SUPPLEMENTARY METHODS

Confocal imaging and data quantification

For data quantification, we used ImageJ to draw regions of interest (ROI) around single secretory events in different confocal planes and determined changes in fluorescence intensity over time. We calculated burst periods by subtracting the time of appearance of secretory events in the first burst from that of the events in the subsequent burst. The temporal pattern of secretion was also examined using cumulative plot of secretory events.

We examined the spatial pattern of granule secretion by plotting fluorescence intensity along cell membranes. To search for sites of preferential secretion, we determined the number of secretory events that reappeared at the same site in intensity plots of two secretory bursts. We compared the number of these observed repeats to the number of predicted repeats that would occur by chance (Fisher's exact test). Predicted repeats were calculated by multiplying the number of observed secretory events with the probability that a secretory event randomly repeats at the same location, which is (adapted from the Birthday Problem)

$$p\,(n, d) = 1 - \frac{\frac{d!}{(d-n)!}}{d^n}$$

where n are the observed secretory events within range (1, d), with d being the number of possible discrete events within the examined membrane section. Based on the profiles we obtained, we assumed that a secretory event takes 2 μm of space. Thus, 25 discrete events fit within a membrane section of 50 μm. The assumption for the size of discrete events

likely overestimated predicted values for a secretory event to appear at the same location. Despite these stringent conditions, we were able to determine that secretory events repeated more often than predicted.

Spatial analyses included determination of coefficient of variation (CV) [1] during prolonged incubation with high glucose (15-20 min). We created temporal stacks of images acquired during 30 s (~ 20 images), a time period that contained all secretory events per burst. In ROIs of cells, we calculated the CV of fluorescence signals according to the formula: CV = SD / mean. Higher CV values indicate higher concentration of fluorescence signals. To determine colocalization of secretory events, we used ImageJ to calculate Pearson's correlation coefficient values of images (30-s temporal stacks) from two bursts.

[1] Hoppa MB, Collins S, Ramracheya R, et al. (2009) Chronic palmitate exposure inhibits insulin secretion by dissociation of Ca(2+) channels from secretory granules. Cell metabolism 10: 455-465