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SOGA1 and SOGA2/MTCL1 are CLASP-interacting proteins required for faithful chromosome segregation in human cells

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Abstract

CLASPs are key modulators of microtubule dynamics throughout the cell cycle. During mitosis, CLASPs independently associate with growing microtubule plus-ends and kinetochores and play essential roles in chromosome segregation. In a proteomic survey for human CLASP1-interacting proteins during mitosis, we have previously identified SOGA1 and SOGA2/MTCL1, whose mitotic roles remained uncharacterized. Here we performed an initial functional characterization of human SOGA1 and SOGA2/MTCL1 during mitosis. Using specific polyclonal antibodies raised against SOGA proteins we confirmed their expression and reciprocal interaction with CLASP1 and CLASP2 during mitosis. In addition, we found that both SOGA1 and SOGA2/MTCL1 are phospho-regulated during mitosis by CDK1. Immunofluorescence analysis revealed that SOGA2/MTCL1 co-localizes with mitotic spindle microtubules and spindle poles throughout mitosis and both SOGA proteins are enriched at the midbody during mitotic exit/cytokinesis. GFP-tagging of SOGA2/MTCL1 further revealed a microtubule-independent localization at kinetochores. Live-cell imaging after siRNA-mediated knockdown of SOGA1 and SOGA2/MTCL1 showed that they are independently required for distinct aspects of chromosome segregation. Thus, SOGA1 and SOGA2/MTCL1 are bona fide CLASP-interacting proteins during mitosis required for faithful chromosome segregation in human cells.

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The authors declare no conflict of interest.

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Keywords

CLASP1; CLASP2; SOGA; MTCL1; mitosis; kinetochore

Introduction

Faithful chromosome segregation relies on the proper assembly and function of a microtubule-based structure called the mitotic spindle. The dynamics of mitotic spindle microtubules is tightly regulated at the entry and progression through mitosis by a complex network of microtubule-associated proteins (MAPs). Among these, CLASPs are conserved microtubule plus-end tracking proteins encoded by two paralogue genes, *CLASP1 and CLASP2*. CLASPs also independently associate with kinetochores, centrosomes and midbodies, and play critical roles in the regulation of microtubule dynamics at the kinetochore-microtubule interface, necessary for proper mitotic spindle function and faithful chromosome segregation (Girao et al. 2020; Logarinho et al. 2012; Maiato et al. 2003; Mimori-Kiyosue et al. 2006; Pereira et al. 2006).

In a proteomic survey to search for CLASP1-interacting proteins in mitosis we have previously identified two uncharacterized proteins of high molecular weight, originally annotated as KIAA0802 and KIAA0889 (Maffini et al. 2009). Both proteins belong to the Suppressor Of Glucose Autophagy-Associated (SOGA) family and share approximately 40% identity (BLAST®- blastp: Query ID: NP_542194.2; Subject ID: NP_001365135.1). Subsequently, KIAA0802 (from hereafter SOGA2) was identified as a PAR-1b-interacting protein in MDCK cells and shown to localize along individual microtubules and accumulate on microtubule bundles through its N-terminal. Disruption of SOGA2 activity, either by siRNA-mediated depletion or by deletion of its N-terminal domain, impaired the formation of apicobasal microtubule bundles in polarized epithelial cells (Sato et al. 2013). On the basis of this microtubule bundling activity, SOGA2 was also named Microtubule Crosslinking Factor 1 (MTCL1). More recently, SOGA2/MTCL1 was shown to localize to the trans- and cis-Golgi membranes, where it associates with CLASPs and AKAP450/CG-NAP, respectively, to promote non-centrosomal microtubule nucleation (Sato et al. 2014). In agreement, SOGA2/MTCL1 depletion reduced the accumulation of perinuclear acetylated microtubules and disrupted the Golgi ribbon structure (Sato et al. 2014). Structurally, SOGA2/MTCL1 contains a long coiled-coil domain that dimerizes and holds two N-terminal domains that bind to microtubules (Sato et al. 2013). KIAA0889, renamed as SOGA1 (Cowherd et al. 2010), was found enriched in a CLASP2 interactome in 3T3-L1 mouse adipocyte and was proposed to be part of a molecular pathway that regulates glucose metabolism in a microtubule-dependent manner (Kruse et al. 2017).

Despite some functional characterization in interphase, the roles of SOGA family proteins during mitosis remain unclear. In the present work, we performed an initial functional characterization of human SOGA1 and SOGA2/MTCL1 in human mitotic cells. By developing specific polyclonal antibodies against both proteins, we confirmed their expression during mitosis and found that they are both phospho-regulated by CDK1. The interaction between SOGA1 and SOGA2/MTCL1 with CLASP1 during mitosis was also

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verified by co-immunoprecipitation in mitotic cell extracts. Immunofluorescence analysis in human cultured cells revealed that SOGA2/MTCL1 co-localizes with mitotic spindle microtubules throughout mitosis and both SOGA proteins are enriched at the midbody during mitotic exit/cytokinesis. GFP-tagging of SOGA2/MTCL1 further revealed a microtubule-independent localization at kinetochores. Phenotypic analysis by live-cell imaging after siRNA-mediated knockdown revealed that SOGA1 and SOGA2/MTCL1 are independently required for distinct aspects of chromosome segregation. Thus, SOGA1 and SOGA2/MTCL1 are bona fide CLASP-interacting proteins during mitosis required for faithful chromosome segregation in human cells.

Results

SOGA1 and SOGA2/MTLC1 are CLASP-interacting proteins during mitosis

To identify human CLASP1-interacting proteins during mitosis we generated a human CLASP1 fusion protein with a localization and affinity purification (LAP) tag (Cheeseman and Desai 2005; Maffini et al. 2009). This CLASP1^{LAP}fusion protein consists of human CLASP1α tagged at its N-terminal with EGFP, followed by a TEV protease cleavage site and an S peptide domain that binds to S protein (Maffini et al. 2009). Affinity purifications performed in HeLa cells stably expressing CLASP1^{LAP} allowed us to identify, among others, two previously uncharacterized proteins, KIAA0889 and KIAA0802 (Maffini et al. 2009), which were subsequently renamed as SOGA1 and SOGA2/MTCL1, respectively (Cowherd et al. 2010; Sato et al. 2013). To investigate the potential roles of SOGA family proteins in mitosis we started by confirming their interaction with both human CLASP1 and CLASP2. To do so, we first generated rabbit polyclonal antibodies against SOGA1 and SOGA2/MTCL1. These antibodies were specific for each protein, since low-homology regions between SOGA1 and SOGA2/MTCL1 were chosen for the preparation of recombinant protein immunogens (Figure S1A), and validated following siRNA-mediated depletion of SOGA1 or SOGA2/MTCL1, as well as by the exclusive detection of the respective EGFP-fusion proteins (Figure S1B). Next, we investigated the capacity of these SOGA1- and SOGA2/MTCL1-specific antibodies to co-immunoprecipitate endogenous CLASPs from mitotic HeLa cell extracts synchronized with the Kinesin-5 inhibitor S-Trytil-L-Cysteine (STLC), which leads to the formation of monopolar spindles and consequent mitotic arrest (Figure 1A-D). We found that both antibodies against SOGA1 and SOGA2/MTCL1 efficiently co-immunoprecipitated CLASP1 and CLASP2, confirming their reciprocal interaction during mitosis [see also (Maffini et al. 2009)].

