Supporting Information

The organization of protein tyrosine kinase-7 on cell membranes characterized by aptamer probe-based STORM imaging

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Tabel of contents

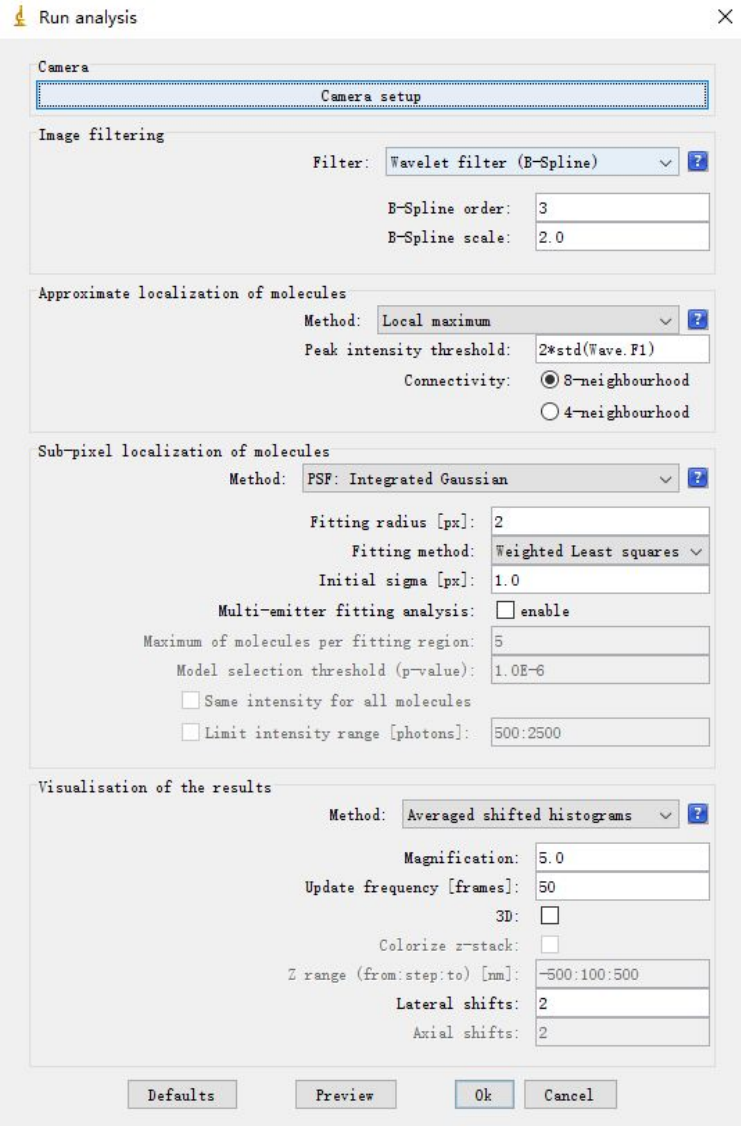

Figure S1. Parameter setting of data analysis in ThunderSTORM. It includes a wavelet B-Spline filter for feature enhancement, local maximum detection to find approximate position of single molecule and a 2D Gaussian function in integrated form using weighted least-squares method.

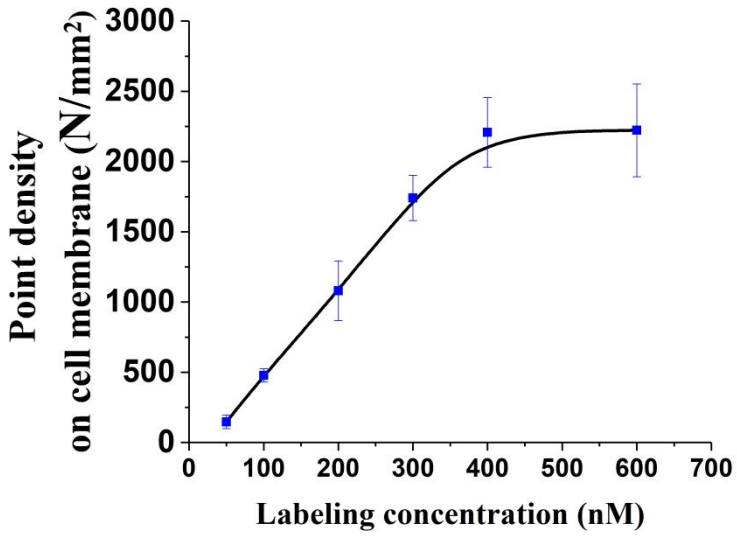

Figure S2. Concentration gradient curve of Sgc8c probe labelling. Data of every labeling concentration were acquired from more than ten cells in three independent experiments. Data are the means ± standard deviation (s.d.)

