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Macrophage migration inhibitory factor promotes immune evasion in multiple myeloma through myeloid-derived suppressor cell immunosuppression and major histocompatibility complex class I downregulation: implications for targeted therapy

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Conceptualization: YZ, JH; Methodology: JW, JH, JX, PZ, XZ, SH, LL, XL, HL, ZM, YZ, QG, HY, YF, XZ, FW, DZ, LZ, EB; Visualization: JW, JH, FW; Funding acquisition: YZ, WT; Project administration: YZ, JH, JW; Supervision: YZ, JH, TN; Writing – original draft: JW, JH; Writing – review & editing: JW, JH, YZ.

Conflict of interest

Authors declare that they have no conflict interests.

Data-sharing statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Short title: MIF-Mediated Immunosuppression and Immune Escape in MM

Abstract

Macrophage migration inhibitory factor (MIF), derived from either tumor cells or the tumor microenvironment, promotes multiple myeloma (MM) progression by suppressing T cell-mediated antitumor responses. Here, we demonstrate that MIF drives immune evasion in MM through both TME-mediated and tumor-intrinsic mechanisms. In the TME, MIF promotes the expansion of myeloid-derived suppressor cells (MDSCs) and enhances the expression of immunosuppressive molecules, including CD84, PD-L1, and CD38, thereby augmenting MDSC-mediated immunosuppression and impairing T cell function. In MM cells, MIF suppresses the COPS5/STAT1/NLRC5 signaling axis, resulting in downregulation of the major histocompatibility complex class I (MHC-I) antigen presentation pathway and reduced T cell-mediated cytotoxicity against MM cells. Therapeutically, combined treatment with the MIF inhibitor 4-IPP and dexamethasone delayed MM progression and prolonged survival in a mouse MM model. In summary, our findings reveal MIF as a critical mediator of immune escape in MM and highlight the therapeutic potential of MIF-targeted strategies for MM treatment.

Introduction

Multiple myeloma (MM) is a prevalent hematologic malignancy defined by the clonal proliferation of plasma cells within the bone marrow. Despite therapeutic advances, MM remains incurable.¹ Interactions between MM cells and the suppressive tumor microenvironment (TME) compromise immune surveillance, enabling malignant plasma cells to evade recognition and elimination.² Antitumor immune dysfunction plays a central role in driving MM initiation, progression, relapse, and resistance to therapy.³ The development of an immunosuppressive TME is a major driver of immune escape in MM.⁴

Macrophage migration inhibitory factor (MIF) is a secreted cytokine produced by a broad range of cells, including myeloid, lymphoid, and epithelial cells, as well as by tumor cells.⁵ Through autocrine and paracrine signaling, MIF orchestrates systemic inflammation⁵ and modulates both innate and adaptive immune responses within the TME across multiple cancer types.⁶ In MM, increased MIF expression in the MM cells and TME has been reported and is significantly correlated with disease progression and unfavorable prognosis.^{7, 8} MIF shows promise as a predictive biomarker of response to proteasome inhibitor therapy in MM.⁸ A recent study using single-cell RNA-seq reports that MIF is highly expressed in malignant plasma cells and myeloid compartments, where it shapes an immunosuppressive microenvironment that supports disease progression. Elevated MIF levels are associated with adverse clinical features and poorer prognosis, underscoring its clinical relevance in MM.⁹ A clinical study found that elevated serum MIF levels in newly diagnosed MM (NDMM) patients are an independent predictor of poor progression-free and overall survival.¹⁰ However, the mechanisms by which MIF regulates tumor immunity in MM remain largely unexplored. Given the pivotal role of MIF in immune regulation, this study aims to elucidate the mechanisms through which MIF shapes the immune microenvironment in MM.

Methods

Clinical Samples

Peripheral blood (PB) samples from newly diagnosed and relapsed/refractory MM patients were obtained from the Department of Hematology, West China Hospital, Sichuan University. The diagnostic criteria were based on the Chinese Guidelines for the Diagnosis and Treatment of

MM.¹¹ The study involving human participants was approved by the Ethics Committee of West China Hospital, Sichuan University (Approval No. 114).

Animal study

The animal experiments were approved by the Experimental Animal Ethics Committee of West China Hospital of Sichuan University (Approval No. 20211248A). All the mice were housed under specific pathogen-free conditions. The mice used in this study included immunocompetent mice C57BL/KaLwRij (RRID: MGI:8212865), C57BL/6NGpt (RRID: MGI:2159965), C57BL/6N-*Mif*^{-/-} and severely immunodeficient NCG mice (NOD/ShiLtJGpt-*Prkdc*^{em26Cd52}*Il2rg*^{em26Cd22}/Gpt; RRID: MGI:6770253).

In the 5TMM model, C57BL/KaLwRij mice were inoculated subcutaneously on the right dorsum or via the tail vein with luciferase-labeled WT 5TGM1 or MIF-KO 5TGM1 cells at a dose of 2.0×10^6 cells/100 μ L per mouse. In the Vk12598 model, C57BL/6N mice received intravenous tail vein injections of Vk12598 MM cells at 1.0×10^6 cells/100 μ L per mouse (Vk model). For the NCG model, mice were subcutaneously injected in the right flank with either WT 5TGM1 or MIF-KO 5TGM1 cells at a dose of 2.0×10^6 cells/100 μ L per mouse.

WT 5TGM1 cells were subcutaneously inoculated into C57BL/KaLwRij mice, and treatments were initiated when the tumor diameter reached approximately 4–5 mm. The regimen consisted of intraperitoneal administration of 4-IPP (0.5 mg/mouse every three days,^{7, 8} and dexamethasone (1 mg/kg on Days 1–5 weekly) (**Table S1**, Detailed information of reagents). Tumor size was measured every 2–3 days and calculated using the formula $\text{volume} = (\text{length}) \times (\text{width})^2 \times 0.52$. The endpoint was defined when the size of the tumor reached 1.5 cm in its longest dimension or 1500 mm³ in volume.

MDSC adoptive transfer

Six- to eight-week-old sex-matched C57BL/6N *Mif*^{-/-} mice were used. From day -15 to day 0, mice in the experimental group received intravenous injections of 2×10^6 MDSCs every other day. On day 0, all mice were injected with Vk12598 MM cells. Mice in the experimental group received intravenous injections of 2×10^6 MDSCs every 4th day thereafter. Disease onset was monitored weekly by measuring serum M protein levels in PB (peripheral blood).

Additional methods

Additional methods and materials are available in the Online Supplementary Appendix.

Results

MIF suppresses the tumor-eliminating functions of T cells

Immunocompetent C57BL/KaLwRij mice were intravenously or subcutaneously injected with CTR-KO or MIF-KO 5TGM1 cells to establish the murine MM 5TMM model (**Table S2**, sgRNA sequences). The results revealed that mice intravenously injected with MIF-KO 5TGM1 cells failed to form tumors (**Figure S1A**). In mice subcutaneously inoculated with MIF-KO 5TGM1 cells, tumors formed one-week post-injection but regressed to disappearance at approximately 3 weeks. In vivo imaging of tumors and tumor volume data are shown in **Figure 1A** and **B**. The regression of subcutaneous MIF-KO tumors suggested that anti-MM immune responses were effectively activated in the inoculated mice. Alternatively, we intravenously injected the Vk*MYC-derived MM cell line Vk12598 MM into MIF knockout (*Mif*^{-/-}) and wild-type (WT) mice (**Figure S1B**). At 30–35 days post-inoculation, serum protein electrophoresis analysis revealed M protein in all the WT mice but not in the *Mif*^{-/-} mice, indicating that MM tumorigenesis depends on microenvironmental MIF (**Figure S1B**). Importantly, knocking out *Mif* did not impair 5TGM1 cell proliferation in vitro (**Figure S1C**). In severely immunodeficient mice, both MIF-KO and CTR-KO MM cells formed tumors without notable differences (**Figure S1D**). Therefore, the results of the aforementioned animal experiments suggested that MIF promoted MM progression via immunosuppression.

Next, we investigated immune cell involvement in MIF-driven tumor growth. Notably, in the subcutaneous 5TMM model, flow cytometry revealed no significant differences in the infiltration or function of NK cells (**Figure S2A**), macrophages (**Figure S2B**), or T cells (**Figure S2C-F**) within tumors between CTR-KO and MIF-KO groups. Further analyses revealed that on day 20 post-inoculation, the MIF-KO-inoculated mice presented a greater ratio of IFN- γ -expressing CD4⁺ and CD8⁺ T cells in the PB than the CTR-KO-inoculated mice did (**Figure 1C**). The same was true for TNF- α -expressing CD4⁺ and CD8⁺ T cells (**Figure 1D**). The gating strategies used to identify T-cell subsets are shown in **Figure S3**. Importantly, the expression levels of IFN- γ and TNF- α in CD4⁺ and CD8⁺ T cells changed in a time-dependent manner following tumor cell

inoculation (**Figure 1E, F**). However, the regulatory T (Treg) frequency exhibited consistent trends between the two groups (**Figure S4A**). In MIF-KO inoculated mice, the frequencies of IFN- γ^+ /TNF- α^+ CD4 $^+$ and CD8 $^+$ T cells peaked around day 20 and then declined, reflected the time course of the anti-tumor immune response and coincided with tumor clearance.

The above results demonstrated that MIF deficiency in MM cells enhanced T cell functions in the 5TMM model. We then assessed how MIF deficiency in the TME affects T cell responses. The transplantable Vk12598 cell line failed to establish MM in *Mif* $^{-/-}$ mice (**Figure S1B**). Following tumor cell inoculation, the *Mif* $^{-/-}$ group exhibited increased IFN- γ secretion by CD8 $^+$ T cells (**Figure S4B**) and increased TNF- α production by CD4 $^+$ T cells compared to WT mice (**Figure S4C**). In conclusion, MIF derived from either tumor cells or the microenvironment suppressed the production of IFN- γ and TNF- α by T cells.

To assess whether the observed functional differences in T cells resulted in enhanced tumor cell killing, we reintroduced antigen stimulation by performing a secondary inoculation of MIF-KO 5TGM1 cells into the contralateral side of MIF-KO-inoculated mice in the 5TMM model. After secondary inoculation, splenic CD3 $^+$ T cells were isolated and cocultured with either CTR-KO or MIF-KO 5TGM1 cells. The results showed that the stimulated T cells exhibited significant cytotoxicity against both CTR-KO and MIF-KO tumor cells, compared to T cells from WT mice (**Figure S5A, B**).

MIF promotes tumor growth by suppressing T cell functions via MDSCs

MDSCs are well-established immunosuppressive cells in the MM microenvironment and are capable of potently inhibiting T cell activity.¹² When subcutaneous tumor growth was significantly slowed in the MIF-KO group (**Figure 1B**, days 15–17 post tumor inoculation), the CTR-KO group presented a markedly increased proportion of total MDSCs (CD11b $^+$ Gr-1 $^+$) in PB (**Figure 2A**), along with a significantly greater proportion of MDSCs among CD11b $^+$ cells in subcutaneous tumors (**Figure 2B**), than did the MIF-KO group. These findings were validated in WT and *Mif* $^{-/-}$ mice using the Vk12598 model, which showed a significantly lower proportion of MDSCs in the PB of *Mif* $^{-/-}$ mice compared to WT mice (**Figure 2C**). These findings suggest that MIF may promote immunosuppression by enhancing MDSC expansion.

We next investigated the role of MIF in MDSC-mediated T cell dysfunction using a trans-well system to generate MDSCs in vitro under two conditions (**Figure S6A**). In protocol 1, tumor cells in the upper chamber were either CTR-KO (WT) or MIF-KO (**Figure S6A**, upper). In protocol 2, bone marrow mononuclear cells (BMMCs) from WT or *Mif*^{-/-} mice were differentiated into MDSCs (**Figure S6A**, lower). The resulting MDSCs were then cocultured with WT splenic CD3⁺ T cells. T cell proliferation assays revealed that MDSCs from a MIF-high TME, whether MIF derived from tumor cells or MDSCs, significantly suppressed T cell proliferation (**Figure S6B**). Similarly, these MDSCs inhibited T cell secretion of IFN- γ and TNF- α , irrespective of MIF source (**Figure 2D, E**). MIF also promoted the immunosuppressive function of MDSCs and suppressed T cell effector function by reducing IFN- γ expression in a human MM ex vivo coculture model (**Figure S7A, B**).

Mif^{-/-} mice did not develop tumors after MM cell inoculation. To further investigate the role of MDSCs in MM initiation and progression, induced MDSCs ($\geq 90\%$ purity; **Figure S6C**) were administered to *Mif*^{-/-} mice (experimental group) intravenously, followed by inoculation with Vk12598 MM cells (**Figure 2F**, upper). Serum protein electrophoresis revealed that none of the control group mice (*Mif*^{-/-} mice inoculated with Vk12598 MM cells only) developed tumors, whereas all the mice in the experimental group (*Mif*^{-/-} mice that received MDSC transfer) formed tumors (**Figure 2F**, lower). Flow cytometry confirmed tumor formation in *Mif*^{-/-} mice after adoptive MDSC transfer (**Figure S6D**), with tumor infiltration in the liver or mesenteric lymph nodes (**Figure S6E**). This provides direct evidence that MDSCs act as key effector cells in MIF-mediated tumor immune escape.

MIF plays a critical role in maintaining the immunosuppressive phenotypes of MDSCs

To investigate the regulatory role of MIF in MDSC function, PB MDSCs were isolated from the 5TMM mouse model for subsequent proteomic analysis. Proteomic results revealed significantly higher expression of IL-18 and CD38 in the CTR-KO group (**Figure 3A**). IL-18 has been shown to promote the expansion and function of MDSCs, thereby accelerating MM progression through MDSC-mediated immunosuppression.¹³ Serum ELISA analysis revealed increased levels of IL-18 in the CTR-KO group of the 5TMM model (**Figure 3B**). Ex vivo culture of MDSCs derived from BMMCs revealed that WT MDSCs secreted higher levels of IL-18 than did those from *Mif*^{-/-} mice (**Figure 3C**, left). MDSCs were isolated from Vk12598 tumor-bearing mice on day 10 post-

inoculation. RT-qPCR analysis revealed significantly lower IL-18 mRNA levels in *Mif*^{-/-} MDSCs compared to WT MDSCs. (**Figure 3C**, right).

CD38 expression on MDSCs has been reported to be correlated with enhanced suppression of T cell function.¹⁴ CD84 and PD-L1 are established markers associated with the immunosuppressive phenotypes of MDSCs.^{15, 16} In the 5TMM model, compared with the MIF-KO group, the CTR-KO group presented significantly higher levels of CD38, CD84 and PD-L1 on MDSCs in the PB (**Figure 3D**). The elevated expression of CD38 was also detected in human derived MDSCs cocultured with human CTR-KO MM cells, comparing with MIF-KO group (**Figure S7C**). Increased PD-L1 expression on MDSCs from the CTR-KO group was also detected in secondary lymphoid organs, including the lymph nodes and spleen (**Figure 3E**). Consistently, PD-1 expression on both CD4⁺ and CD8⁺ T cells in the PB (**Figure S8A, B**) and spleen (**Figure S8C, D**) was significantly greater in the CTR-KO group than in the MIF-KO group at the experimental endpoint. Moreover, in the Vk12598 model, the expression levels of CD38, CD84, and PD-L1 on MDSCs in WT mice were significantly greater than those in *Mif*^{-/-} mice (**Figure 3F**). Correspondingly, PD-1 expression was higher on CD4⁺ and CD8⁺ T cells in the PB of WT mice (**Figure S8E, F**). In summary, MIF maintains the immunosuppressive functions of MDSCs.

Peripheral blood MIF levels positively correlate with MDSC frequency in MM patients

We evaluated the correlation between the PB MIF concentration and MDSC levels in MM patients. The median PB MIF level in relapsed/refractory MM patients (RRMM, n=15) was 198.25 (179.10–259.88) ng/mL, which was significantly higher than that in NDMM patients (NDMM, n = 82; 172.06 [33.26–244.43] ng/mL; *P*=0.009) and patients in remission (n=25; 179.46 [55.65–199.60] ng/mL; *P*=0.024). Healthy controls (n=12) had a mean PB MIF concentration of 54.60 ± 23.00 ng/mL. MIF levels in NDMM, RRMM, and remission patients were significantly elevated compared with those in healthy controls (*P* < 0.0001) (**Figure 4A**).

Unlike murine MDSCs, which are characterized by CD11b⁺Gr-1⁺ expression, human MDSCs lack a Gr-1 homolog and thus have no pan-MDSC marker.¹⁶ Human monocytic MDSCs (M-MDSCs) are identified as CD11b⁺CD14⁺HLA-DR^{-/low}CD15⁻ cells.¹⁷ The concentration of PB MIF was moderately correlated with the proportion of M-MDSCs (CD11b⁺CD14⁺HLA-DR^{-/low}CD15⁻) among CD11b⁺ cells (*r*_s=0.545, *P*=0.000; **Figure 4B**), the proportion of CD84⁺ M-MDSCs among CD11b⁺ cells (*r*_s=0.508, *P*=0.000; **Figure 4C**), and the proportion of PD-L1⁺ M-MDSCs among

CD11b⁺ cells ($r_s=0.411$, $P=0.000$; **Figure 4D**). CD38 expression on MDSCs was simultaneously detected in 31 patients with NDMM. The ratio of CD38⁺M-MDSCs to CD11b⁺ cells was positively correlated with the ratio of CD84⁺M-MDSCs to CD11b⁺ cells ($r_s=0.623$, $P=0.000$; **Figure 4E**) and the ratio of PD-L1⁺M-MDSCs to CD11b⁺ cells ($r_s=0.581$, $P=0.001$; **Figure 4F**). The analyses of the aforementioned clinical samples indicated that serum MIF levels in MM patients were positively correlated with both the tumor burden and PB MDSC levels.

MIF deficiency promotes the expansion of dendritic cells

To identify additional immune cell populations involved in MIF-mediated immunosuppression, we performed mass cytometry on subcutaneous tumors from the CTR-KO and MIF-KO 5TMM models, revealing a differentially abundant cluster (Cluster 13, **Figure 5A**, left) that was significantly enriched in MIF-KO tumors (**Figure 5A**, right). This cluster exhibited a CD11b⁺CD206^{high}CD196^{high}MHC-II⁺ signature (**Figure S9**), which is consistent with previously described dendritic cells (DCs).¹⁸ Increased frequencies of this population were also observed in both the PB and spleen in the MIF-KO group (**Figure 5B**). In the draining lymph nodes, the DC frequency was moderately greater in the MIF-KO group than in the CTR-KO group (**Figure 5B**). CD8⁺ DCs are a specialized subset capable of cross-presenting antigens via major histocompatibility complex class I (MHC-I) to CD8⁺ T cells.¹⁹ In our study, the proportions of CD8⁺ DCs among CD45⁺ cells were significantly elevated in the draining lymph nodes and spleen in the MIF-KO group (**Figure 5C**). This increase in DCs was further confirmed in the Vk12598 model, where *Mif*^{-/-} mice presented significantly more CD8⁺ DCs in the PB and spleen compared to WT controls (**Figure 5D**). These results showed that MIF deficiency promoted the expansion of DCs.

MIF suppresses MHC-I expression and the associated antigen presentation pathway through transcriptional inhibition of NLRC5

Given the established role of MIF in immune evasion, we hypothesized it also regulates tumor-intrinsic immunogenicity. Proteomics of MIF-KO cells confirmed this, showing significant upregulation of the antigen processing and presentation (APP) pathway (**Figure S10A**) and enrichment of MHC-I-related molecules (**Figure S10B**). The significantly increased expression of MHC-I in the MIF-KO 5TGM1 cells was confirmed by flow cytometry (**Figure 6A**). The impact

of MIF on MHC-I expression was further validated in vivo. On day 14 of the subcutaneous 5TMM model, flow cytometry of tumor tissues revealed significantly greater MHC-I expression on plasma cells (CD138⁺) in the MIF-KO group than in the CTR-KO group (**Figure 6B**).

To investigate the clinical relevance of our findings, we analyzed human MM cell lines and patient transcriptomic datasets. A modest inverse correlation between MIF and HLA-A, HLA-B, and HLA-C expression was observed in DepMap (Expression Public 26Q1, <https://depmap.org/portal>) MM cell lines ($r = -0.24$) (**Figure S11A**), and between MIF and HLA-A expression in the GSE58133 cohort ($r = -0.218$) (**Figure S11B**). However, similar associations were not consistently detected in other patient cohorts (**Figure S11C**), indicating that the relationship between MIF and MHC-I expression may be context-dependent.

