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SUPPLEMENTARY ONLINE DATA

Calcium- and polyphosphate-containing acidic granules of sea urchin eggs are similar to acidocalcisomes, but are not the targets for NAADP

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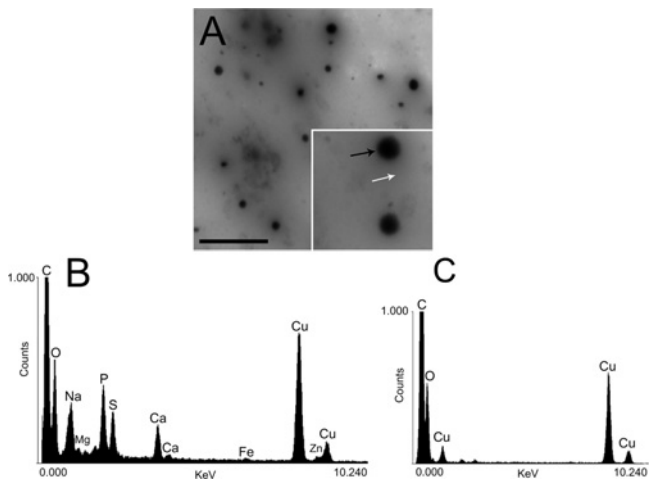

Figure S1 Electron microscopy and X-ray microanalysis of the dense granule fraction

(**A**) Direct observation of unfixed and unstained dense granules air-dried directly on to microscope grids. (**B**) Typical X-ray microanalysis spectrum of dense granules (black arrow). (**C**) Control spectrum of an adjacent area (white arrow). Scale bars in (**A**) are 0.5 μm.

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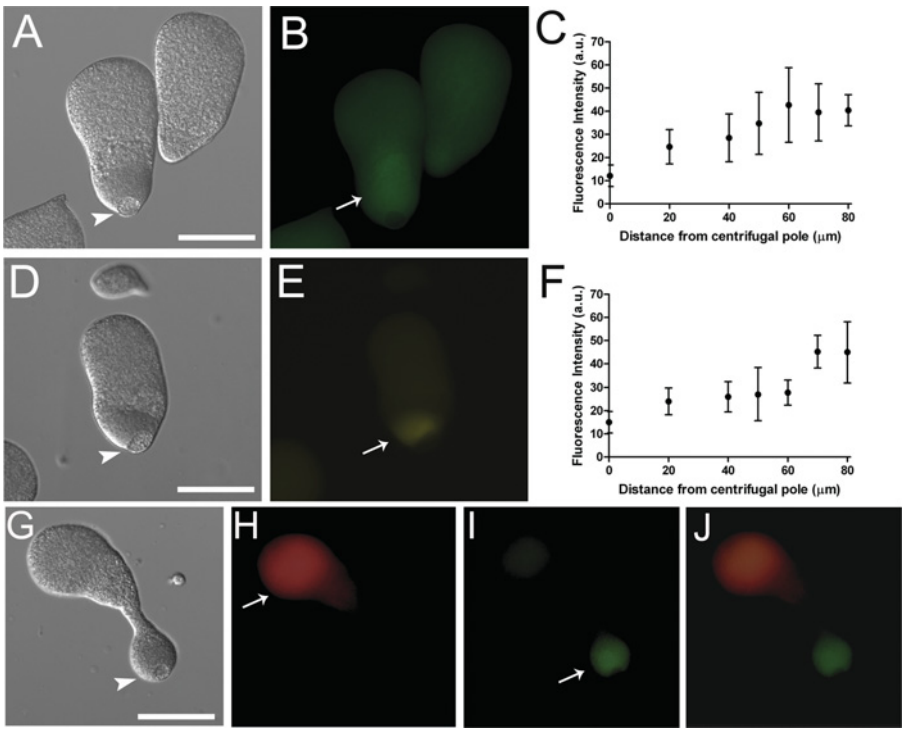

Figure S2 Segregation of poly P stores in stratified sea urchin eggs

(**A**, **B** and **C**) Sea urchin eggs were stratified and poly P stores were localized using the recombinant PPBD of *E. coli* PPX linked with an Xpress epitope tag. (**D, E** and **F**) Stratified eggs after DAPI incubation for poly P localization. (**C** and **F**) Fluorescence intensity profile plots of PPBD and DAPI staining respectively. Graphs show means ± S.D. for five eggs. (**G, H, I** and **J**) Sea urchin eggs were incubated with LysoTracker Red, stratified and then fixed and stained with PPBD as described in the Materials and methods section of the main text. (**G**) Differential interferential contrast microscopy image of one stratified egg. (**H, I** and **J**) LysoTracker, PPBD and merged corresponding images of (**G**) respectively. Arrows in (**B, E, H** and **I**) indicate staining near the centripetal pole, where the nucleus and lipid droplets (arrowheads in **A**, **D** and **G**) are also located. Images were not deconvolved. Scale bars are 50 μm.

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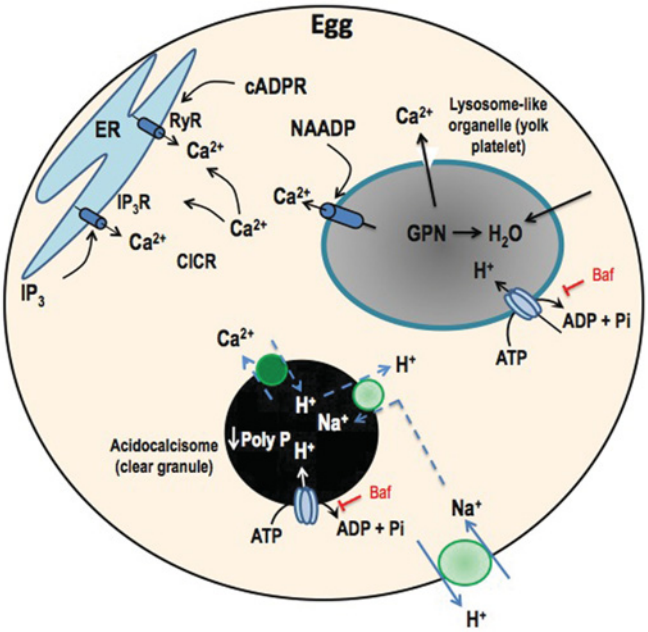

Figure S3 Proposed Ca^{2+} mobilization pathways in sea urchin eggs

ER, acidocalcisomes and yolk platelets are major Ca^{2+} storage organelles. IP_3 receptors (IP_3R) and ryanodine receptors (RyR) mediate Ca^{2+} release from the ER in response to increases in IP_3 and cADPR respectively. Both receptors types are, in addition, activated by Ca^{2+} via the so-called Ca^{2+}-induced Ca^{2+}-release mechanism (CICR). NAADP triggers Ca^{2+} release from yolk platelets and this in turn triggers ER Ca^{2+} mobilization through Ca^{2+}-induced Ca^{2+} release via IP_3R and RyR. Na^+ entry after fertilization leads to alkalinization of the cytosol and stimulates Ca^{2+} release from acidocalcisomes by coupling the activity of Na^+/H^+ and Ca^{2+}/H^+ exchangers. GPN is hydrolysed by cathepsin C increasing the osmolarity of yolk platelets, attracting water and leading to osmotic lysis and Ca^{2+} release. Both acidocalcisomes and yolk platelets possess bafilomycin A_1 (Baf)-sensitive vacuolar-type H^+-ATPases to acidify the organelles. Poly P is hydrolysed after alkalinization of acidocalcisomes.

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