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SERCA-mediated endoplasmic reticulum stress facilitates hematopoietic stem cell mobilization

Lijun Li[1†], Danhua Xu[1†] and Xinxin Huang[1*]

Abstract

Background Hematopoietic stem cell (HSC) transplantation is widely recognized as an effective treatment for various malignant diseases. Enhancing HSC mobilization can improve transplantation outcomes and ultimately increase patient survival rates. Recent studies suggest that mild endoplasmic reticulum (ER) stress promotes HSC self-renewal, anti-apoptotic, and anti-aging capabilities. This led us to investigate whether inducing mild ER stress could facilitate HSC mobilization.

Methods The phenotype changes in cells treated with ER stress inducers and Sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) inhibitors were assessed using flow cytometry. The efficacy of these agents on HSC mobilization was evaluated in C57Bl/6 mice, with colony forming unit (CFU) assays used for quantification. Knockdown Jurkat cell lines were constructed to validate the role of SERCA in the mobilization mechanism. Molecular and protein expression levels associated with the pathway were analyzed through quantitative reverse-transcription PCR and western blotting.

Results Our findings revealed that BHQ, a SERCA inhibitor, efficiently enhanced HSC mobilization in vivo. Mechanistically, BHQ regulated the CaMKII-STAT3-CXCR4 pathway by suppressing SERCA activity. This inhibition led to a reduction in CXCR4 expression on the surface of HSCs, facilitating their migration from the bone marrow into peripheral circulation.

Conclusions Our study provides novel insights into the role of the SERCA-ER stress pathway in HSC mobilization. By targeting SERCA activity with BHQ, we observed a significant enhancement in the mobilization of HSCs, facilitated by the modulation of the CaMKII-STAT3-CXCR4 signaling pathway. This research highlights the potential of utilizing mild ER stress as a strategy to promote HSC mobilization, with significant implications for improving stem cell-based therapies, including those used in HSC transplantation.

Keywords ER stress, SERCA, HSC, Mobilization, CXCR4

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Introduction

Hematopoietic stem cell (HSC) transplantation is an effective treatment for various hematopoietic malignancies and genetic diseases. In clinical practice, peripheral blood (PB) stem cells have gradually replaced bone marrow (BM) as the primary source for stem cell transplantation due to safer and more convenient collection procedures, faster hematological recovery, a lower risk of transplant failure, and improved disease-free survival and overall survival rates [1]. However, in the steady state, HSCs are primarily anchored within the BM microenvironment, with only a small number circulating in PB, rendering it insufficient for collection purposes [2]. HSCs can be mobilized from the BM into the PB through the administration of exogenous cytokines. Transplantation of a sufficient number of mobilized HSCs enhances the graft-versus-tumor effect by promoting hematopoietic reconstitution and reduces the risk of both acute and chronic graft-versus-host disease as well as infection. The clinical outcomes of HSC transplantation largely depend on the quantity and quality of hematopoietic stem/progenitor cells (HSPCs) in the graft.

According to the National Comprehensive Cancer Network (NCCN) guidelines and clinical experience, a minimum of 5×10^6 $CD34^+$ cells per kilogram of recipient body weight is required for successful transplantation and rapid hematopoietic reconstitution [3, 4]. Therefore, effective mobilization is a crucial step for successful HSC transplantation and hematopoietic recovery. Current methods of HSC mobilization primarily involve the continuous administration of granulocyte colony-stimulating factor (G-CSF) to the donor, with apheresis typically starting on the 5th day of G-CSF administration. To achieve the target cell harvest, it is necessary to stimulate the donor for at least 5 days [5]. While G-CSF has benefited many patients, there is still a failure rate ranging from 10–60% [6]. Repeated mobilization attempts also increase the incidence of side effects and the burden on patients or donors. Another newly developed auxiliary mobilization strategy is the use of the C-X-C chemokine receptor type 4 (CXCR4) inhibitor, Plerixafor (AMD3100), which can enhance mobilization when combined with G-CSF. However, even with this combined regimen, approximately 24% of patients still fail to achieve adequate mobilization [7]. A clinical trial involving multiple myeloma patients demonstrated that, despite receiving up to 8 G-CSF injections, 4 plerixafor injections, and 4 apheresis procedures, 15–35% of patients still failed to mobilize an adequate number of $CD34^+$ cells, leading to significant patient distress [5, 8]. These limitations underscore the urgent need to develop more rapid and effective mobilization protocols that minimize side effects and reduce donor discomfort.

Among the currently known regulatory pathways, the chemotactic regulator stromal cell-derived factor 1 (SDF-1) and its receptor CXCR4 are recognized as the primary mediators of HSC homing, quiescence, and maintenance [9]. Therefore, the SDF-1/CXCR4 axis remains the most important target for the development of mobilization strategies. CXCR4 belongs to the G-protein-coupled receptor (GPCR) superfamily, and its function is dynamically regulated by the specific phosphorylation of multiple serine residues in the C-terminal domain of the receptor [10–12]. Phosphorylation at S339, in particular, is associated with receptor endocytosis [13], and studies have shown that CXCR4 endocytosis reduces its surface expression [14]. The rapid phosphorylation and degradation of CXCR4 may be linked to hematopoietic cell stress.

Among the various types of stress, endoplasmic reticulum (ER) stress has been strongly associated with HSC function [15, 16]. Mild ER stress is a characteristic of HSCs [15, 17], as it promotes cell protection and self-renewal capacity [15, 18]. The induction of ER stress is often accompanied by impaired Sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) function and the disruption of intracellular Ca^{2+} homeostasis [19, 20]. SERCA is a key protein responsible for transporting Ca^{2+} from the cytosol into the lumen of the sarco/endoplasmic reticulum (SR/ER), thus maintaining intracellular Ca^{2+} homeostasis. SERCA is encoded by three genes (*SERCA1*, *SERCA2*, and *SERCA3*), which generate several tissue-specific SERCA isoforms (*SERCA1-3*) through alternative splicing [21]. *SERCA2* and *SERCA3* are the primarily expressed subtypes in blood cells [22]. Previous studies have reported that ER stress-induced increases in intracellular calcium can activate calmodulin-dependent protein kinase II (CaMKII) [23]. Jisu et al. found that vitamin D deficiency-induced ER stress increased the phosphorylation of CaMKII in human macrophages, without altering the total CaMKII expression. Inhibiting phosphorylated CaMKII (pCaMKII) under conditions of vitamin D deficiency can, in turn, inhibited ER stress [24].

Despite ER stress has been extensively studied in relation to stem cell self-renewal, anti-apoptosis, and anti-aging, its role in HSC mobilization remains poorly understood [15–17, 25, 26]. HSC mobilization is a complex process regulated by multiple signaling pathways and microenvironmental cues. Given the well-established link between ER stress, intracellular calcium homeostasis, and cell migration, we hypothesized that modulating SERCA activity, a crucial regulator of ER calcium balance, might serve as a novel approach to enhance HSC mobilization. In this study, we demonstrate that pharmacological inhibition of SERCA using BHQ induces ER stress and leads to a significant downregulation of CXCR4 surface expression on HSCs. This reduction in CXCR4 expression facilitates the migration of HSCs

from the BM into the peripheral circulation, effectively enhancing their mobilization. Moreover, our findings suggest that BHQ-mediated SERCA inhibition acts through the CaMKII-STAT3-CXCR4 signaling axis, providing mechanistic insight into how ER stress influences HSC trafficking. These findings offer novel insights into the molecular mechanisms governing HSC mobilization and suggest a potential therapeutic strategy for enhancing HSC mobilization in clinical settings.

Materials and methods

Key small molecular compounds

Animals

Adult female C57Bl/6 mice (6–8 weeks old) were purchased from Shanghai SLAC Laboratory Animal Company. Animal anaesthesia was not administered during the experimental procedures in this study. All experimental procedures were carried out in accordance with institutional guidelines, and approved by the Animal Care and Use Committee of Fudan University School of Medicine. The work has been reported in line with the ARRIVE guidelines 2.0.

Cell isolation and culture

Mice were euthanized by cervical dislocation, and bone marrow cells were isolated by flushing the femur with sterile PBS. Lineage-negative (Lin^-) cells were enriched using the Lineage Cell Depletion Kit (130-090-858, Miltenyi Biotec) according to the manufacturer's instructions and previously published studies [27, 28]. Lin^- cells were cultured in vitro in SFEM II medium (StemCell, 09655), supplemented with 100 U/mL penicillin/streptomycin, and 50 ng/mL each of recombinant mouse SCF, TPO, and FLT3L for 4 days.

The Jurkat cell line was maintained in RPMI-1640 medium (Gibco, 11875093), and the HEK293T cell line was cultured in DMEM (Gibco, 11965092). Both media were supplemented with 10% FBS (Gibco, 10099158) and 100 U/mL penicillin/streptomycin. All cells were cultured at 37 °C in a humidified incubator with 5% CO_2.

Flow cytometry analysis

Cells were washed at least once with FACS buffer. They were then resuspended in Fc-block (1:100, BD) for 10 min, followed by a 30-minute incubation with an appropriately concentrated antibody staining mixture (if applicable) at 4 °C in the dark. After a final wash, cells were resuspended in 400 µL of FACS buffer. All flow cytometry experiments were performed using a BD Canto II flow cytometer, and data analysis was conducted using FlowJo version 10.4.0.

Mice mobilization assay

C57Bl/6 mice were subcutaneously injected (SC) with 125 µg/kg of G-CSF (Qilu Pharmaceutical) twice daily in 100 µL PBS, or an equivalent volume of PBS alone, for 4 days. BHQ, BD, and KN93 were also administered SC at specific doses in 100 µL of corn oil (See Table 1). At the final time point, the mice were euthanized by cervical dislocation, and peripheral blood was collected via cardiocentesis into anticoagulant tubes containing 18 U/mL heparin. Red blood cells (RBC) were lysed to obtain mononuclear cells (MNCs) for flow cytometry analysis and colony forming unit (CFU) assays [29–32]. For the hematopoietic progenitor CFU assay, RBC-lysed MNCs were plated in methylcellulose medium (Stem Cell Technologies, M3434) [33] in 24-well culture plates. The plates were then incubated in a humidified incubator for 7 days before the colonies were scored.

