Supporting Information

SI Materials and Methods

Generation of cells expressing H2AX-WT (H2AX-rescued cells or Revertant: REV)

For retroviral infection, HEK 293T were used for virus packaging according to the manufacturer's instructions. Briefly, retroviral constructs p-BABE-puro or pBABE-puro-H2AX-WT, pVPack-VSVG and pVPack-GP were transfected into HEK 293T cells using Lipofectamine 2000 according to the manufacturer's instructions. Viral particles were harvested at 48 h post-transfection. Cells were infected with virus for 48 h in the presence of DEAE-dextran (10 μg. ml^{-1}). Infected cells were either harvested for gene and protein expression analysis or selected to establish stable expression. For the plasmid pBABE-puro-H2AX construct, H2A.X was amplified between *Bam*HI and *Eco*RI sites from pCR2.1 vector using the following primers: forward primer: 5'-GTCGGATCCATGTCGGGCCGCGG-3' and reverse primer: 5'-GTAGAATTCTTAGTACTCCTGGGAGGCCTGG-3'. The PCR product was digested and subcloned into p-BABE-puro vector.

Western blots

Cells were washed twice with PBS, directly solubilized in denaturing sample buffer and then subjected to SDS-PAGE. Proteins were electrotransferred to 0.2 μm Protran BA 83 nitrocellulose sheets (Invitrogen, Carlsbad, CA, USA) for immunodetection with the following primary antibodies: H2AX (1:5000; ab20669, Abcam, Cambridge, MA, USA); PGC-1α (1:2000; ab54481, Abcam, Cambridge, MA, USA); OXPHOS (1:5000; ab110413, Abcam, Cambridge, MA, USA), TH (1:5000; MAB318, Millipore Sigma, Burlington, MA, USA), GAPDH-HRP (1:20,000; A00192, Genscript, Piscataway, NJ, USA) . Immune complexes were detected with

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horseradish peroxidase coupled anti-rabbit or anti-mouse IgG antibodies (Amersham^{TM}, GE Healthcare, Pittsburgh, PA, USA).

Cell viability assay

Cell viability assays were performed according to the Vybrant MTT Cell Proliferation Assay Kit (V13154, Invitrogen) protocol. Briefly, mouse embryonic fibroblasts (MEFs) were grown to 70-80% confluence in 24-well plates and subjected to treatment with either MPP+, rotenone or 3-nitropropionic acid (3-NP) for 48 hours. The MTT solution (5mg/ml) was then added to the medium to reach the final concentration of 0.5 mg/ml. The cells were cultured for another 4 hours. At the end of the incubation, the medium was carefully removed and the MTT formazan crystals were dissolved in 500 µL DMSO by incubation at room temperature in darkness for 15 minutes. The absorbance was measured at 570 nm by using a microplate reader.

Transmission Electron Microscopy (TEM)

Mouse embryonic fibroblasts (MEFs)

Mouse embryonic fibroblasts (MEF) from both wild-type and H2AX mutant mice were fixed in 2.5% (vol/vol) glutaraldehyde, 3 mM $MgCl_2$, in 0.1 M sodium cacodylate buffer, pH 7.2, for 1 h at room temperature. After buffer rinse, samples were postfixed in 1% osmium tetroxide in buffer (1 h) on ice in the dark. The cells were stained with 2% (wt/vol) aqueous uranyl acetate (0.22 µm filtered, 1 h in the dark), dehydrated in a graded series of ethanol solutions, and embedded in Eponate 12 (Ted Pella) resin. Samples were polymerized at 37 °C for 2–3 d before moving to 60 °C overnight. Thin sections of 70–90 nm were cut with an ultramicrotome (Leica EM UC6, Leica Microsystems, Buffalo Grove, IL), stained with uranyl acetate and lead citrate,

lightly carbon coated, and observed with a Hitachi 7600 TEM. Images were captured with an AMT CCD XR50 (2K × 2K) camera.

