

Figure S1. Early leaf senescence in SIR impaired tomato plants at the age of 2 and 3 months compared with the wild type (WT).

A — The appearances of the tomato plants and their leaves at the age of two months. The third (3L), the fifth (5L) and the seventh (7L) leaves from the plant tops are shown. Chlorophyll content and maximal quantum yield in the two-month-old tomato plants are shown in the left and the middle inserts, respectively. The bars are the average values \pm SE (n=5 for WT, SIR Ri40, n=3 for SIR Ri37 in the chlorophyll assay; n=9 for WT, n=5 for SIR Ri37, n=14 for SIR Ri40 in the Fv/Fm assay). Quantification of *clpD* transcript in the two-month-old tomato plants is presented in the right panel. The bars are the average values \pm SE (n=3). Transcript quantification was performed by real-time PCR using *TFIID* (*SGN-U571616*) as a house-keeping gene. The values were normalized to the third leaf of the wild type. B – Phenotype of SIR Ri mutants and wild type plants at the age of 3 months. The plants were propagated vegetatively and grew in a greenhouse under the light intensity of 400-500 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Chlorophyll content and the maximal quantum yield were measured in the third (3L) and the sixth (6L) leaves counted from the top of the plants and presented in the left and right panels, respectively. Scale bars are shown. The values denoted with different letters are significantly different according to the Turkey-Kramer HSD test (JMP 8.0; $p < 0.05$). The upper-case letters reflect differences between leaves of the same genotype; the lower-case letters distinguish different genotypes.

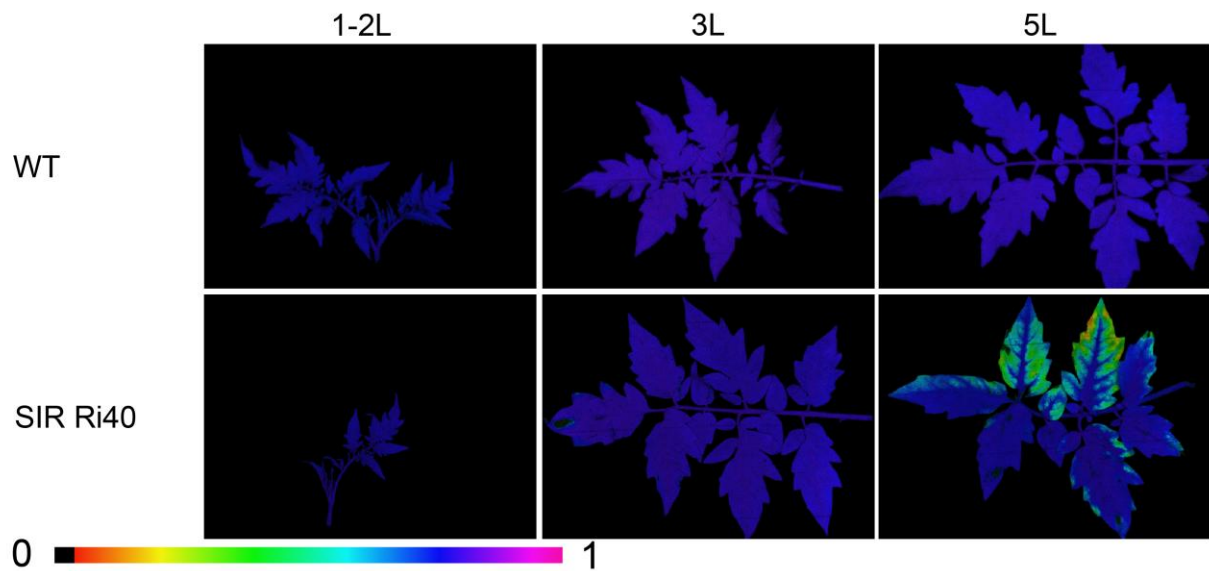


Figure S2. The uneven decline of maximal quantum yield (F_v/F_m) in leaves of SIR Ri plants. The top (1-2L), the third (3L) and the fifth (5L) leaves (counted from the tops) were collected from one-month-old tomato SIR Ri plants and analyzed using IMAGING-PAM M-Series using the MAXI Version (Heinz Walz GmbH). The corresponding leaves of the wild type plants (WT) are shown for comparison. The F_v/F_m imaging is shown as the heatmap with the range from 0 to 1. The color scale is presented.

