

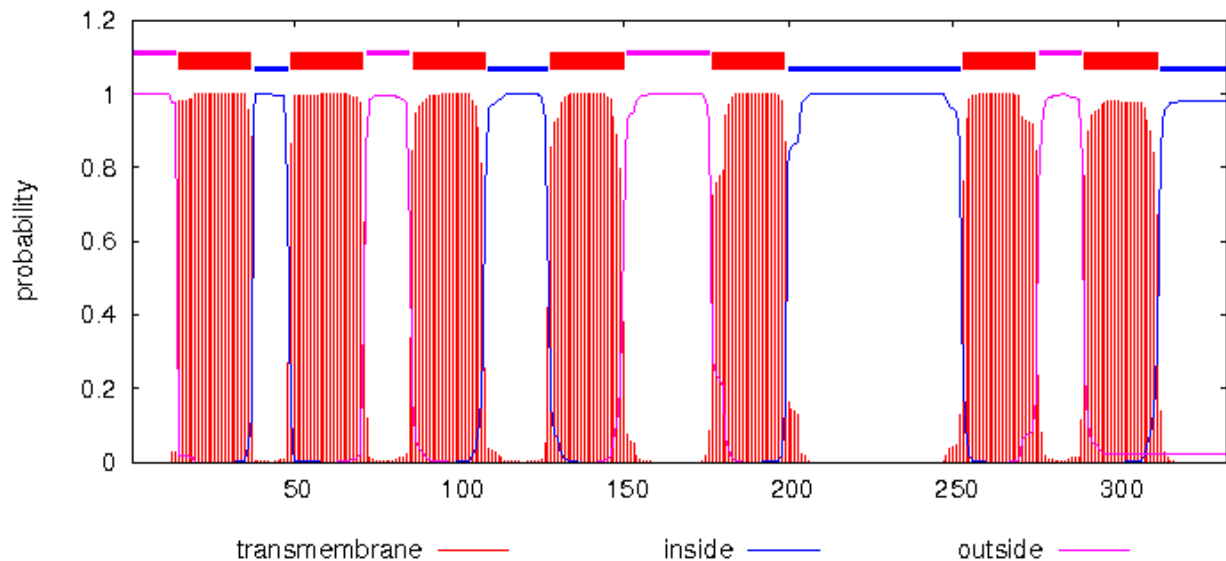
Identification, Functional Characterization, and Pharmacological Analysis of Two Sulfakinin
Receptors in the Medically-Important Insect *Rhodnius prolixus*

Supplementary Figures S1 to S4 and
Supplementary Tables S1 to S4

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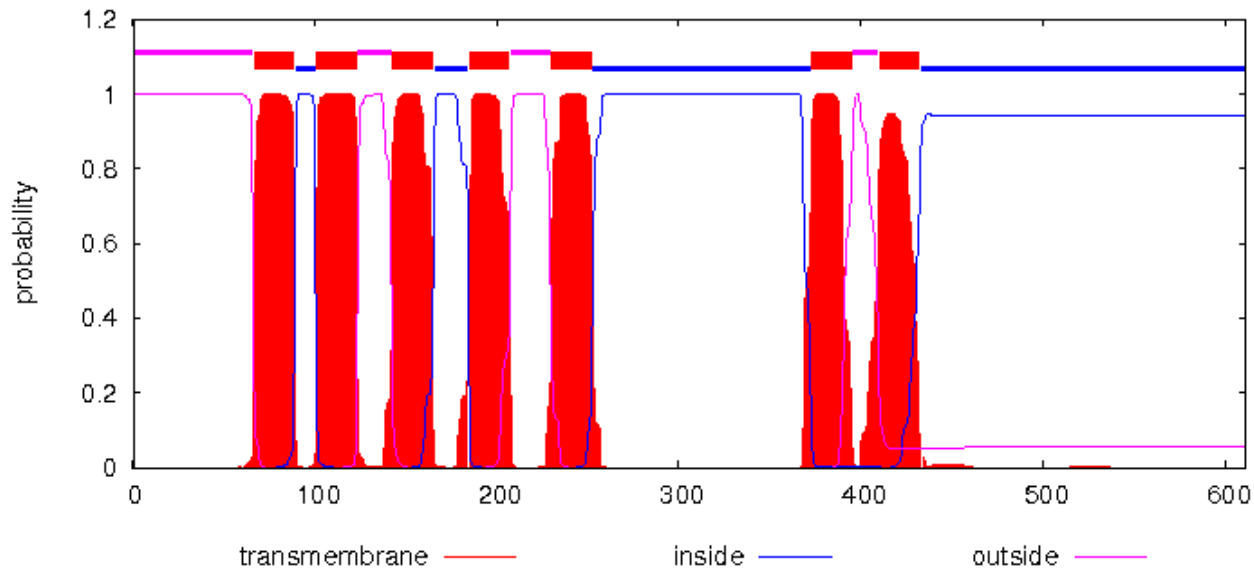
Supplementary Figure S1. Predicted membrane topology of Rhopr-SKR-1's seven transmembrane domains in *Rhodnius prolixus* (via TMHMM server 2.0).

TMHMM posterior probabilities for WEBSEQUENCE



Supplementary Figure S2. Predicted membrane topology of Rhopr-SKR-2's seven transmembrane domains in *Rhodnius prolixus* (via TMHMM server 2.0).

