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1 **Deregulation of the p19/CDK4/CDK6 axis in Jak2^{V617F} megakaryocytes accelerates the**
2 **development of myelofibrosis**

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25 **Running title:** "Palbociclib treatment of myelofibrosis"

26

27 Primary and secondary myelofibrosis (MF) present the worst prognosis in the group of
28 *BCR::ABL*-negative myeloproliferative neoplasms and are driven in half of cases by *JAK2*^{V617F}.
29 In mouse models, MF occurs mainly secondary to essential thrombocythemia (ET)- or
30 polycythemia vera (PV)-like disorders induced by *Jak2*^{V617F} or *CALR*^{del52} mutation. Direct and
31 strong activation of the thrombopoietin receptor MPL signaling by *MPL*^{W515L} or high
32 thrombopoietin levels also results in rapid MF development.¹⁻⁸ All these models together with
33 the topology of the bone marrow (BM) at the onset of MF underscore the key role of the
34 megakaryocytes (MKs) in the disease. Presently, there is no curative therapy in MF, except
35 allogenic BM transplantation; JAK inhibitors being not selective of the oncogenic JAK2
36 signaling.⁹ Therefore, it is of great interest to identify and inhibit specific downstream targets
37 deregulated by the abnormal JAK2 activation.

38 Targeting CDK6, which is up-regulated in MPN hematopoietic progenitors, either
39 pharmacologically by Palbociclib or by its genetic deletion in *Jak2*^{V617F} and *MPL*^{W515L} mice
40 significantly inhibits MPN development.^{10,11} In addition, the knock-out (KO) of *p19*^{Ink4d}, a
41 CDK4/CDK6 inhibitor that regulates the endomitotic arrest of MKs,¹² leads to the amplification
42 of hyperploid MKs, increased levels of TGF-β1 and fibrosis development with aging.¹³

43 Here, we first investigated *p19*^{Ink4d} and *CDK6* expressions in MKs of induced
44 *Jak2*^{FLEXV617F/+}*SCL-Cre*^{+/-} (KI thereafter) mice². The *p19*^{Ink4d} expression level decreased unlike
45 that of *CDK6* in MKs from KI mice as compared to controls (**SF 1A**) and correlated with their
46 higher ploidy (major peak at 32N *versus* 16N) (**SF 1B**). These results suggest that *p19*^{Ink4d}
47 downregulation could contribute to the abnormal megakaryopoiesis and MF development in
48 the *Jak2*^{V617F} context.

49

50 To further support this hypothesis, the $p19^{Ink4d^{-/-}}$ ($p19$ KO)¹⁴ and KI mice were crossed and
51 heterozygous expression of $Jak2^{V617F}$ was induced in native $p19^{Ink4d^{-/-}}/Jak2^{FLEXV617F/+}SCL-$
52 $Cre^{+/-}$ (KOKI thereafter) and KI mice. No marked difference in survival curve and blood
53 parameters was detected between KI and KOKI mice (**SF2A, B**). However, as soon as 1-week
54 post- $Jak2^{V617F}$ induction, there were a significant reduction of BM cellularity and a faster
55 development of splenomegaly in KOKI compared to KI mice (**Fig 1A, B**). Moreover, after 3
56 weeks of $Jak2^{V617F}$ induction, a grade 1 BM MF was already present in KOKI mice (**Fig 1C,**
57 **SF 2C**). At 8 weeks, we detected a grade 2/3 MF in the BM of all KOKI mice compared to a
58 grade 1 MF in some, but not all KI mice (**Fig 1C, SF 2C**). A similar accelerated progression of
59 fibrosis was seen in the spleen (**SF 2D**). Numerous dysplastic MKs in both BM and spleen were
60 visible (**SF 3A, B**). $P19^{Ink4d}$ deletion accelerated the development of $Jak2^{V617F}$ -dependent
61 extramedullary hematopoiesis (EMH) as illustrated by the increased erythropoiesis and
62 granulopoiesis in the KOKI spleens as early as 1-week post- $Jak2^{V617F}$ induction (**SF 4A, B, D,**
63 **E**). At 3 weeks, we observed a marked decrease in all the BM erythroid populations of KOKI
64 mice in accordance with the establishment of MF, compared to KI mice (**SF 4C**).

65 We next focused on MKs as they represent the major source of TGF- β 1 involved in the MF
66 development. The numbers of MK progenitors (MK-Ps) and MKs gradually increased from 1-
67 week post- $Jak2^{V617F}$ induction (data not shown) to be significant at 3 weeks in the BM and
68 spleen of KI and KOKI mice (**Fig 1D, E, SF 5A, B**) suggesting that $p19^{INK4D}$ depletion
69 predominantly increases the proliferation of MK-Ps during differentiation. Importantly, a
70 significant increase of MK-Ps and MKs was found in the spleen of KOKI compared to KI mice,
71 consistent with an enhanced EMH (**SF 5C, D**). As expected, the study of MK ploidy level
72 showed a major peak at 16N in WT mice and at 64N in $p19$ KO mice (**SF 5E**)¹² and the mean
73 ploidy level (>4N) was significantly higher in KOKI than in $p19$ KO and KI mice (55.9N,
74 43.7N and 40.0N, respectively) (**Fig 1F**). These polyploid KOKI MKs were dysplastic and

75 diffuse von Willebrand factor (vWF) and P-selectin staining (**SF 5F**) was observed in BM
76 suggesting a spontaneous activation of MKs with premature release of granule content in KOKI
77 mice. This was confirmed at 1-week post-*Jak2*^{V617F} induction. Indeed, the KOKI mice showed
78 the presence of numerous abnormal MKs (**Fig 1G**) with an increased P-selectin expression at
79 cell surface (**Fig 1H**) showing that p19 loss accelerates the development of an abnormal
80 megakaryopoiesis. Of note, electron microscopy revealed an abnormal localization of granules,
81 mitochondria and exosome-like particles close to the cell membrane only in KOKI MKs due to
82 a loss of the marginal zone. In addition, a more important emperipoiesis was observed in KOKI
83 than in KI MKs (**SF6A-D**). Interestingly, KOKI MKs significantly harbored higher levels of
84 soluble vWF measured in BM supernatants compared to KI mice, confirming their activation
85 (**Fig 1I**). Total and active TGF- β 1 concentration measured in BM supernatants were increased
86 in both genotypes without significant difference between KOKI and KI mice (**SF 6E**).
87 Nevertheless, only BM supernatants from KOKI mice co-cultured with stromal MS-5 cells
88 induced a significant increase in TGF- β 1-mediated target genes such as Actin alpha2 (*Acta2*),
89 Plasminogen Activator Inhibitor 1 (*Pai1*) and an increased trend in Connective tissue growth
90 factor (*Ccn2*) (**SF 6F**). These results suggest that active TGF- β 1 levels might be underestimated
91 in KOKI BM potentially due to its rapid receptor binding, signaling and internalization in
92 stromal cells, leading to a decreased bioavailability.

93 Overall, these data show that KOKI mice present an accelerated development of MF as
94 compared to KI mice that were shown to lose approximately half of p19 expression. This
95 accelerated MF in KOKI as compared to KI mice is associated with numerous dysplastic,
96 activated, and hyperploid MKs leading to premature release of granule content such as vWF
97 and TGF- β 1.

98 P19^{INK4d} is an inhibitor of CDK4/6, but has also other functions. Thus, we used Palbociclib
99 (PB), a CDK4/6 inhibitor, to investigate if CDK4/6 activation was involved in the MF triggered
100 by *Jak2*^{V617F} and *p19*^{Ink4d} loss.

101 After 2 weeks of treatment, PB induced a decrease in the blood parameters (**Fig 2A**) of the
102 KOKI mice, and only slightly decreased their already low BM cellularity. In contrast, a 2.1-
103 fold decrease was observed in WT mice due to a reduction of all types of progenitors (**Fig 2B**,
104 **SF 7A-D**). Interestingly, PB restored normal spleen weight in KOKI mice (**Fig 2C**) due to a
105 decrease in the numbers of all progenitors (**SF 7D-G**). In KOKI BM, PB treatment induced an
106 increase in LSK number and a decrease in MK-Ps with no change in erythroid progenitors (pre-
107 CFU-E) and granulo-monocytic progenitors (GMP) (**SF 7A-D**). Although MK number in
108 KOKI BM did not change (**SF 8A**), PB decreased their modal ploidy (64N versus 32N) (**SF**
109 **8B**) and mean ploidy (65.3N versus 35.2N) (**Fig 2D**). PB-treated KOKI MKs were of smaller
110 size with a less diffuse vWF staining in the cytoplasm as compared to untreated condition (**SF**
111 **8C**). Importantly, PB treatment prevented the development of a reticulin fiber network (**Fig 2E**)
112 and restored basal active TGF- β 1 levels (**Fig 2F**) in the KOKI BM. Overall, the PB treatment
113 prevented the occurrence of EMH and the development of MF in KOKI mice through inhibition
114 of MK endomitosis and TGF- β 1 activation.

115 To further confirm the effect of PB on KOKI and evaluate its role in KI mice, we used a model
116 of primary transplanted mice, with BM cells of the different genotypes (WT, KI and KOKI)
117 being engrafted into lethally-irradiated WT mice. In both KI and KOKI models, PB
118 administration decreased blood parameters (**SF 9A**), BM cellularity in all the mice except in
119 KOKI mice (**SF 9B**), and spleen weight particularly in KI and KOKI mice (**SF 9C**). A decrease
120 in MK-Ps due to an inhibition of cell cycle and in mean ploidy level of MKs was detected in
121 both KI and KOKI animals while MK numbers were only reduced in KI mice (**SF 9D-G**). The
122 MKs were smaller as shown by vWF staining, and the MF development in the BM of KOKI

123 mice was prevented by PB treatment (**SF 9H**). Overall, these results show a similar effect of
124 PB on hematopoietic parameters in KI and KOKI mice. To further study the specificity of PB
125 treatment on *Jak2*^{V617F} cells, a competitive engraftment between 75% WT-GFP⁺ cells and 25%
126 of KI- or KOKI-GFP⁻ cells was performed. In both WT/KI and WT/KOKI engrafted mice, PB
127 treatment once again significantly reduced the blood parameters (**SF 10A**). To know whether
128 this effect was due to the specific targeting of *Jak2*^{V617F} cells, the frequencies of GFP⁻ cells were
129 analyzed. The PB treatment significantly decreased the proportion of *Jak2*^{V617F} granulocytes,
130 platelets and erythrocytes in mice engrafted with WT/KI and WT/KOKI cells (**Fig 2G, SF**
131 **10B**). A significant decrease in the BM cellularity (**SF 10C**) and spleen weight (**SF 10D**) was
132 associated with a decrease in the frequencies and numbers of *Jak2*^{V617F} MKs, erythroblasts and
133 granulocyte precursors (**Fig 2H**). A specific decrease in the frequencies and numbers of
134 *Jak2*^{V617F} SLAM and MK-Ps in both WT/KI and WT/KOKI mice was also detected (**SF 10E**).
135 Finally, to test whether these last results could be extrapolated to human, we tested the effect
136 of PB treatment on the clonogenicity of *JAK2*^{V617F} progenitors. We observed a decrease in the
137 clonogenicity of the *JAK2*^{V617F} CD34⁺ cells isolated from peripheral blood of 3 ET and 2 PV
138 patients (*JAK2*^{V617F} variant allele frequency 4.4-40.5%) (**Fig 2I**).

139 Overall, these results afford evidence that p19^{Ink4d} deregulation is involved in the abnormal
140 megakaryopoiesis of *Jak2*^{V617F} mice and that its loss accelerates the development of a severe
141 myelofibrosis. The effects of p19^{Ink4d} deregulation are mediated through CDK4/6
142 overactivation. Therefore, CDK4/CDK6 inhibitors appear as potent therapeutic molecules to
143 prevent the progression of MPN into MF. Preclinical studies have shown their synergic effect
144 with ruxolitinib, which predominantly targets the inflammation.^{10,15}

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159 **Authorship Contributions**

160 HD, DM, LG, ALC, MEK, AK and IAD performed and analyzed experiments. FP provided
161 patient samples. HD, DM, IAD, LG, ND, AK, FP, CM, RG, MW, VW, IP, HR discussed
162 results. HR supervised the work. HD, DM, LG, VW, IP and HR wrote the article. DM and LG
163 equally contributed to this work.

164 **Disclosure of Conflict of Interest:** The authors declare that there are no conflicts of interest.

