The transcription factor Zt107320 affects the dimorphic switch, growth and virulence of the fungal wheat pathogen *Zymoseptoria tritici*

Michael Habig, Sharon Marie Bahena-Garrido[#], Friederike Barkmann, Janine Haueisen and Eva Holtgrewe Stukenbrock[*]

Environmental Genomics, Christian-Albrechts University of Kiel and Max Planck Institute for Evolutionary Biology, Plön, Germany,

Current address:

#National Research Institute of Brewing, 3-7-1 Kagamiyama, Higashi-Hiroshima, 739-0046 Japan

*corresponding author: estukenbrock@bot.uni-kiel.de

Running head: Zt107320 regulates virulence in *Z. tritici*

Keywords: Fungal dimorphism, incompatible host, Septoria leaf blotch, gene expression, pycnidia formation

Word count: 6136

Summary

Zymoseptoria tritici is a filamentous fungus causing Septoria tritici blotch in wheat. The pathogen has a narrow host range and infections of grasses other than susceptible wheat are blocked early after stomatal penetration. During these abortive infections the fungus shows a markedly different expression pattern. However, the underlying mechanisms causing differential gene expression during host and non-host interaction are largely unknown, but likely include transcriptional regulators responsible for the onset of an infection program in compatible hosts. In the rice blast pathogen *Magnaporthe oryzae*, MoCOD1, a member of the fungal $Zn(II)_2Cys_6$ transcription factor family, has been shown to directly affect pathogenicity. Here, we analyse the role of the putative transcription factor Zt107320, a homolog of MoCOD1, during infection of compatible and incompatible hosts by *Z. tritici*. We show for the first time that *Zt107320* is differentially expressed in host versus non-host infections and that lower expression corresponds to an incompatible infection of non-hosts. Applying reverse genetics approaches we further show that Zt107320 regulates the dimorphic switch as well as the growth rate of *Z. tritici* and affects fungal cell wall composition *in vitro*. Moreover, Δ*Zt107320* mutants showed reduced virulence during compatible infections of wheat. We conclude that Zt107320 directly influences pathogen fitness and propose that Zt107320 regulates growth processes and pathogenicity during infection. Our results suggest that this putative transcription factor is involved in discriminating compatible and non-compatible infections.

Introduction

The fungus *Zymoseptoria tritici* (synonym *Mycosphaerella graminicola*) infects wheat and causes the disease Septoria tritici blotch. The pathogen is found worldwide where wheat is grown and can cause severe reduction in yield (Fones and Gurr, 2015). Upon infection, the fungus enters the leaf through stomata and establishes a hyphal network in the mesophyll. It propagates without causing visual symptoms for 7-14 days before inducing necrosis and producing pycnidia – asexual fructifications, where pycnidiospores are produced that can spread via contact or rain-splash to neighbouring leaves (Brading *et al.* 2002; Kema, G. H. J. *et al.* 1996; Ponomarenko A. S.B. Goodwin, G.H.J. Kema, 2011). *Z. tritici* has a heterothallic mating system and meiosis leads to the production of wind-borne ascospores that are considered to be the main primary inoculum (Kema *et al.* 1996; Morais *et al.* 2016; Ponomarenko A. S.B. Goodwin, G.H.J. Kema, 2011). Recently, we could show that chromosomal inheritance is characterised by frequent chromosome losses and rearrangements during mitosis and a drive of accessory chromosomes during meiosis (Habig *et al.* 2018; Möller *et al.* 2018), which may contribute to the genomic variation observed for *Z. tritici* (Grandaubert *et al.* 2017; Hartmann *et al.* 2017). Under experimental conditions, the fungus has a narrow host range infecting wheat and shows abortive infections on closely related non-host grass species like *Triticum monococcum* (Jing *et al.* 2008) and *Brachypodium distachyon* (Kellner *et al.* 2014; O'Driscoll *et al.* 2015). However, the underlying determinants of host specialisation and host specificity of *Z. tritici* are largely unknown.

A previous study comparing the expression profiles of *Z. tritici* between early infection (4 days post infection) of the compatible host *T. aestivum* and the non-host *B. distachyon* revealed a set of 289 genes that were similarly expressed in the two hosts, but differentially expressed compared to growth in axenic culture (Kellner *et al.* 2014). These genes are likely crucial for *Z. tritici* during stomatal penetration that occurs in same way in both hosts. However, 40 genes showed differential expression between host and non-host infections (Kellner *et al.* 2014) and are possibly involved in the discrimination of compatible and non-compatible host-pathogen interactions. The signalling and

regulatory networks responsible for these differential expression patterns are however unknown. One of the differentially expressed genes encodes the putative transcription factor Zt107320. Expression of *Zt107320* was significantly increased during infection of *T. aestivum* compared to the early infection of *B. distachyon* (Kellner *et al.* 2014) suggesting a host-dependent regulation of the gene.

Zt107320 encodes a putative transcription factor belonging to the $Zn(II)_2Cys_6$ family. This gene family of transcription factors is exclusive to fungi (MacPherson *et al.* 2006; Pan and Coleman, 1990) and many members play an important role in the regulation of fungal physiology. For example, $Zn(II)_2Cys_6$ transcription factors in *Magnaporthe oryzae*, *Fusarium oxysporum*, *Leptosphaeria maculans, Parastagonospora nodorum* and *Pyrenophora tritici-repentis* are involved in the regulation of fungal growth and pathogenicity (Fox *et al.* 2008; Galhano *et al.* 2017; Imazaki *et al.* 2007; Lu *et al.* 2014; Rybak *et al.* 2017). Interestingly, the homolog of *Zt107320* in the rice blast pathogen *M. oryzae*, MoCOD1, was shown to affect conidiation and pathogenicity (Chung *et al.* 2013). *MoCOD1* was found to be upregulated during conidiation and appressorium formation at 72h post infection. Furthermore, the deletion mutant Δ*MoCOD1* showed defects in conidial germination and appressorium formation. *In planta*, the mutant Δ*MoCOD1* was attenuated in extending growth from the first-invaded cells and caused markedly reduced symptoms when compared to the wildtype (Chung *et al.* 2013).

Transcription factors, in general, regulate expression by integrating various signalling pathways and represent interesting targets for dissecting causes and mechanisms of pathogenicity and host specificity. In *M. oryzae,* a systemic approach was applied to characterise all 104 members of the $Zn(II)_2Cys_6$ family of transcription factors. Of these, 61 were shown to be involved in fungal development and pathogenicity (Lu *et al.* 2014). Similarly, in the head blight causing fungus *Fusarium graminearum* 26% of 657 tested transcription factors had an effect on the tested phenotypes mycelial growth, conidia production and toxin production (Son *et al.* 2011). In summary, a number of transcription factors, which play important roles in host infection and pathogenesis, have been

identified for several important crop pathogens (Chen *et al.* 2017; Okmen *et al.* 2014; Xiong *et al.* 2015; Zhang *et al.* 2018; Zhuang *et al.* 2016). In *Z. tritici* however, only few regulatory genes have been characterised. Recently, two transcription factors ZtWor1 (Mirzadi Gohari *et al.* 2014) and ZtVf1 (Mohammadi *et al.* 2017) were shown to be important regulators of development and virulence of *Z. tritici* during compatible infections of wheat, highlighting how transcription factors can be used to identify and dissect aspects of pathogenicity. ZtWor1 has been functionally characterised and appears to be involved in the cAMP-dependent pathway, upregulated during the initiation of colonization and involved in regulating effector genes (Mirzadi Gohari *et al.* 2014). ZtVf1, a transcription factor belonging to the C_2-H_2 subfamily is required for virulence and its deletion leads to lower pycnidia density within lesions. Decreased virulence appears to be due to a reduced penetration frequency and impaired pycnidia differentiation (Mohammadi *et al.* 2017)

Based on the close homology of *Zt107320* to *MoCOD1* and its significantly different expression profile during host and non-host infections, we hypothesized that Zt107320 plays an important role during early wheat infection of *Z. tritici*. Our results confirm that Zt107320 affects virulence of *Z. tritici* during compatible infections and regulates the dimorphic switch as well as growth rate and cell wall properties of this important fungal plant pathogen.

Results

Phylogenetic analysis of Zt107320

In order to determine the distribution of homologs of Zt107320 we performed a similarity search on the protein level among putative fungal transcription factors. Among the 30 best matches 17 are found among either plant pathogens or plant associated fungal organisms, however this association does not appear to be monophyletic (fig 1 a). Among the thirty best hits, the *M. oryzae* homolog MoCod1 and the *Alternaria brassicola* homolog AbPf2 have been functionally analysed. Deletion of the *AbPf2* resulted in non-pathogenic strains (Cho *et al.* 2013). In the wild-type expression of *AbPf2* decreased after initial colonization of host tissues and the authors conclude that AbPf1 regulates pathogenesis (Cho *et al.* 2013). Among these highly conserved putative transcription factors two protein domains are shared: Zn(2)-C6 fungal-type DNA binding domain superfamily (IRP036864) and the transcription factor domain (IRP007219), indicating a functional role of the putative transcription factors (see fig. 1 b). Zt1073120 is therefore homologous to several predicted transcription factors in other fungal species, including some that have been associated with pathogenicity in plant pathogenic species, indicating a similar regulatory role of this protein in *Z. tritici*.

