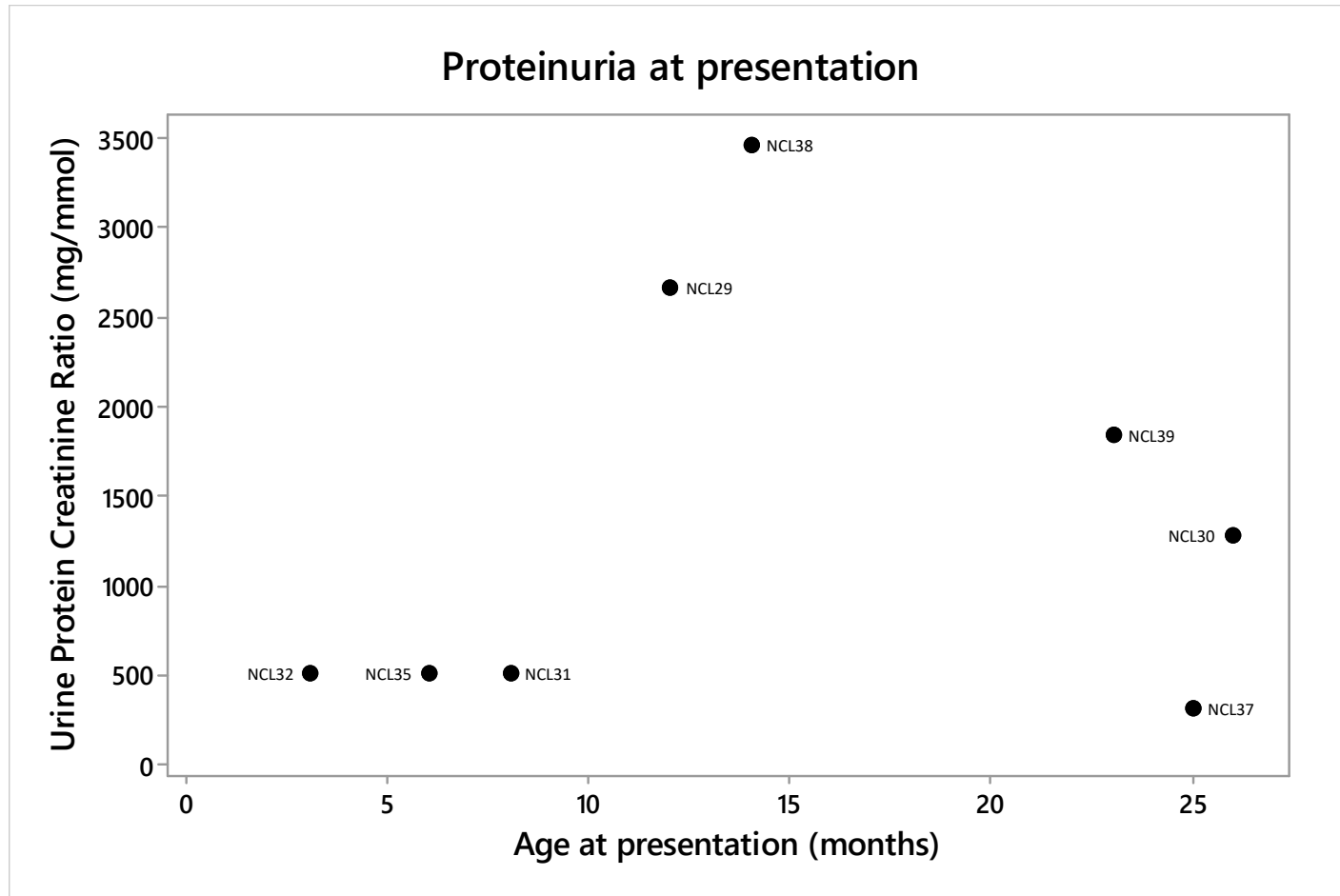
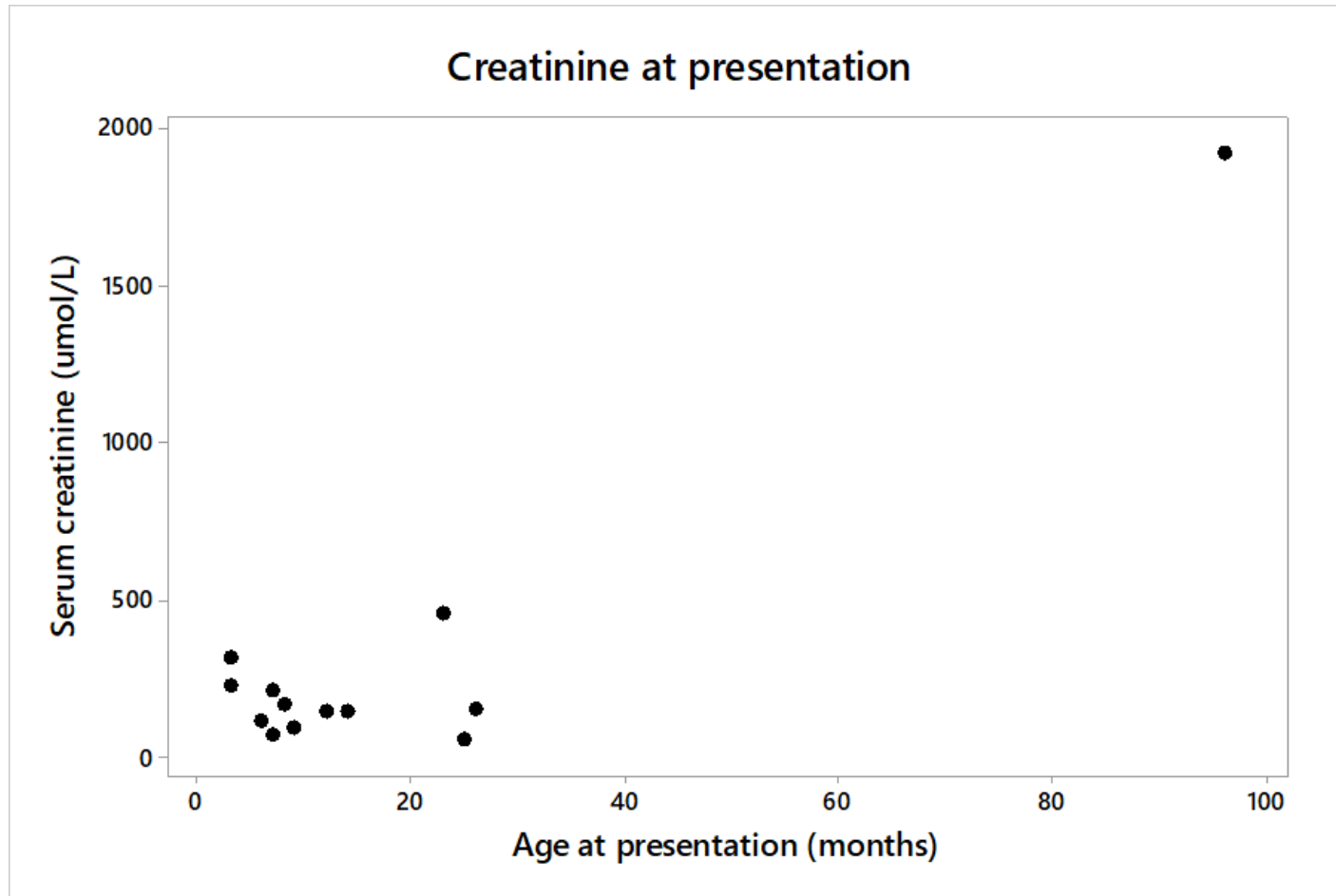


