Structural perspective on ancient neuropeptide Y -like system reveals hallmark features for peptide recognition and receptor activation

AUTHORS

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SUPPLEMENTARY INFORMATION

Table S1. Activity of NPR-1 mutants in cAMP reporter gene assays ($G_{i/o}$). EC_{50} shifts are given relative to the wild type receptor. All variants tested were full agonists with E_{max} values within the statistical error of the wild type receptor-peptide pairing. Data represent mean ± SEM of n ≥ 3 independent experiments. n.d., activation not determinable up to 10 µM peptide concentration.

		peptide	
		FLP-21	
		EC_{50} [nM] logEC_{50} ± SEM	x-fold of wt
receptor	**NPR-1 wt**	0.8 (-9.10 ± 0.05)	(1)
	Nluc-NPR-1	0.2 (-9.79 ± 0.12)	0.3
	T2.61A	139 (-6.86 ± 0.11)	176
	T2.61S	26 (-7.59 ± 0.13)	33
	Q3.32H	37 (-7.43 ± 0.11)	49
	Y4.60A	5 (-8.30 ± 0.11)	6.3
	F5.24L	25 (-7.61 ± 0.61)	32
	E5.27A	n.d.	n.d.
	E5.27Q	n.d.	n.d.
	E5.27D	495 (-6.31 ± 0.10)	627
	T5.39A	2.7 (-8.57 ± 0.13)	3.4
	W6.48Y	2.94 (-8.53 ± 0.19)	3.7
	W6.48H	10 (-8.00 ± 0.12)	13
	I6.58A	3.6 (-8.45 ± 0.18)	4.6
	E6.59A	20 (-7.70 ± 0.11)	25
	E6.59D	0.1 (-9.88 ± 0.15)	0.13
	D6.61A	8.3 (-8.10 ± 0.08)	10
	D6.62A	2.8 (-8.55 ± 0.18)	3.5
	E6.59A+D6.61A	1469 (-5.83 ± 0.29)	1859
	E6.59A+D6.61A +D6.62A	n.d.	n.d.
	D7.26A	1.2 (-8.92 ± 0.16)	1.5
	D7.27A	3.6 (-8.45 ± 0.26)	4.5
	Y7.28A	1.8 (-8.74 ± 0.11)	2.3
	Y7.32A	19 (-7.72 ± 0.09)	24

Table S2. Activity of FLP-21 mutants in cAMP reporter gene assays ($G_{i/o}$). EC_{50} shifts are given relative to the corresponding wild type receptor. All variants tested were full agonists with E_{max} values within the statistical error of the wild type receptor-peptide pairing. n.d., activation not determinable up to 10 µM peptide concentration. Data represent mean ± SEM of n ≥ 3 independent experiments.

		receptor			
		NPR-1		NPR-11	
		EC_{50} [nM] $logEC_{50}$ ± SEM	x-fold of wt	EC_{50} [nM] $logEC_{50}$ ± SEM	x-fold of wt
peptide	**FLP-21**	0.8 (-9.10 ± 0.05)	(1)	51 (-7.30 ± 0.05)	(1)
	Ac-FLP-21	2.6 (-8.58 ± 0.18)	3.3	120 (-6.92 ± 0.12)	2.4
	[TAMRA]-FLP-21	9.4 (-8.03 ± 0.16)	12	212 (-6.67 ± 0.10)	4.2
	[R5A]-FLP-21	290 (-6.54 ± 0.15)	367	925 (-6.03 ± 0.17)	18
	[R5hArg]-FLP-21	5.2 (-8.28 ± 0.13)	6.6	295 (-6.53 ± 0.10)	5.8
	[P6A]-FLP-21	27 (-7.57 ± 0.35)	34	925 (-6.03 ± 0.18)	18
	[P6G]-FLP-21	464 (-6.33 ± 0.18)	587	n.d.	n.d.
	[L7A]-FLP-21	393 (-6.41 ± 0.15)	497	478 (-6.32 ± 0.19)	9.4
	[R8A]-FLP-21	n.d.	n.d.	n.d.	n.d.
	[R8Q]-FLP-21	n.d.	n.d.	n.d.	n.d.
	[R8hArg]-FLP-21	771 (-6.11 ± 0.17)	976	6868 (-5.16 ± 0.50)	135
	[F9A]-FLP-21	n.d.	n.d.	n.d.	n.d.
	[F9L]-FLP-21	n.d.	n.d.	n.d.	n.d.
	[F9Cha]-FLP-21	478 (-6.32 ± 0.20)	605	1078 (-5.97 ± 0.13)	21
	[F9Y]-FLP-21	11 (-7.96 ± 0.21)	14	370 (-6.43 ± 0.10)	7.3
	FLP-21-COOH	n.d.	n.d.	n.d.	n.d.
	[F9tyramide]-FLP-21	n.d.	n.d.	n.d.	n.d.

Table S3. Binding affinities of ligand variants at NPR-1 and NPR-11. K_i values were obtained from displacement binding assays using a fixed concentration of 100 nM [TAMRA]-FLP-21/-FLP-34-1. -, not tested; n.d., binding not determinable up to 10 µM peptide concentration. Data represent mean ± SEM of n ≥ 3 independent experiments.

		receptor			
		NPR-1		NPR-11	
		K_i [nM] logK_i ± SEM	x-fold of wt FLP-21	K_i [nM] logK_i ± SEM	x-fold of wt FLP-21
peptide	**FLP-21**	4.7 (-8.33 ± 0.07)	(1)	79 (-7.10 ± 0.15)	(1)
	[R5A]-FLP-21	1730 (-5.76 ± 0.19)	368	1020 (-5.99 ± 0.14)	13
	[R5hArg]-FLP-21	34 (-7.47 ± 0.03)	7.2	310 (-6.51 ± 0.10)	3.9
	[L7A]-FLP-21	753 (-6.12 ± 0.15)	160	302 (-6.52 ± 0.34)	3.8
	[R8A]-FLP-21	11600 (-4.94 ± 6.45)	2468	n.d.	n.d.
	[R8Q]-FLP-21	16600 (-4.78 ± 17.56)	3532	n.d.	n.d.
	[R8hArg]-FLP-21	1040 (-5.98 ± 0.10)	221	2370 (-5.63 ± 0.46)	30
	[F9L]-FLP-21	8280 (-5.08 ± 2.75)	1762	n.d.	n.d.
	[F9Cha]-FLP-21	906 (-6.04 ± 0.24)	193	1210 (-5.92 ± 0.67)	15
	[F9Y]-FLP-21	51 (-7.29 ± 0.12)	11	107 (-6.97 ± 0.46)	1.4
	FLP-21-COOH	7760 (-5.11 ± 2.66)	1651	-	-
	[F9tyramide]-FLP-21	1370 (-5.86 ± 0.16)	291	3650 (-5.44 ± 0.32)	46
		NPR-11			
	FLP-34-1	107 (-6.97 ± 0.11)	(1)		
	[R15A]-FLP-34-1	10600 (-4.97 ± 2.04)	99		
	[R15hArg]-FLP-34-1	334 (-6.48 ± 0.10)	3.1		
	[L16A]-FLP-34-1	2890 (-5.54 ± 0.67)	27		
	[L16Q]-FLP-34-1	1710 (-5.78 ± 0.41)	16		
	[R17A]-FLP-34-1	18000 (-4.74 ± 5.48)	168		
	[R17hArg]-FLP-34-1	1180 (-5.93 ± 0.17)	11		

Table S4. Identification of direct interacting partners between NPR-1 (receptor) and FLP-21 (ligand), measured by double-cycle mutagenesis in cAMP reporter gene assay ($G_{i/o}$). EC_{50} values as well as EC_{50} shifts are given. The first column of EC_{50} shifts shows the relative shift of the corresponding mutant (mut) compared to the wild type (wt) receptor stimulated with wt FLP-21. The second column then normalizes the EC_{50} shift of the respective receptor variant to wt FLP-21, i.e., is set to 1. All other -fold-EC_{50} shifts relate to this column. Reduced EC_{50} shifts of a peptide at a particular receptor variant compared to the shift at the wild type receptor (top row) indicate a direct interaction. All variants tested were full agonists with E_{max} values within the statistical error of the wild type receptor-peptide pairing. -, not tested; n.d., activation not determinable up to 10 µM peptide concentration. Data represent mean ± SEM of n ≥ 3 independent experiments.

