# Figure S1. Related to Figure 1.

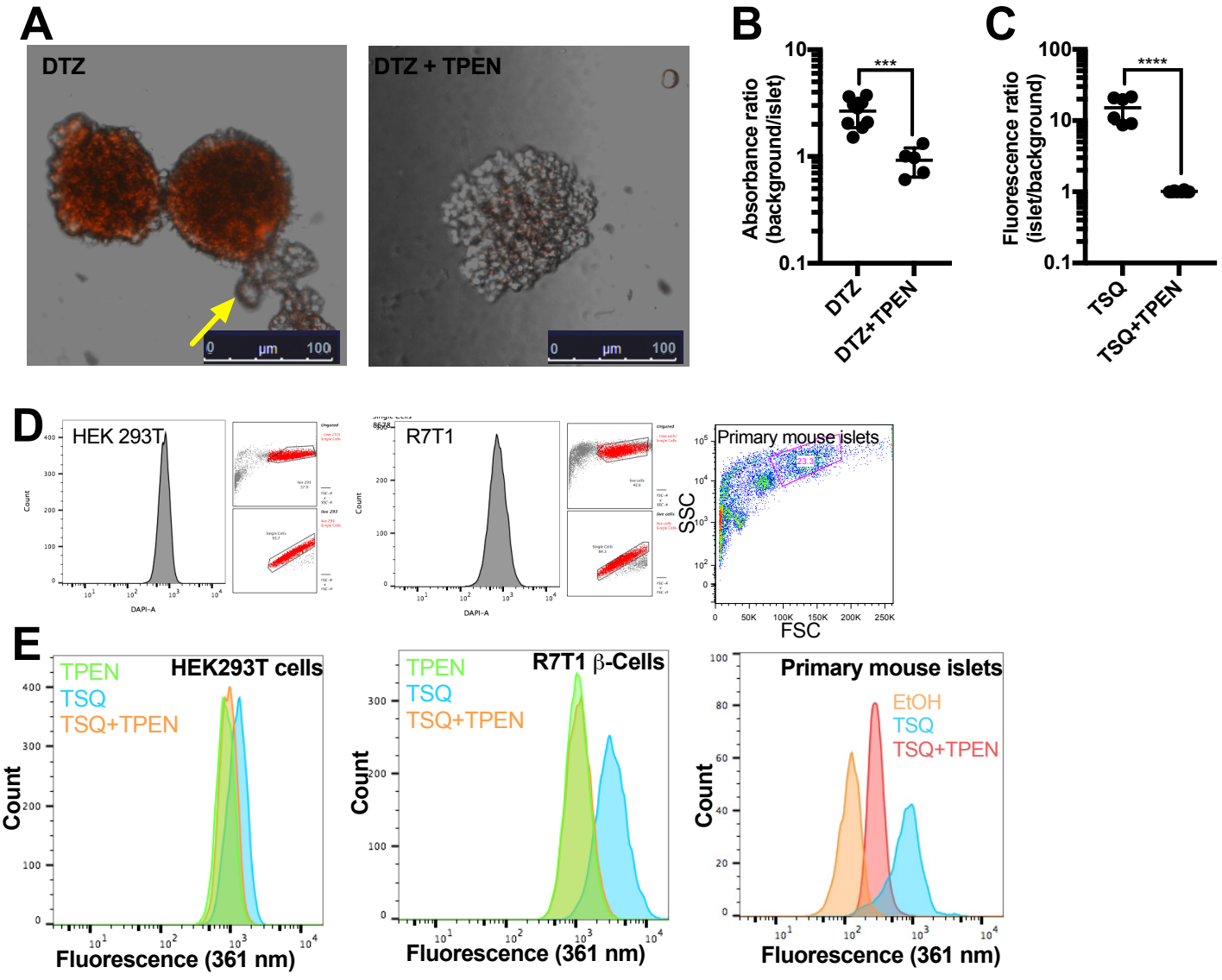


**Figure S1, related to Figure 1. Islet Dithizone staining and cellular TSQ florescence are zinc-dependent. (A)** Representative images of DTZ-treated (177 µM) islets in the absence (left) and presence (right) of the $Zn^{2+}$ chelator TPEN (1 mM). Staining was conducted in 6-well format with 50 islets per well, and at least 5 images per condition were taken. Yellow arrows indicate unstained exocrine tissue. **(B)** Quantification of ratio of total absorbance outside versus inside the islet area of islets treated with DTZ and DTZ+TPEN (n=5-9). **(C)** Quantification of ratio of total absorbance inside versus outside the islet area of islets in cell culture treated with TSQ and TSQ+TPEN (n=6-8). **(D)** Fluorescence-Activated Cell Scanning (FACS) gating strategy for HEK 293T (left), R7T1 (center), and primary mouse islet (right) cells. The endocrine population was selected based upon high forward and side scatter and retrospectively validated as the $TSQ^{bright}$ population. **(E)** Representative histogram plots for HEK 293T (left), R7T1 (center), and primary mouse islet (right) cells following treatment with TSQ, TPEN or the combination of TSQ and TPEN. In all cases TSQ fluorescence is decreased with TPEN treatment. Data are represented as mean ± S.D. Comparisons with $p > 0.05$ are indicated by NS (not significant); $0.01<p<0.05$ by *; $0.001<p<0.01$ by **; $0.0001<p<0.001$ by ***; and $p<0.0001$ by ****.

