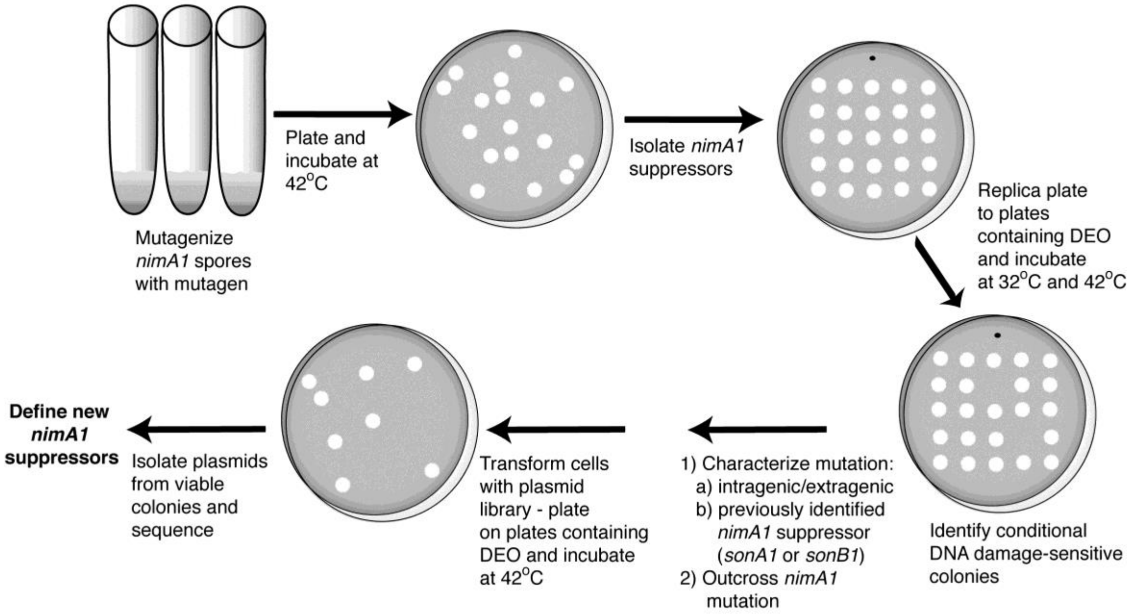
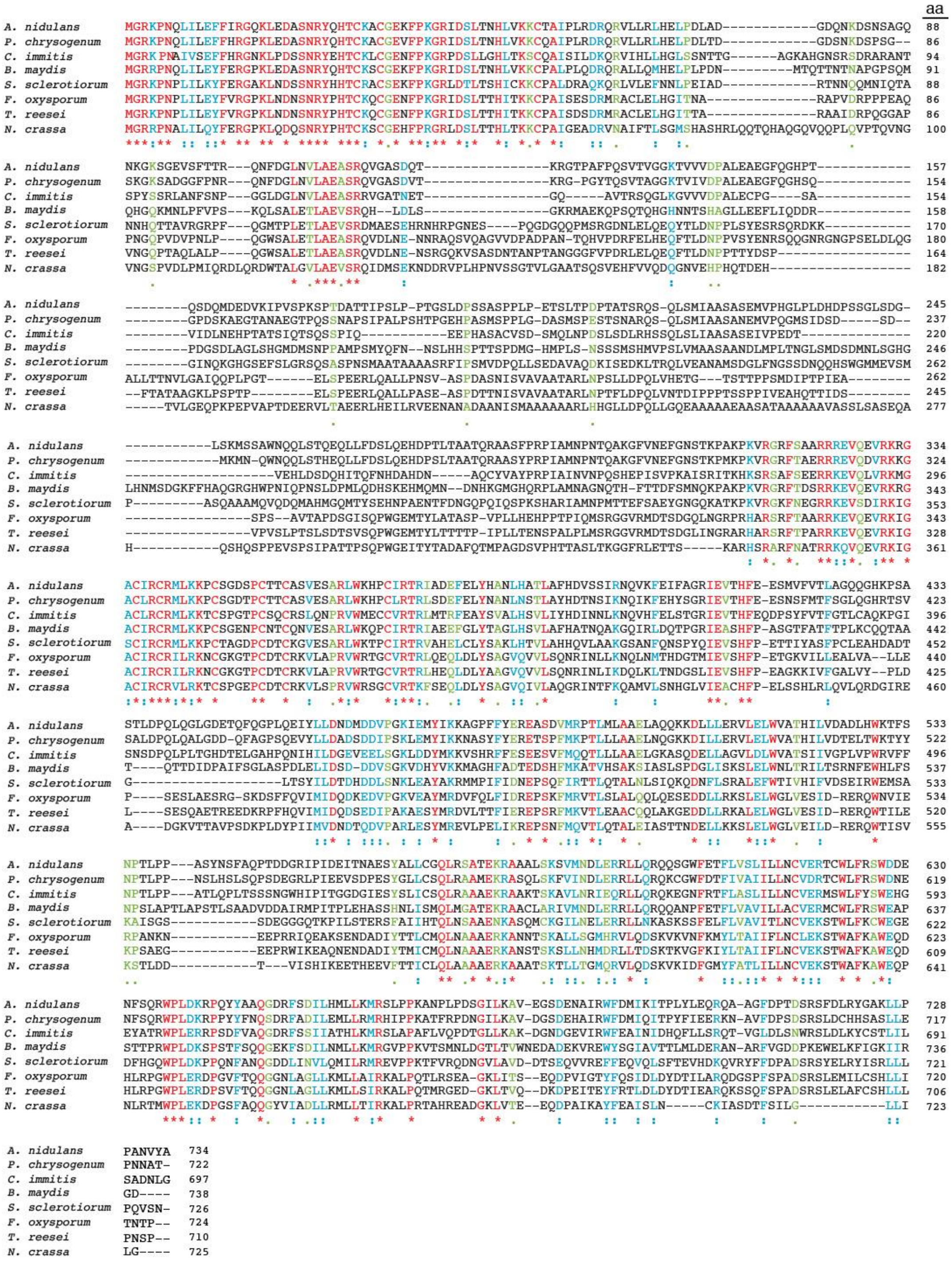


