

New putative phenoloxidase in ascidian blood cells

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Supplementary information

Domains 
Sru_Tuph MKKYKKDTSNFGFHNAAGTHVFPYQCMGPDGNMAGCCYHGARLNFAMWHRAELLNFEEGL
peptides -----AELLNFEEGL


Domains  TYR
Sru_Tuph TRHLKDKTLGLPYWDWLKYPEPPALVWSKKLYGKENPFTSMTIRYDTPHTLTQRSPTVY
peptides TR-----ENPFTSMTIRYDTPHTLTQRSPTVY

Domains 
Sru_Tuph PFMVKVSKDNYYVAQKLMQSHTIHEFSEALENSHNTIHAGICWGKESAAGKCTYSMGS LT
peptides PFMVK-----

Domains 
Sru_Tuph YAGFDPVFFFHHANVDRLFSVFNWMSAHGEEAWTRQSVLEPQENIFHF
peptides -----

Supplementary Figure S1. Peptides of 26 kDa band of *Styela rustica*.

Alignment of tryptic peptides from 26 kDa protein band with *in silico* translated *Tuphoxin* transcript of *S. rustica* (Sru_Tuph). Tryptic peptides are merged in longer sequences. Predicted conserved domain of tyrosinase (TYR) is marked by orange rectangle.

Domains 

Sca_Tuph KDSSTFGFHNAGGTHEFPFQCMGSSGEDKGCCYHGARLNFVMWHRAELLNFEEGLSRHLR
 peptides -----AELLNFEEGLTR

Domains  TYR

Sca_Tuph DKTLGLPYWDWLTYPNPPPLVWSKKLYNKANPFTSGLVRYDTKPHTLTQRSPTVDPFMVK
 peptides -----SPTVDPFMVK

Domains 








Sca_Tuph MSKDNYVARKLMQSHSIEHFSEALENSHNTIHSSICWGAQQKAGKCEFSMGGLTYAAFD
 peptides -----

Domains 

Sca_Tuph PVFFFHHTNVDRLFVSFQNWMTNNNEVAWTRQSVLEPQENIFHFNQPFLPFNNRSLTPFK
 Peptides -----

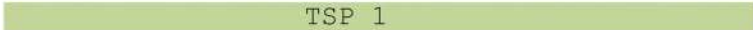






Supplementary Figure S2. Peptides of 26 kDa band of *Styela canopus*.

Alignment of tryptic peptides from 26 kDa band with *in silico* translated *Tuphoxin* transcript of *Styela canopus* (Sca_Tuph). Tryptic peptides are merged in longer sequences, with aa substitutions compared to translated transcript highlighted in yellow. Predicted conserved domain of tyrosinase (TYR) is marked by orange rectangle.

Hau_Tuph1	QRDTRNKLVVERLEGPEIDIESEESPFRRDDRENSLDEDEDNVLVYKGEHDHLTKALPGATCE	60
Peptides	-----	
Hau_Tuph1	AGYQIKPGCIFCQDIDECTTGTEKTLTVDQLLNADSDGLNNEVTKDQILTFHSEKALAIG	120
Peptides	-----	
Hau_Tuph1	KPLTPVKLYYRQPCSTLGSMTMVQNLHLNKKDKIPDMIITYVCVRSBSTNMYRIKSVFDKEA	180
Peptides	-----	
Domains	 TSP	
Hau_Tuph1	KWTEWTTWTACKPTIWGGLQTRARICKKGLVGYGKCLGSASAYRECYRGKKEALRVREA	240
Peptides	-----	
Domains	 *	
Hau_Tuph1	NLTTLTAQKSDLIQAMKKYKDDLSTYGYHQAGTHFWPNECVKPNGVSGACCYHGAKVNFV	300
Peptides	-----	
Domains	 * TYR	
Hau_Tuph1	MWHRAELLYYEQGLTRHLKDKKLGAPYWDWLVPNPPQLVWAKTIGGKPNPFAGSKIRYD	360
Peptides	-----KTIGGKPNPFAGSKI---	
Domains	 *	
Hau_Tuph1	LPVVISTARSPAVSSSSVSMAKSIFQTITSKLARSHMIHEYSESSLESSHNNIHSVCHAD	420
Peptides	-----	
Domains	 *	
Hau_Tuph1	TGCDYSMGELEYAAYDPIFFFHANVDRLFSVVFQSHMVKKKDMAWTRQGFLEPSENIYHF	480
Peptides	-----	
Hau_Tuph1	NEPYQPFNRKTLTPFKKLRVANSIADLLYYEQLLGYKYDSLKAHNTKLNTKDAIEEKSHV	540
Peptides	-----RVANSIADLLYYEQLLGYKYDSLKA-----	
Hau_Tuph1	PTKTDVVVLAQYEQSTGLNVKMRYFYAGGTTPLPPCASIADSNAAYLGTLTILGSRKERA	600
Peptides	-----	
Hau_Tuph1	WTAKQPTLLDITSKLTELEATLTSNIKIKACYTHGMGCQSKCKEIFATVRTKIPAPQTIL	660
Peptides	-----	
Domains		
Hau_Tuph1	YPSNIDVDIIKFRWDVGHRRMPIGKIVVNAGKEYIYFWGKEAKNVVQVGRTCYDSCSAMGG	720
Peptides	-----	
Domains	 CuOx	
Hau_Tuph1	KTVNCPYGCALPAGSHFFVNKKYCKSGLKLGILSVI	756
Peptides	-----	

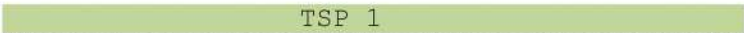






Supplementary Figure S3. Peptides of 48 kDa band of *Halocynthia aurantium*.

Alignment of tryptic peptides from 48 kDa band of morula cells with *in silico* translated *Tuphoxin* transcript of *H. aurantium* (Hau_Tuph1). Tryptic peptides are merged in longer sequences. Predicted conserved domains are marked by rectangles: green (thrombospondin first type repeat - TSP1), orange (tyrosinase - TYR) and blue (domain of cupredoxin family – CuOx). Asterisks (*) indicate the active site amino acids.

Hau_Tuph1	QRDTRNKLVERLEGPEDIESEESPFRDDRENSLDEDEDNVLVYKGEHDHLTKALPGATCE	60
Peptides	-----	
Hau_Tuph1	AGYQIKPGCIFCQDIDECTTGTEKTLTVDQLLNADSDGLNNEVTKDQILTFHSEKALAIG	120
Peptides	-----ALAIG	
Hau_Tuph1	KPLTPVKLYYRQPCSTLGSTMVQNLHLNKDKIPDMIITYVCVRSNSTMYRIKSVFDKEA	180
Peptides	KPLTPVK----QPCSTLGSTMVQNLHLNKDKIPDMIITYVCV-----IKSVFDKEA	
Domains	 TSP 1	
Hau_Tuph1	KWTEWTTWTACKPTIWGGLQTRARICKKGLVGYGKCLGSASAYRECYRGKKEALRVRREA	240
Peptides	K-----KGLVGYGKCLGSASAYR-----REA	
Domains		
Hau_Tuph1	NTLTTAQKSDLIQAMKKYKDDLSTYGYHQAAGTHFWPNECVKPNGVSGACCYHGAKVNFV	300
Peptides	NTLTTAQKSDLIQAMKKYKDDLSTYGYHQAAG-----GVSGACCYHGAKVNFV	
Domains	 TYR	
Hau_Tuph1	MWHRAELLYYEQGLTRHLKDKKLGAPYWDWLVYPNPPQLVWAKTIGGKPNPFAGSKIRYD	360
Peptides	MWHRAELLYYEQGLTR-----TIGGKPNPFAGSKIRYD	
Domains		
Hau_Tuph1	LPVVISTARSPAVSSSSVSMKSIFFQITITSKLARSHMIHEYSESLESSHNNIHSVCHAD	420
Peptides	LPVVISTARSPAVSSSSVSMKSIFFQITITSK-----	
Domains		
Hau_Tuph1	TGCDYSMGELEYAAYDPIFFFHHANVDRLFVSFQSHMVKKKDMAWTRQGFLEPSENIYHF	480
Peptides	-----LFSVFQSHMVK-----QGFLEPSENIYHF	
Hau_Tuph1	NEPYQPFNRKTLTPFKLRVANSIADLLYYEQLLGYKYDSLKAHNTKLNTKDAIEEKSHV	540
Peptides	NEPYQPFNR-----	
Hau_Tuph1	PTKTDVVVLAQYEQSTGLNVKMRIFYAGGTTPLPPCASIADSNAAYLGTTLTILGSRKERA	600
Peptides	-----	
Hau_Tuph1	WTAKQPTLLDITSKLLEATLTSNIKIKACYTHGMGCQSKCKEIFATVRTKIPAPQITIL	660
Peptides	-----	
Domains		
Hau_Tuph1	YPSNIDVDIIKFRWDVGHMPIGKIVVNAGKEYIYFWGKEAKNVVQVGRTCYDSCSAMGG	720
Peptides	-----	
Domains	 CuOx	
Hau_Tuph1	KTVNCPYGCALPAGSHFFVNKKYCKSGLKLGILSVI	756
Peptides	-----	



