A CD63 homologue specially recruited to the fungi-contained phagosomes is involved in the cellular immune response of oyster *Crassostrea gigas*

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Supplementary Material

1 Supplementary Data

Supplementary Data 1. The homologs of cgCD63 employed in phylogenetic analysis

Mouse

>Mm.CD151_CD151.Mm__NP_033972

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Frog

>Xt.CD53_ENSXETP00000033554

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>Xt.CD81_NP_989271

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>Xt.CD82_NP_989191

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VPKY

>Xt.CD37__NP_001015801

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Zebrafeish

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>Dr.CD63__NP_955837

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Other vertebrates

>NP_001134074.1 CD63 antigen Salmo salar

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>BAM36397.1 CD63 antigen Oplegnathus fasciatus

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Athropoda

>FBgn0033629_CD63_ Drosophila melanogaster

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MAWDNIQQKL MCCGVDSPADWRTLSANKTLPGSCCQPQYIDSTVGHCLESPALGKDKYFQ
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>AJF38198.1 cd63 Locusta migratoria

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>CDF77374.1 CD63 protein Tenebrio molitor

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Mollusca

>APM87502.1 CD63 Haliotis discus discus

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VKEGWKFIQQSFKCCGADNYTSWEQFLPQPPESCCKSIGSIECKDRSYNKTDGIYTKSCTKGI
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>XP_005097532.1 PREDICTED: CD63 antigenlike Aplysia californica

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HHEGVTKSWNKLQEEFSCCGVNNYTDWNTTRGLSAPPVSCCAKIPCTPSFDDPSSIYTDPCA
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>XP_013069835.1 PREDICTED: CD63 antigenlike Biomphalaria glabrata

MVEGGMKCVKYLLFVFNLIFVIAGIGLIAAGAYVKVKLDQYYDFFGSDYVGPGILLIIVGVII
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VSKFEDWLKDKVAIIGGVGIGLAFVQVVGILFACCLARAIKKEYEVV

>XP_025084302.1 CD63 antigenlike Pomacea canaliculata

MVEGGMKCIKFLLFAFNLIFVIAAIAIIAVGAYVQIKLTEYYDFFGNQYAGPGILLIVVGVFIFI
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NPGVTDAWNVLQQEFQCCGVTNSSDWKQASDLNTTYPWSCCMDSEACKTKQNITYDDIYK
QACESAFIGWMKDKVAIIGGVGIGLAFVMVVGVLFACCLARAIRKEYEVV