RNA extraction and quantitative reverse transcription (qRT)-PCR assay

Total RNA was extracted from cells using Trizol Reagent (TransStart, ET111-01-V2) according to the manufacturer's instructions. cDNA synthesis was performed using a FastKing-RT Kit (Tiangen, KR118). Real-time PCR amplification was carried out using SYBR-Green I qPCR SuperMix (TransStart, AQ141-01) and detected on a Roche LightCycler® 480 (Roche Diagnostics) following the manufacturer's recommendations. β-actin was amplified as a housekeeping gene to ensure equal baseline analysis across samples. Primer sequences for *β-actin*, *SERCA1*, *SERCA2*, *SERCA3*, and *XBP1s* are listed in Supplemental Table 1. The relative quantification of genes was performed using the $2^{-\Delta\Delta Ct}$ method.

Table 1 Compounds and abbreviation

Compounds	Abbreviation	Source	CatLog
2,5-di-t-butyl-1,4-benzohydroquinone	BHQ	APExBIO	B6648
CDN1163	CDN	Selleck	S6815
Calcium gluconate monohydrate	Ca	Aladdin Scientific	C2201503
Thapsigargin	TG	Sigma	T9033
Isoprenaline hydrochloride	ISO	Sigma	I0599990
Tris(2-chloroethyl) phosphate	TCEP	Sigma	96,382
Tunicamycin	TM	APExBIO	B7417
Brefeldin A	BFA	MCE	HY-16,592
Berbamine dihydrochloride	BD	MCE	HY-N0714A
KN-93 Phosphate	KN93	Selleck	S7423

Construction of SERCA2/3-knockdown Jurkat cell line

Lentivirus production and packaging for stable knockdown of SERCA2/3 were performed using pMD2.G and psPAX2 with the hU6-pLKO.1-EGFP vector. The primers used are listed in Supplemental Table 2. To establish a stable SERCA2/3-knockdown Jurkat cell line, GFP-positive cells were sorted for further assays.

Western blot

Equal numbers of cells from each group were washed with ice-cold PBS and lysed in RIPA buffer containing a protease inhibitor cocktail (Roche, 4693132001) for 10 min at 4 °C, with ultrasonic assistance. After centrifugation at 14,000 g for 15 min, the supernatant was collected as the protein extract.

Proteins were separated by polyacrylamide gel electrophoresis (PAGE) and transferred onto a polyvinylidene fluoride (PVDF) membrane (Biosharp, BS-PVDF-45). The membranes were then blotted with primary antibodies against CXCR4, phospho-CXCR4 (S339), CaMKII, phospho-CaMKII (T286), STAT3, phospho-STAT3 (Y705), and GAPDH, as listed in Supplemental Table 3. Peroxidase-conjugated secondary antibodies (anti-mouse, 1:5000; Immunoway, RS2108; anti-rabbit, 1:5000; Immunoway, RS0002) were applied at room temperature for 1 h. Protein bands were detected using an enhanced chemiluminescence (ECL) system and visualized with a chemiluminescence imaging system (Tanon 4600).

Statistics

Data were analyzed using GraphPad Prism 9 (GraphPad). All data are presented as mean ± SEM. The sample size (n) represents the number of mice per experiment, as specified in the figure legends. Statistical significance was determined using an unpaired, two-tailed Student's t-test to compare two groups. For all data, differences were considered statistically significant when $p < 0.05$. Specifically, the following notation was used: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$.

Results

ER stress inducers down-regulate CXCR4 surface expression

Previous studies have reported ER stress leads to the activation of inositol-requiring enzyme 1 (*IRE1*), which subsequently cleaves X-box-binding protein 1 (*XBP1*) mRNA, producing the spliced form of *XBP1* (*XBP1s*). Since *XBP1s* is a widely recognized marker of ER stress [34–36], we firstly assessed the mRNA level of *XBP1s* in Jurkat cells by qPCR method and found that under the action of ER stress inducers BHQ [37] and TG [38], the level of *XBP1s* significantly increased, with the upregulation becoming more pronounced over time (Fig. 1A, B). These results confirm that BHQ and TG effectively induce ER stress in Jurkat cells.

To examine the correlation between ER stress and HSPC mobilization, we evaluated the effects of various ER stress inducers (BHQ, TG, and BFA [39]) on the surface expression of CXCR4 in Jurkat cells. Flow cytometry analysis demonstrated that all three agents significantly reduced CXCR4 surface expression (Fig. 1C-E). Moreover, BHQ exhibited both concentration- and time-dependent effects on CXCR4 expression (Figure. S1D-G). To further verify whether this inhibitory effect extended to primary cells, we isolated Lin^- cells from C57Bl/6 mice and assessed CXCR4 surface expression on LSK($Lin^-Sca1^+c\text{-}Kit^+$) and SLAM LSK cells (phenotypic HSCs). We found that BHQ, TG, and BFA significantly reduced the surface expression of CXCR4 on LSK cells and HSCs (Fig. 1F-J, Fig. S2). In addition, other ER stress inducers have also been shown to significantly inhibit the surface expression of CXCR4 on Jurkat cells (Figure S1A-C), indicating that ER stress inducers may serve as potential mobilizing agents for HSPCs. Given that BHQ exhibited the strongest phenotypic effect with minimal cytotoxicity (Figure S3), it was primarily used as the representative agent in subsequent experiments.

BHQ promotes HSPC mobilization in mice and enhances the mobilization effect of G-CSF

To assess the in vivo mobilization potential of BHQ, C57Bl/6 mice were administered a single subcutaneous injection (SC) of BHQ at a dose of 20 mg/kg. Two hours post-injection, PB samples were collected to enumerate HSPCs (Fig. 2A). Colony forming unit (CFU) assays revealed a significant increase in hematopoietic colony formation in BHQ-treated mice compared to the control group (Fig. 2B). Flow cytometry analysis, which quantified the absolute numbers of LSKs and phenotypic HSCs per milliliter of PB, further demonstrated a greater mobilization of HSPCs in BHQ-treated mice compared to the control group (Fig. 2C-E).

To further investigate the potential of BHQ in augmenting the conventional G-CSF mobilization strategy, mice were initially administered subcutaneous injections of G-CSF (125 μg/kg, twice daily) for four consecutive days. Two hours before PB collection, a single BHQ injection (20 mg/kg) was administered. The mobilization of HSPCs into the peripheral circulation was then assessed by CFU assay and flow cytometry (Fig. 2F). Notably, the combination of BHQ and G-CSF significantly enhanced HSPC mobilization compared to G-CSF alone (Fig. 2G-I). Collectively, these results indicate that BHQ, either alone or in combination with G-CSF, markedly promotes the mobilization of HSPCs in vivo.