Treatment of CTR-KO 5TGM1 cells with the MIF antagonist ISO-1 (100 μ M, 48 h) resulted in increased MHC-I expression (**Figure 6C**, left). This effect was verified in a human MM cell line, as treatment of KMS-11 cells with 200 or 400 μ M ISO-1 for 48 h enhanced MHC-I expression (**Figure S11D**). Conversely, supplementation with recombinant murine MIF (rmMIF) protein (200 ng/ml, 24 h) reduced MHC-I levels in MIF-KO 5TGM1 cells (**Figure 6C**, right). To investigate the mechanism underlying elevated MHC-I expression in MIF-KO cells, we performed proteomic analysis, which revealed significant upregulation of NLRC5, a key transcriptional activator of MHC-I (**Figure S12A**).²⁰ Western blot analysis confirmed that the MIF-KO 5TGM1 cells expressed higher levels of NLRC5 than the CTR-KO cells (**Figure 6D**, left). The RT-qPCR results demonstrated that MHC-I-associated genes (differentially expressed proteins identified via proteomics, including *H2-D1*, *H2-K1*, *H2-M3*, *H2-Q7*, *H2-Q8*, *B2M* and *NLRC5*) (**Table S3**, RT-qPCR primers) were significantly upregulated in MIF-KO cells compared with CTR-KO cells at transcriptional level (**Figure 6D**, right). Notably, the expression of the transcriptional regulator *NLRC5* was increased by 12.7-fold in MIF-KO cells compared with that in CTR-KO cells (**Figure 6D**, right).

CTR-KO-OVA and MIF-KO-OVA cells were generated to elucidate the role of MIF in MHC-I antigen presentation. In this model, intracellular OVA is processed into antigenic peptides (SIINFEKL) that are presented by H-2Kb, enabling the evaluation of MHC-I-dependent antigen presentation.²¹ Flow cytometry analysis revealed significantly higher basal MHC-I expression in MIF-KO-OVA cells (**Figure S12B**), along with increased surface presentation of the processed

OVA peptide in complex with H2-Kb (**Figure 6E**). Treatment of CTR-KO-OVA cells with ISO-1 increased the presentation of processed OVA peptides on the cell surface (**Figure 6F**, left). Conversely, exogenous rmMIF reduced peptides presentation on MIF-KO-OVA cells (**Figure 6F**, right), suggesting that MIF negatively regulates MHC-I-mediated antigen presentation in 5TGM1 cells. To assess whether the observed alterations in antigen presentation led to enhanced activation of antigen-specific T cells, we employed a B3Z-based MHC class I antigen presentation assay. B3Z cells are CD8⁺ T cell hybridomas carrying an NFAT-driven lacZ reporter that expresses β -galactosidase following T-cell receptor recognition of the SIINFEKL:H-2Kb complex (**Figure S12C**).²² B3Z cells cocultured with MIF-KO-OVA cells exhibited significantly higher β -galactosidase activity than those cocultured with CTR-KO-OVA cells (**Figure S12D**), providing functional evidence that MIF deficiency enhances MHC-I-dependent antigen presentation and promotes antigen-specific T-cell activation. Consistent with these functional findings, western blot analysis revealed that compared with CTR-KO cells, MIF-KO 5TGM1 cells exhibited decreased PSMB5 expression but increased levels of immunoproteasome subunits PSMB8 and PSMB9, together with TAPBP (**Figure S12E**). PSMB8, PSMB9, and TAPBP are essential components of antigen processing and MHC-I peptide loading, and are transcriptionally regulated by NLRC5.²³ These findings provide a mechanistic connection between MIF deficiency, NLRC5 activation, enhanced antigen processing, and improved MHC-I antigen presentation.

MIF deficiency enhances MHC-I expression through activation of the COPS5/STAT1/NLRC5 signaling axis

We next sought to determine how MIF regulates *NLRC5* expression in MM cells. Proteomic analysis revealed significant upregulation of STAT1 in MIF-KO cells (**Figure 7A**, left). Since STAT1 is a known transcriptional regulator of *NLRC5*,²⁴ we hypothesized that STAT1 mediated the MIF-dependent suppression of MHC-I expression and the MHC-I-associated APP pathway in MM cells. Western blot analysis confirmed that the MIF-KO 5TGM1 cells presented increased STAT1 expression (**Figure 7A**, middle and right). Consistently, STAT1 expression was upregulated in CTR-KO cells following treatment with ISO-1, whereas supplementation with rmMIF in MIF-KO cells reduced STAT1 levels (**Figure 7B**, left). Together, these results demonstrated that the loss of MIF in MM cells led to STAT1 upregulation. ChIP-qPCR analysis comparing CTR-KO and MIF-KO 5TGM1 cells revealed increased STAT1 enrichment at the *NLRC5* promoter region in MIF-KO cells (**Figure 7B**, right) (**Table S4**, ChIP-qPCR primers).

MIF signals through receptors CD74, CXCR4, CD44, and COPS5.^{25, 26} Despite their unchanged expression in MIF-KO cells (**Figure S13A**), we individually knocked them down to identify which mediates MIF's suppression of NLRC5 expression (**Table S2**, shRNA sequences). shRNA-mediated knockdown of MIF receptors revealed that reducing *CXCR4* (**Figure S13B**), *CD74* (**Figure S13C**), or *CD44* (**Figure S13D**) had no significant effect on *NLRC5* mRNA levels, whereas *COPS5* knockdown (**Figure S13E**) tended to decrease *NLRC5* levels. These findings suggest that MIF may regulate *NLRC5* expression through COPS5. In COPS5-KD 5TGM1 cells, both STAT1 and p-STAT1 levels, as well as MHC-I expression (**Figure 7C**), were significantly reduced. Since MIF inhibits COPS5 function,²⁶ we reasoned that MIF may downregulate *NLRC5* via COPS5. To test this, we treated cells with the COPS5 inhibitor thiolutin (THL).²⁷ Treatment of MIF-KO 5TGM1 cells with 3 μ M THL for 12 h significantly downregulated *NLRC5* mRNA levels and MHC-I expression (**Figure 7D**, left and middle). Additionally, treatment with THL significantly reduced STAT1, p-STAT1, and *NLRC5* protein levels (**Figure 7D**, right). The mRNA levels of STAT1 remained unchanged in both the MIF-KO and COPS5-KD 5TGM1 cells (**Figure S14A**) and were slightly upregulated following THL treatment (**Figure S14B**). Given the observed discrepancy between the STAT1 mRNA and protein expression levels, cycloheximide chase assays were conducted, which revealed accelerated STAT1 protein degradation in CTR-KO cells (**Figure S14C, D**). Given COPS5's role as the COP9 signalosome catalytic subunit that suppresses cullin-RING ligase activity via deneddylation,²⁸ we investigated how functional COPS5 stabilizes STAT1 protein in MIF-KO cells. Based on these evidences, we compared STAT1 ubiquitination levels in CTR-KO and MIF-KO cells via ubiquitination analysis, which revealed increased polyubiquitination of STAT1 in CTR-KO cells (**Figure 7E**).

In MIF-KO MM cells, the removal of MIF-mediated negative regulation enhanced the function of COPS5. This led to suppressed polyubiquitination of STAT1, resulting in the upregulation of the downstream *NLRC5*/MHC-I axis (**Figure 7F**). Therefore, these results suggest that COPS5 acts as a key downstream effector of MIF in regulating MHC-I expression.

Targeting MIF for the treatment of MM

Previous studies have reported that the MIF inhibitor 4-IPP enhances the antitumor effects of melphalan or proteasome inhibitors in immunodeficient MM mouse models; however, it lacks therapeutic efficacy as a monotherapy.^{7, 8} On the basis of our findings regarding the role of MIF in

mediating the immunosuppressive effects of MM, the therapeutic potential of 4-IPP was evaluated in the immunocompetent 5TMM model. Given that dexamethasone (Dex) is a backbone agent in virtually all combination chemotherapy regimens for MM and exerts synergistic effects with multiple novel agents,²⁹ we further assessed the efficacy of combining 4-IPP with Dex, thereby strengthening the translational rationale for this combinatorial strategy.

In the 5TMM model, 4-IPP alone inhibited subcutaneous tumor growth and showed therapeutic efficacy comparable to that of Dex alone, as demonstrated by reductions in tumor volume and circulating monoclonal protein levels (**Figure 8A, B**). Compared with the PBS control or any monotherapy, the 4-IPP+Dex combination resulted in significantly enhanced tumor growth inhibition (**Figure 8A, B**) and prolonged survival (**Figure 8C**). Two weeks after treatment, the frequency of immunosuppressive cells in the PB was analyzed. Although the frequency of MDSCs in the 4-IPP + Dex group did not differ significantly from that in the PBS control group, the combination treatment resulted in a marked reduction in MDSC levels relative to those in the Dex alone group (**Figure 8D**). The dexamethasone-induced expansion of MDSCs may be attributed to their lymphodepleting effects.³⁰ The addition of 4-IPP could mitigate this effect. Compared with the PBS control or Dex alone, treatment with 4-IPP alone reduced PD-1 expression on both CD4⁺ and CD8⁺ T cells, but the combination treatment did not further enhance these effects (**Figure 8E, F**). At the end of the experiment, routine blood parameters, liver function (total protein, albumin, alanine aminotransferase, total bilirubin), and renal function (creatinine) were measured in each group. No significant differences were observed between the groups (**Figure S15**).

Discussion

The cancer immunoediting theory posits that the immune system interacts with tumors through three phases—elimination, equilibrium, and escape—that shape tumor progression and immune evasion.³¹ Many studies have demonstrated that MM immune escape is a key mechanism of tumor progression.^{4, 32} Although evidence implicating MIF in tumor immune escape has been identified, the underlying molecular mechanisms remain unclear.³³

The precise mechanisms by which MIF modulates the immunoregulatory network within the MM microenvironment remain incompletely elucidated. Current evidence indicates that MIF promotes the expansion, recruitment, and immunosuppressive function of MDSCs, and together with CCL5 secreted by MM cells, is required for MDSC induction.^{34, 35} MIF further facilitates the

differentiation of bone marrow progenitors into MDSCs and increases their abundance, while MDSCs in turn enhance MIF and CCL5 production by MM cells, establishing a vicious cycle that further exacerbates immunosuppression.³⁴ In addition, MIF induces CD84 expression within the bone marrow microenvironment, which upregulates PD-L1 expression on MDSCs and suppresses T cell function.^{15, 34, 36} Beyond MDSCs, MIF has also been implicated in promoting the polarization of tumor-associated macrophages toward a pro-tumor M2 phenotype.^{9, 37}

Current understanding of MIF-mediated regulation of T-cell function remains limited. A lymphoma study showed that splenocyte cultures treated with MIF-neutralizing antibodies exhibited enhanced antitumor T-cell responses and increased IFN- γ production compared with control cultures, suggesting that immune cell-derived MIF can directly suppress T-cell immunity.³⁸ In addition, tumor-derived MIF has been reported to inhibit T-cell activation.³⁹ Consistent with these findings, our immunocompetent MM models demonstrated that MIF deficiency in either tumor cells or the TME enhanced CD4⁺ and CD8⁺ T-cell-mediated antitumor immunity, indicating that MIF promotes MM progression through T-cell suppression. Cancer-associated T-cell dysfunction is mediated by both TME-driven immunosuppressive mechanisms, including MDSCs, Tregs, and PD-L1, and tumor-intrinsic mechanisms, such as MHC-I downregulation, defective antigen presentation, and inhibitory ligand expression.⁴⁰

MDSCs are a heterogeneous population of immature myeloid cells that expand under pathological conditions, including cancer and chronic inflammation, where they promote immune evasion and tolerance.¹⁶ MDSCs are conventionally divided into monocytic (M-MDSCs) and polymorphonuclear (PMN-MDSCs) subsets according to their distinct myeloid lineage origins.¹⁷ In MM, elevated M-MDSC levels before autologous stem cell transplantation (ASCT) independently predict inferior clinical outcomes and reduced post-transplant responses, whereas lower levels are associated with prolonged time to progression.^{41, 42} Consistently, increased M-MDSC abundance correlates with MM relapse and poor therapeutic responses.^{36, 41} Therapeutically, immunomodulatory agents such as lenalidomide and pomalidomide can suppress MDSC generation,⁴³ while daratumumab may enhance antitumor immunity partly through MDSC reduction.⁴⁴ Functionally, increased expression of immunosuppressive molecules, including CD84 and CD38, contributes to MDSC-mediated immune suppression.^{14, 45}

In this study, we demonstrated that MIF promotes MDSC expansion and enhances the expression

of immunosuppressive markers, including CD38, on MDSCs. Proteomic analysis further revealed reduced IL-18 expression in MIF-deficient MDSCs. Previous studies have shown that IL-18 can regulate MDSC accumulation and immunosuppressive function in a tumor context-dependent manner, promoting M-MDSC expansion in a murine hepatocellular carcinoma model⁴⁶ and being associated with PMN-MDSC enrichment in MM patients.¹³ However, the detailed signaling mechanisms underlying MIF-mediated regulation of MDSCs remain to be further elucidated.

In our study, MIF deficiency led to an increased population of DCs, particularly the CD8⁺ DC subset. The CD8⁺ conventional dendritic cell (cDC1) subset plays a key role in cross-presenting tumor antigens to CD8⁺ T cells.^{19, 47} This aligns with reports that MIF knockdown enhances intratumoral CD11c⁺CD8⁺CD103⁺ DCs in breast cancer,⁴⁸ and that disrupting MIF-CD74 signaling in DCs boosts their ability to activate cytotoxic T cells in melanoma.⁴⁹ Together, these results suggest that MIF downregulation enhances the functional capacity of APCs, potentially promoting more robust antitumor immunity.

Our results demonstrated that MIF deficiency relieved the transcriptional inhibition of NLRC5, thereby activating NLRC5 downstream MHC-I class gene expression and enhancing the MHC-I-associated antigen-presenting capacity of tumor cells. To our knowledge, we are the first to reveal that the MIF/COPS5/STAT1/NLRC5 signaling axis regulates the MHC-I-associated antigen-presenting pathway. Our findings reveal a mechanism by which MIF regulates MM antigen processing and presentation. For a detailed investigation of this pathway, we are conducting studies to identify the specific ubiquitin ligases responsible for COPS5-regulated STAT1 polyubiquitination. Although MIF deficiency consistently increased MHC-I expression in our experimental models, the inverse association between MIF and MHC-I transcript levels was relatively weak and was not uniformly observed across independent MM patient cohorts. This discrepancy likely reflects the complexity of clinical specimens, in which MHC-I expression is regulated by multiple factors, including genetic alterations, molecular subtypes, interferon signaling, prior treatment, and bone marrow microenvironment composition. Therefore, transcript-level correlation analyses in heterogeneous patient cohorts may not fully capture the tumor cell-intrinsic regulatory effect revealed by genetic perturbation experiments.

Previous studies have demonstrated that the MIF inhibitor 4-IPP has therapeutic efficacy in mouse models of solid tumors, including neuroblastoma and melanoma.^{50, 51} Furthermore, 4-IPP has been

shown to enhance the antitumor effects of melphalan or proteasome inhibitors in immunodeficient MM mouse models.^{7, 8} Here, we evaluated the therapeutic efficacy of 4-IPP in treating MM in an immunocompetent mouse model, which was capable of dampening the immunosuppressive effects of immune cells. Our results suggested that the combination of 4-IPP and dexamethasone suppressed tumor growth and prolonged survival in a mouse MM model. Recently, a novel MIF inhibitor, IPG1094, was registered for a clinical trial (Phase I/II, ClinicalTrials.gov, ID: NCT06212076) in patients with advanced solid tumors. This is the first MIF small-molecule inhibitor to enter clinical trials. A related publication showed that this inhibitor was capable of inhibiting MDSC differentiation in vitro and reducing tumor growth in an orthotopic pancreatic cancer model, which was associated with decreased numbers of MDSCs and increased infiltration of CD8⁺ T cells in the TME.⁵² On the basis of our findings, IPG1094 holds promise for MM treatment, potentially exerting therapeutic effects by enhancing antitumor immunity.

In conclusion, our findings elucidate how MIF drives immune evasion in MM. MIF sustains the immunosuppressive phenotypes of MDSCs and inhibits T-cell cytotoxicity through MDSCs. Furthermore, MIF mediates the immune escape of MM cells by inhibiting MHC-I expression and associated antigen presentation through the COPS5/STAT1/NLRC5 signaling axis. These results highlight the therapeutic potential of targeting the immunosuppressive functions of MIF in MM treatment.

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Figure legends

Figure 1. MIF suppresses the tumor-eliminating functions of T cells. (A) Establishment of the 5TMM model in the CTR-KO and MIF-KO groups. In vivo imaging confirmed successful tumor engraftment in both groups on day 7. By week 3, tumor regression was observed specifically in the MIF-KO group. Representative images from each group (n=4) are shown. (B) Subcutaneous tumor growth curves for the CTR-KO and MIF-KO groups. (C, D) Flow cytometry of PB T-cell cytokine secretion: (C) IFN- γ ⁺ CD4⁺ T cells and IFN- γ ⁺ CD8⁺ T cells; (D) TNF- α ⁺ CD4⁺ T cells and TNF- α ⁺ CD8⁺ T cells; n=5 and 6 mice, respectively. Student's t test was performed. (E, F) IFN- γ (E) and TNF- α (F) secretion by CD4⁺ and CD8⁺ T cells in CTR-KO and MIF-KO mice during tumor progression. Two-way ANOVA with Tukey's post hoc test was performed. *P* values are shown as * *P* ≤0.05, ** *P* <0.01.

Figure 2. MIF promotes tumor growth by suppressing T cell functions via MDSCs. (A-B) 5TMM model: (A) Representative FACS plot of MDSCs (CD11b⁺Gr1⁺) among CD11b⁺ cells in PB and quantitative analysis, n=5/group. Student's t test was performed. (B) Tumor-infiltrating MDSCs among the CD11b⁺ population; n=4 and 5 mice, respectively. Student's t test was performed. (C) Percentage of PB MDSCs (gated within the CD45⁺ population) in the Vk model, n=6/group. Student's t test was performed. (D-E) MDSCs were induced following Protocol 1 and Protocol 2 (Figure. S6A) and then cocultured with T cells. Under both conditions, MDSCs significantly suppressed the secretion of IFN- γ and TNF- α by both CD4⁺ and CD8⁺ T cells. Student's t test was performed. (F) Experimental procedure for MDSC adoptive transfer in *Mif*^{-/-} mice (upper). Serum protein electrophoresis was used to detect M protein changes following tumor inoculation (lower).[#]Mice that died from the tumor burden. *P* values are shown as * *P* ≤0.05, ** *P* <0.01, **** *P* <0.0001.

Figure 3. MIF plays a critical role in maintaining the immunosuppressive phenotypes of MDSCs. (A-E) 5TMM model: (A) Proteomic heatmap of PB MDSCs from CTR-KO vs MIF-KO tumor-bearing mice (Day 14) showing elevated CD38 and IL-18 levels in the CTR-KO group. (B) Serum IL-18 levels (via ELISA) in the CTR-KO and MIF-KO groups. (C) IL-18 secretion (ELISA) in culture supernatants of MDSCs induced in vitro from WT or *Mif*^{-/-} mouse bone marrow. IL-18 mRNA levels (RT-qPCR) in spleen-sorted MDSCs from C57BL/6N WT (n=5) vs *Mif*^{-/-} mice (n=4) bearing Vk12598 MM tumors. (D) Percentage of CD38⁺ (n=5/group), CD84⁺

(n=4/group), and PD-L1⁺ MDSCs (n=4/group) in PB. (E) Frequency of PD-L1⁺ MDSCs in tumor-draining lymph nodes (n=4 and 5 mice, respectively) and spleen (n=4/group). (F) Flow cytometry was performed to evaluate MDSCs after tumor engraftment in the Vk model. Percentages of CD38⁺ (n=5/group), CD84⁺ (n=3 and 5 mice, respectively), and PD-L1⁺ MDSCs (n=5/group) in PB. Student's t test was performed. *P* values are shown as * *P* ≤0.05, ** *P* <0.01, *** *P* <0.001.

