Supporting information:

Identification of key regions mediating human melatonin type 1 receptor functional selectivity revealed by natural variants

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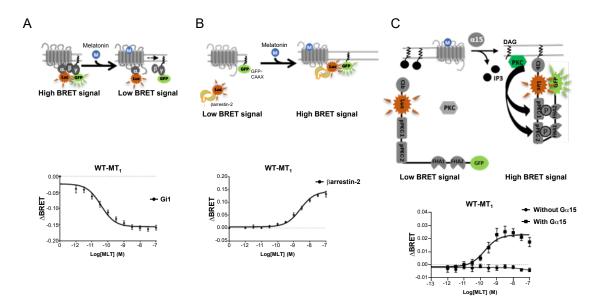


Figure S1. Representation of the different BRET-based biosensors used for MT1 characterization.

(A) In basal condition, MT1 is bound to inactive complex $\alpha\beta\gamma$ (left part). In our case, we used α proteins fused to Rlucll and Gy proteins fused to GFP10. When melatonin is added, it binds to and activates the MT1 receptor leading to the dissociation of the complex (upper panel). Rlucll too distant from GFP10 to transfer its energy, a decrease of BRET is observed (bottom panel). (B) For this experiment, we used βarrestin-2 fused to RlucII (βarrestin-2-RlucII) and the CAAX part of Kras protein which anchors to the plasma membrane fused to rGFP (CAAX-rGFP). In basal state, Barrestin-2 is not present at the plasma membrane (upper left panel). Upon MT1 activation by melatonin, β arrestin-2 is recruited to MT1 at the cell surface leading to a proximity between Barrestin-2-RlucII and CAAX-rGFP leading to an increase of EbBRET signal (upper right panel) as shown by the dose-response curve on the bottom panel. (C) The last BRET sensor used was the unimolecular biosensor of PKC activation. This biosensor contains the c1b domain of PKC δ able to bind DAG, Rlucll, two specific phospho-substrate sequences (pPKC1 and pPKC2), two phosphosensing domains (FHA1 and FHA2) and a rGFP (upper left panel). Upon MT1 activation by melatonin, $G\alpha 15$ is activated leading to the activation of PLC, producing DAG and IP3 and PKC activation. This activated PKC and our biosensors are then able to bind to DAG present at the plasma membrane (upper right panel). PKC phosphorylates the biosensor, leading to its conformational change increasing the proximity of RlucII with rGFP and an increase of EbBRET (bottom panel).

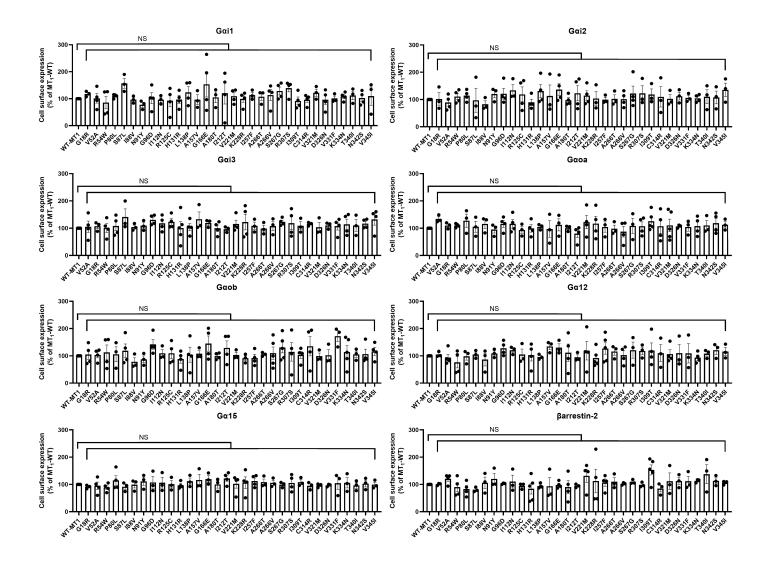


Figure S2. MT1 variant receptors are as expressed at the cell surface as the MT1-WT.

Cell surface expression of MT1 variants measured by enzyme-linked immunosorbent assay (ELISA) in HEK293 cells in parallel of BRET experiments for every G protein activation or β arrestin-2 recruitment. Cell surface expression of each variant was adjusted to MT1-WT. Statistical analysis was performed using one-sample t test. Data represents means ± SEM of 3 to 6 experiments. Data were fitted in GraphPad Prism 9.

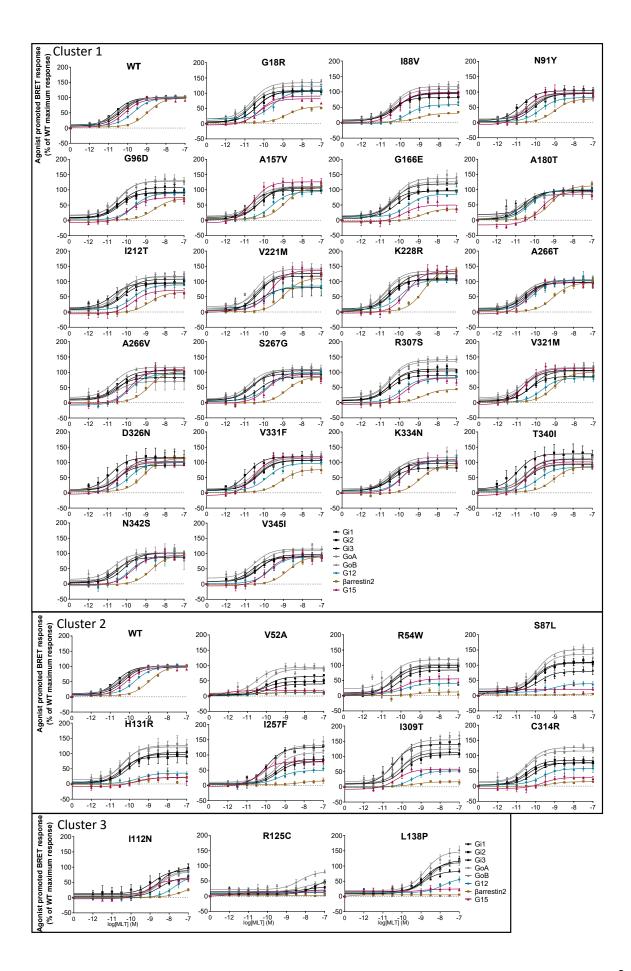


Figure S3. Melatonin concentration-response curves for G protein activation and βarrestin-2 recruitment of the MT₁-WT and the different variant profiles grouped in 3 clusters.

Functional profile of the MT_1 -WT receptor and the first cluster corresponding to the 21 variants with a similar profile to the MT_1 -WT receptor. The cluster 2 groups 7 variants with a total loss of βarrestin-2 recruitment and generally an impairment of Gα12 and/or Gα15 activation. The cluster 3 groups 3 variants with a loss of βarrestin-2 recruitment and a defect for every maximal efficacy (Max) but with similar potencies (EC50) to the MT_1 -WT. Cell surface expression of each variant was adjusted to MT_1 -WT and monitored by ELISA. Experiments were repeated at least 3 times. Data were plotted using non-linear regression with a fixed Hill slope equal to 1. Data points represent means ± SEM of 3 to 15 experiments. WT; wild-type. Data were fitted in GraphPad Prism 9.

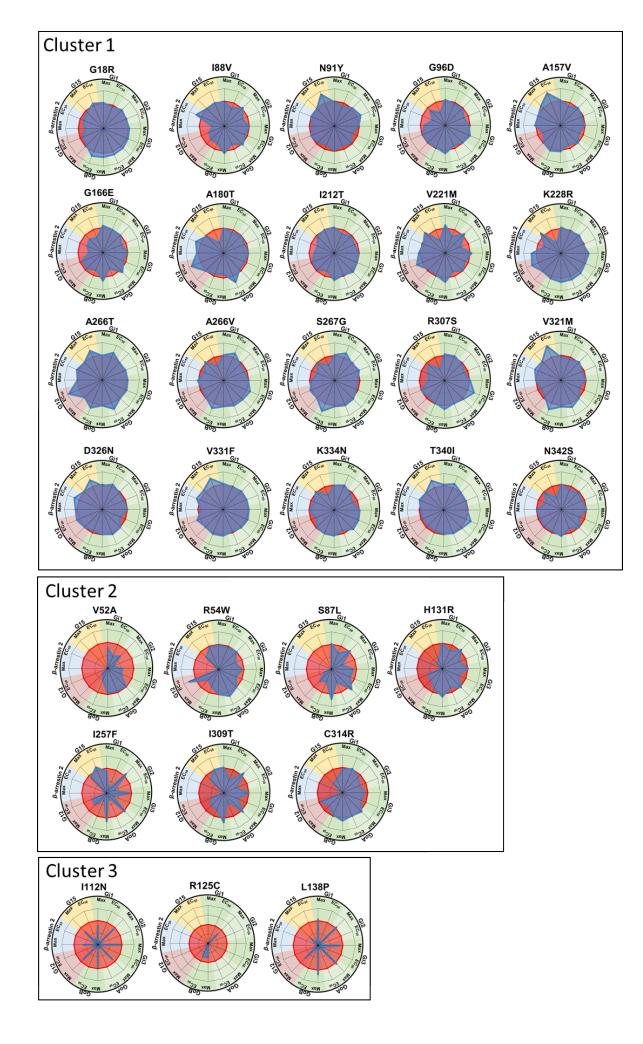
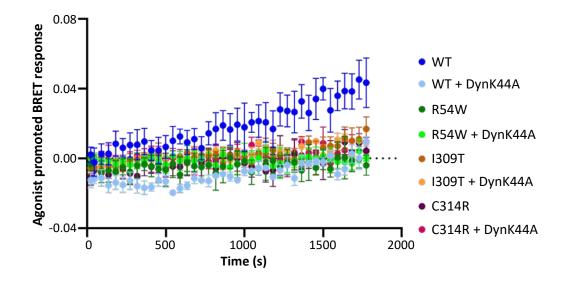


Figure S4. Radial graph representation of the different variant profiles grouped in 3 clusters.

