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Susheel K Gunasekar  
*Washington University School of Medicine in St. Louis*

Litao Xie  
*Washington University School of Medicine in St. Louis*

Ashutosh Kumar  
*Washington University School of Medicine in St. Louis*

Juan Hong  
*Washington University School of Medicine in St. Louis*

Chen Kang  
*Washington University School of Medicine in St. Louis*

See next page for additional authors

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Small molecule SWELL1 complex induction improves glycemic control and nonalcoholic fatty liver disease in murine Type 2 diabetes

Susheel K. Gunasekar1,13, Litao Xie1,13, Ashutosh Kumar1,13, Juan Hong1, Pratik R. Chheda2, Chen Kang1, David M. Kern1,3,4, Chau My-Ta5, Joshua Maurer1, John Heebink1, Eva E. Gerber3,4, Wojciech J. Grzesik6, Macaulay Elliot-Hudson7, Yanhui Zhang8, Phillip Key1, Chaitanya A. Kulkarni2, Joseph W. Beals9, Gordon I. Smith9, Isaac Samuel10, Jessica K. Smith10, Peter Nau10, Yumi Imai7, Ryan D. Sheldon11, Eric B. Taylor1,11, Daniel J. Lerner12, Andrew W. Norris6, Samuel Klein9, Stephen G. Brohawn3,4, Robert Kerns2 & Rajan Sah1

Type 2 diabetes is associated with insulin resistance, impaired pancreatic β-cell insulin secretion, and nonalcoholic fatty liver disease. Tissue-specific SWELL1 ablation impairs insulin signaling in adipose, skeletal muscle, and endothelium, and impairs β-cell insulin secretion and glycemic control. Here, we show that ICLSWELL and SWELL1 protein are reduced in adipose and β-cells in murine and human diabetes. Combining cryo-electron microscopy, molecular docking, medicinal chemistry, and functional studies, we define a structure activity relationship to rationally-design active derivatives of a SWELL1 channel inhibitor (DCPIB/SN-401), that bind the SWELL1 hexameric complex, restore SWELL1 protein, plasma membrane trafficking, signaling, glycemic control and islet insulin secretion via SWELL1-dependent mechanisms. In vivo, SN-401 restores glycemic control, reduces hepatic steatosis/injury, improves insulin-sensitivity and insulin secretion in murine diabetes. These findings demonstrate that SWELL1 channel modulators improve SWELL1-dependent systemic metabolism in Type 2 diabetes, representing a first-in-class therapeutic approach for diabetes and nonalcoholic fatty liver disease.

1Department of Internal Medicine, Cardiovascular Division, Washington University School of Medicine, St. Louis, MO, USA. 2Department of Pharmaceutical Sciences and Experimental Therapeutics, University of Iowa, College of Pharmacy, Iowa City, IA, USA. 3Department of Molecular & Cell Biology, University of California Berkeley, Berkeley, CA, USA. 4Helen Wills Neuroscience Institute, University of California Berkeley, Berkeley, CA, USA. 5Feinberg School of Medicine, Northwestern University, Chicago, IL, USA. 6Stead Family Department of Pediatrics, Endocrinology and Diabetes Division, Fraternal Order of Eagles Diabetes Research Center, University of Iowa, Iowa City, IA, USA. 7Department of Internal Medicine, Cardiovascular Division, University of Iowa, Iowa City, IA, USA. 8Xiamen Cardiovascular Hospital, Xiamen University, Xiamen, China. 9Center for Human Nutrition, Washington University School of Medicine, St. Louis, MO, USA. 10Department of Surgery, University of Iowa, Carver College of Medicine, Iowa City, IA, USA. 11Department of Biochemistry, University of Iowa, Iowa City, IA, USA. 12Senseion Therapeutics Inc, BioGenerator Labs, St Louis, MO, USA. 13These authors contributed equally: Susheel K. Gunasekar, Litao Xie, Ashutosh Kumar. ✉email: rajan.sah@wustl.edu
Type 2 diabetes mellitus (T2D) is a globally ubiquitous metabolic disease characterized by hyperglycemia that is caused by reduced insulin sensitivity in target tissues and impaired insulin secretion from pancreatic $\beta$-cells\textsuperscript{1–3}. T2D accounts for 90–95% of all diabetes mellitus in the United States, or about 24 M people\textsuperscript{4}. It is associated with increased risk of cardiovascular disease, renal disease, liver disease, cancer, and infection and a hazard ratio for all-cause mortality of 1.80 compared to patients without T2D\textsuperscript{5,6}. The cost of medical care for patients with diabetes is 2.3-fold the cost in non-diabetics. In 2017, the direct medical cost of diabetes in the United States was $237B\textsuperscript{7}.

There are at least ten distinct classes of medications approved to treat T2D: sulfonylureas, meglitinides, amylin mimetics, biguanides, alpha-glucosidase inhibitors, thiazolidinediones, glucagon-like peptide-1 analogs (GLP-1a), dipeptidyl peptidase-4 inhibitors (DPPi), sodium-glucose co-transporter (SGLT)\textsuperscript{-2} inhibitors (SGLT2i), and insulin. Despite this diverse array of T2D medications, there are several reasons why new medications for T2D are needed. First, cardiovascular disease (CVD) is the leading cause of death in diabetics\textsuperscript{8,9}, and although newer T2D medications like SGLT2i and GLP-1a affect a reduction in CVD mortality, significant residual CVD mortality remains\textsuperscript{10}, which presents a therapeutic opportunity for T2D medications with novel mechanisms of action. Second, 25–33% of patients with T2D have inadequate glycemic control, with HbA1c levels above guideline recommendations\textsuperscript{6,11–14}. This poor glucose control is associated with an increased risk of death from vascular causes, non-vascular causes, and cancer\textsuperscript{15}. Third, T2D medication-induced hypoglycemia remains a significant problem for patients with T2D, especially with patients on multiple T2D medications\textsuperscript{15,16}. For all these reasons, there remains sustained interest in developing new T2D and metabolic syndrome therapeutics, especially with novel mechanisms of action\textsuperscript{17}.

SWELL1 or LRRC8a (leucine-rich repeat-containing protein 8a) encodes a transmembrane protein first described in 2003 as the site of a balanced translocation in an immunodeficient child with agammaglobulinemia and absent B-cells\textsuperscript{18,19}. Subsequent work revealed that this condition was caused by impaired SWELL1-dependent GRB2-PI3K-AKT signaling in lymphocytes, resulting in a developmental block in lymphocyte differentiation\textsuperscript{20}. So, for about a decade, SWELL1 was considered a membrane protein that regulates PI3K-AKT mediated signaling in lymphocytes, and it was not until 2014 that SWELL1/LRRC8a was discovered to also form an essential component of the volume-regulated anion channel (VRAC)\textsuperscript{21,22}, forming hetero-hexamers with LRRC8b\textsuperscript{22,23}. Therefore, historically, the SWELL1/LRRC8 complex was first described as a membrane protein that participated in non-ion channel-mediated protein–protein signaling (non-conductive signaling) and then later found to form an ion channel complex with ion conductive signaling properties. Indeed, prior work highlights each of these modes of SWELL1-LRRC8 channel complex signaling. We previously showed SWELL1 to mediate insulin-PI3K-AKT signaling in adipocytes, skeletal muscle, and endothelium via non-conductive signaling mechanisms\textsuperscript{24–27}. Also, we and others showed SWELL1-LRRC8 channel activity (conductive signaling) in the pancreatic $\beta$-cell is required for normal insulin secretion\textsuperscript{28,29}. Thus, SWELL1-LRRC8 loss-of-function both downregulates insulin signaling in target tissues\textsuperscript{24,30} and insulin secretion from the pancreatic $\beta$-cell\textsuperscript{28,29,29} inducing a state of glucose intolerance\textsuperscript{24,28,30}. Since T2D is characterized by both a loss of insulin sensitivity of target tissues (fat, skeletal muscle, liver) and ultimately, impaired insulin secretion from the pancreatic $\beta$-cell\textsuperscript{1–3}, these data raised the question: could impaired SWELL1-mediated signaling contribute to T2D pathogenesis, and if so, could this be corrected pharmacologically to improve systemic glycemia?

In this study, we provide evidence that SWELL1-mediated currents and SWELL1 protein are reduced in murine and human adipocytes and pancreatic $\beta$-cells in the setting of T2D and hyperglycemia suggesting that dysfunctional SWELL1-mediated signaling could contribute to T2D pathogenesis by impairing insulin sensitivity and insulin secretion. Next, we identify a small molecule modulator, DCPiB (renamed SN-401), as a tool compound that binds the SWELL1-LRRC8 complex\textsuperscript{31}, and potentially functions as a chemical chaperone to augment SWELL1 expression and plasma membrane trafficking at concentrations 5-fold lower than its IC\textsubscript{50} of ~5 µM for I\textsubscript{CL,SWELL}\textsuperscript{32}. In vivo, SN-401 normalizes glucose tolerance by increasing insulin sensitivity and secretion in insulin-resistant T2D mouse models, while augmenting tissue glucose uptake, suppressing hepatic glucose production, inducing serum FGF21 levels, and reducing hepatic steatosis and hepatocyte damage (ballooning) in obese T2D mice. Importantly, while SN-401 normalizes glycemia in diabetic mice, it has very mild glucose-lowering effects on non-obese euglycemic mice—indicating a low risk of hypoglycemic events associated with other commonly used anti-diabetic therapies, including sulfonylureas and insulin. Combining cryo-EM structure data of SN-401 bound to its target SWELL1/LRRC8a\textsuperscript{33} with molecular docking simulations, and cryo-EM structure data of an active SN-40X congener bound to SWELL1 hexameric channels in lipid nanodiscs, we validate a structure-activity relationship (SAR) based approach to generate SN-401 congeners with subtle molecular changes to “tune” on-target activity, both in vitro and in vivo. This approach allows us to attribute the cellular and systemic SN-40X effects to drug-target binding while controlling for off-target effects. We propose small molecule SWELL1 modulators may represent a first-in-class therapeutic approach to treat metabolic syndrome and associated diseases by restoring SWELL1 signaling across multiple organ systems that are dysfunctional in T2D.

**Results**

I\textsubscript{CL,SWELL} and SWELL1 protein are reduced in T2D $\beta$-cells and adipocytes. SWELL1/LRRC8a ablation impairs insulin signaling in target tissues\textsuperscript{24,30} and insulin secretion from the pancreatic $\beta$-cell\textsuperscript{28,29}, inducing a pre-diabetic state of glucose intolerance\textsuperscript{24,28,30}. These recent findings suggest that reductions in SWELL1 may contribute to Type 2 diabetes (T2D). To determine if SWELL1-mediated currents are altered in T2D we measured I\textsubscript{CL,SWELL} in pancreatic $\beta$-cells freshly isolated from T2D mice raised on HFD for 5–7 months (Fig. 1a, c) and from individuals with T2D (Fig. 1b, d and Supplementary Table S1) compared to non-T2D controls. In both mouse and human T2D $\beta$-cells, the maximum I\textsubscript{CL,SWELL} current density (measured at +100 mV) upon stimulation with hypotonic swelling is significantly reduced (90% in murine; 63% in human, Fig. 1c, d) compared to non-T2D controls, similar to reductions observed in SWELL1 knockout (KO) and knockdown (KD) murine and human $\beta$-cells\textsuperscript{28}, respectively. As SWELL1/LRRC8a is a critical component of I\textsubscript{CL,SWELL}/VRAC\textsuperscript{21,22} in both adipose tissue\textsuperscript{24,30} and $\beta$-cells\textsuperscript{28,29}, we asked whether these reductions in I\textsubscript{CL,SWELL} in the setting of T2D\textsuperscript{33} are associated with reductions in SWELL1 protein expression. Total SWELL1 protein in murine islets (largely representing $\beta$-cells) isolated from T2D mice raised on HFD for 33 weeks (40 weeks old) is significantly reduced 66% compared to islets from lean, non-T2D control mice (Fig. 1e). Similarly, diabetic human cadaveric islets (representing numerous islet cell types) also shows a trend toward being reduced 50% compared to islets from non-diabetics (Fig. 1f and...
Supplementary Table S2), suggesting that reduced SWELL1 protein may underlie these reductions in $I_{\text{CL,SWELL}}$ currents.

