A novel sub-precision detection method (MCS-DETECT) identifies shape complexity of mitochondria-ER contacts (MERCs) in 3D STED super-resolution microscopy

Ben Cardoen[*1], Kurt Vandevoorde [2*], Guang Gao [2*], Parsa Alan [2], William Liu [2], A. Wayne Vogl [2], Ghassan Hamarneh [1#], Ivan Robert Nabi [2#]

1 Simon Fraser University, British Columbia, Canada
2 University of British Columbia, British Columbia, Canada
*,# Equal contribution

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With the inter-organelle distance of mitochondria-ER contacts (MERCs) below the resolution of 3D fluorescence and super-resolution microscopy, identification and morphological analysis of MERCs is restricted. We illustrate how a novel Membrane Contact Site detection algorithm (MCS-DETECT) is able to accurately reconstruct sub-precision MERCs from 3D STED super-resolution microscopy and describe their morphological diversity. Our approach reconstructs the sub-precision MERC interface using a windowed Spearman correlation of the 2nd intensity differential, making the approach robust against inherent fluctuations in fluorescence markers across channels and datasets. To enable quantitative analysis, we compute shape features of the produced contacts, as well as a confidence map to report on reliability of contact detection. We validate MCS-DETECT by a parallel electron microscopy (EM) study of elongated ribosome-studded MERCs (riboMERCs), present in HT-1080 but not COS-7 cells. MCS-DETECT reconstructs large, tubular riboMERCs selectively in HT-1080 cells and identifies morphological differences between riboMERCs and large contacts induced by expression of an ER-mitochondria linker in COS-7 cells. MCS-DETECT registers decreased large riboMERCs in Gp78 knockout HT-1080 cells, and increased riboMERCs, that retain the elongated, tubular morphology, upon overexpression in COS-7 cells of wild-type Gp78 but not a Ring finger mutant Gp78 lacking ubiquitin ligase activity. Gp78-dependent riboMERCs present complex tubular shapes that intercalate between and contact multiple mitochondria. MCS-DETECT applied to whole cell 3D super-resolution microscopy therefore shows that Gp78 ubiquitin ligase activity regulates the formation of novel tubular shaped riboMERCs.
Supported by CIHR grants PJT-148698 and PJT-183625.

Novel Sub-Precision Detection Method (MCS-DETECT) Identifies Shape Complexity of Mitochondria-ER contacts (MERCS) in 3D STED Super-resolution Microscopy

P1032 - B33

Ben Cardoen†, #, Kurt Vandevoorde‡, #, Guang Gao‡, #, William Liu‡, Ellia Tiliakou‡, A. Wayne Vogl‡, Ghassan Hamarneh†*, Ivan Robert Nabi ‡, §*

† Medical Image Analysis Laboratory, School of Computing Science, Simon Fraser University
‡ Department of Cellular and Physiological Sciences, Life Sciences Institute, University of British Columbia
§ School of Biomedical Engineering, University of British Columbia, Vancouver, British Columbia, Canada

