Supplymental Information

Mechanical force regulation of YAP by F-actin and GPCR revealed by super-resolution imaging

Jing Gao,[a] Lingli He,[b] Lulu Zhou,[a] Yingying Jing,[a,d] Feng Wang,[c] Yan Shi,[a] Mingjun Cai,[a] Jiayin Sun,[a] Haijiao Xu,[a] Junguang Jiang,[a] Lei Zhang [b] and Hongda Wang *[a,e]

[a.] *State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Research Center of Biomembranomics, Changchun, Jilin, 130022, P.R. China.*

[b.] *State Key Laboratory of Cell Biology, CAS Center for Excellence in Molecular Cell Science, Innovation Center for Cell Signaling Network, Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, Shanghai, 200031, P.R. China.*

[c.] *Institute of Immunology, The First Bethune Hospital Academy of Translational Medicine, Jilin University, Changchun, Jilin, 130021, P.R. China.*

[d.] *University of Science and Technology of China, Hefei, Anhui, 230027, P.R. China.*

[e.] *Laboratory for Marine Biology and biotechnology, Qingdao National Laboratory for Marine Science and Technology, Qingdao, Shandong, 266237, P.R. China.*

* Correspondence: hdwang@ciac.ac.cn

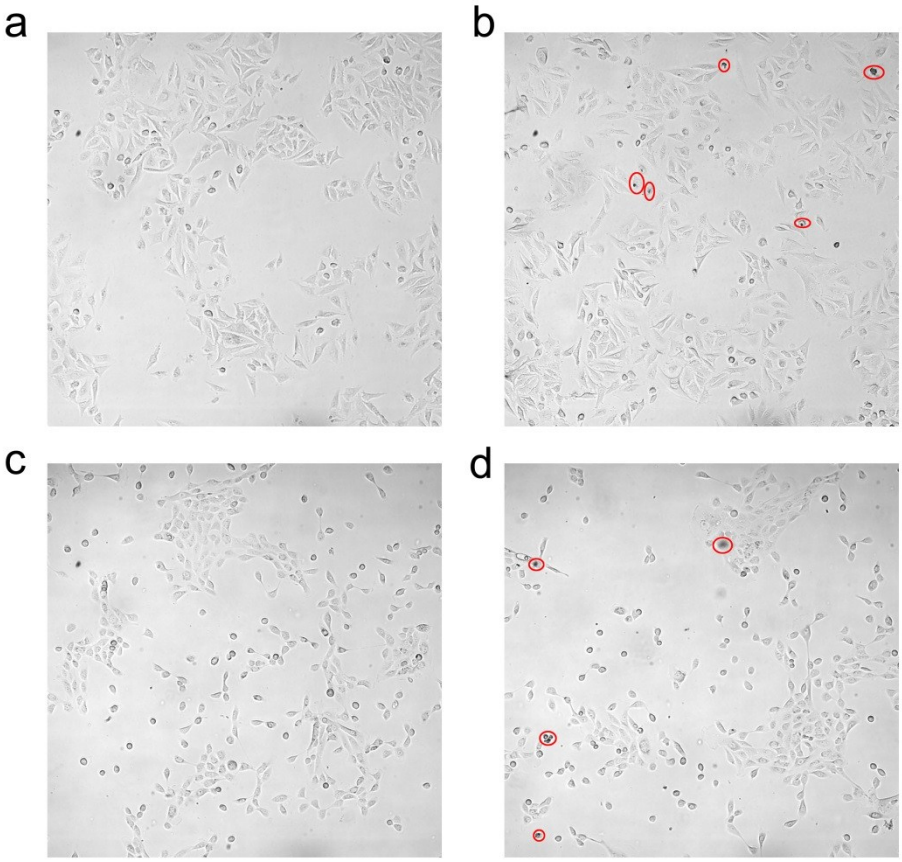

Figure S1. Cell viability assay by trypan blue staining. Sparsely cultured cells with or without press treatment were stained with 0.04% trypan blue solution for 10 min, and observed under a 10× microscope after removing the solution. (a, c) Control groups of HeLa (a) and MCF-10A (c) cells. (b, d) The corresponding pressed groups. Cells were pressed with PDMS for 1 h, stained by trypan blue and then imaged. Dead cells were marked by red circles.

