Supporting Information

Mechanistic insights into Trop2 clustering on lung cancer cell membranes revealed by super-resolution imaging

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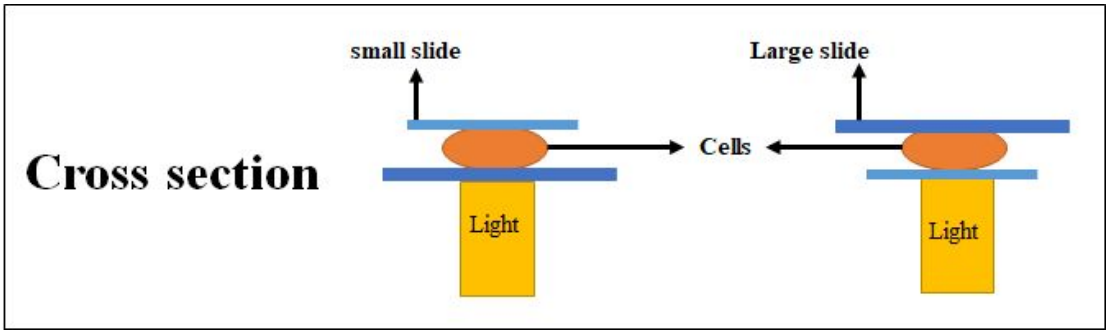

Figure S1. The schematic diagram of imaging the apical and basal membranes with TIRF illumination. The cross section demonstrating how to image the apical membranes (left) and the basal membranes (right).

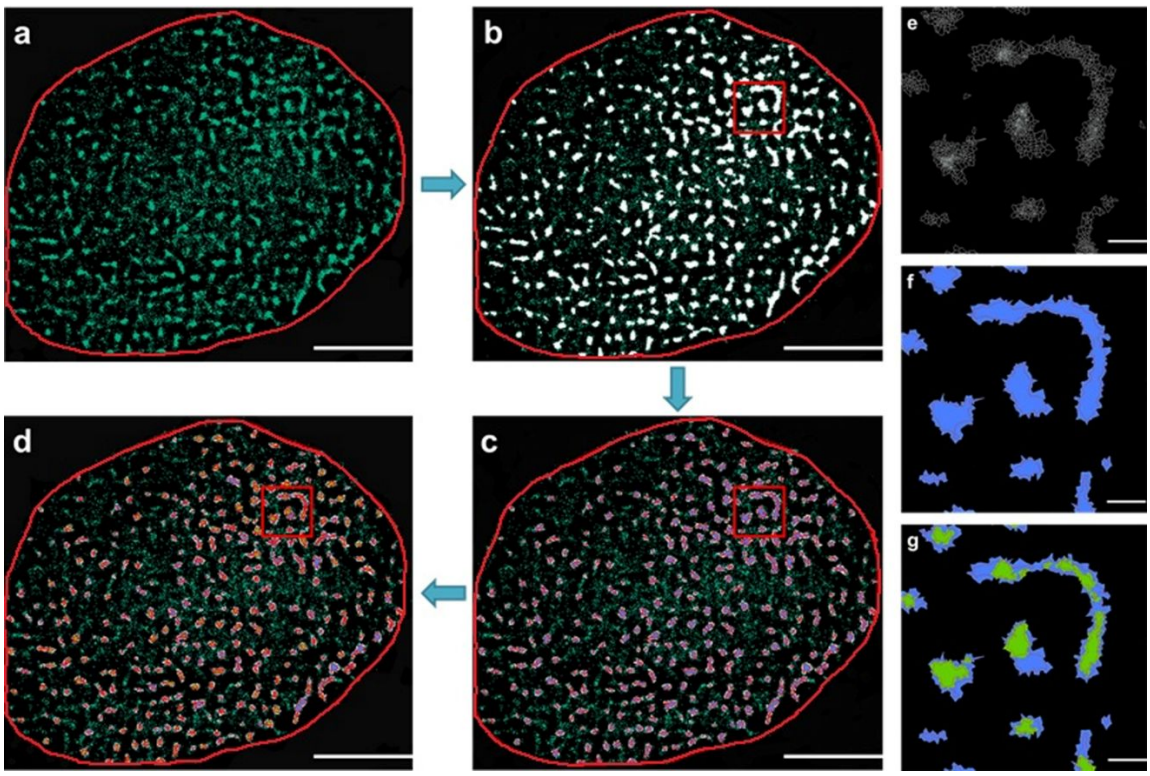

Figure S2. The flow diagram of analyzing clusters by SR-Tesseler method. (a) The reconstructed dSTORM image of Trop2 on an A549 cell. The outline of the cell membrane was circled by red line and set as an "ROI". (b) The localization map was segmented into many polygons, whose edges were bisectors between the nearest localizations (white line). (c) The extracted objects (blue) satisfied that the localization density of the polygon δ_i was higher than the average localization density of the cell δ. (d) The extracted clusters (green regions) which had a higher localization density than the average localization density of all objects. (e-g) The enlarged images corresponding to the red boxed regions in (b-d), respectively. Scale bars: 5 μm in (a-d), 200 nm in (e-g).

