

Supplementary data files for the article:

Identification of Novel Protein Targets of Dimethyl Fumarate Modification in Neurons and Astrocytes Reveal Actions Independent of Nuclear Factor (erythroid-derived 2)-Related Factor 2 Stabilization

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Running Title: Novel Targets of Dimethyl Fumarate Action in Neuronal Cells

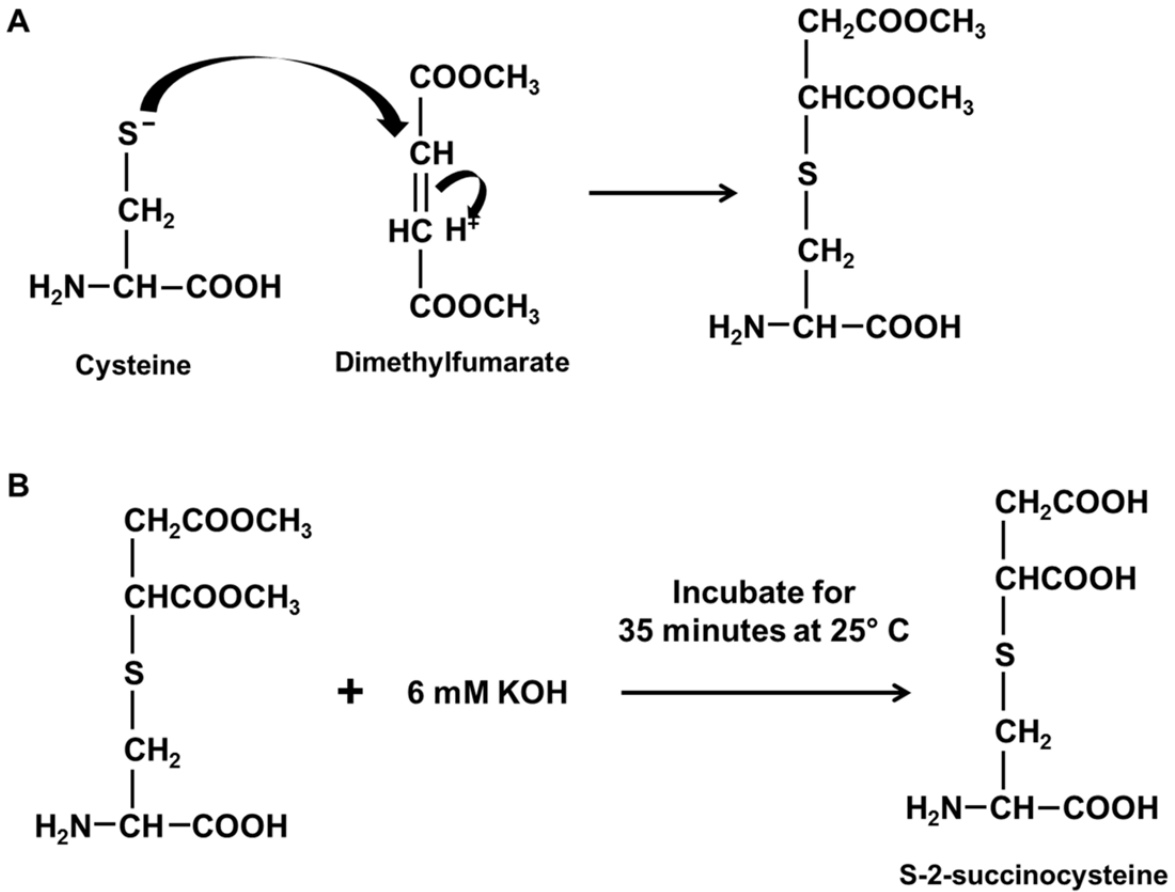
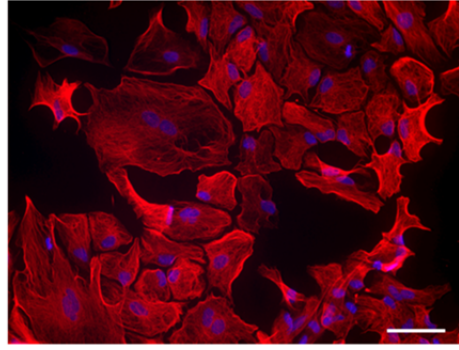


Figure S1: Immunodetection of DMF cysteine adducts. (A) Reaction of dimethyl fumarate (DMF) with cysteine residues forms an adduct that is not recognized by an antibody that detects protein succination (see Figure 1A). (B) Removal of the methyl ester groups by alkaline treatment converts the adduct formed into S-2-succinocysteine (2SC), which can be immunodetected by the anti-2SC antibody (see Figure 1B).

