# Supplementary material

## Hypomorphic PCNA mutation underlies a novel human DNA repair disorder

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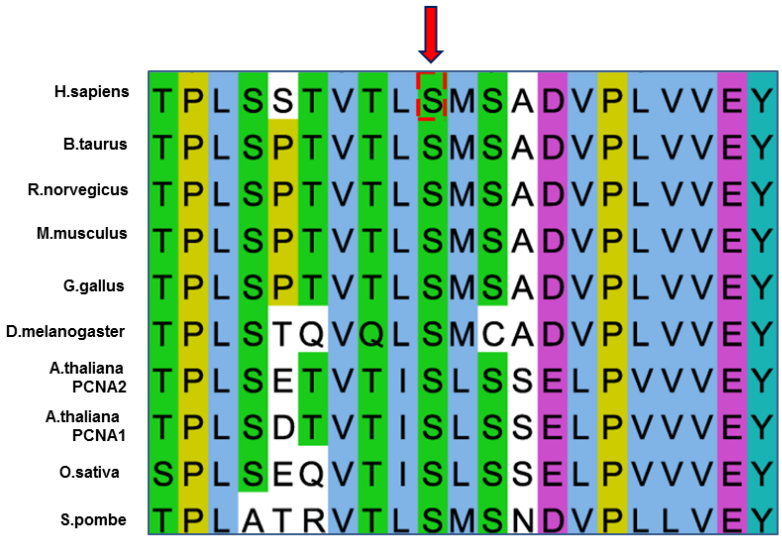
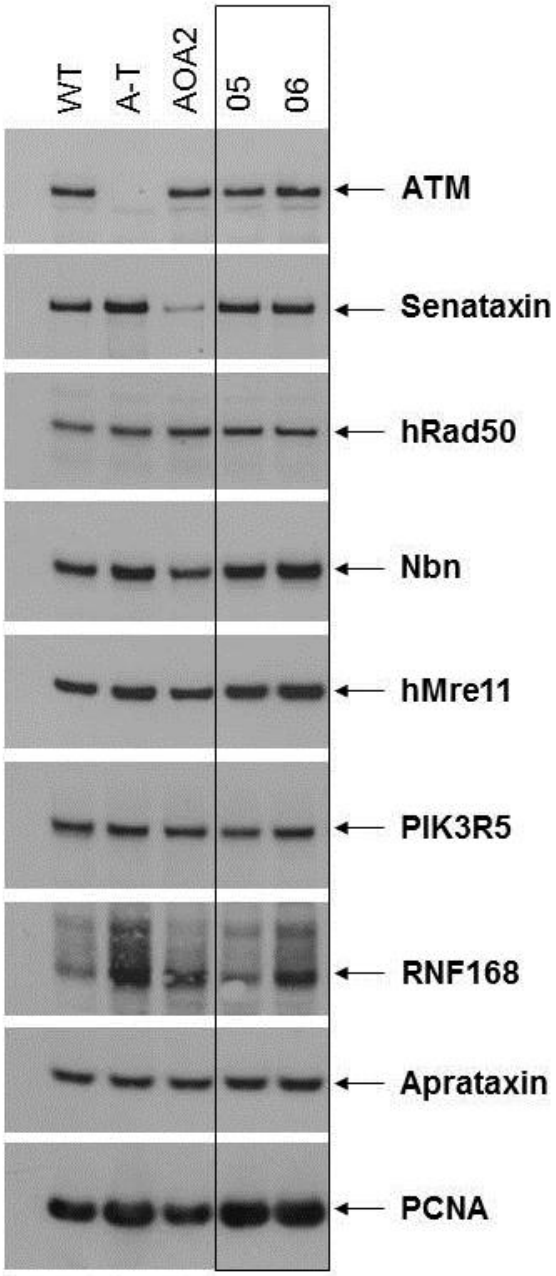
**Figure S1**




**Conservation of Ser228**. A ClustalW2 alignment of amino acid sequences of PCNA in Homo sapiens and 8 other species, illustrating stringent conservation of the Ser228 residue, indicated with the red arrow (created using Jalview software).

