SUPPLEMENTARY MATERIAL

In the footsteps of sea stars: deciphering the catalogue of proteins involved in underwater temporary adhesion

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Published in Open Biology, 2022, Doi:10.1098/rsob.2016xxxx

(1) Whole mount *in situ* hybridization for transcript comp179_c0_seq3 (coding for Sfp11)

RNA probe synthesis and whole-mount in situ hybridization were performed as previously described (Lengerer et al., 2019). Sequence and primers are listed in Table S2. Briefly, transcript-specific primers were designed and a T7 promoter region was added at the 5' end of the reverse primers. The purified polymerase chain reaction (PCR) product was then used to produce single stranded digoxigenin-labelled RNA probes. RNA probes were used at a concentration of 0.1–0.2 ng μl^{-1} and detected with alkaline phosphatase conjugated anti-digoxigenin Fab fragments (Roche) at a dilution of 1: 2000. The signal was developed using the NBT/BCIP system (Roche) at 37°C. Images were taken with a Zeiss Axioscope A1 microscope.

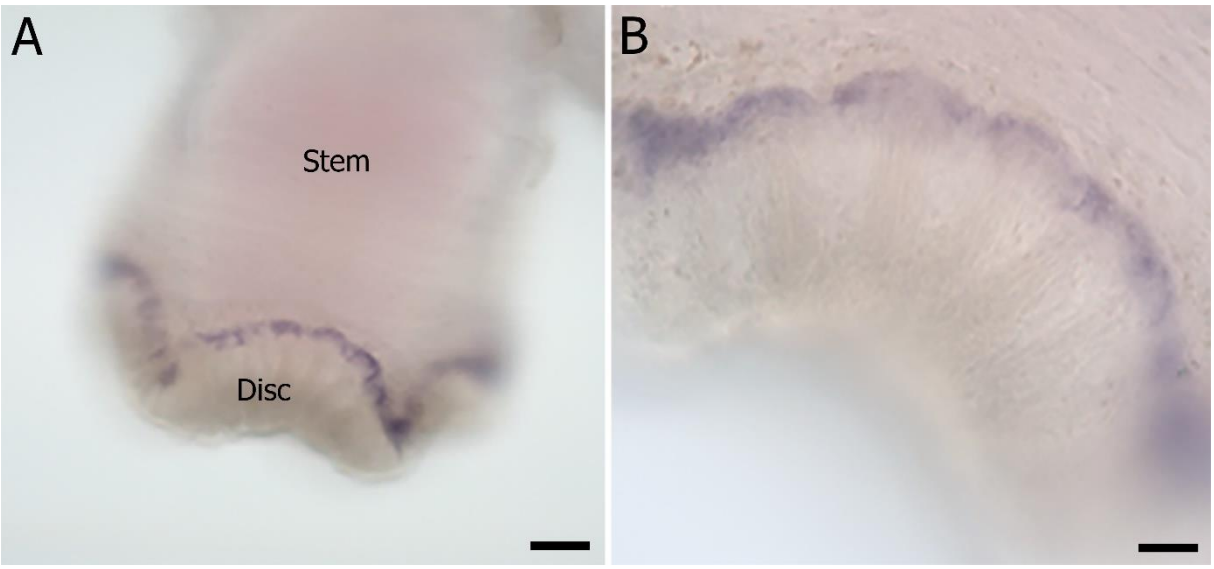
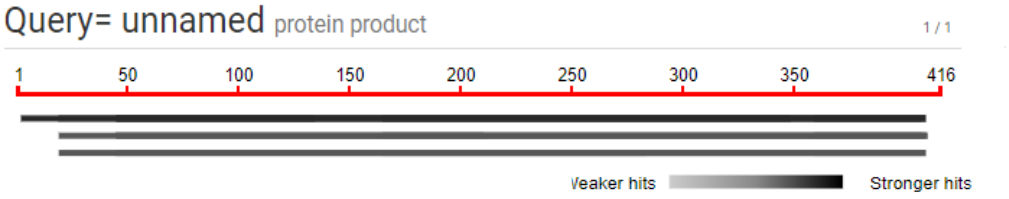


Figure S1. Whole mount *in situ* hybridization for comp179_c0_seq3 (coding for Sfp11) in *A. rubens.* Scale: 100 µm (A) and 25 µm (B).

(2) Sfp sequence elongation based on *Pisaster ochraceus* transcriptomic data

Partial Sfp sequences were elongated using publicly available transcriptomic and genomic resources. For example, the sequence of Arub-12 was originally identified in the tube foot transcriptome of *A. rubens* (Hennebert et al., 2015; Lengerer et al., 2019) but the N-terminal part was missing. To elongate the sequence, a tBLASTn search was performed in the transcriptome of *P. ochraceus* using the translated partial sequence (Fig. S2), and the best hit was the transcript 639619 from *P. ochraceus*, with an e-value of 2.50 10^{-122}. We aligned the translated sequence from *P. ochraceus* with Arub-12 (Fig. S3) and Arub-12 matched with the C-terminal end of the protein encoded by *P. ochraceus* transcript. We then performed a reciprocal tBLASTn search in the tube foot transcriptome of *A. rubens* using the N-terminal end of *P. ochraceus* transcript as query (Fig. S4). Arub-12 (comp15624_c0_seq2), and the other sequences with the best e-values (comp15624_c0_seq6, comp15624_c0_seq4, comp15624_c0_seq5) were aligned. Comp15624_c0_seq4 and seq6 were almost identical, only the 25 first nucleotides did not match. Comp15624_c0_seq5 was almost identical to the transcript coding for Arub-12, with only the 11 last nucleotides different. The comp15624_c0_seq4 sequence matched the *Pisaster* sequence better than seq6 at the N-terminal end, so this sequence was suspected to correspond to the C-terminal end of Arub-12. PCR (list of primers available in Table S1) and Sanger sequencing were performed to validate this elongation. The resulting sequence presented a perfect alignment with both comp15624_c0_seq2 and comp15624_c0_seq4 (elongated protein). Moreover, the reanalysis of the elongated protein sequence with the original mass spectrometry data (Hennebert et al., 2012, 2015) revealed a sequence coverage of 73% (Fig. S5). We concluded that the protein sequence encoded by comp15624_c0_seq4 is the elongation of Arub-12. The corresponding protein sequence is still missing the start, but could be elongated from 416 aa up to 747 aa and the protein was named Sfp10.



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Number	Sequences producing significant alignments	Total score	E value
1.	639619-Pisaster-ochraceus	1106.21	2.50×10^{-122}
2.	639618-Pisaster-ochraceus	893.59	8.24×10^{-77}
3.	639620-Pisaster-ochraceus	910.54	2.36×10^{-78}

Figure S2. tBLASTn search for Arub-12 in the transcriptome of *P. ochraceus*

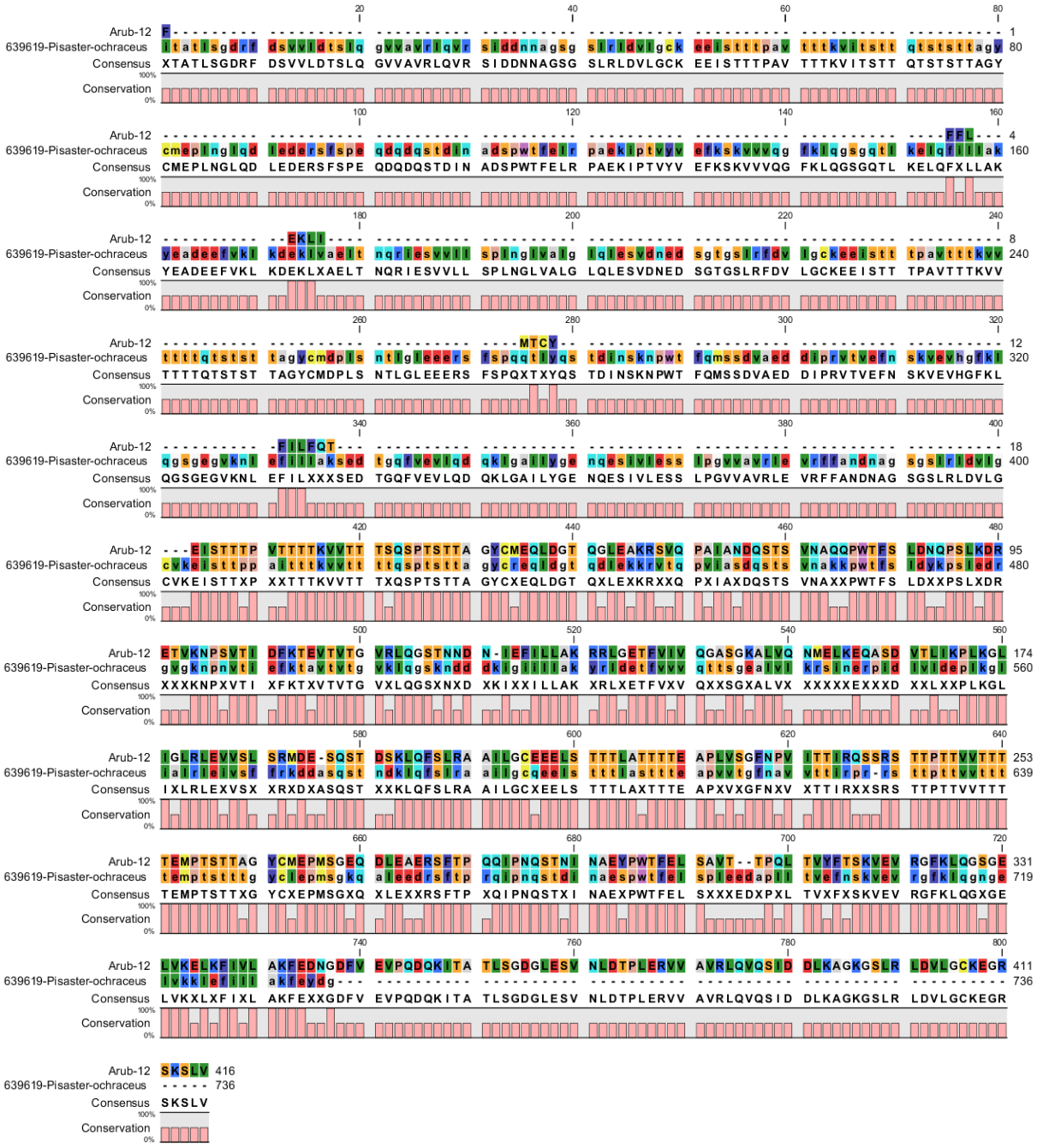
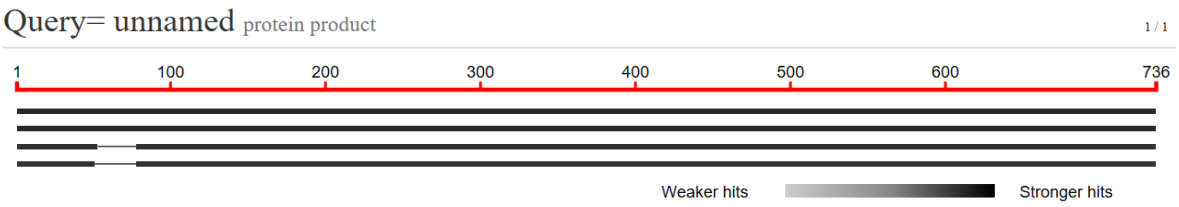


Figure S3. Alignment of Arub-12 and the protein sequence translated from transcript 63619 from *Pisaster ochraceus*.