# Supplemental Figure 1: Individual value plots for laboratory findings

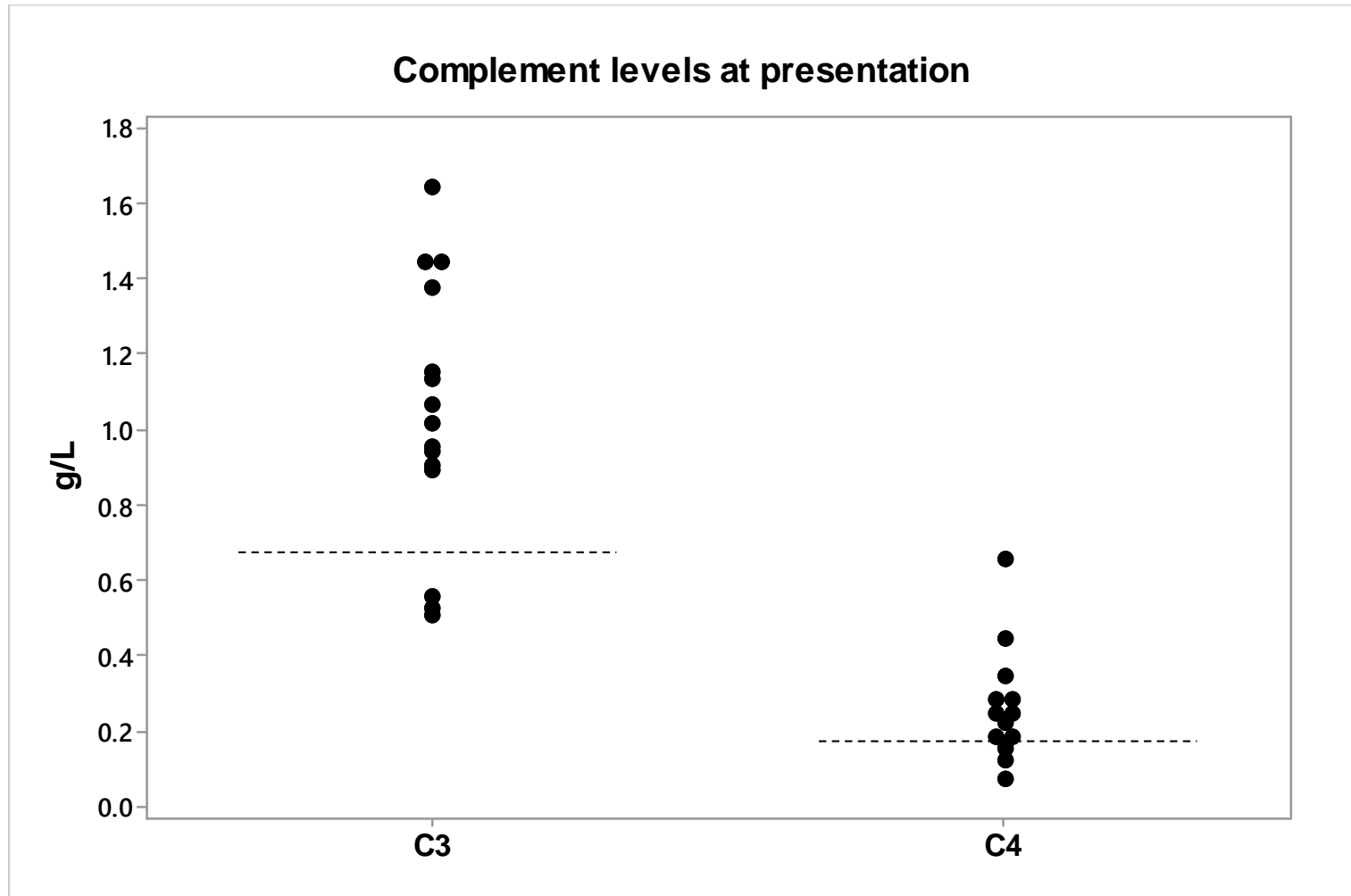
## A. Proteinuria at presentation



## B. Creatinine at presentation

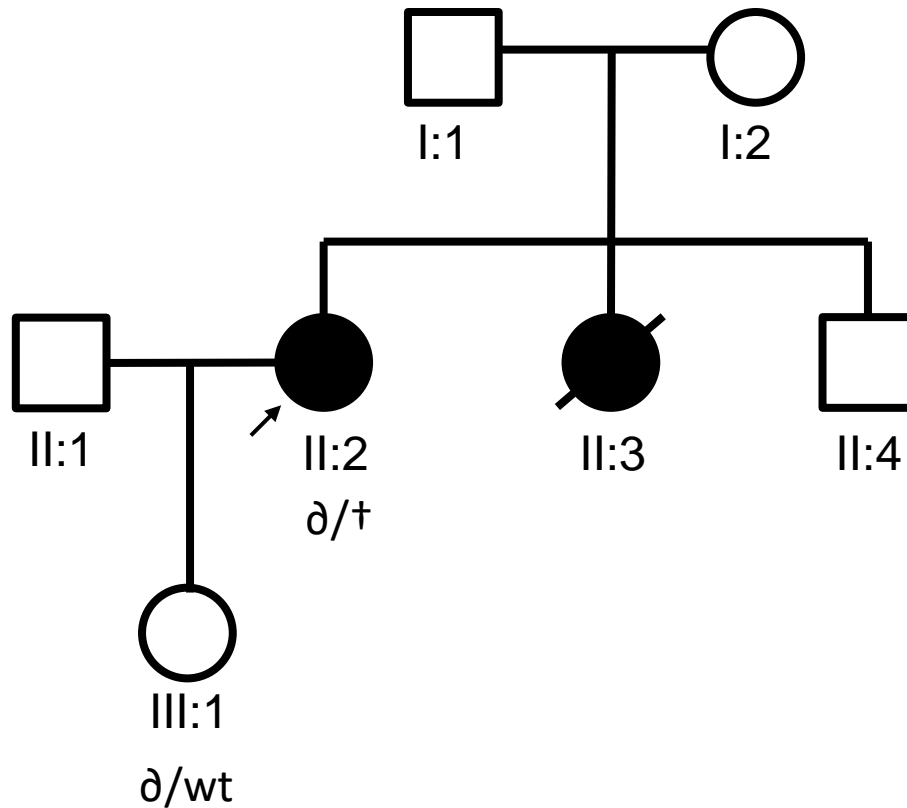


### C. Complement levels at presentation



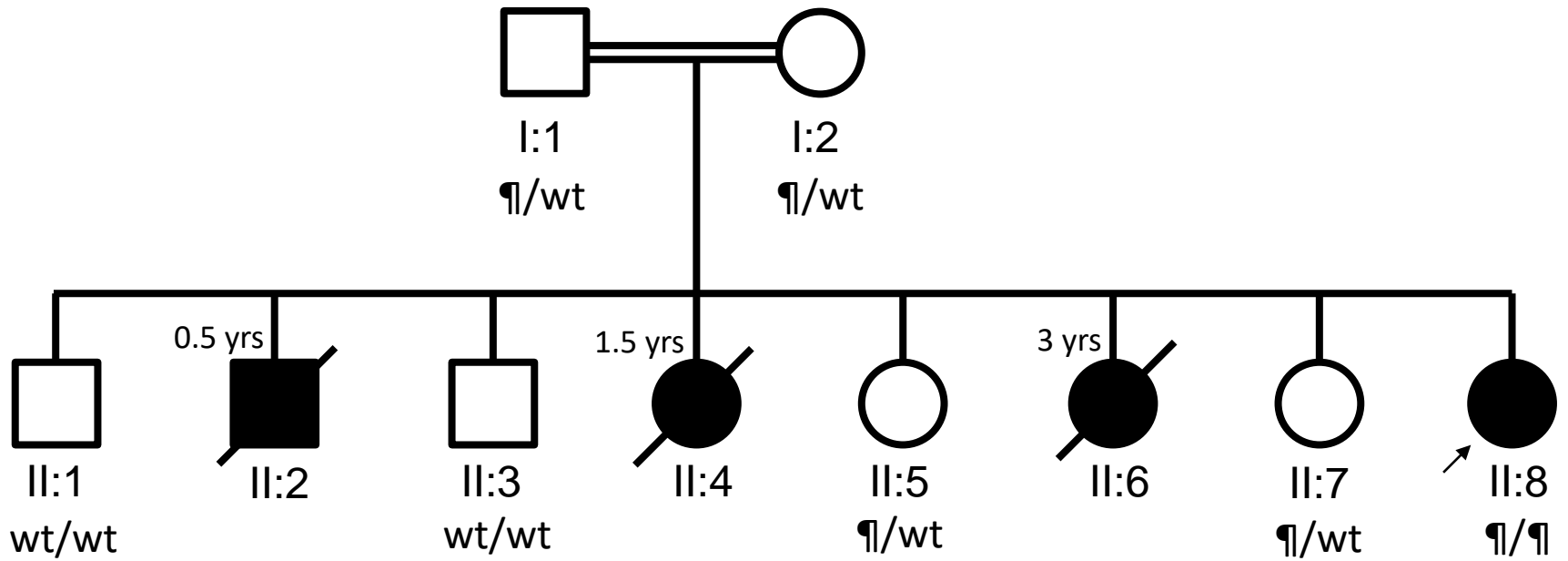
## Supplemental Figure 2: Pedigrees for familial cases

A: NCL25 (II:2)



$\delta$  *DGKE* c.463A>G p.(Arg155Gly)  
 $\dagger$  *DGKE* c.1427T>C p.(Leu476Pro)  
wt: wild type

B: NCL26 (II:8)



■ aHUS

yrs = age (years) when died

$\eta$  *DGKE* c.826delG p.(Val276Phefs\*8)

wt: wild type

## NCL26 pedigree: additional clinical information

### II:2

- Died aged 5 ½ months
- Death certificate recorded “Recurrent haemolytic uraemic syndrome and malignant hypertension”
- Blood tests before his death showed anaemia, kidney failure with a few fragments and burr cells, and thrombocytopenia
- Kidney biopsy showed very large quantities of fibrillary material between the basement membranes and endothelial cells, extending into mesangial areas and also some ‘formed, spherical, electron dense particles’ in places; appearances were felt to be consistent with HUS

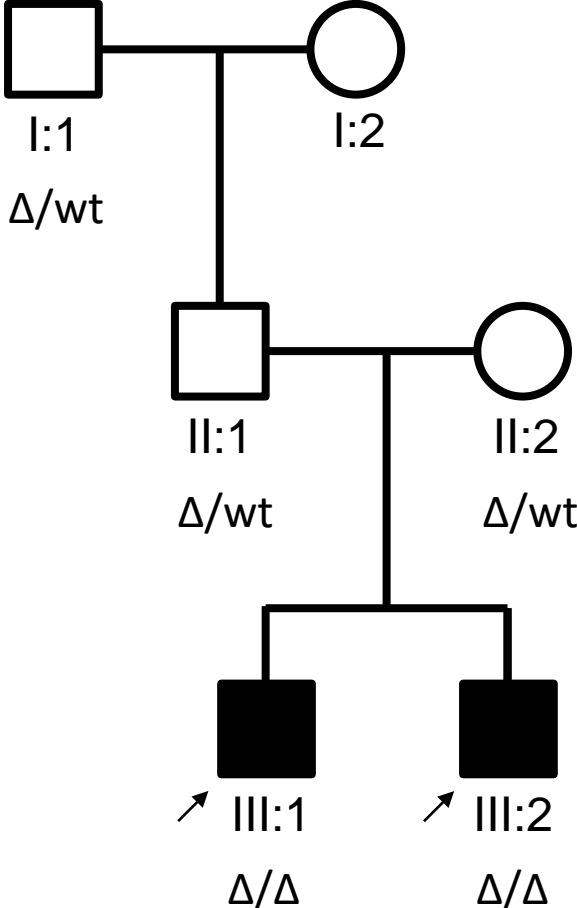
### II:4

- Presented aged 3 ½ months with anaemia
- Presented aged 10 months with oedema and proteinuria; diagnosed with ‘mild HUS’
- Episode of oedema after a measles vaccination
- Presented aged 18 months with febrile episode, oedema and vomiting
  - Haemolysis and AKI
  - Consent was not given for dialysis
  - Deteriorated with convulsions, anuria, and AKI
  - Then had peritoneal dialysis but remained ill with poorly controlled hypertension and heart failure
  - Subsequently had a cardiac arrest and died

### II:6

- Presented aged 7 months with proteinuria and haemolysis
- HUS ‘gradually came under control’
- Relapse aged 3 years: presented with vomiting and diarrhoea
  - HUS was diagnosed 10 days later, complicated by pulmonary oedema, heart failure and *E. coli* urinary tract infection
- Relapse aged 3 years 6 months
  - Consent was not given for dialysis
  - Died 1 month later

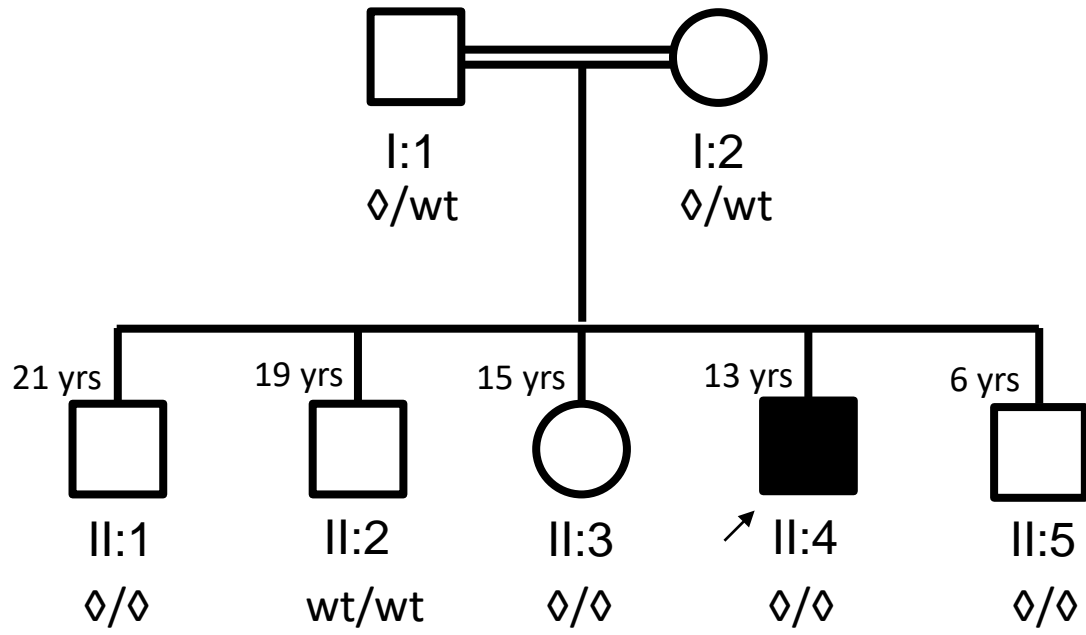
C: NCL27 (III:1) and NCL29 (III:2)



■ aHUS/MPGN

Δ *DGKE* c.1597A>C p.(Thr533Pro)  
wt: wild type

D: NCL34 (II:4)



■ aHUS

yrs = current age (2019)

$\diamond$  *DGKE* c.1647\_1650delAACA p.(T550Mfs\*13)



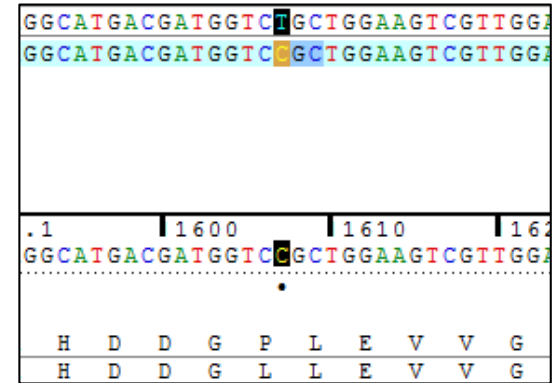
# Supplemental Figure 3: Sanger sequencing traces

A. NCL25. a. *DGKE* exon 2. b. *DGKE* exon 11.

a.



b.



