Supplemental Tables and Figures for Martin et al.

Supplementary Table S1. Percentage of mice that unexpectedly died following systemic administration of β-blockers. All doses are in mg/kg.

Drug	Dose (mg/kg)	Mortality Ratio	Percentage (%)
Propranolol	10	0/5	0.0
	30	0/5	0.0
	60	2/7	28.6
(S)-(-)-Propranolol	10	0/5	0.0
	30	0/5	0.0
	60	0/5	0.0
(R)-(+)-Propranolol	10	0/5	0.0
	30	1/6	16.6
	60	3/8	37.5
Bupranolol	60	0/5	0.0
	90	0/4	0.0
	120	0/5	0.0
(S)-(-)-Bupranolol	60	0/4	0.0
	90	0/4	0.0
	120	0/5	0.0
(R)-(+)-Bupranolol	60	0/4	0.0
	90	2/5	40.0
	120	4/4	100

Table S2. RMSD of propranolol and bupranolol enantiomers.

	β2–AR (RMSD in Å)[§]	β1–AR (RMSD in Å)[§]	β3–AR (RMSD in Å)[§]
R-propranolol S-propranolol	0.58	0.95	0.78
R-bupranolol S-bupranolol	0.43	0.62	2.6[*]

[§]RMSD values are computed over the heavy atoms of R/S propranolol and R/S bupranolol docking solutions. [*]The higher RMSD value is due to the flipping of the aromatic moiety that causes the opposite orientation of the substituents on the ring without altering the hydrophobic interaction with F309$^{6.52}$ in β3–AR binding site (Fig. S2).

Table S3. EC_{50} and 95% confidence interval (CI) data for dose-response curves of isoproterenol for all three β–ARs (A), and the antagonistic effects (IC_{50} and 95% CI) of the four inhibitors used at β1 (B), β2 (C), and β3 (D) adrenergic receptors (ARs). Data correspond to Fig. 5 in the main text.

A. Isoproterenol DRC

Receptor	EC_{50} (mol/l)	95% CI
β1–AR	1.5 E^{-10}	1.1 E^{-10} – 2.0 E^{-10}
β2–AR	9.7 E^{-12}	7.6 E^{-12} – 1.2 E^{-11}
β3–AR	1.5 E^{-9}	1.3 E^{-9} – 1.8 E^{-9}

B. β1–AR

Inhibitor	IC_{50} (mol/l)	95% CI
ICI 118,551	7.0 E^{-7}	4.8 E^{-7} – 1.0 E^{-6}
Propranolol	1.1 E^{-8}	8.5 E^{-9} – 1.6 E^{-8}
(S)-bupranolol	8.0 E^{-9}	5.7 E^{-8} – 1.1 E^{-8}
(R)-bupranolol	2.6 E^{-7}	1.7 E^{-7} – 4.1 E^{-7}

C. β2–AR

Inhibitor	IC_{50} (mol/l)	95% CI
ICI 118,551	1.7 E^{-8}	1.0 E^{-8} – 2.8 E^{-8}
Propranolol	1.6 E^{-8}	1.1 E^{-8} – 2.2 E^{-8}
(S)-bupranolol	6.7 E^{-9}	5.5 E^{-9} – 8.1 E^{-9}
(R)-bupranolol	2.9 E^{-8}	2.3 E^{-8} – 3.7 E^{-8}

D. β3–AR

Inhibitor	IC_{50} mol/l)	95% CI
ICI 118,551	1.3 E^{-6}	3.2 E^{-11} – 5.4 E^{-2}
Propranolol	5.5 E^{-3}	0.0 E^{0} – 2.2 E^{34}
(S)-bupranolol	1.1 E^{-7}	7.8 E^{-8} – 1.5 E^{-7}
(R)-bupranolol	---	---

Table S4. pA_2, slope and r^2 values for the different inhibitors at β1, β2, and β3 adrenergic receptors (ARs). 95% confidence intervals are in parentheses.

