Single-cell RNA sequencing reveals heterogeneity of mucosa-associated invariant T cells in donor grafts and its diagnostic relevance in gastrointestinal graft-*versus*-host disease

Mengge Gao,[1] Kainan Zhang,[1] Siqi Li,[1] Huidong Guo,[1] Yuqian Sun,[1] Jun Kong,[1] Tingting Han,[1] Yingjun Chang,[1,2] Xiaojun Huang[1,2,3,4] and Xiaosu Zhao[1,2,4]

[1]Peking University People's Hospital, Peking University Institute of Hematology, National Clinical Research Center for Hematologic Disease, Beijing Key Laboratory of Cell and Gene Therapy for Hematologic Malignancies, Peking University; [2]Research Unit of Key Technique for Diagnosis and Treatments of Hematologic Malignancies, Chinese Academy of Medical Sciences, 2019RU029; [3]Peking-Tsinghua Center for Life Sciences, Academy for Advanced Interdisciplinary Studies, Peking University and [4]Collaborative Innovation Center of Hematology, Peking University, Beijing, China

Correspondence: X. Zhao
zhao.xiaosu@outlook.com

Received: December 28, 2024.
Accepted: July 22, 2025.
Early view: July 31, 2025.

https://doi.org/10.3324/haematol.2024.287114

Abstract

Granulocyte colony-stimulating factor (G-CSF) enhances acute graft-*versus*-host disease (aGVHD) prophylaxis in allogeneic hematopoietic stem cell transplantation (allo-HSCT) by inducing T-cell tolerance and altering graft cell composition. Previous studies have shown that the number of mucosa-associated invariant T (MAIT) cells in G-CSF-induced graft was associated with a low incidence of gut aGVHD. However, the effect of G-CSF mobilization on MAIT cell and its role in MAIT-mediated protection against gut GVHD remain unclear. Here, using single-cell RNA sequencing, we found that the interaction of G-CSF with its receptor CSF3R enhances immunosuppression and tissue repair functions of MAIT cells, contributing to the anti-gut aGVHD effect. The chemokine receptor CXCR6 was identified as potentially crucial for recruiting these functional MAIT cells to gut tissues. Furthermore, we simulated the dynamic distribution of MAIT cells from donor G-CSF-mobilized peripheral blood stem cells (G-PBSC) grafts in recipient mouse, and further confirmed that the circulating MAIT cells migrated into gut tissue in a CXCR6-CXCL16-dependent manner. To further validate these findings, we developed a flow cytometry panel that effectively predicts the gut aGVHD occurrence following allo-HSCT by analyzing the frequency and functional markers of MAIT cells in G-PBSC. The predictive model shifts aGVHD prediction and intervention to the pre-transplant stage and offers a new strategy for the prevention and management of gut aGVHD in clinical practice.

Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is an effective treatment for hematological malignancies. However, graft-*versus*-host disease (GVHD) remains a major obstacle, leading to transplantation failure and reduced overall survival. The overactivation of donor-derived T cells is recognized as a primary instigator of GVHD.[1-2] Granulocyte colony-stimulating factor (G-CSF) is widely used to mobilize hematopoietic stem/progenitor cells and exerts immunomodulatory effects in allo-HSCT, particularly in allo-HSCT strategies without T-cell depletion. G-CSF promotes T-cell tolerance and alters graft cell composition, which can help to prevent GVHD.[3-5] In healthy donors, G-CSF administration induces a shift in T cells from a Th1 to Th2 phenotype while preserving the graft-*versus*-leukemia effect.[4-6] Additionally, G-CSF modulates graft composition by promoting immune tolerance, suppressing NK cell activity, and expanding regulatory T cells.[5-7] However, its impact on unconventional T cells, particularly mucosal-associated invariant T (MAIT) cells, is poorly understood.

MAIT cells, a subset of multifunctional innate-like T cells, are abundant in human blood (1-10% of T cells) and mucosal tissues, including the gut (10%), lungs, and liver (20-40%).[8-11] A distinctive feature of MAIT cells is their recognition of the non-polymorphic MR1 molecule presenting riboflavin metabolite derivatives such as 5-OP-RU.[12,13] Once activated, MAIT cells exhibit both conventional effector and tissue-reparative functions.[14-17] This dual role highlights their importance in protecting mucosal surfaces from microbial threats.[17] In mice, MAIT cells differentiate into T-bet$^+$ MAIT1 and RORγt$^+$ MAIT17 subsets, which contribute to infection control and tissue repair, respectively.[10,18-23] Our previous work shows that MAIT cells in grafts are involved in suppressing gut aGVHD,

potentially via interactions with specific gut microbiota. Furthermore, G-CSF mobilization enhances the expression of RORγt in MAIT cells from donor peripheral blood stem cell (PBSC) grafts.[24] However, how G-CSF mobilization affects graft-derived MAIT cell function in recipients and their role in regulating gut GVHD is still unclear.

In adult human blood, MAIT cells express tissue-homing chemokine receptors such as CCR5, CCR6, CXCR6, and CCR9.[14-17] Increasing evidence suggests that chemotaxis is critical for MAIT cell-mediated antimicrobial and tissue repair.[25,26] Studies have shown that MAIT cells can be recruited from circulation to inflamed tissues, such as skin wounds.[12] In the context of allo-HSCT, the reconstruction of MAIT cells in recipients largely depends on their abundance in grafts, due to the pre-transplant myeloablative conditioning regimen.[21,27,28] Therefore, both the tissue migration capability and the abundance of MAIT cells in target tissues are crucial for mucosal protection and the prevention of gut aGVHD. However, the dynamics of MAIT cell distribution and their chemotactic heterogeneity during allo-HSCT are not well understood.

To investigate the role of G-CSF in the anti-gut aGVHD effects of MAIT cells, we integrated single-cell RNA sequencing (scRNA-seq) with *in vitro* and *in vivo* analyses. G-CSF-induced MAIT (G-MAIT) cells exhibited a transcriptional shift toward immunosuppressive and reparative profiles. In murine models, donor-derived G-MAIT cells showed dynamic tissue distribution and chemotactic targeting. These findings reveal a G-CSF-driven mechanism underlying MAIT cell-mediated protection against gut aGVHD and highlight their potential as biomarkers and therapeutic targets in allo-HSCT.

Methods

Human samples

Peripheral blood (PB) samples were obtained from four healthy donors before and after G-CSF administration (filgrastim, 5 μg/kg/day for 5 days) for scRNA-seq (*Online Supplementary Table S1*). For multiparameter flow cytometry (FCM), the PB of 16 healthy donors was collected before and after G-CSF administration. A clinical cohort of 80 G-CSF-mobilized PBSC grafts (G-PBSC) was also analyzed (*Online Supplementary Tables S2, S3*) (see the *Online Supplementary Appendix*).

All procedures were approved by the Ethics Committee of Peking University People's Hospital (2018PHB222-01), and informed consent was obtained per the Declaration of Helsinki.

Mucosa-associated invariant T-cell isolation and single-cell RNA analysis

MAIT cells ($CD3^+CD161^{hi}V\alpha7.2^+$) were isolated from PBMC before and after G-CSF via fluorescence-activated cell sorting (FACS). scRNA-seq libraries were generated using 10x Genomics and sequenced on the Illumina NovaSeq 6000. Data were processed using Cell Ranger (v6.0.2) and analyzed with Seurat (v3.2.2)[29] in R. UMAP was used for dimensionality reduction and canonical markers for cluster annotation. Differential expression was analyzed using the Wilcoxon rank-sum test with Benjamini–Hochberg correction. Gene set enrichment analysis (GSEA) was performed using clusterProfiler (v4.10.0)[30] (see the *Online Supplementary Appendix*).

Signature scoring

Gene set scores were calculated using Seurat's AddModuleScore. Gene sets included immunosuppression (61 genes),[31,32] cytotoxicity (*GZMA, GZMB, NKG7, IFNG, KLRD1, FGFBP2, GNLY*) and tissue repair genes (*VEGFB, VEGFA, IL17A, IL17RE, TGFB1, CCL3, FURIN, AREG, CSF1, TNF)*[20] (details in *Online Supplementary Table S4*).

Flow cytometry

Flow cytometry (FACS Canto II, BD Biosciences) assessed MAIT cell frequency and function. Antibodies included CD45, CD3, CD161, Vα7.2, CCR6, CXCR6, CD114, IL-17A, IL-4, IFN-γ (details in *Online Supplementary Table S5*).

In vitro suppression and cytotoxicity assays

PBMC from donors were cultured with or without G-CSF (2 μL/well, 24 hours [h]), followed by sorting of MAIT and $CD4^+CD25^-$ T cells. CFSE-labeled $CD4^+$ T cells were co-cultured with MAIT cells at 1:1, 2:1, 4:1 in RPMI-1640 with CD3/CD28 beads for 96 h, followed by proliferation assessment via FCM. For cytotoxicity, G-CSF–treated or untreated MAIT cells were co-cultured with K562 cells (1:1) under IL-12/IL-18 and CD3/CD28 stimulation. Apoptosis was evaluated by Annexin V/7-AAD staining (see the *Online Supplementary Appendix*).

Graft-*versus*-host disease mouse model and CXCL16 blockade

NOD-SCID-IL2Rγ-/- (NSG) mice received 180 cGy irradiation followed by 2×10^6 human G-PBSC to establish xenogeneic GVHD. Mice received intraperitoneal injections of anti-CXCL16 antibody (100 μg) or PBS twice in the first week post-transplant. Survival, body weight, and GVHD scores (0-6) were monitored. Intestinal tissues were analyzed by immunofluorescence for CD3, CD4, CD8, and CD161 (see the *Online Supplementary Appendix*).

Diagnosis of acute graft-*versus*-host disease and predictive score calculation

Gut aGVHD was diagnosed clinically and histologically per established criteria.[33,34] MAIT cells and their subsets ($CD114^+$, $IL\text{-}17A^+$, $IL\text{-}4^+$, $CXCR6^+$) were quantified by FCM. Bootstrap resampling established median thresholds for each subset. Binary scores (1 or 0) per subset were summed into

a composite predictive score (range, 0-5) (see the *Online Supplementary Appendix*).

