Supporting Information

FoxA2 and RNA Pol II Mediate Human Islet Amyloid Polypeptide Turnover in ER-stressed Pancreatic β-cells

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Supporting information contains data demonstrating ectopic expression of hIAPP (Fig. S1), hIAPP nuclear/cytosolic accumulation (Figs. S2-S5, movies S1/S2), RNA polymerase-mediated hIAPP expression (Fig. S5) and FoxA2-dependent hIAPP promoter activation by prolonged high glucose treatment (Fig. S6). List of primers (Table S1) and figure legends for movies S1 and S2 are also provided.

Name	Primer Sequences
hIAPP	F: 5'-AGCTACACCCATTGAAAGTCATC-3' R: 5'-GATGAGAGAATGGCACCAAAGT-3'
Human Insulin	F: 5'-TGTCCTTCTGCCATGGCCCT-3' R: 5'-TTCACAAAGGCTGCGGCTGG-3'
Human TXNIP	F: 5'-ACAGAAAAGGATTCTGTGAAGGTGAT-3' R: 5'-GCCATTGGCAAGGTAAGTGTG-3'
Human Ki67	F: 5'-ATAAACACCCCAACACACACAA -3' R: 5'-GCCACTTCTTCATTCCAGTTACA-3'
Human 47S	F: 5'-CCTGTCGTCGGAGAGGTTGG-3' R: 5'-ACCCCACGCCTTCCCACAC-3'
Human 18S	F: 5'-GGCCCTGTAATTGGAATGAGTC-3' R: 5'-CCAAGATCCAACTACGAGCTT-3'
Human BIP	F: 5'-GAACGTCTGATTGGCGATGC-3' R: 5'-ACCACCTTGAACGGCAAGAA-3'
Human FoxA2	F: 5'-TCTCCATCAACAACCTCATGTC-3' R: 5'-GTAGTGCATCACCTGTTCGTAG-3'
Human PDX1	F: 5'-CCTTGTGCTCGGGTTATGTT-3' R:5'-ATCATCCCACTGCCAGAAAG-3'
Human β-Actin	F: 5'-AGGCACCAGGGCGTGAT-3' R: 5'-GCCCACATAGGAATCCTTCTGAC-3'
hIAPP promoter-cloning primer	F: 5'-AAAAAGATCTACAGCTCTGGCATTTATAAC-3' R: 5'-AAAACCATGGCTTTTAATGTTTCAATGTCAGC-3'
Site directed mutagenesis primers	F: 5'-TTAACTCATCAGTACATATTAACG-3' R: 5'-CGTTAATATGTACTGATGAGTTAA-3'

Table S1. List of primers used in gene expression studies.