A



B

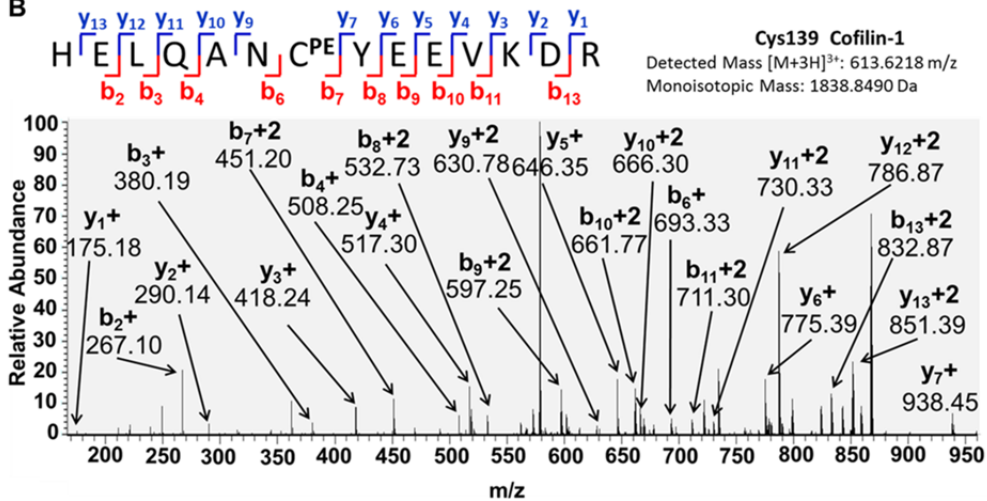


Figure S2: (A) Astrocytes isolated from P1 rat cortices were stained with a GFAP antibody (red) and DAPI (blue) at DIV 12. The staining shows that the culture is enriched in astrocytic cells, though some cells not clearly associated with GFAP fluorescence may reflect a minor presence of microglia. Scale bar: 75 μm . (B) MS/MS spectrum from astrocyte protein following DMF treatment showing the unmodified (pyridylethylated) Cys139 in the peptide HELQANC^{PE}YEEVKDR from cofilin 1, detected after alkylation with 4-vinylpyridine. The spectrum of the MMF succinated Cys139 of cofilin 1 from the same extract is shown in Figure 2A. See Table 1 for the entire list of succinated proteins in primary rat astrocytes after treatment with DMF.

