

IL-1 β promotes MPN disease initiation by favoring early clonal expansion of *JAK2*-mutant hematopoietic stem cells

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Key Points

- In a mouse model of oligoclonal MPN, IL-1 β favors disease initiation by promoting early expansion of a subclinical *JAK2*-V617F clone.
- Anti-IL-1 β antibody treatment during the early expansion phase of the *JAK2*-mutant clone reduced the frequency of MPN disease initiation.

JAK2-V617F is the most frequent somatic mutation causing myeloproliferative neoplasm (MPN). *JAK2*-V617F can be found in healthy individuals with clonal hematopoiesis of indeterminate potential (CHIP) with a frequency much higher than the prevalence of MPNs. The factors controlling the conversion of *JAK2*-V617F CHIP to MPN are largely unknown. We hypothesized that interleukin-1 β (IL-1 β)–mediated inflammation can favor this progression. We established an experimental system using bone marrow (BM) transplantations from *JAK2*-V617F and *GFP* transgenic (*VF*;*GFP*) mice that were further crossed with *IL-1 β ^{-/-}* or *IL-1R1^{-/-}* mice. To study the role of IL-1 β and its receptor on monoclonal evolution of MPN, we performed competitive BM transplantations at high dilutions with only 1 to 3 hematopoietic stem cells (HSCs) per recipient. Loss of *IL-1 β* in *JAK2*-mutant HSCs reduced engraftment, restricted clonal expansion, lowered the total numbers of functional HSCs, and decreased the rate of conversion to MPN. Loss of *IL-1R1* in the recipients also lowered the conversion to MPN but did not reduce the frequency of engraftment of *JAK2*-mutant HSCs. Wild-type (*WT*) recipients transplanted with *VF*;*GFP* BM that developed MPNs had elevated IL-1 β levels and reduced frequencies of mesenchymal stromal cells (MSCs). Interestingly, frequencies of MSCs were also reduced in recipients that did not develop MPNs, had only marginally elevated IL-1 β levels, and displayed low GFP-chimerism resembling CHIP. Anti-IL-1 β antibody preserved high frequencies of MSCs in *VF*;*GFP* recipients and reduced the rate of engraftment and the conversion to MPN. Our results identify *IL-1 β* as a potential therapeutic target for preventing the transition from *JAK2*-V617F CHIP to MPNs.

Introduction

Myeloproliferative neoplasms (MPNs) are clonal disorders of the hematopoietic stem cell (HSC) in most cases caused by activating mutations in *JAK2*, *CALR*, or *MPL* that increase the proliferation of

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supplemental Material. Requests for original data, resources, and reagents should be addressed to the corresponding author, Radek C. Skoda (radek.skoda@unibas.ch).

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erythroid, megakaryocytic, and myeloid lineages.¹⁻⁷ MPNs can be subclassified as polycythemia vera, essential thrombocythemia, and primary myelofibrosis.^{8,9} *JAK2-V617F* is the most frequent driver gene mutation in patients with MPN, but *JAK2-V617F* can also be detected as clonal hematopoiesis of indeterminate potential (CHIP) in healthy individuals, with a frequency much higher than the incidence of MPN.¹⁰⁻¹³ Thus, the acquisition of *JAK2-V617F* is not the rate-limiting step for developing MPN, and there are factors that can suppress or promote the expansion of the *JAK2* mutated clone and thereby control the transition from CHIP to MPN.

Inflammation is a hallmark of advanced MPN.¹⁴ Chronic immune stimulation in patients with a history of infectious or autoimmune diseases was associated with increased risk of myeloid malignancies including MPN.¹⁵⁻¹⁷ Therefore, we hypothesized that inflammation might be one of the factors promoting the transition from *JAK2-V617F*-positive CHIP to MPN. Interleukin-1 β (IL-1 β), a pleiotropic cytokine with diverse innate and adaptive immune functions, is a master regulator of inflammatory state^{18,19} and has been implicated in promoting several hematological malignancies.^{20,21} Previous studies have shown that IL-1 β is favoring progression to myelofibrosis at advanced stages of MPN.^{22,23}

Here, we examined the role of IL-1 β in the very early stages of MPN initiation by *JAK2-V617F* using competitive bone marrow (BM) transplantations at high dilutions (1:100), with very few (1-3) long-term HSCs (LT-HSCs). The initiation of MPN from a single mutant HSCs is fundamentally different from the progression of chronic phase MPN to myelofibrosis, which is mediated by profibrotic cytokines such as transforming growth factor β and is linked to augmented megakaryopoiesis and the emergence of myofibroblasts and monocyte-derived profibrotic fibrocytes.

We found that IL-1 β secreted by *JAK2*-mutant hematopoietic cells promotes the early expansion of the *JAK2-V617F* clone and requires the presence of the IL-1 receptor type 1 (IL-1R1) on the responding BM stromal cells. Genetic ablation of *IL-1 β* or *IL-1R1* and treatment with a neutralizing IL-1 β antibody reduces MPN disease initiation.

Methods

Mice

We used conditional *JAK2-V617F* transgenic mice,²⁴ *ScfCre^{ER}* mice,²⁵ *Ubc-GFP* mice,²⁶ *IL-1 β* -knockout mice,²⁷ and *IL-1R1*-knockout mice.²⁸ *ScfCre^{ER};V617F (VF)* mice were characterized previously.²⁹ *Cre^{ER}* was activated by intraperitoneal injections of 2 mg tamoxifen (Sigma Aldrich) for 5 consecutive days. All mice were of pure C57BL/6N background and kept under specific pathogen-free conditions with free access to food and water in accordance with Swiss federal regulations. All animal experiments were approved by the Cantonal Veterinary Office of Basel-Stadt, Switzerland.

Blood samples and clinical data of patients with MPN were collected at the University Hospital Basel, Switzerland. Blood samples from healthy controls were obtained from the local blood donation center (Stiftung Blutspendezentrum SRK beider Basel). The study was approved by the local ethics committee (Ethik Kommission Beider Basel). Written informed consent was obtained from all patients in accordance with the Declaration of Helsinki.

BM transplantations and drug treatment

BM from transgenic mice was harvested 6 to 8 weeks after tamoxifen induction, mixed with wild-type BM competitor cells, and injected into the tail vein of lethally irradiated recipient mice (12 Gy). Blood was drawn from the tail vein every 4 to 6 weeks. Green fluorescent protein (GFP)-chimerism was assessed by flow cytometry, and complete blood counts were performed on Advia120 Hematology Analyzer with Multispecies software version 5.9.0-MS (Bayer). Aspirin (Sigma) was added to drinking water (150 μ g/mL) and bottles were changed every 3 to 4 days. Mouse immunoglobulin G2a (IgG2a) anti-mouse IL-1 β antibody (01BSUR; Novartis Pharma AG, Basel, Switzerland),³⁰⁻³² or mouse IgG2a isotype were injected intraperitoneally once per week (10 mg/kg every week, intraperitoneal).

Results

Loss of *IL-1 β* from *JAK2*-mutant hematopoietic cells reduces the rate of MPN disease initiation

To test the hypothesis that IL-1 β favors MPN disease initiation by promoting the early expansion of the *JAK2-V617F* clone, we used competitive BM transplantations at limiting dilutions that mimic the monoclonal origin of MPN.³³ As BM donors, we used triple-transgenic *ScfCre^{ER};JAK2-V617F;Ubc-GFP (VF;GFP)* mice that allow tracking of the transplanted BM cells by detection of GFP. *VF;GFP* mice were crossed with *IL-1 β ^{-/-}* mice to obtain *VF;IL-1 β ^{-/-};GFP* mice. Expression of the *VF* transgene was induced with tamoxifen. After 6 to 8 weeks, 2×10^4 unfractionated BM cells containing only 1 to 3 *JAK2*-mutant LT-HSCs were mixed with a 100-fold excess of *IL-1 β ^{-/-}* BM competitor cells and transplanted into recipient mice (Figure 1A).³³ Within 36 weeks, ~60% of these recipients developed MPN phenotype characterized by elevated hemoglobin and/or platelet counts and splenomegaly (Figure 1B). Mice with MPN phenotype also showed increased IL-1 β levels in the plasma and BM, whereas mice without MPN phenotype displayed very low IL-1 β levels (Figure 1C). Very similar results were obtained in experiments when *WT* BM competitor cells were used instead of *IL-1 β ^{-/-}* competitors (supplemental Figure 1), indicating that *WT* competitor cells did not contribute to the increased IL-1 β production.

