

## **Supplementary Appendix for**

### **Enhancing regulatory T cell function via inhibition of high mobility group box 1 protein signaling in immune thrombocytopenia**

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## **Supplementary Methods**

### **Strains and backgrounds of mice for the establishment of ITP murine models**

Wild-type (WT) C57BL/6 mice (male, 8-12 weeks old) as platelet donors were obtained from the Centre for New Drug Evaluation of Shandong University (Jinan, China). C57BL/6 CD61-knockout mice (B6.129S2-Itgb3tm1Hyn/JSemJ, Stock No: 008819) were originally from Jackson Laboratory. Severe combined immunodeficient (SCID) mice with a C57BL/6 background (B6.Cg-Prkdc<sup>scid</sup>/SzJ, Stock No: 001913, 6-8 weeks old) as spleen-cell transfer recipients were purchased from Jackson Laboratory (Bar Harbor, Maine, USA).

### **Treg cell-depletion in ITP murine models**

CD4<sup>+</sup> CD25<sup>+</sup> T cells were first depleted from splenocytes of immunized CD61-knockout mice with CD61<sup>+</sup> platelets before splenocyte infusion to SCID mice using Magnetic cell sorting kits (Miltenyi Biotec, Bergisch Gladbach, Germany). The deletion efficiency was over 95% measured by flow cytometry. Splenocytes except Tregs were collected for infusion to establish the ITP murine models.

### **CD8<sup>+</sup> T cells or CD19<sup>+</sup> B cell-depletion in ITP murine models**

Splenocytes from immunized CD61-knockout mice with CD61<sup>+</sup> platelets were first depleted of either CD8<sup>+</sup> T cells, or CD19<sup>+</sup> B cells before transfer by the procedures and reagents provided by the Magnetic cell sorting kits (Miltenyi Biotec, Bergisch Gladbach, Germany). The deletion efficiency was over 95% measured by flow cytometry. Splenocytes except CD8<sup>+</sup> T cells or CD19<sup>+</sup> B cells were collected for infusion to establish the ITP murine models.

### **Detection of serum anti-platelet CD61 specific antibodies in ITP mice**

To determine the levels of anti-platelet antibodies,  $5 \times 10^6$  platelets from C57BL/6 mice were incubated with 4  $\mu$ L of undiluted serum from ITP mice(1) for 30 min at

room temperature. Next, 2  $\mu$ L of PE-conjugated goat anti-mouse IgG (BETHYL; 1/200 final) was added and incubated in the dark for 30 min at room temperature, then for detection and analysis using flow cytometry.

### **Cytokine analysis of ITP mice**

Serum samples of ITP mice at day 35 were tested for cytokines using the Meso Scale Discovery kit (Meso Scale Diagnostics, Rockville, MD, USA) following the manufacturer's instructions.

### **Cell isolation and culture from ITP patients and healthy controls**

Peripheral blood mononuclear cells (PBMCs) from ITP patients and healthy controls were isolated using Ficoll–Hypaque centrifugation (Amersham Biosciences, Piscataway, NJ, USA). Isolation of circulating CD4<sup>+</sup> T cells and naïve CD4<sup>+</sup> T cells from patients with ITP or healthy controls was performed using anti-CD4 and anti-naïve CD4 magnetic beads separation (Miltenyi Biotec, Bergisch Gladbach, Germany). The purity of the isolated cells was found to be >90% by flow cytometry.

Cells were treated with either 18 $\beta$ -GA at various concentrations (12.5, 25, 50, and 100  $\mu$ M), recombinant human HMGB1 protein (rhHMGB1, 100 ng/mL, Sigma-Aldrich, USA), or HSF1 inhibitor KRIBB11 (2 $\mu$ M) (2) for 3 days. DMSO (0.1 %) was used as the vehicle control.