	peptide																
	FLP-21			[R5A]-FLP-21		[R5hArg]-FLP-21		[P6G]-FLP-21		[L7A]-FLP-21		[R8hArg]-FLP-21		[F9Cha]-FLP-21		[F9Y]-FLP-21	
receptor	EC_{50} [nM] $logEC_{50}$ ± SEM	x-fold of wt NPR-1 /FLP-21	x-fold of mut /FLP-21	EC_{50} [nM] $logEC_{50}$ ± SEM	x-fold of mut /FLP-21	EC_{50} [nM] $logEC_{50}$ ± SEM	x-fold of mut /FLP-21	EC_{50} [nM] $logEC_{50}$ ± SEM	x-fold of mut /FLP-21	EC_{50} [nM] $logEC_{50}$ ± SEM	x-fold of mut /FLP-21	EC_{50} [nM] $logEC_{50}$ ± SEM	x-fold of mut /FLP-21	EC_{50} [nM] $logEC_{50}$ ± SEM	x-fold of mut /FLP-21	EC_{50} [nM] $logEC_{50}$ ± SEM	x-fold of mut /FLP-21
NPR-1 wt	0.8 (-9.10±0.05)	(1)	(1)	290 (-6.54 ± 0.15)	367	5.2 (-8.28 ± 0.13)	6.6	464 (-6.33 ± 0.18)	580	393 (-6.41 ± 0.15)	497	771 (-6.11 ± 0.17)	976	478 (-6.32 ± 0.20)	605	11 (-7.96 ± 0.21)	14
T2.61A	139 (-6.86 ± 0.11)	174	(1)	-	-	-	-	-	-	n.d.	n.d.	-	-	-	-	1493 (-5.83 ± 0.41)	11
T2.61S	26 (-7.59± 0.13)	33	(1)	-	-	-	-	n.d.	n.d.	2199 (-5.66 ± 0.25)	84	-	-	1964 (-5.71 ± 0.16)	76	-	-
Q3.32H	37 (-7.43 ± 0,11)	46	(1)	-	-	-	-	-	-	n.d.	n.d.	-	-	-	-	451 (-6.35 ± 0.47)	17
Y4.60A	5 (-8.30 ± 0.11)	6.3	(1)	-	-	-	-	-	-	-	-	-	-	-	-	57 (-7.24 ± 0.16)	11
E5.27D	495 (-6.31 ± 0.10)	627	(1)	n.d.	n.d.	1809 (-5.74 ± 0.15)	3.7	-	-	-	-	16600 (-4.78 ± 0.35)	34	-	-	-	-
E6.59A	20 (-7.70 ± 0.11)	25	(1)	4.8 (-8.32 ± 0.11)	0.24	9.4 (-8.03 ± 0.11)	0.5	-	-	-	-	1820 (-5.74 ± 0.22)	91	-	-	-	-
E6.59D	0.1 (-9.88 ± 0.15)	0.13	(1)	-	-	0.6 (-9.26 ± 0.14)	6.0	-	-	-	-	157 (-6.81 ± 0.14)	1570	-	-	-	-
D6.61A	8.3 (-8.10 ± 0.08)	10	(1)	971 (-6.01 ± 0.18)	117	49 (-7.31 ± 0.06)	5.9	-	-	-	-	-	-	-	-	-	-
D6.62A	2.8 (-8.55 ± 0.18)	3.5	(1)	1153 (-5.94 ± 0.35)	412	16 (-7.81 ± 0.15)	5.7	-	-	-	-	3810 (-5.42 ± 0.26)	1360	-	-	-	-
E6.59A+ D6.61A	1469 (-5.83 ± 0.29)	1859	(1)	-	-	303 (-6.52 ± 0.23)	0.2	-	-	-	-	-	-	-	-	-	-
Y7.32A	19 (-7.72 ± 0.09)	24	(1)	-	-	-	-	n.d.	n.d.	n.d.	n.d.	-	-	-	-	381 (-6.42 ± 0.18)	20

Table S5. Identification of direct interacting partners between NPR-11 (receptor) and FLP-21 (ligand), measured by double-cycle mutagenesis in cAMP reporter gene assay ($G_{i/o}$). EC_{50} values as well as EC_{50} shifts are given. The first column of EC_{50} shifts shows the relative shift of the corresponding mutant (mut) compared to the wild type (wt) receptor stimulated with wild type FLP-21. The second column then normalizes the EC_{50} shift of the respective receptor variant to wt FLP-21, i.e., is set to 1. All other -fold-EC_{50} shifts relate to this column. Reduced EC_{50} shifts of a peptide at a particular receptor variant compared to the shift at the wild type receptor (top row) indicate a direct interaction. All variants tested were full agonists with E_{max} values within the statistical error of the wild type receptor-peptide pairing. -, not tested; n.d., activation not determinable up to 10 µM peptide concentration. Data represent mean ± SEM of n ≥ 3 independent experiments.

		peptide													
		FLP-21			[R5A]-FLP-21		[R5hArg]-FLP-21		[L7A]-FLP-21		[R8hArg]-FLP-21		[F9Cha]-FLP-21	
		EC_{50} [nM] logEC_{50} ± SEM	x-fold of wt NPR-11 /FLP-21	x-fold of mut /FLP-21	EC_{50} [nM] logEC_{50} ± SEM	x-fold of mut /FLP-21	EC_{50} [nM] logEC_{50} ± SEM	x-fold of mut /FLP-21	EC_{50} [nM] logEC_{50} ± SEM	x-fold of mut /FLP-21	EC_{50} [nM] logEC_{50} ± SEM	x-fold of mut /FLP-21	EC_{50} [nM] logEC_{50} ± SEM	x-fold of mut /FLP-21
receptor	**NPR-11 wt**	51 (-7.30 ± 0.05	(1)	(1)	925 (-6.03 ± 0.17)	18	295 (-6.53 ± 0.10)	5.8	478 (-6.32 ± 0.19)	9.4	6868 (-5.16 ± 0.50)	135	1078 (-5.97 ± 0.13)	21
	T2.61A	203 (-6.69 ± 0.07)	4.0	(1)	-	-	-	-	1647 (-5.78 ± 0.13)	8.1	-	-	-	-
	M2.68N	502 (-6.30 ± 0.14)	9.8	(1)	-	-	-	-	3315 (-5.48 ± 0.60)	6.6	-	-	-	-
	Q3.32A	0.1 (-10.02±0.06)	0.002	(1)	-	-	-	-	30 (-7.53 ± 0.09)	300	-	-	-	-
	L4.51A	315 (-6.50 ± 0.13)	6.2	(1)	-	-	-	-	n.d.	n.d.	-	-	-	-
	E5.23A	n.d.	n.d.	(1)	n.d.	n.d.	-	-	-	-	-	-	-	-
	E5.23D	3926 (-5.41 ± 0.24)	77	(1)	-	-	12200 (-4.91 ± 0.20)	3.1	-	-	6030 (-5.22 ± 0.33)	1.5	-	-
	E5.26D	89 (-7.05 ± 0.06)	1.7	(1)	-	-	226 (-6.65 ± 0.09)	2.5	-	-	2808 (-5.55 ± 0.16)	32	-	-
	F7.35A	n.d.	n.d.	(1)	-	-	-	-	n.d.	n.d.	-	-	-	-
	F7.35H	15620 (-4.81 ±0.19)	306	(1)	-	-	-	-	-	-	-	-	n.d.	n.d.
	F7.35L	12660 (-4.90 ± 0.61)	248	(1)	-	-	-	-	-		-	-	n.d.	n.d.

Table S6. Activity of FLP-34-1 mutants in cAMP reporter gene assays ($G_{i/o}$). EC_{50} shifts are given relative to the corresponding wild type receptor. All variants tested were full agonists with E_{max} values within the statistical error of the wild type receptor-peptide pairing. n.d., activation not determinable up to 10 µM peptide concentration. Data represent mean ± SEM of n ≥ 3 independent experiments.

		receptor	
		NPR-11	
		EC_{50} [nM] logEC_{50} ± SEM	x-fold of wt
peptide	**FLP-34-1**	1.1 (-8.96 ± 0.05)	(1)
	[TAMRA]-FLP-34-1	0.4 (-9.37 ± 0.12)	0.4
	[S8K]-FLP-34-1	0.2 (-9.75 ± 0.06)	0.2
	[G14A]-FLP-34-1	2.0 (-8.70 ± 0.16)	1.8
	[G14E]-FLP-34-1	0.8 (-9.08 ± 0.14)	0.7
	[R15A]-FLP-34-1	1006 (-6.00 ± 0.13)	915
	[R15hArg]-FLP-34-1	2.2 (-8.65 ± 0.08)	2
	[L16A]-FLP-34-1	161 (-6.79 ± 0.15)	146
	[L16Q]-FLP-34-1	147 (-6.83 ± 0.24)	134
	[R17A]-FLP-34-1	1061 (-5.97 ± 0.39)	965
	[R17hArg]-FLP-34-1	85 (-7.07 ± 0.10)	77
	[R15,17A]-FLP-34-1	4201 (-5.38 ± 0.61)	3819
	[Y18A]-FLP-34-1	n.d.	n.d.
	[Y18F]-FLP-34-1	1.3 (-8.88 ± 0.19)	1.2
	[cycK8-Cterm] -FLP-34-1	393 (-6.41 ± 0.12)	357

Table S7. Activity of NPR-11 mutants in cAMP reporter gene assays ($G_{i/o}$). EC_{50} shifts are given relative to the corresponding wild type receptor. All variants tested were full agonists with E_{max} values within the statistical error of the wild type receptor-peptide pairing. -, not tested; n.d., activation not determinable up to 10 µM peptide concentration. Data represent mean ± SEM of n ≥ 3 independent experiments.

		peptide			
		FLP-21		FLP-34-1	
		EC_{50} [nM] logEC_{50} ± SEM	x-fold of wt	EC_{50} [nM] logEC_{50} ± SEM	x-fold of wt
receptor	**NPR-11 wt**	51 (-7.30 ± 0.05)	(1)	1.1 (-8.96 ± 0.05)	(1)
	Nluc-NPR-11	27 (-7.57 ± 0.16)	0.5	3.1 (-8.50 ± 0.14)	2.8
	T2.61A	203 (-6.69 ± 0.07)	4.0	2.3 (-8.63 ± 0.08)	2.1
	T2.64A	90 (-7.05 ± 0.20)	1.8	2.0 (-8.70 ± 0.15)	1.8
	M2.68A	88 (-7.05 ± 0.07)	1.7	1.2 (-8.92 ± 0.11)	1.1
	M2.68N	502 (-6.30 ± 0.14)	9.8	2.7 (-8.57 ± 0.06)	2.5
	Q3.32A	0.1 (-10.02 ± 0.06)	0.002	0.05 (-10.30 ± 0.08)	0.05
	Q3.32H	44 (-7.35 ± 0.14)	0.9	8.3 (-8.08 ± 0.15)	7.5
	L4.51A	315 (-6.50 ± 0.13)	6.2	84 (-7.08 ± 0.08)	76
	I5.20A	38 (-7.42 ± 0.10)	0.7	17 (-7.77 ± 0.10)	15
	E5.23A	n.d.	n.d.	955 (-6.02 ± 0.18)	868
	E5.23Q	n.d.	n.d.	1852 (-5.73 ± 0.40)	1684
	E5.23D	3926 (-5.41 ± 0.24)	77	101 (-7.00 ± 0.12)	92
	E5.26A	n.d.	n.d.	n.d.	n.d.
	E5.26Q	17 (-7.21 ± 0.08)	0.3	1.1 (-8.95 ± 0.15)	1
	E5.26D	89 (-7.05 ± 0.06)	1.7	1.1 (-8.97 ± 0.07)	1
	E5.29A	94 (-7.03 ± 0.17)	1.8	-	-
	T5.39A	498 (-6.30 ± 0.08)	9.8	-	-
	Q5.46A	18 (-7.75 ± 0.09)	0.4	0.5 (-9.33 ± 0.13)	0.5
	W6.48A	96 (-7.02 ± 0.11)	1.9	4.9 (-8.31 ± 0.18)	4.5
	N6.58A	58 (-7.24 ± 0.06)	1.1	2.0 (-8.69 ± 0.21)	1.8
	T6.59A	17 (-7.76 ± 0.10)	0.3	0.2 (-9.69 ± 0.16)	0.2
	E6.61A	81 (-7.09 ± 0.15)	1.6	3.1 (-8.51 ± 0.17)	2.8
	F7.35A	n.d.	n.d.	842 (-6.07 ± 0.15)	765
	F7.35H	15620 (-4.81 ±0.19)	306	58 (-7.24 ± 0.11)	53
	F7.35L	12660 (-4.90 ± 0.61)	248	117 (-6.93 ± 0.06)	106