Number	Sequences producing significant alignments	Total score	E value	Length
1.	comp15624_c0_seq6	997.21	1.19×10^{-120}	1056
2.	comp15624_c0_seq4	999.52	2.69×10^{-120}	1261
3.	comp15624_c0_seq5	982.57	1.25×10^{-108}	1260
4.	comp15624_c0_seq2	974.10	1.76×10^{-108}	1327

Figure S4. tBLASTn search of the N-terminal end of the sequence 63619 from *Pisaster ochraceus* in the tube foot transcriptome of *A. rubens*.

YKNPCLICFAVITTTTQTSTSTTAGECMEPLNGLLDLEERRFISPQQNQSTGINADNPWIFELLPYVD
AENKPTVTVEFKSKVEVHGFKLQGSGDIVKELQFVVSAKYEVDGLFVELEDEKLVADLTNQRVETVAL
PSPLSGLLALSLQLESVDQEDAGFGSLRFDVLGCEEEISTTTPVVTTTKVVTTTTQTSTSTTAGYCMD
PLSELDNLEQRSFAPQQTLENQSTLINSENPWTFQLSSDAGEEYDNPWVIVQYQSKVEVRGFKLQGSG
EDVITLEFILSAKSEDNEQFVDVLEGEKLGATLYQDNLKSITLDSPLPGVVAVKLQVIKKQNGGTGSL
RIEVLGCEKEISTTTPVTTTTKVVTTTSQSPTSTTAGYCMEQLDGTQGLEAKRSVQPAIANDQSTSVN
AQQPWTFSLDNQPSLKDRETVKNPSVTIDFKTEVTVTGVRLQGSTNNDNIEFILLAKRRLGETFVIVQ
GASGKALVQNMELKEQASDVTLIKPLKGLIGLRLEVVSLSRMDESQSTDSKLQFSLRAAILGCEEELS
TTTLATTTTEAPLVSGFNPVITTIRQSSRSTTPTTVVTTTTEMPTSTTAGYCMEPMSGEQDLEAERSF
TPQQIPNQSTNINAEYPWTFELSAVTTPQLTVYFTSKVEVRGFKLQGSGELVKELKFIVLAKFEDNGD
FVEVPQDQKITATLSGDGLESVNLDTPLERVVAVRLQVQSIDDLKAGKGSLRLDVLGCKEGRSKSLV*

Fig. S5. Protein pilot analysis of the elongated protein (Sfp10) with the original mass spectrometry data (Hennebert et al., 2012, Hennebert et al., 2015). The green color in the protein sequence indicates peptides matching the mass spectrometry data with high confidence (≥95%).

(3) Sequences elongation based on *Asterias rubens* genomic data

Using a local genome browser, we mapped the transcripts from the tube foot transcriptome and the corresponding Illumina sequencing raw reads (Hennebert et al, 2015) to the genome of *A. rubens* (eAstRub 1.3). Additionally, we mapped the sequences of the coding regions (CDS) predicted by the Sanger institute. As a typical example, transcript comp7100_c0_seq1 and transcript comp19_c0_seq1 were found to align to the same CDS, now named Sfp8 (Fig. S6). That the transcripts are indeed part of one longer sequence was also confirmed by a PCR and sequencing. Moreover, all elongated proteins were reanalysed with the original mass spectrometry data (Hennebert et al., 2012, 2015).

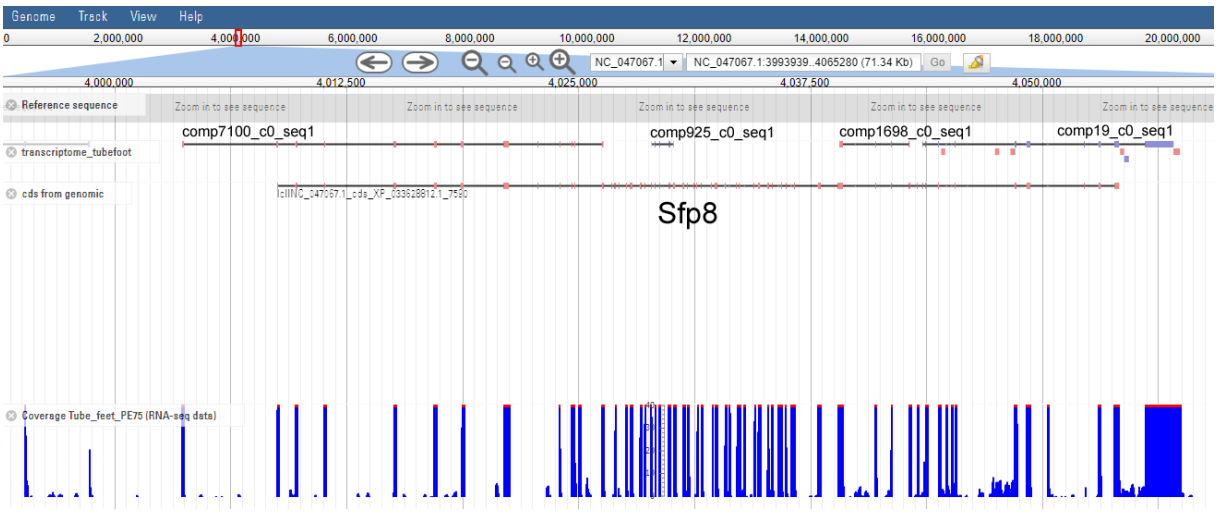


Figure S6. Screenshot from the genome browser in the region of the genome comprising the gene coding for Sfp8. The transcripts sequences from the tube foot transcriptome, the predicted CDS from the *A. rubens* genome, and the Illumina reads were all aligned to the genome sequence. The localization of the transcripts is represented as black line with pink/blue boxes indicating the position of the exons. The coverage of aligned Illumina reads is visualized with blue peaks at the bottom. Note that the predicted CDS from the *A. rubens* genome spans four separate sequences from the tube foot transcriptome.

(4) Protein sequences of Sfps

All Sfp sequences elongated using the *Pisaster ochraceus* transcriptome and the *Asterias rubens* genome were translated *in silico* and reanalyzed with the mass spectrometry data resulting from tube foot and footprint analysis (Hennebert et al., 2012, Hennebert et al., 2015) using the software ProteinPilot 5.0. All peptides identified with high confidence (>95%) in these extracts and present in the sequences are underlined. Amino acid sequences in bold show the parts of the proteins that were confirmed by PCR amplification and sequencing of the corresponding cDNA (to see Table S1 "primers and sequences elongation") and those in italics indicate the signal peptide identified with SignalP 5.0. Peptides used to raise polyclonal antibodies are highlighted in yellow in the corresponding sequences (Sfp7/8, Sfp10 and Astacin-like Sfp).

>Sfp2

*MASAPMIVLLPLLLLALTRTVVTS*DLKMVYVCEGDTLRLNCGEDRFIKMWSALYGRANPTYCPDDTVKASVHHCR
ADKSNFETMRGCDGKNSCEIEASDAVFGDPCPDETSKWYLQVEYYCRSERPS**ECNPKDSPKYQSAHACDDKELVL**
ECPTPGEVVKILDANYGRQGSDYCGYRPGIDDVFCDDDSGESFEYVCLQCRGKDYCMIPVNPLEFKDRCVNTRKY
LSVRYVCVQTEPEIEEEDDSEEEGPEVGDVDLRVTTIAPRRGRTQPATDFPFMMFPVGSAGASGKIKVGEAEHEG
ASFKFEGAPSIIGSANANGKLEVGEIDHEGGSFMFEGSPSRTKTIPGLSSIYTLPAGLVGQSAVLMKITVKYNEH
GEKYFTLTPFDGTSNTKTDGTEFLMKIDLNKILRDDSLQYTFRRLLENYNRGTTYYNNNDGVNTPFQFPVGNGLE
SFFSTLFQMIIETDSSGKMVTTPTNPSSNFQVILNGGNKAIVDYLMTFGSNTMDQNLRTELINHRLFISTSFYNT
LTQKTEEIKIPFYVNFDGLDGSLSTLFKSTSRPLTQFGMKYQVVGPGGAKQIFTMPFTLIEYQGRKRQFLTLPTC
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ALRSVSDMCSQEMTHECVQKSTHFVNGKAHIWWESLDGVKRENWGTKTEEKMCHCGVNNACSDANSLCNCDSRGG
VSDWGTLTDKDTLPVGKVVFQKPVATDSEDNLKVGQFRCGGDQSSSTLQFFIFMNQMLANPKKTCHEFKELGYEL
DGVYLIDPDEKEGERPFTVFCEMEKQNEGITVVHHDLSDQRTQVNGYEEPGSYARKINYRATSRQIDNLKAVSGK
CEQELMYECQGSAFHVGTVLNHWWVSFNGDKMPNWGGAPCDFDGCACKSDSSCVDNLKCNCDKDDNVMRKDGGKL
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TGKMYTWWESRTGVKMMNWGEVADGCQCSVSNTCKGGPSTKCNCDVNTQSYMTDEGYLTDKTKLPVSGIRSGDVS
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RQDGATHLEPKYFTFSTEEAISWKFDCPSFKYICSVKDAITCADKKPRIKTVRYGVPAANPCNEAVDYSQCSGMS
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VEVILDLVVIEMKQGGEIVVDDVPQSSPTVIEDTFSITDKGDIIELVAPEFQLTVTWNNKNNVEVFLPKYSGDIC
GLCGNTIGEPEVKDPVLFSNRWQVGECKSNTAVGERSNPAESAETLAFRRSIAETFCKAISVSDPFERICPQLYR
DMKSKYLNWCIFDNTQKSIQTYCSYIQEYKRKCDEESSEMFPTNWMKENTECYQGCPSHLNSTTCKSCESTCASL
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RYIRIRPKNPRRKKIGLRVEFLGCPELDPCASSPCEQGGTCKQASGLNPDGYVCKCPQGWTGKNCDAEEGVCHSA
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TNAACGECAECILPKPCQQYPDKLTKARALCKPILDTAGPFADCHHVLKPTFIYGDCLEDICGSDLISIVPVCTQ
LAEYADLCAMHGVVIKSWRKTELCSITCPVGMMYNPCGSACPATCGDPHAPNTCTKPCEETCECPDGLLKEGNKM
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SVEDDASLLVCEGEELSIFCAPGETIVIIEAVYGRSEDFQKCPSATQPRGVCDDGSALSVTRTKCQGKESCILPV
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IQSTDCVAASSLLVVQEMCQGEHKCTGLQAASHVFGANPCEGTFKYLEVRYMCNAEVLPKESIIVNPCALEPCEN
GGQCVTVHTVEGFMCKCPHDWTGPTCENEEVSCRISGLSHFIDFNQQSFTFLGDSKYILAEGCLGSEHTFKVMVE
MSRSPINNEVTSVKDVHVHYDDRIVQLKHEHRVLVDGEKASSSVNLNFGKIKICRVGNYLLLRTDFGMIVAWDAL
NRVQVRIPDIVARKSCGLCGTYQGVSDEPVGFVNSDGNKVDDLHAFGSSWRVAADPPAFVHTNLIDTPACIKWPE
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LSINCGSEYVDIISGTYSVFDRSSAGSPTDRDCPTDNTLDVLANHIIGGSKLKVISSAMQFGDSCQPENNLLNVT
YLCSEELPVHEVEHGRVVHMRACENRMVQLKCKKGQKMEILSAKYGRNAGSEVCPSKQIRTTSCLAPQDRTLKKL
EKHCRGRDKCIIHVNGRRFFRNDPCSGTHKYLDVTFKCNDY*

