MATERIALS AND METHODS

Lingulodinium polyedra

The dinoflagellate *Lingulodinium polyedra* (MCCV 130) was originally collected from the "Baie de Vilaine" and cultured at the Mediterranean Culture Collection of Villefranche (MCCV). Cultures were maintained in 75 mL of L1 medium [1] prepared with sterile and aged sea water adjusted to a salinity of 34. Cultures were maintained at 22°C, under a 14/10 light/dark cycle with a light intensity of 250 $\mu mol.m^{-2}.s^{-1}$. Cells of *L. polyedra* used for the experiments were collected from the cultures after 14 days. They were counted in 12 mL aliquots (triplicates) fixed with acidic lugol (4% v/v) using a liquid particle counter (HIAC/Royco 9703, Pacific Scientific Instruments) with a size range of 2-80 μm^2.

Copepods

The harpacticoid copepod *Sarsamphiascus* cf. *propinquus* (Sars, 1906) was collected from the Marinières site (Bay of Villefranche-sur-Mer, N-W Mediterranean, 43°42'21.51"N-7°19'07.44"E) using a WP2 net towed over the macroalgal cover. Copepods were maintained in 10 L tanks in 0.2-µm filtered aged seawater (salinity 38) at 22°C in the dark. They were fed three times a week with a mixture of the microalgae *Dunaliella salina* (MCCV 020) and *Tisochrysis lutea* (CCAP 927/14).

cDNA sequencing and expression studies

For RNA extraction, L. polyedra were flash-frozen and ground using the TissueLyser LT (Qiagen Inc.) for 2 min at 50Hz. Samples were centrifuged at 13000 g, for 20 min, at 4°C to remove debris. Total RNA was extracted with the PureLink™ RNA Mini Kit (Ambion-Life Technologies/Thermo Fisher Scientific) following the manufacturer's instructions and were quantified using a Nanodrop (Life Technologies/Thermo Fisher Scientific). RNAs were reverse-transcribed using iScript™ cDNA Synthesis Kit (Bio-Rad Laboratories) for 30 min at

42 °C. The reverse transcriptase was inactivated for 5 min at 85 °C. The cDNAs were amplified by Phusion DNA Polymerase (New England Biolabs, Ipswich,UK) with the 5X Phusion GC Buffer (New England Biolabs, Ipswich,UK) and specific primers designed according to the GenBank sequence JO713726.1. Amplified cDNA was sequenced by Eurofins and deposited as LpMIF under the GenBank accession number MN911288.

For RT-qPCR, transcripts were amplified by Takyon™ qPCR SYBR® MasterMix (Eurogentec, Seraing, Belgium, #UF-NSMT-B0701) following the manufacturer's instructions. Primers for RT-qPCR were designed with Primer3Plus at www.bioinformatics.nl [2]. Expression levels of LpMIF were normalized to expression levels of internal reference genes encoding, *L. polyedra* actin (Lpactin, GenBank accession number AY423582.1), and glyderaldehyde-3-phosphate dehydrogenase (LpGAPDH, GenBank accession number AY028562.1). Amplification efficiencies were assessed for each amplicon and the relative expression ratios were calculated using the relative quantification method [3].

Sequence analysis

The physico-chemical parameters of molecular weight and theoretical isoelectric point (pI) of the deduced protein were computed using the ProtParam tool (http://web.expasy.org/protparam/) [4]. The functional domains and important sites of the protein were predicted by InterPro [5] and PFAM [6] softwares. The transmembrane regions and their topology were predicted using a set of 5 transmembrane prediction algorithms: TMHMM Server [7], HMMTOP [8], TMpred [9], ΔG Prediction server [10]and Phobius [11].