TMHMM posterior probabilities for WEBSEQUENCE



Supplementary Figure S3. Amino acid sequence alignment for the *R. prolixus* sulfakinin receptors' transcripts, aligned via Clustal Omega, with homologous receptors. Following the 60% majority rule, identical amino acids are highlighted in black, whilst conserved amino acids are shaded in grey. The seven putative transmembrane domains are indicated via red bars. Dashes were utilized to introduce gaps to maximize homologies. The sequenced used consisted of *Rhodnius prolixus* receptor 1 (GenBank: MK513659) and receptor 2 (GenBank: MK513660), *Tribolium castaneum* sulfakinin receptor 1 (GenBank: AGK29938.1) and receptor 2 (GenBank: XP_972750.1), *Drosophila melanogaster* sulfakinin receptor 1 (GenBank: NP_001097023.1) and receptor 2 (GenBank: NP_001097021.1), *Anopheles gambiae* sulfakinin receptor (GenBank: AAR28375.1), *Periplaneta americana* sulfakinin receptor (GenBank: AAX56942.1), *Nilaparvata lugens* CCK-like receptor (GenBank: XP_022184688.1), *Dendroctonus ponderosae* CCK-like receptor (GenBank: XP_019756917.1), *Nicrophorus vespilloides* CCK-like receptor (GenBank: XP_017773275.1), *Athalia rosae* CCK-like receptor (GenBank: XP_012254014.1), *Neodiprion lecontei* CCK-like receptor (GenBank: XP_015517254.1), *Pediculus humanus* CCK-like receptor (GenBank: XP_002433137.1), *Bactrocera dorsalis* CCK-like receptor (GenBank: XP_011198391.1), *Ooceraea biroi* CCK-like receptor (GenBank: EZA56385.1), *Drosophila persimilis* CCK-like receptor (GenBank: XP_002026780.1), *Homo sapiens* CCK receptor 1 (GenBank: NP_000721.1) and receptor 2 (GenBank: AAA91831.1).

Rhodniuspro-SKR1	1	-----
Rhodniuspro-SKR2	1	-----
Tricas-SKR1	1	-----
Tricas-SKR2	1	-----
Drom-SKR1	1	-----
Drom-SKR2	1	MLP-----RLCADACRQCFAKIARRDTHR-GTRTPYGC-----ADTQS
Anog-SKR	1	-----
Pera-SKR	1	-----
Nill-CCK-like	1	-----
Denp-CCK-like	1	-----
Nicv-CCK-like	1	-----
Athr-CCK-like	1	-----
Neol-CCK-like	1	-----
Pedh-CCK-like	1	-----
Bacd-CCK-like	1	-----
Oocb-CCK-like	1	-----
Drop-CCK-like	1	MLPCCCGELAAAAVGPTCCGSRCLRATEGANNGASASVLHEGYEARDTPRTVSSVSVLG
Homos-CCKR1	1	-----
Homos-CCKR2	1	-----

Rhodniuspro-SKR1	1	-----
Rhodniuspro-SKR2	1	-----
Tricas-SKR1	1	-----MSEV---EM
Tricas-SKR2	1	-----
Drom-SKR1	1	-----MFNYEE
Drom-SKR2	38	RPKPNFLLREVDEVCCCTAASA---SPRLLVLFRD-----HKRASFFGLTIDAFYHYLR
Anog-SKR	1	-----MTDGPFFVYGI
Pera-SKR	1	-----MEMMDDN---SI
Nill-CCK-like	1	-----
Denp-CCK-like	1	-----
Nicv-CCK-like	1	-----
Athr-CCK-like	1	-----
Neol-CCK-like	1	-----
Pedh-CCK-like	1	-----
Bacd-CCK-like	1	-----MFIYTR
Oocb-CCK-like	1	-----MSIQ---GH
Drop-CCK-like	61	GSLPTFFLAYFSTPLCSSVSRRVASARLLQARADSSDQQGRIRGDPRDSLITIGTFYRYLR
Homos-CCKR1	1	-----
Homos-CCKR2	1	-----

Rhodniuspro-SKR1	1	-----
Rhodniuspro-SKR2	1	-----
Tricas-SKR1	7	NFTN-----NV-----
Tricas-SKR2	1	-----
Drom-SKR1	7	GDADQAAMAAAAAYRALLDYYANAPSAAGHIVSLNVAPYNG-TGNGGTVSLAGNA-----
Drom-SKR2	88	QALP---LAKEAAIHLNAS-----NEISA-----VG DGVTITGT-----
Anog-SKR	11	ELRD-----PPTAPTELTQ-----YDLLFGPGSLLYRPPN---SM
Pera-SKR	10	EMLD-----SEV-----NTSYN-----TS
Nill-CCK-like	1	-----
Denp-CCK-like	1	-----
Nicv-CCK-like	1	-----
Athr-CCK-like	1	-----
Neol-CCK-like	1	-----
Pedh-CCK-like	1	-----M-----
Bacd-CCK-like	7	EYSEKFS--SYSYELKSKSTSPTPTYATGISVTSVEPAAA-LVAAGTL-----
Oocb-CCK-like	7	NLSN-----NPV-----TTEYF-----GA
Drop-CCK-like	121	QLLSALAPLATDAVLQLNGT-----YAAQELYGGSPGG-LGGGGSGSGSGSGMAEQD
Homos-CCKR1	1	-----
Homos-CCKR2	1	-----

Rhodniuspro-SKR1	1	-----M-----RNNTEATVQPKS-TSTT
Rhodniuspro-SKR2	1	-----M-----RNNTEATVQPKS-TSTT
Tricas-SKR1	13	---FGSE-----FALN----SN-----VQISAADFHNFT-TRFN
Tricas-SKR2	1	-----MDWA--ENSTL-----WN-----ISQTLTLLNEVTTPET
Drom-SKR1	61	TSSYGDDDDRDGYMDTEPSDLVTE-----LA-----FSLGTS--SSPSPS
Drom-SKR2	119	----PGDLLN----YSGLELDLGL-DLD-----LNLDMDLATTPSSSTLA-PAVTVRT
Anog-SKR	43	AGDYGDELYGTNLSLALGELLRDNISATVVGTHN----L---SGNGRSAAGNLPP-ATGT
Pera-SKR	24	SVLWGES--VASTPYSLN----TV-----TSGSVSVVTNVS-NISA
Nill-CCK-like	1	-----
Denp-CCK-like	1	-----MFDMNWT--FNESN-----WT-----AA-----T
Nicv-CCK-like	1	-----ME--ANLSF-----LE-----VSNGTLNATSDGDG
Athr-CCK-like	1	-----MKMEKLEG
Neol-CCK-like	1	-----MEKSDS
Pedh-CCK-like	2	ETIYGNDTNIFDYYS--DNLTS-----FS-----LK-----
Bacd-CCK-like	53	SRELLNE----DLNMTVNVENVRL-----ILPQAPSPLTFVFQNESAFGDN
Oocb-CCK-like	21	SRTDDDDLLIITELPQFS----TV-----TSGI-----A-NVTS
Drop-CCK-like	172	VSISGIDLNYNSYNYTGNGLEFAL-EADGLGSRPGFGLLSSTLSAPTANAAA-PS-SNHS
Homos-CCKR1	1	-----MDVVDs-LLVNGSNITPP---CEL--G---LE---NET-LFCL
Homos-CCKR2	1	-----MELLKLNRSVQGTGPGPGASLCRP--GAPLLNSSSVGN-LSCE

