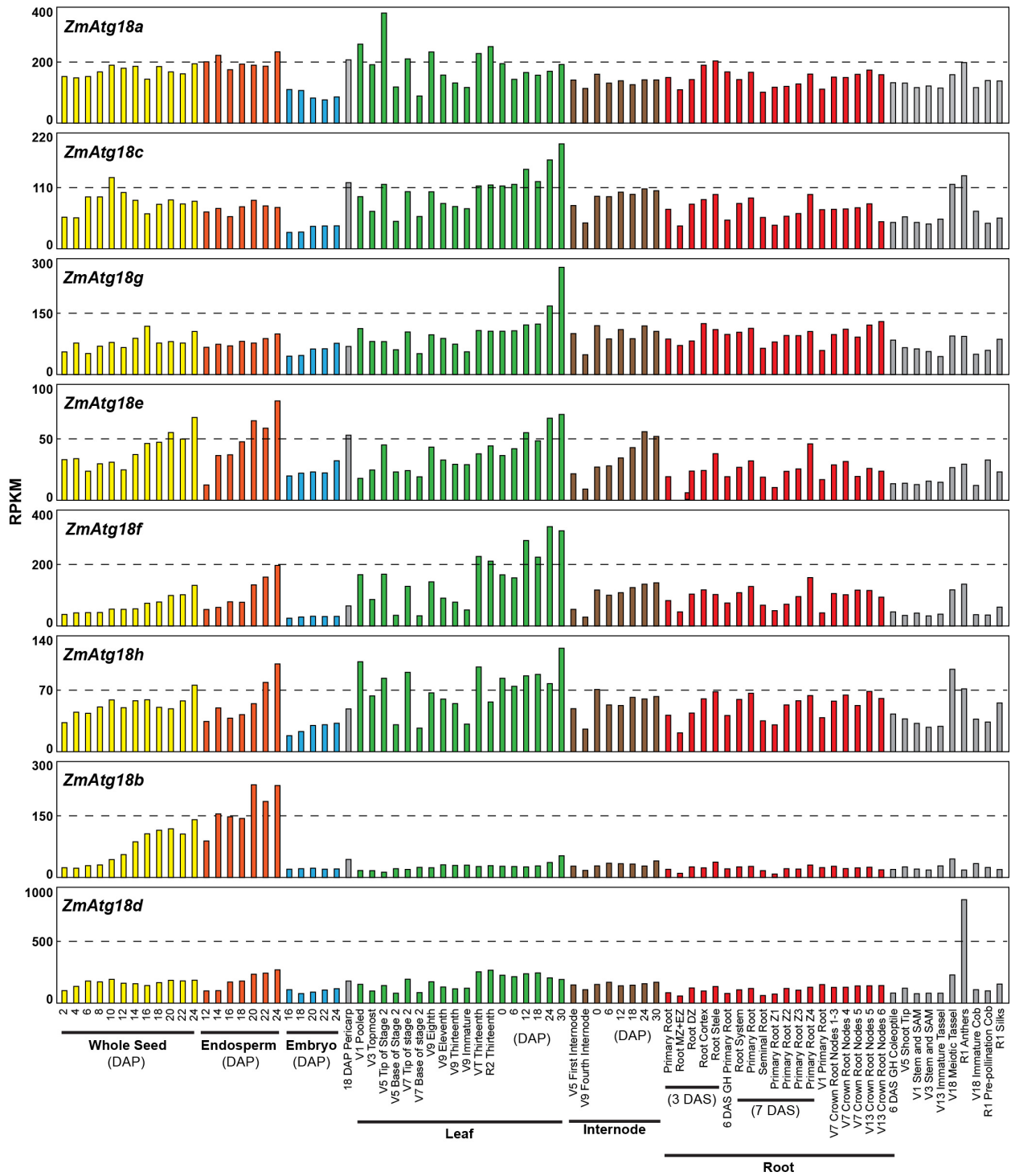




Supplemental Figure 1. Transcript Abundance from Selected Maize *Atg* Genes Increases as Leaves Mature or Senesce.