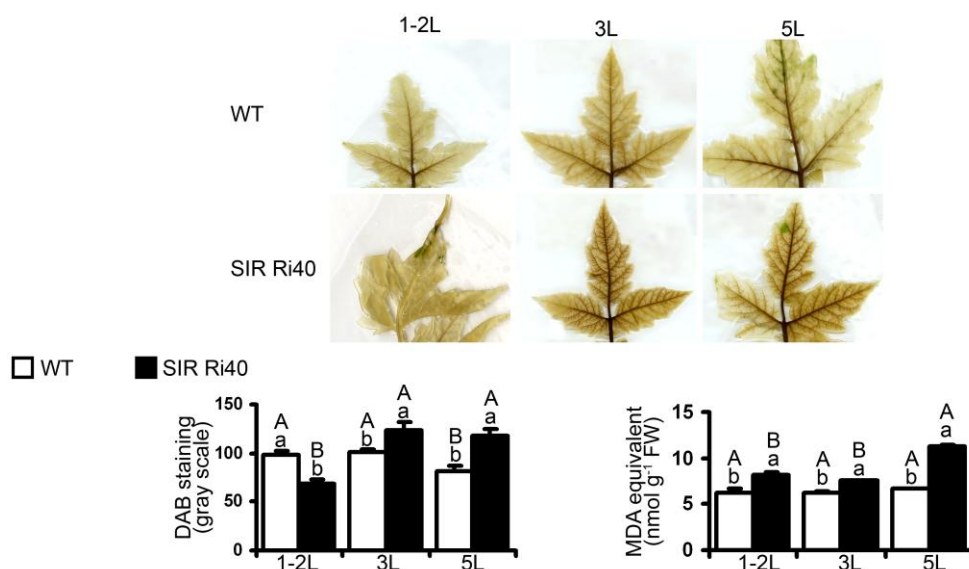


Figure S3. Enhanced markers of oxidative stress in SiR impaired tomato plants. The top (1-2L), the third (3L) and the fifth (5L) leaves (counted from the top) were cut from one-month-old SIR Ri and wild type (WT) tomato plants. The leaf petioles were dipped in 3,3'-diaminobenzidine solution and incubated for 4 h under light in order to allow the solution to penetrate into leaves with transpiration. The leaves were afterwards destained by several ethanol washings and photographed. The representative leaves are shown in the upper panel. The pictures were turned to grayscale and intensity of DAB staining was quantified by using ImageJ software (<http://rsbweb.nih.gov/ij/>; shown in the left bottom panel). The gray scale was ranged from 0 (white) to 255 (complete black). The values are average \pm SE (n=4). Malondialdehyde (MDA), the marker of lipid peroxydation, was detected in wild type and SIR Ri tomato plants as described in the "Materials and Methods" section. The results are shown in the right bottom panel as average \pm SE (n=4). The values denoted with different letters are significantly different according to the Turkey-Kramer HSD test (JMP 8.0; p<0.05). The upper-case letters reflect differences between leaves of the same genotype; the lower-case letters distinguish different genotypes.