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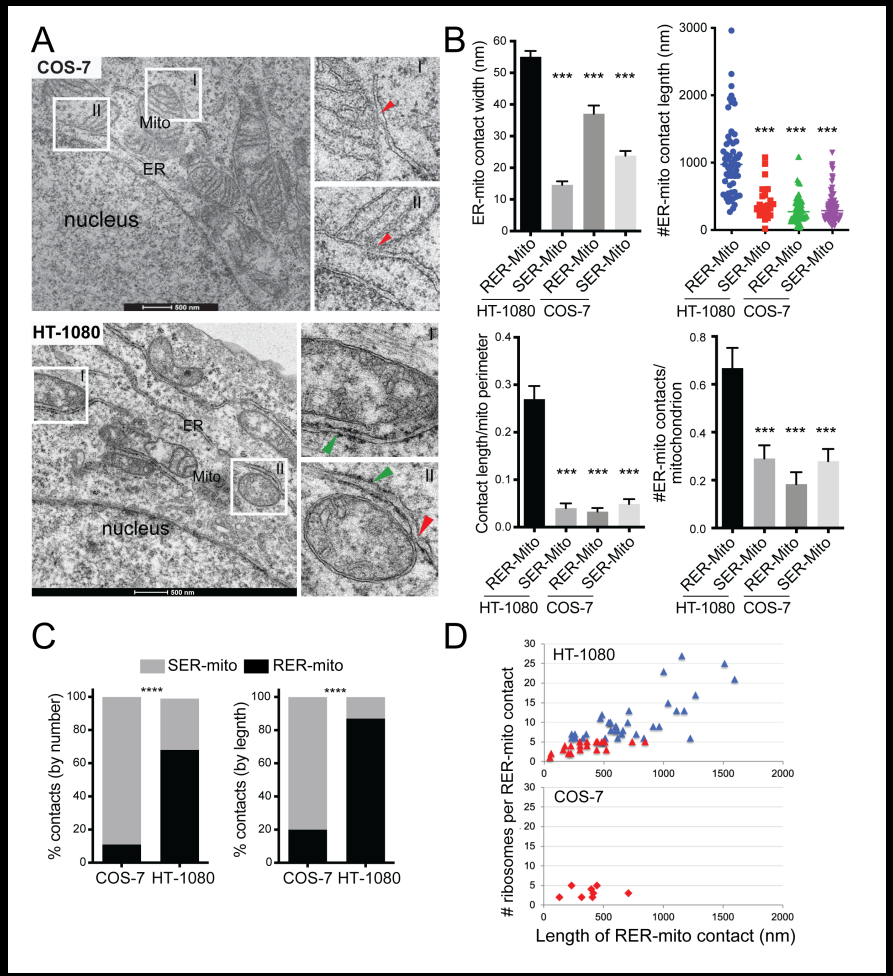
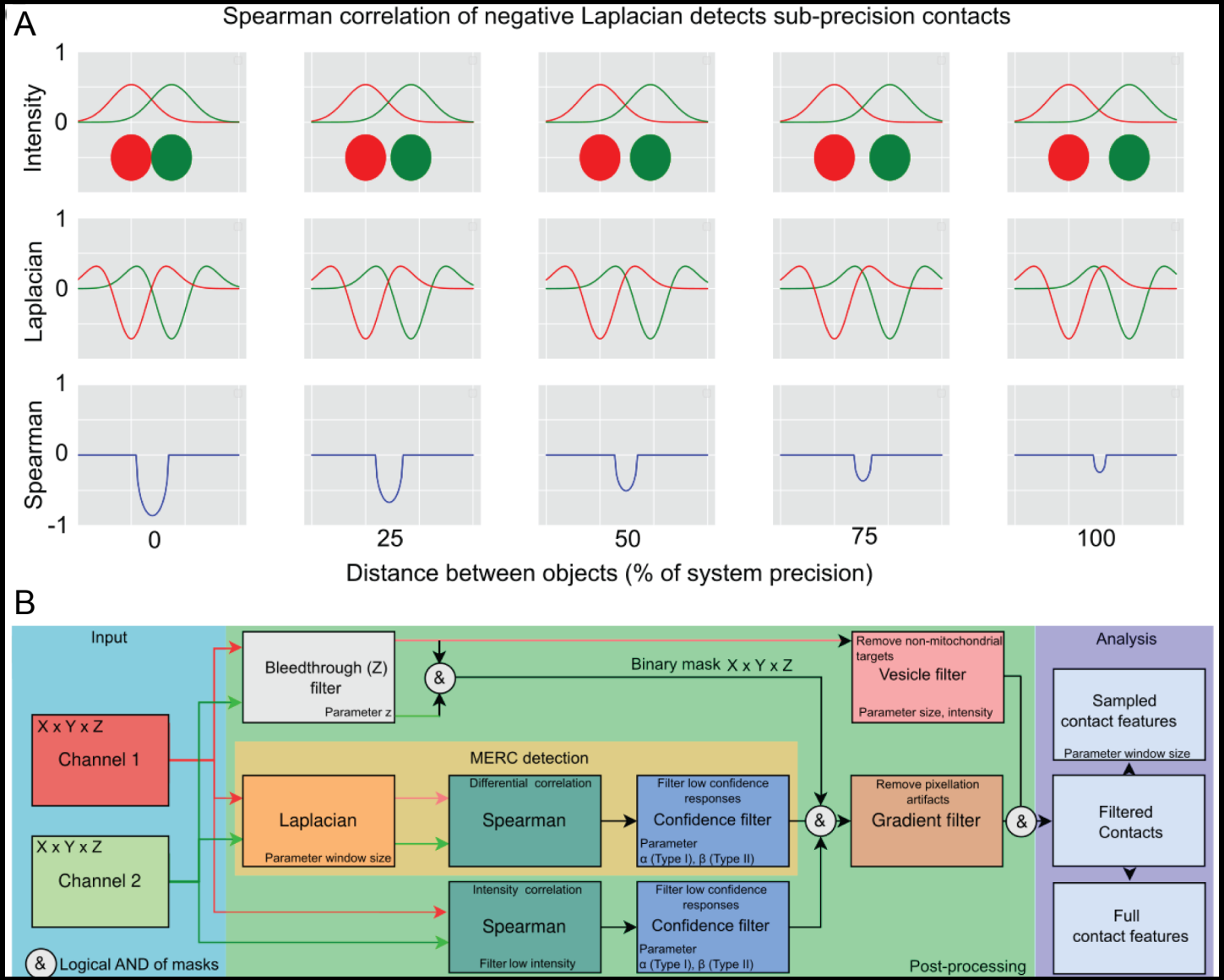
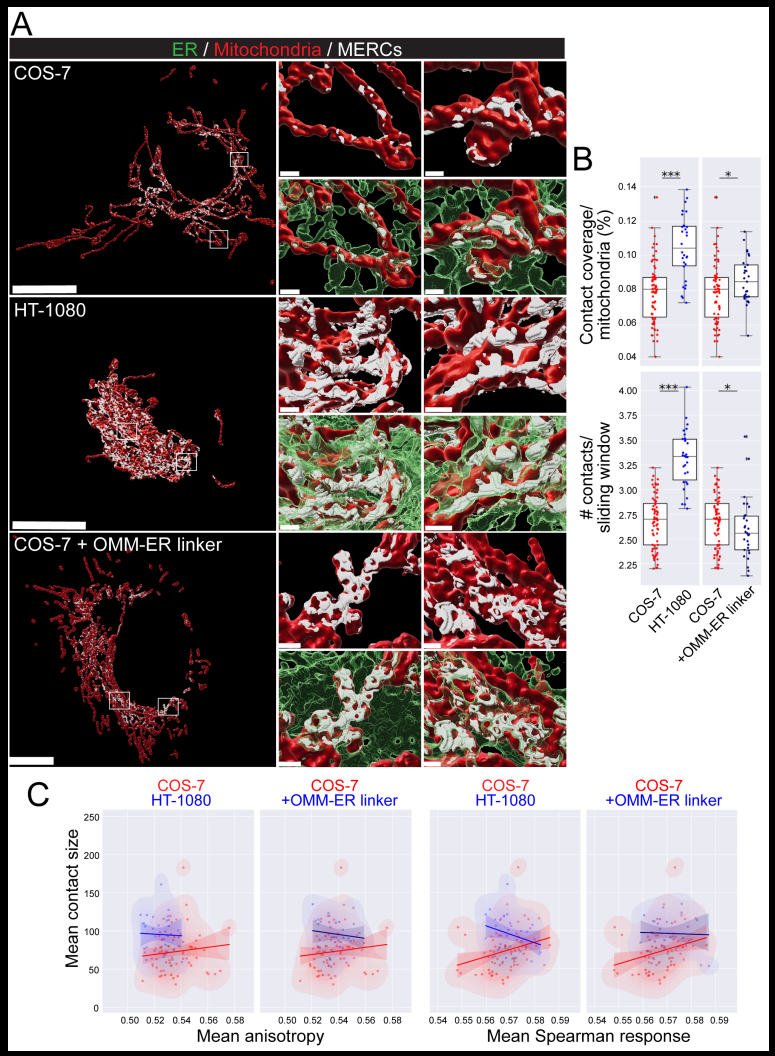
EM: MERCs in HT-1080 and COS-7 cells shows distinct differences



