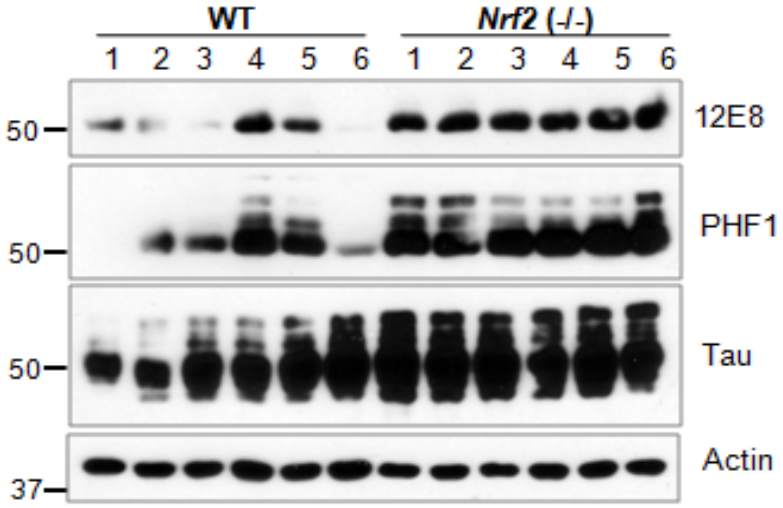
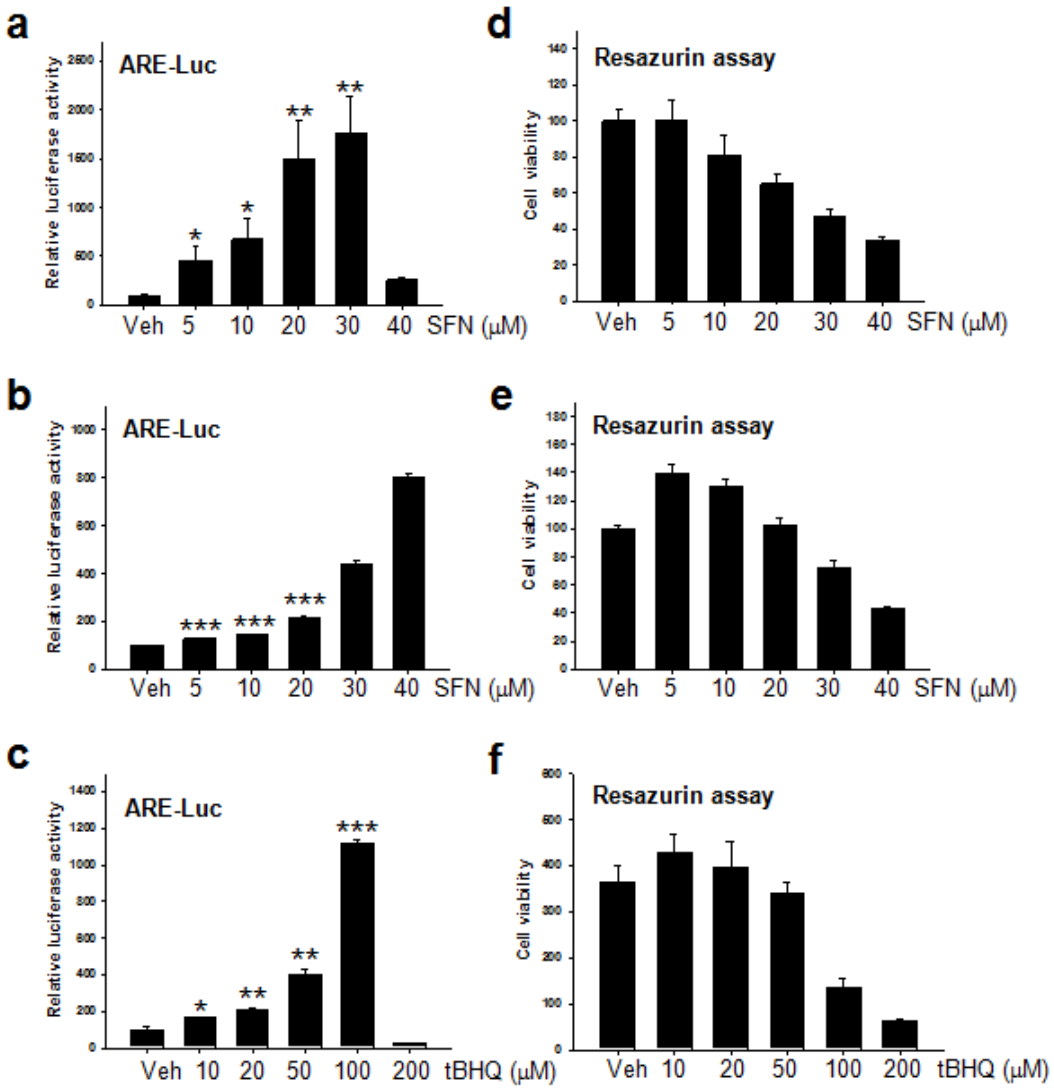
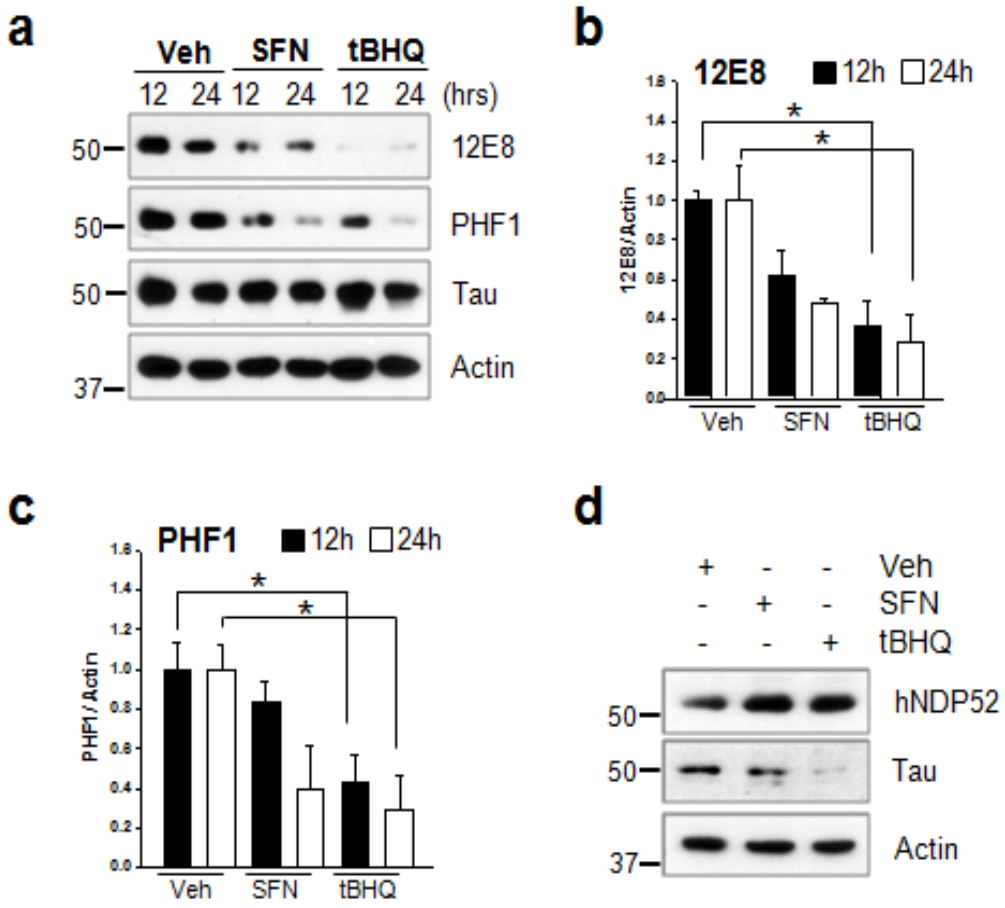


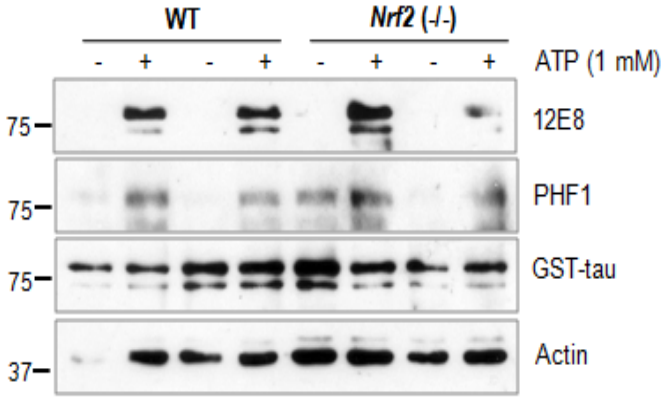

**Supplementary Figure 1** Phosphorylated tau accumulates in *Nrf2* (-/-) mice. Hippocampal tissues obtained from *Nrf2* (-/-) (10 months old, 4 male; 2 female) or wild-type (5 months old, 1 male; 11 months old, 1 male; 5 months old, 1 female; 12 months old, 3 female) mice were immunoblotted with 12E8 (pSer262/Ser356), PHF1 (pSer396/Ser404), a polyclonal to total tau (Tau) or an actin antibody. The relative molecular masses (kD) are indicated to the left of each blot.