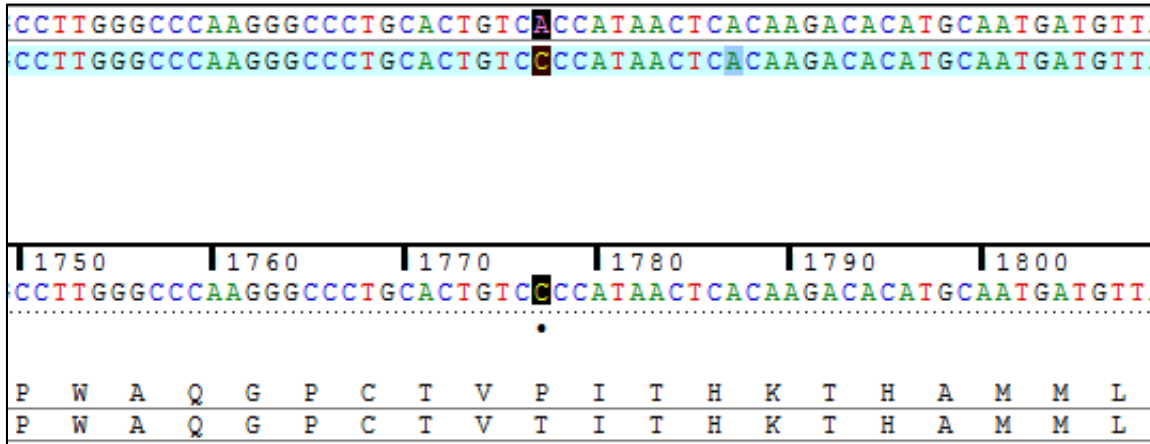
Reference sequence chromatogram

NCL25 chromatogram

*DGKE* c.463A>G p.(R155G)

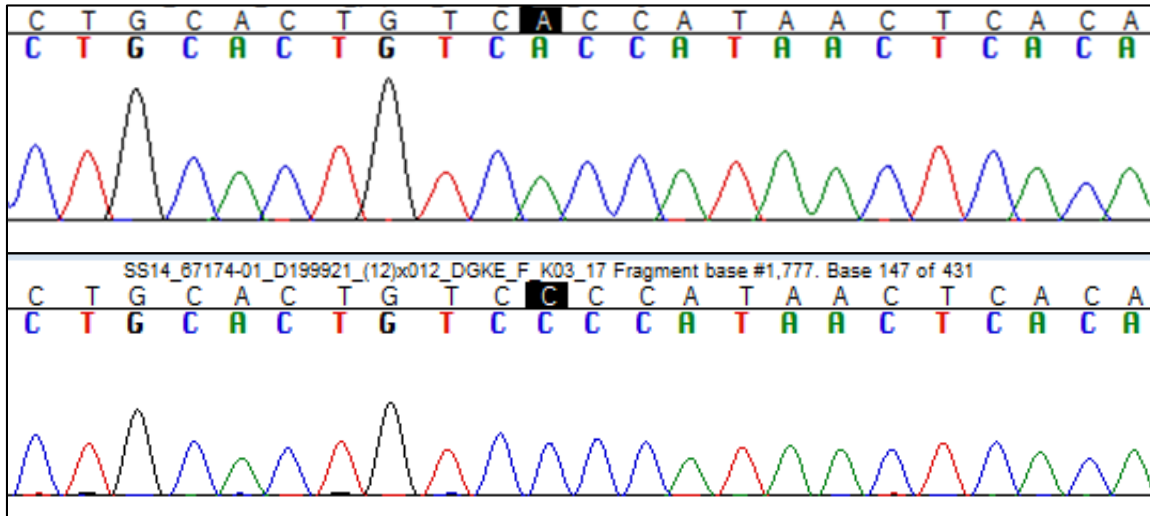
*DGKE* c.1427T>C p.(L476P)

## B. NCL27. *DGKE* exon 12



Reference base sequence  
NCL27 base sequence

NCL27 amino acid sequence  
Reference amino acid sequence

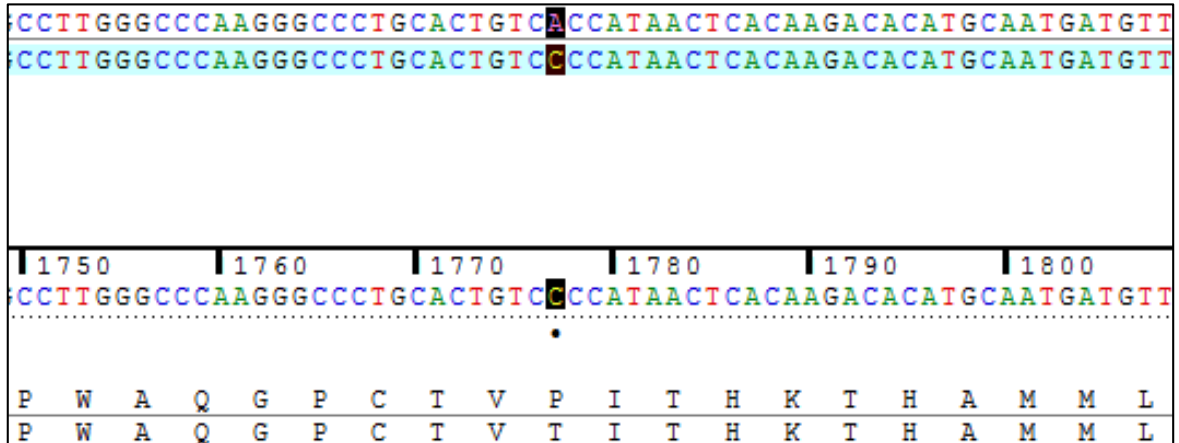


Reference sequence chromatogram

NCL27 chromatogram

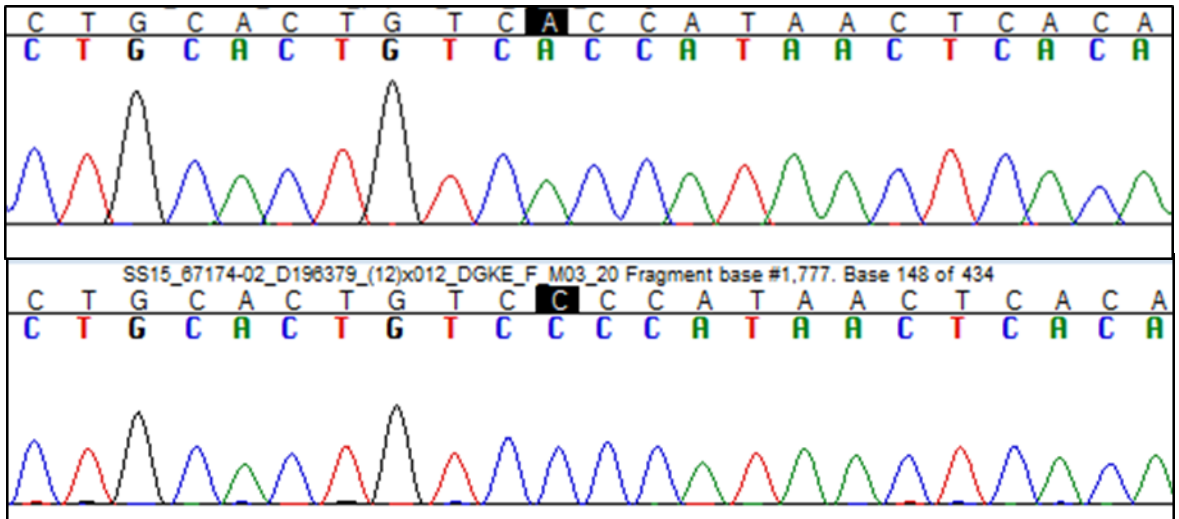
↑  
*DGKE* c.1597A>C p.(T533P)

# C. NCL29. *DGKE* exon 12



Reference base sequence  
 NCL29 base sequence

NCL29 amino acid sequence  
 Reference amino acid sequence



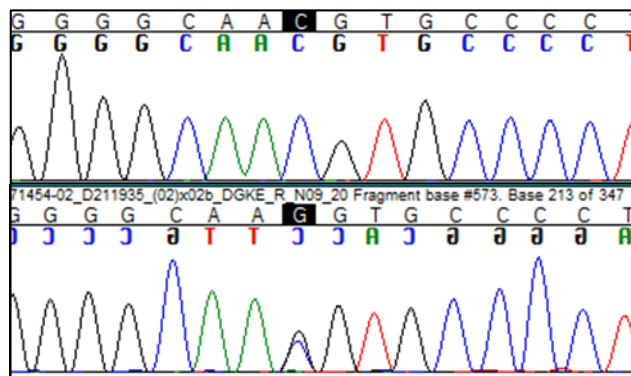
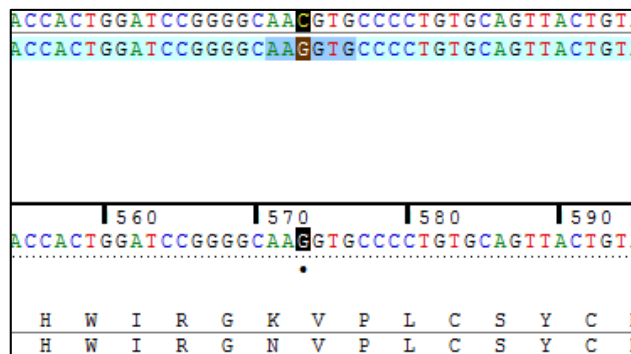
Reference sequence chromatogram

NCL29 chromatogram

↑  
*DGKE* c.1597A>C p.(T533P)

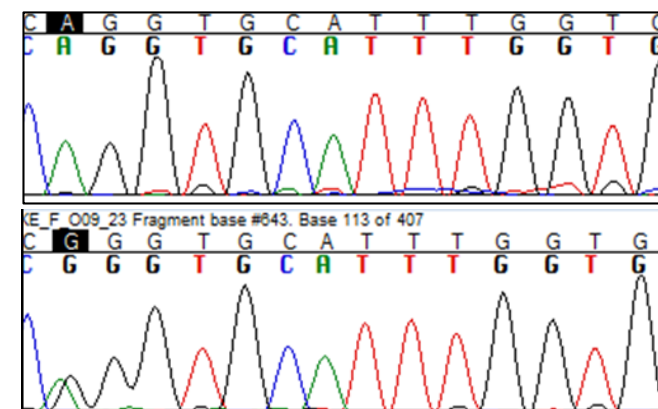
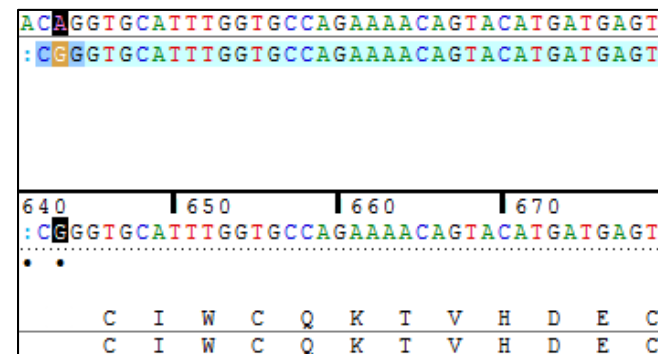
**D. NCL30. a. *DGKE* exon 2. b. *DGKE* exon 3.**

