Manuscript Title:

Glutathionylation potentiates benign superoxide dismutase 1 variants to the toxic forms responsible for amyotrophic lateral sclerosis progression

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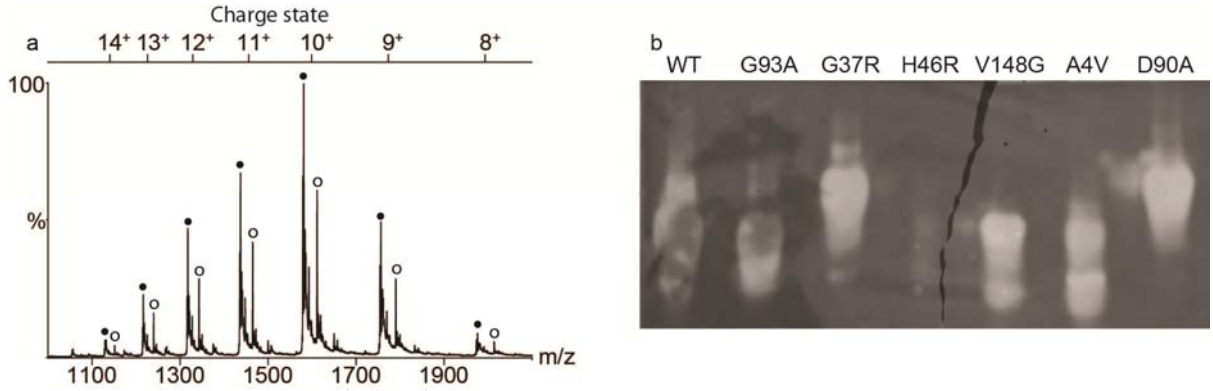
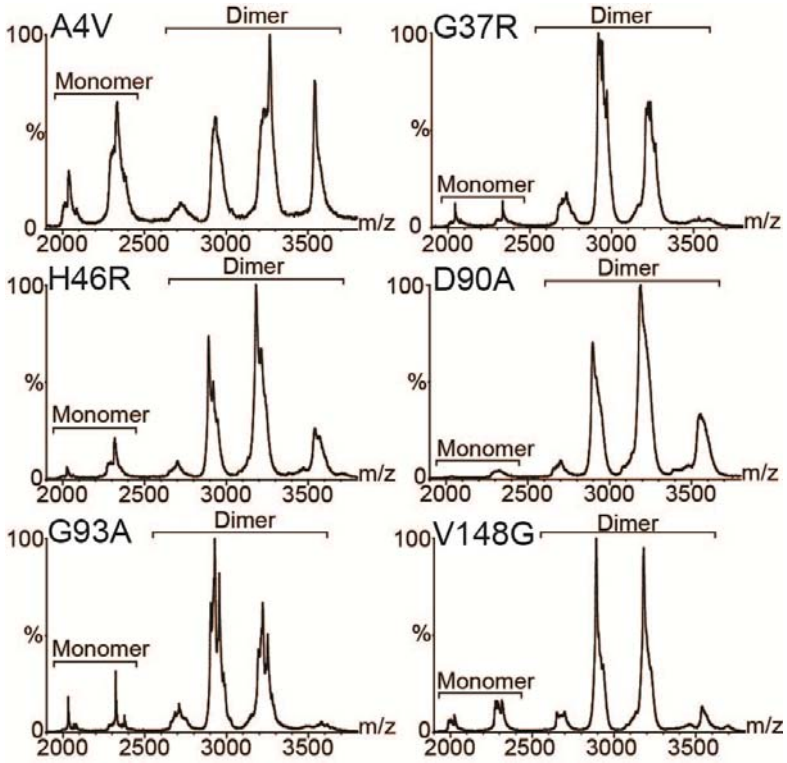
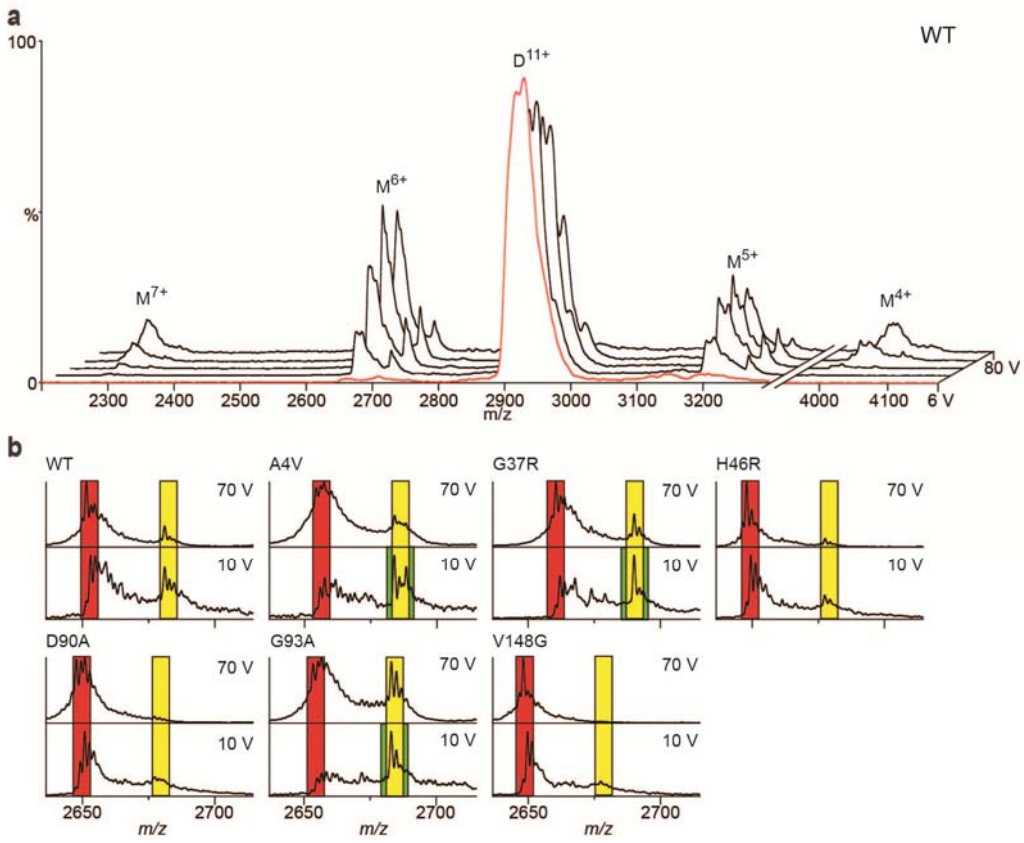