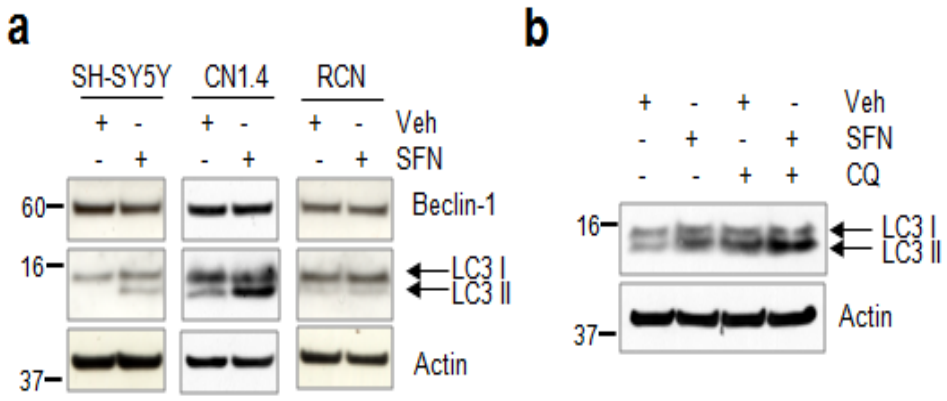

**Supplementary Figure 2** Sulforaphane (SFN) and *tert*-butylhydroquinone (tBHQ) activate Nrf2. (**a, c**) Primary cortical neurons (DIV 3) were transiently co-transfected with the ARE-Luc and TK-renilla plasmids using Lipofectamine 2000 (Invitrogen). (**b**) CN1.4 cortical neurons were kept in the presence of doxycycline (1 μg/ml) to induce the expression of tau for 24 h, and transfected with the same plasmids. On the next day, both cell types were treated with the indicated concentrations of SFN or tBHQ for 12 h, and assayed for luciferase activity. (**d, f**) Primary cortical neurons (DIV 3) and (**e**) CN1.4 cortical neurons were treated with the indicated concentrations of SFN or tBHQ for 24 h, and their viability measured using the resazurin assay. n=4. Data shown are mean±SE and were analyzed using Student's *t* test. (*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$)



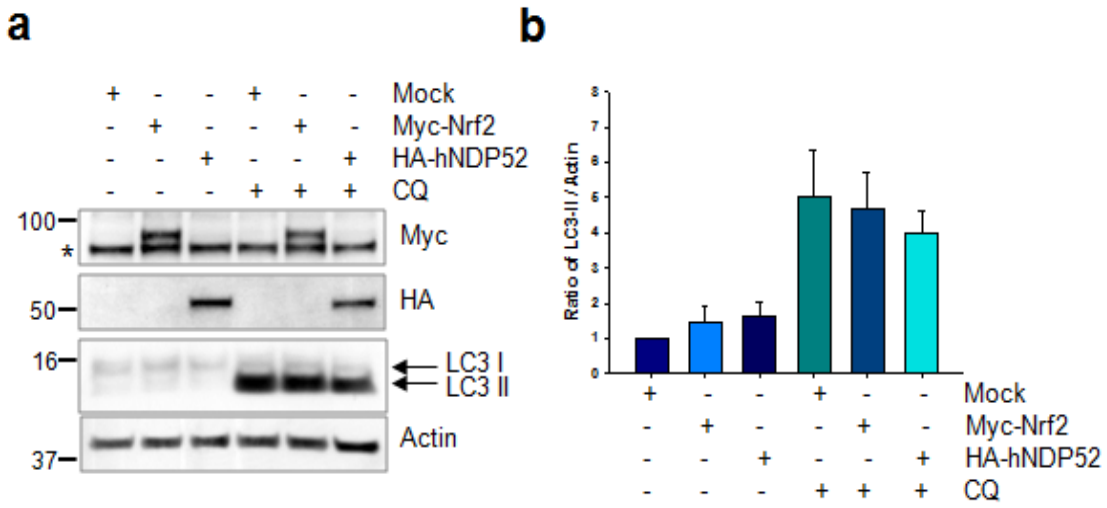

**Supplementary Figure 3** Nrf2 activation enhances the degradation of phosphorylated tau in neurons. (**a**) Primary cortical neurons (DIV 5) were treated with sulforaphane (10 μM, SFN) or *tert*-butylhydroquinone (50 μM, tBHQ) for 12 h or 24 h. The levels of tau phosphorylated at Ser262/Ser356 and Ser396/Ser404 were analyzed by immunoblotting using a 12E8- and PHF1-specific antibody, respectively. Tau was detected with a polyclonal phospho-independent tau antibody (Tau). The relative molecular masses (kD) are indicated to the left of each blot. (**b, c**) Bar graph of the relative optical density of phosphorylated tau normalized to actin. (**d**) SH-SY5Y cells were treated with of SFN (10 μM) or tBHQ (50 μM) for 24 h, and the expression levels of human NDP52 examined by immunoblotting using anti-NDP52 antibody. Tau was detected with a polyclonal phospho-independent tau antibody (Tau). n=3. Data shown are mean±SE and were analyzed using Student's *t* test. (*, $p<0.05$)



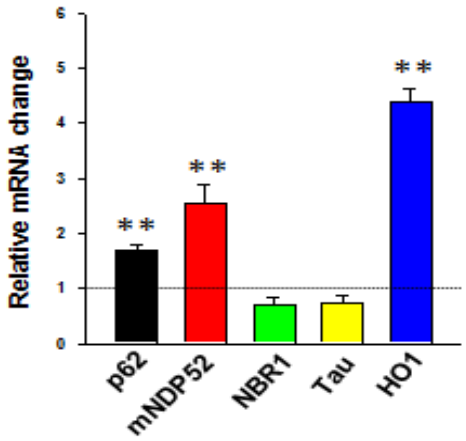

**Supplementary Figure 4** There is no significant difference in tau kinase activities between wild-type and *Nrf2* (-/-) mice. Mouse brain tissues obtained from wild-type (11 months old, 1 male; 12 months old, 1 female) and *Nrf2* (-/-) (10 months old, 1 male; 1 female) mice were homogenized in lysis buffer, and 10 μg of lysates was incubated with 2 μg of GST-tau protein at 37°C for 30 min in the presence or absence of 1 mM ATP. The levels of tau phosphorylated at Ser262/Ser356 and Ser396/Ser404 were analyzed by immunoblotting using a 12E8- and PHF1-specific antibody, respectively. GST-tau was detected with a monoclonal GST antibody (GST-tau). The relative molecular masses (kD) are indicated to the left of each blot. Data were analyzed using Student's *t* test.



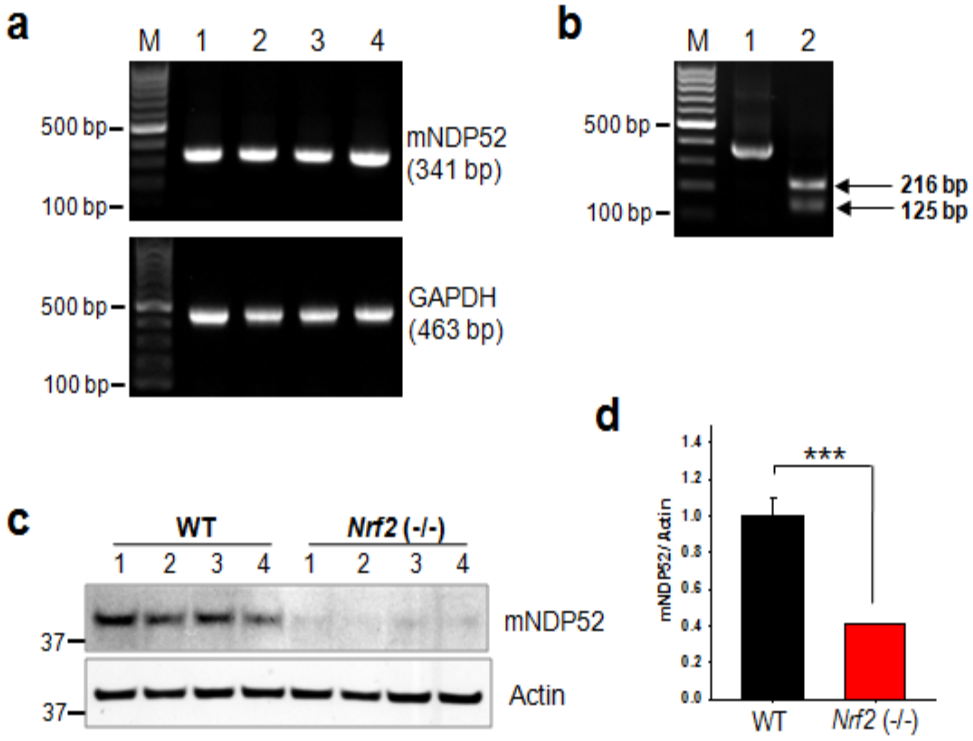

**Supplementary Figure 5** Sulforaphane induces autophagy in neurons. (**a**) SH-SY5Y cells, CN1.4 mouse cortical cells or rat cortical neurons (RCN) were treated with sulforaphane (SFN, 10 μM) for 24 h. The protein levels of beclin-1 and LC3 were analyzed by immunoblotting. (**b**) CN1.4 mouse cortical neurons treated with vehicle or SFN were incubated with chloroquine (50 μM, CQ), a lysosomal inhibitor for 12 h. The protein level of LC3 was analyzed by immunoblotting. The relative molecular masses (kD) are indicated to the left of each blot.



