Figure S1

A

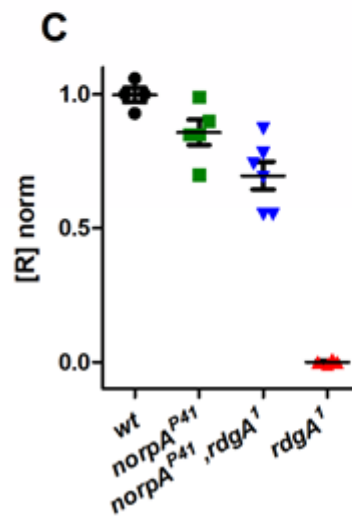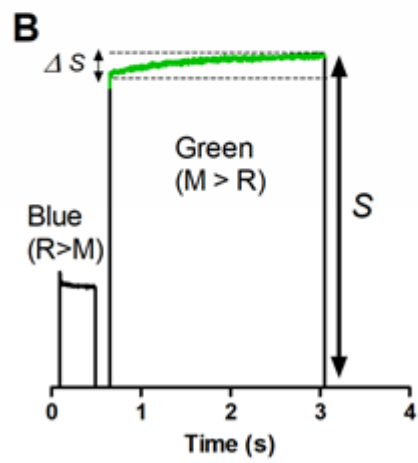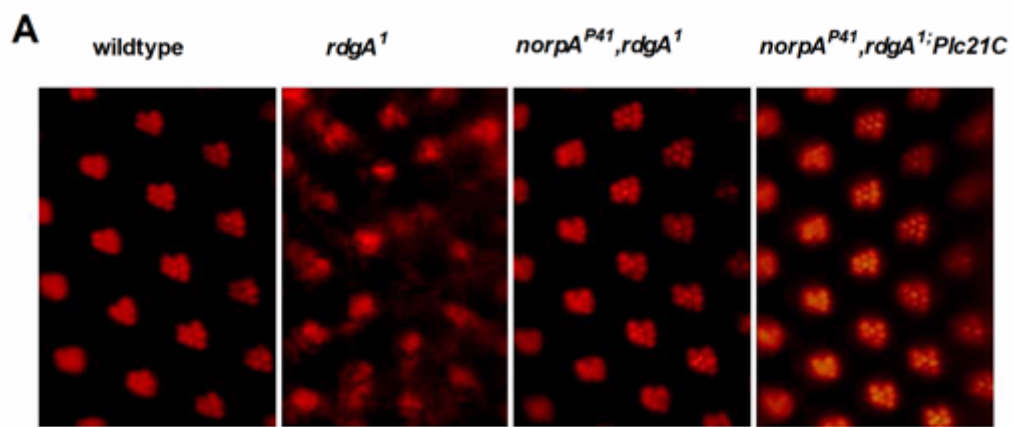| wildtype | *rdgA¹* | *norpA^P41^,rdgA¹* | *norpA^P41^,rdgA¹;Plc21C* |

