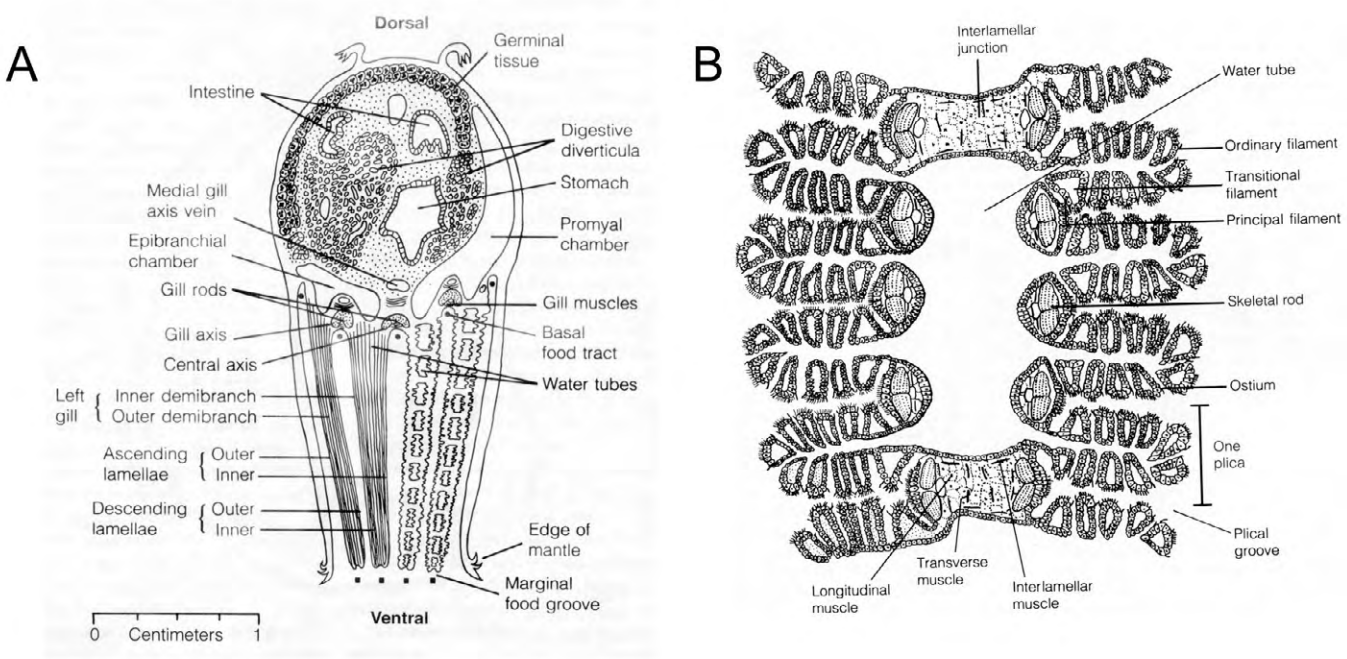

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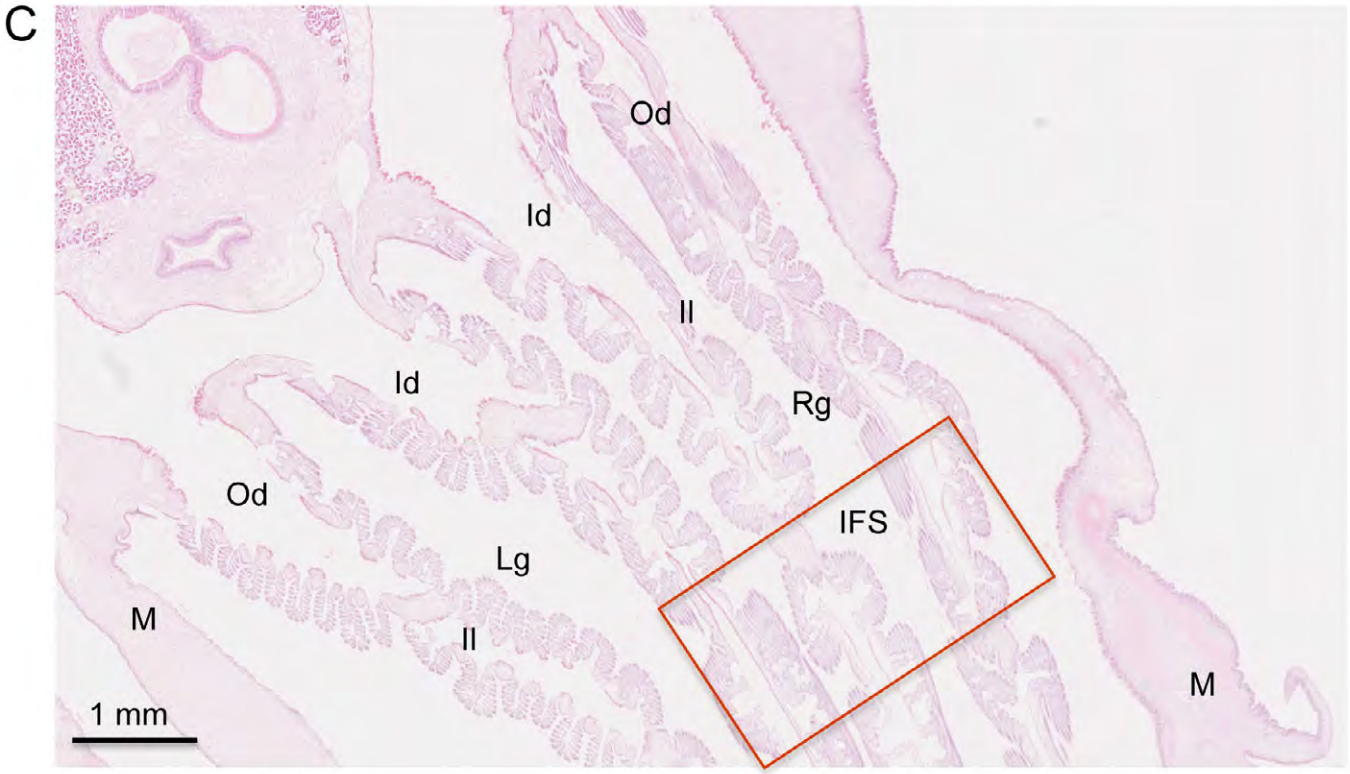

Fig. S1. General structure of the oyster gill. (A) Oyster gills are made of two V-shaped demi-branches, each composed of an ascending and a descending lamella delimiting a water tube and linked by inter-lamellar junctions. (B) Each lamella is a succession of folded structures, termed plicas, which are made of the repetition of a tubular structural unit called filament. The central part of the filament is occupied by a sinus, a space filled with hemolymph, while the basal part of the filament consists of a more or less regular layer of tightly-packed and non-ciliated cells usually referred to as epithelium. (C) Hematoxylin/eosin stained cross-section of the oyster gill and its attachment region scanned at low magnification using a Nanozoomer (Hamamatsu, Massy, France). Id, inner demi-branch; IFS, irregularly folded structure; Il, inter-lamellar junction; M, mantle; Lg, left gill; Od, outer demi-branch; Rg right gill. Credit for the drawings to Maryland Sea Grant, The American oyster *Crassotrea virginica*, editors: VS Kennedy, RIE Newell, AF Eble, Mechanisms and physiology of feeding, RIE Newell and CJ Langdon, illustrated by D Kennedy, modified from Galtsoff (1964).