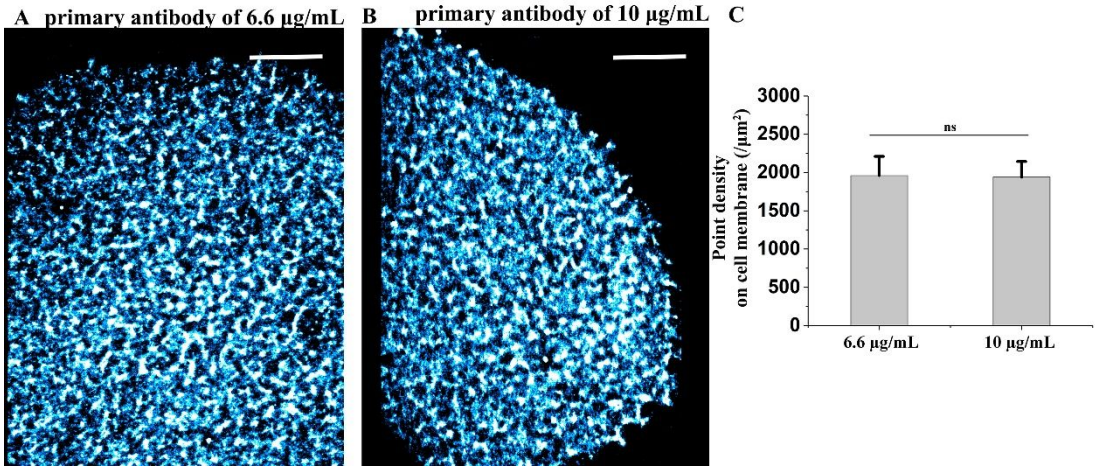

Figure S3. The similar imaging of PTK7 by different concentrations of antibody-probe. The similar dSTORM images of PTK7 distribution on the MCF10A basal membranes with using indirect immunofluorescence labeling method at the primary antibody of an appropriate concentration (A, 6.6 μg/mL) and the higher one (B, 10 μg/mL). Scale bars are 5 μm. (C) The histogram of point density on cell membrane obtained from these two concentration labeling. Statistical data was acquired from ten cells in three independent experiments. Significant difference analyses were applied with unpaired two-tailed t test, with "ns" meaning no significant difference.

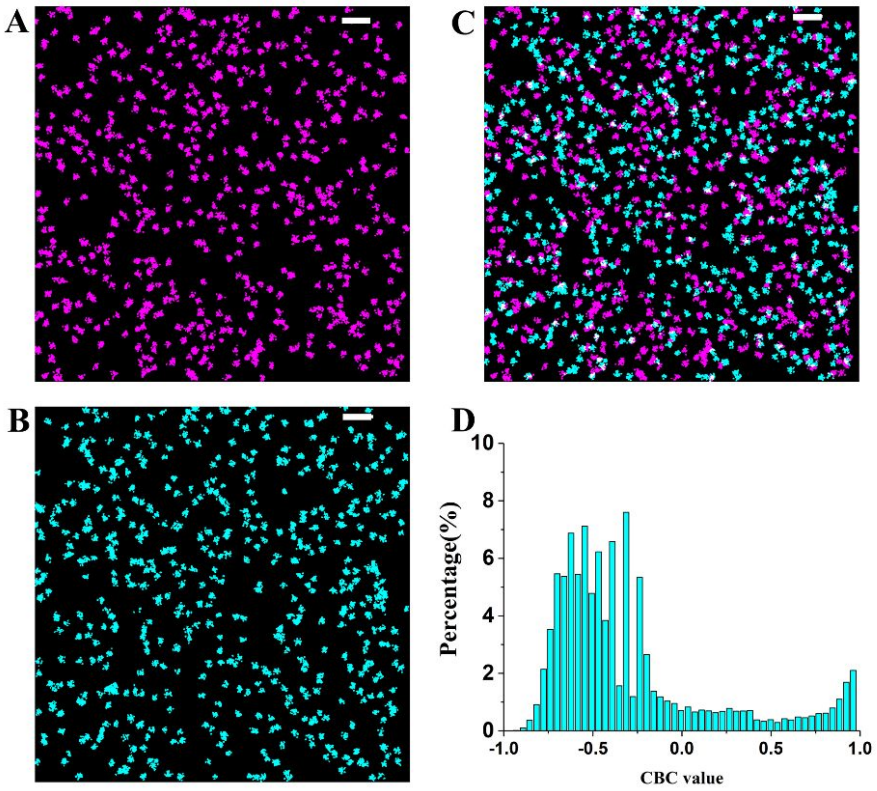

Figure S4. The CBC analysis on simulated clusters with random distribution. (A and B) The images of random distribution of simulated clusters yielded by DBSCAN. (C) The merged image of A and B shows every few colocalization distribution of these two random clusters. (D) The distribution of CBC value of the clusters in cyan color indicating that these two clusters are mainly in the anti-correlation (C_{Ai} = -1) or non-correlated distribution (C_{Ai} = 0). Scale bars are 2 μm in A-C.

