INSTITUTO POTOSINO DE INVESTIGACIÓN CIENTÍFICA Y TECNOLÓGICA, A.C.

POSGRADO EN CIENCIAS EN BIOLOGIA MOLECULAR

Abf1 es una proteína esencial y participa en el silenciamiento subtelomérico en *Candida glabrata*

Tesis que presenta

Grecia Hernández Hernández

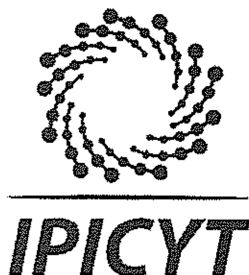
Para obtener el grado de

Maestra en Ciencias en Biología Molecular

Directora de la Tesis:

Dra. Irene B. Castaño Navarro

San Luis Potosí, S.L.P., Agosto de 2017



Constancia de aprobación de la tesis

La tesis ***"Abf1 es una proteína esencial y participa en el silenciamiento subtelomérico en Candida glabrata"*** presentada para obtener el Grado de Maestra en Ciencias en Biología Molecular fue elaborada por Grecia Hernández Hernández y aprobada el **dieciocho de agosto de dos mil diecisiete** por los suscritos, designados por el Colegio de Profesores de la División de Biología Molecular del Instituto Potosino de Investigación Científica y Tecnológica, A.C.

Dra. Irene Beatriz Castaño Navarro
Directora de la tesis

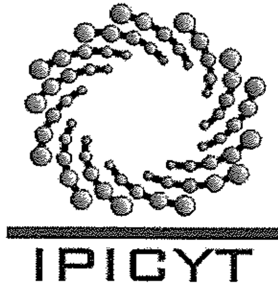
Dr. J. Sergio Casas Flores
Miembro del Comité Tutoral

Dr. Samuel Lara González
Miembro del Comité Tutoral

Créditos Institucionales

Esta tesis fue elaborada en el Laboratorio de Microbiología Molecular de la División de Biología Molecular del Instituto Potosino de Investigación Científica y Tecnológica, A.C., bajo la dirección de la Dra. Irene B. Castaño Navarro, apoyada por el proyecto No. CB-2014-239629 del Fondo de Ciencia Básica.

Durante la realización del trabajo el autor recibió una beca académica del Consejo Nacional de Ciencia y Tecnología (No. 590366) y del Instituto Potosino de Investigación Científica y Tecnológica, A. C.



Instituto Potosino de Investigación Científica y Tecnológica, A.C.

Acta de Examen de Grado

El Secretario Académico del Instituto Potosino de Investigación Científica y Tecnológica, A.C., certifica que en el Acta 166 del Libro Primero de Actas de Exámenes de Grado del Programa de Maestría en Ciencias en Biología Molecular está asentado lo siguiente:

En la ciudad de San Luis Potosí a los 18 días del mes de agosto del año 2017, se reunió a las 12:00 horas en las instalaciones del Instituto Potosino de Investigación Científica y Tecnológica, A.C., el Jurado integrado por:

Dr. J. Sergio Casas Flores	**Presidente**	**IPICYT**
Dr. Samuel Lara González	**Secretario**	**IPICYT**
Dra. Irene Beatriz Castaño Navarro	**Sinodal**	**IPICYT**

a fin de efectuar el examen, que para obtener el Grado de:

MAESTRA EN CIENCIAS EN BIOLOGÍA MOLECULAR

sustentó la C.

Grecia Hernández Hernández

sobre la Tesis intitulada:

Abf1 es una proteína esencial y participa en el silenciamiento subtelomérico en Candida glabrata

que se desarrolló bajo la dirección de

Dra. Irene Beatriz Castaño Navarro

El Jurado, después de deliberar, determinó

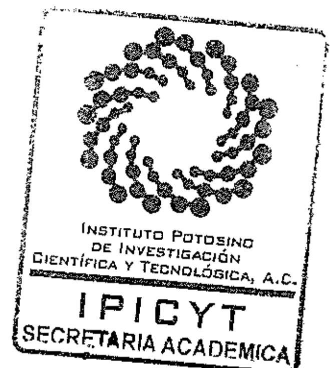
APROBARLA

Dándose por terminado el acto a las 13:30 horas, procediendo a la firma del Acta los integrantes del Jurado. Dando fe el Secretario Académico del Instituto.

A petición de la interesada y para los fines que a la misma convengan, se extiende el presente documento en la ciudad de San Luis Potosí, S.L.P., México, a los 18 días del mes de agosto de 2017.

Dr. Horacio Flores Zúñiga
Secretario Académico

Mtra. Ivonne Lizette Cuevas Vélez
Jefa del Departamento del Posgrado



Dedicatorias

A mis padres Rosario y Horacio, por sus enseñanzas y apoyo, por guiarme y ayudarme a convertirme en una mejor persona cada día, por dar lo mejor de ellos para que yo llegara hasta aquí.

A mi hermana Cielo, por escucharme e impulsarme en todo, por siempre estar ahí, por ser un ejemplo de perseverancia y dedicación.

A Fermín, por ser, por estar, por existir.

Agradecimientos

Al Instituto Potosino de Investigación Científica y Tecnológica por el apoyo institucional.

Al Consejo Nacional de Ciencia y Tecnología por el apoyo económico para la realización de mis estudios de Maestría.

A la Dra. Irene Castaño Navarro por guiarme y enseñarme en este proyecto, por ser un ejemplo para mi como científica y persona.

Al Dr. Alejandro De Las Peñas por sus enseñanzas, ideas y sugerencias en este proyecto.

Al Dr. Sergio Casas Flores y Dr. Samuel Lara González por sus comentarios, sugerencias y apoyo.

A la Dra. Guadalupe Gutiérrez Escobedo por su trabajo y apoyo técnico en el laboratorio.

Al Dr. Nicolás Gómez Hernández y Dra. Norma Angélica Ramírez Pérez por su apoyo técnico.

A Gloria López por la ayuda brindada en el laboratorio.

A mis compañeros de Laboratorio 6, en especial a Karina, Lupita, Yamille, Oscar y Eunice, por brindarme su amistad y enseñarme siempre.

Table of Contents

Constancia de aprobación de la tesis ii

Créditos Institucionales iii

Acta de Examen iv

Dedicatorias v

Agradecimientos vi

Abbreviations ix

Resumen xi

Abstract xii

1. Abstract 2

2. Introduction 3

3. Materials and Methods 5

3.1. Strains, plasmids, and primers 5
3.2. Media 5
3.3. Yeast transformation 6
3.5. Construction of null mutant, and truncated allele of ABF1 gene 7
3.6. Plasmid loss assay to determine if ABF1 is essential in C. glabrata 8
3.7. Plasmid for tagging Abf1 with c-Myc under the inducible MT1 promoter 9
3.8. Reporter URA3 gene expression assays (5-FOA sensitivity assays) 9

4. Results 10

5. Discussion 14

6. Literature cited 19

7. Tables, figure legends and figures 25

Figures

Figure 1 Abf1 has a role in silencing at the *C. glabrata* telomere E-$_R$ 29
Figure 2 *ABF1* is essential for cell viability in *C. glabrata* 30
Figure 3 Abf1 has an important role during growth in different media and at high temperature 31
Figure 4 *ABF1* mutation is complemented with cMyc-Abf1 version 32
Figure 5 Overexpression of *ABF1* is toxic in *Candida glabrata* 33
Figure 6 Abf1 has a role in silencing at different subtelomeric regions in *C. glabrata* 34
Figure 7 Model for telomere loop formation at the E-$_R$ telomere in *C. glabrata* 35

Supplementary Figures

Fig. S 1 Schematic representation of N-terminal tagged version of *ABF1*. 48
Fig. S 2 Abf1 does not play a detectable role in transcriptional regulation of P$_{EPA1}$ by the NE. 49
Fig. S 3 Alignment of Abf1 from *C. glabrata* (*Cg*Abf1) and *S. cerevisiae* (*Sc*Abf1). 52

Supplementary Tables

Table S 1 *C. glabrata* and *E. coli* strains used in this study. 38
Table S 2 Plasmids used in this study. 44
Table S 3 Oligonucleotides used in this study. 47

Abbreviations

EPA Epithelial Adhesin

E-R Right telomere, chromosome E

Abf1 ARS-Binding Factor 1

Rap1 Repressor-Activator Protein 1

Sir Silent Information Regulator Proteins

MT1 Metallothionein 1

$\mathbf{P}_{\mathbf{\mathit{MT1}}}$ *MT1* promoter

$\mathbf{P}_{\mathbf{\mathit{EPA1}}}$ *EPA1* promoter

c-Myc MyeloCytomatosis proto-oncogen protein

Flag Polypeptide protein tag

Kb Kilobases

C-terminal Carboxyl terminal

SC Synthetic complete media

$\mathbf{NH_2SO_4}$ Ammonium sulfate

$\mathbf{CuSO_4}$ Copper sulfate

CAA Casamino Acids

5-FOA 5-Fluoroorotic acid

YPD Yeast extract-Peptone-Dextrose

YNB Yeast Nitrogen Base

LB Luria-Bertani media

OD Optical Density

LiAc Lithium acetate

SS Salmon sperm DNA

PEG Polietilenglicol

NAT Nourseothricin resistance marker

$\mathbf{Nat^R}$ Nourseothricin-resistance strain

UTR Untranslated region

ARS Autonomously replicating sequence

ORF Open reading frame

FRT Flp Recombination Targets

CEN Centromere

kDa Kilodalton

aa aminoacids

bp base pair

Resumen

Abf1 es una proteína esencial y participa en el silenciamiento subtelomérico en *Candida glabrata*

Candida glabrata es un comensal de humanos capaz de causar infecciones en individuos inmunocomprometidos, y un factor importante para su virulencia es su habilidad de adherirse a las células epiteliales. Esta capacidad se debe principalmente a Epa1 codificada por el gen *EPA1*, que pertenece a una gran familia de genes que codifican para proteínas de pared celular. La mayoría de estos genes se encuentran en regiones subteloméricas, por lo que están sujetos a silenciamiento subtelomérico. Los genes *EPA1*, *EPA2* y *EPA3* forman un clúster en el teloméro derecho del cromosoma E (E_{-R}). El silenciamiento subtelomérico en *C. glabrata* esta regulado por el complejo SIR (Sir2, Sir3 y Sir4), Rap1 y Rif1. Además, se han identificado elementos en *cis* que regulan de forma negativa la expresión de *EPA1* independientemente del silenciamiento subtelomérico, uno de ellos es el protosilenciador Sil2126, el cual puede silenciar un gen reportero insertado a 32 Kb alejado del teloméro. En un análisis *in silico*, encontramos varios sitios putativos de unión para Abf1 en Sil2126 y muchas otras regiones a lo largo de la región subtelomérica del teloméro E_{-R}. En este trabajo demostramos que la actividad de Sil2126 depende del dominio C-terminal de *Cg*Abf1, cuando medimos el silenciamiento del gen reporter *URA3* a 32 Kb de distancia del teloméro E_{-R}. El dominio C-terminal de *Cg*Abf1 se requiere también para silenciamiento en otros telomeros. El efecto en el silenciamiento por *Cg*Abf1 solo se puede detectar cuando el reportero es insertado a una distancia mayor de 10 Kb del teloméro. De manera interesante, encontramos que *Cg*Abf1 es esencial para la viabilidad celular y su sobreexpresión afecta el crecimiento de forma negativa. ***Palabras clave:*** *Candida glabrata,* Abf1*,* silenciamiento subtelomérico*,* gen esencial.

Abstract

Abf1 is an essential protein in *Candida glabrata* and is required for subtelomeric silencing

Candida glabrata is a commensal of humans capable of causing infection in immunocompromised individuals that is able to adhere to host epithelial cells, which is an important factor for its virulence. This ability is primarily mediated by the Epa1 protein encoded by *EPA1* gene, which belongs to a large family of cell wall protein-encoding genes. Most of these are localized in subtelomeric regions, and are subject to subtelomeric silencing. *EPA1*, *EPA2* and *EPA3* form a cluster close to the right telomere of chromosome E ($E\text{-}_R$). In *C. glabrata* the subtelomeric silencing is mediated by the SIR complex (Sir2, Sir3 and Sir4), Rap1 and Rif1. In addition, we have identified *cis*-acting elements that negatively regulate *EPA1* expression independently of subtelomeric silencing, one of these is a protosilencer Sil2126, which can silence a reporter gene inserted 32 Kb away from this particular telomere. In an *in silico* analysis, we found several putative binding sites for *Cg*Abf1 in Sil2126 and in several other regions throughout the subtelomeric region of telomere $E\text{-}_R$. Here we describe that Sil2126 activity depends on the C-terminal domain of *Cg*Abf1, as measured by silencing of the *URA3* reporter gene 32 kb away from the telomere $E\text{-}_R$. The C-terminal domain of *Cg*Abf1 is also required for silencing at other telomeres. The effect in silencing of *Cg*Abf1 can only be detected when the reporter is inserted at a distance of >10 kb from the telomere. Importantly, we show that *Cg*Abf1 is essential for cell viability, and its overexpression affects growth negatively.

