Supplementary information, Data S1

Materials and Methods

Mice

DB mice (BKS.Cg-*Dock7*^{m}+/+*Lepr*^{db}/Nju mice in C57BLKS/JNju background), NOD mice (NOD/ShiLtJNju mice), and C57BL/6J mice were purchased from the Nanjing Biomedical Research Institute of Nanjing University. To establish the mouse model of diet/streptozotocin (STZ)-induced obesity, eight-week-old male C57BL/6J mice were fed a diet containing 15% sucrose, 15% lard, 5% yolk, and 5% whole milk powder for 4 weeks, injected intraperitoneally with STZ in citrate buffer at 100 mg/kg body weight, and examined 2 weeks later. Blood samples were taken from the mouse tail vein, and glucose levels were measured using a glucometer (Sinocare). For the treatment of mice with BZX, this agent was dissolved in phosphate-buffered saline (PBS) and dropped into mouse eyes 5 times per day for 15 days. All mouse experiments were performed in accordance with the protocols and complied with the relevant ethical regulations approved by the Animal Care and Use Committee of Nankai University.

Antibodies, chemicals, and siRNAs

Antibodies against OGT (Santa Cruz Biotechnology and Abcam), OGA (Santa Cruz Biotechnology), IFT88 (Proteintech), centrin (Millipore), acetylated α-tubulin, β-actin, *O*-GlcNAc, γ-tubulin (Sigma-Aldrich), α-tubulin, and γ-tubulin (Abcam) were purchased from the indicated sources. Alexa Fluor 488 and 568 secondary antibodies were from Life Technologies. DAPI, TMG, and STZ were from Sigma-Aldrich. BZX was synthesized following the procedures described previously.[1] The sequences for OGT siRNAs were 5′-GGAUGGAAUUCAUAUCCUU-3′ (#1) and 5′-AUACGAUGGCAUCUUCUGGUAACCC-3′ (#2). The sequences for OGA siRNAs were 5′-GCAAGAAGAUUGUAUUAGU-3′ (#1) and 5′-GGCACUUUCUGUUAUCCAA-3′ (#2).

Cell culture and transfection

RPE-1 cells were obtained from the American Type Culture Collection and grown in the DMEM/F12 medium supplemented with 10% fetal bovine serum. To induce ciliary formation, RPE-1 cells were cultured in serum-free DMEM/F12 medium. siRNAs were transfected using Lipofectamine RNAiMAX (Invitrogen).

Immunofluorescence microscopy

Mouse tissues were fixed in 4% paraformaldehyde, embedded in Tissue-Tek OCT (Sakura), and quick-frozen in liquid nitrogen. Thin sections were fixed with 4% paraformaldehyde for 30 min and permeabilized in 0.5% Triton X-100/PBS for 25 min. Then tissues were blocked in 4% bovine serum albumin (BSA) for 1 hr and stained with primary antibodies, fluorescein-conjugated secondary antibodies and then DAPI. Sections were subsequently mounted onto slides and examined with an LSM710 confocal microscope (Carl Zeiss). Cells grown on glass coverslips were fixed with cold

methanol for 3 min, blocked in 4% BSA and then incubated with primary antibodies, secondary antibodies and DAPI. The length of cilia and the percentage of ciliated cells were measured with ImageJ (National Institutes of Health), and only cilia with distinguishable ends were included for the measurement of ciliary length. OGT localization at basal bodies was quantified as the intensity of OGT at basal bodies divided by the intensity of total cellular OGT.

Electron microscopy

For scanning electron microscopy, mouse tracheal tissues were isolated and fixed with 2.5% glutaraldehyde in 0.1 M PBS at 4°C overnight. Samples were post-fixed in 1% osmium tetroxide for 1 hr, dehydrated in ethanol gradient buffer, and dried by critical point drying. The samples were then gold-coated by the sputter technique and examined by a QUANTA 200 scanning electron microscope (Thermo Fisher Scientific) at an accelerating voltage of 15 kV.

Centrosome isolation

Centrosomes were isolated following the procedures described previously.[2] Briefly, RPE-1 cells were treated with 10 μM nocodazole (Sigma-Aldrich) and 10 μM cytochalasin D (Sigma-Aldrich) for 1 hr at 37°C. After being washed gently with PBS and 8% sucrose, cells were lysed on a rotating platform at 4 °C for 20 min. The swollen nuclei, chromatin aggregates, and nonlysed cells were removed by centrifugation at 1,500 g for 3 min. The supernatants were then applied on the Ficoll cushion (GE Healthcare) and centrifuged at 25,000 g for 15 min at 4°C. The supernatant above the Ficoll cushion was collected, applied onto the sucrose density gradient, and centrifuged at 130,000 g for 90 min at 4°C. The gradient was fractionated manually, and the fractions between 40%-60% sucrose were analyzed by immunoblotting.

Statistical analysis

All quantitative data were presented as the mean ± SEM of at least three independent experiments by Student's *t*-test.

REFERENCES

1 Jiang, J., Lazarus, M. B., Pasquina, L., Sliz, P. & Walker, S. A neutral diphosphate mimic crosslinks the active site of human O-GlcNAc transferase. *Nat. Chem. Biol.* **8**, 72-77 (2011).

2 Reber, S. Isolation of centrosomes from cultured cells. *Methods Mol. Biol.* **777**, 107-116 (2011).