**Figure S1** Methods used to identify conditional drug-sensitive suppressors of *nimA1*. Diagram of the genetic screen performed and the general steps involved to identify suppressors of *nimA1* temperature sensitivity.



```
                                                                                                                       aa
A. nidulans      MGRKPNQLILEFFIRGQKLEDASNRYQHTCKACGEKFPKGRIDSLTNHLVKKCTAIPLRDRQRVLLRLHELPDLAD------------GDQNKDSNSAGQ  88
P. chrysogenum   MGRKPNQLILEFFHRGPKLEDASNRYQHTCKACGEVFPKGRIDSLTNHLVKKCQAIPLRDRQRVLLRLHELPDLTD------------GDSNKDSPSG--  86
C. immitis       MGRKPNAIVSEFFHRGNKLPDSSNRYEHTCKLCGENFPKGRPDSLLGHLTKSCQAISILDKQRVIHLLHGLSSNTTG------AGKAHGNSRSDRARANT  94
B. maydis        MGRRPNQLILEYFERGPKLEDASNRYQHTCKSCGEKFPKGRIDSLTNHLVKKCPALPLQDRQRALLQMHELPLPDN---------MTQTTNTNAPGPSQM  91
S. sclerotiorum  MGRKPNPLILKYFERGAKLNDSSNRYHHTCRACSEKFPKGRLDTLTSHICKKCPALDRAQKQRLVLEFNNLPEIAD------------RTNNQQMNIQTA  88
F. oxysporum     MGRKPNPLILEYFVRGPKLNDNSNRYPHTCKQCGENFPKGRIDSLTTHITKKCPAISESDRMRACLELHGITNA-------------RAPVDRPPPEAQ  86
T. reesei        MGRKPNPLILEYFVRGPKLNDNSNRYPHTCKQCGENFPKGRIDSLTTHITKKCPAISDSDRMRACLELHGITTA-------------RAAIDRPQGGAP  86
N. crassa        MGRRPNALILQYFERGPKLQDQSNRYPHTCKSCGEHFPRGRLDSLTTHLTKKCPAIGEADRVNAIFTLSGMSHASHRLQQTQHAQGQVQQPLQVPTQVNG  100
                 ***:** :: ::* ** ** * **** ***: *.* **:** *:*  *: *.* *:   :: .    :  :.                    .

A. nidulans      NKGKSGEVSFTTR---QNFDGLNVLAEASRQVGASDQT----------------KRGTPAFPQSVTVGGKTVVVDPALEAEGFQGHPT------------  157
P. chrysogenum   SKGKSADGGFPNR---QNFDGLNVLAEASRQVGASDVT----------------KRG-PGYTQSVTAGGKTVIVDPALEAEGFQGHSQ------------  154
C. immitis       SPYSSRLANFSNP---GGLDGLNVLAEASRRVGATNET----------------GQ-----AVTRSQGLKGVVVDPALECPG----SA------------  154
B. maydis        QHGQKMNLPFVPS---KQLSALETLAEVSRQH--LDLS----------------GKRMAEKQPSQTQHGHNNTSHAGLLEEFLIQDDR------------  158
S. sclerotiorum  NNHQTTAVRGRPF---QGMTPLETLAEVSRDMAESEHRNHRPGNES----PQGDGQQPMSRGDNLELQEQYTLDNPPLSYESRSQRDKK-----------  170
F. oxysporum     PNGQPVDVPNLP----QGWSALETLAEASRQVDLNE-NNRAQSVQAGVVDPADPAN-TQHVPDRFELHEQFTLDNPPVSYENRSQQGNRGNGPSELDLQG  180
T. reesei        VNGQPTAQLALP----QGWSALETLAEASRQVDLNE-NSRGQKVSASDNTANPTANGGGFVPDRLELQEQFTLDNPPTTYDSP-----------------  164
N. crassa        VNGSPVDLPMIQRDLQRDWTALGVLAEVSRQIDMSEKNDDRVPLHPNVSSGTVLGAATSQSVEHFVVQDQGNVEHPHQTDEH------------------  182
                    .                 * .***.**     :                                 :    ..

A. nidulans      --------QSDQMDEDVKIPVSPKSPTDATTIPSLP-PTGSLDPSSASPPLP-ETSLTPDPTATSRQS-QLSMIAASASEMVPHGLPLDHDPSSGLSDG-  245
P. chrysogenum   --------GPDSKAEGTANAEGTPQSSNAPSIPALPSHTPGEHPASMSPPLG-DASMSPESTSNARQS-QLSMIAASANEMVPQGMSIDSD-----SD--  237
C. immitis       --------VIDLNEHPTATSIQTSQSSPIQ-----------EEPHASACVSD-SMQLNPDSLSDLRHSSQLSLIAASASEIVPEDT-------------  220
B. maydis        --------PDGSDLAGLSHGMDMSNPPAMPSMYQFN--NSLHHSPTTSPDMG-HMPLS-NSSSMSHMVPSLVMAASAANDLMPLTNGLSMDSDMNLSGHG  246
S. sclerotiorum  --------GINQKGHGSEFSLGRSQSASPNSMAATAAAASRFIPSMVDPQLLSEDAVAQDKISEDKLTRQLVEANAMSDGLFNGSSDNQQHSWGMMEVSM  262
