Actin-Spectrin Scaffold Supports Open Fenestrae in Liver Sinusoidal Endothelial Cells

Supplementary materials

Bartlomiej Zapotoczny[1,*], Filip Braet[2], Edyta Kus[3], Katarzyna Ginda-Mäkelä[4], Beata Klejevskaja[4], Roberto Campagna[3], Stefan Chlopicki[3,5], Marek Szymonski[1]

[1] Centre for Nanometer-Scale Science and Advanced Materials, NANOSAM, Faculty of Physics, Astronomy, and Applied Computer Science, Jagiellonian University, Krakow, Poland.

[2] School of Medical Sciences (Discipline of Anatomy and Histology) – Cellular Imaging Facility, Charles Perkins Centre – Australian Centre for Microscopy & Microanalysis, The University of Sydney, NSW 2006, Australia.

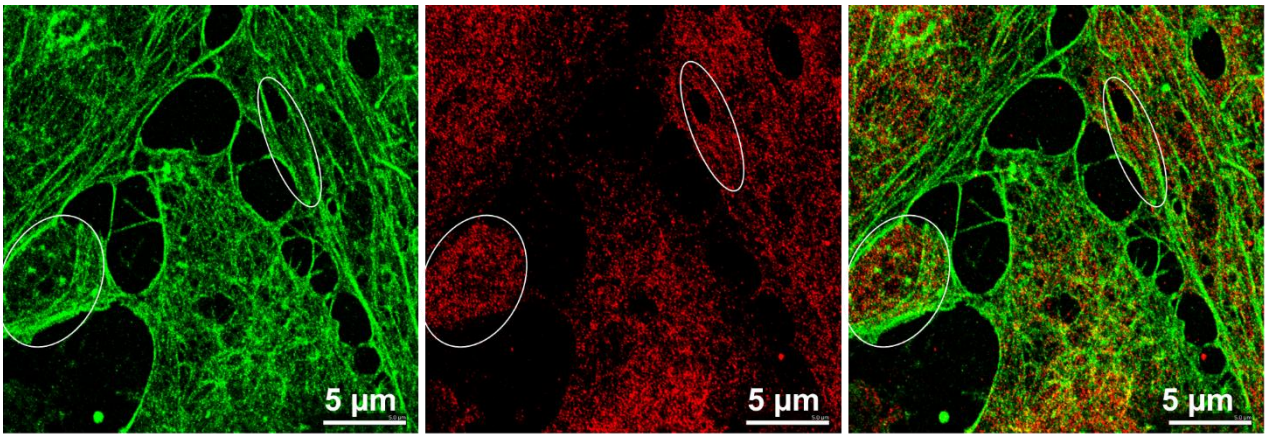
[3] Jagiellonian Centre for Experimental Therapeutics (JCET), Jagiellonian University, Krakow, Poland.

[4] ONI (Oxford Nanoimaging Ltd), Oxford, United Kingdom.

