

Interaction Analysis of Single Molecule Localization Microscopy Reveals Cavin1 Regulation of Caveolin-1 8S Complex Organization

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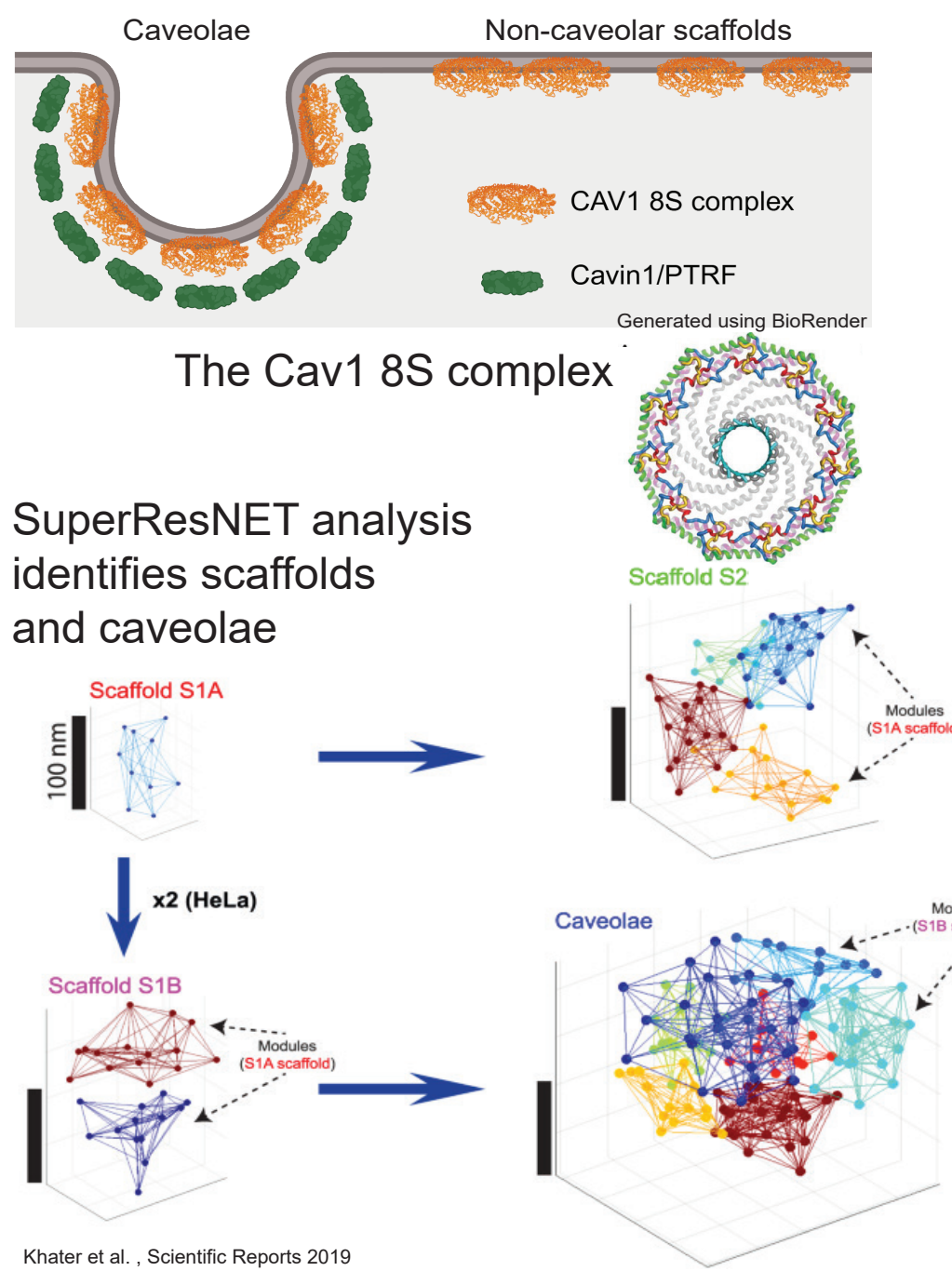
Abstract