Transplantation of *VF;IL-1 β ^{-/-};GFP* BM with *IL-1 β ^{-/-}* competitor cells at a 1:100 ratio produced considerably fewer recipients with MPN phenotype than mice that received *VF;GFP* BM, and only 1 mouse showed thrombocytosis (Figure 1B, lower panel). Recipients with MPN phenotype did not show increased IL-1 β levels, suggesting that excess IL-1 β was produced by the *JAK2-V617F* expressing cells (Figure 1C). Recipients transplanted with *VF;GFP* BM showed higher GFP chimerism (Figure 1D), higher percentage of engraftment (90% vs 66%) defined as GFP chimerism of >1% in peripheral blood Gr1⁺ granulocytes, and a higher percentage of MPN initiation (48% vs 12%) than mice transplanted with *VF;IL-1 β ^{-/-};GFP* BM (Figure 1E). We have previously shown that *VF* and *VF;IL-1 β ^{-/-}* mice have comparable frequencies of HSCs in the BM.²²

When only recipients with MPN phenotype were compared, blood counts and peripheral blood GFP chimerism were similar, except for slightly lower platelet count and GFP chimerism in platelets of

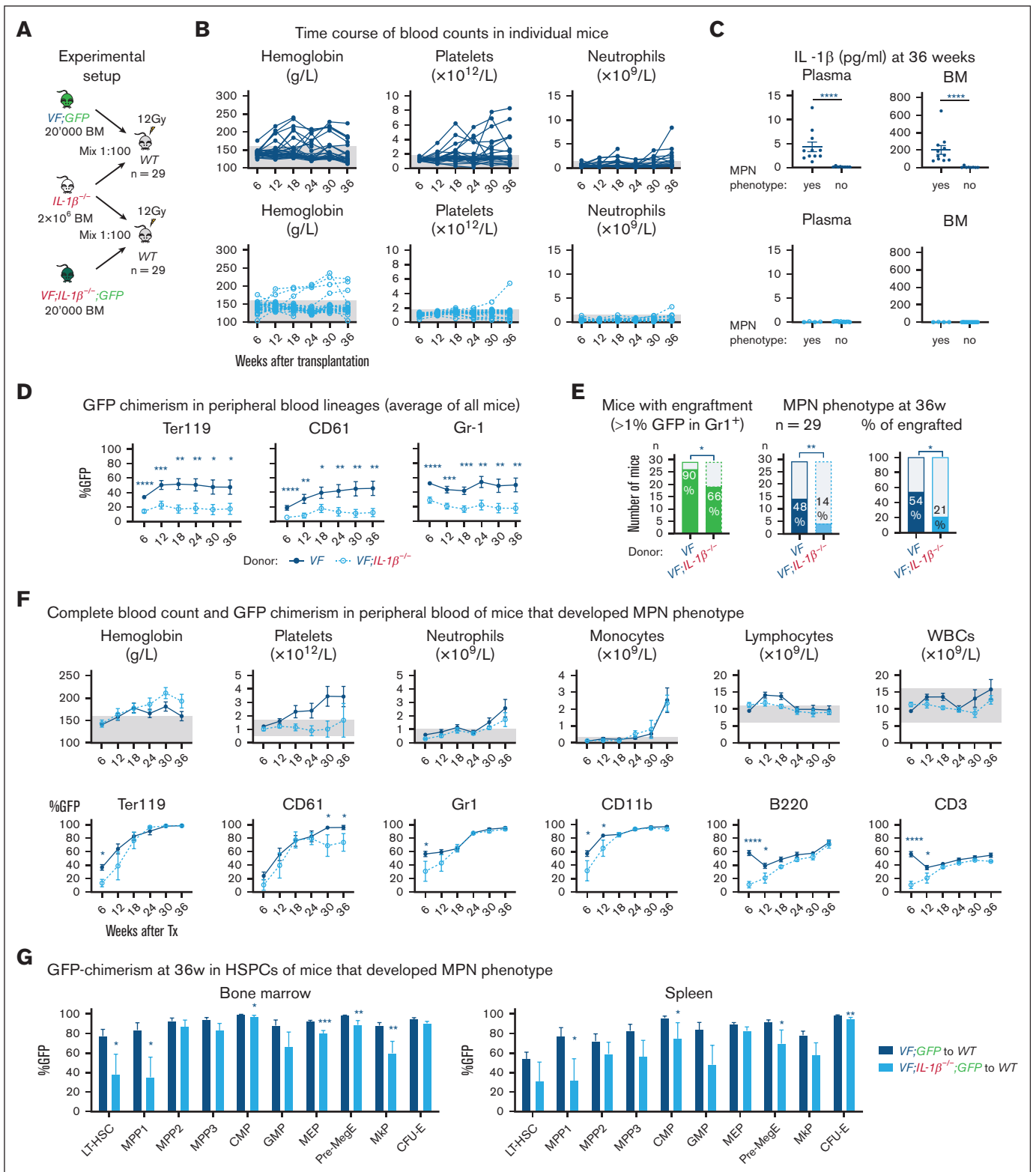


Figure 1. Loss of *IL-1 β* from *JAK2*-mutant hematopoietic cells reduces MPN disease initiation. (A) Schematic drawing of the experimental setup for competitive transplantation at 1:100 dilution. BM from *VF;GFP* or *VF;IL-1 β ^{-/-};GFP* donor mice was mixed with a 100-fold excess of BM competitor cells from an *IL-1 β ^{-/-}* donor. (B) Time course of blood counts from individual mice that received BM from *VF;GFP* (upper panel) or *VF;IL-1 β ^{-/-};GFP* donors (lower panel). (C) IL-1 β protein levels in plasma and BM lavage (1 femur and 1 tibia) of mice with or without MPN phenotype is shown (right panel). Nonparametric Mann-Whitney 2-tailed *t* test was performed for statistical comparisons. (D) Time courses of GFP chimerism in the peripheral blood are shown. Multiple *t* tests were performed for statistical analyses. (E) Bar graphs show the percentages of mice that

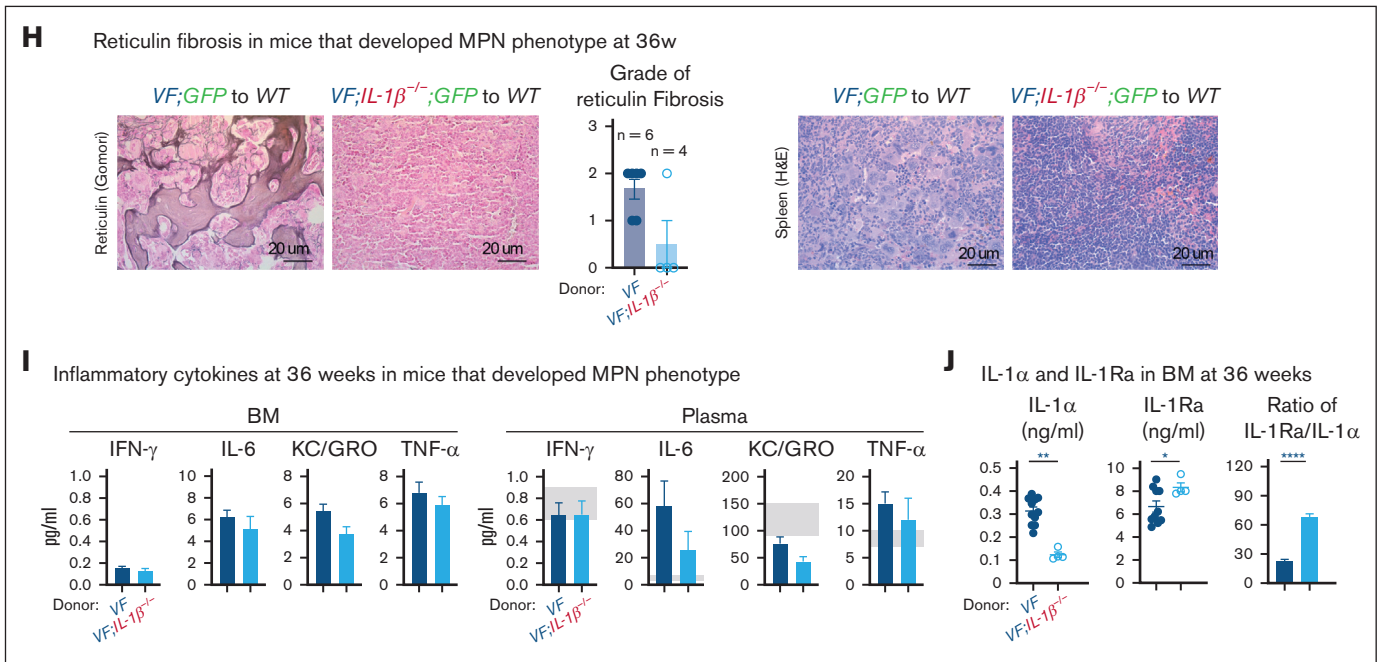


Figure 1 (continued) showed engraftment defined as GFP chimerism of >1% at 18 weeks after transplantation and the percentages of mice that developed MPN phenotype (elevated hemoglobin and/or platelet counts). *P* values in lower panel were computed using Fisher exact test. (F) Time course of mean blood counts and GFP chimerism in the peripheral blood of *WT* mice transplanted with BM from *VF;GFP* or *VF;IL-1 β ^{-/-};GFP* and *IL-1 β ^{-/-}* competitor cells that developed MPN phenotype during 36-weeks follow-up. Multiple *t* tests were performed for statistical analyses. (G) GFP chimerism in HSPCs at 36 weeks after transplantation in the BM (left) and spleen (right) of *WT* mice transplanted with BM from *VF;GFP* or *VF;IL-1 β ^{-/-};GFP* and *IL-1 β ^{-/-}* competitor cells that developed MPN phenotype. Multiple *t* tests were performed for statistical analyses. (H) Representative images of reticulin fibrosis staining in the BM (left panel) and hematoxylin and eosin staining in the spleen (right panel) of mice showed MPN phenotype at 36 weeks after transplantation. Histological grade of reticulin fibrosis in BM is shown in a bar graph (right). (I) Levels of inflammatory cytokines in BM lavage (1 femur and 1 tibia) and plasma of mice that displayed MPN phenotype at 36 weeks after transplantation. (J) Levels of IL-1 cytokines in the BM. Gray shaded area represents normal range. All data are presented as mean \pm standard error of the mean (SEM); **P* < .05; ***P* < .01; ****P* < .001; and *****P* < .0001. See also supplemental Figures 1 and 2.

