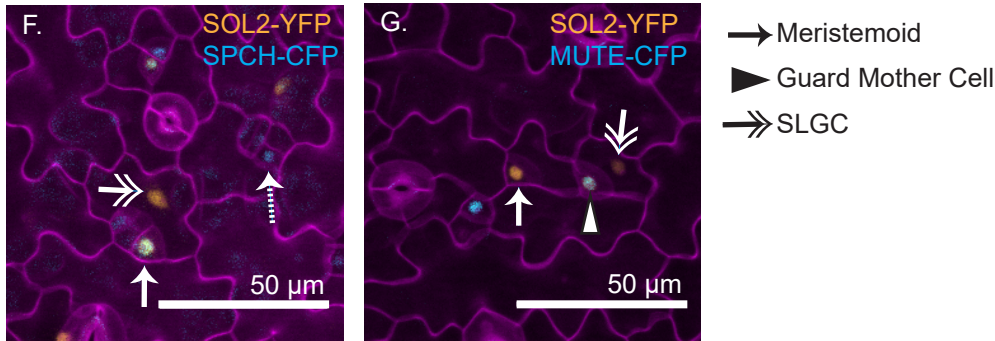
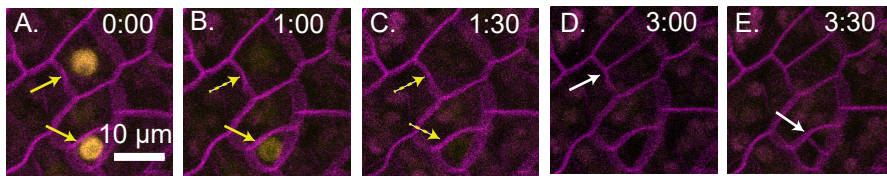
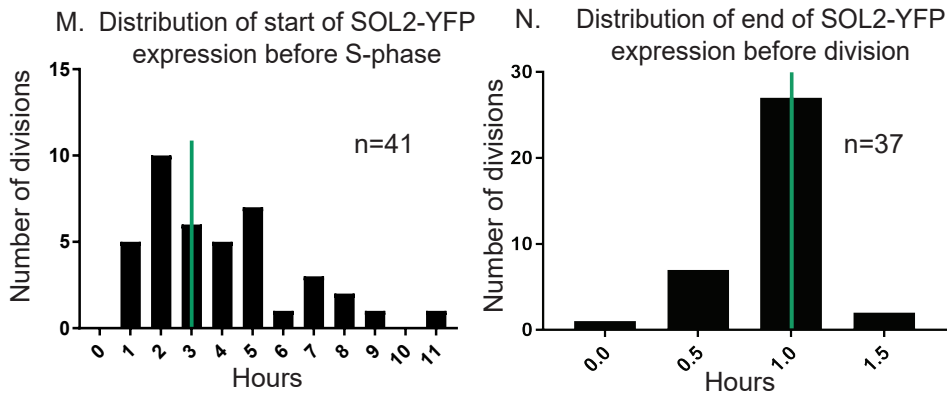
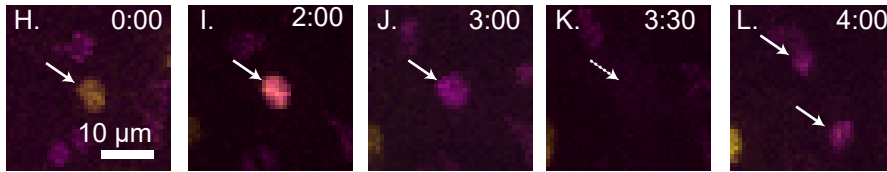


