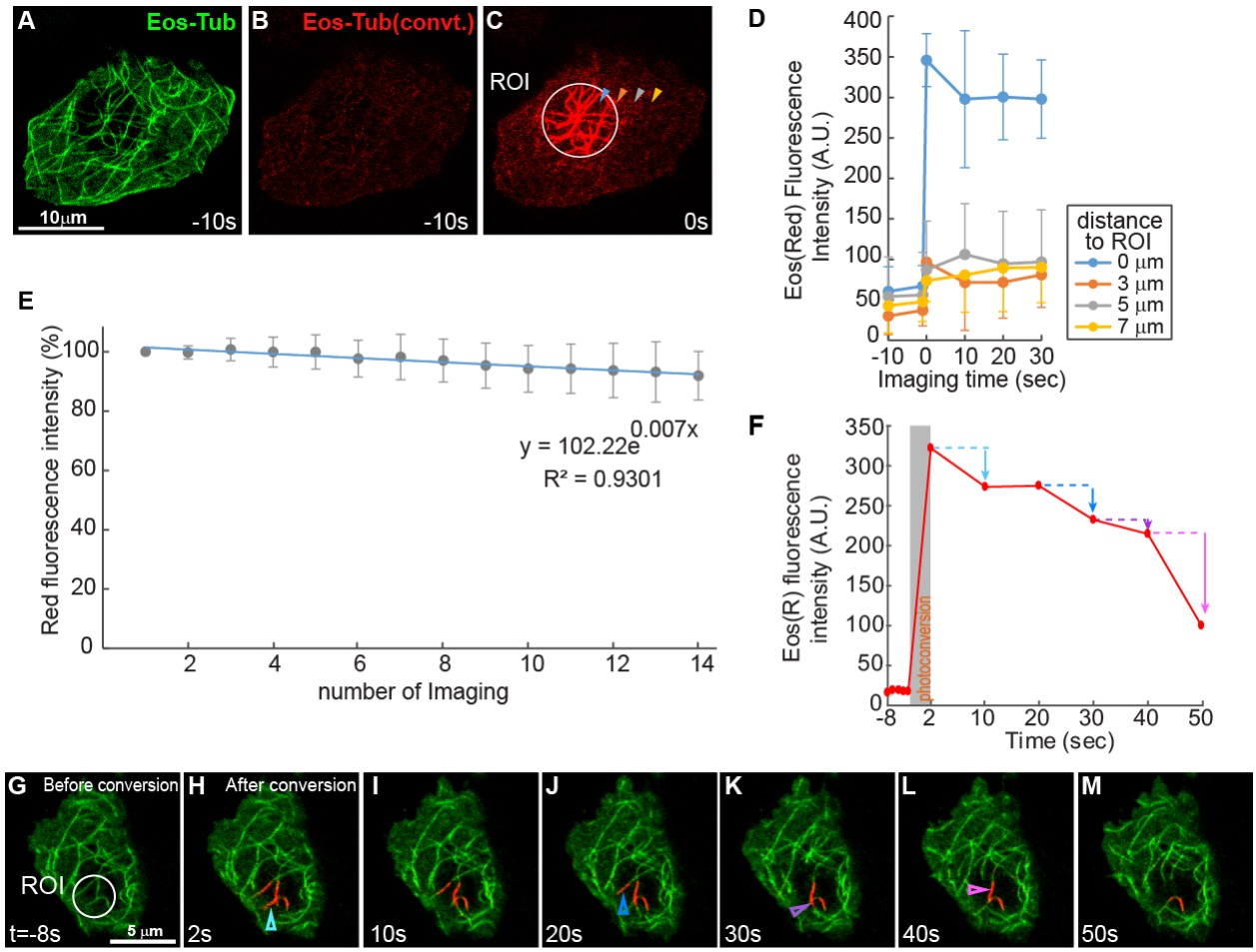
Online Suppl. Materials

Summary:

