Supplemental information

NOX4 promotes ferroptosis of astrocytes by oxidative stress-induced lipid peroxidation via the impairment of mitochondrial metabolism in Alzheimer's diseases.

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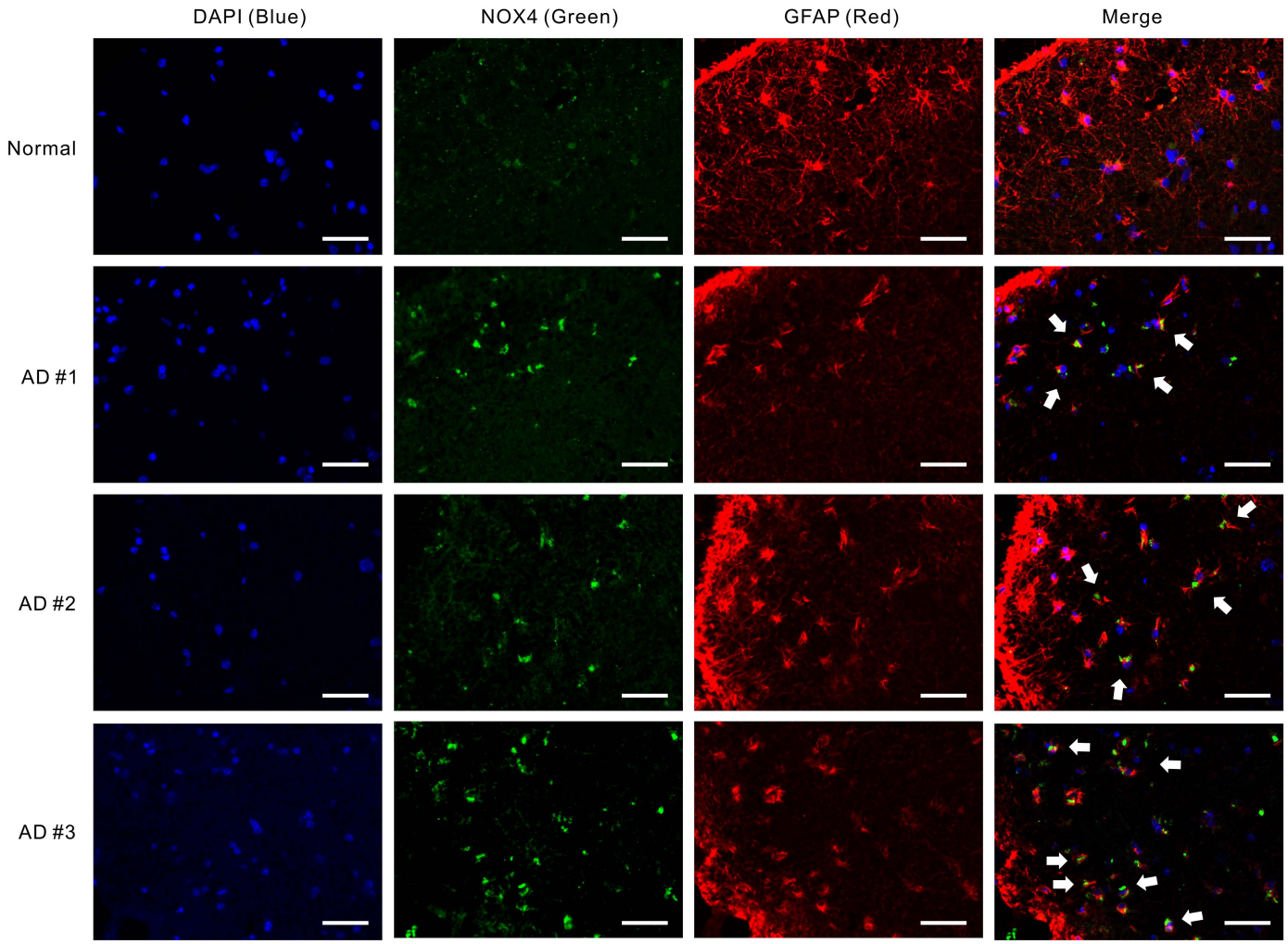
Supplemental Table S1. The characteristics of patients with Alzheimer's diseases (AD) and non-AD donor (Normal).