The isolated cells were resuspended in RPMI-1640 medium (Life Technologies, Paisley, UK) supplemented with 10% heat-inactivated fetal bovine serum (Gibco, Grand Island, NY, USA), 1% penicillin and streptomycin (Solarbio, Beijing, China). PBMCs, (naïve) CD4<sup>+</sup> T cells and Tregs were cultured with recombinant human IL-2 (10 ng/mL, R&D Systems, Minneapolis, MN, USA), anti-human CD3 and anti-human CD28 antibodies (1  $\mu$ g/mL; eBioscience, San Diego, CA, USA). Naïve CD4<sup>+</sup> T cells were supplemented with anti-TGF $\beta$ 1 antibodies in addition (5 ng/ml, R&D Systems, Minneapolis, MN, USA).

## **Cell characterization and apoptosis**

Tregs cultured from human, or Tregs from ITP mice's single-cell suspensions were stained and detected using anti-human or anti-mouse CD4, CD25, and Foxp3 conjugated-antibodies (eBioscience) as per manufacturer's instructions. Macrophages from ITP mice were stained using anti-mouse F4/80-, CD80-, CD86-conjugated antibodies (eBioscience). For apoptosis analysis, peripheral blood mononuclear cells (PBMCs) from ITP patients were stained with FITC-Annexin V and PI using a Cell Apoptosis Kit (Bestbio, Shanghai, China). Data analysis was carried out using a FACS Calibur cytometer equipped with Kaluza Flow Cytometry Analysis Software (Beckman Coulter).

## **Suppression capacity of Tregs on CD4<sup>+</sup> CD25<sup>-</sup> effector T cells in ITP patients**

CD4<sup>+</sup> CD25<sup>-</sup> T cells (effector T cells) and CD4<sup>+</sup> CD25<sup>+</sup> CD127<sup>-</sup> Tregs were isolated from ITP patients using a CD4<sup>+</sup> CD25<sup>+</sup> CD127<sup>-</sup> Regulatory T Cell Isolation Kit (Stemcell Technologies, Vancouver, Canada) according to the manufacturer's instructions. Tregs were incubated with 25 µmol/L of 18β-GA or 0.1 % DMSO for 3 days and then the drug was eluted. CD4<sup>+</sup> CD25<sup>-</sup> T cells were labeled with CFSE (5(6)-carboxyfluorescein diacetate N-succinimidyl ester, 5 µmol/L; Sigma-Aldrich), seeded at  $2 \times 10^5$  cells/well and treated with DMSO or 18β-GA, and then co-cultured with or without Tregs at a ratio of 4:1. Samples were acquired for flow cytometry analysis after 5 days of co-culture. Data were analysed using the Flow-Jo software. Division index of effector T cells is the average number of cell divisions that a cell in the original population has undergone.(3) The lower the division index, the stronger the inhibition of Tregs on effector T cells.

## **Suppression capacity of Tregs on CD8<sup>+</sup> cytotoxic T lymphocytes induced platelet apoptosis in ITP patients**

Inhibition of Tregs towards cytotoxic T lymphocytes-induced platelet apoptosis is detailed as previously described.(4) CD8<sup>+</sup> cytotoxic T lymphocytes (CTLs) were isolated from ITP patients using anti-CD8 magnetic beads (Miltenyi Biotec, Bergisch Gladbach, Germany). Autologous platelets were prepared and used as target cells. After isolated Tregs from ITP patients treated with 18β-GA (25μM) / DMSO for 3 days, Tregs and CD8<sup>+</sup> T cells were co-cultured at a ratio of 1:4 for 5 days. Then CD8<sup>+</sup> T cells and platelets were adjusted to 10<sup>5</sup>/mL:10<sup>6</sup>/mL and co-cultured for 4 hours, and the cells were harvested to analyse the platelet apoptosis. Platelets were stained with JC-1 using mitochondrial membrane potential assay kit (Beyotime, Nantong, China). JC-1 aggregates exhibiting red fluorescence represented the live platelets and JC-1 monomer showing green fluorescence represented the apoptotic platelets. The percentage of JC-1 monomer on platelets was defined as the apoptosis rate. The level of spontaneous platelet apoptosis was determined in the tubes holding only culture media and platelets.