# Figure S2. Related to Figure 2.

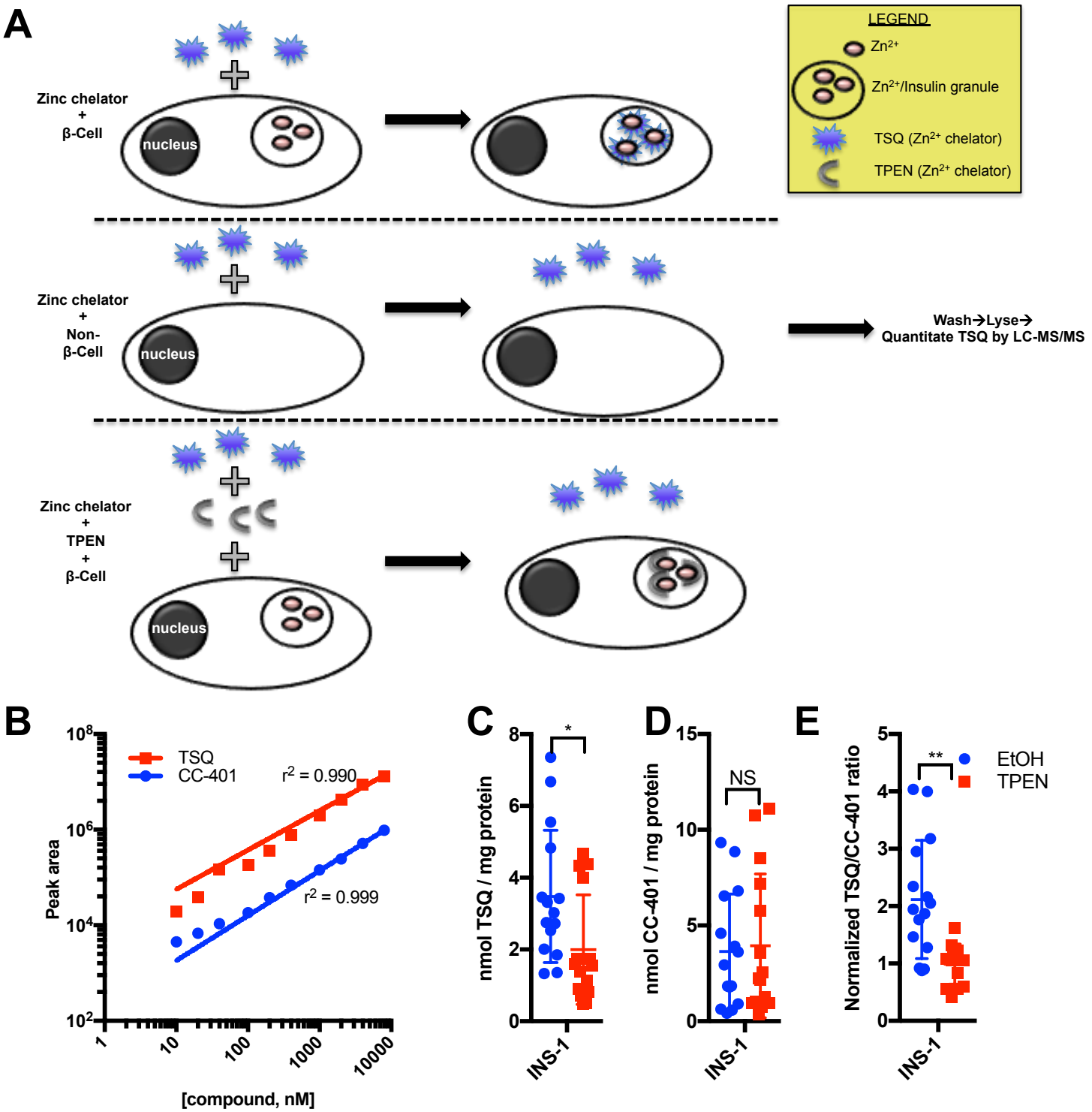


**Figure S2, related to Figure 2. Quantification by liquid chromatography/tandem mass spectrometry (LC-MS/MS) and INS-1 cells accumulation. (A)** Workflow schematic for LC-MS/MS experiments, showing behavior of TSQ in β-cells (top), non-β-cells (middle), and β-cells with TPEN (bottom). **(B)** Representative linear calibration curves for TSQ and CC-401 (range= [10-8000 nM]). **(C)** INS-1E rat β-cells exposed to TSQ and CC401 (10 μM each) in the absence (blue) and presence (red) of TPEN (1 mM) and TSQ (n=14), **(D)** CC-401 (n=14), and **(E)** the molar ratio TSQ/CC-401 (n=14) were quantified by LC-MS/MS. Data are represented as mean ± S.D. Comparisons with $p > 0.05$ are indicated by NS (not significant); $0.01<p<0.05$ by *; $0.001<p<0.01$ by **; $0.0001<p<0.001$ by ***; and $p<0.0001$ by ****.