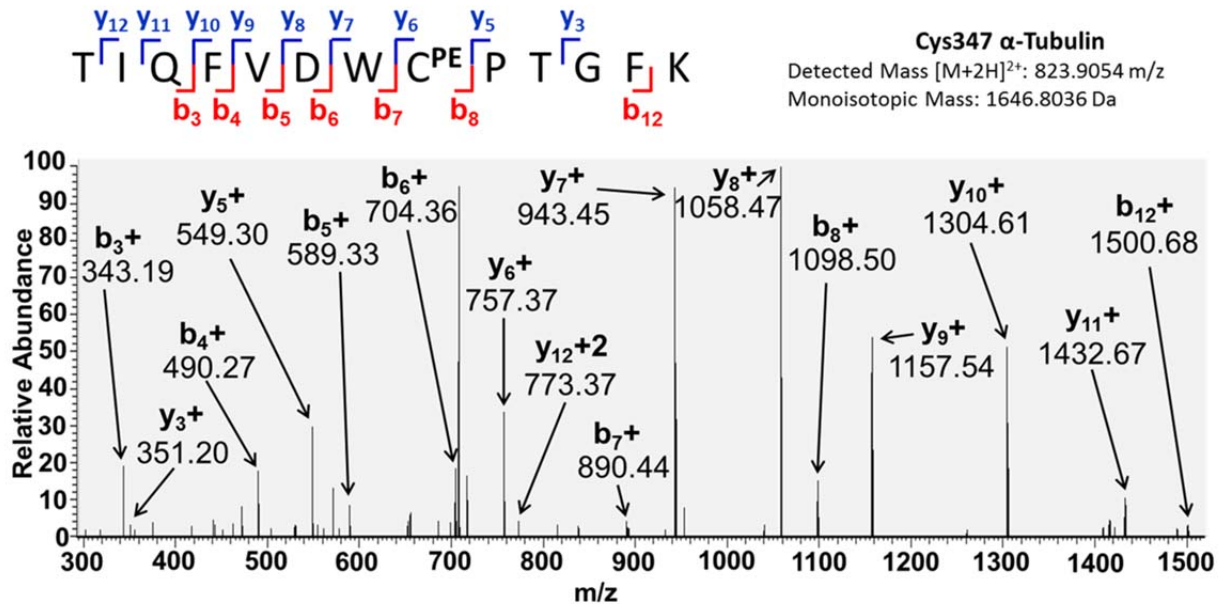


Figure S3: MS/MS spectrum from N1E-115 neuron extracts after DMF treatment showing the unmodified (pyridylethylated) Cys347 of α -tubulin in the peptide TIQFVDWC^{PE}PTGFK. See Figure 3A for the MS/MS spectrum showing the DMF succinated Cys347 of α -tubulin in the same peptide.

