Xenopus Shugoshin 2 regulates the spindle assembly pathway mediated by the chromosomal passenger complex

Teresa Rivera[1*], Cristina Gheniou[2,3], Miriam Rodríguez-Corsino[1], Satoru Mochida[4], Hironori Funabiki[2] and Ana Losada[1]

Supplementary Figure Legends

Supplementary Figure 1. Characterization of XSgo2. (A) Sequence alignment of *H. sapiens* Sgo2 (hSgo2), *M. musculus* Sgo2 (mSgo2) and *X. laevis* Sgo2 (XSgo2). Identical and similar residues appear in black and grey, respectively. Serine or Threonine residues within a putative Aurora B phosphorylation motif (R/K-X-S/T) are boxed in red for hSgo2/mSgo2 and blue for XSgo2. The red arrowhead points to the only site common to the three proteins whereas orange arrowheads indicate conservation between human and mouse Sgo2. (B) Immunoblot analysis of mitotic and interphase egg extracts with an affinity-purified rabbit polyclonal antibody against XSgo2 (left) and Coomassie Brilliant Blue-stained gel of immunoprecipitates obtained with the same antibody or RbIgG as control (right). (C) Immunoblot analysis of increasing amounts of a mock depleted extract (expressed as percentage of a 1-μl aliquot, lanes 1-5), and 1-μl aliquots of an extract depleted of XSgo2 (lane 6, Δ XSgo2) or depleted of XSgo2 and reconstituted with 0.2 mg/ml of XSgo2 mRNA (lane 7, Δ XSgo2 + XSgo2 mRNA). Tubulin was used as a loading control.

Supplementary Figure 2. XSgo1 and XSgo2 bind to microtubules. We performed a microtubule pelleting assay to assess the ability of XSgo proteins to bind to microtubules. Frozen mitotic extracts were thawed, diluted, precleared, incubated with 10 μM taxol or 33 μM nocodazole for 30 min and pelleted through a sucrose cushion as

previously described (Tseng et al., 2010). The pellets were analyzed by immunoblot with the indicated antibodies. XKid and Asf1 are the positive and negative controls for microtubule binding, respectively. Both XSgo1 and XSgo2 were detected in the microtubule pellet in the presence of taxol (lanes 3 and 4), but not if microtubule polymerization was prevented by nocodazole (lanes 5 and 6). An exogenously added XSgo2 protein (GFP-XSgo2) behaved as the endogenous in this assay.

Supplementary Figure 3. XSgo2 does not regulate bipolar spindle length. (A) Quantitation of the average spindle length measured as the distance between the two spindle poles of bipolar spindles assembled in Mock, XSgo1, XSgo2 or XSgo1 and XSgo2 depleted extracts. 50 spindles were measured in each condition from three independent experiments. Error bars, SEM. No significant differences were found. (B) Spindles assembled in cycled extracts containing Rhodamine-tubulin (red) that had been depleted as indicated were fixed and analyzed for immunofluorescence with antibodies against NuMa (green). DNA was counterstained with DAPI (blue). Scale bar, 10 μm. Localization of NuMa at spindle poles was observed in all conditions.

Supplementary Figure 4. XSgo2 regulates MCAK and MCAK pS196 targeting to centromeres independently of the presence of microtubules or XSgo1, respectively. (A) Mitotic chromosomes were assembled in Mock or Δ XSgo2 extracts in the presence or in the absence of nocodazole and immunostained with anti-MCAK (green), anti-CENPC (red) and DAPI (blue). MCAK accumulates at the centromeric regions labelled by CENPC in mock, but not in the absence of XSgo2, independently of the presence of microtubules. The fluorescence intensity of MCAK staining at individual centromeres is plotted on the right. Data come from n>10 nuclei per condition from a representative experiment. Mean intensity, red line. (B) Mitotic chromosomes were assembled in Mock, Δ XSgo1, Δ XSgo2 or Δ XSgo1+Δ XSgo2 extracts cycled through interphase and immunostained with anti-MCAKpS196 (red), anti-CENPC (green) and DAPI (blue). Phosphorylation of MCAK at S196 is defective in the absence of XSgo2 and is not rescued by double depletion of XSgo1 and XSgo2. The fluorescence intensity of MCAKpS196 staining at individual centromeres is plotted on the right. Data come from n>10 nuclei per condition from a representative experiment. Mean intensity, red line. Scale bar, 10 μm.