Figure 4. Peripheral blood MIF levels positively correlate with MDSC frequencies in MM patients. (A) Plasma MIF levels in RRMM patients (n=15, median 197.99 ng/mL) were significantly elevated compared with those in NDMM patients (n=82, 172.06 [33.26–244.43] ng/mL), remission patients (n=25, 179.46 [55.65–199.60] ng/mL), and healthy controls (n=12) at 54.60 ± 23.00 ng/mL (rank-sum test). Nonnormally distributed data were analyzed via the rank-sum test, with Dunnett's method applied for pairwise comparisons. (B–D) Nonparametric Spearman correlation analysis was performed in 85 MM patients (74 NDMM patients and 11 RRMM patients). PB MIF levels were moderately positively correlated with (B) M-MDSCs (% of CD11b⁺ cells), (C) CD84⁺ M-MDSCs (% of CD11b⁺ cells), and (D) PDL1⁺ M-MDSCs (% of CD11b⁺ cells). (E–F) In 31 NDMM patients with concurrent CD38⁺ MDSC assessment: (E) CD84⁺ M-MDSCs (% of CD11b⁺ cells) positively correlated with CD38⁺ M-MDSCs (% of CD11b⁺ cells); (F) PDL1⁺ M-MDSCs (% of CD11b⁺ cells) positively correlated with CD38⁺ M-MDSCs (% of CD11b⁺ cells). Nonnormally distributed data were analyzed via the Mann–Whitney U test for group comparisons and Spearman's rank correlation for correlation analysis. *P* values are shown as * *P* ≤0.05, ** *P* <0.01, **** *P* <0.0001.

Figure 5. MIF deficiency promotes the expansion of dendritic cells. (A–C) 5TMM model: (A) t-SNE visualization of mass cytometry (CyTOF) data from day 5 subcutaneous tumors identified cluster 13 as a dendritic cell (DC) population. Compared with that in CTR-KO tumors, the abundance of this DC cluster in MIF-KO tumors was significantly greater (n=3/group). (B–C) Flow cytometry analysis of DCs 3–5 days post-inoculation: (B) Frequency of DCs among CD45⁺ cells in PB (n=3/group), spleen (n=4/group), and tumor-draining lymph nodes (n=4 and 5 mice, respectively). (C) Frequency of CD8⁺ DCs in tumor-draining lymph nodes (n=4 and 6 mice, respectively) and spleen (n=4/group). (D) Vk model: Frequency of CD8⁺ DCs in PB (n=5 and 6 mice, respectively) and spleen (n=6/group). Student's t test was performed. *P* values are shown as

* $P \leq 0.05$, ** $P < 0.01$. **** $P < 0.0001$.

Figure 6. MIF suppresses MHC-I expression and the associated antigen presentation pathway through the transcriptional inhibition of NLRC5. (A) Flow cytometry histograms of MHC-I in CTR-KO vs MIF-KO 5TGM1 cells, $n=3/\text{group}$. (B) MHC-I expression on CD138⁺ cells from 5TMM subcutaneous tumors. Left, representative histograms; right, Mean fluorescence intensity (MFI) quantification ($n=3$ and 4 , respectively). (C) Upregulation of MHC-I expression in CTR-KO 5TGM1 treated with the MIF antagonist ISO-I ($100 \mu\text{M}$, 48 h), as assessed by flow cytometry, $n=3/\text{group}$ (left). Downregulation of MHC-I expression in MIF-KO 5TGM1 cells after treatment with recombinant murine MIF protein (200 ng/ml , 24 h), as detected by flow cytometry, $n=3/\text{group}$ (right). (D) Western blot results of NLRC5 expression in CTR-KO and MIF-KO 5TGM1 cells (left). Relative mRNA levels of MHC-I-related genes (*B2M*, *H2-D1*, *H2-K1*, *H2-M3*, *H2-Q7*, *H2-Q8*, *Nlrc5*) determined via RT-qPCR in CTR-KO and MIF-KO 5TGM1 cells. (E, F) MHC-I-SIINFEKL expression on OVA-transfected cells: (E) Left, representative flow cytometry histograms; right, MFI quantification ($n=3/\text{group}$). (F) MFI of MHC-I-SIINFEKL on 5TGM1-OVA (CTR-KO) cells treated with ISO-1 ($400 \mu\text{M}$, 48 h ; left) and on 5TGM1-OVA (MIF-KO) cells treated with recombinant MIF (200 ng/mL , 48 h ; right); $n=3$ per condition. Student's t test was performed. P values are shown as ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

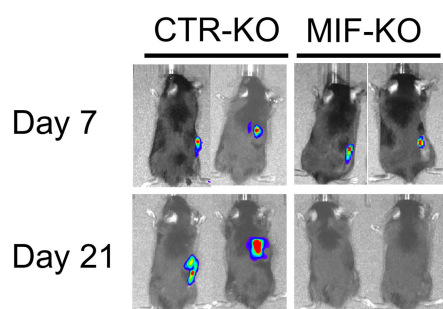
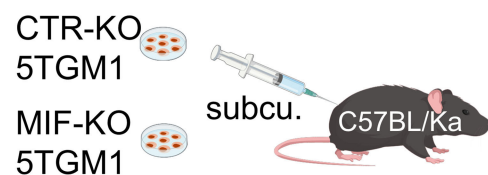
Figure 7. MIF deficiency enhances MHC-I expression through activation of the COPS5/STAT1/NLRC5 signaling axis. (A) Proteomic analysis revealed differential STAT1 expression between CTR-KO and MIF-KO cells (left). STAT1 protein expression was measured by western blotting (middle). Densitometric analysis across three independent western blot assays of STAT1, β -Actin as loading control. Student's t test was performed (right). (B) STAT1 in CTR-KO cells after ISO-1 ($100 \mu\text{M}$, 1 h), STAT1 in MIF-KO cells after recombinant MIF (200 ng/mL , 1 h) (left). ChIP analysis of STAT1-*Nlrc5* promoter binding in CTR-KO vs. MIF-KO 5TGM1. Two-way ANOVA with Tukey's post hoc test was performed (right). (C) Western blot confirmed COPS5 knockdown in 5TGM1 cells, with downregulated STAT1 and p-STAT1 compared with CTR-KD (left); flow cytometry revealed lower MHC-I expression in COPS5-KD cells (right). (D) Effects of the COPS5 inhibitor THL on MIF-KO 5TGM1 cells: THL decreased relative *Nlrc5* mRNA levels, $n=3/\text{group}$ (left); flow cytometry revealed that treatment with THL reduced MHC-I

expression; western blot results showed decreased protein levels of STAT1, p-STAT1 (Tyr701) and NLRC5 after THL treatment (right). (E) STAT1 was immunoprecipitated from MG132-treated CTR-KO and MIF-KO 5TGM1 cell lysates and immunoblotted with anti-ubiquitin or anti-STAT1 antibody, normal rabbit IgG as a negative control for immunoprecipitation, whole cell lysates were used as the input controls for immunoblotting. Molecular weight markers were indicated on the blots. (F) Schematic illustration of MIF-mediated MHC-I regulation via the COPS5/STAT1/NLRC5 signaling axis. This image was created with BioGDP.com.⁵³ *P* values are shown as * *P* ≤ 0.05, ** *P* < 0.01, **** *P* < 0.0001.

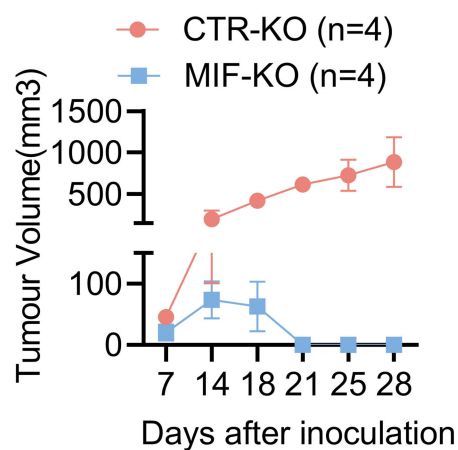
Figure 8. Targeting MIF for the treatment of multiple myeloma. (A) Tumor growth curves of mice subjected to different treatments. Two-way ANOVA with Tukey's post hoc test was performed. (B) Monoclonal protein (mouse IgG2b secreted by 5TGM1) levels in mouse PB were examined via ELISA. One-way ANOVA with a post hoc LSD *t* test was performed. (C) Survival curves of mice subjected to different treatments. The Mantel-Cox test was performed. (D-F) Flow cytometry analysis of MDSCs and PD-1 expression on T cells in the PB. One-way ANOVA with a post hoc LSD *t* test was performed. *P* values are shown as * *P* ≤ 0.05, ** *P* < 0.01, *** *P* < 0.001, **** *P* < 0.0001. ns: not significant, *P* > 0.05.

Figure 1.

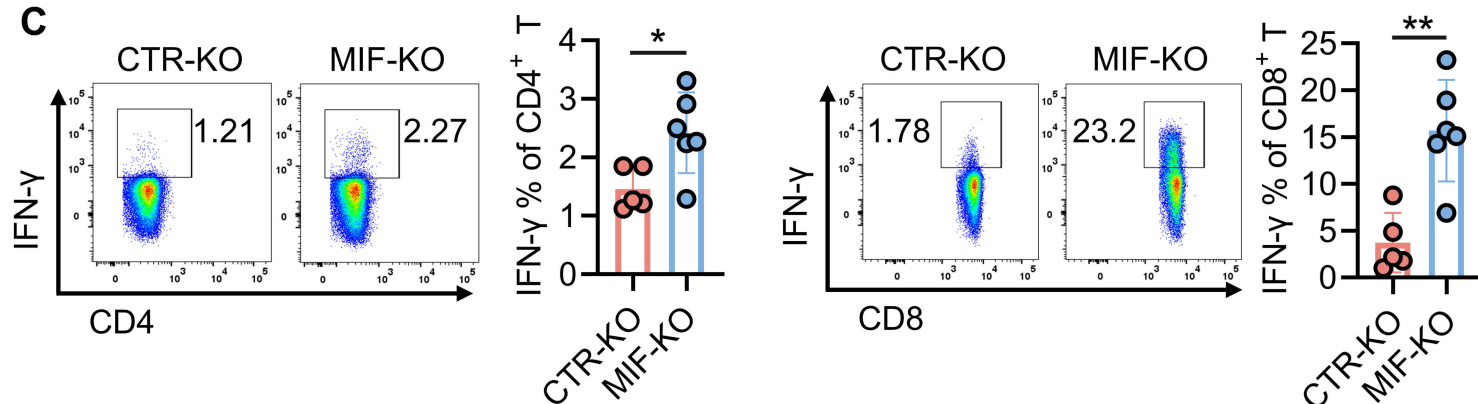
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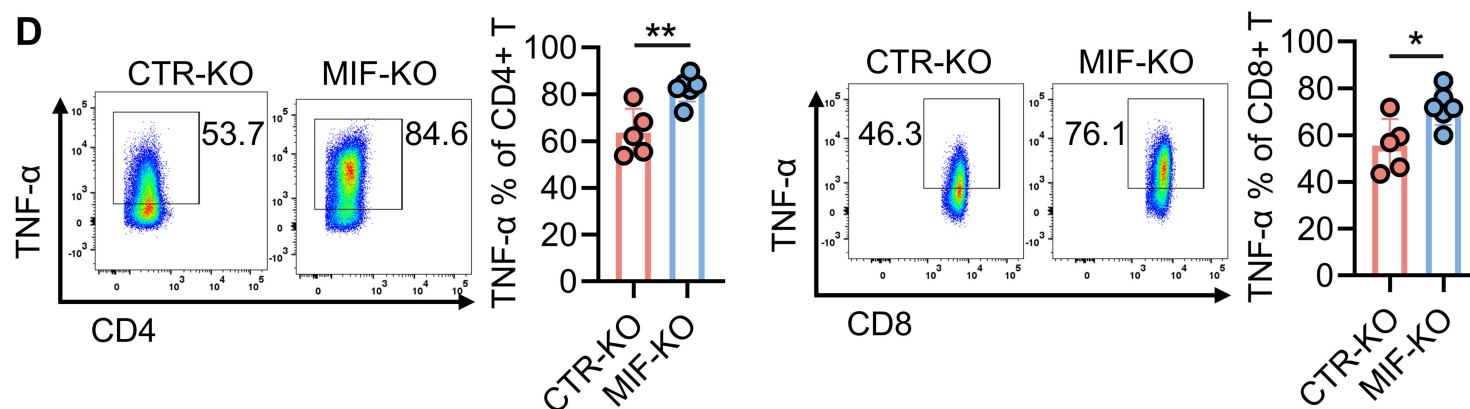
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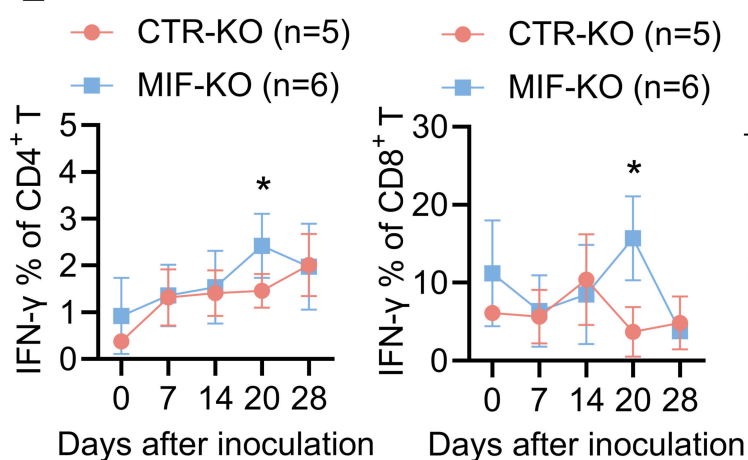
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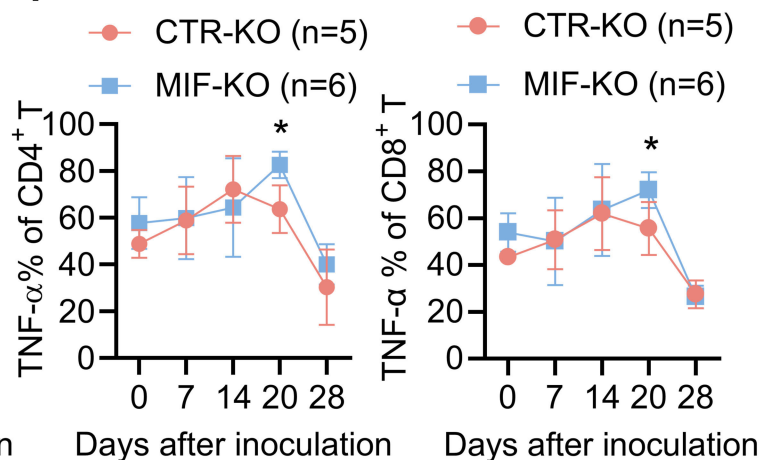


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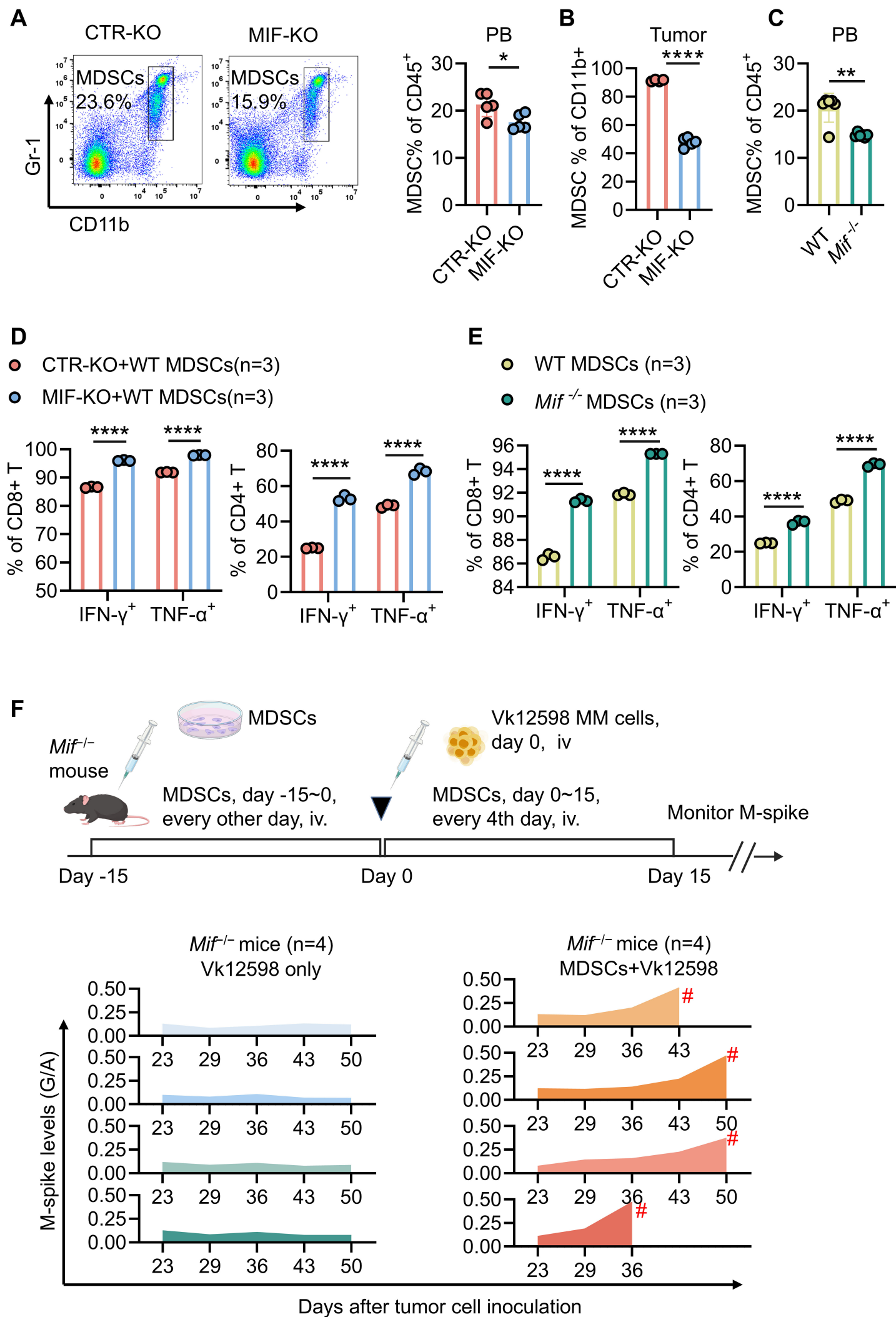