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231

232 **Legends**

233

234 **Figure 1. *P19^{Ink4d}* depletion favors the rapid development of splenomegaly and MF in**

235 ***Jak2^{V617F}* context through deregulation of megakaryopoiesis**

236 **A-B.** Rapid splenomegaly and MF development in absence of *p19^{Ink4d}*. **A.** Total BM cellularity

237 for 2 femurs and 2 tibias ($\times 10^6$) is shown for each genotype after 1 or 3 weeks (W) post-

238 *Jak2^{V617F}* induction. Data represent mean \pm SEM, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$,

239 **** $p < 0.001$, nonparametric Mann-Whitney test. WT (n=11, 16), KO p19 (n=11, 17),

240 *Jak2^{V617F}* KI (KI, n=12, 15) and *p19* KO / *Jak2^{V617F}* KI (KOKI, n=16, 21). **B.** Spleen weight

241 (mg) is shown for the four genotypes at 1, 3 and 8 weeks post-*Jak2^{V617F}* induction. Data

242 represent mean \pm SEM, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$, **** $p < 0.001$, nonparametric Mann-

243 Whitney test. WT (n=12, 22, 9), KO p19 (n=9, 20, 8), *Jak2^{V617F}* KI (KI, n=13, 16, 4) and *p19*

244 KO / *Jak2^{V617F}* KI (KOKI, n=19, 19, 5). **C.** Representative pictures of BM stained with a

245 standard histological silver kit to underscore reticular fibers in hematopoietic organs at 3- and

246 8- weeks post *Jak2^{V617F}* induction for WT, KO p19, KI or KOKI mice. Scale bar = 20 μ m. **D-**

247 **F.** BM analysis of MK lineage 3 weeks post-*Jak2^{V617F}* induction. **D.** Number of CFU-MKs in

248 semi-solid conditions per 100×10^3 of total BM cells seeded in duplicate for each mouse (n=6

249 for each genotype) and counted at 7 days of culture after acetylcholinesterase staining. Data

250 represent mean \pm SEM, ** $p < 0.01$, nonparametric Mann-Whitney test. **E.** Frequency of MKs

251 (CD41⁺CD42⁺). Data represent mean \pm SEM, *** $p < 0.005$, nonparametric Mann-Whitney test.

252 WT (n=15), KO p19 (n=13), KI (n=15), KOKI (n=19). **F.** Mean ploidy $> 4N$ for BM MKs for

253 each genotype. Data represent median with 95%CI, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$,

254 nonparametric Mann-Whitney test. WT (n=8), KO p19 (n=10), KI (n=12), KOKI (n=9). **G.**

255 Immunofluorescence staining of MKs in BM of WT, KI or KOKI mice 1-week post-*Jak2^{V617F}*

256 induction. CD41 is in red, P-selectin in green and nucleus (DAPI) in blue color. Scale bar = 50

257 μ m or 20 μ m. The lower panels show the white squares of the panels at the top by higher

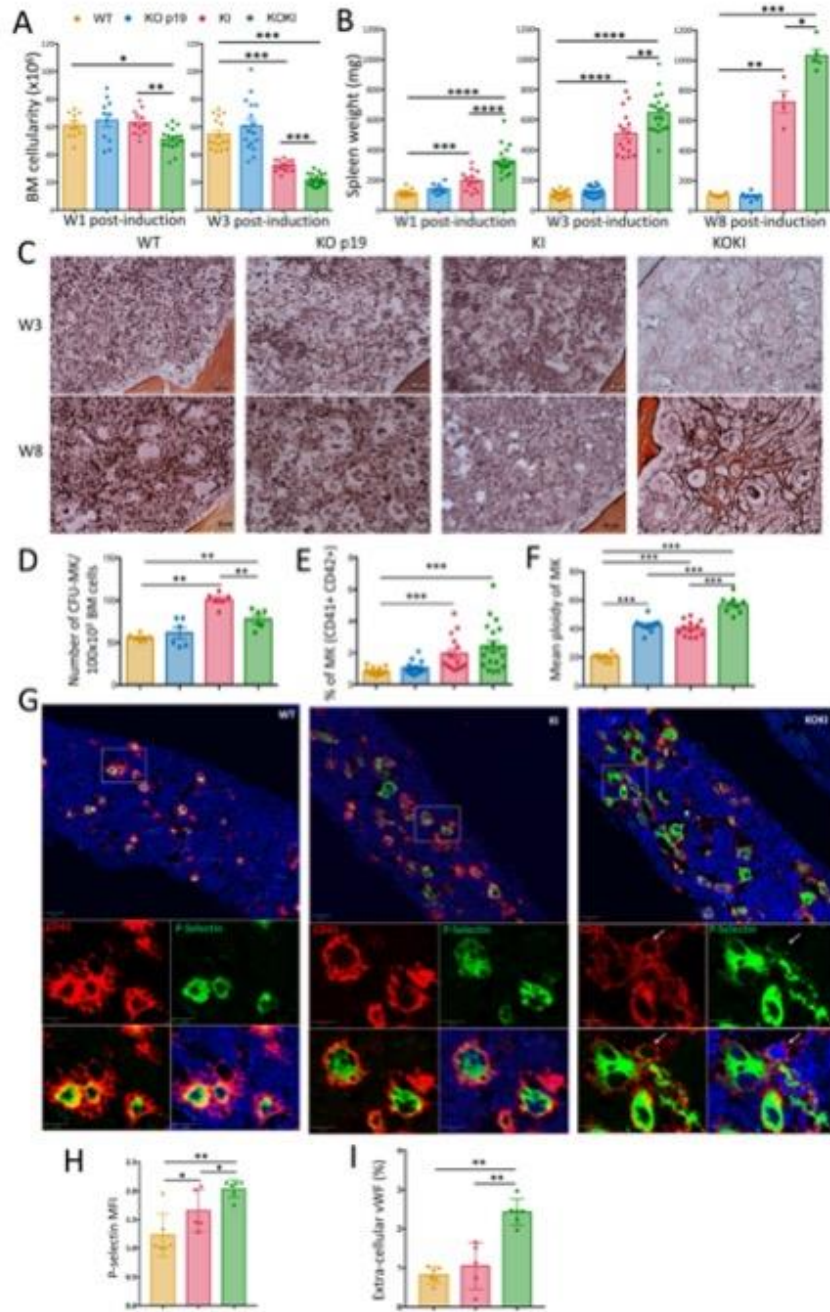
258 magnification. The white arrow indicates the MK with disorganized cytoplasm. **H.** P-selectin
259 expression on cell surface of mature BM MKs (CD41⁺CD42⁺). The median fluorescence
260 intensity (MFI) for each mouse of each genotype was normalized to the MFI measured for P-
261 selectin on MKs of one WT mouse. **I.** % of vWF measured by ELISA in BM supernatants of
262 WT, KI and KOKI mice. **H-I.** Data represent mean±SEM, *p<0.05; **p<0.01, nonparametric
263 Mann-Whitney test. WT (n=6), KI (n=5), KOKI (n=6).

264

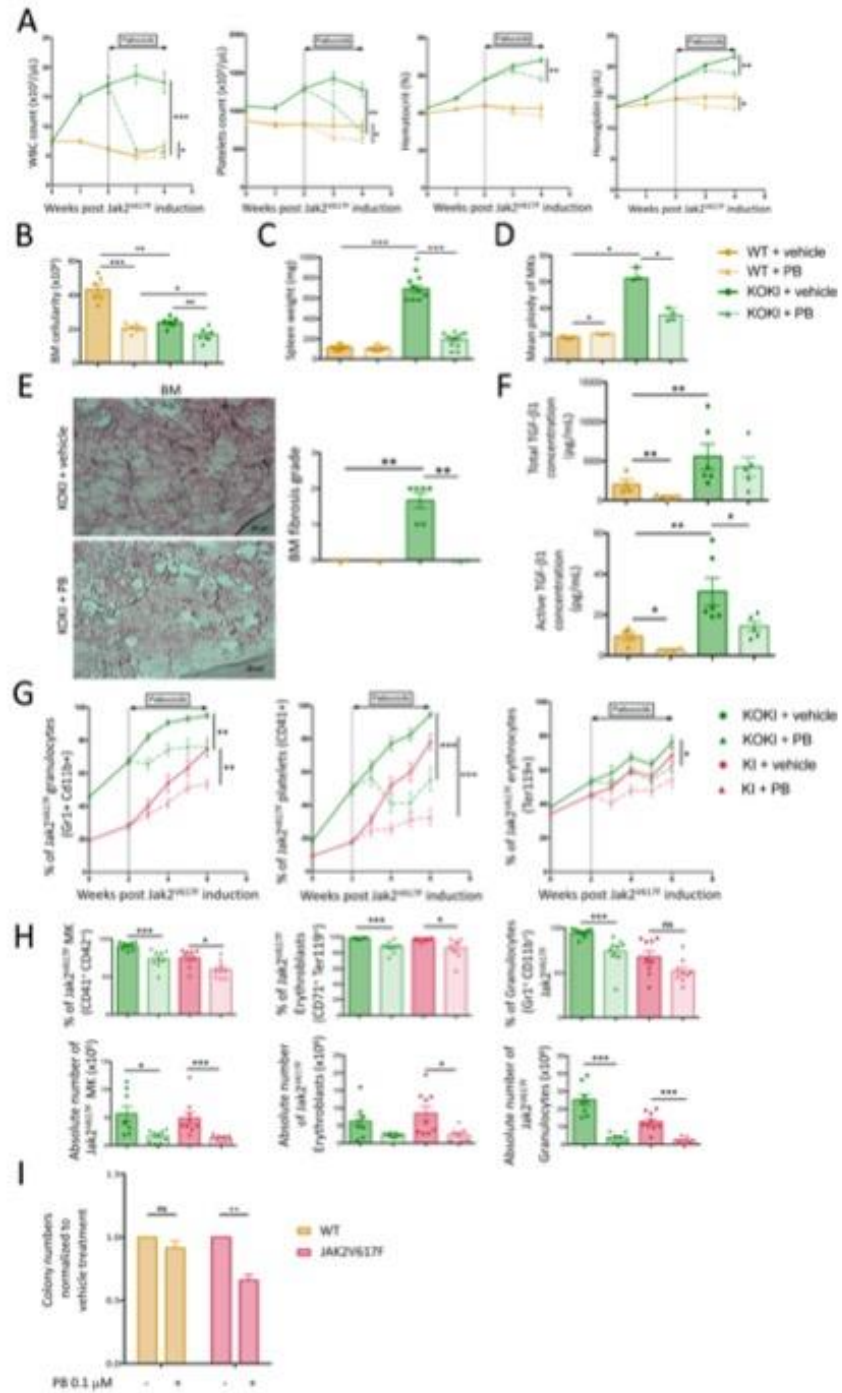
265 **Figure 2. Palbociclib treatment prevents myeloproliferation, splenomegaly and MF**
266 **development in *Jak2*^{V617F} context.**

267 **A-F.** Two weeks after *Jak2*^{V617F} induction, KOKI mice were treated daily for 2 weeks with PB
268 150 mg/kg, a dose that did not affect the mouse weight (**SF 11**). The WT mice were used as
269 controls. **A.** White blood cells count (WBC) (x10³/μL), platelet count (x10³/μL), Hematocrit
270 (%) and Hemoglobin (g/dL) were analyzed weekly from week 0 to week 4 post-*Jak2*^{V617F}
271 induction (end of treatment and sacrifice). Data represent mean±SEM, *p<0,05; **p<0.01;
272 ***p<0.005, one-way ANOVA coupled to Bonferroni test. WT + vehicle (n=11) or PB (n=11),
273 KOKI + vehicle (n=9) or PB (n=11). **B.** Total BM cellularity for 2 femurs and 2 tibias (x10⁶).
274 **C.** Spleen weight (mg). **B-C.** Data represent mean ± SEM, *p<0.05; **p<0.01; ***p<0.005,
275 nonparametric Mann-Whitney test. WT+vehicle (n=6, 9); WT+PB (n=8,11), KOKI+vehicle
276 (n=6, 9), KOKI+PB (n=7, 11). **D.** Mean ploidy >4N for BM MKs. Data represent median with
277 95% CI, *p<0.05, nonparametric Mann-Whitney test. n=3-4 for each condition. **E.** Left panel:
278 Representative pictures of KOKI BM stained with a standard histological silver kit to
279 underscore reticular fibers. Scale bar = 20 μm. Right panel: Fibrosis grade scored in BM. Data
280 represent mean± SEM, **p<0.01, nonparametric Mann-Whitney test. WT+vehicle (n=3),
281 WT+PB (n=3), KOKI+vehicle (n=6), KOKI+PB (n=3). **F.** Total and active concentration of
282 TGF-β1 in BM supernatant. Total concentration is measured after latent TGF-β1 activation

283 with HCl. Data represent mean±SEM, *p<0.05; **p<0.01, nonparametric Mann-Whitney test.
284 WT+vehicle (n=4) or PB (n=5), KOKI+vehicle (n=6) or PB (n=5). **G-I.** PB specifically targets
285 the *Jak2*^{V617F} clone. **G-H.** The irradiated (9.5 Gy) WT mice were engrafted with 3x10⁶ cells
286 (75% WT-GFP⁺ + 25% KI- or KOKI-GFP⁻). After 4 weeks of hematopoietic reconstitution,
287 *Jak2*^{V617F} was induced by tamoxifen. From week 2 to 6 post-*Jak2*^{V617F} induction, mice were
288 treated daily with vehicle or 125 mg/kg PB (dose not toxic for engrafted animals, **SF 11**) and
289 analyzed at the end of treatment. **G.** Frequency of *Jak2*^{V617F} (GFP⁻) granulocyte precursors (Gr-
290 1⁺CD11b⁺), platelets (CD41⁺), and erythrocytes (Ter-119⁺) were analyzed weekly from week
291 0 to week 6 post-*Jak2*^{V617F} induction. Data represent mean±SEM, **p<0.01; ***p<0.005, one-
292 way ANOVA coupled to Bonferroni test. KOKI+vehicle (n=8), KOKI+PB (n=9), KI+vehicle
293 (n=9), KI+PB (n=9). **H.** Frequencies and absolute numbers of BM *Jak2*^{V617F} MKs
294 (CD41⁺CD42⁺), erythroblasts (CD71⁺Ter-119⁺) and granulocyte precursors (Gr-1⁺CD11b⁺).
295 Absolute numbers are calculated according to the total frequency of the population of interest
296 and the total BM cellularity. Data represent mean±SEM, ns: non-significant *p<0.05;
297 **p<0.01; ***p<0.005, nonparametric Mann-Whitney test. KOKI+vehicle (n=8), KOKI+PB
298 (n=9), KI+vehicle (n=9), KI+PB (n=9). **I.** CD34⁺ cells isolated from ET and PV patients were
299 cultured at 1 progenitor/well in 96-well plates in presence of EPO, TPO, FLT3-L, IL-3, G-CSF,
300 IL-6, SCF and GM-CSF without or with PB (0.1 μM) for 14 days. Then the individual colonies
301 were genotyped for *JAK2*^{V617F} or wild-type (WT) *JAK2* status for 3 ET and 2 PV patients with
302 heterozygous *JAK2*^{V617F} mutation with a VAF 4-40.5%. Shown is a ratio of the colony numbers
303 grown in presence of PB normalized to the colony numbers grown in absence of PB (vehicle).
304 Data represent mean±SEM, ns: non-significant; **p<0.01, nonparametric Mann-Whitney test.
305



308 **Figure 2**



309

310

311 **Supplementary Informations**
312

313 **MATERIAL AND METHODS**

314

315 **Patients**

316 Peripheral blood samples were collected from patients with their written informed consent in
317 accordance with the Declaration of Helsinki and the study was approved by the Ethics
318 Committee from Gustave Roussy; from Comité de Protection des Personnes (CPP) Ile de
319 France IV- Institutional review board (agreement from US Department of Health and Human
320 Services n°IRCB 00003835- Protocol 2015/59-NICB and then n°IRCB 2021-A03067-34.) The
321 $JAK2^{V617F}$ VAF (4.4-40.5%) was determined on granulocytes by PCR (patient N#1) or by NGS
322 (patients N#2-5). The sampling was done during the treatment with hydroxyurea or aspirin and
323 the additional mutations are listed in **ST1**.