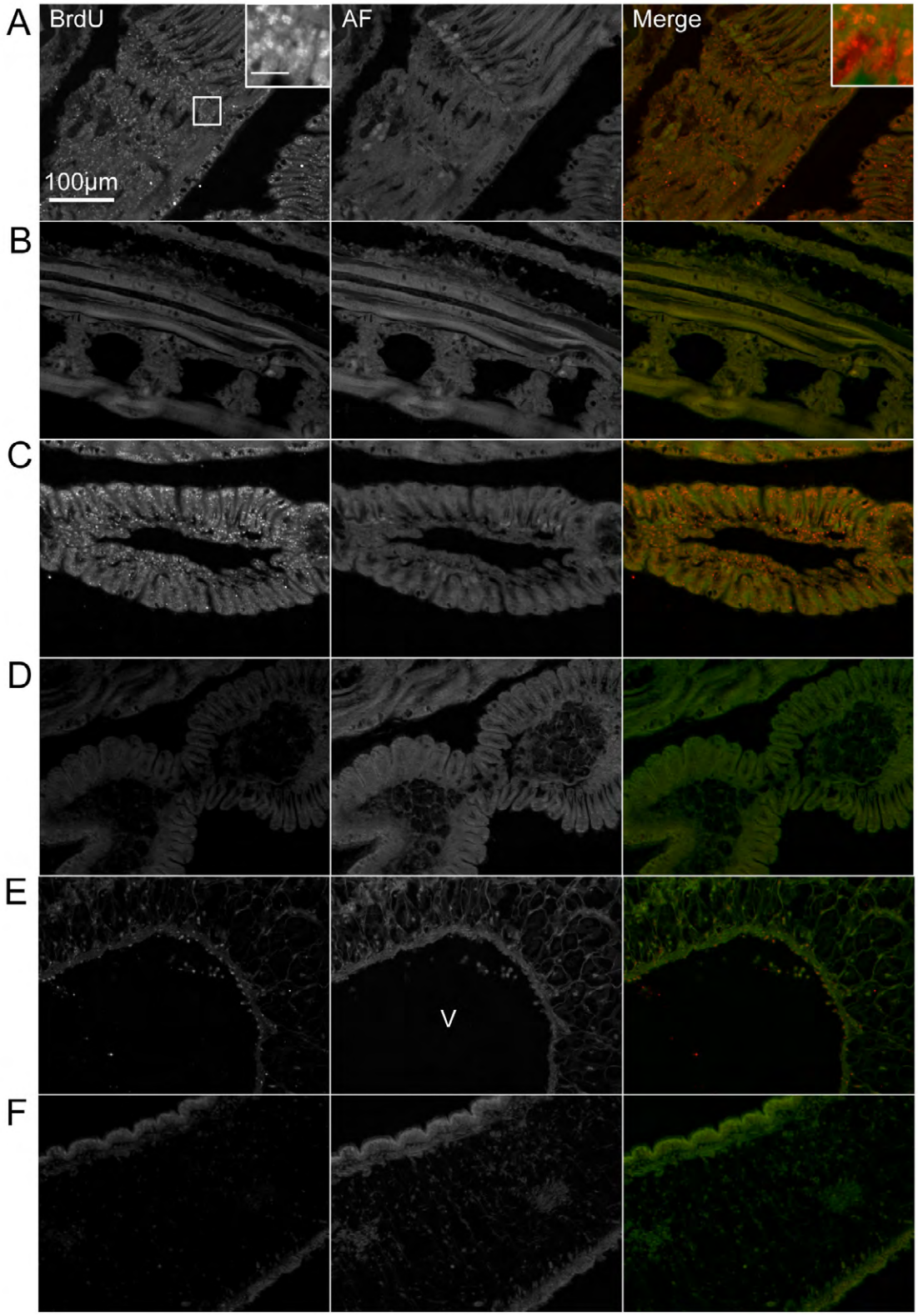

Fig. S2. Relative BrdU incorporation in the oyster gill and mantle cells. Oysters were injected with BrdU (n=3) or seawater (controls, n=3) into the sinus of the adductor muscle. The animal body was fixed in Davidson's fixative 16 hours later. Fluorescence micrographs were recorded at low magnification (x20) in the Cy3 channel for BrdU while the GFP channel is used to outline the tissues through autofluorescence (AF). Images (A,C,E) are representative of the BrdU-injected animals (n=3) and (B,D,F) of the control animals (n=3) (A) BrdU-labeling appears evenly spread in IFS section. (Inset) Intense and punctuated BrdU-labeling shown at higher magnification. Inset scale bar, 20 µm. (B) No BrdU signal on this IFS section from a control oyster injected with seawater. (C) Labeling of a region of the regularly folded gill. (D) No BrdU signal on section of the regularly folded gill from a control oyster injected with seawater. (E) BrdU labeling of a mantle area including a large vessel (V) section. A few scattered positive cells appear on the vessel vicinity. (F) No BrdU signal on this section of the mantle from a control oyster injected with seawater. V, vessel.

