

# Supplementary

## Signal transduction by VIP and PACAP receptors

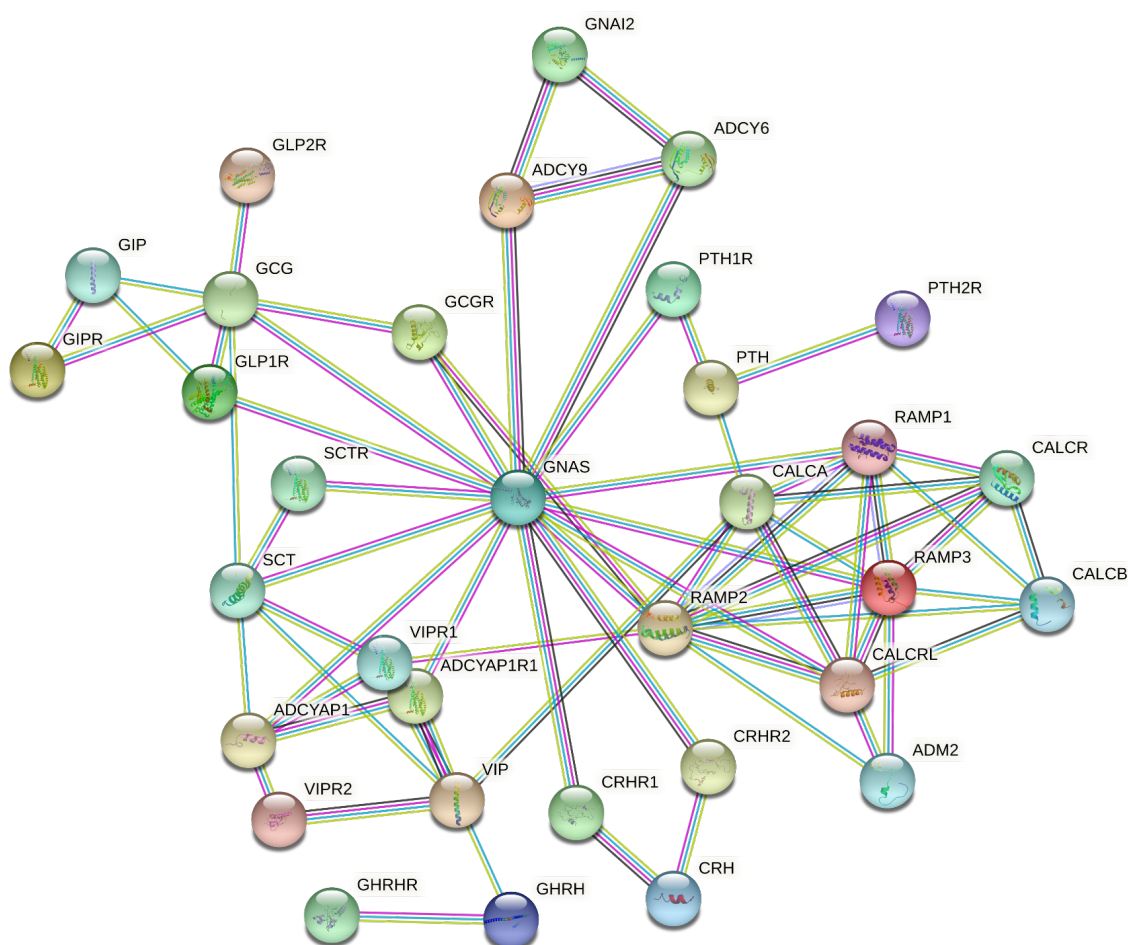
Ingrid Langer<sup>1</sup>, Jérôme Jeandriens<sup>1</sup>, Alain Couvineau<sup>2</sup>, Swapnil Sanmukh<sup>3</sup>, Dorota Latek<sup>3,\*</sup>

<sup>1</sup>Institut de Recherche Interdisciplinaire en Biologie Humaine et Moléculaire (IRIBHM),  
Université libre de Bruxelles, B-1070 Brussels, Belgium

<sup>2</sup> Université de Paris, UMR 1149 Inserm - Centre de Recherche sur l'Inflammation (CRI),  
75018 Paris, France

<sup>3</sup>Faculty of Chemistry, University of Warsaw, 02-093 Warsaw, Poland

**Figure S1.** Network of all class B receptors and their peptide endogenous agonists. This network of associations in Homo Sapiens was obtained from Szklarczyk et al. (the highest confidence level setting). Close associations between: VIP and GHRH; SCT and PACAP (ADCYAP1) and VIPR1; VIP and CALCA were detected.



