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Supplementary Materials for

Islet primary cilia motility controls insulin secretion

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

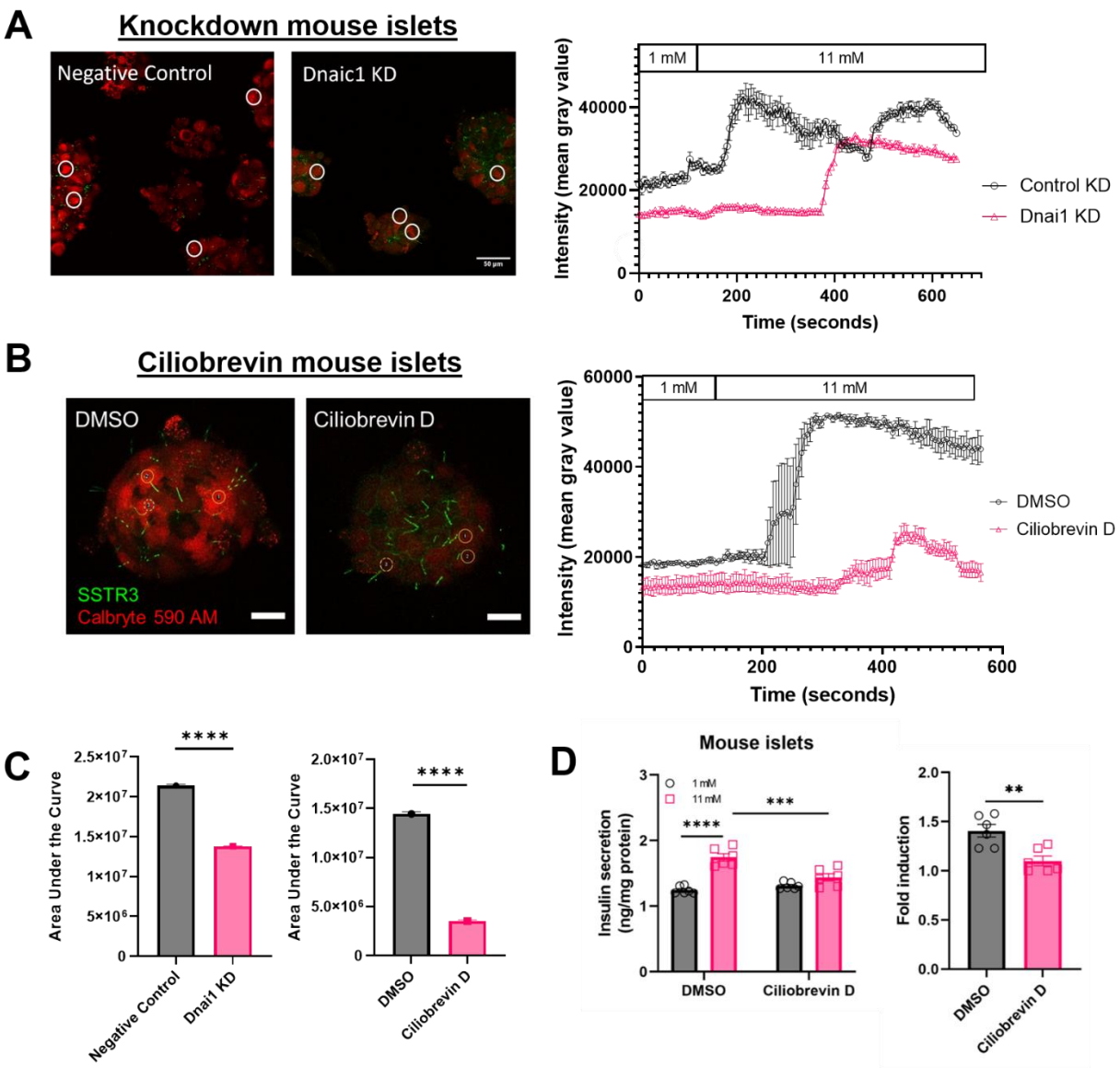

Figure S1. Dynein loss-of-function inhibits calcium entry and insulin secretion in mouse islets.

