Supporting Information

Super-resolution microscopy reveals the reorganization of GLUT4 on plasma membrane regulated by insulin resistance

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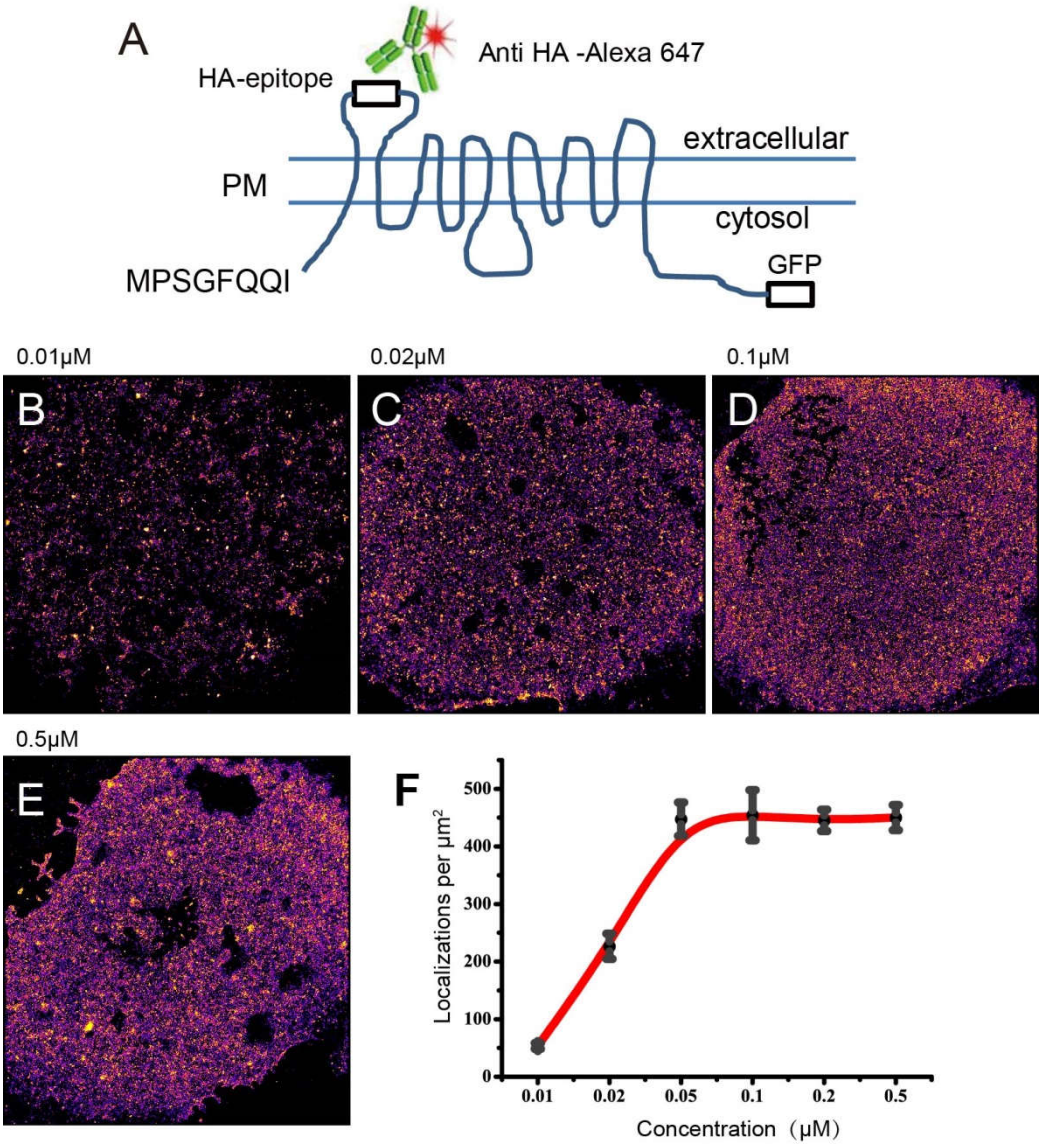

Figure S1. A. Structural diagram of the HA-GLUT4-GFP protein. The F^5QQI motif is indicated, and the positions of the HA-epitope tag and GFP are shown. B-E. dSTORM images GLUT4 on the PM of insulin-stimulated adipocytes treated with different concentrations of anti-HA 1.1-Alexa Fluor 647 (0.01- 0.5 μM). F. Localization density of GLUT4 on the PM of insulin-stimulated adipocytes treated with different concentrations of anti-HA 1.1-Alexa Fluor 647 (0.01- 0.5 μM); at an anti-HA 1.1-Alexa Fluor 647 concentration of about 0.05 μM, the number of localizations per unit of cell membrane area, as determined by dSTORM, was saturated.

