Figure S1, Piroli et al.

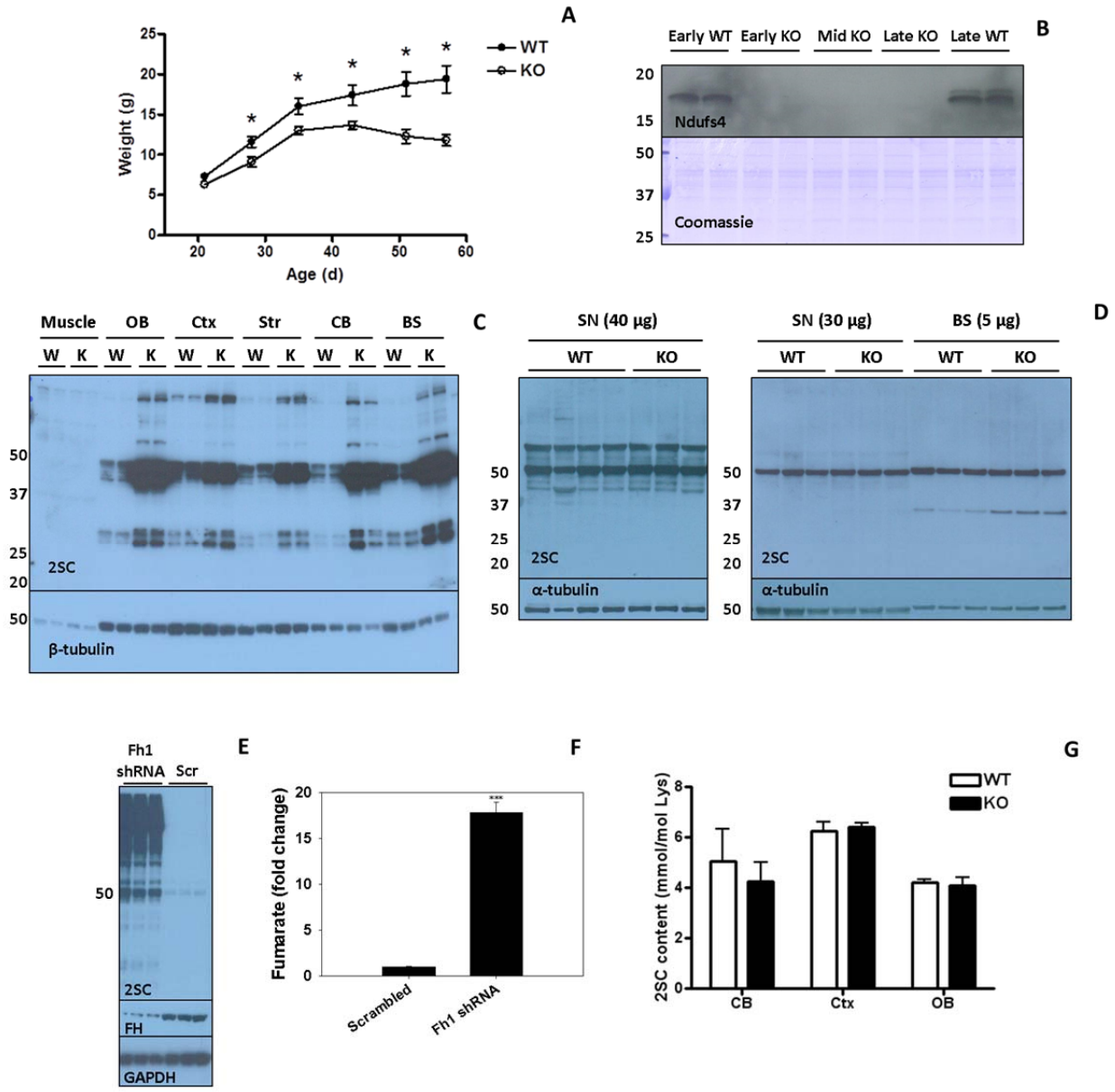

Figure S1: (A) Growth curve of WT and Ndufs4 KO littermates, showing the failure to thrive in KO mice. (B) Expression of Ndufs4 protein in the BS of early, middle and late WT and Ndufs4 KO mice, confirming the absence of the protein in KO animals. (C) Protein succination in skeletal muscle and brain areas of WT (W) and Ndufs4 KO (K) mice. Brain areas included are olfactory bulb (OB), cortex (Ctx), striatum (Str), cerebellum (CB) and brainstem (BS). Succination (2SC, upper panel) is weak and unchanged in muscle at this exposure, but increases in all the brain areas of KO mice, with stronger labeling for OB, CB and BS. β-tubulin (lower panel) was used as a loading control. (D) Protein succination in sciatic nerve (SN). Upper