>Sfp3

*MARLWLAAALCALVFIA***LANDAEGHGHHYGHRHGYHHHKSKGACKSKGRNIGHHHARKSYPRGNIGVLGSLYAKK**
GCSKRRHKSHSGHKHGHGLFKAFGDIKKCGSRRRKHGLSVSGNKKSHSHSHSGGRSHKLNINNKRRNRSRKRQKH
GGSGSKDSSGSSSHHVNKNNKRRNGTRKRQKHGGSGSKDSSGSSSHHVNKNNKRRNRTRRQKKRGSSSSSSSSSG
GNSDHQQTNTVDKKRPVPSKKEHPCRSNPCQNGAECYEKPNGDYTCSCKAGFRGTRCEQKIVVKVRPCDSNPCLN
HGQCIPSGSGYRCRCERGFAGENCQIVPKSFDPCAGVVCHNGGTCYAENGEAKCKCRTGFKGTLCRNKVDRSGAC
ANNPCKHNGDCHEIDNGNTFYCNCMGDWEGVTCKIAIDHSGPCDNNPCAHGHCVPSRNIFRCLCSGGYTGDLCDV
PPKKDPCLQNPCLNNGQCISLTTGDFNCKCRSGFEGAFCETGKMRPGLCPISNTGNCVRGIDGCNSDSECPGEDE
KCCSTGCSRVCRKPCRGVCYNFCPNGFVYDNDGCPTCQCKELPLPPKEEVVPPKPKVADQVGKENIAFNTKIDLS
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LFRRDGSVSVVYEDTQTEFITYSIIEPREGSFNVFISSGHGGQASWKVHQSTTIQEDTITLKPHPIEQNDKTWTF
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WIGWSDGNLCVGDIDNYKKNDALACIAVPVKDQVFTWAGFKGDMMDYIIYYSFEYAVSYGIFSTYDSNMAYPLRF
KRARMTNNVLEFGVMTDSDAHMAVSNCQGADTPTAPMYELVMGTQGSEGCLFRRCKGCEAVAEGACPGLLDETEP
KDYTFEFNNGHLKLHKGRGVSKKLLLEWDDPNPIQNVGHLATATGFQSSGTSRWILYHP*

>Sfp4a

TQNPTKSASKENRPSSPGSSSHTDPDHVNSHASGSKSGSGSGRGSGSNERRGYYRSPCEKNPCKRGSCKEFGKTF
KCKCPSGFKGRRCQYTKRPRTTHTHGSKSSGSSSSAEIEAPQPCDYNPCWNGGTCLPEGDPYTCNCAPGYEGRRC
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YEGTNCEQVQPGPCEDSPCTNGGTCFPDGQDFFCQCPKQFEGRRCEIALEVGPCQTEPCFNGGTCTPTATDFECA
CKPSYTGKRCEDSPDVNDHVILESTNGEMEDVDIPLLAFYTHSSYLLRFSVMCYDSVRIGFKSAVYSHVYYYVYL
GKTSARIEHCENTCQSVSEVDISGQLSATAFADYYIRFTSTGSLIVGILDASFTELMSFNGEPPTCPLLVFVSSG
DGYVATWKIYEAITEQVNTITWRTVNVNLRSDQYTYLGESFQVYDRQLRLGLAIDSEVTVLLSKGYDDANNYEIV
FRAAGNTKLQIKKNGEVVKEGDCVVNSSSLDDFEDFWISFAKSTISVGRVGLDMQPAFVSWSDTSSLEVSLAAFK
GASSSYLVYYKFEYQTARGIFWTFDCDMAYPLPFEKIKMPQTALEIGLTADCDAHFSIAATDEDSPDAPMYEVVI
GAEDNTKCLIRKCKECECMAEVDCPHILDKNKQVDFTFEFEFGHLKLFKGKGASKSAQPIMSCEDPEPLIGLEYL
ALSTGFESDGESHWRTYNV*

>Sfp4b

*MGRFCFDIAFCSLVCLFLAIYSVDGY*SISRGGSGSQESTKGSGGSSTGGSHEQHAPSPQPCDSHPCQHGGICHNH
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ALGDTQNPTKSASKENRPSSPGSSSHTDPDHVNS*

>Sfp5

*MAKITLLVVLLVSVLPAI*ARKDQSYKCNFLESNGDGAYVFDDKQLNGGKFSVSFNVIANRDVRIALSPIKEEHNS
MIVISIGGPNNDDSTIGKSDGTILSEYLMTTGSRESELQCLTTCMNNYWICYEDNMLNVGRGGEIEPFLKYSVDA
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SPCSGLTCSETCANGYEVDDHGCFTCDCKSGPCDSQPCQNDGYCVSEDADEYRCICVNGFTGTNCERDEVPPTIE
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PQMTCPVDMDVPTDEGLPTSSFDFENPTATDNDGGEPSVVCTADNSPMVLGENTITCTATDSGGLSVECSYTVTV
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YTITAKKKK

>Sfp6

*MLRTLILVTLLGYVVPAAVM*QRCEECAPPPPPPKRCHWAKSTPSECCPSKLVCEEPLTACRSGAKVAKYCPDSLC
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PAQEEPDQEQNKPTLGETVHQQENELVQRETESVEEIEPSTGAVESQEESEPVGKVEPSTGAVESQEESEPVGEV
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ADGEKWKEGCVDCECSNGIPSCKLIPCALFMPPEGTQCKVRPGTENDCCPQFDCEDVGSHCVDKEGNKYAEGEKW
KDGCAECECLTGFSHICKSTSCPIYEVLEGSVCKVRPGTEEDCCPQYDCEEVESHCVDKEGKKYADGEKWKEGCV
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QCKIRPGTEEDCCPQYDCEEAPSNKPCIDANGNTRKHREEYFLGKCDPCVCIDGIPVCAVVDCVHHPPVSPFEIC
HVRPRTEDQCCPEYDCTVKRERRKSVGANQHEVIDQPEEIDQPTDINQAGYTEQPDIA**GIEQPIETEQLVDSEHE**
DSEQPLVIEHTVGEQNPSSKLDCSTVRCKLARTKLPPLCRSIVLPGECCADGMVCEEPLSDCPIAVSVPESCPDD
LCLKATCESHPEAKCIISRCGGCFTKFFDDSGTVIEECSKDFANKTEMCPPPPLSFWCISPPCAYGQHCKAYPDA
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FVCEEPISACGDGVNTLQLDCPDDLCQRATCENHPGAKCKISHCGKCHTVFYDNDGNKVDCGNDPVESDTYCPRN
EWGRCSEPACPLPILPLPGLILICPRCFLDWCDSCKAYNFDAEFKLMDCDKEPCQCTMLIEGTEQPVDIEQPVVS
EQEDSEPPIVMEHSVGQQNMGGIEHQVDTEQLVDSEQEDSEQQLVIEHTVGEQNPSSKLDCSTVRCKLARTKLPP
LCRSIVLPGECCADGMVCEESLSKCPIAVSVPESCPDDLCLKATCESHPEAKCIISRCGGCFTKFFDDSGTAIEE
CNKDFSDLISCPPLPDTISCVSPPCAYGQHCKAYPDAICHSTYCSIKCGLPEIFHDKYNNEIKDCSIEGFKNKCE
EMGPCEDPPKGVLPMFCRWIVQPEECCPRDFGPNGFVCEEPISACGDEVNTLQLDCPDDLCQRATCENHPGAKCK
ISHCGKCHTVFYNCNGNKVDCGNNPVESDTFCPTNFWGRCAEPACPIPPPPQPGLICPRCFLDWCDSCKAYNYNA
EFKLMDCNNESCHCIMSQVIEQPVDNEQLVDEQEDSKQPIVMEHSVGDKMIH*

>Sfp7

*MFQLLLLIMVTVPASSLVQS*DVADMTCGVIRE**TGHSEEFMWDLPQLHNDPVVVEFSVYAKSEVYLALTDKPGPNL**
PAKATVYYIVIGGDGNTASYISRCRGCQPLTPASTEVLQCDRLNNYWLHFEDGFIKLGLRGKLEPITTYEVPEKD
FQPRYLGYSSNMLSQGMFTFSFDKFGYHTYSEHVYYTGEAFCLDWFLKEIPVGVKQFCFQAQVEQGPIYVVLSPQ
RFRNPGEIAYEIVIGEEENSKTFVFDKVRSGEQAIKEVFTPSVLNPNSFVEYCFTFNEKVITFGPASRRDLVVWV
RDESIQIKHVGFAGSKKPAQFLFPVDFIPSYYQGVIDLPPESPAGSDKCLEKPCAENGKCYVDHSFSHGYRCECS
NGYTGKNCDILPPETVPAVEESAVKSTKAPEKRPTPKPDTTPPTIDCNVELGDNYVTSADSKTMQVPFSFPRAED

NADKNP**YVVCVPSSNSEFPLGTSTVKCTATDESGNEATCDLVVEVKNSCENNPCGLHGTCVVDVFQPSEFNCVCI**
DDYEGELCTKAPKVDEEPLVEESDVKSSTKAPGRSSTTPKPPKPDVDEGETFTTSGDSSQEIKRTLDTKVISAFE
ISPFDDKVSKEFQIGCEIGEFKLDVNSETFSLKNGKGEVVDSETTPETSGSLKFSIEITESAFIISYILDQDFTT
LIEYSPPGREDGKELNCKAFTVKNVDKRRQRGCQIKYTKITQHKWRSYHRKPRRTRTRNIEKGETYVRKTSDEKT
RQRRPRTDRTKTRTIEKGVSERKKSSSGSNSGSSSEENKAISVGDTQVLPTNGQTSGQRRRPRTDRSKTRTIEKG
VSERKKSSSGSNSGSSSEENKAISVGDTQVLPTNGQTSGKRRRPRTDRTKTRTIEKGVSGRQKSGSRSSSGSSSE
EKNAISVGATVNKNTDVTTRRRTPAAKVKSSSATLVSGDTE