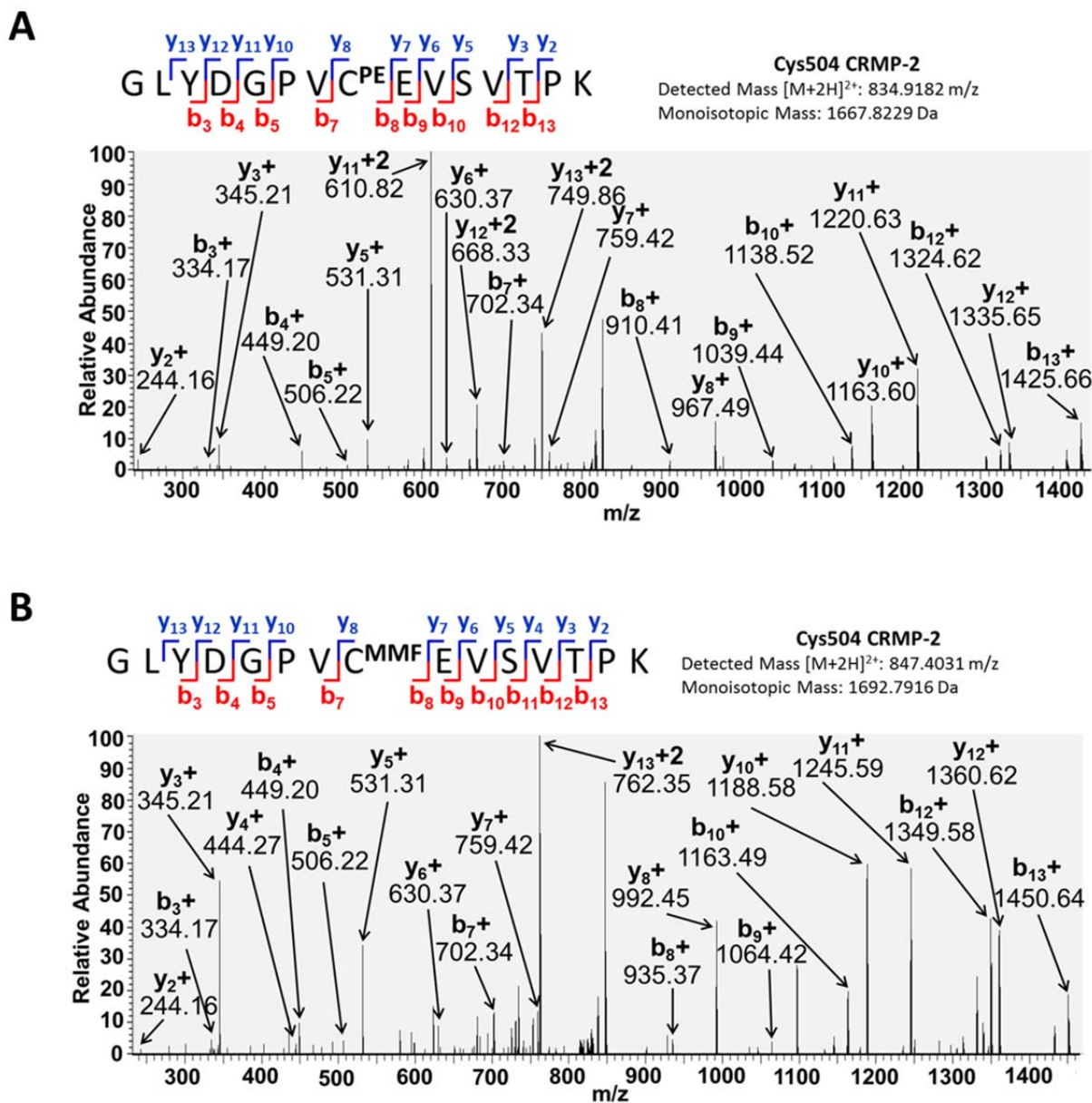


Figure S4: Additional spectra for Cys504 of CRMP-2 in DMF treated neural cells. (A) MS/MS spectrum from rat primary neuron extracts after DMF treatment showing the unmodified (pyridylethylated) Cys504 of CRMP-2 in the peptide GLYDGPV^{PE}EVSVTPK. (B) MS/MS spectrum from N1E-115 neuron extracts after DMF treatment showing the MMF succinated Cys504 of CRMP-2 in the peptide GLYDGPV^{MMF}EVSVTPK.