Statistical analyses

Data are presented as mean ± standard error of the mean. Continuous variables were compared using the Mann-Whitney U test or one-way ANOVA, as appropriate. Cumulative incidences were compared using Gray's test. *P* values <0.05 were considered significant. Analyses were performed using GraphPad Prism 9.0 and SPSS v23 (Chicago, IL, USA).

Results

Granulocyte colony-stimulating factor induces MAIT cells to enhance immunosuppression and tissue repair

We first assessed the impact of G-CSF on MAIT cells via FCM (*Online Supplementary Figure S1A)*. No significant change in MAIT cell frequency was observed in PB of donors before and after G-CSF mobilization (Figure 1A). Considering IFN-γ, IL-4, and IL-17A as hallmark effector cytokines of Th1, Th2, and Th17 cells, respectively, we evaluated their expression in MAIT cells. G-MAIT cells exhibited a marked increase in IL-4 expression relative to IFN-γ and IL-17A (Figure 1B; *Online Supplementary Figure S1B*), indicating G-CSF-induced type 2 polarization and enhanced immune tolerance, consistent with previous reports of G-CSF-mediated T-cell polarization.[2]

To better characterize G-MAIT cells, we collected paired PB samples from four healthy donors before and after 5-day G-CSF treatment. Using FACS, we isolated MAIT cells for subsequent scRNA-seq (Figure 1C). After quality control, 80709 MAIT cells were clustered into 14 clusters reflecting functional heterogeneity (Figure 1D; *Online Supplementary Table S6*). Notably, cluster 7 was enriched for cytotoxicity-related genes (*NKG7*, *IFNG*, *GZMB*), cluster 5 expressed naïve markers (*CCR7*, *SELL*, *LEF1*), and cluster 13 displayed a proliferative signature (*MKI67*, *STMN1*) (*Online Supplementary Figure S1C*; *Online Supplementary Table S6*).

Contrary to murine MAIT cell subsets, human MAIT1 and MAIT17 cells demonstrated a mixed expression of *TBX21* and *RORC* at transcript and protein levels (*Online Supplementary S1D*), corroborating prior studies.[8,9] Although distinct Th1, Th2 and Th17 phenotypes were not isolated, G-MAIT cells showed upregulation of genes related to Th2 differentiation (IL-4 and IL-13 signaling) (Figure 1E) and a higher G-CSF score (Figure 1F) compared to MAIT cells without G-CSF administration, this suggested that G-CSF induces IL-4-driven polarization and enhances immune tolerance. Functional analyses revealed that G-MAIT cells exhibited enhanced immunosuppressive and tissue repair gene signatures, accompanied by reduced cytotoxic profiles (Figure 1G). G-CSF also enhanced expression of migration and tissue residency markers (*Online Supplementary Figure S1E*), supporting their role in tissue repair. Furthermore, G-MAIT cells showed decreased expression of cytotoxic genes (*NKG7*, *GNLY*, *FGFBP2*, *GZMA*), increased expression of naïve-associated genes (*SELL*, *LEF1*) (*Online Supplementary Figure S1F*). Immune checkpoint molecules (*PDCD1*, *TIGIT*, *LAG3*, *CTLA4*), proliferation markers (*MKI67*, *CDK1*), and co-stimulatory molecules (*CD28*, *TNFRSF9*, *TNFRSF14*) were also upregulated, though FCM results were not statistically significant (*Online Supplementary Figure S2A*).

To validate the immunoregulatory capacity of G-MAIT cells, *in vitro* co-culture assays with $CD4^+$ T cells were performed. G-MAIT cells exhibited significantly stronger suppression of $CD4^+$ T-cell proliferation compared to controls at MAIT/CD4 ratios of 1:1 (*P*=0.023) and 4:1 (*P*=0.043) (Figure 1H). Cytotoxicity against K562 cells under IL-12/IL-18 or CD3/CD28 stimulation was comparable between G-MAIT and control MAIT cells (*Online Supplementary Figure S2B*). These results demonstrate that G-CSF enhances MAIT cell immunoregulatory capacity without impairing cytotoxic function.

G-CSF-CSF3R interaction mediates the functions of MAIT cells

To elucidate how G-CSF modulates MAIT cell function, we performed differentially expressed gene (DEG) analysis, which revealed upregulation of genes associated with immunosuppression (*NFKBIA*, *ZFP36*, *DUSP2*),[31,38,39] Th2 developmental (*JUNB* and *MT2A*)[31] and IL-17 signaling (*JUN* and *FOS*), as well as tissue residency (*CD69*, *H3F3B*, *JUN*, *TNFAIP3*) following G-CSF mobilization (Figure 2A; *Online Supplementary Figure S3A*) (details in *Online Supplementary Table S7*).[40,41] Notably, these genes were enriched in cluster 12, characterized by high expression of *CSF3R* (G-CSF receptor), which the proportion and G-CSF score of cluster 12 significantly increased after G-CSF mobilization (Figure 2B; *Online Supplementary Figure S3B*). These findings suggested that G-CSF could mediate MAIT cell function through CSF3R of cluster 12.

Previous studies have shown that *G-CSF*, by binding to G-CSFR, directly regulate downstream targeted genes related to anti-inflammation and immunosuppression (*ZFP36* and *DUSP2*).[31] Consistently, *CSF3R* and its targets were upregulated after G-CSF stimulation (Figure 2C). Cluster 12 exhibited a stronger immunosuppressive signature than other clusters (*Online Supplementary Figure S3C*), suggesting that its hyporesponsiveness may result from the direct G-CSF–CSF3R signaling. GSEA of cluster 12 showed the enrichment of IL-17 and TNF signaling pathway related to tissue repair (Figure 2D; *Online Supplementary Figure S3D*), supporting that cluster 12 may be involved in tissue repair of G-MAIT cells. Additionally, downregulation of the GVHD pathway (Figure 2D; *Online Supplementary Figure S3D*) supports a role for the G-CSF–CSF3R axis in cluster 12 in mediating the anti-GVHD effects of G-MAIT cells.

FCM confirmed the presence of $CD114^+$ MAIT cell subset (CD114 encoded by *CSF3R*), which expanded after G-CSF induction (Figure 2E; *P*=0.048). In a clinical G-PBSC cohort,

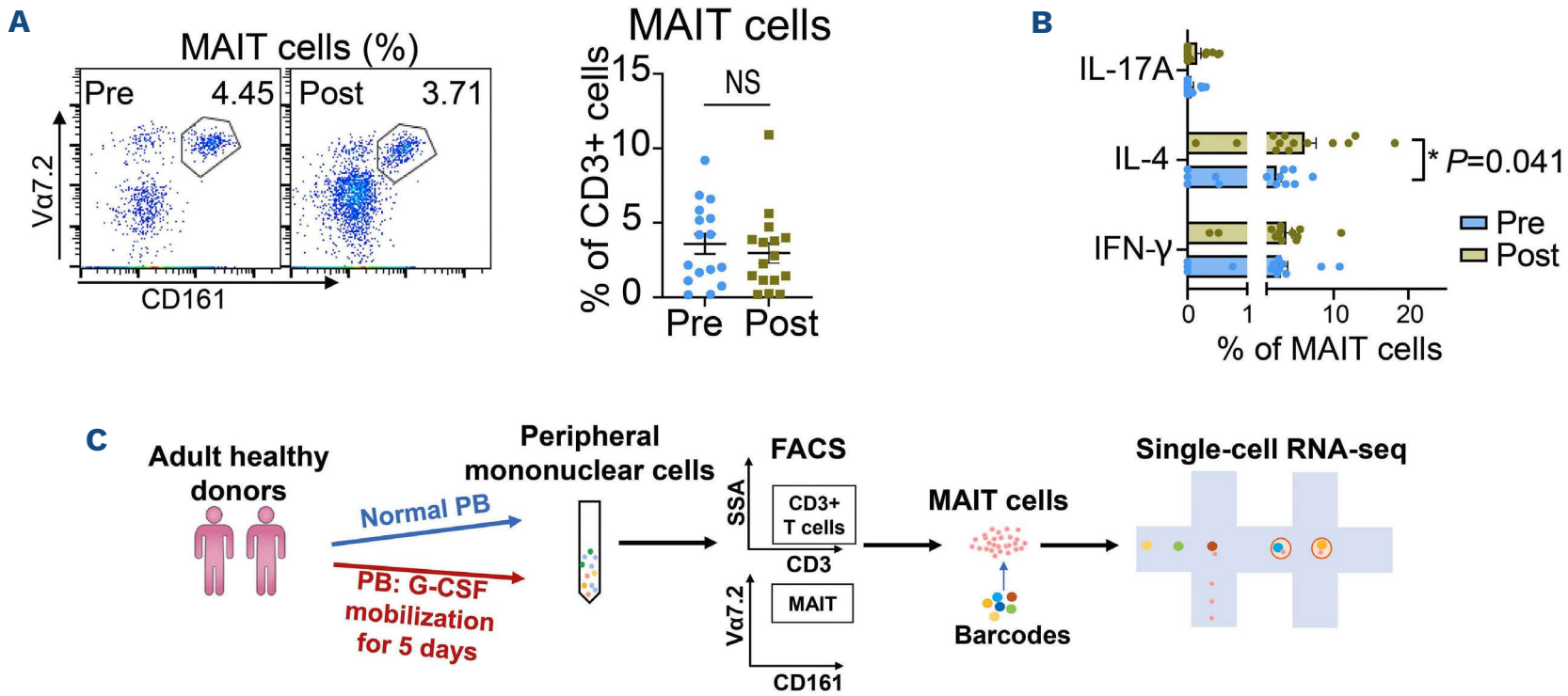

Figure 1. Characteristics of MAIT cells before and after granulocyte colony-stimulating factor administration. (A) Flow cytometric analysis showing mucosa-associated invariant T- (MAIT) cell frequency in donor peripheral blood stem cells (PBSC) before and after granulocyte colony-stimulating factor (G-CSF) mobilization. (B) Flow cytometric analysis (FACS) showing the expression of interleukin (IL)-4 (*P*=0.041), interferon (IFN)-γ and IL-17A in MAIT cells from donor PBSC (N=16) before and after G-CSF mobilization. (C) Workflow showing the processing of MAIT cells of 8 PB samples from 4 healthy donors before and after 5-day G-CSF

Continued on following page.

treatment for single-cell RNA sequencing (scRNA-seq). (D) Uniform manifold approximation and projection (UMAP) visualization of 14 MAIT cell clusters identified in human PB based on scRNA-seq. Each dot represents a single cell, with colors indicating different clusters. (E) Violin plot showing the expression of genes related to IL-4/IL-13 pathway before and after G-CSF mobilization. (F) Violin plot showing the G-CSF score of MAIT cells before and after G-CSF mobilization (****P<0.0001). (G) Dot plot showing functional signatures of MAIT cells before and after G-CSF mobilization. (H) Representative flow cytometry results showing the immunosuppressive effect of MAIT cells on $CD4^+$ T cells under C-CSF mobilization. MAIT and $CD4^+$ T cells were isolated from the mobilized and unmobilized PB of 3 healthy donors. 5,6-carboxy-fluorescein diacetate succinimidyl ester (CFSE)-labeled $CD4^+$ T cells were stimulated with CD3/CD28 beads and co-cultured with MAIT cells at ratios of 1:1, 2:1, 4:1 for 4 days. PBS: phosphate-buffered saline; NS: not significant.