Key words: *Candida glabrata,* Abf1*,* subtelomeric silencing, essential gene*.*

1 Running title: **Abf1 is required for subtelomeric silencing in**

2 ***Candida glabrata***

3 **Key words:** *Candida glabrata,* Abf1*,* subtelomeric silencing, essential gene*.*

4 Grecia Hernández[1], Leonardo Castañedo-Ibarra[2], Eunice López-Fuentes[1],

5 Alejandro De Las Peñas[1], and Irene Castaño[1]*

6 [1]División de Biología Molecular, Instituto Potosino de Investigación Científica y

7 Tecnológica. Camino a la Presa San José 2055, San Luis Potosí, S. L. P. 78216,

8 Tel. (444) 834 2038

9 [2]Faculty of Biology and Biotechnology, Ruhr University Bochum

10 Universitätsstrasse. 150 ND3/30, D-44801 Bochum, Germany.

11 * Corresponding author:

12 Irene Castaño

13 Mailing address: Camino a la Presa San José # 2055. División de Biología

14 Molecular

15 Instituto Potosino de Investigación Científica y Tecnológica. San Luis Potosí, San

16 Luis Potosí

17 78216, México

18 Phone (52) 444-834-2000 ext. 2038

19 Fax: (52) 444-834-2010

20 e-mail: icastano@ipicyt.edu.mx

1. Abstract

Candida glabrata is a commensal of humans capable of causing infection in immunocompromised individuals that is able to adhere to host epithelial cells, which is an important factor for its virulence. This ability is primarily mediated by the Epa1 protein encoded by *EPA1* gene, which belongs to a large family of cell wall protein-encoding genes. Most of these are localized in subtelomeric regions, and are subject to subtelomeric silencing. *EPA1*, *EPA2* and *EPA3* form a cluster close to the right telomere of chromosome E (E-$_{R}$). In *C. glabrata* the subtelomeric silencing is mediated by the SIR complex (Sir2, Sir3 and Sir4), Rap1 and Rif1. In addition, we have identified *cis*-acting elements that negatively regulate *EPA1* expression independently of subtelomeric silencing, one of these is a protosilencer Sil2126, which can silence a reporter gene inserted 32 Kb away from this particular telomere. In an *in silico* analysis, we found several putative binding sites for *Cg*Abf1 in Sil2126 and in several other regions throughout the subtelomeric region of telomere E-$_{R}$. Here we describe that Sil2126 activity depends on the C-terminal domain of *Cg*Abf1, as measured by silencing of the *URA3* reporter gene 32 kb away from the telomere E-$_{R}$. The C-terminal domain of *Cg*Abf1 is also required for silencing at other telomeres. The effect in silencing of *Cg*Abf1 can only be detected when the reporter is inserted at a distance of >10 kb from the telomere. Importantly, we show that *Cg*Abf1 is essential for cell viability, and its overexpression affects growth negatively.

42 ## 2. Introduction

43 In eukaryotes, temporal and spatial arrangement of chromatin plays an
44 important role in regulation of gene expression, and many other nuclear functions
45 (Workman and Kingston, 1998). One important mechanism of gen regulation is
46 silencing, which is only present at certain chromosomal regions, and depends on
47 gene position, post-translational modification of histone tails (like methylation,
48 phosphorylation, deacetylation, etc.) at specific regions and the absence of
49 boundary elements that block the propagation of silencing. Silencing of chromatin
50 depends on some specific DNA sequences (called, *cis*-acting elements), remodeling
51 proteins and transcription factors (*trans*-acting elements) (Yarragudi et al., 2004b;
52 Strahl-Bolsinger et al., 1997; Rine and Herskowitz, 1987; Bi et al., 1999)

53 The proteins involved in such mechanisms vary depending on the organism,
54 and also on certain chromosomal regions in the same organism. Such is the case of
55 the yeast *Saccharomyces cerevisiae* that has been studied to elucidate the
56 mechanism of silencing. For example, the silent copies of the loci responsible for
57 mating (*MAT*), called *HML* and *HMR*, are subject to silencing that is mediated by the
58 complex formed by Sir proteins (Sir1, Sir2, Sir3 and Sir4) and by silencers (*cis*-acting
59 elements) that flank the silent loci *HML* and *HMR* (Rine and Herskowitz, 1987).
60 *Candida glabrata,* which is an opportunistic fungal pathogen closely related to *S.*
61 *cerevisiae*, has a similar silencing mechanism in the subtelomeric regions that also
62 depends on the SIR complex, which in the case of *C. glabrata* is formed only by Sir2,
63 Sir3 and Sir4 (*C. glabrata* does not contain the *SIR1* gene). Silencing in *C. glabrata*
64 regulates gene expression of the orthologous mating locus (*MTL3*) and the
65 expression of the majority of the *EPA* genes that encode cell wall proteins important

66 for adhesion and virulence (Castaño *et al*., 2005; Cormack *et al.*, 1999; De Las
67 Peñas *et al.*, 2003). Most of these *EPA* genes, are located at subtelomeric regions,
68 and are subject to subtelomeric silencing.

69 In *C. glabrata* subtelomeric silencing depends also on Rap1 and Rif1
70 (Castaño *et al.*, 2005; De Las Peñas *et al*., 2003; Rosas-Hernández *et al*., 2008). In
71 addition, we have described different *cis*-acting sequences that negatively regulate
72 *EPA1* expression independently of subtelomeric silencing, such as the protosilencer
73 Sil2126 found in the right telomere of chromosome E ($E\text{-}_R$), between *EPA3* and the
74 telomere. Sil2126 depends on yKu70 and yKu80 proteins, and is capable of
75 mediating silencing at a distance of 31.9 Kb away from its telomere, but not when it
76 is removed from the telomere context or when it is inverted with respect to the its
77 relative position in the chromosome (Juárez-Reyes *et al*., 2012). Another *cis*-acting
78 element is found in the intergenic region between *EPA1* and *EPA2* in the
79 chromosome $E\text{-}_R$, called the negative element (NE). This element represses the
80 transcription of *EPA1*, and is independent of the telomere context, but it depends on
81 yKu70 and 80 proteins (Gallegos-García *et al.*, 2012). In a *in silico* analysis we have
82 also found putative binding sites for *Cg*Abf1 (ARS-binding factor 1) along the
83 chromosome $E\text{-}_R$ of *C. glabrata*, which could function as *cis*-acting elements (Fig. 1).

84 *C. glabrata* encodes the *ABF1* gene, orthologous to *ScABF1*, which is
85 required for silencing of the mating type silent loci *HML* and *HMR. ScABF1* is an
86 essential gene, which is involved in numerous cellular functions such as DNA
87 replication, DNA repair and chromatin reorganization that can enhance the
88 accessibility for other transcription factors and establish an active or inactive
89 conformation of the chromatin. Many of these activities require the C-terminal

90 domain of *Sc*Abf1 (Miyake et al., 2002, 2004; Yarragudi et al., 2004b; Fermi et al.,
91 2016; Buchman et al., 1988). However, *CgABF1* has not been characterized and it
92 is not known whether it is involved in silencing and or if it is an essential gene.

93 In this study, we showed that *CgABF1* is essential for cell viability in *C.*
94 *glabrata*. Interestingly, we found that the absence of the last 43 amino acids at the
95 C-terminal end of *Cg*Abf1 (*Cg*Abf1-43) that correspond to the conserved domain
96 responsible for silencing in *Sc*Abf1, decreases the level of silencing mediated by the
97 protosilencer Sil2126 as well as the subtelomeric silencing at this telomere and
98 others (Tel $B\text{-}_L$). Additionally, we found that the mutant *abf1-43* displays a slower
99 growth rate in different media. We also constructed an amino terminal, cMyc-tagged
100 version of Abf1, under the control of an inducible promoter (P_{MT1}), which is induced
101 with copper ions. The tagged version (cMyc-*Cg*Abf1) is functional since it
102 complements a chromosomal *abf1*Δ allele.

103 3. Materials and Methods

104 3.1. Strains, plasmids, and primers

105 All strains, plasmids and oligonucleotides used are listed in Table S1, Table S2,
106 and Table S3 respectively.

107 3.2. Media

108 Yeast were grown in standard yeast media as described previously with 2% agar
109 added for plates (Sherman *et al*, 1986). Synthetic complete (SC) medium contains
110 1.7 g/L yeast nutrient base (neither contains NH_2SO_4 and amino acids), 5 g/L
111 NH_2SO_4 and supplemented with 0.6% casamino acids (CAA) and 2% glucose. To
112 prepare 5-fluoroorotic acid (5-FOA; Toronto Research Chemicals) media, 0.9 g of 5-

113 FOA compound that is toxic to cells when are expressing *URA3* gene (and 25 mg of
114 uracil/L were added to the SC. Yeast extract-peptone-dextrose (YPD) medium
115 contains 10 g/L yeast extract, 20 g/L peptone, and supplemented with 2% glucose.
116 When required, YPD plates were supplemented with Nourseothricin (Invitrogen™)
117 at 100 µg/mL.
118 Bacteria were grown in LB medium as described previously (Ausubel *et al*, 2001).
119 LB medium contained 5 g/L yeast extract, 10 g/L tryptone, 5 g/L NaCl. All plasmid
120 constructs were introduced into strain DH10 by electroporation, and 100 µg/mL
121 carbenicillin (Invitrogen™) was added to select for plasmids. For plates, 1.5% agar
122 was used.

123 ### 3.3. Yeast transformation

124 Yeast transformations with digested or supercoiled plasmids were performed as
125 previously described using the LiOAc/salmon sperm carrier DNA/PEG method
126 (Castaño *et al*., 2003; Gietz, 2014).

127 ### 3.4. Growth assays in liquid and solid media

128 Cells were grown to stationary phase for 48 h in YPD, CAA or YNB. To determine
129 duplication time, cells of each strain were grown in the appropriate liquid media.
130 Stationary phase cultures were adjusted to an OD_{600} of 0.01 in the corresponding
131 media and 300 µL dispensed in a 100-well plate. Growth was automatically recorded
132 using Bioscreen C analyser at 30 ºC (Thermic Labsystems Oy, Finland) with
133 constant shaking and OD measurements taken every 15 minutes during a period of
134 48 h; the doubling time of each strain was calculated as described elsewhere
135 (Gutiérrez-Escobedo *et al.*, 2013).

136 For solid media experiments, stationary phase cultures were adjusted to an OD_{600}
137 of 1.0 with sterile water, and 10-fold serial dilutions were made in a 96-well plate. 5
138 µL of each dilution were spotted on to YPD plates. For temperature sensitivity assay,
139 a total of 5 µL of each dilution was spotted onto YPD, then incubated at 30, 37 or 45
140 ºC, and photographed every 24 h during three days.

141 **3.5. Construction of null mutant, and truncated allele of *ABF1* gene**

142 Since *ScABF1* is essential for cell viability in *S. cerevisiae*, we designed a strategy
143 to determine whether *CgABF1* is an essential gene in *C. glabrata*. To do this, we first
144 constructed a replicative plasmid containing the complete ORF of *CgABF1* plus the
145 5' and 3' flanking regions of this gene (1 Kb upstream and 800 bp downstream,
146 primers #1589 and #1881, Table S3), and the *URA3* selection marker (pCI12). We
147 also generated a disruption plasmid for *CgABF1*. Briefly, the 5' (primers #1589 and
148 #1881) and 3' (primers #1561 and #1562, Table S3) untranslated regions of the gene
149 to be deleted were PCR amplified and cloned into pYC44 integrative plasmid
150 (Yáñez-Carrillo *et al.*, 2015), flanking the nourseothricin expression cassette
151 (conserving the relative orientation of the chromosomal locus of *ABF1*) (pCI42). The
152 plasmid generated was used to construct an allele replacement of *CgABF1* by
153 homologous recombination in a one-step gene replacement procedure. For this, we
154 first transformed the parental strain BG14 (Table S1) with the plasmid with the wild-
155 type *CgABF1* gene and *URA3* marker (pCI12). This strain was then transformed with
156 the linearized fragment from the knockout plasmid (pCI42). The plasmid was
157 previously digested with enzymes that cut within both ends of the cloned 5' and 3'
158 flanking fragments, generating homologous ends to *ABF1* gene in the *C. glabrata*

159 genome. Transformants were selected on plates supplemented with nourseothricin
160 (Invitrogen™) at 100 µg/mL. Homologous recombination and allele replacement of
161 the gene was verified by PCR analysis using primers annealing within the
162 noursothricin cassette and outside the cloned 5' and 3' flanking regions. We also
163 verified the absence of gene deletion by the inability to amplify by PCR an internal
164 fragment from *ABF1*.
165 To construct the truncated allele of *ABF1* that lacks the last 43 amino acids that
166 correspond to the CS2 domain important for silencing in *S. cerevisiae* (Miyake *et al,*
167 2002), we designed primers to amplify the truncated ORF of *ABF1* ending at amino
168 acid 436 using primers (Table S3) #1559 and #1880 (Figure 1B*)*. The fragment
169 obtained was cloned into the integrative vector pYC44, generating plasmid pCI30. A
170 714 bp fragment containing the 3' UTR region of *ABF1* was cloned into pCI30
171 flanking the nourseothricin cassette (pCI32). Plasmid pCI32 was digested with *Bsg*I
172 within the region of *C. glabrata* homology and transformed into the parental BG14
173 selecting for Nat^R.

174 **3.6. Plasmid loss assay to determine if *ABF1* is essential in *C. glabrata***

175 We used the null mutant described above, which contains a *URA3* plasmid carrying
176 *ABF1* (pCI12) and a deletion of the chromosomal copy of *ABF1* (*abf1*∆). This strain
177 as well as the BG14 parental strain transformed with pCI12, and BG14 with empty
178 vector were grown at 30 ºC for 48 h in YPD and diluted into fresh media and grown
179 for another 24 h as described previously (Gutiérrez-Escobedo *et al.*, 2013). This was
180 repeated three times. Tenfold serial dilutions were plated on YPD plates for viable
181 counts and on SC + 5-FOA plates to select for the loss of the *URA3* plasmid. Ura^+

182 cells die on SC + 5-FOA plates, therefore only cells that have lost the *URA3* plasmid
183 can grow on SC + 5-FOA. The percentage of cells without plasmid was calculated
184 by counting the number of colonies on SC + 5-FOA divided by the number of colonies
185 on YPD (viable count).

186 **3.7. Plasmid for tagging Abf1 with c-Myc under the inducible *MT1***
187 **promoter**

188 We generated a replicative vector to tag Abf1 at the N-terminal end with c-Myc
189 epitope separated by a linker (5 repetitions of GA) in order not to interfere with the
190 protein folding, and under the control of the promoter of the *MT1* gene
191 (metallothionenin 1), which is inducible with $CuSO_4$. We amplified the full-length
192 *ABF1* gene with primers #2353 and #2354 (Table S3) both of which contain a *Cla*I
193 restriction site to clone it into the plasmid pGH3, which contains the P_{MT1} promoter
194 fusion with the c-Myc tag and the GA linker to generate translational fusions at the
195 N-terminal end of the protein of interest (Figure S1). The final plasmid (pGH8) was
196 transformed into *E. coli*, and after verifying that the gene fragment was cloned in the
197 correct orientation, it was transformed into *C. glabrata* (see yeast transformation
198 section).