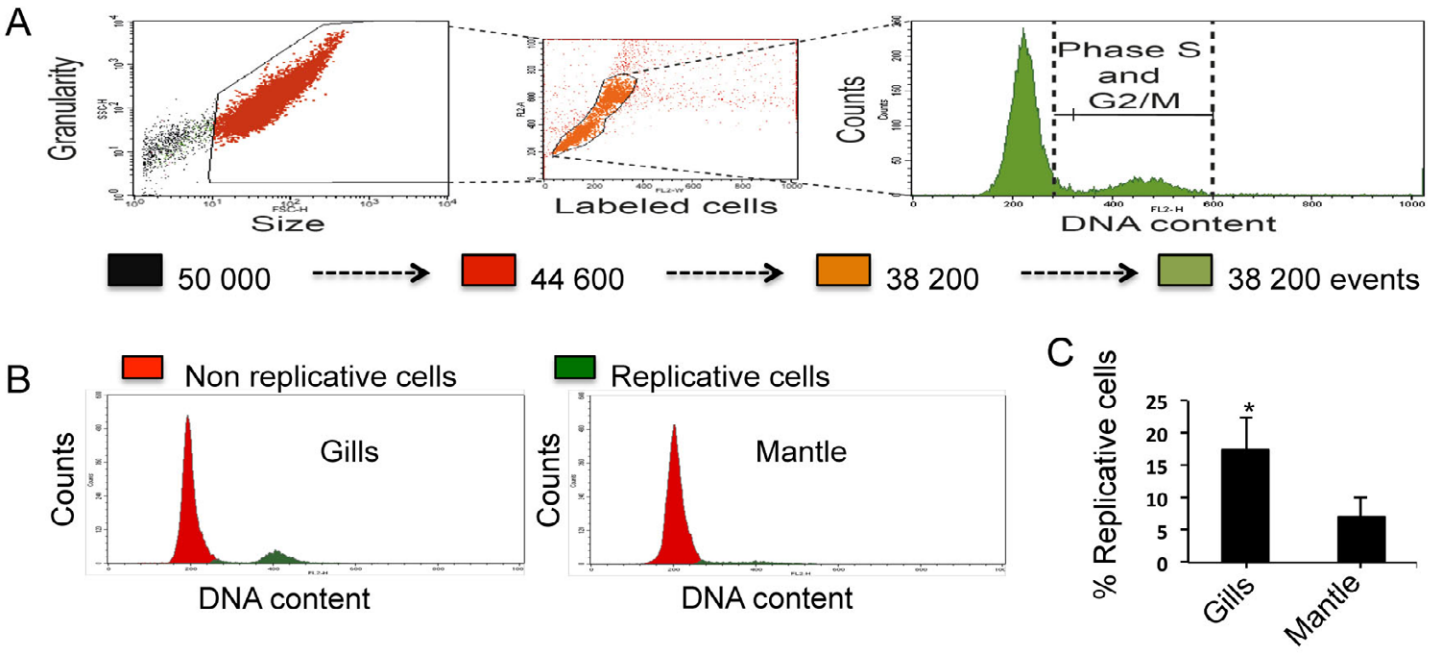

Fig. S3. **FACS analysis of the cell cycle distribution of the oyster gill and mantle cells.** Gills and mantles from oysters (n=6) were minced and digested with Pronase in Hank's buffer for dissociation into individual cells. Cells were fixed and DNA was stained with propidium iodide (PI). (A) Samples were analyzed through Flow Activated Cell Sorting. The gating procedure to isolate a population of individual cells consists in sequentially separating events. Cells were sorted through a normal light scattering to exclude bacteria and debris (granularity versus size; left panel) (50.000 to 44.600 events). Then fluorescent cells were gated according to their size in order to exclude doublets (central panel) (44.6000 to 38.200 events). Gated populations of fluorescent cells are figured according to their cell cycle profile (right panel), the peak of replicative cells corresponding to S and G2/M. (B) Cell cycle profiles for gill and mantle, which are representative of the data obtained through 5 independent experiments. Green peak, replicative cells. (C) Percentage of replicative cells in the gill and mantle cells, which are 17.3±5% and 7.2±3% for gills and mantles (n=6), respectively. Results are reported as means ± s.d. *P<0.01(two-tailed Student's t-test).

