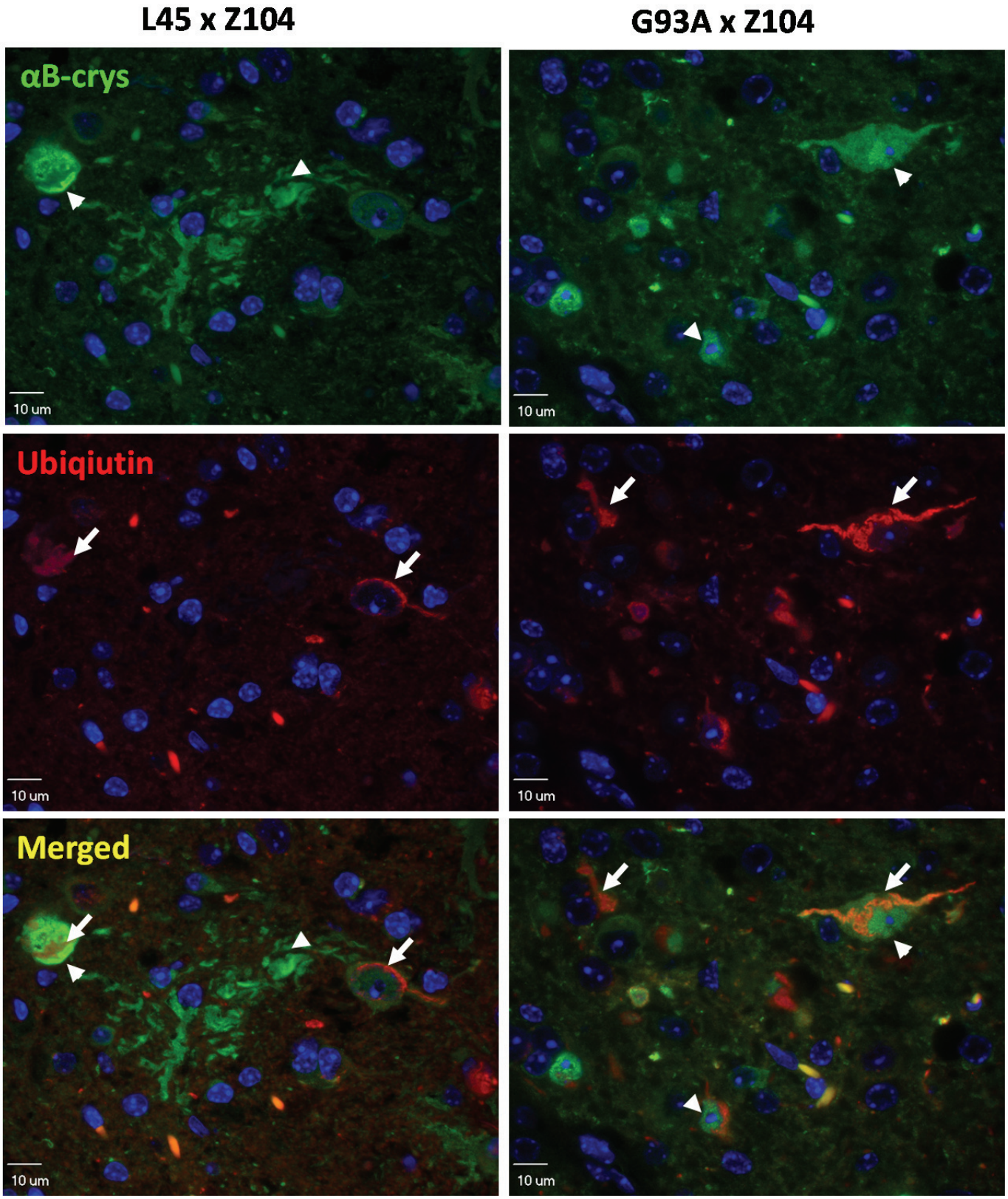

Figure S3. αB-crys does not exclusively co-localize with ubiquitin immunoreactive aggregates in spinal cords of paralyzed bigenic mice. Tissue sections from paralyzed bigenic mice were immunostained with antibodies to ubiquitin and αB-crys as described in Materials and Methods. We commonly observed ubiquitin immunoreactive inclusion-like structures in which αB-crys immunoreactivity did not seem to specifically co-localize even though the cell possessed diffusely distributed immunoreactivity.

