Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information for

Super-resolution imaging of STAT3 cellular clustering during the nuclear transport

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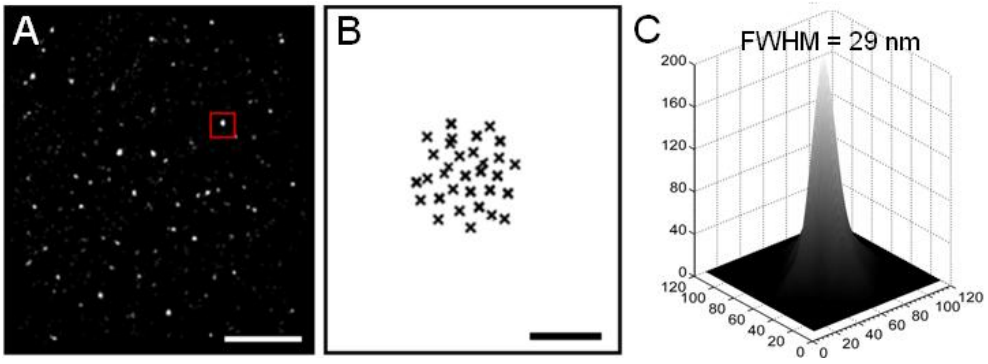

Fig. S1 The localization precision of single Alexa Fluor 647-conjugated STAT3 antibody on fixed and permeablized HeLa cells. (A) The dSTORM image of adequately diluted Alexa Fluor 647-STAT3 antibody molecules on HeLa cells. (B) Repetitive localizations of a single Alexa Fluor 647-STAT3 antibody boxed in (A) and the number of localizations in this molecule is 31. (C) The average value of singe molecule localization precision is 29 nm. The two-dimensional histograms of localizations were generated by aligning 100 fluorescent molecules with their center of mass and fitted to a Gaussian function to determine the full-width at half-maximum (FWHM). Scale bars represent 1 μm in (A) and 20 nm in (B).

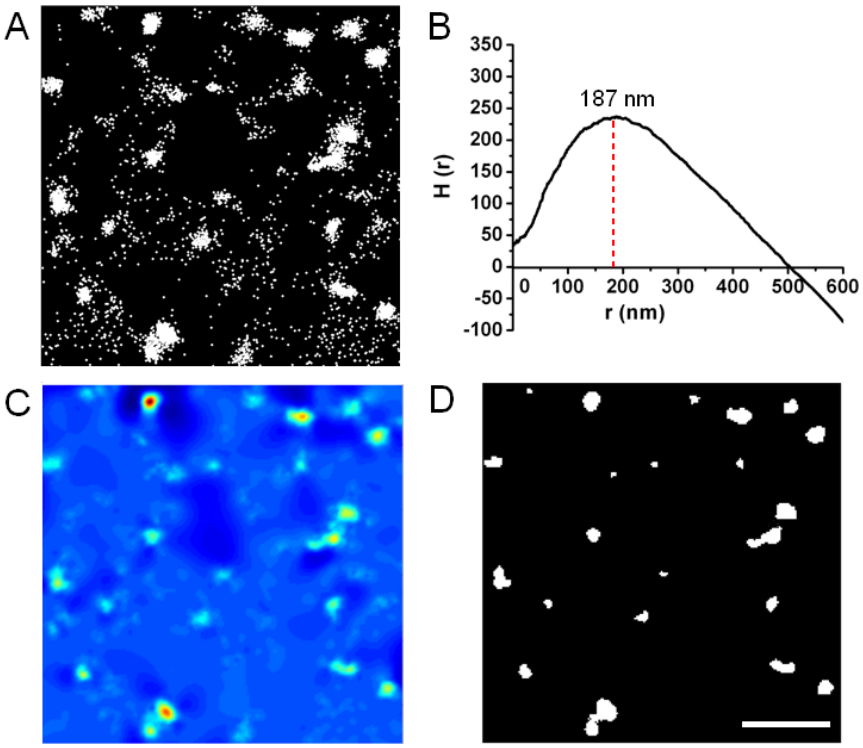

Fig. S2 Illustration of Ripley's K-function analysis for STAT3 clustering. (A) A 4×4 μm^2 region of the reconstructed dSTORM image of STAT3 in the cytoplasm of the unstimulated HeLa cell. (B) The corresponding Ripley's K-function plot showing that the radius of maximal aggregation is 187 nm and clustering range on length scales at 500 nm. (C) The interpolated cluster map based on Ripley's K-function analysis indicating a significant clustering distribution. (D) The binary cluster image generated from the color-coded cluster map through a defined threshold, from which the cluster number, size and other information can be extracted. Scale bar represents 1 µm.

