

Supplementary Information for:

**Bub1 kinase- and H2A phosphorylation-independent regulation of Shugoshin proteins under glucose-restricted conditions**

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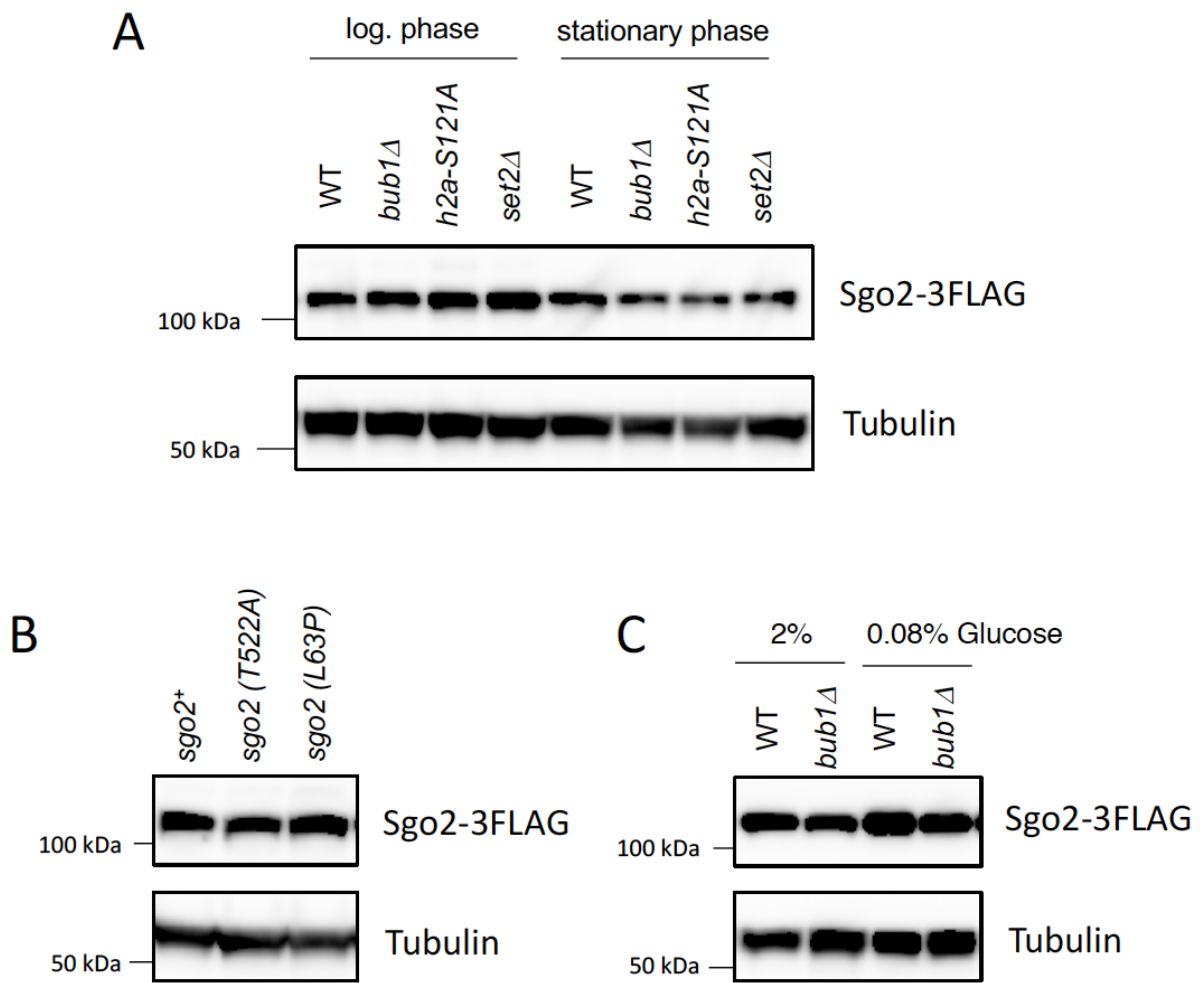
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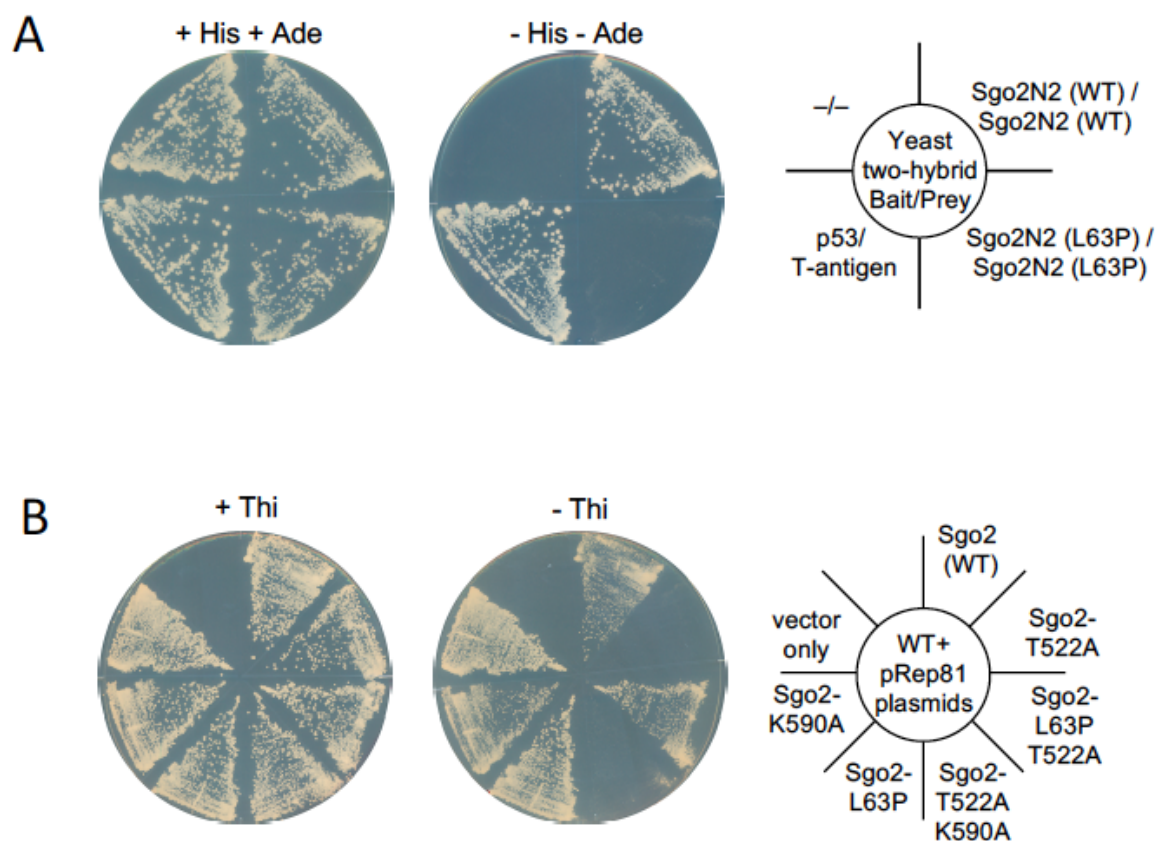
Email: [skawashima@mol.f.u-tokyo.ac.jp](mailto:skawashima@mol.f.u-tokyo.ac.jp)