SOGA1 and SOGA2/MTCL1 are phosphorylated during mitosis in a CDK1-dependent manner

To investigate whether the expression of SOGA1 and SOGA2/MTCL1 is regulated during mitosis, we synchronized human HeLa cells with STLC and released them into medium containing the proteasome inhibitor MG132 for 1h after STLC washout, to allow spindle bipolarization while preventing anaphase onset. When compared with extracts from asynchronous HeLa cells, western blot analyses after SDS-PAGE revealed a marked increase in SOGA1 and SOGA2/MTCL1 expression in mitosis (Figure 2A). Moreover, the electrophoretic mobility of SOGA2/MTCL1 was reduced in mitotic extracts, suggesting

phospho-regulation in mitosis (Figure 2A). This was confirmed by the loss of the observed mobility shift after treatment with λ phosphatase (Figure 2A). We also detected a very minor but reproducible decrease in the electrophoretic mobility of SOGA1 in mitotic extracts that disappeared after treatment with λ phosphatase (Figure 2A). To determine which kinase activities account for SOGA2/MTCL1 (and possibly SOGA1) phosphorylation in mitosis we treated mitotic HeLa cells with specific small-molecule inhibitors of well-established mitotic kinases, including CDK1 (RO3306), PLK1 (BI2536), Aurora B (ZM447439), MPS1 (Mps1-IN-1) and GSK3β (lithium chloride) (Coghlan et al. 2000; Ditchfield et al. 2003; Kwiatkowski et al. 2010; Lenart et al. 2007; Vassilev 2006). Western-blot analyses after SDS-PAGE revealed that only CDK1 inhibition markedly affected the electrophoretic mobility of SOGA2/MTCL1 in mitotic extracts, to a similar extent as that observed after λ phosphatase treatment. Of note, this was not due to mitotic exit, as cells remain arrested in mitosis in the presence of MG132 upon Cdk1 inactivation (Maia et al. 2012; Moutinho-Pereira et al. 2009; Skoufias et al. 2007). Likewise, the very subtle band shift observed in SOGA1 during mitosis was also abolished after CDK1 inhibition (Figure 2A). Consistent with these observations, *in silico* analyses of the respective protein sequences using the PhosphoSitePlus database (see Methods for details) revealed many putative phosphorylation sites for CDK1 in both SOGA1 and SOGA2/MTLC1. Noteworthy, the putative CDK1 phosphorylation sites on SOGA2/MTCL1 were abundant and widely distributed along the protein sequence, whereas in the case of SOGA1 they were concentrated in the C-terminal, thereby explaining their distinct electrophoretic mobility observed during mitosis (Figure 2A,B). Overall, these data suggest that, similar to their interacting partner CLASP2 (Maia et al. 2012), both SOGA1 and SOGA2/MTCL1 are phospho-regulated during mitosis in a CDK1-dependent manner.

SOGA1 and SOGA2/MTCL1 are microtubule-associated proteins that localize to defined mitotic structures

In order to investigate the mitotic roles of SOGA1 and SOGA2/MTCL1 we sought to determine their cellular distribution throughout mitosis by indirect immunofluorescence in HeLa cells using our newly generated antibodies. We found that during interphase both SOGA proteins accumulated in the cytoplasm in areas of high microtubule density where they partially co-localized with interphase microtubules (Figure 3A and 4A). Despite the abundant cytosolic fraction causing low signal/noise, SOGA2/MTCL1 exhibited a distinguishable enrichment on spindle microtubules and spindle poles throughout mitosis (Figure 3B and Figure S2). In contrast, no clear localization of SOGA1 on the mitotic spindle was observed until late anaphase/telophase (Figure 4B). However, both SOGA proteins were found enriched at midbodies (Figure 3B and 4B). The specificity of our antibodies against SOGA1 and SOGA2/MTCL1 was validated by the significant reduction of spindle and midbody localization after their respective knockdown by RNAi (Figure 3B and 4B). To further validate these results, we transfected HeLa cells with mammalian expression vectors encoding for SOGA1/2 tagged with EGFP at the N-termini under the control of a CMV promoter. HeLa cells transiently overexpressing EGFP-SOGA1/2 exhibited exuberant bundles, which co-localized with a fraction of interphase microtubules (Figure 5A, 6A) and persisted upon microtubule depolymerization with 1 μM of nocodazole for 4h (Figure S3). The exact co-localization between the direct EGFP signal and the

indirect signal obtained with our SOGA antibodies supports their specificity (Figure S3). Noteworthy, significant cell death was observed upon SOGA protein overexpression (data not shown), which precluded the use of these constructs for RNAi-rescue experiments. As result, only a few cells expressing low levels of the fusion proteins were observed in mitosis. In these cells, the expression pattern observed by the direct EGFP signal detection (Figure 5B and 6B) coincides with the pattern of the respective endogenous protein obtained by indirect immunofluorescence (Figure 3B and 4B), except for a stronger accumulation of the fusion proteins at the spindle poles. Given that CLASPs also accumulate on unattached kinetochores in mitosis, we next investigated the kinetochore localization of EGFP-tagged SOGA proteins after their ectopic expression in HeLa cells (to improve signal/noise), upon depolymerization of mitotic spindle microtubules with nocodazole and mild hypotonic shock to spread the chromosomes. We found that EGFP-SOGA2/MTCL1 formed corona-like crescents on unattached outer kinetochores, as inferred by co-localization with anti-centromere antibodies (Figure 5C). In contrast, EGFP-SOGA1 was undetectable at unattached kinetochores (data not shown). Collectively, our data shows that SOGA1 and SOGA2/MTCL1 are microtubule-associated proteins that localize to defined mitotic structures, albeit in a distinct manner.

SOGA1 and SOGA2/MTLC1 are independently required for chromosome segregation fidelity

Given the localization of both SOGA1 and SOGA2/MTCL1 on well-defined mitotic structures, next we investigated the resulting mitotic phenotypes after their individual knockdown by RNAi in HeLa cells stably expressing histone H2B-GFP and mRFP-α-tubulin to directly visualize chromosomes and mitotic spindle microtubules, respectively. Live-cell analyses by spinning-disk confocal microscopy revealed that either SOGA1 or SOGA2/MTCL1 depletion resulted in a significant mitotic delay, as quantified by the interval between nuclear envelope breakdown (NEB) and anaphase onset or death (Figure 7A, B). SOGA2/MTCL1 knockdown caused a penetrant phenotype in which 37% of the cells displayed a prolonged metaphase delay with the subsequent scattering of the chromosome/chromatids along the spindle (Figure 7A-E). In contrast, SOGA1 depletion caused a more heterogeneous phenotype: while some cells displayed a prolonged metaphase delay, followed by chromosome/chromatids scattering along the spindle (17%), other cells exited mitosis after a short metaphase delay with missegregated chromosomes and/or chromatin bridges (18%), while some cells failed cytokinesis (Figure 7A-E). Noteworthy, efficient depletion of SOGA2/MTCL1 was only achieved with a pool of two different siRNAs (see Materials and Methods) and mitotic phenotypes were observed only upon pooling these two siRNAs, decreasing the chances of possible off-targeting effects.