F. oxysporum     ALLTTNVLGAIQQPLPGT------ELSPEERLQALLPNSV-ASPDASNISVAVAATARLNPSLLDPQLVHETG---TSTTPPSMDIPTPIEA--------  262
T. reesei        --FTATAAGKLPSPTP--------ELSPEERLQALLPASE-ASPDTTNISVAVAATARLNPTFLDPQLVNTDIPPPTSSPPIVEAHQTTIDS--------  245
N. crassa        -----TVLGEQPKPEPVAPTDEERVLTAEERLHEILRVEENANADAANISMAAAAAARLHHGLLDPQLLGQEAAAAAEAASATAAAAAAVASSLSASEQA  277
                                           .                .               .

A. nidulans      -----------LSKMSSAWNQQLSTQEQLLFDSLQEHDPTLTAATQRAASFPRPIAMNPNTQAKGFVNEFGNSTKPAKPKVRGRFSAARRREVQEVRKRG  334
P. chrysogenum   ------------MKMN-QWNQQLSTHEQLLFDSLQEHDPSLTAATQRAASYPRPIAMNPNTQAKGFVNEFGNSTKPMKPKVRGRFTAERRREVQDVRKKG  324
C. immitis       -------------------VEHLDSDQHITQFNHDAHDN-----AQCYVAYPRPIAINVNPQSHEPISVPKAISRITKHKSRSAFSEERRKEVQLVRKMG  296
B. maydis        LHNMSDGKFFHAQGRGHWPNIQPNSLDPMLQDHSKEHMQMN--DNHKGMGHQRPLAMNAGNQTH-FTTDFSMNQKPAKPKVRGRFTDSRRKEVQEVRKRG  343
S. sclerotiorum  P---------ASQAAAMQVQDQMAHMGQMTYSEHNPAENTFDNGQPQIQSPKSHARIAMNPMTTEFSAEYGNGQKATKPKVRGKFNEGRRKEVSDIRKIG  353
F. oxysporum     ----------------SPS--AVTAPDSGISQPWGEMTYLATASP-VPLLHEHPPTPIQMSRGGVRMDTSDGQLNGRPRHARSRFTAARRKEVQEVRKIG  343
T. reesei        ----------------VPVSLPTSLSDTSVSQPWGEMTYLTTTTP-IPLLTENSPALPLMSRGGVRMDTSDGLINGRARHARSRFTPARRKEVQEVRKIG  328
N. crassa        H-----------QSHQSPPEVSPSIPATTPSQPWGEITYTADAFQTMPAGDSVPHTTASLTKGGFRLETTS-----KARHSRARFNATRRKQVQEVRKIG  361
                                                                                                : *. *.  **::*. :** *

A. nidulans      ACIRCRMLKKPCSGDSPCTTCASVESARLWKHPCIRTRIADEFELYHANLHATLAFHDVSSIRNQVKFEIFAGRIEVTHFE-ESMVFVTLAGQQGHKPSA  433
P. chrysogenum   ACLRCRMLKKPCSGDTPCTTCASVESARLWKHPCLRTRLSDEFELYNANLNSTLAYHDTNSIKNQIKFEHYSGRIEVTHFE-ESNSFMTFSGLQGHRTSV  423
C. immitis       ACLRCRMLKKTCSPGTPCSQCRSLQNPRVWMECCVRTRLMTRFEAYSVALHSVLIYHDINNLKNQVHFELSTGRIEVTHFEQDPSYFVTFGTLCAQKPGI  396
B. maydis        ACIRCRMLKKPCSGENPCNTCQNVESARLWKQPCIRTRIAEEFGLYTAGLHSVLAFHATNQAKGQIRLDQTPGRIEASHFP-ASGTFATFTPLKCQQTAA  442
S. sclerotiorum  SCIRCRMLKKPCTAGDPCDTCKGVESARLWKTPCIRTRVAHELCLYSAKLHTVLAHHQVLAAKGSANFQNSPYQIEVSHFP-ETTIYASFPCLEAHDADT  452
F. oxysporum     ACIRCRILRKNCGKGTPCDTCRKVLAPRVWRTGCVRTRLQEQLDLYSAGVQVVLSQNRINLLKNQLNMTHDGTMIEVSHFP-ETGKVILLEALVA--LLE  440
T. reesei        ACIRCRILRKNCGKGTPCDTCRKVLAPRVWRTGCVRTRLHEQLDLYAAGVQVVLSQNRINLIKDQLKLTNDGSLIEVSHFP-EAGKKIVFGALVY--PLD  425
N. crassa        ACIRCRVLRKTCSPGEPCDTCRKVLSPRVWRSGCVRTKFSEQLDLYSAGVQIVLAQGRINTFKQAMVLSNHGLVIEACHFP-ELSSHLRLQVLQRDGIRE  460
                 :*:***:*:* *    **  *  :  .*:*   *:**:.  .:  * . :: .*        :    :      **. **         :

A. nidulans      STLDPQLQGLGDETQFQGPLQEIYLLDNDMDDVPGKIEMYIKKAGPFFYEREASDVMRPTLMLAAELAQQKKDLLLERVLELWVATHILVDADLHWKTFS  533
P. chrysogenum   SALDPQLQALGDD-QFAGPSQEVYLLDADSDDIPSKLEMYIKKNASYFYERETSPFMKPTLLLAAELNQGKKDILLERVLELWVATHILVDTELTWKTYY  522
C. immitis       SNSDPQLPLTGHDTELGAHPQNIHILDGEVEELSGKLDDYMKKVSHRFFESEESVFMQQTLLLAAELGKASQDELLAGVLDLWVATSIIVGPLVPWRVFF  496
B. maydis        T----QTTDIDPAIFSGLASPDLELIDSD-DDVSGKVDHYVKKMAGHFADTEDSHFMKATVHSAKSIASLSPDGLISKSLELWNLTRILTSRNFEWHLFS  537