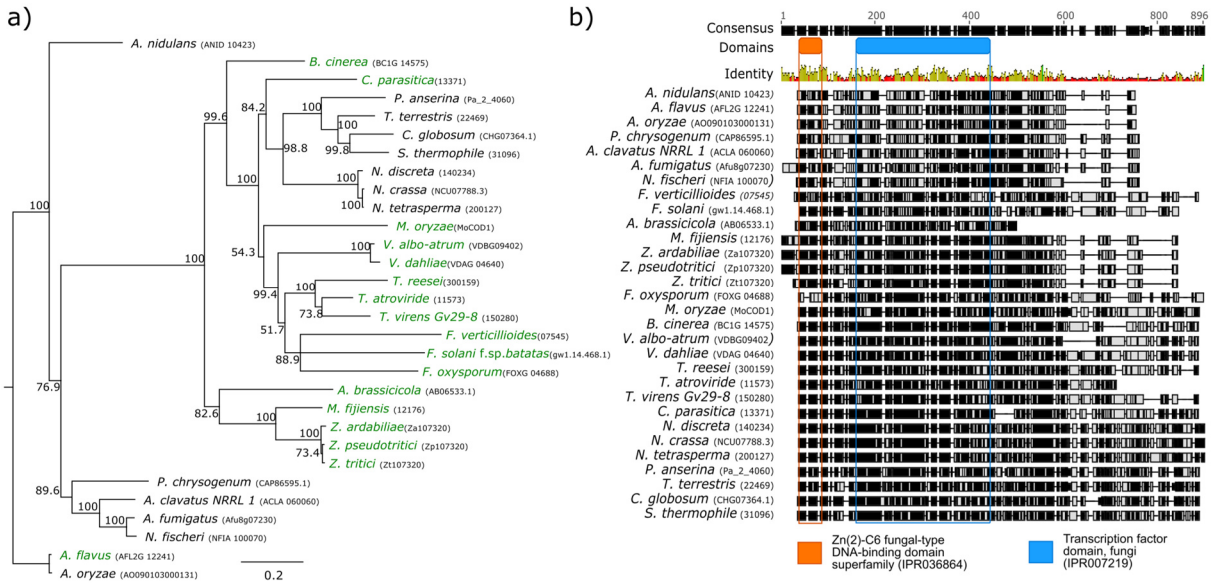


Figure 1. Homology of Zt107320 among fungal transcription factors. a) Phylogenetic tree based on 30 sequences showing the highest similarity with Zt107320 on the protein level, including the orthologs in the sister species *Z. pseudotritici* and *Z. ardabiliae*. The phylogenetic tree was

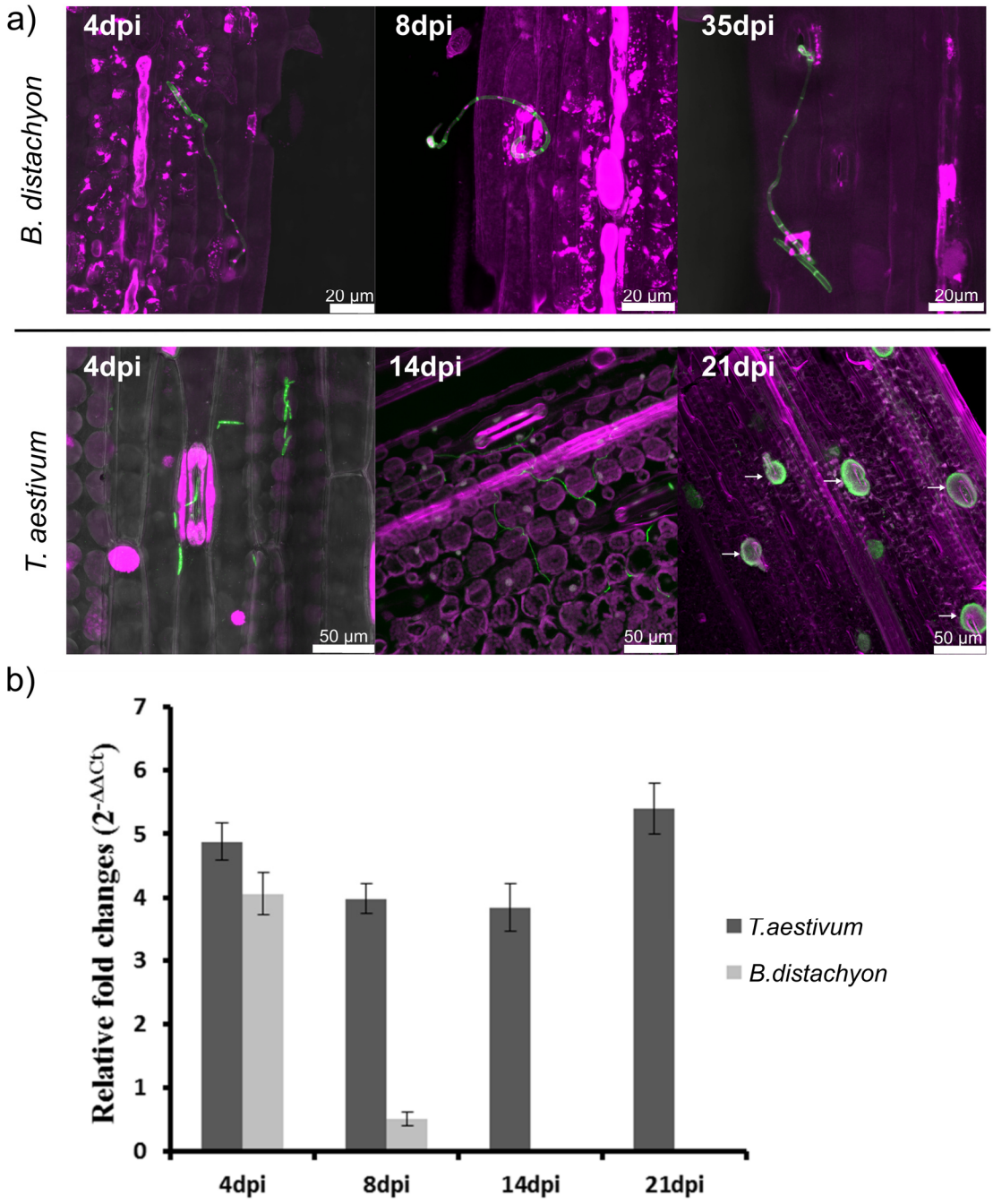
constructed using MUSCLE alignments (Edgar, 2004) with Neighbour-Joining upon a consensus tree with 1000 bootstrapping iterations. Support of nodes by percentage of bootstrapping iterations is indicated. Species considered to be associated with plants are indicated in green. B) MUSCLE alignment of homologous sequences to Zt107320 indicating regions according to their identity with the consensus sequence. The localisations of two functional domains, identified using InterProScan, in the consensus sequence are indicated.

Infections of *Z. tritici* are blocked in the substomatal cavities during incompatible interaction with *B. distachyon* coinciding with reduced expression of *Zt107320*

To study compatible and incompatible infections of *Z. tritici* in more detail, we inoculated leaves of 12-14 days old seedlings of *T. aestivum* (cultivar Obelisk) and *B. distachyon* (ecotype Bd21), and analysed the infection development by confocal microscopy. Fungal cells germinated upon contact with the leaf surface and developed infection hyphae. In contrast to previous observations (O'Driscoll *et al.* 2015), we found that *Z. tritici* infection hyphae entered into open *B. distachyon* stomata at four days post inoculation (dpi). However, further infection development of *Z. tritici* was blocked in the substomatal cavities of *B. distachyon* leaves (fig 2a), similar to phenotypes previously observed in incompatible interactions with einkorn wheat (Jing *et al.* 2008). Consequently, *Z. tritici* hyphae did not colonize the mesophyll tissue of *B. distachyon*, no necrotic lesions developed, and no asexual pycnidia formed. Interestingly, fungal growth was completely halted and no further growth was observed, even when leaves were examined five weeks post inoculation (fig 2a). In contrast, compatible infection of *Z. tritici* on the wheat cultivar Obelisk was characterised by previously described infection stages (Haueisen *et al.* 2019). Penetration of leaf stomata at 4 dpi was followed by the establishment of a hyphal network in the mesophyll tissue until 7-11 dpi and subsequently the switch to necrotrophy when biomass increased substantially resulting in the formation of pycnidia (fig 2a).

158 Previously, comparative transcriptome analyses during *Z. tritici* infection of the host *T. aestivum* and
159 the non-host *B. distachyon* identified 40 differentially expressed genes at 4 dpi (Kellner *et al.* 2014).
160 Building on this expression analysis, we focused on the putative transcription factor Zt107320 and
161 validated the expression kinetics of *Zt107320* by RT-qPCR. We confirmed differential expression at 8
162 dpi (fig 2b). Expression in the non-host *B. distachyon* was greatly reduced at 8 dpi compared to
163 expression in axenic cultures, whereas the expression of *Zt107320* during a compatible infection of
164 the host *T. aestivum* was strongly upregulated during the course of the infection until 21 dpi.