Rhodniuspro-SKR1	1	---MLPNESWWEAGKVOIPTYSIIFLLGLVGNILVILVLVKNKGMRTVTNVFLLNLAVSD
Rhodniuspro-SKR2	18	N--TGSDNGSGISEIMPLYMIFILAVVGNSLVILATITRNKRMRMTVTNVVIFENLAVAD
Tricas-SKR1	39	KTRGSGASSGVFESELIIPLYATIFVLSIVGNSLVILVTLVRNKRMRMTVTNVVILLNLAI
Tricas-SKR2	26	ATKTGSVPQWYETGRITIPLYAVIFMLAVIGNTLVILTLVKNQRMRTITNLFLLNLAVSD
Drom-SKR1	98	STPASSSSTSTGMPVWLIPSYSMILLEFAVLGNLVLISITLVQNRMRMTITNVFLLNLAI
Drom-SKR2	162	PGNRSVVRVSADVPPIWVIPCYSAILLCVAVGNLVLVTLVQNRMRMTITNVFLLNLAI
Anog-SKR	95	GTAGSRGGDGTGFQNOIIPLYATIFLLSVVGNLVLILTIAQNKRMRMTVTNVVILLNLAI
Pera-SKR	58	GAGGRGGGGGVFESDLIIPLYVVFVLSIVGNSLVILITIAQNKRMRMTVTNVVILLNLAVSD
Nill-CCK-like	1	-----MSGSWNVQIPLYAGIFLLAVAGNALVILTLVRNOSMRTITNIFLLNLAVSD
Denp-CCK-like	18	APEDPQGGPPWYVTTKIQTITLYATIFLLAVIGNSLIILITIAQNRRMRMTVTNIFLLNLAVSD
Nicv-CCK-like	24	LLGAASVSQWYEMGKVOIPLYTVIFLLAVVGNTLVIITLVQNRMRMTITNIFLLNLAI
Athr-CCK-like	9	--LNATMYSRWDMYVQIPLYSLIFLAGVVGNTLVILTLVQHORMRTVTNVFLLNLAI
Neol-CCK-like	7	SFSTRIANARWDMYVQIPLYSLIFLAGVIGNILVILTLVQHORMRTVTNVFLLNLAI
Pedh-CCK-like	27	-YSKTLLETLLWKNGKWEIPLYSIIFLLAVVGNTLVILTLVKNIRMRMTITNVFLLNLAVSD
Bacd-CCK-like	94	GTSAVTPRISSEIPIWLIIPCYCVIFFAIFGNLVLISITLVQNRMRMTNVFLLNLAI
Oocb-CCK-like	50	PVIRQSSGQASILENIIPLYGTIIFLLSVGNSLVILITIAQNKRMRMTVTNVVILLNLAI
Drop-CCK-like	229	HRSGGVAKVSADVPPIWVIPCYSIILLCAVVGNTLVVTLVQNRMRMTITNVFLLNLAI
Homos-CCKR1	31	D---QPRPSKEWQPAVOILLYSIIFLLSVIGNTLVITVLIRNKRMRMTVTNIFLLSLAVSD
Homos-CCKR2	41	PPRIRGAGTRELELAIRITLYAVIFLMSVGGNMLIIVVLGLSRRIRTVTNAFLLSLAVSD

Rhodniuspro-SKR1	58	ILLGVLCMPFTLVGSLKDKDFVFGHFMCRILIPYMQACSVAVSGWTLVCLSVERYYAICHPL
Rhodniuspro-SKR2	76	ILLGVFCMPFTLIGQLLRNFVFGRIKCLIPYFQAVSVSVAVWTLVAISLERYFAICRPL
Tricas-SKR1	99	LLLGVFCMPFTLVGOVLRNFIFGATMCRILIPYFQAVSVSVGVWTLVAISLERYFAICRPL
Tricas-SKR2	86	LLLGVLCIPFTLIGTLRHRFVFGVMCKLIPFLOACSVSVGVWTLVAISVERYYAICHPL
Drom-SKR1	158	MLLGVLCMPVTLVGTLLRNFIFGEFTCKLIQESQAASVAVSSWTLVAISCERYYAICHPL
Drom-SKR2	222	ILLGVFCMPVTLVGTLLRHFIFGELLCKLIQEAQAASVAVSSWTLVAISCERYYAICHPL
Anog-SKR	155	LLLGVFCMPFTLAGOVLRRFVFGSMCKLIPYFQAVSVSVAVWTLVAISLERYFAICRPL
Pera-SKR	118	LLLGVFCMPFTLVGOVLRNFVFGAAMCKLIPFQAVSVSVGVWTLVAISLERYFAICRPL
Nill-CCK-like	52	LLLGVVCMPTLVGNILRDFVFGDIMCRILIPFLOATSVAVSAWTLVAISVERYYAICHPL
Denp-CCK-like	78	LLLGVLCMPFTLTGYILRDFVFGAAMCKLIPFLOACTVAVSAWTLVAISVERYYAICHPL
Nicv-CCK-like	84	LLLGVLCMPFTLIGALLRDFVFGVMCKLIPFLOACSVSVGVWTLVAISVERYYAICHPL
Athr-CCK-like	67	IMLGVLCMPFTLVGAILRDFVFGVMCKLIPYLOACTVSVSAWTLVAISVERYYAICHPL
Neol-CCK-like	67	IMLGVLCMPFTLVGAILRDFVFGVMCKLIPYLOACTVSVSAWTLVAISVERYYAICHPL
Pedh-CCK-like	86	LILAVLCMPFTLIGTLRDRFVFGETMCKLIPYLOATSVAVSVWTLVAISLERYYAICHPL
Bacd-CCK-like	154	MLLGVLCMPITLVGTLLRHFIFGEFTCKLIQESQAGSVAVSSWTLVAISCERYYAICHPL
Oocb-CCK-like	110	LLLGVFCMPFTLIGQILKNFVFGLTMCKLIPYFQAVSVSVGVWTLVAISLERYFAICRPL
Drop-CCK-like	289	ILLGVFCMPVTLVGTLLRHFIFGEFTCKLIQEAQAASVAVSSWTLVAISCERYYAICHPL
Homos-CCKR1	88	IMLCIFCMPFNLIIPNLLKDFIFGSAVCKTTTYFMGTSVSVSTENLVAISLERYGAICKPL
Homos-CCKR2	101	LLLAVACMPFTLIPNLMGTIFFGTVICKAVSYLMGVSVSSTLSLVAIALERYSAICRPL

