

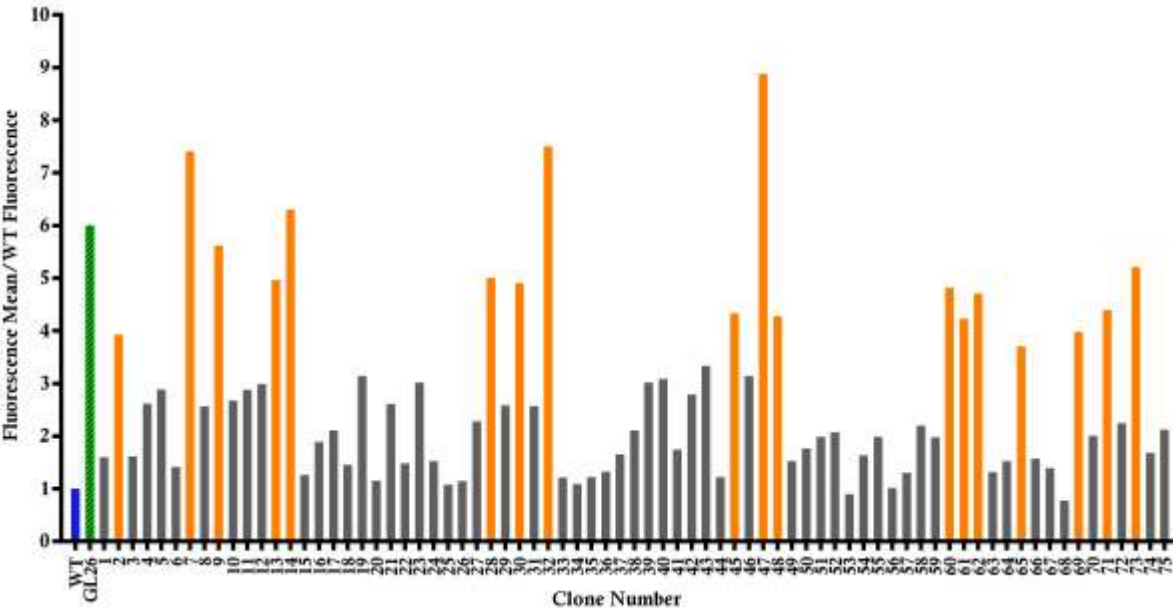
Supplementary information

Yeast-based directed-evolution for high-throughput structural stabilization of G protein-coupled receptors (GPCRs)

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Supplementary Fig. S1. OG resistance profile of clones randomly selected from YDDS library.

Encoding DNA isolated by PCR from the OG-resistant cell population present in the A2aR library was reintroduced into yeast by homology recombination to yield a sub-library enriched in OG-resistant A2aR variants (the YDDS library). Seventy five randomly selected clones from the YDDS library were analyzed using CA200623 (20 nM) binding assay in the presence of 2% OG, and the extent of residual florescence was measured by flow cytometry (as described in the Methods). Eighteen clones with the highest residual florescence (geometric MFI across the population) were selected for further analyses (colored orange). The bars representing A2aR-WT and thermostable mutant GL26 are colored in blue and green, respectively.



Supplementary Fig. S2. Amino acid sequence alignment of OG resistant A2aR variants retrieved by YDDS. The amino acid sequence alignment of A2aR-WT, thermostable GL26 mutant and 18 highly OG resistant A2aR variants present in the YDDS library was generated by using T-Coffee multiple sequence alignment server: <http://tcoffee.crg.cat/apps/tcoffee/do:regular>. The positions of structure-stabilizing substitutions, the transmembrane helices (TMH) and the cytoplasmic helix 8 are indicated.