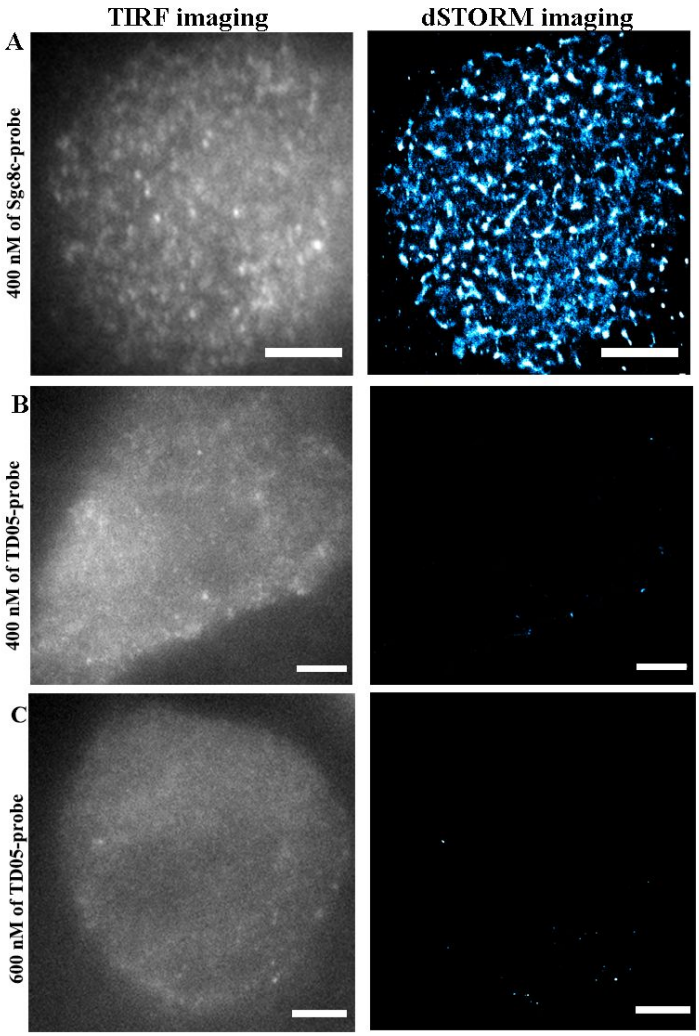

Figure S5. The control imaging of MCF10A membrane with the control aptamer-probe staining. (A-C) The TIRF image (left) and dSTORM image (right) of MCF10A cell apical membranes stained with Sgc8c-probe at 400 nM and TDO5-TAMRA at 400 nM and 600 nM. Scale bars are 5 μm.

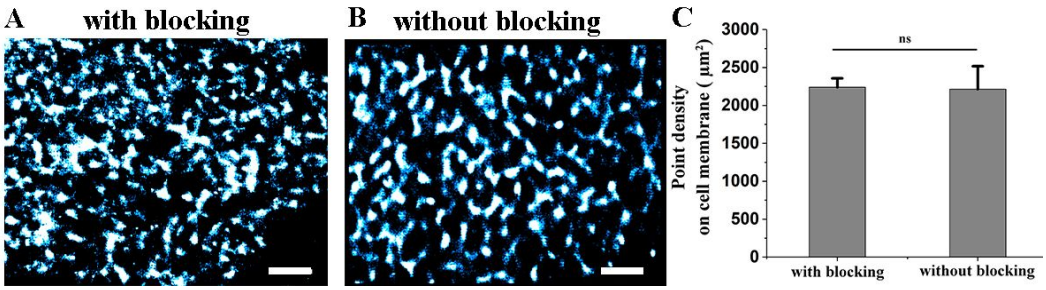

Figure S6. Blocking experiment of Dextran sulfate has no effect on the probe labeling. (A and B) The similar dSTORM imaging results of PTK7 on MCF10A basal membrane by Sgc8c-TAMRA labeling with (A) or not (B) blocking agent of Dextran sulfate. Scale bars are 2 μm. (C) The histogram of point density on cell membrane from imaging with or not blocking agent. Statistical data was acquired from ten cells of three independent experiments. Significant difference analyses were applied with unpaired two-tailed t test, with "ns" meaning no significant difference.

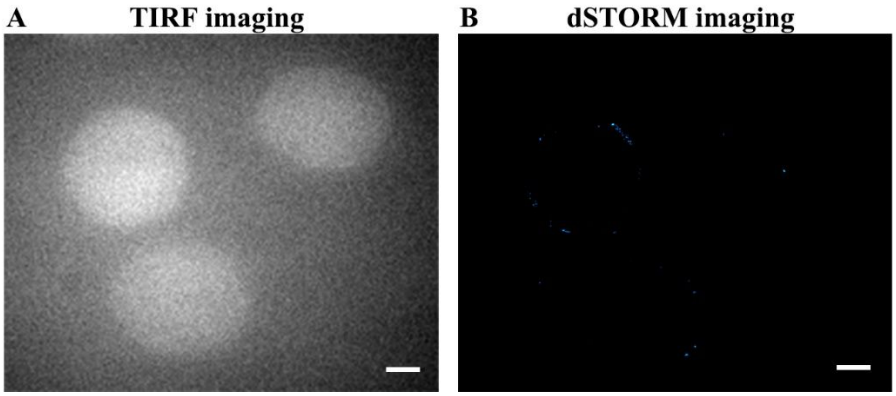

Figure S7. The control imaging of RBC membrane with Sgc8c-TAMRA staining. (A-B) The TIRF image and reconstructed dSTORM image of RBC cells with Sgc8c-TAMRA labeling. Scale bar are 2 μm.

