Figure S1

$B$


Fig. S1 Full cDNAs of TaAGL6 genes.
(A) Results of RACE. (B) Alignment of TaAGL6 full cDNAs. Rectangular boxes indicate the start and stop codons, respectively.

Figure S2


Fig. S2 Protein sequence and the effect of SNPs.
(A) Multiple allignment of TaAGL6 proteins. Rectangular boxes indicated MADS-box, K domains, AGL6 motifs I and II, respectively. (B and C) The effect of SNPs to the protein functions. Default threshold is -2.5 , that is: The variant with a score equal to or below -2.5 is considered "deleterious," the variants with a score above -2.5 are considered "neutral."

Figure S3


Fig. S3 The expression of TaAGL6-A (A), TaAGL6-B (B) and TaAGL6-D (C) in different floral organs.

Mean and SD values were obtained from three replications. Different letters indicate a significant difference ( $P<0.05$ by Student's $t$ test).

Figure S4


Fig.S4 Phenotypes of transgenic Arabidopsis over-expressing TaAGL6 genes
(A-D) Four-week plants of wild type (A) and transgenic Arabidopsis overexpressing TaAGL6-A (B), TaAGL6-B (C), and TaAGL6-D (D) to show the early flowering phenotype of transgenic lines. (E) The number of rosette leaves in the control and in transgenic line overexpression TaAGL6-B at flowering time. (F-I) Wild type (F), transgenic Arabidopsis overexpressing TaAGL6-A (G), TaAGL6-B (H), and TaAGL6-D (I) to show the multi-shoot phenotypes. (J) The number of main stems in the control and transgenic Arabidopsis overexpressing TaAGL6-B. (K) The expression level of $A t F T$ in different transgenic lines. Bars $=1 \mathrm{~cm}$ in A to $\mathrm{D}, \mathrm{F}$ to I . In E and J , mean and SD values were obtained from 30 replications ( $n=30$ ), and letters $a$ and $b$ indicated the significant difference between the control and transgenic plants according to Student's $t$-test ( $P<0.05$ ). Mean and SD values in K were obtained from three replications, and different letters in K indicated a significant difference ( $P<0.05$ by Student's $t$ test).

Figure S5


Fig. S5 The expression of TaAGL6 genes in transgenic Arabidopsis (A), transgenic wheat (B) overexpressing TaAGL6-B, and TaAGL6 RNAi wheat (C).

Mean and SD values were obtained from three replications, and different letters indicated significant differences ( $P<0.05$ by Student's $t$ test).

Figure S6


Fig. S6 Phenotype of transgenic wheat over-expressing TaAGL6-B.
(A-C) One plant of control (A) and transgenic Line 11 (B) and Line 18 (C) photographed at the same time. (D and E) One inflorescence of control (D) and one inforescence of transgenic plants (E) collected at the same time. (F) The expression of TaFT in different transgenic lines at vegetative stage. Bars $=1 \mathrm{~cm}$ in A-C, $50 \mu \mathrm{~m}$ in D and E . Mean and SD values in F were obtained from three replications, and different letters in F indicated a significant difference ( $P<0.05$ by Student's $t$ test).

Figure S7


Fig. S7 Self-activation assays of TaAGL6, TaAP3, TaAG, and TaMADS13.

Figure S8


Fig. S8 Interactions between TaAGL6-B/TaAGL6-D and TaAP3, TaAG, TaMADS13. (A and B) Interactions between TaAGL6-B and TaAP3, TaAG, TaMADS13 in yeast cells (A), and tobacco leaf cells (B). (C and D) Interactions between TaAGL6-D and TaAP3, TaAG, TaMADS13 in yeast cells (C), and tobacco leaf cells (D). In B and D, left, YFP; middle, Bright; right, Merged.

Figure S9


Fig. S9 The expression of wheat floral genes and TaMGH3 in wild type and

## TaAGL6 RNAi stamens.

Mean and SD values in were obtained from three replications, and different letters indicated significant differences ( $P<0.05$ by Student's $t$ test).

Figure S10

|  | ProtaAP3-B M2 |  |  | ProTaAP3-D M3 |  |  | ProTaAP3-D M4 |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| TaAGL6-B-GST | - | - | $+$ | - | - | + | - | - | $+$ |
| GST | - | + | - | - | $+$ | - | - | + | - |
| Biotin-Probes | + | + | + | + | $+$ | + | + | + | $+$ |



Fig. S10 Results of EMSA to show that AGL6 proteins could not bind to CArG motifs 2-4.

