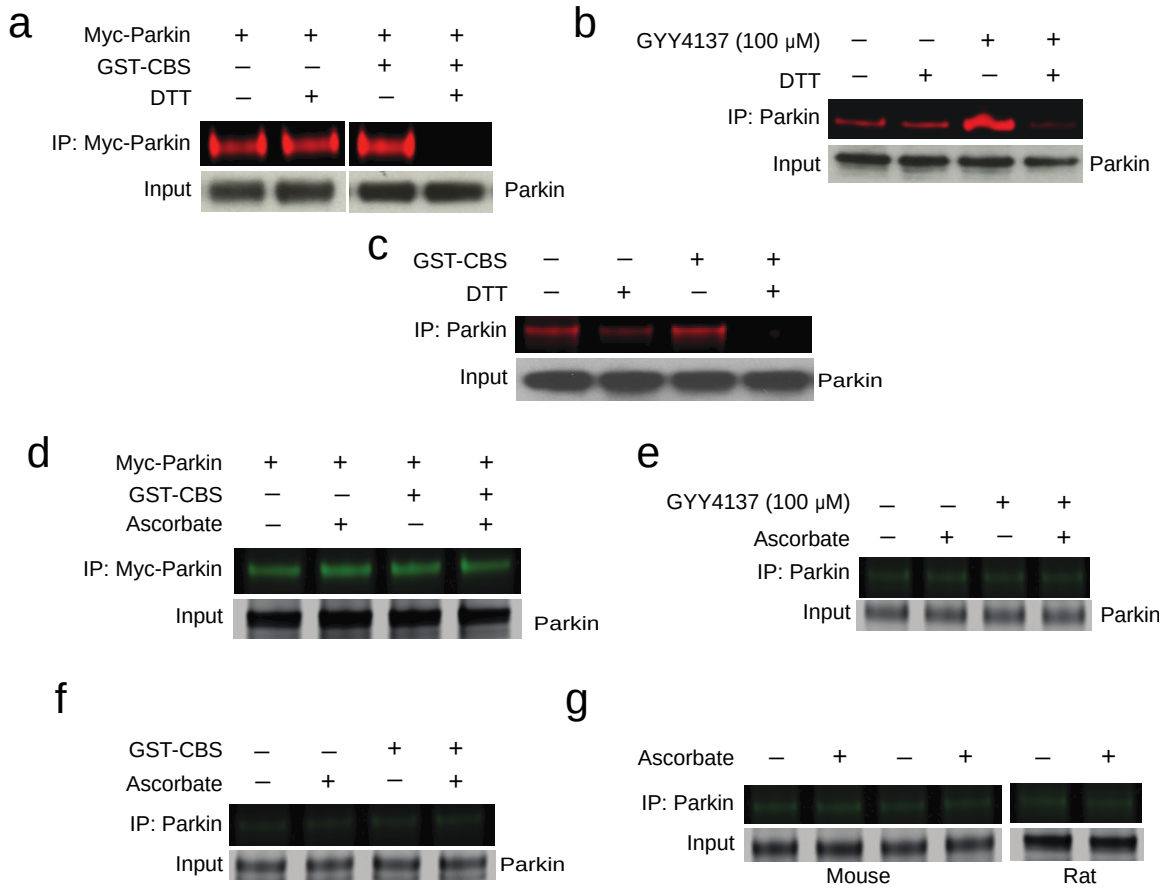
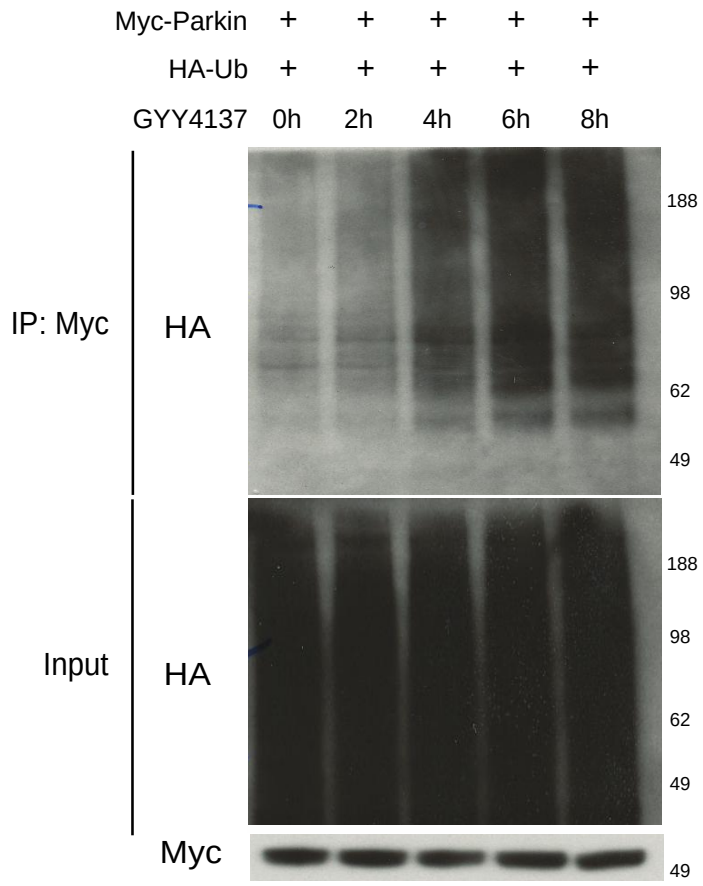
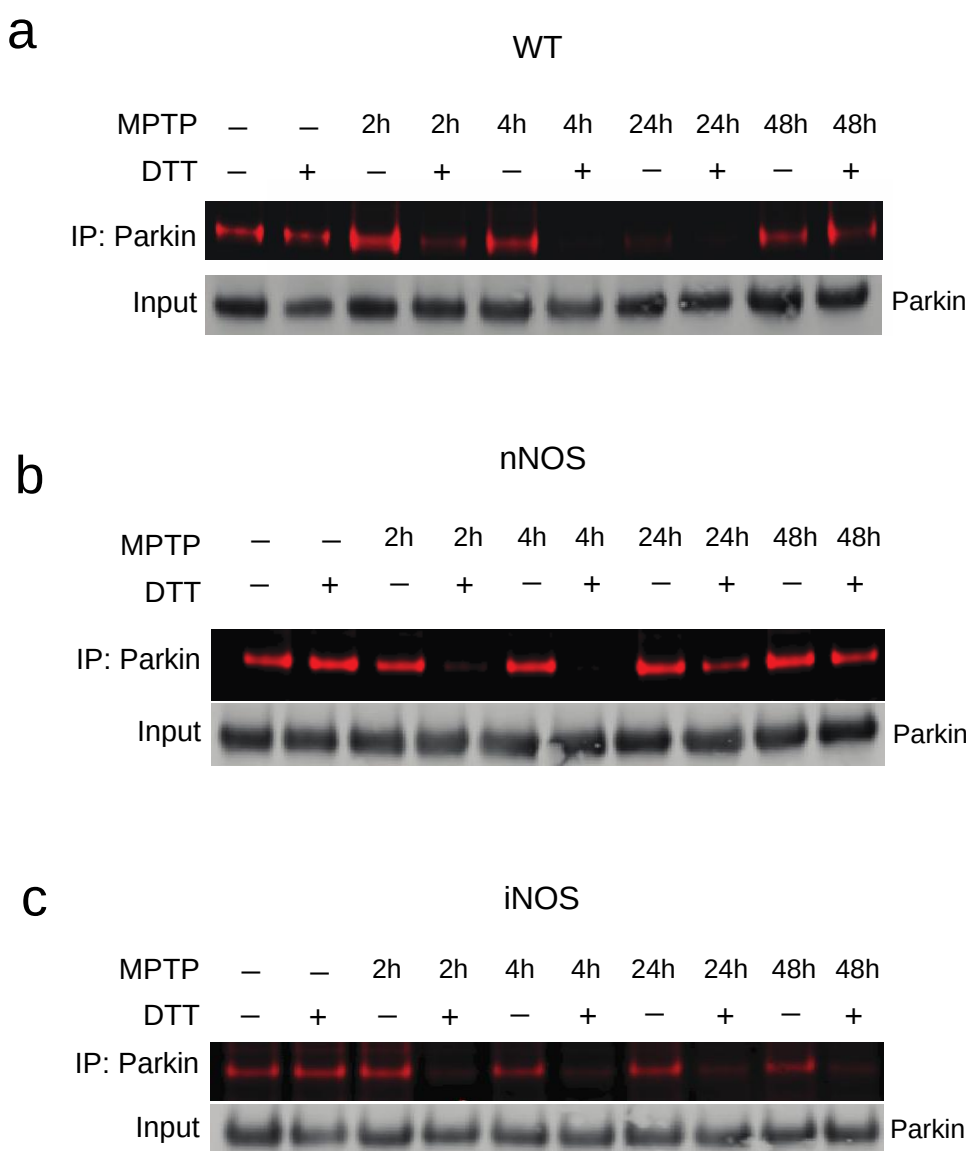


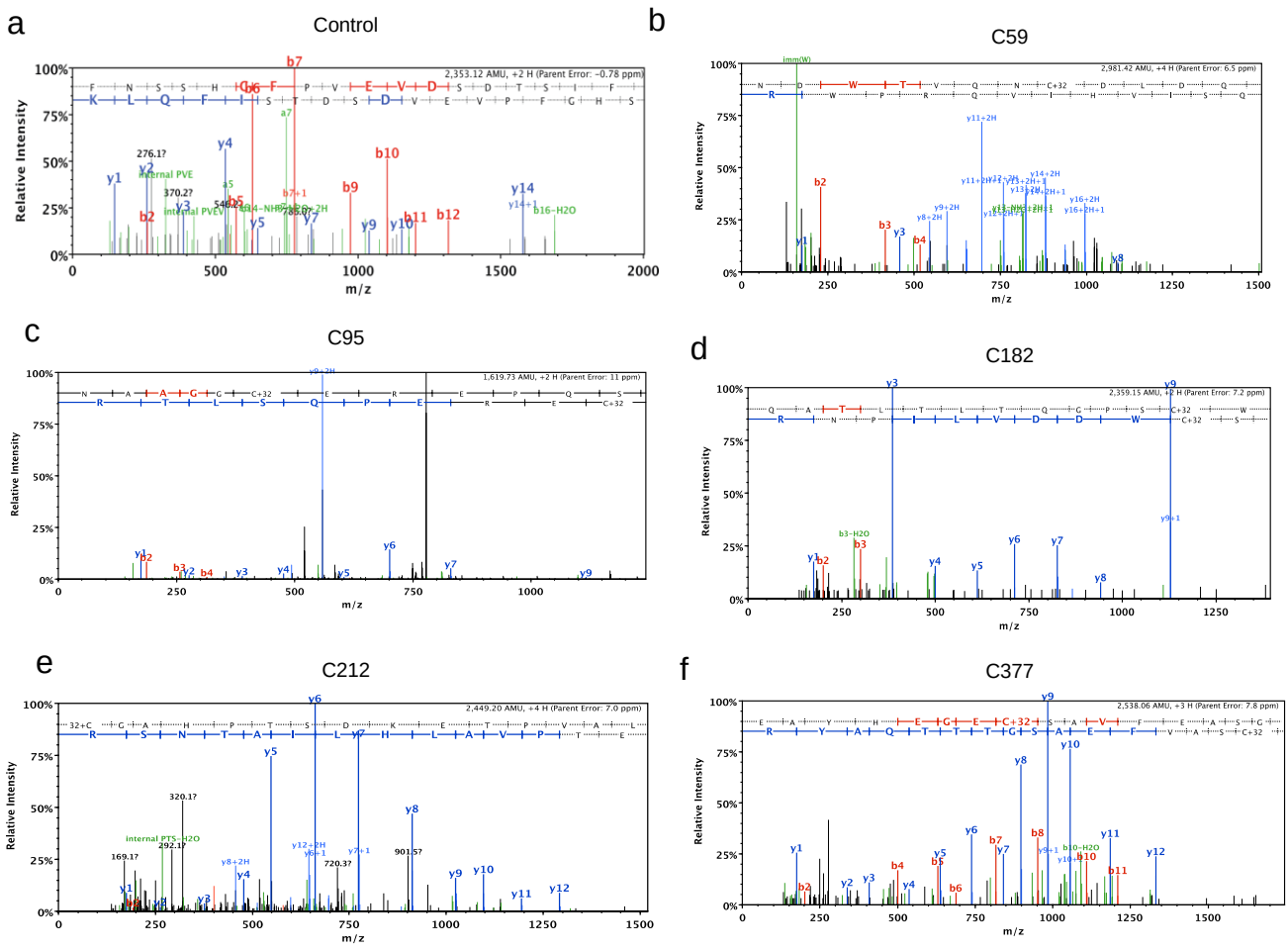
Supplementary Figure S1 | Representative maleimide assays for experiments involving sulfhydration and nitrosylation. Sulfhydration was measured with the red channel and nitrosylation quantified via the green channel. A decrease in signal intensity with DTT treatment in the red channel signifies increased sulfhydration