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The Microtubule Plus-End Tracking Protein CLASP2 Is Required for Hematopoiesis and Hematopoietic Stem Cell Maintenance

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SUMMARY

Mammalian CLASPs are microtubule plus-end tracking proteins whose essential function as regulators of microtubule behavior has been studied mainly in cultured cells. We show here that absence of murine CLASP2 in vivo results in thrombocytopenia, progressive anemia, and pancytopenia, due to defects in megakaryopoiesis, in erythropoiesis, and in the maintenance of hematopoietic stem cell activity. Furthermore, microtubule stability and organization are affected upon attachment of *Clasp2* knockout hematopoietic stem-cell-enriched populations, and these cells do not home efficiently toward their bone marrow niche. Strikingly, CLASP2-deficient hematopoietic stem cells contain severely reduced mRNA levels of *c-Mpl*, which encodes the thrombopoietin receptor, an essential factor for megakaryopoiesis and hematopoietic stem cell maintenance. Our data suggest that thrombopoietin signaling is impaired in *Clasp2* knockout mice. We propose that the CLASP2-mediated stabilization of microtubules is required for proper attachment, homing, and maintenance of hematopoietic stem cells and that this is necessary to sustain *c-Mpl* transcription.

INTRODUCTION

Cells of the blood are generated in a complex process called hematopoiesis. Production begins with hematopoietic stem cells (HSCs), which are present in a very limited number in the adult bone marrow. HSC maintenance is achieved by self-renewal, whereas HSC differentiation eventually generates cells of all the blood lineages. Attachment, migration, and (a)symmetric cell division are important for the maintenance, proliferation, and differentiation of all hematopoietic cells, including HSCs. These processes are regulated by the microtubule (MT) cytoskeleton. MTs are dynamic polymers assembled from heterodimers of α- and β-tubulin. MTs perform many of their cellular tasks by changing their organization and stability in response to the needs of the cell. This process is largely controlled by MT-associated proteins. Some of these factors are localized specifically at the ends of growing MTs and are called "plus-end tracking proteins," or +TIPs (for review, see Galjart, 2010).

Mammalian CLASP1 and CLASP2 are +TIPs that can stabilize MTs in specific regions of the cell, thereby regulating both mitosis and interphase. In motile fibroblasts, for example, CLASP2 acts downstream of PI3K and GSK-3β to enhance MT stabilization at the leading edge and support directed motility (Akhmanova et al., 2001; Drabek et al., 2006). The C terminus of CLASP2 is required for association with the cell cortex, through LL5β and ELKS (Lansbergen et al., 2006), proteins that might form a PI3K-regulated cortical platform to which CLASPs attach distal MT ends. Cortex localization and clustering of LL5β

