Research Square

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STAT3 S-Glutathionylation Regulates Th17 Differentiation in Experimental Immune Thrombocytopenia

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Abstract

Background

Immune thrombocytopenia (ITP) is an autoimmune condition marked by an abnormal response of T cells, particularly driven by splenic T helper cells under the regulation of STAT3. Protein S-glutathionylation (PSSG), a vital post-translational modification, significantly impacts the function of numerous proteins. However, the role of S-glutathionylation in modulating STAT3 activation and T helper cells in ITP has not been previously investigated. This study reveals that S-glutathionylation, regulated by the deglutathionylation enzyme glutaredoxin 1 (Grx1), is essential in the pathogenesis of ITP.

Methods

Levels of S-glutathionylation in ITP patients were evaluated using Western blotting and GSSG/GSH assay kits. To explore the role of S-glutathionylation in ITP, we employed wild-type (WT) and glutaredoxin 1 knockout (Grx1 KO) mice to create a passive ITP mouse model. The differentiation of Th17 cells was assessed both in vitro and in vivo via flow cytometry. Additionally, STAT3 activation was examined using Western blotting, GSSG assays, and immunofluorescence.

Results

An elevation in protein S-glutathionylation (PSSG) was observed in ITP patients. In our mouse model, Grx1 KO mice displayed higher PSSG levels, which were associated with a reduction in ITP symptoms. Mechanistically, Grx1 KO enhanced STAT3 S-glutathionylation, thereby inhibiting its activation and decreasing the production of inflammatory cytokines. Moreover, STAT3 S-glutathionylation significantly suppressed Th17 cell differentiation, underscoring its role in modulating immune responses in ITP.

Conclusions

Elevated PSSG levels due to Grx1 KO enhance STAT3 S-glutathionylation, which subsequently inhibits STAT3 activation and Th17 cell differentiation, thereby alleviating ITP symptoms.

Introduction

Immune thrombocytopenia (ITP) is an autoimmune disease marked by heightened platelet destruction and diminished platelet production due to immune system imbalances [1]. T helper cells (Th cells), such as Th1, Th2, Th3, Th17, and Treg cells, are central to autoimmune reactions. In particular, Th17 and Treg cells are vital in the progression of ITP. Studies have indicated that a higher proportion of Th17 cells among CD4 + T cells can hasten the development of ITP [2]. Therefore, understanding the mechanisms behind Th17 cell differentiation could provide new therapeutic strategies for ITP.

Th17 cells are integral to adaptive immunity and play a significant role in defending the host against pathogens. They safeguard mucosal surfaces from bacterial and fungal infections by secreting cytokines like interleukin (IL)-17A and IL-17F [3, 4]. However, improper regulation of Th17 cells has been linked to the development and progression of several autoimmune diseases, including inflammatory bowel disease, multiple sclerosis, and rheumatoid arthritis [5–7]. The differentiation of Th17 cells from naïve CD4 + T cells is tightly controlled at the transcriptional level. This process involves the coordinated actions of the signal-responsive transcription factor Signal Transducer and Activator of Transcription 3 (STAT3), the pioneering factors IRF4 and BATF, and the Th17 lineage-specific regulator RORγ [8]. These factors work together to regulate the gene transcription program of Th17 cells, supporting their immune functions. Importantly, STAT3, a key regulator of Th17 cells, also functions as a crucial transcriptional regulator in many other cell types. However, the specific mechanisms regulating STAT3 in Th17 cells are still not well understood.

Signal Transducer and Activator of Transcription 3 (STAT3), part of the transcription factor family, is recognized as a major factor in cancer progression [9]. Lately, though, there has been growing interest in its regulatory functions in autoimmune conditions, including immune thrombocytopenia (ITP) [10]. Research comparing the peripheral whole blood of ITP patients with that of healthy individuals has shown a notable increase in STAT3 levels in the plasma of ITP patients [11]. It has also been found that knocking out STAT3 in T cells prevents Th17 cell differentiation [12], while overexpressing STAT3 enhances it [13]. Collectively, these findings suggest that STAT3 is a crucial controller of Th17 cell differentiation and that it contributes to ITP development by boosting Th17 differentiation. However, the upstream regulator of STAT3 in Th17 differentiation is still unclear.

The post-translational modification called S-glutathionylation (PSSG) has been linked to various diseases involving redox imbalances [14]. PSSG is a distinctive redox-driven process where the cysteine residue of glutathione forms a disulfide bond with the -SH group of a target cysteine in the protein. This modification is reversible and causes temporary structural and functional changes in the target proteins. PSSG occurs spontaneously in response to oxidative stress, but its rate and extent are controlled by specific enzymes [15, 16]. One key enzyme is glutaredoxin-1 (Grx1), a cytosolic thioltransferase that efficiently reduces PSSG [17]. As a result, Grx1-regulated PSSG is important in redox signaling and has significant implications in disease states [18]. However, the role of Grx1-regulated STAT3 S-glutathionylation in ITP is not yet fully understood.

