Structurally distinct oligomers of islet amyloid polypeptide mediate toxic and non-toxic membrane poration

(Supporting Information)

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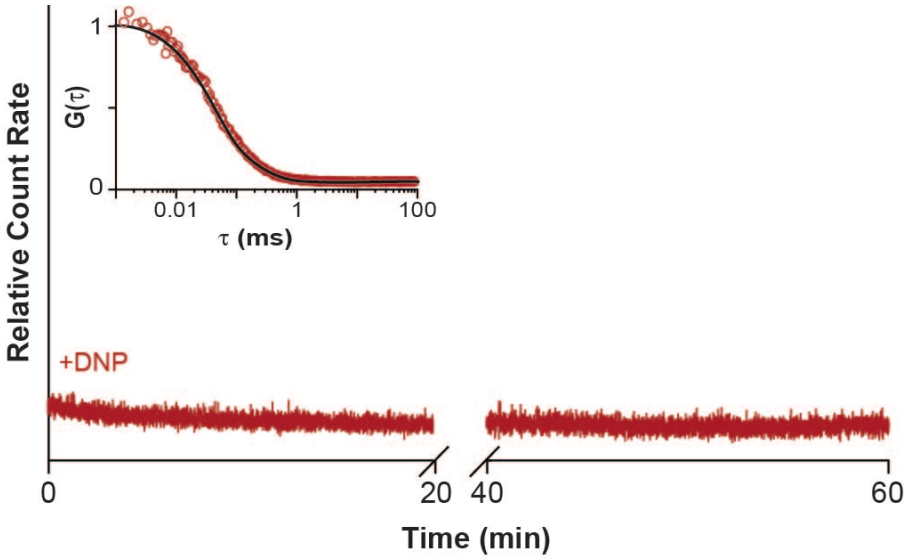

Figure S1. Related to Figure 1; DNP mediated poration monitored by FCS. Representative photon bursts from fluorophore labeled materials exiting GPMVs after addition of 10 μM DNP. **Inset**: autocorrelation of the last 20 minutes of data acquisition overlaid with a fit that includes only a single diffusing species.

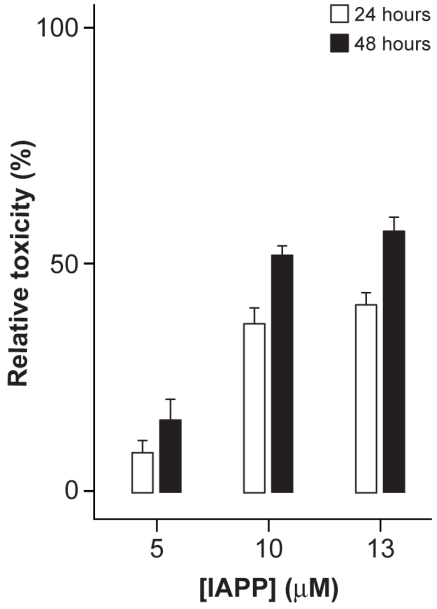

Figure S2. Related to Figure 2 and Figure 6; Cytotoxicity of IAPP. Colorimetric measure of toxicity 24 and 48 hours after introduction of the indicated concentration of human IAPP to culture media. Data is expressed relative to vehicle-only addition prepared on the same well-plates. Each histogram bar is the average of eight, on-plate repeats across each of three independently performed replicates (n = 24).

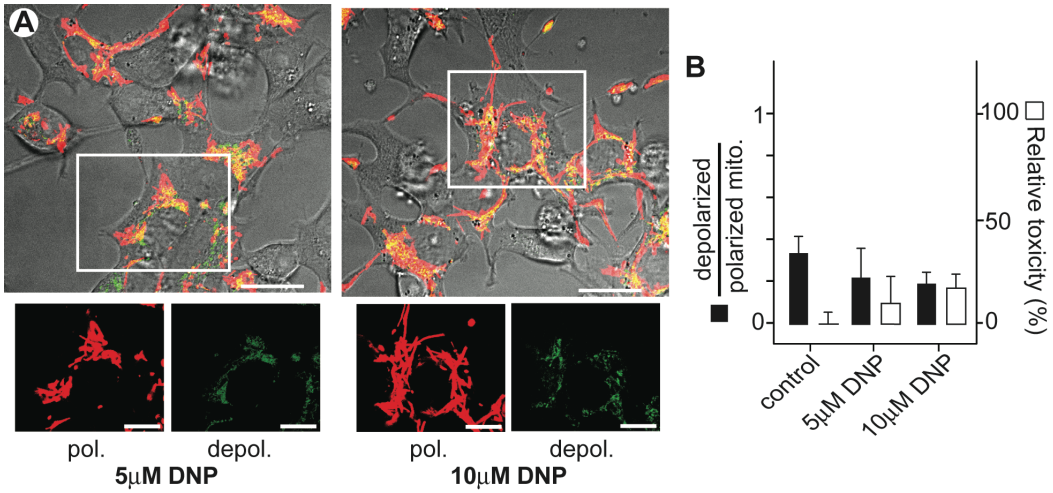

Figure S3. Related to Figure 2; DNP does not depolarize mitochondria.