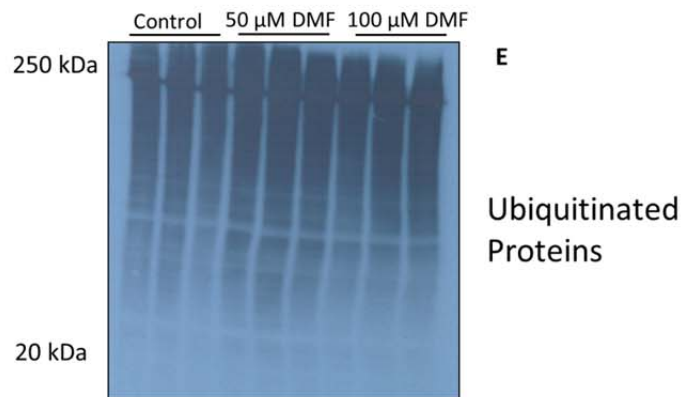
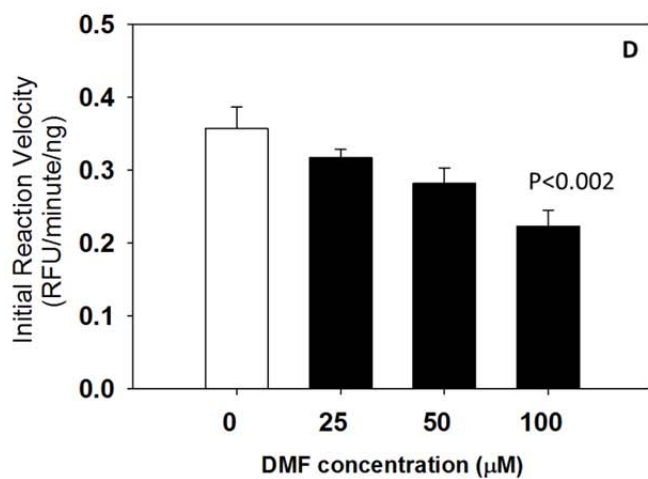
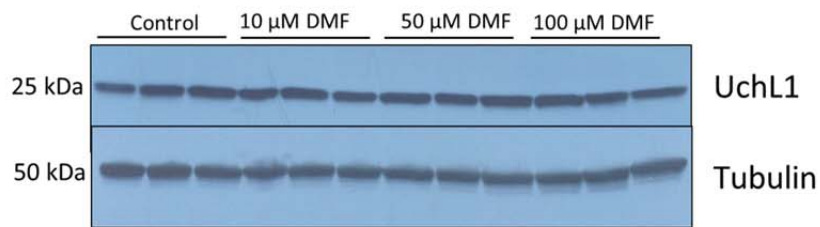
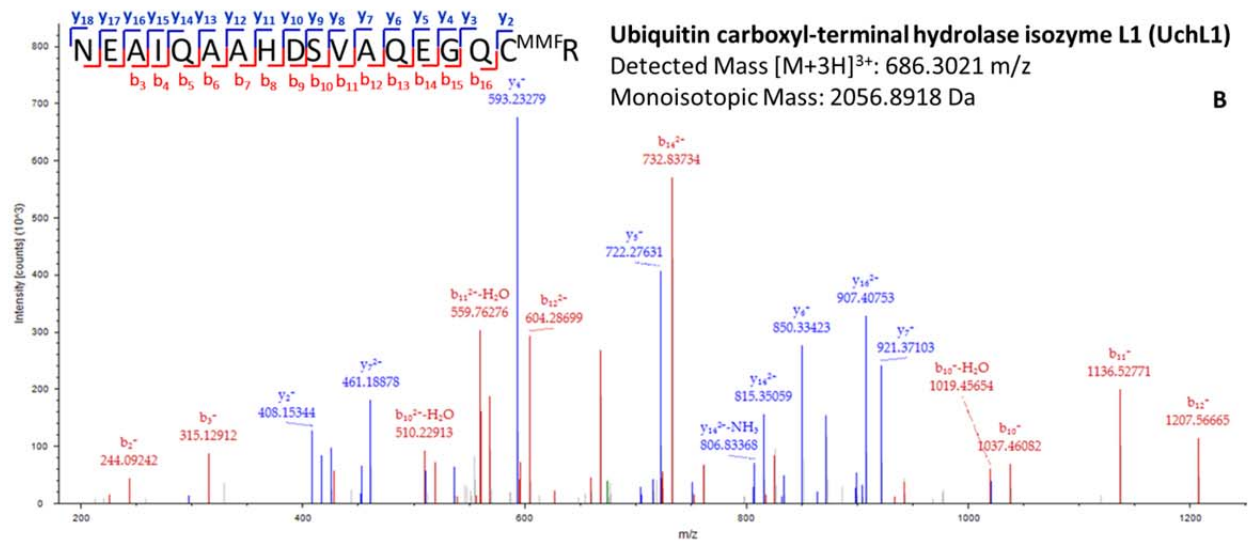
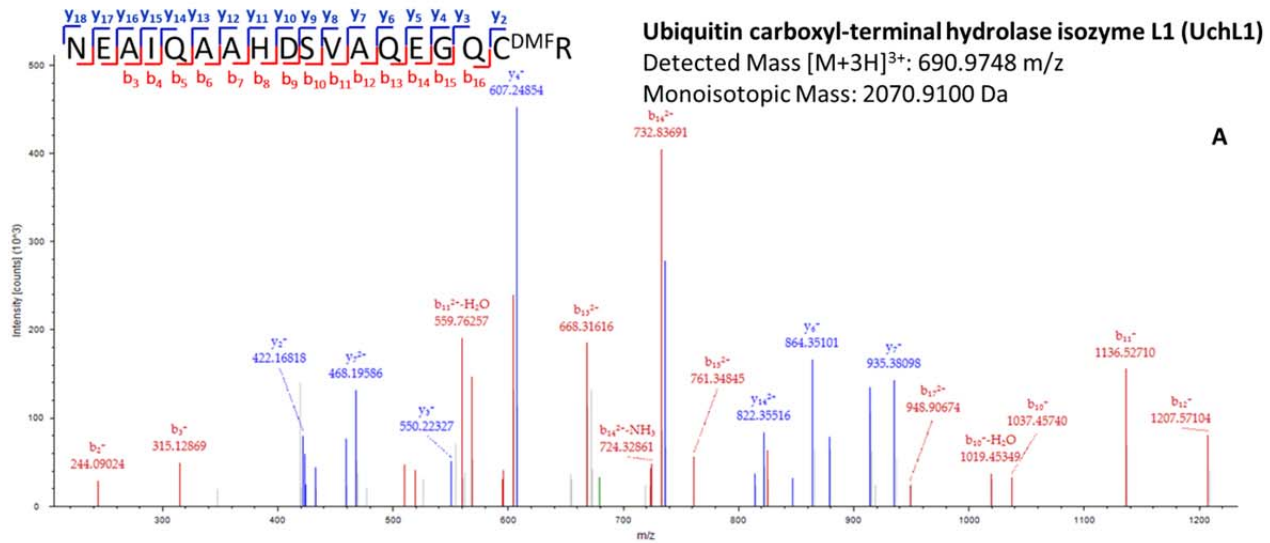


Figure S5: Spectra for Cys152 of Uchl1 in DMF treated N1E-115 cells demonstrating that (A) DMF and (B) MMD adducts were present on Cys152 on peptide NEAIQAAHDSVAQEGQCR. The masses and charge states detected are shown on the spectrum labels. (C) Total protein levels of Uchl1 and tubulin were measured in N1E-115 cells treated with 0-100 μ M DMF for 24 hrs. (D) Initial reaction velocity of control and DMF modified Uchl1 protein in a fluorescent deubiquitinase assay, n=5 per group, p<0.002 control versus 100 μ DMF treatment. (E) Total levels of polyubiquitinated proteins were measured in N1E-115 cells treated with 0-100 μ M DMF for 24 hrs.