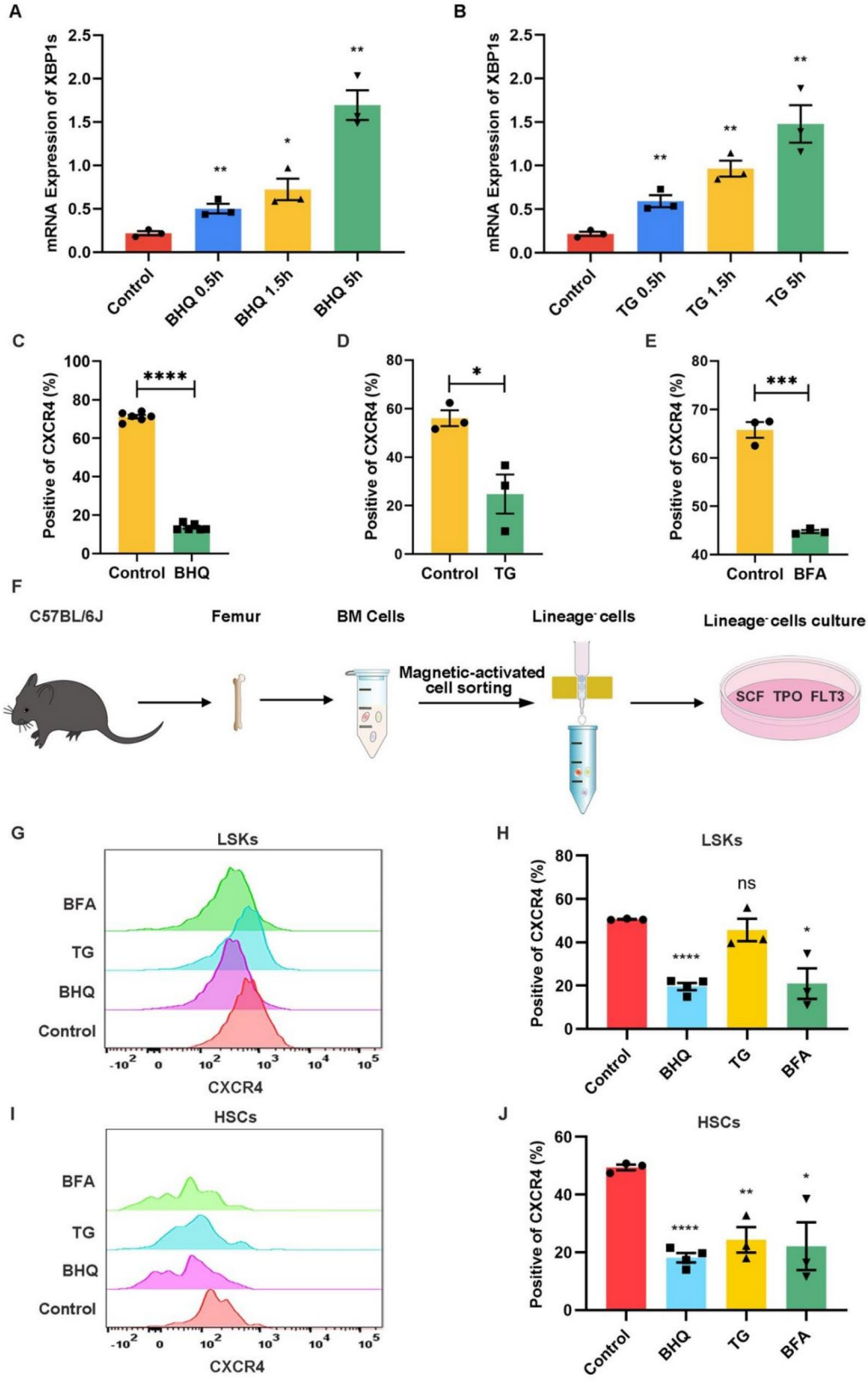

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Fig. 1 ER stress inducers suppress CXCR4 surface expression. (**A-B**) Induction of *XBP1s* mRNA by ER stress inducers. Jurkat cells were treated with 100 μM BHQ or 100 nM TG for 0.5, 1.5, and 5 h, and *XBP1s* mRNA levels were quantified by qPCR ($n = 3$). (**C-E**) CXCR4 surface expression in Jurkat cells. Cells were treated with 100 μM BHQ for 1.5 h, 100 nM TG for 5 h, or 10 μM BFA for 5 h, and CXCR4 expression was analyzed by flow cytometry ($n = 3$–6). (**F**) Schematic diagram of mouse Lin$^-$ cells sorting and culture. (**G-H**) Downregulation of CXCR4 surface expression in mouse LSK cells following treatment with ER stress inducers (100 μM BHQ, 100 nM TG, and 10 μM BFA) ($n = 3$). (**I-J**) Downregulation of CXCR4 surface expression in mouse HSCs following treatment with ER stress inducers (100 μM BHQ, 100 nM TG, and 10 μM BFA) ($n = 3$)

BHQ induces ER stress by inhibiting SERCA activity, suppressing camkii and subsequently reducing CXCR4 expression

In line with previous studies [40, 41], BHQ inhibited SERCA activity, leading to calcium release from the ER and triggering ER stress. To examine the role of SERCA in hematopoietic cells, we conducted gene knockdown experiments targeting SERCA isoforms. Given the tissue-specific expression of SERCA isoforms, we first assessed the expression levels of *SERCA1*, *SERCA2*, and *SERCA3* in Jurkat cells. Our analysis revealed that *SERCA2* and *SERCA3* were the predominant isoforms expressed (Fig. S4A). Subsequently, we used shRNA to individually knock down these two isoforms (Fig. S4B-C).

Flow cytometry analysis revealed that knockdown of *SERCA2* and *SERCA3* significantly decreased the surface expression of CXCR4 in Jurkat cells (Fig. 3A-B), despite no significant changes in CXCR4 mRNA levels (Fig. S5). To further verify the role of SERCA in CXCR4 regulation, we treated Jurkat cells, both with and without SERCA knockdown, with the SERCA agonist CDN11633. Interestingly, CXCR4 expression increased significantly in non-knockdown cells, whereas no significant change in CXCR4 expression was observed in the knockdown cells (Fig. 3C). These findings provide evidence that the expression and activity of SERCA play a crucial role in regulating CXCR4 surface expression.

As BHQ inactivated SERCA, intracellular Ca^{2+} failed to enter the ER for storage, resulting in a sudden increase in cytoplasmic Ca^{2+} concentration. This surge (within 30 min) initially upregulated CaMKII activity, but was followed by a sharp decline in intracellular Ca^{2+} levels, which dropped below the baseline. This fluctuation generated a transient calcium wave (Fig. 3D), followed by a decrease in CaMKII levels (Fig. 3E). The level of phosphorylated CaMKII at T286 (CaMKII-T286) continued to decrease, possibly due to the inhibition of phosphorylation triggered by the stress response. These results indicate that SERCA inactivation in hematopoietic cells induces calcium transients, resulting in negative feedback inhibition of downstream CaMKII activity.

To verify whether CaMKII activity plays a key role in the change of CXCR4 surface expression, we employed two classic CaMKII inhibitors, BD [42] and KN93 [43]. Both inhibitors significantly reduced CXCR4 expression in Jurkat cells, mirroring the effect of BHQ (Fig. 3F-G). Further verification in mouse primary cells revealed that BD and KN93 could also significantly inhibit CXCR4 expression in primary LSKs (Fig. 3H) and HSCs (Fig. 3I). Collectively, these findings indicate that the inhibition of CaMKII activity is a critical mechanism driving the reduction of CXCR4 expression.

CaMKII inhibitors promote HSPC mobilization and enhance G-CSF mobilization in mice

To investigate whether CaMKII inhibition also affects HSPC mobilization in vivo, we administered SC injections of BD (30 mg/kg, 3 h) and KN93 (20 mg/kg, 12 h) to C57Bl/6 mice, and then collected PB to analyze HSPC counts. CFU assays revealed that mice injected with BD or KN93 exhibited a significant increase in hematopoietic colony formation compared to the control group (Fig. 4A, D). This effect was also observed in the counts of LSKs and phenotypic HSCs, as detected by flow cytometry (Fig. 4B-C, E-F).

To further determine whether BD or KN93 synergizes with G-CSF, mice were initially administered continuous subcutaneous injections of G-CSF (125 μg/kg, twice daily) for 4 days. Subsequently, BD (30 mg/kg) or KN93 (20 mg/kg) was administered 3–12 h before PB collection. CFU assays and flow cytometry analysis revealed that both BD and KN93 significantly enhanced G-CSF-induced HSPC mobilization (Fig. 4G-L). These findings indicate that CaMKII inhibitors efficiently promote HSPC mobilization in vivo and exhibit a synergistic effect with G-CSF.

BHQ and KN93 induce CXCR4 phosphorylation and alter its membrane localization by inhibiting STAT3 phosphorylation

To elucidate how ER stress regulates CXCR4 level on the cell surface, we examined STAT3 and its phosphorylated form, pSTAT3 (Y705), a downstream target of CaMKII. Following 1.5 to 5 h of BHQ stimulation and 0.5 to 5 h of KN93 stimulation in Jurkat cells, total STAT3 protein level remained unchanged. However, phosphorylated STAT3 (pSTAT3 Y705) levels were significantly reduced, indicating that CaMKII inhibition affects STAT3 activation. Previous studies have shown that the expression of CXCR4 is regulated by STAT3 [44]. Therefore, we examined the total CXCR4 protein levels and found that in Jurkat cells, the total CXCR4 protein level was significantly downregulated after 0.5 to 5 h of BHQ and KN93 stimulation, which is consistent with literature reports.

