New Phytologist Supporting Information Figs S1–S10 and Table S1

Article title: SMALL LEAF AND BUSHY1 controls organ size and lateral branching by modulating the stability of BIG SEED1 in *Medicago truncatula*

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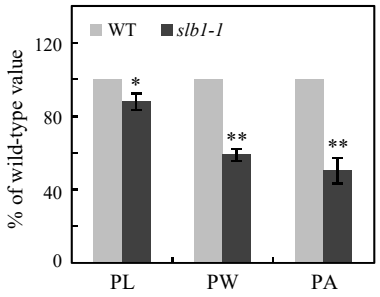

Fig. S1 Comparsion of vexillum petal size of *M. truncatula* wild type and *slb1-1*.
Vexillum petal length (PL), vexillum petal width (PW), vexillum petal area (PA) of the wild type (WT) and *slb1-1*. Bars represent means ± SD (n = 15); asterisks indicate significant differences from WT (*$P < 0.05$, **$P < 0.01$, Student's t test).

Fig. S2 The *M. truncatula slb1* mutant shows defects in floral organ development.
(a-d) The mature flower (a), dissected vexillum petal (b), the fused alae and keel petals (c), and the stamens and carpel (d) of wild-type (WT) *M. truncatula*. The inset is the magnification of anthers. Red arrow indicates the stigma. Bars = 2 mm. (e-h) The mature flower (e), dissected vexillum petal (f), the alae and keel petals (g), and the stamens and carpel (h) of *slb1-1*. The inset is the magnification of anthers. Red arrow indicates the stigma. Bars = 2 mm. (i, j) Pollen staining in WT (i) and *slb1-1* (j). Bars = 0.5 mm. (k, l) *In vitro* pollen tube germination of WT (k) and *slb1-1* (l). Bars = 0.5 mm. (m-p) The carpels (m, n) and ovules (o, p) of WT. Bars = 1 mm in (m-o), and 0.2 mm in (p). (q-t) The carpels (q, r) and ovules (s, t) of the *slb1-1* mutant. Bars = 1 mm in (q-s), and 0.2 mm in (t).

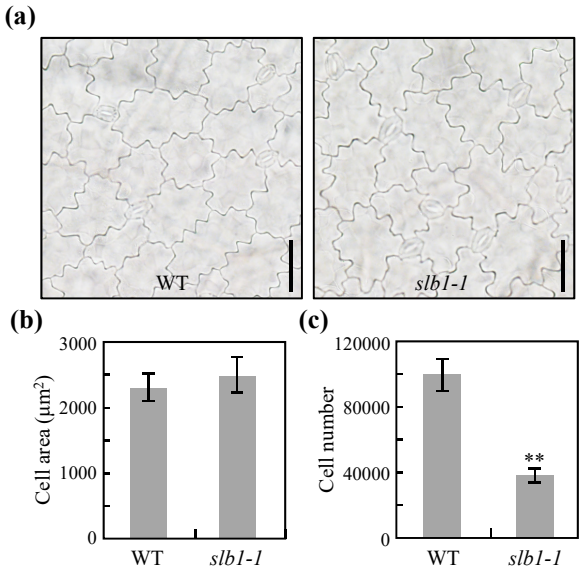

Fig. S3 Disruption of *SLB1* leads to significant decreases in leaf size, primarily by affecting cell proliferation in *M. truncatula*.

(a) Micrographs of cleared wild-type (WT) and *slb1-1* leaves. Bars = 50 μm. (b) Comparison of cell area in WT and *slb1-1* leaves. Bars represent means ± SD (n = 15). (c) Comparison of cell number in WT and *slb1-1* leaves. Bars represent means ± SD (n = 15); asterisks indicate significant differences from the WT (**P < 0.01, Student's t test).