(**A**) Calcium imaging using the cell-permeable dye Calbryte in mouse islets after siRNA against the outer dynein arm gene *Dnai1*. (**B**) Treatment with the dynein inhibitor ciliobrevin D produces similar effect on calcium dynamics as dynein knockdown. (**C**) Area under the curve showing decreased cumulative $[Ca^{2+}]_i$ as reported by fluorescence intensity in both dynein knockdown and ciliobrevin treatments. (**D**) Ciliobrevin D treatment inhibits GSIS in mouse islets. Fold induction by glucose was significantly reduced by ciliobrevin D. Statistical significance was analyzed by two-way ANOVA, ***p < 0.01*, ****p < 0.001*, *****p< 0.0001*. Corresponding **Movies S9-10**.

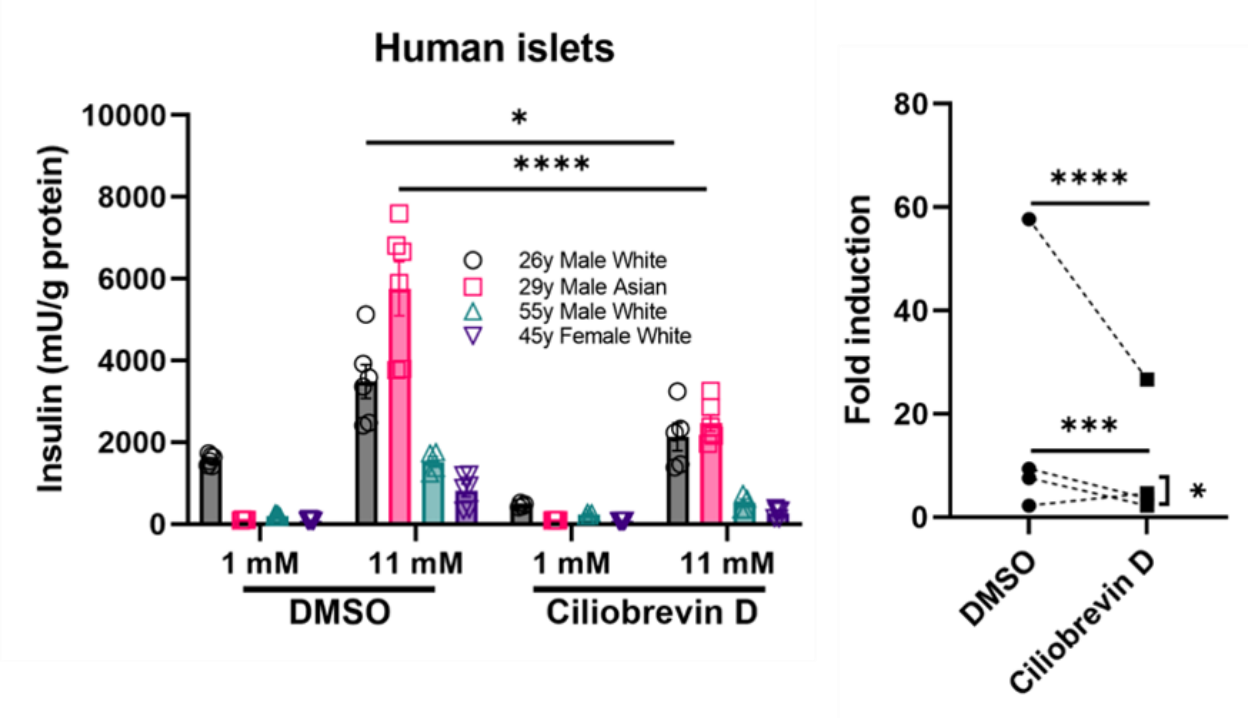

Figure S2. Ciliobrevin inhibits insulin secretion from human islets.
Ciliobrevin D treatment inhibits GSIS in intact islets; n = 4 healthy male human donors aged 26 to 62 years old. One-hour static incubation in 11 mM glucose induced insulin secretion in all human islet samples and to a greater extent in younger donor islets. Fold induction by glucose was significantly reduced by co-treatment with 50 μM ciliobrevin D. Statistical significance was analyzed by two-way ANOVA, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p< 0.0001$.

