iScience, Volume *26*

Supplemental information

CAMSAP2 localizes to the Golgi in islet β-cells and facilitates Golgi-ER trafficking

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CAMSAP2 localizes to the Golgi apparatus in pancreatic β-cells to facilitate Golgi-endoplasmic reticulum trafficking and support robust insulin secretion

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Supplemental Figure Titles and Legends

Figure S1

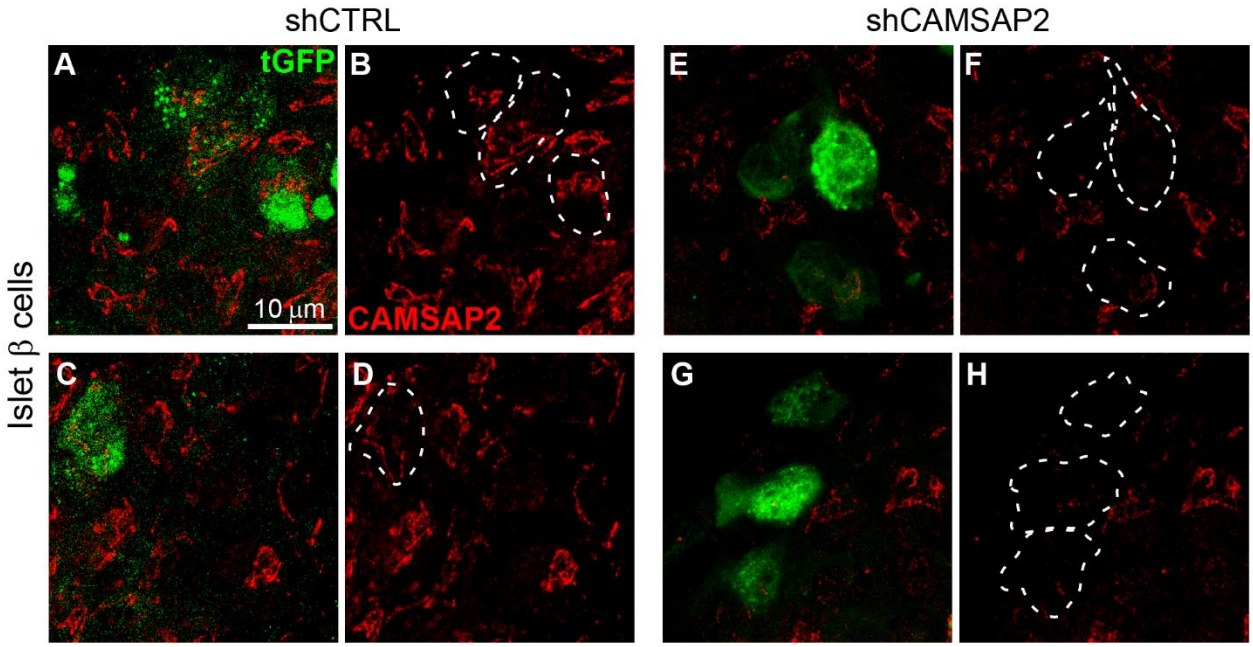

Figure S1. Knockdown of CAMSAP2 by shRNA in primary β-cells in mouse islets. Related to Figure 1.

Immunofluorescence staining of CAMSAP2 (red) in islet cells expressing non-targeting shRNA (shCtrl, A-D) or shRNA targeting CAMSAP2 #1 (shCAMSAP2, E-H). Intact mouse islets were incubated with lentivirus and individual transduced β-cells on the surface of each islet were quantified and presented in Figure 1C. Green, tGFP to indicate successful transduction.

