**Supplemental Figure legends**

**Figure S1. SART1 is a microtubule-associated protein.**

A. Endogenous Xenopus SART1 binds to MTs. Xenopus CSF egg extract was incubated with taxol-stabilized MTs. MTs and MAPs were spun down. MAPs are eluted from MTs with high salt and separated by centrifugation. The eluate and MT pellet were immunoblotted for SART1 and a negative control XCAP-G.

B. His Acidic-Target Tag (HisATT)-fused SART1 was incubated with the indicated concentrations of taxol-stabilized pure MTs. After centrifugation, supernatant (s) and pellet (p) were analyzed by SDS-PAGE and Coomassie staining. Note that HisATT-fused TRIM21, not a MAP, was not sedimented in this assay.

C. HisATT-SART1 was incubated with 20 μM MTs in the presence or absence of importin  complex and RanGTP. After centrifugation, supernatant (s) and pellet (p) were analyzed by SDS-PAGE and Coomassie staining.

**Figure S2. SART1 downregulation in HeLa cells causes spindle defects and cell death.**

A. Western blot of HeLa cells treated for 3 d with 10 μM of three different siRNAs targeting SART1.

B. Frequency of abnormal spindles in HeLa cells treated as in A. Spindles were stained for α-tubulin and DNA. Error bars: SD. N = 2 experiments, n > 50 prometaphase and metaphase-like cells per experiment.

C. siRNA-transfected HeLa cells as in A were stained with Annexin V and Propidium iodide (PI) and analyzed by flow cytometry. Annexin V positive cells, shown with %, indicate dead cells. Cells in early apoptosis are Annexin V positive and PI negative, and cells in late apoptosis or already dead are both Annexin V and PI positive.

D. Scheme of the Sendai Virus (SeV) harboring FLAG-tagged human SART1, resistant to siSART1 #3, and Dasher GFP (DGFP).

E. siRNA-treated HeLa cells (as in A) were stained as indicated. Inter-kinetochore distance was measured based on Ndc80 dots along the CREST rods. n > 50 kinetochore pairs. p values (student’s test, two tailed). Scale bar, 10 m

F. Chromosome spreads prepared from siRNA-transfected HeLa cells (as in A). Scale bar, 10 m.

G. The spindle checkpoint is activated in the absence of SART1. siRNA-transfected HeLa cells (as in A) were stained for a spindle checkpoint protein BubR1, α-tubulin, and DNA. Scale bar, 10 m

**Figure S3. SART1-depleted HeLa cells frequently die as consequence of mitotic collapse.**

A. HeLa cells expressing H2B-mCherry were transfected with 20 μM of the indicated siRNA oligos and after 30h imaged for 70h. Up to 20 randomly chosen dead cells, determined by chromatin fragmentation, per condition were manually tracked back in order to determine the last healthy cell cycle phase (see color legend). Red bars indicate the period since chromatin collapse starts until the first clear chromatin fragmentation appears.

B. Example of time lapse sequence from A showing the collapse and death of a mitotic HeLa cells expressing H2B-mCherry transfected with the indicated siRNA. Scale bars, 10 m.

 C. Percentage of dead cells 70 hours post-transfection automatically identified based on the chromatin morphology by CellCognition analysis of live cell imaging records.

**Figure S4. SART1 localization at centrosomes in the course of mitotic progression.**

A. HeLa cells were fixed, and stained for SART1 (red), MTs (green), and DNA (blue). Mitotic stages were classified based on MTs and DNA morphology.

B. HeLa cells were fixed, and stained with an SART1 mouse monoclonal antibody (#2, Santa Cruz), pericentrin rabbit polyclonal antibody, and DAPI.

C. In contract to SART1, γ-tubulin centrosomal staining is unaffected by taxol and nocodazole treatment. HeLa cells were incubated in the presence of either drug for 10 min, fixed, and stained for γ-tubulin and DNA.

Scale bars, 10 m

**Figure S5. Immunoprecipitation of SART1 from Xenopus egg extract.**

A. Production of Xenopus SART1 antibody. Full length Xenopus laevis SART1 was expressed in bacteria, purified with Ni-NTA under denaturing condition, and used for antibody production in rabbits. The purified antibody recognizes a SART1 in egg extracts by Western blot. The specificity was confirmed by disappearance of the respective signal after SRAT1 depletion form egg extarcts using SART1 antibody-immobilized beads.

B. Immunoprecipitation (IP) of SART1 was conducted from Xenopus egg extract using antibody beads. Interacting proteins were eluted with triethylamine at pH11.5. The eluate and proteins left on the beads were analyzed by SDS-PAGE and Coomassie staining as well as Western blotting.

C. Cep192 mRNA level was unchanged upon SART1 downregulation. Quantitative real-time PCR was performed and gene expression was normalized by GAPDH levels. Error bars: SD. N = 4 experiments. p value (student’s test, two tailed). NS (not significant).