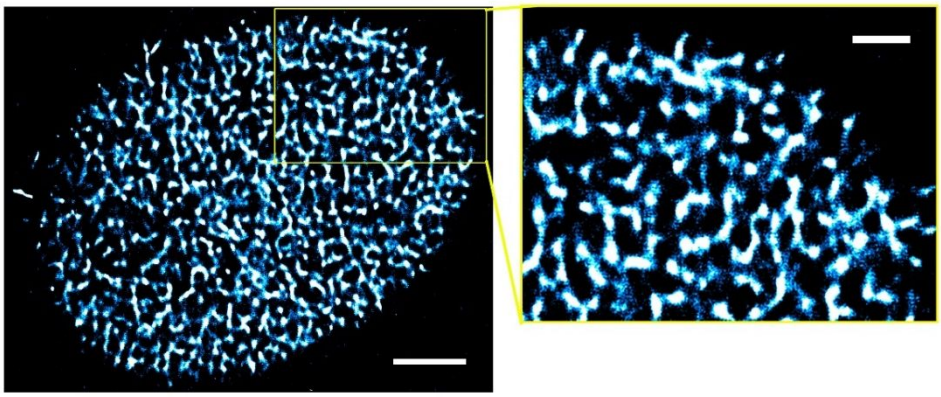

Figure S8. dSTORM imaging of PTK7 on MCF 10A basal membrane by aptamer-probe labeling. The original (left) and the corresponding enlarged (right) dSTORM reconstructed images of PTK7 distribution on the MCF10A basal membrane by applying the Sgc8c-TAMRA probe. Scale bar is 5 μm in original image and 2 μm in enlarged image.

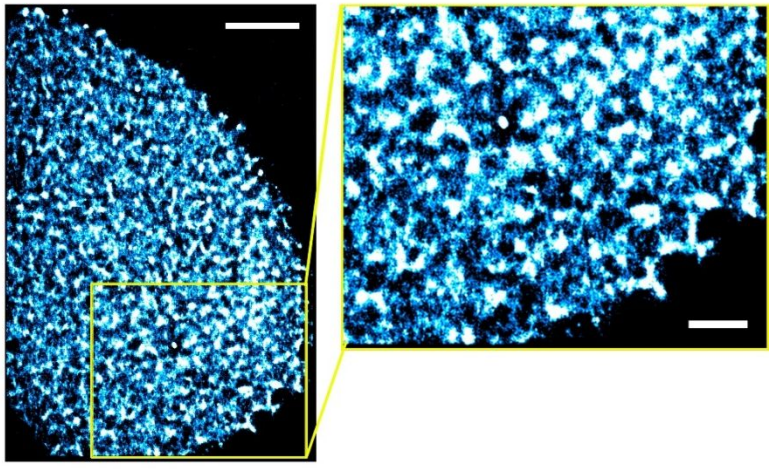

Figure S9. dSTORM imaging of PTK7 on MCF 10A basal membrane by antibody-probe labeling. The original (left) and the corresponding enlarged (right) dSTORM reconstructed images of PTK7 distribution on the MCF10A basal membrane by applying indirect immunofluorescence labeling. Scale bar is 5 μm in original image and 2 μm in enlarged image.

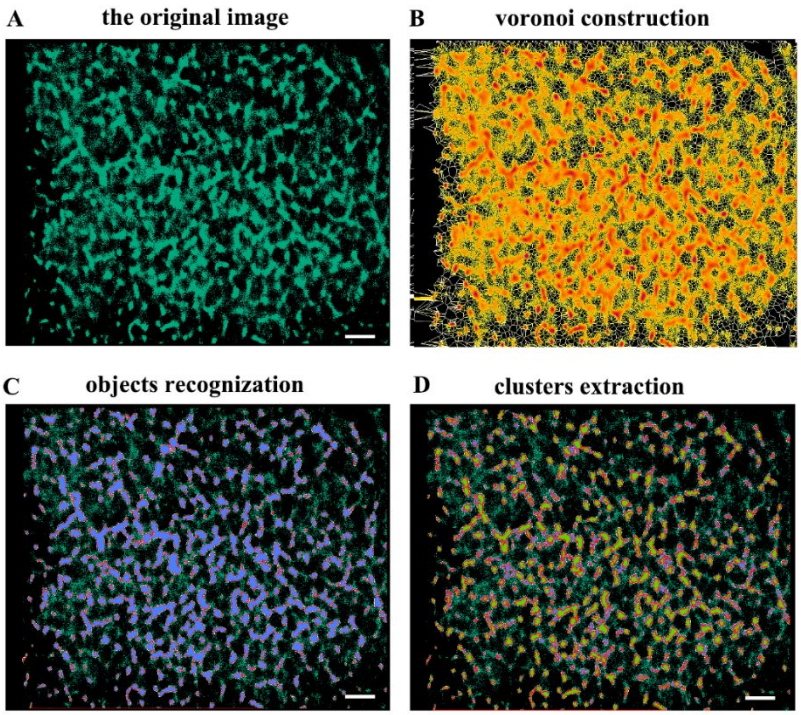

Figure S10. Graphical representation of the SR-Tesseler analysis process. (A) The original image rendered after the localization data was imported. (B) The Voronoï diagram was reconstructed by creating polygons based on local density. (C) The qualified objects were recognized from the figure B. (C) The qualified clusters were further extracted from the figure C with setting density factor and cut distance. Scale bars are 2 μm in A-D.

