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Supplementary Materials for

ATM directs DNA damage responses and proteostasis via genetically separable pathways

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:
(available at www.sciencesignaling.org/cgi/content/full/11/512/eaan5598/DC1)

Table S1 (Microsoft Excel format). Phosphorylated peptides observed in AT1ABR cells with inducibly expressed wild-type, CL, 2RA, or 2RA/CL *ATM* alleles.

Table S2 (Microsoft Excel format). Proteins detected in aggregate fractions.

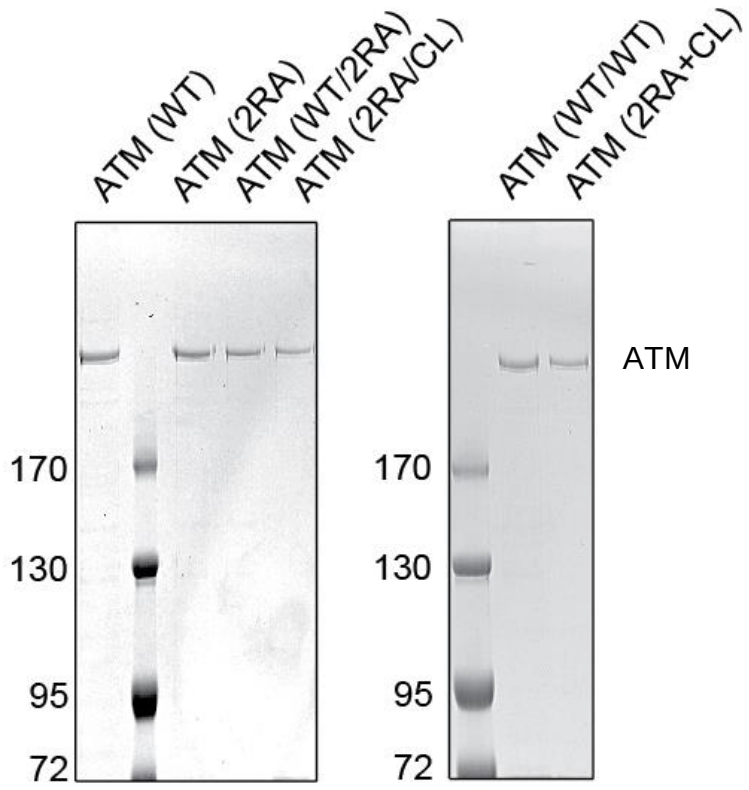


Figure S1. Recombinant ATM protein expression. Recombinant ATM proteins as indicated were expressed in human HEK-293T cells and purified to homogeneity. SDS-PAGE separation of the proteins was performed and the gel stained with Coomassie blue.