>XP_021342561.1 CD63 antigenlike Mizuhopecten yessoensis

MVEGGMKCVKLLLFAFNFIFVVVGCVLIGVGTYIQVQFKDYLNFVGQFSSGPGALLIAVGVII
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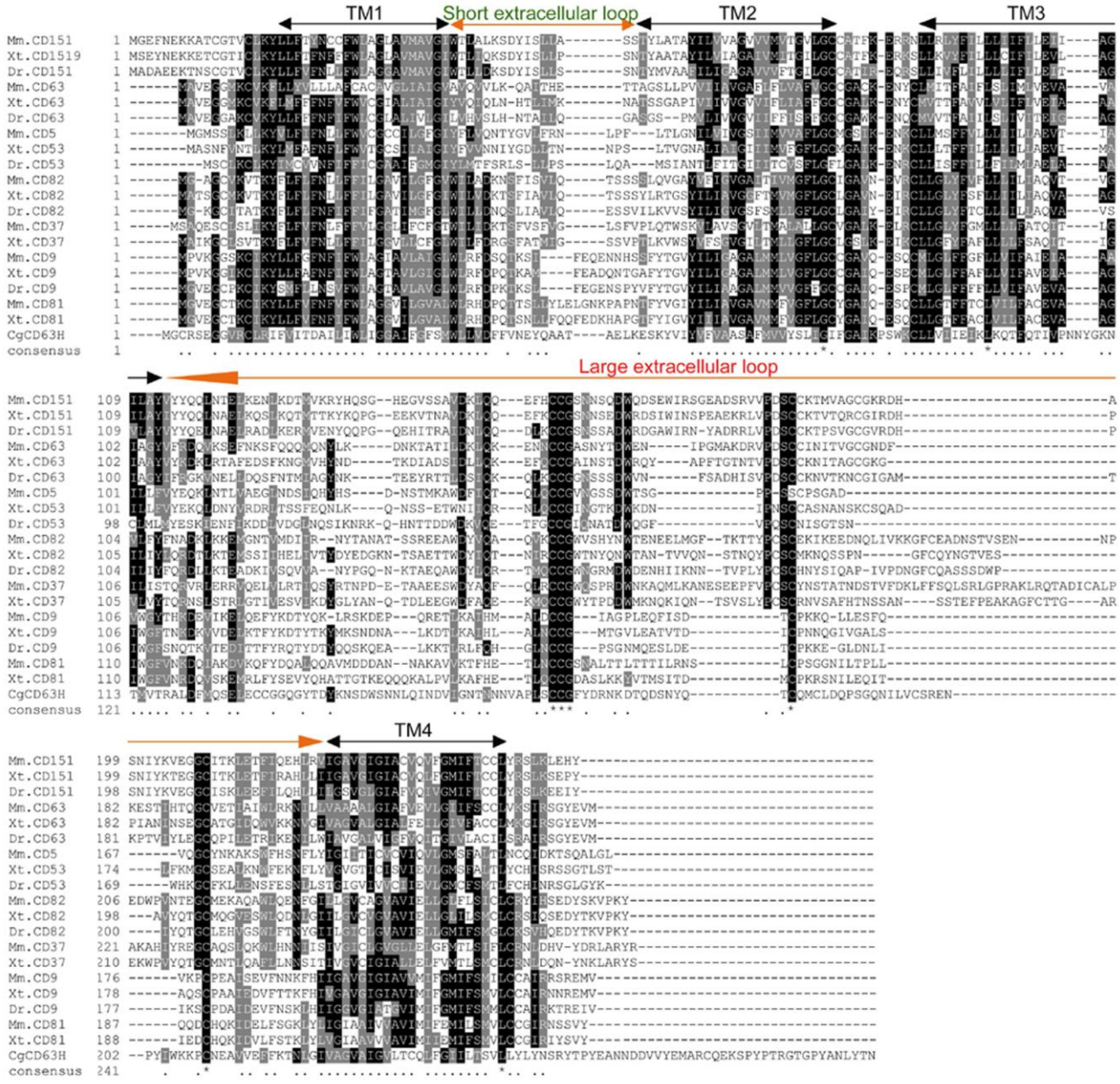
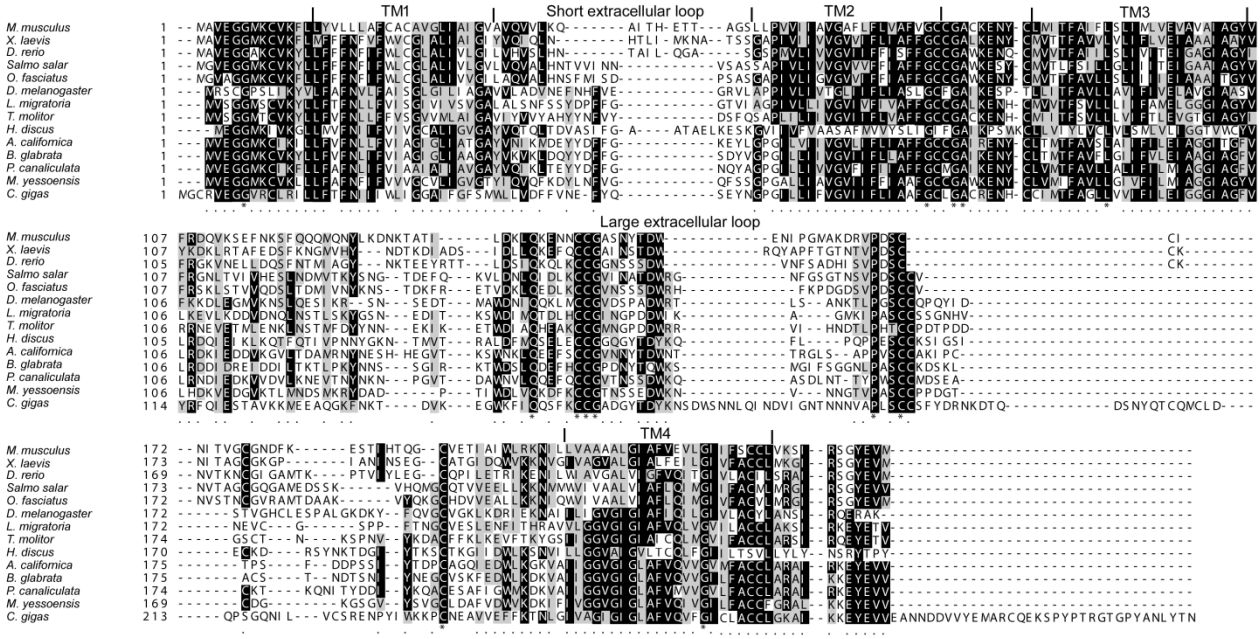
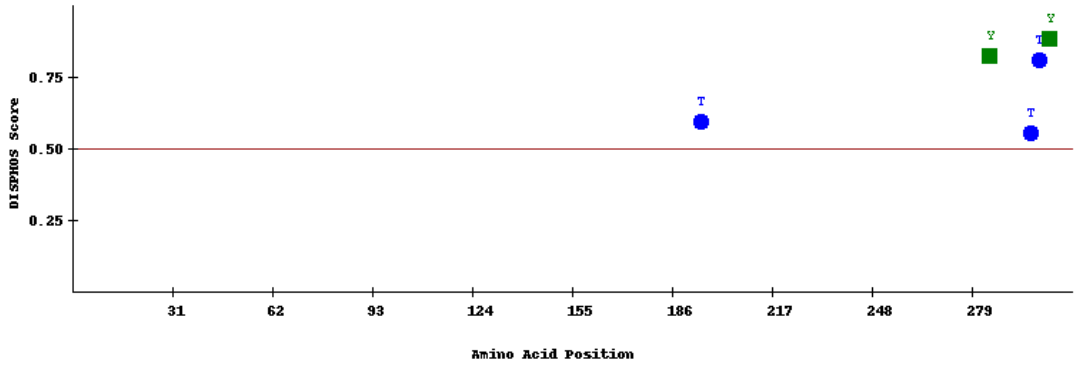
2 Supplementary Figures