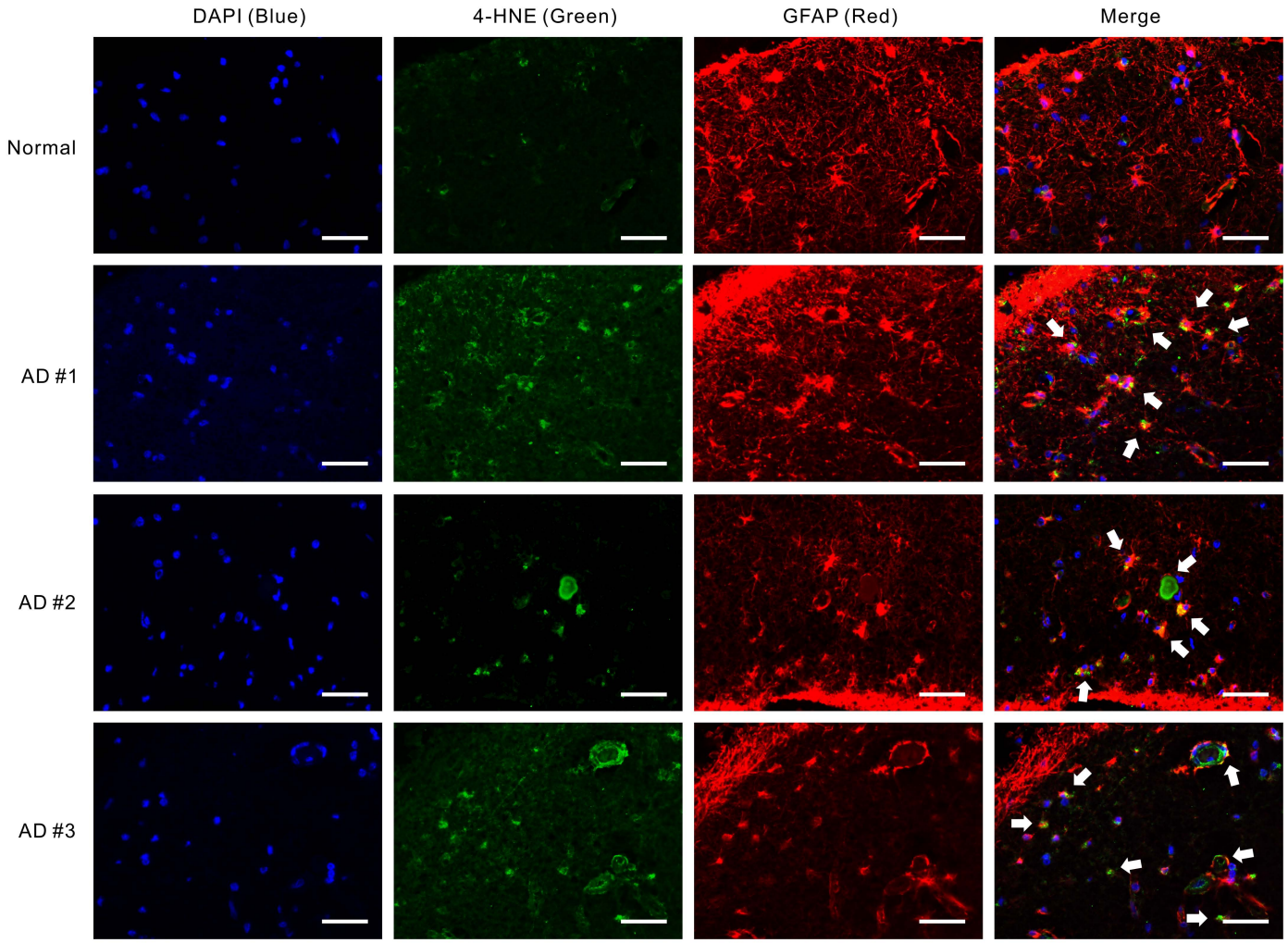
	Normal (n= 3)	AD (n= 3)
Age, years	65 ± 16	72 ± 11
Sex, male/female	2 / 1	0 / 3
Brain weight (g)	1300 – 1400	848 – 928

