STIM2 interacts with AMPK and regulates calcium-induced AMPK activation

Anoop Singh Chauhan,*1,2 Xiaoguang Liu,*1,2 Ji Jing,‡ Hyemin Lee,* Raj Kumar Yadav,* Jindou Liu,†,3 Yubin Zhou,†,‡ and Boyi Gan,*8,†,‡

*Department of Experimental Radiation Oncology, †Department of Molecular and Cellular Oncology, and ‡UTHealth Graduate School of Biomedical Sciences, The University of Texas M. D. Anderson Cancer Center, Houston, Texas, USA; †Institute of Biosciences and Technology, Texas A&M University Health Science Center, Houston, Texas, USA; and §Department of Medical Physiology, College of Medicine, Texas A&M University, Temple, Texas, USA

ABSTRACT: AMPK is a crucial regulator of energy homeostasis that acts downstream of its upstream kinase liver kinase B1 (LKB1) and calcium/calmodulin-dependent protein kinase 2 (CaMKK2). LKB1 primarily phosphorylates AMPK after energy stress, whereas calcium-mediated activation of AMPK requires CaMKK2, although the regulatory mechanisms of calcium-mediated AMPK activation remain unclear. Using biochemical, microscopic, and genetic approaches, we demonstrate that the stromal interaction molecule (STIM)2, a calcium sensor, acts as a novel regulator of CaMKK2-AMPK signaling. We reveal that STIM2 interacts with AMPK and CaMKK2 and that the increase in intracellular calcium levels promotes AMPK colocalization and interaction with STIM2. We further show that STIM2 deficiency attenuates calcium-induced but not energy stress-induced AMPK activation, possibly by regulating the CaMKK2-AMPK interaction. Together, our results identify a previously unappreciated mechanism that modulates calcium-mediated AMPK activation. — Chauhan, A. S., Liu, X., Jing, J., Lee, H., Yadav, R. K., Liu, J., Zhou, Y., Gan B. STIM2 interacts with AMPK and regulates calcium-induced AMPK activation. FASEB J. 33, 2957–2970 (2019). www.fasebj.org

KEY WORDS: CaMKK2 · energy stress · LKB1 · STIM1

Metazoans have evolved adaptive mechanisms that help maintain their survival under altered physiologic conditions. To achieve this, an intricate signaling network has evolved to sense, respond, and adapt to changes in intra- and extracellular environments. AMPK is one such master regulator of cellular homeostasis. AMPK plays a key role in regulating energy homeostasis in response to energy stress (1, 2). Energy deprivation results in depletion of cellular ATP levels with concomitant increases of cellular AMP levels, inducing energy stress. AMPK can sense cellular energy status by detecting fluctuations of cellular AMP levels. AMPK exists as a heterotrimeric complex, consisting of 3 subunits: AMPK-α, AMPK-β, and AMPK-γ (3). The catalytic kinase activity of AMPK resides in its α subunit, whereas the β and γ subunits function as regulatory subunits (4). AMP can directly bind to the γ subunit of AMPK, resulting in conformational change and allosteric activation of the AMPK complex (5, 6). Energy stress–induced AMPK activation also requires the phosphorylation of Thr172 in its α catalytic subunit by the upstream kinase liver kinase B1 (LKB1) (7–9). Energy stress–induced AMPK activation involves other regulatory mechanisms. For example, in response to energy stress, long noncoding RNA neighbor of BRCA1 gene 2 (nBRCA2) interacts with the AMPK-α subunit, promotes AMPK kinase activity, and potentiates AMPK activation during energy stress (10–12). In addition, the scaffolding axis inhibition protein (AXIN) regulates the complex formation between LKB1 and AMPK and thus promotes energy stress–induced AMPK activation (13, 14). These regulatory mechanisms serve to fine-tune AMPK activity in response to energy stress.

Once activated, AMPK phosphorylates a number of downstream substrates, which shift from ATP-consuming
anabolic processes to ATP-generating catabolic processes, restoring energy homeostasis under energy stress (2). For example, in response to energy stress, AMPK suppresses mechanistic target of rapamycin complex 1 (mTORC1, also called mammalian target of rapamycin complex 1)-mediated protein synthesis and cell growth, an anabolic process that is highly energy consuming. AMPK inhibits mTORC1 by directly phosphorylating mTORC1 component Raptor and its upstream regulator tuberous sclerosis complex (TSC)1–TSC2 complex (15, 16). Dysregulation of AMPK, its upstream regulators such as LKB1, or its downstream effectors such as TSC1 and TSC2 results in defective cellular response to energy stress and leads to diseases such as cancer and diabetes (17, 18).

In addition to LKB1, calcium/calmodulin-dependent protein kinase 2 (CaMKK2) can phosphorylate and activate AMPK. The basal phosphorylation of AMPK Thr172 is significantly reduced but not completely abolished in LKB1-deficient cells, leading to the identification of CaMKK2 as another upstream kinase of AMPK (19–21). Unlike LKB1, CaMKK2 does not appear to mediate energy stress–induced AMPK phosphorylation but phosphorylates AMPK at Thr172 in response to increased intracellular calcium levels (19–21). It has been proposed that increases of intracellular calcium levels upon calcium ionophore treatment cause a conformational change in CaMKK2, resulting in AMPK activation (22). Although energy stress–mediated and LKB1–mediated AMPK activation has been extensively characterized, the regulatory mechanism of calcium- and CaMKK2-mediated AMPK activation is still poorly understood.

Calcium plays essential roles in regulating many signaling pathways and cellular processes, such as cell growth and differentiation (23). To maintain appropriate levels of intracellular calcium, cells have evolved elegant mechanisms to sense and adjust intracellular calcium levels, prominent among which is the stromal interaction molecule (STIM)1 and ORAI1-mediated store-operated calcium entry (SOCE) (24). When calcium levels in the endoplasmic reticulum (ER) lumen are high, calcium can bind to the luminal EF hand motif of STIM1, a single-transmembrane protein that mainly localizes on ER. As calcium in the ER lumen is depleted, calcium dissociates from the luminal side of STIM1, leading to STIM1 conformational change, oligomerization, and translocation to ER–plasma membrane junctions, where STIM1 couples with and gates ORAI1 and subsequently triggers ORAI1-mediated extracellular calcium influx (24, 25). Thus, similar to AMPK serving as an energy sensor to maintain energy homeostasis, STIM1 functions as a calcium sensor to maintain cellular calcium homeostasis. STIM2 is a homolog of STIM1 (26). Despite extensive sequence homology and predominant localization on ER, STIM1 and STIM2 appear to have very different functions in SOCE. Previous studies have shown that, whereas STIM1 knockdown largely eliminated SOCE, STIM2 knockdown had only a minimal effect on SOCE, and its overexpression even inhibited SOCE (27–32). It has been suggested that STIM2 is a much weaker activator of SOCE, with poor ORAI1-coupling efficacy compared with STIM1 (26). Whether STIM2 play any additional role in mediating calcium signaling remains unknown.

