

1 **Supplementary Information**

2 ***Aspergillus fumigatus* colonization of lungs is controlled by ZNF77**

3 Gago et al

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Supplementary Note 1:

Initial Variant selection

To investigate genetic susceptibility factors to ABPA, DNA from 96 patients with ABPA and 167 asthmatic controls were whole exome sequencing as described in [1] and compared with the European controls in the 1000 genomes dataset. This discovery experiment, demonstrated a possible association of the genetic variant rs35699176 (Chr19:2936537, ZNF77) (Supplementary Table 1) with ABPA. Although we failed to replicate this variant using HiSeq, in a different population of 96 ABPA and 96 asthmatics, we found that it was present in ABPA patients carrying high fungal loads in their respiratory airways (30 % of patients (9/32) with an sputum *Aspergillus* positive PCR and 0% (0/13) patients with an *Aspergillus* negative PCR ($p=0.036$); and 60% (4/6 vs 0/15) of patients with fungal loads higher than 100 genome counts in bronchoalveolar lavage ($p=0.0006$). This variant was therefore associated with increased fungal burden rather than with ABPA per se (Tables 1 and 2, Figure 4). Functional analysis on this variant in the ZNF77 gene was performed as rs35699176 results in a premature stop codon which made it a good candidate for phenotypic characterization.

Supplementary Tables

Supplementary Table 1: Association analysis for the rs35699176 genetic variant between ABPA (n =97, asthma (n= 167)and the 1000 genome dataset. FET: Fischer’s Exact Test P-value. AF: Allele Frequency

			Original population			Replication population			24
Rs number (dbSNP)	Ref/Alt	Gene	Alt allele freq in ABPA	Alt allele freq in Asthma	FET	Alt allele freq in ABPA	Alt allele freq in Asthma	FET	25
rs35699176	G/A	ZNF77	9.3%	0.8%	1.52×10^{-5}	3.1%	4.7%	n/s	26

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30 **Supplementary Table2**

31 Sequence of the CRISPR guides, repair template and primers used in this study. RT: Repair
 32 template, Rs35699176-1 and Rs35699176-2 CRISPR guides.

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Name	Aim	Sequence 5-3'	Reference
Rs35699176-1	CRISPR guide	TCAGATGCCTCTGTGGGATTGTTTT	This work
Rs35699176-1	CRISPR guide	AATCCACAGAGGGCATCTGACGGTG	This work
RT_rs35699176	Repair template	CGGAGAGGCCCATCCCTCCGGTTGAGCGCCACTCACCTC AGATGCCTCTATG GGATTGATGCTGATCTCCAGTGTATCAAATCTCCAATTTT CTCAA	This work
Rs35699176-3	ZNF77 genotyping	TCTCTGTGGGTCTGAAATTGTGT	This work
Rs35699176-4	ZNF77 genotyping	TGAAAAGAAATCCGTATGTGGTCT	This work
ZNF77_2934825	ZNF77 expression	CAGCTGACTTCTCACCTCAG	This work
ZNF77_2936594	ZNF77 expression	CACAGGAAGTGATTCCTGGT	This work
GAPDH_F	Gene expression	GTCTTCACCACCATGGAGA	[2]
GAPDH_R	Gene expression	CCAAAGTTGTCATGGATGACC	[2]
CAV	Gene expression	N/A	Biorad
ECAD	Gene expression	N/A	Biorad
OCLUD	Gene expression	N/A	Biorad

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40 **Supplementary Table 3**

41 Fungal strains used in this work.

Species	Strain	Origin
<i>Aspergillus fumigatus</i>	CEA10	CBS144.89 (Isolated by JP Latge)
<i>Aspergillus fumigatus</i>	A1160	CEA10 DeltaKU80 pyrG-
<i>Aspergillus fumigatus</i>	Af293	University Hospital of South Manchester
<i>Aspergillus fumigatus</i>	3MD	Strain 9.20152 National Aspergillosis Center, Manchester UK.
<i>Aspergillus fumigatus</i>	Δ Asp f 13	Michael Bromley gift
<i>Aspergillus fumigatus</i>	Δ Asp f 5	Michael Bromley gift
<i>Aspergillus nidulans</i>	KE12	Michael Bromley gift
<i>Aspergillus terreus</i>	MRCM67020	University Hospital of South Manchester
<i>Aspergillus niger</i>	MRCM44001	University Hospital of South Manchester

