## Supplemental material

## JCB



Starfish XenopusArpp19 XenopusEnsa HumanArpp19 HumanEnsa Drosophila C.elegans	1 1 1 1 1 1	10   20   30   40   50   60   70     MSDNSVKAEKPQATEQEPKPEAQVTAEEETTQEPEVIEDKP   1
Starfish XenopusArpp19 XenopusEnsa HumanArpp19 HumanEnsa Drosophila C.elegans	71 30 30 25 30 30 23	80   90   100   110   120   130   140     EKME   AKYGKL - KPGG - SDFLRKRLNKGVKYFDSGDYQMEQQSG KMKLRPNSGKPMGLAG   132   130   130     EKSEE   IKLKARYPNLGPKPGG - SDFLRKRLNKGVKYFDSGDYQMEQQSG KMKLRPNSGKPMGLAG   132   132   130     EKSEE   IKLKARYPNLGPKPGG - SDFLRKRLQKGQKYFDSGDYNMAKAKM KNKQLPTAA KNKQLPCAG   86   86   86     EKAEEQKLKAKYPNLGQKPGG - SDFLMKRLQKGQKYFDSGDYNMAKAKM KNKQLPCAG   86   86   86   86     EKAEEAKLKARYPHLGQKPGG - SDFLMKRLQKGQKYFDSGDYNMAKAKM KNKQLPCAG   86   86   86   86     EKAEEAKLKAKYPSLGQKPGG - SDFLMKRLQKGQKYFDSGDYNMAKAKM KNKQLPSAG   87   86   86   86     EKAEEAKLKAKYPSLGQKPGG - SDFLMKRLQKGQKYFDSGDYNMAKAKM KNKQLPSAG   87   86   86   86     EKAEEAKLKAKYPSLGQKPGG - SDFLMKRLQKGQKYFDSGDYNMAKAKM KNKQLPSAG   87   87   87   87     EKAEEAKLKAKYPSLGQKPGG - SDFLMKRLQKGQKFFDSGDYNMAKAKM GOVKQVFAN 87   87   87   87   87     EKAEEAKLKAKYPSGGRAFGARAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAG
Starfish XenopusArpp19 XenopusEnsa HumanArpp19 HumanEnsa Drosophila C.elegans	133 86 86 81 86 87 92	150   160   170   180   190   200   210     ARVGGAM
Starfish XenopusArpp19 XenopusEnsa HumanArpp19 HumanArps19 HumanEnsa Drosophila C.elegans	173 117 121 112 121 119 162	220      173      117     DLHHV   125      112      121      119     QHHDAASPNATSE   174

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), muman 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.

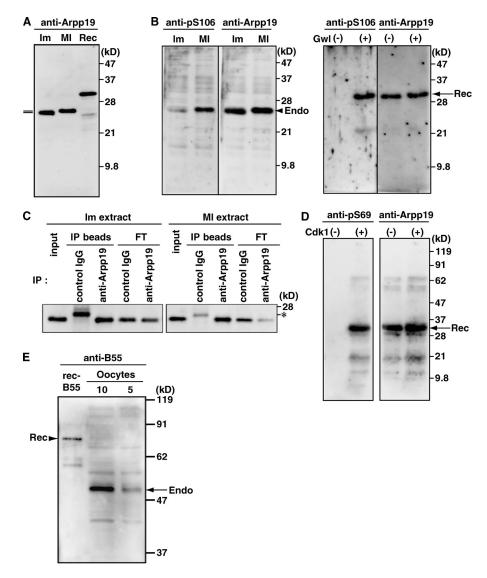


Figure S2. **Specific anti-Arpp19, anti-pSer106 of Arpp19, anti-pSer69 of Arpp19, and anti-B55 subunit of PP2A antibodies.** (A) Anti-Arpp19 antibodies were raised against the full-length recombinant starfish Arpp19 protein (N-terminal His-tagged and C-terminal FLAG-tagged) and affinity purified. Whole 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 immunobloted 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 antibody 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 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 the anti-B55 antibody were performed on GST-tagged B55 recombinant protein and 10 and 5 immature oocytes.

