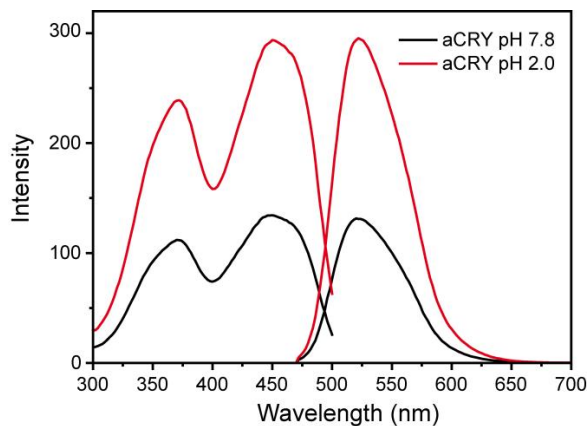


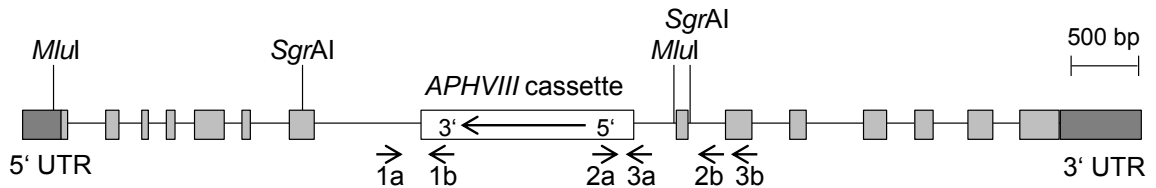
Supplemental Figure 2. Fluorescence Excitation and Emission of the aCRY Chromophore before and after Acidification.



To reveal the identity of the flavin chromophore, heterologously expressed aCRY with $OD_{447} = 0.5$ was denatured by heat (10 min, 95°C). The denatured protein was pelleted by centrifugation for 2×10 min at 15,000 g. Emission spectra with excitation at 447 nm and excitation spectra with emission at 520 nm were taken of the supernatant. Upon acidification with 50 mM phosphoric acid to a pH of 2.0, an increase of fluorescence typical of FAD was detected. This rise has been attributed to a loss of interaction between the adenine and flavin ring systems upon protonation of the adenine ring.

Supplemental Figure 3. Sequences in *acry* Mutant Strains and Primers for Characterization

(A) Position of the *APHVIII* insertion in the *acry* mutant



The position of the *APHVIII* cassette in the *acry* mutant is shown. Boxes in light gray depict exons, black lines introns. Boxes in dark gray show the 5'- and 3'-UTRs, respectively. The *APHVIII* cassette (white box) is inserted in intron 7 in reverse direction relative to *APHVIII*. Arrows show primer-binding sites. Mutant line CRMS101 was identified using primer pair 1a/1b (see Supplemental Figure 3D). DNA regions spanning the borders of the insertion were amplified with primer pairs 1a/1b and 2a/2b, and sequenced (Supplemental Figure 3B and 3D). In the *acry* mutant in an SAG73.72 background (SAG73.72:*acry*1A), the full *APHVIII* cassette was amplified with primer pair 1a/3b and sequenced with primers 1a, 3a and 3b (Supplemental Figure 3C and 3D). The restriction sites that are shown were used to characterize the *acry* mutant by Southern blot analysis.