In our research, we explored how the removal of Grx1 impacts the STAT3 S-glutathionylation process in mice with immune thrombocytopenia (ITP). We suggest that eliminating Grx1 boosts STAT3 S-glutathionylation, which in turn blocks STAT3 activation and the development of Th17 cells, ultimately improving ITP symptoms. Overall, the deletion of Grx1 appears to have a protective role in the ITP disease process and could be a promising target for ITP therapies.

Methods

Patients

Peripheral bloods were obtained from individuals newly diagnosed with ITP [19] (Table 1; n = 6; 3 males and 3 females). Control blood samples were collected from healthy donors (HDs) (n = 6; 3 males and 3 females). Patients with severe dysfunction in cardiac, renal, pulmonary, or hepatic systems, as well as those with primary immunodeficiency, were not included in the study. Pregnant or breastfeeding individuals were also excluded.

Table 1
Clinical characteristics of Immune thrombocytopenia patients (ITP) and health donor (HD)

Patient no.	Sex	Age (years)
ITP1	M	27
ITP2	M	24
ITP3	M	31
ITP4	F	29
ITP5	F	33
ITP6	F	28
HD 1	M	32
HD 2	M	35
HD3	M	38
HD 4	F	35
HD 5	F	31
HD 6	F	36

All procedures conformed to the ethical principles set forth in the Declaration of Helsinki. The investigation received prior approval from the Institutional Review Board of the First Affiliated Hospital of Xinjiang Medical University (IRB, No.: 20210226-11) and was carried out only after each participant—or their legally designated proxy—provided signed informed consent.

ITP Mice

A standardized passive model of ITP was induced following a previously validated protocol [20]. Grx1-deficient mice on the C57BL/6J background (Grx1-/-) were obtained from Prof. Chunbao Guo at Chongqing Medical University; wild-type C57BL/6 mice of both sexes, 6–8 weeks old, were purchased from Xinjiang Medical University. All animals were housed in a specific-pathogen-free barrier facility

under the university's standard husbandry conditions. Protocol approval was granted by the Xinjiang Medical University Animal Ethics Committee (No.: 20210301-11).

For disease induction, animals were matched by age and sex and then given intraperitoneal injections of anti-CD41 monoclonal antibody (BD Biosciences, USA). The regimen consisted of an initial 68 μg/kg load delivered over the first 48 h, followed by 34 μg/kg daily for four additional days. Twenty-four hours after the final injection (study day 6), mice were euthanized with CO_2, consistent with American Veterinary Medical Association guidelines. Disease course was tracked by daily platelet enumeration: 5 μL tail-vein blood was collected into 45 μL heparin (2 mg/mL) and analyzed immediately on a Mindray BC-5300 hematology analyzer.

Iron Assays

Fe^{2+} and total iron in mouse spleen homogenates were quantified with a commercial ferrous-ion detection kit (Abcam), strictly adhering to the supplier's instructions.

GSH and GSSG Measurement

Oxidized (GSSG) and reduced (GSH) glutathione contents were measured by spectrophotometry using a glutathione assay kit (Beyotime), following the provided protocol.

Immunoblotting

Protein extracts were prepared by homogenizing tissue samples in lysis buffer as described elsewhere [17]. After centrifugation, supernatant protein levels were quantified with a BCA kit (Beyotime) following the vendor's protocol. Equal aliquots (30 μg) were resolved on SDS-PAGE gels and electro-transferred onto PVDF membranes. Membranes were probed overnight with the primary antibodies listed in Table 2, then incubated for 2 h at ambient temperature with HRP-linked secondary antibodies. Band intensities were measured using ImageJ.

Table 2
List of mouse antibodies used in the study

Antibodies	Source	Identifier
β-actin	Proteintech	20536-1-AP
Histone H3	zenbio	R24572
Na, K-ATPase	abcam	ab76020
GSS	Proteintech	15712-1-AP
Anti-Glutathione (GSH)	abcam	ab19534
p-STAT3	zenbio	R381552
STAT3	abcam	ab119352
Grx1	Proteintech	15804-1-AP
JAK2	abcam	ab108596

Immunofluorescence

For cellular assays, splenocytes were seeded on glass coverslips in 12-well plates, fixed with 4% paraformaldehyde, and made permeable with 0.1% Triton X-100 before overnight primary-antibody incubation at 4°C. For tissue sections, paraffin-embedded spleen slices were deparaffinized, subjected to heat-induced antigen retrieval in Tris-EDTA-Tween (pH 8.0), treated with 3% H_2O_2 to suppress endogenous peroxidases, and blocked with 3% BSA, followed by overnight primary-antibody exposure at 4°C. Both cell and tissue specimens were then probed with fluorochrome-conjugated secondary antibodies (1 : 500) for 1 h at room temperature, counterstained with DAPI, and examined by confocal microscopy.

Immunoprecipitation

Co-immunoprecipitation (Co-IP) was executed following an established protocol [17]. Fresh mouse spleen samples were homogenized in ice-cold RIPA lysis buffer (Beyotime) containing a protease inhibitor cocktail and kept on ice for 30 min to achieve complete solubilization. After centrifugation to remove debris, equal protein aliquots were gently rotated overnight at 4°C with STAT3-specific primary antibody. Antigen-antibody complexes were captured with Protein A-Sepharose beads (Invitrogen), then stringently washed to eliminate non-specific binders. Precipitated proteins were resolved on 5% SDS-PAGE gels and electro-blotted onto PVDF membranes. Membranes were blocked with 3% BSA in TBS-T to quench residual binding sites, followed by incubation with an anti-glutathione antibody to assess S-glutathionylation levels. After extensive washing, membranes were probed with HRP-linked anti-mouse IgG for chemiluminescent detection. Antibody details are provided in Table 2.

