

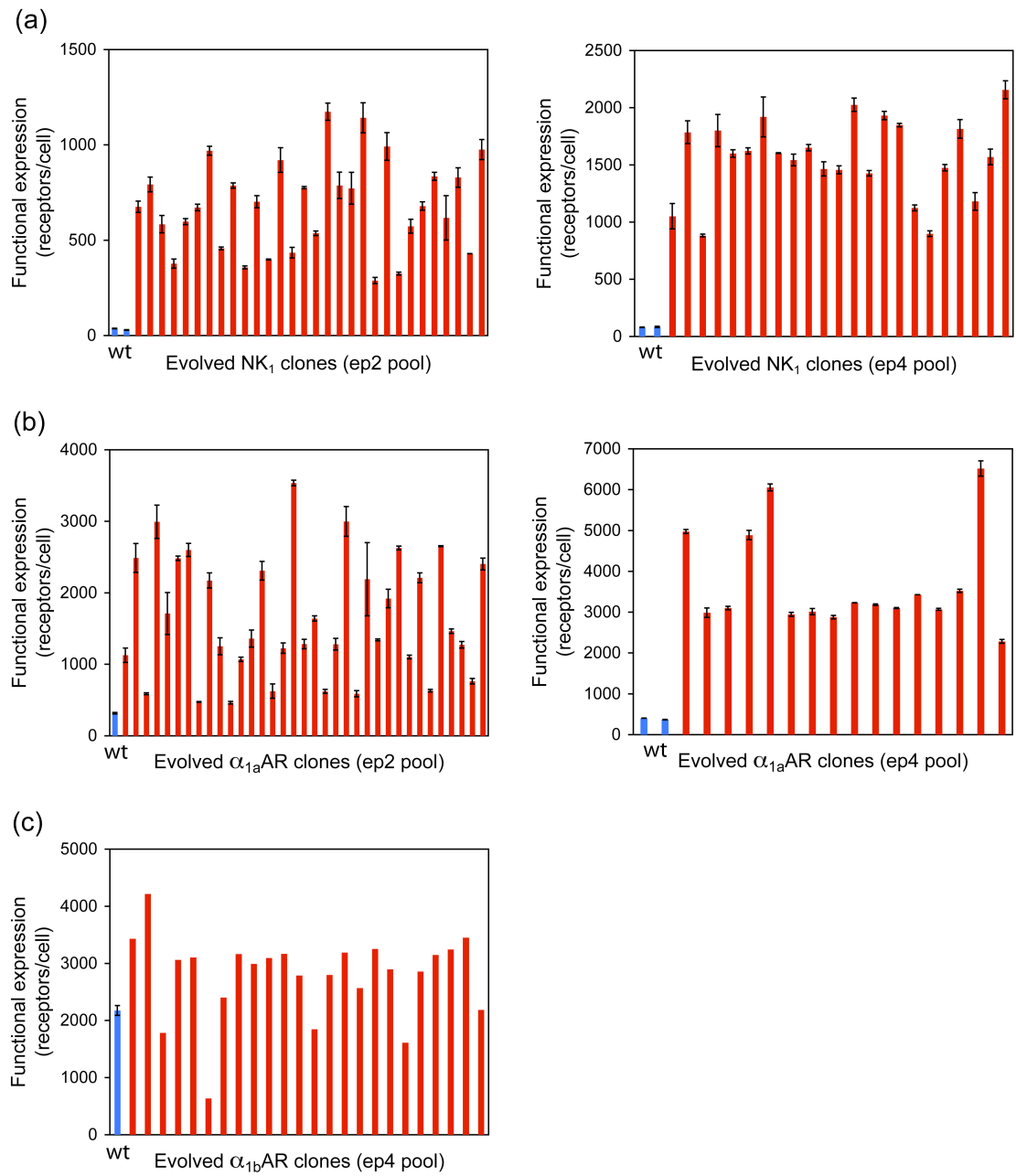
Supporting Material

Evolution of three human GPCRs for higher expression and stability

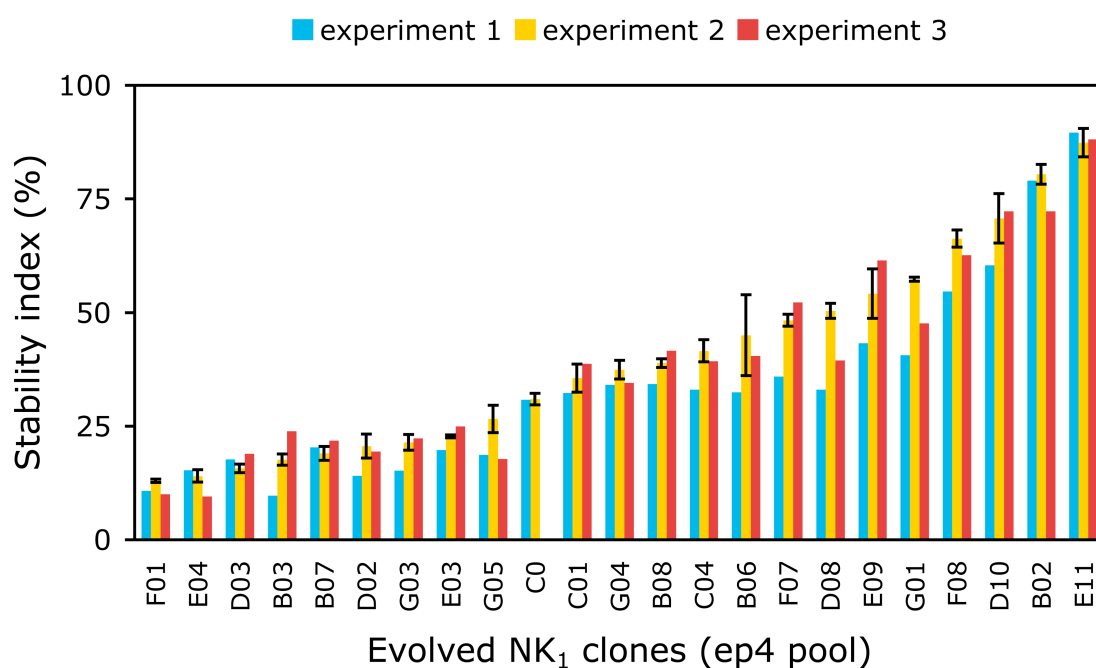
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Supplementary Figure 1



Supplementary Figure 1: Increased functional expression level of evolved receptor variants of NK₁, α_{1a} AR and α_{1b} AR after two or four rounds of evolution. One round of evolution consists of one error prone PCR followed by four to six rounds of sorting by FACS. The expression level of single clones was measured by radioligand binding assays on whole cells expressing evolved receptors (red bars) and the corresponding wt receptors (blue bars). **(a)** Single evolved NK₁ receptors isolated after two or four rounds of evolution (ep2 pool and ep4 pool respectively). **(b)** Single evolved α_{1a} AR receptors isolated after two or four rounds of evolution. **(c)** Single evolved α_{1b} AR receptors isolated after two rounds of evolution. The α_{1b} AR receptor was evolved for two rounds only.



