Medicine

Mechano-regulation of GLP-1 production by Piezo1 in intestinal L cells

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Abstract

Glucagon-like peptide 1 (GLP-1) is a gut-derived hormone secreted by intestinal L cells and vital for postprandial glycemic control. As open-type enteroendocrine cells, whether L cells can sense mechanical stimuli caused by chyme and thus regulate GLP-1 synthesis and secretion is unexplored. Our study showed expression of Piezo1 in intestinal L cells. Its level varied in different energy status and correlates with blood glucose and GLP-1 levels. Mice with L cell-specific loss of Piezo1 (*IntL-Piezo1*$^{-/-}$) exhibited impaired glucose tolerance, increased body weight, reduced GLP-1 production and decreased CaMKKβ/CaMKIV-mTORC1 signaling pathway under normal chow diet or high fed diet. Activation of the intestinal Piezo1 by its agonist Yoda1 or intestinal bead implantation increased the synthesis and secretion of GLP-1, thus alleviated glucose intolerance in diet-induced-diabetic mice. Overexpression of Piezo1, Yoda1 treatment or stretching stimulated GLP-1 production and CaMKKβ/CaMKIV-mTORC1 signaling pathway, which could be abolished by knockdown or blockage of Piezo1 in primary cultured mouse L cells and STC-1 cells. These findings suggest a previously undiscovered mechano-regulation of GLP-1 production in L cells, which may shed new light on the treatments of diabetes.

eLife Assessment

This study focuses on the regulation of GLP-1 in enteroendocrine L cells and how this may be stimulated by the mechanogated ion channel Piezo1 and the CaMKKbeta-CaMKIV-mTORC1 signaling pathway. The work is innovative and is considered **valuable**, as the hypothesis that is being tested may have significant mechanistic and translational implications. Data to support the proposed mechanism were considered **incomplete**, yet data to support the overall physiological characterization were considered **solid**.

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Introduction

The gastrointestinal (GI) tract represents the largest endocrine organ in the human body. The enteroendocrine cells (EECs) located throughout the GI tract secrete a large number of gastrointestinal hormones to regulate a variety of physiological processes and are key regulators for energy homeostasis (Bany Bakar et al., 2023). GLP-1 is one of the gut-derived peptide hormones essential for postprandial glycemic control (Song et al., 2019). It is produced from Proglucagon (Gcg) by proprotein convertase in the intestinal L cells, a group EECs predominantly situated in the distal gut (Drucker, 2006; Rouillé et al., 1997). The circulating GLP-1 levels rapidly increase after meal and reduce postprandial blood glucose fluctuations by augmenting insulin secretion, suppressing glucagon secretion and slowing gastric emptying (Drucker, 2006; Willms et al., 1996). Nowadays, GLP-1-based therapy is well-recognized and commonly used in treatment of Type 2 Diabetes Mellitus (T2DM) (Saxena et al., 2021; Tan et al., 2022). Elucidation of the mechanism that regulates GLP-1 production is essential for the development of new drug targets for the treatment of diabetes.

EECs can be divided into two categories according to its morphology: open type and closed type. The open type EECs possess microvilli protruding into the gut lumen and have direct contact with the luminal contents. In contrast, the closed type EECs are located basolaterally without direct contact with the lumen (Gribble & Reimann, 2016). Both types of EECs synthesize and store peptides or hormones in secretory granules and release them by exocytosis at the basolateral membrane upon mechanical, chemical or neural stimulation (Atanga et al., 2023). As open-type EECs, L cells received both chemical and mechanical signals from the luminal contents, and neural signals from the nerves (Furness et al., 2013). It has been well-documented that nutrients such as glucose, lipids, and amino acids in the intestinal lumen can stimulate the secretion of GLP-1 from L cells (Diakogiannaki et al., 2012). GLP-1 secretion can also be stimulated by intrinsic cholinergic nerves (Anini et al., 2002; Drucker, 2006). However, whether and how L cells coordinate mechanical stimuli from intestinal lumen to regulate GLP-1 production remain poorly understood.

Piezo channels, including Piezo1 and Piezo2 have recently been identified as mechanosensitive ion channels involved in the sensation of multiple mechanical stimuli, such as shear stress, pressure, and stretch (Gudipaty et al., 2017; Li et al., 2014; Romac et al., 2018). They allow the influx of cations such as Ca^{2+} and Na^{+} in response to mechanical tension and converts mechanical stimuli into various electrical and chemical signals. Piezo1 plays a crucial role in blood pressure regulation, red blood cell volume regulation, bone homeostasis, pulmonary and cardiac functions (Cahalan et al., 2015; Lai et al., 2022; Wang et al., 2023; Wang et al., 2016). Previous studies have reported that Piezo1 is expressed in the intestinal epithelium, regulating gut

peristalsis, barrier function, mucus secretion, and inflammation (Jiang et al., 2021; Liu et al., 2022; Sugisawa et al., 2020; Xu et al., 2021). Interestingly, accumulating evidence demonstrates the regulation of insulin and ghrelin secretion by Piezo1 (Deivasikamani et al., 2019; Ye et al., 2022; Zhao et al., 2024). Recent studies have also reported that Piezo2 is expressed in a population of EECs and convert force into serotonin release (Alcaino et al., 2018; Treichel et al., 2022). These findings suggest a critical role of Piezo channels in the mechano-regulation of hormone production. However, whether Piezo channels are expressed L cells and play a role in GLP-1 production remain unknown.

Here, we present evidence that Piezo1 channels on intestinal L cells mediate the mechanosensing from intestinal contents and trigger the synthesis and secretion of GLP-1 through CaMKKβ/CaMKIV-mTORC1 signaling pathway, thus regulating glucose homeostasis. Our research provides new insights for the treatment of T2DM and a new theoretical basis for hypoglycemic medicines targeting Piezo1.

Methods

Collection of Human Intestine Samples

Male obese participants with type 2 diabetes (n=6, BMI=45.87±4.889 kg/m^2) and one-year post-RYGB patients (n=6, BMI=25.48±1.085 kg/m^2) were recruited in current study. Written informed consent was obtained from each donor. The study protocol was approved by the Institutional Review Board of Jinan University. Mucosal biopsies were obtained from human intestines by using a colonoscopy (CF-HQ290I; Olympus).

Genetic Mouse Generation

Villin-Flippase (Vil-Flp) mice

Vil-Flp knock-in mouse model was developed by Shanghai Model Organisms Center, Inc. The targeting construct was designed to insert a 2A-Flp-WPRE-pA coexpression cassette into the stop codon of mouse *Vil1* gene via homologous recombination using CRISPR/Cas9 system. 5'-AGCCCCTACCCTGCCTTCAA-3' was chosen as Cas9 targeted guide RNA (sgRNA). The donor vector, sgRNA and Cas9 mRNA was microinjected into C57BL/6J fertilized eggs. F0 generation mice positive for homologous recombination were identified by long PCR. The primers (I-IV) used for detection of the correct homology recombination were I: 5'-ACTTCAGGCCTAACGCTCAC-3' and II: 5'-TGTCCTGCAGGCAGAGAAAG-3' for the correct 5' homology arm recombination, and III: 5'-GTGCCGTCTCTAAGCACAGT-3'and IV:5'-TGTTGGTGCTTCGGAGTGTT-3'for the correct 3' homology arm recombination. The PCR products were further confirmed by sequencing. F0 mice were crossed with C57BL/6J mice to obtain *Vil-Flp* heterozygous mice.

Flp-dependent Glucagon-Cre (FGC) mice

FGC mouse model was developed by Shanghai Model Organisms Center, Inc. The targeting construct was designed to insert an IRES-F3-Frt-Wpre-pA-Cre-Frt-F3 expression cassette into the 3' UTR of mouse *Gcg* gene of via homologous recombination using CRISPR/Cas9 system. 5'-ATGCAAAGCAATATAGCTTC-3' was chosen as Cas9 targeted guide RNA (sgRNA). The donor vector, sgRNA and Cas9 mRNA was microinjected into C57BL/6J fertilized eggs. F0 generation mice positive for homologous recombination were identified by long PCR. The primers (I-IV) used for detection of the correct homology recombination were I: 5'-TGCTACACAGGAGGTCTGTC-3' and II: 5'-AGGCATGCTCTGCTATCACG-3' for the correct 5' homology arm recombination, and III: 5'-

CCCTCCTAGTCCCTTCTCAGT-3' and IV: 5'-GCCAAGGACATCTTCAGCGA-3' for the correct 3' homology arm recombination. The PCR products were further confirmed by sequencing. F0 mice were crossed with C57BL/6J mice to obtain *FGC* heterozygous mice.

IntL-Cre mice

Vil-Flp mice were crossed with *FGC* mice to obtain Intestinal L cell-specific Cre (*IntL-Cre*) mice.

IntL-Piezo1$^{-/-}$ mice

Piezo1$^{loxp/loxp}$mice (B6.Cg-Piezo1$^{tm2.1Apat}$/J) purchased from Jackson laboratory were crossed with IntL-Cre mice to generate *IntL-Piezo1*$^{-/-}$mice.

PCR is used to identify the genotype of mice during the subsequent mating and breeding process. The primers required for mouse genotyping are shown in the Supplementary information, Table S1.

Mouse Validation

mT/mG reporter mice were purchased from Shanghai Model Organisms Center, Inc. *IntL-Cre* mice were bred with *mT/mG* reporter mice to further validate Cre recombinase activity and specificity. Every single *IntL-Cre* mouse was confirmed by *mT/mG* reporter mice before breeding with *Piezo1*$^{loxp/loxp}$ mice to generate Intestinal L cell-Piezo1$^{-/-}$ (*IntL-Piezo1*$^{-/-}$) mice.

Frozen Tissue Confocal Imaging

IntL-Cre-mT/mG reporter mice were sacrificed. Fresh ileum and pancreas tissues were collected and embedded in O.C.T. compound for histological analysis immediately. Slices of the tissues were cut for confocal imaging, which was protected from light. Fluorescence was detected by laser scanning confocal microscopy (Li et al., 2022).

Animal Housing and Treatment

Male mice were maintained on a 12-hour light/12-hour dark cycle environment. Normal chow diet (NCD) or a high-fat diet (HFD) and water were available ad libitum unless specified otherwise. The animal protocols were approved by the Animal Care and Use Committee of Jinan University.

Male *IntL-Piezo1*$^{-/-}$mice and age-matched control littermates (*Piezo1*$^{loxp/loxp}$,

Vil-Flp, *FGC*, *IntL-Cre* mice) fed with NCD or HFD were used in the experiments.

Male *Piezo1*$^{loxp/loxp}$and *IntL-Piezo1*$^{-/-}$ mice fed with 10 week-high fat diet were intraperitoneally injected with normal saline (NS) or the GLP-1R agonist Exendin-4 (100 ug/kg body weight) for 7 consecutive days.

High fat diet induced diabetic mice were randomly divided into 3 groups. When indicated, animals were injected intraperitoneally with Vehicle, Yoda1 (2 μg per mouse) or GsMTx4 (250 μg/kg) plus Yoda1 for 7 consecutive days.

High fat diet treated *IntL-Piezo1*$^{-/-}$ mice were randomly divided into 2 groups. When indicated, animals were injected intraperitoneally with Vehicle, Yoda1 (2 μg per mouse) for 7 consecutive days.

Diet induce diabetic C57BL/6J mice were divided into sham and intestinal bead implantation groups.

Food and water intake detection

The food and water intake were quantified using metabolic cages (Cat 41853, Ugo Basile, Comerio, Italy). The mice were individually housed in these specialized cages and given a period of 3 days to acclimate before data collection began. They had unrestricted access to food and water, which was continuously monitored throughout the study. The 41850-010 software/interface package, consisting of EXPEDATA (for data analysis) and METASCREEN (for data collection) software, along with the IM-2 interface module, was employed to record and analyze the data.

Intraperitoneal Glucose Tolerance Test

Mice were fasted for 12 hours before measuring their fasting glucose levels. An intraperitoneal glucose tolerance test (IPGTT) was performed by administering 1.5 g/kg body weight of glucose. Blood glucose concentrations were measured at specified time points using a glucometer by collecting tail vein blood samples.

Insulin Tolerance Test

Mice were subjected to a 4-hour fast before measurement of fasting glucose were taken. Insulin tolerance tests (ITT) were conducted with a dose of 0.75 U/kg body weight of insulin. Blood glucose levels were measured at specified time points.

Intestinal Bead implantation

High-fat diet-induced type 2 diabetic C57BL/6J mice were fasted 6 to 8 h before the operation. Standard aseptic procedures were used throughout the operation. Intestinal bead implantation was similar to gastric bead implantation described in our previous study (Zhao et al., 2024). Briefly, a 1cm incision was made on the abdominal wall to expose the intestine. A 1 cm incision was made approximately 1cm above the ileocecal region. A 2.5 mm diameter bead was implanted into the ileum of the mouse through an incision. Then the wound was closed with suture. Finally, the abdominal wall was closed with suture. For sham operation, all the procedures were the same as the bead implantation except that the bead was not implanted.

Stretching of isolated ileum

About 2cm ileum was isolated from control and *IntL-Piezo1*$^{-/-}$ mice and kept in the specimen chamber filled with Tyrode's solution (KCl 0.2g/L, NaCl 8g/L, $CaCl_2$ 0.2g/L, $MgCl_2$ 0.1g/L, $NaHCO_3$ 1g/L, NaH_2PO_4 0.05g/L, Glucose 1g/L) of 37[gassed with oxygen. The specimen was connected to the force transducer of organ bath system (HW200S, Techman, Chengdu, CN). Adjust the transducer to apply traction force of 1.5 grams on the tissue and maintained for two hours.

Measurement of GLP-1 Secretion

The measurement of GLP-1 secretion was carried out according to previously described methods (Zhai et al., 2018). Samples were collected in the presence of aprotinin (2 μg/mL), EDTA (1 mg/mL) and diprotin A (0.1 mmol/L), and stored at −80 °C before use. GLP-1 levels were assayed using enzyme immunoassay kits following the manufacturer's instructions.

Histological Analysis

Tissues were collected, fixed with 4% paraformaldehyde, embedded in paraffin, and cut into 4μm sections. Standard protocols were followed for staining the sections with hematoxylin-eosin. Photomicrographs were captured under an inverted microscope (Leica, Germany).

Immunofluorescence

Paraffin sections were dewaxed and rehydrated. After antigen retrieval in citrate buffer (pH6.0), sections were blocked with normal serum and then incubated with rabbit anti-Piezo1 (1:400) and mouse anti-GLP-1 (1:500) antibodies at 4°C overnight. The sections were then incubated with a mixture of secondary antibodies. Images were taken by laser scanning confocal microscopy (Leica SP8). Fluorescence intensity was quantified by ImageJ software.

In situ hybridization

Paraffin sections were dewaxed and rehydrated. After antigen retrieval in in citrate buffer (pH6.0), the sections were incubated with Proteinase K (5ug/ml) at 37°C for the 15 minutes. Then the sections were hybridized with the probes overnight in a temperature-controlled chamber at 40°C. The Piezo1 probe sequences were as follows: 5'-CTGCAGGTGGTTCTGGATATAGCCC-3',5'-AAGAAGCAGATCTCCAGCCCGAAT-3', 5'-GCCATGGATAGTCAATGCACAGTGC-3'. After washing with SSC buffers, the sections were hybridized in pre-warmed branch probes at 40°C for 45 minutes. After washing with SSC buffers, the sections were hybridized with signal probe at 42°C for 3 hours. After washing with SSC buffers, the sections were blocked with normal serum and then incubated with mouse anti-GLP-1 (1:500) antibody at 4°C overnight followed by secondary antibody. Images were taken laser scanning confocal microscopy and the fluorescence signals were quantified by ImageJ.

Western Blot Analysis

Tissues and cells were harvested. Ileal mucosa was scraped for protein extraction. Protein extraction was performed by using RIPA lysis buffer (50mM Tris PH 7.4, 150mM NaCl, 1% Triton X-100, 0.1% SDS, 1% Sodium deoxycholate, 1mM PMSF and protease inhibitor cocktail.), then 40 μg of proteins were loaded onto an SDS-PAGE gel for separation. After the separation, the proteins were transferred onto a nitrocellulose membrane. The membrane was then incubated in blocking buffer at room temperature for 1 hour. For overnight incubation, the membrane and primary antibody (at the recommended dilution as stated in the product datasheet) were immersed in primary antibody dilution buffer, with gentle agitation, at 4°C. Subsequently, the membrane was incubated with a secondary antibody that specifically recognizes and binds to the primary antibody. Finally, Western blotting luminol reagent was used to visualize bands. The grey scale values of the bands were measured using ImageJ software.

