**Supplementary Figure Legends.**

**Figure S1. Mitochondrial DNA content is slightly decreased in the absence of ATM.** Genomic DNA was extracted from either WT or ATM-deficient samples and a small fragment of the mitochondrial genome quantitatively amplified by QPCR; data were normalized to nuclear DNA and all samples were compared to their respective WT control. Results were reproduced at least 3 independent times; error bars represent ±SEM, statistical significance was evaluated using Student's *t*-Test.

**Figure S2. Mitochondrial membrane depolarization is more evident in A-T cells.** WT and A-T cells were submitted to 60 min treatment with 200 μM $H_2O_2$. Immediately after treatment cells were collected and mitochondrial membrane polarization judged using JC-1 (10 μM for 10 min at 37°C) by FACS. Dot plots are representative; experiments were reproduced 3 independent times.

**Figure S3. Kinetics of $H_2O_2$ decomposition is comparable in the presence or absence of ATM**. WT and A-T cells were submitted to 60 min treatment with 200 μM $H_2O_2$ and the concentration of $H_2O_2$ present in the medium was measured using Amplex red. Aliquots were taken at the indicated times and samples analyzed in triplicate. Experiments were reproduced independently 3 times.

**Figure S4. Mitochondrial clearance is not defective in the absence of ATM.** (A) Total and phosphorylated AMPK were probed by Western blotting in WT and A-T cells prior to and after exposure to 200 μM $H_2O_2$ for 60 min (left blot). Right panel shows robust AMPK phosphorylation in an unrelated fibroblast cell line, indicating that our experimental conditions can induce AMPK activation. C = control, T = $H_2O_2$-treated. Blots are representative of 3 independent experiments. (B) Immediately after $H_2O_2$ treatment (0h) and 24 later WT and A-T

cells were loaded with 200 nM of Mitotracker Green for 30 min prior to FACS analysis. Results represent the mean change in fluorescence compared to non-treated control. N=3 independent experiments, error bars represent ± SD, statistical significance was evaluated with Student's *t* test. (C) Representative Mitotracker Green traces; upper panels depict results obtained with WT cells and lower panels with A-T patient-derived cells. (D) Lysates from WT and A-T cells were fractionated by differential centrifugation and mitochondrial extracts were evaluated for the levels of the mitochondrial matrix protein mtHSP70 prior to and after $H_2O_2$ exposure. C = control, T = $H_2O_2$-treated. ND-1, which is encoded by the mtDNA, was used as loading control. Ponceau-stained membrane (below) depicts comparable amounts of proteins between control and treated samples of each cell type.

**Figure S5. Ligase activity is decreased in mitochondria from ATM-deficient samples.** Lig3 activity in cerebrum and cerebellum from 3 WT and 3 ATM KO animals was assayed using pUC18 cut with PstI. The plasmid was incubated with 10 µg of nuclear or mitochondrial extracts at 16°C overnight. The DNA was then purified and ran on agarose gels. T4 ligase was included as positive control. Arrow indicates linearized plasmid; red asterisk indicates the expected ligated product. Results are representative of 3 independent assays using 2 distinct lysate preparations.

**Figures S6. Levels of Lig3 are reduced in the absence of ATM.** (A) protein levels of Lig3 in nuclear extracts (30 µg) were probed by Western blotting in patient-derived cells (left) and in mouse tissue (right); Ponceau-stained membranes were used to monitor even loading of samples. Graphs depict levels of Lig3 normalized to total protein loading (based on Ponceau) obtained in 3 independent experiments. *=$p \leq 0.05$. (B) Total levels of Lig 3 were probed in WT and A-T lymphoblastoid cell lines, (C) in HME-CC where ATM was stably depleted using shRNA (for

details on these cells see Palii et al., 2013) and (D) in pre-B cells from ATM KO animals. β-actin was used as loading control; results are representative of 2 independent biological replicates.