

SUPPLEMENTARY MATERIAL**Supplementary Methods****Study subjects and Clinical Procedure**

Ten randomly selected DCM patients were included, who presented in the outpatient clinic for regular follow up between January 2007 and January 2008. In all patients presented heart failure symptoms equivalent to New York Heart Association stage II – IV with left ventricular ejection fraction (LVEF) <35% and left ventricular end-diastolic diameter (LVEDD) >50mm. Incidentally, clinical data from three of the patients were published earlier.¹ Coronary heart disease was previously excluded by angiography. Patients were not included if they had suffered from active infectious diseases, known autoimmune diseases, cancer, chronic alcoholism, or heart failure due to known origins (e.g., primary valvular disease). In all patients, acute myocarditis was excluded by endo-myocardial biopsy according to Dallas criteria. All patients had received stable oral heart failure medication for more than three months before blood sampling. Ten healthy volunteers with no symptoms of heart disease and exclusion of left ventricular dysfunction by echocardiography were selected as controls based on age and gender. Detailed clinical characteristics of patients and matched volunteers are listed in *Supplementary Table 1*. The investigation conforms to the principles of the Declaration of Helsinki. Written consent was taken from each patient. The study protocol was approved by the Ethics Committee of the University of Greifswald, Germany.

Ligand binding

Receptor expression in intact cells was measured using the β AR antagonist [³H]-(-)-CGP12,177 (Amersham Biosciences, Freiburg, Germany). $0.5 - 1 \times 10^4$ cells were incubated with [³H]-(-)-CGP12,177 ($5 - 1200 \times 10^{-12}$ mol/l) in a final volume of 0.2 ml of HBSS buffer supplemented with HEPES (pH 7.4). Affinity of isoproterenol was determined under the same conditions by displacement of [³H]-(-)-CGP12,177 (5.67×10^{-9} mol/l) by unlabeled isoproterenol ($10^{-9} - 10^{-3}$ mol/l). Binding at 37

°C was terminated after 45 min by threefold medium exchange. Non-specific binding was determined as < 2.5 % of total binding. Maximum specific binding (B_{max}) and the dissociation constant (K_D) were determined using non-linear regression and assuming a K_D -value of [³H]-(-)-CGP12,177 for β_1 ARs of 2×10^{-10} mol/l.² For competition binding studies with the β_1 -selective adrenergic antagonist CGP20,712A³ and the β_2 -selective adrenergic antagonist ICI 118,551,⁴ cells were homogenized in 2.5×10^{-2} mol/l TRIS, pH 7.5, containing a standard protease inhibitor cocktail (Sigma, Munich, Germany). Homogenates were sedimented (40,000 x g for 20 min at 4 °C), resuspended in 7.5×10^{-2} mol/l TRIS, pH 7.4, 1.2×10^{-3} mol/l MgCl₂, 2×10^{-3} mol/l EDTA, and 5-20 μ g of protein were incubated with 5×10^{-11} mol/l [¹²⁵I]-(-)-cyanopindolol (Amersham Biosciences, Freiburg, Germany), 500 μ M Gpp(NH)p (Sigma, Munich, Germany), and various concentrations of displacing ligand. Nonspecific binding was determined in the presence of 10^{-5} mol/l isoproterenol and was < 15% of total binding. Binding reactions were carried out for 90 min at 25°C and terminated by rapid filtration through Whatman GF/C glass fiber filters presoaked in PBS containing 0.3 % polyethyleneimine.

Confocal imaging and determination of FRET by donor/acceptor emission ratio

Cells grown on coverslips were maintained in serum-free media (DMEM) at precisely 37° C under an LSM 510 Meta inverted confocal microscope placed inside an XL-incubator and equipped with a 63x/1.3 DIC oil immersion objective (all Carl Zeiss, Jena, Germany). To monitor conformational changes of the receptors by changes in intramolecular FRET-efficiency, the donor CFP was excited at 458 nm and fluorescent emission was recorded using the Meta detector. CFP and YFP emission spectra were separated by emission fingerprinting and corrected for bleaching during image

acquisition. FRET efficiency was expressed as the ratio of emission intensity $\frac{(I_{YFP})}{(I_{CFP})}$.

Fluorescence lifetime measurements and fluorescence lifetime imaging

Cells grown on coverslips were maintained in serum free media (DMEM) at precisely 37°C under a DCS-120 Confocal Scanning FLIM system (Becker & Hickl GmbH, Berlin, Germany) mounted on an IX-71 inverted microscope equipped with a 60x (NA 1.2) water immersion objective (Olympus

Deutschland GmbH, Hamburg, Germany) and placed inside a cage incubator (Okolab, Naples, Italy). The FRET donor CFP was excited using a 405 nm diode laser (Becker & Hickl GmbH) with a pulse repetition rate of 50 MHz. Fluorescence signals in the CFP channel (BP 460–500 nm) and the YFP channel (BP 520–550 nm) were detected using H7422P-40 photomultipliers (Hamamatsu, Herrsching, Germany) connected to two TCSPC-150 modules (Becker & Hickl GmbH).

Fluorescence lifetime imaging (FLIM) of live cells was performed by continuous fast scanning. To describe fluorescence lifetime, TCSPC data were fitted by a double exponential decay function using SPC Image software (version 2.9.1, Becker & Hickl GmbH). Mean lifetime τ_m was calculated from

multi exponential decay of each pixel in a lifetime image according to: $\tau_m = \frac{\sum_{i=1}^N a_i \tau_i}{\sum_{i=1}^N a_i}$ with the intensity with the intensity coefficient a_i given as relative amplitude.