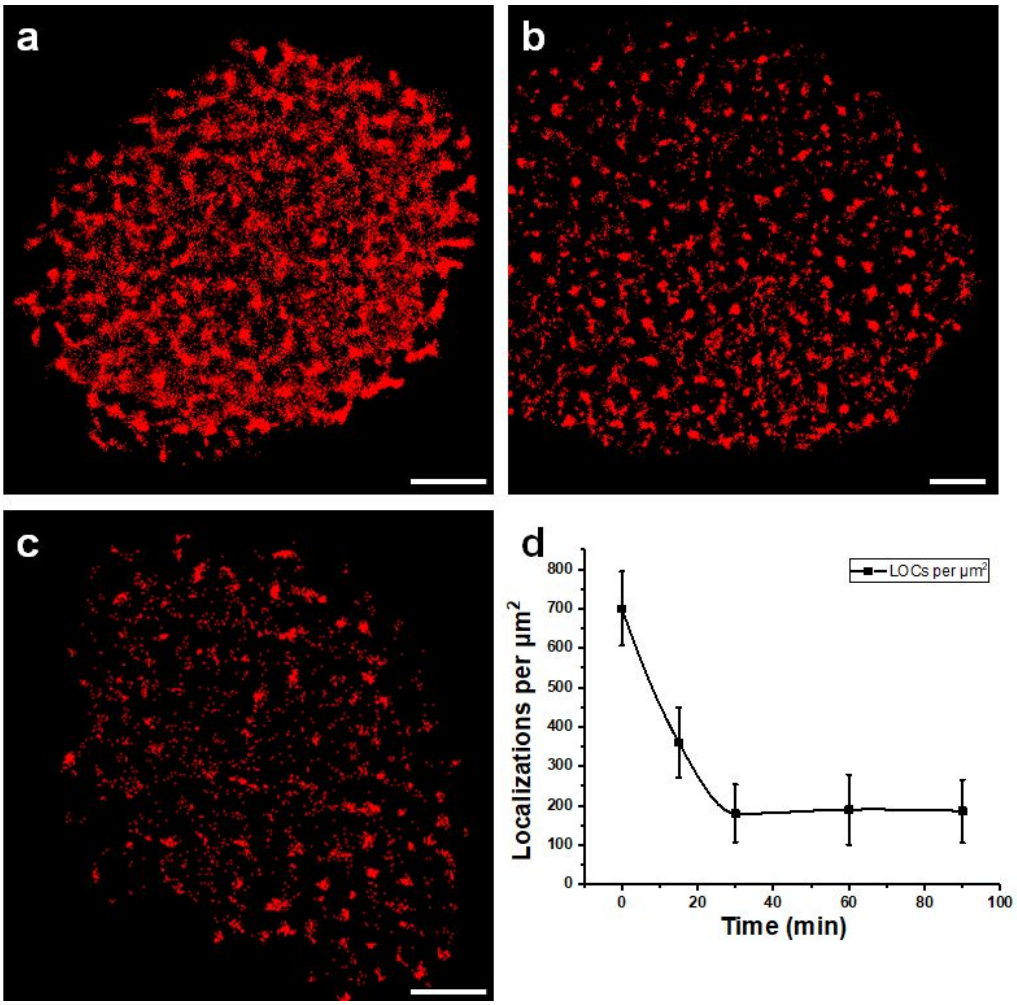

Figure S3. Effects of cholesterol depletion on the distribution of lipid rafts. The dSTORM reconstructed images acquired when the MβCD treatment time is (a) 0 min, (b) 15 min and (c) 30 min, respectively. Scale bars are 5 μm. (d) The localizations of lipid rafts per μm^2 under different treatment time. Data are obtained from 10 cells in 3 independent experiments (mean ± SD).

