

Supplementary Information

The VPS33B-VPS16B protein complex facilitates Golgi ribbon formation and Golgi-endosome trafficking

Helen HY Yao^{1,2}, Tanya YT Shi^{1,2}, Seoyoun Jung^{1,2}, Chang Hua Chen^{1,2}, Ryan W Li¹, Richard Leung¹, Ling Li¹, Fred G Pluthero¹, David Chitayat^{3,4,5}, Brian Raught⁶, Walter HA Kahr^{*1,2,7}

¹ Cell and Systems Biology, Research Institute, Hospital for Sick Children, Toronto, ON, M5G 0A4, Canada.

² Department of Biochemistry, University of Toronto, Toronto, ON, M5S 1A8, Canada

³ Division of Clinical and Metabolic Genetics, Department of Pediatrics, The Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada.

⁴ Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada.

⁵ The Prenatal Diagnosis and Medical Genetics Program, Department of Obstetrics and Gynecology, Mount Sinai Hospital, University of Toronto, Toronto, Ontario, Canada.

⁶ Princess Margaret Cancer Centre, University Health Network, and Department of Medical Biophysics, University of Toronto, Toronto, ON, Canada

⁷ Division of Haematology/Oncology, Department of Paediatrics, The Hospital for Sick Children, University of Toronto, Toronto, ON, M5G 1X8, Canada

Corresponding author:

Walter HA Kahr

Departments of Paediatrics & Biochemistry, University of Toronto

Division of Haematology/Oncology,

Cell Biology Program,

The Hospital for Sick Children,

555 University Avenue

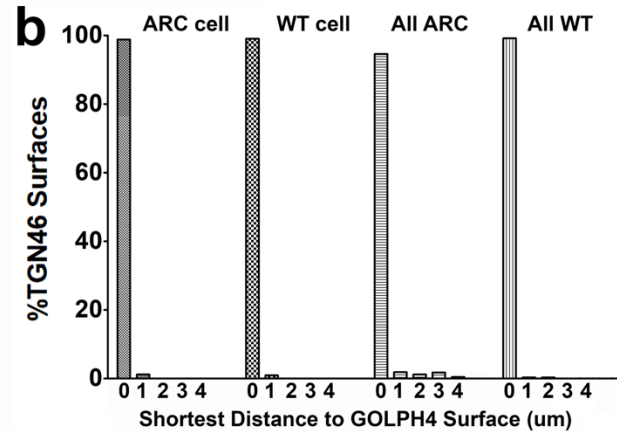
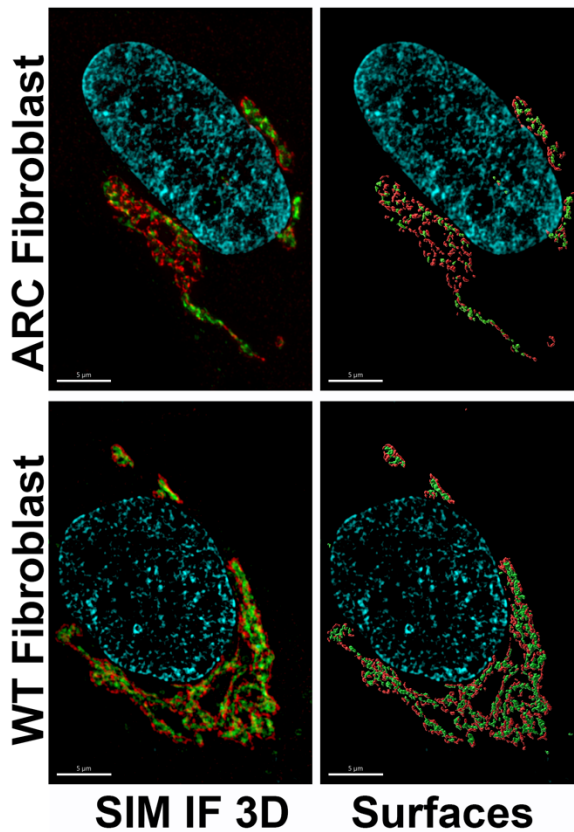
Toronto, ON, M5G 1X8, CANADA