Supplemental Figure 3. continued (2/4)

(B) Sequenced regions in the *acry* mutant CRMS101

CGGGCAGTGGTGGCAGCCACCAGACCCCTGCCCCCTACGCCGGAGCCCCAACAGACACGCGACACAACCTAAAGCCACAGCAACCAC
CGCGCCCGCGGCTCCCAAGTCCACGAGAGCGAAGCCACCAGACGGGGCGGGCAAGAAGCGCCGCCCGCGAACTTTCCATCG
GCCAGCAACATCCCCGCGCCCTGGTCGGGATGTCGCCGACCTGAGAATTCGATATCTCAGAAGAACTCGTCCAACAGCCGGTA
AAACGCCAGCTTTTCTCCGATACCGCCCCATCCACCCGCGCCGTACTCCCGCAGGAACGCCGCGGAACACTCCGGCCCGAACC
ACGGGTCTCTCGTGGGCCAGCTCGCGCAGCACCAGCGGAGATCGGAGTGCCGGTCCCGCACGGCCGACCCGCCCCACGTCGATC
AGCCCGGTCACTCGCAGGTACGAGGGTCGAGCAGCAGTGTGCCGGCAGAGTGACCGTGGCAAACCGCCAGATCCTCGTCCGC
AGGCCGAGTCCGCTCCAGCTCGGCGAGAAGCCGCTCCCCCGACCACCCCTTCCGCTCCTCGTCCAGATCCTCCAAGTCCGAGCTCC
CTTCAGCGACAGCACGGGCCCTGCGGCACCGTCACCGCGAGACTGCGATCGAACGGACACCGCTCCAGTCCAGCGGTGCAGC
GAACGAGCGAGCCCCGCGAGCCGCCACCGCCACGTCCAGCCGCTGCTCCCGCGGCCACCGCGCACTGGCCGGACGCCCGGAACCGC
TTCCGGTGACCAACCAGGCGACCTCTCGTCCCCACCACCTCCACAACACGAGGTACGGGAATCCCCACCTCCGCCAACACACCA
GCCGCTCAGCCTCACCAACAAGCCACCCCGCCCCAGAGCTGCCACCTTGACAAACAACCTCCCGCCACCACCCGAAGCCGA
TAAACACCAGCCCCGAGGCCCATCTCCACACAACCCACTCACAACCGGGATACCACCCCGCAGTGCACGCAACCGCATCGTC
CATATGGGCTTGTGTGAGTAGCAGTGGGGTCTAGAAATGCACAACGAGTCAAGAGCGCAACACCTAACCCCTGGCTTGCTCGGGCA
GGAAACCTCCCCGAGCAAGCCATCTCGGTGCTACCTCCAATTCCAGATCCCCCTCCCGAGCCCGCAGGAGCCCTCGCACGAGT
GCCGAACGCAACAATATTGATACATAATCGTCCCTGGCTGGGGAAAGGGCCGCTAACGCGCCGGGCGCTCGCGTAAATACCAAT
AATCACGCCGCTCCACTTTGCTCTCTCGCCTGCAACTTAAAAGCCTACTGCCTCGCCAGATTGCTCCAATTGTGTACAAAT
GACAATTGCGTCCGAATATGGGGCCGAGCGCTCAGGAAAGGTGGCACTGCGATCTGCTAACATGCTCGGAACGACGACAAGAAG
CGGCTTTTTAAGGACTCCGAGTTCGGGCAAACATGAGCATTGCTGCCTTCACGCATCGGTAGGTGTGGAGGCGCGCTGGAGAA
AGGCACCCGAACCTGGCGGCGAGCCCTTCGAACAGCCAGGCCGCTGCTCCGCCCTTCGCTTTCGCATGCGCTTCCAAGCGATCAC
CAGCACAAAGGGCACCGCTGGCACGAGTACGGGTGTTGAGGCATGCTGAGAGCCCTGGGTCTGTCGGTGGCCTAGGAAGGGCAAA
AGGGCTGCGGGTTCGGCCGTGAGAGGGAGCGTGGCGGAGACGTGTTTCTGACGAGGGTCTGTGT*ACGATTGGTCCGCACCGCT
GGGCGGTG*GCAAGCGAGGCCGCGGCTCCAGAGACCAGGACACCTGGGGCGGGGCGAGCCGGAAGGCTCGCGGGCGGGAACA
GGAGGGCTTGGTCAATAATACCCTCAACGAAGCGGTGAGAGCTGCTACAGAGAGGCAGAATGCCGAACGGCTGAAGCAGCGAACA
CAAATGCTTCGGCCGTCTTGTACGGCTCCATGTAACTCCGCTTAGCCTTAATCTCGCTCCGCTCCGCTTCGCTCCGCTACGCT
CAAACCAGGCTAACCGTAATTTCAATGCACCTTTGCATGCAGCATGCAAGATTCCCTTTGAAGTAACAGATCATGTGTGTACGCG
TGTGTGTGCATTACAGGTTGGTGTGAGACGGAGGCTCTGGCCAGACTGGAGCGCGGTTCCAAGACCCCAAATGGGTGGCGGGGTTTC
CAGGTGAGAGGCGAGGCTGGGGCGGTGCACCGGT
GCGATGTTACAACCAATGCCGTGATGGAATGGAGTAAGCTGGCTGGACAGAGTGGACCGGCCGCACTCCCGCGGCAAGCCTATG
CAGGGTG