**a.**



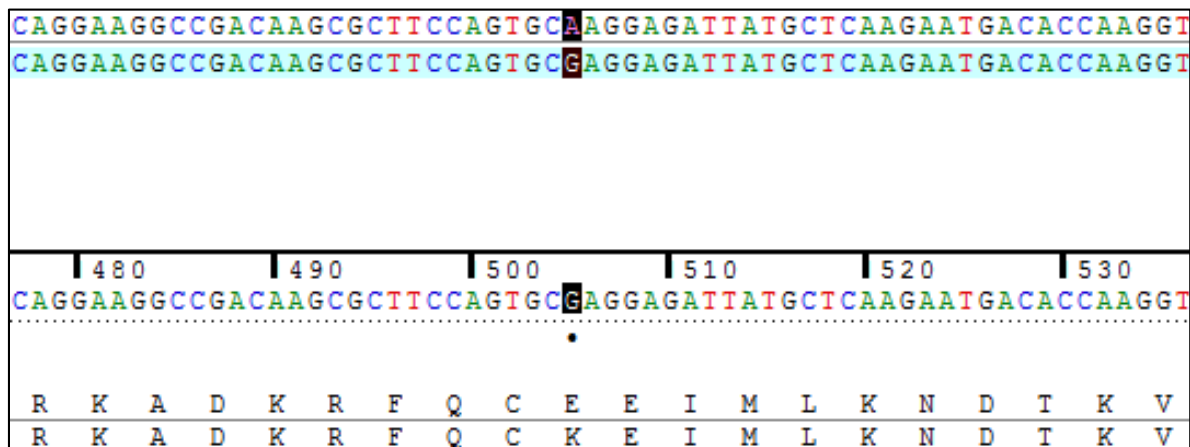
*DGKE* c.393C>G p (N131K)

**b.**



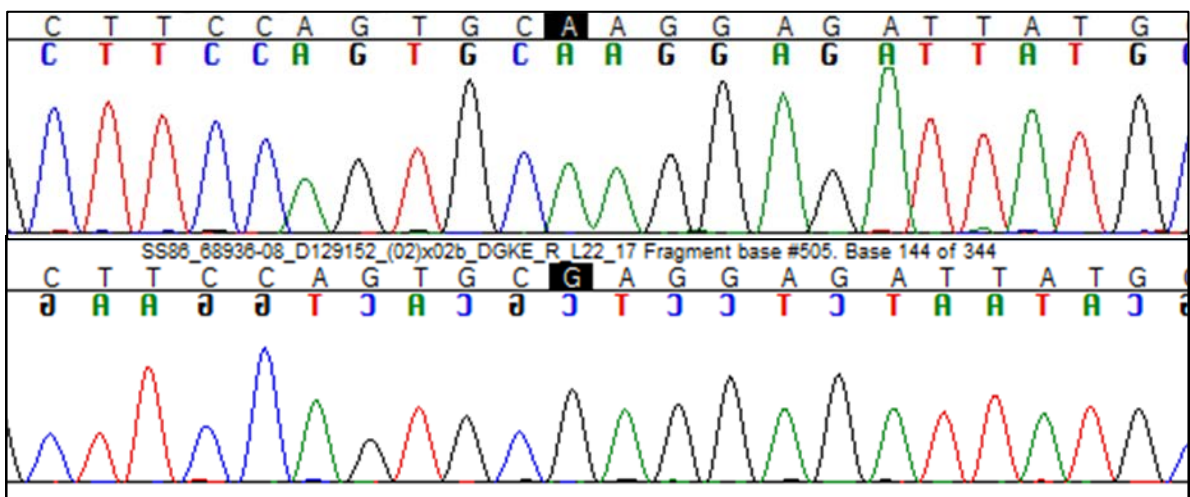
*DGKE* c.465-2A>G

# E. NCL31. *DGKE* exon 2



Reference base sequence  
NCL31 base sequence

NCL31 amino acid sequence  
Reference amino acid sequence

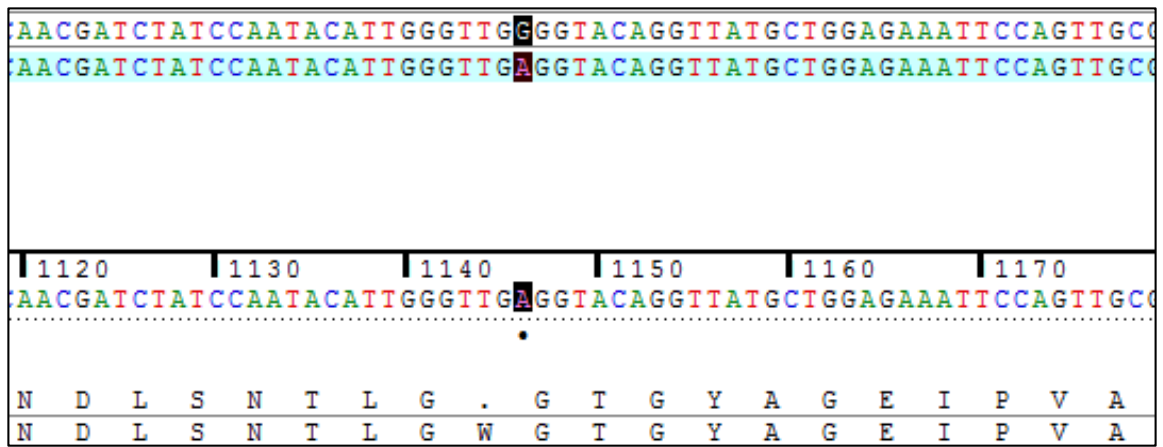


Reference sequence chromatogram

NCL31 chromatogram

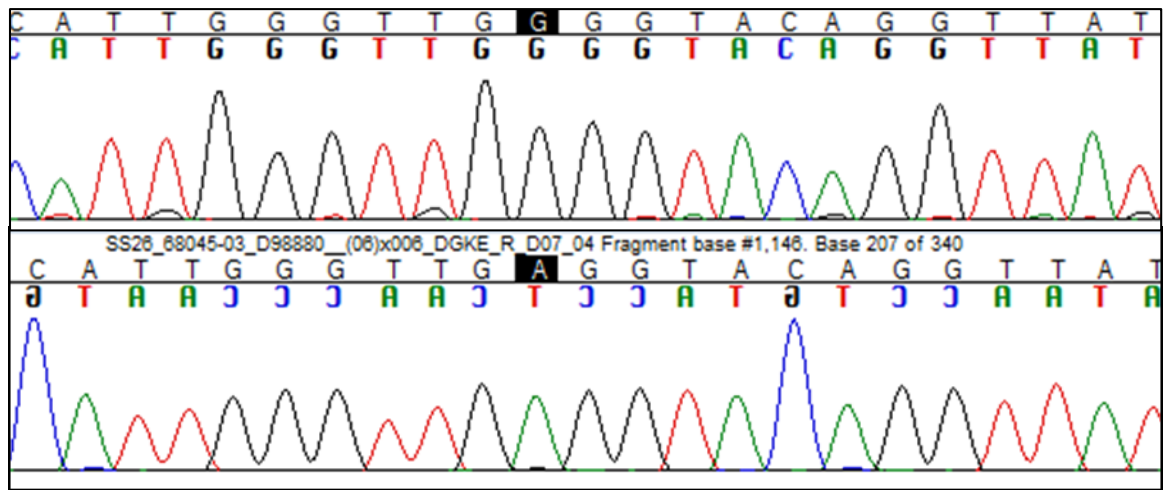
↑  
*DGKE* c.325A>G p.(K109E)

# F. NCL33. *DGKE* exon 6



Reference base sequence  
 NCL33 base sequence

NCL33 amino acid sequence  
 Reference amino acid sequence

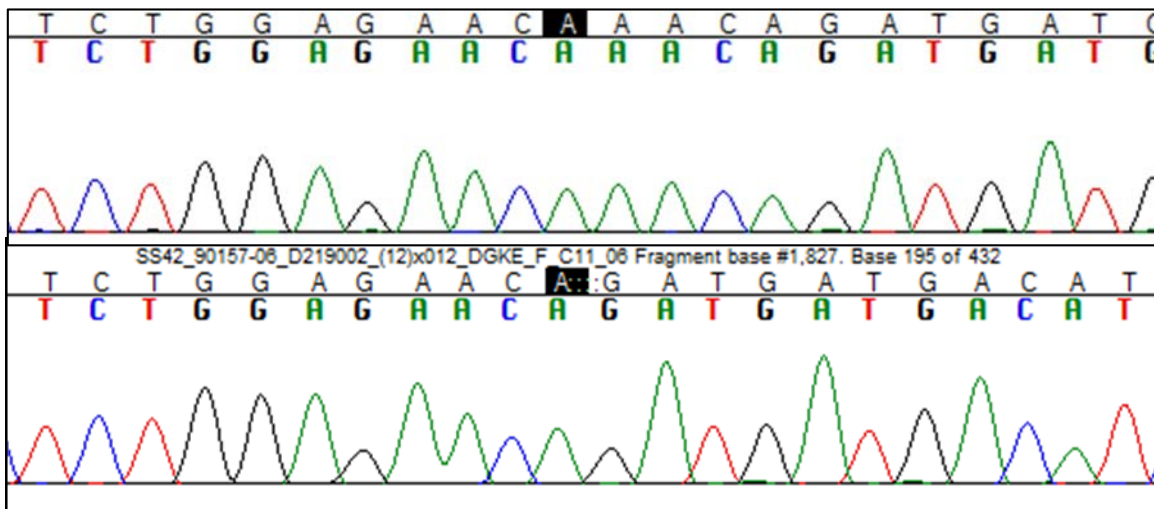


Reference sequence chromatogram

NCL33 chromatogram

↑  
*DGKE* c.966G>A p.(Trp322\*)

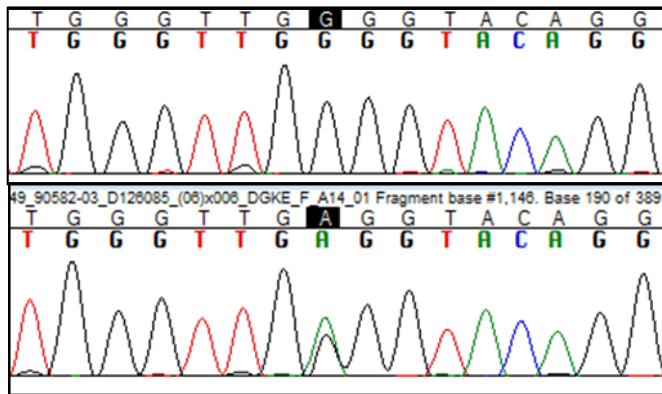
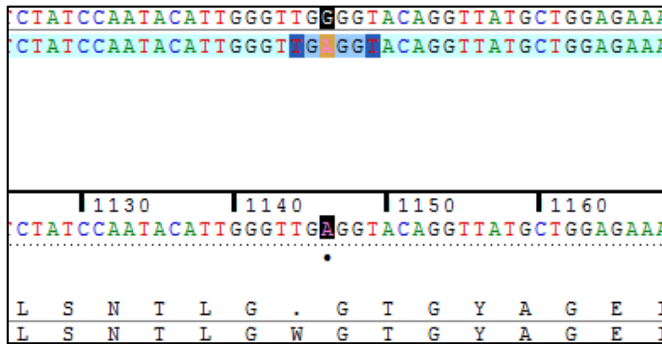
# G. NCL34. *DGKE* exon 12



↑  
*DGKE* c.1647\_1650delAACA p.(T550Mfs\*13)

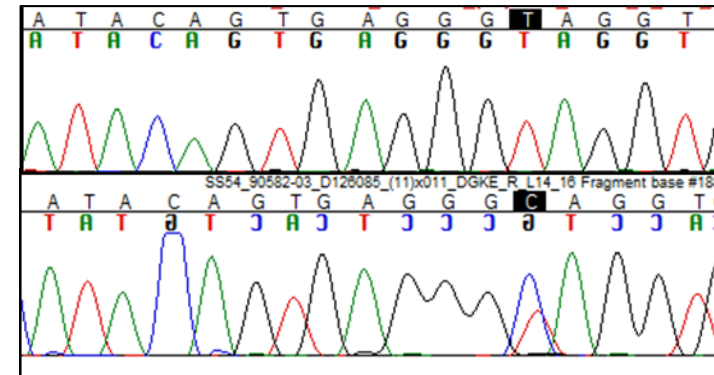
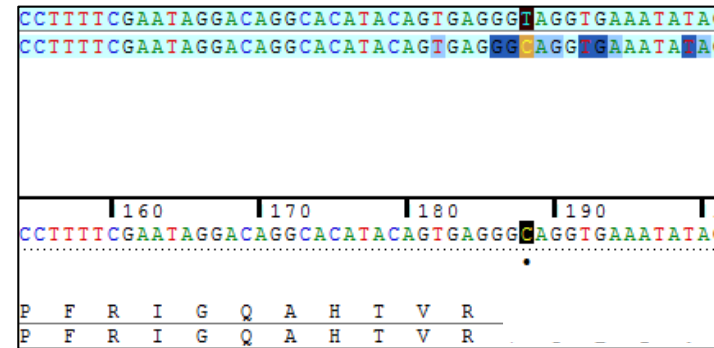
H. NCL36. a. *DGKE* exon 6. b. *DGKE* exon 11.

a.



