Fig. S1. Detection of NGFFamide in an extract of *Strongylocentrotus purpuratus* body tests using HPLC-MS. A. Full mass spectrum showing detection of synthetic NGFFamide peptide (630.3) at 32.0 to 32.9 minutes, marked by an asterisk. B. Magnified view of the NGFFamide peak in (A) showing peak characteristics. C. Full mass spectrum showing detection of NGFFamide (630.3; at 32.0 to 32.9 minutes, marked by an asterisk) in an HPLC-separated extract of *S. purpuratus* body tests. D. Magnified view of the NGFFamide peak in (C) showing peak characteristics.
Fig. S2. Strongylocentrotus purpuratus NGFFamide receptor. The cDNA nucleotide sequence (lowercase, 1514 bases) encoding the NGFFamide receptor protein (uppercase, 444 amino acid residues) is shown. The positions of introns in the gene encoding this protein are shown by highlighting the pairs of bases (bold and underlined) in the sequence that are interrupted by an intron. The asterisk shows the position of the stop codon. This sequence has been deposited in the GenBank database under accession number KP171538.
**Fig. S3.** Diagram showing that a vasopressin/oxytocin-type receptor gene (SPU_021290) is located adjacent to the NGFFAmide receptor gene (SPU_021291) on scaffold 114 of the *Strongylocentrotus purpuratus* genome. Exons are represented by white-filled rectangles and introns are represented by the lines. The diagram was generated as an output from the *Strongylocentrotus purpuratus* genome browser, which can be accessed at: [http://www.spbase.org/cgi-bin/gb2/gbrowse/chado3.1/](http://www.spbase.org/cgi-bin/gb2/gbrowse/chado3.1/).
Fig. S4. *Apostichopus japonicus* candidate NGIWYamide receptor (isotig 14190; Du et al., 2012). The nucleotide sequence (lowercase, 1646 bases) encoding the receptor protein (uppercase, 423 amino acid residues) is shown. The asterisk shows the position of the stop codon.
Fig. S5. *Asterias rubens* candidate NGFFYamide receptor (contig 1116923). The nucleotide sequence (lowercase, 1994 bases) encoding the receptor protein (uppercase, 449 amino acid residues) is shown. The asterisk shows the position of the stop codon. This sequence has been deposited in the GenBank database under accession number KP171535.
Fig. S6. Ophionotus victoriae NGFFamide/NGFFYamide precursor (contig 205045). The nucleotide sequence (lowercase, 1597 bases) encoding the precursor protein (uppercase, 248 amino acid residues) is shown. The predicted signal peptide is shown in blue, in tandem copies of the sequences NGFFF and NGFFY are shown in red, with C-terminal glycine (G) residues that are putative substrates for amidation shown in orange. Putative dibasic cleavage sites are shown in green. The C-terminal region of the precursor comprises a neurophysin domain (purple), with fourteen cysteine residues (underlined) that are a characteristic and conserved feature of neurophysins. The asterisk shows the position of the stop codon. This sequence has been deposited in the GenBank database under accession number KP171536.
Figure S7. Antedon mediterranea candidate NG peptide receptor (contig 1796804). The nucleotide sequence (lowercase, 1746 bases) encoding the receptor protein (uppercase, 394 amino acid residues) is shown. The asterisk shows the position of the stop codon. This sequence has been deposited in the GenBank database under accession number KP171537.
>Branchiostoma floridae SFRNGVamide precursor
MMQTPIFLCSVVLGAVCGQLSETNNFPQNGNRRLSPERSATVRQFLHLEGAVGSPVSP
SDGRALETGDKRSFRNGVngkrrdseeELPONRAGATELKAETATIFSONQGDHPDEGAKAAS
EKRSFRNGVCRTHFRIVADASLSGGLDEPEALQRQGDANSPLSRDLWAVEQGGKDEEQ
PACGSGDGCVLKVGCRLDSGCVLRKDCSSLPPRALCALSQYSATCTDGGCKVAPGV
CCRAADHSCFLPDECD