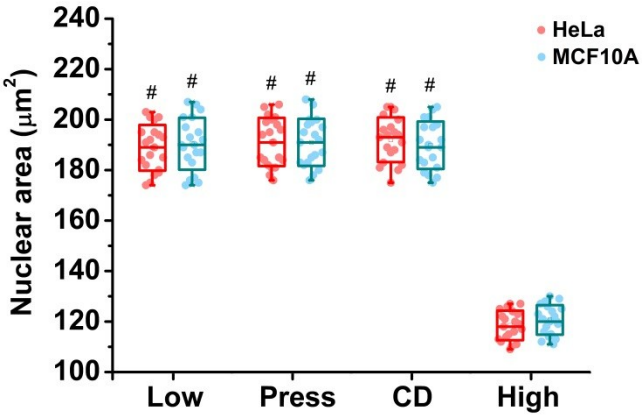

Figure S2. Nuclear areas of HeLa and MCF-10A cells cultured at low or high cell density without treatment, and at low cell density with pressure or CD treatment. #P<0.01, Low versus High, Press versus High and CD versus High in each cell line, two tailed unpaired t-test.

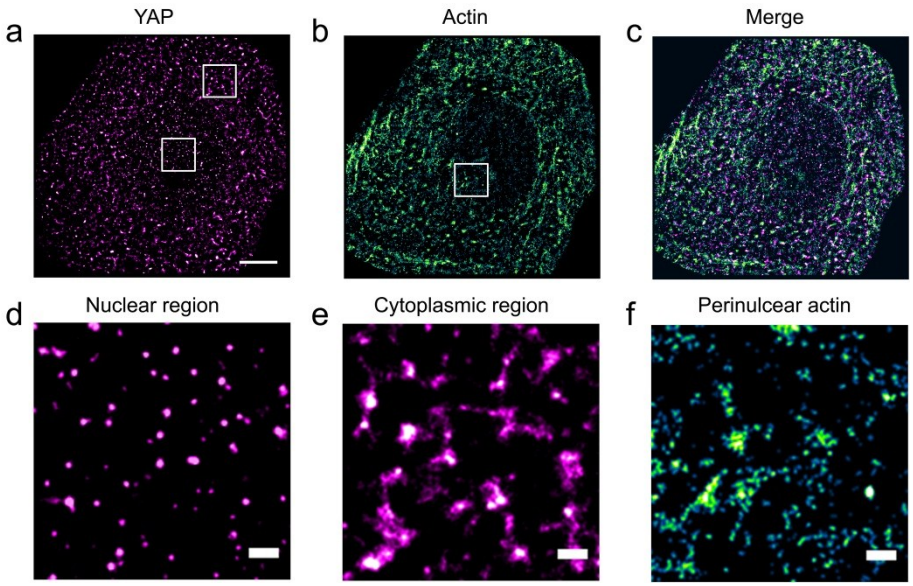

Figure S3. Dual-color dSTORM imaging of YAP and F-actin in MCF-7 cells with press treatment. (a-c) YAP, actin and their merged dSTORM images in sparsely cultured MCF-7 cells after press. (d-f) The enlarged view of nuclear and cytoplasmic regions of YAP, and perinuclear regions of actin, which are boxed with white in (a) and (b). Scale bars are 5 μm in (a-c) and 500 nm in (d-f).

