

## **Supporting Information**

### **Newly Identified *Aplysia* SPTR-Gene Family-Derived Peptides: Localization and Function**

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## Supporting Information:

There are a total of one supplemental table and 5 supplemental figures (Figure S1-5). The figures are included in this document. The supplemental table is included as a separate Excel files (Table S1.xlsx), whereas the legends and references for the table are provided below.

**Table S1.** A list of SPTR-Gene Family precursor sequences in mollusca and annelida, and proctolin precursor sequences in arthropoda used for sequence alignment in Figure S1-3. We also indicated whether each precursor has been aligned in previous publications.

## References for Table S1

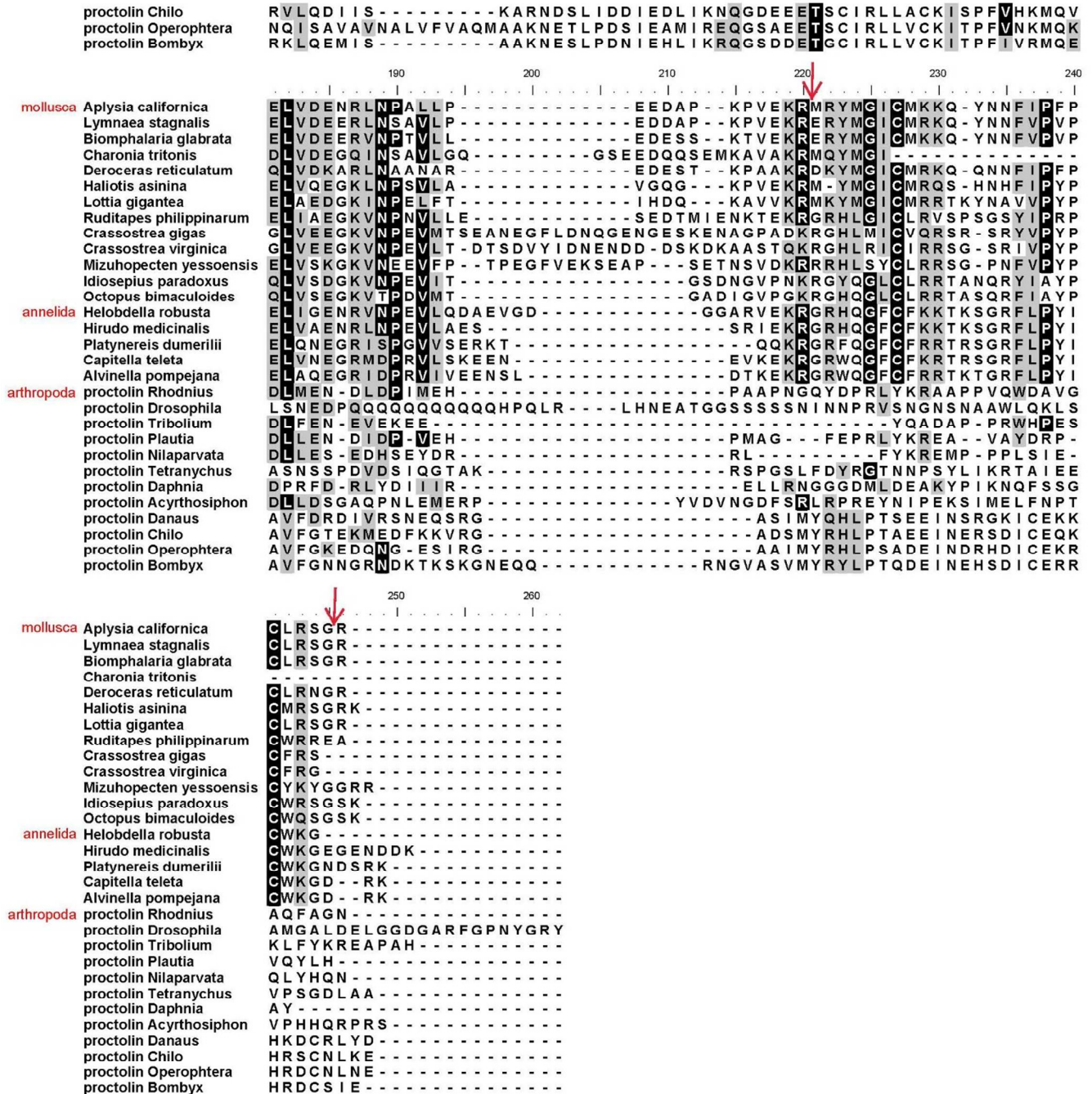
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and Schroder, R., and Tautz, D., and Zdobnov, E. M., and Muzny, D., and Gibbs, R. A., and Weinstock, G. M., and Attaway, T., and Bell, S., and Buhay, C. J., and Chandrabose, M. N., and Chavez, D., and Clerk-Blankenburg, K. P., and Cree, A., and Dao, M., and Davis, C., and Chacko, J., and Dinh, H., and Dugan-Rocha, S., and Fowler, G., and Garner, T. T., and Garnes, J., and Gnirke, A., and Hawes, A., and Hernandez, J., and Hines, S., and Holder, M., and Hume, J., and Jhangiani, S. N., and Joshi, V., and Khan, Z. M., and Jackson, L., and Kovar, C., and Kowis, A., and Lee, S., and Lewis, L. R., and Margolis, J., and Morgan, M., and Nazareth, L. V., and Nguyen, N., and Okwuonu, G., and Parker, D., and Richards, S., and Ruiz, S. J., and Santibanez, J., and Savard, J., and Scherer, S. E., and Schneider, B., and Sodergren, E., and Tautz, D., and Vattahil, S., and Villasana, D., and White, C. S., and Wright, R., and Park, Y., and Beeman, R. W., and Lord, J., and Oppert, B., and Lorenzen, M., and Brown, S., and Wang, L., and Savard, J., and Tautz, D., and Richards, S., and Weinstock, G., and Gibbs, R. A., and Liu, Y., and Worley, K., and Weinstock, G., and Elsik, C. G., and Reese, J. T., and Elhaik, E., and Landan, G., and Graur, D., and Arensburger, P., and Atkinson, P., and Beeman, R. W., and Beidler, J., and Brown, S. J., and Demuth, J. P., and Drury, D. W., and Du, Y. Z., and Fujiwara, H., and Lorenzen, M., and Maselli, V., and Osanai, M., and Park, Y., and Robertson, H. M., and Tu, Z., and Wang, J. J., and