FIG S1

A

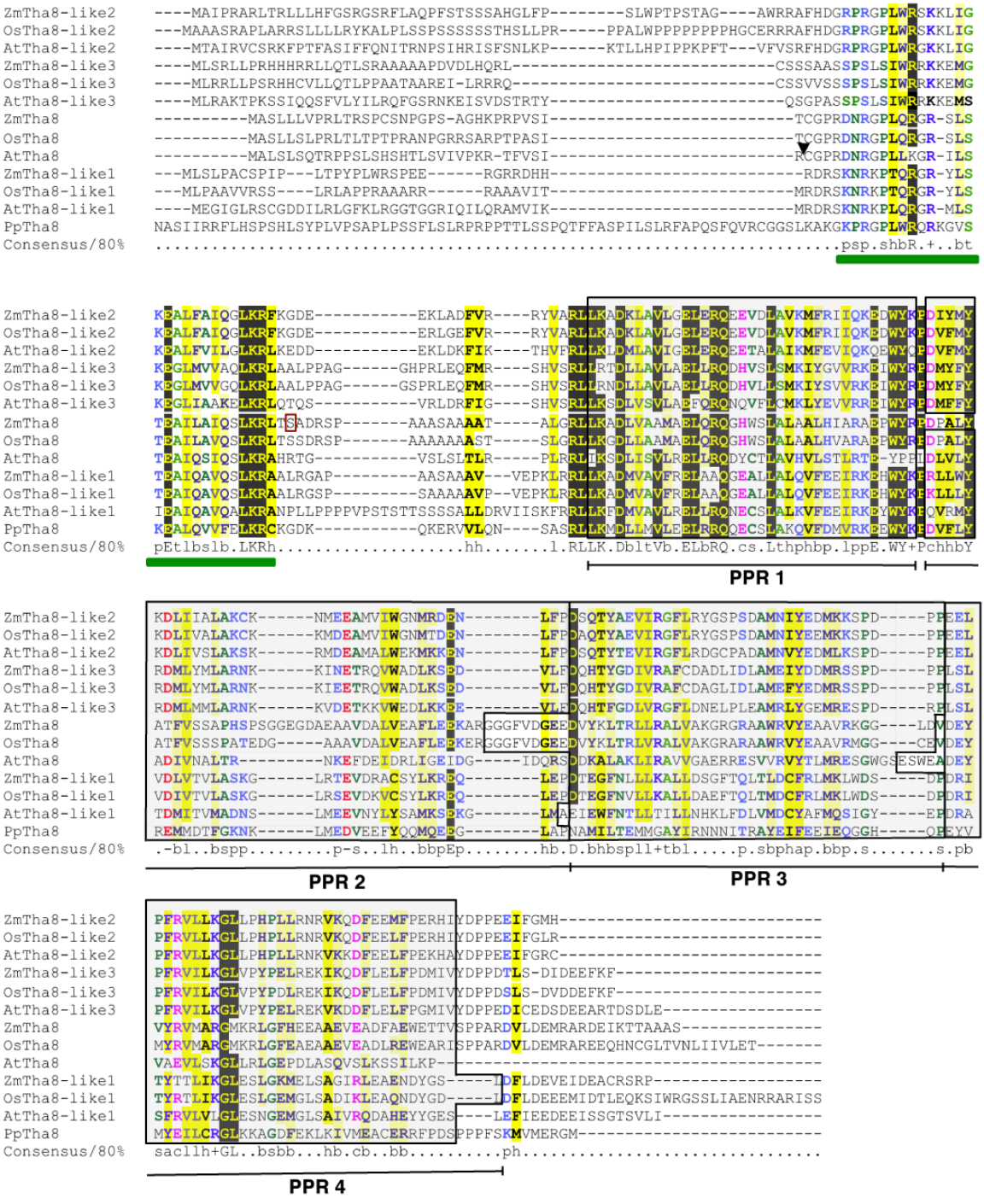
ZmTha8-like2
OsTha8-like2
AtTha8-like2
ZmTha8-like3
OsTha8-like3
AtTha8-like3
ZmTha8
OsTha8
AtTha8
ZmTha8-like1
OsTha8-like1
AtTha8-like1
PpTha8
Consensus/80%
PPR 1
PPR 2
PPR 3
PPR 4