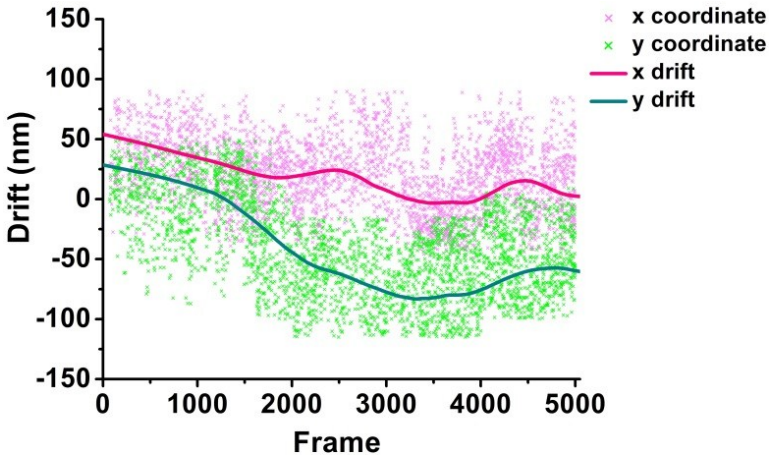

Figure S4. Lateral drift correction during the acquisition of 5000 frames in analyzing the raw data of F-actin.

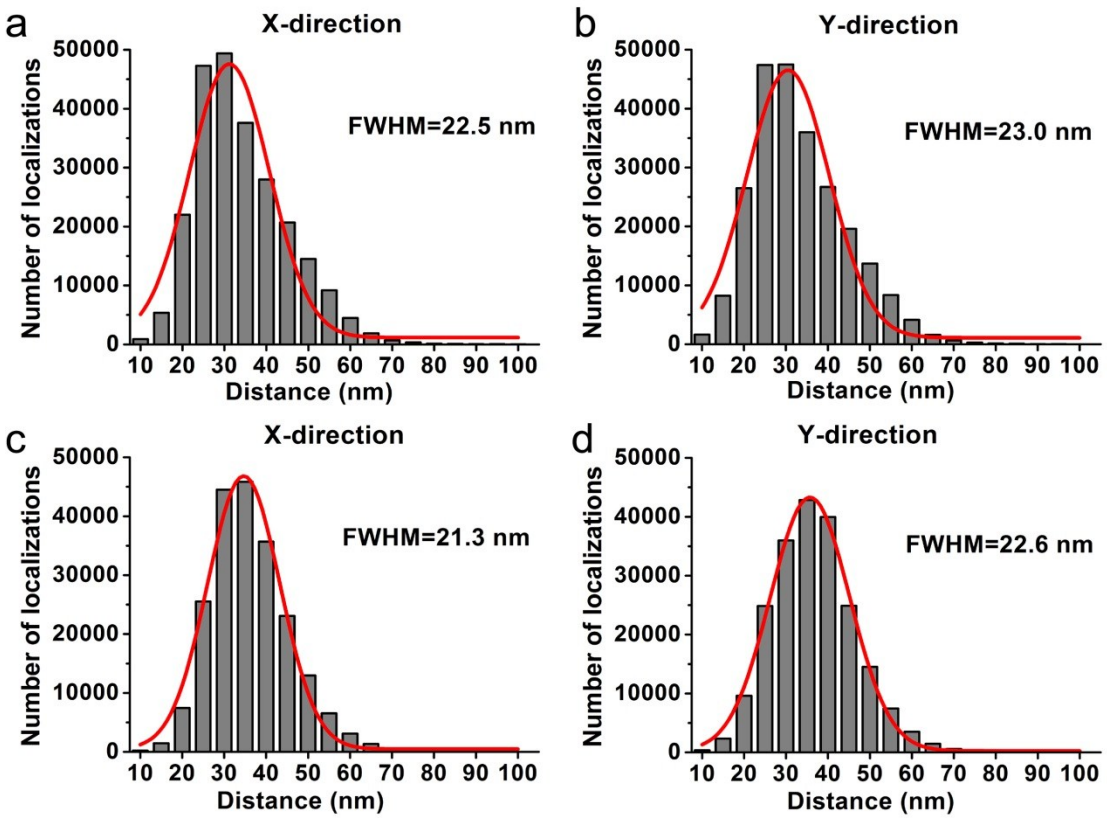

Figure S5. Measurement of the resolution of YAP dSTORM images in two cell lines. (a) The distribution of x coordinates of YAP localizations from 5000 frames in a typical HeLa cell cultured at low cell density. The histogram was fitted to a Gaussian function and its full width at half-maximum (FWHM) was calculated to determine the resolution, which was 22.5 nm. (b) The distribution of y coordinates of YAP localizations in the same cell. The FWHM was 23.0 nm. (c, d) The distribution of x and y coordinates of YAP localizations in a MCF-10A cell. The FWHM was 21.3 and 22.6 nm, respectively.