pA_2 (95 % CI)	**β1–AR**	**β2–AR**	**β3–AR**
ICI 118,551	6.7 (6.4 – 7.3)	8.3 (7.6 – 8.7)	5.5 (5.5 – 6.0)
Propranolol	8.7 (6.2 – 9.5)	8.6 (8.4 – 8.9)	7.4 (6.7 – 8.3)
R-bupranolol	7.3 (6.9 – 7.8)	8.7 (8.3 – 9.4)	5.9 (5.4 – 6.5)
S-bupranolol	8.7 (8.4 – 9.2)	8.7 (8.4 – 9.0)	7.1 (6.7 – 7.8)
Slope	**β1–AR**	**β2–AR**	**β3–AR**
ICI 118,551	- 1.3 ± 0.2 (-1.7 to -0.9)	- 2.2 ± 0.3 (-2.8 to -1.7)	- 1.7 ± 0.1 (-2.0 to -1.4)
Propranolol	- 1.1 ± 0.2 (-1.5 to -0.8)	- 1.9 ± 0.1 (-2.2 to -1.7)	- 0.8 ± 0.1 (-1.0 to -0.5)
R-bupranolol	- 1.0 ± 0.1 (-1.2 to -0.7)	- 1.2 ± 0.2 (-1.6 to -0.9)	- 0.8 ± 0.1 (-1.1 to -0.6)
S-bupranolol	- 1.2 ± 0.1 (-1.4 to -0.9)	- 2.8 ± 0.3 (-3.3 to -2.2)	- 1.1 ± 0.1 (-1.4 to -0.8)
r^2	**β1–AR**	**β2–AR**	**β3–AR**
ICI 118,551	0.72	0.73	0.88
Propranolol	0.59	0.87	0.59
R-bupranolol	0.76	0.68	0.61
S-bupranolol	0.80	0.79	0.63

Supplemental Figures

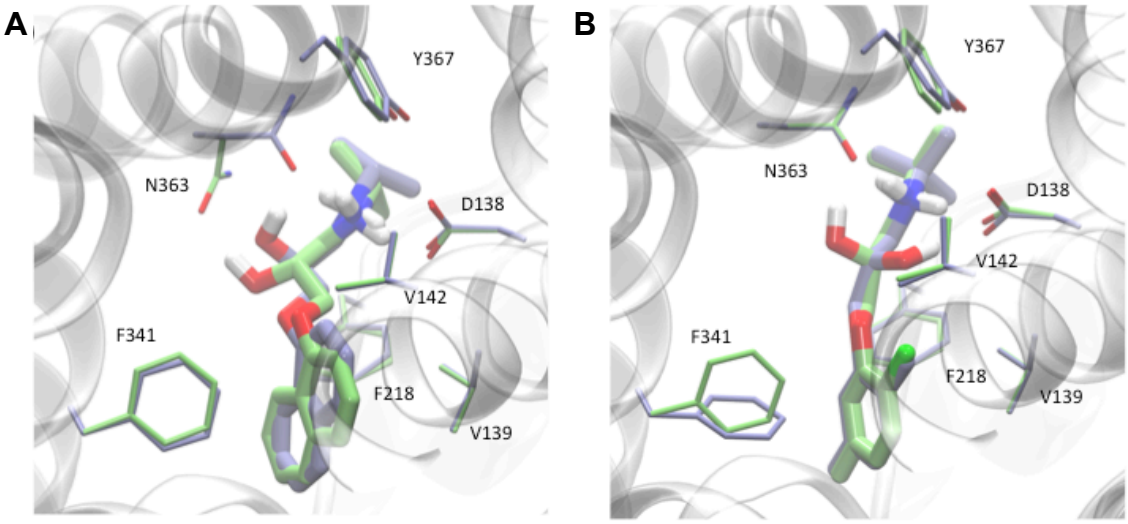

Fig. S1. R/S propanolol and bupranolol docking solutions in β1–AR binding site. (A) Zoomed view of R- and S-propranolol and of (B) R- and S- bupranolol. The structure of β1–AR is shown in grey cartoon; carbon atoms are represented in green and blue for R and S enantiomers, respectively. The same color code is adopted to indicate the side chains of β1–AR amino acids when in complex with the two enantiomers.