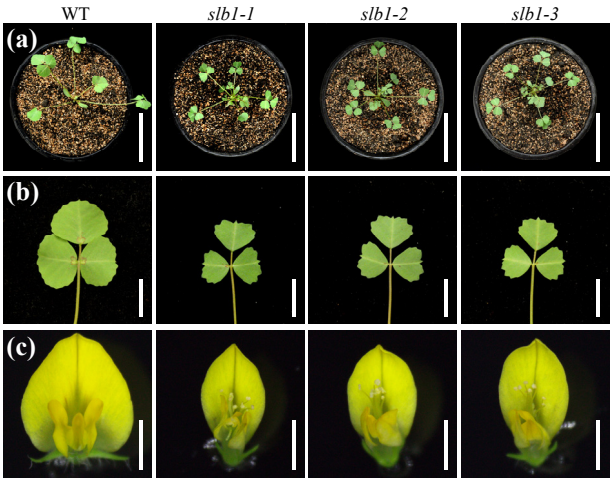

Fig. S4 Phenotypes of the *M. truncatula* wild type and *slb1* alleles.
(a) 3-week-old seedlings of the wild type (WT) and *slb1* alleles. Bars = 5 cm. (b, c) Phenotypes of leaves (b) and flowers (c) of WT and *slb1* mutants. Bars = 1 cm for leaves and 2 mm for flowers.

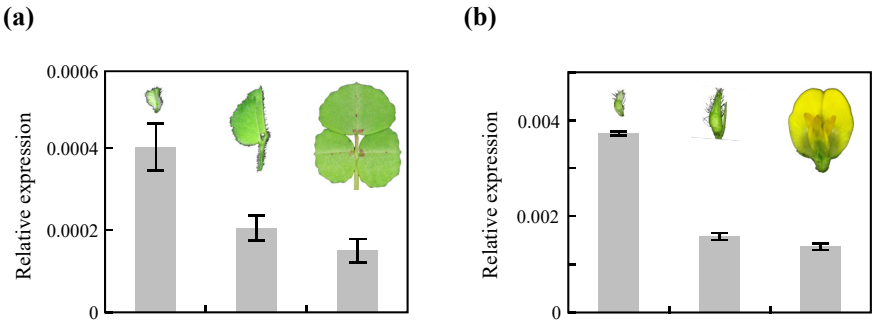

Fig. S5 Transcript abundance of *SLB1* in *M. truncatula* leaves and flowers at different developmental stages.

Quantitative RT-PCR analysis of *SLB1* expression in leaves (a) and flowers (b) at different developmental stages. Bars represent means ± SD (n = 3).

