Okumura et al., http://www.jcb.org/cgi/content/full/jcb.201307160/DC1

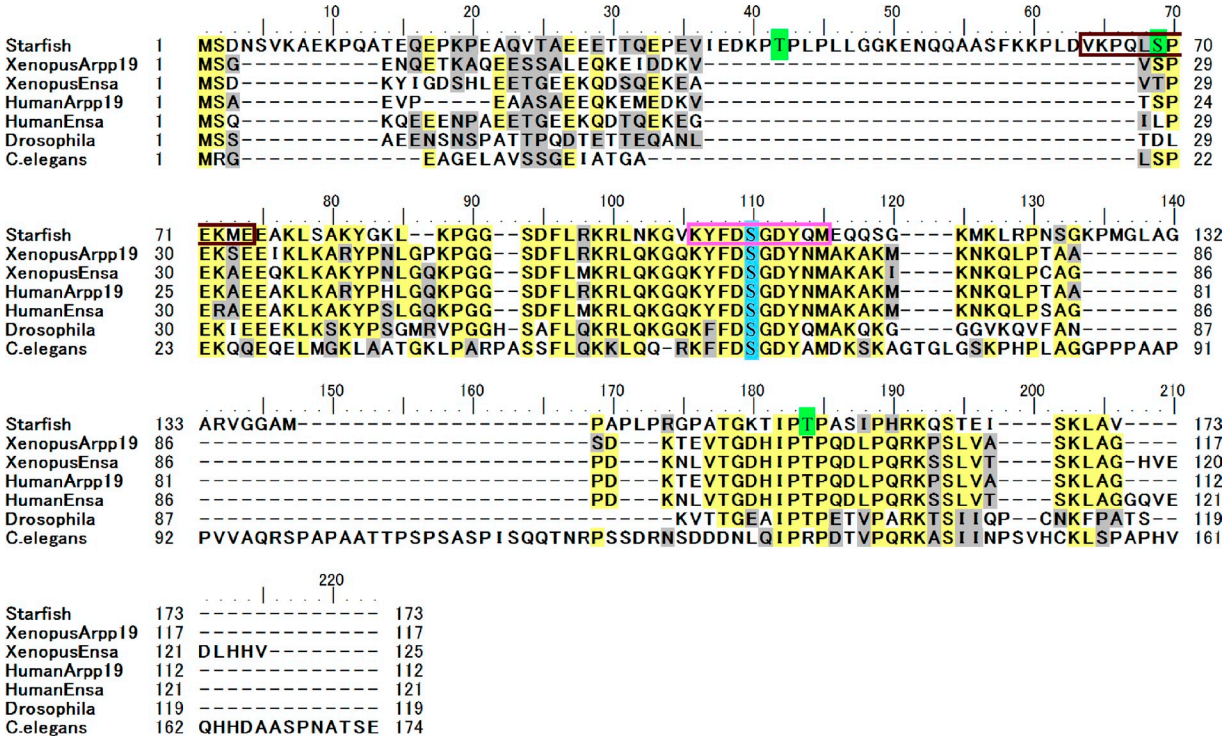

Figure S1. **Deduced amino acid sequences of starfish Arpp19 .** The cDNA of the starfish *A. pectinifera* Arpp19 (ApArpp19; GenBank accession no. AB818897) contains an open reading frame coding for 173 amino acids; the predicted molecular mass is 19 kD. The deduced amino acid sequence of ApArpp19 was aligned with *X. laevis* Arpp19 (XenopusArpp19; NM_001093165.1), *X. laevis* Ensa A (XenopusEnsa: NM_001086605.1), human Arpp19 (HumanArpp19; NM_001093165.1), human Ensa (HumanEnsa; NM_004436.2), fruit fly Endos (Drosophila; NM_140427.1), and nematoda *C. elegans* Ensa-1 (C. elegans; NM_060208) by ClustalW. Identical and closely related amino acids are shaded in yellow and light gray, respectively, and gaps introduced for optimal alignment are indicated by dashes. Conserved Ser of the Gwl target site are shaded in blue. Three putative cyclin B–Cdk1 target sites in starfish Arpp19 are shaded in green. Peptide antigens including phospho-Ser106 and phospho-Ser69 are enclosed with a magenta square and a brown square, respectively.