In order to investigate whether the mitotic delay resulted from the incapacity to fully satisfy the spindle assembly checkpoint (SAC) once chromosomes have completed metaphase alignment, we silenced the SAC by inhibiting MPS1 with 5 μM of MPS1-IN-1 (Kwiatkowski et al. 2010) in SOGA1/2-depleted cells. Shortly after MPS1 inhibition (up to 20 min after NEB), cells exited mitosis, indicating that the observed mitotic delay resulted from the incapacity to satisfy the SAC (Figure 7F). Interestingly, co-depletion of SOGA1 and SOGA2/MTCL1 resulted in a combination of the individual phenotypes but without

worsening each phenotype, suggesting that SOGA1 and SOGA2/MTCL1 play both independent and overlapping roles that contribute to mitotic fidelity (Figure 7A-E).

Discussion

Faithful chromosome segregation relies on the tight regulation of spindle microtubule dynamics throughout mitosis and is orchestrated by a complex network of MAPs. We have previously identified (Maffini et al. 2009) and now characterized two new MAPs, SOGA1 and SOGA2/MTCL1, that interact with CLASPs during mitosis. SOGA proteins were also independently identified as CLASPs interactors in interphase and were proposed to be high-molecular weight MAPs based on their co-localization with microtubules (Kruse et al. 2017; Sato et al. 2013). In agreement with our findings, SOGA2/MTCL1 was previously shown to localize to the spindle poles and midbody in mitotic cells (Sato et al. 2013). SOGA2/MTCL1 appears to form a two-stranded structure in the long coiled-coil region that may dimerize and hold two N-terminal microtubule-binding domains that crosslink microtubules (Sato et al. 2013). This structural property is conserved among microtubule crosslinkers, such as NuMA, and is consistent with the potent induction of microtubule bundling upon overexpression of SOGA proteins. *In silico* modeling of SOGA1 structure revealed similarities with some domains of dynein-2, including the microtubule-binding domain (Kruse et al. 2017). In the future, the potent microtubule bundling induced upon SOGA protein overexpression may be used to identify or refine their respective microtubule-binding domains. In this work, we independently confirmed previous work reporting co-localization of both SOGA proteins with interphase microtubules, while providing evidence that SOGA2/MTCL1 decorates spindle microtubules and poles throughout mitosis, with both SOGA proteins accumulating on midbodies. Additionally, we show that EGFP-SOGA2/MTCL1 localizes to the outer kinetochore in the absence of microtubules. Functional studies in living cells revealed a consistent mitotic phenotype upon the individual knockdown of each SOGA protein. While depletion of each protein resulted in a prolonged metaphase arrest, followed by chromatid scattering and death in mitosis, this was somewhat more penetrant in the case of SOGA2/MTCL1 depletion, accounting for almost 40% of the mitotic defects (Figure 7A, C, D). The loss of sister-chromatid cohesion before anaphase onset resulting in the scattering of single chromatids and subsequent accumulation at spindle poles is highly suggestive of a phenomenon called cohesion fatigue, described as the endpoint phenotype caused by the progressive loss of sister-chromatid cohesion (Daum et al. 2011). In the case of SOGA1 depletion, there was also an increase in chromosome missegregation events (lagging chromosomes and chromatin bridges) in anaphase and cytokinesis failure. Anaphase lagging chromosomes and metaphase delay are mitotic defects frequently associated with unstable or defective kinetochore-microtubule attachments. Moreover, it was recently shown that a prolonged metaphase deregulates Separase activity and causes chromosome non-disjunction in anaphase (Shindo et al. 2021). Curiously, we found that SOGA2/MTCL1 is highly phosphorylated during mitosis in a CDK1-dependent manner. This led us to speculate that, similar to the phospho-regulation of CLASP2 in mitosis (Maia et al. 2012), CDK1-dependent phosphorylation of SOGA2 might contribute to the stabilization of kinetochore-microtubule attachments required for timely SAC satisfaction. However, further conclusions with respect to the impact of SOGAs in regulating

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kinetochore microtubule dynamics await proper quantitative measurements. Moreover, while anaphase lagging chromosomes and bridges might prevent the completion of abscission (Mullins and Biesele 1977), SOGA proteins might also play more specific roles in cytokinesis due to their association with midbodies.

Large scale CRISPR-Cas9 screens indicate that neither SOGA protein is individually required for viability across more than 780 different cell lines (https://depmap.org/). This suggests that SOGA proteins play at least some partially redundant roles during mitosis that ensure cell viability upon their independent inactivation. A non-mutually exclusive possibility is that clonal expansion following CRISPR-Cas9-mediated inactivation of SOGA proteins caused rewiring of cell cycle control networks enabling efficient proliferation. Curiously, mouse embryonic fibroblasts (MEFs) derived from CLASP2 KO mice are fully viable, but show a background of mitotic errors that only marginally impact cell proliferation (Pereira et al. 2006). Moreover, ectopic expression of either CLASP is able to rescue the minor mitotic abnormalities observed in CLASP2 KO MEFs, suggesting functional redundancy. More recently, we found that human cells quickly adapt to the loss of tubulin tyrosine ligase (TTL) upon CRISPR-mediated inactivation, and suppress chromosome missegregation events observed after short-term depletion of TTL by RNAi (Ferreira et al. 2020). Because rescue experiments with our CMV promoter constructs were not possible due to strong deleterious effects associated with SOGA protein overexpression, at this stage we cannot completely rule out possible off-targeting effects. However, the localization to particular mitotic structures, the observation of mitotic phenotypes only upon the use of an siRNA pool in the case of SOGA2/MTCL1, and the partially overlapping phenotypes between SOGA1 and SOGA2/MTCL1, strongly suggest that these proteins play specific roles in chromosome segregation. The development of constructs where SOGA protein expression is under control of inducible promoters will be important tools for future validation of SOGA proteins' roles in mitosis.

Similar to CLASPs, SOGA proteins are highly and almost exclusively expressed in the brain/cerebellum (https://gtexportal.org). However, this does not exclude that, as for CLASPs (Dillon et al. 2017; Drabek et al. 2012; Drabek et al. 2006; Sayas et al. 2019), SOGA proteins play important roles during both mitosis (our study) and interphase (Sato et al. 2013; Sato et al. 2014) in other tissues. Although the physiological relevance of the interaction between SOGAs and CLASPs in mitosis has not been addressed in the present work, distinct mitotic defects have been associated with interference of CLASPs and SOGAs' function in human cells. While interference with CLASPs' function has been reported to cause monopolar, short and/or multipolar spindles, as well as chromosome alignment defects (Girao et al. 2020; Logarinho et al. 2012; Maiato et al. 2003; Mimori-Kiyosue et al. 2006; Pereira et al. 2006), the earlier have not been observed upon SOGA1 and/or SOGA2/MTCL1 depletion (Figure 7A,C). Further interdependency studies are required to fully understand the relationship between CLASPs and SOGAs in mitosis and beyond.