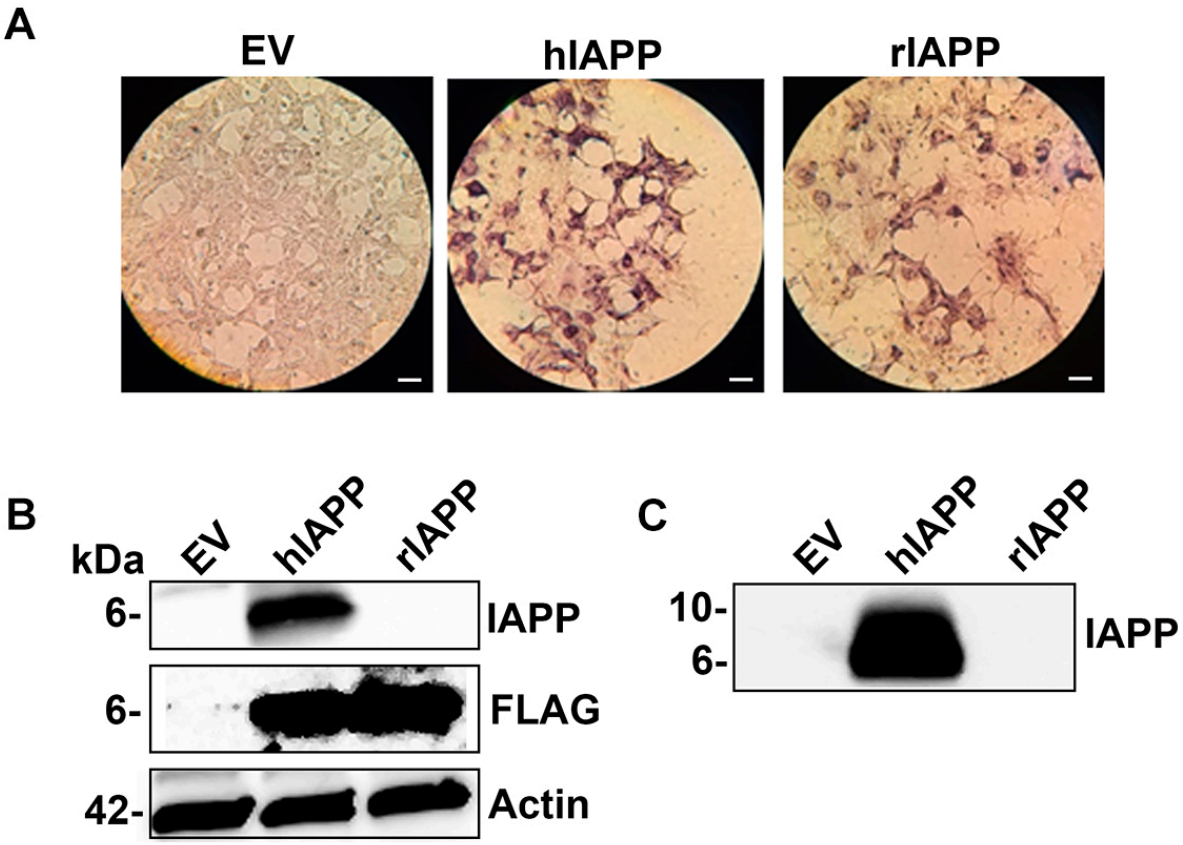

Figure S1. Lentivirus-mediated expression of human and rat IAPP in RIN-m5f cells. RIN-m5f cells were transduced with empty vector (EV), hIAPP and rIAPP encoding lentivirus for 5h as described in the method section. Transfection medium was replaced and cells cultured for an additional 48 h. (A) Immunocytochemistry using anti-FLAG antibody followed by alkaline phosphatase staining was used to test the efficacy of hIAPP and rIAPP expression in transduced RIN-m5f cells. Bars, 5 μm. Western blot analysis of hIAPP and rIAPP protein levels in cell extracts (B) and hIAPP levels in conditioned media (C) of RIN-m5f cells after 48 h of lentivirus-mediated transduction.