ERK1/2 phosphorylation

HEK 293 cells were grown to 80% confluency, kept for 24h in serum free medium and then subjected to treatment with receptor ligands (for 10 min at 37°C, unless stated otherwise). Subsequent steps followed published procedures⁶. Phosphorylation was analyzed by immunoblotting using mouse monoclonal antibodies against phospho-p42/p44 MAP kinase (Thr 202/Tyr 204) and polyclonal ERK antibodies (Cell Signaling, Boston, USA).

Supplemental Results

Biofluorescent human β_1 ARs stably expressed in HEK293 cells.

Human β_1 AR bearing in frame the sequence of CFP in the third intracellular loop and/or the sequence of YFP appended to the C-terminus (*Supplementary Figure S1A*) were expressed in HEK 293 cells. For each construct, several cell clones supporting constitutive expression of biofluorescent receptors in the cell membrane were isolated and characterized. C-terminally YFP-fused receptors were 1000-fold more abundant than endogenous binding sites, and receptors bearing in addition or instead an internal CFP moiety in the third intracellular loop were 600-fold more abundant. All heterologous receptors exhibited β_1 AR ligand binding characteristics, whereas endogenous binding sites accounting for less than 0.2 % of B_{\max} in the transfected cell lines exhibited β_2 AR binding characteristics (*Supplementary Table 2*).

In GFP-directed immunoblotting (*Supplementary Figure S1B*), the recombinant receptors exhibited the expected electrophoretic mobility (given the addition of one or two GFP-like moieties) and a double banded pattern known to reflect physiological heterogeneity in glycosylation.⁷ Receptor associated fluorescence was exclusively located in the outer cell membrane. Double labeled constructs exhibited a complete overlap in the patterns of the two labels (*Supplementary Figure S1C*, top) and brief exposure to isoproterenol induced internalization of the receptors into vesicles (*Supplementary Figure S1C*, bottom). Time frame (10 min) and extent (30%) of receptor internalization were as reported by others.⁸⁻¹⁰

Exposure of cells expressing the construct 1 or 3 to isoproterenol induced increases in intracellular cAMP up to 20-fold. 10-fold increases were induced by the phosphodiesterase inhibitor IBMX. cAMP levels were marginally (< 1.5 -fold) altered by CGP20712A. cAMP response to isoproterenol showed a stringent dose dependence. The lowest concentration of isoproterenol detectable as a > 2-fold increase in intracellular cAMP was 5×10^{-9} mol/l (*Supplementary Figure S1E*), attesting to a high sensitivity of the cells for β_1 -adrenergic receptor stimulation. In untransfected HEK293 cells, cAMP was not significantly stimulated by CGP20,712A or IBMX alone, and marginally (< 2-fold) increased

by isoproterenol (*Supplementary Figure S1D*), indicating that in the transfected cells cAMP responses were highly specific for stimulation through the heterologously expressed β_1 AR.

Binding affinity of isoproterenol assessed in cells transfected with β_1 AR (construct 3) by competition with [3 H]-(-)-CGP12,177 is shown in *Supplementary Figure S1F*. To avoid depletion of radioligand by extensive binding to overexpressed receptors a high concentration of radioligand ($> 5 \times 10^{-9}$ mol/l) was used in a high volume of buffer (2×10^4 l) resulting in a simple logistic curve. K_D values for isoproterenol were estimated by non-linear regression of data as $90.5 \pm 4.9 \times 10^{-9}$ mol/l. Similar experiments were done with cells transfected with the construct 1 and 2 are summarized in *Supplementary Table 2*.

In summary, these control experiment ascertained that the heterologous receptors were of the proper size and subtype, appropriately folded and inserted into the cell membrane and fully functional with respect to ligand binding. They exerted an overruling effect on intracellular cAMP levels and underwent normal agonist induced receptor cycling (given the known restrictions imposed by the incorporation of fluorescence proteins).⁹

FRET sensor of intramolecular conformation changes of human β_1 ARs.

Exposure of CFP/YFP-labeled receptors (construct 3 in *Supplementary Figure S1A*) to isoproterenol (10^{-5} mol/l) triggered a rapid (response time < 1 sec) drop in YFP/CFP emission ratio (I_{YFP}/I_{CFP}) corresponding to a Δ_{max} value of 0.09. A similar response was not inducible by the antagonist CGP20,712A or buffer alone (*Supplementary Figure S1G*). Agonist induced changes in FRET efficiency were quantitatively correlated to isoproterenol dose and cAMP stimulation (*Supplementary Figure S1J and K*, respectively). Similar decreases in the donor/acceptor emission ratio were previously observed upon activation of comparable receptor constructs^{11, 12} and interpreted to reflect a decrease in FRET efficiency due to an increase in the distance between the two labeled receptor domains caused by activation associated conformational changes. Several control experiments were performed to assure that this was indeed the case.

Firstly, we ascertained that decreases in FRET efficiency were due to intra-molecular conformation changes as opposed to receptor-receptor interactions. It has been shown that activation of human

β_1 ARs can involve receptor homo-dimerization.^{13,14} To check whether such interactions contributed to the observed decrease in donor/acceptor fluorescence emission ratio, the two fluorophores were placed in separate receptor molecules co-expressed in the same cell. In other words: Cells co-expressing the constructs 1 and 2 were compared to cells expressing the construct 3 (constructs see *Supplementary Figure S1A*). In the cells coexpressing FRET donor and acceptor in separate receptor molecules, exposure to isoproterenol (10^{-5} mol/l) did not trigger a similar drop in YFP/CFP emission ratio as in the cells expressing the double-labeled FRET sensor receptor (*Supplementary Figure S1H*). Thus, the decrease in FRET efficiency could be assigned solely to intramolecular conformation changes, and a contribution of receptor-receptor interactions to the signal could be excluded.