CD114 expression on G-MAIT cells was significantly lower in donors whose recipients developed gut aGVHD compared to those without aGVHD or with skin aGVHD (Figure 2F), further underscoring a potential role of $CD114^+$ G-MAIT cells against gut GVHD.

Functional heterogeneity of G-MAIT cells correlates with gut acute graft-*versus*-host disease outcomes

To investigate whether the functional states of G-MAIT cells influence gut aGVHD development, we stratified G-CSF-treated scRNA-seq samples by whether recipients developed gut aGVHD post-transplant or not (DEG for details, see *Online Supplementary Table S8*). G-MAIT cells from the no gut aGVHD group exhibited higher expression of *CSF3R* and its target genes (Figure 3A), indicating donor heterogeneity in G-MAIT cells response to G-CSF. They also exhibited enhanced immunosuppressive and tissue repair signatures compared to the gut aGVHD group (Figure 3B), with increased expression of pro-repair mediators[18,20] including *TNF*, *FURIN*, *TGFB1*, *AREG* and *IL-17A* (*Online Supplementary Figure S4A*), along with enhanced tissue residency and migration signatures (Figure 3C). These findings imply that, beyond immunosuppression, the enhanced tissue repair, migration, and residency capabilities of G-MAIT cells are critical for maintaining intestinal mucosa integrity and preventing gut aGVHD.

Key markers associated with tissue residency, repair, immune tolerance, and cytotoxicity-CD69, IL-17A, IL-4, and IFN-γ were analyzed by flow cytometry in a clinical G-PBSC cohort (Figure 3D, E). Results confirmed lower IL-4 and IL-17A expression in G-MAIT cells from donors whose recipients developed gut aGVHD, while other effector factors showed no significant differences (Figure 3E). These results further highlighted the importance of immunosuppressive and tissue repair functions of G-MAIT cells in mediating protection against gut aGVHD.

Given that tissue repair by circulating MAIT cells depends on chemokine receptor-mediated migration, we analyzed chemokine signaling and receptor expression. Chemokine signaling pathways were significantly downregulated in the gut aGVHD group (Figure 3F). To rigorously evaluate this, we analyzed expression of chemokine receptor genes in G-MAIT cells, previously used to evaluate MAIT chemotaxis.[12,17,41] scRNA-seq revealed distinct receptor profiles across MAIT clusters, reflecting functional heterogeneity. For example, CXCR3 was enriched in cytotoxic cluster 7, *CXCR6* in cluster 3 and 6, *CCR7* in naïve cells, and *CCR1/CCR2* in cluster 12 (*Online Supplementary Figure S4B*). The heterogeneity suggested differential migration, distribution and function among MAIT subsets under various microenvironment or pathological conditions. Comparing no gut aGVHD and gut aGVHD groups (Figure 3G), G-MAIT cells from the no gut aGVHD group showed higher *CCR1*, *CCR5*, *CCR7*, *CXCR3*, and *CXCR6* expression, while *CCR2* and *CCR6* were relatively upregulated in the gut aGVHD group (Figure 3G). FCM validation confirmed significantly reduced expression of CXCR6 and CCR7 in the gut aGVHD group, with no significant differences for other receptors (Figure 3H; *Online Supplementary Figure S4C*). Overall, above results indicated that heterogeneous expression of key functional mediators of G-MAIT cells could be associated with the occurrence of gut aGVHD.

G-MAIT cells exhibit gut-specific chemotaxis and anti-gut acute graft-*versus*-host disease effects *in vivo*

To assess the tissue-homing behavior of G-MAIT cells after allo-HSCT, we used a humanized NSG mouse model (Figure 4A). Engraftment of human white blood cell (WBC) was detected from PB samples of mice at 8-14 days after transplantation. With GVHD progressed, MAIT cell abundance increased within organ tissues, particularly in the intestine, as compared to PB and bone marrow (Figure 4B-D). On day 14 after transplantation, mice were divided into two groups based on their GVHD score (≥3 or <3). Notably, mice with lower GVHD scores exhibited higher intestinal MAIT cell infiltration (Figure 4E), indicating that MAIT cells preferentially migrated to the gut and contributed to mitigating gut aGVHD. These findings highlight the gut-directed chemotactic capacity of G-MAIT cells as a key mechanism underlying their protective effect.

CXCR6 is required for G-MAIT cell recruitment to gut tissue

CXCR6, a key receptor for liver-intestinal targeting,[42] emerged as the most differentially expressed chemotactic receptor gene between no gut aGVHD and gut aGVHD group (Figure 3G, H). We therefore focused on its function in intestinal chemotaxis. To assess its role in intestinal homing, we blocked CXCR6-CXCL16 interaction using CXCL16 blocking antibody in NSG mice (Figure 5A). CXCL16 blockade led to a

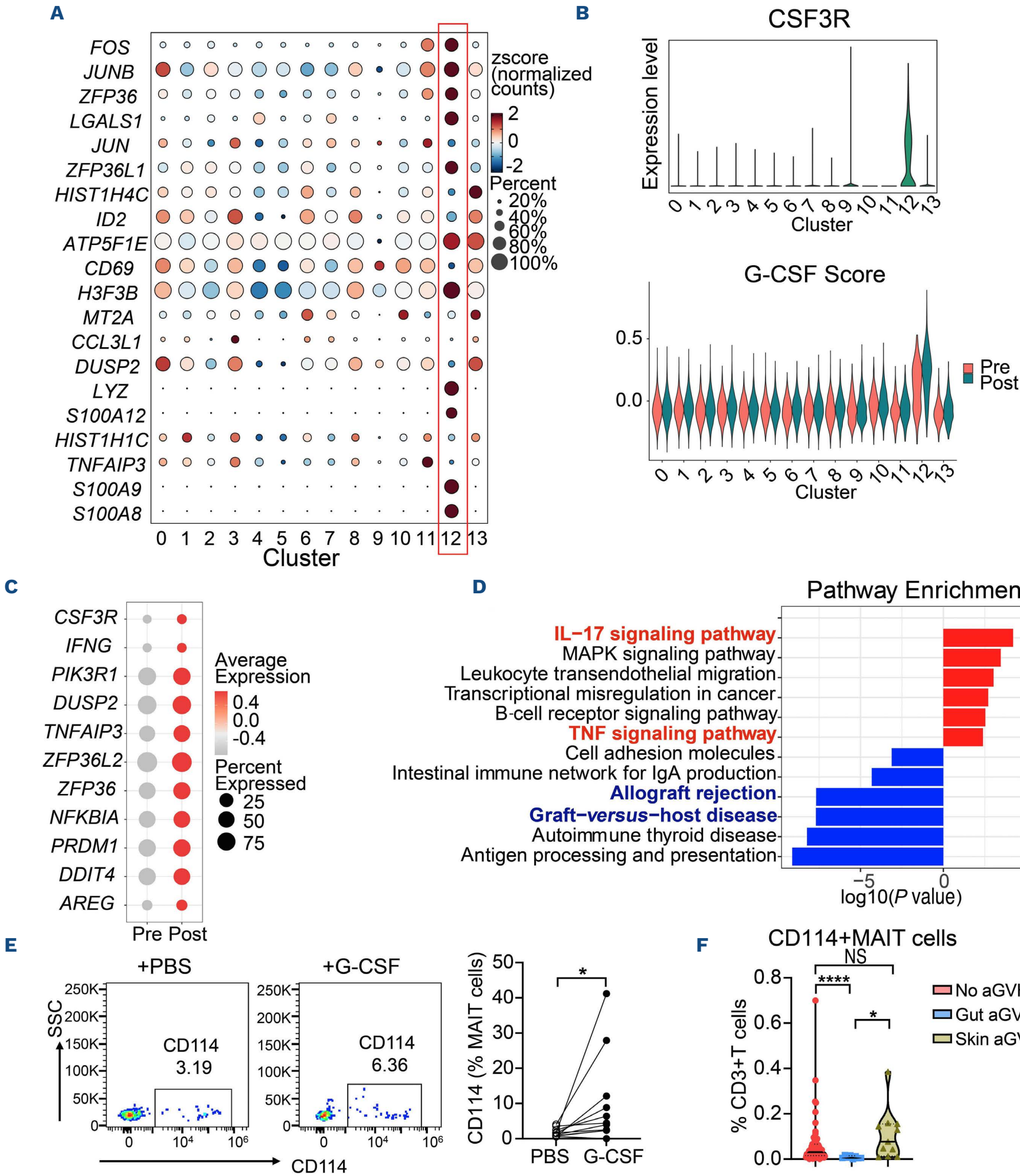

Figure 2. G-CSF-CSF3R interaction mediates MAIT cell functions. (A) Dot plot showing the distribution of immune regulation-related genes among the top 50 differentially expressed genes (DEG) in mucosa-associated invariant T- (MAIT) cell clusters. (B) Violin Box plot showing the distribution of *CSF3R* expression and granulocyte colony-stimulating factor (G-CSF) score across different clusters. (C) Dot plot showing the expression of *CSF3R* and its target genes in MAIT cells. (D) Bar plots from gene set enrichment analysis (GSEA) analysis showing the major enriched terms of DEG before (left) and after (right) G-CSF administration. (E) Flow cytometric analysis of CD114 expression in donor MAIT cells from peripheral blood (PB) before and after G-CSF mobilization. (F) Flow cytometric analysis showing CD114 expression of MAIT cells in G-CSF-mobilized peripheral blood stem cells (G-PBSC) [G-MAIT]) between groups of gut acute graft-*versus*-host disease (aGVHD) (N=9), skin aGVHD (N=13) and no aGVHD (N=55) (*P=0.042;****P<0.0001). PBS: phosphate-buffered saline; NS: not significant.