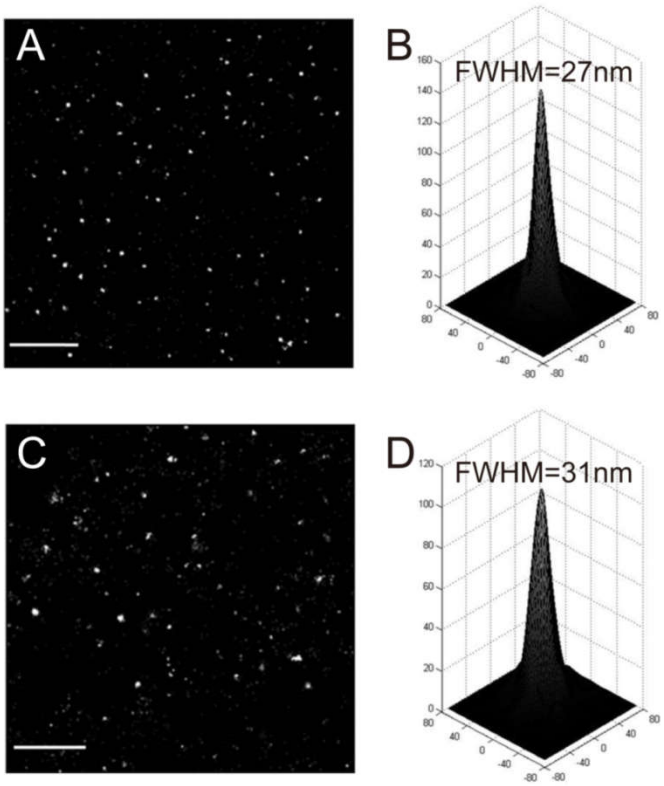

Figure S2. Measurement of the localization precision of a single Alexa Fluor 647-conjugated anti-HA.11 monoclonal antibody (mAb) molecule on a coverslip or the cell surface. (A) Typical dSTORM image of Alexa Fluor 647-conjugated anti-HA.11 mAbs at an appropriate concentration (~7 nM) on a clean coverslip. Each spot in the image represents a cluster of localizations from a single Alexa Fluor 647-conjugated antibody molecule. (B) Two-dimensional histograms of the localizations were generated by aligning 50 single antibody molecules, and the localization precision of 27 nm was determined by measuring the full-width at half-maximum (FWHM). (C，D) The localization precision of a single Alexa Fluor 647-conjugated anti-HA.11 mAb molecule on the 3T3-L1 adipocyte membrane. The FWHM was 31 nm. Scale bar: 2 μm.

