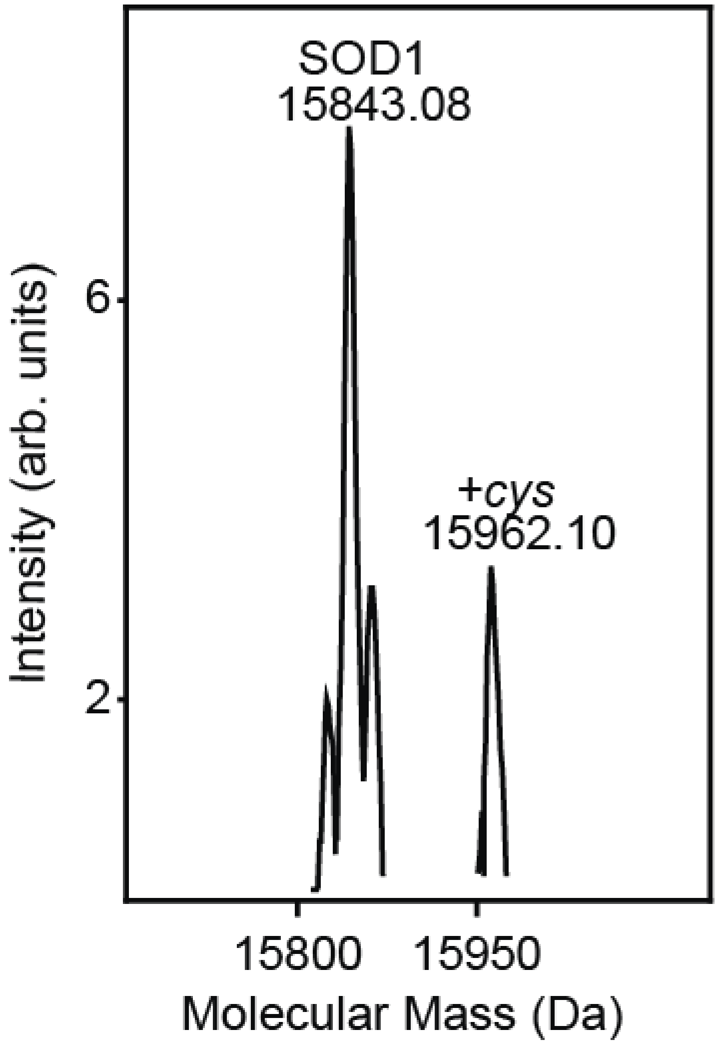
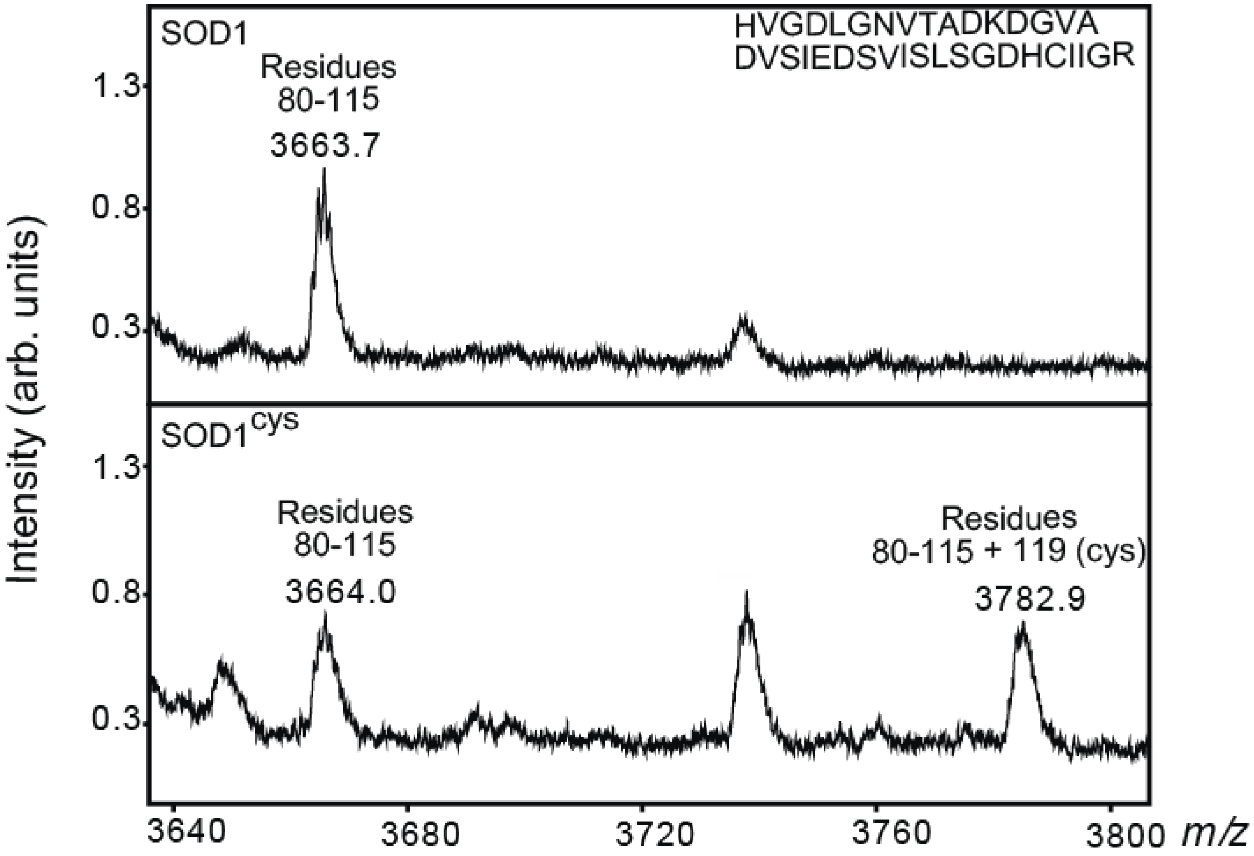
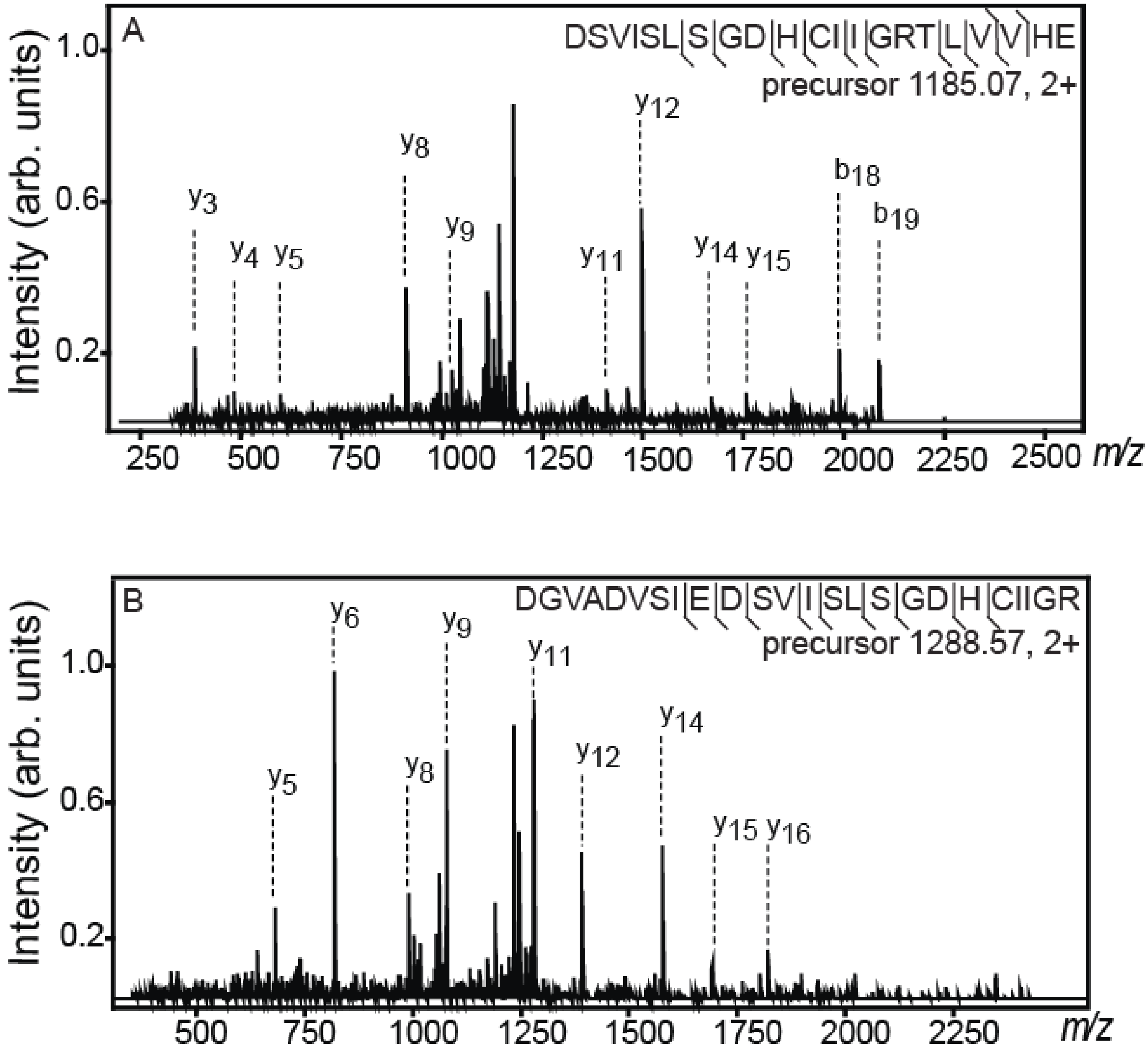
Supplemental Figures:



Supplemental Figure 1: Cysteinylation is a prevalent post-translational modification of human SOD1 expressed and purified from mouse tissue. hSOD1 was isolated from a mouse brain using an SOD1 antibody column, 40 % acetonitrile was added to improve MS signal, and then infused directly into the Bruker Daltonics HCT Ultra ion trap [14]. Deisotoped and Deconvoluted data for the entire mass range analyzed showing the average mass for unmodified (15843.08 Da) and modified (15962.10 Da) SOD1, the delta mass being 119.02 Da, which is consistent with cysteinylation. Note that the solvents and declustering potentials used were such that native metals were not observed. This is a representative spectrum of the 2 mice analyzed.