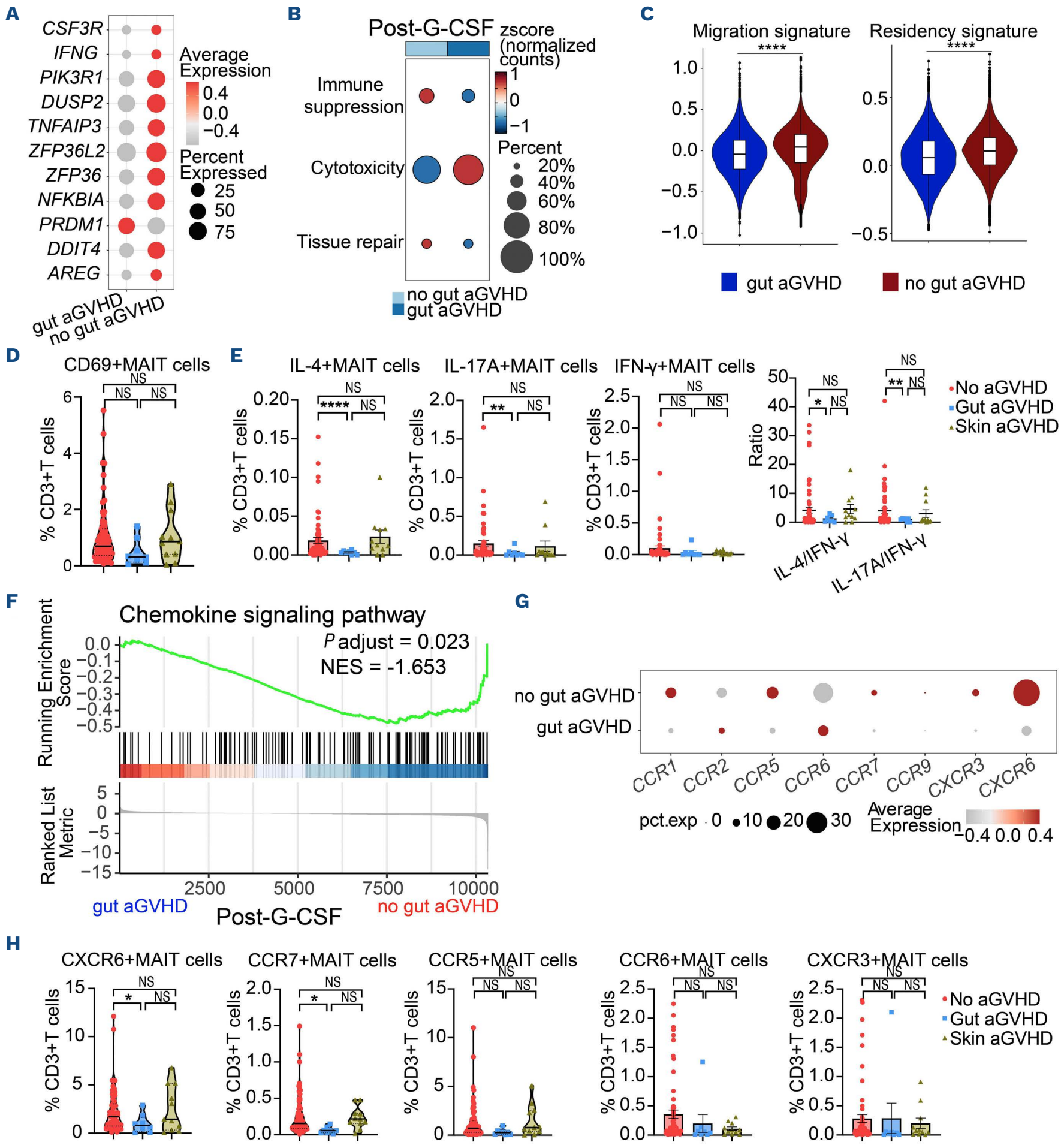

Figure 3. Granulocyte colony-stimulating factor-induced MAIT cells contribute to anti-gut acute graft-*versus*-host disease effects. (A) Dot plot showing the expression of *CSF3R* and its target genes in granulocyte colony-stimulating factor-induced mucosa-associated invariant T (G-MAIT) cells between groups of gut acute graft-*versus*-host disease (aGVHD) (N=1) and no gut aGVHD (N=3). (B) Dot plot illustrating the functional signatures of G-MAIT cells between the two groups. (C) Violin box plot showing the expression of migration- and tissue residency-related signatures between the 2 groups (****P<0.0001). (D) Flow cytometric analysis showing CD69 expression in G-MAIT cells between groups of gut aGVHD (N=9), skin aGVHD (N=13) and no aGVHD (N=55). (E) Flow cytometric analysis showing interleukin (IL)-4, IL-17A and interferon (IFN)-γ expression in G-MAIT cells among the 3 groups (*P<0.05; ***P<0.001; ****P<0.0001). (F) Gene set enrichment analysis (GSEA) plots showing downregulation of the chemokine signaling pathway in G-MAIT cells from the no gut aGVHD group compared to the gut aGVHD group. (G) Dot plot showing chemokine receptor expression in G-MAIT cells between gut aGVHD and no gut aGVHD group. (H) Flow cytometric analysis showing chemokine receptor expression in G-MAIT cells between groups of gut aGVHD (N=9), skin aGVHD (N=13) and no aGVHD (N=55) (*P<0.05). NS: not significant. pct.exp.: percent expressed.

marked reduction in gut-infiltrating MAIT cells post-transplantation, with minimal impact on PB, bone marrow, or other tissues (Figure 5B), indicating that CXCR6-CXCL16 was critical for gut recruitment of G-MAIT cells. Additionally, mice in the anti-CXCL16 group showed more severe GVHD symptoms such as hair loss, visceral congestion and had higher GVHD scores, especially on day 16 after transplantation (Figure 5C). Histological examination confirmed

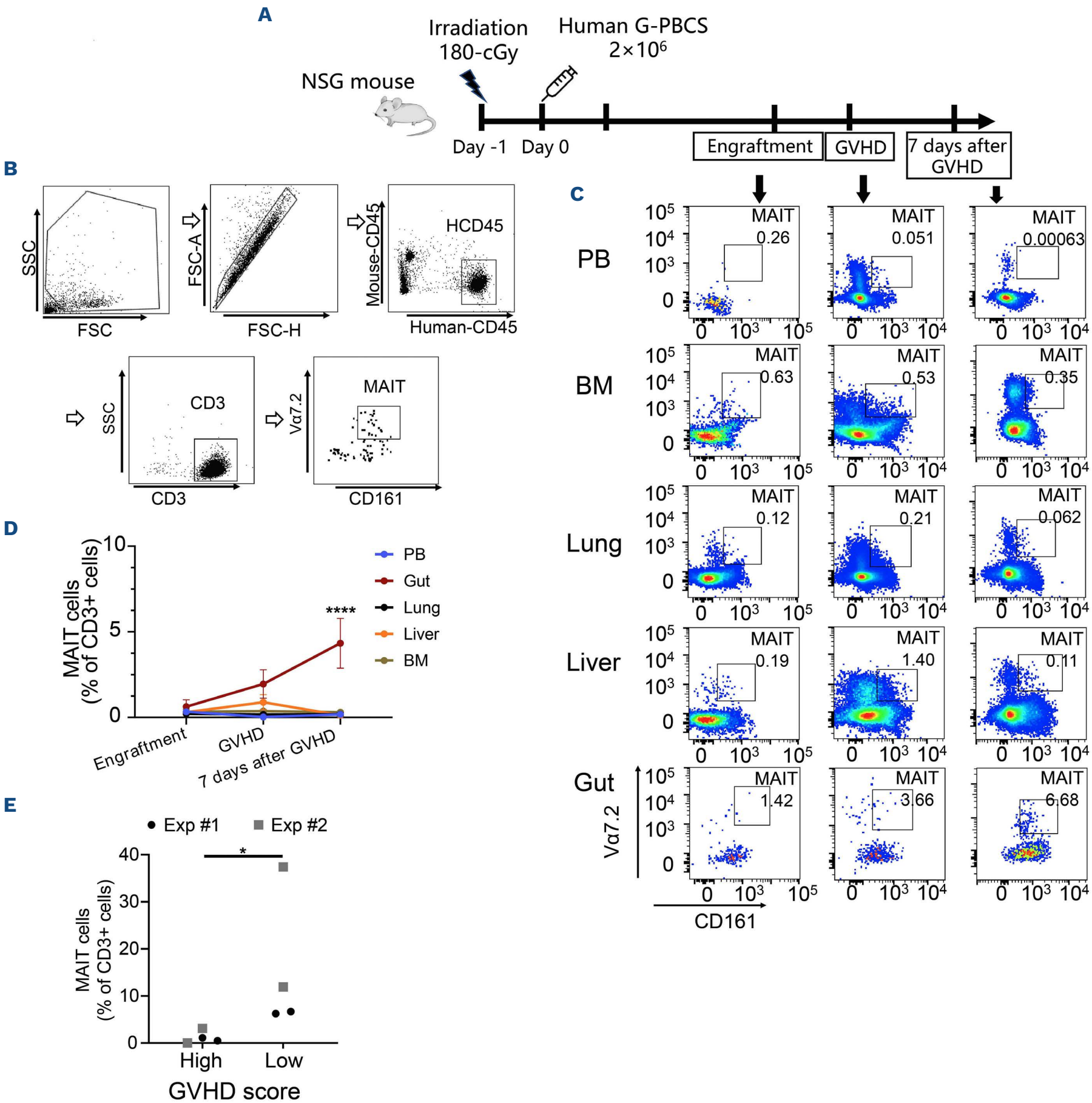

Figure 4. Specific chemotactic migration of MAIT cells from granulocyte peripheral blood stem cells to the gut. (A) Protocol for establishing a graft-*versus*-host disease (GVHD) model in NSG mice by injecting human granulocyte colony-stimulating factor-mobilized peripheral blood stem cells (G-PBSC). (B) Gating strategy for identifying human mucosa-associated invariant T- (MAIT) cells in NSG mice. (C) Flow cytometric analysis showing the dynamic distribution of human MAIT cells after transplantation in peripheral blood (PB), bone marrow (BM), gut, liver and lung tissues of NSG mice. (D) Line graphs showing the dynamic changes in human MAIT cells post-transplantation in PB, BM, lung, gut and liver tissues of NSG mice. (E) Flow cytometric analysis showing the frequency of MAIT cells in gut tissues between mice with high GVHD score (GVHD score ≥3) and low GVHD (GVHD score<3) score on day 14 post-hematopoietic stem cell transplantation (HSCT). Exp: experiment.

