Molecular basis of ligand recognition and activation of succinate receptor

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Abstract

Succinic acid, a tricarboxylic acid (TCA) cycle intermediate, significantly influences mitochondrial reactive oxygen species homeostasis through the G protein-coupled succinate receptor (SUCR1, also called GPR91), linking it to various physiological and pathological processes. Despite SUCR1's pivotal role in mediating effects leading to liver fibrosis, hypertension, angiogenesis, inflammation, and offering a therapeutic target for multiple diseases, its activation mechanism by diverse ligands and interaction with downwards G protein remains poorly understood. This study presents the cryo-electron microscopy (cryo-EM) structures of SUCR1 in complex with inhibitory G protein (Gi) bound to succinic acid, maleic acid, and compound 31, a high-affinity agonist. These structures elucidate the distinct ligand binding modes, uncover the activation signal cascade, and detail the G protein coupling mechanism of SUCR1. Our findings provide a comprehensive structural basis for SUCR1 activation, paving the way for structure-based drug design aimed at SUCR1-related pathologies.

Full Text

Molecular Basis of Ligand Recognition and Activation of the Succinate Receptor

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Abstract

Succinic acid, a tricarboxylic acid (TCA) cycle intermediate, significantly influences mitochondrial reactive oxygen species homeostasis through the G protein-coupled succinate receptor (SUCR1, also called GPR91), linking it to various physiological and pathological processes. Despite SUCR1's pivotal role in mediating effects leading to liver fibrosis, hypertension, angiogenesis, inflammation, and its potential as a therapeutic target for multiple diseases, the molecular mechanisms underlying its activation by diverse ligands and interaction with downstream G proteins remain poorly understood. This study presents the cryo-electron microscopy (cryo-EM) structures of SUCR1 in complex with inhibitory G protein (Gi) bound to succinic acid, maleic acid, and compound 31, a high-affinity agonist. These structures elucidate distinct ligand binding modes, uncover the activation signal cascade, and detail the G protein coupling mechanism of SUCR1. Our findings provide a comprehensive structural basis for SUCR1 activation, paving the way for structure-based drug design aimed at SUCR1-related pathologies.

Introduction

Succinic acid, once considered merely a tricarboxylic acid (TCA) cycle intermediate[1], has been recognized for its significant role in influencing mitochondrial reactive oxygen species (ROS) homeostasis[2]. This process is largely mediated through the G protein-coupled succinate receptor (SUCR1, also known as GPR91), which has emerged as a vital link connecting metabolic status to a myriad of physiological and pathological processes.

SUCR1 is intricately involved in the regulation of blood pressure[3], angiogenesis[4], and inflammation[5], and has been implicated in the pathogenesis of liver fibrosis, hypertension, and rheumatic arthritis[6]. These multifaceted roles highlight the receptor's potential as a promising therapeutic target for a wide spectrum of diseases. However, the molecular underpinnings of SUCR1's activation by various ligands and its subsequent interaction with the inhibitory G protein (Gi) have remained elusive, obscuring our understanding of its broad physiological significance and therapeutic potential.

Results

Cryo-EM Structures of SUCR1-Gi Complexes

To address this knowledge gap, we determined three cryo-electron microscopy (cryo-EM) structures of human SUCR1-Gi complexes bound to the endogenous ligand succinic acid, the analog maleic acid, and the synthetic agonist compound 31, which exhibits higher affinity. These structures were solved using combinatorial approaches including BRIL fusion, Gi engineering, and stabilizing antibody scFv16—methods previously successful for solving several GPCR-G protein complexes[7],[8] (Supplementary information, Fig. S1). The succinic acid-, maleic acid-, and compound 31-bound SUCR1-Gi complexes were resolved at global resolutions of 2.75 Å, 2.69 Å, and 2.48 Å, respectively (Fig. 1a [Figure 1: see original paper]; Supplementary information, Figs. S2-S4 and Table S1).

