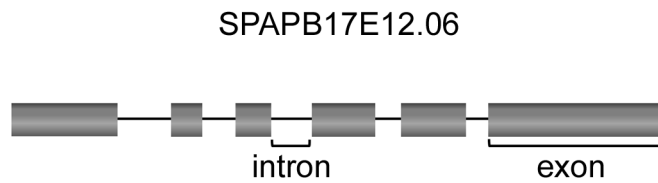


A

c-DNA (Bp)
1-174
264-314
369-436
497-598
641-747
786-1079

B

```

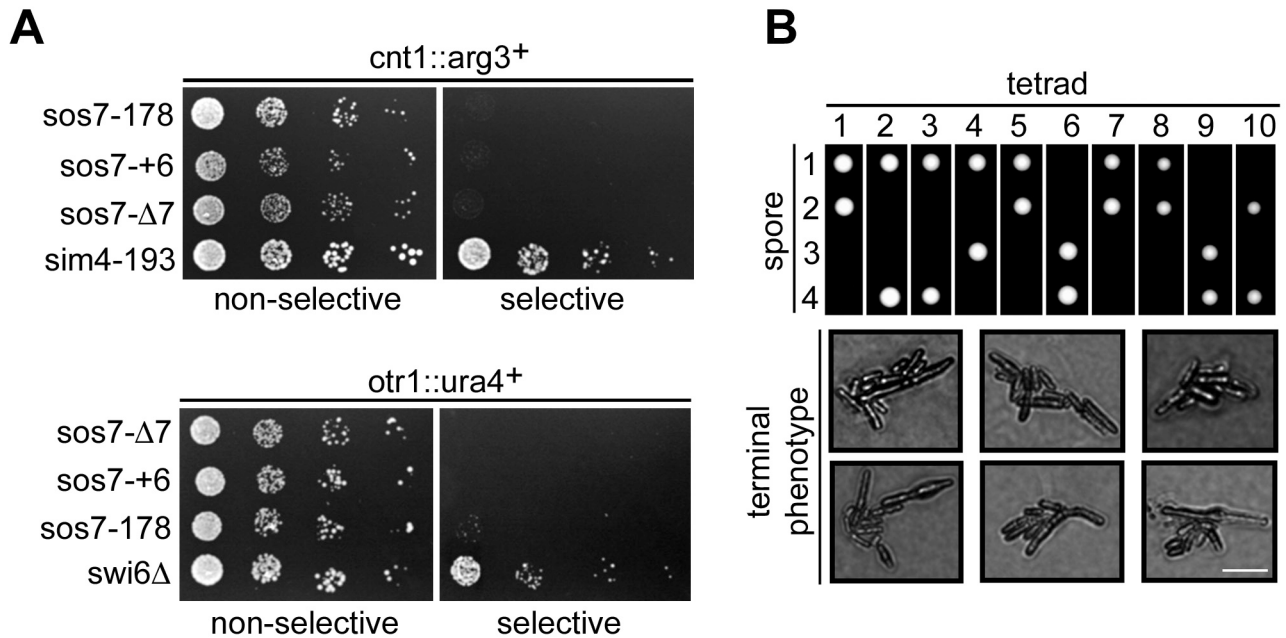
1 MDQNKSTQAT GNGKKTEEIN ELLESFIKEG PKLLWGSTNL KYEDQISRSS 50
51 EHELQQYREL FTRLKFSYIE QGTKERYLRA ILDDPPMLVE AEDNEKLETT 100
101 NSSLKGR LKS EKREVDLLTE ELKTTSRELS SNYESVMEEC KNTKSTLSKL 150
151 ESLESELLKL QQDSSTKTPI LPEVEAAIHD LESELNITNE SIETIDGKID 200
      ⋮
      N (Sos7-178)
201 NDEKYFIQLT KNLSLLEKEY KIASERSNQI KAAIHTRTPD ADAKKQVQNW 250

251 YTSMLEIYDQ LLQK IMRRYR 264
      |         |
      | deleted | additional |
      |         |
      Sos7-Δ7  Sos7-+6