Secondly, we ascertained that decreases in YFP/CFP emission ratio were due to FRET and not to unrelated artifacts such as channel bleed-through and bleaching.¹⁵ To exclude such artifacts, we investigated the lifetime of donor fluorescence (CFP), which is decreased by FRET but not by other mechanisms altering $I_{\text{YFP}}/I_{\text{CFP}}$. CFP expressed alone, or as a fusion with the third intracellular loop of the human β_1 AR (construct 2 in *Supplementary Figure S1A*), or together with, but not fused to the potential acceptor fluorophor YFP had a mean fluorescence lifetime (τ_m) between 2.80 and 2.89 ns (*Supplementary Table 3*, lines 1-3). Significantly lower τ_m values (2.28 ± 0.07 ns) were measured when CFP and YFP were expressed as a fusion protein supporting constitutive intramolecular FRET (*Supplementary Table 3*, line 3). Similarly decreased τ_m values (2.32 ± 0.04 ns) were observed in cells expressing β_1 ARs bearing the CFP/YFP-FRET sensor (construct 3 in *Supplementary Figure S1A*) (*Supplementary Table 3*, line 5), whereas CFP-fused receptors lacking a C-terminal YFP (construct 2 in *Supplementary Figure S1A*) exhibited normal τ_m values (2.81 ± 0.03 ns) (*Supplementary Table 3*, line 4). Thus, the τ_m decrease in the dual labeled receptor construct indicates constitutive FRET between the C-terminal YFP and the CFP in the third intracellular receptor domain. Fluorescence lifetime images of a representative cell cluster expressing the dual labeled receptor construct are shown in *Supplementary Figure S2A*. τ_m values of CFP ranging from 1.5 to 3.0 ns are encoded orange to blue. Without stimulation (*Supplementary Figure S2A*, top), CFP fluorescence of the receptors was exclusively localized in the outer cell membrane and exhibited τ_m values of 2.32 ± 0.04 ns (encoded

green in *Supplementary Figure S2A*, top; quantification in *Supplementary Table 3*, row 5) indicative of FRET. Upon exposure to 10^{-5} mol/l μ M isoproterenol (*Supplementary Figure S2A*, middle and bottom), encoding of lifetime of receptor-associated CFP fluorescence in the membrane shifted towards longer τ_m values (encoded blue). However, this shift was inapparent in quantitative assessments of the entire field of vision (*Supplementary Table 3*, lines 6 and 7). Subcellular analysis (*Supplementary Figure S2B* and *Supplementary Table 3*, lines 8-10) revealed a significant increase in τ_m in the outer cell membrane, which shifted from 2.37 ± 0.04 to 2.51 ± 0.03 ns upon exposure to the agonist isoproterenol indicating a decrease in FRET efficiency in this receptor subpopulation. In contrast, receptors sequestered into intracellular vesicles (green and orange spots within the circle in *Supplementary Figure S2B*, right) exhibited a shortening of CFP fluorescence lifetime ($\tau_m = 2.11 \pm 0.18$ ns) possibly reflecting a more dense conformation (due to partial denaturation of the internalized receptors suggested to play a role in uncoupling of ligand and receptor^{16, 17}), or a change in the basic properties of the fluorophor (due to pH-alterations inside the vesicles). In receptors localized in the outer cell membrane, the drop in I_{YFP}/I_{CFP} triggered by 10^{-5} mol/l isoproterenol (*Supplementary Figure SIG and H*) corresponds to increases in fluorescence life time of the donor fluorophor, and therefore, represents a genuine decrease in FRET efficiency.

In conclusion, the drop in YFP/CFP emission ratio of the receptor construct 3 can be used as a quantitative measure for intra-molecular conformational changes of the receptor involving an increase in distance between the two labeled receptor domains. τ_m values of activated receptors are still smaller than those of CFP alone (*Supplementary Table 3*) suggesting a decrease in FRET efficiency, consistent with a minor repositioning of the labeled receptor domains. A similar conclusion was previously drawn from experiments with purified β_2 AR bearing a different FRET pair incorporated at similar positions.¹⁸ These control experiments ascertain that the dual labeled receptors were capable of reporting the activation associated conformational switch by a decrease in FRET efficiency (measurable by I_{YFP}/I_{CFP} and τ_m), thus allowing sensitive detection and quantification of conformational changes related to the activation of human β_1 ARs.

No evidence of MAP-kinase signaling.

In a recent study it was shown that a significant proportion of β_1 AR-autoantibodies stimulate ERK1/2-kinases independently of cAMP⁶. Stimulation of MAP kinases by β -adrenergic receptors involves direct recruitment, activation, and scaffolding of cytoplasmic signaling complexes *via* β -arrestin.¹⁹ Our observation that conformational changes induced by receptor autoantibodies in some cases failed to be correlated to cAMP stimulation (*Main Figure 4B*) could have indicated that the associated conformational switches were coupled to the alternative pathway of MAP kinase activation in cAMP independent manner. This was investigated and the typical result presented in *Supplementary Figure S5* shows that in HEK293 cells overexpressing YFP-fused β_1 AR ERK1/2 kinase could be readily stimulated by EGF but not by isoproterenol saturation nor by any of the autoantibodies, irrespectively of whether they induced receptor conformations linked (e.g. “P4”) or not linked (e.g. “H5”) to cAMP stimulation. We concluded that in our system changes in receptor conformations induced by isoproterenol or autoantibodies did not promote MAP-kinase signaling.