Sequenced regions are depicted in black and unsequenced regions in gray. The sequence of the *APHVIII* insertion cassette within the *acry* gene is shown in italics. The *APHVIII* ORF is underlined. The cassette is inserted in the 3' → 5' direction. Nucleotides, which have been incorporated when compared to the theoretical sequence, are marked in bold. One asterisk shows the position of one deleted nucleotide (G). Two asterisks show the position of 11 missing nucleotides (ACGCGGGCCGC) from intron 7 of wild-type *aCRY*, which have been deleted in the mutant.

Supplemental Figure 3. continued (3/4)

(C) Oligonucleotides used for PCR and sequencing reactions in the *acry* mutant

<u>Abbreviated name</u>	<u>Original name</u>	<u>Sequence (5' → 3')</u>
1a	OMM845	ACAGACACGCGACACAACCTAAAGC
1b	RB2	TACCGGCTGTTGGACGAGTTCTTCTG ¹
2a	RIM5-2	GCTGGCACGAGTACGGGTTG
2b	OMM854	CCAGCCAGCTTACTCCATTCCATC
3a	OMM1025	CGAGCCCTCGTCAGAAACAC
3b	OMM757	ACTTGAGGTAGGGCGACAGCACAGTGG

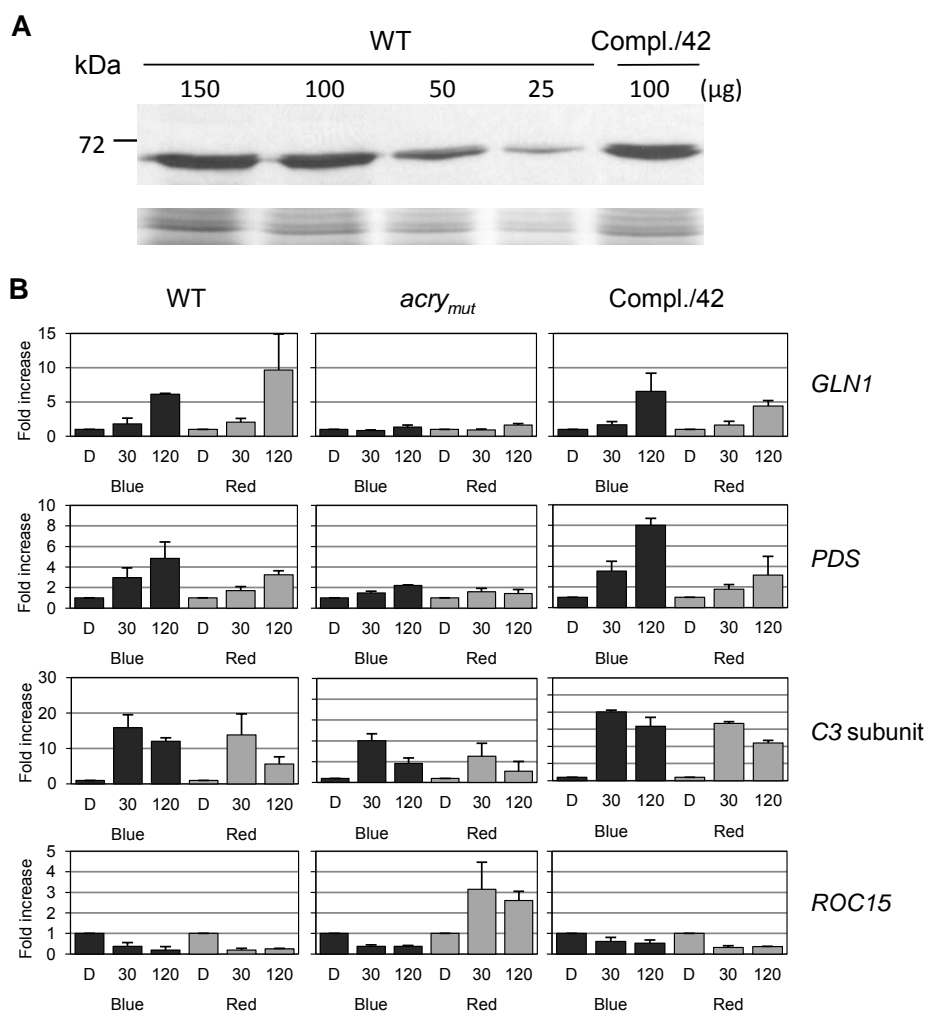
Supplemental Figure 3. continued (4/4)

