In vivo applicability of *Neosartorya fischeri* antifungal protein 2 (NFAP2) in treatment of vulvovaginal candidiasis

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Running title: Treatment of vulvovaginal candidiasis with NFAP2

TABLE S1 Composition of media used in this study.

Description	Composition
R10 (Fibroblast cell culture medium)	RPMI 1640 (Lonza BE12-167F), 10 mM HEPES buffer (Biochrom-Merck L1613), 0.1% gentamicin (Gibco 15750-037), 10% heat-inactivated fetal calf serum (PAN Biotech P30-1502), 2 mM L-alanyl-L-glutamine (GlutaMAX, Gibco 35050-038)
SD (Sabouraud dextrose)	4% glucose, 1% peptone, and 2% agar (w/v) if necessary
YEGK (Yeast extract glucose medium with KH_2PO_4)	1% glucose; 1% KH_2PO_4; 0.5% yeast extract, and 2% agar (w/v) if necessary
YPD (Yeast extract peptone dextrose medium)	1% yeast extract, 2% bacteriological peptone, 2% D-(+)-glucose, and 2 % (w/v) agar if necessary

Table S2 Significance values from murine VVC model.

Treatment 1	**Treatment 2**	**p-value**	**Significance**
35 mg/kg FLC	untreated	>0.9999	non significant
5 mg/kg/day FLC	untreated	>0.9999	non significant
800 mg/kg/day NFAP2	untreated	0.0177	significant (*)
800 mg/kg/day NFAP2 + 5 mg/kg/day FLC	untreated	0.0017	significant (**)
5 mg/kg/day FLC	35 mg/kg FLC	>0.9999	non significant
800 mg/kg/day NFAP2	35 mg/kg FLC	0.0016	significant (**)
800 mg/kg/day NFAP2 + 5 mg/kg/day FLC	35 mg/kg FLC	0.0001	significant (***)
800 mg/kg/day NFAP2	5 mg/kg/day FLC	0.0687	non significant
800 mg/kg/day NFAP2 + 5 mg/kg/day FLC	5 mg/kg/day FLC	0.0084	significant (**)
800 mg/kg/day NFAP2 + 5 mg/kg/day FLC	800 mg/kg/day NFAP2	>0.9999	non significant

Abbreviations: FLC, fluconazole; NFAP2: *Neosartorya fischeri* antifungal protein 2.

* : $p \leq 0.05$, ** : $p \leq 0.005$, *** : $p \leq 0.0001$.