In the present study, we identified STIM2, but not STIM1, as a novel AMPK-binding protein. Our results demonstrated that STIM2 regulates calcium-mediated AMPK signaling in an LKB1-independent manner. Our data further suggested that STIM2 might function as a scaffolding protein to maintain the AMPK–CaMKK2 interaction and to regulate calcium-mediated, but not energy stress–mediated, AMPK activation. Our study thus uncovered a hitherto unrecognized regulatory mechanism of calcium-regulated AMPK signaling.

MATERIALS AND METHODS

Cell culture studies

Human embryonic kidney (HEK) 293T, 786-O, and HeLa cells were obtained from American Type Culture Collection (Manassas, VA, USA). All cell lines were free of mycoplasma contamination (tested by the vendor). No cell lines used in this study are found in the International Cell Line Authentication Committee (ICLAC) database (http://iclac.org) of commonly misidentified cell lines, based on short tandem repeat profiling performed by the vendor. All cell lines were cultured and maintained in DMEM (D6429; MilliporeSigma, Burlington, MA, USA) supplemented with 10% (v/v) fetal bovine serum (F10435; MilliporeSigma). Plasmid transfection in cells was carried out using Lipofectamine 3000 (Thermo Fisher Scientific, Waltham, MA, USA). For experiments to induce energy stress, 786-O and HeLa cells were cultured in either glucose-containing or glucose-free DMEM supplemented with 10% diazoyl fetal bovine serum (F0392; MilliporeSigma) or with DMEM containing 2 deoxy-glucose (5 mM) (D6134; MilliporeSigma) as previously described (10, 33, 34). For experiments to induce intracellular calcium levels, cells were treated with ionomycin (1 μM) (I9657; MilliporeSigma) or thapsigargin (1 μM) (T9033; MilliporeSigma) for 15 or 30 min.

Constructs and viruses

Full-length STIM2 and its truncated mutants were amplified using PCR and cloned in entry vector using a pENTR/D-TOP Cloning Kit (K240020; Thermo Fisher Scientific) following the manufacturer’s instructions. Subsequently, STIM2-containing entry vector was used to generate gateway vectors with N-terminal myc-tag using the generate gateway-based LR reaction (12538; Thermo Fisher Scientific). To construct AMPK–CaMKK2 entry clones were obtained from the pEF4 vector (Clontech Laboratories, Mountain View, CA, USA). The STIM1 Myc-His construct was generated by inserting mouse STIM1 cDNA between the BamHI and XhoI sites in pEF4 vector. AMPK-α, AMPK-β, AMPK-γ, and CaMKK2 entry clones were obtained from the Human ORFeome V5.1 library. These entry clones were used to generate gateway vectors with N-terminal myc-tag using the gateway-based LR reaction (12538; Thermo Fisher Scientific). To construct AMPK-α–mCherry vector, AMPK-α was amplified using PCR and cloned into the mCherry-N1 vector (S4157; Addgene, Cambridge, MA, USA) using XhoI and EcoRI restriction sites. To generate knockout (KO) cells using the clustered regularly interspaced short palindromic repeats (CRISPR)–CRISPR-associated protein 9 (Cas9) approach, single guide RNAs (sgRNAs) targeting STIM1 and STIM2 were

2958 Vol. 33 February 2019 The FASEB Journal • www.fasebj.org CHAUHAN ET AL.
cloned in the lentiviral pLenti-CRISPR-V2 plasmid (52961; Addgene) following the manufacturer’s instructions. HEK 293T cells were transfected with sgRNA vector along with the ViraPower Lentiviral Expression System (K496000; Thermo Fisher Scientific) to generate lentiviral supernatant. Forty-eight hours after transfection, the cell supernatant was collected, filtered using 0.45-µm filters (SLHV033RS; Thermo Fisher Scientific), and used to infect target cells. Stably transfected cells were selected using 2 µg/ml puromycin (ant-pr-1; Invitrogen, San Diego, CA, USA).

Immunoprecipitation

Cultured cells were washed with PBS and lysed using NP40 lysis buffer [50 mM Tris (pH 7.4), 250 mM NaCl, 5 mM EDTA, 50 mM NaF, 1 mM Na3VO4, 1% Nonidet P40 (NP40) (pH 8.0)] containing complete mini protease inhibitors (11836153001; Roche, Basel, Switzerland) and phosphatase inhibitor cocktail (524625; Calbiochem, San Diego, CA, USA) as previously described (36). Cell lysates were then centrifuged at 12,000 g for 10 min at 4°C to clear the cellular debris. For immunoprecipitation of SFB-tagged proteins, 30 µl S-protein agaropectin beads (69704; MilliporeSigma) were washed twice with NP40 lysis buffer and incubated with the cell lysate overnight at 4°C. Immunoprecipitates were washed 3 times with NP40 lysis buffer and subjected to denaturation in 30 µl LDS sample buffer (1610747; Bio-Rad, Hercules, CA, USA). Coimmunoprecipitated proteins were then resolved on 10% SDS-PAGE gel and analyzed by immunoblotting after Western blotting. For immunoprecipititation of endogenous AMPK proteins or Myc tag STIM1 proteins, 30 µl Protein G magnetic beads (90065; Cell Signaling Technology, Danvers, MA, USA) were washed twice with NP40 lysis buffer and incubated with the cell lysate with anti-AMPKα1+α2 antibody (ab80039; Abcam, Cambridge, MA, USA) or anti-Myc antibody (22765; Cell Signaling Technology, Danvers, MA, USA) overnight at 4°C. Immunoprecipitates were washed 3 times with NP40 lysis buffer and subjected to denaturation in 30 µl LDS sample buffer. Coimmunoprecipitated proteins were resolved on 10% SDS-PAGE gel and analyzed by immunoblotting.

Western blotting analysis

For Western blotting analysis, 20–40 µg cell lysate or immunoprecipitated samples were denatured in 3× LDS sample buffer and separated on either 10 or 12% SDS-PAGE gel. Separated proteins were transferred onto PVDF membrane (1620177; Bio-Rad). The following antibodies were used in our study: phospho-AMPK-α (25335S, 1:1000 dilution; Cell Signaling Technology) rabbit mAb, AMPK-α (58325, 1:1000 dilution; Cell Signaling Technology) rabbit mAb, AMPK-β (4150S, 1:1000 dilution; Cell Signaling Technology) rabbit mAb, AMPK-γ (4187S, 1:1000 dilution; Cell Signaling Technology) rabbit polyclonal Ab, phospho-ACC (3661S, 1:1000 dilution; Cell Signaling Technology) rabbit pAb, acetylCoA carboxylase (ACC) (36625, 1:1000 dilution; Cell Signaling Technology) rabbit pAb, phospho-S6K (9205S, 1:1000 dilution; Cell Signaling Technology) rabbit pAb, S6K (9202S, 1:1000 dilution; Cell Signaling Technology) rabbit pAb, phospho-S6 (2215S, 1:3000 dilution; Cell Signaling Technology) rabbit pAb, phospho-GSK3 (9204S, 1:1000 dilution; Cell Signaling Technology) rabbit pAb, GSK3 (9202S, 1:1000 dilution; Cell Signaling Technology) rabbit pAb, phospho-GSK3 (2460S, 1:1000 dilution; Cell Signaling Technology) rabbit pAb, c-Myc (5605P, 1:1000 dilution; Cell Signaling Technology) rabbit mAb, and Flag-tag (14793S, 1:5000 dilution; Cell Signaling Technology) rabbit mAb.