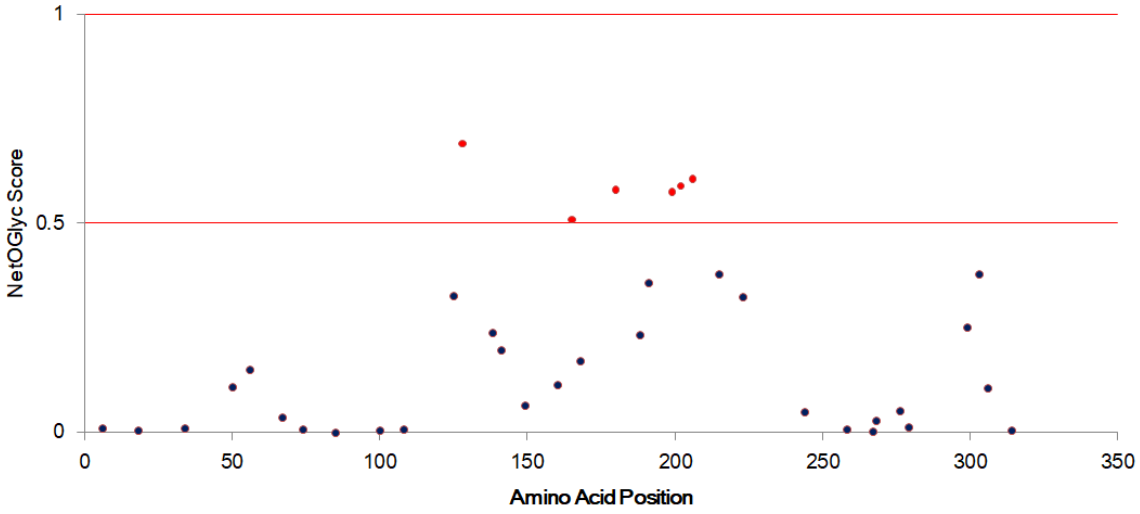
Supplementary Figure 1. Multiple sequence alignment of the tetraspanins by ClustalW2. The tetraspanin homologs of CgCD63H from Mus musculus, Xenopus tropicalis and Danio rerio were employed for multiple sequence alignment and sequence information was described in supplementary 1. Amino acid residues that are conserved in at least 60% sequences are shaded in dark, and similar amino acids are shaded in gray. Asterisks below indicate the CCG motif and Cys residues that are conserved throughout the tetraspanins. TM domains (TM1, TM2, TM3, and TM4), short extracellular loop and long extracellular loop were indicated in arrows.



