

Supplementary information

Survival factor A (SvfA) contributes to *Aspergillus nidulans* pathogenicity

Joo-Yeon Lim¹, Ye-Eun Jung², Hye-Eun Hwang³, Cheol-Hee Kim³, Nese Basaran Akgul¹, Sri Harshini Goli¹, Steven P. Templeton^{1*} and Hee-Moon Park^{2*}

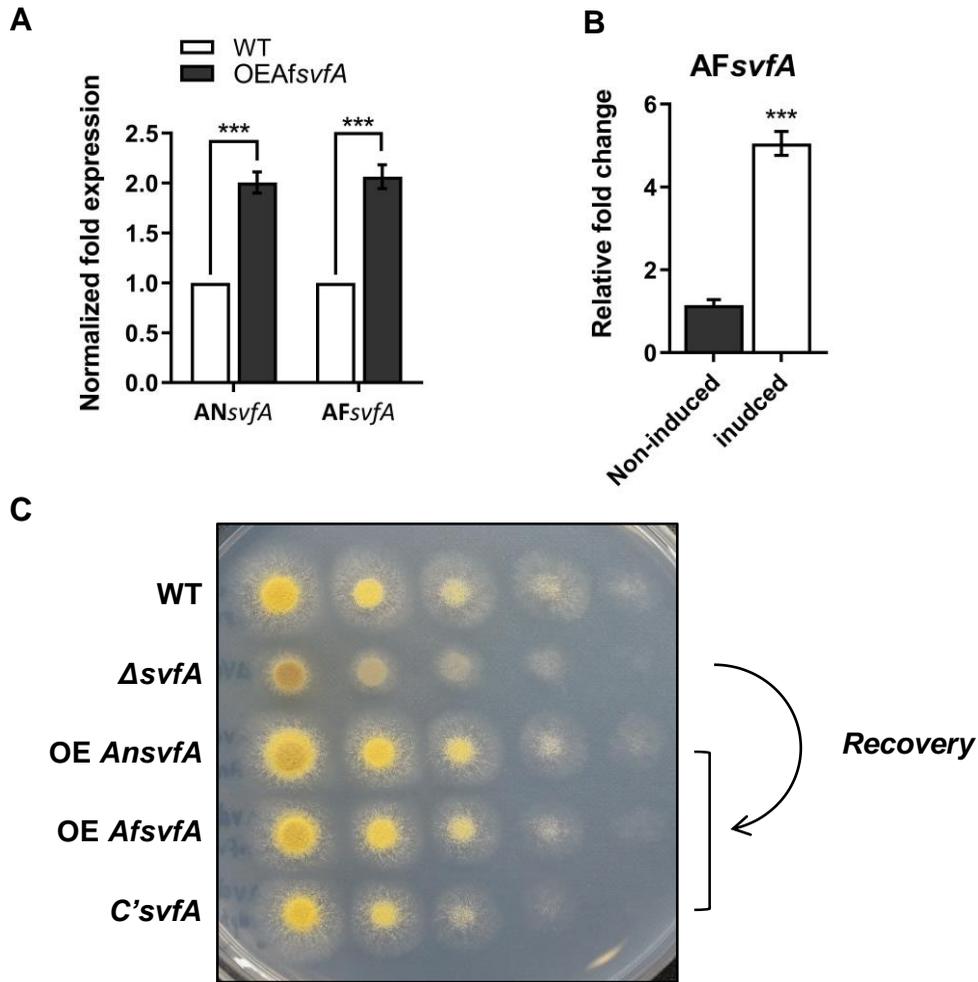
¹ Department of Microbiology and Immunology, Indiana University School of Medicine-Terre Haute, Terre Haute, IN; jooyeonlim1220@gmail.com (JY Lim), nbasaran@iu.edu (NB Akgul), sgoli@sycamores.indstate.edu (SH Goli) and sptemple@iupui.edu (S Templeton)

² Laboratory of Cellular Differentiation, Department of Microbiology and Molecular Biology, College of Bioscience and Biotechnology, Chungnam National University, Daejeon 34134, Republic of Korea; jye6865@naver.com (YE Jung)