Brain Tissue Preparation for TEM

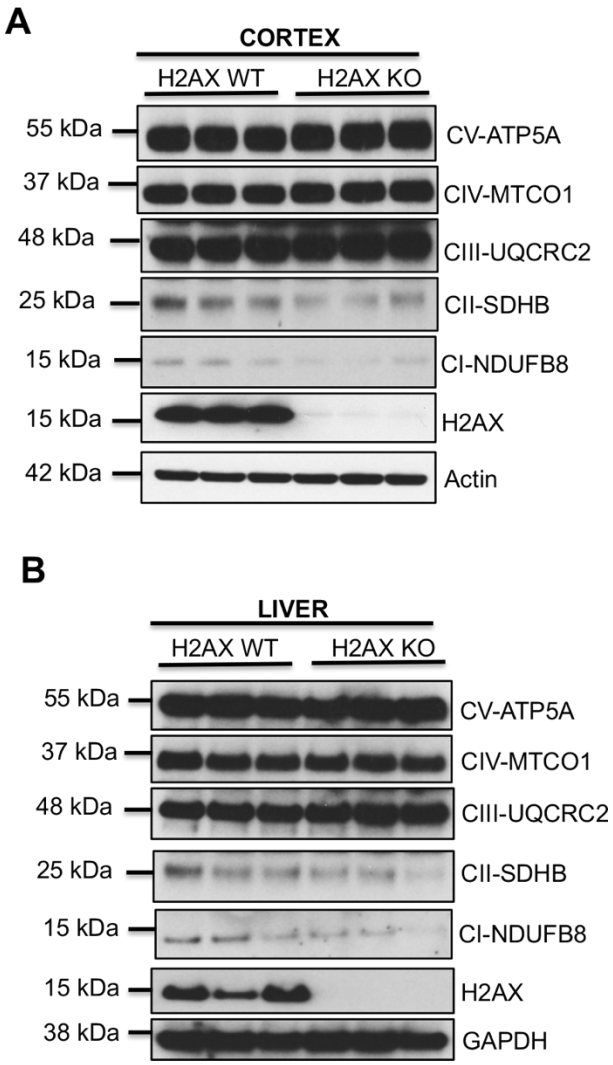
Mice were perfused with 10% Neutral Buffered Formalin (vol/vol) for 30 min at a rate of 2 mL/min, then post-fixed overnight. Striata from wild-type and H2AX mutant mice were dissected and samples were washed in 0.1 M sodium cacodylate buffer with 3 mM $MgCl_2$ and 3% (wt/vol) sucrose. Samples were postfixed in reduced 2% (wt/vol) osmium tetroxide, 1.6% (wt/vol) potassium ferrocyanide in buffer (2 h) on ice in the dark. Samples were stained with 2% (wt/vol) aqueous uranyl acetate (0.22 μm filtered, 1 h in the dark), dehydrated in a graded series of ethanol propylene oxide solutions, and embedded in Eponate 12 (Ted Pella) resin. Samples were polymerized at 60 °C overnight. Thin sections (60–90 nm) were cut with a diamond knife on a Reichert-Jung Ultracut E ultramicrotome and picked up with 2 × 1 mm copper slot grids. Grids were stained with 2% (wt/vol) uranyl acetate in 50% (vol/vol) methanol and lead citrate at 4 °C and observed with a Hitachi 7600 TEM. Images were captured with an AMT CCD XR50 (2K × 2K) camera.