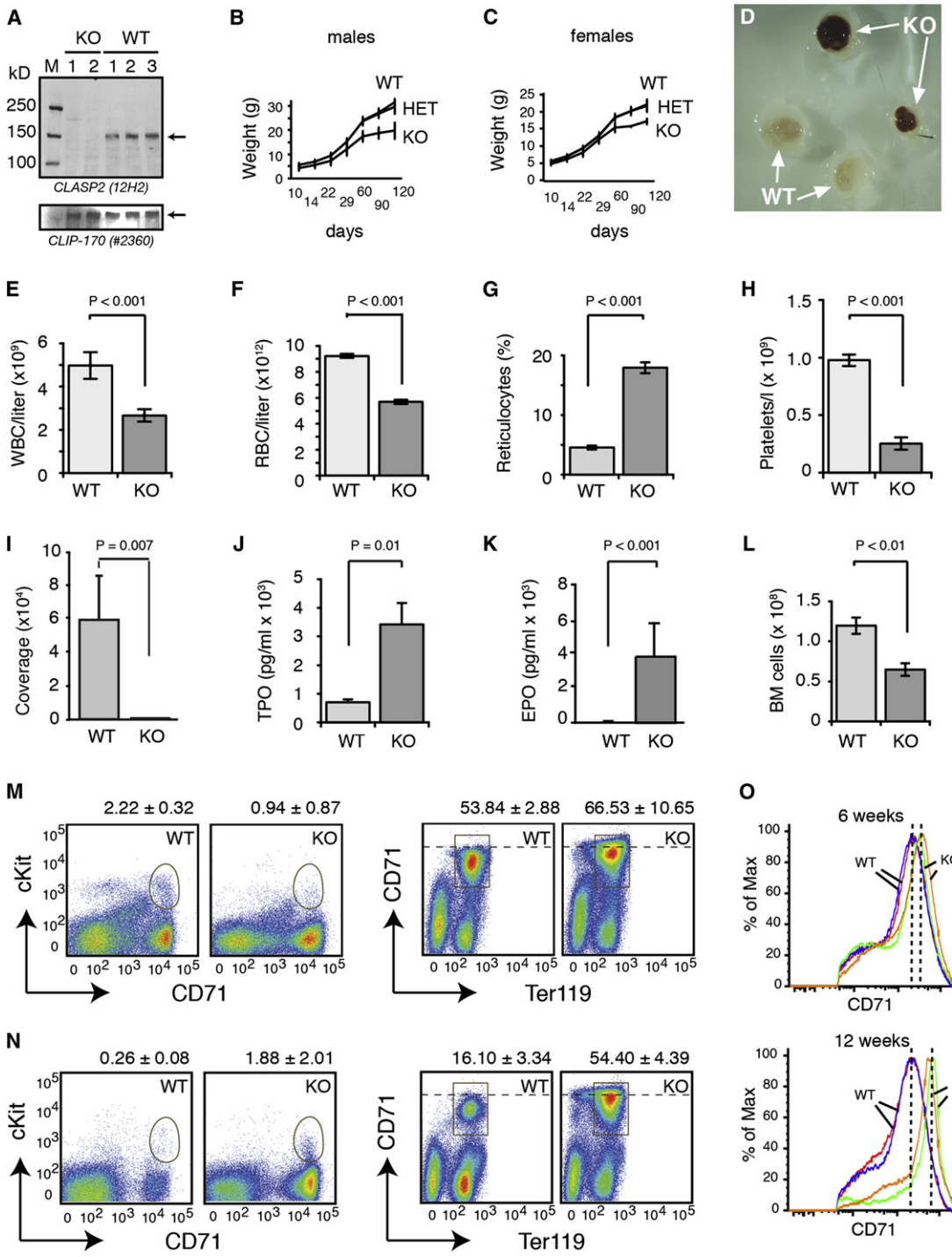

Figure 1. Hemorrhages, Pancytopenia, and Erythroid Defects in Clasp2 KO Mice

(A) Western blot analysis. Protein extracts from the bone marrow of WT and *Clasp2* KO mice were analyzed on western blot using anti-CLASP2 (12H2) and anti-CLIP-170 (#2360) antisera.

(B and C) Growth curves. Body weights of WT, heterozygous (HET), and homozygous *Clasp2* KO mice are shown at different time points. Groups consisted of 5 KO, 10 HET, and 9 WT males (B) and 4 KO, 14 HET, and 9 WT females (C).

(D) Hemorrhages in *Clasp2* KO mice. Ovaries from WT and *Clasp2* KO mice were isolated. In both KO ovaries, severe hemorrhages are present.

(E and F) WBC and RBC counts. Blood was isolated from 12 WT and 16 *Clasp2* KO mice.

(G) Reticulocyte counts. Blood from three WT and three *Clasp2* KO mice was deposited on glass slides. Approximately 2,000 RBCs were counted per mouse, and the percentage of reticulocytes was measured.

in epithelial cells are regulated by integrins and the cell-adhesion molecule laminin (Hotta et al., 2010), providing a link among cell adhesion, integrin signaling, CLASPs, and MTs.

To examine the in vivo role of CLASP2, we generated *Clasp2* knockout (KO) mice. These mice display multiple defects, including problems in megakaryopoiesis, erythropoiesis, and the maintenance of the HSC bone marrow pool. We show that the MT network is affected in CLASP2-deficient HSC-enriched bone marrow cells and that these cells do not home efficiently toward their niche. Interestingly, expression of *c-Mpl*, which encodes the thrombopoietin (TPO) receptor (Kaushansky, 2009), is reduced in CLASP2-deficient HSCs. Our data reveal a link between the mechanical action of CLASP2 at MT ends and the expression of a hematopoietic factor that is important for megakaryocyte development and HSC maintenance.

RESULTS

Pancytopenia in Clasp2 KO Mice

We disrupted the murine *Clasp2* gene (Figures S1A and S1B) and showed abrogation of CLASP2 expression (Figures 1A and S1). Matings between heterozygous *Clasp2* KO mice yielded less homozygous offspring than expected (29% wild-type [WT], 50% heterozygous, and 22% homozygous *Clasp2* KOs in 729 mice counted). The body weight of homozygous *Clasp2* KO mice was approximately 30% lower than that of WT and heterozygous littermates (Figures 1B and 1C). Patho-anatomic examination of adult *Clasp2* KO mice revealed severe anomalies in male and female reproductive organs but no gross alterations in other tissues (data not shown). These results indicate that CLASP2 plays a role in germ cell development as well as in multiple somatic tissues.

We observed hemorrhages in different *Clasp2* KO organs, including the reproductive system (in 50% of the animals; Figure 1D) and stomach (in 67% of the mice). Petechiae, small red spots caused by a minor hemorrhage, were found in the brain, bladder, and lymph nodes (in 20%, 17%, and 11% of the KOs, respectively). Furthermore, white blood cell (WBC) and red blood cell (RBC) numbers were reduced in *Clasp2* KO blood (Figures 1E and 1F), as were hematocrit and hemoglobin levels (data not shown). By contrast, we detected more reticulocytes in *Clasp2* KOs (Figure 1G), indicating that immature RBCs are extruded into the blood to compensate for the erythrocyte deficiency.