## SOL1-YFP

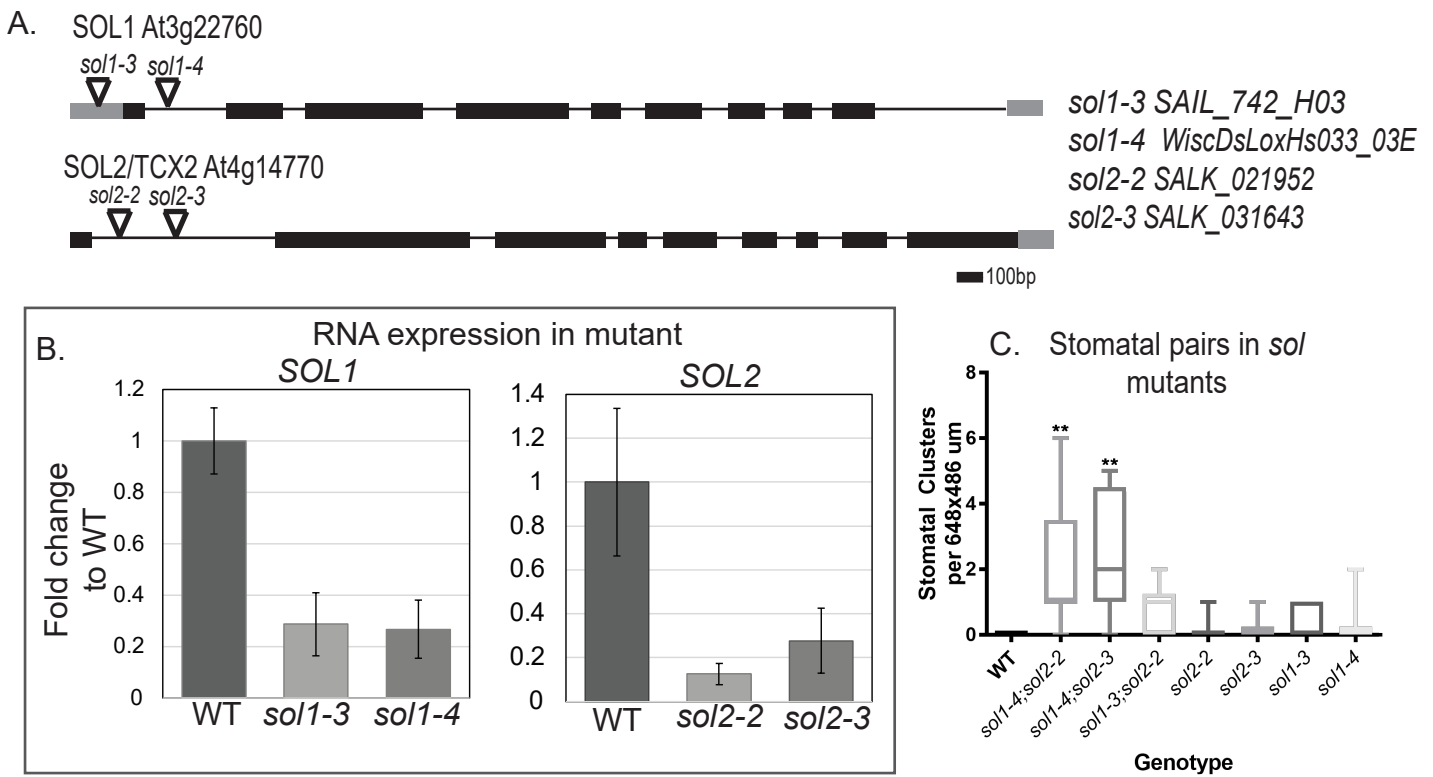


## SOL2-YFP, CDT1a-RFP



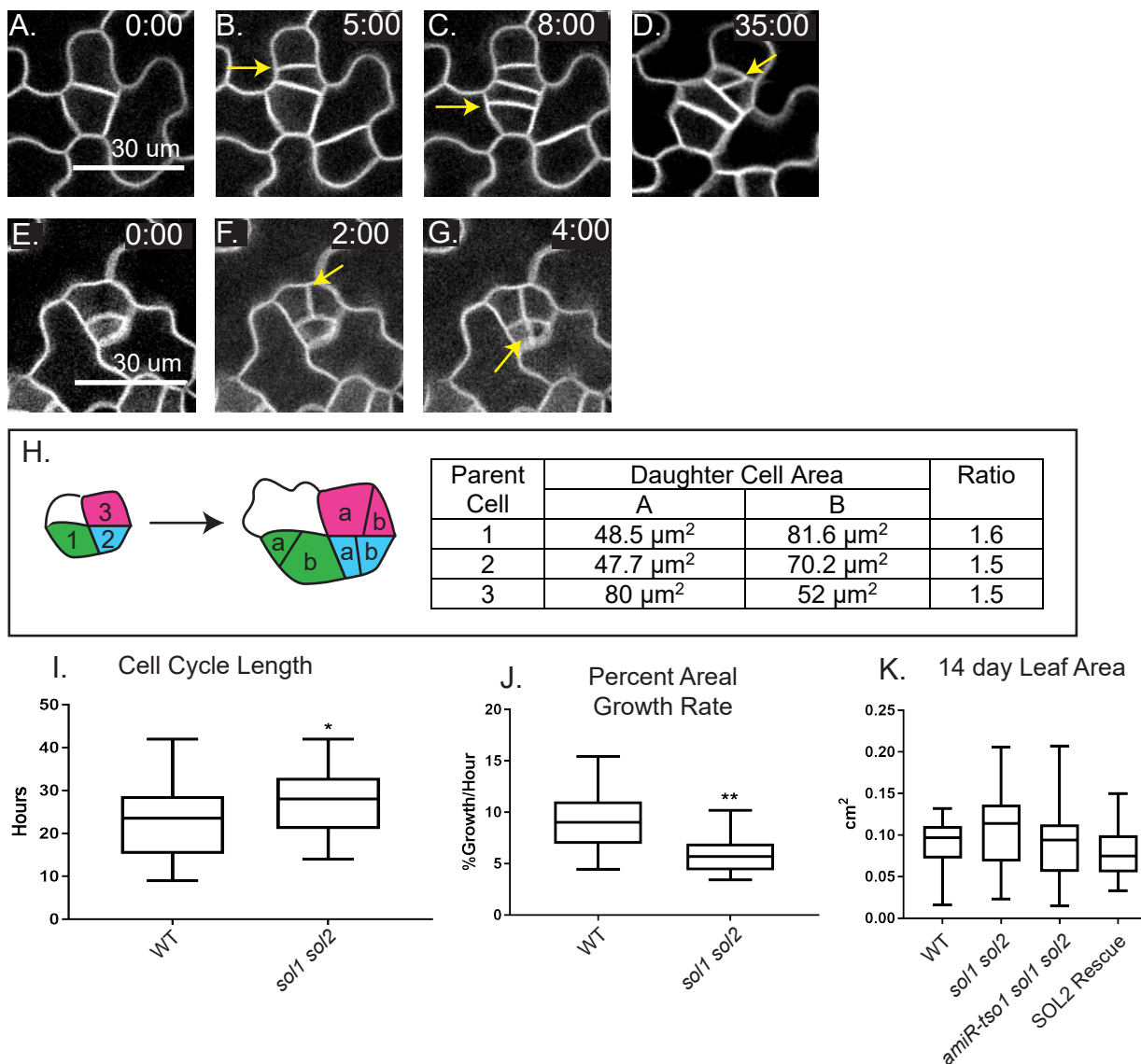
**Figure S1. Additional analysis of SOL1-YFP and SOL2-YFP, emphasizing connections between their protein expression and cell cycle stage**

(A-E) Time-lapse confocal imaging of SOL1p:SOL1-YFP in wildtype 6dpg true leaf; plasma membrane visualized with ML1p:RCI2A-mCherry, image captured every 30 min. SOL1 is expressed in two cells (A, yellow arrows). It turns off in the upper cell (B, dotted yellow arrow) then the lower cell (C, dotted yellow arrow). Each cell divides 2 hrs after SOL1-YFP expression is last seen (D, upper cell, white arrow) (E, lower cell, white arrow). (F-G) SOL2p:SOL2-YFP in wildtype is co-expressed with SPCHp:SPCH-CFP in some (white arrow), but not all meristemoids (white dotted arrow) and with MUTEp:MUTE-CFP in GMCs (arrowhead). SOL2 is also expressed in pavement cells (double arrows) that don't express SPCH or MUTE. Cell outlines (purple) visualized with propidium iodide. (H-L) Representative images from time-lapse of SOL2p:SOL2-YFP, HTR2p:CDT1a(C3)-RFP, in wildtype. SOL2-YFP is visible first (H), then co-expressed with CDT1a-RFP (I). CDT1a-RFP is not visible for one frame (K) presumably during nuclear envelope breakdown, however, it persists into both daughter cells (L). (M) Quantification of length of time from when YFP is first detected until before RFP is detected, green line indicates median at 3 hours. (N) Quantification of length of time from when YFP is last visible until cell divides, green line indicates median at 1 hour.



