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**CABLES1 deficiency impairs quiescence and stress responses of hematopoietic stem cells in intrinsic and extrinsic manners.**

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## Summary

Bone marrow (BM) niche cells help keeping adult hematopoietic stem cells (HSC) in a quiescent state via secreted factors and induction of cell cycle inhibitors. Here, we demonstrate that the adapter protein CABLES1 is a key regulator of long-term hematopoietic homeostasis during stress and aging. Young mice lacking *Cables1* displayed hyperproliferation of hematopoietic progenitor cells. This defect was cell intrinsic, since it was reproduced in BM transplantation assays using wild-type animals as recipients. Overexpression and shRNA mediated depletion of CABLES1 protein resulted in p21<sup>Cip/waf</sup> up and down regulation, respectively. Aged mice lacking *Cables1* displayed abnormalities in peripheral blood cell counts accompanied by a significant reduction in HSC compartment, concomitant with an increased mobilization of progenitor cells. In addition, *Cables1*<sup>-/-</sup> mice displayed increased sensitivity to the chemotherapeutic agent 5-fluorouracil due to an abnormal microenvironment. Altogether, our findings uncover a key role for CABLES1 in HSC homeostasis and stress hematopoiesis.

## Introduction

Maintenance of the hematopoietic system requires continuous cellular replenishment from a rare population of hematopoietic stem cells (HSC) endowed with self-renewal and multilineage differentiation capacities (Orkin and Zon, 2008). At steady state, in the bone marrow (BM) the majority of HSC remain quiescent in the G<sub>0</sub> phase of the cell cycle (Bernitz et al., 2016; Foudi et al., 2009). When a stress occurs and the number of mature cells is reduced in the blood circulation (e.g. bleeding, infection or chemical/radiation injury), HSC rapidly enter the cell cycle to give rise to progenitor cells with robust proliferative potential, allowing replenishment of the hematopoietic system (Pietras et al., 2014). However, when HSC are submitted to frequent proliferative stress situations, self-renewal is severely impeded. Similarly, HSC accumulate changes with age that include a decrease in the regenerative potential and a preferential differentiation into myeloid cells with a loss of support of B-cell lineage. Although aging of HSC was initially thought to be solely influenced by stem cell intrinsic mechanisms, several recent data indicate that aging of the niche and the microenvironment contributes to aging-associated phenotypes of HSC (Florian et al., 2012; Rossi et al., 2005).

The cyclin-dependent kinase (Cdk) 5 and Abl enzyme substrate 1 (CABLES1), also called Ik3-1, is a Cdk interacting protein which binds to multiple Cdks including Cdk2 (Wu et al., 2001), Cdk3 (Yamochi et al., 2001) and Cdk5 (Zukerberg et al., 2000). Probably due to its potential to interact with Cdk, CABLES1 inhibits proliferation in cell line models by enhancing the inhibitory function of Cdk2 mediated by Wee1 (Wu et al., 2001). It also regulates cell proliferation by stabilizing p21<sup>Cip/waf</sup> (p21) (Shi et al., 2014). In addition to an impact on proliferation, CABLES1 can induce cell death by regulating stability and proteasomal degradation of several members of the p53 family including p53, p73 and TAp63a (Matsuoka et al., 2003; Tsuji et al., 2002). In addition, CABLES1 links Robo-bound Abl kinase to N-cadherin

regulating  $\beta$ -catenin function and transcription (Rhee et al., 2007). Likely due in part to its ability to negatively regulate proliferation and to induce apoptosis, loss of CABLES1 is associated with a high frequency in multiple types of cancer in humans, including endometrial cancer (DeBernardo et al., 2005), colorectal cancer (Park et al., 2007), ovarian cancer (Dong et al., 2003) and non-small lung cancer (Huang et al., 2017). In mouse models, loss of *Cables1* results in a high incidence of endometrial adenocarcinoma (Zukerberg et al., 2004). In addition, *Cables1* acts as tumor suppressor regulating intestinal tumor progression in *Apc<sup>Min</sup>* mice (Arnason et al., 2013). Despite its well-recognized role in cancer, only a few studies have addressed its function in physiologic settings. Current studies indicates a role of CABLES1 in neural differentiation and neurite outgrowth by interacting with Cdk5 (a non-cell cycle associated kinase) and Abl (Zukerberg et al., 2000). Moreover, CABLES1 is required for embryonic neural development in the zebrafish model (Groeneweg et al., 2011). Finally, loss of CABLES1 enhances oogenesis associated with reduced oocyte quality (Lee et al., 2007).

Previous studies reported that loss of *Cables1* results in an increase of BM hematopoietic progenitor cells, suggesting that CABLES1 could be a potent regulator of hematopoiesis (Lee et al., 2007). Here, we broaden our understanding of CABLES1 function(s) in hematopoiesis using a *Cables1<sup>-/-</sup>* mouse model. We first report that CABLES1 is predominantly expressed in the progenitor cell compartment, suggesting that CABLES1 is a stemness marker. We also show that absence of *Cables1* in mice markedly impacts progenitor cell proliferation. Under stress conditions, absence of *Cables1* delays hematopoietic recovery, while during aging the HSC number was impaired. Finally, the number of mesenchymal stromal is reduced in *Cables1<sup>-/-</sup>* mice. Thus, CABLES1 participates in the control of HSC maintenance during aging and under hematopoietic stress.

## Results

### **CABLES1 is expressed in hematopoietic stem and progenitor cells and in niche cells**

The experimental strategy to analyze CABLES1 function in hematopoiesis is depicted in **Figure 1A**. The mRNA expression levels of *Cables1* in cells of the hematopoietic and BM microenvironment lineages were analyzed by quantitative RT-PCR. We isolated different subsets of primitive hematopoietic progenitor cells (Kiel et al., 2005; Morita et al., 2010) and used the mouse brain as reference as previously described (Zukerberg et al., 2000). *Cables1* mRNA expression level was substantially higher in LSK (Lin<sup>-</sup>Kit<sup>+</sup>Sca-1<sup>+</sup>) cells and SLAM (CD150<sup>+</sup>CD48<sup>-</sup> LSK) compared to differentiated cells, such as B220<sup>+</sup>, CD4<sup>+</sup>, CD8<sup>+</sup> and Gr-1<sup>+</sup> cells (**Figure 1B**). We also performed analysis of *Cables1* expression in BM niche cells such as osteoblasts, endothelial cells and mesenchymal stem cells (MSCs) (Mendez-Ferrer et al., 2015). All three sorted cell populations expressed *Cables1* mRNAs (**Figure 1B**). Of note, the expression of *Cables1* mRNA was not modified during aging in mice (Figure S1). *CABLES1* was also expressed in human CD34<sup>+</sup> progenitor cells from cord blood (CB-CD34<sup>+</sup>), mobilized peripheral blood (PB-CD34<sup>+</sup>) and human MSCs, in contrast to mature cell populations (**Figure 1C**). These results were confirmed at the protein level (**Figure 1D, Figures S2A, B**). In addition, the localization of CABLES1 protein in CB-CD34<sup>+</sup> cells was mainly nuclear (**Figure 1E**). These findings suggest that CABLES1 is expressed in the adult BM.

### **Steady state hematopoiesis in young mice is not affected by *Cables1* deficiency**

To address the impact of loss of *Cables1* in hematopoiesis, we assessed complete blood counts in *Cables1*<sup>-/-</sup> and wild-type mice. Numbers of white blood cells (WBC), red blood cells (RBC) and platelets (PLTs) were within normal ranges in 10 to 12-week-old animals (**Figures S3 A, B, C**) as well as the percentages of B-, T-lymphocytes and neutrophils (**Figure S3 D**). BM and spleen

cellularities were also comparable between *Cables1*<sup>-/-</sup> mice and their normal counterparts (**Figures S4 A, B**). No significant differences in the number of colony forming unit-cells (CFU-Cs) (**Figure S4 C**) and phenotypically defined progenitor cells including granulomonocytic progenitors (GMP), common myeloid progenitors (CMP), common lymphoid progenitors (CLP) and mega-erythroid progenitors (MEP) were noted between *Cables1*<sup>-/-</sup> and wild type mice (**Figures S4, D, E**). Finally, the numbers of phenotypically defined LT-HSC (CD150<sup>+</sup>CD48<sup>-</sup>KSL) were similar between the two groups (**Figure S4 F**). These data demonstrate that loss of *Cables1* does not have a major impact on steady state hematopoiesis and suggest that blood production is not affected by *Cables1* deficiency.

### ***Cables1* is an intrinsic cell cycle inhibitor in mouse progenitor cells**

As CABLES1 is highly expressed in hematopoietic progenitor cells and involved in cell cycle regulation and cell death as shown in previous in-vitro studies (Huang et al., 2017), we evaluated its impact on apoptosis, the levels of mitochondrial reactive oxygen species (ROS) and cell cycle status of hematopoietic progenitor cells under steady state conditions. In 10 to 12-week-old *Cables1*<sup>-/-</sup> mice, the proportions of Annexin V<sup>+</sup> DAPI<sup>-</sup> LSK and SLAM cells and mitochondrial ROS levels were not altered compared to *Cables1*<sup>+/+</sup> mice (**Figures 2A, 2B**). However, as illustrated in **Figure 2C**, the fraction of cycling cells (Ki67<sup>+</sup>) among LSK cells was significantly higher in the BM of *Cables1*<sup>-/-</sup> mice compared to WT mice (**Figure 2D**). Of note, within the more immature subset of HSC, namely the SLAM population, the proportion of Ki67<sup>+</sup> cells was not affected (**Figure 2E**). To confirm these results, we performed in-vivo BrdU incorporation assays. In accordance with the Ki67 assays, a higher number of *Cables1*<sup>-/-</sup> LSK cells were BrdU positive (**Figure 2F**), whereas the proportion of BrdU<sup>+</sup> cells in the SLAM cell population was not altered (**Figure 2G**). As CABLES1 is a cell cycle inhibitor for LSK progenitor cells, we compared its

expression between G0 and cycling LSK cells. We sorted LSK cells in the G0, G1, and S/G2/M phase using Pyronin Y and Hoechst 33342 staining and tested the relative expression of *Cables1* by q-PCR. LSK cells in the G0 phase showed the highest expression level of *Cables1* compared to LSK cells in the G1 and S/G2/M phase (**Figure 2H**). In addition, *Cables1* expression sharply decreased upon culture of freshly isolated LSK cells in cytokine containing medium (**Figure 2I**). Similarly, in vivo treatment of mice with LPS, leading to quiescence loss of hematopoietic progenitor cells (**Figure 2J**), was associated with a sharp decrease in *Cables1* expression (**Figure 2K**). Altogether, these results suggest that *CABLES1* expression negatively regulates the cell cycle status of hematopoietic progenitor cells.