Rhodniuspro-SKR1	118	RSRTWQTLTTHAYRLIGAIWVCSLLIMTPISVISELIPTS-G---GHRKCRELWPN--EDI
Rhodniuspro-SKR2	136	KSRRWQTQFHAYKMIAIVWAMSLVWNSPILFVSRLLAMG-GK--GRHKCREVWPG--RRS
Tricas-SKR1	159	KSRRWQTQFHAYKMIAVWVLASIFWSAPVLAVSSIKAMK-GR---GHKCREEWPS--KSS
Tricas-SKR2	146	RSLRWQTITSHAYKLIVGIWIGSLICMAPIALLSQLKPTK-Q---GNYKCREDWPS--LDY
Drom-SKR1	218	RSRSWQTITSHAYKLIIGFIWLGGLICMTPIAVFSQLIPTS-RP--GYCKCREFWPD--QGY
Drom-SKR2	282	RSRTWQTINHANKIIAIIWLGSLVCMTPIAAFSOLMPTS-RP--GLRKCREQWPADSLNY
Anog-SKR	215	SSRRWQTQFHAYKMIGLVWTVSFLANSPLGYVQRLIPVGRST--GOMKCREEWPS--PAW
Pera-SKR	178	KSRRWQTQFHAYKTIVVWVLASLIWNSPIFVVSRLQAIAK-ET--ERHKCREDWPS--KSS
Nill-CCK-like	112	RSRRWQTITSHAYRLIAVIWLASFVTMLPISLISRLIPTN-Q---GLKKCREIWPD--ADY
Denp-CCK-like	138	RSLAWQTITSHAYKTILGIWICSFICMLPIAFLSELKPTI-K---GNRKCRENWAS--LEY
Nicv-CCK-like	144	RSLRWQTITSHAYKLIITAIWAGSVICMGPIALLSQLKPTN-Q---GNHKCREDWHT--LDY
Athr-CCK-like	127	RSRRWQTITSHSYRLIAVIWTGSLILMSPIAVLSELKPTS-N---GHHKCRENWPGH-GEY
Neol-CCK-like	127	RSRRWQTITSHSYRLIAVIWTGSLILMSPIAVLSELKPTS-N---GHQKCRENWPGH-GEY
Pedh-CCK-like	146	RSRRWQTITSHAYHLICYIWTGSTITMLPIFILTELQPTN-K---GRHKCREKWPN--GDY
Bacd-CCK-like	214	RSRTWQTINHAYRIIGFIWFGSLICMTPIALFSQLIPTS-RQ--GLRKCRDQWPEDTIAY
Oocb-CCK-like	170	KSRRWQTQFHAYKMIAVWVTLISLTWNMPILVVSRLKSLR-G---GRRKCREEWPS--VGS
Drop-CCK-like	349	RSRTWQTINHANKIIAIIWLGSLICMTPIALFSQLMPTS-RP--GLRKCREQWPADSLNY
Homos-CCR1	148	QSRVWQTKSHALKVIAATWCLSFITIMTPYPIYSNLVPFTKNNQTANMCRFLIPN--DVM
Homos-CCR2	161	QARVWQTRSHPARVIVATWLLSGLILMVPYPVYTVVQPVGPR---VLQCVHRWPS--ARV

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Rhodniuspro-SKR1	172	EKTYNLLLDLFIILLVPLIVMVTYTLVAKTLWRVMKTKQKPGNEMGLKD-----
Rhodniuspro-SKR2	191	EGAYITFLDIVLLMIPLLIMSLAYSILVLKLWKGQLRELKHSNSCLQTVDR-SASLPTMT
Tricas-SKR1	213	EQIENLFLDAMLILLIPVLIMSLAYSILMTKLWKGRLREIQHNNSSFQAQMIQRSNSSPTIN
Tricas-SKR2	200	EKAYNLFLDVLLVPLLVGLVGTYSLITRTLCCKGMKTERALRDHTVNGAVDVYINL----
Drom-SKR1	273	ELEYNILLDFILLVPLLVLCVAYILITRTLYVCGMAKDSGRILQQSLPVSATTAGG-SAP
Drom-SKR2	339	ERAYNLFLDLIALLVPLLLALSFTYLFITRTLYVSMRNERAMNFGSSGPEVTTSSSA-AVA
Anog-SKR	271	EKAYVLFHDMGLLFLPLLLTMGFAYSMIVSKLWRGLRHEIKHSSLYQQTSRQHGTGGQGSS
Pera-SKR	233	ERAYNIFLDAMLILLVPLIIMTLAYSILTVSKLWKGRLREIRHNSSCRRQLER-TSSSATVN
Nill-CCK-like	166	ERCYNMLDLAVLLVPLFLFILIVTYSLITITLWRGIRPSNQSGQELMNNGCISHIEV----
Denp-CCK-like	192	EKAENIVLDIVLMVFPLLVLAATYSLIIRTTLWKGIKTERASRSTGSTM--EIYVNL----
Nicv-CCK-like	198	EKAENIFLDVLLVPLFVLAVTYSLITINTLCKGMQTERTLRDSGAGGVVEVYINM----
Athr-CCK-like	182	EKAYNLILDVALLIFPLVILVTYTLITITPLQSSVDPAVVRHSANGSVVSEVYLGS----
Neol-CCK-like	182	EKAYNLILDVALLIFPLIILVTYTLITITPLQSSVDPT-----VYLGS----
Pedh-CCK-like	200	ERIYNLLLDLILLLLIPLIALGVTYYLISITLWRDL SKKSEDVSYQRHEENSKREKN----
Bacd-CCK-like	271	ERFYNIFLDITLLVPLFVLVCVAYILITRTLYVGMRAERALVLGGNSTNGSTPAKQ----
Oocb-CCK-like	224	ERAYNLFLDGTLLLVPLLIMSLAYSILIAIKLWRGLKLEIRQSSTRTKRHLS-----
Drop-CCK-like	406	ERAYNLFLDLIALLVPLLLALSFTYLFITRTLYVSMRNERAMNFGSSGPE-----
Homos-CCR1	206	QQSWHTFLLLILFLIPGVMMVAYGLISLELYQGKFEASQKKSARKERP-----
Homos-CCR2	215	RQTWSVLLLLILFFIPGVMMVAVAYGLISRELYLGVRFDDGSDSDSQSRVRNQG-GL---P