Figure S2

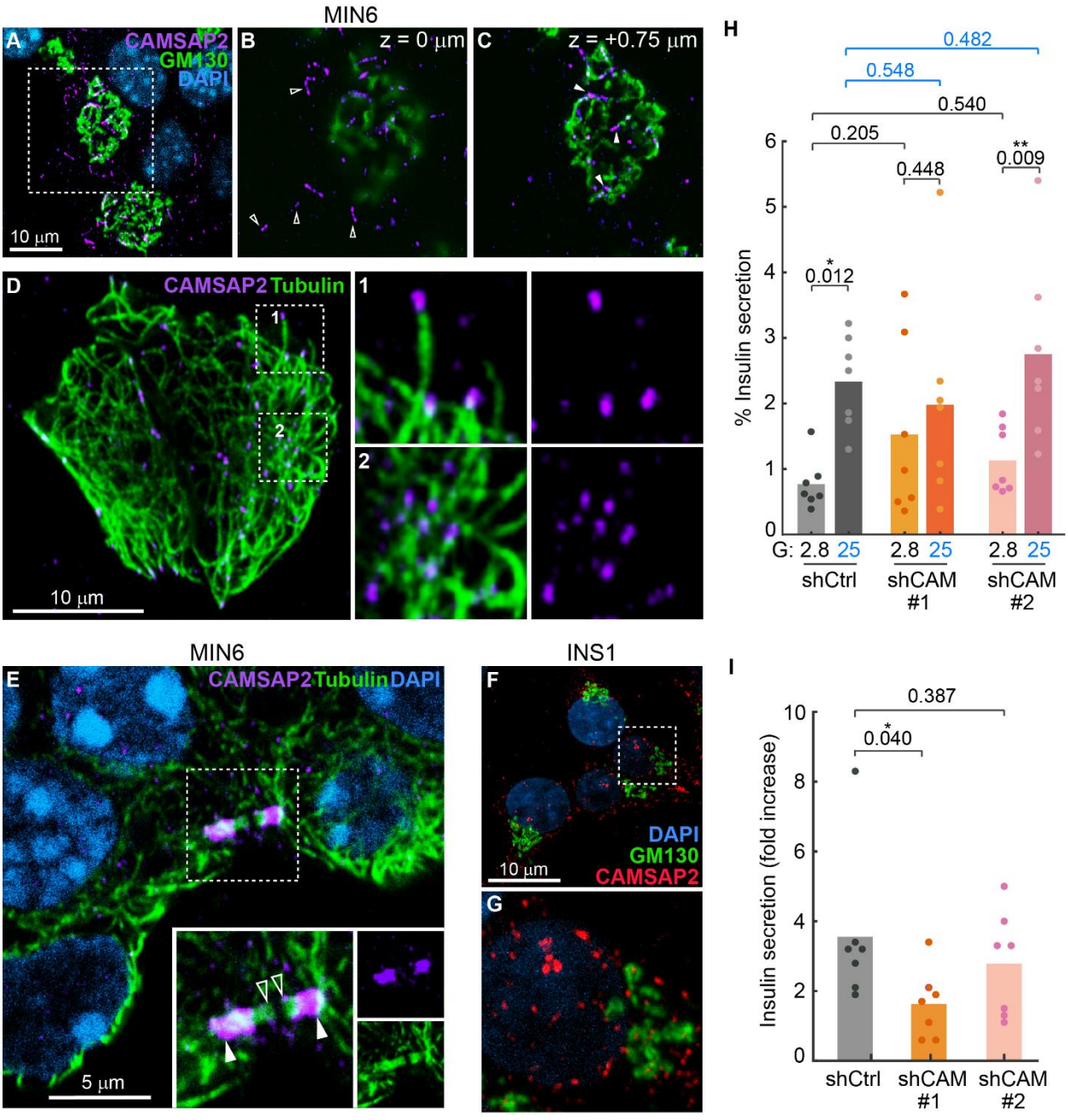

Figure S2. CAMSAP2 binds to microtubule minus ends in MIN6 and in INS1 cells, whereas CAMSAP2 knockdown in MIN6 cells does not significantly compromise GSIS. Related to Figure 3.

(A-C) Immunofluorescence staining of GM130 (green) and CAMSAP2 (magenta) in

MIN6 cells. Cyan, DAPI. Panel B and C are at the same x-y position and 0.75 μm apart on the z-axis. Panel A is the maximum intensity projection of 7 z-slides between 0 and +0.75 μm. Open arrow heads, CAMSAP2 stretches in cell periphery; closed arrow heads, CAMSAP2 stretches associated with the Golgi. (D-E) Immunofluorescence staining of tubulin (green) and CAMSAP2 (magenta) in MIN6 cells. Cyan, DAPI. Closed arrow heads, minus ends of microtubules in the midbody; open arrow heads, plus ends. (F-G) Immunofluorescence staining of GM130 (green) and CAMSAP2 (red) in INS1 cells. Cyan, DAPI. (H-I) Insulin secretion of MIN6 cells expressing non-targeting (shCtrl) or CAMSAP2-targeting (shCAM) shRNAs. MIN6 cells were incubated with 2.8 glucose for 45 minutes, and then with 25 mM glucose for another 45 minutes. Panel I shows the fold increase of insulin secretion (stimulated over basal secretion). Dots represent individual wells that grown MIN6 cells. Columns represents three independent repeats. * $p<0.05$, ** $p<0.01$ (multiple comparisons corrected by False discovery rate of Benjamini and Hochberg).

