

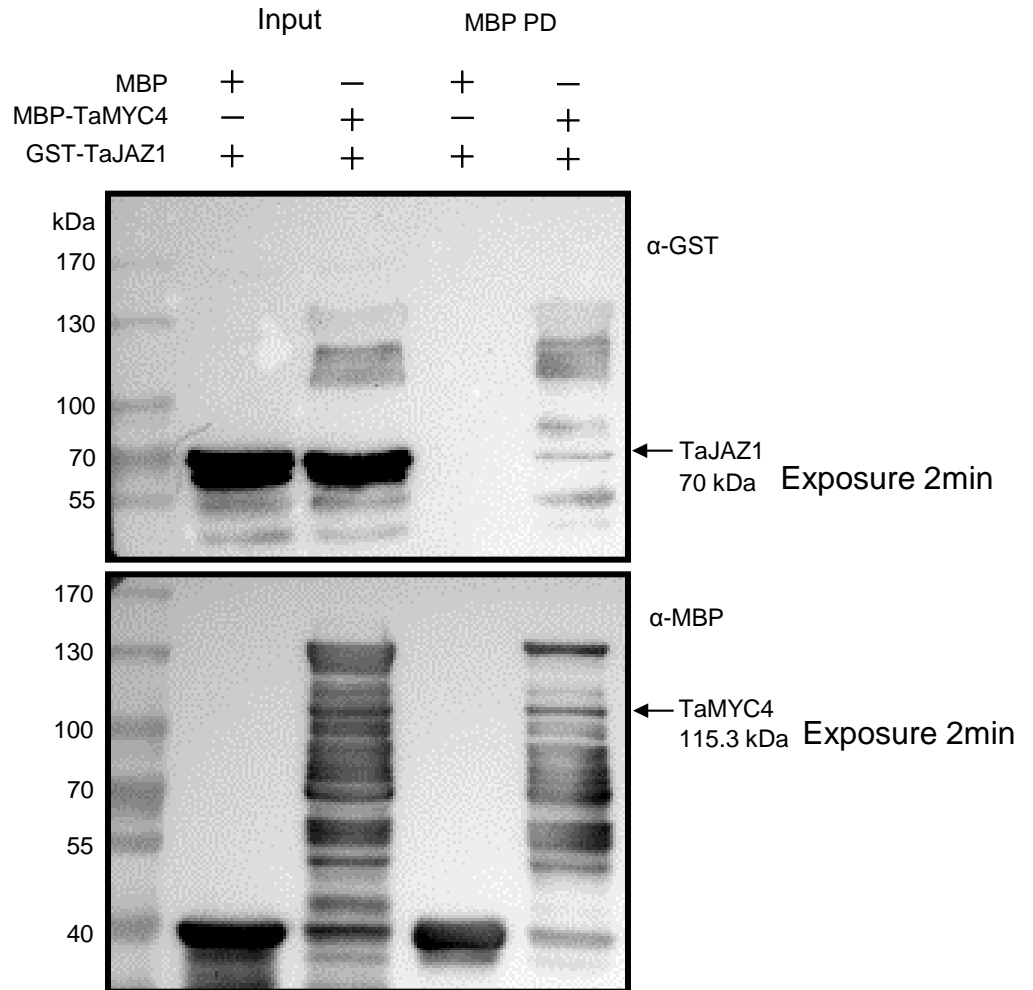
Supplemental Figure 2

TaMYC4-1B

5'-
ATGAACCTGTGGACGGACGACAACGCCTCCATGATGGAGGCCCTTCATGGCCTCCGCCGAC
ATGCCGGCCTTCCCCTGGGGCGCTGCGGCCACCCCGCCGCCGCCGCCGCGCCGTGCCGC
AGCAGCCGGCCTTCAACCAGGACACGCTGCAGCAGCGCCTGCAGGCCATCATCGAGGGC
TCCAGGGAGACCTGGACCTACGCCATCTTCTGGCAGTCCTCCACCGACGCCGGCGCCTC
GCTCCTCGGCTGGGGCGACGGCTACTACAAGGGCTGCGACGACGCCGACAAGCGCCGC
CAGCAGCCCACCCCGGCCTCCGCCGCCGAGCAGGAGCACCAGCAAGCGCGTCTCCGGG
AGCTCAACTCGCTCATAGCCGGGGGAGGCGCCGCCGCCGCCGACGAGGCCGTGAGGA
GGAGGTCACGGACACCGAGTGGTTCTTCTCGTCTCCATGACCCAGTCCTTCCCCAACGG
GATGGGCTTGCCGGGCCAGGCGCTCTTCGCCGGCCAGGCCACCTGGATCGCCACGGGG
CTCGCCAGCGCGCCCTGCGAGCGGGCCAGGCAGGCCTACACCTTCGGCCTCCGCACCAT
GGTCTGCATCCCCCTCGGCACCGCGTGCTCGAGCTCGGCGCCACCGAGGTCATCTTCC
AGACCAACGATAGCTTGGGGAGGATCCGCTCGCTCTTCAACCTCAACGGCGGAGGAGGG
GGCTCTGGATCCTGGCCGCCATCGCGCCGCCGCCGCCGAGGAGCGGAGACGGATCCGT
CCGTGCTCTGGCTCGCCGACGCGCCGGCCGGGGACATGAAGGAGTCGCCGCCGTCCGT
CGAGATCTCCGTCTCAAACCGCCGCCACAACCGCCGCAGATCCATCACTTCGAGAA
CGGGAGCACCAGCAGCTCACGGAGAACCCAGTCTCTCCGTGCACGCGCAGCAGCCTC
CGCCCAGCAGGCGGCTGCGGCGGCGCAGAGGCAGAACCAGCACCAGCAGCAGCTCCA
GCATCAGCACCAGCTCCAGCTCCAGCACCAGCACAACCAGGGTCTTTCCGCCGGGAGC
TCAATTTCTCAGATTTTCGCGTCCAACGCATCCGTACGGTGACCCCGCCTTTCTTCAAGCC
CGAGTCTGGTGAGATCCTAAACTTTGGCGCTGACAGCACCAGCCGGAGGAACCCTTCGCC
GGCGCCCCCGCCGCGACGGCCAGCCTCACCACCGCGCCCGGGAGCCTATTCTCCAG