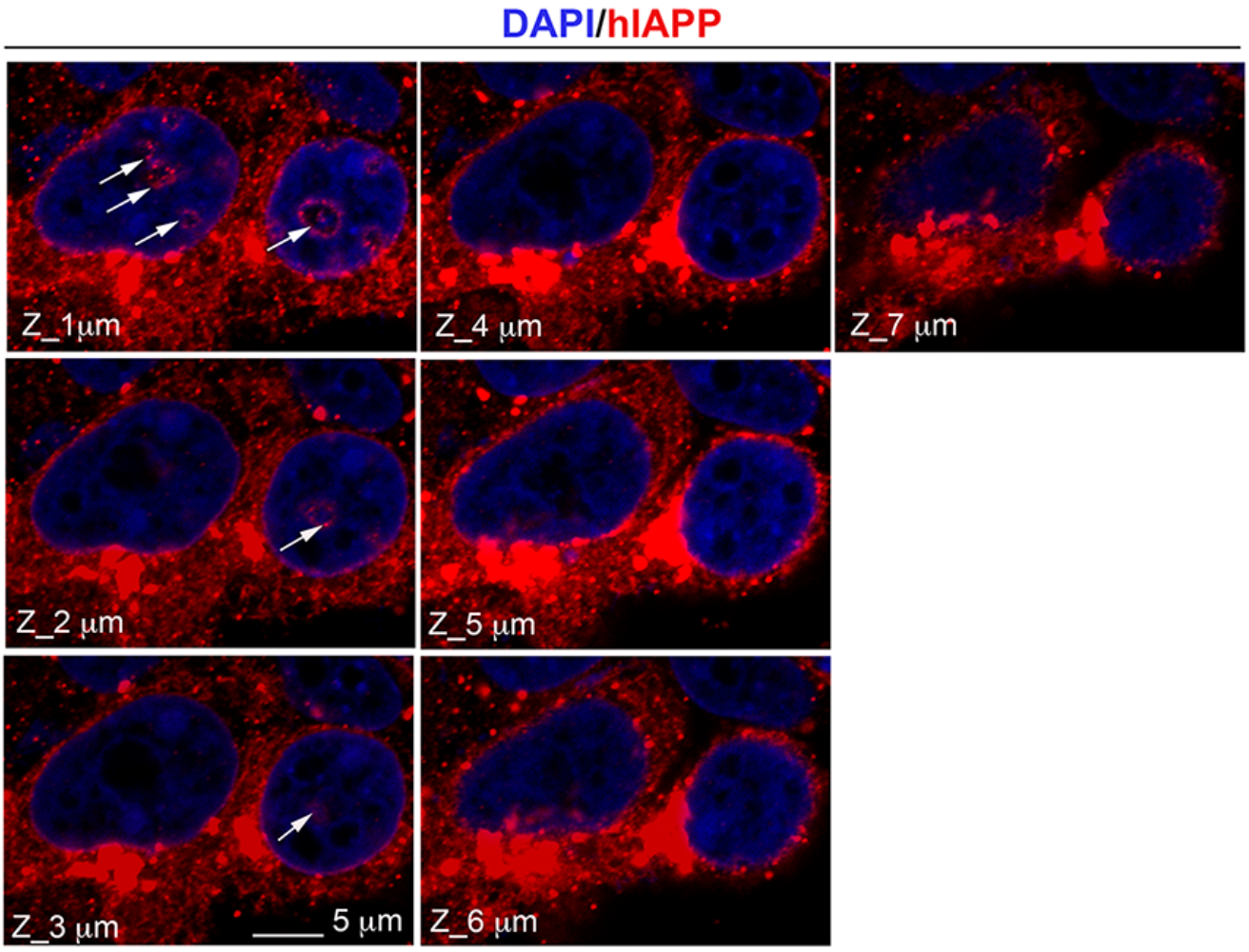

Figure S2. 3-D LSCM analysis of human amylin expression in transfected RINm5F cells. Immuno-confocal microscopy of RIN-m5f cells transduced with hIAPP encoding lentivirus. Cells were co-stained with hIAPP-specific IAPP monoclonal antibody (red) and DAPI (blue). 3-D fluorescence optical sections (Z plane-1μm) of amylin intracellular distribution in hIAPP lentivirus-transfected cells. Arrows point to amylin accumulation sites within the cell nucleus.