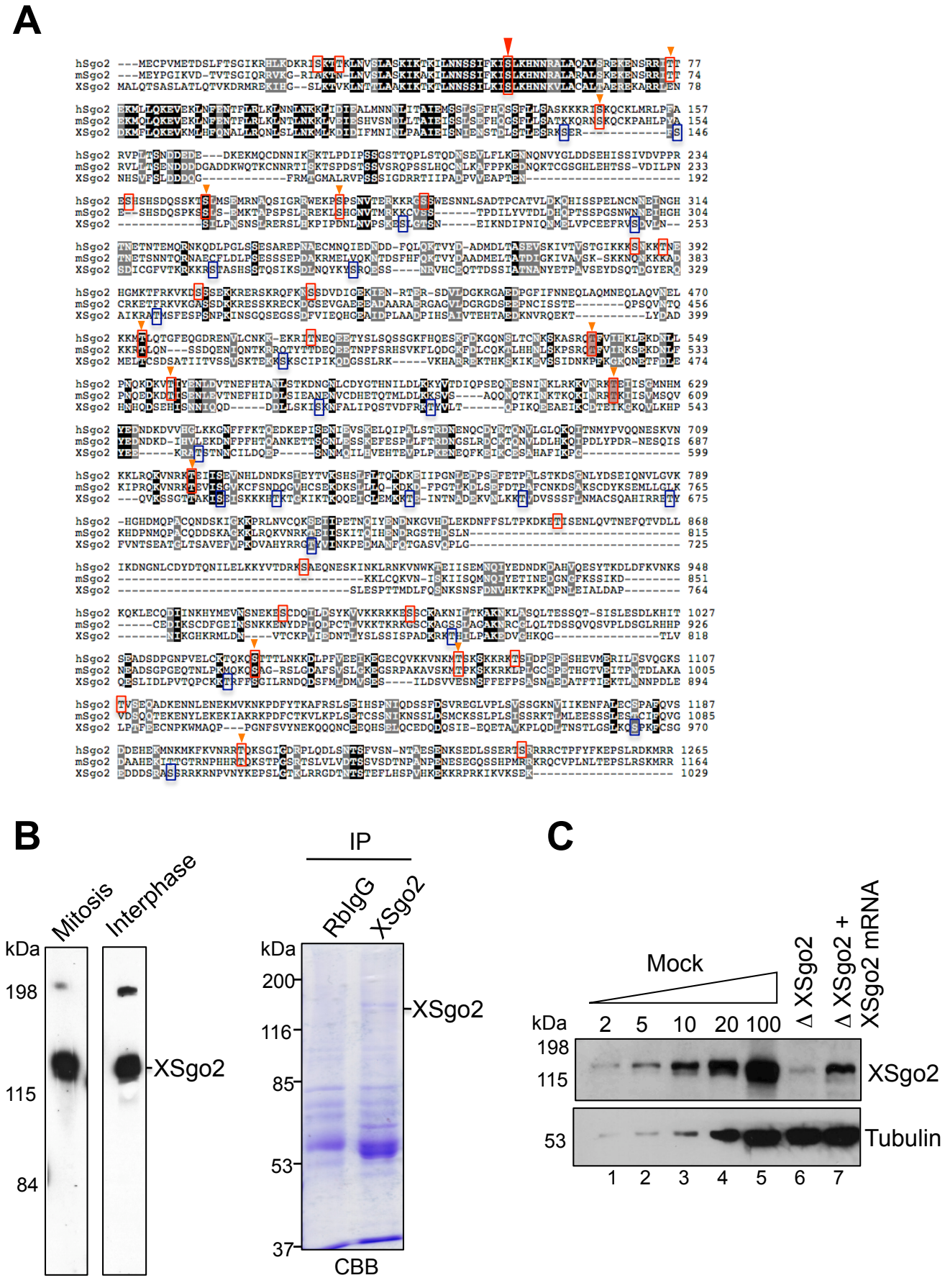
Supplementary Figure 5. XSgo2 regulates neither assembly nor activity of the soluble CPC in the extract. Quantitative immunoblot of aliquots from Δ XSgo1 or Δ XSgo2 extracts were analyzed alongside different amounts of the mock depleted extract (expressed as percentage of 1 μl). Aurora B levels were analyzed in each condition. No change in Aurora B levels can be observed in the XSgo2-depleted extracts. (B) Mitotic chromosomes were assembled in mock, Δ XSgo1 or Δ XSgo2 extracts and immunostained with anti-INCENP (green) and CENPA (red) antibodies. Blue, DAPI. Scale bar, 10 μm. (C) The CPC was immunoprecipitated with anti-Aurora B from mock, Δ XSgo1 or Δ XSgo2 extracts (lanes 3-5) and analyzed by immunoblotting. A control immunoprecipitation reaction with RbIgG was performed in mock depleted extracts (lane 2). All the components of the complex were present in all the conditions. Input represents 1% of the reaction (lane 1). (D) Mock, Δ XSgo1, or Δ XSgo2 mitotic extracts were incubated with γ-^{32}P-ATP for 1 h and then the CPC was purified from these extracts using Aurora B antibody bound to beads. A control immunoprecipitation reaction was carried out from mock depleted extracts also containing γ-^{32}P-ATP using Rb IgG. The isolated proteins were separated by SDS-PAGE and analyzed by Coomassie staining (CBB) and autoradiography. Arrowheads indicate the position of INCENP, Aurora B, Dasra A and Survivin from top to bottom. Radioactive signals in the bands corresponding to INCENP were quantified in eight independent experiments, normalized against the amount of protein (according to CBB staining) and expressed as a percentage of the signal in the mock depleted extract. Error bars, SEM. No significant differences were found, indicating that XSgo2 does not regulate the activity of the soluble CPC present in the extract.