#### **Lentiviral interference of the expression of *HMGB1* in Tregs of ITP patients**

Tregs from ITP patients were isolated and transduced with an mCherry-tagged lentivirus encoding the human HMGB1 short hairpin RNA (shRNA) target sequence: TTCCTCTTCTGCTCTGAGTAT (Genechem, Shanghai, China). The transduced cells were sorted by Moflo Astrios EQ (Beckman Coulter). HMGB1 downregulation was measured using RT-PCR analysis. HMGB1/control shRNA–transfected Tregs treated with 18β-GA or 0.1% DMSO for 3 days were then co-culture with CD4<sup>+</sup> CD25<sup>-</sup> T cells for 5 days. Division index was analysed as described before.

#### **Quantitative real time-PCR**

The mRNA expression of Foxp3 and HMGB1 of patients and mice was measured by real-time reverse transcription polymerase chain reaction (RT-PCR) (Supplementary Table 3). Relative gene expressional levels were finally measured on a LightCycler®

480 System (Roche Applied Science, Mannheim, Germany). GAPDH was used as the internal standard. Gene expression was calculated by the  $2^{-\Delta Ct}$  method.

### **Western blotting**

Isolated CD4<sup>+</sup> T cells from ITP patients and healthy controls cultured for 3 days were extracted. Immunoblots were performed using monoclonal rabbit anti-human GAPDH, monoclonal rabbit anti-human NF- $\kappa$ B (p65) / phosphor-NF- $\kappa$ B (p65) and rabbit anti-human HSF1 (Cell Signaling Technology, USA), rabbit anti-HSF1 (phospho S326) antibody and polyclonal rabbit anti-human HMGB1 (Abcam, USA). Semi-quantitative evaluation of total protein level was analysed by calculating the ratio of the band of HMGB1 / NF- $\kappa$ B / phosphor-NF- $\kappa$ B to the housekeeping GAPDH signal.

### **Determination of acetylation level of plasma HMGB1 in ITP patients**

Plasma of ITP patients were immunoprecipitated with the HMGB1 polyclonal antibody or control IgG (Proteintech, USA) using protein A/G-magnetic beads (MedChemExpress, USA). Samples were then used for western blotting to detect the acetylation level of HMGB1 with the polyclonal rabbit anti-human HMGB1 antibody (Abcam, USA) and acetylated-lysine antibody (Cell Signaling Technology, USA).

### **Preparation of fully reduced or oxidized HMGB1**

Plasma of ITP patients and healthy controls were immunoprecipitated with the HMGB1 polyclonal antibody as described above with DSS crosslinker (MedChemExpress, USA). HMGB1 from ITP patients was exposed to either 5 mM DTT (MedChemExpress, USA) or 100 mM H<sub>2</sub>O<sub>2</sub> for 1 hours to get fully reduced or oxidized HMGB1, and dialyzed with 96-well microdialysis plates (Thermo Fisher, USA) before the addition to CD4<sup>+</sup> T cells isolated from ITP patients(5).

### **Enzyme-linked immunosorbent assay (ELISA)**

The content of HMGB1 in human and mouse plasma was determined using HMGB1 ELISA kits (OM533113, OM450958, OmnimAbs, USA) following the manufacturer's instructions.

### **Phagocytosis of macrophages in ITP patients and mice**

For ITP patients, antibody-mediated phagocytosis assays were performed as described(6) with slight modifications. CD14<sup>+</sup> monocytes from ITP patients are isolated and induced to macrophages by phorbol 12-myristate 13-acetate (PMA, Multisciences, China) for 1 hour, and stimulated with 18 $\beta$ -GA or 0.1% DMSO for 24 hours. Platelets from healthy controls were incubated with CD41 monoclonal antibody or an isotype control antibody (eBioscience, USA), and labeled with 5-chloromethylfluorescein diacetate (CMFDA, Invitrogen). Then monocyte-derived macrophages were incubated with CD41 or isotype control antibody-opsonized platelets at 37 °C for 1 hour at a 1: 10 ratio (macrophages: platelets). APC-anti human CD61-conjugated antibody was used to label the platelets that were not devoured by macrophages. The phagocytic index was calculated as the mean fluorescence intensity (MFI) of CMFDA obtained in the group of CD41-sensitized platelets divided by the MFI of CMFDA in the group of isotype control antibody-sensitized platelets by flow cytometry. Furthermore, the platelet retention could be visualized by immunofluorescence imaging using the EVOS™ FL Auto 2 Imaging System (Thermo Fisher) under 200 $\times$  magnification, and the value of endocytic platelet numbers divided by macrophage numbers was counted using Imagepro Plus 6.0 software.