>Sfp8

*MARTFVCAIVVAIFVTIPAVA*SQLCQLYQTERNIGVQWLPETLPAERFEITFRVLALGDVYIYLGDGPHARLAIG
IGVDQNSATIIKGNSGKTYAKTPGIIKCDTFSEFWLLYSPSFVKLGRVGQTSPIVTLFHSESYVPTRLGFSTSSV
SVGLFSFDPFGKTAPTFFEQEFNTCTGFCYDWRLNPMPLGERICFQARTEGVAAIALSPMREPNAGQSLYEIVFG
YDENSMIVLRRHMHGEEIEVVEAKDTLNCDEFVSYCIEASGPHIQITRLGDPKILMDFTDSVGIPINYRYIGFAG
GFDHCVEWRWPVDIIPKLILETGTQLTEETGEAPAVDPVLPDAGETSIVVPDLPAAETDVQPPLDESNNPSGSQG
CAPVRCKKFCPFGR**ATDKNGCEKCKCNRKGKSSEEDPEISVGETDVQPPSDKPDTPRPRKDRPNNNDKPKTETDV**
QPPSDETDTPSGNQAVCPEVPEGVFGICSEECGADKKCDDGQLCCSNGCGHVCKKGCSPVRCRRYCPFGWATDED
GCDMCKCKRKGGSKGKKENRRRGESSEEDPEISVGETDVQPPSDKPDTPRPRKDRPNNNNKPKKETDVQPPSDET
DTPSGNQAVCPEVPEGVFGICSEECGADKKCDDGQLCCSNGCGHVCKKGCSPVRCRRNCPFGWATDEDGCDMCKC
KRKGGSKGKNKNPRRGESSEEDPEISVGETDVQPPSDKPDTPRPRKDRPNNNNKPKKETDVQPPSDETDTPSGNQ
AVCPEVPEGVFGICSEECGADKKCDDGQLCCSNGCGHVCKKGCSPVRCRRYCPFGWATDEDGCDMCKCKRKGGSK
GKKENRRRGESSEEDPEISVGETDVQPPSDKPDTPRPRKDRPNNNNKPKKETDVQPPSDETDTPSGNQVVCPEVP
EGVFGICSEECGADKKCDDGQLCCSNGCGHVCKKGCSPVRCRRYCPFGWATDEDGCDMCKCKRKGGSKGKNKNPR
RGESSEEDPEISVGETDVQPPSDKPDTPRPRKDRPNNNNKPKKGESSEEDPEISVGETDVQPSSDDPSVPRGNQD
GDEGETFTTSGDSSQEIKRTLDKKVISVFEISPFDDKVSKEFQIGCEVGDFKLDVNSETFSLKNGKGEEVDSQTT
PDTSGRLKFSIEITESAFIISYILDQDFTTLIEYSPPGREDGKELNCKAFTVKNVDKRRQRGCQIKYTKISQTRW
RSYNRRNRPSNTRKVQTGATYVPKKTSGSKPRRVREHSRGSGSGSGNSKGSKSGGGRRGRGSGSGSGKKDKILSA
GETFVPKKTSGSRPRPTVRERSSGSGSGSGNSKGSKSGGGRRGRGSGSGSGKKDKILSAGETFVPKKTSGSRPRP
TVRERSSGSESRRKKKS*

>Sfp9

*MGLSKSGTRAWIVVVCVIYVLPYATG*QNLKIQPVVDEPLIMPAPMPPAPVPPVDIDNEEAEEKIALEKQAEIRNR
AFGAGYLAIAPKVMCAGGHEKVFLTFTNFTQPVDVQFAVLDKNDESVGTSKLETFSTPCGCLDVHLNPSDSPRKT
VTLVMYAKRTLMQCEEFEIVKKIKVYLDACYTETFIETDKPMYKAGQNVHFRVLTLHPDLRPDISEVDKIWIEAP
GGVHMAQWLGVETNQGLIDETMKLSTNPTQGEWTIKVLHHNRDFSQKFTVGEYVLPKYEVMVQGPDYVVVDVEQI
SVVVCGRYTHGQPVVGSIILKVGVLNYPDKSVETSAEAKAGCFTFEVDMTKLDLMSSSYNVWNAKLHLEATFEEA
STNHIMLKTSDSTKIVNKPFHLEFSAPSTFKPGLSYSGALLFTDPSDKPLADTKSEINIEADGVSIFKETVISDA
KGMAFFSVTTIPSGSKSVTLKAVSKGYFRQSTWHPKNGGTHGIQDPTAYDVAQPQISPSNSFLHIDPVLETAGVN
SFQSLTIHLTSDKPEYNGVYLHTVMMSRGNILLSVESIIPPEDIDGFRRPGTQGVYPVDTDEYGRKEYPTRTNME
VTDIPVNILNKGQSKTTRRKIRILYEMAPAVSVMVYYIREDGEVVADTITIPVEEVFQNEVDVSFDEKTAAPGDS
AVLSVRAKASSLCAFGVVDKSVHLLGGDNRLTKKKVFEALEGLQLSSEGGSAEPSRCSSGGGMFRGYMPPQNLND
ASQAFKDLGVVYLTNLKVDTAPCPEVNPYMYFRGEVQNDGGDMMMAMPMMMMAVMEDSAIEGAVMKGAESDAAGP
TIRSYFPETWLYQLVEIDDSGHKDINVHVPHTITKWIGHGFCTSTKYGAGVSDLTSITAFQAFFIDMRLPYSVIR
YELVPVTVVVFNYITECLMVELTLEASSSYEIDDQNRVEKLCVCAGESQSVSFYVKAIELGDIPIQVAAVSVEAS
GACIGREMDDSLLGVSDTVLKKLLVKPEGIETEQVFSTFFCPVDYENGVYIEDIILKDLPENFVSGSERGIITLT
GDMMGPSISNLDQLLRMPTGCGEQNMIGFVPNIFVLHYLTGINKLTPGVEAKAKQHMEIGYQRELTYQHTDGSYS
AFGMSDPSGSTWLTAFVVRSFAQASKYIFIDPEELAKSKVWLLSLQQKDGCFKSVGKVIHKDMMGNVNDQATLTA
YVMISLIEAGDASEAEHIQKGVQCLNKEVDIQTADMYTLALSSYAYILAGSPHTDTLLERLNELAVESEGLMHWE
GAEVLNTDKRSPYLHRQASSQNIEITSYVLLTYVRSYDKSQALVKGNPVARWIVGQRNAEGGFSSTQDTVMALQA
LADYSMMAYSGGNLDISLGIKFSCDDDIRKLHVTQENSLLLQIEQIPEVPVEIKMTATGSGCALTQVIVTYNIIP
EVSAEPPFTIDISSVDNRNPRQFPCISYSMTICAHYNGEDEFSNMAVIQVRMVSGFEPESNSLQALEQEEGGPKM
VEHEGKEVNFYYDQLEKEDTCVSFMVTREEFVKEPRKGIVTVFDYYEKSLSVSELYDLECKR*

>Sfp10

YKNPCLICFAVITTTTQTSTSTTAGECMEPLNGLLDLEERRFISPQQNQSTGINADNPWIFELLPYVDAENKPTV
TVEFKSKVEVHGFK**LQGSGDIVKELQFVVSAKYEVDGLFVELEDEKLVADLTNQRVETVALPSPLSGLLALSLQL**
ESVDQEDAGFGSLRFDVLGCEEEISTTTPVVTTTKVVTTTTQTSTSTTAGYCMDPLSELDNLEQRSFAPQQTLEN
QSTLINSENPWTFQLSSDAGEEYDNPWVIVQYQSKVEVRGFKLQGSGEDVITLEFILSAKSEDNEQFVDVLEGEK
LGATLYQDNLKSITLDSPLPGVVAVKLQVIKKQNGGTGSLRIEVLGCEKEISTTTPVTTTTKVVTTTSQSPTSTT
AGYCMEQLDGTQGLEAKRSVQPAIANDQSTSVNAQQPWTFSLDNQPSLKDRETVKNPSVTIDFKTEVTVTGVRLQ
GSTNNDNIEFILLAKRRLGETFVIVQGASGKALVQNMELKEQASDVTLIKPLKGLIGLRLEVVSLSRMDESQSTD
SKLQFSLRAAILGCEEELSTTTLATTTTEAPLVSGFNPVITTIRQSSRSTTPTTVVTTTTEMPTSTTAGYCMEPM
SGEQDLEAERSFTPQQIPNQSTNINAEYPWTFELSAVTTPQLTVYFTSKVEVRGFKLQGSGELVKELKFIVLAKF
EDNGDFVEVPQDQKITATLSGDGLESVNLDTPLERVVAVRLQVQSIDDLKAGKGSLRLDVLGCKEGRSKSLV*

>Sfp11

*MEFFILVLLFSLLTTKGAVA*QDDLDWDGFGEADGSLYELKPLSIIGFHTEQSSDYDSNLYPSSKGVDGNVDHGFF
HTQNDEHSFWTVQLKEEHCIGKVTLFNRKDCCGARLANAVVRAGVSPFYSLNQRCGSPVESHLGNGGVIDVYCDP
PLLASEVNVVLPNREYLHLHEVQVEEYPIQRCHQIERRISILNSPTEQSSGYDDTKYPAERAADGFIGKFVGFSH
TGHDFDPWWRVD**LLDTHCISKVVLFNRYDCCSERLSNAVVRAGTSKDIHTNTRCGSAITAEQAKIKGGVLAVYCG**
PPLSGRYVSVNIPGRNEYLQLREVEIYELDVNECESDERHLSIIGKPTEQSSYYDNRYTPDKAADGNLDTKLSPE
FICTHTKEEDDPWWIVDLEDEHCISKVVLLNRGDCCSERLTNAVVRAGTSHNIQSNPQCGDVVSRAMAAPRGGTI
ELVCDPPLVARYISVDIPSDEHSFLQLCEVTVKEFIGEDACREPEPIVIKAPEPQQPVEEPKEAVEEDNQKKVLG
GGVKIQQDVPDDMGP*

>Sfp12a

CAEEFSTTEYVTTKSPTT**KGLPTGDTFLSSTTINSRHPPTTTATTTKTSSTTTHQLSTTQGYCLENMDGEEGLQE**
GFTPTSDLITLQVENGTTYIKFESLPLEVGDNNLPSVDGILTRSASIRAISIRLVPSSSSSTPSPTGNEELVSVT
INFFTKKERNDDFKPLLLEDNQEPKVITFTPVSDQPLQKFLKIGNEVIEGVVAFRVTVLEVTGVSSATLELHVLG
CAEEFTTTQVTTTAVSVTTPTTTTEESTSTVAVTTPTQTTTERTTTSTTSLVSTTEGYCLQNMDSESGVETKDQT
RGNLLTDESGFLTFSELPIEQSVGNSAVLERKFNSNINVRGVLITLKKITSSVTSGQAEGMTTPETGSTSPQSVK
FTFTLLAKRVDETDFKQVVIKNTITEFTLTIEGIQSEVKVFTEDEPSLQGITELQIKFIDVSGVSGA