Materials and methods

Cell lines, plasmids and RNAi transfections

All cell lines were cultured at 37°C in 5% CO_2 atmosphere in DMEM (Gibco, Thermo Fisher Scientific) containing 10% FBS (Gibco, Thermo Fisher Scientific). Parental U2OS cells were kindly provided by S. Geley (Innsbruck Medical University, Innsbruck, Austria). EGFP-SOGA1 and EGFP-SOGA2/MTCL1 cell lines were generated by transient expression of pIC113-EGFP-SOGA1 and pIC133-EGFP-SOGA2/MTCL1, respectively, under control of a CMV promoter, and selected with G418. pIC113-EGFP-SOGA1 was generated by cloning cDNA of isoform 1 of KIAA0889 into pIC113 backbone, between *Spe*I and *Sal*I restriction sites. pIC133-EGFP-SOGA2/MTCL1 was generated by cloning the cDNA of isoform 2 of KIAA0802 (NM_015210, Kazuza institute) into a pIC113 backbone between *Xho*I and *Hind*III restriction sites. Human SOGA1 was depleted using the small interfering RNA (siRNA) oligonucleotide 5′-GUGAGAAGAUCCACGACAA-3′; and SOGA2/MTCL1 was depleted using a combination of two siRNAs, 5′-GCGCUAAAGCUGAGGAUGA-3′ and 5′-ACUCUCCGACAGCAGAUGA-3', at 5 nM each.

Drug treatments

Mitotic enriched extracts were obtained from parental HeLa cells incubated for 12-16 h in media containing 5 μM of STLC (S-trityl-L-cysteine, Sigma) before harvesting by mitotic shake off. For inhibition of different mitotic kinases the following drugs were used for 1 h at 37ºC: 10 μM RO 3306 (Cdk1 inhibitor, Enzo Life Sciences), 100 nM BI 2536 (Plk1 inhibitor, Axon Medchem), 2 μM ZM 447439 (AurkB inhibitor, AstraZeneca), 5 μM MPS1-IN-1 (Kwiatkowski et al. 2010) and 40 mM LiCl (GSK3β inhibition). Mitotic exit was blocked with 10 μM of the proteasome inhibitor MG132 (EMD Millipore).

Antibody generation

The cDNA regions encoding the amino acid sequence between 1265-1423 of SOGA1 (GSTES...PPSE; isoform 1) and between 915-1273 of the SOGA2/MTLC1 (KENSP...QTNGS, isoform 2) were amplified and cloned into pENTR™/D-TOPO® vector (TOPO® cloning, ThermoFisher Scientific) followed by recombination with a pDEST17™ destination vector (Gateway®, ThermoFisher Scientific). The resulting his-tagged recombinant proteins were expressed in BL21 Rosetta and solubilized inclusion bodies fractions used for affinity chromatography with nickel-agarose columns. Purified recombinant proteins were dialyzed against phosphate-buffered saline (PBS) and used to immunize rabbits. The sera from immunized animals were further subjected to antigen-affinity purification (Abgent).

Immunoprecipitation

Immunoprecipitation experiments for validation of the reciprocal interaction between CLASP and SOGA proteins was performed using native protein extracts (1.5 mg of total protein in a total volume of 500 mL of IP buffer: 150 mM NaCl, 20 mM HEPES [pH 7.5], 1 mM EGTA, 1 mM EDTA, 1.5 mM MgCl2, 0.1% NP40 and protease, 0.1% Triton and

protease inhibitors) prepared from HeLa parental cell cultures enriched for mitotic cells by incubation with 5 μM of STLC. 15 μL of each antibody were used for immunoprecipitation (polyclonal rabbit anti-SOGA1 (1.7 μg/μL), polyclonal rabbit anti-SOGA2/MTCL1 (1 μg/μL). Normal unconjugated rabbit IgGs (Santa Cruz Biotechnology) were used as control for non-specific interactions (data not shown). Precipitated proteins were subjected to electrophoresis followed by western blot with the appropriate antibodies: polyclonal rabbit anti-SOGA1 (1:1,000), polyclonal rabbit anti-SOGA2/MTCL1 (1:1,000), monoclonal rat anti-CLASP1 (1A6, 1:25) (Maffini et al. 2009), monoclonal rat anti-CLASP2 (6E3, 1:25) (Maffini et al. 2009). Total α-tubulin was detected using a mouse monoclonal antibody against α-tubulin (clone B512, 1:10,000; Sigma-Aldrich) and used as loading control.

Western blotting

Cell pellets were washed with pre-warmed PBS and resuspended in ice-cold lysis buffer (50 mM Tris HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 0.5% NP-40, and 0.5% Triton X-100) supplemented with a cocktail of protease inhibitors (Roche). Protein samples were denatured in Laemmli buffer at 95°C for 5 min, and 50 μg of total protein were separated by 7.5% (vol/vol) SDS-PAGE electrophoresis to better resolve phosphorylation-dependent electrophoretic mobility shifts and 10% (vol/vol) for all other purposes. Proteins were transferred to a nitrocellulose membrane using an iBlot Dry Blotting System (Invitrogen). SOGA1/2 were detected using polyclonal rabbit antibodies against SOGA1 (1:100) and SOGA2/MTCL1(1:200), respectively. Total tubulin was detected using mouse monoclonal antibody against α-tubulin B512 clone (1:10,000; Sigma-Aldrich) and used as loading control. HRP-conjugated secondary antibodies (1:5,000–10,000, Jackson Immunoresearch) were visualized using an ECL system (Bio-Rad).

In silico prediction of phosphorylation sites

To assess the putative site of phosphorylation by CDK1 in SOGA proteins we took advantage of the resource PhosphoSitePlus (http://www.phosphosite.org/), a knowledgebase that predicts site phosphorylation, to identify the optimal consensus sequence (S/T-P-X-R/K). Only sites found in 5 or more independent high-throughput analysis using proteomic discovery mass spectrometry were represented.

Immunofluorescence

HeLa cells were fixed with cold methanol (–20°C) for 4 min. SOGA1 and SOGA2/MTCL1 were immunostained using newly generated polyclonal rabbit anti-SOGA1 (1:100) and anti-SOGA2/MTCL1 (1:100). Tyrosinated α-tubulin and centromeres were immunostained using rat monoclonal anti-tyrosinated α-tubulin clone YL1/2 (1:100–500, Bio-Rad) and human anti-centromere antibodies (ACA, 1:100–2,000, kind gift from B. Earnshaw, Welcome Trust Centre for Cell Biology, University of Edinburgh, Edinburgh, UK), respectively. GFP-tagged constructs were visualized by means of direct fluorescence. Alexa Fluor 488 (1:200–2,000, Themofisher), STAR-580, and STAR-RED (1:200, Abberior) were used as secondary antibodies and DNA was counterstained with 1 μg/ml DAPI (Sigma-Aldrich).

Chromosome Spreads

Chromosome spreads were obtained upon incubation with 3.3 μM of nocodazole (Sigma-Aldrich) for 4 h followed by incubation with hypotonic solution (75 mM KCL) in the presence of nocodazole at 37ºC for 30 min, then fixed with cold methanol (-20ºC) for 4 min. Subsequent immunofluorescence was performed as described above.

Image acquisition

Confocal images were acquired using Abberior Instruments “Expert Line” gated-STED coupled to a Nikon Ti microscope. An oil-immersion 60× 1.4 NA Plan-Apo objective (Nikon, Lambda Series) and pinhole size of 0.8 Airy units were used in all confocal acquisitions.