## Nodes:

Network nodes represent proteins  
splice isoforms or post-translational modifications are collapsed, i.e. each node represents all the proteins produced by a single, protein-coding gene locus.

## Node Color

colored nodes:  
query proteins and first shell of interactors  
white nodes:  
second shell of interactors

## Node Content

empty nodes:  
proteins of unknown 3D structure  
filled nodes:  
some 3D structure is known or predicted

## Edges:

Edges represent protein-protein associations  
associations are meant to be specific and meaningful, i.e. proteins jointly contribute to a shared function; this does not necessarily mean they are physically binding to each other.

## Known Interactions

from curated databases  
experimentally determined

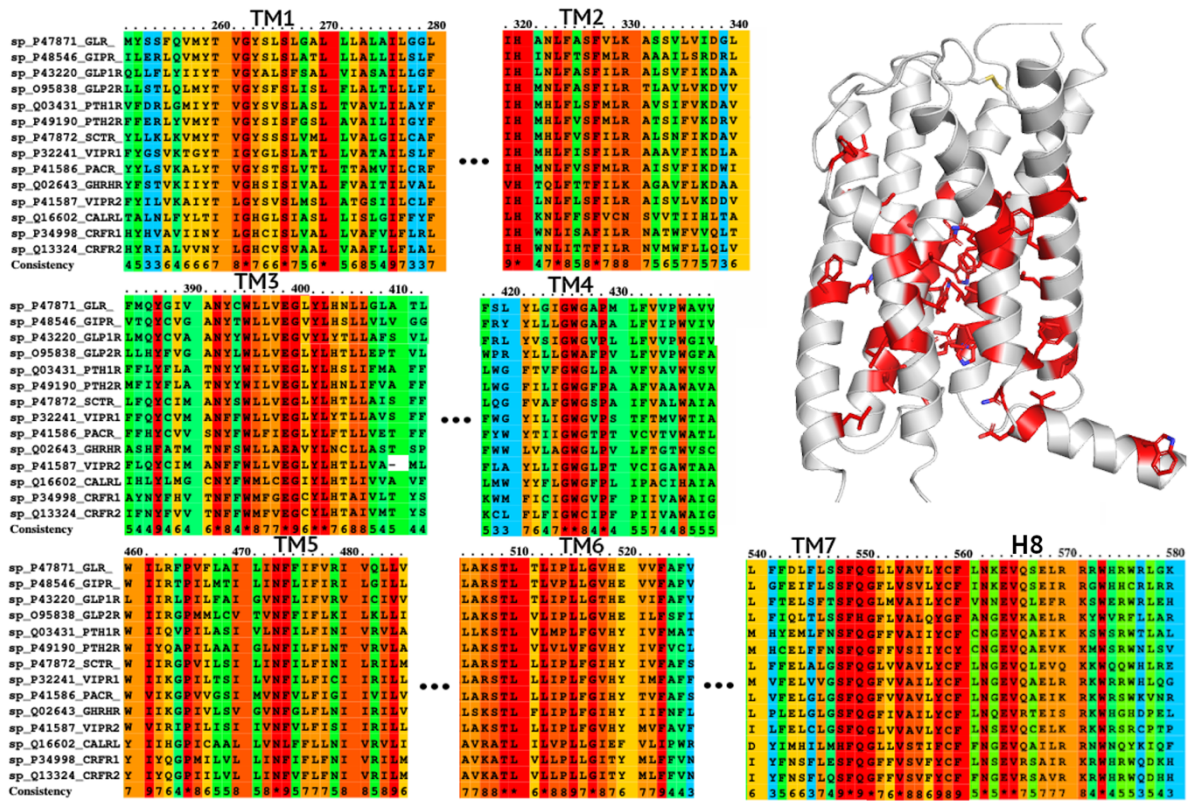
## Predicted Interactions

gene neighborhood  
gene fusions  
gene co-occurrence

## Others

textmining  
co-expression  
protein homology

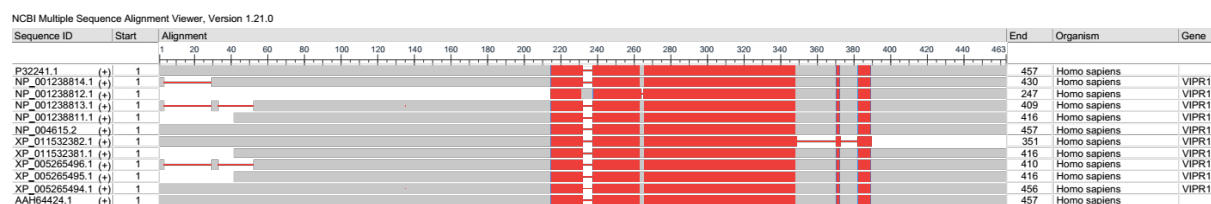
**Figure S2.** Sequence variation in TM helices of class B GPCRs. Amino acids were marked from the least conserved among class B GPCR sequences (blue) to the most conserved (red). The most conserved amino acids were marked in the right panel representing an inactive conformation of VPAC1 based on 5VEW.



**Figure S3.** Co-occurrence of class B GPCRs and their peptide agonists in various species obtained from Szklarczyk et al. 2021.