		TMH1	TMH2
WT	1	MPIMGSSVYITVELAIAVLAILGNVLVCWAVWLNSNLQNV	TNYFVVS
GL26	1	MPIMGSSVYITVELAIAVLAILGNVLVCWAVWLNSNLQNV	TNYFVVS
c2	1	MPIMGSSVYITVELAIAVLAILGNVLVCWAVWLNSNLQNV	TNYFVVS
c7	1	MPIMGSSVYITVELAIAVLAILGNVLVCWAVWLNSNLQNV	TNYFVVS
c9	1	MPIMGSSVYITVELAIAVLAILGNVLVCWAVWLNSNLQNV	TNYFVVS
c13	1	MPIMGSSVYITVELAIAVLAILGNVLVCWAVWLNSNLQNV	TNYFVVS
c14	1	MPIMGSSVYITVELAIAVLAILGNVLVCWAVWLNSNLQNV	TNYFVVS
c28	1	MPIMGSSVYITVELAIAVLAILGNVLVCWAVWLNSNLQNV	TNYFVVS
c30	1	MPIMGSSVYITVELAIAVLAILGNVLVCWAVWLNSNLQNV	TNYFVVS
c32	1	MPIMGSSVYITVELAIAVLAILGNVLVCWAVWLNSNLQNV	TNYFVVS
c45	1	MPIMGSSVYITVELAIAVLAILGNVLVCWAVWLNSNLQNV	TNYFVVS
c47	1	MPIMGSSVYITVELAIAVLAILGNVLVCWAVWLNSNLQNV	TNYFVVS
c48	1	MPIMGSSVYITVELAIAVLAILGNVLVCWAVWLNSNLQNV	TNYFVVS
c60	1	MPIMGSSVYITVELAIAVLAILGNVLVCWAVWLNSNLQNV	TNYFVVS
c61	1	MPIMGSSVYITVELAIAVLAILGNVLVCWAVWLNSNLQNV	TNYFVVS
c62	1	MPIMGSSVYITVELAIAVLAILGNVLVCWAVWLNSNLQNV	TNYFVVS
c65	1	MPIMGSSVYITVELAIAVLAILGNVLVCWAVWLNSNLQNV	TNYFVVS
c69	1	MPIMGSSVYITVELAIAVLAILGNVLVCWAVWLNSNLQNV	TNYFVVS
c71	1	MPIMGSSVYITVELAIAVLAILGNVLVCWAVWLNSNLQNV	TNYFVVS
c73	1	MPIMGSSVYITVELAIAVLAILGNVLVCWAVWLNSNLQNV	TNYFVVS

		TMH2	TMH3	TMH4
WT	61	PFAITISTGFCAACHGCLFIACFVLVLTQSSIFSL	LAIADRYIAIRIPLRYNGLVTGTR	
GL26	61	PFAITISTGFCAACHGCLFIACFVLVLTQSSIFSL	LAIADRYIAIRIPLRYNGLVTGTR	
c2	61	PFAITISTGFCAACHGCLFIACFVLVLTQSSIFSL	LAIADRYIAIRIPLRYNGLVTGTR	
c7	61	PFAITISTGFCAACHGCLFIACFVLVLTQSSIFSL	LAIADRYIAIRIPLRYNGLVTGTR	
c9	61	PFAITISTGFCAACHGCLFIACFVLVLTQSSIFSL	LAIADRYIAIRIPLRYNGLVTGTR	
c13	61	PFAITISTGFCAACHGCLFIACFVLVLTQSSIFSL	LAIADRYIAIRIPLRYNGLVTGTR	
c14	61	PFAITISTGFCAACHGCLFIACFVLVLTQSSIFSL	LAIADRYIAIRIPLRYNGLVTGTR	
c28	61	PFAITISTGFCAACHGCLFIACFVLVLTQSSIFSL	LAIADRYIAIRIPLRYNGLVTGTR	
c30	61	PFAITISTGFCAACHGCLFIACFVLVLTQSSIFSL	LAIADRYIAIRIPLRYNGLVTGTR	
c32	61	PFAITISTGFCAACHGCLFIACFVLVLTQSSIFSL	LAIADRYIAIRIPLRYNGLVTGTR	
c45	61	PFAITISTGFCAACHGCLFIACFVLVLTQSSIFSL	LAIADRYIAIRIPLRYNGLVTGTR	
c47	61	PFAITISTGFCAACHGCLFIACFVLVLTQSSIFSL	LAIADRYIAIRIPLRYNGLVTGTR	
c48	61	PFAITISTGFCAACHGCLFIACFVLVLTQSSIFSL	LAIADRYIAIRIPLRYNGLVTGTR	
c60	61	PFAITISTGFCAACHGCLFIACFVLVLTQSSIFSL	LAIADRYIAIRIPLRYNGLVTGTR	
c61	61	PFAITISTGFCAACHGCLFIACFVLVLTQSSIFSL	LAIADRYIAIRIPLRYNGLVTGTR	
c62	61	PFAITISTGFCAACHGCLFIACFVLVLTQSSIFSL	LAIADRYIAIRIPLRYNGLVTGTR	
c65	61	PFAITISTGFCAACHGCLFIACFVLVLTQSSIFSL	LAIADRYIAIRIPLRYNGLVTGTR	
c69	61	PFAITISTGFCAACHGCLFIACFVLVLTQSSIFSL	LAIADRYIAIRIPLRYNGLVTGTR	
c71	61	PFAITISTGFCAACHGCLFIACFVLVLTQSSIFSL	LAIADRYIAIRIPLRYNGLVTGTR	
c73	61	PFAITISTGFCAACHGCLFIACFVLVLTQSSIFSL	LAIADRYIAIRIPLRYNGLVTGTR	