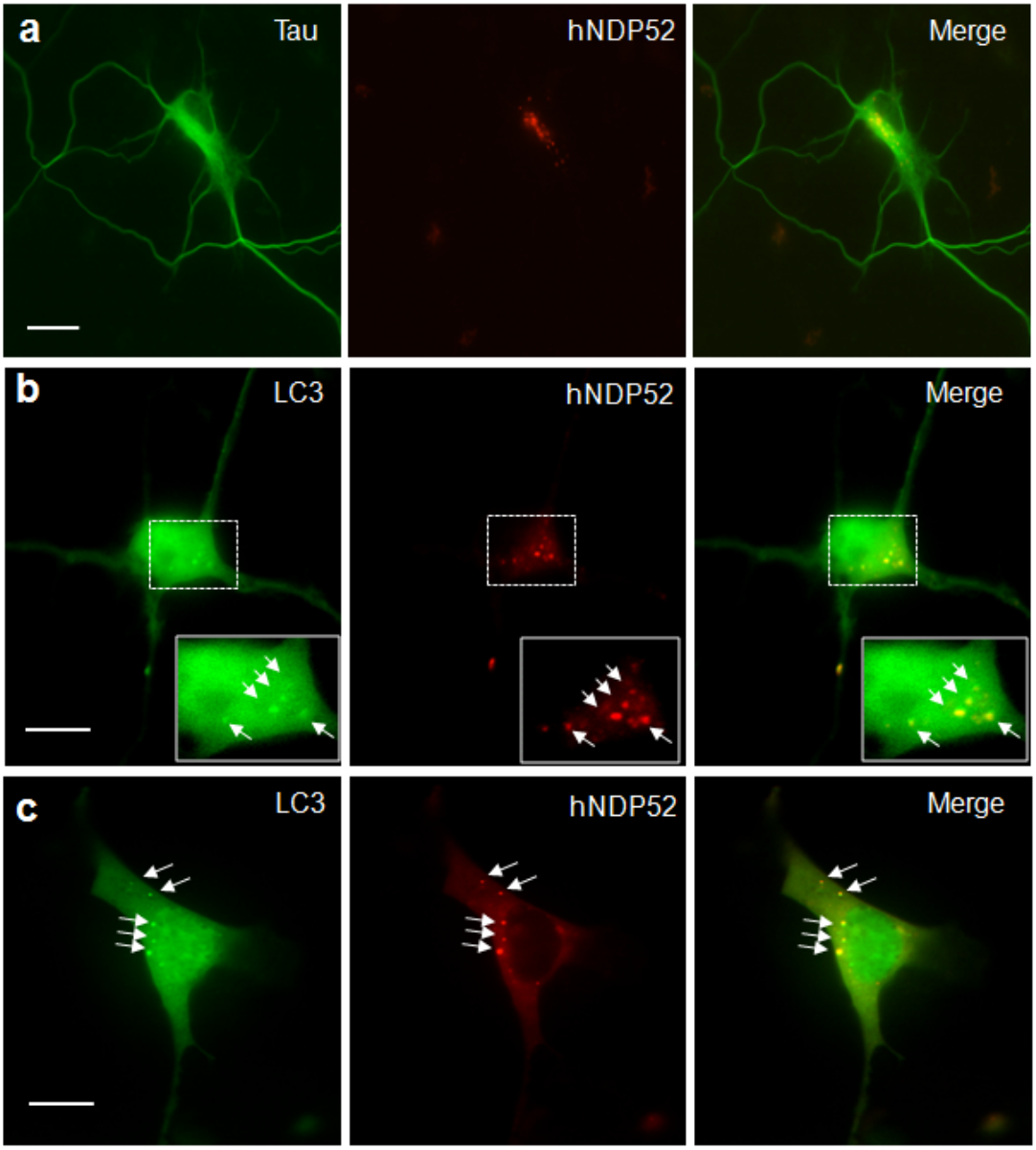

**Supplementary Figure 6** Neither Nrf2 nor NDP52 directly induces autophagy. (**a**) HEK293-TN cells were transiently transfected with pcDNA3.1 (mock), Myc-Nrf2 or HA-hNDP52 using Lipofectamine 2000. Half of the cells were incubated with chloroquine (50 μM, CQ), a lysosomal inhibitor for 18 h followed by determination of the protein levels of Nrf2, transfected hNDP52 and LC3 by immunoblotting using Myc (9B11), HA and LC3 antibodies, respectively. The asterisk to the left of the Nrf2 blot designates the position at which a non-specific band migrated. The relative molecular masses (kD) are indicated to the left of each blot. (**b**) Bar graph represents the relative optical density of LC3-II normalized with that of actin. n=3. Data shown are mean±SE.



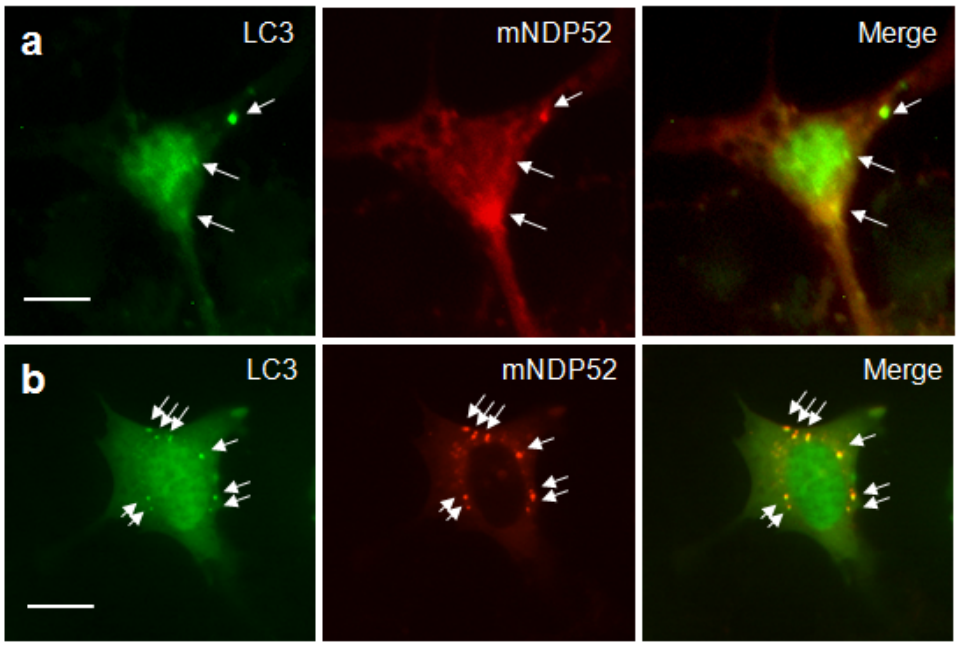

**Supplementary Figure 7** Mouse NDP52 is induced by the activation of Nrf2. CN1.4 mouse cortical cells stably expressing inducible human tau maintained in the presence of doxycycline (1 μg/ml) for 24 h to induce tau were treated with sulforaphane (SFN, 10 μM) for 12 h. Bar graphs represent the relative mRNA level of each mouse gene compared to that of cells not treated with SFN. n=6. Data shown are mean±SE and were analyzed using Student's *t* test. (**, $p<0.01$)



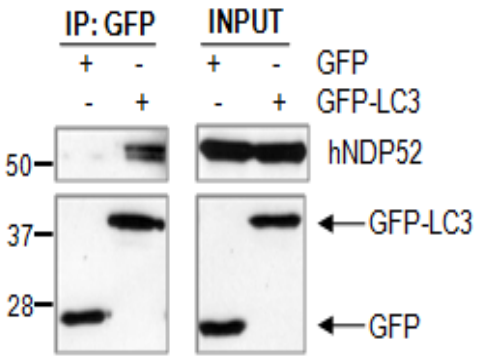

**Supplementary Figure 8** The expression of mouse NDP52 is significantly reduced in *Nrf2* (-/-) mice. (**a**) To investigate whether NDP52 is expressed in mouse brain, RT-PCR was performed using mouse NDP52 gene specific primers and cDNA prepared from hippocampal tissues of wild-type (8 months old, 2 male; 2 female) mice as described in Methods. PCR products were analyzed with 1.2% agarose gel electrophoresis. M, DNA marker. n=4. (**b**) To confirm whether mouse NDP52 gene was specifically amplified by RT-PCR, *Hin*dIII digestion was used since a *Hin*dIII recognition site exists within the PCR product of mouse NDP52 gene. Two DNA fragments with expected size were observed following the incubation with *Hin*dIII at 37°C overnight. M, DNA marker; lane 1, PCR product of mouse NDP52 gene purified with the Qiaquick PCR product purification kit (Qiagen); lane 2, *Hin*dIII-digested DNA fragments. (**c**) The level of NDP52 in *Nrf2* (-/-) (7 months old, 2 male; 2 female) or wild-type (8 months old, 2 male; 2 female) mice was examined by immunoblotting using anti-NDP52 antibody. The relative molecular masses (kD) are indicated to the left of each blot. (**d**) Bar graphs represent the relative optical density of mouse NDP52 normalized with that of actin. n=4. Data shown are mean±SE and were analyzed using Student's *t* test. (***, $p<0.001$)