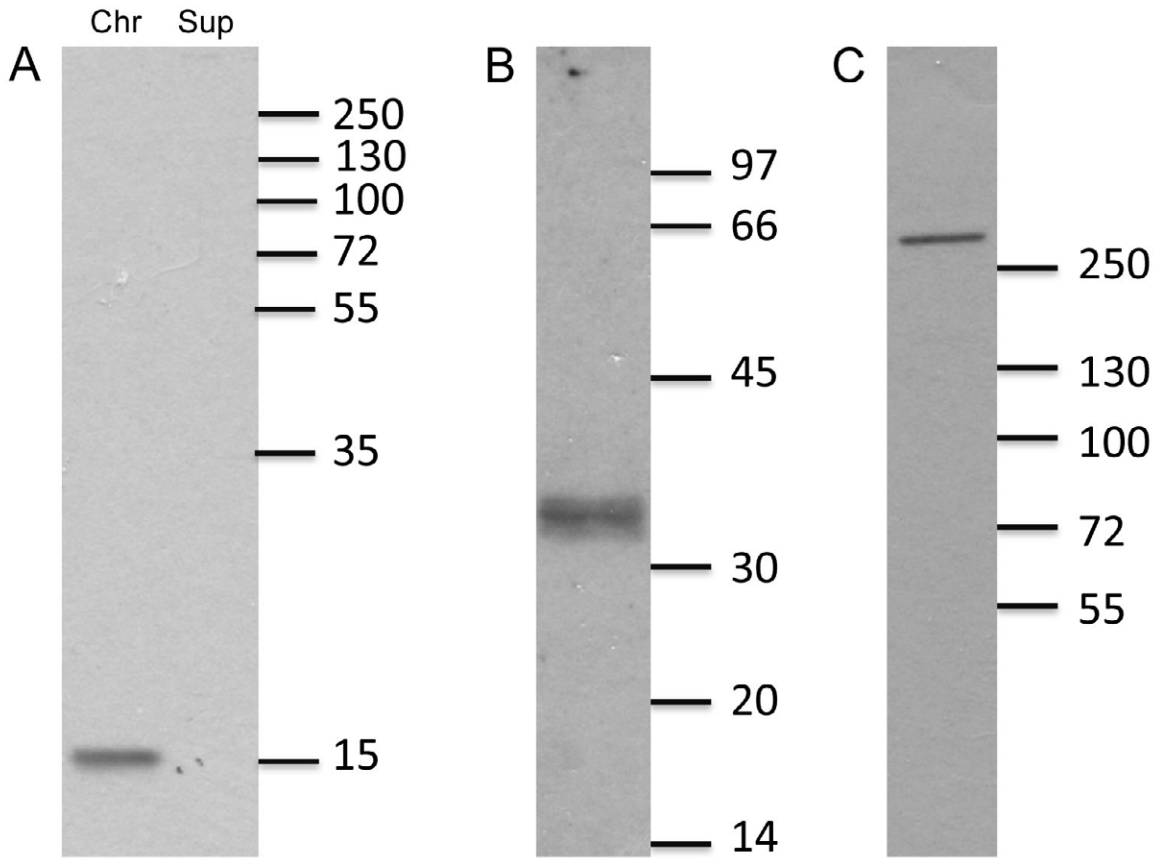

Fig. S4. Antibody specificity for oyster proteins. Immunoblots were carried out against oyster chromatin (A) or tissue extracts (B and C) (50 and 10 µg of total proteins, respectively) transferred to PVDF membrane. (A) H3P rabbit antibody revealed a unique band for histone H3 (15 kDa) on oyster chromatin extract (Chr) while no band was detected on the cytosolic extract (Sup). (B) Rabbit Sox2 antibody revealed a unique band (36 kDa) of the predicted molecular weight for Sox2 (EKC24855) on protein extract. (C) An in-house immuno-purified FLN rabbit antibody revealed a unique band migrating well over the 250 kDa marker in agreement with the predicted molecular weight for the oyster FLN (323 kDa; EKC28512).

