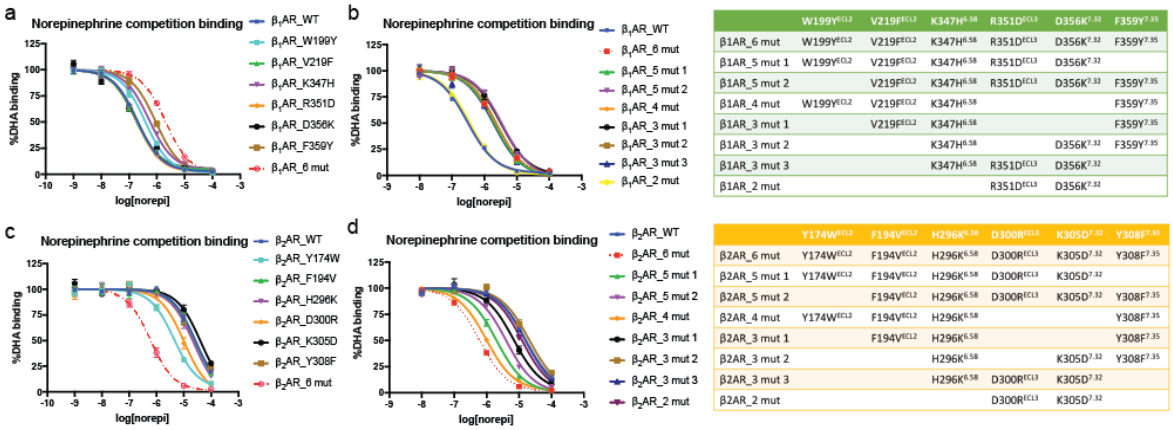

	W199Y^{ECL2}	V219F^{ECL2}	K347H$^{6.58}$	R351D^{ECL3}	D356K$^{7.32}$	F359Y$^{7.35}$
β1AR_6 mut	W199Y^{ECL2}	V219F^{ECL2}	K347H$^{6.58}$	R351D^{ECL3}	D356K$^{7.32}$	F359Y$^{7.35}$
β1AR_5 mut 1	W199Y^{ECL2}	V219F^{ECL2}	K347H$^{6.58}$	R351D^{ECL3}	D356K$^{7.32}$	
β1AR_5 mut 2		V219F^{ECL2}	K347H$^{6.58}$	R351D^{ECL3}	D356K$^{7.32}$	F359Y$^{7.35}$
β1AR_4 mut	W199Y^{ECL2}	V219F^{ECL2}	K347H$^{6.58}$			F359Y$^{7.35}$
β1AR_3 mut 1		V219F^{ECL2}	K347H$^{6.58}$			F359Y$^{7.35}$
β1AR_3 mut 2			K347H$^{6.58}$		D356K$^{7.32}$	F359Y$^{7.35}$
β1AR_3 mut 3			K347H$^{6.58}$	R351D^{ECL3}	D356K$^{7.32}$	
β1AR_2 mut				R351D^{ECL3}	D356K$^{7.32}$	

	Y174W^{ECL2}	F194V^{ECL2}	H296K$^{6.58}$	D300R^{ECL3}	K305D$^{7.32}$	Y308F$^{7.35}$
β2AR_6 mut	Y174W^{ECL2}	F194V^{ECL2}	H296K$^{6.58}$	D300R^{ECL3}	K305D$^{7.32}$	Y308F$^{7.35}$
β2AR_5 mut 1	Y174W^{ECL2}	F194V^{ECL2}	H296K$^{6.58}$	D300R^{ECL3}	K305D$^{7.32}$	
β2AR_5 mut 2		F194V^{ECL2}	H296K$^{6.58}$	D300R^{ECL3}	K305D$^{7.32}$	Y308F$^{7.35}$
β2AR_4 mut	Y174W^{ECL2}	F194V^{ECL2}	H296K$^{6.58}$			Y308F$^{7.35}$
β2AR_3 mut 1		F194V^{ECL2}	H296K$^{6.58}$			Y308F$^{7.35}$
β2AR_3 mut 2			H296K$^{6.58}$		K305D$^{7.32}$	Y308F$^{7.35}$
β2AR_3 mut 3			H296K$^{6.58}$	D300R^{ECL3}	K305D$^{7.32}$	
β2AR_2 mut				D300R^{ECL3}	K305D$^{7.32}$	

Supplementary information, Fig. S8| Norepinephrine competition curves of the human β_1AR and β_2AR mutations.

a, The norepinephrine competition curves of single mutations of the β_1AR. b, The norepinephrine competition curves of multiple mutations (containing 2 – 6 mutations) of the β_1AR. c,The norepinephrine competition curves of single mutations of the β_2AR. d, The norepinephrine competition curves of multiple mutations (containing 2 – 6 mutations) of the β_2AR. Data are given as mean ± SEM of 3 – 12 independent samples. The concentration of [^{3}H]-DHA used for competition binding is 2 nM. The β_2AR-Y174W and β_2AR-D300R single mutations showed increased norepinephrine affinity compared to wild type β_2AR, however, the corresponding mutations in the β_1AR only show small (β_1AR-W199Y) or no (β_1AR-R351D) decreases on norepinephrine affinity. While the β_1AR-K347H and β_1AR-F359Y showed decreased norepinephrine affinity compared to the wild type β_1AR; the corresponding mutations (β_2AR-H296K or β_2AR-Y308F) did not show any increase in norepinephrine affinity. Furthermore, although the β_1AR-F359Y showed the largest decrease on norepinephrine affinity among the 6 single mutations of the β_1AR a, a triple mutant (β_1AR-3mut-3) without the F359Y mutation showed a comparable effect in decreasing norepinephrine affinity as the β_1AR-6mut b, The complex behaviors of the mutants suggest that norepinephrine selectivity is determined by a combination of residues that cooperate to form the ligand binding pathway.