Platelets were also reduced in *Clasp2* KOs (Figure 1H). Because a reduction in platelet number is by itself not sufficient to explain a bleeding phenotype, we analyzed whether defects in the endothelium that lines the blood vessels could explain the internal bleedings, but we detected no obvious endothelial damage in hematoxylin/eosin-stained sections of *Clasp2* KO mice (data not shown). Because platelets need to adhere in order to form a clot, we examined platelet adhesion to collagen under physiological shear rate. Strikingly, adhesion was almost completely inhibited in CLASP2-deficient platelets (Figure 1I), indicating that this underlies the hemorrhages in *Clasp2* KO mice. Taken together, our data reveal a severe pancytopenia in *Clasp2* KO mice.

TPO is a major regulator of platelet homeostasis in vivo (Kaushansky, 2009), whereas erythropoietin (EPO) controls the levels of RBCs (Richmond et al., 2005). TPO values were about four times higher, and EPO levels were even more increased in *Clasp2* KO mice compared to WT littermates (Figures 1J and 1K, respectively). These results indicate that the thrombocytopenia and anemia are sensed by the *Clasp2* KO mice; however, despite elevated TPO and EPO levels, platelet and RBC numbers are not restored to normal values. Furthermore, in addition to reduced WBC, RBC, and platelet numbers in the blood, we also observed fewer nucleated cells in the bone marrow of *Clasp2* KO mice (Figure 1L). We therefore investigated the various hematopoietic lineages in *Clasp2* KO mice in more detail.

CLASP2 Is Required for Erythropoiesis

During erythropoiesis, committed erythroid progenitors (burst-forming unit erythroid [BFU-E] and colony-forming unit erythroid [CFU-E]) differentiate toward the erythroblast stage, which develops further into enucleated reticulocytes and, finally, into mature erythrocytes, or RBCs. In case of anemia, erythroid progenitors relocate to the spleen and commence stress erythropoiesis in order to increase RBC output in the blood. As a result, the spleen increases in size. Clonogenic culture assays showed reduction of bone marrow-derived BFU-E and CFU-E colonies in the absence of CLASP2 (Figure S1F). By contrast, in the spleen, a slight increase in the number of these colonies was observed (Figure S1G), indicative of stress erythropoiesis.

Further analysis of the erythroid system revealed a significant decrease in early c-kit$^+$CD71$^+$ erythroblasts in the *Clasp2* KO bone marrow, whereas the amount of later-stage CD71$^+$ TER119$^+$ erythroblasts was similar to WT (Figure 1M). By contrast, in the *Clasp2* KO spleens, both early- and late-stage erythroblasts were increased (Figure 1N). Taken together, our data suggest that a CLASP2 deficiency limits erythroid

(H) Platelet counts. Blood was isolated from 18 WT and 22 *Clasp2* KO mice, and platelets were counted.
(I) Platelet adhesion to collagen under physiological shear rate. Whole blood from WT and *Clasp2* KO mice was perfused over collagen-coated slides. Adhered platelets were visualized with CD41-PE- or CD61-FITC-conjugated antibodies, and immunofluorescence coverage of slides was quantified.
(J and K) Cytokine production. TPO (J) and EPO (K) levels were determined in the serum of WT and *Clasp2* KO mice. TPO levels were measured in 12 WT and 10 KO mice; EPO levels were measured in 6 WT and 6 KO mice (SD indicated).
(L) Analysis of bone marrow. Nucleated cells were counted in bone marrow (BM) from seven WT and seven *Clasp2* KO mice. In (A)–(L), SEM is shown (unless stated otherwise) together with t test results.
(M and N) Erythroid differentiation. Early erythroid progenitors were identified by flow cytometry using c-kit and CD71 in bone marrow (M) and spleen (N) from WT and *Clasp2* KO mice. Single-flow cytometry plots are shown with the area outlined used to calculate c-kit$^+$/CD71$^+$ cells. Percentages (n = 6 in all groups, SD indicated) of cells are indicated above the plots.
(O) Age-dependent increase in CD71 expression. Cells of four WT and four *Clasp2* KO mice, of 6 and 12 weeks, were gated on the basis of the erythroid marker CD71. Vertical striped lines show approximate CD71 peak positions. % of Max, percentage of maximum.
See also Figure S1.

differentiation in the bone marrow. This induces stress erythropoiesis in the spleen, which is consistent with an increased spleen/body weight ratio in *Clasp2* KO mice (Figure S1E). Stress erythropoiesis is, however, not sufficient to compensate for the RBC loss in the blood (Figure 1F), which explains the highly increased EPO levels (Figure 1K).