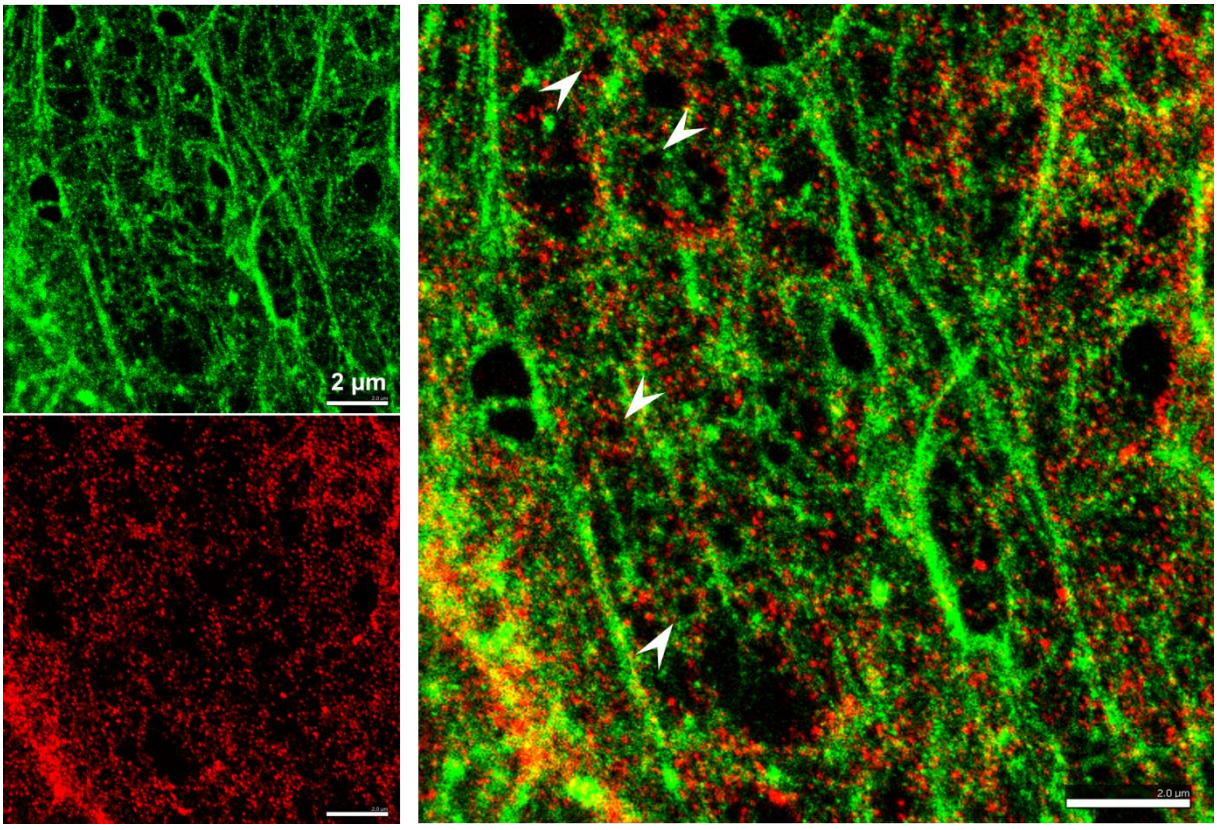
[5] Chair of Pharmacology, Jagiellonian University Medical College, Krakow, Poland.

*Contact information

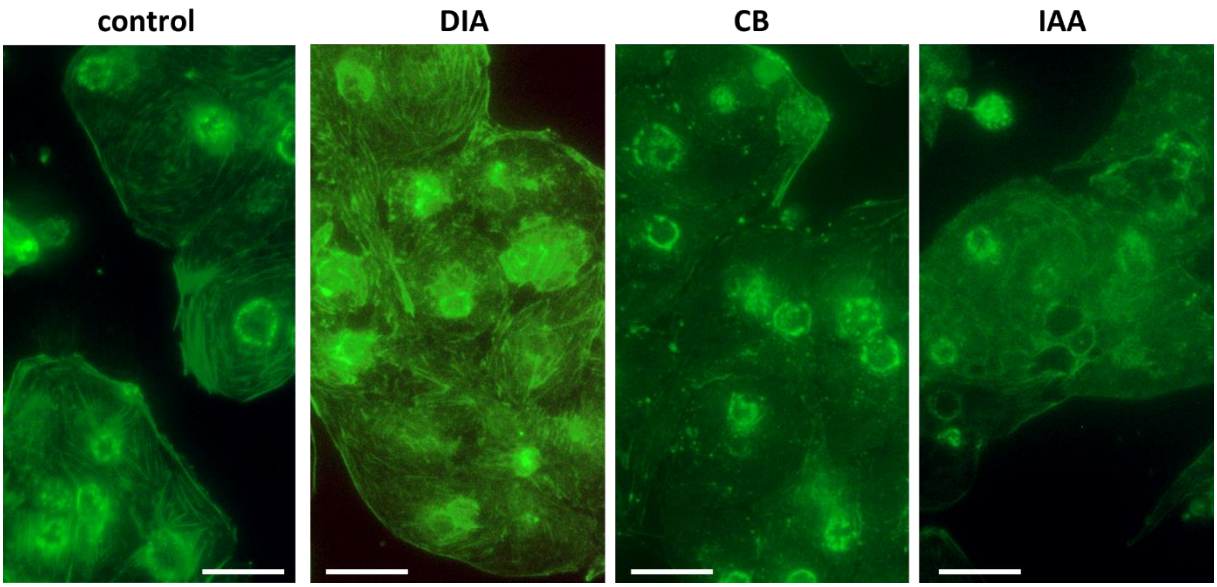
Corresponding author Bartlomiej Zapotoczny[1]. Tel.: +48 12 664 48 63; fax: +48 12 664 49 05. E-mail address: bartlomiej.zapotoczny@uj.edu.pl, (B. Zapotoczny).