Protein structures are now being resolved at the atomic level, but deciphering their molecular organization in the cell remains a challenge. SuperResNET is a machine learning-based analysis software for visualizing and quantifying 3D point cloud data acquired by single molecule localization microscopy (SMLM) approaches. Integrated HDCluster enables analysis of multiplex DNAPaint data and nanometer resolution MINFLUX. Caveolin-1 (Cav1) forms stable 11-mer disk-shaped 8S complexes whose oligomerization and interaction with Cavin1 are necessary for caveolae biogenesis. However, structural organization of Cavin1 and how it contributes to the organization of Cav1 8S complexes in caveolae and scaffolds remains unclear. SuperResNET analysis of spectral demixing dual-channel dSTORM and MINFLUX SMLM now shows that Cavin1 interacts with Cav1 predominantly in caveolae but also in heterologous scaffolds where it promotes 8S complex oligomerization. Within both caveolae and heterologous scaffolds, proximity to Cavin1 reduces inter-8S complex distance. Cavin1 organizes to form linear networks similar to the striations of the caveolar coat observed by deep etch electron microscopy. This suggests that close interaction of Cav1 8S complexes with striated Cavin1 promotes caveolae biogenesis and clustering of 8S complexes within caveolae.

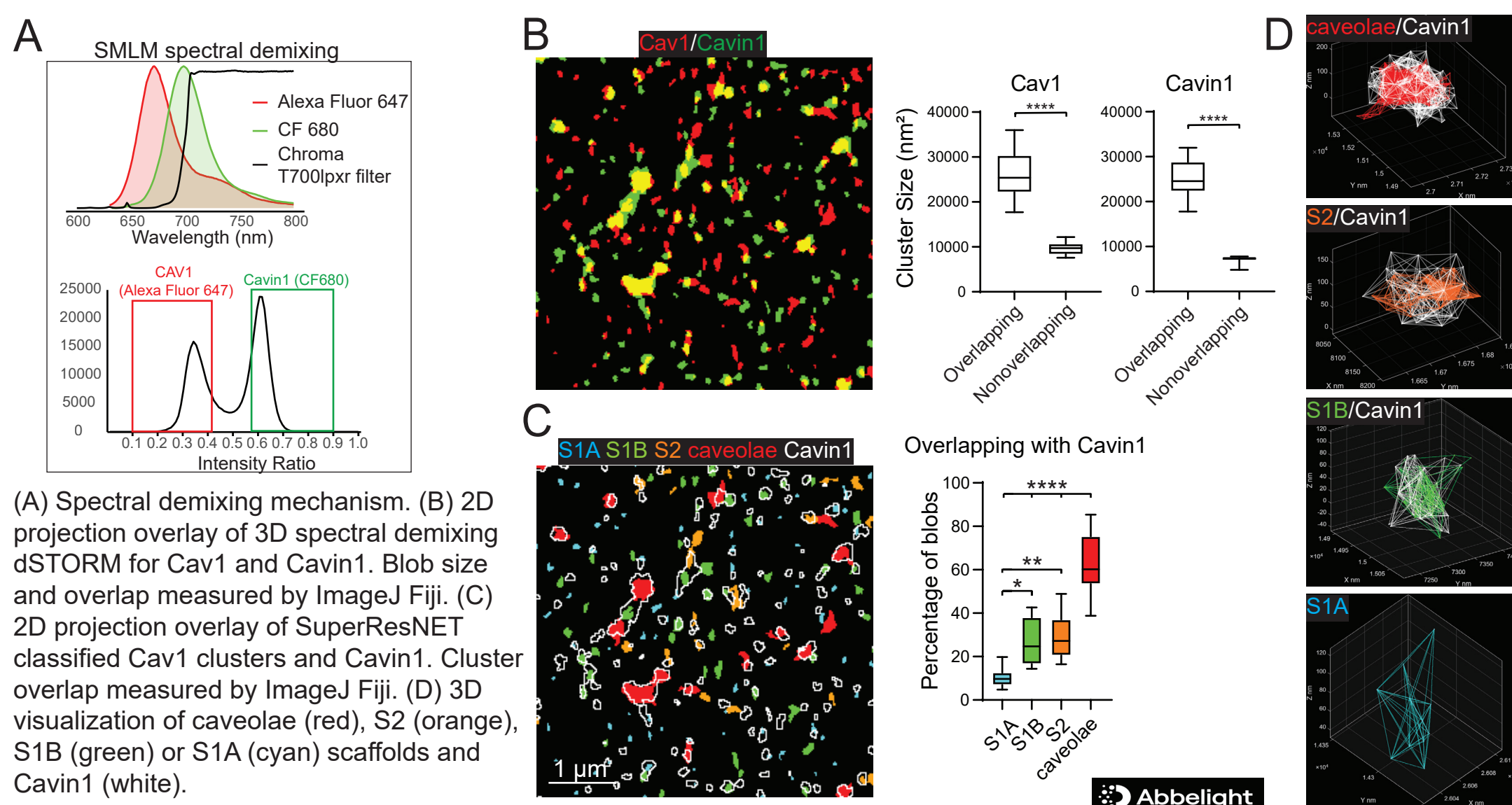
Supported by CIHR, Mitacs, FCRF and CNRS IRP.