RNA extraction, quantitative real-time PCR

RNA was extracted and reverse-transcribed into cDNAs using RT-PCR kit. Real-time PCR was performed as previously described (Zhai et al., 2018). Sequences for the primer pairs used in this study were shown in Supplementary information, Table S2.

Isolation of Mouse Intestinal L Cells

A 5~6cm long ileum segment was collected from the *IntL-cre-mT/mG* mouse. The tissue was washed with ice-cold PBS twice to remove the chyme in the lumen. The tissue was minced into 0.5mm^3 pieces in ice-cold PBS and then digested in 100mIU collagenase I and 0.01g/mL trypsin at 37°C for 30min with rotation. After digested tissue was passed through 40μm and 30μm cell strainers sequentially, then centrifuged for 7min at 4°C. The cell pellet was resuspended in red cell lysis buffer and incubated for 10min at room temperature. The unlysed cells were collected by centrifugation and resuspended with 1mL cold PBS. The GFP positive cells was sorted by fluorescence-activated cell sorting (FASC) on Beckman Coulter MoFlo XDP cell sorter system.

Cell Culture and Treatments

STC-1 cells were maintained in DMEM medium supplemented with 2.5% fetal bovine serum and 10% equine serum at 37 °C with 5% CO_2 air. L cells were maintained in DMEM medium supplemented with 10% fetal bovine serum.

For cell transfection, cells were plated at optimal densities and grown for 48 h. Cells were then transfected with GFP, Piezo1-GFP, CaMKKβ, CaMKIV by using lipofectamine reagent according to the manufacturer's instructions.

For stable knockdown of Piezo1 in STC-1 cells, short hairpin RNA (shRNA) sequences for mouse Piezo1 interference were cloned in to pLKO.1 vector. To produce lentivirus, psPAX2, pMD2G and pLKO.1 or pLKO.1-shPiezo1 plasmids (siPiezo1: CCAACCTTATCAGTGACTT) were co-transfected into 293T cells with lipofectamine 2000 reagent. Supernatant containing lentivirus was collected 48 hours after transfection and filtered through 0.45μm filter. The virus-containing supernatant was used to infect STC-1 cells. Forty-eight hours after infection, the STC-1 cells were subjected to 1ug/mL puromycin selection for 2-3 days.

For cell stretching, cells were grown in silicone elastic chambers coated by 0.1 % gelatin solution. After incubated at 37 °C for 24-48 hours, The chambers were subjected to mechanical stretch to 120% of their original length.

Calcium Imaging

Cells were plated onto confocal dishes at optimal densities and grown for 24 h. Cells were loaded with the calcium fluorescent probe fluo-4 AM (1 μM) for 1 h at 37 °C, then the cells were treated with Yoda1 (5 μM) or GsMTx4. The intracellular calcium ions were measured at room temperature using a laser confocal microscope with an excitation wave length of 494 nm and an emission wave length of 516 nm. The change of fluorescent signal was presented as $\Delta F/F_0$ and plotted against time.

Whole-cell Patch-Clamp recording

Borosilicate glass-made patch pipettes (BF150-86-7.5, Sutter Instrument Co, USA) were pulled with micropipette puller (P-1000, Sutter Instrument Co, USA) to a resistance of 3–5[MΩ after being filled with pipette solution: 138mM KCl, 10mM NaCl, 1mM $MgCl_2$, 10mM Glucose and 10mM HEPES (pH 7.4). Cells were bathed in Margo-Ringer solution: 130mM NaCl, 5mM KCl, 1mM $MgCl_2$, 2.5mM $CaCl_2$, 10mM Glucose, 20mM HEPES (pH7.4). Whole-cell calcium currents of STC-1 cells were recorded with the EPC10 USB patch-clamp amplifier (HEKA, Germany) controlled by PatchMaster software.

Key resource

Key reagents or resources are listed in the Supplementary information, Table S3.

Statistical Analysis

All data were expressed as mean ± S.E.M. Statistical differences were evaluated by one-way ANOVA or Student's t-test. The correlation was determined by Pearson analysis. $P < 0.05$ was considered significant. (*$p < 0.05$, **$p < 0.01$, *** $p < 0.001$, ns=not significance). In our study, the data collection and analysis processes were not conducted in a blinded manner with respect to the experimental conditions. For the administration of drugs to animals, we allocated mice of the same genetic background to various experimental cohorts using a randomization protocol. No data were excluded during the data analysis.

Results

Assessment of Piezo1 in human and mouse intestine in different energy status

Piezo1 mRNA was found to be highly expressed in both mouse ileal mucosa and STC-1 cells (Figure1-figure supplement 1A). Moreover, Piezo1 was co-localized with GLP-1 in immunofluorescent staining on NCD fed mouse ileal sections, indicating its expression in L cells (Figure1-figure supplement 1B). Interestingly, increased body weight and impaired glucose tolerance were observed in high-fat diet-induced diabetic mice, while Piezo1 and Proglucagon expression levels in the ileal mucosa of diabetic mice were significantly lower than that in mice feed with normal chow diet (Figure1-figure supplement 1C-F). Moreover, ileal mucosal *Piezo1* mRNA levels were positively correlated with *Proglucagon* mRNA levels (Figure1-figure supplement 1G), but negatively correlated with the AUC of glucose tolerance test (Figure1-figure supplement 1H). Obese T2MD patients who underwent Roux-en-Y gastric bypass (RYGB) surgery showed decreased BMI (Figure1-figure supplement 1 I) and increased *Piezo1* and GLP-1 in ileal mucosa (Figure1-figure supplement 1J and K) compared to that before surgery. These findings indicated that Piezo1 is expressed in intestinal L cells and its level varies in different energy status.

Generation and characterization of *IntL-Piezo1*$^{-/-}$ mice

To investigate the potential role of Piezo1 in GLP-1 production, we tried to knockout *Piezo1* in L cells by Cre-loxP system driven by an L cell-specific promoter. Proglucagon (encoded by *Gcg* gene) is mainly expressed in both L cells and pancreatic α cells (Jin, 2008). Villin-1 (encoded by *Vil1* gene) is expressed in gastrointestinal epithelium, including L cells, but not in pancreatic α cells (Maunoury et al., 1992; Rutlin et al., 2020). Since neither Gcg nor Villin are specific markers for L cells, we tried to generate a new line of mice enabling loss of Piezo1 expression specifically in the intestine L cell by combination of Flp-Frt and Cre-loxP system. We inserted a Flippase (Flp) expression cassette in the 3'UTR of *Vil1* to generate a *Vil1* promoter-driven Flp mice (*Vil-Flp*) (**Figure 1A**). Then, we generated Flippase-dependent Gcg promoter driven-Cre (*FGC*) mice by inserting an Frt-flanked Cre expression cassette in reverse orientation within the 3'- UTR of *Gcg* gene (**Figure 1A**). We further crossed the *Vil-Flp* mice with *FGC* mice to obtain L cell specific Cre mice (*IntL-Cre*), in which *Vil1* promoter-driven Flippase flipped the reverse Cre cassette into a correct orientation in Villin positive cells (including L cells, but not pancreatic α cells), and thus Cre can only be expressed under the *Gcg* promoter in L cells. The genotypes of the *Vil-Flp*, *FGC* and *IntL-Cre* mice were identified by PCR with specific primers (**Figure 1B**). The flipping of the reverse Cre cassette was validated by PCR, which confirmed that the flipping only occurred the intestine, but not in the pancreas (**Figure 1C**). To confirm the cell type specificity of Cre activity, we crossed *IntL-Cre* mice to *mT/mG* reporter mice. All tissues and cells of *mT/mG* mice express red fluorescence (membrane-targeted tdTomato; mT) at baseline, and switch to membrane-targeted EGFP in the presence of cell-specific Cre (**Figure 1D**). EGFP expression was only observed scatteredly in the intestine, but not in the pancreas, indicating the intestine-specific Cre activity in the *IntL-Cre* mice (**Figure 1E**). Finally, we bred *IntL-Cre* mice with *Piezo1*$^{loxp/loxp}$ mice to generate *IntL-Piezo1*$^{-/-}$ mice (**Figure 1F**).

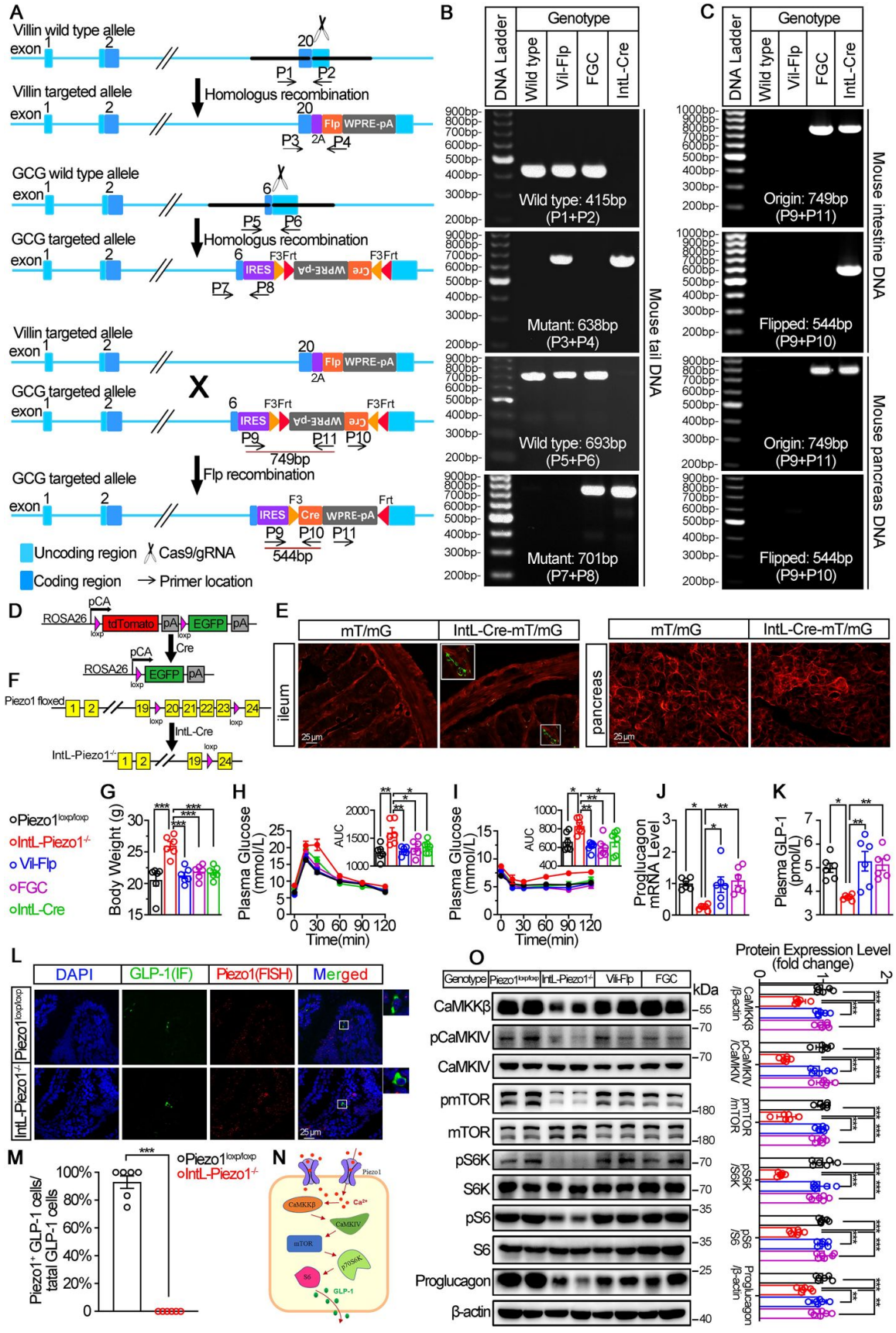

A
Villin wild type allele
exon 1 2 20
P1 P2
Villin targeted allele
Homologus recombination
Flp WPRE-pA
P3 2A P4
GCG wild type allele
6
P5 P6
GCG targeted allele
Homologus recombination
F3Frt IRES Cre WPRE-pA F3Frt
P7 P8
Villin targeted allele
GCG targeted allele
749bp
Flp recombination
F3 Cre Frt
P9 P10 P11
544bp
Uncoding region
Cas9/gRNA
Coding region
Primer location
B
DNA Ladder
Genotype
Wild type
Vil-Flp
FGC
IntL-Cre
Wild type: 415bp (P1+P2)
Mutant: 638bp (P3+P4)
Wild type: 693bp (P5+P6)
Mutant: 701bp (P7+P8)
Mouse tail DNA
C
Origin: 749bp (P9+P11)
Flipped: 544bp (P9+P10)
Origin: 749bp (P9+P11)
Flipped: 544bp (P9+P10)
Mouse intestine DNA
Mouse pancreas DNA
D
ROSA26
pCA
tdTomato
EGFP
pA
loxp
Cre
E
mT/mG
IntL-Cre-mT/mG
ileum
pancreas
25μm
F
Piezo1 floxed
IntL-Piezo1-/-
IntL-Cre
G
Piezo1loxp/loxp
IntL-Piezo1-/-
Vil-Flp
FGC
IntL-Cre
Body Weight (g)
H
Plasma Glucose (mmol/L)
AUC
Time(min)
I
Plasma Glucose (mmol/L)
AUC
Time(min)
J
Proglucagon mRNA Level
K
Plasma GLP-1 (pmol/L)
L
DAPI
GLP-1(IF)
Piezo1(FISH)
Merged
Piezo1loxp/loxp
IntL-Piezo1-/-
25μm
M
Piezo1+ GLP-1 cells/ tatal GLP-1 cells
Piezo1loxp/loxp
IntL-Piezo1-/-
N
Piezo1
Ca2+
CaMKKβ
CaMKIV
mTOR
pS6K
S6
GLP-1
O
Genotype
Piezo1loxp/loxp
IntL-Piezo1-/-
Vil-Flp
FGC
kDa
CaMKKβ
pCaMKIV
CaMKIV
pmTOR
mTOR
pS6K
S6K
pS6
S6
Proglucagon
β-actin
Protein Expression Level (fold change)
CaMKKβ /β-actin
pCaMKIV /CaMKIV
pmTOR /mTOR
pS6K /S6K
pS6 /S6
Proglucagon /β-actin

Figure 1

Generation, Validation and Characterization of *IntL-Piezo1*$^{-/-}$ mice

(A) Schematic description for the generation of Villin-Flippase (*Vil-Flp*) and Flippase-dependent Gcg-Cre (*FGC*) mice. Vil-Flp flip the inverted Cre gene in the Gcg-Cre cassette in *IntL-Cre* mice to restrict Cre expression in intestinal L cells. As shown, Locations of genotyping primers are also indicated.

(B) Tail DNA genotyping PCR results using genotyping primer for *Vil-Flp*, *FGC* and Flippase-activated Cre (*IntL-Cre*) mice.

(C) Intestine and pancreas DNA genotyping results. The "Original" band represents the original FGC cassette with inverted Cre, while the "Flipped" band represents recombined FGC cassette with Cre flipped into the correct direction.

(D) Schematic description for the validation of *IntL-Cre* efficacy by crossing with *mT/mG* reporter mice.

(E) Fluorescence was detected in the ileal and pancreatic tissues from *mT/mG* and *IntL-Cre-mT/mG* mice by frozen tissue confocal microscopy. Green fluorescence represents successful deletion of TdTomato and reactivation of EGFP in the Cre-expressing cells.

(F) Schematic description for the generation of Intestinal L cell-Piezo1$^{-/-}$ mice (*IntL-Piezo1*$^{-/-}$) by crossing *Piezo1*$^{loxp/loxp}$ mice with *IntL-Cre* mice.