```

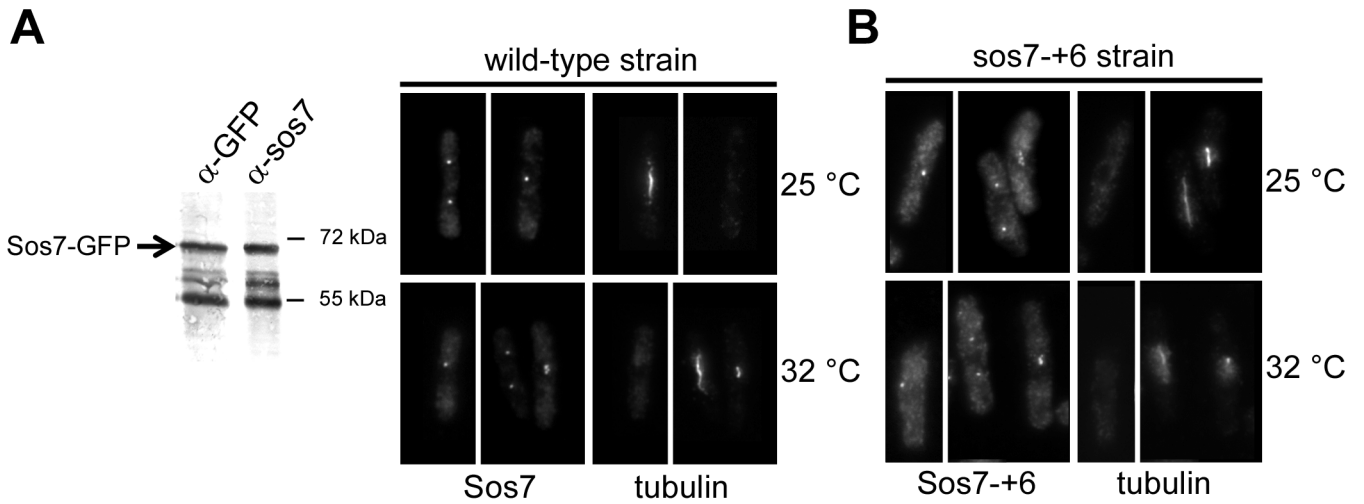
Supplementary Figure S1

(A) Diagrammatic representation of the *sos7*⁺ (systematic name SPAPB17E12.06) gene showing intron and exon regions. The exon regions were confirmed by our cDNA. (B) Amino acid sequence of Sos7. The amino acid alterations of the 3 Sos7 variants are indicated.