Introduction

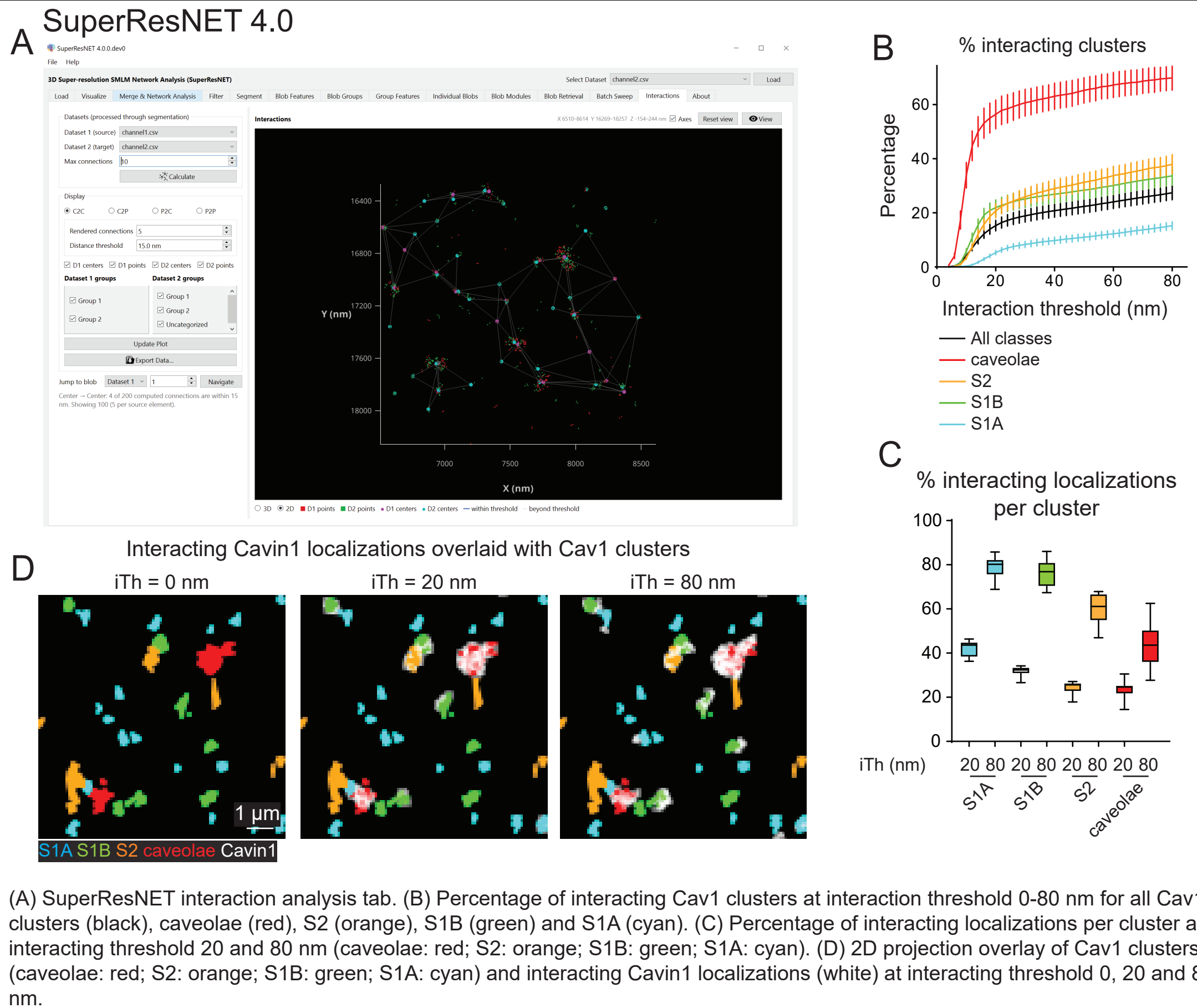
- Protein structures are now being resolved by CryoEM, AlphaFold modeling and molecular simulation at the atomic level, but deciphering their molecular organization in the cell remains a challenge
- Single molecule localization microscopy (dSTORM, DNA PAINT, RESI, MINFLUX) bridges the gap between structural biology and fluorescent microscopy and provide molecular resolution in the cell
- Caveolin-1 (Cav1) is an integral membrane protein that localizes to caveolae in presence of Cavin1 and to non-caveolar scaffolds in absence of Cavin1
- The Cav1 8S complex is the building block of caveolae and non-caveolar scaffolds but how 8S complexes are organized in relation to Cavin1 remains unclear