Wang, S., and Richards, S., and Song, H., and Zhang, L., and Sodergren, E., and Werner, D., and Stanke, M., and Morgenstern, B., and Solovyev, V., and Kosarev, P., and Brown, G., and Chen, H. C., and Ermolaeva, O., and Hlavina, W., and Kapustin, Y., and Kiryutin, B., and Kitts, P., and Maglott, D., and Pruitt, K., and Sapojnikov, V., and Souvorov, A., and Mackey, A. J., and Waterhouse, R. M., and Wyder, S., and Zdobnov, E. M., and Zdobnov, E. M., and Wyder, S., and Kriventseva, E. V., and Kadowaki, T., and Bork, P., and Aranda, M., and Bao, R., and Beermann, A., and Berns, N., and Bolognesi, R., and Bonneton, F., and Bopp, D., and Brown, S. J., and Bucher, G., and Butts, T., and Chaumot, A., and Denell, R. E., and Ferrier, D. E., and Friedrich, M., and Gordon, C. M., and Jindra, M., and Klingler, M., and Lan, Q., and Lattorff, H. M., and Laudet, V., and von Levetzow, C., and Liu, Z., and Lutz, R., and Lynch, J. A., and da Fonseca, R. N., and Posnien, N., and Reuter, R., and Roth, S., and Savard, J., and Schinko, J. B., and Schmitt, C., and Schoppmeier, M., and Schroder, R., and Shippy, T. D., and Simonnet, F., and Marques-Souza, H., and Tautz, D., and Tomoyasu, Y., and Trauner, J., and Van der Zee, M., and Vervoort, M., and Wittkopp, N., and Wimmer, E. A., and Yang, X., and Jones, A. K., and Sattelle, D. B., and Ebert, P. R., and Nelson, D., and Scott, J. G., and Beeman, R. W., and Muthukrishnan, S., and Kramer, K. J., and Arakane, Y., and Beeman, R. W., and Zhu, Q., and Hogenkamp, D., and Dixit, R., and Oppert, B., and Jiang, H., and Zou, Z., and Marshall, J., and Elpidina, E., and Vinokurov, K., and Oppert, C., and Zou, Z., and Evans, J., and Lu, Z., and Zhao, P., and Sumathipala, N., and Altincicek, B., and Vilcinskis, A., and Williams, M., and Hultmark, D., and Hetru, C., and Jiang, H., and Grimmekhuijzen, C. J., and Hauser, F., and Cazzamali, G., and Williamson, M., and Park, Y., and Li, B., and Tanaka, Y., and Predel, R., and Neupert, S., and Schachtner, J., and Verleyen, P., and Raible, F., and Bork, P., and Friedrich, M., and Walden, K. K., and Robertson, H. M., and Angeli, S., and Foret, S., and Bucher, G., and Schuetz, S., and Maleszka, R., and Wimmer, E. A., and Beeman, R. W., and Lorenzen, M., and Tomoyasu, Y., and Miller, S. C., and Grossmann, D., and Bucher, G. (2008) The genome of the model beetle and pest *Tribolium castaneum*, *Nature* 452, 949-955.