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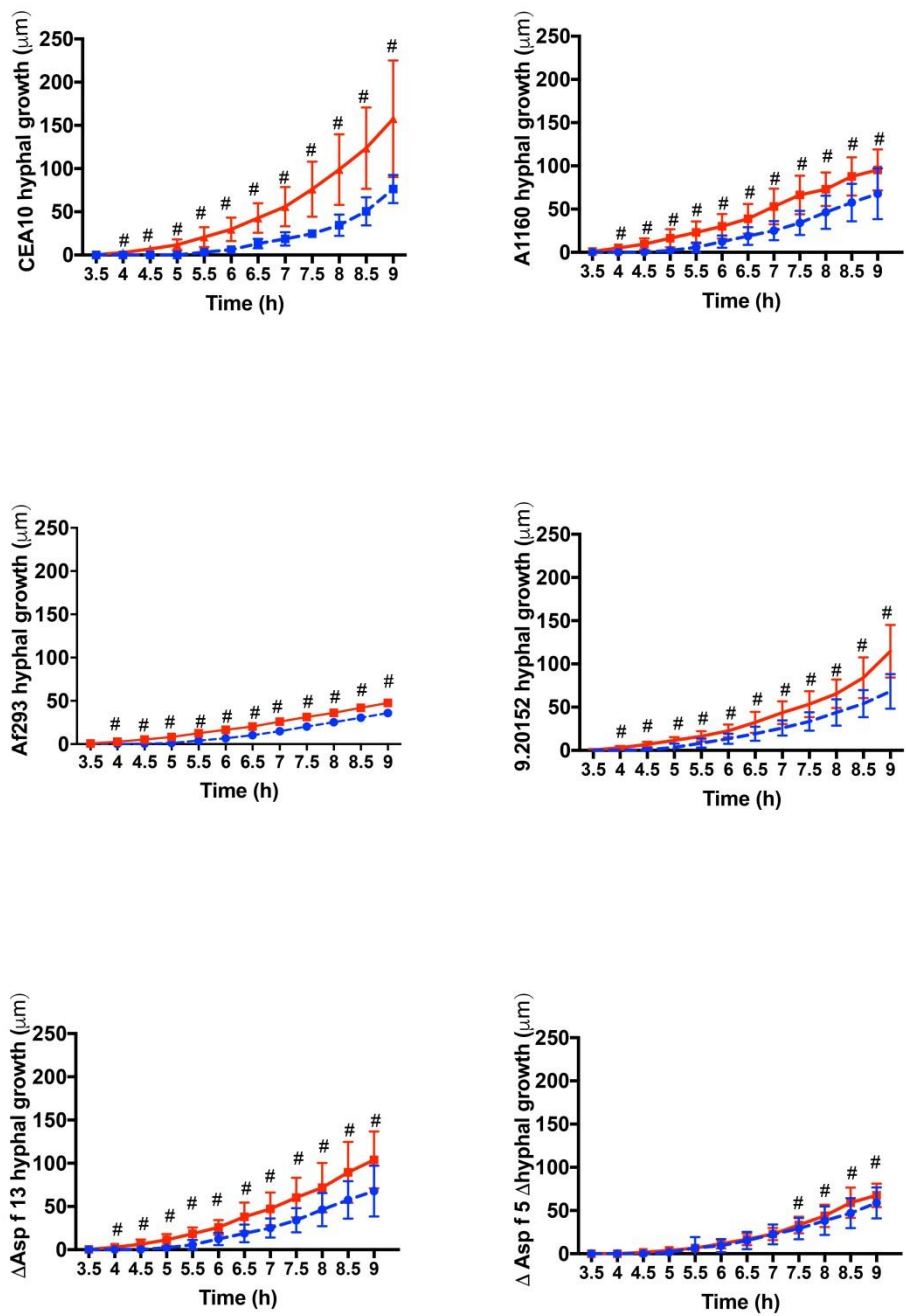
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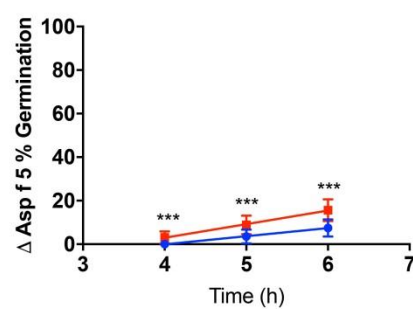
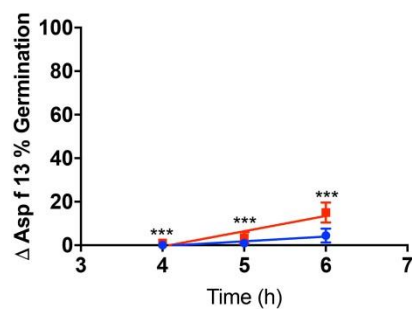
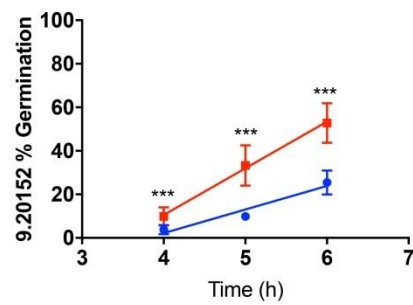
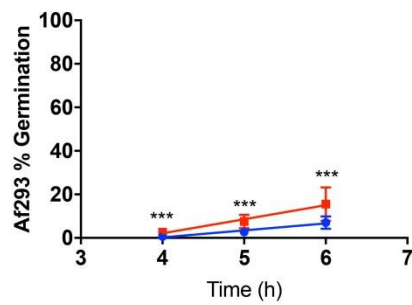
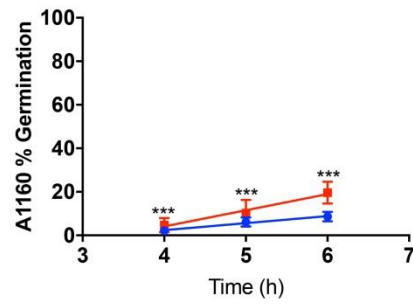
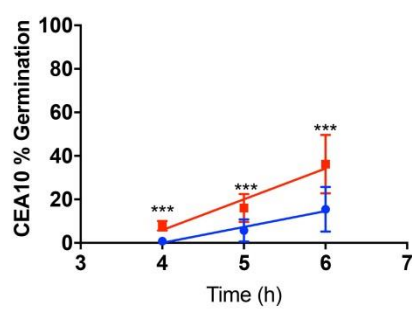
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55 **Supplementary Figures:**