and increases in signal in the green channel with ascorbate treatment represents enhanced nitrosylation. (**a**) Overexpressed parkin in HEK293 cells is sulfhydrated in the presence of GST-cystathionine beta-synthase (CBS). (**b**) Sulfhydration of endogenous parkin in SH-SY5Y cells is enhanced by the H_2S donor GYY4137 (100 µM). (**c**) Basal sulfhydration of endogenous parkin in SH-SY5Y cells is increased by the addition of GST-CBS. Representative maleimide assays and loads for experiments involving nitrosylation of parkin. (**d**) Overexpressed parkin in HEK293 cells is not nitrosylated basally or in the presence of GST-cystathionine beta-synthase (CBS). (**e**) Nitrosylation of endogenous parkin in SH-SY5Y cells is not present or affected by the H_2S donor GYY4137 (100 µM). (**f**) The negligible levels of basal nitrosylation of endogenous parkin in SH-SY5Y cells is unaffected by the addition of GST-CBS. (**g**) Addition of ascorbate does not reveal an increase in signal for basal nitrosylation in mouse brain and rat striatum suggesting that there is negligible basal sulfhydration of parkin.



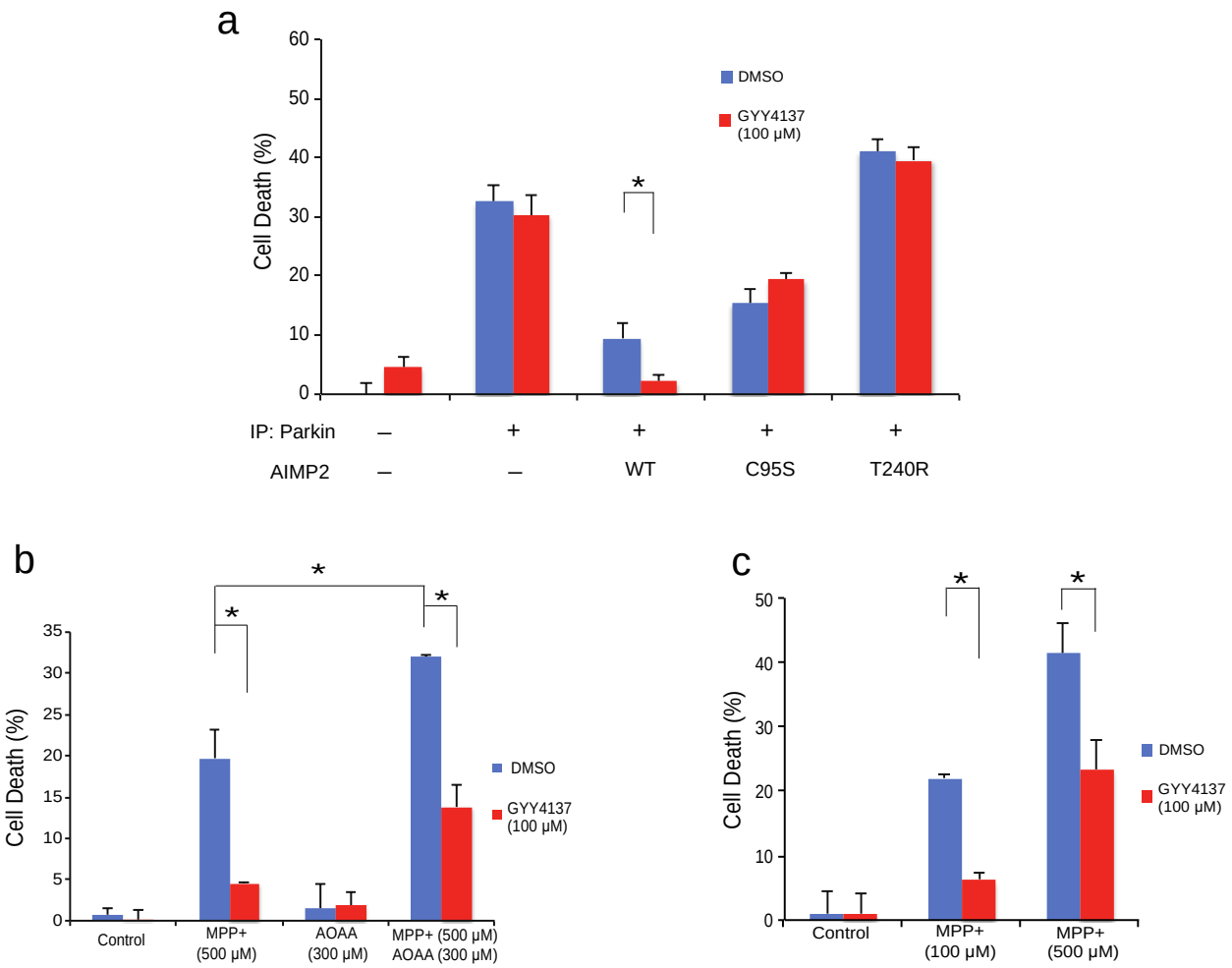
Supplementary Figure S2 | Parkin E3 ligase activity in HEK293 cells is augmented by GYY4137. HEK293 cells were transfected with parkin and treated with GYY4137 (100 μM) over a time course of 2-8 hrs. Parkin ubiquitination was monitored over time as a measure of its E3 ligase activity. GYY4137 