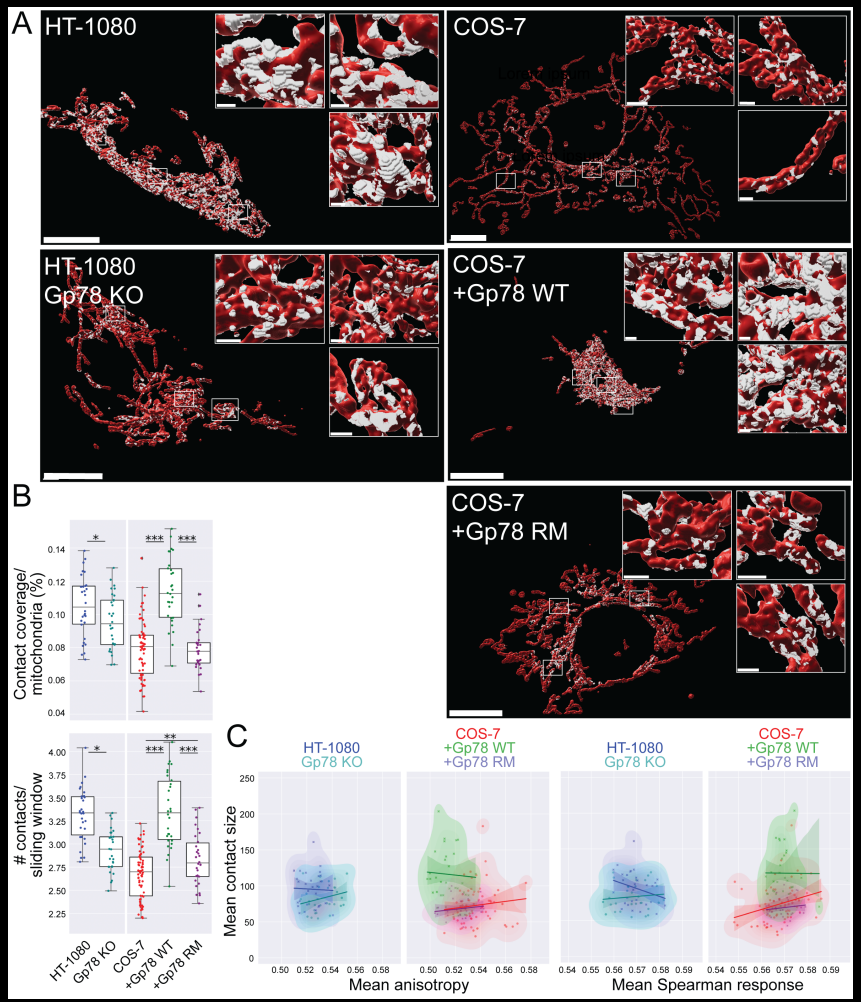
MCS: Reconstructing sub-precision contacts