Figure S3

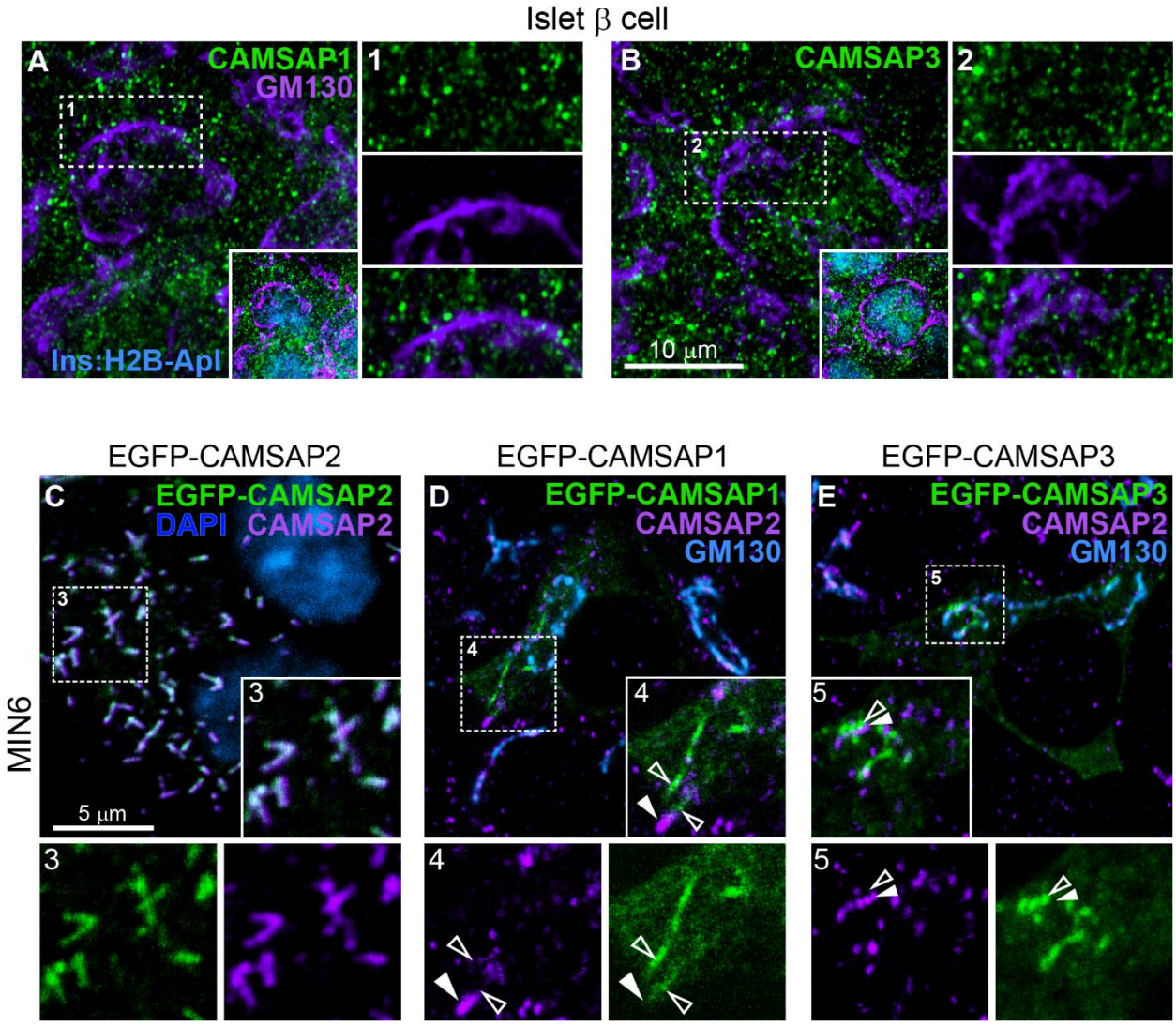

Figure S3. CAMSAP1 and CAMSAP3 do not localize to the Golgi in primary β-cells and their signal do not co-localizes with CAMSAP2. Related to Figure 3.

(A-B) Immunofluorescence staining of CAMSAP1 (green in A), CAMSAP3 (green in B) and GM130 (magenta) in primary β-cells in mouse islets. Cyan, *Ins2* promoter-driven H2B-mApple to label β-cells in islets. (C) Immunofluorescence staining of CAMSAP2 (magenta) in MIN6 cells ectopically expressed EGFP-CAMSAP2 (human CAMSAP2 transcript variant 1, green). Blue, DAPI. (D-E) Immunofluorescence staining of

CAMSAP2 (magenta) and GM130 (cyan) in MIN6 cells ectopically expressed EGFP-CAMSAP1 (D) or EGFP-CAMSAP3 (E) in green. Closed arrowheads, CAMSAP2; open arrowhead, EGFP-CAMSAP1 or EGFP-CAMSAP3.