Time-lapse microscopy

For phenotypic analysis of SOGA proteins knockdown by RNAi transfection, HeLa H2B-GFP/mCherry-α-tubulin cells were cultured in 35 mm glass-bottom dishes (14 mm, No 1.5 coverglass; MatTek Corporation). Cell culture medium was replaced with Leibovitz's-L15 medium (GIBCO, Life Technologies) supplemented with 10% FBS. Time-lapse imaging was performed in a heated chamber (37°C) using a 100× 1.4 NA Plan-Apochromatic differential interference contrast objective mounted on an inverted microscope (TE2000U; Nikon) equipped with a CSU-X1 spinning-disk confocal head (Yokogawa Corporation of America) and with two laser lines (488 nm and 561 nm). Images were acquired with an iXon + EM-CCD camera (Andor Technology). Eleven 1-μm-separated z-planes covering the entire volume of the mitotic spindle were collected every 2 min.

Statistical analysis

An unpaired two-tailed Student’s *t* test was used to determine the significance of differences between two groups (time from NEB to anaphase onset/death). All statistical analysis was performed using Prism, version 7.0a.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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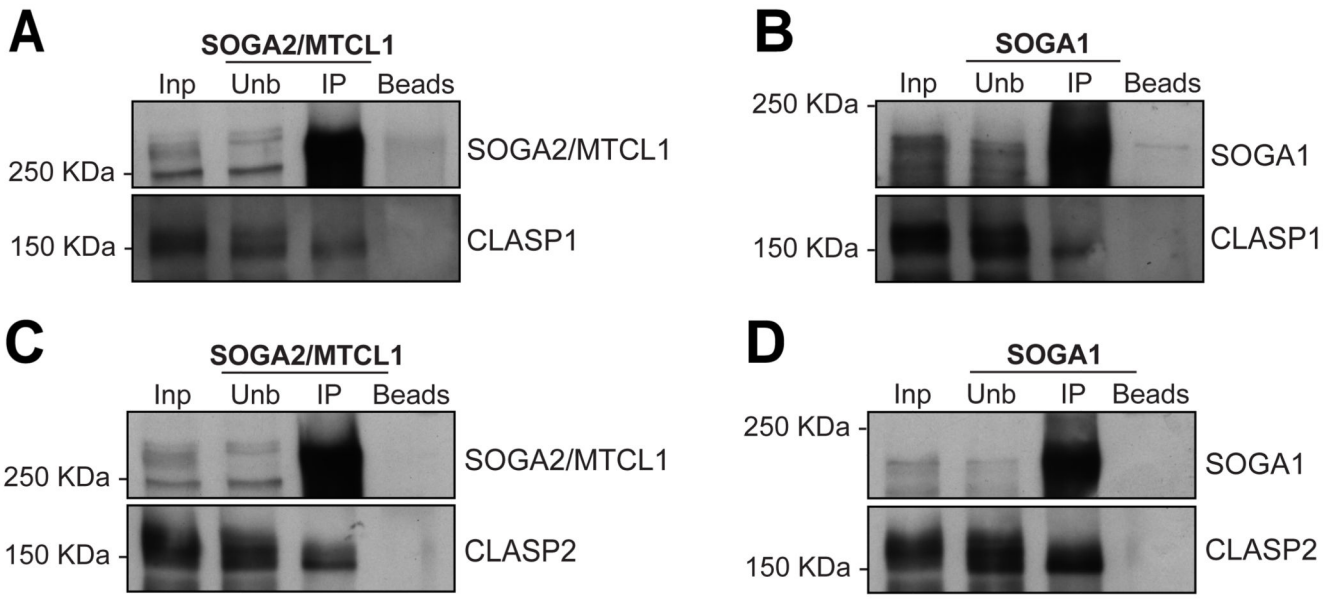

Figure 1. Human SOGA1 and SOGA2/MTCL1 interact with CLASP1 and CLASP2 during mitosis.

A-D. Immunoprecipitation performed in mitotic extracts of HeLa cells using antibodies against SOGA2/MTCL1 and SOGA1. Immunoprecipitates were analyzed by western-blot using antibodies against SOGA2/MTCL1, SOGA1, CLASP1 and CLASP2. Native input material (Inp), unbound fraction (Unb), immunoprecipitated fraction (IP) and fraction bound to beads during pre-clearing (Beads).

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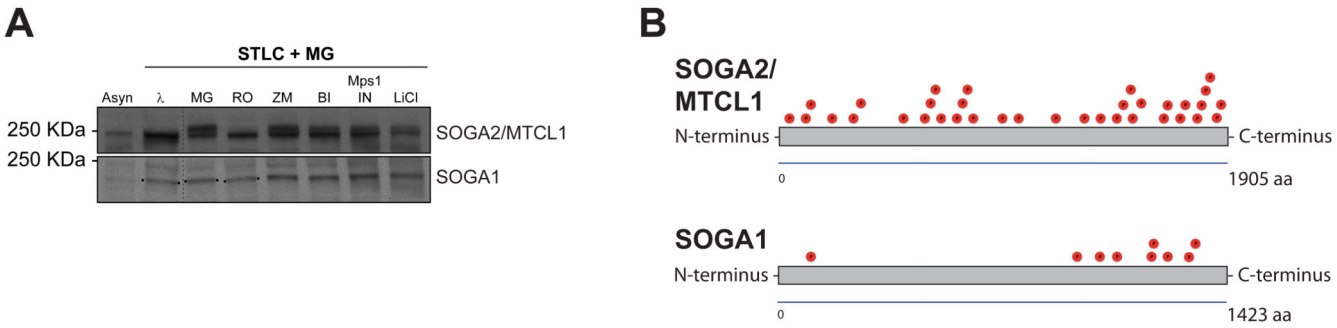

Figure 2. SOGA1 and SOGA2/MTCL1 are expressed and phospho-regulated by CDK1 during mitosis.

A. Protein lysates of HeLa cells synchronized in mitosis with STLC and treated with different kinase inhibitors: CDK1 (RO), Aurora B (ZM), Plk1 (BI), MPS1 (Mps) and GSK3β (LiCl). Drugs: MG132 (MG), RO 3306 (RO), ZM447439 (ZM), BI 2536 (BI), Mps1-IN-1 (Mps) and lithium chloride (LiCl). Treatment with λ phosphatase was used as negative control. Dashed lines indicate discontinuous lanes. Black dots highlight the slight change in mobility of SOGA1 in the indicated conditions. **B.** *In silico* analysis for putative consensus sites for CDK1 phosphorylation in SOGA2/MTCL1 and SOGA1.

