Intra-S phase checkpoint kinase Chk1 dissociates replication proteins Treslin and TopBP1 through multiple mechanisms during replication stress

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Supporting Information:

Supporting Tables S1 - S3

Supporting Figures S1 – S7
## Supporting Table S1 - Primary antibodies used in this study

| Antibody         | Species     | Company                                           | Dilution |
|------------------|-------------|---------------------------------------------------|----------|
| CDK2             | Rabbit mAb  | ab32147, Abcam                                    | 1:1000   |
| pChk1 S296       | Rabbit mAb  | D3O9F, Cell Signaling Technology, Inc.            | 1:1000   |
| pChk1 S345       | Rabbit mAb  | 133D3, Cell Signaling Technology, Inc.            | 1:1000   |
| Chk1             | Mouse mAb   | sc-8408, Santa Cruz Biotechnology, Inc.           | 1:1000   |
| FLAG (DYKDDDDK Tag) | Rabbit mAb  | D6W5B, Cell Signaling Technology, Inc.            | 1:1000   |
| HSP90            | Mouse mAb   | H9010, D. Toft, Mayo Clinic                       | 1:20,000 |
| Mouse IgG        | Mouse       | 0107-01, Southern Biotech                         | IP - 1:50 |
| TopBP1           | Rabbit pAb  | A300-111A, Bethyl Laboratories, Inc.              | 1:500    |
| Treslin          | Rabbit pAb  | this publication                                  | 1:1000   |
| Treslin          | Mouse mAb   | this publication                                  | IP - 1:50 |
| Treslin          | Rabbit pAb  | A303-472A, Bethyl Laboratories, Inc.              | 1:1000   |
| S-Tag            | Mouse mAb   | Hackbarth et al.(1)                               | 1:10,000 |

## Supporting Table S2 - Secondary antibodies used in this study

| Antibody                     | Company                                           | Dilution |
|------------------------------|---------------------------------------------------|----------|
| Anti-rabbit IgG HRP-linked   | 7074S, Cell Signaling Technology, Inc.            | 1:5000   |
| Anti-mouse IgG HRP-linked    | 7076S, Cell Signaling Technology, Inc.            | 1:5000   |

## Supporting Table S3 – Peptides for Treslin rabbit antibody production

| Antibody          | Synthetic peptide          | Treslin amino acids |
|-------------------|---------------------------|---------------------|
| Total Treslin     | SVSQPKSRSVQRVHSFQ - C     | 1030-1046           |
| pTreslin T968     | LLTKSVAE(p)TPVHKQIS -C    | 960-975             |
| pTreslin S1000    | DIGVVEE(p)SPEKGDEI -C     | 993-1007            |
| pTreslin S1114    | KKSHQKSL(p)SFSKTPPRR - C  | 1106-1122           |
Supporting Figure S1

Supporting Figure S1. K562 cells were transfected with either empty vector (EV) or pIRES SFB Treslin WT, incubated for 24 hours, treated with either no treatment or 5 µM Aphidicolin (APH), 10 µM hydroxyurea (HU) with or without 2 µM MK-8776 for 4 hours, and lysed. Lysates were incubated with streptavidin agarose beads for pulldown of SFB Treslin. Pulldowns and lysates were immunoblotted for the indicated antigens. *, nonspecific band.
Supporting Figure S2.

U2OS cells were transfected with siRNA targeting luciferase (siLuc, negative control) or two different Chk1 siRNAs. After 48 hours, cells were treated for 2 hours with AraC and lysed. Lysates were immunoprecipitated (IP) with mouse immunoglobulin G (IgG) control antibody or Treslin monoclonal antibody (mAb) pre-bound to protein G agarose beads. Immunoprecipitates and lysates were immunoblotted for the indicated antigens.
Supporting Figure S3

**Supporting Figure S3 A-B.** K562 cells were lysed 24 hours after transfection with pIRES SFB Treslin WT, and lysates were incubated with streptavidin agarose beads. After washing, beads were incubated in phosphatase buffer for 30 minutes at 30 °C with or without lambda phosphatase (pptase) as indicated. SFB Treslin was eluted from beads, and membranes were immunoblotted with anti-phospho-S1000 Treslin (pTreslin S1000) (A) and anti-phospho-T968 tresp (pTreslin T968) (B) antibodies. **C.** Lysates from K562 cells transfected with pIRES SFB Treslin 900-1100 WT or the indicated mutants were immunoblotted for Treslin phospho-T968 and phospho-S1000 Treslin. **D.** pIRES SFB Treslin WT, T968A, S1000A, and S6A were overexpressed in K562 cells. 24 hours later, cells were lysed, and lysates were incubated with streptavidin agarose beads for pulldown of the SFB-tagged protein. Pulldowns were immunoblotted for Treslin phosphorylation sites: phospho-T968 (pTreslin T968), phospho-S1000 (pTreslin S1000), and phospho-S1114 (pTreslin S1114). **E.** U2OS cells were treated for 4 hours with the indicated agents prior to lysis. Lysates were incubated with protein G agarose beads pre-bound with mouse IgG monoclonal control antibody or Treslin monoclonal antibody (mAb). Immunoprecipitates (IP) and lysates were immunoblotted for the indicated antigens.
Supporting Figure S4. K562 cells were transfected with pIRES SFB Treslin WT or pIRES SFB Treslin 7A. 24 hours later, cells were treated for 4 hours with the indicated agent prior to lysis. Lysates were incubated with streptavidin agarose beads. SFB Treslin and interacting proteins were eluted from beads, run on a gel, and blotted for the indicated antigens.
Supporting Figure S5

A. Sequence of Treslin from amino acids 882-1257 with T968 and S1000 indicated in green and with six Chk1 consensus sites (S937, S1019, S1025, S1038, S1044, S1114) that are mutated to alanines in Treslin S6A, indicated in red.

B. K562 cells transfected with pIRES SFB Treslin WT were lysed and lysates were incubated with streptavidin agarose beads. After washing, beads were incubated in phosphatase buffer for 30 minutes at 30 °C with or without lambda phosphatase as indicated. Fragments were eluted from beads, run on a gel, and blotted with pTreslin S1114 antibody.

C. pIRES SFB Treslin WT or pIRES SFB Treslin S1114 were overexpressed in K562 cells. Cells were lysed and lysates were incubated with streptavidin agarose beads for pulldown of the overexpressed protein. Pulldowns were immunoblotted for Treslin phospho-S1114 (pTreslin S1114).
Supporting Figure S6. HeLa cells were co-transfected with either empty vector, pIRES SFB Treslin WT, or pIRES SFB Treslin S6A along with pCMV CDK2 HA AF, as indicated. 48 hours later, cells were treated with AraC for 2 hours and collected for lysis. Lysates were incubated with streptavidin agarose beads to pulldown SFB-tagged proteins. Lysates and bead-bound proteins were immunoblotted for the indicated antigens.
Supporting Figure S7

Example image of DNA Fiber Labeling events:

ongoing fork: red-green

initiation: green-red-green

termination: red only, or red-green-red

new origin: green only
1. Hackbarth, J. S., Lee, S. H., Meng, X. W., Vroman, B. T., Kaufmann, S. H., and Karnitz, L. M. (2004) S-peptide epitope tagging for protein purification, expression monitoring, and localization in mammalian cells. *BioTechniques* **37**, 835-839