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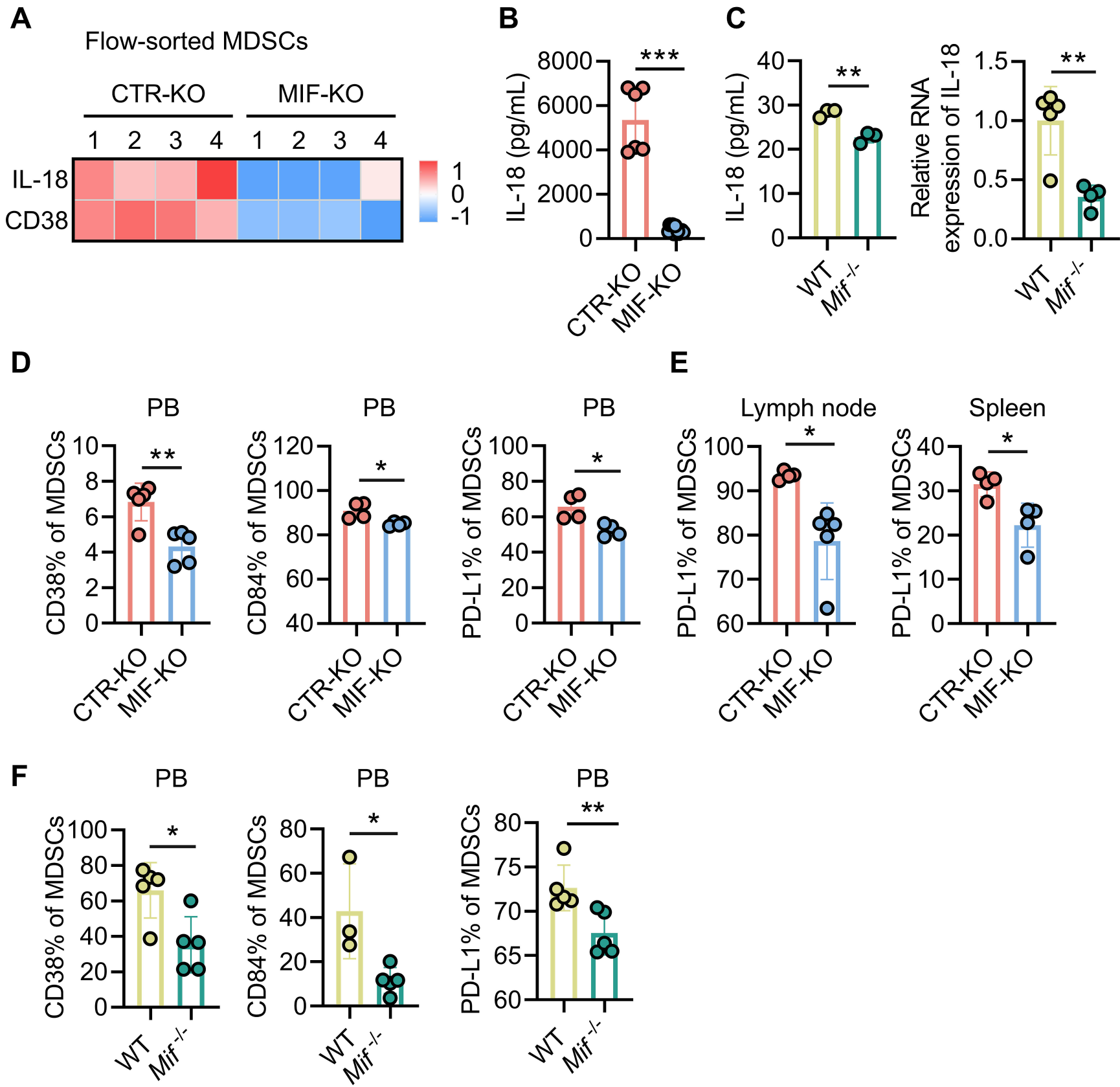
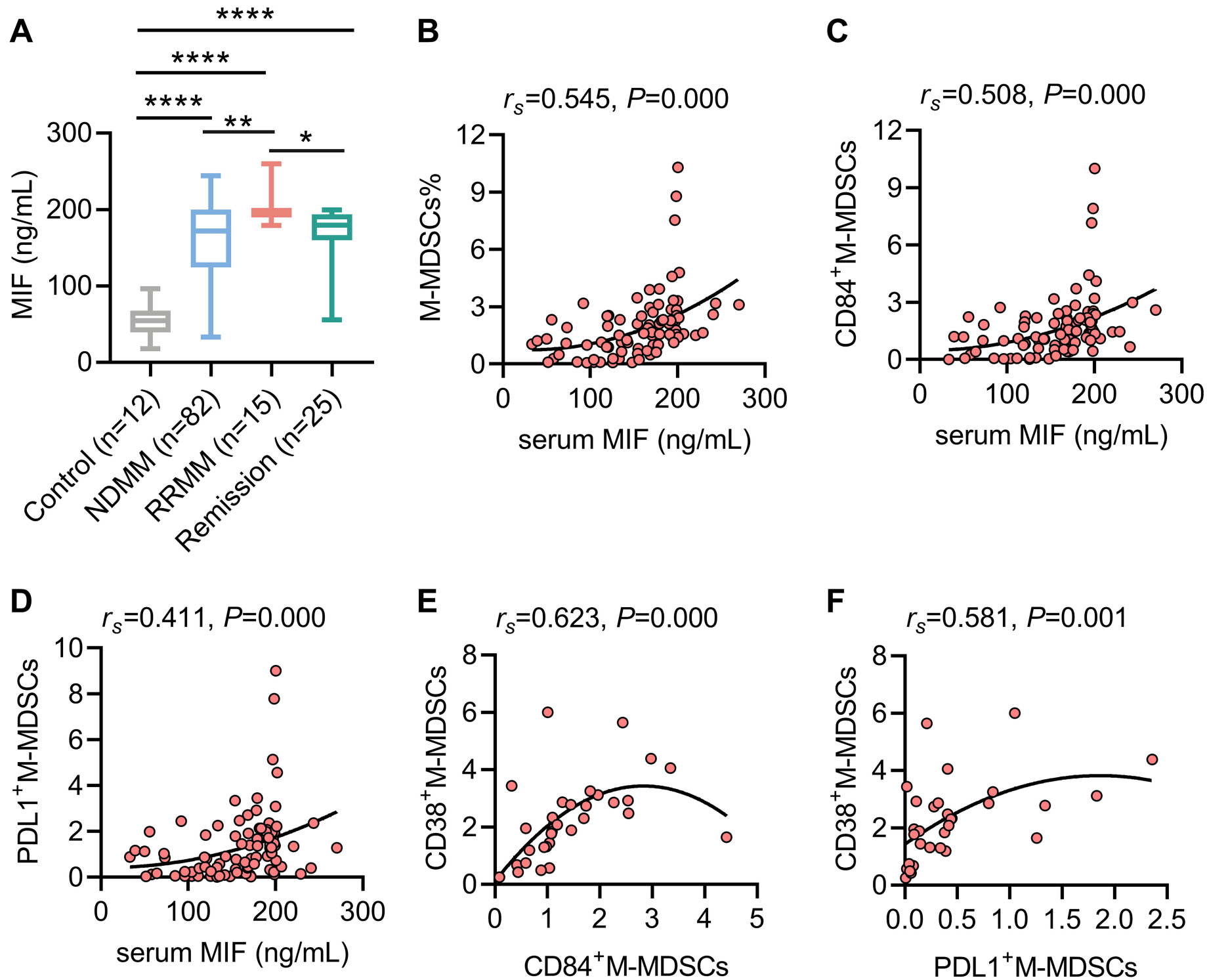


Figure 4.



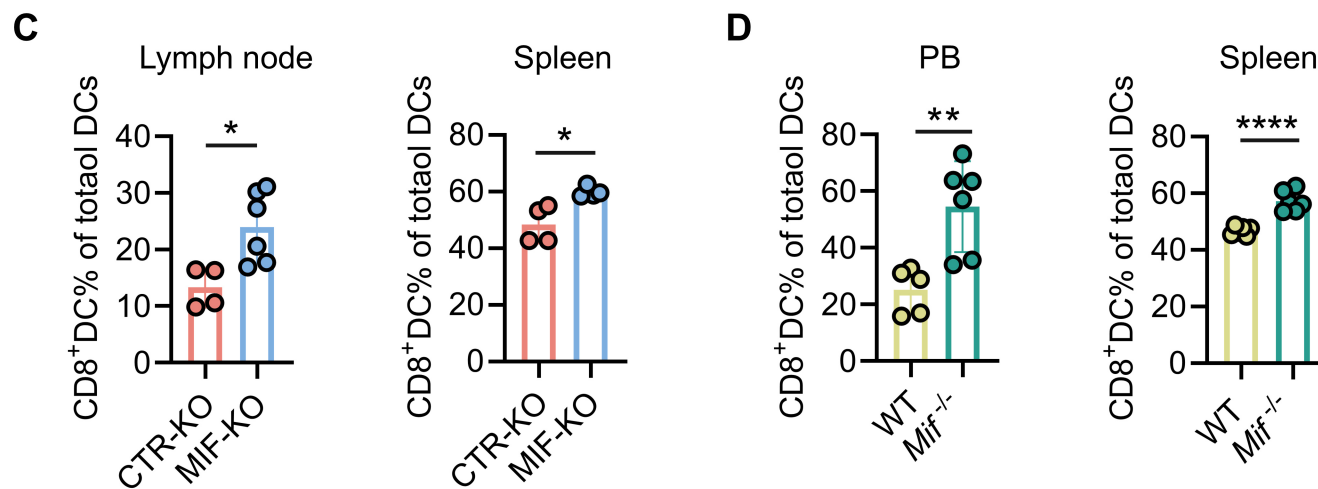
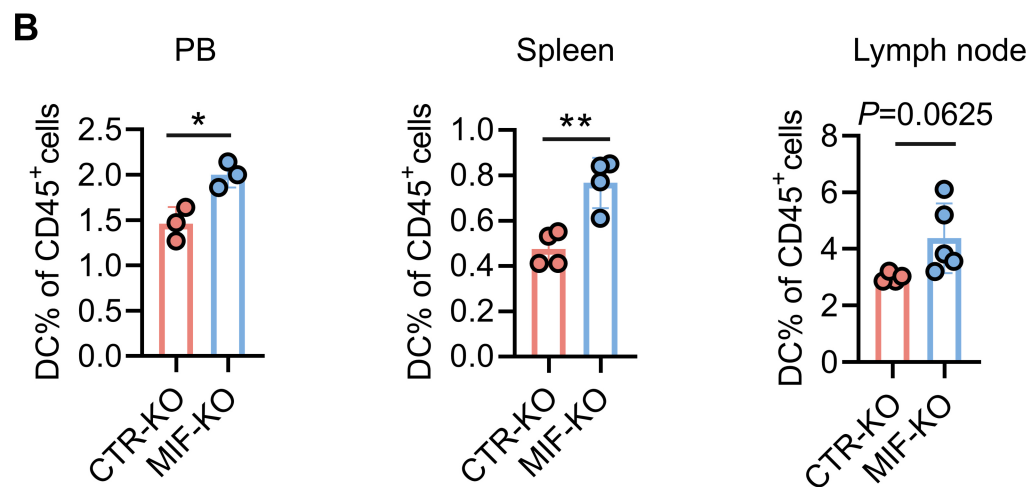
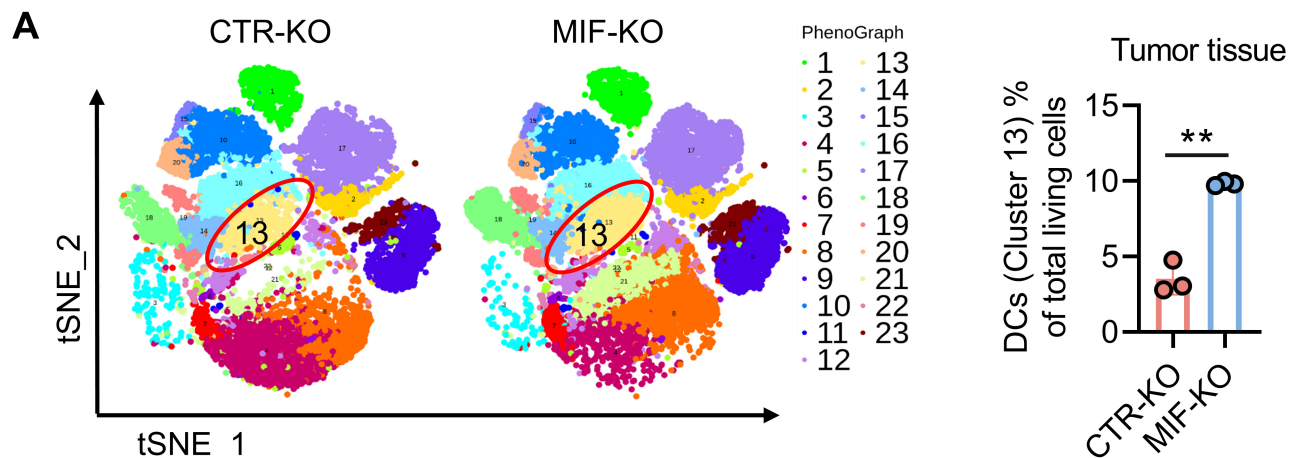


Figure 6.

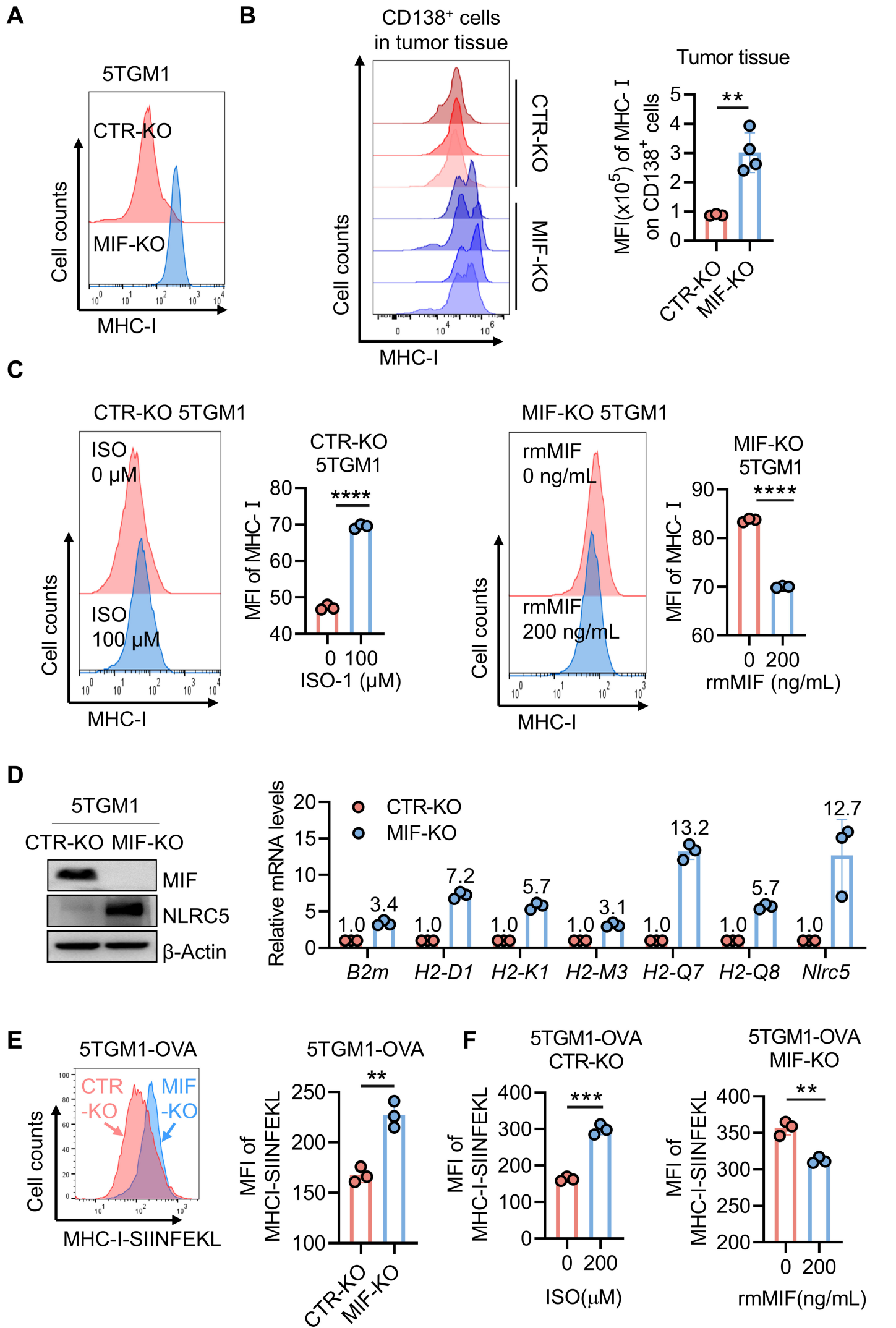


Figure 7.

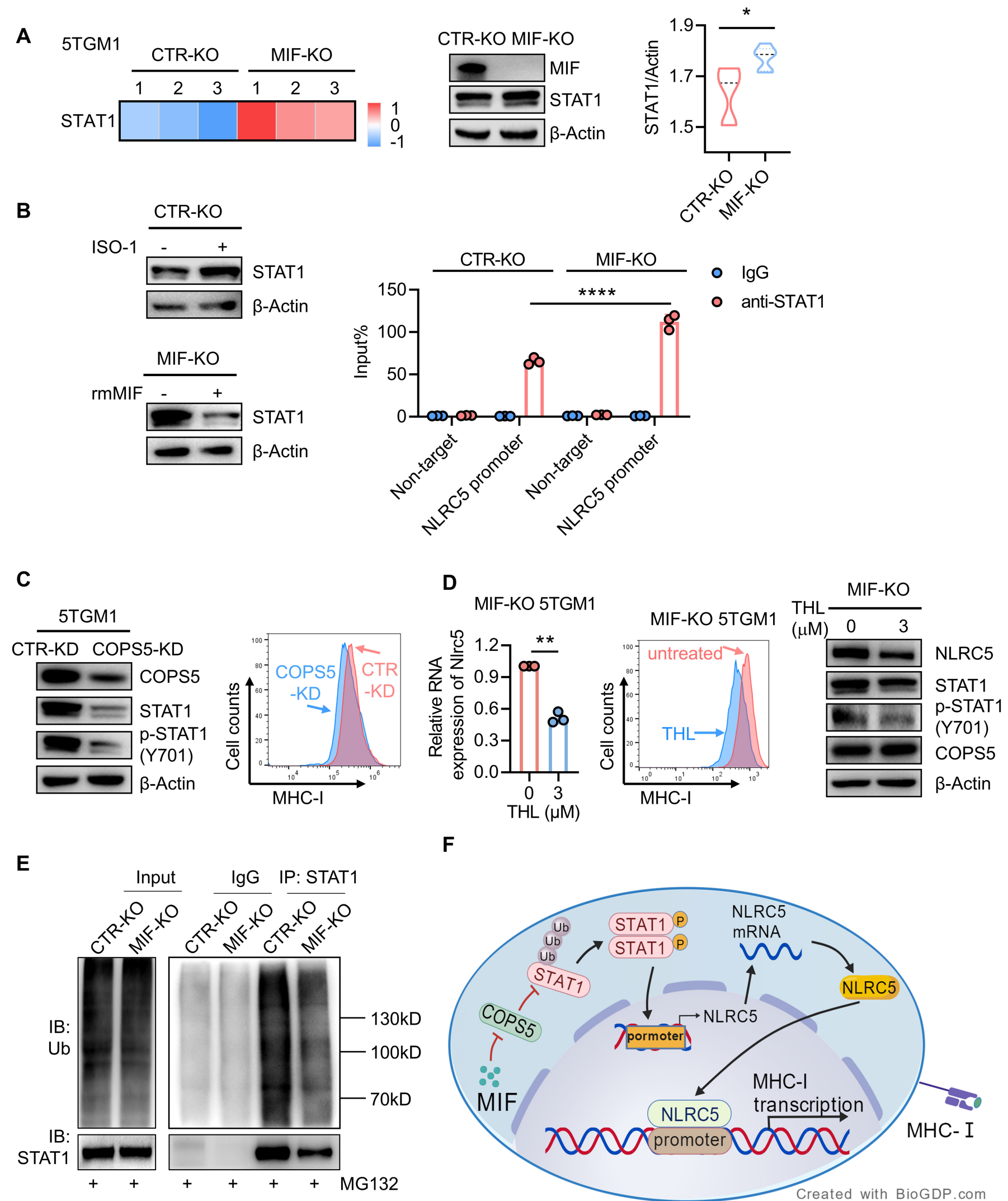
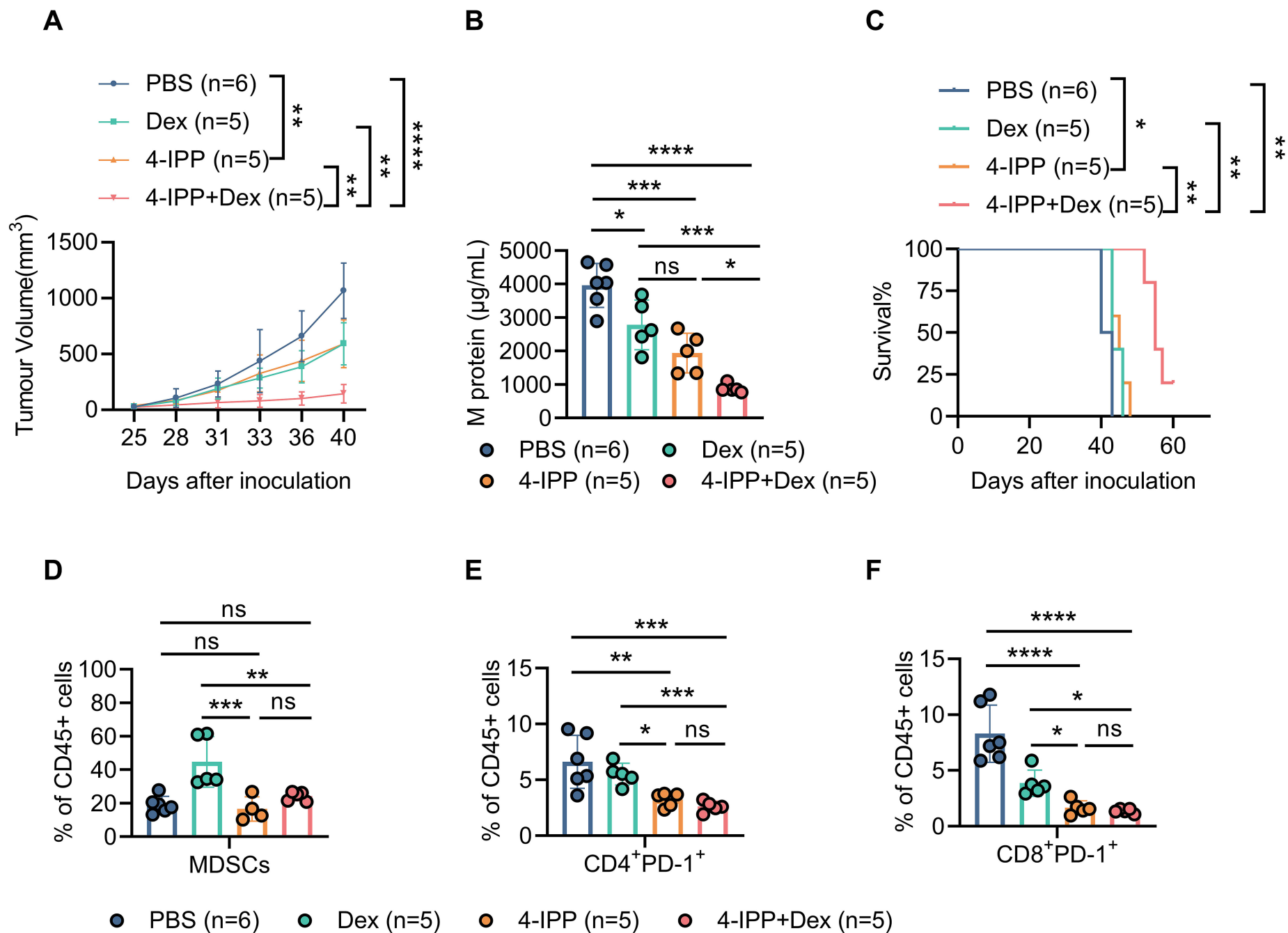


Figure 8.