# Figure S3. Related to Figure 3.

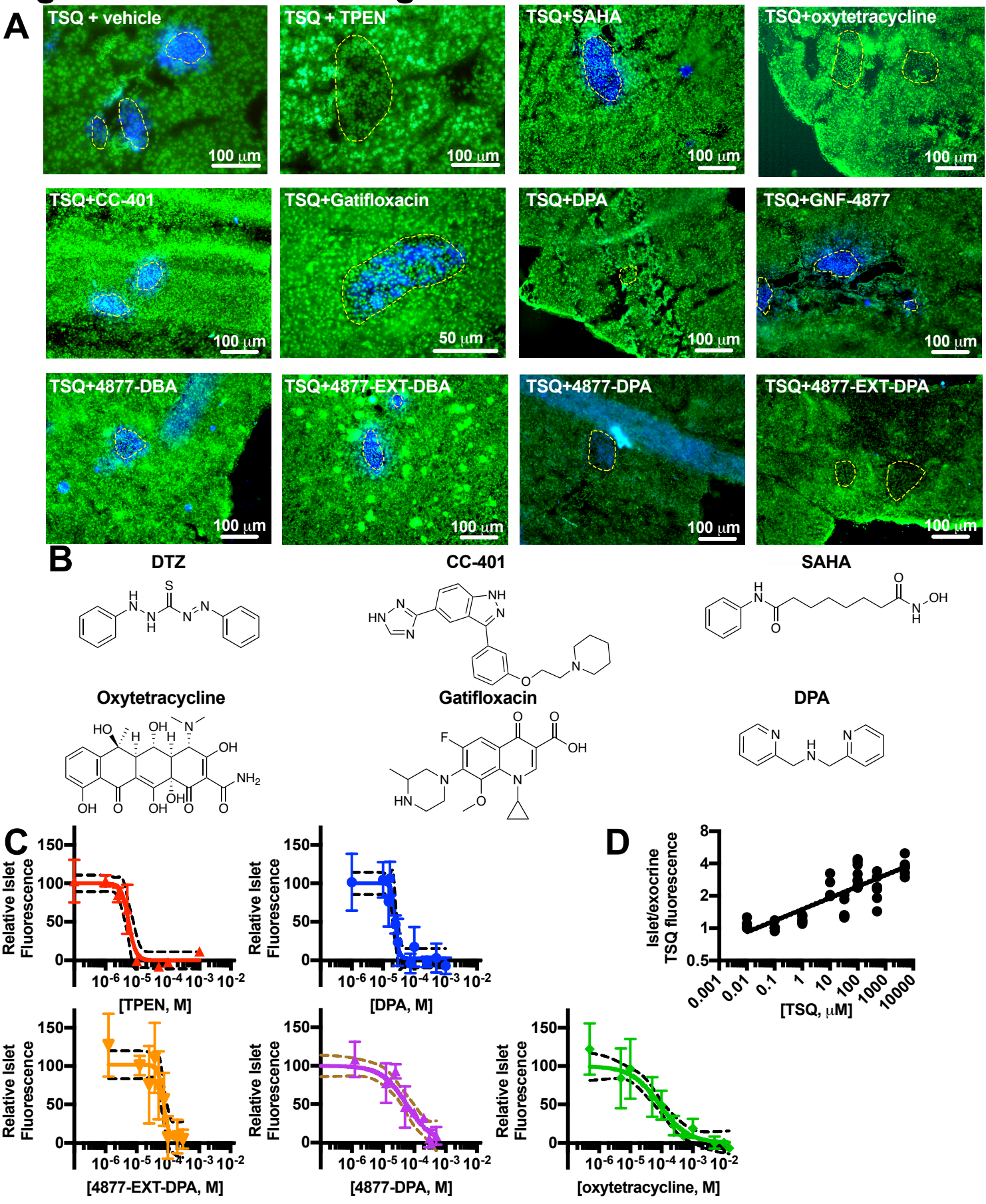

**Figure S3, related to Figure 3. TSQ fluorescence in islets in fresh frozen pancreatic sections directly correlates with TSQ concentration and can be competed away by zinc chelators. (A)** Fresh frozen murine pancreatic sections (10 μm thickness) exposed to TSQ (10 μM) and vehicle, TPEN, or experimental compounds (1 mM) (right) were counterstained with Nuclear Green (DSC1) (333 nM) to identify the islet area (yellow outline shows islet) and the amount of TSQ fluorescence was quantified. **(B)** Dithizone (DTZ), CC-401, SAHA, Oxytetracycline, Gatifloxacin, DPA, GNF-4877, 4877-DBA, 4877-EXT-DBA, 4877-DPA, and 4877-EXT-DPA were tested for competition with TSQ in islets. **(C)** Islet fluorescence on fresh frozen pancreas sections treated with DSC-1 (333 nM) and TSQ (10 μM) in competition with varying concentrations of zinc chelators (TPEN, DPA, oxytetracycline, 4877-DPA, and 4877-EXT-DPA) was quantified and plotted to yield $IC_{50}$, Hill slope ($n_H$), and $R^2$. (n=3-12 per concentration per compound). **(D)** TSQ fluorescence (y-axis) within islets (defined by DSC-1 staining) divided by exocrine (background) fluorescence follows a log-log relationship with TSQ concentration (x-axis). Fluorescence = 10^(0.106*log[TSQ]+0.176 ($R^2$ = 0.533; n=4-9 per concentration). Data are represented as mean ± S.D.