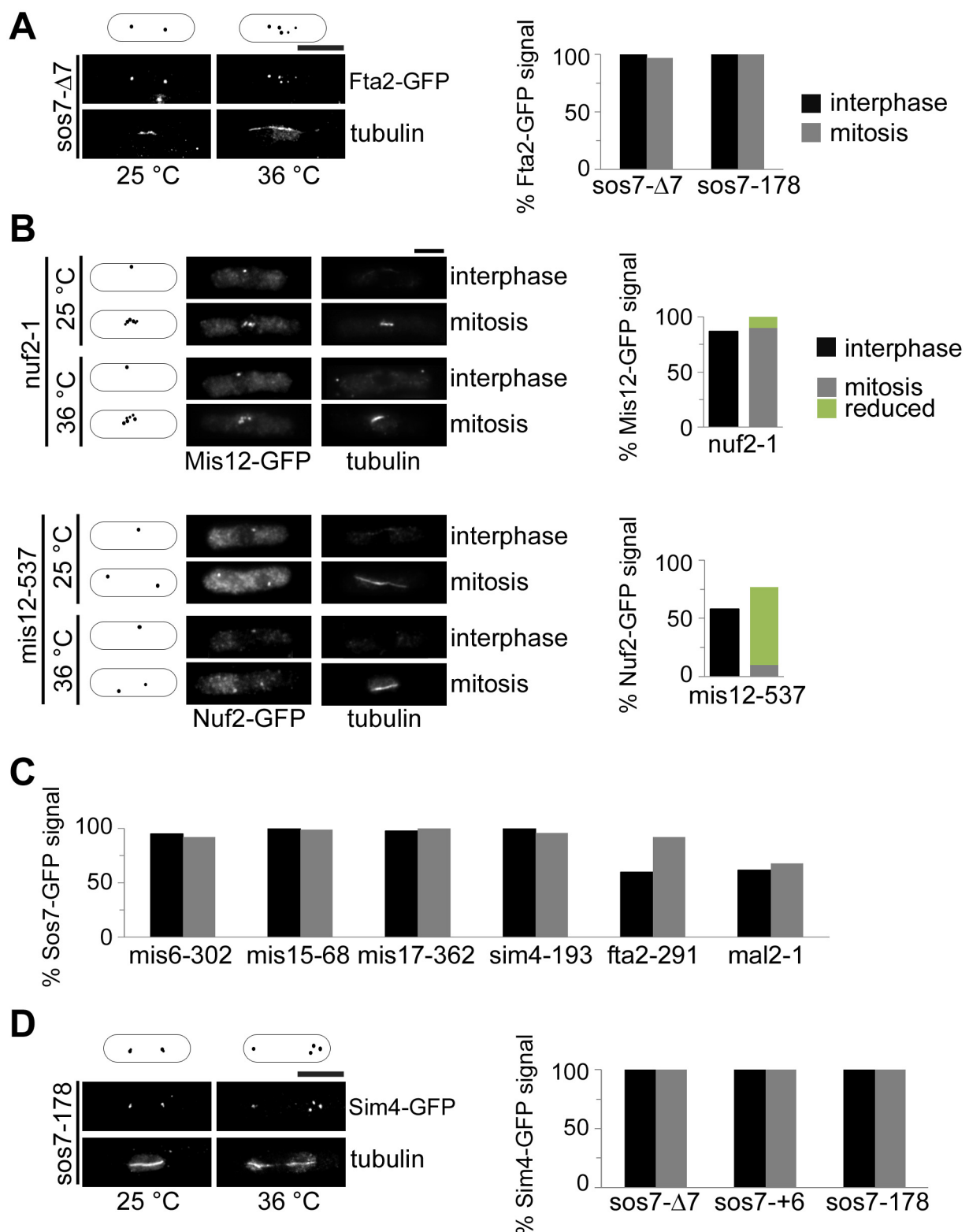
Supplementary Figure S2

Sos7 is an essential protein and not required for centromere silencing. (A) Shown are serial dilution patch tests (10^4 - 10^1 cells) of the indicated *sos7^{ts}* strains that have the *arg3⁺* gene inserted at *cnt* of chromosome I or the *ura4⁺* gene inserted at *otr1*. Alleviation of silencing leads to growth on selective arginine-minus plates or selective uracil-minus plates as shown for the positive control strain *sim4-193* or *swi6Δ*, respectively. Plates were incubated for 5 d at 25 °C. (B) Tetrad analysis of a heterozygous diploid *sos7⁺/Δsos7::his3⁺* strain revealed that *sos7⁺* is essential for growth. Only 2 spores per tetrad were able to form colonies (top panel) and these were *his⁻*. *Δsos7* spores germinated; cells divided up to 3 times and then arrested with an elongated phenotype (bottom panel). Bar, 10 μm.