Supplementary Figure S4. Peptides of 35 kDa band of *Halocynthia aurantium*.

Alignment of tryptic peptides from 35 kDa band of morula cells with *in silico* translated *Tuphoxin* transcript of *H. aurantium* (Hau_Tuph1). Tryptic peptides are merged in longer sequences. Predicted conserved domains are marked by rectangles: green (thrombospondin first type repeat - TSP1), orange (tyrosinase - TYR) and blue (domain of cupredoxin family – CuOx).

Hau_Tuph1	QRDTRNKLVERLEGPEIDIESEESPFRDDRENSLDEDEDNVLVYKGEHDHLTKALPGATCE	60
Hau_Tuph2	-----	0
Peptides	-----	
Hau_Tuph1	AGYQIKPGCIFCQDIDECTTGTEKTLTVDQLLNADSDGLNNEVTKDQILTFHSEKALAIG	120
Hau_Tuph2	-----	0
Peptides	-----ALAIG	
Hau_Tuph1	KPLTPVKLYYRQPCSTLSTLMVQNLHLNKKDKIPDMIITYVCVRSRGSTNMYRIKSVFDKEA	180
Hau_Tuph2	-----MVQNLHLNKKDKIPDMIITYVCVRSRGSTNMYRIKSVFDKE-	39
Peptides	KPLTPVK-----	
Domains	 TSP 1	
Hau_Tuph1	KWTEWTTWTACKPTIWGGLQTRARICKKGLVGYGKCLGSASAYRECYRGKKEALRVRREA	240
Hau_Tuph2	-----ALRVRREA	
Peptides	-----	
Domains	 *	
Hau_Tuph1	NLTTLTAQKSDLIQAMKKYKDDLSTYGYHQAAGTHFWPNECVKPNGVSGACCYHGAKVNFV	300
Hau_Tuph2	NLTTLTAQKSDLIQAMKKYKDDLSTYGYHQAAGTHFWPNECVKPNGVSGACCYHGAKVNFV	107
Peptides	-----	
Domains	 *	
Hau_Tuph1	MWHRAELLYEQGLTRHLKDKKLGAPYWDWLVPNPPQLVWAKTIGGKPNPFAGSKIRYD	360
Hau_Tuph2	MWHRAELLYEQGLTRHLKDKKLGAPYWDWLVPNPPQLVWAKTIGGKPNPFAGSKIRYD	167
Peptides	----AELLYEQGLTR-----	
Domains	 TYR * *	
Hau_Tuph1	LPVVISTARSPAVSSSSVSMAKSIFQTITSKLARSHMIHEYSESLESSHNNIHSVCHAD	420
Hau_Tuph2	LPVVISTARSPAVSSSSVSMAKSIFQTITSKLARSHMIHEYSESLESSHNNIHSVCHAD	227
Peptides	-----	
Domains	 *	
Hau_Tuph1	TGCDYSMGELEYAAYDPIFFFHHANVDRLFVSVFQSHMVKKKDMAWTRQGFLEPSENIYHF	480
Hau_Tuph2	TGCDYSMGELEYAAYDPIFFFHHANVDRLFVSVFQSHMVKKKDMAWTRQGFLEPSENIYHF	287
Peptides	-----	
Hau_Tuph1	NEPYQPFNRKTLTPFKKLRVANSIADLLYEQLLGYKYDSLKAHNTKLNKDAIEEKSHV	540
Hau_Tuph2	NEPYQPFNRKTLTPFKKLRVANSIADLLYEQLLGYKYDSLKAHNTKLNKDAIEEKSHV	347
Peptides	-----SHV	
Hau_Tuph1	PTKTDVVVLAQYEQSTGLNVKMRIFYAGGTTPLPPCASIADSNAAYLGTTLILGSRKERA	600
Hau_Tuph2	PTKTDVVVLAQYEQSTGLNVKMRIFYAGGTTPLPPCASIADSNAAYLGTTLILGSRKERA	407
Peptides	PTKTDVVVLAQYEQSTGLNV-----	
Hau_Tuph1	WTAKQPTLLDITSKLTELEATLTSNIKIKACYTHGMGCQSKCKEIFATVVRTKIPAPQTIL	660
Hau_Tuph2	WTAKQPTLLDITSKLTELEATLTSNIKIKACYTHGMGCQSKCKEIFATVVRTKIPAPQTIL	467
Peptides	---KQPTLLDITSKLTELEATLTSNIK-----TKIPAPQTIL	
Domains		
Hau_Tuph1	YPSNIDVDIIKFRWDVGHHRMPIGKIVVNAGKEYIYFWGKEAKNVVQVGRTCYDSCSAMGG	720
Hau_Tuph2	YPSNIDVDIIKFRWDVGHHRMPIGKIVVNAGKEYIYFWGKEAKNVVQVGRTCYDSCSAMGG	527
Peptides	YPSNIDVDIIK--WDVGH-----EYIYFWGK---NVVQVGR-----	
Domains	 CuOx	
Hau_Tuph1	KTVNCPYGCALPAGSHFFVNKKYCKSGLKLGILSVI	756
Hau_Tuph2	KTVNCPYGCALPAGSHFFVNKKYCKSGLKLGILSVI	563
Peptides	-TVNCPYGCALPAGSHFFVNK-----	

Supplementary Figure S5. Alignment of two *Tuphoxin* transcripts of *Halocynthia aurantium*.

Alignment of tryptic peptides from 26 kDa band of morula cells with two *in silico* translated *Tuphoxin* transcripts of *H. aurantium* (Hau_Tuph1 and Hau_Tuph2). Tryptic peptides are merged in longer sequences. Predicted conserved domains are marked by rectangles: green (thrombospondin first type repeat - TSP1), orange (tyrosinase - TYR) and blue (domain of cupredoxin family – CuOx). Asterisks (*) indicate the active site amino acids.

Hau_Tuph1	WTAKQPTLLDITSKLTELEATLTSNIKIKACYTHGMGCQSKCKEIFATVVRTKIPAPQTIL	660
Peptides	-----LTELEATLTSNIK-----	
Domains		
Hau_Tuph1	YPSNIDVDIIKFRWDVGHHRMPIGKIVVNAGKEYIYFWGKEAKNVVQVGRTCYDSCSAMGG	720
Peptides	-----EYIYFWGK-----	
Domains	 CuOx	
Hau_Tuph1	KTVNCPYGCALPAGSHFFVNKKYCKSGLKLGILSVI	756
Peptides	-----	

Supplementary Figure S6. Peptides of 26 kDa band of *Halocynthia aurantium* hyalinocytes.

Alignment of tryptic peptides from 26 kDa band of hyalinocytes with *in silico* translated *Tuphoxin* transcript of *H. aurantium* (Hau_Tuph1). Predicted conserved domain of cupredoxin family (CuOx) is marked by blue rectangle.