199 **3.8. Reporter *URA3* gene expression assays (5-FOA sensitivity assays)**

200 We designed a collection of mutants that contains the *URA3* reporter gene that allow
201 us to measure silencing at different positions throughout specific telomeres as shown
202 in the relevant Figs. The experiment was done using a plate growth assay as
203 described previously (De Las Peñas *et al.*, 2003; Castaño *et al*., 2005). Strains were
204 grown in YPD for 48 h to stationary phase and then were adjusted to an OD_{600} of 1.0

205 with sterile water, and 10-fold serial dilutions were made in 96-well plates.
206 Subsequently, 5 µL of each dilution was spotted onto YPD, SC -uracil, and SC + 5-
207 FOA plates (with or without $CuSO_4$ for the induction of P_{MT1} containing plasmids),
208 with the replica-plating tool (Frogger) (NUNC™). The plates were incubated for 48 h
209 at 30 ºC, and then photographed.

210 **3.9. FACS analysis of GFP expression**

211 Strains that contains the reporter gene GFP (Table S1) were grown for 48 h at 30º
212 in CAA media. Cells were diluted into fresh media to induce the *EPA1* promoter.
213 *GFP* was used as reporter gene to measure the activity of *EPA1* promoter in
214 presence of *abf1-43* mutant. Stationary phase cells (48 h cultures) were diluted into
215 fresh media and 500 µL samples of these cultures (logarithmic phase), were
216 analyzed for GFP expression by fluorescence cytometry (FACS) using BD
217 FACSCalibur Flow Cytometer with Cell Quest Pro software.

218 ## 4. Results

219 **4.1. Abf1 is essential for cell viability in *Candida glabrata***

220 In order to determine if *ABF1* is essential for cell viability in *C. glabrata*, we performed
221 a plasmid loss assay (see methods). We introduced a replicative plasmid in the
222 parental strain (BG14) with the *ABF1* gene with its own promoter and the selectable
223 marker *URA3* (pCI12). In this strain, we deleted the native *ABF1* gene by
224 homologous recombination of a knock-out plasmid (pCI42). Loss of the plasmid
225 containing the wild-type *ABF1* gene in this strain during growth in absence of
226 selective pressure (rich media) indicates that *ABF1* is not required for cell viability.
227 In this experiment, we can identify the loss of the plasmid by growth on SC + 5-FOA

228 plates, since cells expressing *URA3* die in the presence of 5-FOA and only cells that
229 have lost the *URA3* plasmid can grow on these plates.
230 If the strain *abf1*Δ/p*ABF1.URA3* cannot lose the plasmid, this indicates that *ABF1* is
231 essential for cell viability. Fig. 2 shows that the strain *abf1*Δ/p*ABF1.URA3* containing
232 the null mutation, cannot lose the complementing plasmid as measured by the
233 absence of 5-FOA^R colonies. This result is in agreement with the fact that *ScABF1*
234 is also essential for viability in *S. cerevisiae* (Miyake et al., 2002; Yarragudi et al.,
235 2004b).
236 The complementing plasmid with *ABF1* is also retained in a background with the
237 truncated version of *ABF1* (*abf1-43*), although as opposed to the null mutant, a small
238 percentage (8%) of cells in the *abf1-43* can lose the plasmid. Also, in the parental
239 strain more than half of the cells retain an extra copy of *ABF1* (the endogenous and
240 the episomal copies).

241 **4.2. *abf1-43* mutant has a longer duplication time than the parental strain**
242 **in different media and confers temperature sensitivity**

243 We determined whether the strain that only expresses the truncated protein *abf1-43*
244 displays a growth defect under different conditions. We analyzed the growth curve
245 of the mutant carrying the truncated version of *ABF1*, the strain with the null allele in
246 the chromosome complemented with the plasmid with the wild-type *ABF1* gene and
247 the parental strain for 48 h in different media using the apparatus Bioscreen C. We
248 found that the *abf1-43* mutant has a longer doubling time in comparison with the
249 other strains in all media tested: rich media (YPD), minimal media supplemented
250 with casaminoacids (CAA) and minimal media (YNB) (Fig. 3A and Table 1). This
251 indicates that the C-terminal end of Abf1 is required for normal cell growth.

252 We also found that the *abf1-43* strain shows a temperature sensitive phenotype at
253 45ºC as measured by growth on solid media using a spot assay (Figure 3B).

254 **4.3. Overexpression of *ABF1* is toxic in *Candida glabrata***

255 To determine whether overexpression of Abf1 results in toxicity in *C. glabrata*
256 as it has been reported for *S. cerevisiae* (Sopko et al., 2006; Stevenson et al., 2001),
257 we generated an epitope tagged version of Abf1 by constructing a plasmid that
258 contains a translational fusion of the c-Myc tag at the 5' end of *ABF1*, separated by
259 a linker and under the control of the copper inducible promoter P_{MT1}. This construct
260 complements the *abf1*Δ strain as determined by the ability to exchange the
261 *ABF1*.*URA3* plasmid for the plasmid carrying the cMyc-*ABF1* tagged version (Fig.
262 4A). This cMyc tagged Abf1 is also functional for silencing activity mediated by the
263 protosilencer Sil2126 when the Sil-reporter system is placed 32 kb away from
264 telomere E_{-R} (See below, Fig. 4B).

265 We evaluated the growth of the parental and the *abf1-43* strains in the
266 presence or absence of the replicative plasmid that contains cMyc-*ABF1* under the
267 promoter of the *MT1* gene, inducible by copper (Supplementary Fig. S1). We used
268 rich media (YPD) without copper and with 50 µM of $CuSO_4$. Fig. 5 and Table 1 show
269 that the basal expression level of the inducible vector in the absence of added
270 copper, decreases the growth rate in both the parental strain and the mutant *abf1-*
271 *43*, moreover, when we addition of 50 µM copper, the decrease in growth rate is
272 more pronounced, particularly in the *abf1-43* strain.

273 **4.4. Subtelomeric silencing at telomeres E_{-R} and B_{-L} is decreased in the**
274 ***abf1-43* mutant**

275 *Sc*Abf1 has been shown to plays a role in silencing of the silent mating-type
276 cassettes (*HML* and *HMR*) in *S. cerevisiae*. Nevertheless, it has not been studied
277 whether *Cg*Abf1 participates in silencing in *C. glabrata*. We constructed different
278 strains containing the *URA3* reporter gene integrated at various distances from
279 different telomeres in the wild-type background and in the *abf1-43* mutant. Silencing
280 activity by *abf1-43* was assayed by the ability of the strains carrying each reporter
281 insertion to grow on plates lacking uracil (SC-ura plates) where only cells that
282 express the *URA3* reporter can grow, and on plates containing 5-flourootic acid (5-
283 FOA plates) where cells expressing *URA3* react with 5-FOA and create a toxic
284 compound. As shown in Fig. 6A, insertions 1 and 2 where the reporter gene *URA3*
285 is closer to the telomere (1.3 and 14.8 kb respectively), were efficiently silenced in
286 the *abf1-43* mutant. On the other hand, when the reporter was inserted 20.6 kb away
287 from the telomere (insertion 3), the level of silencing was decreased in the *abf1-43*
288 mutant. This mutant strain is also defective for silencing at telomere B_{-L} where the
289 silenced *MTL3* locus is localized. Fig. 6D shows that the *URA3* insertions placed
290 further away than 11.2 kb from this telomere (between alpha1 and alpha3 genes and
291 downstream from alpha3) are not efficiently silenced in the *abf1-43* mutant, while the
292 insertion placed closer to the telomere (9.9 kb from the telomere) is efficiently
293 silenced in this strain.
294 Interestingly, the *abf1-43* strain is also defective in silencing mediated by the
295 protosilencer Sil2126 when it is inserted 32 kb away from the telomere E_{-R} (Fig. 6B
296 and C).

297 Instead, silencing at the Chr I-$_R$ where *EPA4* and *EPA5* form a 15 kb inverted repeat,
298 silencing of the reporter *URA3* at different positions was as efficient in the parental
299 strain as in the *abf1-43* mutant (Fig. 6E).

300 ## 5. Discussion

301 Arrangement of DNA into chromatin is one of the main processes in the
302 regulation of gene expression. This arrangement depends on several factors, *cis*-
303 and *trans*- acting elements, that respond to different environmental changes to which
304 cells are exposed at various times (Margueron and Reinberg, 2010; Probst et al.,
305 2009). Some *trans*-acting elements like Abf1 in *S. cerevisiae* are capable not only to
306 function as transcription factors, but also to participate in replication, DNA repair,
307 and gene silencing (Miyake et al., 2002). This paper describes for the first time that
308 *Cg*Abf1 has a role in silencing in *C. glabrata*. In particular we found that the C-
309 terminal 43 amino acids of the protein are required for silencing of a reporter gene
310 inserted at distances over 10 kb away from two different telomeres subject to
311 subtelomeric silencing (telomeres E$_{-R}$ and B-$_L$). We also show that *Cg*Abf1 is
312 required for silencing mediated by the unique protosilencer Sil2126, which is
313 naturally present between *EPA3* and the telomere E$_{-R}$. Both of these telomeres and
314 Sil2126 depend also on the SIR complex, Rif1 and Rap1 and differentially on yKu70
315 and yKu80 (Castaño et al., 2005; De Las Peñas et al., 2015; Rosas-Hernández et
316 al., 2008; Ramírez-Zavaleta et al., 2010; Juárez-Reyes et al., 2012). Collectively, the
317 data presented in this work indicates that *Cg*Abf1 is required for cell viability,
318 contributes to the regulation of cell growth and has a role in silencing in a distance-
319 dependent manner from the telomere.

320 **6.1. *Cg*Abf1 is essential for viability in *C. glabrata* and may be implicated**
321 **in cell cycle progression**

322 It is well known that *Sc*Abf1 is a multifunctional protein that plays an important
323 role in transcriptional activation and repression, telomere structure, ribosome
324 biogenesis (since it regulates the expression of genes encoding ribosomal proteins),
325 establishment of prereplicative complex (pre-RC) during the cell cycle and is also
326 part of the nucleotide excision repair subcomplex (Fermi et al., 2016; Reed et al.,
327 1999; Kawasaki et al., 2006). Involvement in all of these processes could explain the
328 fact that of *Sc*Abf1 is essential for cell viability and growth rate under different
329 conditions in *S. cerevisiae* (Miyake et al., 2002; Yarragudi et al., 2004a). In the case
330 of *C. glabrata*, *CgABF1* is also required for cell viability since the *abf1Δ* strain
331 containing a plasmid with the full-length *CgABF1,* is not able of losing the
332 complementing plasmid; unless this strain is also transformed with a second plasmid
333 containing an epitope tagged version of *CgABF1* (Fig. 4). This suggests that
334 *CgABF1* might function in similar way to *S. cerevisiae,* and therefore have a role in
335 the regulation of many important cellular processes.

336 In our experiments, we also observed that overexpression of *CgABF1* with an
337 inducible promoter (P_{MT1},), it strongly inhibits normal cell growth of both, the parental
338 and the *abf1-43* mutant (Table 1 and Fig. 5). When the promoter is not induced, *i.e.*
339 in rich media (YPD) without added copper there is also a slight increase in the
340 doubling time in the parental and in the *abf1-43*. In *S. cerevisiae* cells that
341 overexpress *ABF1*, contain twice the amount of DNA, which is indicative of a G2-M
342 phase arrest of the cell cycle (Stevenson et al., 2001). We have not yet tested

343 whether *C. glabrata* is also arrested at this point of the cell cycle when *CgABF1* is
344 overexpressed but we are currently addressing this question.

345 On the other hand, we observed that the absence of the C-terminal domain
346 of Abf1 (*abf1-43* allele) decreases growth rate significantly in different media (Figure
347 3A). This indicates that *Cg*Abf1-43 is not completely functional, as shown also by the
348 fact that growth at high temperatures is impaired, although it maintains cell viability
349 at 30°C (Figure 3B). The C-terminal domain of *Sc*Abf1 is important for its activity as
350 transcriptional regulator of genes involved in diverse cellular processes such as
351 nitrogen and carbon utilization, all of which require a functional *Sc*Abf1 (Miyake et
352 al., 2002; Kovari et al., 1993; Planta et al., 1995; Della Seta et al., 1990; Chen et al.,
353 1988). It is possible that *Cg*Abf1-43 allele is also defective in the regulation of
354 expression of genes involved in assimilation of different nutrients and/or adaptation
355 at higher temperatures, in comparison with the null-mutant is able to support cell
356 viability. One possibility for this could be that the *CgAbf-43* allele conserved other
357 domains that allows to mediated partially some activities like transcription activation
358 and repression, also replication.

359 **6.2. C-terminal of Abf1 contributes to subtelomeric silencing**

360 In this work we describe for the first time that *Cg*Abf1 is involved in
361 subtelomeric silencing in *Candida glabrata* (Fig. 6) as well as other, essential
362 funcitons. We found that the last 43 aa of the C-terminus (437-479) are required for
363 silencing function of Abf1 at two different subtelomeric regions. *Cg*Abf1 has a modest
364 contribution to subtelomeric silencing because this effect is only visible at relatively
365 long distances (>10 kb) from each telomere. We propose that this is due to the fact
366 that silencing propagating from the telomeres in *C. glabrata* is strong, and a relatively

367 small contribution by Abf1 is masked by the strong silencing at distances shorter
368 than 10 kb. Interestingly, at telomere I_{-R} where silencing propagates over 23 kb from
369 the telomere, the effect in silencing of the *URA3* reporter by *Cg*Abf1-43 is not
370 observed. This is probably because silencing at this telomere is even stronger than
371 at other telomeres since a stem and loop structure may be formed, as *EPA4* and
372 *EPA5* form an almost perfect inverted repeat, contributing to a strong silencing at
373 this telomere. This would mask the possible contribution of *Cg*Abf1 silencing at this
374 telomere (Fig. 6E).

375 It is possible that *Cg*Abf1 could be recruited at some specific *cis*-acting
376 elements to establish boundaries between regions of silent and permissive
377 chromatin regions by recruiting other *trans*-acting elements, such as Sir3 and Sir4
378 and in this way spreading the silencing as has been proposed for *S. cerevisiae* (Fox
379 and McConnell, 2005; Fourel et al., 2002; Sekinger and Gross, 2001).