For ITP mice, spleens and livers were removed to prepare single cell suspensions at day 35. Cells from spleen and liver were incubated with CD41 antibody or an isotype control antibody (eBioscience, USA)-opsonized platelets at 37 °C for 1 hour at a 1: 10 ratio (macrophages: platelets). APC-anti mouse CD61 antibody was used to label the platelets that were not devoured by macrophages. The phagocytic index of spleen and liver in ITP mice was calculated as the MFI of CMFDA in the group of CD41-

opsonized platelets divided by the that in the group of an isotype control antibody-opsonized platelets by flow cytometry.

### **Lentiviral interference of *HMGB1* in monocyte-derived macrophages of ITP patients**

Macrophages from ITP patients were transfected in vitro with lentivirus encoding the human HMGB1 shRNA and sorted by Moflo Astrios EQ. HMGB1 downregulation was measured using RT-PCR analysis. HMGB1/control shRNA-transfected macrophages treated with 18 $\beta$ -GA or 0.1% DMSO for 24 hours were then co-culture with CD41-sensitized platelets. Phagocytic index was analysed as described above.

### **Platelet retention analysis using living liver and spleen imaging of ITP mice**

Platelets from wild-type C57BL/6 mice were incubated with 1,1'-Dioctadecyl-3,3,3',3'-Tetramethylindotricarbocyanine Iodide (DIR, Sigma-Aldrich, USA), and injected into two groups of ITP mice at day 35 described as before(7) via the caudal vein. Mice were euthanized, and their spleens and livers were removed 30 min after injection. Platelet retention analysis was assessed by total radiant efficiency (TRE) at the region of interest (ROI) with an in vivo living imaging system (PerkinElmer, Boston, US) of liver and spleen.

### **Statistical analysis**

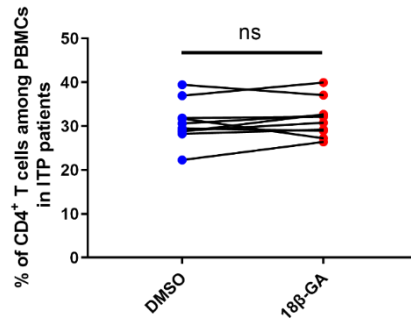
Data are reported as the mean  $\pm$  SD or median with range. One-Way ANOVA, Two-Way ANOVA, and Friedman's test were performed for comparisons among the groups. Differences between the control and drug treatment groups were determined using Student's t test and Wilcoxon matched-pairs signed rank test. Correlation analysis was used to evaluate the relationship between platelets and HMGB1. Statistical analyses were performed using SPSS 23. A *P* values < 0.05 was considered statistically significant.



## Supplementary Figure 1

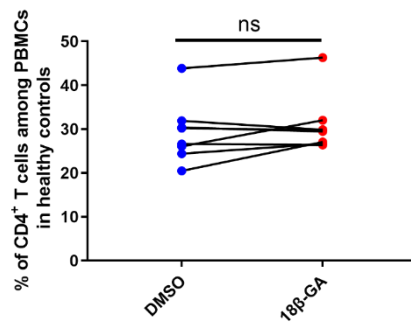
The percentages of CD4<sup>+</sup> T cells among the PBMCs remained unchanged after 18 $\beta$ -GA treatment in ITP patients and healthy controls.

A



The percentages of CD4<sup>+</sup> T cells among the PBMCs remained unchanged after treated with 18 $\beta$ -GA at the dose of 25  $\mu$ M in ITP patients. Significance between the two groups of ITP patients was determined by Paired t test. n=10.