324

325 **Patient cell purification**

326 Mononuclear cells were purified from peripheral blood cells by a Ficoll density gradient, and
327 $CD34^+$ cells were purified by a double-positive magnetic cell sorting system (AutoMACS;
328 Miltenyi Biotec).

329 **Mice**

330 Heterozygous $p19^{Ink4d+/-}$ mice with a C57BL/6 x Sv129j genetic background were kindly
331 provided by Martine Roussel (Memphis, TN) and crossed to obtain homozygous $p19^{Ink4d+/+}$ and
332 $p19^{Ink4d-/-}$ mice. Homozygous $p19^{Ink4d-/-}$ mice were crossed with the conditional
333 $Jak2^{FLEXV617F/+SCL-Cre+/-}$ KI mice available in the laboratory. Transgenic mice expressing GFP
334 (UBI-GFP/BL6)¹ were used in competitive assays.

335 All animal experiments were performed in accordance with 2010/63/UE European legislation
336 and decree n°2013-118 of French legislation; and recorded under protocol number 2012_060
337 and 2020_007_23589. Mice were housed in pre-clinical platform of Gustave Roussy Animal
338 Facilities PFEP (Villejuif, France) (ministerial approval n° 94-076-11) under 12-hour light/12-
339 hour dark cycles and monitored ambient temperature (21 ± 1 °C) and humidity range (20–70%).
340

341 **Mice genotyping**

342 Mice were genotyped using the Mouse Direct PCR Kit according to manufacturer's protocol
343 (Bimake) with the different sets of primers available upon request.

344 **$Jak2^{V617F}$ induction by tamoxifen**

345 Tamoxifen (Sigma) solution was administrated to 6-8- weeks-old mice at 200 mg/kg by force-
346 feeding during 4 days. Mice were subsequently fed during 2-4 weeks with tamoxifen food
347 (Ssniff Diet + 400 mg/kg tamoxifen (Sigma)).

348 **Blood parameters and mouse experimentation**

349 Blood parameters were determined using an automated blood counter (MS9, Schloessing
350 Melet). BM cells were collected by flushing the femurs and two tibiae of individual mice.
351 Cellularity was calculated using the 2 femurs and 2 tibiae or 1 femur of mice as indicated in
352 legends. Spleens were weighted and dissociated to prepare single-cell suspensions. The BM
353 and spleen cell number was determined by Sysmex Cell Counter.

354

355 **Cell analysis by flow cytometry**

356 BM lineage-negative (Lin⁻) cells were identified for their absence of labeling with the following
357 FITC-conjugated antibodies (Ab) against CD3, Ter-119, CD11b, Gr-1, B220 (BioLegends).
358 APC-conjugated antibodies for GFP positive mice were used in competitive engraftment
359 experiments. BM and spleen cell content were investigated including: progenitor cell-enriched
360 fraction Lin⁻Sca-1⁺c-Kit⁺ (LSK), hematopoietic stem cell (HSC)-enriched population Lin⁻Sca-
361 1⁺c-Kit⁺CD48⁻CD150⁺ (SLAM), and progenitors from the megakaryocytic (MK-P: Lin⁻Sca-1⁻
362 c-Kit⁺CD150⁺CD41⁺), erythroid (pre-CFU-E: Lin⁻Sca-1⁻c-Kit⁺CD41⁻CD150⁺FcγRII/III⁻
363 CD105⁺) and granulocytic (GMP: Lin⁻Sca-1⁻c-Kit⁺CD41⁻FcγRII/III⁺CD150⁻) lineages, using
364 the anti-c-Kit-PerCP/Cy5.5, -Sca-1-PE/Cy7, -CD150-PE, -CD41-BV421, -CD105-APC, -
365 CD16/32-APC/Cy7 and -CD48-Pacific Blue Ab (all purchased from BioLegend).

366 Precursor and mature cells including MKs (CD41⁺CD42⁺), granulocytes (Gr1⁺CD11b⁺) and the
367 erythroid lineage (CD71⁺Ter119⁻, CD71⁺Ter119⁺, CD71⁻Ter119⁺), were analyzed with Ab
368 against CD41-PerCP/Cy5.5, CD42d-APC, Gr1-FITC, Cd11b-PE, Ter119-FITC (BioLegend),
369 CD71-PE (BD).

370 Activation of MKs, was investigated by determining the level of P selectin on the cell surface.
371 In this purpose, the Median Fluorescent Intensity (MFI) of anti-P-selectin-PE Ab (Emfret) and
372 of IgG-PE at cell surface (without activation) was measured on cells expressing CD41 and
373 CD42 (anti CD41-PerCP/Cy5.5 and CD42d-APC antibodies) after Lin positive depletion.
374 Treatment of cells with TRAP 4 at 10mM was used as a positive control of activation.

375 **Progenitors cell cycle analysis by flow cytometry**

376 BM progenitors were first labelled with non-conjugated antibodies against CD3-FITC, Ter-
377 119-FITC, Cd11b-FITC, Gr-1-FITC, B220-FITC, then with c-kit PerCP/Cy5.5, Sca-1-PE/Cy7,
378 CD41-AF700R, CD48-APC/Cy7 and CD150-PE (BioLegend). After extracellular staining,
379 cells were fixed and permeabilized with Cytofix/Cytoperm and cytofix plus (BD Biosciences).
380 The intracellular staining was then performed with a Ki-67-APC Ab (Biolegend) and with
381 DAPI (5 µg/mL). Different cell cycle phases were analyzed by flow cytometry.

382 **Flow Cytometry analysis and cell sorting**

383 FACSCanto II, FACS Canto X and LSR Fortessa (BD Biosciences) were used for analysis. All
384 data were collected on Diva software. INFLUX (BD Biosciences) was used for cell sorting.

385 **MK ploidy analysis**

386 BM MKs were labeled with anti-CD41-FITC and anti-CD42d-APC Ab (BioLegend). After
387 hypotonic lysis with a citrate solution containing 50 µg/mL propidium iodide (PI) (Sigma-
388 Aldrich), ploidy was determined using a FACSCanto II cytometer (BD Bioscience). The mean
389 ploidy in >4N MKs was calculated as (%8Nx0.08) + (%16Nx0.16) + (%32Nx0.32) +
390 (%64Nx0.64) + (%128Nx1.28) + (%256Nx2.56).

391 **Histology and immunohistochemistry**

392 BM (femur) and spleen samples were fixed in 4% paraformaldehyde (PFA), decalcified and
393 embedded in paraffin. Sections were stained with hematoxylin, eosin and safran. MK were
394 revealed with immunohistochemistry using a rabbit anti-von Willebrand Factor (vWF) antibody
395 (Dako). Reticulin fibers were stained following the Gordon and Sweet's silver staining

396 procedure (black stain). Slides were examined using a Zeiss Axiophot microscope (Carl Zeiss)
397 with 2.5×1 (magnification ×2.5) or 10×/1 (magnification ×10) numeric aperture objectives.

398 **Immunofluorescence**

399 BM (femur) and spleen samples were fixed in 4% paraformaldehyde (PFA), decalcified and
400 embedded in paraffin. After deparaffinization and rehydration, the antigen retrieval was
401 performed (Tris EDTA pH8, 98°C, 30min). Slides were cooled 30 min at room temperature and
402 after washing in distilled water they were permeabilized (4% Triton X-100 in PBS (PBS-T))
403 and blocking of non-specific binding was done with 5% BSA in PBS-T for 10 min. Then the
404 slides were incubated with specific anti-vWF-FITC (Abcam) and P-selectin-PE (Emfret)
405 antibodies diluted in blocking solution (1% BSA in PBS-T) for 1h at room temperature. Nuclear
406 labelling was done with DAPI (Molecular Probes). The slides were examined using Vectra
407 Polaris Automated Quantitative Pathology Imaging System (AKOYA) with a x20 objective.

408 **Transmission electron microscopy**

409 Tibias were flushed with 2% glutaraldehyde in 0.1M phosphate buffer for 1h at 4°C for fixation
410 and were postfixed with 2% osmium tetroxide in 0.1M phosphate buffer for 1h at room
411 temperature. Following dehydration through a graded ethanol series, samples were embedded
412 in Epon™ 812. Polymerization was complete after 48 h at 60 °C. Ultrathin sections were
413 stained with standard uranyl acetate and lead citrate and observed with FEI Tecnai 12 electron
414 microscope. Digital images were taken with a SIS MegaviewIII CCD camera.
415

416 **In vitro growth of MKs in semi-solid conditions**

417 To quantify MK progenitors (CFU-MK), 1×10^5 total BM cells were seeded in serum-free fibrin
418 clot media as previously described² supplemented with TPO (10 ng/mL, Kirin Brewery, Tokyo,
419 Japon), mSCF (25ng/mL, Biovitrum AB, Stockholm, Sweden) and IL-6 (10ng/mL, Peprotech,
420 France).

421 After 7 days of culture, cells were fixed with glutaraldehyde 5% and MK colonies were stained
422 with acetylcholinesterase solution (acetylcholine iodide dissolved in sodium phosphate buffer
423 pH 6.3, 0.1 M sodium citrate, 30 mM copper sulfate, 5mM potassium ferricyanide).

424 **TGF-β1 and vWF level determination in BM supernatant**

425 BM cells were recovered by flushing one femur in 1 mL of PBS and centrifuged.

426 ***TGF-β1***

427 a) TGF-β1 level in the supernatant was detected using the Quantikine ELISA Kit (R&D system,
428 Bio-Techne SAS). The kit detects active TGF-β1 levels. In order to measure latent TGF-β1
429 levels, we followed the manufacturer's instruction to activate the latent form by addition of
430 20% of 1N HCl followed with neutralization with 15% of 1.2N NaOH/0.5 M HEPES. Total
431 TGF-β1 represents the addition of latent and active forms.

432 b) 40×10^3 of BM stromal murine MS-5 cells were co-cultured in 6-well plates with 200μl of
433 BM supernatants and 800 μl of DMEM supplemented with 10% FCS at 37°C in 5% CO₂ for 5
434 hours. The expression levels TGF-β1 responding genes (Ccn2, Acta1 and Pai1) were then
435 measured by qPCR.³

436 **vWF**
437 vWF antigen levels in BM supernatants were measured with an in-house enzyme-linked
438 immunosorbent assay essentially as previously described⁴. A pooled plasma prepared from 20
439 (males and females) C57Bl/6 mice was used as reference.
440

441 **Transplant procedures**

442 Recipient are WT mice irradiated at 9.5Gy with XRAD 320 KV irradiator. These mice were
443 grafted with 3×10^6 (15×10^6 cells/mL in PBS) WT or non-induced KOKI BM cells by retro
444 orbital injection. For competitive procedure mice have received 75% of WT-GFP cells + 25%
445 of non-induced KI $Jak2^{V617F}$ or 25% of non-induced KOKI cells. Proportions of cells have been
446 verified by following the GFP by flow cytometry. Four weeks after engraftment, $Jak2^{V617F}$
447 expression was induced by tamoxifen.

448 **Palbociclib administration**

449 Palbociclib isethionate salt (#P-7766, LC Laboratories, USA) was suspended in sterile water
450 (15 or 12.5 mg/mL) and 200 μ L/20g were administered to the mice every 24 h by force-feeding.
451 Preventive treatment was administrated between weeks 2 and 4 post- $Jak2^{V617F}$ induction at
452 150mg/kg for native mice. For engrafted mice, PB was administrated between weeks 2 and 6
453 post- $Jak2^{V617F}$ induction at 125 mg/kg (or at the indicated concentration in the legend). Control
454 mice received vehicle (water). The toxicity of Palbociclib was tested in both native and
455 engrafted mice (SF11).

