Cell Reports, Volume 30

## **Supplemental Information**

## Structures of $G\alpha$ Proteins in Complex

## with Their Chaperone Reveal

## **Quality Control Mechanisms**

Alpay Burak Seven, Daniel Hilger, Makaía M. Papasergi-Scott, Li Zhang, Qianhui Qu, Brian K. Kobilka, Gregory G. Tall, and Georgios Skiniotis



**Supplementary Figure 1.** Overview of protein purification strategies and cryoEM imaging. (A) Representative elution profile of purified Ric-8A-G $\alpha_{i1}$  and Ric-8A-G $\alpha_q$  complexes on Superdex200 Increase 10/300 size-exclusion column. (B) Representative cryoEM micrographs and (C) 2D class averages of full-length Ric-8A bound to full-length G $\alpha_{i1}$  (left panels) and G $\alpha_q$  (right panels) for ~200,000 total particle projections with each class representing between 500 to 2500 particle projections. Related to Figure 1.



**Supplementary Figure 2.** CryoEM workflow for the Ric-8A-G $\alpha$  complexes. (A) CryoEM processing flow-chart, (B) local resolution estimation and (C) angular distribution of the projections contributing to the refined cryoEM map of the Ric-8A-G $\alpha_{i1}$  complex. (D) CryoEM processing flow-chart, (E) local resolution estimation and (F) angular distribution of the projections contributing to the refined cryoEM map of the Ric-8A-G $\alpha_{q}$  complex. Related to Figure 1.



**Supplementary Figure 3.** CryoEM map quality and validation. (A) Representative cryoEM densities of  $G\alpha_q$  (magenta) and  $G\alpha_{i1}$  (gold) bound to Ric-8A (blue - left panels for  $G\alpha_q$  and right panels for  $G\alpha_{i1}$ ). (B) Fourier shell correlation plots between the model and full map and between the half maps generated using Mtriage in PHENIX and 3DFSC. Dashed lines represent the resolution values at 0.143 FSC for half maps and 0.5 FSC for the model to full map. (D) Structural variability in Ric-8A-G $\alpha$  complexes. Ric-8A-G $\alpha_{i1}$  particles were subjected to iterative 3D classification rounds followed by refinement to elucidate variation across reconstructions. Corresponding Ric-8A and G $\alpha_{i1}$  densities are colored blue and gold respectively. Four representative classes are shown to highlight the structural variability (indicated by arrows) of the extended C-terminal loop region of Ric-8A, the C-terminal helix of Ric-8A interacting with the switch2 motif of G $\alpha_{i1}$ , the Ras-like domain secondary structure elements that interact with guanine nucleotide and the AHD, and the loop between  $\alpha$ 5 and  $\beta$ 6 of G $\alpha_{i1}$  including the TCAT motif respectively. Related to Figure 1.



**Supplementary Figure 4.** Comparison of Ric-8A structures and topology of the C-terminal tail of Ric-8A. (A) Domain architecture of Ric-8A, color coded by ARM/HEAT repeats and the extended C-terminal loop, in complex with  $G\alpha_{i1}$  (grey). (B) Comparison of the Ric-8A structures bound to  $G\alpha_{i1}$  (tan) and  $G\alpha_q$  (blue), aligned according to the first two HEAT repeats of Ric-8A to show the more compact conformation of the core ARM/HEAT repeats of Ric-8A bound to  $G\alpha_q$ . (C) Ric-8A- $G\alpha_{i1}$  complex structure (tan/grey) compared with the crystal structure of Ric-8A 1-453 alone (red, PDB: 6NMG); the arrow indicates the sulfate ion bound to Ric-8A in the crystal structure. (D) Ric-8A- $G\alpha_{i1}$  complex structure (tan/grey) compared with the crystal structure. (D) Ric-8A- $G\alpha_{i1}$  complex structure (tan/grey) compared with the crystal structure. (D) Ric-8A- $G\alpha_{i1}$  complex structure (tan/grey) compared with the crystal structure. (D) Ric-8A- $G\alpha_{i1}$  complex structure (tan/grey) compared with prediction of Ric-8A (cyan) bound to a  $G\alpha$  transducin ( $G\alpha_t$ ) peptide (purple), (PDB:6N85). (E) Secondary structure prediction of Ric-8A residues 401-530 and experimentaly identified phosphorylation sites. Related to Figure 3.





**Supplementary Figure 5.** Comparison of Gα structures in complex with GTPγS, Ric-8A or a GPCR. Comparison of (A) the structure of Gα<sub>i1</sub> (gold) bound Ric-8A (blue) aligned with the nucleotide free structure of Gα<sub>i1</sub> (purple) bound to Cannabinoid Receptor 1 (CB1) (PDB:6N4B) and (B) the structure of Gα<sub>q</sub> (magenta) bound to Ric-8A (blue) to the nucleotide free structure of Gα<sub>11/i</sub> chimera (green) bound to the Muscarinic Acetylcholine Receptor 1 (M1)

tures in complex with GTPyS, Ric-8A or a GPCR. Comparison of (A) the structure of  $G\alpha_{i1}$  (gold) bound to Ric-8A (blue) aligned with the nucleotide free structure of Ga<sub>i1</sub> (purple) bound to Cannabinoid Receptor 1 (CB1) (PDB:6N4B) and (B) the structure of  $G\alpha_{\scriptscriptstyle q}$ (magenta) bound to Ric-8A (blue) to the nucleotide free structure of  $G\alpha_{11/i}$  chimera (green) bound to the Muscarinic Acetylcholine Receptor 1 (M1) (PDB:60IJ). (C-D) Comparison of the Ga<sub>i1</sub> structure bound to GTPyS (PDB ID: 1GIA) or to Ric-8A. The G  $\alpha_{i1} \alpha 5$  helix (gold) adopts a distinct conformation when bound to Ric-8A compared to that of the canonical GTP bound state (white). The α5 helix in the Ric-8A-G  $\alpha_{i1}$  complex is displaced to interact with the concave inner surface of Ric-8A. The canonical α5 helix position is occupied by the second helix of the final HEAT repeat (aB9) of Ric-8A. (E) Ric-8A prevents

premature association of  $G_{\beta\gamma}$  with  $G_{\alpha}$  by interacting with the switch2 motif. Overlay of the  $G\alpha_{i1}\beta_1\gamma_2$  crystal structure (PDB ID:1GP2) with the cryoEM model of  $G\alpha_{i1}$  (gold) bound to Ric-8A (blue). The switch2 motif interacts with Ric-8A in both  $G\alpha_{i1}$  and  $G\alpha_{q}$ . The switch2 motif of  $G\alpha_{i1}$  bound to Ric-8A adopts a different conformation than switch2 motif in either  $G\alpha_{i1}$  in complex with  $\beta_1\gamma_2$  (slate grey/grey) or  $G\alpha_{i1}$  alone. The conformation of  $G\alpha$  switch2 in Ric-8A-G $\alpha$  structures does not appear to be compatible with canonical  $\beta_1\gamma_2$  interaction. Related to Figure 3.



**Supplementary Figure 6.** Multiple sequence alignment of Ric-8, thermal stability of Ric-8A and expression control of Ric-8A mutants in the cell based Ric-8A complementation assay. (A) The sequence alignment of Ric-8 includes Ric-8A *Rattus norvegicus* (NP\_001093990.1), Ric-8A *Homo sapiens*, (NP\_001273063.1), Ric-8B *Homo sapiens* (NP\_001317074.1), Ric-8 *Drosophila melanogaster* (NP\_001285048.1), Ric-8 *Caenorhabditis elegans* (NP\_001023561.1) and RIC-8 *Xenopus tropicalis* (NP\_989159.1). (B) Thermal stability of full-length Ric-8A (Ric-8A FL), phosphorylated/full-length Ric-8A (p-Ric-8A FL) and truncated Ric-8A (Ric-8A 1-452), and phosphorylated/truncated Ric-8A (p-Ric-8A 1-452). The stability was analyzed using SYPRO Orange fluorescence over increasing temperatures. (C) Knockout of RIC-8A in HEK293T cells results in a Ga<sub>q</sub> abundance defect (Papasergi-Scott et al., 2018). The rescue of Ga<sub>q</sub> abundance upon stable expression of Ric-8A mutants was analyzed by quantitative immunoblotting of cell membrane samples. Representative western blot of membrane-bound Ga<sub>q</sub> is shown in upper panel and Ric-8A in lower panel. Related to Figure 4 and 6.



**Supplementary Figure 7.** Comparison of interaction profiles of the  $\alpha$ 5 helices with the Ric-8 core domain. Interaction profile of the  $\alpha$ 5 helix of (A) G $\alpha_{i1}$  or (B) G $\alpha_{q}$  with Ric-8A identified in the cryoEM structures, and the  $\alpha$ 5 helix of G $\alpha_{s}$  with Ric-8B predicted by homology modeling. Residue numbering is based on the "common G $\alpha$  numbering (CGN) system in superscript" (Flock et al., 2015). Related to Figure 5.