>Sfp12b

Q**GITELQIKFIDVSGVSGASFELTVLGCAEEVSTTTQTTTSSTVSTTTPQLSTTPGYCLENMDGEEGLQEGFTPT**
SDLITLQVENGTTYIKFESLPLEVGDNNLPSVDGILTRSASIRAISIRLVPSSSSSTPSPTGNEELVSVTINFFT
KKERNDDFKPLLLEDNQEPKVITFTPVSDQPLQKFLKIGNEVIEGVVAFRVTVLEVTGVSSATLELHVLGCAEEF
TTTQVTTTAVSVTTPTTTTEESTSTVAVTTPTQTTTERTTTSTTSLVSTTEGYCLQNMDSESGVETKDQTRGNLL
TDESGFLTFSELPIEQSVGNSAVLERKFNSNINVRGVLITLKKITSSVTSGQAEGMTTPETGSTSPQSVKFTFTL
LAKRVDETDFKQVVIKNTITEFTLTIEGIQSEVKVFTEDEPSLQGITELQIKFIDVSGVSGA

>Sfp12c

PTTTTEESTSTVAVTTPTQTTTERTTTSTTSLVSTTEGYCLQNMDSESGVETKDQTRGNLLTDESGFLTFSELPI
EQSV**GNSAVLERKFNSNINVRGVLITLKKITSSVTSGQAEGMTTPETGSTSPQSVKFTFTLLAKRVDETDFKQVV**
IKNTITEFTLTIEGIQSEVKVFTEDEPSLQGITELQIKFIDVSGVSGASFELTVLGCAEEVSTTTQTTTSSTVST
TTPQLSTTPGYCLENMDGEEGLQEGFTPTSDLITLQVENGTTYIKFESLPLEVGDNNLPSVDGILTRSASIRAIS
IRLVPSSSSSTPSPTGNEELVSVTINFFTKKERNDDFKPLLLEDNQEPKVITFTPVSDQPLQKFLKIGNEVIEGV
VAFRVTVLEVTGVSSATLELHVLGCAEEFTTTQVTTTAVSVTTPTTTTEE

>Sfp13

MEEQVVCMLEFKTSAEVSCAPVLVTKLNAQGSIKVSTGDISQSQK**LPFEMKLKDGSTLIARQVSSIFSRMTIADL**
GIDIQLGLDKRIYVTLNPSLQGKVSGLCGNYNNKQIDDFTTSSGVNAKANINKFGNSWKVSLHCGDVEEKNLRPC
DLKSQQRPLAEEICRKLREEPFASCNQEIPVDKYVEQCEQDVCGCPPSDKECQCLAFATYARECSVAGFDFLWRG

LHNCPIKCPVGQVYKMCGSNCHVNCKDFLTQQPCHEECVEGCQCPEDKVRDDESGQCVKVDYCPCIVDGIVYQSH
QSWKKGCNDCFCSSGTHTCTELDCSTYETCADGMEWIPCLQCERTCQSANKACSIRSGCTPGCGCPKGLVLHKGS
CIDVSDCTCEYNDKSYKPFDTTAMDCHTCMCHTDYRWICDEDQCPSTCRSYGESHFQTLDAKWYSFQGNCEYTLV
ENFCGGSNVEGFFRVTIENVPCGRNGVTCTKAVKFTLHDTTIHLVRGAEYTVAKDPRVTTKARFKMEDAGLFLVI
KTAEGILLKWDYGTSVLVTLDPSHAGQVCGLCGNFNGDSSDDFYTRSGAMESSPQLFGRSWRTSENCPDPKELTL
PCKSEPHRRAWAESVCSIIKKSIFASCHNLVDSEPFFQACVSDTCFCDRGADCECMCTAVTAYATACNENNVAIA
WRAEGYCPLQCESGRVYQACGKICPDKCYPSHAEQNYGCEERCVEGCHCPEGTVLFRDECIERKLCPCFINNGQE
EIRHGALVVEDCMQCMCNGGHLECTGRNCTESVVAETTTPCAHKTKQCSCAPNFSCCADKSVCIHDSKFCDGEYN
CKDFSDEVHCNCIYNDIVYLTGDNWPGVGACEECKCEAGKTTCYKKCQLTCEFPEILNLYEDDDRKCCSCEGGED
TVRRTTTPPRTRPPRSPPTTTQSTTQECVDPFVVGEPTCCQYLDVCAINTKFSGSENCNACREPLVFNGTDCVRP
NECLCKDKVGYHLPGDTWENTEKACSVCTCFNNNIECSDVMCDDTMLDCSKYGEVFAYGACEECTCLDEIVCVKS
ECKLTLEDCEEGEVVFPIGDSCCECRAIETTTIVPTTTLPACVAPFVYRESCERHVMCETLGDDTNEVNCEEGCF
CPEGKLMSEKECIPSHQCPCTDIFGIKREFEEMWAQSDCQQCVCYENMTIHCTKECDLKCDESEEVLVNPDGSMC
CYCEPIPETTTMFIPETTTATTTPSGECFGCYDDKSDTCHAVDTSWKDDVCTSCECKYKNEFIMNCEHMQCGKDL
ECKEGHVLVKDDQECCYHCVPDICVGDFNCNSTFCIPNKWVCDGNMDCANEYDEENCPLPPTLPPPSPACAKTEE
RAFCKDCRERVCHDMECFEMPQELCCECKQGTVYNGTQCVHPKECQCLDENGNIRLPFDKWETADCQECKCHYNR
ITCYETGLCHSTTPSTSTLPPRPYYCTETCTCNLDCEGDFIDEKCLFAVPQECNDTCLCLPPMKKDDVAGRSCIY
QPSQEECIKATTDATTTRELHPISFIEKQIEIGKEKQGKRLQPGSSTPYHL*

>Sfp14

LITGGVQIKL**EGEQRVFLEYVTQGRKSKCTRVRETYTRTKSTVQQGSRRRVYSRQRGGEGGSGSRGGSNSGSSSE**
ENKAISVGATVNKNTDVSTRRRTPAATVQSSSATLVPGEMQQVDWTQKKYEVNFVDKIEDILDLTFQDGSVLTID
VTRTRMTIKDQSGKELKSESLRGKVRDNRLVFTIYLITGGVKIKLEGGQQTFLEYVKAGRTSKCNRVRESYKSSY
RRYL*

>Sfp15

*MWVLRG**FVVLLVIAECLC**RSPPFMKMCPVVPEDVGGICVEECGPDKKCDDGQLCCSNGCGHVCKAACPPLICGLT*
GCLYGRVTDENGCETCSCRGPPAVCPKAPEGVFGICVEECGPNKKCDDGQLCCPNGCGHVCKAGCAPVLCRMGCR
NGWATDEDGCEICQCKRPAQ*

>Astacin-like Sfp

*MKTLLITLLLVGFTLA*FVKNRGMRDDFNSKAVEFLSGRDGEAARAEMTPSPGCVEGDMELTSEQKDVYFAMREAT
AKNRARVRRDAQAAFSIWPNKTLVYRFDASLDNGIKQVFREAIEIYREHTGLSFEEAFDDYSGAIHPLVVYGLND
GCSSSVGYRERSGGRMHLETEKCGALGTILHELGHALGRFHEQTRSDRDDYITVIESNILPGFEHNFRKADRPIL
ALYDLQSIMHYSRDAFSFDRRSSTIMPHNAKDSEKMGTQRRLSRLDVFTVNAAYGVDALCPADLWDKCQRGGVPN
KHCSCDCLPEFEGPYCEIVVIQTECSGHYQLEESGLISSPGYPNTYSSNAKCSYVIECAKDEVVQLDFLEFSLEG
WGCFFDKMNMMTGDLVTEEYPDEYCDDNLKGESVFSKSNVALMTFESDDMFNYEGYQIFYHCIHSDLVSK*

Coding nucleotide sequences of Sfps

All Sfp cDNA coding sequences are listed here. Sequences in bold show the parts of the sequences that were confirmed by PCR amplification and sequencing of the corresponding cDNA (see Table S1 “primers and sequences elongation”). Primers used for ISH probe production are listed in Table S2. Note that some primers were designed in the non-coding UTR and therefore cannot be found in the coding sequences.

