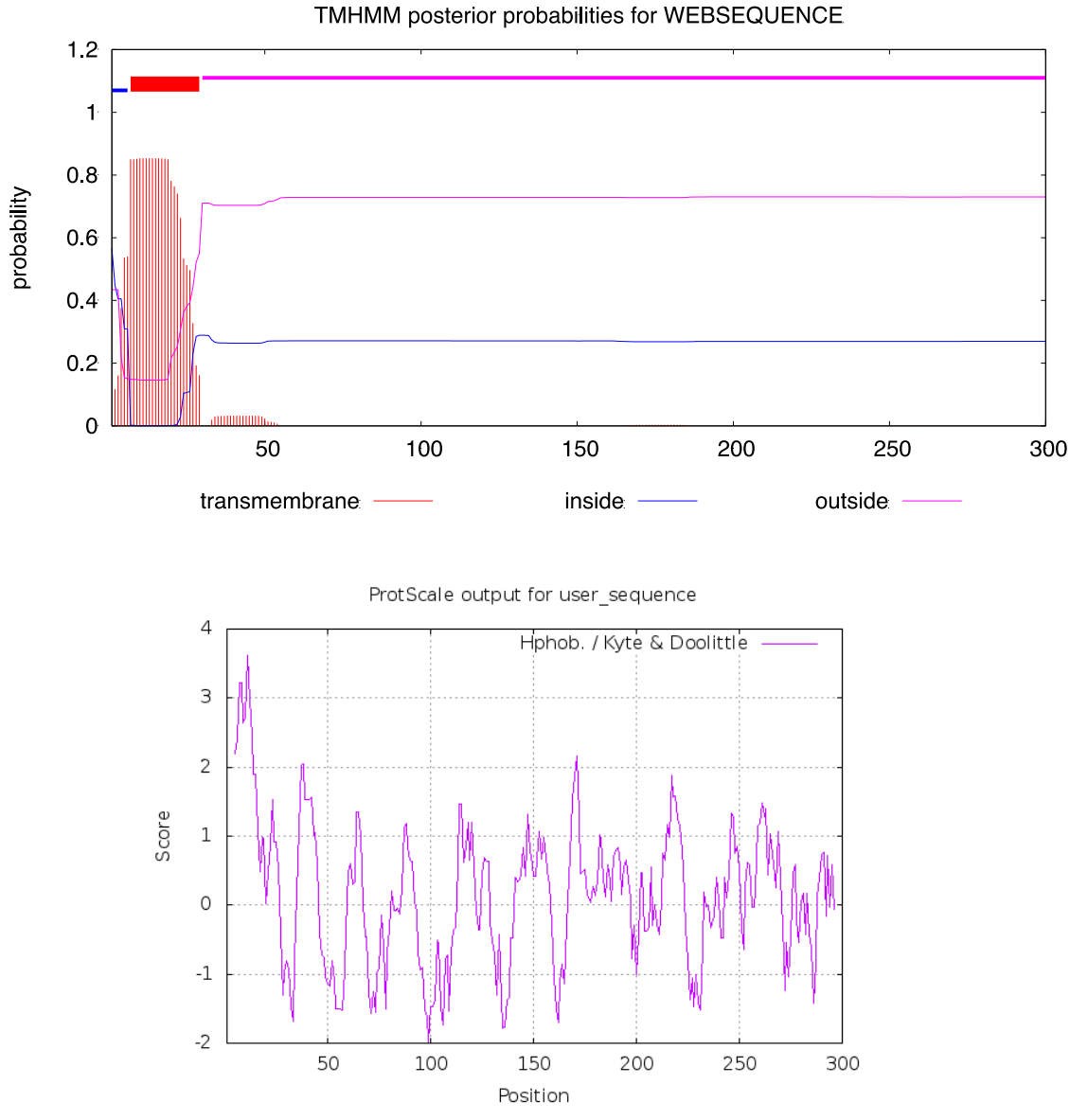
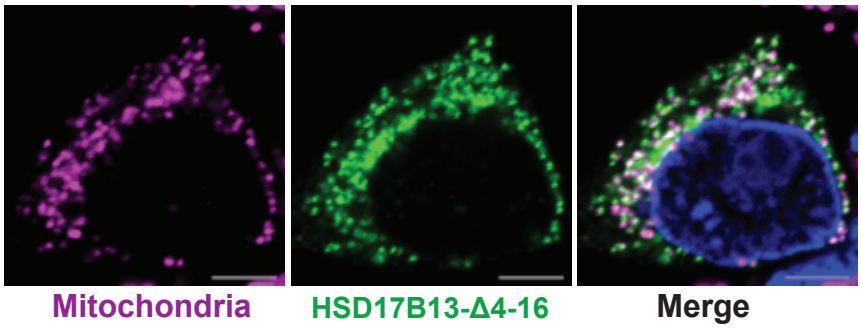
Supplemental Figure S1



