**SUPPLEMENTARY INFORMATION**

# Impaired biosynthesis of ergosterol confers resistance to complex sphingolipid biosynthesis inhibitor aureobasidin A in a *PDR16*-dependent manner

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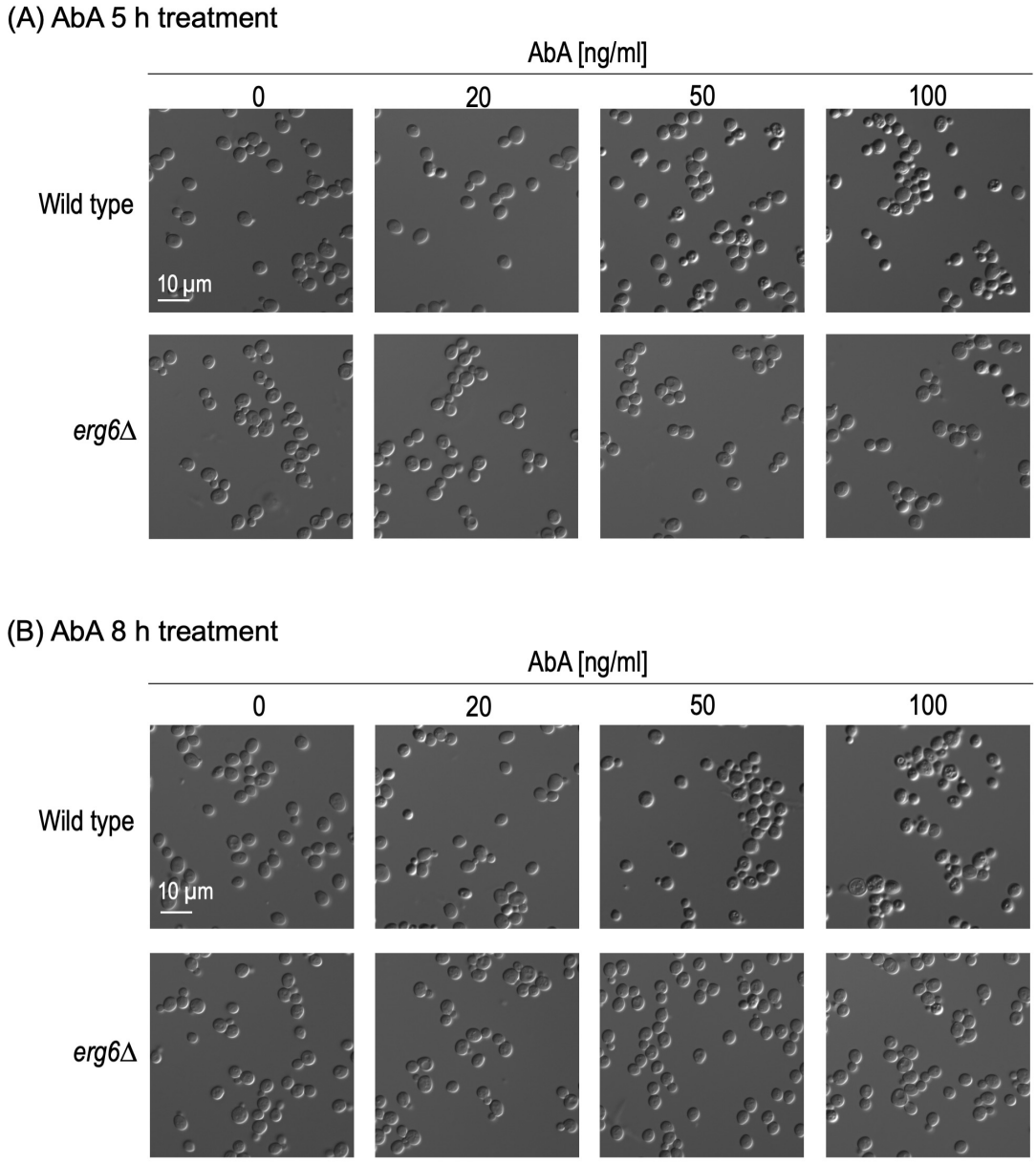



**Figure S1. Morphological changes in AbA-treated wild-type and *erg6*Δ cells.**

Cells were cultured overnight in YPD medium, diluted (0.05 $OD_{600}$ units/ml) in fresh YPD medium, incubated for 3 h at 30°C, and then treated with 0, 20, 50, or 100 ng/ml AbA for 5 or 8 h at 30°C. Cells were observed under a differential interference contrast microscope.