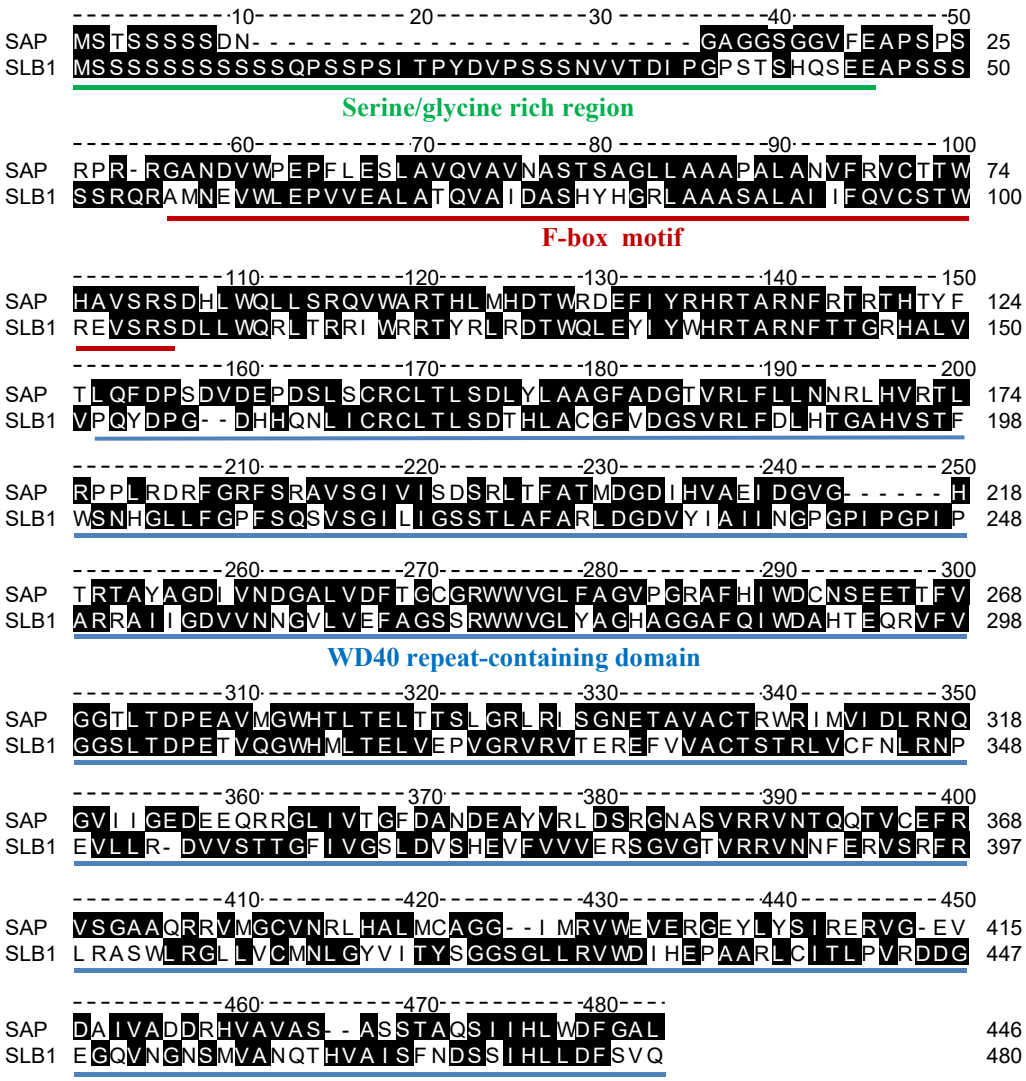

Fig. S6 Sequence alignment of *M. truncatula* SLB1 and Arabidopsis SAP.
The conserved serine/glycine rich region, F-box motif, and WD40 repeat-containing domain sequences are underlined in green, red, and blue, respectively.