Curiously, reductions in β-cell $I_{\text{CL,SWELL}}$ observed in the setting of T2D (Fig. 1a–d) are consistent with previous measurements of VRAC/$I_{\text{CL,SWELL}}$ in the adipocytes of the murine KKA^Y^ T2D model^3^, which are reduced by 60% in T2D KKA^Y^ mice compared to KKA^a^ controls^3^ (Fig. 1g, data plotted from Inoue et al., 2010^3^). Similarly, SWELL1-mediated $I_{\text{CL,SWELL}}$ measured in isolated human adipocytes from a diabetic individual with obesity (BMI = 52.3, HgbA1c = 6.9%; Fasting glucose = 148–151 mg/dl) show a trend toward being reduced 48% compared to non-diabetic individuals with obesity that we reported previously^24, and not different from $I_{\text{CL,SWELL}}$ in adipocytes from lean individuals (Fig. 1h and Supplementary Table S3). Consistent with reductions observed in murine and human adipocyte $I_{\text{CL,SWELL}}$, SWELL1 protein is also reduced (38%) in adipose tissue of T2D KKA^Y^ mice as compared to parental control KKA^a^ mice (Fig. 1i), and 66% in obese T2D mice raised on HFD for 33 weeks (40 weeks old) compared to lean mice (Fig. 1j). Similarly, SWELL1 protein is 50% lower in adipose tissue from
individuals with hyperglycemic T2D and obesity (HgbA1c > 6.0%) compared to adipose tissue from normoglycemic individuals (HgbA1c < 6.0% Fig. 1k and Supplementary Table S4). Taken together, these findings suggest reduced SWELL1 activity in adipocytes and β-cells (and possibly other tissues) may underlie insulin resistance and impaired insulin secretion associated with T2D. Moreover, SWELL1 protein expression increases in both adipose tissue and liver in the setting of early euglycemic obesity 30, and shRNA-mediated suppression of this SWELL1 induction exacerbates insulin resistance and glucose intolerance 30. Similar to these prior findings, hepatic SWELL1 protein is induced ninefold in mice raised on HFD for 33 weeks compared to lean, non-T2D mice (Supplementary Fig. 1a), and increased ~3.2-fold in T2D KKAy mice compared to non-T2D control KKAa mice (Supplementary Fig. 1b). This is in contrast to the reductions of SWELL1 protein, and ICLSWELL observed in T2D adipose and islets/β-cells. Therefore, we speculate that maintenance or induction of SWELL1 expression/signaling in peripheral tissues may support insulin sensitivity and secretion to preserve systemic glycaemia in the setting of T2D.

SWELL1 protein expression regulates insulin-stimulated PI3K-AKT2-AS160 signaling. To test whether SWELL1 regulates insulin signaling, we re-expressed Flag-tagged SWELL1 (SWELL1 O/E) in SWELL1 KO 3T3-F442A adipocytes and measured insulin-stimulated phosphorylated AKT2 (pAKT2; Ser474) and phosphorylated AS160 (pAS160; Ser1177) as a readout of insulin signaling (Fig. 2a, b). SWELL1 KO 3T3-F442A adipocytes exhibit significantly blunted insulin-mediated pAKT2 and pAS160 signaling compared to WT adipocytes, similar to described previously 22,26, and this is fully rescued by reexpression of SWELL1 in SWELL1 KO adipocytes (KO + SWELL1 O/E, Fig. 2a, b) 26, SWELL1 reexpression also recapitulates SWELL1-mediated ICLSWELL in SWELL1 KO cells in response to hypotonic stimulation (Fig. 2c and Supplementary Fig. S2a-c), which is consistent with restoration of SWELL1-LRRC8a signaling complexes at the plasma membrane. Notably, the reductions in total AKT2 protein expression observed in SWELL1 KO adipocytes is not rescued by SWELL1 reexpression, indicating that transient changes in SWELL1 protein expression in adipocytes regulate pAKT2 signaling, as opposed to total AKT2 protein expression. We confirmed FLAG-tagged SWELL1 traffics normally to the plasma membrane when expressed in both WT and SWELL1 KO adipocytes visualized by immunofluorescence (IF) using anti-FLAG and SWELL1 KO-validated anti-SWELL1 antibodies, respectively (Supplementary Fig. S2d, e). FLAG-tagged SWELL1 overexpressed in WT and SWELL1 KO adipocytes assume a punctate pattern at the cell periphery, similar to endogenous SWELL1 in WT adipocytes. Overall, these data indicate that SWELL1 expression levels regulate insulin-PI3K-AKT2-AS160 signaling in adipocytes—potentially by modulating GRB2 signaling 20,24-26. Furthermore, these data imply that pharmacological SWELL1 induction in peripheral tissues in the setting of T2D may enhance insulin signaling and improve systemic insulin sensitivity and glycemic control.

A small molecule binds SWELL1-LRRC8 channel complex, increases adipocyte SWELL1 protein expression, and SWELL1-dependent insulin signaling. The small molecule 4-[(2-Butyl-6,7-dichloro-2-cyclopentyl-2,3-dihydro-1-oxo-1H-inden-5-yl)oxy]butanoic acid (DCPIB, Fig. 2d) is among a series of structurally diverse (acylarylxylo)acetic acid derivatives, that were synthesized and studied for diuretic properties in the late 1970s 34,35 and evaluated in the 1980s as potential treatments for brain edema 36,37. While DCPIB was derived from the FDA-approved diuretic ethacrynic acid, it has minimal diuretic activity 38 and has instead been used as a selective VRAC/ICLSWELL inhibitor 24,28,32 (Fig. 2e), binding at a con- striction point within the SWELL1-LRRC8 hexamer 39,40. Having demonstrated that SWELL1 regulates insulin-AKT2 signaling in multiple cell types, including adipocytes 24,25,30, skeletal muscle 26, and endothelium 27, we anticipated pharmacological inhibition of VRAC/ICLSWELL with DCPIB, which we here re-name SN-401, would decrease insulin signaling. Unexpectedly, SN-401 increased SWELL1 protein expression in 3T3-F442A preadipocytes (threefold control expression; Fig. 2f, g) and adipocytes (1.5-fold control expression; Fig. 2h) when applied for 96 h, and was associated with enhanced insulin-stimulated levels of pAKT2 (Fig. 2i, g, i, j), and insulin-stimulated levels of pAS160 (Fig. 2i, j). These SN-401-mediated effects on insulin-AKT2-AS160 signaling are absent in SWELL1 KO 3T3-F442A adipocytes, consistent with an on-target SWELL1-mediated mechanism of action for SN-401 (Fig. 2i, j). The SN-401-mediated increases in SWELL1 protein expression are not associated with increases in SWELL1 mRNA, nor in the mRNA for other LRRC8 subunits: LRRC8b, LRRC8c, LRRC8d, or LRRC8e that form the SWELL1 channel complex (Supplementary Fig. S3a-c), implicating post-transcriptional mechanisms for increased SWELL1 expression and SWELL1-LRRC8 associated signaling.

SN-401 increases SWELL1 and improves systemic glucose homeostasis in murine T2D models by enhancing insulin sensitivity and secretion. To determine if SN-401 improves insulin signaling and glucose homeostasis in vivo we treated two T2D mouse models: obese, HFD-fed mice and the polygenic-T2D KKAy mouse model with SN-401 (5 mg/kg i.p. for 4–10 days). In vivo, SN-401 augmented SWELL1 expression twofold in adipose tissue of HFD-fed T2D (Fig. 3a), threefold in adipose tissue of...
KKAY T2D (Fig. 3b) mice, and was associated with improved fasting blood glucose (FG), glucose tolerance (GTT), and insulin tolerance (ITT) in both T2D models (Fig. 3c–f). These metabolic improvements were not associated with significant differences in body weight (Supplementary Table S5). Adipocyte size was mildly reduced 9% in KKAy mice (Fig. 3g) and unchanged in the HFD-fed mice (Fig. 3h) after SN-401 treatment for 5 days as compared to vehicle. Remarkably, treating the control KKAa parental strain with SN-401 at the same treatment dose (5 mg/kg × 4–10 days) does not cause hypoglycemia, nor does it alter glucose and insulin tolerance (Fig. 3d–f). Similarly, lean, non-T2D, glucose-tolerant mice treated with SN-401 have similar FG, GTT and ITT compared to vehicle-treated mice (Fig. 3i, j and Supplementary Fig. S4a–c). However, when made insulin-resistant and diabetic after 16 weeks of HFD-feeding, these same mice (from Fig. 3i, j) treated with SN-401 show marked improvements in FG (Fig. 3k), GTT, and ITT (Fig. 3l) as compared to vehicle. These data show that SN-401 restores glucose homeostasis in the setting of T2D, but has little effect on glucose homeostasis in non-T2D mice. Importantly, this portends a low risk for inducing hypoglycemia.
SN-401 was well-tolerated during chronic i.p. injection protocols, with no overt signs of toxicity with daily i.p. injections for up to 8 weeks, despite striking effects on glucose tolerance (Supplementary Fig. S4d).

To examine SN-401-mediated enhancements in insulin secretion from pancreatic β-cells, we next measured glucose-stimulated insulin secretion (GSIS) in SN-401 treated mice subjected to 21 weeks of HFD. We found that the impairments in GSIS commonly observed with long-term HFD (21 weeks HFD) are significantly improved in SN-401-treated HFD mice based on serum insulin measurements (Fig. 3m) and perfusion GSIS from isolated islets (Fig. 3n), consistent with the predicted effect of SWELL1 induction in pancreatic β-cells28,29. Similar results were obtained from islets isolated from SN-401 treated T2D KKAy mice compared to vehicle-treated mice (Fig. 3o). Collectively, these data suggest that SN-401-mediated improvements in systemic glycemia in T2D occur via augmentation of both peripheral insulin sensitivity and β-cell insulin secretion—the inverse phenotype observed in vivo in loss-of-function studies24,28–30.

SN-401 improves systemic insulin sensitivity, tissue glucose uptake, and nonalcoholic fatty liver disease in murine T2D models. To more rigorously evaluate SN-401 effects on insulin sensitization and glucose metabolism in T2D mice we performed euglycemic hyperinsulinemic clamps traced with 3H-glucose and 14C-deoxyglucose in T2D KKAy mice treated with SN-401 or vehicle. SN-401 treated T2D KKAy mice require a higher glucose-infusion rate (GIR) to maintain euglycemia compared to vehicle, consistent with enhanced systemic insulin sensitivity (Fig. 4a).

The rate of glucose appearance (Rg), which reflects hepatic glucose production from gluconeogenesis and/or glycogenolysis, was reduced 40% in SN-401-treated T2D KKAy mice at baseline (Basal, Fig. 4b), and further suppressed 75% during glucose/insulin infusion (Clamp, Fig. 4b), revealing SN-401 increases hepatic insulin sensitivity—similar to thiazolidinediones (TZD)43.

As the SN-401-mediated increase in SWELL1 signaling is expected to enhance insulin-pAKT2-pAS160 signaling, GLUT4 plasma membrane translocation, and tissue glucose uptake24, we next measured the effect of SN-401 on tissue glucose uptake in KKAy mice using 2-deoxyglucose (2-DG). SN-401 enhanced insulin-stimulated 2-DG uptake into inguinal white adipose tissue (iWAT), gonadal white adipose tissue (gWAT), and myocardium (Fig. 4c). Similarly, we found previously that SWELL1 ablation markedly reduces insulin-pAKT2-pGSK3β signaling24,26,27 and cellular glyogen content24, and accordingly asked whether the SN-401-mediated increase in SWELL1 signaling would increase glucose incorporation into tissue glycogen in the setting of T2D. Indeed, SN-401 markedly increased glucose incorporation into glycogen in liver, adipose, and skeletal muscle (Fig. 4d).

Consistent with improved glucose metabolism in peripheral tissues, we observe a trend toward SN-401 mediated SWELL1 induction in skeletal muscle (Supplementary Fig. 5a, b) in HFD-T2D mice (18 weeks HFD), associated with increases in downstream pAKT2, pAS160, and pGSK3β (Ser9) signaling (Supplementary Fig. 5a, b). In contrast to adipose and skeletal muscle, SN-401 does not induce hepatic SWELL1 protein in T2D KKAy mice (Supplementary Fig. 5c), nor when applied for 24–48 h to cultured primary hepatocytes (Supplementary Fig. 5d–i). However, SN-401 application in vitro to cultured primary hepatocytes robustly induces pAS160 in a SWELL1-dependent manner, but without significant pAKT2 activation (Supplementary Fig. 5d, e, g). Collectively, these data suggest that SN-401 mediated effects on systemic metabolism in vivo occur via SWELL1 modulation in multiple tissues, including adipose, skeletal muscle, liver, and pancreatic islets.

Nonalcoholic fatty liver disease (NAFLD), like T2D, is associated with insulin resistance43. NASH is an advanced form of nonalcoholic liver disease defined by three histological features: hepatic steatosis, hepatic lobular inflammation, hepatocyte damage (ballooning) and can be present with or without fibrosis. NAFLD and T2D likely share at least some pathophysiologic mechanisms because more than one-third of patients (37%) with T2D have NASH44 and almost one-half of patients with NASH (44%) have T2D45. Mice raised on HFD for 16 weeks followed by intermittent dosing with SN-401 over the course of 5 weeks (Fig. 4e) were noted to have grossly smaller livers (Fig. 4f), lower hepatic triglyceride concentrations (Fig. 4g), and experienced a mild 14% reduction in body weight compared to vehicle-treated mice (Fig. 4f). Histologic evaluation revealed significant reductions in hepatic steatosis and hepatocyte damage compared to vehicle-treated mice (Fig. 4f). Histologic evaluation revealed significant reductions in hepatic steatosis and hepatocyte damage compared to vehicle-treated mice (Fig. 4f). Histologic evaluation revealed significant reductions in hepatic steatosis and hepatocyte damage compared to vehicle-treated mice (Fig. 4f).