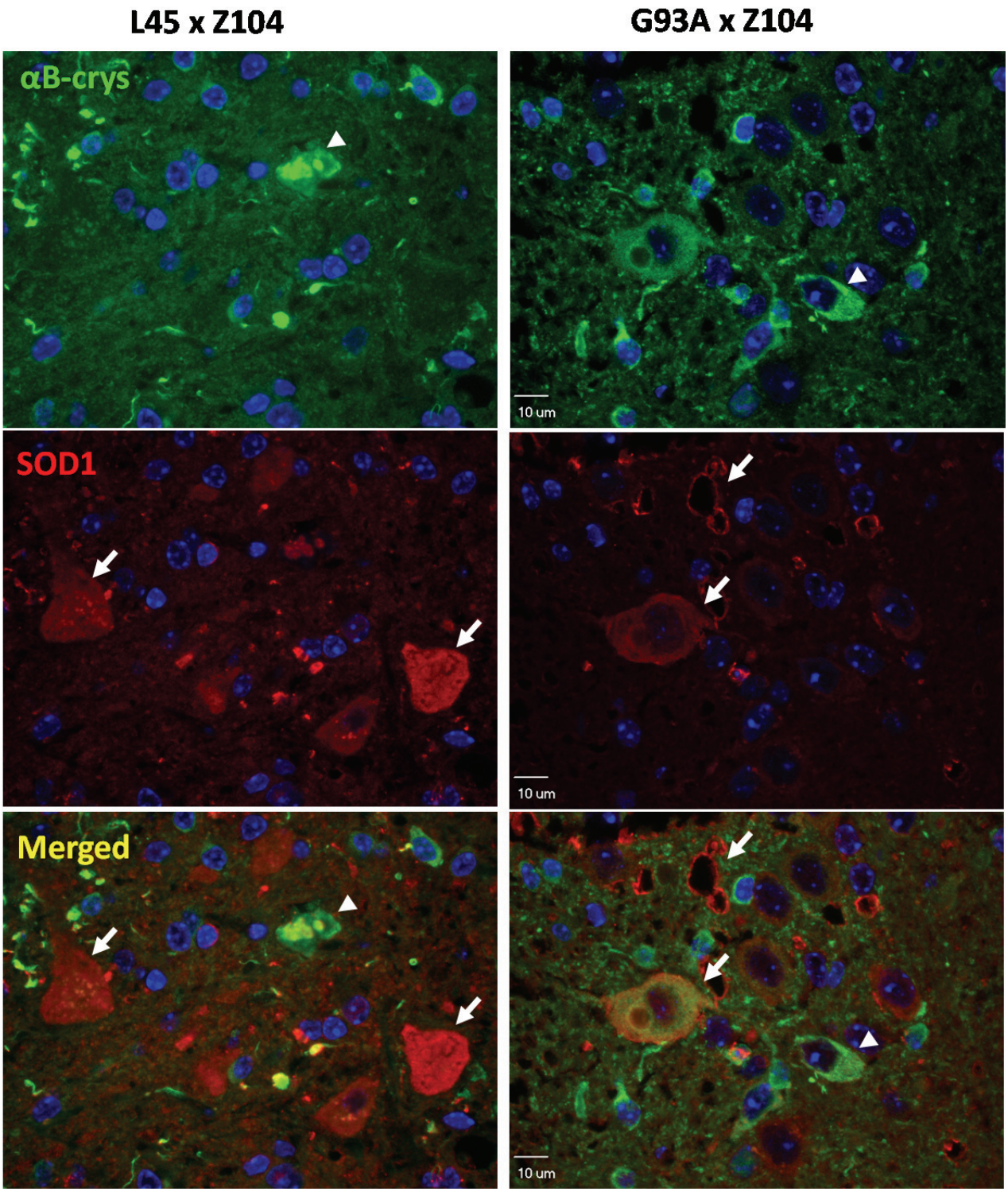

Figure S4. αB-crys does not exclusively co-localize with SOD1 immunoreactive inclusions in spinal cords of paralyzed bigenic mice. Tissue sections from paralyzed bigenic mice were immunostained with antibodies to SOD1 and αB-crys as described in Materials and Methods. In the L126Z/αB-crys bigenic mice, we commonly observed accumulations of αB-crys immunoreactivity that did not appear to contain mutant SOD1. In bigenic G93A/αB-crys mice, we commonly observed vacuoles rimmed withSOD1 immunoreactivity that were not similarly rimmed by αB-crys immunoreactivity. Neurons in which mutant SOD1 accumulated did appear to co-express αB-crys but we could not discern specific co-localization.