B

C

Figure S1. Full rescue of *rdgA¹*-induced photoreceptor degeneration by *norpA^P41*. A) Rhabdomeres visualized by optical neutralization from red-eyed wild-type, *rdgA¹* single mutant, *norpA^P41,rdgA¹* and *norpA^P41,rdgA¹;Plc21C^P319* double and treble mutants. Heads of dark-reared young flies were illuminated with antidromic white light under oil immersion. B) Spectrophotometric method for determination of relative rhodopsin content in vivo in white-eyed flies (Randall et al., 2015): first blue light (470 nm) is delivered to convert the majority of (blue-absorbing) rhodopsin to green/yellow absorbing metarhodopsin. Subsequently, green light (540 nm) is delivered to convert M back to R) and the light scattered back out of the retina is measured (S). As M is photoreisomerised, the green light backscattered out of the retina increases with an exponential time course representing the rate of photoreisomerisation (M>R). The relative increase in back-scatter ($\Delta S/S$) is a measure of the rhodopsin content. C) $\Delta S/S$ values normalized to wild-type (*w^1118*) give relative rhodopsin (R) content. There was no detectable increase in backscatter (i.e no detectable rhodopsin) in *rdgA¹* mutants, but levels were near normal in *norpA^P41* and *norpA^P41,rdgA¹*.
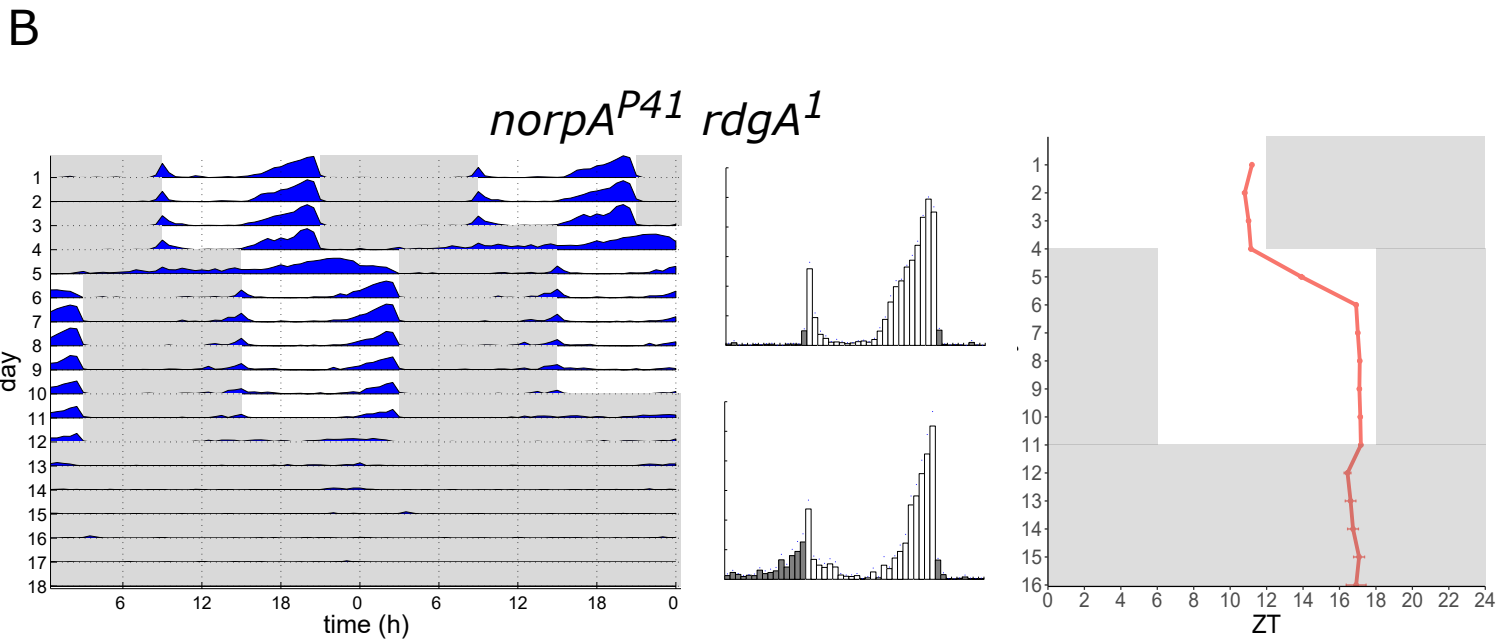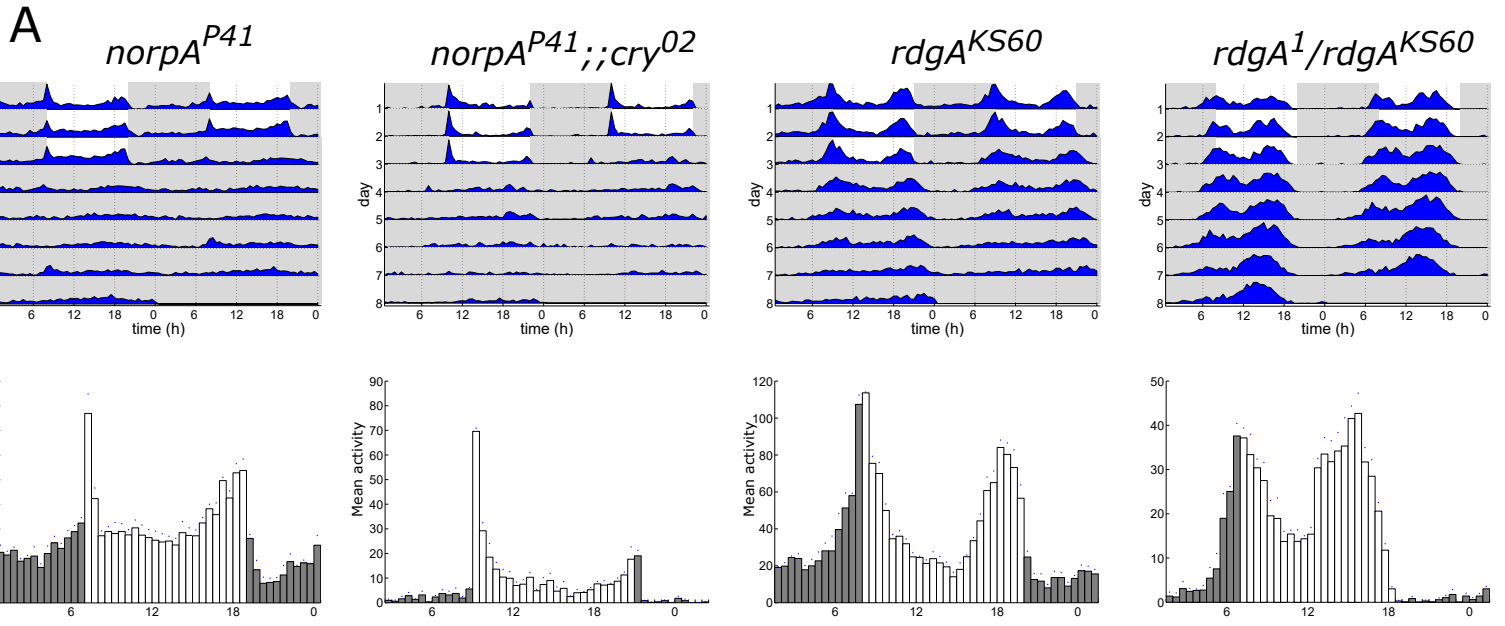
Figure S2