CACACGGCGACTGTGACGGCCCCGTCAAACGACGCCAAGAACAACCCGAAGCGGTCCAT
GGAGGCCACCTCCCGCGCGAGCAACACCAACCACCACCAGAACGCCACAGCCAACGAGG
GGATGCTGTCTTCTCGTTCGGCGCCGACGACGCGGCCGTCCACCGGCACGGGCGCGCC
AGCCAAGTCGGAGTCCGACCACTCCGACCTGGAGGCGTCCGTCCGCGAGGTGGAGAGCA
GCCGCGTGGTGCCTCCGCCGGAGGAGAAGCGGCCGCGCAAGCGCGGGCGCAAGCCGG
CGAACGGGCGCGAGGAGCCCCTGAACCACGTGGAGGCGGAGCGGCAGCGGCGGGAGA
AGCTGAACCAGCGGTTCTACGCCCTCCGCGCCGTGGTGCCCAACGTGTCCAAGATGGAC
AAGGCCTCGCTGCTGGGCGACGCCATCTCCTACATCAACGAGCTCCGCGGCAAGATGAC
GGCGCTGGAGTCGGACAAGGAGACGCTCCACTCCCAAATCGAGGCGCTCAAGAAGGAGC
GCGACGCCCGGCCGGCCGCGCCGTTCGTCGGGGATGCACGACAACGGGGCGCGGTGCC
ACGCGGTCGAGATCGAGGCCAAGATCCTGGGGCTGGAGGCGATGATCCGCGTGCAGTGC
CACAAGCGCAACCACCCGGCGGCGAAGCTGATGACGGCGCTGCGGGAGCTGGACCTGG
ACGTGTACCACGCCAGCGTGTCCGTGGTGAAGGACATCATGATCCAGCAGGTGGCGGTG
AAGATGGCCACCCGGGTCTACTCCAGGACCAGCTCAACGCGGCGCTCTACGGCCGCCT
CGCCGAGCCGGGCACCGCGATGCAAATCCGGTAA-3'

Supplemental Figure 2. The coding sequence of *TaMYC4* from bread wheat cultivar KN199.
The full length CDS of *TaMYC4* derived from the B subgenome is shown.

Supplemental Figure 3



Supplemental Figure 3. Pull-down assay showing the interaction between TaJAZ1 and TaMYC4. GST-TaJAZ1 protein was pulled down by MBP-TaMYC4 protein, and detected using an anti-GST antibody. MBP and MBP-TaMYC4 were detected using anti-MBP antibody. Arrow indicates specific band.

