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Delineating Transcription Factor Networks Governing Virulence of a Global Human Meningitis Fungal Pathogen, *Cryptococcus neoformans*

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Cryptococcus neoformans causes life-threatening meningoencephalitis in humans, but the treatment of cryptococcosis remains challenging. To develop novel therapeutic targets and approaches, signaling cascades controlling pathogenicity of *C. neoformans* have been extensively studied but the underlying biological regulatory circuits remain elusive, particularly due to the presence of an evolutionarily divergent set of transcription factors (TFs) in this basidiomycetous fungus. In this study, we constructed a high-quality of 322 signature-tagged gene deletion strains for 155 putative TF genes, which were previously predicted using the DNA-binding domain TF database (http://www.transcription-factor.org/). We tested in vivo and in vitro phenotypic traits under 32 distinct growth conditions using 322 TF gene deletion strains. At least one phenotypic trait was exhibited by 145 out of 155 TF mutants (93%) and approximately 85% of the TFs (132/155) have been functionally characterized for the first time in this study. Through high-coverage phenome analysis, we discovered myriad novel TFs that play critical roles in growth, differentiation, virulence-factor (melanin, capsule, and urease) formation, stress responses, antifungal drug resistance, and virulence. Large-scale virulence and infectivity assays in insect (*Galleria mellonella*) and mouse host models identified 34 novel TFs that are critical for pathogenicity. The genotypic and phenotypic data for each TF are available in the *C. neoformans* TF phenome database (http://tf.cryptococcus.org). In conclusion, our phenome-based functional analysis of the *C. neoformans* TF mutant library provides key insights into transcriptional networks of basidiomycetous fungi and ubiquitous human fungal pathogens.