ITPR2, AN ER CALCIUM CHANNEL, REGULATES ER STRESS AND INFLAMMATORY RESPONSE IN PRE-CANCEROUS KIDNEY TUBULE CELLS

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GENERAL INFORMATION

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ABSTRACT

Renal cell carcinoma (RCC) is the 16th most common cause of cancer death worldwide, with clear cell renal cell carcinoma (ccRCC) being the most lethal subtype. Despite advancements in diagnosis and management, ccRCC remains highly fatal. Additionally, over 70% of ccRCC cases exhibit defects in the *VHL* tumor suppressor gene. In previous study, we reported that endoplasmic reticulum (ER) stress is one of the key regulators of *VHL* mutant ccRCC progression by inducing inflammatory responses via IRE1α signaling. Interestingly, *ITPR2*, an ER membrane protein and a second messenger intracellular calcium release channel, was found downregulated in RCC tumors compared to healthy kidney tissue, yet recent studies identified *ITPR2* as a susceptibility locus for ccRCC on chromosome 12p11.23, a region frequently amplified in *VHL*-related RCC. These outcomes indicate that *ITPR2* somehow affects the ccRCC progression with various influences in different stages. That leads us to the hypothesis that the alteration of *ITPR2* could impact on the ER stress response, which in turn influences inflammation and ccRCC development. Here, we generated *ITPR2* knockdown in proximal tubule epithelial cell line (HK-2) with or without *VHL* knockdown and used calcium flow as the reporter for *ITPR2* activation to investigate the role of this gene in ER stress and inflammation. Our results showed that loss of *ITPR2* expression reduces the ER stress burden and ER stress-induced inflammation. Besides, we also found that double knockdown *ITPR2* and *VHL* in HK-2 diminishes macrophages recruitment compared with *VHL* single knockdown HK-2 in in vitro extravasation assay. These findings offer insights into ccRCC pathogenesis and suggest a novel therapeutic approach for kidney cancer.

1. INTRODUCTION

Kidney cancer is the sixteenth leading cause of cancer-related deaths globally, characterized by 338,000 new cases and 144,000 deaths in 2018 (Ferlay et al., 2015; Mahdavifar et al., 2016). Renal cell carcinoma (RCC), comprising over 90% of kidney cancers (Cairns, 2011), exhibits varying incidence rates based on geography and gender, with North America and the Czech Republic reporting the highest rates (Jonasch et al., 2014; Nabi et al., 2018). Among three subtypes of RCC, clear cell renal cell carcinoma (ccRCC) is the most common one, accounting for 75% of cases (Linehan & Ricketts, 2019). Over 80% of ccRCC cases lack the short arm of chromosome 3 (3p), leading to the absence of von *Hippel–Lindau (VHL)* gene region crucial in ccRCC tumorigenesis. *pVHL* controls hypoxia pathways by targeting HIF for degradation in normal oxygen conditions, but in *VHL* deficiency or hypoxia, stabilized HIF activates genes linked to angiogenesis, glycolysis, and erythropoiesis in the nucleus. (Gossage & Eisen, 2010).

The multifunctional endoplasmic reticulum (ER) plays a vital role in protein synthesis, modification, and transport, crucial for organismal homeostasis, with imbalances or misfolded protein accumulation leading to the unfolded protein response (UPR) and ER stress if not resolved. (Bahar et al., n.d.). The UPR reduces protein translation, manages unfolded proteins, and improves protein folding and degradation via IRE1, PERK, and ATF6. Activated during ER stress when BiP binds to unfolded proteins, this process involves phosphorylation events like p-PERK inhibiting mRNA translation and p-eIF2α activating ATF4 for gene transcription. Phosphorylated IRE1 splices XBP1 mRNA, enhancing ER chaperone gene transcription for protein folding and stress relief. (Graner et al., 2015). IRE1 slso impacts inflammation and apoptosis via TRAF2, JNK, ASK1, and caspase 12 pathways. In ER stress, ATF6 is released from BiP, cleaved in the Golgi, becoming an active transcription factor that enhances ER chaperone gene expression, ER-associated degradation (ERAD), and protein folding. (Fu et al., 2014).

Calcium ions (Ca^{2+}) act as vital intracellular second messengers, particularly affecting protein folding mediated by ER chaperones. These chaperones are crucial for cellular protein balance by aiding in folding and removing misfolded proteins, their function being tied to proper calcium levels (Ashby & Tepikin, 2001; Edu, n.d.; Saibil, 2013; Strehler & Treiman, 2005). Transporting calcium into the ER is crucial for maintaining high calcium levels needed for chaperone function. Inhibitors of calcium influx, like thapsigargin (a SERCA inhibitor), can trigger the unfolded protein response (UPR) by depleting ER calcium. (Coe & Michalak, 2009). The depletion of calcium in the ER disrupts protein folding and maturation, leading to ERstress, which has been linked to various disorders including kidney disease (Berridge et al., 2003; Braakman & Bulleid, 2011; Corbett et al., 1999; Torres et al., 2011; Vidal et al., 2011). ER stress responses are linked to sunitinib resistance in renal cell carcinoma (RCC) and are involved in inflammation, tumorigenesis, angiogenesis, immune modulation, and cell survival. (Auf et al., n.d.; Bettigole & Glimcher, 2014; Garg et al., n.d.; Ghosh et al., n.d.; Hu et al., n.d.)