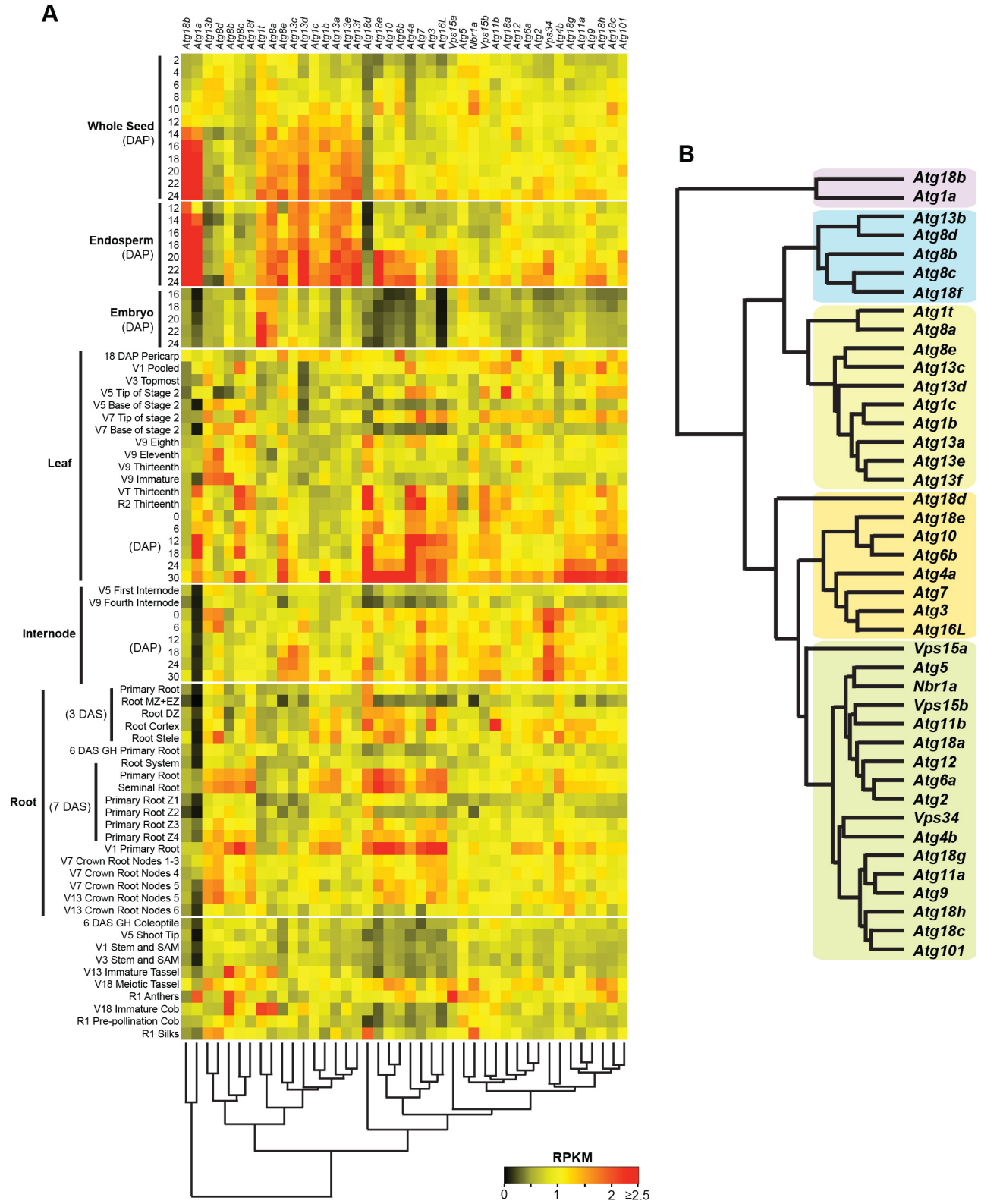
Total RNA was isolated from the leaves at the various positions in a mature plant (leaf 1 younger to leaf 12 older at pollination, green color), or from the base (B), middle (M), and tip (T) sections of the seventh leaf (orange color), and subjected to quantitative real time PCR using the comparative cycle threshold method. Values represent the means (\pm SD) of three biological replicates, each with three technical replicates, which were normalized to that determined for *UBC9*.



Supplemental Figure 2. Developmental and Tissue-Specific Expression Profiles of Maize *Atg18* Genes.

RNA-seq experiments representing 80 developmentally or anatomically distinct maize samples were analyzed for members of the *Atg18* gene family based on reads per kilobase per one million reads (RPKM). Vegetative (V1-18) and reproductive (R1-2)

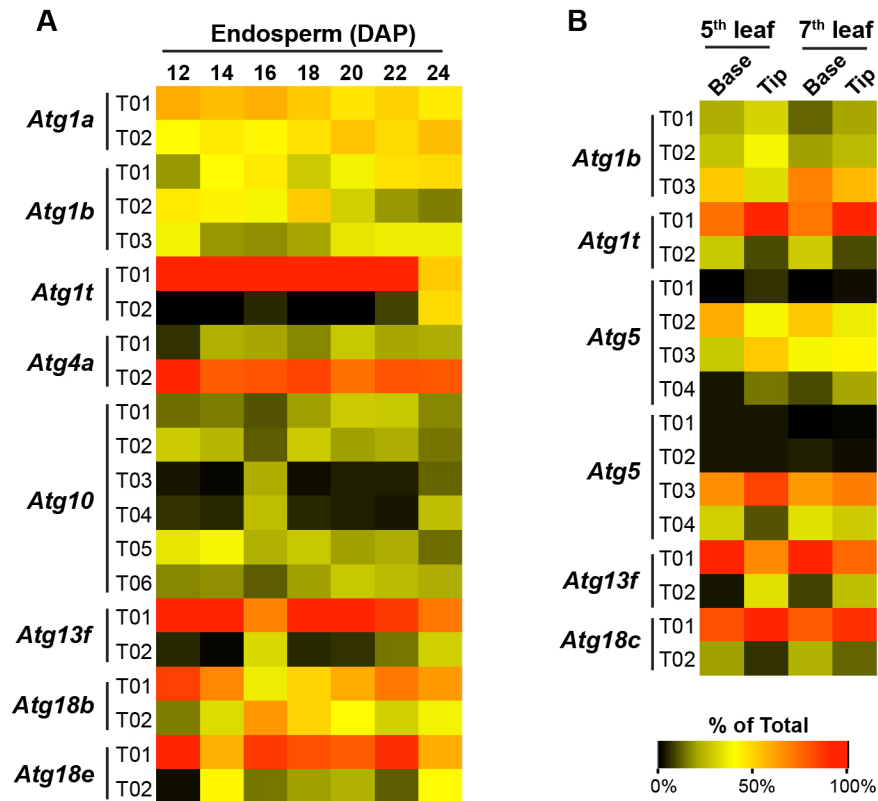
growth stages were defined based on the Corn Field Guide published by Iowa State University Extension (Abendroth et al., 2011). Dissected primary root- Z1 = Zone 1 (1st cm of root tip); Z2 = Zone 2 (from end of Z1 to the point of root hair/lateral root initiation); Z3 = Zone 3 (lower half of differentiation zone); Z4 = Zone 4 (upper half of differentiation zone). DAP, days after pollination. DAS, days after sowing. DZ, differentiation zone. EZ, elongation zone. MZ, meristematic zone. SAM, shoot apical meristem. More complete descriptions of the tissues are available in Supplemental Table 2 online.



Supplemental Figure 3. Developmental and Tissue-Specific RNA-seq Expression Profiles of Maize Autophagy Genes Clustered by Co-expression.