A

B

Figure S2. Control genotypes for resynchronization experiments to 6-hr delayed LD cycles.

(A) Representative actograms (top) and histograms (bottom) of 16 flies of the indicated genotypes subjected to 3 days in LD at 25 °C followed by 5 days in DD at 25°C. (B) Results for *norpA^{P41} rdgA^1* double mutants subjected to similar "jetlag" conditions as in Figure 3, where flies were exposed to 4 days of combined LDTC, followed by a 6 hr delay of the LD cycle at constant 25°C. Average actogram (left), histograms (middle) and phase quantification (right). The histograms represent the first 3 days before the phase shift (top) and the last 3 days after the shift (bottom).
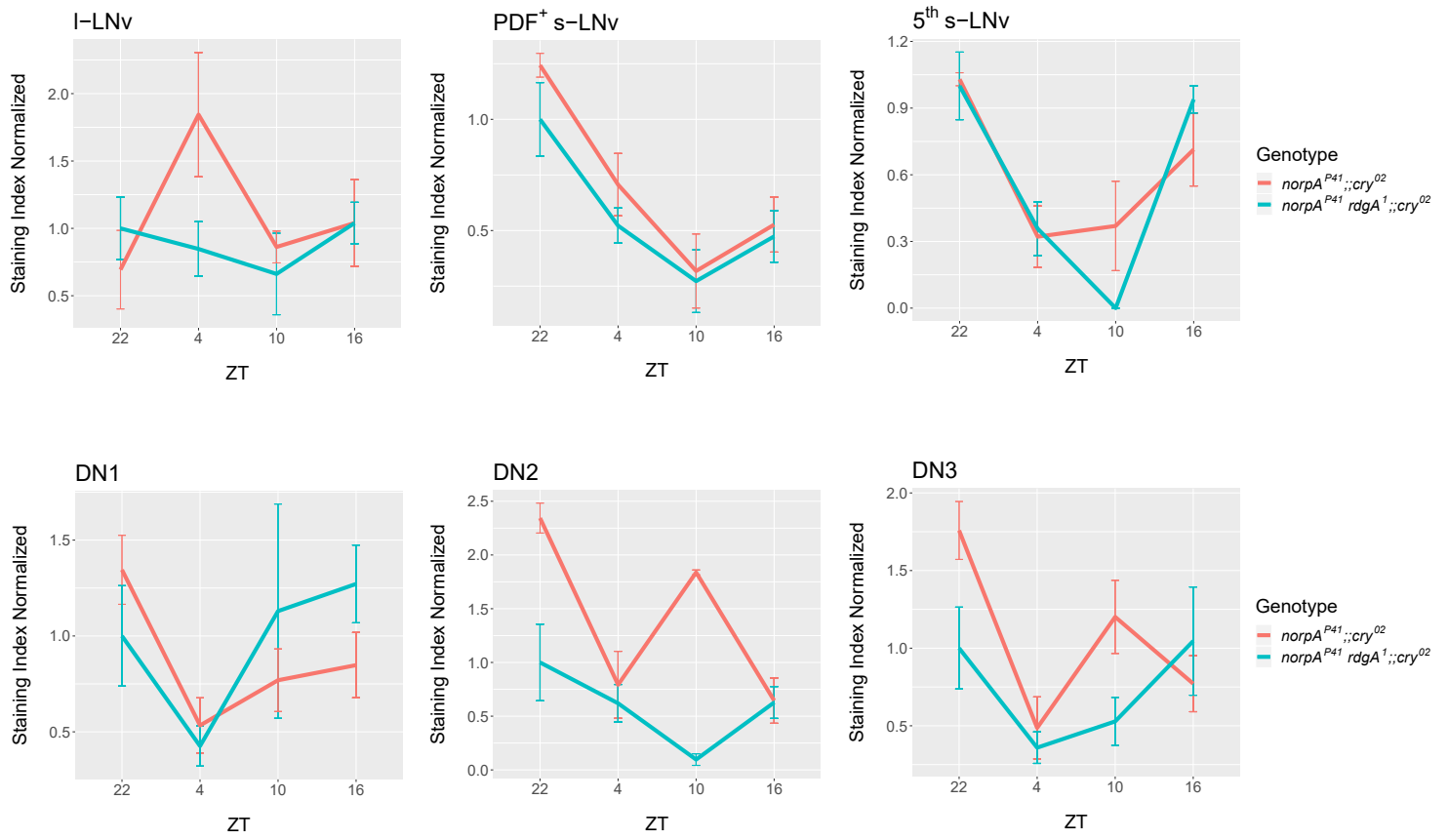
Figure S3

Figure S3. **Improved PER synchronization in clock neurons of *norpA^{P41}rdgA^1 cry^{02}* treble, compared to *norpA^{P41} cry^{02}* double-mutants.** Quantification of PER expression in individual clock neuronal groups (A), and in all groups combined (B) for the indicated genotypes. Brains were dissected at the indicated time points on day 2 of a 6-hr delayed LD cycle in a jetlag experiment (e.g., see Figure 3A). Between 3 and 6 brain hemispheres were analyzed for each genotype and time point.