Cell death assay

Cell death was measured by propidium iodide (PI) staining as previously described (34, 37, 38). Briefly, 1 × 10⁶ cells were counted and washed twice with PBS. Cells were resuspended in 100 µl PBS containing 2 µg/ml PI and incubated for 30 min at room temperature. Percentages of PI-stained cells were analyzed using a flow cytometer (Accuri C6; BD Biosciences, San Jose, CA, USA) and compared with unstained cells.

Light microscopy and immunofluorescence

For light microscopy, cells were cultured in 6-well plates and incubated in glucose-containing and glucose-free medium for the indicated time points. After treatment, phase contrast images were acquired using an EVO51 microscope (Advanced Microscopy Group, Bothell, WA, USA) with ×10 phase contrast objective. For each condition, images from at least 3 different fields were taken, and representative images from the single field were shown. For immunofluorescence microscopy, HeLa cells (5 × 10⁵) were plated on glass coverslips in 6-well plates and transfeacted with AMPK-α-mCherry (2 µg) and STIM2-YFP (2 µg). After 36 h, cells were incubated with ionomycin (1 µM) or thapsigargin (1 µM) containing medium for 30 min before proceeding to fixation. Cells were washed 3 times with PBS and fixed using 4% paraformaldehyde (sc-281692; Santa Cruz Biotechnology, Dallas, TX, USA) for 15 min at room temperature. Cells were washed 3 times after fixation, and coverslips were mounted on glass slides using Vectashield (H-1200; Vector Laboratories, Burlingame, CA, USA) containing DAPI. In other experiments, HeLa cells were transfected with CaMKK2-myc (2 µg). Thirty-six hours after transfection, cells were treated with thapsigargin (1 µM) for 30 min before washing and fixation. After fixation, cells were permeabilized with 0.5% Triton X-100 (T9284; MilliporeSigma) in PBS for 5 min at room temperature. Cells were then washed and blocked with Odyssey locking buffer (927-40100; Li-Cor Biosciences, Lincoln, NE, USA) for 60 min and incubated with the following primary antibodies: rabbit anti-myc (ab9106, 1:500 dilution; Abcam) mAb and anti-AMPKα (NBP2-22127, 1:200 dilution; Novus Biologic, Littleton, CO, USA) mouse mAb prepared in Odyssey blocking buffer overnight at 4°C. After incubation with primary antibodies, cells were washed 3 times and incubated with the following secondary antibodies: Alexa Fluor 488 goat anti-mouse IgG antibody (A-11001, 1:500 dilution; Thermo Fisher Scientific) and Alexa Fluor 633 goat anti-rabbit IgG antibody (A21070, 1:50 diluted in 100 µl PBS containing 2 % SDF-PAGE gel and analyzed by immunoblotting after Western blotting. For immunoprecipitation of endogenous AMPK proteins or Myc tag STIM1 proteins, 30 µl Protein G magnetic beads (90065; Cell Signaling Technology, Danvers, MA, USA) were washed twice with NP40 lysis buffer and incubated with the cell lysate with anti-AMPKα1+α2 antibody (ab80039; Abcam, Cambridge, MA, USA) or anti-Myc antibody (22765; Cell Signaling Technology, Danvers, MA, USA) overnight at 4°C. Immunoprecipitates were washed 3 times with NP40 lysis buffer and subjected to denaturation in 30 µl LDS sample buffer. Coimmunoprecipitated proteins were resolved on 10% SDS-PAGE gel and analyzed by immunoblotting.

Western blotting analysis

For Western blotting analysis, 20–40 µg cell lysate or immunoprecipitated samples were denatured in 3× LDS sample buffer and separated on either 10 or 12% SDS-PAGE gel. Separated proteins were transferred onto PVDF membrane (1620177; Bio-Rad). The following antibodies were used in our study: phospho-AMPK-α (25335S, 1:1000 dilution; Cell Signaling Technology) rabbit mAb, AMPK-α (58325, 1:1000 dilution; Cell Signaling Technology) rabbit mAb, AMPK-β (4150S, 1:1000 dilution; Cell Signaling Technology) rabbit mAb, AMPK-γ (4187S, 1:1000 dilution; Cell Signaling Technology) rabbit polyclonal Ab, phospho-ACC (3661S, 1:1000 dilution; Cell Signaling Technology) rabbit pAb, acetylCoA carboxylase (ACC) (36625, 1:1000 dilution; Cell Signaling Technology) rabbit pAb, phospho-S6K (9205S, 1:1000 dilution; Cell Signaling Technology) rabbit pAb, S6K (9202S, 1:1000 dilution; Cell Signaling Technology) rabbit pAb, phospho-S6 (2215S, 1:3000 dilution; Cell Signaling Technology) rabbit pAb, phospho-GSK3 (9204S, 1:1000 dilution; Cell Signaling Technology) rabbit pAb, GSK3 (9202S, 1:1000 dilution; Cell Signaling Technology) rabbit pAb, phospho-GSK3 (2460S, 1:1000 dilution; Cell Signaling Technology) rabbit pAb, c-Myc (5605P, 1:1000 dilution; Cell Signaling Technology) rabbit mAb, and Flag-tag (14793S, 1:5000 dilution; Cell Signaling Technology) rabbit mAb.

STIM2 INTERACTS WITH AND REGULATES AMPK 2959
Figure 1. STIM2 interacts with AMPK. A, B) Coimmunoprecipitation (Co-IP) of STIM2 and AMPK subunits in HEK 293T (A) and HeLa (B) cells. Protein extracts from cells stably expressing STIM2-SFB were immunoprecipitated using S-protein beads after immunoblotting with the indicated antibodies. Cells expressing empty vector (EV) were used as the control. C) Endogenous interaction of STIM2 and AMPK. HeLa cell lysates were subjected for immunoprecipitation using mouse anti-AMPK-α antibody and IgG control. Coprecipitated STIM2 was detected after immunoblotting. The arrow denotes STIM2 in AMPK immunoprecipitation. D–F) STIM2 interacts with all 3 subunits of AMPK. HEK 293T cells were cotransfected with STIM2-SFB or EV along with myc–AMPK-α (D), myc–AMPK-β (E), or myc–AMPK-γ (F), and then subjected for STIM2-SFB immunoprecipitation. Coprecipitated proteins were detected by immunoblotting with anti-myc antibody. G) STIM2 interacts with AMPK independent of STIM1. STIM1 wild-type (WT) or knock-out (KO) cells expressing STIM2-SFB or control vector were immunoprecipitated with S-protein beads. Coprecipitated proteins were probed by indicated antibodies. H) STIM1 does not interact with AMPK. Protein extracts from transiently transfected HEK293T cells with STIM1-myc were immunoprecipitated using S-protein beads after immunoblotting with the indicated antibodies. Cells expressing EV were used as control.
Figure 2. STIM2 interaction with AMPK promotes AMPK activation. A) Schematic representation of STIM2 full-length (FL) and deletion mutants used in this study. B) STIM2 interacts with AMPK through its C-terminal region. Protein extracts of HEK 293T cells expressing FL and truncated mutants of STIM2 were immunoprecipitated with S-protein beads and analyzed for AMPK binding. C) Detailed analysis of AMPK binding to different STIM2 mutants. D) Quantification of AMPK protein levels in cell lysates and immunoprecipitates. E) Western blot analysis of AMPK protein levels in HEK 293T cells expressing different STIM2 constructs. (continued on next page)
Real-time intracellular Ca^{2+} measurements