*DGKE* c.966G>A p.(Trp322\*)

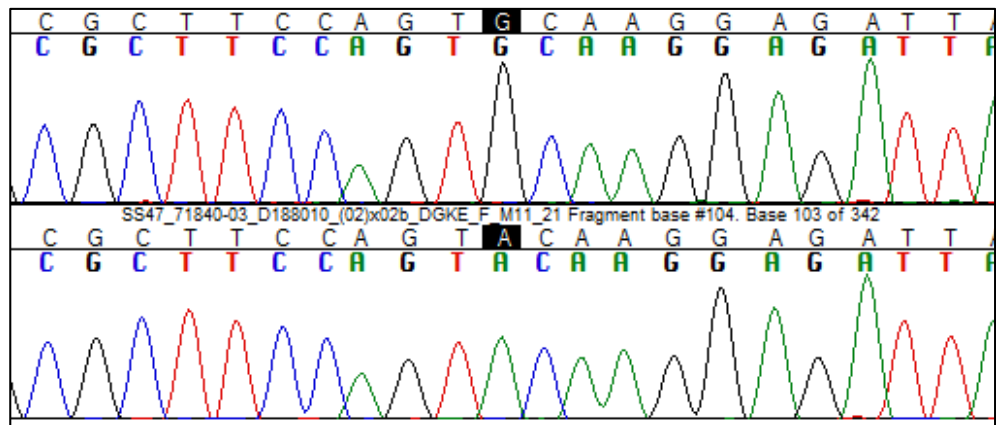
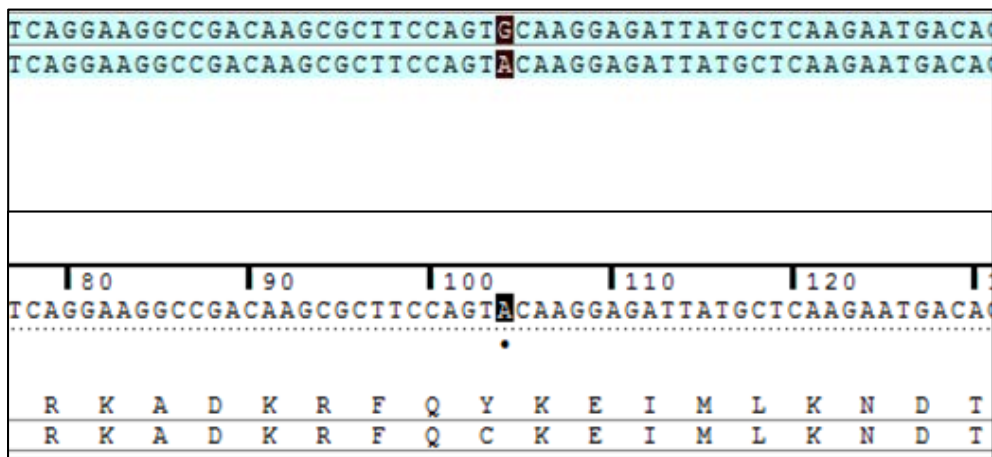
b.



*DGKE* c.1524+2T>C

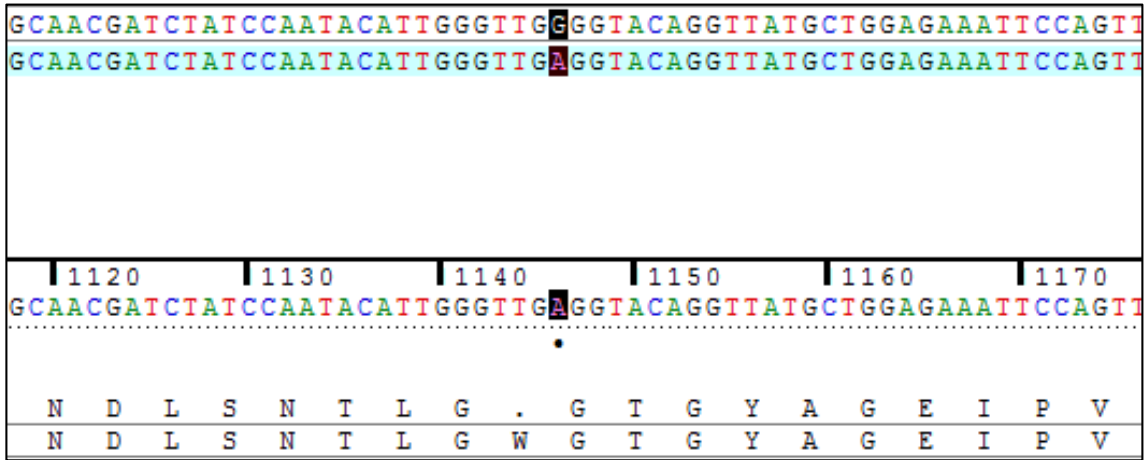


# I. NCL37. *DGKE* exon



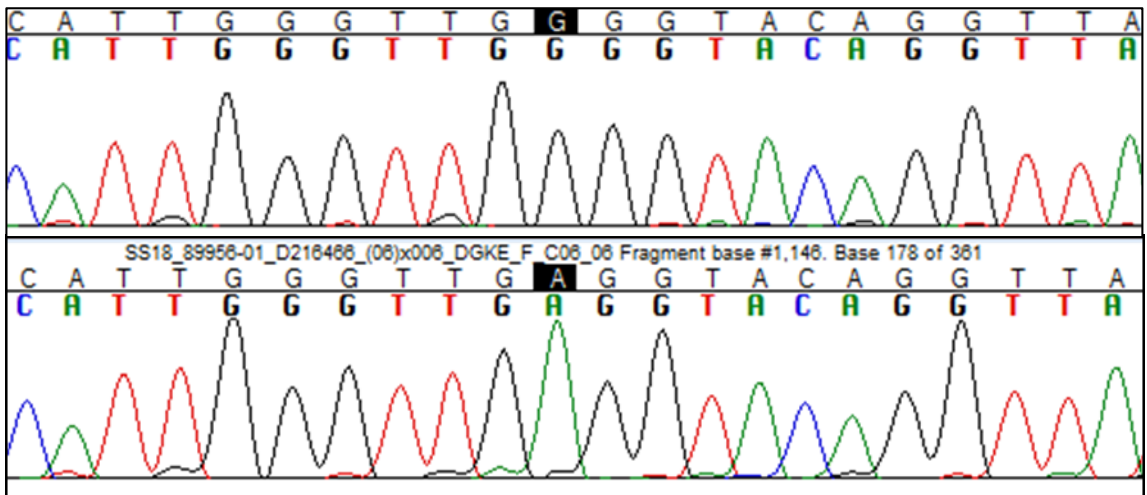
↑  
*DGKE* c.323G>A p.(C108Y)

# J. NCL38. *DGKE* exon 6



Reference base sequence  
 NCL38 base sequence

NCL38 amino acid sequence  
 Reference amino acid sequence



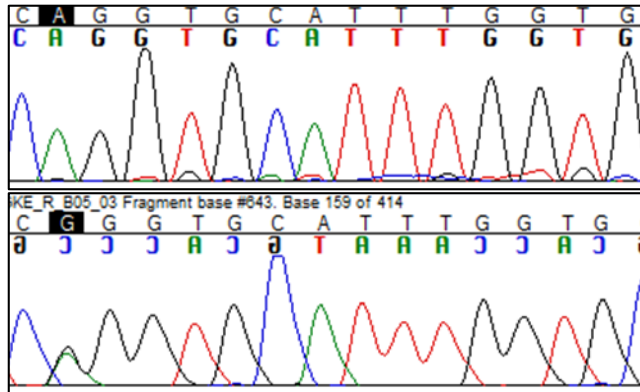
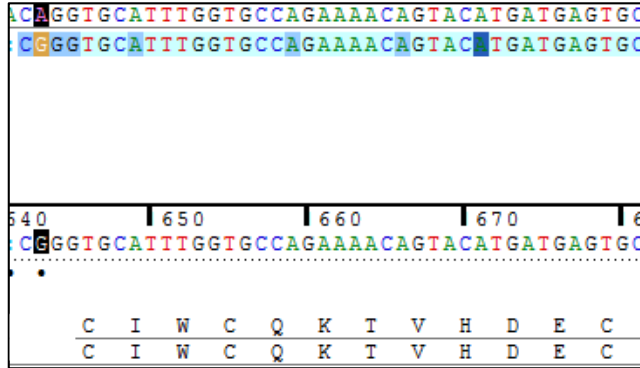
Reference sequence chromatogram

NCL38 chromatogram

DGKE c.966G>A p.(Trp322\*)

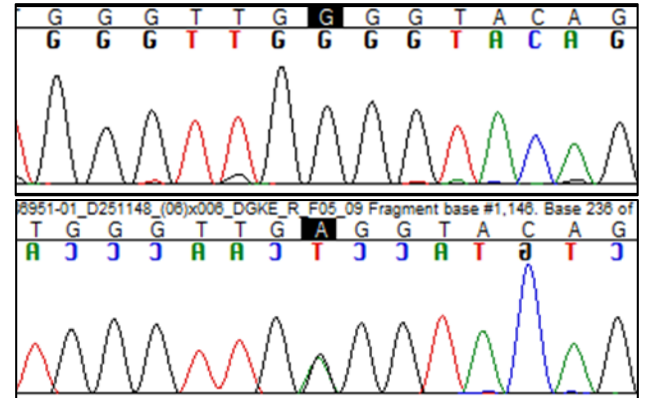
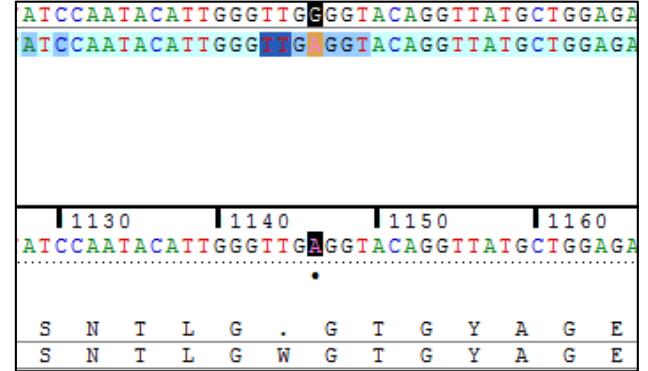
**K. NCL39. a. *DGKE* exon 3. b. *DGKE* exon 6.**