As cells from the hematopoietic microenvironment also express *CABLES1*, we sought to know whether the role of *CABLES1* in progenitor cell proliferation was intrinsic or extrinsic. Thus, we conducted transplantation assays using donor BM cells from *Cables1*<sup>-/-</sup> or *Cables1*<sup>+/+</sup> (Ly5.2) mice that were transplanted into lethally irradiated recipient mice (Ly5.1) (**Figure 2L**). Three months after transplantation, donor contribution was over 90% for each group (**Figures S3, E, F, G, H**). *Cables1*-null derived LSK and SLAM cells had significantly higher proportions of Ki67-proliferating cells than their wild type counterparts (**Figure 2M**). This illustrates the intrinsic role of *CABLES1* in regulating the proliferative status of hematopoietic progenitor cells.

### ***Cables1*<sup>-/-</sup> HSCs harbor higher reconstitution fitness**

We next evaluated the impact of *Cables1* deficiency in competitive transplantation. BM cells from *Cables1*<sup>-/-</sup> (Ly5.2) or *Cables1*<sup>+/+</sup> (Ly5.2) mice were mixed 1:1 with age-matched BM competitor cells (Ly5.1) and injected into lethally irradiated recipient mice (Ly5.1) (**Figure 3A**). During five continuous months post transplantation, recipients were analyzed monthly for the percentage of Ly5.2 donor derived cells in blood. There was significantly 5%, 10%, 13.4%, 13%

and 13% more *Cables1*<sup>-/-</sup> derived cells in the blood of Ly5.1 recipients, 1, 2, 3, 4 and 5 months post reconstitution, respectively (**Figure 3B**). A similar higher reconstitution level was observed when *Cables1*<sup>-/-</sup> LSK cells were transplanted (Figure 3C). The percentages of Ly5.2 donor-derived B cells (**Figure 3D**), T cells (**Figure 3E**) and granulocytes (**Figure 3F**) were similar between the two groups.

The frequency of *Cables1*<sup>-/-</sup> BM LSK and SLAM cells tended to be higher than that of *Cables1*<sup>+/+</sup> LSK and SLAM cells in transplanted animals, but this was not significant (**Figure 3G**). In order to characterize the enhanced competitive properties of *Cables1*<sup>-/-</sup> HSC, we measured the apoptotic rate of LSK and SLAM cells after transplantation. LSK and SLAM cells from *Cables1*<sup>-/-</sup> mice had similar apoptotic rates compared to *Cables1*<sup>+/+</sup> LSK and SLAM cells (**Figures 3H, I**). We also conducted homing experiments. CFSE marked *Cables1*<sup>-/-</sup> or *Cables1*<sup>+/+</sup> BM cells were injected into irradiated recipient mice. The numbers of CFSE<sup>+</sup> *Cables1*<sup>-/-</sup> or *Cables1*<sup>+/+</sup> cells in the BM, spleen and blood of recipients were comparable (**Figure 3J**). The number of progenitor cells that homed to the BM was similar between *Cables1*<sup>-/-</sup> or *Cables1*<sup>+/+</sup> genotypes (**Figure 3K**). Thus, the enhanced competitive capacity of *Cables1*<sup>-/-</sup> HSC is neither due to increased homing capacity nor decreased apoptosis and could be related to their enhanced cell cycle entry under transplantation settings.

### **CABLES1 slows cell proliferation of human CD34<sup>+</sup> cells and regulates p21 level**

To examine whether CABLES1 could regulate the proliferation of human hematopoietic progenitor cells, CB CD34<sup>+</sup> cells were transduced using lentiviral vectors encoding either GFP alone or human CABLES1 (**Figure S5 A**). Transduced cells were sorted based on GFP expression (**Figure S5 B**) and CABLES1 overexpression was verified by Western blotting (**Figure 4A**). GFP<sup>+</sup> cells were seeded in methylcellulose to assess the effect of CABLES1 on

proliferation of hematopoietic progenitor cells. CABLES1 overexpression not only reduced the number of colonies but also diminished their size (**Figures 4B, C**). Thus, CABLES1 could slow the proliferation of hematopoietic progenitor cells.

To further investigate the effect of CABLES1 on cell proliferation, we next silenced *CABLES1* expression in CB-CD34<sup>+</sup> cells using a short hairpin RNA (shRNA) strategy. A sharp reduction of *CABLES1* messenger RNA was observed using two different shRNAs (**Figure 4D and Figure S5C**) and decreased protein levels (**Figure 4E**) were confirmed in *CABLES1* shRNA-transduced CD34<sup>+</sup> cells compared to control shRNA-transduced CD34<sup>+</sup> cells. The proliferation rate of CD34<sup>+</sup> cells transduced with *CABLES1* shRNA was higher compared to control shRNA-transduced cells (**Figure 4F**). CABLES1 has previously been demonstrated to interact with the master regulator CDKI p21, inducing inhibition of cell proliferation and apoptosis (Shi et al., 2014). Thus, we investigated the potential role of p21 in CABLES1 effects on cell proliferation. Similarly to murine progenitor cells, CABLES1 expression decreased upon cytokine stimulation of CD34<sup>+</sup> cells (**Figure 4G**). This decrease was accompanied by a parallel reduction in the level of p21 protein expression (**Figure 4H**). Strikingly, an increase in p21 protein expression was found in CB-CD34<sup>+</sup> cells overexpressing CABLES1 compared to control GFP expressing cells (**Figure 4I**). Furthermore, a reduction in p21 protein level was observed in *CABLES1* shRNA-transduced CD34<sup>+</sup> cells compared with control shRNA-transduced CD34<sup>+</sup> cells (**Figure 4J**). No change in p21 mRNA expression was noted in *CABLES1* shRNA-transduced CD34<sup>+</sup> cells compared to control shRNA-transduced CD34<sup>+</sup> cells (data not shown). To assess whether our in vitro results on human cells were representative for the CABLES1-p21 link, we evaluated p21 protein level in murine *Cables1* WT and KO LSK and SLAM cells. In accordance with our in vitro strategy using shRNA in human cells, our analysis showed that the protein levels of p21 was decreased in *Cables1*<sup>-/-</sup> LSK (**Figure 4 K**) and LSK SLAM cells (**Figure**

**4 L**). Thus, our results suggest a role of CABLES1 in the regulation of p21 protein expression.

#### **Loss of *Cables1* delays hematopoietic regeneration after 5-FU treatment**

Recent reports suggest that p21 regulates progenitor cell proliferation during periods of stress. As CABLES1 expression is correlated to p21 protein level, we reasoned that *Cables1*<sup>-/-</sup> mice might be more sensitive to hematopoietic injury, for example the chemotherapy agent 5-fluorouracil (5-FU) or irradiation. Therefore, 3-month-old *Cables1*<sup>-/-</sup> and littermate control mice were treated with a single dose of 5-FU (250 mg/kg body weight). In *Cables1*<sup>-/-</sup> treated mice, WBC (**Figure 5A**) recovery was delayed. In addition, 10 days after 5-FU challenge, the BM of *Cables1*<sup>-/-</sup> mice was hypocellular, characterized by a paucity of hematopoietic clusters in both osteoblastic and vascular zones compared to the BM of *Cables1*<sup>+/+</sup> mice (**Figures 5B, C**). Hematopoietic clusters were seen in close contact to the bone osteoblastic zone. In line with an impaired hematopoietic recovery, progenitor cell numbers were lower in the BM (**Figure 5D**), spleen (**Figure 5E**), and blood (**Figure 5F**) of *Cables1*<sup>-/-</sup> mice. Similar results were obtained when mice were challenged with cyclophosphamide (**Figures S6A, B, C, D**) or using moderate doses of ionizing radiation (**Figure S 6E**). Therefore, CABLES1 expression favors hematopoietic recovery in response to hematopoietic stress.

#### **Loss of *Cables1* is associated with defective hematopoiesis over time**

Given the impact of CABLES1 on hematopoietic recovery under hematopoietic stress conditions and the inability of *Cables1*-deficient HSC to tolerate stress, we expected that CABLES1 might play a prominent role during aging. Interestingly, aged (21 month-old) *Cables1*<sup>-/-</sup> mice displayed a significant increase in WBC counts compared to age-matched controls, while young (3 month-old) or middle aged (8 month-old) *Cables1*<sup>-/-</sup> mice had similar WBC numbers as their

counterparts (**Figure 6A**). No significant changes in RBC and platelet counts were observed between *Cables1* deficient mice and their controls during the process of aging (data not shown). It has been documented that the frequencies of B cell precursors within the BM are markedly diminished by 4-5 months of age in mice. However, paradoxically, this is not accompanied by a similar decline in the mature cells at the periphery due to compensatory mechanisms such as increased lifespan of B cells (Kline et al., 1999) (Johnson et al., 2002). In line with this, differential analysis of blood cells of *Cables1<sup>+/+</sup>* animals showed no significant variations in the percentage of circulating B-lymphocytes during aging (**Figures 6B, C** and **Figure S 3D**). However, we observed a significant decrease in the B cell percentages in 21-month-old *Cables1<sup>-/-</sup>* deficient mice compared to age-matched controls (**Figure 6B**). In contrast, the percentage of circulating mature myeloid cells (Gr-1<sup>+</sup>) was significantly increased, making this cell population accountable for the increase in total leukocyte counts. No significant differences in WBC subtypes in middle-aged animals were observed between both genotypes (**Figure 6C**). We next wondered whether *Cables1<sup>-/-</sup>* progenitor cell compartments were likewise perturbed during aging. There were no differences in BM cellularity between WT and *Cables1<sup>-/-</sup>* mice during aging (**Figure S7**). However, BM clonogenic progenitor, LSK and SLAM cell numbers were increased in middle-aged *Cables1<sup>-/-</sup>* mice compared to age-matched controls (**Figures 6D, E, F**). Strikingly, the number of these progenitors and LSK and LSK SLAM cells was reduced in aged *Cables1<sup>-/-</sup>* mice compared to age-matched controls (**Figures 6D, E, F**). Consistent with the results on young animals, LSK cells from middle-aged *Cables1<sup>-/-</sup>* mice displayed significantly higher proliferation rate than their WT counterparts (**Figure 6G**). Of importance, the frequency of dividing cells (BrdU<sup>+</sup>) within BM SLAM cells was higher in *Cables1<sup>-/-</sup>* mice compared to WT controls (**Figure 6H**).

