## **Supplementary Information**

Journal name: Biomolecules

Title: Characterization of matrix metalloprotease-9 gene from Nile tilapia (*Oreochromis niloticus*) and its high-level expression induced by *Streptococcus agalactiae* challenge

Author's names: Fu-Rui Liang<sup>1†</sup>, Qin-Qing Wang<sup>1,2†\*</sup>, Yun-Lin Jiang<sup>1</sup>, Bei-Ying Yue<sup>1</sup>, Qian-Zhi Zhou<sup>1</sup>, Jiang-Hai Wang<sup>1,2,3\*</sup>

## Affiliations:

<sup>1</sup>Guangdong Provincial Key Laboratory of Marine Resources and Coastal Engineering, School of Marine Sciences, Sun Yat-Sen University, Guangzhou 510006, China

<sup>2</sup>Southern Marine Science and Engineering Guangdong Laboratory (Zhuhai), Zhuhai 519000, China

<sup>3</sup>South China Sea Bioresource Exploitation and Utilization Collaborative Innovation Center, School of Marine Sciences, Sun Yat-Sen University, Guangzhou 510006, China

<sup>†</sup>The authors contributed equally to this work.

\* Corresponding authors: wangjhai@mail.sysu.edu.cn (J.-H. Wang); wangqq39@mail.sysu.edu.cn (Q.-Q. Wang).

Tel.: +86 20 39332212.

## **Supplementary figures**



Figure S1. Reconstruction of the expression vector pET-28a/aNtMMP-9



**Figure S2.** Identification of positive transformants containing pET-28a/*aNtMMP-9* vector amplified by PCR.

M, DNA marker DL2000; Lanes 1-4, the bands (1,716 bp) were amplified from different transformants.