¹Abbreviations in Supplementary Data

β_1 AR, β_1 -adrenergic hormone receptor; β_2 AR, β_2 -adrenergic hormone receptor; CFP, cyan fluorescence protein; [³H]-(-)-CGP12,177, (4-(3-t-butylamino-2-hydroxypropoxy)- [5,7-³H] bezimidazol-2-one); CGP 20,712A, (\pm)-2-Hydroxy-5-[2-[[2-hydroxy-3-[4-[1-methyl-4-(trifluoromethyl)-1H-imidazol-2-yl]phenoxy]propyl] amino]ethoxy]-benzamide methanesulfonate salt; FLIM, fluorescence lifetime imaging microscopy; FRET, Fluorescence Energy Resonance Transfer (Förster effect); GFP, green fluorescence protein; Gpp(NH)p, Guanosine 5'-[β , γ -imido]triphosphate trisodium salt hydrate; ICI 118.551, (\pm)-1-[2,3-(Dihydro-7-methyl-1H-inden-4-yl)oxy]-3-[(1-methylethyl)amino]-2-butanol hydrochloride; ¹²⁵I-CYP, [¹²⁵I]-iodocyanopindolol; ISO, (-)-isoproterenol; IBMX, 1-methyl-3-isobutylxanthine; TCSPC, time correlated single photon counting; YFP, yellow fluorescence protein.

Supplemental References

1. Trimpert C, Herda LR, Eckerle LG, Pohle S, Muller C, Landsberger M *et al.* Immunoabsorption in dilated cardiomyopathy: long-term reduction of cardiodepressant antibodies. *Eur J Clin Invest* 2010;**40**:685-691.
2. Lemoine H. beta-adrenoceptor ligands: characterization and quantification of drug effects. *Quant Struct-Act Relat* 1992;**11**:211-218.
3. Lemoine H, Kaumann AJ. Regional differences of beta 1- and beta 2-adrenoceptor-mediated functions in feline heart. A beta 2-adrenoceptor-mediated positive inotropic effect possibly unrelated to cyclic AMP. *Naunyn-Schmiedeberg's Arch Pharmacol* 1991;**344**:56-69.
4. Lemoine H, Ehle B, Kaumann AJ. Direct labelling of beta 2-adrenoceptors. Comparison of binding potency of 3H-ICI 118,551 and blocking potency of ICI 118,551. *Naunyn-Schmiedeberg's Arch Pharmacol* 1985;**331**:40-51.
5. Christensen MO, Krokowski RM, Barthelmes HU, Hock R, Boege F, Mielke C. Distinct effects of topoisomerase I and RNA polymerase I inhibitors suggest a dual mechanism of nucleolar/nucleoplasmic partitioning of topoisomerase I. *J Biol Chem* 2004;**279**:21873-21882.
6. Tutor AS, Penela P, Mayor F, Jr. Anti-beta1-adrenergic receptor autoantibodies are potent stimulators of the ERK1/2 pathway in cardiac cells. *Cardiovasc Res* 2007;**76**:51-60.
7. Jahns R, Siegmund C, Jahns V, Reilander H, Maidhof A, Muller-Esterl W *et al.* Probing human beta 1- and beta 2-adrenoceptors with domain-specific fusion protein antibodies. *Eur J Pharmacol* 1996;**316**:111-121.
8. Burgi S, Baltensperger K, Honegger UE. Antidepressant-induced switch of beta 1-adrenoceptor trafficking as a mechanism for drug action. *J Biol Chem* 2003;**278**:1044-1052.
9. McLean AJ, Milligan G. Ligand regulation of green fluorescent protein-tagged forms of the human beta(1)- and beta(2)-adrenoceptors; comparisons with the unmodified receptors. *Br J Pharmacol* 2000;**130**:1825-1832.
10. Xiang Y, Devic E, Kobilka B. The PDZ binding motif of the beta 1 adrenergic receptor modulates receptor trafficking and signaling in cardiac myocytes. *J Biol Chem* 2002;**277**:33783-33790.

11. Rochais F, Vilardaga JP, Nikolaev VO, Bunemann M, Lohse MJ, Engelhardt S. Real-time optical recording of beta1-adrenergic receptor activation reveals supersensitivity of the Arg389 variant to carvedilol. *J Clin Invest* 2007;**117**:229-235.
12. Vilardaga JP, Bunemann M, Krasel C, Castro M, Lohse MJ. Measurement of the millisecond activation switch of G protein-coupled receptors in living cells. *Nature Biotechnol* 2003;**21**:807-812.
13. Mercier JF, Salahpour A, Angers S, Breit A, Bouvier M. Quantitative assessment of beta 1- and beta 2-adrenergic receptor homo- and heterodimerization by bioluminescence resonance energy transfer. *J Biol Chem* 2002;**277**:44925-44931.
14. Lavoie C, Mercier JF, Salahpour A, Umapathy D, Breit A, Villeneuve LR *et al.* Beta 1/beta 2-adrenergic receptor heterodimerization regulates beta 2-adrenergic receptor internalization and ERK signaling efficacy. *J Biol Chem* 2002;**277**:35402-35410.
15. Biskup C, Zimmer T, Kelbauskas L, Hoffmann B, Klocker N, Becker W *et al.* Multi-dimensional fluorescence lifetime and FRET measurements. *Microsc Res Tech* 2007;**70**:442-451.
16. Gagnon AW, Kallal L, Benovic JL. Role of clathrin-mediated endocytosis in agonist-induced down-regulation of the beta2-adrenergic receptor. *J Biol Chem* 1998;**273**:6976-6981.
17. Lohse MJ. Molecular mechanisms of membrane receptor desensitization. *Biochimica et biophysica acta* 1993;**1179**:171-188.
18. Granier S, Kim S, Shafer AM, Ratnala VR, Fung JJ, Zare RN *et al.* Structure and conformational changes in the C-terminal domain of the beta2-adrenoceptor: insights from fluorescence resonance energy transfer studies. *J Biol Chem* 2007;**282**:13895-13905.
19. Azzi M, Charest PG, Angers S, Rousseau G, Kohout T, Bouvier M *et al.* Beta-arrestin-mediated activation of MAPK by inverse agonists reveals distinct active conformations for G protein-coupled receptors. *Proc Natl Acad Sci* 2003;**100**:11406-11411.
20. Jahns R, Boivin V, Siegmund C, Inselmann G, Lohse MJ, Boege F. Autoantibodies activating human beta1-adrenergic receptors are associated with reduced cardiac function in chronic heart failure. *Circulation* 1999;**99**:649-654.