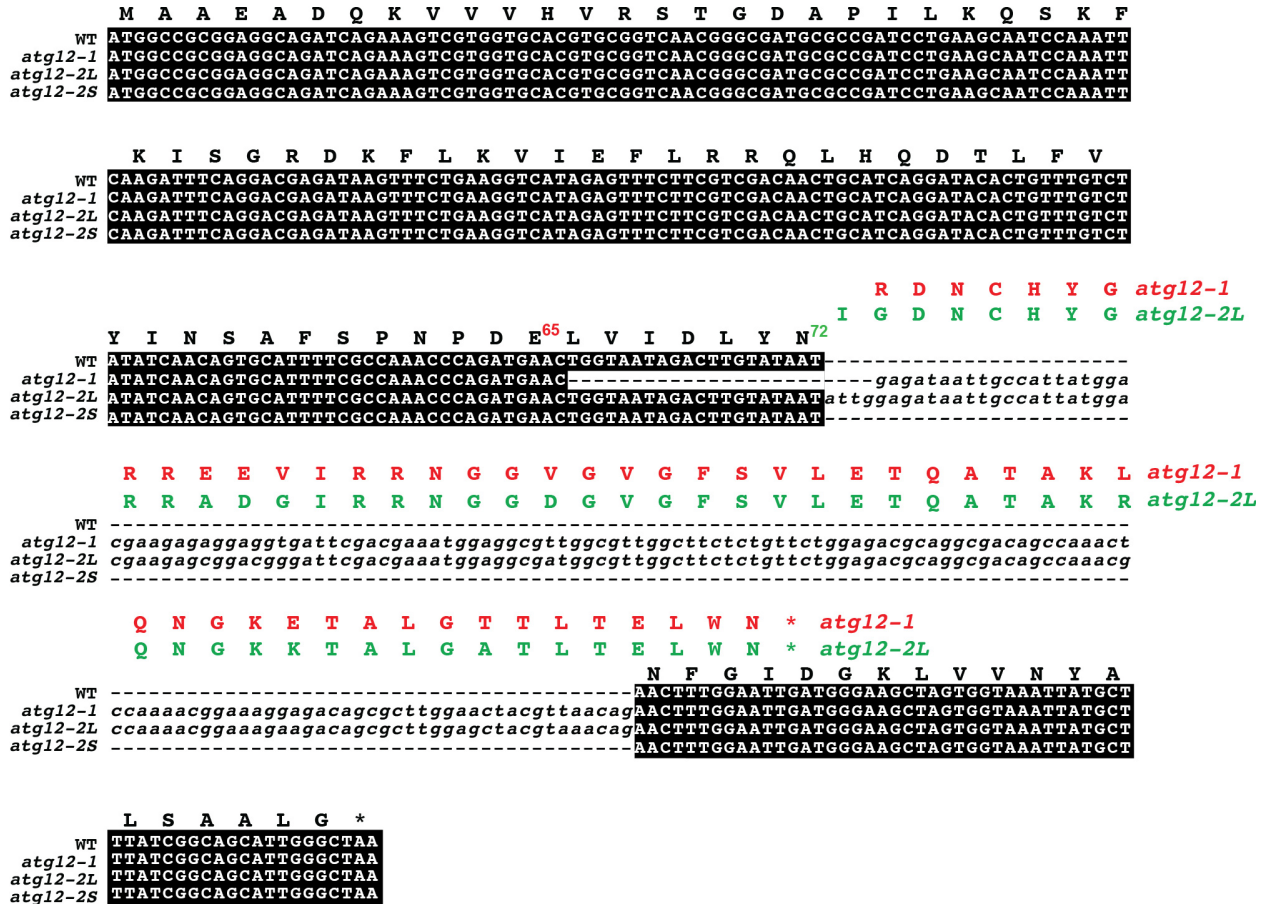
(A) Heat map of *Atg* genes showing the spatio-temporal expression pattern after hierarchical clustering. RNA-seq experiments representing 80 developmentally or anatomically distinct maize samples were analyzed for autophagy-related genes based on reads per kilobase per one million reads (RPKM) and clustered in R based on co-expression. The color indicates the degree of fold change: red, high; black, low. Vegetative (V1-18) and reproductive (R1-2) growth stages were defined based on the Corn Field Guide published by Iowa State University Extension (Abendroth *et al.*, 2011). Dissected primary root- Z1 = Zone 1 (1st cm of root tip); Z2 = Zone 2 (from end of Z1 to the point of root hair/lateral root initiation); Z3 = Zone 3 (lower half of differentiation zone); Z4 = Zone 4 (upper half of differentiation zone). DAP, days after pollination. DAS, days after sowing. DZ, differentiation zone. EZ, elongation zone. MZ, meristematic zone. SAM, shoot apical meristem. See Supplemental Table 2 online for full descriptions of the tissues analyzed.

(B) Hierarchical cluster display of maize *Atg* genes analyzed in (A).



