Electronic Supplementary Material (ESM)

ESM 1: List of parameters measured in the high-content analysis

GRANULES: x, y, asymmetry, border index, border length, compactness, distance to cell border, distance to nucleus border, elliptic fit, length, length to width, main direction, intensity, radius of largest enclosed ellipse, radius of smallest enclosing ellipse, roundness.1shape index; CELL: width, cell area, cell length, cell width, nuclei area, nuclei length, nuclei width, cytoplasm area.

ESM 2: Reconstruction of the TSD from the empirical OSD

1) Take the bin i corresponding to the largest ISG size (D_i) in the OSD with amplitude A_i.
2) Scale the refSD for a sphere of size D_i such that the amplitude of its last bin equals A_i.
3) Assume that the number of ISGs under the scaled refSD represents ISGs of size D_i and add it to the corresponding bin in the TSD.
4) Subtract the scaled refSD from the OSD (note that bin i of the OSD becomes empty and also smaller bins of the OSD are reduced by this).
5) Restart with 1) using the reduced OSD starting from bin i=i-1 until the OSD is empty.

This algorithm worked also for bimodal distributions, as those found in chemically fixed samples. As the slice thickness is finite, the ISG equator is intersected more frequently. Thus, the reconstructed TSD was corrected for this skewing artifact.

ESM 3: Kolmogorov–Smirnov test (KS test)

Assuming $F_1(x)$ and $F_2(x)$ as empirical distribution functions of two tested samples, D is defined as the supremum of the distances between two empirical distribution functions: $D=supr\left|F_1(x)-F_2(x)\right|$. KS test was calculated with MatLab (MathWorks Ltd., USA). For all measured quantities we calculated the standard deviation to determine the distribution spread

around the corresponding mean value. For a set of n measured values $x_1,..., x_n$ with the mean value the standard deviation σ. As most distributions exhibited a single peak the standard deviation was a good indicator of how much the data were scattered, even when the distribution was not perfectly normal.

ESM 4: Validation of HCA on 2D sections for extrapolation of granule number in 3D.

As the HCA protocol underestimated the number of ISGs by 17% compared to the manual counting, it was necessary to test whether this error would led to a much larger error when converting the 2D results to 3D.

A key point to consider is that sections for EM have a thickness. Thus, the values obtained from EM images are not truly 2D measurements and counting granules in a slice provides a measurement of granules density. Therefore, the error of the automated counting versus the manual counting should not change when extrapolating values from sections to the whole cell.

In order to verify this statement, we made a counter experiment. We placed 6,000 granules in an *in-silico* HPF beta cell, generated 100 random slices and then measured the observed granule density in 2D slices. Next, we modified the number of granules to 5,400 and 4,800 granules, corresponding to 10% and 20% decrease in granule number and investigated how this translated into the counting of granules in the quasi-2D slices. The number of granules in the slices was decreased by 9.7% and 20.15%, respectively. Thus, changes in the "2D" slices reflected exactly the changes in the 3D cell. In other words, any loss of granules in the slices (i.e. in the automated granule recognition) corresponds with a high accuracy to the same fractional loss in the estimated total number of granules.

ESM 5: Impact of the size of the nucleus on the estimated total number of granules.

The nucleus occupies ~12% of the cytoplasmic volume. However, granule density decreases from the PM towards the nucleus and reaches its minimum value in the vicinity of the nucleus (Fig. 5D). Hence, deviations in the nuclear observed size in "2D" slices should not have a major impact on the estimated granule number, as only few granules are found in proximity of the nucleus.

To verify this assumption we performed two *in-silico* experiments on the same HPF beta cell, cutting 70 slices in random directions in each case. In the first experiment we choose only those slices in which the nucleus was visible and its major axis measured ≧50% of its diameter, whereas in the second experiment the minimum required axis was set to ≧25% of its diameter. The estimated true granules density in these two experiments differed <1%. Thus, the size of the nucleus in the slices had a minor impact on the ultimate results, i.e. for the estimation of the total granule number.

ESM 6: Impact of the shape (spherical vs. ellipsoidal) of the beta cell model on the estimated average number of granules/beta cell.

An interesting question that can be raised is how the shape of the beta cell model affects the estimated average number of granules/cell. The answer to this question is rather complex because it depends on how the experimental data, which are not compatible with the modeling of beta cells as spheres, are nevertheless forced into the generation of a spherical model of a beta cell. We have tested three scenarios and compared the resulting granule numbers with the number of 4,950 granules (see Table 2 in the manuscript) that we have found:

1) We generated slices from an *in silico* spherical HPF beta cell having the same volume as estimated from the measured minor and major axis of the *in silico* ellipsoidal HPF beta cell. In this case the granule number was 5,500, thus only ~10% larger than the indicated value of 4,950

granules.

2) We generated slices from an *in silico* spherical HPF beta cell such that the diameters of these slices would correspond to the average values between the minor and major axes measured in the experimental sections. In this case the granule number was 4,500, i.e. even less that the estimated value of 4,950 granules. This was not unexpected, since the volume of this *in silico* spherical HPF beta cell is smaller than the volume of the corresponding *in silico* ellipsoidal HPF beta cell.

3) We proceeded as in case 2 but now the diameter of the *in silico* slices corresponded to the average major axis measured in the experimental sections. In this case the granule number was 9,500. This value (~11,100 taking into account the 17% underestimation of ISG count by HCA) is in good agreement with the values reported in the literature (references 1-4 in the manuscript), in which beta cells were considered to be spheres with a diameter comparable to the one used in this third simulation.

Based on these simulations, we can conclude that a major reason why our estimated average granule number/rat beta cell deviates from previous accounts is a better estimation of the average beta cell volume. However, as illustrated by the outcome of the first simulation and specified in the discussion, other factors of our improved analysis did contribute to the estimated granule number.