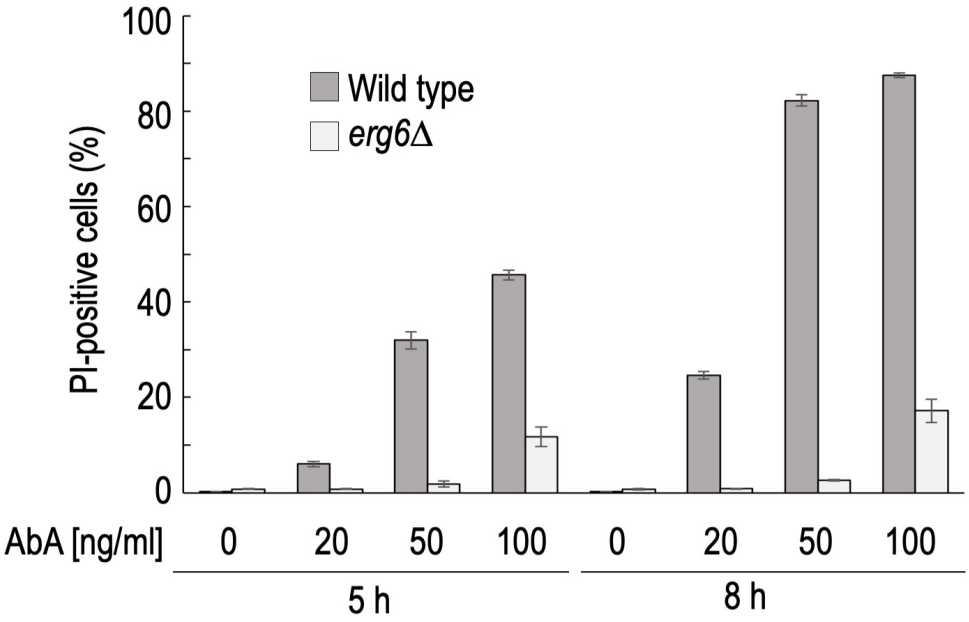



**Figure S2. Detection of dead cells by propidium iodide (PI) staining.**

Cells were cultured overnight in YPD medium, diluted (0.05 $OD_{600}$ unit/ml) in fresh YPD medium, and incubated for 3 h at 30°C, and then treated with 0, 50, 100 ng/ml AbA for 4 h 40 min or 7 h 40 min at 30°C. Cells were stained with 2.5 μg/ml of PI for 20 min, and then PI-positive cells were detected by flow cytometry. Briefly, fluorescence intensity of 10,000 cells was measured per sample using a flow rate of 10 μl/min. Wild-type cells treated with 70% ethanol for 5 min were used to generate a fluorescence threshold to identify PI-positive cells. The threshold was set to the fluorescence at which >99% but <100% of 70% ethanol-treated cells were at least that fluorescent. Data represent means ± SD for one experiment (triplicate) representative of three independent experiments.

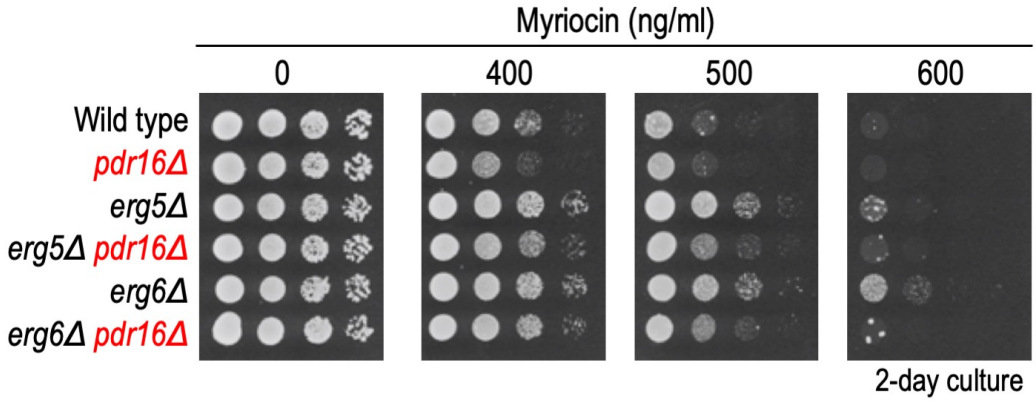



**Figure S3. Effects of deletion of *PDR16* on myriocin sensitivity of *ERG5*- or *ERG6*-deleted cells.**

Cells cultured overnight in YPD medium were spotted onto YPD plates with or without the indicated concentrations of myriocin. All plates were incubated at 30°C and photographed after 2 days. The details are given in Methods.