The NAFLD-activity score (NAS), which integrates histologic scoring of hepatic steatosis, lobular inflammation, and hepatocyte ballooning46 (Fig. 4i), also improved >2 points in SN-401-treated mice compared to vehicle-treated mice. These SN-401 mediated reductions in hepatic steatosis and hepatocyte damage are consistent with the observed increases in hepatic insulin sensitivity and consequent reductions in hepatic glucose production via gluconeogenesis available for hepatic de novo lipogenesis (Fig. 4b), as observed with other insulin sensitizers, such as metformin and TZDs47 that have both primary effects on liver and secondary effects on other peripheral tissues.

To evaluate other possible mechanisms for the observed improvements in systemic metabolism we measured serum adiponectin and Fibroblast Growth Factor 21 (FGF21) in KKAy mice treated with SN-401 for 5 days (Fig. 4j, k). Adiponectin is an adipokine that is increased by other anti-diabetic agents, such as TZDs, and is thought to improve glycemic control and lipid...

Fig. 2 SWELL1 protein expression regulates insulin-stimulated PI3K-AKT2-AS160 signaling. a, b Western blots detecting SWELL1, pAKT2Ser474, AKT2, pAS160Thr642, AS160, and β-actin in wildtype (WT, black), SWELL1 knockout (KO, red), and adenoviral reexpression of SWELL1 in KO (KO + SWELL1 O/E, blue) 3T3-F442A adipocytes stimulated with 0 and 10 nM insulin for 15 min (a) and densitometric quantification for pAKT2/β-actin and pAS160/β-actin (b) (n = 3 independent experiments for each condition). c Mean insulin and onward current densities recorded at −100 and +100 mV from WT (black, n = 5 cells), KO (red, n = 4 cells), and KO + SWELL1 O/E (blue, n = 4 cells) 3T3-F442A preadipocytes. d SN-401/DCFPI8 chemical structure. e LC3β insulin and onward current over time upon hypotonic (210 mosm) stimulation and subsequent inhibition by 10 µM SN-401 in a HEK-293 cell. f, g Western blots detecting SWELL1, pAKT2Ser474, and β-actin in WT 3T3-F442A adipocytes treated with either vehicle or SN-401 (10 µM) for 96 h (f) (n = 2 each) and the corresponding densitometric ratio (g). h Western blots detecting SWELL1 and β-actin in WT 3T3-F442A adipocytes treated with either vehicle or SN-401 (10 µM) for 96 h (n = 6 each) and the corresponding densitometric ratio below. i, j Western blots detecting pAKT2Ser474, AKT2, pAS160Thr642, AS160, and β-actin in WT (n = 9 each) and SWELL1 KO (n = 3 each) 3T3-F442A adipocytes treated with either vehicle or SN-401 (500 nM) for 96 h and stimulated with 0 and 10 nM insulin for 15 min (i) and the corresponding densitometric ratio for pAKT2/β-actin and pAS160/β-actin respectively (j). Data were represented as mean ± SEM. Two-tailed unpaired t-test was used in b, c, g, h, and j where *", **", and ***" represent p values of <0.05, <0.01, and <0.001, respectively. ns difference did not exceed the threshold for significance.
metabolism via effects in the liver, pancreatic β-cells, and adipocytes. FGF21 is an endocrine hormone that is expressed in the liver, pancreas, and adipose tissue, but under physiological conditions is largely a liver-derived hepatokine, and is a potent regulator of glucose and lipid metabolism. Curiously, we found that serum adiponectin was unchanged in SN-401 treated KKAy mice as compared to vehicle (Fig. 4j), while serum FGF21 was increased 2.8-fold by SN-401 (Fig. 4k). Taken together, these data reveal that SN-401 augments SWELL1 protein and SWELL1-mediated signaling in some tissues, while also increasing serum FGF21 to concomitantly enhance both systemic insulin sensitivity and pancreatic β-cell insulin secretion, thereby normalizing glycemic control in T2D mouse models. This improved metabolic state can reduce ectopic lipid deposition,
hepatocyte damage, and NAFLD that is associated with obesity and T2D.

Chemical synthesis, molecular docking, and cryo-EM reveal specific SN-401-SWELL1 interactions important for on-target binding. To confirm that SN-401-induced increases in SWELL1 protein and signaling are mediated by direct binding to the SWELL1-LRRC8 channel complex, as opposed to off-target effects, we designed and synthesized SN-401 congeners (Fig. 5a) with subtle structural changes predicted to either enhance (SN-403, SN-406, SN-407), or reduce (Inactive 1, Inactive 2, Inactive 3) SN-401-SWELL1 complex binding (Fig. 5a). The cryo-EM structure of SN-401/DCP1B bound within the SWELL1 homo-hexamer, published by Kern and colleagues31 revealed that SN-401 binds at a constriction point in the pore wherein the electronegative SN-401 carboxylate group interacts electrostatically with the R103 residue in one or more of the SWELL1 monomers31. Moreover, SN-401 appeared to stabilize the pore region of the SWELL1 hexamer in lipid nanodiscs31. To characterize the structural features of SN-401 responsible for binding to SWELL1-LRRC8, we performed molecular docking simulations of SN-401 and its analogs into the SWELL1 homo-hexamer (PDB: 6NZZ), and identified two molecular determinants predicted to be critical for SN-401-SWELL1-LRRC8 binding (Fig. 5b i, ii): (1) the length of the carbon chain leading to the anionic carboxylate group predicted to electrostatically interact with one or more R103 guanidine groups (found in SWELL1/LRRC8a and LRRC8b; Fig. 5b-solid circles); and (2) proper orientation of the hydrophobic cyclopentyl group that slides into a hydrophobic cleft at the interface of LRRC8 monomers (conserved among all LRRC8 subunit interfaces; Fig. 5b-broken circles). Docking simulations predicted shortening the carbon chain leading to the carboxylate by 2 carbons would yield a molecule, Inactive 1, that could either interact with R103 through the carboxylate group (Fig. 5c(i)) or have the cyclopentyl ring occupy the hydrophobic cleft (Fig. 5c(ii)), but unable to simultaneously participate in both interactions (Fig. 5c). Similarly, docking simulations predicted removing the butyl group of SN-401 would yield a molecule, Inactive 2, unable to orient the cyclopentyl group into a position favorable for interaction with the hydrophobic cleft without introducing structural strain in the molecule (Supplementary Fig. S7a). Finally, replacing the carboxylic acid with boronic acid, yielded Inactive 3, a molecule predicted to be unchanged at physiological pH, thereby chemically removing the predicted electrostatic interaction of SN-401 with R103. Indeed, docking simulations of Inactive 3 revealed no poses that included an electrostatic interaction with R103, with the molecule largely sitting at the mouth of the pore (Fig. 5d i, ii). Importantly, all of these structural modifications, predicted to abrogate either carboxylic-R103 electrostatic binding (Inactive 1, Inactive 3) or cyclopentyl-hydrophobic pocket binding (Inactive 2) were insufficient to impair SWELL1 channel binding as demonstrated by a rightward shift in the IC50 for ICLSWELL inhibition in vitro for Inactive 1 (Fig. 6b, e; IC50 = 35.5 μM), Inactive 2 (Fig. 6c, e; IC50 = 10.6 μM), and Inactive 3 (Fig. 6d, e; IC50 = 18.3 μM) as compared to SN-401 (Fig. 6a, e; IC50 = 3.9 μM)32.

Conversely, lengthening the carbon chain attached to the carboxylate group of SN-401 by 1–3 carbons (Fig. 5a: SN-403, SN-406, and SN-407) was predicted to enhance the R103 electrostatic interactions (Fig. 5e–g; black solid circle), and better orient the cyclopentyl group to bind within the hydrophobic cleft (Fig. 5e–g, broken circle). Additional binding interactions for SN-403, SN-406 and SN-407 are also predicted along the channel, due to the longer carbon chains affording additional hydrophobic interactions with side-chain carbons of the R103 residues (Fig. 5e–g; purple dashes). As anticipated, SN-403, SN-406, and SN-407 had increased ICLSWELL inhibitory activity compared to SN-401 (Fig. 6f-i and Supplementary Fig. S7b, c), with SN-406 and SN-407 having IC50 of 3.1 and 1.6 μM, respectively, and SN-401 IC50 = 3.9 μM (Fig. 6f–i). Overexpressing a SWELL1 mutant in WT HEK cells wherein the endogenous SWELL1 R103 is replaced with an electronegative E103 (R103E-SWELL1) functioned as a partial dominant negative for ICLSWELL current, reducing ICLSWELL 84% (Fig. 6i). This residual current in R103E-SWELL1 expressing cells is less sensitive to both SN-401 and SN-407 inhibition, as demonstrated by a rightward shift in the IC50 of SN-401 from 3.9 to 6.0 μM (Fig. 6k–m), and SN-407 from 1.6 to 4.2 μM (Fig. 6n–p). Molecular docking simulations also reveal that R103E mutant SWELL1 homomers are unable to dock SN-401 as predicted in WT homomers (Supplementary Fig. S7d i, ii), and as demonstrated in the published cryo-EM structure33, unless at least 1 LRRC8 subunit contains an arginine at amino acid 103 (Supplementary Fig. S7d(iii), further supporting the requirement of R103 for SN-401 binding determined experimentally (Fig. 6k–m). A similar docking result is obtained with SN-407 binding to R103E mutant SWELL1 homomers (Supplementary Fig. S7e), also consistent with experimental data (Fig. 6n–p).

To further test the predictions of our molecular docking simulations, we used cryo-EM to determine the structure of the SWELL1 homo-hexamer in lipid nanodiscs in the presence of SN-407 (Fig. 7, Supplementary Figs. S8–10, and Supplementary Table. S6). In initial maps using sixfold symmetry, SN-407 density was less apparent than for DCP1B/SN-40131, potentially due to a reduction in SN-407 occupancy that is a consequence of lower compound solubility or the presence of multiple drug poses in different particles (Supplementary Fig. S9). Therefore, we
utilized sixfold symmetry expansion and symmetry relaxation and were able to resolve two distinct poses for SN-407 with similar occupancy. Pose 1 shows the drug oriented vertically in the channel’s selectivity filter in a manner that is similar to that observed for DCPIB/SN-401, but with the lengthened carboxylate chain coiling to maintain its interaction with R103 (Fig. 7a). In Pose-2, SN-407 is tilted off the SWELL1 central axis, positioning its cyclopentyl group closer to the hydrophobic cleft between SWELL1 subunits (Fig. 7b). These data confirm lengthening the carboxylate chain in SN-407 preserves electrostatic interactions with R103 and enables additional contacts between the carboxylate chain and upper hydrophobic moieties in distinct binding poses, consistent with the results of our docking simulations. We note that while these models represent the best
SN-401 and SWELL1-active congener SN-406 inhibit \( I_{\text{CL,SWELL}} \) but promote SWELL1-dependent signaling at sub-micromolar concentrations. To test this hypothesis, we compared the potency of SN-401/SN-406 to inhibit \( I_{\text{CL,SWELL}} \) when applied to closed as compared to open SWELL1-LRR8 channels. SN-401/SN-406 concentrations of 6–10 \( \mu M \) are required to effectively inhibit channels when first opened by hypotonic activation, while concentrations of 1 \( \mu M \) are relatively ineffective (Fig. 6f, g, i)\(^{32}\). In contrast, application of 1 \( \mu M \) of SN-401 or SN-406 to SWELL1-LRR8 channels in the closed state (i.e., for 30 min prior to hypotonic activation) markedly suppressed and delayed subsequent hypotonic activation of \( I_{\text{CL,SWELL}} \) compared to either vehicle alone, or Inactive compounds (Fig. 8a, b). SN-401 and SN-406 at concentrations as low as 250 \( nM \) had a similar effect (Fig. 8c, d). These data support the notion that SN-40X compounds bind with higher affinity to SWELL1-LRR8 channels in the closed/resting state than the open/activated state, and stabilize the closed conformation at less than one-tenth the concentration required to inhibit activated SWELL1-LRR8 channels.