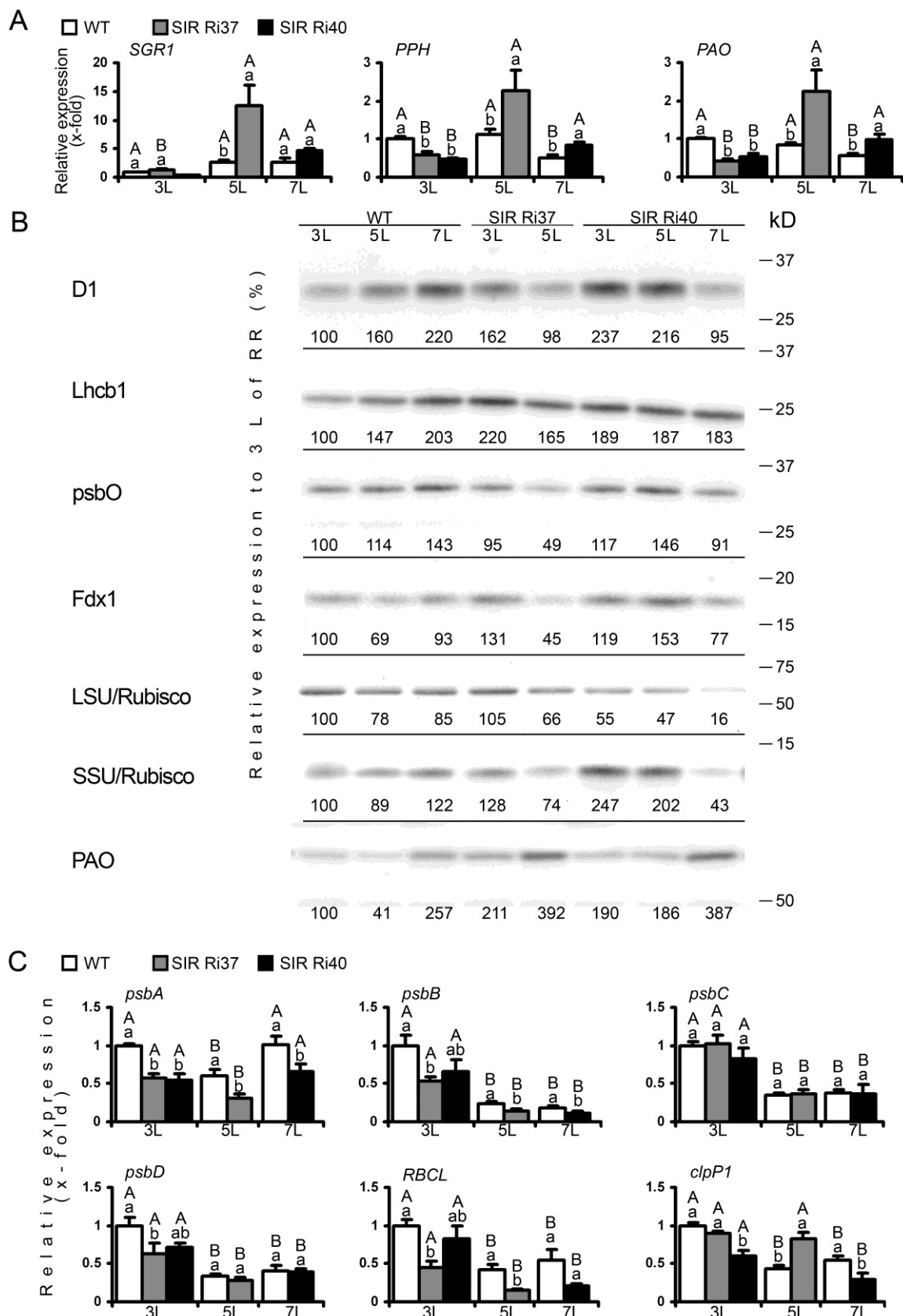


Figure S4. Expression of plastidic transcripts and proteins in wild type (WT) and SIR Ri plants at the age of two months.

A – Expression of genes related to chlorophyll degradation. The third (3L), the fifth (5L) and the seventh (7L) leaves (counted from the tops) were collected and analyzed by quantitative real-time PCR using *TFIID* (*SGN-U571616*) as a house-keeping gene. The values are the

