 

**Supplementary Figure 1** [Left image] To visualise intracellular levels of calcium [Ca2+]i pre- and post- activation, the calcium indicator Fluo-3 AM was used. Coelomic fluid from *Psammechinus miliaris* was extracted into an equal volume of anti-coagulant (20mM Tris-HCl, 0.5M NaCl, 70mM EDTA, pH 7.5) and incubated with 5 μM Fluo-3 AM and 0.1% PluronicTM F68 for 30 min at 4oC, followed by centrifugation at 170 x *g* for 5 min and re-suspended in ACF to a final cell number of 1x 106 mL-1. The non-ionic detergent, PluronicTM F68, facilitates dispersion of AM esters in the medium[1], and, incubating the coelomocytes at 4oC helps to prevent non-specific labelling of intracellular organelles. Labelled coelomocytes (200 μL) were placed into chambers of an Ibidi µ-slide and allowed to settle for 20 min at room temperature. Ionomycin (10 μM) was perfused into the chamber and calcium was visualised as an increased green fluorescence signal that results from it binding to Fluo-3. [Right image] Living coelomocytes removed from PBS-injected *Paracentrotus lividus*. An intact red spherule cell can be seen alongside a small phagocyte. Scale bar represents 10 μm. As the cell flattens the individual granules become more distinct.

[1] Masiera, N., Buczyńska, J., Orzanowska, G., Piwoński, H. and Waluk, J. (2014). Enhancing fluorescence by using pluronic block copolymers as carriers of monomeric porphycenes. *Methods and Applications in Fluorescence*, *2*(2), 024003.

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**Supplementary Figure 2** Degranulation response of red spherule cells from *Psammechinus miliaris* (*n = 3*) following treatment with microbial ligands (25 µM) and ionomycin (10 µM) *in vitro* under different calcium regimes. Following removal and enumeration of coelomocytes (see methods) samples were re-suspended in regular artificial coelomic fluid (ACF) containing 10 mM Ca2+, ACR without Ca2+ added (i.e. nominal) and ACF with 5 mM EGTA and no Ca2+. Data are expressed as mean values + 95% CI. Unshared letters and an asterisk (control vs. ionomycin) indicate significant differences (p < 0.05).



**Supplementary Figure 3** Antimicrobial activities of red spherule cell lysates in the presence/absence of ferric iron (Fe3+). Data from the main text (Figure 6) is expanded to include values where RSC lysate and ferric ammonium citrate (0.05% w/v) are exposed to microbes prior to plating on agar (orange bars). In this instance, the presence of iron appeared to prevent/inhibit the putative antimicrobial properties of echinochrome. Bars represent mean values with 95% CI, *n = 3*. An asterisk indicates a significant difference when compared to the control (p < 0.05).

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**Supplementary Figure 4** Echinochrome A assay. Absorbance of *Paracentrotus lividus* coelomocyte samples (*n = 5*) following treatment with microbial ligands (25 µM) and ionomycin (2 µM) *in vitro*. These two wavelengths (346 and 480 nm) correspond to the peak absorbance values of echinochrome a (derived from red spherule cells). Data are expressed as mean values + 95% CI.