Next, we applied SWELL1-active (SN-401 and SN-406), and Inactive (Inactive 1 and Inactive 2) compounds to differentiated 3T3-F442A adipocytes under basal culture conditions for 4 days and then measured SWELL1 protein after 6 h of serum starving. At both 10 and 1 \( \mu M \), SN-401 and SN-406 markedly augmented SWELL1 protein to levels 1.5–2.1-fold greater than vehicle-treated controls, while SWELL1-inactive congeners Inactive 1 and Inactive 2 did not significantly increase SWELL1 protein levels (Fig. 8e–h). SN-401 and SN-406 also enhanced plasma membrane trafficking of SWELL1 (Fig. 8i–k). These findings are consistent with the results of SN-401/SN-406 \( I_{\text{CL,SWELL}} \) inhibition when pre-applied to closed SWELL1-LRR8 channels (Fig. 8a–d) and also with our observations that 500 \( nM \) SN-401 is sufficient to augment SWELL1-dependent insulin-AKT2-AS160 signaling in 3T3-F442A adipocytes (Fig. 2i, j). As we have recently shown that SWELL1 regulates AKT-eNOS signaling in endothelium and endothelium-targeted SWELL1 depletion contributes to vascular dysfunction in the setting of T2D\(^{27}\), we next examined SN-401 mediated signaling in human umbilical vein endothelial cells (HUVECs). SN-401 applied at 500 \( nM \) to HUVECs increases eNOS phosphorylation (Ser1177), a downstream AKT target, and this effect is abrogated by small interfering/short hairpin mediated SWELL1 knockdown in HUVECs, supporting a SWELL1-dependent mechanism (Supplementary Fig. S12a–d). Furthermore, SN-406 applied to HUVECs at 100 \( nM \) was sufficient to induce SWELL1 1.5-fold, basal pAKT2 2.8-fold, and downstream p-eNOS 5.5-fold as compared to vehicle (Fig. 8k, l), while Inactive 1 had no effect at 500 \( nM \) (Fig. 8m, n). Indeed, SN-401 exhibits dose-dependent induction of p-eNOS in HUVECs that saturates beyond 100 \( nM \) (Fig. 8o, p).

SWELL1-active compounds prevent reductions in SWELL1 protein and rescue SWELL1-dependent islet insulin secretion under glucolipotoxic conditions. We next asked whether endoplasmic reticulum (ER) stress associated with glucolipotoxicity in metabolic syndrome may promote SWELL1 protein degradation, and thereby reduce \( I_{\text{CL,SWELL}} \) and SWELL1 protein in T2D (Fig. 1). In this context, we hypothesized that SN-401 and SN-406 might assist with SWELL1-LRR8 assembly and rescue SWELL1-LRR8 from degradation. To test this concept in vitro, we first treated 3T3-F442A adipocytes with either vehicle, SN-401, SN-406, or Inactive 2, and then subjected these cells to 1 \( \mu M \) palmitate + 25 \( mM \) glucose to induce glucolipotoxic stress (Fig. 9a, b). We found that SWELL1 protein was reduced by 50\% upon palmitate/glucose treatment, consistent with ER stress-mediated SWELL1 degradation, and this reduction was entirely prevented by both SWELL1-active SN-401 and SN-406, but not by Inactive 2 (Fig. 9a, b). These data were consistent with the...
notion that SN-401 and SWELL1-active congeners are functioning to stabilize SWELL1-LRR8 assembly and signaling under glucolipotoxic conditions associated with T2D and metabolic syndrome.

To examine whether these protective effects of SN-401 under glucolipotoxic conditions is also capable of rescuing islet insulin secretion and whether this effect occurs via target engagement, we measured dynamic glucose-stimulated insulin secretion (GSIS) by perifusion in human and murine islets ± palmitate ± SN-401 and ±SWELL1 as outlined in Fig. 9c. In both human and murine islets, we found that 16 h of 1 mM palmitate treatment reduces GSIS compared to baseline (Fig. 9d–g). However, when islets are treated with SN-401 (10 µM) for 4 days prior to palmitate treatment, then maintained during palmitate treatment, and
Fig. 5 Molecular docking of SN-401 and synthesized congeners to SWELL1 reveal specific drug-target binding interactions. a Chemical structures of SN-401/DCPIB, SN-403, SN-406, SN-407, Inactive 1, Inactive 2, and Inactive 3. b top (i) and side (ii) view of binding poses of SN-401; SN-401 carboxylate groups interact with R103 residue guanidine groups (solid black circle), the SN-401 cyclopentyl group occupies a shallow hydrophobic cleft at the interface of two monomers formed by SWELL1 D102 and L101 (black broken circle). c Binding poses for Inactive 1 (i) side view of the first binding pose of Inactive 1 showing potential electrostatic interaction with R103 (solid black circle) but unable to reach into and occupy the hydrophobic cleft (black broken circle); (ii) side view of the second pose with guanidine groups (solid black circle) that the carboxylate group unable to reach and interact with R103 (solid black circle). d Binding pose for Inactive 3; (i) side and (ii) top view of Inactive 3 on the top of the pore not able to occupy the hydrophobic cleft (black broken circle) or electrostatically interact with R103 (solid black circle). e-g (i) top and (ii) side view of binding poses of SN-403 (e), SN-406 (f), and SN-407 (g); the cyclopentyl group occupies a shallow hydrophobic cleft at the interface of two monomers formed by D102 and L101 (black broken circle) but the alkyl side chain SN-403/SN-406/SN-407 interacts with the alkyl side chain of R103 (purple broken circle). h Poses generated for respective compounds by docking into PDB 6NZZ using Molecular Operating Environment 2016 (MOE) software package. SN-401 congeners are depicted as yellow sticks and R103, D102, and L101 are depicted as green sticks.

subsequently, SN-401/palmitate washed off during GSIS, insulin secretion is normalized (Fig. 9d–g and Supplementary Table S2). Importantly, this SN-401 mediated GSIS normalization under glucolipotoxic conditions is SWELL1-dependent, since this rescue is completely abrogated in SWELL1 KO human islets (Fig. 9d, e) and β-cell-targeted SWELL1 KO murine islets (Fig. 9f, g).

SWELL1-active SN-401 congeners improve glycemic control in murine T2D. To determine if the effects of SN-401 observed in vivo in T2D mice are attributable to SWELL1-LRRC8 binding, as opposed to off-target effects, we next measured fasting blood glucose and glucose tolerance in HFD-T2D mice treated with either SWELL1-active SN-403 or SN-406 as compared to SWELL1-inactive Inactive 1 (all at 5 mg/kg/day × 4 days). In mice treated with HFD for 8 weeks, SN-403 significantly reduced fasting blood glucose and improved glucose tolerance compared to Inactive 1 (Fig. 10a). In cohorts of mice raised on HFD for 12–18 weeks, with more severe obesity-induced T2D, SN-406 also markedly reduced fasting blood glucose and improved glucose tolerance (Fig. 10b). Similarly, in a separate experiment, SN-406 significantly improved glucose tolerance in HFD-T2D mice, compared to Inactive 1 (Fig. 10c), and this is associated with a trend toward improved insulin sensitivity based on the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR)37 (Fig. 10d), and significantly augmented insulin secretion in perfusion GSIS (Fig. 10e). Finally, based on the GTT AUC, SN-407 also improved glucose tolerance in T2D KKAy mice, compared to Inactive 1 (Fig. 10f) and increased GSIS (Fig. 10g). These data reveal the in vivo anti-hyperglycemic action of SN-401 and its bioactive congeners require SWELL1-LRRC8 binding and thus support the notion of SWELL1-on-target activity in vivo.

An important feature of the hypothesized mechanism of action of SN-40X is that these active compounds bind to SWELL1-LRRC8 channel complexes in vivo in the ~100–500 nM range to augment SWELL1-dependent signaling (Figs. 2i, j, 8k–p and Supplementary Fig. S12a–d) without achieving the serum concentrations necessary for open channel SWELL1 current inhibition (~5–10 µM)32, followed by unbinding. As such, SWELL1 function may be rescued without significant SWELL1-LRRC8 or VRAC inhibition. Consistent with this hypothesis, in vivo pharmacokinetics (PK) of SN-401 and SN-406 in mice following i.p. or p.o. administration of 5 mg/kg of SN-401 or SN-406 reveals plasma concentrations that either transiently approach (Supplementary Fig. S13a, i.p. dosing), or remain well below IC50SWELL inhibitory concentrations (Supplementary Fig. S13b, p.o. dosing) while exceeding concentrations sufficient for induction of SWELL1 signaling activity (~100 nM) for 8–12 h. Importantly, SN-401 PK in a target tissue, adipose, also closely tracks serum concentrations via both i.p. and p.o. administration (Supplementary Fig. S13c). Finally, these in vivo PK studies demonstrate that SN-401 has high oral bioavailability (AUCp.o./AUCi.v. = 79%, Supplementary Table S7), and when administered via oral gavage to HFD-fed T2D C57 mice at 5 mg/kg/day SN-401 fully retains in vivo therapeutic efficacy (Supplementary Fig. S13d). Collectively, these PK data reveal that appropriate SN-401 concentrations are attained to achieve the observed therapeutic effect while remaining insufficient to inhibit activated VRAC.

Discussion

Our current working model is that the transition from compensated obesity (pre-diabetes, normoglycemia) to decompensated obesity (T2D, hyperglycemia) reflects, among other things, a relative reduction in SWELL1-dependent signaling in peripheral insulin-sensitive tissues30,33 and in pancreatic β-cell58,59—metabolically phenocopying SWELL1-loss-of-function models.24,26–30. This contributes to the combined insulin resistance and impaired insulin secretion associated with poorly-controlled T2D and hyperglycemia. SWELL1 forms a macro-molecular signaling complex that includes hetero-hexamers of SWELL1 and LRRC8b-e,22,23, with stoichiometries that likely vary from tissue to tissue. We propose SWELL1-LRRC8 signaling complexes are inherently unstable, and thus a proportion of complexes succumb to disassembly and degradation. Glucolipotoxicity and ensuing ER stress associated with T2D states provide an unfavorable environment for SWELL1-LRRC8 complex assembly, contributing to SWELL1 degradation and reductions in SWELL1 protein and SWELL1-mediated IC50SWELL observed in T2D in some tissues. We speculate that small molecules SN-401 and active congeners (SN-40X) serve as pharmacological chaperones32 to stabilize the formation of the SWELL1-LRRC8 complex. This reduces SWELL1 degradation and enhances the passage of SWELL1-LRRC8 heteromers through the ER and Golgi apparatus to the plasma membrane—thereby rectifying the SWELL1-deficient state in multiple metabolically important tissues in the setting of metabolic syndrome to improve glycemic control via both insulin sensitization24,25,30 and secretion28,29 mechanisms. Indeed, the concept of small molecule inhibitors acting as therapeutic molecular chaperones to support the folding, assembly, and trafficking of proteins (including ion channels) has been demonstrated for Niemann-Pick C disease and congenital hyperinsulinism (SUR1-KATP channel mutants).63–65. Similarly, in the case of congenital hyperinsulinism, the SUR1-KATP chemical chaperones are also themselves, paradoxically, KATP channel inhibitors63–65. Also, this therapeutic mechanism is analogous to small molecule correctors for another chloride channel, CFTR (VX-659/VX-445, Vertex Pharmaceuticals)55,56, which is proving to be a breakthrough therapeutic approach66,67 for cystic fibrosis. In vivo, we hypothesize SN-40X compounds bind to SWELL1-LRRC8
complexes in the closed state, within the concentration range between $C_{\text{max}}$ and ~100 nM. This shifts the balance toward maintaining stable SWELL1-LRRC8 complexes to preserve normal levels and localization (trafficking) within the T2D glucotoxic milieu. SN-40X may then unbind from the SWELL1-LRRC8 complex, thereby restoring insulin signaling in target tissues, and permitting SWELL1-mediated β-cell insulin secretion. This seemingly paradoxical mechanism may rely on the phasic SN-40X concentrations observed in vivo (see in vivo PK data) to allow for SN-401 binding, resultant chaperone-mediated rescue, followed by unbinding; as opposed to tonic SN-40X-SWELL1 binding. Another prediction of this model is that lower-affinity SN-40X compounds may be preferable to very high-affinity congeners, to provide the appropriate pharmacodynamics.
Fig. 6 SN-401 congener I_CSWELL inhibitory activity support predicted structural requirements for on-target activity. a–d I_CSWELL inward/outward current over time upon hypotonic (210 mOsm) stimulation and subsequent inhibition with 10 μM of SN-401 applied at 30 pA/pF outward I_CSWELL current density (a), Inactive 1 (b), Inactive 2 (c), and Inactive 3 (d) in HEK-293 cells. e Percentage inhibition of outward current blocked by SN-401/Inactive 1/Inactive 2/Inactive 3 at varying concentrations (n = 4–10 cells) in HEK-293 cells and the corresponding IC50 values. f–h I_CSWELL inward/outward current over time upon hypotonic (210 mOsm) stimulation and subsequent inhibition with 3 μM SN-401 applied at 30 pA/pF outward I_CSWELL current density (f), SN-406 (g), and SN-407 (h) in HEK-293 cells. i Percentage inhibition of outward current blocked by SN-401/SN-406/SN-407 at varying concentrations (n = 4–10 cells) in HEK-293 cells and the corresponding IC50 values. j Mean outward I_CSWELL current densities at +100 mV from HEK-293 (wildtype, n = 6) and R103E overexpression in HEK-293 (R103E O/E, n = 70) cells. k, l I_CSWELL inward/outward current over time upon hypotonic (210 mOsm) stimulation and subsequent inhibition with 10 μM of SN-401 applied at 30 pA/pF outward I_CSWELL current density in HEK-293 (wildtype, k) and at maximum current density in R103E expressing HEK-293 (R103E O/E, l) cells. m Percentage inhibition of outward current blocked by SN-401 at varying concentrations in wild-type and R103E O/E HEK-293 cells and the corresponding IC50 values (n = 4–10 cells). n, o I_CSWELL inward/outward current over time upon hypotonic (210 mOsm) stimulation and subsequent inhibition with 3 μM of SN-407 at 30 pA/pF outward I_CSWELL current density in HEK-293 (wildtype, n) and at maximum current density in R103E expressing HEK-293 (R103E O/E, o) cells. p Percentage inhibition of outward current blocked by SN-407 at different concentrations in wild-type and R103E O/E HEK-293 cells and the corresponding IC50 values (n = 4–10 cells). Data were presented as mean ± SEM. A two-tailed unpaired t-test was used in j.

