

IPD Project Details

Project ID: IPD7282

Project Title: Label-free quantitative plasma membrane proteome analysis of *Candida glabrata* wild-type and Cgvps34⁻ (lacks phosphatidylinositol 3- phosphate kinase, CgVps34) strains. Label-free quantitative plasma membrane proteome analysis of *Candida glabrata* wild-type and Cgvps34⁻ (lacks phosphatidylinositol 3- phosphate kinase, CgVps34) strains.

Description: The project is aimed at characterizing the effect of loss of phosphatidylinositol 3- phosphate kinase (CgVps34) on the plasma membrane proteome of *Candida glabrata*.

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Sample Preparation: *C. glabrata* wild-type and Cgvps34⁻ strains were grown in YPD medium till logarithmic-phase. Next, cells were harvested and washed twice using ice-cold water. The cells were normalized to 200 OD600 and suspended in homogenization buffer [50 mM Tris (pH 7.5), EDTA (2.5 mM)] containing 1 mM PMSF, 10 mM sodium fluoride, 1 mM sodium orthovanadate and 1X protease inhibitor cocktail. The 0.5 mm acid-washed glass beads were added to the cells, and cells were lysed using mechanical force. The supernatant was collected after centrifugation at 2000 rpm for 15 min at 4°C, and an aliquot was saved as total cell lysate fraction. After ultracentrifugation (SW 41 Ti rotor) of the remainder supernatant at 25000 rpm for 35 min at 4°C, the pellet was suspended in protease inhibitor-containing buffer [10 mM Tris (pH 7.5), 0.5 mM EDTA, and 10% glycerol and protease inhibitors]. One aliquot was saved as total membrane fraction. Next, the discontinuous sucrose gradient [43.5 and 53.5% (w/v)] ultracentrifugation was carried out at 35000 rpm for 5 h. The plasma membrane fraction was collected from the middle ring, and ultracentrifuged at 38000 rpm for 30 min. The PM pellet was suspension in solution containing 6 M Gn-HCl and 0.1 M Tris (pH 8.8). The PM preparation quality was tested using anti-Pma1 antibody. Samples were collected in two biological replicates, and sent on dry ice to Valerian Chem Pvt Ltd, New Delhi, India.

Peptide Separation: At the Valerian Chem facility, the label-free quantitative mass

spectrometry analysis was conducted on Ultimate 3000 RSLCnano system coupled with an Orbitrap Eclipse. This analysis involved sample reduction with 5 mM TCEP, followed by alkylation with 50 mM iodoacetamide, and digestion with Trypsin (1:50, Trypsin/lysate ratio) for 16 h at 37°C. Samples with the search name S1 refers to *C. glabrata* wild-type replicate 1, S2 refers to *C. glabrata* wild-type replicate 2, S3 refers to Cgvps34? replicate 1, and S4 refers to Cgvps34? replicate 2.

Protein Characterization: RAW files were analysed with the Proteome Discoverer (v2.2) against the Uniprot *C. glabrata* reference proteome database. For Sequest search, precursor and fragment mass tolerances were set at 10 ppm and 0.5 Da, respectively. Both peptide spectrum match and protein false discovery rate were set to 0.01 FDR.

Experiment Type: Shotgun proteomics

Species: Data in species_details No Data

Tissue: Unknown No Data

Cell Type: Unknown No Data

Disease: Unknown No Data

Instrument Details: Data in instrument_details Data in instrument_details

Protein Modifications: iodoacetamide derivatized residue

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