Supplementary Material

Cell-Free expression and Photo-Crosslinking of the Human Neuropeptide Y2 Receptor

Lisa Maria Kögler1, Jan Stichel1, Anette Kaiser1, Annette G. Beck-Sickinger1

1Institute of Biochemistry, Faculty of Life Sciences, Leipzig University, Brüderstr. 34, D 04103 Leipzig, Germany

**\* Correspondence:**Annette G. Beck-Sickinger  
abeck-sickinger@uni-leipzig.de

U:\Publications\Front_Pharmacol Cell-Free Y2 Expression\Figures_Publication_3\LisaKoegler_SI_Figure_2.tif

**Supplementary Figure S1.** Summary of IP accumulation assays with increasing concentrations of NPY, 5(6)-TAMRA-Ahx(5-24)NPY, biotin-(Ahx)2-NPY or [K22[(Ahx)2-biotin]Bpa27]NPY at Y2R\_cysteine\_deficient. EC50 values were determined using GraphPad Prism 5.0 nonlinear regression (curve fit), normalized to the associated NPY curves. n ≥ 2.

**U:\Publications\Front_Pharmacol Cell-Free Y2 Expression\Figures Publication 2\LisaKoegler_SI_Figure_1A.tifU:\Publications\Front_Pharmacol Cell-Free Y2 Expression\Figures Publication 2\LisaKoegler_SI_Figure_1B.tif**

**Supplementary Figure S2.** Restriction sites in [K22[(Ahx)2-biotin]Bpa27]NPY and Y2R\_cysteine\_deficient after digestion with the endoproteinase chymotrypsin. **(A)** Amino acid sequence of [K22[(Ahx)2-biotin]Bpa27]NPY with highlighted restriction sites. **(B)** Amino acid sequence of Y2R\_cysteine\_deficient with a C-terminal deca-histidine-tag. N terminus and extracellular loops are highlighted in cyan, transmembrane helices in black and intracellular loops and the modified C terminus in gray. Restriction sites of chymotrypsin are indicated.

**U:\Publications\Front_Pharmacol Cell-Free Y2 Expression\Figures_Publication_3\LisaKoegler_Supplementary_Figure_2.tif**

**Supplementary Figure S3. Western Blots of the optimization of cell-free Y2R\_eGFP expression**. **(A)** To monitor soluble receptor expression the crude reaction (1,3,5) was centrifuged for 2 min at 13,000 rpm and the supernatant (2,4,6) was used for analysis. Y2R\_eGFP expression was performed without detergents (1,2) or in presence of DDM and Chaps (3,4), which completely inhibit the receptor expression. As expression control, human chemerin was used, expressed without detergents (5,6). **(B)** Soluble expression of Y2R\_eGFP (1,2) or Y2R\_eYFP (3) without detergents (1) or in presence of Brij-35 (2,3). Nonsoluble protein was removed after expression by centrifugation. As control the RM without DNA was plotted (4). **(C)** Soluble expression of Y2R\_eGFP in presence of 0.5 % (w/v) (1,2) or 0.2 % (w/v) Brij-58. Crude reaction and supernatant after centrifugation for 2 min at 13,000 rpm were plotted to monitor soluble receptor expression. **(D)** The Y2R\_eGFP expression in presence of oxidized (GSSG) and reduced (GSH) glutathione was monitored by Western Blot analysis. A higher amount of GSH promotes receptor expression (1), while an increased amount of GSSG strongly inhibits it (2). A change in the redox potential during expression by omitting DTT during expression (3) completely inhibits receptor expression. The RM without DNA was used as control (4). **(E)** Buffer pH was lowered towards pH 7.4 (2) and the effect of GSH and GSSG (1) or CHS (3) addition during expression was monitored. The negative control contents the RM without DNA (4). Arrows indicate synthesized receptors. Brij-35 – polyoxyethylene(23)laurylether, Brij-58 – polyoxyethylene(20)cetyl-ether, Chaps – 3-[(3-cholamidopropyl)dimethylammonio]-1-propansulfonat, CHS – cholesteryl hemisuccinate; DDM – n-dodecyl β-D-maltoside, DTT – dithiothreitol**,** RM – reaction mix; n ≥ 2.

U:\Publications\Front_Pharmacol Cell-Free Y2 Expression\Figures_Publication_3\LisaKoegler_SI_Figure_4A.tif

U:\Publications\Front_Pharmacol Cell-Free Y2 Expression\Figures_Publication_3\LisaKoegler_SI_Figure_4B.tif

U:\Publications\Front_Pharmacol Cell-Free Y2 Expression\Figures_Publication_3\LisaKoegler_SI_Figure_4C.tif

**Supplementary Figure S4:** Analysis of tandem MS/MS spectra by Biotools, allowing crosslinking to occur between Bpa at position 27 of NPY and one amino acid sequence of the identified receptor fragment at a time. (A) Parent ion 2665.5. (B) Parent ion 3345.0 (C) Parent ion 3655.1.