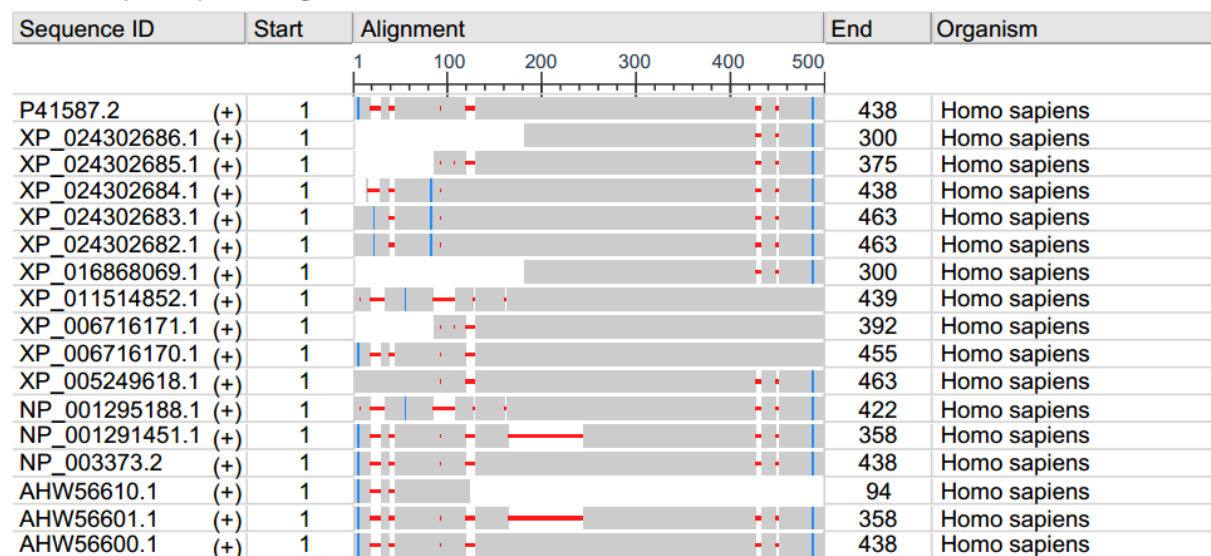


**Figure S4.** Isoforms of *VPAC1* deposited in NCBI.



**Figure S5.** Isoforms of *VPAC2* deposited in NCBI.

NCBI Multiple Sequence Alignment Viewer, Version 1.21.0



**Figure S6. Multiple sequence alignment (MSA) of five VPAC1 sequences deposited in Uniprot.**

<u>P32241</u>	VIPR1_HUMAN	1	MRPPSPLP-----ARWLCLVLAG---ALAWAL-----GPA---G-----	27
<u>P32241-2</u>	VIPR1_HUMAN	1	MPPPLLSLRRLGGGSVAVTRLVVAAAGARSRGGRGSGRAGGGGRRGVARRRRLLELRAA	60
<u>P32241-3</u>	VIPR1_HUMAN	1	-----	0
<u>P32241-4</u>	VIPR1_HUMAN	1	-----	0
<u>P32241-5</u>	VIPR1_HUMAN	1	-----	0
<u>P32241</u>	VIPR1_HUMAN	28	--GQAARLQEECDYVQMIIEVQHKQCLEEAQLENETIGCSKMWDNLTCPATPRGQVVVLA	85
<u>P32241-2</u>	VIPR1_HUMAN	61	RSLGSSSLQEECDYVQMIIEVQHKQCLEEAQLENETIGCSKMWDNLTCPATPRGQVVVLA	120
<u>P32241-3</u>	VIPR1_HUMAN	1	-----	0
<u>P32241-4</u>	VIPR1_HUMAN	1	-----MRAGRRPRLGFWAGGCSKMWDNLTCPATPRGQVVVLA	38
<u>P32241-5</u>	VIPR1_HUMAN	1	-----MIEVQHKQCLEEAQLENETIGCSKMWDNLTCPATPRGQVVVLA	44
<u>P32241</u>	VIPR1_HUMAN	86	CPLIFKLFSSIQGRNVSRSCDTDEGWTHTLEPGPYPIACGLDDKAASLDEQQTMYFGSVKGTG	145
<u>P32241-2</u>	VIPR1_HUMAN	121	CPLIFKLFSSIQGRNVSRSCDTDEGWTHTLEPGPYPIACGLDDKAASLDEQQTMYFGSVKGTG	180
<u>P32241-3</u>	VIPR1_HUMAN	1	-----	0
<u>P32241-4</u>	VIPR1_HUMAN	39	CPLIFKLFSSIQGRNVSRSCDTDEGWTHTLEPGPYPIACGLDDKAASLDE-QTMYFGSVKGTG	97
<u>P32241-5</u>	VIPR1_HUMAN	45	CPLIFKLFSSIQGRNVSRSCDTDEGWTHTLEPGPYPIACGLDDKAASLDEQQTMYFGSVKGTG	104
<u>P32241</u>	VIPR1_HUMAN	146	YTIGYGLSLATLLVATAILSLFRKLHCTRNYIHMHLFISFILRAAAVFIKDLALFDSGES	205
<u>P32241-2</u>	VIPR1_HUMAN	181	YTIGYGLSLATLLVATAILSLFRKLHCTRNYIHMHLFISFILRAAAVFIKDLALFDSGES	240
<u>P32241-3</u>	VIPR1_HUMAN	1	-----	0
<u>P32241-4</u>	VIPR1_HUMAN	98	YTIGYGLSLATLLVATAILSLFRKLHCTRNYIHMHLFISFILRAAAVFIKDLALFDSGES	157
<u>P32241-5</u>	VIPR1_HUMAN	105	YTIGYGLSLATLLVATAILSLFRKLHCTRNYIHMHLFISFILRAAAVFIKDLALFDSGES	164
<u>P32241</u>	VIPR1_HUMAN	206	DQCSEGSVGCKAAMVFFQYCVCM-----ANFFWLLVEGLYLYTLLAVSFFSERKYFWGYI	259
<u>P32241-2</u>	VIPR1_HUMAN	241	DQCSEGSVGCKAAMVFFQYCVCM-----ANFFWLLVEGLYLYTLLAVSFFSERKYFWGYI	294