On each radial graph for each MT_1 variant, maximal agonist-induced efficacy (Max) and potency (EC50) obtained by BRET are indicated. The three clusters were made regarding the profile of each variant. The first cluster corresponds to the 21 variants with a similar profile to the MT_1 -WT receptor. The cluster 2 groups 7 variants with a total loss of βarrestin-2 recruitment and generally an impairment of Gα activation potencies, especially for Gα12 and/or Gα15. The cluster 3 groups 3 variants with a defect for every efficacy (Max) but generally with similar potencies (EC50) to the MT_1 -WT. WT profile is represented in red and mutant profiles are in blue. A loss of potency or efficacy for a specific protein results in the decrease of the blue area. Cell surface expression of each variant was adjusted to MT_1 -WT and monitored by ELISA. Data were fitted in Microsoft Excel 2016.





Kinetic of melatonin-induced BRET response between MT1-WT (Blue), R54W (Green), I309T (Brown) and C314R (Purple) receptors fused to RlucII and the early endosome marker FYVE fused to rGFP, in absence (Dark curves) or presence (Clear curves) of the dominant negative Dynamin K44A (DynK44A) to inhibit endocytosis. Only MT1-WT receptor internalizes contrary to R54W, I309T and C314R. Data points represent means ± SEM of 3 experiments. WT; wild-type. Data were fitted in GraphPad Prism 9.

Supporting Tables

Table S1. Coding variants of *MTNR1A* (NM_005958.4) which were accurately detected in 8,687 individuals. 2,102 cases and 4,008 controls were involved in the case-control study for type 2 diabetes; 991 cases and 985 controls were involved in the case-control study for childhood obesity; 1,301 cases and 2,621 controls were involved in the case-control study for adulthood obesity.

						Minor Allele Count						
Chr_Pos	Def	Mut Conse- quence		AA	C	Type 2 diabetes		Obesity C	Obesity Childhood		Obesity Adulthood	
(hg19)	Ref			change GnomAL		Control	Case	Control	Case	Control	Case	
4_187476468	С	Т	NS	p.G18R	1	0	0	2	0	0	0	
4_187476365	А	G	NS	p.V52A	1	3	0	3	1	1	1	
4_187476360	G	А	NS	p.R54W	1	0	0	1	1	0	0	
4 187476335	С	А	SD	p.?	1	0	1	0	0	0	0	
4_187455657	G	А	NS	p.P80L	1	1	0	0	0	0	1	
4_187455636	G	А	NS	p.S87L	1	0	1	0	0	0	0	
4_187455634	т	С	NS	p.188V	1	1	0	0	0	1	0	
4_187455625	т	А	NS	p.N91Y	1	0	1	0	0	0	1	
4_187455617	С	т	SG	p.W93*	1	1	0	0	0	1	0	
4_187455609	С	т	NS	p.G96D	1	12	10	2	5	5	1	
4 187455561	А	т	NS	p.I112N	0	0	1	0	0	0	1	
	G	А	NS	p.R125C	1	0	1	0	0	0	0	
_ 4_187455504	т	С	NS	p.H131R	1	1	0	1	0	0	1	
4 187455483	А	G	NS	p.L138P	1	0	1	0	1	1	0	
4_187455426	G	А	NS	p.A157V	1	0	1	0	0	0	0	
4_187455399	С	т	NS	p.G166E	1	159	64	19	28	103	40	
4_187455386	G	С	SG	p.Y170*	1	2	2	1	0	2	0	
4_187455358	С	т	NS	p.A180T	1	1	1	0	0	2	0	
4_187455261	А	G	NS	p.I212T	1	1	29	1	1	6	5	
4_187455235	С	Т	NS	p.V221M	1	0	0	0	1	0	1	
4_187455213	Т	С	NS	p.K228R	0	0	1	0	0	0	0	
4_187455127	Т	А	NS	p.1257F	1	1	0	0	0	1	0	
4_187455100	С	Т	NS	p.A266T	1	3	0	3	0	2	1	
4_187455099	G	А	NS	p.A266V	1	152	93	42	26	102	49	
4_187455097	Т	С	NS	p.S267G	0	0	1	0	0	0	1	
4_187454975	С	G	NS	p.R307S	0	1	0	1	0	0	0	
4_187454956	А	G	NS	p.C314R	1	1	0	0	0	1	0	
4_187454935	С	Т	NS	p.V321M	1	1	1	0	0	0	0	
4_187454920	С	Т	NS	p.D326N	1	0	1	0	1	0	0	
4_187454905	С	Α	NS	p.V331F	1	0	1	0	0	0	0	
4_187454894	Т	Α	NS	p.K334N	1	26	13	4	4	16	4	
	CAGT											
4_187454882	GGAG	طما	ГС	n \\/222*	1	1	1	0	0	2	0	
4_10/404662	ACGG	del	FS	p.W333*	Т	1	1	U	0	2	U	
	TTTC											
4_187454877	G	Α	NS	p.T340I	1	2	0	0	0	1	0	
4_187454871	Т	С	NS	p.N342S	0	1	0	0	0	0	0	
4_187454863	С	т	NS	p.V345I	1	5	2	2	0	4	3	
4_187454843	Т	Α	SL	p.*351Yext*?	0	1	0	0	0	0	0	

AA, amino acid; Chr, chromosome; Del, deletion; FS, frameshift variant; GnomAD, genome aggregation database browser (v2.1.1; 1: present, 0: absent); Mut, mutated allele; NS, non-synonymous variant; Pos, position (according to the human alignment hg19/GRCh37); Ref, reference allele; SD, splice-donor variant; SG, stop-gain variant; SL, stop-loss variant.

Study	Variant	OR [CI]	P _π	Pτ	Р
CC T2D	Cluster of rare variants	1.17 [0.759-1.78]	0.473	0.373	0.483
CC ObC	Cluster of rare variants	0.669 [0.302-1.47]	0.315	0.464	0.427
CC ObA	Cluster of rare variants	0.706 [0.406-1.19]	0.201	0.383	0.275
CC T2D	p.G166E (rs28383653)	0.941 [0.676-1.30]	NA	NA	0.712
CC ObC	p.G166E (rs28383653)	2.28 [1.03-5.16]	NA	NA	0.0445
CC ObA	p.G166E (rs28383653)	0.804 [0.546-1.16]	NA	NA	0.257
CC T2D	p.A266V (rs28383652)	1.15 [0.855-1.54]	NA	NA	0.347
CC ObC	p.A266V (rs28383652)	0.757 [0.364-1.56]	NA	NA	0.451
CC ObA	p.A266V (rs28383652)	0.855 [0.596-1.21]	NA	NA	0.387

Table S2. Association between *MTNR1A* coding variants and the risk of T2D, childhood obesity and adulthood obesity.

CC, case-control study; CI, confidence interval; NA, not applicable; ObC, obesity childhood; ObA, obesity adulthood; OR, odds ratio; π , mean effect of the cluster; τ , heterogeneous effect of the cluster

Table S3. Data from the functional characterization of variants. Summary of the functional profiling of (A) Gai1, Gai2, Gai3, (B) GaoA, GaoB, (C) Ga12 and Ga15 activation and β arrestin-2 recruitment by MT₁-WT and MT₁ variants. Data represent the mean ± SEM of 3-13 independent experiments with repeats in quadruplicate. Data were analyzed by comparing independent fits.