165



166

167 **Figure 2. *Z. tritici* infection hyphae are blocked in the substomatal cavities of *B. distachyon* which**
168 **coincides with reduced expression of *Zt107320*.** a) Micrographs showing *Z. tritici* cells and emerging
169 hyphae during penetration of *B. distachyon* stomata and infection of *T. aestivum*. On *T. aestivum*, *Z.*
170 *tritici* germinated and penetrated the stomata at 4 dpi. Intercellular mesophyll colonization of *Z.*
171 *tritici* occurred until 14 dpi resulting in the formation of pycnidia (indicated by arrows) at 21 dpi. In
172 contrast, *Z. tritici* germinated on *B. distachyon* and penetrated stomata, but growth was halted below
173 the stomata with no further leaf colonization (8 dpi - 35 dpi). Maximum projections of confocal image
174 z-stacks. Nuclei and grass cells displayed in *purple* and fungal hyphae or septae respectively in *green*.
175 b) Transcription of *Zt107320* in compatible (*T. aestivum)* and incompatible (*B. distachyon*) hosts. *In*
176 *planta* expression of *Zt107320* is displayed relative to expression during axenic growth. Values are

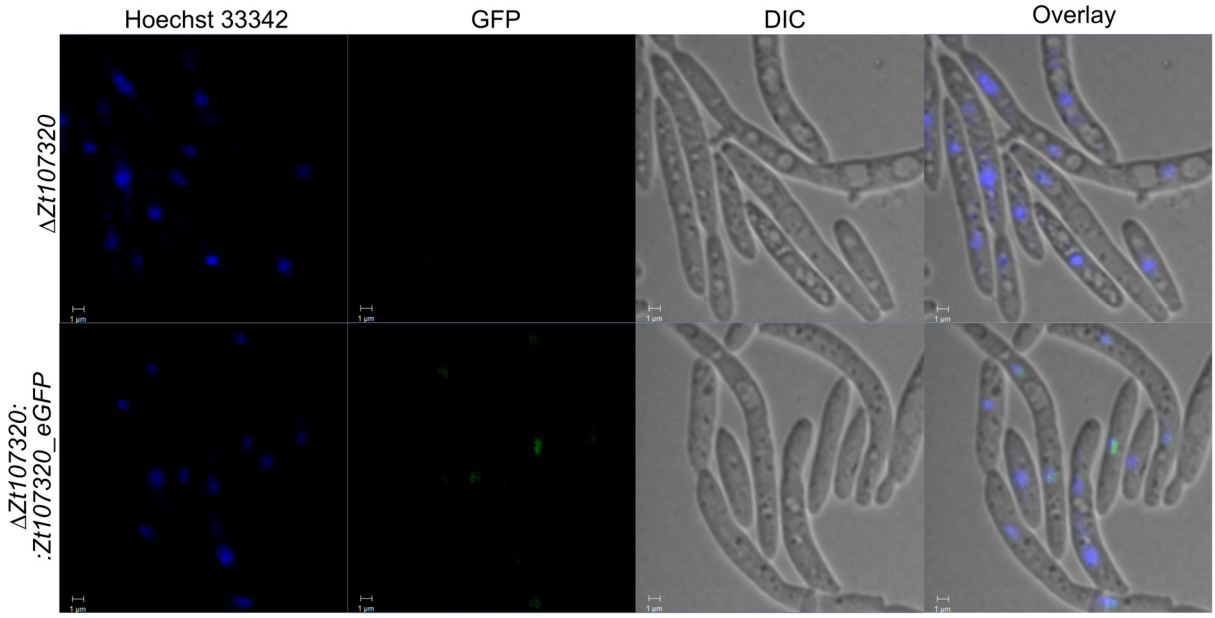
177 normalized to the expression of the gene encoding the housekeeping protein GAPDH. Error bars
178 indicate the standard error of the mean (SEM) of three independent biological replicates per sample.

179

180 **Zt107320 is located with the nucleus during yeast-like growth**

181 The localization of Zt107320 within fungal cells was analysed using complementation strains that
182 expressed a Zt107320_eGFP fusion protein regulated by the native promotor. During yeast-like
183 growth on YMS media, the GFP-fusion protein appeared to be located in the nucleus or in its
184 immediate vicinity (see fig 3). This localization is similar to the reported nuclear localization of the
185 homolog AbPf1 (Cho *et al.* 2013) and supports the putative functional role of Zt107320 as a
186 transcription factor. The observed localisation of the Zt107320_eGFP fusion protein also verifies the
187 predicted nuclear localization of Zt107320 using the program WoLFPsort which implements an
188 algorithm for prediction of subcellular locations of proteins based on sequence composition and
189 content (Horton *et al.* 2007).

190



191

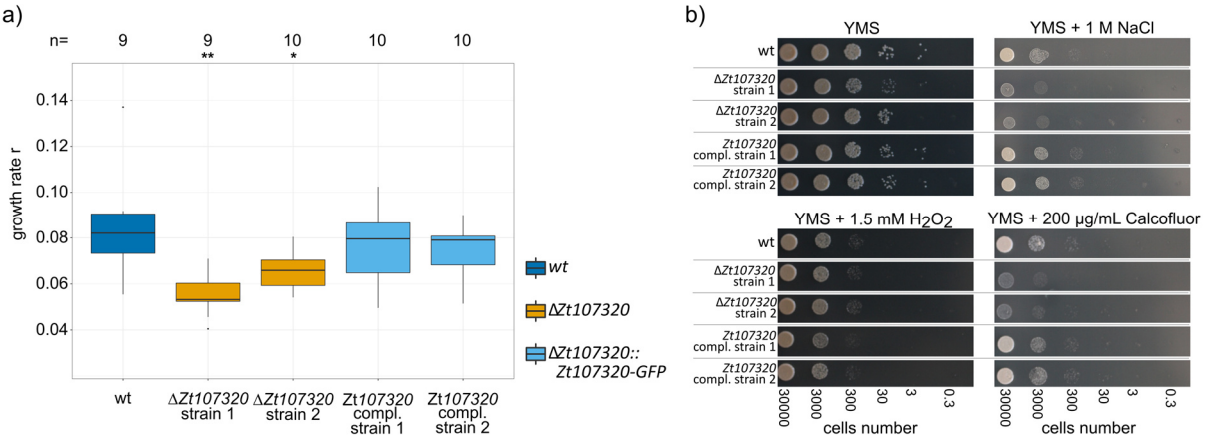
192 **Figure 3. Localization of the Zt107320_eGFP fusion protein detected by fluorescence microscopy**.
193 Nuclei were counterstained using the DNA-specific dye Hoechst33342. eGFP fluorescence co-
194 localized with the Hoechst33342 signal indicating nuclear localization of the Zt107320_eGFP fusion
195 protein. eGFP fluorescence is restricted to the nuclei (scale bars = 1 µm).
196

197 **Zt107320 regulates growth and affects cell wall properties**

198 Based on the coinciding halted growth and development and down-regulation of *Zt107320* in
199 incompatible infections, we next asked whether Zt107320 is involved in the regulation of growth of
200 *Z. tritici in vitro*. We determined the maximum growth rate r of *Z. tritici* in liquid cultures assuming a
201 logistic growth curve model. For two independently generated *Zt107320* deletion mutants, we
202 observed a significant reduction in the maximum growth rate compared to the wildtype. We thereby
203 confirm the relevance of *Zt107320* as a putative regulator for growth, as two complementation
204 strains showed no significant difference in the growth rate compared to the wildtype (fig 4a)

205 The effect of *Zt107320* deletion was not restricted to the growth rate but also included cell wall
206 properties. Compared to the wildtype, we observed reduced growth *in vitro* when challenging the
207 deletion mutants with high osmotic stresses (0.5 M and 1 M NaCl; 1 M, 1.5 M and 2 M Sorbitol) as
208 well as with cell wall stress agents (300 µg/mL and 500 µg/mL Congo red; 200 µg/mL Calcofluor).
209 Again, the wildtype phenotype was restored in both complementation strains (fig 4b and fig S1).
210 Interestingly, temperature stress (28°C) did not affect the wildtype and the deletion mutants
211 differently, as well as the exposure to H_2O_2 (1.5 mM and 2 mM). This indicates a specific effect of
212 *Zt107320* on the cell wall properties but not on the ability of the fungus to counteract reactive
213 oxygen species which are produced by the plant during activation of immune responses (Jones and
214 Dangl, 2006).

215



216

Figure 4. Zt107320 regulates growth and affects cell wall properties of *Z. tritici*. a) Maximum growth rate in liquid culture. Statistical significance inferred through an ANOVA and subsequent post hoc Tukey's HSD comparing the deletion and complementation strains to the wildtype, is indicated as *= p <0.05; **= p<0.005; ***= p<0.0005. b) *In vitro* growth of the wildtype (wt), two deletion strains (Δ*Zt107320*), two complementation strains (Δ*Zt107320*::*Zt107320*_eGFP) on YMS media including the supplemented compounds to assess the role of osmotic stress (1 M NaCl), reactive oxygen species (1.5 mM H_2O_2) and cell wall stressors (200 µg/mL Calcofluor).

