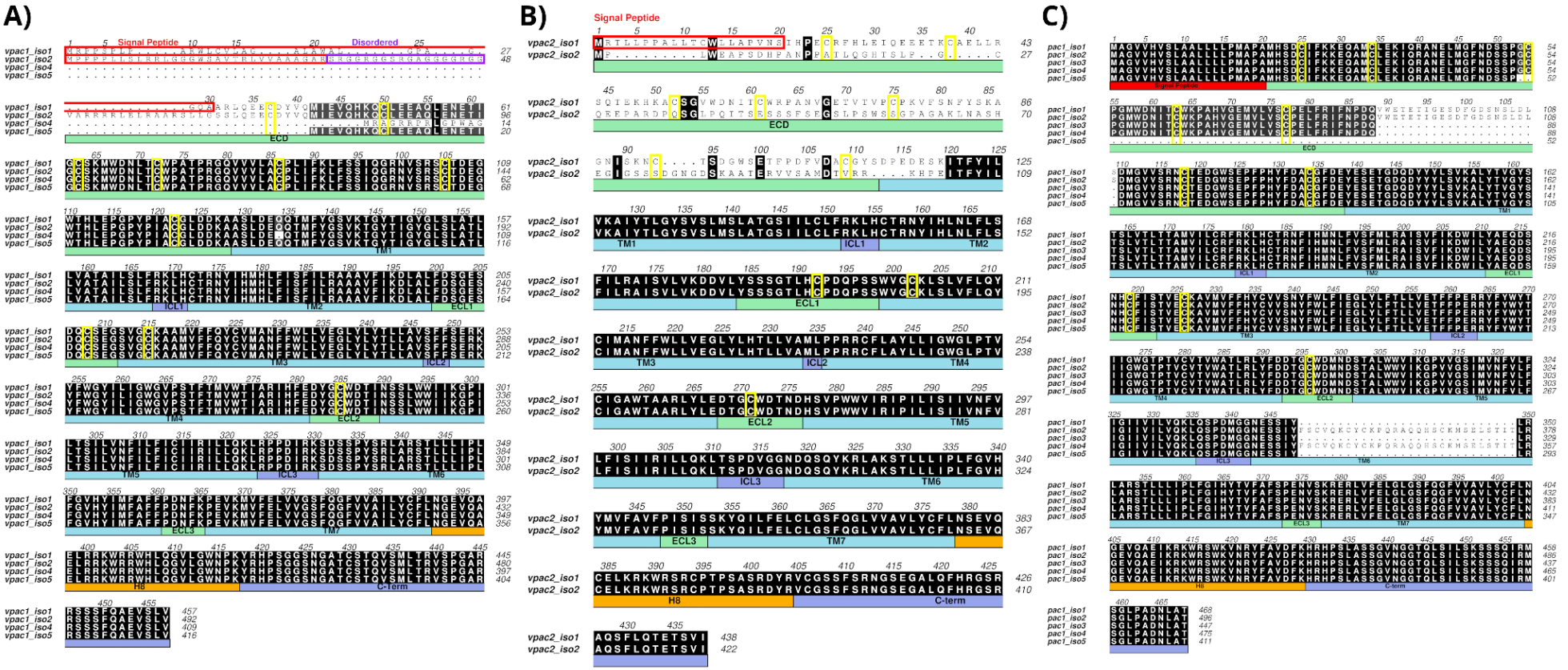
Extracellular domain stability determines VIP affinity in VPAC1, VPAC2, and PAC1 Variants