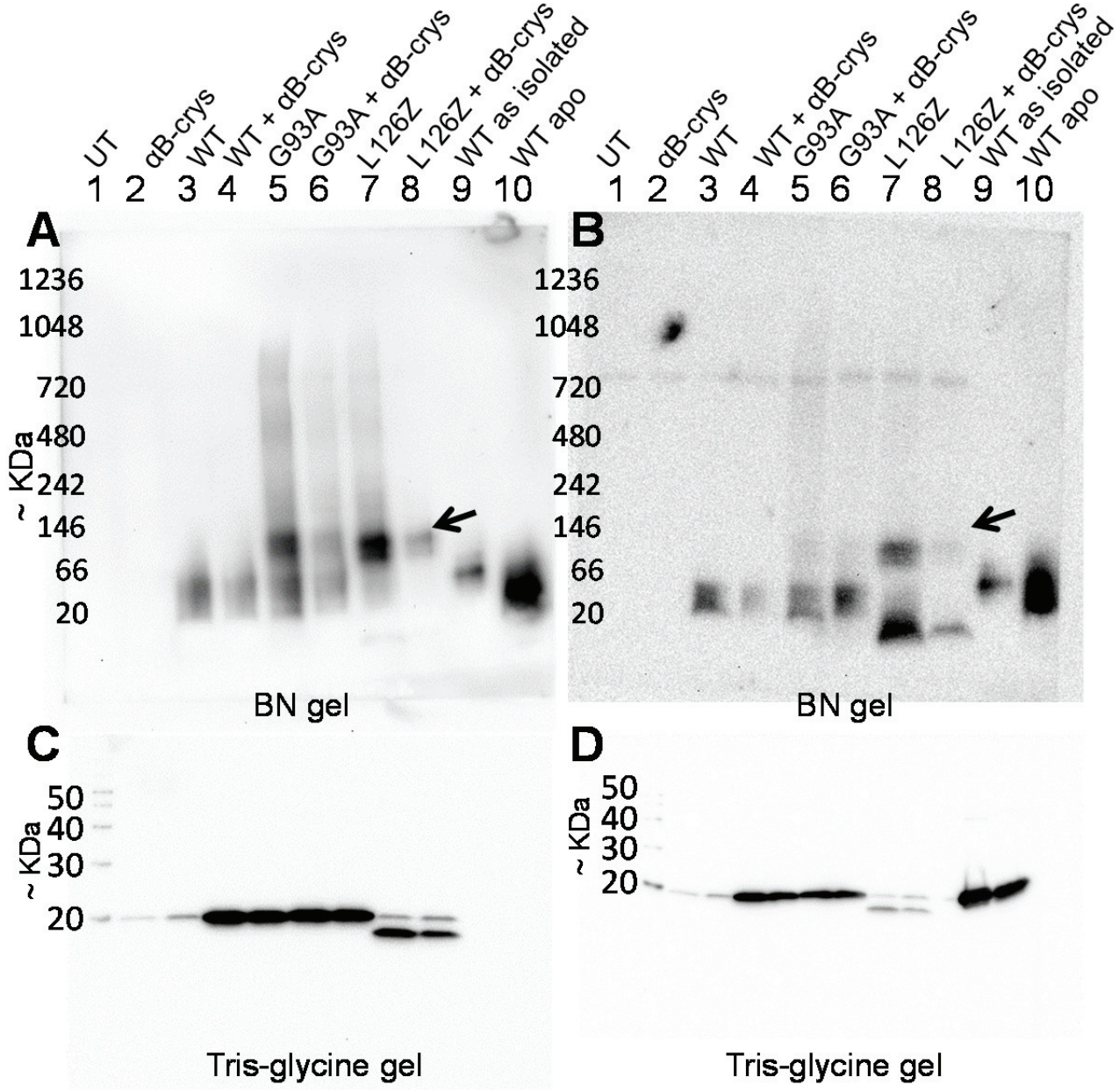

Figure S5. Effect of αB-crys over-expression on the oligomerization of G93A and L126Z SOD1 in cultured cells. As described in Materials and Methods, HEK293FT cells were transiently transfected with expression plasmids for SOD1 and αB-crys as noted above the image. The image shown is representative of at least 3 independent replications.