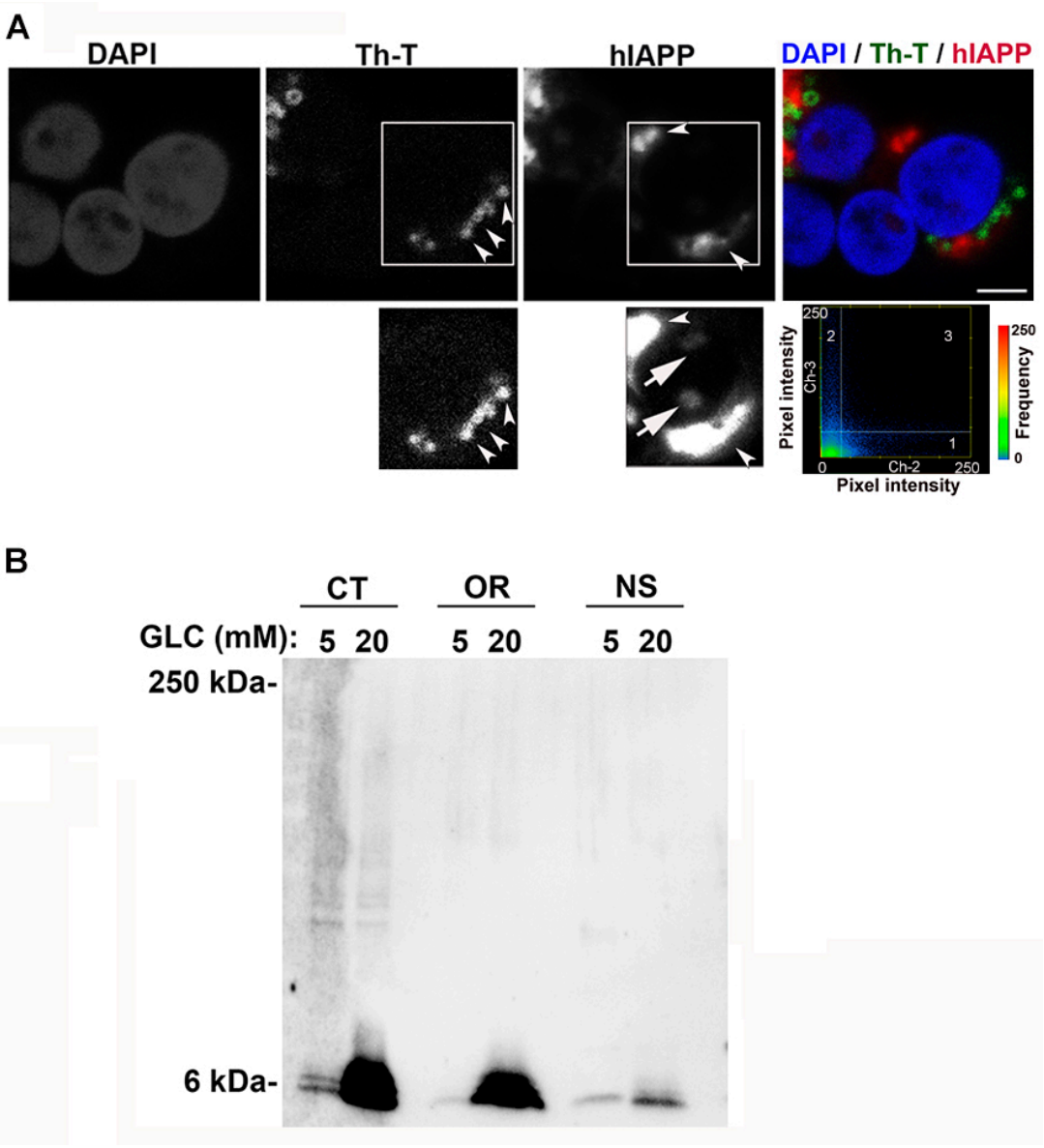

Figure S3. The nucleus accumulates hIAPP's monomers but not aggregates. (A) Immuno-confocal microscopy of hIAPP accumulation in T2DM islet cells. Cultured human islets from T2DM patients were fixed, blocked and incubated with hIAPP specific antibody (red), nuclear dye, DAPI (blue) and amyloid stain, Thioflavin-T (Th-T, green). Stack of optical sections (Z-5 μm) was acquired with the confocal microscope. Representative single optical sections were selected to show locations of Th-T and hIAPP-positive cytosolic structures (arrowheads denote Th-T and hIAPP-positive perinuclear clusters, Fig. 6A). At higher imaging gains, nuclear hIAPP assemblies (denoted by arrows) were detected in the same micrographs (Fig. 6A, hIAPP bottom micrograph). Th-T positive structures were not detected in the nuclei even at higher imagining settings (Fig. 6A, Th-T bottom micrograph). Bar, 5μm. Colocalization analysis is performed on a pixel by pixel basis with ZEN software and following the Zeiss colocalization protocol. Every pixel in the image was plotted in the scatter diagram based on its intensity level from each channel. The pseudo-color in the scatterplot represents the number of pixels from individual channels (quadrants 1 and 2) and colocalized pixels (quadrant 3). Th-T (Ch-2) intensity distribution is shown on the x-axis and hIAPP (Ch-3) intensity distribution is shown on the y-axis. The lower left (unlabeled) quadrant in the scatterplot represents background-pixels that have low-intensity levels in both channels, which were excluded from colocalization analysis. (B) Western blot analysis of hIAPP intracellular distribution in human islets cultured under basal (5mM) and high (20mM) glucose (GLC) conditions. Blot was developed using an HRP-conjugated monoclonal anti-hIAPP monoclonal antibody. Cell fractions were prepared and resolved using SDS-PAGE as described in the method section. CT, cytoplasmic fraction, OR, organelle fraction, NS, nuclear soluble fraction.