Figure S1



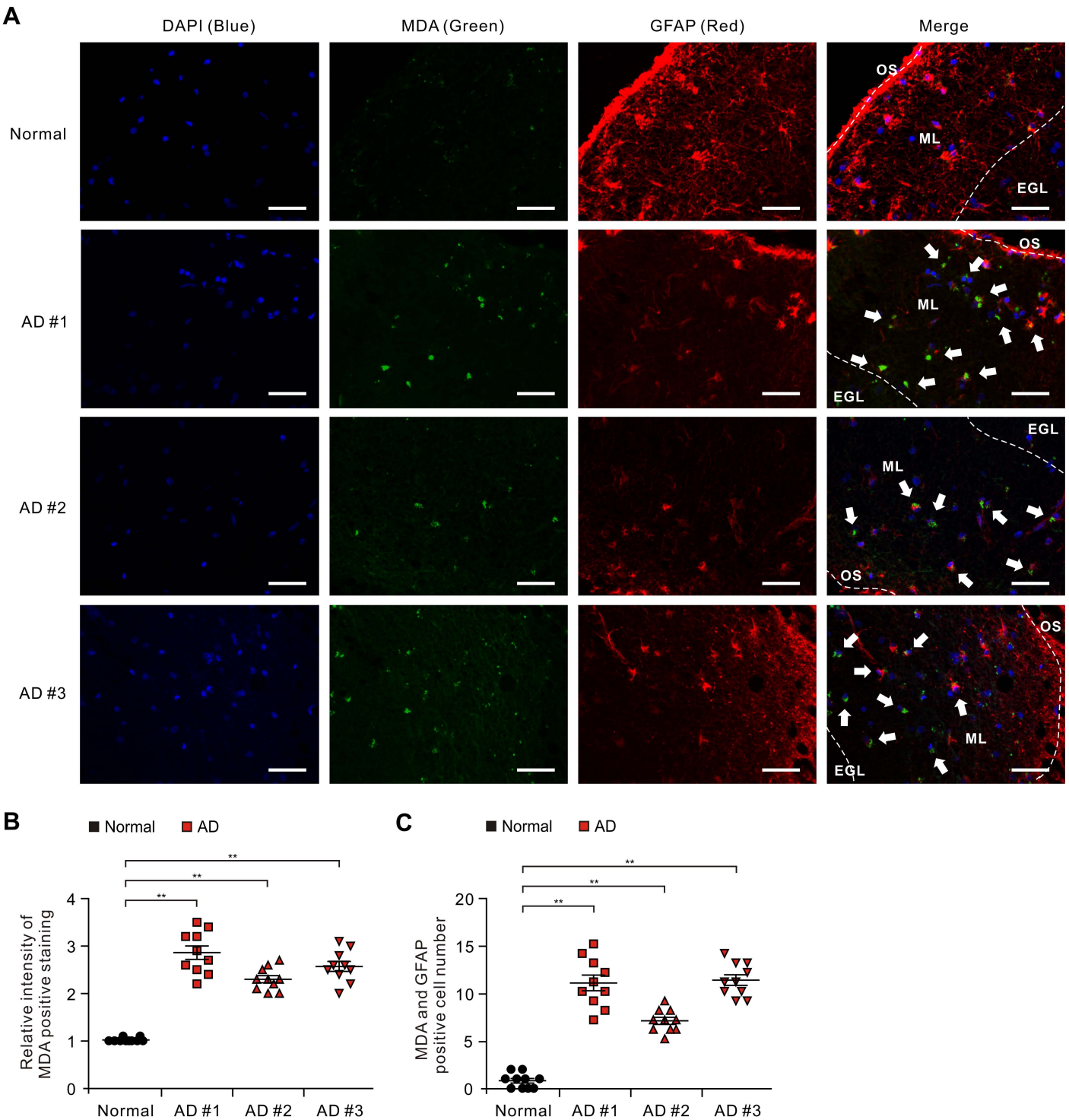
Supplemental Figure S1. The protein levels of NOX4 were elevated in impaired astrocytes of cerebral cortex region from patients with Alzheimer's diseases. Representative immunofluorescence images of NOX4 expression in cerebral cortex region from patients with AD (AD) or non-AD (Normal) showing NOX4 (green) in astrocytes expressing the astrocytes marker GFAP (red). DAPI-stained nuclei are shown in blue. Scale bars, 20 μM. White arrows indicates NOX4 and GFAP positive cells.