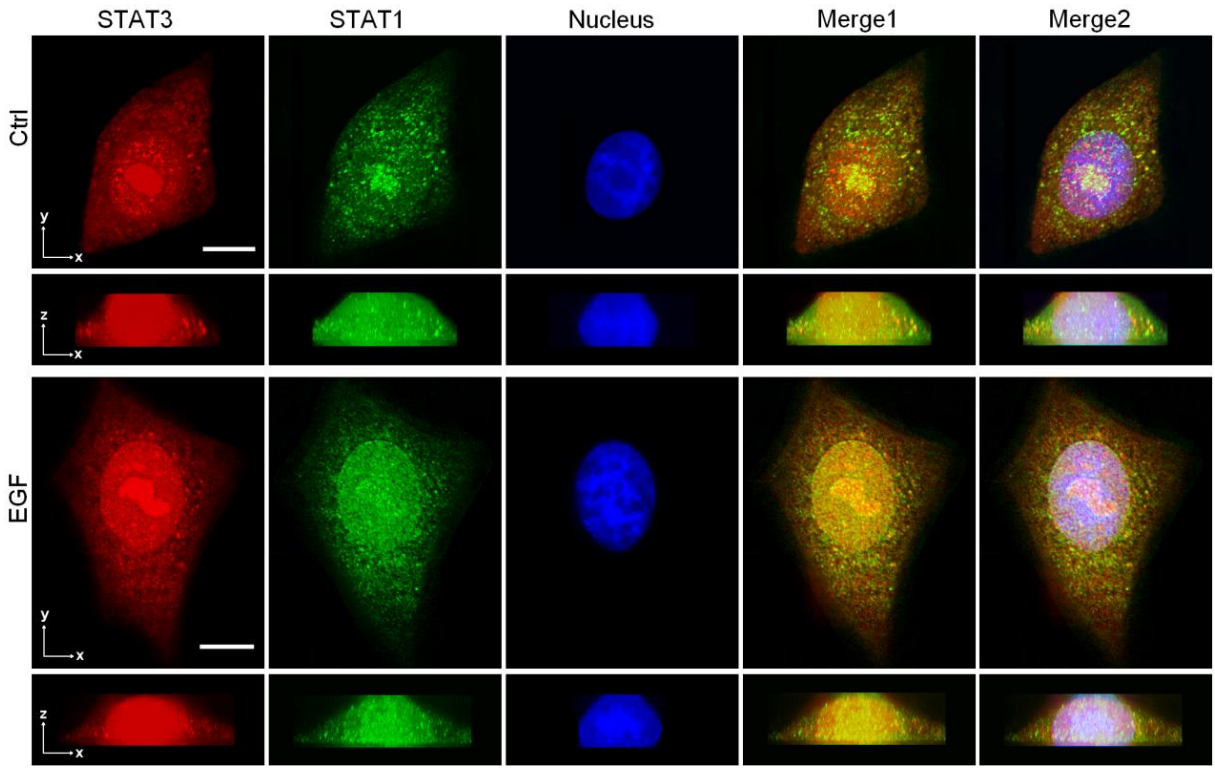

Fig. S3 3D fluorescence imaging of STAT3 and STAT1 in HeLa cells. HeLa cells, without stimulation and with 30 min-EGF stimulation, were fixed, permeated and stained with Alexa Fluor 647-conjugated STAT3 antibodies, Alexa Fluor 532-conjugated STAT1 antibodies and Hoechst 33342. The fluorescence images of control HeLa cell are shown in the first row, which were captured at approximately 3 μm above the bottom of the cell. The 3D images of control Hela cell were shown in the second row, which were constructed by successive z-stacks spaced by 200 nm from the bottom to the top of the cell. The total distance of scanned z-axis is 8 μm. The third and fourth rows are the results of EGF-stimulated HeLa cell. From left to right: STAT3, STAT1, the nucleus, merged panels of STAT3 and STAT1 (Merge 1) and merged panels of STAT3, STAT1 and the nucleus (Merge 2). Scale bars represent 10 μm.

Table S1 The parameters of STAT3 clustering by Ripley's K-function analysis.

Time	Cytoplasm			Nucleoplasm		
(min)	r_{ave} [a)] (nm)	$H(r)_{max}$ [b)]	r_{max} [c)] (nm)	r_{ave} (nm)	$H(r)_{max}$	r_{max} (nm)
0	180 ± 14	242 ± 22	498 ± 35	105 ± 13	96 ± 10	340 ± 27
20	166 ± 10	287 ± 20	551 ± 42	204 ± 18	154 ± 13	386 ± 31
40	177 ± 13	309 ± 27	549 ± 44	191 ± 15	165 ± 17	405 ± 39
60	209 ± 19	292 ± 24	538 ± 37	158 ± 11	122 ± 14	320 ± 24
120	193 ± 16	245 ± 22	526 ± 31	121 ± 10	113 ± 12	316 ± 21
240	188 ± 17	265 ± 23	502 ± 39	87 ± 8	88 ± 9	302 ± 20

[a)] the r value corresponding to the maximum of H(r); [b)] the vertex of the curve; [c)] the intercept of the curve at the horizontal axis.

Ripley's K function is applied in the regions (4×4 μm^2) of STAT3 dSTORM images at different stimulation times. Every group includes 20 cells from five independent experiments, and each cell is chosen three regions in both the cytoplasm and nucleoplasm. The values of every parameter are the mean ±s.d.

Table S2 The parameters of EGF-induced STAT3 and STAT1 clustering by Ripley's K-function analysis.

Name	Treatment	Cytoplasm			Nucleoplasm		
		r_{ave} (nm)	$H(r)_{max}$	r_{max} (nm)	r_{ave} (nm)	$H(r)_{max}$	r_{max} (nm)
STAT3	Ctrl	179 ± 13	251 ± 24	504 ± 36	108 ± 10	105 ± 10	352 ± 28
	EGF	175 ± 12	289 ± 26	543 ± 47	214 ± 23	172 ± 13	411 ± 40
STAT1	Ctrl	184 ± 14	263 ± 25	515 ± 40	113 ± 11	112 ± 13	362 ± 31
	EGF	181 ± 13	294 ± 27	559 ± 46	210 ± 17	183 ± 15	407 ± 38

Ripley's K function is applied in the regions (4×4 μm^2) of STAT3 and STAT1 dSTORM images before and after EGF addition. Every group includes 20 cells from five independent experiments, and each cell is selected three regions in both the cytoplasm and nucleoplasm. The values of every parameter are the mean ±s.d.