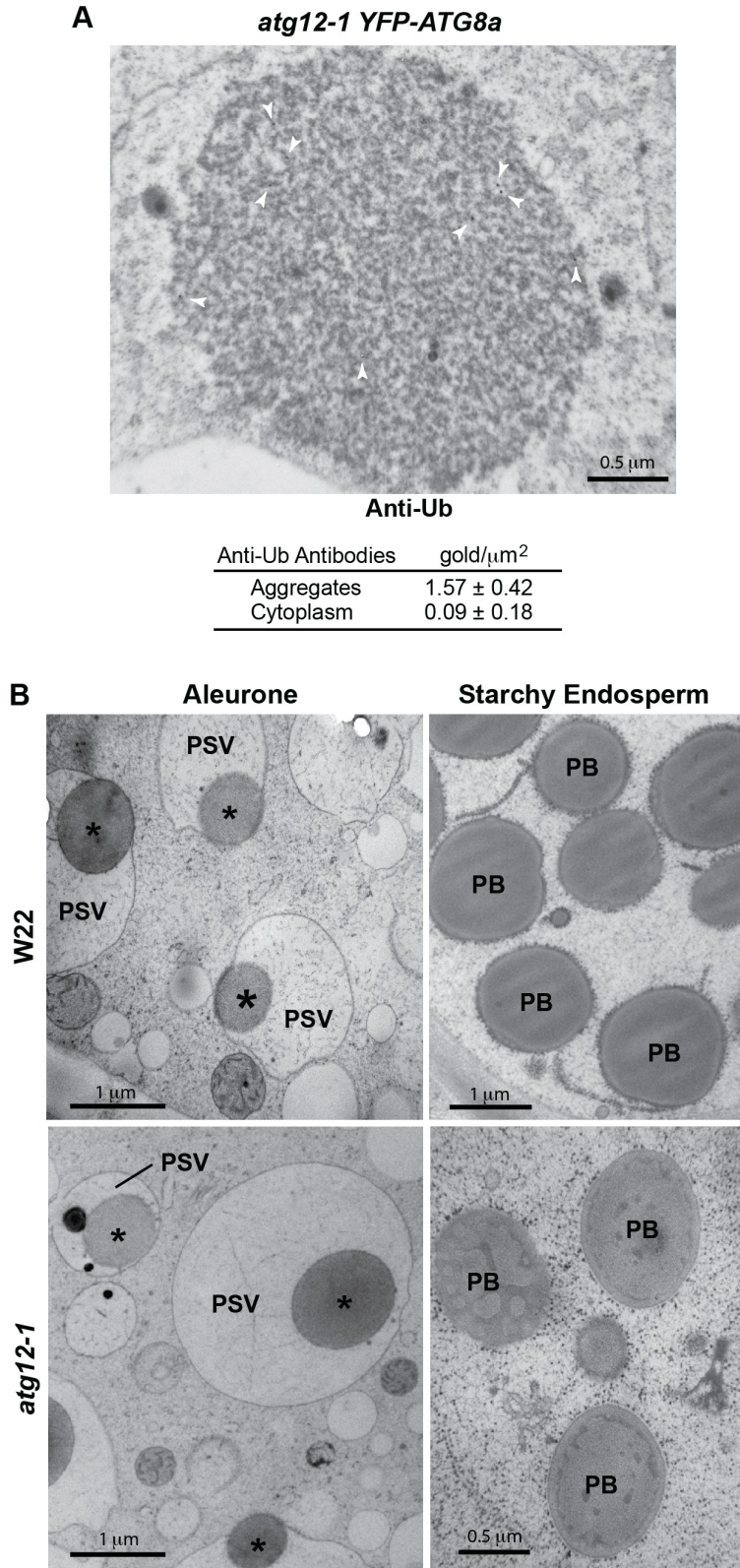
Supplemental Figure 4. Developmental and Tissue-Specific Alternative Splicing of Maize *Atg* Gene Isoforms in Endosperm and Leaf Tissues.

RNA-seq experiments spanning **(A)** endosperm development (DAP 12 to 24) and **(B)** dissected fifth and seventh leaves (Base and Tip) were analyzed for splice-specific isoforms from various *Atg* loci. Gene families encoding particular ATG factors are highlighted on the left along with the number for each transcript isoform. The color indicates the degree of fold change based on reads per kilobase per one million reads (RPKM): red, high; black, low. DAP, days after pollination.



Supplemental Figure 5. Nucleotide and Amino Acid Sequence Alignment of Wild-Type *ATG12* and the *atg12-1* and *atg12-2* Mutants.

The amino acid sequence of wild-type B73 *ATG12* and the regions changed by the *atg12-1* and *atg12-2* mutations are shown in black, red, and green letters above the nucleotide sequences. Nucleotides not shaded in black represent those introduced by the *UniformMu* insertion. The *atg12-1* transcript contains a 156-bp insertion derived from *UniformMu* that introduced a random 51-amino acid sequence (red color) followed by a stop codon after Glu-65. A 159-bp insertion was appended by *UniformMu* to the *atg12-2* long transcript (*atg12-2L*) that also introduced a 52-amino acid sequence (green color) followed by a stop codon after Asn-72. Asterisk indicates stop codon.