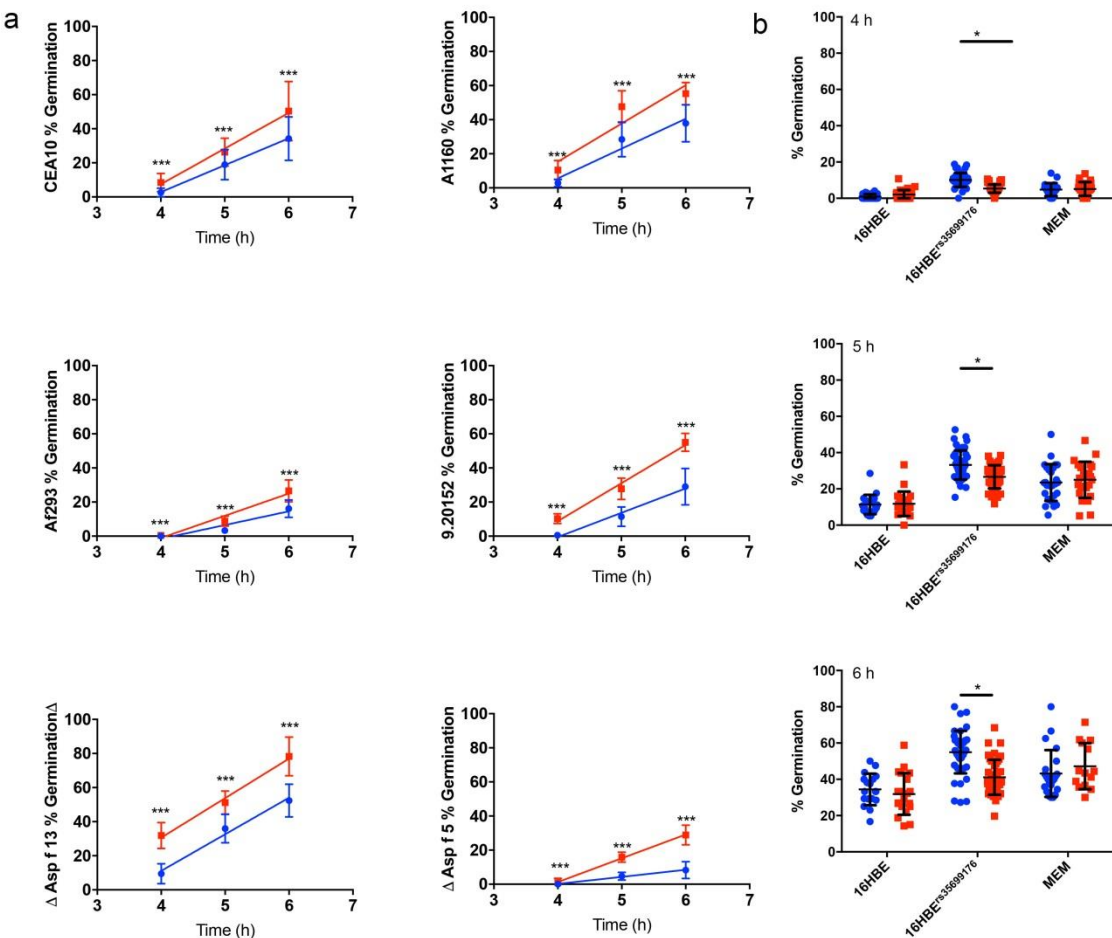
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57 **Supplementary Figure 1: *A. fumigatus* hyphal extension during infection 16HBE and**
58 **16HBE^{rs35699176} of bronchial epithelial cells; Multiple comparison t test were used to detect**
59 **differences in growth for each time point represented as mean and standard deviation #**
60 **indicates P < 0.05.**