Real-time intracellular [Ca^{2+}] was measured with Fura-2 AM as previously described (39, 40). In brief, 786-O or HeLa cells were seeded and cultured on a glass-bottom plate for 24 h before imaging. The cells were loaded with 2 μM Fura-2 AM in the dark for 30 min at room temperature. Intracellular [Ca^{2+}] levels are shown as the F340/F380 ratio, which was collected every 5 s by the Nikon A1 confocal microscope.

Oligonucleotide and primer sequences

Primers used for protein expression:

- STIM2 forward: 5’-CACCCTTGCTGTGCTGGCCTG-3’
- STIM2 reverse: 5’-CTTAGATTTCTCTTAAAAGGC-3’
- STIM2-242 forward: 5’-CACCAAGAAGATGTGCAAA-AATGATG-3’
- STIM2-341 forward: 5’-CATTTCAATCTTITTTGGCC-3’
- STIM2-441 reverse: 5’-ACAGATCTTCTCAAATTTTGCC-3’
- STIM2-P/H forward: 5’-TTTCTCTCCATTATGACAAAGG-3’
- STIM2-P/H reverse: 5’-CACCGTGCAGACCATTCA-CATGCTCGCCGTGAA-3’
- STIM2-730 reverse: 5’-TTTCTCCTCATATGACAAAGG-TCC-3’
- AMPKa1 forward: 5’-CGGCTCGAGATGCAGATCCTACGTCTCCG-3’
- AMPKa1 reverse: 5’-GGGAAATTCTGTGTGCAAGATA-CTTAG-3’

Guide RNA sequences used for generation of CRISPER knockout cells:

- STIM1 sg: 5’-GATATCCCTCCGCTCTGCTGC-3’
- STIM2 sg1: 5’-GCCCCCTTGAGACACTCTCTCG-3’
- STIM2 sg2: 5’-GACCCTGAGAGGTCTCTCA-3’

Statistical analysis and reproducibility

All experiments were performed at least 3 times. A Student’s t-test was used to calculate P values. A value of P < 0.05 was considered statistically significant.

RESULTS

STIM2 interacts with AMPK

To identify potential novel regulators of AMPK signaling, we mined the BioPlex protein–protein interaction database (41) to search for potential AMPK-interacting proteins. Analysis of AMPK interactome identified STIM2 as a putative AMPK binding protein. AMPK consists of 1 catalytic subunit (α subunit) and 2 regulatory subunits (β and γ subunits), which together form a stable heterotrimeric complex (4). Further analysis of the BioPlex database revealed that immunoprecipitation of overexpressed STIM2 followed by mass spectrometry identified all 3 subunits of the AMPK complex as potential STIM2-binding proteins (Table 1). To confirm STIM2 interaction with AMPK, we generated stable cells expressing STIM2-SFB (S protein–Flag-SFB tagged at the C-terminal cytoplasmic region of STIM2). Pulldown of overexpressed STIM2 coprecipitated all 3 subunits of endogenous AMPK complex in both HEK 293T and HeLa cells (Fig. 1A, B). To further validate our findings, we immunoprecipitated endogenous AMPK using anti-AMPK-α antibody and detected coprecipitated endogenous STIM2 in HeLa cells, confirming the AMPK-STIM2 interaction at the endogenous level (Fig. 1C). Because HeLa is an LKB1-deficient cell line (10), our data also indicated that the STIM2–AMPK interaction is independent of LKB1.

We carried out experiments to identify the regions in STIM2 that mediate its interaction with AMPK. STIM2 is a coprecipitated AMPK subunits after immunoblotting with the indicated antibodies. C) Schematic representation of STIM2 FL and other deletion mutants in this study. D) HEK 293T cells expressing STIM2 FL (1–746) and STIM2 1–441 were immunoprecipitated using S-protein beads and analyzed for coprecipitated proteins using the indicated antibodies. E) HEK 293T cells expressing FL STIM2, STIM2 deletion mutants for the P/H domain (ΔP/H) or the K domain (ΔK) were immunoprecipitated using S-protein beads and analyzed for coprecipitated proteins using indicated antibodies. F) Transient overexpression of STIM2 FL, but not its AMPK-nonbinding mutant, promotes AMPK phosphorylation. Cell lysates from HEK 293T cells expressing either empty vector, STIM2 FL, or its AMPK-nonbinding mutant (STIM2 1–441) were analyzed by immunoblotting for the phosphorylated and total levels of AMPK.

STIM2 interaction with AMPK modulates AMPK activation

We carried out experiments to identify the regions in STIM2 that mediate its interaction with AMPK. STIM2 is a
Figure 3. STIM2 does not regulate AMPK-mTORC1 signaling upon energy stress. A) Glucose starvation does not alter AMPK binding to STIM2. Cells stably expressing STIM2-SFB were cultured in glucose-containing and glucose-free medium for 6 h and subjected for immunoprecipitation by S-protein beads. Coprecipitated proteins were analyzed after Western blotting. B, C) STIM2 levels in 786-O (B) and HeLa (C) cells infected with control sgRNA (Ctl sg), STIM2 sgRNA-1 (STIM2 sg1), and STIM2 sgRNA-2 (STIM2 sg2) were measured by Western blotting. D, E) Energy stress does not alter AMPK and mTORC1 activation in STIM2-depleted cells. STIM2-decient 786-O cells were cultured in glucose-containing or glucose-free medium for 24 h (D) or treated with 2-deoxy-glucose (5 mM) for 18 h (E). Cell lysates were analyzed by immunoblotting for the phosphorylated and total levels of indicated proteins. F–I) STIM2 depletion does not affect glucose starvation–induced cell death. STIM2-deficient 786-O (F, H) and HeLa (G, I) cells were cultured in glucose-containing or glucose-free medium for 48 or 6 h, respectively. Representative images (F, G) and quantification of the cell death (H, I) were analyzed after glucose starvation. Ns, not significant. P > 0.05.
single transmembrane protein and consists of a luminal N-terminal region, a transmembrane region, and a cytoplasmic C-terminal region that contains a coiled-coil domain/STIM1 Orai1-activating region, a Pro/His-rich (P/H) domain, and a Lys-rich (K) domain (Fig. 2A) (26, 43, 44). Because AMPK mainly localizes in cytoplasm, we generated a series of truncation mutants of the cytoplasmic region in STIM2 (Fig. 2A) and examined their interactions with endogenous AMPK. Such analyses revealed that the C-terminal portion of the STIM2 cytoplasmic region (442–776 aa, which contains P/H and K domains) was both required and sufficient for mediating STIM2 interaction with AMPK (Fig. 2A, B). We then truncated 442–776 aa in the context of full-length STIM2 (1–441 aa; Fig. 2C) and confirmed that full-length STIM2, but not the 1–441 mutant, interacted with AMPK (Fig. 2D). To further characterize the STIM2 interaction with AMPK, we generated STIM2 truncation mutants lacking either the P/H domain (ΔP/H) or the K domain (ΔK). Coimmunoprecipitation experiment revealed that ΔP/H and ΔK mutants of STIM2 could still interact with AMPK (Fig. 2E). These data suggest that the P/H and K domains are not required for STIM2 interaction with AMPK. Because the SOAR, P/H, and K domains are conserved between STIM1 and STIM2, our data showing that none of these domains is critical for STIM2 interaction with AMPK explains why AMPK selectively interacts with STIM2 but not STIM1 and suggests that a divergent region in the C-terminal portion of STIM2, such as the linker region between P/H and K domains, might be involved in STIM2 interaction with AMPK.