>Sfp2

ATGGCTTCAGCCCCTATGATTGTCCTGCTTCCTCTGCTGCTGCTGGCACTAACTCGAACGGTCGTTACTAGTGAC
CTCAAGATGGTCTACGTCTGCGAAGGAGATACTCTTCGACTCAACTGCGGGGAGGACAGATTTATCAAGATGTGG
AGCGCTCTCTACGGGCGTGCCAACCCGACTTACTGCCCAGACGACACCGTCAAGGCCTCTGTGCATCACTGCAGA
GCAGATAAGTCCAATTTCGAAACCATGCGTGGATGTGACGGAAAGAATAGCTGCGAGATCGAGGCAAGTGATGCA
GTCTTTGGTGACCCTTGTCCGGACGAGACGAGCAAGTGGTATCTCCAGGTGGAATACTACTGTCGCTCCGAGAGA
CC**ATCTGAATGCAACCCAAAGGATTCACCAAAATACCAGAGCGCGCATGCATGCGACGACAAAGAGTTGGTTCTG**
GAATGTCCGACCCCAGGAGAGGTTGTCAAGATCCTTGATGCCAATTACGGTCGCCAGGGATCCGACTACTGCGGG
TACAGACCGGGGATCGATGACGTCTTTTGCGACGACGATTCTGGGGAATCGTTTGAGTATGTTTGTCTTCAATGT
CGAGGGAAGGACTACTGCATGATTCCCGTGAACCCACTGGAGTTCAAAGACCGCTGTGTCAACACAAGGAAGTAC
CTCTCTGTTCGCTATGTGTGTGTACAAACAGAGCCGGAAATTGAAGAGGAAGATGATTCAGAGGAAGAGGGGCCA
GAGGTTGGTGATGTCGATCTTCGTGTTACAACCATAGCTCCAAGACGAGGAAGAACTCAACCTGCTACCGATTTT
CCTTTCATGATGTTTCCAGTTGGTTCAGCTGGTGCCAGTGGAAAGATCAAAGTGGGAGAAGCTGAACACGAAGGG
GCTTCATTTAAGTTTGAAGGTGCACCAAGCATAATTGGTTCAGCTAACGCCAATGGAAAGCTTGAGGTAGGAGAA
ATTGACCACGAAGGAGGCTCATTTATGTTCGAGGGTTCACCATCAAGAACTAAAACTATTCCTGGATTGTCAAGT
ATTTACACATTGCCTGCAGGTTTGGTGGGCCAGTCAGCGGTGCTGATGAAGATCACAGTGAAGTATAATGAACAC
GGAGAGAAGTATTTTACCTTGACACCCTTTGACGGAACTTCAAATACCAAAACAGATGGGACTGAATTTTTAATG
AAGATTGACCTGAATAAGATACTCCGAGATGACTCCTTACAGTACACCTTCAGGAGATTGTTGGAAAACTACAAT
AGAGGAACAACTTACTACAATAACAACGACGGGGTGAACACGCCGTTCCAGTTTCCCGTCGGTAATGGACTGGAG
AGTTTCTTCTCAACACTGTTTCAGATGATCATTGAAACGGACTCCAGCGGGAAGATGGTTACAACTCCAACCAAC
CCATCGTCCAACTTCCAAGTTATTCTGAACGGAGGCAACAAGGCCATCGTGGACTACCTCATGACCTTTGGCTCC
AACACAATGGATCAAAATCTACGAACTGAACTGATCAACCATAGGCTTTTTATATCAACAAGTTTCTACAATACG
TTGACCCAAAAAACTGAAGAAATTAAAATTCCCTTCTATGTCAACTTTGATGGATTGGACGGCTCGCTCTCGACT
TTATTCAAATCAACTTCAAGACCTCTGACCCAATTTGGAATGAAGTATCAAGTTGTGGGACCGGGCGGAGCCAAG
CAAATCTTCACCATGCCTTTTACTCTGATTGAATATCAGGGAAGAAAGAGGCAGTTTCTGACCCTGCCAACATGT
ACTGCTTTCAAGATGTATGGATTTGTGTCTGATAAAGAGTACCTCATTGATCCAGATGGGATCAACAACGGAGAG
GAACCATTTGTGGTAAACTGCAACATGGAAAAAGGTGAAACTGTTGTCAATCACGACCGCGCTGAATCCATGGTC
GCCAGTTACCAAGCCGGCAAGAATACAGTGGACGTTAATATCAACTACGAGGGCGCTACAAAGAAACAGCTCCTC
GCTCTGCGGAGCGTCTCGGATATGTGCAGCCAGGAGATGACGCACGAATGCGTGCAGAAGTCGACTCATTTTGTC
AACGGGAAAGCGCATATCTGGTGGGAGAGTCTGGACGGGGTCAAAAGAGAGAATTGGGGCACGAAAACTGAAGAA
AAGATGTGTCACTGTGGTGTTAACAATGCCTGCAGTGATGCCAACAGCCTCTGTAACTGTGACAGTAGAGGAGGT
GTCAGCGATTGGGGAACCCTCACAGACAAAGACACCCTTCCTGTCGGAAAGGTCGTCTTTCAGAAACCTGTGGCT
ACGGACAGTGAGGACAATCTCAAAGTCGGGCAATTCAGATGCGGCGGAGATCAGTCCTCAAGCACTCTTCAGTTT
TTCATCTTCATGAATCAGATGCTAGCCAATCCCAAGAAGACTTGCCATGAGTTTAAGGAGCTCGGTTACGAGTTG
GACGGTGTTTACTTAATCGACCCGGATGAGAAGGAAGGAGAACGACCTTTCACTGTCTTCTGTGAGATGGAAAAG
CAGAATGAAGGAATCACCGTTGTCCACCATGACCTCAGCGACCAACGAACCCAAGTCAATGGCTACGAAGAACCA
GGTAGCTACGCCAGGAAAATTAACTATCGAGCTACCTCGAGACAAATCGATAACTTGAAGGCTGTTTCCGGTAAA
TGTGAACAGGAGCTCATGTACGAGTGTCAAGGGTCTGCGTTTCACGTCGGCACGGTTCTCAACCATTGGTGGGTG
TCATTCAATGGGGATAAAATGCCAAACTGGGGTGGGGCCCCGTGCGATTTCGATGGGTGTGCTTGCAAGTCTGAT
TCGAGTTGCGTCGACAACCTCAAATGCAACTGTGACAAAGATGACAATGTCATGCGTAAGGACGGTGGCAAGCTG
GATGACAAGGACAAGCTACCCGTCTCCGAGCTTCGCTTCGGTGACACTGGCAGCGCTGAGGAGATGGGGTACTAC
ACTCTAGGAGCATTGAAATGCTGGGATGCCTCTGAAAAGAAAATAACAAGGCGCTTCTTGCTCAAATCTTGCAGT
GAAATCAAAAGGGATGATCCTTTTGCGCTGAGTGGTAACTACCATATAAGACCTTCAGACTCCAAGGATGAATTC
CTCGTCTATTGTGACATGTCCGGCACTGAAGGAGTGACGGTCGTTTACCCCGACAAAGGAGAGACTCTTCAAATC
GATGGTTTCTACGAGGAGCCCGGAACCTGGTCGCGAACGATCCAATATGGCGCATCGGAGCCGCAGCTAAAAGCC
CTGACAACGGTTTCTACTACCTGTAAACAATACATCAGCCACGGTTGTTACGGCTCACTCCATCACAGCAGCGAG
ACTGGCAAGATGTACACATGGTGGGAGTCTAGAACGGGAGTGAAGATGATGAATTGGGGGGAGGTGGCTGATGGC
TGCCAGTGCTCTGTCAGTAACACTTGTAAGGGAGGTCCAAGCACAAAGTGTAACTGTGACGTCAACACTCAGAGC
TACATGACGGACGAAGGTTACCTGACCGATAAAACTAAACTGCCTGTGAGTGGGATCCGCTCTGGTGATGTCAGC

GATGATAAGGGACTGGAATATGGAACGGCCAAGCTGGGGCCGCTGATGTGTCAAGACGCCGGTTCAAATGAACAG
ACCAGTAGAGCTCTCGCCGAAGCAGATGACGTCTGTGACGAAATCACAACGGAAGGCAACGTAGAACGTTATCTC
TCGGTAGGAGACTTTAAAGATTTTCACTTTGACCTGTACGGTTTGGGAAAAGCTACTGTGAATCTAGCCGAAGCT
AGTTCTTTGTCTGCGTCTTACAAAATTGAAATTGGAACGGACTTGGACAAACAAAAAGTGATCCTGAGCCTGTGC
AATGACGACAGCAAGTGTTCTGTCGCAGAAGAGAAATCCTTTACTTCCTCCACCAGTAGTGATGGTGTGGAACGT
AGCTTCACTGTCAACGTTAAGAGCGAGAACATCGTGCTGCAATCTGGAGACGAGGCCTTGAGTTTTACCAGGCCT
CGTCAGGATGGAGCGACACACTTGGAGCCCAAGTATTTCACCTTCTCAACAGAAGAAGCGATCAGCTGGAAGTTT
GACTGTCCATCTTTCAAATATATCTGCAGTGTAAAAGACGCAATCACATGCGCGGATAAAAAACCACGCATCAAG
ACGGTCCGCTACGGAGTCCCGGCCGCGAACCCGTGCAACGAAGCAGTAGATTATTCACAGTGCAGCGGCATGTCA
CTGCTGAACAAAAAATCTGTGAGTCAATTGTGTTCGGGGAAGTCCTGTTTCTACATGAAGAAATCTTTGGAGGAT
GGCCAGTGCACTGCTCAAGGGATGGTTCTTAAGGTGGAATATGAATGCGACTACAAGGAGGGTAGGACGTTAGAC
TTGGCCAACCCATGCAATAGTAACCCATGCCAGAACTCTGGGACGTGTGACTCCCCAGACGGTGAAGACACCTAC
AGTTGCGAGTGTGGACAATACTTTACGGGACTCAACTGTGAAACAGAACTGGTGTATTGCAGTGCCTGGGGCGAC
CCTCACTACCAAAACTTCGCAGGGCAGAATTATGACTTCATGGGTAATTGCACATACGTGTTAGCCCAAGACTGC
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>Astacin-like Sfp

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(5) Expression of Sfps in secretory cells of *Asterias rubens*

Primers used for ISH probe production are listed in Table S2. Note that the ISH primers were designed based on the transcriptomic data (Lengerer et al 2019).

