Supporting Information for

Quantitatively Mapping the Assembly Pattern of EpCAM on Cell Membranes with Peptide Probes

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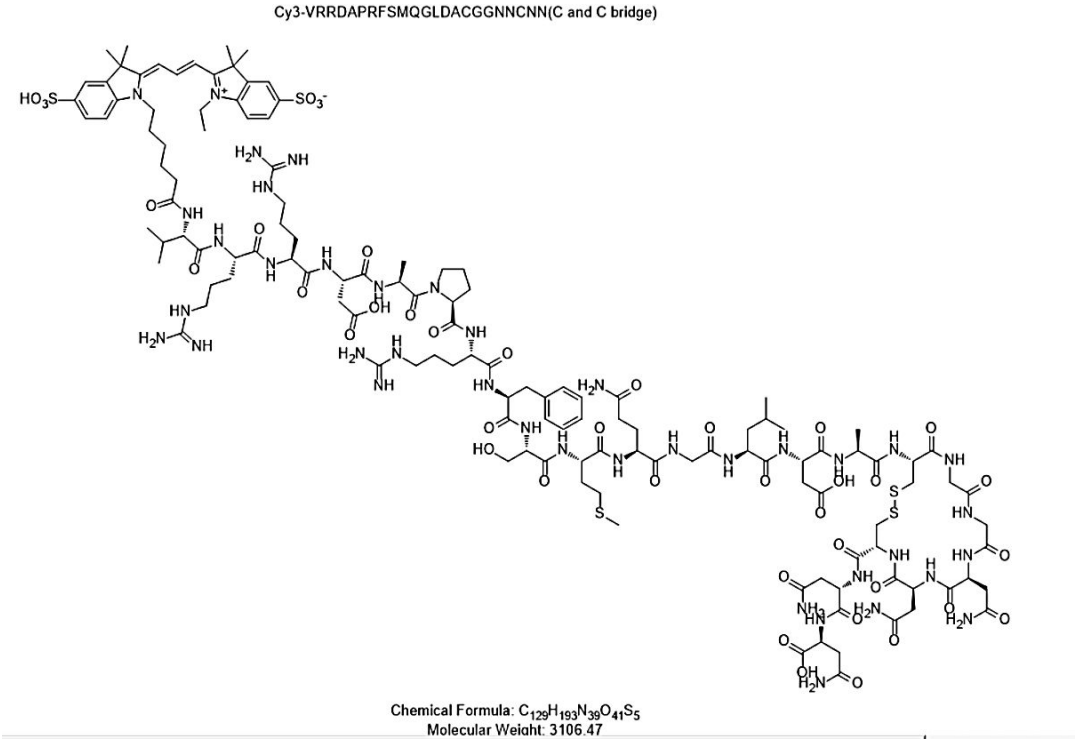

Figure S1. Structure of Cy3-conjugated peptides.

