**(13) Supporting Information**

**Supplemental Table S1 - Amino-acid sequences and denomination of cyclopeptides.**

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| **Denomination** | **Amino acid sequence** |
| 18 C/C/S18 C/S/C |  **A**DEARRCYNDPKCSDFV**Q** **A**DEARRCYNDPKSCDFV**Q**  |
| β1ECII201-220β2ECII183-202 |  AESDEARRCYNDPKCCDFVT A**THQ**EA**IN**CY**ANET**CCDF**F**T |
| 22 C/C/B-β122 C/C/D-β2E202AS203AD204AR207AR208AN211AD212AP213AK214A | RAESDEARRCYNDPKCBDFVT**G**RATHQEAINCYANETCDDFFT**G**RAASDEARRCYNDPKCBDFVT**G**RAEADEARRCYNDPKCBDFVT**G**RAESAEARRCYNDPKCBDFVT**G**RAESDEAARCYNDPKCBDFVT**G**RAESDEARACYNDPKCBDFVT**G**RAESDEARRCYADPKCBDFVT**G**RAESDEARRCYNAPKCBDFVT**G**RAESDEARRCYNDAKCBDFVT**G**RAESDEARRCYNDPACBDFVT**G** |

Amino-acids added to the primary human 1AR-sequence (to allow for cyclisation between N- and C-terminal residues) are typeset in **bold**. Amino-acid sequences not conserved between 1/2-adrenoceptors are typeset in **green**. Mutated residues are typeset in red. Cysteines are typeset in **blue**. Amino-acids are denominated by the standard one-letter-code. Letter “B” stands for the synthetic amino-acid *α-amino-butyrate* introduced to prevent aberrant C209↔C216 disulfide-bridging.

**Supplemental Table S2 - Primer for site directed mutagenesis by nested PCR**

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| **Primer** | **Sequence (5‘ → 3‘)** |
| OuterPri1 forOuterPri2 revE202A forE202A revR207A forR207A revD212N forD212N revP213A forP213A revV219A forV219A rev | GACCTGGTCATGGGGCTGCTGGTGGTGCCGCGGGGACGGGCGAGGGCGAGGGCGAACTGGTGGCGGGCG**GCT**AGCGACGAGGCGCCTCGTCGCT**AGC**CGCCCGCCACCAGTGCGACGAGGCG**GCA**CGCTGCTACAACGACCCGGGTCGTTGTAGCAGCG**TGC**CGCCTCGTCGCCCGCTGCTACAAC**GCT**CCCAAGTGCTGCGACTTCGAAGTCGCAGCACTTGGG**AGC**GTTGTAGCAGCGGGCTGCTACAACGAC**GCC**AAGTGCTGCGACTTCGCGAAGTCGCAGCACTT**GGC**GTCGTTGTAGCAGCAGTGCTGCGACTTC**GCT**ACCAACCGGGCCTACGCGTAGGCCCGGTTGGT**AGC**GAAGTCGCAGCACT |

Primers (for: forward; rev: reverse) designed for the directed mutagenesis (point mutations) of the native human 1-AR, with the respective nucleotide-changes underscored. Successful introduction of the mutations in the 1AR-cDNA was checked by sequencing.

Single-AA mutant human 1-AR were expressed in HEK293-cells and their functionality was ascertained by radioligand-binding and by adequate cAMP-responses following agonist (-)isoproterenol-stimulation (see suppl. Table S3).

**Supplemental Table S3 - Characteristics of β1AR-mutants expressed in HEK293-cells**

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| --- | --- | --- |
| **Construct** | **Ligand binding**Ki CGP20712A (nM) | **cAMP-stimulation**-Log EC50 (-)- Isoproterenol (nM) |
| β1AR wildtype | 7.56  | 0.694 ± 0.027 |
| β1AR R207A | 7.03 (n.s.)1 | 0.592 ± 0.036 (n.s.)2 |
| β1AR D212N | 8.49 (n.s.)1 | 0.628 ± 0.019 (n.s.)2 |
| β1AR P213A | 9.95 (n.s.)1 | 0.402 ± 0.090 (n.s.)2 |
| β1AR V219A | 4.55 (n.s.)1 | 0.651 ± 0.031 (n.s.)2 |

1Significance of deviation from wildtype, Dunnett's Multiple Comparison Test, n.s.= not significant

2Significance of deviation from wildtype, Dunnett's Multiple Comparison Test, n.s.= not significant

**Supplemental Figure S1**

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**Supplemental Figure S1 - Neutralization of a monoclonal mouse anti-1ECII-antibody by 22mer-cyclopeptides corresponding to the human 1-ECII, each having a different non-conserved amino-acid (compared to the amino-acids constituting the ECII-loop of the β2-AR) sequentially replaced with alanine.** Monoclonal mouse anti-1ECII-abs were pre-absorbed with the indicated cyclopeptides (40 mol/ mol IgG, 4°C, 16 h). Grey columns: mouse anti-1ECII-Mab binding to a linear 25AA-1ECII-peptide (residues 199-223) as determined by ELISA. Black columns: 1AR-mediated cAMP-stimulation as measured in cells expressing the native 1-AR functionally coupled to a FRET-sensor for intracellular cAMP. Decreases in immuno-reactivity (grey bars) or cAMP-stimulation (black bars) after pre-absorption with the indicated cyclopeptides (40 mol CP/ mol IgG) normalized to the values obtained without pre-absorption. Columns represent mean values ±SEM of triplicate determinations. Differences between the non-mutated 22 C/C/B-1-cyclopeptide and CP-mutations were analyzed by one-way ANOVA with subsequent Dunnett's post-hoc test for multiple comparisons; **\*** p< 0.05; **\*\*** p< 0.01). Internal negative control: non-mutated 22C/C/D-beta2 (sequence and alignment see Tab S1).

**Supplemental Figure S2**

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LVED

LVES

**Supplemental Figure S2 - Time course of left ventricular diameters from rats immunized against 1ECII or receiving a monoclonal rat anti-1ECII** **and corresponding control animals, determined by echocardiography.** The graphs show the time-course of the LV end-diastolic and end-systolic diameters (LVED/LVES) over 12 months of Lewis-rats immunized subcutaneously every 4 weeks with 1ECII/GST fusion-proteins **(beta1-ECII, black circles, n=10)** as previously described in detail ([11](file:///D%3A%5CPrograms%5CWBMetaXMLCreator%5CINPUTOUTPUT%5CEHF2_12747%5Cdoc%5CAlaScan%20paper%20_REV2_ESCHF.doc#_ENREF_11)), or receiving a monoclonal rat anti-1ECII intravenously every 4 weeks **(rat Mab 13F6, black triangles, n=8)**. Untreated Lewis rats **(controls, white circles, n=10)** or animals receiving a rat IgG isotype-control every 4 weeks **(isotype control, white triangles, n=5)** served as the respective control groups.

Error bars indicate mean ±SEM in the indicated groups; \*\*\*p<0.001, \*\*\*\*p<0.0001 (two-way ANOVA and Dunnett's post-hoc test for multiple comparisons, asterisks are indicated for the Mab 13F6 transfer-group *versus* control animals).