To investigate the consequences of quiescence loss in *Cables1<sup>-/-</sup>* HSC, we studied the

effect of a weekly injection of 5-FU. 5-FU injection induces myeloablation by preferentially eliminating actively cycling cells, which in turn activates quiescent stem cells to restore the ablated hematopoietic system. Thus, weekly injection of 5-FU leads to HSC depletion resulting in death of the mice due to BM failure. Strikingly, *Cables1*<sup>-/-</sup> mice had an increased sensitivity to serial 5-FU treatment and showed a decreased median survival compared to WT animals (**Figure 6I**). Finally, no significant differences in the number of apoptotic Annexin V<sup>+</sup>/DAPI<sup>-</sup> cells within hematopoietic progenitor cells were detectable (data not shown), excluding that differences in apoptosis contributed to the elevated number of HSC found in the BM of middle aged *Cables1*<sup>-/-</sup> mice. Preferential differentiation into myeloid cells with loss of support for the B-cell lineage is one of the characteristics of aged hematopoiesis (Dykstra et al., 2011). Therefore, we asked whether *Cables1*<sup>-/-</sup> HSCs displayed an aging-like phenotype using the CD41 and CD150 markers as previously described (Gekas and Graf, 2013). Of note, the relative frequency of myeloid-biased HSC phenotypically defined by positive expression of CD41 in CD150<sup>+</sup> LSK cell population was higher in aged *Cables1*<sup>-/-</sup> mice compared to WT mice (**Figure 6J**). We also analyzed the frequency of LSK/CD34<sup>+</sup>/CD135<sup>+</sup> cell population, which is enriched for the lymphoid-primed multipotent progenitors (LMPP). No difference was found between the two genotypes (**Figure 6K**). Similarly, the numbers of CLP were comparable between the two genotypes (**Figure 6L**). In search for the source of hematopoietic abnormalities, we assessed CFC frequency in blood and an extramedullar hematopoietic site, the spleen. Notably, both middle aged and 21-month-old *Cables1*-deficient mice displayed increased CFU-C frequencies in blood (**Figure 6M**) and spleen (**Figure 6N**) compared to age-matched controls. Since increased mobilization could be explained by an abnormal response to CXCL12 (Foudi et al., 2006), the main chemokine involved in retention of progenitor cells within the BM, in vitro chemotactic assays in response to CXCL12 were performed. No significant difference was observed between

the two genotypes (**Figure S5 D**). In addition, CABLES1 overexpression in U937 and HL-60 cells did not modify their chemotactic response to CXCL12 (**Figures S5 E, F**). Overall, our data demonstrate that the loss of *Cables1* is associated with defects in blood counts, HSC activation and HSPC mobilization during aging.

### **Environmental defects are involved in the defective hematopoiesis of *Cables1*<sup>-/-</sup> mice**

Since our results showed significant CABLES1 expression in BM niche cells, we next investigated the putative role of the BM microenvironment in abnormal hematopoiesis of *Cables1*<sup>-/-</sup> mice. We thus conducted transplantation assays in which lethally irradiated *Cables1*<sup>-/-</sup> or littermate control mice were used as recipients for WT CD45.1<sup>+</sup> transplant BM cells (**Figure 7A**). Four months after reconstitution, *Cables1*<sup>-/-</sup> recipient mice displayed a significant decrease in relative B cell donor counts with a significant increase in the frequency of mature myeloid cells (**Figures 7B, C**). We also determined, whether the increased susceptibility of *Cables1*<sup>-/-</sup> mice to serial 5-FU injection was cell autonomous or related to an abnormal microenvironment. *Cables1*<sup>-/-</sup> or WT BM cells were transplanted into lethally irradiated recipients of both genetic backgrounds in order to obtain chimeric mice. Four months after hematopoietic reconstitution, 5-FU was injected three times at 10-days intervals and survival was monitored (**Figure 7D**). As expected, most (12/13) *Cables1*<sup>-/-</sup>/*Cables1*<sup>-/-</sup> mice died after 5-FU treatment while less WT/WT mice (4/12) succumbed, recapitulating our observation in middle aged straight *Cables1*<sup>-/-</sup> mice. Conversely, *Cables1*<sup>-/-</sup> recipient mice either reconstituted with *Cables1*<sup>+/+</sup> or *Cables1*<sup>-/-</sup> BM cells showed a decreased median survival, compared with *Cables1*<sup>+/+</sup> recipient mice reconstituted with WT or *Cables1*<sup>-/-</sup> BM cells. Of note, *Cables1*<sup>-/-</sup> recipient mice either reconstituted with *Cables1*<sup>-/-</sup> or WT BM cells showed no significant difference survival curves and this was also observed for WT recipient groups transplanted with *Cables1*<sup>-/-</sup> or littermate control BM cells.

Overall, these results indicate that the lack of *Cables1* in hematopoietic cells is not sufficient to provide a disadvantage to mice treated with repeated cycles of 5-FU and suggest that the increased susceptibility of *Cables1*<sup>-/-</sup> mice to 5-FU is mainly related to an abnormal hematopoietic microenvironment. To substantiate this finding, we quantified the mesenchymal stromal progenitor cells in the BM of *Cables1*<sup>-/-</sup> and *Cables1*<sup>+/+</sup> mice by CFU-F assays. CFU-F frequencies were comparable in the BM of 3 month-old mice (**Figures 7E**). However, middle-aged and aged *Cables1*<sup>-/-</sup> mice displayed a lower frequency of CFU-F compared to their age-matched counterparts (**Figures 7F, G**). These data show that BM microenvironment is altered in *Cables1*<sup>-/-</sup> during aging. Altogether, our results indicate that CABLES1 plays both intrinsic and extrinsic roles in hematopoiesis as summarized in **Figure 7H**.

## Discussion

Here, we report that the CABLES1 plays at least two important roles in the regulation of HSC pool. One of these is cell autonomous, mainly evidenced in stress conditions; the second role relates to the microenvironment and underscores the role of CABLES1 during aging of HSC.

The first preliminary evidence for a role of CABLES1 in the regulation of hematopoiesis came from the observation that the number of progenitor cells was slightly increased in the BM of *Cables1<sup>-/-</sup>* mice (Lee et al., 2007). Our data revealed that the cell cycle status of HSC was not altered in steady state young animals. In contrast, we revealed an enhanced proliferative status of HSC when transplanted into WT recipients, suggesting that enhanced proliferation of *Cables1<sup>-/-</sup>* HSC may operate preferentially during hematopoietic stress. This could explain the slightly higher competitive characteristics of *Cables1<sup>-/-</sup>* HSC over their WT counterparts. *Cables1<sup>-/-</sup>* HSC displayed higher reconstitution fitness despite hyperproliferation under transplantation settings. This indicates that hyperproliferation per se does not necessarily inhibit stem cell function. In line with this, the knockout of some genes including cell cycle regulatory genes such as *Cdkn2c* or *Cdkn2a* enables preservation or higher repopulation potential despite increased HSC proliferation (Janzen et al., 2006; Yu et al., 2006; Yuan et al., 2004). Thus, our results suggest that CABLES1 controls progenitor proliferation through a cell autonomous mechanism and that it is required for quiescence of HSC especially under stress conditions.

Several studies have previously demonstrated that CABLES1 is present in a multimolecular complex containing p21/Cip (Shi et al., 2015). The cyclin-dependent kinase inhibitor p21<sup>Cip1/Waf1</sup> is a G1 checkpoint regulator that selectively regulates HSC and progenitor cell behavior under stress conditions (Cheng et al., 2000; van Os et al., 2007). Using overexpression experiments, we showed that enhanced CABLES1 expression in human progenitor cells clearly correlated with an increase in p21 protein expression. Inversely, when an

shRNA strategy was used, decreased CABLES1 expression correlated with a decrease in p21 protein expression. Mechanistically, CABLES1 interferes with the proteasome subunit alpha type-3 (PMSA3) binding to p21 and protects p21 from proteasomal degradation (Shi et al., 2014). In line with these studies, we showed that p21 mRNA expression is not impacted by CABLES1 expression, suggesting that CABLES1 may control p21 levels in hematopoietic cells through a post-transcriptional mechanism.