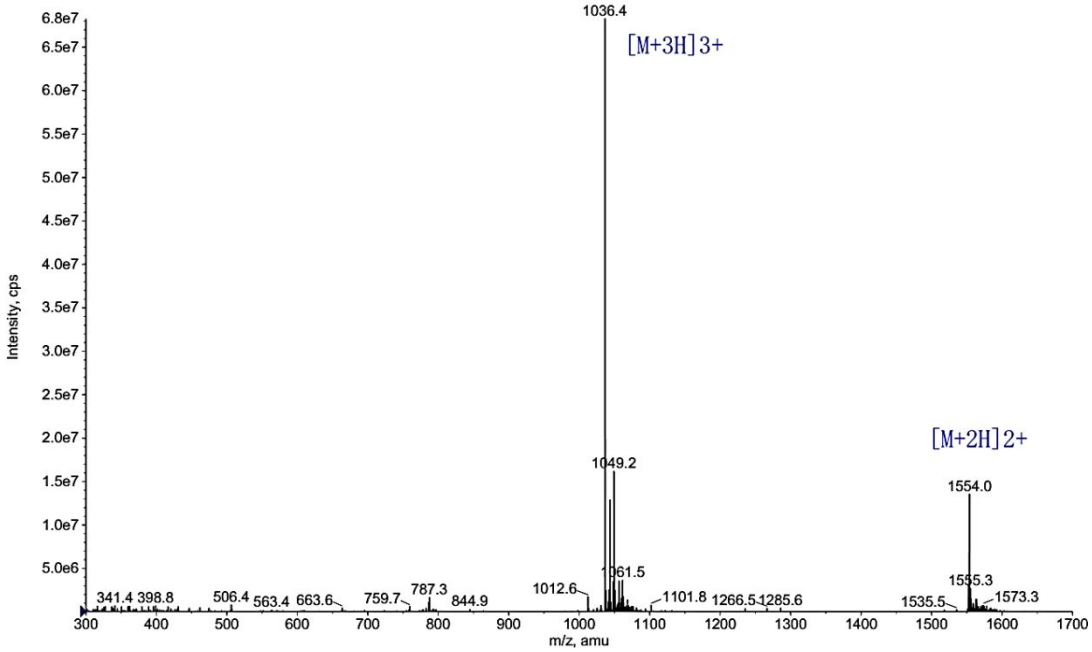

Figure S2. Mass spectrum of Cy3-conjugated peptides.

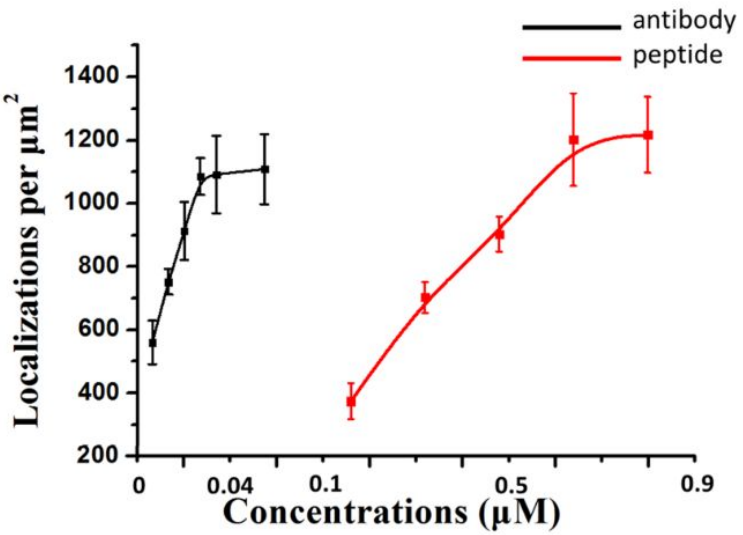

Figure S3. The saturated concentration curves of peptides and antibodies calculated by localization density. Data shown are means ± standard deviation (s. d.). The statistical results were obtained from ten cells in five independent experiments.