Data collection and	Ric-8A-Gα <sub>q</sub>	Ric-8A-Gα <sub>i1</sub>
processing		
Magnification	165,000	130,000
Voltage (kV)	300	300
Electron exposure (e <sup>-</sup> /Å <sup>2</sup> )	62	65
Defocus range (µm)	1.0-2.0	1.0-2.0
Pixel size (Å)	0.82	1.06
Symmetry imposed	C1	C1
No. Initial particle images	4,723,551	4,960,082
No. Final particle images	70,439	153,110
Map resolution (Å)	3.5	4.0
FSC threshold	0.143	0.143
Map resolution range (Å)	3.2 – 4.2	3.8-4.5
Map sharpening <i>B</i> factor (Å <sup>2</sup> )	-90	-180
Refinement		
Initial model used (PDB code)	6NMG (Ric-8A)/3AH8	6NMG (Ric-8A)/1GP2
	(Gaq)	(Gα <sub>i1</sub> )
Supplied resolution (Å)	3.5	4.0
FSC threshold	0.5	0.5
Model composition		
Chains	2	2
Non-hydrogen atoms	4,657	5249
Protein residues	603	669
<i>B</i> factors (Å <sup>2</sup> )	71.51	142.16
R.m.s. deviations		
Bond lengths (Å)	0.006	0.005
Bond angles (°)	0.934	1.023
Validation		
MolProbity score	1.98	1.97
Clashscore	8.28	7.78
Poor rotamers (%)	0.00	0.00
Ramachandran plot		
Favored (%)	90.53	90.08
Allowed (%)	9.47	9.92
Disallowed (%)	0	0