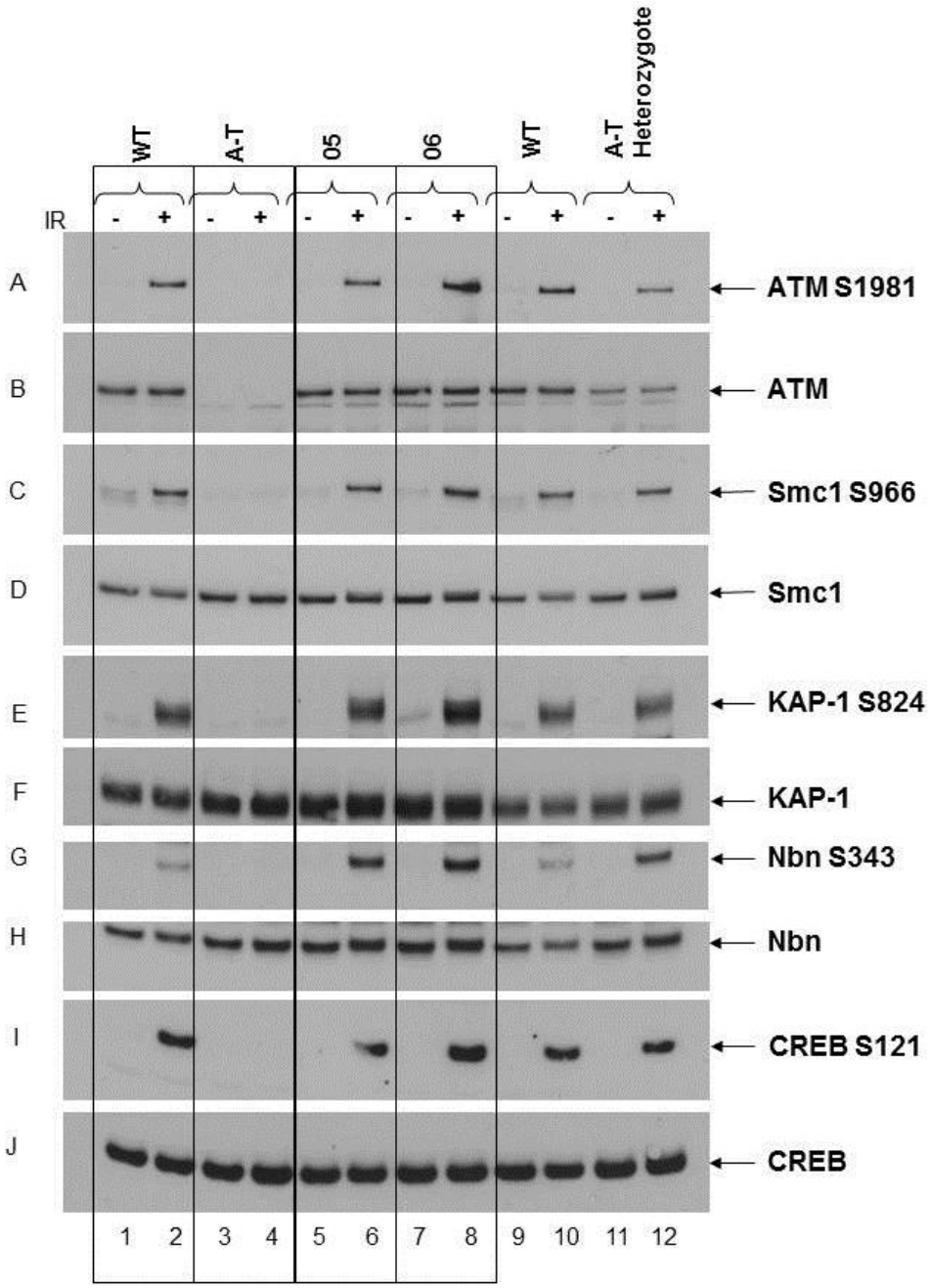
**Figure S2**




**Cells homozygous for PCNA p.Ser228Ile have normal levels of proteins mutated in AT-like disorders**. Western blot of extracts from EBV transformed control cells (WT), Ataxia telangiectasia cells (A-T), AOA2 cells (AOA2) and affected individuals homozygous for PCNA p.Ser228Ile (05 and 06). Levels of PCNA, ATM and additional

proteins which when mutated cause AT-like disorders in cells derived from affected individuals are normal.