Subcellular Compartment Isolation

Fresh splenocytes were suspended in ice-cold fractionation buffer containing a protease-inhibitor mix [21]. After homogenization, the lysate was centrifuged at 1 000 × g for 5 min to pellet nuclei. The post-nuclear supernatant was spun at 6 000 × g for 5 min, and the resulting supernatant further ultracentrifuged at 20 000 × g for 2 h. The final pellet (membrane) and supernatant (cytosol) were resuspended in lysis buffer and analysed by immunoblotting.

In Vitro T-Cell Polarization

Naive $CD4^+CD25^-$ T cells were purified from splenic single-cell suspensions using an EasySep Mouse $CD4^+CD25^+$ Regulatory T Cell Isolation Kit II (Stemcell Technologies). Cells were plated at 1×10^5 per well on anti-CD3/anti-CD28-coated plates (1 µg/ml each, BD Biosciences). For Th17 skewing, cultures were supplemented with 2.5 ng/ml TGF-β, 20 ng/ml IL-6, and 20 ng/ml IL-23 (PeproTech).

Flow-Cytometric Analysis

Spleens were mechanically dissociated to yield single-cell suspensions at 1×10^6 cells/ml. Samples were stained for 1 h in the dark with fluorochrome-conjugated antibodies against CD3, CD4, and IL-17A (Abcam) and analysed on an FACS Canto II (BD).

ELISA

Inflammatory mediators were quantified with commercial ELISA reagents (R&D Systems, Minneapolis, MN) following the vendor's standard operating procedures.

RT-PCR

Total RNA from splenocytes was isolated using an AG RNA kit (China) and reverse-transcribed with AG reverse-transcription reagents (China). Real-time PCR was performed on a CFX96 platform (Bio-Rad, Hercules, CA) with SYBR Premix Ex Taq™ (AG, China). Transcript abundance relative to β-actin was calculated by the ΔΔCt method; primer sequences are provided in Table 3.

Table 3
List of mouse primers used in the study.

Gene	Forward primer	Reverse primer
Rora	TCTCCCTGCGCTCTCCGCAC	TCCACAGATCTTGCATGGA
Rorc	GAGTTTGCCAAGCGGCTTT	TCCATTGCTCCTGCTTTCAGT
Il23r	GCCAAGAAGACCATTCCCGA	TCAGTGCTACAATCTTCTTCAGAGGACA
Il17a	GCTCCAGAAGGCCCTCAG	CTTTCCCTCCGCATTGACA
Csf2	TTTACTTTTCCTGGGCATTG	TAGCTGGCTGTCATGTTCAA
β-actin	TAGGAGCCAGAGCAGTAATC	ACAGGTGAGGTTCACTGTTTCT

Statistical

Data are expressed as mean ± SEM. Two-group comparisons used an unpaired two-tailed Student's t-test; multi-group comparisons employed one-way ANOVA. Each experiment was repeated 2–3 times. Analyses were performed with GraphPad Prism 8.0, and $P < 0.05$ was considered statistically significant.

Results

S-glutathionylation is implicated in regulating ITP

Growing evidence indicates that oxidative stress is a crucial element in the development of ITP, though the exact mechanisms are still not fully understood [22, 23]. The ratio of oxidized glutathione (GSSG) to reduced glutathione (GSH) is a dependable measure of the cellular redox state. Increased GSSG/GSH ratios were detected in the plasma of ITP patients (Fig. 1A) and in the spleens of ITP mice (Fig. 1B). Compared with healthy controls, ITP mice showed higher expression levels of glutathione synthetase (GSS) (Fig. 1C). Moreover, levels of ferrous iron (Fe^{2+}) and total iron, which serve as markers of oxidative stress and early ferroptosis, were notably higher in ITP mice (Fig. 1D, E). These observations suggest that oxidative stress promotes an environment conducive to protein S-glutathionylation (PSSG). To delve deeper into this, we assessed intracellular PSSG levels, a post-translational modification tightly controlled by redox status, using immunoblotting. PSSG levels were markedly elevated in the plasma of ITP patients (Fig. 1F) and in ITP mice (Fig. 1G). Together, these results underscore the link between redox regulation and PSSG in ITP.

Grx1 Knockout Mitigates ITP through S-Glutathionylation

Glutaredoxin 1 (Grx1) is a pivotal enzyme responsible for regulating protein S-glutathionylation (PSSG) levels by reversing this post-translational modification. To explore the impact of S-glutathionylation on ITP, Grx1 knockout (KO) mice were employed (Fig. 2A). In the spleens of ITP mice, Grx1 KO led to a substantial increase in the GSSG/GSH ratio (Fig. 2B). Western blot analysis using anti-GSH antibodies demonstrated a significant elevation in overall protein S-glutathionylation in the spleens of ITP mice following Grx1 KO (Fig. 2C, D). Immunofluorescence studies also confirmed a pronounced increase in protein S-glutathionylation in the spleens of ITP-exposed Grx1 KO mice compared to controls (Fig. 2E). Importantly, Grx1 deficiency notably reduced ITP symptoms (Fig. 2F). Moreover, the levels of inflammatory cytokines in the spleens of ITP-exposed Grx1 KO mice were markedly decreased (Fig. 2G-L).