TMH4

TMH5

WT	121	AKGIIAICWVLSFAIGLTPMLGWNNCGQPKEGKNHSQGC GEGQVACLFEDVVP MN YMVYF
GL26	121	AKGIIAICWVLSFAIGLTPMLGWNNCGQPKEGKNHSQGC GEGQVACLFEDVVP MN YMVYF
c2	121	AKGIIAICWVLSFAIGLTPMLGWNNCGQPKEGKNHSQGC GEGQVACLFEDVVP MN YMVYF
c7	121	AKGIIAICWVLSFAIGLTPMLGWNNCGQPKEGKNHSQGC GEGQVACLFEDVVP MN YMVYF
c9	121	AKGIIAICWVLSFAIGLTPMLGWNNCGQPKEGKNHSQGC GEGQVACLF ^K EDVVP MN YMVYF
c13	121	AKGIIAICWVLSFAIGLTPMLGWNNCGQPKEGKNHSQGC GEGQVACLFEDVVP MN YMVYF
c14	121	AKGIIAICWVLSFAIGLTPMLGWNNCGQPKEGKNHSQGC GEGQVACLFEDVVP MN YMVYF
c28	121	AKGIIAICWVLSFAIGLTPMLGWNNC ^D QPKEGKNHSQGC GEGQVACLFEDVVP MN YMVYF
c30	121	AKGIIAICWVLSFAIGLTPMLGWNNCGQPKEGKNHSQGC GEGQVACLFEDVVP MN YMVYF
c32	121	AKGIIAICWVLSFAIGLTPMLGWNNCGQPKEGKNHSQGC GEGQVACLFEDVVP MN YMVYF
c45	121	AKGIIAICWVLSFAIGLTPMLGWNNCGQPKEGKNHSQGC GEGQVACLFEDVVP MN YMVYF
c47	121	AKGIIAICWVLSFAIGLTPMLGWNNCGQPKEGKNHSQGC GEGQVACLFEDVVP MN YMVYF
c48	121	AKGIIAICWVLSFAIGLTPMLGWNNCGQPKEGKNHSQGC GEGQVACLFEDVVP MN YMVYF
c60	121	AKGIIAICWVLSFAIGLTPMLGWNNCGQPKEGKNHSQGC GEG ^S QVACLFEDVVP MN YMVYF
c61	121	AKGIIAICWVLSFAIGLTPMLGWNNCGQPKEGKNHSQGC GEGQVACLFEDVVP MN YMVYF
c62	121	A ^E GIIAICWVLSFAIGLTPMLGWNNCGQPKEGKNHSQGC GEGQ ^E ACLFEDVVP MN YMVYF
c65	121	AKG ^N I ^A ^F ICWVLSFAIGLTPMLGWNNCGQPKEGKNHSQGC GEGQVACL ^I EDVVP ^S MN YMVYF
c69	121	AKGIIAICWVLSFAIGLTPMLGWNNCGQPKEGKNHSQGC GEGQVACLFEDVVP MN YMVY ^L
c71	121	AKG ^I ^N AICWVLSFAIGLTPMLGWNNCGQPKEGKNHSQGC GEGQ ^E ACL ^Y EDVVP MN YMVYF
c73	121	AKGIIAICWVLSFAIGLTPMLGWNNCGQPKEGKNHSQGC GEGQVACLFEDVVP MN YMVYF

