Supporting Material

Evolution of three human GPCRs for higher expression and stability

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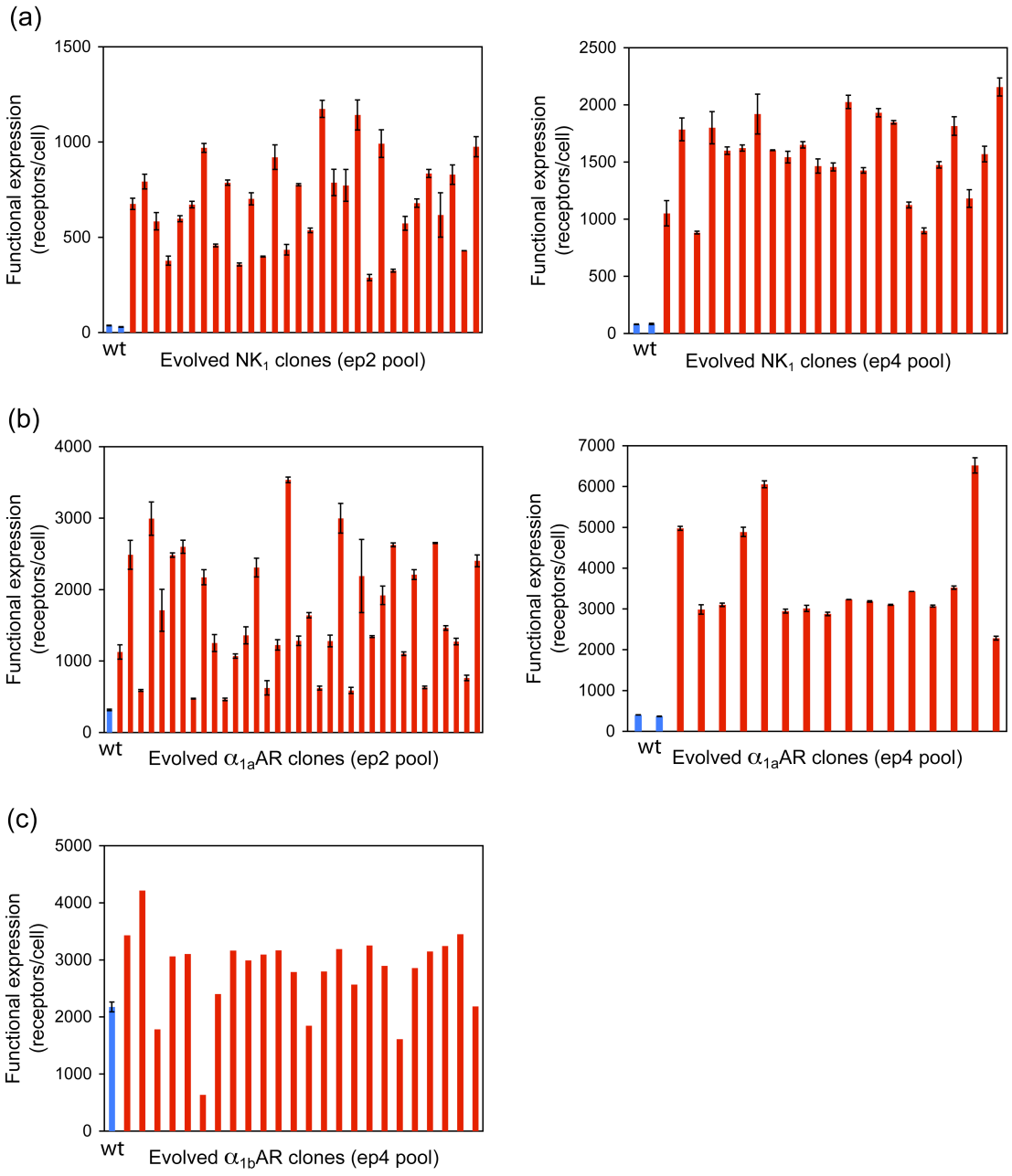
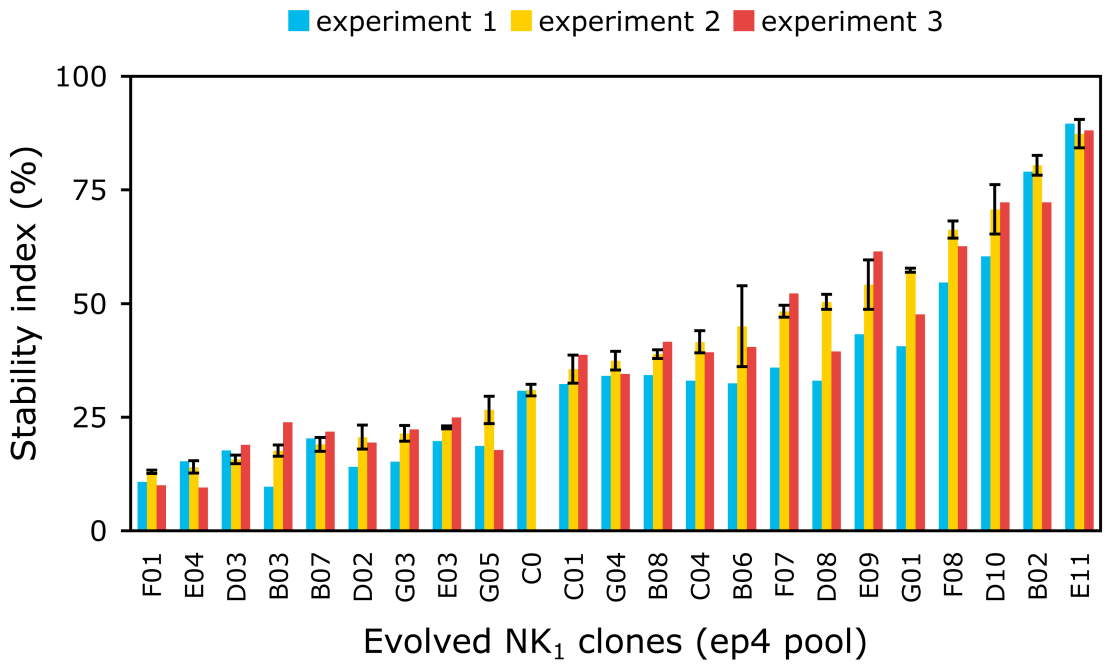
Supplementary Figure 1