To determine any possible role of STIM2 in regulating AMPK signaling, we overexpressed full-length STIM2 and its AMPK nonbinding mutant (1–441) in HEK 293T cells. Overexpression of full-length STIM2, but not its

Figure 4. Cytosolic calcium influx regulates AMPK-STIM2 colocalization. HeLa cells transiently expressing AMPKα-mCherry and STIM2-YFP were incubated with medium containing ionomycin (1 μM) or thapsigargin (1 μM) for 30 min and processed for immunofluorescence imaging. Represented images from each condition are presented. Overlap coefficients for the STIM2 and AMPK signal are presented in the upper right corner of the merge panels (n = 12 for DMSO- or ionomycin-treated cells; n = 15 for thapsigargin-treated cells). In all images, insets display the selected area along with the overlays. Scale bars, 5 μm. P < 0.0001.
Figure 5. STIM2 regulates calcium-induced AMPK activation. A, B) Cytosolic calcium influx promotes STIM2–AMPK interaction. HEK 293T (A) and HeLa (B) cells stably expressing STIM2-SFB were incubated with medium containing ionomycin (1 μM) or thapsigargin (1 μM) for 30 min. Cell lysates were subjected to immunoprecipitation using S-protein beads. Coprecipitates were (continued on next page)
AMPK nonbinding mutant, increased AMPK phosphorylation (Fig. 2F), suggesting that STIM2 promotes AMPK activation likely through its interaction with AMPK. Together, our results revealed that the C-terminus of the STIM2 cytoplasmic region mediates its interaction with AMPK and suggested that STIM2 interaction with AMPK promotes AMPK activation.

**STIM2 does not regulate AMPK-mTORC1 signaling upon energy stress**

Given the well-established role of AMPK in mediating energy stress response (2), we studied the potential role of STIM2 in regulating AMPK signaling in response to energy stress. Previous studies have shown that glucose starvation–induced energy stress modulates AMPK interaction with its regulators, such as LKB1 and NBR2 (10, 14). Thus, we examined whether glucose starvation also affected STIM2–AMPK complex formation. Our analysis showed that glucose starvation did not obviously affect STIM2 interaction with any of the AMPK subunits (Fig. 3A). We then studied the potential role of STIM2 in regulating energy stress–induced AMPK activation. To this end, we used CRISPR-Cas9 technology to generate STIM2 KO cells in both 786-O and HeLa cells by 2 independent guide RNAs (Fig. 3B, C). Consistent with our previous report (10), glucose starvation in 786-O cells potently induced phosphorylation of AMPK and the AMPK substrate ACC and inhibited phosphorylation of S6K and S6, the biochemical markers of mTORC1 activation; however, STIM2 deficiency did not affect glucose starvation–induced AMPK activation or mTORC1 inhibition (Fig. 3D). Similarly, STIM2 deletion did not affect AMPK or mTORC1 signaling upon treatment of 2 deoxy-glucose, another energy stress inducer that inhibits hexokinase and blocks glycolysis (10) (Fig. 3E). Because energy stress–induced AMPK activation is known to be defective in the HeLa cell line (which is LKB1 deficient) (10), we did not further study whether STIM2 deficiency affects energy stress–induced AMPK activation in HeLa cells. In response to energy stress, AMPK promotes catabolic processes and inhibits anabolic processes to conserve ATP expenditure and to maintain cell survival during energy stress; deficiency of AMPK or its activators, such as LKB1 or NBR2, results in defective energy stress response and impaired survival under energy deprivation (2, 10). Consistent with these data, we did not observe any significant difference in glucose starvation–induced cell death in both control and STIM2 KO 786-O or HeLa cells (Fig. 3F–I). Together, these results suggested that STIM2 is not required for energy stress–induced AMPK activation.

**STIM2 regulates calcium-induced AMPK signaling**

Similar to energy stress–mediated AMPK activation, alteration in intracellular calcium levels can also modulate AMPK signaling (45). Given the established role of STIM2 as a calcium sensor (26), we studied the potential involvement of STIM2 in calcium-induced AMPK activation. We first analyzed the subcellular localization of STIM2 and AMPK by immunofluorescence and observed an increase in colocalization between YFP-tagged STIM2 and mCherry-tagged AMPK-α after treatment of ionomycin or thapsigargin (Fig. 4). Further supporting our observation, coimmunoprecipitation analysis revealed an increase in the interaction between STIM2 and AMPK upon treatment of calcium ionophores (Fig. 5A, B). Next, we examined the effect of STIM2 deficiency on calcium-induced AMPK activation. As expected, ionomycin or thapsigargin treatment increased phosphorylation levels of both AMPK and AMPK substrate ACC. STIM2 deletion significantly attenuated ionomycin- or thapsigargin–induced AMPK activation (Fig. 5C, D). We made a similar observation in HeLa cells, an LKB1-deficient cell line (Fig. 5E, F), thus suggesting that STIM2 regulation of calcium-induced AMPK activation is independent of LKB1. We also examined the effect of STIM2 deficiency on SOCE in the cell lines used in our study. Consistent with previously reported results (32), our data revealed that STIM2 deletion did not affect SOCE in either 786-O or HeLa cells (Fig. 5G, H), suggesting that it is less likely that STIM2 regulates AMPK indirectly through SOCE. Collectively, our results indicated that an increase in the cytosolic calcium levels promotes complex formation between STIM2 and AMPK and that STIM2 promotes calcium-induced AMPK activation independent of SOCE.