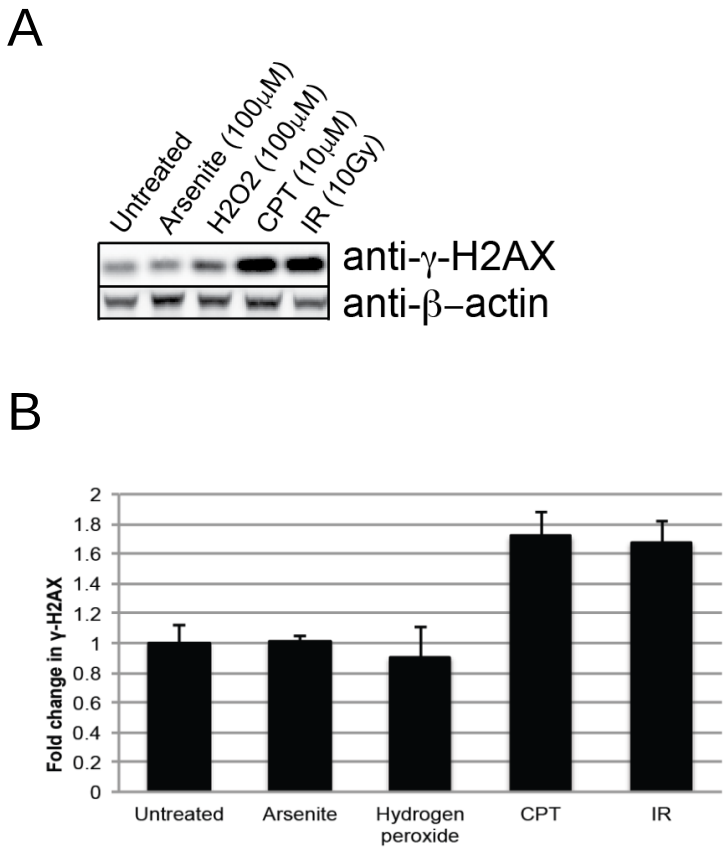
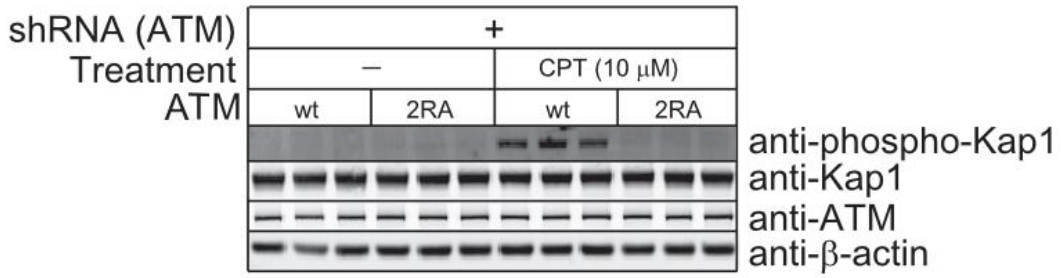


Figure S2. Arsenite and peroxide treatments do not induce DSBs in ATM-inducible U2OS cell lines. (A) U2OS cells were treated with arsenite, hydrogen peroxide, or CPT as indicated for 60 min, or with ionizing radiation. Cell lysates were analyzed by western blotting for phosphorylated H2AX or actin as a loading control. **(B)** Results from 3 experiments were quantitated and presented here as the fold change in γ-H2AX signal with treatment over the signal in untreated cells, normalized by the actin control.

A



B

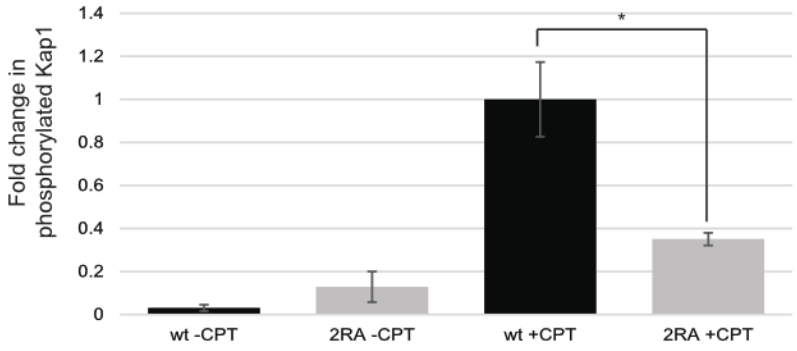


Figure S3. The 2RA ATM mutant is deficient in CPT-induced KAP1 phosphorylation. (A) Human U2OS osteosarcoma cells were depleted for endogenous ATM using shRNA and induced to express either wild-type (wt) or 2RA (2RA) ATM alleles with doxycycline as indicated, and exposed to CPT (10 μM) for 1hour. ATM activity was examined using antibodies directed against phospho-KAP1 Ser-^{824} and total KAP1, ATM, and β-actin abundance were assessed by western blotting. Blot shows samples from each of three independent experiments. **(B)** Quantitation of the phosphorylated KAP1 signal, normalized with total KAP1 measured from blot in (A) with error bars showing standard deviation. * indicates p-value < 0.05.