**a.**



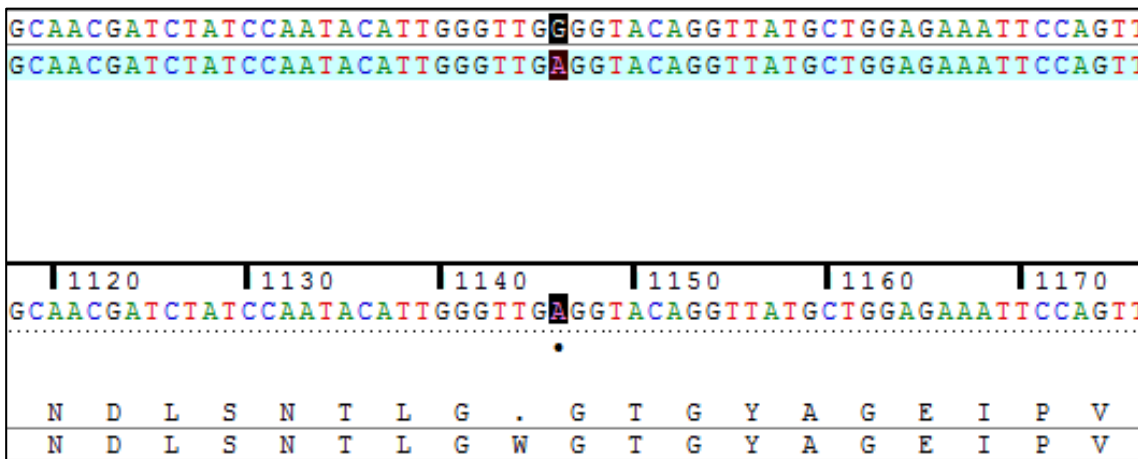
***DGKE* c.465-2A>G**

**b.**



***DGKE* c.966G>A p.(Trp322\*)**

# L. NCL40. *DGKE* exon 6

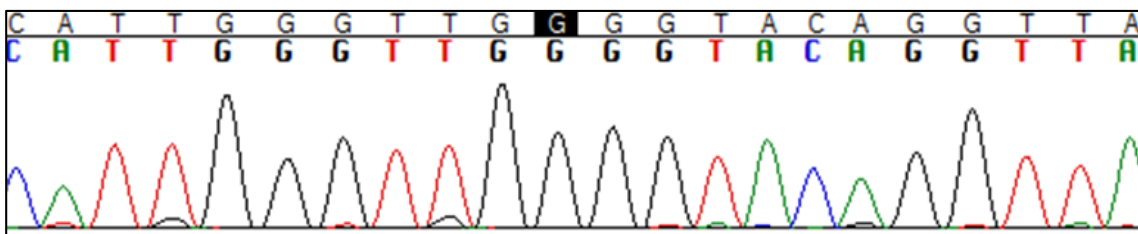


Reference base sequence

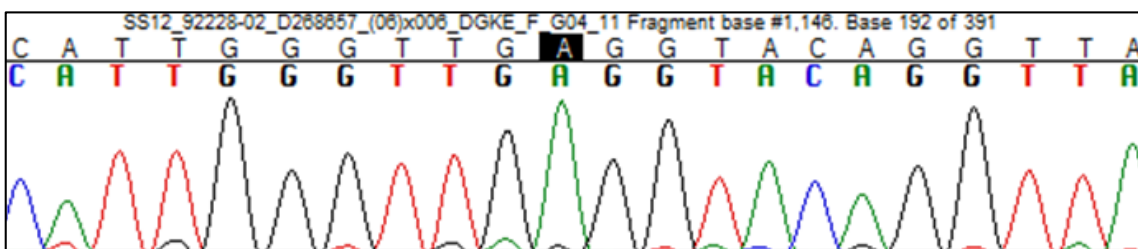
NCL40 base sequence

NCL40 amino acid sequence

Reference amino acid sequence



Reference sequence chromatogram



NCL40 chromatogram

*DGKE* c.966G>A p.(Trp322\*)

# Supplemental Figure 4: DGKE amino acid conservation alignments for newly described missense mutations

## A. NCL25. i. R155G. ii. L476P

i.

Species	Amino acid sequence										
<b>NCL25</b>	K 150	L 151	C 152	D 153	Y 154	<b>G</b> <b>155</b>	C 156	I 157	W 158	C 159	Q 160
Human	K	L	C	D	Y	R	C	I	W	C	Q
Chimp	K	L	C	D	Y	R	C	I	W	C	Q
Gorilla	K	L	C	D	Y	-	-	-	-	-	-
Orangutan	K	L	C	D	Y	R	C	I	W	C	Q
Rhesus Monkey	K	L	C	D	Y	R	C	I	W	C	Q
Mouse	K	L	C	D	Y	R	C	I	W	C	Q
Rat	K	L	C	D	Y	R	C	V	W	C	Q
Rabbit	K	L	C	D	Y	R	C	I	W	C	Q
Dolphin	K	L	C	D	Y	R	C	I	W	C	Q
Dog	K	L	C	D	Y	R	C	I	W	C	Q
Opossum	K	L	C	D	Y	R	C	I	W	C	Q
Platypus	K	L	C	D	Y	R	C	I	W	C	Q
Chicken	K	L	C	D	Y	R	C	V	W	C	Q
Zebrafish	K	L	C	D	Y	R	C	V	W	C	Q

ii.

Species	Amino acid sequence										
<b>NCL25</b>	R 471	H 472	D 473	D 474	G 475	<b>P</b> <b>476</b>	L 477	E 478	V 479	V 480	G 481
Human	R	H	D	D	G	L	L	E	V	V	G
Chimp	R	H	D	D	G	L	L	E	V	V	G
Gorilla	R	H	D	D	G	L	L	E	V	V	G
Orangutan	R	H	D	D	G	L	L	E	V	V	G
Rhesus Monkey	R	H	D	D	G	L	L	E	V	V	G
Mouse	R	H	D	D	G	L	L	E	I	V	G
Rat	R	H	D	D	G	L	L	E	V	V	G
Rabbit	R	H	D	D	G	L	L	E	V	V	G
Dolphin	R	H	D	D	G	L	L	E	V	V	G
Dog	R	H	D	D	G	L	L	E	V	V	G
Opossum	S	H	D	D	G	L	L	E	V	V	G
Platypus	R	H	D	D	G	L	L	E	V	V	G
Chicken	R	H	D	D	G	L	L	E	V	V	G
Zebrafish	<b>R</b>	<b>V</b>	D	D	G	L	L	E	V	V	G

## B. NCL27 and NCL29. T533P

Species	Amino acid sequence										
NCL27 and NCL29	G 528	P 529	C 530	T 531	V 532	<b>P</b> <b>533</b>	I 534	T 535	H 536	K 537	T 538
Human	G	P	C	T	V	T	I	T	H	K	T
Chimp	G	P	C	T	V	T	I	T	H	K	T
Gorilla	G	P	C	T	V	T	I	T	H	K	T
Orangutan	G	P	C	T	V	T	I	T	H	K	T
Rhesus Monkey	G	P	C	T	V	T	I	T	H	K	T
Mouse	G	P	C	T	V	T	I	T	H	K	T
Rat	G	P	C	T	V	T	I	T	H	K	T
Rabbit	G	P	C	T	V	T	I	T	H	K	T
Dolphin	G	P	C	T	V	T	I	T	H	K	T
Dog	G	P	C	T	V	T	I	T	H	K	T
Opossum	G	P	C	I	V	T	I	T	H	K	T
Platypus	G	P	C	T	V	T	I	T	H	K	T
Chicken	G	P	C	T	V	T	I	T	H	K	T
Zebrafish	G	P	C	T	I	T	I	T	H	K	T

## C. NCL28. M1L

Species	Amino acid sequence					
NCL36	L 1	E 2	A 3	E 4	R 5	R 6
Human	M	E	A	E	R	R
Chimp	M	E	A	E	R	R
Gorilla	M	E	A	E	R	R
Orangutan	M	E	A	E	R	R
Rhesus Monkey	M	E	A	E	R	R
Mouse	M	E	G	D	Q	R
Rat	M	E	G	D	Q	R
Rabbit	M	E	G	E	R	R
Dolphin	M	E	G	E	K	R
Dog	M	E	A	E	R	R
Opossum	M	E	G	R	K	Q
Platypus	M	E	R	G	E	P
Chicken	M	L	P	E	A	A
Zebrafish	M	E	E	N	N	E

## D. NCL30. N131K

Species	Amino acid sequence										
NCL30	H 126	W 127	I 128	R 129	G 130	<b>K</b> <b>131</b>	V 132	P 133	L 134	C 135	S 136
Human	H	W	I	R	G	N	V	P	L	C	S
Chimp	H	W	I	R	G	N	V	P	L	C	S
Gorilla	H	W	I	R	G	N	V	P	L	C	S
Orangutan	H	W	I	R	G	N	V	P	L	C	S
Rhesus Monkey	H	W	I	R	G	N	V	P	L	C	S
Mouse	H	W	I	R	G	N	V	P	L	C	S
Rat	H	W	I	R	G	N	V	P	L	C	T
Rabbit	H	W	I	R	G	N	V	P	L	C	S
Dolphin	H	W	I	R	G	N	V	P	L	C	S
Dog	H	W	I	R	G	N	V	P	L	C	S
Opossum	H	W	I	R	G	N	V	P	L	C	S
Platypus	R	W	I	R	G	N	V	P	L	C	S
Chicken	H	W	I	R	G	N	V	P	L	C	S
Zebrafish	Q	W	V	K	G	N	V	P	L	A	S

## E. NCL32. Q79P

Species	Amino acid sequence										
<b>NCL32</b>	C 74	C 75	V 76	C 77	A 78	<b>P</b> 79	H 80	I 81	L 82	Q 83	G 84
Human	C	C	V	C	A	Q	H	I	L	Q	G
Chimp	C	C	V	C	A	Q	H	I	L	Q	G
Gorilla	C	C	V	C	A	Q	H	I	L	Q	G
Orangutan	C	C	V	C	A	Q	H	I	L	Q	G
Rhesus Monkey	C	C	V	C	A	Q	H	I	L	Q	G
Mouse	C	C	V	C	A	Q	H	I	L	Q	G
Rat	C	C	V	C	A	Q	H	I	L	Q	G
Rabbit	C	C	V	C	A	Q	H	I	L	R	G
Dolphin	C	C	V	C	A	Q	H	I	L	Q	G
Dog	C	C	V	C	A	Q	H	I	L	L	G
Opossum	C	C	V	C	T	Q	H	I	L	Q	G
Platypus	C	C	L	C	A	Q	H	I	L	R	G
Chicken	C	C	V	C	A	Q	H	I	L	R	G
Zebrafish	C	C	V	C	C	Q	P	I	L	Q	G

## F. NCL37. C108Y

Species	Amino acid sequence										
<b>NCL37</b>	D 103	K 104	R 105	F 106	Q 107	<b>Y</b> 108	K 109	E 110	I 111	M 112	L 113
Human	D	K	R	F	Q	C	K	E	I	M	L
Chimp	D	K	R	F	Q	C	K	E	I	M	L
Gorilla	D	K	R	F	Q	C	K	E	I	M	L
Orangutan	D	K	R	F	Q	C	K	E	I	M	L
Rhesus Monkey	D	R	R	F	Q	C	K	E	I	M	L
Mouse	D	K	R	F	P	C	K	E	I	M	L
Rat	D	K	R	F	P	C	K	E	I	M	L
Rabbit	D	R	R	F	A	C	K	E	I	V	L
Dolphin	D	K	R	F	H	C	K	E	I	M	L
Dog	D	K	R	F	P	C	K	E	I	M	L
Opossum	D	R	Q	F	Q	C	K	E	I	M	L
Platypus	D	R	R	F	P	C	K	E	I	M	L
Chicken	D	Q	H	F	L	C	K	E	I	V	M
Zebrafish	D	R	I	L	S	C	K	E	I	M	T