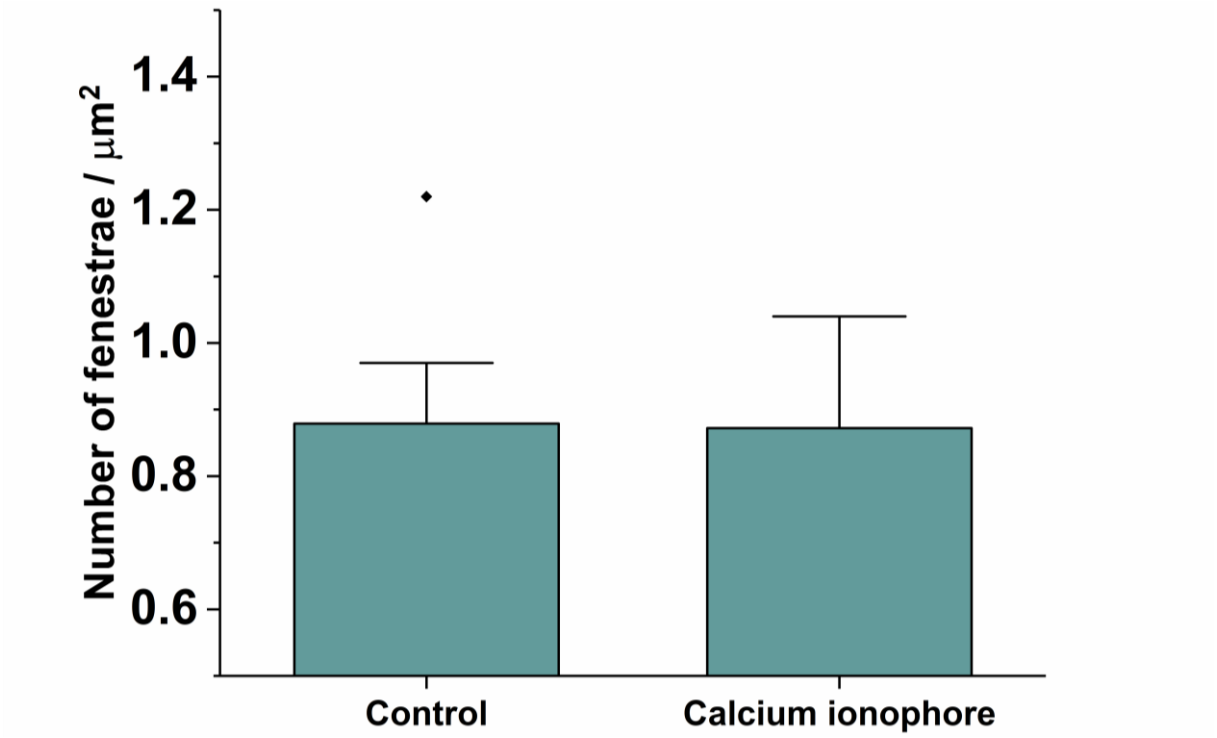
Supplementary Fig. 1 Two-colored dSTORM imaging revealing the organization of actin and spectrin in the cytoskeleton of intact, control LSECs. The left panel represents spectrin (red, spectrin β II Antibody conjugated with Alexa Fluor 647), middle actin (green, phalloidin Atto488), and right merged signals on the interconnection of three LSECs. Encircled areas indicate example sieve plates.