Gi1 Gi2 Gi3 Receptor (% of WT) LogEC50 Log(Tau/Ka) (% of WT) LogEC50 Log(Tau/Ka) Emax (% of WT) LogEC50 Log(Tau/Ka) LogEC50 Log(Tau/Ka) (% of WT) LogEC50 Log(Tau/Ka) (% of WT) LogEC50 Log(Tau/Ka) (% of WT) LogEC50 LogEC50	Α									
Receptor (% of WT) LogECS0 LogITau/Ka) (% of WT) LogITau/Ka) (% of WT) LogECS0 LogITau/Ka) (% of WT) LogITau/Ka) LogI			Gi1			Gi2		Gi3		
G18R 104.9±3.9 -10.47±0.11 10.39±0.08 108.3±2.3 -10.72±0.06 106.5±0.07 106.1±2.5 -10.45±0.06 10.83±0.06 I8SV 92.9±4.5 -10.3±0.13 10.21±0.09 108.7±2.4 -10.55±0.06 10.45±0.08 95.67±4.0 -10.3±0.12 10.9±0.07 N91Y 92.7±2.7 -10.4±0.08 10.4±0.09 10.3±3.3 -10.85±0.09 10.4±0.08 91.5±3.1 -10.4±0.01 10.3±0.08 G96D 106.1±3.3 -10.4±0.04 10.3±0.08 10.15±6.0 10.4±0.07 94.4±2.0 -10.2±0.05 10.1±0.07 G166E 116.9±3.8 -10.3±0.01 95.6±1.2 -10.6±0.07 10.5±0.09 93.2±2.2 -10.3±0.07 10.2±0.08 10.2±0.07 10.2±0.09 93.2±2.2 -10.3±0.07 10.2±0.00 10.2±0.01 10.2±0.01 10.4±0.07 10.2±0.01 10.4±0.07 10.2±0.01 10.4±0.07 10.4±0.07 10.4±0.09 10.4±0.07 10.4±0.08 10.3±0.01 10.4±0.07 10.4±0.07 10.4±0.08 10.3±0.01 10.4±0.07 10.4±0.01 10.4±0.09 10.4±0.01<	Receptor		LogEC50	Log(Tau/Ka)		LogEC50	Log(Tau/Ka)		LogEC50	Log(Tau/Ka)
IS8V 92.9±4.5 -10.3±0.13 10.2±0.09 108.7±2.4 -10.5±0.06 10.4±0.08 95.67±4.0 -10.3±0.12 10.1±0.07 N91V 92.7±2.7 -10.4±0.085 10.7±0.09 10.8±0.09 10.4±0.09 91.3±2.9 -10.8±0.09 10.4±0.18±0.07 10.05±0.07 G96D 106.1±3.3 -10.4±0.14 10.2±0.08 10.1±3.6 -10.6±0.07 94.4±2.0 -10.3±0.07 10.1±0.07 G166E 116.9±3.8 -10.5±0.01 10.3±0.09 95.8€±1.7 -10.5±0.05 95.9±1.24 -10.3±0.07 10.1±0.07 G166E 116.9±3.8 -10.3±0.10 10.3±0.09 91.0±2.2 -10.6±0.07 10.4±0.07 10.2±0.08 L121T 106.2±4.9 -10.3±0.07 10.4±0.09 105.7±4.0 -10.7±0.11 10.3±0.06 110.3±4.8 -10.2±0.06 10.2±2.4 -10.4±0.09 10.2±0.08 V221M 126.5±2.7 -10.5±0.07 10.4±0.09 105.7±4.0 -10.7±0.11 10.6±0.20 10.5±2.2±4.0 -10.4±0.09 10.2±0.06 10.2±2.10.02 10.2±0.06 10.2±2.2±0.06	WT-MT1		-10.54 ± 0.04	10.44 ± 0.05		-10.75 ± 0.03	10.64 ± 0.04		-10.4 ± 0.03	10.29 ± 0.03
N91Y 92.7±2.7 -10.4±0.085 10.27±0.09 103.6±3.3 -10.87±0.09 10.78±0.08 94.15±2.6 -10.8±0.07 10.05±0.07 G96D 106.1±3.3 -10.4±0.14 10.32±0.08 10.15±3.6 -10.69±0.10 10.4±0.08 91.5±3.1 -10.4±0.10 10.33±0.09 A157V 103.9±2.0 -10.4±0.14 10.32±0.08 10.15±3.6 -10.69±0.10 10.4±0.00 95.9±1.24 -10.3±0.05 10.1±0.07 G166E 116.9±3.8 -10.5±0.10 10.3±0.09 95.8±1.7 -10.5±0.05 10.4±0.07 10.5±0.09 93.2±2.2 -10.3±0.05 10.2±0.07 V221M 126.8±2.5 -10.2±0.05 10.2±0.08 10.7±0.4±0.07 10.5±0.07 10.5±0.09 93.2±2.2 -10.3±0.06 10.2±0.00 V221M 126.8±2.5 -10.2±0.05 10.4±0.07 10.5±0.08 10.7±0.4±0.07 10.5±0.08 10.3±0.06 10.2±0.00 10.2±0.00 10.2±0.00 10.2±0.00 10.2±0.00 10.2±0.00 10.2±0.00 10.2±0.00 10.2±0.00 10.2±0.00 10.2±0.00 10.2±0.00 10.2±0.00	G18R	104.9 ± 3.9	-10.47 ± 0.11	10.39 ± 0.08	108.3 ± 2.3	-10.72 ± 0.06	10.65 ± 0.07	106.1 ± 2.5	-10.45 ± 0.06	10.38 ± 0.06
G96D106.1±3.3-10.48±0.0910.4±0.0991.39±2.9-10.58±0.0910.43±0.0891.5±3.1-10.46±0.1010.33±0.09A157V103.9±5.0-10.4±0.1410.3±0.0810.15±3.6-10.69±0.1010.6±0.0794.4±2.0-10.23±0.0510.11±0.07G166E116.9±3.8-10.45±0.1010.3±0.0995.66±1.7-10.58±0.0510.46±0.0995.91±2.4-10.3±0.0710.19±0.08A180T97.3±3.4-10.45±0.1010.3±0.0991.0±2.2-10.67±0.0610.5±0.0993.22±2.2-10.39±0.0610.2±0.08V221M126.8±2.5-10.28±0.0510.24±0.08117.8±4.8-10.41±0.1110.3±0.06110.3±4.8-10.2±0.06K228R108.7±2.7-10.5±0.0710.4±0.09105.7±4.0-10.7±0.1210.4±0.07125.2±4.0-10.4±0.0910.4±0.07A266T95.0±4.3-10.7±0.1310.57±0.0895.8±2.6-10.76±0.0810.6±1.0910.4±0.0710.4±0.0710.4±0.0710.4±0.0710.4±0.0710.4±0.0710.4±0.0710.4±0.0710.4±0.0910.4±0.0710.4±0.0710.4±0.0910.4±0.0710.4±0.0910.4±0.0710.4±0.0910.4±0.0710.4±0.0910.4±0.0710.4±0.0910.4±0.0710.4±0.0910.4±0.0710.4±0.0910.4±0.0710.4±0.0910.4±0.0710.4±0.0910.4±0.0710.4±0.0910.4±0.0710.4±0.0910.4±0.0710.4±0.0910.4±0.0710.4±0.0910.4±0.0710.4±0.0910.4±0.0710.4±0.0910.4±0.0710.5±0.0880.9±2.3-10.2±0.08	188V	92.9 ± 4.5	-10.34 ± 0.13	10.21 ± 0.09	108.7 ± 2.4	-10.55 ± 0.06	10.48 ± 0.08	95.67±4.0	-10.31 ± 0.12	10.19 ± 0.07
A157V 103.9±5.0 -10.4±0.14 10.32±0.08 101.5±3.6 -10.69±0.10 10.6±0.07 94.42±2.0 -10.23±0.05 10.1±0.07 G166E 116.9±3.8 -10.53±0.09 10.49±0.09 95.6±2.0 -10.55±0.08 98.3±2.3 -10.3±0.07 10.9±0.08 A180T 97.3±3.4 -10.45±0.10 10.3±0.09 91.02±2.2 -10.67±0.06 10.55±0.08 98.3±2.2 -10.3±0.07 10.2±0.08 V221M 126.8±2.5 -10.28±0.05 10.28±0.08 117.8±4.8 -10.41±0.11 10.3±6.8 -10.27±0.12 10.22±0.06 K228R 108.7±2.7 -10.5±0.07 10.4±0.09 105.7±0.8 95.8±2.6 -10.64±0.07 10.6±1.09 95.8±2.8 10.3±1.8 -10.2±0.08 10.3±0.05 10.4±0.07 10.4±0.09 10.4±0.07 A266V 103.5±2.2 -10.69±0.06 10.6±0.08 85.8±2.8 -10.6±0.07 10.4±0.07 10.5±0.08 80.9±3.2 -10.6±0.07 10.4±0.07 10.5±0.08 10.3±2.4 -10.6±0.07 10.4±0.07 10.5±0.08 10.3±0.08 10.3±0.08 10.3±0.08 </td <td>N91Y</td> <td>92.7 ± 2.7</td> <td>-10.4 ± 0.085</td> <td>10.27 ± 0.09</td> <td>103.6 ± 3.3</td> <td>-10.87 ± 0.09</td> <td>10.78 ± 0.08</td> <td>94.15 ± 2.6</td> <td>-10.18 ± 0.07</td> <td>10.05 ± 0.07</td>	N91Y	92.7 ± 2.7	-10.4 ± 0.085	10.27 ± 0.09	103.6 ± 3.3	-10.87 ± 0.09	10.78 ± 0.08	94.15 ± 2.6	-10.18 ± 0.07	10.05 ± 0.07
G166E 116.9±3.8 -10.53±0.09 10.49±0.09 95.86±1.7 -10.58±0.05 10.46±0.09 95.91±2.4 -10.3±0.07 10.19±0.08 A180T 97.3±3.4 -10.45±0.10 10.34±0.10 95.66±2.0 -10.67±0.06 10.55±0.08 98.31±2.3 -10.32±0.07 10.21±0.08 V221M 126.8±2.5 -10.28±0.05 10.28±0.08 117.8±8 -10.41±0.01 10.3±4.8 -10.41±0.07 125.2±4.0 -10.44±0.09 10.22±0.06 K228R 108.7±2.7 -10.5±0.07 10.44±0.09 105.7±4.8 -10.7±0.11 10.64±0.07 104.6±2.9 -10.45±0.08 10.37±0.08 A266T 95.0±4.3 -10.7±0.13 10.57±0.08 95.8±2.4 -10.6±0.08 10.6±0.07 10.46±2.9 -10.45±0.08 10.41±0.09 S267G 10.3.7±2.7 -10.5±0.07 10.5±0.08 85.2±1.7 -10.6±0.05 10.45±0.08 10.3±2.7 -10.6±0.07 10.4±0.09 S267G 10.3.7±2.7 -10.5±0.01 10.2±0.10 106±4.2 -10.67±0.11 10.5±0.08 10.3±2.7 -10.6±0.07 10.5±0.0	G96D	106.1 ± 3.3	-10.48 ± 0.09	10.4 ± 0.09	91.39 ± 2.9	-10.58 ± 0.09	10.43 ± 0.08	91.5 ± 3.1	-10.46 ± 0.10	10.33 ± 0.09