Plasmid design for Agrobacterium-mediated transformation-subcellular localization in an heterologous system

LpMIF with and without the transmembrane domain, were amplified from *L. polyedra* cDNA with Gateway-compatible adaptor primers, cloned into the entry vector pDON207

(Invitrogen) and transferred into the destination GFP fusion vector pK7WGF2 (Plant Systems Biology, VIB, Gant,Belgium, [12]) using the BP and LR reaction protocols (Invitrogen™/ Thermo Fisher Scientific, #11789020 and #11791100). All the constructs were validated by sequencing (Eurofins GATC, Konstanz, Germany) and transformed into *Agrobacterium tumefaciens* strain GV3301. Transformants were selected using gentamycin (25µg/mL (Sigma-Aldrich, #G1264)), rifampicin (50µg/mL (Sigma-Aldrich, #R7382)) and spectinomycin (100µg/mL (Duchefa Biochemies, #S0188)). Recombinant strains were grown in YEB medium with the above-mentioned antibiotics at 28°C to an OD600 of 1.5. After centrifugation, the pellet was recovered in infiltration buffer (150µM MES hydrate pH5.7 (Sigma-Aldrich, #M2933), 100mM MgCl2, 100µM acetosyringone (Sigma-Aldrich # D134406)[13].

Agrobacterium-mediated transient expression was carried in *N. benthamiana.* Epidermal leaf cells were syringe-infiltrated with *A. tumefaciens* at a final OD_{600} = 0.1, according to a classical procedure described classically such in Evangelisti at al.[14]. Two days after infiltration, leaf discs were observed by a laser confocal microscope (Carl Zeiss MicroImaging GmbH, Jena, Germany, #LSM880) using an excitation at 488 nm.

Western blots

L. polyedra cells were transferred to 300µl of protein extraction buffer (100 mM Tris pH7.4, 10µM KCl, 10µM EDTA, 0.1% Tergitol (Sigma-Aldrich, Saint-Louis, USA #NP40S) and Plant Protease Inhibitor Mix (Sigma-Aldrich #P9599). Samples were ground using the TissueLyser LT (Qiagen Inc., Valencia, CA, USA) during 2 min at 50Hz. After 20 min under gentle rotation on ice, tubes were centrifuged at 13000g, during 20min, at 4°C, to remove cell debris. The supernatant was transferred to an ultracentrifugation tube and centrifuged at 100 000g in a fixed angle rotor, during 1h at 4°C to pellet. The pellets, containing cell

membranes, were washed with 1mL of extraction buffer and resuspended in 100 µl of 10% SDS and 250µl of extraction buffer. For the immunodetection of proteins excreted in the sea water, 5000 cells of *L. polyedra* were separated from sea water after a centrifugation (4,500xg, 2 mins). 50 mL sea water, were desalted and concentrated by successive centrifugations on Centricon tubes (Vivaspin-6, 5,000 MWCO, Sartorius, Göttingen, Germany, #VS0611), to a final volume of 200µl. Protein concentrations were measured using the Pierce™ 660nm Protein Assay (#22662) according to manufacturer's instructions. Ten micrograms of proteins were loaded on 12% Mini-PROTEAN® TGX™ precast gels (Bio-Rad Laboratories, Munich, Germany, #4561045) using Laemmli buffer 2X without reducing agent (Bio-Rad Laboratories, #1610737), and separated under non-denaturing conditions, to allow potential visualization of MIF oligomers. Proteins were then transferred to a 2µm PVDF membrane (Bio-Rad Laboratories, #1704157EDU) using a semi-dry blotting system. Blots were blocked 1h at room temperature (RT) with 5% milk/ PBS-T and then incubated in 5% milk/ PBS-T, over-night at 4°C with a custom-made polyclonal antibody raised against two LpMIF peptides at 1:5000 or 1:1000. The design and synthesis of antigenic peptides as well as polyclonal antibody production and purification were performed by Proteogenix (http://www.proteogenix.fr/). After 3 washes with PBS-T, blots were incubated in 5% milk/PBS-T, 45 min at RT with a secondary antibody at 1:10 000 (goat anti-rabbit IgG-HRP, #AS09 602 Agrisera, Vännäs, Sweeden). Proteins were visualized using a chemiluminescence detection kit, Luminata™ Forte Western HRP substrate (Merck Millipore, Watford, UK, #WBLUF0100) following the manufacturer's instructions.