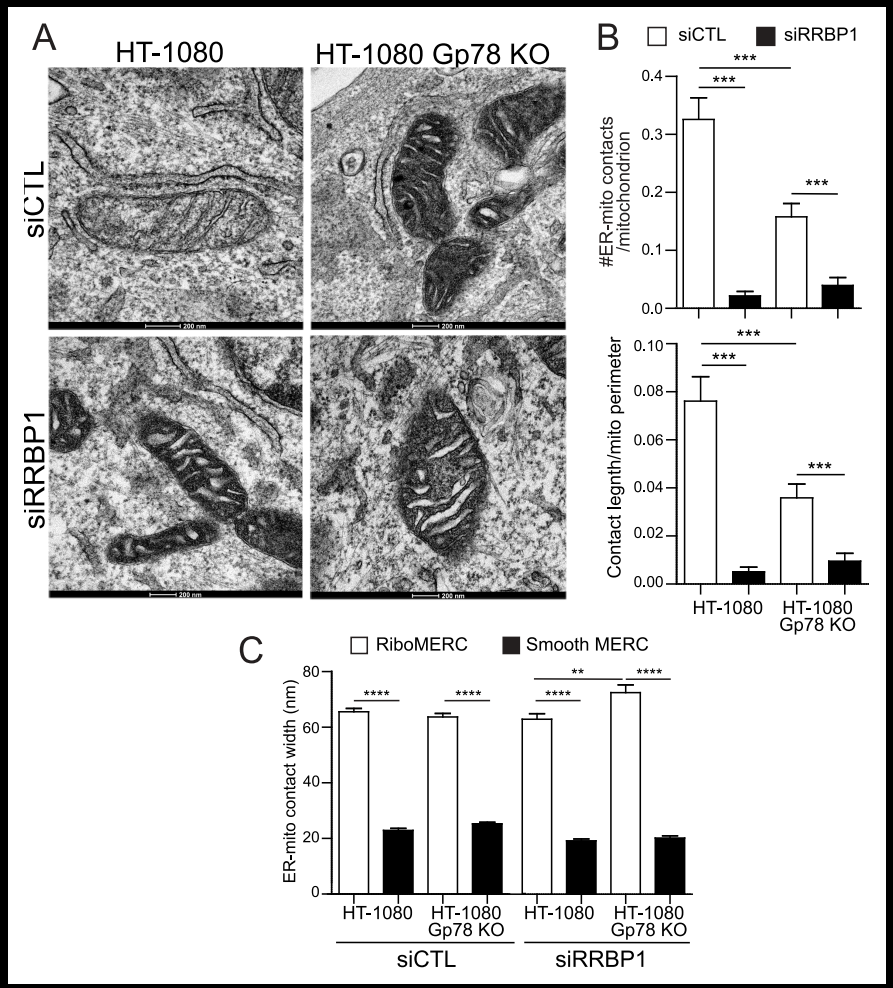
MCS-Detect recovers distinct MERC signatures in HT-1080 and COS-7 in STED and shows they are regulated by Gp78



