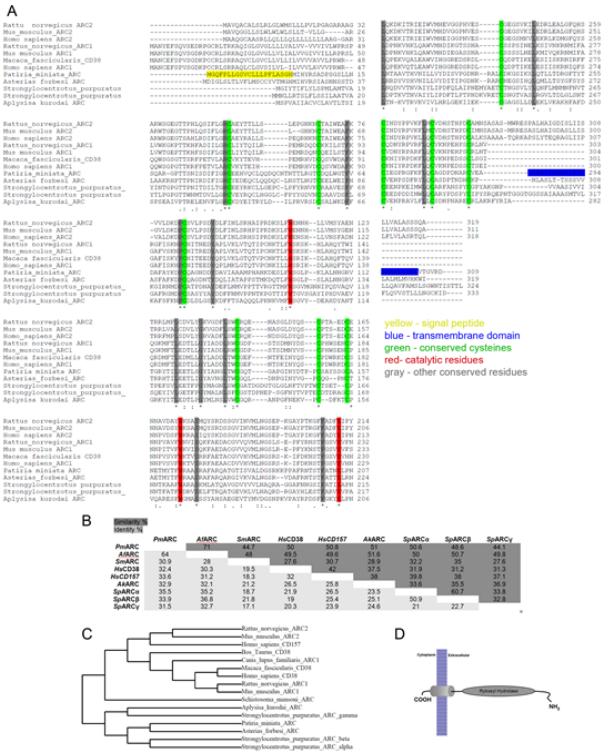

Figure S1: *P. miniata* ADP ribosyl cyclase (PmARC). One isoform of ARC was found in ovarian transcriptomes of *P. miniata*. **A,** Sequence alignment of ARC family proteins by ClustalW. Catalytic residues (red), conserved cysteines (green), identical residues (dark grey). The signal peptide (yellow) was predicted by SignalP 4.0 and the C-terminal transmembrane domain (blue) was predicted by TMHMM2. Putative sites for N-glysosylation (squares) were predicted by NetNGlyc 1.0. **B,** Percentage of identical (light grey) and similar (dark grey) residues amongst ARC proteins across species. Values relate to the alignment in (A). **C,** Phylogenetic relationship of cloned ARC proteins across different species using the Neighbor-Joining method by Phylotree. **D,** Predicted topology of mature *P. miniata* ARC protein. The ribosyl-hydrolase domain (Ribhydrolase) was predicted by Pfam (accession number PF02267) and transmembrane segment (cylinder) predicted by TMHMM2. *Hs: Homo sapiens, Sp: S. purpuratus, At: A. thaliana, Pm: P. miniata*, Af: *A. forbesi*, Ak: *A. kurodai*, Rn: *R. norvegicus*, Sm: *S. mansoni*, Mm, *M. musculus*, Mf: *M. fascicularis.*