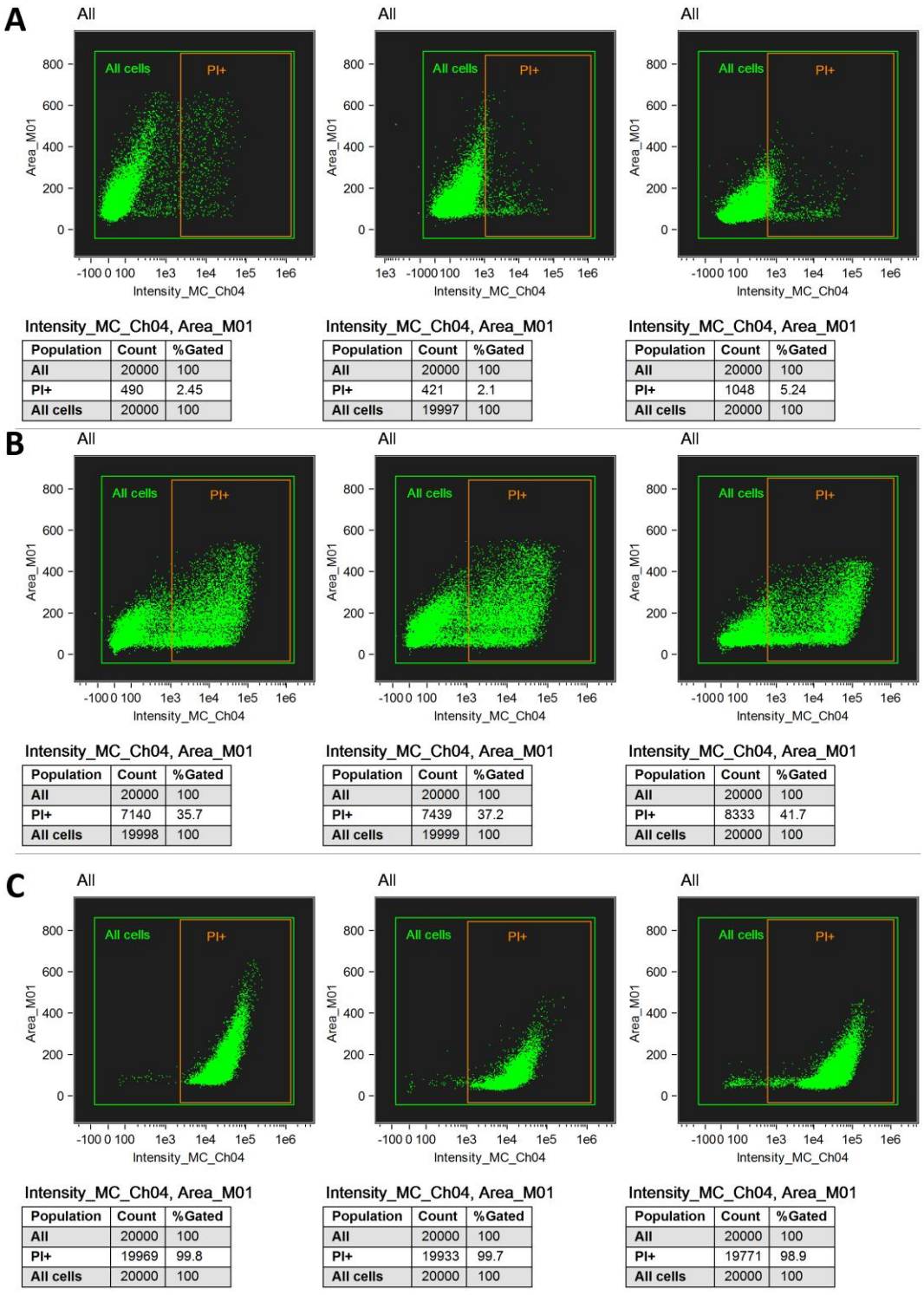

FIG S1 FACS analysis and quantification of PI-positive *C. albicans* 27700 cells after incubation for 24 hours at 30 °C under continuous shaking at 160 rpm (A) in RPMI 1640 (Sigma-Aldrich, St Louis, MO, USA), (B) in RPMI 1640 supplemented with MIC of NFAP2 (800 µg/ml), and (C) after treatment with 70% (v/v) ethanol for 10 min at 4 °C. Data represent the results from three independent experiments.

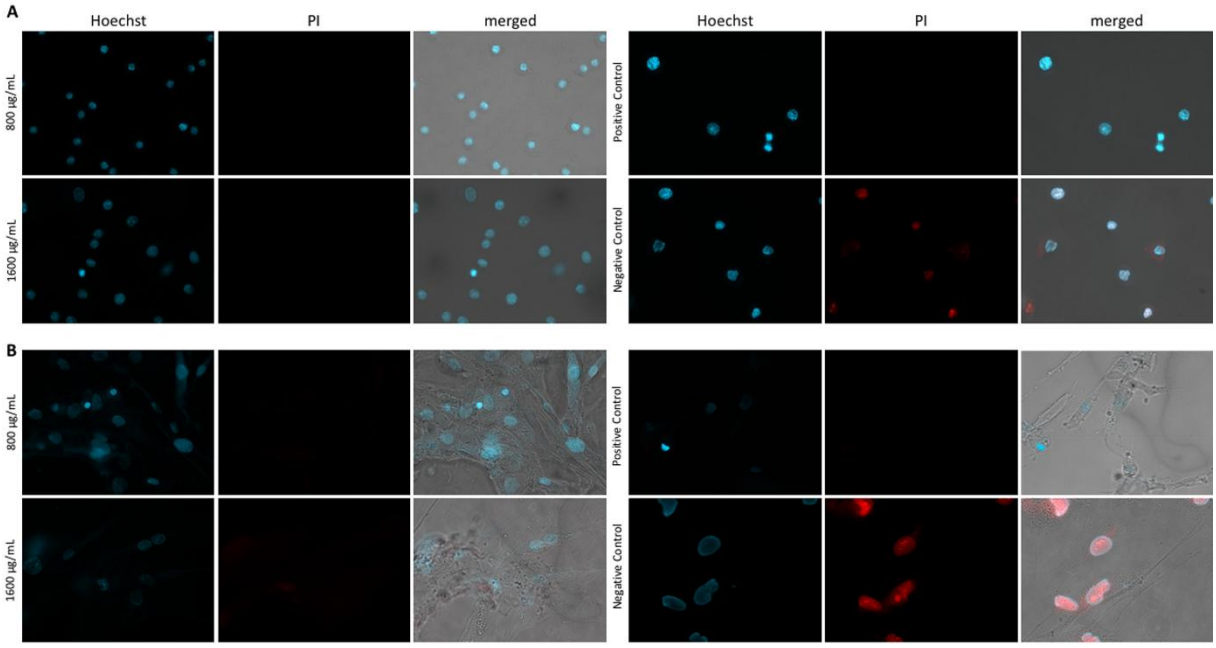

FIG S2 *In vitro* toxicity-testing of NFAP2 on primary HKC and HDF. Fluorescence staining with PI (red) and counterstaining with Hoechst (blue) of primary (A) HKC and (B) HDF after 24 hours exposure to 800 µg/ml and 1600 µg/ml NFAP2. Untreated cells were used as living controls, and 50% ethanol-treated as dead control.