Supplementary materials

Supplementary methods

Mice and Genotyping

C57BL/6N-*Mif*^{em1Cya} mice were obtained from Cyagen Biotechnology Co. Ltd. Six- to eight-week-old wild-type NCG and C57BL/6NGpt mice were obtained from GemPharmatech Co., Ltd. C57BL/KaLwRij mice were obtained from Envigo. C57BL/6N-*Mif*^{-/-} mice were obtained by mating C57BL/6N-*Mif*^{em1Cya} (*Mif*^{+/-}; RRID: MGI:7837948) F0 generation mice with C57BL/6NGpt mice. Genotyping was performed via PCR with the standard protocol. The primers used for genotyping were as follows: (i) F: 5'-GAATGCCTCGACAAACCTAATCAC-3'; R: 5'-CATATTACCGGCTCCATTTGCCAC-3'. (ii) F: 5'-CTATTACGACATGAACGCTGCCAAC-3'; R: 5'-CATATTACCGGCTCCATTTGCCAC-3'.

Cell lines and primary cells

Murine myeloma cell line 5TGM1(RRID: CVCL_VI66), 5TGM1-luc (expressing the luciferase gene) and human myeloma cell line KMS-11 (RRID: CVCL_2989) were kindly provided by the Institute of Hematology, West China Hospital, Sichuan University, and verified via STR profiling. MIF knockout in 5TGM1 and KMS-11 cells was achieved via CRISPR/Cas9 gene editing technology. Human and mouse MIF sgRNA (**Table S2**) was purchased from GENEWIZ Biotechnology Co., Ltd. MIF receptors (CD74, CD44, CXCR4, and COPS5) were knocked down in 5TGM1 cells via shRNA lentiviral infection. The shRNAs (**Table S2**) were purchased from Tsingke Biotechnology Co., Ltd. Lentivirus-infected cells were selected by puromycin treatment to generate stable cell lines with consistent target gene knockdown. All the cell lines were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% fetal bovine serum (FBS).

The primary mouse MM cells used in this study were Vk12598 (RRID: CVCL_D5FT) MM cells kindly provided by the P. Leif Bergsagel research group at the Mayo Clinic¹. Vk12598 MM primary cells were obtained via in vivo passage in C57 mice.

T cell in vitro cytotoxicity assay

C57BL/KaLwRij mice bearing established MIF-KO 5TGM1 tumors received a subcutaneous inoculation of 1×10^6 MIF-KO 5TGM1 cells in the contralateral flank. After 6 days of in vivo stimulation, splenocytes from C57BL/6N mice were harvested as an additional source of T cells. CD3⁺ T cells were isolated from these splenocytes via the EasySep™ Mouse T-Cell Isolation Kit according to the manufacturer's instructions. The isolated splenic CD3⁺ T cells were cocultured with tumor cells at effector-to-target ratios of 15:1. To evaluate T-cell-mediated tumor killing efficacy, a reporter gene transfection-based cytotoxicity assay was performed. Briefly, luciferase-transduced WT 5TGM1 or MIF-KO 5TGM1 cells (10^4 cells/well in 100 μ L) were seeded into opaque 96-well plates. After 24 hours of coculture with T cells, potassium luciferin was added to each well, and the relative light units (RLUs) were measured via a multifunctional microplate luminometer. Higher RLU values correspond to greater numbers of surviving tumor cells, indicating lower killing activity.

T-Cell Isolation and CFSE Labeling

Murine CD3⁺ T cells were isolated from the spleens of WT C57BL/6N mice via the EasySep™ Mouse T-Cell Isolation Kit (STEMCELL, Cat. #19851) according to the manufacturer's instructions. To evaluate T-cell proliferation, isolated CD3⁺ T cells were labeled with 5 μ M CFSE (Merck, Cat. #SCT110) for 10–15 minutes at 37°C in the dark and then stimulated with anti-CD3 (Thermo Fisher, Cat. #14-0032-82; RRID: AB_467053) and anti-CD28 (Thermo Fisher, Cat. #14-0281-82; RRID: AB_467190) antibodies.

Human T cells were derived from peripheral blood mononuclear cells (PBMCs) from healthy donors. The study involving human participants was approved by the Ethics Committee of West China Hospital, Sichuan University (Approval No. 1688). PBMCs were stimulated with ActiveMax® Human T Cell Activation/Expansion CD3/CD28 Beads (ACROBiosystems, Cat. # MBS-C001) at a 1:1 ratio in GMP T Cell Expansion Medium (ACROBiosystems, Cat. # GMP-CM3103), supplemented with 50 U/mL GMP Human IL-2 Protein (ACROBiosystems, Cat. # GMP-L02H14).

Flow cytometry analysis

For sample preparation, 40–50 μ L of heparin-anticoagulated whole blood was collected. Single-cell suspensions were prepared from the spleen, lymph nodes, and bone marrow by mechanical

grinding, followed by filtration through a 70 µm strainer. Red blood cells were lysed with RBC lysis buffer when needed. For cell surface staining, the cells were incubated with Live/Dead Fixable Viability Stain 700 for 10 minutes at room temperature, followed by Fc blocking with an anti-mouse CD16/CD32 antibody (BD Pharmingen, Cat. #553141; RRID: AB_394656) for 10 minutes on ice. The cells were subsequently stained with surface marker antibodies for 20 minutes at room temperature in the dark. For intracellular cytokine staining, the cells were stimulated with eBioscience™ Cell Stimulation Cocktail (plus protein transport inhibitors) for 5 hours. After stimulation, the cells were fixed and permeabilized via either the Intracellular Fixation & Permeabilization Buffer Set or the Foxp3/Transcription Factor Staining Buffer Set according to the manufacturer's instructions, followed by intracellular antibody staining. Flow cytometry was performed on a BD FACS Celesta, and the data were analyzed via FlowJo (RRID:SCR_008520) software (version 10.0).

Enzyme-Linked Immunosorbent Assay

The concentration of MIF in mouse PB was measured via an ELISA kit (Proteintech, Cat. #KE10027). The human MIF level was detected via an ELISA kit from R&D Systems (Cat. #DMF00B). IL-18 concentrations in both mouse serum and in vitro culture supernatants were quantified via an ELISA kit (Multi sciences, Cat. #EK218–96). ELISA was performed according to the manufacturer's instructions.

Quantitative real-time PCR

RNA was extracted with an RNA Extraction Kit (Accurate Bio, Inc., Cat. #AG21023) and converted to single-strand cDNA via HiScript® II Q RT SuperMix for qPCR (Vazyme Biotech Co., Ltd., Cat. #R223-01) according to the manufacturer's protocol. Amplification was performed with the Bio-Rad CFX Maestro using ChamQ SYBR qPCR Master Mix (Vazyme Biotech Co., Ltd., Cat. #Q311-02). Relative gene expression was quantified via normalization. The primer sequences are provided in **Table S3**. The formula for calculating relative gene expression for quantitative reverse transcription PCR is as follows: Fold gene expression = $2^{-(\Delta\Delta Ct)}$, $\Delta\Delta Ct = \Delta Ct (\text{experimental sample}) - \Delta Ct (\text{control sample})$, $\Delta Ct = Ct (\text{gene of interest}) - Ct (\text{housekeeping gene})$.

Western blotting

The cells were lysed with General Protein Lysis/Extraction Reagent (Aidlab Biotechnologies Co., Ltd.) supplemented with a protease inhibitor cocktail. Protein concentrations were measured via a bicinchoninic acid protein assay kit (CWBIO). Equal amounts of protein were subjected to SDS–polyacrylamide gel electrophoresis and then transferred to nitrocellulose membranes (Millipore) for immunoblotting. The membranes were blocked and incubated with the indicated primary and secondary antibodies. Images were visualized with a chemiluminescent imaging system (Tanon 5200).

Ubiquitination analysis

CTR-KO and MIF-KO 5TGM1 cells were treated with the proteasome inhibitor MG132 (MCE, Cat. #HY 13259; CAS# 133407-82-6) 1 μ M for 3.5 hours. The cells were then harvested, washed twice with PBS, and lysed. Whole cell lysates were prepared, and a portion was saved as the input for immunoblotting. Protein A/G magnetic beads (MCE, Cat. #HY-K0202) were preincubated with either an anti-STAT1 antibody or control rabbit IgG on a rotator at 4°C for 2 hours. The antibody-bead complexes were subsequently incubated with the cell lysates overnight at 4°C with rotation. After washing, the bound proteins were eluted under denaturing conditions and analyzed by western blotting using an anti-ubiquitin antibody or an anti-STAT1 antibody.

Public dataset analysis

Using MM gene expression profiling (GEP) datasets (GSE2658, GSE9782, GSE58133, GSE26760, and GSE4204) obtained from the NCBI Gene Expression Omnibus (GEO; RRID:SCR_005012) database, Pearson correlation analyses were performed between expression levels of MIF and HLA-A. The expression profile of human MM cell lines was obtained from the DepMap RNA-seq datasets (Expression Public 26Q1, <https://depmap.org/portal>).² Pearson correlation analyses were performed between expression levels of MIF and the mean expression levels of HLA-A, HLA-B, and HLA-C.

MDSC generation

MDSCs derived from mouse bone marrow mononuclear cells

MDSCs were generated in vitro following a previously established protocol.³ Briefly, BM cells (2.5×10^6) isolated from WT healthy C57BL/6N mice were cultured in 10 cm dishes with 10 mL

of RPMI-1640 GlutaMAX medium supplemented with 10% heat-inactivated FBS, 1% penicillin/streptomycin, 10 mM HEPES, 1 mM sodium pyruvate, 50 μ M β -mercaptoethanol, 1 $\times$ MEM nonessential amino acids (all from Thermo Fisher), 40 ng/mL GM-CSF (Yeasten, Cat. #91115ES60), and 40 ng/mL IL-6 (Yeasten, Cat. #90146ES6) for 4 days. MDSCs (Gr-1⁺CD11b⁺) were then isolated from the single-cell suspensions by flow cytometry (purity >90%).

MDSCs derived from human peripheral blood mononuclear cells

Mononuclear cells isolated from the peripheral blood of healthy donors by Ficoll-Paque density gradient centrifugation. The study involving human participants was approved by the Ethics Committee of West China Hospital, Sichuan University (Approval No. 1688). Seed cells at 1×10^6 cells/mL density in 2 mL in a 24-well plate in RPMI-1640 medium with 10% FBS and 40 ng/mL GM-CSF (R&D systems, Cat. #215-GM-010) and 20 ng/mL IL-6 (Sino Biological, Cat. #10395-HNAE) for 6 days.⁴

Suppressive effects of MDSCs on T-cell proliferation and function

Myeloid-derived suppressor cells (MDSCs) were generated from WT C57BL/6N mouse BMDCs through indirect coculture with 5TGM1 cells in a 6-well trans-well plate (Corning, 0.4 μ m pores). Briefly, 0.5×10^5 5TGM1 cells were seeded in the upper chamber, and 5×10^5 BMDCs were placed in the lower chamber. After 5 days of indirect coculture, the MDSC frequency was assessed via flow cytometry.⁵ For T-cell proliferation and functional assays, MDSCs were cocultured with CD3⁺ T cells (1:1 ratio) in RPMI 1640 medium containing L-glutamine, penicillin/streptomycin, and 10% heat-inactivated FBS. After 72 hours, CD8⁺ T-cell proliferation was assessed by flow cytometry via CFSE dilution.⁶ T cell function was assessed by flow cytometry after gating on live CD4⁺ or CD8⁺ T cells.

Derived Human MDSCs were indirectly cocultured with KMS-11 cells (CTR-KO or MIF-KO) in a 12-well trans-well plate (Corning, 0.4 μ m pore). Following 48 h of indirect coculture, autologous human T cells were seeded into the lower chamber to enable direct coculture with MDSCs. After an additional 48 h, T cell function was evaluated by flow cytometry of viable CD3⁺ T cells.

ChIP assay

Chromatin immunoprecipitation (ChIP) was carried out via the Magna ChIP™ A/G Kit (EMD Millipore Corporation, Cat. #17-10085) according to the manufacturer's protocol. Briefly, the cells were crosslinked with 1% formaldehyde for 10 min at room temperature, and the reaction was quenched with glycine. The cell pellet was resuspended in 0.5 mL of Cell Lysis Buffer supplemented with 2.5 µL of 1× Protease Inhibitor Cocktail II, followed by resuspension in 0.5 mL of Nuclear Lysis Buffer containing the same protease inhibitors. Chromatin was sheared via sonication (5 s pulse, 9 s pause, 35% power, 90 s total). For immunoprecipitation, sheared chromatin was incubated overnight at 4°C with rotation with 5 µg of antibody and 20 µL of protein A/G magnetic beads. Protein–DNA complexes were eluted and reverse crosslinked to release DNA, which was then quantified via real-time quantitative PCR. Primers targeting a genomic region on murine chromosome 8 (harboring the *Nlr5* locus) lacking predicted STAT1-binding motifs and ChIP-seq evidence of STAT1 occupancy were used as a negative control for ChIP-qPCR. The sequences of primers used for ChIP-qPCR were provided in **Table S4**.

Proteomic Analysis of 5TGM1 cells and MDSCs

Proteomic analysis of 5TGM1 cells: CTR-KO and MIF-KO 5TGM1 cells were harvested, and three replicates of each cell type (1×10^7 cells per replicate) were collected. The cell pellets were then washed twice with ice-cold PBS. Proteomic analysis of MDSCs: A 5TMM mouse model was established by subcutaneously inoculating mice with either CTR-KO or MIF-KO 5TGM1 MM cells, with four mice per group. On day 14 post-tumor inoculation, peripheral blood (PB) MDSCs were isolated by fluorescence-activated cell sorting (Beckman CytoFLEX SRT) using CD11b and Gr-1 antibodies. Four sorted MDSC samples were obtained from CTR-KO and MIF-KO group. All samples were submitted to Shanghai Bioprofile Biotechnology Co. Ltd (Shanghai, China) for DIA-based quantitative proteomic analysis. Protein extracts from samples were prepared and digested into peptides using the FASP method. Peptide mixtures were then analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) on an Orbitrap Astral mass spectrometer coupled with Vanquish Neo UHPLC system. Data were acquired using a data-independent acquisition (DIA) method, with full MS scans from 380-980 m/z and DIA MS/MS scans from 150-2000 m/z. Acquired spectra were processed and searched against the UniProtKB/Swiss-Prot database using DIA-NN software (version 1.8.1), with strict filtering at a

1% false discovery rate (FDR) at both peptide and protein levels. Subsequent bioinformatic analyses, including Gene Ontology (GO) enrichment, Kyoto Encyclopedia of Genes and Genomes (KEGG; RRID:SCR_012773) pathway analysis, and protein-protein interaction (PPI) network construction, were conducted using R statistical software and the Cytoscape (RRID:SCR_003032) platform.

Experiment method of Cytot

The samples were collected and Live/Dead stained with 2 μ M cisplatin (Fluidigm) for 2 minutes before quenching with CSB (Fluidigm). A Fix-I buffer (Fluidigm) was then used to fix cells for 15 minutes at room temperature, followed by washing three times with 1x PBS. The samples were stained with Cell-ID™ 20-Plex Pd Barcoding Kit (Fluidigm) to minimize internal cross reaction between samples. MaxPar $\times$ 8 Polymer Kits (Fluidigm) were used to conjugate with purified antibodies according to the manual. All metal-conjugated antibodies were titrated for optimal concentrations before use. For the surface protein staining, cells were counted, diluted into 1×10^6 cell/ml in PBS and cultured with antibodies cock-tail in a total 50 μ L CSB for 30 minutes at RT. After that, cells were washed, underwent permeabilization with 80% methanol for 15 minutes at 0°C and stained with an intracellular antibody cocktail for 30 minutes. After triple washes in CSB, cells were triple washed in CSB and incubated with 0.125 μ M iridium intercalator in fix and perm buffer (Fluidigm) at 4 °C overnight. After cultured with intercalator, cells were washed with ice cold PBS and deionized water three times separately. Prior to acquisition, samples were resuspended in deionized water containing 10% EQ 4 Element Beads (Fluidigm) and cell concentrations were adjusted to 1×10^6 cell/ ml. Data acquisition was performed on a Helios mass cytometer (Fluidigm). The original FCS data were normalized and .fcs files for every sample were collected. All .fcs files were uploaded into Cytobank (RRID:SCR_014043), data cleaning and populations of single living cells were exported as .fcs files for further analysis.

MHC Class I antigen presentation assay using B3Z reporter cells

B3Z cells (kindly provided by Prof. Guido Kroemer and Dr. Hui Pan, Université Paris Cité, Sorbonne Université, Paris, France) are CD8⁺ T-cell hybridomas engineered to express β -galactosidase under TCR-dependent NF-AT signaling upon recognition of the OVA257–264 (SIINFEKL) peptide presented by H-2Kb.⁷ Cells were maintained in RPMI-1640 supplemented with 10% fetal bovine serum, 2 mM Glutamax, 50 μ M 2-mercaptoethanol, 1 mM sodium pyruvate,

and 10mM HEPES. For antigen presentation assay, 5TGM1-OVA cells (CTRO-KO and MIF-KO) were harvested, washed twice with PBS and seeded in 96-well plates at 2×10^4 per well in 100 μ L complete RPMI-1640 medium without phenol red. B3Z cells were subsequently added at 1×10^5 per well (E: T=5:1), followed by brief centrifuge at 150g for 2 min to facilitate cell-cell contact. Cell were then cocultured for 16 h at 37°C. Three control groups were included: (i) B3Z cells cultured alone to determine background β -galactosidase activity; (ii) OVA-negative 5TGM1-wt cells co-cultured with B3Z cells to confirm antigen-specific activation; and (iii) 5TGM1-wt cells pulsed with SIINFEKL peptide (0.15 μ g/mL) for 1 h at 37°C, washed twice with PBS to remove excess peptide, and subsequently co-cultured with B3Z cells to serve as a positive control for peptide presentation independent of endogenous antigen processing. Following coculture, 50 μ L of freshly prepared CPRG lysis buffer was added directly to each well. The assay was performed using optimized final concentrations of 0.15 mM chlorophenol red- β -D-galactopyranoside (CPRG), 0.2% NP-40, 9 mM MgCl_2 , and 100 mM 2-mercaptoethanol.⁸ Plates were incubated at 37°C until color development reached a stable, quantifiable absorbance. β -Galactosidase activity was quantified by measuring absorbance at 570 nm with background correction at 630 nm using a microplate reader. Antigen presentation activity was expressed as OD570–OD630 after subtraction of the background signal obtained from the B3Z-only control.