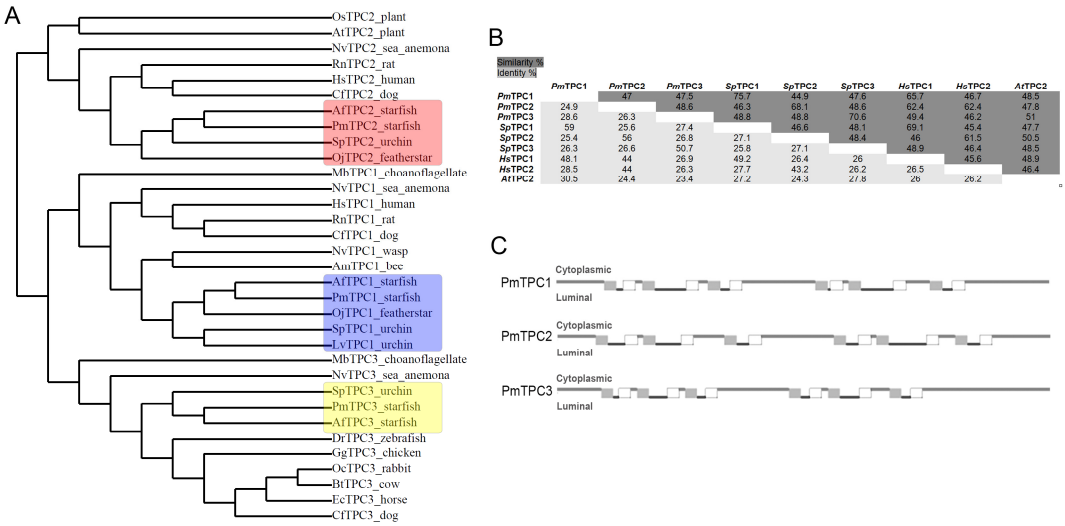

Figure S2: *P. miniata* Two-Pore Channels (PmTPCs). Three isoforms of TPCs were found in ovarian transcriptomes of *P. miniata* (PmTPC1, PmTPC2 and PmTPC3). **A,** Phylogenetic relationship of cloned PmTPCs across different species using the Neighbor-Joining method by Phylotree. **B,** Percentage of identical (light grey) and similar (dark grey) residues amongst TPC proteins across species. Values relate to the alignment in (A). **C,** Predicted topology of mature *P. miniata* TPCs. Transmembrane segments (cylinders) were predicted by TopCons server. *Hs: Homo sapiens, Sp: S. purpuratus, At: A. thaliana, Pm: P. miniata*, Af: *A. forbesi*, Oj: *O. japonicus*, Lv: *L. variegatus*, Mb: *M. brevicollis,* Nv: *N. vectensis,* Rn: *Rattus norvegicus,* Cf: *C. familiaris,* Am: *A. mellifera,* Dr: *D. rerio,* Gg: *G. gallus,* Oc: *O. cuniculus,* Bt: *B. Taurus,* Ec: *E. caballus.*

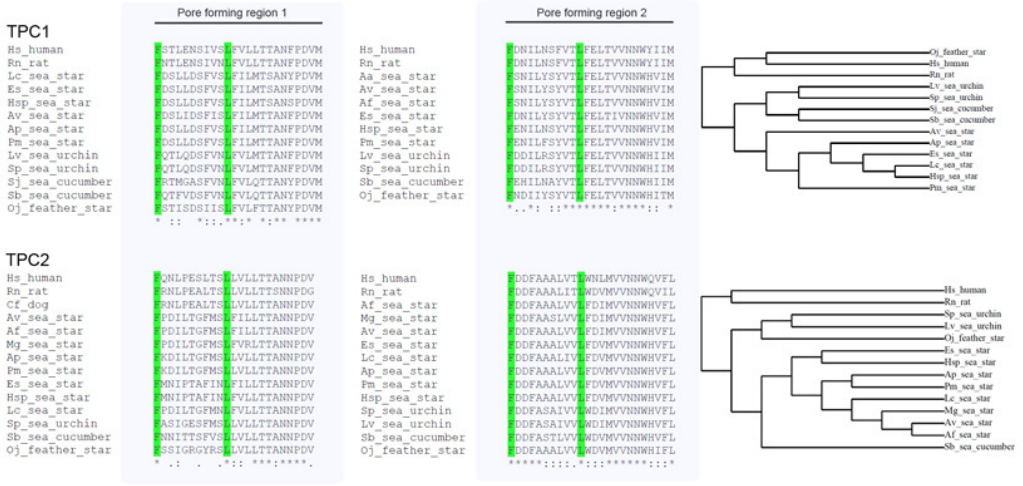

Figure S3: Alignment of TPCs 1 and 2 putative pore forming regions from human, rat and different echinoderms. Residues in green are conserved between the pores and across the species. Lc, *L. clathrata*; Es, *E. spinulosus*; Av, *A. vulgaris (A. rubens)*; Ap, *A. pectinifera (P. pectinifera)*; Pm, *P. miniata*; Lv, *L. variegatus*; Sp, *S. purpuratus*; Sj, *S. japonicus (A. japonicus)*; Sb, *S. briareus*; Oj, *O. japonicus*; Hsp, *Henricia* species; Af, *A. forbesi*; Rn, *R. norvegicus*; Cf, *C. familiaris*, Hs, *H. sapiens.*