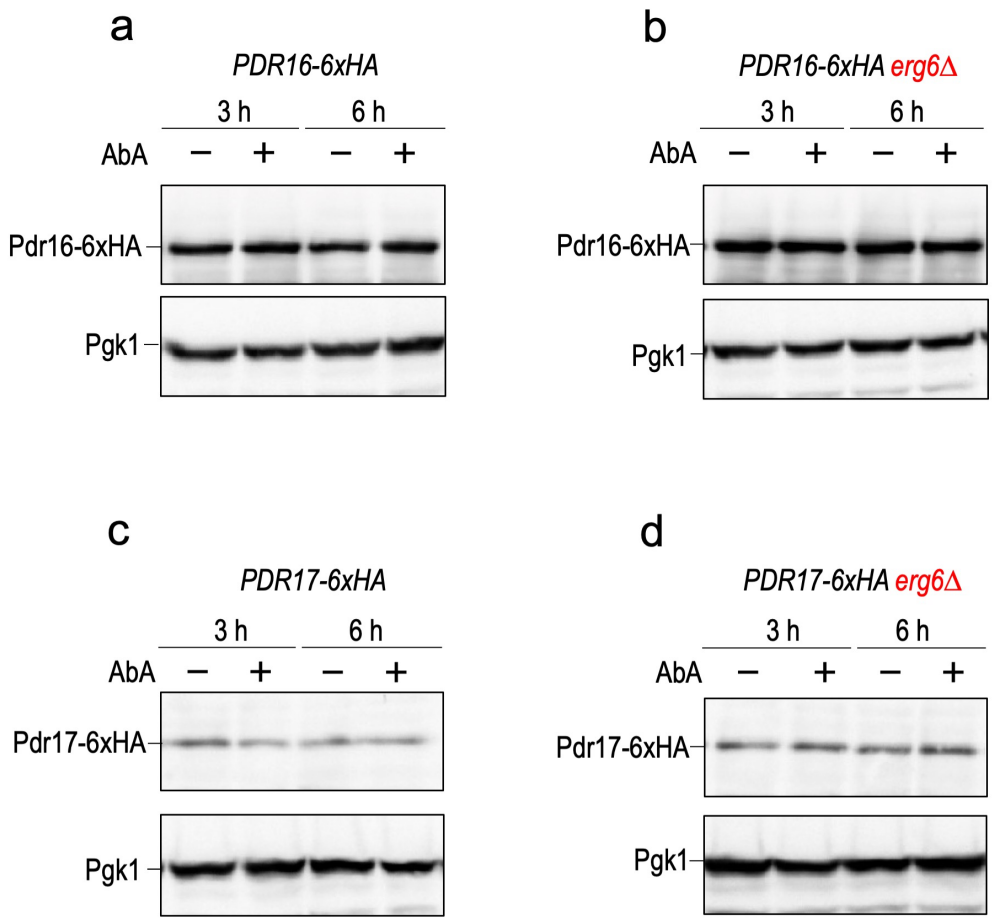



**Figure S4. Effects of AbA on the protein expression levels of Pdr16-6xHA and Pdr17-6xHA.**

Cells expressing Pdr16-6xHA or Pdr17-6xHA with the native promoter were cultured overnight in YPD medium, diluted (0.05 $OD_{600}$ units/ml) in fresh YPD medium, and then incubated for 3 h at 30°C. Cells were treated with 0 or 50 ng/ml of AbA for 3 or 6 h at 30°C. Yeast cell extracts were immunoblotted using anti-HA or anti-Pgk1. The details are given in Methods. The original blots are presented in Fig. S9 and 10.

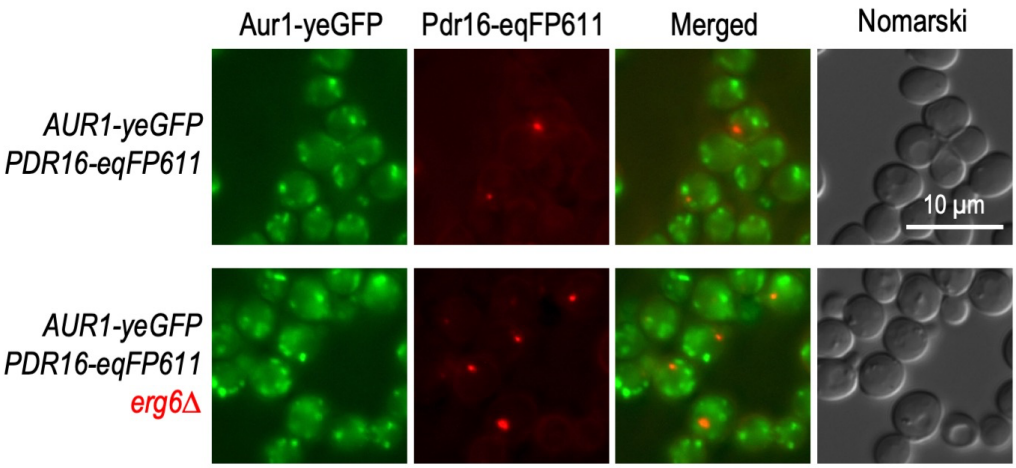



**Figure S5. Localization of Aur1-yeGFP and Pdr16-eqFP611.**

Cells expressing Aur1-yeGFP and Pdr16-eqFP611 were cultured overnight in YPD medium, diluted (0.3 $OD_{600}$ units/ml) in fresh YPD medium, and then incubated for 5 h at 30°C. GFP and RFP fluorescence was observed by fluorescence microscopy.