In all structures, SUCR1 adopts an active-like conformation typical of GPCR-G protein coupling. The overall structure of SUCR1 is similar to that of the medium-chain fatty acid receptor GPR84 (PDB: 8J18)[9], with a root mean square deviation (RMSD) of 0.36 Å for Cα atoms of SUCR1 (residues 10-309) and GPR84 (residues 8-395). In both receptors, extracellular loop 2 (ECL2) forms a lid over the ligand-binding pocket (Fig. 1b). Due to the smaller ligands and more compact ligand-binding pockets, the SUCR1 structure is leaner compared to other class A GPCRs including GPR84, with the extracellular domains of TM1, TM5, and TM6 shifting inward toward the ligand binding pocket (Fig. 1b). Additionally, structural comparisons indicate that TM1 of SUCR1 is significantly longer than in GPR84, which may contribute to receptor stability (Fig. 1b).

Ligand Recognition Mechanisms

In the succinic acid-SUCR1 complex structure, succinic acid is securely held within a binding pocket formed by TM1, TM2, TM3, TM7, and ECL2 that exhibits a C-shaped architecture. The pocket is highly electropositive to accommodate the negatively charged succinic acid. The two carboxylic acid groups of succinic acid are directed toward the extracellular side of the ligand binding pocket (Supplementary information, Fig. S5a), forming hydrogen bonds with Y30$^{1.39}$ and Y83$^{2.64}$ and ionic bonds with R99$^{3.29}$ and R281$^{7.39}$, respectively (Fig. 1c). Additionally, Y30$^{1.39}$ also forms a hydrogen bond with R281$^{7.39}$, stabilizing the hydrophilic environment of the binding pocket (Fig. 1c). As mentioned above, ECL2 is positioned above succinic acid and covers the entry to the ligand-binding pocket. The main chain amine of F175 ^{2} forms a hydrogen bond with a carboxylic acid group of succinic acid (Supplementary information, Fig. S5a). Simultaneously, the alkyl portion in the middle of the ligand predominantly orients itself toward the bottom of the ligand binding pocket, establishing hydrophobic interactions with residues including L79$^{2.60}$, L102$^{3.32}$, and F285$^{7.43}$ (Fig. 1c).

Maleic acid, an analog of succinic acid containing an alkene in the alkyl portion,

aligns almost identically with succinic acid in the SUCR1 binding pocket. In the maleic acid-bound structure, the carboxylic acids on the same side form polar interactions with SUCR1 residues including Y30$^{1.39}$, Y83$^{2.64}$, R99$^{3.29}$, H103$^{3.33}$, and R281$^{7.39}$ (Fig. 1d). Maleic acid forms a hydrogen bond with D174 ^{2} (Supplementary information, Fig. S5b). Alanine substitution at D174 ^{2} reduces receptor activation by maleic acid, underscoring ECL2's significance in SUCR1 activation by endogenous ligands (Supplementary information, Fig. S6b). In the maleic acid-SUCR1-Gi structure, the alkene of maleic acid also forms hydrophobic interactions with L79$^{2.60}$, L102$^{3.32}$, and F285$^{7.43}$, similar to succinic acid (Fig. 1d). Alanine mutations in the binding pocket, including Y30$^{1.39}$, L79$^{2.60}$, Y83$^{2.64}$, R99$^{3.29}$, and R281$^{7.39}$, significantly impair the activation potential of both succinic acid and maleic acid, underscoring their essential roles in SUCR1 function (Fig. 1e, 1f; Supplementary information, Fig. S6 and Tables S2, S3). These findings further highlight the intricate molecular interactions that govern SUCR1 regulation and activation by succinic acid and maleic acid.