VF;IL-1 β ^{-/-};GFP recipients (Figure 1F). Furthermore, mice transplanted with *VF;IL-1 β ^{-/-};GFP* BM that developed MPN phenotype showed lower GFP chimerism in hematopoietic stem and progenitor cells (HSPCs; Figure 1G), less reticulin fibrosis and osteosclerosis in the BM, and partial restoration of splenic architecture (Figure 1H). Inflammatory cytokines were largely unchanged, except for a trend toward lower IL-6 levels (Figure 1I). Loss of *IL-1 β* in *VF* donor cells resulted in reduced levels of IL-1 α in BM of mice that developed MPN (Figure 1J). Interestingly, loss of *IL-1 β* in *VF* donor cells also increased the levels of IL-1R antagonist (IL-1Ra). As a result, the ratio of IL-1Ra to IL-1 α increased in *VF;IL-1 β ^{-/-}* recipients with MPN phenotype, correlating with the less severe MPN phenotype and less myelofibrosis than recipients of *VF* BM.

Finally, BM transplantations into *IL-1 β ^{-/-}* recipients using *IL-1 β ^{-/-}* competitor cells demonstrated that *VF* BM cells alone were the source for the increased IL-1 β levels in the plasma and BM (supplemental Figure 2A, upper panel). Unexpectedly, *IL-1 β ^{-/-}* recipients transplanted with *VF;IL-1 β ^{-/-}* BM showed higher frequencies of engraftment and MPN disease initiation than expected and almost equal to *IL-1 β ^{-/-}* recipients transplanted with *VF* BM (supplemental Figure 2A, middle panel). GFP chimerism was reduced when all recipient mice were considered (supplemental Figure 2A, lower panel), but when only mice that developed MPN were compared, very few alterations were noted (supplemental Figure 2B). At 36 weeks, *IL-1 β ^{-/-}* recipients transplanted with

VF;IL-1 β ^{-/-} BM showed a trend toward reduced reticulin fibrosis, reduced GFP chimerism in HSPCs, and partial restoration of splenic architecture (supplemental Figure 2C-D), and a trend toward reduction of IL-6 and KC/GRO levels (supplemental Figure 2E). The reason why engraftment and MPN disease initiation increased when IL-1 β was absent in both donors and recipients is currently unknown. No increase in IL-1Ra or in the ratio between IL-1 α and IL-1Ra was found in *IL-1 β ^{-/-}* recipients transplanted with *VF;IL-1 β ^{-/-}* BM (supplemental Figure 2F), suggesting that lack of upregulation of IL-1Ra might contribute to the more efficient MPN disease initiation. Complete loss of *IL-1 β* partially prevented this outcome, possibly because of diminished anti-IL-1 inflammatory responses in the BM.

Overall, our data show that loss of *IL-1 β* in hematopoietic cells reduced MPN initiation, suggesting that IL-1 β produced by the *JAK2*-mutant cells is promoting early expansion of the *JAK2*-V617F clone and conversion to MPN.

JAK2-V617F mutant HSCs need IL-1 β for optimal expansion of the HSC population with long-term repopulation capacity

To examine the effects of *IL-1 β* loss on mutant HSC function, we performed secondary transplantations into *WT* recipients (Figure 2). As donors, we selected 3 recipients of *VF;GFP* BM

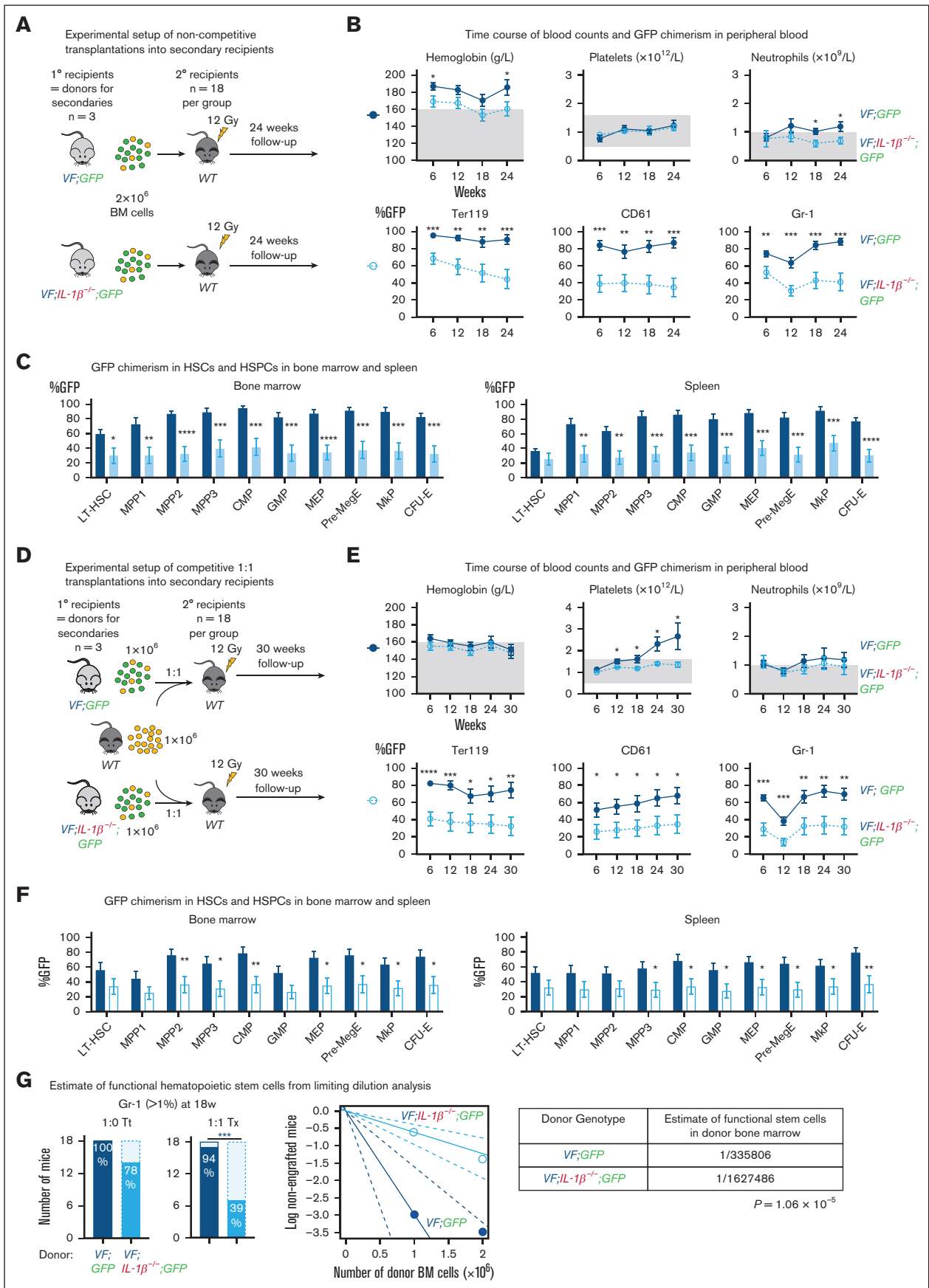


Figure 2.

and 3 recipients of *VF;IL-1 β ^{-/-};GFP* BM that displayed MPN phenotype (supplemental Figure 3). BM cells were pooled and used for noncompetitive (Figure 2A-C) and competitive secondary transplantations (Figure 2D-F). Secondary recipients of *VF;IL-1 β ^{-/-};GFP* BM showed significantly reduced blood counts and lower GFP chimerism in the peripheral blood, BM, and spleen than recipients of *VF;GFP* BM (Figure 2B-C,E-F; supplemental Figure 4A-B). By comparing the frequencies of engraftment (defined as >1% in Gr1⁺ granulocytes at 18 weeks) between the competitive and noncompetitive secondary transplantations, and using the "extreme limiting dilution analysis" algorithm,³⁴ we estimated that the numbers of functional HSCs were approximately fivefold reduced in donor mice that initially received *VF;IL-1 β ^{-/-}* BM compared with in donors initially transplanted with *VF* BM (Figure 2G). Overall, the HSC pool in primary recipient mice transplanted with 1 to 3 HSCs from *VF* donors expanded to a total of ~300 functional HSCs after 36 weeks, whereas primary recipients transplanted with 1 to 3 HSCs from *VF;IL-1 β ^{-/-}* donors had only ~50 functional HSCs. These results indicate that *JAK2-V617F* mutant HSCs require IL-1 β for optimal expansion of the HSC population with long-term repopulation capacity.