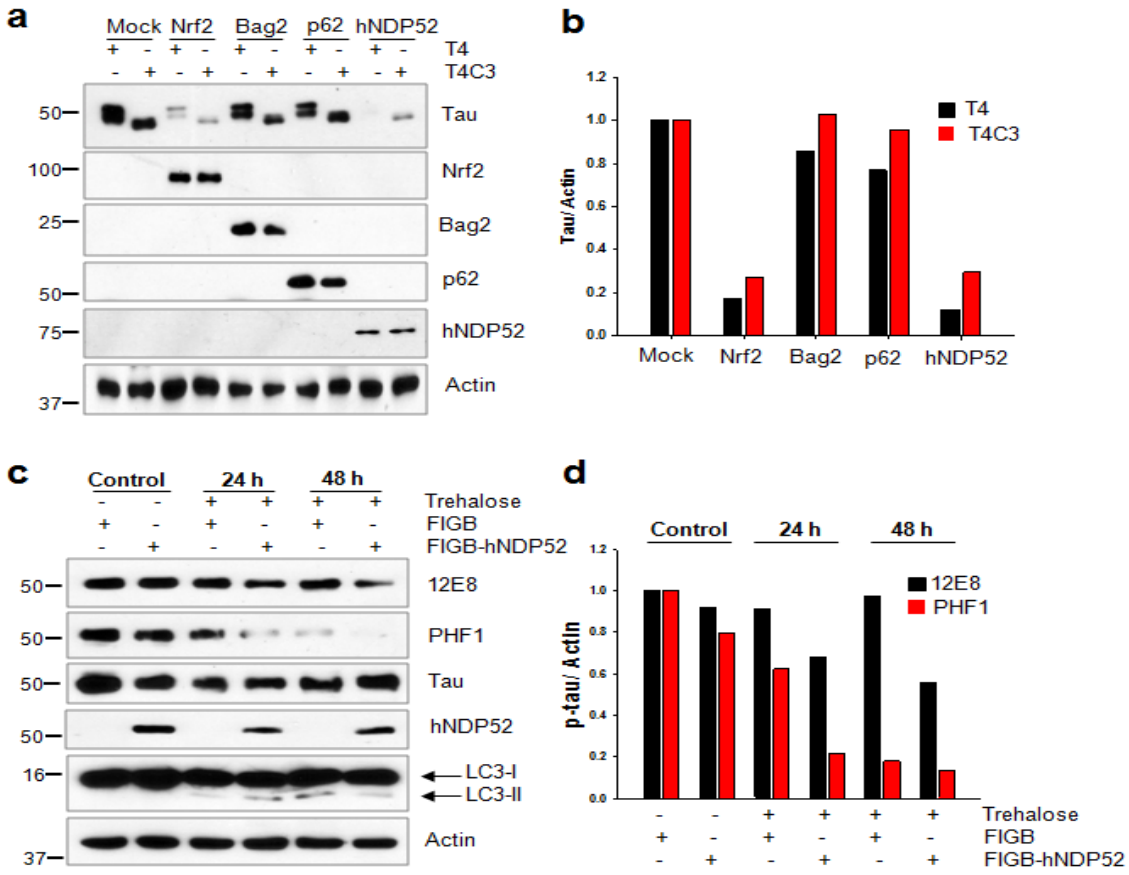

**Supplementary Figure 9** Human NDP52 colocalizes with LC3 at autophagic vesicles (AVs) in neurons. (**a**) Primary cortical neurons (DIV 3) were co-transfected with the pmCherry-hNDP52 and pEGFP-tau plasmids. The next day, images were captured using a fluorescence microscope (Carl Zeiss AX10, x400 magnifications). (**b**) Primary cortical neurons (DIV 3) and (**c**) CN1.4 naïve cells were co-transfected with pmCherry-hNDP52 and pEGFP-LC3 plasmids. The next day, images were captured using a fluorescence microscope (x630 magnifications). The area indicated by the dotted lined boxes in (**b**) is enlarged and shown in the right corner of the image. Arrows indicate autophagic vesicles (AVs). Scale bar = 20 μm.



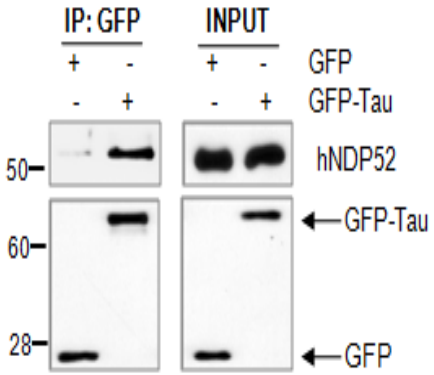

**Supplementary Figure 10** Mouse NDP52 marks autophagic vesicles (AVs) co-localizing with LC3 in neurons. (**a**) Primary cortical neurons (DIV 3) and (**b**) CN1.4 naive cells were co-transfected with pmCherry-mNDP52 and pEGFP-LC3 plasmids. The next day, images were captured using a fluorescence microscope (x630 magnifications). Arrows indicate autophagic vesicles (AVs). Scale bar = 20 μm.



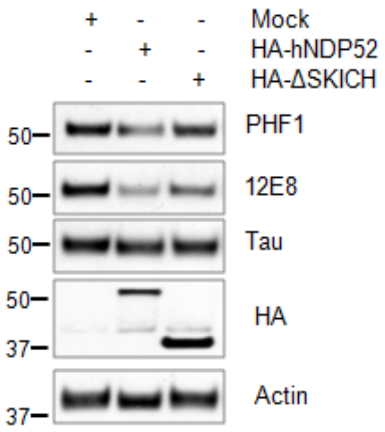

**Supplementary Figure 11** Human NDP52 associates with LC3. HEK 293-TN cells were transfected with a plasmid expressing GFP or GFP-LC3. On the next day, co-precipitation of endogenous NDP52 with LC3 was examined by immunoblotting using anti-NDP52 antibody following the immunoprecipitation with a monoclonal GFP antibody. Homogenates (Input) used for co-immunoprecipitation were analyzed by immunoblotting using anti-NDP52 or GFP antibody. The relative molecular masses (kD) are indicated to the left of each blot.



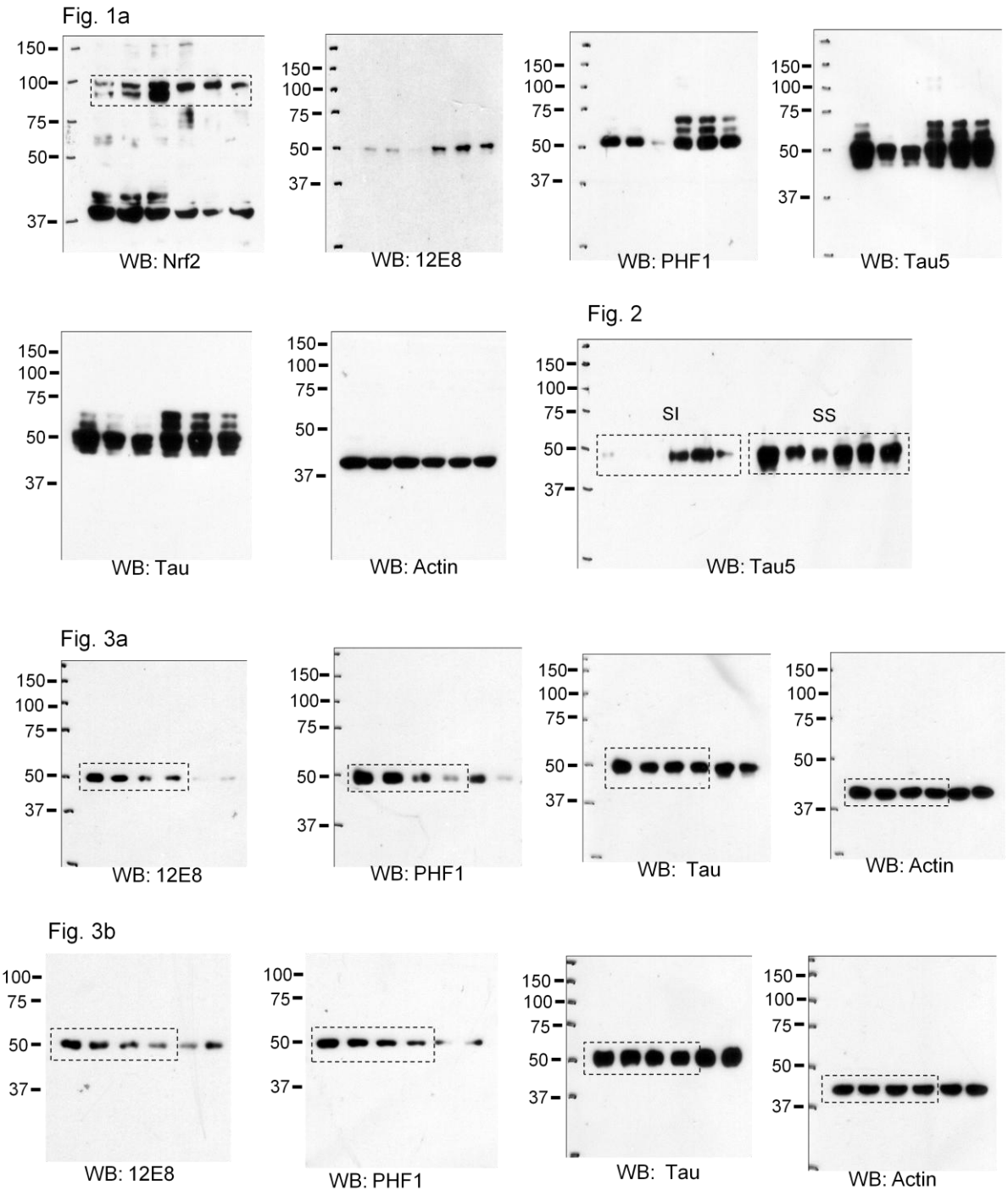

**Supplementary Figure 12** Human NDP52 facilitates the degradation of tau. (**a**) HEK 293-TN cells were co-transfected with the plasmids expressing T4 (wild-type tau) or T4C3 (caspase-cleaved tau) together with a mock plasmid (pcDNA3.1) or a plasmid expressing Myc-Nrf2, Myc-p62/SQSTM1, HA-Bag2 or GFP-hNDP52. The next day, the levels of T4 or T4C3 were analyzed by immunoblotting using a monoclonal tau antibody (Tau5). The expression of Nrf2, p62/SQSTM1, Bag2 and NDP52 was confirmed with immunoblotting using a monoclonal Myc, HA and GFP antibody, respectively. (**b**) Histogram represents the optical density of tau normalized with that of actin. (**c**) Primary cortical neurons were transduced with a control lentivirus (FIGB) or with one expressing hNDP52 at DIV 1. To induce autophagy, trehalose (150 mM) was added at DIV 5 and the neurons incubated for 24 h (DIV 6) or 48 h (DIV 7). The levels of tau phosphorylated at Ser262/Ser356 and Ser396/Ser404 were analyzed by immunoblotting using 12E8- and PHF1-specific antibodies, respectively. Total tau, hNDP52 and LC3 were detected with polyclonal anti-tau (Tau), anti-NDP52 and anti-LC3 antibodies, respectively. (**d**) Histogram represents the optical density of phosphorylated tau normalized with that of actin. The relative molecular masses (kD) are indicated to the left of each blot.