456 **Patient progenitor cultures**

457 To study the effect of Palbociclib, CD34⁺ cells were sorted (anti-CD34-APC Ab (BD) and
458 DAPI) at 1 progenitor/well in 96-well plates with a BD Influx cell sorter. Clones were
459 expanded in serum-free medium with a cocktail of human recombinant cytokines containing 1
460 U/mL EPO (Amgen), 20 ng/mL TPO (generous gift from Kirin), 10 ng/mL FLT3-L (Celldex
461 Therapeutics), 10 ng/mL IL-3, 20 ng/mL G-CSF, 10 ng/mL IL-6 (Miltenyi Biotech), 25 ng/mL
462 SCF and 5 μ g/mL GM-CSF (Peprotech) without or with Palbociclib (0.1 μ M). Fourteen days
463 later, individual colonies corresponding to the progeny of each progenitor were lysed and DNA
464 was genotyped for homozygous, heterozygous or wild-type (wt) $JAK2^{V617F}$ status as previously
465 described⁵.

466 **qPCR**

467 Sorted BM MKs and MS-5 cells cocultured with BM supernatants were kept in Trizol and RNA
468 was extracted with Direct zol RNA Microprep. $p19^{Ink4d}$, $CDK6$, $Ccn2$, $Acta2$ and $Pail$
469 expression levels were measured by RT-qPCR (Superscript IV Vilo and Takara Sybr Green).
470 HPRT was used as housekeeping gene. Following murine primers were used: $p19^{Ink4d}$: F: CAA-
471 GAT-GCC-TCC-GGT-ACT-AG and R: CAA-CTC-CAG-GGG-AGT-GAG-AC; $Cdk6$: F:
472 GGCACCTGGAGACCTTCGA and R: CGTGACACTGTGCACACATCA; $Ccn2$: F: AGG
473 ACC GCA CAG CAG TTG and R: GCA GTT GGC TCG CAT CAT AG; $Acta2$: F: TCA GGG
474 AGT AAT GGT TGG AAT GG and R: GTT GGT GAT GAT GCC GTG TTC; $Pail$: F: CTC
475 CTC ATC CTG CCT AAG TTC and R: GTC CCG CTC TCG TTT ACC.

476 **Cytometry data and statistical analyses.**

477 Cytometry data were analyzed on FlowJo software and statistical analyses were performed
478 using the Prism software. If not otherwise mentioned, tests used an unpaired design with two-
479 tailed nonparametric Mann-Whitney test. Blood parameters were analyzed with the One-way

480 ANOVA with the Bonferroni correction test. Results are presented as means \pm SEM. * $p < 0.05$,
481 ** $p < 0.01$ or *** $p < 0.001$; **** $p < 0.0001$; “n =” stands for individual mice in all experiments.

482 **Data sharing statement**

483 Other material and methods will be provided upon request by corresponding author.

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485

486 **Supplementary references**

487

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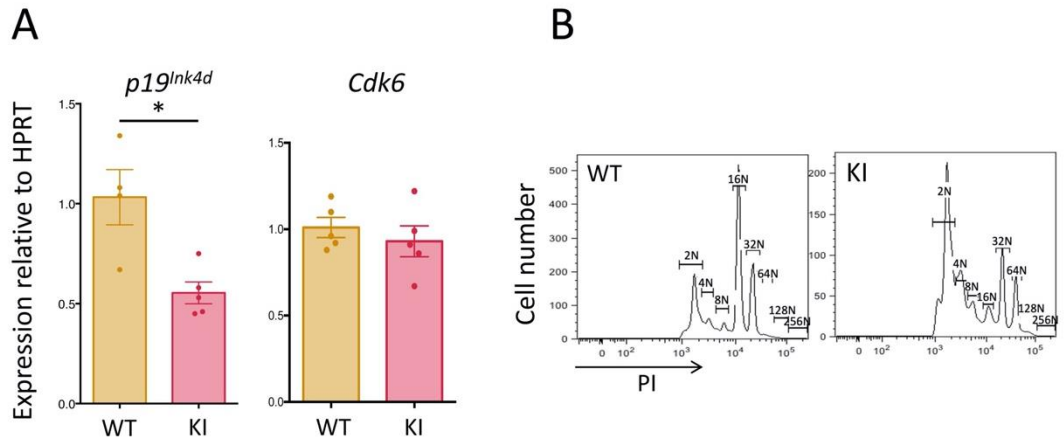
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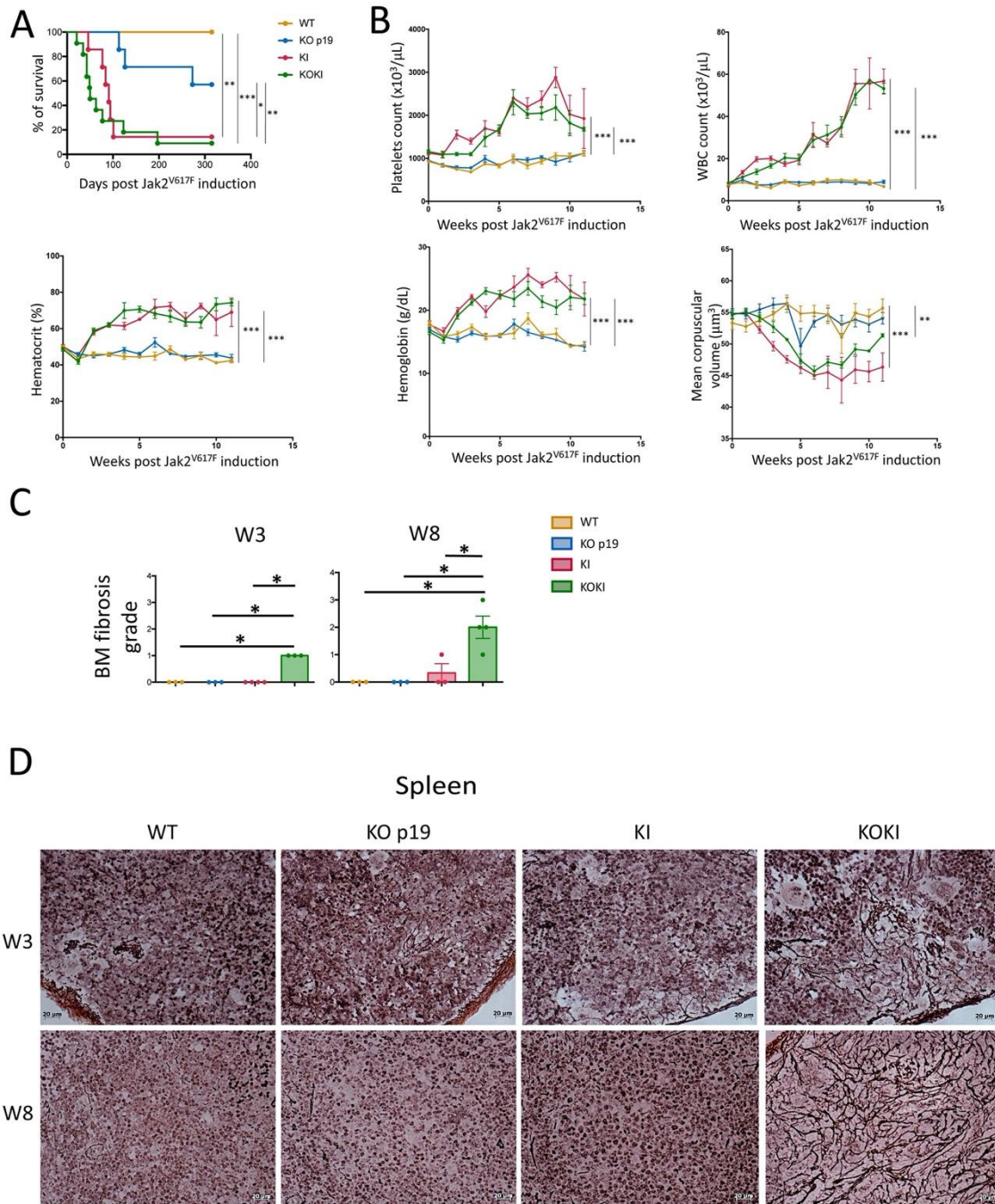
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509 **Supplementary figures**
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 511 **Supplementary figure 1**
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513 **SF1: Ploidy and *p19^{Ink4d}* and *CDK6* expression levels in WT and *Jak2^{V617F}* (KI) MKs.**
 514
 515 **A.** *p19^{Ink4d}* and *Cdk6* expression relative to HPRT in WT and KI CD41⁺CD42⁺ MKs after 3
 516 weeks post-*Jak2^{V617F}* induction. Data represent mean±SEM, *p<0.05; **p<0.01; ***p<0.005,
 517 nonparametric Mann-Whitney test. WT (n=4, 5) and KI (n=5). **B.** The representative picture of
 518 BM MK ploidy for WT and KI mice.
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 522 **Supplementary figure 2**
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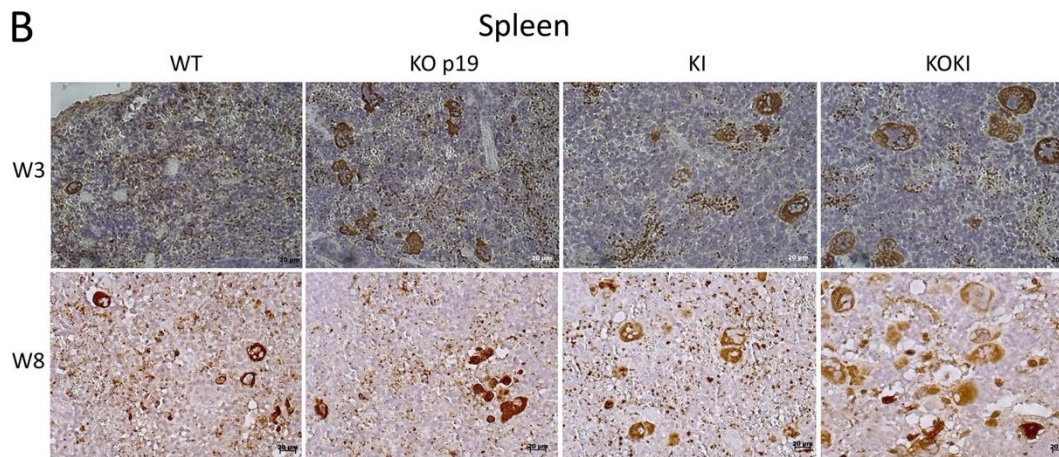
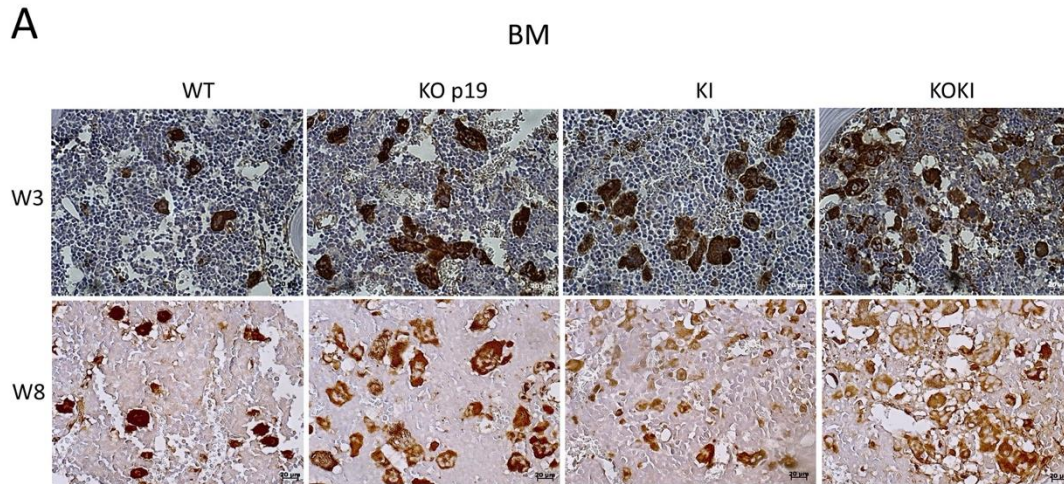
524 **SF2. *P19^{Ink4d}* depletion favors the rapid development of splenomegaly and myelofibrosis**
 525 **in *Jak2^{V617F}* context**
 526

527 **A.** Survival curve (315 days follow-up) for the WT, *p19* KO (KO p19), *Jak2^{V617F}* KI (KI) or
 528 *p19* KO/*Jak2^{V617F}* KI (KOKI) mice according to the days post-*Jak2^{V617F}* induction. n=7-11 for
 529 each genotype. Log-rank test was used, *p<0.05; **p<0.005; ***p<0.001. **B.** Platelet count
 530 (x10³/μL), white blood cells count (WBC, x10³/μL), hematocrit (%), hemoglobin (g/dL) and
 531 mean corpuscular (μm³) were analyzed weekly for 11 weeks post-*Jak2^{V617F}* induction. Data
 532 represent mean±SEM, *p<0.05; **p<0.01; ***p<0.005, one-way ANOVA coupled to
 533 Bonferroni test, (n=4 to 29). **C.** Fibrosis grade scored in bone marrow (BM) of WT (n=3 or 4),

534 KO p19 (n=3), KI (n=3 or 4) and KOKI (n=3 or 4) mice at different weeks (W) post-*Jak2*^{V617F}
535 induction. Data represent mean±SEM, *p<0.05, nonparametric Mann-Whitney test. **D.**
536 Representative pictures of spleen stained with a standard histological silver kit to reveal
537 reticular fibers at 3 and 8 weeks post-*Jak2*^{V617F} induction. Scale bar = 20µm.