Flow cytometry analysis showed increased amounts of CD71, the transferrin receptor, on the cell surface of CLASP2-deficient erythroblasts (Figures 1M–1O). Strikingly, CD71 expression was higher in 12-week-old than in 6-week-old *Clasp2* KO mice (Figure 1O). Because CD71 is a target of EPO (Sivertsen et al., 2006), the age-dependent increase in CD71 expression in *Clasp2* KO mice indicates that EPO levels also increase with age. EPO levels, in turn, correlate with severity of anemia. We therefore propose that lack of CLASP2 results in progressive anemia.

CLASP2 Is Required for Megakaryopoiesis

Histological sections suggested a deficit of megakaryocytes, the cells that give rise to platelets, in *Clasp2* KO bone marrow (Figures 2A and 2B). Flow cytometry analysis suggested impairment in all stages of megakaryopoiesis (Figures 2C and S2A). Megakaryocytes acquire high DNA ploidy levels by endomitosis, allowing them to grow very large and form many platelets from a single megakaryocyte. DNA staining indicated that *Clasp2* KO megakaryocytes skew toward a lower ploidy status (Figure 2D). Analysis in fetal liver-derived megakaryocyte cultures showed that *Clasp2* KO megakaryocytes were smaller than WT (Figures 2E and 2F). In addition, in bone marrow-derived cultures, 10-fold fewer megakaryocytes developed (Figure 2G), and the fraction of KO megakaryocytes with >8N DNA content was lower (Figure 2H). Furthermore, we observed a decrease in *Clasp2* KO megakaryocyte progenitor cells (colony-forming unit megakaryocyte [CFU-MK]) in clonogenic assays (Figure 2I). Thus, CLASP2 deficiency affects megakaryocytes at the level of progenitors and at later stages. The block in maturation leads to fewer and smaller megakaryocytes with reduced DNA content. Absence of CLASP2 did not appear to impede (pro)platelet formation (Figures S2B–S2E), indicating that CLASP2 is not involved in the final stages of megakaryopoiesis. Platelet activation upon thrombin stimulation was also not affected by the lack of CLASP2 (Figures S2F–S2M),

Because endomitosis requires the MT-based movement of chromosomes, we asked if CLASP2 localizes to the mitotic spindle machinery. Immunofluorescence analysis showed that in WT megakaryocytes from 6 day cultures, CLASP2 colocalizes with the CREST kinetochore marker (Figures 2J–2M). Thus, CLASP2 might have a role in endomitosis as a kinetochore protein. Although this is a novel finding, it is consistent with a role for CLASP2 at kinetochores in fibroblast mitosis (Pereira et al., 2006).

CLASP2 Is Required for HSC Maintenance and Homing

Flow cytometry analysis in HSC-enriched and early progenitor populations revealed a reduction in all fractions in *Clasp2* KO mice (Figures 3A and S3A–S3C), including $Lin^-/Kit^+/Sca1^+$ (LSK) cells, which contain both long- and short-term HSCs, and $CD48^-/CD150^+$ LSK cells, which are further enriched for long-term HSCs (Kiel et al., 2005). Thus, a deficiency of CLASP2 affects HSCs and early progenitors.

To examine whether the stem cell defect in *Clasp2* KO mice is due to malfunctioning HSCs themselves or to an abnormal stromal environment, we performed transplantation assays. We injected bone marrow cells derived from *Clasp2* KO mice and WT littermates into sublethally irradiated recipients and examined donor HSC progeny in the blood of recipient mice after short (i.e., 1 month) and long-term (i.e., 4 months) reconstitution. Bone marrow cells from the *Clasp2* KOs failed to fully reconstitute irradiated recipient mice even with the highest dose of cells injected (Figure 3B). By contrast, bone marrow cells from WT donors reconstituted efficiently, even when few cells were injected (Figure 3B). These data reveal a cell-autonomous HSC defect in *Clasp2* KO mice.

To examine whether CLASP2 plays a role in HSC homing in vivo, we isolated bone marrow cells from WT and *Clasp2* KO mice, loaded these with the fluorescent compound CFSE, and injected cells into sublethally irradiated recipients. Fifteen hours after injection, we examined the number of $CFSE^+$ LSK cells in the bone marrow of the recipient mice and compared this value to the number of original $CFSE^+$ LSK cells injected. The efficiency with which CLASP2-deficient LSK cells homed to their niche in recipient bone marrow was approximately half of that of WT cells (Figure 3C). We conclude that, akin to its role in persistent motility in fibroblasts (Drabek et al., 2006), CLASP2 is involved in the homing of LSK cells in vivo.

CLASP2 Deficiency Affects c-Mpl Transcription

To investigate whether the hematopoietic phenotype in CLASP2-deficient mice might be explained by a differential expression of CLASP1 and CLASP2, we examined *Clasp1* and *Clasp2* mRNA levels in different bone marrow fractions by RT-PCR. This revealed that *Clasps* are expressed at similar levels throughout the hematopoietic system (Figure S3D). Thus, CLASP1 and CLASP2 are not redundant in vivo.