<u>P32241-3</u>	VIPR1_HUMAN	1	-----MTRQRVWMRWAVRQPWSFSNIVSWLTSSGCWWRASCTCPCLPSPSSLS--G	49
<u>P32241-4</u>	VIPR1_HUMAN	158	DQCSEGSVGCKAAMVFFQYCVCM-----ANFFWLLVEGLYLYTLLAVSFFSERKYFWGYI	211
<u>P32241-5</u>	VIPR1_HUMAN	165	DQCSEGSVGCKAAMVFFQYCVCM-----ANFFWLLVEGLYLYTLLAVSFFSERKYFWGYI	218
			. *:::.* . ** .* : : . . : . . :	
<u>P32241</u>	VIPR1_HUMAN	260	LIGWGPSTFTMVWTIARIHFEDYGCWDTINSSLWMIKGPILTSILNVFILFICIRIL	319
<u>P32241-2</u>	VIPR1_HUMAN	295	LIGWGPSTFTMVWTIARIHFEDYGCWDTINSSLWMIKGPILTSILNVFILFICIRIL	354
<u>P32241-3</u>	VIPR1_HUMAN	50	STSGGVPSTFTMVWTIARIHFEDYGCWDTINSSLWMIKGPILTSILNVFILFICIRIL	109
<u>P32241-4</u>	VIPR1_HUMAN	212	LIGWGPSTFTMVWTIARIHFEDYGCWDTINSSLWMIKGPILTSILNVFILFICIRIL	271
<u>P32241-5</u>	VIPR1_HUMAN	219	LIGWGPSTFTMVWTIARIHFEDYGCWDTINSSLWMIKGPILTSILNVFILFICIRIL	278
			*****	
<u>P32241</u>	VIPR1_HUMAN	320	LQKLRPPDIRKSDSSPYSLARSTLLIPLFGVHYIMFAFFPDNFKPEVKMVFELVVGSGF	379
<u>P32241-2</u>	VIPR1_HUMAN	355	LQKLRPPDIRKSDSSPYSLARSTLLIPLFGVHYIMFAFFPDNFKPEVKMVFELVVGSGF	414
<u>P32241-3</u>	VIPR1_HUMAN	110	LQKLRPPDIRKSDSSPYSLARSTLLIPLFGVHYIMFAFFPDNFKPEVKMVFELVVGSGF	169
<u>P32241-4</u>	VIPR1_HUMAN	272	LQKLRPPDIRKSDSSPYSLARSTLLIPLFGVHYIMFAFFPDNFKPEVKMVFELVVGSGF	331
<u>P32241-5</u>	VIPR1_HUMAN	279	LQKLRPPDIRKSDSSPYSLARSTLLIPLFGVHYIMFAFFPDNFKPEVKMVFELVVGSGF	338
			*****	
<u>P32241</u>	VIPR1_HUMAN	380	QGFWVAILYCFNLGEVQAEELRRKWRWHLQGVLGWNPKYRHPSGGSNGATCSTQVSMLTR	439
<u>P32241-2</u>	VIPR1_HUMAN	415	QGFWVAILYCFNLGEVQAEELRRKWRWHLQGVLGWNPKYRHPSGGSNGATCSTQVSMLTR	474
<u>P32241-3</u>	VIPR1_HUMAN	170	QGFWVAILYCFNLGEVQAEELRRKWRWHLQGVLGWNPKYRHPSGGSNGATCSTQVSMLTR	229
<u>P32241-4</u>	VIPR1_HUMAN	332	QGFWVAILYCFNLGEVQAEELRRKWRWHLQGVLGWNPKYRHPSGGSNGATCSTQVSMLTR	391
<u>P32241-5</u>	VIPR1_HUMAN	339	QGFWVAILYCFNLGEVQAEELRRKWRWHLQGVLGWNPKYRHPSGGSNGATCSTQVSMLTR	398
			*****	
<u>P32241</u>	VIPR1_HUMAN	440	VSPGARRSSSFQAEVSLV	457
<u>P32241-2</u>	VIPR1_HUMAN	475	VSPGARRSSSFQAEVSLV	492
<u>P32241-3</u>	VIPR1_HUMAN	230	VSPGARRSSSFQAEVSLV	247
<u>P32241-4</u>	VIPR1_HUMAN	392	VSPGARRSSSFQAEVSLV	409
<u>P32241-5</u>	VIPR1_HUMAN	399	VSPGARRSSSFQAEVSLV	416
			*****	