Table S8. Identification of direct interacting partners between NPR-11 (receptor) and FLP-34-1 (ligand), measured by double-cycle mutagenesis in cAMP reporter gene assay ($G_{i/o}$). EC_{50} values as well as EC_{50} shifts are given. The first column of EC_{50} shifts shows the relative shift of the corresponding mutant (mut) compared to the wild type (wt) receptor stimulated with wild type FLP-21. The second column then normalizes the EC_{50} shift of the respective receptor variant to wt FLP-21, i.e., is set to 1. All other -fold-EC_{50} shifts relate to this column. Reduced EC_{50} shifts of a peptide at a particular receptor variant compared to the shift at the wild type receptor (top row) indicate a direct interaction. All variants tested were full agonists with E_{max} values within the statistical error of the wild type receptor-peptide pair. -, not tested; n.d., activation not determinable up to 10 µM peptide concentration. Data represent mean ± SEM of n ≥ 3 independent experiments.

	peptide																
	FLP-34-1			[R15A]-FLP-34-1		[R15hArg]-FLP-34-1		[L16A]-FLP-34-1		[L16Q]-FLP-34-1		[R17A]-FLP-34-1		[R17hArg]-FLP-34-1		[Y18F]-FLP-34-1	
receptor	EC_{50} [nM] logEC_{50} ± SEM	x-fold of wt NPR-11 /FLP-34-1	x-fold of mut /FLP-34-1	EC_{50} [nM] logEC_{50} ± SEM	x-fold of mut /FLP-34-1	EC_{50} [nM] logEC_{50} ± SEM	x-fold of mut /FLP-34-1	EC_{50} [nM] logEC_{50} ± SEM	x-fold of mut /FLP-34-1	EC_{50} [nM] logEC_{50} ± SEM	x-fold of mut /FLP-34-1	EC_{50} [nM] logEC_{50} ± SEM	x-fold of mut /FLP-34-1	EC_{50} [nM] logEC_{50} ± SEM	x-fold of mut /FLP-34-1	EC_{50} [nM] logEC_{50} ± SEM	x-fold of mut /FLP-34-1
NPR-11 wt	1.1 (-8.96 ± 0.05)	(1)	(1)	1006 (-6.00 ± 0.13)	915	2.2 (-8.65 ± 0.08)	2	161 (-6.79 ± 0.15)	146	147 (-6.83 ± 0.24)	134	1061 (-5.97 ± 0.39)	965	85 (-7.07 ± 0.10)	77	1.3 (-8.88 ± 0.19)	1.2
T2.61A	2.3 (-8.63 ± 0.08)	2.1	(1)	-	-	-	-	638 (-6.20 ± 0.25)	277	-	-	-	-	-	-	-	-
M2.68N	2.7 (-8.57 ± 0.06)	2.5	(1)	-	-	-	-	676 (-6.17 ± 0.20)	250	-	-	-	-	-	-	-	-
Q3.32A	0.05 (-10.30±0.08)	0.05	(1)	-	-	-	-	6.6 (-8.18 ± 0.13)	132	29 (-7.54 ± 0.36)	580	-	-	-	-	0.07 (-10.15±0.21)	1.4
Q3.32H	8.3 (-8.08 ± 0.15)	7.5	(1)	-	-	-	-	432 (-6.37 ± 0.17)	52	1104 (-5.96 ± 0.17)	133	-	-	-	-	-	-
L4.51A	84 (-7.08 ± 0.08)	76	(1)	-	-	-	-	n.d.	n.d.	-	-	-	-	-	-	-	-
E5.23A	955 (-6.02 ± 0.18)	868	(1)	n.d.	n.d.	-	-	-	-	-	-	n.d.	n.d.	-	-	-	-
E5.23D	101 (-7.00 ± 0.12)	92	(1)	n.d.	n.d.	159 (-6.80 ± 0.07)	1.6	-	-	-	-	-	-	95 (-7.02 ± 0.07)	0.9	-	-
E5.26D	1.1 (-8.97 ± 0.07)	1	(1)	-	-	3.7 (-8.43 ± 0.07)	3.4	-	-	-	-	-	-	163 (-6.79 ± 0.10)	148	-	-
F7.35A	842 (-6.07 ± 0.15)	765	(1)	-	-	-	-	n.d.	n.d.	-	-	-	-	-	-	-	-

Table S9. Alignment of NPR-1 and NPR-11 sequences to the structural templates used in Rosetta comparative modeling (RosettaCM). Template alignments were obtained from GPCRdb [1], and NPR-1 and NPR-11 sequences were aligned to the template alignment using ClustalW [2]. The sequence alignment was further manually adjusted as previously described [3]. The N- and C-termini of the receptors as well as parts of the ICL3 were truncated because of lack of coordinates in the template structures: NPR-1, residues 1-7, 228-265, 362-458; NPR-11, residues 1-16, 248-259, 345-402 (truncation indicated by /). PSIPRED [4] and OCTOPUS [5] were used to predict transmembrane regions. **Bold**, conserved residue x.50 (BW numbering); yellow, cysteines forming a disulfide bridge; green, transmembrane region predicted by PSIPRED; grey, transmembrane region in structure; red, β-sheet in structure (for more information, see Method section)

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                  1                                                TM1           ICL1   60
NPR-1_C.elegans   ----/DCQVYWKVYPDPSQSIYAIVPFLTVYLFLFFLGLFGNVTLIYVTCSH-KALL-SVQ
NPR-11_C.elegans  /KINYFFRDDQVINGTEYSPKEFGYFITFAYMLIILFGAIGNFLTIIVVILN-PAMR-TTR
Y1R(5ZBQ)         -FSEKNAQLLAFENDDCHLPLAMIFTLALAYGAVIILGVSGNLALIIIILKQ-KEMR-NVT
ET_BR(5GLI)       -------ISPPPCQGPIEIKETFKYINTVVSCLVFVLGIIGNSTLLYIIYKN-KCMR-NGP
K-OR-1(4DJH)      -------------------SPAIPVIITAVYSVVFVVGLVGNSLVMFVIIRY-TKMK-TAT

                  61      TM2                        ECL1           TM3           120
NPR-1_C.elegans   NIFILNLAASDCMMCILSLPITPITNVY-K-NWYF-GNLLCHLIPCIQGISIFVCTFSLG
NPR-11_C.elegans  NFFILNLALSDFFVCIVTAPTTLYTVLY-M-FWPF-SRTLCKIAGSLQGFNIFLSTFSIA
Y1R(5ZBQ)         NILIVNLSFSDLLVAIMCLPFTFVYTLM-D-HWVF-GEAMCKLNPFVQCVSITVSIWSLV
ET_BR(5GLI)       NILIASLALGDLLHIVIAIPINVYKLLA-E-DWPF-GAEMCKLVPFIQKASVGITVLSLC
K-OR-1(4DJH)      NIYIFNLALADALVT-TTMPFQSTVYLM-N-SWPF-GDVLCKIVLSIDYYNMFTSIFTLT

                  121               ICL2                        TM4                180
NPR-1_C.elegans   AIALDRYILVVR-PHS----TPL-SQRGAFLTTVLLWILSFVVTLPYAFNMQMIEY-TEE
NPR-11_C.elegans  SIAVDRYVLIIF-PTK----RER-QQNLSFCFFIMIWVISLILAVPLLQASDLTPV-FVE
Y1R(5ZBQ)         LIAVERHQLIIN-PRG----WRP-NNRHAYVGIAVIWVLAVASSLPFLIYQVMTDE-PFQ
ET_BR(5GLI)       ALSIDRYRAVAS-WSR----IKG-VPKWTAVEIVLIWVVSVVLAVPEAIGFDIITM-DYK
K-OR-1(4DJH)      MMSVDRYIAVCH-PVKA--LDFR-TPLKAKIINICIWLLSSSVGISAIVLGGTKVR-ED-

                  181           ECL2                                TM5           240
NPR-1_C.elegans   ------RIC-GYFCTE--KWE-------S-AKSRRAYTMIVMLAQFVVPFAVMAFCYANI
NPR-11_C.elegans  P---SCDLA-LYICHE--QNE---IWEKM-IISKGTYTLAVLITQYAFPLFSLVFAYSRI
Y1R(5ZBQ)         NVTLDAYKD-KYVCFD--QFP-------S-DSHRLSYTTLLLVLQYFGPLCFIFICYFKI
ET_BR(5GLI)       ------GSY-LRICLL--HPV--QKTAFM-QFYATAKDWWLFSFYFCLPLAITAFFYTLM
K-OR-1(4DJH)      -------VD-VIECSL--QFP----DDDY-SWWDLFMKICVFIFAFVIPVLIIIVCYTLM