Figure S11


Fig. S11 Proposed model to illustrate the function and mechanism of TaAGL6 in stamen development
(A) In wild type wheat, TaAGL6 transcription factors directly and indirectly regulate the expression of TaAP3 and TaMGH3, respectively. TaAP3 and TaMGH3 function in stamen development. Meanwhile, TaAP3 represses the expression of TaDL in stamens. As a result, stamens develop normally. (B) In TaAGL6 RNAi plants, the expression of TaAGL6 genes is down-regulated. Consequently, the expression of TaAP3 and TaMGH3 is down-regulated, while TaDL is ectopically expressed in stamens. As a result, the stamens develop abnormally, and display the potential to transform into carpels.

Table S1. Primers used in this research.

| Name | Sequence ( $5^{\prime}-3 \prime$ ) |
| :---: | :---: |
| 3GSP | ATGGGGAGGGGAAGGGTCGAG |
| 5GSP | ATCTTTCCAATGTTTTTGTTGTGCC |
| TaAGL6ADLF | TAGTAGGTTGCGCGTCAGAA |
| TaAGL6ADLR | CCTCCTTCCAAGCAAGCAAGAAAAT |
| TaAGL6BDLF | GAAGGCAGCAACAGCAACAA |
| TaAGL6BDLR | GGAATGATTAGGGTGTTGCTGA |
| TaAGL6DDLF | GTACCCTCATCAGTTCGCGG |
| TaAGL6DDLR | ACAAGCTAACCAGCAGCTCGCT |
| TaAGL6DLF | CTTGCTTGGGGAGGACCTTGGA |
| TaAGL6DLR | CATCATAAGTTGTGTCTTTCGCTGT |
| TaAGL6RNAiPF | GGTGGTAAGCTTGCGGCCGCTGAAGGCAGCAACAGCAACAACTA |
| TaAGL6RNAiPR | GGTGGTGAATTCGGATCCATGGACAGCTTGGAACTTGCA |
| T7 promoter sequence | TAATACGACTCACTATAGGG |
| TaAGL6T7-F | TAATACGACTCACTATAGGGCTGAAGGCAGCAACAGCAACAACTA |
| TaAGL6T7-R | TAATACGACTCACTATAGGGATGGACAGCTTGGAACTTGCAA |
| TaActinPF | TATGCCAGCGGTCGAACAAC |
| TaActinPR | GGAACAGCACCTCAGGGCAC |
| TaAGL6OF | GGTGGTCCATGGGGAGGGGAAGGGTCGAG |
| TaAGL6OR | GGTGGTCACGTGTCAGAGAATCCACCCCAGCAT |
| 1301PF | GTGATATCTCCACTGACGTAAGGG |
| 1301PR | GATAATCATCGCAAGACCGGCA |
| pBSKR | GACAGCAGCAGTTTCATCAATCA |
| AtGAPCPF | TCAGACTCGAGAAAGCTGCTACC |
| AtGAPCPR | GATCAAGTCGACCACACGGGAA |
| TaAGL6PF | ATGGGGAGGGGAAGGGTC |