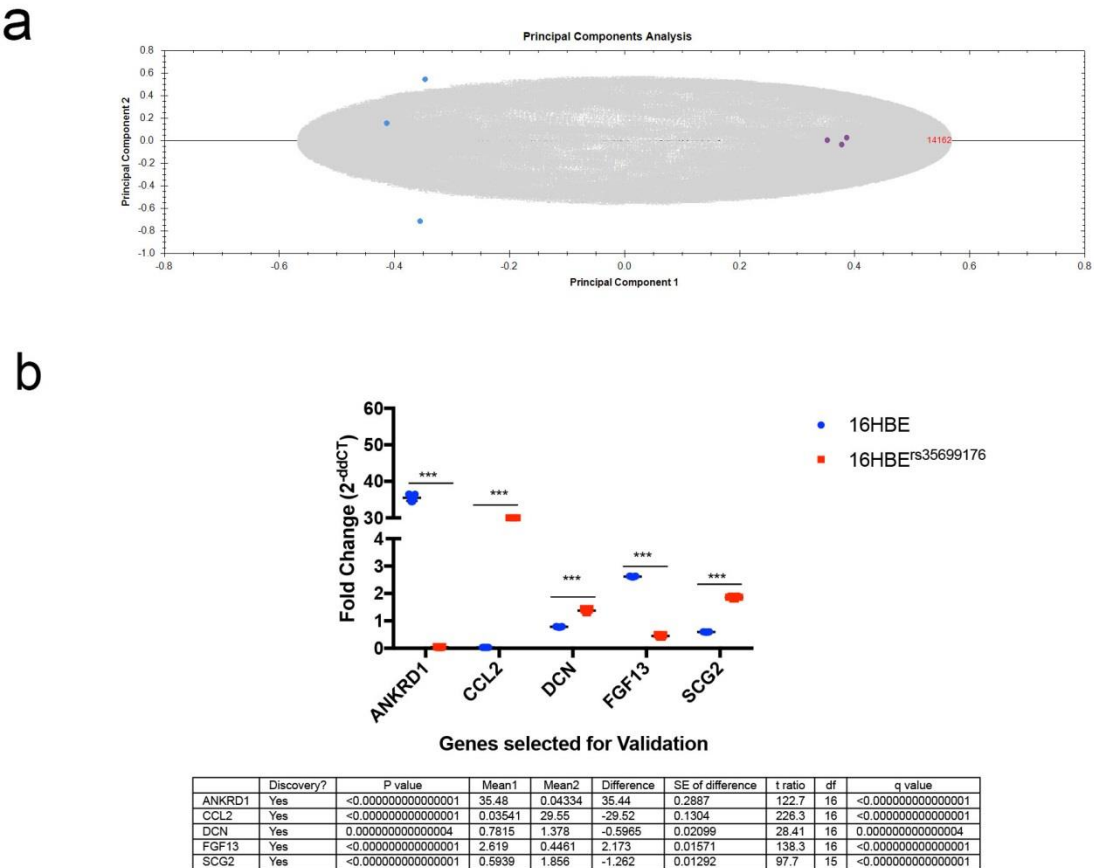
Supplementary Figure 2: Percentage *A. fumigatus* germination during infection of bronchial epithelial cells.