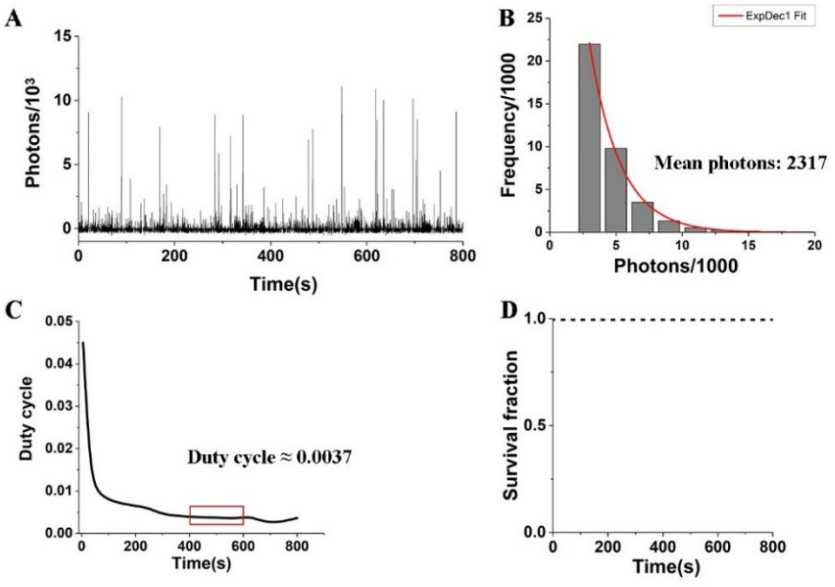

Figure S11. Characterization of photophysical properties of Sgc8c-linked TAMRA. (A) Single-molecule fluorescence time traces measured in the presence of βME and an oxygen-scavenging system (glucose oxidase with catalase (GLOX)) for Sgc8c-linked TAMRA. (B) From traces in A, the histogram of the number of detected photons for each switching event, the data was acquired from many events from many molecules, with showing the mean

value derived from the single exponential fit of the distribution (red curves). (C) The on-off duty cycle value was plotted versus time, with showing the average duty cycle between 400–600 s (red box). (D) The fraction of molecules that survived photobleaching.

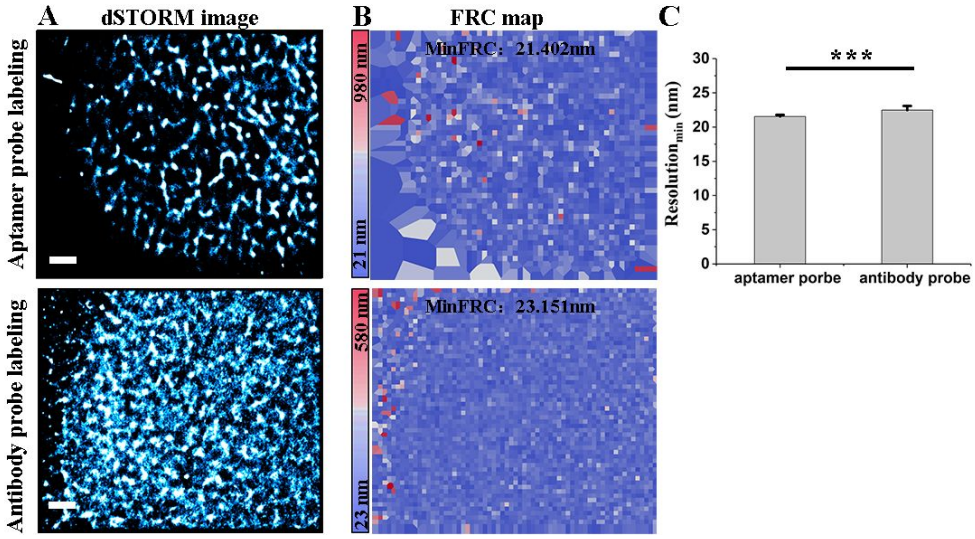

Figure S12. The compared minimum resolution of aptamer-probe and antibody-probe labeling. (A) The reconstructed dSTORM images of PTK7 on MCF10A cell membranes by aptamer-probe (up) and antibody-probe labeling (below). (B) The FRC maps of these two types of probes labeling. (C) The histogram of the minimum resolution of these two kinds of probes labeling. All statistics resulted from 10 cells in three independent experiments. Data are the means ± standard deviation (s.d.). Significant difference was measured by two-tailed unpaired t test, with "***"means $P < 0.001$. Scale bar 2 μm.