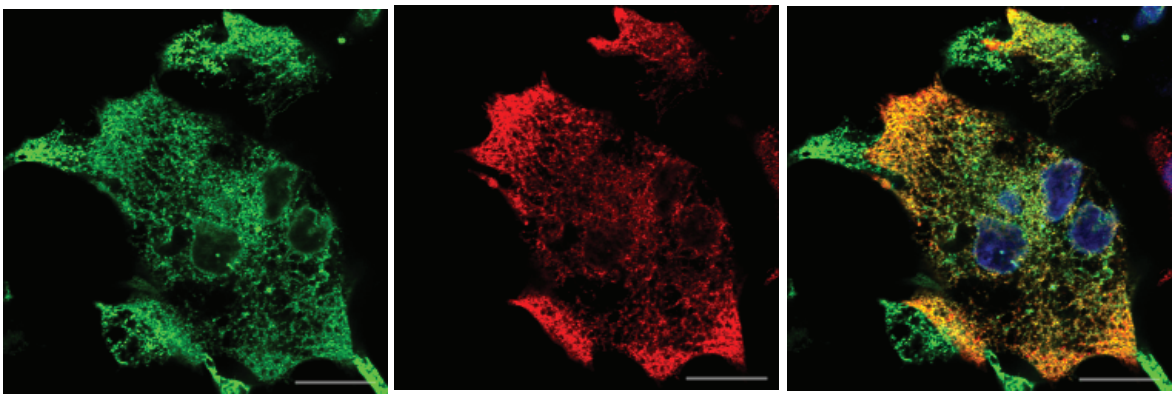
Supplemental Figure S1. Transmembrane domain prediction of HSD17B13. Transmembrane domains in wild type HSD17B13 were predicted using TMHMM Server v. 2.0 (http://www.cbs.dtu.dk/services/TMHMM/) and Protscale-ExPASy (https://web.expasy.org/protscale/). N-terminal sequences of HSD17B13 are identified by both tools as putative transmembrane domains.

Supplemental Figure S2



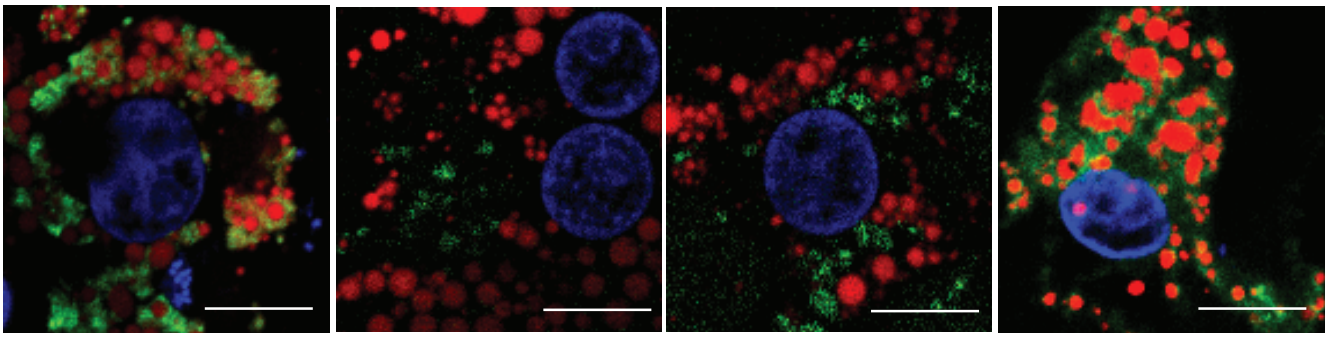
Supplemental Figure S2. Mitochondrial localization of HSD17B13-Δ4-16. HepG2 cells were transiently transfected with HSD17B13-Δ4-16 plasmids originated from HSD17B13-FLAG plasmid. Transfected cells were treated with fatty acids to induce LDs. Immunofluorescence staining was used to determine the cellular localization of mitochondria (Far red) and HSD17B13-Δ4-16 (green). Nuclei are counter stained with Hoechst (Blue). Images were analyzed by confocal microscopy. Bar indicates 10 μM.

