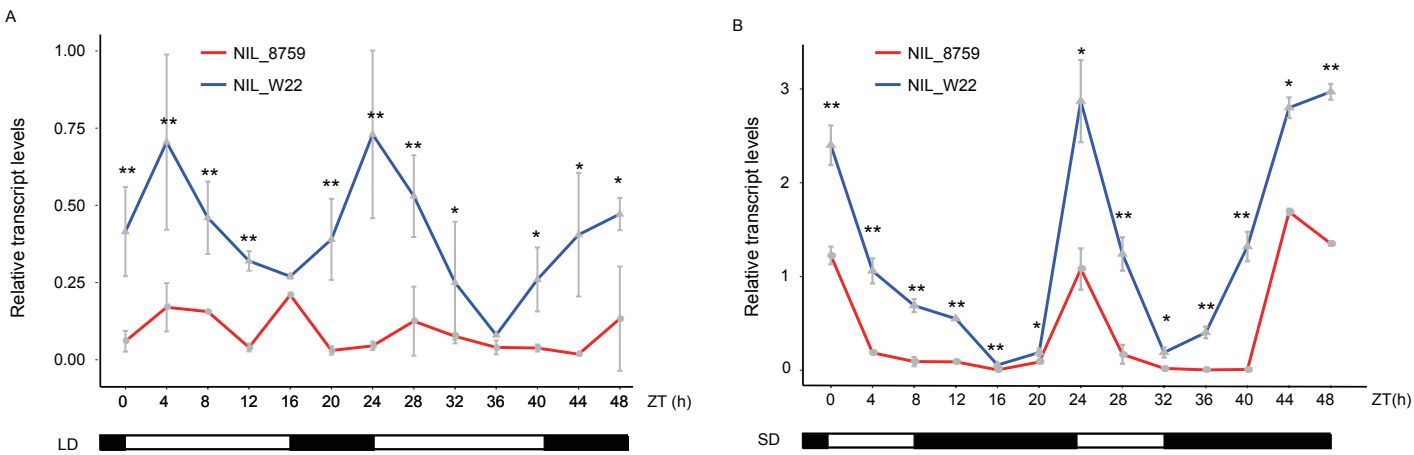

Figure S1.Diurnal expression pattern of *ZCN8* in mature leaves of NIL(W22) and NIL(8759) under natural long day condition (A) and short day condition (B). Related to Figure2.
The black bars and white bars indicate the dark period and the light period, respectively. ZT, zeitgeber time. All data are expressed relative to the control gene *ZmTubulin1* and represent as the means ± s.d. of three biological replicates. The single and double asterisks represent significant differences determined by Student's *t* test at $P < 0.05$ and $P < 0.01$,respectively.