The dimorphic switch from yeast-like to hyphal growth is considered to be central for pathogenicity during the early stages of infection (Kema, G. H. J. *et al.* 1996; Yemelin *et al.* 2017). We therefore next asked whether Zt107320 is involved in regulating this morphological switch of *Z. tritici.* To promote hyphal growth we used minimal medium and supplied different carbon sources in order to compare carbon utilisation and growth between the mutants and wildtype. Without carbon sources as well as in the presence of cellulose as exclusive carbon source, *Z. tritici* showed solely hyphal growth, indicating that carbon sources are required for yeast-like growth and that cellulose cannot be utilized by the fungus. The wildtype *Z. tritici* strain showed markedly increased yeast-like growth in the presence of the monosaccharides glucose, galactose, fructose and mannose as well as the sugar alcohols sorbitol and mannitol. Increased growth was also observed in the presence of the disaccharides sucrose and maltose (fig 5, fig S2). Interestingly, xylose as sole carbon source led to predominant hyphal growth in the wild type strain, which contrasts to the mainly yeast-like growth observed in the presence of all other tested carbohydrates. For xylose as sole carbon source, overall growth is markedly increased compared to the minimal medium lacking a carbon source indicating that xylose can be utilized by *Z. tritici* as a carbon source. The deletion of *Zt107320* affected fungal growth morphology on all tested carbon sources. Compared to the wildtype and the complementation strains, we observed increased hyphal growth for the Δ*Zt107320* strains on all carbon sources after 14 days of incubation, except for cellulose (fig 5, fig S2). In particular, supplementation of the monosaccharides fructose, glucose and xylose caused substantially pronounced hyphal growth when *Zt107320* was deleted. The wildtype phenotype was restored in

both complementation strains confirming that *Zt107320* plays a role in the regulation of growth in *Z. tritici*.

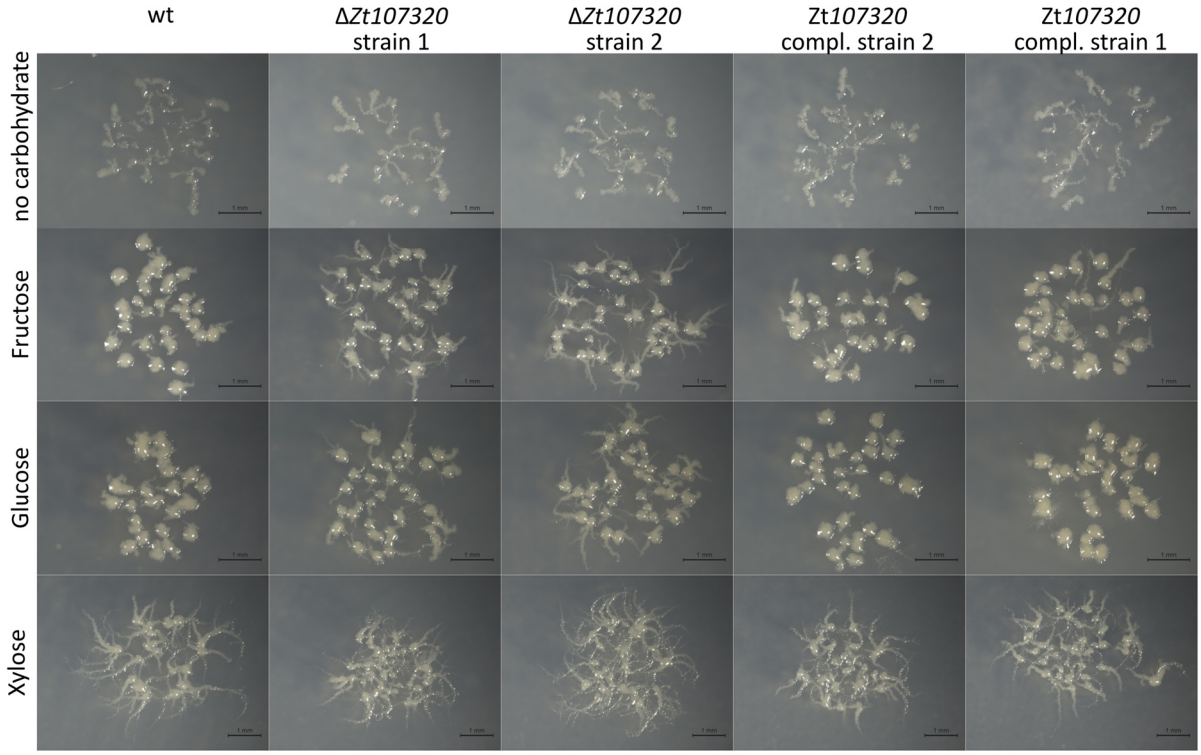
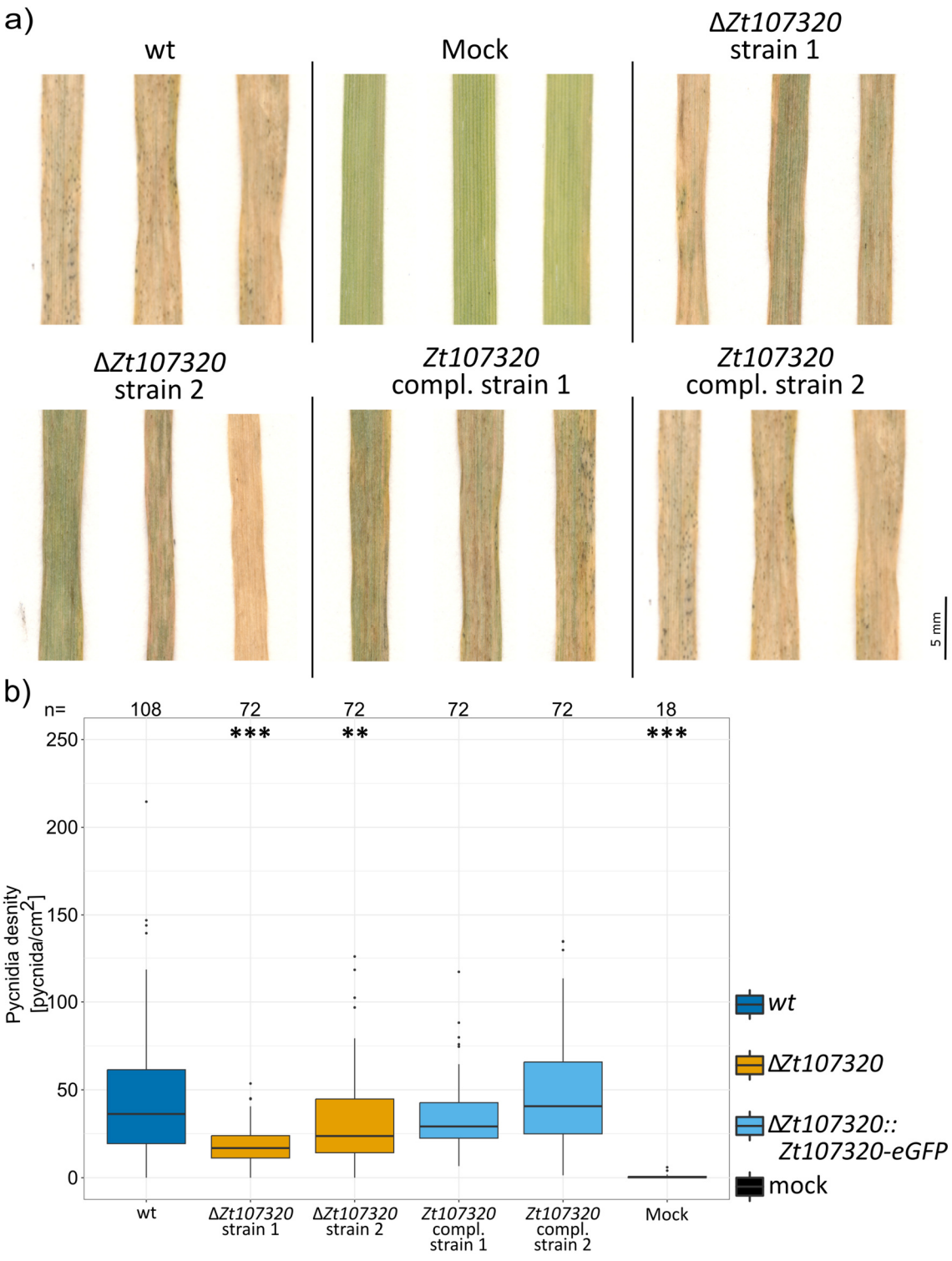


Figure 5. Zt107320 regulates the dimorphic switch of *Z. tritici*. Pictures showing morphologies of colonies originating from single cells after 14 days of growth at 18°C on minimal media containing the indicated carbohydrates as carbon sources. Fructose, glucose and xylose can be utilized by the fungus and led to increased growth. The two strains in which *Zt107320* was deleted independently showed an increase in hyphal growth compared to the wildtype, while in the two complementation strains the wildtype colony morphology was restored.

Zt107320 affects the ability of *Z. tritici* to produce pycnidia

We next addressed whether the impact of *Zt107320* deletion on growth rate, the dimorphic switch and cell wall properties also influence the ability of *Z. tritici* to infect its host, *T. aestivum*. We inoculated a predefined area of the second leaf of the susceptible wheat cultivar Obelisk and measured the number of pycnidia 21 days post infection. We observed a pronounced reduction in the production of pycnidia for the *Zt107320* deletion strains. The density of pycnidia per cm^2 of infected leaf area was reduced significantly for both independent deletions of *Zt107320* (ANOVA, $p<1*10^{-7}$, p=0.002) (fig 6 a, b) compared to the wildtype. Complementing the *Zt107320* in-locus fully

263 restored the wildtype phenotype *in planta*. As the density of pycnidia is considered to be a
264 quantitative measure for virulence (Stewart *et al.* 2016), significantly decreased pycnidia production
265 indicates reduced virulence of mutants. Thus, we conclude that the putative transcription factor
266 Zt107320 by its effect on the growth rate, the dimorphic switch and the cell wall properties affects
267 the fitness of *Z. tritici* during infection of the compatible wheat host.