(G) Body weight of 14- to 16-week-old male mice of the indicated genotypes fed NCD (n=6/group)

(**H-I**) IPGTT (**H**) and ITT (**I**) and associated area under the curve (AUC) values of 14- to 16-week-old male mice of the indicated genotypes fed NCD (n=6/group).

(J) *Proglucagon* mRNA levels in ileum of 14- to 16-week-old male mice of the indicated genotypes fed NCD. (n=6/group).

(K) The plasma GLP-1 levels in 14- to 16-week-old male mice of the indicated genotypes fed NCD (n=6/group).

(L) Representative images for Piezo1 RNA-FISH and GLP-1 immunofluorescent staining in the ileum of 14-week-old male mice of indicated genotypes fed NCD (n=6/group).

(M) Percentage of Piezo1-positive GLP-1 cells in total GLP-1 cells in the ileal mucosa of 14-week-old male mice of indicated genotypes fed NCD (n=6/group).

(N) A schematic diagram depicting the potential mechanisms linking the CaMKKβ/CaMKIV-mTOR signaling pathway and GLP-1 production.

(O) Representative western blots are shown for indicated antibodies in the ileal mucosa (n = 6/group).

Data are represented as mean ± SEM. Significance was determined by Student's t test for comparison between two groups, and by one-way ANOVA for comparison among three groups or more, *p < 0.05, **p < 0.01, ***p < 0.001.

Under normal chow diet, *IntL-Piezo1*$^{-/-}$ mice exhibited increased body weight (**Figure 1G**) and greater glycemic excursions compared to control groups (*Piezo1*$^{loxp/loxp}$, *Vil-Flp*, *FGC* and *IntL-cre*) (**Figure 1H and I**), while the food and water intake were not changed (**Figure 1**-figure

supplement 2A and B). The morphology of islet (**Figure 1**-figure supplement 3A) and ileum (Figure 1-figure supplement 4A) were not affected. Ileal mucosal Proglucagon expression and plasma GLP-1 level were significantly lower in *IntL-Piezo1*$^{-/-}$ mice than that in all littermate controls such as *Piezo1*$^{loxp/loxp}$, *Vil-Flp*, *FGC* and *IntL-Cre* mice (**Figure 1J and K**), while no significant alteration was observed in the expression of pancreatic Piezo1 and Proglucagon (Figure 1-figure supplement 3B-D). According to in situ hybridization of Piezo1 and GLP-1, the expression of Piezo1 disappeared in GLP-1 positive cells, suggesting successful knockout of Piezo1 in L cells in *IntL-Piezo1*$^{-/-}$mice (**Figure 1L and M**). Also depicted in Figure 1-figure supplement 5, Piezo1 is expressed in GLP-1-positive cells of the duodenum, jejunum, ileum, and colon in control mice, but not in *IntL-Piezo1*$^{-/-}$ mice. However, Piezo1 remains expressed in intestinal ghrelin positive cells and pancreatic glucagon positive cells of *IntL-Piezo1*$^{-/-}$ mice (Figure 1-figure supplement 6). Moreover, while GLP-1 levels were reduced in L cells of *IntL-Piezo1*$^{-/-}$ mice, levels of PYY, another hormone secreted by L cells, were unaffected (Figure 1-figure supplement 7A-D). Additionally, ileal mucosal cholecystokinin (CCK), a hormone secreted by I cells with metabolic effects similar to GLP-1, was also unchanged in *IntL-Piezo1*$^{-/-}$ mice (Figure 1-figure supplement 7E). Previous study showed that Piezo1 affected intestinal tight junctions and epithelial integrity (Jiang et al., 2021). To access whether loss of Piezo1 in L cells affect epithelial integrity of the intestine, we examined the expression of tight junction proteins, including ZO-1 and Occludin. As shown in Figure 1-figure supplement 8, the expression of ZO-1 and Occludin remained unchanged in *IntL-Piezo1*$^{-/-}$mice when compared to littermate controls.

Piezo1 is a non-selective cationic channel that allows passage of Ca^{2+} and Na^{+}. CaMKKβ is the main calcium/calmodulin dependent protein kinase kinase involved in the regulation of metabolic homeostasis (Marcelo et al., 2016). It is activated by binding calcium-calmodulin (Ca^{2+}/CaM), resulting in downstream activation of kinases CaMKIV. The activation of CaMKIV modulate the gene expression of nutrient- and hormone-related proteins (Ban et al., 2000; Chen et al., 2011; Takemoto-Kimura et al., 2017). Previous studies have reported that Ca^{2+} and mTOR signaling regulate the production of GLP-1 (Tolhurst et al., 2011; Xu et al., 2015; Yu & Jin, 2010). Drawing from these findings, we hypothesized that Piezo1 might regulate GLP-1 synthesis through the CaMKKβ/CaMKIV-mTOR signaling pathway (**Figure 1N**). As shown in **Figure 1O**, abrogated GLP-1 production was associated with decreased CaMKKβ/CaMKIV-mTOR signaling in the ileal mucosa of *IntL-Piezo1*$^{-/-}$mice (**Figure 1O**).

Derangements of glucose metabolism and GLP-1 production were induced by HFD in *IntL-Piezo1*$^{-/-}$ mice, which was mitigated by Exendin-4

We next assessed the effect of L cell-specific *Piezo1* gene deletion on GLP-1 and glucose tolerance in diet-induced diabetic mice. *IntL-Piezo1*$^{-/-}$ and control mice were exposed to HFD for 10 weeks. Compared to the controls, higher body weight (**Figure 2A**), greater glucose excursions (**Figure 2B**) were observed in *IntL-Piezo1*$^{-/-}$ mice exposed to HFD. Ileal mucosal Proglucagon expression levels were lower in *IntL-Piezo1*$^{-/-}$ than control mice (**Figure 2C-F**). Impaired CaMKKβ/CaMKIV-mTORC1 signaling pathway in ileal mucosa as evidenced by a decrease in CaMKKβ, reduced phosphorylation levels of CaMKIV, mTOR, S6K, and S6 was also observed in *IntL-Piezo1*$^{-/-}$mice (**Figure 2F**). No significant alteration in morphology, Piezo1 or Proglucagon levels were observed in the pancreas of *IntL-Piezo1*$^{-/-}$mice (Figure 2-figure supplement 3E-H). Together these data demonstrate that *IntL-Piezo1*$^{-/-}$ mice with prolonged HFD feeding exhibit impaired glucose metabolism phenotype and reduced GLP-1. Injection of GLP-1 analog Exendin-4 (Ex-4) decreased the body weight (**Figure 2G**) and improved both glucose tolerance (**Figure 2H**) and insulin resistance (**Figure 2I**) in control and *IntL-Piezo1*$^{-/-}$ mice, while endogenous synthesis of GLP-1 was not changed by Ex-4 injection in *IntL-Piezo1*$^{-/-}$ mice (**Figure 2J and K**). These data suggested that decreased GLP-1 synthesis and secretion contribute to impaired glucose metabolism in *IntL-Piezo1*$^{-/-}$ mice.

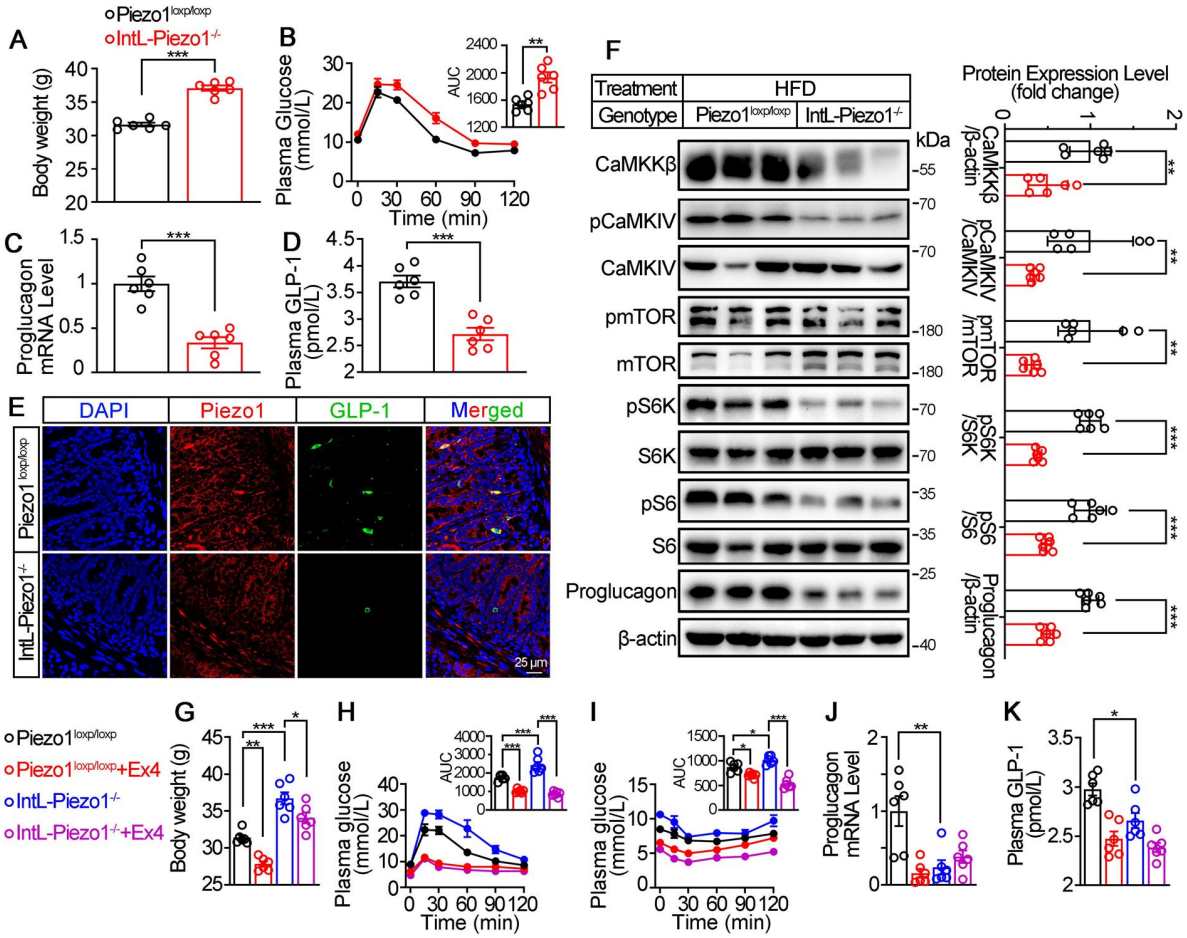


Figure 2

Validation and phenotype of *IntL-Piezo1*$^{-/-}$ mice fed with high-fat diet

(A) Body weight of 14- to 16-week-old male *Piezo1*$^{loxp/loxp}$ and *IntL-Piezo1*$^{-/-}$ mice fed with HFD for 10 weeks (n=6/group).
(B) IPGTT and associated area under the curve (AUC) values of 14- to 16-week-old male *Piezo1*$^{loxp/loxp}$ and *IntL-Piezo1*$^{-/-}$ mice fed with HFD (n=6/group).
(C) *Proglucagon* mRNA levels in the ileal mucosa of 14- to 16-week-old male *Piezo1*$^{loxp/loxp}$ and *IntL-Piezo1*$^{-/-}$ mice fed with HFD (n=6/group).
(D) The plasma GLP-1 level in 14- to 16-week-old male *Piezo1*$^{loxp/loxp}$ and *IntL-Piezo1*$^{-/-}$ mice fed with HFD (n=6/group).
(E) Double immunofluorescent staining of Piezo1, and GLP-1 in the ilea of 14- to 16-week-old male *Piezo1*$^{loxp/loxp}$ and *IntL-Piezo1*$^{-/-}$ mice fed HFD (n=6/group).
(F) Representative western blots are shown for indicated antibodies in the ileal mucosa (n=6/group).
(G) Body weight after 7 consecutive days infusion of saline or Ex-4 (100ug/kg body weight) in 14- to 16-week-old male *Piezo1*$^{loxp/loxp}$and *IntL-Piezo1*$^{-/-}$ mice fed with HFD (n=6/group).
(**H**-**I**) IPGTT (**H**) and ITT (**I**) and associated area under the curve (AUC) values after consecutive infusion of saline or Ex-4.
(J) *Proglucagon* mRNA levels in the ileal mucosa (n=6/group) after consecutive infusion of saline or Ex-4.
(K) The plasma GLP-1 level after consecutive infusion of saline or Ex-4 (n=6/group). Data are represented as mean ± SEM. Significance was determined by Student's t test for comparison between two groups, and by one-way ANOVA for comparison among three groups or more, *p < 0.05, **p < 0.01, ***p < 0.001.

The pharmacological and mechanical activation of ileal Piezo1 stimulates GLP-1 synthesis

We next examined whether activation of Piezo1 could rescued the impaired glucose metabolism in diet-induced diabetic mice. Injection of Piezo1 activator Yoda1 after 10 weeks of high-fat diet, led to reduced body weight and improved the impaired glucose metabolism significantly in diabetic mice, while Piezo1 antagonist GsMTx4 reversed the weight loss and glucose-lowering effect of Yoda1 (**Figure 3A and B**). Yoda1 remarkably induced an increase in GLP-1 synthesis and secretion (**Figure 3C and D**), as well as an increment of CaMKKβ/CaMKIV-mTORC1 signaling in ileal mucosa (**Figure 3E**), while GsMTx4 abolished the effect of Yoda1 (**Figure 3C-E**). However, weight loss, improved plasma glucose and increased GLP-1 production induced by Yoda1 were not observed in *IntL-Piezo1*$^{-/-}$ mice (**Figure 3F-J**).