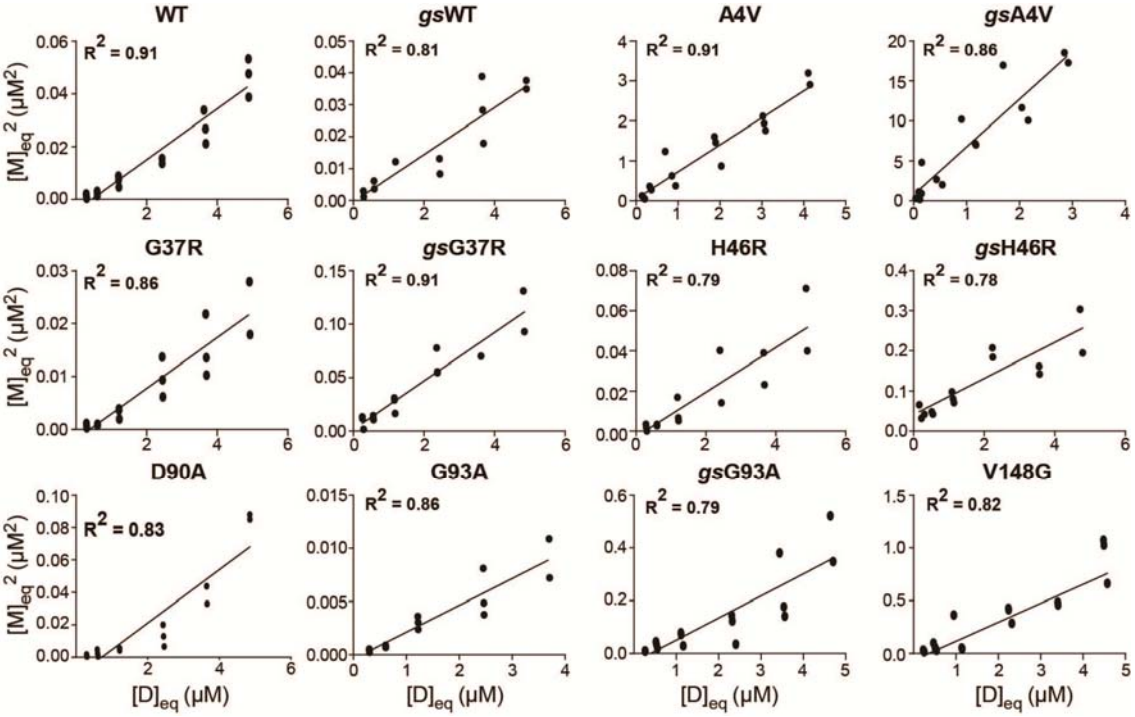
Supplementary Figure 1 | Mass analysis of SOD1 and in-gel-zymography. (**A**)SOD1 was fully unfolded in acidic solvent before mass analysis for the accurate mass identification of covalent modifications. The two major species observed were *u*SOD1 (closed circle) and *gs*SOD1 (open circle). (**B**) Native-PAGE electrophoresis of SOD1. The gel was assayed for dismutase activity. Dismutase activity is denoted by achromatic bands.