Rhodniuspro-SKR1	220	-----
Rhodniuspro-SKR2	250	EVVISKN-LNTN-SEAI-----HRVI-----PAD
Tricas-SKR1	273	GELNKSTSPQSTSEPSGMN-HLRPT-----NRLL-----PPSHNKAHSRV
Tricas-SKR2	256	-HG---S-----STT-----SRWSK-----
Drom-SKR1	332	NPG---T---SSSSNCILVLTAT-----AVYNENSN-----
Drom-SKR2	398	EAG---SQRRANGSHCQSLDTIVPHQHNPQQHHHHSQYYYDYGHGCGSKRRLISGGGPCE
Anog-SKR	331	-VG---GAPTG--AAAGSV-GT-----ASGEQHCT
Pera-SKR	292	EVVMATSNINGN-STTGSA-TITPL----RQHRLKQQQM-----LNRQSAGSTTLCA
Nill-CCK-like	222	-TR---S-----SSS-----FK-----
Denp-CCK-like	246	-QM---N-----SRT-----SWRIKNK-----
Nicv-CCK-like	254	-QS---T-----SS-----KRWSR-----
Athr-CCK-like	238	-RG---D-----TLT-----R---CSL-----
Neol-CCK-like	225	-RG---D-----TLT-----R---CSL-----
Pedh-CCK-like	256	-SS---Y-----DLS-----DEYNPNNN-----
Bacd-CCK-like	327	--H---D-----
Oocb-CCK-like	275	-----QGPR-----IQI-----
Drop-CCK-like	455	-----
Homos-CCR1	256	-----S-----
Homos-CCR2	271	GAV-----HQ-----NGRCR-----

Rhodniuspro-SKR1	220	-----
Rhodniuspro-SKR2	272	SQQGQFCNKNPVMWLLKVKLEE-----
Tricas-SKR1	312	KDAKHSKKTESVKMWMFMKGIVQVRLPASIKKGYTCNTQTKSTLVPRCELTT---PSS-EH
Tricas-SKR2	267	-----
Drom-SKR1	357	-----
Drom-SKR2	455	GRRHLYC-----MRS-----ASVK-----
Anog-SKR	354	GDTGKPPKPPGG-----KRHEPG-FVLTSSLKKAPFKSSTAV
Pera-SKR	339	GRDGN NRAATSVKLWLFKGMVQVRLPSSSSNAQSR YRERPGSLSSSKSVTIAPLP TATS A
Nill-CCK-like	230	-----
Denp-CCK-like	259	-----
Nicv-CCK-like	264	-----
Athr-CCK-like	248	-----
Neol-CCK-like	235	-----
Pedh-CCK-like	270	-----
Bacd-CCK-like	329	-----
Oocb-CCK-like	282	-----
Drop-CCK-like	455	-----
Homos-CCKR1	257	-----TTSS-----
Homos-CCKR2	281	-----PETG-----

Rhodniuspro-SKR1	220	-----NRV-T-W--KQN--
Rhodniuspro-SKR2	295	-----GIAQR-----SGTP
Tricas-SKR1	368	CSY-----NELCPS-----NDASY
Tricas-SKR2	267	-----RHNP NWRQLRH-N-W--SQESS
Drom-SKR1	357	-----NNNGNSEGSAGGGSTNMATTTLTTRPTAPT VITT-T-T-TTTVT L
Drom-SKR2	469	-----SLRHQQINGGGGTLSGTGAGNGECCS-RVHRMR-QQMQLQQQGYVSDNESRR
Anog-SKR	390	VNFASNNTINKSNFSGAG--SGSSSNGAGSSGSS--NGS-----NGGGCNGSGAD
Pera-SKR	399	GTYN TS---PETSRLIG-----SSNGNGAACRDDKDLGG-----AGATVDGEDVN
Nill-CCK-like	230	-----FNS
Denp-CCK-like	259	-----TN-----MSRQQSNWTT-LR-N-W--SDES N
Nicv-CCK-like	264	-----TSRGTSLRQLRQ-N-W--SQDST
Athr-CCK-like	248	-----RT-----LERTPSSRRSLRL-V-TKPSLNAV
Neol-CCK-like	235	-----RT-----LDRTPSSRRSLRL-V-AKSSVGGM
Pedh-CCK-like	270	-----NN-----IT-TGRNKKK LKR-E-I ILNDVTK
Bacd-CCK-like	329	-----NSSL-----GAI
Oocb-CCK-like	282	-----RANGSGCLTRETKDNSN-----E---D-Q---
Drop-CCK-like	455	-----
Homos-CCKR1	261	-----GKYEDSDG CYLQKTRPP---RKLEL-----
Homos-CCKR2	285	-----AVGEDSDG CYVQLPRSR---PALEL-----