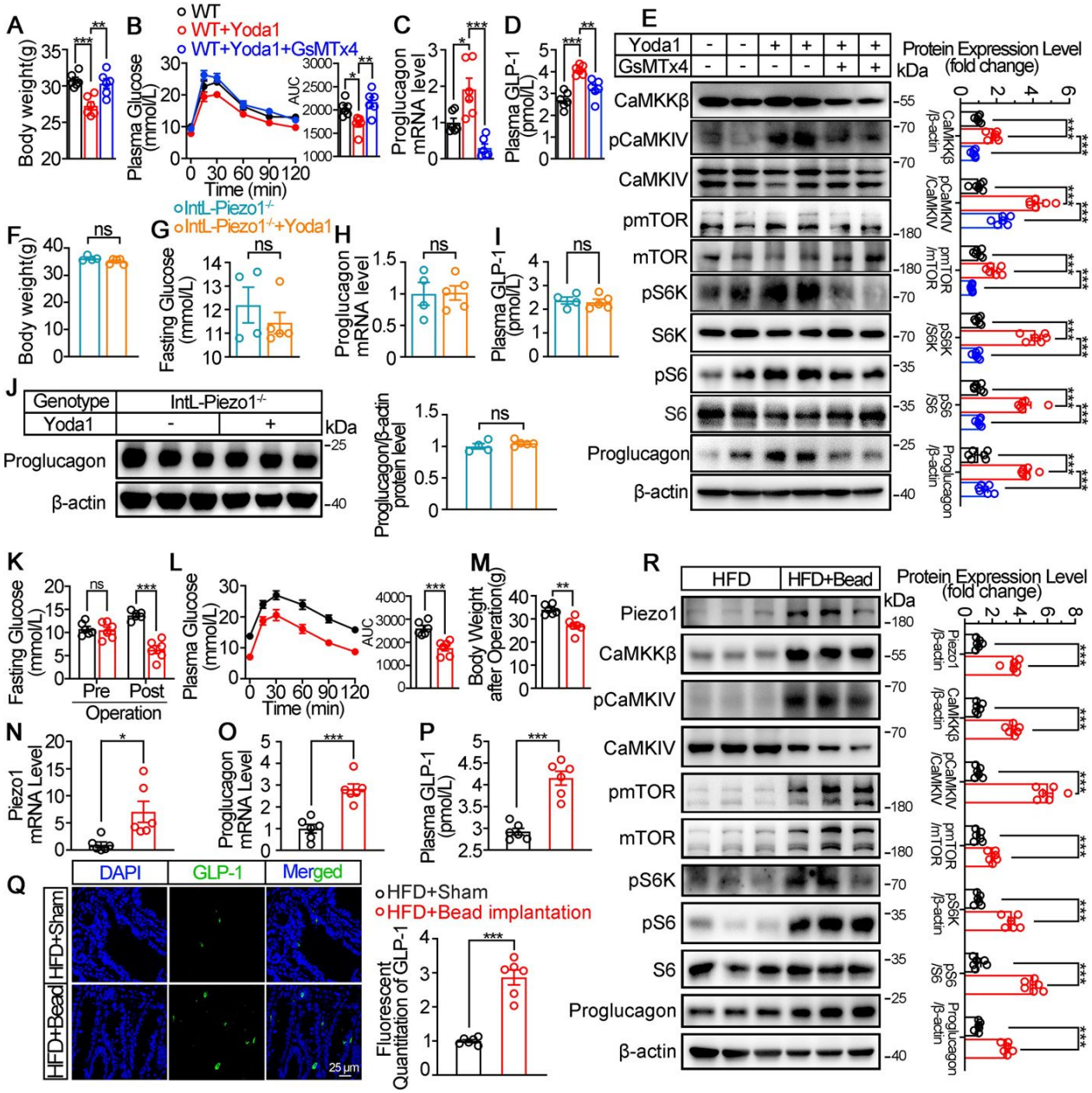

A
Body weight(g)
B
WT
WT+Yoda1
WT+Yoda1+GsMTx4
Plasma Glucose (mmol/L)
Time (min)
AUC
C
Proglucagon mRNA level
D
Plasma GLP-1 (pmol/L)
E
Yoda1
GsMTx4
kDa
CaMKKβ
pCaMKIV
CaMKIV
pmTOR
mTOR
pS6K
S6K
pS6
S6
Proglucagon
β-actin
Protein Expression Level (fold change)
F
Body weight(g)
G
IntL-Piezo1-/-
IntL-Piezo1-/-+Yoda1
Fasting Glucose (mmol/L)
H
Proglucagon mRNA level
I
Plasma GLP-1 (pmol/L)
J
Genotype
IntL-Piezo1-/-
Yoda1
Proglucagon
β-actin
Proglucagon/β-actin protein level
K
Fasting Glucose (mmol/L)
Pre
Post
Operation
L
Plasma Glucose (mmol/L)
Time (min)
AUC
M
Body Weight after Operation(g)
N
Piezo1 mRNA Level
O
Proglucagon mRNA Level
P
Plasma GLP-1 (pmol/L)
Q
DAPI
GLP-1
Merged
HFD+Sham
HFD+Bead
25 μm
HFD+Sham
HFD+Bead implantation
Fluorescent Quantitation of GLP-1
R
HFD
HFD+Bead
Piezo1
CaMKKβ
pCaMKIV
CaMKIV
pmTOR
mTOR
pS6K
pS6
S6
Proglucagon
β-actin
Protein Expression Level (fold change)

Figure 3

Chemical and mechanical interventions of Piezo1 regulates GLP-1 synthesis in mice

(**A**-**E**) 14- to 16-week-old male C57BL/6J mice fed with HFD for 10 weeks were infused with vehicle, Yoda1 (2 μg per mouse) or GsMTx4 (250 μg/kg) by i.p. for 7 consecutive days. (n=6/group).

(A) Body weight after consecutive drug infusion.

(B) IPGTT and associated area under the curve (AUC) values.

(C) *Proglucagon* mRNA levels in the ileal mucosa.

(D) Plasma GLP-1.

(E) Representative western blots are shown for indicated antibodies in the ileal mucosa

(**F**-**J**) 14- to 16-week-old male *IntL-Piezo1*$^{-/-}$ mice fed with HFD for 10 weeks were infused with vehicle, Yoda1 (2 μg per mouse) by i.p. for 7 consecutive days. (n=4 or 5/group)

(F) Body weight after 7 consecutive days' drug infusion.

(G) Fasting blood glucose levels.

(H) Ileal mucosal *Proglucagon* mRNA levels.

(I) Plasma GLP-1 levels.

(J) Ileal mucosal Proglucagon protein levels.

(**K-R**) 14- to 16-week-old male C57BL/6J mice fed with HFD were subjected to sham operation, or intestinal bead implantation (n=6/group).

(K) Fasting blood glucose levels.

(L) IPGTT and associated area under the curve (AUC) values.

(M) Body weight.

(**N** and **O**) *Piezo1* (**N**) and *Proglucagon* (**O**) mRNA levels in the ileal mucosa.

(P) The plasma GLP-1 levels.

(Q) Immunofluorescence staining of GLP-1 in ileum and quantification of GLP-1 positive cells.

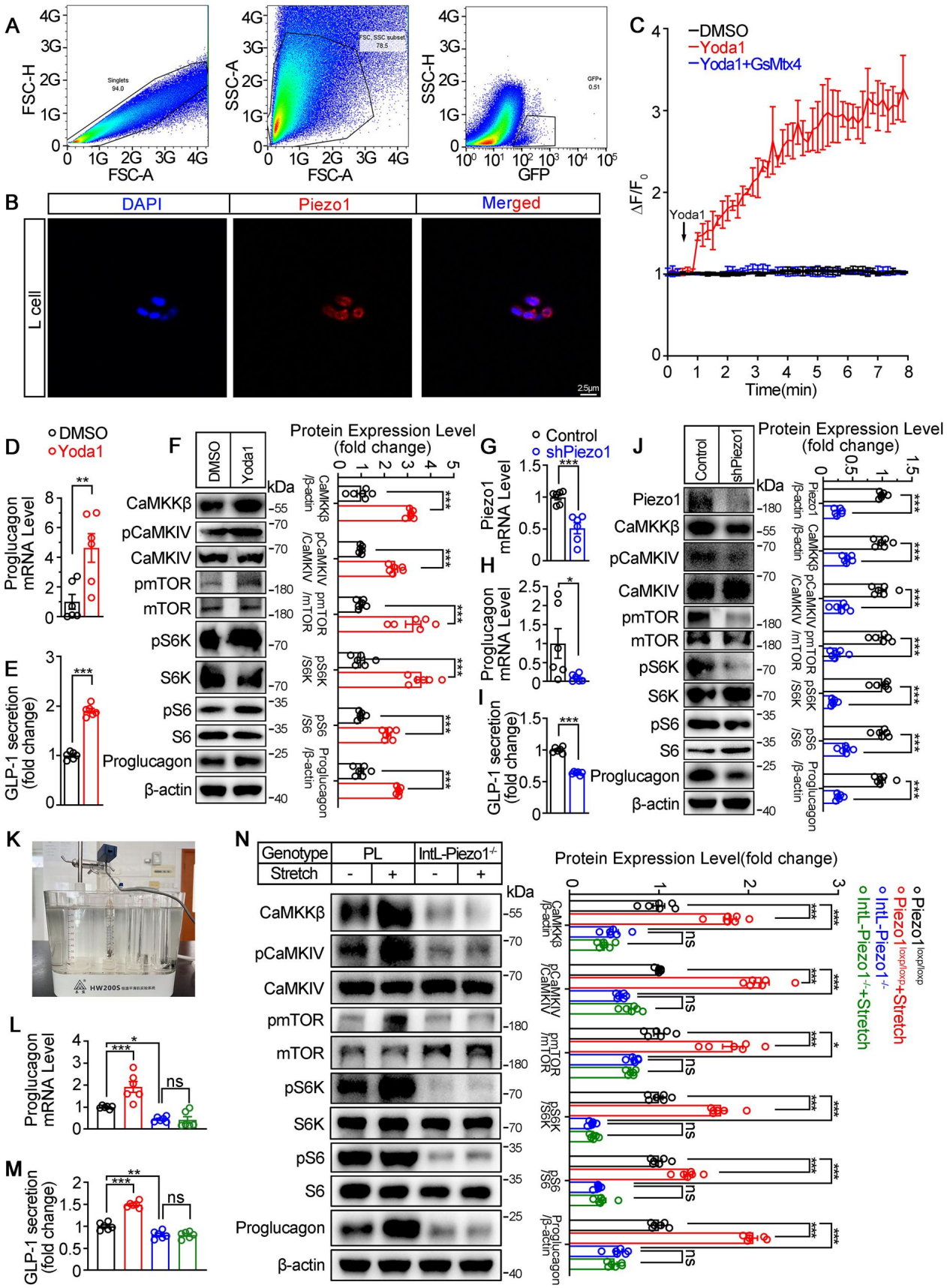
(R) Representative western blots images and densitometry quantification for indicated antibodies in the ileal mucosa.

Data are represented as mean ± SEM. Significance was determined by Student's t test for comparison between two groups, and by one-way ANOVA for comparison among three groups or more, *p < 0.05, **p < 0.01, ***p < 0.001.

The intestine receives mechanical stimulation from the chyme, which may activate Piezo1 in the intestine epithelium, including L cells. To mimic the mechanical pressing and stretching induced by intestinal contents, a small silicon bead was implanted into the high-fat diet-induced diabetic mouse ileum. To exclude the possibility of bowel obstruction and abdominal pain caused by bead implantation, we measured the fecal mass and gastrointestinal transit time, and accessed abdominal mechanical sensitivity in both sham and bead-implanted mice. As shown in Figure3-figure supplement 9A-B, there was no significant difference in fecal mass and gastrointestinal transit time between the sham-operated mice and those implanted with beads. The results of abdominal mechanical sensitivity indicated that no difference in abdominal pain threshold was observed between sham and bead implanted mice (Figure3-figure supplement 9C). Intestinal bead implantation improved the impaired glucose metabolism in diabetic mice (**Figure 3K and L**). Body weight loss, activated ileal mucosal CaMKKβ/CaMKIV-mTOR signaling, increased mRNA and protein levels of ileal mucosal Piezo1 and Proglucagon, as well as the circulating levels of GLP-1 were observed in diabetic mice after operation (**Figure 3M-R**). The above data suggest that mechanical stimuli induced by intestinal bead implantation activates ileal Piezo1 in diabetic mice, stimulating GLP-1 production via CaMKKβ/CaMKIV-mTOR signaling axis, thus improving glucose homeostasis.

Piezo1 regulates GLP-1 synthesis and secretion in primary cultured mouse L cells and isolated mouse ileum

To obtain primary L cells, we isolated cell from the ileum of *IntL-Cre-mT/mG* mice, in which tdTomato expression switched to EGFP expression in L cells as shown in **Figure 1E**. EGFP positive cells (mouse L cells) were then sorted from isolated single cells (**Figure 4A**). Immunofluorescence showed that the sorted EGFP+ cells were Piezo1 positive (**Figure 4B**).


A
FSC-H
FSC-A
SSC-A
SSC-H
GFP
Singlets
94.0
FSC, SSC subset
78.5
GFP+
0.51
B
DAPI
Piezo1
Merged
L cell
2.5μm
C
DMSO
Yoda1
Yoda1+GsMtx4
ΔF/F0
Yoda1
Time(min)
D
DMSO
Yoda1
Proglucagon mRNA Level
E
GLP-1 secretion (fold change)
F
DMSO
Yoda1
kDa
CaMKKβ
pCaMKIV
CaMKIV
pmTOR
mTOR
pS6K
S6K
pS6
S6
Proglucagon
β-actin
Protein Expression Level (fold change)
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Piezo1 mRNA Level
H
Proglucagon mRNA Level
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K
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M
GLP-1 secretion (fold change)
N
Genotype
PL
IntL-Piezo1-/-
Stretch
Protein Expression Level(fold change)
Piezo1loxp/loxp
Piezo1loxp/loxp+Stretch
IntL-Piezo1-/-
IntL-Piezo1-/-+Stretch

Figure 4

Piezo1 regulates GLP-1 synthesis and secretion in primary cultured mouse L cells and isolated mouse ileum.

(A) Isolation of mouse L cells (GFP positive) from ileal tissue by FACS. The gating in flowcytometry for sorting of GFP positive cells.

(B) Immunofluorescent staining of Piezo1 in sorted GFP positive L cells.

(C) Intracellular Ca^{2+} imaging by fluo-4-AM calcium probe. The change of fluorescent intensity (ΔF/F0) was plotted against time

(**D**-**F**) L cells were treated with vehicle or Yoda1 (5μM) for 24 hours.

(D) *Proglucagon* mRNA expression.

(E) GLP-1 concentrations in the culture medium.

(F) Western blot images and densitometry quantification for the indicated antibodies. (**G**-**J**) Knockdown of Piezo1 in L cells by shRNA for 48 hours.

(G) *Piezo1* mRNA expression.

(H) *Proglucagon* mRNA expression.

(I) GLP-1 levels in the culture medium.

(J) Western blot images and densitometry quantification for the indicated antibodies. (**K**-**N**) Ileal tissues from *Piezo1*$^{loxp/loxp}$ and *IntL-Piezo1*$^{-/-}$ mice were subjected to tension force (n=6/group).

(K) A representative photograph showing the traction of isolated ileum.

(L) *Proglucagon* mRNA levels.

(M) GLP-1 concentrations in the medium.

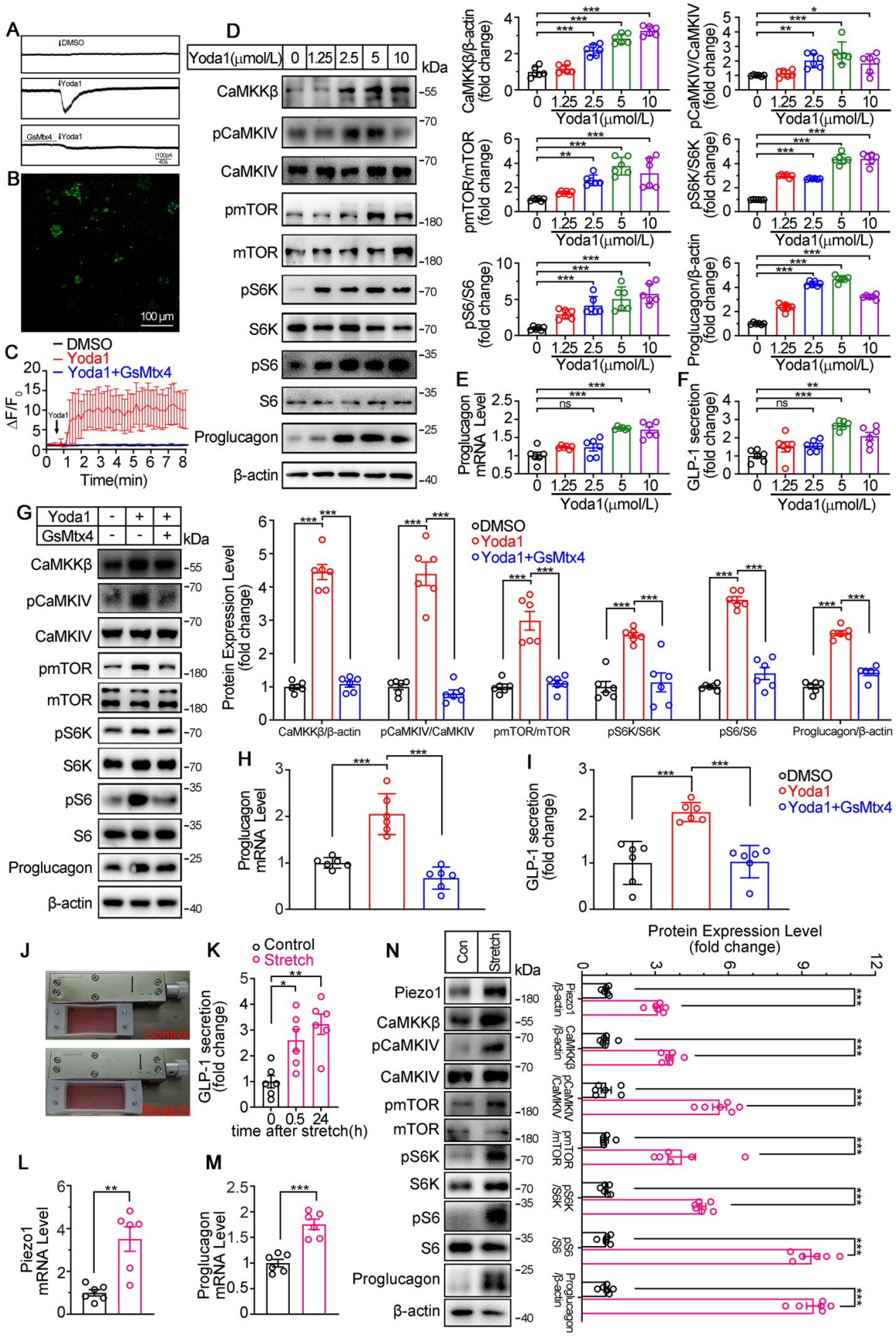
(N) Western blot images and densitometry quantification for the indicated antibodies. Data are represented as mean ± SEM and are representative of six biological replicates. Significance was determined by Student's t test for comparison between two groups, and by one-way ANOVA for comparison among three groups or more, *p < 0.05, **p < 0.01, ***p < 0.001.