268

Figure 6. *Zt107320* deletion affects the pycnidia production during a compatible infection of wheat. a) Pictures of representative leaves infected with the *Z. tritici* wildtype (wt), two deletion strains (Δ*Zt107320*), two complementation strains (Δ*Zt107320*::*Zt107320-eGFP*) and mock-treated leaves. b) Boxplot depicting the pycnidia density (pycnidia per cm^2 leaf) pooled for two independent experiments. Number of leaves (N) for each strain is indicated on top. Statistical significance, inferred through an ANOVA and subsequent post hoc Tukey's HSD comparing the deletion and complementation strains to the wildtype, is indicated as *= p <0.05; **= p<0.005; ***= p<0.0005.

Discussion

Here, we show that the putative transcription factor Zt107320, belonging to the fungal $Zn(II)_2Cys_6$ family, is involved in the infection program of *Z. tritici* during a compatible interaction with the host plant *T. aestivum*. Transcription of *Zt107320* is specifically induced during infection of wheat but not during the infection of the non-host *B. distachyon*. This suggests a functional role of this gene in the regulation of the infection program during a compatible host-pathogen interaction that allows the fungus to overcome host defences and propagate in the mesophyll tissue.

To date, only a small number of genes have been shown to be involved in virulence of this important wheat pathogen (Orton *et al.* 2011; Poppe *et al.* 2015). These genes encode the chitin binding LysM effector Mg3LysM (Lee *et al.* 2014; Marshall *et al.* 2011) , two transcription factors ZtWor1 and ZtVf1 (Mirzadi Gohari *et al.* 2014; Mohammadi *et al.* 2017) and three rapidly evolving small proteins (Hartmann *et al.* 2017; Poppe *et al.* 2015; Zhong *et al.* 2017). Indeed, multiple effector candidate genes have been deleted but for most mutants no or small effects on pathogenicity were observed (Gohari *et al.* 2015; Rudd *et al.* 2015). Therefore, a high level of functional redundancy seems to be present in *Z. tritici*. The deletion of *Zt107320* results in a relatively small, nevertheless significant, reduction of the pycnidia density which is in contrast to the fully or partially avirulent phenotype demonstrated for the two previously deleted transcription factors ZtWor1 and ZtVf1, respectively (Mirzadi Gohari *et al.* 2014; Mohammadi *et al.* 2017). Similar to our observation in the *Zt107320* deletion mutant, deletion of *MoCOD1*, the rice blast homolog of *Zt107320*, led to a quantitative reduction in lesion size and number (Chung *et al.* 2013). This partial, but incomplete, quantitative

298 reduction in virulence of *ΔZt107320* strains corresponds with the reduced, but still substantial
299 growth rate of these deletion mutants *in vitro*. Therefore, other mechanisms, independent of
300 Zt107320 are involved in the regulation of growth and development *in vitro* and *in planta*. Similarly, a
301 partial, but incomplete reduction of invasive growth *in planta* as observed in Δ*MoCOD1* (Chung *et al.*
302 2013). The impact of Zt107320 on fungal growth and pathogenicity is therefore similar to the effect
303 caused by other members of the $Zn(II)_2Cys_6$ transcription factor family: TPC1 regulates invasive,
304 polarized growth and virulence in the rice blast fungus *M. oryzae* (Galhano *et al.* 2017), and the
305 transcription factor FOW2 is known to control the ability of *F. oxysporum* to invade roots and
306 colonize plant tissue (Imazaki *et al.* 2007).

307 Zt107320 appears to be involved in the morphological switch from yeast-like to hyphal growth as
308 deletion of *Zt107320* led to an increase in hyphal growth. The switch to filamentous growth is
309 considered to be essential for plant infection (Kema, G. H. J. *et al.* 1996; Yemelin *et al.* 2017).
310 Therefore, the involvement of Zt107320 in regulating this dimorphism underscores its importance for
311 pathogenicity. Based on RT-qPCR data we observe that *Zt107320* is differentially expressed between
312 non-host and host infections and is further highly expressed during infections of compatible hosts.
313 Other RNAseq-based transcriptome studies found that *Zt107320* is upregulated during later stages of
314 wheat infection associated to necrotrophic host colonization, indicating a possible function for
315 pycnidia formation (Haueisen *et al.* 2019; Rudd *et al.* 2015). Together, these findings support a role
316 of Zt107320 in regulation of growth and pathogenicity of *Z. tritici*.

317 Interestingly, xylose the main product of hemicellulose degradation by fungal xylanases had a
318 pronounced effect on the growth pattern of Z. *tritici*. Xylose supplementation not only increased
319 overall growth compared to pure minimal medium but also led to an increase in the development of
320 filaments spanning larger distances. In *Z. tritici*, the switch to necrotrophic growth after an initial
321 phase of symptomless infection, overall disease severity and quantitative pycnidiospore production
322 are associated with the activity of endo-β-1,4-xylanase (Siah *et al.* 2010; Somai-Jemmali *et al.* 2017).
323 During the switch to necrotrophy *Z. tritici* rapidly develops large hyphal networks and uses plant-

derived nutrients (Haueisen *et al.* 2019; Rudd *et al.* 2015). The observed effect of xylose on the growth morphology suggest that xylose – next to its role as a carbon source – may also direct growth and promote the spatial expansion of the intrafoliar hyphal network during the fungal lifestyle switch to necrotrophic growth. Indeed, genes encoding xylanases were shown to evolve under positive selection (Brunner *et al.* 2013), indicating an important functional role of this class of enzymes for adaptation to wheat infection. Although functional analyses of several xylanases in other plant pathogenic fungi, resulted in no direct phenotypic effect (Brunner *et al.* 2013; Douaiher *et al.* 2007) xylanases have been proposed as virulence factors (Douaiher *et al.* 2007). However, the results presented here indicate a possible role of xylose as a host infection-associated signal molecule for *Z. tritici* and should warrant further analysis.

In conclusion, we showed that Zt107320 affects the fitness of the wheat pathogen *Z. tritici*. *Zt107320* is differentially expressed in host and non-host environments, being upregulated during the early stages of infection on compatible hosts and down-regulated on non-hosts. This down-regulation corresponds to a considerably reduced growth and halted infections of *Z. tritici* after stomatal penetration of the non-host *B. distachyon*. In addition, we could confirm that Zt107320 has a nuclear localisation, consistent with its putative function as a transcription factor and that it further regulates the dimorphic switch between yeast-like and hyphal growth that is considered to be essential for pathogenicity. We therefore hypothesize that the putative transcription factor Zt107320 is part of to the regulatory network that controls host-associated growth and development, integrating signals that differ between compatible and non-compatible infections. Future studies should address the specific target genes of Zt107320 and their expression pattern during compatible and non-compatible interactions. Furthermore, the transcriptional regulation of *Zt107320* suggests that specific signals in the compatible host-pathogen interaction in wheat are responsible for the up-regulation of this particular transcription factor-encoding gene. Identification of these host-derived signals will provide fundamental insight into the molecular basis of host-pathogen interaction and host specificity in *Z. tritici*.

Experimental Procedures

Fungal and plant strains

The Dutch isolate IPO323 was kindly provided by Gert Kema (Wageningen, The Netherlands) and is available from the Westerwijk Institute (Utrecht, The Netherlands) with the accession number CBS 115943. The strain used in our experiments lacked accessory chromosome 18, presumably lost during culture maintenance *in vitro* (Kellner et al. 2014). Strains were maintained in either liquid Yeast Malt Sucrose (YMS) broth (4 g/L Yeast extract. 4 g/L Malt extract, 4 g/L sucrose) at 18°C on an orbital shaker or on solid YMS (+20 g/L agar) at 18°C. The *T. aestivum* cultivar Obelisk was obtained from Wiersum Plantbreeding BV (Winschoten, The Netherlands). *B. distachyon* inbred line Bd21 was kindly provided by Thierry Marcel (Bioger, INRA, France).

Sequence analysis

Phylogenetic analysis of Zt107320 was conducted using the software Geneious Prime 2019.0.4 (https://www.geneious.com). Homologues sequences to protein sequence of Zt107320 were retrieved from the Fungal Transcription Factor Database (Park *et al.* 2008). Including Zt107320 and its homologs from the sister species *Z. pseudotritici* and *Z. ardabiliae* a total of the 30 best matches were retrieved. Alignments were constricted using MUSCLE (Edgar, 2004) and trees constructed using Neighbour-Joining algorithm building a consensus tree using 1000 bootstrapping replicates. Protein domains of the consensus sequence were identified using InterProScan (Quevillon *et al.* 2005). Prediction of the nuclear localisation of the Zt107320 was conducted using the WoLF PSORT predictor (Horton *et al.* 2007).

Analysis of *Z. tritici* during its compatible and non-compatible infections by confocal microscopy

374 Morphology and development of *Z. tritici* inside and on the surface of leaves of B*. distachyon* inbred
375 line Bd21 were analysed by confocal laser-scanning microscopy (CLSM) as described previously
376 (Haueisen et al. 2019). Analyses of compatible *Z. tritici* infections on *Triticum aestivum* cultivar
377 Obelisk were conducted by combining microtomy and CLSM as previously described (Rath *et al.*
378 2014). Distinct areas of the second leaf of 12-day-old (Bd21) and 14-day-old (wheat) seedlings were
379 brush-inoculated with $1x10^7$ cells/ml in 0.1% Tween 20. Plants were incubated at 22°C [day]/ 20°C
380 [night] and 100% humidity with a 16-h light period for 48 h. Subsequently, humidity was reduced to
381 70%. Microscopy was conducted using a Leica TCS SP5 and analysis of image z-stacks was done using
382 Leica Application Suite Advanced Fluorescence (Leica Microsystems, Germany) and AMIRA® (FEITM
383 Visualization Science Group, Germany).