S. sclerotiorum  G-------------------LTSYILDTDHDDLSNKLEAYAKRMMPIFIDNEPSQFIRTTLQTALNLSIQKQDNFLSRALEFWTIVHIFVDSEIRWEMSA  533
F. oxysporum     P----SESLAESRG-SKDSFFQVIMIDQDKEDVPGKVEAYMRDVFQLFIDREPSKFMRVTLSLALQQLQESEDDLLRKSLELWGLVESID-RERQWNVIE  534
T. reesei        L----SESQAETREEDKRPFHQVIMIDQDSEDIPAKAESYMRDVLTTFIEREPSRFMKVTLEAACQQLAKGEDDLLRKALELWGLVESID-RERQWTILE  520
N. crassa        A----DGKVTTAVPSDKPLDYPIIMVDNDTQDVPARLESYMREVLPELIKREPSNFMQVTLQTALEIASTTNDELLKKSLELWGLVEILD-RERQWTISV  555
                                         ::* : :::. : : * :     : . * * .:: *:  * .      * ::   *::*  .  :      *

A. nidulans      NPTLPP---ASYNSFAQPTDDGRIPIDEITNAESYALLCGQLRSATEKRAAALSKSVMNDLERRLLQRQQSGWFETFLVSLILLNCVERTCWLFRSWDDE  630
P. chrysogenum   NPTLPP---NSLHSLSQPSDEGRLPIEEVSDPESYGLLCSQLRAAMEKRASQLSKFVINDLERRLLQRQKCGWFDTFIVAIILLNCVDRTCWLFRSWDNE  619
C. immitis       NPTLPP---ATLQPLTSSSNGWHIPITGGDGIESYSLICSQLRAAAEKRAAKTSKAVLNRIEQRLLQRQKEGNFRTFLASLILLNCVERMSWLFYSWEHG  593
B. maydis        NPSLAPTLAPSTLSAADVDDAIRMPITPLEHASSHNLISMQLMGATEKRAACLARIVMNDLERRLLQRQQANPFETFLVAVILLACVERMCWLFRSWEAP  637
S. sclerotiorum  KAISGS-----------SDEGGGQTKPILSTERSFAIIHTQLNSAAENKASQMCKGILNELERRLLNKASKSSFELFLVAVITLNCVEKSTWLFKCWEGE  622
F. oxysporum     RPANKN-----------EEPRRIQEAKSENDADIYTTLCMQLNAAAERKANNTSKALLSGMHRVLQDSKVKVNFKMYLTAIIFLNCLEKSTWAFKAWEQD  623
T. reesei        KPSAEG-----------EEPRWIKEAQNENDADIYTMICMQLNAAAERKANSTSKSLLNHMDRLLTDSKTKVGFKIYLTAIIFLNCVEKSTWAFKAWEQD  609
N. crassa        KSTLDD-----------T---VISHIKEETHEEVFTTICLQLAAAAERKAAATSKTLLTGMQRVLQDSKVKIDFGMYFATLILLNCVEKSTWAFKAWEQP  641
                 ..                                .  :  ** .* *.:*   .: ::. :.: * :      *  ::.::* * *:::  * * .*:

A. nidulans      NFSQRWPLDKRPQYYAAQGDRFSDILHMLLKMRSLPPKANPLPDSGILKAV-EGSDENAIRWFDMIKITPLYLEQRQA-AGFDPTDSRSFDLRYGAKLLP  728
P. chrysogenum   NFSQRWPLDKRPPYYFNQSDRFADILEMLLRMRHIPPKATFRPDNGILKAV-DGSDEHAIRWFDMIQITPYFIEERKN-AVFDPSDSRSLDCHHSASLLE  717
C. immitis       EYATRWPLERRPSDFVAQGDRFSSIIATHLKMRSLAPAFLVQPDTGLLKAK-DGNDGEVIRWFEAINIDHQFLLSRQT-VGLDLSNWRSLDLKYCSTLIL  691
B. maydis        STTPRWPLDKSPSTFSQQGEKFSDILNMLLKMRGVPPKVTSMNLDGTLTVWNEDADEKVREWYSGIAVTTLMLDERAN-ARFVGDDPKEWELKFIGKIIR  736
S. sclerotiorum  DFHGQWPLDKPPQNFANQGDDLINVLQMILRMREVPPKTFVRQDNGVLAVD-DTSEQVVREFFEQVQLSFTEVHDKQVRYFFDPADSRSYELRYISRLLL  721
F. oxysporum     HLRPGWPLERDPGVFTQQGGNLAGLLKMLLAIRKALPQTLRSEA-GKLITS--EQDPVIGTYFQSIDLDYDTILARQDGSPFSPADSRSLEMILCSHLLI  720
T. reesei        HLRPGWPLERDPSVFTQQGGNLAGLLKMLLSIRKALPQTMRGED-GKLTVQ--DKDPEITEYFRTLDLDYDTIEARQKSSQFSPADSRSLELAFCSHLLL  706
N. crassa        NLRTMWPLEKDPGSFAQQGYVIADLLRMLLTIRKALPRTAHREADGKLVTE--EQDPAIKAYFEAISLN-----CKIASDTFSILG-----------LLI  723
                      ***:: *  :  *.  : .::   * :*   *        * * .     :     ::  : :       :     :   .           ::

A. nidulans      PANVYA  734
P. chrysogenum   PNNAT-  722
C. immitis       SADNLG  697
B. maydis        GD----  738
S. sclerotiorum  PQVSN-  726
F. oxysporum     TNTP--  724
T. reesei        PNSP--  710
N. crassa        LG----  725
```