Supplemental Figure 4

TaJAZ1-5A-promoter

5'-

TACGCTAAATGCTAAGCCGCTATCATCACGCCCTAATGCAACCGGGCGTCGCCACGTAGGGA
GACAAACCTGCATCATGTACAGTAATTAAGTACTGCTCCACTACGCAACCGATGAGCACCTTG
TTAAAGTAATCAACAGGGTTCGATCAGCATGCTAACTGCTCCTCCTCCTAGTAGAGTGGCGTGG
AGTATACTATTGCTAATACTGCTAGCCGGTAGCTCCACATGGCCTGTACCTTATCTCCCTAGAT
TAACATTTAACAAAGTACTACTACTACTTAATCGTGGGATGCAGACGGGAATCTTGCACAG
TGTCTCGGACAAGTTTGACAAGACTCTGCTTGTATCACTAAATGATGTTATGGAAAGTACTCCA
TCTAGTCATCGTTTTACTTTTTCATGGGTGCCAGTACAGTAGAGTATAGTCCATCACATAAAAAC
TTGGGAGATAAACAAAGCTATCTCAGTGTACCAAGTGATGCTACGAGTACCGTAGGAATTGAC
AAGAAGACGTGTTTGAATAGGAAACCATAATTTTCGGGGCAAAAAGAGTTAACACACCATGATT
TGGTACTACTACCAGTAGTACTCCTATCCAATAGGTCACGGTCCGTGGTTCATGTTGATTTGAT
ATTTACTCCAAAGTGGACCATACAAAAAACTCTCATTTTAAAGCCGACATACAAAAGCTGTAAA
TTTGATCAAAAAGAGCACCTGAATGTTGCGTCGATAGATTCCTAAAAAATGCTACTCCACGAAG
AACAAATTCATTTGAAAATCCACGAAAGGCCCTCGAACTCGCCTCGAAAGGCCACGCACGGC
CGTCTCCTTTCCGAAAAGGCAACGTCACCATCTCTCTAATCTACTACCGAACCTATAAAACTT
CGCTAAACAAATCCCGCAGCCTAAGCAAGGAATAGTACGTACAAGTCCAGCAGATGACCACAT
TAGCTCAAAAAGAAAAGCAGATGACCACATAGAGAAGAAAAACAACATGGCGCGCACATGG
CACATGGTCATGGCCACAAGACATAAACAAAACCGGCGAAGCACGCCAGCCCCCACCAC
CCCAACCCCAACCCCGTGGACGCAAATTTTTTAATCATCAGAAGACACAAAAAAAGGAACA
TCACGGGATTAGTATTATTATTCTACCCATAAATCCAAAAGTCTACTGTAAGCTCGGGCGCCG
GGCCCCCGCGACGCGCAGCACGTGGTCTTCCCCCGGACCCACGCGCAGGGAATCCCGC
GGGCCACAGCGTCAGCGACGGCCCGCTCCCCACCTGGTAATTCGGCTCGTGTGACCCAGC
CGGGGCTGCACGTGTGCCACTGCAGCCGCGCCAGGGCCCTGTGCGCGGCTACCCGCA
CCGGGCGGCACTCACCGACGCGCGGGGCGAGGGTCTGGGGTTTGACCCGGCTGTCAGTGGC
AGTGGGCGGCGTGGGAGCCTCGCGAGGCGGGATGCGCTCGTGCCCGACCACGTGGGCGC
GACCGGGCTGGGCCAGCGCGAGAGGAGAGGGCGCCCCGATCCGCTCACCGCGGTGGCCC
ACCGCCGTGCTCGCCACACCTTTCCTCTCGGTTGATTATCACTCGGTTTTGCTTGTTTATTTAT
CTATAGTTTGTATTTATTTAATTAATCAATCATCTGCCACCCGATCTAACCGCCTGGGCCGA
AAATAAATAAGCGATTAGCGGCACCACAAGCGCACGCACACCCGCTAGAGGCCATGCTTGGC
TAGGAGGAGTACTGGAGCAGTATACATACGAGAGAGAGAGAGGCAGGAAGGGAAAGGCAGA
GCGGTCAAGGCTGGGAGAGGGAGAGGGAGAGCATCGTGTGTCATCATTGCTCGTTTAC
AGAGGAGTGAGAGAGTCAGAGGAGGAAGGAAGGTTTCAGGGGAGTGAGAGACGAAATTTCC-3'

Supplemental Figure 4. Promoter sequence of *TaJAZ1* from bread wheat cultivar KN199.