At steady state, in young animals, *Cables1* deletion impacted essentially the cell cycle of progenitor cells with only few effects on HSC numbers and cell cycle. Deletion of p21 has also a limited role on steady state hematopoiesis and its impact on HSC was only observed under stress conditions, such as 5-FU treatment and irradiation (Cheng, 2009; van Os et al., 2007) similarly to what we observed in *Cables1*<sup>-/-</sup> mice. Despite these similarities, our experiments revealed that the deletion of *Cables1* does not completely recapitulate the p21 phenotype. Indeed, under transplantation settings, *Cables1*<sup>-/-</sup> HSC displayed a higher proliferative status while the deletion of p21 either led to decreased (Cheng, 2009) or normal reconstitution (van Os et al., 2007). Thus, although these observations are consistent with the model that inhibition of proliferation by CABLES1 operates at least in part through an increase of p21 activity, other CABLES1 targets could be involved. Indeed, CABLES1 has a cyclin-like domain, which is a key element in its interactions with multiple Cdks including Cdk3, Cdk2 and Cdk5 (Matsuoka et al., 2000; Zukerberg et al., 2000). CABLES1 is phosphorylated by Cdk2 or Cdk3 bound to cyclin A and to cyclin E (Yamochi et al., 2001). In addition, CABLES1 enhances Cdk2 tyrosine phosphorylation at residue 15 (Y15) by Wee1 family kinase leading to the inhibition of Cdk2 activity and suppression of cell proliferation (Wu et al., 2001). Therefore, regulation of these targets by CABLES1 can account for increased proliferative capacity of *Cables1* deficient cells under transplantation.

By further investigating *Cables1*<sup>-/-</sup> mice, we observed that loss of *Cables1* is associated with defective hematopoiesis over time. At 21 months of age, *Cables1*<sup>-/-</sup> mice displayed a significant increase in myeloid cell numbers in the periphery without significant alterations in B cell counts. This phenotype is not transplantable, suggesting that this feature is non HSC-autonomous.

To understand why *Cables1*<sup>-/-</sup> mice display increased 5-FU sensitivity, we conducted transplantation assays in which lethally irradiated *Cables1*<sup>-/-</sup> or littermate control mice were used as recipients for WT transplant BM cells. We also generated reverse chimera by transplanting *Cables1*<sup>-/-</sup> BM cells to irradiated *Cables1*<sup>-/-</sup> or littermate control mice. Using this strategy, we demonstrated that the increased susceptibility of *Cables1*<sup>-/-</sup> mice to 5-FU is mainly dependent on the BM microenvironment. Indeed, 5-FU not only acts on HSC but also on niche cells that become stimulated thus inducing a stimulating effect on HSC (Jeong et al., 2018). Our hypothesis is supported by our results showing that niche cells, including MSC express high levels of *CABLES1* mRNA and protein. In addition, we have afforded evidence that BM from *Cables1*<sup>-/-</sup> mice have lower numbers of MSC, indicating that *CABLES1* is important for stromal cells. MSC are the principal cells that mediate retention of progenitor cells within the BM, and their deficiency is often associated with a marked exhaustion of HSC and increased mobilization of progenitor cells. The phenotype of middle-aged *Cables1*<sup>-/-</sup> mice strikingly mimicked transgenic mice with such MSC depletion. It remains to determine how *CABLES1* regulates the dynamic adaptation of niche cells to replicative stress and how this adaptation influences the regeneration of HSC. One potential mechanism could be stress or age-associated decreases in the adhesion potential of *Cables1*<sup>-/-</sup> MSC (Rhee et al., 2007). The use of in vitro and in vivo models as described recently will help to deeply understand how *CABLES1* regulates the dynamic adaptation of niche cells to replicative stress and how this adaptation influences the regeneration

of HSC (Jeong et al., 2018).

In conclusion, we report here an important role of CABLES1 in the direct regulation of progenitor cell cycle and long-term hematopoiesis. Our results suggest that the observed defects in *Cables1*<sup>-/-</sup> mice may be due in part to the potential role of CABLES1 to regulate p21 levels. The present results also underscore the protective role of CABLES1 during genotoxic stress and aging.

## **Experimental Procedures**

### **Animals**

Heterozygous *Cables1*<sup>+/-</sup> C57BL/6 (CD45.2) background mice were previously described and kindly provided by Bo. R. Rueda (Zukerberg et al., 2004). All experiments were approved by an appropriate institutional review committee (C2EA-26, Animal Care, Villejuif, France).

### **Culture of human cord blood (CB) CD34<sup>+</sup> cells and mesenchymal stem (MSC)**

CB samples from normal full-term newborn infants were collected after normal vaginal delivery with informed written consent. CD34<sup>+</sup> cells separated using a magnetic cell sorting system (miniMACS; Miltenyi Biotec) were cultured as previously described (Abdelouahab et al., 2017). MSC were obtained by culture of BM from healthy donors in plastic dishes in MSC expansion medium (Miltenyi Biotec). Chemotaxis assays in response to CXCL12 were performed as previously described (Rivière et al., 1999).

### **Flow cytometry**

BM cells were analyzed on FACS CANTO II (BD) and sorted on Influx flow cytometer (BD). HSC cell cycle analysis was performed with either Ki-67/Hoechst 33342 or BrdU/Hoechst 33342 costaining. Intracellular p21 was assessed with anti p21 antibody (Thermo Fisher Scientific) followed by a goat anti-rabbit Alexa Fluor 488 antibody, according to the supplied protocol of fixation/ permeabilization BD Cytotfix/Cytoperm™. Mitochondrial ROS detection was assessed after staining for 20 min with 10 μM MitoSox at 37°C as previously described (Zhang et al., 2016). BM cells stained for surface markers were incubated with Alexa Fluor 647–conjugated Annexin V (Invitrogen) and DAPI, according to the supplied protocol. To avoid false-positive annexin V staining, cells were processed at 2–8°C and analyzed within 4 hours after recovery from mice.

### **Reconstitution assays**

To establish chimeric mice, 12-14 weeks old *Cables1<sup>-/-</sup>* and *Cables1<sup>+/+</sup>* mice were lethally irradiated. 24 hours later, mice were reconstituted with  $5 \times 10^6$  *Cables1<sup>-/-</sup>* or *Cables1<sup>+/+</sup>* BM cells from 8-10 weeks old mice. The reconstitution was analyzed by sampling blood once per month. Mice were euthanized five months after transplantation for analyzing test cell-derived HPSCs and their cycling status by Ki67 assay. Half of the spleen and one femur per mouse were kept for histoimmunocytochemistry.

For competitive assays, cells ( $3 \times 10^6$  BM cells/host) from *Cables1<sup>-/-</sup>* and *Cables1<sup>+/+</sup>* donor mice (CD45.2) and an equal number of CD45.1 BM cells (competitor cells) were co-transplanted into lethally irradiated (9.5 Gy) CD45.1 recipients. Blood was collected monthly post transplantation and the relative contribution of test cells (CD45.2) to competitor cells (CD45.1) in the reconstituted host was quantified by flow cytometry. For reconstitution with purified cells, WT or KO BM LSK cells (8000 cells per mouse) were injected together with  $1.5 \times 10^5$  CD45.1 BM cells.

### **Treatment of mice with 5-FU or LPS**

Mice were injected intra-peritoneally with 5-FU (Fluorouracil Dakota Pharm, Paris, France) at 150 mg/kg body weight for survival rates of mice or at 250 mg/kg body weight for hematopoietic regeneration analysis. Mice were treated with 4 mg of CY followed by 5 days of 5  $\mu$ g per day s.c. rhG-CSF. *Cables1<sup>-/-</sup>* and *Cables1<sup>+/+</sup>* mice were subjected to TBI (6 Gy). Mice were injected with LPS (200  $\mu$ g/kg body weight) and sacrificed 24 hours after.

### **Transduction of human CB CD34<sup>+</sup> cells**

Human *CABLES1* cDNA sequence was cloned into the lentiviral vector pTrip-MND-IRES-GFP vector (kindly provided by F. Pflumio, CEA, L'Hay-les-Roses, France). *CABLES1* shRNAs (417758, 433848) and the control shc002, all from Sigma were cloned into a lentiviral vector

(pRRLsin-PGK-eGFP-WPRE, Genethon). Cell transduction was performed as previously described (Hirsch et al., 2016). To test the effect of CABLES1 overexpression on CD34<sup>+</sup> cell proliferation, 4,000 GFP<sup>+</sup> cells were sorted 48 hours post transduction and seeded in human methycellulose supplemented with recombinant human (rh) rhSCF (50 ng/ml, Biovitrum AB, Stockholm, Sweden), rhIL-3 (100 U/ml, Peprotech, France) and rh erythropoietin (1 U/ml, Amersham). Colonies were counted after 2 weeks.

### **Data processing and statistical analysis**

Flow cytometry results were analyzed by BD FACS Diva software. Results were evaluated using Student's unpaired t test by GraphPad Prism version 5.0 software (GraphPad Software, San Diego, CA, USA). Results are presented as means  $\pm$  SEM and the value of  $p < 0.05$  was determined as significant, and  $p < 0.01$  and  $p < 0.001$  as highly significant.

### **Authorship contributions**

HL performed experiments, analyzed data and wrote the paper. FB, VB, MD, YZ and MW performed experiments and analyzed data. AF, VJ and PG analyzed data. FL designed and supervised the study, analyzed data and wrote the manuscript. All authors contributed to writing and editing.

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The authors declare no conflict of interest.

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endometrial cancer. *Cancer Res.* 64, 202–208.