The inositol 1,4,5-trisphosphate receptors (ITPRs or IP3Rs) are ER Ca^{2+} release channels crucial for G protein-coupled receptor signaling. Activated by receptor-ligand binding, they trigger PLC to convert PIP2 into IP3 and DAG. IP3 then binds to ITPRs, releasing Ca^{2+} into the cytosol and impacting cell processes such as division, proliferation, apoptosis, fertilization, development, behavior, memory, and learning (Daniel Mak & Kevin

Foskett, 2014). Structurally, the three ITPR isoforms (*ITPR1*, *ITPR2*, and ITPR3) share structural similarities in their ligand binding domain (LBD), coupling and modulatory domain, and transmembrane domain (TMD) (Bezprozvanny, 2005; Chandrasekhar et al., n.d.)(Bosanac et al., 2002; Chandrasekhar & Yule, n.d.; Foskett et al., 2007). *ITPR2*, with its higher affinity and selective activation by ATP at high IP3 concentrations, is predominantly expressed in tissues like the heart, pancreas, liver, and salivary glands (Daniel Mak & Kevin Foskett, 2014). Notably, *ITPR2's* involvement in renal cell carcinoma (RCC) tumorigenesis has been highlighted, although the precise mechanisms linking *ITPR2* to RCC remain unclear. Studies also suggest a significant impact of *ITPR2* on kidney function, especially regarding Ca^{2+} dependent pathways like osteoclastogenesis mediated by IRE1α and XBP1 (Kopacek et al., 2009; *The Journal of Clinical Investigation,* n.d.).

Here, we demonstrated that reduced *ITPR2* expression, with or without *VHL* knockdown, alleviates ER stress and associated inflammation in HK-2 cells, suggesting a potential therapeutic target for ccRCC and kidney cancer.

2. METHODOLOGY

2.1. Cell culture

Human kidney proximal tubule epithelial cells (HK-2, BCRC) were cultured in KSFM (GIBCO) with BPE and EGF. HEK293T cells (BCRC) for Lentivirus production were cultured in DMEM (Life Technologies) with 10% FBS.

Human dermal microvascular endothelial cells (HMEC-1, ATCC) were cultured in MCDB131 without L-glutamine, supplemented with EGF, hydrocortisone, glutamine, and FBS. THP-1 cells were cultured in RPMI-1640 with L-glutamine and FBS. All cultures were maintained at 37°C in 5% CO_2.

2.2 Vectors and gene transfer

Two shRNAs targeting *ITPR2* were bought from the RNA Technology Platform and Gene Manipulation Core (at Academia Sinica, Taiwan) with these sequences:

sh*ITPR2* C3:
AGGGAATGAAAGGGCAATTAA

sh*ITPR2* H1: CCTGGCTGTGTTCATCAATTT

The sh*ITPR2* shRNAs in the pKLO1 vector were then transfected into HK-2 by lentivirus transfection.

2.3. Quantitative RT-PCR

Total RNA was extracted using the GeneDirex Total RNA Isolation Kit. 500 ng of RNA was reverse-transcribed into cDNA using the GScript First-Strand Synthesis Kit (MB305-0050) from GeneDirex. The qPCR reaction was performed using GoTaq® qPCR Master Mix (Promega) and run on a ViiATM 7 Real- Time PCR System from Thermo Fisher Scientific Inc. All reactions were performed in triplicate, and mRNA levels were quantified using the comparative Cp method normalized against GAPDH. The primers used are listed in the Table 1.

2.4. Western blot analysis

Protein extracts were loaded onto SDS-polyacrylamide gel electrophoresis and transferred to PVDF membranes (Immobilon®-P). Membranes were blocked in TBS with 5% nonfat milk, then probed overnight at 4°C with appropriate antibodies (see Table 2 for details). Afterward, membranes were washed three times with TBST and incubated with secondary antibodies (diluted 1:200) in 5% nonfat milk/TBS for 1 hour at room temperature. Finally, membranes were washed again and protein bands were visualized using an ECL kit.

Table 1. Primer sequences used for qPCR.

Gene name	Forward sequence (5'-3')	Reverse sequence (5'-3')
GAPDH	ATGACAACAGCCTCAAGAT	AGTCCTTCCACGATACCAAA
ITPR2	AACCGGGAATTGCAGAACTT	CACCGGTTGTACTTCCACAA
BiP	AAGCCCGTCCAGAAAGTGTT	ACGCTACAGCTTCATCTGGG
CHOP	TTAAAGATGAGCGGGTGGCA	AAGCTGGTCTGATGCCTGTT
sXBP1	CTGAGTCCGCAGCAGGT	TCCAGAATGCCCAACAGGAT
IL1α	GTAGCAACCAACGGGAAGGT	AGGTGCTGACCTAGGCTTGA
IL2	AACCTCAACTCCTGCCACAA	TCCAGCAGTAAATGCTCCAGT
IL6	GCTAGCATGAACTCCTTCTCC ACAA	GAATTCCTACATTTGCCGAAG AGCC
IL8	TGGACCCCAAGGAAAACTGG	TGGCATCTTCACTGATTCTTGGA
IL12α	CCAGAAGGCCAGACAAACTCTA	TCTCTCTGGAATTTAGGCAACTCT
TNFα	CCTGCTGCACTTTGGAGTGA	TCACTCGGGGTTCGAGAAGA
IFNγ	AGGCTTTATCTCAGGGGCCA	CAGGCATATTTTCAAACCGGCA

Table 2. Primary antibodies used for Western blot

Primary antibody	Company	Dilution ratio
α-tubulin	Sigma-Aldrich (T5168)	1:4000
GAPDH	Cell signaling (ab9485)	1:1000
ITPR2	Santa Cruz (sc-398434)	1:200
p-IRE1	Abcam (ab48187)	1:500
IRE1	Cell signaling (3294S)	1:1000
p-PERK	Santa Cruz (sc-32577)	1:200
PERK	Cell signaling (5683S)	1:1000
BiP	Cell signaling (3177S)	1:1000
p-JNK	Cell signaling (4668S)	1:1000
JNK	Cell signaling (9252S)	1:1000
p-NFκB	Cell signaling (3033S)	1:1000
VHL	Cell signaling (68547S)	1:1000

2.5. Intracellular calcium measurement

Cells (5x105) in a 6-well plate were stained with Fluo-4 AM (2034141, Invitrogen) the next day. The Fluo-4 AM Loading Solution, comprising Fluo-4 AM (Component A), PowerLoad™ concentrate (Component B), and 20 mM Glucose Stock, was prepared, with optional Probenecid stock added for specific cell types. Following incubation at 37°C and room temperature, the loading solution was removed, cells were PBS-washed, and membrane proteins were digested with trypsin-EDTA. Neutralization, centrifugation, and resuspension in Glucose Stock were done, and cells were split for baseline and calcium measurement post-IP3 addition.