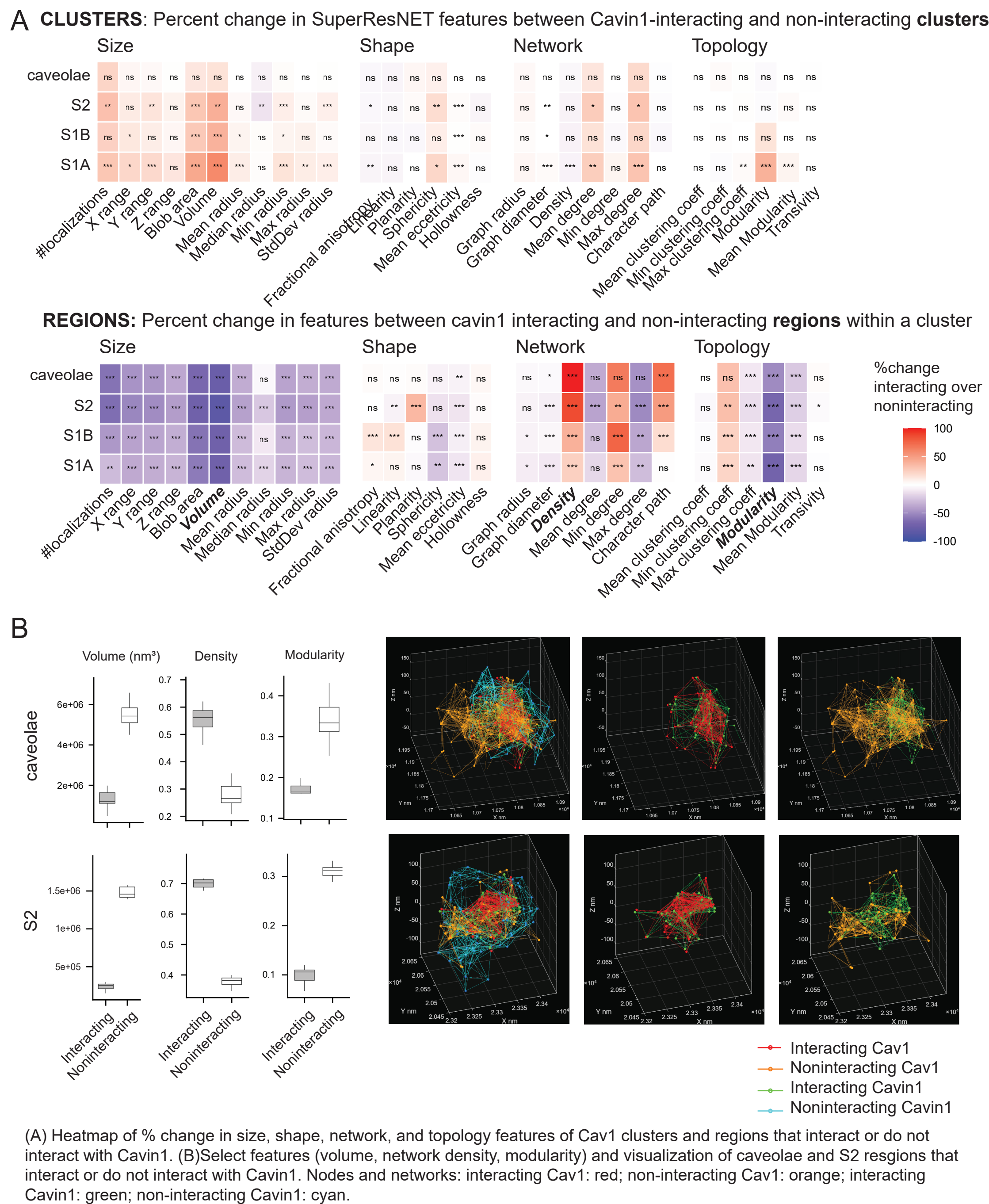
Spectral demixing dSTORM shows overlap of caveolin-1 and Cavin1