A180T97.3 ± 3.4-10.45 ± 0.1010.34 ± 0.1095.66 ± 2.0-10.67 ± 0.0610.55 ± 0.0898.31 ± 2.3-10.32 ± 0.0710.21 ± 0.081212T106.2 ± 4.9-10.38 ± 0.1310.3 ± 0.0991.0 ± 2.2-10.64 ± 0.0710.5 ± 0.0993.2 ± 2.2-10.39 ± 0.0610.26 ± 0.09V221M126.8 ± 2.5-10.28 ± 0.0510.28 ± 0.08117.8 ± 4.8-10.41 ± 0.1110.58 ± 0.06110.3 ± 4.8-10.27 ± 0.1210.22 ± 0.06K228R108.7 ± 2.7-10.5 ± 0.0710.44 ± 0.09105.7 ± 0.0-10.71 ± 0.1110.64 ± 0.07104.6 ± 2.9-10.44 ± 0.0910.44 ± 0.07A266T95.0 ± 4.3-10.7 ± 0.1310.57 ± 0.0895.8 ± 2.6-10.76 ± 0.0810.64 ± 0.07104.6 ± 2.9-10.45 ± 0.0810.31 ± 0.06A266V103.5 ± 2.2-10.69 ± 0.0810.6 ± 0.1092.13 ± 1.9-10.79 ± 0.0610.64 ± 0.0710.46 ± 0.0710.56 ± 0.08S267G103.7 ± 2.9-10.65 ± 0.0110.2 ± ± 0.0510.4 ± 1.010.6 ± 0.0884.38 ± 2.0-10.33 ± 0.0610.16 ± 0.09R307S109.4 ± 2.7-10.56 ± 0.0810.6 ± 0.1092.4 ± 1.5-10.67 ± 0.1110.58 ± 0.08103.1 ± 2.7-10.64 ± 0.0710.56 ± 0.08V321M85.6 ± 3.0-10.51 ± 0.1010.4 ± 0.0910.3 ± 0.1010.5 ± 1.110.55 ± 0.110.1 ± 2.5-10.44 ± 0.0710.55 ± 0.08V331F105.9 ± 3.6-10.51 ± 0.0910.4 ± 0.0710.64 ± 0.0710.68 ± 0.08105.3 ± 3.1-10.45 ± 0.0810.3 ± 0.08	A157V	103.9 ± 5.0	-10.4 ± 0.14	10.32 ± 0.08	101.5 ± 3.6	-10.69 ± 0.10	10.6 ± 0.07	94.42 ± 2.0	-10.23 ± 0.05	10.11 ± 0.07
1212T 106.2 ± 4.9 -10.38 ± 0.13 10.3 ± 0.09 91.02 ± 2.2 -10.64 ± 0.07 10.5 ± 0.09 93.2 ± 2.2 -10.39 ± 0.06 10.26 ± 0.09 V221M 126.8 ± 2.5 -10.2 ± 0.05 10.2 ± 0.08 117.8 ± 4.8 -10.41 ± 0.11 10.3 ± 4.8 -10.7 ± 0.12 10.2 ± 0.06 K228R 108.7 ± 2.7 -10.5 ± 0.07 10.44 ± 0.09 105.7 ± 4.0 -10.7 ± 0.11 10.64 ± 0.07 125.2 ± 4.0 -10.44 ± 0.09 10.44 ± 0.07 A266T 105.5 ± 2.2 -10.69 ± 0.06 10.6 ± 0.08 85.2 ± 3.4 -10.5 ± 0.07 10.44 ± 0.09 95.8 ± 2.8 -10.5 ± 0.08 10.4 ± 0.09 S267G 103.7 ± 2.9 -10.69 ± 0.08 10.6 ± 0.10 92.13 ± 1.9 -10.79 ± 0.06 10.6 ± 0.08 84.3 ± 2.0 -10.33 ± 0.08 10.1 ± 0.09 R3075 109.4 ± 2.7 -10.58 ± 0.07 10.51 ± 0.09 10.6 ± 4.2 -10.67 ± 0.05 10.45 ± 0.08 10.5 ± 1.0 10.43 ± 0.08 10.5 ± 1.1 10.5 ± 0.18 10.1 ± 2.5 -10.19 ± 0.06 10.1 ± 0.28 V321M 95.7 ± 2.3 -10.6 ± 0.07 10.44 ±	G166E	116.9 ± 3.8	-10.53 ± 0.09	10.49 ± 0.09	95.86 ± 1.7	-10.58 ± 0.05	10.46 ± 0.09	95.91 ± 2.4	-10.3 ± 0.07	10.19 ± 0.08
V221M 126.8±2.5 -10.28±0.05 10.28±0.05 117.8±4.8 -10.41±0.11 10.38±0.06 110.3±4.8 -10.27±0.12 10.22±0.06 K228R 108.7±2.7 -10.5±0.07 10.44±0.09 105.7±4.0 -10.71±0.11 10.64±0.07 125.2±4.0 -10.44±0.09 10.44±0.07 A266T 95.0±4.3 -10.7±0.13 10.57±0.08 95.8±2±6 -10.76±0.11 10.64±0.07 104.6±2.9 -10.45±0.08 10.37±0.06 A266V 103.5±2.2 -10.69±0.08 10.6±0.01 95.8±2.3 -10.79±0.06 10.66±0.08 84.38±2.0 -10.33±0.06 10.16±0.09 R307S 109.4±2.7 -10.58±0.07 10.28±0.10 10.28±1.7 -10.65±0.05 10.45±0.08 96.09±2.3 -10.29±0.06 10.09±0.08 V321M 85.6±3.0 -10.45±0.10 10.3±0.08 105.3±3.1 -10.45±0.08 10.3±4.2 -10.67±0.01 10.1±2.5 -10.95±0.08 10.3±1.27 -10.4±0.07 10.3±0.08 V331F 105.9±3.6 -10.5±0.07 10.4±0.09 81.2±1.3 -10.67±0.01 10.5±0.13	A180T	97.3 ± 3.4	-10.45 ± 0.10	10.34 ± 0.10	95.66 ± 2.0	-10.67 ± 0.06	10.55 ± 0.08	98.31 ± 2.3	-10.32 ± 0.07	10.21 ± 0.08
K228R 108.7±2.7 -10.5±0.07 10.44±0.09 105.7±4.0 -10.71±0.11 10.64±0.07 125.2±4.0 -10.44±0.09 10.44±0.07 A266T 95.0±4.3 -10.7±0.13 10.57±0.08 95.8±2.6 -10.76±0.08 10.64±0.07 104.6±2.9 -10.45±0.08 10.37±0.06 A266V 103.7±2.9 -10.69±0.06 10.6±0.08 85.2±2.6 -10.76±0.05 10.64±0.09 95.8±2.8 -10.52±0.08 10.41±0.08 S267G 103.7±2.9 -10.69±0.07 10.5±0.09 92.13±1.9 -10.79±0.05 10.45±0.08 84.38±2.0 -10.32±0.07 10.5±0.08 V321M 85.6±3.0 -10.45±0.10 10.28±0.10 10.6±4.2 -10.67±0.05 10.45±0.08 103.1±2.7 -10.64±0.07 10.5±0.08 V321M 85.6±3.0 -10.45±0.10 10.28±0.10 10.6±4.2 -10.67±0.05 10.45±0.08 103.1±2.7 -10.64±0.07 10.5±0.08 V321M 95.7±2.3 -10.6±0.07 10.45±0.10 10.45±0.10 10.45±0.10 10.3±0.08 10.5±1.1 10.1±0.08 10.5±0.12 10.44±	I212T	106.2 ± 4.9	-10.38 ± 0.13	10.3 ± 0.09	91.02 ± 2.2	-10.64 ± 0.07	10.5 ± 0.09	93.22 ± 2.2	-10.39 ± 0.06	10.26 ± 0.09
A266T 95.0 ± 4.3 -10.7 ± 0.13 10.57 ± 0.08 95.8 ± 2.2 -10.76 ± 0.08 10.64 ± 0.07 104.5 ± 2.9 -10.45 ± 0.08 10.37 ± 0.08 A266V 103.5 ± 2.2 -10.69 ± 0.06 10.6 ± 0.08 85.2 ± 3.4 -10.55 ± 0.12 10.48 ± 0.09 95.8 ± 2.8 -10.52 ± 0.08 10.41 ± 0.08 S267G 103.7 ± 2.9 -10.69 ± 0.08 10.6 ± 0.10 92.13 ± 1.9 -10.79 ± 0.05 10.66 ± 0.08 84.38 ± 2.0 -10.33 ± 0.06 10.6 ± 0.08 R307S 109.4 ± 2.7 -10.58 ± 0.07 10.51 ± 0.09 89.15 ± 1.7 -10.67 ± 0.05 10.35 ± 0.08 103.1 ± 2.7 -10.64 ± 0.07 10.52 ± 0.08 V321M 85.6 ± 3.0 -10.45 ± 0.09 10.46 ± 0.10 90.44 ± 1.5 -10.67 ± 0.05 10.53 ± 0.1 101.1 ± 2.5 -10.44 ± 0.07 10.35 ± 0.08 103.7 ± 0.08 103.7 ± 0.08 103.7 ± 0.08 103.7 ± 0.08 103.7 ± 0.04 10.68 ± 0.08 105.3 ± 3.1 -10.45 ± 0.08 10.37 ± 0.06 10.53 ± 0.11 10.35 ± 0.08 10.3 ± 0.07 10.34 ± 0.07 10.34 ± 0.07 10.34 ± 0.07 10.35 ± 0.08 10.34 ± 0.07<	V221M	126.8 ± 2.5	-10.28 ± 0.05	10.28 ± 0.08	117.8 ± 4.8	-10.41 ± 0.11	10.38 ± 0.06	110.3 ± 4.8	-10.27 ± 0.12	10.22 ± 0.06
A266V 103.5 ± 2.2 -10.6 9 ± 0.06 10.6 ± 0.08 85.2 ± ± 3.4 -10.6 5 ± 0.12 10.48 ± 0.09 95.8 ± 2.8 -10.5 ± 0.08 10.4 ± 1.08 S267G 103.7 ± 2.9 -10.6 9 ± 0.08 10.6 ± 0.10 92.1 ± 1.9 -10.7 9 ± 0.06 10.6 ± 0.08 84.3 ± 2.0 -10.3 ± 0.06 10.1 ± 0.09 R3075 109.4 ± 2.7 -10.5 8 ± 0.07 10.5 ± 1.0.9 88.1 ± 1.7 -10.6 ± 0.05 10.4 ± 5.06 103.1 ± 2.7 -10.6 ± 0.07 10.5 ± 0.08 V321M 85.6 ± 3.0 -10.5 ± 0.09 10.4 ± 0.01 10.2 ± 0.01 10.6 ± 0.05 10.5 ± 0.10 10.1 ± 0.28 10.6 ± 0.11 10.5 ± 0.05 10.1 ± 1.2.7 -10.6 ± 0.07 10.4 ± 0.09 V331F 105.9 ± 3.6 -10.5 ± 0.01 10.4 ± 0.09 10.5 ± 1.1 -10.7 ± 0.04 10.6 ± 0.08 105.3 ± 3.1 -10.4 ± 0.07 10.3 ± 0.08 K334N 95.7 ± 2.3 -10.6 ± 0.07 10.4 ± 0.09 10.3 ± 0.10 88.4 ± 2.2 -10.6 ± 0.07 10.4 ± 0.07 10.3 ± 0.08 10.3 ± 0.10 10.4 ± 0.07 10.3 ± 0.08 10.4 ± 0.07 10.3 ± 0.08 <td< td=""><td>K228R</td><td>108.7 ± 2.7</td><td>-10.5 ± 0.07</td><td>10.44 ± 0.09</td><td>105.7 ± 4.0</td><td>-10.71 ± 0.11</td><td>10.64 ± 0.07</td><td>125.2 ± 4.0</td><td>-10.44 ± 0.09</td><td>10.44 ± 0.07</td></td<>	K228R	108.7 ± 2.7	-10.5 ± 0.07	10.44 ± 0.09	105.7 ± 4.0	-10.71 ± 0.11	10.64 ± 0.07	125.2 ± 4.0	-10.44 ± 0.09	10.44 ± 0.07
S267G 103.7 ± 2.9 -10.69 ± 0.08 10.6 ± 0.10 92.13 ± 1.9 -10.79 ± 0.06 10.66 ± 0.08 84.38 ± 2.0 -10.33 ± 0.06 10.16 ± 0.09 R307S 109.4 ± 2.7 -10.58 ± 0.07 10.51 ± 0.09 89.5 ± 1.7 -10.6 ± 0.05 10.4 ± 5.08 103.1 ± 2.7 -10.64 ± 0.07 10.56 ± 0.08 V321M 85.6 ± 3.0 -10.45 ± 0.10 10.28 ± 0.10 106 ± 4.2 -10.67 ± 0.05 10.53 ± 0.1 101.1 ± 2.5 -10.94 ± 0.08 10.9 ± 0.08 V321M 6.0.51 ± 0.10 10.44 ± 0.10 90.44 ± 1.5 -10.67 ± 0.05 10.53 ± 0.1 101.1 ± 2.5 -10.19 ± 0.08 10.3 ± 0.08 10.5 ± 1.3 -10.45 ± 0.08 10.3 ± 0.08 10.3 ± 0.08 10.5 ± 1.3 -10.45 ± 0.08 10.3 ± 0.08 10.3 ± 0.08 10.3 ± 0.08 10.3 ± 0.08 10.3 ± 0.08 10.3 ± 0.08 10.3 ± 0.08 10.3 ± 0.07 10.3 ± 0.08 10.3 ± 0.07 10.3 ± 0.08 10.3 ± 0.08 10.3 ± 0.08 10.3 ± 0.07 10.3 ± 0.08 10.3 ± 0.08 10.3 ± 0.08 10.3 ± 0.08 10.3 ± 0.08 10.3 ± 0.08 10.4 ± 0.07 10.3 ± 0.08 10.4 ± 0.07	A266T	95.0 ± 4.3	-10.7 ± 0.13	10.57 ± 0.08	95.82 ± 2.6	-10.76 ± 0.08	10.64 ± 0.07	104.6 ± 2.9	-10.45 ± 0.08	10.37 ± 0.06