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**Figure S1.** Alignment of 18 SPTR-Gene family precursor sequences and all the known arthropod proctolin precursors sequences using Bioedit. Threshold (%) for shading: 50. Similar: grey; identical: black.

apSPTR-GF-DP2 sequence is marked with red arrows.

		10	20	30	40	50	60
<b>mollusca</b>	<i>Aplysia californica</i>	---	MELQACN	---	IFALFVVVVV	TL	SVASSLPASRTDDVLQEASG
	<i>Lymnaea stagnalis</i>	---	MELPTYH	---	IFALFVA	AAVALSVVTGSP	TRTDEVLQEASG
	<i>Biomphalaria glabrata</i>	---	---	---	VFDP	---	VSVLSNSLP
	<i>Charonia tritonis</i>	---	---	---	MEQSMMA	---	MSAVLLAVLVSCASALP
	<i>Deroceras reticulatum</i>	---	---	---	MKFQLHYT	IAVFTLITCAV	TNGTSAETGRQVAAYS
	<i>Haliotis asinina</i>	---	---	---	MDVTTTTV	LGSLLLALLT	VATSSGLP
	<i>Lottia gigantea</i>	MA	IA	RI	KMEMRSSTIL	SLLVLL	LAAPTFC
	<i>Ruditapes philippinarum</i>	---	---	---	MVAMFV	ACMVVTQSLA	IPISS
	<i>Crassostrea gigas</i>	---	---	---	MPMLLCT	SLVVLV	ISTECSVP
	<i>Crassostrea virginica</i>	---	---	---	MELNMAC	FLCSSLV	LVLIASVSSVP
	<i>Mizuhopecten yessoensis</i>	---	---	---	MPAL	---	SIP
	<i>Idiosepius paradoxus</i>	---	---	---	MDSRLLAF	VS	VCLFLLT
	<i>Octopus bimaculoides</i>	---	---	---	MDCRLIA	LVSVCL	FLLTSP
<b>annelida</b>	<i>Helobdella robusta</i>	---	---	---	AMHTSLS	HVI	IVSVL
	<i>Hirudo medicinalis</i>	---	---	---	MLATHSN	LNIFV	VVVV
	<i>Platynereis dumerilii</i>	---	---	---	MESKI	IMMSLAV	LAALCL
	<i>Capitella teleta</i>	---	---	---	MEI	RLTLV	LALLVA
	<i>Alvinella pompejana</i>	---	---	---	MDMCR	LSA	ILVILY
<b>arthropoda</b>	<i>proctolin Rhodnius</i>	---	---	---	MATTG	QSKVMS	REVIV
	<i>proctolin Drosophila</i>	---	---	---	MGVPR	SHGTG	IGKSG
	<i>proctolin Tribolium</i>	---	---	---	MFDR	---	KL
	<i>proctolin Plautia</i>	---	---	---	MYKQ	SLACL	LALMMLLA
	<i>proctolin Nilaparvata</i>	---	---	---	MGLRA	VL	MVA
	<i>proctolin Tetranychus</i>	---	---	---	MLNFG	SKQY	HHILL
	<i>proctolin Daphnia</i>	---	---	---	MLKST	SLKALL	TFMVT
	<i>proctolin Acyrthosiphon</i>	---	---	---	MAGK	FSAL	FLVGF

