Fig. S1. Sequence motifs found in bZIP28. The positions of the bZIP, transmembrane (TM) domain, and putative S1P and S2P sites are indicated. Predicted glycosylation sites are indicated with asterisks. Numbers represent amino acid residue positions.
Fig. S2. Up-regulation of bZIP28 expression by heat. WT seedlings were heat treated for the indicated periods of time. RNA was extracted from each plant, and the expression levels of bZIP28 (At3g10800) were analyzed using semiquantitative RT-PCR as described in the text.
Fig. S3. Three-dimensional reconstruction of a tobacco leaf epidermal cell expressing YFP-bZIP28 after heat shock at 42°C for 15 min. Images were collected as single planes with 1 airy unit pinhole and stacked as maximum projection for three-dimensional reconstruction. The ER is clearly visible in the cortical regions of the cell (arrow). (Scale bar, 5 µm.)
Fig. S4. RT-PCR analysis of the bZIP28 T-DNA insertion allele. (A) Diagram of a bZIP28 insertion allele. Black boxes represent exons, gray boxes represent the 5'- and 3'-untranslated regions, and the hatched box represents an intron. The first base of the 72-bp 5' UTR is defined as +1. The location of the T-DNA insertion in the bZIP28 null allele (i.e., SALK_132285) is indicated with an arrow. (B) PCR-based detection of a T-DNA insertion in bZIP28. DNA extracted from WT (1) and Salk.132285 (2) were analyzed by PCR using the following oligonucleotides: 132285-LP, 132285-RP, and LBb1. (Left) 1-kb DNA ladder (New England BioLabs) is shown. (C) Analysis of bZIP28 expression in the bZIP28 mutant and a rescued line. The bZIP28 T-DNA mutant (Mutant) and the same mutant containing a transgene in which a bZIP28 promoter fragment drives the expression of YFP-bZIP28 (Comp 1) were heat treated for the indicated periods of time. RNA was extracted from each plant, and the expression level of bZIP28 (At3g10800) was analyzed using semiquantitative RT-PCR with oligonucleotides that bind upstream and downstream of the T-DNA insertion site. Comp 1 was also used in Fig. 4. (D) Expression analysis of the bZIP28 mutant upstream and downstream of the T-DNA insertion site. The T-DNA insertion mutant was incubated at 42°C for the indicated periods of time. RNAs transcribed from upstream (bZIP28–2) and downstream (bZIP28–3) of the T-DNA insertion site were detected using semiquantitative PCR.
Fig. S5. Effect of Δ301-675 on genes that are heat induced and coexpressed with bZIP28 or genes that are induced by ER stress. The expression of the indicated genes was quantitated in the bZIP28 mutant and line A (Fig. 1B) by RT-PCR, as described in the text. These data are from two biological replicates and were normalized to UBQ10 expression. Error bars represent SD. Genes were identified as described in supporting information (SI) Table S2 and Table S3.
Fig. S6. The effect of estradiol and experimental manipulations on BiP2 expression. The bZIP28 mutant was subjected to treatment with estradiol as described in Materials and Methods or to a mock treatment that was identical except for the omission of estradiol. BiP2 mRNA levels were quantitated by RT-PCR at 0, 1, 2, or 4 h, as described in Materials and Methods.
Table S1. Heat-induced genes that are coexpressed with bZIP28

<table>
<thead>
<tr>
<th>AGI locus identifier</th>
<th>Encoded protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>At1 g07350</td>
<td>Putative transformer serine/arginine-rich ribonucleoprotein</td>
</tr>
<tr>
<td>At1 g16030</td>
<td>HSP70B</td>
</tr>
<tr>
<td>At1 g54050</td>
<td>17.4-kDa class III HSP</td>
</tr>
<tr>
<td>At1 g74310</td>
<td>HSP101</td>
</tr>
<tr>
<td>At2 g18510</td>
<td>Embryo defective 2444</td>
</tr>
<tr>
<td>At2 g20560</td>
<td>DNAJ heat shock family protein</td>
</tr>
<tr>
<td>At3 g01210</td>
<td>Nucleic acid–binding protein</td>
</tr>
<tr>
<td>At3 g12050</td>
<td>Aha1 domain-containing protein</td>
</tr>
<tr>
<td>At3 g25230</td>
<td>Rotamase FKB 1</td>
</tr>
<tr>
<td>At3 g57810</td>
<td>OTU-like cysteine protease family protein</td>
</tr>
<tr>
<td>At4 g03320</td>
<td>TIC20-IV</td>
</tr>
<tr>
<td>At5 g09590</td>
<td>HSP70</td>
</tr>
<tr>
<td>At5 g27660</td>
<td>Serine-type peptidase/trypsin</td>
</tr>
<tr>
<td>At5 g51740</td>
<td>Peptidase M48 family protein</td>
</tr>
</tbody>
</table>

We identified the genes in this list by screening publicly available genome expression data for genes that are heat induced and coexpressed with bZIP28, as described in Materials and Methods.
Table S2. The effect of Δ301–675 on genes induced by heat and ER stress

<table>
<thead>
<tr>
<th>AGI locus identifier</th>
<th>Encoded protein</th>
<th>Induced/not induced</th>
</tr>
</thead>
<tbody>
<tr>
<td>At4 g21810</td>
<td>Similar to DER1</td>
<td>–</td>
</tr>
<tr>
<td>At5 g42020</td>
<td>Bip2</td>
<td>+</td>
</tr>
<tr>
<td>At2 g47180</td>
<td>Putative galactin synthase</td>
<td>–</td>
</tr>
<tr>
<td>At5 g61790</td>
<td>Calnexin 1</td>
<td>+</td>
</tr>
<tr>
<td>At4 g24190</td>
<td>AtHsp90-7</td>
<td>+</td>
</tr>
<tr>
<td>At2 g32920</td>
<td>Protein disulfide isomerase</td>
<td>+</td>
</tr>
<tr>
<td>At5 g03160</td>
<td>P58IPK</td>
<td>+</td>
</tr>
<tr>
<td>At1 g18260</td>
<td>Similar to SEL1/HRD3</td>
<td>+</td>
</tr>
<tr>
<td>At5 g47120</td>
<td>BAX inhibitor 1</td>
<td>+</td>
</tr>
</tbody>
</table>

These genes were obtained from a larger list of genes that are induced by heat and during ER stress (29). +, induced; –, induced in line A relative to the bZIP28 mutant (Fig. S3).
Table S3. The effect of Δ301–675 on heat-induced genes that encode ER-localized proteins

<table>
<thead>
<tr>
<th>AGI locus identifier</th>
<th>Encoded protein</th>
<th>Induced/not induced</th>
</tr>
</thead>
<tbody>
<tr>
<td>At1 g14360</td>
<td>UDP-galactose transporter 3</td>
<td>+</td>
</tr>
<tr>
<td>At1 g05170</td>
<td>Galactosyltransferase family protein</td>
<td>+</td>
</tr>
<tr>
<td>At1 g13080</td>
<td>Cytochrome P450 71B2</td>
<td>+</td>
</tr>
</tbody>
</table>

We identified the genes in this list by screening publicly available genome expression data for genes that are induced by heat, are coexpressed with bZIP28, and encode ER-localized proteins as described in Materials and Methods. +, induced; –, not induced in line A relative to the bZIP28 mutant (Fig. S3).