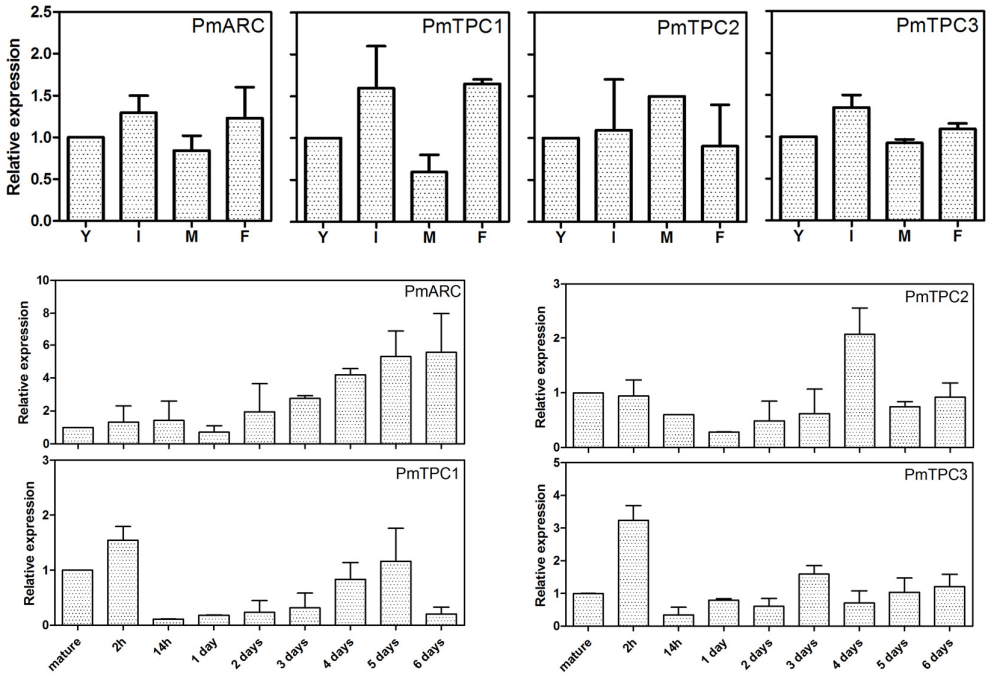

Figure S4: qPCR for PmARC and PmTPCs isoforms throughout oogenesis and early embryogenesis of *P. miniata*. RNA was extracted from different stages of oogenesis and embryogenesis and gene expression was accessed by Real-Time PCR. Values are normalized by the levels of *P. miniata* Ubiquitin cDNA. Oogenesis: (Y) young oocyte 50-100 μm in diameter, (I) full grown immature oocyte, (M) mature egg, (F) egg 30 min after fertilization. Embryogenesis: mature egg, followed by embryos hours and days after fertilization as indicated in the image. Graphs show the mean (± 1 S.D.) of at least three experiments.

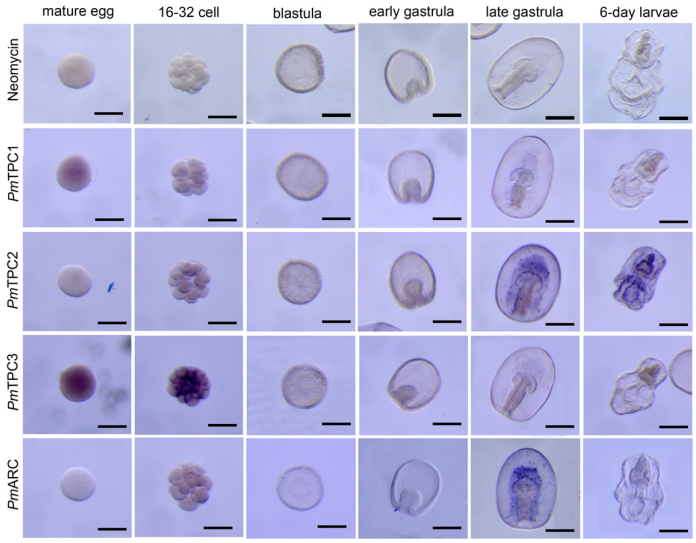

Figure S5: Whole mount *In situ* hybridizations for PmARC and PmTPC isoforms in *P. miniata* eggs and embryos. Neomycin was used as a negative control for staining. Embryonic stages are indicated in the image. Bars: 180 μm.