		70	80	90	100	110	120
<b>mollusca</b>	<i>Aplysia californica</i>	MDTR	---	RDLDV	FKDLV	LISIQE	LV
	<i>Lymnaea stagnalis</i>	MDTR	---	RDMDV	FKDLV	LMSIQE	LV
	<i>Biomphalaria glabrata</i>	MDTR	---	RELDM	FKDLV	LMSIQE	LV
	<i>Charonia tritonis</i>	MDTR	---	DLDI	FKYML	MASIRD	LV
	<i>Deroceras reticulatum</i>	MDTR	---	RDLDI	LQGL	LISIQE	LV
	<i>Haliotis asinina</i>	MDTRQ	---	EDFEMV	KQTVL	IAMEEL	LV
	<i>Lottia gigantea</i>	MDTR	---	EELS	SVL	KDMVY	IVL
	<i>Ruditapes philippinarum</i>	MDTR	---	DLK	DML	MYALE	LEEL
	<i>Crassostrea gigas</i>	MDTR	---	DL	DAFEK	L	VYAL
	<i>Crassostrea virginica</i>	MDTR	---	DL	DAFEK	L	VYAL
	<i>Mizuhopecten yessoensis</i>	MDTR	---	ELG	DMQEL	VYNAL	KEEL
	<i>Idiosepius paradoxus</i>	MDTR	---	DPQD	I	FKDLV	YLT
	<i>Octopus bimaculoides</i>	MDTR	---	DPQD	I	FKDLV	YLT
<b>annelida</b>	<i>Helobdella robusta</i>	LETR	---	NLENN	FKELV	YLSIQE	LV
	<i>Hirudo medicinalis</i>	LETR	---	NLENN	FKELV	YLSIQE	LV
	<i>Platynereis dumerilii</i>	LDTR	---	DGLD	EDQ	FKELV	YLA
	<i>Capitella teleta</i>	LETR	---	DLED	DFKELV	YLT	IEEL
	<i>Alvinella pompejana</i>	LETR	---	DLEND	FKDLV	YLT	IEEL
<b>arthropoda</b>	<i>proctolin Rhodnius</i>	LPTR	---	GADDR	I	RLRQL	L
	<i>proctolin Drosophila</i>	LPTR	---	SHGDD	L	DKLREL	M
	<i>proctolin Tribolium</i>	LPTR	---	NGDR	I	EKLREL	L
	<i>proctolin Plautia</i>	LPTR	---	SQDDR	L	LLRQL	L
	<i>proctolin Nilaparvata</i>	LPTRR	---	SQDDR	L	LDRLREL	L
	<i>proctolin Tetranychus</i>	LPTR	---	NDDTR	K	EQIKEL	L
	<i>proctolin Daphnia</i>	LPTR	---	SDPL	S	PIGPPR	G
	<i>proctolin Acyrthosiphon</i>	LPTR	---	GND	R	LTRL	L