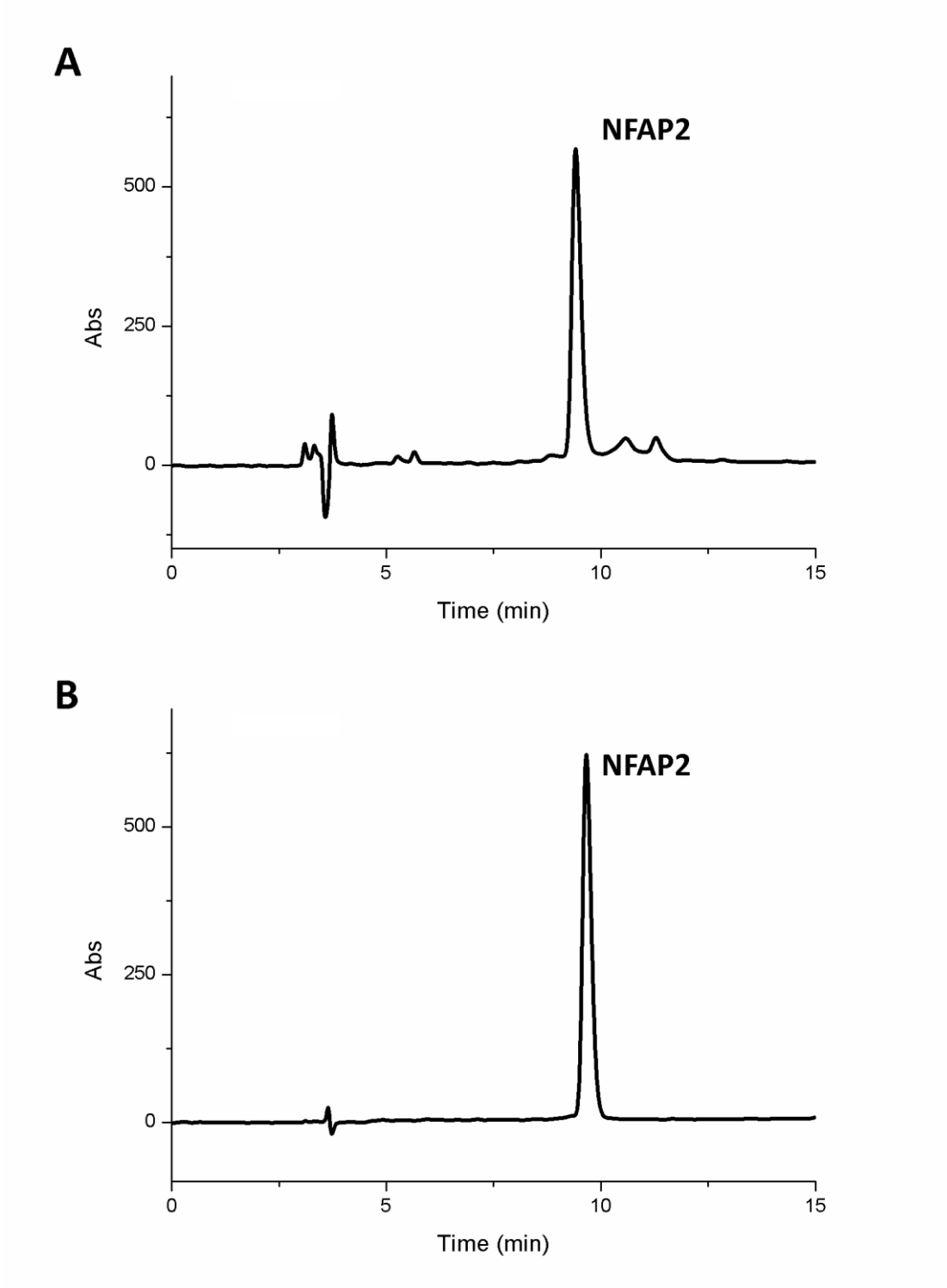

Fig S3 RP-HPLC chromatogram of recombinant NFAP2 produced by *P. chrysogenum* (A) after cation-exchange chromatography, and (B) after the additional semipreparative RP-HPLC purification step to reach the 100% purity.