**Table S1.** CryoEM data collection, refinement and validation statistics. Related to Figure 1.

Oligonucleotides           Gar-AF354, Fw: 5'-AAAGATTGTGGTCTCTAAGC         Stanford Pan Facility         Custom synthesis           TCTGAGATCCGGCTGC-3'         Stanford Pan Facility         Custom synthesis           ATCTTTAGATACTTITTATGATGACACATCGTTACA-3'         IDT         Customer synthesis           Quickchange PCR forward primer for R75E: 5'-         IDT         Customer synthesis           GGCAATCCTATCCGAAGACCGCAGCTGCC-3'         IDT         Customer synthesis           GGCACCTGCGGTCTTCGGATAGGATTCGG-3'         IDT         Customer synthesis           GUickchange PCR reverse primer for R75E: 5'-         IDT         Customer synthesis           CGAGTCCAGGCACAAGAGCACAAGGCATTGG-3'         IDT         Customer synthesis           Quickchange PCR reverse primer for R158: 5'-         IDT         Customer synthesis           GGAAGTCTAAGTCAGCAACAGAGCTCG-3'         IDT         Customer synthesis           Quickchange PCR reverse primer for L174W: 5'-         IDT         Customer synthesis           CCTGCAAAGGCCAGCGCAGCTGATGGC-3'         IDT         Customer synthesis           GUickchange PCR reverse primer for L174W: 5'-         IDT         Customer synthesis           GCAAGCTCGCAGCGCAGCGGATGACGA'         IDT         Customer synthesis           GATGTTAAAGAGCACTGCGAGGAGATTCCA'G'         IDT         Customer synthesis	REAGENT or RESOURCE	SOURCE	IDENTIFIER
Gar-AF354         Fw: 5'-AAAGATTGGGTCTCTAAGC         Stanford Pan Facility         Custom synthesis           TTCTGAGATCCGGCTGC-3'         Gar-AF354         Rev: 5'-TCTCAGAAGCTAGAGACCACA         Stanford Pan Facility         Custom synthesis           CGGAACCTATTTTTTATGATAGACATCTGTTAC-3'         IDT         Customer synthesis         Customer synthesis           CGGAACCTACCGAGCCGCAGCGCC-3'         IDT         Customer synthesis         Customer synthesis           GGCAGCTGCGGTCTTCGGATAGGATTCGG-3'         IDT         Customer synthesis         Customer synthesis           Cuckchange PCR forward primer for N123A: 5'-         IDT         Customer synthesis         Customer synthesis           Cuckchange PCR forward primer for F163A: 5'-         IDT         Customer synthesis         Customer synthesis           Cuckchange PCR reverse primer for F163A: 5'-         IDT         Customer synthesis         Customer synthesis           GAACCCTAACAGCCAGGCACTGACTGGACTTCG-3'         IDT         Customer synthesis         Customer synthesis           GCACATCAGCGGCGCACGGACTGGACTGAGTGTCC-3'         IDT         Customer synthesis         Customer synthesis           GCACATCAGCGGCGCACGGACTGACTGGACTGAGTGTCC-3'         IDT         Customer synthesis         Customer synthesis           GCACATCAGCGGCGCACGGACTGACTGACTGC-3'         IDT         Customer synthesis         Cactomer synthesis	Oligonucleotides		
TTCTGAGATCCGGCTGC-3'       Stanford Pan Facility       Custom synthesis         ATCTTTTAGATTATTTTTTATGATGACATCGTTAC-3'       IDT       Customer synthesis         Quickchange PCR forward primer for R75E: 5'-       IDT       Customer synthesis         Quickchange PCR reverse primer for N123A: 5'-       IDT       Customer synthesis         Quickchange PCR reverse primer for N123A: 5'-       IDT       Customer synthesis         Quickchange PCR reverse primer for N123A: 5'-       IDT       Customer synthesis         CGGAAGCCAGACACAGAGCCACACAGG-3'       IDT       Customer synthesis         Cuickchange PCR reverse primer for N123A: 5'-       IDT       Customer synthesis         CGGCAGCTGACTGACTAAGGCCACACAGGCACTTGG-3'       IDT       Customer synthesis         CGAAGCCCAAGGCAGCACAGGCACTTGG-3'       IDT       Customer synthesis         GAAGTCCAGGTGGCGCACTGGACTTGAGCATGTG-3'       IDT       Customer synthesis         Quickchange PCR reverse primer for L174W: 5'-       IDT       Customer synthesis         GCGCACTCAGGCCAGGCGTGTGCACAGGAG3'       IDT       Customer synthesis         Quickchange PCR reverse primer for K225A: 5'-       IDT       Customer synthesis         GCTGCTAAAGCCGCAGCGGCACTGCAGGAGATTCACAG-3'       IDT       Customer synthesis         GATGTTAAGAGACACTGCGAGGAGTTCACAG-3'       IDT       Customer synthesis	Gα <sub>i1</sub> -ΔF354_Fw: 5'-AAAGATTGTGGTCTCTAAGC	Stanford Pan Facility	Custom synthesis
Gai-AF354 Rev: 5'-TCTCAGAAGCTTAGAGACCACA       Stanford Pan Facility       Custom synthesis         ATCTTTTAGATATTTTTATAGACACATCTGTACA-3'       IDT       Customer synthesis         CGAATCCTATCCGAAGACCGCAGCGCGCC3'       IDT       Customer synthesis         GGCAGCTGCGGTCTCGGATAGGATTCGG-3'       IDT       Customer synthesis         GGCAGCTGCGGTCTTGTGGCTCAGCAG-3'       IDT       Customer synthesis         CMickchange PCR reverse primer for N123A: 5'-       IDT       Customer synthesis         CGAAGTCCACAGAGCACACAGGCCATTG-3'       IDT       Customer synthesis         Quickchange PCR forward primer for F163A: 5'-       IDT       Customer synthesis         GGAAGCCACACAGGCACACGGCCCACTG-3'       IDT       Customer synthesis         GGAAGTCCAGTTCAGCGACACTGGACTTGC-3'       IDT       Customer synthesis         Cuckchange PCR reverse primer for F163A: 5'-       IDT       Customer synthesis         CGCACTCAGTCAGCCAGGCGCGCACGAGGCGC-3'       IDT       Customer synthesis         CCTGCTAACAGCCGCGCAGGCGCGTTAGCAGG-3'       IDT       Customer synthesis         CGCACACAGAGGCGCCCC-3'       IDT       Customer synthesis         CGCACACAGAGCGCGCACAGGGCGCCC-3'       IDT       Customer synthesis         CGTCAAGTGGCGCCAGGGCGCGCCCC-3'       IDT       Customer synthesis         CGCACACGGCGCCACGCGGCGCCCCC-3' <t< td=""><td>TTCTGAGATCCGGCTGC-3'</td><td></td><td></td></t<>	TTCTGAGATCCGGCTGC-3'		
ATCTTTTAGATTATTTTTATGATGACATCTGTTAC-3       IDT       Customer synthesis         Cuckchange PCR forward primer for R75E: 5'-       IDT       Customer synthesis         GCAACCTGCGGTCTCGGATAGGATTCGG-3'       IDT       Customer synthesis         Quickchange PCR forward primer for N123A: 5'-       IDT       Customer synthesis         Quickchange PCR reverse primer for N123A: 5'-       IDT       Customer synthesis         Quickchange PCR reverse primer for N123A: 5'-       IDT       Customer synthesis         CGAAGTCCAGGCACAAGAGCACACAGCGCATTGG-3'       IDT       Customer synthesis         GGAAGCTTAGTGGCGGACTGACTTAGGGCTCC-3'       IDT       Customer synthesis         GGACCTTAGTCAGCGACATGACATTGG-3'       IDT       Customer synthesis         Quickchange PCR reverse primer for L174W: 5'-       IDT       Customer synthesis         GCTGGTAACAGCCTGGCACGGACTGTAGCG-3'       IDT       Customer synthesis         Quickchange PCR reverse primer for K225A: 5'-       IDT       Customer synthesis         CATGGAAATCCTCGCAAGGCTCTTTACATC-3'       IDT       Customer synthesis         Quickchange PCR forward primer for K225A: 5'-       IDT       Customer synthesis         CATGGAAATCCTGGCAAGTGGACATTCCATG-3'       IDT       Customer synthesis         CATGGAAAGCGCCCACGAGGACTTCCAGG-3'       IDT       Customer synthesis	Gα <sub>i1</sub> -ΔF354_Rev: 5'-TCTCAGAAGCTTAGAGACCACA	Stanford Pan Facility	Custom synthesis
Quickchange PCR forward primer for R75E: 5'-       IDT       Customer synthesis         GCGAATCCTATCCGAAGACCGCAGCTGCC-3'       IDT       Customer synthesis         GGCAGCTGCGGGTTTCGGAAGAGATTCGG-3'       IDT       Customer synthesis         GUickchange PCR reverse primer for N123A: 5'-       IDT       Customer synthesis         CMickchange PCR reverse primer for N123A: 5'-       IDT       Customer synthesis         CGAATCCAGTTGGCTGACTACAGAGGCAC-3'       IDT       Customer synthesis         CGACTCCAGTTGGCTGACTTAGGGCTCC-3'       IDT       Customer synthesis         GGAGCCTAGGTGACTGGACTTGGACTTGG-3'       IDT       Customer synthesis         Quickchange PCR reverse primer for L174W: 5'-       IDT       Customer synthesis         CGCTGCTAACGCGCACGGCGCACTGGACTGGAG-3'       IDT       Customer synthesis         CGAACTCCAGCGCAGCTGTTAGCAGG-3'       IDT       Customer synthesis         CGAACTCCAGCGCAGCTGTTAGCAGG-3'       IDT       Customer synthesis         CACACTCAGTGCCCAGGCTGTTAGCAGG-3'       IDT       Customer synthesis         CATGGAAATCCTCGCAGCAGTGTTCCATG -3'       IDT       Customer synthesis         CATGGAAATCCTCGCAGGATTTCCATG -3'       IDT       Customer synthesis         CGTCAAAGGCCTGCGCGAGTGCTAGC-3'       IDT       Customer synthesis         CGTCAAAGGCCCTGGAGCTCTGCAGGA-3'       IDT </td <td>ATCTTTTAGATTATTTTTTATGATGACATCTGTTAC-3'</td> <td></td> <td></td>	ATCTTTTAGATTATTTTTTATGATGACATCTGTTAC-3'		