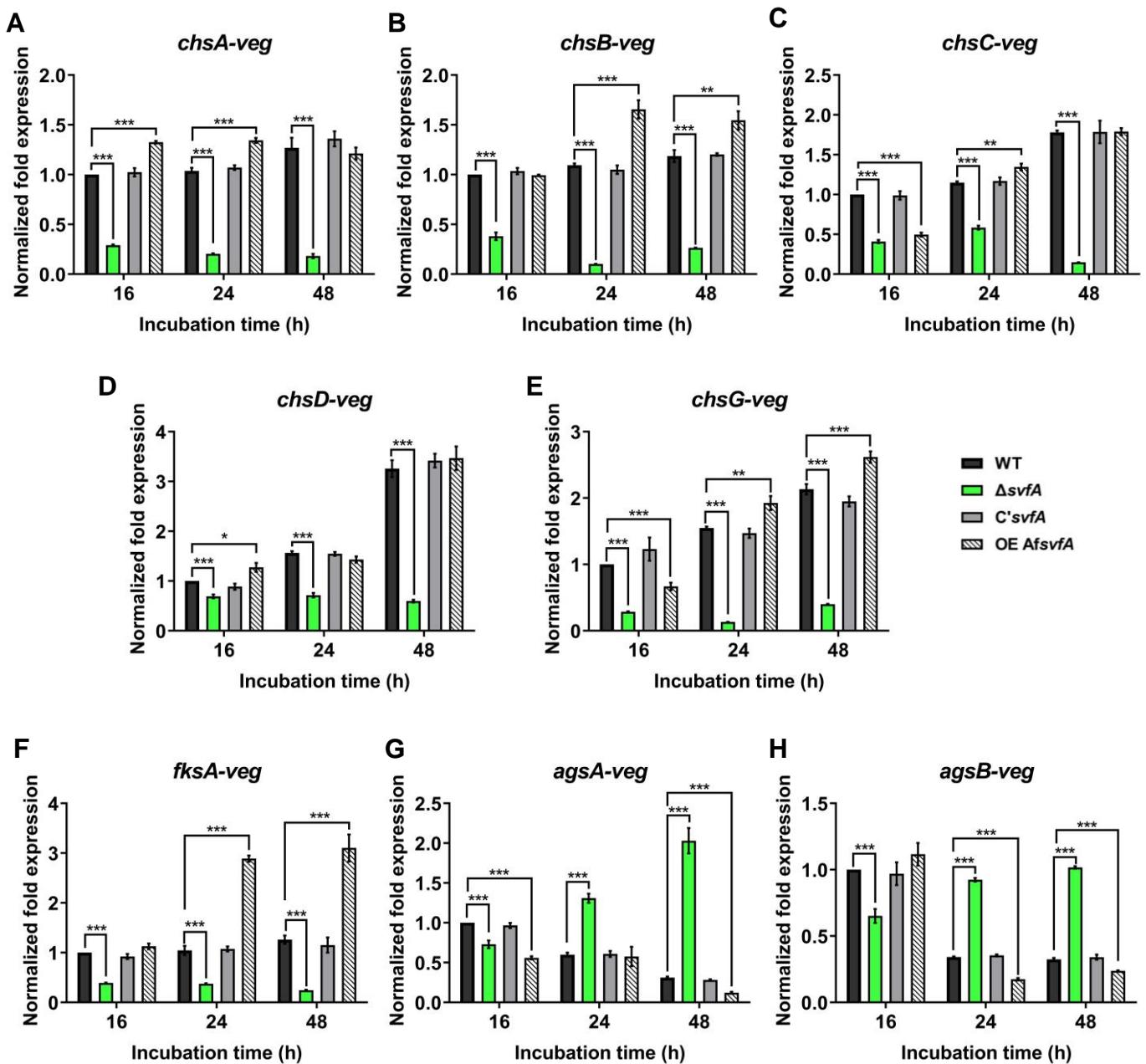
³ Laboratory of Developmental Genetics Department of Biology, College of Bioscience and Biotechnology, Chungnam National University, Daejeon 34134, Republic of Korea; gwj03068@naver.com (HE Hwang) and zebrakim@cnu.ac.kr (CH Kim)

* To whom correspondence should be addressed.

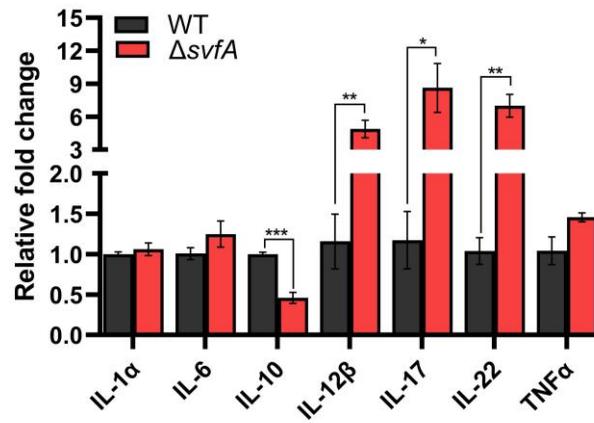
Hee-Moon Park, Tel: 042-821-7553/ Fax : 042-822-7367, E-mail: hmpark@cnu.ac.kr