Sru_Tuph	-----	0
Sca_Tuph	MREALVITLLWVCVAVTQVEGRKKTCAVNFVGGCDKSRGLCWNTDGGKSSMCSCKAGYRFR	60
Hau_Tuph1	-----	0
Hau_Tuph2	-----	0
Domains	+ + EGF_CA1 +	
Sru_Tuph	-----	0
Sca_Tuph	LPHTRRGRCVDINECRANLHSCDTKNGGCTNTIGSHRCYCKKGFVLDYTKTKLRRKHIK	120
Hau_Tuph1	-----	0
Hau_Tuph2	-----	0
Domains	+ +	
Sru_Tuph	-----	0
Sca_Tuph	GTCRGRRCGRRCNRFRYDAEDEDASKFLLRLLRRRKGRCVCEPGFAKRRGCKYQCINE	180
Hau_Tuph1	-----	0
Hau_Tuph2	-----	0
Domains	EGF_CA2 +	
Sru_Tuph	-----CGFKCYEPTKRPSTCNSESFQSSSL	26
Sca_Tuph	CRLRISMCDLRRGYCHNSDGGYFCSCRKGYVLGPCGFKCYRPPKRPSCNSEIFKSSSLA	240
Hau_Tuph1	-----	0
Hau_Tuph2	-----	0
Sru_Tuph	KGDS DGLNDETTKAVLLDYITEKIAKSGGKLSLFHTLMYKTCKTGGAQRVQRFVDVDGDGK	86
Sca_Tuph	KGDS DGLNDETTKAVLLDYITEKIAKAGGRLTLFHTLMYKTCKTGQKKRIQRFDINRDGK	300
Hau_Tuph1	-----MVQNLHLNKKDKI	12
Hau_Tuph2	-----MVQNLHLNKKDKI	12
	:*.:*.:* :*	
Domains	TSP 1	
Sru_Tuph	TDIIINYVCVGL---KWTVKNVFDTEAKWETWSCWNTCKPTCSGGIQTRTRICKHGLVG	142
Sca_Tuph	DDLVIHYVCVGN---KWTVKAVYEEEARWAPWTCWTSCKPTCSGGIQTRVRICKNGLVG	356
Hau_Tuph1	PDMIITYCVRSNSTMYRIKSVFDKEAKWETWTTWTACKPTIWGGLQTRARICKKGLVG	72
Hau_Tuph2	PDMIITYCVRSNSTMYRIKSVFDKE-----	39
	.: *.* * : * *.:* *	
Domains		
Sru_Tuph	EGRCKGPATEYKECYTGRKCKLRVREAHSLNRVQKKDLIQAMKKYKDDTSTNFGFHNAAG	202
Sca_Tuph	EGKCKGRATQYKTCYTGKCCPLRVREAHSLNTRQKKDLIQAMEKYKDDSTSTFGFHNAAG	416
Hau_Tuph1	YGKCLGSASAYRECYRGKKEALRVREANTLTTAQKSDLIQAMKKYKDDLSTYGYHQAAG	132
Hau_Tuph2	-----ALRVREANTLTTAQKSDLIQAMKKYKDDLSTYGYHQAAG	79
	*****:*. *.*****:***. *.:*.:*.*	
Domains		
Sru_Tuph	THVFPYQCMGPDGNMAGCCYHGARLNFAMWHRRAELLNFEEGLTRHLKDKTLGLPYWDWLK	262
Sca_Tuph	THEFPFQCMGSSGEDKGCYHGARLNFVMWHRRAELLNFEEGLSRHLRDKTLGLPYWDWLT	476
Hau_Tuph1	THFWPNECVKPNGVSGACCYHGAKVNFVMWHRRAELLYEQGLTRHLKDKKLGAPYWDWL	192
Hau_Tuph2	THFWPNECVKPNGVSGACCYHGAKVNFVMWHRRAELLYEQGLTRHLKDKKLGAPYWDWL	139
	** : * : * : * . * .*****:*. ***** : * : * : * : * : * : * : * : * : *	
Domains	TYR	
Sru_Tuph	YPEPPALVWSKKLYGKENPFTSMTIRYDTPHTLTQRSPTVYPFMVKSKDNYY-VAQKL	321
Sca_Tuph	YPNPPPLVWSKKLYNKANPFTSGLVRYDTPHTLTQRSPTVDPFMVKMSKDNYY-VARKL	535
Hau_Tuph1	YPNPPQLVWAKTIGGKPNPFAGSKIRYDLPVVISSTARS PAVSSSSVSMKSI FQTITSKL	252
Hau_Tuph2	YPNPPQLVWAKTIGGKPNPFAGSKIRYDLPVVISSTARS PAVSSSSVSMKSI FQTITSKL	199
	** : * * : * : * . * * : * : * : * : * : * : * : * : * : * : * : * : *	
Domains		
Sru_Tuph	MQSHTIHEFSEALENSHNTI HAGICWGKESAAGKCTYSMGLTYAGFDPVFFFHHANVDR	381
Sca_Tuph	MQSHTIHEFSEALENSHNTI HSSICWGAQQKAGKCEFSMGGLTYAADFDPVFFFHHTNVDR	595
Hau_Tuph1	ARSHMIHEYSESLSSHNNIHS AVCHA----DTGCDYSMGELEYAAYDPIFFFHHANVDR	308
Hau_Tuph2	ARSHMIHEYSESLSSHNNIHS AVCHA----DTGCDYSMGELEYAAYDPIFFFHHANVDR	255
	: * * : * : * : * . * : * : * * * : * : * : * : * : * : * : * : *	
Domains		
Sru_Tuph	LFSVFNWMSAHGEEAWTRQSVLEPQENIFHFNEPYLPFRNKTLTPFKKLQELASIQNMF	441
Sca_Tuph	LFSVFNWMTNNEVAWTRQSVLEPQENIFHFNQPF LFPNRRSLTPFKKLHALSTIQDLF	655
Hau_Tuph1	LFSVFNWMSAHGEEAWTRQSVLEPQENIFHFNEPYLPFRNKTLTPFKKLQELASIQNMF	368
Hau_Tuph2	LFSVFNWMSAHGEEAWTRQSVLEPQENIFHFNEPYLPFRNKTLTPFKKLQELASIQNMF	315
	*****. * : : *****.***.***:***. * : * : * : * : * : * : * : *	
Sru_Tuph	YYRELLGYRYDNLAV-----	456
Sca_Tuph	FNRELLGYRYDSLKVPKEKKTKKVGDDEEKAHIPSSGDKLMLGQFERNIRTNVKITYFFAN	715
Hau_Tuph1	YYEQLLGYKYDSLKAHNTKLNKDAIEEKSHVPTKTDVVVLAQYEQSTGLNVKMRYFYAG	428
Hau_Tuph2	YYEQLLGYKYDSLKAHNTKLNKDAIEEKSHVPTKTDVVVLAQYEQSTGLNVKMRYFYAG	375
	: . : * : * : * : * .	
Sru_Tuph	-----	456
Sca_Tuph	KIKGFKDIKTC SKLNEDETRYLASAQILGSKRERAWKSRQPTVVDI SNLFGKLTWQQRRT	775
Hau_Tuph1	G---TTPLPPCASIADSNAAYLGTLTILGSRKERAWTAKQPTLLDITSKLTELE-----	479
Hau_Tuph2	G---TTPLPPCASIADSNAAYLGTLTILGSRKERAWTAKQPTLLDITSKLTELE-----	426
Sru_Tuph	-----	456
Sca_Tuph	SGLSATSNIQIRACYTHNLS CRKNCKQIRSRVKKLIPSPQLILYPKNIRTDIQKIEWAND	835
Hau_Tuph1	--ATLTSNIKIKACYTHGMGCQSKCKEIFATVVRTKI PAPQTILYPSNIDVDI IKFRWDVG	537
Hau_Tuph2	--ATLTSNIKIKACYTHGMGCQSKCKEIFATVVRTKI PAPQTILYPSNIDVDI IKFRWDVG	484
Domains	CuOx	
Sru_Tuph	-----	456
Sca_Tuph	VRLPTWTIDAVSGTRWVFWGSEAKNVVQVARRAYLTCNTRGARKVRCETACVLPSTGIH	895
Hau_Tuph1	HRMPIGKIVVAGKEYIYFWGKEAKNVVQVG-RTCYDSCSAMGGKTVNCPYGCALPAGSH	596
Hau_Tuph2	HRMPIGKIVVAGKEYIYFWGKEAKNVVQVG-RTCYDSCSAMGGKTVNCPYGCALPAGSH	543
Domains		
Sru_Tuph	-----	456
Sca_Tuph	YFVHKTRCRRGMKLSVLAHL	915
Hau_Tuph1	FFVNKKYCKSGLKLGILSVI	616
Hau_Tuph2	FFVNKKYCKSGLKLGILSVI	563

Supplementary Figure S7. Alignment of *Tuphoxin* sequences belonging to *Styela rustica*, *S. canopus* and *Halocynthia aurantium*.