CCGAATCCTATCCGAAGACCGCAGCTGCC-3'       IDT       Customer synthesis         GGCAGCTGCGGTCTTCGGATAGGATTCGG-3'       IDT       Customer synthesis         GCAATCCTGTGTCCTTCTGCTCAGCA6-3'       IDT       Customer synthesis         Quickchange PCR reverse primer for N123A: 5'-       IDT       Customer synthesis         CTGCTGAGCACAAGAGCACACAGGCATTTG-3'       IDT       Customer synthesis         Quickchange PCR reverse primer for F163A: 5'-       IDT       Customer synthesis         GGAAGCTCACATCGCGGACTGGACTTCG-3'       IDT       Customer synthesis         GGAGCCTTAAGTCAGCGAACTGGACTTGC-3'       IDT       Customer synthesis         GGAGCCTAAGTCAGCGAACTGGACTTGC-3'       IDT       Customer synthesis         GCACATCAGTCGCGCACTGATGTGC-3'       IDT       Customer synthesis         Quickchange PCR reverse primer for L174W: 5'-       IDT       Customer synthesis         GCACATCAGTGCGCACGGCGACTGTTAGCAGG-3'       IDT       Customer synthesis         Quickchange PCR reverse primer for K225A: 5'-       IDT       Customer synthesis         GATGTAAAGAGCACTGGCAACTGCCAGGAGATTTCACT-3'       IDT       Customer synthesis         Quickchange PCR reverse primer for F228W: 5'-       IDT       Customer synthesis         GATGTTAAAGAGCACTGGAACATCACCTACC-3'       IDT       Customer synthesis         Quickchange PCR reverse	Quickchange PCR forward primer for R75E: 5'-	IDT	Customer synthesis
Quickchange PCR reverse primer for R75E: 5'-       IDT       Customer synthesis         GGCAGCTGGCGGCTTCTGGGTAGCAGATCGGA3'       IDT       Customer synthesis         Quickchange PCR reverse primer for N123A: 5'-       IDT       Customer synthesis         CGGAGCTCGGGCTCTGGCTAGCTAAGGCAC-3'       IDT       Customer synthesis         Quickchange PCR reverse primer for F163A: 5'-       IDT       Customer synthesis         GGAGCCTCAGCTTAAGGCCTGC-3'       IDT       Customer synthesis         Quickchange PCR reverse primer for F163A: 5'-       IDT       Customer synthesis         GGAGCCTAAGCGAGCAGCACTGGACTTGGC-3'       IDT       Customer synthesis         Quickchange PCR reverse primer for L174W: 5'-       IDT       Customer synthesis         CCTGCTAACAGCCTGGCGCAGCTGTTAGCAGG-3'       IDT       Customer synthesis         Quickchange PCR reverse primer for K225A: 5'-       IDT       Customer synthesis         GATGGAAATCCTGCGAGGAGTTCACAG-3'       IDT       Customer synthesis         Quickchange PCR reverse primer for F228W: 5'-       IDT       Customer synthesis         CCTAAAGTGCAGGACTCACGACCTGGAGCAGCTTGAGG-3'       IDT       Customer synthesis         Quickchange PCR reverse primer for F228W: 5'-       IDT       Customer synthesis         CTTAACAGCCCTGGACCTTGAGGAGCTTGAGG-3'       IDT       Customer synthesis	CCGAATCCTATCCGAAGACCGCAGCTGCC-3'		-
GeCAGC1 GCGG1C11CGGA1AGGA1CGG-3'       IDT       Customer synthesis         Cuickchange PCR forward primer for N123A: 5'-       IDT       Customer synthesis         CGGCAGCAGCACAGCAGCACGCAGCGCATTG-3'       IDT       Customer synthesis         GGAAGTCCAGTTGGCTGACTTAAGGCTCC-3'       IDT       Customer synthesis         GGAAGTCCAGTTCGCTGACTTAAGGCTCC-3'       IDT       Customer synthesis         GGAGCCTTAAGTCGCGACTGACTTAAGGCTCC-3'       IDT       Customer synthesis         GGAGCCTTAAGTCAGCGACTGGACTTGC-3'       IDT       Customer synthesis         GCGCACTCACGTGCCCACGGCTTAGCGGC3       IDT       Customer synthesis         GCACATCAGTGCCCAGGCGTTAGCAGC-3'       IDT       Customer synthesis         GCACATCAGTGCCCAGGCGTTAGCAGC-3'       IDT       Customer synthesis         GCACATCAGTGCCAGGCGTTAGCAGC-3'       IDT       Customer synthesis         GATGGAAATCCTCGCAGGTGTTAGCAGC-3'       IDT       Customer synthesis         GATGGAAATCCTCGCAGGTGCTCTTAACATC-3'       IDT       Customer synthesis         GATGGAAATCCTCGCAGGGACTTCCATGCAG-3'       IDT       Customer synthesis         GCTCAAAGTCCTCGGAACATCACCTACCAG-3'       IDT       Customer synthesis         GGAGATTCAAGGCCATGGAGGACTTCCAG-3'       IDT       Customer synthesis         GUickchange PCR reverse primer for F232A: 5'-       IDT       Cus	Quickchange PCR reverse primer for R75E: 5'-	IDT	Customer synthesis
Quickchange PCR forward primer for N123A: 5'-       ID1       Customer synthesis         Quickchange PCR reverse primer for N123A: 5'-       IDT       Customer synthesis         CTGCTGAGCACAAGACCACACAGGCATTTG-3'       IDT       Customer synthesis         CGAAGTCCAGTTCGCTGACTAAGCCTCC-3'       IDT       Customer synthesis         CGAAGTCTAAGTCAGCGAACTGGACTTCG-3'       IDT       Customer synthesis         Quickchange PCR reverse primer for F163A: 5'-       IDT       Customer synthesis         CGAGCCTTAAGTCAGCGAACTGGACTTCG-3'       IDT       Customer synthesis         Quickchange PCR reverse primer for L174W: 5'-       IDT       Customer synthesis         GCACATCAGTGCGCCAGGCGCATGTGCG-3'       IDT       Customer synthesis         GAAATCCTCGCAGTGCTCTTTAACAGCAGG-3'       IDT       Customer synthesis         GAAGTCACGGAATCGCGAGGGATTTCAACTG-3'       IDT       Customer synthesis         GATGTAAAGAGCACTGCGAGGACTTTCAACTG-3'       IDT       Customer synthesis         Quickchange PCR reverse primer for F228W: 5'-       IDT       Customer synthesis         CTTAACTACGGCGGCACTTTGAGG-3'       IDT       Customer synthesis         CTTAACAGGCGGGCGGGTGTTAAGAG-3'       IDT       Customer synthesis         CTTAACAGGCGGGGGGGGTGTTAAGAG-3'       IDT       Customer synthesis         GTTAGTAGTCACGGGGGGGGGTATTGAG-3' <td>GGCAGCTGCGGTCTTCGGATAGGATTCGG-3</td> <td></td> <td></td>	GGCAGCTGCGGTCTTCGGATAGGATTCGG-3		
LAAATGCCTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGGAGAC-3       IDT       Customer synthesis         CTGCTGAGCACAAGAGCACAGGCATTTG-3'       IDT       Customer synthesis         Quickchange PCR forward primer for F163A: 5'-       IDT       Customer synthesis         GGAAGTCCAGTTCGCGTGACTTAAGGCTCC-3'       IDT       Customer synthesis         GGAAGTCAGCGGCACTGACTGGACTTGG-3'       IDT       Customer synthesis         GCTGCTAACAGCCTGGCGCACTGATGTGC-3'       IDT       Customer synthesis         CGCACTCAGTGCGCCAGGCGCACTGATGTGC-3'       IDT       Customer synthesis         GCACATCAGTGCGCCAGGCGCACTGATGTGC-3'       IDT       Customer synthesis         Quickchange PCR reverse primer for L174W: 5'-       IDT       Customer synthesis         GCACATCAGTGCGCAGGGCACTGATGCGG-3'       IDT       Customer synthesis         Quickchange PCR reverse primer for K225A: 5'-       IDT       Customer synthesis         GATGTTAAGAGCACTGCGAGGAGTTTCCATG -3'       IDT       Customer synthesis         GATGGTAAGGCACTGGAGAGATTTGCAGG -3'       IDT       Customer synthesis         GUickchange PCR reverse primer for F228W: 5'-       IDT       Customer synthesis         CGTCAAGTGCTCTGAGACATCCTGAGGAGCATTTGAGG -3'       IDT       Customer synthesis         CTTAAGTGCACCGAGCGGCATGTGTAAGG -3'       IDT       Customer synthesis         GGAGTGCACA	Quickchange PCR forward primer for N123A: 5'-	IDT	Customer synthesis
Quickchange PCR reverse primer for N123A: 5-       IDT       Customer synthesis         CGTGCTGAGCAACAGACCACCACAGGCATTTG-3'       IDT       Customer synthesis         Quickchange PCR reverse primer for F163A: 5'-       IDT       Customer synthesis         GGAGCCTTAACTCAGCGAACTGGACTTCG-3'       IDT       Customer synthesis         GGAGCCTTAACTCAGCGACCTGGACTTCG-3'       IDT       Customer synthesis         GCACACTCAGTGCGCCACTGATGTGC-3'       IDT       Customer synthesis         CCTGCTAACACCCTGCGCGCACTGATGTGC-3'       IDT       Customer synthesis         GCACATCAGTGCGCCAGGCGTTAGCAGG-3'       IDT       Customer synthesis         GCACATCAGTGCGCCAGGGCTCTTAACCAG-3'       IDT       Customer synthesis         GATGTTAAAGAGCACTGCGAGGACTTCCATG-3'       IDT       Customer synthesis         Quickchange PCR reverse primer for F228W: 5'-       IDT       Customer synthesis         GCTCAAAGTGCTCTGGAACACCTACCACCTACG-3'       IDT       Customer synthesis         Quickchange PCR forward primer for F228W: 5'-       IDT       Customer synthesis         CTTTAACAGCCGCGACTTGGTAAGAG-3'       IDT       Customer synthesis         CTTTAACAGCGCGCGCGCGCACTTGTAAGAG-3'       IDT       Customer synthesis         GGAGTTCACCAGCGCGGGGGGGGATGTTAAGAG-3'       IDT       Customer synthesis         GGAGTTCACCAGCGGCGGGGGGGGGGATGTTAAG			