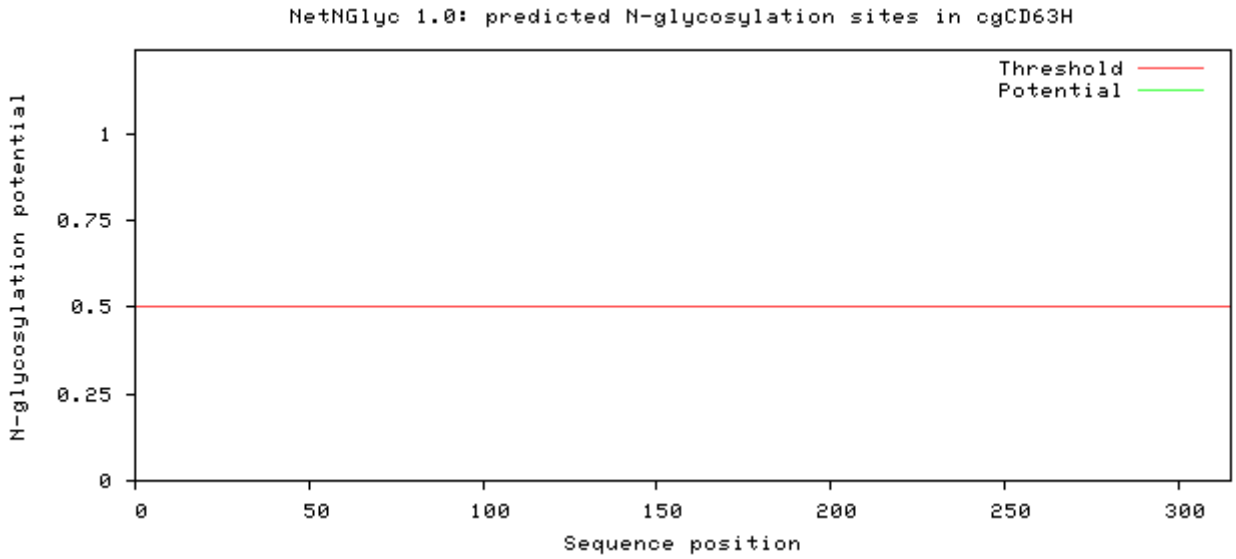
Supplementary Figure 2. Multiple sequence alignment of the CD63 homologs by ClustalW2. The homologs of CgCD63H_were employed for multiple sequence alignment and sequence information was described in supplementary data 1. Amino acid residues that are conserved in at least 60% sequences are shaded in dark, and similar amino acids are shaded in gray. Asterisks below indicate the CCG motif and Cys residues that are conserved throughout the tetraspanins. TM domains (TM1, TM2, TM3, and TM4), short extracellular loop and long extracellular loop were indicated upon the alignment.



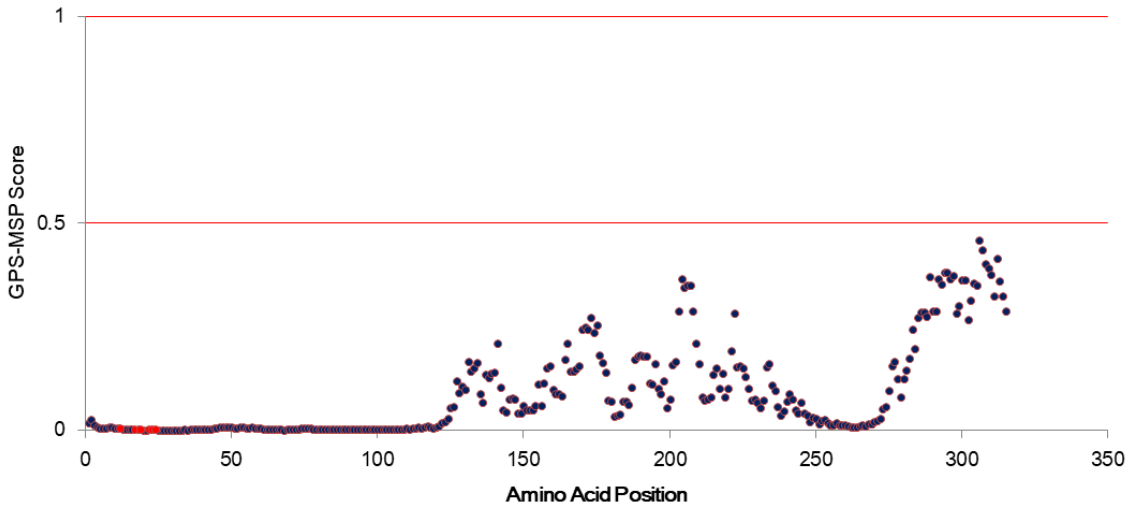
Supplementary Figure 3. The phosphorylation modification sites of *Cg*CD63H predicted by DISPHOS 1.3. Sites with score > 0.5 were deemed as positive signals.