B

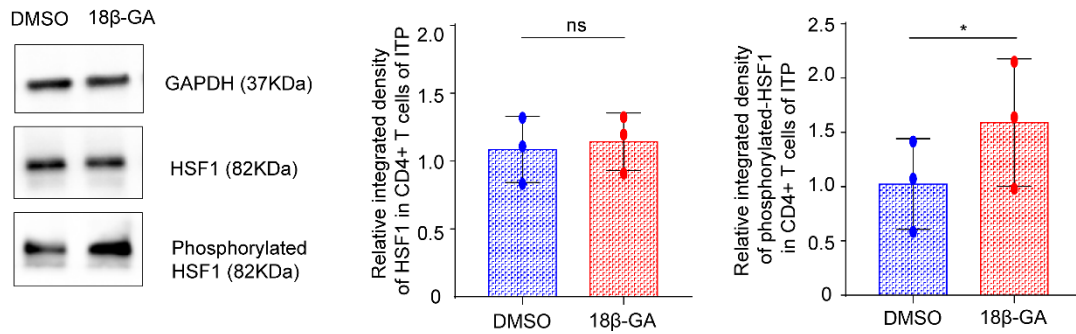


The percentages of CD4<sup>+</sup> T cells among the PBMCs remained unchanged after treated with 18 $\beta$ -GA at the dose of 25  $\mu$ M in healthy controls. Significance between the two groups of healthy controls was determined by Wilcoxon matched-pairs signed rank test. n=8.

## Supplementary Figure 2

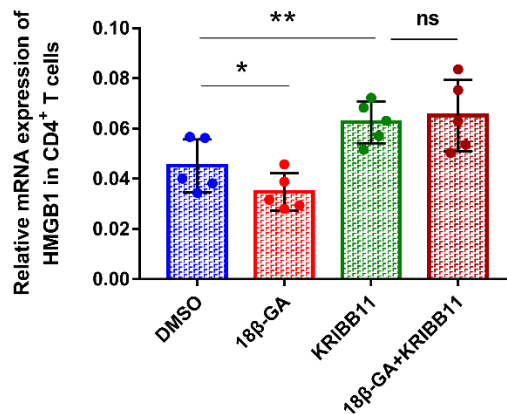
### 18 $\beta$ -GA inhibited the mRNA expression of HMGB1 via phosphorylation of HSF1.

**A**



The phosphorylation level of HSF1 in CD4<sup>+</sup> T cells of ITP patients was significantly increased after 18 $\beta$ -GA treatment for 3 days. Significance between the two groups was determined by Paired t test. n=3. \*P=0.0286.

**B**

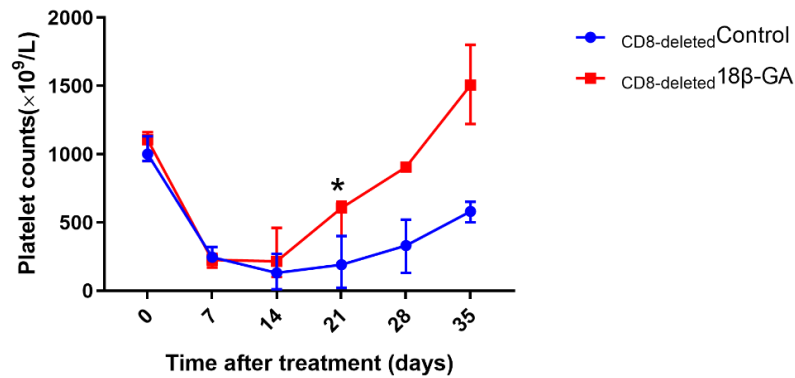


The mRNA expression of HMGB1 was significantly increased after the addition of HSF1 inhibitor KRIBB11 for 3 days, and the effect of KRIBB11 could not be reversed by 18 $\beta$ -GA. Significance between the two groups was determined by One-way ANOVA. n=5. \*\*P=0.0055. Multiple comparisons: DMSO vs. 18 $\beta$ -GA, \*P=0.0372; DMSO vs. KRIBB11, \*\*P=0.0025.