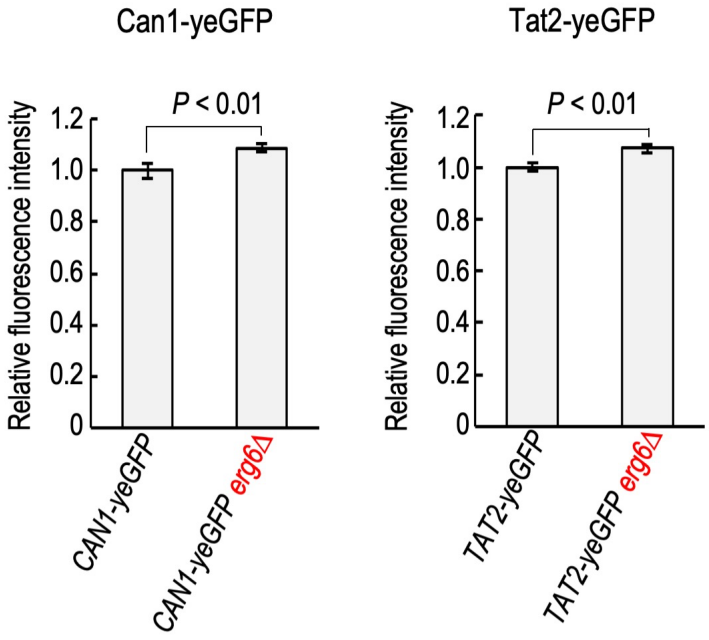



**Figure S6. Expression level of Can1-yeGFP and Tat2-yeGFP.**

For tagging of the C-terminus of Can1 or Tat2 with a yeast enhanced green fluorescent protein (yeGFP), a yeGFP fusion cassette with the *kanMX4* marker from pKT127 (*Yeast* **21**, 661-670 (2004)) was introduced immediately upstream of the stop codon of each chromosomal gene Cells expressing Can1-yeGFP and Tat2-yeGFP were cultured overnight in YPD medium, diluted (0.3 $OD_{600}$ units/ml) in fresh YPD medium, and then incubated for 5 h at 30°C. Fluorescence intensity of GFP in individual cells was measured with a flow cytometer. Each value is the average of the fluorescence intensity of 10,000 cells from one sample. The fluorescence intensity in *CAN1-yeGFP* or *TAT2-yeGFP* cells was taken as 1, respectively. Data represent means ± SD for three samples representative of three independent experiments.