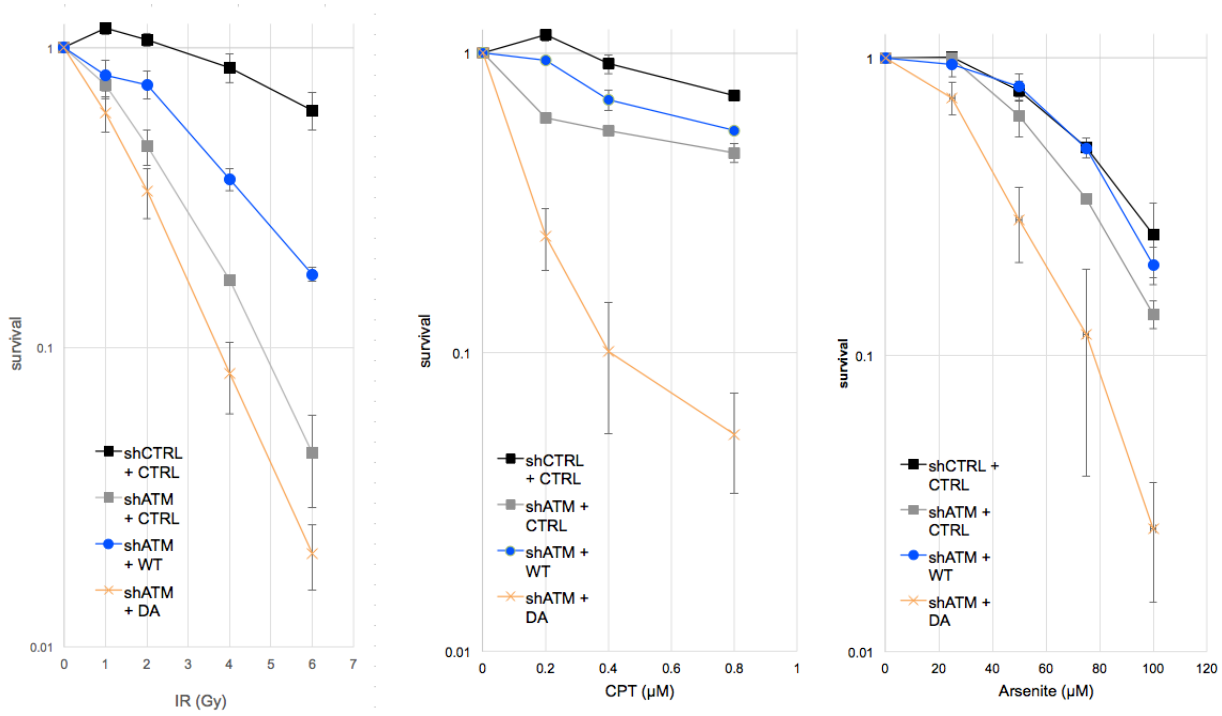


Figure S4. Expression of kinase-deficient ATM sensitizes cells to exogenous stress. U2OS cells depleted for endogenous ATM (shATM) or treated with a control shRNA (shCTRL) were induced with 1 μg/ml doxycycline to express vector only (CTRL), wild-type ATM (WT), or kinase-deficient ATM (D2889A, "DA") as indicated, and were analyzed for cell survival after treatment with IR (A), CPT (B), or arsenite (C) as indicated. Quantitation is shown from 3 independent experiments and error bars show standard error. Cell lines were identical to those used in Fig. 2, where the levels of ATM depletion with shRNA are shown.