**Figure S2** ClustalW alignment (http://workbench.sdsc.edu) of SonC and its orthologs. Identical (*), conserved strong groups (:), and conserved weak groups (.) are indicated. Accession numbers for the sequences are: *A. nidulans* (AN1232; *Aspergillus* Genome Database, http://www.aspgd.org/07-16-13), *Penicillium chrysogenum* (Pc16g03990), *Coccidioides immitis* (CIMG_10322), *Bipolaris maydis* (COCC4DRAFT_161203, Δ641-688), *Sclerotinia sclerotiorum* (SS1G_09964), *Fusarium oxysporum* (FOXB_15136, Δ1-67), *Trichoderma reesei* (hypothetical protein, locus EGR51802), *Neurospora crassa* (NCU07692). Sequences were modified as noted in Fig. 5C before performing the alignment.

## File S1

## Time-lapse imaging of Mag1-GFP

Video shows time-lapse imaging of Mag1-GFP during mitosis (Fig. 2B). Images were collected every 1 min and are displayed at 2 frames per second (fps). File S1 is available for download as an AVI at http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.113.156745/-/DC1.

## File S2

## Time-lapse imaging of SonC-GFP

Video shows time-lapse imaging of SonC-GFP during mitosis (Fig. 6C). Images were collected every 1 min and are displayed at 2 fps. File S2 is available for download as an AVI at http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.113.156745/-/DC1.