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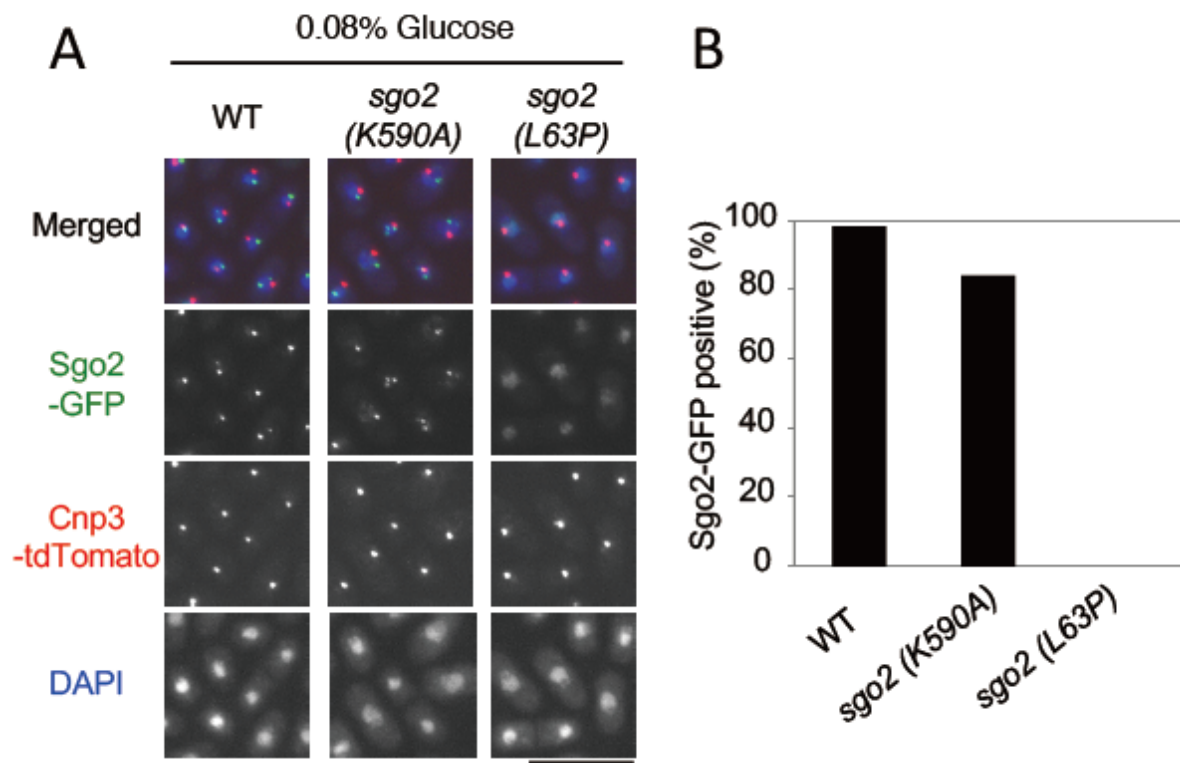
1. Supplementary Figures
2. Supplementary Tables