Alignment of four translated sequences of *Tuphoxins* belonging to *S. rustica* (Sru_Tuph), *S. canopus* (Sca_Tuph), and *H. aurantium* (Hau_Tuph1, Hau_Tuph2). Predicted conserved domains are marked by rectangles: grey (calcium-binding EGF-like – EGF_CA), green (thrombospondin first type repeat - TSP1), orange (tyrosinase - TYR), blue - cupredoxin family domain (CuOx).

A Adhesion G protein-coupled receptor B3 of *Homo sapiens* - O60242 (AGRB3_HUMAN)
Expect = 6e-10 Identities = 21/46 (46%)

```
TSP1    1    WETWSCWNTCKPTCSGGIQTRTRICKHGLVGEGRCKGPATEYKEYC 46
          W+ WS W  C  TCS G Q R+R C      G  C+GP  E +ECY
AGRB3  403 WQEWSSWSQCSVTCSNGTQQRSRQCTAAAHGGSECRGPWAESRECY 448
```

B Zinc metalloproteinase nas-36 OS of *Caenorhabditis briggsae* - Q61EX6 (NAS36_CAEBR)
Expect = 3e-06 Identities = 23/53 (43%)

```
TSP1    1    WAPWTCWTSCKPTCSG-GIQTRVTRICKNGLVGEGKCKGRATQYKTCYTGKKCP 52
          WA W  W+ C  TC G GI++RVR C+      KC+GR  ++ TC  K CP
NAS36  509 WADWGEWSMCSRTCGGCGIRSRVRSRCS-----KKCEGRRQEFGTCNL-KACP 555
```

Supplementary Figure S8. Results of similarity search for TSP1 domain.

Alignment of Taphoxins sequences of TSP1 belonging to *S. rustica* (*Sru_Tuph*) (A) and *S. canopus* (*Sca_Tuph*) (B) with best hit sequences in UniProtKB/Swiss-Prot database.

Supplementary Table S1. Sequences of *Tuphoxin* homologues used for phylogeny construction.

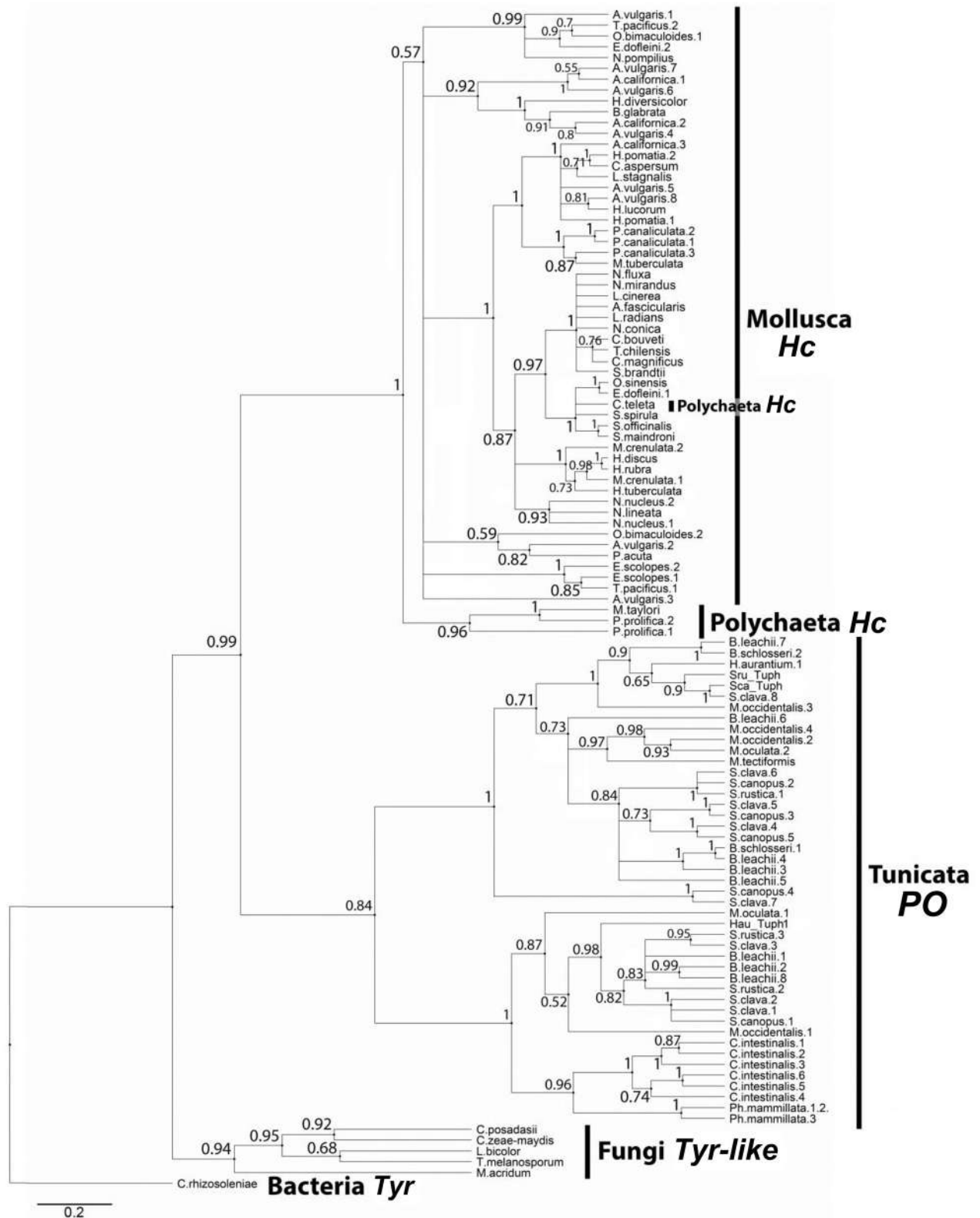
Colour codes				
Ascidacea	Annelida	Mollusca	Fungi	Cyanobacteria
Database ID	DB	Name	Species	
Contig_28777 a)	de novo	Sru_Tuphoxin	<i>Styela rustica</i>	
Contig_67444 a)	de novo		<i>Styela rustica</i>	
Contig_7091	de novo	S.rustica.1	<i>Styela rustica</i>	
Contig_7704	de novo	S.rustica.2	<i>Styela rustica</i>	
Contig_15578	de novo	S.rustica.3	<i>Styela rustica</i>	
SC_m.117002.p1	GitHub	Sca_Tuphoxin	<i>Styela canopus</i>	
SC_m.152480.p1	GitHub	S.canopus.1	<i>Styela canopus</i>	
SC_m.16402.p1	GitHub	S.canopus.2	<i>Styela canopus</i>	
SC_m.86302.p1	GitHub	S.canopus.3	<i>Styela canopus</i>	
SC_m.42250.p1	GitHub	S.canopus.4	<i>Styela canopus</i>	
SC_m.26137.p1	GitHub	S.canopus.5	<i>Styela canopus</i>	
AA49_m.7122.p1	GitHub	S.clava.1	<i>Styela clava</i>	
AA49_m.7119.p1	GitHub	S.clava.2	<i>Styela clava</i>	
AA49_m.30831.p1	GitHub	S.clava.3	<i>Styela clava</i>	
AA49_m.87151.p1	GitHub	S.clava.4	<i>Styela clava</i>	
AA49_m.2942.p1	GitHub	S.clava.5	<i>Styela clava</i>	
AA49_m.30764.p1	GitHub	S.clava.6	<i>Styela clava</i>	
AA49_m.72475.p1	GitHub	S.clava.7	<i>Styela clava</i>	
AA49_m.3326.p1	GitHub	S.clava.8	<i>Styela clava</i>	
Haaura.CG.MTP2014.S1292.g08050.02.p	Aniseed	Hau_Tuphoxin1	<i>Halocynthia aurantium</i>	
Haaura.CG.MTP2014.S1209.g07864.01.p	Aniseed	H.aurantium.1	<i>Halocynthia aurantium</i>	
Phfumi.CG.MTP2014.S5765.g07096.01.p b)	Aniseed	Ph.mammillata.1	<i>Phallusia mammillata</i>	
Phfumi.CG.MTP2014.S5765.g07097.01.p b)	Aniseed	Ph.mammillata.2	<i>Phallusia mammillata</i>	
CAB3267405.1	GenBank	Ph.mammillata.3	<i>Phallusia mammillata</i>	
Moocul.CG.ELv1_2.S110138.g11514.01.p	Aniseed	M.oculata.1	<i>Molgula oculata</i>	
Moocul.CG.ELv1_2.S61057.g03850.01.p	Aniseed	M.oculata.2	<i>Molgula oculata</i>	
Moocci.CG.ELv1_2.S255256.g05916.01.p	Aniseed	M.occidentalis.1	<i>Molgula occidentalis</i>	
Moocci.CG.ELv1_2.S565922.g22193.01.p	Aniseed	M.occidentalis.2	<i>Molgula occidentalis</i>	
Moocci.CG.ELv1_2.S454880.g14754.01.p	Aniseed	M.occidentalis.3	<i>Molgula occidentalis</i>	
Moocci.CG.ELv1_2.S568724.g22364.01.p	Aniseed	M.occidentalis.4	<i>Molgula occidentalis</i>	
CJ410700.1	GenBank	M.tectifformis	<i>Molgula tectifformis</i>	
XP_026691523.1	GenBank	C.intestinalis.2	<i>Ciona intestinalis</i>	
XP_002122867.2	GenBank	C.intestinalis.1	<i>Ciona intestinalis</i>	
XP_018672468.2	GenBank	C.intestinalis.3	<i>Ciona intestinalis</i>	
XP_018671739.2	GenBank	C.intestinalis.4	<i>Ciona intestinalis</i>	
XP_002119675.4	GenBank	C.intestinalis.5	<i>Ciona intestinalis</i>	
XP_002128425.2	GenBank	C.intestinalis.6	<i>Ciona intestinalis</i>	
Boleac.CG.SB_v3.S604.g12287.01.p	Aniseed	B.leachii.1	<i>Botrylloides leachii</i>	
Boleac.CG.SB_v3.S604.g12288.01.p	Aniseed	B.leachii.2	<i>Botrylloides leachii</i>	
Boleac.CG.SB_v3.S227.g05332.01.p	Aniseed	B.leachii.3	<i>Botrylloides leachii</i>	
Boleac.CG.SB_v3.S227.g05333.01.p	Aniseed	B.leachii.4	<i>Botrylloides leachii</i>	
Boleac.CG.SB_v3.S227.g05334.01.p	Aniseed	B.leachii.5	<i>Botrylloides leachii</i>	
Boleac.CG.SB_v3.S227.g05338.01.p	Aniseed	B.leachii.6	<i>Botrylloides leachii</i>	
Boleac.CG.SB_v3.S195.g04406.01.p	Aniseed	B.leachii.7	<i>Botrylloides leachii</i>	
Boleac.CG.SB_v3.S281.g06648.01.p	Aniseed	B.leachii.8	<i>Botrylloides leachii</i>	
JG332329.1	GenBank	B.schlosseri.1	<i>Botryllus schlosseri</i>	
JG338731.1	GenBank	B.schlosseri.2	<i>Botryllus schlosseri</i>	
AWK28007.2	GenBank	P.prolifica.1	<i>Phyllochaetopterus prolifica</i>	
AWK28006.2	GenBank	P.prolifica.2	<i>Phyllochaetopterus prolifica</i>	