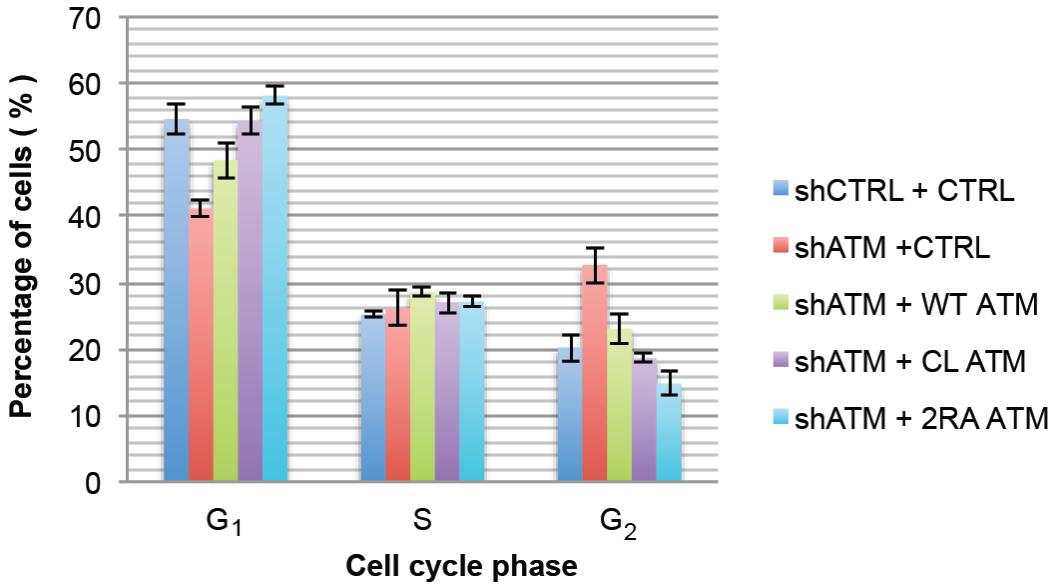


Figure S5. U2OS cells expressing wild-type and mutant alleles of *ATM* have equivalent ratios of cells in S phase. U2OS cells with either shRNA directed against ATM ("shATM") or control shRNA ("shCTRL") were induced for wild-type (WT), C2991L (CL), or R2579A/R2580A ("2RA") ATM expression. Cells were stained with propidium iodide and assessed for DNA content by FACS. Approximately 10,000 cells were measured for each cell line. Error bars indicate standard deviation from 3 biological replicates.