We recorded calcium signal in untreated cells as the baseline. Then, IP3 was added immediately (not more than 15s) and the

calcium signal was captured within 300s by flow cytometry (ACEA Novocyte1000).

2.6 Macrophage recruitment assay

The macrophage endothelial extravasation model was established by co-culturing HK2 cells with single *VHL* knockdown or *VHL* and *ITPR2* double knockdown, THP-1 monocyte precursors differentiated into macrophages using phorbol myristate acetate (PMA), and human dermal microvascular endothelial cells (HMEC-1) in a transwell system. THP-1 cells were differentiated with 50 ng/ml PMA on the first day, followed by medium refreshment without PMA after 24 hours. HMEC-1 cells were cultured on the upper side of the transwell on the second day, while HK2 cells with *VHL* knockdown or *VHL* and *ITPR2* double knockdown were seeded in the lower chamber. Upon HMEC-1 confluency on the third day, macrophages from THP-1 were added to the upper transwell with a 1:1 ratio of HMEC-1 and THP-1 media, while the bottom well contained serum-free HK-2 medium with 1% BSA. After 48 hours of co-culture, the membrane was stained with crystal violet to analyze macrophage extravasation through the endothelial layer model, with cells on the upper side of the membrane removed

2.7 Immunohistochemistry staining

Mouse kidney tissue sections from formalin-fixed, paraffin-embedded samples were uesed to detect ITPR2 signal. Antigen retrieval was achieved with sodium citrate buffer (pH 6) in a microwave. Slides were washed with water and then treated with 3% hydrogen peroxide to block endogenous peroxidase activity. Non-specific binding was blocked with 15% normal goat serum and 1% bovine serum albumin (BSA) in PBS for 1 hour. Primary antibodies against IP3 receptor 2 (1:400) and rabbit IgG isotype control (1:200) were applied and incubated overnight at 4°C. After washing, secondary anti-rabbit IgG (1:200) was applied, followed by DAB substrate incubation. Sections were counterstained with hematoxylin, dehydrated, and mounted. Imaging was done with an Olympus IX83, and images were edited using Adobe Photoshop CS6 without bias.

2.8 Statistical Analysis

Summarized data were presented as means ± SEM. Statistical significance between independent groups was assessed using a two-tailed unpaired Student's t-test, with significance set at $p < 0.05$. Student's t-tests and graphs were generated using GraphPad Prism 8.0.2.

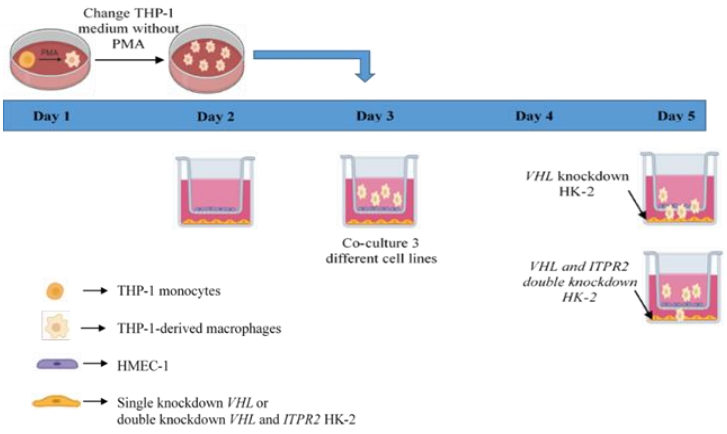

Figure 1. Schematic diagram of transwell assay

3. FINDINGS AND DISCUSSION

3.1. Single knockdown *ITPR2* and double knockdown *VHL* and *ITPR2* HK-2 were established as experiments material

We established *ITPR2* knockdown cell lines in HK-2 cells using lentivirus-delivered shRNAs, achieving over 80% reduction in *ITPR2* protein expression compared to controls (Figure 2A). Subsequently, we performed double knockdown experiments targeting *VHL* and *ITPR2*, confirming successful knockdown and observing increased *ITPR2* expression in *VHL*-deficient HK-2 cells, providing initial evidence for the *VHL-ITPR2* correlation in kidney cells (Figure 2B).