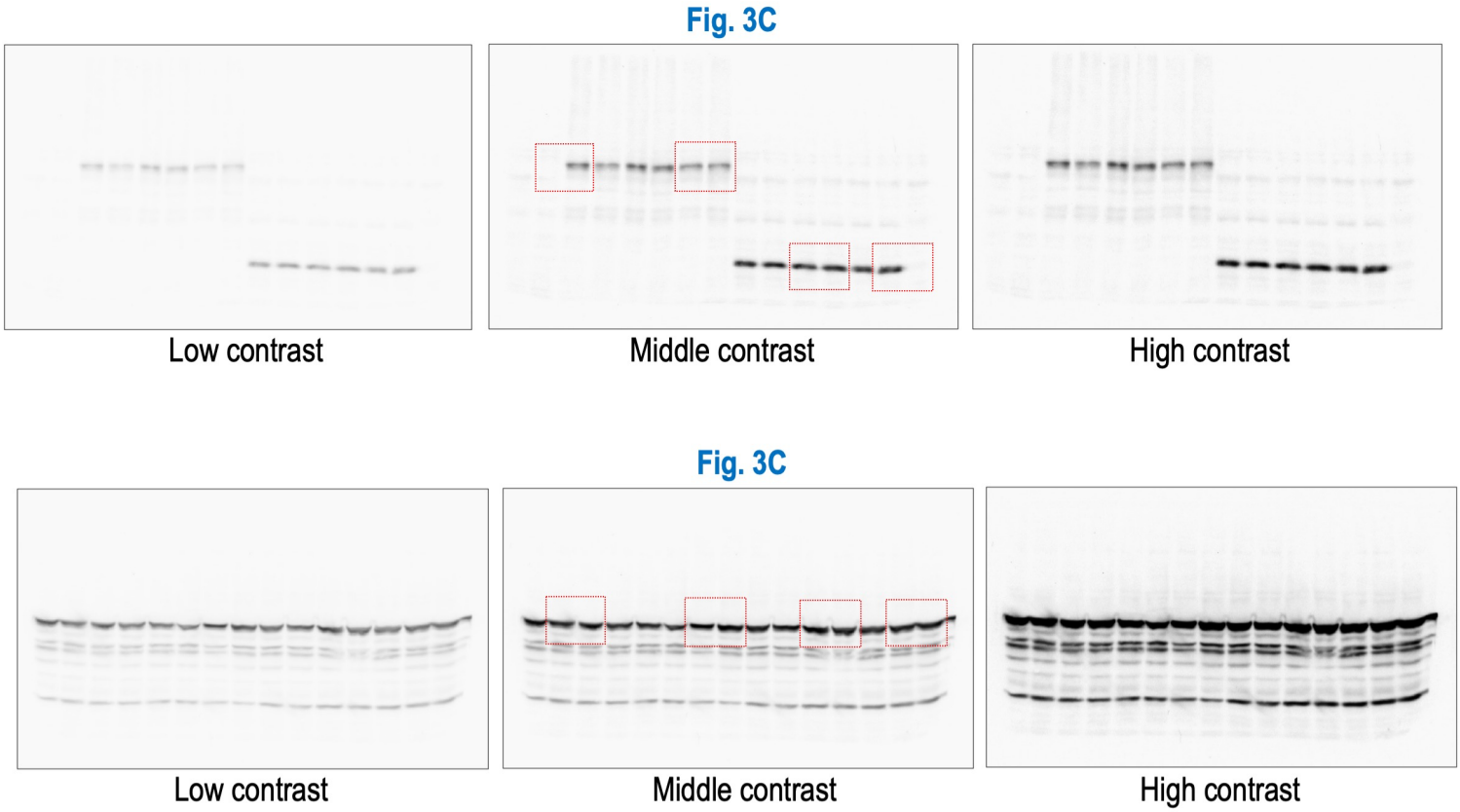
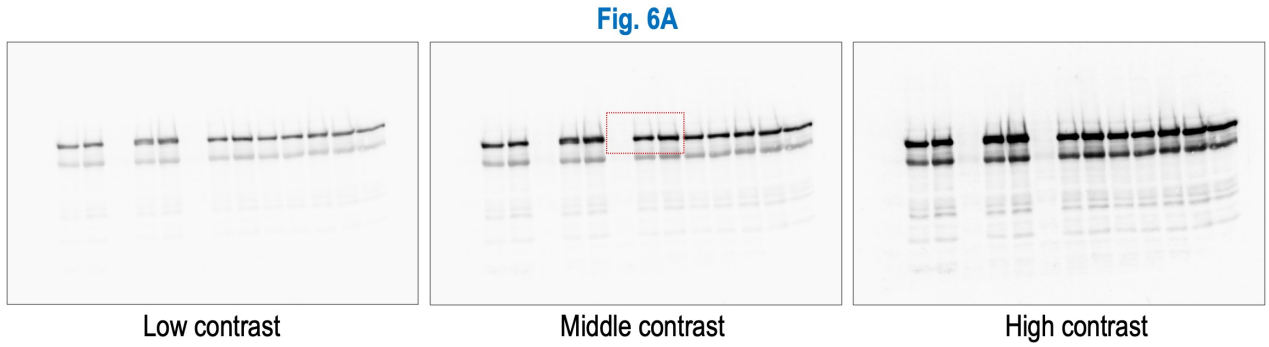
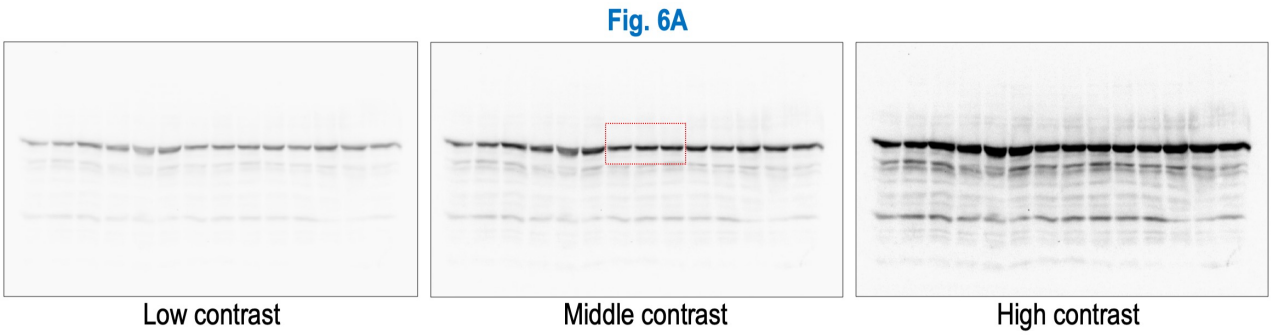
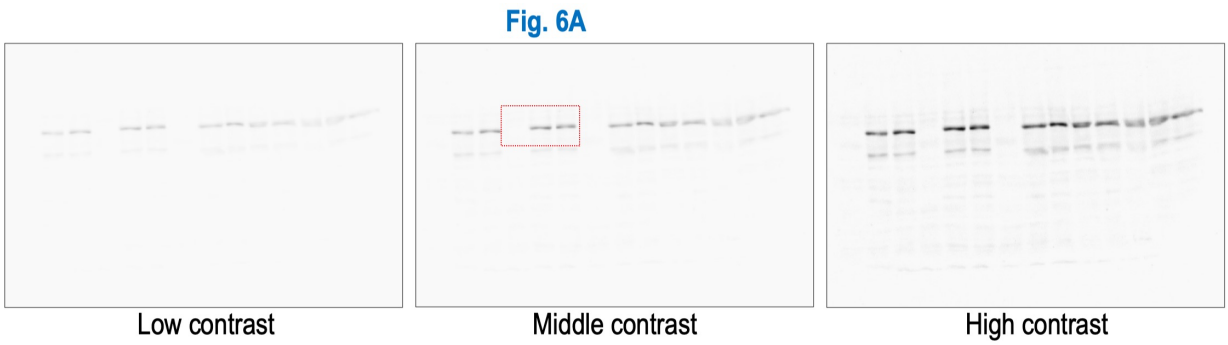



**Figure S7. Full Western blot images in Fig. 3C.**

Membranes were cut prior to hybridization with antibodies.



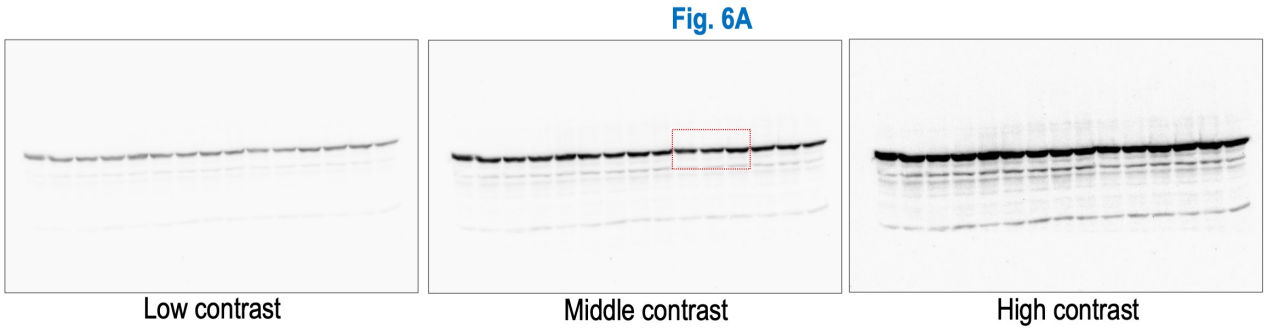




**Figure S8. Full Western blot images in Fig. 6A.**

Membranes were cut prior to hybridization with antibodies.