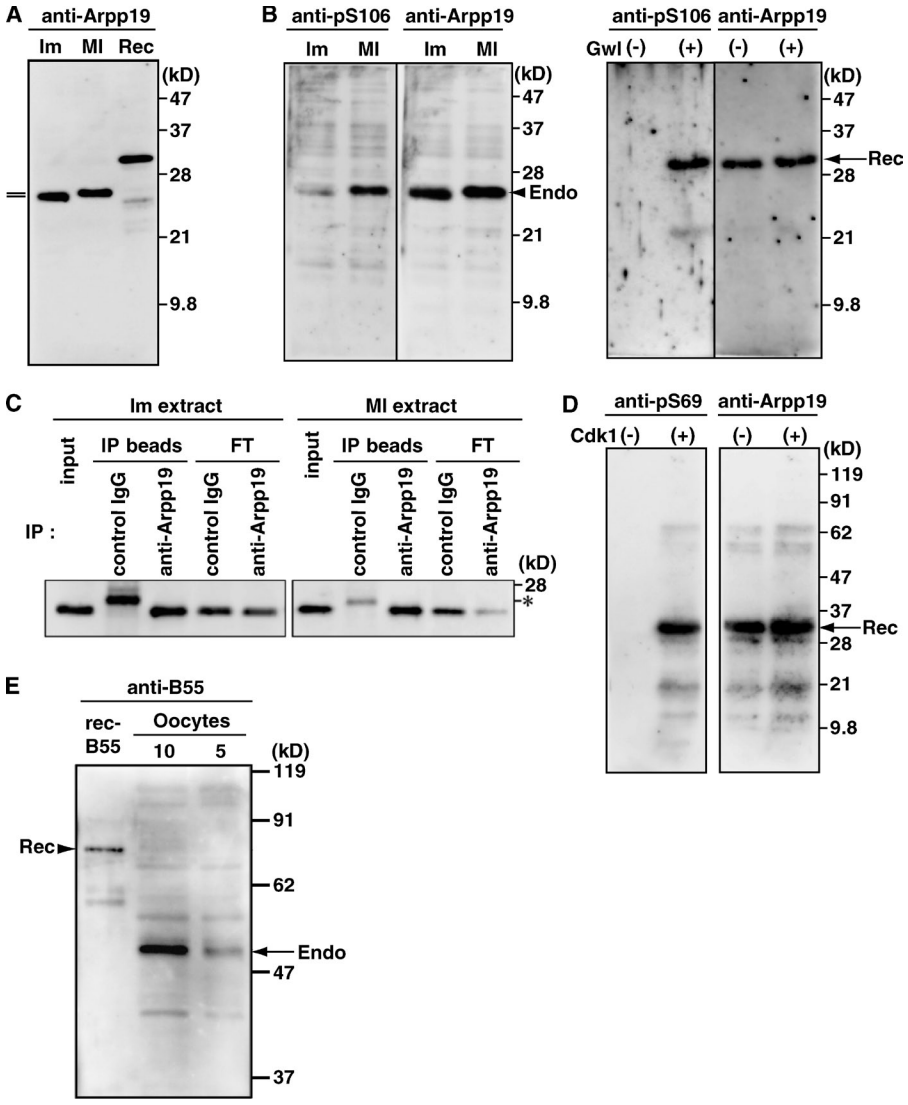

Figure S2. **Specific anti-Arpp19, anti-pSer106 of Arpp19, anti-pSer69 of Arpp19, and anti-B55 subunit of PP2A antibodies.** (A) Anti-Arpp19 antibodies were raised against the full-length recombinant starfish Arpp19 protein (N-terminal His-tagged and C-terminal FLAG-tagged) and affinity purified. Whole cell lysate of five immature oocytes (Im), five maturing oocytes at metaphase of meiosis I (MI), and 500 pg of recombinant protein (Rec) were separated by 12.5% SDS-PAGE and immunoblotted with the anti-Arpp19 antibody. The antibody reacted with bands, high mobility in Im lysate and low mobility in MI lysate, each of which is indicated by a bar. (B) Anti–phospho-Ser106 of Arpp19 antibodies were raised against a peptide containing phospho-Ser106 of starfish Arpp19 and affinity purified. Immunoblots with the anti-pS106 antibody and after stripping and reprobing the same membrane with the anti-Arpp19 antibody were performed in lysates of Im and MI oocytes (left), and in recombinant starfish Arpp19 protein that was treated with (+) or without (−) Gwl kinase (right). (C) Immunoprecipitation with anti-Arpp19 antibody. Im and MI oocyte extracts (input) were mixed with control IgG or purified anti-Arpp19 antibody and then mixed with Protein A–Sepharose beads. Flowthrough fractions from beads (FT) and the washed beads were each analyzed by westen blot with anti-Arpp19 antibody. Asterisk indicates a nonspecific band resulting from control IgG solution. (D) Anti–phospho-Ser69 of Arpp19 antibodies were raised against a peptide containing phospho-Ser69 of starfish Arpp19 and affinity purified. Immunoblots with the anti-pS69 antibody and after stripping and reprobing the same membrane with anti-Arpp19 antibody were performed on recombinant starfish Arpp19 protein treated with (+) or without (−) cyclin B–Cdk1. Brightness, contrast, and gamma settings were adjusted in the image presentation. (E) Anti-B55 antibodies were raised against His-tagged starfish B55 recombinant protein and affinity purified using GST-tagged B55 recombinant protein. Immunoblots with the anti-B55 antibody were performed on GST-tagged B55 recombinant protein and 10 and 5 immature oocytes.