Yoda1 at the dose of 5μM triggered an increase in intracellular Ca^{2+} level in primary cultured mouse L cells, which was blocked by pre-incubation of cells with GsMTx4 (0.1 μM) for 15 min (**Figure 4C**). Yoda1 also stimulated Proglucagon expression and GLP-1 secretion, as well as CaMKKβ/CaMKIV-mTOR signaling pathway in primary cultured mouse L cells (**Figure 4D-F**). In contrast, knockdown of Piezo1 by shRNA led to significant decrease in Proglucagon expression and GLP-1 secretion, as well as inhibition of CaMKKβ/CaMKIV/mTOR signaling pathway (**Figure 4G-J**).

Given the ability of Piezo1 in sensing mechanical force, tension of 1.5g was applied to the isolated mouse ileum bathed in Tyrode's solution for two hours. Tension stimulated Proglucagon expression, GLP-1 secretion and activated CaMKKβ/CaMKIV-mTOR signaling pathway in the ileum of control mice, but not in *IntL-Piezo1*$^{-/-}$ mice (**Figure 4K-N**), suggesting the involvement of Piezo1 of the L cells in mediating the force-induced GLP-1 production and CaMKKβ/CaMKIV-mTOR signaling.

Pharmacological, mechanical and genetic activation of Piezo1 stimulates GLP-1 synthesis and secretion in STC-1 cells

To further validate the role of Piezo1 in regulating GLP-1, we examined the effect of manipulating Piezo1 on GLP-1 production in an intestinal neuroendocrine cell line STC-1. Pharmacological activation of Piezo1 by Yoda1 triggered an inward current in STC-1 cell recorded by whole cell patch-clamp, which could be inhibited by pre-incubation of GsMTx4 (**Figure 5A**). Yoda1 also triggered an increase in intracellular Ca^{2+} level in STC-1 cells. Pre-incubation of cells with GsMTx4 (0.1 μM) for 15 min inhibited $[Ca^{2+}]_i$ increase (**Figure 5B and C**). Yoda1 induced a concentration-dependent activation of CaMKKβ/CaMKIV-mTOR pathway and GLP-1 synthesis and secretion (**Figure 5D-F**). GsMTx4 blocked the effect of Yoda1 on STC-1 cells in both GLP-1 and CaMKKβ/CaMKIV-mTOR activation (**Figure 5G-I**).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GsMtx4
Yoda1
100pA
40s
B
100 μm
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Yoda1
Yoda1+GsMtx4
ΔF/F0
Yoda1
Time(min)
D
Yoda1(μmol/L) 0 1.25 2.5 5 10
kDa
CaMKKβ
pCaMKIV
CaMKIV
pmTOR
mTOR
pS6K
S6K
pS6
S6
Proglucagon
β-actin
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pCaMKIV/CaMKIV (fold change)
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pS6K/S6K (fold change)
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F
GLP-1 secretion (fold change)
G
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GsMtx4
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Yoda1+GsMtx4
CaMKKβ/β-actin
pCaMKIV/CaMKIV
pmTOR/mTOR
pS6K/S6K
pS6/S6
Proglucagon/β-actin
H
Proglucagon mRNA Level
I
GLP-1 secretion (fold change)
DMSO
Yoda1
Yoda1+GsMtx4
J
Control
Stretch
K
Control
Stretch
GLP-1 secretion (fold change)
time after stretch(h)
L
Piezo1 mRNA Level
M
Proglucagon mRNA Level
N
Con
Stretch
kDa
Piezo1
CaMKKβ
pCaMKIV
CaMKIV
pmTOR
mTOR
pS6K
S6K
pS6
S6
Proglucagon
β-actin
Protein Expression Level (fold change)
Piezo1/β-actin
CaMKKβ/β-actin
pCaMKIV/CaMKIV
pmTOR/mTOR
pS6K/S6K
pS6/S6
Proglucagon/β-actin

Figure 5

Modulation of GLP-1 synthesis and secretion by pharmacological and mechanical activation of Piezo1 in STC-1 cells

(A) Whole-cell currents induced by Yoda1 (5μM) were recorded from STC-1 cells or STC-1 cells pretreated with GsMTx4 for 30 min

(**B** and **C**) Intracellular calcium imaging in STC-1 cells. (**B**) STC-1 cells were loaded with fluo-4 AM for 1 h. The representative time-lapse image showing the intracellular Ca^{2+} signals. (**C**) The change of fluorescent intensity (ΔF/F0) was plotted against time.

(**D-F**) STC-1 cells were treated with various concentrations of Yoda1 for 24 h. (**D**) Whole-cell extracts underwent western blot with indicated antibodies. (**E**) *Proglucagon* mRNA levels. (**F**) GLP-1 concentrations in the culture medium.

(**G-I**) STC-1 cells were treated with Yoda1 (5 μM) in the presence or absence of GsMTx4 (0.1 μM) for 24 h. (**G**) Whole-cell extracts underwent western blot with indicated antibodies. (**H**) *Proglucagon* mRNA levels. (**I**) GLP-1 concentrations in the culture medium

(**J-N**) STC-1 were subjected to mechanical stretch. (**J**) STC-1 cells were cultured in elastic chambers and the chambers were subjected to mechanical stretch by 120% extension of their original length. (**K**) The medium GLP-1 concentrations were detected at indicated time. (**L**) *Piezo1* mRNA levels. (**M**) *Proglucagon* mRNA levels.

(**N**) Whole-cell extracts underwent western blot with indicated antibodies.

Data are represented as mean ± SEM and are representative of six biological replicates. Significance was determined by Student's t test for comparison between two groups, and by one-way ANOVA for comparison among three groups or more, *p < 0.05, **p < 0.01, ***p < 0.001.

To mimic the activation of Piezo1 by mechanical stretching in vivo, STC-1 cells grown on elastic chambers were subjected to mechanical stretch to 120% of their original length. Mechanical stretch upregulated Piezo1 and Proglucagon expression, promoted GLP-1 secretion (**Figure 5J-N**), and activated CaMKKβ/CaMKIV- mTOR signaling pathways (**Figure 5N**).

Consistent to the pharmacological and mechanical activation of Piezo1, over-expression of Piezo1 in STC-1 cells resulted in a significant increase in GLP-1 production, as well as activation of the CaMKKβ/CaMKIV-mTOR signaling pathway (**Figure 6A-D**). Conversely, knockdown of Piezo1 by shRNA led to a significant decrease in GLP-1 production and inhibition of CaMKKβ/CaMKIV-mTOR signaling pathways (**Figure 6E-H**).

Piezo1 regulates GLP-1 production through CaMKKβ/CaMKIV and mTOR in STC-1 cells

Next, we examined whether CaMKKβ/CaMKIV and mTOR signaling mediates the effects of Piezo1 on GLP-1 production. Overexpression of CaMKKβ or CaMKIV increased CaMKKβ/CaMKIV and mTOR signaling activity, resulting in increased synthesis and secretion of GLP-1 (**Figure 7A-C**). In contrast, the CaMKKβ inhibitor STO-609, downregulated CaMKKβ/CaMKIV and mTOR signaling, as well as GLP-1 synthesis and secretion (**Figure 7D-F**). Inhibition of mTORC1 activity by rapamycin suppressed GLP-1 production induced by Yoda1, which was associated with inhibition of mTOR signaling (**Figure 7G-I**).

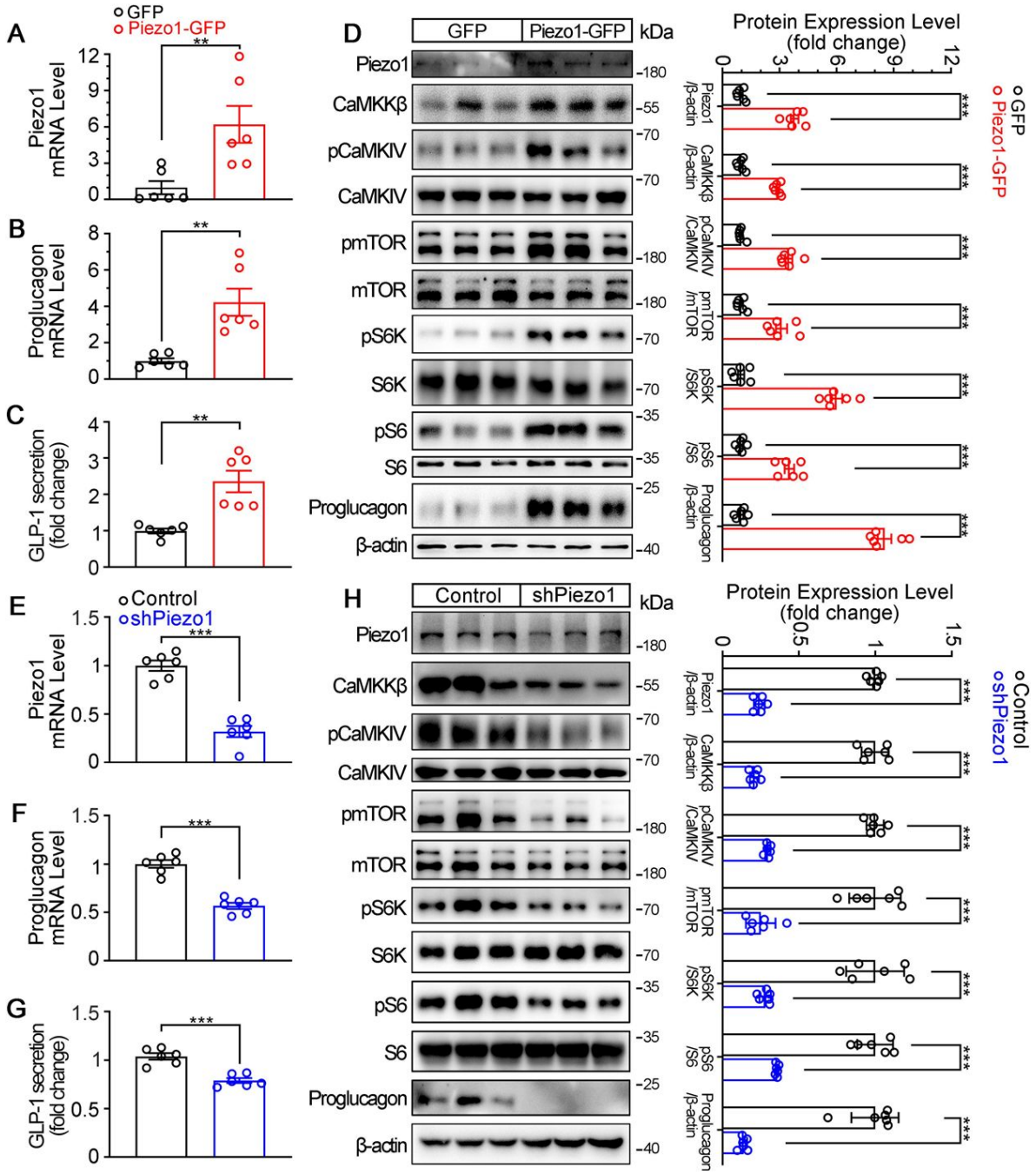


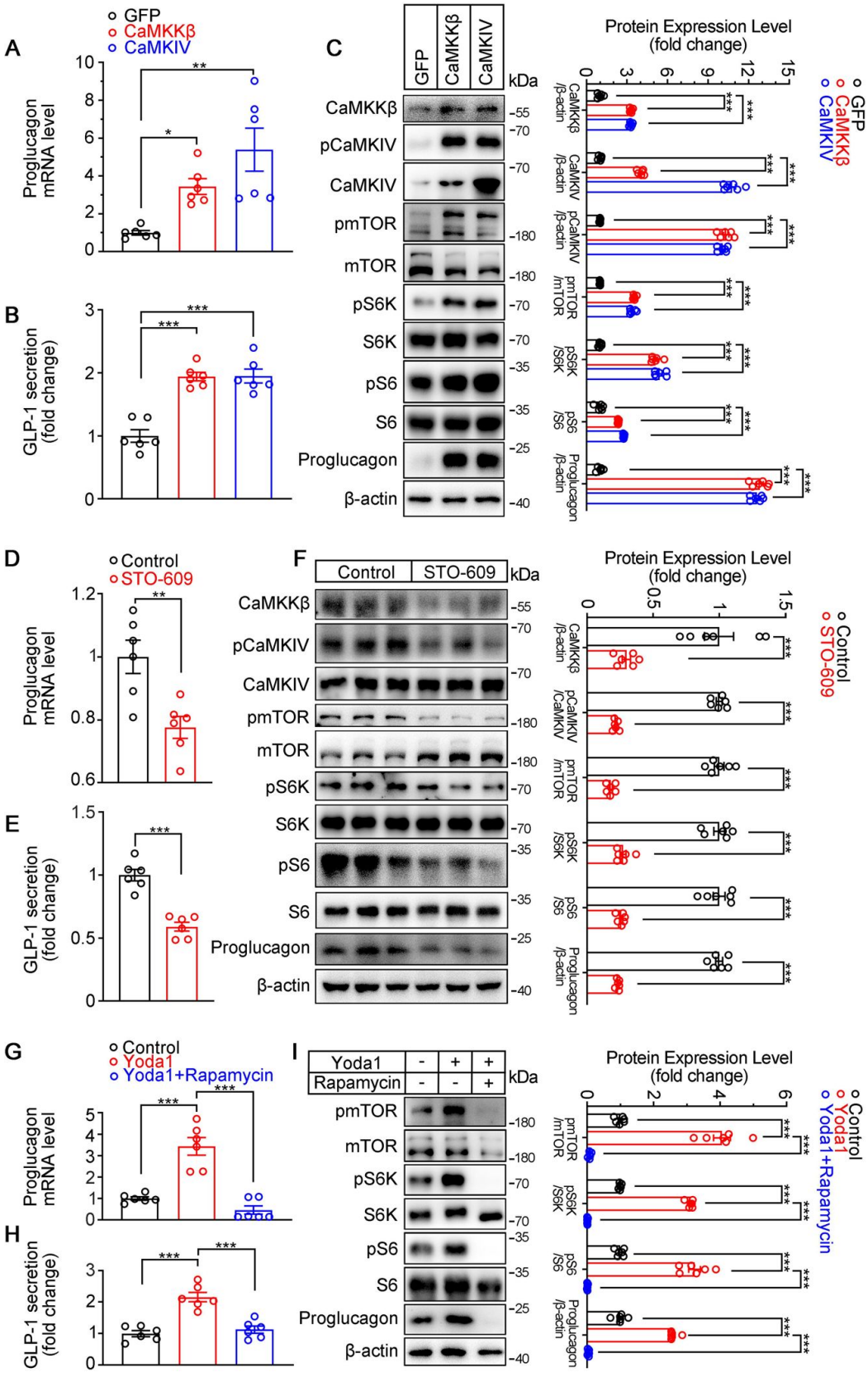
Figure 6

Genetic interference of Piezo1 regulates GLP-1 production in STC-1 cells

(**A-D**) STC-1 cells were transfected with mouse control or *Piezo1* expression plasmids for 48h. *Piezo1* (**A**) and *Proglucagon* (**B**) mRNA levels in STC-1 cells. (**C**) GLP-1 concentrations in culture medium. (**D**) Whole-cell extracts underwent western blot with indicated antibodies.

(**E-H**) Stable knockdown of Piezo1 in STC-1 cells. *Piezo1* (**E**) and *Proglucagon* (**F**) mRNA levels in STC-1 cells. (**G**) GLP-1 concentrations in culture medium. (**H**) Whole-cell extracts underwent western blot with indicated antibodies.