**Figure S1.** The level of Sgo2-3FLAG in the indicated strains was estimated by western blotting with anti-FLAG antibody. Tubulin is the loading control. (A) log-phase vs stationary phase in YE medium. (B) log-phase in YE medium. (C) EMM medium with 2% or 0.08 % glucose.



**Figure S2.** Additional analysis of Sgo2 mutants. (A) Yeast two-hybrid assay for dimerization of the Sgo2N2 peptide (aa1–206). The p53/T-antigen was used as a positive control. (B) Cells in which wild-type Sgo2 (WT), the indicated Sgo2 mutant, or vector control (pRep81) were overexpressed from the nmt81 promoter were streaked on plates. Thiamine (Thi) addition represses transcription from the nmt81 promoter.



**Figure S3.** Analysis of Sgo2 mutants under glucose-restricted conditions. (A–B) Sgo2-GFP signals in WT, *sgo2-L63P*, or *sgo2-T522A* cells at the log phase in medium containing 0.08% glucose were examined. (A) Representative images are shown. Cnp3-tdTomato signals indicate kinetochores. DNA was stained with DAPI. Scale bar, 10  $\mu$ m. (B) The percentage of cells showing Sgo2-GFP signals were analyzed (n >200).

**Table S1. Strain list**

|            |         |          |                                                      |
|------------|---------|----------|------------------------------------------------------|
| Fig. 1     | SAK856  | h-       | cnp3-tdtomato<<kanr sgo2-GFP<<hygr                   |
|            | SAK885  | h-       | cnp3-tdtomato<<kanr sgo2-GFP<<hygr bub1::natr        |
| Fig. 2B, C | SAK3672 | h-       | sgo2-3flag<<kanr                                     |
|            | SAK3745 | h-       | ade6 leu1 bub1::ura4+ sgo2-3flag<<kanr               |
|            | SAK3746 | h-       | ade6 leu1 hta1(S121A) hta2(S121A) sgo2-3flag<<kanr   |
| Fig. 2D    | SAK3672 | h-       | sgo2-3flag<<kanr                                     |
| Fig. 2E, F | SAK3755 | h90      |                                                      |
|            | SAK3758 | h90      | bub1::natr                                           |
|            | SAK3768 | h90      | sgo2::bsdr bub1::natr                                |
|            | SAK3769 | h90      | sgo2::bsdr                                           |
| Fig. 3     | SAK3672 | h-       | sgo2-3flag<<kanr                                     |
|            | SAK3824 | h-       | sgo2-3flag<<kanr set2::natr                          |
| Fig. 4B, C | SAK856  | h-       | cnp3-tdtomato<<kanr sgo2-GFP<<hygr                   |
|            | SAK885  | h-       | cnp3-tdtomato<<kanr sgo2-GFP<<hygr bub1::natr        |
|            | SAK3843 | h90      | cnp3-tdtomato<<kanr sgo2(590KA)-GFP<<hygr            |
|            | SAK3847 | h+ or h- | cnp3-tdtomato<<kanr bub1::natr sgo2(590KA)-GFP<<hygr |
| Fig. 4D, E | SAK856  | h-       | cnp3-tdtomato<<kanr sgo2-GFP<<hygr                   |
|            | SAK3840 | h90      | cnp3-tdtomato<<kanr sgo2(522TA)-GFP<<hygr            |
|            | SAK3848 | h90      | cnp3-tdtomato<<kanr sgo2(63LP)-GFP<<hygr             |