# Figure S4. Related to Figure 4.

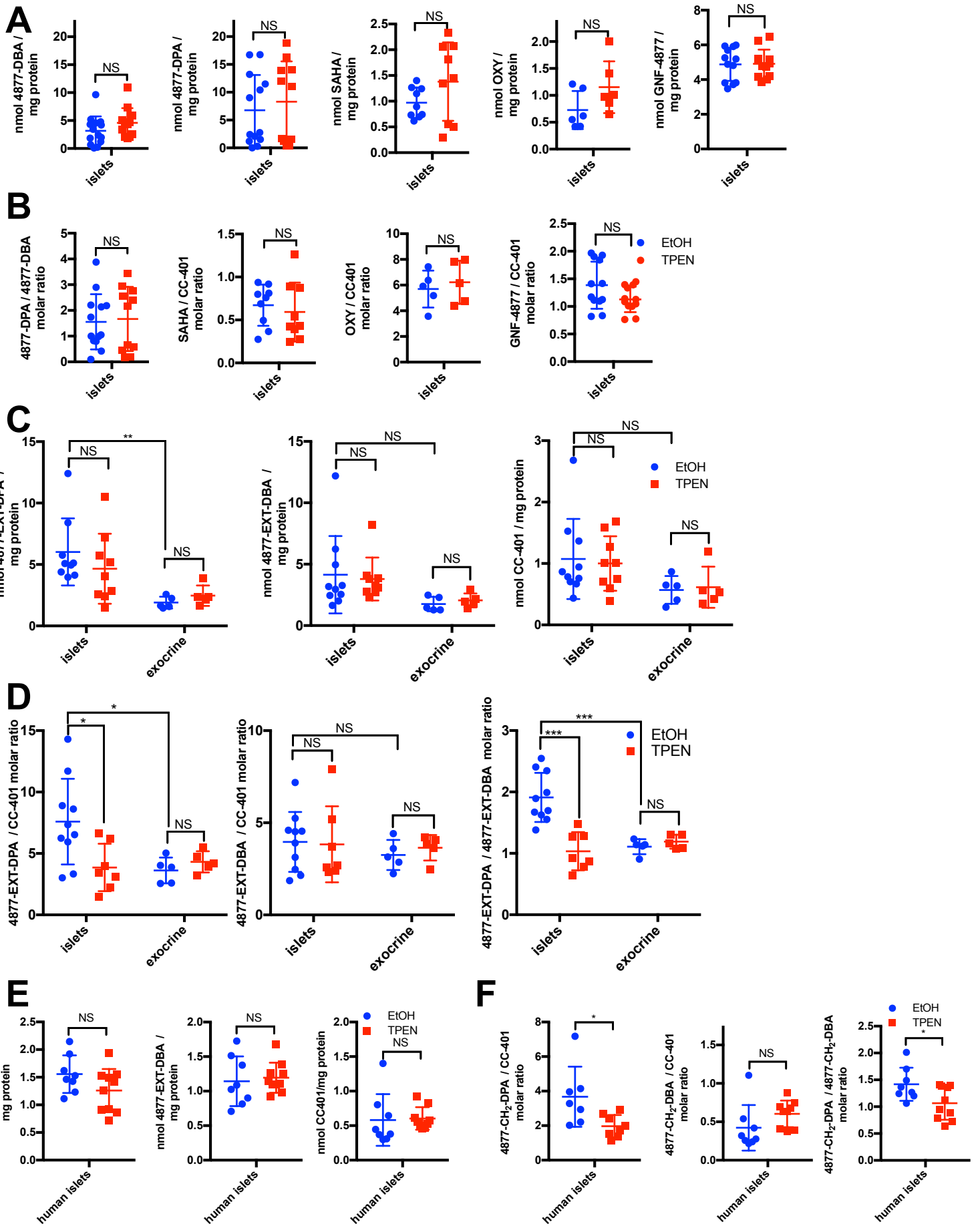

**Figure S4, related to Figure 4. A subset of chelators do not exhibit $Zn^{2+}$-dependent response to TPEN in rodent islets, and normalization to CC-401 is similar to normalization to 4877-EXT-DBA. (A)** Quantification (LC-MS/MS) in rodent islets of 4877-DBA, 4877-DPA, SAHA, Oxytetracycline, and GNF-4877 in the absence (blue) and presence (red) of TPEN (n=6-14). **(B)** Molar ratios of 4877-DPA:4877-DBA, SAHA:CC-401, Oxytetracycline:CC-401, and GNF-4877:CC-401 in the absence (blue) and presence (red) of TPEN (n=6-14). **(C)** 4877-EXT-DPA, 4877-EXT-DBA, and CC-401 (10 μM each)-treated mouse islets and exocrine tissue (n=5-9), without and with TPEN co-treatment. **(D)** Data from *(C)* normalized to CC-401 concentration (n=5-9). **(E)** Human islets treated with 4877-EXT-DPA, 4877-EXT-DBA, and CC-401 (10 μM each), without and with TPEN co-treatment (n-8-10). **(F)** Data from *(E*) normalized to CC-401 concentration (n=8-10), with normalization of 4877-EXT-DPA to 4877-EXT-DBA as a comparison.