AWK28003.1	GenBank	M. taylori	<i>Mesochaetopterus taylori</i>
EY523895.1	GenBank	C. teleta	<i>Capitella teleta</i>
CAQ64614.1	GenBank	T. chilensis	<i>Tonicia chilensis</i>
CAQ64609.1	GenBank	N. mirandus	<i>Nuttallochiton mirandus</i>
CAQ64607.1	GenBank	N. conica	<i>Notoplax conica</i>
CAQ64615.1	GenBank	A.fascicularis	<i>Acanthochitona fascicularis</i>
CAQ64608.1	GenBank	N.fluxa	<i>Nuttallina fluxa</i>
CAQ64600.1	GenBank	C.magnificus	<i>Chiton magnificus</i>
CAQ64603.1	GenBank	L.radians	<i>Lepidozona radians</i>
CAQ30429.1	GenBank	C.bouveti	<i>Callochiton bouveti</i>
CAQ30427.1	GenBank	N.lineata	<i>Nierstraszella lineata</i>
CAQ64612.1	GenBank	S.brandtii	<i>Schizoplax brandtii</i>
AYO86686.1	GenBank	H.pomatia.1	<i>Helix pomatia</i>
AYO86688.1	GenBank	H.pomatia.2	<i>Helix pomatia</i>
AEO51768.1	GenBank	H.lucorum	<i>Helix lucorum</i>
AYO86691.1	GenBank	L.stagnalis	<i>Lymnaea stagnalis</i>
CEK95569.1	GenBank	A.vulgaris.1	<i>Arion vulgaris</i>
CEK81230.1	GenBank	A.vulgaris.2	<i>Arion vulgaris</i>
CEK79687.1	GenBank	A.vulgaris.3	<i>Arion vulgaris</i>
CEK74026.1	GenBank	A.vulgaris.4	<i>Arion vulgaris</i>
CEK95583.1	GenBank	A.vulgaris.5	<i>Arion vulgaris</i>
CEK74025.1	GenBank	A.vulgaris.6	<i>Arion vulgaris</i>
AYO86690.1	GenBank	A.vulgaris.7	<i>Arion vulgaris</i>
AYO86689.1	GenBank	A.vulgaris.8	<i>Arion vulgaris</i>
DW255933.1	GenBank	E.scolopes.1	<i>Euprymna scolopes</i>
DW256698.1	GenBank	E.scolopes.2	<i>Euprymna scolopes</i>
DAC71535.1	GenBank	A.californica.1	<i>Aplysia californica</i>
CAD88977.1	GenBank	A.californica.2	<i>Aplysia californica</i>
XP_035826072.1	GenBank	A.californica.3	<i>Aplysia californica</i>
AYO86685.1	GenBank	C.aspersum	<i>Cornu aspersum</i>
PVD31865.1	GenBank	P.canaliculata.1	<i>Pomacea canaliculata</i>
PVD32161.1	GenBank	P.canaliculata.2	<i>Pomacea canaliculata</i>
PVD32038.1	GenBank	P.canaliculata.3	<i>Pomacea canaliculata</i>
CAH10287.1	GenBank	N.nucleus.1	<i>Nucula nucleus</i>
CAH10286.1	GenBank	N.nucleus.2	<i>Nucula nucleus</i>
CAG28308.1	GenBank	M.crenulata.1	<i>Megathura crenulata</i>
4BED_B	GenBank	M.crenulata.2	<i>Megathura crenulata</i>
CAC82192.1	GenBank	H.tuberculata	<i>Haliotis tuberculata</i>
ANE23704.1	GenBank	H.rubra.1	<i>Haliotis rubra</i>
CX726143.1	GenBank	H.discus	<i>Haliotis discus</i>
GT869640.1	GenBank	H.diversicolor	<i>Haliotis diversicolor</i>
ABD47516.1	GenBank	S.officinalis.1	<i>Sepia officinalis</i>
CBW46988.1	GenBank	L.cinerea	<i>Lepidochitona cinerea</i>
AGX25261.1	GenBank	M.tuberculata	<i>Melanoides tuberculata</i>
O61363.1	GenBank	E.dofleini.1	<i>Enteroctopus dofleini</i>
AAA29391.1	GenBank	E.dofleini.2	<i>Enteroctopus dofleini</i>
4YD9_A	GenBank	T.pacificus.1	<i>Todarodes pacificus</i>
4YD9_B	GenBank	T.pacificus.2	<i>Todarodes pacificus</i>
ADT91166.1	GenBank	S.spirula	<i>Spirula spirula</i>
AGV74427.1	GenBank	S.maindroni	<i>Sepiella maindroni</i>