>Saccoglossus kowalevskii NGFWNamid/NGFYNamide precursor
MLRKIAVVFVFLVAICTLSRATFGTQAVKLHLYWPKEDISELGSSTSGDSGNE
EAVKMFGWAVDGKRGFWNGKRGFWNGKRNFDLRLIFGTNKKHSINNNQKRGFWNGK
NFDDLFEEPKELPQHWRDEKKNGFWNGKRNFDNFKTDSDLQPSIEDKRNGFWNGKRN
SMNKKASASTESKRNFGYNGKRSVDNTRNISSEYNHYKESAKKCTTGCPCGKQCYQLGVC
CSLEIGCSMKTETEECTTSPLVECEGDRSDVQCGNGGCCVANGVCTKETQSCSKIDQEC

>Strongylocentrotus purpuratus NGFFFamide precursor
MGYERRILRLLSILILASFTVYGERDSNMQQKFRRNIVPSPLIQLKwRENRMGPAEE
KTSNEQWREDLLSNLRLKHNASPSSRSDRDTDITAYGLQEMQQLPADVTAQLFLIL
EGAVNSPENEYEEETPIDEDKRNGFFFGKRNFFGGKRSDFSSSTKMDDRLPKYESSG
SFDKCRPCGPGQRGQCTGMMGVTCCSLFLGCYLTRTPEAAACMTEDVSCPQLNAPSCGLAGKC
VAQGICCSAAEGCHLDPTCTMSL

>Apostichopus japonicus NGIWYamide precursor
MAVEAKIVSLCWLSTTVYSQNTGRTHDELSKAADVTFDLILMDEENFDDVNNIES
WETVLKEDNPKIRLALAHMRSSLSPDTSRLEQVFPSDYRSLQEIITDEQPFWDES
SPKLPISLTPEDQIKASADERNNFSNWDPNHRPPEGIFSAEGGSEKRNGIWYGRKSSL
DGEAKRNIWYGRKASSPPVDDKRNIGIWyGRKRNIGIWyGRKRDSLYSEEMM

>Asterias rubens NGFFFamide precursor
MTMGSRIBLVLTIVITVIPISIWAGIAIAGAQTQKIRRESRESGKYPWNSVGISDQQLRQLL
AHSLADSIYTSCEASHIRGDQDGADYIYDSRDQVDDDGARTNEEQEGERVSKEVSRSRDSQNGT
SKRNNFYGGKRNGFFGKRSATPGANAEVTQICPCPGQNNQCMFGTCCSLYELGCEF
LITEALPCVSKSSLCELSLPCGDEGYGRECVASVCLPQEGSCHINATCGGKMTQF

>Ophionotus victoriae NGFFFamide/NGFFYamide precursor
MAVGIRRNLHVILVLVYARTILLGEIDTYKVRRYTDGSPVNSNHWTQSDINELRKEIFA
SLPADLPGIIKSKTHDSRSGRHDSLLEQLNQVAKGDGANDAKDLAYLNFMSKOT
RNNLGDLDKRNGFFGGKRNFFGKRGDVEAIVNEDSCVRCGPENGQCMFGTCPPSOF
GCYLMKTEACSMNHIHGTCRNLDMAPQCGSTGVCAKVGVCSCPQDGACHIDVTSCLSD
NTTIDKSL

Fig. S8 NG peptide precursors in FASTA format
>Branchiostoma floridae NPS/CCAP-type receptor 1
TEQLVTLWVLFAFIVVGNSLVLLVMWMERHKKTRMNFFITNLAIADLGAGLFNVLPDLVH
RFTVEWIAAGDFMCKLYIQGGVLYGSTYVLYVALSVRDYAIHPMRFAHDRKSKAMICV
AWGLAALFSIPSVFAQTCLDNGEQCWATPEDWYWIPYMTVTTLLVFIPLVIISTC
YIFIVKIVRWRQVLESQFVFKGLKLSSQLGIIIPKAKIKTIKLSSLAIITAFIACWVYF
VFDMITNFSLDESETKRRASLIIQNLPAINSNAIPPIYIFGFTSKNGKFR

>Branchiostoma floridae NPS/CCAP-type receptor 2
NYLVTLQTEQLVTLWVLFVIFVGVNSLVLLIMWLERNKTSRMNFFINLAVADLSAGLFN
VLDPHHRVFVVEIAGNCLTYQAVLLYASTYVLYVALSIDRDAIVHYPNMFGKRMNIMIVW
SKVMSAVGLALLFSVPSPVIFAVARRPQNGEQCWAWEPEDWYWIPYMTVAAVFFIP
LGIISICAIIVIKIWRQKGMAYEDHPIRSASSGKNFNYFRIHLINIINFGTSSRAKART
IKLSVAIAIFCICWVSFLFDFILDNYDLQPDTQAKKEASLIINQNLPAINSNAIPPIYIFG
FSTKLYRKLR