FIG S1

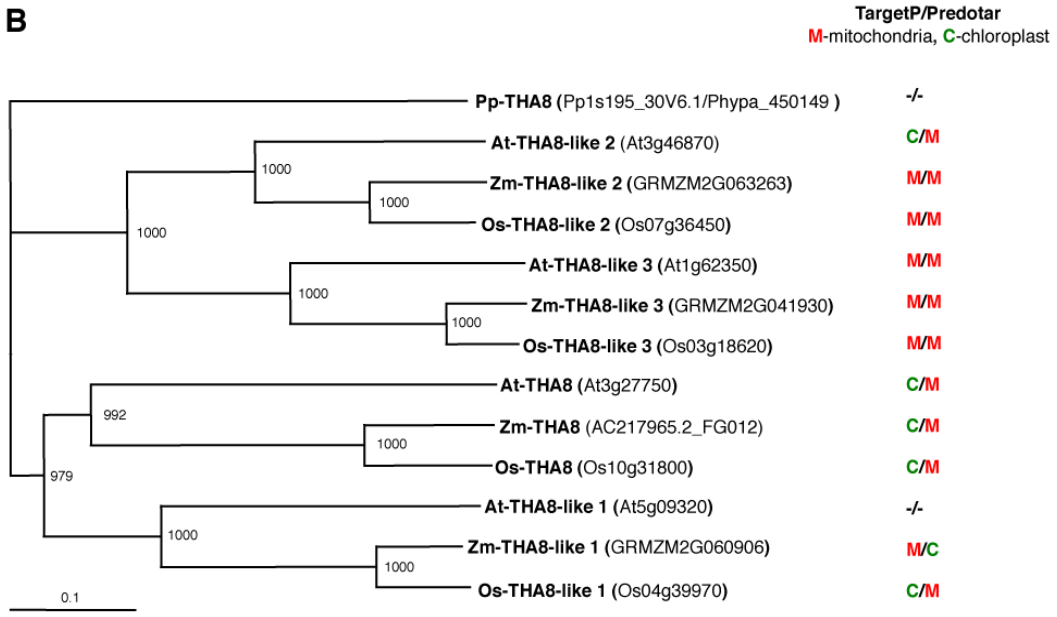

FIG S1 THA8-like proteins in land plants.

(A) Alignment of THA8 and THA8-like proteins from *Arabidopsis thaliana* (At), rice (*Oryza sativa*, Os), maize (*Zea mays*, Zm) and moss (*Physcomitrella patens*, Pp). These protein sequences were identified as the top BLAST hits with a THA8 query at http://www.ncbi.nlm.nih.gov/, http://www.maizesequence.org/, and http://www.cosmoss.org/. Gene identifiers for the aligned proteins are listed in panel B. The transit peptide cleavage site predicted for Zm-THA8 by ChloroP (Emanuelsson et al. 1999) is marked with a red box. However this cleavage site would remove conserved amino acids and is unlikely to be correct. The predicted cleavage site for At-THA8 is marked with a black triangle. The alignment was generated using Clustal X (Larkin et al. 2007) and formatted using CHROMA (http://www.llew.org.uk/chroma/index.html). The 120 N-terminal amino acids from Pp-THA8 were omitted from the alignment. The PPR motifs predicted by TPR-pred (Karpenahalli et al. 2007) are marked. The annotated start codons for several of these proteins are likely to be incorrect, as similarity among family members could be improved substantially by using alternative start codons. We believe that annotated AtTHA8-like 3 (At1g62350) lacks 56 amino acids at the N-term, annotated OsTha8-like1 (Os04g39970) lacks 29 amino acids at the N-term, and annotated ZmTha8-like1 (GRMZM2G060906) lacks 30 amino acids at the N-term. In addition, At5g09320 (AtTha8-like1) is misannotated in TAIR as a gene fusion that connects VSP9B (N-term part) to AtTha8-like1. The alignment here uses the corrected gene models.

(B) Phylogenetic tree constructed using Clustal X and the neighbour-joining (NJ) method with a seed number of 111 and 1000 bootstrap trials. The resulting tree was visualized using TreeView (Page 1996). PpTHA8 was chosen as an outgroup. Subcellular localisations predicted by TargetP (Emanuelsson et al. 2000) and Predotar (Small et al. 2004) are shown to the right. M - mitochondria, C - chloroplast.