R3075 109.4 ± 2.7 -10.58 ± 0.07 10.51 ± 0.09 89.15 ± 1.7 -10.6 ± 0.05 10.45 ± 0.08 103.1 ± 2.7 -10.64 ± 0.07 10.56 ± 0.08 V321M 85.6 ± 3.0 -10.45 ± 0.10 10.28 ± 0.10 106 ± 4.2 -10.67 ± 0.11 10.59 ± 0.08 96.09 ± 2.3 -10.2 ± 0.06 10.09 ± 0.08 D326N 90.0 ± 2.7 -10.61 ± 0.09 10.46 ± 0.10 90.44 ± 1.5 -10.67 ± 0.05 105.3 ± 0.1 101.1 ± 2.5 -10.19 ± 0.06 10.1 ± 0.08 V331F 105.9 ± 3.6 -10.51 ± 0.10 10.44 ± 0.09 81.25 ± 3.3 -10.76 ± 0.04 10.68 ± 0.08 105.3 ± 1.1 101.1 ± 2.5 -10.44 ± 0.07 10.35 ± 0.08 T3401 108.5 ± 2.5 -10.68 ± 0.07 10.48 ± 0.09 81.25 ± 3.3 -10.75 ± 0.07 10.62 ± 0.08 92.25 ± 3.8 -10.58 ± 0.12 10.44 ± 0.07 N342S 100.9 ± 3.4 -10.4 ± 0.09 10.3 ± 0.10 86.4 ± 2.1 -10.77 ± 0.07 10.62 ± 0.08 95.68 ± 1.9 -10.39 ± 0.05 10.27 ± 0.08 V3451 88.5 ± 4.0 -10.53 ± 0.13 10.3 ± 0.11 93.8 ± 0.12	A266V	103.5 ± 2.2	-10.69 ± 0.06	10.6 ± 0.08	85.24 ± 3.4	-10.65 ± 0.12	10.48 ± 0.09	95.83 ± 2.8	-10.52 ± 0.08	10.41 ± 0.08
V321M 85.6 ± 3.0 -10.45 ± 0.10 10.2 ± 0.10 10.6 ± 4.2 -10.67 ± 0.11 10.59 ± 0.08 96.09 ± 2.3 -10.2 ± 0.06 10.09 ± 0.08 D326N 90.0 ± 2.7 -10.61 ± 0.09 10.46 ± 0.10 90.44 ± 1.5 -10.67 ± 0.05 10.53 ± 0.1 101.1 ± 2.5 -10.19 ± 0.06 10.1 ± 0.08 V331F 105.9 ± 3.6 -10.51 ± 0.10 10.43 ± 0.08 105.8 ± 1.5 -10.76 ± 0.04 10.68 ± 0.08 105.3 ± 3.1 -10.44 ± 0.07 10.34 ± 0.08 103.7 ± 0.06 10.3 ± 2.5 -10.44 ± 0.07 10.34 ± 0.07 10.48 ± 0.09 10.3 ± 2.3 -10.56 ± 0.07 10.49 ± 0.01 10.35 ± 0.12 10.44 ± 0.07 N342S 100.9 ± 3.4 -10.4 ± 0.09 10.3 ± 0.10 86.4 ± 2.2 -10.66 ± 0.07 10.49 ± 0.10 100.8 ± 2.1 -10.19 ± 0.05 10.1 ± 0.08 V34SI 88.5 ± 4.0 -10.53 ± 0.13 10.37 ± 0.16 49.33 ± 4.2 -9.81 ± 0.22 9.401 ± 0.16 39.25 ± 1.7 -9.88 ± 0.12 9.47 ± 0.18 V52A 64.0 ± 1.7 -10.07 ± 0.07 9.77 ± 0.16 49.33 ± 4.2 -9.81 ± 0.22 9.401 ± 0.1	S267G	103.7 ± 2.9	-10.69 ± 0.08	10.6 ± 0.10	92.13 ± 1.9	-10.79 ± 0.06	10.66 ± 0.08	84.38 ± 2.0	-10.33 ± 0.06	10.16 ± 0.09
D326N 90.0±2.7 -10.61±0.09 10.46±0.10 90.44±1.5 -10.67±0.05 10.53±0.1 10.1±2.5 -10.19±0.06 10.1±0.8 V331F 105.9±3.6 -10.51±0.10 10.43±0.08 105.8±1.5 -10.66±0.04 10.68±0.08 105.3±3.1 -10.45±0.08 10.33±0.06 K334N 95.7±2.3 -10.6±0.07 10.48±0.09 81.25±3.3 -10.56±0.01 10.37±0.11 103±2.5 -10.44±0.07 10.35±0.08 T3401 108.5±2.5 -10.64±0.09 10.3±0.10 86.42±2.2 -10.66±0.07 10.49±0.10 100.8±2.1 -10.19±0.05 10.29±0.10 10.49±0.01 10.49±0.01 10.29±0.11 10.49±0.01 10.29±0.11 10.49±0.	R307S	109.4 ± 2.7	-10.58 ± 0.07	10.51 ± 0.09	89.15 ± 1.7	-10.6 ± 0.05	10.45 ± 0.08	103.1 ± 2.7	-10.64 ± 0.07	10.56 ± 0.08
V331F 105.9±3.6 -10.51±0.10 10.43±0.08 105.8±1.5 -10.76±0.04 10.68±0.08 105.3±3.1 -10.45±0.08 10.37±0.10 K334N 95.7±2.3 -10.6±0.07 10.48±0.09 81.25±3.3 -10.56±0.12 10.37±0.11 103±2.5 -10.44±0.07 10.35±0.08 T340I 108.5±2.5 -10.68±0.07 10.61±0.08 100.3±2.3 -10.75±0.09 92.25±3.8 -10.58±0.12 10.44±0.07 N342S 100.9±3.4 -10.4±0.09 10.3±0.10 86.42±2.2 -10.66±0.07 10.49±0.19 10.8±2.1 -10.19±0.05 10.1±0.08 V345I 88.5±4.0 -10.5±0.13 10.3±0.10 88.46±2.1 -10.77±0.07 10.6±2±0.08 95.68±1.19 -10.39±0.05 10.27±0.08 V52A 64.0±1.7 -10.07±0.07 9.77±0.16 49.3±4.2 -9.81±0.22 9.40±0.16 39.25±1.7 -9.98±0.12 9.47±0.18 R54W 91.8±3.0 -10.49±0.09 10.3±0.11 10.82±0.08 84.4±3.0 -10.16±0.10 9.99±0.10 S871 104.9±3.4 -10.2±0.10	V321M	85.6 ± 3.0	-10.45 ± 0.10	10.28 ± 0.10	106 ± 4.2	-10.67 ± 0.11	10.59 ± 0.08	96.09 ± 2.3	-10.2 ± 0.06	10.09 ± 0.08
K334N 95.7±2.3 -10.6±0.07 10.48±0.09 81.25±3.3 -10.56±0.12 10.37±0.11 103±2.5 -10.44±0.07 10.35±0.8 T340I 108.5±2.5 -10.68±0.07 10.61±0.08 100.3±2.3 -10.73±0.06 10.63±0.09 92.25±3.8 -10.58±0.12 10.44±0.07 N342S 100.9±3.4 -10.4±0.09 10.3±0.10 86.42±2.2 -10.66±0.07 10.49±0.10 100.8±2.1 -10.19±0.05 10.1±0.08 V34SI 88.5±4.0 -10.53±0.13 10.3±0.10 88.46±2.1 -10.77±0.07 10.62±0.08 95.68±1.9 -10.39±0.05 10.27±0.08 V52A 64.0±1.7 -10.07±0.07 9.77±0.16 49.33±4.2 -9.81±0.22 9.401±0.16 39.25±1.7 -9.98±0.12 9.47±0.18 S87L 104.9±3.4 -10.2±0.09 10.7±0.11 108.7±4.0 -10.4±0.07 10.1±0.10 9.99±0.10 S87L 104.9±3.4 -10.2±0.09 10.7±0.10 111.6±3.1 -10.4±0.07 10.2±5.1 -10.09±0.08 10.0±0.08 10.0±0.10 10.1±0.10 10.1±0.10 10.1±0.10	D326N	90.0 ± 2.7	-10.61 ± 0.09	10.46 ± 0.10	90.44 ± 1.5	-10.67 ± 0.05	10.53 ± 0.1	101.1 ± 2.5	-10.19 ± 0.06	10.1 ± 0.08
T340I 108.5±2.5 -10.68±0.07 10.61±0.08 100.3±2.3 -10.73±0.06 10.63±0.09 92.25±3.8 -10.58±0.12 10.44±0.07 N342S 100.9±3.4 -10.4±0.09 10.3±0.10 86.42±2.2 -10.66±0.07 10.49±0.10 100.8±2.1 -10.19±0.05 10.1±0.08 V345I 88.5±4.0 -10.53±0.13 10.37±0.10 88.46±2.1 -10.77±0.07 10.62±0.08 95.68±1.9 -10.39±0.05 10.27±0.08 V52A 64.0±1.7 -10.07±0.07 9.77±0.16 49.33±4.2 -9.81±0.22 9.401±0.16 39.25±1.7 -9.98±0.12 9.47±0.18 R54W 91.8±3.0 -10.29±0.07 10.7±0.1 108.7±4.0 -10.2±0.10 10.3±0.08 84.4±3.0 -10.16±0.10 9.99±0.10 S87L 104.9±3.4 -10.29±0.07 10.17±0.10 111.6±3.1 -10.47±0.08 10.42±0.07 10.22±0.14 10.01±0.10 H131R 97.6±2.6 -10.29±0.07 10.17±0.10 111.6±3.1 -10.47±0.08 10.42±0.07 102.5±3.1 -10.09±0.08 10.01±0.08 1257F	V331F	105.9 ± 3.6	-10.51 ± 0.10	10.43 ± 0.08	105.8 ± 1.5	-10.76 ± 0.04	10.68 ± 0.08	105.3 ± 3.1	-10.45 ± 0.08	10.37 ± 0.06
N3425 100.9±3.4 -10.4±0.09 10.3±0.10 86.42±2.2 -10.66±0.07 10.49±0.10 100.8±2.1 -10.19±0.05 10.1±0.08 V3451 88.5±4.0 -10.53±0.13 10.37±0.10 88.46±2.1 -10.77±0.07 10.62±0.08 95.68±1.9 -10.39±0.05 10.27±0.08 V52A 64.0±1.7 -10.07±0.07 9.77±0.16 49.33±4.2 -9.81±0.22 9.401±0.16 39.25±1.7 -9.98±0.12 9.47±0.18 R54W 91.8±3.0 -10.49±0.09 10.35±0.11 98.79±3.9 -10.48±0.11 10.38±0.08 84.4±3.0 -10.16±0.10 9.99±0.10 S87L 10.49±3.4 +10.2±0.07 10.17±0.10 111.6±3.1 -10.47±0.08 10.42±0.07 102.5±3.1 -10.09±0.08 10.01±0.08 I307 7.7.2±5.1 -9.48±0.16 9.26±0.12 124.3±3.1 -9.998±0.06 9.991±0.07 85.21±3.7 -9.57±0.10 9.40±0.08 I309T 104.1±2.8 -10.09±0.07 10±0.08 126.5±2.8 -10.3±0.06 10.3±0.07 97.78±2.5 -9.99±0.06 9.88±0.08	K334N	95.7 ± 2.3	-10.6 ± 0.07	10.48 ± 0.09	81.25 ± 3.3	-10.56 ± 0.12	10.37 ± 0.11	103 ± 2.5	-10.44 ± 0.07	10.35 ± 0.08