Compound 31 is a synthetic small molecule that activates SUCR1 with high potency[10]. In this study, we found that compound 31 activated SUCR1 via the Gi signaling pathway with approximately 100-fold greater potency than succinic acid and maleic acid (Supplementary information, Fig. S6). The structure of compound 31 comprises a "succinic acid" motif (part 1) and a "two-ring" motif (part 2) (Supplementary information, Fig. S7a). Comparison of the compound 31-bound SUCR1 structure with the succinic acid-bound structure reveals that the sidechain of R99$^{3.29}$ is deflected, leading to formation of an extended pocket (Supplementary information, Fig. S7b). In the ligand binding pocket of the compound 31-bound structure, the "succinic acid" motif of compound 31 partially aligns with the position of succinic acid or maleic acid (Supplementary information, Fig. S7b), forming polar interactions with surrounding amino acids such as Y30$^{1.39}$, Y83$^{2.64}$, and R281$^{7.39}$, and nonpolar interactions with residues including L79$^{2.60}$, L102$^{3.33}$, and F285$^{7.43}$ (Fig. 1g). Additionally, similar to the maleic acid-SUCR1 structure, the carboxylic acid in the "succinic acid" motif of compound 31 also forms a hydrogen bond with D174 ^{2} (Supplementary information, Fig. S7c). While the "two-ring" motif of compound 31 occupies the expanded pocket, the pyridine ring is constrained by L79$^{2.60}$, W88 ^{1}, N98$^{3.28}$, and R99$^{3.29}$ (Fig. 1h). Furthermore, the benzene ring in compound 31 is clamped by Y83$^{2.64}$ and W88 ^{1} (Fig. 1h). The formation of these additional interactions with surrounding SUCR1 residues by the "two-ring" motif likely accounts for the significantly greater potency of compound 31 compared to succinic acid and maleic acid. Mutation of Y30$^{1.39}$, L79$^{2.60}$, N87 ^{1}, N98$^{3.28}$, R99$^{3.29}$, and R281$^{7.39}$ in SUCR1 reduced compound 31-induced signaling responses, confirming the essential roles of these residues in ligand binding and receptor activation (Fig. 1i, 1j; Supplementary information, Fig. S6 and Table S4). These intricate details reveal the comprehensive mechanism underlying SUCR1 activation by the synthetic agonist compound 31.

Activation Mechanism

Comparisons of the three complex structures revealed that SUCR1 maintains similar overall structural arrangements, with RMSD values for Cα atoms of the entire receptor ranging between 0.33-0.50 Å (Fig. 1k, 1l). Given the similarity among the three complexes, we selected the succinic acid-SUCR1 complex for detailed analysis of SUCR1 activation. Comparing the active succinic acid-SUCR1 structure with the inactive $ 2*ARstructure*(*PDB* : 8*JJO*)^{11}$, the cytoplasmic end of TM6 in SUCR1 displays a pronounced outward movement while the cytoplasmic portion of TM7 shifts inward. This rearrangement accommodates the C-terminal $ 5*helixofthe**G* $i subunit, a key feature of class A GPCR activation (Fig. 1m). In general, many GPCRs sense ligand binding via a conserved toggle switch, exemplified by W6.48 in $ 2*AR*.*Attheequivalentpositionofthistoggleswitch*^{12}, *SUCR1hasF245*{6}.^{48}, *whichdoesnotdirectlycontactanyofthea* and F285$^{7.43}$ directly contact the alkyl portion of the ligands (Fig. 1l), which pushes F285$^{7.43}$ downward to pack against L106$^{3.36}$ and F245$^{6.48}$, leading to rotations of the P5.50-I3.40-F6.44 motif. This chain of conformational changes ultimately results in outward bending of TM6 to accommodate Gi coupling.

Discussion

In this study, we have determined cryo-EM structures of SUCR1 in complex with succinic acid, maleic acid, and compound 31. The detailed structural analysis provided herein not only enhances our understanding of SUCR1-ligand interactions but also underscores the potential of structure-based drug design in developing targeted therapies for diseases associated with dysregulated succinic acid signaling, such as hypertension, inflammation, and metabolic disorders. The identification of key residues involved in ligand binding and receptor activation presents specific targets for the development of drugs aimed at modulating SUCR1 activity with high specificity and efficacy.

In conclusion, this study not only contributes to the expanding repository of GPCR structural biology but also represents a pivotal step forward in the quest for targeted therapeutics. The comprehensive structural basis for SUCR1 activation provided herein highlights the receptor's potential as a versatile and valuable target for drug design and discovery. As we continue to unravel the complex signaling networks mediated by SUCR1, the prospects for developing novel therapeutic strategies that harness the power of structure-based drug design become increasingly tangible, offering hope for the effective management and treatment of a wide range of diseases.