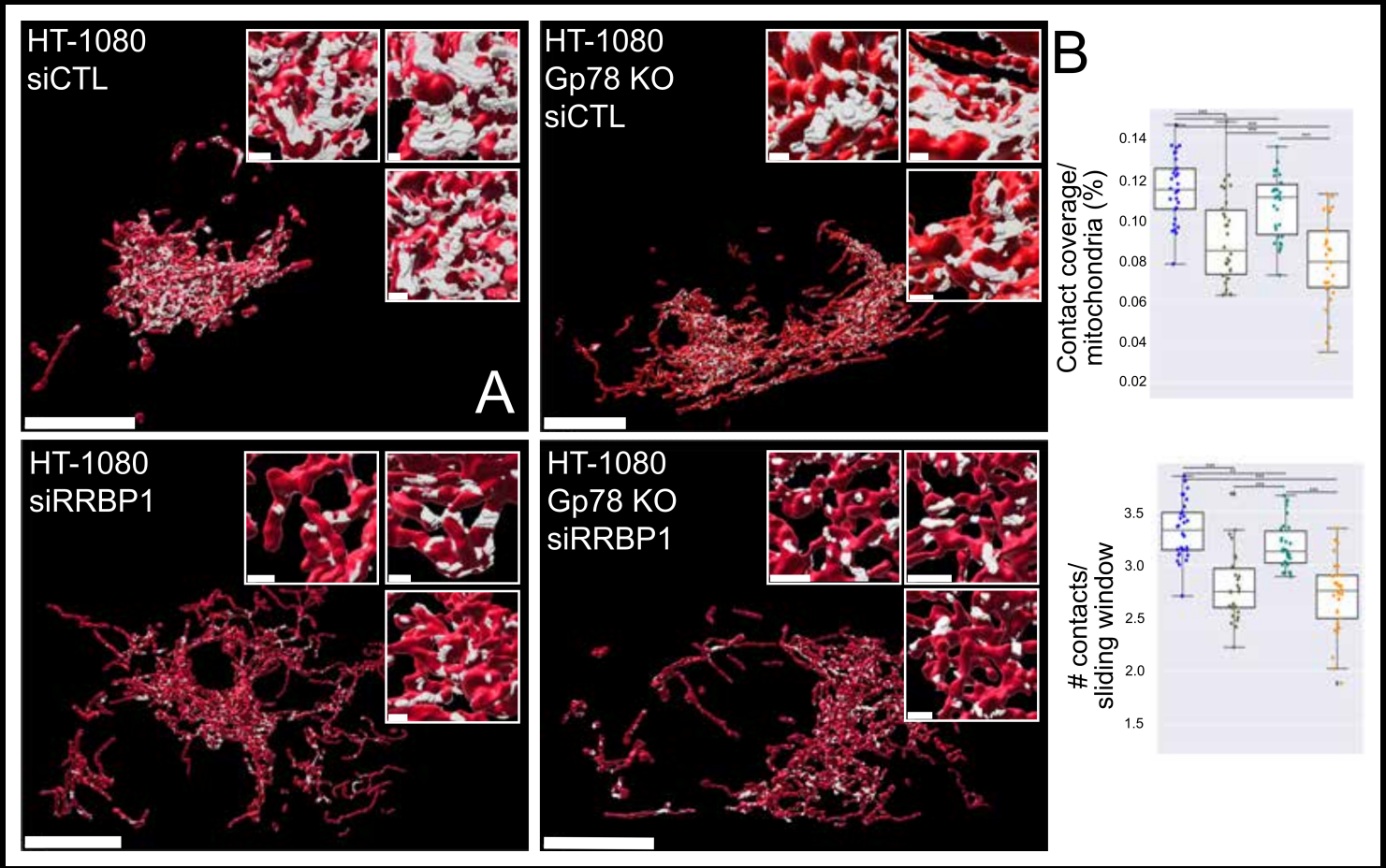
Gp78 regulates contact profiles in HT-1080 and COS-7 (STED)



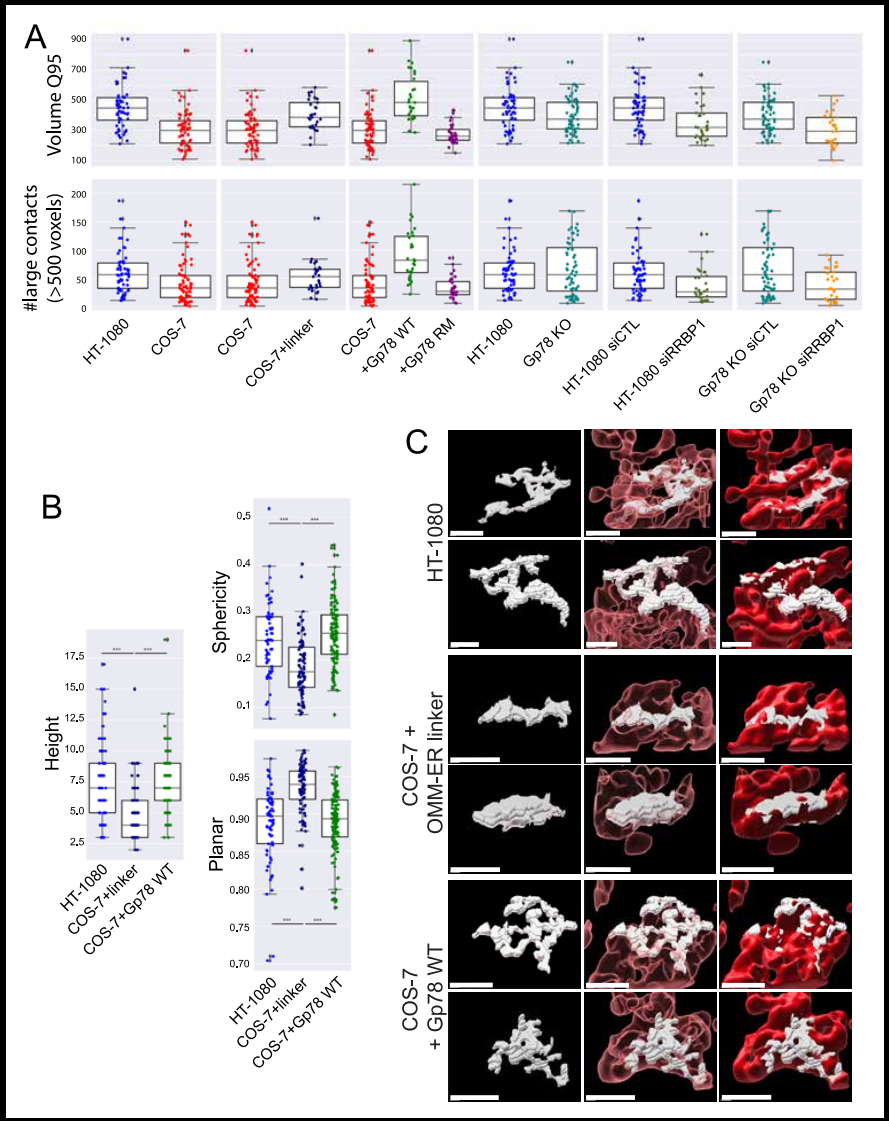
RRBP1 knockdown reduces riboMERCS independent of Gp78