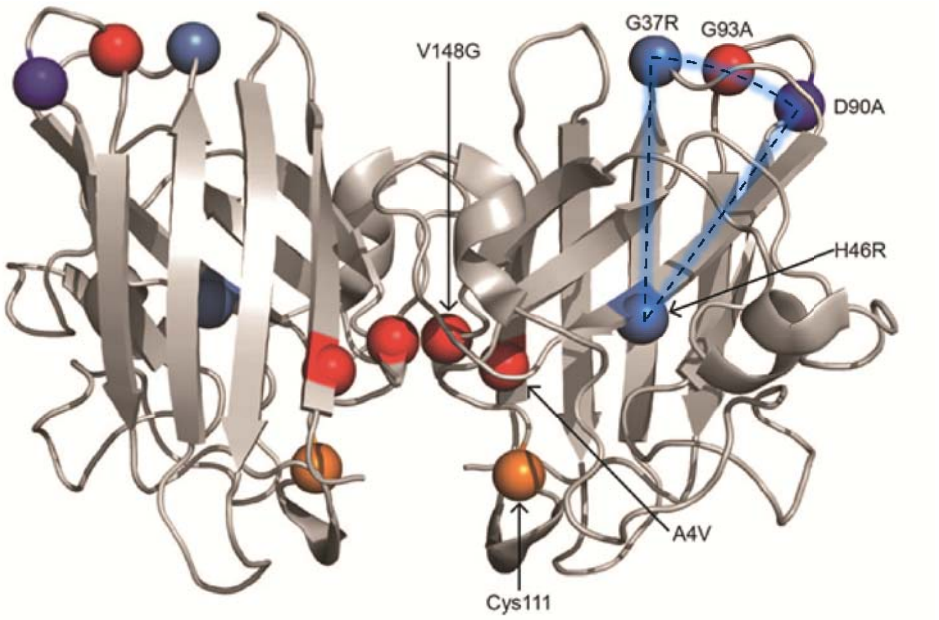
Supplementary Figure 2 | Mass of native SOD1 variants. SOD1 variants were analysed using mass spectrometry under gentle conditions to maintain their native state. All SOD1 variants showed an abundance of dimer in the 10+ and 11+ charge states, with substantially lower abundance in the 9+ and 12+ charge states. Solution phase monomer was confined to the 7+ and 8+ charge states.



Supplementary Figure 3 | Tandem mass spectrometry of SOD1 variants. (**a**) Progressive dissociation of the 11+ charge state of the dimer resulted in the evolution of monomer primarily in the 6+ and 5+ charge states, with minor amounts in 7+ and 4+ charge states. (**b**) Analysis of the M^{6+} ion at collision energies of 10 and 70 V, comparing *u*SOD1 (red) to *gs*SOD1 (yellow). Variants that have a greater *gs*SOD1 ion abundance relative to *u*SOD1 at 10 V (i.e., *gs*SOD1 subunit dissociation is more facile) are marked with a green border (A4V, G37R, and G93A).



Supplementary Figure 4 | Determination of K_d values. Plots of $[D]_{eq}$ against $[M]_{eq}^2$ revealing K_d values for *u*SOD1 and *gs*SOD1 variants. Least squares regression (R^2) is shown. K_d is derived from the line of best fit gradient (See supplementary table 1.)



Supplementary Figure 5| Location of mutations on the SOD1 dimer. Residues are colour coded according to severity of disease progression associated with the mutation. Red represents rapid disease progression (< 5 years), and blue represents slow (>5 years). Residue Cys111 is marked orange. (PDB file: 2V0A adapted from Strange et. al. 2007). Residues of the mutants tested, which have wildtype-like K_d prior to glutathionylation, are seen to be arranged in a two-dimensional molecular plane orthogonal to the β-barrel polar axis.