380 In *C. glabrata*, we have described the *cis*-acting element Sil2126, which is a
381 protosilencer that can mediate silencing of a reporter when inserted 31.9 Kb from
382 the telomere E_{-R}. We defined two essential regions for Sil2126 activity, the first region
383 (Region 1), contains one putative binding site for Abf1 (Juárez-Reyes et al. 2012).
384 We found that Sil2126 silencing activity at -32 kb depends on Abf1, more specifically,
385 on the C-terminal of the protein (Figure 6C). The C-terminus of *Cg*Abf1 could be
386 important to mediate the interaction with other proteins of the silencing machinery.
387 Protosilencers act as propagators of silencing, and silencers are composed of
388 combinations of binding sites for several proteins like Abf1, ORC (origin replication
389 complex) and Rap1, which in turn interact with Sir proteins, allowing nucleation of
390 protein complexes and the assembly of silent chromatin in a continuous manner.

391 Also, silencers and protosilencers can propagate silencing discontinuously by the
392 formation of chromatin loops between the telomere and the protosilencers nearby
393 through the interaction of different proteins that bind DNA such as Rap1, Abf1 and
394 ORC (Cockell et al., 1995; Hecht et al., 1995; Zaman et al., 2002; Fourel et al., 1999;
395 Lebrun et al., 2001).

396 We propose that the protosilencer Sil2126, which can propagate silencing that
397 comes from the telomere as far as 32 Kb, binds *Cg*Rap1, *Cg*Abf1, and possibly
398 *Cg*Rif1, and in this way interacts with other silencing proteins like the SIR complex,
399 to spread silencing. We propose that silencing could be favored by the formation of
400 a chromatin loop through the interaction between silencing proteins bound at the
401 telomere and at Sil2126 inserted 32 kb from the telomere and possibly at other *cis*-
402 acting elements in the subtelomeric region of chromosome E_{-R}, as shown in Fig. 7.
403 We are currently testing this model by first determining whether *Cg*Abf1 physically
404 interacts with *Cg*SIR complex, *Cg*Rap1 and *Cg*Rif1.

405 ## 6. Literature cited

406 Ausubel, F., R. Bent, R.E. Kingston, Moore, J.G. Seidman, J.A. Smith, and K. Struhl.
407 2001. Current protocols in molecular biology. Wiley & Sons, Inc., New York, NY.
408 Bi, X, Braunstein, M, Shei, GJ, Broach, JR. 1999. The yeast HML I silencer defines
409 a heterochromatin domain boundary by directional establishment of silencing.
410 Proc. Natl. Acad. Sci. U. S. A. 96: 11934–9.
411 Buchman, AR, Kimmerly, WJ, Rine, J, Kornberg, RD. 1988. Two DNA-Binding
412 Factors Recognize Specific Sequences at Silencers , Upstream Activating
413 Sequences , Autonomously Replicating Sequences , and Telomeres in
414 Saccharomyces cerevisiae. 8: 210–225.
415 Castaño, I, Kaur, R, Pan, S, Cregg, R, De Las Peñas, A, Guo, N, Biery, MC, Craig,
416 NL, Cormack, BP. 2003. Tn7-based genome-wide random insertional
417 mutagenesis of Candida glabrata. Genome Res. 13: 905–915.
418 Castaño, I, Pan, SJ, Zupancic, M, Hennequin, C, Dujon, B, Cormack, BP. 2005.
419 Telomere length control and transcriptional regulation of subtelomeric adhesins
420 in Candida glabrata. Mol. Microbiol. 55: 1246–1258.
421 Chen, WJ, Padmanabha, R, Glover, C. 1988. Isolation, sequencing and disruption
422 of the CKAI gene encoding the alpha subunit of yeast casein kinase II. Mol Cell
423 Bio 8: 4981–4990.
424 Cockell, M, Palladino, F, Laroche, T, Kyrion, G, Liu, C, Lustig, AJ, Gasser, SM. 1995.
425 The carboxy termini of Sir4 and RAP1 affect Sir3 localization: Evidence for a
426 multicomponent complex required for yeast telomeric silencing. J. Cell Biol. 129:
427 909–924.

428 Cormack, BP, Ghori, N, Falkow, S. 1999. An adhesin of the yeast pathogen Candida
429 glabrata mediating adherence to human epithelial cells. Science 285: 578–582.
430 Fermi, B, Bosio, MC, Dieci, G, Bioscienze, D, Area, P. 2016. Promoter architecture
431 and transcriptional regulation of Abf1-dependent ribosomal protein genes in
432 Saccharomyces cerevisiae. 1–14.
433 Fourel, G, Miyake, T, Defossez, PA, Li, R, Gilson, É. 2002. General Regulatory
434 Factors (GRFs) as genome partitioners. J. Biol. Chem. 277: 41736–41743.
435 Fourel, G, Revardel, E, Koering, CE, Gilson, Éric. 1999. Cohabitation of insulators
436 and silencing elements in yeast subtelomeric regions. EMBO J. 18: 2522–2537.
437 Fox, CA, McConnell, KH. 2005. Toward biochemical understanding of a
438 transcriptionally silenced chromosomal domain in Saccharomyces cerevisiae.
439 J. Biol. Chem. 280: 8629–8632.
440 Gallegos-García, V, Pan, SJ, Juárez-Cepeda, J, Ramírez-Zavaleta, CY, Martin-del-
441 Campo, MB, Martínez-Jiménez, V, Castaño, I, Cormack, B, Peñas, A de Las.
442 2012. A novel downstream regulatory element cooperates with the silencing
443 machinery to repress EPA1 expression in candida glabrata. Genetics 190:
444 1285–1297.
445 Gietz, RD. 2014. Yeast transformation by the LiAc/SS carrier DNA/PEG method.
446 Methods Mol. Biol. 1205: 1–12.
447 Gutiérrez-Escobedo, G, Orta-Zavalza, E, Castaño, I, De Las Peñas, A. 2013. Role
448 of glutathione in the oxidative stress response in the fungal pathogen Candida
449 glabrata. Curr. Genet. 59: 91–106.
450 Hecht, A, Laroche, T, Strahl-Bolsinger, S, Gasser, S, Grunstein, M. 1995. Histone
451 {H}3 and {H}4 {N}-termini interact with {SIR3} and {SIR4} proteins - a molecular

452 model for the formation of heterochromatine in yeast. Cell 80: 583.
453 Juárez-Reyes, A, Ramírez-Zavaleta, CY, Medina-Sánchez, L, de Las Peñas, A,
454 Castaño, I. 2012. A protosilencer of subtelomeric gene expression in Candida
455 glabrata with unique properties. Genetics 190: 101–111.
456 Kawasaki, Y, Kim, HD, Kojima, A, Seki, T, Sugino, A. 2006. Reconstitution of
457 Saccharomyces cerevisiae prereplicative complex assembly in vitro. Genes to
458 Cells 11: 745–756.
459 Kovari, LZ, Kovari, I, Cooper, TG. 1993. Participation of RAP1 protein in expression
460 of the Saccharomyces cerevisiae arginase (CAR1) gene. J. Bacteriol. 175: 941–
461 951.
462 De Las Peñas, A, Pan, SJ, Castaño, I, Alder, J, Cregg, R, Cormack, BP. 2003.
463 Virulence-related surface glycoproteins in the yeast pathogen Candida glabrata
464 are encoded in subtelomeric clusters and subject to RAP1- and SIR-dependent
465 transcriptional silencing. Genes Dev. 17: 2245–2258.
466 De Las Peñas, A, Juárez-Cepeda, J, López-Fuentes, E, Briones-Martín-del-Campo,
467 M, Gutiérrez-Escobedo, G, Castaño, I. 2015. Local and regional chromatin
468 silencing in Candida glabrata: Consequences for adhesion and the response to
469 stress. FEMS Yeast Res. 15: 1–9.
470 Lebrun, É, Revardel, E, Boscheron, C, Li, R, Gilson, E, Fourel, G. 2001.
471 Protosilencers in Saccharomyces cerevisiae subtelomeric regions. Genetics
472 158: 167–176.
473 Margueron, R, Reinberg, D. 2010. Chromatin structure and the inheritance of
474 epigenetic information. Nat. Rev. Genet. 11: 285–96.
475 Miyake, T, Loch, CM, Li, R. 2002. Identification of a Multifunctional Domain in

476 Autonomously Replicating Sequence-Binding Factor 1 Required for
477 Transcriptional Activation , DNA Replication , and Gene Silencing. 22: 505–516.
478 Miyake, T, Reese, J, Loch, CM, Auble, DT, Li, R. 2004. Genome-wide Analysis of
479 ARS (Autonomously Replicating Sequence) Binding Factor 1 (Abf1p) -
480 mediated Transcriptional Regulation in. 279: 34865–34872.
481 Planta, RJ, Goncalves, PM, Mager, WH. 1995. Global regulators of ribosome
482 biosynthesis in yeast. Biochem. Biol. 73: 825–834.
483 Probst, A V., Dunleavy, E, Almouzni, G. 2009. Epigenetic inheritance during the cell
484 cycle. Nat. Rev. Mol. Cell Biol. 10: 192–206.
485 Ramírez-Zavaleta, CY, Salas-Delgado, GE, de Las Peñas, A, Castaño, I. 2010.
486 Subtelomeric silencing of the MTL3 locus of Candida glabrata requires yKu70,
487 yKu80, and Rif1 proteins. Eukaryot. Cell 9: 1602–1611.
488 Reed, SH, Akiyama, M, Stillman, B, Friedberg, EC. 1999. Yeast autonomously
489 replicating sequence binding factor is involved in nucleotide excision repair. 4:
490 3052–3058.
491 Rine, J, Herskowitz, I. 1987. Four genes responsible for a position effect on
492 expression from HML and HMR in Saccharomyces cerevisiae. Genetics 116:
493 9–22.
494 Rosas-Hernández, LL, Juárez-Reyes, A, Arroyo-Helguera, OE, De Las Peñas, A,
495 Pan, SJ, Cormack, BP, Castaño, I. 2008. yKu70/yKu80 and Rif1 regulate
496 silencing differentially at telomeres in Candida glabrata. Eukaryot. Cell 7: 2168–
497 2178.
498 Sekinger, EA, Gross, DS. 2001. Silenced chromatin is permissive to activator binding
499 and PIC recruitment. Cell 105: 403–414.

500 Della Seta, F, Ciafré, S a, Marck, C, Santoro, B, Presutti, C, Sentenac, A, Bozzoni,
501 I. 1990. The ABF1 factor is the transcriptional activator of the L2 ribosomal
502 protein genes in \textit{Saccharomyces cerevisiae}. Mol. Cell. Biol. 10: 2437–
503 2441.
504 Sopko, R, Huang, D, Preston, N, Chua, G, Papp, B, Kafadar, K, Snyder, M, Oliver,
505 SG, Cyert, M, Hughes, TR, Boone, C, Andrews, B. 2006. Mapping pathways
506 and phenotypes by systematic gene overexpression. Mol. Cell 21: 319–330.
507 Stevenson, LF, Kennedy, BK, Harlow, E. 2001. A large-scale overexpression screen
508 in Saccharomyces cerevisiae identifies previously uncharacterized cell cycle
509 genes. 98.
510 Strahl-Bolsinger, S, Hecht, A, Luo, K, Grunstein, M. 1997. SIR2 and SIR4
511 interactions differ in core and extended telomeric heterochromatin in yeast.
512 Genes Dev. 11: 83–93.
513 Workman, JL, Kingston, RE. 1998. Alteration of Nucleosome Structure As a
514 Mechanism of Transcriptional Regulation. Annu. Rev. Biochem. 67: 545–579.
515 Yáñez-Carrillo, P, Orta-Zavalza, E, Gutiérrez-Escobedo, G, Patrón-Soberano, A, De
516 Las Peñas, A, Castaño, I. 2015. Expression vectors for C-terminal fusions with
517 fluorescent proteins and epitope tags in Candida glabrata. Fungal Genet. Biol.
518 80: 43–52.
519 Yarragudi, A, Miyake, T, Li, R, Morse, RH. 2004a. Comparison of ABF1 and RAP1
520 in Chromatin Opening and Transactivator Potentiation in the Budding Yeast
521 Saccharomyces cerevisiae. 24: 9152–9164.
522 Yarragudi, A, Miyake, T, Li, R, Morse, RH. 2004b. Comparison of ABF1 and RAP1
523 in Chromatin Opening and Transactivator Potentiation in the Budding Yeast

524 Saccharomyces cerevisiae Comparison of ABF1 and RAP1 in Chromatin
525 Opening and Transactivator Potentiation in the Budding Yeast Saccharomyces
526 cerevisiae. Mol. Cell. Biol. 24: 9152–9164.
527 Zaman, Z, Heid, C, Ptashne, M. 2002. Telomere looping permits repression “at a
528 distance” in yeast. Curr. Biol. 12: 930–933.

529 # 7. Tables, figure legends and figures

530 ## 7.1. Tables

531 **Table 1 Doubling times for mutant strains in different media.**

	Duplication time (min) [a]				
Strain	**YPD**	**CAA**	**YNB**	**YPD + NAT**	**YPD + 50µM $CuSO_4$**
Parental	**52.7±0.6**	**52.0±2.7**	**64.9±1.1**	**-**	**-**
abf1Δ/(pP$_{ABF1}$::ABF1.URA3)	**56.2±0.1**	**52.1±0.2**	**66.5±1.0**	**-**	**-**
abf1-43	**64.4±1.3**	**66.1±2.0**	**81.6±0.1**	**-**	**-**
Parental/(pP$_{MT1}$::MYC-ABF1.NAT)	**-**	**-**	**-**	**66.1±0.4**	**89.6±0.4**
abf1-43/(pP$_{ABF1}$::ABF1.URA3)	**57.06±2.7**	**-**	**-**	**-**	**55.08±0.9**
abf1-43/(pP$_{MT1}$::MYC-ABF1.NAT)	**-**	**-**	**-**	**70.6±0.6**	**106±1.5**

532 - Experiment was not performed due to the presence of nourseothricin

533 [a] The values correspond to the mean of three different biological replicates, each one with two

534 technical replicates.