                  241           ICL3                                TM6           300
NPR-1_C.elegans   VSVLSK-RAQTKIR/QRVV-LQNRRTTSILVTMVVWFGITWLPHNVISLIIEYD-DTQSF
NPR-11_C.elegans  AHRMKL-RFANRNQ/RSVV-ERQRRTHLLLVCVVAVFAVAWLPLNVFHIFNTFE-L----
Y1R(5ZBQ)         YIRLKR-R------------SETKRINIMLLSIVVAFAVCWLPLTIFNTVFDWN-HQII-
ET_BR(5GLI)       TCEMLR-KL------NDHL-KQRREVAKTVFCLVLVFALCWLPLHLARILKLTL-YNQND
K-OR-1(4DJH)      ILRLKS-VRLLSGR--EKD-RNLRRITRLVLVVVAVFVVCWTPIHIFILVEALG------

                  301     ECL3                    TM7                    H8     354
NPR-1_C.elegans   FRLYGRDDY-DISYLLNLFTHSIAMSNNVLNPVLYAWLNPSFRQLVIKTYFGDR/
NPR-11_C.elegans  -------VN-SFSVTTFSICHCLAMCSACLNPLIYAFFNHNFRIEFMHLFDRVG/
Y1R(5ZBQ)         -------AT-CNHNLLFLLCHLTAMISTCVNPIFYGFLNKNFQRDLQFFFNF---
ET_BR(5GLI)       P--NRCELL-SFLLVLDYIGINMASLNSCANPIALYLVSKRFKNAFKSALCC---
K-OR-1(4DJH)      ----------SAALSSYYFCIALGYTNSSLNPILYAFLDENFKRCFRDFCFP---
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Table S10. Affinity of TAMRA-labeled peptides (FLP-21 and FLP-34-1) to NPR-1 and NPR-11 variants in a nanoBRET binding assay. The fluorophore is attached at the N terminus of the peptides, which still retains high potency (see Table S2 and S6). The peptide concentrations were varied over 7 orders of magnitude and affinities were obtained by a sigmoidal concentration-response fit with fixed Hill slope n_H =1. *, biphasic behavior with $n_{H, 1,2}$ = 1. n.d., not detectable. Data represent mean ± SEM of n ≥ 3 independent experiments.

		peptide		
		[TAMRA]-FLP-21		
		K_D [nM] logK_D ± SEM	x-fold of wt	$BRET_{max}$ ± SEM
receptor	**NPR-1 wt**	59 (-7.23 ± 0.08)	(1)	0.093 ± 0.004
	NPR-1 Q3.32H	1135 (-5.95 ± 0.12)	19	0.10 ± 0.011
	NPR-11 wt*	biphasic $K_{D,1}$: 15 (-7.82 ± 0.69) $K_{D,2}$ > 5000 (> -5.3)	$K_{D,1}$ (1)*	0.005 ± 0.001 > 0.04
	NPR-11 Q3.32H	biphasic n.d > 5000 (> -5.3)	n.d.	> 0.04
	NPR-11 Q3.32A	biphasic n.d. 190 (-6.72 ± 0.07)	13	0.15 ± 0.005
		[TAMRA]-FLP-34-1		
		K_D [nM] logK_D ± SEM	x-fold of wt	$BRET_{max}$ ± SEM
	NPR-11 wt	388 (-6.41 ± 0.05)	(1)	0.29 ± 0.009
	NPR-11 Q3.32H	1400 (-5.85 ± 0.05)	3.6	0.29 ± 0.014
	NPR-11 Q3.32A	49 (-7.31 ± 0.07)	0.1	0.25 ± 0.009

Table S11. Binding affinities of ligand variants at wild type and $Q^{3.32}$ mutants of NPR-1 and NPR-11. K_i values were obtained from displacement binding assays using a fixed concentration of 100 nM [TAMRA]-FLP-21/-34-1. Comparison of the K_i values at different receptor variants enables identification of direct ligand−receptor contacts in the same way as using receptor activation data (double-cycle mutagenesis, cf. Figures 3-5 and Tables S4, S5, S8). Reduced K_i shifts of a peptide at a particular receptor variant compared to the shift at the wt receptor (top row) indicate a direct interaction. Data represent mean ± SEM of n ≥ 3 independent experiments.

		peptide								
		FLP-21			**[L7A]-FLP-21**		**[F9Cha]-FLP-21**		**[F9Y]-FLP-21**	
		K_i [nM] logK_i ± SEM	x-fold of NPR-1/ FLP-21	x-fold of mut/ FLP-21	K_i [nM] logK_i ± SEM	x-fold of mut/ FLP-21	K_i [nM] logK_i ± SEM	x-fold of mut/ FLP-21	K_i [nM] logK_i ± SEM	x-fold of mut/ FLP-21
receptor	**NPR-1 wt**	4.6 (-8.34 ± 0.06)	(1)	(1)	915 (-6.04 ± 0.09)	199	976 (-6.01 ± 0.13)	212	49 (-7.31 ± 0.09)	11
	NPR-1 Q3.32H	133 (-6.88 ± 0.26)	29	(1)	> 10,000 > -5	> 75	1580 (-5.80 ± 0.31)	12	186 (-6.73 ± 0.19)	1.4
	NPR-11 wt	107 (-6.97 ± 0.21)	(1)	(1)	417 (-6.38 ± 0.27)	3.9	1810 (-5.74 ± 0.60)	17	169 (-6.77 ± 0.37)	1.6
	NPR-11 Q3.32H	157 (-6.80 ± 0.36)	1.5	(1)	521 (-6.28 ± 0.41)	4.6	452 (-6.34 ± 0.39)	4	78 (-7.11 ± 0.34)	0.7
	NPR-11 Q3.32A	120 (-6.93 ± 0.14)	1.1	(1)	> 10,000 > -5	> 83	1736 (-5.76 ± 0.16)	14	176 (-6.75 ± 0.13)	1.5
		FLP-34-1			**[L16A]-FLP-34-1**		**[L16Q]-FLP-34-1**			
		K_i [nM] logK_i ± SEM	x-fold of NPR-11/ FLP-34-1	x-fold of mut/ FLP-34-1	K_i [nM] logK_i ± SEM	x-fold of mut/ FLP-34-1	K_i [nM] logK_i ± SEM	x-fold of mut/ FLP-34-1		
	NPR-11 wt	142 (-6.84 ± 0.09)	(1)	(1)	> 10,000 > -5	> 70	> 10,000 > -5	> 70		
	NPR-11 Q3.32H	345 (-6.46 ± 0.03)	2.4	(1)	> 10,000 > -5	> 29	> 10,000 > -5	> 29		
	NPR-11 Q3.32A	41 (-7.39 ± 0.01)	0.3	(1)	> 10,000 > -5	> 244	> 10,000 > -5	> 244		

Table S12. *C. elegans* strains used in this study.

Strain	Abbreviation used in main text	Genotype	Origin
N2	wild type	*C. elegans* wild isolate	[6]
CX4148	*npr-1*	*npr-1(ky13) X*	[7]
APR576	*Ex[npr-1]*	*npr-1 (ky13) X; aprEx229 [pnpr-1::npr-1::gfp, pmyo-3::mcherry, pBSK]*	[8]
APR657	*Ex[E6.59A]*	*npr-1(ky13) X; aprEx248 [pSP154, pmyo-3::mCherry, pBSK]*	*Generated in this study*
APR659	*Ex[E6.59A + D6.61A]*	*npr-1(ky13) X; aprEx250 [pSP155, pmyo-3::mCherry, pBSK]*	*Generated in this study*
APR661	*Ex[E6.59A + D6.61A + D6.62A]*	*npr-1(ky13) X; aprEx252 [pSP156, pmyo-3::mCherry, pBSK]*	*Generated in this study*

Table S13. Sequence of primers used to generate constructs presented in the study.

Primer	Sequence (5'- 3')
MluI_secNluc_for	AAAACGCGTGCCACCATGAACTCCTTCTCCACAAGC
Nluc_SGGGGS_rev	ACTACCTCCACCGCCTGACGCCAGAATGCGTTCGCAC
QC_NPR1-delNT-C9_for	CCACGCGTGCCACCATG CAAGTATATTGGAAAGTGTATCC
QC_NPR1-delNT-C9_rev	TTCCAATATACTTGCATGGTGGCACGCGTGGATCC
QC_NPR1_C9A_for	GAAAATTTTACCGACGCTCAAGTATATTGGAAAGTG
QC_NPR1-C9A_rev	CAATATACTTGAGCGTCGGTAAAATTTTCAAC
QC_NPR-1-T2.61A_f	CCAATCGCCCCAATCACAAATGTGTACAAAAACTGGTAC
QC_NPR-1-T2.61A_r	GATTGGGGCGATTGGAAGCGATAATATGCACATCATGC
QC_NPR-1-T2.61S_f	CTTCCAATCAGTCCAATCACAAATGTGTACAAAAACTGGTAC
QC_NPR-1-T2.61S_r	GATTGGACTGATTGGAAGCGATAATATGCACATCATGC
QC_NPR-1-W2.70A_f	CAAAAACGCCTACTTTGGAAATCTACTCTGCCATTTGATACC
QC_NPR-1-W2.70A_r	CCAAAGTAGGCGTTTTTGTACACATTTGTGATTGGAGTGATTG
QC_NPR-1_C3.25A_f	ATCTACTCGCCCATTTGATACCATGCATTCAAGG
QC_NPR-1_C3.25A_r	AAATGGGCGAGTAGATTTCCAAAGTACCAGTTTTTG
QC_NPR-1-Q3.32A_f	ATGCATTGCCGGTATCAGCATTTTCGTATGCACATTCAG
QC_NPR-1-Q3.32A_r	TGATACCGGCAATGCATGGTATCAAATGGCAGAGTAG
QC_NPR-1-Q3.32L_f	ATGCATTCTGGGTATCAGCATTTTCGTATGCACATTCAG
QC_NPR-1-Q3.32L_r	TGATACCCAGAATGCATGGTATCAAATGGCAGAGTAG
QC_NPR-1-Q3.32H_f	ATGCATTCACGGTATCAGCATTTTCGTATGCACATTCAG
QC_NPR-1-Q3.32H_r	TGATACCGTGAATGCATGGTATCAAATGGCAGAGTAG
QC_NPR-1-M4.57A_f	ATATGCAAGCCATTGAATACACAGAAGAGAGAATATGCGGC
QC_NPR-1-M4.57A_r	GTATTCAATGGCTTGCATATTGAACGCATAGGGTAGAGTTAC
QC_NPR-1-Y4.60A_f	GATTGAAGCCACAGAAGAGAGAATATGCGGCTACTTTTG
QC_NPR-1-Y4.60A_r	CTTCTGTGGCTTCAATCATTTGCATATTGAACGCATAGGG
QC_NPR-1_C5.21A_f	GAATAGCCGGCTACTTTTGCACTGAAAAGTGGG
QC_NPR-1_C5.21A_r	GTAGCCGGCTATTCTCTCTTCTGTGTATTCAATC
QC_NPR-1-F5.24A_f	GCTACGCCTGCACTGAAAAGTGGGAATCTGCCAAG
QC_NPR-1-F5.24A_r	AGTGCAGGCGTAGCCGCATATTCTCTCTTCTGTGTATTC