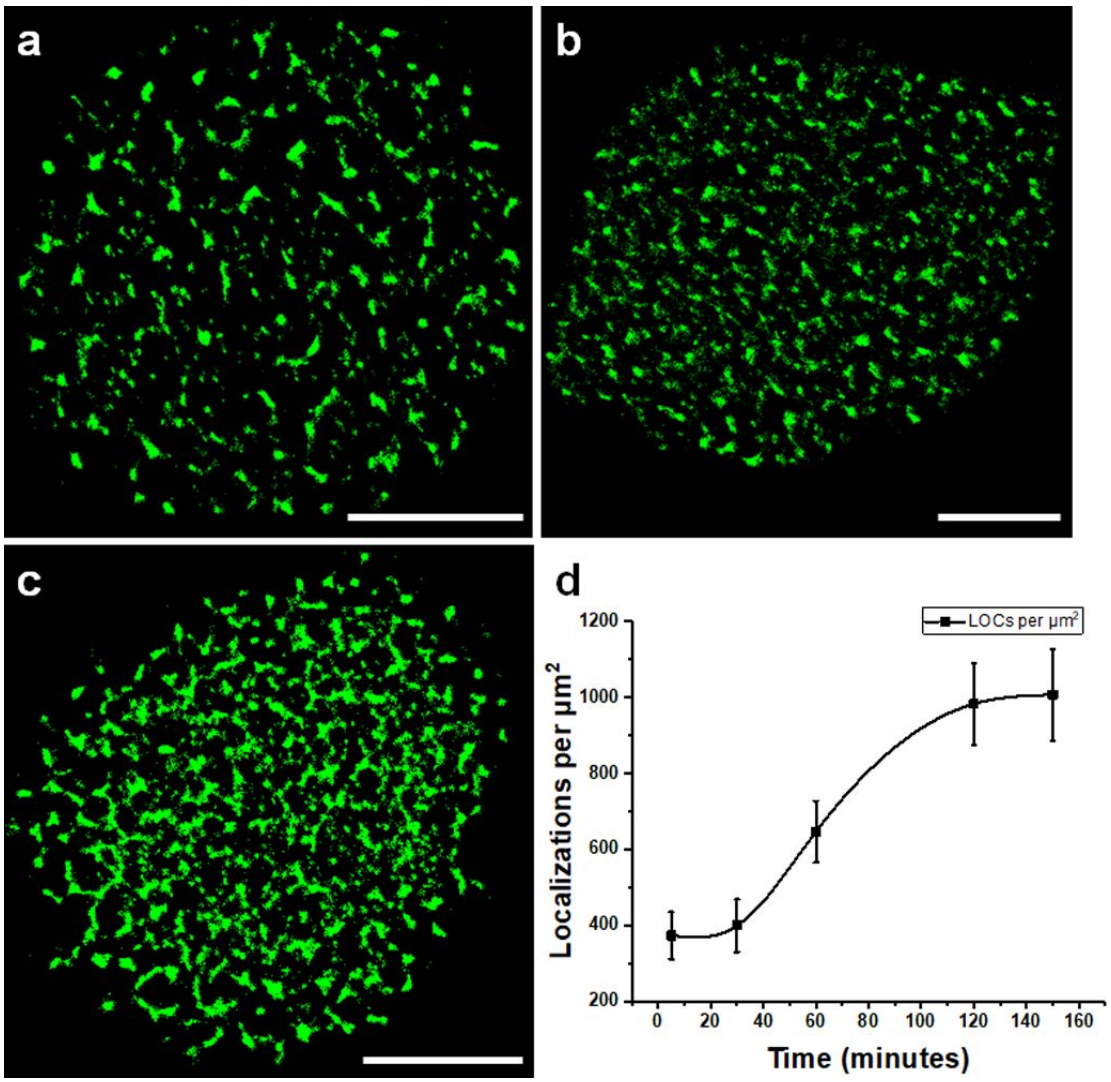

Figure S4. Optimizing the stimulation time of IGF-1. (a–c) The reconstructed dSTORM images of Trop2 on HBE cells after IFG-1 stimulation for 30 min (a), 60 min (b) and 120 min (c), respectively. Scale bars are 5 μm. (d) The plot of localization density with different stimulation time showed that the appropriate stimulation time is 120 min. Data shown are means ± standard deviation (s. d.). The statistical results were obtained from 10 cells.