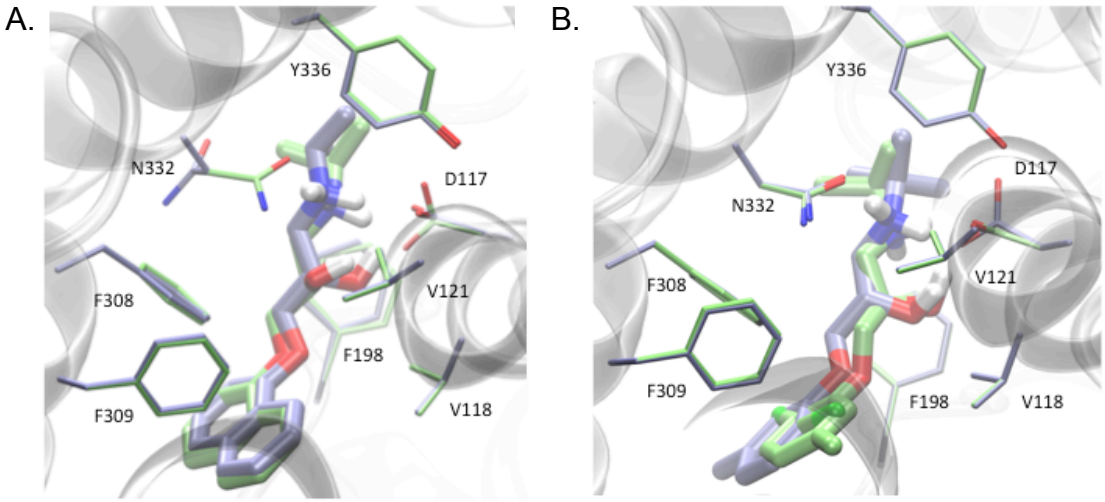

Fig. S2. R/S propanolol and bupranolol docking solutions in β3–AR binding site. (**a**) Zoomed view of R- and S-propanolol and of (**b**) R- and S- bupranolol. The structure of β3–AR is shown in grey cartoon; carbon atoms are represented in green and blue for R and S enantiomers, respectively. The same color code is adopted to indicate the side chains of β3–AR amino acids when in complex with the two enantiomers.