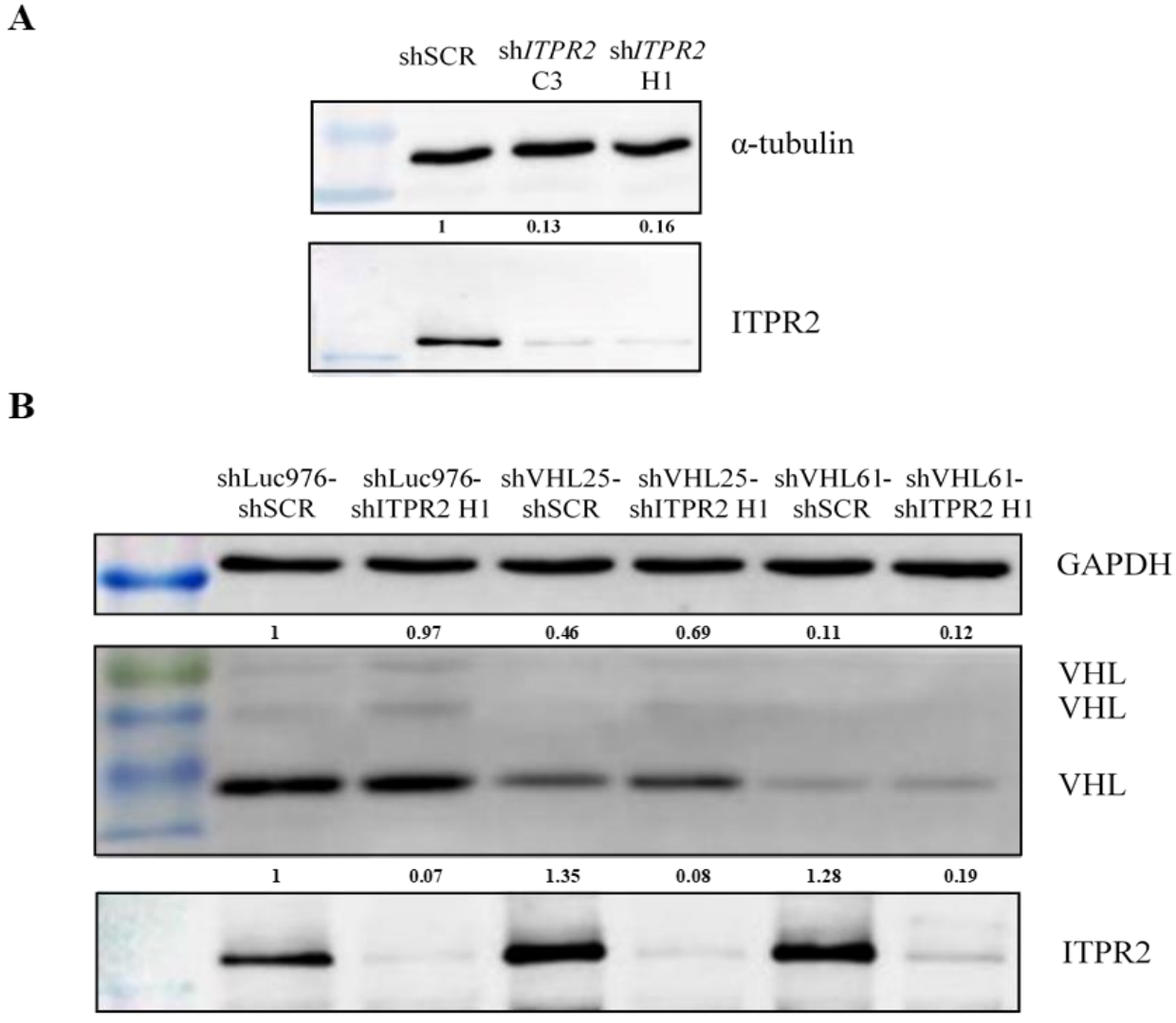

Figure 2. Knockdown ITPR2 efficiency (A) HK-2 cells were infected with control (shSCR) or two *ITPR2*-targeting shRNA vectors (shITRP2 C3 and shITRP2 H1), selected with puromycin (2 µg/ml) for 72 hours, and analyzed by Western blot. (B) Lentiviral transfection of Scramble and sh*ITPR2* into Luc976 and *VHL*-inactivated HK-2 cells, selected with blasticidin (2 µg/ml) for 86 hours, followed by Western blot analysis. shSCR denotes shScramble, and C3, H1, 25, and 61 denote specific sh*ITPR2* and sh*VHL* sequences as mentioned earlier.

3.2. VHL deletion increases ITPR2 expression

In our prior study, we demonstrated that *VHL* inactivation drives kidney tumorigenesis in ccRCC by inducing an inflammatory response through IRE1α signaling in ER stress (Kuo et al., n.d.), leading us to investigate a potential correlation between *VHL* and *ITPR2* in ER stress by examining *ITPR2* expression in *VHL* knockdown HK-2 cells and VHL$^{-/-}$ mice. Then, we further confirmed that outcome in Hoxb7–Cre–EGFP-driven *VHL*h$^{fl/fl}$ mice by immunohistochemistry (IHC) experiment. Mouse strains used have been described previously (Pritchett et al., 2015).

We observed strong ITPR2 signals in all cells on slides from both wild-type and mutant samples, with significantly stronger signals in mutant sections compared to wild-type samples (Figure 3). Confirmation of anti-*ITPR2* antibody specificity was demonstrated by the absence of *ITPR2* signal in negative control slides stained with rabbit IgG. This finding supports the overexpression of *ITPR2* in *VHL*-depleted conditions, consistent with the Western outcome in Figure 2B and other previous studies (Beroukhim et al., 2009; Moore et al., 2012; Wu et al., 1173), highlighting the impact of *VHL* loss-of-function on *ITPR2* levels in kidney cells.