Methods

Cell Lines

Trichoplusia ni (High Five, Thermo Fisher) cells were grown in SIM HF Expression Medium (Sino Biological, MHF1) at 27°C and 120 rpm. HEK293T

cells were maintained in a humidified 37°C incubator with 5% CO_2 using media supplemented with 100 I.U./mL penicillin and 100 mg/mL streptomycin (Invitrogen). The human cell line HEK293T was cultured in DMEM (VWR) containing 10% fetal bovine serum (FBS, VWR).

Protein Complex Expression and Purification

Wild-type human SUCR1, modified with an N-terminal thermally stabilized BRIL as a fusion protein along with an HA signal peptide, Flag tag, and His tag epitope, was cloned into a pFastBac transfer plasmid (Invitrogen). All constructs were generated using Phanta Max Super-Fidelity DNA Polymerase (Vazyme Biotech Co., Ltd) and verified by DNA sequencing (Genewiz). A dominant-negative (DN) Gαi format containing mutations G203A/A326S was constructed to increase the stability of the Gαi$ 1 2*complex^{1}$.

SUCR1, Gαi, G$ 1, *G* 2, *and scFv16 were co-expressed in Trichoplusiani (Hi5) insect cells. Baculoviruses were prepared to-Bac Expression System (Invitrogen). Cells were infected with viruses at a density of* 3.0×10^{6}$ cells/mL, and the cell culture was collected by centrifugation 48 h post-infection and stored at -80°C until use.

For purification of succinic acid-, maleic acid-, and compound 31-bound SUCR1-Gαi complexes, cell pellets were thawed in 20 mM HEPES pH 7.4, 100 mM NaCl, 5 mM $MgCl_2$, 5 mM $CaCl_2$, and protease inhibitor cocktail (TargetMol, USA). The suspensions were incubated for 1.5 h at room temperature supplemented with 50 mM/L succinic acid (TargetMol, USA), 50 mM/L maleic acid (TargetMol, USA), or 50 M/L compound 31 and 25 mU/ml apyrase (Sigma-Aldrich), respectively. Subsequently, 0.5% (w/v) n-dodecyl-β-d-maltoside (DDM, Anatrace) and 0.1% (w/v) cholesteryl hemisuccinate (CHS, Anatrace) were added to solubilize complexes for 2.5 h at 4°C. Insoluble material was removed by centrifugation at 30,000 g for 30 min, and the supernatant was immobilized by batch binding to Nickel Sepharose resin (GE Healthcare). The resin was then packed and washed with 20 column volumes of 20 mM HEPES pH 7.4, 100 mM NaCl, 20 mM imidazole, 0.01% (w/v) LMNG, and 0.002% (w/v) CHS, with corresponding ligands at the concentrations described above. Finally, the complex was eluted in buffer containing 250 mM imidazole and concentrated using an Amicon Ultra Centrifugal Filter (MWCO 100 kDa). Complexes were loaded onto a Superdex 200 Increase 10/300 column (GE Healthcare) with buffer containing 20 mM HEPES pH 7.4, 100 mM NaCl, 0.00075% (w/v) LMNG, 0.00025% (w/v) GDN, 0.0002% (w/v) CHS, and corresponding ligands at the concentrations described above to separate the complex from contaminants. Eluted fractions containing the SUCR1-Gαi protein complex were pooled and concentrated for electron microscopy experiments.

Cryo-EM Grid Preparation and Data Collection

For cryo-EM grid preparation of SUCR1-Gi complexes, 3 l of protein at ~10 mg/mL were loaded onto a glow-discharged holey carbon grid (Quantifoil Au

300 mesh R1.2/1.3) and subsequently plunge-frozen in liquid ethane using a Vitrobot Mark IV (Thermo Fisher Scientific). Cryo-EM imaging was performed on a Titan Krios at 300 kV using a Gatan K3 Summit detector and Falcon 4 direct electron detection device at the Cryo-Electron Microscopy Research Center, Shanghai Institute of Materia Medica, Chinese Academy of Sciences (Shanghai, China).

