SUPPLEMENTARY MATERIAL

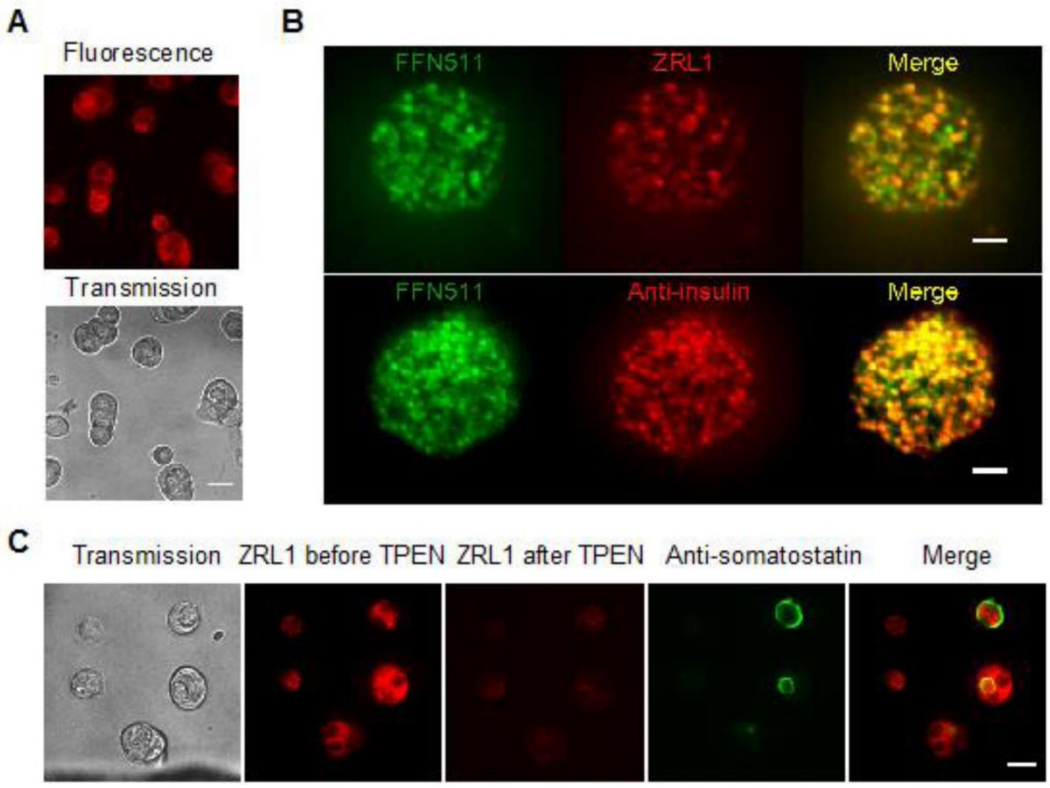


Fig. S1. ZRL1 could label all dissociated islet cells. (A) A representative image of dissociated islet cells labeled with ZRL1 under the wide-field microscope. Scale bars represent 20 μm. **(B)** ZRL1 labels insulin granules in primary β cells. Upper, confocal imaging of FFN511 (left) and ZRL1 (middle) signals in an EYFP negative living islet cell from GYY mice after sequential incubation with ZRL1 and FFN511. Lower, confocal imaging of FFN511 (left) and immunofluorescence with anti-insulin antibody (middle) in an EYFP negative fixed islet cell from GYY mice after FFN511 labeling followed with immunostaining. Scale bars represent 2 μm. **(C)** ZRL1 labels primary δ cells, which fluorescence could be quenched by TPEN. A representative example of immunofluorescent staining of dissociated mouse pancreatic islet cells with anti-somatostatin antibodies after ZRL1 labeling, with and without TPEN quenching. The scale bar represents 20 μm.