Statistical analysis

Significance was assessed via SPSS 26.0 (RRID:SCR_002865) or GraphPad Prism 8.0 (RRID:SCR_002798), with the latter also used for generating graphs. Normally distributed continuous variables are expressed as the means $\pm$ standard deviations, whereas nonnormally distributed data are presented as medians (ranges). For normally distributed continuous variables, Student's t test (for two groups) or one-way ANOVA (for three or more groups) was applied. Two-way ANOVA was performed to compare the differences between groups over time. Nonnormally distributed data were analyzed via the rank-sum test. The optimal cutoff value was determined via receiver operating characteristic (ROC) curve analysis. Overall survival (OS) was analyzed via the Kaplan–Meier method, and between-group differences were assessed via the log-rank test. Correlation analysis was performed via Spearman's rank correlation. P value ≤ 0.05 was considered statistically significant.

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Supplementary Figures

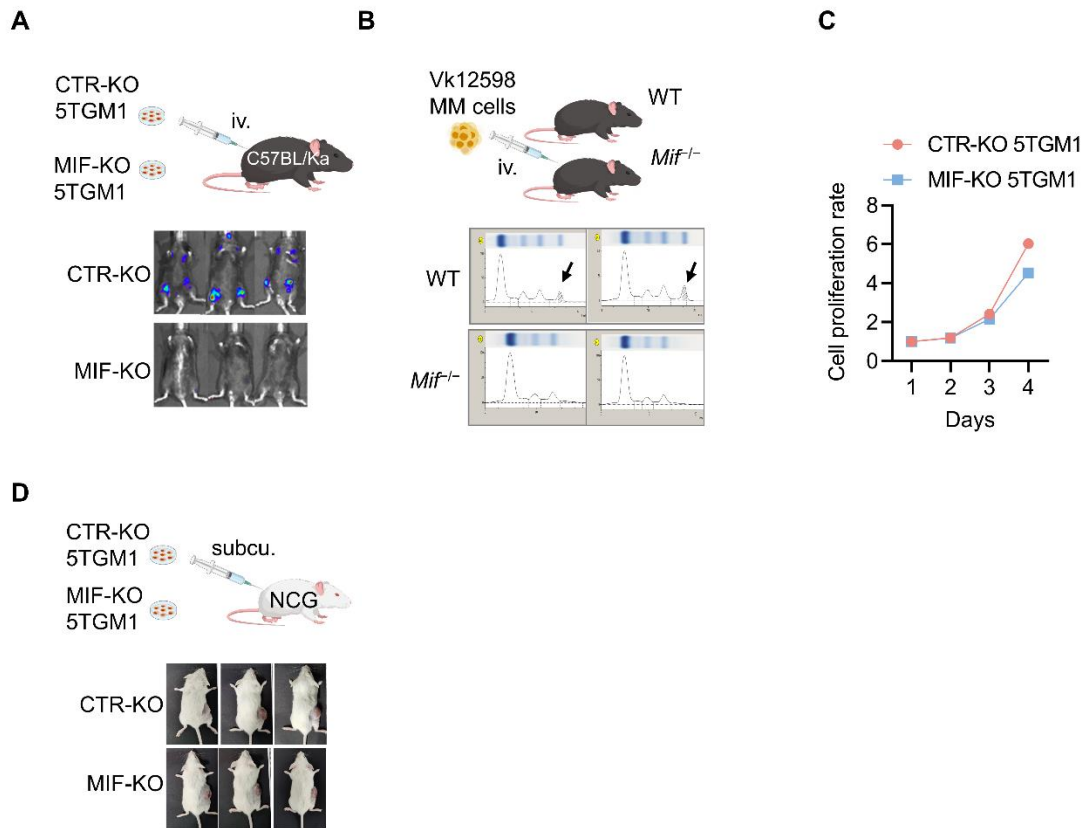


Figure S1.

MIF deficiency in tumor cells or host reduces myeloma progression in immunocompetent mice. (A) Establishment of the 5TMM model: CTR-KO 5TGM1 or MIF-KO 5TGM1 cells were intravenously injected into immunocompetent C57BL/6 mice. Tumor formation was observed in the CTR-KO group but not in the MIF-KO group. (B) Vk12598 MM development in C57BL/6N (WT) and MIF-knockout (*Mif*^{-/-}) mice. Serum protein levels were assessed post-inoculation (representative results, n=3/group). (C) Proliferation curves of CTR-KO and MIF-KO 5TGM1 cells. (D) Schematic diagram of the tumor-bearing model in NCG mice: CTR-KO 5TGM1 cells and MIF-KO 5TGM1 cells were subcutaneously injected. The tumors at the ethical endpoint in each group are shown.

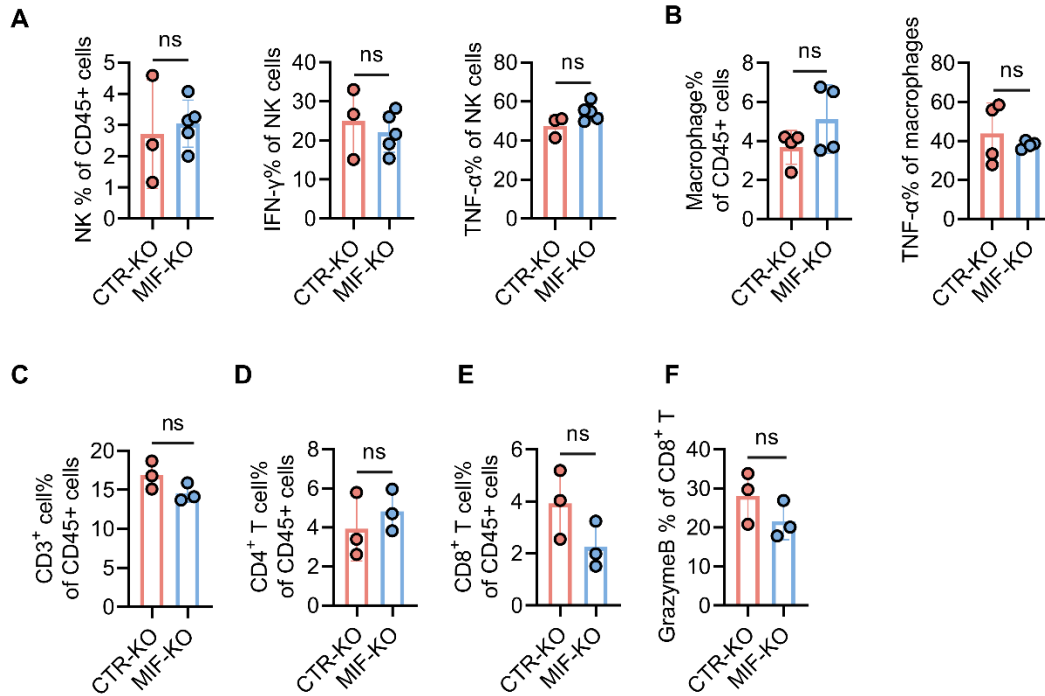


Figure S2.

Immune cell infiltration within subcutaneous 5TMM tumors. Subcutaneous tumors from mice inoculated with CTR-KO 5TGM1 cells (CTR-KO group) or MIF-KO 5TGM1 cells (MIF-KO group) were harvested at 24 h after engraftment for flow cytometry. **(A)** NK cell proportions among CD45⁺ cells and percentages of IFN- γ ⁺ and TNF- α ⁺ subsets among NK cells (CTR-KO group, n=3; MIF-KO group, n=5). **(B)** Macrophage abundance in CD45⁺ populations and ratios of TNF- α ⁺ macrophages (n=4/group). **(C-E)** Proportions of **(C)** CD3⁺ T cells, **(D)** CD4⁺ T cells, and **(E)** CD8⁺ T cells within the CD45⁺ population (n=3/group). **(F)** Frequency of Granzyme B⁺ (GB⁺) cells among CD8⁺ T cells (n=3/group). Student's t test was performed. ns: not significant, $P>0.05$.

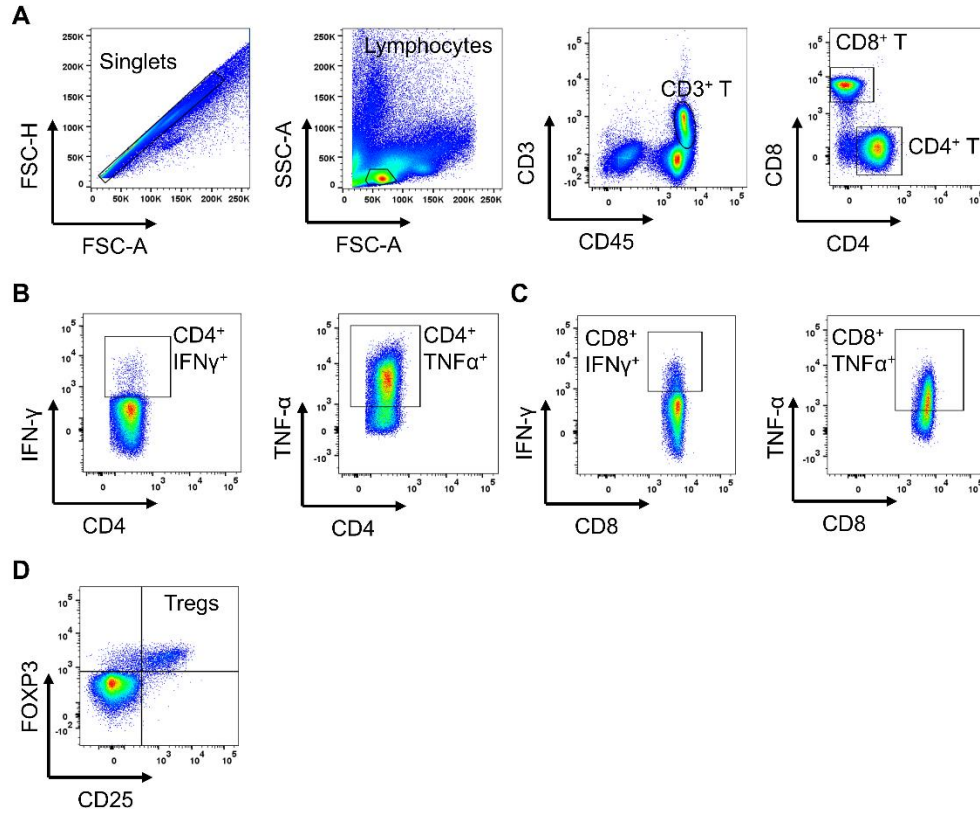


Figure S3.

Flow cytometry gating strategy for the analysis of T cell subsets. (A) CD45⁺ cells were gated first, followed by selection of CD3⁺ T cells, which were further divided into CD4⁺ and CD8⁺ T cell subsets. (B) Analysis of the expression of IFN- γ ⁺ and TNF- α ⁺ in CD4⁺ T cells. (C) Evaluation of IFN- γ ⁺ and TNF- α ⁺ expression in CD8⁺ T cells. (D) Identification of regulatory T cells (Tregs) as CD25⁺FOXP3⁺ cells within the CD4⁺ T cell population.

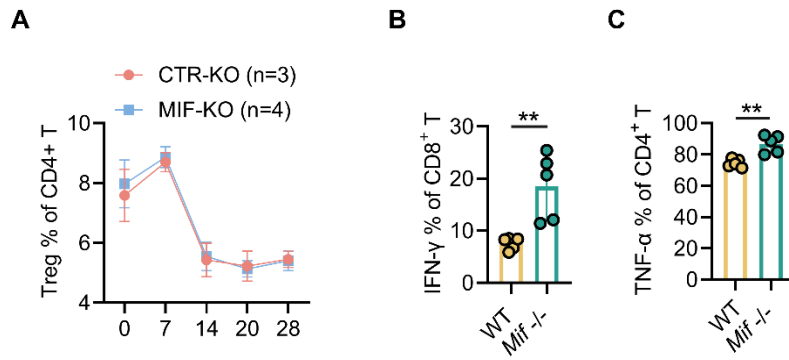


Figure S4.

MIF deficiency enhances effector T cell cytokine production without altering Treg frequency.

(A) MM models were established via the inoculation of CTR-KO or MIF-KO 5TGM1 cells, and the Treg frequency was analyzed via flow cytometry. Changes in Tregs during tumor progression. (B–C) PB T cell function was assessed in the Vk model. (B) IFN- γ secretion by CD8⁺ T cells on day 4, n=5/group. (C) TNF- α secretion by CD4⁺ T cells on day 4; n=5/group. Student's t test was performed. *P* values are shown as ** *P*<0.01.

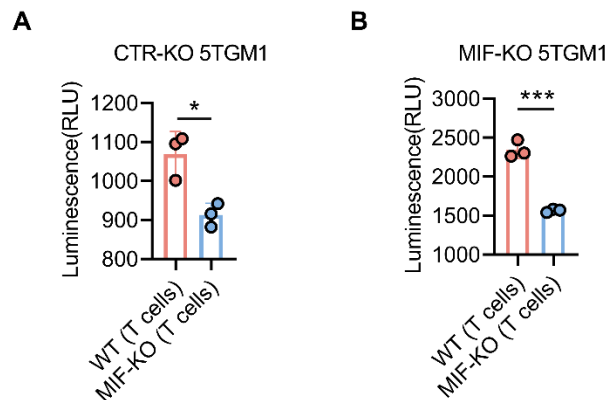


Figure S5.

T cells from mice inoculated with MIF-KO 5TGM1 cells mediate anti-MM cytotoxicity. Splenic CD3⁺ T cells were isolated from MIF-KO-inoculated mice following secondary inoculation with MIF-KO 5TGM1 cells, whereas T cells in the control group were derived from WT mice. The isolated T cells were cocultured with CTR-KO or MIF-KO 5TGM1 cells to evaluate their cytotoxic activity. Tumor cell death was quantified via a luciferase reporter assay (with luciferase-expressing 5TGM1 cells), where decreased luminescence corresponded to increased tumor cell death. **(A)** Target cells: CTR-KO 5TGM1 cells (E: T=15:1, n=3/group). **(B)** Target cells: MIF-KO 5TGM1 cells (E: T=15:1, n=3/group). E:T ratio, effector: target ratio. Student's t test was performed. *P* values are shown as * *p*<0.05, *** *p*<0.001.

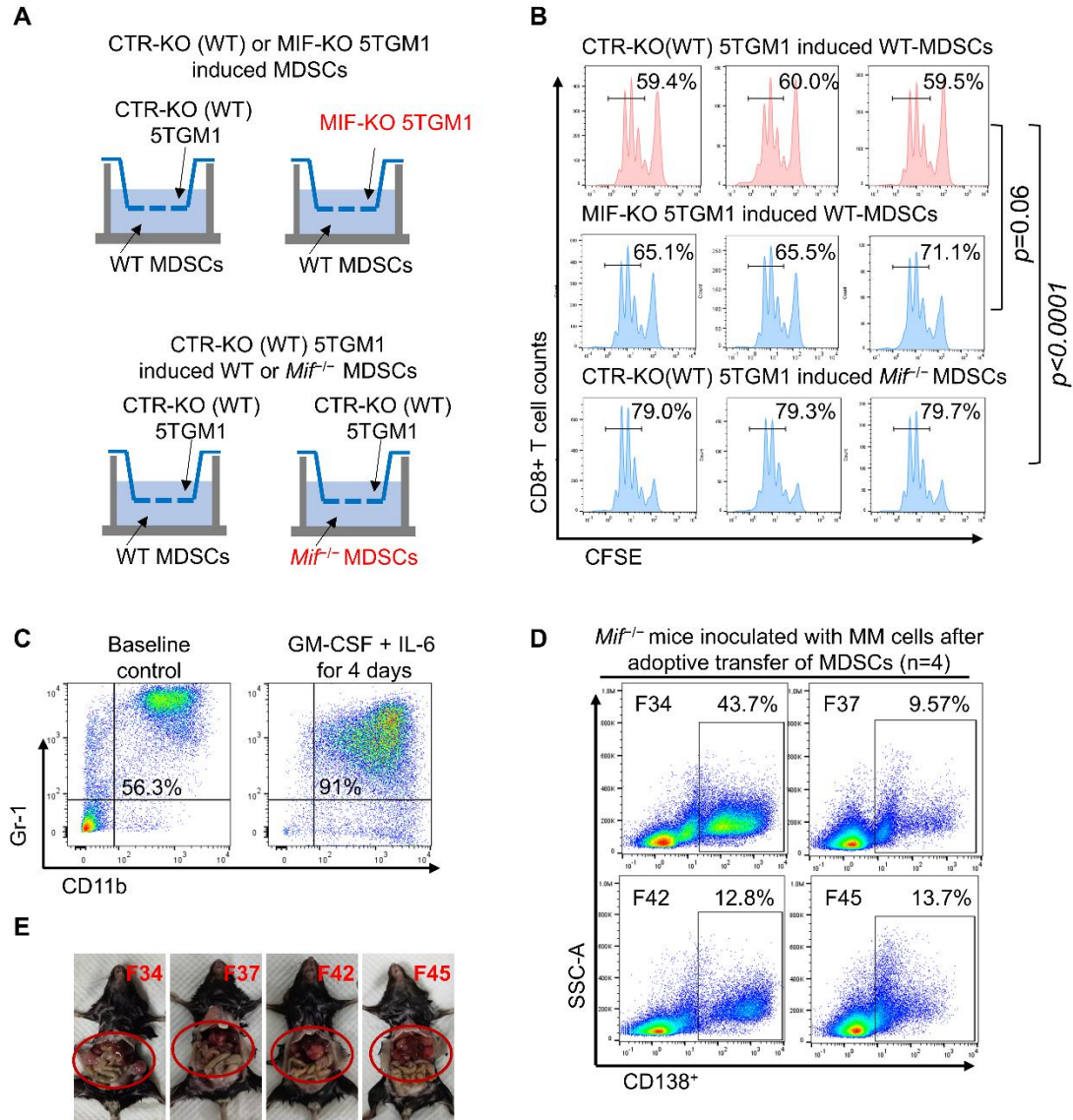


Figure S6.

Ex vivo MDSC function and tumor formation in *Mif*^{-/-} mice after MDSC transfer. (A) Two MDSC induction protocols: Protocol 1, coculture of WT mouse bone marrow mononuclear cells (BMMCs) with either CTR-KO (WT) or MIF-KO 5TGM1 cells via transwell for 5 days (upper); Protocol 2, coculture of CTR-KO (WT) 5TGM1 cells with BMMCs derived from either WT or *Mif*^{-/-} mice (lower). (B) Murine splenic T cells were cocultured with MDSCs induced from Protocol 1 or Protocol 2. Histogram showed CFSE-labeled CD8⁺ T-cell proliferation. (C) MDSCs derived from mouse bone marrow mononuclear cells. Left: MDSCs in the bone marrow before induction. Right: Proportion of MDSCs after induction (>90% indicates successful induction). (D) Flow cytometry analysis of CD138⁺ cells in *Mif*^{-/-} mice receiving adoptive transfer of MDSCs. (E) Abdominal tumors in *Mif*^{-/-} mice receiving adoptive transfer of MDSCs.