535 ## 7.2. Figure legends

536 **Fig. 1 Abf1 has a role in silencing at the *C. glabrata* telomere E-$_R$.**

537 A) Schematic representation of the subtelomeric region of the telomere E-$_R$ in *C.*
538 *glabrata*. Pink bars indicate the putative binding sites for Abf1 throughout the region.
539 Bars shown above the chromosome indicate that the putative binding sites are in the
540 top strand while bars below the chromosome are in the bottom strand (analyzed with
541 JASPAR program) (Lopéz-Fuentes *et al*, in preparation) B) Schematic
542 representation of Abf1 from *S. cerevisiae* and *C. glabrata*, *Cg*Abf1 has 479 aa and
543 *Sc*Abf1 731 aa; the black bars correspond to the DNA binding domains, grey bars to
544 the CS2 domain responsible for chromatin remodeling and transcription. Below is
545 shown the 43 C-terminal amino acids that where eliminated in the *abf1-43* mutant.
546 In red are represented the last 15 aa that belong to the CS2 domain which are
547 deleted in the *abf1-43* truncation mutant, and that are identical to the orthologous
548 amino acids in *Saccharomyces cerevisiae*.

549 **Fig. 2 *ABF1* is essential for cell viability in *C. glabrata*.**

550 Strains were grown in YPD rich media (without selection) during 72 hours, diluting in
551 fresh media every 12 h. The graph shows the percentage of cells that lost the
552 plasmid in each culture after 48 h of growth without selection. Vector corresponds to
553 pGRB2.0 empty plasmid; p*ABF1* corresponds to the vector with the complete
554 *CgABF1* gene with its own promoter.

555 **Fig. 3 Abf1 has an important role during growth in different media and at high**
556 **temperature**

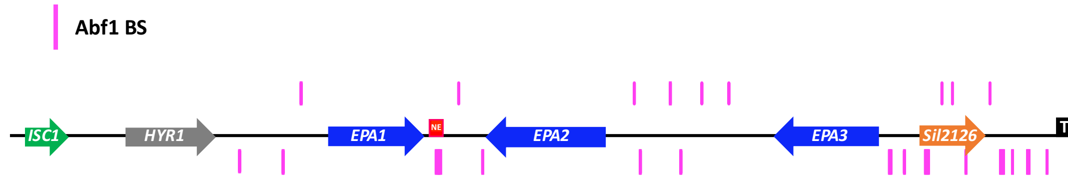
557 A) Strains were grown in three different media [rich media (YPD), minimal media
558 supplemented with casaminoacids (CAA) and minimal media (YNB) during 48 h at
559 30 ºC in a Bioscreen C apparatus. OD_{600} was recorded every 15 min. For statistical
560 analysis, One-way ANOVA and Tukey's test was performed using InStat Graph Pad
561 software (InStat Graph Pad Inc., v. 5.0. San Diego, CA, USA). Error bars represent
562 the standard deviation (SD). $p<0.05$ was considered statistically significant. B) Cells
563 from stationary phase cultures were serially diluted in sterile water to 10^{-6} and 5 µL
564 drops of each dilution was spotted on rich solid media and incubated at different
565 temperatures (30ºC, 37ºC and 45ºC) during 48 h and photographed. We compared
566 the growth of parental strain (BG14) and the *hdf1Δ* mutant as a control of a
567 temperature sensitive strain (Juaréz-Reyes et al, 2012).
568 **Fig. 4 *ABF1* mutation is complemented with cMyc-Abf1 version**
569 A) Representation of plasmid shuffling between *ABF1* full-length and the amino-
570 terminal tagged version of *ABF1* in the *abf1Δ* background. B) Silencing in the *abf1-*
571 *43* mutant at a distance of 32 Kb from the telomere $E\text{-}_R$ in *C. glabrata* is restored
572 when an N-terminal tagged version of *ABF1* is reintroduced in a plasmid. Expression
573 of the tagged version of *ABF1* is under the P_{MT1} inducible with copper.
574 **Fig. 5 Overexpression of *ABF1* is toxic in *Candida glabrata***
575 Cells from the indicated strains were grown to stationary phase in rich media (YPD)
576 and diluted to an OD_{600} 0.01. Solid color bars correspond to strains that grew on
577 YPD during the experiment. Empty color bars correspond to growth in YPD + 50 µM
578 $CuSO_4$ to induce the P_{MT1} promoter. Plasmid pMyc-*ABF1* contains the full length
579 *ABF1* tagged with cMyc at the N-terminus under the inducible *MT1* promoter.

580 Plasmid p*ABF1* contains the full length of *ABF1* under its own promoter. For
581 statistical analysis, One-way ANOVA and Tukey's test was performed using InStat
582 Graph Pad software (InStat Graph Pad Inc., v. 5.0. San Diego, CA, USA). Error bars
583 represent the standard deviation (SD). $p<0.05$ was considered statistically significant
584 **Fig. 6 Abf1 has a role in silencing at different subtelomeric regions in *C.***
585 ***glabrata*.**
586 A) Schematic representation of the subtelomeric region of the Chr E_{-R} in *C. glabrata*.
587 The red triangles represent the position of three independent insertions of the *URA3*
588 reporter. The numbers correspond to the insertions in the assay below. Ten-fold
589 dilutions of cells grown to stationary phase were spotted onto the media indicated,
590 incubated at 30 °C for 48 h and photographed. B) and C) *abf1-43* mutant has an
591 effect in silencing mediated by the protosilencer Sil2126 at a distance of 31.9 Kb.
592 Cells of the indicated strains were diluted, spotted, incubated and photographed as
593 described in Fig. 5A. D) Schematic representation of subtelomeric region of
594 chromosome B_{-L}. Green arrows represent the alpha1 and alpha3 genes and their
595 orientation at the subtelomeric region of the Chr B_{-L}. Red triangles represent the
596 insertions of the *URA3* reporter. E) Schematic representation of the subtelomeric
597 region of Chr I_{-R}. Blue arrows represent *EPA4* and *EPA5* genes and their relative
598 orientation at the Chr I_{-R}. Red triangles represent the insertions of *URA3* reporter.
599 **Fig. 7 Model for telomere loop formation at the E_{-R} telomere in *C. glabrata***
600 In this model we propose that Abf1 and Rap1 recognize *cis*-acting sequences, like
601 Sil2126 and others at the intergenic regions at this telomere, and in this way recruit
602 SIR complex, spreading the silencing by the interaction of this proteins and possibly
603 assemblying a loop.

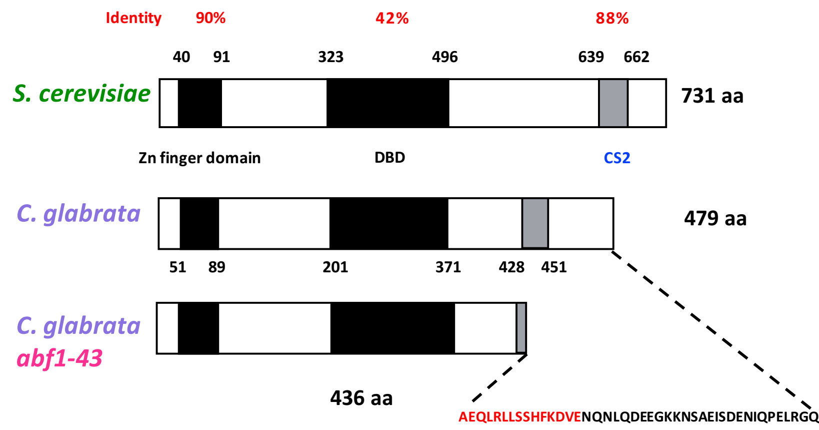
604 ## 7.3. Figures

605 **Figure 1**

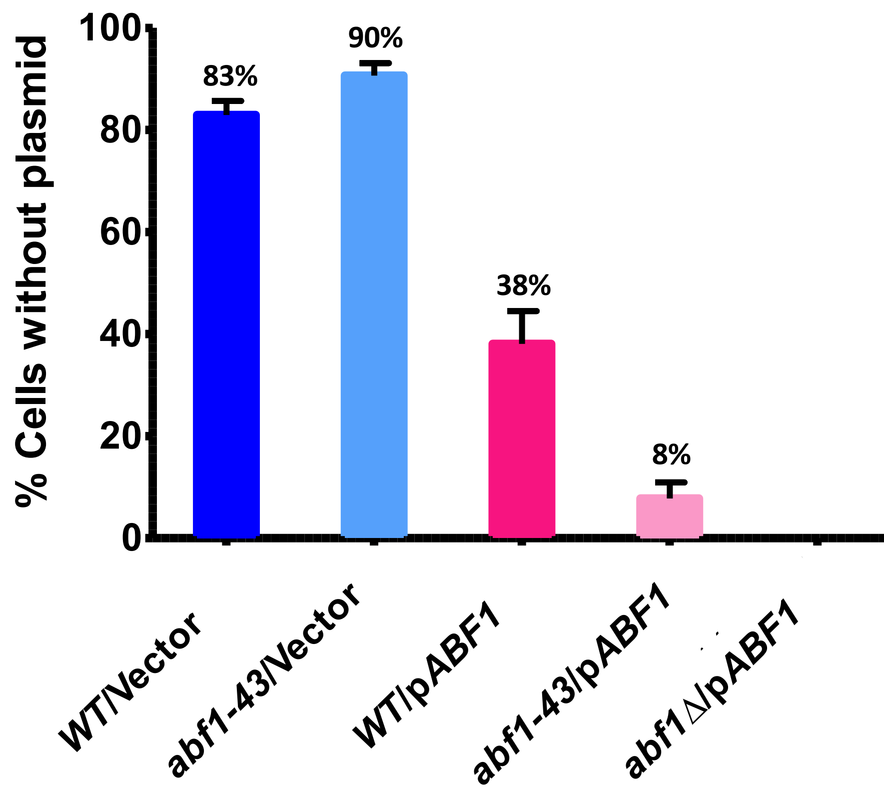
A)



B)

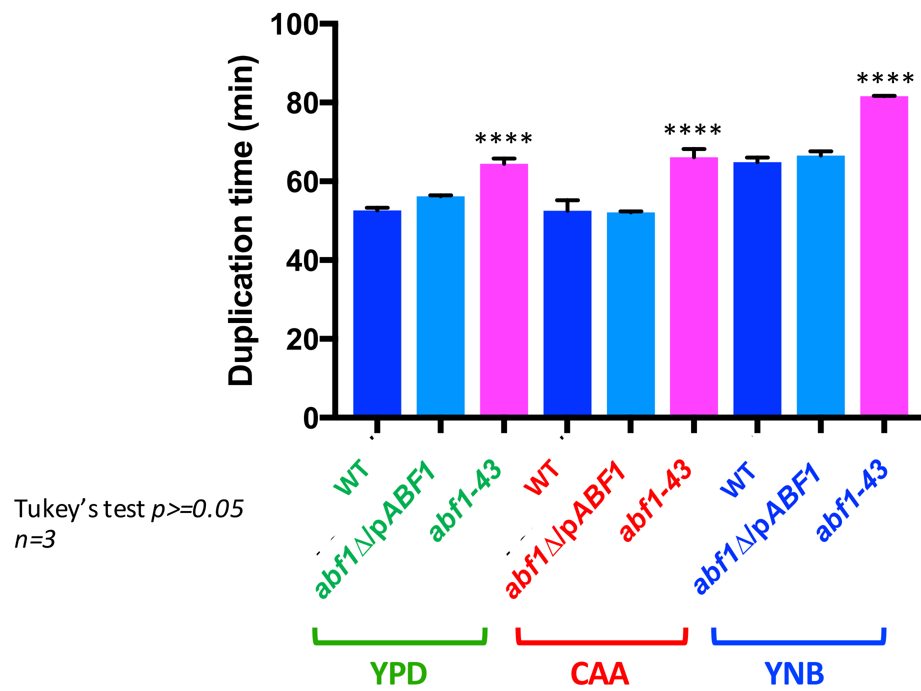


606 **Figure 2**



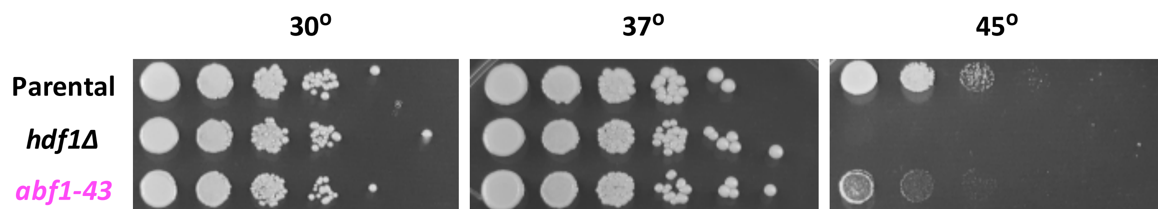
607 **Figure 3**

A)



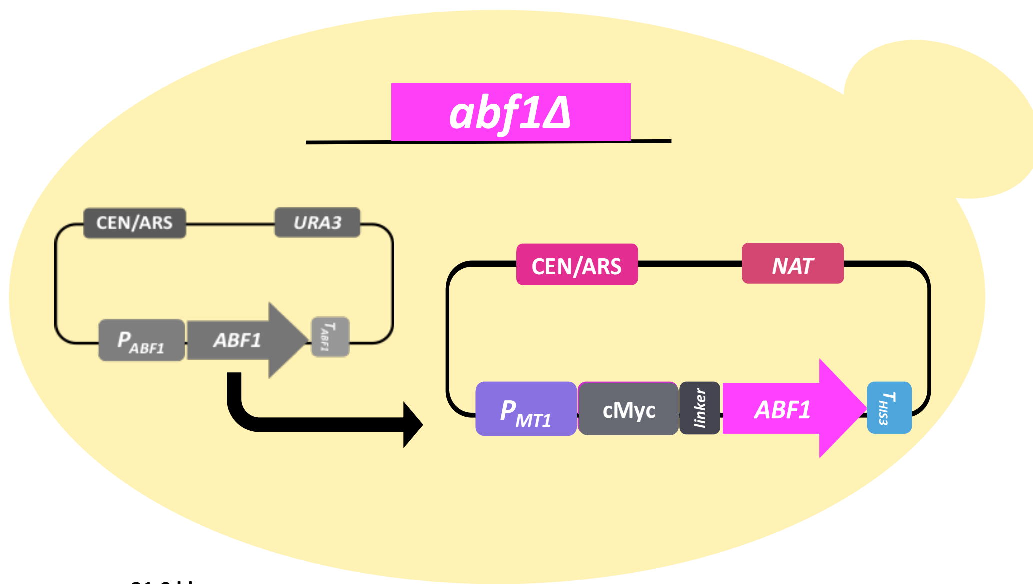
Tukey's test *p>=0.05*
n=3

B)