MCS-DETECT captures changes induced by RRBP1 knockdown



Gp78 induces convoluted tubular MERCs



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- SFU Research Computing Group

University of British Columbia

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- Kurt Vandevoorde
- Dr. Guang Gao
- Parsa Alan
- William Liu
- Ellie Tiliakou
- Prof. A. Wayne Vogl

Preprint (under revision JCB Tools) ↓

https://www.biorxiv.org/content/10.1101/2022.06.23.497346v2

Poster ↓

https://bit.ly/mercs_2022

Code ↓

https://github.com/bencardoen/SubPrecisionContactDetection.jl

Contact ↓

bcardoen@sfu.ca

NOVEL SUB-PRECISION DETECTION METHOD (MCS-DETECT) IDENTIFIES SHAPE COMPLEXITY OF MITOCHONDRIA-ER CONTACTS (MERCS) IN 3D STED SUPER-RESOLUTION MICROSCOPY

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[†]School of Computing Science, Simon Fraser University, Burnaby, British Columbia, Canada
[‡]Life Sciences Institute, University of British Columbia, Vancouver, British Columbia, Canada
[§]School of Biomedical Engineering, University of British Columbia, Vancouver, British Columbia, Canada

#, * equal contribution

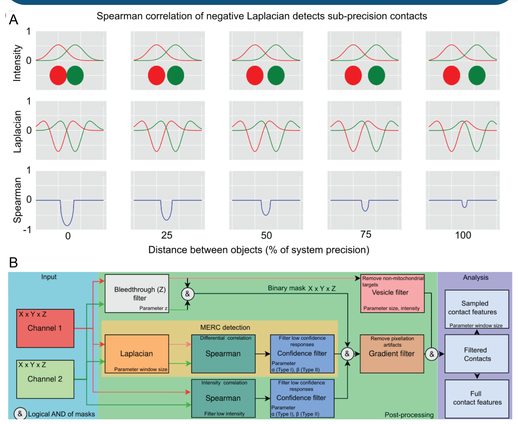
bcardoen@sfu.ca
https://github.com/bencardoen/SubPrecisionContactDetection.jl

Abstract

With the inter-organelle distance of mitochondria-ER contacts (MERCs) below the resolution of 3D fluorescence and super-resolution microscopy, identification and morphological analysis of MERCs is restricted. We illustrate how a novel Membrane Contact Site detection algorithm (MCS-DETECT) is able to accurately reconstruct sub-precision MERCs from 3D STED super-resolution microscopy and describe their morphological diversity. Our approach reconstructs the sub-precision MERC interface using a windowed Spearman correlation of the 2nd intensity differential, making the approach robust against inherent fluctuations in fluorescence markers across channels and datasets. To enable quantitative analysis, we compute shape features of the produced contacts, as well as a confidence map to report on reliability of contact detection. We validate MCS-DETECT by a parallel electron microscopy (EM) study of elongated ribosome-studded MERCs (riboMERCs), present in HT-1080 but not COS-7 cells. MCS-DETECT reconstructs large, tubular riboMERCs selectively in HT-1080 cells and identifies morphological differences between riboMERCs and large contacts induced by expression of an ER-mitochondria linker in COS-7 cells. MCS-DETECT registers decreased large riboMERCs in Gp78 knockout HT-1080 cells, and increased riboMERCs, that retain the elongated, tubular morphology, upon overexpression in COS-7 cells of wild-type Gp78 but not a Ring finger mutant Gp78 lacking ubiquitin ligase activity. Gp78-dependent riboMERCs present complex tubular shapes that intercalate between and contact multiple mitochondria. MCS-DETECT applied to whole cell 3D super-resolution microscopy therefore shows that Gp78 ubiquitin ligase activity regulates the formation of novel tubular shaped riboMERCs.