Mass spectrometry

Mass spectrometry was performed by the Protein Science Facility (SFR Biosciences, Lyon, France). Briefly, following protein precipitation step (using trichlororoacetic acid 20% in volume, overnight at 4°C), samples were washed twice in acetone and solubilized in 8M

urea. Samples were reduced (Tris(2-CarboxyEthyl)Phosphine (Sigma-Aldrich, #C4706), 5 mM, 57°C, 1h), alkylated (iodoacetamide (Sigma-Aldrich, #I1149) 10 mM, RT, 45 min), and digested overnight at 37°C with trypsin (1/100 ratio). Peptides digest was next desalted using C18 spin column (Thermo Fisher Scientific). Peptides were dried in a speed-vac and suspended in 50 µL 0.1% HCOOH before nanoLC-MS/MS analysis. Samples were analyzed using an Ultimate 3000 nano-RSLC (Thermo Fisher Scientific) coupled on line with a Q Exactive HF mass spectrometer via a nano-electrospray ionization source (Thermo Fisher Scientific). 1 µL of peptide mixtures was loaded on a C18 PepMap100 trap-column (300 µm ID x 5 mm, 5 µm, 100Å, Thermo Fisher Scientific) for 3.0 minutes at 20 µL/min with 2% ACN, 0.05% TFA in H_2O and then separated on a C18 Acclaim PepMap100 nano-column, 50 cm x 75 µm i.d, 2 µm, 100 Å (Thermo Fisher Scientific) with a 60 minutes linear gradient from 3.2% to 40% buffer B (A: 0.1% FA in H_2O, B: 0.1% FA in ACN) and then from 40 to 90% of B in 2 min, hold for 10 min and returned to the initial conditions in 1 min for 15 min. The total duration was set to 90 minutes at a flow rate of 300 nL/min. The oven temperature was kept constant at 40°C. Sample were analysed with TOP20 HCD method: MS data were acquired in a data dependent strategy selecting the fragmentation events based on the 20 most abundant precursor ions in the survey scan (350-1600 Th). The resolution of the survey scan was 60,000 at m/z 200 Th. The Ion Target Value for the survey scans in the Orbitrap and the MS^2 mode were set to 3E6 and 1E5 respectively and the maximum injection time was set to 60 ms for both scan modes. Parameters for acquiring HCD MS/MS spectra were as follows; collision energy = 27 and an isolation width of 2 m/z. The precursors with unknown charge state or a charge state of 1 were excluded. Peptides selected for MS/MS acquisition were then placed on an exclusion list for 20 s using the dynamic exclusion mode to limit duplicate spectra.

Proteins were identified by database searching using Sequest HT with Proteome Discoverer 2.2 software (Thermo Fisher Scientific) against the uniprot *Lingulodinium polyedra* database (50 entries, June 2019) and LpMIF sequence. Precursor mass tolerance was set at 10 ppm and fragment mass tolerance was set at 0.02 Da, and up to 2 missed cleavages were allowed. Oxidation (M), acetylation (Protein N-terminus) were set as variable modification, and Carbamidomethylation (C) as fixed modification. Proteins were filtered with a fixed value PSM validator.

Stress bio-assays

To investigate *L. polyedra* stress responses, three experimental samples were compared: (i) *L. polyedra* cells; (ii) *L. polyedra* exposed to the copepods for 24 hours; and (iii) *L. polyedra* exposed to the copepods for 24 hours and then maintained under normal conditions (without copepods) for a week. Each treatment was replicated 3 times. All samples were maintained in 75 mL flasks containing 50 mL of autoclaved, aged and 0.2-µm filtered seawater (salinity 34), at 22°C. Prior to the experiments, copepods (adults and late copepodites) were collected from the culture tanks, rinsed twice in seawater and transferred to each flask (50 individuals per flask). They were left for 2 days without food to allow gut clearance and salinity acclimation before the experiments. *Lingulodinium polyedra* cells were added in each flask at a final concentration of 100 cells.mL^{-1}. All samples were maintained in 75 mL flasks containing 50 mL of autoclaved, aged and 0.2-µm filtered seawater (salinity 3.4%), at 22°C. After 24 hours of exposure, 2 mL of *L. polyedra* were sampled for each replicate to study the photosystem II (PSII) function using a Multi-Color Pulse-Amplitude-Modulated analyser (MC-PAM, Heinz Walz Gmbh, Effeltrich, Germany) by measuring the maximum quantum yield of PSII (Fv/Fm ratio) [15,16]. Prior to fluorescence measurement, samples were dark-acclimated at 24 °C for 15 min to re-open PSII reaction centres and relax non-photochemical quenching.