**Figure S7. MSA of two VPAC2 sequences deposited in Uniprot.**

<u>P41587</u>	VIPR2_HUMAN	1	MRTLLPALLTCWLLAPVNSIHPECRFHLEIQEEETKCAELLRSQTE-----KHKACSG	
<u>P41587-2</u>	VIPR2_HUMAN	1	-----MPLWEAPSDHPANPPATLQG--HTSLPGQEEFARDPQSGLPQITSESSSF	
			: * : : * . : : : . * * : : : . : . *	
<u>P41587</u>	VIPR2_HUMAN	55	VWDNITCWRPANVGETVTVPCKVFSNFYISKAGNISKNCTSDGWSETFPDFVDACGYSDP	
<u>P41587-2</u>	VIPR2_HUMAN	50	SEGSLPSWSSGPAAGLNASHE-----GIGSSSDGNGDSKAATERVVSAMDTVR	
			..: .* . .* :.. . *... :.*::: : * :. :	
<u>P41587</u>	VIPR2_HUMAN	115	EDESKITFYILVKAIYTLGYSVSLMSLATGSIILCLFRKLHCTRNYIHLNLFSLFILRAI	
<u>P41587-2</u>	VIPR2_HUMAN	99	RKHPEITFYILVKAIYTLGYSVSLMSLATGSIILCLFRKLHCTRNYIHLNLFSLFILRAI	
			... :*****	
<u>P41587</u>	VIPR2_HUMAN	175	SVLVKDDVLYSSSGTLHCPDQPSWVGCKLSLVFLQYCIANFFWLLVEGLYLHTLLVAM	
<u>P41587-2</u>	VIPR2_HUMAN	159	SVLVKDDVLYSSSGTLHCPDQPSWVGCKLSLVFLQYCIANFFWLLVEGLYLHTLLVAM	
			*****	
<u>P41587</u>	VIPR2_HUMAN	235	LPPRRCFLAYLLIGWGLPTVCIGAWTAARLYLEDTCGWDNDHSPVWVIRIPILISIIIV	
<u>P41587-2</u>	VIPR2_HUMAN	219	LPPRRCFLAYLLIGWGLPTVCIGAWTAARLYLEDTCGWDNDHSPVWVIRIPILISIIIV	
			*****	
<u>P41587</u>	VIPR2_HUMAN	295	NFVLFISIIIRILLQKLTS PDVGGNDQSQYKRLAKSTLLIPLFGVHYMVFAVFPISISSK	
<u>P41587-2</u>	VIPR2_HUMAN	279	NFVLFISIIIRILLQKLTS PDVGGNDQSQYKRLAKSTLLIPLFGVHYMVFAVFPISISSK	
			*****	
<u>P41587</u>	VIPR2_HUMAN	355	YQILFELCLGSGFQGLVAVLYCFLNSEQCELKRKWRSRCPTPSASRDYRVCGSSFSRNG	
<u>P41587-2</u>	VIPR2_HUMAN	339	YQILFELCLGSGFQGLVAVLYCFLNSEQCELKRKWRSRCPTPSASRDYRVCGSSFSRNG	
			*****	
<u>P41587</u>	VIPR2_HUMAN	415	SEGALQFHRGSRASQFLQTETSVI	
<u>P41587-2</u>	VIPR2_HUMAN	399	SEGALQFHRGSRASQFLQTETSVI	
			*****	