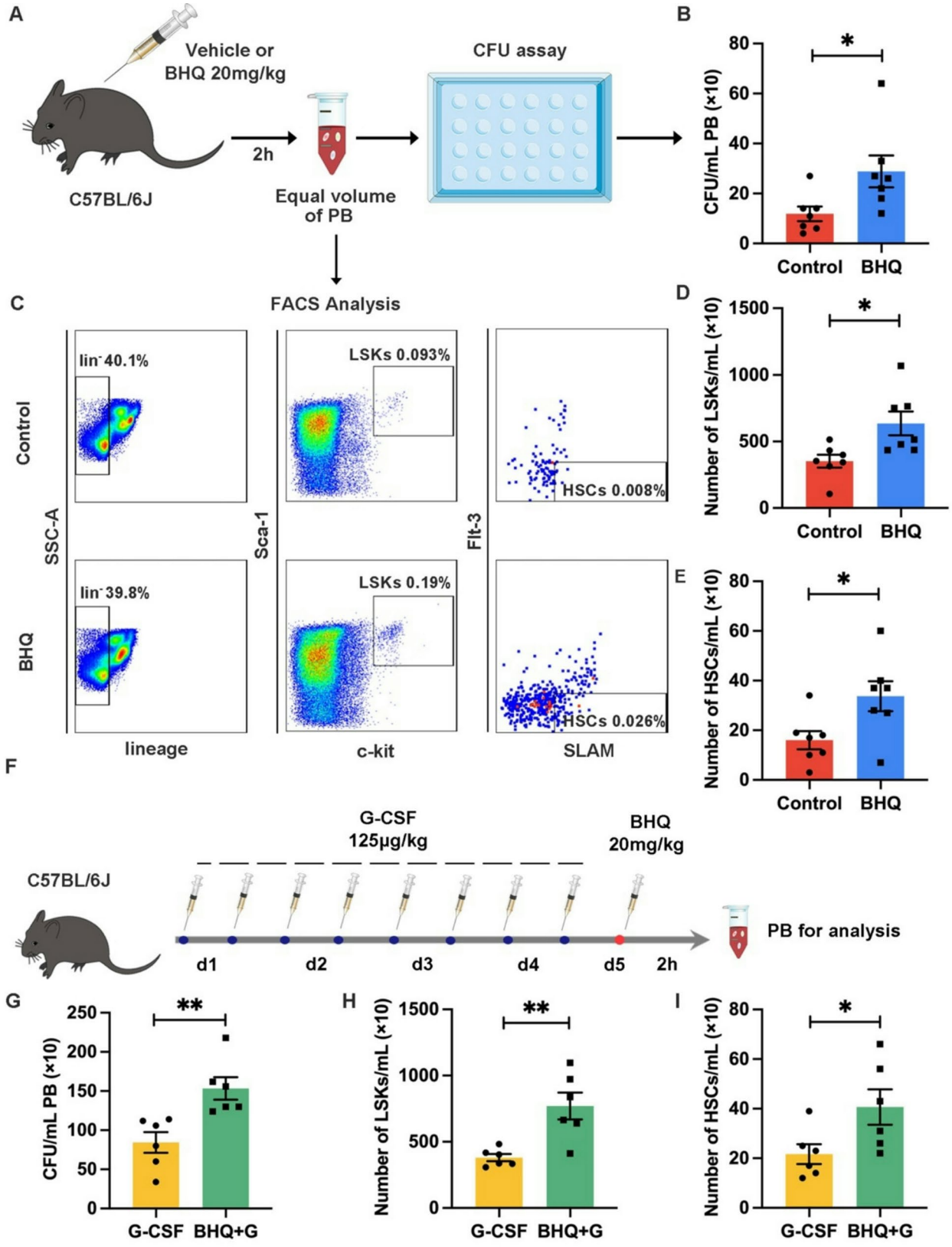

Fig. 2 BHQ promotes HSPC mobilization in vivo. (**A**) Schematic representation of the mouse mobilization model. (**B**) CFU counts in 1 mL of PB following BHQ treatment. (**C**) Gating strategy for flow cytometry analysis ($n=7$). (**D-E**) Absolute numbers of mobilized LSK cells and HSCs in 1 mL of PB following BHQ administration ($n=7$). (**F**) Schematic diagram of the combined mobilization experiment using BHQ and G-CSF. (**G-I**) Synergistic effect of BHQ and G-CSF on mouse HSPC mobilization ($n=6$). n indicates the number of mice used per group

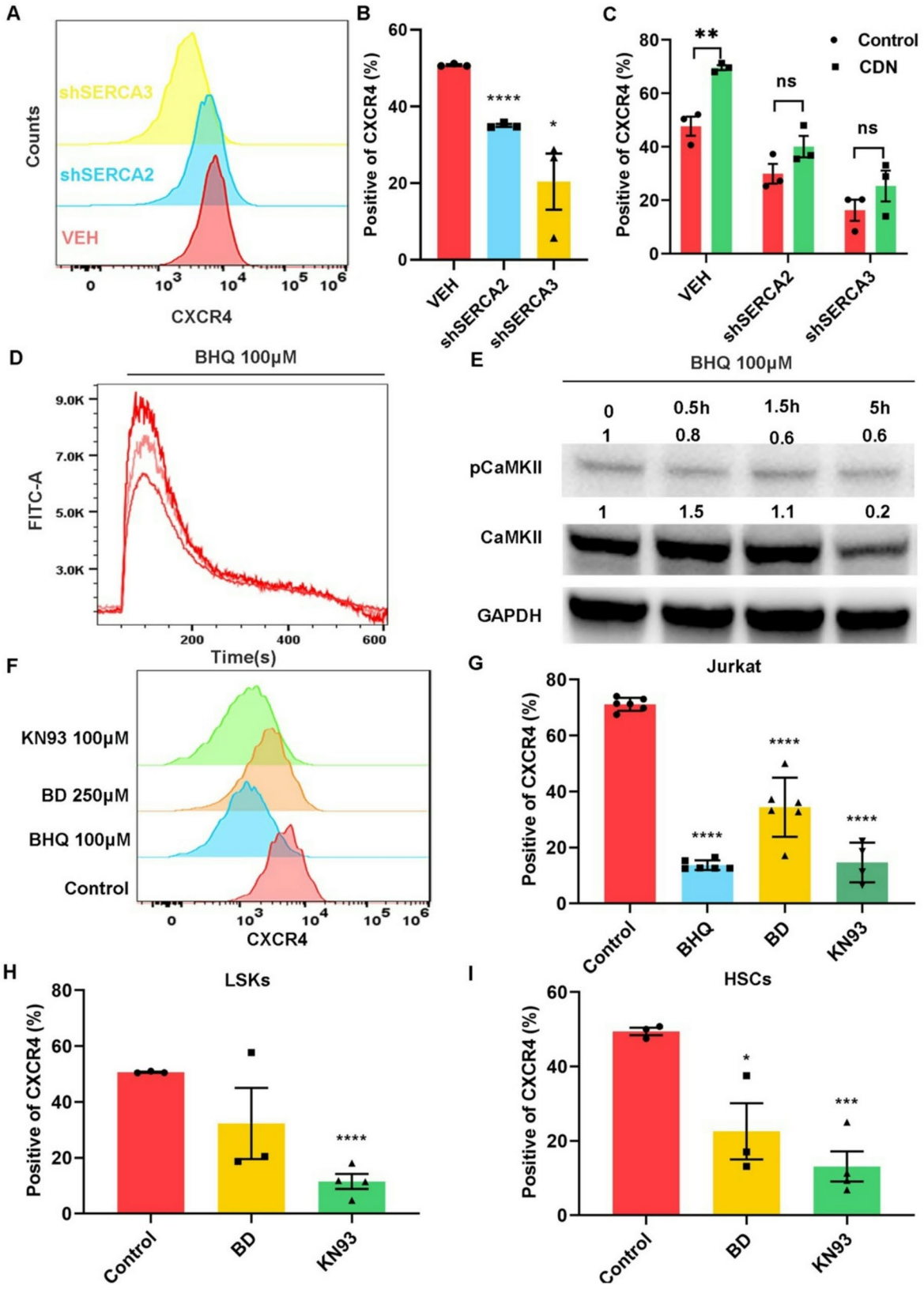

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Fig. 3 ER Stress triggers calcium signaling pathway to regulate CXCR4 expression. (**A-B**) Downregulation of CXCR4 surface expression in SERCA2/3 knockdown Jurkat cells ($n=3$). (**C**) The reduced CXCR4 expression could not be restored by the SERCA agonist (CDN1163) in SERCA2/3 knockdown Jurkat cells ($n=3$). (**D**) Flow cytometric analysis of intracellular calcium transients in BHQ-treated Jurkat cells ($n=3$). (**E**) Western blot analysis of p-CaMKII and CaMKII protein levels following BHQ treatment. Full-length blots are presented in Supplementary Fig. 6. (**F-G**) Suppression of CXCR4 expression in Jurkat cells treated with BD (250 μM for 1.5 h) and KN93 (100 μM for 5 h), similar to the effect of BHQ ($n=3$). (**H-I**) Suppression of CXCR4 expression in mouse LSKs and HSCs by CaMKII inhibitors ($n=3$)

Additionally, we observed a significant increase in the phosphorylation of CXCR4 at S339, suggesting that CXCR4 phosphorylation plays a crucial role in its membrane localization (Fig. 5).

To validate these findings in primary cells, we treated mouse LSK cells with BHQ (100 μM, 5 h). Flow cytometry revealed significant suppression of pSTAT3 (Y705) (Fig. 6A) and elevation of pCXCR4 (S339) (Fig. 6B). These results implicate ER stress in rewiring STAT3-CXCR4 signaling, where CaMKII inhibition dampens STAT3 activity while enhancing CXCR4 phosphorylation—a dual mechanism likely facilitating HSPC mobilization under stress conditions.

Exogenous calcium supplementation restores CXCR4 expression

To investigate whether the expression of CXCR4 can be rescued by replenishing ER calcium reserves with calcium supplementation, we first supplemented Jurkat cells with calcium gluconate (600 μg/mL) prior to BHQ (100–400 μM) treatment. The cells were then incubated together at 37 °C for 30 min. We found that calcium gluconate significantly mitigated the effect of BHQ, thereby restoring CXCR4 surface expression (Fig. 7B). To verify whether calcium supplementation has a similar effect in mouse LSKs, Lin^- cells were isolated from C57Bl/6 mice and stimulated with calcium gluconate along with BHQ at 37 °C for 30 min. Flow cytometry analysis demonstrated that calcium gluconate restored CXCR4 expression in LSK cells. Notably, calcium gluconate alone significantly increased the expression of CXCR4 (Fig. 7C). Moreover, in SERCA2/3 knockdown Jurkat cells, additional supplementation with calcium gluconate (600 μg/mL) also significantly restored the expression of CXCR4 (Fig. 7D). These results collectively indicate that calcium supplementation counteracts SERCA inhibition-induced suppression of CXCR4, highlighting the critical role of ER calcium homeostasis in CXCR4 regulation.

Discussion

This study elucidates that inducing ER stress in hematopoietic cells leads to a reduction in intracellular CaMKII activity. Consequently, the downstream STAT3-CXCR4 pathway is inhibited, resulting in a rapid decline in CXCR4 expression on the cell membrane. This process promotes the migration of HSPCs out of the BM, thereby facilitating their mobilization. These findings suggest a potential strategy for enhancing the mobilization of HSPCs through targeted modulation of ER stress.

In the BM microenvironment, stromal cells secrete CXCL12, which binds to CXCR4 on the hematopoietic cell surface, thereby anchoring them within the BM niche [45]. A reduction in CXCR4 surface receptor disrupts the CXCL12-CXCR4 axis, leading to the migration of HSPCs from the BM. Based on this principle, various mobilization strategies for HSPCs have been developed. Our study demonstrates that ER stress inducers significantly reduce CXCR4 expression on hematopoietic cells, suggesting a potential link between ER stress and HSPC mobilization. To verify this hypothesis, we performed relevant in vivo experiments in which mice received an appropriate dose of BHQ via SC injection for short-term stimulation (2 h). PB was then collected for CFU assays and absolute counts of LSKs/HSCs. Under these conditions, we observed a significant increase in the number of mobilized HSPCs in circulation. Furthermore, BHQ enhanced the mobilization effect of G-CSF, a well-established HSPC mobilization agent, leading to a greater yield of HSPC grafts. These results provide preliminary evidence that ER stress plays a regulatory role in HSPC migration, offering potential avenues for optimizing mobilization strategies.