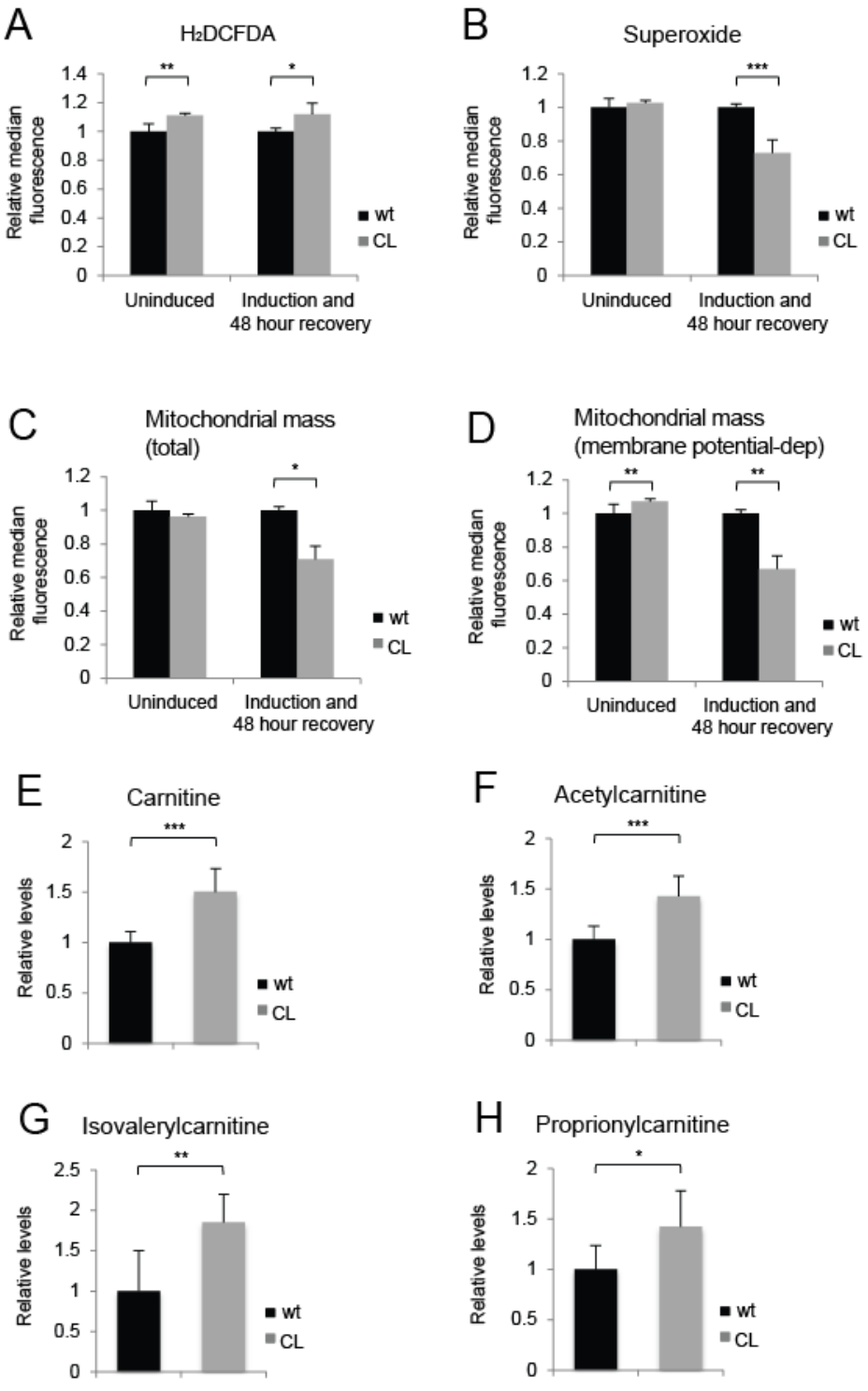


Figure S6. Mitochondrial dysfunction in AT1ABR cells expressing CL ATM. (A and B) The ROS levels of AT1ABR cells cells were measured by H2DCFDA (A) or DHE (B) staining

followed by analysis using flow cytometry and normalized with data from cells expressing wt ATM in each group, uninduced and induced. **(C)** Mitochondrial mass in AT1ABR cells was measured by MitoTracker Green staining followed by analysis using flow cytometry and normalized with data from cells expressing wt ATM in each group, uninduced and induced. **(D)** The mitochondrial membrane potential of AT1ABR cells were measured by MitoTracker Red staining followed by analysis using flow cytometry and normalized with data from cells expressing wt ATM in each group, uninduced and induced. (A-D) Median values from independent replicates (n =4) were compared and used for t-tests. Error bars represent standard deviation. * P< 0.05; ** P< 0.005; *** P< 0.0005. **(E to F)** metabolic profiles of carnitine (E) and its derivatives, acetylcarnitine (F), isovalerylcarnitine (G), and proprionylcarnitine (H) in AT1ABR cells expressing wt and CL ATM normalized to cells expressing wild-type (wt) ATM. Mean values from 6 biological replicates are shown; error bars represent standard deviation. * P< 0.05; ** P< 0.005; *** P< 0.0005.