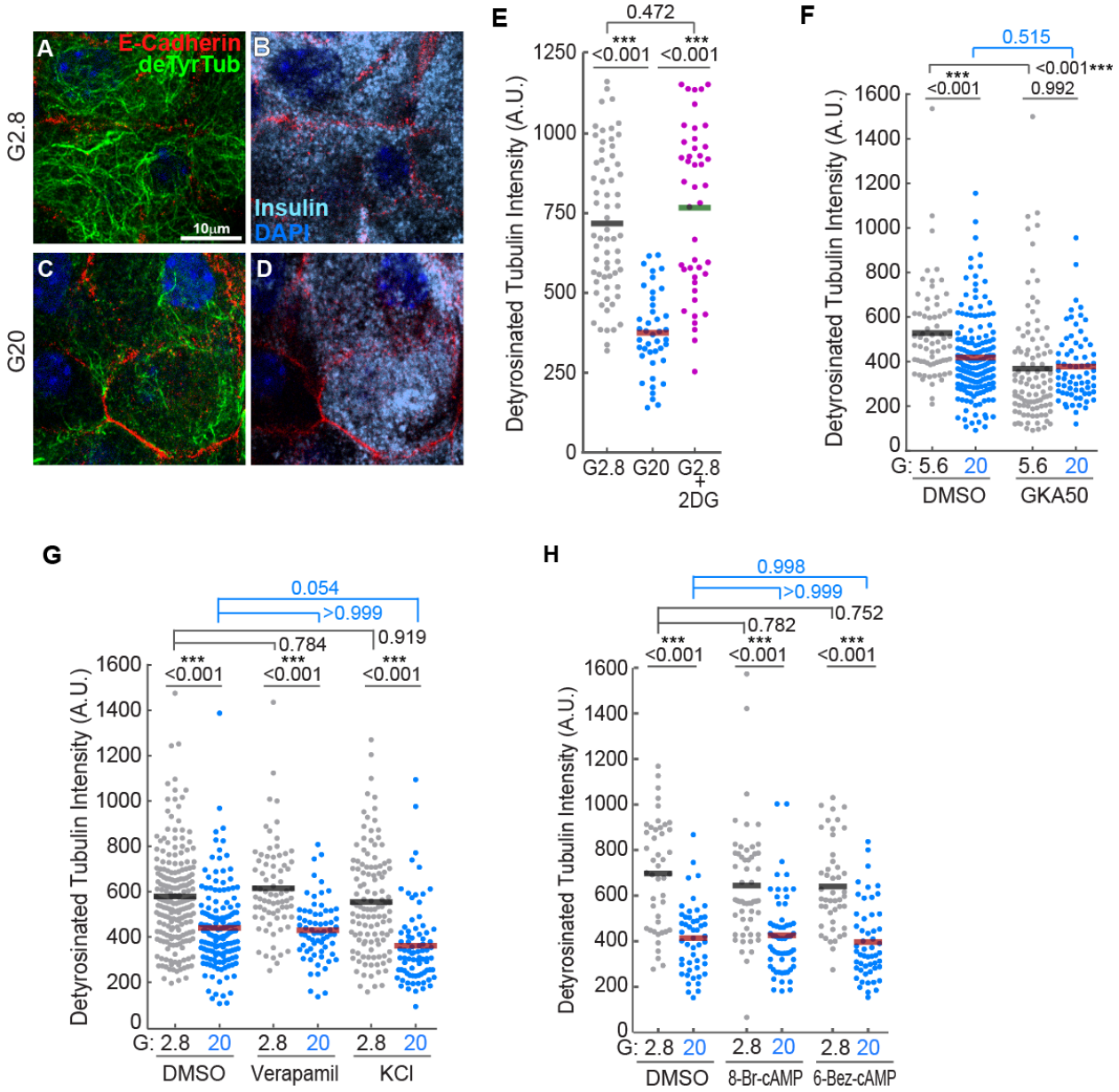
Supplemental Movie 1. Detection of microtubule disassembly in live islet β-cells in the photoconversion assay. Volume projection of islet β-cells expressing Eos-Tubulin and *Ins2*::H2B-Apple. Images were taken with a 0.6 µm z-step at 10 seconds before photoconversion, 10 seconds after, and 145 seconds after photoconversion. Green, Eos-tubulin; red, photoconverted Eos-tubulin, dim red, H2B-Apple.

