Supporting Information

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SI Text

A fraction of anti–microtubule-associated protein 1 light chain 3 (LC3)-immunostaining puncta were costainable with LAMP1 antibody, reflecting lysosomal localization, whereas the non-colocalizing puncta are likely, based on previous studies (1), to be associated with autophagosomes. The fraction of LC3 puncta colocalizing with LAMP1 did not seem different between motor neurons from WT Cu/Zn superoxide dismutase (SOD1)YFP and G85R SOD1YFP.

Note that we did not perform Western blots for LC3 or sequestosome 1 (SQSTM1) on whole spinal cord from the mouse strains because motor neurons are well under 5% of the total cells in a cord. Western analysis would thus comprise a convolution of cell types, mainly astrocytes and other neuron types, so we would not be able to interpret what cell types are contributing to any particular result. In addition, a motor-specific result might not be observable because the "signal" might be too small or buried beneath signals from other cell types.

1. Kabeya Y, et al. (2000) LC3, a mammalian homologue of yeast Apg8p, is localized in autophagosome membranes after processing. *EMBO J* 19(21):5720–5728.

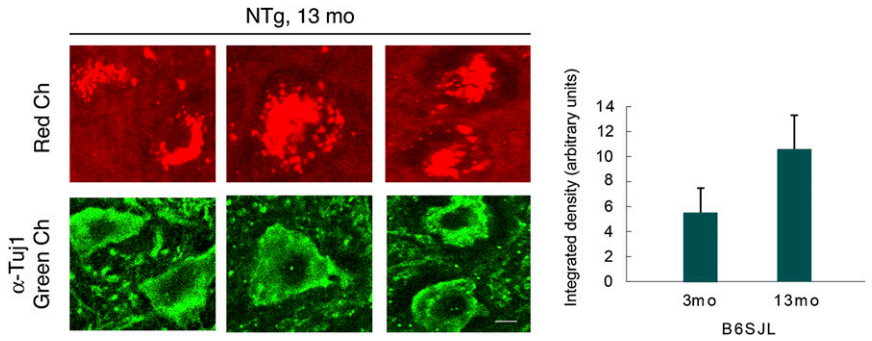

Fig. S1. Increase in lipofuscin auto-fluorescence intensity with age in nontransgenic mice. Spinal cord motor neurons from a 13-mo-old nontransgenic mouse (strain B6SJL) were identified by their position in the ventral horn and by staining with anti-Tuj1 antibody and Alexa Fluor 488 secondary (Green Ch). Corresponding auto-fluorescent images (Red Ch) are shown. Auto-fluorescence images were acquired with the same microscope settings as used for sections shown in Fig.1 for a 3-mo-old nontransgenic animal. Total intensity of auto-fluorescence (integrated density) for each cell body was calculated using a function in ImageJ (*Materials and Methods*). The bar graph on the right shows the mean and SD of this value for 26 cells (3 mo) and 29 cells (13 mo). Both the images on the left and the bar graph suggest that the amount of lipofuscin in neuron cell bodies increases with age of the animal. (Scale bar, 10 μm.)