We next investigated at a genome-wide level whether lack of CLASP2 affects distinct genes and/or signaling cascades. Next-generation sequencing of LSK-derived mRNA showed that many mRNAs, including *Actin*, *Clasp1*, and *Slam1* (CD150), were not affected by a lack of CLASP2 (Figures 3D and S3F; Table S1). However, the levels of *Meis1*, which encodes an important transcription factor for megakaryopoiesis and HSC survival (Hisa et al., 2004; Simsek et al., 2010), and of *c-Mpl*, which encodes the TPO receptor (Kaushansky, 2009), were severely reduced in the absence of CLASP2 (Figures 3D and S3F). Real-time PCR confirmed the reduction in *Meis1* (Figure S3E).

One reason for the reduced expression of *Meis1* and *c-Mpl* in LSK cells could be that long-term HSCs are underrepresented in the *Clasp2* KO. We therefore also sequenced $CD150^+$ LSK fractions, which are highly enriched for HSCs (Kiel et al., 2005). Whereas *Meis1* was only moderately affected in *Clasp2* KO $CD150^+$ LSK cells, *c-Mpl* expression was still severely decreased (Figure 3E). Moreover, the expression of *Prdm16*, an HSC marker (Forsberg et al., 2010) and target of c-Mpl in HSCs (Heckl et al., 2011), was also downregulated (Figure 3E). These results suggest that absence of CLASP2 leads to aberrant *c-Mpl* transcription and c-Mpl signaling.

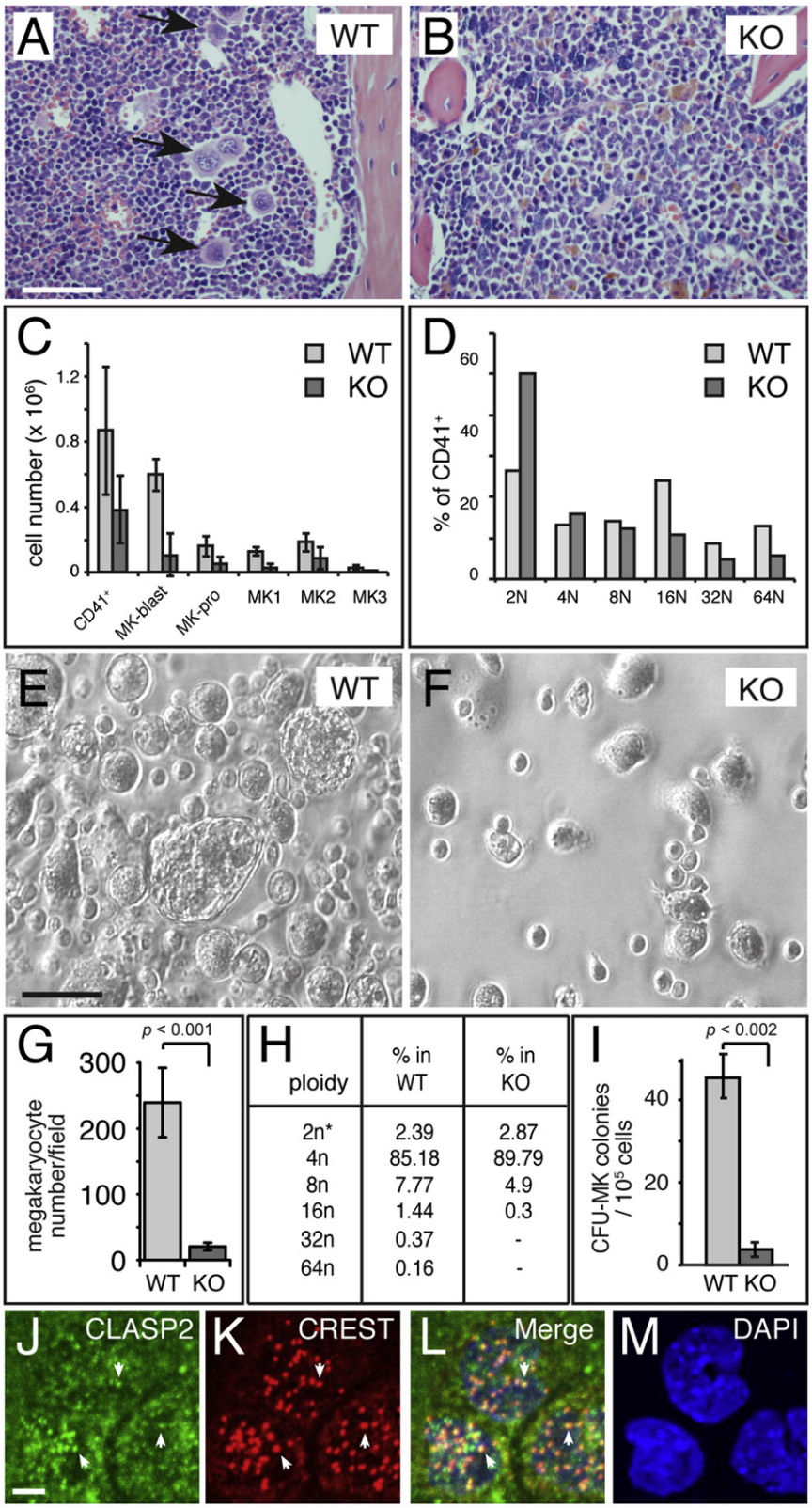

Figure 2. Megakaryocyte Defects in Clasp2 KO Mice

(A and B) Hematoxylin and eosin-stained sections of the bone marrow. (A) WT and (B) *Clasp2* KO bone marrow is shown. Megakaryocytes are indicated with arrows. Scale bar, 100 μm.