Supplementary Figure 6. Depletion of PP2A-B56γ does not affect XSgo2 localization or function at centromeres. Mitotic chromosomes were assembled in mock and Δ B56γ extracts and immunostained with antibodies against XSgo1 (red) and MCAK (green) in (A) and against XSgo1 (green) and CENPC (red) in (B). Blue, DAPI. Scale bar, 10 μm. Targeting of XSgo1 is dramatically decreased, consistent with the fact that this factor is removed from the extract along with PP2A-B56γ. In contrast, the centromeric localization of XSgo2 and its function, at least regarding MCAK recruitment, are not affected under this condition.

Protein Name	peptides	Score	MW	# Aas	# Accession
Shugoshin 2 [Xenopus laevis]	10	237,29	115,8	1030	
Protein phosphatase 2, catalytic subunit, beta isoform [Xenopus laevis]	4	88,31	35,6	309	gi148234623
Protein phosphatase 2, regulatory subunit A, beta [Xenopus laevis]	3	48,22	65,5	589	gi148230849

Supplementary Table 1. Proteins detected by mass spectrometry analysis of an affinity purified fraction of XSgo2 from mitotic extracts (fraction E2 in Figure 7B). The number of peptides identified for each protein and the score are shown. Aa, amino acids; MW, molecular weight.