CTGCTGAGCACACAGGCATTTG-3       IDT       Customer synthesis         Quickchange PCR roward primer for F163A: 5'-       IDT       Customer synthesis         GGAGCTTAAGTCAGCGAACTGAGCTCG-3'       IDT       Customer synthesis         Quickchange PCR roward primer for L174W: 5'-       IDT       Customer synthesis         CCTGCTAACCAGCCTGGCGCACTGATGTGC-3'       IDT       Customer synthesis         Quickchange PCR rowerse primer for L174W: 5'-       IDT       Customer synthesis         GCACATCAGTGCGCCAGGCTGTTAGCAGG-3'       IDT       Customer synthesis         Quickchange PCR rowerse primer for K225A: 5'-       IDT       Customer synthesis         GATGGAAACCTCCCAGGGCACTTAGCAGG-3'       IDT       Customer synthesis         Quickchange PCR roward primer for K225A: 5'-       IDT       Customer synthesis         GATGTAAAGACACATCACCAGGGATTTCCATG-3'       IDT       Customer synthesis         Quickchange PCR roward primer for F228W: 5'-       IDT       Customer synthesis         CTTCAAAGTCCTCGGAGCACTTGAGGG-3'       IDT       Customer synthesis         Quickchange PCR roward primer for F232A: 5'-       IDT       Customer synthesis         CTTTAACATCACCGCGCGACTGTTGTAAGAG-3'       IDT       Customer synthesis         Quickchange PCR roward primer for H2375: 5'-       IDT       Customer synthesis         GGA	Quickchange PCR reverse primer for N123A: 5'-	IDT	Customer synthesis
Quickchange PCR reverse primer for F163A: 5-       ID1       Customer synthesis         Quickchange PCR reverse primer for F163A: 5'-       IDT       Customer synthesis         Quickchange PCR reverse primer for L174W: 5'-       IDT       Customer synthesis         Quickchange PCR reverse primer for L174W: 5'-       IDT       Customer synthesis         GCACCTGACTGACCGACCTGATGTGC-3'       IDT       Customer synthesis         Quickchange PCR reverse primer for L174W: 5'-       IDT       Customer synthesis         GCACATCAGTGCGCCAGGCTGTTAGCAGG-3'       IDT       Customer synthesis         Quickchange PCR reverse primer for K225A: 5'-       IDT       Customer synthesis         Quickchange PCR reverse primer for F228W: 5'-       IDT       Customer synthesis         Quickchange PCR reverse primer for F228W: 5'-       IDT       Customer synthesis         CTTCAAAGTGCTCCGGAGCACTTGAGG-3'       IDT       Customer synthesis         CTTTAACAGTCCCGGCGACTTTGAGG-3'       IDT       Customer synthesis         CTTTAACAGCCCCGACTCTTGAGGAG-3'       IDT       Customer synthesis         CTTTAACAGCCCGGCGACTGTTAAGGAG-3'       IDT       Customer synthesis         Quickchange PCR reverse primer for F232A: 5'-       IDT       Customer synthesis         CTCTTAACAGACTCAGGCGGACATGAGACTCCA3'       IDT       Customer synthesis <tr< td=""><td>CIGCIGAGCACAAGAGCACACAGGCAIIIG-3</td><td></td><td></td></tr<>	CIGCIGAGCACAAGAGCACACAGGCAIIIG-3		
Caractic Control Contect Contro Control Contect Control Control Control Control Control		IDT	Customer synthesis
GGAGCCTTAAGTCAGCGAACTGGACTTCG-3'       IDT       Customer synthesis         GGAGCCTTAAGTCAGCGAACTGGACTGGC-3'       IDT       Customer synthesis         Quickchange PCR forward primer for L174W: 5'-       IDT       Customer synthesis         Quickchange PCR reverse primer for L174W: 5'-       IDT       Customer synthesis         Quickchange PCR reverse primer for K225A: 5'-       IDT       Customer synthesis         Quickchange PCR reverse primer for K225A: 5'-       IDT       Customer synthesis         GATGTTAAGAGCACTGCGAGGATTTCCATC-3'       IDT       Customer synthesis         Quickchange PCR reverse primer for F228W: 5'-       IDT       Customer synthesis         CATGGGAATGTTCCAGGAGCACTTGGGACACTTGGGGAG       IDT       Customer synthesis         CATAGGTGATGTTCCAGAGCACTTGGGAGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG	CGAAGTCCAGTTCGCTGACTTAAGGCTCC-3		
Guickchange PCR forward primer for L174W: 5'-       IDT       Customer synthesis         Quickchange PCR reverse primer for L174W: 5'-       IDT       Customer synthesis         GCACATCAGTGCGCCAGGCTGTTAGCAGG-3'       IDT       Customer synthesis         Quickchange PCR reverse primer for K225A: 5'-       IDT       Customer synthesis         Quickchange PCR reverse primer for K225A: 5'-       IDT       Customer synthesis         Quickchange PCR forward primer for K225A: 5'-       IDT       Customer synthesis         Quickchange PCR forward primer for K225A: 5'-       IDT       Customer synthesis         Quickchange PCR forward primer for F228W: 5'-       IDT       Customer synthesis         CCTCAAAGTGCTGCAGACATCACCTACG-3'       IDT       Customer synthesis         Quickchange PCR reverse primer for F228W: 5'-       IDT       Customer synthesis         CCTCAAAGTGCTGCAGCACCTTGAGAG -3'       IDT       Customer synthesis         Quickchange PCR reverse primer for F228A: 5'-       IDT       Customer synthesis         CTTTAACATCACCGCGCGCGCGCGGGACCTTGAGAG -3'       IDT       Customer synthesis         Quickchange PCR reverse primer for H273F: 5'-       IDT       Customer synthesis         GGAGTTCCATGGCTGCGCGGTGATGTTAAAG -3'       IDT       Customer synthesis         GGAGATTCCACGGCGTGAAGCCATGGACACTCC -3'       IDT       Cust		וטו	Customer synthesis
Cutocharge PCK followard primer for L174W. 5'-       IDT       Cutocharge PCR reverse primer for L174W. 5'-         Quickcharge PCR reverse primer for L174W. 5'-       IDT       Customer synthesis         Quickcharge PCR reverse primer for K225A: 5'-       IDT       Customer synthesis         Quickcharge PCR reverse primer for K225A: 5'-       IDT       Customer synthesis         Quickcharge PCR reverse primer for K225A: 5'-       IDT       Customer synthesis         Quickcharge PCR forward primer for F228W: 5'-       IDT       Customer synthesis         CCTGCTAAGGCACTGCGGAGCACTCACG-3'       IDT       Customer synthesis         Quickcharge PCR reverse primer for F228W: 5'-       IDT       Customer synthesis         CGTAGGTAGTGTCCAGAGCACTTGAGG -3'       IDT       Customer synthesis         Quickcharge PCR forward primer for F232A: 5'-       IDT       Customer synthesis         CTTTAACAGAGTCGCGCGGGGACTTGGTAAGGAG-3'       IDT       Customer synthesis         Quickcharge PCR forward primer for F232A: 5'-       IDT       Customer synthesis         CTTTAACAGAGTCGCGCGGGGAACTCTCC3'       IDT       Customer synthesis         Quickcharge PCR forward primer for H273F: 5'-       IDT       Customer synthesis         GGAGATTCACGGCGGTGAACTCCC3'       IDT       Customer synthesis         GGAGATTCACGGCGGTGAACCTGCAGGGGGGGACCT       GAGATTCACA	Quickehongo DCP forward primar for L174W: 5'		Customor synthesis
CurickChange PCR reverse primer for L174W: 5'-       IDT       Customer synthesis         GCACATCAGTGCGCCAGGCTGTTAGCAGG-3'       IDT       Customer synthesis         QuickChange PCR forward primer for K225A: 5'-       IDT       Customer synthesis         GATGGAAATCCTCGCAGTGCAGGAGGATTTCCATG -3'       IDT       Customer synthesis         GATGTTAAAGAGCACTGCGAGGAGATTTCCATG -3'       IDT       Customer synthesis         GATGTTAAAGAGCACTGCGAGGAGATTTCCATG -3'       IDT       Customer synthesis         Quickchange PCR reverse primer for F228W: 5'-       IDT       Customer synthesis         CCTCAAAGTGCTCGGAACATCACCTACG-3'       IDT       Customer synthesis         Quickchange PCR reverse primer for F228W: 5'-       IDT       Customer synthesis         CTTAACAGTGCTCGGAGCACTTTGAGAG -3'       IDT       Customer synthesis         Quickchange PCR reverse primer for F232A: 5'-       IDT       Customer synthesis         CTTTAACAGAGTCGCGCGGGGAGATGTTAAGAG -3'       IDT       Customer synthesis         Quickchange PCR reverse primer for H273F: 5'-       IDT       Customer synthesis         GGAGTTCACGGTGAAGCCATGGAACTCC -3'       IDT       Customer synthesis         GGAGATTCACGGTGAAGCCATGGAACTCC -3'       IDT       Customer synthesis         GGAGATTCACGGTGAAGCCATGGAACTCC -3'       IDT       Customer synthesis		וטו	Customer synthesis
GuickChange PCR forward primer for K225A: 5'-       IDT       Customer synthesis         Quickchange PCR forward primer for K225A: 5'-       IDT       Customer synthesis         Quickchange PCR forward primer for K225A: 5'-       IDT       Customer synthesis         Quickchange PCR forward primer for K225A: 5'-       IDT       Customer synthesis         Quickchange PCR forward primer for F228W: 5'-       IDT       Customer synthesis         CCTCAAAGTGCTCTGGAACATCACCTACG-3'       IDT       Customer synthesis         Quickchange PCR forward primer for F228W: 5'-       IDT       Customer synthesis         CGTAGGTGATGTTCCAGGCGCACTTTGAGG -3'       IDT       Customer synthesis         Quickchange PCR forward primer for F232A: 5'-       IDT       Customer synthesis         CTTTAACATCACCGCGCGGCGATGTTAAGG-3'       IDT       Customer synthesis         Quickchange PCR forward primer for H273F: 5'-       IDT       Customer synthesis         GGAGATTCACGGGTGAAGCCATGGAACTCC -3'       IDT       Customer synthesis         GGAGATTCACGGTGAAGCCATGGAACTCC -3'       IDT       Customer synthesis         GGAGATTCACGGTGAAGCCATGGAACTCC -3'       IDT       Customer synthesis         GGAGATTCACGGTGAAGCCATGGAACTCC -3'       IDT       Customer synthesis         GGAGATTCACGGTGAAGCCATGGAGTGCTGCGGTGGGGGACCT       IDT       Customer synthesis	Ouickchange PCP reverse primer for L174W: 5'-		Customer synthesis