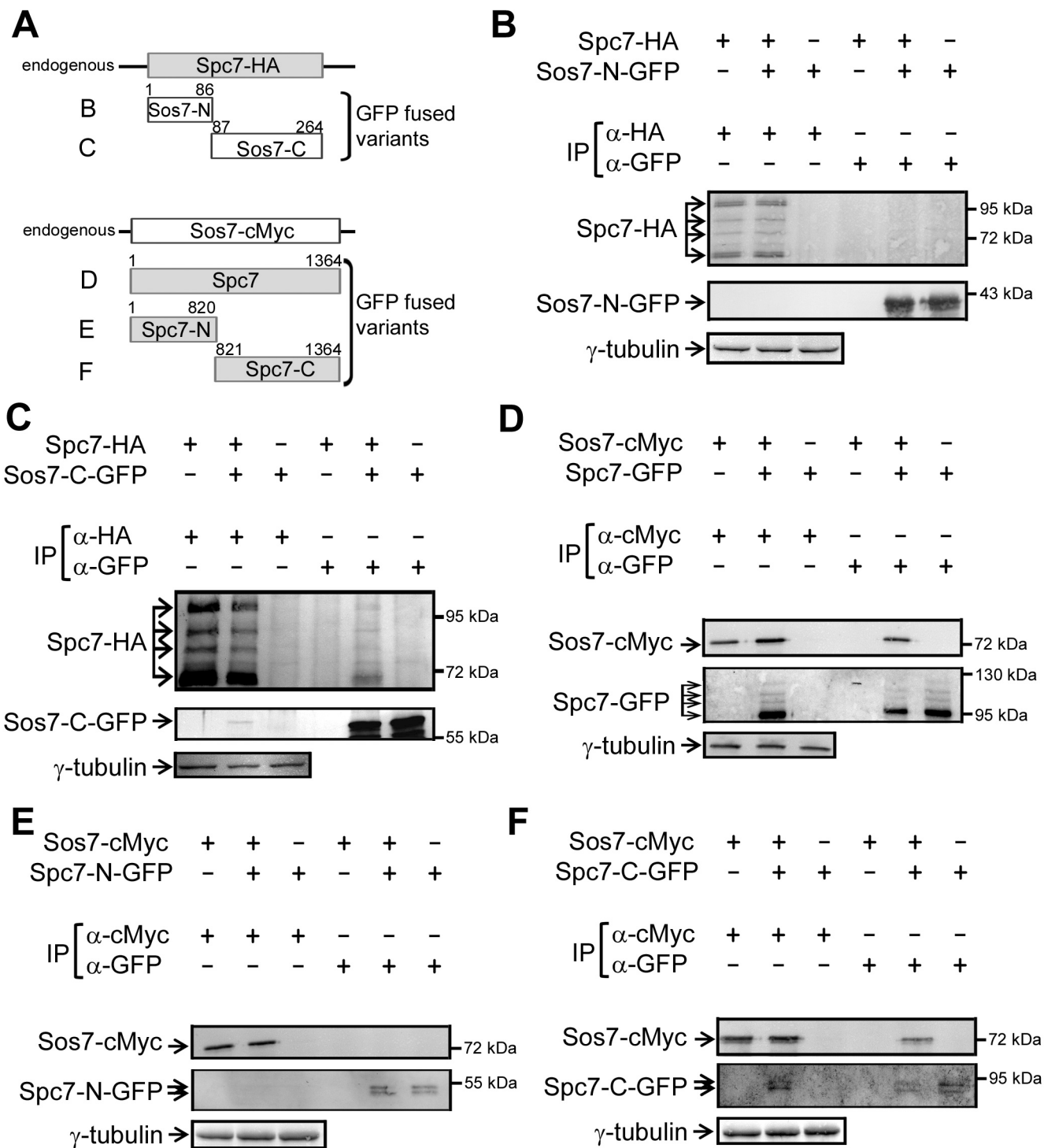
Supplementary Figure S3

Immunofluorescence analysis using our polyclonal Sos7 antibody. (A) Left: A protein extract prepared from the Sos7-GFP strain was used for immunoprecipitation using anti-GFP antibody followed by western blotting with an anti-GFP (left panel) or the anti-Sos7 antibody (right panel). The 58 kDa Sos7 fusion protein runs at approximately 67 kDa. Right: Photomicrographs of fixed wild-type cells grown at the indicated temperatures followed by incubation with the anti-Sos7 antibody and the anti-tubulin antibody TAT-1. Fixation did not preserve interphase microtubules. (B) Photomicrographs of fixed *sos7-+6* cells incubated at the indicated temperatures followed by incubation with the anti-Sos7-antibody and the anti-tubulin antibody TAT-1.