V3451 88.5 ± 4.0 -10.53 ± 0.13 10.37 ± 0.10 88.46 ± 2.1 -10.77 ± 0.07 10.62 ± 0.08 95.68 ± 1.9 -10.39 ± 0.05 10.27 ± 0.08 V52A 64.0 ± 1.7 -10.07 ± 0.07 9.77 ± 0.16 49.33 ± 4.2 -9.81 ± 0.22 9.401 ± 0.16 39.25 ± 1.7 -9.98 ± 0.12 9.47 ± 0.18 R54W 91.8 ± 3.0 -10.49 ± 0.09 10.35 ± 0.11 98.79 ± 3.9 -10.48 ± 0.11 10.38 ± 0.08 84.44 ± 3.0 -10.16 ± 0.10 9.99 ± 0.10 S87L 104.9 ± 3.4 -10.25 ± 0.09 10.17 ± 0.10 111.6 ± 3.1 -10.47 ± 0.08 10.42 ± 0.07 102.5 ± 3.1 -10.09 ± 0.08 10.01 ± 0.08 I257F 77.2 ± 5.1 -9.48 ± 0.10 9.26 ± 0.12 124.3 ± 3.1 -9.998 ± 0.06 9.991 ± 0.07 85.21 ± 3.7 -9.57 ± 0.10 9.40 ± 0.08 I309T 104.1 ± 2.8 -10.09 ± 0.07 10 ± 0.08 126.5 ± 2.8 -10.39 ± 0.07 10.2 ± 0.07 9.78 ± 2.5 -9.99 ± 0.06 9.88 ± 0.08 C314R 84.2 ± 1.1 -10.56 ± 0.14 10.38 ± 0.12 75.89 ± 2.8 -10.49 ± 0.07 10	T340I	108.5 ± 2.5	-10.68 ± 0.07	10.61 ± 0.08	100.3 ± 2.3	-10.73 ± 0.06	10.63 ± 0.09	92.25 ± 3.8	-10.58 ± 0.12	10.44 ± 0.07
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	N342S	100.9 ± 3.4	-10.4 ± 0.09	10.3 ± 0.10	86.42 ± 2.2	-10.66 ± 0.07	10.49 ± 0.10	100.8 ± 2.1	-10.19 ± 0.05	10.1 ± 0.08
R54W 91.8 ± 3.0 -10.49 ± 0.09 10.35 ± 0.11 98.79 ± 3.9 -10.48 ± 0.11 10.38 ± 0.08 84.44 ± 3.0 -10.16 ± 0.10 9.99 ± 0.10 S87L 104.9 ± 3.4 -10.25 ± 0.09 10.17 ± 0.1 108.7 ± 4.0 -10.2 ± 0.10 10.13 ± 0.08 77.33 ± 4.1 -10.22 ± 0.14 10.01 ± 0.10 H131R 97.6 ± 2.6 -10.29 ± 0.07 10.17 ± 0.10 111.6 ± 3.1 -10.47 ± 0.08 104.2 ± 0.07 102.5 ± 3.1 -10.09 ± 0.08 10.01 ± 0.08 I257F 77.2 ± 5.1 -9.48 ± 0.16 9.26 ± 0.12 124.3 ± 3.1 -9.998 ± 0.06 9.991 ± 0.07 85.21 ± 3.7 -9.57 ± 0.10 9.40 ± 0.08 I309T 104.1 ± 2.8 -10.09 ± 0.07 10 ± 0.08 126.5 ± 2.8 -10.3 ± 0.07 9.77 ± 2.5 -9.99 ± 0.06 9.88 ± 0.08 C314R 84.2 ± 4.1 -10.56 ± 0.14 10.38 ± 0.12 75.89 ± 2.8 -10.49 ± 0.01 10.27 ± 0.11 76.46 ± 1.7 -10.2 ± 0.06 9.88 ± 0.08 I112N 65.8 ± 55 8.105 8.27 ± 0.15 83.08 ± 3.2 -8.71± 0.07 8.528 ± 0.11 89.65 ± 3.2 </td <td>V345I</td> <td>88.5 ± 4.0</td> <td>-10.53 ± 0.13</td> <td>10.37 ± 0.10</td> <td>88.46 ± 2.1</td> <td>-10.77 ± 0.07</td> <td>10.62 ± 0.08</td> <td>95.68 ± 1.9</td> <td>-10.39 ± 0.05</td> <td>10.27 ± 0.08</td>	V345I	88.5 ± 4.0	-10.53 ± 0.13	10.37 ± 0.10	88.46 ± 2.1	-10.77 ± 0.07	10.62 ± 0.08	95.68 ± 1.9	-10.39 ± 0.05	10.27 ± 0.08
S87L 104.9±3.4 -10.25±0.09 10.17±0.1 108.7±4.0 -10.2±0.10 10.13±0.08 77.33±4.1 -10.22±0.14 10.01±0.10 H131R 97.6±2.6 -10.29±0.07 10.17±0.10 111.6±3.1 -10.47±0.08 10.42±0.07 102.5±3.1 -10.09±0.08 10.01±0.08 I257F 77.2±5.1 -9.482±0.16 9.26±0.12 124.3±3.1 -9.998±0.06 9.99±0.07 85.21±3.7 -9.57±0.10 9.40±0.08 I309T 104.1±2.8 -10.09±0.07 10±0.08 126.5±2.8 -10.3±0.06 10.3±0.07 97.78±2.5 -9.99±0.06 9.88±0.08 C314R 84.2±4.1 -10.5±0.14 10.3±0.12 75.89±2.8 -10.49±0.01 10.2±0.11 76.4±1.7 -10.2±0.06 9.98±0.10 1112N 65.8±5.6 8.27±0.15 83.08±3.2 8.71±0.07 8.52±0.11 8.65±3.2 -8.49±0.06 8.34±0.08 R125C NR NR 53.06±6.8 -7.78±0.17 7.42±0.22 NR NR N3±0.11 1138P 111.7±6.5 8.94±0.12 8.89±0.1	V52A	64.0 ± 1.7	-10.07 ± 0.07	9.77 ± 0.16	49.33 ± 4.2	-9.81 ± 0.22	9.401 ± 0.16	39.25 ± 1.7	-9.98 ± 0.12	9.47 ± 0.18
H131R 97.6±2.6 -10.29±0.07 10.17±0.10 111.6±3.1 -10.47±0.08 10.42±0.07 10.09±0.08 10.01±0.08 I257F 77.2±5.1 -9.482±0.16 9.26±0.12 124.3±3.1 -9.998±0.06 9.991±0.07 85.21±3.7 -9.57±0.10 9.40±0.08 I309T 104.1±2.8 -10.99±0.07 10±0.08 126.5±2.8 -10.3±0.06 10.3±0.07 97.8±2.5 -9.99±0.06 9.88±0.08 C314R 84.2±4.1 -10.5±0.14 10.38±0.12 75.89±2.8 -10.49±0.01 10.27±0.11 76.4±1.7 -10.2±0.06 9.88±0.08 I112N 65.8±5.6 -8.555±0.16 8.27±0.15 83.08±3.2 -8.71±0.07 8.52±0.11 8.4±0.08 8.4±0.08 R12SC NR NR 53.06±6.8 -7.798±0.17 7.42±0.22 NR NR NR L138P 111.7±6.5 -8.948±0.12 8.89±0.1 109.1±4.0 -9.015±0.07 8.95±0.08 78.6±3.7 -9.23±0.11 9.03±0.11	R54W	91.8 ± 3.0	-10.49 ± 0.09	10.35 ± 0.11	98.79 ± 3.9	-10.48 ± 0.11	10.38 ± 0.08	84.44 ± 3.0	-10.16 ± 0.10	9.99 ± 0.10
1257F 77.2 ± 5.1 -9.482 ± 0.16 9.26 ± 0.12 124.3 ± 3.1 -9.998 ± 0.06 9.991 ± 0.07 85.21 ± 3.7 -9.57 ± 0.10 9.40 ± 0.08 1309T 104.1 ± 2.8 -10.09 ± 0.07 10 ± 0.08 126.5 ± 2.8 -10.3 ± 0.06 10.3 ± 0.07 97.78 ± 2.5 -9.99 ± 0.06 9.88 ± 0.08 C314R 84.2 ± 4.1 -10.56 ± 0.14 10.3 ± 0.12 75.89 ± 2.8 -10.49 ± 0.10 10.27 ± 0.11 76.46 ± 1.7 -10.2 ± 0.06 9.88 ± 0.08 1112N 65.8 ± 5.6 8.255 ± 0.16 82.7 ± 0.15 83.08 ± 3.2 -8.71 ± 0.07 8.52 ± 0.11 89.65 ± 3.2 -8.49 ± 0.06 8.34 ± 0.08 R125C NR NR 53.06 ± 6.8 -7.78 ± 0.17 7.42 ± 0.22 NR NR NR L138P 111.7 ± 6.5 8.94 ± 0.12 8.99 ± 1 109.1 ± 4.0 -9.015 ± 0.07 8.951 ± 0.08 78.62 ± 3.7 -9.23 ± 0.11 9.03 ± 0.11	S87L	104.9 ± 3.4	-10.25 ± 0.09	10.17 ± 0.1	108.7 ± 4.0	-10.2 ± 0.10	10.13 ± 0.08	77.33 ± 4.1	-10.22 ± 0.14	10.01 ± 0.10
I309T 104.1±2.8 -10.09±0.07 10±0.08 126.5±2.8 -10.3±0.06 10.3±0.07 97.78±2.5 -9.99±0.06 9.88±0.08 C314R 84.2±4.1 -10.56±0.14 10.38±0.12 75.89±2.8 -10.49±0.10 10.27±0.11 76.46±1.7 -10.2±0.06 9.88±0.08 I112N 65.8±5.6 -8.555±0.16 8.27±0.15 83.08±3.2 -8.71±0.07 8.528±0.11 89.65±3.2 -8.49±0.06 8.34±0.08 R125C NR NR S3.06±6.8 -7.798±0.17 7.42±0.22 NR NR NR L138P 111.7±6.5 -8.948±0.12 8.89±0.1 109.1±4.0 -9.015±0.07 8.951±0.08 78.62±3.7 -9.23±0.11 9.03±0.11	H131R	97.6 ± 2.6	-10.29 ± 0.07	10.17 ± 0.10	111.6 ± 3.1	-10.47 ± 0.08	10.42 ± 0.07	102.5 ± 3.1	-10.09 ± 0.08	10.01 ± 0.08
C314R 84.2 ±4.1 -10.56 ±0.14 10.38 ±0.12 75.89 ±2.8 -10.49 ±0.10 10.27 ±0.11 76.46 ±1.7 -10.2 ±0.06 9.98 ±0.10 I112N 65.8 ±5.6 -8.555 ±0.16 8.27 ±0.15 83.08 ±3.2 -8.71 ±0.07 8.528 ±0.11 89.65 ±3.2 -8.49 ±0.06 8.34 ±0.08 R125C NR NR NR 53.06 ±6.8 -7.798 ±0.17 7.42 ±0.22 NR NR NR L138P 111.7 ±6.5 -8.948 ±0.12 8.89 ±0.1 109.1 ±4.0 -9.015 ±0.07 8.951 ±0.08 78.62 ±3.7 -9.23 ±0.11 9.03 ±0.11	1257F	77.2 ± 5.1	-9.482 ± 0.16	9.26 ± 0.12	124.3 ± 3.1	-9.998 ± 0.06	9.991 ± 0.07	85.21 ± 3.7	-9.57 ± 0.10	9.40 ± 0.08
I112N 65.8±5.6 -8.555±0.16 8.27±0.15 83.08±3.2 -8.71±0.07 8.528±0.11 89.65±3.2 -8.49±0.06 8.34±0.08 R125C NR NR NR 53.06±6.8 -7.798±0.17 7.42±0.22 NR NR NR L138P 111.7±6.5 -8.948±0.12 8.89±0.1 109.1±4.0 -9.015±0.07 8.951±0.08 78.62±3.7 -9.23±0.11 9.03±0.11	1309T	104.1 ± 2.8	-10.09 ± 0.07	10 ± 0.08	126.5 ± 2.8	-10.3 ± 0.06	10.3 ± 0.07	97.78 ± 2.5	-9.99 ± 0.06	9.88 ± 0.08
R125C NR NR NR 53.06±6.8 -7.798±0.17 7.42±0.22 NR NR NR NR L138P 111.7±6.5 -8.948±0.12 8.89±0.1 109.1±4.0 -9.015±0.07 8.951±0.08 78.62±3.7 -9.23±0.11 9.03±0.11	C314R	84.2 ± 4.1	-10.56 ± 0.14	10.38 ± 0.12	75.89 ± 2.8	-10.49 ± 0.10	10.27 ± 0.11	76.46 ± 1.7	-10.2 ± 0.06	9.98 ± 0.10
L138P 111.7±6.5 -8.948±0.12 8.89±0.1 109.1±4.0 -9.015±0.07 8.951±0.08 78.62±3.7 -9.23±0.11 9.03±0.11	I112N	65.8±5.6	-8.555 ± 0.16	8.27 ± 0.15	83.08 ± 3.2	-8.71 ± 0.07	8.528 ± 0.11	89.65 ± 3.2	-8.49 ± 0.06	8.34 ± 0.08
	R125C	NR	NR	NR	53.06 ± 6.8	-7.798 ± 0.17	7.42 ± 0.22	NR	NR	NR
P80L NR NR NR NR NR NR NR NR NR	L138P	111.7 ± 6.5	-8.948 ± 0.12	8.89±0.1	109.1 ± 4.0	-9.015 ± 0.07	8.951 ± 0.08	78.62 ± 3.7	-9.23 ± 0.11	9.03 ± 0.11
	P80L	NR	NR	NR	NR	NR	NR	NR	NR	NR