		130	140	150	160
<b>mollusca</b>	<i>Aplysia californica</i>	KRMRYMGI	CMKKQ	YNNF	IFPFC
	<i>Lymnaea stagnalis</i>	KRERYMGI	CMRKQ	YNNFV	VPVPC
	<i>Biomphalaria glabrata</i>	KRERYMGI	CMKKQ	YNNFV	VPVPC
	<i>Charonia tritonis</i>	KRMQYMG	---	---	---
	<i>Deroceras reticulatum</i>	KRDKYMGI	CMRKQ	QNNF	IFPFC
	<i>Haliotis asinina</i>	KRM	YMGIC	CMRQS	HNFIP
	<i>Lottia gigantea</i>	KRMKYMGI	CMRRT	KYNA	AVVPC
	<i>Ruditapes philippinarum</i>	KRGRHLGI	CLRV	SPSGSY	IPRCP
	<i>Crassostrea gigas</i>	DKRGHLM	ICVQ	RSR	SRYV
	<i>Crassostrea virginica</i>	QKRGHLR	ICIR	RSG	SRIV
	<i>Mizuhopecten yessoensis</i>	KRRRHLS	YCLRR	SG	PNFV
	<i>Idiosepius paradoxus</i>	NKRGYQGL	CLRR	TANQ	RYIAY
	<i>Octopus bimaculoides</i>	GKRGHQGL	CLRR	TASQ	RFIAY
<b>annelida</b>	<i>Helobdella robusta</i>	KRGRHQGF	CFKKT	KSGR	FLPY
	<i>Hirudo medicinalis</i>	KRGRHQGF	CFKKT	KSGR	FLPY
	<i>Platynereis dumerilii</i>	KRGRFQGF	CFRR	TRSGR	FLPY
	<i>Capitella teleta</i>	KRGRWQGF	CFKRT	RSGR	FLPY
	<i>Alvinella pompejana</i>	KRGRWQGF	CFRR	TQGR	FLPY
<b>arthropoda</b>	<i>proctolin Rhodnius</i>	NGYD	PRLYK	RAAPP	VQWDA
	<i>proctolin Drosophila</i>	SN	INNPRV	SGNS	NAAWL
	<i>proctolin Tribolium</i>	---	---	---	---
	<i>proctolin Plautia</i>	---	---	---	---
	<i>proctolin Nilaparvata</i>	---	---	---	---
	<i>proctolin Tetranychus</i>	KR	SPGSL	FDYR	G
	<i>proctolin Daphnia</i>	R	NGGD	M	DEAK
	<i>proctolin Acyrthosiphon</i>	V	NGD	F	S

**Figure S2.** Alignment of 18 SPTR-Gene family precursor sequences and the classical arthropod proctolin (RYLPT) precursor sequences using Bioedit. Threshold (%) for shading: 50. Similar: grey; identical: black. apSPTR-GF-DP2 sequence is marked with red arrows.



