

Figure S1 caption

The typical distribution of the rS1 IRs in dead oyster tissues (digestive glands, gills, heart, and mantle). Live oysters are quickly frozen in liquid nitrogen and subsequently thawed slowly in a 4-degree refrigerator. This ensures that the oysters are killed with minimal ice crystal damage to the oyster tissue. The dead oysters were then placed in the same acclimation system as the experimental group for the rS1 bioaccumulation experiment. The panels on the left column show a low-magnification view of the tissue. The squared panels in the other two columns show the microscope enlargement of the boxed regions in the panels on the left. The nuclei of the hematoxylin-stained cells are blue, and the positive expression of DAB is brownish yellow. The black arrows indicate the positive IRs spots, where the rS1 exist.

Figure S1

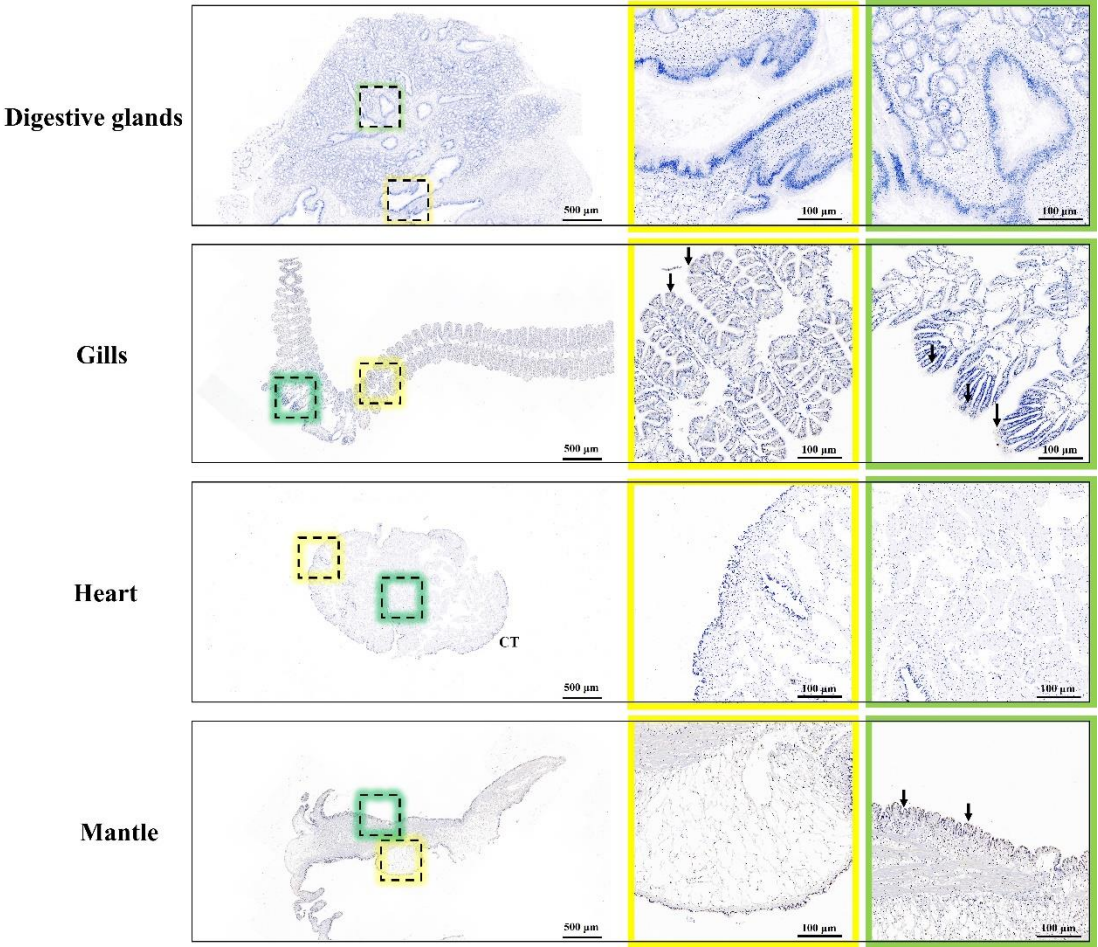


Table S1 Summary information of all reagents

ELISA reagents	Company	City	Country
Recombinant ACE 2	Frdbio	Wuhan	China
Porcine gastric mucin	Sigma	St. Louis	USA
Recombinant oHSP 70	Self-made	Shanghai	China
Bovine serum albumin	Yeason Biotech	Shanghai	China
Recombinant SARS-CoV-2 spike protein S1 subunit	Frdbio	Wuhan	China
SARA-CoV-2 spike protein rabbit monoclonal antibody	Frdbio	Wuhan	China
HRP conjugated goat anti-rabbit IgG	Sangon Biotech Co.	Shanghai	China
3,3',5,5'-tetramethylbenzidine (TMB)	Frdbio	Wuhan	China
rS1 and ligands localization reagents			
Primary antibody			
SARA-CoV-2 spike protein rabbit monoclonal antibody	Frdbio	Wuhan	China
Mouse anti-ACE 2 monoclonal antibody	Frdbio	Wuhan	China
Mouse anti-A-type HBGs monoclonal antibody	Covance	Emeryville	USA
Mouse anti-oHSP 70 polyclonal antibodies	Frdbio	Wuhan	China
Secondary antibody			
HRP conjugated goat anti-mouse IgG	Servicebio	Wuhan	China
HRP conjugated goat anti-rabbit IgG	Servicebio	Wuhan	China
Other reagents			
Instant ocean salt	Yi'er BE Co., Ltd.	Guangzhou	China
4% paraformaldehyde fixing solution	Sangon Biotech Co.	Shanghai	China
Recombinant SARS-CoV-2 spike protein S1 subunit	Frdbio	Wuhan	China
3, 3-diaminobenzidine (DAB)	Servicebio	Wuhan	China
Hematoxylin	Servicebio	Wuhan	China
Eosin	Servicebio	Wuhan	China
Co-localization of rS1 and potential ligands			
Primary antibody			
SARA-CoV-2 spike protein rabbit monoclonal antibody	Frdbio	Wuhan	China
Mouse anti-ACE 2 monoclonal antibody	Frdbio	Wuhan	China
Mouse anti-A-type HBGs monoclonal antibody	Covance	Emeryville	USA
Mouse anti-oHSP 70 polyclonal antibodies	Frdbio	Wuhan	China
Secondary antibody			
HRP conjugated goat anti-mouse IgG	Servicebio	Wuhan	China
Cy5 conjugated goat anti-mouse IgG	Servicebio	Wuhan	China
Alexa Fluor® 594 conjugated goat anti-rabbit IgG	Jackson ImmunoResearch	West Grove	USA