147 **Immunolocalization**

148 *L. polyedra* cells were centrifuged to remove sea water at 1000g for 5min. They were
149 fixed in a 4% formaldehyde/PBS solution (Sigma-Aldrich, #252549) at RT for 45min.
150 Following fixation, cells were washed twice in PBS (Corning-Mediatech, Inc., Manassas,
151 USA #21-040-CM) with 0.1% Triton and permeabilized during 10min with 0.25%
152 Triton/PBS (Sigma-Aldrich #T9284). After 3 washes with 0.1% Triton/PBS (5 min, 1mL
153 each), the samples were blocked with 1% BSA (Sigma Aldrich, #A2153)/PBS at least 1h at
154 RT, with gentle rotation. They were incubated with MIF antibody (1:1000) over night at 4°C
155 in1% BSA/PBS under agitation. *L. polyedra* were washed three times in PBS and incubated
156 with 1:1000 goat anti-rabbit secondary antibody conjugated to Alexa-Fluor 488 (Invitrogen-
157 Life Technologies/Thermo Fisher Scientific, Waltham, USA, #A11008). After washing three
158 times with PBS, the samples were mounted in chambered cover glass Lab-Tek II (Life
159 Technologies/Thermo Fisher Scientific #A155411) and observed by confocal microscopy
160 (Carl Zeiss MicroImaging GmbH, Jena, Germany, #LSM880). To control for potential non-
161 specific labelling, two additional samples were systematically prepared: samples where the
162 primary antibody was either omitted, or replaced by pre-immune serum.

163 Images of fluorescent cells were converted by the LSM Image Browser software, ZEN
164 2011, Blue edition (Carl Zeiss) into JPEG files (8 bit black and white quality). Fluorescence
165 was measured with ImageJ (cut off > 10 pixels) [17]. Results are shown as the ratio of
166 fluorescent vs total area. Nine images were analysed per condition, corresponding to the
167 examination of approximately 20 independent dinoflagellates. All images, including those
168 converted for ImageJ analyses, have been deposited in Mendeley Data
169 (http://dx.doi.org/10.17632/5j4k6r79ys.1).

170 ***Immunogold labelling and Transmission Electron Microscopy (TEM)***

For ultrastructural immunocytochemistry, cells were fixed with 4% paraformaldehyde (PAF) or 4% PAF and 0.2% glutaraldehyde (PG) in phosphate buffer 0.1 M (pH 7.4) (Sigma Aldrich). They were then dehydrated in ethanol series and embedded in acrylic resin (LR-WHITE (Electron Microscopy Sciences- EMS) before sectioning. For immunogold labelling, grids were deposited face down on the top of small drops of the following solutions: PBS containing 50 mM NH4Cl for 10 min, PBS containing 1% BSA and twin 20 for 10 min, PBS containing the relevant mAbs in 1% BSA and tween 20 for 1 h, PBS containing 0.1% BSA for 10 min, PBS 10 min, PBS containing 1% BSA and tween 20 with 15 nm protein-A gold conjugated (PAG-15nm Cell Microscopy Core, Utrecht, Netherland) for 30 min, PBS containing 0.1% BSA for 5 min, PBS for 5 min twice, PBS containing 1% glutaraldehyde for 5 min and distilled water for 5 min. For controls, only the primary antibody was omitted. The sections were then contrasted with uranyl acetate (4% in water) and visualized using a JEM 1400 Electron Microscope (JEOL, Tokyo, Japan) operating at 100kV equipped with a MORADA SIS camera (Olympus, Tokyo, Japan).

