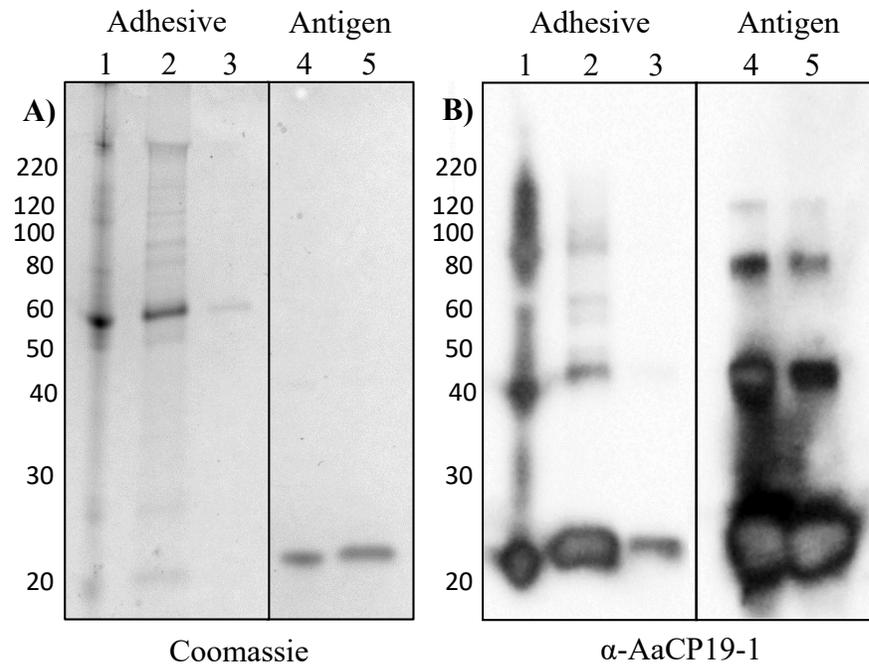
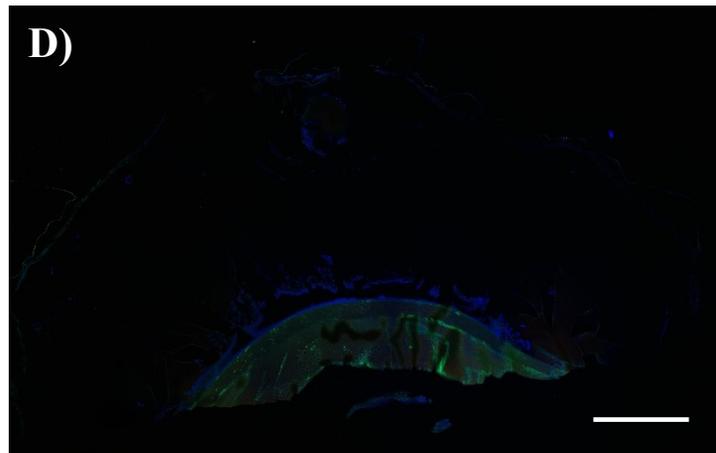
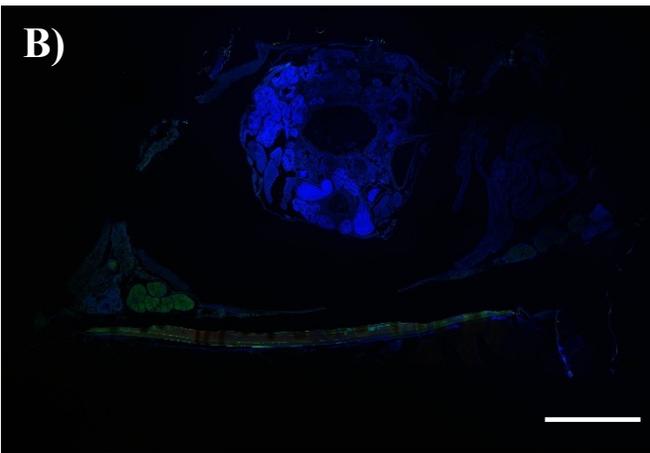
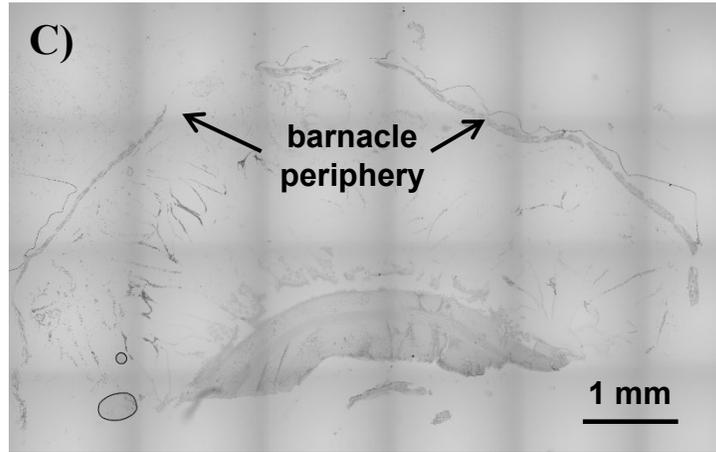
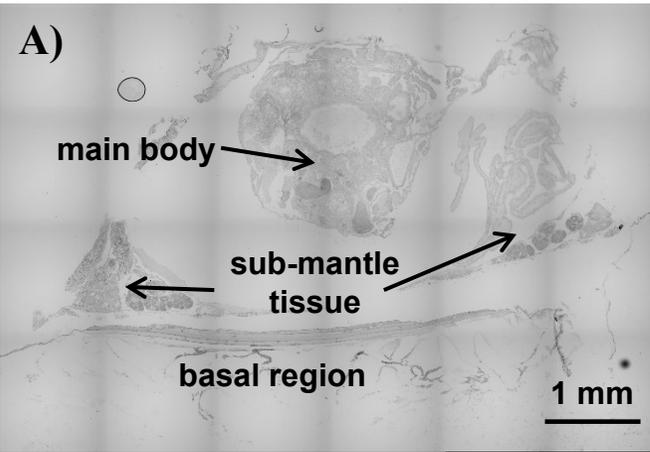


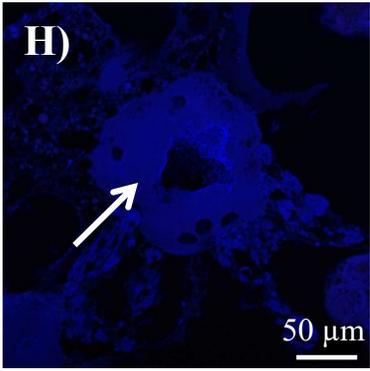