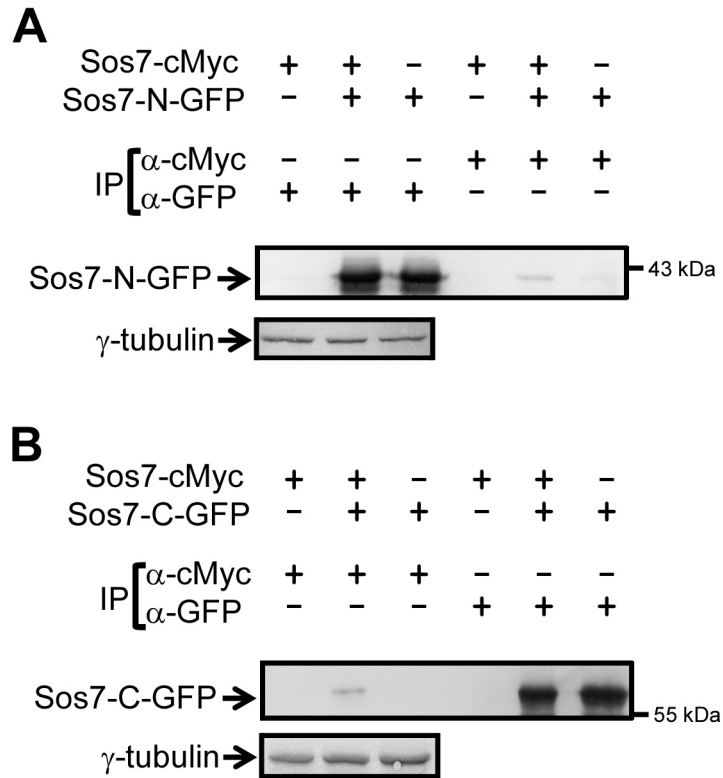
Supplementary Figure S4

Localization of certain kinetochore proteins in specific kinetochore mutants. (A) Fta2-GFP localization in *sos7^{ts}* strains grown at 25 °C or incubated for 6 h at 36 °C. Left: photomicrographs of fixed *sos7-Δ7 fta2-gfp* mitotic cells incubated at the indicated temperatures. Cells were incubated with anti-GFP antibody and the anti-tubulin antibody TAT-1. Diagrams above photomicrographs show position of GFP-signal. Right: Diagrammatic representation of Fta2-GFP kinetochore localization in the indicated strains incubated at the restrictive temperature. N/strain= 100. (B) Kinetochore localization of Mis12-GFP in the *nuf2-1* strain and Nuf2-GFP in *mis12-537* strain was analyzed in interphase and mitotic cells. Paraformaldehyde-fixed cells were incubated with anti-GFP antibody and the anti-tubulin antibody TAT-1. Diagram: The kinetochore signals were analyzed in the indicated strains incubated at the restrictive temperature. N/strain= 100. (C) Diagrammatic representation of Sos7-GFP kinetochore localization in the indicated strains. The Sos7-GFP signal was analyzed in strains incubated for 6 h at the restrictive temperature. N/strain= 100. (D) Sim4-GFP localization in *sos7^{ts}* strains grown at 25 °C or incubated for 6 h at 36 °C. The analysis was carried out as described in (A).



Supplementary Figure S5

Co-immunoprecipitation of Sos7 and Spc7 variants. This figure shows all western blot data that were summarized diagrammatically in Figure 5A. (A) Diagrammatic representation of the Spc7 and Sos7 variants used. GFP-tagged variants were over-expressed via the *nmt1*⁺ promoter. Loading control, γ -tubulin. (B) Protein extracts from strains expressing endogenous Spc7-HA or Spc7 and transformed with a vector control (depicted as Sos7-N-GFP -) or a plasmid expressing Sos7-N-GFP (depicted as Sos7-N-GFP +) were incubated with anti-HA antibody (depicted as +) or anti-GFP antibody (depicted as +). Co-immunoprecipitates were split in two and analyzed by western blot analysis using anti-HA or anti-GFP antibodies. Sos7-N-GFP (predicted size 38 kDa) runs at approximately 40 kDa. (C) Experimental set-up as described in (B) but this time the strains were transformed with a plasmid expressing Sos7-C-GFP. Sos7-C-GFP (runs at 60 kDa, predicted size 48 kDa). (D) Protein extracts from strains expressing endogenous Sos7-cMyc or Sos7 and transformed with a vector control or a plasmid expressing Spc7-GFP were incubated with anti-cMyc antibody or anti-GFP antibody. Co-immunoprecipitates were split in two and analyzed by western blot analysis using anti-cMyc or anti-GFP antibodies. Sos7-cMyc (predicted size 51 kDa) runs at approximately 72 kDa. (E) Experimental set-up as described in (D) but this time the strains were transformed with a plasmid expressing Spc7-N-GFP. (F) Experimental set-up as described in (D) but this time the strains were transformed with a plasmid expressing Spc7-C-GFP (run at predicted size 90 kDa).



Supplementary Figure 6

Co-immunoprecipitation of tagged Sos7 variants. This figure shows the western blot data that were summarized diagrammatically in Figure 5C. (A) Protein extracts from strains expressing endogenous Sos7-cMyc or Sos7 transformed with a vector control (depicted as Sos7-N-GFP -) or a plasmid expressing Sos7-N-GFP (depicted as Sos7-N-GFP +) were incubated with anti-cMyc antibody (depicted as +) or anti-GFP antibody (depicted as +). Co-immunoprecipitates were analyzed by western blot analysis using anti-GFP antibody. (B) Experimental set-up as described in (A) but this time the strains were transformed with a plasmid expressing Sos7-C-GFP.