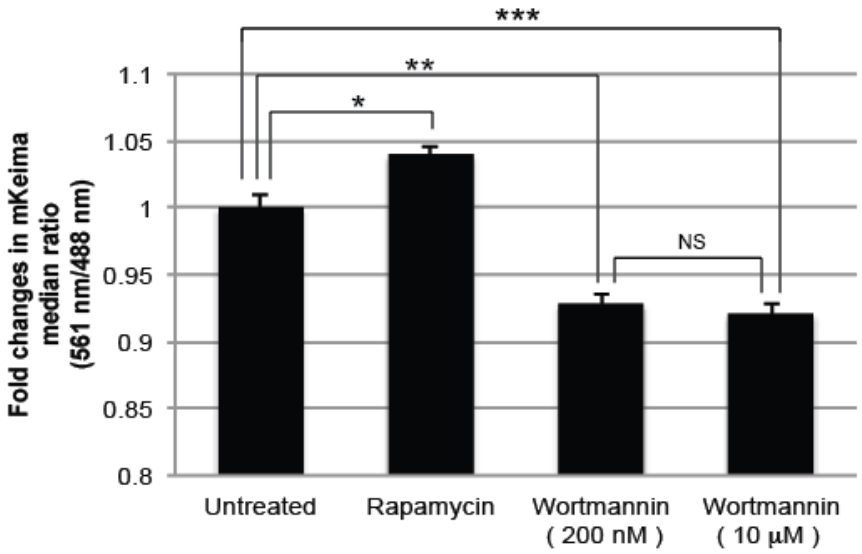


Figure S7. The mKeima mitochondria-targeted pH probe responds to induction or repression of mitophagy. U2OS cells expressing wild-type ATM were transduced with m-Keima lentivirus. After 2 days, cells were exposed to 500 nM Rapamycin, 200 nM wortmannin, or 10 μM wortmannin for 18 hours, and the fold change in the ratio of mKeima emission was determined for 3 biological replicates as in Figure 4. Error bars indicate standard deviation.