S-Glutathionylation Suppresses STAT3 Signaling in ITP

The JAK–STAT axis, essential for rapid regulation of $CD4^+$ T-cell balance, is frequently hyper-activated in ITP, resulting in elevated phosphorylated STAT3 (p-STAT3) [24]. Earlier studies indicate that S-

glutathionylation can block STAT3 phosphorylation [25], prompting us to test whether this redox modification mitigates ITP by restraining STAT3 activity.

Spleen tissue from ITP mice showed markedly increased STAT3 S-glutathionylation in Grx1-KO animals versus WT controls (Fig. 3A). Consistently, Grx1 deletion strongly lowered p-STAT3 levels in splenic lysates (Fig. 2B), underscoring the regulatory impact of Grx1 and S-glutathionylation on STAT3 in ITP.

Since STAT3 must traffic to the plasma membrane to engage JAK2, we investigated whether S-glutathionylation influences this localization. In splenic cells, Grx1 loss reduced membrane-associated STAT3 while enhancing its nuclear presence (Fig. 3C, D). Moreover, co-localization of STAT3 with JAK2 was more pronounced in WT than in Grx1-KO cells (Fig. 3E). Together, the data imply that S-glutathionylation hampers STAT3 membrane recruitment and JAK2 coupling, thereby attenuating the JAK–STAT cascade in ITP.

STAT3 S-Glutathionylation Suppresses Th17 Development

Because STAT3 is indispensable for Th17 lineage commitment, we examined whether its S-glutathionylation alters Th17 generation in murine splenocytes. Under Th17-polarizing conditions, genetic deletion of Grx1 diminished both STAT3 phosphorylation and its transcriptional output (Fig. 4A–C). Flow-cytometric analysis mirrored this reduction, revealing fewer IL-17–producing cells in Grx1-deficient cultures (Fig. 4D). Correspondingly, key Th17 genes—Csf2, Il23r, and Rora—were significantly suppressed (Fig. 4E–G), and the lineage-defining transcription factor RORγt was expressed at lower levels (Fig. 4H). These data highlight S-glutathionylation as a potent brake on STAT3-driven Th17 programming.

In an ITP model, Grx1 ablation likewise curtailed splenic Th17 expansion (Fig. 4I). Furthermore, re-activating STAT3 with Colivelin reversed this protective effect, lowering platelet counts in Grx1-null ITP mice (Fig. 4J). Together, the results implicate STAT3 S-glutathionylation as a safeguard against ITP by restraining both STAT3 signaling and subsequent Th17 differentiation.

Discussion

Immune thrombocytopenia (ITP) is a prevalent autoimmune bleeding disorder hallmarked by low circulating platelet numbers [26]. While STAT3-dependent Th17 programming is recognized as a pivotal driver of disease, the upstream redox mechanisms that fine-tune STAT3 activity in this context remain unclear. We now report that, in Grx1-deficient mice, STAT3 becomes S-glutathionylated; this covalent modification blocks its membrane anchoring and subsequent JAK2-mediated phosphorylation. Consequently, Grx1 loss dampens STAT3 signaling, restrains Th17 development, and mitigates ITP severity.

S-glutathionylation is a dynamic, thiol-reversible adduct formed when reduced glutathione (GSH) attaches to cysteine residues; its extent is dictated by the local GSH-to-GSSG ratio [27]. Cytosolic glutaredoxin-1 (Grx1) is the principal deglutathionylating enzyme [28], and Grx1-controlled S-

glutathionylation has been implicated in disorders ranging from acute lung injury to atherosclerosis [17, 29, 30]. Previous work documented diminished GSH and elevated GSSG in ITP patients [31, 32]. In the current study, we observed a similarly decreased GSH/GSSG ratio and heightened global S-glutathionylation in these individuals. Genetic ablation of Grx1 further elevated protein S-glutathionylation and, importantly, conferred protection in an ITP model, underscoring the central role of Grx1-regulated redox signaling in the disease.

Covalent changes to proteins provide swift, switch-like control of intracellular communication; phosphorylation and ubiquitination are textbook examples. S-glutathionylation—discovered decades ago—adds glutathione to cysteines, yet its signaling relevance is still emerging. The first hint came in 2009, when glutathionylated STAT3 was reported to resist phosphorylation and to blunt downstream transcription in HepG2 cells [33]. Extending this concept, we now show that STAT3 glutathionylation similarly curbs its phosphorylation in splenocytes from mice with immune thrombocytopenia (ITP), underscoring the broader regulatory potential of this redox modification.

Multiple lines of evidence designate Th17 cells as key accelerators of ITP. Flow-cytometric analyses have consistently revealed an expanded Th17 compartment in patient peripheral blood [34, 35], and Sun et al. corroborated this in a CD41-antibody mouse model, noting splenic Th17 accumulation [36]. Thus, excessive Th17 skewing is a defining immunologic signature of the disease.