Data are represented as mean ± SEM Data are represented as mean ± SEM and are representative of six biological replicates. Significance was determined by Student's t test, *p < 0.05, **p < 0.01, ***p < 0.001.


A
GFP
CaMKKβ
CaMKIV
Proglucagon mRNA level
B
GLP-1 secretion (fold change)
C
GFP
CaMKKβ
CaMKIV
kDa
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pCaMKIV
CaMKIV
pmTOR
mTOR
pS6K
S6K
pS6
S6
Proglucagon
β-actin
Protein Expression Level (fold change)
D
Control
STO-609
E
F
Control
STO-609
G
Control
Yoda1
Yoda1+Rapamycin
H
I
Yoda1
Rapamycin

Figure 7

Modulation of GLP-1 production by CaMKKβ/CaMKIV and mTOR signaling activity in STC-1 cells

(**A-C**) STC-1 cells were transfected with GFP, CaMKKβ or CaMKIV plasmids for 48h. (**A**) *Proglucagon* mRNA levels in STC-1 cells. (**B**) GLP-1 concentrations in culture medium. (**C**) Whole-cell extracts underwent western blot with indicated antibodies.

(**D-F**) STC-1 cells were treated with CaMKKβ inhibitor STO-609 (10 μmol/L) for 24 h. (**D**) *Proglucagon* mRNA levels in STC-1 cells. (**E**) GLP-1 concentrations in culture medium. (**F**) Whole-cell extracts underwent western blot with indicated antibodies. (**G-I**) STC-1 cells were pretreated with Rapamycin (50 nmol/L) for 1 h, then treated with Yoda1 (5 μmol/L) for 24 h. (**G**) *Proglucagon* mRNA levels in STC-1 cells. (**H**) GLP-1 concentrations in the culture medium. (**I**) Whole-cell extracts underwent western blot with indicated antibodies.

Data are represented as mean ± SEM Data are represented as mean ± SEM and are representative of six biological replicates. Significance was determined by Student's t test for comparison between two groups, and by one-way ANOVA for comparison among three groups or more, *p < 0.05, **p < 0.01, ***p < 0.001.

Discussion

It has been known for decades that GLP-1 secretion from the intestinal L cells is stimulated by meal intake and is essential for postprandial glycemic control (Drucker, 2006; Song et al., 2019). However, the mechanism underlying the regulation of GLP-1 production is not completely understood. One of the problems that impeded the investigation of regulation mechanism of GLP-1 is the lack of an L cell-specific genetically engineered animal model. Here, we generated an L cell-specific Cre mouse line for the first time by combination of the Flp-Frt and Cre-LoxP systems, which allows genetic manipulation specifically in the L cells and thus creates a useful tool to investigate molecular mechanisms in L cells.

Previous studies have shown that L cells are able to sense nutrients in the intestinal lumen such as glucose and other carbohydrates, lipids and amino acids, which induce GLP-1 secretion through different mechanisms, including membrane depolarization-associated exocytosis, Ca^{2+}/Calmodulin (Tolhurst et al., 2011), cAMP (Yu & Jin, 2010), mTORC1 (Xu et al., 2015) and AMPK (Jiang et al., 2016) signaling pathways. However, it is innegligible that as open type endocrine cells, L cells not only receive the chemical stimulations from the nutrients, but also mechanical stimulation when the chyme passing through the intestine, including stretching, pressure and shear force (Sensoy, 2021). While the food needs to be digested and nutrients absorbed before L-cells can detect the nutritive signals, mechanical stimulation may be more direct and faster. Here, we showed the expression of the mechano-sensitive ion channel Piezo1 in L cells of human and mouse intestinal sections, mouse primary L cell culture and an intestinal neuroendocrine cell line STC-1, suggesting the mechano-sensing ability of L cells and potential regulation of GLP-1 in response to mechanical stimulation. Indeed, our study showed that the mechano-regulation of GLP-1 secretion did exist as demonstrated by the increased GLP-1 secretion by intestinal bead implantation, intestinal tissue stretching or STC-1 cell stretching. Moreover, mice with selective loss of Piezo1 expression in intestinal L cell (*IntL-Piezo1*$^{-/-}$) exhibited reduced circulating levels of GLP-1, increased body weight and impaired glucose homeostasis, while pharmacological activation of Piezo1 on mice, primary L cells and STC-1 cells did the reverse. More importantly, *IntL-Piezo1*$^{-/-}$ mice was unable to response to the tension-induced GLP-1 production. These further suggested a Piezo1-mediated mechanical sensing mechanism in L cells that regulates GLP-1 production and glucose metabolism by sensing the stimulation of intestinal luminal contents.

Therefore, our study provides a mechano-regulation mechanism in addition to the existing known nutritive regulation for GLP-1 production. However, the relationship between mechano-regulation and nutritive regulation remains to be explored.

Interestingly, this intestinal Piezo1-mediated mechanical sensing mechanism may severely impaired in diabetic patients and rodents. We observed a decreased Piezo1 expression in the ileal mucosa of diet-induced diabetic mice accompanied by reduced GLP-1 production. When challenged with high-fat, *IntL-Piezo1*$^{-/-}$ mice exhibited more severe symptoms of diabetes which was mitigated by Ex-4. These findings suggest that the impairment of Piezo1-mediated mechanical sensing function in the intestine is an important mechanism for the pathogenesis of T2DM. It is noteworthy that RYGB, a commonly performed weight-loss and hypoglycemic surgery (Cummings et al., 2004), significantly increased Piezo1 expression in L cells of obese diabetic patients. Yoda1 treatment or intestinal bead implantation enhanced GLP-1 production and improved glucose metabolism in the diet-induced diabetic mouse model, suggesting that restore the mechano-sensing or enhance the function of Piezo1 either pharmacologically or mechanically, may be a new strategy to improve the secretion of GLP-1, thus alleviate T2DM. However, in our study, the Piezo1-mediated regulation of GLP-1 production is only demonstrated in transgenic mice, mouse primary L cells and an intestinal neuroendocrine cell line derived from mouse. Whether Piezo1 plays the same role in human L cells awaits to be investigated. A number of studies have generated L cells culture from human intestinal organoid culture or human intestinal stem cell monolayer culture by manipulating the growth factors in the media (Goldspink et al., 2020; Petersen et al., 2014; Villegas-Novoa et al., 2022). It is worthy to validate our finding in human L cells in order to prove its translational potential in T2DM treatment.

The intragastric balloon is a current noninvasive clinical weight loss measure that involves placing a space-occupying balloon in the stomach to reduce food intake and generate satiety signals, thus maintaining satiety. Investigations illustrated that intragastric balloon alter the secretion of hormones such as cholecystokinin and pancreatic polypeptide, delay the emptying of food in the stomach and reduce the appetite (Mathus-Vliegen & de Groot, 2013). Intragastric balloon provides a feasible weight loss intervention for obese people (Kim et al., 2016). In this study, a new intestinal implantation surgery of beads was adopted, which may offer a novel approach for weight loss and glucose control by activating the intestinal Piezo1-GLP-1 signaling pathway in the future.

Mechanistically, our study suggest that Piezo1 regulates GLP-1 production through a CaMKKβ/CaMKIV-mTOR signaling pathway. CaMKKβ/CaMKIV has been reported to mediate the Ca^{2+} signaling in many metabolic processes, including liver gluconeogenesis and de novo lipogenesis, adipogenesis, insulin sensitivity and β cell proliferation (Anderson et al., 2012; Lin et al., 2011; Liu et al., 2012; J. Liu et al., 2022). mTOR plays a central role in nutrient and energy sensing and regulates cellular metabolism and growth in response to different nutrient and energy status (Howell & Manning, 2011). Here we showed that mTOR can also response to mechanical stimuli through a mechano-sensitive Ca^{2+} channel mediated CaMKKβ/CaMKIV activation. Although we did not demonstrate direct phosphorylation of mTOR or S6K by CaMKIV in L cells, previous study reported that CaMKKβ could serve as a scaffold to assemble CaMKIV with key components of the mTOR/S6K pathway and promote liver cancer cell growth (Lin et al., 2015), which lend support to the CaMKKβ/CaMKIV-mTOR signaling in our study. Recently, Knutson et. al. found that ryanodine and IP3-triggered calcium release from intracellular calcium store could amplified the initial Peizo2 - Ca^{2+} signal triggered by mechanical stimulation, and was required for the mechanotransduction in the serotonin release from enterochromaffin cells (Knutson et al., 2023). In our study, we also observed long-lasting intracellular Ca^{2+} increase triggered by Yoda1 in primary L cells and STC-1 cells, which also suggested an involvement of intracellular Ca^{2+} store in the Ca^{2+} relay. Beside Ca^{2+}, cyclic AMP (cAMP) is another signaling molecule that active *Gcg* gene expression and GLP-1 production (Drucker et al., 1994; Jin, 2008; Simpson et al., 2007). cAMP was found to play a critical role in nutrients-induced GLP-1

secretion, including glucose (Ong et al., 2009), lipids (Hodge et al., 2016), and amino acids (Tolhurst et al., 2011). Previous study reported that Ca^{2+} can activate soluble adenylyl cyclase (sAC) to increase intracellular cAMP (Jaiswal & Conti, 2003). Whether sAC-cAMP can be activated by Piezo1-mediated Ca^{2+} influx and whether it is an alternative signaling pathway that mediates the Piezo1-regulated GLP-1 production remain to be explored.

In summary, our study reveals a previously undiscovered Piezo1-mediated mechano-sensing property of intestinal L cell, which plays an essential role in the regulation of GLP-1 production and glucose metabolism. This finding also suggests a new mechano-regulation in enteroendocrine cells, in addition to chemical and neuronal regulation, which may shed new light on the strategy for metabolic diseases such as diabetes and obesity.

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Conflicts of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Author contribution

Yanling Huang, Data curation, Software, Formal analysis, Validation, Investigation, Methodology, Writing - original draft;

Haocong Mo, Data curation, Software, Formal analysis, Validation, Investigation, Methodology, Writing - original draft;

Jie Yang, Data curation, Formal analysis, Validation, Investigation, Methodology, Funding acquisition;

Luyang Gao, Data curation, Formal analysis, Validation, Investigation, Methodology, Writing - original draft;

Tian Tao, Data curation, Investigation, Methodology; Qing Shu, Formal analysis, Validation, Investigation; Wenying Guo, Formal analysis, Validation, Investigation; Yawen Zhao, Formal analysis, Validation, Investigation; Jingya Lyu, Resources, Formal analysis, Validation; Qimeng Wang, Formal analysis, Validation;

Jinghui Guo, Resources, Investigation; Hening Zhai, Resources, Investigation;

Linyan Zhu, Resources, Investigation, Methodology;

Hui Chen, Conceptualization, Resources, Formal analysis, Supervision, Investigation, Software, Visualization, Methodology, Writing - review and editing;

Geyang Xu, Conceptualization, Resources, Formal analysis, Supervision, Funding acquisition, Investigation, Visualization, Methodology, Writing - review and editing, Project administration.

Ethical Statement

Animals used in this study were handled in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publications No. 8023, revised 1978). All animal protocols were approved by the Animal Care and Use Committee of Jinan University.

Human subject research was approved by the Institutional Review Board of Jinan University.

Data availability

All of the data supporting the findings of this study are included in the article and supplementary information.

Abbreviations

- CaMKKβ: Ca^{2+}/calmodulin-dependent protein kinase kinase β
- CaMKIV: Ca^{2+}/calmodulin-dependent protein kinases IV
- CCK: Cholecystokinin
- EECs: Enteroendocrine cells
- mTOR: Mechanistic target of rapamycin
- PYY: Peptide tyrosine tyrosine
- RYGB: Roux-en-Y gastric bypass
- S6K: p70 S6 Kinase
- S6: S6 Ribosomal Protein
- T2DM: Type 2 Diabetes Mellitus

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Editors

Reviewer #1 (Public review):

Summary:

In this manuscript, authors intended to prove that gut GLP-1 expression and secretion can be regulated by Piezo1, and hence by mechanistic/stretching regulation. For this purpose, they have assessed Piezo1 expression in STC-1 cell line (a mouse GLP-1 producing cell line) and mouse gut, showing the correlation between Piezo1 level and Gcg levels (Fig. S1). They then

aimed to generate gut L cell-specific Piezo1 KO mice and claimed the mice show impaired glucose tolerance and GLP-1 production, which can be mitigated by Ex-4 treatment (Fig. 1-2). Pharmacological agents (Yoda1 and GsMTx4) and mechanic activation (intestinal bead implantation) were then utilized to prove the existence of ileal Piezo1-regulated GLP-1 synthesis (Fig. 3). This was followed by testing such mechanism in a limited amount of primary L cells and mainly in the STC-1 cell line (Fig. 4-7).

While the novelty of the study is somehow appreciable, the bio-medical significance is not well demonstrated in the manuscript. The authors stated (in lines between lines 78-83) a number of potential side effects of GLP-1 analogs, how can the mechanistic study of GLP-1 production on its own be essential for the development of new drug targets for the treatment of diabetes. Furthermore, the study does not provide a clear mechanistic insight how the claimed CaMKKbeta/CaMKIV-mTORC1 signaling pathway upregulated both GLP-1 production and secretion. This reviewer also has concerns about the experimental design and data presented in the current manuscript, including the issue of how can proglucagon expression can be assessed by Western blotting.

Strengths:

Novelty of the concept.

Weaknesses:

Experimental design and key experiment information.

https://doi.org/10.7554/eLife.97854.2.sa3

Reviewer #2 (Public review):

Summary:

The study by Huang and colleagues focuses on GLP-1 producing enteroendocrine (EEC) L-cells and their regulation of GLP-1 production by a mechanogated ion channel Piezo1. The study describes Piezo1 expression by L-cells and using an exciting intersectional mouse model (villin to target epithelium and Gcg to target GLP-1 producing cells and others like glucagon producing pancreatic endocrine cells), which allows L-cell specific Piezo1 knockout. Using this model, they find an impairment of glucose tolerance, increased body weight, reduced GLP-1 content, and changes to the CaMKKbeta-CaMKIV-mTORC1 signaling pathway using normal diet and then high fat diet. Piezo1 chemical agonist and intestinal bead implantation reversed these changes and improved the disrupted phenotype. Using primary sorted L-cells and cell model STC-1, they found that stretch and Piezo1 activation increased GLP-1 and altered the molecular changes described above.

Strengths:

This is an interesting study testing a novel hypothesis that may have important mechanistic and translational implications. The authors generated an important intersectional genetics mouse model that allowed them to target Piezo1 L-cells specifically, and the surprising result of impaired metabolism is intriguing.

Weaknesses:

However, there are several critical limitations that require resolution before making the conclusions that the authors make. (1) A potential explanation for the data, and one that is consistent with existing literature [see for example, PMC5334365, PMC4593481], is that epithelial Piezo1, which is broadly expressed by the GI epithelium, impacts epithelial cell density and survival, and as such, if Piezo1 is involved in L-cell physiology, it may be through

regulation of cell density. Thus, it is critical to determine L-cell densities and epithelial integrity in controls and Piezo1 knockouts systematically across the length of the gut, since the authors do not make it clear which gut region contributes to the phenotype they see. Current immunohistochemistry data are not convincing. (2) Calcium signaling in L-cells is implicated in their typical role of being gut chemosensors, and Piezo1 is a calcium channel, so it is not clear whether any calcium-related signaling mechanism would phenocopy these results. (3) Intestinal bead implantation, while intriguing, does not have clear mechanisms - and is likely to provide a point of intestinal obstruction and dysmotility. (4) previous studies, some that are very important, but not cited, contradict the presented results (e.g., epithelial Piezo1 role in insulin secretion) and require reconciliation.
Overall, this study makes an interesting observation but the data are not currently strong enough to support the conclusions.