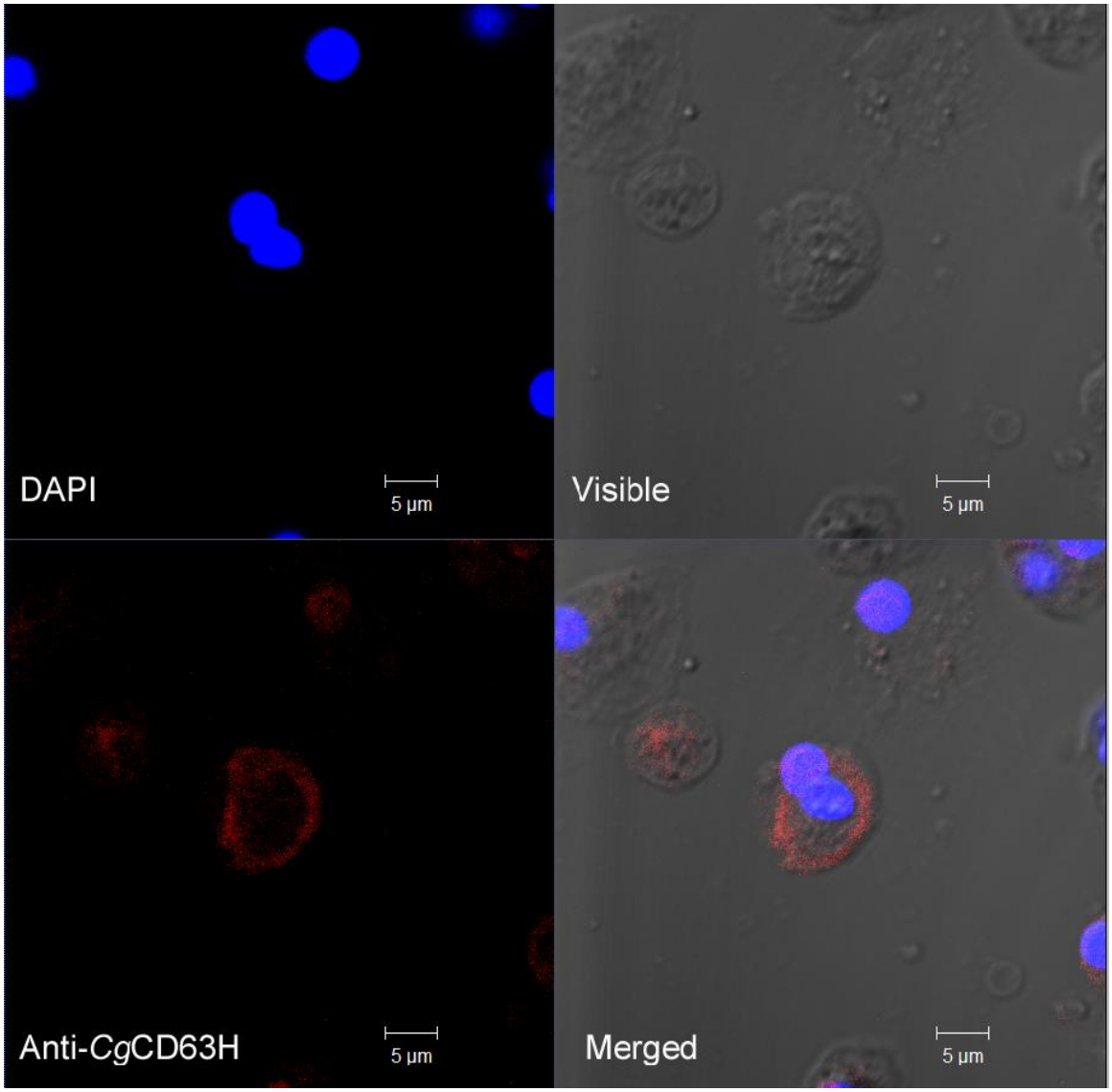
Supplementary Figure 4. The O-linked glycosylation modification sites of *Cg*CD63H predicted by NetOGlyc 4.0 Server. Sites with score > 0.5 were deemed as positive signals.