Rivera_Figure S1R



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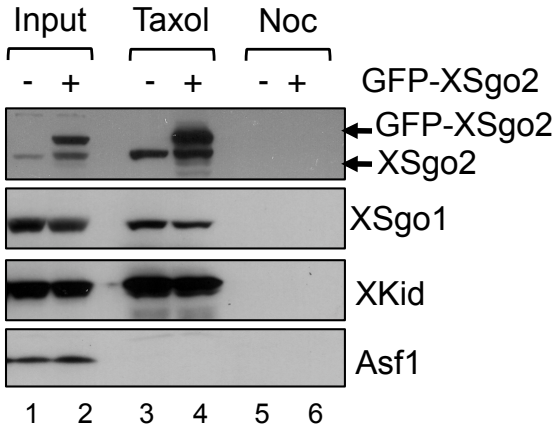

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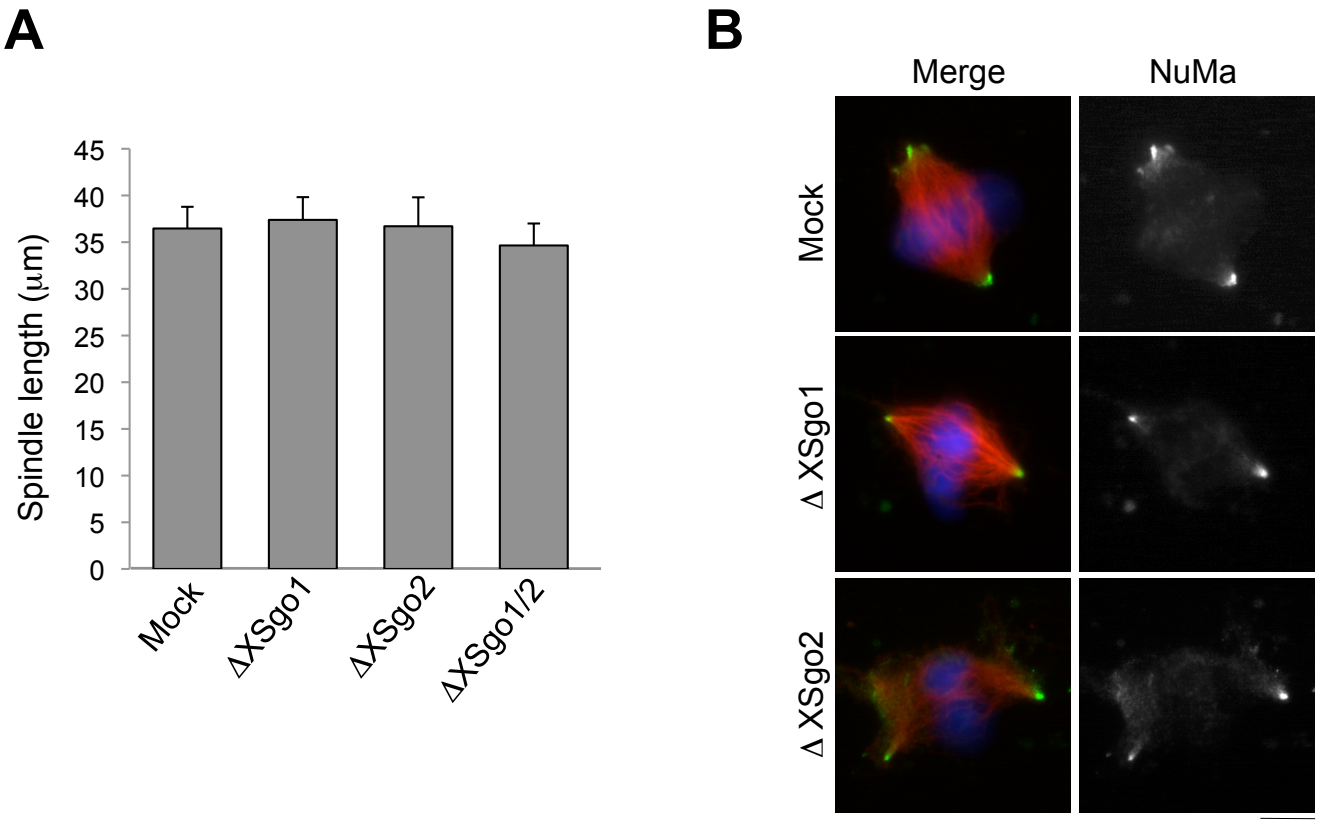