**Figure S6. Centrosomal signal of Pericentrin, but not γ-tubulin, decreases upon SART1 downregulation.**

A. siRNA-treated HeLa cells (as in Fig 4A) were stained for pericentrin (red), α-tubulin (green), and DNA (blue). Pericentrin fluorescence intensity was quantified. N = 2 experiments, n = 10 cells per experiment. p values (student’s test, two tailed). NS (not significant).

B. Ninein, PCM1, and pericentrin mRNA levels were not reduced upon SART1 downregulation. Quantitative real-time PCR was performed and gene expression was normalized by GAPDH levels. Error bars: SD. N = 4 experiments. p value (student’s test, two tailed). NS (not significant).

C. siRNA-treated HeLa cells were stained for γ-tubulin (green), α-tubulin (red), and DNA (blue). N = 2 experiments, n = 10 cells per experiment. p values (student’s test, two tailed). NS (not significant).

D. Centrosome duplication is not impeded by SRAT1 downregulation. siRNA-treated HeLa cells were stained for the centriole marker centrin (red), α-tubulin (green), and DNA (blue).

Scale bars, 10 m

**Figure S7. SART1 downregulation in iPS cells does not affect spindle assembly and cell viability.**

A. RPE1 cells are not transformed by SART1 overexpression. Cells were infected by overnight incubation with SeV harboring SART1 or c-Myc. After 1 month with occasional medium change the cell population as analyzed by phase contrast microscopy. Scale bar, 100 m

B. SART1 downregulation in RPE1 cells neither affects spindle assembly nor causes cell death. RPE1 cells were treated with 20 nM siRNAs for 3 d, and stained with α-tubulin and DAPI for microcopy (left) or with Annexin V and Propidium iodide for flow cytometry (right). Annexin V positive cells, shown with %, indicate dead cells.

C. RPE1 cells die upon SSX2IP depletion. RPE1 cells were treated with 20 nM siRNAs for 3 d, and stained for Annexin V and Propidium iodide and analyzed by flow cytometry.

D. SART1 depletion prevents indefinite growth induced by c-Myc overexpression. RPE1 cells are treated with 20 nM siRNAs and infected with SeV harboring c-Myc, and incubated for 2 weeks. The culture medium in each well is shown and the yellow medium indicates higher cell proliferation. The cells recovered after 2 weeks were immunoblotted for SART1.

**Table S1. Top 10 Proteins identified in SART1 immunoprecipitation from Xenopus egg extractsa**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Protein | Full name | Accession | MW (kD) | Quantitative Value |
| SF3B3b,c | Splicing factor 3B subunit 3 | A0A1L8GF54 | 136 | 123 |
| SF3B1b,c | Splicing factor 3B subunit 1 | A0A1L8EPY7 | 146 | 100 |
| Cep192d  | Centrosomal protein 192 | A0A1L8FYG2 | 289 | 92 |
| CPSF1e | Cleavage and polyadenylation specific factor 1 | A0A1L8FU85 | 140 | 64 |
| SONc | SON DNA And RNA Binding Protein | A0A1L8HCI1 | 527 | 58 |
| Symplekine | Symplekin | A0A1L8F3F9 | 134 | 54 |
| CSPP1d | Centrosome and spindle pole associated protein 1 | A0A1L8FZ71 | 149 | 53 |
| TNRC6B | Trinucleotide repeat-containing adaptor 6B | A0A1L8GH02 | 195 | 50 |
| TRIM37 | Tripartite motif-containing 37 | A0A1L8H898 | 114 | 50 |
| CPSF2e | Cleavage and polyadenylation specificity factor 2 | A0A1L8F0H9 | 84 | 48 |

a We show proteins specifically found in SART1 immunoprecipitates but not in control IgG immunoprecipitates.

bComponent of the Splicing factor 3B (SF3B) complex

cSplicing relevant

dCentrosome relevant

eComponent of the cleavage and polyadenylation specificity factor (CPSF) complex

**Table S2. Target sequences of primers and probes used for quantitative real-time PCR**

|  |  |  |
| --- | --- | --- |
| Gene name | Primer name | Target sequences (5'-3') |
| Cep192 | Forward | CACCGTCACTCTCACTGCCATTGCCG |
| Reverse | GAGACCATCGTACAGGCAGAAG |
| Probe | CGTCTTTCTTTTCTGTTTCTACCTCA |
| Ninein | Forward | TCATACTCCTCACTGCGTTGCGTCTTCCA |
| Reverse | GACGGTGATTGAGCCACTGG |
| Probe | GTTCCAAAACCTTAACTGGCCTTC |
| PCM1 | Forward | TGTGATACTGACGCCAGATAAGCTACCTGC |
| Reverse | TGCAGTGATGGATGATTCTGTTG |
| Probe | CGCTGAATTAAGTCATTCAATTCTTC |
| Pericentrin | Forward | TTCTCCACAGCCGTCCTCTGCACATG |
| Reverse | GTTCCCCAGGCGTGTCTG |
| Probe | GGATGTTGTGATAAAATCTTCGACA |