Hyperactive STAT3 is the molecular engine that drives Th17 commitment; when unchecked, it worsens immune pathology [37, 38]. Pharmacologic or genetic dampening of STAT3 has already proven effective in limiting Th17 expansion and restoring platelet counts in pre-clinical ITP [10]. While phosphatases, endogenous inhibitors, and ubiquitin ligases are known STAT3 brakes, our work identifies reversible S-glutathionylation as an additional layer of control. By restraining STAT3 activity and consequently Th17 development, Grx1-directed deglutathionylation emerges as a druggable node. Because Th17 hyperactivity is a shared driver in disorders ranging from ITP to colitis and arthritis, modulating STAT3 glutathionylation could offer therapeutic leverage across a spectrum of autoimmune diseases.

Conclusion

Heightened oxidative stress and widespread protein S-glutathionylation (PSSG) emerge as key drivers of immune thrombocytopenia. Loss of Grx1 amplifies PSSG, leading to reversible glutathione tagging of STAT3. This redox switch impedes STAT3 activation and subsequent Th17 polarization, ultimately easing disease severity. Targeting the Grx1–STAT3 glutathionylation axis could therefore offer a novel therapeutic avenue for ITP and related autoimmune conditions.

Declarations

Competing Interests

The authors affirm that no financial or non-financial conflicts of interest exist.

Ethical Clearance

All procedures were reviewed and sanctioned by both the Human Research Ethics Committee and the Laboratory Animal Care and Use Committee of the First Affiliated Hospital of Xinjiang Medical University. Written informed consent was secured from every participant or an authorized proxy prior to enrollment.

Data Availability

Underlying datasets are available from the corresponding author upon reasonable inquiry.

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Author Contributions

GM originated and directed the project; YC collected the data and assisted with data analysis; YY performed data analysis and interpretation; DL prepared the manuscript and provided substantive intellectual edits; YZ wrote the manuscript and gave final approval to the submitted version.