### Supplementary Figure 3

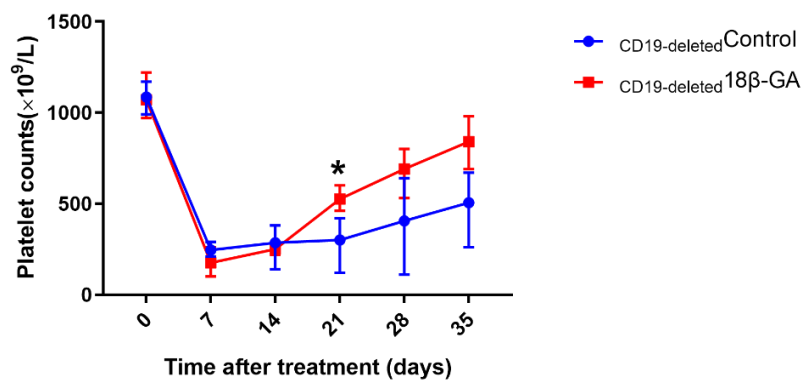
**18 $\beta$ -GA was still capable to raise platelet counts in ITP mice after CD19<sup>+</sup> B / CD8<sup>+</sup> T cell-depletion.**

**A**



Platelet counts increased in the 18 $\beta$ -GA group compared with those in the control group in mice receiving CD8<sup>+</sup> T cell-depleted splenocytes. Significant difference between groups emerged on day 21. The lines denoted the median (with range) in each group as the data is not normally distributed. Significance among groups was determined by two-way ANOVA. n=4 for each group. \*P=0.0424.

**B**



Platelet counts increased in the 18 $\beta$ -GA group compared with those in the control group in mice receiving CD19<sup>+</sup> B cell-depleted splenocytes. Significant difference between

groups emerged on day 21. The lines denoted the median (with range) in each group as the data is not normally distributed. Significance among groups was determined by two-way ANOVA. n=4 for each group. \*P=0.0440.

**Supplementary Table 1. The key clinical information of ITP patients.**

| Patient Number | Sex | Age (years) | Platelet counts ( $\times 10^9/L$ ) | Symptoms            | Antiplatelet antibodies |              | Bleeding score(8, 9) |
|----------------|-----|-------------|-------------------------------------|---------------------|-------------------------|--------------|----------------------|
|                |     |             |                                     |                     | Anti-GPIIb/IIIa         | Anti-GPIb/IX |                      |
| 1              | F   | 47          | 19                                  | FT, PT, EC          | -                       | -            | 1                    |
| 2              | F   | 59          | 4                                   | GIH                 | +                       | +            | 3                    |
| 3              | F   | 27          | 30                                  | EP, GH              | -                       | -            | 2                    |
| 4              | F   | 46          | 30                                  | EC                  | -                       | -            | 1                    |
| 5              | F   | 25          | 14                                  | None                | +                       | -            | 0                    |
| 6              | M   | 66          | 9                                   | FT, EP, OMH         | ND                      | ND           | 3                    |
| 7              | M   | 60          | 12                                  | EC, GH              | -                       | -            | 3                    |
| 8              | F   | 44          | 16                                  | PT                  | -                       | -            | 1                    |
| 9              | F   | 54          | 5                                   | PT, EC              | ND                      | ND           | 1                    |
| 10             | F   | 48          | 38                                  | HM                  | -                       | -            | 3                    |
| 11             | F   | 56          | 12                                  | None                | -                       | +            | 0                    |
| 12             | M   | 23          | 2                                   | OMH                 | -                       | -            | 2                    |
| 13             | M   | 49          | 10                                  | None                | +                       | -            | 0                    |
| 14             | F   | 65          | 4                                   | EC, GH              | ND                      | ND           | 4                    |
| 15             | F   | 49          | 1                                   | PT, EP, GH, GIH, HM | +                       | +            | 3                    |
| 16             | M   | 48          | 25                                  | PT, EC              | -                       | -            | 1                    |
| 17             | F   | 59          | 23                                  | None                | -                       | +            | 0                    |
| 18             | M   | 24          | 6                                   | PT, GH              | -                       | -            | 2                    |
| 19             | F   | 27          | 12                                  | None                | ND                      | ND           | 0                    |
| 20             | F   | 18          | 17                                  | None                | -                       | +            | 0                    |
| 21             | F   | 53          | 1                                   | PT, EC              | -                       | -            | 1                    |
| 22             | F   | 53          | 3                                   | PT, EC              | ND                      | ND           | 1                    |
| 23             | F   | 33          | 6                                   | None                | -                       | -            | 0                    |
| 24             | F   | 63          | 23                                  | PT, EC, GH          | -                       | -            | 3                    |
| 25             | M   | 40          | 31                                  | PT                  | +                       | -            | 1                    |
| 26             | M   | 20          | 2                                   | GH                  | -                       | -            | 2                    |
| 27             | M   | 23          | 21                                  | PT, EP, GH          | -                       | +            | 2                    |
| 28             | F   | 55          | 26                                  | GH                  | -                       | -            | 2                    |
| 29             | F   | 52          | 1                                   | PT, OMH             | ND                      | ND           | 2                    |