District of the construction of the			Customer synthesis
CATGGAAATCCTCGCAGTGCTCTTTAACATC-3'IDTCustomer synthesisQuickchange PCR reverse primer for K225A: 5'- Quickchange PCR forward primer for F228W: 5'- CCTCAAAGTGCTCTGGAACATCACCTACG-3'IDTCustomer synthesisQuickchange PCR reverse primer for F228W: 5'- CCTCAAAGTGCTCCAGAGACATCACCTACG-3'IDTCustomer synthesisQuickchange PCR reverse primer for F228W: 5'- CCTCAAGGCGATGTTCCAGAGCACCTTGAGG -3'IDTCustomer synthesisQuickchange PCR reverse primer for F228W: 5'- CTTTAACATCACCGCCGCGCACTCTGTTAAGAG-3'IDTCustomer synthesisQuickchange PCR forward primer for F232A: 5'- Quickchange PCR reverse primer for F232A: 5'- Quickchange PCR forward primer for H273F: 5'- GGAGTTCCACGGCGGGGGATGTTAAAGA-3'IDTCustomer synthesisQuickchange PCR reverse primer for H273F: 5'- Quickchange PCR reverse primer for R365A+E367A: 5'- Quickchange PCR forward primer for R365A+E367A: 5'- Quickchange PCR forward primer for R365A+E367A: 5'- Quickchange PCR forward primer for R365A+E367A: 5'- Quickchange PCR reverse primer for T381V: 5'- Quickchange PCR forward primer for R389A: 5'- Quickchange P	Ouickchange PCR forward primer for K225A: 5'-		Customer synthesis
Direction of the former for K225A: 5'- GATGTTAAAGAGCACTGCGAGGATTTCCATG -3'IDTCustomer synthesisQuickchange PCR forward primer for K225A: 5'- CCTCAAAGTGCTCTGGAACATCACCTACG-3'IDTCustomer synthesisQuickchange PCR reverse primer for F228W: 5'- Quickchange PCR forward primer for F228W: 5'- Quickchange PCR forward primer for F232A: 5'- Quickchange PCR reverse primer for F232A: 5'- Quickchange PCR reverse primer for F232A: 5'- IDTIDTCustomer synthesisQuickchange PCR reverse primer for F232A: 5'- Quickchange PCR reverse primer for F232A: 5'- Quickchange PCR forward primer for H273F: 5'- IDTIDTCustomer synthesisQuickchange PCR reverse primer for H273F: 5'- GGAGTTCCATGGCTTCACCGTGAATCTCC-3'IDTCustomer synthesisQuickchange PCR reverse primer for R365A+E367A: 5'- GGAGTTCCACGGTGAAGCCATGGAGCACTGCGGGGGGACCT GCTC-3'IDTCustomer synthesisQuickchange PCR reverse primer for R365A+E367A: 5'- GGAGATGTGAGGACTGCGCCTGCGGTGGGGGGACCT GCTC-3'IDTCustomer synthesisQuickchange PCR reverse primer for R365A+E367A: 5'- GGAGTGCCCCCACGCAGGCGCAGTCCTCACAT CCC -3'IDTCustomer synthesisQuickchange PCR reverse primer for R365A+E367A: 5'- GAGCAGGTCCCCCACGCAGGCGCAGTCCTCACAT CCC -3'IDTCustomer synthesisQuickchange PCR reverse primer for T381V: 5'- Quickchange PCR reverse primer for T381V: 5'- Quickchange PCR forward primer for R389A: 5'- Quickchange PCR forward primer for R389A: 5'- Quickchange PCR reverse primer for R389A: 5'- Quickchange PCR forward primer for R389A: 5'- Quickchange PCR forward primer for R389A: 5'- Quickchange PCR reverse primer for R389A: 5'- Quickchange PCR reverse primer for R389A: 5'- Quic	CATGGAAATCCTCGCAGTGCTCTTTAACATC-3'		Oustomer synthesis
GATGTTAAAGAGCACTGCGAAGGATTTCCATG -3'IDTCustomer synthesisQuickchange PCR forward primer for F228W: 5'- CGTAGGTGATGTTCCAGAGCACTCACCTACG-3'IDTCustomer synthesisQuickchange PCR reverse primer for F228W: 5'- CGTAGGTGATGTTCCAGAGCACTTGAGG -3'IDTCustomer synthesisQuickchange PCR forward primer for F228W: 5'- CGTAGCTGACGCGCGCGCGCGCTGTTAAGAG-3'IDTCustomer synthesisQuickchange PCR forward primer for F232A: 5'- CTTTAACATCACCGCCGACTCTGTTAAGAG-3'IDTCustomer synthesisQuickchange PCR forward primer for F232A: 5'- GGAGTTCCATGGCTTCACCGTGAATGTTAAAG -3'IDTCustomer synthesisQuickchange PCR forward primer for H273F: 5'- GGAGTTCCATGGCTTCACCGTGAATCTCC-3'IDTCustomer synthesisQuickchange PCR forward primer for H273F: 5'- GGAGATTCACGGTGAAGCCATGGAACTCC -3'IDTCustomer synthesisQuickchange PCR forward primer for R365A+E367A: 5'- GCTC-3'IDTCustomer synthesisQuickchange PCR forward primer for R365A+E367A: 5'- GCTC-3'IDTCustomer synthesisQuickchange PCR forward primer for T381V: 5'- CTTGTCCGCCTCATGGTACACCTGACAGCACGCCTIDTCustomer synthesisQuickchange PCR forward primer for T381V: 5'- CTGTATCCAGGTGTACACAGAGCGCGACACGACGCAGCACGCAGCACGACACGCACAGCACGCACAGCACGACAGCACGCACAGCACGACACAGCA	Quickchange PCR reverse primer for K225A: 5'-	IDT	Customer synthesis
Quickchange PCR forward primer for F228W: 5'- CCTCAAAGTGCTCTGGAACATCACCTACG-3'IDTCustomer synthesisQuickchange PCR reverse primer for F228W: 5'- CGTAGGTGATGTTCCAGAGCACTTTGAGG -3'IDTCustomer synthesisQuickchange PCR reverse primer for F232A: 5'- CTTTAACATCACCGCCGACTCTGTTAAGAG-3'IDTCustomer synthesisQuickchange PCR reverse primer for F232A: 5'- CTTTAACATCACCGCCGACTCTGTTAAGAG-3'IDTCustomer synthesisQuickchange PCR reverse primer for F232A: 5'- CTCTTAACAGAGTCGGCGGTGATGTTAAGA -3'IDTCustomer synthesisQuickchange PCR reverse primer for H273F: 5'- GGAGTTCCATGGCTGAAGCCATGGAACTCC -3'IDTCustomer synthesisQuickchange PCR reverse primer for H273F: 5'- GGAGATTCACGGTGAAGCCATGGAACTCC -3'IDTCustomer synthesisQuickchange PCR reverse primer for R365A+E367A: 5'- GGAGATTCACGGTGAGGCCTGCGCTGCGGTGGGGGACCT GCTC-3'IDTCustomer synthesisQuickchange PCR reverse primer for T381V: 5'- CTTGTCCGCCTATGGTACACGCGGAGCACTGCGCAGGCGCAGTCCTCACAT CCC -3'IDTCustomer synthesisQuickchange PCR forward primer for T381V: 5'- CTTGTCCAGGTGTACCATGAGCGGACACTGGGCGAACTCGGGACACAG -3'IDTCustomer synthesisQuickchange PCR forward primer for T381V: 5'- CTGTATCCAGGTGTACCATGAGCGGACACAG -3'IDTCustomer synthesisQuickchange PCR reverse primer for T381V: 5'- CTGTATCCAGGTGTACCATGAGCGGACAAG -3'IDTCustomer synthesisQuickchange PCR forward primer for T381V: 5'- CTGTATCCAGGTGTACCATGAGCGGACAAG -3'IDTCustomer synthesisQuickchange PCR forward primer for T381V: 5'- CTGTATCCAGGTGTACCATGAGCGGACAAG -3'IDTCustomer synthesis <td>GATGTTAAAGAGCACTGCGAGGATTTCCATG -3'</td> <td></td> <td></td>	GATGTTAAAGAGCACTGCGAGGATTTCCATG -3'		
CCTCAAAGTGCTCTGGAACATCACCTACG-3'IDTQuickchange PCR reverse primer for F228W: 5'- CGTAGGTGATGTTCCAGAGCACTTTGAGG -3'IDTQuickchange PCR forward primer for F232A: 5'- Quickchange PCR reverse primer for F232A: 5'- Quickchange PCR reverse primer for F232A: 5'- Quickchange PCR reverse primer for F232A: 5'- CTTTAACAGAGTCGCGCGGTGATGTTAAGAG-3'IDTQuickchange PCR reverse primer for F232A: 5'- Quickchange PCR reverse primer for H273F: 5'- GGAGTTCCATGGCTTCACCGTGAATCTCC-3'IDTCustomer synthesisQuickchange PCR reverse primer for H273F: 5'- GGAGTTCCATGGCTGCAACCCGGAATCTCC-3'IDTCustomer synthesisQuickchange PCR reverse primer for H273F: 5'- GGAGTTCCACGGTGAAGCCATGGAACTCC-3'IDTCustomer synthesisQuickchange PCR reverse primer for R365A+E367A: 5'- GCTC-3'IDTCustomer synthesisQuickchange PCR reverse primer for R365A+E367A: 5'- GCTC-3'IDTCustomer synthesisQuickchange PCR reverse primer for T381V: 5'- CTTGTCCGCCTCATGGTACACCTGGATACAG-3'IDTCustomer synthesisQuickchange PCR reverse primer for T381V: 5'- CTGTATCCAGGTGTACCATGAGCGGACAAG-3'IDTCustomer synthesisQuickchange PCR reverse primer for T381V: 5'- CTGTATCCAGGTGTACCATGAGCGGACAAG-3'IDTCustomer synthesisQuickchange PCR reverse primer for T381V: 5'- CTGTATCCAGGTGTACCATGAGCGGACAAG-3'IDTCustomer synthesisQuickchange PCR forward primer for T381V: 5'- CTGTATCCAGGTGTACCATGAGCGGACAAG-3'IDTCustomer synthesisQuickchange PCR forward primer for T381V: 5'- CTGTATCCAGGTGAAGGCAGTAGCTGCTGAGTC-3'IDTCustomer synthesisQuickchange PCR forward primer for T381	Quickchange PCR forward primer for F228W: 5'-	IDT	Customer synthesis
Quickchange PCR reverse primer for F228W: 5'- CGTAGGTGATGTTCCAGAGCACTTTGAGG -3'IDTCustomer synthesisQuickchange PCR forward primer for F232A: 5'- CTTTAACATCACCGCCGACTCTGTTAAGAG-3'IDTCustomer synthesisQuickchange PCR reverse primer for F232A: 5'- CTCTTAACAGAGTCGGCGGTGATGTTAAGAG-3'IDTCustomer synthesisQuickchange PCR reverse primer for F232A: 5'- CTCTTAACAGAGTCGGCGGTGATGTTAAGAG -3'IDTCustomer synthesisQuickchange PCR reverse primer for H273F: 5'- GGAGTTCCATGGCTTCACCGTGAATCTCC-3'IDTCustomer synthesisQuickchange PCR reverse primer for H273F: 5'- GGAGATTCACGGTGAAGCCATGGAACTCC -3'IDTCustomer synthesisQuickchange PCR reverse primer for R365A+E367A: 5'- GGGATGTGAGGACTGCGCCTGCGGTGGGGGGACCT GCTC-3'IDTCustomer synthesisQuickchange PCR reverse primer for R365A+E367A: 5'- GGGATGTCACCACGCACGCAGGCGCAGTCCTCACAT CCC -3'IDTCustomer synthesisQuickchange PCR reverse primer for T381V: 5'- CTTGTCCGCCTCATGGTACACCTGGATACAG-3'IDTCustomer synthesisQuickchange PCR reverse primer for T381V: 5'- CTGTATCCAGGTGTACCATGAGGCGGACAAG -3'IDTCustomer synthesisQuickchange PCR reverse primer for T381V: 5'- CTGTATCCAGGTGTACCATGAGGCGGACAAG -3'IDTCustomer synthesisQuickchange PCR forward primer for R389A: 5'- CTGTATCCAGGTGACATGGAAGCCAGGCGACAAG -3'IDTCustomer synthesisQuickchange PCR forward primer for R389A: 5'- CTGTATCCAGGTGAAGGCAGTAGCTGCTGAGTTC-3'IDTCustomer synthesisQuickchange PCR forward primer for R389A: 5'- CATACAGATGTGAAGGCAGTAGCTGCTGAGTTC-3'IDTCustomer synthesisQuickchang	CCTCAAAGTGCTCTGGAACATCACCTACG-3'		