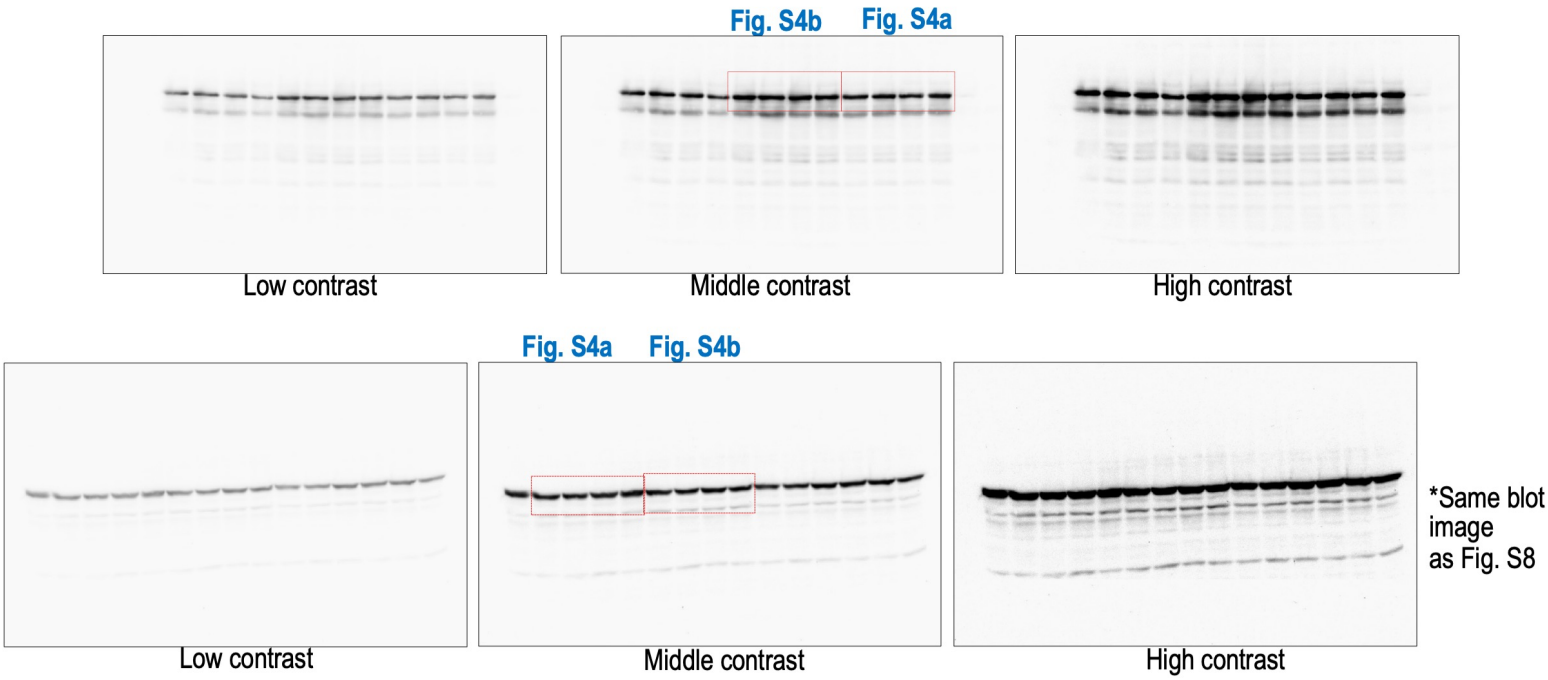



**Figure S9. Full Western blot images in Fig. S4a and b.**

Membranes were cut prior to hybridization with antibodies.