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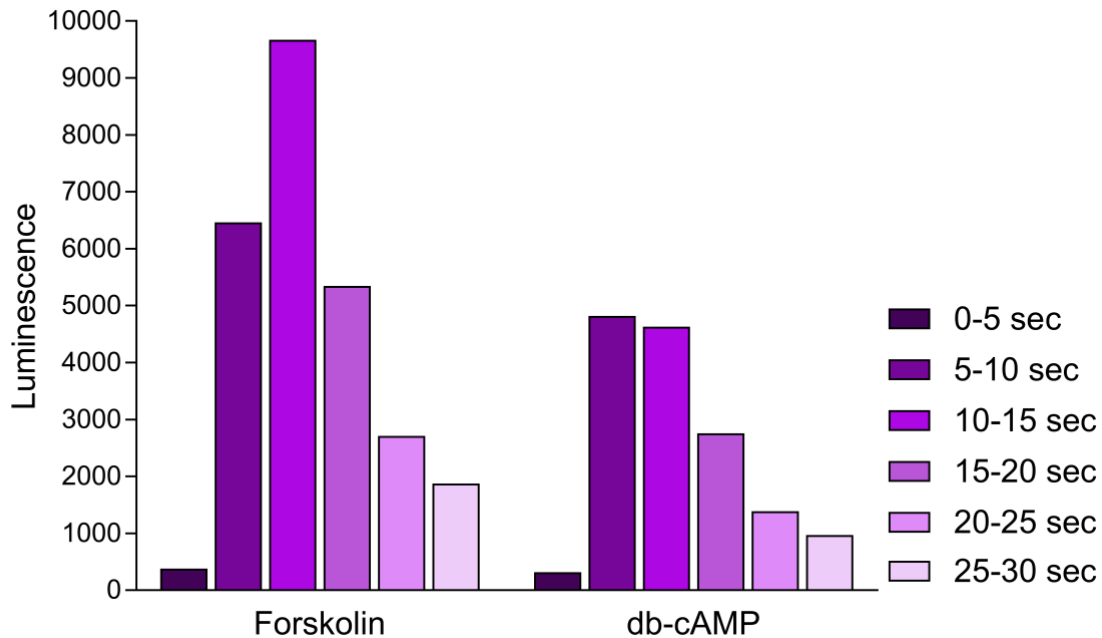
Rhodniuspro-SKR1	228	-----SRGSPHLRRSNT EKALKKKKRVVKMLFAVVLEFFVCWTPLYVINTITL F
Rhodniuspro-SKR2	304	LEPL-ESPGPK-FTRHAIRS NYMDKSI EAKKKVIRMLFVVVAEFFFCWAPLHVINTWYQ F
Tricas-SKR1	382	ATSSVDETTYH-FTRHAIRS NYMDKSI EAKKKVIRMLFVVVAEFFFCWAPLHVINTWYLF
Tricas-SKR2	285	S-PGTGS---QKYTPGLRR TN AERSLLNKKRVIKMLFAVVLEFFFCWTPLYVINTIVLF
Drom-SKR1	399	A-KTSSP--SIRVHDAALRRSNEAKTLESKKRVVKMLFVIVLEFFFCWTPLYVINTMVML
Drom-SKR2	519	K-SLSQP--SLRITEAGLRRSNETKSLESKKRVVKMLFVIVLEFFFCWTPLYVINTMTML
Anog-SKR	437	Q----RTHYCGGAGCETRPGR LRGRHF FFAK VIRMLFVIVLEFFFCWAPLHVINTVYLY
Pera-SKR	441	IPPGESPNTYT-FTRHAIRS NYMDKSI EAKKKVIRMLFVVVAEFFFCWAPLHVINTWYLF
Nill-CCK-like	233	T-VGTTS---SSSPGLRR TNTEKALLKKKRVIKMLCAVVLEFFFCWTPLYVINTVTLF
Denp-CCK-like	280	S-PVGSN--SSKRLVSGLRRTNAERSLCNKKRVIKMLCVVLEFFFCWSPLYTINTIVLF
Nicv-CCK-like	283	S-PIGS-----QRTGLRRSNAERSL TNKKRVIKMLFAVVLEFFFCWTPLYVINTIVLF
Athr-CCK-like	272	K-PWDES---DLSQHTTRGRSALLKRKRKRRI VEMLCVVVLEFFFLFWTPLYVINTVALF
Neol-CCK-like	259	K-PWDDQ---GEASQRSTRGRSAMLKRKRKRRI VEMLCVVVLEFFFLFWTPLYVINTVALF
Pedh-CCK-like	293	L-EVFQS--IKSCSSISLRHN NYERSLRKKQLVIKMLFVVVLEFFFCWTPLYVINTIALF
Bacd-CCK-like	336	A-TV-TK--STAAAPSATLQSNEAKNLESKKRVVKMLFVIVLEFFFCWTPLYVINTISMF
Oocb-CCK-like	302	----QNSICPL-SRQHVIRSNYMGKSI EAKKKVIRMLFVIVLEFFFCWAPLHVINTWYLF
Drop-CCK-like	455	-----SKKRVVKMLFVIVLEFFFCWTPLYVINTMTML
Homos-CCKR1	283	----RQLSTGSSSRANRIRSNSSAANIMAKKRVIRMLTIVIVLFFLCWMPHISANTWRA Y
Homos-CCKR2	307	----TALTAPGPGS----GSRPTQAKLLAKKRVIRMLTIVIVLFFLCWLPVYSANTWRA F