CAF03590.1	GenBank	N.pompilius	<i>Nautilus pompilius</i>
XP_014789836.1	GenBank	O.bimaculoides.1	<i>Octopus bimaculoides</i>
XP_014779647.1	GenBank	O.bimaculoides.2	<i>Octopus bimaculoides</i>
XP_029636914.2	GenBank	O.sinensis	<i>Octopus sinensis</i>
HX972596.1	GenBank	P.acuta	<i>Physella acuta</i>
ES748875.1	GenBank	B.glabrata	<i>Biomphalaria glabrata</i>
JK740481.1	GenBank	M.acridum	<i>Metarhizium acridum</i>
FP450128.1	GenBank	T.melanosporum	<i>Tuber melanosporum</i>
FG247591.1	GenBank	C.zea-maydis	<i>Cercospora zea-maydis</i>
CO033831.1	GenBank	C.posadasii	<i>Coccidioides posadasii</i>
EX805973.1	GenBank	L.bicolor	<i>Laccaria bicolor</i>
WP_088241413.1	GenBank	C.rhizosoleniae	<i>Calothrix rhizosoleniae</i>

Footnotes:

- a) - overlapping sequences, merged to give Tuphoxin transcript of *Styela rustica*
- b) - overlapping sequences, merged under the name of *Ph.mammillata.1.2*



Supplementary Figure S9. Phylogenetic analysis of tyrosinase domains of *Tufphoxin* homologues. Phylogenetic tree based on Bayesian Inference (BI) rooted by bacterial sequence. Statistical support (posterior probabilities) are indicated at the nodes. Only posterior probabilities >0.5 are shown. Accession numbers of the proteins used in this tree can be found in Supplementary Table S1.

Supplementary Table S2 Arthropod-like phenol oxidases of ascidians used for phylogeny construction.

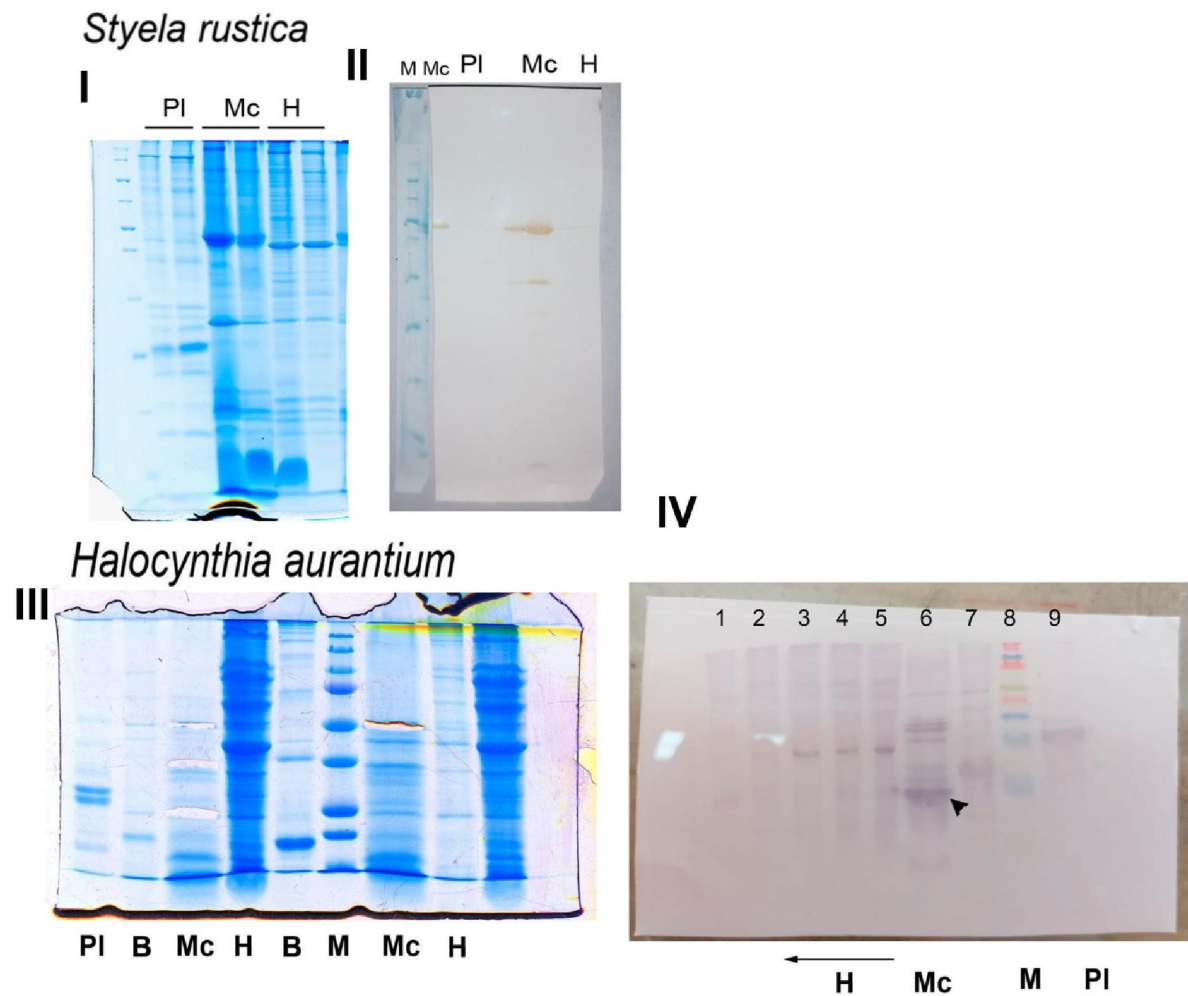
Database ID	Sequence name	Species	Protein name	Citation
XP_018671030.1	C.intestinalis.7	<i>Ciona intestinalis</i>	tyr1	Aguilera et al., 2013
NP_001029009.2	C.intestinalis.8	<i>Ciona intestinalis</i>	tyr3	Aguilera et al., 2013
CAD68058.1	C.intestinalis.9	<i>Ciona intestinalis</i>	tyr4	Aguilera et al., 2013
AJ547814	C.intestinalis.10	<i>Ciona intestinalis</i>		Immesberger, Burmester, 2004
ALM04212.1	B.schlosseri.3	<i>Botryllus schlosseri</i>	HLP	Ballarin et al., 2012

Supplementary Table S3. Number of unique alleles of *Tuphoxin* homologues present in Tunicata. Eleven ascidian species discussed in the present study are listed with number of alleles in each of two clades of Tunicata homologues. Clades are supported by maximum likelihood (ML) inference of phylogenetic relations.

species	number of unique alleles	
	clade I	clade II
<i>Styela rustica</i>	2	2
<i>Styela canopus</i>	5	1
<i>Styela clava</i>	5	3
<i>Halocynthia aurantium</i>	1	1
<i>Phallusia mammillata</i>	0	1
<i>Molgula oculata</i>	1	1
<i>Molgula occidentalis</i>	3	1
<i>Molgula tectiformis</i>	1	0
<i>Ciona intestinalis</i>	0	6
<i>Botryllus schlosseri</i>	2	0
<i>Botrylloides leachii</i>	5	3

Supplementary Table S4. Presence or absence of TSP1 domain and/or cupredoxin-like sequence (CuOx-like) in full length homologues of *Tuphoxin* belonging to Tunicata.