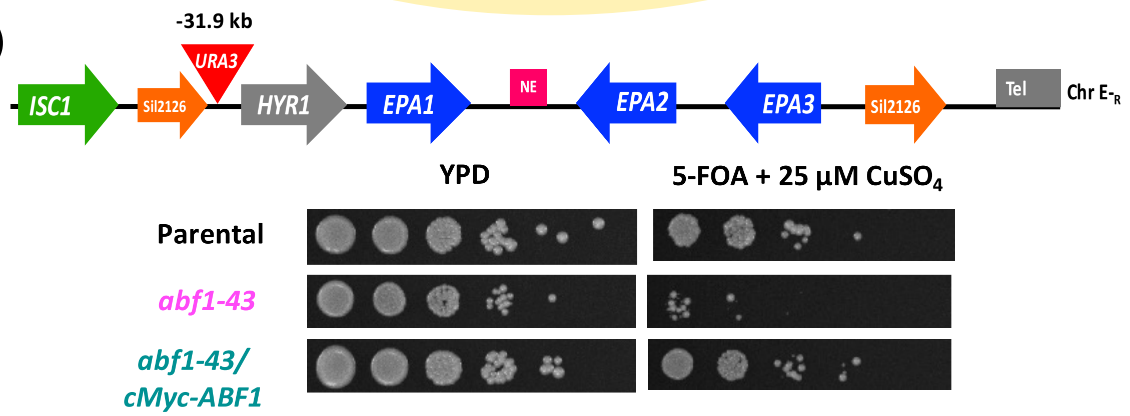


608 **Figure 4**

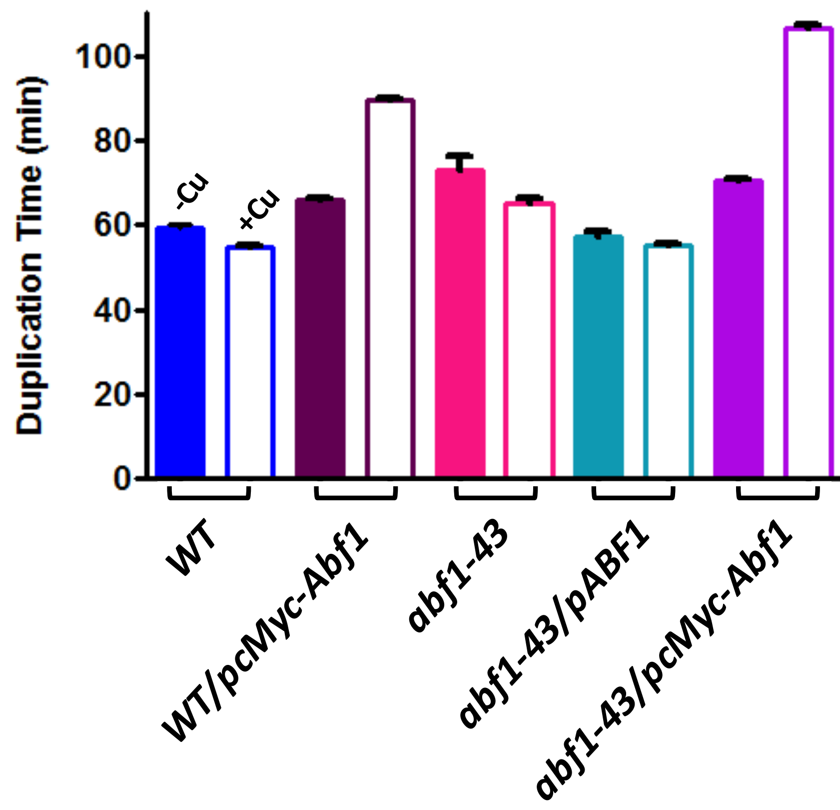
A)



B)



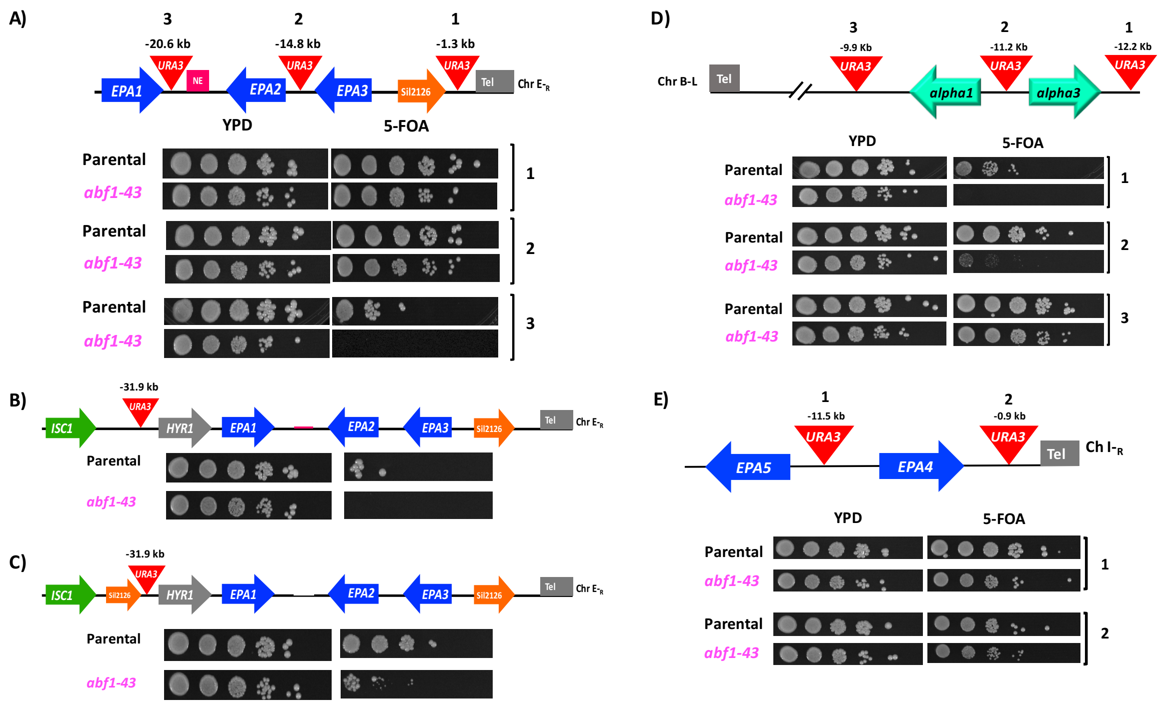
609 **Figure 5**



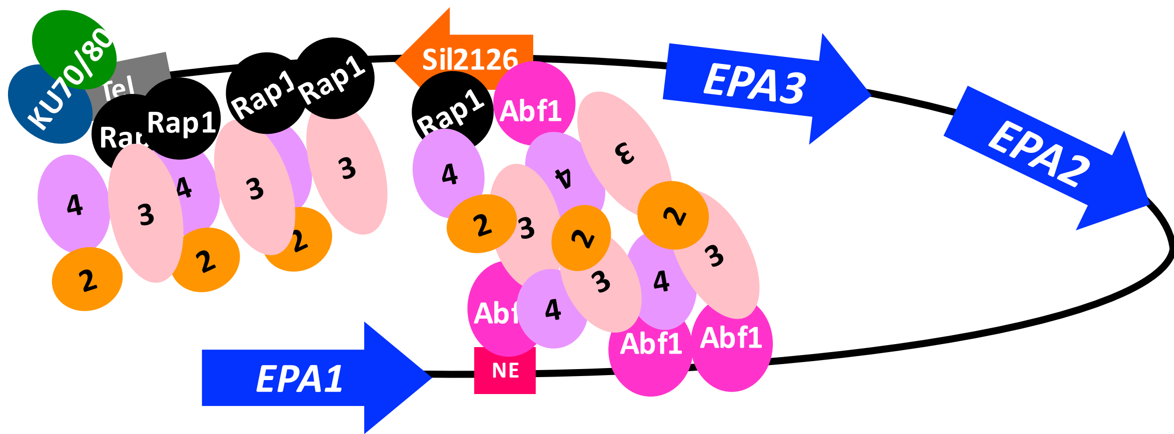
Tukey's test *p>=0.05*
n=3

610

Figure 6



611 **Figure 7**



612 # 8. Supplementary Data

613 ## 8.1. Expression of P_{EPA1} that requires the negative element (NE) is not
614 affected by *ABF1*

615 In *C. glabrata,* adherence is mediated primarily by Epa1 adhesin. *EPA1* transcription
616 is regulated by two different mechanisms: 1) subtelomeric silencing that requires SIR
617 complex, Rap1, Rif1, yKu70 and yKu80; 2) repression immediately after lag phase
618 that involves a *cis*-acting regulatory negative element (NE) located at the 3'
619 intergenic region of *EPA1*, which requires yKu70 and yKu80 and is independent from
620 subtelomeric silencing (Gallegos-García *et al*., 2012). Bioinformatic analysis showed
621 that there are putative binding sites for Abf1 in the NE, therefore we tested if Abf1
622 participates in this repression mechanism by using a transcriptional fusion of the
623 *EPA1* promoter with GFP and followed the promoter activity by flow cytometry
624 (FACS).

625 We used a *URA3* CEN-ARS plasmid (pGBR2.0) that has the P_{EPA1}::GFP
626 transcriptional fusion followed by the entire 3.1 kb *EPA1-EPA2* intergenic region that
627 contains the NE (pAP385). As a control we used a plasmid where we replaced this
628 intergenic region with the *HIS3* 3'UTR (pAP354) (Gallegos-García *et al.*, 2012). We
629 transformed these plasmids in the parental strain (WT) and in the *abf1-43* mutant.
630 GFP fluorescence is measured in cells in stationary phase (SP) and upon dilution
631 into fresh media by flow cytometry (FACS). We observed that expression is highly
632 induced immediately after dilution in fresh media in all the backgrounds as previously
633 described, and in the presence of the NE (the intergenic region) GFP fluorescence
634 rapidly decreases, but not in its absence. This regulation however does not depend

635 on Abf1 since in the truncated mutant strain (*abf1-43*), the same promoter activity
636 was detected (Figure S2).
637 We also measured silencing of the *EPA1* promoter by constructing reporter strain in
638 which we replaced the *EPA1* ORF in its chromosomal position with the *URA3* ORF.
639 Separately, a fragment about 1.0 Kb that carries the NE from *EPA1-EPA2* intergenic
640 region with a 1.2 Kb fragment containing the chloramphenicol acetyl transferase
641 gene (*cat*) from a bacterial plasmid that allows us to maintain the same distance at
642 this region (Gallegos-García *et al*., 2012). In these strains we replaced the *ABF1*
643 with the truncated version (*abf1-43*) to assess the impact in silencing. These
644 constructs enable the assessment of silencing of the *EPA1* locus as well as the
645 impact of the NE on silencing at this region.
646 The strains were grown in YPD media and spotted onto YPD (viable count), SC-ura
647 (to assess activity of the *EPA1* promoter) and 5-FOA (to assess silencing of the
648 P_{EPA1}::*URA3* locus). Suppl. Fig. 2 shows that the strain with the NE grows poorly on
649 SC-ura media, and that this depends on the presence of the NE, in agreement with
650 the repressor role of the NE on the expression of *EPA1* (Gallegos-García *et al*.,
651 2012). However, the presence of the *abf1-43* mutation does not affect the negative
652 regulation of *EPA1* promoter by the NE.

653 ## 9. Supplementary tables

654 **Table S 1 *C. glabrata* and *E. coli* strains used in this study.**

***E. coli* strain**	**Use**	**Genotype**	**Reference**
DH10B	Electrocompetent cells	F⁻ *mcrA* Δ(*mrr-hsdRMS-mcrBC*) 80d*lacZ*Δ*M15* Δ*lacX74 deoR recA1 endA1 araD139* Δ(*ara,leu*)7697 *galU galK* ⁻ *rpsL nupG*	Calvin and Hanawalt 1988
***C. glabrata* strains**	**Parental**	**Genotype**	**Reference**
BG14	BG2	*ura3*Δ::Tn*903* G418^R	Cormack and Falkow 1999
abf1Δ* and *abf1-43			
CGM2746	BG14	*abf1Δ::NAT (*pCI45 integrated*)* pCI12 (pP_{ABF1}::*ABF1. URA3)*	This work
CGM3068	BG14	*abf1-43::NAT (*pCI32 integrated *BsgI)*	This work
CGM3113	CGM3068	*abf1-43::FRT* (pCI32 integrated *Bsg*I)	This work
***URA3* reporter gene integrated at *EPA1* locus**			
CGM147	BG14	*ura3*Δ::Tn*903* G418^R Tn*7* at intergenic region between *EPA1* and *EPA2* (pAP508 *Spe*I/*Bcg*I). Insertion 1	De Las Peñas *et al.* 2003
CGM148	BG14	*ura3*Δ::Tn*903* G418^R Tn*7* at intergenic region between *EP A2* and *EP A3* (pAP559 *Bsr*GI/*Sph*I). Insertion 2	De Las Peñas *et al.* 2003

CGM149	BG14	*ura3*Δ::Tn*903* G418^{R} Tn7 at intergenic region between *EPA3* and telomere (pAP553 *Pst*I/*Eco*RI). Insertion 3	De Las Peñas *et al.* 2003
BG1124	BG1212	*ura3*Δ::Tn*903* G418^{R} *epa1Δ::URA3. EPA1* replaced by *URA3*. (P_{EPA1}*::URA3)*	Gallegos-García et al, 2012
BG1132	BG14	*ura3*Δ::Tn*903* G418^{R} *epa1Δ::URA3. neΔ::cat. EPA1* replaced by *URA3* and NE (negative element) replaced by the bacterial *cat* gene, chloramphenicol acetyl transferase from pACYC184. *(*P_{EPA1}*::URA3 neΔ::cat)*	Gallegos-García et al, 2012
***URA3* reporter gene integrated at *EPA4* and *EPA5* locus**			
CGM159	BG14	*ura3*Δ::Tn*903* G418^{R} Tn7 at intergenic region between *EPA5* and *EPA4* (pAP534/*BcgI*)	De Las Peñas et al, 2003
CGM160	BG14	*ura3*Δ::Tn*903* G418^{R} Tn7 at unique region between *EPA5* and *EPA4* (pAP534 *Bcg*I) Insertion 4	De Las Peñas et al, 2003
Sil2126-*URA3* reporter system at -31.9 kb			
CGM399	BG14	*ura3*Δ::Tn*903* G418^{R} pAP509/*Spe*I integrated between *ISC1* and *HYR1*.	Rosas-Hernández et al. 2008
CGM397	BG14	*ura3*Δ::Tn*903* G418^{R} (pAP430/*Spe*I) integrated in the chromosome	Rosas-Hernández et al. 2008
***URA3* reporter gene integrated at the *MTL3* locus**			
CGM454	BG14	*ura3*Δ::Tn*903* G418^{R}	Ramirez-Zavaleta et al. 2010