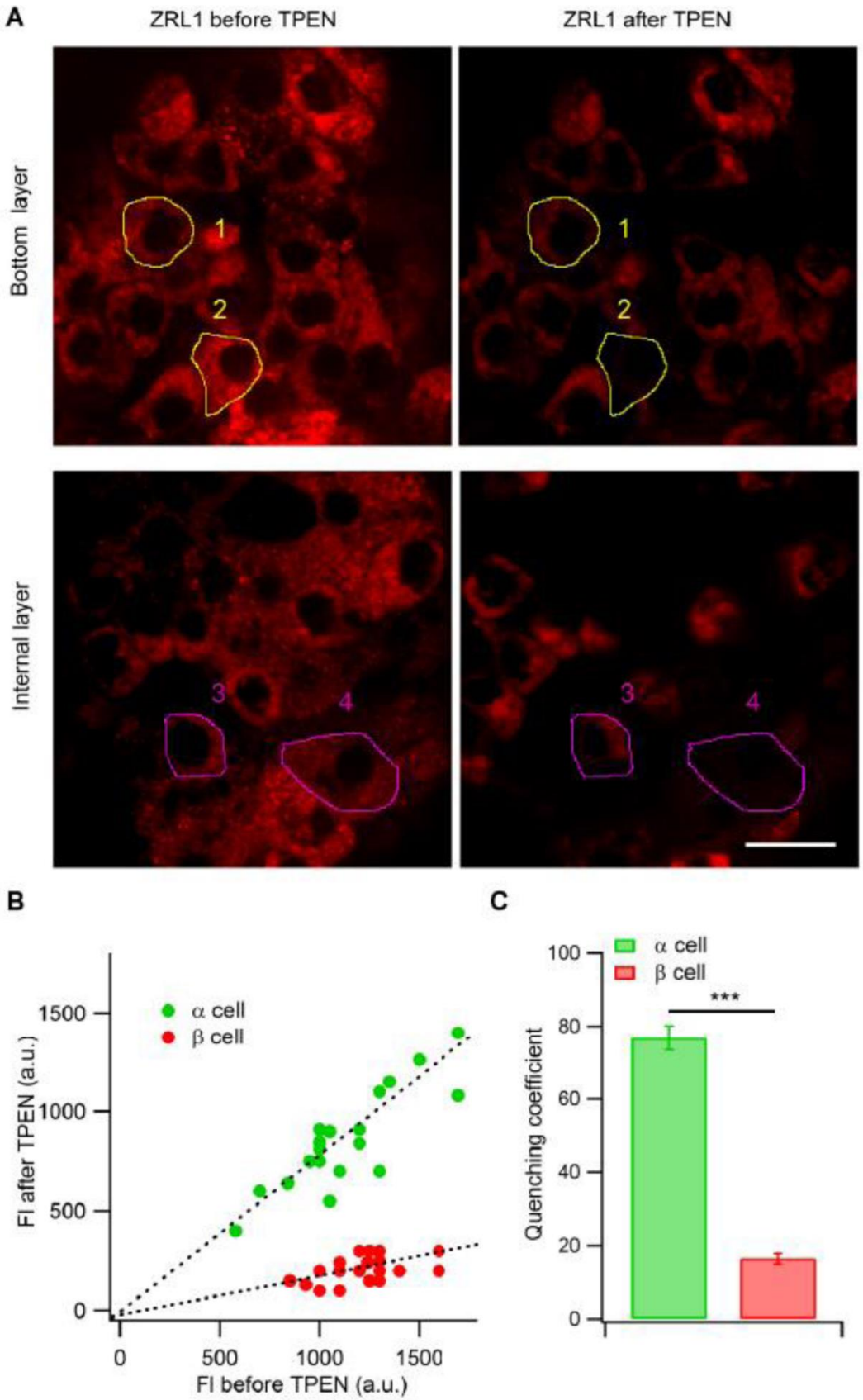


Fig. S2. Calculation of ZRL1 quenching coefficients in α-cells and β-cells. (A) Representative bottom layer and internal layer (40 μm inside) of ZRL1 imaging of islets before and after TPEN quenching. Cell 1 and 3 are two α-cell examples, while cell 2 and 4 are two β-cell examples. ZRL1 fluorescence intensity of each cell before and after TPEN quenching was measured in the selected area. The scale bar represents 20 μm. **(B)** ZRL1 fluorescence intensity after TPEN quenching positively linearly correlated with that before TPEN quenching in α-cells or in β-cells. Each spot represents data from one cell. $n = 20$ for each cell type. **(C)** The average quenching coefficient calculated based on **(B).**