## Legends to Figures

**Figure 1: CABLES1 expression in human and murine hematopoietic and niche cells.** (A) Experimental strategy used to probe functions of CABLES1 in hematopoiesis. (B) *Cables1* mRNA expression in FACS-sorted mouse cells: B cells (B220<sup>+</sup>), T cells (CD4<sup>+</sup> and CD8<sup>+</sup>), myeloid (Gr-1<sup>+</sup>) cells, Lin<sup>-</sup> (lineage markers negative cells, namely CD3<sup>-</sup>B220<sup>-</sup>Ter119<sup>-</sup>Gr-1<sup>-</sup>), LSK (Lin<sup>-</sup>c-Kit<sup>+</sup>Sca-1<sup>+</sup>), c-kit<sup>+</sup> (Lin<sup>-</sup>c-Kit<sup>+</sup>Sca-1<sup>-</sup>), SLAM (CD150<sup>+</sup>CD48<sup>-</sup>LSK), CMP (Lin<sup>-</sup>Sca-1<sup>+</sup>c-Kit<sup>+</sup>FcR<sup>-</sup>CD34<sup>+</sup>), GMP (Lin<sup>-</sup>Sca-1<sup>+</sup>c-Kit<sup>+</sup>FcR<sup>+</sup>CD34<sup>+</sup>), MEP (Lin<sup>-</sup>Sca-1<sup>+</sup>c-Kit<sup>+</sup>FcR<sup>-</sup>CD34<sup>-</sup>) and in cellular components of the BM microenvironment : osteoblasts (CD45<sup>-</sup>Ter119<sup>-</sup>CD31<sup>-</sup>Sca-1<sup>-</sup>CD51<sup>+</sup>), endothelial cells (CD45<sup>-</sup>Ter119<sup>-</sup>CD31<sup>+</sup>) and MSC (CD45<sup>-</sup>Ter119<sup>-</sup>CD31<sup>-</sup>Sca<sup>+</sup>CD51<sup>+</sup>). Data are normalized to HPRT transcript levels and mouse brain is used as reference. Data represents a pool from 10 mice and are the mean  $\pm$  SEM of triplicates (See also Figure S1). (C) CABLES1 expression in human CD34<sup>+</sup> cells from cord blood (CB-CD34<sup>+</sup>), mobilized peripheral blood (PB-CD34) and mature blood cells (CD4<sup>+</sup>, CD8<sup>+</sup>, CD14<sup>+</sup>, CD19<sup>+</sup>, CD56<sup>+</sup>). Data are normalized to HPRT transcript levels and CD34<sup>+</sup> cells are used as reference. (D) Expression level of human CABLES1 in CB-CD34<sup>+</sup>, CD3<sup>+</sup>, CD14<sup>+</sup> and MSC by Western blot. Two different MSC samples are shown as MSC1 and MSC2 (See also **Figure S2**). (E) Immunolocalization of CABLES1 in CB-CD34<sup>+</sup> cells. Staining with the anti-CABLES1 antibody of human CD3<sup>+</sup> cells that do not express significant levels of *CABLES1* mRNA, is presented in the lower panel.

## **Figure 2: CABLES1 is an intrinsic cell cycle inhibitor in HPSC of young mice**

(A) Frequencies of AnnexinV<sup>+</sup> cells in BM LSK and SLAM populations. (B) Mitosox staining in BM LSK and SLAM cells of 12-week-old WT and *Cables1*<sup>-/-</sup> mice. Data are mean  $\pm$  SEM from six mice per group, pooled from two independent experiments. (C) Representative flow cytometric images of cell cycle analysis by KI67/DAPI costaining. (D, E) Frequencies of Ki-67<sup>+</sup>

cells in BM LSK and SLAM cells. **(F, G)** Frequencies of BrdU<sup>+</sup> cells in LSK and SLAM cells. **(A-F)**: Five mice were analyzed for each genotype. Data represent mean  $\pm$  SEM. **(H)** *Cables1* mRNA expression in LSK cells in the G0, G1 or S/G2/M phase. Each data represents a pool of 10 mice and is the mean  $\pm$  SEM of three independent experiments. **(I)** *Cables1*<sup>-/-</sup> mRNA expression in LSK cells cultured in cytokine containing medium (mouse Stem Cell Factor, IL3, Flt3L) for 24 hours; 0 H represents isolated LSK cells before culture. Data are mean  $\pm$  SEM of three independent experiments. **(J)** Frequencies of LSK cells in the G0 phase (Ki-67/DAPI) 24 hours after mice treatment with LPS (n=6). **(K)** *Cables1*<sup>-/-</sup> mRNA expression in LSK cells from LPS-treated mice. **(L)** Schematic representation of transplantation: *Cables1*<sup>-/-</sup> or WT BM donor cells were transplanted into lethally irradiated Ly5.1 mice (n=5). **(M)** Frequencies of Ki-67<sup>+</sup> LSK and SLAM cells three months after reconstitution (See also **Figure S3, E, F, G, H**). q-PCR are normalized to expression of HPRT. Data are mean  $\pm$  SEM, unpaired Student's two-tailed t-test was performed for statistical analysis. LPS, lipopolysaccharides.

**Figure 3: *Cables1*<sup>-/-</sup> HSC harbor higher reconstitution fitness.** **(A)** Schematic representation of competitive repopulation assays. **(B)** Contributions of donor cells (CD45.2<sup>+</sup>) in blood of recipient mice after transplantation of unfractionated BM cells. **(C)** Contributions of donor cells (CD45.2<sup>+</sup>) in blood of recipient mice after transplantation of purified BM LSK cells. **(D)** Frequencies of donor-derived B cells, **(E)** T cells and **(F)** granulocytes. **(G)** Frequencies of donor-derived CD45.2<sup>+</sup> LSK and SLAM cells in recipients' BM two months after reconstitution (n=5 per group). **(H)** Percentages of apoptotic donor-derived LSK and **(I)** SLAM cells. **(J)** Numbers of CFSE-labelled *Cables1*<sup>-/-</sup> or WT BM cells, evaluated 16 hours after homing to BM and spleen of lethally irradiated recipients (n=6). **(K)** CFU-C numbers per femur in the BM 4 hours after injection. All values are mean  $\pm$  SEM, unpaired Student's two-tailed t-test was performed for statistical analysis.

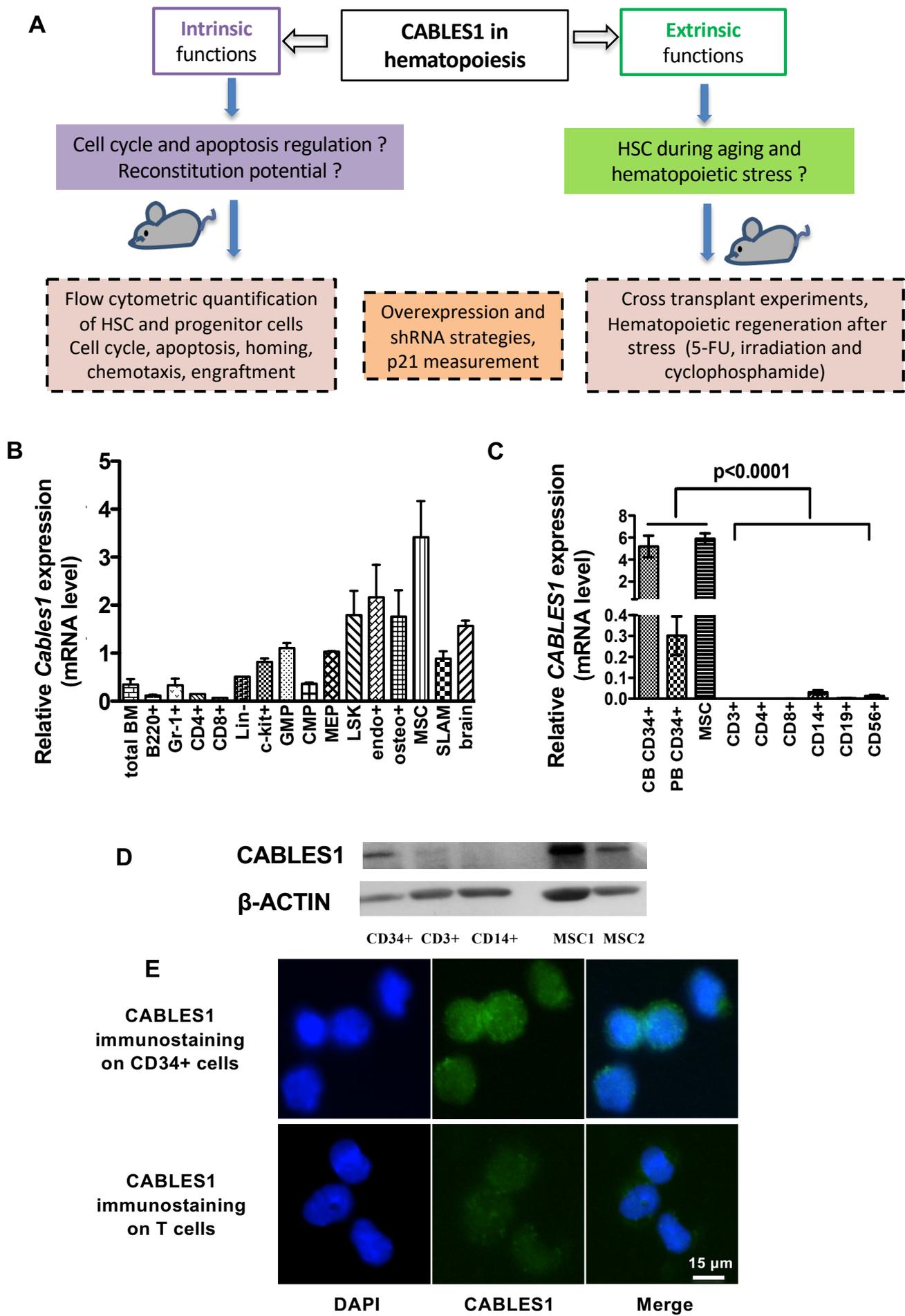
**Figure 4: CABLES1 slows cell growth of human CD34<sup>+</sup> cells and regulates p21 level.** (A) Lentiviral overexpression of human CABLES1 in CB CD34<sup>+</sup> cells (See also **Figure S5**). (B) 48 hours after transduction, GFP<sup>+</sup> CD34<sup>+</sup> cells were seeded in semisolid media for CFU-C determination. (C) Microscopic picture of colonies formed by control vector transduced CB-CD34<sup>+</sup> cells (left) and CABLES1-overexpressing CB-CD34<sup>+</sup> cells (right). (D) CABLES1 expression in *CABLES1* shRNA-transduced CB-CD34<sup>+</sup> cells compared to cells transduced with control shRNA (shC002) and to non-transduced cells (NT). (E) CABLES1 protein level after transduction of CB-CD34<sup>+</sup> cells with sh417758 compared to shC002. (F) Proliferation of CB-CD34<sup>+</sup> cells after transduction with sh417758 compared to shC002. Data represent the mean  $\pm$  SEM of three independent experiments. (G) *CABLES1* mRNA expression in cultured CB-CD34<sup>+</sup> cells. (H) Protein levels of CABLES1 and p21 in CB-CD34<sup>+</sup> cells 24 hours after culture. (I) Protein levels of CABLES1 and p21 in CB-CD34<sup>+</sup> cells overexpressing CABLES1. (J) Protein levels of CABLES1 and p21 in CB-CD34<sup>+</sup> cells transduced with *CABLES1* shRNAs. All values are expressed as mean  $\pm$  SEM, Student's unpaired two-tailed t-test was performed for statistical analysis. Experiments were performed twice. (K) Mean Fluorescence Intensity (MFI) of p21 in BM LSK and SLAM (L) cells of 12-week-old WT and *Cables1*<sup>-/-</sup> mice. Data are mean  $\pm$  SEM from six mice per group, pooled from two independent experiments.