For succinic acid- and compound 31-bound SUCR1-Gi complexes, cryo-EM imaging was collected on a Titan Krios at 300 kV using a Gatan K3 Summit detector with a pixel size of 0.824 Å. Images were recorded at a dose rate of approximately 8.0 e/Å^2/s with defocus values ranging from -1.0 to -2.0 m using EPU software (FEI Eindhoven, Netherlands). The total exposure time was 8 s, with 36 frames recorded per micrograph. For the maleic acid-bound SUCR1-Gi complex, cryo-EM imaging was collected on a Titan Krios equipped with a Falcon 4 direct electron detection device at 300 kV. Images were acquired with a pixel size of 0.73 Å, defocus ranging from -1.0 to -2.0 m using EPU software (FEI Eindhoven, Netherlands), with a total dose of 50 e/Å^2/s over 2.5 s exposure per EER-format movie.

Image Processing and Map Construction

For the succinic acid-bound SUCR1-Gi complex, particles auto-picked by blob picker were subjected to interactive 2D and 3D classifications, yielding 1,979,364 particles with complete complex and good quality. The selected particles were further processed through several rounds of ab initio reconstruction and heterogeneous refinement, resulting in a well-defined subset. Subsequent heterogeneous refinement, non-uniform refinement, and local refinement generated a map with an indicated global resolution of 2.75 Å reconstructed from 573,312 particles.

For the maleic acid-bound SUCR1-Gi complex, particles auto-picked by blob picker were subjected to interactive 2D and 3D classifications, producing 1,436,056 particles with complete complex and good quality. The selected particles were further processed through several rounds of ab initio reconstruction and heterogeneous refinement, resulting in a well-defined subset. Subsequent heterogeneous refinement, non-uniform refinement, and local refinement generated a map with an indicated global resolution of 2.69 Å reconstructed from 394,879 particles.

For the compound 31-bound SUCR1-Gi complex, particles auto-picked by blob picker were subjected to interactive 2D and 3D classifications, yielding 775,432 particles with complete complex and good quality. The selected particles were further processed through several rounds of ab initio reconstruction and heterogeneous refinement, resulting in a well-defined subset. Subsequent heterogeneous refinement, non-uniform refinement, and local refinement generated a map with an indicated global resolution of 2.48 Å reconstructed from 385,083 particles. The overall resolution was estimated based on gold-standard Fourier

shell correlation of independently refined half maps using the 0.143 cutoff criterion. Local resolution was estimated in cryoSPARC using default parameters.

Model Building and Refinement

Predicted models of the active-state SUCR1 receptor from AlphaFold were used as initial models for rebuilding and refinement against the electron microscopy density maps. UCSF Chimera-1.14[2] was used to dock the models into the electron microscopy density maps, followed by iterative manual adjustment and rebuilding in COOT-0.9.6[3] and ISOLDE-1.2[4]. Models were then further refined and validated in Phenix-1.20[5] programs (Table S1). Structural figures were generated using UCSF Chimera-1.14, ChimeraX-1.2[6], and PyMOL-2.0 (https://pymol.org/2/).

GloSensor cAMP Assay

The GloSensor cAMP assay (Promega) was performed to monitor real-time cAMP variation induced by SUCR1. Full-length SUCR1 was fused downstream of an HA signal peptide and Flag tag. The constructs were cloned into a pcDNA3.0 vector for HEK293T expression. Before transfection, HEK293T cells were plated onto 6-well plates at a density of 1.5$×10^{5}$ cells/mL. After 16 h, cells were transfected with 1.5 g receptor and 1.0 g GloSensor-22F (Promega). After 24 h, transfected cells were digested and transferred to 96-well plates with 50 L suspension at a density of 3$×10^{5}$ cells/mL. Following another 16 h incubation, cells were starved with 50 L Hank's balanced salt solution for 30 min and then incubated in 50 L CO_2-independent media containing 2% GloSensor cAMP Reagent (Promega) for 1 h. After incubation, 5.5 L test ligands at various concentrations were added and incubated for 10 min at room temperature. For Gi-coupled SUCR1, an additional 5.5 L forskolin was added to the cells at a final concentration of 1 M. All luminescence signals were measured using an EnVision multi-plate reader according to the manufacturer's instructions. All data were analyzed using Prism 9 (GraphPad) and presented as means ± S.E.M. from at least three independent experiments performed in technical duplicates or triplicates. Non-linear curve fitting was performed using a three-parameter logistic equation [log (agonist vs response)]. Final curves were shown as normalized values compared to wild-type. Statistical significance was determined using two-sided one-way ANOVA followed by Fisher's LSD test compared with WT. *P<0.05;* ***P<0.01; and*** P<0.001 were considered statistically significant.