Starfish B55 1 Xenopus B55 alpha 1 Xenopus B55 delta 1 Human B55 alpha 1 Human B55 delta 1 Rat B55 delta 1 Human B55 beta 1 Human B55 gamma 1	MAGVGGGND MAGAGGGND MAGAGGGGCPAGGND MAGAGGGGCPTGGND MEEDI	IQWCFSQVKGAVEDD FQWCFSQVKGAIDED IQWCFSQVKGAVDDD FQWCFSQVKGAIDED FQWCFSQVKGAVDED DTRKINNSFLRDHSY	0 40 VTDAD LISTVEFNHD VISEAD LISTVEFNHS VAEAD LISTVEFNHS VAEAD LISTVEFNKS VAEAD LISTVEFNYS VAEAD LISTVEFNYS VAEAD LISTVEFNHT KTPAD LISTVEFNHT	GELLATGDKGGRVVI GELLATGDKGGRVVI GELLATGDKGGRVVI GDLLATGDKGGRVVI GDLLATGDKGGRVVI GELLATGDKGGRVVI	FQQESKS 61 FQREQENKSR 64 FQQEQENKIQ 64 FQREQENKGR 70 FQREQENKGR 70 FQREQESKNQ 60
Starfish B5562Xenopus B55 slapha62Xenopus B55 slapha65Human B55 alpha65Human B55 delta71Rat B55 delta71Human B55 delta61Human B55 gamma61	PYHRGEYSVYSTFQS PHSRGEYNVYSTFQS SHSRGEYNVYSTFQS PHSRGEYNVYSTFQS AHSRGEYNVYSTFQS VHRRGEYNVYSTFQS	HEPEFDYLKSLEIEE HEPEFDYLKSLEIEE HEPEFDYLKSLEIEE HEPEFDYLKSLEIEE HEPEFDYLKSLEIEE HEPEFDYLKSLEIEE	0 110 KINKI KWLNRQNYAH KINKI RWLPQKNAAQ KINKI RWLPQKNAAQ KINKI RWLPQQNAAN KINKI RWLPQQNAAH KINKI RWLPQQNAAH	FLLSTNDKTIKLWKI FLLSTNDKTIKLWKI FLLSTNDKTIKLWKI FLLSTNDKTIKLWKI FLLSTNDKTIKLWKI FLLSTNDKTVKLWKV	SERDKRPEGY 131 SERDKRVEGY 134 SERDKRPEGY 134 SERDKRAEGY 140 SERDKRAEGY 140 SERDKRAEGY 130
Starfish B55     132       Xenopus B55 alpha     132       Xenopus B55 alpha     135       Human B55 alpha     135       Human B55 delta     141       Rat B55 delta     141       Human B55 beta     131       Human B55 delta     141       Human B55 beta     131       Human B55 gamma     131	NLKDDDGRLRDPFRI NLKEEDGRYRDPTTV NLKDEDGRLRDPFRI NLKDEDGRLRDPFRI NLKDEEGRLRDPATI	TTLRVPVFRPMDLMV TSLRVPILKPMDLMV TTLRVPVFRPMDLMV TALRVPILKPMDLMV TALRVPILKPMDLMV TTLRVPVLRPMDLMV	0 180 ICASPRRVFSNAHTYH VEASPRRIFANAHTYH VEASPRRIFANAHTYH VEASPRRIFANAHTYH VEASPRIFANAHTYH VEASPRIFANAHTYH VEASPRIFANAHTYH VEASPRRIFANAHTYH	INSISVNSDYETYLS INSISVNSDHQTYLS INSISINSDYETYLS INSISVNSDHETYLS INSISVNSDHETYLS INSISVNSDYETYMS	ADDLRINLWH 201 ADDLRVNLWH 201 ADDLRVNLWH 204 ADDLRINLWH 204 ADDLRINLWH 210 ADDLRINLWH 210 ADDLRINLWH 200
	LEITDRSFNIVDIKP LEITDRSFNIVDIKP LEITDRSFNIVDIKP LEITDRSFNIVDIKP	ANMEELTEVITAAEF ANMEELTEVITAAEF ANMEELTEVITAAEF ANMEELTEVITAAEF ANMEELTEVITAAEF ANMEELTEVITAAEF		TIRLCDMRESALCDR TIRLCDMRDAALCDR TIRLCDMRASALCDR TIRLCDMRSSALCDR TIRLCDMRSSALCDR TIRLCDMRSSALCDR	HSKLFEEPED 271 HSKFFEEPED 274 HSKLFEEPED 274 HSKFFEEPED 280 HAKFFEEPED 280 HTKFFEEPED 270