CGTAGGTGATGTTCCAGAGCACTTTGAGG -3'IDTCustomer synthesisQuickchange PCR forward primer for F232A: 5'- CTTTAACATCACCGCCGACTCTGTTAAGAG-3'IDTCustomer synthesisQuickchange PCR reverse primer for F232A: 5'- CTCTTAACAGAGTCGGCGGTGATGTTAAGA -3'IDTCustomer synthesisQuickchange PCR forward primer for H273F: 5'- GGAGTTCCATGGCTCACCGTGAAGCCATGGAACTCC -3'IDTCustomer synthesisQuickchange PCR reverse primer for H273F: 5'- GGAGATTCACGGTGAAGCCATGGAACTCC -3'IDTCustomer synthesisQuickchange PCR forward primer for R365A+E367A: 5'- GGAGTGTGAGGACTGCGCCTGCGGTGGGGGGACCT GCTC-3'IDTCustomer synthesisQuickchange PCR reverse primer for R365A+E367A: 5'- GGACAGGTCCCCACCGCAGGCCGCAGTCCTCACAT CCC -3'IDTCustomer synthesisQuickchange PCR reverse primer for R365A+E367A: 5'- GAGCAGGTCCCCACCGCAGGCGCAGTCCTCACAT CCC -3'IDTCustomer synthesisQuickchange PCR reverse primer for R365A+E367A: 5'- GAGCAGGTCCCCACCGCAGGCGCAGTCCTCACAT CCC -3'IDTCustomer synthesisQuickchange PCR reverse primer for T381V: 5'- CTTGTCCGCCTCATGGTACACCTGGATACAG-3'IDTCustomer synthesisQuickchange PCR reverse primer for T381V: 5'- CTGTATCCAGGTGTACCATGAGGCGGACAAG -3'IDTCustomer synthesisQuickchange PCR forward primer for R389A: 5'- GATACAGATGTGAAGCCATGCAGCGCGCGAGCTC-3'IDTCustomer synthesisQuickchange PCR forward primer for R389A: 5'- GATACAGATGTGAAGCCATGCAGCTGCTGAGTTC-3'IDTCustomer synthesisQuickchange PCR forward primer for R389A: 5'- GATACAGATGTGAAGCCATGCAGCAGCTGCTGAGTTC-3'IDTCustomer synthesis	Quickchange PCR reverse primer for F228W: 5'-	IDT	Customer synthesis
Quickchange PCR forward primer for F232A: 5'- CTTTAACATCACCGCCGACTCTGTTAAGAG-3'IDTCustomer synthesisQuickchange PCR reverse primer for F232A: 5'- CTCTTAACAGAGTCGGCGGGGGATGTTAAAG -3'IDTCustomer synthesisQuickchange PCR forward primer for H273F: 5'- GGAGTTCCATGGCTTCACCGTGAATCTCC-3'IDTCustomer synthesisQuickchange PCR reverse primer for H273F: 5'- GGAGATTCACGGTGAAGCCATGGAACTCC -3'IDTCustomer synthesisQuickchange PCR reverse primer for H273F: 5'- GGAGATTCACGGTGAAGCCATGGAACTCC -3'IDTCustomer synthesisQuickchange PCR reverse primer for R365A+E367A: 5'- GGATGTGAGGACTGCGCCTGCGGTGGGGGGACCT GCTC-3'IDTCustomer synthesisQuickchange PCR reverse primer for R365A+E367A: 5'- GGATGTCACGCTCCCACCGCAGGCGCAGTCCTCACAT CCC -3'IDTCustomer synthesisQuickchange PCR reverse primer for R365A+E367A: 5'- GAGCAGGTCCCCCACCGCAGGCGCAGTCCTCACAT CCC -3'IDTCustomer synthesisQuickchange PCR reverse primer for R365A+E367A: 5'- GAGCAGGTCCCCCACCGCAGGCGCAGTCCTCACAT CCC -3'IDTCustomer synthesisQuickchange PCR reverse primer for T381V: 5'- CTTGTCCGCCTCATGGTACACCTGGATACAG-3'IDTCustomer synthesisQuickchange PCR reverse primer for T381V: 5'- CTGTATCCAGGTGTACCATGAGGCGGACAAG -3'IDTCustomer synthesisQuickchange PCR reverse primer for R389A: 5'- CGTATCCAGGTGTACCATGAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGC	CGTAGGTGATGTTCCAGAGCACTTTGAGG -3'		
CTTTAACATCACCGCCGACTCTGTTAAGAG-3'IDTQuickchange PCR reverse primer for F232A: 5'- CTCTTAACAGAGTCGGCGGTGATGTTAAAG -3'IDTCustomer synthesisQuickchange PCR forward primer for H273F: 5'- GGAGTTCCATGGCTTCACCGTGAATCTCC-3'IDTCustomer synthesisQuickchange PCR reverse primer for H273F: 5'- GGAGATTCACGGTGAAGCCATGGAACTCC -3'IDTCustomer synthesisQuickchange PCR reverse primer for H273F: 5'- GGAGATTCACGGTGAAGCCATGGAACTCC -3'IDTCustomer synthesisQuickchange PCR forward primer for R365A+E367A: 5'- GGATGTGAGGACTGCGCCTGCGGTGGGGGACCTIDTCustomer synthesisQuickchange PCR reverse primer for R365A+E367A: 5'- GAGCAGGTCCCCCACCGCAGGCGCAGTCCTCACAT CCC -3'IDTCustomer synthesisQuickchange PCR forward primer for T381V: 5'- CTTGTCCGCCTCATGGTACACCTGGATACAG-3'IDTCustomer synthesisQuickchange PCR reverse primer for T381V: 5'- CTGTATCCAGGTGTACCATGAGGCGGACAAG -3'IDTCustomer synthesisQuickchange PCR forward primer for T381V: 5'- CTGTATCCAGGTGTACCATGAGGCGGACAAG -3'IDTCustomer synthesisQuickchange PCR forward primer for R389A: 5'- GATACAGATGTGAAGCAGTAGCAGTAGCAGTAGCAGTTC-3'IDTCustomer synthesisQuickchange PCR forward primer for R389A: 5'- GATACAGATGTGAAGCAGTAGCAGTAGCAGTAGCAGTAGCAGTACGCAGTACTC3'IDTCustomer synthesisQuickchange PCR forward primer for R389A: 5'- GATACAGATGTGAAGCAGTACCACAIDTCustomer synthesis	Quickchange PCR forward primer for F232A: 5'-	IDT	Customer synthesis
Quickchange PCR reverse primer for F232A: 5'- CTCTTAACAGAGTCGGCGGTGATGTTAAAG -3'IDTCustomer synthesisQuickchange PCR forward primer for H273F: 5'- GGAGTTCCATGGCTTCACCGTGAATCTCC-3'IDTCustomer synthesisQuickchange PCR reverse primer for H273F: 5'- GGAGATTCACGGTGAAGCCATGGAACTCC -3'IDTCustomer synthesisQuickchange PCR reverse primer for H273F: 5'- GGAGATTCACGGTGAAGCCATGGAACTCC -3'IDTCustomer synthesisQuickchange PCR forward primer for R365A+E367A: 5'- GGATGTGAGGACTGCGCCTGCGGTGGGGGGGACCT GCTC-3'IDTCustomer synthesisQuickchange PCR reverse primer for R365A+E367A: 5'- GAGCAGGTCCCCCACCGCAGGCGCAGTCCTCACAT CCC -3'IDTCustomer synthesisQuickchange PCR forward primer for T381V: 5'- CTTGTCCGCCTCATGGTACACCTGGATACAG-3'IDTCustomer synthesisQuickchange PCR reverse primer for T381V: 5'- CTGTATCCAGGTGTACCATGAGGCGGACAAG -3'IDTCustomer synthesisQuickchange PCR forward primer for T381V: 5'- CTGTATCCAGGTGTACCATGAGGCGGACAAG -3'IDTCustomer synthesisQuickchange PCR forward primer for R389A: 5'- GATACAGATGTGAAGGCAGTAGCTGCTGCAGGTTC-3'IDTCustomer synthesisQuickchange PCR forward primer for R389A: 5'- GATACAGATGTGAAGGCAGTAGCTGCTGCAGGTTC-3'IDTCustomer synthesisQuickchange PCR forward primer for R389A: 5'- GATACAGATGTGAAGGCAGTAGCTGCTGCAGGTTC-3'IDTCustomer synthesis	CTTTAACATCACCGCCGACTCTGTTAAGAG-3'		
CTCTTAACAGAGTCGGCGGTGATGTTAAAG -3'Quickchange PCR forward primer for H273F: 5'- GGAGTTCCATGGCTTCACCGTGAATCTCC-3'IDTCustomer synthesisQuickchange PCR reverse primer for H273F: 5'- GGAGATTCACGGTGAAGCCATGGAACTCC -3'IDTCustomer synthesisQuickchange PCR forward primer for R365A+E367A: 5'- GGGATGTGAGGACTGCGCCTGCGGTGGGGGGACCT GCTC-3'IDTCustomer synthesisQuickchange PCR reverse primer for R365A+E367A: 5'- GAGCAGGTCCCCCACCGCAGGCGCAGTCCTCACAT CCC -3'IDTCustomer synthesisQuickchange PCR reverse primer for R365A+E367A: 5'- GAGCAGGTCCCCCACCGCAGGCGCAGTCCTCACAT CCC -3'IDTCustomer synthesisQuickchange PCR forward primer for T381V: 5'- CTTGTCCGCCTCATGGTACACCTGGATACAG-3'IDTCustomer synthesisQuickchange PCR reverse primer for T381V: 5'- CTGTATCCAGGTGTACCATGAGGCGACAAG -3'IDTCustomer synthesisQuickchange PCR forward primer for T381V: 5'- CTGTATCCAGGTGTACCATGAGGCGGACAAG -3'IDTCustomer synthesisQuickchange PCR reverse primer for T381V: 5'- CTGTATCCAGGTGTACCATGAGGCGGACAAG -3'IDTCustomer synthesisQuickchange PCR forward primer for R389A: 5'- GATACAGATGTGAAGGCAGTAGCTGCTGAGTTC-3'IDTCustomer synthesis	Quickchange PCR reverse primer for F232A: 5'-	IDT	Customer synthesis
Quickchange PCR forward primer for H273F: 5'- GGAGTTCCATGGCTTCACCGTGAATCTCC-3'IDTCustomer synthesisQuickchange PCR reverse primer for H273F: 5'- GGAGATTCACGGTGAAGCCATGGAACTCC -3'IDTCustomer synthesisQuickchange PCR forward primer for R365A+E367A: 5'- GGGATGTGAGGACTGCGCCTGCGGTGGGGGGACCT GCTC-3'IDTCustomer synthesisQuickchange PCR reverse primer for R365A+E367A: 5'- GAGCAGGTCCCCACCGCAGGCGCAGTCCTCACAT CCC -3'IDTCustomer synthesisQuickchange PCR reverse primer for R365A+E367A: 5'- GAGCAGGTCCCCCACCGCAGGCGCAGTCCTCACAT CCC -3'IDTCustomer synthesisQuickchange PCR forward primer for T381V: 5'- CTTGTCCGCCTCATGGTACACCTGGATACAG-3'IDTCustomer synthesisQuickchange PCR reverse primer for T381V: 5'- CTGTATCCAGGTGTACCATGAGGCGGACAAG -3'IDTCustomer synthesisQuickchange PCR forward primer for T381V: 5'- CTGTATCCAGGTGTACCATGAGGCGGACAAG -3'IDTCustomer synthesisQuickchange PCR forward primer for T381V: 5'- CTGTATCCAGGTGACCATGAGGCGGACAAG -3'IDTCustomer synthesisQuickchange PCR forward primer for R389A: 5'- GATACAGATGTGAAGGCAGTAGCTGCTGAGTTC-3'IDTCustomer synthesis	CTCTTAACAGAGTCGGCGGTGATGTTAAAG -3'		