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Author Contributions

H.E.X. initiated the project. C.L. designed and screened SUCR1 expression constructs, prepared protein samples of succinic acid-SUCR1-Gi, maleic acid-SUCR1-Gi, and compound 31-SUCR1-Gi complexes for cryo-EM data collection, participated in cryo-EM grid inspection, and executed functional studies. H.L. performed cryo-EM grid preparation, data acquisition, structure determination, and model building, and prepared the manuscript draft and figures. H.Z. synthesized compound 31 for structural studies. J.L. performed cell-based functional assays and participated in figure preparation. W.F. guided H.Z. in synthesizing compound 31. H.E.X. wrote the manuscript with input from all authors.

Data Availability

Competing interests are declared in the Supplementary Information.

Competing Interests

The authors declare no competing interests.

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Supplementary Information

Supplementary References

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Supplementary Figure Legends

Figure S1. Cartoon models of the constructs used in this study.

Figure S2. Structure determination of succinic acid-SUCR1-Gi complex. (a,b) Size exclusion chromatography (SEC) profiles (a) and SDS-PAGE analysis (b) of SUCR1-Gi complex activated by succinic acid. (c,d) Representative cryo-EM image (c) and 2D classification averages (d) of succinic acid-SUCR1-Gi. (e) Cryo-EM data processing flowcharts of succinic acid-SUCR1-Gi. (f) The global density map of succinic acid-SUCR1-Gi colored by local resolutions. (g) Fourier shell correlation (FSC) curves of succinic acid-SUCR1-Gi. The global resolution of the final processed density map estimated at FSC = 0.143 is 2.75 Å. (h) Density maps of helices TM1-TM7 of the transmembrane domain and succinic acid in succinic acid-SUCR1-Gi.

Figure S3. Structure determination of maleic acid-SUCR1-Gi complex. (a,b) Size exclusion chromatography (SEC) profiles (a) and SDS-PAGE analysis (b) of SUCR1-Gi complex activated by maleic acid. (c,d) Representative cryo-EM image (c) and 2D classification averages (d) of maleic acid-SUCR1-Gi. (e) Cryo-EM data processing flowcharts of maleic acid-SUCR1-Gi. (f) The global density map of maleic acid-SUCR1-Gi colored by local resolutions. (g) Fourier shell correlation (FSC) curves of maleic acid-SUCR1-Gi. The global resolution of the final processed density map estimated at FSC = 0.143 is 2.68 Å. (h) Density maps of helices TM1-TM7 of the transmembrane domain and maleic acid in maleic acid-SUCR1-Gi.

Figure S4. Structure determination of compound 31-SUCR1-Gi complex. (a,b) Size exclusion chromatography (SEC) profiles (a) and SDS-PAGE analysis (b) of SUCR1-Gi complex activated by compound 31. (c,d) Representative cryo-EM image (c) and 2D classification averages (d) of compound 31-SUCR1-Gi. (e) Cryo-EM data processing flowcharts of compound 31-SUCR1-Gi. (f) The global density map of compound 31-SUCR1-Gi colored by local resolutions. (g) Fourier shell correlation (FSC) curves of compound 31-SUCR1-Gi. The global resolution of the final processed density map estimated at FSC = 0.143 is 2.68 Å. (h) Density maps of helices TM1-TM7 of the transmembrane domain and compound 31 in compound 31-SUCR1-Gi.

Figure S5. Interaction of D174 ^{2} with succinic acid (a), maleic acid (b), and compound 31 (c) in SUCR1 structures.

Figure S6. Evaluation of mutational effects on SUCR1 induced by three ag-

onists measured by GloSensor assay. Concentration-response curves of wild-type/mutant SUCR1 induced by succinic acid (a), maleic acid (b), and compound 31 (c). Every mutant response was normalized to the corresponding wild-type as 100%. Data are shown as means ± S.E.M. from a minimum of three independent experiments performed in technical duplicates.