Supplementary Figure 2: Reproducibility of the new stability screening method in a 96-well format. The comparison of three independent stability screens of a set of evolved NK₁ receptors shows that the new screening method yields highly reproducible results. The error bars in experiment 2 indicate the standard error of duplicate measurements of the stability index of a solubilized receptor sample.

Supplementary Figure 3

(a)

Clone	Expression (rec/cell)
NK1 wt	30
NK1-C0	750
NK1-09	790
NK1-02	793
NK1-13	920
NK1-07	970
NK1-30	980
NK1-22	1000
NK1-20	1150

(b)

Clone	Expression (rec/cell)	Stability Index (%)
NNK1 wt	80	0
NNK1-F01	1790	11
NNK1-E04	880	13
NNK1-F04	1600	15
NNK1-B03	990	17
NNK1-D03	890	17
NNK1-D02	1060	18
NNK1-F09	1480	18
NNK1-G03	1640	20
NNK1-B07	2030	24
NNK1-E03	1620	24
NNK1-C0	1130	31
NNK1-G04	1540	37
NNK1-C01	1050	38
NNK1-H02	1600	38
NNK1-C04	1920	39
NNK1-B10	1820	39
NNK1-B06	1460	40
NNK1-D08	1850	42
NNK1-F07	1430	47
NNK1-G01	890	50
NNK1-E09	900	54
NNK1-F08	1120	61
NNK1-D10	1180	68
NNK1-H04	1650	70
NNK1-B02	1800	78
NNK1-E11	1570	91