**STIM2 interacts with CaMKK2 and regulates CaMKK2 colocalization with AMPK**

The aforementioned data prompted us to further study the potential mechanism by which STIM2 regulates calcium-mediated AMPK activation. Because CaMKK2 functions as the main upstream kinase for calcium-induced AMPK phosphorylation (19, 21), we analyzed the potential interaction of STIM2 with CaMKK2. Our results showed that ectopically expressed STIM2 interacted with CaMKK2 (Fig. 6A). Similar to our observation with calcium-stimulated STIM2 interaction with AMPK (Fig. 5A, B), treatment of cells with thapsigargin also increased STIM2 interaction with CaMKK2 in both HEK 293T and HeLa cells (Fig. 6B, C).
Figure 6. STIM2 recruits CaMKK2 to regulate calcium-induced AMPK activation. A) STIM2 interacts with CaMKK2. HEK 293T cells were cotransfected with STIM2-SFB [or empty vector (EV)] and myc-CaMKK2. STIM2-SFB was immunoprecipitated using S-protein beads, and coprecipitated myc-CaMKK2 was detected after immunoblotting using anti-myc antibody. B, C) (continued on next page)
To map the regions of STIM2 responsible for its interaction with CaMKK2, we cotransfected various truncation mutants of STIM2 with CaMKK2 into cells and examined their binding with CaMKK2. Coimmunoprecipitation analysis revealed that STIM2 interacted with CaMKK2 through its CAD/SOAR domain spanning residues 349–441 (Fig. 6D). The CaMKK2-binding region in STIM2 is different from and adjacent to its AMPK-interacting region (residues 442–746; Fig. 2A, B), indicating that STIM2 might act as a scaffolding protein for AMPK and CaMKK2. To test this hypothesis, we examined whether STIM2 deficiency affected AMPK interaction with CaMKK2. Consistent with a previous report (46), we failed to detect an interaction between CaMKK2 and AMPK by coimmunoprecipitation analysis (Fig. 6E), likely due to a weak and/or transient interaction between CaMKK2 and AMPK, which is common for interactions between kinases and substrates. As more indirect evidence, our analysis by immunofluorescence microscopy showed that CaMKK2 partially colocalized with AMPK upon thapsigargin treatment, and the CaMKK2–AMPK colocalization was significantly attenuated in STIM2 KO cells (Fig. 6F). In summary, our results showed that STIM2 also interacts with CaMKK2 and suggest that STIM2 might serve as a scaffolding protein to promote CaMKK2 interaction with and phosphorylation of AMPK in response to calcium stimulation.

**Discussion**

Regulation of AMPK activation is central to the maintenance of cellular homeostasis. AMPK activation by alterations of intracellular energy or calcium levels is mediated through distinctive mechanisms: LKB1 mediates energy stress–induced AMPK activation, whereas CaMKK2 mainly mediates calcium-induced AMPK activation. Our study identifies STIM2 as a regulator of calcium-induced, but not energy stress–induced, AMPK activation and suggests that the increase in intracellular calcium levels promotes STIM2 interaction with both AMPK and CaMKK2 and that STIM2 functions as a scaffolding protein to tether CaMKK2 to phosphorylate AMPK and thus promotes calcium-induced AMPK activation (Fig. 7). This model is supported by several lines of evidence. The rise in intracellular calcium level, either through depletion of ER calcium levels (*via* thapsigargin treatment) or influx from extracellular space (*via* ionomycin treatment), promotes the interaction between STIM2 and AMPK (as well as CaMKK2) (Figs. 5A, B and 6B, C); AMPK- and CaMKK2-interacting regions in STIM2 are adjacent to each other, raising the possibility that STIM2 can tether CaMKK2 to interact with AMPK (Figs. 2A, B and 6D); and CaMKK2 colocalization with AMPK and calcium-induced AMPK activation is significantly compromised in STIM2-deficient cells (Figs. 5C–F and 6F). Our model is thus analogous to another model wherein energy stress promotes AXIN–LKB1–AMPK complex formation and the scaffolding protein AXIN tethers LKB1 to phosphorylate AMPK in response to energy stress (14).

The underlying mechanisms by which intracellular calcium regulates STIM2 interaction with AMPK (or CaMKK2) remain elusive. STIM2 proteins contain a luminal EF-hand motif that can associate with calcium in ER. It has been proposed that, after depletion of ER calcium stores, the dissociation of calcium from the luminal EF-hand motif in STIM triggers STIM conformational changes to a more extended state, promoting its oligomerization and interaction with plasma membrane–localized ORAI1, thereby opening ORAI1 for calcium entry (26). STIM2 is a more sensitive calcium sensor but is a much weaker activator of SOCE than STIM1. It has been shown that phenylalanine residue (Phe-394) of STIM1 is crucial for its interaction with ORAI1; however, this residue is replaced by leucine in STIM2, resulting in much reduced affinity for STIM2 to interact with ORAI1 (47). Our study raises an intriguing hypothesis that the release of calcium from the luminal EF-hand motif in STIM2 might cause a conformational change of STIM2 and expose the AMPK- and CaMKK2-interacting sites located in the cytosolic region of STIM2, resulting in STIM2 interaction with AMPK and CaMKK2 and subsequent CaMKK2-mediated AMPK phosphorylation (Fig. 7). This hypothesis awaits further investigation in future studies.

Our study has convincingly showed the interaction between STIM2 and AMPK; however, which subunit of the AMPK complex mediates STIM2 interaction with CaMKK2 and AMPK is unknown. Further investigation is necessary to identify the specific domains of STIM2, CaMKK2, and AMPK that are involved in their interaction.

**Cytosolic calcium influx promotes STIM2 interaction with CaMKK2.** HEK 293T (B) and HeLa (C) cells stably expressing STIM2-SFB alone were transiently transfected with myc-CaMKK2 and then incubated with medium containing thapsigargin (1 μM) for 30 min. Cell lysates were subjected to immunoprecipitation using S-protein beads. Coprecipitates were analyzed by Western blotting with anti-myc antibody. D) STIM2 interacts with CaMKK2 through its CAD/SOAR region. Upper panel: Schematic representation of STIM2 full-length and deletion mutants used in this study. Lower panel: Protein extracts of HEK 293T cells expressing full-length and truncated mutants of STIM2-SFB alone with myc-CaMKK2 were immunoprecipitated with S-protein beads and analyzed for coprecipitated myc-CaMKK2 using immunoblotting with anti-myc antibody. E) AMPK does not form a stable complex with CaMKK2. Protein extracts of HEK 293T cells expressing full-length CaMKK2-SFB were immunoprecipitated with S-protein beads and analyzed for coprecipitated proteins using indicated antibodies. F) Calcium-induced colocalization between AMPK and CaMKK2 depends on STIM2. Control and STIM2-depleted HeLa cells were transiently transfected with myc-CaMKK2, incubated with medium containing thapsigargin (1 μM) for 30 min, and processed for immunofluorescence imaging. Rabbit anti-myc antibody, goat anti-rabbit IgG Alexa Fluor 633 secondary antibody for myc (red), and mouse anti–AMPKα goat anti-mouse IgG Alexa Fluor 488 secondary for AMPKα (green) were used. Representative images from each condition are presented. Overlap coefficients for the AMPK and CaMKK2 signal are presented in the upper right corner of the merge panels (n = 19 for Ctl sg + DMSO, n = 22 for Ctl sg + thapsigargin, and n = 18 for STIM2 sg + thapsigargin). In all images, insets display the selected area along with the overlays. Scale bars, 5 μm. P < 0.0001.
AMPK remains unclear. Our coimmunoprecipitation analysis showed that STIM2 interacted with all 3 ectopically expressed AMPK subunits (Fig. 1D–F). Thus, it is likely that STIM2 can directly interact with all 3 subunits of AMPK. However, because the cell lines used in our experiments (HEK 293T or HeLa cells) express endogenous AMPK, it is possible that STIM2 interaction with some AMPK subunits might be indirect and mediated through another AMPK subunit or a third partner. Future studies will be directed to further characterize the interaction between STIM2 and AMPK.