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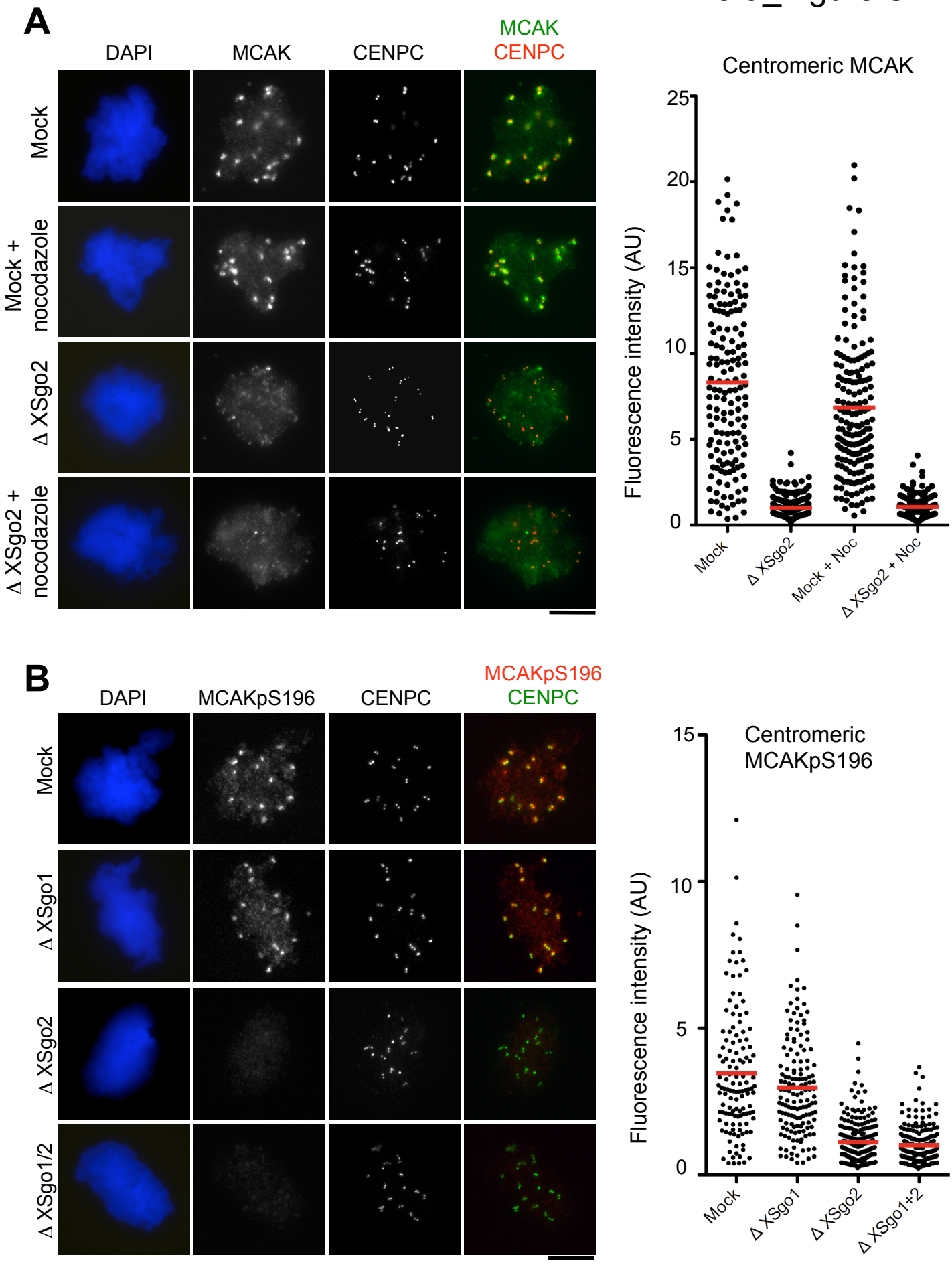