TMH5

TMH6

WT	181	NFFACVLVPLLLMLGVYLRIFLAARRQLKQMESQPLPGERARSTLQKEVHAAKSLAIIVG
GL2	181	NFFACVLVPLLLMLGVYLRIFLAARRQLKQMESQPLPGERARSTLQKEVHAAKSLAIIVG
c2	181	N ^F ^Y ACVLVPLLLMLGVYLRIFLAARRQLKQME ^R QPLPGERARSTLQKEVHAAKSLAIIVG
c7	181	NFFACVLVPLLLMLGVYLRIFLAARRQLKQMESQPLPGERARSTLQKEVHAAKSLAIIVG
c9	181	NFFACVLVPLLLMLGVYLRIFLAARRQLKQMESQPLPGERARSTLQKEVHAAKSLAIIVG
c13	181	NFFACVLVPL ^L MLGVYLRIFLAARRQLKQMESQPLPGERARSTLQKEVHAAKSLAIIVG
c14	181	NFFACVLVPLLLMLGVYLRIFLAARRQLKQMESQPLPGERARSTLQKEVHAAKSLAIIVG
c28	181	NFFACVLVPLLLMLGVYLRIFLAARRQLKQMESQPLPGERA ^W STLQKEVHAAKSLAIIVG
c30	181	NFFACVLVPLLLMLGVYLRIFLAARRQLKQMESQPLPGERARSTLQKEVHAAKSLAIIVG
c32	181	NFFACVLVPLLLMLGVYLRIFLAARRQLKQMESQPLPGERARSTLQKEVHAAKSLAIIVG
c45	181	NFFACVLVPLLL ^M MGVYLRIFLAARRQLKQMESQPLPGERARSTLQKEVHAAKSLAIIV ^E
c47	181	NFFACVLVPLLLMLGVYLRIFLAARRQLKQMESQPLPGERARSTLQKEVHAAKSLAIIVG
c48	181	NFFACVLVPLLLMLGVYLRIFLAARRQLKQMESQPLPGERARSTLQKEVHAAKSLAIIVG
c60	181	NFFACVLVPLLLMLGVYLRIFLAARRQLKQMESQPLPGERARSTLQKEVHAAKSLAIIVG
c61	181	NFFACVLVPLLLMLGVYLRIFLAARRQLKQMESQPLPGERARSTLQKEVHAAKSLAIIVG
c62	181	DFFACV ^R VPLLLMLGVYLRIFLAARRQLKQ ^I ESQPLP ^W ERARSTLQKEVHAAKSLAIIVG
c65	181	NFFACVLVPL ^Q MLGV ^F LRI ^C LAARRQLKQMESQPLPGERARSTLQKEVHAAKSLAIIVG
c69	181	NFFACVLVPLLLMLGVYLRIFLAARRQLKQMESQPLPGERARSTLQKEVHAAKSLAIIVG
c71	181	NFFACVLVPLLLMLGVYLRIFLAARRQLKQMESQPLPGERARSTLQKEVHAAKSLAIIVG
c73	181	NFFACVLVPLLLMLGVYLRIFLAARRQLKQMESQPLPGERARSTLQKEVHAAKSLAIIVG