Supplemental Figure 5

TaNINJA-1A

5'-

ATGGAGGATGGCCTTGAGCTTAGTTTAGGCCTCTCTTTGGGTGGTGGGTCTTCTGGGAAGT
CTAAGGCAAGAGATGCTTCTCTAGAGCCTAAAGCAGAACCTCAAGTGGGAAGAGAGCAGTAG
CAAAGGCGTCTCGCAACCTCCCGATGCTCCCTTTGCGCATTACTATCAAGCAACTGCTGAGA
ACCAGGAACACAGTAGCAAGCAGAGGCATAGCCCTGCTACCCCGCCATTTGGAACTTCTG
GGGACAACACGGTGGCTCCTCTGCTCCAGTGGCAGATGGATCAAGTGAACATAAGCCCAC
CAATCTCAGCTTCCCCAGTATCAGGAGGGGGCGGACTCCAATAATATTGGAAATAATTCGGA
GGAAACATGCCAGTCTCAAGTAAACGAAAGCTGCTTTCTGAAGAGACAAGTTTTTCAGAAGA
AGCATCATTCTGCTGCTGACCAGCCTGATGCATTAGTAAGAGCTCTGAGGGAGGTGTGAA
AAATGCACCGATTTCAATTAGCACGGATGATGGTTCAACAGGTGAAAATGAGGATGTTGCAG
ACTCGGAAGCAGAAGGCTCAAACCTTTGGTTGGTTGCACAGCGTGAAGACAGTGCCAAGGG
CTCTGTGGTAAACAGAGGATCCGATATAAAGAGATCCAGTGATGCTACAGGTGGATTTTC
AAGGAAAAGGCAACCGAGCTTTTCAGGAAGTGAATCTAGCTCTGGAAAGTTGCCGCATGG
AAATCCCTTACAAGCATCAAATGTAGTGACTGCACAATATCAAGGCCAGACTCAGGTTTTCAG
CACCCCTGGGCATAACTAATGCACCCAATTTTCTCCAATGTATACAGTGCAATTGAGGCCA
CCCGTAAACAATGGACCAGCGGTCCAGACGATGGGTGGTGCCTCCCAGGTTTCTTTTGGTT
ACCCAACAGGTCAGCTACCAATACTTGAACGAGCTCTTCATGGGCATTTGGTACTCCGCCT
CAGGCTATTTCTTCTTTGCTGCAAAGCAAGCCGAACGAGCAGGAGCCAAACAAGCTGATG
ATGGAAAGAAACCTCAAGAGGCTGGCGCGTCTCCTCTGCTCTTTTGGAGGATGGTAAGGT
CGTTGAGAAGGTATTGCCTCTCATGGGCTCGGGCTCTGGTATAAGGCCGGGCATCGCCCC
GAATGTCAAATTCGGAGGGTCCGGATCATAACCCTGATCTTCCCTGGGTATCCACAACCGGG
TCTGGACCAAATGGCAGGACCATATCGGGCGTAACATACAACTTCGGCAGAAACGAAGTGA
AGATCGTGTGTGCTTGCCATGGCACACACATGACGCCTGAGGAGTTCACGCGACACGCTAG
CGTGGACGCAACAGGCCAGGAGAACAATGCTACCATGTCCGGCGTTTCTGTCTGGGAATCAA
GCGGCCTCAGCCCAAACCTAA-3'

Supplemental Figure 5. The coding sequence of TaNINJA from bread wheat cultivar KN199

Supplemental Figure 6

(a)



KN199

#7

#16

pUbi:TaJAZ1ΔJas

(b)



KN199

#7

#16

pUbi:TaJAZ1ΔJas

Supplemental Figure 6. Overexpression of TaJAZ1ΔJas have no defects on plant growth phenotypes.