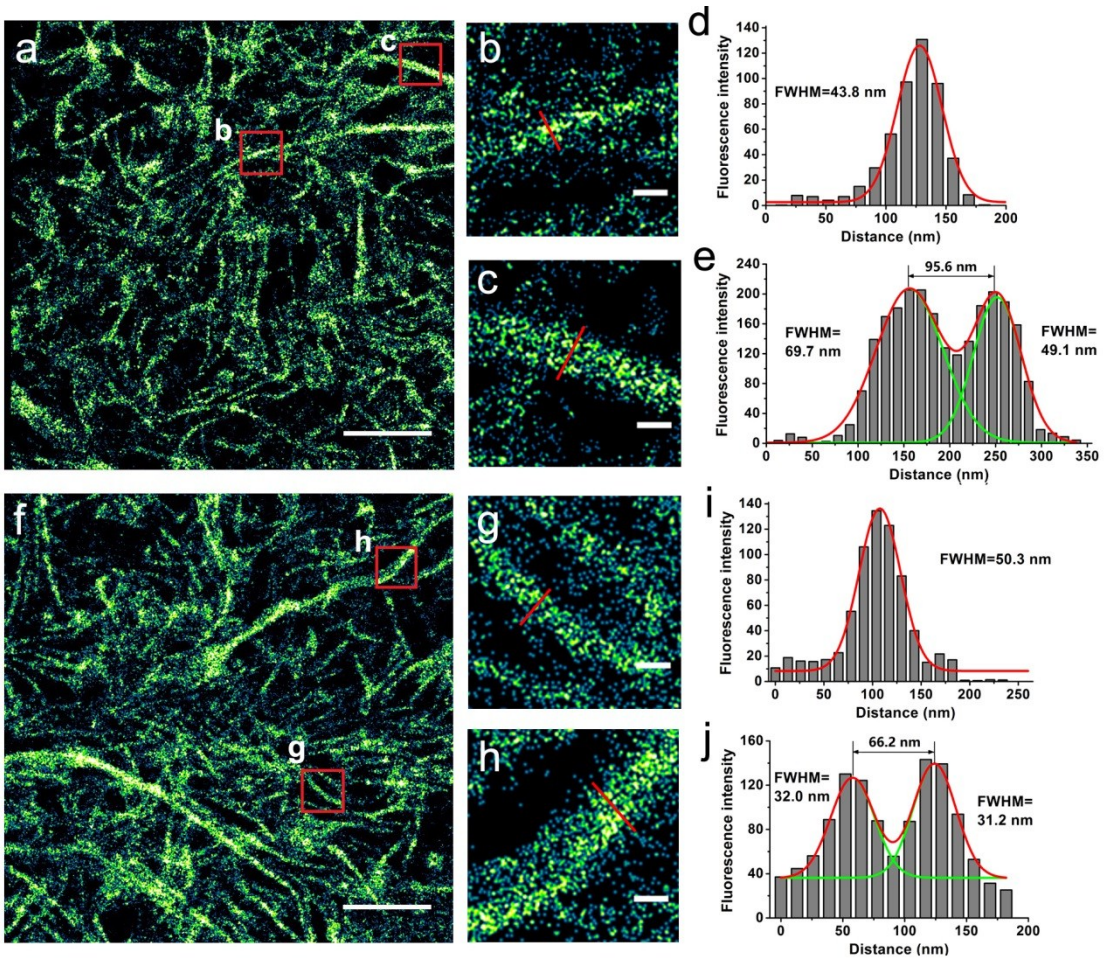

Figure S6. Quantitative size analysis of F-actin in two cell lines. (a, f) dSTORM imaging of F-actin in sparsely cultured HeLa and MCF-10A cells, respectively. (b, c, g, h) The enlarged areas of the ROIs (red boxes) in (a) and (f). (d, e, i, j) The corresponding transverse profiles of the F-actin labeled with red lines show the width and the distances between two actin filaments. Scale bars, 2 um in (a, f), 100 nm in (b, c, g, h).