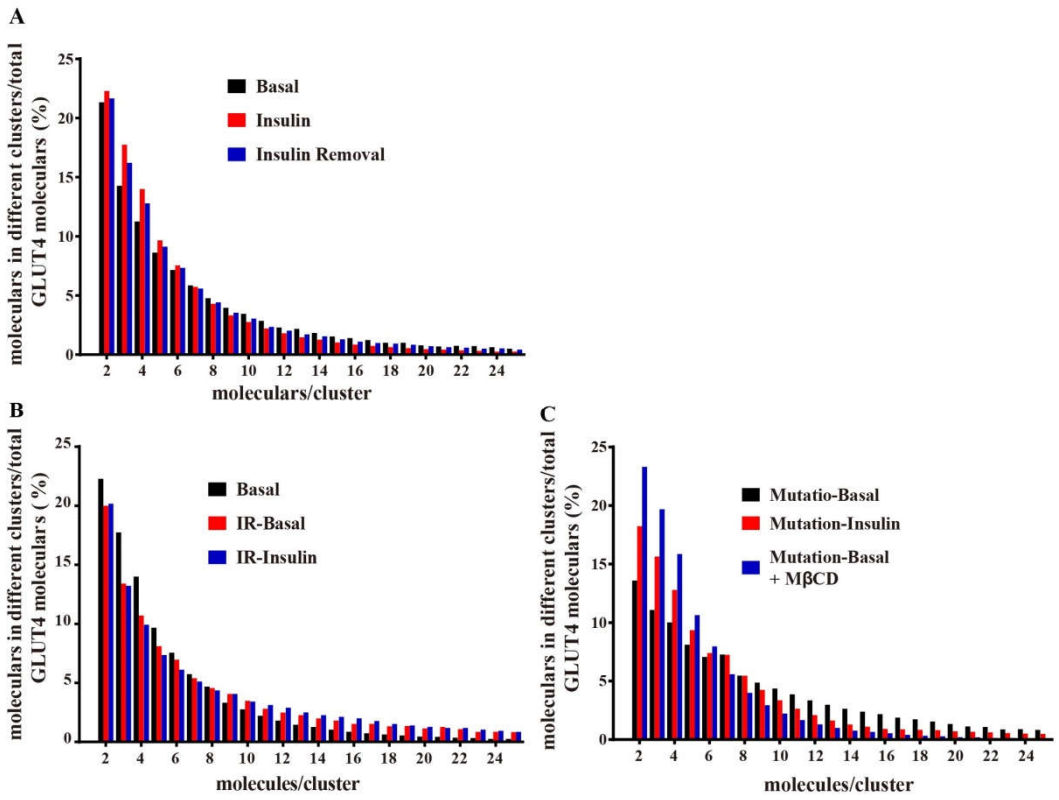

Figure S3. The entire GLUT4 molecules distribution of different sizes of clusters in all conditions studied: (A) wide-type GLUT4 on the PM of normal cells; (B) wide-type GLUT4 on the PM of insulin-resistant cells; (C) F^5QQI-GLUT4 on the PM of normal cells.

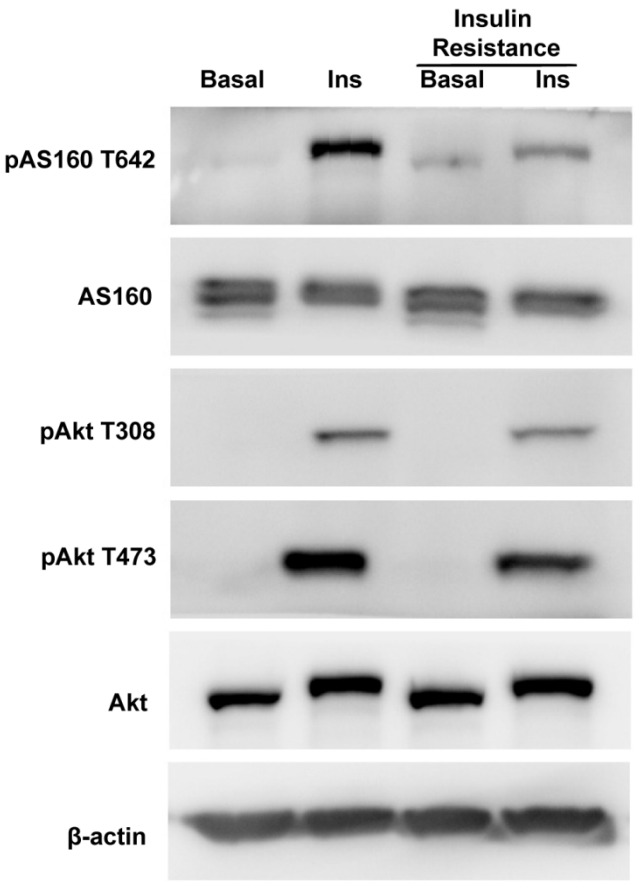

Figure S4. Representative western blot of screened proteins involved in the insulin signaling pathway in insulin-resistant adipocytes. Insulin signaling was monitored by measuring phosphorylation of Akt at Thr-308 and Ser-473 and AS160 at Thr-642 in response to 100 nM insulin. Total levels of Akt and AS160 were assessed in all conditions, and β-actin was used as a control.

Table S1. Compare of corresponding data in Figure 1 and Figure 3.

	Widetype GLUT4		GLUT4 (F^5QQA)	
	Normal-Basal	Normal-Insulin	Normal-Basal	Normal-Insulin
Molecules Density	90 ± 41	229 ± 48	184 ± 37	363 ± 123
Clusters Density	31.4 ± 13.2	79.6 ± 12.7	49.8 ± 11.0	108.9 ± 33.4
Average molecules per cluster	2.79 ± 0.39	2.87 ± 0.36	3.73 ± 0.36	3.44 ± 0.33
Molecules in clusters/total molecules	78% ± 5%	66% ± 2%	80% ± 4%	63% ± 5%