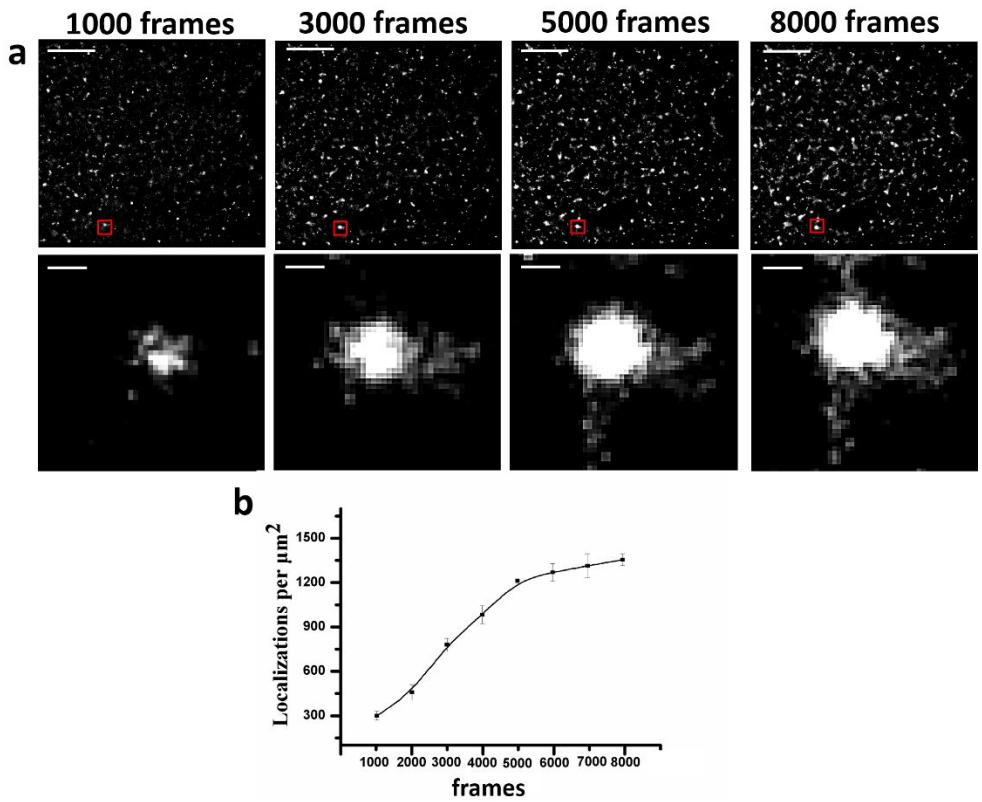

Figure S4. Reconstruction of EpCAM with increasing frame number. (a) The representative dSTORM images of EpCAM which were reconstructed from 1000, 3000, 5000, 8000 frames, respectively. The magnified boxed region shows the detailed morphology of individual cluster with the increasing frame number. Scale bars, 5 μm in original images and 50 nm in zoomed-in images. (b) The number of localizations per μm^2 with increasing frame number. Data shown are means ± standard deviation (s. d.). The statistical results were obtained from five cells.

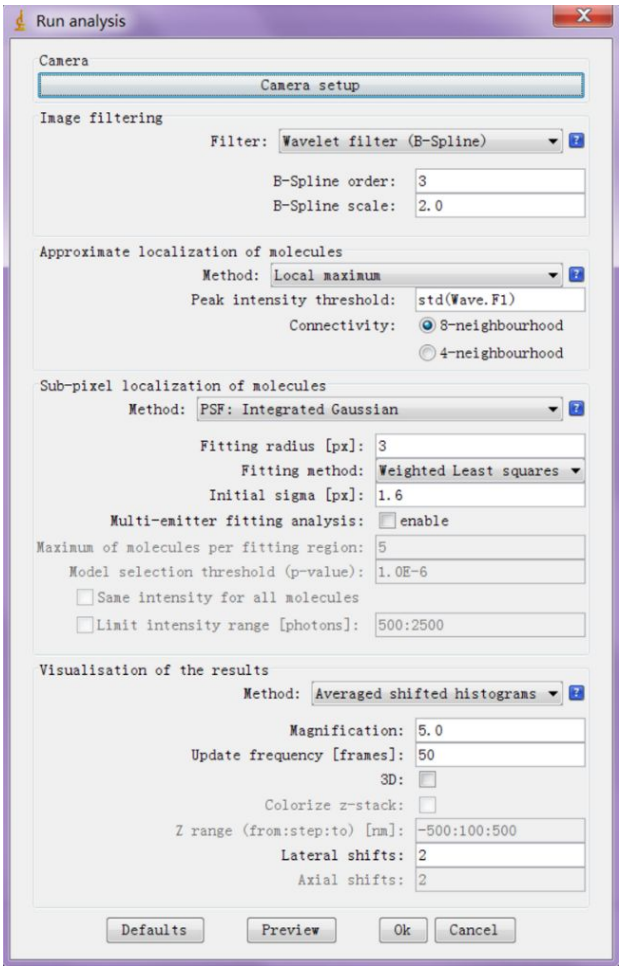

Figure S5. The parameter setting in ThunderSTORM. It includes a wavelet B-Spline filter for feature enhancement, local maximum detection to find approximate positions of single molecules and a 2D Gaussian function in integrated form using maximum likelihood methods.