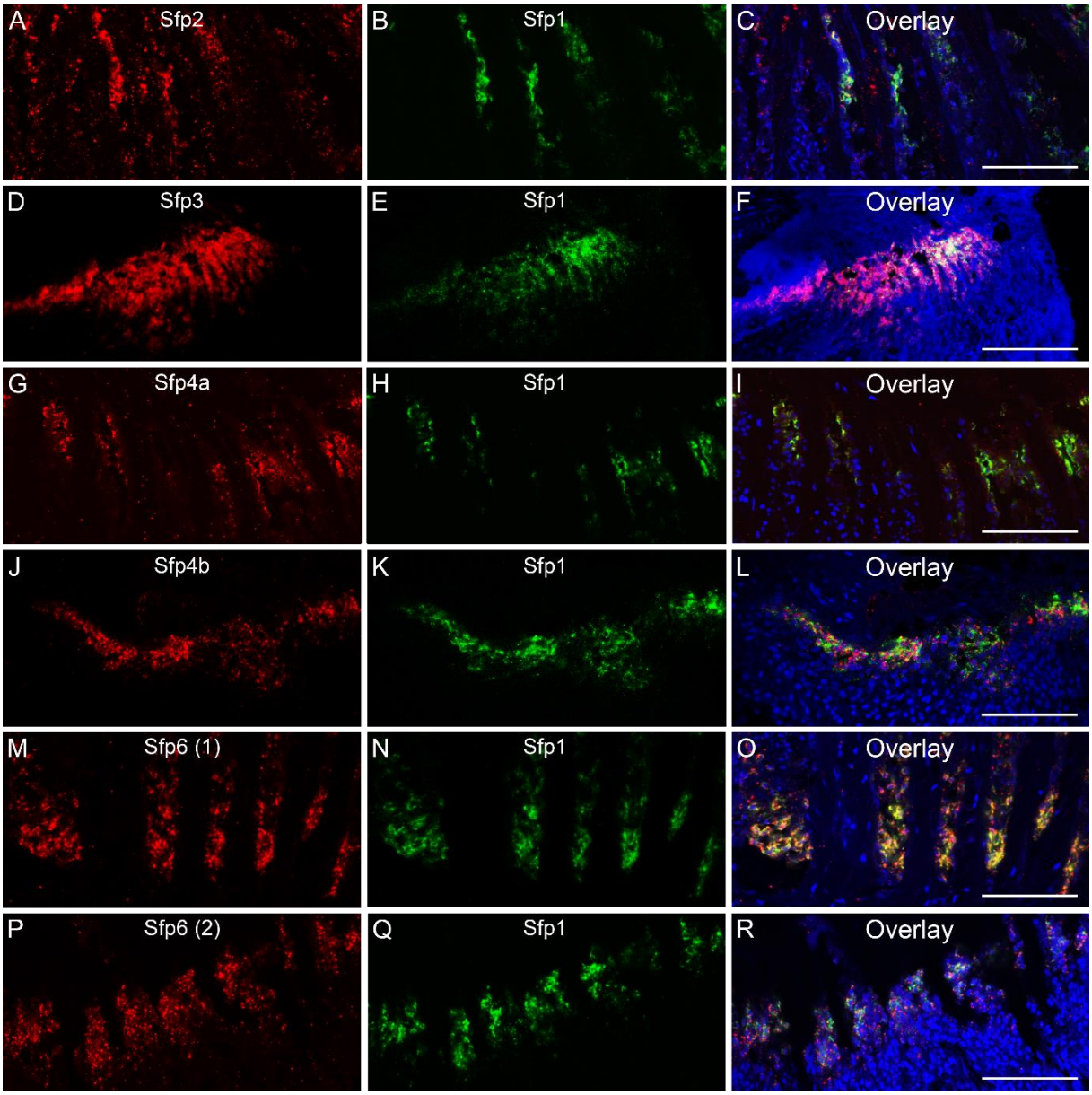

Figure S7. Localization of the expression sites of mRNA coding for Sfp1 (in green) and five Sfps candidates (Sfp2, 3, 4a, 4b, and 6) (in red) on tube foot sections of *Asterias rubens* by double ISH. Co-localization of mRNAs coding for the proteins Sfp1 and Sfp2 (A-C), Sfp1 and Sfp3 (D-F), Sfp1 and Sfp4a (G-I), Sfp1 and Sfp4b (J-L), Sfp1 and Sfp6 (M-R). Two different ISH probes were used for Sfp6, e.g. Sfp6 (1) (M-O) and Sfp6 (2) (P-R) (see Table S2). Scale bar: 50µm.

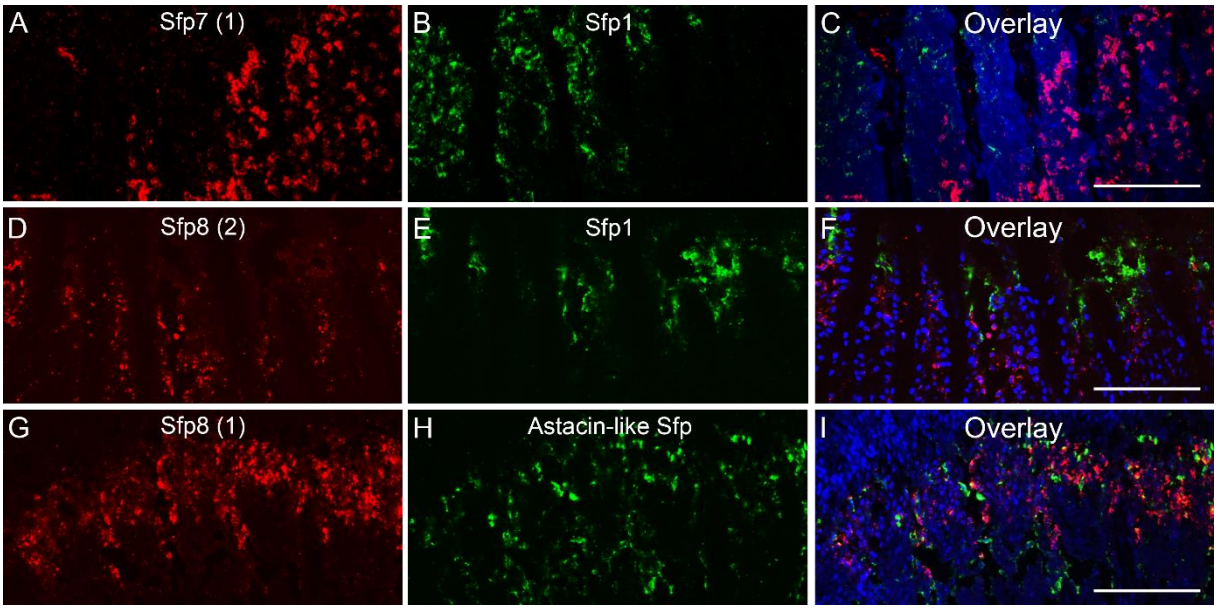

Figure S8. Localization of the expression sites of mRNA coding for Sfp1 or Astacin-like Sfp (in green) and two Sfps candidates (Sfp7 and 8) (in red) on tube foot sections of *Asterias rubens* by double ISH. Co-localization of mRNAs coding for the proteins Sfp1 and Sfp7 (A-C), Sfp1 and Sfp8 (D-F), Astacin-like Sfp and Sfp8 (G-I). Two different ISH probes were used for Sfp8, e.g., Sfp8 (1) (D-F) and Sfp8 (2) (G-I) (see Table S2). Scale bar: 50µm.

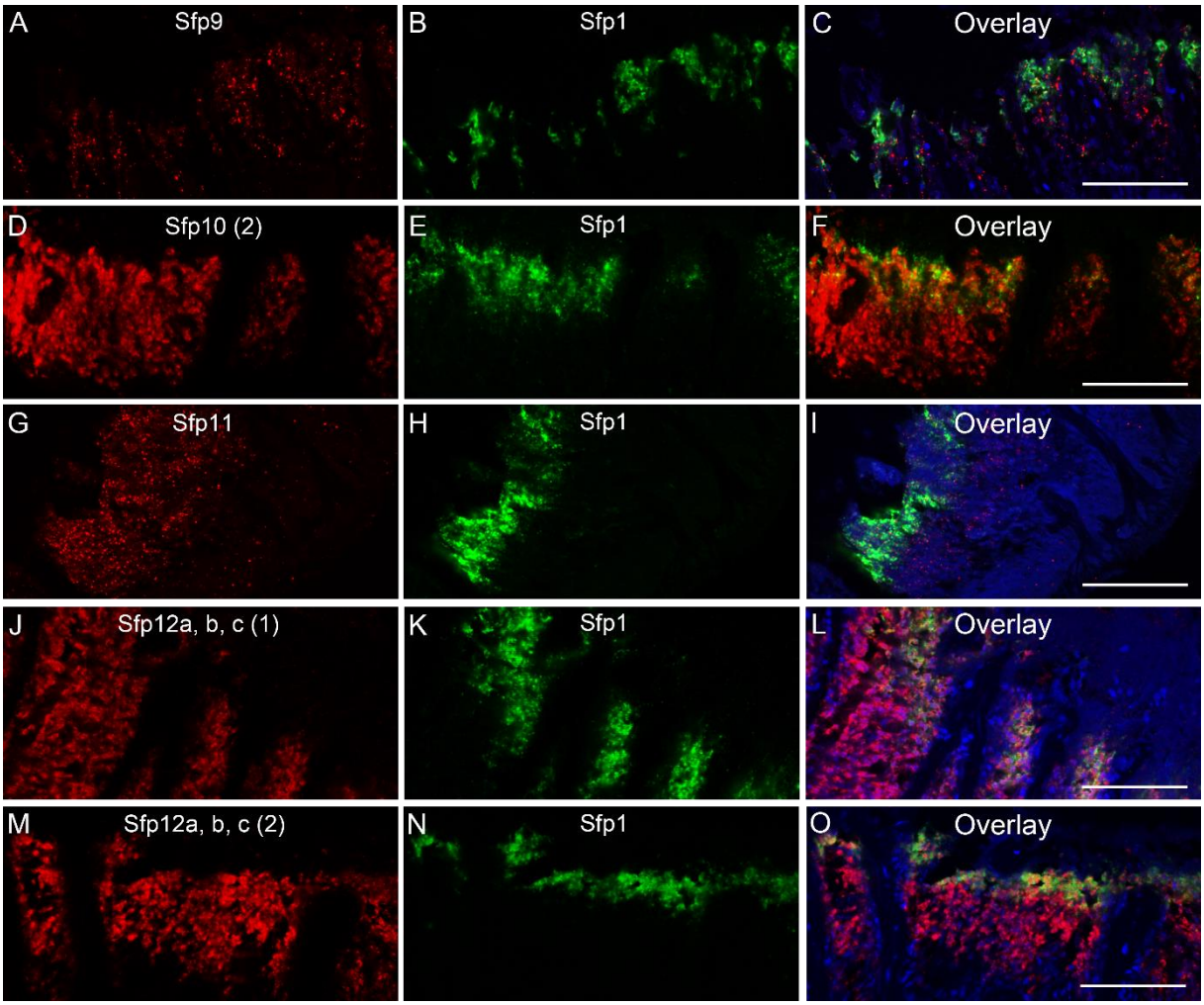

Figure S9. Localization of the expression sites of mRNA coding for Sfp1 (in green) and four Sfps candidates (Sfp9, 10, 11 and 12a, b, c) (in red) on tube foot sections of *Asterias rubens* by double ISH. Co-localization of mRNAs coding for the proteins Sfp1 and Sfp9 (A-C), Sfp1 and Sfp10 (D-F), Sfp1 and Sfp11 (G-I), Sfp1 and Sfp12a, b, c (J-O). Two different ISH probes were used for Sfp12a, b, c, e.g. Sfp12a, b, c (1) (J-L) and Sfp12a, b, c (2) (M-O) (see Table S2). Scale bar: 50µm.