В							
		GoA			GoB		
Receptor	Emax (% of WT)	LogEC50	Log(Tau/Ka)	Emax (% of WT)	LogEC50	Log(Tau/Ka)	
WT-MT1		-10.65 ± 0.04	10.53 ± 0.04		-10.68 ± 0.04	10.5 ± 0.05	
G18R	121.9 ± 2.1	-10.75 ± 0.05	10.71 ± 0.07	132.4 ± 2.8	-10.84 ± 0.06	10.79 ± 0.06	
188V	106.8 ± 3.0	-10.43 ± 0.08	10.34 ± 0.08	116.7 ± 3.3	-10.43 ± 0.08	10.33 ± 0.07	
N91Y	89.74 ± 2.1	-10.49 ± 0.06	10.33 ± 0.12	94.97 ± 2.5	-10.5 ± 0.07	10.31 ± 0.09	
G96D	95.62 ± 5.7	-10.54 ± 0.17	10.4 ± 0.11	126.5 ± 2.9	-10.64 ± 0.06	10.58 ± 0.08	
A157V	99.66 ± 2.1	-10.5 ± 0.06	10.38 ± 0.10	108.5 ± 2.6	-10.76 ± 0.07	10.62 ± 0.08	
G166E	124.9 ± 4.8	-10.36 ± 0.11	10.34 ± 0.08	128.7 ± 4.2	-10.44 ± 0.09	10.38 ± 0.07	
A180T	82.55 ± 2.6	-10.88 ± 0.11	10.68 ± 0.14	72.56 ± 1.9	-10.6 ± 0.08	10.29 ± 0.15	
I212T	113.7 ± 4.8	-10.6 ± 0.12	10.54 ± 0.09	116.3 ± 2.8	-10.46 ± 0.06	10.35 ± 0.09	
V221M	95.85 ± 5.8	-10.46 ± 0.18	10.33 ± 0.11	136.7 ± 3.9	-10.63 ± 0.09	10.6 ± 0.07	
K228R	124.4 ± 3.0	-10.76 ± 0.08	10.73 ± 0.09	133.5 ± 3.7	-10.75 ± 0.09	10.71 ± 0.08	
A266T	102.7 ± 2.8	-10.87 ± 0.08	10.77 ± 0.08	101.5 ± 1.9	-10.85 ± 0.05	10.69 ± 0.08	
A266V	115.3 ± 3.0	-10.72 ± 0.08	10.67 ± 0.09	106.8 ± 3.5	-10.85 ± 0.10	10.71 ± 0.08	
S267G	108.1 ± 3.6	-10.72 ± 0.10	10.64 ± 0.10	107.3 ± 2.4	-10.97 ± 0.07	10.83 ± 0.09	
R307S	111.5 ± 3.8	-10.63 ± 0.18	10.56 ± 0.09	132.8 ± 3.2	-10.65 ± 0.07	10.6 ± 0.07	
V321M	102 ± 2.4	-10.79 ± 0.07	10.69 ± 0.09	112.6 ± 3.0	-10.91 ± 0.08	10.79 ± 0.08	
D326N	102.5 ± 2.9	-10.71 ± 0.08	10.6 ± 0.09	97.87 ± 2.6	-10.59 ± 0.07	10.41 ± 0.09	
V331F	104.6 ± 2.6	-10.6 ± 0.07	10.5 ± 0.10	110.9 ± 3.2	-10.73 ± 0.08	10.61 ± 0.08	
K334N	115 ± 4.2	-10.52 ± 0.10	10.46 ± 0.09	104.2 ± 2.0	-10.69 ± 0.05	10.54 ± 0.08	
T340I	110.6 ± 2.8	-10.68 ± 0.07	10.61 ± 0.08	94.81 ± 2.6	-10.6 ± 0.08	10.41 ± 0.09	
N342S	100.9 ± 2.7	-10.48 ± 0.07	10.36 ± 0.10	112.4 ± 3.7	-10.61 ± 0.09	10.5 ± 0.08	
V345I	109.3 ± 2.5	-10.47 ± 0.06	10.39 ± 0.09	112.4 ± 2.9	-10.85 ± 0.08	10.15 ± 0.07	
V52A	93.59 ± 3.2	-10.59 ± 0.10	10.45 ± 0.09	88.61 ± 2.4	-10.18 ± 0.07	9.95 ± 0.10	
R54W	98.04 ± 6.2	-10.77 ± 0.20	10.65 ± 0.10	96.33 ± 4.3	-10.45 ± 0.13	10.27 ± 0.09	
S87L	131.2 ± 4.4	-10.21 ± 0.09	10.21 ± 0.08	147.3 ± 4.6	-10.22 ± 0.08	10.22 ± 0.07	
H131R	108.2 ± 5.8	-10.47 ± 0.16	10.39 ± 0.09	124.4 ± 5.0	-10.52 ± 0.12	10.45 ± 0.07	
1257F	105.9 ± 4.1	-9.58 ± 0.09	9.48 ± 0.09	130.1 ± 4.4	-9.83 ± 0.09	9.78 ± 0.07	
1309T	118.6 ± 2.6	-9.92 ± 0.05	9.88 ± 0.09	143.3 ± 3.8	-10.42 ± 0.07	10.41 ± 0.07	
C314R	113.8 ± 2.1	-10.63 ± 0.05	10.57 ± 0.09	125.3 ± 3.0	-10.64 ± 0.07	10.57 ± 0.08	
1112N	71.23 ± 4.5	-8.34 ± 0.11	8.08 ± 0.14	87.96 ± 3.5	-8.63 ± 0.07	8.41 ± 0.11	
R125C	NR	NR	NR	55.41 ± 4.5	-10.44 ± 0.23	10 ± 0.18	
L138P	109.2 ± 5.5	-8.82 ± 0.10	8.74 ± 0.10	140 ± 7.2	-9.12 ± 0.11	9.10 ± 0.07	
P80L	NR	NR	NR	NR	NR	NR	