A. fumigatus germination time points (h) determined by time-lapse microscopy. Mean $\pm$ SD is represented. Linear regression analysis and the Pearson correlation test were performed to compare germination time between cell lines. Data points represent mean and standard deviation; *** indicates $P < 0.001$.



73 **Supplementary Figure 3: *A. fumigatus* challenge assays in cell culture supernatants. Data**
74 **points represent mean and standard deviation.**

75 A. *A. fumigatus* germination time points (h) in cell culture supernatants from 16HBE
76 and 16HBE^{rs35699176}. Mean $\pm$ SD is represented. Linear regression analysis and the
77 Pearson correlation test were performed to compare germination time between cell
78 lines; *** indicates P < 0.001. Blue: 16HBE, Red 16HBE^{rs35699176}
79 B. Heat treatment delays *A. fumigatus* CEA10 germination time in 16HBE^{rs35699176} cell
80 lines. Results are expressed as mean $\pm$ SD of three experiments assayed in triplicate.
81 Comparisons were performed using two way anova with multiple comparison test.
82 Statistically significant differences: * P < 0.05. Blue: native conditions, Red: heat
83 treatment



Supplementary Figure 4: Principal component analysis and RNA-seq validation

- (A) Principal component analysis matrix for 16HBE and 16HBE^{rs35699176} indicates no differences within but a significant difference between groups. Three biological replicates were assessed: 16HBE (purple) and 16HBE^{rs35699176} (blue) cells.
- (B) RNA-seq validation. Five genes were selected for replication based on p-adjusted value and function: Ankyrin Repeat Domain 1 (ANKRD1), C-C Motif Chemokine Ligand 2 (CCL2), decorin (DCN), fibroblast growing factor (FGF3) and secretogranin (SGG2). Two way ANOVA with multiple comparison test were used for comparison; Data represents mean and standard deviation *** = $p < 0.0001$. Normalization was done using GAPDH as a housekeeping gene. Blue: 16HBE, Red 16HBE^{rs35699176}

99 **Supplementary References:**

- 100 1. Overton NLD, Brakhage AA, Thywißen A, Denning DW, Bowyer P. Mutations in EEA1 are
101 associated with allergic bronchopulmonary aspergillosis and affect phagocytosis of *Aspergillus*
102 *fumigatus* by human macrophages. *PLoS ONE* 2018 **13**(3): e0185706.
- 103 2. O'Driscoll KE, Hatton W, Burkin HR, Leblanc N, Britton F. Expression, localization, and functional
104 properties of Bestrophin 3 channel isolated from mouse heart. *Am J Physiol Cell Physiol* 2008, **295**
105 (6): C1610-C1624
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