Supplementary Table 1. Clinical characterization of DCM patients and healthy volunteers (controls).

	Controls (n=10)	DCM Patients (n=10)
Mean Age \pm SEM (y)	51.5 \pm 3.9	52.7 \pm 2.7
Age range (y)	36-78	39-66
Sex (m/f)	9/1	9/1
LVEF (%)	61.9 \pm 1.4	27.3 \pm 1.5
LVEDD (mm)	48.7 \pm 1.3	71.3 \pm 3.2
NYHA	N/A	2.6 \pm 0.2
CI	N/A	2.0 \pm 0.15
PAP	N/A	20.1 \pm 1.6
PCWP	N/A	12.8 \pm 1.7
Medication		
ACE-I (%)	0	100
ARB (%)	0	10
β -Blocker (%)	0	100
Diuretics (%)	0	90
Myocardial Biopsy		
CD68 ⁺ / μ m	N/A	13.4 \pm 1.2
CD3 ⁺ / μ m	N/A	0.22 \pm 0.18
viral RNA present (%)	N/A	30

LVEF: left ventricular ejection fraction; LVEDD: left ventricular end-diastolic diameter; NYHA: New York Heart Association; CI: cardiac index; PAP: pulmonary artery pressure; PCWP: pulmonary capillary wedge pressure; ARB: angiotensin receptor blocker.

Supplementary Table 2. Ligand binding characteristics of biofluorescent human β_1 ARs stably expressed in HEK 293 cell clones (mean \pm SEM, n =3).

	[³ H]-(-)-CGP12,177	[³ H]-(-)-CGP12,177	CGP20,712A	ICI118,551	ISO
	B _{max}	K _D	K _i	K _i	K _i
	10 ⁶ sites×cell ⁻¹	pM	nM	nM	nM
untransf.	10 ⁻⁴ \pm 2×10 ⁻⁵	512.0 \pm 22	687.1 \pm 1.5	0.21 \pm 0.14	125 \pm 5.8
Construct 1*	8.0 \pm 0.66	189.0 \pm 64	8.6 \pm 1.1	144.0 \pm 1.1	111.4 \pm 1.3
Construct 2	4.8 \pm 0.89	243.0 \pm 38	7.9 \pm 2.8	186.0 \pm 2.8	133.0 \pm 6.1
Construct 3	4.4 \pm 0.52	198.0 \pm 42	8.8 \pm 2.1	175.0 \pm 8.2	129.0 \pm 9.2 [†]

*) Numbering of constructs see Supplementary Figure S1A

[†]) Maximum specific binding and dissociation constant (K_D) of isoproterenol (ISO) were also determined in intact cells by displacement of [³H]-(-)-CGP12,177 as 3.42 \pm 0.38 \times 10⁶ sites per cell and 100 nM, respectively.

Supplementary Table 3. Measurements of fluorescence lifetime of CFP

Constructs and Conditions*	mean lifetime τ_m [ns][†]
CFP only	2.89 ± 0.03
CFP and YFP (not fused)	2.80 ± 0.04
CFP-YFP (fused, constitutive FRET)	2.28 ± 0.07
β_1 AR-CFP (construct 2 in Supplementary Figure S1A)	2.81 ± 0.03
β_1 AR-CFP-YFP (construct 3 in Supplementary Figure S1A)	
not stimulated, whole field of vision	2.32 ± 0.04
1 sec ISO (10 μ M), whole field of vision	2.39 ± 0.04
10 min ISO (10 μ M), whole field of vision	2.34 ± 0.05
not stimulated, focus on cell membrane	2.37 ± 0.04
1 sec ISO (10 μ M), focus on cell membrane	2.48 ± 0.02
10 min ISO (10 μ M), focus on cell membrane	2.51 ± 0.03
10 min ISO (10 μ M), focus on internal vesicle	2.11 ± 0.18

^{*}) Constructs were stably expressed in HEK293 cells.

[†]) Mean ± SEM of five measurements at 37°C with independent cell samples.