Recent studies have shown that an appropriate level of ER stress and a moderate unfolded protein response (UPR) can help HSCs stimulate the "hormesis effect"—a phenomenon where low levels of stress enhance the organism's resistance to subsequent stress, promoting life extension or rejuvenation under various stress conditions [15]. This effect enables HSCs to activate their self-repair, renewal, and maintenance capabilities. Under low-dose ER stress signals, the UPR can induce the activation of IRE1. IRE1 possesses both kinase and endoribonuclease activities, which lead to the splicing of *XBP1* mRNA into its active form, *XBP1s* [37]. *XBP1s* is then translated into a transcription factor that induces the expression of genes encoding molecular chaperones, folding enzymes, and endoplasmic reticulum-associated degradation (ERAD) components, thereby participating in cell protection programs [15, 46]. In this study, we detected a significant increase in the mRNA levels of *XBP1s*, the spliced form of *XBP1*, under the stimulation of low-dose BHQ and TG. This indicates that the ER stress-induced process activated an "hormesis effect" in hematopoietic cells.

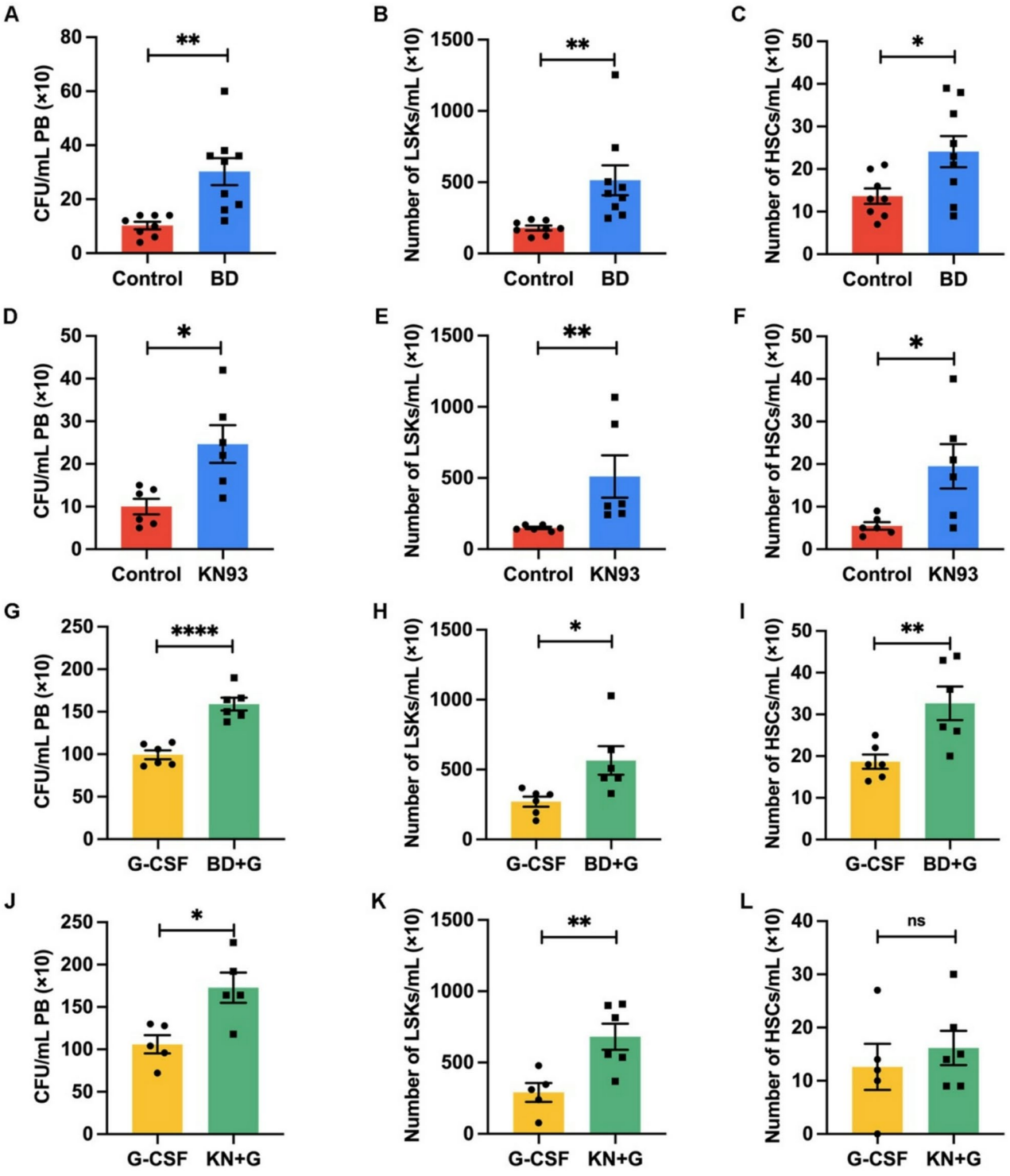

Fig. 4 CaMKII inhibitors promote HSPC mobilization in vivo. (**A**, **D**) CFU counts per milliliter of PB following treatment with BD ($n=9$) or KN93 ($n=6$). (**B-C**, **E-F**) Absolute numbers of LSKs and HSCs per milliliter of PB, as determined by flow cytometry, following treatment with BD ($n=9$) or KN93 ($n=6$). (**G-L**) Enhanced HSPC mobilization when G-CSF is combined with BD ($n=6$) or KN93 ($n=5$). n indicates the number of mice used per group

BHQ has been widely used as a potent SERCA inhibitor in previous studies [37, 47]. During the ER stress response, inhibition of SERCA prevents intracellular Ca^{2+} from entering the ER for storage through conventional pathways, resulting in an instantaneous decrease in ER calcium reserves while increasing cytoplasmic Ca^{2+} levels. This increase stimulates the ER stress response, and the excess Ca^{2+} in the cytoplasm activates the

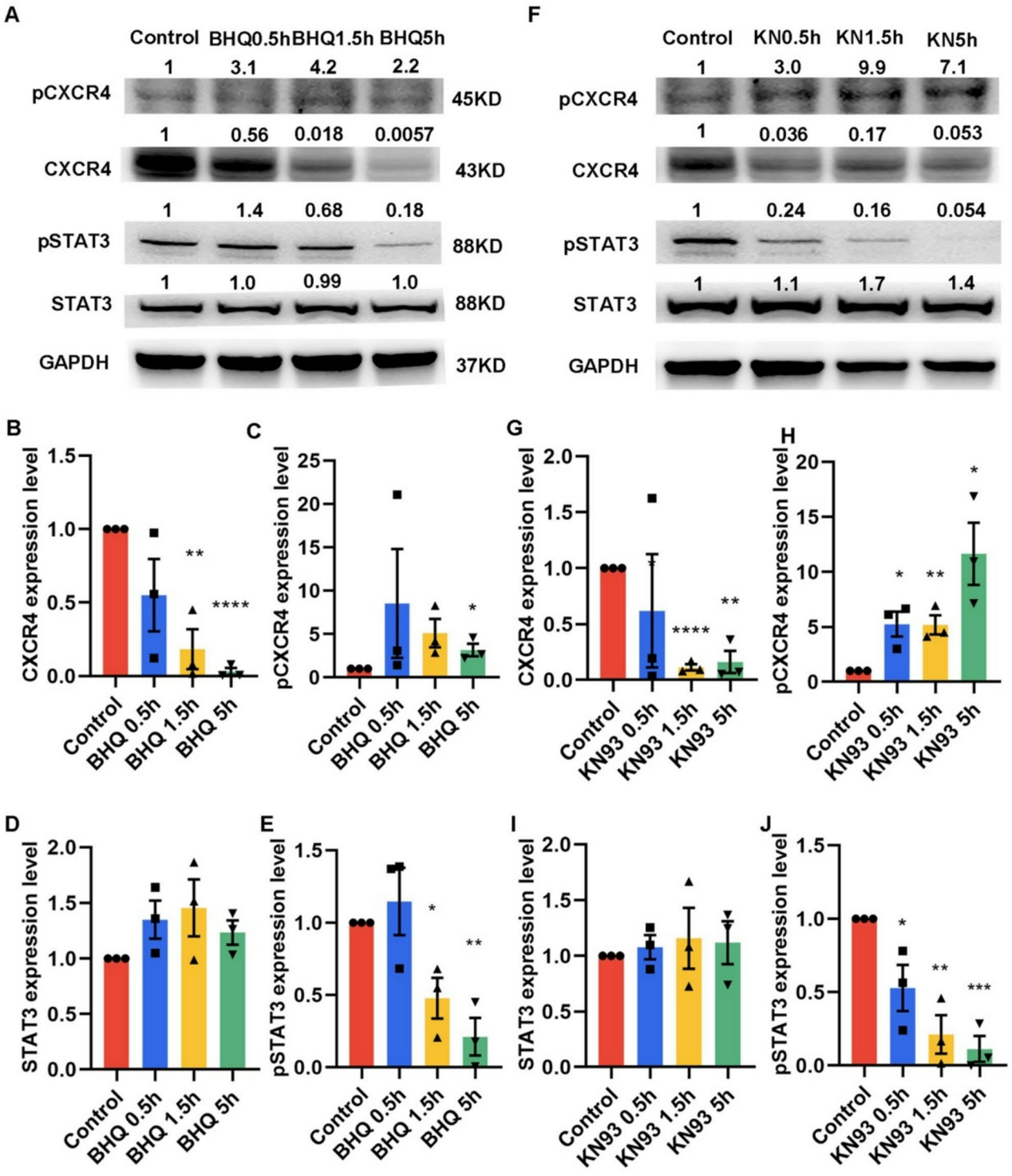

Fig. 5 STAT3 and CXCR4 phosphorylation in Jurkat cells following BHQ or KN93 treatment. (**A-E)** Western blot analysis of protein levels of STAT3, pSTAT3 (Y705), CXCR4, and pCXCR4 (S339) in Jurkat cells treated with BHQ for 0.5, 1.5, and 5 h. (**F-J**) Western blot analysis of protein levels of STAT3, pSTAT3 (Y705), CXCR4, and pCXCR4 (S339) in Jurkat cells after KN93 stimulation for 0.5, 1.5, and 5 h. Full-length blots are presented in Supplementary Fig. 6

mitochondrial calcium transport channel and enters the mitochondria via the mitochondrial Ca^{2+} uniporter (MCU) [48–50]. When cytoplasmic Ca^{2+} levels increase, CaMKII activity also increases immediately. As Ca^{2+} is taken up by the mitochondria, CaMKII activity decreases in a negative feedback manner. Consequently, the overall effect of this ER stress response is a prolonged inhibition of CaMKII activity. To investigate whether CaMKII