-MRCCTLLVCLLLG1ST0VG%SLPLKSVFVTFPGD1PKNLTDSELAENYLKNPGYMNTVERSGF0SMVSTAKALKRM0R0LGLEETGVLNKETLDAMKKPRCGVPDVANY0T1EGDLK Oreochromis niloticus Ctenopharyngodon Idella -MRLG-VVAFLvLGTCSLRAWCVPVKS---VTFPGDV1KNMTD1QLADEYLKRYGY1DVLQKSGLQAVVSPSKALKELQRQLGLEETGSLDQPT1DAMKQPRCGVPD1RNYQTFDGDLK Hypophthalmichthys molitrix -MRLG-VVAFLVLGTCSLRAWCVPLKSVYVTFPGDV1KNWTDK0LADEVLKRYGYTDVL0KSGL0AVVSPSKALKKL0R0LGLEETGSLDKPTTDAMK0PRCGVPDTRNY0TFDGDLK MRLG-WWWLVLGTCSLRAWSAPLKSVFVTFPGDVIKNMTDNQLAEEYLKRYGYIDVLQKSGLQAWVSTSKALMKLQQQLGLEETGSLDQPTIDAMKQPRCGVPDIRNYQTFDGDLK Cyprinus carpio Schizothorux prenanti -WRLG-VLAFLVLGTCSLRAWCVPLKSVVVTPPCDVTKSWTSTOLADRYLKRYGYTDVLQKSGLQAVVSPSKSLKKLQRQLGLEPTGSLDLPTTDAWKQPRCGVPDTRNYQTPPGDLK MRLG-VLAFLVLGTCSLRAWCLPLKSVFVTFPGDVTKNMTNTQLADEVLKRYGVDVLQRSGLQAVTSNAKALKKLQRQLGLEETGLLDQPTVDANKQPRCGVPDTRNYKTFDGDLK Danio rerio Fundulus heteroclitus MMGCCGLVVCLLLGIVAQSGWSLPVKSIFVTFPGDIVKNVTDTELAESYLKKPGYMETEQRSGPQSMVSTSKALKKMQRQMGLEETGELDKPTLEAMKHPRCGVPDVANYKTFDGDLK -MRCCALAVCLVLGISMQDGWSLPLKSVFVTFPGD11KNMTDSELAESYLKKFGYMDTLQRSGFQSMVSTSKALKRMQRQMGLEETGELDKSTLEAMKHPRCGVPDVANYKTFDGDLK Siniperca chuatsi Boleophthalmus pectinirostris -MRCRAFAVCVLLGVCLQNGWSLPLKSVFVTFPGD1VKNVTDTEMAENYLKRFGYMESVQRSGFQS1VSTSKALKRMQRQMGLEETGELDKSTLEAMKHPRCGVPDVANYKTFDGDLK consensus \*. \*:\*\* ::\*:,\*\*\*,:\*\*;; ::\*\*:\*:\*:\*:\*:\*:\*:\* ::\*\* □ \*\* : \* : Oreochromis niloticus #DHNDVTYRTLNYSPDMDSATTDDAFARAFKV#SDVTPLTFTRLYDGTADTMTSFGKKDHGDPYPFDGKDGLLAHAYPPGEGT0GDAHFDDDFFWTLGKGAVVKTRFGNANGATCHFP Ctenopharyngodon Idella ©DISEVTYRILNYSPDMESSLIDDAFARAFKV%SDVTPLTFTRLYDGTADIMISFGRADHGDPYPFDGKDGLLAHAYPPGQGIQGDAHFDDDEYWTLGSGPAIQTRYGNAEGALCHF Hypophthalmichthys molitrix 8DUSEVTYRTI NYSPDMEASI. IDDAFARAFKV#SD I TPL TEKRI. PDG TAD IMI SEGKENI I CDPVPEDGKDCI LAHAVPPGEG I OCDAHEDDDEV#TL CSCPA I KTY GNAEGALCHE #DITDVTYRTLNYSPDMEAPLIDDAFARAFKV#SDVTPLTFTRL/DGTADIMISFCREN/GDPYPFDGKDGLLVIAVPPGEGIQGDA/#DDDDEV#TLGSGPAIQTRY GNAEGAMCHFI Cyprinus carpio Schizothorax prenanti #DHNDVTYRVLNYSPDMDASL1DDAFARAFKV#SAVTPLTFTRLYDGTAD1M1SFGKADHCDPYPFDGKDGLLAHAYPPGKGVQCDAHFDDDEY#TLGSGPa1QTRYCNAEGAMCHFI \*DHTDVTYRLLNYSPDMEASLIDDAFARAFKV%SDVTPLTFTRLFDGIADIMISFGKLDHGDPYPFDGKDGLLAHAYPPGEGTQGDAHFDDDEY#TLGSGPALQTRYGNAEGANCHFI Danio rerio Fundulus heteroclitus  $\label{eq:constraint} with the second seco$ #DSHDVTYRILNYSPD1qSSLTDDAFARAFKV#SDVTPLTFTRLFDGTADIMISFGKADHGDPYPFDGKDGLLAHAYPPGKGVQGDAHFDDDEY#TLGTGPVVKTLYGNADGAMCHFI Siniperca chuatsi Boleophthalmus pectinirostris WDHIDTTYRTLNYSPDMESSLTDDAFARAFKYWSAVTPLSFTRLFDGTADIMISFGKKDHCDPYPFDGKDGLLAHAYPPGEGMQGDAHFDDDEHWTLGKGAVVKTSY<mark>DNANGALCHFP</mark> sidiple - sidiplektiek - -consensus  $\label{eq:Fibronectintype2domain} \underbrace{Fibronectintype2domain} \leftarrow Fibronectintype2domain} \leftarrow Fibronectintype2domain} \underbrace{Fibronectintype2domain} \leftarrow Fibronectintype2domain} \\ FSFGGKSYSTCTTEGREDGLP4CSTTADYDKDKYGFCPSELLTHDGNSREAPCVFPF1FIGEEVDSCTTEGREDGV34CATTANFDDKYGFCPSRD1AVIGGSSEGEPCGFPF1FIGESSSCTTEGREDGLP4CATTANFDKKYGFCPSRD1AVIGGSSEGEPCGFPF1FIGESSSCTTEGREDGLP4CATTANFDTKKYGFCPSRD1AVIGGSSEGEPCGFPF1FIGESSCTTEGREDGLP4CATTANFDTKKYGFCPSRD1AVIGGSSEGEPCGFPF1FIGESSCTTEGREDGLP4CATTANFDTKKYGFCPSRD1AVIGGSSEGEPCGFPF1FIGESSCTTEGREDGLP4CATTANFDTKKYGFCPSRD1AVIGGSSEGEPCGFPF1FIGESSCTTEGREDGLP4CATTANFDTKKYGFCPSRD1AVIGGSSEGEPCGFPF1FIGESSCTTEGREDGLP4CATTANFDTKKYGFCPSRD1AVIGGSSEGEPCGFPF1FIGESSCTTEGREDGLP4CATTANFDTKKYGFCPSRD1AVIGGSSEGEPCGFPF1FIGESSCTTEGREDGLP4CATTANFDTKKYGFCPSRD1AVIGGSSEGEPCGFPF1FIGESSCTTEGREDGLP4CATTANFDTKKYGFCPSRD1AVIGGSSEGEPCGFPF1FIGESSCTTEGREDGUPSCTTEGREDGUPSCTTGGSSGGY34CATTANFDTKKYGFCPSRD1AVIGGSSEGEPCGFPF1FIGESSCTTEGREDGUPSCTTGGSSGTACATTANFDTKKYGFCPSRD1AVIGGSSEGEPCGFPF1FIGESSCTTEGREDGUPSCTTGGSSGTACATTANFDTKKYGFCPSRD1AVIGGSSEGEPCGFPF1FIGESSCTTEGREDGUPSCTTGGSSGTACATTANFDTKKYGFCPSRD1AVIGGSSEGEPCGFPF1FIGESSCTTEGREDGUPSCTTGGSSGTACATTANFDTKKYGFCPSRD1AVIGGSSEGEPCGFPF1FIGESSCTTEGREDGUPSCTTGGSSGTACATTANFDTKKYGFCPSRD1AVIGGSSGTACATTANFDTKKYGFCPSRD1AVIGGSSGTACATTANFDTKKYGFCPSRD1AVIGGSSGTACATTANFDTKKYGFCPSRD1AVIGGSSGTACATTANFDTKKYGFCPSRD1AVIGGSSGTACATTANFDTKKYGFCPSRD1AVIGGSSGTACATTANFDTKKYGFCPSRD1AVIGGSSGTACATTANFDTKKYGFCPSRD1AVIGGSSGTACATTANFDTKKYGFCPSRD1AVIGGSSGTACATTANFDTKKYGFCPSRD1AVIGGSSGTACATTANFDTKKYGFCPSRD1AVIGGSSGTACATTANFDTKKYGFCPSRD1AVIGGSSGTACATTANFDTKKYGFCPSRD1AVIGGSSGTACATTANFDTKKYGFCPSRD1AVIGGSSGTACATTANFDTKKYGFCPSRD1AVIGGSSGTACATTANFDTKKYGFCPSRD1AVIGGSGTACATTANFDTKKYGFCPSRD1AVIGSGTACATTANFDTKKYGFCPSRD1AVIGGSTACATTANFDTKKYGFCPSRD1AVIGSGTACATTANFDTKKYGFCPSRD1AVIGGSTACATTANFDTKKYGFCPSRD1AVIGFCPSRD1AVIGFCPSRD1AVIGSGTACATTANFDTKKYGFCPSRD1AVIGFCPSRD1AVIGFCPSRD1AVIGSGTACATTANFDTKKYGFCPSRD1AVIGFCPSRD1AVIGSGTACATTANFDTKKYGFCPSRD1AVIGSTACATTANFDTKKYGFCPSRD1AVIGSTGTACATTANFDTKKYGFCPSRD1AVIGFCPSRD1AVIGFCPSRD1AVIGFCPSRD1AVIGFCP$ Oreochromis niloticus Ctenopharyngodon Idella Hypophthalmichthys molitrix Cyprinus carpio FLFEGTSYTSCTTEGREDGLPACATTADYDRORKYGFCPSELLFTHJOANSNQAPCVFPYPJEGVRYNSCTTDGRSDGYRACATTANFDTDRYGGCPNRDTAYTGGNSDGEPCRFPF FLFEGTSYSSCTTDGRTDGTPRCATTADYDRDR TFGFCPSELLFTHJOANSNGAPCVFPFVFLGVRYDSCTTDGRTDGYRRCATTANFDTDRYGCPNRDTAYTGGNSEGEPCQFPF LFEGKSYSTCTTEGRTDDLL#CATTADYDKDKKFGFCPSELLFTHDGNSNEAPCVFPFvFLGEKYDSCTTDGRNDGYR#CATTAMFDTDKKYGFCPNRDTAVLGGNSEGEPC0FPF Schizothorax prenanti LFEGTSYSTCTTEGRTDGLP%CSTTADYDKDKKFGPCPSELLFTFDGNSNEAPCVFPFVFDGKKYDSCTTEGRNDGYR%CSTTANFDTDKKYGFCPNRDTAMIGGNSEGEPCHFPF Danio rerio Fundulus heteroclitus FTFEGKSYTSCTTEGRTDNLPWCATTEDYGRDKKYGHCPSFLJ.VTHOGNADGAPCVFPFTFLGKEVDSCTTEGRSDGYR#CATTSNFUTDKKYGFCPSRUTAVLGGNSEGEPCHEPFTFEGKSYSTCTTDGRPDNLP#CATTADYSTDQKYGFCPSELLYTFGGNADGAKCVFPFIFLGETYDSCTTEGRSDGYR#CATTDNFDRDVKYGFCPSRDTAVTGGNSEGEPCHFPFI Siniperca chuatsi Boleophthalmus pectinirostris CIFEGKSYTTCTTEGRIDNLP#CGTTADYDKDXKYGECPSELLYTIGGNSXXXXXFICIFIGKEYNSCTTEGRADDYR#CATTDNFDKDKKYGCPNRDTAVTGGNAEGESCHFPFY consensus \* \* \*\*::\*\*\*:\*\* \* : \*\* \*\* \*\*: \*: \*\*\* \*:\*\*\*\*:\*\*: \*: \*\*\*\*:\*\* \*\*\* Oreochromis niloticus LGQSYTSCTSEGRSDGKLØCGTTSNYDTDKKV<mark>G</mark>FCPDRGYSLFLVAAHEFGHALGLDHSVVKDALMFPNYKYTEDFSLNQDDTAGTQYLYGPKTGPDPTPPKPKTTTSSPTGPTKPT