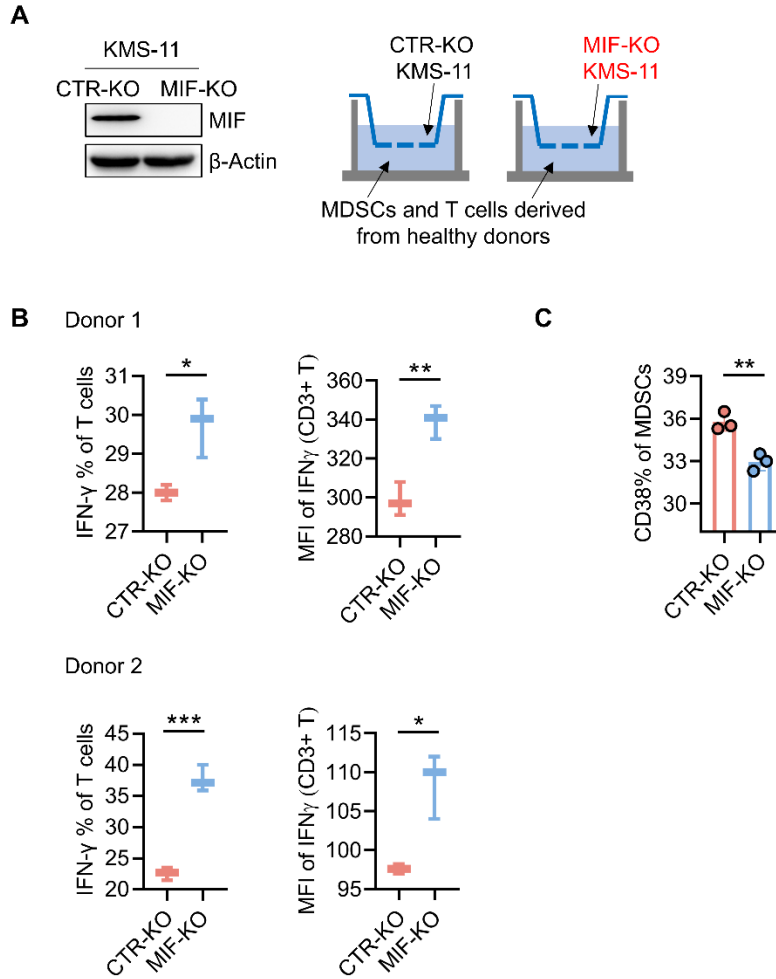


Figure S7.

MIF promotes the immunosuppressive phenotype of MDSCs and suppresses T cell function in a human ex vivo culture model. (A) Western blot showed the protein level of MIF in CTR-KO and MIF-KO KMS-11 cells (left), diagram displayed the coculture protocol of human derived MDSCs and T cell with CTR-KO and MIF-KO KMS-11 cells respectively (right). (B) The expression of IFN γ by T cells was suppressed by MDSCs cocultured with CTR-KO cells, comparing with MIF-KO cells (from two different donors). (C) Flow cytometry analysis showed the frequency of CD38⁺ was higher in MDSCs cocultured with CTR-KO KMS-11 cells, comparing with MIF-KO cells. Student's t test was performed. *P* values are shown as * $P \leq 0.05$, ** $P < 0.01$, *** $P < 0.001$.

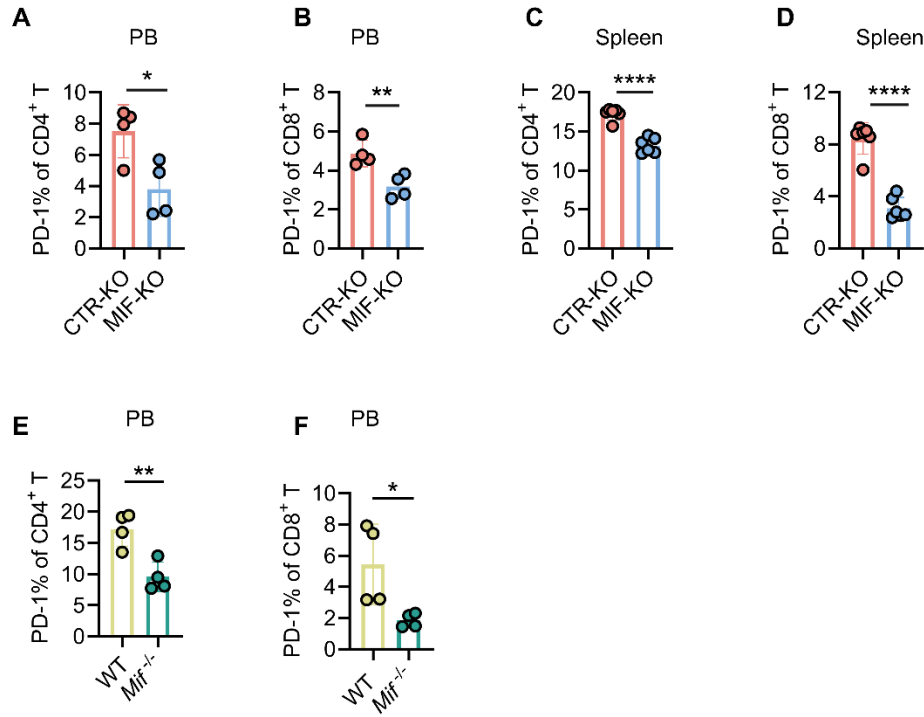


Figure S8.

PD-1 expression on T cells in the 5TMM and Vk12598 models. (A-D) 5TMM model was established via subcutaneous inoculation of CTR-KO or MIF-KO 5TGM1 cells, and T-cell function was analyzed via flow cytometry at the endpoint (Day 28). (A) PD-1 expression on PB CD4⁺ T cells, n=4/group. (B) PD-1 expression on PB CD8⁺ T cells, n=4/group. (C) PD-1 expression on splenic CD4⁺ T cells, n=6/group. (D) PD-1 expression on splenic CD8⁺ T cells, n=6/group. (E-F) The MM model was established by intravenously injecting the Vk12598 MM cell line into WT and *Mif*^{-/-} mice. PB T-cell function was assessed by flow cytometry on day 10 post-tumor inoculation: (E) PD-1 expression on CD4⁺ T cells on day 10, n=4/group; (F) PD-1 expression on CD8⁺ T cells on day 10, n=4/group. Student's t test was performed. *P* values are shown as * *P* ≤ 0.05, ** *P* < 0.01, **** *P* < 0.0001.

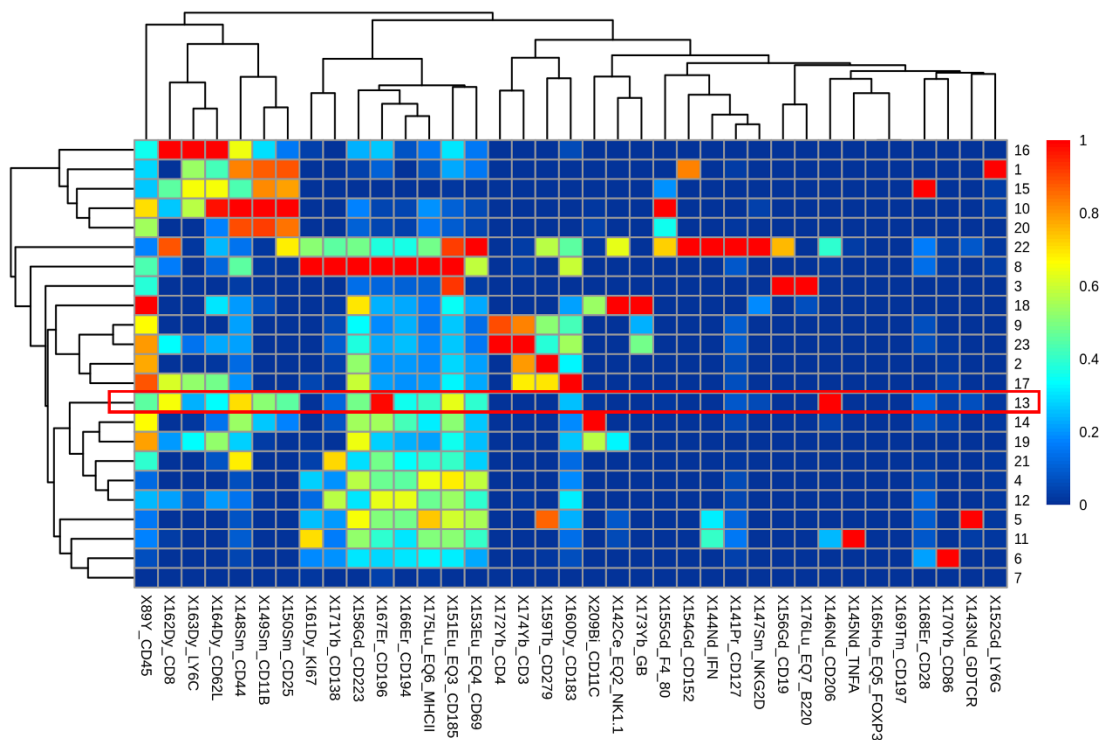


Figure S9.

Mass cytometry-based heatmap of cell clusters in subcutaneous 5TMM tumors. 5TMM model was established via subcutaneous inoculation of CTR-KO or MIF-KO 5TGM1 cells. Heatmap of signal values for cellular subpopulation antigens in subcutaneous tumors analyzed by mass cytometry 5 days post-inoculation. Cluster 13 expressed CD11b⁺CD206^{high}CD196^{high}MHC-II⁺ cells.

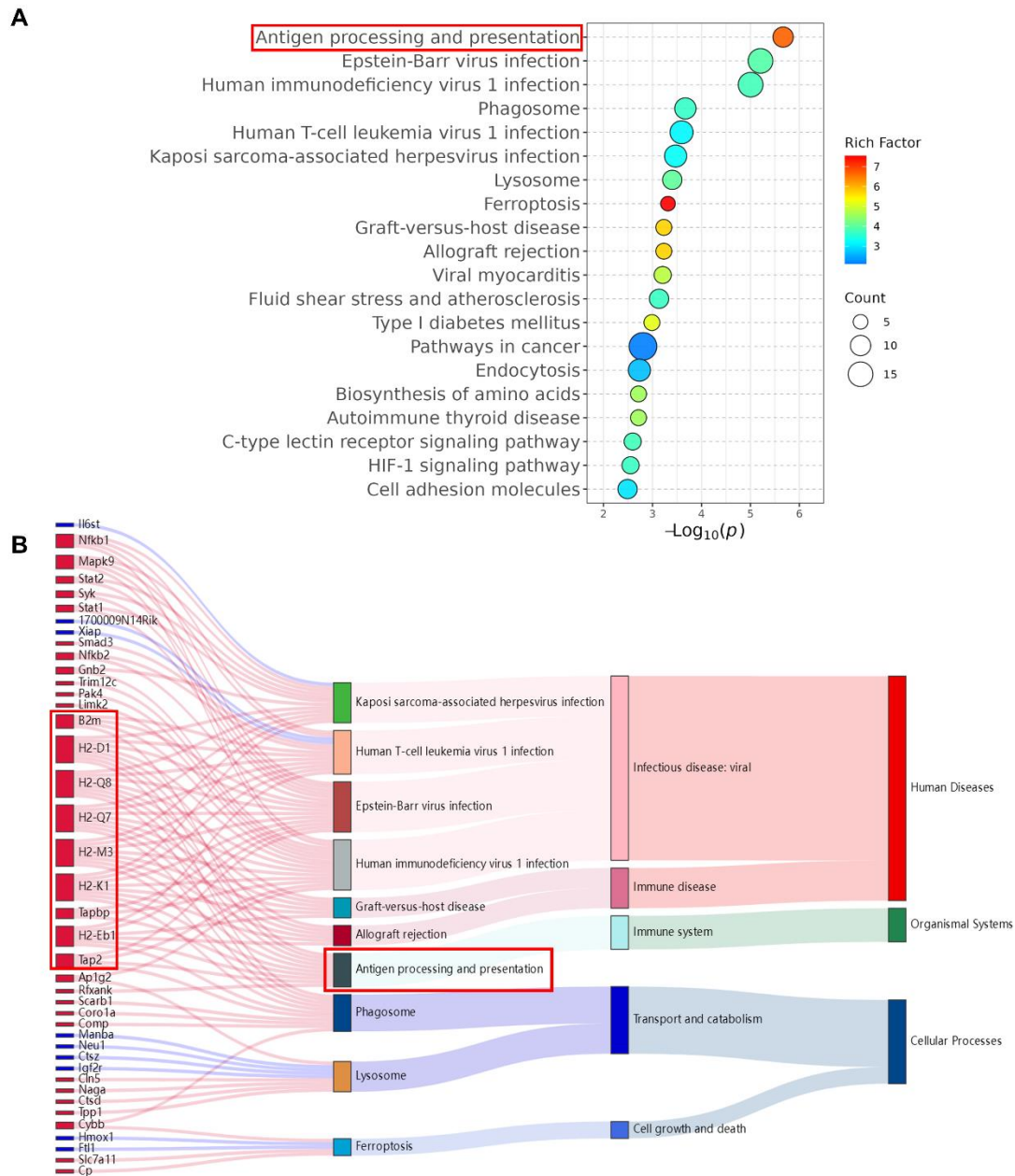


Figure S10.

Pathway enrichment analyses of proteomic data from CTR-KO and MIF-KO 5TGM1 cells. (A) The most prominent difference in KEGG signaling pathways, marked with a red box, was antigen processing and presentation. (B) Sankey diagram displaying the top 10 enriched pathways and their associated differentially expressed proteins, with red boxes highlighting key proteins enriched in antigen presentation pathways.

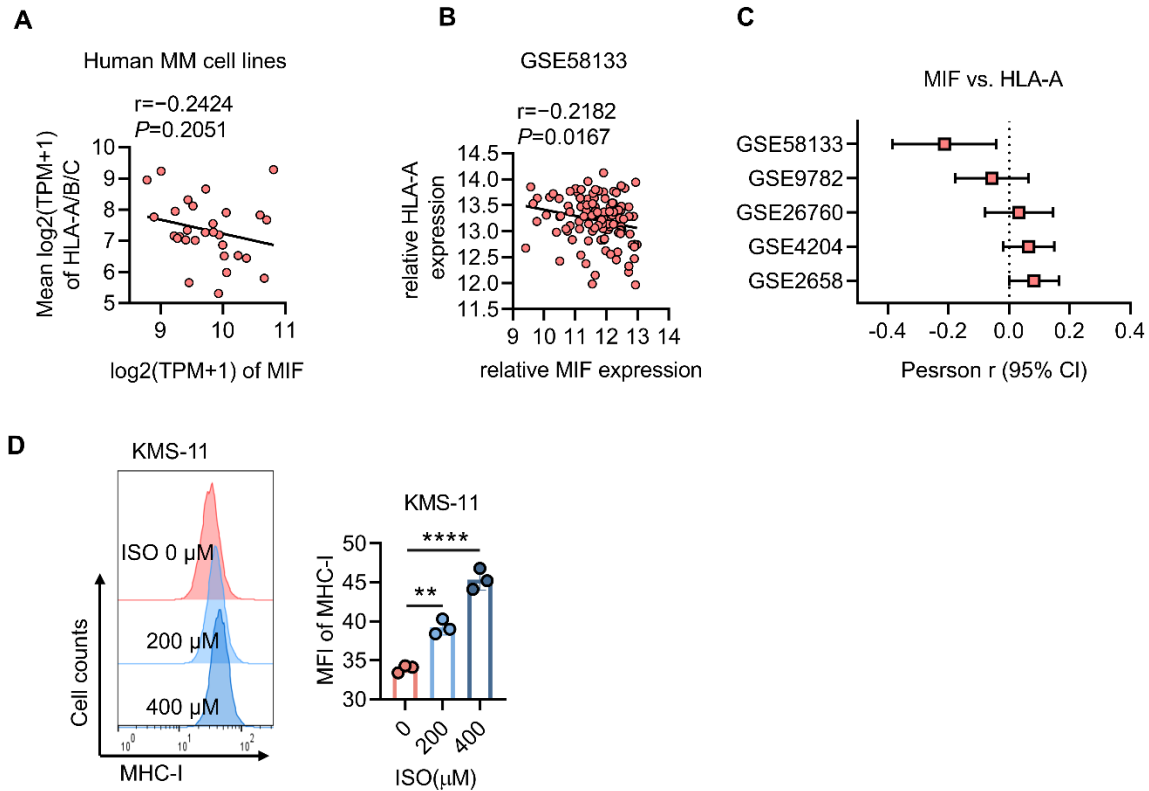


Figure S11.

The expression of MIF and MHC-I class genes in human MM samples. (A) Pearson correlation analysis of MIF expression with HLA-A, HLA-B, and HLA-C expression in MM cell lines from the DepMap RNA-seq data ($r = -0.2424$). (B) MIF and HLA-A expression in the GSE58133 MM patient cohort, showing a weak negative correlation ($r = -0.218$). (C) Summary of Pearson correlation analyses across independent MM patient datasets. (D) Human MM cell line KMS-11 treated with ISO-I (200 or 400 μ M, 48 h), flow cytometry analysis demonstrated upregulation of MHC-I (representative histograms, left), mean fluorescence intensity (MFI) of MHC-I significantly increased (right), $n = 3/\text{group}$. One-way ANOVA with post hoc Tukey's test was performed. P values are shown as ** $P < 0.01$, **** $P < 0.0001$.

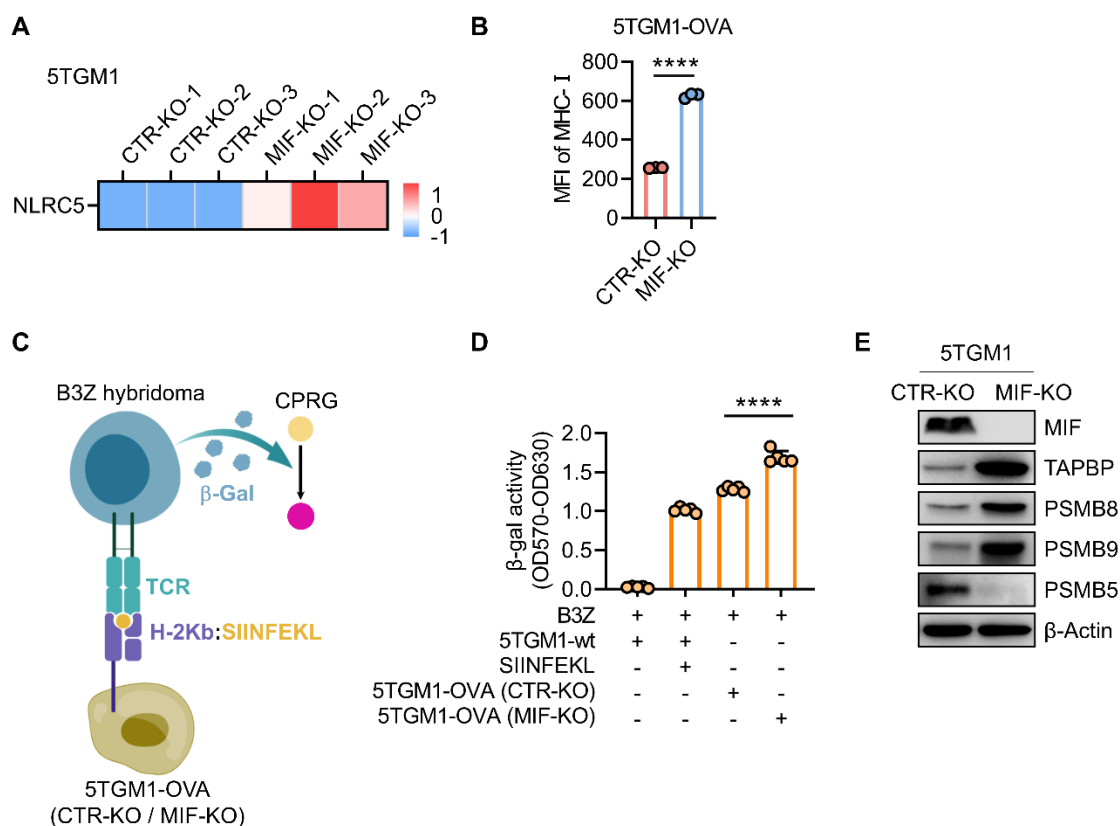


Figure S12.