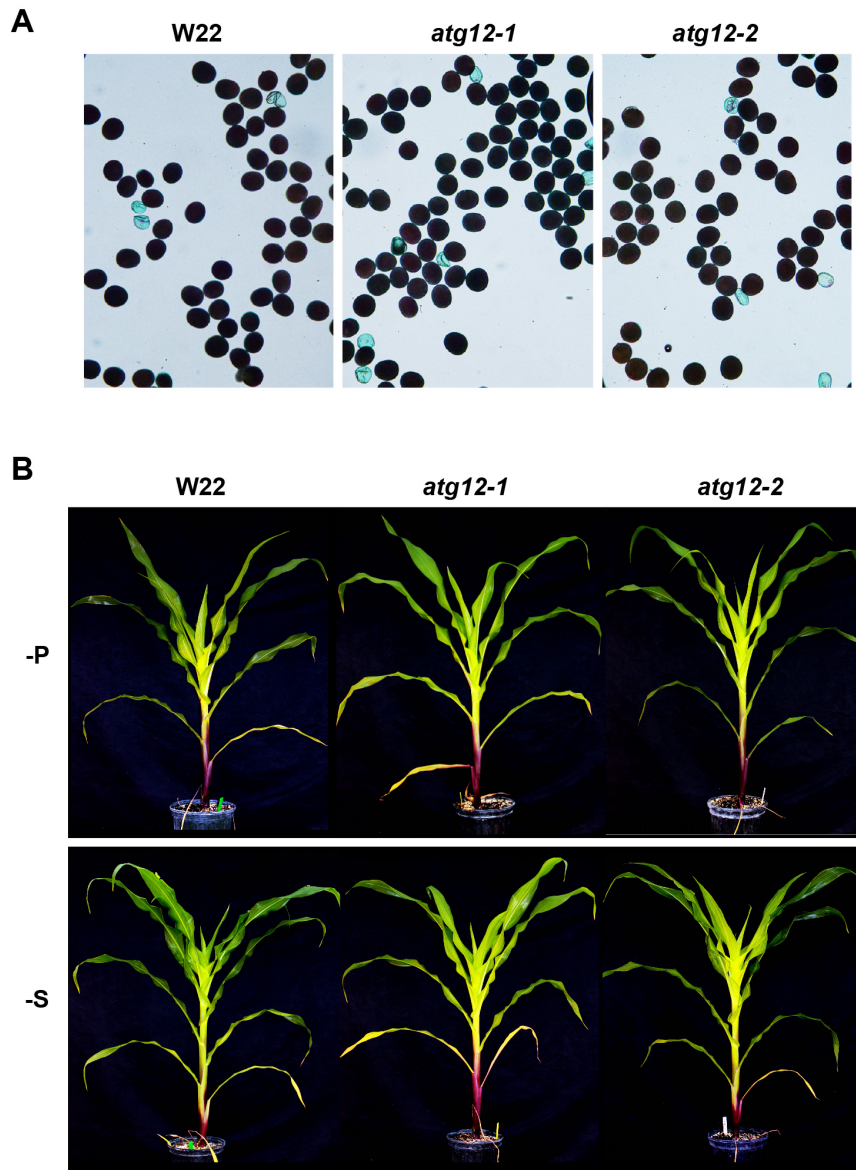


Supplemental Figure 6.

Electron Microscopy Images of Cells from *atg12* Mutants.

(A) Images of N-starved *atg12-1* root cells pretreated with ConA showing that the YFP-ATG8a-containing aggregates also contain ubiquitin (arrowheads) as determined by immunogold-labeling with anti-ubiquitin antibodies. Table shows quantification of gold particles in six independent aggregates and 15 random areas of surrounding cytoplasm. The data indicate a strong enrichment (17 fold) of ubiquitin in the YFP-ATG8a aggregates.

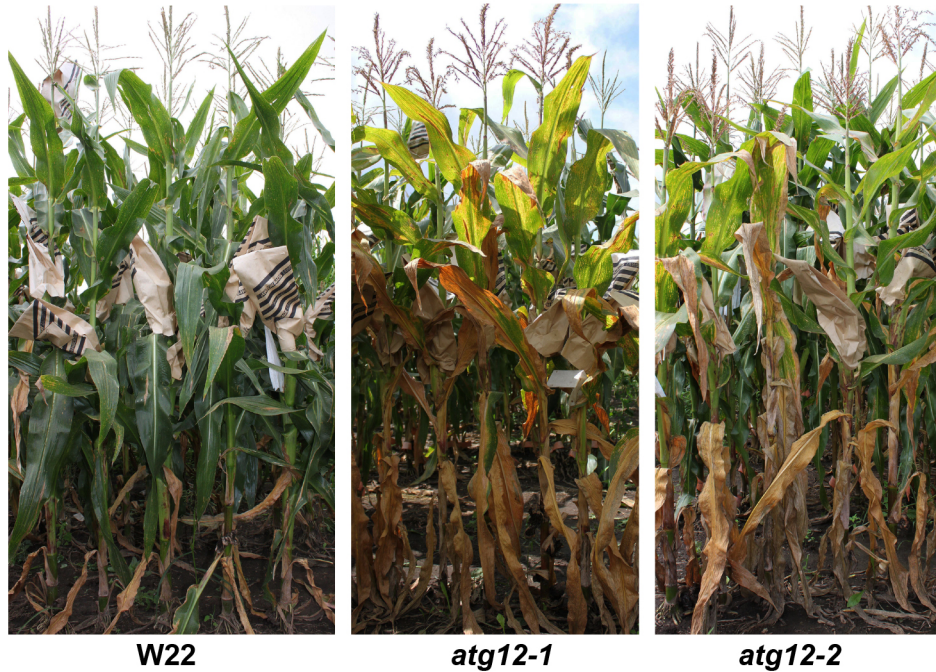
(B) The *atg12-1* mutant accumulates seed storage proteins normally. Shown are transmission EM pictures after cryofixation of aleurone and starchy endosperm cells from wild-type (WT) W22 and mutant seeds at 24 DAP. PSV, protein storage vacuole. PB, ER-associated protein body. Asterisks, storage protein-rich aggregates.



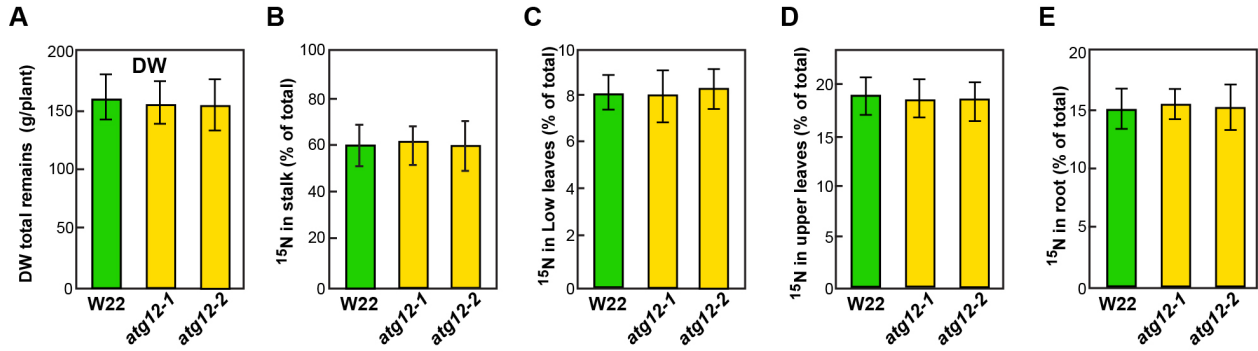
Supplemental Figure 7. *atg12* Mutants Produce Viable Pollen and are not Hyper-sensitive to Phosphorus or Sulfur Limitation.