Figure S4

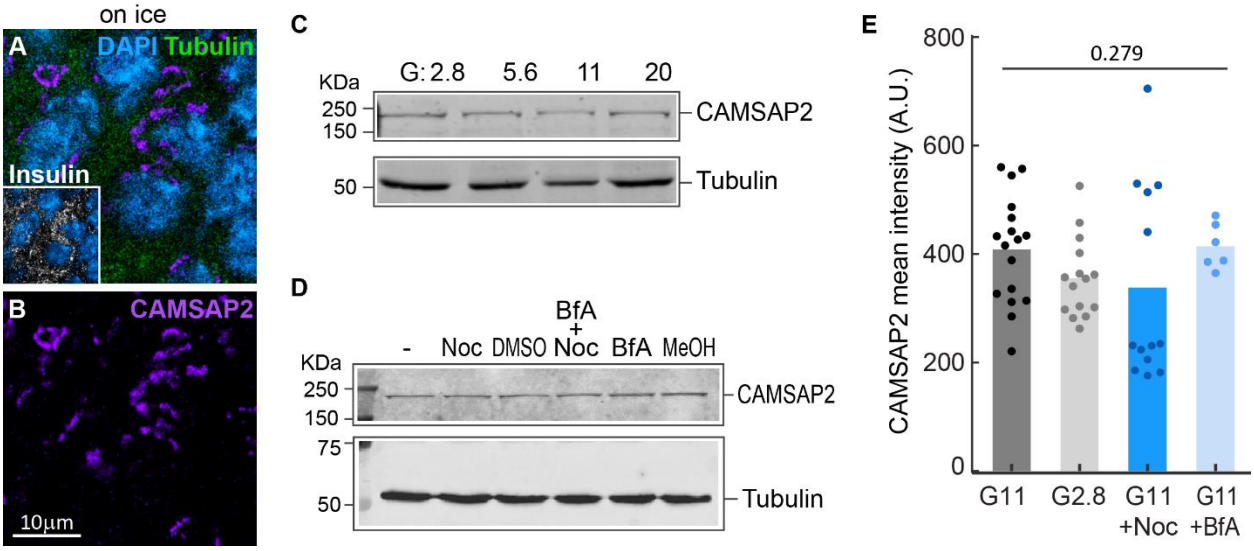

Figure S4. Microtubule depolymerization does not disrupt CAMSAP2 localization and CAMSAP2 protein level is not changed by glucose stimulation, Brefeldin A, or nocodazole treatment. Related to Figure 4.

(A-B) Immunofluorescence staining of tubulin (green) and CAMSAP2 (magenta) in primary β-cells in mouse islets incubated in ice-cold media for 30 minutes. Cyan, DAPI. (C) Immunoblotting against CAMSAP2 and tubulin in lysates from mouse islets. Islets were incubated in media containing 2.8, 5.6, 11, or 20 mM glucose for 2 hours prior to lysis. (D) Immunoblotting against CAMSAP2 and tubulin in MIN6 cultured for 2 hours in media containing 25 mM glucose plus 0.05% methanol, 0.05% DMSO, 5 μg/mL nocodazole, and/or 10 μg/ml Brefeldin A. (E) Quantification of CAMSAP2 immunofluorescence intensity of primary β-cells in mouse islets. Dots represent individual β-cells. Columns represent the average of three animals (One-way ANOVA).