Figure S4
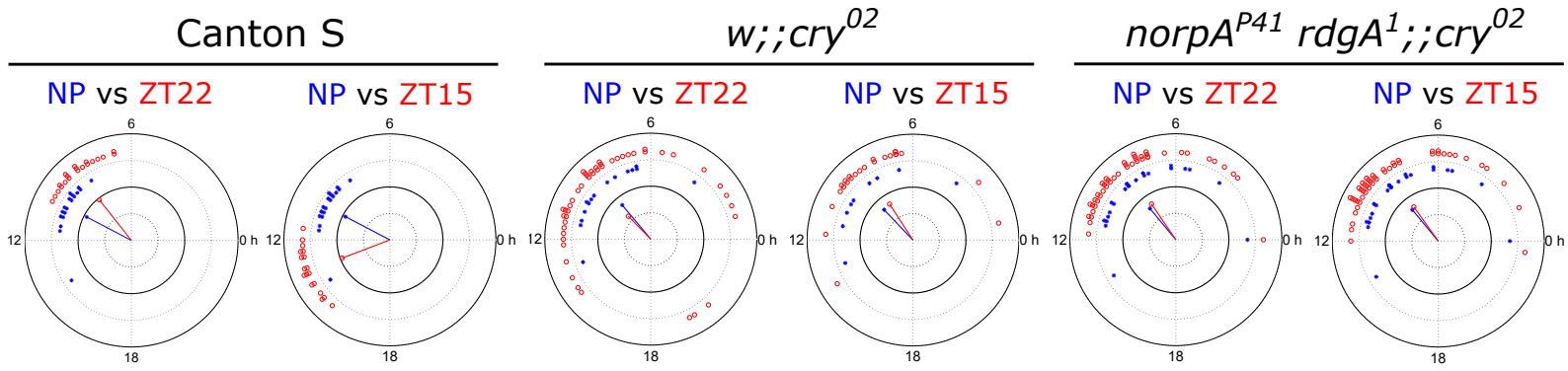
Figure S4. Visual system function in *norpA^P41^ rdgA^1^* double mutants is not sufficient for behavioral clock synchronizatio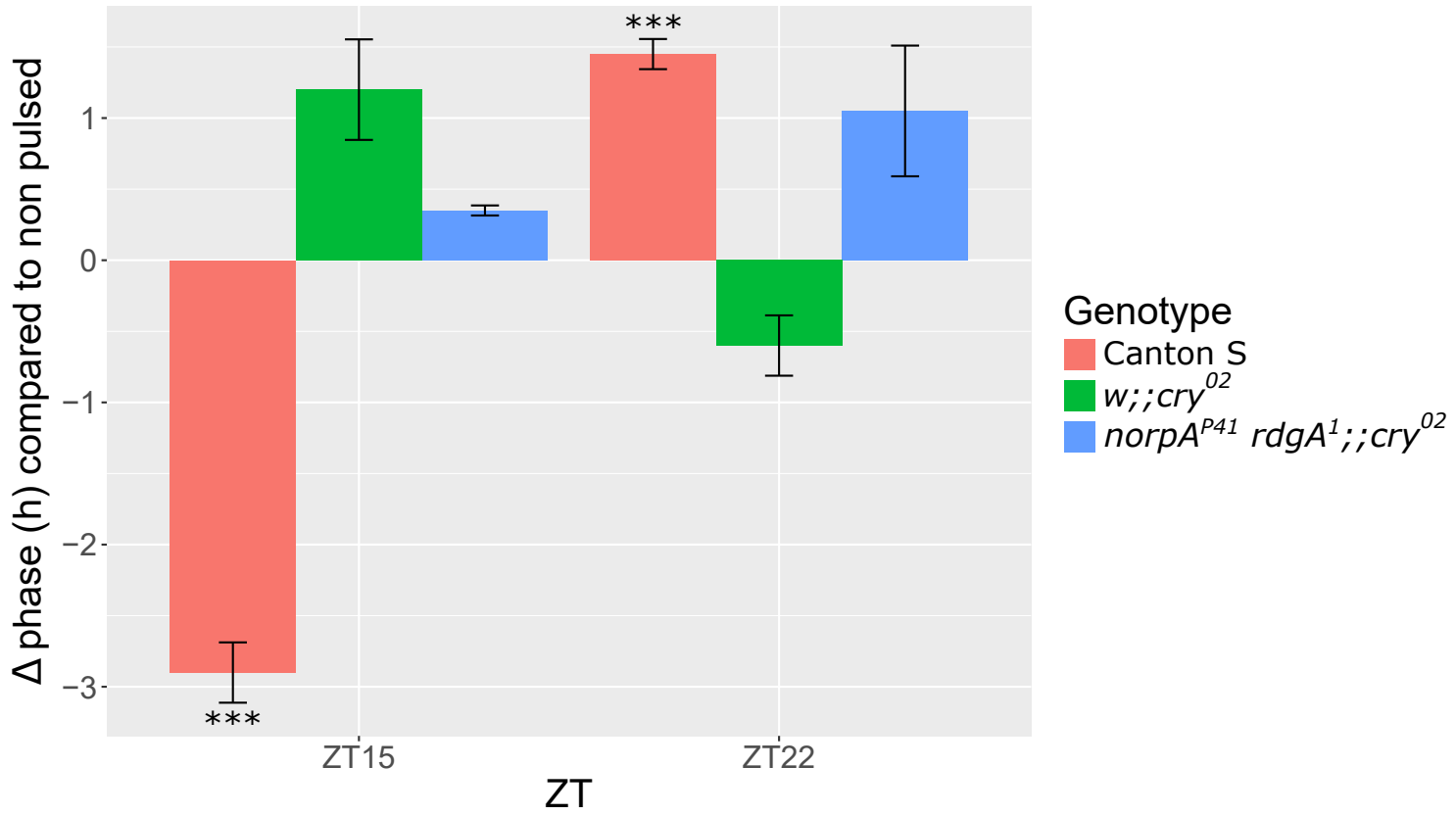n to 30 min Light pulses. Comparison of peak activity phase after a 30 min light pulse (LP) given at ZT15 and ZT22 in subsequent constant conditions. (A) Circular phase plots showing the phase of non-pulsed (blue) and pulsed flies (red) of the indicated genotypes at ZT22 (left) and ZT15 (right) 1 day after the pulse. Dots represent phase of individual flies. The direction of the vector indicates the mean phase of a genotype and the magnitude of the vector indicates the coherence of the group (variance around the mean) (Levine et al., 2002). (B) Bar graph showing the difference of peak activity of the indicated genotypes compared to that of non-pulsed flies of the same genotype. Error bars represent SEM. Δ phase and statistical significance was determined using circular statistics. Canton S, non-pulsed n=34, ZT22 n=40, ZT15 n=41; *w;;cry^02^*, non-pulsed n=37, ZT22 n=69, ZT15 n=68; *norpA^P41^,rdgA^1^;;cry^02^*, non-pulsed n=50, ZT22 n=67, ZT15 n=67