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Figures

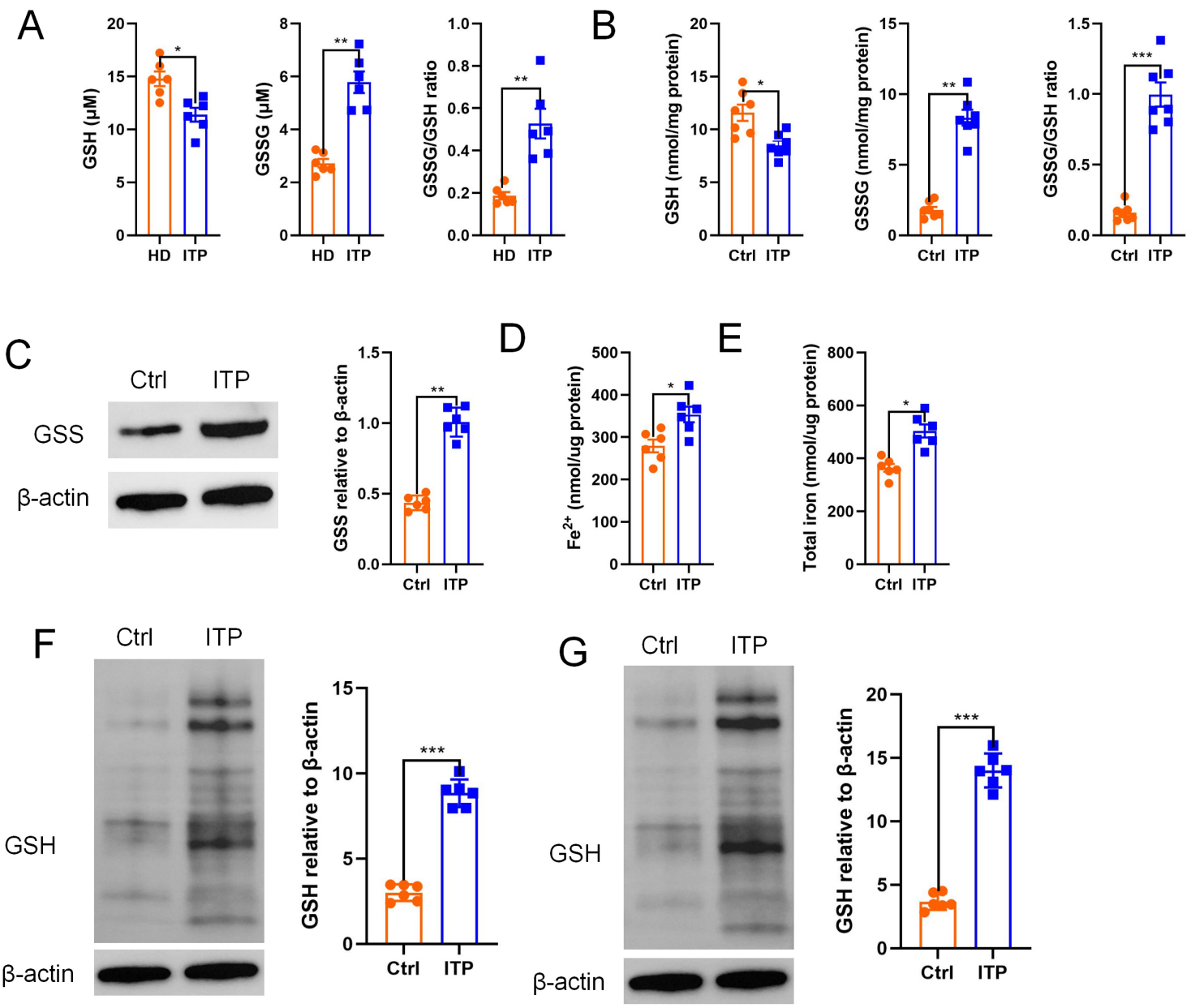


Figure 1

Heightened Redox Imbalance and Elevated Protein S-Glutathionylation in ITP

(A) Plasma GSH, GSSG and the GSSG/GSH ratio in six ITP patients versus six healthy donors (HD).

(B) Equivalent redox measurements in mouse spleen.

(C) Western blot showing up-regulated GSH synthetase (GSS) in ITP spleens.

(D, E) Intracellular Fe^{2+} and total iron content in splenocytes.

(F, G) Immunoblot detection of global protein S-glutathionylation (PSSG) in human plasma (F) and mouse spleen (G).

Bars = mean ± SEM from 2–3 experiments; t-test, *P < 0.05, **P < 0.01, ***P < 0.001.

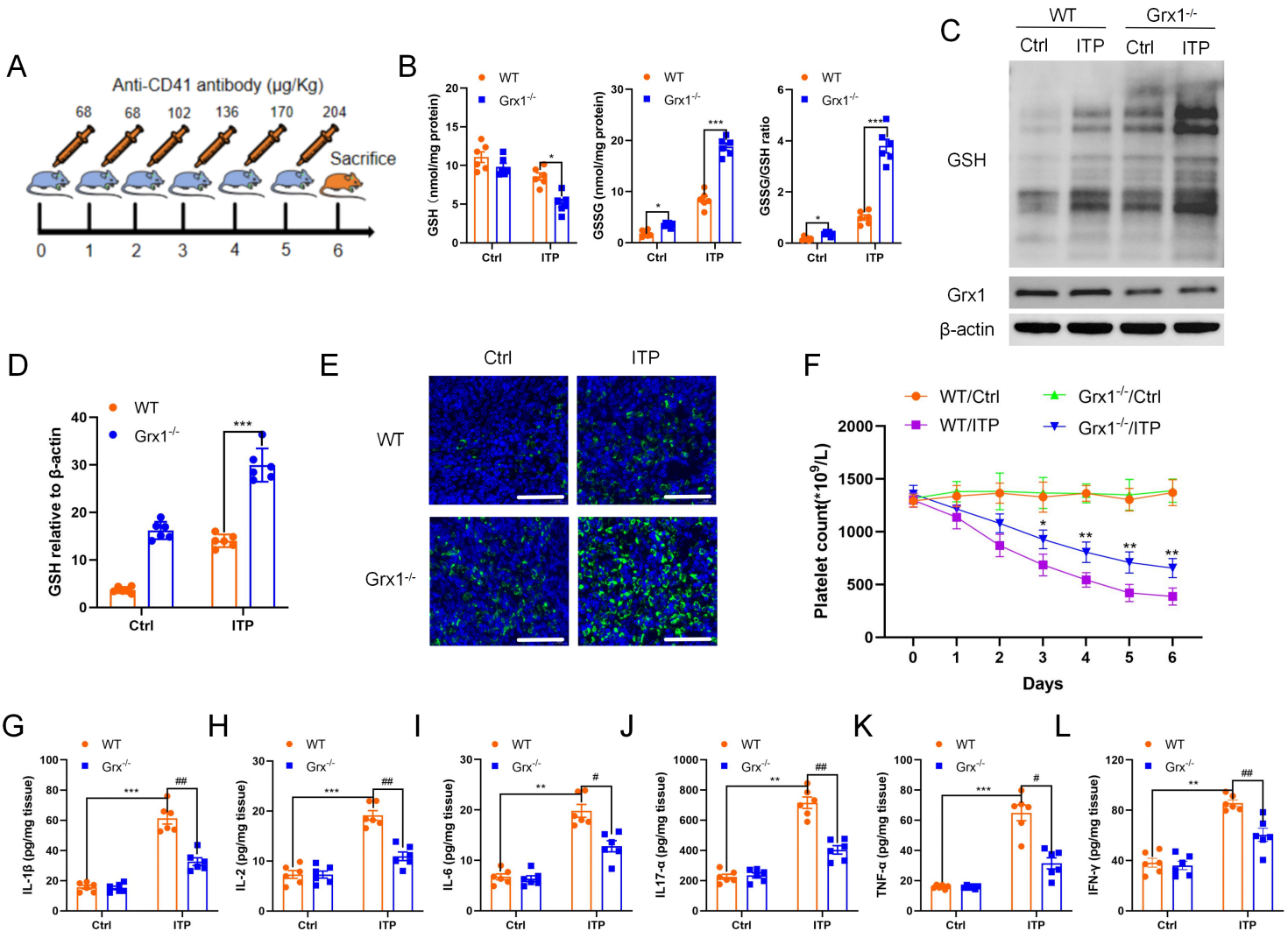


Figure 2

Grx1 Deletion Intensifies S-Glutathionylation and Mitigates ITP

(A) Schema of the passive ITP model.