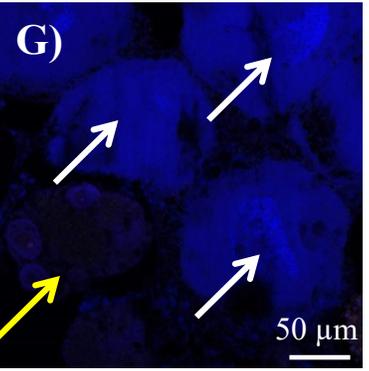
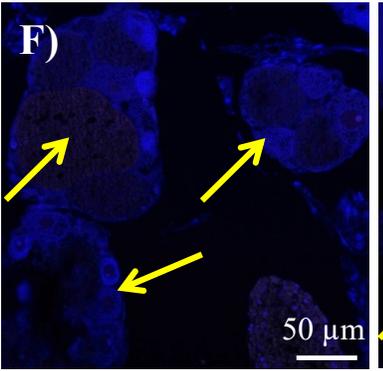
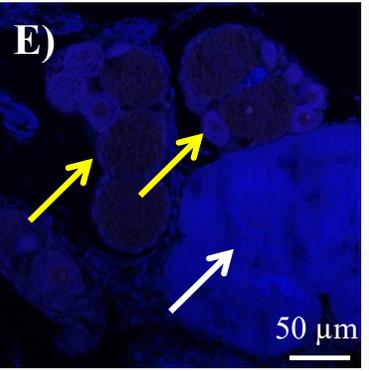
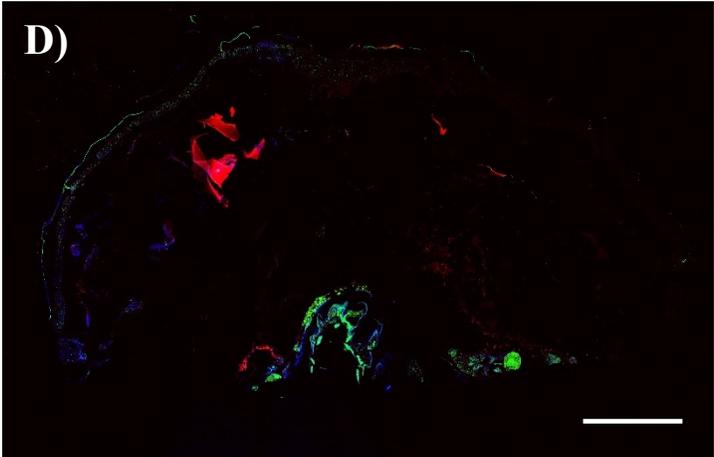
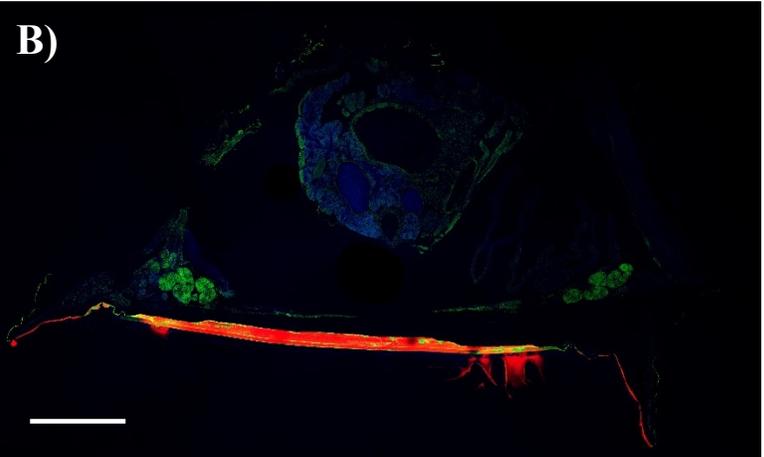
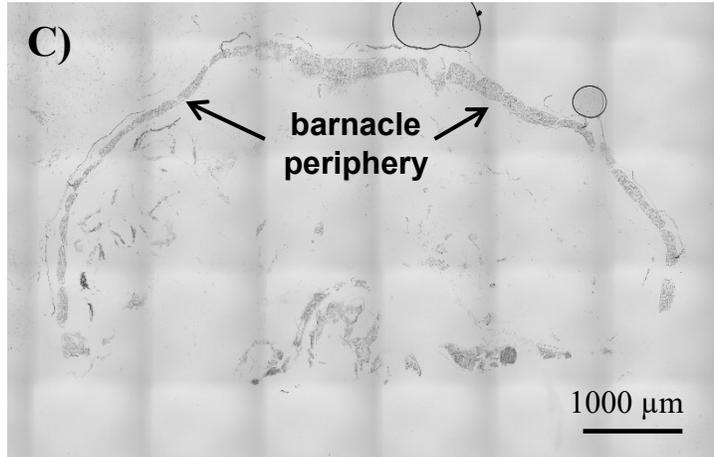
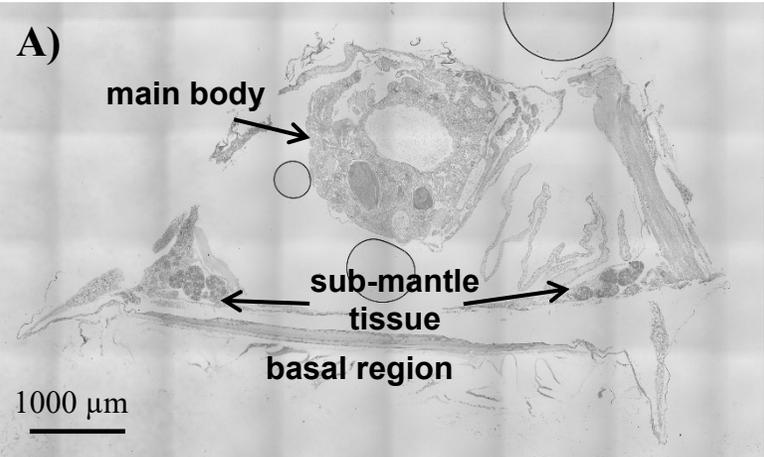