		TMH6	TMH7	Helix-8
WT	241	LFALCWLPLHIINCFTFFCPDCSHAPLWLMYLAIVLSHTNSVVPFIYAYRIREFRQTFR		
GL26	241	LFALCWLPLHIINCFTFFCPDCSHAPLWLMYLAIVLSHTNSVVPFIYAYRIREFRQTFR		
c2	241	LFALCWLPLHIINCFTFFCPDCSHAPLWLMYLAIVLSHTNSVVPFIYAYRIREFRQTFR		
c7	241	LFALCWLPLHIINCFTFFCPDCSHAPLWLMYLAIVLSHTNSVVPFIYAYRIREFRQTFR		
c9	241	LFALCWLPLHIINCFTFFCPDCSHAPLWLMYLAIVLSHTNSVVPFIYAYRIREFRQTFR		
c13	241	LFALCWLPLHIINCFTFFCPDCSHAPLWLMYL T IVLSHTNSVVPFIYAYRIREFRQTFR		
c14	241	LFALCWLPLHIINCFTFFCPDCSHAPLWLMYLAIVLSHTNSVVPFIYAYRIREFRQTFR		
c28	241	LFALCWLPLHIINCFTFFCPDCSHAPLWLMYLAIVLSHTNSVVPFIYAYRIREFRQTFR		
c30	241	LFALCWLPLHIINCFTFFCPDCSHAPLWLMYLAIVLSHTNS D VNPFIYAYRIREFRQTFR		
c32	241	LFALCWLPLHIINCFTFFCPDCSHAPLWLMYLAIVLSHTNSVVPFIYAYRIREFRQTFR		
c45	241	LFALCWLPL R IINCFTFFCPDCSHAPLWLMYLAIVLSHTNSVVPFIYAYRIREFRQTFR		
c47	241	LFALCWLPLHIINCFT I FCPDCSHAPLWLMYLAIVLSHTNSVVPFIYAYRIREFRQTFR		
c48	241	LFALCWLPLHIINCFTFFCPDCSHAPLWLMYLAIVLSHTNSVVPFIYAYRIREFRQTFR		
c60	241	LFALCWLPLHIINC F IFFCPDC G HAPLWLMYLAIVLSHT D SVVPFI T YAYRIREFRQTFR		
c61	241	LFALCWLPLHIINCFTFFCPDCSHAPLWLMYLAIVLSHTNSVVPFIYAYRI C EFRQTFR		
c62	241	LFALCW P PLHIINCFT L FCPDCSHAPLWLMYLAIVLSHTNSVVPFIYAYR N REFRQTFR		
c65	241	LFALCWLPLHIINCFTFFCPDCSHAPLWLMYLAIVLSHTNSVVPFIYAYRIREFRQTFR		
c69	241	LFALCWLPLHIINCFTFFCPDCSHAPLWLMYLAIVLSHTNSVVPFIYAYRIREFRQTFR		
c71	241	LFALCWLPLHIINCFTFFCPDCSHAPLWLMYL D IVLSHTNSVVPFIYAYRIREFRQTFR		
c73	241	LFALCWLPLHIINCFTFFCPDCSHAPLWLMYLAIVLSHTNSVVPFIYAYRIREFRQTFR		