Supplementary Figure Legends

Figure S1 Experimental model and set up. (A) Human β_1 AR was fused to YFP at the C-terminal end (constructs 1 and 3). Alternatively (construct 2) or in addition (construct 3), CFP was inserted into the third intracellular loop replacing residues 293-303. (B) GFP-directed immunoblotting of untransfected cells (lane 1) or cells expressing constructs 1 (lane 2), 2 (lane 3), or 3 (lane 4). (C) Cells expressing the construct 3 were subjected to confocal imaging in mid plane of yellow (left) and blue fluorescence (right) before (top) and 10 min after (bottom) exposure to 10^{-5} mol/l isoproterenol. (D) cAMP stimulation (10 min, 37 °C) of cells not transfected (white bars) or expressing the construct 1 (grey bars) with 1 mM IBMX, 10^{-5} mol/l isoproterenol (iso), or 10^{-5} mol/l CGP20,712A (CGP, green). cAMP values were normalized to total protein and fold increments were calculated by the ratio of stimulated and unstimulated cells. (E) Dose-response of cAMP stimulation by isoproterenol measured in cells expressing the construct 1. (F) Displacement of [3 H]-(-)-CGP12,177 binding by unlabelled isoproterenol in intact cells expressing the construct 3. (G) Typical recording of FRET ratios (I_{YFP}/I_{CFP}) recorded over time in cells stably expressing construct 3 and exposed (arrow) to 10^{-5} mol/l isoproterenol (Iso, red), 10^{-5} mol/l CGP20,712A (cgp, green), or buffer alone (black). Ratios were averaged from time lapsed fluorescent images of approximately 40 cells and corrected for bleaching and signal offset due to reagent addition. (H) Comparison of FRET responses to 10^{-5} mol/l isoproterenol (arrow) between cells expressing the construct 3 (red) and cells coexpressing the constructs 1 and 2 (black). Ratios were averaged from time lapsed fluorescent images of approximately 40 cells and corrected for bleaching and signal offset due to reagent addition. (J) Maximal decreases in FRET-efficiency derived from recordings in cells expressing the construct 3 (done as in G) in response to isoproterenol (5×10^{-12} - 5×10^{-4} mol/l). Mean values of three independent experiments are normalized to the maximal effect at ligand saturation (5×10^{-4} mol/l). (K) Linear correlation ($r^2 = 0.92$) of cAMP- and FRET responses to isoproterenol (5×10^{-11} - 5×10^{-4} mol/l).

Figure S2. FLIM-FRET of β_1 ARs during activation and internalization. (A) FLIM of cells expressing human β_1 ARs bearing a FRET sensor (construct 3) before (top), 30 seconds after (middle) and 10 minutes after (bottom) addition of 10^{-5} mol/l isoproterenol. Corresponding color histograms of quantitative distribution of fluorescence lifetime of CFP measured across the entire field of vision are shown on the right. The size bar (30 μ M) in the top panel applies to all three panels. The white rectangle indicates the area of subcellular analysis in B. (B) FLIM of a single cell expressing the construct 3. Quantitative values of fluorescence lifetime (τ) of CFP were averaged in a membrane section (boxes) or an intracellular vesicle containing internalized receptors (circle). A corresponding color histogram of quantitative distribution of fluorescence lifetime of CFP measured across the entire field of vision is at the bottom. The size bar (30 μ M) in the left panel applies to all three panels.

Figure S3 Autoantibody detection by peptide ELISA versus β_1 AR-IgG colocalization. Scores of β_1 AR – IgG colocalization (data in *Main Figure 1D*) are plotted over corresponding ELISA signals of the binding of autoimmune IgG (10 mg/L) to the peptide analog of the second extracellular loop of the human β_1 AR (Pep 2) measured by published procedures.²⁰ Linearity and sensitivity of the ELISA was controlled by serial dilution of a positive control antibody; the horizontal dotted line indicates the lower detection limit defined by the threshold, above which specific signals were significantly different from background noise. The vertical dotted line indicates specificity cut-off for the colocalization assay as defined in *Main Figure 1C*. Circles indicate mean values of triplicate measurements of individual IgG samples from healthy volunteers (open symbols) and DCM patients (closed symbols). SEM is not shown, as it was less than one scoring step for the colocalization assay and < 20% for the ELISA.