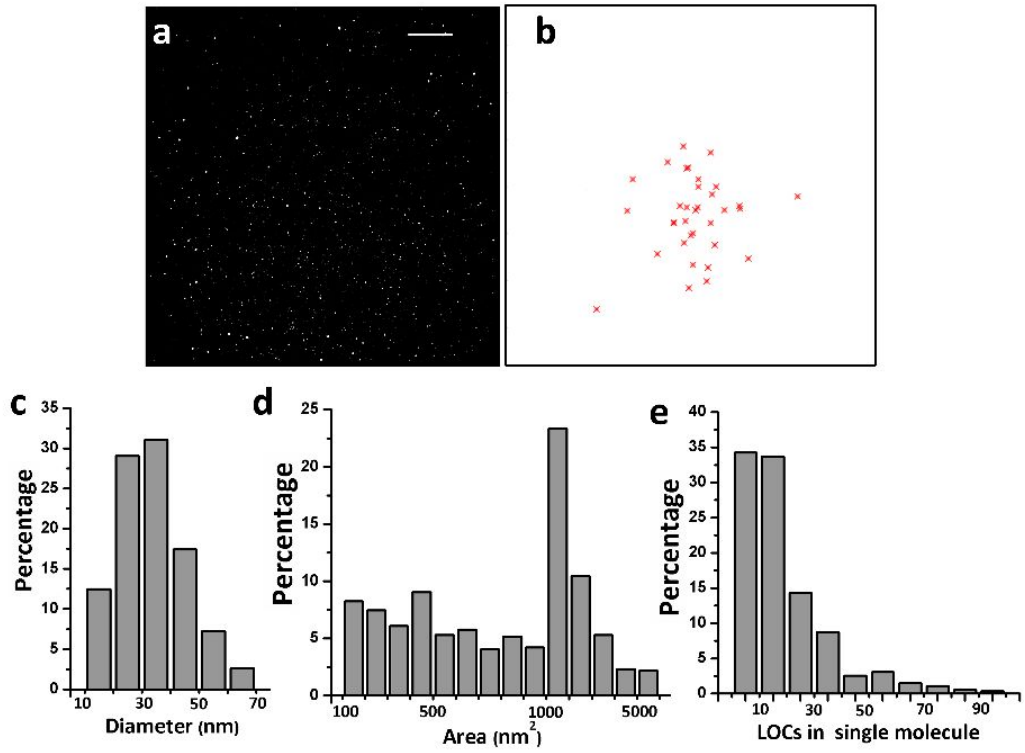

Figure S6. Characterization of single Cy3-conjugated peptides by SR-Tesseler method. (a) Representative dSTORM images of Cy3-conjugated peptides on the empty coverslip. Scale bar, 5 μm. (b) The distribution of repeated localizations from a single emitter. (c-e) Quantitative characterization of single blinking molecules. The distribution of diameter (c), area (d), and localizations in single molecules (e). Statistics were from ten cells in five independent experiments. LOCs is the abbreviation for localizations.