average values \pm SE (n=9). The values were normalized to the third leaf of the wild type. B - Immunoblotting analysis of wild type and SIR Ri tomato plants. Leaf samples were collected according to their positions from the plant top. Extracted proteins were separated using SDS-PAGE, transferred to PVDF membranes and incubated with protein-specific antibodies as described in "Material and Methods" section. Protein extracts were loaded in the amount of 0.5 mkg per lane for D1, chlorophyll a/b-binding protein 1 (Lhcb1), psbO (one of the subunits that construct the water splitting system of PSII) and Rubisco (LSU and SSU – large and small subunits, respectively) or 10 μ g per lane for PHEIDE a OXYGENASE (PAO) and ferredoxin 1 (Fdx1). Relative band intensities are shown normalized to the third leaves of the wild type plants. The positions of Precision Plus Protein™ Standards (Bio-Rad) are shown. C – Expression of genes encoded by chloroplast genome in the two-month-old SIR Ri and wild type plants. Quantitative real-time PCR analysis was performed as described in panel A. The values denoted by different letters are significantly different according to the Turkey-Kramer HSD test (JMP 8.0; $p < 0.05$). The upper-case letters reflect differences between leaves of the same genotype; the lower-case letters distinguish different genotypes.

□ WT ■ SIR Ri37 ■ SIR Ri40

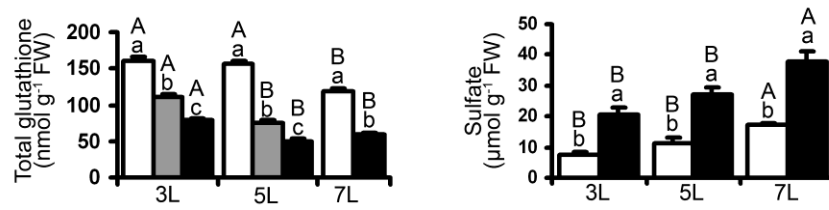


Figure S5. Accumulation of total glutathione (left panel) and sulfate (right panel) in two-month-old wild-type and SIR Ri tomato plants.

The metabolites were quantified in the third (3L), the fifth (5L) and the seventh (7L) leaves counted from the tops of the SIR Ri and wild type (WT) plants. The bars are average \pm SE (n=4). The values denoted by different letters are significantly different according to the Turkey-Kramer HSD test (JMP 8.0; $p < 0.05$). The upper-case letters reflect differences between leaves of the same genotype; the lower-case letters distinguish different genotypes.

□ WT ■ SIR Ri40

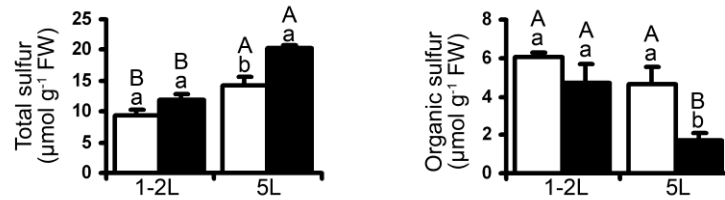


Figure S6. Accumulation of total sulfur and organic sulfur in one month old tomato plants. The top leaves (1-2L) and the 5th leaves (from the top) of SIR Ri and wild type (WT) plants were collected. Total sulfur was measured by inductive coupled plasma spectrophotometry as described in (Yarmolinsky et al., 2013). The results of total sulfur measurements are presented as µmol per g FW. Organic sulfur was determined by subtraction of sulfite and sulfate concentrations (presented in Fig. 3) from total sulfur values. The bars are average \pm SE (n=3). The values denoted by different letters are significantly different according to the Turkey-Kramer HSD test (JMP 8.0; $p < 0.05$). The upper-case letters reflect differences between leaves of the same genotype; the lower-case letters distinguish different genotypes.