(D) Sequenced regions in the SAG73.72:*acry1A* mutant

ACAGACACGCGACACAACCTAAAGCCACAGCAACCACCGCGCCGCGGCTCCCCAAGTCCACGAGAGCGAAGCCACCAGACGGGGC
 GGGCAAGAAGCGCCGCGCGCG**GAACTTTCCATCGGGCCAGCAACATCCCCGCGCCCTGGTCGGGATGTCCCGACCTGAGAA**
TTTCGATATCTCAGAAGAACTCGTCCAACAGCCGGTAAAACGCCAGCTTTTCTCCGATACCGCCCCATCCCACCCGCGCCCGTACT
CCCGCAGGAACGCGCGGAACTCCGGCCCAACCACGGGTCTCTCGTGGGCCAGCTCGCGCAGCACCAGCGCGAGATCGGAG
TGGCGGTCCGCACGGCCGACCCGCCACGTTCGATCAGCCGGTACCTCGCAGGTACGAGGGTCGAGCAGCAGTGTGTCGGGCA
CAGGT**CACCGTGGCAAACGCCAGATCTCTCGTCCGAGGCCGAGTCCGCTCCAGCTCGGCGAGAAGCCGCTCCCCGACCAACCCCT**
TCCGCTCCTCGTCCAGATCTCTCAAGTCGACGCTCCCTTCAGCGACAGCACGGGCCCTGCGGCACCGTACCGTGAGACTGCGA
TCCGACCGGACACCGCTCCCAAGTCCAGCGGTGCAGCGAACGAGCGGCCCGCGAGCGCCACCGCCAGTCCAGCCGCTGCCG
CGGCCACGCGCACTGGCCGAGCAGCCCGGAACCGCTTCGGTGACCAACCAGGCGACCTCTCGTCCCCACCACCTCCACAACAC
GAGGTACGGGAATCCCCACCTCCGCCAACACACCAGCCGCTCAGCCTCACCAACAAGCCACCCCGCCCCAGAGTGCACCC
TTGACAAACAACTCCCGCCACCAACCCGAAGCCGATAAAACACAGCCCGAGGCCCATCTCCACAACAACCACACTCACAACC
GGGATACCGACCCCGCAGTGCACGCAACGCATCGTCCATATGGGCTTGTGTGAGTAGCAGTGGGGTCTAGAATGCACAACGAGT
 CAAGAGCGCAACACCTAACCTGGCTTGTCTCGGCGAGGAAACCTCCCCCGAGCAAGCCATCTCGGTCTACCTCCAATTCACAGA
 TCCCCCTCCCCAGCCCGCAGGAGCCCTCGCACGAGTGCACGCAACAATATTGATACATAATCGTCCCTGGCCTGGGGGAAGG
 GCCGCTAACGCGCGGGCGTCCGTAATAACCAATAATCACGCGCGTCCCCTTTGCTCTCTCGCCTTGAACCTAAAGCCCTA
 CTGCCCTGCCAGATTTGTCCAATTTGTGTACAATGACAATTCGGTCCGAATATGGGGCCGAGCGCGTCAAGAAAGGTGGCACTG
 CGATCTGCTAACATGTCTCGGAACGACGACAAGAAGCGGCTTTTAAGGACTCCGAGTTGGGGCAACATGAGCAATTTGCTGCC
 TCACGCATCGGTAGGTGTGGAGGCGCGGTGGAGAAAGGCACCCGAACGGCGCGAGCCCTCGAACAGCCAGGCCCTGCTCC
 GCCCTTCGTCTTCGCATGCGCTTCCAAGCGATCACCAGCACAAGGGCACCGCTGGCAGGAGTACGGGTTGTTGAGGCATGCTGAG