Fluorescent dye			
DAPI	Servicebio	Wuhan	China
FITC-TSA	Servicebio	Wuhan	China
CY3-TSA	Servicebio	Wuhan	China
Other reagents			
4% paraformaldehyde fixing solution	Sangon Biotech Co.	Shanghai	China
Spontaneous fluorescence quenching agent	Servicebio	Wuhan	China

Anti-fluorescence quenching sealer	Servicebio	Wuhan	China
Hydrogen peroxide solution	Sinopharm Chemical Reagent	Shanghai	China
PBS	Servicebio	Wuhan	China

The detail of the SARS-CoV-2 S1 recombinant protein (His tag, HEK293)

Protein Construction:

A DNA sequence encoding the SARS-CoV-2 spike protein S1 subunit was expressed with a polyhistidine tag at the C-terminus.

Expression of hosts: HEK293 Cells

Purity : > 95% as determined by SDS-PAGE.

Endotoxin : < 1.0 EU per µg protein as determined by the LAL method.

Bio activity: measured by its binding ability with human ACE 2 protein (His tag) in ELISA assay.

Amino acid sequence:

VNLTRTRQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVSQTNGT
KRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQSLIVNNATNVVIKVCEFQFCND
PFLGVYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNI
DGYFKIYSKHTPINLVRDLPGGFSALEPLVDLPIGINITRFQTLLALHRSYLTPGDSSSGWTA
GAAAYYVGYLQPRTFLLKYNENGTITDAVDCALDPLSETKCTLKSFTVEKGIYQTSNFRV
QPTESIVRFPNITNLCPFGEVFNATRFASVYAWNKRISNCVADYSVLYNSASFSTFKCYGV
SPTKLNLDLCTNVYADSFVIRGDEVQRQIAPGQTGKIADYNYKLPPDDFTGCVIAWNSNNLD
SKVGGNYYNYLYRLFRKSNLKPFERDISTEIQAGSTPCNGVEGFNCYFPLQSYGFQPTNGV
GYQPYRVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQ
QFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQDVNCTEVPVAIH
ADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVNNSEHHHHHHH

BLAST

ACE2 VS CgACE

10 20 30 40 50 60 70 80 90

100

.....|

ACE2 -----MSSSSWLLLSLVAVT----

AAQSTIEEQAKTFLDKFNHEAEDLFYQSSLASWNYNINITEENVQNMNAGDKWSAFLKEQSTL

CgACE --

MKALWRLSVLCVLCTCVIARHRHDSQQRLRQVLQQRQRIDTEVSEWPTTKNEELRRWLEKISHASWNYETDLS

EENAEKLNKVNIDFSQWKSTCVKE

↑

110 120 130 140 150 160 170 180 190

200

.....|

ACE2 AQMYPLQEIQNLT--VKLQLQALQQNGSSVLEDKSKRLNTILNTMSTIYSTGKVCNPDNP--

QECLLLEPGLNEIMANSLDYNERLWAWESWRSEVCK

210 220 230 240 250 260 270 280 290

300

	310	320	330	340	350	360	370	380	390
400									

410 420 430 440 450 460 470 480 490

500

ED

AAAAAA A

CgACE ANVSEEEKKSDINFLNQALKLAFLEPGY---LVDKWRWRVFSGEITP-----
DKYNEEWWMKMRLEYQGVTSVPRTNSDFDPGAKFHIVAANSQYI

610 620 630 640 650 660 670 680 690

700

.....|

ACE2 RYVTRILYQFQFQEA LCQA AKHEGP-----
LHKCDISNSTEAGQKLFNMLRLCKSEPWTLALENVVCAKNMNVRLNLYEPLFTWLKDQN--KNSFVCG
CgACE IYFASFLQFQFYESM CQASGHTGP-----
LYTCDFYRSKEAGGKLLNMLKLGGSKHWKEALKQMTGDTNIRTEPFKKYFQPLIDFLMNEN--KGDVCG

710 720 730 740 750 760 770 780

.....|

ACE2 WSTDWSPYAD-----
CgACE WAKAGINWERA-----