ACKNOWLEDGMENTS

The authors thank all members of the B.G. laboratory for their advice and technical assistance. This research was supported by the Andrew Sabin Family Fellow Award, the Sister Institution Network Fund, the Anna Fuller Fund, U.S. National Institutes of Health (NIH) National Cancer Institute Grant CA181196 (to B.G.), NIH National Institute of General Medical Sciences Grant R01GM112003, Welch Foundation Grant BE-1913, American Cancer Society Grant RSG-16-215-01 TBE (to Y.Z.), and NIH Cancer Center Support Grant P30CA016672 to The University of Texas M. D. Anderson Cancer Center. The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

X. Liu performed the experiments shown in Figs. 1G, H, 2C–F, 5E, and 6E; J. Jing performed experiments shown in Fig. 5G, H and generated AMPKα-mCherry construct; A. S. Chauhan performed most of the other experiments with assistance from H. Lee and R. K. Yadav; J. Liu generated the STIM1 KO cells used in Fig. 1G; B. Gan and Y. Zhou supervised the study; A. S. Chauhan, Y. Zhou, and B. Gan designed the experiments and wrote the manuscript; and all authors commented on the manuscript.

REFERENCES

1. Hardie, D. G., Schaffer, B. E., and Brunet, A. (2016) AMPK: an energy-sensing pathway with multiple inputs and outputs. *Trends Cell Biol.* 26, 190–201
2. Mihaylova, M. M., and Shaw, R. J. (2011) The AMPK signaling pathway coordinates cell growth, autophagy and metabolism. *Nat. Cell Biol.* 13, 1016–1025
3. Ross, F. A., MacKintosh, C., and Hardie, D. G. (2016) AMP-activated protein kinase: a cellular energy sensor that comes in 12 flavours. *FEBS J.* 283, 2987–3001
4. Lin, S. C., and Hardie, D. G. (2018) AMPK: sensing glucose as well as cellular energy status. *Cell Metab.* 27, 299–313
5. Ross, F. A., Jensen, T. E., and Hardie, D. G. (2016) Differential regulation by AMP and ADP of AMPK complexes containing different subunit isoforms. *Biochem.* J. 473, 189–199
6. Xiao, B., Sanders, M. J., Underwood, E., Heath, R., Mauer, F. V., Carmena, J., Jing, C., Walker, P. A., Eccleston, J. F., Haire, L. F., Sáu, P., Howell, S. A., Aasland, R., Martin, S. R., Carling, D., and Gamblin, S. J. (2011) Structure of mammalian AMPK and its regulation by ADP. *Nature* 472, 290–293
7. Shaw, R. J., Kosmatka, M., Bardeesy, N., Hurley, R. L., Witters, L. A., DePinho, R. A., and Cantley, L. C. (2004) The tumor suppressor LKB1 kinase directly activates AMP-activated kinase and regulates apoptosis in response to energy stress. *Proc. Natl. Acad. Sci. USA* 101, 3329–3335
8. Woods, A., Johnstone, S. R., Dickerson, K., Leiper, F. C., Fryer, L. G., Neumann, D., Schlattner, U., Wallimann, T., Carlson, M., and Carling, D. (2003) LKB1 is the upstream kinase in the AMP-activated protein kinase cascade. *Curr. Biol.* 13, 2004–2008
9. Hawley, S. A., Boudeau, J., Reid, J. I., Mustard, K. J., Udd, L., Mäkela, T. P., Alesi, D. R., and Hardie, D. G. (2005) Complexes between the LKB1 tumor suppressor, STRAD alpha/beta and MO25 alpha/beta are upstream kinases in the AMP-activated protein kinase cascade. *J. Biol.* 2, 28
10. Liu, X., Xiao, Z. D., Han, L., Zhang, J., Lee, S. W., Wang, W., Lee, H., Zhuang, L., Chen, J., Lin, H. K., Wang, J., Liang, H., and Gan, B. (2016) LncRNA NBR2 engages a metabolic checkpoint by regulating AMPK under energy stress. *Nat. Cell Biol.* 18, 431–442
11. Liu, X., Xiao, Z. D., and Gan, B. (2016) An lncRNA switch for AMPK activation. *Cell Cycle* 15, 1948–1949
12. Xiao, Z. D., Liu, X., Zhuang, L., and Gan, B. (2016) NBR2: a former junk gene emerges as a key player in tumor suppression. *Mol. Cell. Oncol.* 3, e1187322
13. Zhang, C. S., Jiang, B., Li, M., Zhu, M., Peng, Y., Zhang, Y. L., Wu, Y. Q., Li, T. Y., Liang, Y., Lu, Z., Lian, G., Liu, Q., Guo, H., Yin, Z., Ye, Z., Han, J., Wu, J. W., Yin, H., Lín, S. Y., and Lin, S. C. (2014) The lysosomal v-ATPase-Ragulator complex is a common activator for AMPK and
mTORC1, acting as a switch between catabolism and anabolism. Cell Metab. 20, 526–540
14. Zhang, Y. L., Guo, H., Zhang, C. S., Lin, S. Y., Yin, Z., Peng, Y., Luo, H., Shi, Y., Lian, G., Zhang, C., Li, M., Ye, Z., Ye, J., Han, J., Li, P., Wu, J. W., and Lin, S. C. (2013) AMP as a low-energy charge signal autonomously initiates assembly of AXIN-AMPK-LKB1 complex for AMPK activation. Cell Metab. 18, 546–555
15. Gwinn, D. M., Shackelford, D. B., Egan, D. F., Mihaylova, M. M., Mery, A., Vasquez, D. S., Turk, B. E., and Shaw, R. J. (2008) AMPK phosphorylation of raptor mediates a metabolic checkpoint. Mol. Cell 30, 214–226
16. Inoki, K., Zha, T., and Guan, K. L. (2003) TSC2 mediates cellular energy response to control cell growth and survival. Cell 115, 577–590
17. Carling, D. (2017) AMPK signaling in health and disease. J. Biol. Chem. 290, 5319–5327
18. Huang, J., and Manning, B. D. (2008) The TSC1-TSC2 complex: a molecular switchboard controlling cell growth. Biochem. J. 412, 179–190
19. Hawley, S. A., Pan, D. A., Mustard, K. J., Ross, L., Bain, J., Edelman, A. M., Frenguelli, B. G., and Hardie, D. G. (2005) Calmodulin-dependent protein kinase kinase-beta is an alternative upstream kinase for AMP-activated protein kinase. Cell 121, 493–505
20. Hurley, R. L., Anderson, K. A., Franzone, J. M., Kemp, B. E., Means, A. R., and Witters, L. A. (2005) The Ca2+/calmodulin-dependent protein kinase kinases are AMP-activated protein kinase kinases. J. Biol. Chem. 280, 29060–29066