treatment very substantially enhances the E3 ligase activity of parkin consistently over time.



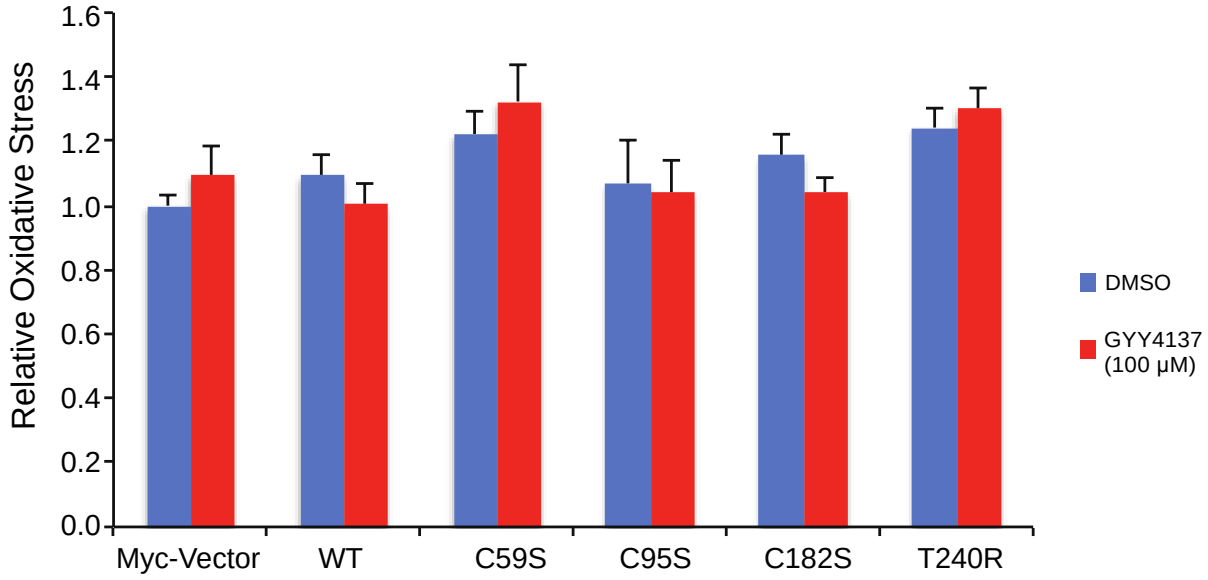
Supplementary Figure S3 | Representative maleimide assays of mice injected with saline or MPTP. Representative maleimide assays whereby parkin sulfhydration was determined by the maleimide technique in WT, nNOS$^{-/-}$, and iNOS$^{-/-}$ mice injected with saline or MPTP and sacrificed at 2 h, 4 h, 24 h, and 48 h after MPTP injection.