538

539 **Supplementary figure 3**



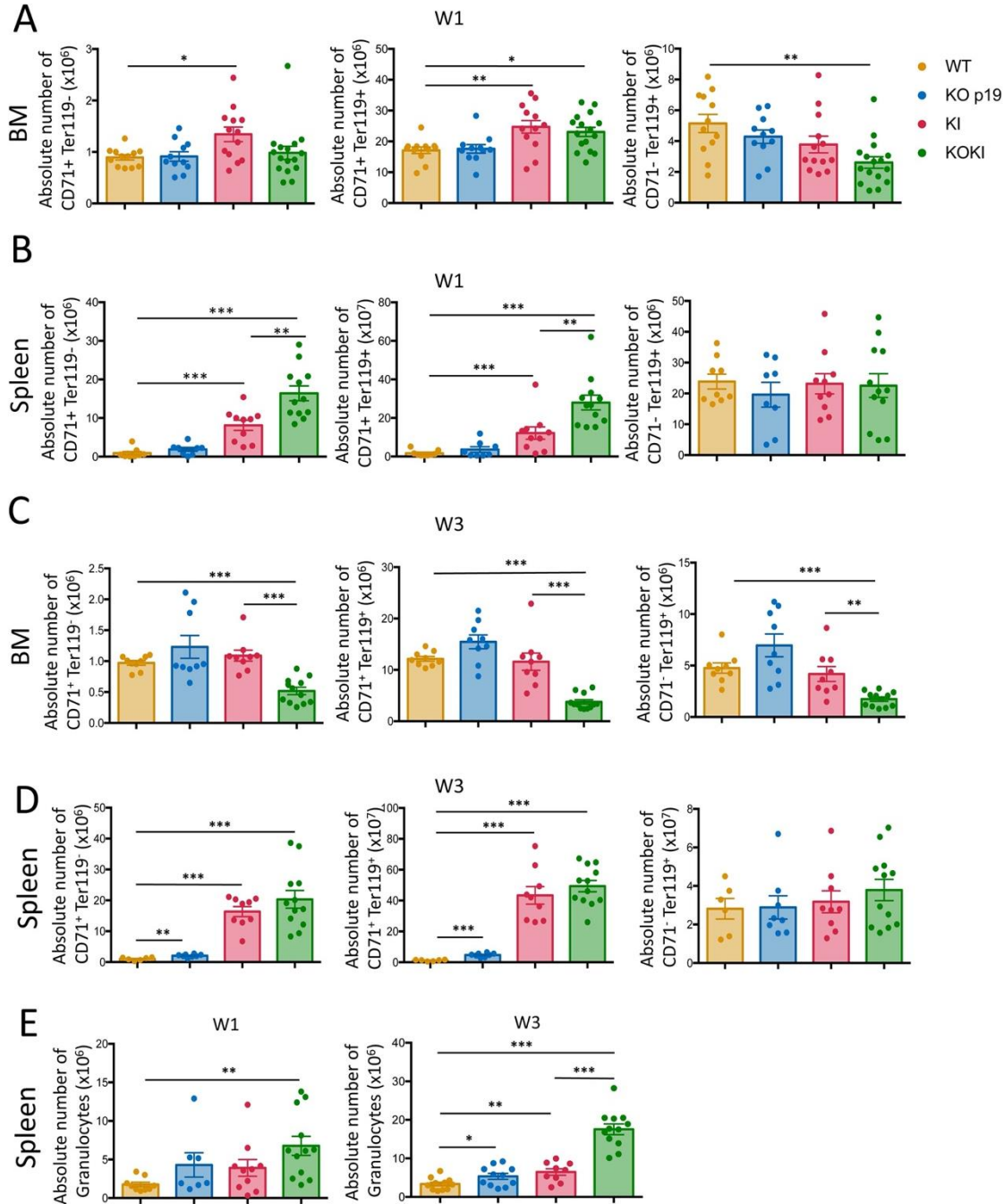
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542 **SF3. A-B.** Representative pictures of BM (**A**) and spleen (**B**) von Willebrand factor staining at
543 3 and 8 weeks post-*Jak2*^{V617F} induction. Scale bar = 20µm.

544

545 **Supplementary figure 4**



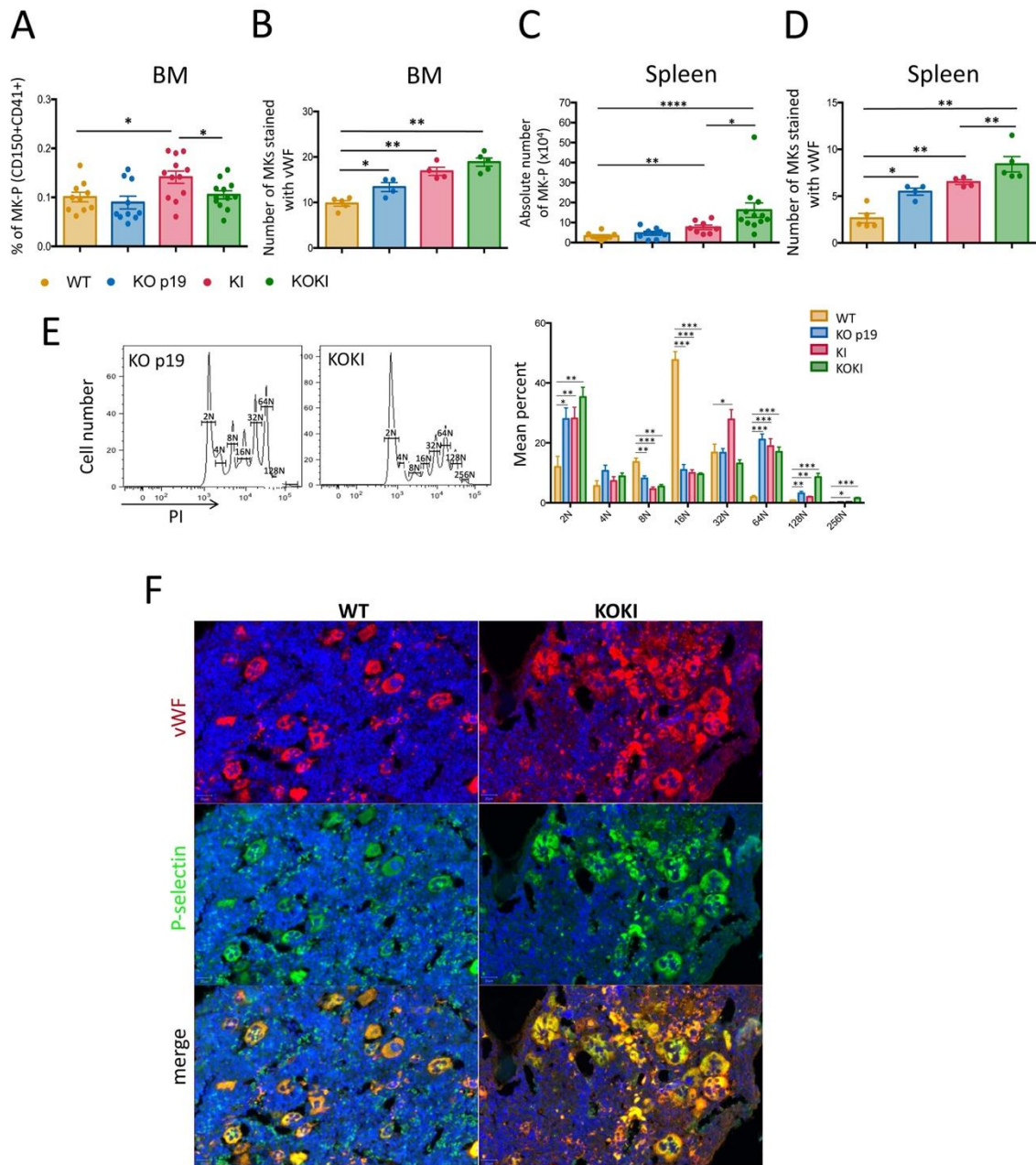
SF4.

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547 ***P19^{Ink4d}* depletion favors the rapid development of extramedullar hematopoiesis in**
 548 ***Jak2^{V617F}* context**

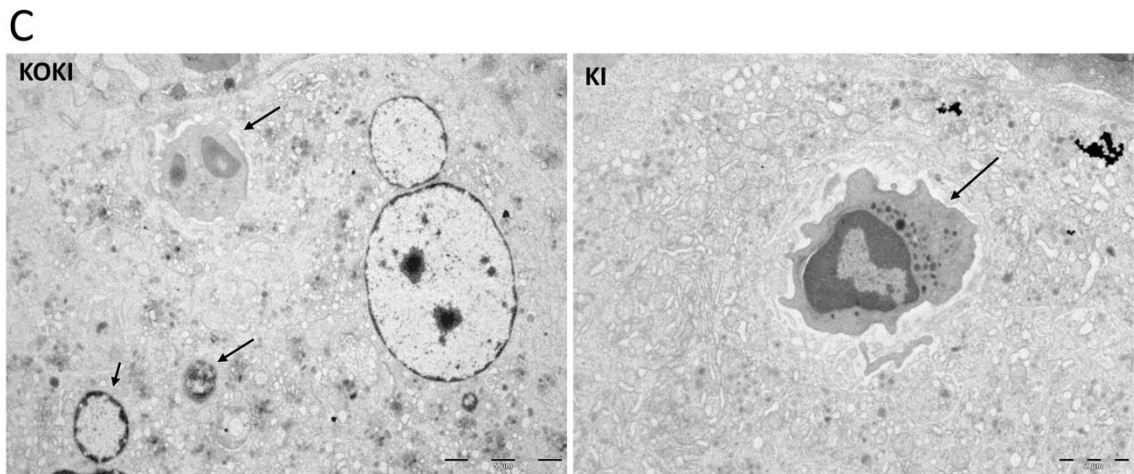
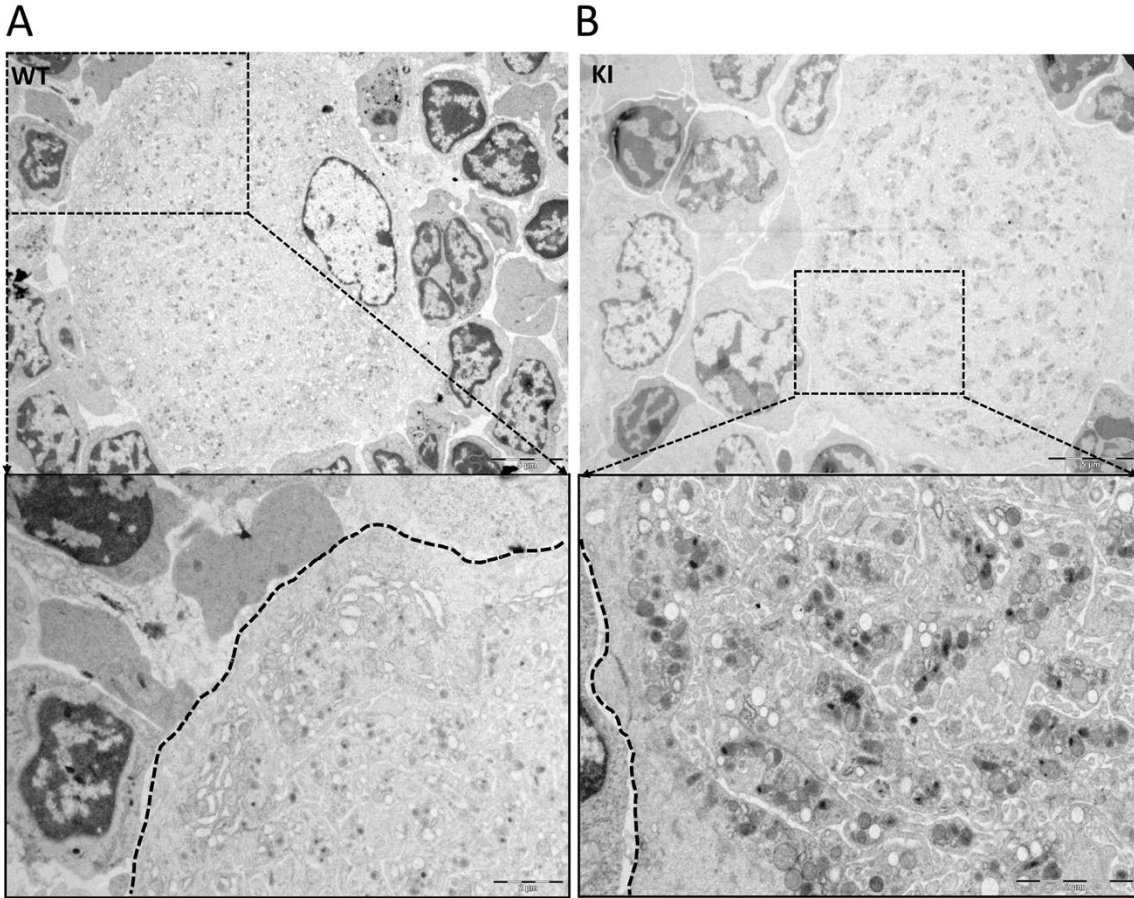
549 Erythroid differentiation followed in bone marrow (A, C) and spleen (B, D) with CD71 and
 550 Ter119 markers at week 1 (W1; A, B) and 3 (W3; C, D) post-*Jak2^{V617F}* induction.
 551 CD71⁺Ter119⁻ cells represent the more immature erythroblasts and the CD71⁻Ter119⁺
 552 population represents the more mature erythroid cells. E. Absolute numbers of granulocytes
 553 (Gr1⁺ CD11b⁺) in spleen at week 1 (W1) and 3 (W3) post-*Jak2^{V617F}* induction. A-E. Absolute
 554 numbers are calculated according to the total frequency of each population and the total BM or
 555 spleen cellularity. Data represent mean±SEM, *p<0.05; **p<0.01; ***p<0.005, nonparametric
 556 Mann-Whitney test. WT (n= 6-12), KO p19 (n=7-11), KI (n= 9-11) and KOKI (n=11-16).