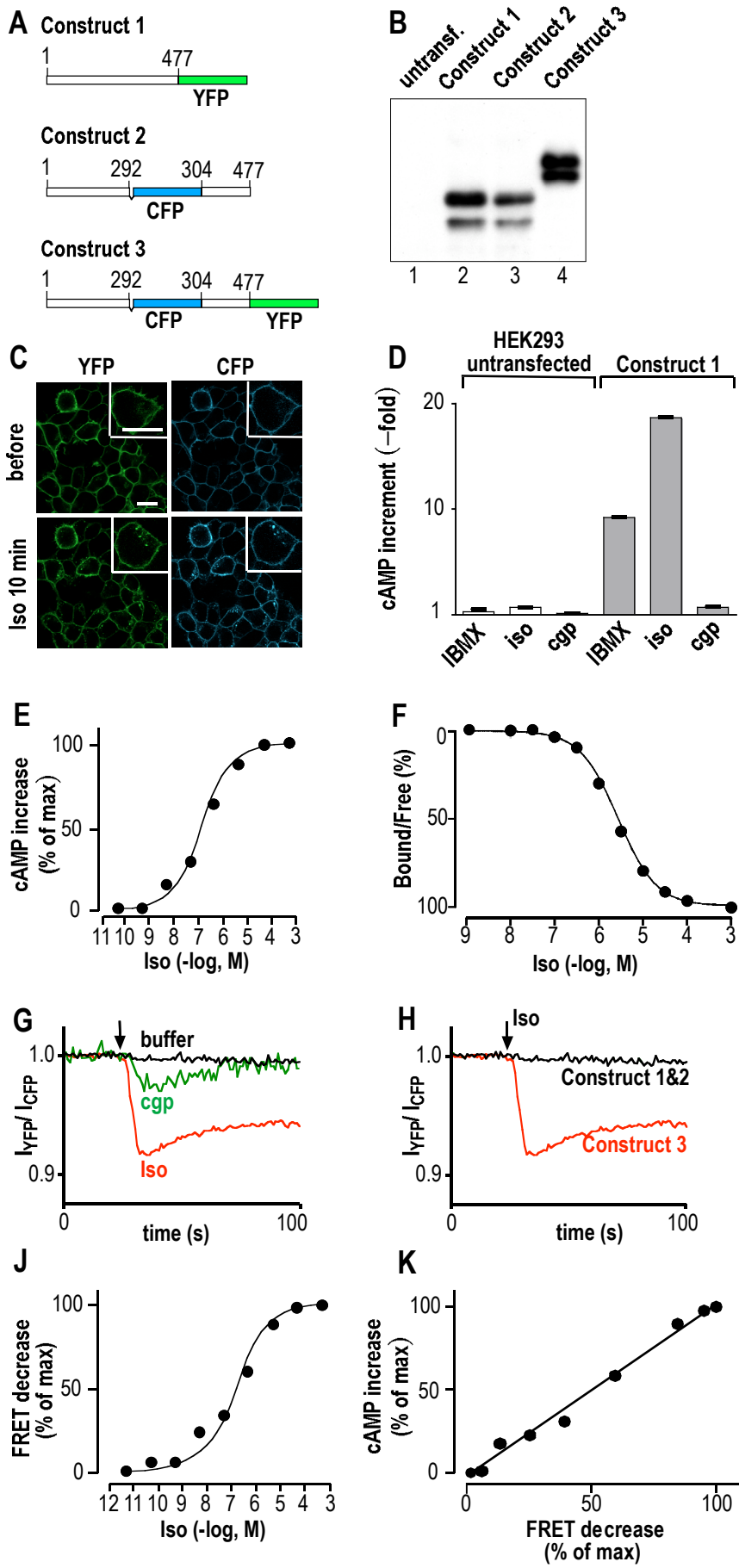
Figure S4 Disruption of IgG co-localization with native biofluorescent β_1 ARs by cell fixation. Receptor- (top) and IgG-associated fluorescence (bottom) visualized by confocal fluorescence microscopy at 400-fold magnification in cells expressing YFP-fused human β_1 ARs and stained with human autoimmune IgG. Prior to the incubation with autoimmune IgG, the cells were exposed (for 5

min at 4°C) to PBS (left), 100% acetone (middle left), 50% methanol in PBS (middle right) or 2% formaldehyde in PBS (right).

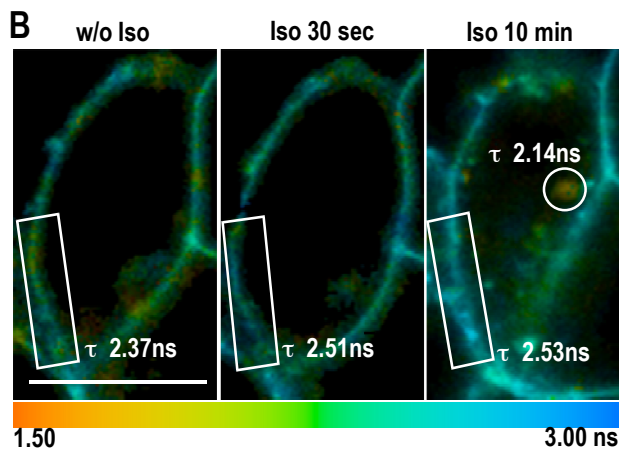
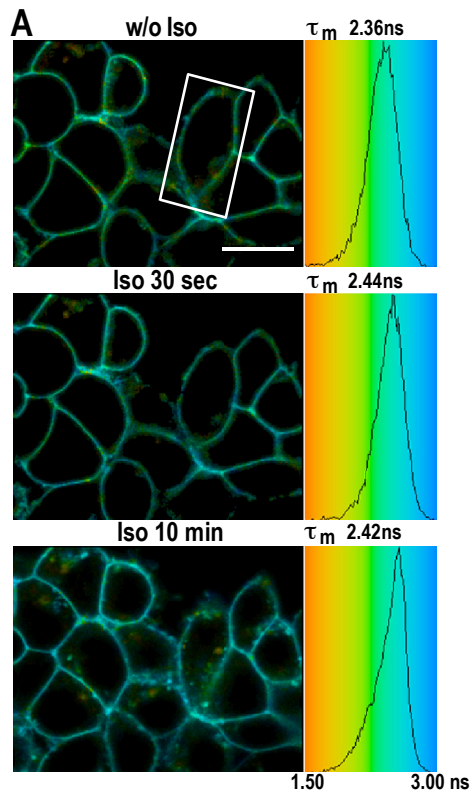
Figure S5 ERK1/2 phosphorylation. Cells expressing the construct 1 were incubated (10 min, 37 °C) with 1 ng/ml of EGF, 10^{-5} mol/l isoproterenol (ISO), 10^{-6} mol/l CGP20,712A (CGP), or 26.7 mg/L of autoimmune IgG or kept without addition (PBS). Following SDS lysis and Western blotting, blots were probed with antibodies specific for total ERK 1 and 2 (top) or specific for ERK 1 and 2 phosphorylated at Thr202/Tyr 204 (bottom).