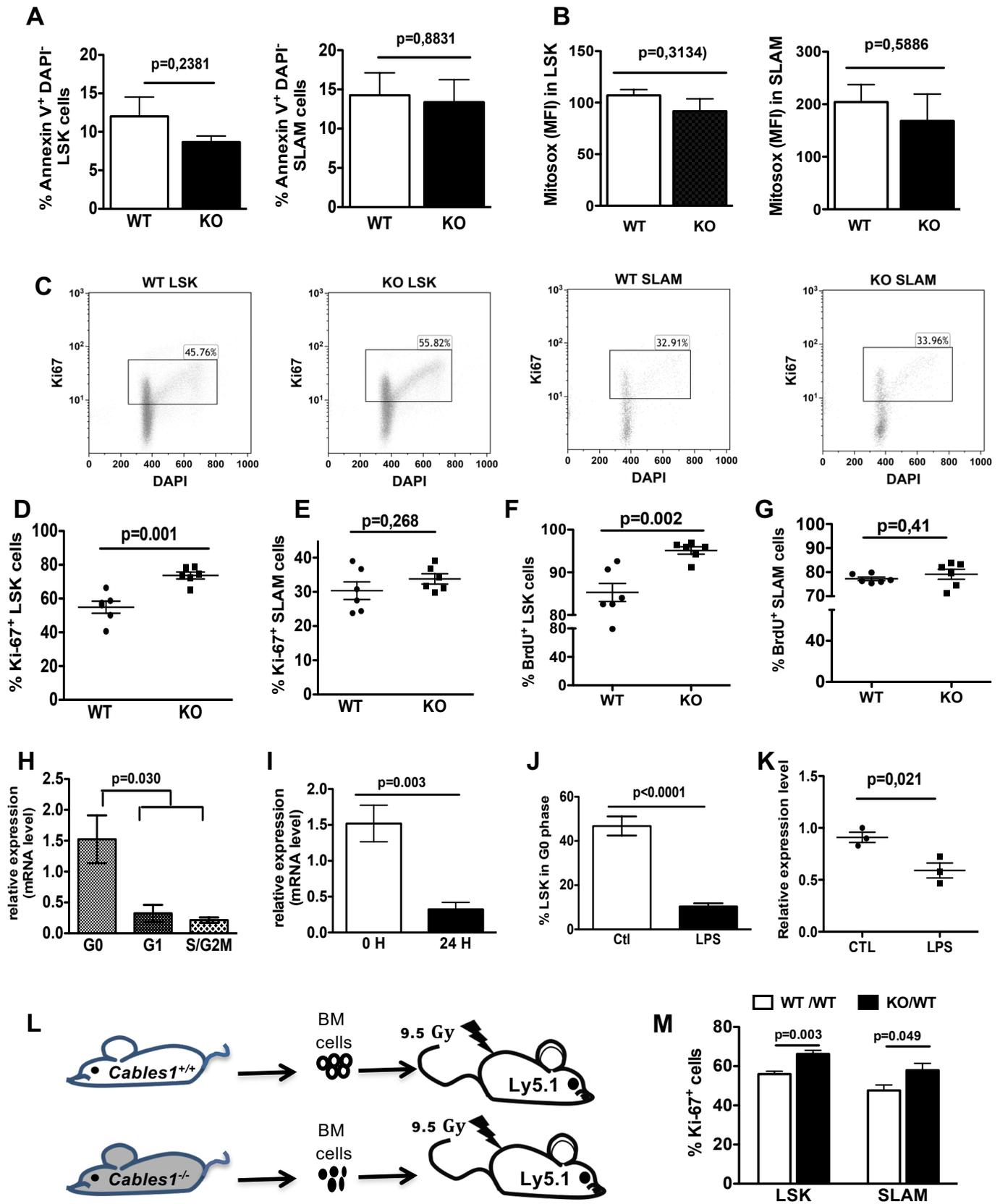
**Figure 5: Loss of *Cables1* delays hematopoietic regeneration after 5-FU treatment.** 3-month-old *Cables1*<sup>-/-</sup> and WT mice were treated with a single dose of 5-FU (250 mg/kg body weight). (A) WBC counts (3 to 5 mice for each time point). (B) BM cellularity of WT and *Cables1*<sup>-/-</sup> mice 10 days after 5-FU treatment. (C) Microscopic images of BM from WT and *Cables1*<sup>-/-</sup> mice 10 days after 5-FU treatment (See also **Figure S6**). (D) Total numbers of CFU-C in BM, (E) spleen and (F) blood of 5-FU treated WT and *Cables1*<sup>-/-</sup> mice. Values are mean  $\pm$  SEM, Student's

unpaired two-tailed t-test was performed for statistical analysis.

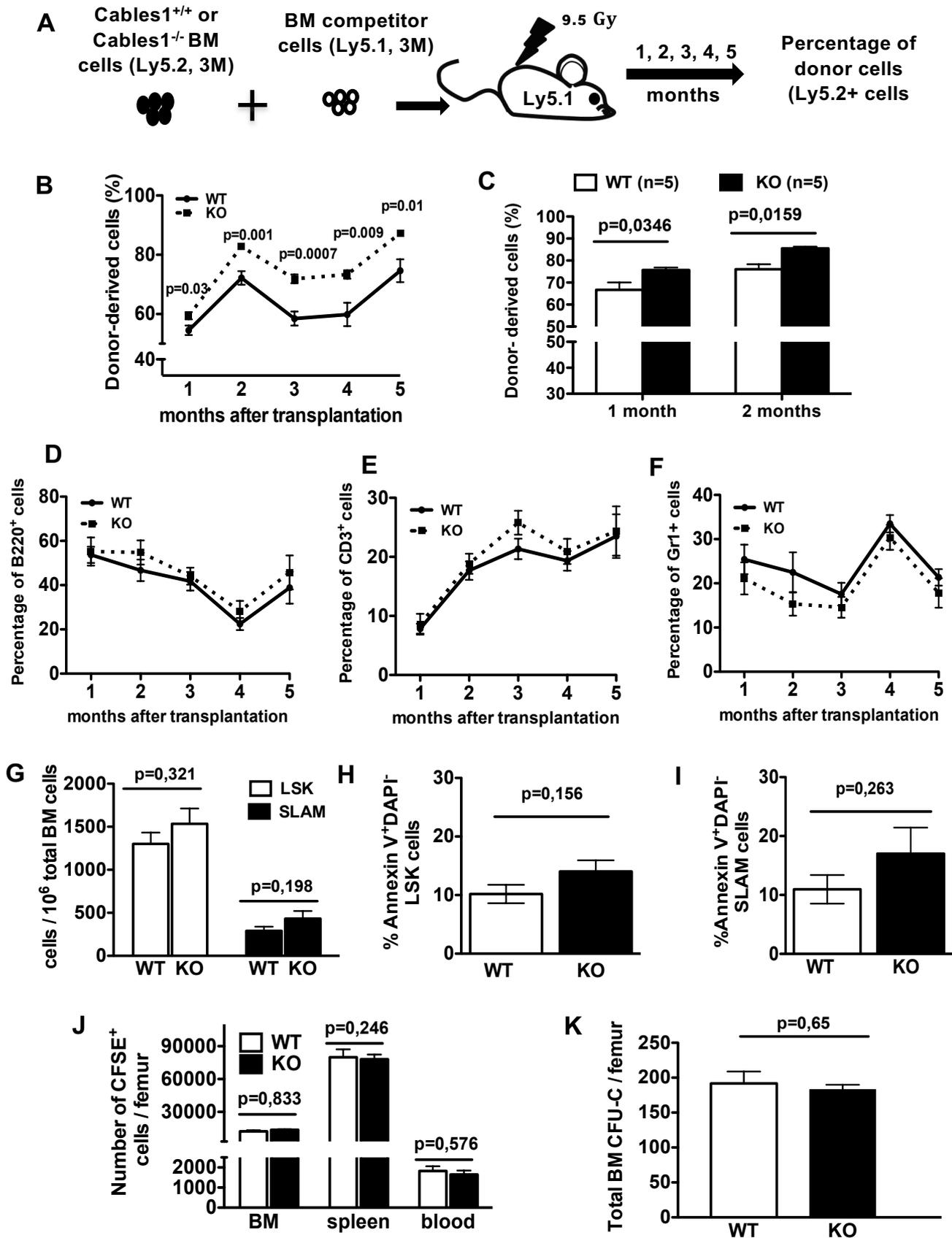
**Figure 6: Loss of *Cables1* is associated with defective hematopoiesis in aged mice.** (A) WBC counts in WT and *Cables1*<sup>-/-</sup> mice at different ages. (B) WBC subtypes (B220<sup>+</sup>, CD3<sup>+</sup>, Gr-1<sup>+</sup>) in 21-month-old (n=6) and (C) 8-month-old mice (n=5) (D) Total BM CFU-C numbers of 3-, 8- and 21-month-old *Cables1*<sup>-/-</sup> and WT mice (n=5-6). (E) Total numbers of LSK and (F) SLAM cells in 3-, 8- and 21-month-old *Cables1*<sup>-/-</sup> and WT mice (n=5-6) (See also **Figure S7**). (G, H) Frequencies of BrdU<sup>+</sup> LSK (G) and SLAM cells (H) in 8-month-old mice after 12 days of BrdU exposure (n=6). (I) Survival of 8-month-old mice treated weakly with 5-FU. (J) Percentages of myeloid-biased HSC (CD41<sup>+</sup>CD150<sup>+</sup>LSK) within the CD150<sup>+</sup>LSK compartment in *Cables1*<sup>-/-</sup> and WT mice (n=10). (K) Frequency of BM LSK/CD34<sup>+</sup>/CD135<sup>+</sup> cells in *Cables1*<sup>-/-</sup> and WT mice (n=10 for 8-month-old mice; n=5 for 21-month-old mice). (L) Frequency of CLP in BM of *Cables1*<sup>-/-</sup> and WT mice (n=5). (M, N) CFU-C numbers in blood and spleen of 3-, 8- and 21-month-old *Cables1*<sup>-/-</sup> and WT mice (n=6), (See also **Figures S5D, E, F**). Data are expressed as mean ± SEM. Log-rank (Mantel-Cox) test was applied for comparing survival between WT and *Cables1*<sup>-/-</sup> mice. Student's unpaired two-tailed t-test was performed for the other experiments.