384

385 **Analysis of Zt107320_eGFP expression using confocal microscopy.**
386 Cells were grown on solid YMS medium for 7 days before being scraped off the media surface and
387 introduced into 10 mM phosphate buffer (pH 7.2) containing 1 µg/µl Hoechst 33342 (Sigma-Aldrich
388 Chemie GmbH, Munich, Germany). Cells were incubated for 15-30 min in the dark and then
389 transferred to a microscope slide and analysed by confocal laser scanning microscopy.
390

391 **RNA isolation and quantitative RT-PCR**

392 We analysed gene expression patterns of *Zt107320* of *Z. tritici* employing a qRT-PCR experiment.
393 Total RNA was extracted from fungal axenic cultures (grown for 72 h in YMS medium at 18°C and 200
394 rpm) and from snap-frozen leaf tissue infected with *Z. tritici* (4, 8, 14 and 21 dpi) using the TRIZOL
395 reagent (Invitrogen, Karlsruhe, Germany), following the manufacturer's instructions. Three biological
396 replicates were included in the experimental set up. The cDNA samples were used in a qRT-PCR
397 experiment employing the iQ SYBR Green Supermix Kit (Bio-Rad, Munich, Germany). PCR was
398 conducted in a CFX96 RT-PCR Detection System (Bio-Rad, Munich, Germany) with the constitutively

399 expressed control gene Glyceraldehyde-3-phosphate dehydrogenase (GAPDH). All primers are listed
400 in table S1.

401 **Generation of Zt107320 deletion mutants and complementation by gene replacement**

402 Fungal transformations and the creation of knock-out and complementation mutants of *Zt107320*
403 were conducted as previously described (Poppe *et al.* 2015). In brief: Gene deletions were created by
404 amplifying an approximately 1 kb region of the 5' and 3' flanking regions of *Zt107320* using PCR. The
405 amplified flanking sequences were fused to a hygromycin resistance (hygR) cassette and an EcoRV
406 cut vector backbone (pES61) using Gibson assembly (Gibson *et al.* 2009). Electro-competent cells of
407 the *Agrobacterium tumefaciens* strains AGL1 were transformed using standard protocols. These
408 transformed *A. tumefaciens* cells were used for the transformation of *Z. tritici* as previously described
409 (Zwiers and De Waard, Maarten A. 2001). The same strategy was applied for the creation of the
410 complementation strains by a C-terminal fusion of the *Zt107320* gene with an eGFP tag and a
411 geneticin resistance cassette as a selection marker (fig. S3). After transformation and homologous
412 recombination in the Δ*Zt107320* strain 1, the hygromycin resistance cassette was replaced by
413 *Zt107320-eGFP* and the geneticin resistance cassette (NeoR) (fig. S3). Homologous recombination
414 and integration was confirmed using PCR and Southern blot analysis by standard protocols. In short,
415 genomic DNA was isolated using Phenol-Chloroform isolation (Sambrook and Russell, 2001).
416 Restriction digestion was performed using PvuII, followed by gel-electrophoresis, blotting and
417 detection using Dig-labelled probes binding to the upstream and downstream flank of *Zt107320* (fig.
418 S2). In total four *ΔZt107320* strains, and two Δ*Zt107320*::*Zt107320*-eGFP strains were created.
419

420 **Isolation of fungal DNA and Southern blot analysis**

421 DNA was isolated using phenol/chloroform applying a protocol previously described (Sambrook and
422 Russell, 2001). Transformants were first screened using a PCR based approach detecting the
423 resistance cassette and the endogenous locus. Candidate transformants were further confirmed by
424 Southern blot analysis using a standard protocol (Southern, 1975). Probes were generated using the

425 PCR DIG labelling Mix (Roche, Mannheim, Germany) according to the manufacturer's instructions
426 (Table S1).
427
428 ***In vitro* phenotyping**
429 The *Z. tritici* strains were grown on YMS solid medium for 5-7 days at 18°C before the cells were
430 scraped from the plate surface. For the determination of the growth rate, the cells were resuspended
431 and counted. The cell density was adjusted to 50000 cells/mL and 175µl of the cell suspension was
432 added to a well of a 96 well plate. Plates were inoculated at 18°C at 200 rpm with the OD600 being
433 measured twice daily on a Multiskan Go plate reader (Thermo Scientific, Dreieich, Germany) (Table
434 S2). Estimation of the growth rate was done by employing the logistic growth equation as
435 implemented in the growthcurver package (version 0.2.1) in R (version R3.4.1) (R Core Team, 2015).
436 For the determination of the *in vitro* phenotypes, the cell number was adjusted to 10^7 cells/mL in
437 ddH_2O and serially diluted to 10^3 cells/mL. 3 µL of each cell dilution was transferred onto YMS agar
438 including the tested compounds and incubated for seven days at 18°C or 28°C. To test high osmotic
439 stresses 0.5 M NaCl, 1 M NaCl, 1M Sorbitol, 1.5 M Sorbitol, 2 M Sorbitol (obtained from Carl Roth
440 GmbH, Karlsruhe, Germany) were added to the YMS solid medium. To test cell wall stresses 300
441 µg/mL and 500 µg/mL Congo red and 200 µg/mL Calcofluor (obtained from Sigma-Aldrich Chemie
442 GmbH, Munich, Germany) were added to the YMS solid medium. Finally, to determine the effect of
443 reactive oxygen species on the mutant growth morphology 2 mM H_2O_2 (obtained from Carl Roth
444 GmbH, Karlsruhe, Germany) was added to the YMS solid medium.
445 To test whether the Zt107320 affects the hyphal growth of *Z. tritici* and the ability of the fungus to
446 use different carbon sources we used minimum media (MM) as described in (Barratt *et al.* 1965).
447 Glucose, fructose, xylose, mannose, galactose, sorbitol, mannitol, sucrose and cellulose were added
448 at a final concentration of 10 g/L and 20g/L agar were included. Strains were grown on YMS solid
449 medium for 7 days and scraped into ddH2O, the cell number adjusted to 10^7, 10^6, 10^5, 10^4, 10^3

cells/mL and 3 µl of each cell concentration added to the surface of the minimum medium plates. Plates were incubated 18°C in the dark and the growth monitored after 7 days and 14 days.

In planta phenotyping

For the *in planta* phenotypic assays, we germinated seeds of the wheat cultivar Obelisk on wet sterile whatman paper for four days before potting using the soil Fruhstorfer Topferde (Hermann Meyer GmbH, Rellingen, Germany). Wheat seedlings were further grown for seven days before inoculation. *Z. tritici* strains were grown in YMS solid medium for five days at 18°C before the cells were scraped from the plate surface. The cell number was adjusted to 10^8 cells/mL in H_2O + 0.1% Tween 20, and the cell suspension was brushed onto approximately five cm on the abaxial and adaxial sides of the second leaf of each seedling. Inoculated plants were placed in sealed bags containing water for 48 h to facilitate infection through stomata. Plants were grown under constant conditions with a day night cycle of 16h light (~200µmol/m^2*s) and 8h darkness in growth chambers at 20°C. Plants were grown for 21 days post inoculation at 90% relative humidity (RH). At 21 dpi the infected leaves were cut and taped to sheets of paper and pressed for five days at 4°C before being scanned at a resolution of 2400 dpi using a flatbed scanner (HP photosmart C4580, HP, Böblingen, Germany). Scanned images were analysed using an automated image analysis in Image J (Schneider *et al.* 2012) adapted from (Stewart *et al.* 2016). The read-out pycnidia/cm^2 leaf surface was used for all subsequent analyses. See Table S3 for summary of *in planta* results.

Statistical analysis

Statistical analyses were conducted in R (version R3.4.1) (R Core Team, 2015) using the suite R Studio (version 1.0.143) (RStudio Team, 2015). Data inspection showed a non-normal distribution for all data sets, including the measured pycnidia density (pycnidia/cm^2). Therefore, we performed an omnibus analysis of variance using rank-transformation of the data (Conover and Iman, 1981)

475 employing the model: pycnidia density ~ strain * experiment and r ~ strain, respectively. Post hoc
476 tests were performed using Tukey's HSD (Tukey, 1949).
477

478 Acknowledgements

479 The study was funded by a personal grant to EHS from the State of Schleswig Holstein and a
480 fellowship from the Max Planck Society, Germany. The funders had no role in study design, data
481 collection and interpretation, or the decision to submit the work for publication. The authors have no
482 conflict of interest.
483