GGAGTTCCATGGCTTCACCGTGAATCTCC-3'Quickchange PCR reverse primer for H273F: 5'- GGAGATTCACGGTGAAGCCATGGAACTCC -3'IDTCustomer synthesisQuickchange PCR forward primer for R365A+E367A: 5'- GGGATGTGAGGACTGCGCCTGCGGTGGGGGGACCT GCTC-3'IDTCustomer synthesisQuickchange PCR reverse primer for R365A+E367A: 5'- GAGCAGGTCCCCACCGCAGGCGCAGTCCTCACAT CCC -3'IDTCustomer synthesisQuickchange PCR reverse primer for T381V: 5'- Quickchange PCR forward primer for T381V: 5'- Quickchange PCR reverse primer for T381V: 5'- CTTGTCCGCCTCATGGTACACCTGGATACAG-3'IDTCustomer synthesisQuickchange PCR forward primer for T381V: 5'- Quickchange PCR reverse primer for T381V: 5'- CTGTATCCAGGTGTACCATGAGGCGGACAAG -3'IDTCustomer synthesisQuickchange PCR forward primer for T381V: 5'- Quickchange PCR reverse primer for T381V: 5'- CTGTATCCAGGTGTACCATGAGGCGGACAAG -3'IDTCustomer synthesisQuickchange PCR forward primer for T381V: 5'- Quickchange PCR forward primer for T381V: 5'- CTGTATCCAGGTGTACCATGAGGCGGACAAG -3'IDTCustomer synthesisCATACAGATGTGAAGGCAGTAGCTGCTGAGTTC-3'IDTCustomer synthesisCustomer synthesisGATACAGATGTGAAGGCAGTAGCTGCTGAGTTC-3'IDTCustomer synthesis	Quickchange PCR forward primer for H273F: 5'-	IDT	Customer synthesis
Quickchange PCR reverse primer for H273F: 5'- GGAGATTCACGGTGAAGCCATGGAACTCC -3'IDTCustomer synthesisQuickchange PCR forward primer for R365A+E367A: 5'- GGGATGTGAGGACTGCGCCTGCGGTGGGGGGACCT GCTC-3'IDTCustomer synthesisQuickchange PCR reverse primer for R365A+E367A: 5'- GAGCAGGTCCCCCACCGCAGGCGCAGTCCTCACAT CCC -3'IDTCustomer synthesisQuickchange PCR forward primer for T381V: 5'- CTTGTCCGCCTCATGGTACACCTGGATACAG-3'IDTCustomer synthesisQuickchange PCR reverse primer for T381V: 5'- CTTGTCCGCCTCATGGTACCACCTGGATACAG-3'IDTCustomer synthesisQuickchange PCR reverse primer for T381V: 5'- CTGTATCCAGGTGTACCATGAGGCGGACAAG -3'IDTCustomer synthesisQuickchange PCR reverse primer for R389A: 5'- GATACAGATGTGAAGGCAGTAGCTGCTGAGTTC-3'IDTCustomer synthesis	GGAGTTCCATGGCTTCACCGTGAATCTCC-3'		
GGAGATTCACGGTGAAGCCATGGAACTCC -3'Quickchange PCR forward primer for R365A+E367A: 5'- GGGATGTGAGGACTGCGCCTGCGGTGGGGGGACCTIDTCustomer synthesisQuickchange PCR reverse primer for R365A+E367A: 5'- GAGCAGGTCCCCCACCGCAGGCGCAGTCCTCACAT CCC -3'IDTCustomer synthesisQuickchange PCR forward primer for T381V: 5'- CTTGTCCGCCTCATGGTACACCTGGATACAG-3'IDTCustomer synthesisQuickchange PCR reverse primer for T381V: 5'- CTTGTCCGCCTCATGGTACACCTGGATACAG-3'IDTCustomer synthesisQuickchange PCR reverse primer for T381V: 5'- CTGTATCCAGGTGTACCATGAGGCGGACAAG -3'IDTCustomer synthesisQuickchange PCR reverse primer for R389A: 5'- GATACAGATGTGAAGGCAGTAGCTGCTGAGCTGCTGCA'IDTCustomer synthesis	Quickchange PCR reverse primer for H273F: 5'-	IDT	Customer synthesis
Quickchange PCR forward primer for R365A+E367A: 5'- GGGATGTGAGGACTGCGCCTGCGGTGGGGGGACCT GCTC-3'IDTCustomer synthesisQuickchange PCR reverse primer for R365A+E367A: 5'- GAGCAGGTCCCCCACCGCAGGCGCAGTCCTCACAT CCC -3'IDTCustomer synthesisQuickchange PCR forward primer for T381V: 5'- CTTGTCCGCCTCATGGTACACCTGGATACAG-3'IDTCustomer synthesisQuickchange PCR reverse primer for T381V: 5'- CTTGTCCGCCTCATGGTACACCTGGATACAG-3'IDTCustomer synthesisQuickchange PCR reverse primer for T381V: 5'- CTGTATCCAGGTGTACCATGAGGCGGACAAG -3'IDTCustomer synthesisQuickchange PCR reverse primer for T381V: 5'- CTGTATCCAGGTGTACCATGAGGCGGACAAG -3'IDTCustomer synthesisQuickchange PCR reverse primer for T381V: 5'- CTGTATCCAGGTGTACCATGAGGCGGACAAG -3'IDTCustomer synthesisQuickchange PCR reverse primer for R389A: 5'- GATACAGATGTGAAGGCAGTAGCTGCTGAGGTCC-3'IDTCustomer synthesisQuickchange PCR forward primer for R389A: 5'- GATACAGATGTGAAGGCAGTAGCTGCTGAGGTCC-3'IDTCustomer synthesis	GGAGATTCACGGTGAAGCCATGGAACTCC -3'		
GGGATGTGAGGACTGCGCCTGCGGTGGGGGGACCT GCTC-3'Customer synthesisQuickchange PCR reverse primer for R365A+E367A: 5'- GAGCAGGTCCCCCACCGCAGGCGCAGTCCTCACAT CCC -3'IDTCustomer synthesisQuickchange PCR forward primer for T381V: 5'- CTTGTCCGCCTCATGGTACACCTGGATACAG-3'IDTCustomer synthesisQuickchange PCR reverse primer for T381V: 5'- CTGTATCCAGGTGTACCATGAGGCGGACAAG -3'IDTCustomer synthesisQuickchange PCR reverse primer for T381V: 5'- CTGTATCCAGGTGTACCATGAGGCGGACAAG -3'IDTCustomer synthesisQuickchange PCR forward primer for R389A: 5'- GATACAGATGTGAAGGCAGTAGCTGCTGAGTTC-3'IDTCustomer synthesis	Quickchange PCR forward primer for R365A+E367A: 5'-	IDT	Customer synthesis
GCTC-3'IDTQuickchange PCR reverse primer for R365A+E367A: 5'- GAGCAGGTCCCCCACCGCAGGCGCAGTCCTCACAT CCC -3'IDTCustomer synthesisQuickchange PCR forward primer for T381V: 5'- CTTGTCCGCCTCATGGTACACCTGGATACAG-3'IDTCustomer synthesisQuickchange PCR reverse primer for T381V: 5'- CTGTATCCAGGTGTACCATGAGGCGGACAAG -3'IDTCustomer synthesisQuickchange PCR reverse primer for T381V: 5'- CTGTATCCAGGTGTACCATGAGGCGGACAAG -3'IDTCustomer synthesisQuickchange PCR forward primer for R389A: 5'- GATACAGATGTGAAGGCAGTAGCTGCTGAGTTC-3'IDTCustomer synthesis	GGGATGTGAGGACTGCGCCTGCGGTGGGGGGACCT		
Quickchange PCR reverse primer for R365A+E367A: 5'-       ID1       Customer synthesis         GAGCAGGTCCCCCACCGCAGGCGCAGTCCTCACAT       ID1       Customer synthesis         Quickchange PCR forward primer for T381V: 5'-       IDT       Customer synthesis         CTTGTCCGCCTCATGGTACACCTGGATACAG-3'       IDT       Customer synthesis         Quickchange PCR reverse primer for T381V: 5'-       IDT       Customer synthesis         CTGTATCCAGGTGTACCATGAGGCGGACAAG -3'       IDT       Customer synthesis         Quickchange PCR forward primer for R389A: 5'-       IDT       Customer synthesis         GATACAGATGTGAAGGCAGTAGCTGCTGAGTTC-3'       IDT       Customer synthesis		107	
GAGCAGGTCCCCCACCGCAGGCGCAGTCCTCACAT       CCC -3'         Quickchange PCR forward primer for T381V: 5'-       IDT         CTTGTCCGCCTCATGGTACACCTGGATACAG-3'       IDT         Quickchange PCR reverse primer for T381V: 5'-       IDT         CTGTATCCAGGTGTACCATGAGGCGGACAAG -3'       IDT         Quickchange PCR forward primer for R389A: 5'-       IDT	Quickchange PCR reverse primer for R365A+E367A: 5-	IDT	Customer synthesis
Quickchange PCR forward primer for T381V: 5'-       IDT       Customer synthesis         CTTGTCCGCCTCATGGTACACCTGGATACAG-3'       IDT       Customer synthesis         Quickchange PCR reverse primer for T381V: 5'-       IDT       Customer synthesis         CTGTATCCAGGTGTACCATGAGGCGGACAAG -3'       IDT       Customer synthesis         Quickchange PCR forward primer for R389A: 5'-       IDT       Customer synthesis         Quickchange PCR forward primer for R389A: 5'-       IDT       Customer synthesis         Quickchange PCR forward primer for R389A: 5'-       IDT       Customer synthesis         Quickchange PCR forward primer for R389A: 5'-       IDT       Customer synthesis			
Quickchange PCR forward primer for T381V: 5-       ID1       Customer synthesis         CTTGTCCGCCTCATGGTACACCTGGATACAG-3'       IDT       Customer synthesis         Quickchange PCR reverse primer for T381V: 5'-       IDT       Customer synthesis         CTGTATCCAGGTGTACCATGAGGCGGACAAG -3'       IDT       Customer synthesis         Quickchange PCR forward primer for R389A: 5'-       IDT       Customer synthesis         GATACAGATGTGAAGGCAGTAGCTGCTGAGTTC-3'       IDT       Customer synthesis	ULU -3		
Quickchange PCR reverse primer for T381V: 5'-       IDT       Customer synthesis         Quickchange PCR forward primer for R389A: 5'-       IDT       Customer synthesis         Quickchange PCR forward primer for R389A: 5'-       IDT       Customer synthesis         Quickchange PCR forward primer for R389A: 5'-       IDT       Customer synthesis         Quickchange PCR forward primer for R389A: 5'-       IDT       Customer synthesis         Quickchange PCR forward primer for R389A: 5'-       IDT       Customer synthesis		וטו	
CTGTATCCAGGTGTACCATGAGGCGGACAAG -3'     IDT     Customer synthesis       Quickchange PCR forward primer for R389A: 5'-     IDT     Customer synthesis       GATACAGATGTGAAGGCAGTAGCTGCTGAGTTC-3'     IDT     Customer synthesis	Ouickchange DCR reverse primer for T2911/: 5'		Customer synthesis
Quickchange PCR forward primer for R389A: 5'-     IDT     Customer synthesis       GATACAGATGTGAAGGCAGTAGCTGCTGAGTTC-3'     IDT     Customer synthesis	CTGTATCCAGGTGTACCATGAGGCGGACAAG 2'	וטו	
GATACAGATGTGAAGGCAGTAGCTGCTGAGTTC-3'	Ouickchange PCR forward primer for P380A+5'	דחו	Customer synthesis
			Sustomer synthesis
LUURKENANGE PUR REVERSE DRIMER FOR RISSUAL 5'- LUU	Ouickchange PCR reverse primer for R380A. 5'-	Т	Customer synthesis
GAACTCAGCAGCTACTGCCTTCACATCTGTATC -3'	GAACTCAGCAGCTACTGCCTTCACATCTGTATC -3'		Sustanter synthesis

 Table S2. Oligonucleotides. Related to Key Resources Table.

Quickchange PCR forward primer for A416W: 5'-	IDT	Customer synthesis
CTACGGGAATGCTTGGGGCCTCCTGGCTG-3'		-
Quickchange PCR reverse primer for A416W: 5'-	IDT	Customer synthesis
CAGCCAGGAGGCCCCAAGCATTCCCGTAG -3'		-
Quickchange PCR forward primer for A420W: 5'-	IDT	Customer synthesis
CTGCTGGCCTCCTGTGGGCCAGGGGCCTCATG -3'		
Quickchange PCR reverse primer for A420W: 5'-	IDT	Customer synthesis
CATGAGGCCCCTGGCCCACAGGAGGCCAGCAG -3'		