# **Supplementary Figures**



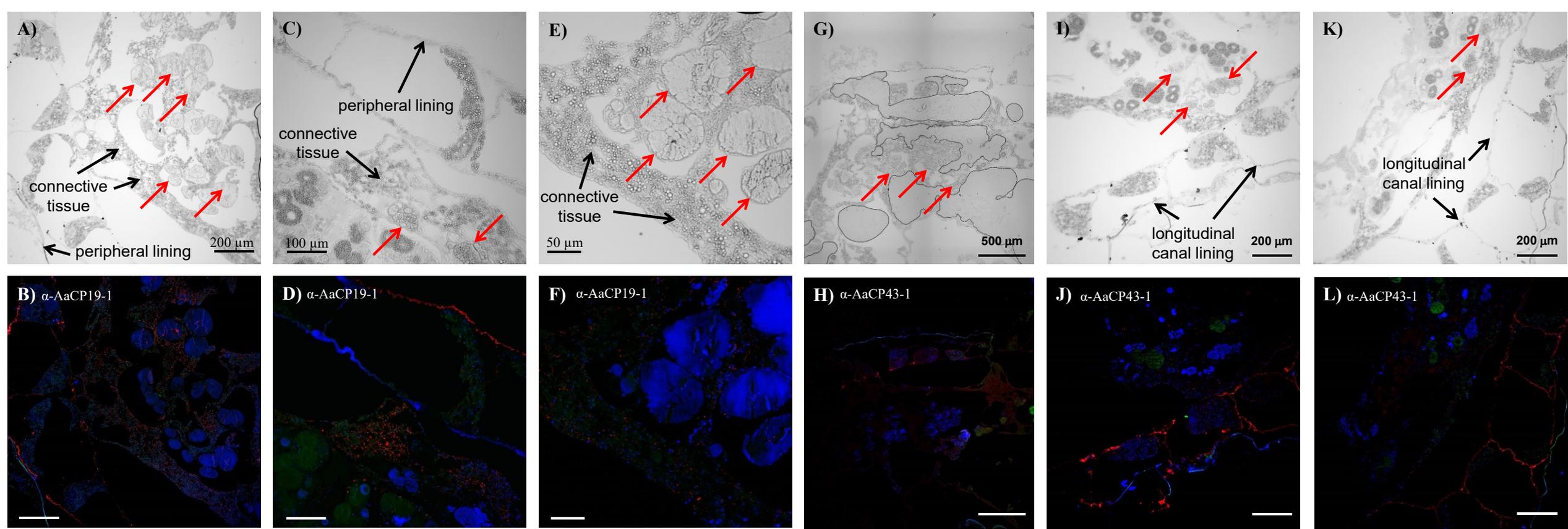
**Supplementary Figure S1.** Immunoblot analysis of *A. amphitrite* adhesive with  $\alpha$ -AaCP19-1. After HFIP solubilization, adhesive proteins were separated by SDS-PAGE gel and stained with Coomassie blue (**A**), then transferred to PVDF membrane and exposed to  $\alpha$ -AaCP19-1 (**B**). Lanes 1-3: adhesive; 1 – undiluted; 2 – 1:100 dilution; 3 – 1:500 dilution. Lanes 4-5: antigen; 4 – 1:400 dilution; 5 – 1:800 dilution.