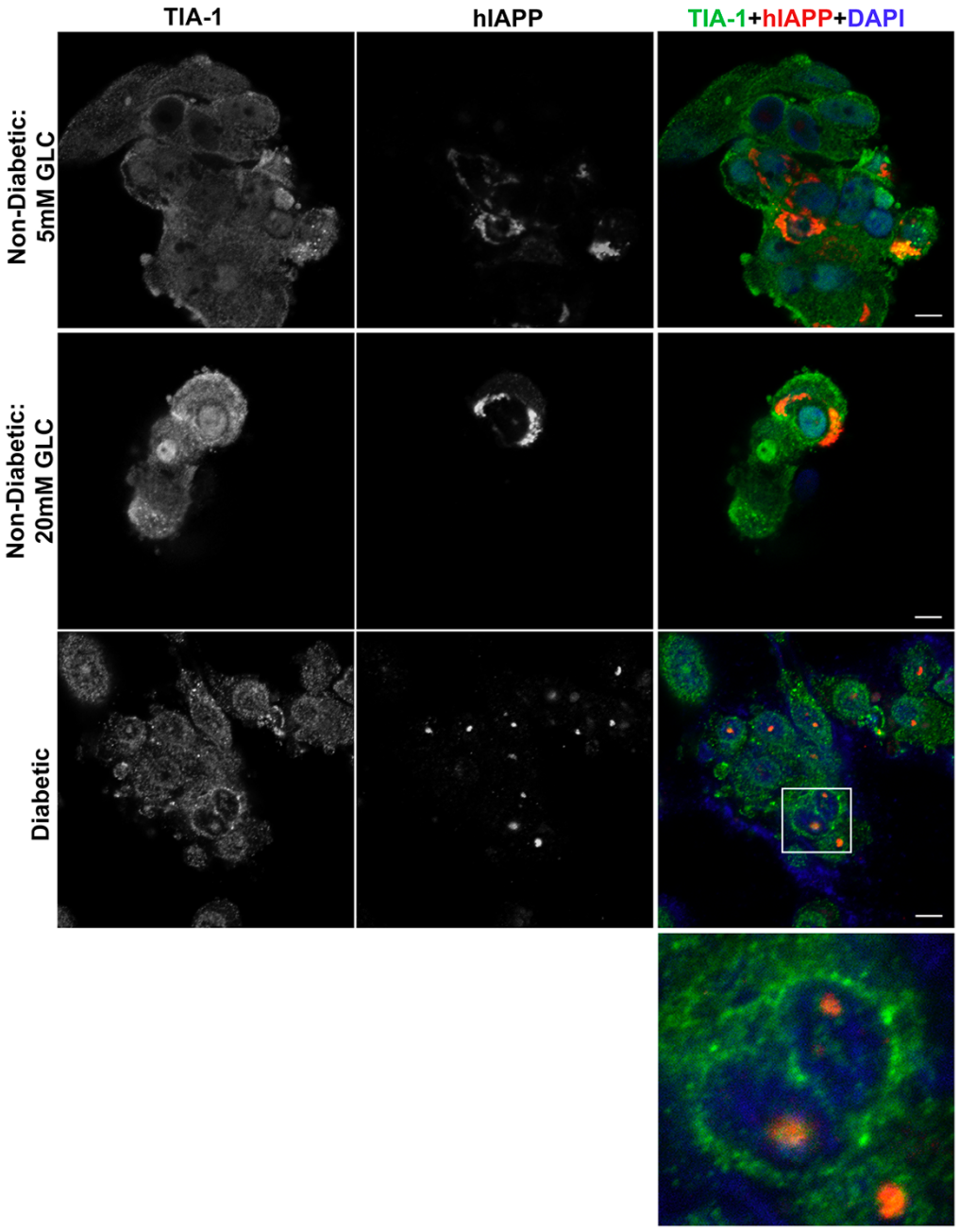

Figure S4. Cellular stress stimulates hIAPP accumulation in the nucleolus of human islet cells. T2DM and non-diabetic human islets were cultured for 4 days in basal (5mM) or high glucose (20mM) media, and hIAPP intracellular locations were determined with immuno-confocal microscopy. Islets were co-stained with hIAPP-specific monoclonal antibody (red), nuclear dye, DAPI (blue) and TIA-1 specific antibody (green). Bar, 10 μm.