**Figure S8. MSA of five PAC1 sequences deposited in Uniprot.**

<u>P41586</u>	PACR_HUMAN	1	MAGVVHVS LAALLLLP MAPAMHSDC I FKKEQAMCLEKIQRANELMGFNDSSPGCGMWDN	60
<u>P41586-2</u>	PACR_HUMAN	1	MAGVVHVS LAALLLLP MAPAMHSDC I FKKEQAMCLEKIQRANELMGFNDSSPGCGMWDN	60
<u>P41586-3</u>	PACR_HUMAN	1	MAGVVHVS LAALLLLP MAPAMHSDC I FKKEQAMCLEKIQRANELMGFNDSSPGCGMWDN	60
<u>P41586-4</u>	PACR_HUMAN	1	MAGVVHVS LAALLLLP MAPAMHSDC I FKKEQAMCLEKIQRANELMGFNDSSPGCGMWDN	60
<u>P41586-5</u>	PACR_HUMAN	1	MAGVVHVS LAALLLLP MAPAMHSDC I FKKEQAMCLEKIQRANELMGFNDSSP-----	52
*****				
<u>P41586</u>	PACR_HUMAN	61	ITCWKPAHV GEMVLVSCPELFRIFNP DQVWETETIGESDFGDSNSLDLSDMGVVS RNCTE	120
<u>P41586-2</u>	PACR_HUMAN	61	ITCWKPAHV GEMVLVSCPELFRIFNP DQVWETETIGESDFGDSNSLDLSDMGVVS RNCTE	120
<u>P41586-3</u>	PACR_HUMAN	61	ITCWKPAHV GEMVLVSCPELFRIFNP DQ-----DMGVVS RNCTE	99
<u>P41586-4</u>	PACR_HUMAN	61	ITCWKPAHV GEMVLVSCPELFRIFNP DQ-----DMGVVS RNCTE	99
<u>P41586-5</u>	PACR_HUMAN	53	ITCWKPAHV GEMVLVSCPELFRIFNP DQ-----DMGVVS RNCTE	63
*****				
<u>P41586</u>	PACR_HUMAN	121	DGWSEPFPHYFDACGFDEYESE TGDQDYY YLSVKALYTVGYSTSLVTLTTAMVILCRFRK	180
<u>P41586-2</u>	PACR_HUMAN	121	DGWSEPFPHYFDACGFDEYESE TGDQDYY YLSVKALYTVGYSTSLVTLTTAMVILCRFRK	180
<u>P41586-3</u>	PACR_HUMAN	100	DGWSEPFPHYFDACGFDEYESE TGDQDYY YLSVKALYTVGYSTSLVTLTTAMVILCRFRK	159
<u>P41586-4</u>	PACR_HUMAN	100	DGWSEPFPHYFDACGFDEYESE TGDQDYY YLSVKALYTVGYSTSLVTLTTAMVILCRFRK	159
<u>P41586-5</u>	PACR_HUMAN	64	DGWSEPFPHYFDACGFDEYESE TGDQDYY YLSVKALYTVGYSTSLVTLTTAMVILCRFRK	123
*****				
<u>P41586</u>	PACR_HUMAN	181	LHCTRNFIH MNLFVSFMLRAISVFIKDWILYAEQDSNHCFISTVECKAVMVFFHYCVVSN	240
<u>P41586-2</u>	PACR_HUMAN	181	LHCTRNFIH MNLFVSFMLRAISVFIKDWILYAEQDSNHCFISTVECKAVMVFFHYCVVSN	240
<u>P41586-3</u>	PACR_HUMAN	160	LHCTRNFIH MNLFVSFMLRAISVFIKDWILYAEQDSNHCFISTVECKAVMVFFHYCVVSN	219
<u>P41586-4</u>	PACR_HUMAN	160	LHCTRNFIH MNLFVSFMLRAISVFIKDWILYAEQDSNHCFISTVECKAVMVFFHYCVVSN	219
<u>P41586-5</u>	PACR_HUMAN	124	LHCTRNFIH MNLFVSFMLRAISVFIKDWILYAEQDSNHCFISTVECKAVMVFFHYCVVSN	183
*****				
<u>P41586</u>	PACR_HUMAN	241	YFWLFIEGLYLFTLLVETFFPERRYFYWYTIIGWGTPTVCVTVWATLRLYFDDTGCWDMN	300