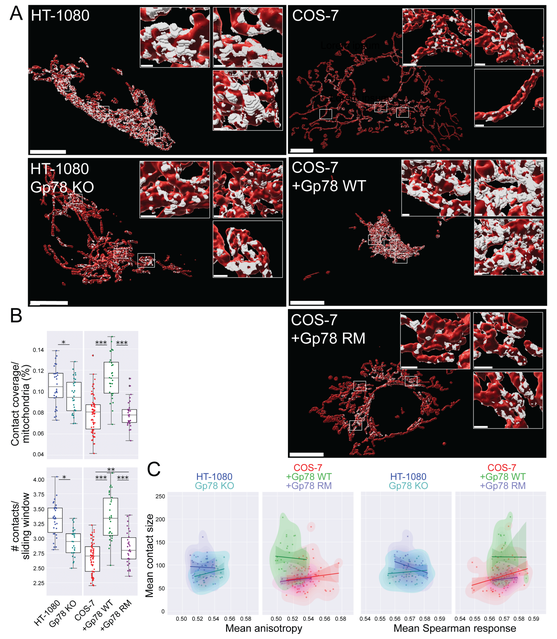
We are grateful the Digital Research Alliance of Canada for computational resources, and the research computing staff at SFU and UBC for their assistance in executing this project. Supported by Canadian Institutes for Health Research (CIHR) Project Grant AWD-022443 (IRN, GH), and for grants (PJT-148698, PJT-183625) that made this work possible.

MCS-DETECT analysis of sub-precision contacts



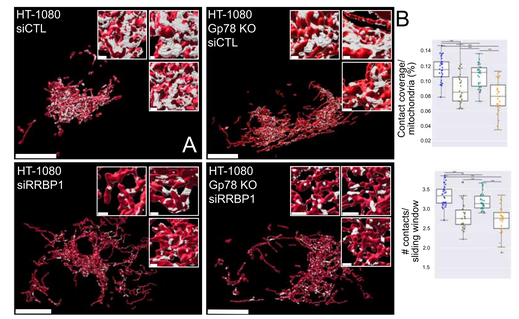
Detection principle on 2 simulated objects (A, red and green discs) at varying sub-precision distances, intensity profiles (top), 2nd derivatives (Laplacian), and Spearman correlations of negative Laplacian (bottom), decreasing with increasing sub-precision distance. Detection algorithm (B) with stages that address specific confounding factors introduced by acquisition (bleedthrough) or sample (vesicle removal).

Gp78 regulation of riboMERCs



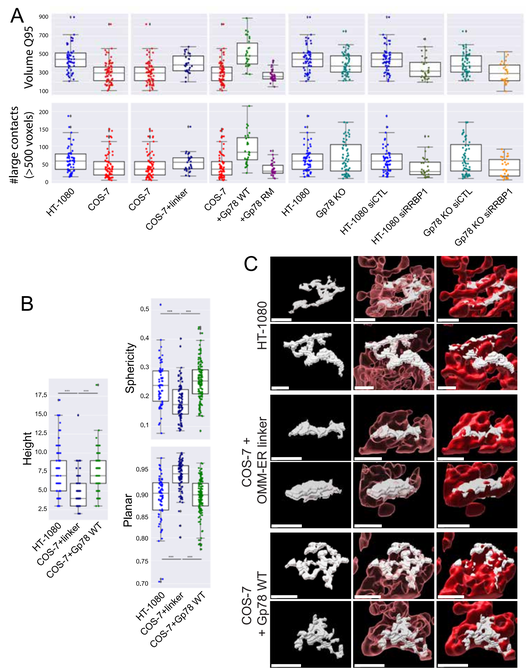
Cells expressing ERmoxGFP and labelled for TOM20 (red) with contact sites (white) for HT-1080 and Gp78 KO HT-1080 cells and for untransfected COS-7 cells and COS-7 cells overexpressing wild-type (WT) Gp78 or RING-finger domain mutated Gp78 (Gp78 RM). Knockout of Gp78 in HT-1080 causes contacts in HT-1080 to acquire feature to those in COS-7, with overexpression (+Gp78 WT) in COS-7 causing a reversal to HT-1080 contacts. Quantification per sampled mitochondria window (B-C), mean/cell, 2 MWU, n=30; Bar = 10 µm whole cell; 1 µm insets)

MCS-DETECT captures changes induced by RRBP1 knockdown



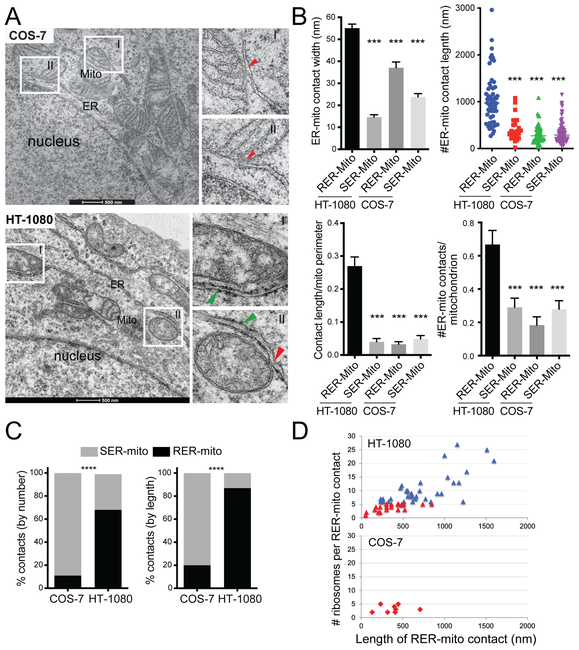
A) MCS-DETECT views of HT-1080 WT and Gp78 KO cells treated with either siControl or siRRBP1. Mitochondria are labelled with TOMM20 (red) and MERCS are visualized in white, with quantification (B-C). (mean/cell, 2-sided MWU, n=30; Bar = 10 µm whole cell; 1 µm insets).