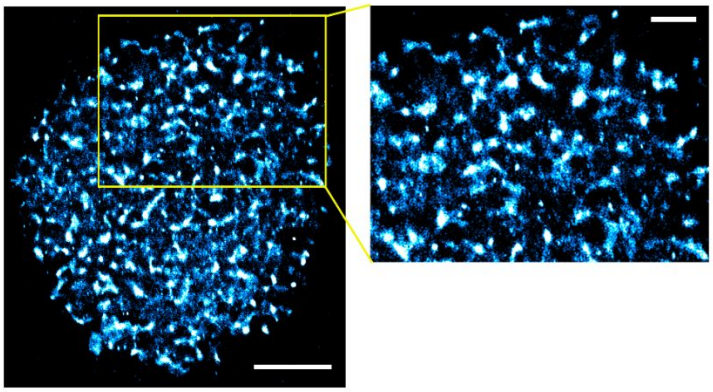

Figure S13. dSTORM imaging of PTK7 on MCF 10A apical membrane by aptamer-probe labeling. The original (left) and the corresponding enlarged (right) dSTORM reconstructed images of PTK7 distribution on the MCF10A apical membrane by applying the Sgc8c-TAMRA probe. Scale bar is 5 μm in original image and 2 μm in enlarged image.

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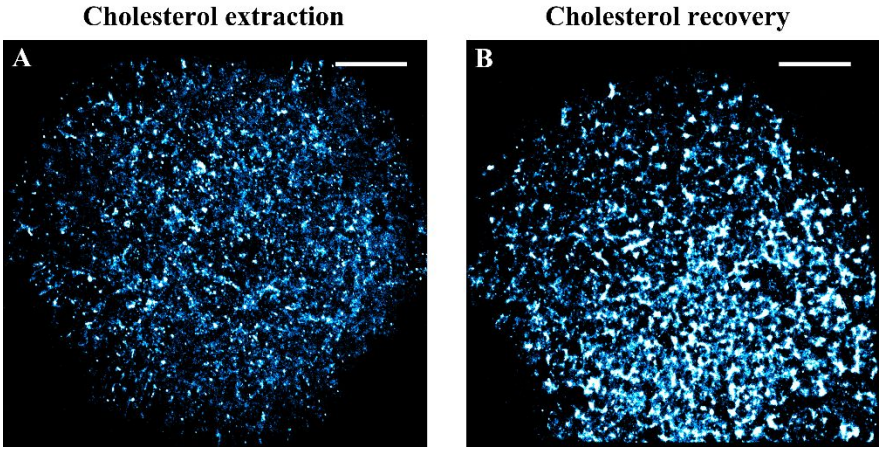

Figure S14. Effect of cholesterol on the distribution of PTK7 on apical membrane. The contrast dSTORM images of PTK7 distribution on the MCF10A apical membranes of cholesterol extraction (A) and cholesterol recovery again (B). Scale bars are 5 μm.

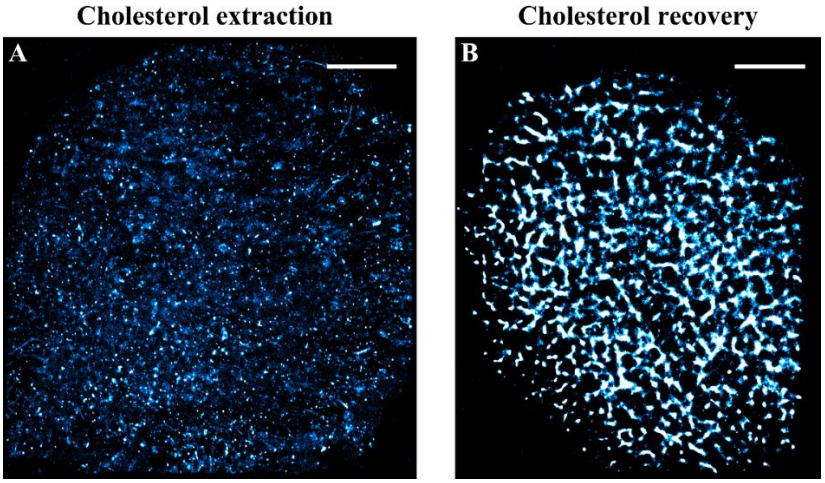

Figure S15. Effect of cholesterol on the distribution of PTK7 on basal membrane. The contrast dSTORM images of PTK7 distribution on the MCF10A basal membranes of cholesterol extraction (A) and cholesterol recovery again (B). Scale bars are 5 μm.

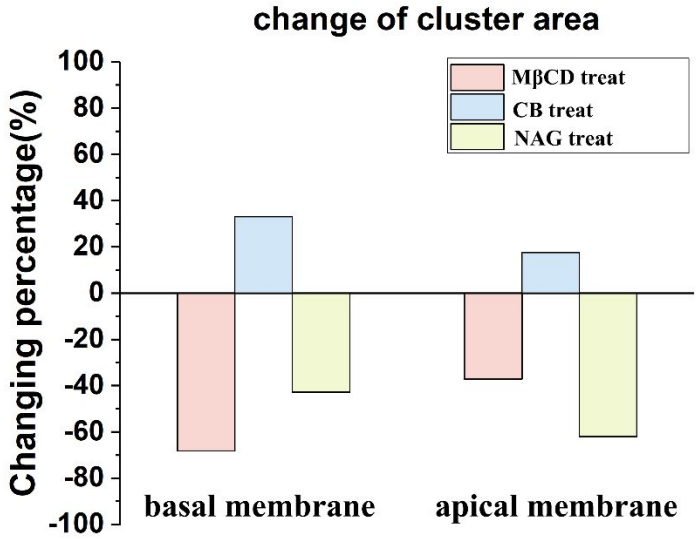

Figure S16. Comparative analysis of the change degree of cluster area under different treatments. The histogram of the change percentage of cluster area of PTK7 on the basal and apical membranes caused by MβCD treat, CB treat and NAG treat, respectively.