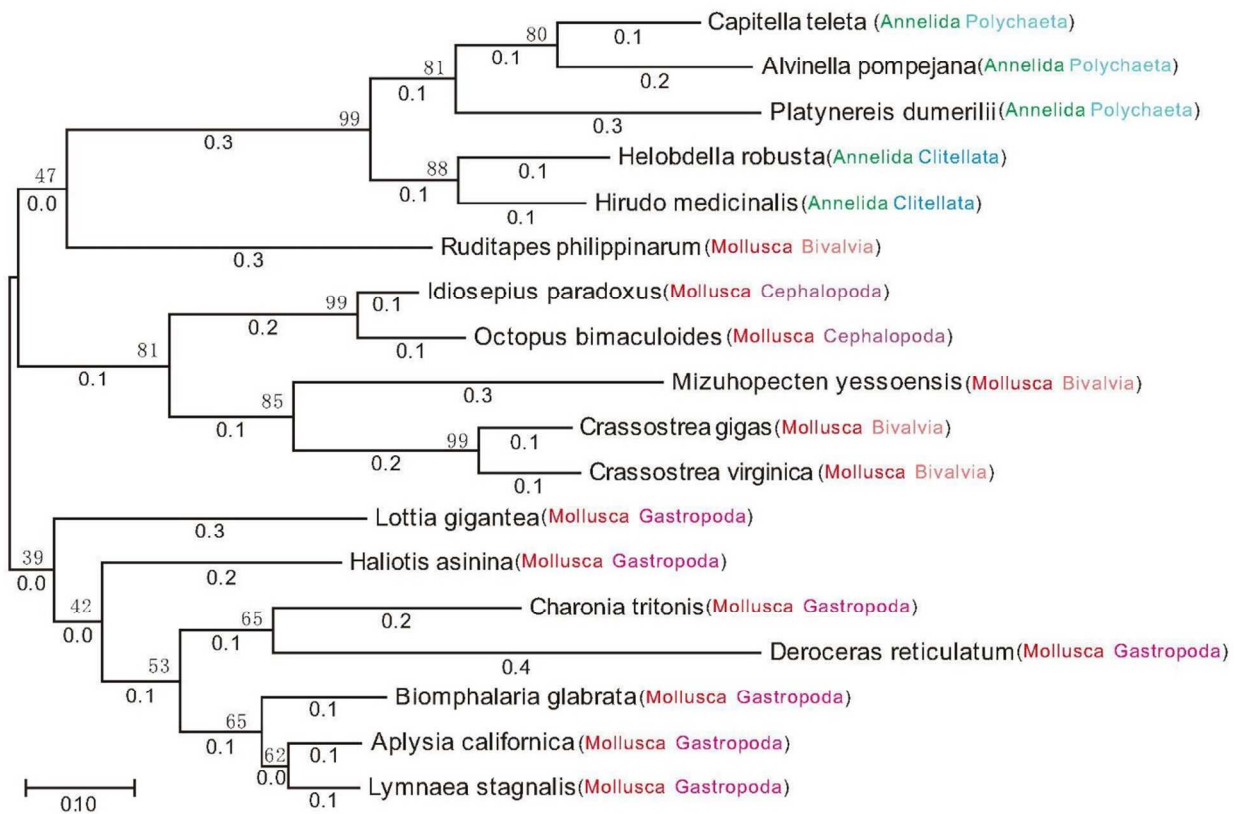
		10	20	30	40	50
<b>mollusca</b>	<i>Aplysia californica</i>	---	MELQACN---	IFALFVVVTVLSVASSLP	PASRTDDVLQEASG---	
	<i>Lymnaea stagnalis</i>	---	MELPTYH---	IFALFVAVALSVVTGSP	TRTDEVLQEASG---	
	<i>Biomphalaria glabrata</i>	---	---	VFDP---	VSVLSNSLP	TTFEDVLQEAN---
	<i>Charonia tritonis</i>	---	MEQSMMA---	MSAVLLAVLVSCASALP	---	TGGSVLQSSG---
	<i>Deroceras reticulatum</i>	---	MKFQLHYTIAVFTLITCAVTNGTSAETGRQVAAYSYNQDDPG---			
	<i>Haliotis asinina</i>	---	MDVTTTTVLGSLLLALLTVATSSGLP	---	ADEG-RLDEAS---	
	<i>Lottia gigantea</i>	MAIARIKMEMRSSTIL	SLLVLLLAAPTFC	LP	---	PDQGSEIDDL---
	<i>Ruditapes philippinarum</i>	---	---	MVAMFVACMVVTQSLAIP	---	ISSADGTS---
	<i>Crassostrea gigas</i>	---	---	MPMLLCTSLVLLVLISTECSVPLEVARETENDHH---		
	<i>Crassostrea virginica</i>	---	MELNMACFLCSSLVLLVLIASVSSVPIDVARETGSDDH---			
	<i>Mizuhopecten yessoensis</i>	---	---	MPAL---	---	SIPLDSQ--ETQEH---
	<i>Idiosepius paradoxus</i>	---	MDSRLLAFVSVCLFLLTSPVFSAP	---	---	AADPKPHLEQSKD---
	<i>Octopus bimaculoides</i>	---	MDCRLIALVSVCLFLLTSPALSAP	---	---	AADARSHLEQSKD---
<b>annelida</b>	<i>Helobdella robusta</i>	---	AMHTSLS-HVIVSVLVTSLCLAV	---	---	PAKKKSSVNMKMSP---
	<i>Hirudo medicinalis</i>	---	MLATHSNLNI	FVVVVVVVMTTFC	SAIP	SKKLNNESSNLG---
	<i>Platynereis dumerilii</i>	---	MESKIIMSSLAVLLAALCLGASAMS	---	---	LPSEVKKHNVQVSES--G-
	<i>Capitella teleta</i>	---	MEI-RLTLVLAALLVAAALGVVANALS	---	---	IPSDALKDNQMDVADKDG-
	<i>Alvinella pompejana</i>	---	MDMCRLSA	ILVILYGTLC	AVTNAMS	IPASQLKDSGVELDSSSENT