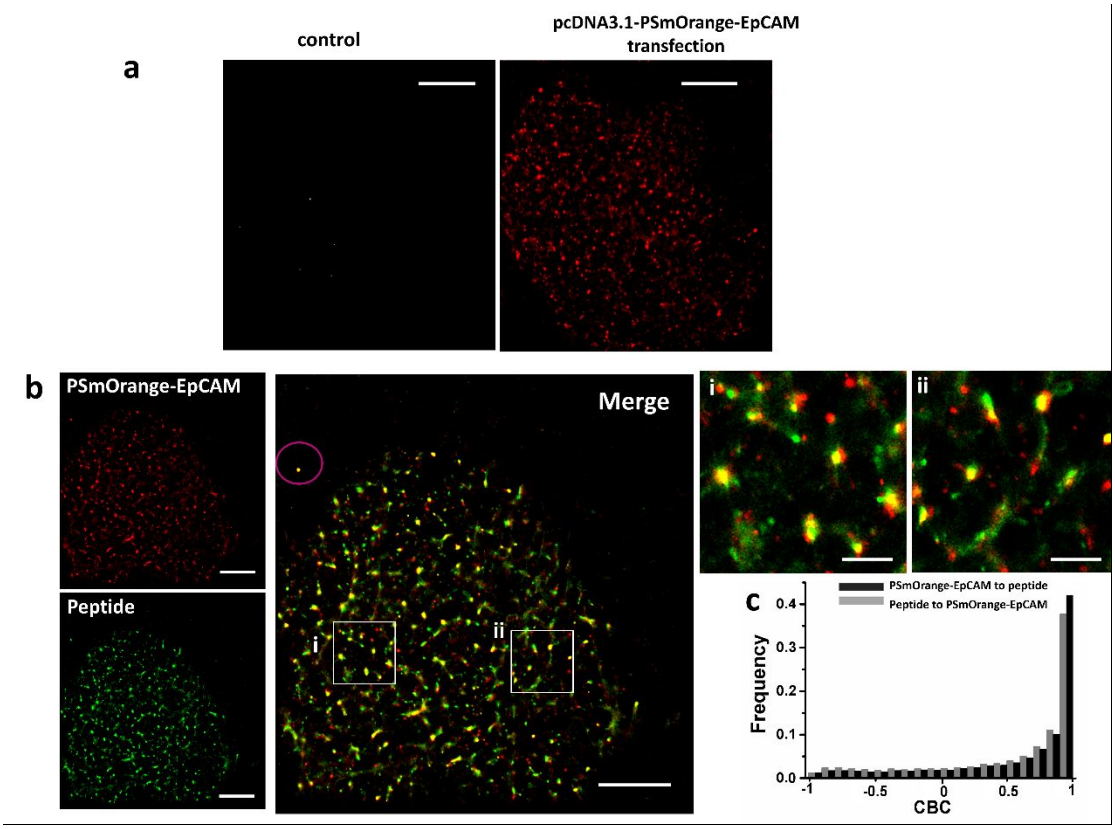

Figure S7. Dual-color imaging of peptide labeling- and PSmOrange-EpCAM. HEK293T cells were transfected with pcDNA3.1-PSmOrange-EpCAM and labeled with Cy3-conjugated peptides. (a) PALM images of pcDNA3.1-PSmOrange-EpCAM on the control and transfected cell membranes. Scale bars, 5 μm. (b) The PALM image of PSmOrange-EpCAM and dSTORM image of peptide-recognized EpCAM, and their merged image. Microspheres were used as fiducial markers to correct the x–y drift and the optical registration between red and green channels. Scale bars are 5 μm in the original images, and 1 μm in the enlarged images (i, ii). (c) The histogram of the colocalization parameter for PSmOrange-EpCAM and peptides by CBC analysis. Data were from ten full-sized cells.

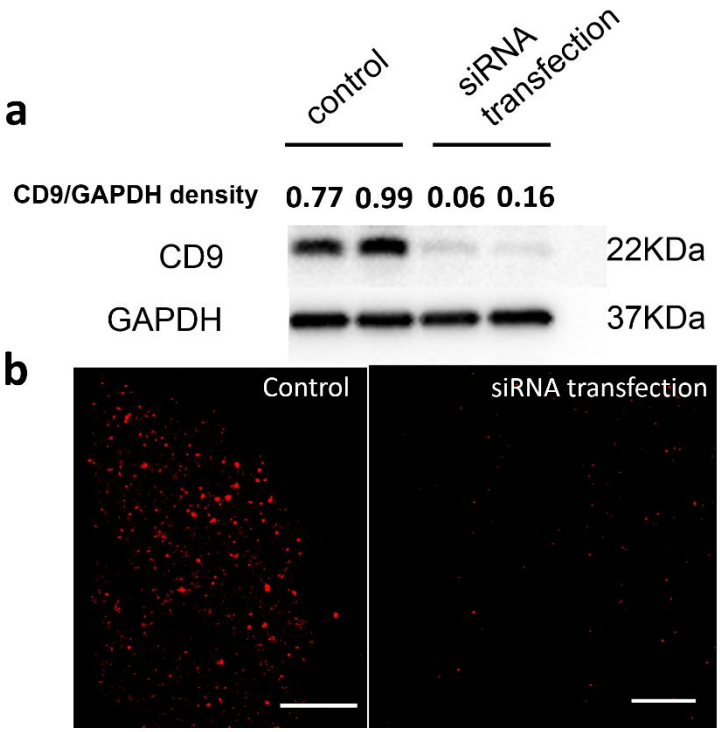

Figure S8. Measurement of the CD9-knockdown efficiency. (a) Western blot analysis of CD9 in the control and siRNA silenced cells. The quantization of the Western blot data after correction for the GAPDH loading control. (b) dSTORM images of CD9 on the control and siRNA silenced cell membranes. Scale bars, 5 μm.