Suppl. Figure S1



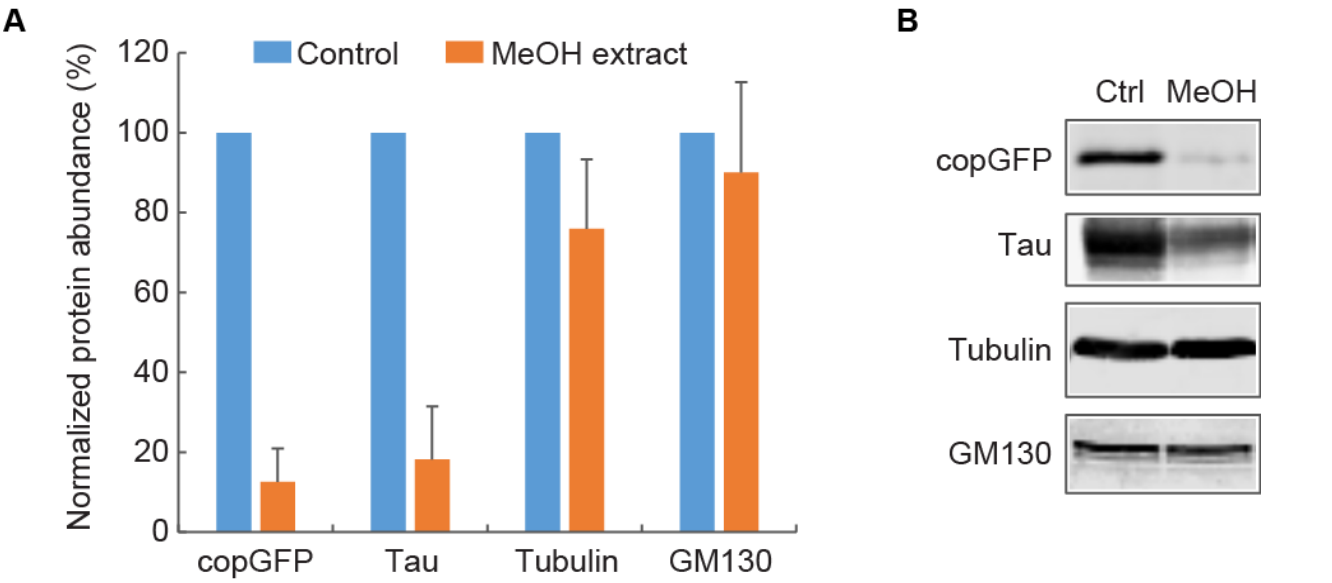
Photoconverted free Eos-tubulin dimer and photobleaching do not interfere with detection of microtubule disassembly. (A-C) Fluorescence images of a live MIN6 cell expressing Eos-tubulin 10 seconds before (-10s) and immediately after (0s) photoconversion. Colored arrowheads label subcellular locations where the cytoplasmic red fluorescence intensity is quantified in (D). Images were brightness- and gamma-adjusted consistently to better present weak signal of photoconverted free Eos-tubulin in the cytoplasm. (D) Quantification of photoconverted free Eos-tubulin dimer in the cytoplasm at different distance to the ROI. Note that the red fluorescence intensity within the ROI (the blue line) drops from 0 to 10-second and stays steady afterward. Image background subtraction used the average fluorescence intensity in an area that has no cells. The data are presented as mean ± SD from three repeats (cells). In each cell, the fluorescence intensity was measured at 4-6 subcellular locations at each distance. (E) The photobleaching curve of red Eos-tubulin in islets. The data are presented as mean ± SD from three repeats (n = 11 cells). The blue line represents an exponential trend line of the photobleaching curve. (F-M) Quantification and time-series images of photoconverted microtubules labeled by Eos-tubulin in a live MIN6 cell. Microtubules within the region of interest (ROI) were photoconverted from green to red by UV irradiation. Arrowheads mark photoconverted microtubules disassembled in the next frame. Note that in (F), arrowheads are color-coded to mark the reduction of red fluorescence corresponding to microtubule disassembly in (G-M).