## File S3

### 3D rotation of SonC-GFP during mitosis

Video shows a 3D rotation over time of SonC-GFP during mitosis (Fig. 6C). Images were collected every 1 min, were rotated in $10^0$ increments, and are displayed at 25 fps. File S3 is available for download as an AVI file at http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.113.156745/-/DC1.

## File S4

### Time-lapse imaging of SonC-GFP during SAC arrest

Video shows time-lapse imaging of SonC-GFP in cells treated with 2.4 μg/ml benomyl during SAC arrest (Fig. 6D). Images were collected every 1 min and are displayed at 2 fps. File S4 is available for download as an AVI at http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.113.156745/-/DC1.

## File S5

### 3D rotation of SonC-GFP during SAC arrest

Video shows a 3D rotation over time of SonC-GFP during SAC arrest in cells treated with 2.4 μg/ml benomyl (Fig. 6D). Images were collected every 1 min, were rotated in $10^0$ increments, and are displayed at 25 fps. File S5 is available for download as an AVI at http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.113.156745/-/DC1.

## File S6

## Time-lapse imaging of SonC-GFP and histone H1-mCherry during mitosis

Video shows time-lapse imaging of SonC-GFP together with histone H1-mCherry during mitosis (Fig. 7A). Images were collected every 1 min and are displayed at 2 fps. File S6 is available for download as an AVI at http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.113.156745/-/DC1.

## File S7

## 3D rotation of SonC-GFP and histone H1-mCherry during mitosis

Video shows a 3D rotation over time of SonC-GFP and histone H1–mCherry during mitosis (Fig. 7A). Images were collected every 1 min, were rotated in $10^0$ increments, and are displayed at 25 fps. File S7 is available for download as an AVI at http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.113.156745/-/DC1.

## File S8

### SonC-GFP and histone H1-mCherry in a hooked projection

Video shows a 3D rotation over time of SonC-GFP together with histone H1-mCherry in nuclei that display a hooked structure (Fig. 7B). Images were collected every 1 min, were rotated in $10^0$ increments, and are displayed at 25 fps.

File S8 is available for download as an AVI at

http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.113.156745/-/DC1.

## File S9

## Time-lapse imaging of SonC-GFP and histone H1-mCherry in lagging chromosome arms

Video shows time-lapse imaging of SonC-GFP together with histone H1-mCherry can sometimes be seen as lagging chromosome arms following mitosis (Fig. 7C). Images were collected every 1 min and are displayed at 2 fps. File S9 is available for download as an AVI at http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.113.156745/-/DC1.

## File S10

### Time-lapse imaging of SonC-GFP and Bop1-mCherry during mitosis

Video shows time-lapse imaging of SonC-GFP together with the nucleolar marker Bop1-mCherry during mitosis (Fig. 7D). Images were collected every 1 min and are displayed at 2 frames per second (fps). File S10 is available for download as an AVI at http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.113.156745/-/DC1.

## File S11

### 3D rotation of SonC-GFP and Bop1-mCherry during mitosis

Video shows a 3D rotation over time of SonC-GFP and Bop1–mCherry during mitosis (Fig. 7D). Images were collected every 1 min, were rotated in $10^0$ increments, and are displayed at 25 fps. File S11 is available for download as an AVI at http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.113.156745/-/DC1.

## File S12

## Time-lapse imaging of SonC-GFP and Pol I-mCherry during mitosis

Video shows time-lapse imaging of SonC-GFP together with the NOR marker Pol I-mCherry during mitosis (Fig. 7E). Images were collected every 1 min and are displayed at 2 frames per second (fps). File S12 is available for download as an AVI at http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.113.156745/-/DC1.