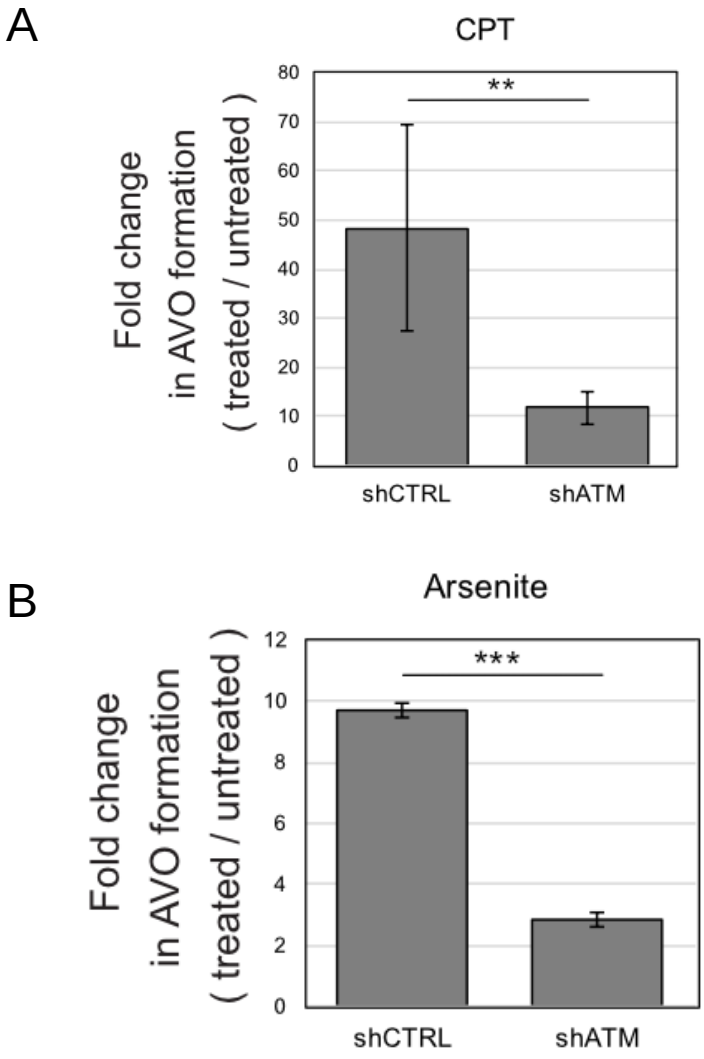


Figure S8. ATM depletion reduces macroautophagy responses of human cells in response to DNA damage or oxidative stress. (A and B) Acridine orange was used to stain acidic vesicular organelles (AVO) in U2OS cells expressing control shRNA or shRNA directed against ATM and cells were treated with CPT (5 μM) (A), or arsenite treatment (100 μM) (B) as indicated. The fold increase in vesicles per cell was quantified by FACS, here showing the fold increase in the percentage of cells with 585 nm emission relative to untreated cells. Results show the average of 6 biological replicates with approximately 5,000 cells measured per replicate. Error bars show standard deviation; ** $P<0.05$, *** $P<0.005$.

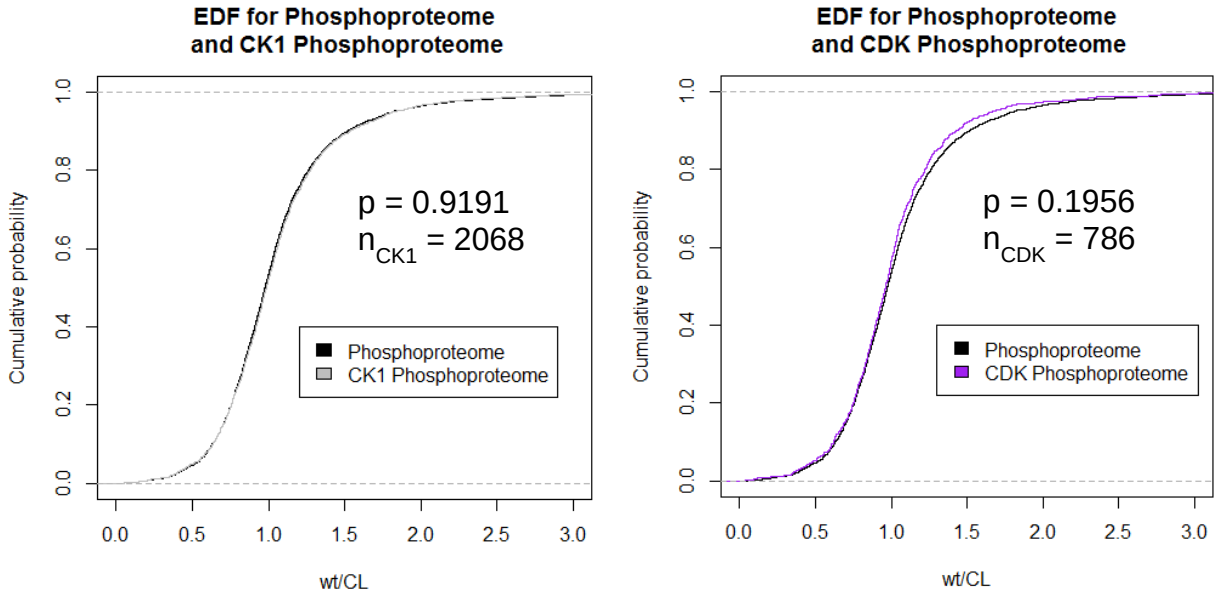


Figure S9. ATM mutant expression does not alter the predicted phosphorylation targets of CK1 or CDK. Empirical cumulative distribution functions of the ratio of phosphopeptide intensities of cells expressing wild-type (wt) ATM or cells expressing the CL allele, comparing the phosphoproteome and the predicted CK1 phosphoproteome (left) or the predicted CDK phosphoproteome (right).