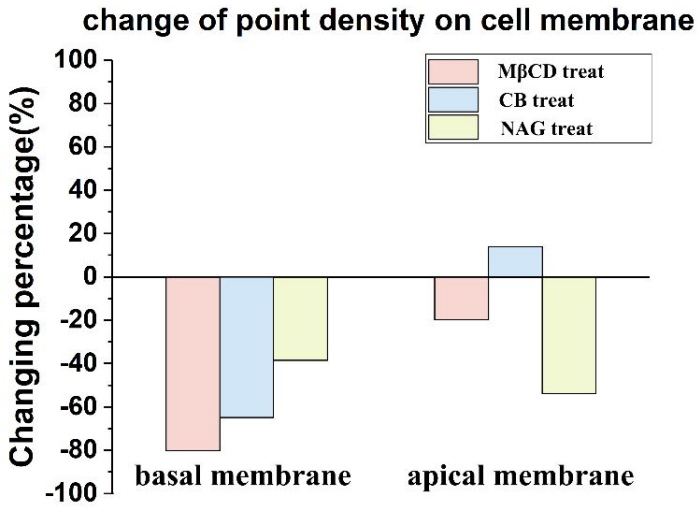

Figure S17. Comparative analysis of the change degree of point density under different treatments. The compared histogram of the change percentage of point density of PTK7 on the basal and apical membranes caused by MβCD treat, CB treat and NAG treat, respectively.

Supplementary Experiment Section

Detial in sample preparation

Cell Culture MCF10A cells were purchased from American Type Culture Collection (ATCC). They were cultured in Dulbecco's Modified Eagle Medium/F12 (DMEM/F12, HyClone) with 5% Horse Serum (FBS, HyClone), 20 ng/mL EGF (Sigma), 0.5 μg/mL Hydrocortisone (Sigma), 100 ng/mL

Cholera toxin (Sigma), 10 μg/mL Insulin (Sigma), 100 μg/mL Penicillin (Sigma) and 100 μg/mL Streptomycin (Sigma), in humidified incubator at 37℃ (with 5% CO_2). For dSTORM imaging, the cells were cultured on a clean cover slip (22 mm × 22 mm, Fisher) in a dish for at least 24 hours to achieve ~60-70% confluence.

Aptamer Probe Labeling 1 OD of Sgc8c-TAMRA was first diluted in 23 μL 100 μM TE buffer containing 10 mM Tris-HCl and 1 mM EDTA (pH = 8.0) to form the stock solution. Well cultured MCF10A cells were washed with pre-warmed PBS for three times and fixed with 4% Paraformaldehyde (PFA) at room temperature for 30 min. Then, with washing for three times with PBS, the cells were stained with the Sgc8c-TAMRA at specific concentration (i.e. 400 nM or 600 nM) in the dark for 10 min at 4°C. Finally, the sample was washed by PBS for 4-5 times to remove away the excess probes for the dSTORM imaging experiments.

Antibody Probe Labeling Well cultured cell was washed and fixed as the steps in the aptamer probe labeling, and then was blocked by 3% Bovine Serum Albumin (BSA) at room temperature for 20 min. Then, the sample was incubated with primary antibody of PTK7 (Monoclone Anti-PTK7, Merck, SAB1404276) solution containing 1% BSA at 4°C overnight. Next, following washing with PBS for 5 min for 3 times, the cells were stained with anti-mouse IgG-Alexa532 (1 μg/mL in 1% BSA; Invitrogen) in the dark at room temperature for 1 h, and then washed 4 times with PBS for 3 min each for the dSTORM imaging experiments.

RBC Sample Preparation Fresh red blood cells were obtained from centrifuging the whole human fingertip blood of healthy donors. In details, RBCs were washed five times with PBS by a low speed centrifuge (1,000 r/min, 1 min) to remove serum and buffy coat. Then, RBCs were resuspended and diluted by PBS into appropriate cell density. 200 μL erythrocyte suspension was dropped on the clean silanized slide to attach for 20 min at room temperature, the unabsorbed RBCs were washed away by PBS. Then, cells were stained as MCF10A cells for dSTORM imaging.