484 References

485 **Barratt, R.W. Johnson, G.B. and Ogata, W.N.** (1965) Wild-type and mutant stocks of Aspergillus
486 nidulans. *Genetics,* **52**, 233.
487 **Brading, P.A. Verstappen, Els C P, Kema, Gert H J and Brown, James K M** (2002) A Gene-for-Gene
488 Relationship Between Wheat and Mycosphaerella graminicola, the Septoria Tritici Blotch
489 Pathogen. *Phytopathology,* **92**, 439–445.
490 **Brunner, P.C. Torriani, S. F. F. Croll, D. Stukenbrock, E.H. and McDonald, B.A.** (2013) Coevolution
491 and Life Cycle Specialization of Plant Cell Wall Degrading Enzymes in a Hemibiotrophic Pathogen.
492 *Molecular Biology and Evolution,* **30**, 1337–1347.
493 **Chen, Y. Le, X. Sun, Y. Li, M. Zhang, H. Tan, X. Zhang, D. Liu, Y. and Zhang, Z.** (2017) MoYcp4 is
494 required for growth, conidiogenesis and pathogenicity in Magnaporthe oryzae. *Mol. Plant Pathol.*
495 **18**, 1001–1011.
496 **Cho, Y. Ohm, R.A. Grigoriev, I.V. and Srivastava, A.** (2013) Fungal-specific transcription factor AbPf2
497 activates pathogenicity in Alternaria brassicicola. *The Plant journal : for cell and molecular biology,*
498 **75**, 498–514.
499 **Chung, H. Choi, J. Park, S.-Y. Jeon, J. and Lee, Y.-H.** (2013) Two conidiation-related Zn(II)2Cys6
500 transcription factor genes in the rice blast fungus. *Fungal Genet. Biol.* **61**, 133–141.
501 **Conover, W.J. and Iman, R.L.** (1981) Rank transformations as a bridge between parametric and
502 nonparametric statistics. *The American Statistician,* **35**, 124–129.
503 **Douaiher, M.-N. Nowak, E. Durand, R. Halama, P. and Reignault, P.** (2007) Correlative analysis of
504 Mycosphaerella graminicola pathogenicity and cell wall-degrading enzymes produced in vitro: the
505 importance of xylanase and polygalacturonase. *Plant Pathology,* **56**.
506 **Edgar, R.C.** (2004) MUSCLE. Multiple sequence alignment with high accuracy and high throughput.
507 *Nucleic Acids Res,* **32**, 1792–1797.
508 **Fones, H. and Gurr, S.** (2015) The impact of Septoria tritici Blotch disease on wheat: An EU
509 perspective. *Fungal genetics and biology : FG & B,* **79**, 3–7.
510 **Fox, E.M. Gardiner, D.M. Keller, N.P. and Howlett, B.J.** (2008) A Zn(II)2Cys6 DNA binding protein
511 regulates the sirodesmin PL biosynthetic gene cluster in Leptosphaeria maculans. *Fungal Genet.*
512 *Biol.* **45**, 671–682.

513 **Galhano, R. Illana, A. Ryder, L.S. Rodríguez-Romero, J. Demuez, M. Badaruddin, M. Martinez-**
514 **Rocha, A.L. Soanes, D.M. Studholme, D.J. Talbot, N.J. and Sesma, A.** (2017) Tpc1 is an important
515 Zn(II)2Cys6 transcriptional regulator required for polarized growth and virulence in the rice blast
516 fungus. *PLoS Pathog,* **13**, e1006516.
517 **Gibson, D.G. Young, L. Chuang, R.-Y. Venter, J.C. Hutchison, C.A. and Smith, H.O.** (2009) Enzymatic
518 assembly of DNA molecules up to several hundred kilobases. *Nature methods,* **6**, 343–345.
519 **Gohari, A.M. Ware, S.B. Wittenberg, Alexander H J, Mehrabi, R. M'Barek, S.B. Verstappen, Els C P,**
520 **van der Lee, Theo A J, Robert, O. Schouten, H.J. de Wit, Pierre P J G M and Kema, G.H.** (2015)
521 Effector discovery in the fungal wheat pathogen Zymoseptoria tritici. *Molecular Plant Pathology,*
522 **16**, 931–945.
523 **Grandaubert, J. Dutheil, J.Y. and Stukenbrock, E.H.** (2017) The genomic determinants of adaptive
524 evolution in a fungal pathogen.
525 **Habig, M. Kema, G. and Holtgrewe Stukenbrock, E.** (2018) Meiotic drive of female-inherited
526 supernumerary chromosomes in a pathogenic fungus.
527 **Hartmann, F.E. Sanchez-Vallet, A. McDonald, B.A. and Croll, D.** (2017) A fungal wheat pathogen
528 evolved host specialization by extensive chromosomal rearrangements. *The ISME journal,* **11**,
529 1189–1204.
530 **Haueisen, J. Möller, M. Eschenbrenner, C.J. Grandaubert, J. Seybold, H. Adamiak, H. and**
531 **Stukenbrock, E.H.** (2019) Highly flexible infection programs in a specialized wheat pathogen.
532 *Ecology and evolution,* **9**, 275–294.
533 **Horton, P. Park, K.-J. Obayashi, T. Fujita, N. Harada, H. Adams-Collier, C.J. and Nakai, K.** (2007)
534 WoLF PSORT. Protein localization predictor. *Nucleic Acids Res,* **35**, W585-7.
535 **Imazaki, I. Kurahashi, M. Iida, Y. and Tsuge, T.** (2007) Fow2, a Zn(II)2Cys6-type transcription
536 regulator, controls plant infection of the vascular wilt fungus Fusarium oxysporum. *Molecular*
537 *microbiology,* **63**, 737–753.
538 **Jing, H.-C. Lovell, D. Gutteridge, R. Jenk, D. Kornyukhin, D. Mitrofanova, O.P. Kema, Gert H. J. and**
539 **Hammond-Kosack, K.E.** (2008) Phenotypic and genetic analysis of the Triticum monococcum-
540 Mycosphaerella graminicola interaction. *New Phytol.* **179**, 1121–1132.
541 **Jones, J.D.G. and Dangl, J.L.** (2006) The plant immune system. *Nature,* **444**, 323–329.
542 **Kellner, R. Bhattacharyya, A. Poppe, S. Hsu, T.Y. Brem, R.B. and Stukenbrock, E.H.** (2014) Expression
543 Profiling of the Wheat Pathogen Zymoseptoria tritici Reveals Genomic Patterns of Transcription
544 and Host-Specific Regulatory Programs. *Genome Biol Evol,* **6**, 1353–1365.
545 **Kema, G.H. Verstappen, E.C. Todorova, M. and Waalwijk, C.** (1996) Successful crosses and molecular
546 tetrad and progeny analyses demonstrate heterothallism in Mycosphaerella graminicola. *Curr*
547 *Genet,* **30**, 251–258.
548 **Kema, G. H. J. Yu, D.Z. Rijkenberg, F. H. J. Shaw, M.W. and Baayen, R.P.** (1996) Histology of the
549 pathogenesis of Mycosphaerella graminicola in wheat. *Phytopathology 86: 777-786*.
550 **Lee, W.-S. Rudd, J.J. Hammond-Kosack, K.E. and Kanyuka, K.** (2014) Mycosphaerella graminicola
551 LysM effector-mediated stealth pathogenesis subverts recognition through both CERK1 and CEBiP
552 homologues in wheat. *Molecular plant-microbe interactions : MPMI,* **27**, 236–243.
553 **Lu, J. Cao, H. Zhang, L. Huang, P. and Lin, F.** (2014) Systematic analysis of Zn2Cys6 transcription
554 factors required for development and pathogenicity by high-throughput gene knockout in the rice
555 blast fungus. *PLoS Pathog,* **10**, e1004432.
556 **MacPherson, S. Larochelle, M. and Turcotte, B.** (2006) A fungal family of transcriptional regulators:
557 the zinc cluster proteins. *Microbiology and molecular biology reviews : MMBR,* **70**, 583–604.
558 **Marshall, R. Kombrink, A. Motteram, J. Loza-Reyes, E. Lucas, J. Hammond-Kosack, K.E. Thomma,**
559 **Bart P H J and Rudd, J.J.** (2011) Analysis of two in planta expressed LysM effector homologs from

the fungus Mycosphaerella graminicola reveals novel functional properties and varying contributions to virulence on wheat. *Plant Physiol.* **156**, 756–769.

Mirzadi Gohari, A. Mehrabi, R. Robert, O. Ince, I.A. Boeren, S. Schuster, M. Steinberg, G. de Wit, Pierre J. G. M. and Kema, Gert H. J. (2014) Molecular characterization and functional analyses of ZtWor1, a transcriptional regulator of the fungal wheat pathogen Zymoseptoria tritici. *Molecular Plant Pathology,* **15**, 394–405.

Mohammadi, N. Mehrabi, R. Gohari, A.M. Mohammadi Goltapeh, E. Safaie, N. and Kema, G.H.J. (2017) The ZtVf1 transcription factor regulates development and virulence in the foliar wheat pathogen Zymoseptoria tritici. *Fungal Genetics and Biology,* **109**, 26–35.

Möller, M. Habig, M. Freitag, M. and Stukenbrock, E.H. (2018) Extraordinary Genome Instability and Widespread Chromosome Rearrangements During Vegetative Growth. *Genetics*.

Morais, D. Gélisse, S. Laval, V. Sache, I. and Suffert, F. (2016) Inferring the origin of primary inoculum of Zymoseptoria tritici from differential adaptation of resident and immigrant populations to wheat cultivars. *Eur J Plant Pathol,* **145**, 393–404.

O'Driscoll, A. Doohan, F. and Mullins, E. (2015) Exploring the utility of Brachypodium distachyon as a model pathosystem for the wheat pathogen Zymoseptoria tritici. *BMC Res Notes,* **8**, 132.

Okmen, B. Collemare, J. Griffiths, S. van der Burgt, Ate, Cox, R. and Wit, P.J.G.M. de (2014) Functional analysis of the conserved transcriptional regulator CfWor1 in Cladosporium fulvum reveals diverse roles in the virulence of plant pathogenic fungi. *Molecular microbiology,* **92**, 10–27.

