

Supplementary Information

Supplementary Video 1 Biphasic insulin secretion during glucose-stimulated insulin secretion (GSIS) in INS-1E β -cells using total internal reflection fluorescence (TIRF) microscopy. The green and red fluorescence represent actin filaments and insulin vesicles, respectively. TIRF microscopy was employed to observe the basal membrane, whose thickness is approximately 120 nm. High glucose stimulation was started at 55 seconds, and samples were collected over a total of 40 minutes. Scale bar: 5 μ m.

Supplementary Video 2 Slice and segment tomograms obtained from the cell periphery under 2.8 Glu - 30 min conditions. Each organelle is labeled with a unique color, including actin filaments (orange), microtubules (violet), insulin secretory granules (blue), endoplasmic reticulum (ER; green), lysosomes (silver), and ribosomes (white).

Supplementary Video 3 Slice and segment tomograms obtained from the cell periphery under 16.7 Glu - 5 min conditions. Each organelle is labeled with a unique color, including actin filaments (orange), microtubules (violet), insulin secretory granules (blue), ER (green), lysosomes (silver), and ribosomes (white).

Supplementary Video 4 Slice and segment tomograms obtained from the cell periphery under 16.7 Glu - 30 min conditions. Each organelle is labeled with a unique color, including actin filaments (orange), microtubules (violet), insulin secretory granules (blue), ER (green), lysosomes (silver), and ribosomes (white).

Supplementary Video 5 Slice and segment tomograms obtained from the cell interior under 2.8 Glu - 30 min conditions. Each organelle is labeled with a unique color, including actin filaments (orange), MTs (violet), ISGs (blue), ER (green), mitochondria (red), lysosomes (silver), and ribosomes (white).

Supplementary Video 6 Slice and segment tomograms obtained from the cell interior under 16.7 Glu - 5 min conditions. Each organelle is labeled with a unique color, including actin filaments (orange), MTs (violet), ISGs (blue), ER (green), mitochondria (red), lysosomes (silver), and ribosomes (white).

Supplementary Video 7 Slice and segment tomograms obtained from the cell interior under 16.7 Glu - 30 min conditions. Each organelle is labeled with a unique color, including actin filaments (orange), MTs (violet), ISGs (blue), ER (green), mitochondria (red), lysosomes (silver), and ribosomes (white).

Supplementary Table 1. Number of tomograms under each condition

	2.8 Glu - 30 min	16.7 Glu - 5 min	16.7 Glu - 30 min
Cell periphery	Eight tomos (four cells from two grids) / Titan Krios G2	Eight tomos (seven cells from three grids) / Titan Krios G4	Eight tomos (five cells from three grids) /Titan Krios G2
Interior of the cell	Six tomos (six cells from two grids) / Titan Krios G4	Six tomos (six cells from two grids) / Titan Krios G4	Six tomos (six cells from two grids) / Titan Krios G4

Supplementary Table 2. Data acquisition parameters

	Titan Krios G2	Titan Krios G4
Magnification	26,000	42,000
Voltage (KV)	300	
Electron exposure (e-/Å ²)	~110-130	
Detector	Gatan K3	Falcon4
Energy filter	Yes	Yes
Slit width	20	20
Phase plate	No	No
Defocus range (µm)	-5 to -7	-3 to -4
Tilt range (min/max, starting angle, step)	-60° to +60°, start from 0°, 2° per step	-60° to +60°, start from 0°, 2° per step, -70° to +50°, start from -10°, 2° per step
Tilt scheme	Dose-symmetric	
Pixel size (Å)	3.353	3.028
Tomograms (no.)	16	26
Cell (no.)	9	25