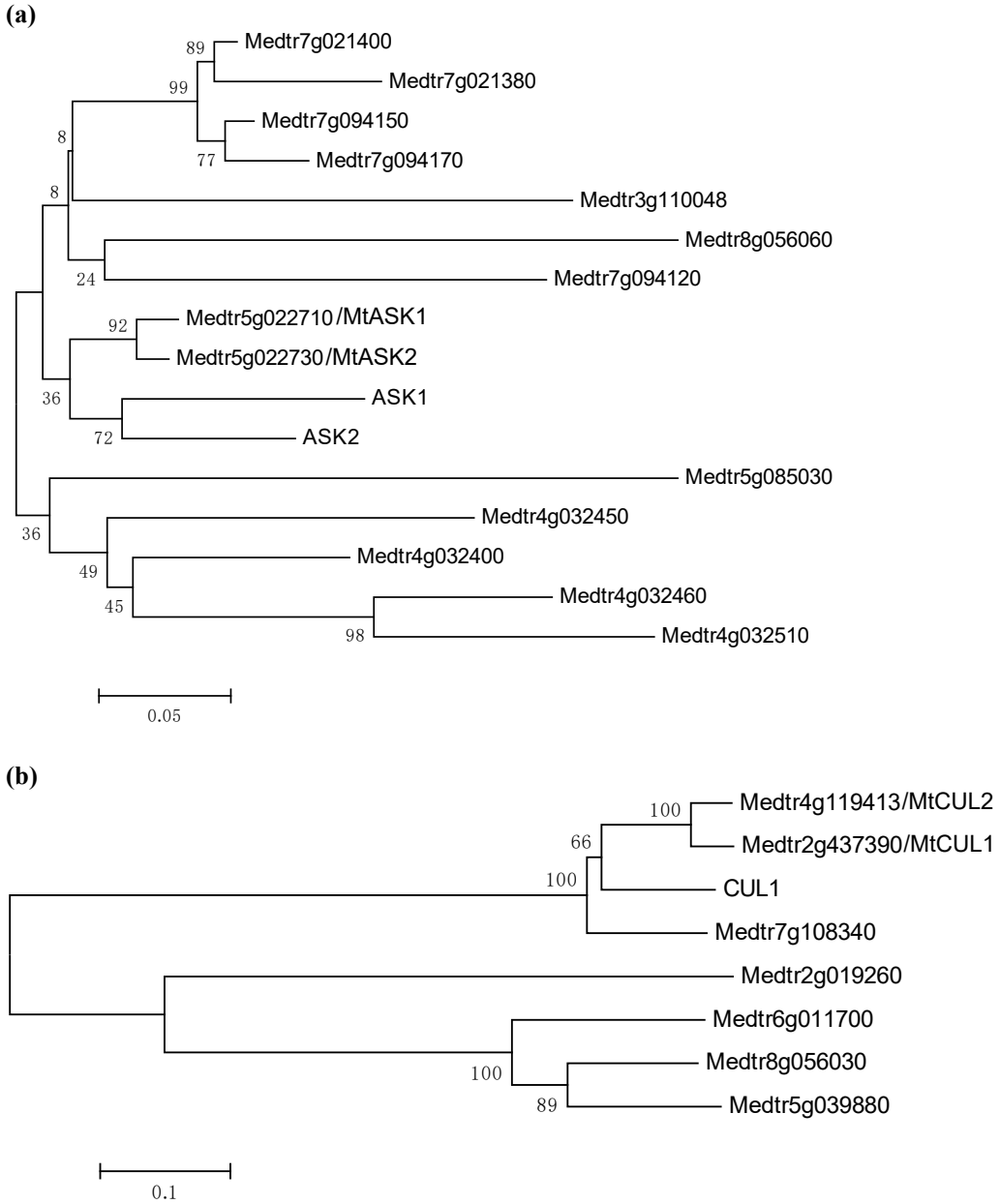

Fig. S7 Phylogenetic analysis of ASK1/2-like and CUL1-like family proteins in *M. truncatula.*

Phylogenetic analysis of *M. truncatula* ASK1/2-like (a) and CUL1-like (b) proteins. The protein sequences were obtained from the *Medicago truncatula* Genome Database (http://www.medicagogenome.org). Full-length amino acid sequences were aligned using ClustalW, and the trees were constructed using MEGA6.0 with 1000 replicates to generate bootstrap values.

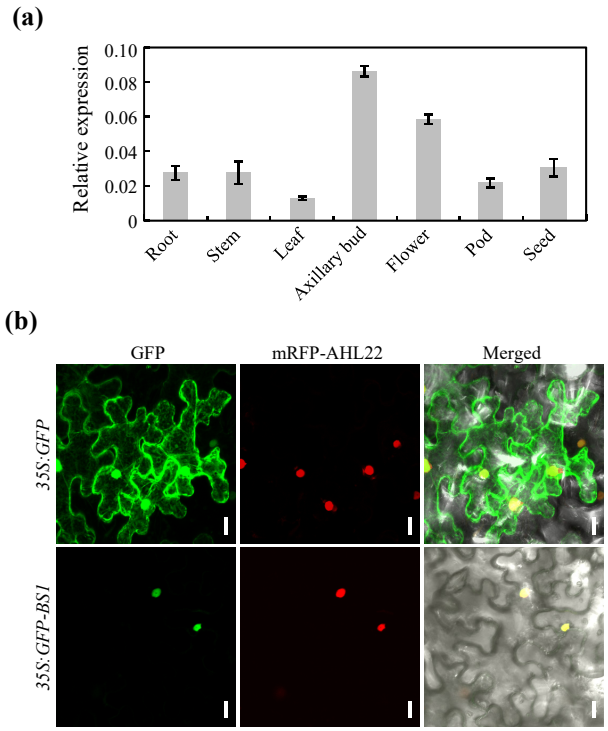

Fig. S8 Expression pattern of *BS1* in *M. truncatula* and subcellular localization of BS1. (a) Transcript levels of *BS1* in different *M. truncatula* tissues, as revealed by quantitative RT-PCR. *MtActin* was used as an internal control. Bars represent means ± SD (n = 3). (b) Subcellular localization of GFP and GFP-BS1 in tobacco leaf epidermal cells. The nuclear protein AHL22 was used as a nuclear localization marker. Bars = 20 μm.