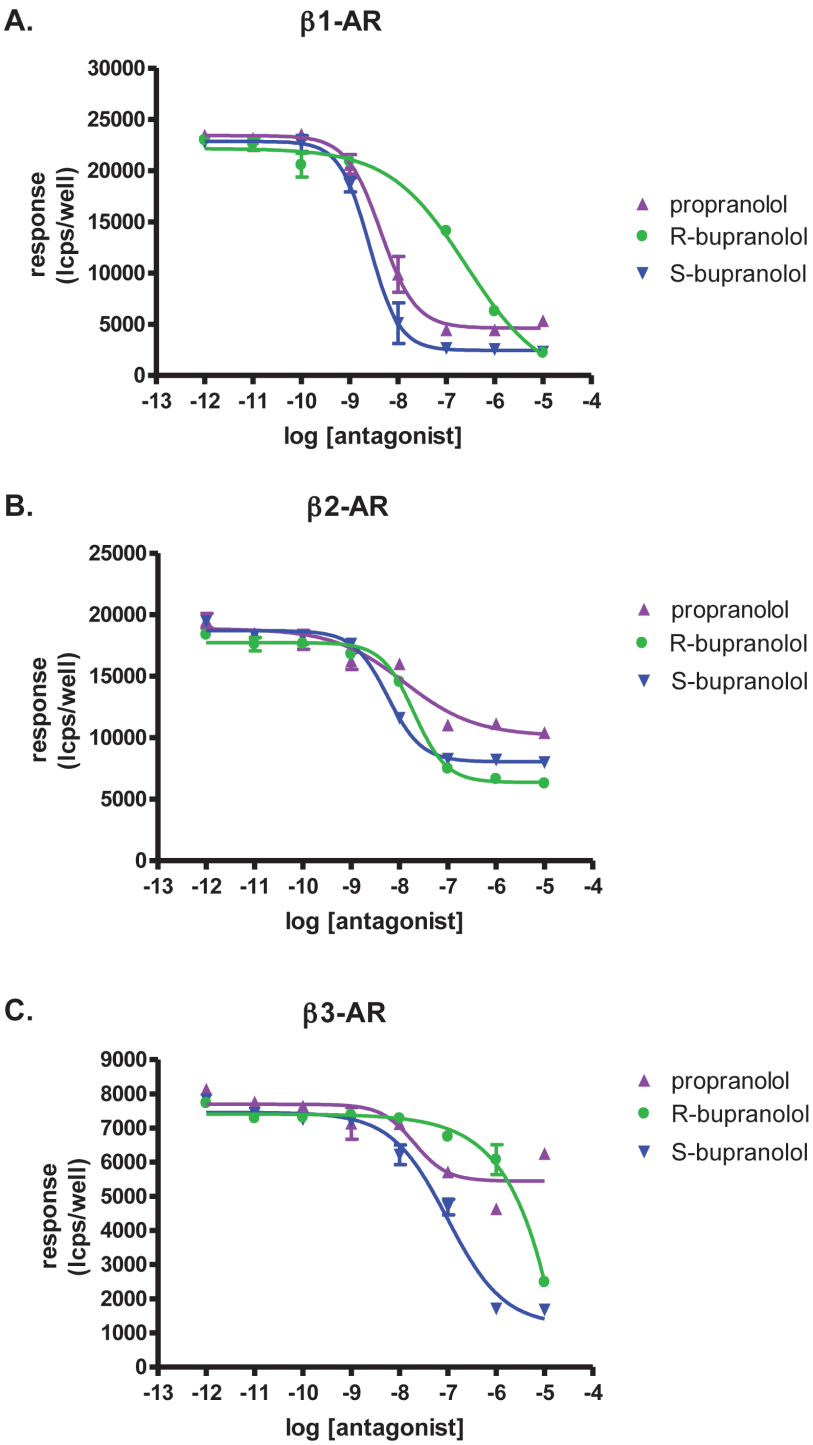

Fig. S3. Examples of raw data representative of dose-response curves of antagonists in cells expressing A) β1–AR (*n*=3), B) β2–AR (*n*=3) or C) β3–AR (*n*=4).