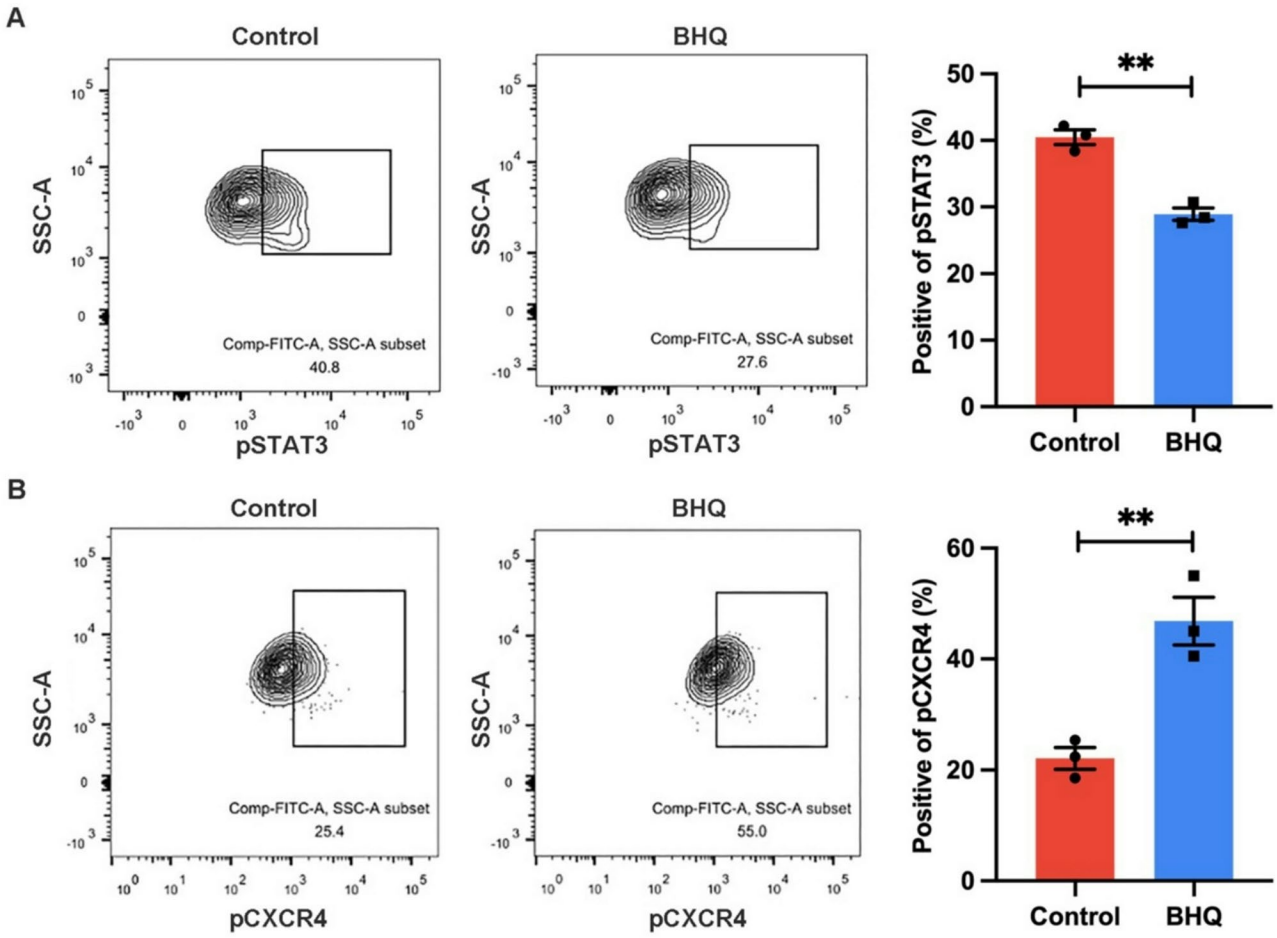

Fig. 6 Phosphorylated protein levels of STAT3 and CXCR4 in mouse LSK cells. (**A**) Flow cytometry analysis of pSTAT3 (Y705) levels in LSK cells after BHQ stimulation for 5 h ($n=3$). (**B**) Flow cytometry analysis of pCXCR4 (S339) levels in LSK cells after BHQ stimulation for 5 h ($n=3$)

inhibition is central to ER stress-induced HSPC mobilization, we employed two CaMKII inhibitors, BD and KN93. Similar to ER stress inducers, these inhibitors suppressed CXCR4 expression and enhanced HSPC mobilization in vivo, strongly supporting our hypothesis that CaMKII acts as a critical mediator in this cascade. This aligns with earlier work identifying CaMKIIγ as a key regulator in hematopoietic cells, where it maintains quiescence in normal HSPCs but drives hyperactivation in leukemic stem cells (LSCs) by modulating NF-κB, Wnt/β-catenin, and STAT3 pathways [42]. Building on these findings, we demonstrate that BHQ- and KN93-mediated CaMKII inhibition reduces phosphorylated STAT3 (pSTAT3) levels, mirroring observations in STAT3-knockout models where IL-30-dependent CXCR4 upregulation was impaired [44]. Our results extend this paradigm by linking ER stress-induced CaMKII suppression to STAT3 inactivation and subsequent CXCR4 downregulation. Furthermore, we uncovered a novel regulatory mechanism: ER stress not only reduced total CXCR4 protein levels but also increased phosphorylation of CXCR4 at S339. This observation aligns with earlier research showing that S339 phosphorylation promotes CXCR4 endocytosis, thereby modulating leukemia cell trafficking in BM niches [13].

Small molecular inhibitors can serve as reversible ER stress triggers, potentially useful in the future development of mobilization strategies to facilitate the regulation of HSPC homing after transplantation. In the current study, to clarify whether the SERCA family is a key component in the ER stress response, we knocked down SERCA2 and SERCA3 using shRNA, which are specifically expressed in hematopoietic cells, to simulate mild ER stress. Notably, simultaneous knockdown of both isoforms resulted in increased apoptosis (data not shown). We found that knocking down either SERCA2 or SERCA3 alone produced a phenotype similar to that induced by BHQ. When treated with SERCA agonists, the surface expression of CXCR4 on non-knockdown Jurkat cells significantly increased. However, in cells expressing shSERCA2 or shSERCA3, the expression of