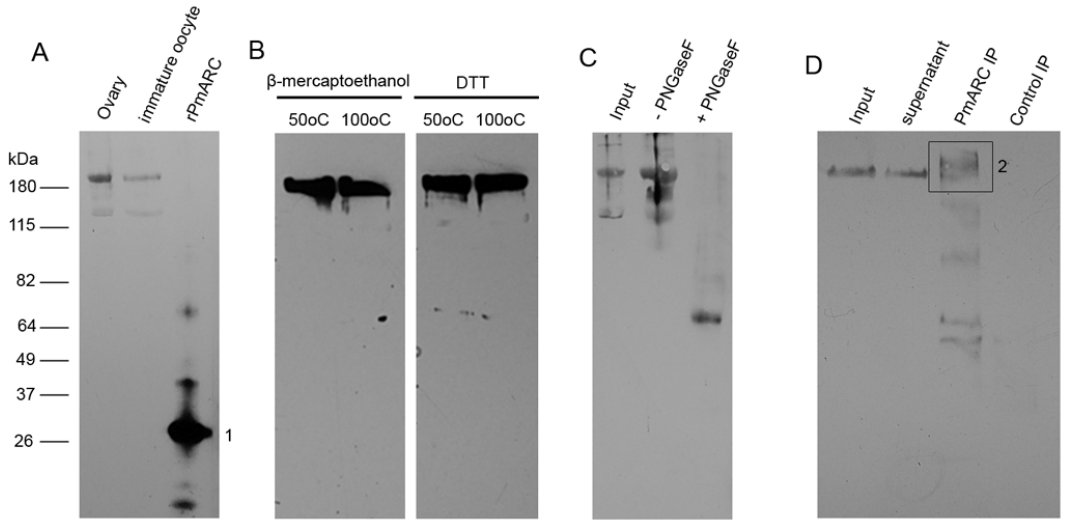

Figure S6: PmARC immunoblotings in different conditions. A, endogenous PmARC from oocytes, ovaries and rPmARC. Note the difference in molecular weight. **B,** immature oocytes samples were prepared using lower heating and different reducing agents before loading in the gel. **C,** PNGase treatment of immature oocytes samples. **D,** immunoprecipitation of PmARC followed by immunoblotting. Samples marked 1 (in **A**) and 2 (in **D**) were excised from the gel and analyzed my mass spectrometry.

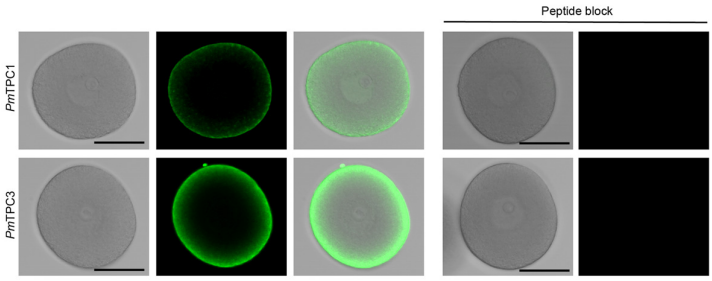

Figure S7: Peptide-block immunolocalization of PmTPCs 1 and 2. Oocytes were pre-incubated with 1 mg/ml of each of the peptides from the cocktail used to raise the antibodies in rabbits and processed for immunolocalization Bars: 150 μm.

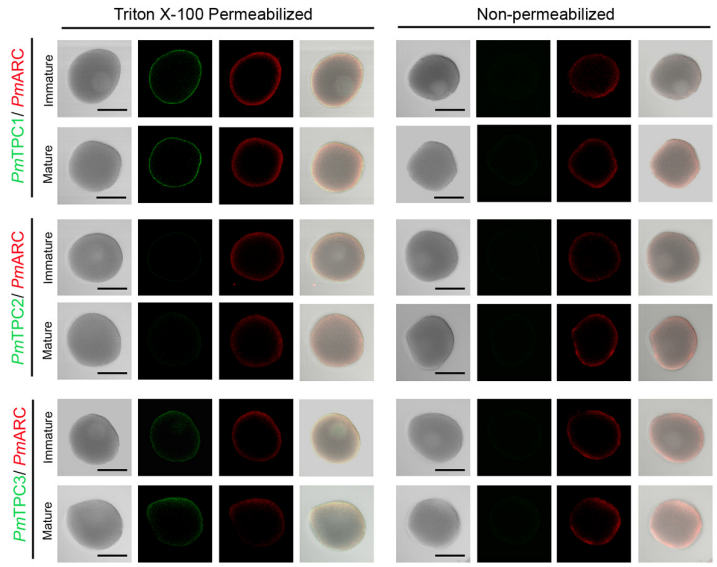

Figure S8: Colocalization of PmTPCs and PmARC in immature oocytes and mature eggs with or without Triton X-100 permeabilization. Note that PmTPCs signals decrease but are still detectable whereas PmARC signals are only slightly affected in non-permeabilized oocytes and eggs. Bars: 150 µm.