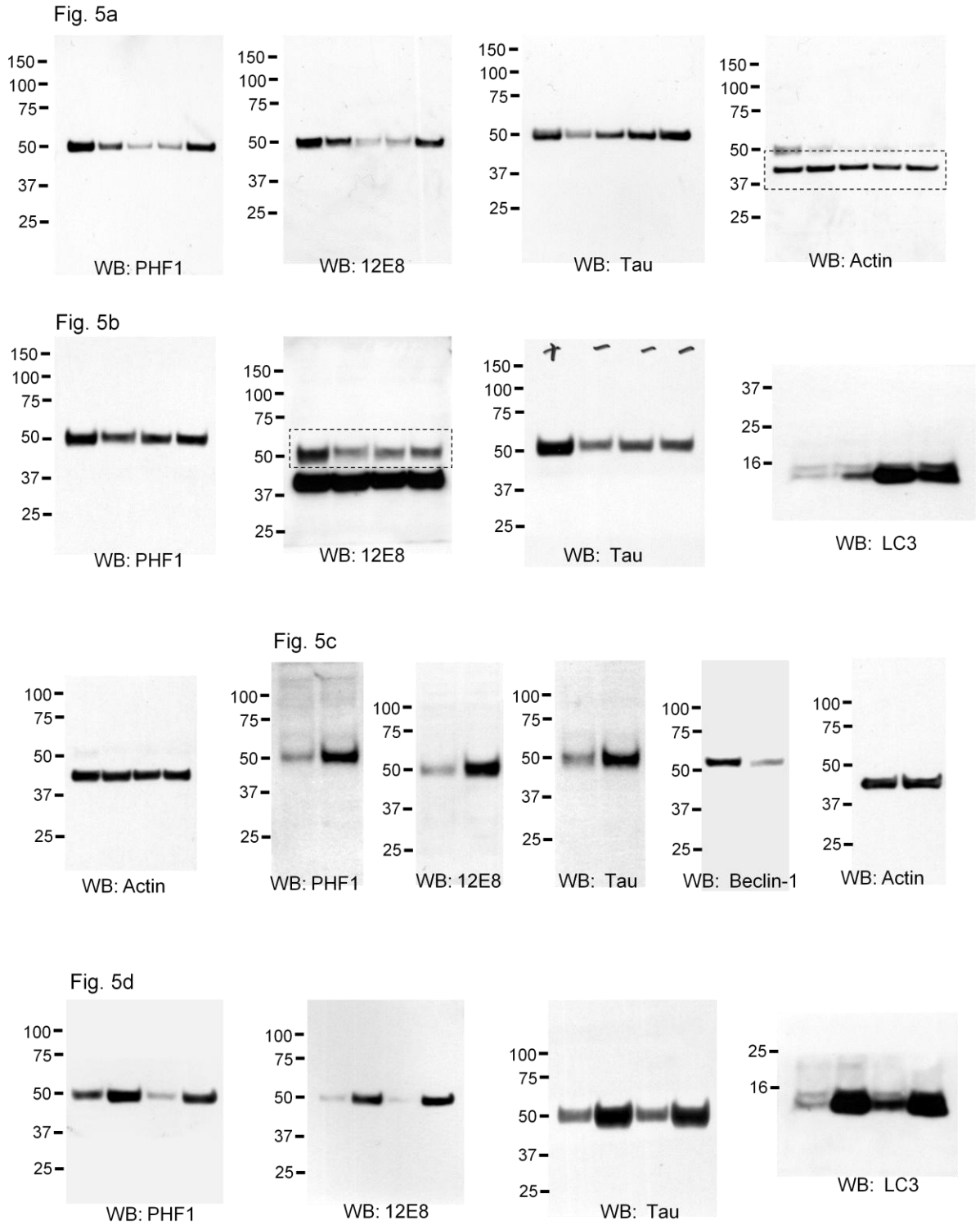

**Supplementary Figure 13** Human NDP52 associates with tau. HEK 293-TN cells were transfected with a plasmid expressing GFP or GFP-tau. On the next day, cell lysates were used for co-immunoprecipitation of NDP52 using a monoclonal GFP antibody. Co-precipitation of endogenous NDP52 was examined by immunoblotting using anti-NDP52 antibody. Homogenates (Input) used for co-immunoprecipitation were analyzed by immunoblotting using anti-NDP52 or GFP antibody. The relative molecular masses (kD) are indicated to the left of each blot.



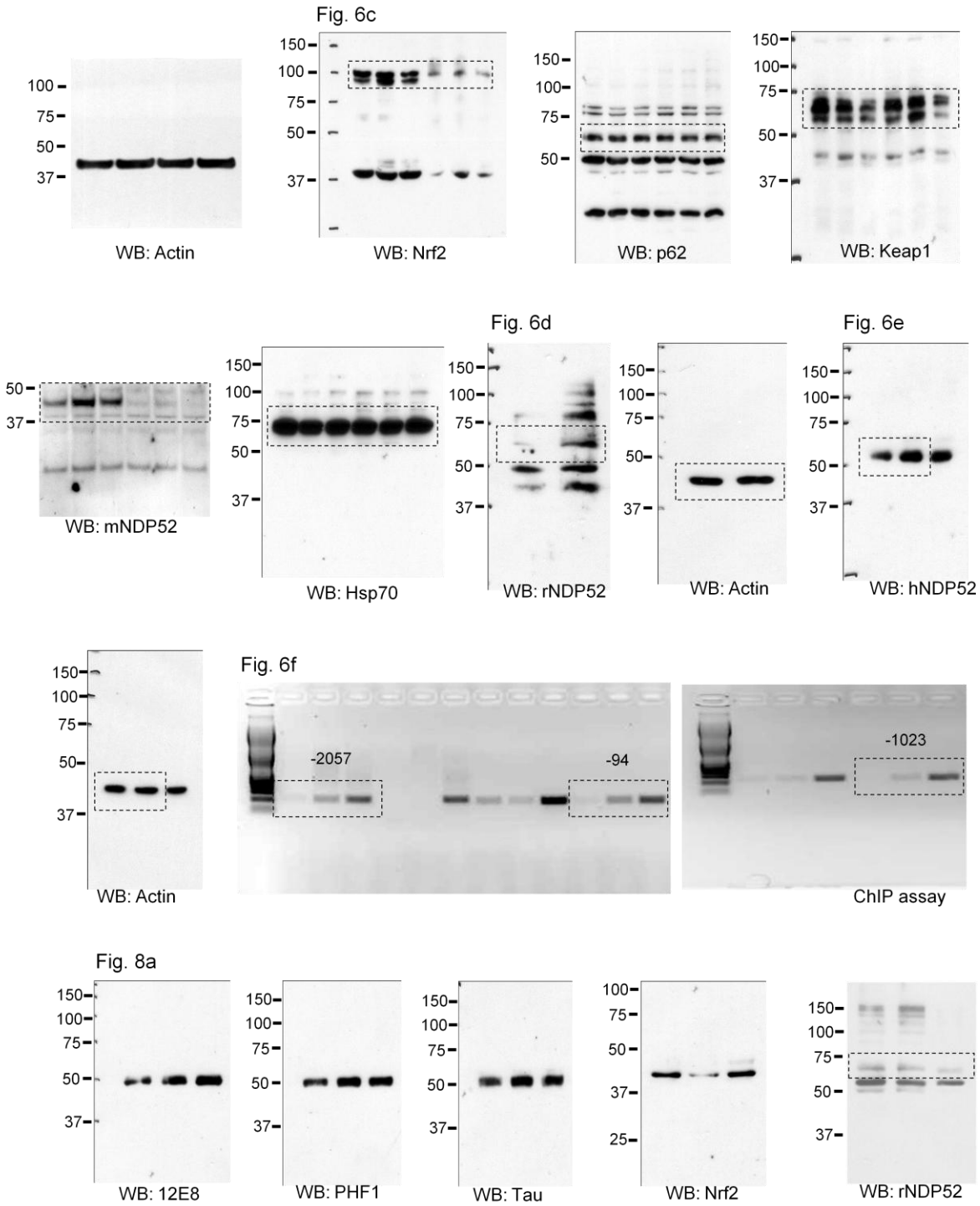

**Supplementary Figure 14** The NDP52 SKICH deletion mutant is unable to enhance the degradation of phosphorylated tau. CN1.4 mouse cortical cells stably expressing inducible tau were treated with doxycycline (1 ug/ml) for 24 h. The cells were then transiently transfected with pcDNA3.1 (mock), HA-hNDP52 or HA-ΔSKICH using Lipofectamine 2000 (Invitrogen). After 24 h, the levels of phosphorylated tau were analyzed by immunoblotting using the antibodies 12E8 or PHF1. Total tau was detected with a polyclonal phospho-independent tau antibody (Tau), and hNDP52 and HA-ΔSKICH were detected with an HA antibody. The relative molecular masses (kD) are indicated to the left of each blot.



