SUPPLEMENTAL RESEARCH DESIGN AND METHODS

Quantitation of β-cell apoptosis rates

β-cell apoptosis rates were determined by detecting DNA fragmentation by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL). Pancreata from 2 months old mice were removed, 4% PFA fixed and embedded in paraffin wax. 7 μm sections were stained by *in situ* cell death detection kit, POD (Roche). A positive control was included by incubating the section with 3U/ml recombinant DNase I prior to labeling. After counterstaining with hematoxylin, islets on the sections were imaged with a Zeiss Axioskop microscope.

shRNA and cell culture

Double-stranded oligonucleotides corresponding (underlined) to the canine Kif5b motor domain (top strand: 5'-GATCCGCCTTATGCATTTGACCGAT TCAAGAGATCGGTCAAATGCATAAGGCTTTTTTGGAAA-3'; bottom strand: 5'-GCGGAATACGTAAACTGGCTAAGTTCTCTAGCCAGTTTACGTATTCCGAAAAA ACCTTTTCGA-3') were ligated into the pSilencer vector (Ambion). The negative control pSilencer hygrovector, that expresses a hairpin siRNA with limited homology to any known sequences in human, mouse, rat and dog genomes, was used as negative control. These shRNA expression vectors were stably transfected into MDCK type II cells under Hygromycin B selection. Control and Kif5b-shRNA stable MDCK cells were cultured in DMEM media (GIBCO) supplemented with 350 μg/ml Hygromycin B (Roche).

Cell proliferation assays

MTT

Cells were trypsinized and diluted into 10^4 cells per ml in complete medium. 100 μl cells were added into each well of a 96-well plate and incubated overnight. All cells were seeded in quintuplet. 20 μl of 5mg/ml MTT was added to each well, including one set of black control, followed by incubation for 3.5 h at 37°C in culture incubation. After incubation, medium was carefully removed, and 150 μl MTT solvent (DMSO) was added into each well. Then the 96-well plate was covered with tinfoil and agitated on orbital shaker for 15 min. Absorbance for each well was read at 570nm on SpectraMAX® 340 microplate spectrophotometer.

Trypan blue staining

10^4 cells were seeded into a 6-well plate in triplicate one day before. 0.4% Trypan blue stain was used to check cell viability. Cells were trypsinized and resuspended PBS at indicated time point. 100 μl of the cells were mixed with 100 μl trypan blue 0.4%. Then the cells were visualized and counted under inverted microscope. Only viable cells that excluded the dye were counted.

SUPPLEMENTAL RESULTS

Pancreatic β-cell apoptosis analysis

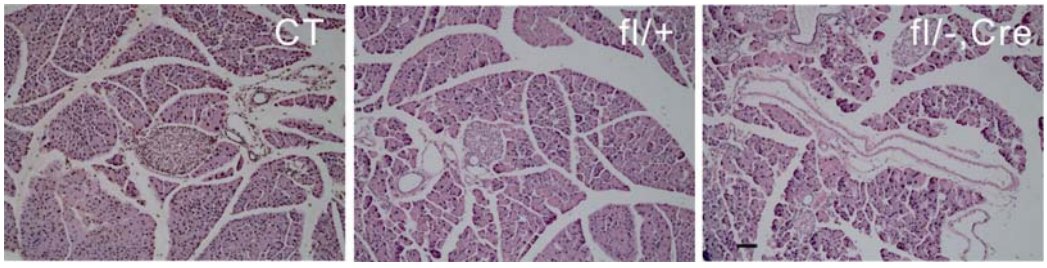
In our positive control, most cells show $TUNEL^+$ positive signal after DNase I treatment. However, we barely detected any islet cell apoptosis signal in sections from both control and mutant mice (supplementary Fig. 1).

Kif5b deficiency cells exhibit a reduced proliferation rate

It was found that conditional knockout of Kif5b in pancreatic β-cell affected cell proliferation and led to reduced islet size *in vivo*. Meanwhile, during the study of Kif5b in epithelial cells, decreased cell proliferation rate was observed in Kif5b deficient MDCK cells. Supplementary Figure 2A was a representative Western blot result for selected clones of stable cells. In the control clones, Kif5b expression levels were comparable to those in wild type MDCK cells. For cells transfected by *Kif5b*-shRNA had variable Kif5b expression levels. Clones sh-6, sh-8 and sh-18 obtained about 80% Kif5b knock down efficiency. Expression levels of several other motor proteins were also analyzed in these stable MDCK cell clones. Kif17 and KLC were expressed in wild type MDCK cells, and Kif5b targeting siRNA had no effect on the expression of these proteins.

MTT assays were carried out to examine cell proliferation rates in several Kif5b knock down clones (MDCK-sh), control cell clones (MDCK-CT) as well as wild type cells (Supplementary Fig.2B). Due to hygromycin selection, MDCK-CT cells had lower proliferation rates compared to wild type cells and there was no difference among different MDCK-CT cell clones. However, after knockdown of Kif5b, cell (MDCK-sh) proliferation rate was significantly reduced and displayed a Kif5b expression level dependent pattern. Growth rate in cell clones sh-2, sh-7, and sh-9, which have a Kif5b level reduced by about 50%, were lower than those of MDCK-CT cells, but higher than those of cell clones sh-6, sh-8 and sh-18, which have more Kif5b reduction. Decreased cell proliferation was further confirmed by counting viable cells after staining with a vital dye trypan blue (Supplementary Fig. 2C). For the first 48 h, there was no significant difference for the cell division rate between CT-1 and sh-6 cells. However, sh-6 cell have progressively reduced cell growth ability comparing to control cell.

Supplemental Fig. 1. Representative photo of TUNEL assay in sections from positive control (CT), wild type mice (fl/+) and mutant mice (fl/-, Cre). Scale bar =50 μm.



Supplemental Fig. 2. Knockdown of Kif5b by shRNA expression in MDCK cells affect cell proliferation. (A) Representative western blot results showed the reduction of Kif5b protein level in *Kif5b*-shRNA transfected cells but not in control cells transfected with a vector expressing an irrelevant shRNA. (B) Cell proliferation rates were analyzed by MTT assays. *Kif5b*-shRNA cells (yellow and red lines) had decreased proliferation rates comparing to control cells (green lines) and wild type cells (black line). Cell proliferation rates in clones sh-2, sh-7 and sh-9 (yellow lines) were higher than in clones sh-6, sh-8 and sh-18 (red lines), which correlated with Kif5b levels in these cells. (C) Control cells (clone 1, green line) and Kif5b deficient cells (clone 6, red line) were seeded at 10^4 cells per well in 6-well plate. Cell numbers were counted at the indicated time point, which further confirmed that cell proliferation was affected in Kif5b depleted cells. (*P< 0.05)