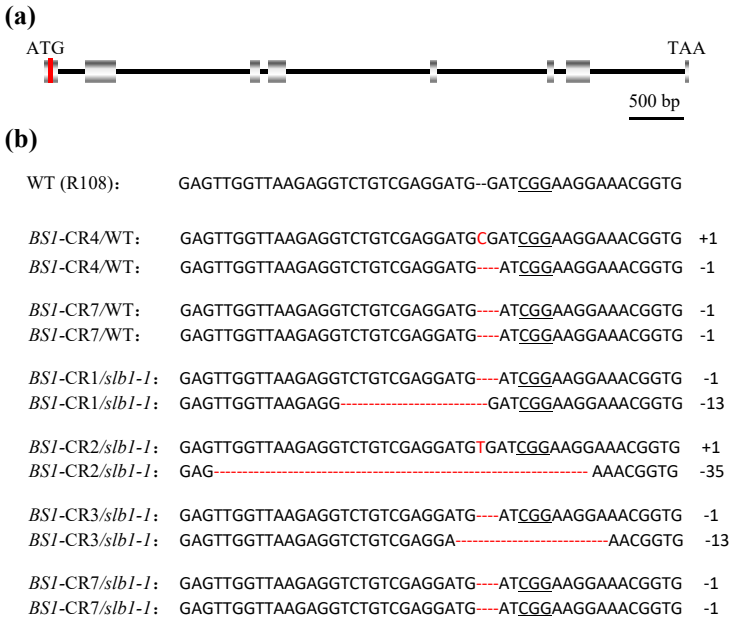

Fig. S9 Targeted mutagenesis of *M. truncatula BS1* using the CRISPR/Cas9 system.
(a) Schematic representation of the gene structure of *BS1*. The red line indicates the CRISPR/Cas9 target site. (b) Nucleotide sequence of wild-type (WT) *BS1* aligned to the sequences of the mutant alleles. Red font indicates base deletions or insertions; the protospacer adjacent motifs are underlined.

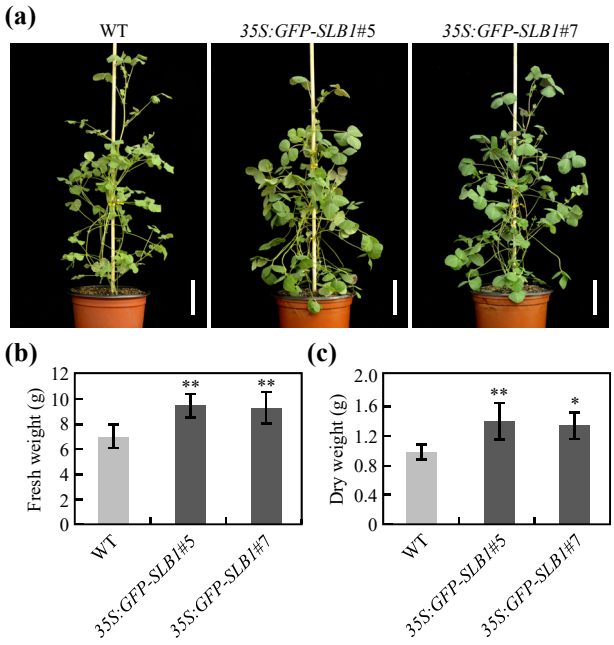

Fig. S10 *SLB1* overexpression improves biomass yield in *M. truncatula*.
(a) Morphology of 9-week-old wild-type (WT) and *SLB1* overexpressing *M. truncatula* plants. Bars = 5 cm. (b, c) Comparison of fresh (b) and dry (c) weights of aboveground biomass in 9-week-old WT and *SLB1* overexpressors. Bars represent means ± SD ($n = 5$); asterisks indicate significant differences from WT (*$P < 0.05$, **$P < 0.01$, Dunnett's test).