		Helix-8
WT	301	KIIRSHVLRQQEPFKAAGTSARVLAAHGSDGEQVSLRLNGHPPGVWANGSAPHPERRPNG
GL26	301	KIIRSHVLRQQEPFKAAGTSARVLAAHGSDGEQVSLRLNGHPPGVWANGSAPHPERRPNG
c2	301	KIIRSHVLRQQEPFKAAGTSARVLAAHGSDGEQVSLRLNGHPPGVWANGSAPHPERRPNG
c7	301	KIIRSHVLRQQEPFKAAGTSARVLAAHGSDGEQVSLRLNGHPPGVWANGSAPHPERRPNG
c9	301	KIIRSHVLRQQEPFKAAGTSARVLAAHGSDGEQVSLRLNGHPPGVWANGSAPHPERRPNG
c13	301	KIIRSHVLRQQEPFKAAGTSARVLAAHGSDGEQVSLRLNGHPPGVWANGSAPHPERRPNG
c14	301	KIIRSHVLRQQEPFKAAGTSARVLAAHGSDGEQVSLRLNGHPPGVWANGSAPHPERRPNG
c28	301	KIIRSHVLRQQEPFKA D TSARVLAAHGSDGEQVSLRLNGHPPGVWANGSAPHPERRPNG
c30	301	KIIRSHVLRQQEPFKAAGTSARVLAAHGSDGEQVSLRLNGHPPGVWANGS R HPERRPNG
c32	301	KIIR G HVLRQQEPFKAAGTSARVLAAHGSDGEQVSLRLNGHPPGVWANGSAPHPERRPNG
c45	301	KIIRSHVLRQQEPFKAAGTSARVLAAHGSDGEQVSLRLNGHPPGVWANGSAPHPERRPNG
c47	301	KIIRSHVLRQQEPFKAAGTSARVLA A HGSDGEQVSLRLNGHPPGVWANGSAPHPERRPNG
c48	301	KIIRSHVLRQQ E SFKAAGTSARVLAAHGSDGEQVSLRLNGHPPGVWANGS A HPERRPNG
c60	301	KIIRSHV L MQQEPFKAAGTSARVLAAHGSDGEQVSLRLNGHPPGVWANGSAPHPERRPNG
c61	301	KIIRSHVLRQQEPFKAAGTSAR F SAAHGSDGEQVSLRLNGHPPGVWANGS A PLERRPNG
c62	301	KI I CSHVLRQQEPFKA S SARVLAAHGSDGEQVSLRLNGHPPGVWANG C APHPERRPNG
c65	301	KIIR S L VL R H EPFKAAGTSARVLAAHGSDGEQVSLRLNGHPPGVWANGSAPHPERRPNG
c69	301	KIIRSHVLRQQEPFKAAGTSARVLAAHGSDGEQVSLRLNGHPPGVWANGSAPHPERRPNG
c71	301	KIIRSHVLRQQEPFKAAGTSARVLAAHGSDGEQVSLRLNGHPPGVWANGSAPHPERRPNG
c73	301	KIIRSHVLRQQEPFKAAGTSARVLAAHGSDGEQVSLRLNGHPPGV V NGSAPHPERRPNG

WT 361 YALGLVSGGSAQESQGNLTGLPDVELLSHELKGVCEPPGLDDPLAQDGAGVS
GL26 361 YALGLVSGGSAQESQGNLTGLPDVELLSHELKGVCEPPGLDDPLAQDGAGVS
c2 361 YALGLVSGGSAQESQGNLTGLPDVELLSHELKGVCEPPGLDDPLAQDGAGVS
c7 361 YALGLVSGGSAQESQGNLTGLPDVELLSHELKGVCEPPGLDDPLAQDGAGVS
c9 361 YALGLVSGGSAQESQGNLTGLPDVELLSHELKGVCEPPGLDDPLAQDGAGVS
c13 361 YALGLVSGGSAQESQGNLTGLPDVELLSHELKGVCEPPGLDDPLAQDGAGVS
c14 361 YALGLVSGGSAQESQGNLTGLPDVELLSHELKGVCEPPGLDDPLAQDGAGVS
c28 361 YALGLVSGGSAQESQGNLTGLPDVELLSHELKGVCEPPGLDDPLAQDGAGVS
c30 361 YALGLVSGGSAQESQGNLTGLPDVELLSHELKGVCEPPGLDDPLAQDGAGVS
c32 361 YALGLVSGGSAQESQGNLTGLPDVELLSHELKGVCEPPGLDDPLAQDGAGVS
c45 361 YALGLVSGGSAQESQGNLTGLPDVELLSHELKGVCEPPGLDDPLAQDGAGVS
c47 361 YALGLVSGGSAQESQGNLTGLPDVELLSHELKGVCEPPGLDDPLAQDGAGVS
c48 361 YALGLVSGGSAQESQGNLTGLPDVELLSHELKGVCEPPGLDDPLAQDGAGVS
c60 361 YALGLVSGGSAQESQGNLTGLPDVELLSHELKGVCEPPGLDDPLAQDGAGVS
c61 361 YALGLVSGGSAQESQGNLTGLPDVELLSHELKGVCEPPGLDDPLAQDGAGVS
c62 361 YALGLVSGGSAQESQGNLTGLPDVELLSHELKGVCEPPGLDDPLAQDGAGVS
c65 361 YALGLVSGGSAQESQGNLTGLPDVELLSHELKGVCEPPGLDDPLAQDGAGVS
c69 361 YALGLVSGGSAQESQGNLTGLPDVELLSHELKGVCEPPGLDDPLAQDGAGVS
c71 361 YALGLVSGGSAQESQGNLTGLPDVELLSHELKGVCEPPGLDDPLAQDGAGVS
c73 361 YALGLVSGGSAQESQGNLTGLPDVELLSHELKGVCEPPGLDDPLAQDGAGVS