Tel: +1-416-813-7977

Fax: +1-416-813-5327

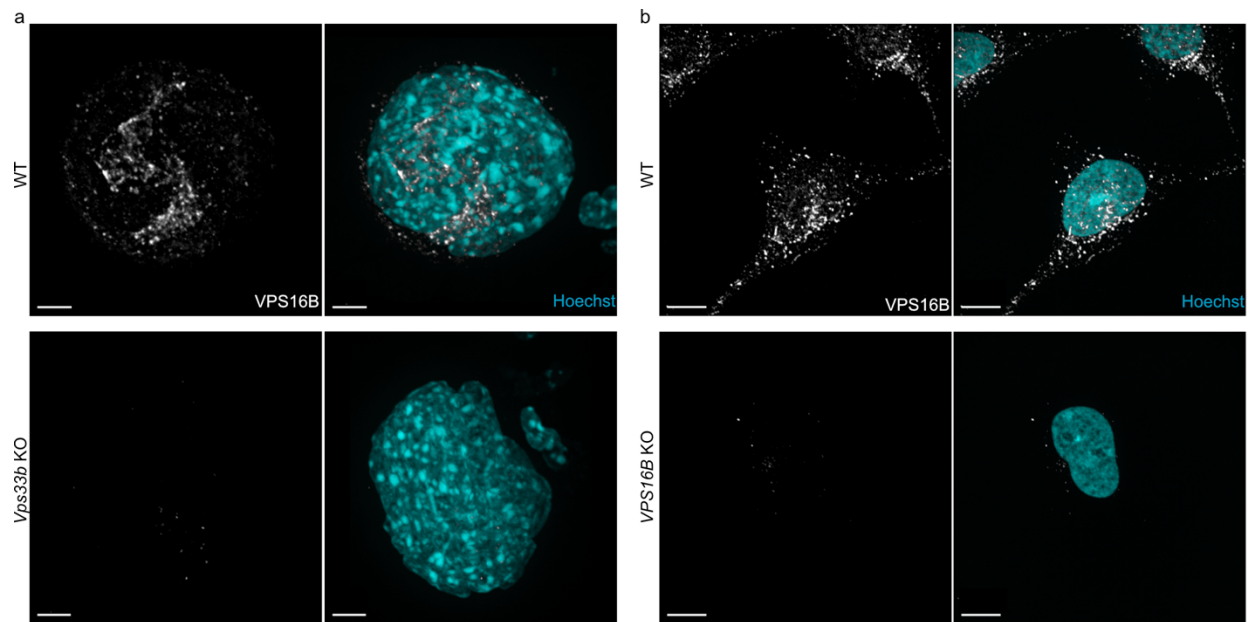
Email: walter.kahr@sickkids.ca

a TGN46 GOLPH4 DNA

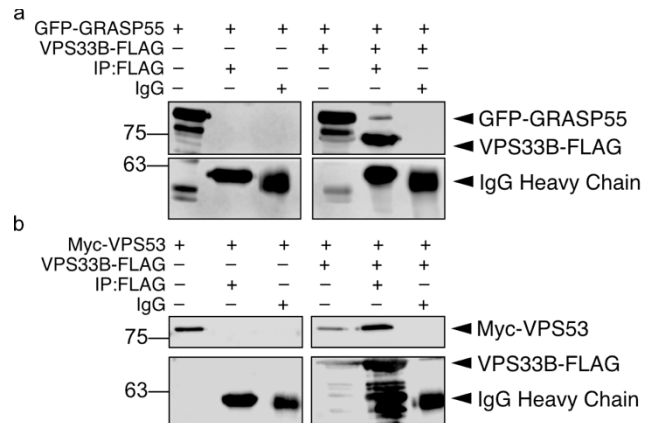


Supplementary Figure 1. Assessment of VPS33B-VPS16B deficiency on Golgi stack formation.