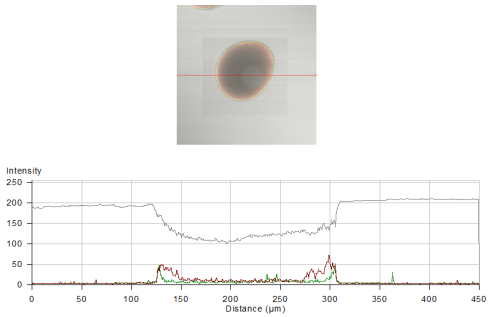

Figure S9: Intensity line Plot for PmTPC3 and PmARC immunolocalization signals. PmTPC3, green signal; PmARC, red signal, Bright field, gray signal.

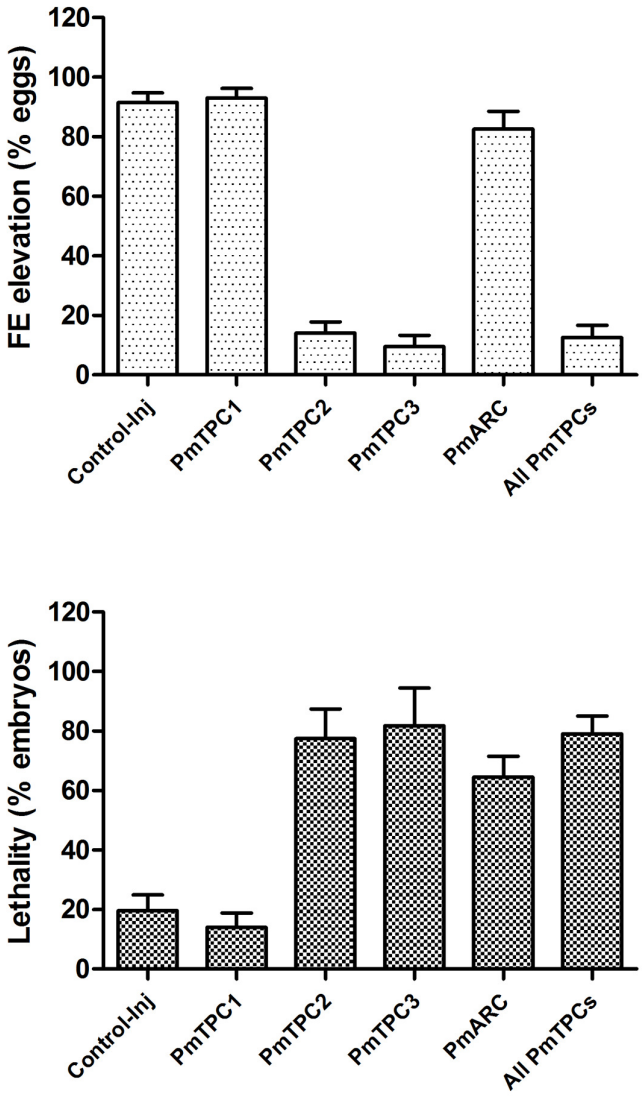

Figure S10: Morpholino phenotypes quantifications. Percentages of embryos presenting the fertilization envelope phenotype and lethality after gastrulation in the different morpholino knockdown experiments (n=6).