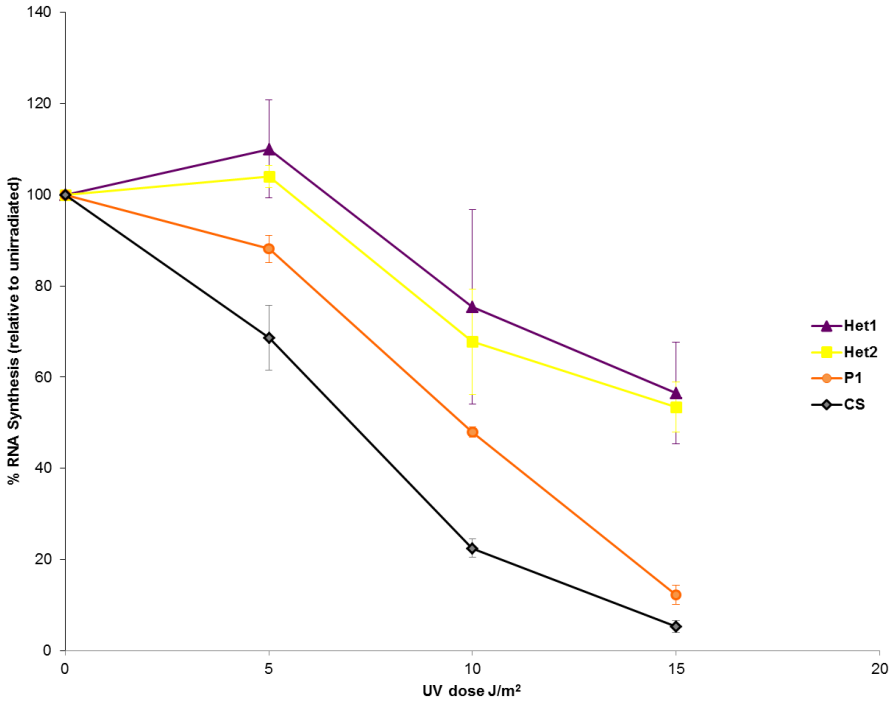
**Figure S3**




**Cells homozygous for PCNA p.Ser228Ile have normal ATM kinase activity.** ATM kinase levels in unirradiated (-) and irradiated (+) lymphoblastoid cell extracts. Lanes 1-2 and 9-10 are from a healthy control (WT) showing autophosphorylation of ATM (B) and phosphorylation of the targets SMC1 ser966 (C), KAP-1 ser824 (E), Nbn1 ser343

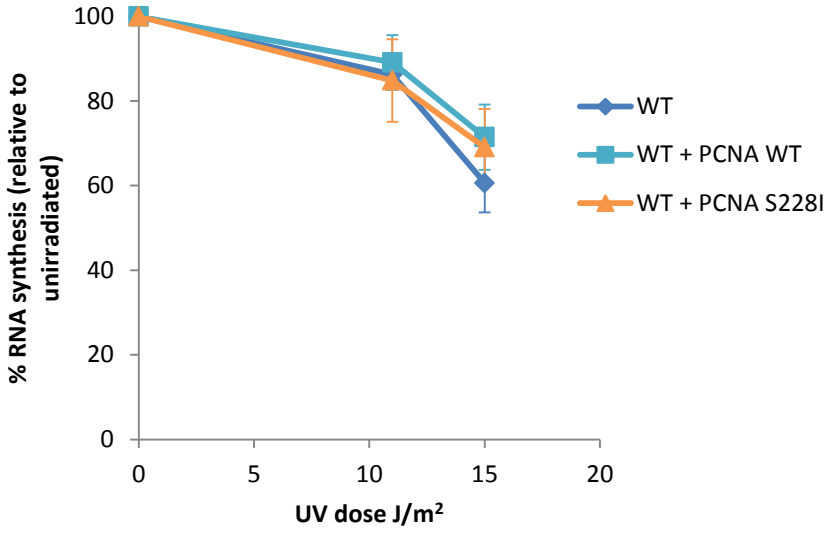
(G) and CREB ser121 (I). Lanes 3-4 and 11-12 show an absence of phosphorylation of these targets in AT cells (A-T). ATM kinase activity is unaffected in cells from a heterozygous ATM mutation carrier (lanes 11-12) and in 05 and 06 cells which are homozygous for PCNA p.Ser228Ile (lanes 5-8).

**Figure S4**




**Deficient RRS in P1 cells**. RNA synthesis measured by the incorporation of radiolabelled UTP 24 h after UVC-irradiation of non-dividing cells with indicated doses. Plotted is the mean ± SEM of three experiments. PCNA-P1 cells are defective in recovering RNA synthesis, to an extent approaching the defect seen in cells from a CS patient, heterozygotes Het1 and 2 cell lines are normal.

**Fig. S5**




**Lentiviral mediated ectopic expression of PCNA has little effect on RRS in wild - type cells.** Ectopic expression of wild-type or p.Ser228Ile PCNA was achieved by lentiviral transduction or GFP– or FLAG-tagged PCNA cDNAs. Virus infection was performed 48hours before RRS assays and viral infection efficiency (>85%) was confirmed by immunofluorescent staining. RNA synthesis was measured fluorescently by the incorporation of 5-ethynyluridine 12h after UVC-irradiation of non-dividing cells with indicated doses, revealed by copper-catalyzed fluorescent azide conjugation reaction (Click reaction). Plotted is the mean ± SEM of three experiments.