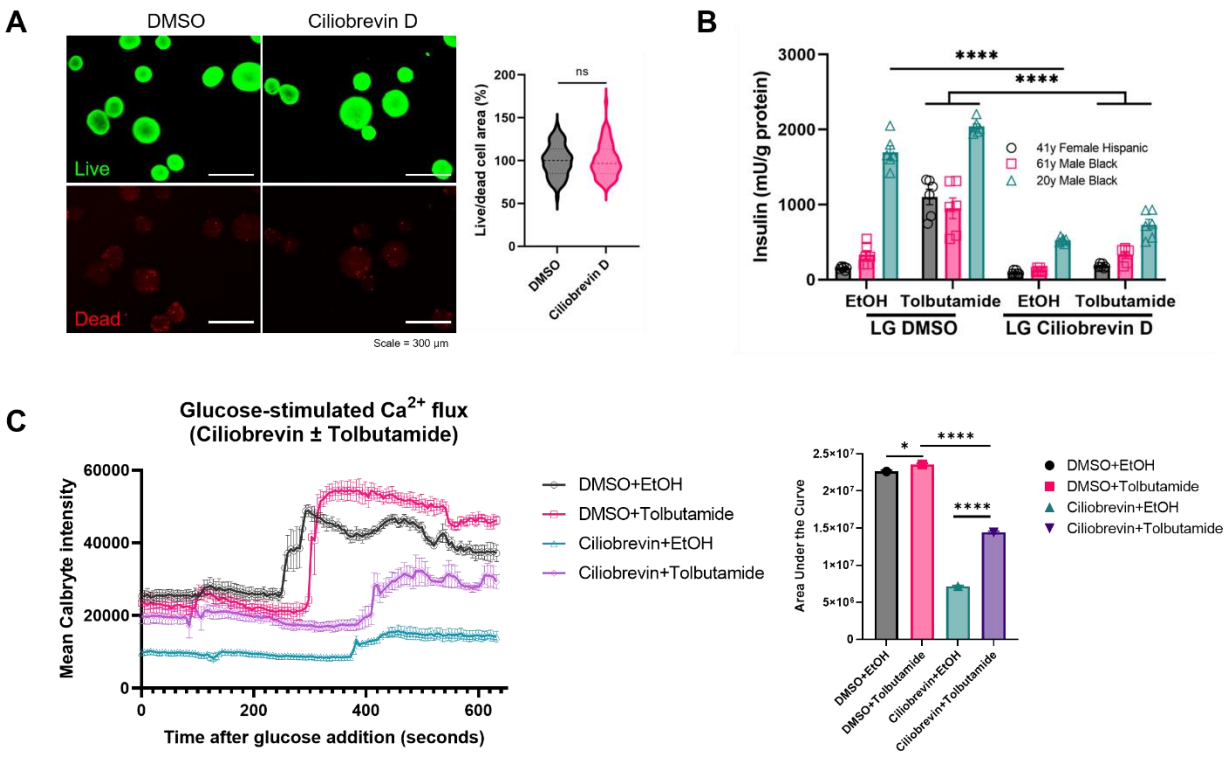

Figure S3. Ciliobrevin inhibition of insulin secretion is partly rescued by tolbutamide. (**A**) Cell viability measured by Cell Counting Kit-8 (CCK-8) showing equal number of live cells in control DMSO versus ciliobrevin D-treated islets. (**B**) Treatment with ciliobrevin D led to a consistent reduction of insulin secretion in human islets, n = 3 individual donors, both female and male. This inhibitory effect was partly overcome by co-treatment with the potassium channel blocker tolbutamide. (**C**) Tolbutamide partly restores calcium influx in islets, consistent with its effect on insulin secretion.