Table S1. Primers used in this study

Primers	Sequences 5'-3'	Application
SLB1-NF11180 /NF19156-F	ATGGTTGGAAAATGCATGC	For genotyping of *slbl-1* and *slb1-3*
SLB1-NF11180 /NF19156-R	ATTATTCACGCGCCGAACA	
SLB1-NF20634-F	TTTGTTCAACATGGAGGGAA	For genotyping of *slb1-2*
SLB1-NF20634-R	AGTTCCAACACCACTTCTTT	
LTR6	GCTACCAACCAAACCAAGTCAA	Primers in *Tnt1* for genotyping of different mutant
LTR31	CTCCTCTCGGGGTCGTGGTT	
gSLB1-F	CCATGATTACGAATTCCAACCGTCAACAT GAAATGAATCCA	To amplify gDNA of *SLB1*
gSLB1-R	GTCGACAGATCCCCGGGACTAATATGTGG TCTTGCTAGCCA	
pSLB1-F	CACCCAACCGTCAACATGAAATGAATCCA	To amplify promoter of *SLB1*
pSLB1-R	GAGGAGTGAGAGAAGTGGAGGG	
GFP-attB1-F	GGGGACAAGTTTGTACAAAAAAGCAGGCT TCATGGGTAAAGGAGAACTTTTCA	To amplify CDS of *GFP*
6*Gly-GFP-R	GCCACCCCCTCCGCCACCGGCATAATCAG GCACATCG	
6*Gly-SLB1-F	GGTGGCGGAGGGGGTGGCATGTCTTCTTC TTCCTCCTCCT	To amplify CDS of *SLB1*
SLB1-attB2-R	GGGGACCACTTTGTACAAGAAAGCTGGGT CTTACTGTACACTAAAATCCAATA	
BS1CDS-F	CACCATGAACGGCGGAAGCACCGTTTCCT	To amplify CDS of *BS1*
BS1CDS-R	TTAGCATTCTTGAACATCTTTATCATTC	
MtU6-F1	GCTTAGGCCTTCTAGAATCCAACATTTCAC TTGAGTTAACT	To amplify *MtU6* promoter
MtU6-R1	AAACCCTGCTGTTCGTCTAG	
BS1-sgRNA-F	CTAGACGAACAGCAGGGTTTGAGGTCTGT CGAGGATGGATGTTTTAGAGCTAGAAATAG	To amplify sg RNA-scaffold fragment
R1	GGCAACGCGTTCTAGAAAAAAAAGCACCG ACTCGGTGC	
6*Gly-BS1-F	GGTGGCGGAGGGGGTGGCATGAACGGCG GAAGCACCGTTTCCT	To amplify CDS of *BS1*
BS1-attB2-R	GGGGACCACTTTGTACAAGAAAGCTGGGT CTTAGCATTCTTGAACATCTTTATCA	
MtASK1-5'UTR-F	AGGGTTAGCGATTTCACAAA	To amplify CDS and UTR of *MtASK1*
MtASK1-3'UTR-R	TCTACATTGCGGAACTTTGA	

MtASK2-5'UTR-F	TCGAAAAGGGGAAGAACAAT	To amplify CDS and UTR of *MtASK2*
MtASK2-3'UTR-R	AGATCCTTGACTTAGATGCT	
MtASK-F	CACCATGTCTTCAACAAGAAAGATCACTCT	To amplify CDS of *MtASK1* and *MtASK2*
MtASK-R	TTCAAATGCCCATTGGTTTTCCCTAC	
MtCUL1-F	CACCATGAGTGAACGGAAGACTATTG	To amplify CDS of *MtCUL1*
MtCUL1-R	AGCCAAGTACTTGAACAAATTTGCATTA	
MtCUL2-F	CACCATGTCAATGAGTGAAAGGAAAA	To amplify CDS of *MtCUL2*
MtCUL2-R	AGCTAAGTACTTGAACATATTTGGATTCTC	
MtWD40-1-attB1-F	GGGGACAAGTTTGTACAAAAAAGCAGGCT TCATGGATAATTCAACACAAGAATCCC	To amplify CDS of *MtWD40-1*
MtWD40-1-attB2-R	GGGGACCACTTTGTACAAGAAAGCTGGGT CAACCCTCAAAAGCTGCATT	
RT-SLB1-F	CACCATGTCTTCTTCTTCCTCCTCCT	For RT-PCR analysis of *SLB1*
RT-SLB1-R	CTGTACACTAAAATCCAATA	
RT-MtActin-F	TCTTACTCTCAAGTACCCCATTGAGC	For RT-PCR analysis of *MtActin*
RT-MtActin-R	GTGGGAGTGCATAACCCTCATAGATT	
qSLB1-F	GGCCACTCAAGTTGCCATTG	For quantitative RT-PCR analysis of *SLB1*
qSLB1-R	ATCCGAACGTGACACTTCCC	
qMtCYCD-F	TCTTGGATGGAAGATGAGTCCAGC	For quantitative RT-PCR analysis of *MtCYCD*
qMtCYCD-R	ACATGAACCATTGTAGCAGTTGCC	
qMtH4-F	AAGGGTGGTGCTAAGCGTCACCGC	For quantitative RT-PCR analysis of *MtH4*
qMtH4-R	TGTTCAGTGTAAGTGACAGCATCACG	
qRT-MtActin-F	TCAATGTGCCTGCCATGTATGT	For quantitative RT-PCR analysis of *MtActin*
qRT-MtActin-R	ACTCACACCGTCACCAGAATCC	
qGmActin11-F	ATCTTGACTGAGCGTGGTTATTCC	For quantitative RT-PCR analysis of *GmActin11*
qGmActin11-R	GCTGGTCCTGGCTGTCTCC	