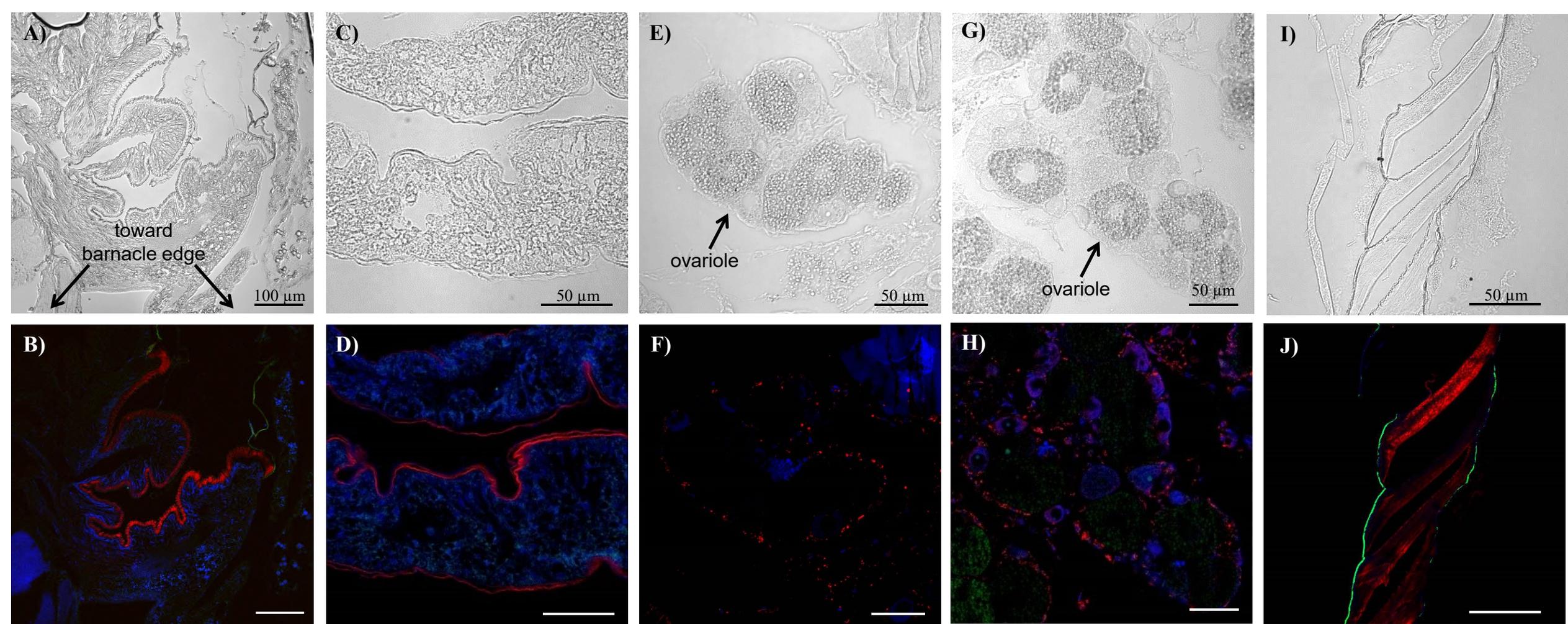
**Supplementary Figure S2.** Bisected *A. amphitrite* images demonstrating no fluorescent bleed-through in the Cy5 (red) channel. **A)** Brightfield and **B)** confocal fluorescent image of a sagittal section. **C)** Brightfield and **D)** confocal fluorescent image of a transverse section close to the barnacle base. In both **B)** and **D)**, DAPI (blue), fluorescein (green), and Cy5 (red) emission channels were collected.



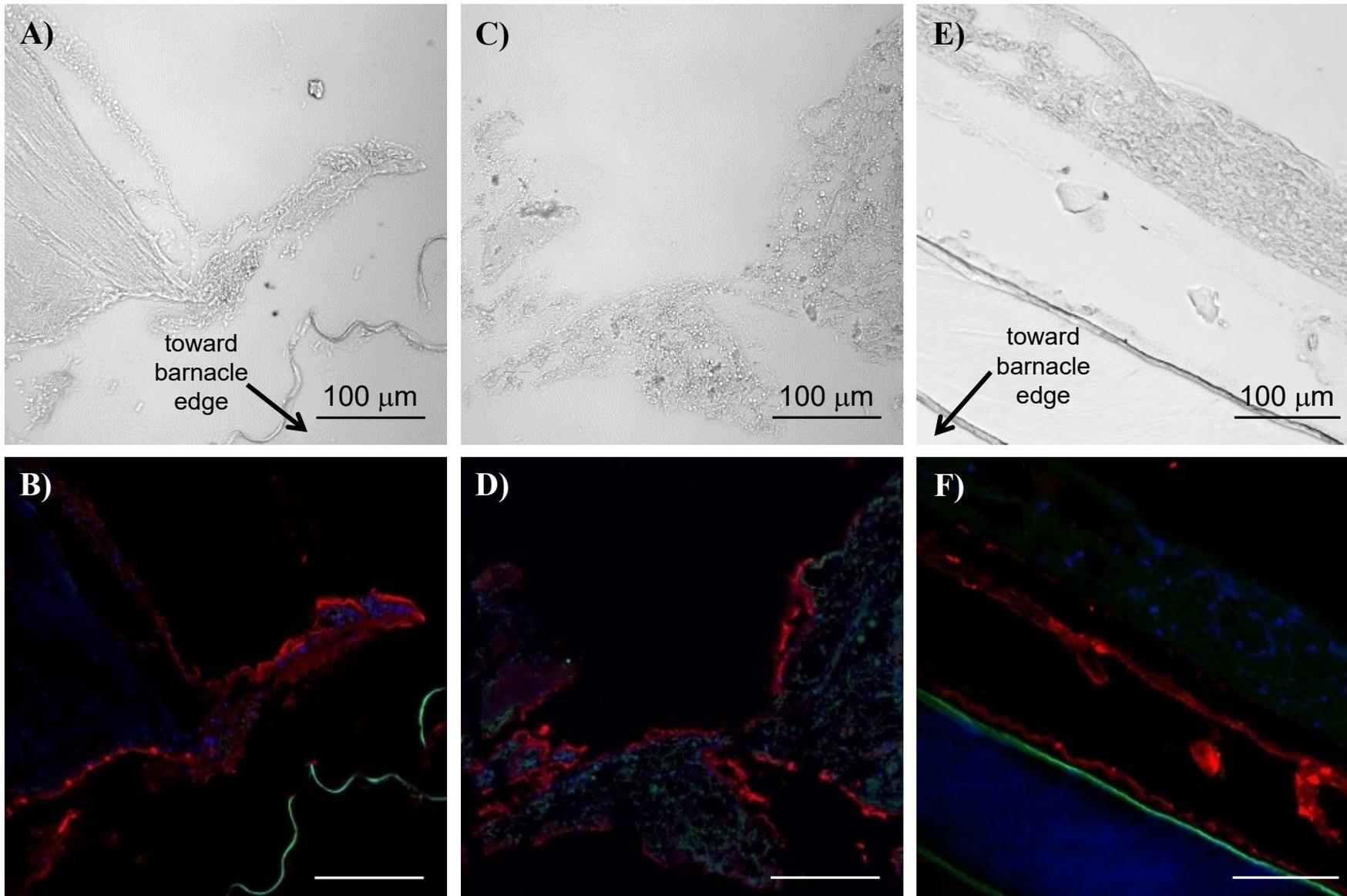
**Supplementary Figure S3.** Cy5-labelled secondary antibody controls on *A. amphitrite* sections. **A)** Brightfield and **B)** confocal fluorescent image of a sagittal section. **C)** Brightfield and **D)** confocal fluorescent image of a transverse section close to the barnacle base. Red fluorescence in **B, D)** indicate secondary antibody labeling of the basal tissue. **E-H)** Sub-mantle tissue labeled with Cy5-conjugated secondary antibody. Ovaries (yellow arrows) and cement glands (white arrows) are indicated. In **B-H)**, DAPI (blue), fluorescein (green), and Cy5 (red) emission channels were collected.



**Supplementary Figure S4.