(A) Representative INS-1 cells stained with JC-1 24 hours after incubation with 5 μM or 10 μM DNP. Two fluorescence channels corresponding to polarized (red) and depolarized (green) mitochondria respectively are shown as a merge with the DIC image. The indicated ROI is shown below the main image as individual fluorescence channels.

(B) Cumulative pixel based analysis of images as in (A) showing the ratio of fluorescence corresponding to depolarized and polarized mitochondria respectively. Parallel experiments to assess DNP toxicity under the same conditions are also shown. Toxicity was measured colorimetrically and is expressed relative to vehicle-only addition prepared on the same well-plates. Each histogram bar is the result of averaging eight on-plate repeats across each of three independently performed replicates (n = 24). Note, the higher proportion of depolarized mitochondria in the control compared to the control in Figure 2E is a consequence of using higher passage number cells.

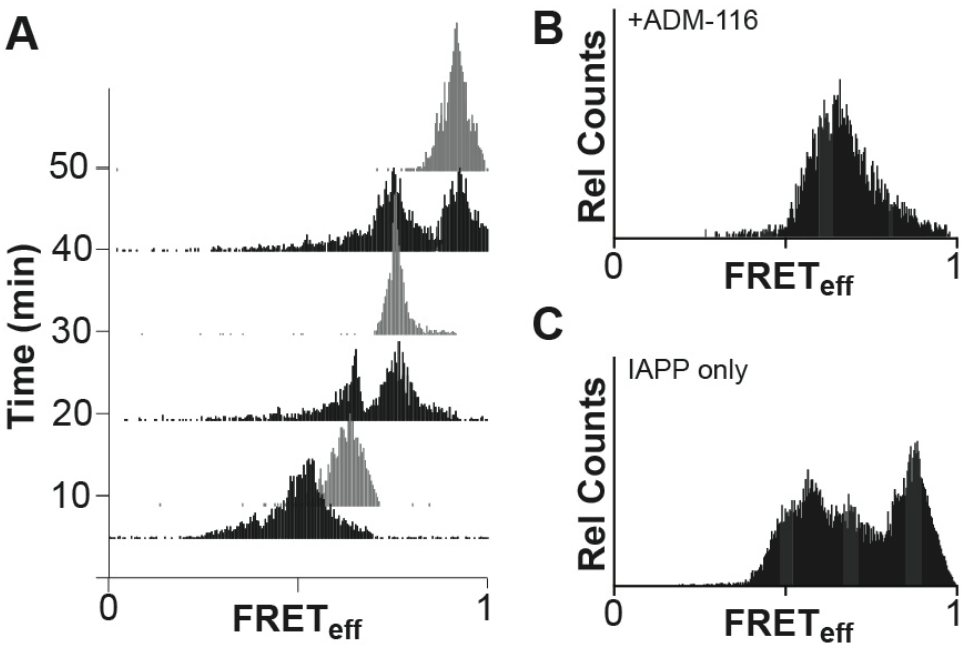

Figure S4. Related to Figure 3 and Figure 6; IAPP oligomer formation measured with an alternative dye pair.

(A) Under conditions matched to those used for Figures 3, intermolecular FRET measured from images of GPMVs under conditions matched to Figure 3D, but using $IAPP_{A488}$ and $IAPP_{A594}$ as the donor/acceptor pair. Representative histograms are shown from a single optical section of a single GPMV as a function of time.

(B) Intermolecular FRET was measured 24 h after exposing INS-1 cells to 13 μM IAPP and equimolar ADM-116. IAPP was doped with 0.1 μM each of $IAPP_{A488}$ and $IAPP_{A594}$.

(C) As in (B), but without addition of ADM-116. For (A) and (B), histograms are cumulative from data from 50 cells across experiments performed on 3 different days.

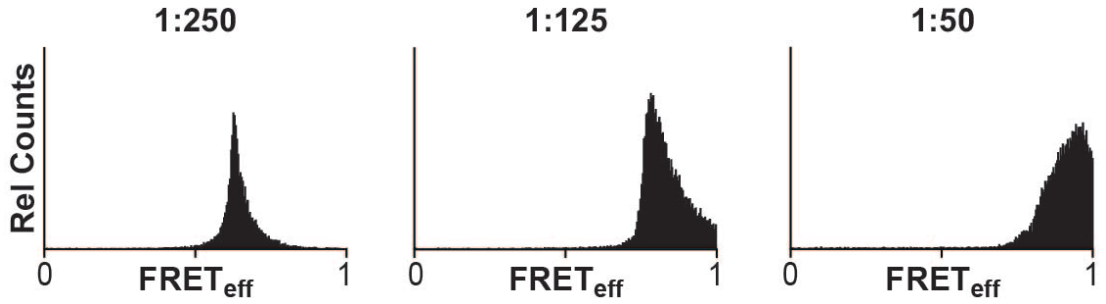

Figure S5. Related to Figure 4: Sensitivity of diluted-FRET to doping ratio. Intermolecular FRET between $IAPP_{A488}$ (donor) and $IAPP_{A647}$ (acceptor) detected on GPMVs at the indicated ratios of (donor+acceptor):unlabeled protein. Solution conditions and time points are otherwise matched, and comparable to those used in Figure 4B (where $FRET_{eff}$ reaches a mostly time invariant value). Each panel represents the sum of observations taken from at least three different GPMVs.