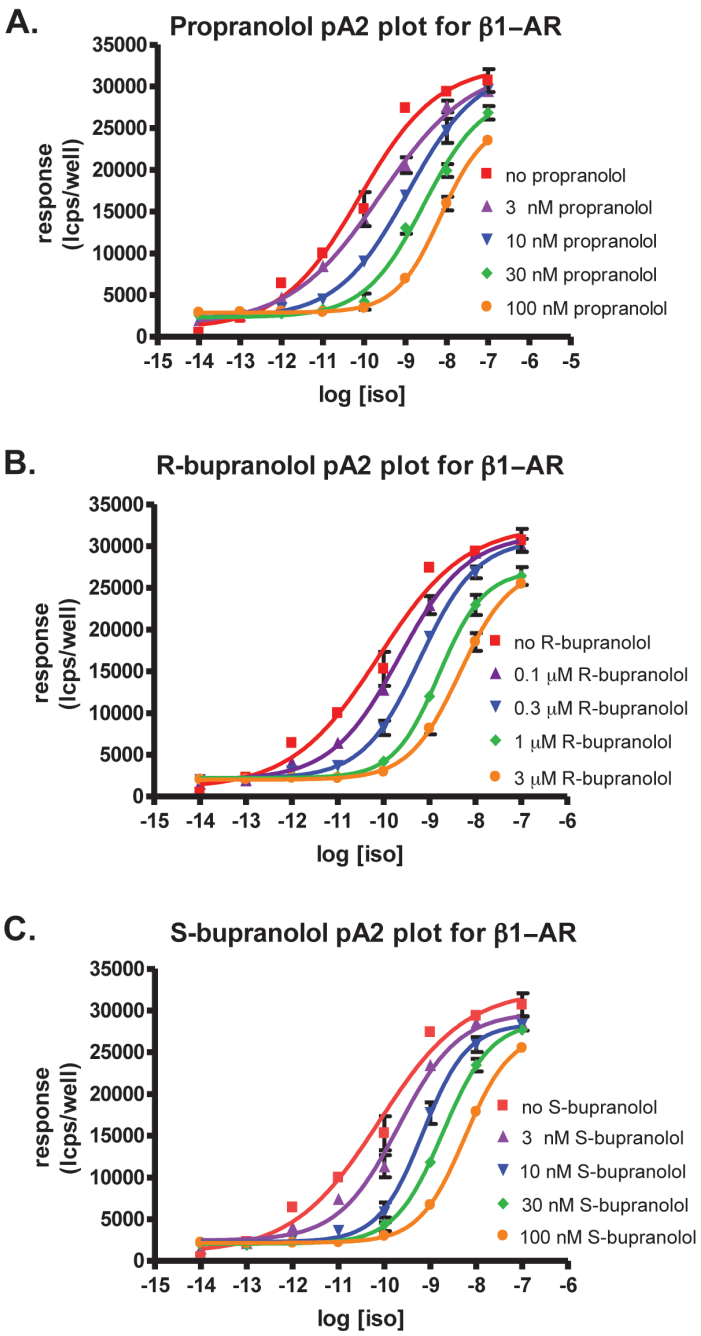

Fig. S4. β1–AR Schild plots with absolute values for pA_2 calculations. Dose-response curves of isoproterenol were generated and EC_{50}-values determined after treatment with various concentrations of A) propranolol, B) R-bupranolol or C) S-bupranolol.