Mitochondrial extraction and immunoblot

Mouse embryonic fibroblasts (MEFs) and striatum from both wild-type and H2AX mutant mice were used. Mitochondrial fractions were prepared using *The Mitochondria Isolation kit* from *Thermo Scientific*. Cells (10^{-7}) were harvested, suspended in 800 μl of buffer A; and incubated for 2 min (cells) or 15 min (striatum) at 4°C under agitation. Buffer B (11 μl) was then added to the mixture and incubated for 5 min. Samples were centrifuged every min for the duration of the

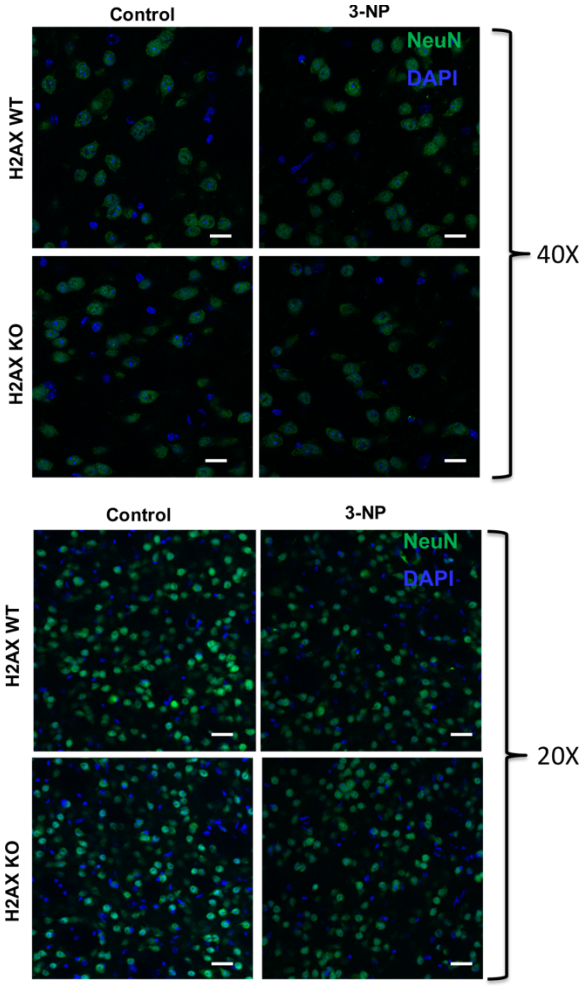
incubation. Buffer C (800 μl) was then added to the mixture, inverted several times followed by centrifugation at 700***g*** for 10 min at 4°C. The pellet was resuspending in RIPA buffer and used for immunoblot of H2AX and GAPDH. The supernatant fraction containing mitochondria was collected for centrifugation at 12,000 g for 15 min, and the pellet was resuspended in buffer C. An additional centrifugation was performed at 12,000 g for 5 min. The final pellet was resuspended in Buffer C and used for protein estimation. Samples were submitted to BN-PAGE in 4–20% gels. The proteins were transferred onto nitrocellulose membranes that were blocked for 1 h with 5% milk in BSA. Then, the membrane was blotted with a cocktail of OXPHOS antibodies (1:5000; ab110413, Abcam, Cambridge, MA, USA). Quantification of the different respiratory complexes was performed with Image J.

Supplementary figures



Supplemental figure 1: Analysis of OXPHOS subunits in the cortex and the liver. (**A-B**) Western blot analysis of OXPHOS subunits in the cortex (A) and the liver (B) of wild-type and H2AX knockout mice. We performed the immunoblot detection using total OXPHOS Human WB Antibody Cocktail enabling concomitant analysis of main proteins of each complex in the electron transport chain including complex I subunit NDUFB8, complex II subunit 30kDa, complex III subunit Core 2, complex IV subunit I, and ATP synthase subunit alpha. H2AX

deletion leads to a partial reduction in the expression of the OXPHOS complexes I and II. Complexes III, IV and V remain relatively unchanged.



Supplemental figure 2: Immunostaining of NeuN in the striatum. Coronal sections of the brain were taken through the striatum and analyzed for NeuN immunoreactivity. Treatment with 3-nitropropionic acid (3-NP) has negligible effect on the general neuronal population in the striatum. Top, 40x magnification, scale bars: 100 µm; Bottom, 20x magnification, scale bars: 20 µm. Green: NeuN, blue: DAPI.