Fig. 7 Cryo-EM of SN-407-SWELL1 complex. a–c Cryo-EM images revealing: Pose-1 (a), Pose-2 (b), and overlay of Pose 1 and 2 (c) views of selectivity filter with SN-407 bound from the membrane plane (side view) and top-down view. The atomic model is represented as ribbons and sticks within the cryo-EM density with three subunits removed in the side view for clarity. Cryo-EM density is represented in transparent gray, nitrogens are colored blue, oxygens red, chlorines green, protein carbons gray, and SN-407 carbons teal (Pose1) or orange (Pose-2).

required for unbinding and optimal therapeutic efficacy. Indeed, this mechanism is reminiscent of the paradoxical use of insulin secretagogue sulfonylurea receptor inhibitors as pharmacological chaperones to rescue K_A TP mutants in congenital hyperinsulinism by binding (and inhibiting) these mutant K_A TP channels63–65 and then unbinding, thereby favoring lower-affinity inhibitors: tolbutamide and carbamazepine, over glibenclamide.

Through structure-activity relationship (SAR), in silico molecular docking studies, and cryo-EM studies, we identified hotspots on opposing ends of the SN-401 molecule that interact with separate regions of the SWELL1-LRRC8 complex: the carboxylate group with R103 in multiple LRRC8 monomers; functioning like a molecular staple or tether to bind loosely associated SWELL1-LRRC8 monomers (especially in the setting of T2D) into a more stable hexameric structure. Indeed, the cryo-EM structure obtained in lipid nanodisks of SN-40131 and derivative SN-407 supports hypothesized binding models of SN-40X with SWELL1 homomer.

Another advantage provided by SAR studies was the identification and synthesis of SN-401 congeners that removed (Inactive 1, 2 and 3) or enhanced (SN-403/406/407) SWELL1 binding, as these provided powerful tools to query SWELL1-on-target activity directly in vitro and in vivo, and also validated the proof-of-concept for developing SN-401 congeners with enhanced efficacy. Indeed, this approach was necessary to prove SWELL1-LRRC8 on-target activity of the SN-40X series in vivo, because SWELL1-LRRC8 is expressed broadly in numerous insulin-sensitive tissues and in islet cells. As the global SWELL1/LRRC8a KO mouse is essentially embryonically lethal20 testing SN-40X compounds in global SWELL1−/− mice is not possible, and generating multi-tissue (adipose, liver, skeletal muscle, and β-cell) SWELL1 KO mice is outside the scope of the current study. Therefore, using the SAR to generate SWELL1-LRRC8 inactive compounds (Inactive 1–3) as a negative control provided an
alternative approach to prove in vivo on-target activity on a broadly expressed signaling molecule. In addition to this medicinal chemistry approach to test on-target activity in vitro and in vivo, we found that SN-40X mediated induction of AKT-AS160 and AKT-eNOS signaling requires SWELL1 in cultured adipocytes, hepatocytes, and HUVECs. Moreover, SN-401 mediated rescue of islet insulin secretion under glucolipotoxic conditions in vitro also requires SWELL1. Finally, it is important to note that the studies demonstrating promiscuity of SN-401/DCPIB with other ion channel targets all applied DCPIB at ~10–200 µM. This is 100–200-fold higher than the concentrations required to potentiate SWELL1-dependent signaling in vitro (Figs. 2i, j, 8k–p and Supplementary Fig. S12a–d), and similarly higher than SN-401 and SN-406 concentrations.
Fig. 8 SN-401 and SWELL1-active congener SN-406 inhibit l_{SWELL} and promote SWELL1-dependent signaling at sub-micromolar concentrations. a-d Representative l_{SWELL} inward/outward current traces over time in HEK-293 cells preincubated for 30 min with vehicle, SN-401, SN-406, Inactive 1, or Inactive 2 at 1 µM (a) and vehicle, SN-401, and SN-406 at 250 nM (c), and subsequent hypotonic stimulation with the compound. Fold changes in mean outward l_{SWELL} current density at +100 mV for 7 min timepoint after hypotonic stimulation are shown in b. e, f Representative western blots for SWELL1 and β-actin (e) and densitometry (f) from 3T3-F442A adipocytes treated with vehicle (n = 8), SN-401 (n = 10), SN-406 (n = 6), or Inactive 1 (n = 3) at 10 µM for 96 h. g, h Representative western blots for SWELL1 and β-actin (g) and densitometry (h) from 3T3-F442A adipocytes treated with vehicle (n = 5), SN-401 (n = 5), SN-406 (n = 6), Inactive 1 (n = 5), or Inactive 2 (n = 4) at 1 µM for 96 h. i, j Representative immunostaining images demonstrating endogenous SWELL1 localization in 3T3-F442A preadipocytes treated with vehicle or SN-401/SN-406/Inactive 1 at 10 µM for 48 h (Scale 20 µm) (i) and mean SWELL1 membrane versus cytoplasm localization fraction from vehicle (n = 19), SN-401 (n = 21), SN-406 (n = 13 for 1 µM and 10 µM), or Inactive 1 (n = 9 for 1 µM and n = 13 for 10 µM) treated cells (j). k-l Representative western blots for SWELL1, p-eNOSSer1177, eNOS, pAKTThr308/Tyr387, AKT2, and β-actin in HUVEC cells treated with vehicle or 100 nM SN-406 for 96 h (k) and densitometry (l, n = 6). m, n Representative western blots for SWELL1, p-eNOSSer1177, eNOS, pAKTThr308/Tyr387, AKT2, and β-actin in HUVEC cells treated with vehicle or 500 nM Inactive 1 for 96 h (m) and densitometry (n, n = 6). o, p Representative western blots for p-eNOSSer1177, eNOS, and β-actin in HUVEC cells treated with vehicle or different concentrations of SN-401 for 96 h (o, n = 6) and densitometry (p). Data were represented as mean ± SEM. Two-tailed unpaired t-test was used in f and h (compared to vehicle), i, j, n. One-way ANOVA was used for b, d, j, and p. *, **, and *** represents p < 0.05, p < 0.01, and p < 0.001. ns not significant.

predominantly attained in vivo (Supplementary Fig. S13a–c) to achieve a therapeutic effect (Figs. 3, 4, 10). Accordingly, these studies are not applicable with respect to putative off-target mechanisms for the therapeutic effects observed from SN-40X compounds. SWELL1-LRR8 complexes are broadly expressed in multiple tissues, and consist of unknown combinations of SWELL1, LRR8b, LRR8c, LRR8d, and LRR8e, indicating SWELL1 complexes may be enormously heterogeneous. However, SWELL1-LRR8 stabilizers like SN-401 may be designed to target many, if not all, possible channel complexes since all will contain the elements necessary for SN-401 binding: at least one R103 (from the requisite SWELL1 monomer: carboxyl group binding site), and the nature of the hydrophobic cleft (cyclcopentyl binding site), which is conserved among all LRR8 monomers. Indeed, traced glucose clamps did reveal insulin sensitization effects in vivo in multiple tissues, including adipose, skeletal muscle, liver, and heart, and this was supported by additional signaling studies in vitro in cultured adipocytes, HUVECs, primary hepatocytes, and isolated islets, as well as in adipose tissue and skeletal muscle. The increased glucose uptake in the heart is particularly interesting, since this may provide salutary effects on cardiac energetics that could favorably impact both systolic (HFReEF) and diastolic (HFpEF) function in diabetic cardiomyopathy, and thereby potentially improve cardiac outcomes in T2D, as observed with SGLT2 inhibitors74–79. Moreover, the finding that SN-401 robustly increases serum FGF21 levels may provide an additional secondary metabolic molecular mechanism for the observed improvements in glucose metabolism, and hepatic steatosis observed in these SN-401 treated T2D models and warrants future investigation.

The current study provides an initial proof-of-concept for pharmacological induction of SWELL1 signaling using SWELL1 modulators (SN-40X) to treat metabolic diseases at multiple homeostatic nodes, including adipose, skeletal muscle, liver, and pancreatic β-cell, whereby SN-40X compounds function to restore both insulin sensitivity and insulin secretion. Hence, SN-401 may represent a tool compound from which a novel drug class may be derived to treat T2D, NAFLD, and other metabolic diseases.

Methods. The University of Iowa Institutional Review Board (approval number 201103721) and the Human Research Protection Office at Washington University School of Medicine in St. Louis, MO (approval number 201808128) approved the studies involving human adipose samples. Written, informed consent was obtained from all participants before participating in this study. Information on participant’s sex, age, body mass index (BMI), HbA1c, and random or fasting plasma glucose concentration was obtained either during an initial screening visit or medical records. Visceral adipose tissue was obtained from esophageal or omental adipose tissue depots in participants with obesity undergoing bariatric surgeries and in normal-weight, control subjects scheduled for non-bariatric surgeries. Fat tissue was kept in ice-cold PBS and transferred to the laboratory within 20 min for patch-clamp experiments, or snap-frozen in liquid nitrogen to assess adipose tissue SWELL1 protein expression.

Animals. All experimental procedures involving mice were approved by the Institutional Animal Care and Use Committee of the University of Iowa and Washington University at St. Louis and performed in accordance with ethical regulations. All C57BL/6 mice involved in this study were purchased from Charles River Labs or Taconic Biosciences. Both KK.Cg-Ay/J (KKAy) and KK.Cg-Aa/J (KKAa) mice involved in the study were obtained from Jackson Labs (Stock No. 0002468). Liver-specific SWELL1 knockout mouse was generated by crossing Albumin-Cre (Jax stock No: 003574) with the SWELL1 allelesmouse24. Mice were gender/age-matched and bred up for experiments. All mice were fed ad libitum with either regular chow (RC, NIH31 irradiated, #7913 or Lab diet, Picolab@ Rodent Diet 20, #5053) or a high-fat diet (Research Diets, Inc., 60 kcal% fat) with free access to water and housed in a 12 h light/dark cycle-, temperature-, and humidity- controlled room. For high-fat diet (HFD) studies, only male mice were used and were started on an HFD regimen at the age of 6–9 weeks. For all experiments involving KKAa and KKAa mice, both males and females were used at ~30/50 ratio. In all experiments, investigators were kept blinded for different treatment groups in mice both during the experiment and subsequent analysis.

Small molecule treatment. All compounds were dissolved in Kolliphor® EL (Sigma, #CS5135). Either vehicle (Kolliphor® EL), SN-401 (DCPIB, 5 mg/kg of body weight/day, Tocris, D1540), SN-403, SN-406, SN-407, or Inactive 1 were administered i.p. as indicated using 1cc syringe/26 × 1/2 inch needle daily for 7–10 days. In one experiment, SN-401 was administered daily for 8 weeks. SN-401, formulated as above, was also administered by oral gavage at 5 mg/kg/day for 5 days using a 20 G × 1.5 inch reusable metal gavage needle.