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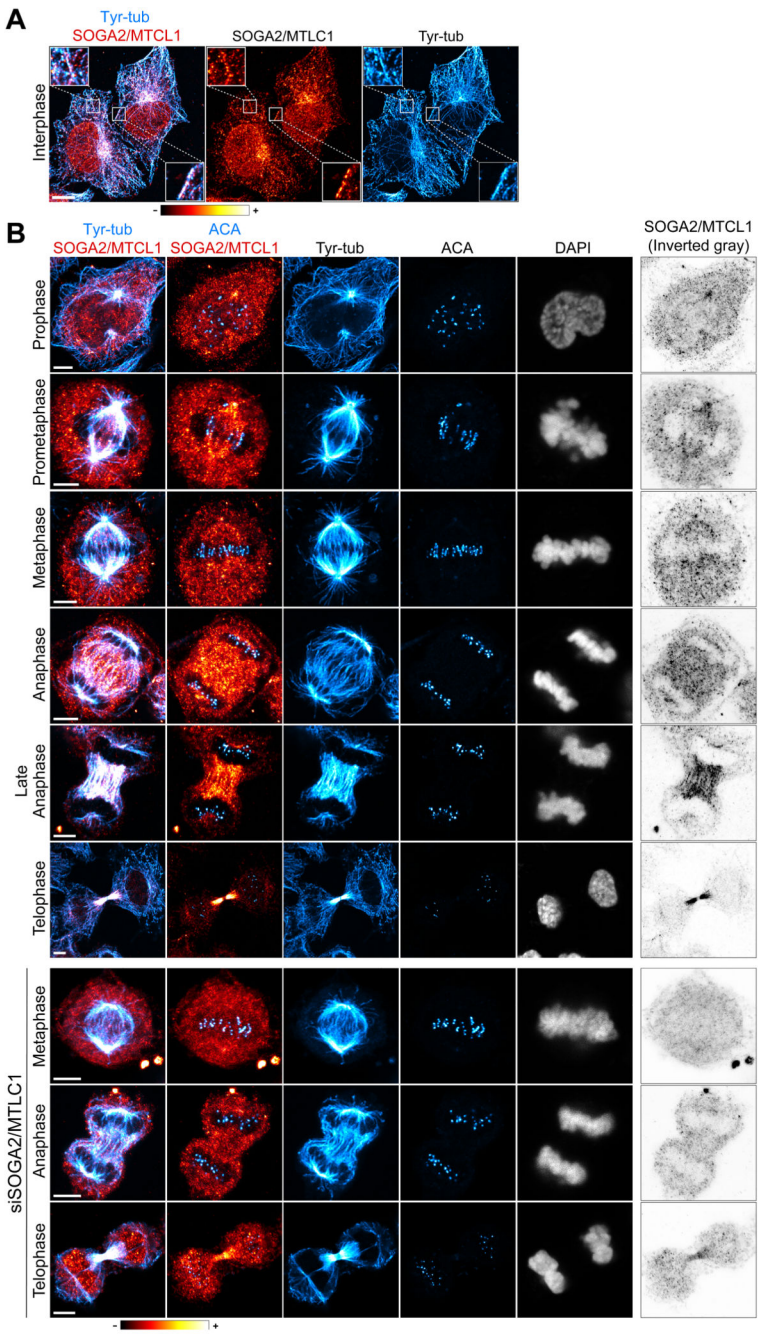

Figure 3. SOGA2/MTCL1 associates with microtubules throughout the cell cycle.

Representative confocal images of interphase **(A)** and mitotic **(B)** HeLa cells immunostained with antibodies against SOGA2/MTCL1, tyrosinated tubulin (Tyr-tub) and centromere proteins (ACA). DNA was counterstained with DAPI. Insets represent 3X magnification of SOGA2/MTCL1 localization to interphase microtubules (A). Red-hot lookup table was used to map extremes in fluorescence intensity for SOGA2/MTCL1. Scale bars, 10 μm (A) and 5 μm (B).

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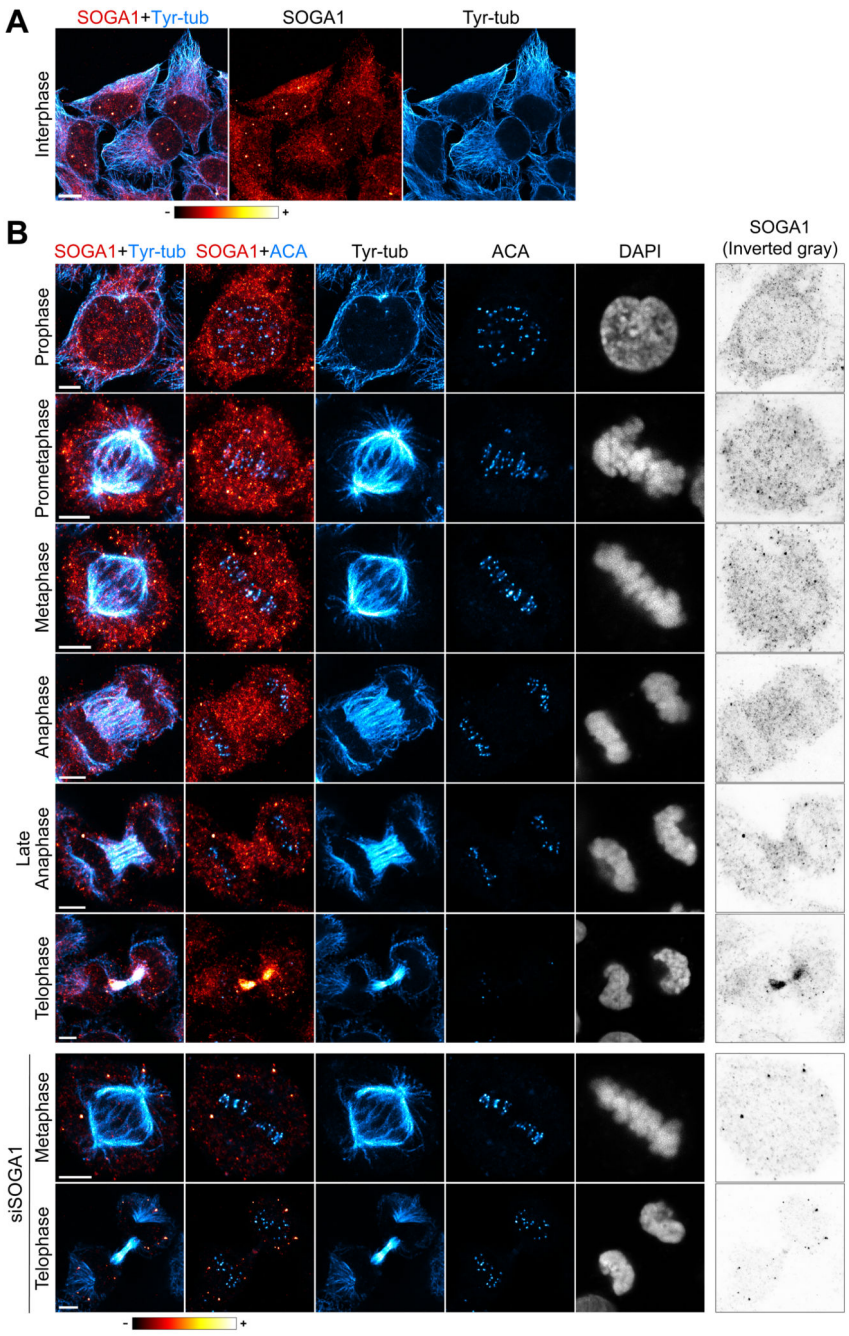

Figure 4. SOGA1 associates with microtubules during late mitosis and interphase.

Representative confocal images of interphase **(A)** and mitotic **(B)** HeLa cells immunostained with antibodies against SOGA1, tyrosinated tubulin (Tyr-tub) and centromere proteins (ACA). DNA was counterstained with DAPI. Red-hot lookup table was used to map extremes in fluorescence intensity for SOGA1. Scale bars, 10 μm (A) and 5 μm (B).