Supplemental Figure S3



Supplemental Figure S3. ER localization of HSD17B13-variant B. HEK293 cells were transiently transfected with HSD17B13-variant B, the exon-2 skipped naturally occuring variant (Δ71-106) and SEC61β-GFP plasmids. Immunofluorescence staining was used to determine the cellular localization of HSD17B13-variant B (Red) and GFP was used to identify ER (green). Nuclei are counter stained with Hoechst (Blue). Images were analyzed by confocal microscopy. Bar indicates 10 μM.

Supplemental Figure S4

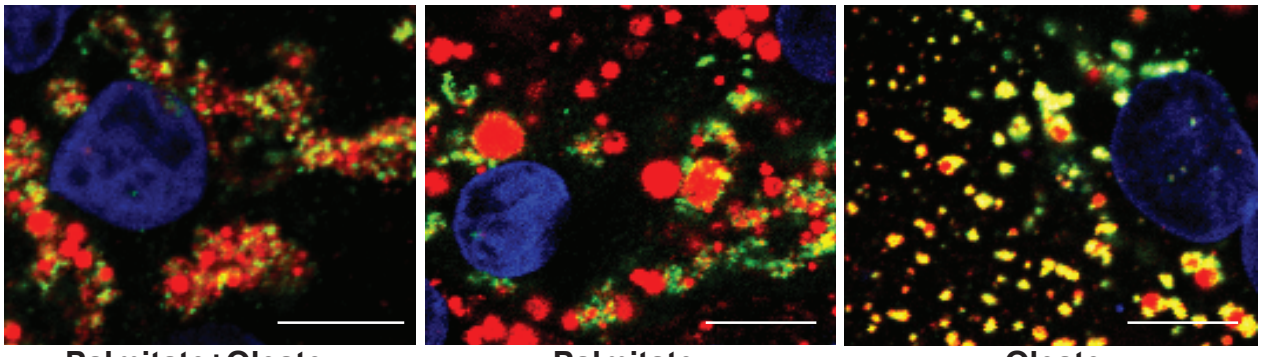


Variant A | Variant B | Δ22-28 | N1-28

Green: HSD17B13
Red: Lipid Droplets
Blue: Nuclei

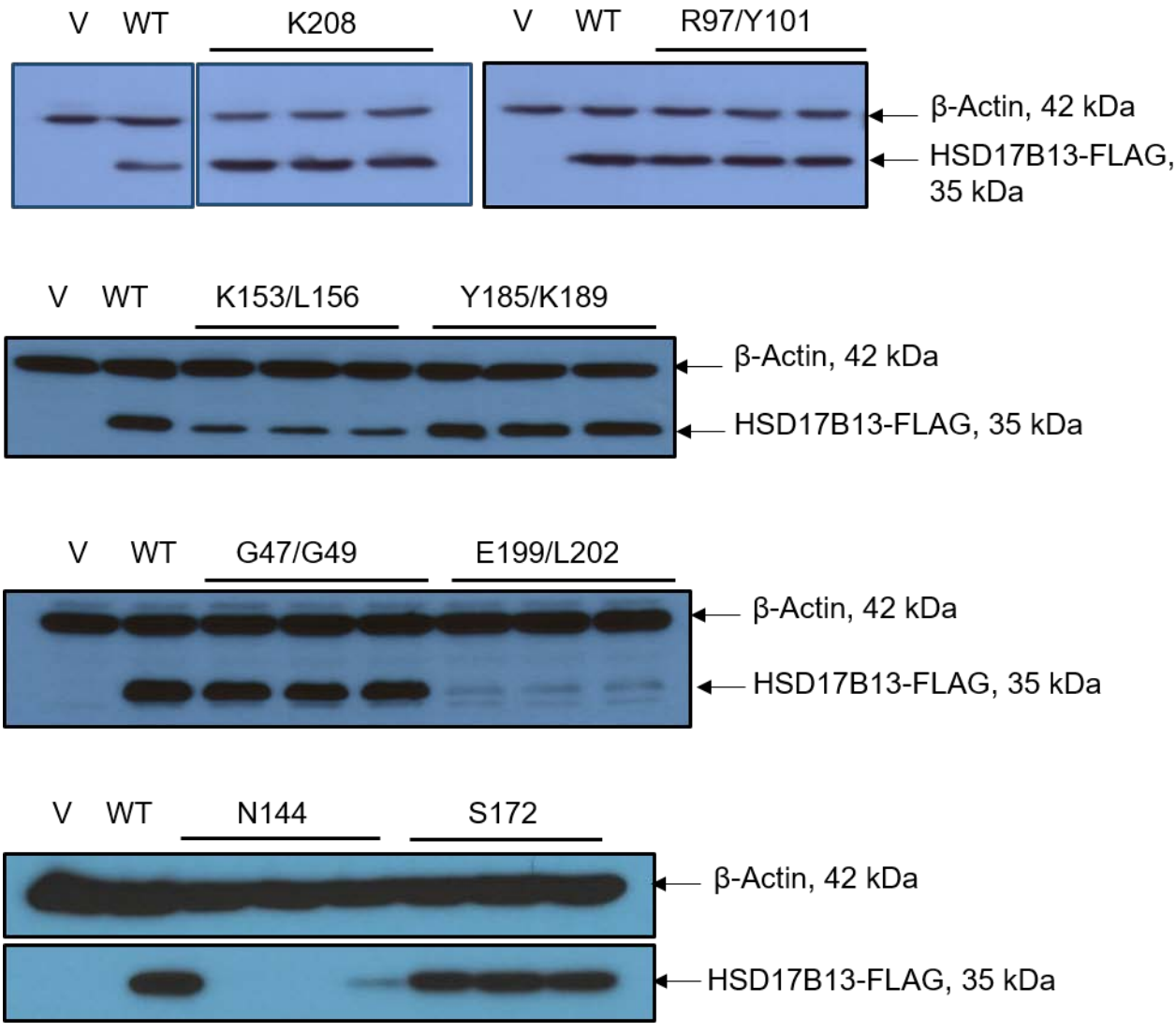
Supplemental Figure S4. Critical domains driving HSD17B13 to target lipid droplets in primary human hepatocytes (PHH). PHH cells (Gibco) were plated on poly-lysine coated 4 well chamber slides and transiently transfected with HSD17B13 plasmids (wild type/variant A, variant B, Δ22-28, and N1-28). Cells were then treated with Fatty acids (200 μM palmitate and 200 μM oleate) to induce LDs. Proteins are C-terminally tagged with GFP, which was used to determine the cellular localization of these proteins (Green). Nuclei are counter stained with Hoechst (Blue), and LDs were stained with LipidTox (Red). Images were analyzed by confocal microscopy. Bar indicates 10 μM.