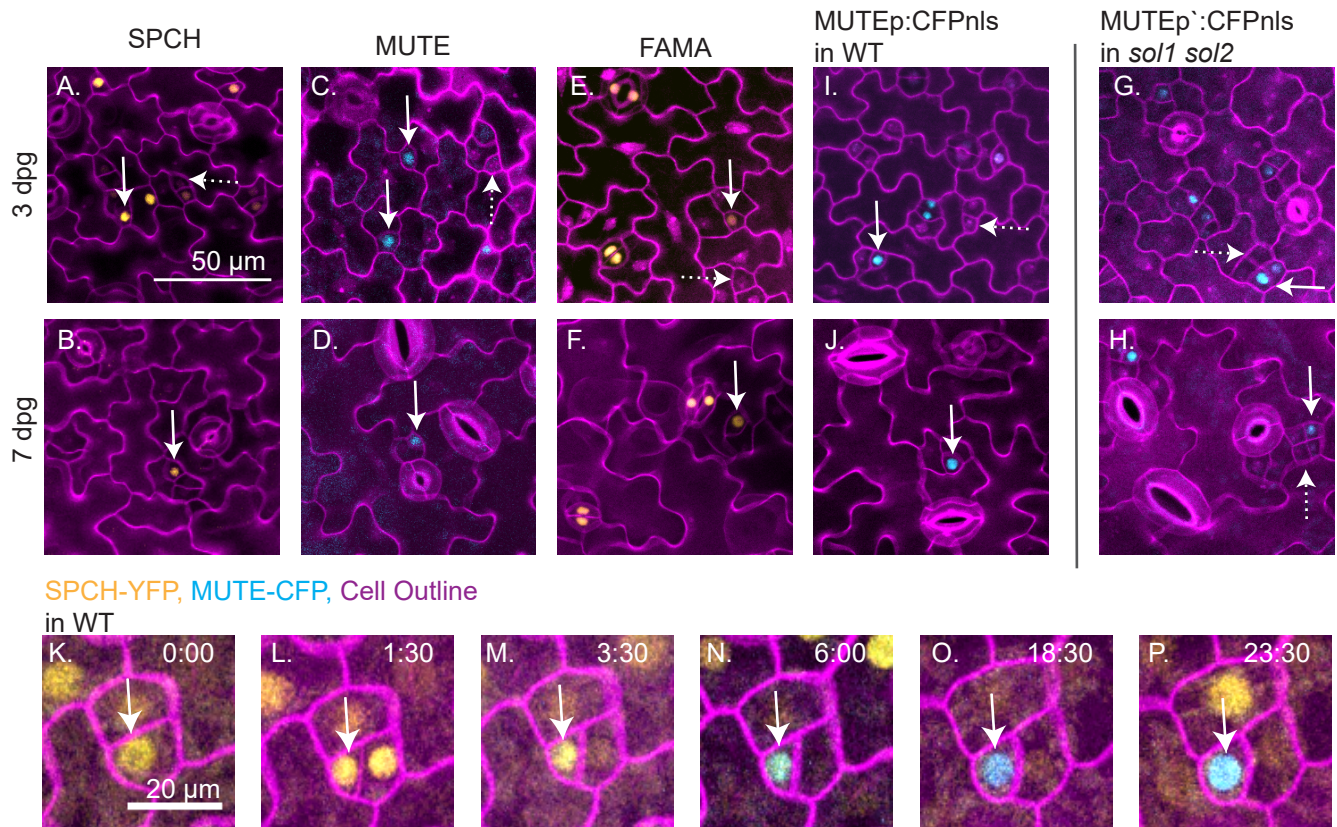
**Figure S2. Supporting information about alleles used for phenotypic analysis**

(A) Diagram of *SOL1* and *SOL2* genomic loci with position of T-DNA alleles indicated by triangles. (B) qRT-PCR analysis of expression levels of *SOL1* and *SOL2* transcripts in mutant seedlings at 9 dpv, levels are normalized to *ACT2* as a reference gene, 3 biological replicates per genotype, error bars indicate standard deviation. (C) Quantification of stomatal pair phenotypes in *SOL* single and double mutants, n = 9-10 plants/genotype. For all box and whisker plots, whiskers extend to minimum and maximum, box indicates interquartile range (25th percentile to 75th percentile) with center line indicating median. Significant difference compared to WT \*\*p<0.01, Dunn's multiple comparison test.