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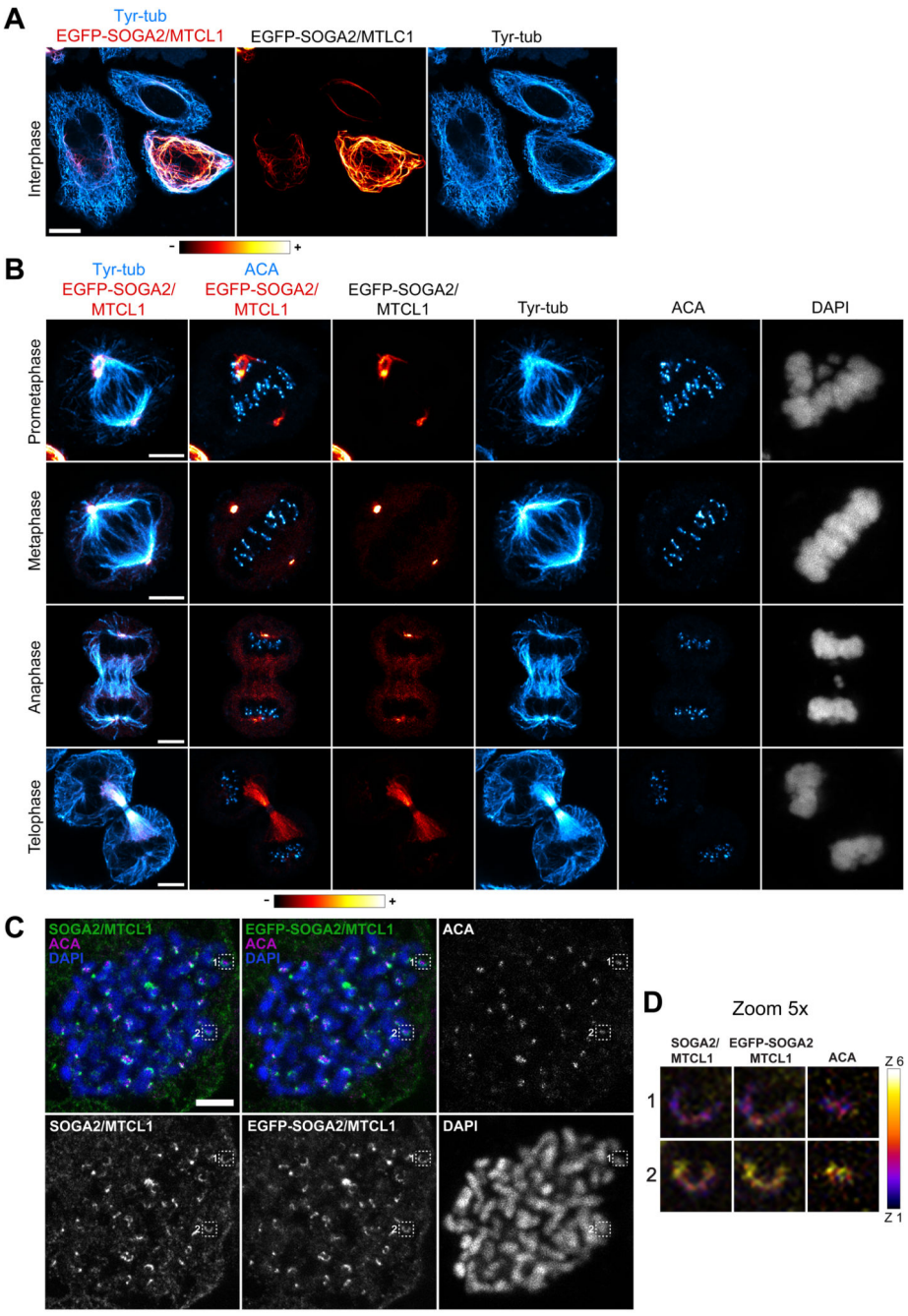

Figure 5. EGFP-SOGA2/MTCL1 localizes at microtubules and the outer-kinetochore.

Representative confocal images of interphase **(A)** and mitotic **(B)** HeLa cells transiently expressing EGFP-SOGA2/MTCL1 and immunostained with antibodies against tyrosinated tubulin (Tyr-tub) and centromere proteins (ACA). DNA was counterstained with DAPI. Red-hot lookup table was used to map extremes in fluorescence intensity for exogenous EGFP-SOGA2/MTCL1. **(C)** Representative confocal images of chromosome spreads of HeLa cells transiently expressing EGFP-SOGA2/MTCL1 and immunostained with antibodies against centromere proteins (ACA). **(D)** 5X magnification of insets 1 and 2 represented in (C).

Chromo-projection with fire lookup table was used as a readout for optical sectioning. Scale bars, 10 μm (A) and 5 μm (B,C).

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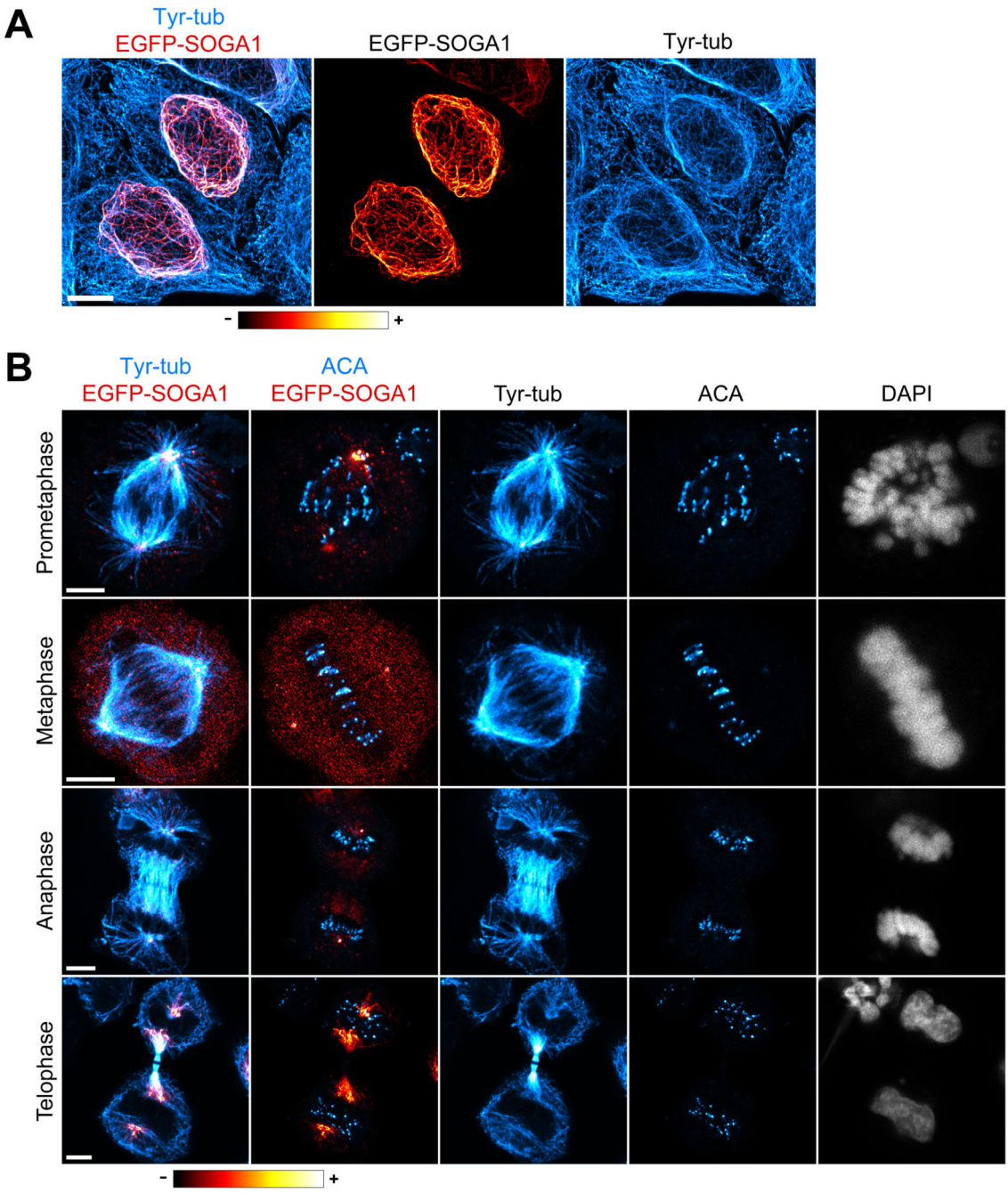