Ctenopharyngodon Idella LGKTYTSCTSEGRSDGKL@CATTSNYDTDQK#GFCSDRGYSLFLVAAHEFGHALGLDHSN1KDALMYPMYKYVEDFSLNQDD1EG1QYLYGPKTGPDPTPPKPKTTTSSP1GPTKPT Hypophthalmichthys molitrix Cyprinus carpio LDKTYTSCTSEGRGDGKL#CATTSNYDTDKK#GFCPDKGYSLFLVAAHEFGIALGLDHSK1KDALMYPMYKY1EDFSLNQDD1EG1QYLYGPKTGPNPTPPKPKTTTSSPVP7KP Schizothorax prenanti I GTFYSSCTSEGRSDGKI #CATTSNYDTDKK#GFCPDRGYSLFI / AAHEFGHALGI DHSNTKDALMYP#YKYVEDFSI HRDDTFGI OYLYGPKTGPDPTPPEPOSTTSSPI MPTKST Danio rerio LGNTYSSCTSEGRNDGKL#CGTTSNYDTDKK#GPCPDRGYSLFLVAAHEFGHALGLDHSN1KDALAYPMYKYVEGFPLHRDDIDGIQYLYGPRTGPEPTAPQPRTTTSSPVVPTKPS Fundulus heteroclitus LGKTYDSCTSEGRGDGKL#CSTTASYDDDKK#GECPDQCYSLFLVAAHEEGHALGLDHSNTRDALMYPMYSYVEDESLHEDDTEGTQYLYGPKTGPTAEPPQPYTPTTVPYPDPDNT Siniperca chuatsi Boleophthalmus pectinirostris  $-\underline{\textit{EGRSDGKLWCATTASYDQDxKW}} GFCPDQGYSLFLVAAHEFGHALGLDHSNIREALMFPWYSYMEDFSLHEDDIEGIQYLYGGRTGPEPTTPQP-TPTTTDYPDTDETFOR STATES STATES$ consensus ← Hemopexin-like repeats → ← Hemopexin-like repeats → ← Hemopexin-like repeats → ← TITTTAPV0PTKDaCKqlfFbTITV1QGELHFFKDGHVKKTSGSSNAELKGPT1SNX0PALJ4V1DSAFEDSLTKKLYFFSGTRF0VYTGQNUGPRS1EkLq Oreochromis niloticus EP--Ctenopharyngodon Idella Hypophthalmichthys molitrix PSE: KTT----KTTTVSTTTHVGPSQDACEINEFDATTEIQKELHFFKDRHYWKISSNG--ERKGPFFISEKWPALFATINSAFEEPLIKKTYFFADKQFWVYTGKEVLGPRKTEKL Cyprinus carpio Schizothorax prenanti -KTTTVSTTVHVDPSQDACQINEFDAITEIQKELHFFKDGHYWKISNKG-ERKGPFLISEKWSALFAVINTAFEDQFTKKIYFFAEKQFWVYTGNEVFGPRKIEKL PSE------KTTTASTTTQVVPSDDACQIMEFDAITEIQKELHFFKDGRYWKISGNG-ERKGPFMISAXWPALIAVINSAFEDHLTKKIYFFSERQFWYSGNDVLGPRKIEKL Danio rerio PSD DATE----DP----STYTPSTVDP'RDACKETKFDTTTVTQGELHPFKDGQF9KYPXKDGGQGQGPSSISEKVSAL AV IDSAFEDLQTKKLYFFSGNF9VYTGSVLGPKSIERLG DPTN----EPRPTEPTTTTRSVDPTKDACKFTKFDATTVTEGELIFFKDGJYVKTSSSSDGGPTGPSSISERRPVLGVVTDSAFEDLISRKLYFFSGTRF9VYTGQNVLGPRSIERLG Fundulus heteroclitus Siniperca chuatsi Boleophthalmus pectinirostris \* \*: \*\*\*: consensus \*\*\* \*\* :\*. \*\*\* \*::\*\*\*: ::\*:\*\*\*: :\*\*\*\*:\*:. ← Hemopexin-like repeats → ← Hemopexin-like repeats → PM(EKVEGALQRGKGXVLLYSGENFVRLDVKAQTLDKGYPKFTDVFGQGFTYFCRDRFVRFVRMSRRQVDrVCYVKt)DLLKCS--SDTRY--Oreochromis niloticus PLDLDRVEGAVQRAKGXVLLFKGENFWRLDVKAQXLDTGYPRLTDSAFGXVPLDSAbQVPLYKGFFYFCRESFYWRRMAKKQVDRVGYVKYDLLKCRDV-PVDLNVEGAVQKGKSXVLLFNGENFWRLDVKAQLDSGYPRFTDSVFGQVPLDSHDVFLYKGFFYFCRESFYWRRNSKRQVDRVGYVKYDLLKC-----Ctenopharyngodon Idella Hypophthalmichthys molitrix F NULINKSENY MANDASALLINGENY NULINAGULINGENY TI DIS YOMYT LIEDIY FULKY FEYDAGAS Y NANISANY YAYO'N Y LULUC PSDLDWYET GYNGRKSYLL I FUCKYNFRU DYNAU I DISGYPEST DSYNPFLYTD TI DISHDYFL HACFYFCRESTYNRUNARRAYDNYGYYNDU L KC-PSNLDRYDG YGRKGYLL LINGENFYRLDYNTO I LIGGYPET DSYPGQYELDSIDYFL YKGFSYFCRENYWRUNARRAYDNYGYYNDU L KC-Cyprimus carpio Schizothorax prenanti Danio rerio Fundulus heteroclitus Siniperca chuatsi Boleophthalmus pectinirostris  $\label{eq:product} PNs we we call a reasonable to the second state of the second sta$ consensus \*\*\*:\* \*:\*\*\*\* ::\* \*\*\*\*: \*\*\*\*\*\*:::\*\*\*\*\*\*\*