 AGCGCTGGGTCTGTGGTGGCTAGGAAAGGGCAAAGGGCTGCGGGTGGCCGTGAGAGGGAGAGCGTGGCGGAGACGTGTT
 TCTGACAGGGGCTCGTGT*ACGATTGGT**CCGCACCGCTGGGCGGTTG*** *GCAAGCGAGGCCGCGGCTCCAGAGACCGAGGACCT
 GGGAGGGGGCGAGCCGAAGGCTCGCGGGCGGAACAGGAGGGCTGGTCAATAATACCGCTCACGAAGCGGTGAGAGCTGCTA
 CAGCAGGCGAGAAATGCCGAACGCTGAAGCAGCGAACACCAAAATGCTTCGGCCGCTTTGTACGGCTCCATGCTAACTCCGCTTAG
 CCTAAATCTCGCTCCGCTCCGCTTCGCTCCGCTACGCTCAAACCAGGCTAACCGGTAATTTCAATGCACCTTTGACCTTTGACATGC
 AAGATTCCCTTTGAAGTAACAGATCATGTGTGTACGCGTGTGTGTGATTACAGGGTGGTGGAGCGGAGGCTCTGGCCAGACTGG
 AGGCGCGGTTCCAAGACCCCAATGGGTGGCGGGTTCAGGTGAGAGGCGAGGGCTGGGGCGGTGCACCGGTGTGTGTGTGT
 ACGTGTGTGATGCCCGAGCCAAGCGGGCAGGTGGGGTGGCGATGTTACAACCAATGCCGTTGATGGAATGGAGTAAGCTGGCTGGA
 CAGAGTGGACCGCGGCACTCCGCGGCAACGCTATGCAGGGTGTGTTATCGACGATGCTTTTACGATACGCCAACAATGCGA
 AACCGAGATAGCTAGGGTGTGCCGCTGTGAGGGCCCCACGGCTGCCGTGCCAACACCCACACAGCCACCCACCCACACAG
 AAGCCCGACAGGACCCAGCGCTGGGAGAAGCCCGGACCACTGTGTGTGCTGCCCTACCTCAAGTTCCGGCTG

Sequenced regions are depicted in black and unsequenced regions in gray. The sequence of the *APHVIII* insertion cassette within the *acry* gene is shown in italics. The *APHVIII* ORF is underlined. The cassette is inserted in the 3' → 5' direction. Two exchanged nucleotides in the *APHVIII* ORF are highlighted in bold and in a gray background. Thereby, a C was replaced by T at position 330, leading to an amino acid change from alanine to threonine. At position 486, a G was replaced by C, thus leading to a swap from histidine to aspartic acid. One asterisk shows the position of one deleted nucleotide (G). Two asterisks show the position of 11 missing nucleotides (ACGCGGGCCGC) from intron 7 of the wild-type *aCRY* gene, which have been deleted in the mutant.