**Supplemental Table 1: *In silico* analysis of newly described missense *DGKE* mutations**

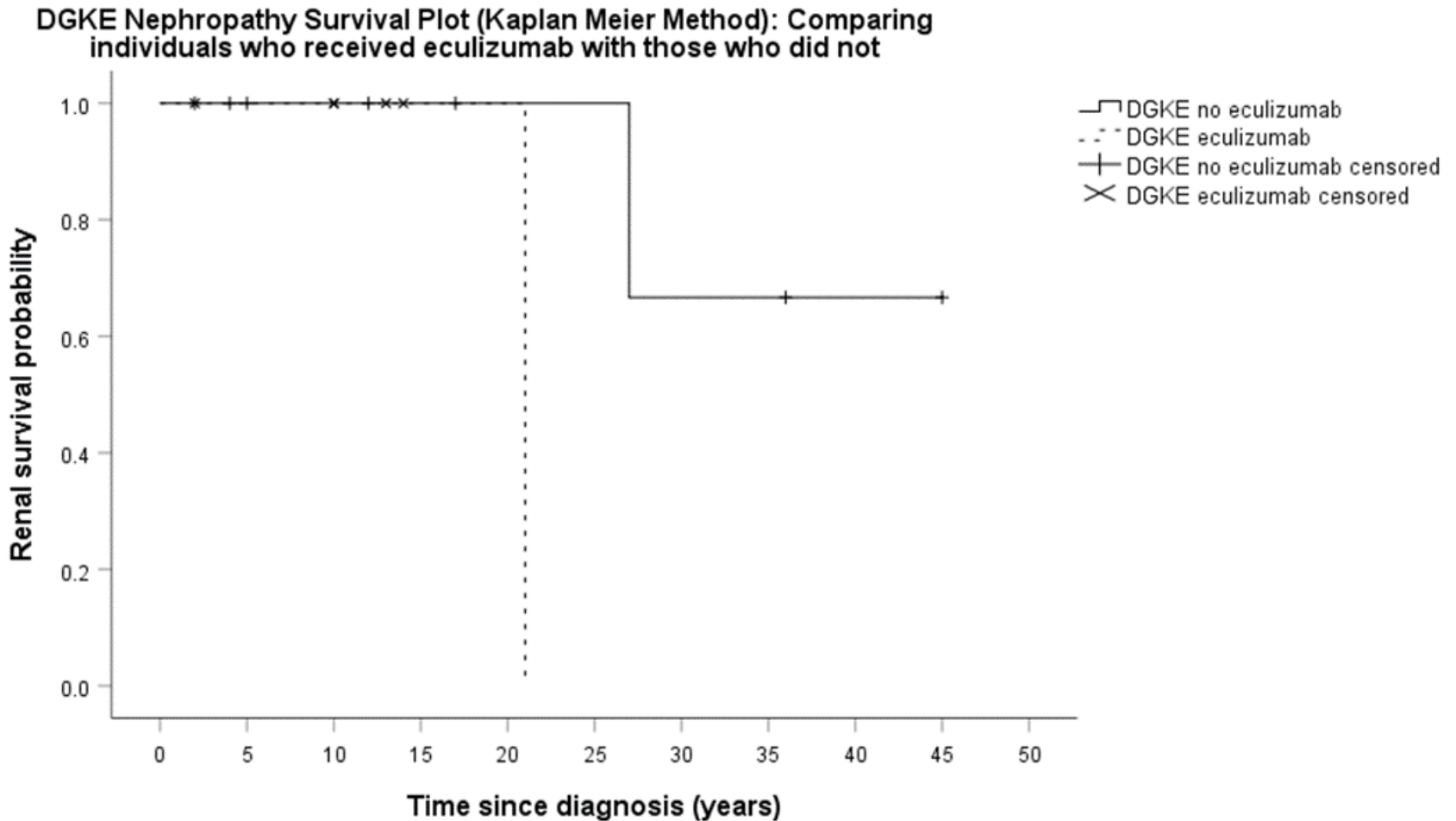
Patient	Inheritance	DGKE variant	ESP frequencies	Align GVGD	SIFT	MutationTaster
<b>NCL25</b>	Compound heterozygous	c.463A>G p.(Arg155Gly)¶	N/A	Class C65 (GV: 0.00 - GD: 125.13)	Deleterious (score: 0)	Disease causing (p-value: 1)
		c.1427T>C p.(Leu476Pro)¶	N/A	Class C45 (GV: 14.30 - GD: 86.59)	Deleterious (score: 0.01)	Disease causing (p-value: 1)
<b>NCL26</b>	Homozygous	c.826delG p.(Val276Phefs*8)¶	N/A	NA: frameshift		
<b>NCL27</b>	Homozygous	c.1597A>C p.(Thr533Pro)¶	N/A	Class C0 (GV: 121.10 - GD: 28.88)	Tolerated (score: 0.23)	Disease causing (p-value: 1)
<b>NCL28</b>	Homozygous	c.1A>T p.(Met1Leu)¶	N/A	NA: start loss		
<b>NCL29</b>	Homozygous	c.1597A>C p.(Thr533Pro) ¶	N/A	Class C0 (GV: 121.10 - GD: 28.88)	Tolerated (score: 0.23)	Disease causing (p-value: 1)
<b>NCL30</b>	Compound heterozygous	c.393C>G p.(Asn131Lys)¶	N/A	Class C65 (GV: 0.00 - GD: 93.88)	Deleterious (score: 0)	Disease causing (p-value: 1)
		c.465-2A>G ¶	N/A	NA: splice site		
<b>NCL32</b>	Homozygous	c.236A>C p.(Gln79Pro)¶	N/A	Class C0 (GV: 261.33 - GD: 0.00)	Tolerated (score: 0.14)	Disease causing (p-value: 1)
<b>NCL34</b>	Homozygous	c.1647_1650delAACA p.(Thr550Metfs*13)¶	N/A	NA: frameshift		
<b>NCL37</b>	Homozygous	c.323G>A p.(Cys108Tyr)¶	N/A	Class C65 (GV: 0.00 - GD: 193.72)	Deleterious (score: 0)	Disease causing (p-value: 1)

**Abbreviations:** DGKE, diacyl glycerol kinase  $\epsilon$ ; ESP, Exome Sequencing Project; GVGD, Grantham Variation Grantham Difference; NA, not applicable; N/A, not available; SIFT, Sorting Tolerant From Intolerant.

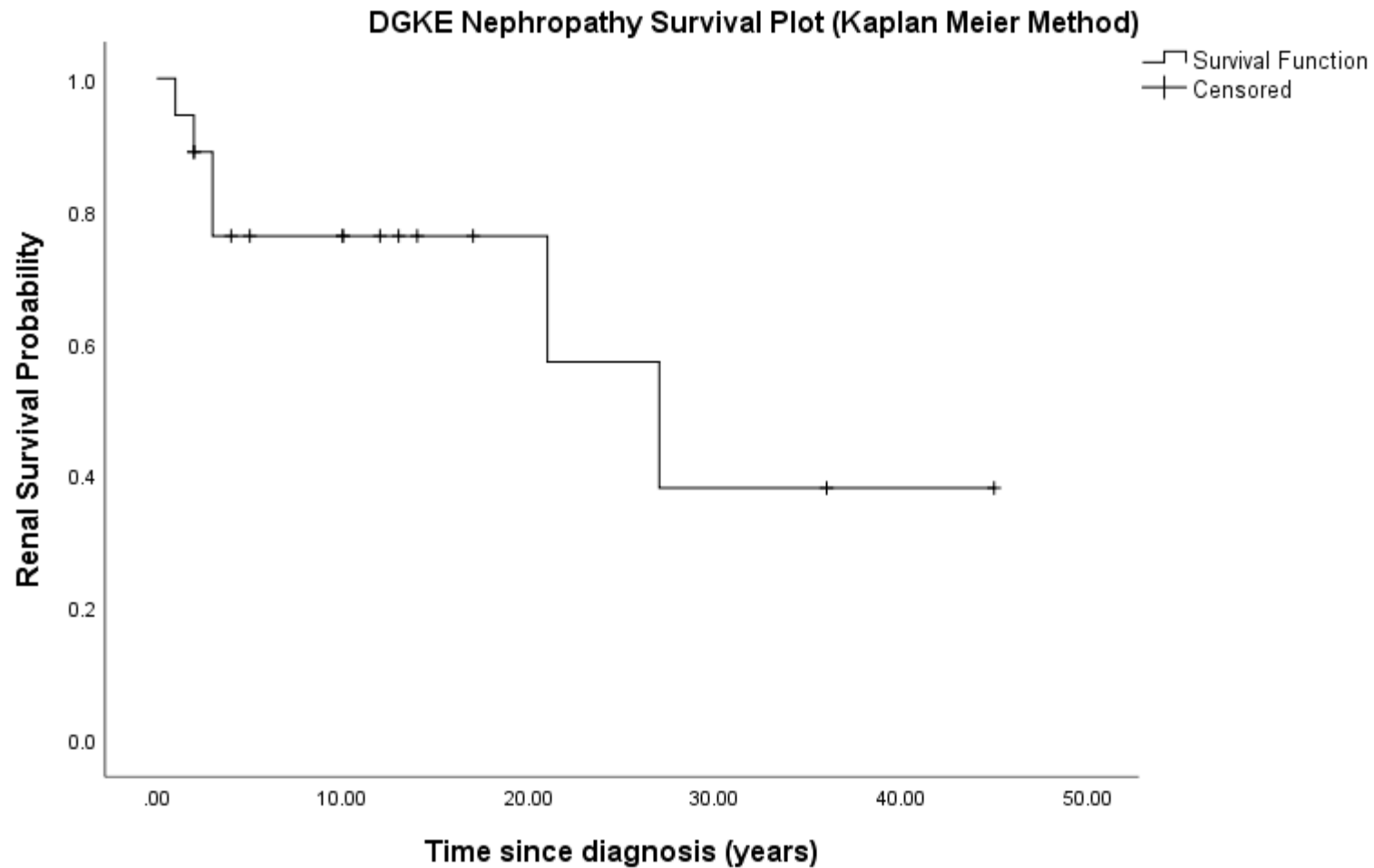


## Supplemental Figure 5: Kaplan Meier Survival Curves

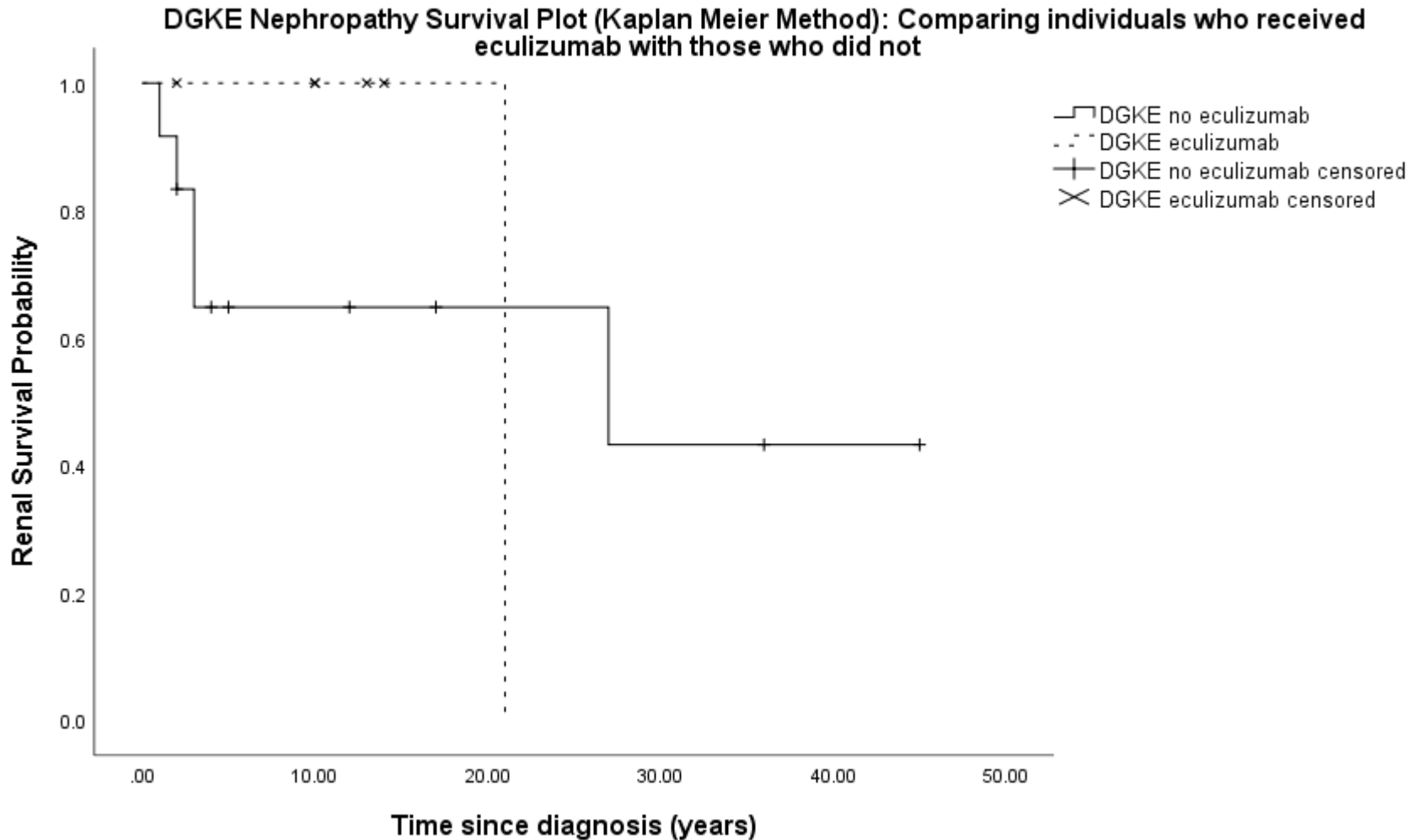
### A. Renal survival comparing individuals who received eculizumab with those who did not