- There needs to be data localizing Piezo1 to L-cells and importantly, this needs to be quantified - are all L-cells (small bowel and colon) Piezo1 positive? This is because several studies show Piezo1 affecting epithelial cell densities. If there are changes in L-cell or other EEC densities in Piezo1 knockout, that shift can potentially explain the changes that the authors see in glucose metabolism and weight.
- The intersectional model for L-cell transduction needs a deeper validation. Images in Fig 1e are not convincing for transduction of GFP in L-cells. The co-localization studies are not convincing, especially because Piezo1 labeling is very broad. There needs to be stronger validation of the intersectional Gcg-Villin-Piezo1 KO model. It is important to determine whether L-cell Piezo1 localization epithelium in small bowel and colon is present (above) and affected specifically in the knockout.
- The authors state that "Villin-1 (encoded by Vill1 gene) is expressed in the gastrointestinal epithelium, including L cells, but not in pancreatic α cells" (line 378-379). However, Villin is highly expressed in whole mouse islets (https://doi.org/10.1016/j.molmet.2016.05.015, Figure 1A).
- There needs to be quantification of L-cells in Piezo1 knockout. This is because several studies show Piezo1 affecting epithelial cell densities. If there are changes in L-cell or other EEC densities in Piezo1 knockout, that shift can potentially explain the changes that the authors see in glucose metabolism and weight.
- L-cells are classically considered to be chemosensors. Do nutritive signals, which presumably also increase calcium compete or complement or dominate L-cell GLP1 synthesis regulation?
- The mechanism of Glp1 synthesis vs release downstream of Piezo1 is not clear. The authors hypothesize that "Piezo1 might regulate GLP-1 synthesis through the CaMKKβ/CaMKIV-mTOR signaling pathway". However, references cited suggest that Ca2+ or cAMP lead to GLP-1-release, while mTOR primarily acts on the regulation of gene expression by promoting Gcg gene expression. These pathways do not clearly link to Piezo1 □ GLP-1 production. These mechanisms need to be reconciled.
- Previous study PMID 32640190 (not cited here) found that Villin-driven Piezo1 knockout, which knocks out Piezo1 from all epithelial intestinal cells (including L-cells), showed no significant alterations in blood glucose or body weight. This is opposite of the presented findings and therefore the current results require reconciliation.

Comments on revised version:

The authors have addressed several comments that were common to the reviewers - specificity and validity of the intersectional model, mechanism of signaling downstream of Piezo1 and reconciliation of the results with previous studies. The authors have provided extensive experiments and revisions which have made the manuscript stronger. However, many important questions remain, and unfortunately, the intersectional mouse model and mechanisms remain unclear.

- I appreciate the authors quantifying the density of L cells in the intersectional Piezo knockout. There is a very clear >50% drop-off in GLP-1+ cells with the Piezo1 knockout (Supp fig 7c, d). Interestingly, there was not a decrease in PYY+ cells, which is curious because GLP1 and PYY are co-expressed in L cells. The mechanism of regulation of one hormone but not the other in the same cell requires clarification and would be relevant for this work. To begin with, co-labeling PYY and GLP1 and showing that one hormone can be found without the other would be useful.
- Piezo1 immunofluorescence has very high background and overall poor specificity (Fig supp 5 and Fig supp 6B are good examples of poor Piezo1 immunofluorescence). Another method for labeling Piezo1 (e.g. via RNAscope) is required - and where tried (e.g., Fig 1L), the results are not convincing.
- The intersectional mouse model requires further validation. The data presented in Fig 1E do not help - the GFP positive cells do not look like L-cells and there appear to be GFP positive cells in the muscle and submucosa.
- Since Piezo1 is known to affect epithelial cell life span, barrier function maybe compromised. While I appreciate that the authors have obtain some images and measured zonular and occluded, this is unfortunately a suboptimal evaluation of barrier function.
- The mechanisms of calcium signaling that will presumably lead to GLP1 release due to Piezo1 activation and mTOR which authors link to GLP1 synthesis remain unreconciled.
- Intestinal bead implantation may provide an important area of obstruction, in addition to potential mechanical stimulation. Unfortunately whole gut transit time and fecal weight do not assay these functions well.
- I believe that the explanation regarding lack of previous findings connecting Piezo1 in the epithelium and glucose tolerance remain poorly reconciled with the current findings.

https://doi.org/10.7554/eLife.97854.2.sa2

Reviewer #3 (Public review):

Summary:

In this work, the authors proposed that the mechano-gated ion channel Piezo1 enhances GLP-1 production and secretion possibly through stimulating Ca2+-CaMKKbeta-CaMKIV-mTORC1 signaling pathway. By using intestinal L cell-specific piezo1 knock-out mice, intestinal bead implantation mice model, and the chemical agonist Yoda1, the authors claimed that piezo1 promotes pro-glucagon expression, GLP-1 production and secretion. In sorted primary intestinal L cells and STC-1 cells, the authors validated that CaMKKbeta-CaMKIV-mTORC1 signaling pathway positively regulated GLP-1 production and secretion. This study provides new evidence about the specific role of piezo1 in intestinal L cells, broadening the understanding of metabolic functions of piezo1.

Strengths:

The new concept and innovative in vivo and in vitro models.

Weaknesses:

Although the authors have addressed most of the issues in the revised manuscript, there are still some questions that need to be clarified.

(1) This study claimed that piezo1 enhances proglucagon expression, GLP-1 production and secretion through Ca2+-CaMKKbeta-CaMKIV-mTORC1 signaling pathway, which is a highly time-consuming process. However, as a mechano-gated ion channel, it should exert functions promptly. Is it possibly that piezo1 directly stimulates GLP-1 release by influx of Ca2+? if so, have authors measured intracellular Ca2+ concentration?

(2) The authors proposed that the CaMKKbeta-CaMKIV-mTORC1 signaling pathway mediated the effects of piezo1. However, the data is not convincing. At least, chemical inhibitors of CaMKKbeta/CaMKIV/mTORC1 should be used in intL-piezo1 KO mice or STC-1 cells to see if piezo1-induced GLP-1 secretion was abrogated by these chemical inhibitors.
(3) According to previous studies of the team, piezo1 could enhance insulin, ghrelin and GLP-1 secretion while inhibit glucagon production in pancreatic α-cells. In a recent work, the authors found that piezo1 in enterocytes suppresses nutrient absorption. Why an ion channel has these various effects in different cells? What is the fundamental and common mechanism underlying its metabolic functions? Its value as a drug target? These questions need to be discussed in more details.

https://doi.org/10.7554/eLife.97854.2.sa1

Author response:

The following is the authors' response to the original reviews.

> ***Public Reviews:***
>
> ***Reviewer #1 (Public Review):***
>
> *Summary:*
>
> *In this manuscript, the authors intended to prove that gut GLP-1 expression and secretion can be regulated by Piezo1, and hence by mechanistic/stretching regulation. For this purpose, they have assessed Piezo1 expression in STC-1 cell line (a mouse GLP-1 producing cell line) and mouse gut, showing the correlation between Piezo1 level and Gcg levels (Figure S1). They then aimed to generate gut L cell-specific Piezo1 KO mice, and claimed the mice show impaired glucose tolerance and GLP-1 production, which can be mitigated by Ex-4 treatment (Figures 1-2). Pharmacological agents (Yoda1 and GsMTx4) and mechanic activation (intestinal bead implantation) were then utilized to prove the existence of ileal Piezo1-regulated GLP-1 synthesis (Figure 3). This was followed by testing such mechanism in a limited amount of primary L cells and mainly in the STC-1 cell line (Figures 4-7).*
>
> *While the novelty of the study is somehow appreciable, the bio-medical significance is not well demonstrated in the manuscript. The authors stated (in lines between lines 78-83) a number of potential side effects of GLP-1 analogs, how can the mechanistic study of GLP-1 production on its own be essential for the development of new drug targets for the treatment of diabetes. Furthermore, the study does not provide a clear mechanistic insight on how the claimed CaMKKbeta/CaMKIV-mTORC1 signaling pathway upregulated both GLP-1 production and secretion. This reviewer also has concerns about the experimental design and data presented in the current manuscript, including the issue of how proglucagon expression can be assessed by Western blotting.*
>
> *Strengths:*
>
> *The novelty of the concept.*
>
> *Weaknesses:*
>
> *Experimental design and key experiment information.*

We appreciate the reviewer's comments. Nowadays, GLP-1-based therapy is well-recognized and commonly used in treatment of Type 2 Diabetes Mellitus (T2DM). Therefore, elucidation of the mechanism that regulates GLP-1 production is essential for the development of new

drug targets for the treatment of diabetes. We have revised the relevant wording in the manuscript.

In our previous studies, we have elucidated the role of mTOR/S6K pathway in regulating GLP-1 production in L cells. Using STC-1 cell line and different mouse models, including *Neurog3-Tsc1*−/− mice, rapamycin or L-lucine treatment to stimulate mTOR activity, we have demonstrated that mTOR stimulates proglucagon gene expression and thus GLP-1 production (Diabetologia 2015;58(8):1887-97； Mol Cell Endocrinol. 2015 Nov 15:416:9-18.). Based on our previous studies, we found that Piezo1 regulated mTOR/S6K pathway and thus proglucagon expression and GLP-1 production through a Ca2+/CaMKKbeta/CaMKIV pathway in our present study. Although we could not exclude involvement of other signaling pathways downstream of Piezo1 in regulating the cleavage of proglucagon, granule maturation and the final release of GLP-1, our present study provided evidence to support the involvement of the Ca2+/CaMKKbeta/CaMKIV/mTOR pathway in mediating the role Piezo1 in proglucagon expression and GLP-1 production.

The reviewer also expressed concerns on the use of western blot to detect proglucagon expression. Proglucagon is encoded by the *GCG* gene and is cleaved by PC1/3 in L cells to form mature GLP-1. In fact, measurement of intestinal proglucagon protein is a common approach for assessing GLP-1 production in the intestine. Here are some examples from other researchers: Diabetes. 2013 Mar;62(3):789-800. Gastroenterology. 2011 May;140(5):1564-74. 2004 Jul 23;279(30):31068-75. The proglucagon antibody used in our study was purchased from abcam (Cat#ab23468), which can detect proglucagon at 21 kDa.

> ***Reviewer #2 (Public Review):***
>
> *Summary:*
>
> *The study by Huang and colleagues focuses on GLP-1 producing entero-endocrine (EEC) L-cells and their regulation of GLP-1 production by a mechano-gated ion channel Piezo1. The study describes Piezo1 expression by L-cells and uses an exciting intersectional mouse model (villin to target epithelium and Gcg to target GLP-1-producing cells and others like glucagon-producing pancreatic endocrine cells), which allows L-cell specific Piezo1 knockout. Using this model, they find an impairment of glucose tolerance, increased body weight, reduced GLP-1 content, and changes to the CaMKKbeta-CaMKIV-mTORC1 signaling pathway using a normal diet and then high-fat diet. Piezo1 chemical agonist and intestinal bead implantation reversed these changes and improved the disrupted phenotype. Using primary sorted L-cells and cell model STC-1, they found that stretch and Piezo1 activation increased GLP-1 and altered the molecular changes described above.*
>
> *Strengths:*
>
> *This is an interesting study testing a novel hypothesis that may have important mechanistic and translational implications. The authors generated an important intersectional genetics mouse model that allowed them to target Piezo1 L-cells specifically, and the surprising result of impaired metabolism is intriguing.*
>
> *Weaknesses:*
>
> *However, there are several critical limitations that require resolution before making the conclusions that the authors make.*
>
> *(1) A potential explanation for the data, and one that is consistent with existing literature [see for example, PMC5334365, PMC4593481], is that epithelial Piezo1, which is broadly expressed by the GI epithelium, impacts epithelial cell density and survival, and as such, if Piezo1 is involved in L-cell physiology, it may be through regulation of cell density.*

> *Thus, it is critical to determine L-cell densities and epithelial integrity in controls and Piezo1 knockouts systematically across the length of the gut, since the authors do not make it clear which gut region contributes to the phenotype they see. Current immunohistochemistry data are not convincing.*

We appreciate the reviewer's comment and agree that Piezo1 may impact L-cell density and epithelial integrity. To address this, we have incorporated quantification of L-cell density in new Figure Supplement 7. The quantitative results demonstrate that the specific deletion of the piezo1 gene in L cells did not significantly impact L-cell density.

Regarding epithelial integrity, we assessed the expression of tight junction proteins (ZO-1 and Occludin). As demonstrated in new Figure Supplement 8, the expression of tight junction proteins such as ZO-1 and Occludin did not show significant changes in *IntL-Piezo1-/-* mice compared to littermate controls.

Furthermore, we conducted double immunofluorescence of Piezo1 and GLP-1 in the duodenum, jejunum, ileum, and colon of control and *IntL-Piezo1-/-* mice. As illustrated in new Figure Supplement 5, Piezo1 is expressed in GLP-1-positive cells of the duodenum, jejunum, ileum, and colon of control mice, but not in *IntL-Piezo1-/-* mice.

> *(2) Calcium signaling in L-cells is implicated in their typical role of being gut chemo-sensors, and Piezo1 is a calcium channel, so it is not clear whether any calcium-related signaling mechanism would phenocopy these results.*

We agree with the reviewer that Piezo1 is a calcium channel (validation of the Ca2+ influx-mediated Piezo1 in primary L cells and STC-1 cells are shown in figure 4A-C and figure 5A-C). According to our study, calcium-related signaling mechanism such as calcium/calmodulin-dependent protein kinase kinase 2 (CaMKKβ) -Calcium/Calmodulin Dependent Protein Kinase IV (CaMKIV) may contribute the phenotype seen in the _IntL-Piezo1-/_mice. In addition, we also discussed other potential calcium-related signaling mechanisms in the article's discussion section (lines645-656).

> *(3) Intestinal bead implantation, while intriguing, does not have clear mechanisms and is likely to provide a point of intestinal obstruction and dysmotility.*

We appreciate the reviewer's comment. To ascertain if intestinal bead implantation led to intestinal obstruction and dysmotility, we conducted a bowel transit time test and detected the postoperative defecation (As shown in new Figure Supplement 9). The results revealed no difference in bowel transit time and fecal mass between the sham-operated mice and those implanted with beads. Furthermore, to assess whether the animals were in pain or under any discomfort after intestinal bead implantation, we performed abdominal mechanical sensitivity test three days after the surgery. As indicated in Figure Supplement 9C, no difference in abdominal pain threshold was observed between sham and bead-implanted mice. These results suggest that the mice did not experience discomfort during the experiment.

> *(4) Previous studies, some that are very important, but not cited, contradict the presented results (e.g., epithelial Piezo1 role in insulin secretion) and require reconciliation.*

Thanks a lot for the point. We have cited more previous studies. The lack of changes in blood glucose seen in *Villin-Piezo1-/-* mice reported by Sugisawa et. al. is not surprising (Cell. 2020 Aug 6;182(3):609-624.e21.). Actually, in another recent study from our group, we found similar results when the *Villin-Piezo1-/-* mice *Piezo1fl/fl* control mice were fed with normal chow diet. Since Villin-1 is expressed in all the epithelial cells of the gut, including enterocytes and various types of endocrine cells, the effect of L-cell Piezo1 loss may be masked by other cell

types under normal condition. However, impaired glucose tolerance was seen in *Villin-Piezo1-/-* mice compared to the *Piezo1fl/fl* control mice after high fat diet for 8 weeks. We further found that Piezo1 in enterocytes exerted a negative effect on the glucose and lipid absorption. Loss of Piezo1 in enterocytes led to over-absorption of nutrients under high-fat diet. （Tian Tao, Qing Shu, Yawen Zhao, Wenying Guo, Jinting Wang, Yuhao Shi, Shiqi Jia, Hening Zhai, Hui Chen, Cunchuan Wang*, Geyang Xu*, Mechanical regulation of lipid and sugar absorption by Piezo1 in enterocytes, Acta Pharmaceutica Sinica B , Accepted, 2024. (https://doi.org/10.1016/j.apsb.2024.04.016).

> *Overall, this study makes an interesting observation but the data are not currently strong enough to support the conclusions.*
>
> ***Recommendations for the authors:***
>
> ***Reviewer #1 (Recommendations For The Authors):***
>
> *Major concerns*
>
> *(1) Figure 1L was labeled wrong, and the co-localization was not clear. The KO leads to such a strong effect on the percentage of GLP-1 positive cells (panel M) but was not clearly demonstrated with immune-staining. Additional experiments are needed to prove tissue-specific knockout in gut GLP-1-producing cells only, but not in other cell lineages or elsewhere. If so, how was the change in gut Gcg mRNA expression? Importantly, this review is not clear on how to use Western blotting to measure proglucagon expression in the tissue samples. What is the size of the product? The antibody information was not provided in the manuscript. Figure 1N, a potential mechanism that affects GLP-1 production involving mTORC and downstream molecules. This comes from nowhere.*

We appreciate the reviewer's feedback. The incorrect label has been corrected in the new Figure 1L. As suggested, we have performed additional experiments to demonstrate tissue-specific knockout of Piezo1 in gut GLP-1-producing cells exclusively, excluding other cell lineages or locations.