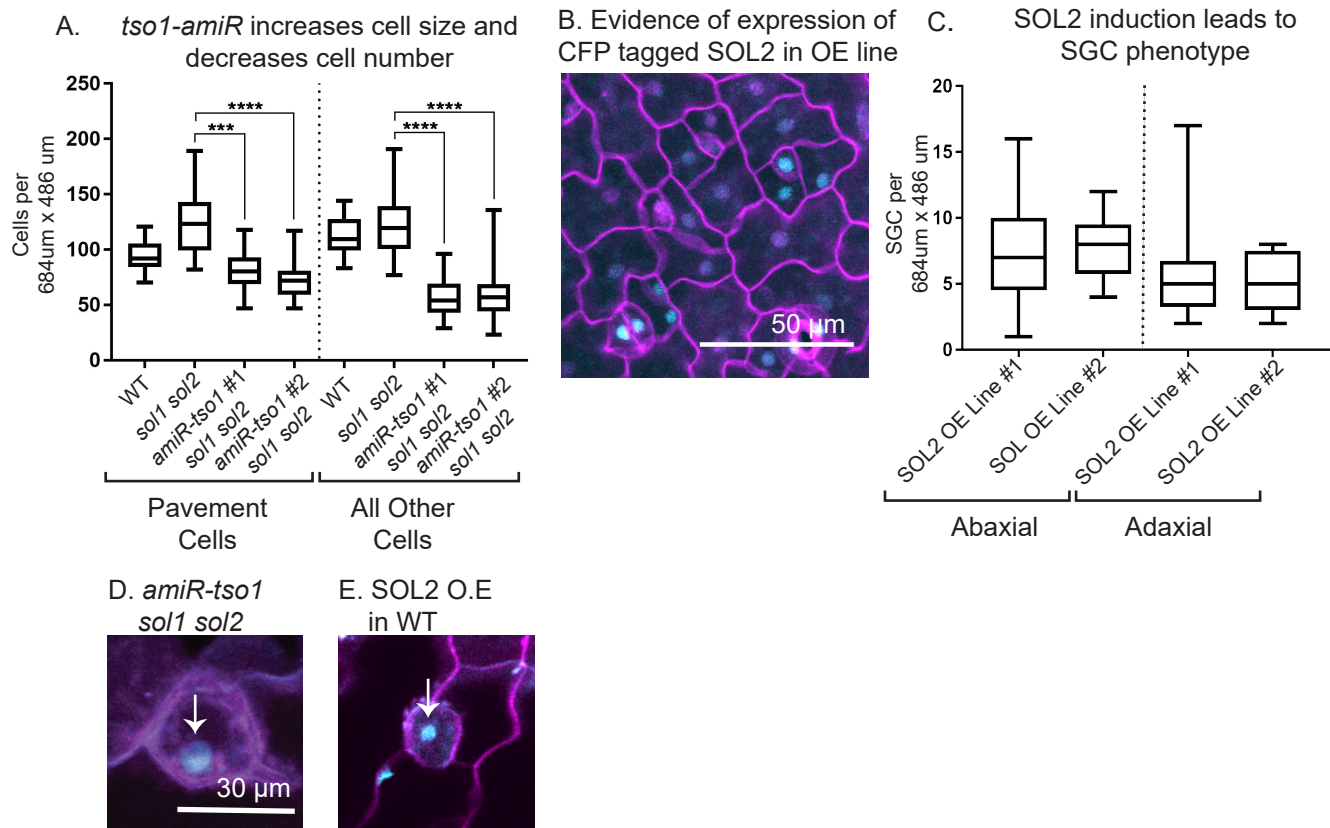
### Figure S3. Evidence that cell cycle times are not decreased, but post-division cell growth is reduced in *sol1 sol2* stomatal lineage cells

(A-G) Confocal time-lapse images of cells dividing in *sol1 sol2* as an example of data quantified in H-J, divisions indicated with yellow arrows. (H) Three cells (from Fig. 4L-M) divide asymmetrically, area of each daughter cell was measured with an ImageJ macro, displayed in table. Ratio of larger cell area/smaller cell area also displayed in table. (I) Cell cycle length is slightly increased in *sol1 sol2* mutants (WT n=24 cells scored, *sol1 sol2* n=22). (J) Percent growth per hour in small cells is reduced in *sol1 sol2* mutants (WT n=14 cells scored, *sol1 sol2* n=13). (K) Overall true leaf area at 14 dpf is not significantly different between WT and *sol1 sol2* mutants (WT n=24, *sol1 sol2* n=31, *amiR-tso1 sol1 sol2* n=17, SOL2 Rescue n=15). For all box and whisker plots, whiskers extend to minimum and maximum, box indicates interquartile range (25th percentile to 75th percentile) with center line indicating median. Significance indicated: \* p<0.05, \*\* p<0.01, \*\*\* p<0.001, Mann Whitney test.