Figure S2



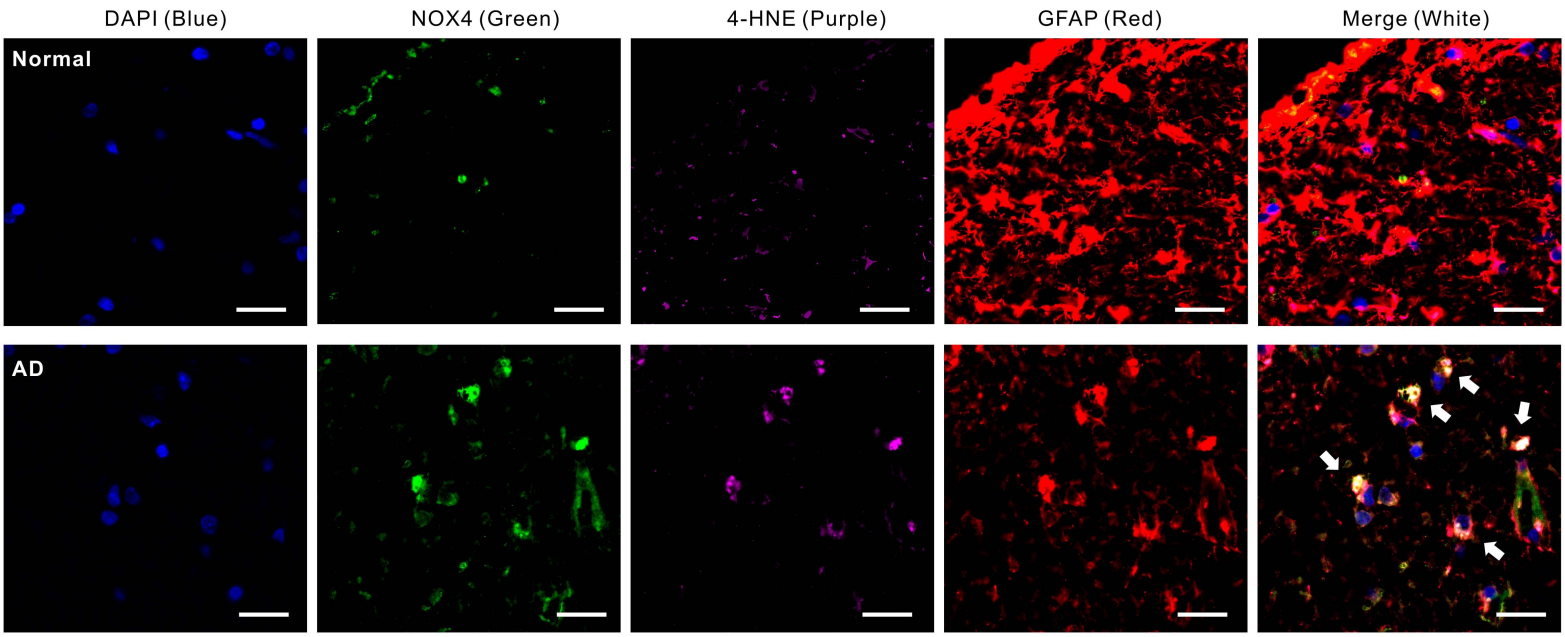
Supplemental Figure S2. The protein levels of 4-HNE were elevated in impaired astrocytes of cerebral cortex region from patients with Alzheimer's diseases. Representative immunofluorescence images of 4-HNE expression in cerebral cortex region from patients with AD (AD) or non-AD (Normal) showing 4-HNE (green) in astrocytes expressing the astrocytes marker GFAP (red). DAPI-stained nuclei are shown in blue. Scale bars, 20 μM. White arrows indicates 4-HNE and GFAP positive cells.