Rhodniuspro-SKR1	277	APQAVYE-RLGYKGISFLQLLAYS	SSCCNPITYCFMNYRFRRAFLKLEFG--CLREEKGSS
Rhodniuspro-SKR2	362	RPDLVHQ-YVGSTGVSLVQLLAYI	SSCCNPITYCFMNYRFRQAFISLENFPR----LCC-
Tricas-SKR1	441	YPEDVYL-YVGSTGISLVQLLAYI	SSCCNPITYCFMNRKFRQAFLAIFNWNVC--YCC-
Tricas-SKR2	340	DSSVIYN-NIGYKAITFFQLLAYC	SSCCNPITYCFMNCGRKSFLENLEK--CLKSRN--
Drom-SKR1	456	IGPVVYE-YVDYTAISFLQLLAYS	SSCCNPITYCFMNASFRRAFVDTHKGLPWRGAGAS
Drom-SKR2	576	LGPTVYE-YVGYTISIFLQLLAYS	SSCCNPITYCFMNASFRRAFVDTHKGMRVCKERLCA-
Anog-SKR	492	SPTFVYQ-YVNSSGIALVQLMAYI	SSCCNPITYCFMNRFRQAFLGVEFSCYRNMPICCC
Pera-SKR	500	NPEAVYS-TVGSTGVSLVQLLAYI	SSCCNPITYCFMNRKFRQAFLGVEDCYRCWSMYCCC
Nill-CCK-like	288	DKYIVYN-ALSEQAISFFQLLAYS	SSCCNPITYCFMNSSFRKAFLKLEFG--CLRGRPKT
Denp-CCK-like	337	TGQYVYD-TIGYTGISLLQLLAYT	SSCCNPITYCFMNGGRKSFLEKLES--CFKRSRYRA
Nicv-CCK-like	335	DAEIVYN-NLGYTVIGYLQLLAYS	SSCCNPITYCFMNCGRKSFMLEFR--CHREAQSNK
Athr-CCK-like	327	NPDALYD-GLTPTWISYFHLLAFC	SSCCNPITYCFMSSGRKSFSGLEFA--CCRDGCRYR
Neol-CCK-like	315	SPDALYD-GLNPTWISYFHLLAFC	SSCCNPITYCFMSSGRKSFSGLEFTGCCSRACGCRK
Pedh-CCK-like	350	WPHLVYRGKIGYKMISFCQLLAYT	SGCCNPITYCFMNGKFRTWKKKKEQ-----
Bacd-CCK-like	392	IGPAIYE-YIDYTSICFFQLLAYS	SSCCNPITYCFMNASFRRAFLDTHKGLRMSSVTSRR
Oocb-CCK-like	357	APDLVYS-VVGSTGISLVQLLAYV	SSCCNPITYCFMNNKFRQAFLEFDCHRCWRVSCRH
Drop-CCK-like	487	IGPVVYE-YVDYTAISFLQLLAYS	SSCCNPITYCFMNASFRRAFVDTHKV-RMCD--GG-
Homos-CKR1	339	DTASAER-RLSGTPISFILLISYTS	SSCCNPITYCFMNRKFRLEGMATE--PCCPNPGPPGA
Homos-CKR2	359	DGPGAHR-ALSGAPISFIHLLSYAS	ACVNPITYCFMHRRFRQACLETCCARCCP--PPRARP

Rhodniuspro-SKR1	334	-----	-----
Rhodniuspro-SKR2	416	WCGIPVES---KLA-----	QRTDTANEPNSLSANDSTLYAGRAN---RSEV
Tricas-SKR1	497	VCMEPKSH---R-TRTKTAQKNGI----	HKIIQNNSDVSCNESTIYIGRQSTIGRS-V
Tricas-SKR2	395	F-----GVTGSEINMETKWTN-R-----	CSENA-----
Drom-SKR1	515	G-----GVGGA-AGGGLSASQAG-----	AGPGAYASANTNIS-----LNPLG-A
Drom-SKR2	634	-----PCCFWRRRSKNE-----	TNLSVAGNSIALANS-V
Anog-SKR	551	FCCASSNGPMEGAESKAAAYLRQNTI--	NQSGAERNNSDMSGNDSLVIYVGRGLVQRSG-K
Pera-SKR	559	CCGDVVAL---RSSRLGGLQRDGAA--	LGGLGANNSDVSGNDSTVFAGRASVGARSEV
Nill-CCK-like	345	H-----F-----	-----
Denp-CCK-like	394	N-----FRMEGSDCQVDLKYSNQR-----	CSEN-----
Nicv-CCK-like	392	S-----LLNGSEFNMDTKCAI-R-----	NEPL-----
Athr-CCK-like	384	S-----GGG-RYR-----	EPP-----
Neol-CCK-like	374	R-----AEG-RYR-----	EPP-----
Pedh-CCK-like		-----	-----
Bacd-CCK-like	451	V-----GEAGFSGWVKRRRRRTTGGGDS	VSCTQLPALGTNNSISLTNNTIIVPNI-N
Oocb-CCK-like	416	ND--V-AM---ATTA-----	NAGQTGNNSELSGNDSAMYLGASLVARSIGI
Drop-CCK-like	542	-----RLCFWRRRSKNE-----	TNLSVAGNSIALANS-V
Homos-CKR1	397	R-----G-----EV--GEE-----	EEGGTTGASLSRFSYSHMSASVPPQ--
Homos-CKR2	418	R-----AL--PDE-----	DPPTPSIASLSRLSYTTISTLPG---

Rhodniuspro-SKR1		-----	
Rhodniuspro-SKR2	456	MVLEKEERV-----	
Tricas-SKR1	546	VVLEAEDRV-----	
Tricas-SKR2	418	-----FCR-----	
Drom-SKR1	552	M---GMGTWRSRSRHEFLNAVVTNSAAA	AVNSPQL-
Drom-SKR2	662	M---SSHTI-----	LESPRL-
Anog-SKR	608	LCARRSGTYPRYYRDAVCNALP	PWDLKM-----
Pera-SKR	613	VVLEAEDRV-----	
Nill-CCK-like		-----	
Denp-CCK-like		-----	
Nicv-CCK-like		-----	
Athr-CCK-like		-----	
Neol-CCK-like		-----	
Pedh-CCK-like		-----	
Bacd-CCK-like	501	T---AMHN-----	SQTPQSN
Oocb-CCK-like	456	IILLN-----	
Drop-CCK-like	570	M---SSHTI-----	LESPRL-
Homos-CKR1		-----	
Homos-CKR2		-----	

Supplementary Figure S4. Luminescence response of forskolin and dibutyryl cAMP (dbcAMP) for Rhopr-SKR-1 transiently expressed in HEK293/CNG cells. Figure displays kinetics of response activation at 5-second intervals for a total of 30 seconds.