left panel shows that succination is unchanged in the SN of Ndufs4 KO mice compared to WT littermates. Upper right panel compares succination in the SN with BS, confirming the lack of changes in SN and the increases in BS (see Figure 1A). A low exposure was used to show slightly increased succination of the tubulin band in BS compared to SN, even when α-tubulin content (lower panel) was higher in SN. (E) 2SC levels (upper panel) are increased when fumarase (Fh1) is knocked down in N1E-115 differentiated neurons following lentiviral transduction (Scr, scrambled control lentivirus). Fumarase (FH) protein levels were significantly decreased in the Fh1 shRNA group versus scrambled controls. GAPDH was used as a loading control, n=3 (F) Fumarate levels, quantified by GC-MS, are increased ~18-fold in Fh1 shRNA knockdown N1E-115 neurons versus scrambled controls, n=3, $p<0.001$ (G) Total 2SC content of CB, Ctx and OB determined by GC-MS/MS. Note that the 2SC content in these brain areas is similar to BS (Figure 1E). No significant differences between genotypes were observed.

In B, C and D, molecular masses of marker proteins are indicated on the left-hand side. In A, * $p<0.05$ vs. KO mice at the same time-point (n = 3-4 mice per group). In E, n=3 mice per group.

Figure S2, Piroli et al.

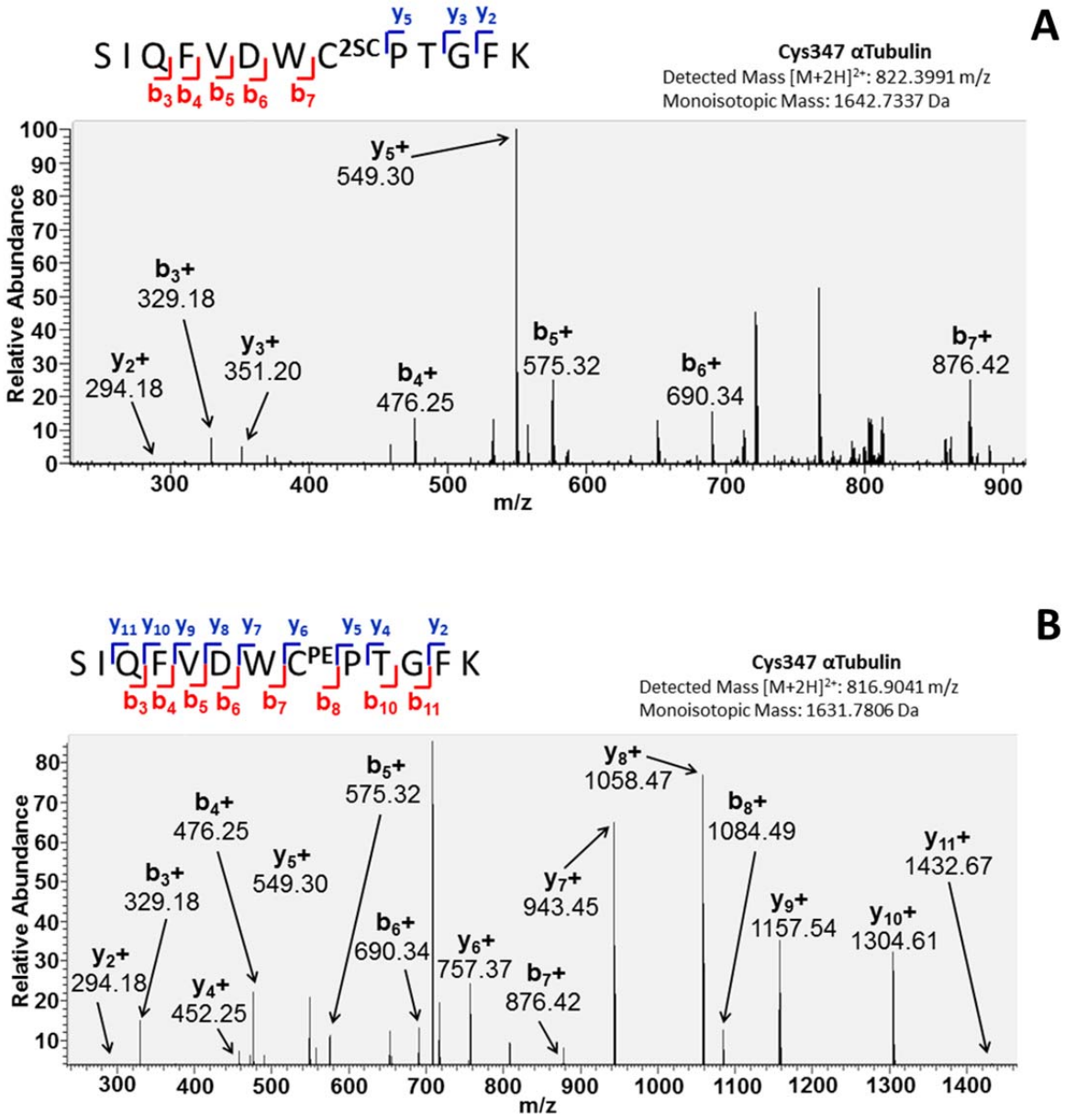

Figure S2: Cys347α is a prominent site of succination in the BS of the mouse. Mt fractions were resolved by SDS/PAGE and the tubulin bands at ~50 kDa were excised and digested with trypsin prior to LC-MS/MS analysis as detailed in Experimental Procedures. (A) MS/MS sequencing showing succination of Cys347α (C^{2SC}) in the peptide SIQFVDWCPTGFK; the pyridylethylated version of Cys347α (C^{PE}) is shown in (B).