>Saccoglossus kowalevskii NPS/CCAP-type receptor
MTSKLNEMKYAAINTVMFGTFDTEGMDTVEPSPVIFAVARRPQNGEQCWAWEPEDWYWIPY
VIGNAIVLVSVLVHHRKSRMNFFINLVAIDVSGLVNLNLIDRIHPATRFYEGGGEICV
LVKYQVAQVYSQSTMALSYLIDRDAIVHYPNMFGKRMNIMIVW

>Strongylocentrotus purpuratus NGFFamide receptor
MATQVNFDPGVTTTEGFDYTEPGSNNGTSNGIVDRWSLDKHIQLAVLWLVFTIIIVNGI
VLIAIVLVRHKSRLNFFINLAVADICGVSFGIDRQTPEFSDIAKCLKYRYQ
AYVVLASSVLYSVLRDFAVYVFMDTNGKRTMLAGGWILPAVGLTSPVVFQVD
PLASPQDMVCSWPAALNSRWSILKYVYYTSRSFVYPLILFCYVTIIVWTR

>Apostichopus japonicus NPS/CCAP-type receptor
 MADEPASLVSTLGTELQRTSENFTTGGTISDGTGFDGFTMLPSTLEGPSYNTSVDDSF
YGGFQILYLWLVFGLTVIGSTVLLAVVYIRHKSLRNFFVHAASDPLLGVGNVNYEV
LYRLEGEFYGGMVFCKIIIFSQAYVINASSSFCQLVALSRDFFAIVFMDMFGSGKKNALM
AVTAWQAPSLASVIFPSWYGKTHCIPPPLVPSGWQKYTVLYVSFGFVPLI
IISTCFMYMISIWRKRKMMMOPKKEVGKAKKNASKEKMPHRASSGLPPIAKIKLT
LMTMSLIAVIFWCPFSSYF/TDAOFVIFIDEANLNTFRASAFIQNPLLIPSANNPFIY
IGFMTSNICQFLRLRRFSVINHATKLRCSCWPSRVYGRSTAGTVMTEFHSHTAIASDNNFRNHFNASNDEKSVRETH

>Asterias rubens NPS/CCAP-type receptor
MATIPAYDHLVTDSLAMYLSNT科室TVMTPGPLSTLEGPSYNTSVDDSF
YGGFQILYLWLVFGLTVIGSTVLLAVVYIRHKSLRNFFVHAASDPLLGVGNVNYEV
LYRLEGEFYGGMVFCKIIIFSQAYVINASSSFCQLVALSRDFFAIVFMDMFGSGKKNALM
AVTAWQAPSLASVIFPSWYGKTHCIPPPLVPSGWQKYTVLYVSFGFVPLI
IISTCFMYMISIWRKRKMMMOPKKEVGKAKKNASKEKMPHRASSGLPPIAKIKLT
LMTMSLIAVIFWCPFSSYF/TDAOFVIFIDEANLNTFRASAFIQNPLLIPSANNPFIY
IGFMTSNICQFLRLRRFSVINHATKLRCSCWPSRVYGRSTAGTVMTEFHSHTAIASDNNFRNHFNASNDEKSVRETH

>Antedon mediterranea NPS/CCAP-type receptor
MDYNMDFNVTDFTENTPTKEVSTRIVLYRDLQLILWLSIISKILGIGAVLFALYTVRH
KRSMNFFMNLSIISGGFVFLVQIHGRYGTWGETGIAKCLLVKOIAASLASSCO
LVAISYRYLIAIYPMNSGRSSHRHAAINWFPSITSLHIERFEGQTCW
VLELENKLLKLYITYIVLICFIFIIPLLVFYILFCYSIITTIFWKKSKMMGPNKIPASNNKKGN
YDNLKAFDSRSRRASSRGLPLAKVKRRQIVTSLIINFVLCWPSIFIFTYLLAOFVQPI
SAKLMVAINLNPASAVNPILYIFGSTNLCRELKRPVIPINVWAGVLPCTARKKAEPGF
TRAMYTRTENTNMDYSVTTCEQDSERIKLGQTSK

Fig. S9 NPS/CCAP-type receptors in deuterostomian invertebrates