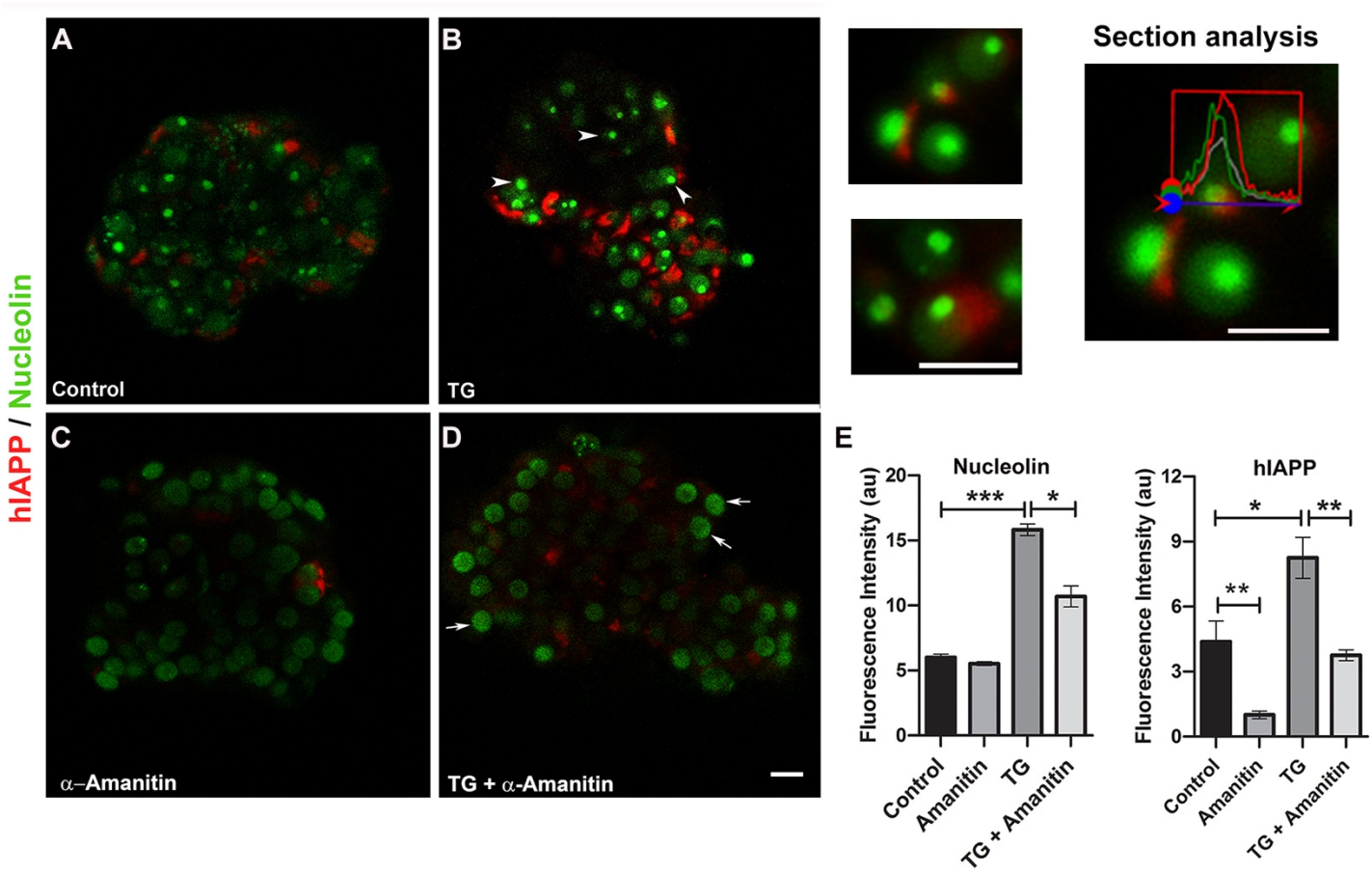

Figure S5. Intact nucleolar organization and functional RNA Pol II are required for hIAPP synthesis in β-cells. Non-dispersed human islets were cultured in the presence or absence of ER-stress inducer, thapsigargin, or RNA Pol II inhibitor, α-amanitin, for 24h. Cells were fixed and process for immune-confocal microscopy as described in the method section. (A-D) Representative LSCM micrographs of hIAPP (red) and nucleolin (green) expression in control and TG/amanitin-treated human islets. Bar, 10 μm. Arrows (D) and arrowheads (B) denote cells with impaired and intact nucleolar organization, respectively. At higher magnification, hIAPP accumulation inside and around the center of nucleolin-positive areas in the TG-treated human islet cells is noticeable (B, insets). FI distribution profile show close overlap between nucleolin and hIAPP fluorescent signals in a representative image (B, section analysis). (E) Quantification of relative protein expression in control and stressed human islets. Data represent mean ± SEM fluorescence intensity signals of nucleolin and hIAPP in human islet cells. Images were captured using constant imaging gains and optical thickness (Z-slice, 5 μm). Significance was established at *p< 0.05, **p< 0.01, ***p< 0.001, ANOVA followed by Tukey's post hoc comparison test.