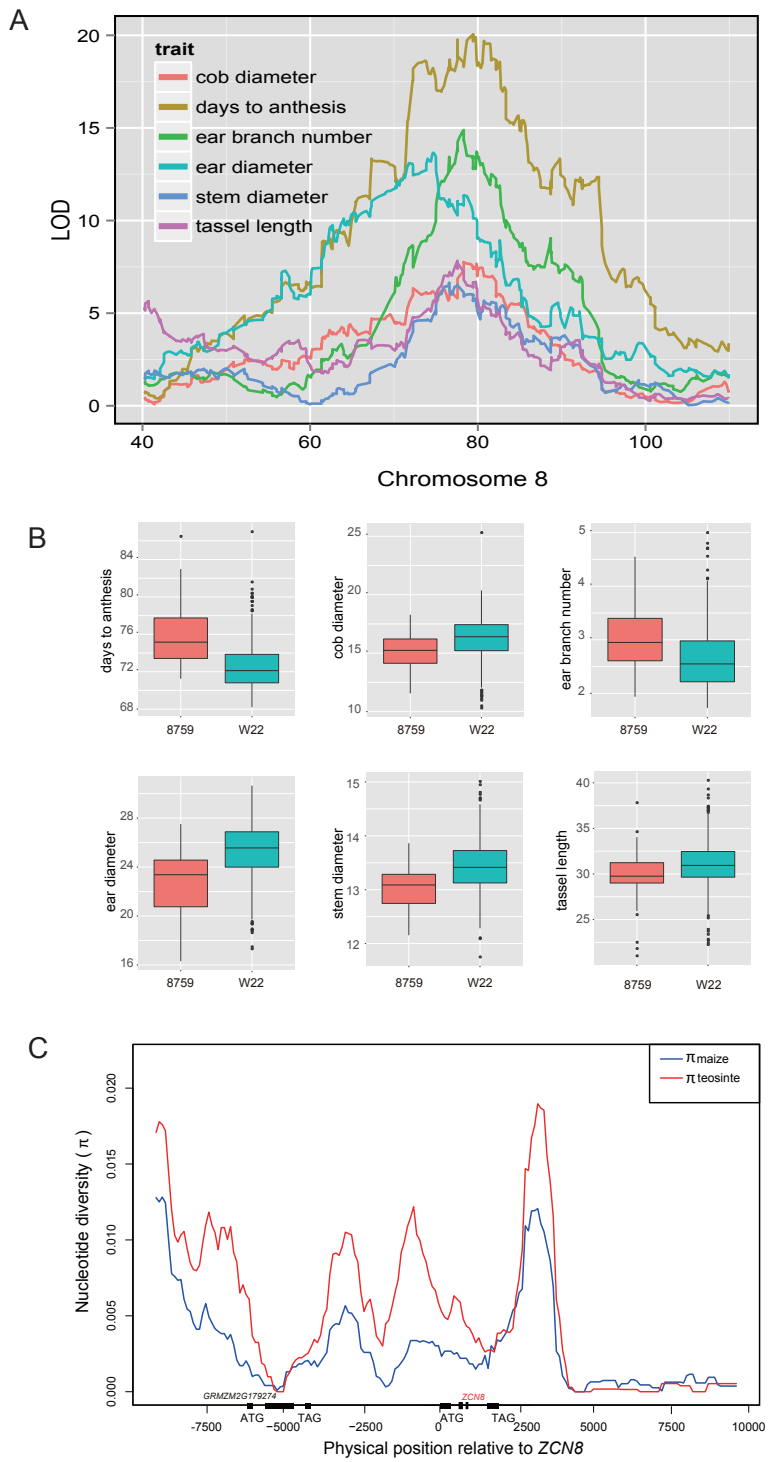

Figure S2. *ZCN8* is located in a QTL hotspot affecting many domestication traits and was targeted by selection. Related to Figure 4.

(A) Logarithm of odds (LOD) scores of scanning positions for different traits. (B) Allele effect at the QTL for different traits. (C) Nucleotide diversity around *ZCN8* region in maize and teosinte (HapMap 3 data)[S1]. The promoter region of *ZCN8* exhibited an obvious reduction of nucleotide diversity in maize relative to teosinte. *GRZM2G179274* is the upstream neighboring gene of *ZCN8*.

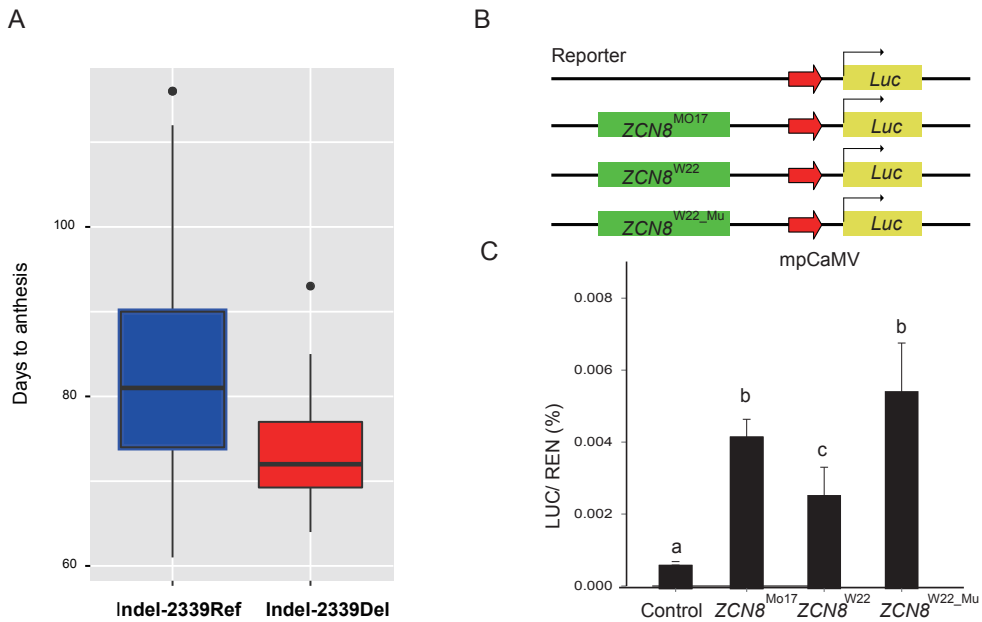

Figure S3. Indel-2339 shows significant association with flowering time and affects gene expression, related to Figure 3.

(A) Flowering time difference at Indel-2339 in the association panel under natural long day conditions. Maize lines carrying the 3-bp deletion (Indel-2339Del) flowered significantly earlier than those carrying the B73 reference allele (Indel-2339Ref). (B) Constructs used to test the effect of the Indel-2339 on gene expression in transient expression assays in maize leaf protoplasts. The luciferase (*LUC*) gene driven by a ~3-kb promoter sequence from W22 or Mo17 was used as the reporter. To examine the effect of Indel-2339, nucleotides GAG at the Indel-2339 site in the W22 construct was site-directed deleted. (C) The effect of Indel-2339 on gene expression. The Mo17 construct exhibited higher luciferase activity than the W22 construct. When nucleotides GAG at the Indel-2339 site in the W22 construct was site-directed deleted, the luciferase activity increased to a level of that of the Mo17 construct. Values are presented as the means ± s.d. (n=5 biological replicates); The differences in luciferase activity were determined by Student's *t* test (P <0.05).