MPN initiation by *JAK2-V617F* is favored by signaling through the IL-1R1 in both hematopoietic and nonhematopoietic cells

Next, we examined the role of *IL-1R1* in MPN disease initiation. *VF;GFP* mice were crossed with *IL-1R1^{-/-}* mice to obtain *VF;IL-1R1^{-/-};GFP* mice that were used as donors for competitive BM transplantations into *WT* recipients (Figure 3A-E), or *IL-1R1^{-/-}* recipients (Figure 3F-J). GFP-chimerism in peripheral blood was higher in mice transplanted with *VF;GFP* BM than in recipients of *VF;IL-1R1^{-/-};GFP* BM, when all mice that showed engraftment were considered (Figure 3D). Transplantations into *WT* recipients resulted in high frequency of engraftment, but fewer recipients transplanted with *VF;IL-1R1^{-/-};GFP* BM developed MPN phenotype (27%) than recipients of *VF;GFP* BM (55%) (Figure 3E). GFP chimerism was similar when only mice with MPN phenotype were compared (supplemental Figure 5A), but loss of *IL-1R1* from *JAK2*-mutant cells resulted in reduced GFP chimerism in HSPCs in the BM and spleen (supplemental Figure 5B). Recipients of *VF;IL-1R1^{-/-};GFP* BM displayed low IL-1 β levels in the plasma and BM, and no differences were noted between mice with or without MPN phenotype (Figure 3C). Nevertheless, IL-1 β levels were higher in *WT* recipients transplanted with *VF;IL-1R1^{-/-}* BM than in those transplanted with *VF;IL-1 β ^{-/-}* BM (supplemental Figure 5C). Although *VF;IL-1 β ^{-/-}* cells are unable to produce IL-1 β , cells from

VF;IL-1R1^{-/-} mice can produce IL-1 β , although at lower levels, because of loss of the positive IL-1 β -feedback mediated by the IL-1R1.^{18,19,35,36} Loss of *IL-1R1* in *JAK2*-mutant cells also reduced reticulin fibrosis and osteosclerosis in the BM and partially corrected the splenic architecture in mice that developed MPN phenotype (supplemental Figure 5D). Cytokine levels did not change, except for decreased IL-1 β in plasma (supplemental Figure 5E).

Transplantations into recipients deficient for *IL-1R1^{-/-}* also resulted in high frequency of engraftment (Figure 3F-J), but MPN disease initiation in *IL-1R1^{-/-}* deficient recipients transplanted with *VF;GFP* BM was reduced to 29% (Figure 3J), compared with 55% in *WT* recipients (Figure 3E). In mice that developed MPN phenotype, blood counts did not change, but GFP chimerism in the peripheral blood was slightly reduced with complete loss of *IL-1R1* (supplemental Figure 6A), whereas GFP chimerism remained unchanged in HSPCs (supplemental Figure 6B). Compared with *WT* recipients, *IL-1R1^{-/-}* recipients transplanted with *VF;GFP* showed reduced grade of reticulin fibrosis in the BM, normalization of splenic architecture, (supplemental Figure 6C), and reduced levels of inflammatory cytokines (supplemental Figure 6D). However, loss of *IL-1R1* in both donor and recipient did not affect reticulin fibrosis or the levels of inflammatory cytokines (supplemental Figure 6C-D).

Taken together, these results show that increased expression of IL-1 β by the *JAK2*-mutant hematopoietic cells and the presence of *IL-1R1* on both mutant hematopoietic cells and *WT* non-hematopoietic cells are required for efficient MPN disease initiation and progression.

Pharmacological inhibition of inflammation can reduce MPN disease initiation in a mouse model of *JAK2-V617F* driven clonal hematopoiesis

Because reducing proinflammatory signaling by *IL-1 β* knockout in *VF* donor cells or deletion of *IL-1R1* in the recipient cells both reduced MPN disease initiation in competitive transplantations at high dilution, we examined whether the same effect can be observed with pharmacologic inhibition of inflammation. We first tested the effects of aspirin, which was added to the drinking water on day 1 after transplantation (supplemental Figure 7). We observed a slight reduction in the frequencies of engraftment (96% in the controls vs 70% in the aspirin-treated mice), but there were no differences in the blood counts or frequencies of MPN disease initiation (supplemental Figure 7).

Figure 2. *JAK2-V617F* HSCs need IL-1 β for long-term stem cell function. (A) Schematic drawing of noncompetitive (1:0) transplantations. Primary recipients of *VF;GFP* or *VF;IL-1 β ^{-/-};GFP* BM were sacrificed at 36 weeks after transplantation and their BM cells (2×10^6) were transplanted into *WT* recipients ($n = 18$ per group). (B) The time course of blood counts from mice that received BM from *VF;GFP* (solid symbols) or *VF;IL-1 β ^{-/-};GFP* donors (open symbols), and their GFP chimerism in the peripheral blood (lower panel) are shown. (C) Analysis of GFP chimerism in HSPCs from the BM and spleen is shown. Multiple *t* tests were performed for statistical analyses. (D-F) Schematic drawing of the identical aforementioned experiment, in which BM competitor cells from a *WT* mouse was mixed to a 1:1 ratio for the transplantations into secondary *WT* recipients. Annotations as in panels B and C. Multiple *t* tests were performed for statistical analyses. (G) Bar graphs show the percentages of mice that showed engraftment defined as GFP chimerism of >1% at 18 weeks after noncompetitive (left) and competitive 1:1 transplantation (right). Middle panel shows the log of nonengrafted mice (GFP chimerism in Gr1 of <1% at 18 weeks) vs the number of donor BM cells transplanted in each group. Estimated frequency of functional stem cells in the BM was calculated using extreme limiting dilution analysis.³⁴ Solid dark blue line represents the estimated frequency of HSCs in mice transplanted with BM from *VF;GFP* donors. Solid light blue line represents the estimated frequency of HSCs in mice transplanted with BM from *VF;IL-1 β ^{-/-};GFP* donors. Dotted lines show 95% confidence interval. Gray shaded areas represent the normal range. All data are presented as mean \pm SEM; **P* < .05; ***P* < .01; ****P* < .001; and *****P* < .0001. See also supplemental Figures 3 and 4.

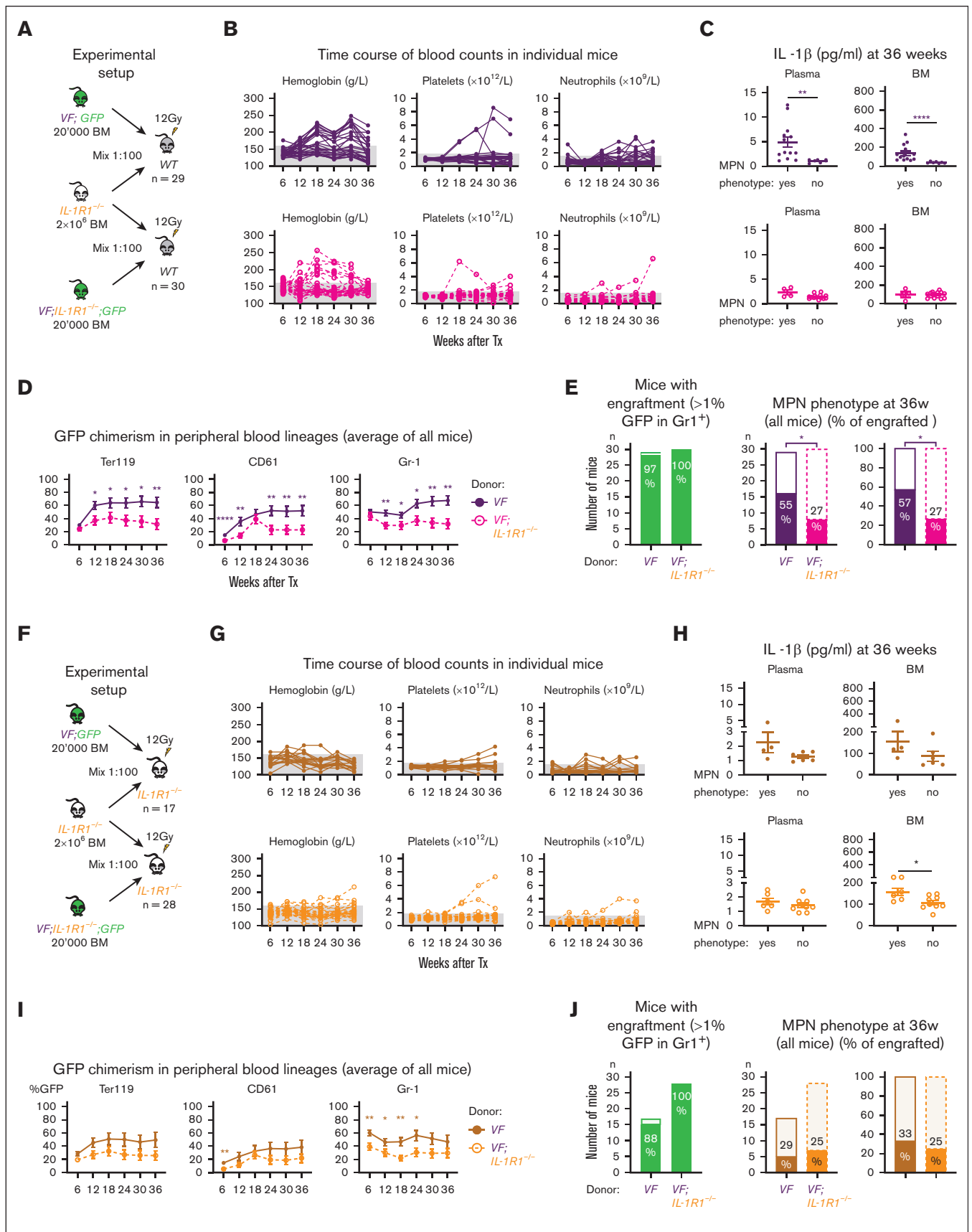


Figure 3.