(A) *atg12* mutants produce healthy pollen. Pollen was obtained from anthers of wild-type W22, *atg12-1* and *atg12-2* plants, and tested for viability with Alexander stain (Alexander, 1969). Non-viable pollen remains translucent in the stain. Bar = 50 μ m.

(B) *atg12* plants grown normally without added phosphate or sulfur. Plants were grown on pre-washed Metro-Mix 360 soil and watered with phosphorus-deficient (-P) (5 mM KNO_3 , 2 mM MgSO_4 , 5 mM $\text{Ca}(\text{NO}_3)_2$, 0.05 mM Fe-EDTA, 1x Micronutrients, and 1 mM KCl), or sulfur-deficient (-S) Hoagland solutions (5 mM KNO_3 , 2 mM MgCl_2 , 1 mM KH_2PO_4 , 5 mM $\text{Ca}(\text{NO}_3)_2$, 0.05 mM Fe-EDTA, 1x Micronutrients) for 7 weeks before the photographs were taken.



Supplemental Figure 8. Field-Grown *atg12* Mutants Display Early Leaf Senescence. Wild type W22, and the homozygous *atg12-1* and *atg12-2* mutants were grown on N-fertilized soil at the West Madison Agricultural Research Station during the summer of 2014. Photographs were taken 3 months after sowing and 20 DAP.



Supplemental Figure 9. ¹⁵N Labeling Efficiency of Wild-Type, *atg12-1*, and *atg12-2* Maize Plants.

Plants at 40 DAG were labeled for 2 days with ¹⁵NO₃⁻, harvested 7 days later, and then measured for dry weight (DW) and ¹⁵N incorporation in various plant parts. Two biological replicates each containing six plants were used for data analysis (n=12). Values are adjusted means (±SD).

(A) Biomass as measured by dry weight of remains (stalk + upper leaves + lower leaves).

(B-E) ¹⁵N accumulation in stalk (B), lower leaves (C), upper leaves (D), and roots (E).

Supplemental Table 1. Collection of Arabidopsis and Maize *Atg* Genes

Gene	Arabidopsis ID	Gene	Maize ID	Splicing variant ^a	No. of Amino Acid Residues	Identity/Similarity to Arabidopsis
ATG1/13 kinase complex						
At <i>ATG1a</i>	AT3G61960	Zm <i>Atg1a</i>	GRMZM2G105415	2	538	34/47%
At <i>ATG1b</i>	AT3G53930	Zm <i>Atg1b</i>	GRMZM2G160428	3	703	47/61%
At <i>ATG1c</i>	AT2G37840	Zm <i>Atg1c</i>	GRMZM2G164160	2	704	48/62%
At <i>ATG1t</i>	AT1G49180	Zm <i>Atg1t</i>	GRMZM2G104658	2 (T01)	283	47/62%
At <i>ATG13a</i>	AT3G49590	Zm <i>Atg13a</i>	GRMZM2G129675	1	536	33/43%
At <i>ATG13b</i>	AT3G18770	Zm <i>Atg13b</i>	GRMZM2G109348	1	533	33/45%
		Zm <i>Atg13c</i>	GRMZM5G825909	1	511	32/44%
		Zm <i>Atg13d</i>	GRMZM2G000973	1	512	32/43%
		Zm <i>Atg13e</i>	GRMZM2G044733	1	606	35/50%
		Zm <i>Atg13f</i>	GRMZM2G125352	2 (T01)	606	34/50%
At <i>ATG11</i>	AT4G30790	Zm <i>Atg11a</i>	GRMZM2G143445	1	1,139	45/64%
		Zm <i>Atg11b</i>	GRMZM2G119571	1	1,143	45/64%
At <i>ATG101</i>	AT5G66930	Zm <i>Atg101</i>	GRMZM2G160174	4 (T03)	213	40/54%
PI3 kinase complex						
At <i>PI3K</i>	AT1G60490	Zm <i>PI3K</i>	GRMZM2G103721	2 (T01)	803	73/85%
At <i>ATG6</i>	AT3G61710	Zm <i>Atg6a</i>	GRMZM2G092112	1	499	55/70%
		Zm <i>Atg6b</i>	GRMZM2G027857	1	579	52/63%
At <i>VPS15</i>	AT4G29380	Zm <i>Vps15a</i>	GRMZM2G179662	2 (T01)	1,480	52/68%
		Zm <i>Vps15b</i>	GRMZM2G111491	1	1,561	49/64%
ATG9/2/18 complex						
At <i>ATG2</i>	AT3G19190	Zm <i>Atg2</i>	GRMZM2G042889	1	1,438	23/38%
At <i>ATG9</i>	AT2G31260	Zm <i>Atg9</i>	GRMZM2G035461	1	888	50/63%
At <i>ATG18a</i>	At3g62770	Zm <i>Atg18a</i>	GRMZM2G122607	1	442	63/73%
At <i>ATG18b</i>	AT4G30510	Zm <i>Atg18b</i>	GRMZM2G146280	2 (T01)	449	61/71%
At <i>ATG18c</i>	AT2G40810	Zm <i>Atg18c</i>	GRMZM2G069177	2 (T01)	417	47/61%
At <i>ATG18d</i>	AT3G56440	Zm <i>Atg18d</i>	GRMZM2G143211	2 (T01)	417	65/77%
At <i>ATG18e</i>	AT5G05150	Zm <i>Atg18e</i>	GRMZM2G018573	2 (T01)	371	48/63%
At <i>ATG18f</i>	AT5G54730	Zm <i>Atg18f</i>	GRMZM2G116700	2 (T01)	865	33/48%
At <i>ATG18g</i>	AT1G03380	Zm <i>Atg18g</i>	GRMZM2G546452	2 (T02)	381	16/22%
At <i>ATG18h</i>	AT1G54710	Zm <i>Atg18h</i>	GRMZM2G078468	2 (T02)	1,557	31/40%
		Zm <i>Atg18i</i>	GRMZM2G301031	n.d.		
		Zm <i>Atg18j</i>	GRMZM2G103793	n.d.		
ATG8/12 conjugation pathway						
At <i>ATG3</i>	AT5G61500	Zm <i>Atg3</i>	GRMZM5G818887	2 (T01)	311	71/84%
At <i>ATG4a</i>	AT2G44140	Zm <i>Atg4a</i>	GRMZM2G064212	2 (T02)	492	50/67%
At <i>ATG4b</i>	AT3G59950	Zm <i>Atg4b</i>	GRMZM2G173682	2	492	50/66%
At <i>ATG5</i>	AT5G17290	Zm <i>Atg5</i>	GRMZM2G098420	4	374	50/68%
At <i>ATG7</i>	AT5G45900	Zm <i>Atg7</i>	GRMZM2G005304	1	1,021	48/63%
At <i>ATG8a</i>	AT4G21980	Zm <i>Atg8a</i>	GRMZM2G336871	2 (T02)	119	85/94%
At <i>ATG8b</i>	AT4G04620	Zm <i>Atg8b</i>	GRMZM2G419694	5 (T04)	120	86/94%
At <i>ATG8c</i>	AT1G62040	Zm <i>Atg8c</i>	GRMZM2G076826	5 (T01)	120	86/94%
At <i>ATG8d</i>	AT2G05630	Zm <i>Atg8d</i>	GRMZM2G134613	2 (T02)	119	86/94%
At <i>ATG8e</i>	AT2G45170	Zm <i>Atg8e</i>	GRMZM2G014975	8 (T02)	119	85/94%
At <i>ATG8f</i>	AT4G16520					
At <i>ATG8g</i>	AT3G60640					
At <i>ATG8h</i>	AT3G06420					
At <i>ATG8i</i>	AT3G15580					
At <i>ATG10</i>	AT3G07525	Zm <i>Atg10</i>	GRMZM2G066059	8	215	44/60%
At <i>ATG12a</i>	AT1G54210	Zm <i>Atg12</i>	GRMZM5G842517	3 (T02)	91	82/89%
At <i>ATG12b</i>	AT3G13970					
At <i>ATG16L</i>	AT5G50230	Zm <i>Atg16L</i>	GRMZM2G078252	2 (T02)	505	54/74%
Cargo receptor						
At <i>NBR1</i>	AT4G24690	Zm <i>Nbr1a</i>	GRMZM2G092447	1	842	34/46%
		Zm <i>Nbr1b</i>	GRMZM2G139846	n.d.		