Figure S6 Determination of IgG effects on basal beating rate and subsequent chronotropic isoproterenol responses of embryonic cardiomyocytes. Cells were plated on E-plate Cardio 96 wells and grown for 5 days until beating rate was stable. Rates of spontaneous synchronised rhythmic cardiomyocyte contraction were monitored with the xCELLigence RTCA Cardio Instrument. For suppression of muscarinic and β_2 -adrenergic chronotropic effects cells were pretreated (2 h) with $10\mu\text{M}$ atropine and $1\mu\text{M}$ ICI 118,551. Cells were then exposed to 50 mg/L IgG and $10\mu\text{M}$ isoproterenol was added after 2 h. A representative recording sequence of one well is shown. Arrows: Reference points for determining changes in the beating frequency in response to IgG exposure (left) and subsequent isoproterenol exposure (right).

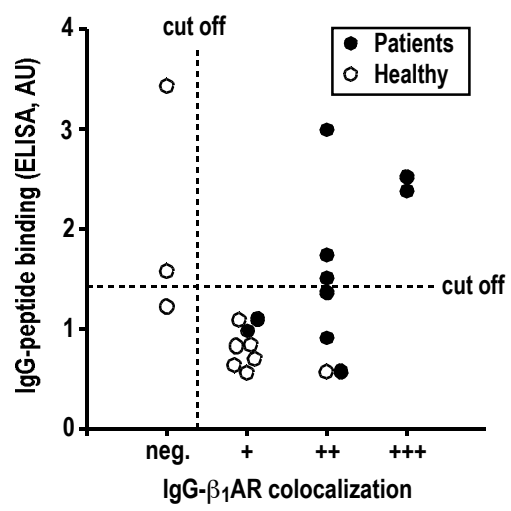
Supplemental Video File Monitoring of agonist-induced receptor cycling by TIRF. Appearance of biofluorescent receptosomes was monitored by TIRF in a 200 nm slice above the basal membrane of cells expressing the construct 1 (*Supplementary Figure S1A*). The movie is a representative recording over 1000 s following addition of 10^{-5} mol/L isoproterenol to the cell medium.



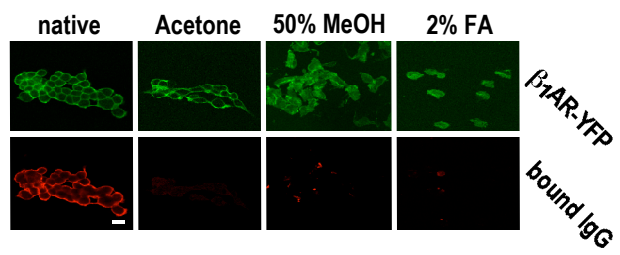
Supplementary Figure S1.



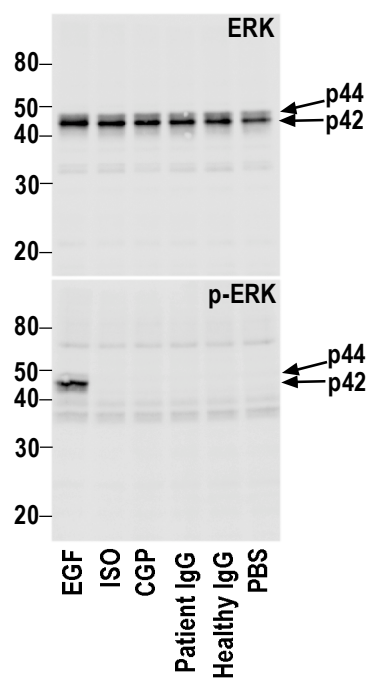
Supplementary Figure S2.



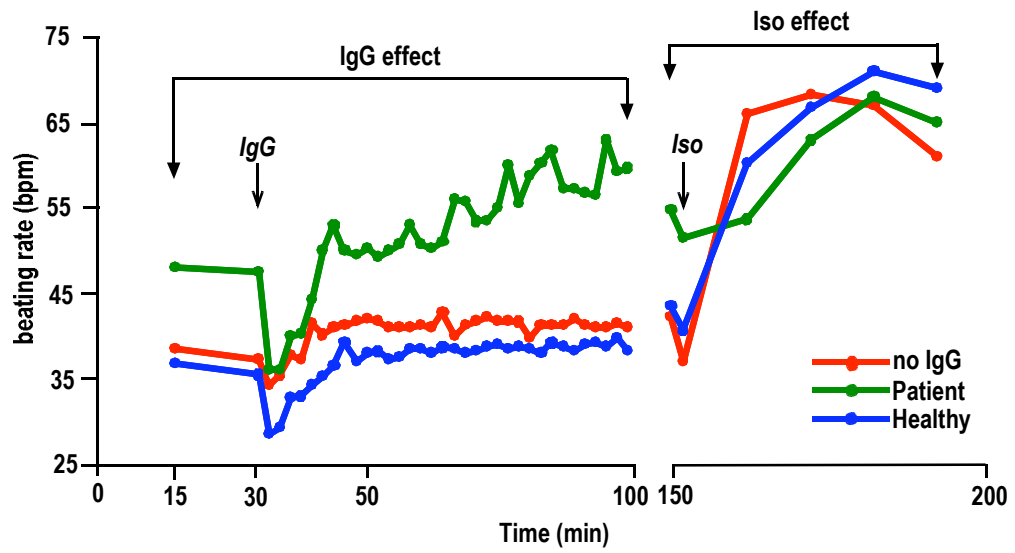
Supplementary Figure S3.



Supplementary Figure S4.



Supplementary Figure S5.



Supplementary Figure S6.