Next, we examined the effects of a neutralizing anti-IL-1 β antibody in mice transplanted with BM from a *VF;GFP* donor (Figure 4A). Treatment with anti-IL-1 β antibody or isotype started on day 1 after transplantation and was continued for 18 weeks. Anti-IL-1 β antibody reduced the percentage of mice with engraftment, defined as >1% GFP at 18 weeks and fewer mice developed MPN phenotype than mice treated with isotype control (Figure 4B). GFP chimerism was already higher at 6 weeks and further increased in *VF* mice that developed MPN phenotype under isotype treatment than in *VF* mice that engrafted but failed to develop MPN (Figure 4C). In contrast, no differences in GFP chimerism were observed after 6 weeks under anti-IL-1 β antibody treatment, but GFP chimerism decreased between week 6 and 18 under anti-IL-1 β antibody treatment in *VF* mice that engrafted but failed to develop MPN (Figure 4D). HSPCs from mice with MPN phenotype displayed high GFP chimerism that did not change by anti-IL-1 β antibody treatment, whereas HSPCs from mice with engraftment >1% but without MPN phenotype, resembling a state of CHIP, showed low GFP chimerism and, in some cases, a decrease in chimerism was noted upon anti-IL-1 β treatment (Figure 4E). Control mice transplanted with BM from *WT;GFP* donor showed no differences in phenotype or percentages of engraftment between the anti-IL-1 β antibody and isotype treatment groups (supplemental Figure 8). Interestingly, anti-IL-1 β antibody treatment did not decrease the GFP chimerism in HSPCs from the BM or spleen compared with isotype control (supplemental Figure 8D).

We confirmed that in our transplantations with *VF;GFP* BM at 1:100, mice that developed MPN phenotype displayed strongly elevated IL-1 β levels in the BM and plasma compared with mice transplanted with *WT;GFP* BM, whereas *VF;GFP* transplanted mice without MPN phenotype had marginally elevated IL-1 β levels only in the BM (Figure 5A). Anti-IL-1 β antibody treatment massively reduced IL-1 β levels in all treated mice. Primitive BM mesenchymal stromal cells (MSCs), sympathetic nerve fibers, and Schwann cells were reduced at 18 weeks in the BM from isotype treated *VF;GFP* mice compared with *WT;GFP* mice. Interestingly, this decrease was also observed in *VF;GFP* transplanted mice without MPN phenotype that display low GFP chimerism and resemble a state of CHIP (Figure 5C-E). Thus, the damage to the niche cells does not appear to require strongly elevated IL-1 β levels that are found in mice that developed MPN phenotype. Anti-IL-1 β antibody largely normalized the frequencies of primitive MSCs and the densities of sympathetic nerve fibers and Schwann cells in all treated mice (Figure 5C-E).

Myelomonocytic cells and megakaryocytes are main sources of IL-1 β production in the BM of *VF* mice

To analyze the distribution of IL-1 β protein in BM sections, we used in situ BM 3D fluorescence imaging with proximity ligation assay

(PLA) that can visualize single IL-1 β molecules.³⁷⁻³⁹ *WT* mice showed low densities of IL-1 β PLA signals, whereas *VF* mice that developed MPN phenotype displayed the highest densities of PLA signals (Figure 6; supplemental Figure 9A). IL-1 β PLA signals were enriched in close proximity with CD11b⁺ myeloid cells in mice of all genotypes, and these gradients were reduced in *VF* mice that were treated with anti-IL-1 β antibody.

In *VF* mice, the IL-1 β PLA signals were also enriched in the vicinity of CD41⁺ megakaryocytes and these signals were strongly reduced in mice treated with anti-IL-1 β antibody (Figure 6). These results show that *JAK2-V617F* upregulates IL-1 β expression not only in myeloid cells but also in megakaryocytes and suggest that the treatment with anti-IL-1 β antibody interrupts the feedback activation of IL-1 β . Consistent with the IL-1 β PLA data, expression of *IL-1 β* messenger RNA was also increased in sorted monocytic and megakaryocytic cells from the BM of *VF* mice compared with that from *WT* mice (supplemental Figure 9B-C).

IL-1 β enhances inflammatory cytokine and chemokine responsiveness in *JAK2*-mutant HSCs

To investigate the cell autonomous mechanisms of IL-1 β in *JAK2*-mutant HSCs and MPN disease initiation, we performed messenger RNA sequencing of *JAK2*-mutant LT-HSCs (Lin⁻ Sca1⁺ cKit⁺ CD48⁻ CD150⁺). BM cells from *VF*, *VF;IL-1 β ^{-/-}*, *VF;IL-1R1^{-/-}*, or *WT* donors that have not been induced with tamoxifen before euthanasia were transplanted into lethally irradiated recipient mice (Figure 7A). These recipient mice received tamoxifen injections starting 16 weeks after transplantation and were euthanized 2 or 4 weeks after tamoxifen induction when they already displayed mild MPN phenotype (Figure 7B).

LT-HSCs from mice transplanted with BM from *VF;IL-1R1^{-/-}* mice displayed significant increase in the expression of *JAK2-V617F* compared with recipients transplanted with BM from *VF* mice, whereas no differences were observed between *VF* and *VF;IL-1 β ^{-/-}* mice (Figure 7C). To identify significantly enriched biological pathways in the RNA sequencing data sets, we used the WebGestalt online tool,⁴⁰ and used the weighted set cover method of WebGestalt to identify the least redundant and most representative gene sets from the data set (Figure 7D; supplemental Figure 10).

At 2 weeks after tamoxifen induction, we observed no significant enrichment (false discovery rate < 0.05) of any biological pathways (supplemental Figure 10). At 4 weeks after tamoxifen, we identified several significantly enriched Gene Ontology pathways (false discovery rate < 0.05) between *VF* vs *WT*, *VF* vs *VF;IL-1 β ^{-/-}*, and *VF* vs *VF;IL-1R1^{-/-}* genotypes (Figure 7D). Interestingly, in all 3 comparisons, LT-HSCs from *VF* mice

Figure 3. *JAK2*-mutant cells need IL-1R1 expression on both hematopoietic and nonhematopoietic cells for optimal MPN initiation. (A) Schematic drawing of the experimental setup for competitive transplantation at 1:100 dilution. BM from *VF;GFP* or *VF;IL-1R1^{-/-};GFP* donor mice was mixed with a 100-fold excess of BM competitor cells from an *IL-1R1^{-/-}* donor. (B) Time course of blood counts from individual mice that received BM from *VF;GFP* (upper panel) or *VF;IL-1R1^{-/-};GFP* donors (lower panel). (C) IL-1 β protein levels in plasma and BM lavage (1 femur and 1 tibia) of mice with or without MPN phenotype is shown. Nonparametric Mann-Whitney 2-tailed *t* test was performed for statistical comparisons. (D) GFP chimerism in the peripheral blood. Multiple *t* tests were performed for statistical analyses. (E) Bar graphs show the percentages of mice that showed engraftment defined as GFP chimerism of >1% at 18 weeks after transplantation and the percentages of mice that developed MPN phenotype (elevated hemoglobin and/or platelet counts). *P* values in lower panel were computed using Fisher exact test. (F-J) Schematic drawing of the identical aforementioned experiment, in which *IL-1R1^{-/-}* mice were used as the recipients instead of *WT* mice. Annotations as in panels B through E. Gray shaded area represents normal range. All data are presented as mean \pm SEM. **P* < .05; ***P* < .01; ****P* < .001; and *****P* < .0001. See also supplemental Figures 5 and 6.