^aNumber of splicing variants were detected by analyzing RNA-seq data; Names of the dominant splice isoforms are shown in parentheses. n.d. = not detected.

Supplemental Table 2. List of Tissues Analyzed by RNA-seq in this Study

Tissue name	Tissue name
1. Whole Seed, 2 DAP	41. Leaf, 18 DAP
2. Whole Seed, 4 DAP	42. Leaf, 24 DAP
3. Whole Seed, 6 DAP	43. Leaf, 30 DAP
4. Whole Seed, 8 DAP	44. First Internode, V5
5. Whole Seed, 10 DAP	45. Fourth, Internode, V9
6. Whole Seed, 12 DAP	46. Internode, 0 DAP
7. Whole Seed, 14 DAP	47. Internode, 6 DAP
8. Whole Seed, 16 DAP	48. Internode, 12 DAP
9. Whole Seed, 18 DAP	49. Internode, 18 DAP
10. Whole Seed, 20 DAP	50. Internode, 24 DAP
11. Whole Seed, 22 DAP	51. Internode, 30 DAP
12. Whole Seed, 24 DAP	52. Primary Root, 3 DAS
13. Endosperm, 12 DAP	53. Root Meristematic Zone (MZ) and Elongation Zone (EZ), 3 DAS
14. Endosperm, 14 DAP	54. Root differentiation zone (DZ), 3 DAS
15. Endosperm, 16 DAP	55. Root Cortex, 3 DAS
16. Endosperm, 18 DAP	56. Root Stele, 3 DAS
17. Endosperm, 20 DAP	57. Primary Root, 6 DAS
18. Endosperm, 22 DAP	58. Root System, 7 DAS
19. Endosperm, 24 DAP	59. Primary Root, 7 DAS
20. Embryo, 16 DAP	60. Seminal Roots 7 DAS
21. Embryo, 18 DAP	61. Primary Root Zone 1 (1st cm of root tip); 7 DAS
22. Embryo, 20 DAP	62. Primary Root Zone 2 (from end of Z1 to the point of root hair/lateral root initiation), 7 DAS
23. Embryo, 22 DAP	63. Primary Root Zone 3 (lower half of differentiation zone), 7 DAS
24. Embryo, 24 DAP	64. Primary Root Zone 4 Zone 4 (upper half of differentiation zone), 7 DAS
25. Pericarp, 18 DAP	65. Primary Root, V1, 4 day after emergence
26. Pooled Leaves, V1, 4 day after emergence	66. Crown Roots, Nodes1-3, V7
27. Topmost Leaf, V3	67. Crown Roots, Node4, V7
28. Leaf Bottom, V5	68. Crown Roots, Node5, V7
29. Leaf Tip, Stage2, V5	69. Crown Roots, Node5, V13
30. Leaf Bottom, V7	70. Brace Roots, Node6, V13
31. Leaf Tip, V7	71. Coleoptile, 6 DAS
32. Eighth Leaf, V9	72. Shoot Tip, V5
33. Eleventh Leaf, V9	73. Stem and SAM, V1, 4 day after emergence
34. Thirteenth Leaf, V9	74. Stem and SAM, V3
35. Immature Leaves, V9	75. Immature Tassel, V13
36. Thirteenth Leaf, VT	76. Meiotic Tassel, V18
37. Thirteenth Leaf, R2	77. Anthers, R1
38. Leaf, 0 DAP	78. Immature Cob, V18
39. Leaf, 6 DAP	79. Pre-pollination Cob, R1
40. Leaf, 12 DAP	80. Silks, R1