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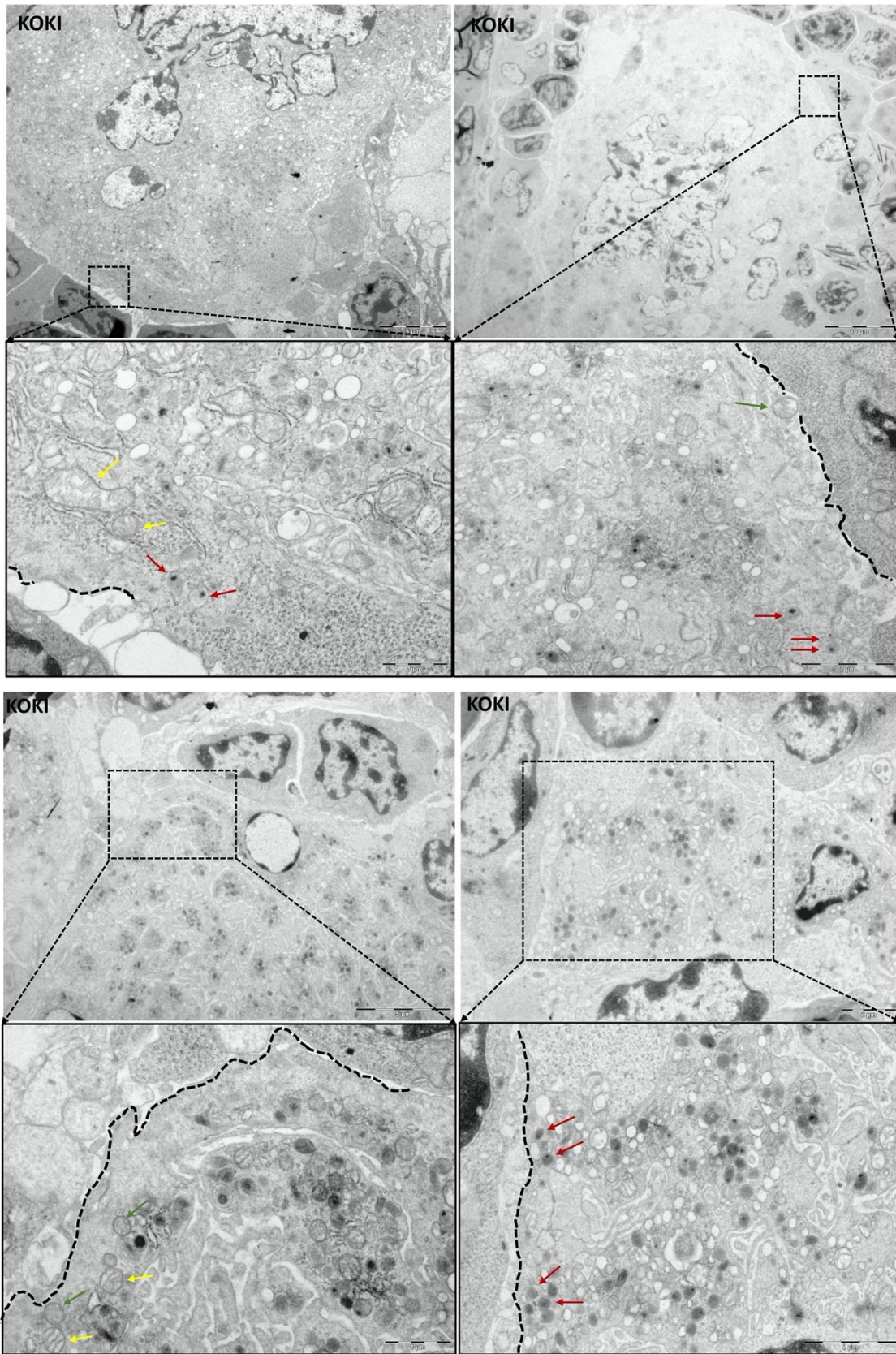


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 560 **SF5. *P19^{Ink4d}* depletion favors the amplification of MK lineage in *Jak2^{V617F}* context**
 561 **A.** Frequency of MK-Ps (Lin⁻ Sca-1⁻c-kit⁺CD41⁺CD150⁺) in BM 3 weeks post-*Jak2^{V617F}*
 562 induction. WT (n=10), KO p19 (n=10), *Jak2^{V617F}* KI (KI, n=12), *p19* KO / *Jak2^{V617F}* KI (KOKI,
 563 n=12). **B, D.** The numbers of MK stained by immunohistochemistry with vWF in BM (**B**) and
 564 spleen (**D**). The MK numbers were counted on equal area of 3 independent BM and 5
 565 independent spleen pictures for each mouse. WT (n=5), KO p19 (n=4), *Jak2^{V617F}* KI (KI, n=4),
 566 *p19* KO / *Jak2^{V617F}* KI (KOKI, n=5). Data represent mean±SEM, *p<0.05; **p<0.01,
 567 nonparametric Mann-Whitney test. **C.** Absolute numbers of MK-Ps in spleen at week 3 post-
 568 *Jak2^{V617F}* induction. Data represent mean±SEM, *p<0.05; **p<0.01; ****p<0.0001,
 569 nonparametric Mann-Whitney test. WT (n=8), *p19* KO (n=8), KI (n=8) and KOKI (n=12). **E.**
 570 Ploidy of BM MKs is calculated for each genotype after 3 weeks of *Jak2^{V617F}* induction. The

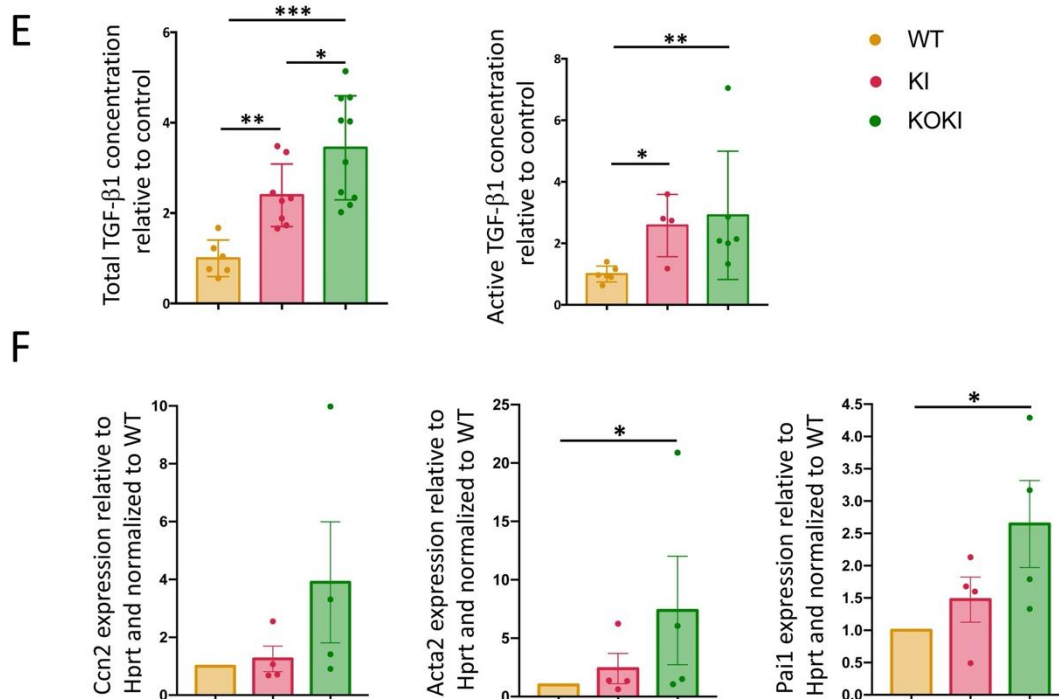
571 representative picture for KO p19 and KOKI genotype is shown on the left panel. The
572 representative picture for WT and KI genotype is shown on SF 1B. The mean percentage for
573 each peak (from 2N to 256N) and for each genotype is shown on the right panel. Data represent
574 mean±SEM, *p<0.05; **p<0.01; ***p<0.005, nonparametric Mann-Whitney test. WT (n=8),
575 *p19* KO (n=10), *Jak2*^{V617F} KI (n=12), *p19* KO / *Jak2*^{V617F} KI (KOKI, n=9). **F.**
576 Immunofluorescence staining of MKs in BM of WT or KOKI mice 3 weeks post-*Jak2*^{V617F}
577 induction. vWF is in red, P-selectin in green and nucleus (DAPI) in blue color. A diffuse
578 localization of vWF and P-selectin are detected in BM of KOKI mice. Scale bar = 20µm.
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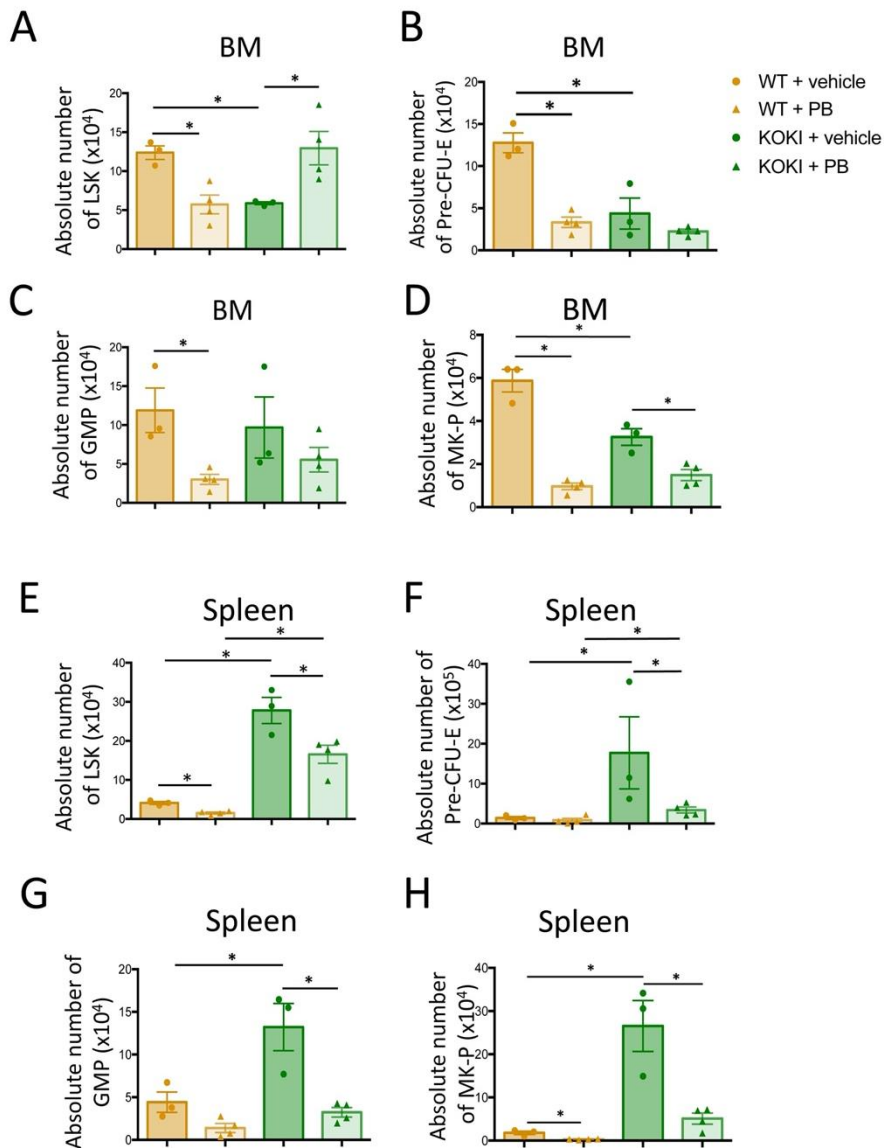
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SF6. Abnormal ultrastructure of MKs in KOKI mice with increased TGFβ1 secretion.