(C) Megakaryocyte differentiation in the bone marrow. Consecutive stages in megakaryocyte differentiation were analyzed by flow cytometry. MK-blast, megakaryoblast; MK-pro, megakaryocyte progenitor; MK1, MK2, and MK3, early and mature megakaryocytes. SD is indicated.

(D) DNA content in bone marrow megakaryocytes. Ploidy levels measured by flow cytometry in two WT and two *Clasp2* KO mice and expressed as percentages of total number of $CD41^+$ cells are presented. Average values are shown.

(E–H) Liquid megakaryocyte cultures. Phase-contrast images (E and F), megakaryocyte counts (G), and DNA content analysis (H) of fetal liver (E and F) and adult (G and H) megakaryocyte cultures from WT and *Clasp2* KO are presented. (G) SEM is indicated; t test reveals significant difference. (H) Ploidy levels in five WT and seven KO cultures expressed as percentages of total number of observed cells are demonstrated. Scale bar, 50 μm.

(I) CFU-MK progenitor assay. Number of colonies (per 50,000 cells seeded) counted after 10 days in culture is shown (SD is indicated; t test reveals significant difference).

(J–M) Localization of CLASP2 in megakaryocytes. WT cells were stained with CLASP2 antibodies (green), the kinetochore marker CREST6 (red), and DAPI (blue). Kinetochore association of CLASP2 is indicated by arrows. Scale bar, 3 μm.

See also Figure S2.

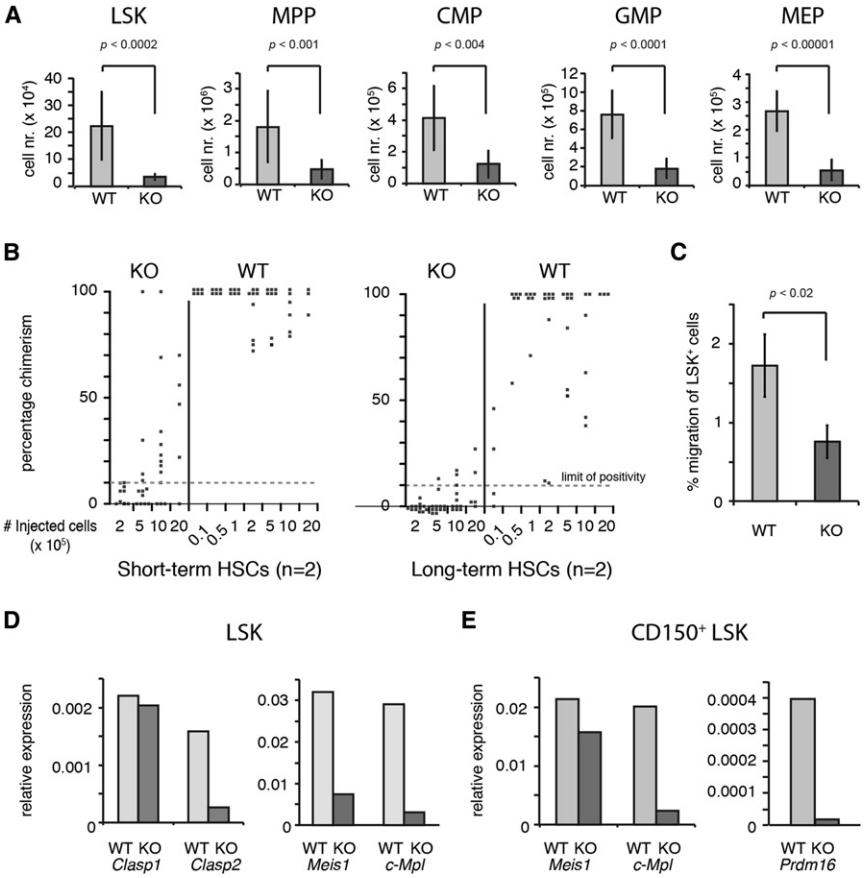

Figure 3. HSC and Progenitor Defects in Clasp2 KO Mice

(A) Analysis of HSC-enriched (LSK) and hematopoietic progenitor (MPP, CMP, GMP, MEP) cells from WT and *Clasp2* KO bone marrow is shown (see Figures S3A and S3B for flow cytometry examples). SD is indicated; t tests reveal significant differences. cell nr., cell number.