Supplementary Tables

Supplementary Table S1. Primer sequences utilized to generate the complete Rhopr-SKR-1 and Rhopr-SKR-2 sequences (5' to 3').

pDNR (Containing cDNA Library) Plasmid-Specific Primers	
pDNR-For1	GTGGATAACCGTATTACCGCC
pDNR-For2	ACGGTACCGGACATATGCC
pDNR-Rev25	GTGGATAACCGTATTACCGCC
Rhopr-SKR-1 5' Region Amplification	
SKR1-Rev1	CGAAATCTTTTAGTAAGGATCCA
SKR1-Rev2	ACACATTTGTTACAGTTCTCATTCC
SKR1-Rev3	CCAGGCCAAGTAAAAAGATGATT
Rhopr-SKR-1 3' Region Amplification	
SKR1-For1	GAAGCTGGTAAAGTACAAATTCC
SKR1-For2	GCAGTTTCTGACATATTACTTGG
SKR1-For3	CTAAAAGATTTCGTGTTTGGAC
Rhopr-SKR-2 5' Region Amplification	
SKR2-For1	TGGTAACAGTGCGCATTCT
SKR2-For2	GCCAAACTAAGGAATTACCAAC
SKR2-For3	CCACTGCCATTATCACTGC
Rhopr-SKR-2 3' Region Amplification	
SKR2-Rev1	TGTCTTTAGTGTGGAATTCGC
SKR2-Rev2	AGCGAAGGAGCGTATATAATATTTC
SKR2-Rev3	GTCCTGTTGATGATTCCGTTAT

Supplementary Table S2. Primers used for the insertion of Rhopr-SKR-1 and Rhopr-SKR-2 into the mammalian expression vector pIRES2-ZsGreen1 with the restriction sites bolded and the kozak sequences underlined (5' to 3').

Rhopr-SKR-1	
Rhopr-SKR-1 Fwd	TACGCAG <u>AGCTCGCCACC</u> ATGTTGCCAAATGAATCTTG
Rhopr-SKR-1 Rvrs	TCAGCTTGATCCTTTTTCTT CCCGCGG TTATTA
Rhopr-SKR-2	
Rhopr-SKR-2 Fwd	ATTATAG <u>AGCTCGCCACC</u> ATGCGGAACAACACTGAA
Rhopr-SKR-2 Rvrs	CTAAACTCGCTCTTCCTTTTCTA CCGCGG TTATTA

Supplementary Table S3. Primer sequences utilized to measure spatial distribution of the transcripts for the Rhopr-SKRs and Rhopr-SKs via Reverse transcriptase quantitative PCR (RT-qPCR), as well as those utilized for the housekeeping genes (5' to 3').

Rhopr-SKR-1	
Rhopr-SKR-1 qPCR Fwd	GAAGCTGGTAAAGTACAAATTC
Rhopr-SKR-1 qPCR Rvrs	CGAAATCTTTTAGTAAGGATCCA
Rhopr-SKR-2	
Rhopr-SKR-2 qPCR Fwd	TCCAAGCTGTTTCAGTATCAGTAG
Rhopr-SKR-2 qPCR Rvrs	GCGAATTCCACACTAAAGACA
Rhopr-Housekeeping Genes	
α -TUB Fwd	GTGTTTGTTGATTGGAACCTACAG
α -TUB Rvrs	CCGTAATCAACAGACAATCTTTCC
β -Actin Fwd	AGAGAAAAGATGACGCAGATAATGT
β -Actin Rvrs	ATATCCCTAACAATTTCACGTTCCG
Rp-49 Fwd	GTGAAACTCAGGAGAAATTGGC
Rp-49 Rvrs	AGGACACACCATGCGCTATC
Rhopr-SK (peptides' transcript)	
Rhopr-SK qPCR Fwd	AGCCAGCTGAAAGGAGATCA
Rhopr-SK qPCR Rvrs	GGTAAAGAATTGGCCATGGTCT

Supplementary Table S4. Primer sequences utilized to generate double stranded RNA templates of Rhopr-SKR-1, Rhopr-SKR-2, and Rhopr-SK, or the ampicillin resistance gene (ARG) (5' to 3').

Rhopr-SKR-1	
T7 Rhopr-SKR-1 Fwd	TAATACGACTCACTATAGGGAGAGAAGCTGGTAAAGTACA AATTC
Rhopr-SKR-1 Rvrs	CGAAATCTTTTAGTAAGGATCCA
Rhopr-SKR-1 Fwd	GAAGCTGGTAAAGTACAAATTC
T7 Rhopr-SKR-1 Rvrs	TAATACGACTCACTATAGGGAGACGAAATCTTTTAGTAAG GATCCA
Rhopr-SKR-2	
T7 Rhopr-SKR-2 Fwd	TAATACGACTCACTATAGGGAGATCCAAGCTGTTTCAGTAT CAGTAG
Rhopr-SKR-2 Rvrs	GCGAATTCCCACTAAAGACA
Rhopr-SKR-2 Fwd	TCCAAGCTGTTTCAGTATCAGTAG
T7 Rhopr-SKR-2 Rvrs	TAATACGACTCACTATAGGGAGAGCGAATTCCCACTAAA GACA
Rhopr-SK (SK peptides)	
T7 Rhopr-SK Fwd	TAATACGACTCACTATAGGGAGAATAATGGGTAGCAGCTTCC
Rhopr-SK Rvrs	GCATACACTCCACTTATAATTCCTCTAATTTAT
Rhopr-SK Fwd	TAATACGACTCACTATAGGGAGAGCATACACTCCACTTATAA TTCC
T7 Rhopr-SK Rvrs	ATAATGGGTAGCAGCTTCCTAATCAC