| Fig. 4F, G | SAK3672 | h-       | sgo2-3flag<<kanr                                     |
|            | SAK3794 | h+       | sgo2(522TA)-3flag<<kanr                              |
|            | SAK3797 | h+       | sgo2(63LP)-3flag<<kanr                               |
| Fig. 5     | SAK856  | h-       | cnp3-tdtomato<<kanr sgo2-GFP<<hygr                   |
|            | SAK885  | h-       | cnp3-tdtomato<<kanr sgo2-GFP<<hygr bub1::natr        |
| Fig. S1A   | SAK3672 | h-       | sgo2-3flag<<kanr                                     |
|            | SAK3745 | h-       | ade6 leu1 bub1::ura4+ sgo2-3flag<<kanr               |
|            | SAK3746 | h-       | ade6 leu1 hta1(S121A) hta2(S121A) sgo2-3flag<<kanr   |
|            | SAK3824 | h-       | sgo2-3flag<<kanr set2::natr                          |
| Fig. S1B   | SAK3672 | h-       | sgo2-3flag<<kanr                                     |
|            | SAK3794 | h+       | sgo2(522TA)-3flag<<kanr                              |
|            | SAK3797 | h+       | sgo2(63LP)-3flag<<kanr                               |
| Fig. S1C   | SAK3672 | h-       | sgo2-3flag<<kanr                                     |
|            | SAK3972 | h        | sgo2-3flag<<kanr bub1::natr c::Padh1-ade6<<bsdr      |

|          |         |    |                                           |
|----------|---------|----|-------------------------------------------|
| Fig. S2B | SAK3905 | h- | leu1 + pRep81-Sgo2(WT)                    |
|          | SAK3906 | h- | leu1 + pRep81-Sgo2(522TA)                 |
|          | SAK3907 | h- | leu1 +pRep81-Sgo2(522TA 63LP)             |
|          | SAK3908 | h- | leu1 +pRep81-Sgo2(522TA 590KA)            |
|          | SAK3909 | h- | leu1 +pRep81-Sgo2(63LP)                   |
|          | SAK3910 | h- | leu1 +pRep81-Sgo2(590KA)                  |
|          | SAK3911 | h- | leu1 +pRep81                              |
| Fig. S3  | SAK856  | h- | cnp3-tdtomato<<kanr sgo2-GFP<<hygr        |
|          | SAK3912 | h- | cnp3-tdtomato<<kanr sgo2(522TA)-GFP<<hygr |
|          | SAK3914 | h- | cnp3-tdtomato<<kanr sgo2(63LP)-GFP<<hygr  |

**Table S2. Primer list for ChIP**

|                | forward              | reverse               |
|----------------|----------------------|-----------------------|
| <i>subtel</i>  | ACTCGCAACTGCTTTTTATG | TGTACGTTTCAAAGCAATGT  |
| <i>ostel-1</i> | AAATCCGAAGTATGAGACAA | AAACATCCTTGTTTTTAACG  |
| <i>ostel-2</i> | ATATTCATCGTCCAAGTAAA | CAAAGTTTTGATACCCTGAT  |
| <i>euch-1</i>  | AACGATTACATCTACGTCT  | ATCAAAAGGACTATCTTAGA  |
| <i>dg</i>      | TTTTCAGCGAGACATGTACC | TCATAAAGCAACACTGGGTG  |
| <i>dh</i>      | GTAAGTATGAGCAACTGGCG | GGAACAAATCAGGAAACCGAG |

**Table S3 Primer list for RT-qPCR**

|              | forward              | reverse              |
|--------------|----------------------|----------------------|
| <i>gal1</i>  | GCGCCTTGTTTTGCTCTCC  | ACGGGCGGTATGGATCAAT  |
| <i>gal10</i> | AAGCAGTTGGGGAATCAATG | ACTGTCTTGACCCGGTGTTG |
| <i>gal7</i>  | GATTTGGCTTCCGCATTAAA | AGCTTGGTGAAGACCCATTG |
| <i>pan5</i>  | GTGGCCGGAAGAACAAAATA | TGGGATTCTTTGGGACTTTG |