		60	70	80	90	100
<b>mollusca</b>	<i>Aplysia californica</i>	LALN	KRPKYMDTR	---	RDLDFVKDLVLI	SIQELVDENR
	<i>Lymnaea stagnalis</i>	LALD	KRPKYMDTR	---	RDMDVFKDLVLM	SIQELVDEER
	<i>Biomphalaria glabrata</i>	MALE	KRPKYMDTR	---	RELD	DFVKDLVLM
	<i>Charonia tritonis</i>	LSAE	KRPKYMDTR	---	DLDFKYM	MLMSTRDLVDEG
	<i>Deroceras reticulatum</i>	LSLD	KRPKYMDTR	---	RDL	DLIQGLILLS
	<i>Haliotis asinina</i>	LAAE	KRPKYMDTR	Q---	EDFEMV	KQTVLIAMEE
	<i>Lottia gigantea</i>	---	DKRPKYMDTR	---	EELS	VLKDMVYIVL
	<i>Ruditapes philippinarum</i>	LSAD	KRPKYMDTR	---	---	DLKDM
	<i>Crassostrea gigas</i>	LEE	KRPKYMDTR	---	DL	DAFEKLVYL
	<i>Crassostrea virginica</i>	LDE	KRPKYMDTR	---	DL	DAFEKLVHL
	<i>Mizuhopecten yessoensis</i>	LEE	KRPKYMDTR	---	ELGDM	MQELVYNAL
	<i>Idiosepius paradoxus</i>	LPISKRPKYMDTR	---	DPQDI	FKDLVYLT	LQQLVSDGKVN
	<i>Octopus bimaculoides</i>	VPLSKRPKYMDTR	---	DPQDI	FKDLVYLT	LQQLVSEGKVT
<b>annelida</b>	<i>Helobdella robusta</i>	QTEET	TRATWLETR	---	NLENN	FKELVYLS
	<i>Hirudo medicinalis</i>	VTDEE	TRATWLETR	---	NLENN	FKELVYLS
	<i>Platynereis dumerilii</i>	LDEK	ERATWLDTR	DLGD	LEDQ	FKELVYLA
	<i>Capitella teleta</i>	---	DQSR	ERATWLETR	---	DLED
	<i>Alvinella pompejana</i>	ALDEGR	ASWLETR	---	DL	ENDFKDLVFL