## B. Renal and patient survival, including individuals for whom no genetic analysis was possible

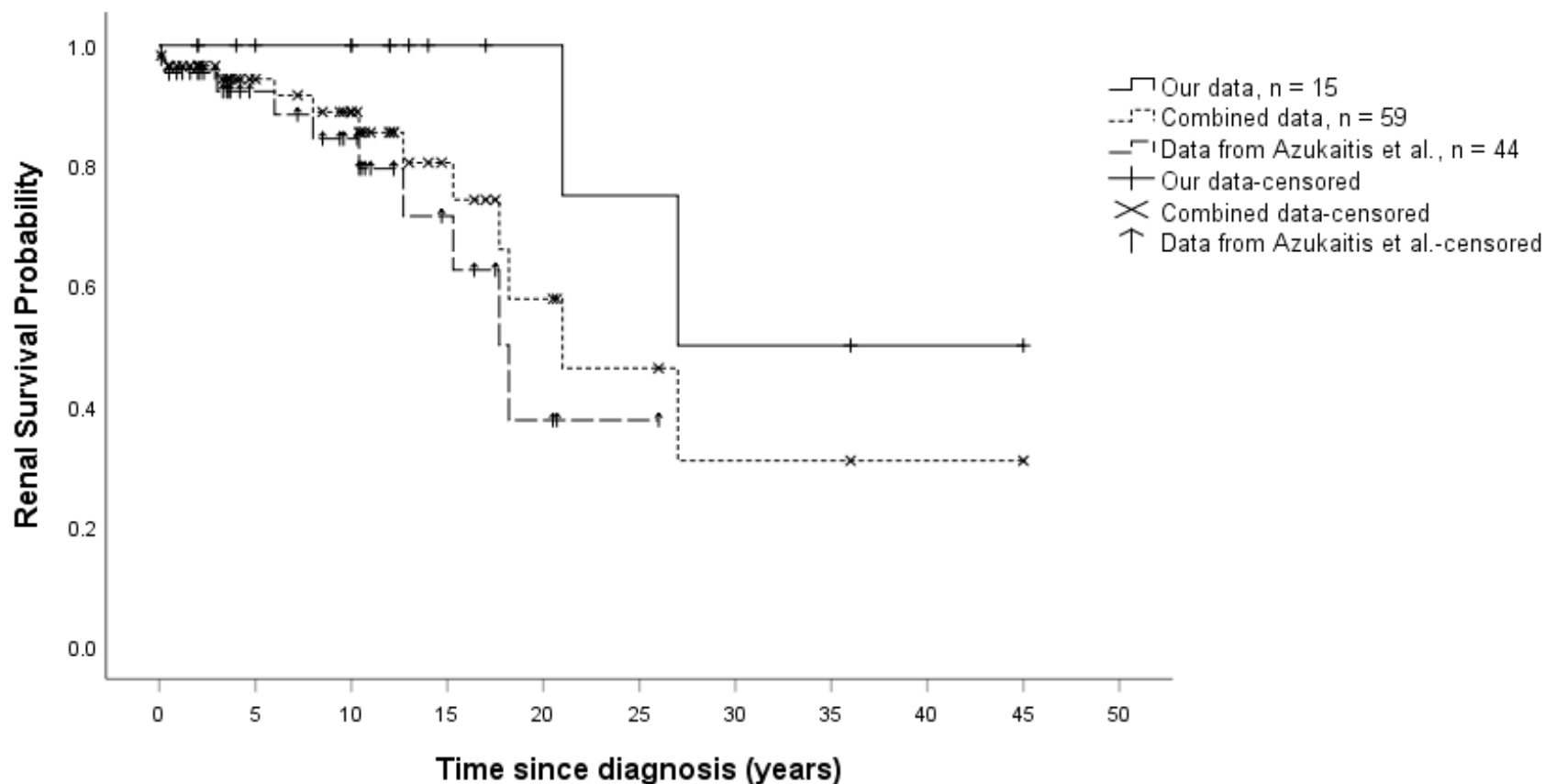


### C. Renal and patient survival, including individuals for whom no genetic analysis was possible, comparing individuals who received eculizumab with those who did not



## D. Renal survival, incorporating data published by Azukaitis et al., for all published DGKA nephropathy cases

DGKE Nephropathy Survival Plot (Kaplan Meier Method): Combining our data with data previously published by Azukaitis et al. (JASN, 28: 3066-3075, 2017)



# Supplemental Figure 6: Primer design for RNA studies

## A. c.1524+2T>C

CTCCAGCTCGCGGCCCGCGCGCCGGATCGGCGTGCCTGCGGCTGGAGCCTTAAGCGTTTCCCCCGCC  
CGGCTTCATCCCTGCTGGCGGCCAGCGTCGTTCTCCTCCTGCGCGAGGCGGCCAAGGCCTGCTGGCC  
CGGAGCCGCGCCTCCACCCGCGCAGGATCGTCTGGAGAAAGATGGAAGCGGAGAGGCGGCCGCG  
CCGGGCTCGCCCTCCGAGGGCCTGTTTGCAGGACGGGCACCTGATCTTGTGGACGCTGTGCTCGGTCT  
GCTGCCGCTGTTTCATCACCTTCTGGTGTAGCCTCCAGCGGTGCGCGCCGAGCTGCACCGCAGGGACA  
TCTTCCGCAAGAGCAAGCACGGGTGGCGCGACACGGACCTGTTTCCAGCCAGCCACCTACTGCTGCGTG  
TGC GCGCAGCACATTCTGCAGGGCGCCTTCTGCGACTGCTGCGGGCTCCGCGTGGACGAGGGCTGCCT  
CAGGAAGGCCGACAAGCGCTTCCAGTGCAAGGAGATTATGCTCAAGGAATGACACCAAGGTCCTGGACG  
CCATGCCCCACCACTGGATCCGGGGCAACGTGCCCTGTGCAGTTACTGTATGGTTTGCAAGCAGCAG  
TGTGGCTGTCAACCAAGCTTTCGATTACAGGTGCATTTGGTGCCAGAAAACAGTACATGATGAGTG  
CATGAAAAATAGTTTTAAAGAATGAAAAATGTGATTTTGGAGAATTCAAAAACCTAATCATTCCACCAA  
GTTATTTAACATCCATTAATCAGATGCGTAAAGACAAAAAACAGATTATGAAGTGCTAGCCTCTAAG  
CTTGGAAAGCAGTGGACCCATTAATAATCCTGGCCAACTCTCGTAGTGGAACTAATATGGGAGAAGG  
ACTGTTGGGAGAATTTAGGATCTTGTGTAATCCAGTCCAGGTTTTTGTGATGTAACATAAACTCCTCCTA  
TCAAAGCCCTACAACTCTGTACTCTTCTCCATATTATCAGCTCGAGTACTTGTGTTGGAGGGGAT  
GGGACTGTAGGGTGGTCTTGATGCAGTTGATGACATGAAGATTAAGGGACAAGAAAAGTACATTCC  
ACAAGTTGCAGTTTTGCCTCTGGGAACAGGCAACGATCTATCCAATACATTGGGTTGGGGTACAGGTT  
ATGCTGGAGAAATCCAGTTGCGCAGGTTTTGCGAAATGTAATGGAAGCAGATGGAATTAACTAGAT  
CG

exon 1 - 162 bp  
exon 2 - 482 bp  
exon 3 - 160 bp  
exon 4 - 120 bp  
exon 5 - 144 bp  
exon 6 - 158 bp

predict exon 3 del - 318 bp  
WT - 478 bp

DGKE\_RNA\_Ex2-5f gtagcgcgacggccagtgAATGACACCAAGGTCCTGG  
DGKE\_RNA\_Ex2-5r cagggcgcagcgatgacTGTAGGGCTTTGATAGGAGG

## B. c.465-2A>G

BCGGTTTACTTATTCTATGGAACCAAAGATTGTTTAGTGCAAGAATGTAAGATTGAATAAAAAAGTTGAGCTA  
GAACTGGATGGTGAGCGAGTAGCACTGCCAGCTTGGAAAGGTATTATAGTTCTGAACATCGGATACTGGGGCGGT  
GGCTGCAGACTATGGGAAGGGATGGGGGACGAGACTTACCCTCTAGCCAGGCATGACGATGGTCTGCTGGAAGTC  
GTTGGAGTATATGGGTCTTTCCACTGTGCTCAGATTCAAGTAAACTGGCTAATCCTTTTCGAATAGGACAGGCA  
CATACAGTGAGGCTGATTTTGAAGTGCTCCATGATGCCAATGCAGGTGGATGGGGAGCCTTGGGCCCAAGGGCCC  
TGCACGTGCACCATAAACTCACAAGACACATGCAATGATGTTATATTTCTCTGGAGAACAAACAGATGATGACATC  
TCTAGTACTTCCGATCAAGAAGATATAAAGGCGACTGAATAGATGGATGAGGGAGTGAAAACTTTGCATAGAATC  
CTCACGCAAGTAGATACATGTTTCATCCAAAAGTATTAATAGAAATTCTCTATCAGCTATTCAGTCTTAATTCAC  
TAGTAGTATAATGGGTATACATTTTTGTAAATAGCATCCCCAAACCAGCCAGCCTTCAGTTATTTACAAATGTTT  
GTTCTTTTTTTCAGCAAAATACTTCAAATGAATAGTATTAACCTTACAAAAAGTCACAAAACTTACATGAGAGTGA  
AAATTTGTTATGACTGTTTTGAGAGTGGGACTCACTCTGAAGTATGTGCTGTCTCATGTCTTATTTTTGAACCAT  
GCATATGATGGACACACAATGGATGGACACATTATATCTCCAACAAGGTGTGGGTGGAAAGATCAAATTAACCTG  
CTTTTTTGAAGGAAATGATTACTGTCAAACCAGCATGGTTAATTTGTGAGCATCTCC

predicted to lose exon 11 = 112bp or get an extra 4 bases on end of exon 11

Expected product

WT = 428 bp

first 43 bases exon 10 to exon 12 = 215 bp

inclusion of intron 11 = 788 bp

DGKE\_RNA\_Ex9f gtagcgcgacggccagtGGAACCAAAGATTGTTTAGTGC

DGKE\_Ex10\_12f gtagcgcgacggccagtTGGATGGTGAGCGAGTAGC

DGKE\_Ex10\_12r cagggcgcagcgatgacTCATTGCATGTGTCTTGTGAG