Database ID	Name	Species	TSP1	CuOx-like
Phfumi.CG.MTP2014.S5765.g07096.01.p	Ph.mammillata.1.2	<i>Phallusia mammillata</i>		
CAB3267405.1	Ph.mammillata.3	<i>Phallusia mammillata</i>		
Moocul.CG.ELv1_2.S110138.g11514.01.p	M.oculata.1	<i>Molgula oculata</i>		
Moocul.CG.ELv1_2.S61057.g03850.01.p	M.oculata.2	<i>Molgula oculata</i>		
Moocci.CG.ELv1_2.S255256.g05916.01.p	M.occidentalis1	<i>Molgula occidentalis</i>		
Moocci.CG.ELv1_2.S565922.g22193.01.p	M.occidentalis2	<i>Molgula occidentalis</i>		
Moocci.CG.ELv1_2.S454880.g14754.01.p	M.occidentalis3	<i>Molgula occidentalis</i>		
Moocci.CG.ELv1_2.S568724.g22364.01.p	M.occidentalis4	<i>Molgula occidentalis</i>		
CJ410700.1.p3	M.tectiformis	<i>Molgula tectiformis</i>		
XP_002122867.2	C.intestinalis.1	<i>Ciona intestinalis</i>		
XP_026691523.1	C.intestinalis.2	<i>Ciona intestinalis</i>		
XP_018672468.2	C.intestinalis.3	<i>Ciona intestinalis</i>		
XP_018671739.2	C.intestinalis.4	<i>Ciona intestinalis</i>		
XP_002119675.4	C.intestinalis.5	<i>Ciona intestinalis</i>		
XP_002128425.2	C.intestinalis.6	<i>Ciona intestinalis</i>		
Boleac.CG.SB_v3.S604.g12287.01.p	B.liachii.1	<i>Botrylloides leachii</i>		
Boleac.CG.SB_v3.S604.g12288.01.p	B.liachii.2	<i>Botrylloides leachii</i>		
Boleac.CG.SB_v3.S227.g05332.01.p	B.liachii.3	<i>Botrylloides leachii</i>		
Boleac.CG.SB_v3.S227.g05333.01.p	B.liachii.4	<i>Botrylloides leachii</i>		
Boleac.CG.SB_v3.S227.g05334.01.p	B.liachii.5	<i>Botrylloides leachii</i>		
Boleac.CG.SB_v3.S227.g05338.01.p	B.liachii.6	<i>Botrylloides leachii</i>		
Boleac.CG.SB_v3.S195.g04406.01.p	B.liachii.7	<i>Botrylloides leachii</i>		
Boleac.CG.SB_v3.S281.g06648.01.p	B.liachii.8	<i>Botrylloides leachii</i>		
AA49_m.7122.p1	S.clava.1	<i>Styela clava</i>		
AA49_m.7119.p1	S.clava.2	<i>Styela clava</i>		
AA49_m.30831.p1	S.clava.3	<i>Styela clava</i>		
AA49_m.87151.p1	S.clava.4	<i>Styela clava</i>		
AA49_m.2942.p1	S.clava.5	<i>Styela clava</i>		
AA49_m.30764.p1	S.clava.6	<i>Styela clava</i>		
AA49_m.72475.p1	S.clava.7	<i>Styela clava</i>		
AA49_m.3326.p1	S.clava.8	<i>Styela clava</i>		
SC_m.117002.p1	Sca_Tuphoxin	<i>Styela canopus</i>		
SC_m.152480.p1	S.canopus.1	<i>Styela canopus</i>		
SC_m.16402.p1	S.canopus.2	<i>Styela canopus</i>		
SC_m.86302.p1	S.canopus.3	<i>Styela canopus</i>		
SC_m.42250.p1	S.canopus.4	<i>Styela canopus</i>		
SC_m.26137.p1	S.canopus.5	<i>Styela canopus</i>		
Contig_28777+NODE_67444	Sru_Tuphoxin	<i>Styela rustica</i>		
Contig_7091	S.rustica.1	<i>Styela rustica</i>		
Contig_7704	S.rustica.2	<i>Styela rustica</i>		
Contig_15578	S.rustica.3	<i>Styela rustica</i>		
Haaura.CG.MTP2014.S1292.g08050.02	Hau_Tuphoxin1	<i>H.aurantium</i>		
Haaura.CG.MTP2014.S1209.g07864.01	H.aurantium.1	<i>H.aurantium</i>		
JG332329.1.p1	B.schlosseri.1	<i>Botryllus schlosseri</i>		
JG338731.1.p1	B.schlosseri.2	<i>Botryllus schlosseri</i>		



Supplementary Figure S12. Examples of the original electrophoresis and immunoblot of the blood cells from the species indicated. I, III - SDS electrophoresis; II, IV - immunoblot. Hyalinocytes (H), morula cells (Mc), blood plasma (PI), bottom fraction of degranulated cells (B) and marker proteins ladder (M) At III the lines visible cut out for MALDI. At IV arrow at the bottom indicate the decreasing loading of hyalinocyte fraction; arrowhead indicate the false positive signal belongs to the debris. The polished image aligned according to the markers is shown at Figure 2 in the text.

>Sru_Tuph_cloned cDNA

CGTATCGCGATGCATCTAGATTGCACTGCGAGGTTGTCATACCTGTATCCCAGCAATTCCTGTAGTAAAACATATTCTGGATGCTGGCGAGTTCCTGAAGCTTTTTGAATGGAGTGAGAG
TCTTGTCTGAAACGGCAGGTATGGTTCATTGAAATGGAAGATGTTTTCTTGAGGTTCAAGCACAGACTGTCTGGTCCAAGCTTCTTCGCCATGAGCTGACATCCAGTTTTGGAAAACGGA
GAAAAGTCTGTCGACGTTTGCATGATGAAAAAGAAGACTGGATCGAATCCCAGCATATGTGAGACTACCCATTGAGTAGGTACATTTGCCAGCGGCTGATTCCCTTTCCCAGCAAATAGCA
GCACGAATGGTGTGTGAGAGTTCTCAAGAGCCTCGGAAAACCTCATGGATGGTGTGTGACTGCATCAACTTTTGTGCAACATAGTAGTTGTCTTCGACACTTTGACCATGAAAGGATAGA
CCGTGGGGGAACGTTGTGTCAGGGTATGCGGGGTAGTGTGTCGATCGAATAGTCATGGACGTAAACGGATTTTCTTGCCGTATAGTTTCTTGCTCCAAACCAATGCAGGCGGCTCAGGATA
TTTAAGCCAGTCCCAGTAAGGCAATCCTAGAGTTTTATCTTTGAGATGCCTTGTAAGTCCTTCTTCAAAGTTCAATAATTCAGCTCTGTGCCACATTGCAAAGTTGAGTCTTGACCATGG
TAGCAACATCCTGCCATATTTCCATCTGGTCCCATGCACTGATAAGGAAAAGACGTGAGTCCCAGCCGATTGTGGAATCCAAAATTGCTAGTATCCTTTTTGTATTTCTCATCGCTTGAA
TAAGATCTTTCTTCTGTACTCGGTTTCAAGTGAATGAGCCTCTCTCCTGACACGCAATTTGCATTTTCTCCCGGTGTAGCATTCCTTGTATTTCAGTTGCTGGTCCCTTTACACCTGCCTTCTCC
CACAAGGCCATGTTTGCAAAATACGAGTCCGGGTCTGAATTCACCTGAACAGGTTGGTTTGCATGTGTTCCAACAGCTCCATGTTTCCCATTTAGCTTCGGTATCAAAGACGTTCTTTACC
GTCCACTTTAGGCCAACGCAGACATAATTGATGATGATGTCAGTTTTGCCATCAACATCTACATCAAACCTTTGCACTCTCTTTGCTCCTCCGGTCTTGCAAGTTTTATACATGAGTGTAT
GAAAA

Supplementary data 1. A sequence of *S.rustica* tuphoxin cloned fragment.