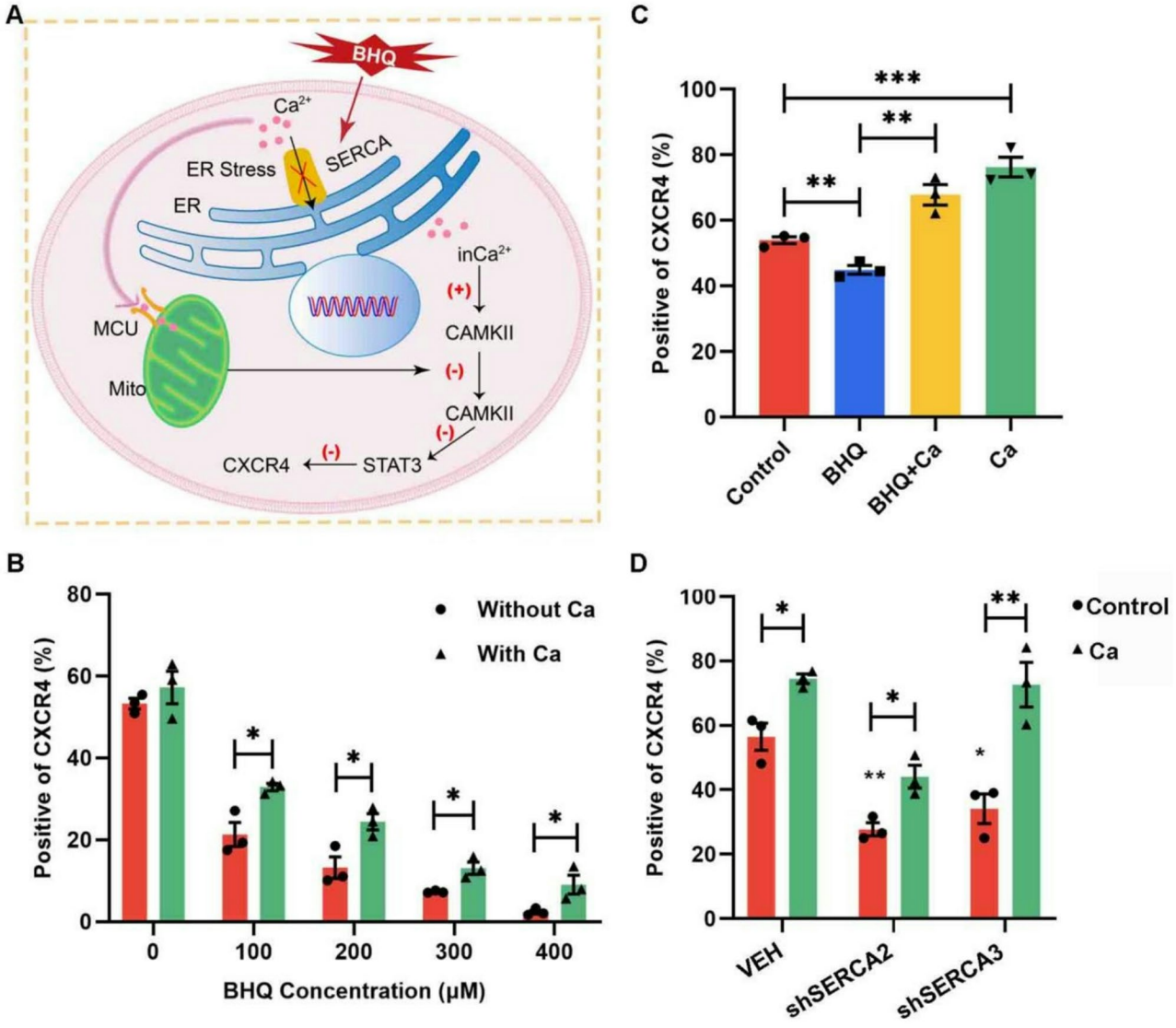

Fig. 7 Calcium gluconate rescues CXCR4 expression in SERCA-inhibited cells. (**A**) Schematic model illustrating ER stress-mediated regulation of CXCR4 expression. (**B**) Flow cytometric analysis showing that calcium gluconate alleviates BHQ-induced suppression of CXCR4 expression in Jurkat cells ($n=3$). (**C**) Flow cytometric analysis demonstrating that calcium gluconate supplementation significantly increases CXCR4 expression in LSK cells ($n=3$). (**D**) Flow cytometric analysis showing that calcium gluconate supplementation significantly increases CXCR4 expression in SERCA2/3 knockdown cells ($n=3$)

CXCR4 did not increase. This indicates a direct correlation between SERCA activity and CXCR4 expression.

In conditions of mild ER stress, exogenous calcium supplementation can activate stromal interaction molecule 1 (STIM1) on the ER membrane. STIM1 then interacts with calcium release-activated calcium channel protein 1 (Orai1) on the plasma membrane, thereby activating Ca^{2+} influx across the plasma membrane. This process, known as store-operated Ca^{2+} entry (SOCE), replenishes the calcium stores in the ER [18, 48, 51, 52]. In this study, CXCR4 expression was significantly restored by supplementing calcium gluconate in BHQ-treated or shSERCA2/3 knockdown cells. These findings suggest that calcium supplementation may enhance the homing ability of mobilized HSPCs when administered at the time of transplantation.

In this study, we utilized the Jurkat cell line as an in vitro model to investigate ER stress-mediated CXCR4 regulation and HSPC mobilization mechanisms. This choice was based on Jurkat cells' well-characterized expression of CXCR4, their responsiveness to calcium-dependent signaling pathways, and their historical utility in studying hematopoietic cell behaviors, including chemotaxis and stress responses. These features allowed us to isolate ER stress-specific effects on CXCR4 dynamics in a controlled experimental system. However, while Jurkat cells share phenotypic and functional similarities with primary HSPCs, they lack the heterogeneity and microenvironmental

interactions inherent to primary hematopoietic cells in vivo. Additionally, immortalized cell lines like Jurkat may exhibit altered stress response pathways compared to quiescent or mobilized HSPCs. We have verified the changes of pSTAT3 and pCXCR4 levels in mouse primary LSK cells following BHQ treatment. Future studies should further validate these findings through in vivo experiments to provide more comprehensive data support. Overall, our results provide foundational mechanistic insights into how ER stress disrupts CXCR4 signaling, sets the stage for developing novel therapeutic strategies aimed at enhancing HSPC mobilization in clinical settings.

Conclusions

In summary, our study reveals that inducing mild ER stress reversibly inhibits the SERCA-CaMKII-STAT3 pathway, leading to a reduction in CXCR4 surface expression on HSPCs and promoting their mobilization into the peripheral circulation. Moreover, calcium supplementation effectively restores CXCR4 expression, suggesting a potential strategy to enhance the homing capacity of mobilized HSPCs post-transplantation. These findings provide mechanistic insights into ER stress-mediated regulation of HSPC mobilization and highlight its potential therapeutic application as an adjunct to existing mobilization regimens.

Abbreviations

HSC	Hematopoietic stem cell
HSPC	Hematopoietic stem/progenitor cell
PB	Peripheral blood
BM	Bone marrow
G-CSF	Granulocyte colony stimulating factor
CXCR4	C-X-C chemokine receptor type 4
SDF-1	Stromal cell-derived factor 1
GPCR	G-protein-coupled receptors
ER	Endoplasmic reticulum
SERCA	Sarco/endoplasmic reticulum Ca^{2+}-ATPase
CaMKII	Calmodulin-dependent protein kinase II
MNC	Mononuclear cells
CFU	Colony forming unit
IRE1	Inositol-requiring protein 1
XBP1	X-box-binding protein 1
SC	Subcutaneous
UPR	Unfolded protein response
ERAD	Endoplasmic reticulum-associated degradation
MCU	Mitochondrial Ca^{2+} uniporter
LSC	Leukemic stem cells
STIM1	Stromal interaction molecule 1
SOCE	Store-operated Ca^{2+} entry

Supplementary Information

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Supplementary Material 1

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Author contributions

All authors participated in the design, interpretation of the studies, analysis of the data, and review of the manuscript; LL and DX conducted the experiments, LL wrote the manuscript, XH conceived the project and revised the manuscript.

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Data availability

All data generated during this study are included in this published article and its supplementary information files. The original data supporting the findings of this study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The project, entitled "The regulating mechanisms of hematopoietic stem cell expansion and homing" was approved by the Animal Care and Use Committee of Fudan University School of Medicine, with approval number 202311021Z granted on November 24, 2023.

Reprducibility

To ensure the reproducibility of our findings, we have provided a detailed description of the methods used in this study.

Conflict of interest

The authors declare no conflicts of interest.

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References

1. Luo C, Wang L, Wu G, Huang X, Zhang Y, Ma Y, Xie M, Sun Y, Huang Y, Huang Z, et al. Comparison of the efficacy of hematopoietic stem cell mobilization regimens: a systematic review and network meta-analysis of preclinical studies. Stem Cell Res Ther. 2021;12(1):310.
2. Itkin T, Gur-Cohen S, Spencer JA, Schajnovitz A, Ramasamy SK, Kusumbe AP, Ledergor G, Jung Y, Milo I, Poulos MG, et al. Distinct bone marrow blood vessels differentially regulate haematopoiesis. Nature. 2016;532(7599):323–8.
3. Bilgin YM. Use of plerixafor for stem cell mobilization in the setting of autologous and allogeneic stem cell transplantations: an update. J Blood Med. 2021;12:403–12.
4. Siena S, Schiavo R, Pedrazzoli P, Carlo-Stella C. Therapeutic relevance of CD34 cell dose in blood cell transplantation for cancer therapy. J Clin Oncol. 2000;18(6):1360–77.
5. Crees ZD, Rettig MP, Jayasinghe RG, Stockerl-Goldstein K, Larson SM, Arpad I, Milone GA, Martino M, Stiff P, Sborov D, et al. Motixafortide and G-CSF to mobilize hematopoietic stem cells for autologous transplantation in multiple myeloma: a randomized phase 3 trial. Nat Med. 2023;29(4):869–79.
6. Crees ZD, Rettig MP, DiPersio JF. Innovations in hematopoietic stem-cell mobilization: a review of the novel CXCR4 inhibitor motixafortide. Ther Adv Hematol. 2023;14:20406207231174304.
7. Ruminski PG, Rettig MP, DiPersio JF. Development of VLA4 and CXCR4 antagonists for the mobilization of hematopoietic stem and progenitor cells. Biomolecules. 2024;14(8).
8. DiPersio JF, Stadtmauer EA, Nademanee A, Micallef INM, Stiff PJ, Kaufman JL, Maziarz RT, Hosing C, Früehauf S, Horwitz M, et al. Plerixafor and G-CSF versus placebo and G-CSF to mobilize hematopoietic stem cells for autologous stem cell transplantation in patients with multiple myeloma. Blood. 2009;113(23):5720–6.
9. Chan KYY, Zhang C, Wong YTS, Zhang X-B, Wang CC, Ng WH, Fok SP, Tang PMK, Kang W, Feng B, et al. R4 RGS proteins suppress engraftment of human