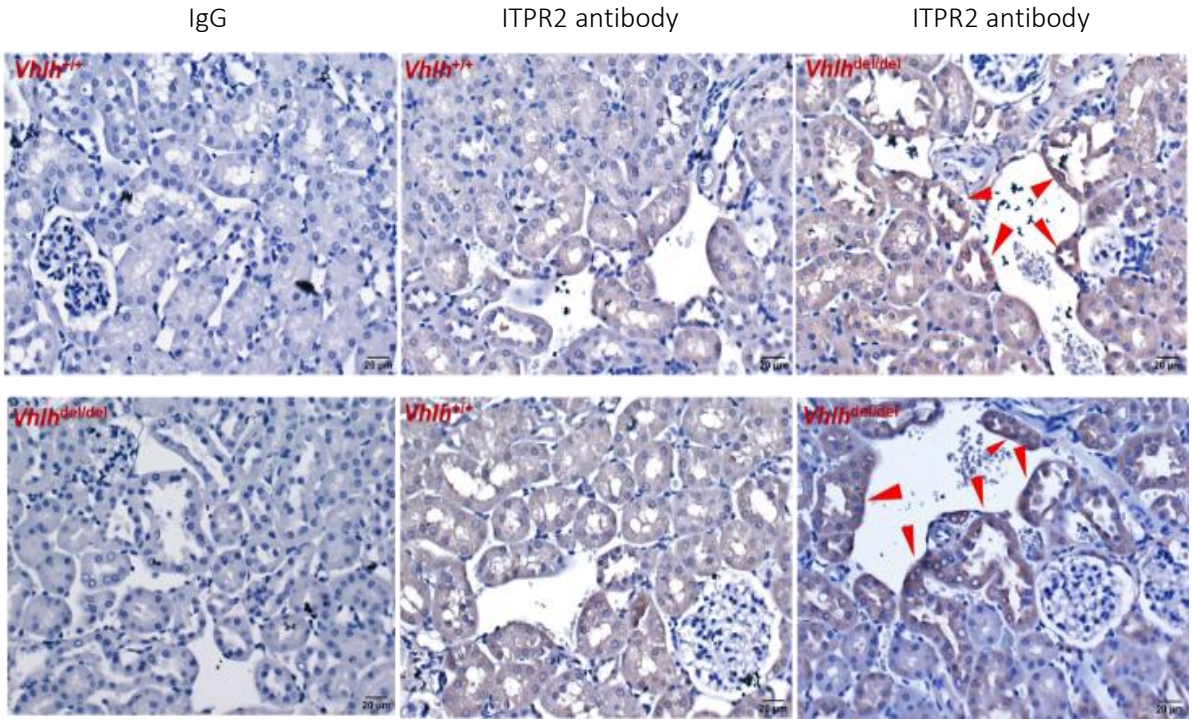

Figure 3. Immunohistochemical analysis of Hoxb7–Cre–EGFP-driven *VHL*h (mouse allele of the *VHL* gene) knockout kidney.

Sections of kidney from wild-type and *VHL*h$^{-/-}$ mice were stained with rabbit IgG as a negative control and rabbit anti-*ITPR2* antibody. Slides were then stained with DAB, counterstained with Hematoxylin, and observed under light microscopy (Scale bar: 20 μm).

3.3 Knockdown ITPR2 rescues ER stress

To test the hypothesis that *ITPR2* deficiency reduces tumor progression by mitigating ER stress, we treated *ITPR2*-

deficient HK-2 cells with the ER stress inducer thapsigargin, which depletes ER calcium by inhibiting SERCA, thereby reducing calcium-dependent ER chaperone activity like calnexin. Then, we examined three ER stress markers (p-IRE1α, p-PERK, and BiP) to evaluate *ITPR2's* impact on ER stress. Additionally, we explored the relationship between *ITPR2* and *VHL* in ER stress by treating cells with single knockdown *VHL* and double knockdown *VHL* and *ITPR2* with thapsigargin.

Our findings revealed increased BiP expression with thapsigargin, indicating ER stress induction, and significantly reduced p-IRE1α levels in *ITPR2*-deficient cells under ER stress, implying reduced ER stress burden. Moreover, the downregulation of p-NFκB suggested a potential role of *ITPR2* in modulating inflammation through p-NFκB pathways. This study elucidates that *ITPR2* inactivation alleviates ER stress via p-IRE1α and mitigates the adverse effects of *VHL* loss on ER stress.