Figure S3, Piroli et al.

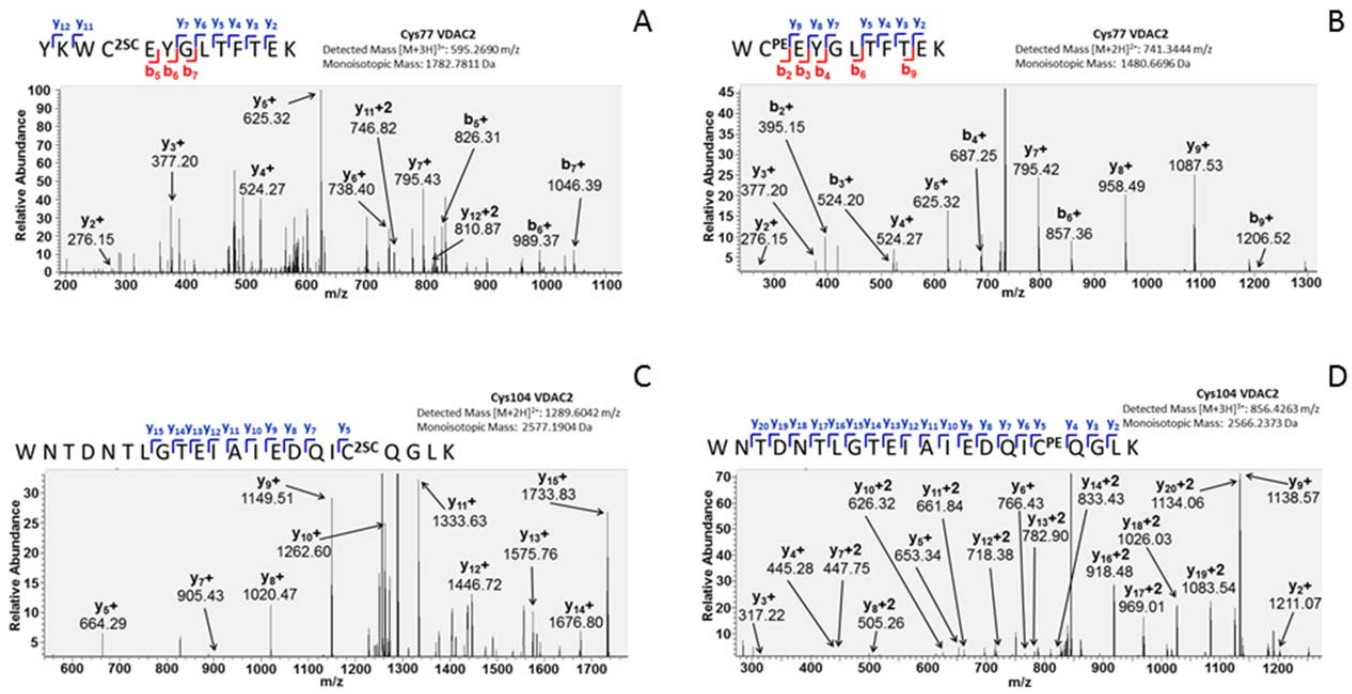

Figure S3: Initial identification of succination sites in VDAC2. Mitochondrial enriched fractions from WT mouse brain cortex (100 µg) were incubated with 100 mM fumarate for 18 h at 37°C, samples were resolved by SDS-PAGE, gels were stained with Coomassie Brilliant Blue, bands at ~30-35 kDa were excised, destained and digested with trypsin as described in Experimental Procedures. Identification of VDAC2 in the digests is shown in Supplemental Table 1. (A, B) MS/MS spectra showing Cys 77 in the peptide YKWC^{2SC}EYGLTFTEK as a succinated residue in VDAC2 (A); the unmodified Cys 77 in the peptide WC^{PE}EYGLTFTEK was also detected (B). (C, D) MS/MS spectra showing Cys 104 in the peptide WNTDNTLGTEIAIEDQIC^{2SC}QGLK as a succinated residue in VDAC2 (C); the unmodified Cys 104 in the peptide WNTDNTLGTEIAIEDQIC^{PE}QGLK was also detected (D).