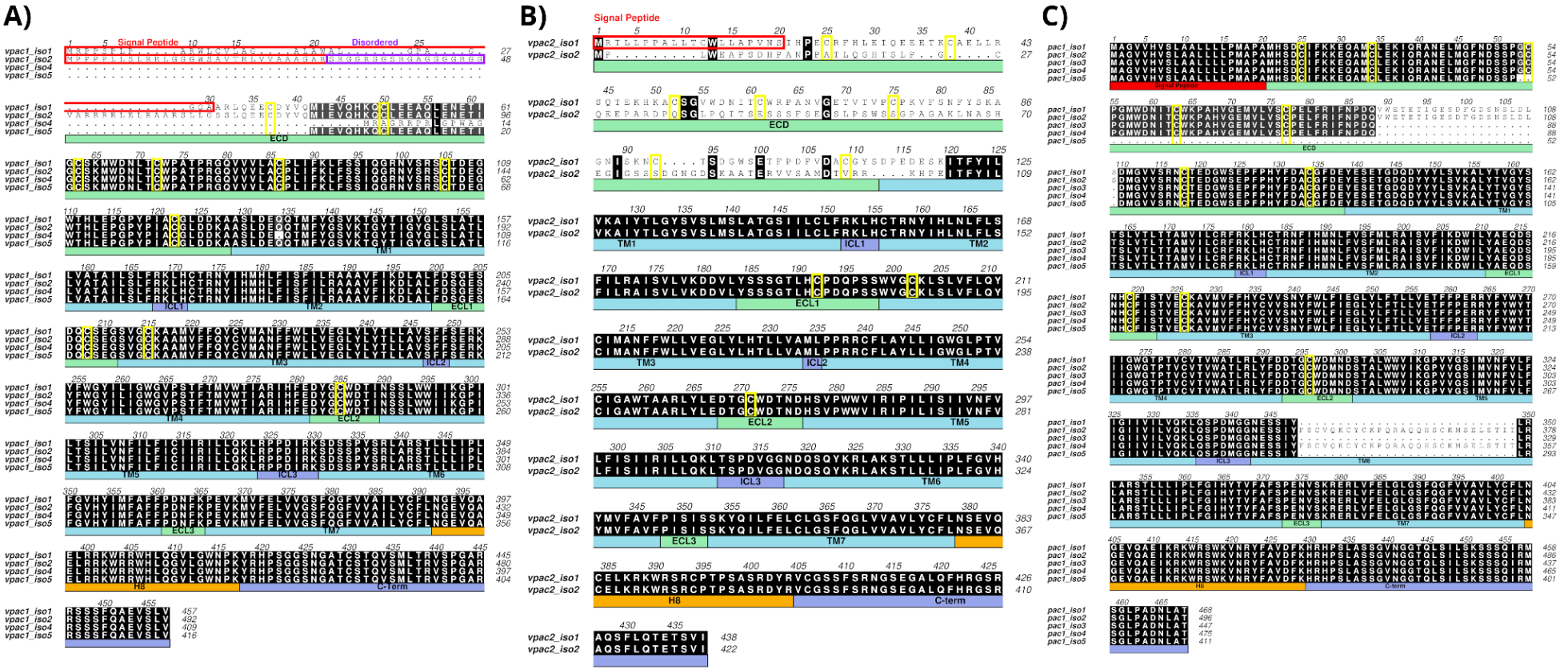
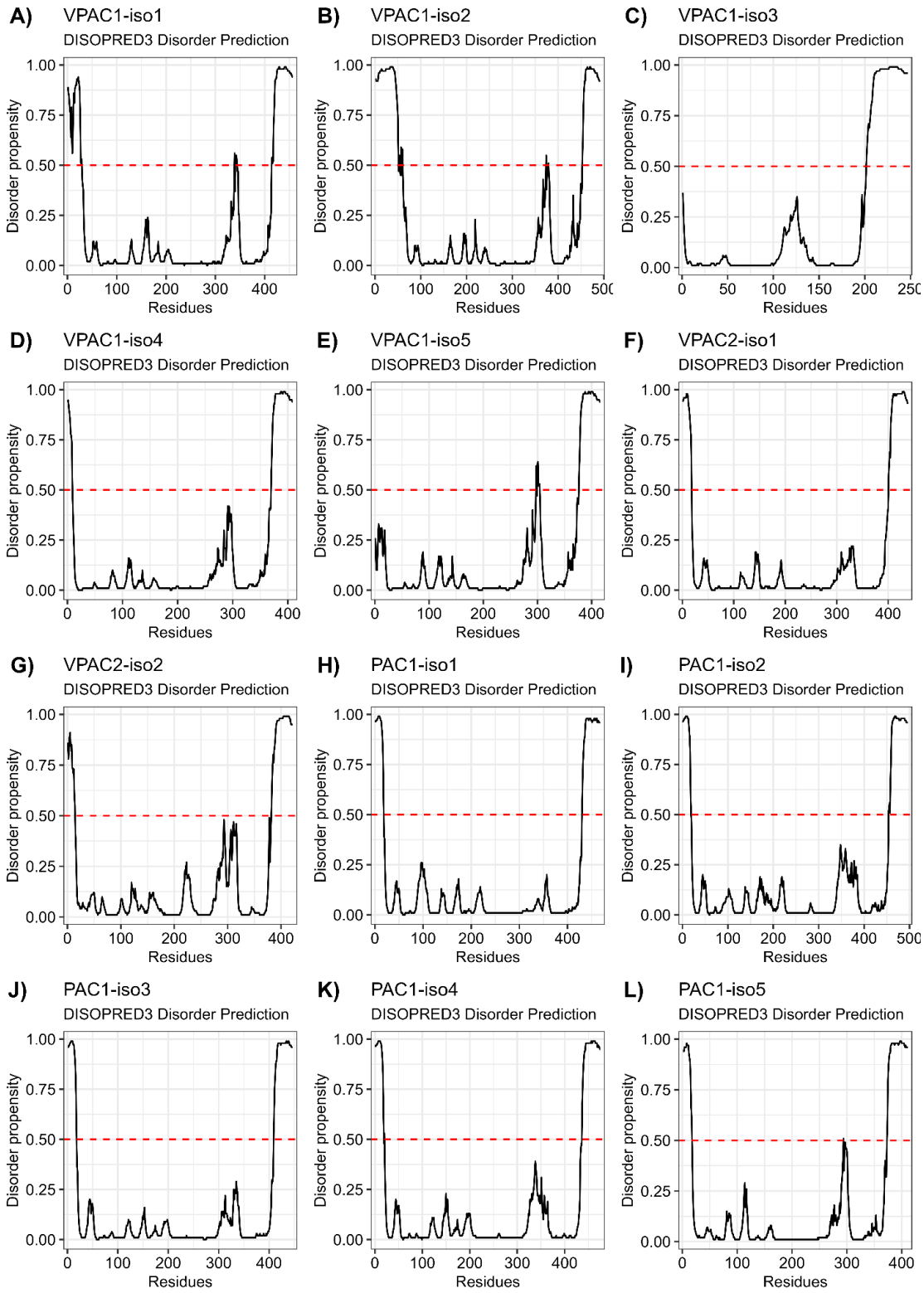
Extracellular domain stability determines VIP affinity in VPAC1, VPAC2, and PAC1 Variants