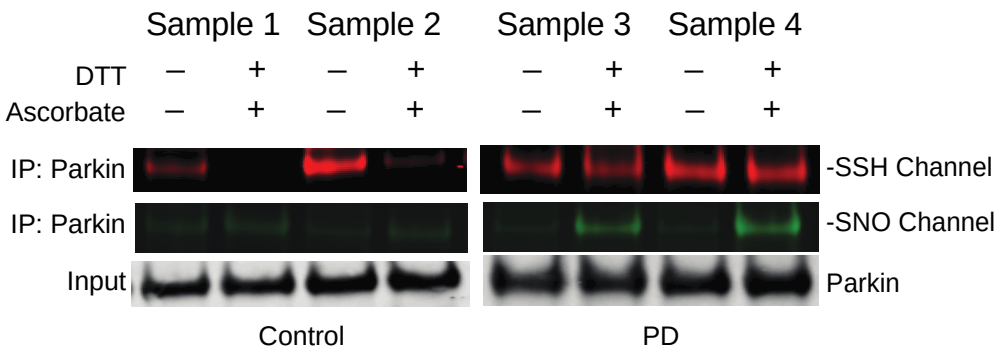
Supplementary Figure S4 | High resolution tandem mass spectrometry was utilized to identify modified cysteine residues. Sufficient resolution was used to differentiate between sulfhydration and sulfinic acid modification Cys-SO_2-H. Annotated, simplified spectra for an untreated (**a**) and thus unmodified control sample and for modifications: C59 (**b**), C95 (**c**), C182 (**d**), C212 (**e**), and C377 (**f**) respectively are shown with the b and y fragments identified. Peptides were analyzed by MS/MS using Mascot and/or Sequest to search for corresponding peptide fragments. Peptide identifications were accepted if they could be established at greater than 95.0% probability as Cys-S-S-H.



Supplementary Figure S5 | H_2S enhances parkin's neuroprotective actions. (**a**) Cell death in tet-repressible AIMP2 expressing PC12 cells is prevented by parkin overexpression and by GYY4137, whose protective effect is not evident in the absence of parkin, with parkin-C95S or with the T240R catalytically inactive parkin mutant n=4-6 with **P*<0.05 via one way ANOVA. (**b**) GYY4137 (100 μM) prevents the cytotoxic actions of MPP+ (0.5 mM/24 h) in PC12 cells. AOAA increases cytotoxicity, an effect reversed by GYY4137 treatment n=3 **P*<0.035 one way ANOVA (**c**) GYY4137 (100 μM) is also protective in the MPP+ model of PD in SH-SY5Y cells (100 μM and 500 μM MPP+) n=3 **P*<0.03 by one way ANOVA. All data expressed as mean ± s.e.m.



Supplementary Figure S6 | GYY4137 does not markedly alter the redox status of AIMP2 cells transfected with parkin. Relative oxidation/ROS status of AIMP2 cells transfected with control, WT parkin or various parkin mutants as measured by fluorimetric assay using general oxidative stress indicator CM-H_2DCFDA. All data expressed as mean ± s.e.m.



Supplementary Figure S7 | Representative maleimide assays from 2 control and 2 PD patient samples. Representative assays are shown for sulfhydration (red channel) and nitrosylation (green channel) in which decreases in signal with DTT treatment in the red channel signify increased sulfhydration and increases in signal in the green channel with ascorbate treatment signify increased nitrosylation. Relative sulfhydration and nitrosylation of parkin was assayed in control and PD patient striatum revealing substantial decreases in sulfhydration and increases in nitrosylation in PD versus control.