Figure S3



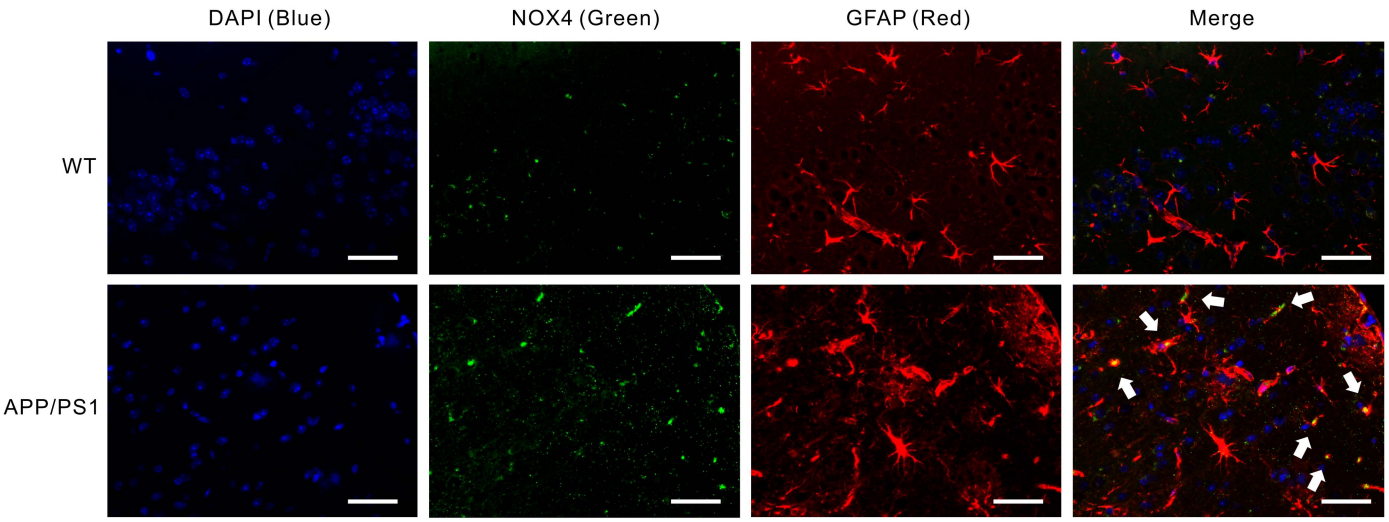
Supplemental Figure S3. The protein levels of MDA were elevated in impaired astrocytes of cerebral cortex region from patients with Alzheimer's diseases. (A) Representative immunofluorescence images of MDA expression in cerebral cortex region from patients with AD (AD) or non-AD (Normal) showing MDA (green) in astrocytes expressing the astrocytes marker GFAP (red) around molecular layer (ML) (n = 3 per group, n = 10 images per individual subject). DAPI-stained nuclei are shown in blue. Scale bars, 20 μM. White arrows indicates MDA and GFAP positive cells. OS, Outer surface; ML, Molecular layer; EGL, External granular layer. Symbols, which are expressed by white dotted line, indicate the distinct area among OS, ML, and EGL. (B) Quantification of intensity of MDA positive staining in astrocytes from immunofluorescence images in the cerebral cortex region from patients with AD (AD #1, AD #2, AD #3) or non-AD (normal) (n = 3 per group, n = 10 images per individual subject). Data are mean ± standard deviation (SD). **, $P < 0.01$ by Student's two-tailed t-test. (C) Quantification of MDA positive astrocytes from immunofluorescence images in the cerebral cortex region from patients with AD (AD #1, AD #2, AD #3) or non-AD (normal) (n = 3 per group, n = 10 images per individual subject). Data are mean ± standard deviation (SD). **, $P < 0.01$ by Student's two-tailed t-test.