С									
		G12		8	βarrestin-2		G15		
Receptor	Emax (% of WT)	LogEC50	Log(Tau/Ka)	Emax (% of WT)	LogEC50	Log(Tau/Ka)	Emax (% of WT)	LogEC50	Log(Tau/Ka)
WT-MT1		-9.84 ± 0.04	9.82 ± 0.04		-9 ± 0.03	8.88 ± 0.02		-9.14 ± 0.05	8.99 ± 0.06
G18R	89.46 ± 5.6	-9.73 ± 0.16	9.67 ± 0.11	55.8 ± 1.9	-8.86 ± 0.09	8.53 ± 0.10	83.5 ± 2.0	-9.20 ± 0.06	8.99 ± 0.11
188V	58.97 ± 2.8	-9.53 ± 0.11	9.29 ± 0.15	30.39 ± 1.7	-9.18 ± 0.12	8.53 ± 0.19	98.43 ± 5.1	-9.15 ± 0.14	9.01 ± 0.11
N91Y	82.38 ± 4.2	-9.84 ± 0.13	9.74 ± 0.09	78.17 ± 2.1	-9.00 ± 0.05	8.76 ± 0.07	97.41 ± 4.6	-9.48 ± 0.13	9.33 ± 0.12
G96D	94.22 ± 2.8	-9.81 ± 0.07	9.77 ± 0.10	71.62 ± 1.7	-8.57 ± 0.05	8.35 ± 0.07	75.22 ± 4.2	-8.70 ± 0.14	8.44 ± 0.14
A157V	93.69 ± 3.4	-9.61 ± 0.09	9.57 ± 0.09	106.2 ± 2.1	-8.98 ± 0.04	8.87 ± 0.05	126.5 ± 4.0	-9.52 ± 0.09	9.49 ± 0.09
G166E	83.89 ± 3.3	-9.76 ± 0.10	9.68 ± 0.10	37.96 ± 1.2	-8.71 ± 0.07	8.21 ± 0.13	60.05 ± 3.3	-8.82 ± 0.14	8.46 ± 0.20
A180T	99.08 ± 2.2	-10.19 ± 0.0€	10.17 ± 0.09	112.6 ± 3.6	-9.17 ± 0.07	9.13 ± 0.05	90.96 ± 5.8	-8.55 ± 0.15	8.37 ± 0.11
1212T	87.93 ± 3.4	-9.79 ± 0.10	9.72 ± 0.10	65.79 ± 1.8	-8.74 ± 0.07	8.48 ± 0.07	88.05 ± 4.1	-9.15 ± 0.12	8.96 ± 0.13
V221M	84.5 ± 4.9	-10.04 ± 0.15	9.96 ± 0.12	110.9 ± 2.5	-8.94 ± 0.05	8.84 ± 0.05	136.2 ± 6.7	-8.71 ± 0.12	8.71 ± 0.08
K228R	102.7 ± 4.0	-10.07 ± 0.10	10.07 ± 0.10	143.5 ± 4.8	-8.72 ± 0.07	8.80 ± 0.05	134.2 ± 6.4	-8.58 ± 0.12	8.57 ± 0.07
A266T	94.77 ± 5.3	-10.24 ± 0.15	10.21 ± 0.09	96.25 ± 2.4	-9.07 ± 0.06	8.94 ± 0.05	97.98 ± 2.4	-9.37 ± 0.07	9.23 ± 0.09
A266V	94.74 ± 2.9	-9.76 ± 0.08	9.72 ± 0.09	105.9 ± 1.9	-8.83 ± 0.04	8.78 ± 0.04	109.6 ± 5.4	-8.77 ± 0.12	8.67 ± 0.09
S267G	98.97 ± 3.8	-9.89 ± 0.10	9.87 ± 0.09	85.42 ± 1.8	-8.75 ± 0.05	8.59 ± 0.05	93.02 ± 4.7	-8.82 ± 0.13	8.65 ± 0.11
R307S	82.27 ± 3.5	-9.84 ± 0.11	9.75 ± 0.10	44.72 ± 1.0	-8.72 ± 0.05	8.31 ± 0.11	80.99 ± 5.2	-8.62 ± 0.16	8.4 ± 0.13
V321M	82.19 ± 4.2	-9.72 ± 0.13	9.62 ± 0.10	99.99 ± 2.6	-9.10 ± 0.08	8.97 ± 0.06	112.4 ± 3.8	-9.59 ± 0.10	9.51 ± 0.10
D326N	98.68 ± 3.2	-9.92 ± 0.08	9.90 ± 0.09	112.5 ± 4.0	-9.17 ± 0.08	9.09 ± 0.05	101.9 ± 3.3	-9.37 ± 0.09	9.25 ± 0.11
V331F	95.75 ± 2.8	-9.94 ± 0.07	9.91 ± 0.09	76.52 ± 3.9	-9.11 ± 0.11	8.87 ± 0.07	118.6 ± 3.3	-9.44 ± 0.08	9.38 ± 0.09
K334N	91.81 ± 3.7	-9.65 ± 0.10	9.60 ± 0.09	93.33 ± 1.8	-8.87 ± 0.05	8.75 ± 0.05	109 ± 5.5	-8.79 ± 0.13	8.69 ± 0.09
T340I	80.05 ± 4.5	-9.77 ± 0.14	9.66 ± 0.12	87.13 ± 4.0	-9.06 ± 0.10	8.87 ± 0.07	102.1 ± 4.9	-9.417± 0.13	9.29 ± 0.11
N342S	89.22 ± 5.6	-9.77 ± 0.16	9.71 ± 0.10	95.11 ± 1.8	-8.68 ± 0.04	8.55 ± 0.05	93.31 ± 3.6	-8.66 ± 0.09	8.49 ± 0.11
V345I	93.51 ± 3.1	-9.7 ± 0.08	9.65 ± 0.09	90.83 ± 2.0	-8.74 ± 0.05	8.62 ± 0.05	93.19 ± 5.7	-8.72 ± 0.15	8.56 ± 0.11
V52A	15.86 ± 5.9	-7.95 ± 0.54	7.14 ± 0.78	NR	NR	NR	NR	NR	NR
R54W	39.75 ± 4.0	-10.08 ± 0.27	9.66 ± 0.25	NR	NR	NR	54.33 ± 4.0	-9.09 ± 0.20	8.69 ± 0.20
S87L	48.06 ± 4.1	-9.06 ± 0.18	8.73 ± 0.22	NR	NR	NR	NR	NR	NR
H131R	45.54 ± 3.8	-9.47 ± 0.20	9.12 ± 0.22	NR	NR	NR	17.84 ± 3.6	-8.50 ± 0.49	7.61 ± 0.58
1257F	47.84 ± 3.6	-9.50 ± 0.18	9.17 ± 0.21	NR	NR	NR	79.14 ± 3.0	-9.25 ± 0.10	9.02 ± 0.14
1309T	52.89 ± 2.9	-9.41 ± 0.13	9.13 ± 0.17	NR	NR	NR	58.42 ± 3.1	-9.14 ± 0.13	8.78 ± 0.20
C314R	57.12 ± 4.9	-9.78 ± 0.22	9.52 ± 0.15	NR	NR	NR	33.11 ± 2.9	-8.75 ± 0.23	8.13 ± 0.36
1112N	75.89 ± 12.5	-7.66 ± 0.20	7.53 ± 0.15	43.35 ± 8.3	-7.27 ± 0.18	6.78 ± 0.21	60.19 ± 3.3	-8.00 ± 0.12	7.65 ± 0.21
R125C	NR	NR	NR	NR	NR	NR	NR	NR	NR
L138P	56.13 ± 7.3	-8.26 ± 0.22	7.99 ± 0.17	NR	NR	NR	26.54 ± 5.1	-7.89 ± 0.40	7.17 ± 0.46
P80L	NR	NR	NR	NR	NR	NR	NR	NR	NR

NR denotes that the experimental parameter could not be determined due to lack of a concentration-response curve.