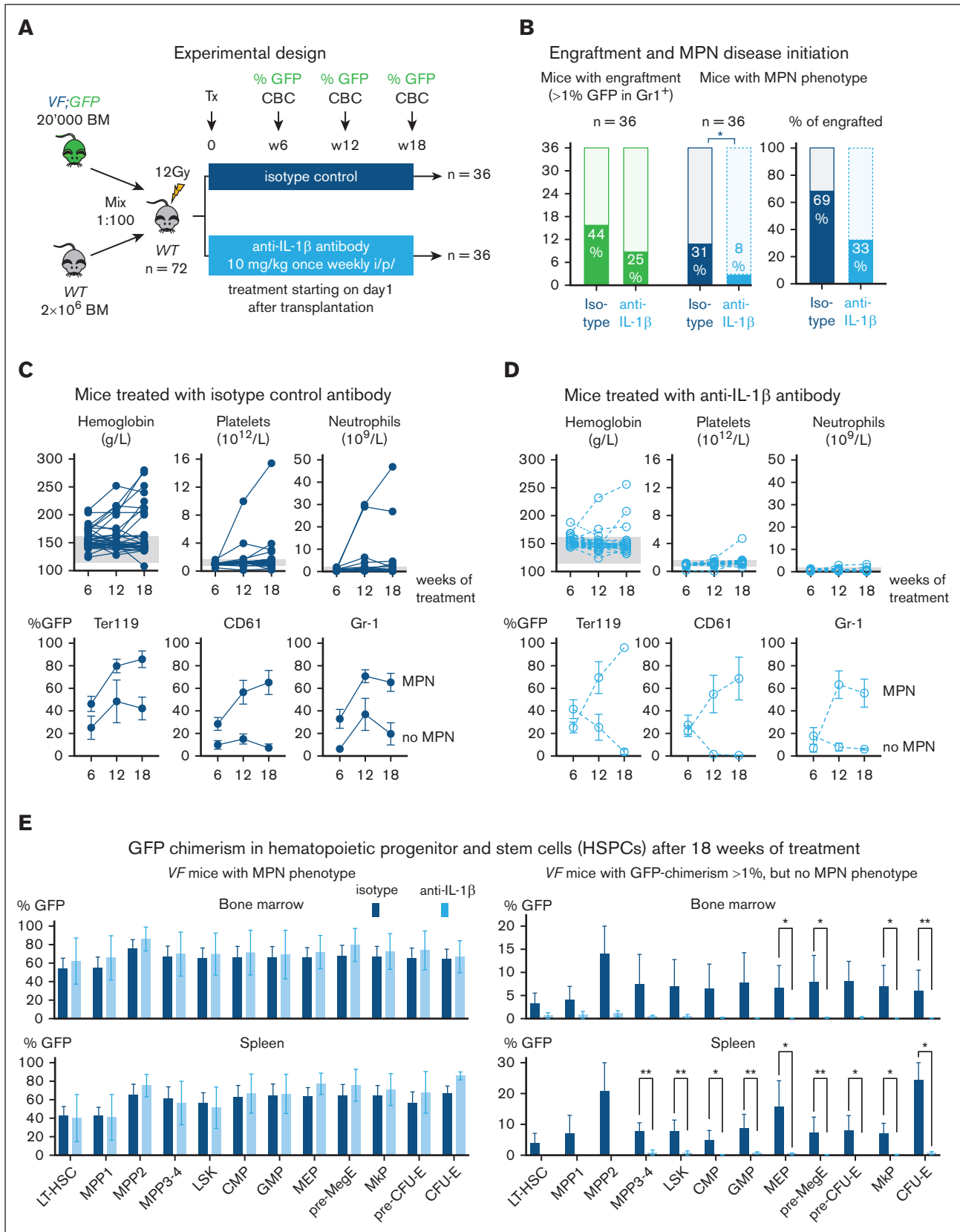


Figure 4. Anti-IL-1 β antibody reduces MPN disease initiation in a mouse model of *JAK2-V617F*-driven clonal hematopoiesis. (A) Schematic drawing of the experimental setup for competitive transplantation at 1:100 dilution. BM cells from a *VF;GFP* donor mouse were mixed with a 100-fold excess of *WT* BM competitor cells and

displayed significantly higher response to interferon signaling than LT-HSCs from *WT*, *VF;IL-1β^{-/-}*, or *VF;IL-1R1^{-/-}* mice. Furthermore, heat map analysis showed increased expression of several genes including *IL-1β*, *Tnf*, *Cxcl4*, *Cxcl3*, *Cxcl10*, *Ccl3*, and *Ccl5* from the top 3 Gene Ontology pathways (response to interferon-β, response to interferon-γ, and cytokine-mediated signaling pathway) that are commonly upregulated in *VF* LT-HSCs compared with LT-HSCs from the other 3 genotypes (Figure 7E). Similarly, the loss of *IL-1β* or *IL-1R1* in *JAK2-V617F*-expressing LT-HSCs resulted in a reduction in the expression of the same genes (Figure 7E). Overall, our results suggest that loss of *IL-1β* or *IL-1R1* in LT-HSCs expressing *JAK2-V617F* reverse the inflammatory signature to make them in this respect more *WT*-like.

***IL1B* polymorphisms are associated with increased serum IL-1β levels in patients with MPN**

Because IL-1β serum levels were increased in patients with MPN and correlated with the *JAK2-V617F* VAF,²² we examined whether genetic polymorphisms located in the *IL1B* gene correlated with higher IL-1β serum levels in patients with MPN (supplemental Figure 11). Several functionally relevant polymorphisms in the *IL1B* gene have been reported.^{41,42} We selected 2 *IL1B* gene polymorphisms that were associated with increased production of IL-1β in nonhematological cancers,⁴³⁻⁴⁵ to compare their frequencies in patients with MPN and normal controls (supplemental Figure 11A). We found that the frequencies of the homozygous AA genotype of rs16944 (G511>A) located in the promoter region of *IL1B* and of the homozygous GG genotype of rs1143627 (A31>G) located closer to the *IL1B* coding regions were almost threefold higher in patients with *JAK2-V617F*-positive MPN than in normal controls. Furthermore, patients with MPN with AA and GG genotypes displayed higher IL-1β serum levels than patients with MPN with GG/GA or AA/AG genotypes (supplemental Figure 11B). Although validation in a larger cohort of patients with MPN is needed, our data support the model that presence of IL-1β favors the expansion of the *JAK2*-mutant clone.

Discussion

In this study, we investigated whether inflammation mediated by IL-1β promotes the early expansion of the *JAK2-V617F* mutant clone and can thereby favor the conversion from a CHIP-like state to MPN. This process is fundamentally different from the progression from chronic phase MPN to myelofibrosis, which we studied previously.²² Competitive BM transplantations at high dilutions allowed us to examine oligoclonal or monoclonal MPN disease initiation starting from 1 to 3 LT-HSCs per transplanted mouse.³³

Genetic ablation of *IL-1β* from *JAK2*-mutant donor HSCs significantly reduced the frequency of engraftment and lowered the frequency of conversion from CHIP to MPN among mice that engrafted (Figure 1), and these HSCs were also less functional in secondary transplantations (Figure 2), consistent with a role of IL-1β for optimal HSC expansion and long-term repopulation capacity. Although the complete genetic loss of *IL-1β* in donor and recipients eliminated the differences in engraftment and MPN disease initiation compared to *VF* (supplemental Figure 2), treatment with anti-IL-1β antibody decreased engraftment and MPN disease initiation (Figure 4). Our data establish IL-1β as an important trigger for the conversion from a CHIP-like state to MPN in carriers of *JAK2-V617F*.

The *IL-1R1*-knockout data are in-line with the *IL-1β*-knockout results. Because the *IL-1R1* is required for the positive autocrine feedback loop that augments IL-1β production,^{18,19,35,36} loss of *IL-1R1* in *VF;IL-1R1^{-/-}* hematopoietic cells prevented the massive overproduction of IL-1β observed in *VF* hematopoietic cells. IL-1β produced by the *VF;IL-1R1^{-/-}* hematopoietic cells was still adequate to assure a high percentage of engraftment, but the levels were not sufficient to reach the same high frequency of conversion from CHIP to MPN observed with *VF* hematopoietic cells (Figure 3A). Loss of *IL-1R1* in the recipients did not reduce the frequency of engraftment but lowered the conversion to MPN (Figure 3B). Thus, IL-1β promotes engraftment in a cell autonomous fashion, also in secondary transplantations, and is not entirely dependent on the presence of the *IL-1R1*, whereas the conversion to MPN required the presence of the *IL-1R1* in the BM niche. Consistent with the genetic deletion of *IL-1β*, treatment with anti-IL-1β antibody reduced both the frequency of engraftment and the conversion to MPN (Figure 4B).

We have previously shown that in the *VF* mouse model, IL-1β mediates loss of sympathetic nerve fibers and the Schwann cells that surround them, resulting in a reduction of nestin-positive BM MSCs,²¹ and these changes were also associated with progression to myelofibrosis.²² The reduction of BM MSCs was also observed in isotype-treated mice transplanted with *VF;GFP* BM that did not develop MPN phenotype and displayed low GFP chimerism resembling a state of CHIP (Figure 5C-E). These results suggest that reducing the frequency of MSCs alone is not sufficient to assure conversion to MPN. Conversely, preserving high frequencies of MSCs by anti-IL-1β antibody treatment did reduce both the rate of engraftment and conversion to MPN. The mechanism of why the presence of MSCs interferes with the expansion of the *JAK2-V617F* clone is currently unknown and will be the subject of future studies.

Recent studies have reported associations between inflammatory cytokines and the expansion of CHIP clones carrying

Figure 4 (continued) transplanted into lethally irradiated *WT* recipient mice. Transplanted mice were randomized into 2 treatment arms ($n = 36$ per group) and treated with anti-IL-1β antibody or isotype control for 18 weeks, starting 1 day after transplantation (Tx). (B) The percentage of mice that showed engraftment defined as GFP chimerism of >1% at 18 weeks after transplantation and the percentages of mice that developed MPN phenotype (elevated hemoglobin and/or platelet counts) are indicated. P value was computed using Fisher exact test. (C-D) The upper panel shows the time course of blood counts from all individual mice for the 2 treatment arms, as indicated. The lower panel shows the mean GFP chimerism in the peripheral blood for mice with MPN phenotype (MPN) and without MPN phenotype (no MPN) that engrafted, defined as GFP chimerism of >1% at 18 weeks. Erythrocytes (Ter119), platelets (CD61), and granulocytes (Gr1) cells are shown separately. Multiple t tests were performed for statistical analyses. (E) GFP chimerism in HSPCs after 18 weeks of treatment. Multiple t tests were performed for statistical analyses. All data are presented as mean \pm SEM; * $P < .05$; ** $P < .01$; *** $P < .001$; and **** $P < .0001$. See also supplemental Figures 7 and 8.