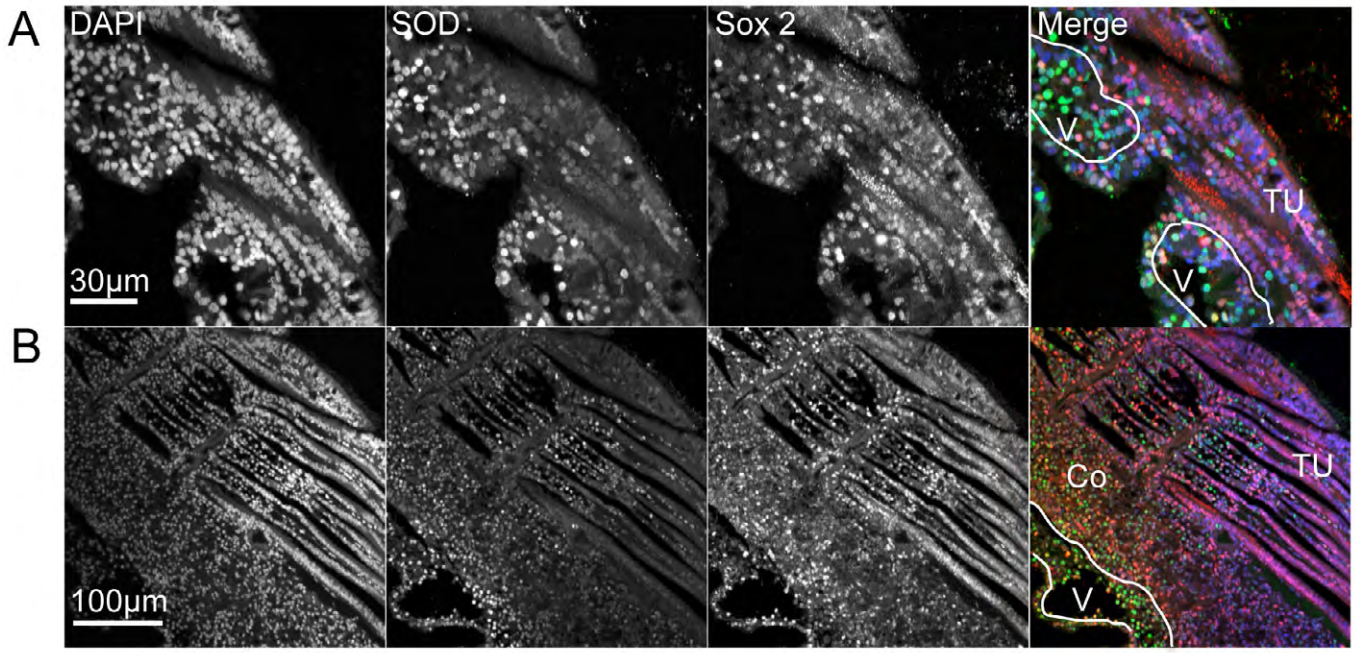

Fig. S5. **Precursor cells differentiate into hemocytes in the oyster gill.** Confocal images of the tubules (A) and convoluted structures (B) of an IFS region using Sox2 (red) and SOD (green) antibodies and DAPI (blue). Sox2-labeled cells are essentially in the tubules region while SOD-labeled cells are essentially in the underlying connective tissue (Co) and vessels (V, white outline). Cells co-labeled for Sox2 and SOD are located in the underlying connective tissue (Co) under the tubules (Tu) and in the neighboring vessel (V). Co connective tissue, V vessel, Tu tubule.