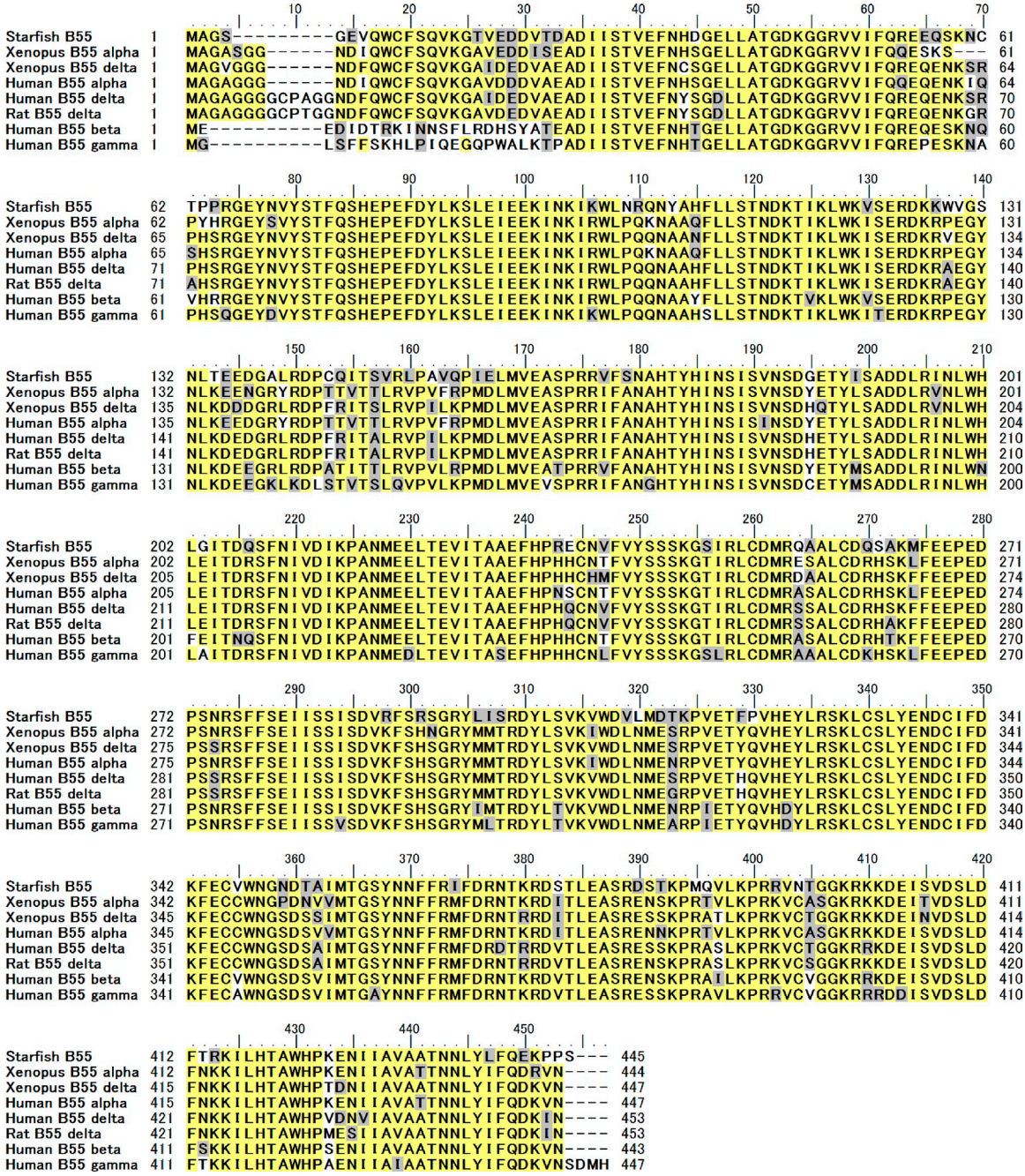

Figure S3. **Deduced amino acid sequence of starfish B55 regulatory subunit of PP2A.** The cDNA of the starfish *A. pectinifera* B55 regulatory subunit of PP2A (starfish B55; GenBank accession no. AB818896) contains an open reading frame coding for 445 amino acids; the predicted molecular mass is 51 kD. The deduced amino acid sequence of starfish B55 was aligned with *X. laevis* B55 α (NP_001084138), *X. laevis* B55 δ (NP_001079618), human B55 α (NP_002708), human B55 δ (NP_060931), rat B55 δ (NP_653347), human B55 β (Q00005), and human B55 γ (NP_870991) by ClustalW. Identical and closely related amino acids are shaded in yellow and light gray, respectively, and gaps introduced for optimal alignment are indicated by dashes. Considering the entire sequence, starfish B55 is most closely related to the δ type, whereas starfish B55 looks more homologous to the α type rather than β, γ, or δ types when the more variable N-terminal region alone is compared between starfish B55 and human B55 subtypes. It is a commonly encountered phenomenon that the starfish genome encodes only a single homologue of vertebrate gene families (Abe et al., 2010).

Reference

Abe, Y., E. Okumura, T. Hosoya, T. Hirota, and T. Kishimoto. 2010. A single starfish Aurora kinase performs the combined functions of Aurora-A and Aurora-B in human cells. *J. Cell Sci.* 123:3978–3988. http://dx.doi.org/10.1242/jcs.076315