ESM Methods

**Cell culture and transfection** Pancreatic islet beta cells from adult Wistar rats (150-250 g) were isolated and cultured as described previously [1]. Briefly, rats were killed by cervical dislocation and islets were collected from the pancreas by collagenase P digestion. After short-term tissue culture (4-24 h) in RPMI 1640 medium, single β-cells were isolated by treating the islets with 0.025% trypsin (Invitrogen) for 5 min at 37°C. Cells were then washed twice in RPMI 1640 and either used for transfection or plated on poly-L-lysine-coated coverslips for culture. Transfection was performed with the Neon™ 10 μl transfection system MPK10096 (Invitrogen) following the manufacturer’s instructions. Cells were cultured for 24-72 h in RPMI 1640. For ADCY8 knockdown, experiments were performed >72 h after transfection.

**Imaging** An upright Zeiss LSM 710 confocal microscope was used for live imaging of the FRET indicator AKAR3 with a 40× oil-immersion objective. A laser at 405 nm was used for excitation of AKAR3, and the simultaneous 2-channel mode was used for emission detection, one channel for cyan (466-489 nm) and the other for yellow (519-535 nm). The FRET ratio ΔR was calculated as \( R = \frac{F_{YFP}}{F_{CFP}} \). ΔR changes were normalized as \( \Delta R = \Delta R' / R \), where \( \Delta R' \) is the absolute value of the FRET ratio change, to avoid variation among the basal FRET ratios in different cells. For simultaneous imaging of \( \text{Ca}^{2+} \) and FRET, cells were preloaded with Rhod-2 AM (5 μmol/l), and the switched-mode of frame-scan was used to detect the FRET and \( \text{Ca}^{2+} \) signals alternately. AKAR3 was excited at 405 nm and the emitted light was detected at
466-489 nm and 519-535 nm, and Ca\(^{2+}\) signals were detected at excitation 543 nm and emission 560-620 nm. Images (256 × 256 pixels) were acquired at 0.5 Hz. Temperature was maintained at 28-32°C using a TempModule S. Control solutions and drugs were puffed locally onto the cells during recording via a multi-channel micro-perfusion system [2].

*Electrophysiology and membrane capacitance recording* Whole-cell and perforated whole-cell configurations were used as described [3, 4], while the latter was used during imaging to avoid loss of fluorescence intensity due to protein leakage into the pipette. For the H-89 inhibition experiments, the intracellular solution contained 15 μmol/l H-89, which was allowed to dialyze into cells for >7 min. Healthy cells with membrane capacitance >4 pF and without Na\(^+\) currents were characterized as beta cells [1]. The standard extracellular solution contained (in mmol/l): 118 NaCl, 20 TEA-Cl, 5.6 KCl, 2.6 CaCl\(_2\)-2H\(_2\)O, 1.2 MgCl\(_2\), 5 D-glucose, 5 HEPES, pH adjusted to 7.4. The intracellular solution for conventional and perforated whole-cell patch clamp recording was (in mmol/l): 152 CsCH\(_3\)SO\(_3\), 10 CsCl, 10 KCl, 1 MgCl\(_2\), 5 HEPES, pH adjusted to 7.35 with CsOH.

*Insulin release detection* Insulin release from β-cells was measured by ELISA as described previously [4]. For ELISA, we used Krebs-Ringer-bicarbonate buffer containing (KRBB, in mmol/l): 5 KCl, 120 NaCl, 15 HEPES, pH 7.4, 24 NaHCO\(_3\), 1 MgCl\(_2\), 2 CaCl\(_2\), and 1 mg/ml BSA. The cells were treated with KCl for 1 min, and the incubating solutions were collected for the subsequent analysis. After 10 min incubation with KRBB (vehicle), PKA blocker (H-89), or ADCY8 blocker
(2',5'-dideoxyadenosine), the cells were treated with KCl for another 1 min. All samples were finally centrifuged at 13 000 rpm for 5 min, and the supernatants processed for insulin measurement.

Statistics All data were collected and analyzed with IGOR software (WaveMetrix, Lake Oswego, OR). The mean ± SEM was calculated, and Student’s t-test was applied to determine significance (*p <0.05; **p <0.01; ***p <0.001).

References