Adenovirus. Human adenoviruses type 5 with hLRRC8A/SWELL1-shRNA (Ad5-mCherry-U6-hLRRC8A/SWELL1-shRNA, 2.2 × 10^10 PFU/ml), a scrambled non-targeting control (Ad5-U6-scramble-mCherry, 1.0 × 10^10 PFU/ml), Ad5-CAG-LoxP-Stop-LoxP-3XFlag-SWELL1 (1 × 10^10 PFU/ml), β-cell-targeted adenovirus type 5 with Ad5-RIP2-GFP (4.1 × 10^10 PFU/ml), GCaMP6s (Ad5-RIP1-GCaMP6s, 4.9 × 10^10 PFU/ml), and GCaMP6s 2A-fCre (Ad5-GCaMP6s-RFP-2A-fCre, 5.8 × 10^9 PFU/ml) were obtained from Vector Biolabs. Adenovirus type 5 with Ad5-CMV-Cre-eGFP (8 × 10^9 PFU/ml) and Ad5-CMV-Cre-mCherry (3 × 10^10 PFU/ml) were obtained from the University of Iowa Viral Vector Core.

Cell culture. 3T3-F442A preadipocytes (Sigma, #00070654) were maintained in 90% DMEM (25 mM d-Glucose and 4 mM l-Glutamine) containing 10% fetal bovine serum (FBS) and 100 IU penicillin and 100 µg/ml streptomycin on collagen-coated (rat tail type I collagen, Corning) plates at 37 °C and 5% CO2. Upon reaching confluency, the cells were differentiated in the above-mentioned media supplemented with 5 µg/ml insulin (Cell Applications) and replenished every other day with the differentiation media. For insulin signaling studies on WT and KO adipocytes with or without SWELL1 overexpression (O/E), the cells were differentiated for 10 days and transduced with Ad5-CAG-LoxP-Stop-LoxP-SWELL1-3XFlag virus (MOI 12) on day 11 in 2% FBS containing differentiation medium. To induce the overexpression, Ad5-CMV-Cre-eGFP (or mCherry) (MOI 12) was added on day 13 in 2% FBS containing a differentiation medium. The cells were then switched to 10% FBS containing differentiation medium from day 15 to 17. On day 18, the cells were
starved in serum-free media for 6 h and stimulated with 0 and 10 nM insulin for 15 min. Either Ad5-CAG-LoxP-stop-LoxP-SWELL1-3XFlag or Ad5-CMV-Cre-eGFP (or mcherry) virus transduced cells alone were used as controls. Based on GFP/mcherry fluorescence, viral transduction efficiency was ~90%.

For SN-401 treatment and insulin signaling studies in 3T3-F442A preadipocytes, the cells were incubated with either vehicle (DMSO) or 10 µM SN-401 for 96 h. The cells were serum-starved for 6 h with vehicle (DMSO) or SN-401 and washed with PBS three times and stimulated with 0, 3, and 10 nM insulin-containing media for 15 min prior to collecting lysates. In the case of 3T3-F442A adipocytes, the WT and KO cells were treated with either vehicle (DMSO), 1 or 10 µM SN-40X (after 7–11 days of differentiation) for 96 h and then stimulated with 0 and 10 nM insulin/serum-containing media with vehicle (DMSO) or SN-40X for 15 min for SWELL1 detection. For AKT and AS160 signaling, the WT and KO cells were treated with either vehicle (DMSO) or 500 nM SN-401 for 96 h and serum-starved in the presence of vehicle or SN-401 (500 nM) for 6 h. The cells were washed twice in hypotonic buffer (240 mOsm; 90 mM NaCl, 5 mM NaHCO₃, 4.8 mM KCl, 1.2 mM KH₂PO₄, 2.5 mM CaCl₂, 2.4 mM CaCl₂).
MgSO₄, 10 mM HEPES, and 25 mM Glucose, pH 7.4) and then incubated at 37 °C in a hypotonic buffer for 10 min followed by a serum-free media wash and subsequent stimulation with insulin- or serum-containing media for 30 min at 37 °C without SN-401 (or vehicle). To simulate glucothropoxia, 8 mM sodium palmitate was dissolved in 15 mL cold DMEM plus 1% P/S media three times. Cells were checked for viability by diluting 1:2 with Trypan blue and batches with >80% viability were selected. The cells were then passed through a 100 µm gel 60 F254, Merck) and visualized under UV light. Flash chromatography was performed using silica gel 60 as stationary phase performed under positive air pressure. 1H NMR spectra were recorded in CDCl₃ on a Bruker Avance spectrometer operating at 300 MHz at ambient temperature unless otherwise noted. All peaks are reported in ppm on a scale downfield from TMS and using the residual solvent peak in CDCl₃, (δ H = 7.26 ppm) or TMS (δ H = 0.00 ppm) as an internal standard. Data for 1H NMR are reported as follows: chemical shift (ppm, scale), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and/or multiplet resonances, dd = double of doublets, dt = double of triplets, br = broad), coupling constant (Hz), and integration. All high-resolution mass spectra (HRMS) were measured on Waters Q TOF Premier mass spectrometer using electrospray ionization (ESI) time-of-flight (TOF).

2-cyclopentyl-1-(2,3-dichloro-4-methoxyphenyl)ethan-1-one (3): Cyclopentyl acetyl chloride (15 g, 102 mmol, 1.1 equiv.) was added to a stirring solution of aluminum chloride (13.64 g, 102 mmol, 1.1 equiv.) in dichloromethane (250 ml) at 0 °C and the resulting solution was allowed to stir for 3 h at ambient temperature. After this was added a solution of 2, 3-dichloro anisole (16.46 g, 92.9 mmol, 1 equiv.) in dichloromethane (50 ml) at 0 °C, and the resulting solution was allowed to warm to room temperature and stirred for 16 h.

Molecular docking
SN-401 and its analogs were docked into the expended electrified state structure of an SWELL1-SN-401 homo-hexamer in MSP1E3D1 nanodisc (PDB ID: 6NZZ, https://www.rcsb.org/structure/6NZZ) using Molecular Operating Environment (MOE) 2016.08 software package [Chemical Computing Group (Montreal, Canada)]. The 3D structure obtained from PDB (PDB ID: 6NZZ) was prepared for docking by first generating the missing loops using the loop generation functionality in the Yasara software package followed by sequentially adding hydrogens, adjusting the 3D protein orientation, and performing energy minimization using Amber10 force-field in MOE. The ligand structures to be docked were prepared by adjusting partial charges followed by energy minimization using Amber10 force-field. The site for docking was defined by selecting the protein residues within 5 Å from a co-crystallized ligand (SN-401). Docking parameters were set as Placement: London DC; Retain P: 0; Refinement: Rigid Receptor; Re-scoring function: GBVI/WSA dG; Retain poses: 5. Binding poses for the compounds were predicted using the above-validated docking algorithm.

Chemical Synthesis
General information. All commercially available reagents and solvents were used directly without further purification unless otherwise noted. Reactions were monitored either by thin-layer chromatography (carried out on silica plates, silica gel 60 F254 Merck) and visualized under UV light. Flash chromatography was performed using silica gel 60 as stationary phase performed under positive air pressure. 1H NMR spectra were recorded in CDCl₃ on a Bruker Avance spectrometer operating at 300 MHz at ambient temperature unless otherwise noted. All peaks are reported in ppm on a scale downfield from TMS and using the residual solvent peak in CDCl₃, (δ H = 7.26 ppm) or TMS (δ H = 0.00 ppm) as an internal standard. Data for 1H NMR are reported as follows: chemical shift (ppm, scale), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and/or multiplet resonances, dd = double of doublets, dt = double of triplets, br = broad), coupling constant (Hz), and integration. All high-resolution mass spectra (HRMS) were measured on Waters Q TOF Premier mass spectrometer using electrospray ionization (ESI) time-of-flight (TOF).
2.43 – 2.22 (m, 1H), 1.96 (s, 1H), 1.73 – 1.48 (m, 5H), 1.46 – 1.33 (m, 1H), 1.17 – 1.00 (m, 1H). LRMS (ESI), m/z calcd for C15H17Cl2O2 [M + H]+ 299.0605, found 299.0614.

2-butyl-6,7-dichloro-2-cyclopentyl-5-methoxy-2,3-dihydro-1H-inden-1-one (5): A stirring suspension of 4 (23 g, 76.8 mmol, 1 equiv.) in anhydrous tert-butanol (220 ml) was allowed to reflux at 95 °C for 30 min. To the resulting solution was added potassium tert-butanol (1 M in tert-butanol) (84 ml, 84.5 mmol, 1.1 equiv.) and the resulting solution was refluxed for 30 min. The reaction was then cooled to room temperature followed by addition of iodobutane (44.2 ml, 384 mmol, 5 equiv.) and the reaction was then allowed to reflux for additional 60 min. The reaction was allowed to cool, concentrated and purified by silica gel chromatography using 0–10% ethyl acetate in hexanes as eluent to furnish compound 5 as clear oil (17.75 g, 65%). 1H NMR (300 MHz, CDCl3) δ 6.89 (s, 1H), 4.09–3.90 (m, 3H), 2.98–2.70 (m, 2H), 2.36–2.18 (m, 1H), 1.89–1.71 (m, 1H), 1.58–1.42 (m, 5H), 1.33–1.09 (m, 4H), 1.09–0.94 (m, 2H), 0.93–0.73 (m, 4H).

HRMS (ESI), m/z calcd for C19H25Cl2O2 [M + H]+ 355.1231, found 355.1231.