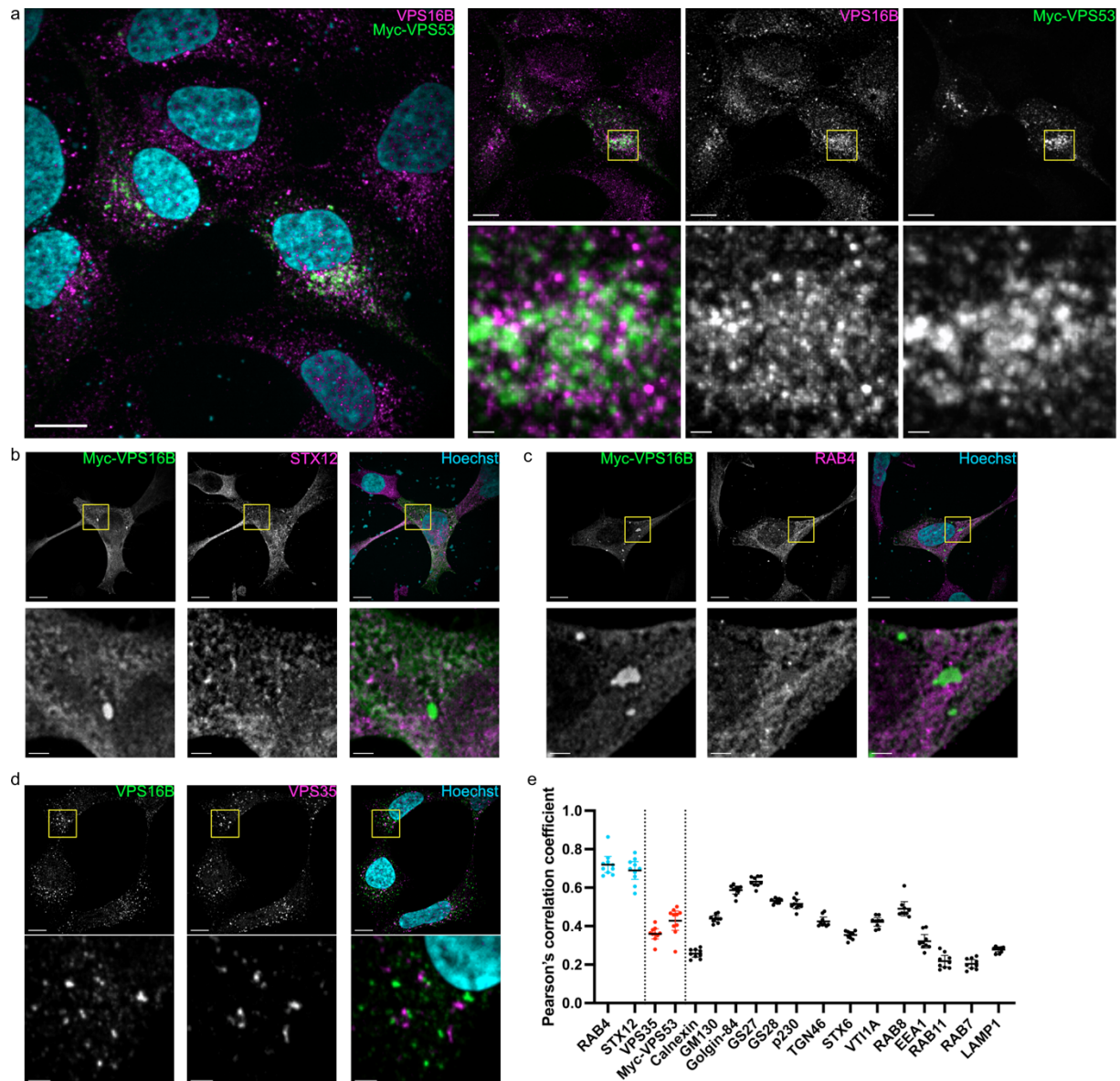
The effects of VPS33B-VPS16B deficiency on Golgi stack formation were assessed by imaging proteins associated with cis (GOLPH4) or trans (TGN46) Golgi (as well as nuclear DNA) in fibroblasts from an ARC patient (top) and wild-type cells (bottom) using immunofluorescence structured illumination microscopy (approximate XY resolution = 100 nm). (a) 3D image renders of Golgi in individual cells (left) were used to generate surface objects (right; bars = 5 μm). (b) The shortest distance to a GOLPH4 surface was measured for individual TGN46 objects via the Imaris 10 statistics function. Data are presented as frequency distributions for the cells shown in (a), and for total TGN46 objects detected in 10 ARC patient cells ("All ARC" - 1019 objects) and 6 WT cells ("All WT" - 1692 objects; a cutoff of 5 μm was used). These comparisons indicate close association of cis and trans Golgi elements in both WT and ARC cells, where a majority of surfaces are closer than the maximum resolving distance of these images (approximately 100 nm in XY dimensions, and 200 nm in Z). Thus, the relatively disordered appearance of Golgi in ARC fibroblasts likely does not reflect gross disruption of Golgi stacks.



Supplementary Figure 2. VPS16B antibody validation in murine megakaryocytes and HEK293 cells. (a) Representative immunofluorescence images of WT (top) or induced hematopoietic lineage-specific *Vps33b* KO (bottom) cultured mouse megakaryocytes stained with anti-VPS16B antibody (Scale bar = 10 μm). Hoechst nuclear staining is shown in cyan. (b) Representative immunofluorescence images of WT or VPS16B KO HEK293 cells stained with anti-VPS16B antibody. Hoechst nuclear staining is shown in cyan. (Scale bar = 10 μm).



Supplementary Figure 3. Co-immunoprecipitation experiments using WT HEK293 cells to assess interactions between VPS33B and GRASP55 or VPS53. Positive interaction are evident between overexpressed GFP-GRASP55 (**a**) or Myc-VPS53 (**b**) and VPS33B-FLAG. Negative controls are included without VPS33B-FLAG expression. IgG Heavy chains are blotted to indicate the presence of anti-FLAG-conjugated beads.



Supplementary Figure 4. Localization of VPS16B relative to VPS53, STX12, RAB4 and VPS35 in HEK293 cells. Representative high-resolution confocal fluorescence microscopy images are shown for HEK293 cells (cyan = DNA) co-stained for: **(a)** endogenous VPS16B (magenta) and Myc-VPS53 (green); **(b)** Myc-VPS16B and endogenous STX12; **(c)** Myc-VPS16B and endogenous RAB4; **(d)** endogenous VPS16B and VPS35; maximum intensity renders, (Scale bar = 10 μ m, inset: 2 μ m). **(e)** Pearson's correlation coefficient values from voxel colocalization analysis of these protein pairs, with data from Figure 2b shown in black. Blue data points indicate overexpressed Myc-VPS16B. Red data points indicate native VPS16B.