**Table S1 Strains Used in This Study**

| NAME | GENOTYPE | SOURCE |
|---|---|---|
| C61 | *yA1 nicB8 pyroA4 niiA4 riboB2 veA1* | (Dowzer and Kelly 1991) |
| CDS36 | *sonB1 nimA1 pyrG89* wA *veA1* | (De Souza *et al.* 2003) |
| CDS364 | *sonB1 pyroA4 wA3 veA1* | (De Souza *et al.* 2006) |
| CDS509 | *sonA1 pyroA4 wA3 yA2*[1] *veA1* | This study |
| JLA1 | *nimA1 pyrG89 wA2/wA3*[1] *yA2*[1] *veA1* | This study |
| JLA73 | *nimA1* mutant D *pyrG89 wA2/wA3? yA2*[1] *veA1* | This study |
| JLA75 | *nimA1* mutant F *pyrG89 wA2/wA3*[1] *yA2*[1] *veA1* | This study |
| JLA77 | *nimA1* mutant C (*sonC1*) *pyrG89 wA2/wA3*[1] *yA2*[1] *veA1* | This study |
| JLA78 | *nimA1* mutant E *pyrG89 wA2/wA3*[1] *yA2*[1] *veA1* | This study |
| JLA184 | *nimA1* mutant 89 *pyrG89 wA2/wA3? yA2*[1] *veA1* | This study |
| JLA185 | *nimA1 sonA2 pyrG89 wA2/wA3*[1] *yA2*[1] *veA1* | This study |
| JLA227 | *sonA2 riboB2 pyroA4 yA2 wA2/wA3*[1] *veA1* | This study |
| JLA255 | *nimA1 sonA3 pyrG89 wA2/wA3*[1] *yA2*[1] *veA1* | This study |
| JLA263 | *mag1-GFP::pyrG*$^{Af}$ *pyrG89 nkuA*$\Delta$*::argB argB2 pyroA4 SE15 nirA14 chaA1 wA3 fwA1 veA1* | This study |
| JLA264 | *mag1*$\Delta$*::pyrG*$^{Af}$ *pyrG89 argB2 nirA14*[1] *wA3 chaA1*[1] *fwA1*[1] *yA2*[1] *veA1* | This study |
| JLA265 | *sonC-GFP::pyrG*$^{Af}$ *pyrG89 argB2 wA3 nirA14*[1] *yA2*[1] *chaA1*[1] *fwA1*[1] *veA1* | This study |
| JLA268 | *sonA3 wA3 yA2*[1] *veA1* | This study |
| JLA319 | *sonC-GFP::pyrG*$^{Af}$ *H1-mCherry::pyro*$^{Af}$ *pyroA4*[1] *pyrG89 argB2 wA3 yA2*[1] *chaA1*[1] *fwA1*[1] *veA1* | This study |
| JLA324 | *sonC-GFP::pyrG*$^{Af}$ *bop1-mCherry::pyrG*$^{Af}$ *pyrG89 argB2 nirA14*[1] *wA3 yA2*[1] *chaA1*[1] *fwA1*[1] *veA1* | This study |
| JLA325 | *sonC-GFP::pyrG*$^{Af}$ *pol I-mCherry::pyrG*$^{Af}$ *pyrG89 argB2 nirA14*[1] *wA3 yA2*[1] *chaA1*[1] *fwA1 veA1* | This study |
| KF018 | *nimA-GFP::pyrG*$^{AF}$ *H1-mCherry::pyro*$^{AF}$ *nirA14*[1] *sE15*[1] *wA3 veA1* | This study |

| | | |
|---|---|---|
| KF110 | *nimA*-GFP*::pyrG*$^{AF}$ *nup170-mCherry::pyroA*$^{AF}$ $\Delta$*nKuA::argB argB2 wA3 veA1* | This study |
| KF120 | *nimA-GFP::pyrG*$^{AF}$ *pyrG89 fib-mCherry::pyrG*$^{AF}$ *wA3 veA1* | This study |
| KF122 | *nimA*-GFP*::pyrG*$^{AF}$ *pyrG89 topo1-mCherry::pyrG*$^{AF}$ *pyroA4 argB2 wA3 veA1* | This study |
| LPW29 | *nimA1 sonA1 riboA1/riboB2*[1] *pyrG89 wA2 veA1* | (Wu *et al.* 1998) |
| LU178 | *cgrA-GFP::pyrG*$^{AF}$ *H1-mCherry::pyrG*$^{AF}$ *pyrG89 argB2 wA3 nirA14*[1] *fwaA1*[1] *chaA1*[1] *veA1* | (Ukil *et al.* 2009) |
| LU193 | *bop1-GFP::pyrG*$^{AF}$ *H1-mCherry::pyrG*$^{AF}$ *pyrG89 argB2 wA3 SE15 pabaA1 pyroA4 nirA14*[1] *fwaA1*[1] *chaA1*[1] *veA1* | (Ukil *et al.* 2009) |
| R153 | *pyroA4 wA3 veA1* | C. F. Roberts |
| SO369 | *sonA1 pyrG89 wA2 nicA2 veA1* | This study |

[1] In some strains we have not confirmed some markers that could be covered by or are recessive to other markers in the strain.