2-butyl-6,7-dichloro-2-cyclopentyl-5-hydroxy-2,3-dihydro-1H-inden-1-one (6): To 5 (3.14 g, 8.87 mmol, 1 equiv.) was added aluminum chloride (2.36 g, 17 mmol, 2 equiv.) and sodium iodide (2.7 g, 17 mmol, 2 equiv.) and the resulting solid mixture was triturated and allowed to stir at 70 °C for 60 min. Once complete, the reaction was diluted with dichloromethane and washed with aqueous saturated sodium thiosulfate solution. The organic fractions were collected and concentrated
to give a beige solid which was then washed multiple times with hexanes to provide compound 6 as white solid (2.87 g, 95%).1H NMR (300 MHz, CDCl3) δ 7.03 (s, 1H), 6.32 (s, 1H), 2.97 – 2.73 (m, 2H), 2.36 – 2.17 (m, 1H), 1.88 – 1.68 (m, 2H), 1.62 – 1.39 (m, 6H), 1.31 – 1.11 (m, 3H), 1.08 – 0.97 (m, 2H), 0.97 – 0.87 (m, 2H), 0.83 (t, J = 7.3 Hz, 3H). HRMS (ESI), m/z calcd for C18H23Cl2O2 [M+H]+ 469.1912, found 469.1906. See Supplementary Fig. S14a. 2-(butyl-6,7-dichloro-2-cyclopentyl-1-oxo-2,3-dihydro-1H-inden-5-yl)-3-ethyl-1H-inden-5-yl)acetate (7) (Inactive 1): To a stirring solution of 6 (170 mg, 0.50 mmol, 1 equiv.) in anhydrous dimethylformamide (1 ml) was added potassium carbonate (1.1 equiv.) and the reaction was allowed to stir at 60 °C for 2 h. Once complete, the reaction was diluted with dichloromethane and washed multiple times with hexanes to provide Inactive 1 as a clear solid (114 mg, 88%).1H NMR (300 MHz, CDCl3) δ 6.85 (s, 1H), 4.21 (t, J = 5.9 Hz, 2H), 2.88 (t, J = 14.4 Hz, 2H), 2.69 (t, J = 7.0 Hz, 2H), 2.26 (dd, J = 12, 6, 1.1 Hz, 3H), 1.87 – 1.73 (m, 2H), 1.64 – 1.44 (m, 6H), 1.35 – 1.10 (m, 4H), 1.08 – 0.95 (m, J = 15.0, 7.7 Hz, 2H), 0.82 (t, J = 7.3 Hz, 3H). HRMS (ESI), m/z calcd for C24H23Cl2O4 [M+H]+ 434.1075, found 434.1089. 5-(((2-butyl-6,7-dichloro-2-cyclopentyl-1-oxo-2,3-dihydro-1H-inden-5-yl)oxy) methyl)-2,3-dihydro-1H-inden-5-yl)butanoic acid (8) (SN-401): To a stirring solution of 6 (100 mg, 0.29 mmol, 1 equiv.) in anhydrous dimethylformamide (1 ml) was added potassium carbonate (45 mg, 0.32 mmol, 1 equiv.) and ethyl 4-bromobutyrate (46 mg, 0.32 mmol, 1 equiv.) and the reaction was allowed to stir at 60 °C for 2 h. Once complete, the reaction was diluted with dichloromethane and washed multiple times with hexanes to provide SN-401 as a clear solid (111 mg, 89%).1H NMR (300 MHz, CDCl3) δ 6.86 (s, 1H), 6.21 (t, J = 5.9 Hz, 2H), 2.88 (t, J = 14.4 Hz, 2H), 2.69 (t, J = 7.0 Hz, 2H), 2.26 (dd, J = 12, 6, 1.1 Hz, 3H), 1.87 – 1.73 (m, 2H), 1.64 – 1.44 (m, 6H), 1.35 – 1.10 (m, 4H), 1.08 – 0.95 (m, J = 15.0, 7.7 Hz, 2H), 0.82 (t, J = 7.3 Hz, 3H). HRMS (ESI), m/z calcd for C21H23BCl2O4 [M+H]+ 416.1300, found 416.1311. 2-(butyl-6,7-dichloro-2-cyclopentyl-1-oxo-2,3-dihydro-1H-inden-5-yl)-3-(3-ethyl-1H-inden-5-yl)-hexanoic acid (9) (SN-403): To a stirring solution of 6 (100 mg, 0.29 mmol, 1 equiv.) in anhydrous dimethylformamide (1 ml) was added potassium carbonate (45 mg, 0.32 mmol, 1 equiv.) and ethyl 6-bromohexanoate (51 mg, 0.32 mmol, 1 equiv.) and the reaction was allowed to stir at 60 °C for 2 h. Once complete, to the reaction was added 4 N NaOH (1 ml) and the reaction was allowed to stir at 100 °C for 60 min. Once complete, reaction was concentrated and purified by column chromatography using 0–10% methanol in dichloromethane as eluent to provide SN-403 as a clear solid (114 mg, 89%).1H NMR (300 MHz, CDCl3) δ 6.89 (s, 1H), 6.10 (t, J = 5.9 Hz, 2H), 2.88 (t, J = 14.4 Hz, 2H), 2.69 (t, J = 7.0 Hz, 2H), 2.26 (dd, J = 12, 6, 1.1 Hz, 3H), 1.87 – 1.73 (m, 2H), 1.64 – 1.44 (m, 6H), 1.35 – 1.10 (m, 4H), 1.08 – 0.95 (m, J = 15.0, 7.7 Hz, 2H), 0.82 (t, J = 7.3 Hz, 3H). HRMS (ESI), m/z calcd for C24H24Cl2O4 [M+H]+ 469.1912, found 469.1906. See Supplementary Fig. S14b. 5-(((2-butyl-6,7-dichloro-2-cyclopentyl-1-oxo-2,3-dihydro-1H-inden-5-yl)oxy) methyl)-2,3-dihydro-1H-inden-5-yl)propionic acid (10) (SN-406): To a stirring solution of 6 (100 mg, 0.29 mmol, 1 equiv.) in anhydrous dimethylformamide (1 ml) was added potassium carbonate (45 mg, 0.32 mmol, 1 equiv.) and ethyl 6-bromohexanoate (55 mg, 0.39 mmol, 1.1 equiv.) and the reaction was allowed to stir at 60 °C for 2 h. Once complete, the reaction was added 4 N NaOH (1 ml) and the reaction was allowed to stir at 100 °C for 60 min. Once complete, reaction was concentrated and purified by column chromatography using 0–10% methanol in dichloromethane as eluent to provide SN-406 as a clear solid (110 mg, 89%).1H NMR (300 MHz, CDCl3) δ 6.86 (s, 1H), 6.12 (t, J = 5.9 Hz, 2H), 2.88 (t, J = 14.4 Hz, 2H), 2.69 (t, J = 7.0 Hz, 2H), 2.26 (dd, J = 12, 6, 1.1 Hz, 3H), 1.87 – 1.73 (m, 2H), 1.64 – 1.44 (m, 6H), 1.35 – 1.10 (m, 4H), 1.08 – 0.95 (m, J = 15.0, 7.7 Hz, 2H), 0.82 (t, J = 7.3 Hz, 3H). HRMS (ESI), m/z calcd for C22H24Cl2O4 [M+H]+ 469.1912, found 469.1906. The intracellular solution contained (in mM): 120L-aspartic acid, 20 CsCl, 1 MgCl2, 1 CaCl2, 10 HEPES, 10 mannitol, pH 7.4 with NaOH (210 mOsm/kg). The swell-activated calcium entry was measured using 0–10 mM butanoic acid as a swell-activated calcium entry indicator. Data were acquired using pClamp 10.4 software. For hypotonic swelling, extracellular solution was composed of the following (in mM): 90 Na2C2O3, 1 MgCl2, 1 CaCl2, 10 HEPES, 10 mannitol, pH 7.4 with NaOH (210 mOsм/kg). The isotonic extracellular solution consisted of the same composition above but with 110 mM instead of 10 mM mannitol (300 mOsм/kg). For hypertonic swelling, extracellular solution was composed of the following (in mM): 120 l-Aspartic acid, 20 CaCl2, 1 MgCl2, 5 EGTA, 10 HEPES, 5 MgATP, 120 CaOH, 0.1 GTP, pH 7.2 with CaOH and had an osmolality of 280–290 mOsм/kg. The patch electrodes were prepared from borosilicate glass capillaries (WRPI) and had a resistance of 2.5–4.8 MΩ when filled with pipette solution. For perforated-patch recordings in isolated human adipocytes, the intracellular solution was as above but without ATP and GTP, and contained 360 mg/ml Amphoterica B (Sigma). The holding potential was 0 mV. NATURE COMMUNICATIONS | https://doi.org/10.1038/s41467-022-28435-0 | www.nature.com/naturecommunications 20
Voltage ramps from $-100$ mV to $+100$ mV (at $0.4$ mV/s) were applied every $4$ s. The sampling interval was $100$ µs and filtered at $10$ kHz. For perforated-patch recordings, internal and extracellular resistance below $1$ GΩ or access resistance above $20$ MΩ were discarded.

3T3-F442A WT and KO preadipocytes were prepared as described in the Cell culture section above. For SWELL1 overexpression recordings, preadipocytes were first transfected with Ad5-CAG-Lex3-stop-Lex3-3Xflag-SWELL1 (MOI 12) in $2\%$ FBS culture medium for $2$ days and then overexpression induced by adding Ad5-CMV-Cre-eGFP (MOI 10-12) in $2\%$ FBS culture medium for $2$ more days and changed to $10\%$ FBS containing culture media and were selected based on GFP expression ($\pm 2$ days).

For measurements, islets were obtained from mice on a regular chow diet between $8$–$13$ weeks of age. For these islets, four had an average body weight of $28.6 \pm 0.51$ g and a glucose blood level of $148 \pm 6.4$ mg/dL respectively. T2D islets were obtained from mice fed with HFD for $4\text{ months}$ and their average body weight and glucose levels were $32.7 \pm 3.0$ g and $229 \pm 21.4$ mg/dL respectively.

For measuring IC_{SWELL} inhibition, which was calculated based on the following equation: (Peak current – current after compound application for $3\text{ min}$)/(peak current – baseline current)$\times 100$. See also Fig. 6 for a schematic representation. For consistency among recordings, SN-401, SN-40X compounds, and Inactive 1 were applied at varying concentrations (1–$5\mu M$) and $10\mu M$ to assess for $\text{IC}_{SWELL}$ inhibition, which was calculated as described above: (Peak current – current after compound application for $3\text{ min}$)/(peak current – baseline current)$\times 100$. See also Fig. 6 for a schematic representation.

For metabolism, mice were fasted for $6\text{ h}$ prior to glucose tolerance tests (GTT). Baseline glucose levels at $0\text{ min}$ time point (fasting glucose, FG) were measured from a blood sample collected from tail snipping using glumeter (Boehringer Mannheim). Either basal blood glucose (BG) and insulin (IN) levels were measured for $5\text{ or }7\text{, }15,\text{ 30, 60, and }90\text{ min}$ time points after injection of insulin. For GTTs, the mice were fasted for $4\text{ h}$. Similar to GTTs, the baseline glucose levels were measured and $15,\text{ 30, and }60\text{ min}$ time points (injection (p.) of insulin (HumulinR, 1 U/kg bodyweight for lean mice or 1.25 U/kg body weight for HFD mice). GTTs or ITTs with vehicle (or SN-401, SN-403, SN-406, and SN-407, and Inactive 1) treated groups were performed approximately $24\text{ h}$ after the last injection. For measuring serum insulin levels, the vehicle (or SN-401, SN-406, and Inactive 1) treated HFD mice were fasted for $6\text{ h}$ and injected (i.p.) with $0.75\% \text{g} \cdot \text{g}^{-1}\text{body mass}$ and blood samples were collected at $0, 7, 15,\text{ and }30\text{ min}$ time points in microvette capillary tubes (SARSTEDT, #16,444) and centrifuged at $2000 \times g$ for $20\text{ min}$ at $4\text{ °C}$. The collected plasma was then measured for insulin content by using Ultra-Sensitive Mouse Insulin ELISA Kit (CrystalChem, #98080). All mouse studies were performed in a blinded fashion. Body weights for all the mice are listed in the supplementary table.

**Adipose tissue morphology and size quantification.** Mice under epidural white adipose tissue (eWAT) was isolated and fixed in $10\%$ neutral buffer formalin for $24\text{ h}$ at $4\text{ °C}$. The formalin-fixed eWAT tissue was washed twice with $2X$ buffer and dehydrated in a series of ethanol gradient. The dehydrated tissue was embedded in paraffin wax and $10\mu m$ sections were cut by automatic microtome and mounted on positively charged slides. Hematoxylin and eosin staining of the slides was performed for histological evaluation. Images (Leica DM5500B) of the paraffin sections were captured with Leica DFC450C camera (Leica Microsystems) and analyzed using ImageJ software.

**Metabolic phenotyping.** For patch-clamp studies involving primary mouse β-cells, mice were anesthetized by injecting Avertin (0.0125 g/ml in H₂O) followed by cervical dislocation. HFD or polygenic KK-Ay mice treated with vehicle or SN-401, SN-406, SN-407, and Inactive 1 were anesthetized with $1–4\%$ isoflurane followed by cervical dislocation. The pancreas was perfused via the common bile duct with $2–3\text{ ml}$ HBSS containing type V collagenase (0.8 mg/ml), removed, and digest at $37\text{ °C}$ for $10\text{ min}$. Islets were then dissociated by gentle agitation, washed in RPMI containing $1\%$ FBS, and purified on Histopaque 1077 (Sigma-Aldrich) followed by centrifugation at $1500 \times g$ for $30\text{ min}$ at $4\text{ °C}$. Islets were further washed in $1X$ Iscove’s Modified Dulbecco’s Medium and transferred to a round bottom tube with a culture medium for short-term culture ($<$4 h) for GSIS experiments, islets were sorted for equal size and cultured in $24\text{-well}$ plates. For isolation of primary β-cells, sorted islets were further incubated in trypsin for $5\text{ min}$, dispersed into single cells, and then transferred to Matrigel-coated coverslips for patch-clamp.

**Islet isolation and perfusion assay.** For patch-clamp studies involving primary mouse β-cells, mice were anesthetized by injecting Avertin (0.0125 g/ml in H₂O) followed by cervical dislocation. HFD or polygenic KK-Ay mice treated with vehicle or SN-401, SN-406, SN-407, and Inactive 1 were anesthetized with $1–4\%$ isoflurane followed by cervical dislocation. The pancreas was perfused via the common bile duct with $2–3\text{ ml}$ HBSS containing type V collagenase (0.8 mg/ml), removed, and digest at $37\text{ °C}$ for $10\text{ min}$. Islets were then dissociated by gentle agitation, washed in RPMI containing $1\%$ FBS, and purified on Histopaque 1077 (Sigma-Aldrich) followed by centrifugation at $1500 \times g$ for $30\text{ min}$ at $4\text{ °C}$. Islets were further washed in $1X$ Iscove’s Modified Dulbecco’s Medium and transferred to a round bottom tube with a culture medium for short-term culture ($<$4 h) for GSIS experiments, islets were sorted for equal size and cultured in $24\text{-well}$ plates. For isolation of primary β-cells, sorted islets were further incubated in trypsin for $5\text{ min}$, dispersed into single cells, and then transferred to Matrigel-coated coverslips for patch-clamp.