SUPPLEMENTAL MOVIES S1-10

Movie S1. Human islet cilia motility as imaged using green cilia cADDis and SiR-Tubulin

Video images of spontaneous cilia movements on human islets were labeled overnight with green cilia cADDis or with 1 µM SiR-Tubulin and 10 µM verapamil for 1 hour in islet media. Images were recorded at 1 mM glucose with a frame time of 2.53 seconds for cADDis-labeled islets and a frame time of 1.27 seconds for SiR-Tubulin-labeled islets. Scale bars = 2 µm.

Movie S2. Beta cell cilia movement in low vs. high glucose.

Video images of cilia on intact islets from Ins1Cre-SSTR3GFP mice incubated in 1 mM glucose were captured with a frame time of 465.45 ms. Glucose level was raised to 11 mM and cells were re-imaged, showing increased cilia motility as measured by amplitude. Scale bar = 5 µm.

Movie S3. Dynein knockdown blocks beta cell cilia motility.

Video images of beta cell cilia on mouse islets after *Dnai1* vs. control siRNA were recorded with a frame time of 20.26 seconds, showing reduced cilia motility after dynein loss-of-function. Scale bar = 10 µm.

Movie S4. ATP supplementation stimulates beta cell cilia motility.

Video images of beta cell cilia on mouse islets after 1-hour treatment with 10 µM ATP in KRBH without glucose stimulation were recorded with a frame time of 20.26 seconds, showing potentiation of beta cell cilia motility by exogenous ATP. Scale bar = 20 µm.

Movie S5. ATP depletion by AA2DG blocks beta cell cilia motility.

Video images of beta cell cilia on mouse islets incubated in EtOH or 20 µM Antimycin A and 10 mM 2-deoxy-D-glucose in PBS with no glucose-containing KRBH for 1 hour were recorded with a frame time of 1.27 seconds, demonstrating that beta cell cilia motility requires ATP and glucose. Scale bar = 5 µm.

Movie S6. Dynein inhibitor ciliobrevin blocks beta cell cilia motility.

Video images of beta cell cilia on mouse islets after 1-hour treatment with 0.5% DMSO or 50 µM ciliobrevin D in 1 mM glucose-containing KRBH were recorded with a frame time of 20.26 seconds, showing inhibition of beta cell cilia motility by ciliobrevin D. Scale bar = 5 µm.

Movie S7. Dynein inhibitor EHNA blocks beta cell cilia motility.

Video images of beta cell cilia on mouse islets incubated in DMSO or 80 mM EHNA in 1 mM glucose-containing KRBH for 1 hour were captured with a frame time of 20.26 seconds, showing that beta cell cilia motility was blocked by EHNA. Scale bar = 5 µm.

Movie S8. Actin inhibitor blebbistatin does not affect beta cell cilia motility.

Video images of beta cell cilia on whole mouse islets incubated in DMSO or 50 µM blebbistatin in 1 mM glucose-containing KRBH for 1 hour were captured with a frame time of 20.26 seconds, showing that beta cell cilia motility does not depend on the actin motor myosin II. Scale bar = 5 µm.

Movie S9. Dynein knockdown disrupts calcium dynamics in cilia reporter beta cells.

Video images of beta cell cilia and calcium influx in cilia reporter mouse islets labeled with 4 µM Calbryte 590 AM in islet media were captured using two-channel time-lapse and z-stack imaging with a frame time of 465.45 ms. Islets received *Dnai1* knockdown or control siRNA. Glucose concentration was raised from 1 mM to 11 mM during image capture, and calcium flashes indicate cell response to glucose. Video shows maximum intensity projection; some cilia appear segmented due to limited z-plane sampling. Scale bar = 50 µm.

Movie S10. Ciliobrevin disrupts calcium dynamics in cilia reporter beta cells.

Video images of beta cell cilia and calcium influx after 1-hour treatment with 50 µM ciliobrevin D, using the same imaging settings as Movie S9. Scale bar = 50 µm.