21. Woods, A., Dickerson, K., Heath, R., Song, S. P., Momcilovic, M., Johnstone, S. R., Carlson, M., and Carling, D. (2005) Ca2+/calmodulin-dependent protein kinase beta acts upstream of AMP-activated protein kinase in mammalian cells. Cell Metab. 2, 21–33
22. Racioppi, L., and Means, A. R. (2012) Calcium/calmodulin-dependent protein kinases: dynamic calcium signal transducers. Nat. Rev. Mol. Cell Biol. 13, 549–565
23. Wedel, B., Boyles, R. R., Putney, J. W., Jr., and Bird, G. S. (2007) Role of the store-operated calcium entry proteins Stim1 and Orai1 in muscarinic cholinergic receptor-stimulated calcium oscillations in human embryonic kidney cells. J. Physiol. 579, 679–689
24. Brandman, O., Liou, J., Park, W. S., and Meyer, T. (2007) STIM2 is a feedback regulator that stabilizes basal cytosolic and endoplasmic reticulum Ca2+ levels. Cell 131, 1327–1339
25. Soboloff, J., Spassova, M. A., Hewavitharana, T., He, L. P., Xu, W., Johnstone, L. S., Dizaduez, M. A., and Gill, D. L. (2006) STIM2 is an inhibitor of STIM1-mediated store-operated Ca2+ Entry. Curr. Biol. 16, 1465–1470
26. Roos, J., DiGregorio, P. J., Yeromina, A. V., Ohlsen, K., Lioudyno, M., Zhang, S., Safina, O., Kozak, J. A., Wagner, S. L., Cahalan, M. D., Velicelciu, G., and Stauderman, K. A. (2005) STIM1, an essential and conserved component of store-operated Ca2+ channel function. J. Cell Biol. 169, 433–445
27. Bird, G. S., Hwang, S. Y., Smith, J. T., Fukushima, M., Boyles, R. R., and Putney, J. W., Jr. (2010) STIM1 is a calcium sensor specialized for digital signaling. Curr. Biol. 19, 1724–1729
28. Zhao, Z. D., Han, L., Lee, H., Zhuang, L., Zhang, Y., Badourou, J., Nagrath, D., Wood, C. G., Gu, J., Wu, X., Liang, H., and Gan, B. (2017) Energy stress-induced lncRNA FILN1 represses c-Myc-mediated energy metabolism and inhibits renal tumor development. Nat. Commun. 8, 783
29. Kopppula, P., Zhang, Y., Shi, J., Li, W., and Gan, B. (2017) The glutamate/cysteine transporter SLC7A11/XTT enhances cancer cell dependency on glucone by exporting glutamate. J. Biol. Chem. 292, 14240–14249
30. Wang, W., Li, N., Li, X., Tran, M. K., Han, X., and Chen, J. (2015) Tankyrase inhibitors target YAP by stabilizing angiomotin family proteins. Cell Rep. 13, 524–532
31. Lin, A., Piao, H. L., Zhuang, L., Sarbassov, D. D., Ma, L., and Gan, B. (2014) FOXO transcription factors promote AKT Ser473 phosphorylation and renal tumor growth in response to pharmacological inhibition of the PI3K-AKT pathway. Cancer Res. 74, 1682–1693
32. Dai, F., Lee, H., Zhang, Y., Zhuang, L., Yao, H., Xi, Y., Xiao, Z. D., You, M. J., Li, W., Su, X., and Gan, B. (2017) BAP1 inhibits the ER stress gene regulatory network and modulates metabolic stress response. Proc. Natl. Acad. Sci. USA 114, 3192–3197
33. Lin, A., Yao, J., Zhuang, L., Wang, D., Han, J., Lam, E. W., and Gan, B. (2014) The FOXO-BNP3 axis exerts a unique regulation of mTORC1 and cell survival under energy stress. Oncogene 33, 3183–3194; erratum: 5930
34. Jing, J., He, L., Sun, A., Quintana, A., Ding, Y., Ma, G., Pan, P., Liang, X., Zheng, X., Chen, L., Shi, X., Zhang, S. L., Zhong, L., Huang, Y., Dong, M. Q., Walker, C. L., Hogan, P. G., Wang, Y., and Zhou, Y. (2015) Proteomic mapping of ER-PM junctions identifies STIMATE as a regulator of Ca2+ influx. Nat. Cell Biol. 17, 1339–1347
35. Zhou, Y., Ramachandran, S., Oh-Hora, M., Rao, A., and Hogan, P. G. (2010) Pore architecture of the ORAI1 store-operated calcium channel. Proc. Natl. Acad. Sci. USA 107, 4896–4901
36. Huttlin, E. L., Bruckner, R. J., Paulo, J. A., Cannon, J. R., Ting, L., Baltier, K., Colby, G., Gergel, F., Gyg, M. P., Parren, H., Szp, J., Tam, S., Zarraga, G., Pontano-Vaites, L., Swarup, S., White, A. E., Schweppe, D. K., Rad, R., Erickson, B. K., Barbar, R. A., Garaharsha, K. G., Li, K., Artavanis-Tsakonas, S., Gyg, F. P., and Harper, J. W. (2017) Architecture of the human interactome defines protein communities and disease networks. Nature 545, 505–509
37. Williams, R. T., Manji, S. S., Parker, N. J., Hancock, M. S., Van Steckebelern, L., Eid, J. P., Senior, P. V., Kazemweld, J. S., Shandala, T., Saint, R., Smith, P. J., and Dizadek, M. A. (2001) Identification and characterization of the STIM (stromal interaction molecule) gene family: coding for a novel class of transmembrane proteins. Biochem. J. 357, 673–685
38. Prakriya, M., and Lewis, R. S. (2015) Store-operated calcium channels. Physiol. Rev. 95, 1383–1436
39. Rosado, J. A., Diez, R., Smani, T., and Jardín, I. (2016) STIM and Orai1 variants in store-operated calcium entry. Front. Pharmacol. 6, 325
40. Fogarty, S., Ross, F. A., Vera Ciruelos, D., Gray, A., Gowans, G. J., and Hardie, D. G. (2016) AMPK causes cell cycle arrest in LKB1-deficient cells via activation of CAMKK2. Mol. Cancer Res. 14, 683–695
41. Fogarty, S., Hawley, S. A., Green, K. A., Saner, N., Mustard, K. J., and Hardie, D. G. (2010) Calmodulin-dependent protein kinase kinase-beta activates AMPK without forming a stable complex: synergistic effects of Ca2+ and AMP. Biochem. J. 426, 109–118
42. Wang, X., Wang, Y., Zhou, Y., Henderson, E., Mancarella, S., Andrzak, M. D., Rothberg, B. S., Soboloff, J., and Gill, D. L. (2014) Distinct Orai coupling domains in STIM1 and STIM2 define the Orai-activating site. Nat. Commun. 5, 3183

Received for publication June 13, 2018. Accepted for publication September 24, 2018.