**Cadicaveric human islets were obtained from the Integrated Islet Distribution Program (IDP), Prodo Laboratories, and the Alberta Diabetes Institute Islet Core. The human islets obtained from the Integrated Islet Distribution program and Prodo laboratories were exempt from Institutional Review Board approval under 45 CFR 46.101 (b) category (4) and 45 CFR 46.102(h), respectively. Human islets were obtained from the Alberta Diabetes Institute IsletCore at the University of Alberta in Edmonton (http://www.bccll/adi/isletcore.html) with the assistance of the Human Organ Procurement and Exchange (HOPE) program, Trillium Gift of Life Network (TGLN), and other Canadian organ procurement organizations. Islet isolation was approved by the Human Research Ethics Board at the University of Alberta. All research protocols were reviewed and approved for the use of pancreatic tissue in research. Human islets were cultured in RPMI media with $2\%$ FBS overnight. The next day each scramble or shSWELL1 adenoviral transduction...
was carried out (final concentration of 5 × 10⁴ PFU/ml) and the islets were incubated for 12 h. The islets were then washed with 1XPBS three times and cultured with fresh 10% FBS RPMI medium. For SNF experiments, human islets were either transduced with an adenoviral short hairpin for control (shScramble) or SWELL1 knockdown (shSWELL1), and murine islets isolated from floxed-SWELL1 mouse (swSWELL1) were either transduced with adenoviral control (Ad-RIP1-GCaMP6s) or Cre-recombinase (Ad-RIP1-GCamP6s-2A-Cre) virus for 12 h, respectively, in 2% FBS containing RPMI media. The islets were then washed in 1XPBS three times and treated with either vehicle or SN-401 (10 μM) for 96 h followed by treatment with 1:3 palmate:BSA with or without SN-401 in 10% FBS containing RPMI media for 16 h (Fig. 3c). The GSIS perfusion assay for islets were performed using a PERA-02 from Biorep Technologies. For each experiment, around 50 freshly isolated islets (all from the same isolation batch) were handpicked to match the size of islets across the samples and loaded into the polycarbonate perfusion chamber between two layers of polycrylamide-microbead slurry (Bio-Gel P-4, Bio-Rad) by the same experienced operator. Perfusion buffer contained (in mM): 120 NaCl, 24 NaHCO₃, 4.8 KCl, 2.5 CaCl₂, 1.2 MgSO₄, 10 HEPS, 2.8 glucose, 27.2 mannitol, 0.25% w/v bovine serum albumin, pH 7.4 with NaOH (300 mOsm/kg). Perfusion buffer kept at 37 °C was circulated at 120 μl/min. After 48 min of washing with 2.8 mM glucose solution for stabilization, islets were stimulated with the following sequence: 16 min of 16.7 mM glucose followed by 8 mM glucose, 10 min of 16.7 mM glucose, and 12.8 μM glucose. Osmolarity was matched by adjusting NaCl concentration when preparing a solution containing 16.7 mM glucose. Serial samples were collected either every 1 or 2 min into 96 wells kept at 4 °C. Insulin concentrations were further determined using a commercially available ELISA kit (Mercodia). The area under the curve (AUC) for the high-glucose-induced insulin release was calculated for times 45–54, 54–63, and 63–72 h. After the completion of the experiment, islets were further lysed by the addition of RIPA buffer and the amount of insulin was detected by ELISA.

**Adiponegin and FGFI21 measurements.** Polymeric-T2D KKA mice (males) between 11–12 weeks old were treated with either vehicle or SN-401 (5 mg/kg/day, i.p.) for 5 days. On day 6, mice were fasted for 6 h and blood samples were collected in microvette capillary tubes (SARSTEDT, #16.444) and centrifuged at 2000g for 20 min at 4 °C. Plasma adiponectin and FGF21 levels were measured using Mouse Adiponectin/Adiponectin Quantitative ELISA (Bio-Techne R⃝ Systems, MRP300) and mouse/ rat FGFI21 ELISA (BioVendor R⃝, RD, 2921108200) kits, respectively, as per the manufacturer’s instructions.

**Drug pharmacokinetics.** The pharmacokinetic studies for SN-401 and SN-406 were performed at Charles River Laboratory as outlined below. Male C57Bl6 mice were used in the study and assessed for a single dose (5 mg/kg) administration. The compounds were prepared in Cremophor for i.v. route at a final concentration of 1 mg/ml. Terminal blood samples were collected via cardiac puncture under anesthesia at time points 0.08, 0.5, 2, 8 h post-dose for i.v and at timepoints 0.25, 2, 8, 24 h post-dose for i.p. and p.o. groups, respectively with a sample size of 3 mice per timepoint. The blood samples were collected in tubes with K2 EDTA anticoagulant and further processed to collect plasma by centrifugation at 13835g for 10 min. Samples were further processed for LC/MS to determine the concentration of the compounds. Non-compartmental analysis was performed to obtain the PK parameters using the PKPlus software package (Simulation Plus). The same method was used for fractionated insulin (SNF) samples: A 10/2% solution of DDM/CHS was added after cell thaw. Cells were then lysed by sonication and centrifuged at 5000×g for 10 min. The supernatant was then used for 20 min at 4 °C to enrich SNF. The enriched SNF fraction was dialyzed against 2.8 mM glucose solution for 80 min before the addition of purification buffer (50 mM HEPES, 150 mM KCl, 10 mM EDTA, 0.025% DDM, pH 7.4). The resin was then washed with 10 mL of Buffer A (20 mM HEPES, 150 mM KCl, 1 mM EDTA, 1% N-Dodecyl-β-D-Maltopyranoside (DDM, Anatrace, Maumee, OH), 0.2% Cholesterol Hemisuccinate Tri Salt (CHS, Anatrace) final pH 7.4). A 10/2% solution of DDM/CHS was first dissolved and clarified by bath sonication on 200 mM HEPES pH 8 prior to addition to buffer to the indicated final concentration. Membrane pellets were homogenized (using a loose-fitting followed by ten strokes with a tight-fitting) and the mixture was then gently stirred at 4 °C for 3 h. The extraction mixture was centrifuged at 33,000×g for 45 min and the supernatant, containing solubilized membrane protein, was bound to 5 mL of sepharose resin column at 4 °C. The resin was then washed with 10 mL of Buffer A (20 mM HEPES, 150 mM KCl, 1 mM EDTA, 0.025% DDM, 0.005% CHS, pH 7.4), 40 mL of Buffer B (A as buffer, but with 500 mM KCl), and then 10 mL of Buffer A. The resin was then resuspended in 8 mL of Buffer A with 0.5 mg Precission protease and rocked gently in the capped column overnight (overnight 2 h of exchange) and the resin was then eluted with an additional 6 mL of Buffer A, spin concentrated to ~500 μl with Amicon Ultra spin concentrator 100 kDa cutoff (Millipore), and then loaded onto a Superose 6 Increase column (GE Healthcare, Chicago, IL) on an NGS system (Bio-Rad, Hercules, CA) equilibrated in Buffer C (200 mM HEPES, 150 mM KCl, 1 mM EDTA, 0.025% DDM, pH 7.4). Peak fractions containing the SWELL1 channel were collected and spin concentrated for nanodisc preparation.

**Nanodisc formation.** Freshly purified SWELL1 from gel filtration in Buffer C (200 mM HEPES, 150 mM KCl, 1 mM EDTA, 0.025% DDM, pH 7.4) was reconstituted into MSPIE1D1 with a lipid mix (2:1 weight ratio of DOPE/POPC/POPS lipids (Avanti, Alabaster, Alabama)) at a final molar ratio of 1:2.5:200 (Monomer Ratio: SWELL1: MSPIE1D1, Lipid Mix). First, solubilized lipid in Column Buffer (200 mM HEPES, 150 mM KCl, 1 mM EDTA, 0.025% DDM, pH 7.4) was mixed with additional DDM detergent, Column Buffer, and SWELL1. This solution was mixed at 4 °C for 30 min before the addition of purified MSPIE1D1. This addition brought the final concentrations to ~10 μM SWELL1, 25 μM MSPIE1D1, 2 mM lipid mix, and 3.3 mM DDM in Column Buffer (1 mL reaction). The solution with MSPIE1D1 was mixed at 4 °C for 10 min before the addition of 130 mg of Biodeads SM2 (Bio-Rad, Hercules, CA). Biodeads (washed into methanol, water, and then Column Buffer) were weighed with liquid removed by P1000 tip (Damp weight). This mix was incubated at 4 °C for 30 min before the addition of another 130 mg of Biodeads (final 260 mg of Biodeads per mL). This final mixture was then mixed at 4 °C (3 times, 5–10 min) and then centrifuged at 17,000 × g for 1 min to remove large beads settle and carefully removing the supernatant with a pipet. The sample was spun for 5 min at 21,000×g before loading onto a Superose 6 column in Column Buffer

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without EDTA. Peak fractions corresponding to SWELL1 in MSp1ED1 were collected, 100 kDa cutoff spin concentrated, and then re-run on the Superose 6. The fractions containing SWELL1 were then pooled and concentrated prior to grid preparation.

Grid preparation. SN-407 in DMSO (Stock 10 mM) was added to the SWELL1-MSp1ED1 sample to give a final concentration of 1 mg/mL. SWELL1-MSp1ED1 and 100 µM SN-407. The drug was allowed to equilibrate and bind complex on ice for ~1 h before use. The sample with the drug was diluted by a 5 µl 21,000 g spin prior to grid making. For freezing grids, a 2 µl drop of protein was applied to freshly glow discharged Holey Carbon, 300 mesh R 1.2/1.3 gold grids (Quantifoil, Großhöchstach, Germany). A Vitrobot Mark IV (Thermo Fisher Scientific, Waltham, MA) was utilized with 22 °C, 100% humidity, one blot force, and a 3 s blot time, before plunging freezing in liquid ethane. Grids were then clipped in autoholder cartridges for collection.

Data collection. SWELL1-MSp1ED1 with SN-407 grids were transferred to a Talos Arctica cryo-electron microscope (Thermo Fisher Scientific) operated at an acceleration voltage of 200 kV. Images were recorded in an automated fashion with SerialEM58 using image shift with a target defocus range of ~0.7 to ~2.2 µm over 5.493 s as 50 subframes with a K3 direct electron detector (Gatan, Pleasanton, CA). Time PCR instrument (Applied Biosystems). Each sample measurement was carried out using an iScript Reverse Transcriptase reaction set up and the amplification containing 0.5 µl of cDNA (per each well) from the above step was used for the qRT-PCR primers (5’ to 3’) were used: GAPDH forward—TGGACACCAACGCCTTAG and reverse—GATCCAGGGATGTGAATGC; SWELL1 forward—AGGCCACAAAC CGTGAGCC and reverse—CGAGACACCTTCGAGTCA and reverse—GGTACGTCTCAATGGGAGG; LRRc8 forward—TCTCTTCTGCGGTATACCT and reverse—AAGCTCTGCAC CGGAATCT; LRRc8 forward—AGGGAGTTCACCTGCAAG; and reverse—CAGAGGTTTGACGCCCTT, respectively.

Liver isolation, triglycerides, and histology. HFD mice treated with either vehicle or SN-401 were anesthetized with 1–4% isoflurane followed by cervical dislocation. Gross liver weights were measured and identical sections from the right medial lobe of the liver were dissected for further examinations. Total triglyceride content was determined by homogenization of 10–50 mg of tissue in 1.5 mL of chloroform:methanol (2:1 v/v) followed by centrifugation at 16,260×g for 10 min at 4 °C. A 20 µl of the supernatant was evaporated in a 1.5 mL microcentrifuge tube for 30 min. Triglyceride content was determined by adding 100 µl of Infinity Triglyceride Reagent (Fisher Scientific) to the dried sample followed by 30 min incubation at RT. The samples were then transferred to a 96-well plate along with standards (0–2000 mg/dl) and absorbance was measured at 540 nm and the final concentration was determined by normalizing to tissue weight. For histological examination, liver sections were fixed in 10% zinc formalin and paraffin-embedded for sectioning. Hematoxylin and eosin (H&E) stained sections were then assessed for steatosis grade, lobular inflammation, and hepatocyte ballooning for nonalcoholic fatty liver disease (NAFLD) scoring.46,95,96.

Quantification and statistical analysis. Standard unpaired or paired two-tailed Student’s t-test were performed while comparing two groups. One-way ANOVA was used for multiple group comparison. For GTTs and ITTs, a two-way analysis of variance (ANOVA) test was used. Data were presented as mean ± SEM. The threshold for significance was 0.05 for all statistical comparisons. *p, **p, ***p represent p values of <0.05, <0.01, and <0.001, respectively. Details of statistical analyses are presented in the figure legends. All data analyses and statistical tests were carried out in GraphPad Prism 7.0 and Microsoft Excel (v2111).

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability. The cryo-EM data generated in this study have been deposited in public databases. For SWELL1-SN-407 in MSp1ED1 nanodiscs pose 1, the final model is in the PDB under 7M17 and the final model is in the Electron Microscopy Data Bank (EMDB) under EMD-23614. For SWELL1-SN-407 in MSp1ED1 nanodiscs pose 2, the final model is in the PDB under 7M19 and the final model is in the Electron Microscopy Data Bank (EMDB) under EMD-23616. The original micrograph movies and final particle stacks are in the EMPIAR database under EMPIAR-10662. 1H NMR spectra for all compounds are included in the supplementary information. All requests for resources, reagents, and additional information that support the findings should be addressed to rajan.sah@vuw.ac.nz and will be fulfilled upon reasonable request. Source data are provided with this paper.

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Author contributions


Competing interests

R.S. is co-founder of Senseon Therapeutics, Inc., a start-up company developing SWELL1 modulators for human diabetes. D.J.L is co-Founder and CEO of Senseon Therapeutics, Inc. The remaining authors declare no competing interests.

Additional information

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Correspondence and requests for materials should be addressed to Rajan Sah.

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