|    |   |    |    |            |    |    |   |
|----|---|----|----|------------|----|----|---|
| 30 | F | 32 | 1  | PT, EP, HM | +  | +  | 3 |
| 31 | M | 64 | 11 | EP, OMH    | -  | -  | 2 |
| 32 | F | 33 | 13 | PT         | +  | +  | 2 |
| 33 | M | 62 | 21 | EC, GH     | ND | ND | 2 |
| 34 | M | 53 | 31 | None       | +  | -  | 0 |
| 35 | F | 52 | 20 | EC, EP     | -  | -  | 2 |
| 36 | M | 45 | 13 | EC         | -  | -  | 1 |
| 37 | M | 51 | 19 | PT, GH     | -  | -  | 2 |
| 38 | M | 44 | 33 | GH         | ND | ND | 2 |
| 39 | M | 40 | 32 | PT, EC     | -  | -  | 1 |
| 40 | F | 39 | 6  | EC         | -  | -  | 1 |
| 41 | F | 34 | 38 | PT, GH, HM | +  | -  | 3 |
| 42 | M | 26 | 3  | PT, OMH    | ND | ND | 2 |

F, female; M, male; FT fatigue; PT petechiae; EC ecchymoses; GIH gastrointestinal hemorrhage; EP epistaxis; GH gingival hemorrhage; OMH oral mucosal hemorrhage; HM hypermenorrhea; ND, not determined;

**Supplementary Table 2. Effect of 18 $\beta$ -GA on cytokines in ITP mice.**

| Cytokines (pg/ml) | Control group            | 18 $\beta$ -GA group       | P       |
|-------------------|--------------------------|----------------------------|---------|
| IFN- $\gamma$     | 3.02 $\pm$ 0.29          | 1.64 $\pm$ 0.92            | 0.0288* |
| IL-10             | 9.33 $\pm$ 6.08          | 13.38 $\pm$ 11.15          | 0.5469  |
| IL-1 $\beta$      | 2.54 $\pm$ 0.72          | 3.09 $\pm$ 0.91            | 0.3782  |
| IL-6              | 202.59 $\pm$ 20.94       | 74.04 $\pm$ 55.11          | 0.0048* |
| TNF- $\alpha$     | 25.02 $\pm$ 6.56         | 14.96 $\pm$ 4.17           | 0.0414* |
| IL-12/IL-23p40    | 4197.77 $\pm$ 862.20     | 2444.46 $\pm$ 991.72       | 0.0371* |
| IL-17a            | 0.62 $\pm$ 0.53          | 0.54 $\pm$ 0.47            | 0.8203  |
| TGF- $\beta$      | 43,264.27 $\pm$ 9,127.85 | 104,416.71 $\pm$ 10,161.79 | 0.0001* |

Data are expressed as mean $\pm$ SD. Unpaired t test, n=4 in each group.