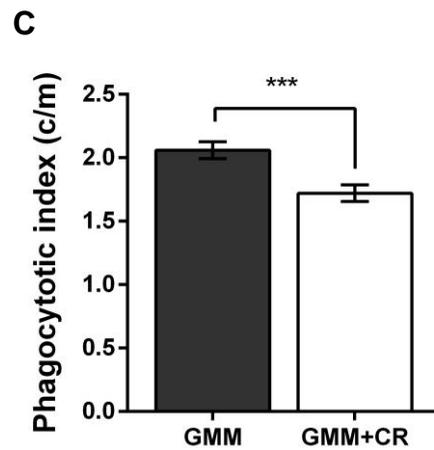
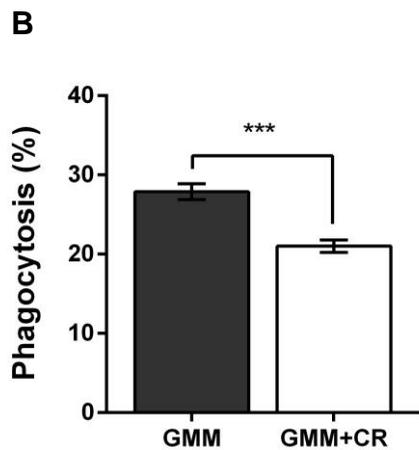
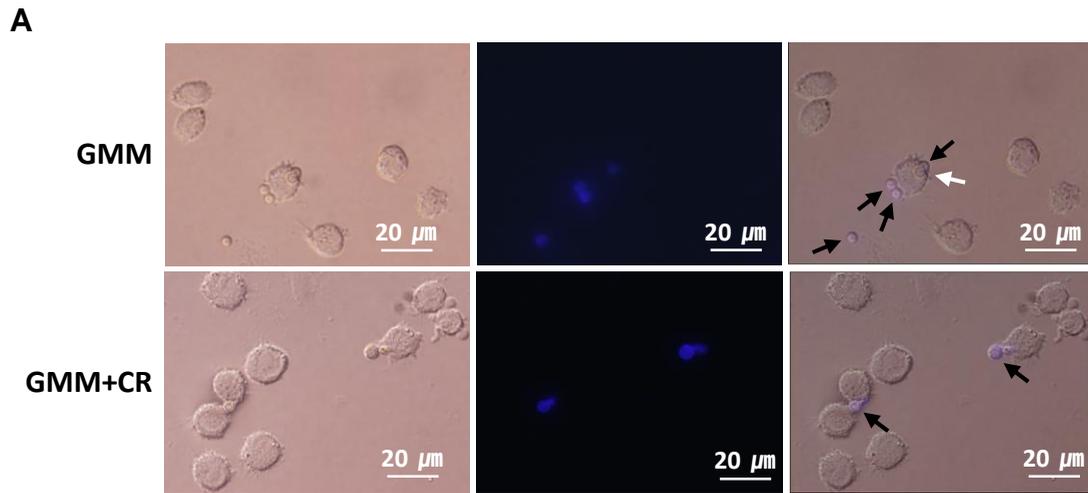
Supplementary Figure S1. Functional complementation of $\Delta svfA$ by overexpression of *AfsvfA* gene. A. Expression level of *ANsvfA* and *AFsvfA* genes in WT and OE *AfsvfA* strains. Spores were inoculated in GMM liquid broth with 0.6% sodium nitrate. *** $P < 0.001$. B. Expression level of *AFsvfA* gene in OE *AfsvfA* strain grown in non-inducing media (GMM containing 0.2% ammonium tartrate as a nitrogen source) or inducing media (GMM containing 0.6% sodium nitrate as a nitrogen source). Total RNA was extracted and RT-qPCR analysis was performed using 18S rRNA gene as an internal control. *** $P < 0.001$. C. Serial diluted conidia from WT, $\Delta svfA$, OE *svfA*, OE *AfsvfA* and *C'svfA* were inoculated on GMM including 0.6% sodium nitrate and incubated for 2 days at 37°C.



Supplementary Figure S2. Expression level of chitin synthases (*chsA*, *chsB*, *chsC*, *chsD*, and *chsG*), β -1,3-glucan synthase (*fksA*) and α -glucan synthase (*agsA* and *agsB*) during vegetative growth. Spores were inoculated in MM liquid broth and incubated for the indicated time. Total RNA was extracted and RT-qPCR analysis was performed using 18S rRNA gene as an internal control. **P < 0.01, *P < 0.001**



Supplementary Figure S3. Expression level of cytokine genes. mRNA was harvested from mouse lung homogenates and analyzed by RT-qPCR for expression of the indicated cytokines. Expression is indicated as a fold change above WT expression. *P < 0.05, **P < 0.01, ***P < 0.001.



Supplementary Figure S4. Alveolar macrophage response to WT conidia grown on GMM containing 200 $\mu\text{g}/\text{mL}$ of Congo red (CR). MH-S murine alveolar macrophage cells were challenged with a three-fold concentration of *A. nidulans* conidia and incubated for 5 h at 37°C in an atmosphere of 5% CO_2 . (A) Microscopic analysis of the uptake of conidia by the macrophages. External conidia (black arrows) were stained by calcofluor white. White arrows indicate conidia endocytosed by macrophage. (B) Phagocytosis of conidia. The percentage of macrophages containing more than one ingested conidia was counted. *** $P < 0.001$. $N = 67-100$. (C) Phagocytic index. The average number of the ingested conidia per macrophage (c/m). $N = 154-185$. *** $P < 0.001$.