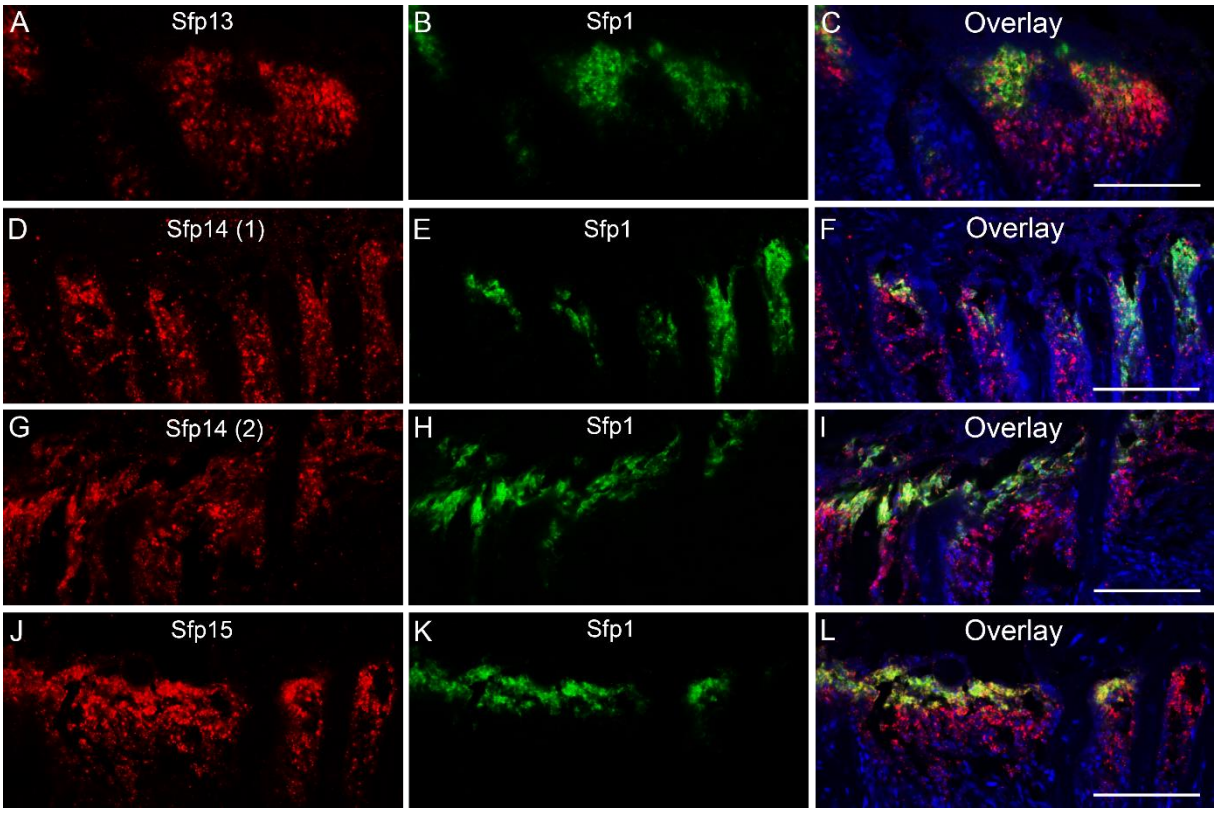

Figure S10. Localization of the expression sites of mRNA coding for Sfp1 (in green) and three Sfps candidates (Sfp13, 14 and 15) (in red) on tube foot sections of *Asterias rubens* by double ISH. Co-localization of mRNAs coding for the proteins Sfp1 and Sfp13 (A-C), Sfp1 and Sfp114 (D-I), Sfp1 and Sfp15 (J-L). Two different ISH probes were used for Sfp14, e.g. Sfp14 (1) (D-F) and Sfp14 (2) (G-I) (see Table S2). Scale bar: 50µm.

(6) Western blot analyses of selected Sfps

Protein extraction and Western blotting

Adhesive footprints were collected as previously described (Hennebert et al., 2012). Tube feet and footprint proteins were extracted as previously described (Hennebert et al., 2014). Briefly, tube feet were rapidly dissected from one sea star and immediately frozen in liquid nitrogen. Freeze-dried footprint material (approximately 2 mg) and tube feet (approximately 400 mg) samples were homogenized in a 1.5 M Tris-HCl buffer (pH 7.8) containing 0.5 M DTT, 5 M urea, 2% (w/v) SDS, and protease inhibitors (cOmplete™, EDTA-free Protease Inhibitor Cocktail, Roche) using a Heidolph Silent Crusher-M homogeniser. The extracts were stored at 4 °C for 15 min to 1 h until use. Footprint and tube foot extracts were centrifuged for 10 min at 19,000 × g and loaded on 10% (w/v) SDS/PAGE gels. After electrophoresis, proteins were transferred onto PVDF membranes (GE Healthcare) using 25 mM Tris, 192 mM glycine, 0.05% (w/v) SDS, 20% methanol (v/v) as transfer buffer. The blots were then immunodetected as previously described (Hennebert et al., 2012) using the anti-Sfp7/8, anti-Sfp10 and anti-

Astacin-like Sfp antibodies diluted 1:1000, followed by HRP-conjugated anti-rabbit antibodies (Invitrogen) and chemiluminescence detection (ECL Western Blotting Substrate or SuperSignal™ West Femto Maximum Sensitivity Substrate, Pierce).

Based on the protein sequences, the predicted sizes are >104 kDa for Sfp7, 149 kDa for Sfp8, >81 kDa for Sfp10 and 48 kDa for Astacin-like Sfp (with propeptide). With the Sfp7/8-antibody, we consistently observed multiple bands in tube foot and footprint extracts (Fig. S11A), presumably deriving from protein fragmentation during protein extraction. With the Sfp10-specific antibody, a large protein band (>200 kDa) was observed in both tube foot and footprint extracts (Fig. S11B). In tube foot extract additionally two bands at around 190 kDa and 110 kDa were present (Fig. S11B). The Astacin-like Sfp-specific antibody detected two protein bands close to the predicted size (55kDa) in the tube foot extract and a faint band at double the size at around 100 kDa in the footprint extract (Fig. S11C).

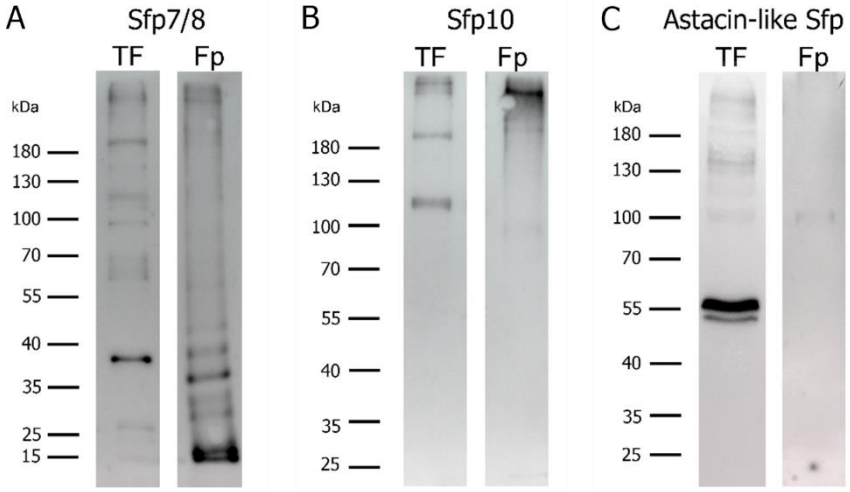

Figure S11. Western blot analysis of selected Sfps in *Asterias rubens* tube feet and footprints. Western blots of the protein extracts immunolabelled with polyclonal antibodies raised against the peptide GDSSQEIKRTLDTK from Sfp7/8 (A), the peptide SLSRMDESQSTDSKL from Sfp10 (B), and the peptide KMNMMTGDLVTEEY from Astacin-like Sfp (C). TF: Tube feet and Fp: Footprint.

(7) Footprint immune-histochemistry

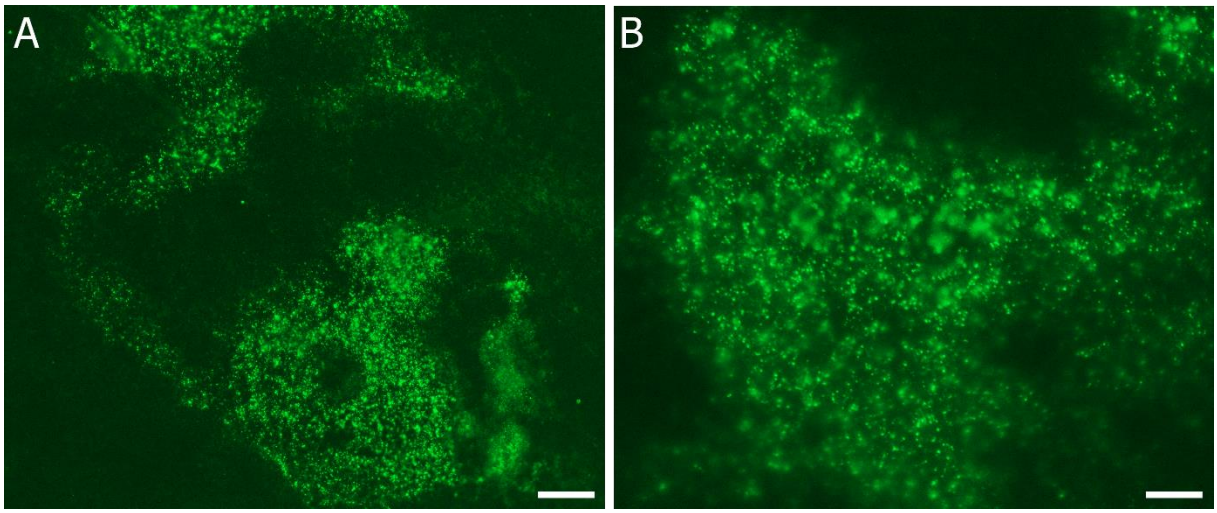

Figure S12. Immunofluorescence localization of Sfp10 in the footprints of *Asterias rubens*. Footprints were labelled with antibodies directed against Sfp10 (A) and high magnification (B). Scale bars: 100 µm (A) and 30 µm (B).

(8) Footprint immune- and lectin histochemistry

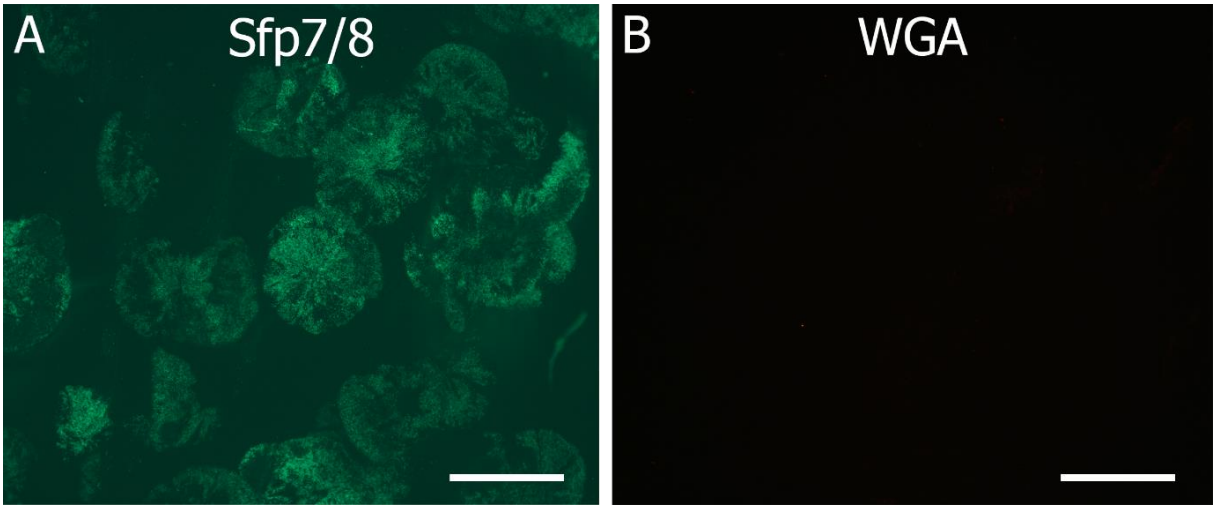

Figure S13. Double immuno- and lectin-histochemistry on footprints of *Asterias rubens*. Footprints obtained from sea stars walking on glass slides were labelled with antibodies directed against Sfp7/8 (A) and with the lectin WGA (B). Scale bars: 500 µm.