QC_NPR-1-F5.24L_f	GGCTACCTCTGCACTGAAAAGTGGGAATCTGCCAAG
QC_NPR-1-F5.24L_r	CAGTGCAGAGGTAGCCGCATATTCTCTCTTCTGTGTATTC
QC_NPR-1_C5.25A_f	CTACTTTGCCACTGAAAAGTGGGAATCTGCCAAG
QC_NPR-1_C5.25A_r	TTCAGTGGCAAAGTAGCCGCATATTCTCTCTTCTG
QC_NPR-1-E5.27A_f	TTGCACTGCCAAGTGGGAATCTGCCAAGTCTAGAAGAG
QC_NPR-1-E5.27A_r	CCACTTGGCAGTGCAAAAGTAGCCGCATATTCTCTCTTC
QC_NPR-1-E5.27Q_f	TTGCACTCAAAAGTGGGAATCTGCCAAGTCTAGAAGAG
QC_NPR-1-E5.27Q_r	CCACTTTTGAGTGCAAAAGTAGCCGCATATTCTCTCTTC
QC_NPR-1-E5.27D_f	TTGCACTGACAAGTGGGAATCTGCCAAGTCTAGAAGAG
QC_NPR-1-E5.27D_r	CCACTTGTCAGTGCAAAAGTAGCCGCATATTCTCTCTTC
QC_NPR1_E183(5.30)D_for	CTGAAAAGTGGGACTCTGCCAAGTCTAGAAG
QC_NPR1_E183(5.30)D_rev	CTTGGCAGAGTCCCACTTTTCAGTGC
QC_NPR1_T192(5.39)A_for	GAAGAGCCTACGCAATGATCGTGATGCTCG
QC_NPR1_T192(5.39)A_rev	CACGATCATTGCGTAGGCTCTTCTAGAC
QC_NPR-1-W6.48A_f	ATAACTGCCCTGCCACATAACGTCATTTCTTTGATTATTGAATA
QC_NPR-1-W6.48A_r	GTGGCAGGGCAGTTATCCCAAACCAGACAACCATG
QC_NPR-1-W6.48Y_f	GATAACTTACCTGCCACATAACGTCATTTCTTTGATTATTGAATA
QC_NPR-1-W6.48Y_r	GTGGCAGGTAAGTTATCCCAAACCAGACAACCATG
QC_NPR-1-W6.48H_f	ATAACTCACCTGCCACATAACGTCATTTCTTTGATTATTGAATA
QC_NPR-1-W6.48H_r	GTGGCAGGTGAGTTATCCCAAACCAGACAACCATG
QC_NPR-1-I6.58A_f	CTTTGATTGCCGAATATGATGACACACAATCGTTTTTCCG
QC_NPR-1-I6.58A_r	CATATTCGGCAATCAAAGAAATGACGTTATGTGGCAGCC
QC_NPR-1-E6.59A_f	ATTGCCTATGATGACACACAATCGTTTTTCCGACTTTATGG
QC_NPR-1-E6.59A_r	TGTGTCATCATAGGCAATAATCAAAGAAATGACGTTATGTGG
QC_NPR-1-E6.59D_f	TTATTGACTATGATGACACACAATCGTTTTTCCGACTTTATGGC
QC_NPR-1-E6.59D_r	GTGTGTCATCATAGTCAATAATCAAAGAAATGACGTTATGTGGC
QC_NPR-1-D6.61A_f	TTGAATATGCCGACACACAATCGTTTTTCCGACTTTATGGC
QC_NPR-1-D6.61A_r	GTGTGTCGGCATATTCAATAATCAAAGAAATGACGTTATGTGG
QC_NPR-1-E6.59A-D6.61A_f	ATTGCCTATGCCGACACACAATCGTTTTTCCGACTTTATGG
QC_NPR-1-E6.59A-D6.61A_r	TGTGTCGGCATAGGCAATAATCAAAGAAATGACGTTATGTGG
QC_NPR-1-D6.62A_f	GAATATGATGCCACACAATCGTTTTTCCGACTTTATGGCAG
QC_NPR-1-D6.62A_r	ATTGTGTGGCATCATATTCAATAATCAAAGAAATGACGTTATGTGG
QC_NPR-1-E6.59A-D6.61A-D6.62A_f:	TGCCTATGCCGCGACACAATCGTTTTTCCGACTTTATGG
QC_NPR-1-E6.59A-D6.61A-D6.62A_r:	GTCGCGGCATAGGCATAATCAAAGAAATGACGTTATGTGG
QC_NPR-1-D7.26A_f	GGCAGAGCCGATTACGATATCAGTTATTTACTGAACCTTTTC
QC_NPR-1-D7.26A_r	GTAATCGGCTCTGCCATAAAGTCGGAAAAACGATTGTGTG
QC_NPR-1-D7.27A_f	CAGAGATGCCTACGATATCAGTTATTTACTGAACCTTTTCACTC
QC_NPR-1-D7.27A_r	GATATCGTAGGCATCTCTGCCATAAAGTCGGAAAAACGATTG
QC_NPR-1-Y7.28A_f	AGATGATGCCGATATCAGTTATTTACTGAACCTTTTCACTCAC
QC_NPR-1-Y7.28A_r	CTGATATCGGCATCATCTCTGCCATAAAGTCGGAAAAAC
QC_NPR-1-Y7.32A_f	GATATCAGTGCCTTACTGAACCTTTTCACTCACAGTATTGC
QC_NPR-1-Y7.32A_r	CAGTAAGGCACTGATATCGTAATCATCTCTGCCATAAAGTC
QC_NPR11-delNT-D9_for	CGTGCCACCATG AATTATGTAGAAATTTTCAACAAAATC
QC_NPR11-delNT-D9_rev	AATTTCTACATAATTCATGGTGGCACGCGTGGATCC
QC_NPR11_C8A_for	CGGTGAATGAATCAGCTGACAATTATGTAGAAATTTTC