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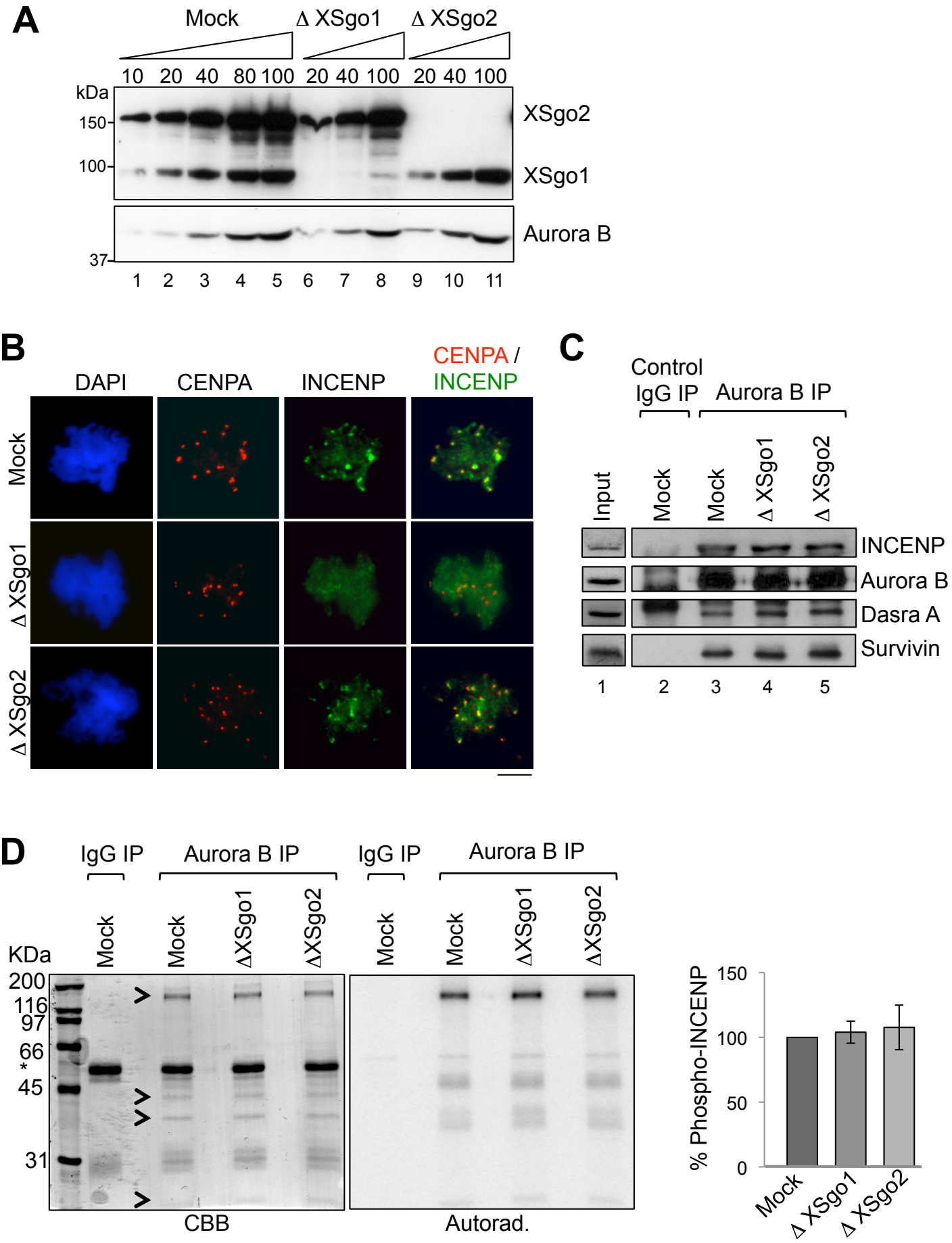

Rivera_Figure S6R