Figure 6. EGFP-SOGA1 localizes at microtubules and distinct mitotic structures.

Representative confocal images of interphase **(A)** and mitotic **(B)** HeLa cells transiently expressing EGFP-SOGA1 and immunostained with antibodies against tyrosinated tubulin (Tyr-tub) and centromere proteins (ACA). DNA was counterstained with DAPI. Red-hot lookup table was used to map extremes in fluorescence intensity for exogenous EGFP-SOGA1. Scale bars, 10 μm (A) and 5 μm (B).

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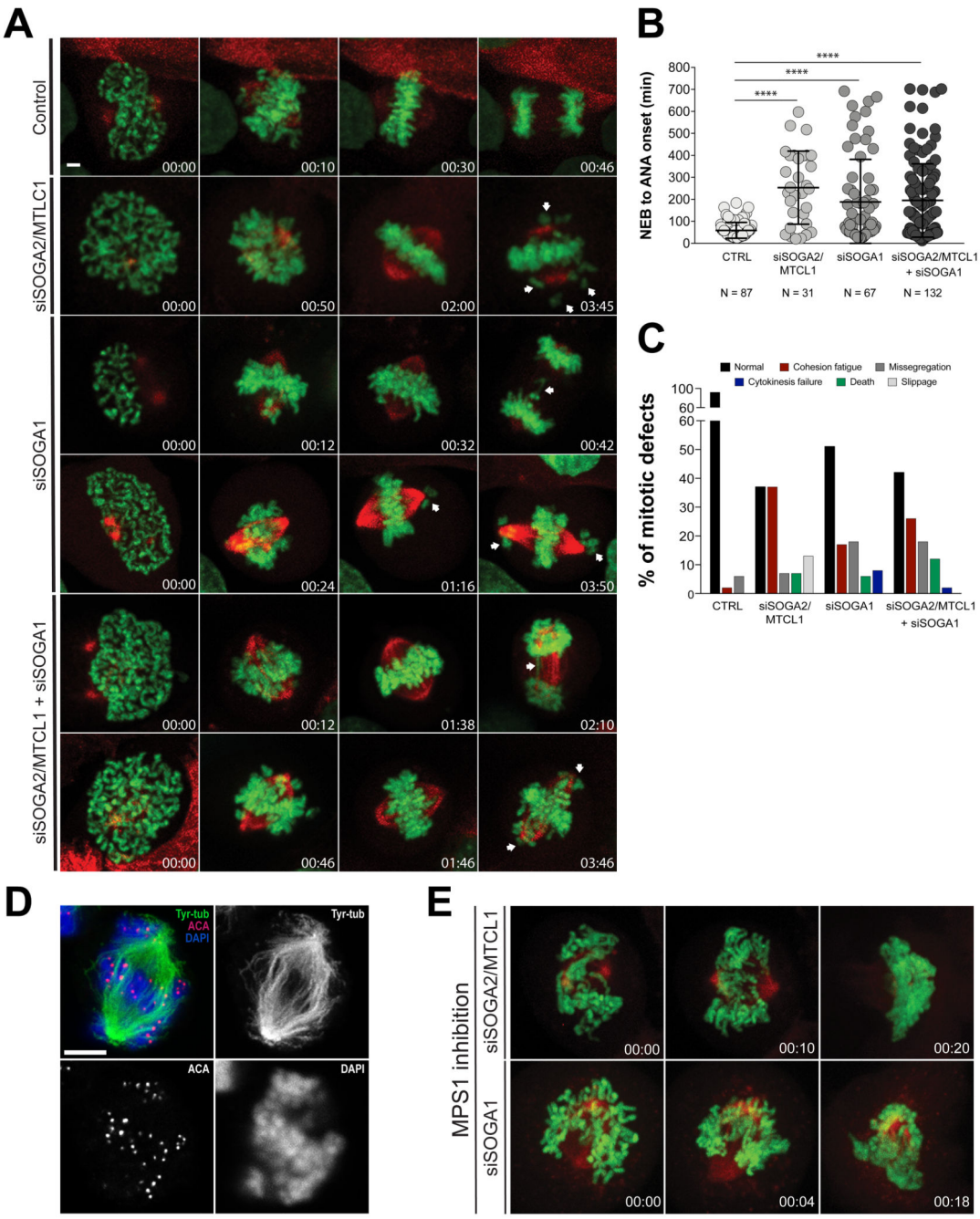

Figure 7. SOGA1 and SOGA2/MTCL1 are required for faithful chromosome segregation.

A. Live-cell imaging HeLa cells stably expressing H2B-GFP/mRFP-α-tubulin in different conditions. Arrows indicate chromosomes/chromatids at the poles in cells exhibiting cohesion fatigue (siSOGA2/MTCL1, siSOGA1 and double depletion). Arrowheads point to a lagging chromosome (siSOGA1) and a DNA bridge (double depletion). Scale bar, 10 μm. Time, hours:minutes. **B.** Elapsed time from nuclear envelope breakdown (NEB) to anaphase onset or death in siSOGA2/MTLC1 (283.66236.5 min, ****p<0.001, unpaired, t-test, N=32, pool of 8 independent experiments), siSOGA1 (188.55192.9 min, ****p<0.001, unpaired, t-

test, N=67, pool of 5 independent experiments), siSOGA2/MTCL1+siSOGA1 (194.88166.7 min, ****p<0.001, unpaired, t-test, N=132, pool of 7 independent experiments) compared to mock transfected (control) (71.89882.7 min, N=90, pool of 5 independent experiments) HeLa cells stably expressing H2B-GFP/mRFP-α-tubulin. **(C)** Quantification of the mitotic defects in HeLa cells stably expressing H2B-GFP/mRFP-α-tubulin subjected to different RNAi conditions. **(D)** Representative image of HeLa cells exhibiting scattered chromatids upon SOGA2/MTCL1 depletion immunostained with antibodies against tyrosinated tubulin (Tyr-tub) and centromere proteins (ACA). DNA was counterstained with DAPI. Scale bar, 5 μm. **(E)** Live-cell imaging HeLa cells stably expressing H2B-GFP/mRFP-α-tubulin treated with MPS1 inhibitor in different conditions (siSOGA2/MTCL1, siSOGA1). Scale bar, 10 μm. Time, hours:minutes.

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