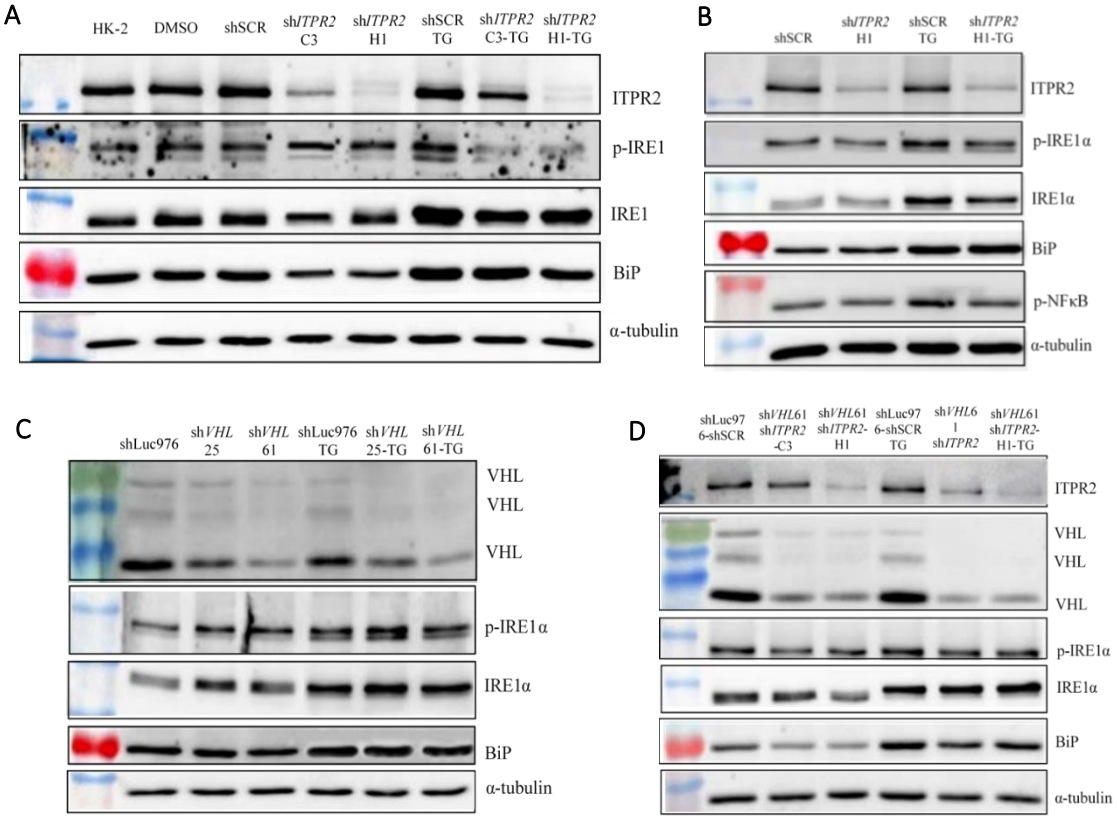

Figure 4. The role of *ITPR2* in ER stress. (A). shScramble and sh*ITPR2*-transfected HK2 cells were treated with 1 μM of thapsigargin (TG) for 6 hours. (B). The repeated experiment for sh*ITPR2* H1 sequence. (C). shScramble or sh*VHL*-transfected HK2 cells were treated with 1 μM of TG for 6 hours. (D). Plasmids containing shScramble or sh*ITPR2* were transfected into sh*Luc*976 or sh*VHL* HK-2 to generate double knockdown stable cells lines. These cells were treated with 1 μM of TG for 6 hours.

3.4 Measurement of intracellular Ca^{2+} concentration as an *ITPR2* activation reporter

We aimed to confirm the role of *ITPR2* depletion in ER stress regulation by assessing its impact on ER calcium flow using the Ca^{2+} indicator Fluo-4 in control and *ITPR2*

knockdown HK-2 cells. Fluo-4, when loaded into cells as Fluo-4 AM, chelates with calcium, increasing fluorescence. Probenecid, an organic anion transporter inhibitor, was used to enhance intracellular retention of the indicators. Flow cytometry detected the emission from calcium-bound Fluo-4 dye (excitation 494 nm/emission 506 nm). Following incubation with Fluo-4, shScramble and sh*ITPR2* HK-2 cells showed no significant difference in intracellular Ca^{2+} levels, suggesting a potential role of *ITPR2*-regulated calcium flow in ER stress modulation, pending further validation.

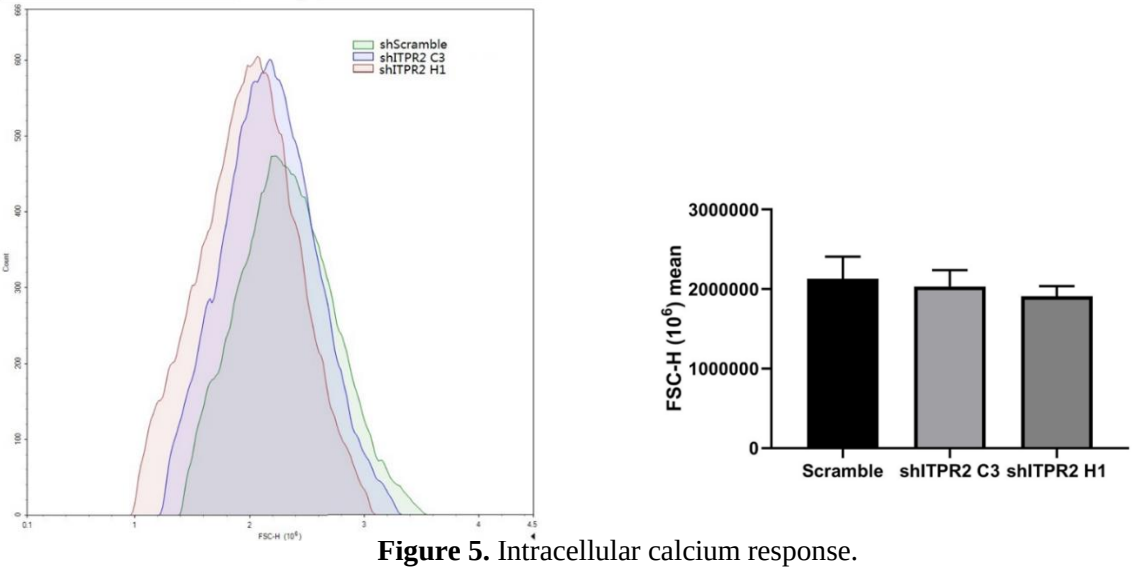

Figure 5. Intracellular calcium response.

Cells were stained with Fluo-4 AM in 20 mM glucose for 30 minutes at 37°C, followed by a 30-minute incubation at room temperature. After three washes with PBS, cells were resuspended and prepared for flow cytometry detection

3.5 Lack of *ITPR2* reduces inflammation via p-IRE1α regulated cytokines production

According to p-JNK and p-NFκB expression in Figure 4, we assume that p-IRE1α expression regulated by *ITPR2* probably leads to alteration of inflammatory response. Therefore, we decided to observe the cytokine production and ER stress marker expression from extracted total mRNA of *ITPR2* knockdown stable cell lines and of these cells treated with two different ER stress inducer, including tunicamycin at 1 µg/ml (a naturally occurring antibiotic, which induces ER stress in cells by inhibiting the first step in the biosynthesis of N-linked glycans in the proteins resulting in many misfolded proteins) and thapsigargin at 1 µM. Every experiment was repeated 3 times and the bar chart shows the mean of those 3 values.