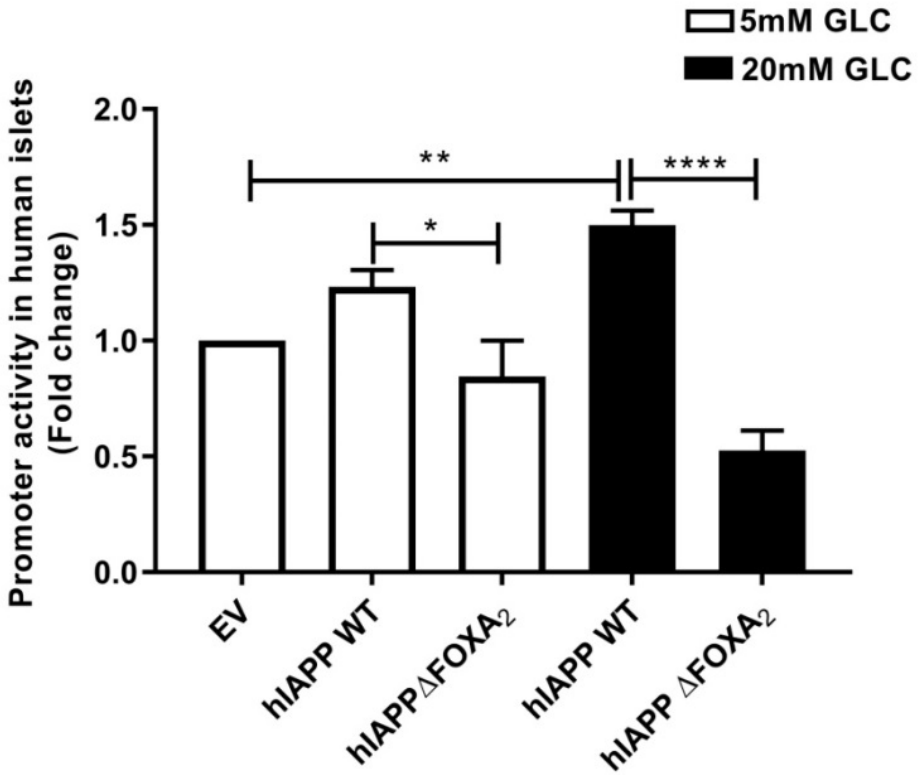

Figure S6. High glucose-induced hIAPP promoter activation requires the functional FoxA2 binding site. Freshly isolated human islets were co-transfected for 24h with renilla (for normalization) and firefly encoding luminescence constructs containing native (WT) or mutated (dominant negative) FoxA2 (ΔFoxA2) hIAPP promoter. Thereafter, cells were incubated with high glucose (HG) for additional 48h. Normalized luminescence signal, reflecting hIAPP promoter activity, is expressed as fold change from empty vector (EV) transfected human islets (arbitrary set to 1), as described in the method section. Significance was established at *p< 0.05, **p< 0.01 and ****p< 0.0001, ANOVA followed by Tukey's post hoc comparison test. Data represent mean ± SEM of three independent experiments.

Movie S1. 3D immuno-confocal analysis of hIAPP intracellular distribution in human islet β-cells. Stacked confocal slices (Z-5 μm) rotated along x-axis reveal accumulation of hIAPP-positive puncta in peri-nuclear and nucleolar regions of control (non-diabetic) human islets. Cultured human islets were co-stained with the hIAPP-specific monoclonal antibody (green) and nuclear dye, DAPI (blue).

Movie S2. 3D immuno-confocal analysis of hIAPP intracellular distribution in human islet β-cells. Stacked confocal slices (Z-5 μm) rotated along y-axis reveal accumulation of hIAPP-positive puncta in peri-nuclear and nucleolar regions of control (non-diabetic) human islets. Cultured human islets were co-stained with the hIAPP-specific monoclonal antibody (green) and nuclear dye, DAPI (blue).