Supplemental Figure 2: Trypsin Proteolytic Digest of SOD1 and SOD1^{Cys} revealed a cysteinylated Cys$_{111}$ peptide. SOD1 or SOD1^{Cys} were heated to 95 °C, cooled to room temperature and incubated with equimolar Trypsin at 37 °C overnight. Peptides were analyzed using a Bruker Microflex MALDI-TOF in reflectron mode. In both the SOD1 and SOD1^{Cys} samples a Cys$_{111}$ containing peptide corresponding to residues 80-115 were observed (*m/z* 3663.7 and 3664.0, respectively). In the SOD1^{Cys} sample a unique peak (*m/z* 3782.9) was observed corresponding to residues 80-115 (*m/z* 3664.0) plus cysteine (119 Da). This experiment was repeated in triplicate.



Supplemental Figure 3: Proteolytic digest and MS/MS data confirmed a cysteinylated Cys_{111} peptide. (A) SOD1^{Cys} was heated at 95 °C, cooled to room temperature, incubated with 1 M urea and equimolar GluC overnight at 30 °C. LC-MS/MS sequence data positively identified an SOD1 Cys_{111} containing peptide from residues 101-121 where the precursor mass (*m/z* 1185.07(2+)) was consistent with cysteinylation of this peptide compared to the uncysteinlyated peptide (*m/z* 1125.58 (2+)). (B) SOD1^{Cys} was heated at 95 °C, cooled to room temperature, incubated with 1

M urea and equimolar Trypsin overnight at 37 °C. LC-MS/MS sequence data positively identified an SOD1 Cys_{111} containing peptide from residues 92-115 and the precursor mass (*m/z* 1288.57(2+)) was consistent with cysteinylation of this peptide compared to the uncysteinlyated peptide (*m/z* 1229.07 (2+)). Peptides were analyzed using a Bruker HCT ultra ion trap in AutoMS(n) mode. These experiments were repeated in triplicate.