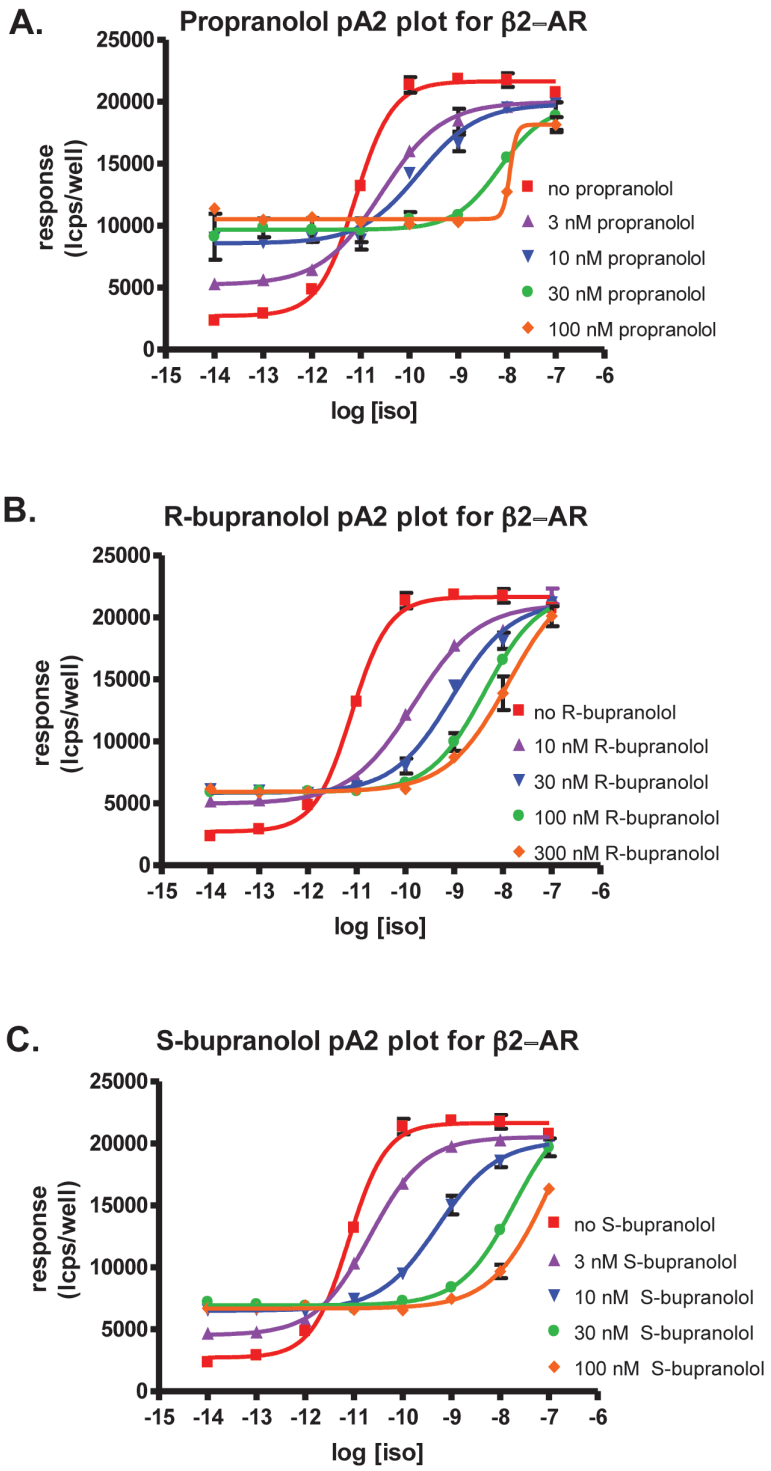

Fig. S5. β2–AR Schild plots with absolute values for pA_2 calculations. Dose-response curves of isoproterenol were generated and EC_{50}-values determined after treatment with various concentrations of A) propranolol, B) R-bupranolol or C) S-bupranolol.