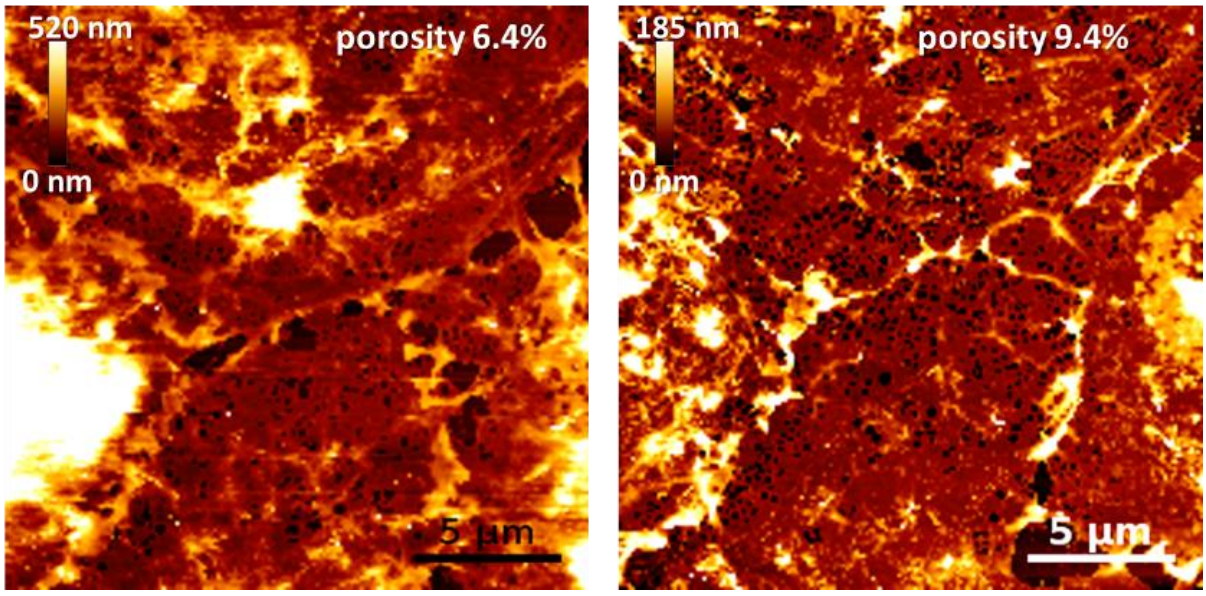
Supplementary Fig. 2 High-magnification of the selected area of intact, control LSEC, visualized using two-colored dSTORM imaging. The left panel represents spectrin (red, spectrin β II Antibody conjugated with Alexa Fluor 647) and actin (green, phalloidin Atto488), and right merged signals. Few fenestrae were indicated with arrowheads.



Supplementary fig. 3. Fluorescence micrographs of LSECs labeled with phalloidin-Atto488 for actin. Similar to elasticity data presented in Fig. 3 - untreated (control) LSECs and LSECs treated with DIA (500 µM), CB (21 µM), IAA (10 µM) were presented. Scale bar: 20 µm.



Supplementary fig. 4. The number of fenestrae per µm^2 calculated for 1% GA fixed LSECs for control (untreated) LSECs and LSECs treated with 1µM calcium ionophore for 60 minutes. n = 8 and n = 5 for control and calcium ionophore group respectively.



Supplementary fig. 5. AFM data illustrating the interconnection of three live LSECs measured before (left) and after (right) 60 minutes of IAA treatment. An overall cell height decreased in the peripheral region of LSECs, which was connected with ~30% increase in porosity as calculated as the ratio of the area occupied by the fenestra to the LSEC cytoplasmic area. The experiment was repeated 4 times with similar results (n = 4). Scan size 20 × 20 µm; 256 × 256 force-distance curves.