**Figure S4. Additional reporter expression in WT and in *sol1 sol2* double mutants**

(A-B) SPCHp:SPCH-YFP in wildtype seedlings. (C-D) MUTEp:MUTE-CFP in wildtype seedlings. (E-F) FAMAp:YFPnls in wildtype seedlings. (I-J) MUTEp:CFPnls in wildtype seedlings. (G-H) MUTEp:CFPnls in *sol1 sol2* seedlings. All images at same scale. (K-P) Images from time-lapse of SPCHp:SPCH-YFP and MUTEp:MUTE-CFP markers in 6 dpv wildtype leaf; plasma membrane visualized with ML1p:RCI2A-mCherry. SPCH expressing cell, indicated by arrow divides (L) and then begins to express MUTE (N).



**Figure S5. Quantification of effects of *tso-1* amiRNA and SOL2-CFP overexpression on cell size and division phenotypes**

(A) Quantification of the changes in cell size and numbers in *tso1*-amiRNA *sol1 sol2* show decreased number of pavement cells and other cells (non-pavement cells, including guard cells) relative to *sol1 sol2* (WT  $n=20$ , *sol1 sol2*  $n=24$ , *amiR-tso1* #1 *sol1 sol2*  $n=18$ , *amiR-tso1* #2 *sol1 sol2*  $n=31$ ). (B) Overexpression of SOL2-CFP (SOL2 O.E.) in 4 dpf seedling is evident throughout epidermis 24 hours after beta-estradiol induction. (C) Incidence of SGCs per field of view in two independent lines of induced seedlings. Seedlings induced at 3 dpf, screened for expression, then collected for analysis at 8 dpf (SOL2 OE Line #1  $n=13$ , SOL2 OE Line #2  $n=14$ ). (D-E) Hoechst 33342 nuclear staining showing that the SGC in both *amiR-tso1 sol1 sol2* (D) and SOL2 OE in wildtype (E) contain a single nucleus. D and E are reproduced from Fig. 6D and H insets, respectively, shown larger. For all box and whisker plots, whiskers extend to minimum and maximum, box indicates interquartile range (25th percentile to 75th percentile) with center line indicating median. Significance indicated: \*\*\*  $p<0.001$ , \*\*\*\*  $p<0.0001$ , Dunn's multiple comparison test.

**Table S1. Primers used in this study**

	<b>Forward primer (5'-3')</b>	<b>Reverse Primer (5'-3')</b>
SOL1 genomic cloning	CACCATGGATACACCCGAAAAGAGTGAAAC	ATGGTGTGGAGTGAGAGAAGGAAAC
SOL1pro cloning	GGGGACAACCTTTGTATAGAAAAGTT GATCCCAAACATTTTATCCCATGGG	GGGGACTGCTTTTTTGTACAAACTTGTTTC TAACTACCAAAAACAATCTC
SOL2 genomic cloning	CACCATGGATACCCCTCAGAAGAGTATTACTCAG	GTGTTGGGGAGTGAGAGAAGGAAAC
SOL2pro cloning	GGGGACAACCTTTGTATAGAAAAGTTGTTACTT GTCCCAACTCAGATCG	GGGGACTGCTTTTTTGTACAAACTTGTTCCA ACACACAAACAAAAAATCAC
UBQ10pro cloning	CATGGCGCGCCAGTCTAGCTCAACAGAGCTTTTAAAC	GAGCTCCTGTTAATCAGAAAAACTCAGATTAA
SOL1 qPCR	CCAAGAAGAAAAGGCGTAAGTCC	CACAGTAAAGCTTCAAACACTTGG
SOL2 qPCR	ATCTTTGACTCACCTGATGCTTCTG	GTGAAACAGCCTCATAAGGAATCG
ACTIN qPCR	TCTTCCGCTCTTTCTTTCCAAGC	ACCATTGTCACACACGATTGGTTG
WiscDsLox- HS033_3E Genotyping	CACACACACACCCACAAAAG	TCTCTGTTGGATTTGGTTTGG
SAIL_742_H03 Genotyping	TGATTAGCAATATTCAGCCAGC	CTTTATGAGAAACCGCGTGAG
SALK_021952 Genotyping	AGATTGCAGACAAAGCAAAGC	TGGAGAATCCTGCATTTTCAG
SALK_031643 Genotyping	AGATTGCAGACAAAGCAAAGC	TGGAGAATCCTGCATTTTCAG
SALK_074231 Genotyping	GCTGGAATAGACCGTAGTATCAGC	GCTCATACCCCCTAGCATCTC