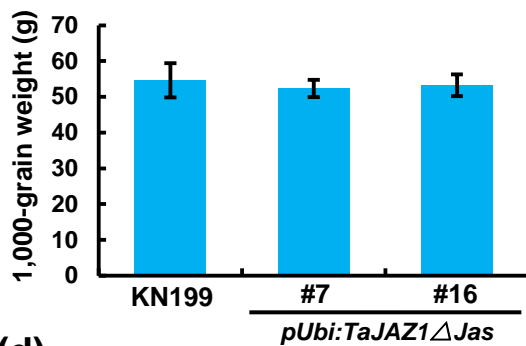
(a) Appearance of a mature plant. Bar = 10 cm. (b) Spike morphology. Bar = 2 cm.

Supplemental Figure 7

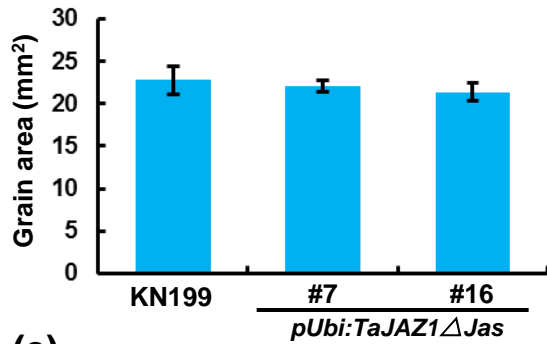
(a)



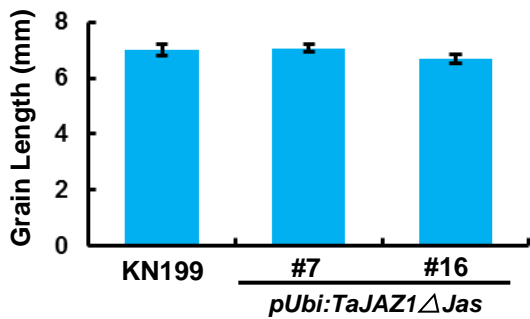
(b)



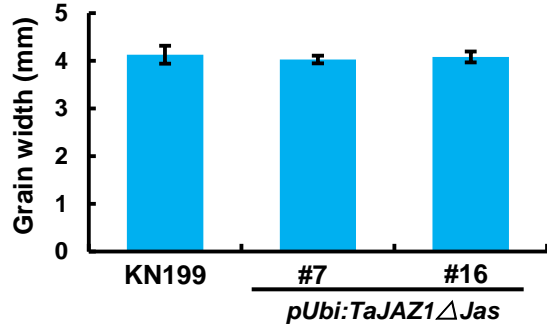
(c)



(d)



(e)



Supplemental Figure 7. Analysis of grain size in KN199 and two *pUbi:TaJAZ1ΔJas* transgenic lines.

(a) Appearance of the grains in KN199 and transgenic plants. Comparisons of 1000-grain weight (b), grain area (c), grain length (d), grain width (e). Error bars denote \pm SD (n = 6).

Supplemental Table 1

Supplemental Table 1. Constructs used in this study.

| Construct name | Vector | Description |
|------------------------------------------|-----------------------|--------------------------------------------------------------|
| <i>pUbi:TaJAZ1Δjas</i> | <i>pUbi:cas</i> | For gene transformation |
| <i>35S:TaJAZ1-GFP</i> | <i>PGWB5</i> | For subcellular localization analysis For LUC activity assay |
| <i>AD-TaJAZ1</i> | <i>pGADT7</i> | For Y2H assay |
| <i>BD-TaMYC4</i> | <i>pGBKT7</i> | For transcriptional activity assay and Y2H assay |
| <i>BD-TaMYC4-NT</i> | <i>pGBKT7</i> | For transcriptional activity assay |
| <i>BD-TaMYC4-CT</i> | <i>pGBKT7</i> | For transcriptional activity assay |
| <i>TaJAZ1pro:LUC</i> | <i>pGWB35</i> | For LUC activity assay |
| <i>TaMYC4-101</i> | <i>pEarlyGate 101</i> | For LUC activity assay |
| <i>MBP-TaMYC4</i> | <i>pMAL-c2X</i> | For pull down assay |
| <i>GST-TaJAZ1</i> | <i>pGEX-4T-1</i> | For pull down assay |
| <i>nYFP-TaJAZ1</i> | <i>pEarly201</i> | For BiFC assay in <i>N. benthamiana</i> |
| <i>cYFP-TaNINJA</i> | <i>pEarly202</i> | For BiFC assay in <i>N. benthamiana</i> |
| <i>nLUC-TaMYC4</i> | <i>p1300-35S-nLUC</i> | For LCI assay in <i>N. benthamiana</i> |
| <i>nLUC-TaMYC4-NT</i> | <i>p1300-35S-cLUC</i> | For LCI assay in <i>N. benthamiana</i> |
| <i>nLUC-TaMYC4-CT</i> | <i>p1300-35S-nLUC</i> | For LCI assay in <i>N. benthamiana</i> |
| <i>nLUC-TaNINJA</i> | <i>p1300-35S-nLUC</i> | For LCI assay in <i>N. benthamiana</i> |
| <i>nLUC-TaNINJA-NT</i> | <i>p1300-35S-nLUC</i> | For LCI assay in <i>N. benthamiana</i> |
| <i>nLUC-TaNINJA-MD</i> | <i>p1300-35S-nLUC</i> | For LCI assay in <i>N. benthamiana</i> |
| <i>nLUC-TaNINJA-CT</i> | <i>p1300-35S-nLUC</i> | For LCI assay in <i>N. benthamiana</i> |
| <i>cLUC-TaJAZ1</i> | <i>p1300-35S-cLUC</i> | For LCI assay in <i>N. benthamiana</i> |
| <i>cLUC-TaJAZ1-NT</i> | <i>p1300-35S-cLUC</i> | For LCI assay in <i>N. benthamiana</i> |
| <i>cLUC-TaJAZ1-CT</i> | <i>p1300-35S-cLUC</i> | For LCI assay in <i>N. benthamiana</i> |