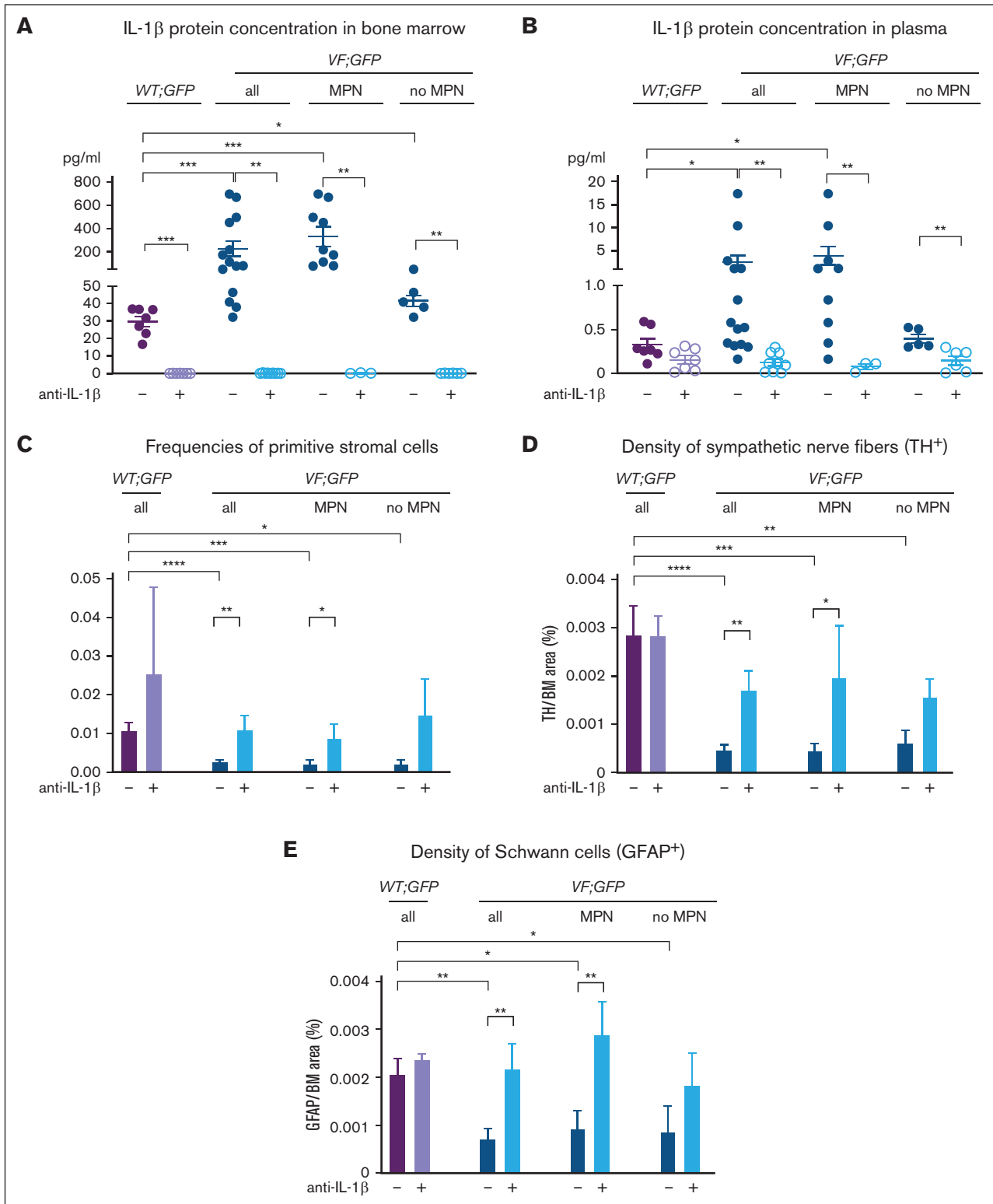


Figure 5. Inhibition of IL-1 β preserves the HSC niche in the BM. (A) IL-1 β protein concentration in BM lavage (1 femur and 1 tibia) and (B) plasma of *WT* mice (from supplemental Figure 8), and *JAK2-V617F* mice (from Fig. 4) with or without MPN phenotype. Nonparametric Mann-Whitney 2-tailed *t* test was performed for statistical comparisons. The lower limit of detection was 0.11 pg/ml. (C) Frequency of primitive stromal cells (CD45⁺CD31⁺Ter119⁺Sca1⁺PDGFR α ⁺) in the skull of mice transplanted with BM from *WT;GFP* or *VF;GFP* donor mouse after 18 weeks of treatment with isotype or anti-IL-1 β antibody (from Figure 4). Two-tailed unpaired *t* tests were performed for statistical comparisons. (D) Bar graphs show the quantification of tyrosine hydroxylase (TH) area and (E) of glial fibrillary acidic protein (GFAP) area in the BM. One-way analysis of variance with uncorrected Fisher least significant difference test was performed for statistical comparisons. All data are presented as mean \pm SEM; **P* < .05; ***P* < .01; ****P* < .001; and *****P* < .0001.

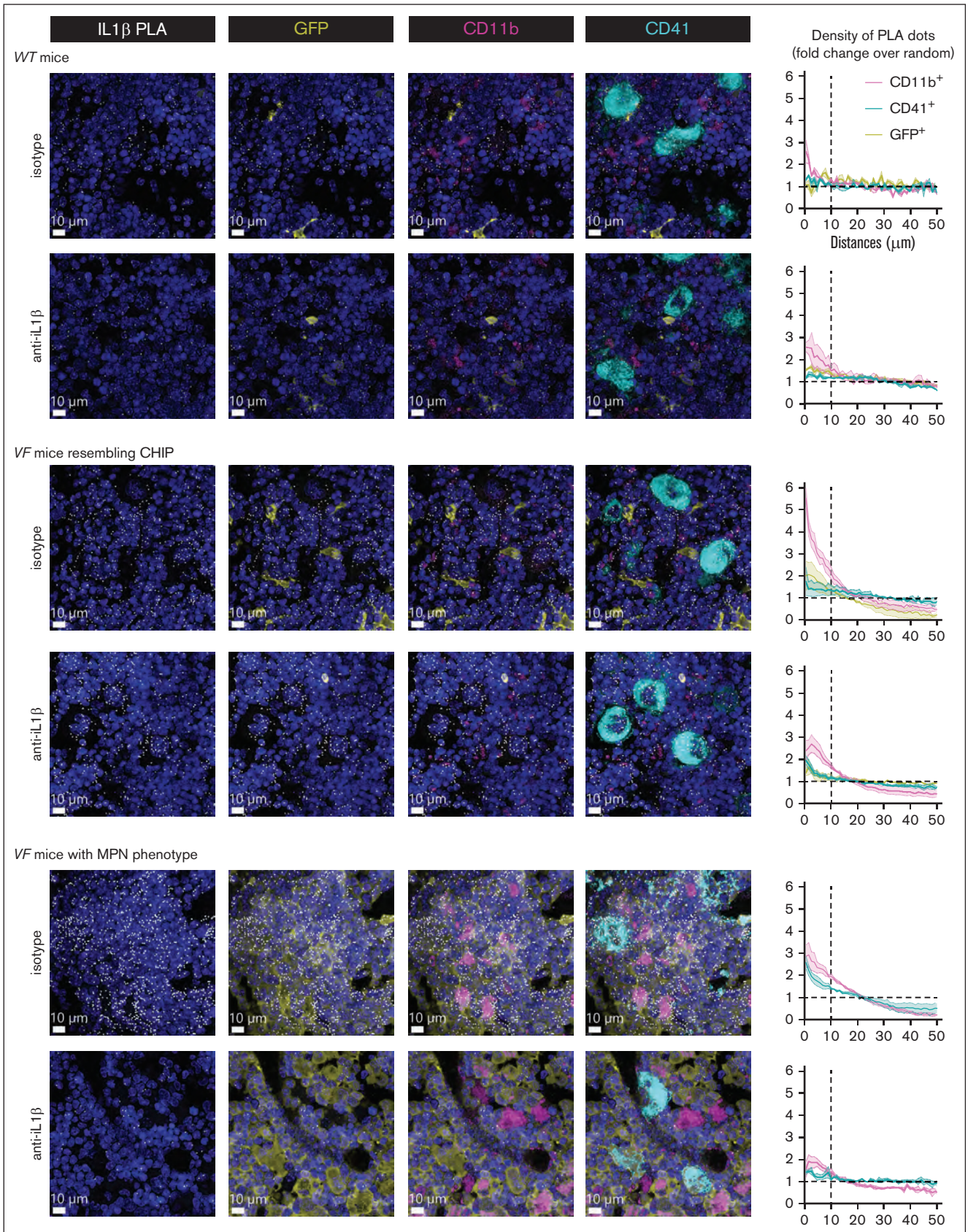


Figure 6.