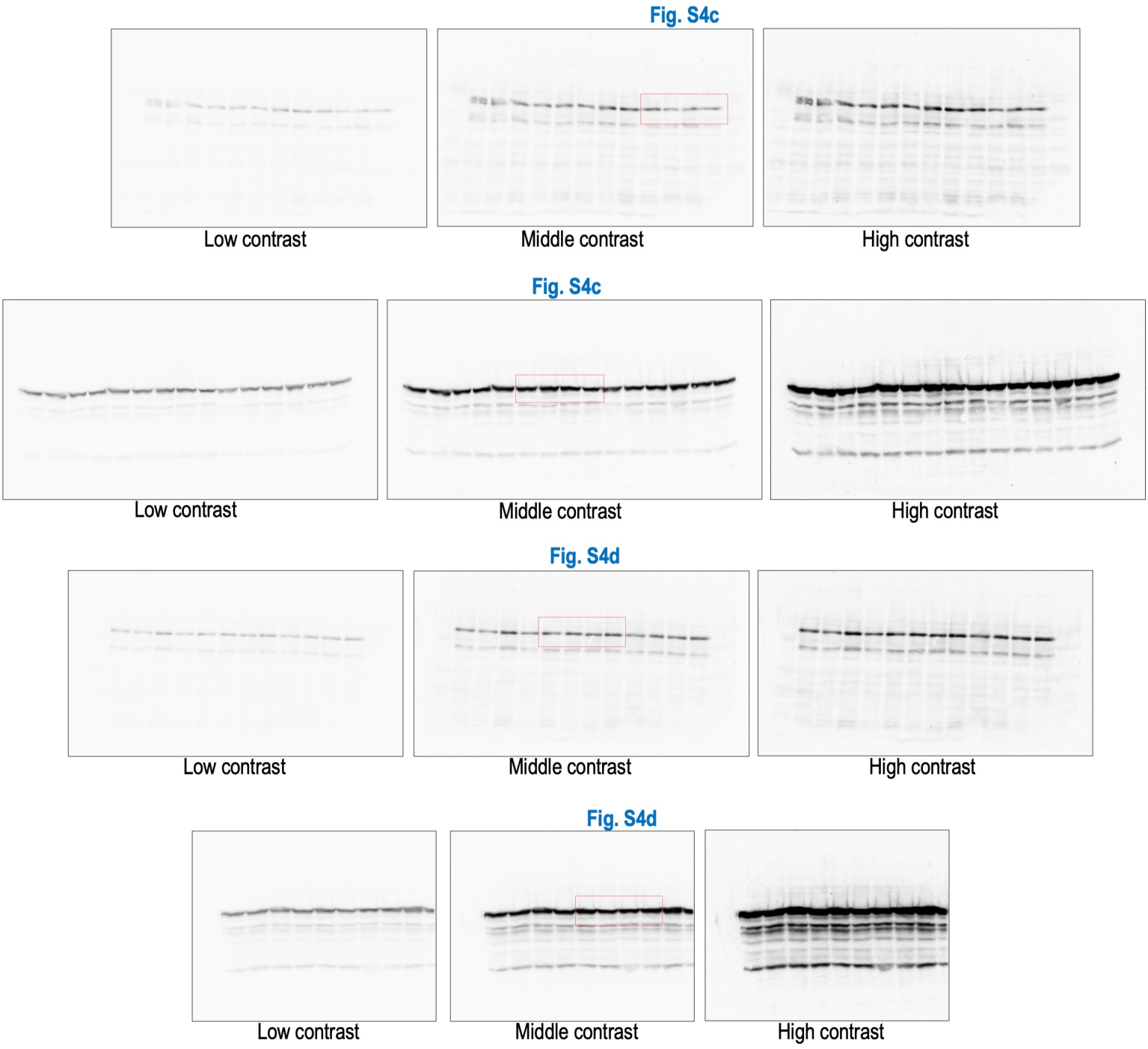



**Figure S10. Full Western blot images in Fig. S4c and d.**

Membranes were cut prior to hybridization with antibodies.

(A)