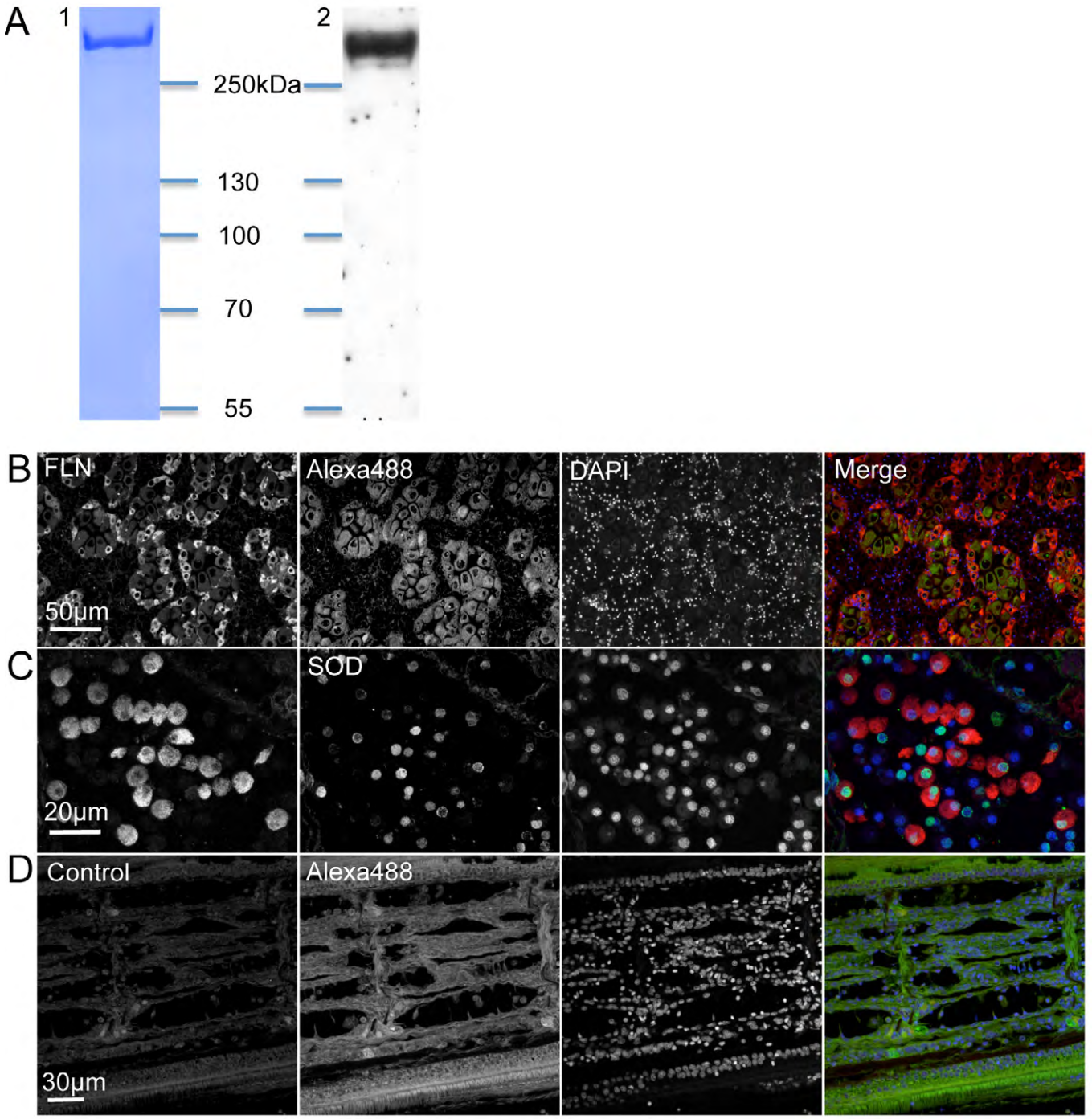

Fig. S6. **Filamin antibody specificity for a sub-population of hemocytes.** (A) Oyster FLN was purified to homogeneity on FPLC. An aliquote of a purified fraction was electrophoresed on a 7% SDS-PAGE. Colloidal Coomassie staining revealed a unique band (panel 1) migrating well above the 250 kDa marker (Thermofisher, Waltham, MA, USA). Western blot using an in-house immuno-purified rabbit FLN antibody (FLNAb) revealed a unique band (panel 2) corresponding to the Coomassie stained band. (B) Oyster cross-section encompassing the gonad submitted to IHC using FLNAb (red) and DAPI (blue). Axillary cells surrounding the oocytes were specifically and intensely labeled for FLN. Autofluorescence (Alexa 488) to outline the tissue structures. (C) Tissue sections stained using the FLN (red) and SOD antibody (green) and DAPI (blue). Image on confocal microscopy of hemocytes in a gill vessel. FLN (red) strongly stained the cytoplasm of a sub-population of hemocytes also stained for SOD (green). (D) Confocal image of a tissue section treated as in B and C but with no primary antibodies.

Table S1. FACS analysis of the DNA content of oyster gill and mantle cells.

Download Table S1

Table S2. Counting of the H3P-positive nuclei on representative regions of the mantle.

Download Table S2

Table S3. Counting of the H3P-positive nuclei on representative regions of the gill.

Download Table S3

Table S4. MS/MS data interpretation. The peak list has been searched against a UniProtKB/Swiss-Prot database of *Crassostrea gigas* combined target-decoy database (created 2013-07-30, containing 26945 target sequences plus the same number of reversed decoy sequences) using Mascot (version 2.4.1, Matrix Science, London, UK). The database contained sequences of human proteins including common contaminants (human keratins and porcine trypsin) and was created using an in-house database generation toolbox (http://msda.u-strasbg.fr). This analysis unambiguously identifies the purified protein as the oyster FLN.

Download Table S4