Supplementary Table S1. Mutations occurring in OG resistant A2aR variants retrieved by YDDS. Positions of the substitutions numbered according to the Ballesteros-Weinstein GPCR numbering scheme are indicated in parentheses. Mutation positions located in either Helix 8 or C-terminus (CT) are highlighted in blue. ICL and ECL are intra- and extra-cellular loops, respectively.

Clone No	Mutations									
GL26	L48A (2x46)	A54L (2x52)	T65A (2x62)	Q89A (3x37)						
c2	F183Y (5x45)	S213R (5x74)								
c7	N34K (1x60)									
c9	T41I (2x39)	E169K (ECL2)	L363Q (CT)	D401V (CT)						
c13	A72D (23x52)	C82R (3x30)	L192I (5x53)	A273T (7x37)	Q372P (CT)					
c14	M4R (1x30)	V40F (2x38)	G379C (CT)							
c28	G147D (ICL2)	R222W (6x24)	G318D (CT)	G376V (CT)						
c30	Y103F (3x51)	V282D (7x47)	P352R (CT)	G364W (CT)						
c32	F62S (2x59)	F79I (3x27)	S305G (8x60)							
c45	G5S (1x31)	L194M (5x55)	G240E (6x42)	H250R (6x52)						
c47	M4E (1x30)	T11A (1x37)	I80T (3x28)	T117N (4x38)	S329R (CT)	D401V (CT)				
c48	P313S (8x68)	P354A (CT)								
c60	F79L (3x27)	S94C (3x42)	I98V (3x46)	G162S (ECL2)	T256I (6x58)	S263G (ECL3)	N280D (7x45)	I287T (7x52)	R309M (8x64)	
	V366A (CT)	N377H (CT)	D401E (CT)	L404P (CT)						
c61	S7P (1x33)	L22R (1x48)	N39S (2x37)	T68A (2x65)	F70S (23x50)	L87R (3x35)	V116E (34x57)	R293C (8x48)	V323F (CT)	
	L324S (CT)	H353L (CT)	L365Q (CT)	T378M (CT)	E384G (CT)	D402V (CT)				
c62	I3Y (1x29)	F79I (3x27)	L96Q (3x44)	V116E (34x57)	T117S (4x38)	K122E (4x43)	V164E (ECL2)	D170V (ECL2)	N181D (5x43)	
	L187R (5x48)	M211I (5x72)	G218W (ICL3)	L247P (6x49)	F257L (6x59)	I292N (8x47)	R304C (8x59)	T319S (CT)	S350C (CT)	
c65	I16V (1x42)	W29R (1x55)	F83L (3x31)	I124N (4x45)	I127F (4x48)	F168I (45x52)	P173S (5x36)	L191Q (5x52)	Y197F (5x58)	
	F201C (5x62)	H306L (8x61)	Q311H (8x66)	Q375L (CT)						
c69	I21N (1x47)	W32R (1x58)	L87P (3x35)	T88S (3x36)	Y103D (3x51)	R111W (34x52)	F180L (5x42)			
c71	Y9F (1x35)	V27M (1x53)	T41I (2x39)	A50V (2x48)	P61S (2x58)	F79S (3x27)	I80N (3x28)	S90C (3x38)	F93Y (3x41)	
	I98F (3x46)	I125N (4x46)	V164E (ECL2)	F168Y (ECL2)	A273D (7x37)	V411A (CT)				
c73	A347V (CT)	V393M (CT)								