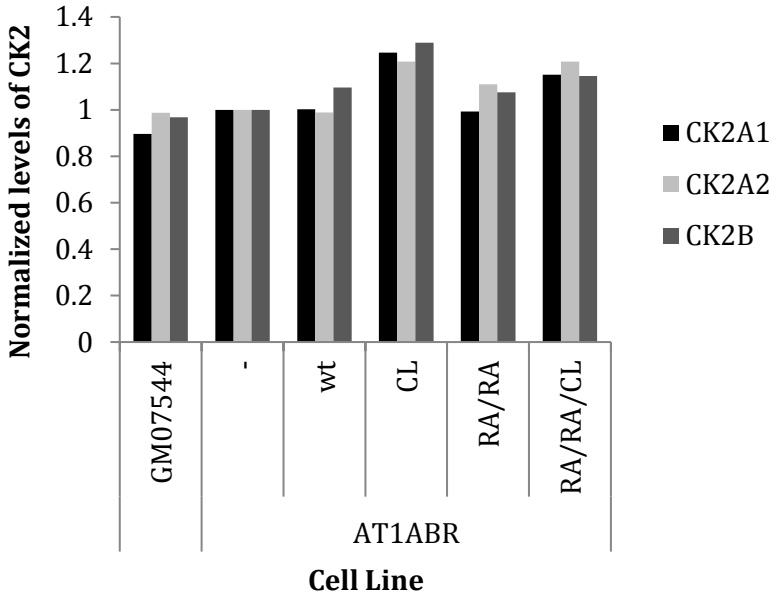


Figure S10. CK2 subunit levels are not reduced in A-T cells expressing the CL ATM allele. Quantitation from mass spectrometry analysis of total CK2 subunit abundance in AT1ABR cells expressing the indicated mutant. CK2 subunit abundance was normalized to the total protein abundance in each cell line and shown in comparison to that in the parental AT1ABR cell line. wt: wild-type; CL: C2991L; RA/RA: R2579A/R2580A, RA/RA/CL: R2579A/R2580A/C2991L.

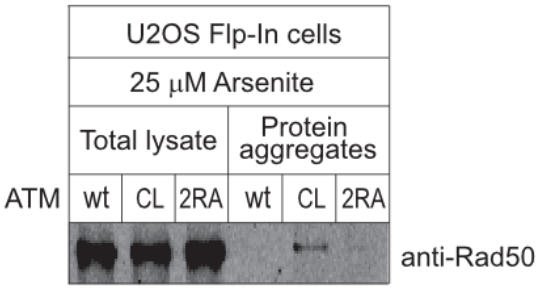


Figure S11. Expression of CL ATM causes Rad50 accumulation in protein aggregates. Analysis of detergent-resistant aggregates in U2OS Flp-In cells expressing wild-type (wt), C2991L (CL), or R2579A/R2580A (2RA) ATM treated with 25 μM arsenite as shown in Figure 6. Total lysates or insoluble aggregates were analyzed by western blotting for Rad50 as indicated.

Table S1. Phosphorylated peptides observed in AT1ABR cells with inducibly expressed wild-type, CL, 2RA, or 2RA/CL *ATM* alleles. Peptides were labeled using an iTRAQ system, enriched for phosphopeptides, and quantified using LC-MS/MS (see Methods). All values shown are log2 values relative to the value in uncomplemented AT1ABR cells. Phosphopeptides listed as "C2991 dependent" were identified by cluster analysis to be lower in the CL and 2RA/CL expressing cell lines relative to the uncomplemented and 2RA-expressing lines. Table is provided in .xls format in the auxiliary online materials.

Table S2. Proteins detected in aggregate fractions. Proteins detected in aggregate fractions using label-free quantification of 3 biological replicates per cell line. Note that undetected proteins were given the value of lowest detectable peptide in the dataset (428254.456) for comparison purposes. Table is provided in .xls format in the auxiliary online materials.