Supplemental Figure S5



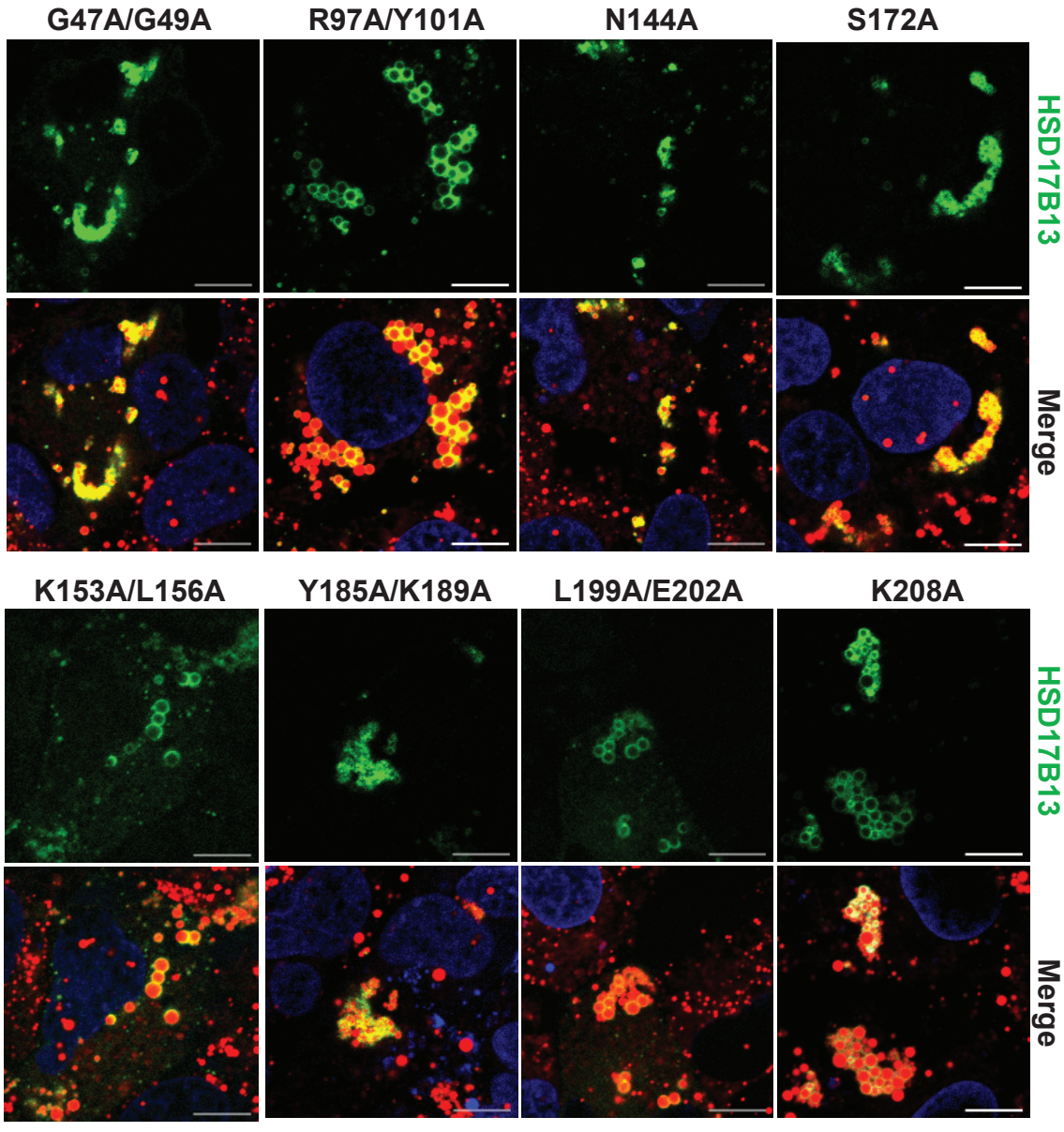
Supplemental Figure S5. HSD17B13 targets lipid droplets induced by different fatty acids. PHH cells (Gibco) were plated on poly-lysine coated 4 well chamber slides and transiently transfected with HSD17B13 plasmids. Cells were then treated with 200 µM palmitate and 200 µM oleate, 200 µM palmitate, or 200 µM oleate to induce LDs. Proteins are C-terminally tagged with GFP, which was used to determine the cellular localization of these proteins (Green). Nuclei are counter stained with Hoechst (Blue), and LDs were stained with LipidTox (Red). Images were analyzed by confocal microscopy. Bar indicates 10 µM.

Supplemental Figure S6



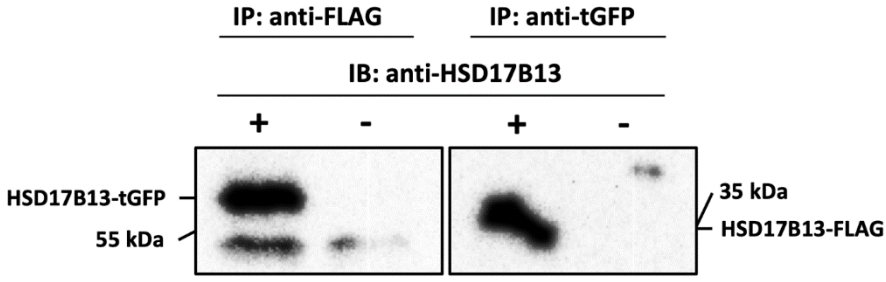
Supplemental Figure S6. Protein expression of HSD17B13 and mutants in HEK293 cells. HEK293 cells were seeded one day before and transiently transfected with HSD17B13 (WT), HSD17B13 mutant, or empty vector (V) plasmids with C-terminus Flag tag. Western blot was performed to determine the protein expression level of HSD17B13 using anti-Flag antibody. β-Actin was used for protein loading control.