Figure S5

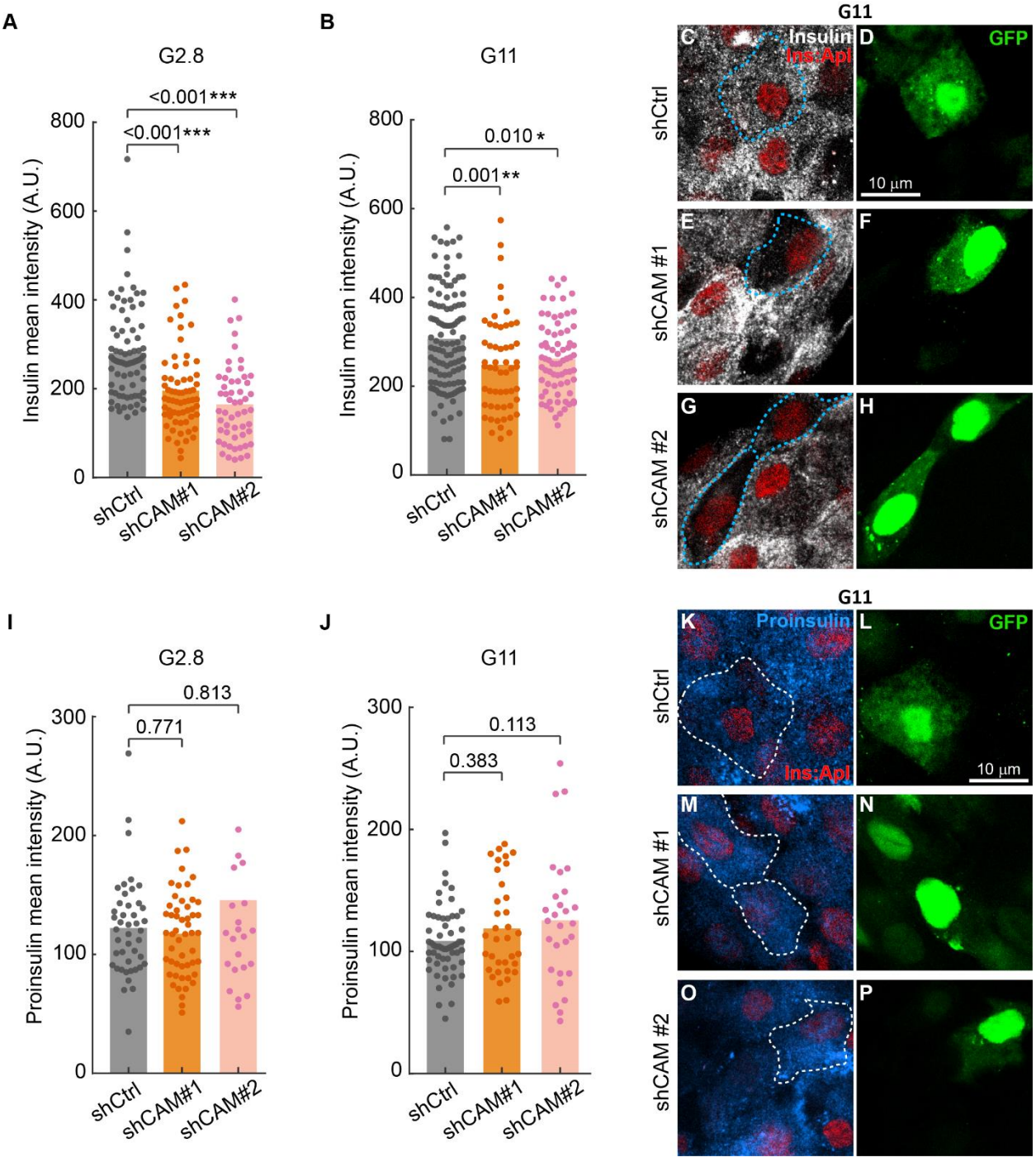

Figure S5. Knockdown of CAMSAP2 reduces total insulin content but not proinsulin level in primary β-cells. Related to Figure 5.

(A-B) Quantification of insulin immunofluorescence intensity of primary β-cells in mouse

islets cultured in media containing 2.8 mM (A) or 11 mM (B) glucose. Dots represent individual β-cells. Bars represent the average of three animals. ** $p<0.01$, *** $p<0.001$ (Dunnett's multiple comparisons test). (C-H) Immunofluorescence staining of insulin (white) in CAMSAP2-KD and control β-cells in mouse islets cultured in media containing 11 mM glucose. Intact mouse islets were incubated with lentivirus and individual transduced β-cells on the surface of each islet were analyzed. Dashed lines delineate transduced cells. Red, *Ins2* promoter-driven H2B-mApple to label β-cells in islets; Green, tGFP to indicate successful transduction. (I-J) Quantification of proinsulin immunofluorescence intensity of primary β-cells in mouse islets cultured in media containing 2.8 mM (I) or 11 mM (J) glucose. Dots represent individual cells. Bars represent the average of three animals. (Dunnett's multiple comparisons test). (K-P) Immunofluorescence staining of proinsulin (cyan) in CAMSAP2-KD and control β-cells in mouse islets cultured in media containing 11 mM glucose. Dashed lines delineate transduced cells. Red, *Ins2* promoter-driven H2B-mApple to label β-cells in islets; Green, tGFP to indicate successful transduction.