### Supplementary Table 3. Quantitative real time-PCR

|              | Forward                      | Reverse                       |
|--------------|------------------------------|-------------------------------|
| <b>Human</b> |                              |                               |
| <i>FOXP3</i> | 5' - GTGGCCCCGGATGTGTGAAG-3' | 5' -GGAGCCCTTGTCGGATGATG-3'   |
| <i>HMGB1</i> | 5' - AGCGAACAGCAGGGTTAGTG-3' | 5' -TCTAAATTGCCTCCCACTTCCA-3' |
| <i>GAPDH</i> | 5' -GCACCGTCAAGGCTGAGAAC-3'  | 5' -TGGTGAAGACGCCAGTGGA-3'    |
| <b>Mice</b>  |                              |                               |
| <i>HMGB1</i> | 5' -AGGCTGACAAGGCTCGTT-3'    | 5' -GATTTTGGGGCGGTACTCAGA-3'  |
| <i>GAPDH</i> | 5' -TGTCTCCTGCGACTTCAACA-3'  | 5' -GGTGGTCCAGGGTTTCTTACT-3'  |

### References

1. Li X, Wang SW, Feng Q, et al. Novel Murine Model of Immune Thrombocytopaenia through Immunized CD41 Knockout Mice. *Thrombosis and haemostasis*. 2019;119(3):377-383. eng. Epub 2019/01/11. doi:10.1055/s-0038-1677032. Cited in: Pubmed; PMID 30630213.
2. Tharp KM, Higuchi-Sanabria R, Timblin GA, et al. Adhesion-mediated mechanosignaling forces mitohormesis. *Cell metabolism*. 2021;33(7):1322-1341.e1313. eng. Epub 2021/05/22. doi:10.1016/j.cmet.2021.04.017. Cited in: Pubmed; PMID 34019840.
3. Coughlin JJ, Stang SL, Dower NA, Stone JC. RasGRP1 and RasGRP3 regulate B cell proliferation by facilitating B cell receptor-Ras signaling. *Journal of immunology (Baltimore, Md : 1950)*. 2005;175(11):7179-7184. eng. Epub 2005/11/23. doi:10.4049/jimmunol.175.11.7179. Cited in: Pubmed; PMID 16301621.
4. Zhou H, Qiu JH, Wang T, et al. Interleukin 27 inhibits cytotoxic T-lymphocyte-mediated platelet destruction in primary immune thrombocytopenia. *Blood*. 2014;124(22):3316-3319. eng. Epub 2014/10/10. doi:10.1182/blood-2014-06-580084. Cited in: Pubmed; PMID 25298039.
5. Venereau E, Casalgrandi M, Schiraldi M, et al. Mutually exclusive redox forms of HMGB1 promote cell recruitment or proinflammatory cytokine release. *The Journal of experimental medicine*. 2012;209(9):1519-1528. eng. Epub 2012/08/08. doi:10.1084/jem.20120189. Cited in: Pubmed; PMID 22869893.
6. Liu XG, Ma SH, Sun JZ, et al. High-dose dexamethasone shifts the balance of stimulatory and inhibitory Fcγ receptors on monocytes in patients with primary immune thrombocytopenia. *Blood*.

2011;117(6):2061-2069. eng. Epub 2010/12/07. doi:10.1182/blood-2010-07-295477. Cited in: Pubmed; PMID 21131591.

7. Zhao Y, Xu P, Guo L, et al. Tumor Necrosis Factor- $\alpha$  Blockade Corrects Monocyte/Macrophage Imbalance in Primary Immune Thrombocytopenia. *Thrombosis and haemostasis*. 2021. eng. Epub 2021/01/21. doi:10.1055/s-0040-1722186. Cited in: Pubmed; PMID 33469903.

8. Liu XG, Bai XC, Chen FP, et al. Chinese guidelines for treatment of adult primary immune thrombocytopenia. *International journal of hematology*. 2018;107(6):615-623. doi:10.1007/s12185-018-2445-z. Cited in: Pubmed; PMID 29619624.

9. [Consensus of Chinese experts on diagnosis and treatment of adult primary immune thrombocytopenia (version 2016)]. *Zhonghua xue ye xue za zhi = Zhonghua xueyexue zazhi*. 2016;37(2):89-93. chi. Epub 2016/03/26. doi:10.3760/cma.j.issn.0253-2727.2016.02.001. Cited in: Pubmed; PMID 27014975.