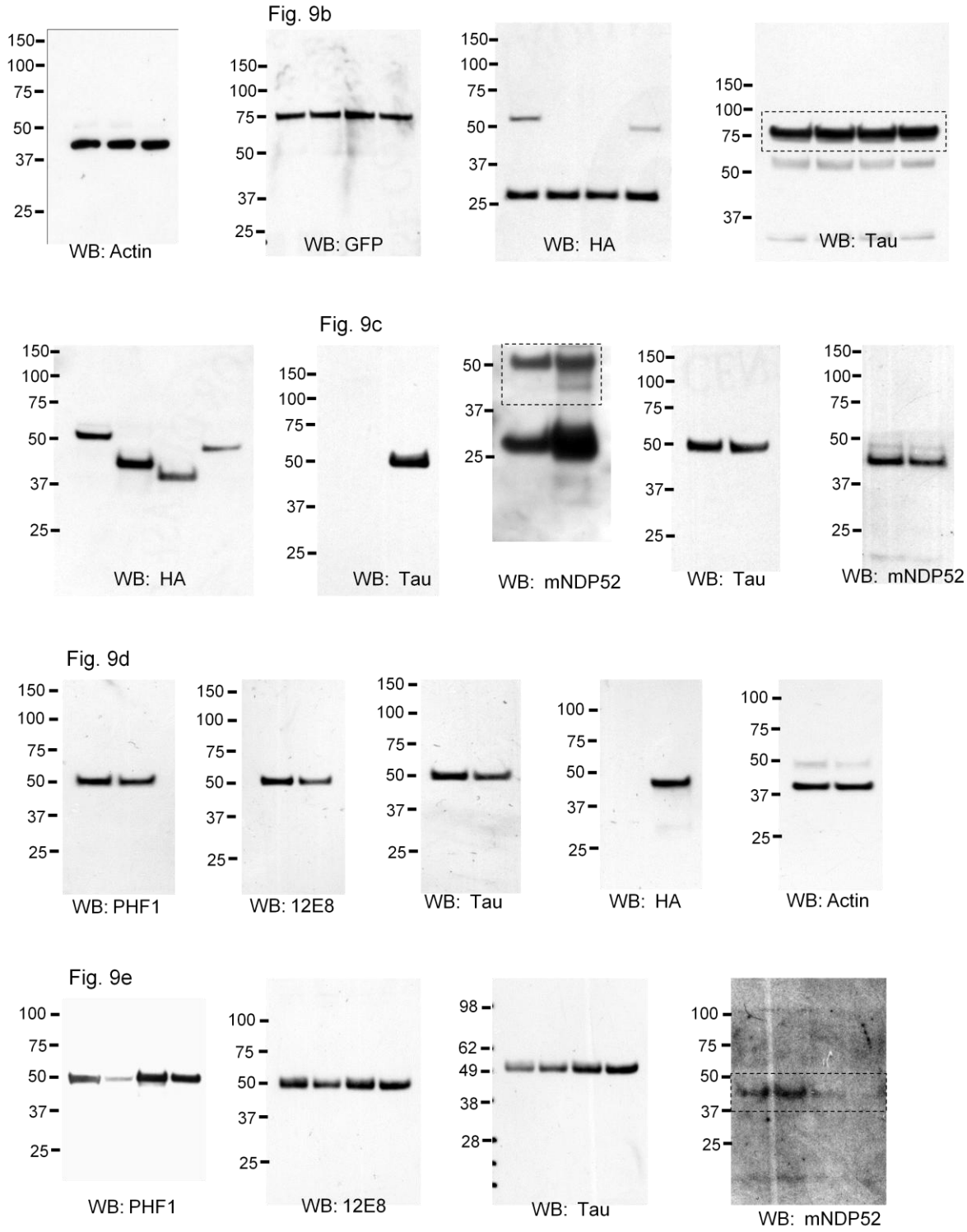

**Supplementary Figure 15** Full scans of uncropped blots and gels.



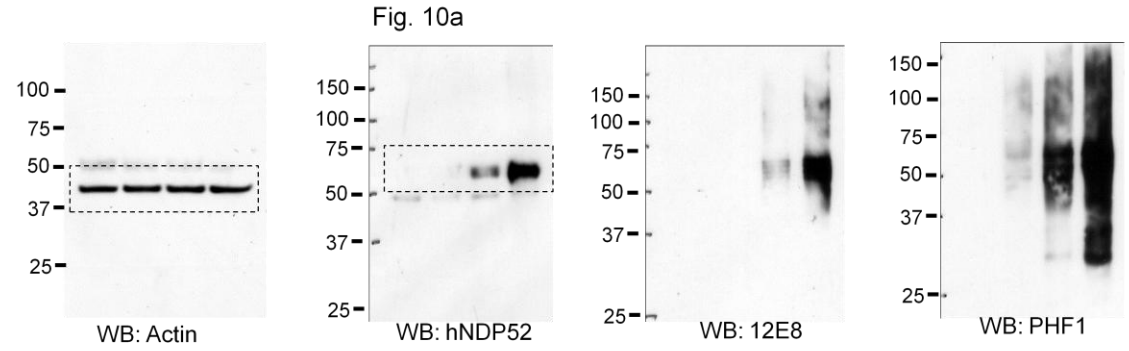

**Supplementary Figure 15** continued.

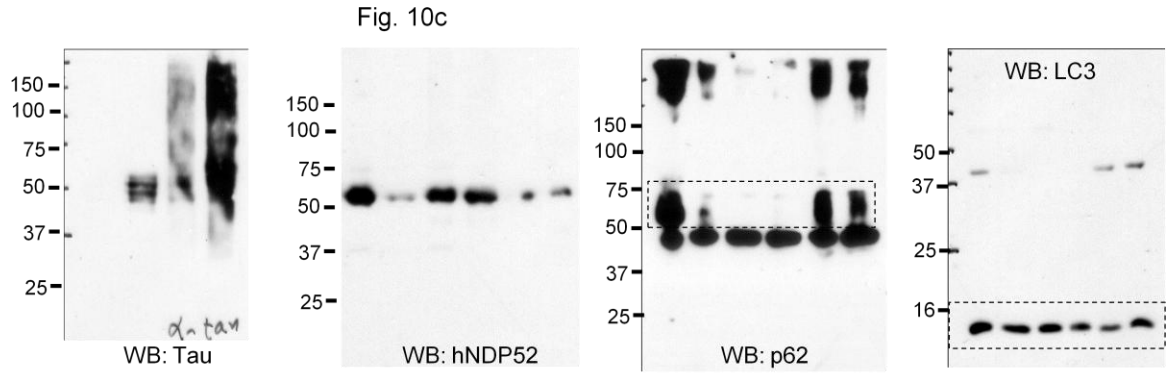


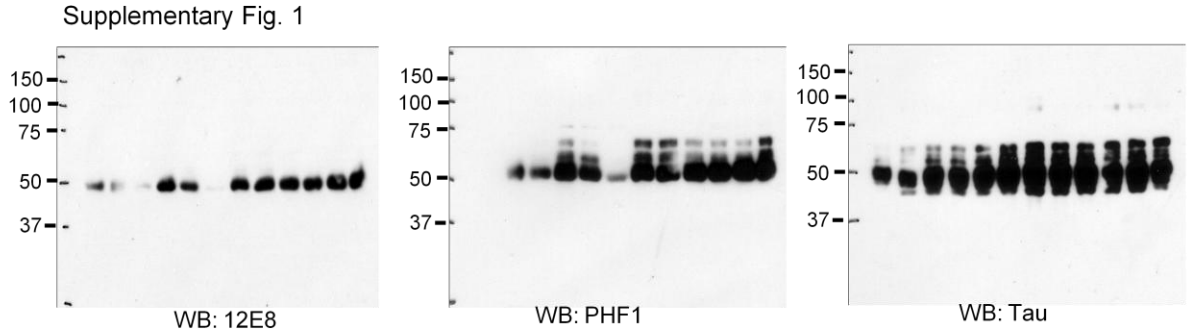

**Supplementary Figure 15** continued.

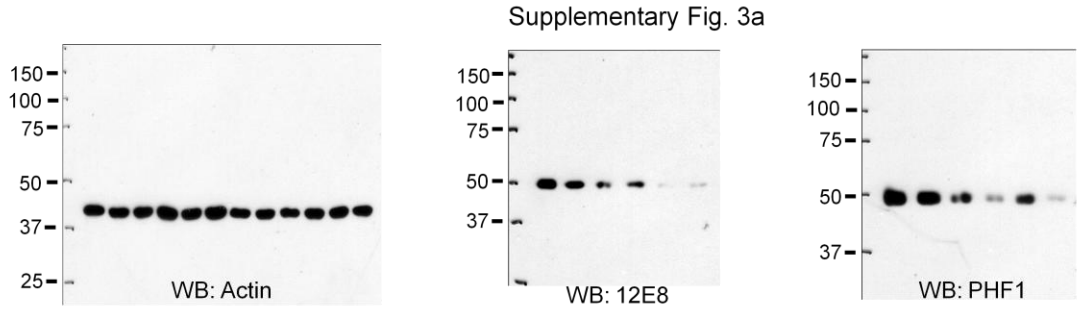