Figure S4



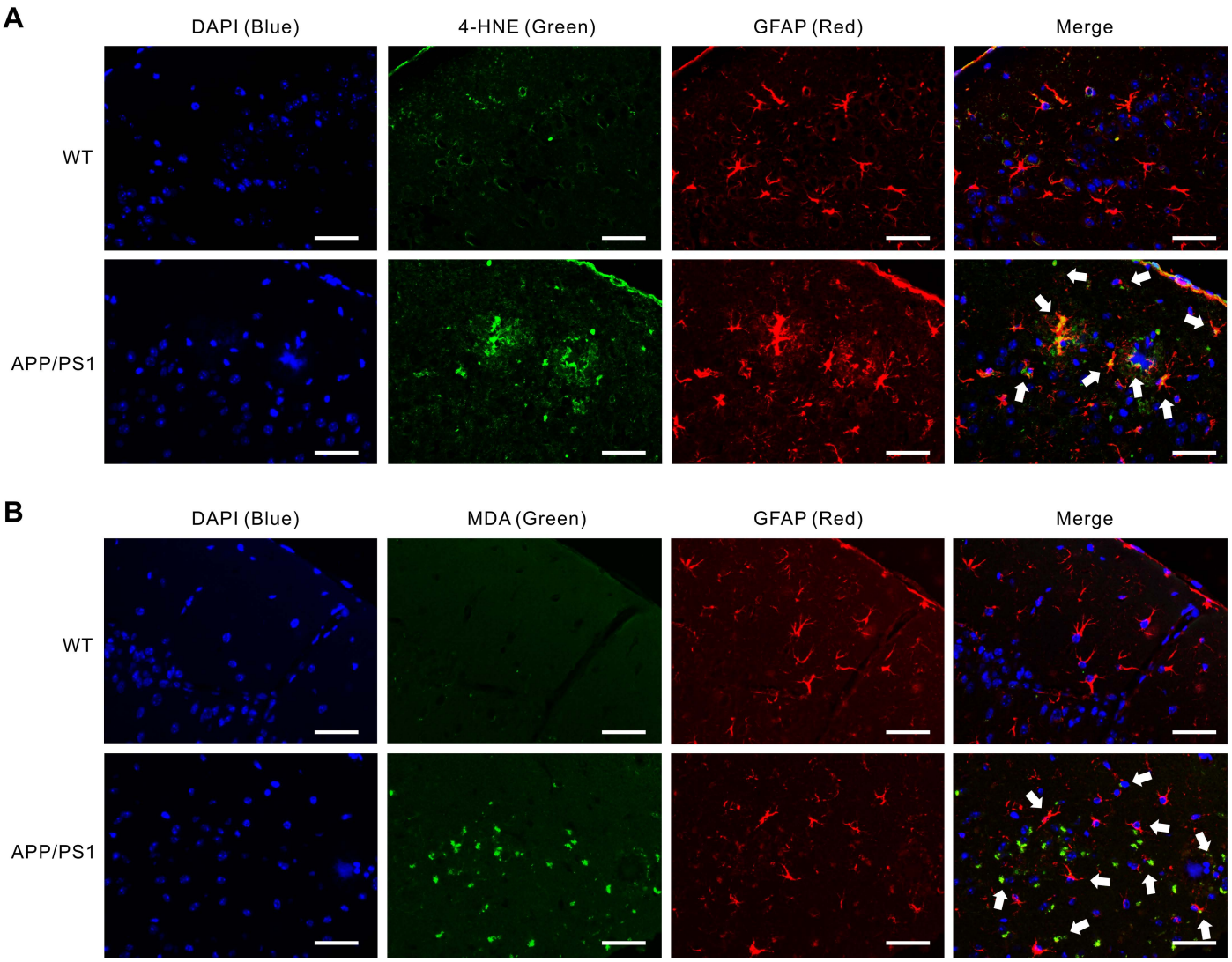
Supplemental Figure S4. The expression of 4-HNE was co-localized in NOX4-positive astrocytes of patients with Alzheimer's diseases. Representative immunofluorescence images of 4-HNE expression (purple) in NOX4 (green)-positive astrocytes (GFAP, red) of cerebral cortex region from patients with AD (AD) or non-AD (Normal). DAPI-stained nuclei are shown in blue. Scale bars, 10 μM. White arrows indicates the co-localization of 4-HNE in NOX4-positive astrocytes.

Figure S5



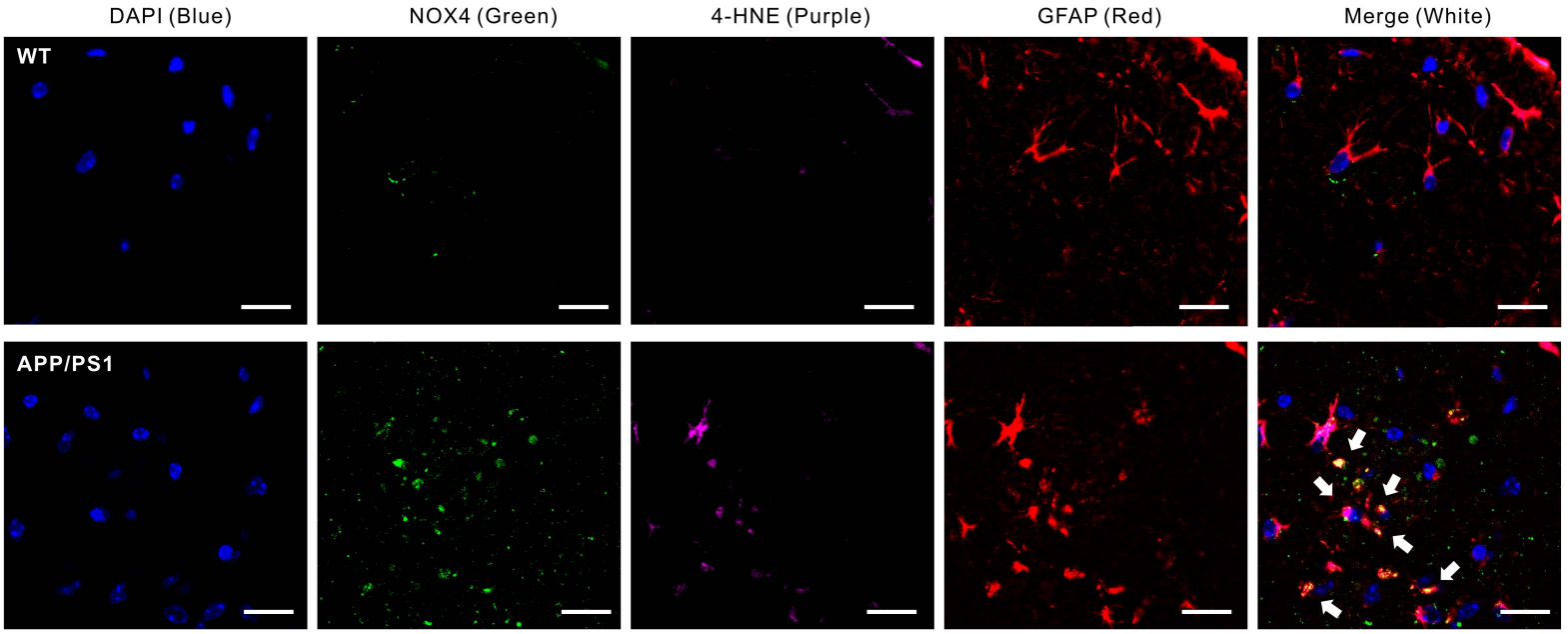
Supplemental Figure S5. The protein levels of NOX4 were elevated in impaired astrocytes of cortex region from brain of APP/PS1 mice. Representative immunofluorescence images of NOX4 expression in cortex region from brain of APP/PS1 mice (APP/PS1) or wild-type (WT) showing NOX4 (green) in astrocytes expressing the astrocytes marker GFAP (red). DAPI-stained nuclei are shown in blue. Scale bars, 20 μM. White arrows indicates NOX4 and GFAP positive cells.