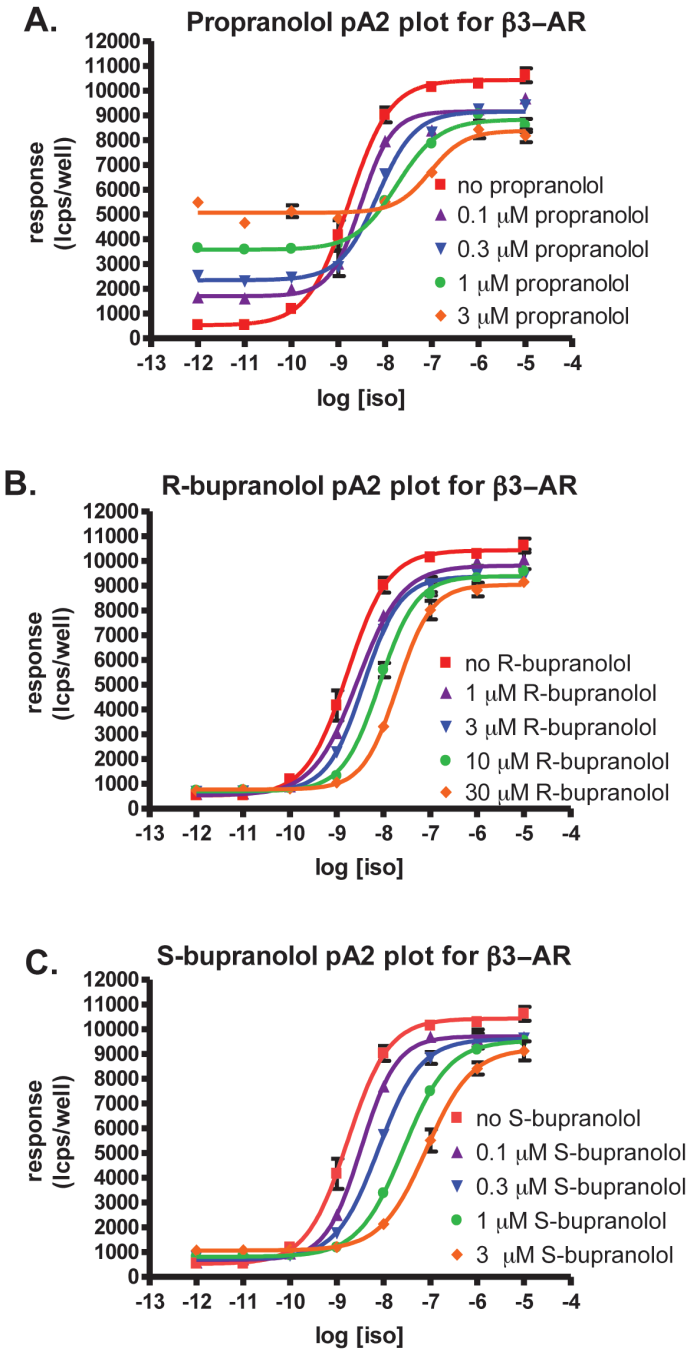

Fig. S6. β3–AR Schild plots with absolute values for pA_2 calculations. Dose-response curves of isoproterenol were generated and EC_{50}-values determined after treatment with various concentrations of A) propranolol, B) R-bupranolol or C) S-bupranolol.

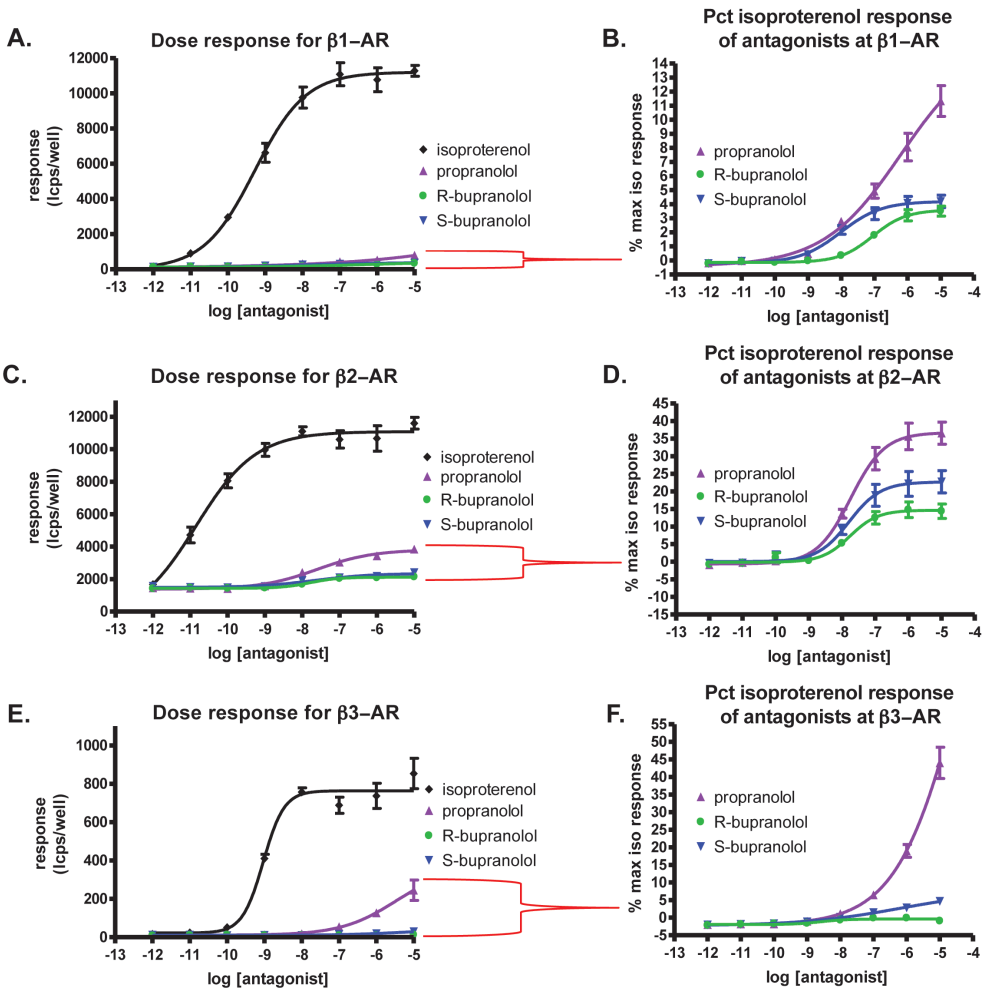

Fig. S7. Comparison of intrinsic sympathomimetic activities of antagonists (n=13) relative to isoproterenol (n=7) response.