## Table S1

| | Repeat 1 | | | Repeat 2 | | | Repeat 3 (reverse) | | |
|---|---|---|---|---|---|---|---|---|---|
| **Protein** | Total spectra | # unique peptides | Ratio h:l normalized | Total spectra | # unique peptides | Ratio h:l normalized | Total spectra | # unique peptides | Ratio h:l normalized |
| **APEX1** | 141 | 28 | 0.11158 | 148 | 21 | 0.10287 | 28 | 15 | 1.5273 |
| **BUB3** | 29 | 14 | 1.0902 | 41 | 13 | 1.0567 | 59 | 17 | 0.7862 |
| **CDK1** | 12 | 8 | 1.0146 | 7 | 4 | 0.90092 | 7 | 6 | 1.0171 |
| **CHTF18** | 31 | 19 | 0.60947 | 30 | 14 | 0.63974 | 13 | 8 | 0.84334 |
| **DDB1** | 84 | 42 | 0.54328 | 38 | 24 | 0.50706 | 9 | 8 | 0.83215 |
| **DNMT1** | 210 | 72 | 0.88423 | 176 | 56 | 0.91885 | 115 | 61 | 0.83092 |
| **FEN1** | 43 | 21 | 11.544 | 63 | 18 | 18.617 | 20 | 14 | 0.10452 |
| **HIST1H1D** | 16 | 6 | 1.5639 | 13 | 5 | 2.8628 | 2 | 2 | 0.90141 |
| **LIG1** | 21 | 17 | 2.8494 | 22 | 11 | 3.2442 | 48 | 33 | 0.18195 |
| **MCM3** | 39 | 20 | 2.5048 | 28 | 21 | 1.7699 | 24 | 22 | 1.1822 |
| **MCM5** | 23 | 16 | 2.6787 | 21 | 9 | 2.0941 | 17 | 15 | 1.1049 |
| **MPG** | 19 | 11 | 0.72417 | 11 | 6 | 0.91538 | 13 | 10 | 0.24551 |
| **NAP1L1** | 27 | 8 | 0.80383 | 26 | 8 | 0.96409 | 71 | 14 | 0.23861 |
| **NAP1L4** | 33 | 11 | 0.79225 | 75 | 14 | 0.97825 | 93 | 18 | 0.25283 |
| **POLD1** | 161 | 58 | 0.41099 | 98 | 44 | 0.41942 | 79 | 46 | 2.5357 |
| **POLD2** | 41 | 19 | 0.46639 | 26 | 13 | 0.43519 | 18 | 13 | 2.1806 |
| **POLD3** | 33 | 13 | 0.42348 | 45 | 15 | 0.42695 | 24 | 12 | 1.7594 |
| **RBBP4** | 10 | 7 | 1.0121 | 6 | 3 | 0.76488 | 3 | 2 | 0.31712 |
| **RFC2** | 17 | 11 | 0.84726 | 17 | 11 | 0.87986 | 83 | 28 | 1.5911 |
| **RFC3** | 12 | 7 | 0.91678 | 5 | 5 | 0.86628 | 19 | 13 | 1.8282 |
| **RFC4** | 25 | 18 | 0.9077 | 29 | 16 | 0.92337 | 36 | 24 | 1.4823 |
| **RNASEH2A** | 7 | 7 | 0.22837 | 7 | 5 | 0.32916 | 8 | 6 | 1.8569 |
| **RNASEH2B** | 17 | 13 | 0.16996 | 17 | 8 | 0.20808 | 12 | 9 | 2.1405 |
| **RNASEH3C** | 14 | 8 | 0.15057 | 12 | 5 | 0.16212 | 12 | 9 | 2.7715 |
| **UNG** | 14 | 9 | 1.4155 | 12 | 5 | 2.0872 | 34 | 14 | 0.3147 |

**MaxQuant outputs from the three SILAC repetitions for the previously characterized PCNA interactors**. A summary of relevant data from the mass spectrometric analysis of proteins eluted from PCNA affinity columns is presented. Repeats 1 and 2 were performed in the forward direction (extracts with “heavy” label on the p.Ser228Ile PCNA column, “light” extracts on the wild-type PCNA column). Repeat 3 was performed in the reverse direction (“heavy” extracts on the WT column, “light” extracts on the p.ser228Ile PCNA column). The number of spectra from each run assigned to each peptide by the MaxQuant software is given, as is the number of unique peptides making up those spectra. The “ratio h:l normalized” column gives the

ratio of signals corresponding to peptides with “heavy” arginine or lysine over unlabeled peptides from the same protein. These ratios are normalized such the total h:l ratio in the entire sample is set to 1, to correct for slight differences in initial protein extract concentrations.