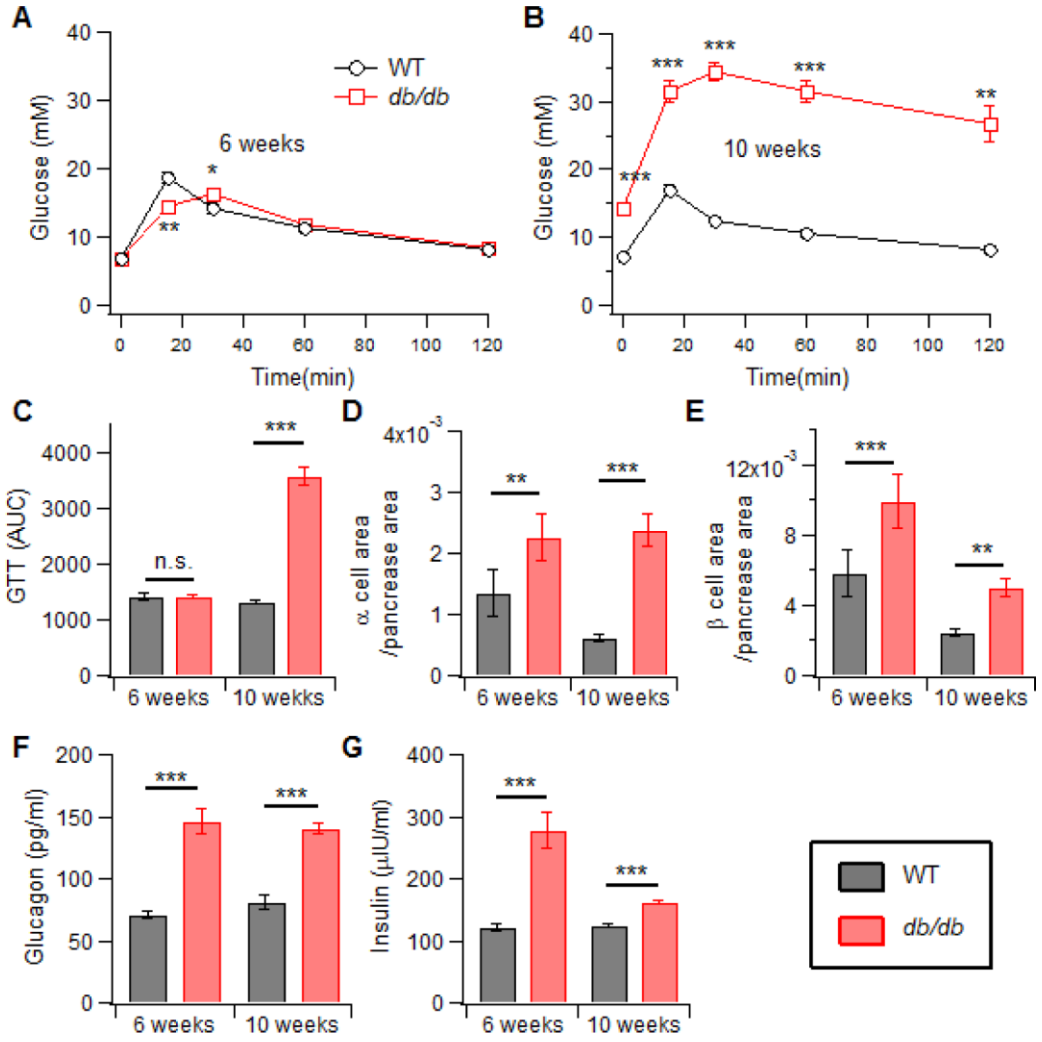
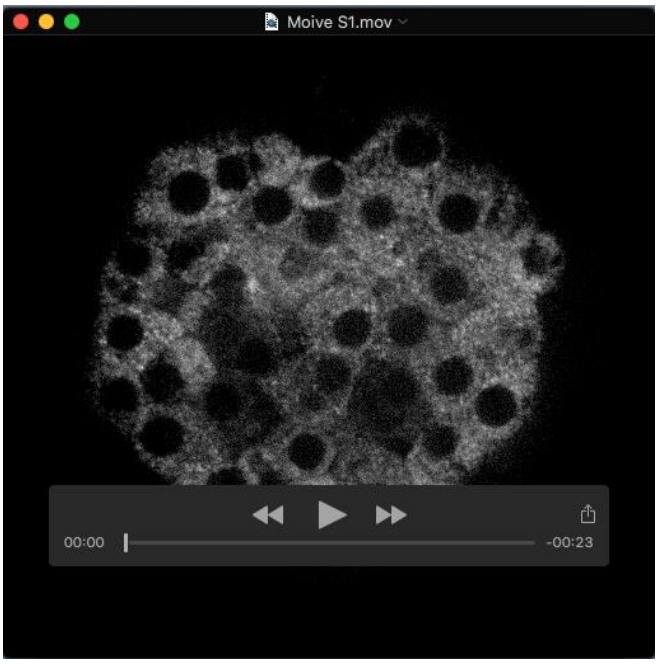


Fig. S3. Changes in the glucose tolerance and α- and β-cell mass and function during diabetes pathogenesis in *db/db* mice. (A, B) IPGTT curves of six- and ten-week-old wild type mice and *db/db* mice (n = 6). **(C)** Area under the curve (AUC) of IPGTT of six- and ten-week-old wild type and *db/db* mice from **(A, B)**. **(D)** The ratio of the α-cell area *versus* the total pancreas area in six- and ten-week-old control and *db/db* mice (n = 6). **(E)** The ratio of the β-cell area *versus* the total pancreas area in six- and ten-week-old control and *db/db* mice (n = 6). **(F)** Glucagon secretion in isolated islets from six- and ten-week-old control and *db/db* mice (n = 6). **(G)** Glucose stimulated insulin secretion in isolated islets from six- and ten-week-old control and *db/db* mice (n = 6).



Movie. S1. A representative time-lapse movie of a ZRL1-labeled islet that is sequentially perfused with TPEN and NH_4Cl.