** Transverse *A. amphitrite* sections of sub-mantle and longitudinal canal tissue stained with either Cy5-conjugated **A-F)**  $\alpha$ -AaCP19-1 or **G-L)**  $\alpha$ -AaCP43-1. All brightfield images in the top row correspond to the confocal fluorescent image in the second row. Red arrows indicate cement glands. **A-F)** Examples of Cy5-conjugated  $\alpha$ -AaCP19-1 staining of the peripheral lining and connective tissue. **G-L)** Examples of Cy5-conjugated  $\alpha$ -AaCP43-1 staining, especially the lining of the longitudinal canal tissue in **J, L)**. In **A-L)**, DAPI (blue), fluorescein (green), and Cy5 (red) emission channels were collected.



**Supplementary Figure S5.** Transverse *A. amphitrite* sections stained with Cy5-conjugated  $\alpha$ -AaCP19-1 highlighting **A-D)** tissue linings, **E-H)** ovarioles, and **I-J)** membranes with positive signal. All brightfield images in the top row correspond to the confocal fluorescent image in the second row. Transverse cuts span the sub-mantle region and higher (tens to hundreds of microns from the base interface). DAPI (blue), fluorescein (green), and Cy5 (red) emission channels were collected.



**Supplementary Figure S6.** Transverse *A. amphitrite* sections stained with Cy5-conjugated  $\alpha$ -AaCP43-1 highlighting various positively stained tissue linings. Brightfield images in the top row correspond to the confocal fluorescent image in the second row. DAPI (blue), fluorescein (green), and Cy5 (red) emission channels were collected.