Supplementary Material

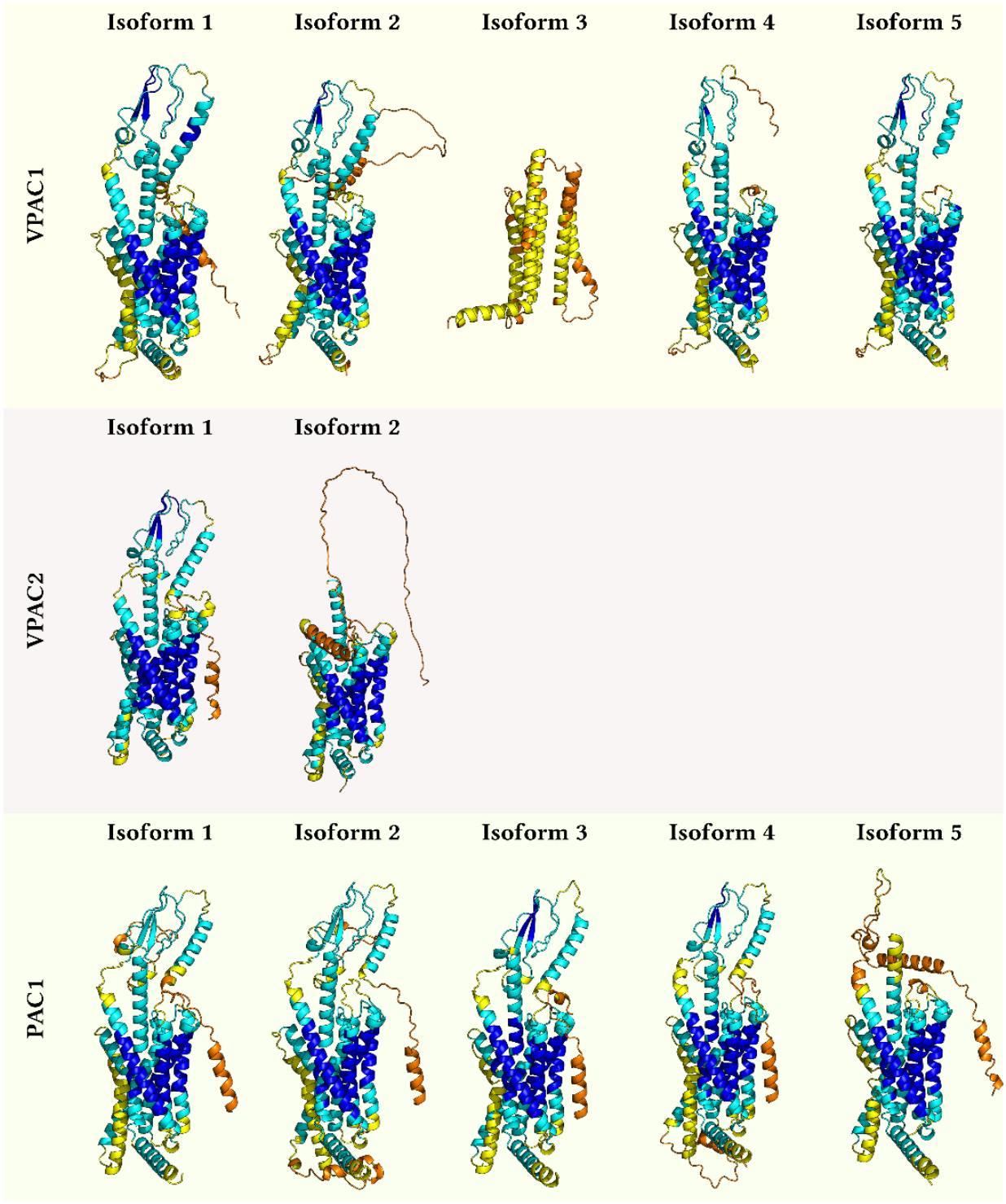


Supplementary Figure S1: Multiple alignments of receptor isoforms. A) VPAC1. B) VPAC2. C) PAC1. Residues are colored according to their similarity, with conserved residues colored black and non-conserved residues uncolored. Conserved GPCR domains are identified according to their position relative to the membrane in the boxes below the sequences: extracellular (green), transmembrane (cyan), intracellular (purple), intracellular α-helix parallel to the membrane (orange). The squares around the residues indicate: signal peptide (red), disordered region (purple), cysteines that form disulfide bonds (yellow).