(B) In vivo hematopoietic repopulation analysis. Irradiated recipient mice were injected with bone marrow cells from adult *Clasp2* KO or WT mice. Analyses were performed at 1 month (short term) and 4 months (long term) post-transplantation (n = 2). Percentage of chimerism is indicated, with each dot representing a transplanted mouse. The number of cells injected per recipient is indicated below the graph. Mice showing greater than 10% donor chimerism (limit of positivity) in peripheral blood are considered as reconstituted.

(C) In vivo homing assay. CFSE-loaded bone marrow cells from two WT and two *Clasp2* KO mice were intravenously injected into the tails of irradiated recipient mice. $CFSE^+$ LSK numbers in WT and KO donor and recipient mice, at the onset and 15 hr after injection, respectively, were calculated (SD is indicated; t test reveals significant difference).

(D and E) Next-generation sequencing of RNA from WT and *Clasp2* KO LSK (D) or $CD150^+$ LSK (E) populations is shown. Expression of indicated genes is relative to *Actin*.

See also Figure S3 and Table S1.

CLASP2 Organizes the MT Network upon Cell Attachment

CLASP2 selectively regulates MT stabilization in motile fibroblasts (Akhmanova et al., 2001). Because fibroblast adherence also results in selective MT stabilization (Palazzo et al., 2004), we asked whether CLASP2 is involved in this process too. Indeed, CLASP2-deficient fibroblasts plated on a fibronectin-coated matrix contained less stable MTs compared to WT cells (Figure S4).

Next, we isolated WT and CLASP2-deficient HSC-enriched (LSK) cells, allowed them to attach, and examined the MT network and associated organelles using specific antibodies. EB1 staining revealed ample-growing MTs, which originated at the centrosome and circumnavigated the nucleus (Figures 4A–4C). No obvious difference was observed in the number of EB1 dots in WT and CLASP2-deficient HSC-enriched cells. Examination of the MT network using anti-α-tubulin antibodies revealed a cage-like organization in most of the WT cells, with MTs originating at the centrosome and surrounding the nucleus (Figures 4D and 4E). The Golgi network, as observed with anti-GM130 antibodies, was clustered around the centrosome; its organization was similar in WT and *Clasp2* KO HSC-enriched cells (Figures 4F and 4G).

In contrast to the Golgi apparatus, the distribution of stable MTs was significantly different in CLASP2-deficient HSC-enriched cells compared to WT cells (Figures 4H–4L). MT organization was also affected, i.e., approximately half of the CLASP2-deficient HSC-enriched cells contained a cage-like MT organization compared to WT cells (Figure 4M). Combined, our data suggest that CLASP2 is required for cell attachment and the subsequent stabilization of the MT network, as well as its organization, both in freshly isolated HSC-enriched cells as well as in cultured MEFs.

DISCUSSION

We show here that CLASP2 is essential for mouse hematopoiesis, acting at multiple steps in erythroid, megakaryocytic, lymphoid, and myeloid differentiation. Shortages in RBCs and platelets in *Clasp2* KO mice lead to increased EPO and TPO levels and induce a stress response. However, these reactions are insufficient, and thus, an enhanced replenishment by HSCs is required. Because CLASP2 is also necessary, in a cell-autonomous manner, for HSC maintenance, the stem cell pool becomes exhausted in *Clasp2* KO mice, explaining the progressive pancytopenia. This phenotype resembles that of patients with congenital amegakaryocytic thrombocytopenia, which starts as a severe thrombocytopenia at birth and then rapidly progresses to pancytopenia (Ballmaier and Germeshausen, 2009). Most patients carry a mutation in the *c-Mpl* gene.