Gp78 induces convoluted tubular MERCs



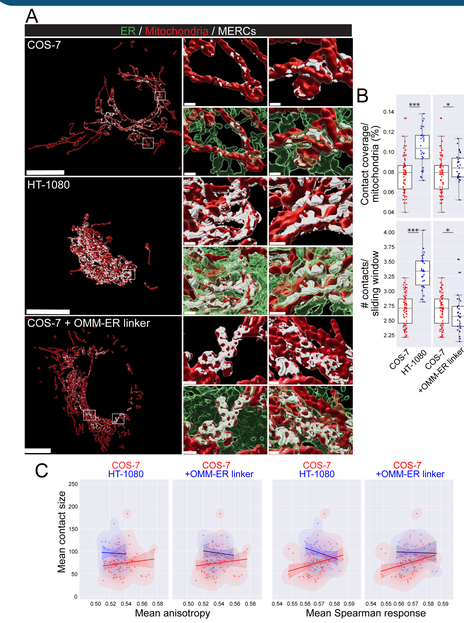
95th quantile of MERC volume/cell (A, Q95V), # of MERCs/cell > mean 500 voxel size of HT-1080 Q95V MERCs, for HT-1080, COS-7, COS-7 overexpressing OMM-ER linker, Gp78 WT , Gp78 RM, Gp78 KO HT-1080, HT-1080 cells transfected with siCTL and siRRBP1, and Gp78 KO HT-1080 cells transfected with siCTL, and siRRBP1. Representative cell whose Q95V is closest to the mean Q95V of the HT-1080 and COS-7 cells overexpressing either the OMM-ER linker or Gp78 WT was selected for analysis (B), showing that COS-7 OMM-ER linker induced contacts have markedly different shape signature compared to those in HT-1080 and COS-7 Gp78OE (i.e. riboMERCS). Q95V MERCs (C, white) from HT- 1080 cells and COS-7 cells expressing OMM-ER linker or wild-type Gp78 are shown alone or adjacent to transparent (pink) or solid mitochondria (red) to highlight intercalation of riboMERCs with mitochondria. Bar = 1 µm.

Quantitative EM analysis of ER-mitochondria contacts in HT-1080 and COS-7 cells



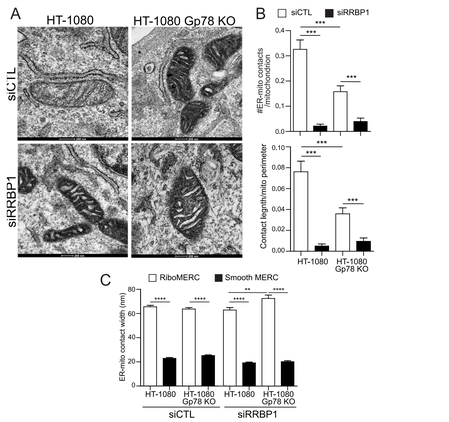
EM images of HT-1080 and COS-7 cells (A), insets showing RER- mitochondria contacts (RER-mito, HT-1080) and SER-mitochondria contacts (SER-Mito, COS-7). B-C) Quantification of contact features (B) and ratios (C). RER-mito contacts with ≤ 5 ribosomes are shown in red; > 5 ribosomes (specific to HT-1080 cells in blue) and defined as riboMERCs. Bar = 500nm).

MCS-DETECT identifies distinct contact profiles in HT-1080 & COS-7 cells



Volume rendered MCS-DETECT views (A) of cells expressing ERmoxGFP (green) and labelled for TOM20 (red) with contact sites overlaid (white) are shown for COS-7, HT-1080 and OMM-ER linker transfected COS-7 cells. COS-7 mito show numerous small contact zones, mito in HT-1080 and OMM-ER linker transfected COS-7 cells show more extended contact zones, quantified in B-C. (mean/cell, 2-sided MWU, n=30 ;Bar = 10 µm whole cell; 1 µm insets).; Bar 10-1 µm whole cell vs insets.)

RRBP1 knockdown reduces riboMERCS independent of Gp78



HT-1080 and HT-1080 Gp78 KO cells treated with either siControl or siRRBP1 (A, EM). Strong presence of riboMERCS in both HT-1080 WT and Gp78 KO cells, near absence in RRBP1 knockdown. Quantification in B-C.