Supplemental Table 3. Oligonucleotide Primers Used in This Study

Zm *Atg12* RT-PCR primers

Primer 1:	ATGGCCGCGGAGGCAGATCAGAAAG
Primer 2:	TTAGCCCCATGCTGCCGATAAAGCA
Primer 3:	CTGAAGGTCATAGAGTTTCTTCGTCTG
Primer 4:	CGACGAAGAACTCTATGACCTTCAG

Primers used for qRT-PCR analysis of wild type-like transcripts in maize *atg12-2* mutant

Primer 5:	CCGCGGAGGCAGATCAGAAA
Primer 6:	TCAATTCCAAAGTTATTATACA

Zm *Atg12* mutant genotyping primers

<i>ZmAtg12-1</i> RP:	GCCCCGATTTTTTTATCCCCAGAT
<i>ZmAtg12-1</i> LP:	CTGAAGGTCATAGAGTTTCTTCGTCTG
<i>ZmAtg12-2</i> RP:	TTAGCCCCATGCTGCCGATAAAGCA
<i>ZmAtg12-2</i> LP:	TGTAATTCCAAGCTCTTTACCTGAGG
TIR6:	AGAGAAGCCAACGCCAWCGCCTCYATTTCTGTC

Primers used for qRT-PCR analysis of maize *Atg* genes

ZMATG1a-RT-F1:	GTGACTTTGGGTTTGCCAGGTC
ZMATG1a-RT-R1:	TGGCGACCCACATATTGTAGCAG
ZMATG2-RT-F1:	CACTTCTTGGGCTAAGGAACAGC
ZMATG2-RT-R1:	CGCCAAAGAATGAACCGACCAC
ZMATG3-RT-F1:	GTACTACCAAACCTCCACGTGTCTG
ZMATG3-RT-R1:	GGCATTAAATGGCATTCTTGACTCG
ZMATG4a-RT-F1:	CCATGTTTCTGTGCTACTGCTAGAC
ZMATG4a-RT-R1:	GCAAGCTCGTCATCACCTAACG
ZMATG4b-RT-F1:	TGGGACGTCAACATACATTGCTG
ZMATG4b-RT-R1:	GCCAAATCTCGGACAACACTGC
ZMATG5-RT-F1:	AGACTCGCAAGGCTGAAGGTAG
ZMATG5-RT-R1:	GCTGAAGAACTCCGGAAGCAATG
ZMATG6-RT-F1:	GCTGTTGCATACCATGGCTCAG
ZMATG6-RT-R1:	GGGTGAATCTTGATCCGGTATTGG
ZMATG7-RT-F1:	ACGTCATTGCTCCTGTCTGACTC
ZMATG7-RT-R1:	AGCGCGTCCTGATGCAATAGAG
ZMATG8a-RT-F1:	AGAACACCTTGCCACCAACTGC
ZMATG8a-RT-R1:	ATTGCTCTAGGCAGAGCCGAAG
ZMATG8b-RT-F1:	TCTTCGTTTCGATCCGTTTCG
ZMATG8b-RT-R1:	ATCACGCTCCTTCCTGCCTTAC
ZMATG8c-RT-F1:	TTCGATCCAACCTGGCAGGAAGG
ZMATG8c-RT-R1:	AGCCTCAGACTGCCTCTTCTCAAG
ZMATG8d-RT-F1:	TCTGTTCCAGGTCGTTCTCTCTG
ZMATG8d-RT-R1:	TTAGCCTCAGCTTGCCTCCTTTTCG
ZMATG8e-RT-F1:	ACTGTCAGCTCTGGGTTGCTTC
ZMATG8e-RT-R1:	TTTGCCACATCGACAAGCTCACG
ZMATG9-RT-F1:	GTGGGAGATGTATGCAGTCTAAGC
ZMATG9-RT-R1:	AGAGCATTGAATGGTGACCCATAG
ZMATG10-RT-F1:	AAGCCGTGCAGCAAATTGGG

ZMATG10-RT-R1: ACAGCTGCGGTACACTCTTTCC
ZMFIP200a-RT-F1: AGAAGAGGGTGAAGGCTTATTCC
ZMFIP200a-RT-R1: TGAGGTGTCCACATCTTTGCTTAG
ZMFIP200b-RT-F1: AGGAATCTGTGCCTTGTTACCC
ZMFIP200b-RT-R1: ACGATCTGCCCGATTATGTACGC
ZMATG12-RT-F1: GGCTCGTGGTAACTTGTTGTCG
ZMATG12-RT-R1: TGCACCACGACTTTCTGATCTGC
ZMATG16-RT-F1: AGGCTGAACTTGAGAAGACAAGCG
ZMATG16-RT-R1: AGCTCGGATTTCACTGTCTGATGC
ZMATG101-RT-F1: AAGGGCTGACAAACATCCAAGC
ZMATG101-RT-R1: TCGTTAATCGACGCCTCCAATGC
ZMATG18a_RT-F1: ACTGCTAGCACCAAGGGAACAC
ZMATG18a_RT-R1: TCAGCACCTCTCCTTACTTCCTG
ZMATG18e_RT-F1: TCCAAATTTCCCTCGCTGAAGAC
ZMATG18e_RT-R1: TTCACGCTCCAACAACCATGAAG
ZmATG18f-RT-F1: ACCAGTCTTCGACTCCCTCCATAC
ZmATG18f-RT-R1: TCAGGAGCGTTCAACCTTGTCTG
ZmUBC-F1: AAGATGCAGGCATCTAGGGCAAGG
ZmUBC-R1: AGGCTCTTGGCTTGGCACATGTTC

Primers used for generating Gly117Ala mutant of maize ATG8a

ZmATG8a_F1: CACCATGGCCAGGACCTCTTTCAAATG
ZmATG8a_R2: TGCTCTAGGCAGAGGCGAAGGTGT