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).

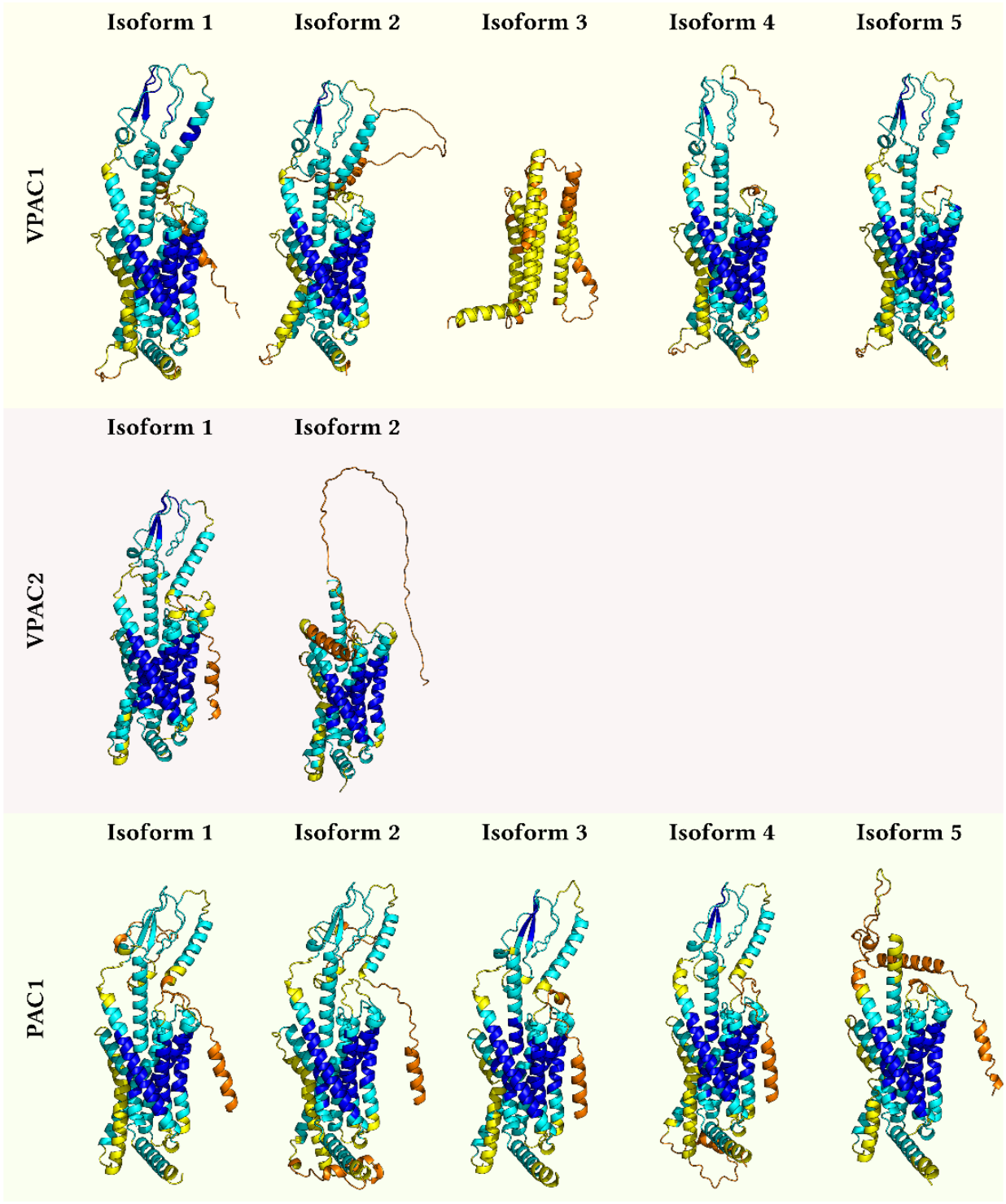


Supplementary Figure S2: Disorder prediction using DISOPRED3 algorithm. A-E) VPAC1 isoforms. F-G) VPAC2 isoforms. H-L) PAC1 isoforms. Horizontal dashed red lines represent the standard cutoff for disorder, regions above this value are assigned as disordered. In general, the regions with more disorder propensity are terminal regions.

Supplementary Table T1: Models stereochemical quality validation

	MolProbity Score	Outliers rotamers	Ramachandran Favorable	Ramachandran Outliers	QMEANDisCo	RMSD (Å) (no refinement cycles)	RMSD (Å) (5 refinement cycles)
Reference Values	≈ 0	< 1%	> 98%	< 0,2%	≈ 1,00	≈ 0	≈ 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 (86,8%)	1,69 (65,1%)
VPAC1-iso2	2,70	12,14	89,92	2,52	0,58	6,62 (86,3%)	1,86 (66,0%)
VPAC1-iso3	2,93	10,54	85,98	4,06	0,47	9,46 (64,0%)	7,10 (57,7%)
VPAC1-iso4	1,94	3,71	91,92	1,62	0,64	4,00 (84,6%)	1,76 (64,4%)
VPAC1-iso5	2,03	5,05	92,05	1,59	0,63	3,78 (87,0%)	1,49 (64,1%)
VPAC2-iso1	2,00	3,49	92,64	1,52	0,61	3,26 (89,0%)	1,42 (69,9%)
VPAC2-iso2	2,70	7,02	81,50	5,75	0,56	9,32 (71,2%)	0,98 (52,3%)
VPAC2-iso2 (Modeller)	2,00	3,23	95,54	0,84	0,54	6,98 (88,8%)	0,32 (61,3%)
PAC1-iso1	2,78	13,69	89,63	2,03	0,58	3,38 (76,6%)	1,72 (63,9%)
PAC1-iso2	2,36	6,00	91,35	2,69	0,55	3,93 (71,0%)	1,77 (59,5%)
PAC1-iso3	2,34	5,85	92,18	1,20	0,61	3,29 (78,9%)	1,76 (66,5%)
PAC1-iso4	2,38	6,10	91,78	1,40	0,59	3,82 (74,1%)	1,78 (61,8%)
PAC1-iso5	1,99	3,83	92,18	1,38	0,60	8,14 (76,9%)	1,38 (56,2%)

Root-mean-square deviation (RMSD) values were calculated for non-hydrogen (non-H) atoms between our structural models and the corresponding experimental PDB structures for each receptor using PyMOL's alignment algorithm. Values in parentheses represent the proportion of atoms used in the RMSD calculation, expressed as (number of aligned non-H atoms)/(total number of non-H atoms).



Supplementary Figure S3: 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