A-D. Representative pictures of MKs in BM of WT, KI or KOKI mice at 1-week post-*Jak2*^{V617F} induction observed by electronic microscopy. WT (**A**) and KI (**B**) MKs with conserved marginal zone are shown. **C.** Emperipolesis in KI and KOKI MKs indicated by black arrows. **D.** KOKI MKs with disrupted marginal zone are shown. Red arrows indicate the granules, yellow arrows the mitochondria and green arrows the exosome-like particles localization on the periphery of cells. The lower panels show zooming in on the black squares of the panels at the top. The interrupted black lines show the edge of the cell wall. Scale bar = 2, 5 or 10μm as indicated. **E.** Total and active concentration of TGF-β1 in BM supernatant after 1 (W1) week post-*Jak2*^{V617F} induction. Total concentration is measured after latent TGF-β1 activation with HCl. Data represent mean±SEM, *p<0.05; **p<0.01; ***p<0.005, nonparametric Mann-Whitney test. WT (n=6), KI (n=8, 4), KOKI (n=10, 5). **F.** *Ccn2*; *Acta2* and *Pai1* expression relative to *Hprt* in MS-5 stromal cells co-cultured for 5h with BM supernatants isolated from BM of WT, KI and KOKI mice at 1-week post-*Jak2*^{V617F} induction. Data represent mean±SEM, *p<0.05; nonparametric Mann-Whitney test. WT (n=4), *Jak2*^{V617F} KI (n=4) and KOKI (n=4).

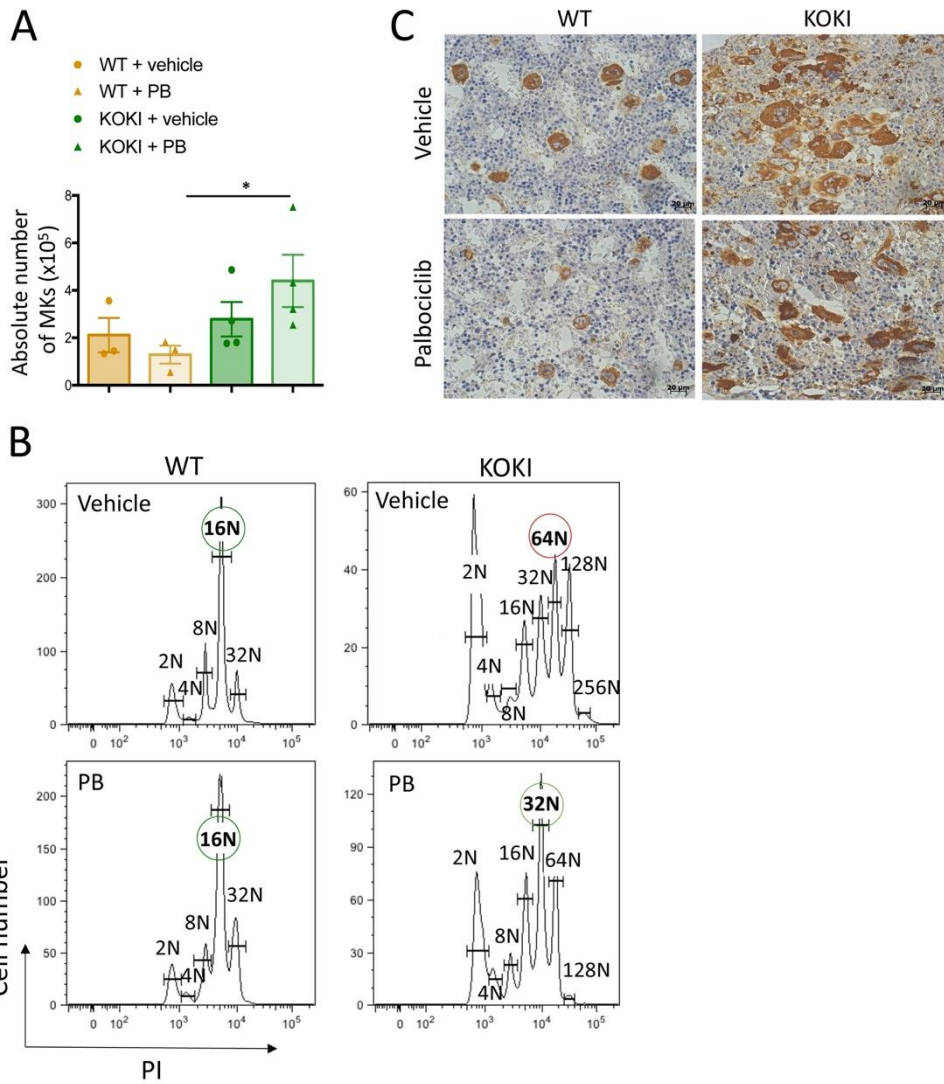


605
 606 **SF7. Palbociclib treatment prevents establishment of extramedullary hematopoiesis 2**
 607 weeks after *Jak2*^{V617F} induction, mice were treated daily with PB (150 mg/kg) for 2 weeks. **A-**
 608 **D.** Absolute numbers of bone marrow progenitors at the end of treatment with vehicle or PB.
 609 **A.** LSK (Lin⁻ Sca-1⁺ c-Kit⁺). **B.** Pre-CFU-E (Lin⁻ c-kit⁺ Sca-1⁻ CD41⁻ FCγR⁻ CD105⁺ CD150⁺).
 610 **C.** GMP (Lin⁻ c-kit⁺ CD41⁻ FCγR⁺ CD150⁻). **D.** MK-P (Lin⁻ Sca-1⁻ c-Kit⁺ CD150⁺ CD41⁺). Data
 611 represent mean±SEM, *p<0.05, nonparametric Mann-Whitney test. WT+vehicle (n=3),
 612 WT+PB (n=4), KOKI+vehicle (n=3), KOKI+PB (n=4). **E-H.** Absolute numbers of progenitors
 613 in the spleen at the end of treatment. **E.** LSK. **F.** Pre-CFU-E. **G.** GMP. **H.** MK-P. Data represent
 614 mean±SEM, *p<0.05, nonparametric Mann-Whitney test. WT+vehicle (n=3), WT+PB (n=4),
 615 KOKI+vehicle (n=3), KOKI+PB (n=4). Absolute numbers were calculated according to the
 616 total BM or spleen cellularity.

617

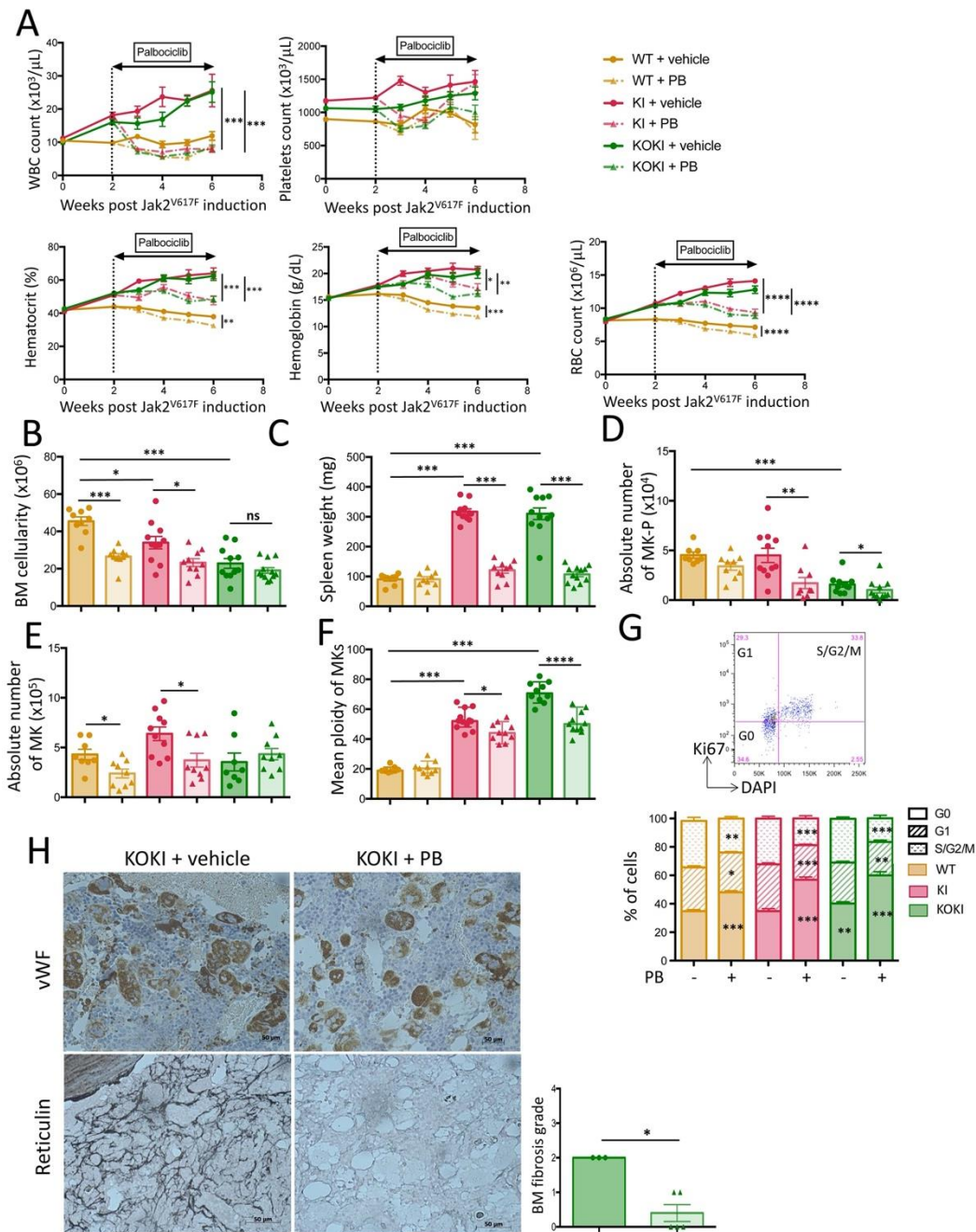
618

619 **Supplementary figure 8**



620
 621 **SF8. Palbociclib treatment partially restores megakaryopoiesis in KOKI mice**
 622 2 weeks after $Jak2^{V617F}$ induction, mice were treated daily with PB (150 mg/kg) for 2 weeks.
 623 **A.** Absolute numbers of mature MKs ($CD41^+CD42^+$) in BM (1 femur). Data represent
 624 mean \pm SEM, * $p < 0.05$, nonparametric Mann-Whitney test. WT+vehicle (n=3), WT+PB (n=3),
 625 KOKI+vehicle (n=4), KOKI+PB (n=4). **B.** Ploidy analysis: representative histograms of BM
 626 MKs for WT or KOKI mice with or without PB treatment. **C.** Representative pictures of BM
 627 von Willebrand factor (vWF) staining for WT or KOKI mice with or without treatment.
 628
 629

630 **Supplementary figure 9**



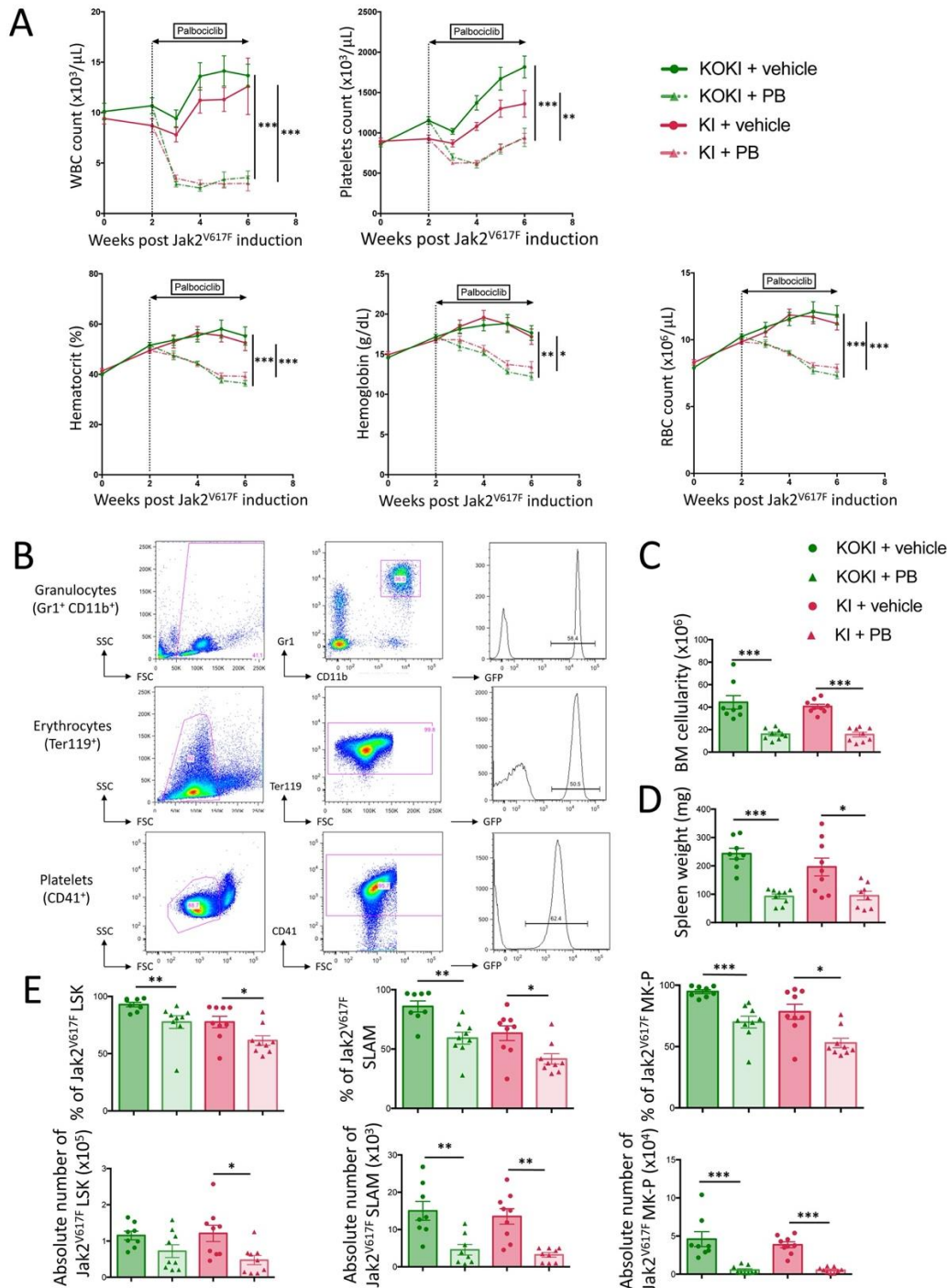
631 **SF9. Palbociclib treatment prevents the myeloproliferation and restores the**
 632 **megakaryopoiesis in $Jak2^{V617F}$ context**
 633

