Figure S1. Dynamic remodeling of cortical F-actin in glucose-stimulated beta cells.

A,B. TIRF microscopy images (top) and recordings (middle) from a glucose-stimulated MIN6 cell expressing mCherry-CH_Utr. Notice the asymmetric formation of cortical actin patches in response to glucose. A kymograph generated from a line drawn across the cell in (A) is shown below.

C. TIRF microscopy recordings of mCherry-CH_Utr (magenta) and Ca²⁺-reporter (GCaMP5G; black) fluorescence from a MIN6 cell stimulated with 11 mM glucose. The three magenta lines correspond to fluorescence intensity changes within the indicated regions in the cell to the right.

D. TIRF microscopy images from a MIN6 cell expressing mCherry-CH_Utr (F-actin) and GFP-Tomm20 (mitochondria) showing the effect of depolarization with 30 mM KCl. Notice the disappearance of mitochondria from sites of cortical actin accumulation. A kymograph generated from a line drawn across an exemplary cell shows the localization of GFP-TOMM20 and mCherry-CH_Utr towards each other. They are not co-localized but seem to rather occupy different locations at the membrane.

E. Representative traces of specific regions in cells from TIRF imaging showing the movement of mitochondria (green) and F-actin (magenta) following depolarization. They move in opposite directions.

F. Quantification of the relative fluorescence change of mCherry-CH_Utr and GFP-Tomm20 at the plasma membrane in response to depolarization (n=12 cells).
Figure S2. Relationship between insulin secretion and cortical mitochondria.
A. Confocal microscopy recording of cytosolic (GCaMP5G, black) and mitochondria (mito-LAR-GECO, red) Ca\(^{2+}\) concentration changes in a glucose-stimulated MIN6 cell.
B. TIRF microscopy recordings of DAG (GFP-C1aC1b\(_{PKC}\), magenta) and mitochondria (mApple-Tomm20, black) in a MIN6 cell in response to glucose stimulation. The boxed area is shown to the right on an expanded time-axis.
Movie 1. This movie relates to the data in Fig. 2G and shows a TIRF microscopy recording of mCh-CHUtr (left; inverted) and GCaMP5G (right, green) fluorescence change in response to 11 mM glucose and subsequent addition, and later washout, of 250 μM diazoxide. The movie plays at 50 times real speed.