QC_NPR11_C8A_rev	CTACATAATTGTCAGCTGATTCATTCACCGATCC
QC_NPR-11_T2.61A_f	CCGACCGCCTTATACACGGTTCTCTACATGTTCTGG
QC_NPR-11_T2.61A_r	GTATAAGGCGGTCGGCGCTGTCACAATACAAACAAAAAAG
QC_NPR-11_T2.64A_f	CATTATACGCCGTTCTCTACATGTTCTGGCCATTTAGC
QC_NPR-11_T2.64A_r	GAGAACGGCGTATAATGTGGTCGGCGCTGTCAC
QC_NPR-11_M2.68A_f	CTCTACGCCTTCTGGCCATTTAGCAGGACATTATGC
QC_NPR-11_M2.68A_r	CCAGAAGGCGTAGAGAACCGTGTATAATGTGGTCG
QC_NPR-11_M2.68N_f	CTCTACAACTTCTGGCCATTTAGCAGGACATTATGC
QC_NPR-11_M2.68N_r	CCAGAAGTTGTAGAGAACCGTGTATAATGTGGTCG
QC_NPR-11_W2.70A_f	ATGTTCGCCCCATTTAGCAGGACATTATGCAAAATTGCG
QC_NPR-11_W2.70A_r	CTAAATGGGGCGAACATGTAGAGAACCGTGTATAATGTG
QC_NPR-11_C3.25A_f	GACATTAGCCAAAATTGCGGGTTCGCTGCAAGG
QC_NPR-11_C3.25A_r	CAATTTTGGCTAATGTCCTGCTAAATGGCCAGAAC
QC_NPR-11-Q3.32A_f	TCGCTGGCCGGCTTTAACATATTTTTATCCACATTCTCG
QC_NPR-11- Q3.32A_r	GCCGGCCAGCGAACCCGCAATTTTGCATAATGTC
QC_NPR-11-Q3.32H_f	TCGCTGCACGGCTTTAACATATTTTTATCCACATTCTCG
QC_NPR-11- Q3.32H_r	GCCGTGCAGCGAACCCGCAATTTTGCATAATGTC
QC_NPR-11_L4.51A_f	TTCCAGCCCTGCAGGCTTCTGATTTGACACCGG
QC_NPR-11_L4.51A_r	CTGCAGGGCTGGAACCGCAAGGATTAGGGAAATC
QC_NPR-11_C5.14A_f	CATCGGCCGATTTGGCTCTTTACATTTGCCATG
QC_NPR-11_C5.14A_r	CAAATCGGCCGATGGCTCAACGAAAACCGG
QC_NPR-11_I5.20A_f	CTTTACGCCTGCCATGAGCAAAATGAGATATGGGAAAAG
QC_NPR-11_I5.20A_r	CATGGCAGGCGTAAAGAGCCAAATCGCACGATGG
QC_NPR-11_C5.21A_f	TTACATTGCCCATGAGCAAAATGAGATATGGGAAAAG
QC_NPR-11_C5.21A_r	CTCATGGGCAATGTAAAGAGCCAAATCGCACGAT
QC_NPR-11-E5.23A_f	TGCCATGCCCAAAATGAGATATGGGAAAAGATGATCATATC
QC_NPR-11-E5.23A_r	TCATTTTGGGCATGGCAAATGTAAAGAGCCAAATCGCAC
QC_NPR-11_E5.23Q_f	TTTGCCATCAACAAAATGAGATATGGGAAAAGATGATCATATCAAAAG
QC_NPR-11_E5.23Q_r	CTCATTTTGTTGATGGCAAATGTAAAGAGCCAAATCGCACG
QC_NPR-11_E5.23D_f	TTTGCCATGACCAAAATGAGATATGGGAAAAGATGATCATATCAAAAGG
QC_NPR-11_E5.23D_r	CTCATTTTGGTCATGGCAAATGTAAAGAGCCAAATCGCACG
QC_NPR-11_E5.26Q_f	GAGCAAAATCAAATATGGGAAAAGATGATCATATCAAAAGGCAC
QC_NPR-11_E5.26Q_r	CCCATATTTGATTTTGCTCATGGCAAATGTAAAGAGCCAAATC
QC_NPR-11_E5.26D_f	GAGCAAAATGACATATGGGAAAAGATGATCATATCAAAAGGCAC
QC_NPR-11_E5.26D_r	CCCATATGTCATTTTGCTCATGGCAAATGTAAAGAGCCAAATC
QC_NPR11_E199(5.26)A_for	CATTTGCCATGCTCAAAATGAGATATGGGAAA
QC_NPR11_E199(5.26)A_rev	CATATCTCATTTTGAGCATGGCAAATGTAAAGAG
QC_NPR11_E202(5.29)A_for	CATGAGCAAAATGCTATATGGGAAAAGATGATC
QC_NPR11_E202(5.29)A_rev	TTTCCCATATAGCATTTTGCTCATGGCAAATGTAAAG
QC_NPR11_T212(5.39)A_for	CAAAAGGCACCTACGCGTTGGCAGTTCTTATC
QC_NPR11_T212(5.39)A_rev	GAACTGCCAACGCGTAGGTGCCTTTTGATATG
QC_NPR-11_Q5.46A_f	ATCACCGCCTACGCATTTCCCCTGTTTTCACTAGTC
QC_NPR-11_Q5.46A_r	TGCGTAGGCGGTGATAAGAACTGCCAACGTGTAGG
QC_NPR-11-W6.48A_f	TCGCCGCCCTGCCACTCAACGTTTTTCATATCTTC
QC_NPR-11- W6.48A_r	GCAGGGCGGCGACGGCGAATACAGCTACAAC
QC_NPR-11_N6.58A_f	TATCTTCGCCACATTCGAGCTGGTCAACAGTTTTTCC
QC_NPR-11_N6.58A_r	CGAATGTGGCGAAGATATGAAAAACGTTGAGTGGCAG

QC_NPR-11_T6.59A_f	CTTCAACGCCTTCGAGCTGGTCAACAGTTTTTCCGTTAC
QC_NPR-11_T6.59A_r	CTCGAAGGCGTTGAAGATATGAAAAACGTTGAGTGGCAG
QC_NPR-11-E6.61A_f:	ATTCGCCCTGGTCAACAGTTTTTCCGTTACAACGTTCAG
QC_NPR-11-E6.61A_r:	GTTGACCAGGGCGAATGTGTTGAAGATATGAAAAACGTTG
QC_NPR-11_F7.35A_f	ACAACGGCCAGCATCTGTCACTGCTTGGCAATGTG
QC_NPR-11_F7.35A_r	GATGCTGGCCGTTGTAACGGAAAAACTGTTGACCAG
QC_NPR-11_F7.35L_f	TTACAACGCTCAGCATCTGTCACTGCTTGGCAATGTG
QC_NPR-11_F7.35L_r	GATGCTGAGCGTTGTAACGGAAAAACTGTTGACCAGC
QC_NPR-11_F7.35H_f	TTACAACGCACAGCATCTGTCACTGCTTGGCAATGTG
QC_NPR-11_F7.35H_r	GATGCTGTGCGTTGTAACGGAAAAACTGTTGACCAGC
QC.npr-1-E6.59A_f	ATTGCCTATGATGACACACAATCGTTTTTCCGACTTTATGG
QC-npr-1-E6.59A_r	TGTGTCATCATAGGCAATAATCAAAGAAATGACGTTATGTGG
QC-npr-1_E6.59A-D6.61A_r	ATTGCCTATGCCGACACACAATCGTTTTTCCGACTTTATGG
QC-npr-1_E6.59A-D6.61A_f	TGTGTCGGCATAGGCAATAATCAAAGAAATGACGTTATGTGG
QC.npr-1-E6.59-D6.61A-D6.62A_f	ATTGCCTATGCCGCGACACAATCGTTTTTCCGACTTTATGG
QC.npr-1-E6.59-D6.61A-D6.62A_r	TGTCGCGGCATAGGCAATAATCAAAGAAATGACGTTATGTGG
SGGGGS_NPR-1_for	TCAGGCGGTGGAGGTAGTATGGAAGTTGAAAATTTTACCGAC
SGGGGS_NPR-11_for	TCAGGCGGTGGAGGTAGTATGGGATCGGTGAATGAATC
YFP-XbaI-NheI-r	TTTGCTAGCGTGTTACCCCTCTAGACCTG

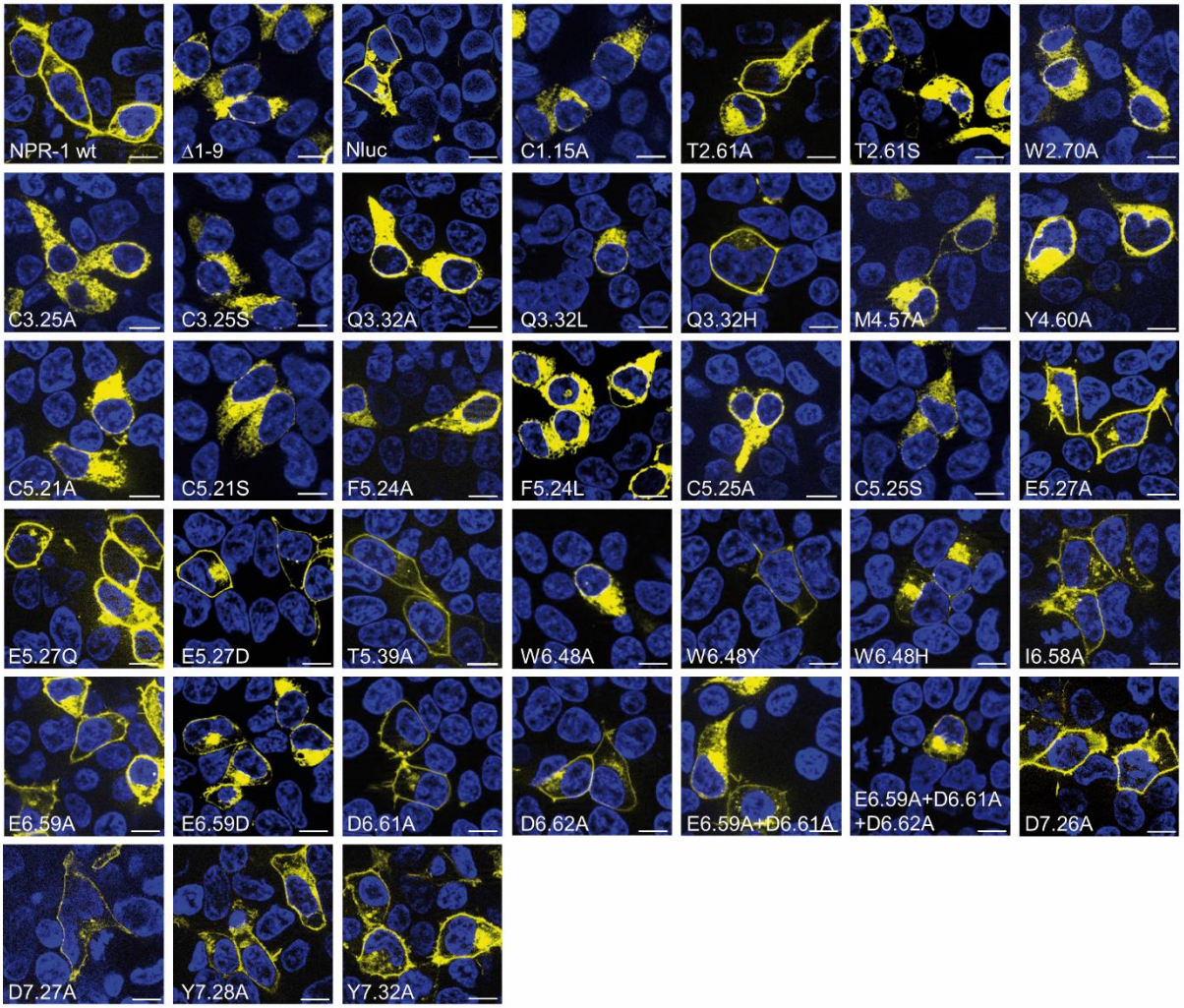

Figure S1. Live cell fluorescence microscopy of *C. elegans* NPR-1 mutants. The receptor variants are genetically fused C-terminally to eYFP (yellow) and transiently transfected into HEK293 cells, nuclei were stained with Hoechst33342 (blue). Several mutants are not correctly folded and exported to the plasma membrane and were not further assessed in functional assays: Δ1-9, C1.15A, W2.70A, C3.25A, C3.25S, Q3.32A, Q3.32L, M4.57A, Y4.60A, C5.21A, C5.21S, F5.24A, F5.24L, C5.25A, C5.25S, W6.48A. Scale bar: 10 µm. Images are representative of three independent experiments.