FIG S2

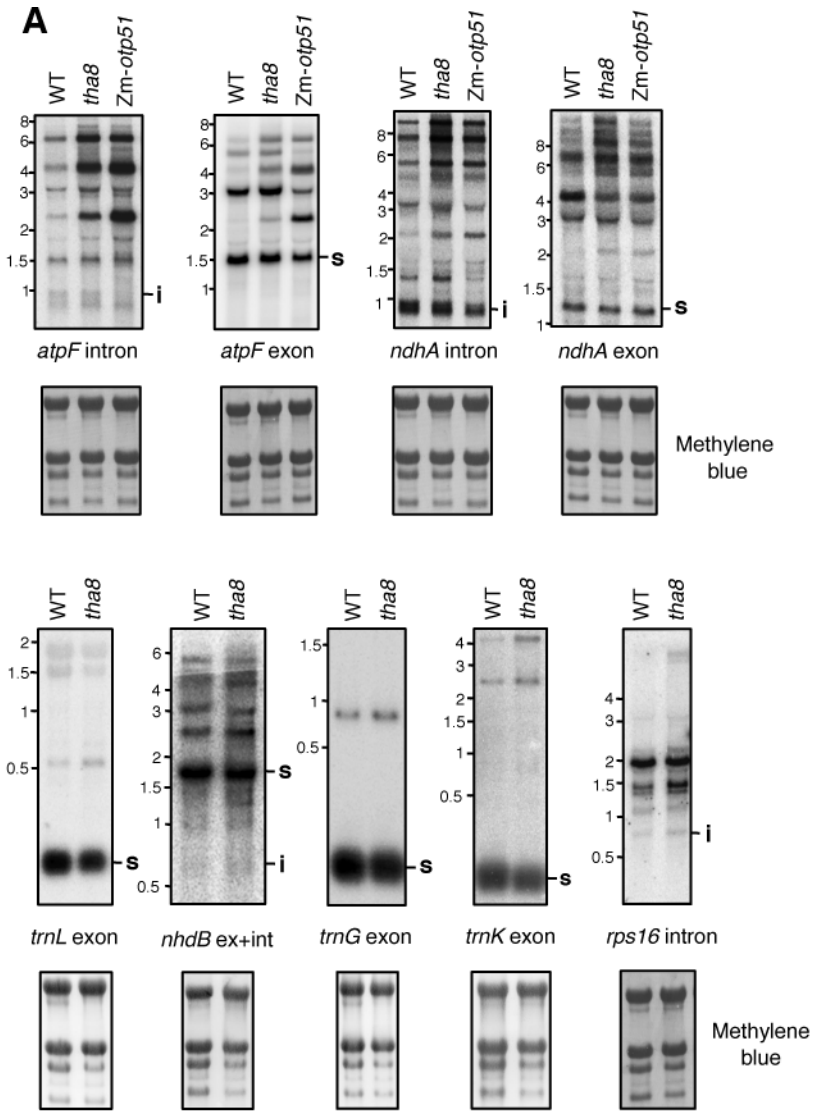
A
WT
tha8
Zm-otp51
atpF intron
atpF exon
ndhA intron
ndhA exon
Methylene blue
trnL exon
nhdB ex+int
trnG exon
trnK exon
rps16 intron
Methylene blue