<u>P41586-2</u>	PACR_HUMAN	241	YFWLFIEGLYLFTLLVETFFPERRYFYWYTIIGWGTPTVCVTVWATLRLYFDDTGCWDMN	300
<u>P41586-3</u>	PACR_HUMAN	220	YFWLFIEGLYLFTLLVETFFPERRYFYWYTIIGWGTPTVCVTVWATLRLYFDDTGCWDMN	279
<u>P41586-4</u>	PACR_HUMAN	220	YFWLFIEGLYLFTLLVETFFPERRYFYWYTIIGWGTPTVCVTVWATLRLYFDDTGCWDMN	279
<u>P41586-5</u>	PACR_HUMAN	184	YFWLFIEGLYLFTLLVETFFPERRYFYWYTIIGWGTPTVCVTVWATLRLYFDDTGCWDMN	243
*****				
<u>P41586</u>	PACR_HUMAN	301	DSTALWWVIKGPVVGSI MNVFLFIGIIVILVQKLQSPDMGNGNESSIY-----	348
<u>P41586-2</u>	PACR_HUMAN	301	DSTALWWVIKGPVVGSI MNVFLFIGIIVILVQKLQSPDMGNGNESSIYFSCVQKCYCKPQ	360
<u>P41586-3</u>	PACR_HUMAN	280	DSTALWWVIKGPVVGSI MNVFLFIGIIVILVQKLQSPDMGNGNESSIY-----	327
<u>P41586-4</u>	PACR_HUMAN	280	DSTALWWVIKGPVVGSI MNVFLFIGIIVILVQKLQSPDMGNGNESSIYFSCVQKCYCKPQ	339
<u>P41586-5</u>	PACR_HUMAN	244	DSTALWWVIKGPVVGSI MNVFLFIGIIVILVQKLQSPDMGNGNESSIY-----	291
*****				
<u>P41586</u>	PACR_HUMAN	349	-----LRLARSTLLLIPLFGIHYTVFAFSPENVSKRERL VFELGLGSFQ	392
<u>P41586-2</u>	PACR_HUMAN	361	RAQQHSCKMSELSTITLRLARSTLLLIPLFGIHYTVFAFSPENVSKRERL VFELGLGSFQ	420
<u>P41586-3</u>	PACR_HUMAN	328	-----LRLARSTLLLIPLFGIHYTVFAFSPENVSKRERL VFELGLGSFQ	371
<u>P41586-4</u>	PACR_HUMAN	340	RAQQHSCKMSELSTITLRLARSTLLLIPLFGIHYTVFAFSPENVSKRERL VFELGLGSFQ	399
<u>P41586-5</u>	PACR_HUMAN	292	-----LRLARSTLLLIPLFGIHYTVFAFSPENVSKRERL VFELGLGSFQ	335
*****				
<u>P41586</u>	PACR_HUMAN	393	GFVVAVLYCFLNGEVQAEIKRKWRSWKVNRYFAVDFKHRHPSLASSGVNGGTQLSILSKS	452
<u>P41586-2</u>	PACR_HUMAN	421	GFVVAVLYCFLNGEVQAEIKRKWRSWKVNRYFAVDFKHRHPSLASSGVNGGTQLSILSKS	480
<u>P41586-3</u>	PACR_HUMAN	372	GFVVAVLYCFLNGEVQAEIKRKWRSWKVNRYFAVDFKHRHPSLASSGVNGGTQLSILSKS	431
<u>P41586-4</u>	PACR_HUMAN	400	GFVVAVLYCFLNGEVQAEIKRKWRSWKVNRYFAVDFKHRHPSLASSGVNGGTQLSILSKS	459
<u>P41586-5</u>	PACR_HUMAN	336	GFVVAVLYCFLNGEVQAEIKRKWRSWKVNRYFAVDFKHRHPSLASSGVNGGTQLSILSKS	395
*****				
<u>P41586</u>	PACR_HUMAN	453	SSQIRMSG L PADNLAT	468
<u>P41586-2</u>	PACR_HUMAN	481	SSQIRMSG L PADNLAT	496
<u>P41586-3</u>	PACR_HUMAN	432	SSQIRMSG L PADNLAT	447
<u>P41586-4</u>	PACR_HUMAN	460	SSQIRMSG L PADNLAT	475
<u>P41586-5</u>	PACR_HUMAN	396	SSQIRMSG L PADNLAT	411
*****				