		Tn7 at 643 bp downstream from alpha1 stop codon (pRZ36/*Spe*I) Insertion 13	
CGM458	BG14	*ura3*Δ::Tn*903* G418^R Tn7 at 166 bp upstream from alpha1 start codon, between alpha1 and alpha2 (pRZ32/*Bcg*I). Insertion 17	Ramirez-Zavaleta et al. 2010
CGM697	BG14	*ura3*Δ::Tn*903* G418^R Tn7 at 131 bp downstream from alpha2 stop codon between alpha2 and *CHAI* (pRZ40/*Bcg*I) Insertion 22	Ramirez-Zavaleta et al. 2010
***GFP* reporter strains**			
BG198	BG14	*ura3*Δ::Tn*903* G418^R *epa1Δ::GFP* *GFP under the control of the EPA1 promoter.* *pAP353*	García-Gallegos et al, 2012
BG201	BG14	*pAP354* *(P_{EPA}::GFP::3'UTR $_{HIS3}$)*	García-Gallegos et al, 2012
CGM2287	BG14	*pAP385* *(P_{EPA1}::GFP::3'UTR $_{EPA1\ NE}$)*	García-Gallegos et al, 2012
CGM2717	CGM520	*pAP353 (GFP::3'UTR $_{HIS3}$)* *abf1-43::NAT* (pCI32/*BsgI integrated)*	This work
CGM2719	CGM522	*pAP354* *(P_{EPA}::GFP::3'UTR $_{HIS3}$)* *abf1-43::NAT* (pCI32/*BsgI integrated)*	This work
CGM2721	CGM2287	*pAP385* *(P_{EPA1}::GFP::3'UTR $_{EPA1\ NE}$)* *abf1-43::NAT* (pCI32/*BsgI integrated)*	This work
***abf1-43* derivatives in *URA3* reporter strains background**			
CGM2485	CGM147	*ura3*Δ::Tn*903* G418^R Tn7 at intergenic region between *EPA1* and *EPA2*. *abf1-43*::*NAT* (pCI32/*BsgI* integrated)	This work

CGM2488	CGM148	*ura3*Δ::Tn*903* G418^R Tn7 at intergenic region between *EPA2* and *EPA3. abf1-43*::*NAT* (pCI32/*BsgI* integrated)	This work
CGM2491	CGM149	*ura3*Δ::Tn*903* G418^R Tn7 at intergenic region between *EPA3* and telomere. *abf1-43*::*NAT* (pCI32/*BsgI* integrated)	This work
CGM3167	CGM159	*ura3*Δ*::*Tn*903* G418^R Tn7 at intergenic region between *EPA5* and *EPA4*. *abf1-43::NAT* (pCI32/*BsgI* integrated)	This work
CGM3150	CGM160	*ura3*Δ*::*Tn*903* G418^R Tn7 at unique region between *EPA5* and *EPA4*. *abf1-43::NAT* (pCI32/*BsgI* integrated)	This work
CGM3152	CGM399	*ura3*Δ::Tn*903* G418^R pAP509/*Spe*I integrated between *ISC1* and *HYR1*. *abf1-43::NAT* (pCI32/*BsgI* integrated)	This work
CGM3151	CGM397	*ura3*Δ::Tn*903* G418^R (pAP430/*Spe*I) integrated in the chromosome. *abf1-43::NAT* (pCI32/*BsgI* integrated)	This work
CGM3168	CGM697	*ura3*Δ::Tn*903* G418^R Tn7 at 131 bp downstream from alpha2 stop codon between alpha2 and *CHAI* (pRZ40/BcgI). *abf1-43::NAT* (pCI32/*BsgI* integrated)	This work
CGM3069	CGM458	*ura3*Δ::Tn*903* G418^R Tn7 at 166 bp upstream from alpha1 start codon,	This work

		between alpha1 and alpha2 (pRZ32/BcgI). *abf1-43::NAT* (pCI32/*BsgI* integrated)	
CGM3180	CGM454	*ura3*Δ::Tn*903* G418^R Tn7 at 643 bp downstream from alpha1 stop codon (pRZ36/SpeI) *abf1-43::NAT* (pCI32/*BsgI* integrated)	This work
CGM3259	BG1124	*ura3*Δ::Tn*903* G418^R *epa1Δ::URA3. EPA1* replaced by *URA3*. *abf1-43::NAT (*pCI32*/BsgI* integrated*)*	This work
CGM3261	BG1132	*ura3*Δ::Tn*903* G418^R *epa1Δ::URA3. neΔ::cat. EPA1* replaced by *URA3* and NE (negative element) replaced by the bacterial *cat* gene. *abf1-43::NAT* (pCI32/*BsgI* integrated)	This work
p*P$_{ABF1}$::ABF1* and pP$_{MT1}$*::MYC::ABF1*			
CGM2391	BG14	*ura3*Δ::Tn*903* G418^R pCI12 (p*P$_{ABF1}$::ABF1. URA3)*	This work
CGM3123	CGM3113	*ura3*Δ::Tn*903* G418^R *abf1-43::FRT* (pCI32/ *Bsg*I integrated) pCI12 (p*P$_{ABF1}$::ABF1. URA3)*	This work
CGM3125	CGM3113	*ura3*Δ::Tn*903* G418^R *abf1-43::FRT* (pCI32/ *Bsg*I integrated) pGBR2.0 *URA3*	This work
CGM3453	BG14	*ura3*Δ::Tn*903* G418^R pGH8 (pP$_{MT1}$*::MYC::ABF1)*	This work
CGM3455	CGM3113	*ura3*Δ::Tn*903* G418^R *abf1-43::FRT* (pCI32/ *BsgI* integrated) pGH8 (pP$_{MT1}$*::MYC::ABF1)*	This work
CGM3457	CGM1107	*ura3*Δ::Tn*903* G418^R pJV22/BsrGI-HpaI (*SIR3*::FLAG::FRT)	This work

		pGH8 (pP$_{MT1}$*::MYC::ABF1)*	
CGM3459	CGM1113	*ura3*Δ::Tn*903* G418^R pJV13/BglII-BcgI (*SIR4*::FLAG::FRT) pGH8 (pP$_{MT1}$*::MYC::ABF1)*	This work
CGM3508	CGM1313	*ura3*Δ::Tn*903* G418^R pGH8 (pP$_{MT1}$*::MYC::ABF1)*	This work
CGM3510	CGM1307	*ura3*Δ::Tn*903* G418^R pGH8 (pP$_{MT1}$*::MYC::ABF1)*	This work
CGM3530	CGM3455	*ura3*Δ::Tn*903* G418^R *abf1-43::FRT* (pCI32/ *BsgI* integrated) pGH8 (pP$_{MT1}$*::MYC::ABF1)* pAP430/*SpeI* integrated between *ISC1* and *HYR1*	This work
CGM3532	CGM3455	*ura3*Δ::Tn*903* G418^R *abf1-43::FRT* (pCI32/ *BsgI* integrated) pGH8 (pP$_{MT1}$*::MYC::ABF1)* pAP509/*SpeI* integrated between *ISC1* and *HYR1*	This work
CGM3221	CGM3113	*abf1-43::FRT* (pCI32/ *BsgI* integrated) pBC34.1 *integrated URA3*	This work
CGM3584	CGM2746	*abf1Δ::FRT ura*$^+$ *NAT*^{s} pCI12 (pP$_{ABF1}$::*ABF1*)	This work
CGM3588	CGM2746	*abf1Δ::FRT* pGH8(pP$_{MT1}$::*MYC::ABF1)*	This work
CGM3594	CGM3584	*abf1Δ::FRT ura*$^+$ *NAT*^{R} pCI12 (pP$_{ABF1}$::*ABF1*)/ pGH8(pP$_{MT1}$::*MYC::ABF1)*	This work

655

656 **Table S 2 Plasmids used in this study.**

Plasmid	Relevant genotype	Reference
Cloning vectors		
pGRB2.0	Cloning replicative vector *URA3* Ap^R pRS406::*C.g. CEN ARS*	Zordan et al. 2013
pMB11	Cloning vector with an *StuI* restriction site added Cm^R Sac^S	Lab collection
pCYC184	Cloning vector Cm^R Tc^R	Chang and Cohen 1978
Replicative and epitope-tagging vectors		
pRS306	Integrative vector Amp^R *URA3*$^+$	Sikoski et al, 1989
pCN-*MET3*	*MET3*pr empty vector Amp^R, *NAT*^{R}	Zordan et al, 2013
pCU-*MET3*	*MET3*pr empty vector Amp^R, *URA3*$^+$	Zordan et al, 2013
pAP599	Cloning, integrative vector with two FRT direct repeats flanking a hygromycin resistance cassette (FRT-P_{PGK1}::*hph::3'*UTR_{HIS3}-FRT) for construction of multiple round of knock-out mutants, Amp^R, Hyg^R, *URA3*$^+$	Domergue et al, 2005
pMZ18	Replicative vector expressing *ScFLP1* (recombinase gene) for removing markers, P_{EPA1}::*FLP1::(3'*UTR_{HIS3}*)Cg CEN ARS*, Amp^R, *URA3*$^+$	Cormack lab collection
pOZ12	A 0.34 Kb fragment (containing *BamHI/BglII* sites) corresponding to the 3'UTR of the *CTA1* gene of *C. glabrata*, with a FRT sequence cloned into a *BamHI/BglII* digested pGEM vector β-$lactam^R$)	Orta-Zavalza et al, 2013
pYC10	pCR-TOPO-NAT (flanked by two FRTs) digested with *SacI/SpeI* and filled with T4 DNA Pol Amp^R	Yáñez-Carrillo et al, 2015
pYC14	pYC10 with a *BamHI* resctriction site removed and filled with T4 DNA Pol Amp^R	Yáñez-Carrillo et al, 2015
pYC22	pYC14 with a *SalI* restriction site removed and filled with T4 DNA Pol Amp^R	Yáñez-Carrillo et al, 2015
pYC23	pYC22 digested with *XhoI* and containing the promoter and 3'UTR of the *TEF* gene of *Ashbya gossypii*. Vector backbone for the amplification of the *NAT* gene,	Yáñez-Carrillo et al, 2015

	(FRT::P_{TEF}::NAT::3'UTR$_{TEF}$::FRT) Amp^R NAT^R	
pYC40	A 1.2 Kb PCR product amplified from pYC23 and digested with *BamHI/XhoI*, cloned into the *BamHI/XhoI* digested pRS306 vector Amp^R NAT^R	Yáñez-Carrillo et al, 2015
pYC44	A 0.34 Kb PCR product, amplified from pOZ12, corresponding to the 3'UTR of the *CTA1* gene, flanked by a FRT site, and digested with *BamHI/BglII*, cloned into pYC40 modified vector, wich has an additional FRT site (FRT::*NAT*::3'UTR$_{CTA1}$::FRT) Amp^R	Yañez-Carrillo et al, 2015
pVA59	Replicative vector c-Myc (amino terminal) pCU-*MET3*::c-Myc-linker Amp^R, *URA3*$^+$	Vidal-Aguiar et al, unpublished
pVA106	P_{MT1} empty vector Amp^R, NAT^R pCN::P_{MT1}	Vidal-Aguiar et al, unpublished
pCI1	A 3.07 Kb PCR product corresponding to the full length *ABF1* gene, cloned into the *StuI* digested pMB11 vector	Castañedo-Ibarra et al, unpublished
pCI12	Full length *ABF1* gene released from pCI1 with *BamHI/SacI*, and cloned into the replicative pGBR2.O vector	Castañedo-Ibarra et al, unpublished
pCI30	A 0.94 Kb PCR product corresponding to the *abf1-43* truncated allele and digested with *BamHI/SacI*, cloned into pYC44 vector	Castañedo-Ibarra et al, unpublished
pCI32	3'UTR of *ABF1* released from pCI9 with *KpnI/XhoI*, cloned into pCI30.	Castañedo-Ibarra et al, unpublished
pCI37	A 1.2 Kb of the 5'UTR of *ABF1*, cloned into the *StuI* digested pMB11 vector.	Castañedo-Ibarra et al, unpublished
pCI42	5'UTR of *ABF1* released with *BamHI/SacI* from pCI37, and cloned into pYC44 digested with *BamHI/SacI.*	Castañedo-Ibarra et al, unpublished
pCI45	*ABF1* deletion vector. 3'UTR of *ABF1* released from pCI9 with *XhoI/KpnI*, and cloned into pCI42 digested with *XhoI/KpnI.*	Castañedo-Ibarra et al, unpublished
pGH3	A 570 bp fragment of cMyc-linker released from pVA59 with *SpeI/ClaI*, and cloned into pVA106 digested *SpeI/ClaI.*	This work
pGH5	A 1.440 Kb PCR product of *ABF1* with *ClaI* sites added, and cloned into pMB11 digested with *StuI.*	This work

pGH8	A 1.44 Kb fragment of *ABF1* released from pGH5 with *ClaI*, and cloned into pGH3 digested with *ClaI*.	This work
pGH9	A 2.1 Kb fragment of *hph* cassette released from pAP599 with *XbaI* and treated with T4 DNA Pol, and cloned into pMZ18 digested with *StuI/SnaBI.*	This work