Figure S3. Multiple amino acid sequences alignment of MMP-9 from Nile tilapia and other species.

Amino acid sequences of MMP-9s from other species, including *Ctenopharyngodon idella* (Accession No. ADU34085.1), *Hypophthalmichthys molitrix* (APO13601.1), *Cyprinus carpio* (BAB39390.1), *Schizothorax prenanti* (AMB72630.1), *Danio rerio* (AAI60656.1), *Fundulus heteroclitus* (XP\_012726094.1), *Siniperca chuatsi* (AKA66298.1), and *Boleophthalmus pectinirostris* (XP\_020785344.1).



**Figure S4.** Relative transcriptional levels of *NtMMP-9* in different tissues from healthy fish. The transcriptional value of *NtMMP-9* in the intestine was used as the internal reference.



Figure S5. Electrophoretic analysis of recombinant aNtMMP-9 expressed from Rosetta-gami 2 (DE3) competent cell.

(A) SDS-PAGE of aNtMMP-9 expressed by Rosetta-gami (DE3) at 16 °C. M, protein maker; Lane 1, the total protein expressed without IPTG; Lane 2, the total protein expressed after IPTG (0.5 mM) induction for 12 h; Lane 3, unbound protein; Lane 4, the protein from last washing with imidazole (200 mM); and Lanes 5–9, the proteins collected using an elution buffer (0.5 mL, 200 mM imidazole) and repeated five times. (B) Western-Blotting analysis for the proteins obtained from lanes 5 and 6 in Figure S4A. (C) Gelatin zymography of the protein harvested from that of Figure S4B. The molecular weight (63.4 kDa) of the obtained aNtMMP-9 was denoted by an arrow.