# Figure S5. Related to Figure 5.

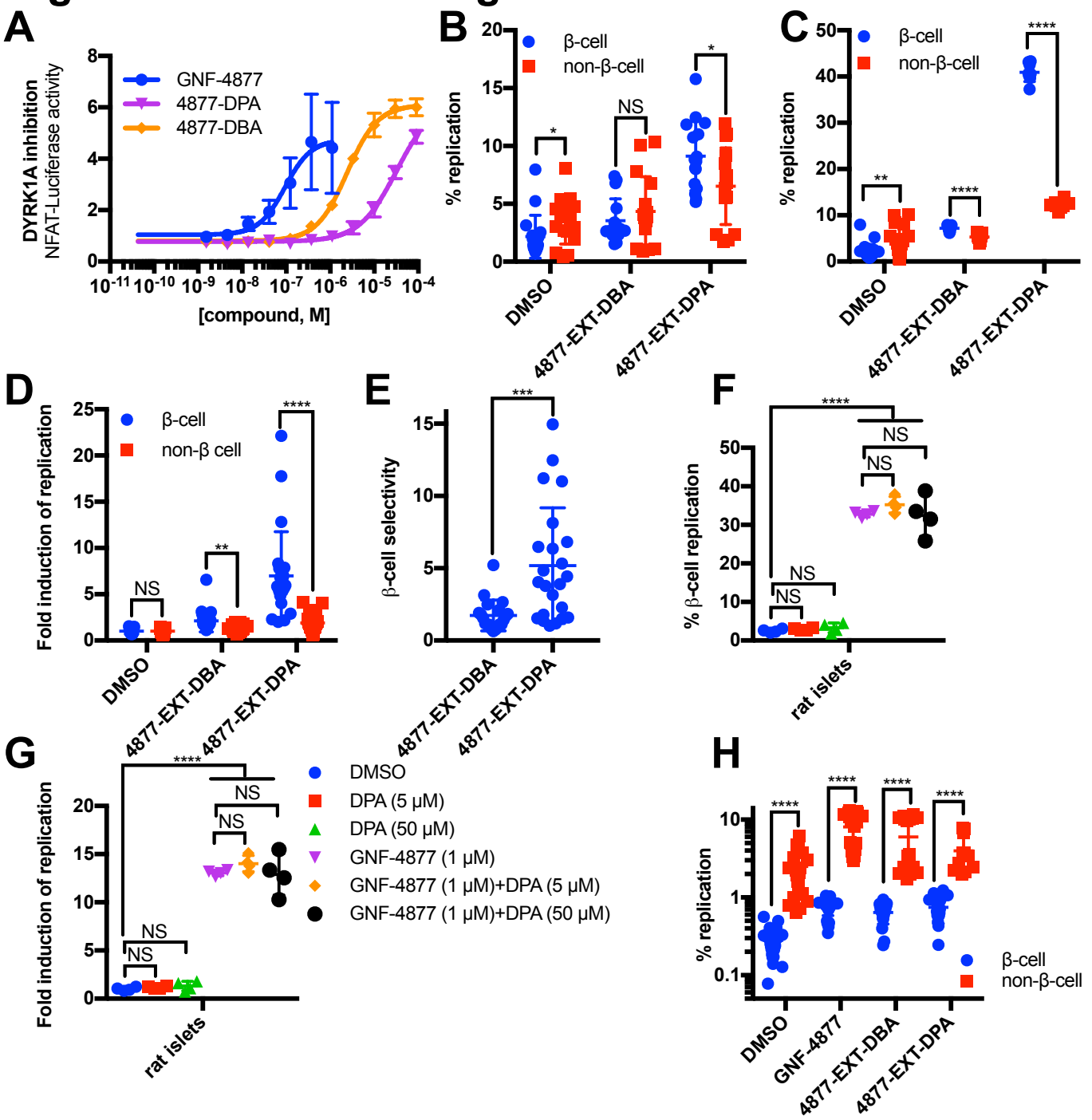


**Figure S5, related to Figure 5. Non-extended GNF-4877 analogues lose much of their potency against DYRK1A in a cell culture system, zinc chelation also enhances β-cell selectivity at 1 μM in rat islets, DPA does not alone cause replication, and percent replication data. (A)** Dose response curves for GNF-4877, 4877-DPA, and 4877-DBA, based on DYRK1A inhibition via a luciferase reporter in transfected HEK 293T cells (n=4 per concentration). **(B)** Dispersed rat islet replication induced by DMSO, 4877-EXT-DBA (1 μM), and 4877-EXT-DPA (1 μM), expressed as a percentage of total cells. **(C)** Dispersed rat islet replication induced by DMSO, 4877-EXT-DBA (3 μM), and 4877-EXT-DPA (3 μM), expressed as a percentage of total cells. **(D)** Replication induced by DMSO, 4877-EXT-DBA (1 μM), and 4877-EXT-DPA (1 μM) in co-cultured rat islet β-cells (blue) and non- β-cells (red). Data are pooled from five independent experiments of one or two rats each (n=11-23). **(E)** β-cell selectivity for the experiment in *(D)*, defined as the ratio of β-cell replication to non-β-cell replication (n=11-23). **(F)** β-cell replication induced by DMSO, DPA (5 μM), DPA (50 μM), in the absence (left) and presence (right) of GNF-4877 (1 μM), expressed as a percent of total β-cells. **(G)** The experiment in *(F)*, expressed as fold of replication in DMSO-treated wells. **(H)** Dispersed human islet replication induced by DMSO, GNF-4877 (1-5 μM), 4877-EXT-DBA (3.3-10 μM), and 4877-EXT-DPA (3.3-10 μM), expressed as a percentage of total cells.