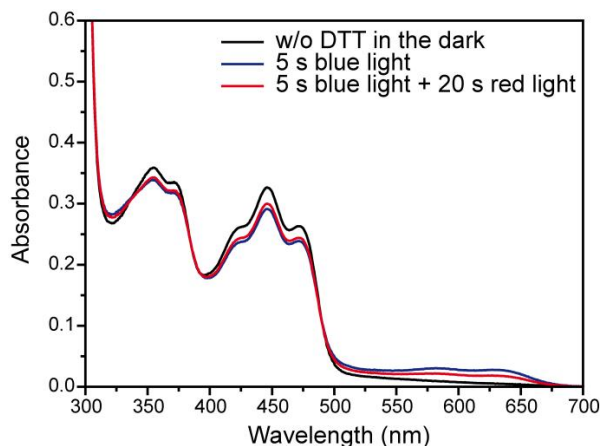
Supplemental Figure 4. Complementation of the *acry* Mutant by Transformation with pKP39 to Generate Transgenic Line 42



(A) Different amounts of protein from a crude extract of WT cells (150, 100, 50 and 25 μg per lane) were separated by 10% SDS-PAGE along with 100 μg of protein from a crude extract of the complemented *acry* mutant strain 42 (Compl./42). The protein samples were used for immunoblotting with anti-aCRY antibodies along with chemoluminescence detection. To control for the different amounts of protein loaded, the PVDF membrane was Coomassie blue-stained after the immunological detection. From this stain, a random band is shown (lower panel). Quantification of aCRY level was done as described in Figure 2B ($n=3$).

(B) Cells of the Compl./42 *acry* mutant strain were grown in a light-dark cycle. At the end of the light period, they were placed in darkness (D) for 60 h before exposure to 30 min and 120 min of either blue or red light (see Methods). Total RNA was extracted and analyzed by RT-qPCR. The changes in transcript levels following exposure of the cells with blue or red light are presented as n-fold change relative to RNA from dark-grown cells. Each experiment was performed in triplicate from at least two different biological samples. Error bars show the mean and standard deviation. Data for WT and the *acry* mutant strain were taken from Figures 3 and 4.

Supplemental Figure 5. UV/Vis Absorption Spectra Showing the Effect of Consecutive Blue- and Red-Light Illumination on aCRY *in Vitro* in the Absence of Reductant.



aCRY was illuminated with blue light (455 nm) for 5 s. Bands rise between 530 nm and 680 nm, which indicates the formation of the neutral radical state of the flavin chromophore. To selectively investigate the light response of the radical state, red light (636 nm) was applied for 20 s. As a consequence, the neutral radical band decreases, whereas the absorption of the oxidized state (band at 447 nm) recovers only to some extent. The loss of both the oxidized state and neutral radical state directly implies the formation of the fully reduced state of FAD by red light. Less pronounced effects are observed than in presence of DTT because of the faster thermal back reaction from the radical to the oxidized state.

Supplemental Table 1. LC-ESI-MS/MS Analysis of Heterologously Expressed aCRY with a C-terminal 6 x His-Tag.