FIG S2

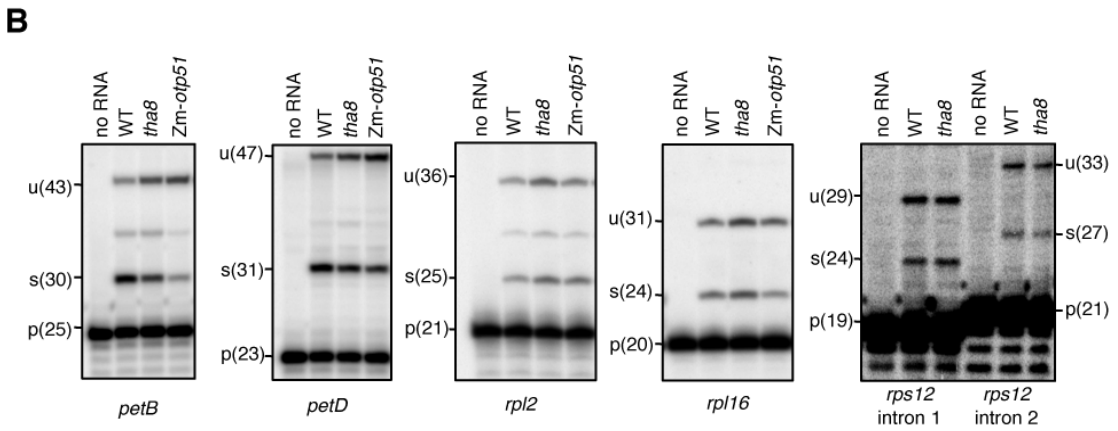

FIG S2 RNA gel blots and poisoned primer extension assays of intron-containing plastid RNAs that are not significantly affected in *tha8* mutants. (A) Northern blots probed to detect the indicated sequences. The corresponding methylene blue stained filters are shown below. s – spliced mature RNA, i – excised intron. (B) Poisoned-primer extension assays. Primer extension reactions were primed with an oligonucleotide binding a short distance downstream of the indicated splice junction, in the presence of a dideoxynucleotide that terminates reverse transcription at different distances on spliced and unspliced templates. u – product of unspliced RNA, s – product of spliced RNA, p – primer. Sizes in nucleotides are indicated.