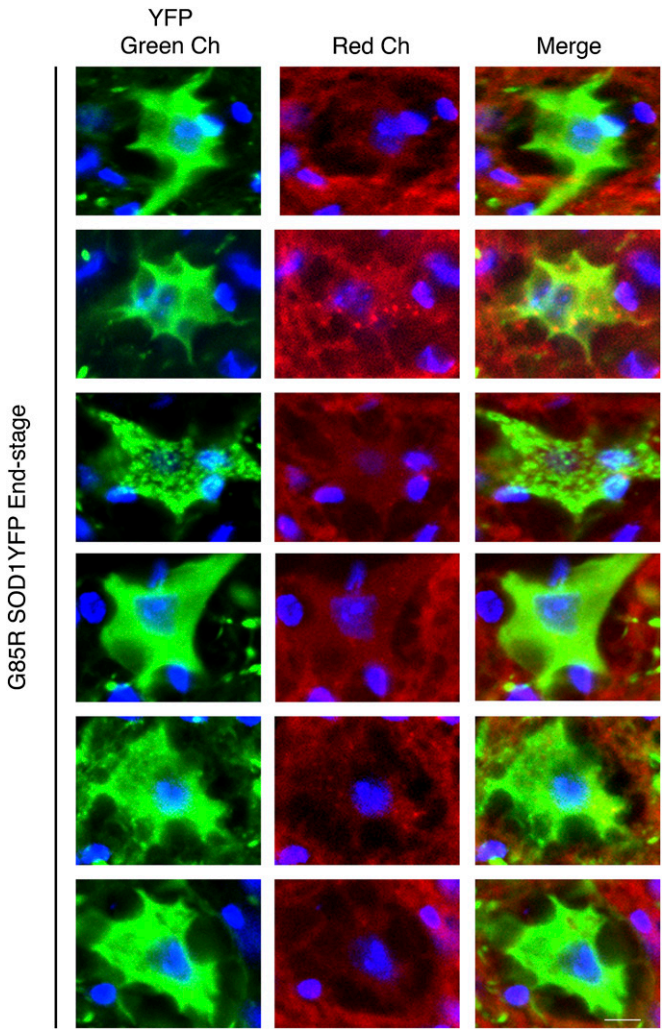

Fig. S2. Lipofuscin auto-fluorescence is strongly reduced or absent in remaining motor neuron cell bodies at the end-stage of neurodegeneration and paralysis in G85R SOD1YFP mice. Six motor neuron cell bodies identified in the ventral horn of the spinal cord of a 5-mo-old G85R SOD1YFP mouse that was paralyzing at the end-stage of its disease were imaged in the green channel for YFP and the red channel for lipofuscin auto-fluorescence; DAPI staining is overlaid in blue. As in presymptomatic 3-mo-old animals of this genotype, most cells have very few or no lipofuscin puncta (Fig. 1). (Scale bar, 10 μm.)

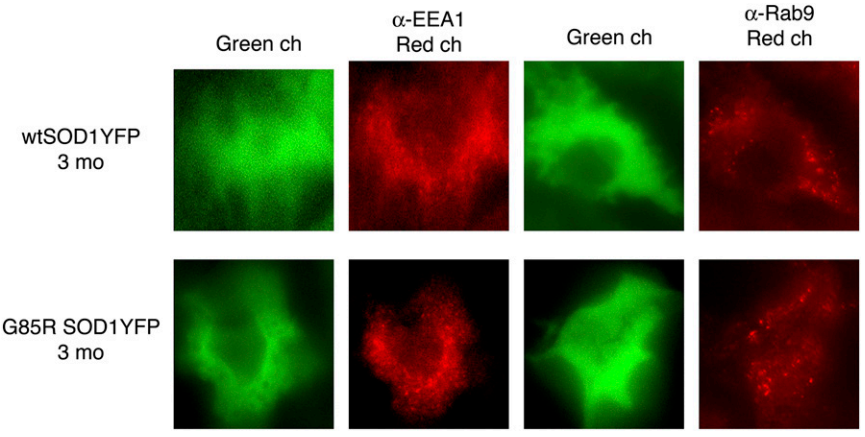

Fig. S3. Endocytic marker immunostaining is unaffected comparing 3-mo-old WT SOD1YFP with ALS G85R SOD1YFP spinal cord motor neurons. Spinal cord sections were prepared and stained using anti-EEA1 (early endosomal marker; mouse mAb 610457, BD Biosciences, 1:100) or anti-RAB9 (late endosomal marker; rabbit mAb 5118 Cell Signaling, 1:100) and an Alexa Fluor 568 secondary antibody used as detailed in *Materials and Methods*. Representative images of motor neuron cell bodies from each strain are shown next to the corresponding YFP staining pattern of the neuron.

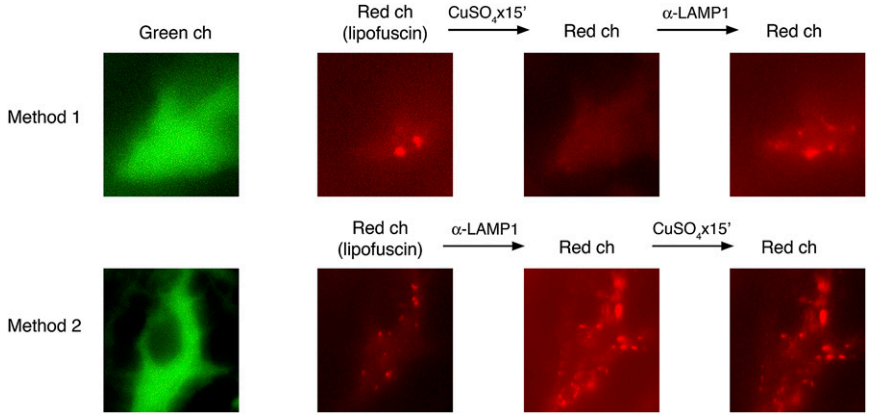

Fig. S4. Copper sulfate ablation of lipofuscin fluorescence can be accomplished by incubation before or after antibody directed against a lysosome-localizing protein, here LAMP1. In method 1, copper sulfate treatment is used first, ablating lipofuscin fluorescence, as shown in the red channel; following anti-LAMP1 immunostaining using an Alexafluor 568 secondary antibody, red puncta appear, two matching the location of the original lipofuscin fluorescence. In method 2, the reverse order of incubation is used. Note that the anti-LAMP1 pattern is extensive, indicating that copper sulfate did not abolish the antibody/Alexafluor 568 signal. Here also, the anti-LAMP1 pattern seems to include the puncta that were originally lipofuscin fluorescent lysosomes.