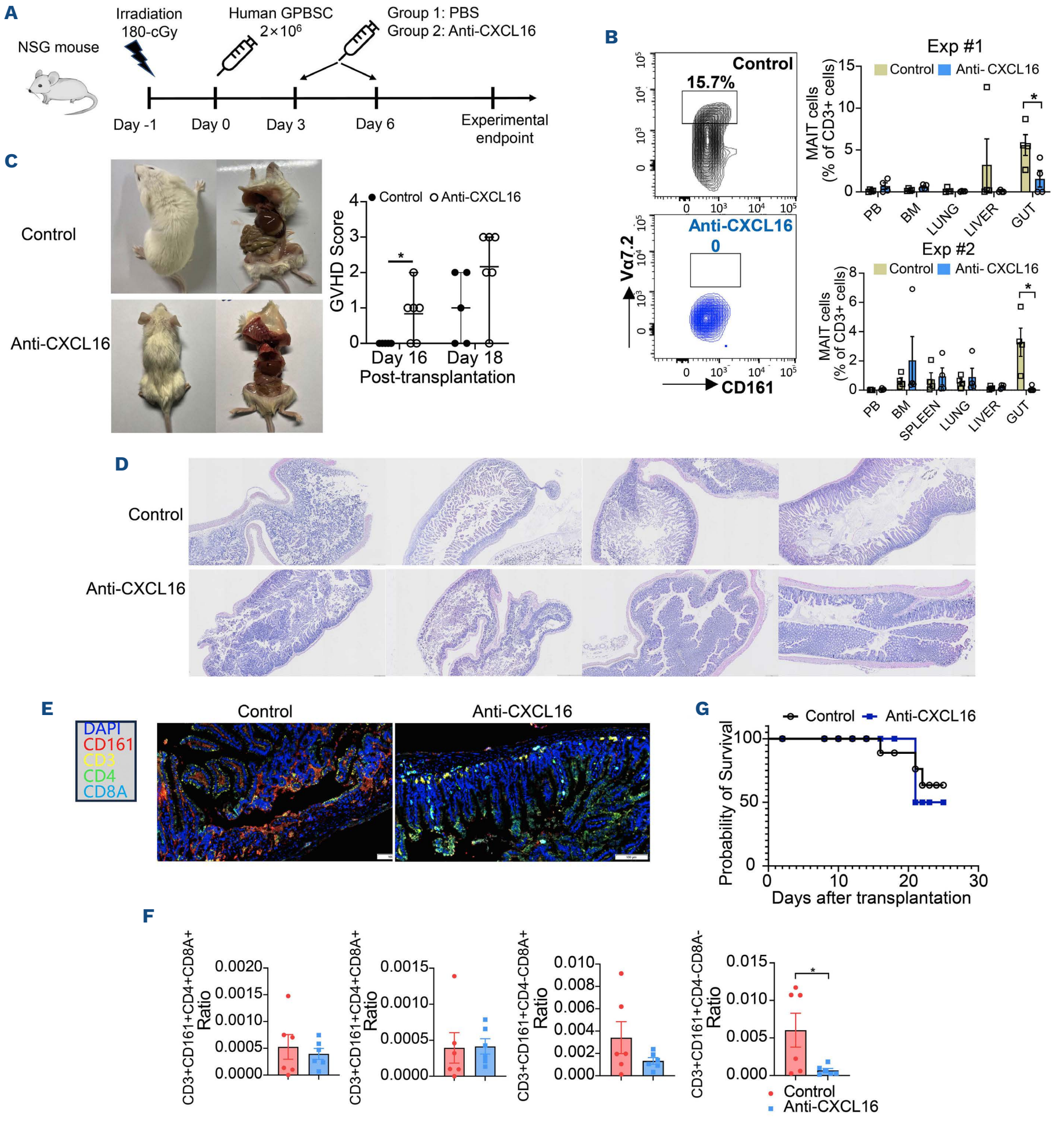

Figure 5. CXCR6 is necessary for G-MAIT cell recruitment to gut tissue. (A) Protocol for the establishment of CXCL16 blocking in NSG mice through injecting human granulocyte colony-stimulating factor-mobilized peripheral blood stem cells (G-PBSC). (B) Representative flow cytometry showing mucosa-associated invariant T- (MAIT) cell frequencies in the gut of control and anti-CXCL16 treatment groups (left), and frequencies of human MAIT cells from PB, bone marrow (BM), gut, liver and lung tissues of NSG mice (right). (C) Graft-*versus*-host disease (GVHD)-related symptoms in control and anti-CXCL16 groups post-transplantation (left), and flow cytometric analysis of GVHD scores on day 16 and day 18 post-hematopoietic stem cell transplantation (HSCT) (right). (D) Hematoxilin and eosin staining showing representative histological features of intestinal tissues harvested from mice at the study endpoint post-transplantation, 10x magnification. (E, F) Multiplex immunofluorescence analysis revealing the target cell populations in intestinal tissues at the study endpoint, comparing control and anti-CXCL16 groups. (G) Survival curve comparing control and anti-CXCL16 groups. G-MAIT: granulocyte colony-stimulating factor-induced MAIT; Exp: experiment.

increased cellular infiltration and mucosal disruption in the intestines of anti-CXCL16-treated mice (Figure 5D), consistent with aggravated gut inflammation.

Immunofluorescence analysis further demonstrated impaired intestinal localization of CD161$^+$ T cells upon CXCL16 blockade, whereas CD3$^+$CD161$^-$T cells were less affected (*Online Supplementary Figure S5A, B*). Previous studies have confirmed that circulating MAIT cells are predominantly CD8$^+$ (approximately 70%) and double-negative (DN, approximately 15%).[17] However, FCM results showed that gut-resident MAIT cells were predominantly CD4$^-$CD8$^-$, followed by CD8$^+$ cells (*Online Supplementary Figure S5C*). Notably, CXCL16 blockade significantly reduced both the frequency and density of intestinal CD3$^+$CD161$^+$CD4$^-$CD8$^-$ T cells, with no apparent changes observed in the CD8$^+$ and CD4$^+$CD8$^-$ MAIT subsets (Figure 5E, F; *Online Supplementary Figure S5D*). Despite these changes, overall survival was not significantly different between groups (Figure 5G). Collectively, these results suggested that G-MAIT cell recruitment to the gut after allo-HSCT depends on the CXCR6-CXCL16 axis.

A predictive MAIT cell-based panel for gut acute graft-*versus*-host disease

scRNA-seq analysis, supported by *in vitro* and *in vivo* experiments, identified a gene set (*CSF3R*, *IL17A*, *IL4*, and *CXCR6*)-potentially involved in mechanism of anti-gut aGVHD (Figure 6A). Gene set scoring revealed significantly higher expression in G-MAIT cells from the no gut aGVHD group, suggesting its predictive potential (Figure 6A).

To validate the clinical translational potential of these findings, we analyzed donor G-PBSC. Consistent with prior work,[21] gut aGVHD onset was associated with a lower frequency of G-MAIT cells, whereas skin aGVHD showed no such correlation (Figure 6B, C). This result further confirmed that the frequency of MAIT cells in G-PBSC can specifically predict the onset of gut aGVHD. Other T-cell subsets, including conventional CD4$^+$, CD8$^+$ T cells and non-conventional γδ T cells, found no significant differences between the gut aGVHD and other groups (Figure 6B; *Online Supplementary Figure S6A*). Further analyses by aGVHD grades showed neither significant differences in frequencies of total T (CD3$^+$ T cells) and T-cell subsets (γδ T, CD4$^+$, CD8$^+$ T and MAIT cells) (*Online Supplementary Figure S6B*) across I, II–IV and no aGVHD groups, nor in expression of functional markers CD114, CXCR6, IL-17A and IL-4 (*Online Supplementary Figure S6C*). These data suggest that the reduction in MAIT cells is more pronounced in gut involvement rather than overall GVHD severity, supporting their potential role as immunological correlates of gut aGVHD.

To enhance predictive power, we developed a composite score integrating MAIT cell frequency and expression of functional markers (CD114, IL-17A, IL-4, CXCR6) (Figure 6D). This score was significantly lower in patients with gut aGVHD than in those with no or skin aGVHD (Figure 6D). Importantly, low-score individuals exhibited a higher incidence of gut aGVHD, whereas no association was observed with skin aGVHD (Figure 6E). The score was neither associated with aGVHD severity grade (*Online Supplementary Figure S6D*), nor did the incidence of grade II-IV aGVHD differ between high- and low-score groups (*Online Supplementary Figure S6E*), indicating its specificity for gut aGVHD prediction.