Supplemental Table 2

Supplemental Table 2. Primers used in this study.

| Primer name | Sequence (5'-3') |
|-------------------------|--------------------------------------------|
| TaJAZ-B-F | AGGGGAGGGGAGGGGAGGGG |
| TaJAZ-B/D-F | CGAAATCCATTTATTCCCCGTC |
| TaJAZ-B/D-R | CCATAGATTCAACCATGATGGCTG |
| TaMYC4-outer-F | CTCCCATCCATTCCCCTTCC |
| TaMYC4-outer-R | CGATCATACGGTGGTGAGTAATAGG |
| TaMYC4-QBV3-F | ATGAACCTGTGGACGGACGA |
| TaMYC4-QBV3-R | CCGGATTTGCATCGCGGT |
| TaNINJA-outer-F | CCACCAGACTGCCTTGTGTTATAAT |
| TaNINJA-outer-R | ATATGGACACAAAGGGAACATCAAG |
| TaNINJA-QBV3-F | ATGGAGGATGGCCTTGAGCT |
| TaNINJA-QBV3-R | GTTTTGGGCTGAGGCCGC |
| TaTPL-outer-F | CAGGCTAGGTTTACGGCCAGG |
| TaTPL-outer-R | CTGAGATGGATACCTTGGTTAGTG |
| TaTPL-F | ATGTCTTCTCTCAGCCGGGAGC |
| TaTPL-R | TTATCTTTCTGGTTGATCAGAACTCGA |
| TaTPL-R(-stop) | TCTTTCTGGTTGATCAGAACTCGA |
| TaJAZ1-promoter-outer-F | CACAGAGAAGAAAGCACATGCAT |
| TaJAZ1-promoter-outer-R | AGTCCCTCTCCATCCAGCC |
| TaJAZ-promoter-QBV3-F | TACGCTAAATGCTAAGCCGCT |
| TaJAZ-promoter-QBV3-R | GGAATAAATGGATTTCTGTCTCTCAC |
| TaJAZ-QBV3-F | ATGGAGAGGGACTTCCTGGG |
| TaJAZ-QBV3-R | GATGTGTAATTTTGTACTCGGGGAC |
| PUBI-BamH1-TaJAZ-F | CGGGATCCATGGAGAGGGACTTCCTGGGC |
| PUBI-Kpn1-TaJAZ-R | GGGGTACCTCAAACAGCTCTTGGCATAATACTTG |
| Real-TaJAZ1-F | GACACGCCGAAGCCAAAGAC |
| Real-TaJAZ1-R | GGCAAAGGAGGTGAAACACG |
| Real-TaMYC4-F | TTTCTCAGATTTTCGCGTCCAAC |
| Real-TaMYC4-R | CTCGTTGATGTAGGAGATGGCG |
| Real-TaNINJA-F | CCTCAGGCTATTTCTTCCTTTGC |
| Real-TaNINJA-R | GGAAGATCAGGGTATGATCCGG |
| nLUC-TaMYC4-F | CACGGGGGACGAGCTCGGTACCATGAACCTGTGGACGGACGA |
| nLUC-TaMYC4-R | ACGCGTACGAGATCTGGTACCGGATTTGCATCGCGGT |
| TaMYC4-N-Kpn1-F | GGGGTACCATGAACCTGTGGACGGACGA |
| TaMYC4-N-Sal1-R | GCGTCGACCGGCCGCTTCTCCTCC |
| TaMYC4-C-Kpn1-F | GGGGTACCATGCGCAAGCGCGGG |
| TaMYC4-C-Sal1-R | GCGTCGACCCGGATTTGCATCGCGG |
| TaJAZ1-N-Kpn1-F | GGGGTACCATGGAGAGGGACTTCCTGGG |
| TaJAZ1-N-Sal1-R | GCGTCGACAGATTTTGGCACTGCGGAAT |
| TaJAZ-C-Kpn1-F | GGGGTACCATGTCAGGCCAGTTGGTTGTG |
| TaJAZ-C-Sal1-R | GCGTCGACGATGTGTAATTTTGTACTCGGGGAC |
| nLUC-TaNINJA-F | CACGGGGGACGAGCTCGGTACCATGGAGGATGGCCTTGAGCT |