M.occidentalis. --MNDTGTHEWPYQCRS----STGINGACCYHGAKLNFLSWHRVEIKNFEMGLTRYL-----KDKTLGMPY
 B.leachii.7 GFWAAGGTHVFPNQCV----HVSNGPGCCYHGARLNFAIWHRAELLNIEEGITRYM-----KDKTLGLPY
 B.schlosseri.2 GFWAAAGTHVFPNQCID---HATGNPGCCYHGARLNFAIWHRAELLNIEEGLIRHL-----KSKTLGLPY
 Hau GYHQAAGTHFWPNECVK----PNGVSGACCYHGAKVNFVMWHRAELLYEQGLTRHL-----KDKKLGAPY
 Sca GFHNAGGTHEFPPQCMG----SSGEDKGCYHGARLNFMWHRAELLNFEEGLSRHL-----RDKTLGLPY
 S.clava.8 GFHNAGGTHEFPPQCMG---SDGKDKGCCFHGAKLNFAMWHRAELLNFEEGLTRHL-----KDKTLGLPY
 M.acridum GYFQISGIHGLPFIENRGGARNDGWWGGYCPHGAL-FLPWHRPFVLLFEQLLVEHAVGIASQYPARYRGQYMAAANNLRSPY
 T.melanosporum GYYQVAGVHGTPYIPWMEKA-DADDRAGDYCTHGAL-FLTWHRPYLLLFEQRIVEEALTIARNFSDKYRAEYEEAALNIRIPY
 C.zae-maydis -VQKCADCOGA-----DGYCTHDSVH-FPAWHRPYMALFEQELMRVAFDIASSFSGAFGDRLKAAAQKLRAPY
 C.posadasii SYDVSSIHGYPFEAWDG---VEGIGGGGYCAHASTI-FPTWHRPYLALFEEMIWNHAQVIARGYRDSLSTYVEAARTLRIPY
 C.rhizosoleniae GYEQIAGLHGFPDW-----ECPHGGLK-FLPWHRAYLLRFEQALQRYV-----PDVTLPLY
 L.bicolor SFFQVGGIHLPLYPWDGVTGIPNPTWGGYCTHGSVL-FPTWHRPYVSAFEQILWTRAQEVAAATYTVD-QAAWKAATTLRQPY
 BLOCKs SFFQVGGIHLPLYPWD-----YCTHGF--FPTWHRPYVSAFEQI-----ATTLRQPY

cons . : * * * * * : *

M.occidentalis. WNWLE-Y---SS---PPPLLV-SKTL-----YGATNPFAEPMEVRLDE-----SNIRK
 B.leachii.7 WNWLE-E---NK---APDFVW-SKTL-----NGKPNPLAH-IIIRHDL-----TPPQP
 B.schlosseri.2 WNWLE-D---NK---APDFVW-SKTY-----NGKPNPFAN-VIIRYDL-----TPHQA
 Hau WDWLV-Y---PN---PPQLVW-AKTI-----GGKPNPFAG-SKIRYDL-----PVVIS
 Sca WDWLT-Y---PN---PPPLVW-SKLL-----YNKANPPTS-GLVRYDT-----KPHTL
 S.clava.8 WDWLT-Y---PN---PPPLVW-SKLL-----FKKDNPPTS-MTIRYDV-----TPNIV
 M.acridum WDWSADS---NV---PPCTVP-ERVS-VNIP-----NGQNLRRIEIRNPLST-YKYPREA---LDGEF---GEFSKTPQMARC
 T.melanosporum WDWATDSD-----VPQSIR-FAETDITLPEVGSAPPV-TRKGVNPMYS-YKFKTSIRRQRDFSIVGVQEMVAWEETKRC
 C.zae-maydis WDWAAVPDPGTPV---LPTVMT-AQQVTI-----EGPN-GQETVDNPLYS-YHFNDSS---DMAY---AVFTTWPDTFRW
 C.posadasii WDWASNP---E---LPRCVV-TPQIRI-----TTPN-GEATIKNPLYS-YTINPTA---GKGFPPRDPLSQYPDTRVA
 C.rhizosoleniae WDWISQE---SQRNGLPNAVA-ESTY-----FDA-EGVEVLNPLYS-AQIPIGG-----ETIRR
 L.bicolor WDWAVNS-----V---PPDEVIKQTVSI-----TGHDGKKKDVANPLYH-YTFHPI---DPSFP---SPYSGWKTTLRQ
 BLOCKs WDWA-----PPDEV-----

cons * : * * * * * :

M.occidentalis. T-----ERIAQPVP-AHVEVARGIYQNETVNL-QSADMIHEFSES-----LESSHNLIHMAVC-PMTPNSKA
 B.leachii.7 L-----QRDNTTQL-SSILQARSIYLTAMKL-MESHTLHEFSEE-----LENAHNWIHTAAC-PY--ETPA
 B.schlosseri.2 V-----QRDTTDF-NFIKLARDIYLTITIRL-MKAHSLFRFSEE-----LENAHNWIHSSAC-PH--ETSA
 Hau T-----ARSPAVSS-SSVSMAKSIFQTITSKL-ARSHMIHEYSES-----LESSHNNIHSAVC----HADTG
 Sca T-----QRSPTVDP-FMVKMSKDNY-YVARKL-MQSHSIHEFSEA-----LENSHNTIHSSICWGAQQKAGK
 S.clava.8 T-----QRSPTVDP-FMVKTSKDNY-YVARKM-MQSHSIHEFSEA-----LENAHNSIHSSICWGAQQNQNK
 M.acridum PDPG--RYPDSANQELQD-----YRLKQATYDIFTTASNHFHQFAVRG-----RVVHLEELHNDVHYSAA-----
 T.melanosporum PDEKGISHPEIVDRQLRIPTVNPTAGSSFRDPIYKLLTLVGSYGAFGNTGWQTGRPGPNNISLEHYHNIHTFTG-TNYIEEKF
 C.zae-maydis PNSNNVDAHSQQDQCID--AFTNSNGNLRDQIYQLLTQCKSYLGFATDA--SGDQ-RCANSLEGIHNTVHLNAG-GPGY-NGI
 C.posadasii PDDEGQSQMEEVQRRLRA-----NGAYFRQATYSLLSSEPNYTIFSTSA--VRDRGNYYNNLETIHGQVHVAVG-GPG-----
 C.rhizosoleniae P-----GQISRLSELENPVAIAM--QENNFVDFSGR-----IENPHNFIHVWVG-GNG-----
 L.bicolor PTSLQPD--ATDNIVRLRQVLGNAQSNIRSNTYNLLTRVTTWPAFSNHT--VGDGGSTSNSLEAIHDGIHVYVG-GNG-----
 BLOCKs -----RVTTWPAFSNH-----EAIHDGIHVYVG-----

cons : . * * . : *

M.occidentalis. CEVSMGSLTYAAFDPFMFHHTNVDRFLFSQF
 B.leachii.7 CTYSMGQLTYAAYDPLFFMHHTNVDRQIARL
 B.schlosseri.2 CTFSMGQLEYAAYDPLFFMHHTNVDRQIARM
 Hau CDYSMGELEYAAYDPIFFFHHANVDRLFSVF
 Sca CEFSMGGLTYAAFDPVFFFHHTNVDRFLFSVF
 S.clava.8 CTYSMAGLTYAAFDPVFFFHHTNIDRLFSVF
 M.acridum CKGQFFDAELSGFEPLFMFHHTQVDRLW---
 T.melanosporum QEGHMSEVGVSAFDPIFWLHHCNVD-----
 C.zae-maydis SGGHMAFLPLASYDPLFFLHHANVDRLVTLY
 C.posadasii --GHMTYVPWSSFDPIFWLHHCNVDRLLLAMW
 C.rhizosoleniae --GSMSNVTFAAFDPIFWMHHSYVDRQWYIW
 L.bicolor ---QMGDPAVAGFDPIFFLHHCNVDRLLSLW
 BLOCKs --GQMGDPAVAGFDPIFFLHHCNVD-----

cons : : : * * : ** : *

Supplementary data 2. Alingment of Tunicate and fungal phenol oxidase proteins. BLOCKs indicate the most conservative regions. Color meaning for alignment: BAD AVG GOOD