**Figure 7: Environmental defects are involved in abnormal hematopoiesis of aged *Cables1*<sup>-/-</sup> mice.** (A) Schematic representation of BM transplantation. (B) Frequencies of donor-derived B cells (B220<sup>+</sup>) and (C) myeloid cells (Gr-1<sup>+</sup>) four months after reconstitution (n=8). (D) Survival of chimeric mice after weekly treatment with 5-FU. Log-rank (Mantel-Cox) test was applied for comparing survival between the different groups. (E) BM CFU-F frequency of 3- (F) 8- and (G) 21-month-old WT and *Cables1*<sup>-/-</sup> mice. Student's unpaired two-tailed t-test was performed for statistical analysis. (H) Illustrative diagram depicting the main identified roles for CABLES1.

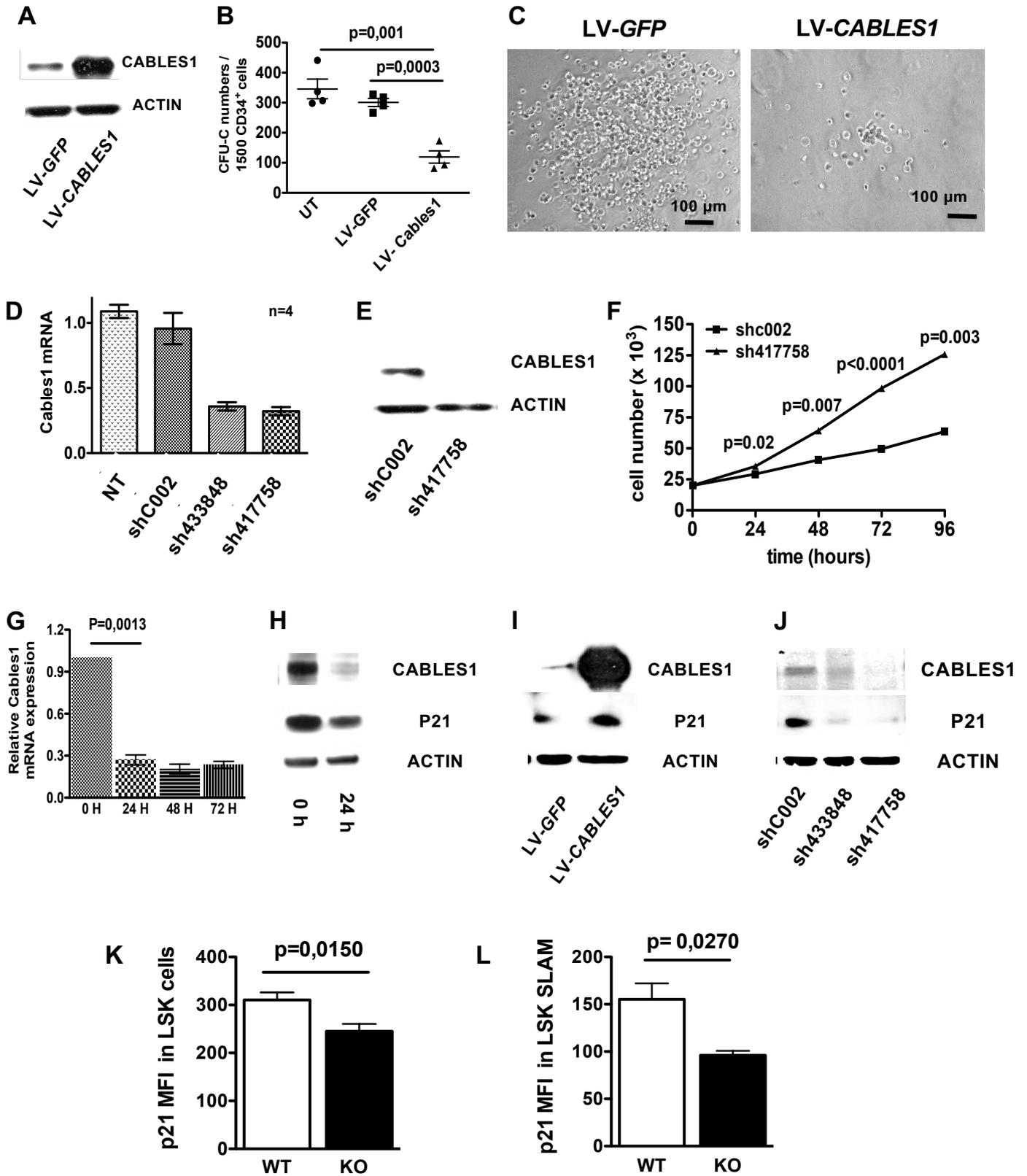




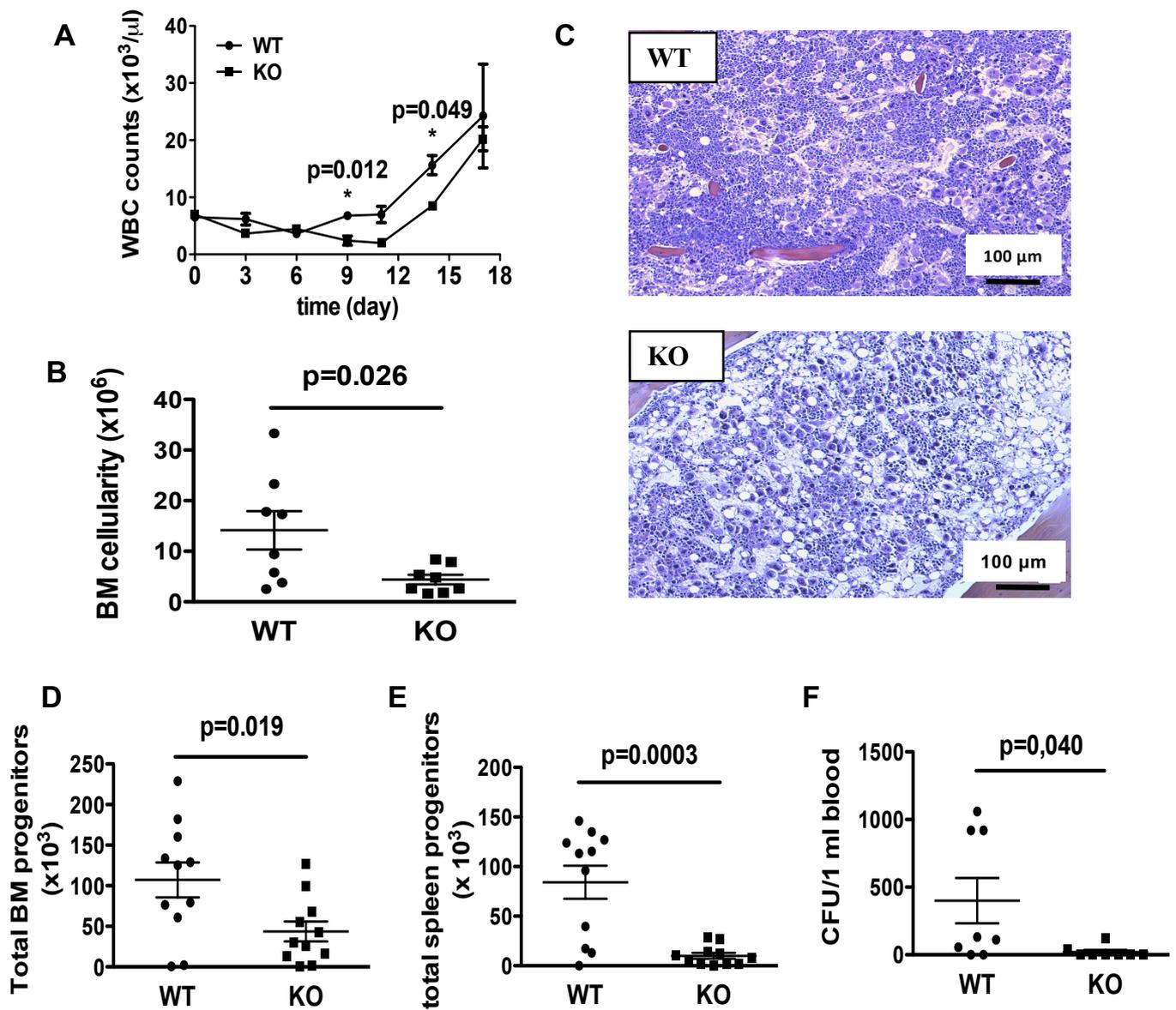
**Figure 2 : CABLES1 is an intrinsic cell cycle inhibitor in mouse HPSCs**