Figure S6



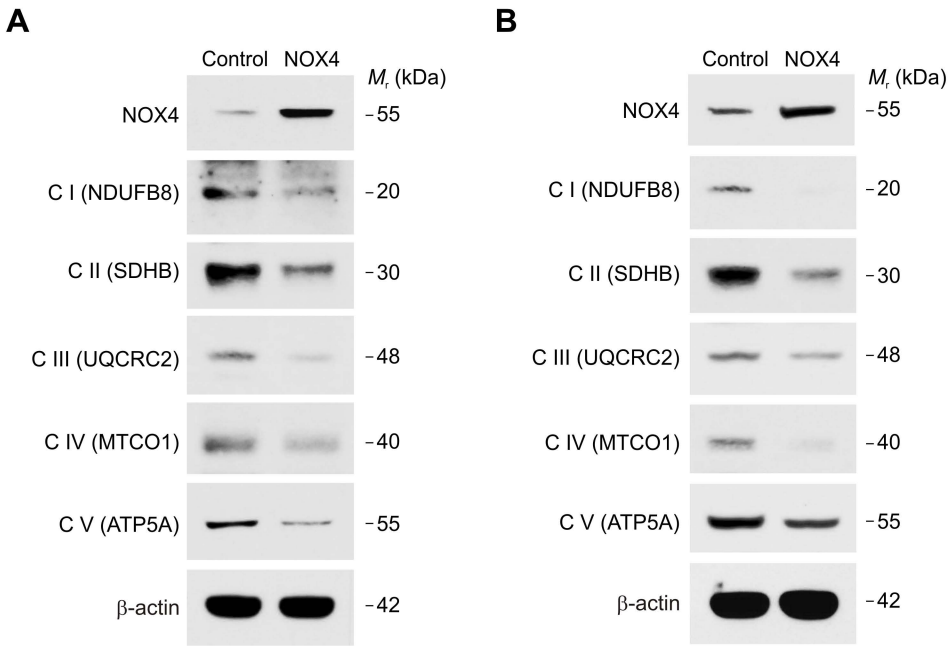
Supplemental Figure S6. The levels of 4-HNE and MDA were elevated in impaired astrocytes of cortex region from brain of APP/PS1 mice. (A) Representative immunofluorescence images of 4-HNE expression in cortex region from brain of APP/PS1 mice (APP/PS1) or wild-type mice (WT) showing 4-HNE (green) in astrocytes expressing the astrocytes marker GFAP (red). DAPI-stained nuclei are shown in blue. Scale bars, 20 μM. White arrows indicates 4-HNE and GFAP positive cells. (B) Representative immunofluorescence images of MDA expression in cortex region from brain of APP/PS1 mice (APP/PS1) or wild-type mice (WT) showing MDA (green) in astrocytes expressing the astrocytes marker GFAP (red). DAPI-stained nuclei are shown in blue. Scale bars, 20 μM. White arrows indicates MDA and GFAP positive cells.