## Supplementary tables

Fish			Fish number in different time points after			
	Fish number	Tank	TankS. agalactiaechallengeforcollectingnumbertheir tissues			
	per tank	number				
			4 h	24 h	48 h	72 h
Healthy group	10	1	Three healthy fish for collecting tissues and identifying the transcriptional levels of <i>NtMMP-9</i> gene			
Control	10	2	2	2	2	2
group	10	3	3	3	3	3
Challenged group	10	3	3	3	3	3

Table S1. Information of experimental fish used in this study

Notes: Eighty fish were purchased, and 76 of them were used in this study. Three fish was used to perform the pathogen-free detection. Three fish was used to extract the total RNA and synthesize cDNA. Others of the 76 fish were divided into three groups according to those described in Materials and Methods.

Abbreviation	Protein/Accession number	Abbreviation	Protein/Accession number	
HB-EGF	Proheparin-binding EGF-like	MMP-14	Matrix metalloproteinase-14	
	growth factor isoform X1		(XP 003457484.1)	
	(XP_019200773.1)			
СҮВА	Cytochrome b-245 light	MMP-15	Matrix metallopeptidase-15	
	chain (XP_003442297.1)		(XP_003437629.1)	
TIMP-2(1)	Metalloproteinase inhibitor 2	DCN	Decorin precursor	
	(XP_003450177.1)		(NP_001266536.1)	
TIMP-2(2)		РТК	Proto-oncogene tyrosine-protein	
	Metalloproteinase inhibitor 2		kinase Src isoform X4	
	(XP_003441786.1)		(XP_003438972.1)	
TIMP-4	Metalloproteinase inhibitor 4	TIMP-3	Metalloproteinase inhibitor 3	
	(XP_003454588.1)		(XP_005939292.1)	
Collagenase 3	Collagenase 3	Thbs2	Thrombospondin-2 isoform X3	
	(XP_013129643.2)		(XP_005474097.1)	
IGF-I	Insulin-like growth factor I	Plat	Tissue-type plasminogen activator	
	(AAC17494.1)		isoform X1 (XP_005473584.2)	
ITGB4	Integrin beta-4 isoform X2	Plasminogen	Plasminogen (XP_005461555.1)	
	(XP_013129071.1)			
Thbs1b	Thrombospondin-1b isoform	Lama4	Laminin subunit alpha-4	
	X1 (XP_005453322.1)		(XP_005457978.1)	
Thbs1a	TT1 1 1' 1	LYVE1	Lymphatic vessel endothelial	
	Inrombospondin-la		hyaluronic acid receptor 1 isoform	
	precursor (NP_001266664.1)		X4 (XP_005471040.1)	
			,	

**Table S2.** Interactions between NtMMP-9 and other proteins in Nile tilapia under S.agalactiae infection.

Note: The data of Nile tilapia were from the STRING 10.5 (http://string-db.org/) of Nile tilapia.

Interaction	NtMMP-9 aa	DCN aa	Length (Å)
Hydrophobic interaction	Cys-376, Cys-561	Trp-335	
TT 1 1 1''' / /'	Phe-538, Trp-540, Val-551,	Leu-327, Phe-328, Val-332,	
Hydrophobic interaction	Gly-552, Tyr-553	Pro-333, Leu-353	
Cation- $\pi$ interaction	Lys-377	Trp-335	
Anion- $\pi$ interaction	Asp-557	Tyr-334	
CH- $\pi$ interaction	Phe-538, Trp-540	Phe-328	
	Lys-377	Glu-336	3.1
Hydrogen bond	Tyr-553	Leu-327	2.3
	Val-551	Leu-353	2.4

Table S3. Putative binding sites of NtMMP-9 interacting with DCN

 Table S4. Putative binding sites of NtMMP-9 interacting with TIMP-2

Interaction			Length
Interaction	INUVIIVIE - 9 aa	T IIVIT -2 ad	(Å)
Hydrophobic interaction	Phe-183, Phe-209, Trp-202	Ala-197	
Underschabig interpotion	Phe-111, Phe-168, Gly-170,	Met-150, Val-194, Tyr-204,	
Hydrophobic interaction	Ala-172, Tyr-200, Tyr-215, Phe-217	Val-207, Ala-208	
Cation- $\pi$ interaction	Tyr-215	Arg-196	
CH- $\pi$ interaction	Tyr-215	Tyr-204	
	Asp-39	Gln-84	2.7
	Ala-31	Thr-83	2.5
Hudeo oon bond	Tyr-12	Gly-81	3.2
Hydrogen bond	Tyr-49	Gln-149	3.4
	Ser-23	Gln-146	3.5
	Arg-196	Met-150	2.8