Figure S7. Chemical formula of compound 31 and structural alignment of SUCR1 structures. (a) Chemical formula of compound 31. (b) Structural alignment indicating the movement of $R99^{3.29}$ during compound 31 insertion.

Supplementary Tables

Table S1. Cryo-EM data collection, refinement and validation statistics.

Table S2. Potency of succinic acid activating WT and mutated SUCR1. The wild type (WT) and mutants of SUCR1 discussed in this manuscript were individually analyzed. Affinities were derived from at least 3 independent experiments using GloSensor functional assays. Expression levels of mutant SUCR1 were normalized to wild-type SUCR1 as 100%. Each data point represents mean ± standard error of the mean (S.E.M.). All data were analyzed by two-sided Student's t-test. *P<0.05,* ***P<0.01,*** P<0.001 vs. WT. Source data are available online. Definitions: NA – not applicable; NT, not tested.

WT and mutants	Succinic acid induced Gi inhibition (pEC50 ± S.E.M)	Span (WT%) ± S.E.M
R252A	5.48$±0.02|21.41±4.08 ∗ ∗||*R*255*A*|5.62±0.20|134.7±5.25||*R*281*A*|5.40±0.06|29.31±5.97∗ ∗|||5.61±0.52|80.60±1.88|||5.29±0.04|28.32±33.32∗ ∗|||6.17±$1.17	

Table S3. Potency of maleic acid activating WT and mutated SUCR1. The wild type (WT) and mutants of SUCR1 discussed in this manuscript were individually analyzed. Affinities were derived from at least 3 independent experiments using GloSensor functional assays. Expression levels of mutant SUCR1 were normalized to wild-type SUCR1 as 100%. Each data point represents mean ± standard error of the mean (S.E.M.). All data were analyzed by two-sided Student's t-test. *P<0.05,* ***P<0.01,*** P<0.001 vs. WT. Source data are available online. Definitions: NA – not applicable; NT, not tested.

WT and mutants	Maleic acid induced Gi inhibition (pEC50 ± S.E.M)	Span (WT%) ± S.E.M
H103A	5.17$±0.08\|58.06±5.45 * * * \|\|$D174A$\|3.78±0.06\|79.56±16.37\|\|$R281A$\|4.63±0.07\|37.54±4.86* * * *\|\|$F285A$|3.20±0.21|38.88±4.82 * *|||3.83±0.35|74.55±5.63|||5.44±0.04|70.66±13.94|||4.57±0.37|61.06±37.02* *|||3.92±$0.22	

Table S4. Potency of compound 31 activating WT and mutated SUCR1. The wild type (WT) and mutants of SUCR1 discussed in this manuscript were individually analyzed. Affinities were derived from at least 3 independent experiments using GloSensor functional assays. Expression levels of mutant SUCR1 were normalized to wild-type SUCR1 as 100%. Each data point represents mean ± standard error of the mean (S.E.M.). All data were analyzed by two-sided Student's t-test. *P<0.05,* ***P<0.01,*** P<0.001 vs. WT. Source data are available online. Definitions: NA – not applicable; NT, not tested.

WT and mutants	Compound 31 induced Gi inhibition (pEC50 ± S.E.M)	Span (WT%) ± S.E.M
L102A	7.05$±0.07\|180.13±17.29 * *\|\|$H103A$\|4.95±0.13 * * * *\|76.57±6.03\|\|$D174A$\|6.38±0.12 * *\|150.9±14.14\|\|$F175A$\|6.88±0.12\|64.53±4.80\|\|$R252A$\|7.17±0.05\|174.7±35.33* *\|\|$R255A$\|5.98±0.04 * * * *\|102.88±20.52\|\|$R281A$\|7.32±0.16\|147.62±39.41\|\|$F285A$|6.08±0.16* * * *|74.90±8.81|||7.03±0.05 * * * *| − 69.74±7.96 * * * *|||6.15±0.32 * * * |64.41±8.08|||7.82±0.11 * * * *|26.46±6.66 * *|||5.55±0.17 * * * *|124.80±15.78|||5.72±0.08 * * * *|36.50±16.26 * |||6.18±0.10 * *|95.27±4.39|||6.40±$0.02	

Note: Figure translations are in progress. See original paper for figures.