As shown in Figure 6, production of some cytokines such as *IL1α, IL6, IL8, TNFα* is dramatically reduced in *ITPR2* deficiency HK-2 cells. However, the decline level of these cytokines in cells without tunicamycin and thapsigargin treatment is more noticeable than treatment group (Figure 6A, 6B). That means *ITPR2* loss-of-function might rescue inflammation in ER stress, but not significantly. This phenomenon could be explained that there are other calcium channels in the ER membrane besides *ITPR2*. Therefore, the elimination of *ITPR2*-mediated calcium flow in cytoplasm can only partially resolve ER stress. Furthermore, mRNA level of *s-XBP1*, which is a downstream target of p-*IRE1α*, seemed to reduce when ITPR2 was silents with and without

tunicamycin treatment although the falling was just significant in sh*ITPR2* C3, not in sh*ITPR2* H1 samples (Figure 6C, 6D). However, other ER stress marker expression, such as BiP and CHOP is reduced only in untreated *ITPR2* knockdown HK-2, but not in ER stress-induced HK-2 (Figure 6C, 6D).

Therefore, the decrease of *ITPR2* may help escape ER stress via p-IRE1α signaling, leading to diminishing cytokine production and inflammation. Together, these results provide evidence for the protective function of *ITPR2* depletion in the precancerous kidney cells

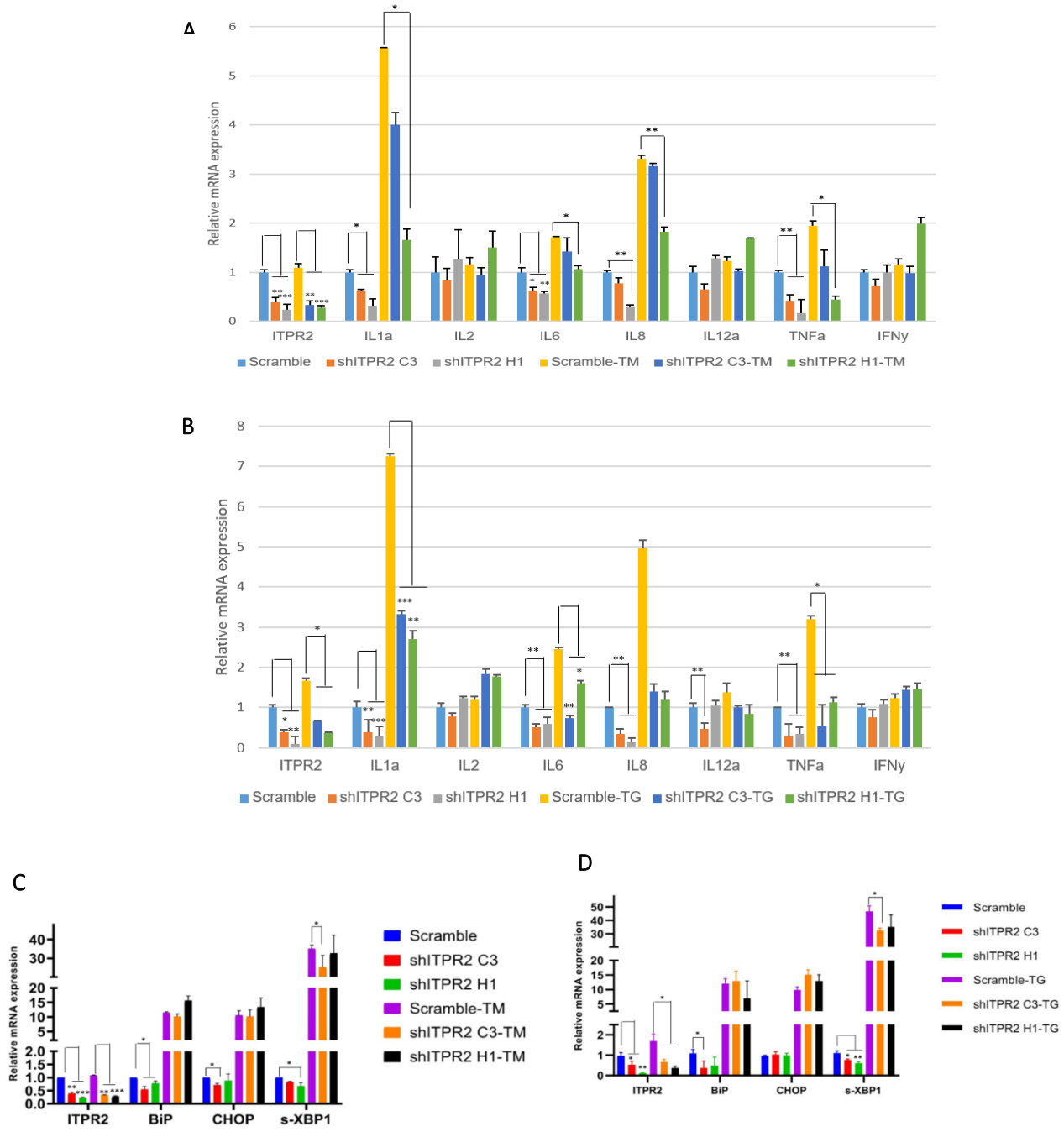

Figure 6. Quantification of mRNA expression levels RT-PCR.

HK-2 cells were transfected with scrambled shRNA or sh*ITPR2* sequences, followed by separate 6-hour treatments with tunicamycin (TM) at 1 µg/ml and thapsigargin (TG) at 1 µM. (A) Cytokine expression after TM treatment. (B) Cytokine expression after TG treatment. (C) ER stress marker expression after TM treatment. (D) ER stress marker expression after TG treatment. Statistical significance was assessed using the two-tailed, unpaired Student t-test (n = 3, ***P < 0.001, **P < 0.01, *P <0.05).