**Table S2 Primers Used in This Study**

| Name | Purpose | Sequence |
|---|---|---|
| 3’sonBcDNA | *sonB* amplification | GCTCGAGTCAGATCTCAGTA |
| 5’UTRsonB | *sonB* amplification | ACGGATGGACGTTGATAACATACTG |
| AF-2 | *nimA* sequencing | GGTGTCACTATGGTACG |
| AF-4 | *nimA* sequencing | GTCATTGCGAGCTGCCT |
| AF-6 | *nimA* sequencing | CTTGAGTCGCCGACGAA |
| AF-7 | *nimA* sequencing | AAGATCCCATCGTCCGC |
| AR-2 | *nimA* amplification | GCAGCAGCGCAAGAAAT |
| HP15 | *nimA* GFP-tagging | GAACACCATTCTACATGTCTCCAG |
| HP16 | *nimA* GFP-tagging | CCTCAAGTTGCGAATCACCTTTC |
| HP17 | *nimA* GFP-tagging | TGAAAACGCAAGATCGTCCTAACC |
| HP18 | *nimA* GFP-tagging | TGGCGTACGAGCGCTTCAAAACTG |
| HP19 | *nimA* GFP-tagging | GGGGAAGAAAGGTGATTCGCAACTTGAGGGGAGCTGGTGCAGGCGCTGGAGCCAAAG |
| HP20 | *nimA* GFP-tagging | ACCGCCGGTTAGGACGATCTTGCGTTTTCACTGTCTGAGAGGAGGCACTGATGCGTG |
| M13 Forward | pRG3-AMA1-*Not*I plasmid library sequencing | CTGGCCGTCGTTTTAC |
| NucP1 | *sonB* sequencing | TATTGTAAGGCTGCATGAAC |
| NucP2 | *sonB* sequencing | AACACTGGCAGCTCACTCTT |
| NucP4 | *sonB* sequencing | GGAGTGGACGACGACACATT |
| NucP8 | *sonB* sequencing | AACAACCGCGCAAACAAGGA |
| NucP11 | *sonB* sequencing | GTCCGCAAGCAAGGTCAGAA |
| NucP12 | *sonB* sequencing | CCTCAGATCGTGAAAGGGCT |
| NucP15 | *sonB* sequencing | GCAGTGGCTCAGGCTTTGGT |
| NucP16 | *sonB* sequencing | GGCATCGTTCCAGTTCTTCC |
| NucP17 | *sonB* sequencing | TTGGTGGATGCCTTGGTTAC |
| NucP20 | *sonB* sequencing | ATGGAAGTGGGCAGGCAGGA |

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|---|---|---|
| NucP21 | *sonB* sequencing | ATTGCTGAGTGTTGCTGCCA |
| NucP23 | *sonB* sequencing | GGCACAGCTGCTACTTCGTC |
| NucP24 | *sonB* sequencing | ACGTGCTCCTCTGCCAATCT |
| NucP26 | *sonB* sequencing | CGGCCAGTGTACCCACACAA |
| NucP27 | *sonB* sequencing | GGCATTACCGCTGATGAACC |
| NucP28 | *sonB* sequencing | GGCCACCTTGGACGGATTCT |
| NucP32 | *sonB* sequencing | CCTGCGAAGGTCTTTGGCTG |
| NucP34 | *sonB* sequencing | ACTCGCTACGACCGATCCTC |
| oMN33 | pRG3-AMA1-*Not*I plasmid library sequencing | AAATAAGCTTGCATGCGC |
| SP204-F | AN4389 amplification | CAAGCATTGACCGATGACACT |
| SP205-R | AN4389 amplification | GCTCTCATTAAGTCAATCCAGCAT |
| SP206-F | AN4389 sequencing | CCGGATTCTGATTGCTAAGGC |
| SP207-F | AN4389 sequencing | GTGGACAACCGAGAATCTCA |
| SP209-F | AN4389 sequencing | CGACAACTGGCACGCTTC |
| SP233-F | *nimA* sequencing | CGACTACTAACCTGCAGA |
| SP234-F | *nimA* amplification | CCCGTGCATTGATATTGACTCC |
| SP251 | *sonC* amplification and deletion | CACCAGAGCACCGAAATTGC |
| SP252 | *sonC* deletion and GFP-tagging | CCAGGTCGAGAATTGTCCTTG |
| SP253-F | *sonC* sequencing | CGGAGCGATAAATCTACGTCT |
| SP254-F | *sonC* sequencing | GGACTAAATGTGCTGGCCG |
| SP255-F | *sonC* amplification, GFP-tagging, and sequencing | CTGCATGGAACCAACAGCT |
| SP256-F | *sonC* sequencing | GATCTGCATGCGACGTTAGC |
| SP257-F | *sonC* sequencing | CGCAGCCTACAGATGACGG |
| SP258-F | *sonC* deletion and GFP-tagging | GCATCAGTGCCTCCTCTCAGACAGATGGCAACAGTTGTGTGAAGTATGC |
| SP259-R | *sonC* deletion | GAAGAGCATTGTTTGAGGCGGACGAGATCGCGTGGCAGAATATAT |
| SP260-R | *sonC* GFP-tagging | GGCTCCAGCGCCTGCACCAGCTCCCGCATAGACATTTGCAGGCGGCAGC |

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|---|---|---|
| SPF1 | *sonA* amplification and sequencing | GCTCTTGATACCCGTCTCTC |
| SPF2 | *sonA* sequencing | GTACATCAATATAGTCGACCT |
| SPF5 | *sonA* sequencing | CTGCTGCTGGTCACCTGTAAG |
| SPR5 | *sonA* amplification and sequencing | CGTGACTGTCTATACGTGCG |
| SPR8 | *sonA* sequencing | CGAGATCGAGCATACGAGCC |