Details in data analysis

dSTORM Imaging For dSTORM imaging, we performed an inverted Nikon Ti-E microscope equipped with an oil-immersion objective (100×, 1.49 NA, Nikon, Japan). Under the total internal reflection fluorescence (TIRF) illuminating mode, the sample was imaged by adjusting the excitation inclination to maximize the signal-to-noise ratio. By flipping the samples slides on the lens to make the large or small slide closer to the lens, we realized the imaging of the basal membrane (the small slide downward) and apical membrane (the large slide downward), respectively. For single color dSTORM imaging, the sample was excited with a 532 nm laser (~200 mW). For dual-color dSTORM imaging, the sample was firstly imaged by 639 nm laser (~150 mW) illumination, then by 532 nm laser excitation. To avoid the color-crosstalk of Alexa 647 by 532 nm laser excitation, a band pass emission filter (FF01-595/34-25, Semrock) was added, in addition to the conventional excitation filter, dichroic mirror and emission filter set. Meanwhile, microspheres (Invitrogen) were added as fiducials to correct the x–y drift and the optical registration between Alexa 647 and the TAMRA channels. Nikon micro imaging equipment also provides a perfect focus system (PFS) to realize the real-time correction of the focus drift in the y axis. Besides, the drift-correct as the post-process in ThouderSTORM can be used to correct the occasional errors. Finally, by combining with Micro-Manager based on ImageJ (U.S. National Institutes of Health), 8000 raw frames were acquired with an EMCCD camera (Photometrics, Cascade II) with 20 ms exposure time.

Reconstruction of dSTORM Image. We implemented ThunderSTORM to analyze and reconstruct dSTORM image. The detailed parameters can see the Figure S1. In short, with setting the private camera parameters according to our experiment conditions and selecting the appropriate analysis method and parameter thresholds (**Figure S1**), the raw data can be analyzed. After obtaining all localizations, we further set the vital parameters thresholds, including the sigma (the range of main peak), the intensity (>100), the offset (removal of the outliers), the uncertainty (<30), and acquired the qualified localizations with removing the "bad localization"; then, with applying the "merge" and "drift correct", a dSTORM image with nanoscale resolution was finally reconstructed in an average shifted histogram mode.

Calculation of Point Density on Cell Membrane The localization density on the cellular membrane was calculated with the method in previous article[1]. In short, one inherent image process in ImageJ was applied to measure the area of the cell membrane of interest, then the MatLab was used to acquire the total number of points on the cell membrane of interest. So that, the point density on the cell membrane was obtained. By statistically analyzing the point density values on the cell membrane at increasing labeling concentrations of the probe, we plotted the concentration gradient curve of aptamer probe (**Figure S2**) and determined the appropriate labeling concentration.

By comparing the point density values on the cell membrane at different labeling concentrations of the antibody-probe, we determined the appropriate labeling concentration.

Cluster Analysis by SR-Tesseler Cluster analysis was performed by SR-Tesseler as the described in our previous studies[1]. SR-Tesseler[2] is an accurate cluster analytical method which is suitable for single-molecule localization data, as it is based on the local density to precisely and automatically segment protein clusters and finally characterize protein organization at different scales. The detailed theoretical basis of this method can refer to the previous relevant literature[2]. Here, for our data, the original dSTORM image is exhibited by importing coordinates of localizations (**Figure S10A**), then the region of interest is selected to create the Voronoï diagram (**Figure S10B**). By setting the density factor, cut distance, min area and min#locs, objects are recognized, and their shape and outline are showed (**Figure S10C**). Then, clusters can be further abstracted from the objects, with setting the density factor and min area and min#locs (**Figure S10D**). Meanwhile, the morphological parameters are yielded, such as the cluster area, the number of points in cluster, and so on.

The simulated clusters with random distribution was analyzed by CBC method. With DBSCAN[3], two sets of cluster data were yielded. Then, after importing the localizations of two sets of data by "import results" and "import ground-truth", CBC analysis is performed to give the corresponding CBC values of every localization from these two images.

Colocalization Analysis by CBC Method The coordinate-based colocalization (CBC) method[4] which is suitable for single-molecule localization data was applied to characterize the spatial association of localizations from two imaging channels. The detailed producers are the similar as described in our previous studies[1]. In this analysis, every location of each species (A or B) is assigned a CBC value C_{Ai}, which can describe the detailed spatial relationship of A to B. After final normalized, the value of C_{Ai} distributes in the range of -1 to 1, which means that the spatial relationship of two species is changed from anti-correlated (C_A = -1) through no colocalization (C_A = 0) to perfectly

correlated distribution ($C_A = 1$). So that, the percentage of C_A greater than 0 can be used to quantify the degree of co-localization of points from two probes labeling.

Resolution Measurement by FRC Mapping. Fourier ring correlation (FRC)[5] is a current standard for measuring image resolution in single molecule localization microscope (SMLM) images. Here, we exploited the NanoJ-SQUIRREL[6] based on ImageJ to calculate the FRC map to measure the imaging resolution from the dSTORM images. This method compares two independently acquired super-resolution images (SRMs) of the same field-of-view, so the SMLM data will firstly be splitted into odd and even frames to acquire two SRM images, then their correlation is measured at different frequencies in Fourier space. When the correlation falls below the set threshold, it indicates the resolution of the image. Finally, the FRC map can be provided to estimate the resolution across the whole image, with the lowest FRC value representing the minimum resolution in the image.

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