**Supplementary Figure 15** continued.

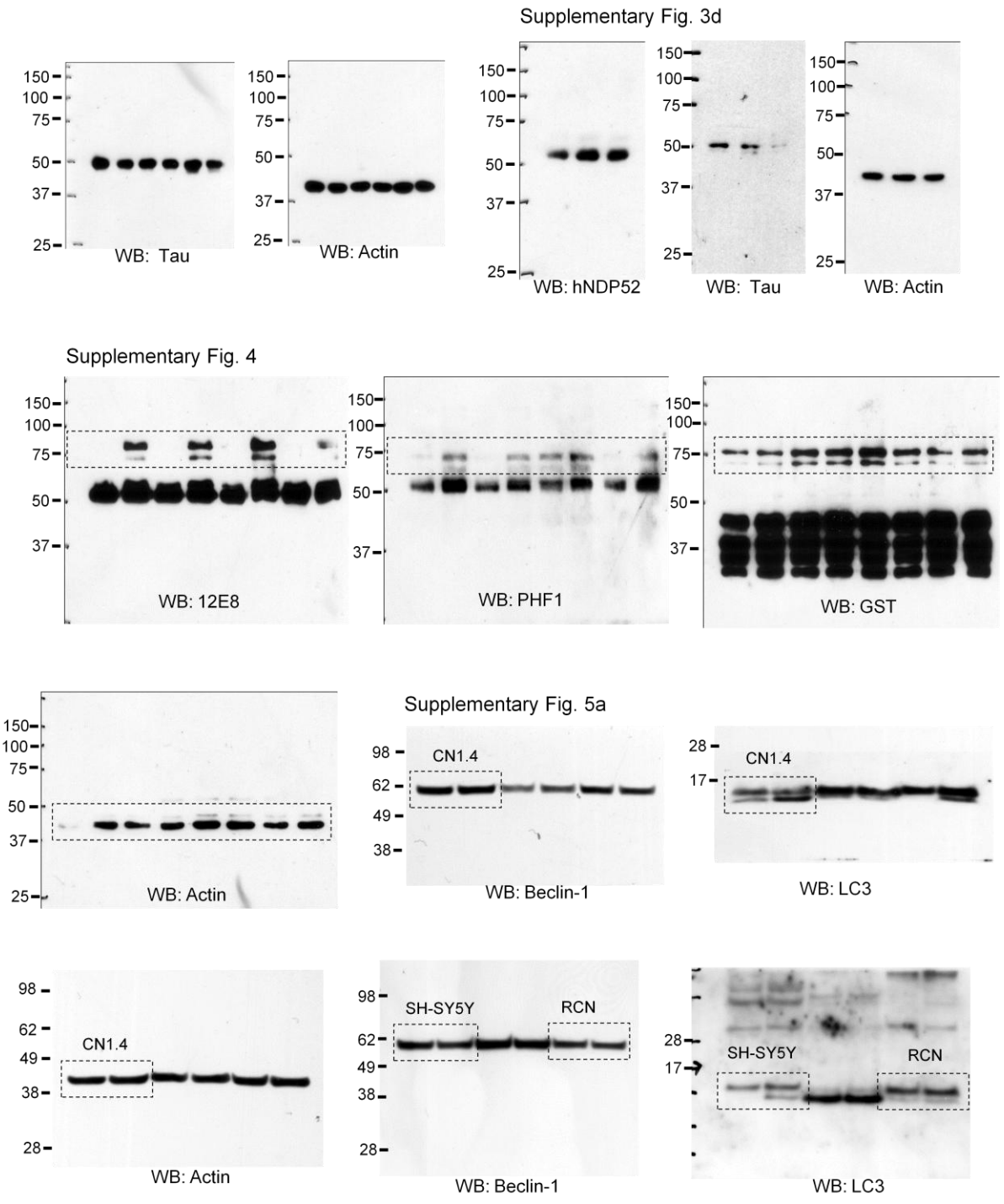



**Supplementary Figure 15** continued.

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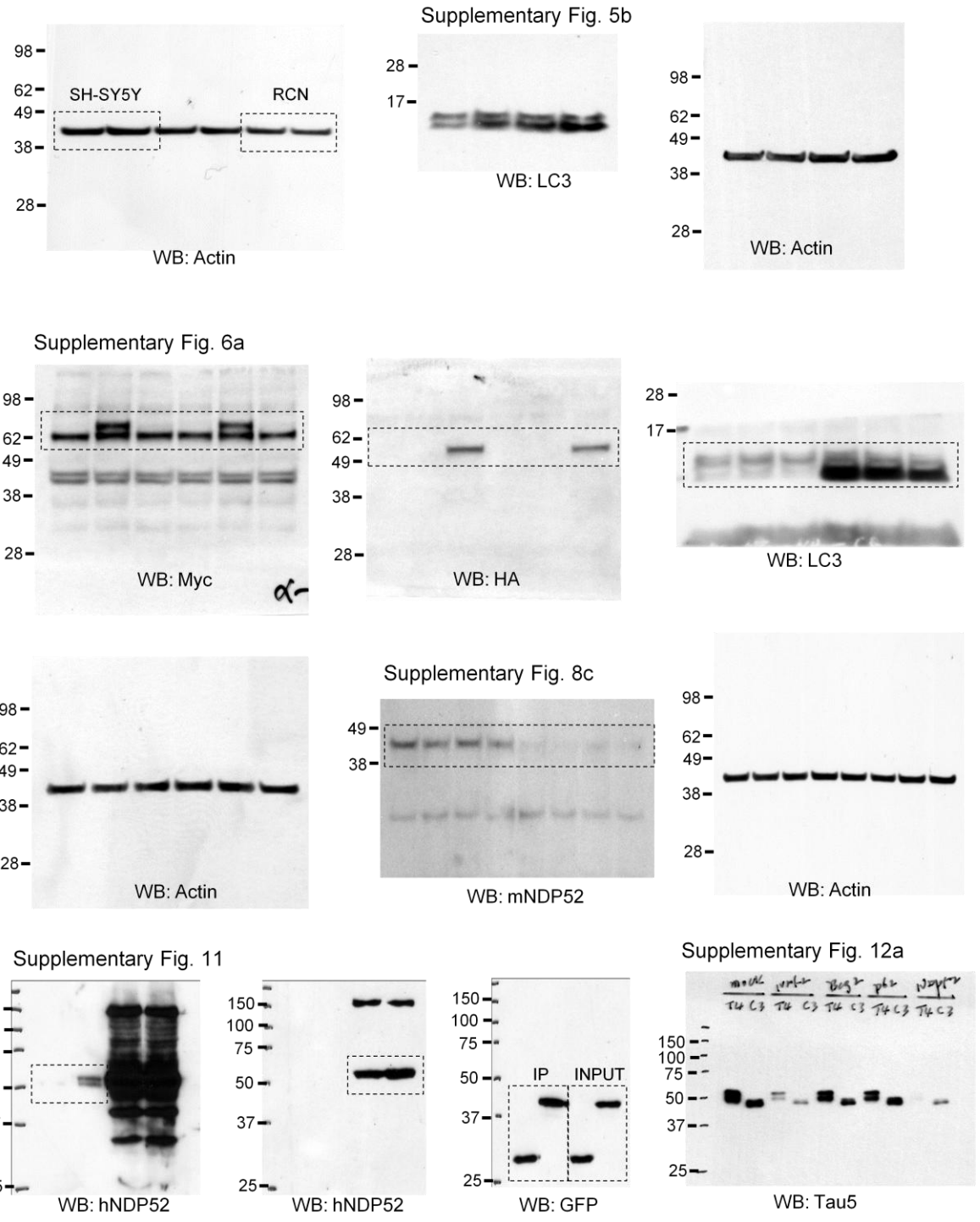


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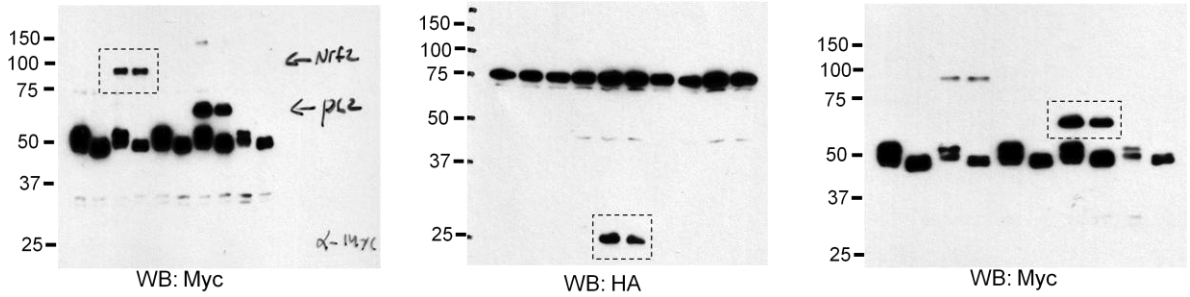
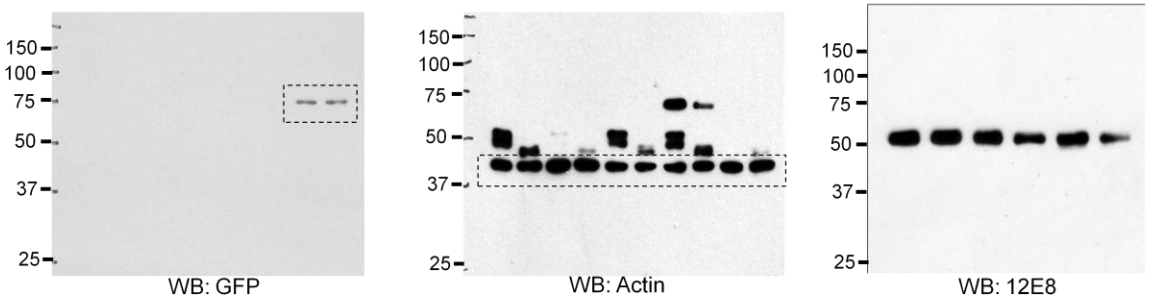
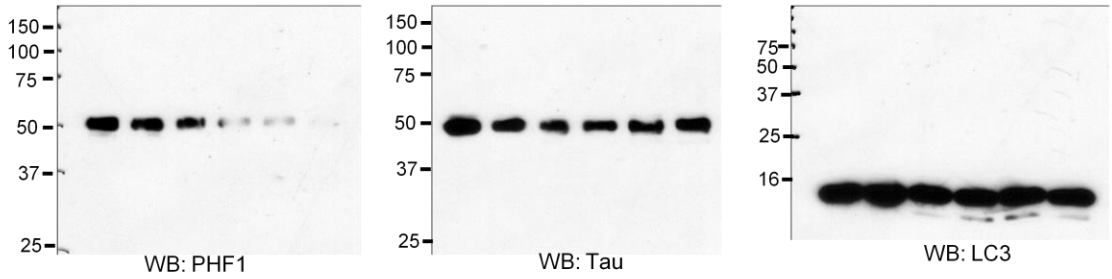
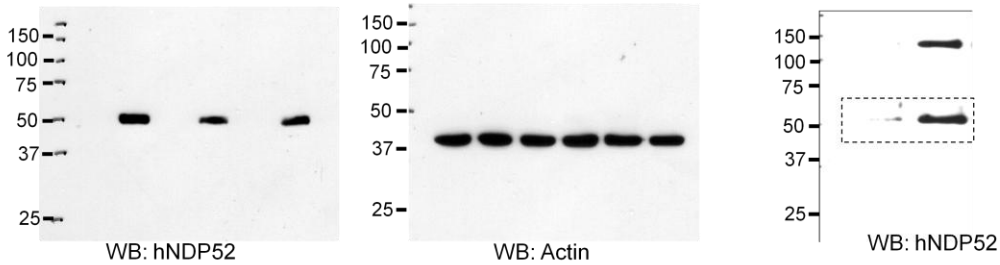