Peptide sequence	Xcorr	z
LAAEAGVEVVTPISHTLYDTDMLVAR	5.73	3
QIDWDDNPEFLAAWR	5.34	2
YNFLLESLEDLQR	5.09	2
YIYEPWTAPLEVQR	4.98	2
GEAGGDGDDSGGEGSGGGAAAGR	4.66	2
HVYPVFLDPHFLQQSSYK	4.49	3
EFFYTVGSTTPNFHR	4.05	2
RHPAHSQPPVSLR	3.87	3
WVAGFQKPDTPSAWEKPATTVLSPYLK	3.86	3
LEAAFQDPK	3.41	2
NGGAAPLTMQSFTK	3.31	2
GDLYVSWER	3.15	2
DYPAPIVDHAVASK	3.07	1
NSIIWFR	3.00	2

Heterologously expressed and purified His-tagged aCRY was digested with trypsin. Peptides were analyzed by LC-ESI-tandem MS (see Methods); z, positive charge; Xcorr, cross correlation factor. The coverage is about 37%.

Supplemental Table 2. Oligonucleotides Used in RT-qPCR

Gene	Gene ID	Forward primer (5' - 3')	Reverse primer (5' - 3')
<i>C1</i>	jgi 182403	GACTGGCCACAAGCGGAAAC	ATCACGCGGCCAACCTTGTC
<i>C3</i>	jgi 99329	CGGAGGCTGAGCTTGCCATC	ATGTGCTGCTCCGTGGTGTG
<i>CDKB1</i>	jgi 59842	CTCCTCTGCGTCGAGCACAC	TTCCATGGCGGTCCATCCAC
<i>CHLD</i>	jgi 134594	CTCTGCTGCCGCCATTGAG	CCGAGCGCATCTTGGTCTTC
<i>CK1</i>	jgi 137286	GGCGTTGGACATTCCGATTG	GCCGGTCTGGATGTTTCGTTT
<i>CO</i>	jgi 159133	GGACTGCGATGTGCGCATCC	CGGCGTCGCACTGCAGTAG
<i>GLN1</i>	jgi 133971	CCATCACGCGCCACTTTCTG	TGGAGGAGCCGTCGTAGTTC
<i>GLN2</i>	jgi 129468	AGTACATCTGGGCGGATGGC	GCAGGATGCAGTCCGAGTTG
<i>GSA</i>	jgi 138524	ACGCCGACTCCTTCCTGGTG	GTTGTAGGTGGCGGTCAGGG
<i>LHCBM6</i>	jgi 184490	GCGAGGCTGTCTGGTTCAAG	CCTCGATCAGGCCATCAGG
<i>PDS</i>	jgi 78128	ACCTTTCGGACGCTGGACAC	GTGCAAGCCAGTCTCGTACC
<i>POR</i>	jgi 136589	GCGCCAAGGCTTACAAGGAC	ACTTCTGGAAGGCGGGAAC
<i>RACK1</i>	jgi 105734	CTTCTCGCCATGACCAC	CCCACCAGGTTGTTCTTCAG
<i>ROC15</i>	AB363964	GCCGGTCCAGCAACAACAAC	GTGTGCATGCGCCAGAAGAG
<i>ROC40</i>	AB363965	ATGAGGAGCATGCCCGATTTC	CCTCGCCATCCTTTGTGGAC
<i>ROC55</i>	AB363966	GTCTGGACGGCTGCTTCTTC	GTCTGGACGGCTGCTTCTTC
<i>ROC66</i>	AB363967	GCCGCTTGTGGGACAACACC	ACCATGCCGAACGCGATGAG
<i>ROC75</i>	AB363968	GGCAACGGCAACGAGAATGG	GCCCTCCGTGCGCATCTTTGG
<i>ROC114</i>	AB363970	GCGGACACCTTGACGGTCAC	GCGAGCAACCCAGCCATTAG

All JGI gene IDs were derived from the Joint Genome Institute (*Chlamydomonas reinhardtii* v4.0). The AB codes are European Molecular Biology Laboratory (EMBL) accession numbers and represent mRNAs, since there were no gene models available in these cases (Matsuo et al., 2008).

¹ **Pootakham, W., Gonzalez-Ballester, D., and Grossman, A.R.** (2010) Identification and regulation of plasma membrane sulfate transporters in *Chlamydomonas*. *Plant Physiol.* **153**: 1653-1668.