Supplemental Figure S7



Supplemental Figure S7. Cellular localization of HSD17B13 mutants. HepG2 cells were transiently transfected with HSD17B13 mutant plasmids and treated with fatty acids to induce LDs. Proteins are C-terminally tagged with GFP, which was used to determine the cellular localization of these proteins (Green). Nuclei are counter stained with Hoechst (Blue), and LDs were stained with LipidTox (Red). Images were analyzed by confocal microscopy. Bar indicates 10 μM.

Supplemental Figure S8



Supplemental Figure S8. Homodimerization of full length variant HSD17B13 when overexpressed in vitro. HepG2 cells stably expressing HSD17B13-tGFP were transfected with HSD17B13-FLAG. Whole cell lysates were processed for immunoprecipitation of either HSD17B13-FLAG or HSD17B13-tGFP with FLAG or turbo GFP monoclonal antibodies bound to protein G magnetic beads. Non-transfected HepG2 lysate served as a negative control. Pulled-down HSD17B13 partners were detected by immunoblot using a polyclonal HSD17B13 antibody (1:2000). "+" indicates immunoprecipitated lysate co-transfected with HSD17B13-FLAG and HSD17B13-tGFP and "-" indicates immunoprecipitated negative control lysate.

Supplemental Table S1-Primers Sequence for Mutagenesis

Name	Sequence	Annealing (°C)
A71-106 dele- Forward	GTGAAGAAAGAAGTGGGTG	60
A71-106 dele- Reverse	CTTATTAATATCCCACAGAACC	
A112-300 dele- Forward	ACGCGTACGCGGCCGCTC	72
A112-300 dele- Reverse	CACTTCTTTCTTCACCTGATTTAGAGAGCGATAGAT CTCTTC	
G47A-G49A- Forward	agccAGGCAGACTACTTATGAATTTG	61
G47A-G49A- Reverse	attgCATGCCCAGCTCCAGTAA	
N144A- Forward	ATTTGAGGTCgcCATCCTAGGACATTTTTG	58
N144A- Reverse	GTCTTGGTAATCTCTTCATC	
S172A- Forward	CACAGTGGCTgCAGTGTGCGG	68
S172A- Reverse	ACGATGTGGCCATGATTTCTCTC	
Y185A-K189A- Forward	cagcgcaTTTGCCGCTGTTGGCTTT	61
Y185A-K189A- Reverse	gaacaagcTGGGATGAGGTAAGGAATC	
R97A-Y101A- Forward	gatcgctCGCTCTCTAAATCAGGTG	60
R97A-Y101A- Reverse	tcttctgcGTTGCTGCAGTCTACCAC	
K153A-L156A-Forward	cttgctCCATCGATGATGGAGAGAAATC	61
K153A-L156A-Reverse	tgctgcTGTGATCCAAAAATGTCCTAG	
L199A-E202A- Forward	tcagcaCTTCAGGCCTTGGGAAAAAC	62
L199A-E202A- Reverse	tgtcgcACCTCTGTGAAAGCCAAC	
K208A- Forward	GGCCTTGGGAgcaACTGGTATCAAAAC	62
K208A- Reverse	TGAAGTTCTGATGTCAGACC	
N1-21 Forward	ACGCGTACGCGGCCGCTC	72
N1-21 Reverse	CGACTCCAAGTAGGAGTAGATGATGGTGATCAG	
N1-28 Forward	ACGCGTACGCGGCCGCTC	72
N1-28 Reverse	AGGAATGAAAAACTTCACCAACGACTCCAAGTAGG	
Delta 22-28 Forward	CAGAGGAGAAAATCTGTGG	60
Delta 22-28 Reverse	CGACTCCAAGTAGGAGTAG	
Delta 36-68 Forward	AATAAGCGCGGTGTGGAG	62
Delta 36-68 Reverse	AGCCACAGATTTTCTCCTC	
Delta 69-84 Forward	GTCACTGCGCATGCGTAT	64
Delta 69-84 Reverse	AATATCCCACAGAACCAATATGC	
Delta 85-93 Forward	TGCAGCAACAGAGAAGAG	62
Delta 85-93 Reverse	GCCTAGTTTTCGGCACTC	
Delta 94-106 Reverse	GTCTACCACATACGCATG	61
DeltaAA4-16 Forward	TCCTACTTGGAGTCGTTG	62

DeltaAA4-16 Reverse	GATGTTCATGGTGGCAGC

Lowercase letters indicate mutagenesis sites