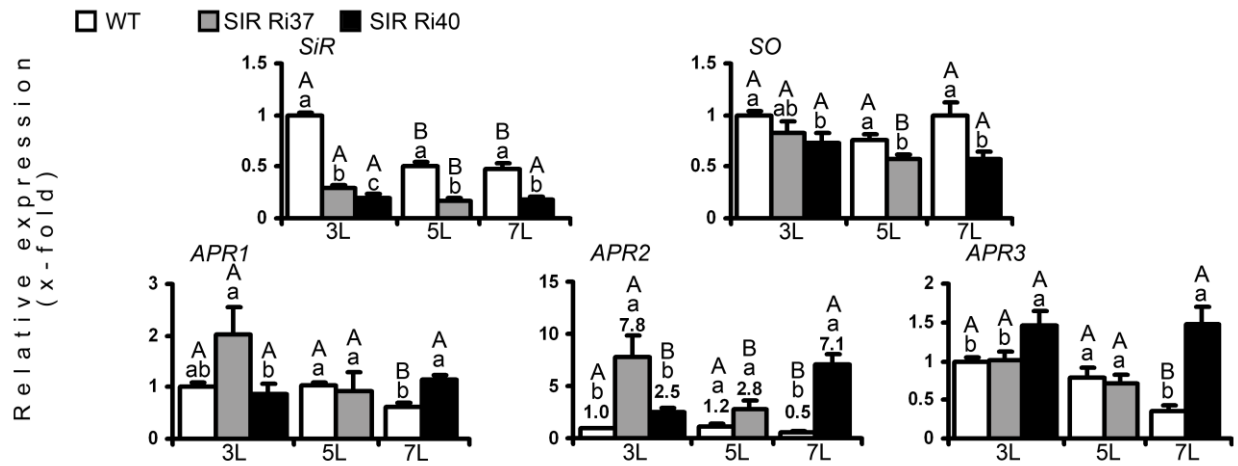


Figure S7. The impact of SiR suppression on transcripts of the sulfite network genes in two-month-old SIR Ri tomato plants

The third (3L), the 5th (5L) and the 7th (7L) leaves (counted from the tops) were collected from SIR Ri and wild type (WT) plants and analyzed by quantitative real-time PCR using *TFIID* (*SGN-U571616*) as a house-keeping gene. The values are the average values \pm SE (n=3). The values were normalized to the third leaf of the wild type. The values denoted by different letters are significantly different according to the Turkey-Kramer HSD test (JMP 8.0; $p < 0.05$). The upper-case letters reflect differences between leaves of the same genotype; the lower-case letters distinguish different genotypes.

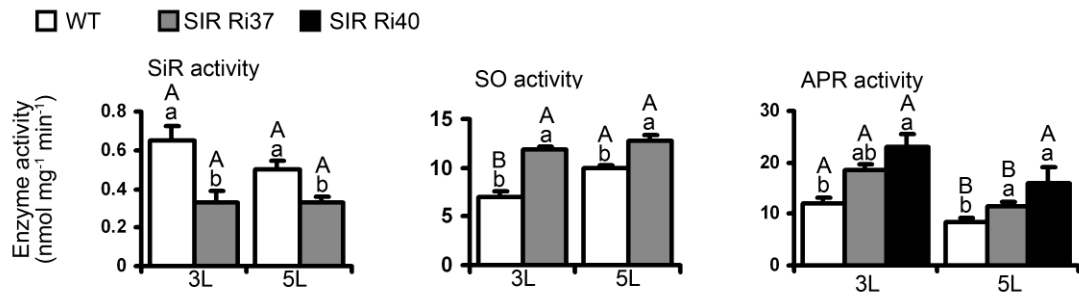


Figure S8. The effect of SiR impairment on SiR (left panel), SO (middle panel) and APR (right panel) activities in the two-month-old tomato plants.

The third (3L), the fifth (5L) and the seventh (7L) leaves (counted from the tops) were collected from SIR Ri and wild type (WT) plants; enzyme assays were performed as described in the “Materials and Methods” section. The values are the average values \pm SE (n=3). The values are average \pm SE (n=4). The values denoted by different letters are significantly different according to the Turkey-Kramer HSD test (JMP 8.0; $p < 0.05$). The upper-case letters reflect differences between leaves of the same genotype; the lower-case letters distinguish different genotypes. SiR activity was expressed as nmol cysteine mg⁻¹ protein min⁻¹, SO and APR activities were expressed as nmol sulfite mg⁻¹ protein min⁻¹.