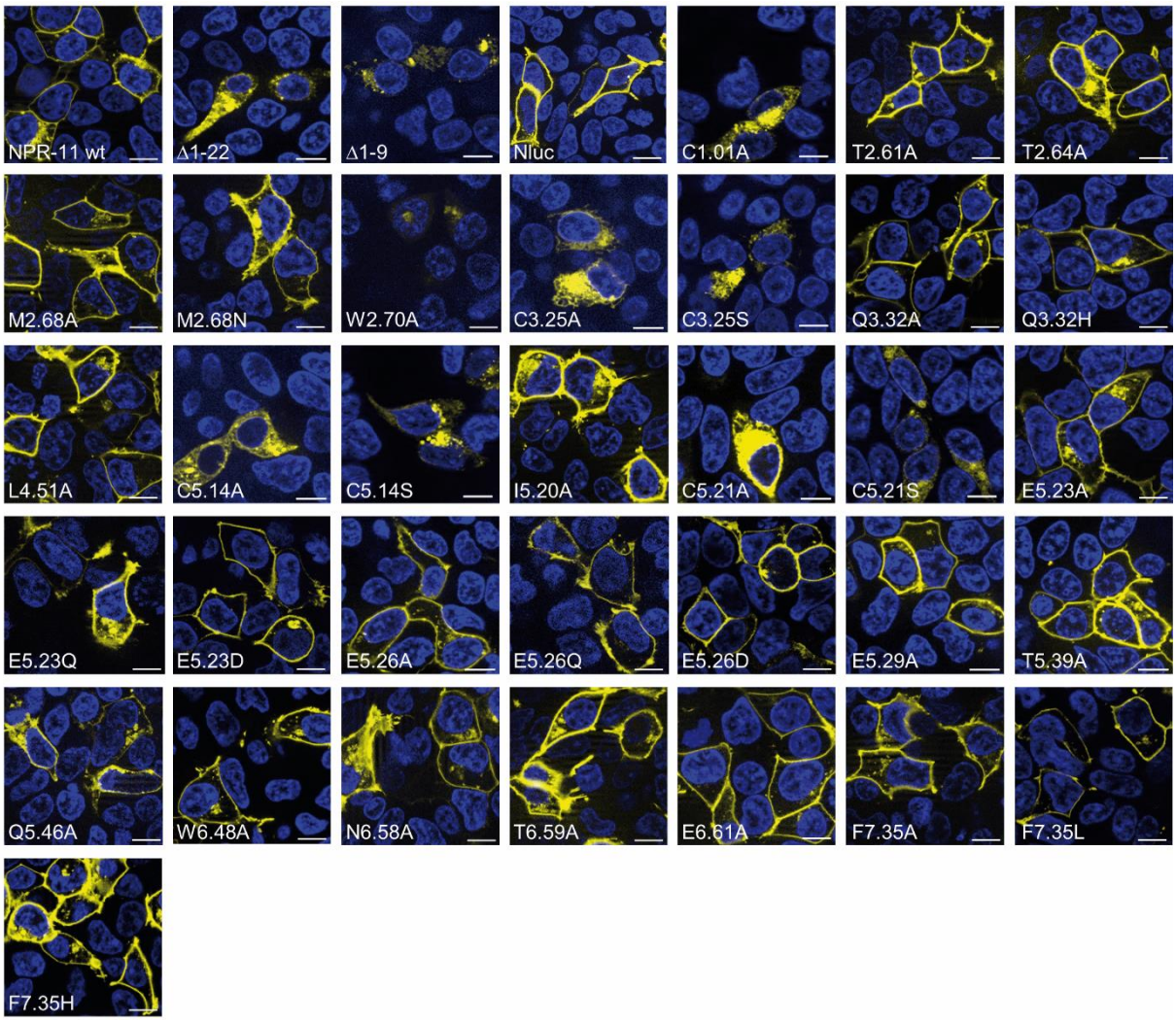

Figure S2. Live cell fluorescence microscopy of *C. elegans* NPR-11 mutants. The receptor variants are genetically fused C-terminally to eYFP (yellow) and transiently transfected into HEK293 cells, nuclei were stained with Hoechst33342 (blue). Several mutants are not correctly folded and exported to the plasma membrane and were not further assessed in functional assays: Δ1-22, Δ1-9, C1.01A, W2.70A, C3.25A, C3.25S, C5.14A, C5.14S, C5.21A, C5.21S. Scale bar: 10 µm. Images are representative of three independent experiments.

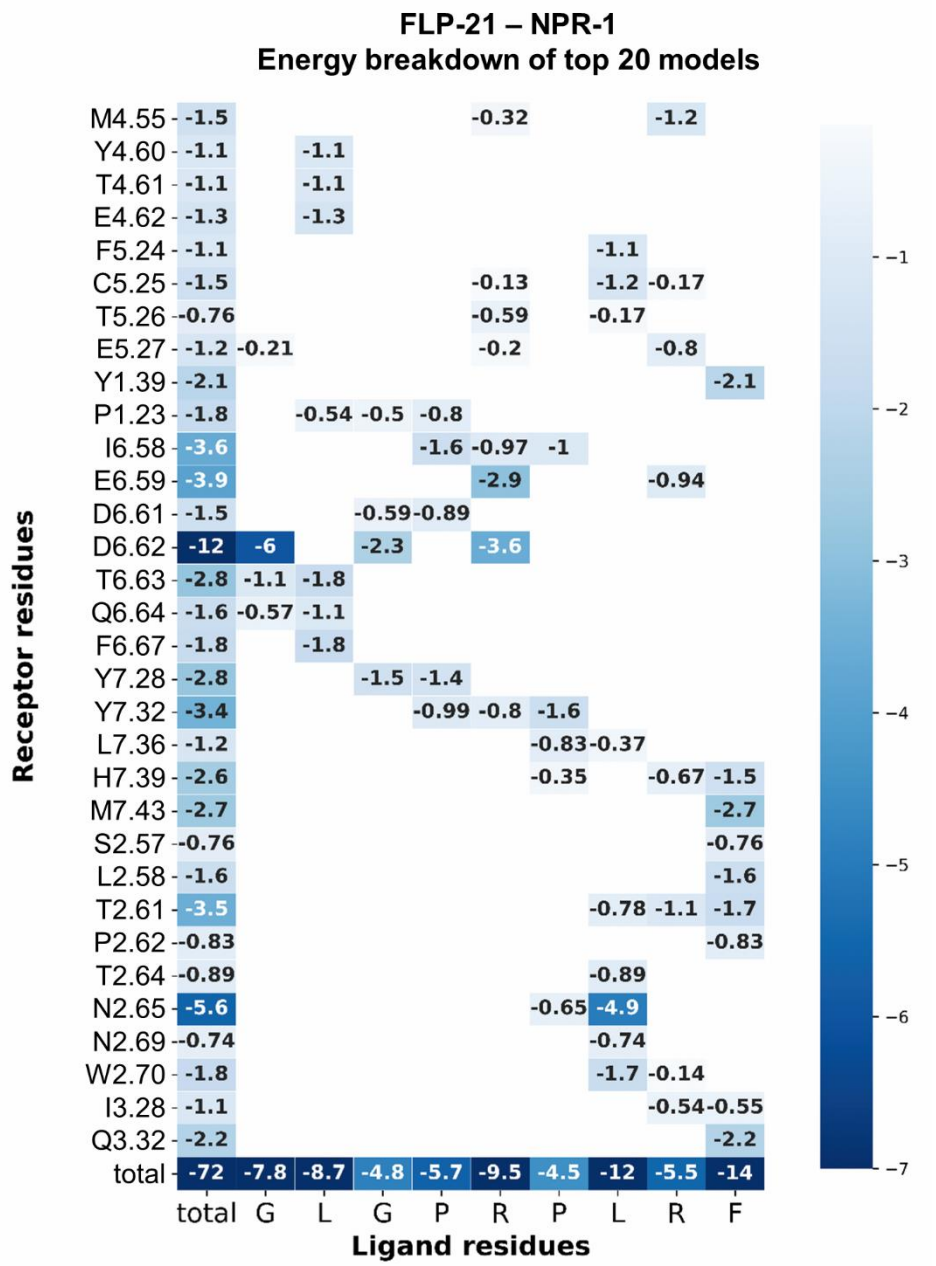

Figure S3. Quantitative analysis of the per-residue energy for the top 20 models of FLP-21 docked into NPR-1. The energetic analysis reflects the contribution of receptor and ligand positions to the binding energy and matches well with the functional data obtained by mutagenesis of the receptor and the ligand. The interface energy is averaged from all models exceeding an interaction energy threshold of 0.1 for this residue. Only receptor residues with an averaged energetic contribution of > 0.5 Rosetta Energy Units (REU) are shown.