As shown in Figure Supplement 6, Piezo1 remains expressed in ileal ghrelin-positive cells and pancreatic glucagon-positive cells of IntL-Piezo1-/mice, suggesting that Piezo1 was specifically knocked out in L cells, but not in other endocrine cell types. Furthermore, the decrease was only observed in GLP-1 levels, but not PYY levels, in L cells of *IntL-Piezo1-/-* mice compared to controls, suggesting that the loss of Piezo1 in L cells affects GLP-1 levels specifically, but not the secretion of other hormones produced by L cells (Figure Supplement 7A-D).

In our previous studies, we have elucidated the role of mTOR/S6K pathway in regulating GLP-1 production in L cells. Using STC-1 cell line and different mouse models, including *Neurog3-Tsc1−/−* mice, rapamycin or L-lucine treatment to stimulate mTOR activity, we have demonstrated that mTOR stimulates proglucagon gene expression and thus GLP-1 production (Diabetologia 2015;58(8):1887-97； Mol Cell Endocrinol. 2015 Nov 15:416:9-18.). Based on our previous studies, we found that Piezo1 regulated mTOR/S6K pathway and thus proglucagon expression and GLP-1 production through a Ca2+/CaMKKbeta/CaMKIV pathway in our present study.

Although we could not exclude involvement of other signaling pathways downstream of Piezo1 in regulating the cleavage of proglucagon, granule maturation and the final release of GLP-1, our present study provided evidence to support the involvement of the Ca2+/CaMKKbeta/CaMKIV/mTOR pathway in mediating the role Piezo1 in proglucagon expression and GLP-1 production.

The reviewer also expressed concerns on the use of western blot to detect proglucagon expression. Proglucagon is encoded by the *GCG* gene and is cleaved by PC1/3 in L cells to form

mature GLP-1. In fact, measurement of intestinal proglucagon protein is a common approach for assessing GLP-1 production in the intestine. Here are some examples from other researchers: Diabetes. 2013 Mar;62(3):789-800. Gastroenterology. 2011 May;140(5):1564-74. 2004 Jul 23;279(30):31068-75. The proglucagon antibody used in our study was purchased from abcam (Cat#ab23468), which can detect proglucagon at 21 kDa.

> *(2) In Figure 2, the LFD control mouse group was missing. Again, I don't understand the detection of proglucagon by Western blotting in this figure.*

We appreciate the reviewer's comments. The figure 1 presents the phenotypic changes of transgenic mice under low-fat diet feeding, while figure 2 focuses on the phenotypic changes of transgenic mice under high-fat diet feeding. As we mentioned before, western blot is often used in detection of the precursor of GLP-1 named proglucagon.

> *(3) Why show body weight change but not body weight itself? How are the changes compared (which one serves as the control)? Again, how to do Western blotting on proglucagon detection?*

We appreciate the reviewer's comments. Body weight has been added in new figure3. Proglucagon is the precursor of GLP-1. Intestinal proglucagon protein measurement is commonly used to assess GLP-1 production in the intestine.

> *(4) After reading the whole manuscript, this reviewer cannot get a clear picture of how the claimed CaMKKbeta-mTORC1 pathway mediates the function of Pieo1 activation (via the utilization of Yoda1 or intestinal bead implantation) on Gcg expression (at the transcription level or mRNA stability level?), hormone production, the genesis of GLP-1 producing cells, and the secretion of the hormone.*

We appreciate the reviewer's comments. Figure 7 showed that overexpression of CaMKKbeta and CaMKIV enhanced mTOR and S6K phosphorylation, proglucagon expression and GLP-1 secretoin, while CaMKKbeta inhibitor STO609 inhibited mTOR and S6K phosphorylation, proglucagon expression and GLP-1 secretoin, suggesting CaMKKbeta and CaMKIV was involved in GLP-1 production. Moreover, mTOR inhibitor rapamycin inhibited Yoda1-induced proglucagon expression and GLP-1 secretion. These results suggested that CaMKKbeta/CaMKIV/mTOR mediated the effect of Piezo1 on GLP-1 production.

> *I strongly suggest that authors focus on more solid findings and dissect the mechanistic insight on something more meaningful, but not on everything (hormone coding gene expression, hormone production, and hormone secretion).*

GLP-1 production involves multiple steps, including proglucagon expression, protein cleavage, granule packaging and final release. In our present study, we focused on how mechanical signals regulated proglucagon expression in L-cells and thus promote GLP-1 production. We did not exclude the possibility that mechanical signals could also affect other step of GLP-1 production and we discussed this possibility in the discussion section.

> *Minor concerns*
>
> *(1) Figure S1A. STC-1 is a Gcg expression cell line, which shows less amount of Peio1 mRNA when compared with most primary tissue samples tested. This does not support the fundamental role of Peio1 in regulating Gcg expression. Maybe qRT-PCR will be more helpful for establishing the correlation.*

Thanks a lot for the comments. As suggested, the results of qRT-PCR have been added in new Figure S1A.

> *(2) There are numerous scientific presentation problems in the written manuscript. Necessary literature citations are missing especially for key methods (such as bean implantation).*

Thank you very much for your comments. We have made every effort to enhance the scientific presentation and have included the necessary literature citations.

> ***Reviewer #2 (Recommendations For The Authors):***
>
> *Overall, this study makes an interesting observation but the data are not currently strong enough to support the conclusions.*
>
> *(1) There needs to be data localizing Piezo1 to L-cells and importantly, this needs to be quantified - are all L-cells (small bowel and colon) Piezo1 positive?*

Thank you very much for your comments. We performed double immunofluorescence of Piezo1 and GLP-1 in the duodenum, jejunum, ileum, and colon of control and *IntL-Piezo1-/-* mice. As shown in new Figure Supplement 5, Piezo1 is expressed in about 90% of GLP-1-positive cells in the duodenum, jejunum, ileum, and colon of control mice, but not in *IntL-Piezo1-/-* mice.

> *(2) The intersectional model for L-cell transduction needs deeper validation. Images in Figure 1e are not convincing for the transduction of GFP in L-cells. The co-localization studies are not convincing, especially because Piezo1 labeling is very broad. There needs to be stronger validation of the intersectional Gcg-Villin-Piezo1 KO model. It is important to determine whether L-cell Piezo1 localization epithelium in the small bowel and colon is present (above) and affected specifically in the knockout.*

Thanks a lot for the comments. In our study, we conducted a double immunofluorescence analysis for Piezo1 and GLP-1 across various segments of the gastrointestinal tract, including the duodenum, jejunum, ileum, and colon, in both control and *IntL-Piezo1-/-* mice. As illustrated in the newly incorporated Figure Supplement 5, it was observed that Piezo1 is indeed expressed within the cells of the aforementioned gastrointestinal segments in control mice, which are also positive for GLP-1 expression. In stark contrast, no evidence of Piezo1 expression was detected in the *IntL-Piezo1-/-* mice. Consistent with these findings, in situ hybridization experiments corroborated the absence of Piezo1 expression within GLP-1 positive cells in the *IntL-Piezo1-/-* mice, offering evidence for the successful knockout of Piezo1 in the L cells of these knockout mice. (Figure 1L and M).

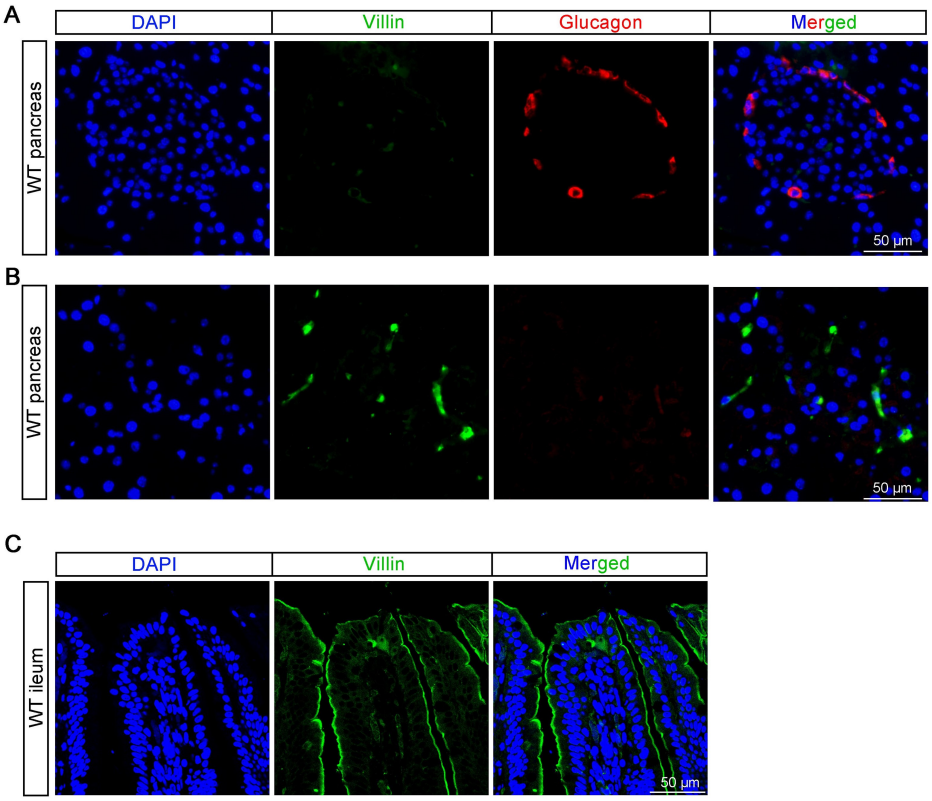
In Figure 1E, IntL-Cre mice were bred with mT/mG reporter mice to further validate Cre recombinase activity and specificity. All tissues and cells of mT/mG mice express red fluorescence (membrane-targeted tdTomato; mT) at baseline, and switch to membrane-targeted EGFP in the presence of cell-specific Cre. EGFP expression was only observed scatteredly in the intestine, but not in the pancreas, indicating the intestinal-specific Cre activity in the IntL-Cre mice (Figure 1E). We have revised the relevant expressions in the main text.

> *(3) The authors state that "Villin-1 (encoded by Vill1 gene) is expressed in the gastrointestinal epithelium, including L cells, but not in pancreatic α cells" (lines 378-379). However, Villin is highly expressed in whole mouse islets (https://doi.org/10.1016/j.molmet.2016.05.015, Figure 1A).*

Thanks a lot for the comments. Although Hassan Mziaut et al. reported that Villin is highly expressed in whole mouse islets, in that article, only the co-localization of insulin cells with Villin is mentioned, while the co-localization of glucagon and Villin is lacking.

According to our research (refer to Author response image 1 below) and previous study (Rutlin, M. et al, 2020, The Villin1 Gene Promoter Drives Cre Recombinase Expression in Extraintestinal Tissues. Cell Mol Gastroenterol Hepatol, 10(4), 864-867.e865.), Villin is sparsely expressed in pancreatic tissue but not highly expressed in islets. We did not observed co-localization of glucagon and Villin in the pancreas (see Author response image 1A and B below). The same antibody was used to stain intestine, which show specific expression on the apical side of the intestinal villi (see Author response image 1C below).

Author response image 1.



> *(4) There needs to be quantification of L-cells in Piezo1 knockout. This is because several studies show Piezo1 affecting epithelial cell densities. If there are changes in L-cell or other EEC densities in Piezo1 knockout, that shift can potentially explain the changes that the authors see in glucose metabolism and weight.*

We appreciate the reviewer's comment. We agree that Piezo1 may affect L-cell density and epithelial integrity.

To assess epithelial integrity we examined the expression of tight junction proteins (ZO-1 and Occludin). As shown in new Figure Supplement 8, the expression of tight junction proteins, including ZO-1 and Occludin, remained unchanged in *IntL-Piezo1-/-* mice when compared to littermate controls.

To assess the L-cell density, we stained PYY, another hormone mainly secreted by L cells, in both control and *IntL-Piezo1-/-* mice. As shown in new Figure Supplement 7A and B, the percentage of PYY positive cells were not significantly different between control and *IntL-Piezo1-/-* mice, suggesting that the L-cell density was not affected by Piezo1 knockout.

> *(5) L-cells are classically considered to be chemosensors. Do nutritive signals, which presumably also increase calcium compete or complement or dominate L-cell GLP1 synthesis regulation?*

We appreciate the reviewer ' s comment and agree that L-cells are traditionally considered to be chemosensors. It is also recognized that nutritive signals regulate L-cell GLP1 synthesis. We have addressed these points in lines 568-595. Both nutritive and mechanical signals regulate GLP-1 production. While the food needs to be digested and nutrients absorbed before L-cells can detect the nutritive signals, mechanical stimulation provides a more direct and rapid response. However, determining whether nutritive signals compete, complement with mechanical signals or dominate in L-cell GLP-1 production will require to be further explored.

> *(6) The mechanism of Glp1 synthesis vs release downstream of Piezo1 is not clear. The authors hypothesize that "Piezo1 might regulate GLP-1 synthesis through the CaMKKβ/CaMKIV-mTOR signaling pathway". However, references cited suggest that Ca2+ or cAMP leads to GLP-1-release, while mTOR primarily acts on the regulation of gene expression by promoting Gcg gene expression. These pathways do not clearly link to Piezo1 GLP-1 production. These mechanisms need to be reconciled.*

Thanks a lot for the point. The effect of Piezo1-mediated Ca2+ increase on GLP-1 production may be two-fold: promote Gcg gene expression through CaMKKβ/CaMKIV-mTOR and promote GLP-1 release by degranulation. Both gene expression and release are important to sustained GLP-1 production.

> *(7) Previous study PMID 32640190 (not cited here) found that Villin-driven Piezo1 knockout, which knocks out Piezo1 from all epithelial intestinal cells (including L-cells), showed no significant alterations in blood glucose or body weight. This is the opposite of the presented findings and therefore the current results require reconciliation.*

We have cited PMID 32640190 in our revised manuscript. The lack of changes in blood glucose seen in *Villin-Piezo1-/-* mice reported by Sugisawa et. al. is not surprising (Cell. 2020 Aug 6;182(3):609-624.e21.). Actually, in another recent study from our group, we found similar results when the _Villin-Piezo1-/_mice *Piezo1fl/fl* control mice were fed with normal chow diet. Since Villin-1 is expressed in all the epithelial cells of the gut, including enterocytes and various types of endocrine cells, the effect of L-cell Piezo1 loss may be masked by other cell types under normal condition. However, impaired glucose tolerance was seen in *Villin-Piezo1-/-* mice compared to the *Piezo1fl/fl* control mice after high fat diet for 8 weeks. We further found that Piezo1 in enterocytes exerted a negative effect on the glucose and lipid absorption. Loss of Piezo1 in enterocytes led to over-absorption of nutrients under high-fat diet (Tian Tao, Qing Shu, Yawen Zhao, Wenying Guo, Jinting Wang, Yuhao Shi, Shiqi Jia, Hening Zhai, Hui Chen, Cunchuan Wang, Geyang Xu, Mechanical regulation of lipid and sugar absorption by Piezo1 in enterocytes, Acta Pharmaceutica Sinica B, Accepted, 2024, https://doi.org/10.1016/j.apsb.2024.04.016).

> ***Reviewing Editor (Recommendations For The Authors):***
>
> *Your paper - while innovative in concept and interesting - has many flaws that in my opinion need to be corrected before the paper and pre-print should be published or*

> *uploaded as pre-print. Can you please make every effort to address the missing data that the Reviewers have asked for and correct the lack of references as noted in the reviews? Thank you.*

Thank you for the invaluable suggestions provided by the editors and reviewers. In response to these suggestions, we have included the missing data as requested and rectified the lack of references to the best of our ability. We hope that these revisions will effectively address the concerns raised by the editors and reviewers.

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