Suppl. Figure S2



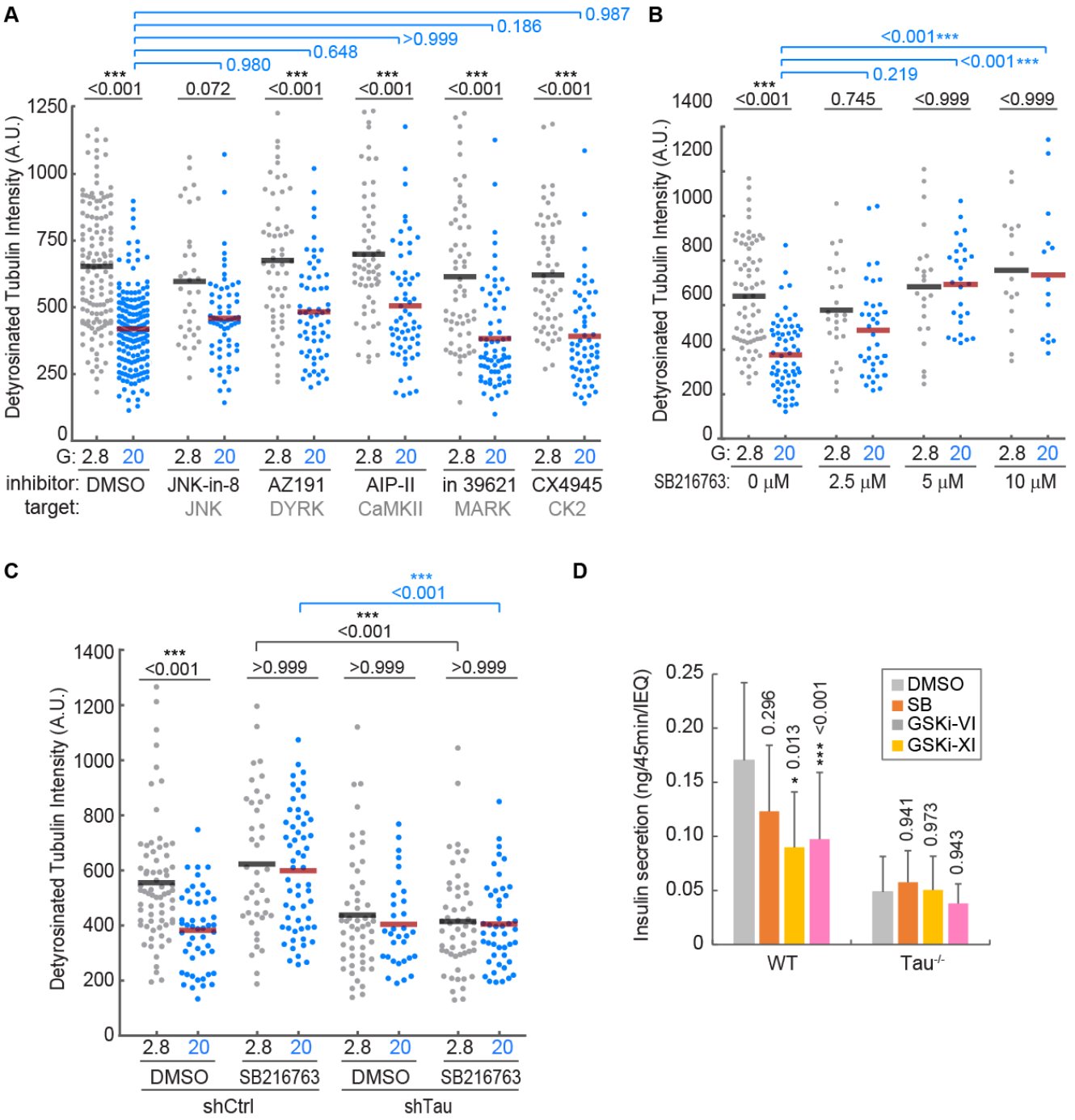
The depolarization of islet β-cells does not induce microtubule disassembly. (A-D) IF images of detyrosinated tubulin in mouse β-cells. Isolated islets were incubated in media with 2.8 mM (A, B) or 20 mM glucose (C, D) for 2 hours. Green, detyrosinated tubulin; red, E-cadherin; blue, DAPI; cyan, insulin. (E) Quantification of detyrosinated tubulin IF in islets β-cells treated with 2.8 mM glucose, 20 mM glucose, or 2.8 mM glucose plus 17.2 mM 2-Deoxy-D-Glucose (2-DG) for 2 hours. Dots represent individual islet β-cells. Bars represent mean; n = 42-65 cells from three repeats (animals). *** p<0.001 (one-way ANOVA). (F-H) Quantification of detyrosinated tubulin IF in β-cells. Islets were pre-treated with 0.05% DMSO, 10 μM Verapamil, 1 μM GKA50, 25 μM 8-Br-cAMP, or 25 μM 6-Bez-cAMP in media containing 2.8 mM (F) or 5.6 mM (G, H) glucose for two hours. Glucose was added to 20mM for two hours and 100 mM KCl was added 15 minutes before fixation. Dots represent individual islet β-cells. Bars represent mean; n = 68-160 (F & G) or 45-60 cells (H) from three repeats (animals). *** p<0.001 (two-way ANOVA).