Supplemental Table 2. Primers used in this study.

(Continued)

| Primer name | Sequence (5'-3') |
|------------------|--------------------------------------------------|
| nLUC-TaNINJA-R | ACGCGTACGAGATCTGGTCGACGTTTTGGGCTGAGGCCGC |
| nLUC-TaNINJA-N-R | ACGCGTACGAGATCTGGTCGACAGCATCGGGAGGTTGCGA |
| nLUC-TaNINJA-M-F | CACGGGGGACGAGCTCGGTACCATGCCTTTTGCGCATTACTATCAA |
| nLUC-TaNINJA-M-R | ACGCGTACGAGATCTGGTCGACTTCGGCTTGCTTTGCAGC |
| nLUC-TaNINJA-C-F | CACGGGGGACGAGCTCGGTACCATGCGAGCAGGAGCCAAACAAG |
| TaJAZ1-4T-1-F | CCGCGTGGATCCCCGGAATTCATGGAGAGGGACTTCCTGGG |
| TaJAZ1-4T-1-R | ATCCGGCCGCTCGABTCGACTCAGATGTGTAATTTTGTACTCGGGGAC |
| TaMYC4-MBP-F | TTCAGAAATTCGGATCCATGAACCTGTGGACGGACGA |
| TaMYC4-MBP-R | TTGCCTGCAGGTGCACTTACCGGATTTGCATCGCGGT |
| TaMYC4-N-BD-F | ATCTCAGAGGAGGACCTGCATATGATGAACCTGTGGACGGACGA |
| TaMYC4-N-BD-R | AGGTCGACGGATCCCCGGGAATTCGGCCGCTTCTCCTCC |
| TaMYC4-C-BD-F | ATCTCAGAGGAGGACCTGCATATGATGCGCAAGCGCGGG |
| TaMYC4-C-BD-R | AGGTCGACGGATCCCCGGGAATTCGGATTTGCATCGCGG |
| TaMYC4-BD-F | ATCTCAGAGGAGGACCTGCATATGATGAACCTGTGGACGGACGA |
| TaMYC4-BD-R | AGGTCGACGGATCCCCGGGAATTCGGATTTGCATCGCGG |
| TaJAZ1-AD-F | CAGATTACGCTCATATGATGGAGAGGGACTTCCTGGG |
| TaJAZ1-AD-R | CACCCGGGTGGAATTCGATGTGTAATTTTGTACTCGGGGAC |
| Real-TaPR1-F | GAGAATGCAGACGCCCAAGC |
| Real-TaPR1-R | CTGGAGCTTGCAGTCGTTGATC |
| Real-TaPR2-F | AGGATGTTGCTTCCATGTTTGCCG |
| Real-TaPR2-R | AAGTAGATGCGCATGCCGTTGATG |
| Real-TaPOX2-F | AGGGGCTTCGGCGTCATC |
| Real-TaPOX2-R | TTGGGCGTCGTCGTGTCC |
| Real-TaSOD-F | CCGAGGTCTGGAACCATCAC |
| Real-TaSOD-R | AGCCGAAATCCTTCTCGATCT |