Figure S7



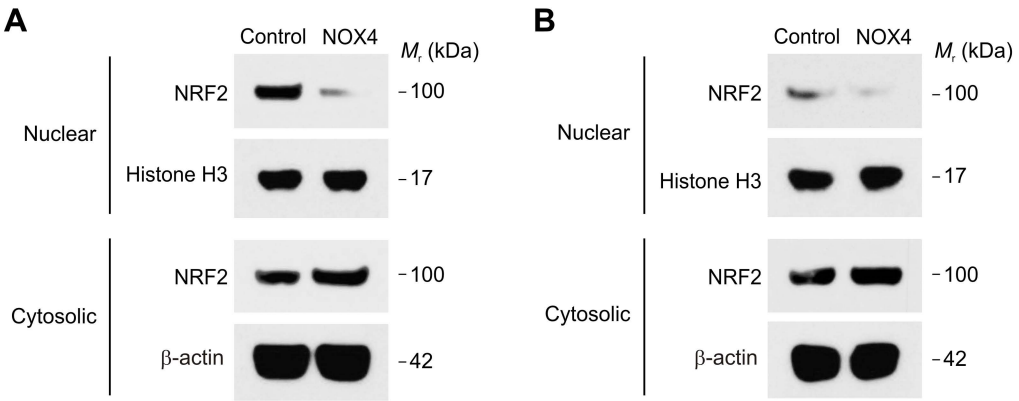
Supplemental Figure S7. The expression of 4-HNE was co-localized in NOX4-positive astrocytes of APP/PS1 mice. Representative immunofluorescence images of 4-HNE expression (purple) in NOX4 (green)-positive astrocytes (GFAP, red) of cortex region from APP/PS1 mice (APP/PS1) or wild-type mice (WT). DAPI-stained nuclei are shown in blue. Scale bars, 10 μM. White arrows indicates the co-localization of 4-HNE in NOX4-positive astrocytes.

Figure S8



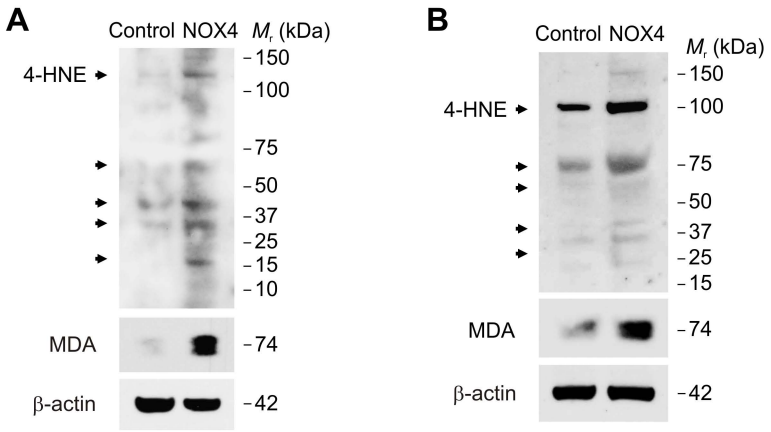
Supplemental Figure S8. The elevation of NOX4 suppresses mitochondrial metabolism by reduction of mitochondrial ETC protein levels. (A, B) Representative immunoblot analysis for five mitochondrial ETC protein levels including NDUFB8 for Complex I (C I (NDUFB8)), SDHB for Complex II (C II (SDHB)), UQCRC2 for Complex III (C III (UQCRC2)), MTCO1 for Complex IV (C IV (MTCO1)), and ATP5F1A for Complex V (C V (ATP5F1A)) in control (Control) and NOX4 overexpressing (NOX4) human astrocytes from two indipendent experiments. For immunoblots, β-actin was used as a loading control.

Figure S9



Supplemental Figure S9. The elevation of NOX4 inhibits nuclear translocation of NRF2 from cytosol. (A, B) Representative immunoblot analysis for nuclear and cytosolic NRF2 in control (Control) and NOX4 overexpressing (NOX4) human astrocytes from two independent experiments. For immunoblots, Histone H3 (nuclear) and β-actin (cytosolic) was used as a loading control.

Figure S10



Supplemental Figure S10. The elevation of NOX4 increases the levels of 4-HNE and MDA in human astrocytes.(A, B) Representative immunoblot analysis for 4-HNE and MDA in control (Control) and NOX4 overexpressing (NOX4) human astrocytes from two independent experiments. For immunoblots, β-actin was used as a loading control.