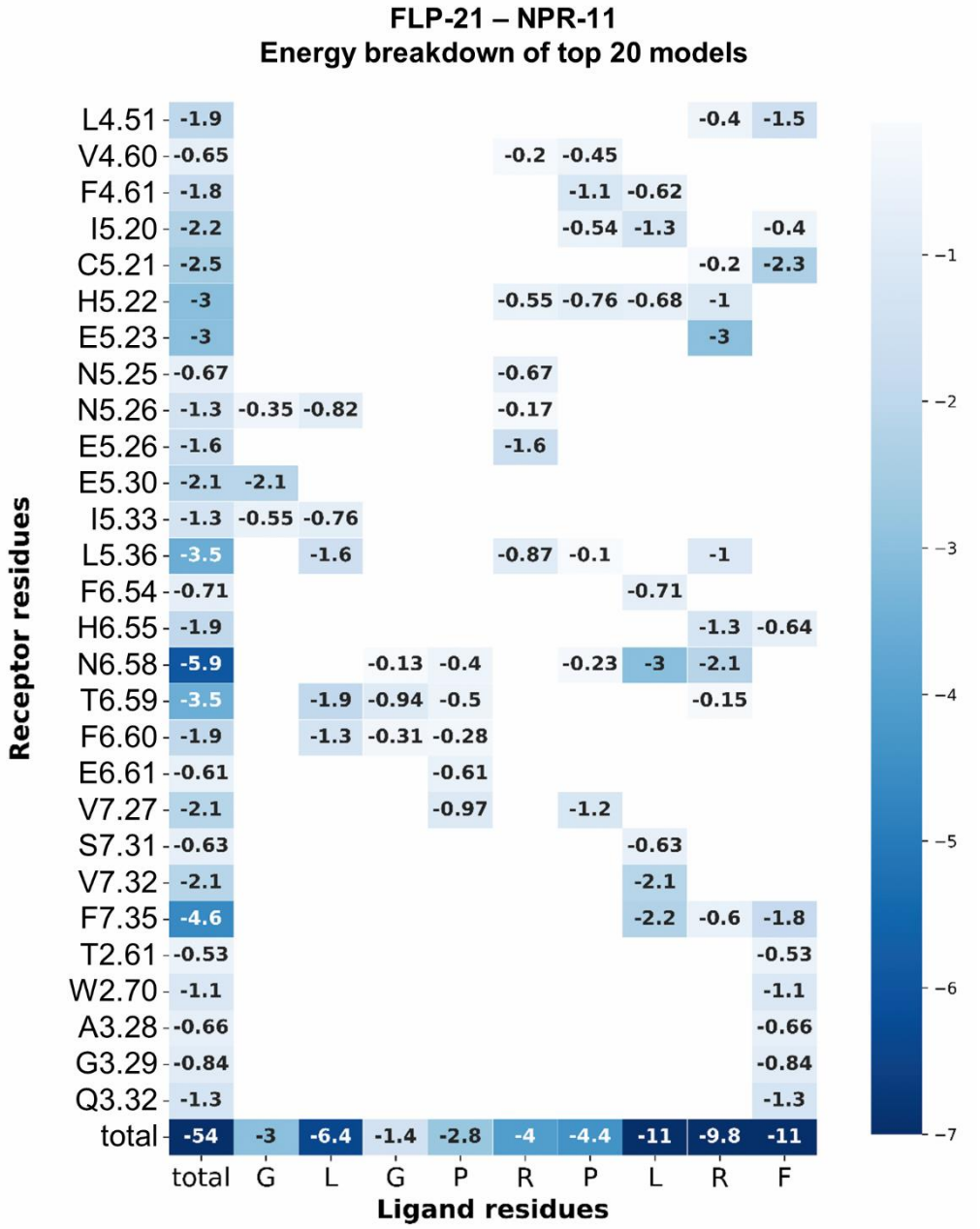

Figure S4. Quantitative analysis of the per-residue energy for the top 20 models of FLP-21 docked into NPR-11. The energetic analysis reflects the contribution of receptor and ligand positions to the binding energy and matches well with the functional data obtained by mutagenesis of the receptor and the ligand. The interface energy is averaged from all models exceeding an interaction energy threshold of 0.1 for this residue. Only receptor residues with an averaged energetic contribution of > 0.5 Rosetta Energy Units (REU) are shown.

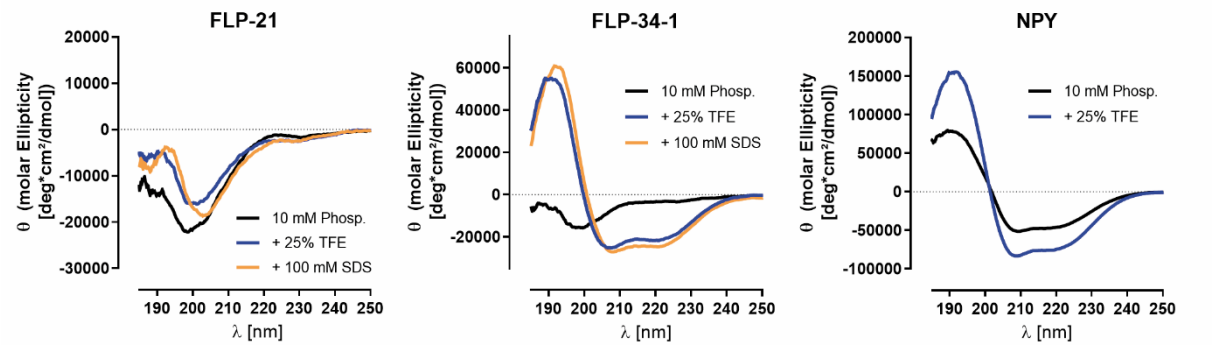

Figure S5. CD spectroscopic analysis of ligands shows that an α-helical shape can be induced in the FLP-34-1 ligand but not in FLP-21. Ligands FLP-21 (left), FLP-34-1 (middle) and NPY (right) were analyzed with CD spectroscopy at a concentration of 25 µM in 10 mM phosphate buffer (pH 7.0) +/- additions to assess their secondary structure. FLP-21 (left) forms a random coil. FLP-34-1 (middle) is a random coil in pure buffer solution, but an α-helical structure with the characteristic minimum at 208 and 222 nm can be induced by addition of 25% trifluoroethanol (TFE) or 100 mM sodium dodecyl sulfate (SDS) as a membrane mimetic. NPY (control, right) forms an α-helix in aqueous solution, which is reinforced by addition of 25% TFE. Data is representative of two independent experiments.

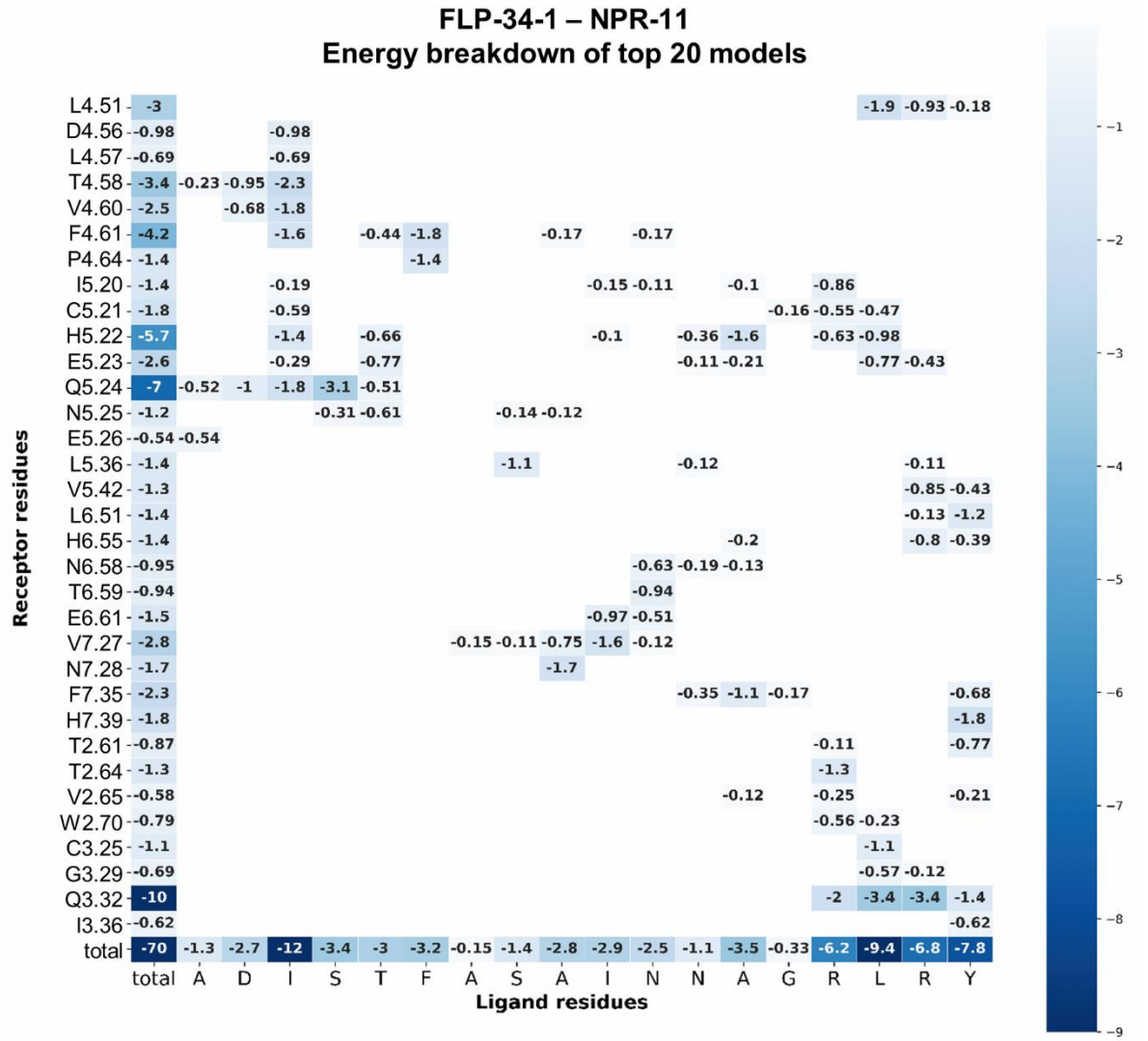

Figure S6. Quantitative analysis of the per-residue energy for the top 20 models of FLP-34-1 docked into NPR-11. The energetic analysis reflects the contribution of receptor and ligand positions to the binding energy and matches well with the functional data obtained by mutagenesis of the receptor and the ligand. The interface energy is averaged from all models exceeding an interaction energy threshold of 0.1 for this residue. Only receptor residues with an averaged energetic contribution of > 0.5 Rosetta Energy Units (REU) are shown.

Supplementary References

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