| TaAGL6PR | TCAGAGAATCCACCCCAGCAT |
| :---: | :---: |
| TaAP3PF | ATGGGGCGGGGGAAGAT |
| TaAP3PR | TTAGCCGAGGCGCAGGTC |
| TaAGPF | ATGCAGATACTCAACGAGCAGCT |
| TaAGPR | TCACCTTCCAACTGAGTT |
| TaMADS13PF | ATGGGGAGGGGAAGGATTG |
| TaMADS13PR | CTAGAACTGATGAGCCACATCGC |
| TaAP3DLF | AGGAGGCATACAAGAATCTGCA |
| TaAP3DLR | GCTAGTAGGAGCGATCGAAGTGA |
| TaAGDLF | TACTCCAACAACAGCGTGAAAGC |
| TaAGDLR | GTATCGCCTATTAGAGTCCTGTTGG |
| TaMADS58DLF | ATCAAGCGCATCGAGAACAC |
| TaMADS58DLR | ATGGTTGCTTTCACGCTGTT |
| TaDLDLF | AACCTCTCCTTTCTCAGCCC |
| TaDLDLR | GGGCTTCACAACAAAGGGAG |
| TaMADS13DLF | TCAGAACCAAGATTGCGGAGGA |
| TaMADS13DLR | CTAGAACTGATGAGCCACATCGC |
| TaSEPDLF | AAGAAGGCCTACGAGCTCTC |
| TaSEPDLR | GGTACTCATTGCGGCTGTTT |
| TaLHS1DLF | CTCAAGCATATCAGGTCAAAAAAGAATCAA |
| TaLHS1DLR | TCAGAAGCCACGTGATCTCTGTT |
| TaMGH3DLF | CCTACATCCAGCGCATTGTC |
| TaMGH3DLR | ACGAACAGGAAGTAGAGGCC |
| TaAP3BCARG1PROBEF | AAAAGATCTTTTCGTTCCAGAAGAA |
| TaAP3BCARG1PROBER | GGTAGCCAAAAAATTCTAAATACCA |
| TaAP3BCARG2PROBEF | TGCCCGTTCTATTCT |
| TaAP3BCARG2PROBER | ATCATTGCTTCGCTGCTTT |
| TaAP3DCARG1PROBEF | GAACGCTAGCTAAGCCATAGG |
| TaAP3DCARG1PROBER | CTGTCCACTTCCAAAAGAGGT |


| TaAP3DCARG2PROBEF | CCTTCTTCCTCCTCCTA |
| :---: | :---: |
| TaAP3DCARG2PROBER | TGGATAGAAGGGGCATTGTCT |
| TaAGL6-BGSTF | CCTGGGATCCCCGGAATTCATGGGGAGGGGAAGGGTC |
| TaAGL6-BGSTR | GTCACGATGCGGCCGCTCGAGTCACCTGTGCTTGAGTTGCCTGTT |
| TaAP3F | AAGCTTGAATTCGAGCTC |
|  | GACTAATTAAAGCAGACTAATTAAAGCAGACTAATTAAAGCA |
|  | GTCGACCTCGAGGCATGT |
| TaAP3R | ACATGCCTCGAGGTCGAC |
|  | TGCTTTAATTAGTCTGCTTTAATTAGTCTGCTTTAATTAGTC |
|  | GAGCTCGAATTCAAGCTT |
| Mut TaAP3F | AAGCTTGAATTCGAGCTC |
|  | GACTCGTTCGCGCAGACTCGTTCGCGCAGACTCGTTCGCGCA |
|  | GTCGACCTCGAGGCATGT |
| Mut TaAP3R | ACATGCCTCGAGGTCGAC |
|  | TGCGCGAACGAGTCTGCGCGAACGAGTCTGCGCGAACGAGTC |
|  | GAGCTCGAATTCAAGCTT |
| TaAGL6-BOE6HAF | GTCGACGGTATCGAT AAGCTT ATGGGGAGGGGAAGGGTCGAG |
| TaAGL6-BOE6HAR | AGAACTAGTGGATCC CCCGGG GAGAATCCACCCCAGCAT |
| TaAP31302GFPF | CATGGTAGATCTG ACTAGT ATGGGGCGGGGGAAGAT |
| TaAP31302GFPR | GCCCTTGCTCACCAT CCTAGG GCCGAGGCGCAGGTC |
| TaAG 1302GFPF | CATGGTAGATCTG ACTAGT ATGCAGATACTCAACGAGCAGCT |
| TaAG 1302GFPR | GCCCTTGCTCACCAT CCTAGG CCTTCCAACTGAGTT |
| TaMADS13GFPF | CATGGTAGATCTG ACTAGT ATGGGGAGGGGAAGGATTG |
| TaMADS13GFPR | GCCCTTGCTCACCAT CCTAGG GAACTGATGAGCCACATCGC |
| TaAGL6-B 62SKF | CGCTCTAGAACTAGT GGATCC ATGGGGAGGGGAAGGGTCGAG |
| TaAGL6-B 62SKR | GTCGACGGTATCGAT AAGCTT TCAGAGAATCCACCCCAGCAT |
| TaAP3B-0800LUCF | GGCCCCCCCTCGAG GTCGACAAAAGATCTTTTCGTTCCAGAAGAA |
| TaAP3B-0800LUCR | GCTCTAGAACTAGT GGATCC GGGGCGGCCGTGGTTTTGA |