MIF inhibits MHC-I-mediated antigen presentation and impairs CD8⁺ T cell activation. (A) Proteomic analysis revealed differential NLRC5 expression between CTR-KO and MIF-KO cells. (B) After stable transduction with OVA, CTR-KO 5TGM1 and MIF-KO 5TGM1 cells were analyzed by flow cytometry for MHC-I mean fluorescence intensity, $n=3/\text{group}$. Student's t test was performed. (C) Schematic of the B3Z CD8⁺ T cell activation assay. B3Z cells express β -galactosidase (β -Gal) upon T cell receptor (TCR) engagement with the OVA257–264 (SIINFEKL) peptide presented by H-2Kb. The pale-yellow substrate CPRG is hydrolyzed by β -Gal to release chlorophenol red, resulting in a distinct purple-red color under assay conditions. This image was created with BioGDP.com.⁹ (D) Bars represent antigen presentation activity, calculated as (OD570 – OD630) after subtraction of the background signal from the B3Z-only control. β -Gal activity was quantified by absorbance at 570 nm with background correction at 630 nm. One-way ANOVA with post hoc Tukey's test was performed. (E) Western blot analysis of the expression of TAPBP, PSMB8, PSMB9, and PSMB5 in CTR-KO and MIF-KO 5TGM1 cells. P values are shown as **** $P<0.0001$.

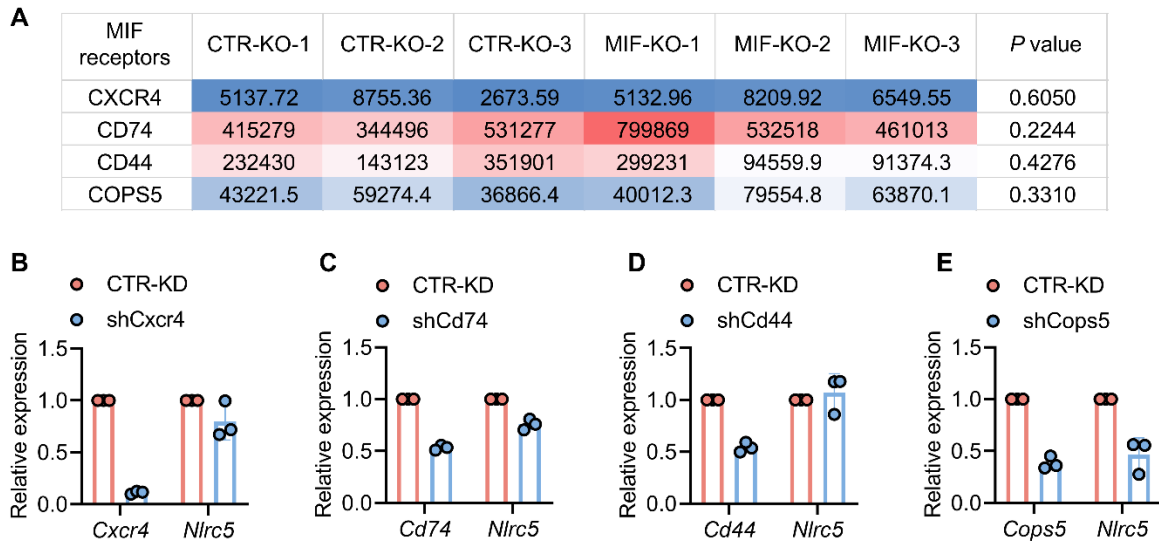


Figure S13.

Impact of MIF receptor knockdown on *Nlr5* expression in 5TGM1 cells. (A) Proteomic analysis of CTR-KO and MIF-KO 5TGM1 cells revealed no statistically significant differences in the expression levels of MIF receptors (*Cxcr4*, *Cd74*, *Cd44*, and *Cops5*). (B–E) shRNA-mediated knockdown of MIF receptors (*Cxcr4*, *Cd74*, *Cd44*, *Cops5*) with RT-qPCR analysis of receptor and *Nlr5* mRNA levels.

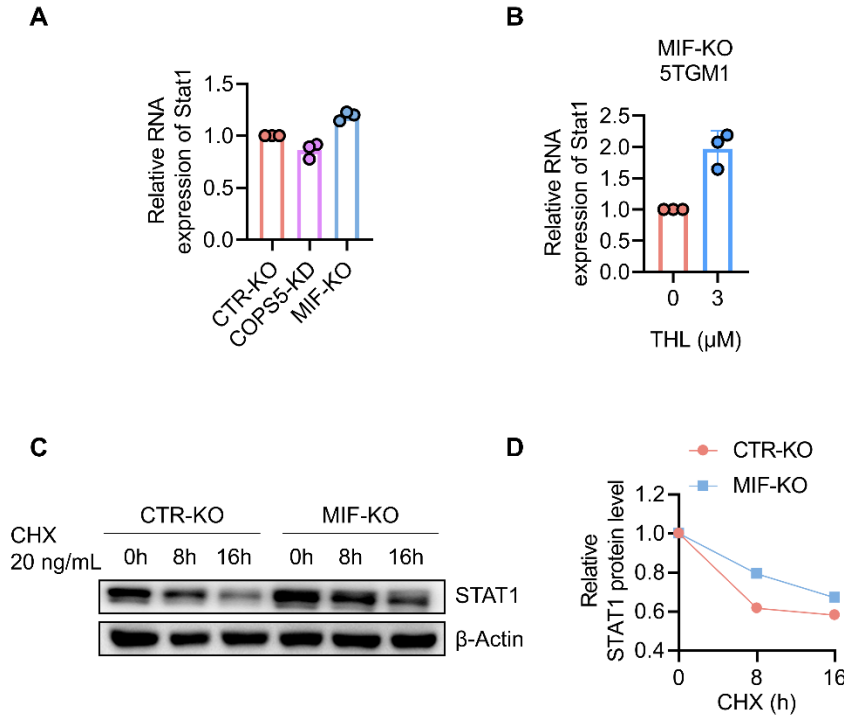


Figure S14.

MIF regulates STAT1 protein stability. (A) STAT1 mRNA levels were quantified by RT-qPCR in CTR-KO, MIF-KO, and COPS5-KD 5TGM1 cells. (B) STAT1 mRNA levels were quantified by RT-qPCR in MIF-KO 5TGM1 cells treated with THL (3μM, 12 h). (C) Western blot analysis of STAT1 expression in CTR-KO and MIF-KO 5TGM1 cells treated with cycloheximide (20 ng/mL) for 0, 8, and 16 hours. (D) Quantification of STAT1 degradation kinetics. Band intensities from (C) were analyzed by ImageJ, with STAT1 signals normalized to β-actin and expressed as relative expression levels to illustrate degradation rates following cycloheximide treatment.

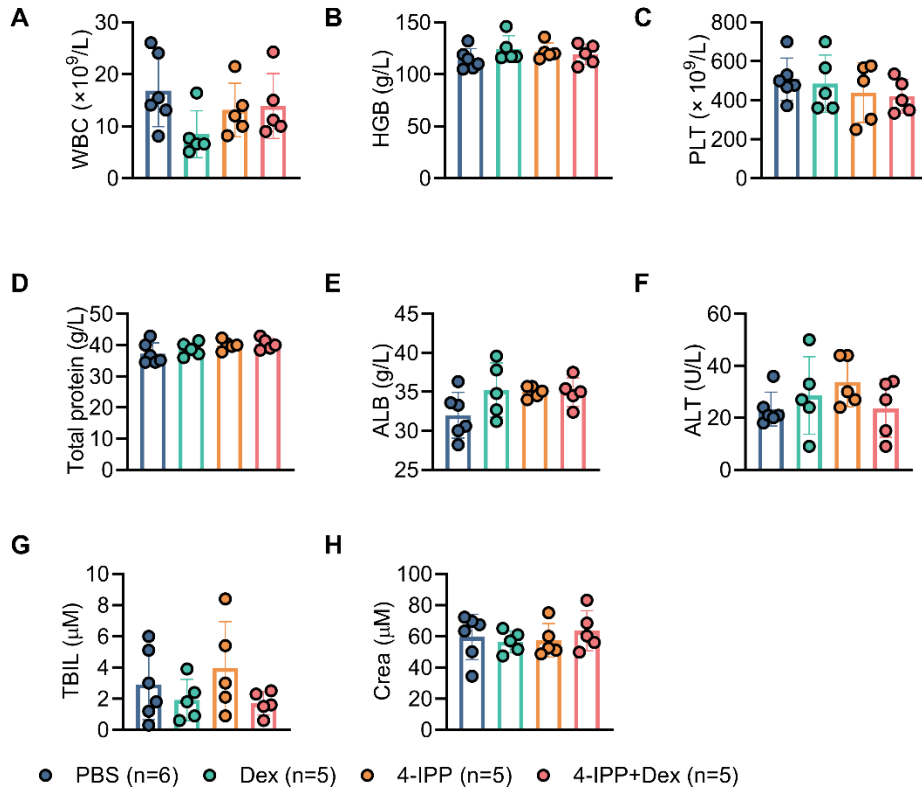


Figure S15.

Treatment-related adverse effects in the 5TMM model. (A-H) Hematologic and biochemical parameter changes at the treatment endpoint: (A) white blood cell (WBC) counts, (B) hemoglobin (HGB) levels, (C) platelet (PLT) counts, (D-E) total protein and albumin (ALB) levels, (F-G) liver function markers: alanine transaminase (ALT) and total bilirubin (TBIL), (H) renal function marker: creatinine. One-way ANOVA with post hoc Tukey's test was performed. There were no statistically significant differences between the groups (all $P > 0.05$).

Supplementary tables

Table S1. Antibodies and reagents

Antibodies	Source
Anti-MIF antibody (ab175189)	Abcam
Beta Actin Monoclonal antibody (66009-1-Ig)	proteintech
TAPBP Polyclonal antibody (30500-1-AP)	Proteintech
JAB1 Polyclonal antibody (27511-1-AP)	Proteintech
IL-18 Polyclonal antibody (10663-1-AP)	Proteintech
Phospho-Stat1 (Tyr701) (58D6) Rabbit mAb (9167)	Cell Signaling Technology
STAT1 Recombinant monoclonal antibody (82016-1-RR)	Proteintech
NLRC5 (E1E9Y) Rabbit mAb (72379)	Cell Signaling Technology
Ubiquitin antibody (P4D1) (sc-8017)	Santa
Antibodies for Murine Flow Cytometry	
FITC anti-mouse CD45 (30-F11)	Thermo Fisher
BV510 anti- mouse CD3e (145-2C11)	BD Pharmingen
PE anti-mouse CD3e (145-2C11)	BD Pharmingen
PE-Cy7 anti-mouse CD3e (145-2C11)	Thermo Fisher
APC-Cy7 anti- mouse CD4 (GK1.5)	BD Pharmingen
APC-eFluor 780 anti- mouse CD4 (GK1.5)	Thermo Fisher
PerCP/Cy5.5 anti-mouse CD8a (53-6.7)	BD Pharmingen
PE-CY7 anti-mouse TNF α (MP6-XT22)	Biolegend
APC anti-mouse IFN γ (XMG1.2)	Thermo Fisher
PE-CY7 anti-mouse Granzyme B (NGZB)	Thermo Fisher
PE anti-mouse FOXP3 (FJK-16s)	Thermo Fisher
APC anti-mouse CD25 (PC61)	Biolegend
BV421 anti-mouse PD1 (J43)	BD Pharmingen
APC anti-Mouse IL-2(JES6-5H4)	BD Pharmingen
PerCP/Cyanine5.5 anti-mouse CD11c (N418)	Biolegend
APC anti-Mouse CD11c (HL3)	BD Pharmingen
PE/Cyanine7 anti-mouse Ly-6G/Ly-6C (Gr-1) (RB6-8C5)	Biolegend
PE anti-mouse CD84 (mCD84.7)	Biolegend
APC anti-mouse/human CD44 Antibody (IM7)	Biolegend
APC anti-mouse CD38 Antibody (90)	Biolegend
APC anti-mouse/human CD45R/B220 Antibody (RA3-6B2)	Biolegend
PerCP/Cyanine5.5 anti-mouse NK-1.1 Antibody (PK136)	Biolegend

Brilliant Violet 510™ anti-mouse Ly-6C (HK1.4)	Biolegend
Brilliant Violet 605™ anti-mouse CD274 (B7-H1, PD-L1) (10F.9G2)	Biolegend
APC-Cy7 Rat Anti-CD11b (M1/70)	BD Pharmingen
PE-CY7 anti-mouse CD206 (MR6F3)	Thermo Fisher
PE anti-mouse CD184 (CXCR4) (2B11)	Thermo Fisher
BV786 anti-mouse CD74 (IN-1)	BD Pharmingen
PerCP-Cy5.5 anti-Mouse Ly-6C (AL-21)	BD Pharmingen
Brilliant Violet 421™ anti-mouse I-A/I-E (MHC II)	Biolegend
FITC anti-mouse F4/80 (BM8)	Thermo Fisher
PE/Cyanine7 anti-mouse H-2Kb/H-2Db (MHC I)	Biolegend
APC anti-Mouse CD138 (281-2)	BD Pharmingen
APC OVA257-264 (SIINFEKL) peptide (25-D1.16)	Thermo Fisher
Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™) (Clone 2.4G2)	BD Pharmingen
Anti mouse CD3 (clone 17A2)	Thermo Fisher
Anti mouse CD28 (clone 37.51)	Thermo Fisher
Fixable Viability Stain 700	BD Pharmingen
Antibodies for Human Flow Cytometry	
FITC anti-human CD45 (HI30)	Thermo Fisher
PE/Cy7 anti-human CD11b (ICRF44)	Biolegend/ BD Pharmingen
PERCP-CYN5.5 anti-human HLA-DR (LN3)	Thermo Fisher
Brilliant Violet 421™ anti-human CD15 (SSEA-1)	Biolegend
V500 anti-human CD15 (HI98)	BD Pharmingen
APC-eFluor™ 780 anti-human CD14 (61D3)	Thermo Fisher
PE anti-human CD84 (CD84.1.21)	Biolegend
APC anti-human CD274 (B7-H1, PD-L1) (29E.2A3)	Biolegend
APC anti-human CD38 (HIT2)	Biolegend
PE anti-human HLA-ABC (W6/32)	Thermo Fisher
PE anti-human CD3(HIT3a)	Biolegend
Reagents	
10× RBC Lysis Buffer (Cat. #CS003)	Leagene
Intracellular Fixation & Permeabilization Buffer Set (Cat. #88-8824-00)	Thermo Fisher
Foxp3/Transcription Factor Staining Buffer Set (Cat. #00-5523-00)	Thermo Fisher
Protease Inhibitor Cocktail (EDTA-Free, 100× in DMSO, Cat. #HY-K0010)	MCE

Cell Stimulation Cocktail (plus protein transport inhibitors, 500×, Cat. #00-4975-93)	Thermo Fisher
Recombinant mouse GM-CSF (Cat. #91115ES60)	Yeasen
Recombinant mouse IL-6 (Cat. #90146ES6)	Yeasen
Recombinant Mouse IL-2(Cat. #CK24)	Novoprotein
Recombinant mouse MIF (Cat. #92509ES10)	Yeasen
Anti mouse CD3 (clone 17A2) (Cat. #16-0032-82)	Thermo Fisher
Anti mouse CD28 (clone 37.51) (Cat. # 16-0281-82)	Thermo Fisher
Recombinant human GM-CSF (Cat. #215-GM-010)	R&D systems
Recombinant human IL-6 (Cat. # 10395-HNAE)	Sino Biological
GMP Human IL-2 Protein (Cat. # GMP-L02H14)	ACROBiosystems
ActiveMax® Human T cell Activation/Expansion CD3/CD28 Beads (Cat. # MBS-C001)	ACROBiosystems
CelThera™ GMP T Cell Expansion Medium (Cat. # GMP-CM3103)	ACROBiosystems
CFSE (Cat. #SCT110)	Merck
MG132 (Cat. #HY-13259)	MCE
Cycloheximide (Cat. #HY-12320)	MCE
GlutaMAX (Cat. #35050061)	Gibco
Chlorophenol red-β-D-galactopyranoside (Cat. # HY-D1224)	MCE
ISO-1 (Cat. #S7732)	Selleck
4-IPP (CAS NO. 41270-96-6) (Cat. #31770)	Cayman Chemical
Dexamethasone Sodium Phosphate Injection (National Medicine Permit Number H37021967)	Cisen Pharmaceutical

Table S2. sgRNA and shRNA sequence

Oligonucleotides	Sequence (5'-3')
<i>Mif</i> sgRNA Forward (mouse)	CACCGTGTGCAGGCGATCGGACAGC
<i>Mif</i> sgRNA Reverse (mouse)	AAACGCTGTCCGATCGCCTGCACAC
<i>MIF</i> sgRNA Forward (human)	CACCGAGCTCGGAGAGGAACCCGTC
<i>MIF</i> sgRNA Reverse (human)	AAACGACGGGTTCCTCTCCGAGCTC
Control sgRNA Forward	CACCGCAACAAGATGAAGAGCACCAA
Control sgRNA Reverse	AAACTTGGTGCTCTTCATCTTGTTGC
<i>Cd74</i> shRNA Forward	CCGGGCGTCCAATGTCCATGGATAACTCGAGTTAT CCATGGACATTGGACGCTTTTGT

<i>Cd74</i>	shRNA	AATTCAAAAAGCGTCCAATGTCCATGGATAACTCG
Reverse		AGTTATCCATGGACATTGGACGC
<i>Cd44</i>	shRNA	CCGGGTGTAGTGCCTACGCCATTAACCTCGAGTTAAT
Forward		GGCGTAGGCACTACACTTTTTG
<i>Cd44</i>	shRNA	AATTCAAAAAGTGTAGTGCCTACGCCATTAACCTCG
Reverse		AGTTAATGGCGTAGGCACTACAC
<i>Cxcr4</i>	shRNA	CCGGGTGTTTCAATTCCAGCATATACTCGAGTATAT
Forward		GCTGGAATTGAAACACTTTTTG
<i>Cxcr4</i>	shRNA	AATTCAAAAAGTGTGTTTCAATTCCAGCATATACTCGA
Reverse		GTATATGCTGGAATTGAAACAC
<i>Cops5</i>	shRNA	CCGGCGTATGAGTATATGGCTGCATCTCGAGATGC
Forward		AGCCATATACTCATACGTTTTTG
<i>Cops5</i>	shRNA	AATTCAAAAACGTATGAGTATATGGCTGCATCTCG
Reverse		AGATGCAGCCATATACTCATACG
Control	shRNA	CCGGCAACAAGATGAAGAGCACCAACTCGAGTTGG
Forward		TGCTCTTCATCTTGTTGTTTTTG
Control	shRNA	AATTCAAAAACAACAAGATGAAGAGCACCAACTCG
Reverse		AGTTGGTGCTCTTCATCTTGTTG

Table S3. Primers for Quantitative Real-Time PCR

Gene	Forward Sequence (5'-3')	Reverse Sequence (5'-3')
<i>Actb</i>	CCAGCCTTCCTTCTTGGGTAT	GGGTGTAAAACGCAGCTCAG
<i>B2m</i>	TTCTGGTGCTTGTCTCACTGA	CAGTATGTTTCGGCTTCCCATTC
<i>H2-D1</i>	GGTGCTGCAGAGCATTACAAGG	GGCAGCTGTCTTCACGCTTTAC
<i>H2-K1</i>	TGAGAAGGAGAAACACAGGTGG	GGGCTCCACAGTTCTTCACA
<i>H2-M3</i>	TATGTGGAGGGAGAGTGCCT	GATTCATCTTTCCCCTGCACCC
<i>H2-Q7</i>	GAGCAGGCTGGTATTGCAGAG	CACCATAAGACCTGGGGTGA
<i>H2-Q8</i>	CCTACACAAGTGGGAGCAGG	TGGAGGAGGCTCCCATCTC
<i>Nlcr5</i>	GAAGCTGCAGGTCAGGGAAT	CAGGCTGTCTTTGCCCTGTA

Table S4. Primers for ChIP q-PCR

Primer	Sequence (5'-3')
<i>Nlrc5</i> ChIP-F	CTGGGACTCCACCATGTGAC
<i>Nlrc5</i> ChIP-R	TACCTGGCTCGCCTCTTACA
Non-target control-F	TGGACCAGGGAAGAGAGATAAGT
Non-target control-R	CTTGAGGGATCGGACTAGCA