Scanning Electron microscopy (SEM)

Cell pellets were fixed with a 1.6% glutaraldehyde solution in 1:1 mixture of 0.2 M sodium cacodylate buffer (pH 7.4) and artificial sea water at room temperature and then stored at 4°C. After three washes in distilled water, cells were filtered on a 0.2 µm isopore filter (Merck Millipore, Carrigtwohill, Ireland). Samples on filters were subsequently dehydrated in a series of ethanol baths (70%, 96 %, 100% three times, 15 min each). After a final bath in hexamethyldisilazane (Carl Roth GmbH, Karlruhe, Germany) (HMDS, 5 minutes), samples were left to dry overnight. Samples on filters were mounted on SEM stubs with silver paint and coated with platinum (3 nm) prior to observation. SEM observations were performed with a JSM-6700F SEM (JEOL) at an accelerating voltage of 3 kV.

Quantification and statistical analysis

All experiments were repeated three times independently. Kruskal-Wallis tests were used to compare the photosystem II activity between the different experimental conditions (control, stress, post-stress). Fluorescence signal intensities between the three different conditions (control, stress, unstressed) were compared using the Kruskal-Wallis statistical test (* = $p \leq 0,05$, ** = $p \leq 0,01$, *** = $p \leq 0,001$). Data are presented as bars of relative fluorescence. Differences in gene expression levels were tested for statistical significance by one-way ANOVA and Tukey-Kramer tests (Software Prism v.5.0, GraphPad). Expression levels of the target genes are presented as a relative expression normalized to internal reference genes and to expression in the control sample.

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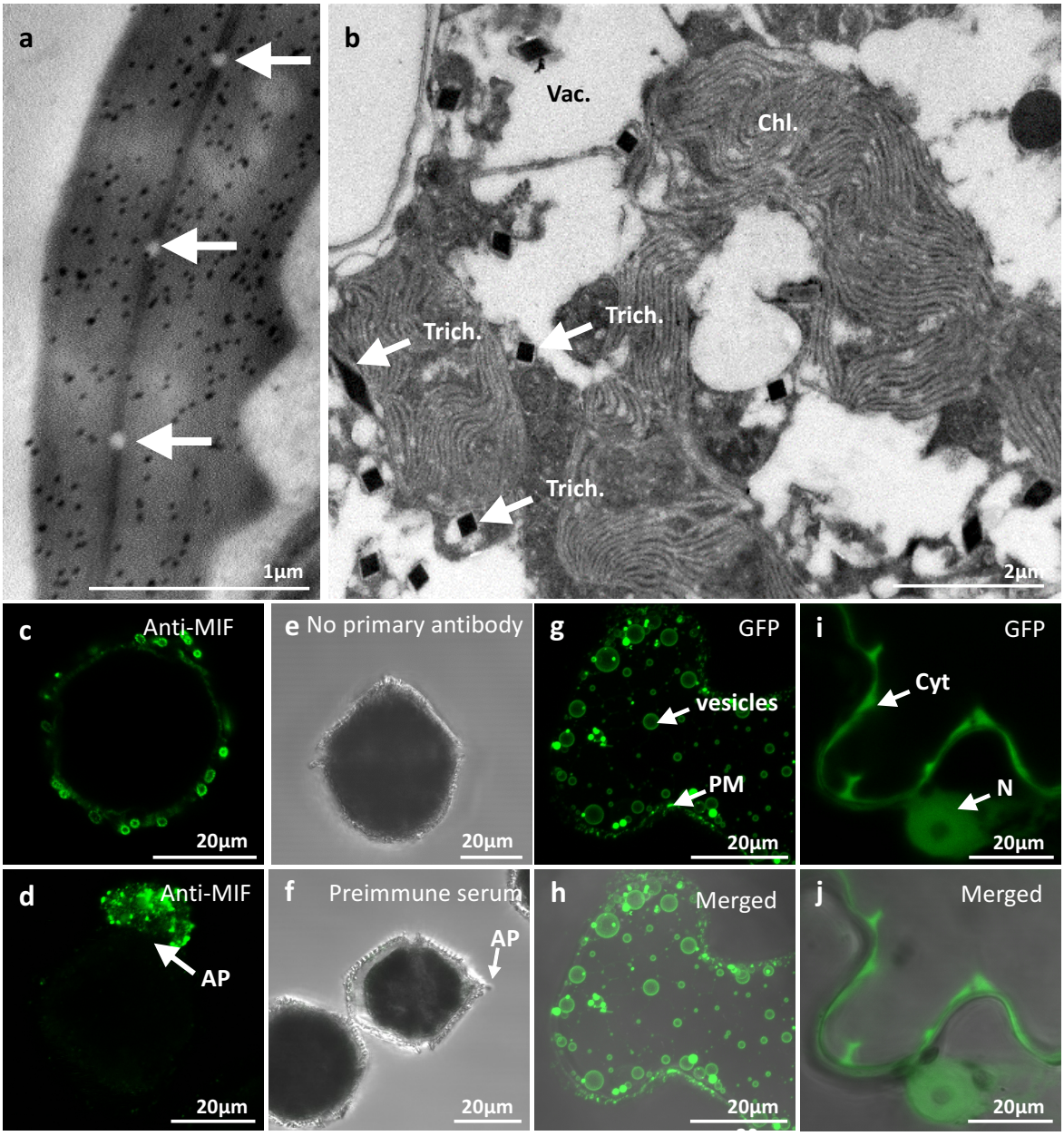