hematopoietic stem/progenitor cells by modulating SDF-1/CXCR4 signaling. Blood Adv. 2021;5(21):4380–92.
10. Orsini MJ, Parent JL, Mundell SJ, Marchese A, Benovic JL. Trafficking of the HIV coreceptor CXCR4. Role of arrestins and identification of residues in the c-terminal tail that mediate receptor internalization. J Biol Chem. 1999;274(43):31076–86.
11. Busillo JM, Armando S, Sengupta R, Meucci O, Bouvier M, Benovic JL. Site-specific phosphorylation of CXCR4 is dynamically regulated by multiple kinases and results in differential modulation of CXCR4 signaling. J Biol Chem. 2010;285(10):7805–17.
12. Fang X, Fang X, Mao Y, Ciechanover A, Xu Y, An J, Huang Z. A novel small molecule CXCR4 antagonist potently mobilizes hematopoietic stem cells in mice and monkeys. Stem Cell Res Ther. 2021;12(1):17.
13. Brault L, Rovó A, Decker S, Dierks C, Tzankov A, Schwaller J. CXCR4-SERINE339 regulates cellular adhesion, retention and mobilization, and is a marker for poor prognosis in acute myeloid leukemia. Leukemia. 2014;28(3):566–76.
14. Ridge LA, Kewbank D, Schütz D, Stumm R, Scambler PJ, Ivins S. Dual role for CXCL12 signaling in semilunar valve development. Cell Rep. 2021;36(8):109610.
15. Luchsinger LL. Hormetic Endoplasmic reticulum stress in hematopoietic stem cells. Curr Opin Hematol. 2021;28(6):417–23.
16. van Galen P, Kreso A, Mbong N, Kent DG, Fitzmaurice T, Chambers JE, Xie S, Laurenti E, Hermans K, Eppert K, et al. The unfolded protein response governs integrity of the Haematopoietic stem-cell pool during stress. Nature. 2014;510(7504):268–72.
17. Liu B, Zhou Y, Wu Q, Fu Y, Zhang X, Wang Z, Yi W, Wang H, Chen Z, Song Z, et al. EVA1A regulates hematopoietic stem cell regeneration via ER-mitochondria mediated apoptosis. Cell Death Dis. 2023;14(1):71.
18. Liu L, Zhao M, Jin X, Ney G, Yang KB, Peng F, Cao J, Iwawaki T, Del Valle J, Chen X, et al. Adaptive Endoplasmic reticulum stress signalling via IRE1α-XBP1 preserves self-renewal of Haematopoietic and pre-leukaemic stem cells. Nat Cell Biol. 2019;21(3):328–37.
19. Viskupicova J, Rezbarikova P. Natural polyphenols as SERCA activators: role in the Endoplasmic reticulum Stress-Related diseases. Molecules. 2022;27(16).
20. Aguayo-Ortiz R, Espinoza-Fonseca LM. Linking biochemical and structural States of SERCA: achievements, challenges, and new opportunities. Int J Mol Sci. 2020;21(11).
21. Bauzá-Thorbrügge M, Banke E, Chanclón B, Peris E, Wu Y, Musovic S, Jönsson C, Strålfors P, Rorsman P, Olofsson CS, et al. Adipocyte-specific ablation of the Ca2 + pump SERCA2 impairs whole-body metabolic function and reveals the diverse metabolic flexibility of white and brown adipose tissue. Mol Metab. 2022;63:101535.
22. Arbabian A, Brouland J-P, Gélébart P, Kovàcs T, Bobe R, Enouf J, Papp B. Endoplasmic reticulum calcium pumps and cancer. BioFactors. 2011;37(3):139–49.
23. Timmins JM, Ozcan L, Seimon TA, Li G, Malagelada C, Backs J, Backs T, Bassel-Duby R, Olson EN, Anderson ME, et al. Calcium/calmodulin-dependent protein kinase II links ER stress with Fas and mitochondrial apoptosis pathways. J Clin Invest. 2009;119(10):2925–41.
24. Oh J, Riek AE, Darwech I, Funai K, Shao J, Chin K, Sierra OL, Carmeliet G, Ostlund RE, Bernal-Mizrachi C. Deletion of macrophage vitamin D receptor promotes insulin resistance and monocyte cholesterol transport to accelerate atherosclerosis in mice. Cell Rep. 2015;10(11):1872–86.
25. Chemaly ER, Troncone L, Lebeche D. SERCA control of cell death and survival. Cell Calcium. 2018;69:46–61.
26. Shahrabi S, Paridar M, Zeinvand-Lorestani M, Jalili A, Zibara K, Abdollahi M, Khosravi A. Autophagy regulation and its role in normal and malignant hematopoiesis. J Cell Physiol. 2019;234(12):21746–57.
27. Farahzadi R, Fathi E, Mesbah-Namin SA, Vietor I. Granulocyte differentiation of rat bone marrow resident C-kit + hematopoietic stem cells induced by mesenchymal stem cells could be considered as new option in cell-based therapy. Regen Ther. 2023;23.
28. Fathi E, Azarbad S, Farahzadi R, Javanmardi S, Vietor I. Effect of rat bone marrow Derived-Mesenchymal stem cells on granulocyte differentiation of mononuclear cells as preclinical agent in cellbased therapy. Curr Gene Ther. 2022;22(2):152–61.
29. Soukup AA, Bresnick EH. Gata2 noncoding genetic variation as a determinant of hematopoietic stem/progenitor cell mobilization efficiency. Blood Adv. 2023;7(24):7564–75.
30. Méndez-Ferrer S, Lucas D, Battista M, Frenette PS. Haematopoietic stem cell release is regulated by circadian oscillations. Nature. 2008;452(7186):442–7.
31. Katayama Y, Battista M, Kao W-M, Hidalgo A, Peired AJ, Thomas SA, Frenette PS. Signals from the sympathetic nervous system regulate hematopoietic stem cell egress from bone marrow. Cell. 2006;124(2):407–21.
32. Karpova D, Rettig MP, Ritchey J, Cancilla D, Christ S, Gehrs L, Chendamarai E, Evbuomwan MO, Holt M, Zhang J, et al. Targeting VLA4 integrin and CXCR2 mobilizes serially repopulating hematopoietic stem cells. J Clin Invest. 2019;129(7):2745–59.
33. Jørgensen AS, Daugvilaite V, De Filippo K, Berg C, Mavri M, Benned-Jensen T, Juzenaite G, Hjortø G, Rankin S, Våbenø J, et al. Biased action of the CXCR4-targeting drug plerixafor is essential for its superior hematopoietic stem cell mobilization. Commun Biol. 2021;4(1):569.
34. Kennedy D, Samali A, Jäger R. Methods for studying ER stress and UPR markers in human cells. Methods Mol Biol. 2015;1292.
35. Luo X, Alfason L, Wei M, Wu S, Kasim V. Spliced or unspliced, that is the question: the biological roles of XBP1 isoforms in pathophysiology. Int J Mol Sci. 2022;23(5).
36. Zhao W, Wang X, Nie W, Jiang M, Zhao Y, Zhang T, Ding Y. Zhimu-Huangbai herb-pair ameliorates hepatic steatosis in mice by regulating IRE1α/XBP1s pathway to inhibit SREBP-1c. Phytomedicine. 2024;134:156017.
37. Pagliaro L, Marchesini M, Roti G. Targeting oncogenic Notch signaling with SERCA inhibitors. J Hematol Oncol. 2021;14(1):8.
38. Zhong W, Chebolu S, Darmani NA. Thapsigargin-induced activation of Ca(2+)-CaMKII-ERK in brainstem contributes to substance P release and induction of emesis in the least shrew. Neuropharmacology. 2016;103:195–210.
39. Yang Y, Fan X, Ji Y, Li J, Dai Z, Wu Z. Glycine represses Endoplasmic reticulum stress-related apoptosis and improves intestinal barrier by activating mammalian target of Rapamycin complex 1 signaling. Anim Nutr. 2022;8(1):1–9.
40. Fusi F, Saponara S, Gagov H, Sgaragli G. 2,5-Di-t-butyl-1,4-benzohydroquinone (BHQ) inhibits vascular L-type Ca(2+) channel via superoxide anion generation. Br J Pharmacol. 2001;133(7):988–96.
41. García-Casas P, Arias-Del-Val J, Alvarez-Illera P, Fonteriz RI, Montero M, Alvarez J. Inhibition of Sarco-Endoplasmic reticulum Ca2 + ATPase extends the lifespan in C. elegans worms. Front Pharmacol. 2018;9:669.
42. Gu Y, Chen T, Meng Z, Gan Y, Xu X, Lou G, Li H, Gan X, Zhou H, Tang J, et al. CaMKII Γ, a critical regulator of CML stem/progenitor cells, is a target of the natural product berbamine. Blood. 2012;120(24):4829–39.
43. Gu Y, Zhang J, Ma X, Kim B-W, Wang H, Li J, Pan Y, Xu Y, Ding L, Yang L et al. Stabilization of the c-Myc protein by CAMKIIγ promotes T cell lymphoma. Cancer Cell. 2017;32(1).
44. D'Antonio L, Fieni C, Ciummo SL, Vespa S, Lotti L, Sorrentino C, Di Carlo E. Inactivation of interleukin-30 in colon cancer stem cells via CRISPR/Cas9 genome editing inhibits their oncogenicity and improves host survival. J Immunother Cancer. 2023;11(3).
45. Crane GM, Jeffery E, Morrison SJ. Adult Haematopoietic stem cell niches. Nat Rev Immunol. 2017;17(9):573–90.
46. Park S-M, Kang T-I, So J-S. Roles of XBP1s in transcriptional regulation of target genes. Biomedicines. 2021;9(7).
47. Yong J, Bischof H, Burgstaller S, Siirin M, Murphy A, Malli R, Kaufman RJ. Mitochondria supply ATP to the ER through a mechanism antagonized by cytosolic Ca2. Elife. 2019;8.
48. Bagur R, Hajnóczky G. Intracellular Ca2 + Sensing: its role in calcium homeostasis and signaling. Mol Cell. 2017;66(6):780–8.
49. Van Keuren AM, Tsai C-W, Balderas E, Rodriguez MX, Chaudhuri D, Tsai M-F. Mechanisms of EMRE-Dependent MCU opening in the mitochondrial calcium uniporter complex. Cell Rep. 2020;33(10):108486.
50. Gutiérrez T, Qi H, Yap MC, Tahbaz N, Milburn LA, Lucchinetti E, Lou P-H, Zaugg M, LaPointe PG, Mercier P et al. The ER chaperone calnexin controls mitochondrial positioning and respiration. Sci Signal. 2020;13(638).
51. Pacheco J, Sampieri A, Vaca L. STIM1: the Lord of the rings? Cell Calcium. 2023;112:102742.
52. Berna-Erro A, Ramesh G, Delgado E, Corbacho AJ, Ferrer-Marín F, Teruel R, Granados MP, Rosado JA, Redondo PC. CAPN1 (Calpain1)-Dependent cleavage of STIM1 (Stromal interaction molecule 1) results in an enhanced SOCE (Store-Operated calcium Entry) in human neonatal platelets. Arterioscler Thromb Vasc Biol. 2023;43(5):e151–70.

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