Fig. 2B

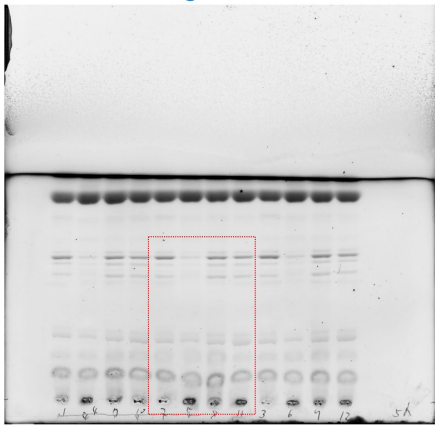


Fig. 2C

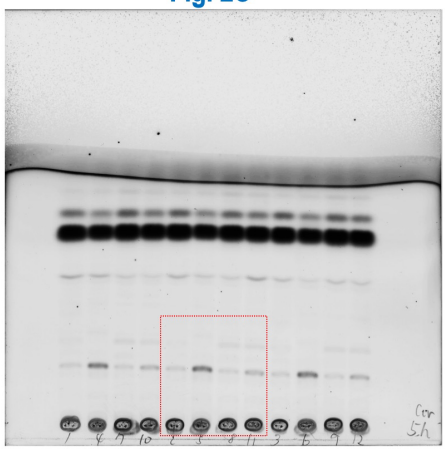


Fig. 2D

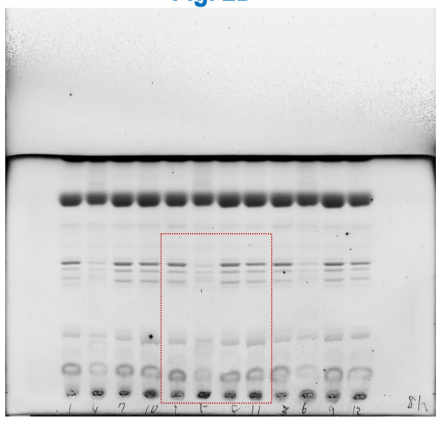


Fig. 2E

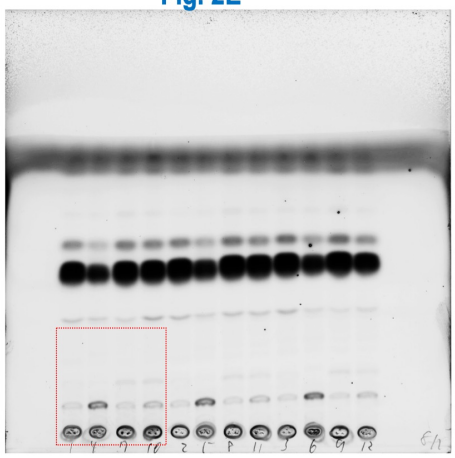
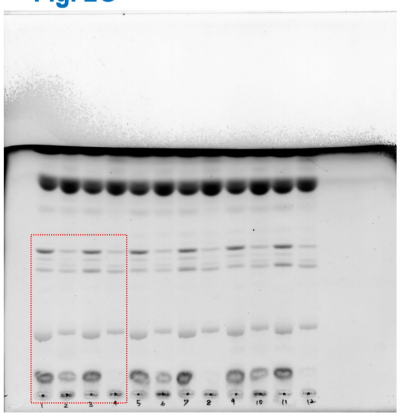


Fig. 2G



(B)

Fig. 3A

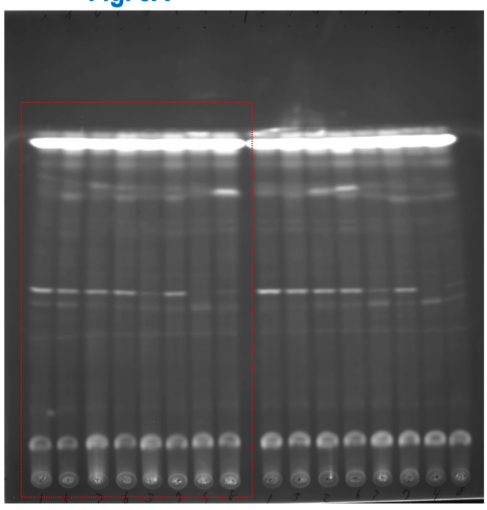


**Figure S11. Full TLC plate images in Fig. 2B-D and G (A), and Fig. 3A (B).**

Fig. 5A

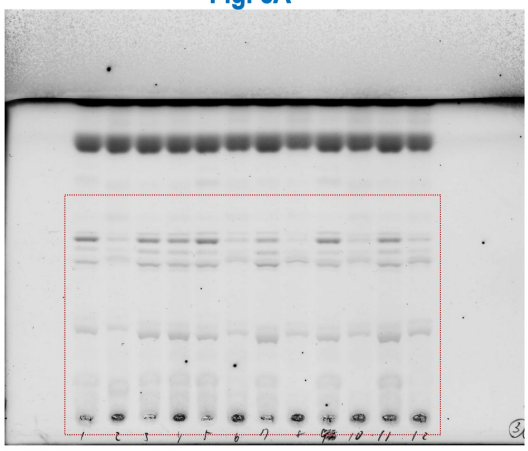


Fig. 5B

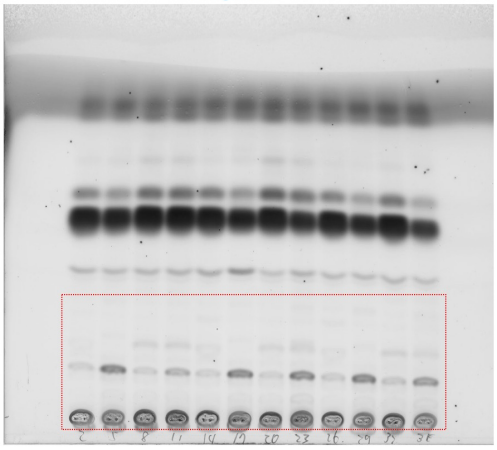


Fig. 5C

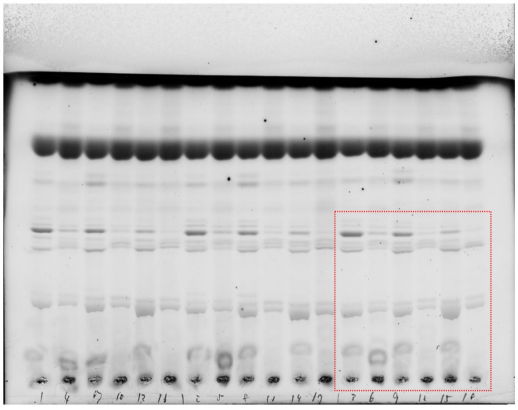


Fig. 5D

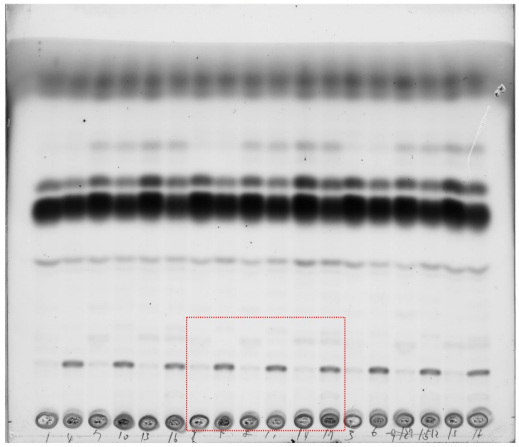


**Figure S12. Full TLC plate images in Fig. 5A-D.**