Figure S1. (a,b) Immunogold-labelled electron micrographs of *L. polyedra*. (a) Representative section of the apical pore showing an important accumulation of gold particles (black dots). The secretory pores are indicated by white arrows. (**b)** Representative image of *L. polyedra* cell content showing a vacuole (vac.), chloroplast (chl.) and the typical sections of the trichocysts (Trich.). Note that no labelled LpMIF (black dots) are observed. **(c,d)** Images corresponding to Figure 2g and h but only with LpMIF labelled in green (Alexa 488®). **(e,f)** Negative control samples directly exposed to the secondary antibody (but no primary antibody) or exposed to pre-immune serum prior to secondary antibody. No labelling was detected in either case. The white arrow indicates the apical pore**. (g-j)** Subcellular localization of LpMIF fused at the N-terminus to GFP, and expressed with (**g,h**) or without the transmembrane domain (**i,j**) in *Nicotiana benthamiana*. (**g,h)** GFP:LpMIF with its endogenous transmembrane domain localizes to vesicle membranes and accumulates in the plasma membrane (PM). **(i,j)** The MIF domain of LpMIF (GFP:LpMIF_TM) without the transmembrane domain localizes to the cytoplasm (Cyt) and diffuses passively into the nucleus (N).

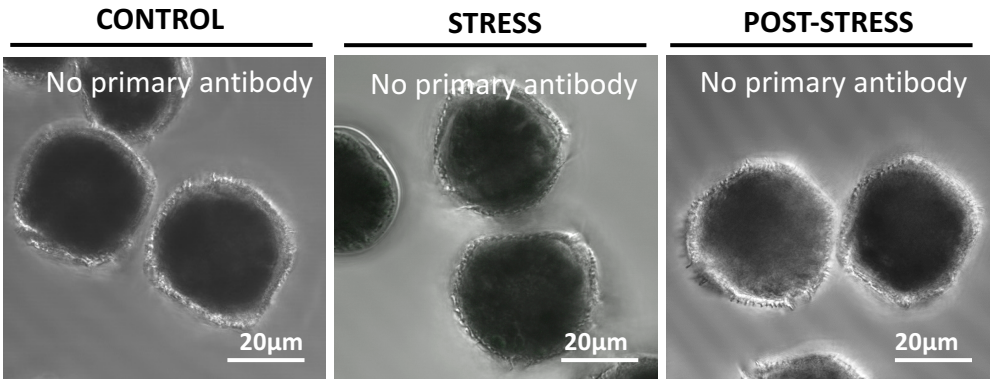

Figure S2. Negative control samples of unstressed (Control) and stressed *L. polyedra* (in the presence of copepods), and after recovery (Post-Stress). Samples were directly exposed to the secondary antibody without the primary antibody.