Figure S5

D. melanogaster	896	VLPL**G**TGND	904
D. suzukii	372	VLPL**G**TGND	380
D. simulans	898	VLPL**G**TGND	906
Danio rerio	394	VLPL**G**TGND	402
M. musculus	453	VLPL**G**TGND	461
rdgA[1] mutants	896	VLPL**S**TGND	904

Figure S5. *rdgA[1]* **encodes a Glycine to Serine change within the catalytic domain of DAG-kinase.** Comparison of the relevant highly conserved amino acid sequence within the catalytic domain of *rdgA* encoded DAG-kinase between different *Drosophila* species, and the orthologs in zebrafish (*Danio rerio*) and mouse (*Mus musculus*) and the mutant *Drosophila melanogaster* DAG-kinase encoded by *rdgA[1]*. Numbers represent the position in the protein sequence according to NCBI.

Table S1. Free running behaviour of the genetic variants used in this study. RS: rhythmic

strength. All values represent the average ± S.E.M.

Genotype	% Rhythmicity	Period	RS	n
Canton S	97.8 ± 1.8	24.3 ±0.1	3.3 ± 0.3	38
$norpA^{P41}$	70.3 ± 3.2	23.4 ± 0.2	2.8 ± 0.2	47
$w;;cry^{02}$	83.3 ± 2.4	23.6 ± 0.4	2.0 ± 0.2	30
$norpA^{P41};;cry^{02}$	64.2 ± 3.6	24.1 ± 0.3	2.5 ± 0.4	50
$rdgA^{1}$	85.1 ± 2.7	23.6 ± 0.2	3.2 ± 0.3	90
$rdgA^{KS60}$	76.8 ± 1.4	24.4 ± 0.2	2.4 ± 0.2	43
$rdgA^{1}/ rdgA^{KS60}$	100	23.5 ± 0.1	4.7 ± 0.1	31
$rdgA^{1};;cry^{02}$	69.4 ± 1.4	23.6 ± 0.6	2.5 ± 0.3	90
$norpA^{P41} rdgA^{1}$	76.7 ± 1.6	23.8 ± 0.2	3.1 ± 0.4	44
$norpA^{P41} rdgA^{1};;cry^{02}$	93.7	23.9 ± 0.2	2.5 ± 0.3	32
$norpA^{P41} rdgA^{1} ;PLC21C^{P319};cry^{02}$	9.6 ± 1.1	23.2 ± 0.3	1.8 ± 0.1	34
$norpA^{P41} rdgA^{1} ;Gq^{V303D};cry^{02}$	28.6	23.6 ± 0.4	1.8 ± 0.6	8

Table S2. List of primers used for sequencing of the *rdgA* CDS

Primers to sequence the common regions between alleles

rdgA primer 1 F	AAGCAGTGCGGCAAGTTCTTT
rdgA primer 1 R	ACAATGACCGGGATTACCTCC
rdgA primer 2 F	GCCCGATTGGACGGAGAATG
rdgA primer 2 R	CCGCCTGCTTCATAGGAAAGAAC
rdgA primer 3 F	AGCACTATTGGAAGCCCACC
rdgA primer 3 R	TTTGCACCGCCTGCTTCATA
rdgA primer 4 F	TTCCTATGAAGCAGGCGGTG
rdgA primer 4 R	CGATCGACATGGTCATCGGT
rdgA primer 5 F	CTCTCGCAATGGGTGACGC
rdgA primer 5 R	ACTGCTCGTACTGTTGCATGG
rdgA primer 6 F	TAAACATTCCCAGCTATGGCG
rdgA primer 6 R	AGCACCAATCCTGTGAGACC
rdgA primer 7 F	AAGGCACTGATGCTGTCGAA
rdgA primer 7 R	GCTCCAGTATAGGGCTCTTCC
rdgA primer 8 F	GAGTGCATTAAAAGCGGCCA
rdgA primer 8 R	TTTGCACCGCCTGCTTCATA

Specific primers for allele A

rdgAA F1	TCGAATGCCAAAACGGAGGA
rdgAA R1	TAGGAAAGAACTTGCCGCACT
rdgAA F2	TGTGGCATAACCTTCACGCT
rdgAA R2	GGTACGCCCGTCTGCATAG
rdgAA F3	GACGAGGCGCGTTGTTCTA
rdgAA R3	TGTTGGACACCAGAAGCCAG
rdgAA F4	CCAGAGAGCCGACCAAGAAC
rdgAA R4	AGGCCGATGATGAGGATTGC
rdgAA F5	GATCACGCACTCCAACAGGT
rdgAA R5	TGCTGTAGCTCAAAGCCGTT
rdgAA F6	ACGGCTTTGAGCTACAGCAA
rdgAA R6	CTCGGACTGGCAAACCCATA

Specific primers for allele C

rdgAC F1	GCAGTGATACAATCTCTGGGC
rdgAC R1	ACGTTTCCCTCATCGTCCAC
rdgAC F2	AATTGTGCAAGTGGCGTTCG
rdgAC R2	CGTGTCTGCGGCTGTCATTA
rdgAC F3	GATTGCTGCACGCTGTTCG
rdgAC R3	GGCCGCTTTTAATGCACTCC

Primers for genomic confirmation of the mutation

rdgA1 F	CGAGCGTTCATTGTGAAGCC
rdgA1 R	TGGGGAGGGGTGAGTAATCTT