657

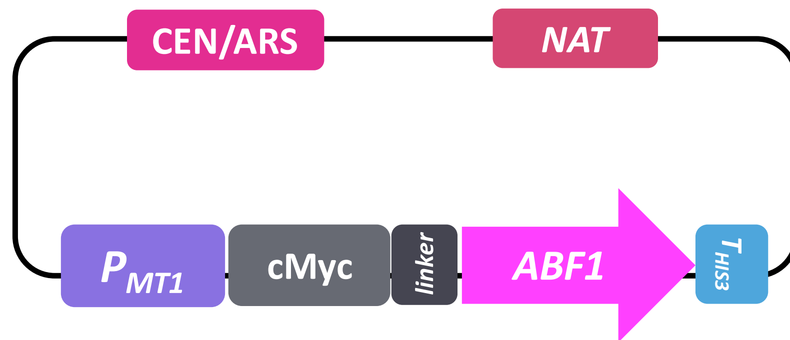
658 **Table S 3 Oligonucleotides used in this study.**

Primer (No.)	Sequence (5'-3')	Site(s) added	Hybridization site (*ABF1*)
2353	TCT**ATCGAT**AAAATGGATTGACGGTATGATTTCTG	*Cla*I	@1 Fw
2354	TCT**ATCGAT**TTATTGTCCTCTTAATTCAGG	*Cla*I	@1440 Rv
1558	GCTACTGCGATTTGCCACTG	None	@-91 Fw
1559	GTT**GAGCTC**TTGTGCAGACGATCCGCAGGTCACCGC	SacI	@385 Fw
1561	CTT**CTCGAG**GCTCCAATTATTAAAATGAATAAAAGG	*Xho*I	@+13 Fw
1562	CTT**GGTACC**TT**GTGCAG**TGCCGCCAACTTAAGCATA	*Kpn*I, *Bsg*I	@+755 Rv
1563	TGTATTCG**GGTACC** GCTAATTCCAG	*Kpn*I	@+933 Rv
1589	CTT**GAGCTC**GATTGTTGTGTAGGCAATATCATAGC	*Sac*I	@-1240 Fw
1590	CTT**GGATCC**CCGAACATTTGGTCAGATCACTG	*BamH*I	@+712 Rv
1834	GGGCCCGCTCCAATTATTAAAATGAATAAAGG	None	@+13 Fw
1835	CTCTGACTCCTCAATCCTTAACC	None	@+1015 Rv
1880	GTT**GGATCC**TTAGACTTCACGAGGAAGCTTGTCGTCGG	*BamH*I	@1308 Rv
1881	CTT**GGATCC**CGTTGTTTGTGTTCTCGTTGG	*BamH*I	@-1 Rv
1884	AGTGCACTTATCCTCCATCC	None	@-2134 Fw
1885	GGATCCACTAGTTCTAGAGCGGCGTTGTTTGTGTTCTCGTTGG	None	@-1 Rv
Primer (No.)	**Sequence (5'-3')**	**Site(s) added**	**Hybridization site**
569	TACAAAGCTTGTTCACCATCGGAAGC	None	Noursothricin resistance cassette Rv
1096	GCTTGCCTCGTCCCCG	None	Noursothricin resistance cassette Fw
1842	CCGCTCTAGAACTAGTGGATCC	None	Noursothricin resistance cassette Fw
1843	GGGCCCCCCCTCGAGGAC	None	Noursothricin resistance cassette Rv

659 *The restriction sites added to the primers are indicated in bold.

660

9.1. Supplementary figures



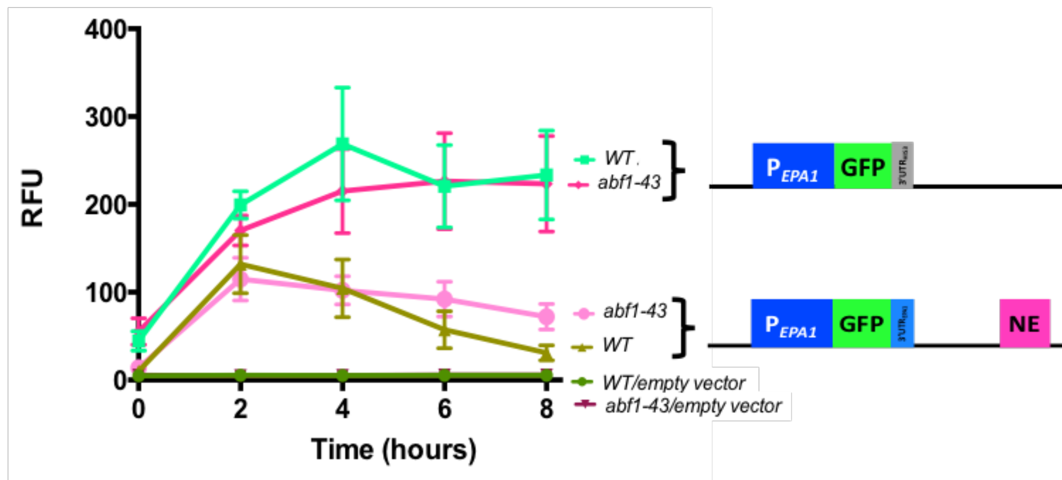
661

662 **Fig. S 1 Schematic representation of N-terminal tagged version of *ABF1*.**

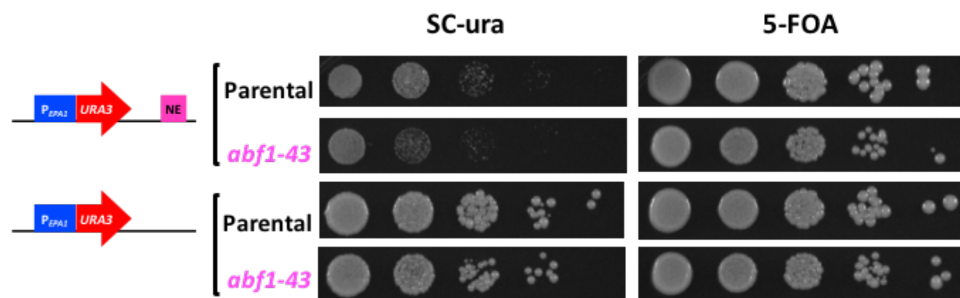
663 Replicative plasmid that contains the inducible P_{MT1} followed by a cMyc epitope

664 separated by a linker from the *ABF1* ORF.

A)



B)



665 **Fig. S 2 Abf1 does not play a detectable role in transcriptional regulation of**

666 **P_{EPA1} by the NE.**

667 A) *EPA1* promoter activity measured by flow cytometry (FACS). Strains were grown

668 for 48 hours at 30º in CAA media. Cells were diluted into fresh media and samples

669 were taken every 2 hours. Fluorescence intensity was measured using a FACS

670 machine. B) *EPA1* was replaced by the *URA3* gene and the NE was replaced by the

671 bacterial *cat* gene and recombined in the chromosome. *URA3* reflects the activity of

672 the *EPA1* promoter. The parental strains with or without the negative element carry

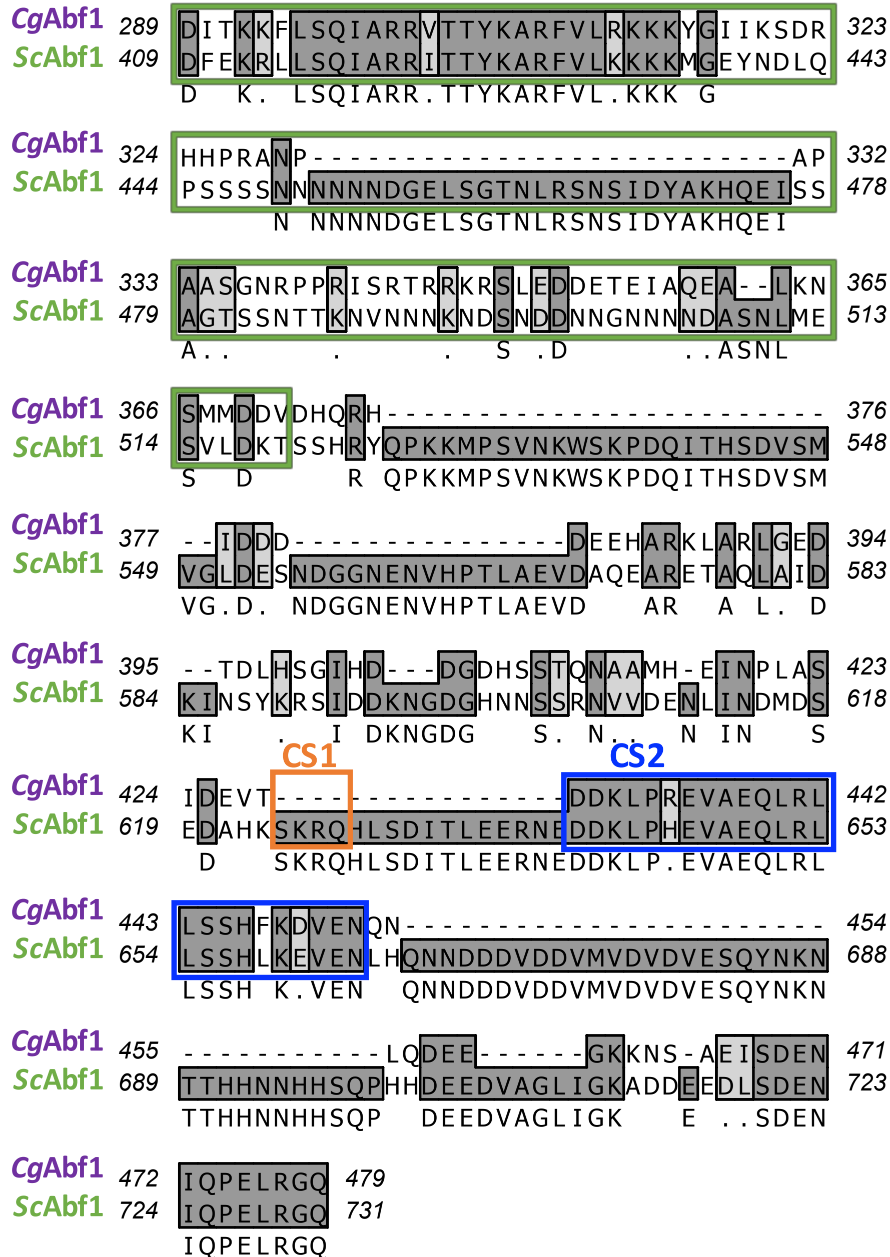
673 separately the *abf1-43* mutation. For statistical analysis, One-way ANOVA test was

674 performed using InStat Graph Pad software (InStat Graph Pad Inc., v. 5.0. San

675 Diego, CA, USA). Error bars represent the standard deviation (SD). $p<0.05$ was

676 considered statistically significant

```
                              Zn
CgAbf1    1  MDDGMISEGVKDVYEYSHPIINNALAVSASEQQGK   35
ScAbf1    1       MDKLVVNYYEYKHPIINKDLAIGAHG--GK   28
             MDDGM    .V    YEY HPIIN  LA. A   QQGK

CgAbf1   36  EVRRFATLADWYDVINDYEFQSRCPIILKNSHRNK   70
ScAbf1   29  ---KFPTLGAWYDVINEYEFQTRCPIILKNSHRNK   60
             EVR.F TL. WYDVIN.YEFQ.RCPIILKNSHRNK

CgAbf1   71  HFTFACHLKNCPFKILLSYS-----------SAG-   93
ScAbf1   61  HFTFACHLKNCPFKVLLSYAGNAASSETSSPSANN   95
             HFTFACHLKNCPFK.LLSY GNAASSETSSPSA N

CgAbf1   94  ----------MHHG---------------------   97
ScAbf1   96  NTNPPGTPDHIHHHSNNMNNEDNDNNNGSNNKVSN  130
             NTNPPGTPDH HH SNNMNNEDNDNNNGSNNKVSN

CgAbf1   98  --------------------HAQDDMYRSKDEDVD  112
ScAbf1  131  DSKLDFVTDDLEYHLANTHPDDTNDKVESRSNEVN  165
             DSKLDFVTDDLEYHLANTHP    D   S.   .V

CgAbf1  113  ALNDG------------------------------  117
ScAbf1  166  GNNDDDADANNIFKQQGVTIKNDTEDDSINKASID  200
             . ND DADANNIFKQQGVTIKNDTEDDSINKASID

CgAbf1  118  --------------AHDHKLEYHD-----------  127
ScAbf1  201  RGLDDESGPTHGNDSGNHRHNEEDDVHTQMTKNYS  235
             RGLDDESGPTHGND   H.     DDVHTQMTKNYS

CgAbf1  128  --VDDPQVTAAIAAAVAAVGKDSSDPHN-----AA  155
ScAbf1  236  DVVNDEDINVAIANAVANVDSQSNNKHDGKDDDAT  270
             DVV D   . .AIA AVA V   S   H GKDDDA

CgAbf1  156  TAAAAAAAAATNGGEDHKNLVQDTQPSAHSQAQAQ  190
ScAbf1  271  NNNDGQDNTNNNDHNNNSNINNNNVGSHGISSHSP  305
                 .      N      N.  .    S
                                DBD
CgAbf1  191  ASVRPNSN-------APETIRGPFVVTKIIPYHDH  218
ScAbf1  306  SSIRDTSMNLDVFNSATDDIPGPFVVTKIEPYHSH  340
              S.R   S NLDVFNSA .  I GPFVVTKI PYH H

CgAbf1  219  PVQDNLSLDKFVLTKIPRILQNELNFDDVLETLVA  253
ScAbf1  341  PLEDNLSLGKFILTKIPKILQNDLKFDQILESSYN  375
             P. DNLSL KF.LTKIP.ILQN.L FD .LE.

CgAbf1  254  EGASDGDVAKFRVSEYVEHSGLLDIIKARYDLMDS  288
ScAbf1  376  N--SNHTVSKFKVSHYVEESGLLDILMQRYGLTAE  408
              GAS   V KF.VS YVE SGLLDI.  RY L
```



677 **Fig. S 3 Alignment of Abf1 from *C. glabrata* (*Cg*Abf1) and *S. cerevisiae***
678 **(*Sc*Abf1).**
679 The alignment was performed with MacVector software; sequences were retrieved
680 from the CGD data base (http://www.candidagenome.org/) or SGD
681 (http://www.yeastgenome.org/). Red boxes indicate zinc finger domain (Zn), green

682 boxes show DNA binding domain (DBD), orange box represent CS1 domain
683 (involved in transcription) present only in *S. cerevisiae*, blue box indicate CS2
684 domain (involve in transcription, replication, chromatin remodeling).