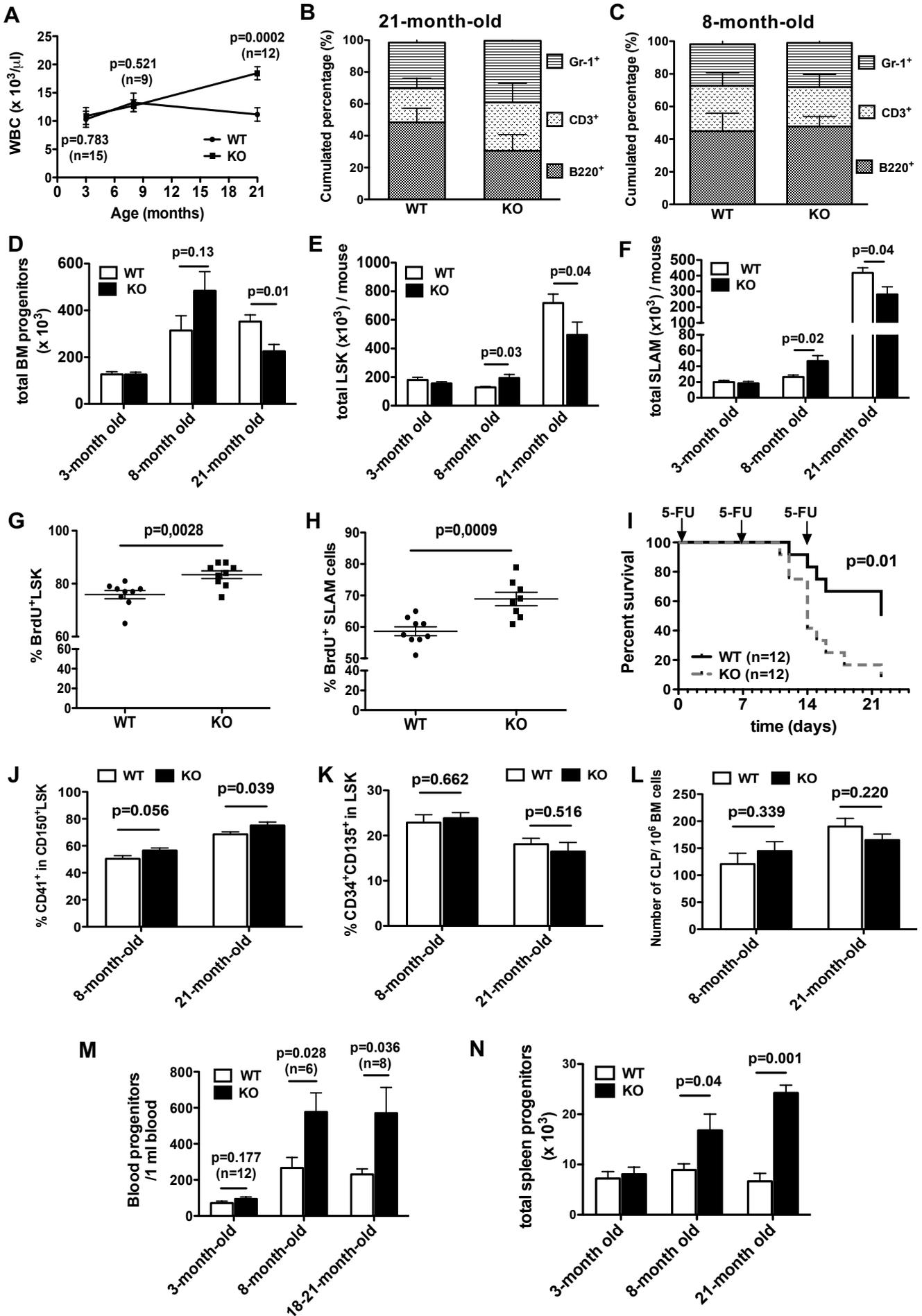
**Figure 3 : Cables1<sup>-/-</sup> HSCs harbor higher reconstitution fitness**



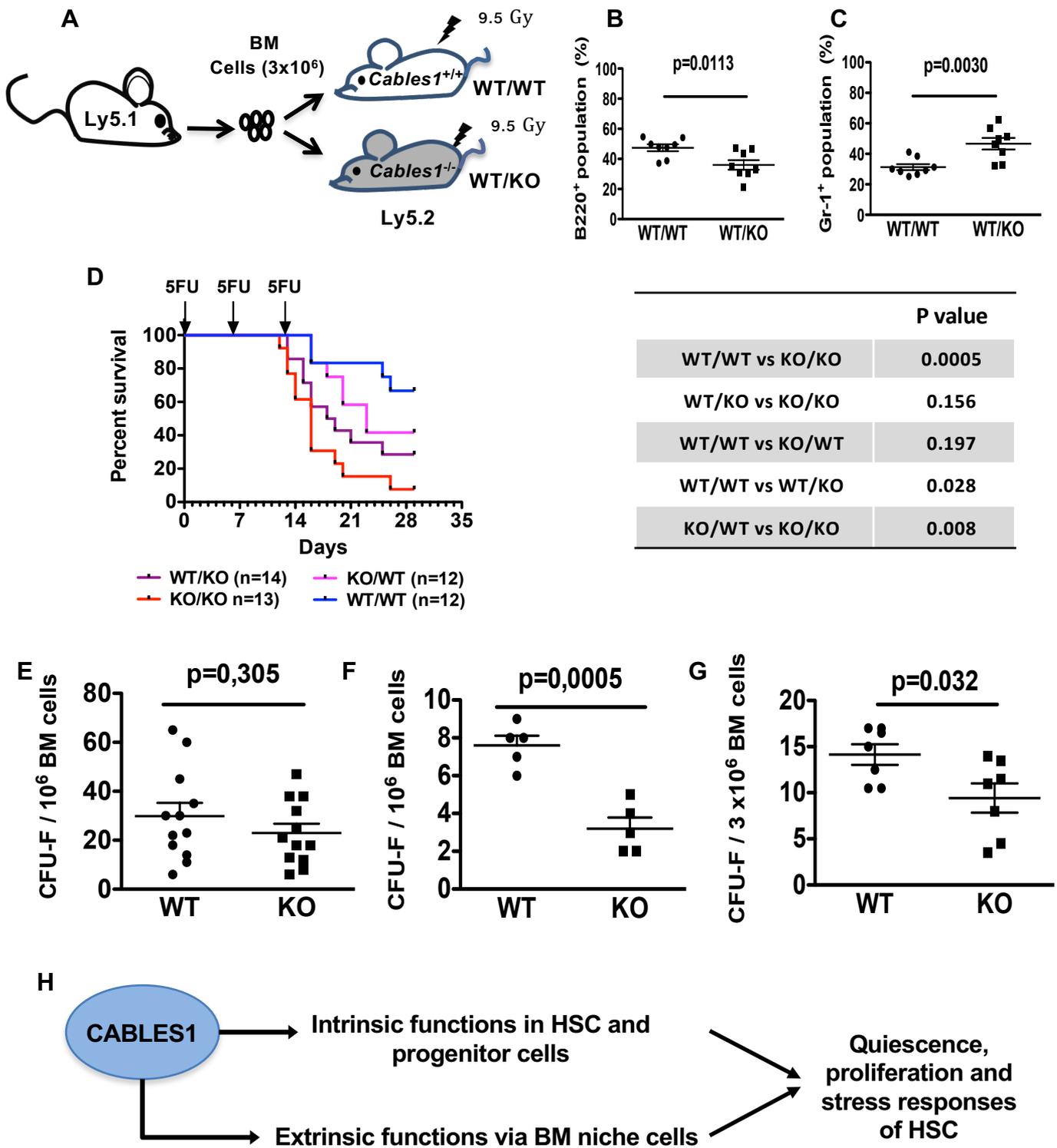
**Figure 4 : CABLES1 slows cell growth of human CD34<sup>+</sup> cells and regulates P21 level**



**Figure 5 : Loss of Cables1 delays hematopoietic regeneration after 5-FU treatment**



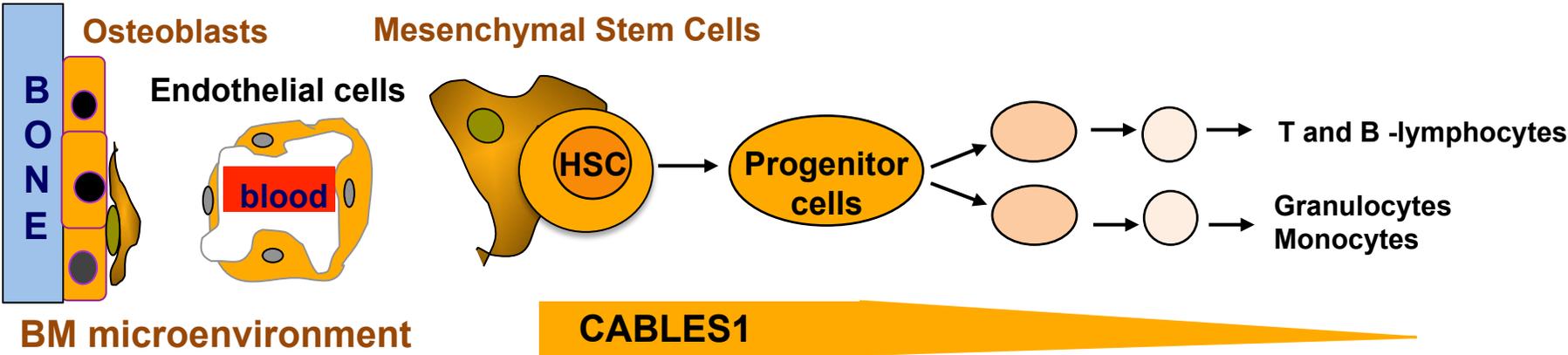
**Figure 6: Altered hematopoiesis, loss of quiescence and increased mobilization of HSC in aged *Cables1*<sup>-/-</sup> mice**



**Figure 7 : Intrinsic and extrinsic mechanisms in abnormal hematopoiesis of  $Cables1^{-/-}$  mice during aging**

# Graphical abstract

1-CABLES1 is expressed in immature hematopoietic progenitor and niche cells



2-CABLES1 has intrinsic and extrinsic functions in hematopoiesis