(B) Spleen GSH, GSSG and GSSG/GSH ratio.

(C, D) Western blot and densitometry of PSSG in spleen lysates; Grx1-KO markedly raises global PSSG.

(E) Representative immunofluorescence of PSSG in spleen sections (50 μm scale bar).

(F) Platelet counts across groups.

(G–L) ELISA quantification of splenic inflammatory cytokines.

Bars = mean ± SEM (n = 6); *P < 0.05, **P < 0.01, ***P < 0.001; #P < 0.05, ##P < 0.01 vs indicated groups.

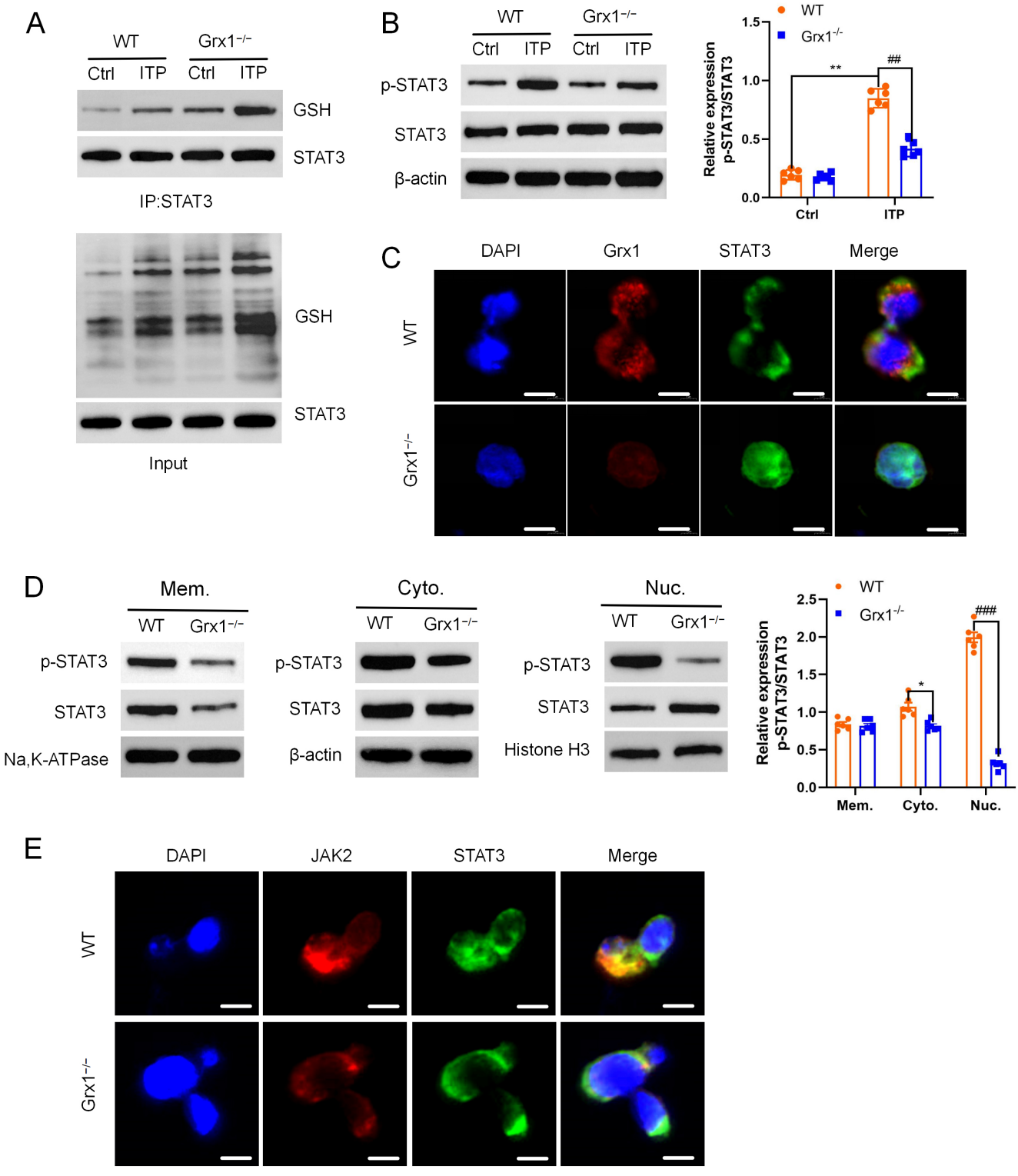


Figure 3

S-Glutathionylation Curtails STAT3 Signaling in Splenocytes

(A) Co-IP reveals elevated STAT3-S-SG adducts in Grx1-KO ITP splenocytes.

(B) Western blot of total and phosphorylated STAT3 (p-STAT3).

(C) Immunofluorescence images of STAT3 distribution (10 μm scale bar).

(D) Sub-cellular fractionation and quantification of STAT3 and p-STAT3.