FIG S3

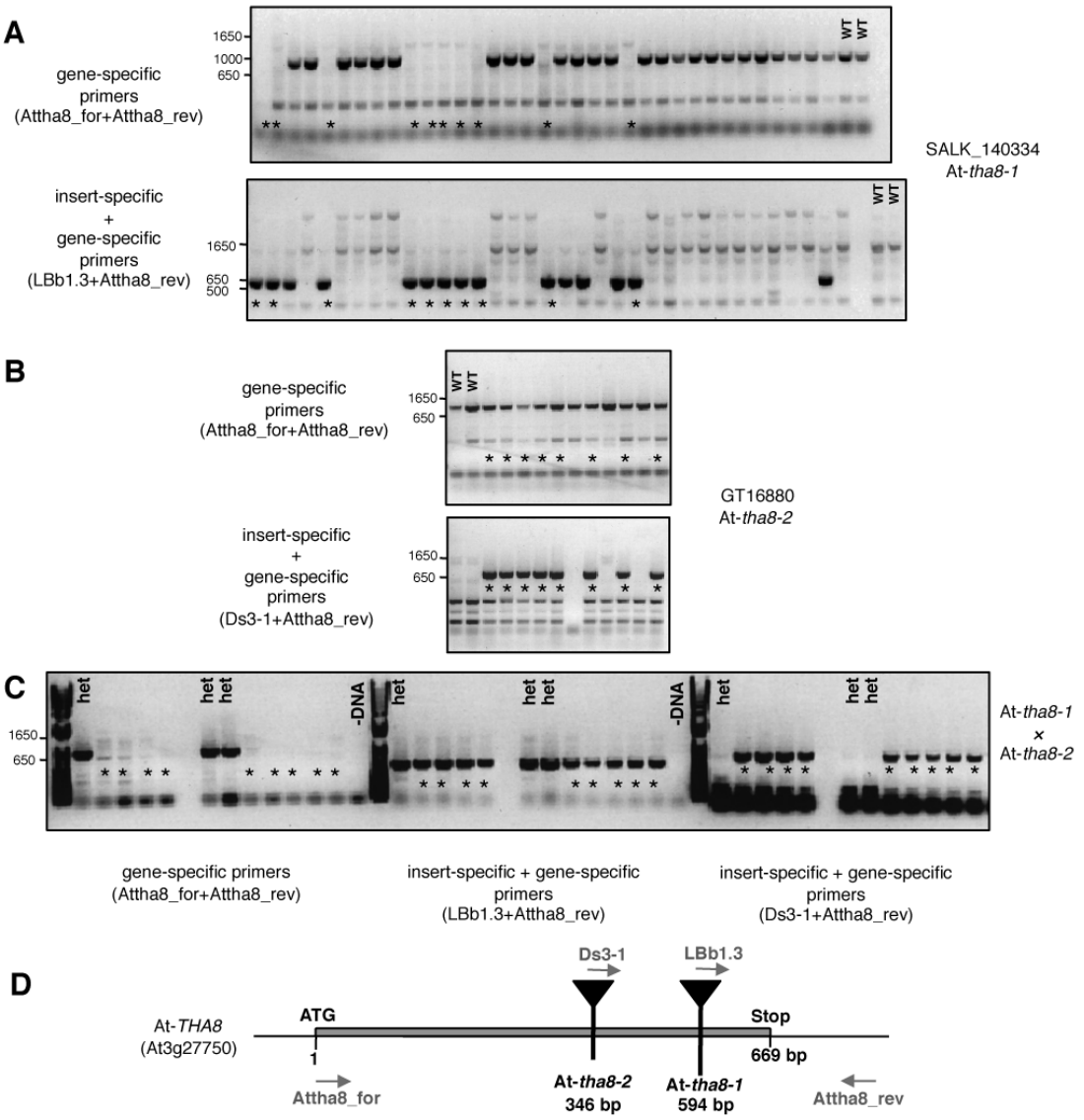

FIG S3 Genotyping of *At-tha8* lines. (A) *At-tha8-1* (SALK_140334). PCR on DNA from phenotypically mutant plants (marked with asterisk) produced no wild-type product with gene specific primers (920 bp) (upper panel) but gave rise to an insert-specific band with T-DNA and gene specific primers (lower panel). (B) *At-tha8-2* (GT16880). PCR with gene-specific primers showed that no homozygous mutant plants were recovered among the plants assayed (PCR on 11 out of 35 assayed plants is shown). PCR on DNA from heterozygous plants (marked with asterisk) give rise to both gene-specific (920 bp, upper panel) and insert specific (lower panel) products. (C) Complementation cross between *At-tha8-1* and *At-tha8-2* mutant lines. Plants with a mutant phenotype (marked with asterisk) have no wild-type allele, but have both *At-tha8-1* and *At-tha8-2* alleles. "Het" – *At-tha8-1*/Ler WT. (D) Schematic of the genotyping primers.

Table S1

Genotyping of Zm-*tha8* and Zm-*otp51* alleles		
RMtha8*15	CGGCAGTGGTTTTGATCTCG	gene-specific primer for Zm-*tha8*
pl1_3_1032	GCAGGGTTCATGTTGCGATT	gene-specific primer for Zm-*otp51*
EOMuMix	(50% GCCTCCATTTCGTCGAATCCC + 50% GCCTCTATTTCGTCGAATCCG)	*Mu*-specific primer mix
Northern probes		
RMtha8*18S-EcoRI	GCGGAATTCCCTGCGGGCCGCGCGA	Zm tha8 probe
RMtha8*19SmodXhoI	GCGCTCGAGCTAGGAGGCAGCGRCAGT	Zm tha8 probe
	ATGCCTAGATCCCGTATAAAT	Zm ycf3 exon probe
RC IRF170Rev-2	GCTATAGCTTGTTTCCAATACTCA	Zm ycf3 exon probe
IRF170 12A	TGATCTGTCATTACGTGCGACTA	Zm ycf3 intron 2 probe
IRF170 12B	CAGTTCCTTTCCCTAGAACCGTA	Zm ycf3 intron 2 probe
IRF170 6	GGATTGAGCCAACATCCGTTA	Zm ycf3 intron 1 probe
IRF170 5	ACTTATTATAGAGATGGTGCGATTTGA	Zm ycf3 intron 1 probe
BT_TRNA5	GGGGATATAGCTCAGTTGGTAGAGCTCCG	Zm/At trnA exon
BT_TRNA3	TGGAGATAAGCGGACTCGAACCGC	Zm/At trnA exon
trnAinF	GGTCGTTGCGATTACGGGTT	Zm trnA intron
trnAinR	TTTCCGCCCGACTCTTTGAT	Zm trnA intron
TRNV5	AGGGCTATAGCTCAGTTCGGT	Zm trnV exon
TRNVT7	TAGGGCTATACGGAT	Zm trnV exon
trnKF	TGGGTTGCCCGGGACTCGAA	Zm trnK exon
trnKR	GGGTTGCTAACTCAATGGTAGA	Zm trnK exon
TRNG5	GCGGGTATAGTTTAGTGGTAAA	Zm trnG exon
TRNGT7	GGGTAGCGGGAATCGA	Zm trnG exon
YA ZmtrnLF	GGGGATATGGCGAAATCGGTA	Zm trnL exon
YA ZmtrnLR	TGGGGATAGAGGGACTTGAACCCT	Zm trnL exon
atpFd1F	GGAGTGTGTGCGAGT	Zm atpF intron
atpFdVIR	AATGAAAGTAGATTATCTTGC	Zm atpF intron
AtpFendR	TTATCTCTTCCATTCTATGG	Zm atpF exon
AtpFc	ATATGAAAAATGTAACCCATTCTT	Zm atpF exon
ndhAint3'	CATAGTCGATGATAACATCACAG	Zm ndhA intron
ndhAint5'	CTAGCAATATCTCTACGTGTGATTCG	Zm ndhA intron
ndhArev	ATTTATAGTGAAACTAGTTGG	Zm ndhA exon
ndhAfor1	CGACTATGATTATCTAATAG	Zm ndhA exon
YA Atycf3-1f	ATGTCGGCTCAATCTGAA	At ycf3 exon 2 probe
YA Atycf3-1r5	CACGTAATGACAGATCACAGCC	At ycf3 exon 2 probe
KW ycf3-2inF	GTGCGACTATCTCCACTATAGAAAAAAAG	At ycf3 intron 2 probe