Suppl. Figure S3



Methanol extraction efficiently removes cytosolic tau that does not bind to microtubules. (A) Quantification of protein abundance in MIN6 cells after methanol extraction. MIN6 cells grown in media containing 25 mM glucose were extracted with cold anhydrous methanol for 5 minutes before lysis. The data are normalized to without extraction and presented as mean ± SD from three repeats. (B) Immunoblotting of MIN6 cells with or without methanol extraction.

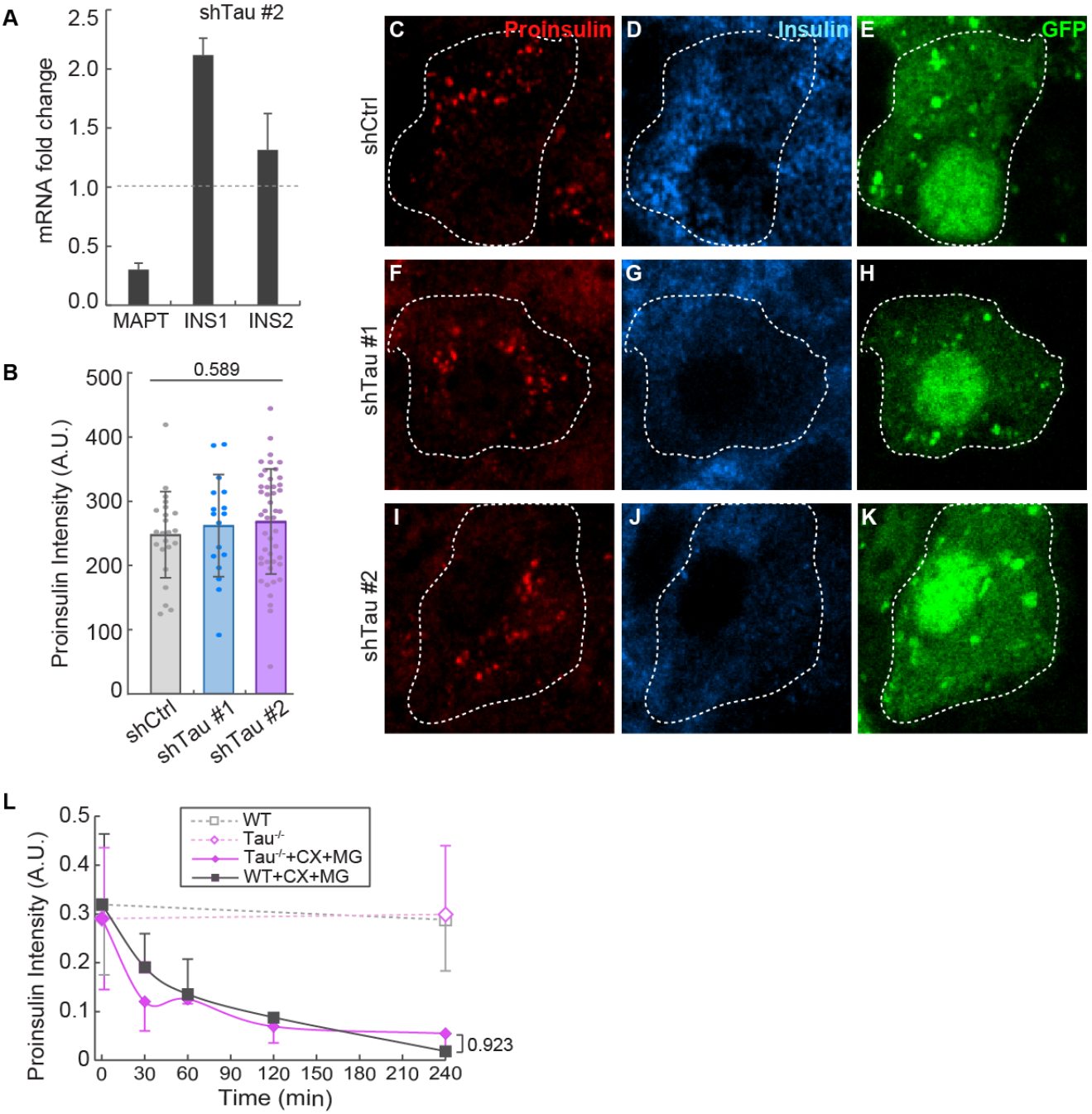
Suppl. Figure S4



The effect of GSK3 inhibitors to suppress microtubule disassembly and insulin secretion in high glucose depends on tau. (A-B) Quantification of detyrosinated tubulin IF. Islets were pre-treated with 0.05% DMSO, 2 µM JNK-IN-8, 10 µM AZ191, 0.4 µM AIP-II, 20 µM MARK inhibitor 39621, 0.1 µM CX-4945, or 2.5-10 µM SB216763 in 2.8 mM glucose for two hours, and glucose was added to half of the islets in each pre-treatment group to 20mM and incubated for two hours before fixation. Dots represent individual islet β-cells. Bars represent mean; n = 70-158 cells (DMSO), 34-68 cells (inhibitor treatment, A), or 15-27 cells (inhibitors treatment, B) from three repeats (animals). *** p<0.001 (two-way ANOVA with Tukey's post hoc test). (C) Quantification of detyrosinated tubulin IF. Islets β-cells expressing