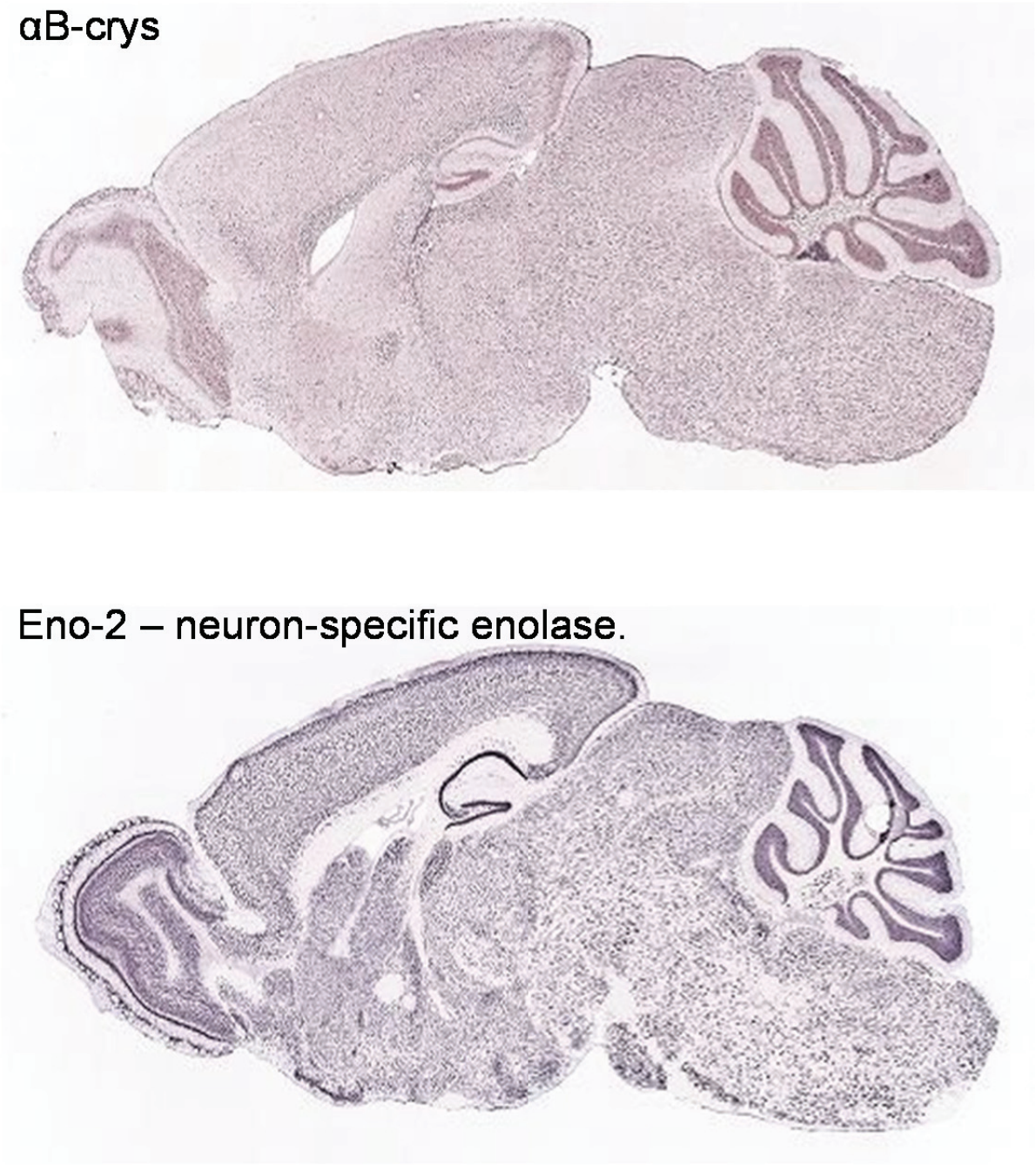

Figure S6. Captured image of in situ hybridization for αB-crys mRNA in the brains of young C57BL/6J mice (www.brain-map.org).