3.6 ITPR2 ablation reduces macrophage recruitment

To confirm ITPR2's role in inflammation related to *VHL* depletion diseases, we co-cultured THP-1-derived macrophages, HMEC-1, and HK-2 cells with *VHL* single knockdown or *VHL* and *ITPR2* double knockdown for 48 hours. We stained the transwell insert with crystal violet and observed the macrophage recruitment under a light microscope. Figure 7 shows significantly higher violet signal in *VHL*-deficient HK-2 cells compared to shScramble HK-2, indicating increased macrophage recruitment due to *VHL* knockdown. However, the number of recruited macrophages decreased dramatically in the absence of both *ITPR2* and *VHL* in HK-2 cells compared to *VHL* single knockdown, suggesting that *ITPR2* depletion can reverse *VHL*-mediated macrophage recruitment, thereby reducing inflammation in kidney cells.

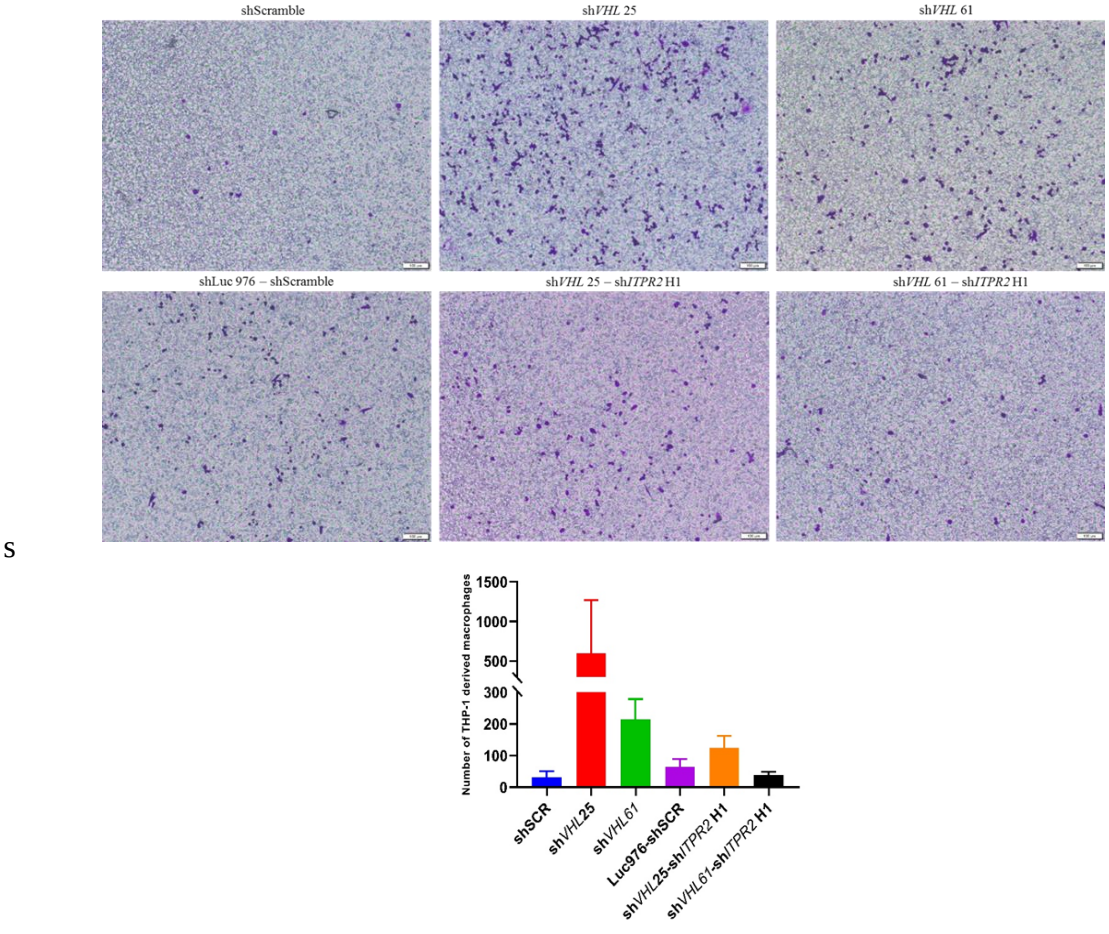

Figure 7. ITPR2 loss of function reverses macrophage recruitment effect of VHL knockdown.

Single *VHL* knockdown or *ITPR2* and *VHL* double knockdown were co-cultred with THP-1 derived macrophages, and endothelial cells, HMEC-1. After co-culture for 48 hours, transwell inserts were stained with crystal violet to observed the number of macrophages migrated under the light microscope. Scale bar: 100 μm.

In conclusion, in this study, we showed that *ITPR2* was upregulated in both *VHL*-inactivated HK-2 and Hoxb7-Cre-EGFP-driven *VHL*h knockout mice. This might be one of the factors contributing to RCC initiation progression. The result agrees with the high expression of *ITPR2* in RCC observed in previous researches (Beroukhim et al., 2009; Moore et al., 2012; Wu et al., 1173).

Additionally, *ITPR2* ablation in human kidney cells, regardless of *VHL* association, demonstrates protective potential. *ITPR2* loss reduces calcium release from the endoplasmic reticulum, alleviating ER stress via p-*IRE1*α signaling and subsequently decreasing inflammation markers such as p-NFκB. This leads to a notable reduction in cytokine production *(IL-1α, IL-6, IL-8, and TNFα)* and diminishes macrophage extravasation, providing kidney cell protection against inflammation through p-*IRE1*α signaling.

Although recent studies have linked downregulated *ITPR2* with poor survival in renal cancer; however, our findings are not entirely contradictory. This discrepancy likely stems from *ITPR2's* varied functions in different aspects of RCC development, highlighting the need for further investigation into its exact role across RCC stages. These findings unveil a new mechanism in clear cell RCC development and suggest *ITPR2* as a promising therapeutic target for ccRCC or kidney cancer broadly.

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