Supplementary Fig. 12c




Supplementary Fig. 13

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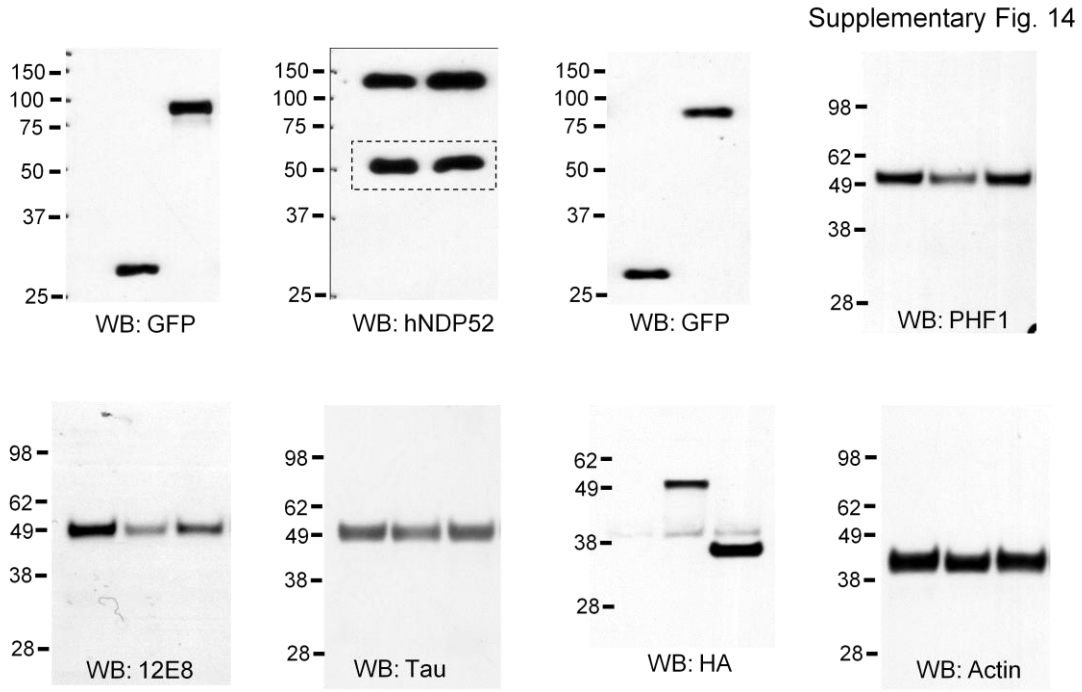


**Supplementary Figure 15** continued.

**Supplementary Table 1** The patient information of human cortical specimens

| Specimen | Age | Sex | PMI (h) | Diagnosis |
|---|---|---|---|---|
| AD1 | 91 | M | 3.5 | AD |
| AD2 | 90 | F | 6 | AD |
| AD3 | 81 | F | 12 | AD |
| AD4 | 66 | F | 16 | AD |
| AD5 | 88 | F | 13 | AD |
| AD6 | 82 | M | 2.4 | AD |

**Supplementary Table 2** The sequence of shRNA used for knock-down of gene expression

| shRNA | Sequence |
|---|---|
| Scramble | 5’-GATCCCCGGAAAGACGATGACGGAAATTCAAGAGATTTCCGTCATCGTCTTTCCTTTTTC-3’ |
| | 3’-GGGCCTTTCTGCTACTGCCTTTAAGTTCTCTAAAGGCAGTAGCAGAAAGGAAAAAGAGCT-5’ |
| Nrf2 | 5’-GATCCCCCCAGCAGGACATGGATTTGATTTCAAGAGAATCAAATCCATGTCCTGCTGGTTTTTC-3’ |
| | 3’-GGGGGTCGTCCTGTACCTAAACTAAAGTTCTCTTAGTTTAGGTACAGGACGACCAAAAAGAGCT-5’ |
| NDP52 | 5’-GATCCCCTTAACAATGTGGAGAAGTTTTCAAGAGAAACTTCTCCACATTGTTAATTTTTC-3’ |
| | 3’- GGGAATTGTTACACCTCTTCAAAAGTTCTCTTTGAAGAGGTGTAACAATTAAAAAGAGCT-5’ |

**Supplementary Table 3** The Sequence of primers used for ChIP assay

| ARE site | Forward | Backward |
|---|---|---|
| -2057 | 5’-ACTTGTCCCTGCAGACCGAGTTTA-3’ | 5’-CCAAAGTGCTGGGATTACAGGCAT-3’ |
| -1023 | 5’-TGTGATCATGGCTTATGGCAGCCT-3’ | 5’-TAGCTCACGCCTGTAATCCCAACA-3’ |
| -94 | 5’-ATTGCTGCTCAGGACTTAGGAGAG-3’ | 5’-CGACAGCAACAGGGCAGAGT-3’ |

**Supplementary Table 4** The Sequence of siRNA used for knock-down of gene expression

| siRNA | Forward | Backward |
|---|---|---|
| Scramble | 5’-CCUACGCCACCAAUUUCGUUU-3’ | 5’-ACGAAAUUGGUGGCGUAGGUU-3’ |
| Mouse NDP52 | 5’-GGAAGGAAGUGAAGGCCUAUU-3’ | 5’-UAGGCCUUCACUUCCUUCCUU-3’ |

**Supplementary Table 5** The sequence of primers used for qRT-PCR

| Gene | Forward | Backward |
|---|---|---|
| Mouse NDP52 | 5’-TGGCAACTTCTCTCAGGTCCTGTT-3’ | 5’-TCCTTGCGTCGAGGGATGAACTTT-3’ |
| Rat NDP52 | 5’-AAAGCAACAGCCAGCTCTCCTCTA-3’ | 5’-AGCTCCTCAGTGCCTTGTTCTCAT-3’ |
| Mouse p62 | 5’-TGAAACATGGACACTTTGGCTGGC-3’ | 5’-ACATTGGGATCTTCTGGTGGAGCA-3’ |
| Rat p62 | 5’-GCACAGCAAGCTCATCTTTCCCAA-3’ | 5’-AAAGTGCCCATGTTTCAGCTTCCG-3’ |
| Mouse NBR1 | 5’-TGACACAGACCAGCCTCAAGACAA-3’ | 5’-AGCTTCTCCTGCAACCTCTGTTCA-3’ |
| Rat NBR1 | 5’-GCGCAGACACAAAGCTCAAGTTCA-3’ | 5’-AACTGCTGGCCTTTGTGAGAGAGA-3’ |
| Mouse HO1 | 5’-TCGTGCTCGAATGAACACTCTGGA-3’ | 5’-ATGTTGAGCAGGAAGGCGGTCTTA-3’ |
| Rat HO1 | 5’-TGCTCGCATGAACACTCTGGAGAT-3’ | 5’-ATGTTGAGCAGGAAGGCGGTCTTA-3’ |
| Mouse Nrf2 | 5’-TAAAGCTTTCAACCCGAAGCACGC-3’ | 5’-TCCATTTCCGAGTCACTGAACCCA-3’ |
| Rat Nrf2 | 5’-TGACTCTGACTCCGGCATTTCACT-3’ | 5’-TCCATTTCCGAGTCACTGAACCCA-3’ |
| Mouse Tau | 5’-AATATCAAACACGTCCCGGGTGGA-3’ | 5’-ATGGATGTTCCCTAACGAGCCACA-3’ |
| Rat Tau | 5’-AACATCCATCACAAGCCAGGAGGT-3’ | 5’-TGGGTGATGTTATCCAAGGAGCCA-3’ |
| Mouse GAPDH | 5’-TCAACAGCAACTCCCACTCTTCCA-3’ | 5’-ACCCTGTTGCTGTAGCCGTATTCA-3’ |
| Rat GAPDH | 5’-TGACTCTACCCACGGCAAGTTCAA-3’ | 5’-ACGACATACTCAGCACCAGCATCA-3’ |

**Supplementary Table 6** The Sequence of primers used for RT-PCR

| Gene | Forward | Backward |
|---|---|---|
| Mouse NDP52 | 5’-AAGGACTGGATTGGCATCTTTA-3’ | 5’-AGGTCAGCGTACTTGTCTTTC-3’ |
| Mouse GAPDH | 5’-GGGTGTGAACCACGAGAAATA-3’ | 5’-GTTGAAGTCGCAGGAGACAA-3’ |