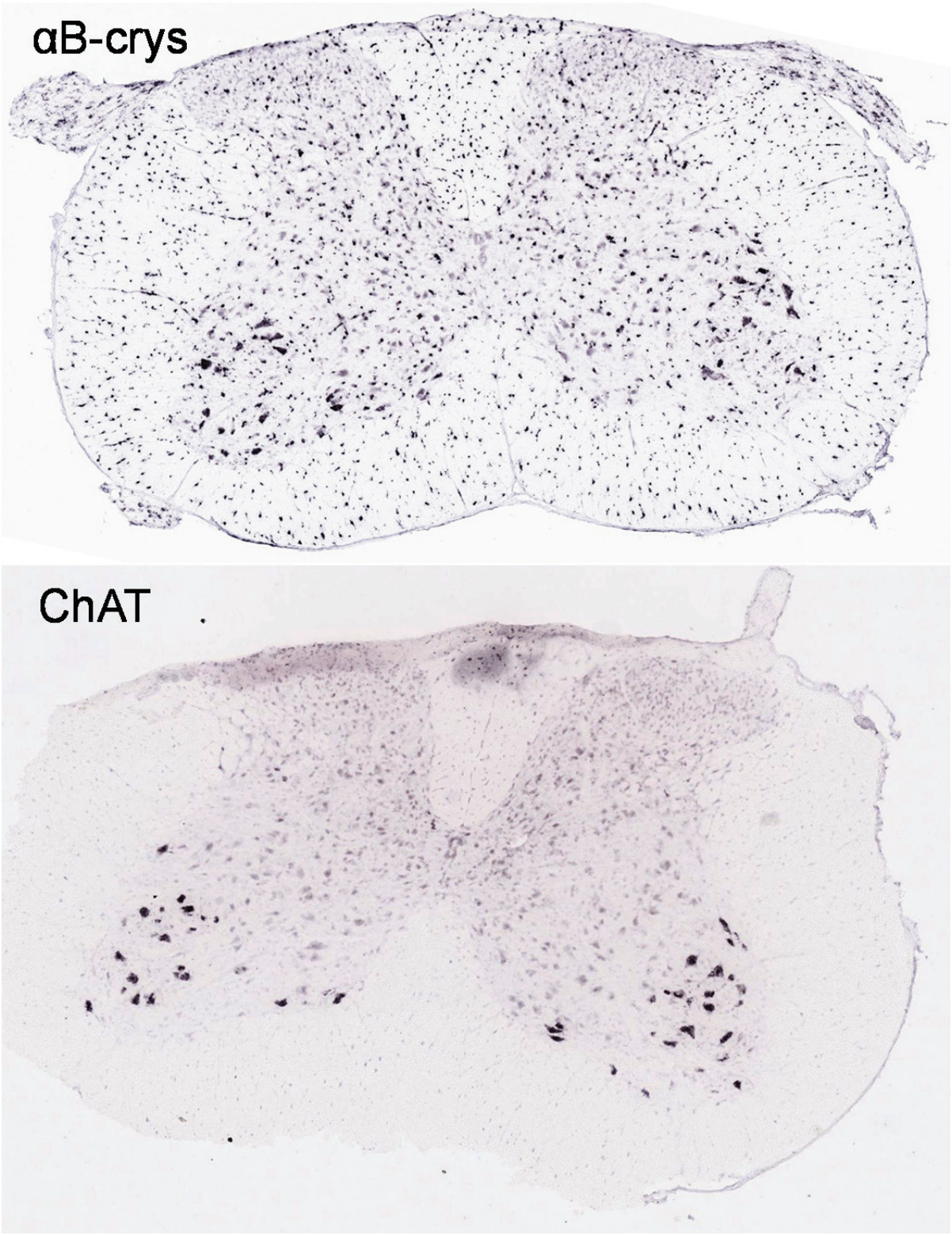

Figure S7. Captured image of in situ hybridization for αB-crys mRNA in the spinal cords of young C57BL/6J mice (www.brain-map.org).