Orton, E.S. Deller, S. and Brown, James K M (2011) Mycosphaerella graminicola: from genomics to disease control. *Mol. Plant Pathol.* **12**, 413–424.

Pan, T. and Coleman, J.E. (1990) GAL4 transcription factor is not a" zinc finger" but forms a Zn (II) 2Cys6 binuclear cluster. *Proc. Natl. Acad. Sci. U.S.A.* **87**, 2077–2081.

Park, J. Park, J. Jang, S. Kim, S. Kong, S. Choi, J. Ahn, K. Kim, J. Lee, S. Kim, S. Park, B. Jung, K. Kim, S. Kang, S. and Lee, Y.-H. (2008) FTFD. An informatics pipeline supporting phylogenomic analysis of fungal transcription factors. *Bioinformatics (Oxford, England),* **24**, 1024–1025.

Ponomarenko A. S.B. Goodwin, G.H.J. Kema (2011) Septoria tritici blotch (STB) of wheat. *10.1094/PHI*.

Poppe, S. Dorsheimer, L. Happel, P. and Stukenbrock, E.H. (2015) Rapidly Evolving Genes Are Key Players in Host Specialization and Virulence of the Fungal Wheat Pathogen Zymoseptoria tritici (Mycosphaerella graminicola). *PLoS pathogens,* **11**, e1005055.

Quevillon, E. Silventoinen, V. Pillai, S. Harte, N. Mulder, N. Apweiler, R. and Lopez, R. (2005) InterProScan. Protein domains identifier. *Nucleic Acids Res,* **33**, W116-20.

R Core Team (2015) R: A Language and Environment for Statistical Computing. Vienna, Austria. https://www.R-project.org.

Rath, M. Grolig, F. Haueisen, J. and Imhof, S. (2014) Combining microtomy and confocal laser scanning microscopy for structural analyses of plant-fungus associations. *Mycorrhiza,* **24**, 293–300.

RStudio Team (2015) RStudio: Integrated Development Environment for R. Boston, MA. http://www.rstudio.com/.

Rudd, J.J. Kanyuka, K. Hassani-Pak, K. Derbyshire, M. Andongabo, A. Devonshire, J. Lysenko, A. Saqi, M. Desai, N.M. Powers, S.J. Hooper, J. Ambroso, L. Bharti, A. Farmer, A. Hammond-Kosack, K.E. Dietrich, R.A. and Courbot, M. (2015) Transcriptome and metabolite profiling of the infection cycle of Zymoseptoria tritici on wheat reveals a biphasic interaction with plant immunity involving differential pathogen chromosomal contributions and a variation on the hemibiotrophic lifestyle definition. *PLANT PHYSIOLOGY,* **167**, 1158–1185.

Rybak, K. See, P.T. Phan, H.T.T. Syme, R.A. Moffat, C.S. Oliver, R.P. and Tan, K.-C. (2017) A functionally conserved Zn2 Cys6 binuclear cluster transcription factor class regulates necrotrophic effector gene expression and host-specific virulence of two major Pleosporales fungal pathogens of wheat. *Mol. Plant Pathol.* **18**, 420–434.

Sambrook, J. and Russell, D.W. (2001) Molecular Cloning. A laboratory manual. Gold Spring Harbor, New York: Gold Spring Harbor Laboratory Pr.

Schneider, C.A. Rasband, W.S. and Eliceiri, K.W. (2012) NIH Image to ImageJ. 25 years of image analysis. *Nat Meth,* **9**, 671–675.

Siah, A. Deweer, C. Duyme, F. Sanssené, J. Durand, R. Halama, P. and Reignault, P. (2010) Correlation of in planta endo-beta-1,4-xylanase activity with the necrotrophic phase of the hemibiotrophic fungus Mycosphaerella graminicola. *Plant Pathology,* **59**, 661–670.

Somai-Jemmali, L. Randoux, B. Siah, A. Magnin-Robert, M. Halama, P. Reignault, P. and Hamada, W. (2017) Similar infection process and induced defense patterns during compatible interactions between Zymoseptoria tritici and both bread and durum wheat species. *Eur J Plant Pathol,* **147**, 787–801.

Son, H. Seo, Y.-S. Min, K. Park, A.R. Lee, J. Jin, J.-M. Lin, Y. Cao, P. Hong, S.-Y. Kim, E.-K. Lee, S.-H. Cho, A. Lee, S. Kim, M.-G. Kim, Y. Kim, J.-E. Kim, J.-C. Choi, G.J. Yun, S.-H. Lim, J.Y. Kim, M. Lee, Y.-H. Choi, Y.-D. and Lee, Y.-W. (2011) A phenome-based functional analysis of transcription factors in the cereal head blight fungus, Fusarium graminearum. *PLoS Pathog.* **7**, e1002310.

Southern, E.M. (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. *Journal of Molecular Biology,* **98**, 503–517.

Stewart, E.L. Hagerty, C.H. Mikaberidze, A. Mundt, C.C. Zhong, Z. and McDonald, B.A. (2016) An Improved Method for Measuring Quantitative Resistance to the Wheat Pathogen Zymoseptoria tritici Using High-Throughput Automated Image Analysis. *Phytopathology,* **106**, 782–788.

Tukey, J.W. (1949) Comparing Individual Means in the Analysis of Variance. *Biometrics,* **5**, 99.

Xiong, D. Wang, Y. Tang, C. Fang, Y. Zou, J. and Tian, C. (2015) VdCrz1 is involved in microsclerotia formation and required for full virulence in Verticillium dahliae. *Fungal Genet. Biol.* **82**, 201–212.

Yemelin, A. Brauchler, A. Jacob, S. Laufer, J. Heck, L. Foster, A.J. Antelo, L. Andresen, K. and Thines, E. (2017) Identification of factors involved in dimorphism and pathogenicity of Zymoseptoria tritici. *PLoS ONE,* **12**, e0183065.

Zhang, W.-Q. Gui, Y.-J. Short, D.P.G. Li, T.-G. Zhang, D.-D. Zhou, L. Liu, C. Bao, Y.-M. Subbarao, K.V. Chen, J.-Y. and Dai, X.-F. (2018) Verticillium dahliae transcription factor VdFTF1 regulates the expression of multiple secreted virulence factors and is required for full virulence in cotton. *Mol. Plant Pathol.* **19**, 841–857.

Zhong, Z. Marcel, T.C. Hartmann, F.E. Ma, X. Plissonneau, C. Zala, M. Ducasse, A. Confais, J. Compain, J. Lapalu, N. Amselem, J. McDonald, B.A. Croll, D. and Palma-Guerrero, J. (2017) A small secreted protein in Zymoseptoria tritici is responsible for avirulence on wheat cultivars carrying the Stb6 resistance gene. *The New phytologist,* **214(2)**, 619–631.

Zhuang, Z. Lohmar, J.M. Satterlee, T. Cary, J.W. and Calvo, A.M. (2016) The Master Transcription Factor mtfA Governs Aflatoxin Production, Morphological Development and Pathogenicity in the Fungus Aspergillus flavus. *Toxins,* **8**.

Zwiers, L.-H. and De Waard, Maarten A. (2001) Efficient Agrobacterium tumefaciens-mediated gene disruption in the phytopathogen Mycosphaerella graminicola. *Current Genetics,* **39**, 388–393.

Supporting Information legends

Figure S1. In vitro phenotype of *Zt107320* mutants. I*n vitro* growth of the wildtype (wt), two independent deletion strains (Δ*Zt107320*), two independent complementation strains (Δ*Zt107320*::*Zt107320*_eGFP) on YMS media including the indicated compounds to assess the effect of osmotic stress (NaCl, Sorbitol) , reactive oxygen species (H_2O_2), cell wall stressors (Calcofluor, Congo Red) and increased temperature (28°C) on growth and morphology of *Z. tritici.*

Figure S2. Growth morphologies of Z. tritici wildtype and Δ*Zt107320* and Δ*Zt107320::Zt107320-eGFP* strains on minimum medium in the presence of different carbon sources. Growth depicted after a) 7 days and b) 14 days**.** After 7 days, and more pronounced after 14 days of incubation, a higher amount of hyphal growth is observed for the Δ*Zt107320* mutants on the carbon sources Fructose, Galactose, Glucose, Maltose, Sorbitol, Sucrose and Xylose.

Figure S3. Generation of *Zt107320* mutants in *Z. tritici*. a) Schematic illustration of the gene replacement strategy of *Zt107320*. Generation of the Δ*Zt107320* strains by homologous recombination between the upstream (UF) and downstream flanking regions (DF) of *Zt107320* in its genomic locus and a plasmid carrying the hygromycin resistance cassette (hygR) located between the UF and DF. Homologous recombination results in the integration of the hygromycin-resistance gene cassette (*hygR*) in the locus of *Zt107320*. Δ*Zt107320::Zt107300*-eGFP were generated by homologous recombination between UF and DF of the Δ*Zt107320* strains and a transformed plasmid containing a c-terminal fusion of *Zt107320* and *eGFP* and a Geneticin resistance cassette (*NeoR*) located between the UF and DF Yellow bars indicate the position of the probes used in the Southern blot analyses. b) Confirmation of *Zt107320* mutants by Southern blot analyses.

677 **Table S1: List of all primers used within this study**

678 **Table S2: Summary of *in vitro* growth data**

679 **Table S3: Summary of *in planta* phenotype**