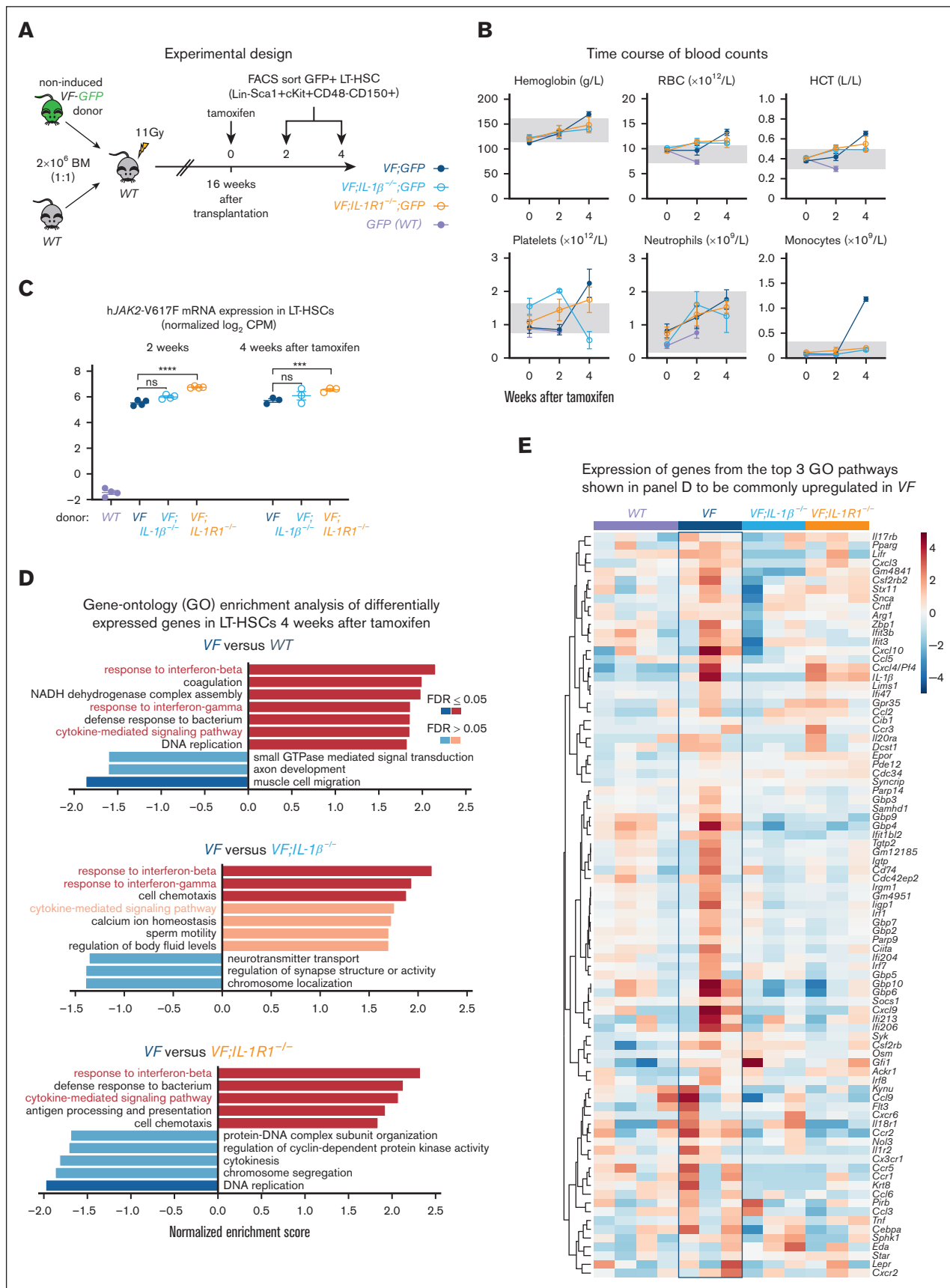


Figure 7.

mutations other than *JAK2-V617F*. Tumor necrosis factor α promoted the expansion of hematopoietic cells with mutations in *Dnmt3a* or *Asx1*,^{46,47} and IL-1 α and IL-1 β mediated expansion of hematopoietic clones carrying *Tet2* mutations.⁴⁸ For example, inhibiting inflammation, by aspirin, could decrease the probability of MPN disease initiation from *JAK2*-mutant HSCs. Although aspirin monotherapy slightly reduced the frequency of engraftment of *VF* BM, it did not decrease the frequency of MPN initiation (supplemental Figure 7). However, aspirin in combination with anti-PD1 showed therapeutic benefit in a *Braf-V600E* melanoma model.⁴⁹ *JAK2-V617F* clones induce strong IL-1-mediated responses, as seen by the relatively high levels of IL-1 β and IL-6 in plasma as compared with other cytokines such as interferon- γ or KC/GRO (Figure 1E). Genetic deletion of *IL-1 β* or *IL-1R1* in LT-HSCs expressing *JAK2-V617F* made them more *WT*-like in respect to *VF*-induced responsiveness to interferon and cytokine signaling (Figure 7D-E). The anti-IL-1 β antibody canakinumab is currently being tested in a clinical trial for clonal cytopenias of unknown significance.⁵⁰

Our results provide evidence that IL-1 β production by *JAK2*-mutant hematopoietic cells generates an inflammatory environment in the BM that promotes *JAK2-V617F* clonal expansion and conversion to MPN. Inhibition of IL-1 β by anti-IL-1 β antibody may be beneficial in individuals with *JAK2-V617F* CHIP who are at increased risk of progression to MPN.

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Authorship

Contribution: S.R. designed and performed the research, analyzed data, and wrote the manuscript; Y. Zhang and E.G. performed research and analyzed data; N.H., Q.K., C.B.S., M.U., H.H.-S., and Y. Zhu performed research; D.L.P. and J.R. analyzed sequencing data; M.S.B. analyzed clinical data; S.D. performed and analyzed histopathology of mouse tissues; C.J.F., T.S., and S.M.-F. designed research and analyzed data; R.C.S. designed the research, analyzed the data, and wrote the manuscript.

Conflict-of-interest disclosure: R.C.S. is a scientific adviser for, scientific advisory board member of, and has equity in Ajax Therapeutics; and has consulted for, and/or received honoraria from, Novartis, Bristol Myers Squibb/Celgene, AOP, GlaxoSmithKline, Baxalta, and Pfizer. N.H. owns stocks in Cantargia. C.J.F. is a full-time employee of Novartis Pharma AG. The anti-IL-1 β antibody studies were carried out in the laboratory of R.C.S. with the antibody provided by Novartis. The remaining authors declare no competing financial interests.

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Figure 6. In situ BM 3-dimensional fluorescence imaging with PLA for IL-1 β single-molecule visualization. Representative images show the distribution of the IL-1 β PLA signals relative to CD11b⁺ myeloid cells and CD41⁺ megakaryocytes in BM sections of *VF* and *WT* mice. The 3-dimensional spatial densities of PLA signals relative to the distances of the closest CD11b⁺ myeloid cells, CD41⁺ megakaryocytes or GFP⁺ *JAK2*-mutant cells are shown in the graphs next to the pictures. See also supplemental Figure 9.

Figure 7. IL-1 β enhances inflammatory cytokine and chemokine responsiveness in *JAK2*-mutant HSCs. (A) Schematic drawing of the experimental setup for competitive transplantation. BM cells from *VF;GFP*, *VF;IL-1 β ^{-/-};GFP*, *VF;IL-1R1^{-/-};GFP*, or *WT;GFP* donor mice that were not induced by tamoxifen were mixed with competitor BM cells from a *WT* donor in a 1:1 ratio. Recipient mice were induced with tamoxifen 16 weeks after transplantation to induce *JAK2-V617F* expression. Mice from each genotype (n = 3-4 per group) were euthanized 2 or 4 weeks after tamoxifen induction and LT-HSCs (Lin⁻Sca1⁺cKit⁺CD48⁻CD150⁺) were purified by fluorescence-activated cell sorter for RNA sequencing. (B) The time course of blood counts of recipient mice after tamoxifen induction. (C) Human *JAK2-V617F* messenger RNA gene expression in LT-HSCs (normalized log2 counts per million [CPM]) at 2 and 4 weeks after tamoxifen induction. Multiple *t* tests were performed for statistical analyses. (D) Pathway analysis 4 weeks after tamoxifen induction performed with WebGestalt online tool⁴⁰, using Gene Ontology as the functional database. Minimum and maximum number of genes for a category was set to 15 and 500, respectively. TOP method (TOP means the categories will be first ranked based on the false discovery rate [FDR] and then the top N most significant categories will be selected) was used to identify significance levels of the enriched categories, and number of permutations was 1000. Bars representing significant pathways (FDR of <0.05) are colored according to their normalized enrichment score and the FDR as indicated. The names of the pathways upregulated in *VF* vs all 3 other genotypes are colored in red or orange according to their FDRs. (E) Expression of genes from the top 3 pathways identified by Gene Ontology in panel D to be commonly upregulated in LT-HSCs from *VF* mice vs mice from all 3 other genotypes. All data are presented as mean \pm SEM; **P* < .05; ***P* < .01; ****P* < .001; and *****P* < .0001. See also supplemental Figure 10.

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