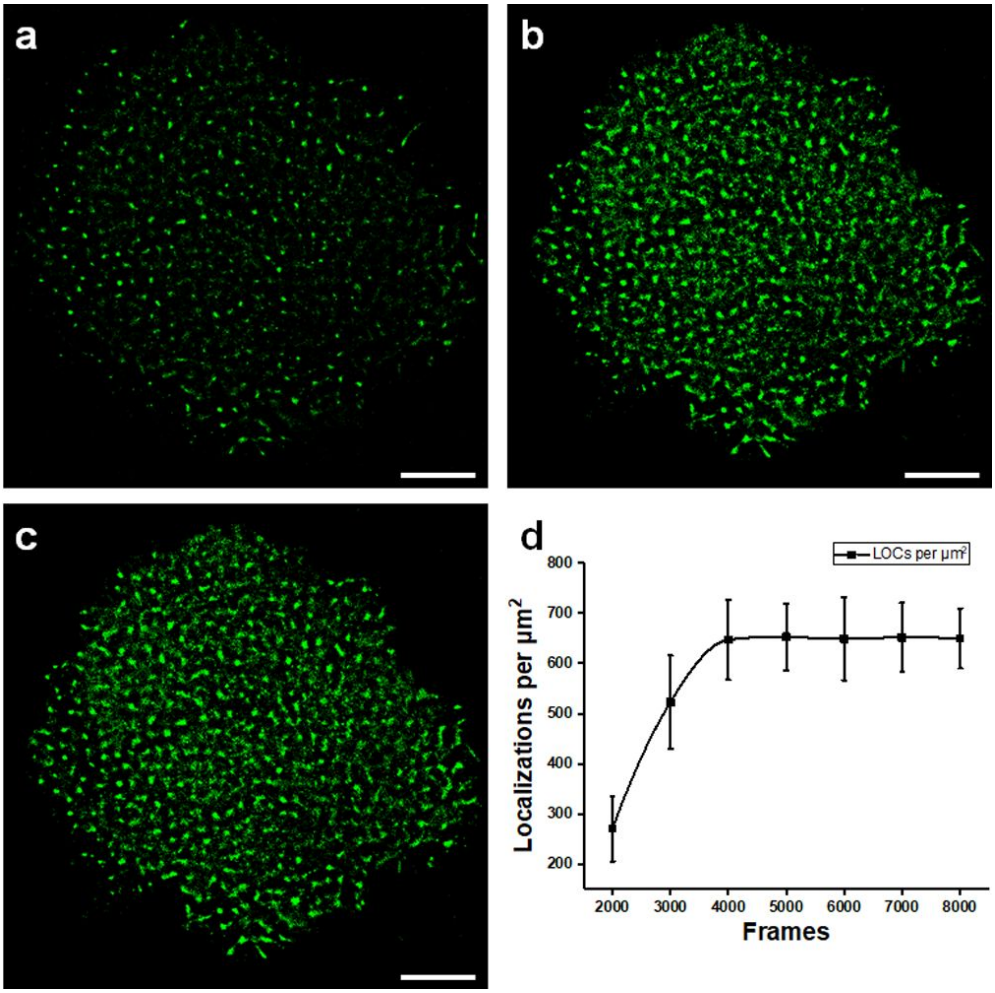

Figure S5. The reconstructed dSTORM images of Trop2 with increasing frame number. (a–c) The dSTORM images of Trop2 on the same A549 cell with different frames, 3000 (a), 5000 (b) and 8000 (c), respectively. Scale bars are 5 μm. (d) The plot of localization density with different frames. Data shown are means ± standard deviation (s. d.). The statistical results were obtained from 10 cells.

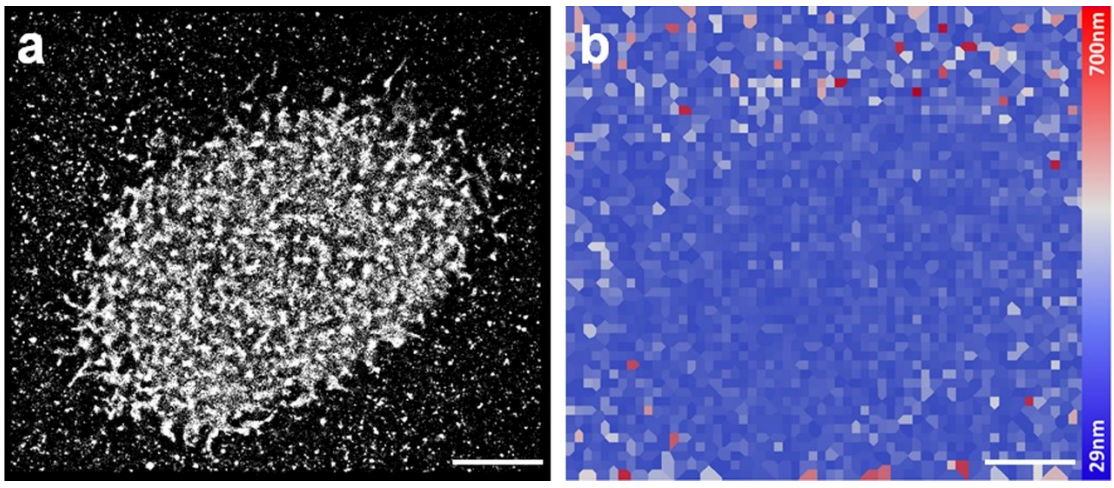

Figure S6. Measurement of the imaging resolution by NanoJ-SQUIRREL. (a) A representative reconstructed dSTORM image of Trop2 on an A549 cell. (b) The corresponding

Fourier ring correlation (FRC) map of the same cell. The highest resolution in the image was 29 nm. Scale bar is 5 μm.