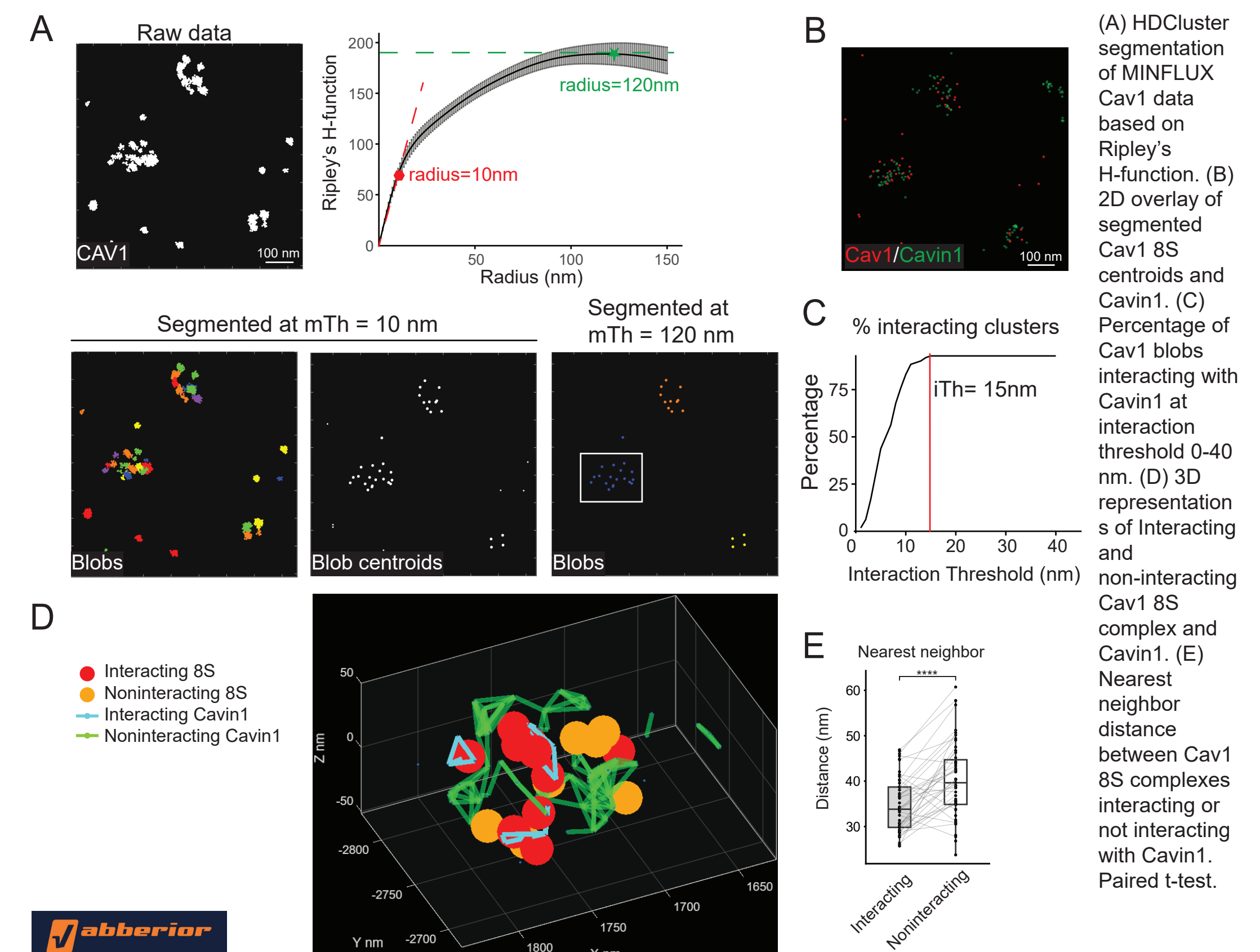
Interaction analysis of SMLM based on localization proximity



Regions of Cav1 clusters in proximity to Cavin1 show increased network density and decreased modularity



Spectral demixing MINFLUX shows closer Cav1 8S complex packing in proximity to Cavin1



Conclusion

SuperResNET:

- Introduce localization proximity-based interaction analysis for spectral demixing dSTORM and MINFLUX data to SuperResNET SMLM network analysis software

dSTORM:

- Caveolae and a smaller proportion of S2 and S1B scaffolds interact with Cavin1
- Regions of Cav1-labeled caveolae and larger scaffolds that interact more closely with Cavin1 are denser and less modular.

MINFLUX:

- Resolve 8S complexes in caveolae
- Cav1 8S complexes cluster in proximity to Cavin1