Notably, this predictive panel outperformed MAIT cell frequency alone in predicting gut aGVHD, achieving a sensitivity of 75% and specificity of 93% (Figure 6F). In univariate analysis, low score, Epstein-Barr virus (EBV) infection, and recipient age were risk factors for gut aGVHD, while multivariate analysis identified the low predictive score as an independent predictor (Figure 6G). Overall, these results support a clinically applicable, FCM-based predictive panel incorporating both MAIT cell frequency and functional phenotype as a specific biomarker for gut aGVHD.

Discussion

This study reveals that G-MAIT cells suppress gut aGVHD by enhancing immunoregulatory and tissue repair functions via the G-CSF–CSF3R axis. The gut homing of these MAIT cells is mediated by the CXCR6-CXCL16 chemokine pathway. We also developed a flow cytometry-based MAIT cell score, which integrates both cell frequency and phenotype in grafts, to predict gut aGVHD risk pre-transplant, providing clinical translational value.

Previous studies have shown that peripheral MAIT cells in both mice and humans exhibit a broad range of phenotypes, spanning homeostatic, effector, cycling, regulatory, and exhausted states.[8,31,43,44] Consistent with the previous studies,[8,44,45] our data showed most MAIT cells in human PB exhibit a mixed MAIT1/MAIT17 transcriptome, co-expressing genes associated with tissue repair, cytotoxicity, type1/17 features. This mixed differentiation program appears to be a conserved feature of innate-like T cells across evolution.[44] A key finding of our study is the identification of a G-CSF–induced MAIT cell subset (cluster 12) characterized by CSF3R expression. These G-MAIT cells showed high expression of genes involved in immunosuppressive and IL-17 signaling pathways, along with enrichment of *CSF3R* target genes, suggesting that G-CSF-CSF3R signaling promotes immunosuppressive and tissue-reparative functions in MAIT cells. This is further supported by the enrichment of transcripts encoding *ZFP36*, *ZFP36L1*, and *ZFP36L2* in cluster 12, which are known to regulate the stability of mRNA for cytokines and immune mediators.[12,46]

MAIT cells are enriched in mucosal tissues and play a key role in mucosal immunity through their unique activation by microbial riboflavin metabolites and their ability to initiate a tissue repair program upon TCR trigger. The reparative function of MAIT cells has been primarily studied in mucosal tissues such as the skin,[12] lungs[48] and colon.[49] Our data

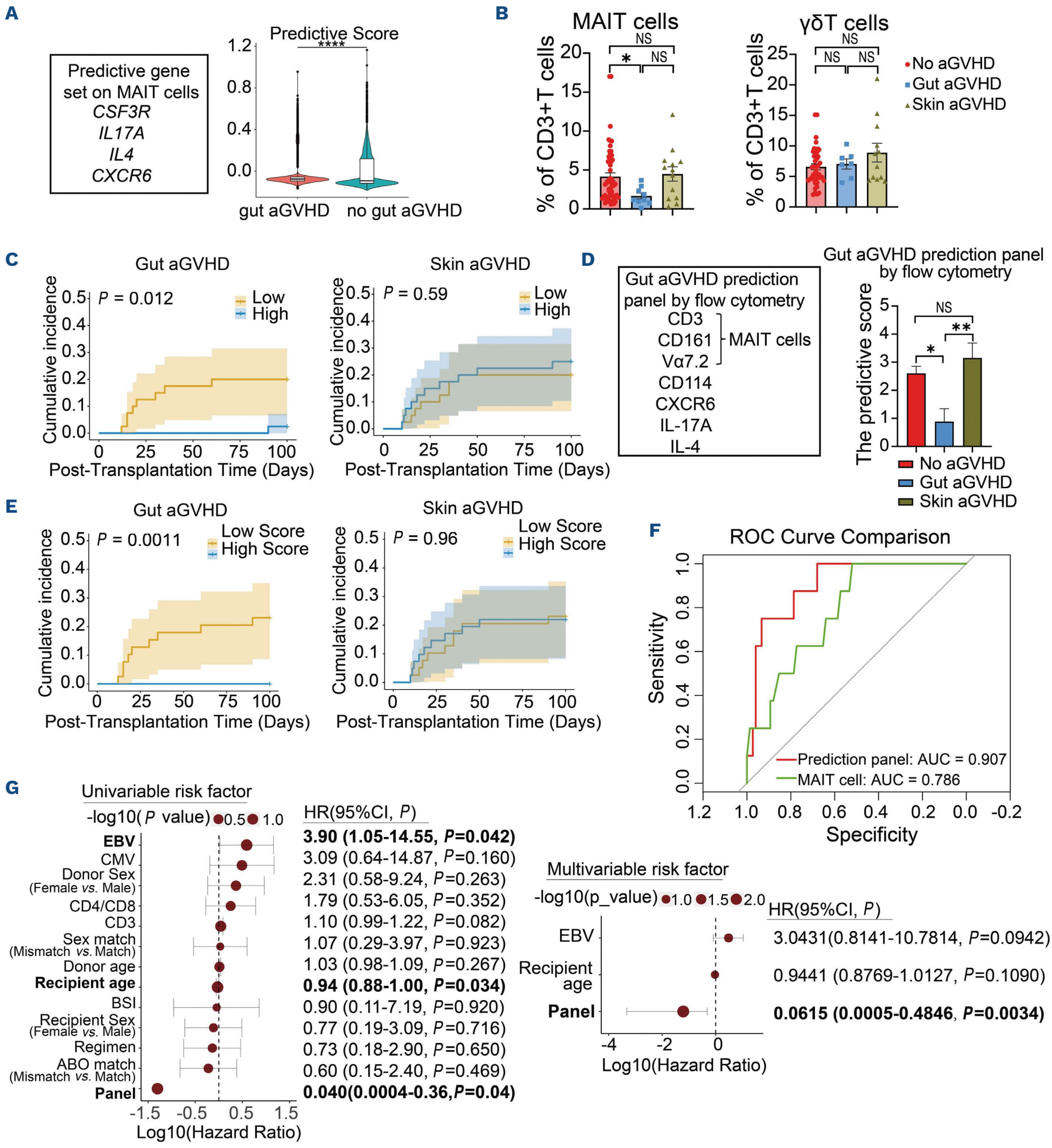

Figure 6. Predictive panel of gut acute graft-*versus*-host disease. (A) Violin box plot showing the expression scores of granulocyte colony-stimulating factor-induced mucosa-associated invariant T (G-MAIT) cell-related gene combination between the gut acute graft-*versus*-host disease (aGVHD) and no gut aGVHD group (****P<0.0001). (B) Flow cytometry analysis showing the frequency of MAIT and γδ T cells among groups of gut aGVHD (N=9), skin aGVHD (N=13) and no aGVHD (N=55) (*P<0.05). (C) The incidence of gut aGVHD and skin aGVHD based on MAIT cell frequency. The cohort of granulocyte colony-stimulating factor-mobilized peripheral blood stem cells (G-PBSC) from donors (N=80) was divided into high (N=40) and low (N=40) MAIT cell frequency groups

Continued on following page.

using a median cutoff of 2.95%. (D) MAIT cell-based scoring panel for donors after granulocyte colony-stimulating factor (G-CSF) mobilization. The panel evaluated 5 parameters: MAIT cell frequency (% $CD3^+$ T cells), CD114, CXCR6, IL-17A, and IL-4. Each parameter was scored based on whether it met or exceeded the median value, with a total score ranging from 0 to 5. (E) The incidence of gut aGVHD and skin aGVHD in high and low score based on the flow cytometry panel in (D). A median score cutoff (>2 or ≤2) was used to define the high (N=41) and low (N=39) score groups. (F) Receiver operating curve (ROC) comparison for MAIT cell proportion and the 5-marker panel combination in predicting gut aGVHD. (G) Univariate and Multivariate analyses of factors associated with gut aGVHD occurrence. CI: confidence interval; EBV: Epstein-Barr virus; HR: hazard ratio; NS: not significant.

demonstrate that circulating MAIT cells also express tissue repair signatures, which are further upregulated following G-CSF mobilization. We hypothesize that this subset, predominantly MAIT17 effector memory cells with a preactivated repair program, can be rapidly recruited to sites of mucosal injury or inflammation, where local environmental cues trigger their reparative functions. This hypothesis is supported by a recent study on skin wound healing using the Kaede photoconvertible mouse, which showed that MAIT cells accumulating in skin wounds originate from the circulation, including lymphoid organs.[12] In addition, the number of MAIT cells in PB of patients was significantly reduced during inflammation or GVHD,[21,49] further indicating their recruitment from the blood to the pathological sites. MAIT cells play a critical role in maintaining intestinal homeostasis through MAIT cells play a critical role in maintaining intestinal homeostasis through their ability to sense microbial-derived riboflavin metabolites and initiate tissue repair responses.[18,47,49] Inflammatory conditions can disrupt the anaerobic environment of the gut, resulting in an increased synthesis of MAIT cell ligands by the microbiota. These MAIT ligands can penetrate the intestinal barrier and activate MAIT cells across various tissues, thereby triggering epithelial repair and enhancing the protection against intestinal inflammation.[49] In the context of allo-HSCT with myeloablative conditioning, our previous work showed that higher levels of MAIT cells in grafts correlate with a reduced incidence of gut aGVHD.[21] This protective effect is likely mediated by both immunoregulatory functions and microbiota-driven activation, resulting in elevated IL-17A production - an essential cytokine for intestinal repair and barrier maintenance.[15,18,47,48] The gut-selective protection of MAIT cells contrasts with their limited impact on skin aGVHD, potentially reflecting differences in MAIT cell abundance (2-10% *vs*. 0.5-2% of $CD3^+$ T cells) and microbial stimulation between tissues.[17] MAIT cell-mediated repair is closely linked to MR1-dependent activation. Compared with $MR1^{+/-}$ NOD mice, increased intestinal permeability was observed in $MR1^{-/-}$ NOD mice.[50] Consequently, the immunosuppression and tissue repair functions triggered by the microbial environment likely constitute key mechanisms through which MAIT cells exert their anti-gut aGVHD effect. CXCR6 has been described to favor recruitment of MAIT cells to pathological tissue sites and is necessary for complete MAIT cell maturation in the thymus.[12,48,50] This mechanism is likely shared for MAIT cell recruitment to other organs, CXCR6-CXCL16 drives MAIT cell accumulation in the skin, lung and kidney.[12,26,41] Furthermore, CXCR6 is enriched in the MAIT17 subset, suggesting selective recruitment from circulation rather than proliferation *in situ*.