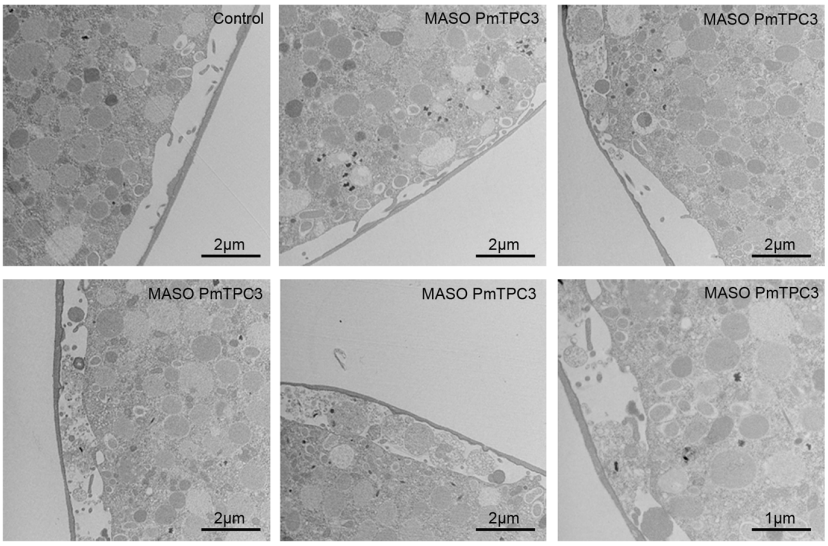

Figure S11: PmTPC3-knockdown eggs 10 min after fertilization. Collection of TEM images of the cortices and fertilization envelopes of PmTPC3 knockdown eggs.

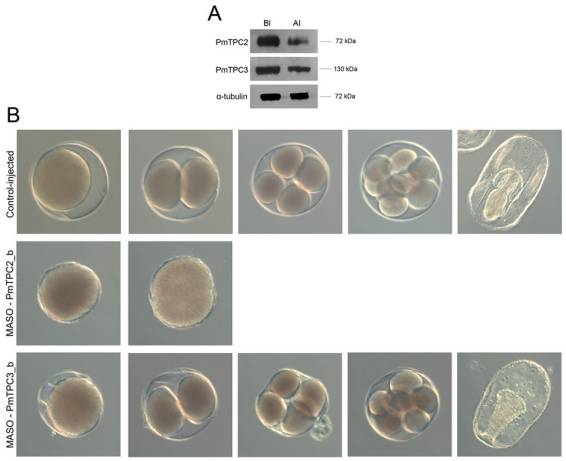

Figure S12: Eggs and embryos after injections of morpholinos directed to different regions of the 5'UTR of PmTPCs 2 and 3. A, Immunoblotting before and after morpholino injections. **B,** Control and injected eggs and embryos at first cleavage, 8-16, 16-32 cell stage, and gastrulation.

Movie 1: Global calcium signals at fertilization in sea stars. Control eggs and eggs co-injected with all three PmTPCs morpholinos. Control calcium signals and the two phenotypes described in Figure 5 are shown.

Table S1: Primers, peptides and morpholinos.

Primers and Peptides	**Sequence**
5´and 3´ RACEs	
PmARC 5'RACE outer	5'- CGGAGCCAAGCAGGGGGAACTGTC-3'
PmARC 5'RACE inner	5'- GACTGGATGAAAGATGCCGGGGGTTG-3´
PmTPC1 5'RACE outer	5'- CGTGATACGAAAATGGCTCCGTTGCCTG-3'
PmTPC1 5'RACE inner	5'- CAAGGACGACGATGGCTTCACAGAAC-3
PmTPC1 3'RACE outer	5'- GGAAACCGGCCACCACAATGACAGCG-3'
PmTPC1 3'RACE inner	5'- GACAATGACAGCACGACCGCGTGCCAGC-3'
PmTPC2 5'RACE outer	5'- CGACTCCGAGGATCTACGACCAGGATGG-3'
PmTPC2 5'RACE inner	5'- GGGATTCATCGTAACTACTCGGTCTGCCT -3'
PmTPC2 3'RACE outer	5'- GACATCTACGCCTCTGATCTCAGGGG-3'
PmTPC2 3'RACE inner	5'- CTCTACGCCGAGACCGAACAGCCAATGC-3'
PmTPC3 5'RACE outer	5'- GCGGAAGACTTTCTCATCACTCCCGTTTCC-3'
PmTPC3 5'RACE inner	5'- CAAGGATGGTGACAACAAGCATATCCAC-3'
PmTPC3 3'RACE outer	5'- GGAATCCGCTTCCGCATTGGAAAGAAATCC-3'
PmTPC3 3'RACE inner	5'- GAAATGTACAGGTGTCGTTGCAGGCCATG-3'
qPCR	
ForPmARC	5'-GCTGTGTGATAAAGGCAGCA-3'
RevPmARC	5'-GCCAGGACCAATGCAATACT-3'
ForPmTPC1	5'-ATCCTGGAGGCCTTTGTCTT-3'
RevPmTPC1	5'-TGCTTTGGTCCTTTTTCTGG-3'
ForPmTPC2	5'-TTGCTAGCGTGCTTTTGCTA-3'
RevPmTPC2	5'-GGCCATTGGCATATGTAACC-3'
ForPmTPC3	5'-CGACAGCAAGTTTGTCCTGA-3'
RevPmTPC3	5'-CGGTGGACAGTCTGACATTG-3'
ForPmUbiquitin	5'-TTCGGTGAAAGCCAAGATTC-3'
RevPmUbiquitin	5'-CCCACCTCTCATGGCTAGAA-3
***In situ* probes**	
ForIsPmARC	5'-GAGGGCCGACCACGTCTAACATAAGC-3'
RevIsPmARC	5'-CTAGAAATGTAGTGAGTTCGGCGATTC-3'
ForIsPmTPC1	5'-TTAGCGGTAGTATACGACACTTTCAC-3'
RevIsPmTPC1	5'-AAAGAACGACTCGTAGGTTGTATTCT-3'
ForIsPmTPC2	5'-GCTGTCAAGTATCGAAGTATAAACCA-3'
RevIsPmTPC2	5'-TGAAGAAAAAGTACAGTCCAATGAC-3'
ForIsPmTPC3	5'-GTACGCCATTCTTTCAGAAGTACATA-3'
RevIsPmTPC3	5'-CCAACTCATCAATCTTCTTTTCAATA-3'
Morpholinos	
PmARC:	5'-GGAACTGTCCCATGACTGGATGAAA-3'
PmTPC1	5'-TGGACTTGTTAAATCGTTCATGGTT-3'
PmTPC2	5'-TGATGTTTTTTCATCAAAGCCAGCA-3'
PmTPC3	5'-GTTTTCTTCACCATCAAGTCATCGC-3'
PmTPC2b	5'-AAAGCCAGCAAACGCAGTGTTCGGA-3'
PmTPC3b	5'-ATTACGTCGTTTCACTTTCAGACTC-3'
Antibodies Peptides	
PmTPC1	NH2-YLKEGENNHNFSTHPKSQDC-CONH2
	NH2-VQLKWKLKQDENRLWFEEC-CONH2
	NH2-ADEIKEWVREQDQTQRQDLQQC-CONH2
PmTPC2:	NH2-ELRGRPSSYDESLDRIHPGRRSSESC-CONH2