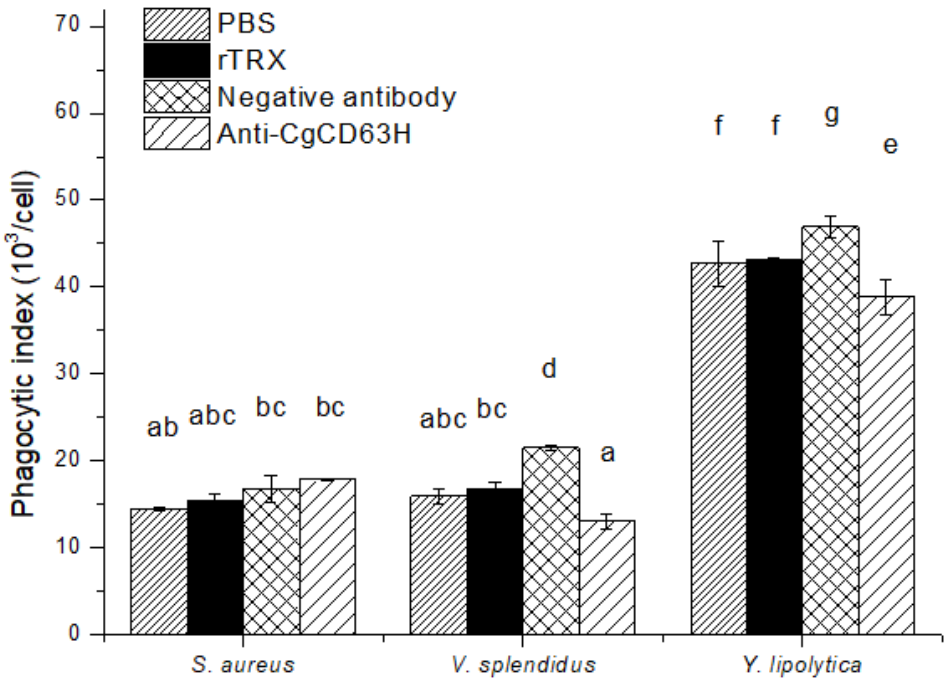
Supplementary Figure 5. The N-linked glycosylation modification sites of *Cg*CD63H predicted by NetNGlyc 1.0 Server. No positive signal was found with score > 0.5.



Supplementary Figure 6. The methylation modification sites of *Cg*CD63H predicted by GPS-MSP Online Service. No positive signal was found with score > 0.5.



Supplementary Figure 7. Subcellular localization of CgCD63H protein in oyster hemocytes (positive and control). Binding of antibody to *Cg*CD63H was visualized by DyLight 594-labeled secondary antibody (red), the nucleus of hemocytes was stained with DAPI (blue), bar = 5 μm.



Supplementary Figure 8. Hemocyte phagocytosis index (mean FITC fluorescent intensity) post the incubation of anti-CgCD63H detected by flow cytometry. Oyster hemocytes were employed to analyze the change of hemocytes phagocytosis rate against *S. aureus*, *V. splendidus* and *Y. lipolytica* post the incubation of anti-CgCD63H. FITC labeled microbe, anti-CgCD63H and hemocytes were mixed and incubated for 1 h in dark. Recombinant protein rTRX and negative antibody were employed as negative controls. For each treatment, assay was performed in three different replicates for statistical analysis ($p < 0.01$).