Notably, heterogeneity among MAIT cells in G-PBSC from different donors was observed. MAIT cells in G-PBSC with gut aGVHD recipients exhibited reduced immunosuppressive and tissue repair features, as well as lower CXCR6 expression. Based on available evidence, the mechanism through which G-MAIT cells provide resistance against gut aGVHD relies on two key factors. Firstly, it depends on the absolute number of MAIT cells in the gut tissues, which is influenced by both the number of MAIT cells transfused from G-PBSC to the recipient,[21] as well as the chemotactic ability of circulating MAIT cells to migrate into the gut tissues. Secondly, it relies on the immunosuppressive and tissue repair capabilities of MAIT cells within G-PBSC, which is modulated by G-CSF responsiveness via CSF3R expression. Thus, the immunosuppression, tissue repair, and chemotactic ability exhibited by G-MAIT cells are key to exerting its anti-gut aGVHD effect.

Of note, discrepancies exist across studies regarding the association between MAIT cells and aGVHD risk. While some have reported that higher MAIT cell abundance correlates with increased aGVHD risk,[51,52] others, including our own[21] and Bhattacharyya *et al*.,[28] have found that early MAIT cell reconstitution is associated with reduced aGVHD incidence. Several factors may contribute to these divergent findings. First, the dynamics of immune reconstitution could influence outcomes: although rapid MAIT cell recovery may indicate effective immune reconstitution, it could also reflect a heightened overall immune activation state, potentially promoting GVHD. Second, infections or endotoxemia may independently activate and expand MAIT cells and other effector T cells while concurrently triggering GVHD. Third, technical variations in MAIT cell identification and subset analysis across studies may account for inconsistent results. These discrepancies highlight the need for future studies that incorporate standardized MAIT cell definitions, synchronized assessments of peripheral and tissue-resident MAIT cells, and integration of single-cell transcriptomic and TCR sequencing analyses to more precisely evaluate the functional quality and reconstitution patterns of MAIT cells. We have developed an innovative flow cytometry panel that integrates both the frequency and phenotypic heterogeneity of MAIT cells in G-PBSC, enabling accurate pre-transplant prediction of gut aGVHD. This panel significantly outperforms the approach that rely solely on MAIT cell frequency.

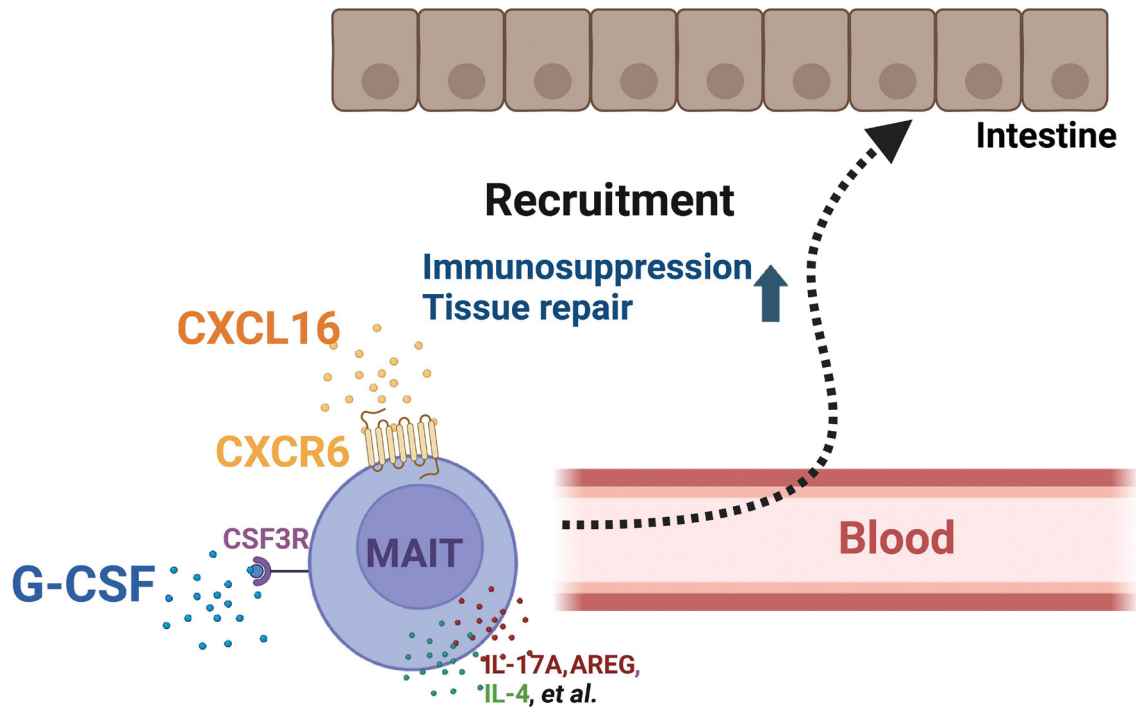

Figure 7. The potential mechanism of anti-gut acute graft-*versus*-host disease by MAIT cells in granulocyte peripheral blood stem cells. Granulocyte colony-stimulating factor (G-CSF) binds to CSF3R on mucosa-associated invariant T (MAIT) cells, stimulating them to secrete immune-modulatory and tissue-repair molecules such as interleukin (IL)-4 and IL-17A. Additionally, the CXCR6 receptor directs these functional MAIT cells to migrate specifically to gut pathological sites. This targeted migration enables MAIT cells to exert their immunosuppressive and tissue-repair functions, thereby countering gut acute graft-versus-host disease (aGVHD). Created with Biorender.com.

In contrast to most current methods, which are applied post-transplant and require multiple time point samples affected by numerous clinical factors, our model allows risk assessment using a single pre-transplant graft sample. This strategy offers practical advantages, including ease of sample collection, operational simplicity, and cost-effectiveness, making it well-suited for clinical application. Nonetheless, further validation with larger sample sizes is necessary, along with comparative evaluations of its accuracy and effectiveness against existing aGVHD prediction methods.

In implementing this model, we employed the widely used $CD3^{+}CD161^{hi}V\alpha7.2^{+}$ gating strategy to identify MAIT cells, given its practicality and broad application in previous studies. However, CD161 expression can be downregulated under certain physiological and pathological conditions,[53] such as activation or infection, potentially leading to an underestimation of MAIT cell frequencies. While MR1 tetramer staining offers greater specificity and is not affected by activation status, its limited accessibility hinders its routine use in large-scale clinical settings. Therefore, our use of CD161-based gating reflects a balance between specificity and feasibility. We acknowledge this methodological limitation and have interpreted our findings with appropriate caution, especially in contexts where MAIT cell activation may vary.

In conclusion our findings identify a novel G-CSF–responsive MAIT cell subset in G-PBSC that suppresses gut aGVHD via enhanced immunoregulation and tissue repair. CXCR6-mediated trafficking to the gut and CSF3R-driven functional regulation are critical to this effect. A graft MAIT cell score incorporating CD114, IL-17A, IL-4, and CXCR6 provides a promising tool for pre-transplant risk stratification. These insights pave the way for MAIT cell-based interventions to prevent gut aGVHD and improve transplant outcomes.

Disclosures

No conflicts of interest to disclose.

Contributions

MG designed and performed flow cytometry and mouse experiment; analyzed the scRNA-seq, flow cytometry, and clinical data; and wrote the manuscript. SL assisted with scRNA-seq data analysis. KZ, XC and HG participated in mouse experiment implementation and sample collection. YS, JK, TH and YC provided critical expertise and collected data. XH, YC and XZ provided experimental platform, guided the project and contributed to the manuscript. XZ designed the project and data analysis strategies, oversaw all raw data, and revised the manuscript.

Funding

The work was supported by the Beijing Municipal Natural Science Foundation (grant number 7242155), the National Natural Science Foundation of China (grant number 62331025), the Beijing Municipal Science and Technology Commission (grant number Z221100007422008), the major Program of the National Natural Science Foundation of China (grant number 82293630, 82293633) and Peking University Medicine Fund for world's leading discipline or discipline cluster development (grant number 71003Y3035).

Acknowledgments

The authors wish to thank all of the health volunteers who donated hematopoietic stem cell transplants, as well as their physicians for the clinical guidance. The authors thank the Peking University Center for Medical and Health Analysis for their support in cell sorting.

Data-sharing statement

All data associated with this study are present in the paper or the Online Supplementary Appendix. The data that support the findings of this study are openly available in The raw sequencing data generated by this project were deposited into the Genome Sequence Archive (https://ngdc.cncb.ac.cn/gsa-human) with accession number GSA-Human: HRA006712. At https://ngdc.cncb.ac.cn/gsa-human, reference number HRA006712.