	TM1	TM2	TM3	TM4	TM5	TM6	TM7	
8	D	S	P	V	V	V	V	R
9	S	P	V	V	V	V	V	R
10	S	P	V	V	V	V	V	R
11	S	P	V	V	V	V	V	R
12	S	P	V	V	V	V	V	R
13	S	P	V	V	V	V	V	R
14	S	P	V	V	V	V	V	R
15	S	P	V	V	V	V	V	R
16	S	P	V	V	V	V	V	R
17	S	P	V	V	V	V	V	R
18	S	P	V	V	V	V	V	R
19	S	P	V	V	V	V	V	R
20	S	P	V	V	V	V	V	R
21	S	P	V	V	V	V	V	R
22	S	P	V	V	V	V	V	R
23	S	P	V	V	V	V	V	R
24	S	P	V	V	V	V	V	R
25	S	P	V	V	V	V	V	R
26	S	P	V	V	V	V	V	R
27	S	P	V	V	V	V	V	R
28	S	P	V	V	V	V	V	R
29	S	P	V	V	V	V	V	R
30	S	P	V	V	V	V	V	R
31	S	P	V	V	V	V	V	R
32	S	P	V	V	V	V	V	R
33	S	P	V	V	V	V	V	R
34	S	P	V	V	V	V	V	R
35	S	P	V	V	V	V	V	R
36	S	P	V	V	V	V	V	R
37	S	P	V	V	V	V	V	R
38	S	P	V	V	V	V	V	R
39	S	P	V	V	V	V	V	R
40	S	P	V	V	V	V	V	R
41	S	P	V	V	V	V	V	R
42	S	P	V	V	V	V	V	R
43	S	P	V	V	V	V	V	R
44	S	P	V	V	V	V	V	R
45	S	P	V	V	V	V	V	R
46	S	P	V	V	V	V	V	R
47	S	P	V	V	V	V	V	R
48	S	P	V	V	V	V	V	R
49	S	P	V	V	V	V	V	R
50	S	P	V	V	V	V	V	R
51	S	P	V	V	V	V	V	R
52	S	P	V	V	V	V	V	R
53	S	P	V	V	V	V	V	R
54	S	P	V	V	V	V	V	R
55	S	P	V	V	V	V	V	R
56	S	P	V	V	V	V	V	R
57	S	P	V	V	V	V	V	R
58	S	P	V	V	V	V	V	R
59	S	P	V	V	V	V	V	R
60	S	P	V	V	V	V	V	R
61	S	P	V	V	V	V	V	R
62	S	P	V	V	V	V	V	R
63	S	P	V	V	V	V	V	R
64	S	P	V	V	V	V	V	R
65	S	P	V	V	V	V	V	R
66	S	P	V	V	V	V	V	R
67	S	P	V	V	V	V	V	R
68	S	P	V	V	V	V	V	R
69	S	P	V	V	V	V	V	R
70	S	P	V	V	V	V	V	R
71	S	P	V	V	V	V	V	R
72	S	P	V	V	V	V	V	R
73	S	P	V	V	V	V	V	R
74	S	P	V	V	V	V	V	R
75	S	P	V	V	V	V	V	R
76	S	P	V	V	V	V	V	R
77	S	P	V	V	V	V	V	R
78	S	P	V	V	V	V	V	R
79	S	P	V	V	V	V	V	R
80	S	P	V	V	V	V	V	R
81	S	P	V	V	V	V	V	R
82	S	P	V	V	V	V	V	R
83	S	P	V	V	V	V	V	R
84	S	P	V	V	V	V	V	R
85	S	P	V	V	V	V	V	R

Supplementary Figure 3: Amino acid mutations, functional expression and stability index of evolved NK₁ receptors. **(a)** Mutations of the highest expressed receptor variants of the ep2 pool. **(b)** Mutations of randomly picked receptor variants of the ep4 pool. The receptors are listed based on increasing expression level. For each mutation the corresponding wt amino acid and the position in the wt NK₁ sequence are indicated on top of the mutation data.

Supplementary Figure 4

Clone	Expression Stability Index	TM1	TM2	TM3	TM4	TM5	TM6	TM7
A1a wt	320							
A1a-43	2650							
A1a-28	3000							
A1a-07	2600							
A1a-23	3540							
A1a-02	2560							
A1a-04	3000							
A1a-41	2210							
A1a-26	620							
A1a-44	1460							
A1a-06	2480							
A1a-11	1250							
A1a-01	1130							
A1a-12	460							
A1a-20	2310							
A1a-08	470							
A1a-29	590							
A1a-31	2190							
A1a-03	600							
A1a-27	1310							
A1a-14	1070							
A1a-22	1230							
A1a-42	630							
A1a-05	1820							

Supplementary Figure 4: Amino acid mutations, functional expression and stability index of evolved α_{1a} AR receptors. The data show randomly picked clones after two rounds of evolution (ep2 pool). The receptors are listed based on increasing expression level. For each mutation the corresponding wt amino acid and the position in the wt α_{1a} AR sequence are indicated on top of the mutation data.

Clone	Expression	Stability	Genetic Map																														
	(rec/cell)	Index (%)	<div><div>TM2</div><div>TM3</div><div></div><div>TM5</div><div>TM6</div><div>TM7</div></div>																														
A1b wt	2020	45	T	T	L	I	F	L	P	D	K	G	V	A	M	S	K	F	L	C	R	R	R	R	C	R	D	P	P	P	R	P	N
A1b-19	1350	91					N	S				M			I									H	S								
A1b-13	1390	79														N	R	L															
A1b-05	1530	82	I	I				M										L															
A1b-14	1590	81									Y							L	I	Y	C	H								S		S	
A1b-22	1600	87			M							E						L						H	S			L					
A1b-C10	2060	86																L															H
A1b-12	2580	83				N						F	G					L					H				E					S	

Supplementary Figure 5: Amino acid mutations, functional expression and stability index of evolved α_{1b} AR receptors. The data show the most stable clones after two rounds of evolution (ep2 pool). For each mutation the corresponding wt amino acid and the position in the wt α_{1b} AR sequence are indicated on top of the mutation data.