Supplementary Table T1: Models stereochemical quality validation

	MolProbity Score	**Outliers rotamers**	**Ramachandran Favorable**	**Ramachandran Outliers**	**QMEANDisCo**	**RMSD (Å)**
Reference Values	≈ 0	< 1%	> 98%	< 0,2%	≈ 1,00	≈ 0
PAC1 (PDB ID 6LPB)	1,68	1,56	95,34	0,00	0,74	-/-
VPAC1 (PDB ID 8E3Z)	1,62	0,32	96,21	0,00	0,77	-/-
VPAC2 (PDB ID 7VQX)	1,65	0,00	95,81	0,00	0,78	-/-
VPAC1-iso1	2,24	6,08	91,48	1,66	0,64	4,51
VPAC1-iso2	2,70	12,14	89,92	2,52	0,58	6,62
VPAC1-iso3	2,93	10,54	85,98	4,06	0,47	9,46
VPAC1-iso4	1,94	3,71	91,92	1,62	0,64	3,99
VPAC1-iso5	2,03	5,05	92,05	1,59	0,63	3,78
VPAC2-iso1	2,00	3,49	92,64	1,52	0,61	3,26
VPAC2-iso2	2,70	7,02	81,50	5,75	0,56	9,32
VPAC2-iso2 (Modeller)	2,00	3,23	95,54	0,84	0,54	6,86
PAC1-iso1	2,78	13,69	89,63	2,03	0,58	3,38
PAC1-iso2	2,36	6,00	91,35	2,69	0,55	3,93
PAC1-iso3	2,34	5,85	92,18	1,20	0,61	3,29
PAC1-iso4	2,38	6,10	91,78	1,40	0,59	3,82
PAC1-iso5	1,99	3,83	92,18	1,38	0,60	8,14

The RMSD was calculated against the PBDs entry for each receptor



Supplementary Figure S2: AlphaFold2-generated structural models of VIP receptor variants colored by confidence scores. Three-dimensional models of all VPAC1 (isoforms 1-5), VPAC2 (isoforms 1-2), and PAC1 (isoforms 1-5) receptor variants generated using AlphaFold2 through ColabFold. Structures are colored according to predicted Local Distance Difference Test (pLDDT) confidence scores: dark blue represents very high confidence (pLDDT > 90), cyan indicates confident regions (pLDDT 70-90), yellow denotes low confidence (pLDDT 50-70), and orange represents very low confidence regions (pLDDT < 50).

Supplementary Table T2: MM/GBSA binding energy estimative in kcal/mol.

Systems	ΔE_{Vdw}	ΔE_{Elec}	ΔE_{GB}	ΔE_{SURF}	ΔG_{GAS}	ΔG_{SOLV}	ΔG_{TOTAL}
VPAC1-iso1	-189 ± 14	-856 ± 70	956 ± 66	-28 ± 2	-1044 ± 76	927 ± 65	-117 ± 17
VPAC1-iso2	-168 ± 9	-519 ± 88	630 ± 78	-24 ± 1	687 ± 93	605 ± 77	-82 ± 19
VPAC1-iso4	-168 ± 15	-350 ± 106	459 ± 103	-24 ± 2	-518 ± 105	435 ± 102	-83 ± 12
VPAC1-iso5	-158 ± 12	-590 ± 99	675 ± 93	-24 ± 1	-748 ± 101	651 ± 92	-97 ± 13
VPAC2-iso1	-151 ± 10	-434 ± 90	517 ± 85	-23 ± 2	-585 ± 95	494 ± 84	-91 ± 16
VPAC2-iso2	-167 ± 14	-195 ± 95	296 ± 95	-25 ± 2	-362 ± 97	271 ± 94	-90 ± 12
PAC1-iso1	-165 ± 9	-770 ± 58	878 ± 57	-25 ± 1	-935 ± 58	853 ± 57	-82 ± 10
PAC1-iso2	-168 ± 11	-997 ± 100	1084 ± 91	-26 ± 2	-1165 ± 104	1058 ± 91	-107 ± 26
PAC1-iso3	-167 ± 10	-769 ± 70	855 ± 63	-25 ± 1	-935 ± 68	829 ± 63	-106 ± 15
PAC1-iso4	-160 ± 20	-780 ± 93	878 ± 101	-23 ± 3	-940 ± 107	854 ± 98	-86 ± 15
PAC1-iso5	-182 ± 15	-858 ± 81	943 ± 79	-27 ± 2	-1040 ± 88	916 ± 78	-124 ± 16