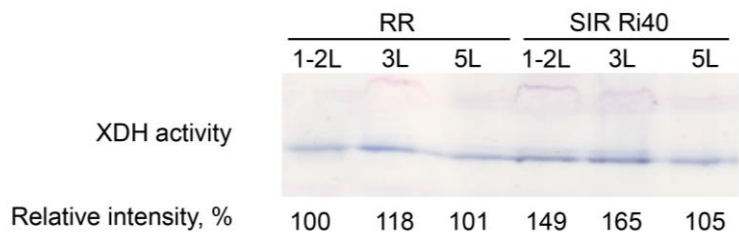


Figure S9. The influence of SiR impairment on xanthine dehydrogenase (XDH) activity in leaves of one-month-old tomato plants.

The top (1-2L), 3rd (3L) and the 5th (5L) leaves (counted from the tops) were collected from SIR Ri and wild type (WT) plants at the age of one month. XDH in-gel activity was performed according to (Yesbergenova et al., 2005). Each lane contained 100 μ g soluble proteins. The intensities of the bands were quantified by using ImageJ software (<http://rsbweb.nih.gov/ij/>) and expressed as relative values to the top leaves of WT plants.

Table S1. List of primers used for quantitative real-time PCR.

Transcript	Accession number	Primer sequence (5'→3')	PCR product, bp
<i>TFIID</i>	SGN-U571616	F ATAGTCCCTACGCTCCAGAATATTGTCTC R CTCCAGTACAAACCATTTTCCCAGAAG	190
<i>Actin Tom41</i>	U60480	F CATGCCATTCTCCGTCTTGA R CGCTCGGTCAGGATCTTCAT	71
<i>SiR</i>	SGN-U577417	F AAGTTGTGAAAGCTCGGAATGATAACT R TTCTCCATCCTCATCAGATACAACAAC	185
<i>SO</i>	SGN-U215342	F CCTGGAGGATGTGAGTGTTGTAAAG R AGTTCTCTGGTATCTGGTGGCTTC	145
<i>SGR1</i>	SGN-U572734	F AAGTTGTTGCAGAGTGGAAGAAAGTAAA R CATGAACAAAAGCCTTGAGAACCA	156
<i>PPH</i>	SGN-U572366	F CTCACAGAACTTGTATGGCAGAAAATTAG R CGAACATTATTGAAGCAAGGGATG	166
<i>PAO</i>	SGN-U580664	F CTCAATGAATAAAAATAGAGATCGACACAAA R CCAGGCACTGTAAACTGGAAGAAG	156
<i>psbA</i>	SGN-U565346	F ATCAGGGAAACCACAGAAAATGAA R AGCTAGGAAGAAGTGTAACGAACGAG	159
<i>psbB</i>	SGN-U565504	F TTATCAGAAGCCTGGTCTAAAATTCC R AAGATAGGGTGTCTAACCATCCAA	146
<i>psbC</i>	SGN-U569465	F AAAGACATACAACCTTGGCAGGAA R GCCACAAATGACCTACAAAGAAGAA	187
<i>psbD</i>	SGN-U593869	F GATTGGCCAGTTCTTATTTGGAAGG R GGACCCCATAGTAACAACAACGAATG	100
<i>RBCL</i>	SGN-U565452	F GCCGAGATAATGGTCTACTTCTTCAC R GCCCAAAGTTATGTCTCTTTCACCT	182
<i>clpP1</i>	SGN-U594203	F GGGCTTCTGTTGCTGACATAAAAAT R CCCTCACGCTAGGGTAATGATACA	200
<i>ClpD</i>	SGN-U577108	F GATGAGCAGCTTAAGAAAAGGGTTG R TGGCAGATTGAGATCCAAAATAAGA	199
<i>APR1</i>	SGN-U580331	F TTCTTCCCATCACCATCTTCTTCTATT R AATTGGGATACTTTGGGTTGTTTCATAA	189
<i>APR2</i>	SGN-U580235	F TAGCAGAGAAATTAATAGAGGCAGAGGA R ATCTTCTGCACCGCTGAAAGCAAT	140
<i>APR3</i>	SGN-U578339	F TTGCTCCTGAGGTGGAACAGAAAGC R GCCACTGAAAGCAATGGCAATTCAC	140
<i>SQD1</i>	FJ711705	F GTTGACAACCTTATCCGTCGATTATTT R GACTACAGCATCAGGTTCAAAGGATTT	195