**Table S1.** Major isoforms of VIP and PACAP receptors according to Uniprot (accession date: 28 Dec 2021).

Receptor	Accession number	Name	Sequence length	Sequence modifications with respect to the reference sequence (isoform 1)
<b>VPAC1</b>	P32241-1	reference sequence, isoform 1	457	
	P32241-2	Isoform 2	492	Additional fragment in N-termini
	P32241-3	Isoform 3	247	5TM sequence
	P32241-4	Isoform 4	409	Short variant according to Ota et al.
	P32241-5	Isoform 5	416	Short variant according to Ota et al.
<b>VPAC2</b>	P41587-1	Reference sequence, isoform 1	438	
	P41587-2	Isoform 2	422	Short variant according to Ota et al.
<b>PAC1</b>	P41586-1	Reference sequence, PAC1 null, isoform N, isoform 1	468	
	P41586-2	PAC1 hop, Isoform N-HOP1	496	insertion hop cassette
	P41586-3	PAC1 $\delta$ 5-6 null, Isoform S	447	deletion exons 5-6
	P41586-4	PAC1 $\delta$ 5-6 hop, Isoform S-HOP1	475	insertion hop cassette + deletion exons 5-6
	P41586-5	PAC1 $\delta$ 4-6 null, Isoform VS	411	deletion exons 4-6
	NCBI ref XP_006715708.1	PAC1 hip, Isoform X1	496	insertion hip cassette