References

1. Roncarolo MG, Battaglia M. Regulatory T-cell immunotherapy for tolerance to self antigens and alloantigens in humans. Nat Rev Immunol. 2007;7(8):585-598.
2. Huang XJ, Chang YJ, Zhao XY. Maintaining hyporesponsiveness and polarization potential of T cells after in vitro mixture of G-CSF mobilized peripheral blood grafts and G-CSF primed bone marrow grafts in different proportions. Transpl Immunol. 2007;17(3):193-197.
3. Xu ZL, Huang XJ. Haploidentical transplants with a G-CSF/ATG-based protocol: Experience from China. Blood Rev. 2023;62:101035.
4. Morris ES, MacDonald KP, Rowe V, et al. Donor treatment with pegylated G-CSF augments the generation of IL-10-producing regulatory T cells and promotes transplantation tolerance. Blood. 2004;103(9):3573-3581.
5. Franzke A, Piao W, Lauber J, et al. G-CSF as immune regulator in T cells expressing the G-CSF receptor: implications for transplantation and autoimmune diseases. Blood. 2003;102(2):734-739.
6. Guo H, Li R, Wang M, et al. Multiomics analysis identifies SOCS1 as restraining T cell activation and preventing graft-versus-host disease. Adv Sci (Weinh). 2022;9(21):e2200978.
7. Minculescu L, Sengelov H, Marquart HV, Ryder LP, Fischer-Nielsen A, Haastrup E. Granulocyte colony-stimulating factor effectively mobilizes TCR γδ and NK cells providing an allograft potentially enhanced for the graft-versus-leukemia effect for allogeneic stem cell transplantation. Front Immunol. 2021;12:625165.
8. Chandra S, Ascui G, Riffelmacher T, et al. Transcriptomes and metabolism define mouse and human MAIT cell populations. Sci Immunol. 2023;8(89):eabn8531.
9. Treiner E, Duban L, Bahram S, et al. Selection of evolutionarily conserved mucosal-associated invariant T cells by MR1. Nature. 2003;422(6928):164-169.
10. Legoux F, Salou M, Lantz O. MAIT cell development and functions: the microbial connection. Immunity. 2020;53(4):710-723.
11. Cogswell DT, Gapin L, Tobin HM, McCarter MD, Tobin RP. MAIT cells: partners or enemies in cancer immunotherapy? Cancers (Basel). 2021;13(7):1502.
12. du Halgouet A, Darbois A, Alkobtawi M, et al. Role of MR1-driven signals and amphiregulin on the recruitment and repair function of MAIT cells during skin wound healing. Immunity. 2023;56(1):78-92.e6.
13. Constantinides MG, Link VM, Tamoutounour S, et al. MAIT cells are imprinted by the microbiota in early life and promote tissue repair. Science. 2019;366(6464):eaax6624.
14. Slichter CK, McDavid A, Miller HW, et al. Distinct activation thresholds of human conventional and innate-like memory T cells. JCI Insight. 2016;1(8):e86292.
15. Schubert K, Karkossa I, Schor J, et al. A multi-omics analysis of mucosal-associated-invariant T cells reveals key drivers of distinct modes of activation. Front Immunol. 2021;12:616967.
16. Dias J, Boulouis C, Gorin JB, et al. The CD4-CD8- MAIT cell subpopulation is a functionally distinct subset developmentally related to the main CD8+ MAIT cell pool. Proc Natl Acad Sci U S A. 2018;115(49):E11513-E11522.
17. Nel I, Bertrand L, Toubal A, Lehuen A. MAIT cells, guardians of skin and mucosa? Mucosal Immunol. 2021;14(4):803-814.
18. Hinks TSC, Marchi E, Jabeen M, et al. Activation and in vivo evolution of the MAIT cell transcriptome in mice and humans reveals tissue repair functionality. Cell Rep. 2019;28(12):3249-3262.e5.
19. Lamichhane R, Schneider M, de la Harpe SM, et al. TCR- or cytokine-activated CD8+ mucosal-associated invariant T cells are rapid polyfunctional effectors that can coordinate immune responses. Cell Rep. 2019;28(12):3061-3076.e5.
20. Leng T, Akther HD, Hackstein CP, et al. TCR and inflammatory signals tune human MAIT cells to exert specific tissue repair and effector functions. Cell Rep. 2019;28(12):3077-3091.e5.
21. Gao MG, Hong Y, Zhao XY, et al. The potential roles of mucosa-associated invariant T cells in the pathogenesis of gut graft-versus-host disease after hematopoietic stem cell transplantation. Front Immunol. 2021;12:720354.
22. Gherardin NA, Loh L, Admojo L, et al. Enumeration, functional responses and cytotoxic capacity of MAIT cells in newly diagnosed and relapsed multiple myeloma. Sci Rep. 2018;8(1):4159.
23. Chen Z, Wang H, D'Souza C, et al. Mucosal-associated invariant T-cell activation and accumulation after in vivo infection depends on microbial riboflavin synthesis and co-stimulatory signals. Mucosal Immunol. 2017;10(1):58-68.
24. Varelias A, Gartlan KH, Wilkinson AN, et al. Expansion of IL-17A-secreting CD8+ mucosa-associated invariant T cells in peripheral blood following stem cell mobilization. Blood Adv. 2019;3(5):718-723.
25. Yu H, Yang A, Liu L, Mak JYW, Fairlie DP, Cowley S. CXCL16 stimulates antigen-induced MAIT cell accumulation but trafficking during lung infection is CXCR6-independent. Front Immunol. 2020;11:1773.
26. Gnirck AC, Philipp MS, Waterhölter A, et al. Mucosal-associated invariant T cells contribute to suppression of inflammatory myeloid cells in immune-mediated kidney disease. Nat Commun. 2023;14(1):7372.
27. Novak J, Dobrovolny J, Brozova J, Novakova L, Kozak T. Recovery of mucosal-associated invariant T cells after myeloablative

chemotherapy and autologous peripheral blood stem cell transplantation. Clin Exp Med. 2016;16(4):529-537.
28. Bhattacharyya A, Hanafi LA, Sheih A, et al. Graft-derived reconstitution of mucosal-associated invariant T cells after allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant. 2018;24(2):242-251.
29. Butler A, Hoffman P, Smibert P, Papalexi E, Satija R. Integrating single-cell transcriptomic data across different conditions, technologies, and species. Nat Biotechnol. 2018;36(5):411-420.
30. Yu G, Wang LG, Han Y, He QY. clusterProfiler: an R package for comparing biological themes among gene clusters. OMICS. 2012;16(5):284-287.
31. You G, Zhang M, Bian Z, et al. Decoding lymphomyeloid divergence and immune hyporesponsiveness in G-CSF-primed human bone marrow by single-cell RNA-seq. Cell Discov. 2022;8(1):59.
32. Rouillard AD, Gundersen GW, Fernandez NF, et al. The harmonizome: a collection of processed datasets gathered to serve and mine knowledge about genes and proteins. Database (Oxford). 2016;2016:baw100.
33. Ramsay NK, Kersey JH, Robison LL, et al. A randomized study of the prevention of acute graft-versus-host disease. N Engl J Med. 1982;306(7):392-397.
34. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus Conference on Acute GVHD Grading. Bone Marrow Transplant. 1995;15(6):825-828.
35. Riffelmacher T, Paynich Murray M, Wientjens C, et al. Divergent metabolic programmes control two populations of MAIT cells that protect the lung. Nat Cell Biol. 2023;25(6):877-891.
36. van Wilgenburg B, Loh L, Chen Z, et al. MAIT cells contribute to protection against lethal influenza infection in vivo. Nat Commun. 2018;9(1):4706.
37. Zhao Z, Wang H, Shi M, et al. Francisella tularensis induces Th1 like MAIT cells conferring protection against systemic and local infection. Nat Commun. 2021;12(1):4355.
38. Taylor GA, Carballo E, Lee DM, et al. A pathogenetic role for TNF alpha in the syndrome of cachexia, arthritis, and autoimmunity resulting from tristetraprolin (TTP) deficiency. Immunity. 1996;4(5):445-454.
39. Dan Lu, Liu L, Sun Y, et al. The phosphatase PAC1 acts as a T cell suppressor and attenuates host antitumor immunity. Nat Immunol. 2020;21(3):287-297.
40. Milner JJ, Toma C, Yu B, et al. Runx3 programs CD8+ T cell residency in non-lymphoid tissues and tumours. Nature. 2017;552(7684):253-257.
41. Legoux F, Gilet J, Procopio E, Echasserieau K, Bernardeau K, Lantz O. Molecular mechanisms of lineage decisions in metabolite-specific T cells. Nat Immunol. 2019;20(9):1244-1255.
42. Gao M, Zhao X. Insights into the tissue repair features of MAIT cells. Front Immunol. 2024;15:1432651.
43. Vorkas CK, Krishna C, Li K, et al. Single-cell transcriptional profiling reveals signatures of helper, effector, and regulatory MAIT cells during homeostasis and activation. J Immunol. 2022;208(5):1042-1056.
44. Bugaut H, El Morr Y, Mestdagh M, et al. A conserved transcriptional program for MAIT cells across mammalian evolution. J Exp Med. 2024;221(2):e20231487.
45. Dias J, Leeansyah E, Sandberg JK. Multiple layers of heterogeneity and subset diversity in human MAIT cell responses to distinct microorganisms and to innate cytokines. Proc Natl Acad Sci U S A. 2017;114(27):E5434-E5443.
46. Makita S, Takatori H, Nakajima H. Post-transcriptional regulation of immune responses and inflammatory diseases by RNA-binding ZFP36 family proteins. Front Immunol. 2021;12:711633.
47. Varelias A, Bunting MD, Ormerod KL, et al. Recipient mucosal-associated invariant T cells control GVHD within the colon. J Clin Invest. 2018;128(5):1919-1936.
48. Lee JS, Tato CM, Joyce-Shaikh B, et al. Interleukin-23-independent IL-17 production regulates intestinal epithelial permeability. Immunity. 2015;43(4):727-738.
49. El Morr Y, Fürstenheim M, Mestdagh M, et al. MAIT cells monitor intestinal dysbiosis and contribute to host protection during colitis. Sci Immunol. 2024;9(96):eadi8954.
50. Rouxel O, Da Silva J, Beaudoin L, et al. Cytotoxic and regulatory roles of mucosal-associated invariant T cells in type 1 diabetes. Nat Immunol. 2017;18(12):1321-1331.
51. Galaverna F, Flamini S, De Luca CD, et al. Mucosal-associated invariant T cells are functionally impaired in pediatric and young adult patients following allogeneic hematopoietic stem cell transplantation and their recovery correlates with clinical outcomes. Haematologica. 2024;109(10):3222-3236.
52. Wang Z, Zhang S, Zhang X, et al. Mucosal-associated invariant T cells predict increased acute graft-versus-host-disease incidence in patients receiving allogeneic hematopoietic stem cell transplantation. Cancer Cell Int. 2022;22(1):297.
53. Leeansyah E, Ganesh A, Quigley MF, et al. Activation, exhaustion, and persistent decline of the antimicrobial MR1-restricted MAIT-cell population in chronic HIV-1 infection. Blood. 2013;121(7):1124-1135.