KW ycf3-2inR	GTCGGAATAGGCGGATCAATTCC	At ycf3 intron 2 probe
trnAinF	GGTCGTTGCGATTACGGGTT	At trnA intron
YA At trnA R	ATAAGCGGACTCGAACCGCTGACAT	At trnA intron
YA At ClpP-2 ex F2	CCCGCTAGTTCGTTTTATGAGG	At clpP exon 3
YA At ClpP-2 ex R3	TTGAACCGCTACAAGATCAAC	At clpP exon 3
PPE primers		
petB	GACGTTCTTAAAACCAATCATATAC	dedioxynucleotide: ddCTP
petD	CAGGATCATTTAAGTCAGGTTTC	dedioxynucleotide: ddCTP
rps12 int.1	GGTTTTTTGGGGTTGATAG	dedioxynucleotide: ddCTP
rps12 int.2	TTGGCTTTTTGGCCCCATATT	dedioxynucleotide: ddCTP
rpl2	GGCCGTGCCTAAGGGCATATC	dedioxynucleotide: ddCTP
rpl16	TACGAAATCTAGTTCTTTTG	dedioxynucleotide: ddCTP
rps16	GCAACGATTCGATAGATAGC	dedioxynucleotide: ddCTP
At-*tha8* mutants genotyping primers		
AtTha8_for	CATACCCTTAGCGTCATCG	gene-specific primer
AtTha8_rev	CGTAAGAGTCAGTGCTCAAGG	gene-specific primer
LBb1.3	ATTTTGCCGATTTCGGAAC	insert-specific for SALK T-DNA insertion
Ds3-1	ACCCGACCGGATCGTATCGGT	insert-specific for Ds transposon insertion
StrepII-ZmOTP51 expression construct		
nco-pl1-tag-for	GCG CCA TGG GTTGGAGCCACCCGCAGTTCGAAAAGGGTGCACTCGTGCTGGAATCTGACGACGAGG	StrepII tag, 1st exon amplification
bam-pl1-rev	GCG GGA TCC TCACTCCTCACTTTCTCTACCATGCCTCTGC	2nd exon amplification
pl1_1exrev	CTGCTGTACCATCCAGCTGTACACCCGGAATGCAGCGGC	1st exon amplification
pl1_2exfor	GCCGCTGCATTCCGGGTGTACAGCTGGATGGTACAGCAG	2nd exon amplification
His-ZmTHA8 expression construct		
Nde_fpet_mTHA	GCG CAT ATG TGCGGGCCGCGCGACAAC	
Hind_rpet_mTHA	GCG AAG CTT CTAGGAGGCAGCAGCAGTGG	
MBP-ZmTHA8 expression construct		
Bam_fmal_mTHA	GCG GGA TCC TGCGGGCCGCGCGACAAC	
Pst_rmal_mTHA	GCG CTG CAG CTAGGAGGCAGCAGCAGTGG	

Table S1 Oligodeoxynucleotide primers used in this study