Starfish     B55     272       Xenopus     B55     alpha     272       Xenopus     B55     delta     275       Human     B55     alpha     275       Human     B55     delta     281       Rat     B55     delta     281       Human     B55     delta     281       Human     B55     delta     281       Human     B55     delta     281       Human     B55     gamma     271	PSNRSFFSELISSIS PSSRSFFSELISSIS PSNRSFFSELISSIS PSSRSFFSELISSIS PSSRSFFSELISSIS PSNRSFFSELISSIS	DVKFSHNGRYMMTRE DVKFSHSGRYMMTRE DVKFSHSGRYMMTRE DVKFSHSGRYMMTRE DVKFSHSGRYMMTRE DVKFSHSGRYIMTRE	0 320 VLSVKVWDVLMDTKP VLSVKVWDLNMESRP VLSVKIWDLNMESRP VLSVKIWDLNMESRP VLSVKVWDLNMESRP VLSVKVWDLNMESRP VLSVKVWDLNMERRP VLTVKVWDLNMEARP	VETYQVHEYLRSKLC VETYQVHEYLRSKLC VETYQVHEYLRSKLC VETHQVHEYLRSKLC VETHQVHEYLRSKLC IETYQVHDYLRSKLC	SLYENDCIFD 341 SLYENDCIFD 344 SLYENDCIFD 344 SLYENDCIFD 350 SLYENDCIFD 350 SLYENDCIFD 340
Starfish B55 342 Xenopus B55 alpha 342 Xenopus B55 delta 345 Human B55 alpha 345 Human B55 delta 351 Rat B55 delta 351 Human B55 beta 341 Human B55 gamma 341	KFECCWNGPDNVVMT KFECCWNGSDSSIMT KFECCWNGSDSVVMT KFECCWNGSDSAIMT KFECCWNGSDSAIMT KFECVWNGSDSVIMT	GSYNNFFRMFDRNTF GSYNNFFRMFDRNTF GSYNNFFRMFDRDTF GSYNNFFRMFDRDTF GSYNNFFRMFDRNTF GSYNNFFRMFDRNTF	0 390 CRDSTLEASRDSTKPM CRDITLEASRENSKPR RDITLEASRENSKPR RDUTLEASRENNKPR RDVTLEASRENSKPR RDVTLEASRENSKPR CRDVTLEASRENSKPR	TVLKPRKVCASGKRK ATLKPRKVCTGGKRK TVLKPRKVCASGKRK ASLKPRKVCTGGKRR ASLKPRKVCSGGKRK AILKPRKVCVGGKRR	KDEITVDSLD411KDEINVDSLD414KDEISVDSLD414KDEISVDSLD420KDEISVDSLD420KDEISVDSLD410
	FNKKILHTAWHPKEN FNKKILHTAWHPTDN FNKKILHTAWHPKEN FNKKILHTAWHPVDN	I I AVATTNNLY I FQD I I AVAATNNLY I FQD I I AVATTNNLY I FQD VI AVAATNNLY I FQD I I AVAATNNLY I FQD I I AVAATNNLY I FQD	RVN 444   KVN 447   OKIN 453   OKIN 453   OKIN 443		

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  $\alpha$  (NP\_001084138), X. *laevis* B55  $\delta$  (NP\_001079618), human B55  $\alpha$  (NP\_002708), human B55  $\delta$  (NP\_060931), rat B55  $\delta$  (NP\_653347), human B55  $\beta$  (Q00005), and human B55  $\gamma$  (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  $\delta$  type, whereas starfish B55 looks more homologous to the  $\alpha$  type rather than  $\beta$ ,  $\gamma$ , or  $\delta$  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