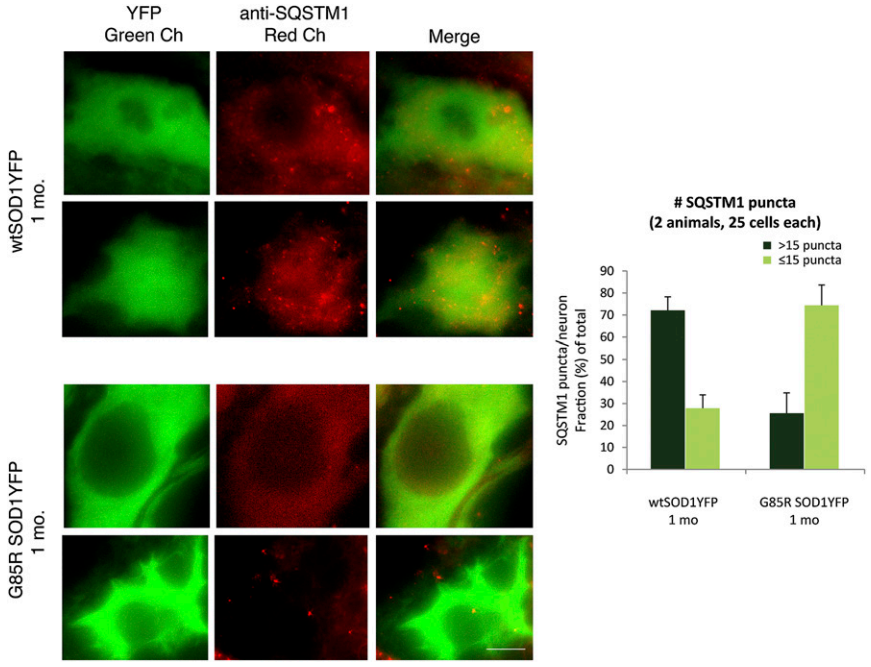

Fig. S5. Puncta of SQSTM1 immunostaining are reduced in G85R SOD1YFP animals compared with WT SOD1YFP even at 1 mo of age. Spinal cord sections were prepared from 1-mo-old WT and mutant SOD1YFP mice and stained using an anti-SQSTM1 antibody and an Alexa Fluor 568 secondary as detailed in *Materials and Methods*. Two representative images of motor neuron cell bodies from each strain are shown (*Left*). (Scale bar, 10 μm.) The bar graph (*Right*) shows the quantitation of the fraction of cells with >15 and ≤15 puncta in the two strains for 25 cells from each of two animals. As observed for the 3-mo-old animals (Fig. 3), most WT cells have a large number of puncta, whereas a similar fraction of mutant cells have few puncta. Note that the SQSTM1 staining in the images from the 1-mo-old animals seems fainter and the puncta seem smaller, even in the WT animal, than in images from 3-mo-old animals (compare images in Fig. 3). This observation was consistent across all animals and all cells examined.

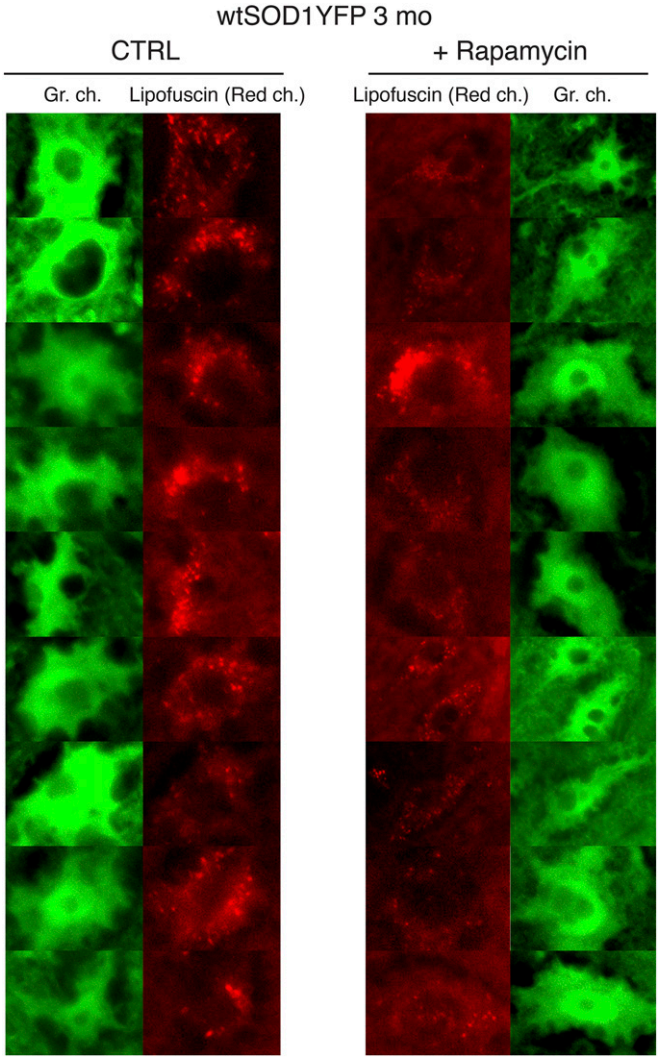

Fig. S6. Rapamycin treatment of WT SOD1YFP mice reduces lipofuscin staining of spinal cord motor neuron cell bodies. Three-month-old mice were treated ($n = 3$) with rapamycin, 5 mg/kg daily, or with vehicle only (CTRL; $n = 3$) for 2 wk and then spinal cord sections were prepared and imaged in the green or red channels, as described.