	NH2-DRDTSRRRKPPIVPKNNHILRKIC-CONH2
	NH2-WDREQQLEASEDPNNQPSYC-CONH2
PmTPC3	NH2-ADLMRERKGSVVRPRSVSFKKC-CONH2
	NH2-NRSTPFFQKYIPSCYNSRVSEFIC-CONH2
	NH2-EENMGPEELDDIDEMNPYENEPIC-CONH2
PmARC	NH2-CEEHVQCLQQDQCNTNT-CONH2
	NH2-GINYDTPCPSAYSSGC-CONH2
	NH2-TMYTFWRAASRAFARQATGC-CONH2

Table S2: Protein Groups and Peptide-Spectrum Matches (PSMs) for the rPmARC and the immunoprecipteted endogenous PmARC after immunoblotting detecetion.

Protein Groups and Peptide-Spectrum Matches (PSMs)				
Sample ID	***Accession Number***	***Protein Name***	***Protein Score***	***Unique PSMs***
1 - rPmARC	Pm56316_0_T_1	Locus_56316.0_Transcript_1/0_Con_3_Len_2051 ~RPKM~2.96~NADA_APLCA~2e-43~ADP-ribosyl_cyclase_maxframe_1080	293.44	K.DESLFWSGLPK.L K.LALNNGR.V R.CEEHVQCLQQDQCNTNTTLR.G K.DLQTLLTDR.G R.QATGTISVALDGSR.T
2 - PmARC IP 182kDa	Pm56316_0_T_1	Locus_56316.0_Transcript_1/0_Con_3_Len_2051 ~RPKM~2.96~NADA_APLCA~2e-43~ADP-ribosyl_cyclase_maxframe_1080	286.78	K.DESLFWSGLPK.L K.LALNNGR.V K.LALNNGRVTGR.Y R.CEEHVQCLQQDQCNTNTTLR.G R.QATGTISVALDGSR.T

Sample ID, numbers 1 and 2 refer to the bands from Figure S6. **Accession Nr.,** accession number from the searched database (DB). **Protein Name,** protein name in the DB. **Protein Score,** sum of the contributing peptide scores. **Unique PSMs,** unique peptide-spectrum matches that contribute to the protein assignment.