shRNA were pre-treated with 0.05 % DMSO or 5 μM SB216763 in 2.8 mM glucose for two hours. Dots represent individual islet β-cells in islets. Bars represent mean; n = 40-70 cells from three repeats (animals). *** $p<0.001$ (three-way ANOVA with Sidak's post hoc test). (D) GSIS assay of wild type (C57BL/6J) or $tau^{-/-}$ islets treated with 0.05% DMSO, 5 μM SB216763, 2 μM GSK3 inhibitor VI, or 0.5 μM GSK3 inhibitor XI for two hours. The data are presented as mean ± SD from four repeats (except for SB216763 treatment, which includes two repeats). * $p<0.05$; *** $p<0.001$ (two-way ANOVA with Dunnett's post hoc test).

Suppl. Figure S5



Tau knock-down does not impair insulin transcription and proinsulin production in high glucose.

(A) QRT-PCR of *Mapt*, *Ins1*, and *Ins2* in tau-KD (shTau #2) MIN6 cells. The transcript levels were normalized to *Gapdh* and presented as fold change compared to control (shCtrl) cells. The data are presented as mean ± SD from three repeats. (B) Quantification of proinsulin fluorescence in islet β-cells expressing shRNA cultured in media containing 11 mM glucose. The data are presented as mean ± SD; n = 20-47 cells from three repeats (animals). Results were examine using one-way ANOVA. (C-K) IF images of isolated mouse islets in 11 mM glucose. Dashed lines delineate individual β-cells expressing shRNA. Red, proinsulin; blue, insulin; green, copGFP (transduction marker). (L) Conversion of proinsulin in wild type

(C57BL/6J) and tau$^{-/-}$ islets. Islets were incubated with 250 µg/mL cycloheximide and 250 µM MG-132 in 2.8 mM glucose for 0, 30, 60, 120, or 240 minutes and the total proinsulin content was determined by proinsulin-specific ELISA assay. Results are examined using two-way ANOVA and presented as mean ± SD of three repeats (animals).