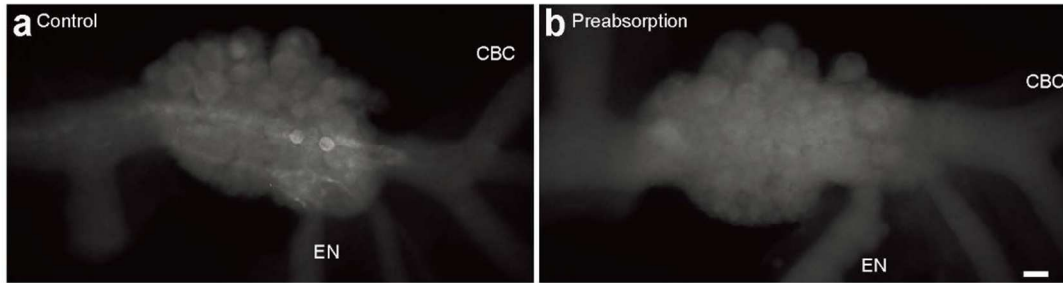
		110	120	130	140	150
<b>mollusca</b>	<i>Aplysia californica</i>	---	EEDAP	---	KPVEKRMRYM	GICM
	<i>Lymnaea stagnalis</i>	---	FDDAP	---	KPVEKRERYM	GICM
	<i>Biomphalaria glabrata</i>	---	EDESS	---	KTVEKRERYM	GICM
	<i>Charonia tritonis</i>	---	GSEEDQ	QSE---	MKAVAKRM	QYMGIL
	<i>Deroceras reticulatum</i>	---	EDEST	---	KPAAKRD	KYMGICM
	<i>Haliotis asinina</i>	---	VGGG	---	KPVEKRM	-YMGICM
	<i>Lottia gigantea</i>	---	IHDQ	---	KAVKRM	KYMGICM
	<i>Ruditapes philippinarum</i>	---	ESED	T---	MIENKTE	KRGRHLGICL
	<i>Crassostrea gigas</i>	EGFL	DNQGENGESKENAGPADKRGHLM	---	CVQRS	SR-SRYVPYPCFRS
	<i>Crassostrea virginica</i>	DVYI	DNENDD-DSKDKA	AS	TQKRGHLR	ICIRRS
	<i>Mizuhopecten yessoensis</i>	EGFV	EKSEAP	---	SETNSV	DKRRRHLSYCLRRSG
	<i>Idiosepius paradoxus</i>	---	GSDNG	V	PNKRGYQGL	CLRRRTANQRYI
	<i>Octopus bimaculoides</i>	---	GADIGV	PGKRGHQGL	CLRRRTASQRF	IAYPCWQSGSK-
<b>annelida</b>	<i>Helobdella robusta</i>	---	DAE	VGD	---	GGARVEK
	<i>Hirudo medicinalis</i>	---	ES	---	SR	TEKRG
	<i>Platynereis dumerilii</i>	---	ERKT	---	---	QKRG
	<i>Capitella teleta</i>	---	KEEN	---	---	EVKE
	<i>Alvinella pompejana</i>	---	EENS	L	---	DTKE

<b>mollusca</b>	<i>Aplysia californica</i>	---
	<i>Lymnaea stagnalis</i>	---
	<i>Biomphalaria glabrata</i>	---
	<i>Charonia tritonis</i>	---
	<i>Deroceras reticulatum</i>	---
	<i>Haliotis asinina</i>	---
	<i>Lottia gigantea</i>	---
	<i>Ruditapes philippinarum</i>	---
	<i>Crassostrea gigas</i>	---
	<i>Crassostrea virginica</i>	---
	<i>Mizuhopecten yessoensis</i>	---
	<i>Idiosepius paradoxus</i>	---
	<i>Octopus bimaculoides</i>	---
<b>annelida</b>	<i>Helobdella robusta</i>	---
	<i>Hirudo medicinalis</i>	DDK
	<i>Platynereis dumerilii</i>	K--
	<i>Capitella teleta</i>	K--
	<i>Alvinella pompejana</i>	K--

**Figure S3.** Alignment of 18 SPTR-Gene family precursor sequences using Bioedit. Threshold (%) for shading: 50. Similar: grey; identical: black. The figure showed part of SPTR-like peptide precursor sequences among different species are highly similar, which may primarily originate from the comparable sequences for the C-terminal apSPTR-GF-DP2. apSPTR-GF-DP2 sequence is marked with red arrows.



**Figure S4.** Phylogenetic tree resulting from the analysis of 18 SPTR-Gene family precursor sequences. Number on the nodes shows the bootstrap scores (percentage) out of 1000. The scale bar indicates the number of substitutions per site. Notably, *Aplysia* SPTR precursor is most closely related to *Lymnaea* SPTR precursor. To a large extent, the phylogenetic relationship matches with the relatedness of the different species.



**Figure S5.** Specificity of apSPTR-GF-DP2 antibody. Preabsorption of the primary antibody with apSPTR-GF-DP2 abolished immunostaining. (a), Rostral surface of a buccal hemiganglion after immunostaining in a normal way, showing two apSPTR-GF-DP2 immunopositive neurons. (b), Rostral surface of a buccal hemiganglion after immunostaining with the primary antibody preabsorbed with  $10^{-4}$  M apSPTR-GF-DP2 overnight, showing a lack of staining. Scale bar: 100  $\mu$ m. Nerve abbreviations as in Fig. 1.