(E) Confocal images of STAT3–JAK2 co-localization (10 μm scale bar).
Bars = mean ± SEM (n = 6); *P < 0.05, **P < 0.01, ##P < 0.01, ###P < 0.001.

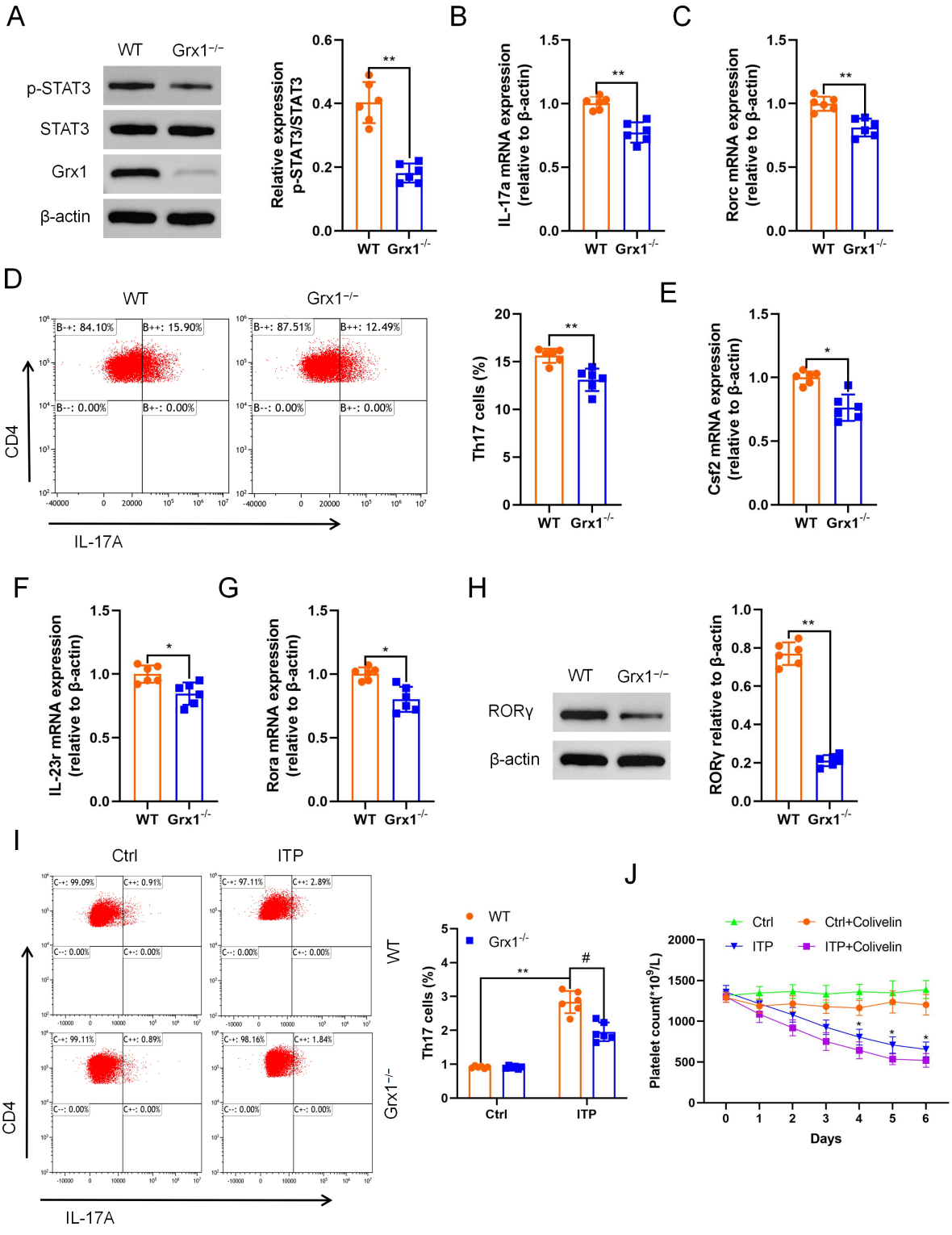


Figure 4

STAT3 S-Glutathionylation Inhibits Th17 Differentiation.

(A) Mouse splenocytes were treated with a cytokine cocktail for 4 days to induce differentiation.

Immunoblot analysis was performed to evaluate the expression of STAT3 and phosphorylated STAT3 (p-STAT3).
(B, C) Levels of IL-17a and Rorc mRNA were analyzed using RT-qPCR.
(D) Mouse splenocytes were treated with a cytokine cocktail for 4 days, and then analyzed by flow cytometry to detect CD4 and IL-17a double-positive cells (Th17 cells).
(E-G) Expression levels of Csf2, Il23r, and Rora were analyzed using RT-qPCR.
(H) Immunoblot analysis was conducted to assess the expression of RORγ.
(I) Th17 cells in mouse spleen were quantified by flow cytometry.
(J) Mice were administered STAT3 activator Colivelin (1 mg/kg) intraperitoneally, and platelet counts were compared among different groups of mice.
Data are presented as mean ± SEM from two to three independent experiments (n = 6). *P < 0.05; **P < 0.01; #P < 0.05.

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