634 The irradiated (9.5 Gy) WT mice were engrafted with 3×10^6 of total BM WT, $Jak2^{V617F}$ KI
 635 (KI) or $p19$ KO / $Jak2^{V617F}$ KI (KOKI) cells. After 4 weeks of hematopoietic reconstitution,
 636 $Jak2^{V617F}$ was induced by tamoxifen. From week 2 to 6 post- $Jak2^{V617F}$ induction, mice were
 637 treated daily during 4 weeks with vehicle or PB (125 mg/kg) and analyzed at the end of
 638 treatment. **A.** Shown are: white blood cells count (WBC) ($\times 10^3/\mu\text{L}$), platelet count ($\times 10^3/\mu\text{L}$),
 639 hematocrit (%), hemoglobin (g/dL) and red blood cells (RBC) count ($\times 10^6/\mu\text{L}$). Data
 640 represent mean \pm SEM, * $p < 0,05$; ** $p < 0,01$; *** $p < 0,005$, **** $p < 0,0001$ one-way ANOVA
 641 coupled to

642 Boneferroni test. WT + vehicle (n=9), WT + PB (n=9), KI + vehicle (n=11), KI + PB (n=10),
643 KOKI + vehicle (n=11), KOKI + PB (n=12). **B.** Total BM cellularity for 2 femurs and 2 tibiae
644 ($\times 10^6$). **C.** Spleen weight (mg). **B-C.** Data represent mean \pm SEM, * $p < 0.05$; ** $p < 0.01$;
645 *** $p < 0.005$, nonparametric Mann-Whitney test. WT+vehicle (n=9), WT+PB (n=9),
646 KI+vehicle (n=11) KI+PB (n=10), KOKI+vehicle (n=11), KOKI+PB (n=12). **D-E.** Absolute
647 numbers of MK progenitors (MK-P, Lin⁻c-Kit⁺Sca-1⁻CD150⁺CD41⁺) (**D**), and mature MKs
648 (CD41⁺CD42⁺) (**E**) in total BM calculated according to their frequency and the total BM
649 cellularity. Data represent mean \pm SEM, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$, nonparametric Mann-
650 Whitney test. WT+vehicle (n=8), WT+PB (n=9), KI+vehicle (n=10,11) KI+PB (n=9),
651 KOKI+vehicle (n=10,8), KOKI+PB (n=11,9). **F.** Mean ploidy level of mature BM
652 CD41⁺CD42⁺ MKs stained with propidium iodide. Data represent mean \pm SEM, * $p < 0.05$;
653 ** $p < 0.01$; *** $p < 0.005$; **** $p < 0.0001$, t-test Mann-Whitney. WT + vehicle (n=9), WT+PB
654 (n=9), KI+vehicle (n=12), KI+PB (n=10), KOKI+vehicle (n=10), KOKI+PB (n=11). **G.** Cell
655 cycle analysis of MK-Ps. Gating strategy is shown on the top panel. Histograms on bottom
656 panel represent different cell cycle phases (G0, G1, S/G2/M) in MK-Ps of all genotypes treated
657 with vehicle or PB (125mg/kg). Data represent mean \pm SEM, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$,
658 nonparametric Mann-Whitney test. Statistical analysis represents the significant difference
659 between each cell cycle phase compare to their respective control: PB condition is compared to
660 vehicle condition for same genotype and for same cell cycle phase. For vehicle, different
661 genotypes are compared to the WT for the same cell cycle phase. WT+vehicle (n=8), WT+PB
662 (n=9), KI+vehicle (n=11), KI+PB (n=9), KOKI+vehicle (n=11), KOKI+PB (n=9). **H.** Left
663 panel: Representative pictures of BM stained with a standard histological silver kit to
664 underscore reticular fibers or von Willebrand factor. Scale bar = 50 μ m. Right panel: Fibrosis
665 grade scored in bone marrow (BM). Data represent mean \pm SEM, * $p < 0.05$, nonparametric Mann-
666 Whitney test. KOKI+vehicle (n=3), KOKI+PB (n=5).

667

668 **Supplementary figure 10**



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670

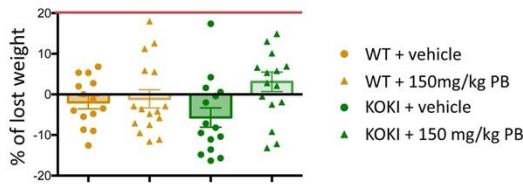
671 **SF10. Palbociclib specifically targets the $\text{Jak2}^{\text{V617F}}$ clone**

672 Irradiated (9.5Gy) WT mice were engrafted with 3×10^6 cells (75% WT-GFP⁺ + 25% KI- or
673 KOKI-GFP). After 4 weeks of hematopoietic reconstitution, $\text{Jak2}^{\text{V617F}}$ was induced by
674 tamoxifen. From week 2 to 6 post- $\text{Jak2}^{\text{V617F}}$ induction, mice were treated daily with vehicle or
675 PB (125mg/kg) and analyzed at the end of treatment. **A**. Shown are: white blood cells count
676 (WBC) ($\times 10^3/\mu\text{L}$), platelet count ($\times 10^3/\mu\text{L}$), hematocrit (%), hemoglobin (g/dL) and red blood
677 cells count (RBC) ($\times 10^6/\mu\text{L}$). Data represent mean \pm SEM, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$,
678 one-way ANOVA coupled to Bonferroni test. KOKI+vehicle (n=8), KOKI+PB (n=9),

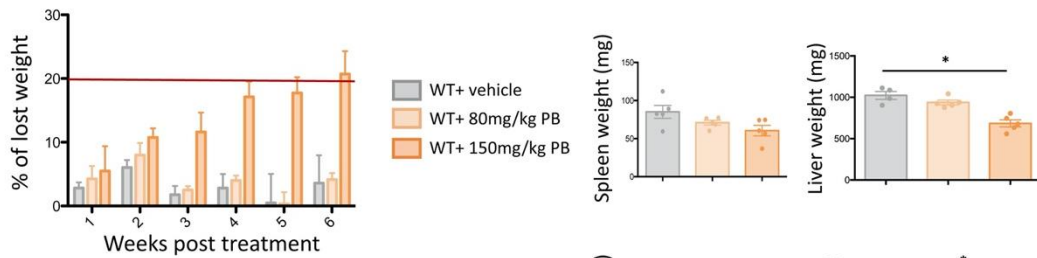
679 KI+vehicle (n=9), KI+PB (n=9). **B.** Gating strategy for the analysis of WT granulocytes
680 (Gr1⁺CD11b⁺GFP⁺), WT erythrocytes (Ter119⁺GFP⁺) and WT platelets (CD41⁺GFP⁺) in
681 peripheral blood. **C.** Total BM cellularity for 2 femurs and 2 tibiae (x10⁶). **D.** Spleen weight
682 (mg). **B, D.** Data represent mean ± SEM, *p<0.05; **p<0.01; ***p<0.005, nonparametric
683 Mann-Whitney test. KOKI+vehicle (n=8), KOKI+ PB (n=9), KI+vehicle (n=9), KI+PB (n=8).
684 **E.** Frequencies and absolute numbers of BM *Jak2*^{V617F} progenitors: LSK (Lin⁻Sca-1⁺c-Kit⁺),
685 SLAM (Lin⁻Sca-1⁺c-Kit⁺CD48⁻CD150⁺) and MK-P (Lin⁻Sca-1⁻c-Kit⁺CD150⁺CD41⁺).
686 Absolute numbers are calculated according to the total frequency of the population of interest
687 and the total BM cellularity. Data represent mean±SEM, *p<0.05; **p<0.01; ***p<0.005,
688 nonparametric Mann-Whitney test. KOKI+vehicle (n=8), KOKI+PB (n=9), KI+vehicle (n=9)
689 KI+PB (n=9).

690 **Supplementary figure 11**

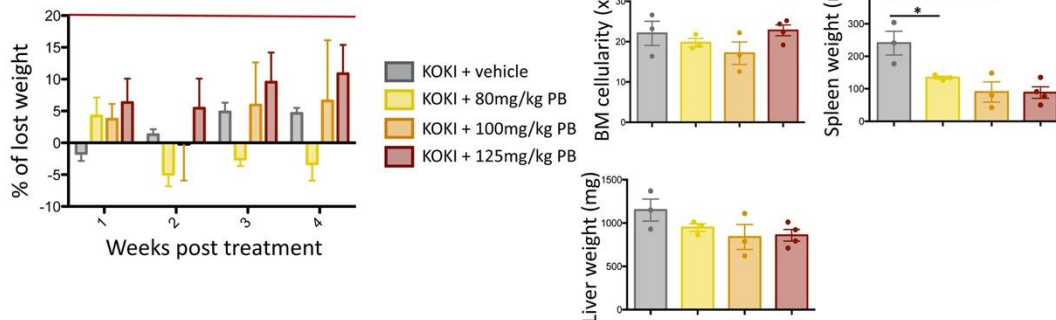
A



B



C



691

692 **SF11. Toxicity test of Palbociclib treatment**

693 **A.** 2 weeks after $Jak2^{V617F}$ induction, WT and $p19$ KO / $Jak2^{V617F}$ KI (KOKI) mice were treated
 694 daily with PB (150 mg/kg) for 2 weeks. Mouse weight was followed each week, the histogram
 695 represents the percentage of weight loss at the end of 2 weeks of treatment compare to the initial
 696 weight. Data represent mean \pm SEM. WT+vehicle (n=14), WT+PB (n=16), KOKI+vehicle
 697 (n=16), KOKI+PB (n=14). **B.** Irradiated (9.5Gy) WT mice were engrafted with 3×10^6 WT
 698 cells. After 4 weeks of hematopoietic reconstitution, mice are treated daily during 6 weeks with
 699 vehicle or PB (80 or 150mg/kg). Mouse weight was followed each week, the histogram on the
 700 left panel represents the percentage of weight loss at the end of each week of treatment compare
 701 to the initial weight. A dose of 150 mg/kg induced a 20% loss of weight and was considered as
 702 a toxic in transplanted mice. Spleen and liver weights are shown on the right panel. Data
 703 represent mean \pm SEM, * $p < 0.05$; nonparametric Mann-Whitney test. WT+vehicle (n=4),
 704 WT+80 mg/kg PB (n=5), WT+150 mg/kg PB (n=5). **C.** The irradiated (9.5Gy) WT mice were
 705 engrafted with 3×10^6 of non-induced KOKI cells. After 4 weeks of hematopoietic
 706 reconstitution, $Jak2^{V617F}$ was induced by tamoxifen. Mice were treated daily from week 2 to
 707 week 6 post- $Jak2^{V617F}$ induction with vehicle or PB (80, 100 or 125 mg/kg). Mice weight was
 708 followed each week, the histogram on the left panel represents the percentage of weight loss at
 709 the end of each week of treatment compare to the initial weight. BM cellularity, spleen and liver
 710 weights are shown on the right panel. A decrease in the spleen weight with PB treatment
 711 correspond to the decrease of splenomegaly in KOKI mice. A dose of 125 mg/kg of PB is not
 712 toxic in the transplanted model and was chosen for further experiments. Data represent

713 mean±SEM, *p<0.05; nonparametric Mann-Whitney test. KOKI+vehicle (n=3), KOKI+80
 714 mg/kg PB (n=3), KOKI+100 mg/kg PB (n=3), KOKI+125 mg/kg PB (n=4).

Patient N# (Polycythemia vera or Essential thrombocythemia)	JAK2 ^{V617F} VAF (%)	Additional mutations (VAF %)
1 (PV)	4.4*	nd
2 (PV)	12	TET2 ^{M508fs*} (23) BCORL1 ^{P810S} (13) TP53 ^{Y220C} (4.5)
3 (ET)	8	SF3B1 ^{K666N} (25)
4 (ET)	11	DNMT3A ^{P700L} (9)
5 (ET)	40.5	TET2 ^{R1452*} (1.5) SF3B1 ^{K666N} (1)

715 **Supplementary table 1**

716

717 **ST1. Polycythemia vera (PV) and Essential Thrombocythemia (ET) patients.**

718 List of PV and ET patients, their Jak2^{V617F} allelic frequency (VAF) and associated additional
 719 mutations. *VAF determined by PCR, the other VAFs were determined by NGS. nd: not done.

720

721