Supplementary Figure 1: Increased functional expression level of evolved receptor variants of NK_1, 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 NK_1 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.

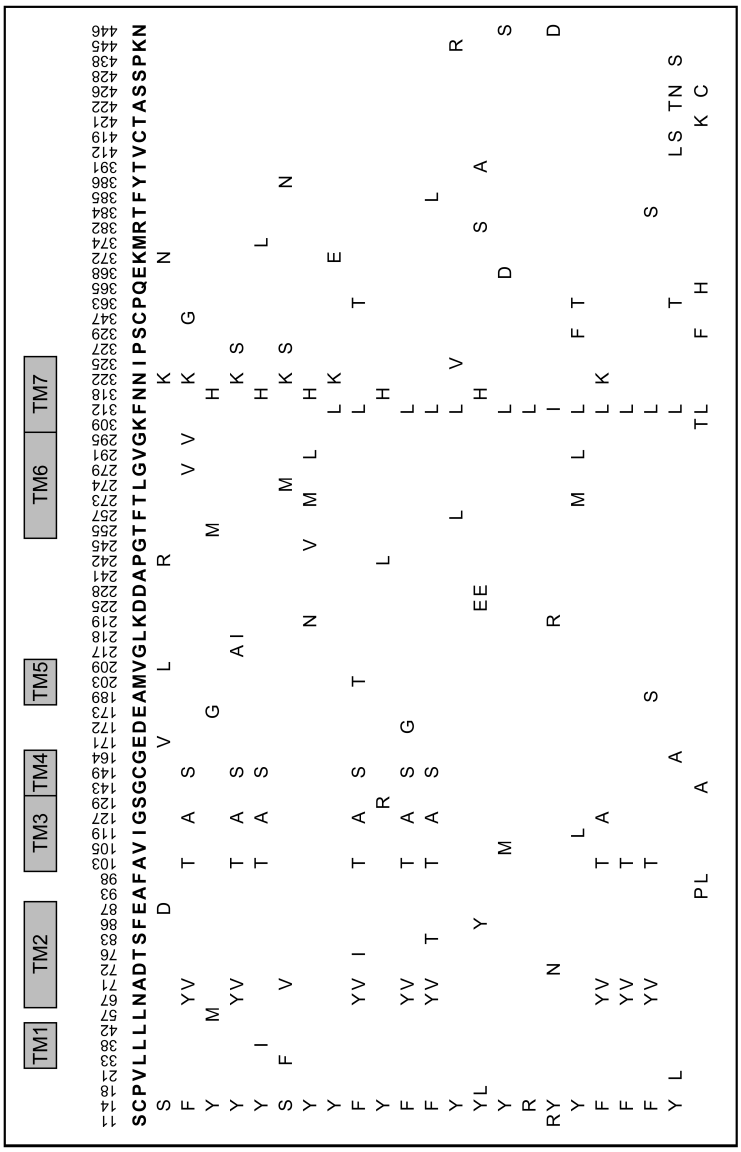


Supplementary Figure 2: Reproducibility of the new stability screening method in a 96-well format. The comparison of three independent stability screens of a set of evolved NK_1 receptors shows that the new screening method yields highly reproducible results. The error bars in experiment 2 indicate the standard error of duplicate measurements of the stability index of a solubilized receptor sample.

Supplementary Figure 3

Supplementary Figure 3: Amino acid mutations, functional expression and stability index of evolved NK_1 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 NK_1 sequence are indicated on top of the mutation data.

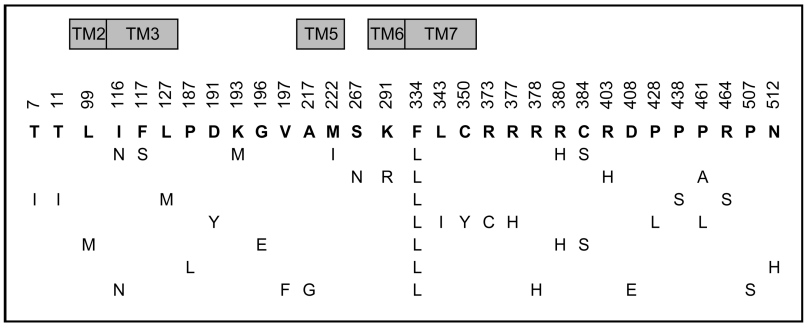
Supplementary Figure 4



Clone	Expression (rec/cell)	Stability Index (%)
A1a wt	320	56
A1a-43	2650	34
A1a-28	3000	45
A1a-07	2600	49
A1a-23	3540	52
A1a-02	2560	57
A1a-04	3000	58
A1a-41	2210	63
A1a-26	620	63
A1a-44	1460	68
A1a-06	2480	69
A1a-11	1250	69
A1a-01	1130	73
A1a-12	460	77
A1a-20	2310	78
A1a-08	470	78
A1a-29	590	80
A1a-31	2190	82
A1a-03	600	83
A1a-27	1310	83
A1a-14	1070	83
A1a-22	1230	89
A1a-42	630	96
A1a-05	1820	96

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 (rec/cell)	Stability Index (%)
A1b wt	2020	45
A1b-19	1350	91
A1b-13	1390	79
A1b-05	1530	82
A1b-14	1590	81
A1b-22	1600	87
A1b-C10	2060	86
A1b-12	2580	83



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.