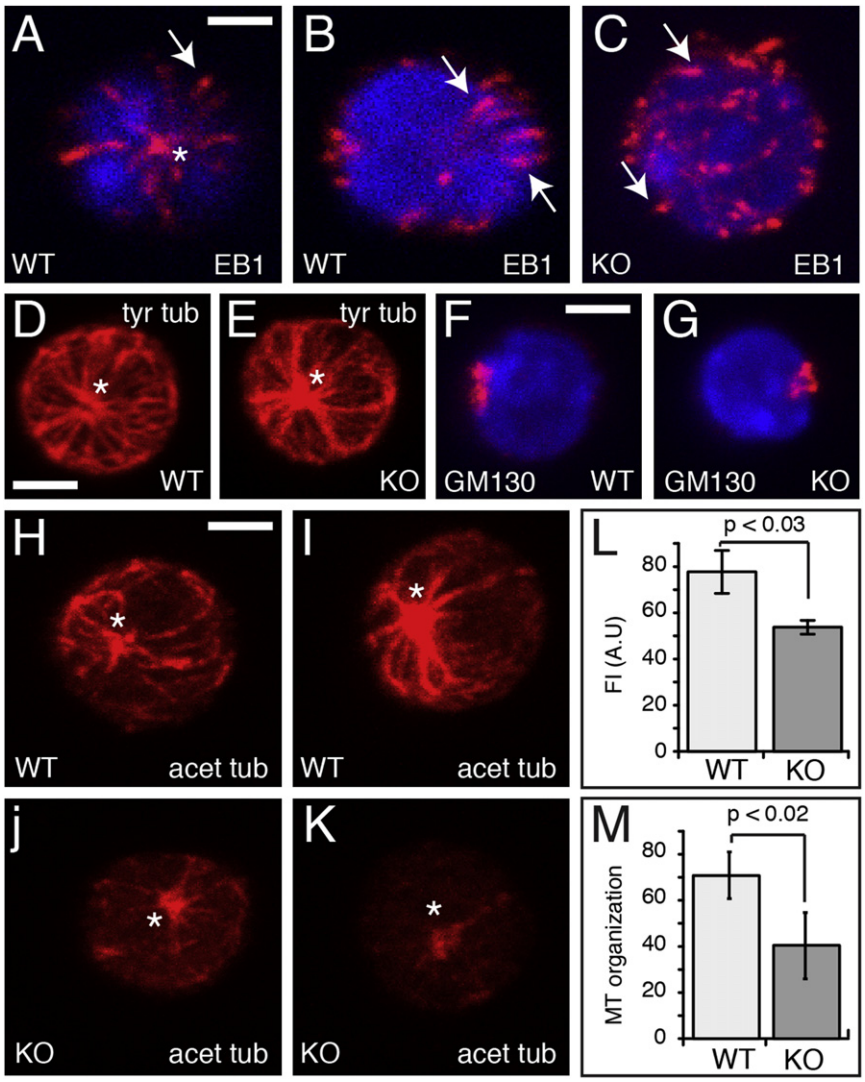

Figure 4. CLASP2 Stabilizes and Organizes MTs in HSC-Enriched Cells

(A–K) Immunofluorescence analysis. HSC-enriched cells from WT and *Clasp2* KO mouse bone marrow were deposited on poly-L-lysine-coated coverslips, incubated for 30 min, and stained with antibodies against EB1 (A–C), tyrosinated tubulin (tyr tub) (D and E), GM130 (F and G), and acetylated tubulin (acet tub) (H–K). Maximum intensity projections of cells are shown, except in (A) and (B), where bottom and top projections, respectively, of the same cell are shown. Centrosomes are indicated by asterisks. Arrows indicate EB1-comets, decorating the ends of growing MTs. Scale bars, 2 μm.

(L and M) Quantification of MT stability and organization. The amount of stable MTs per HSC-enriched cells, and of HSC-enriched cells with a "cage-like" MT network, was measured using fluorescence intensity (FI) of antibodies (SD is indicated; t tests reveal significant differences). AU, arbitrary unit.

See also Figure S4.

Strikingly, one of the most downregulated genes in *Clasp2* KO HSC-enriched fractions is *c-Mpl*. Thus, a major molecular mechanism underlying the HSC maintenance defect in *Clasp2* KO mice might be impaired TPO signaling through c-Mpl.

How is it possible that CLASP1, which is similar to CLASP2 and also expressed in the hematopoietic system, cannot compensate for a CLASP2 deficiency? One explanation is that CLASP levels are critical, such that in the absence of CLASP2, there is not enough CLASP1 to compensate for a lack of CLASP2. Alternatively, CLASP2 might be recruited by signaling pathways that do not act on CLASP1, and vice versa. Recent work has indeed shown that CLASP1, and not CLASP2, is important for maintaining spindle position and a correct cell division axis in human cells (Samora et al., 2011). CLASPs may therefore coexist in cells, but distinct cascades, acting through phosphorylation (Kumar et al., 2009), might selectively affect their activity toward MTs.

Our data show that despite their round shape and small cytoplasm attached HSC-enriched cells are polarized, with centrosome and Golgi located at one side of the nucleus and the plus ends of MTs emanating toward the other side. Both in these cells and in fibroblasts, CLASP2 stabilizes and organizes the MT network. Furthermore, CLASP2 is required for the proper attachment of fibroblasts, HSC-enriched cells, and platelets, indicating a general role for this protein in cell attachment. It remains to be shown whether an integrin-LL5-CLASP2-MT connection is operative in these systems as it is in epithelial cells (Hotta et al., 2010). We hypothesize that in the absence of CLASP2, long-term HSCs do not recognize their bone marrow niche anymore because of faulty attachment and/or migration, turn off *c-Mpl* transcription due to deregulated signaling and thereby lose stem cell potential.

EXPERIMENTAL PROCEDURES

Miscellaneous Assays

We have obtained all the necessary permissions from local and national review boards for the mice used in our research. Gene targeting and immunohistochemistry techniques (Hoogenraad et al., 2002), generation of CLASP2-deficient fibroblasts and immunofluorescent staining assays (Drabek et al., 2006), and standard molecular biology techniques (Sambrook et al., 1989) have been described. See Extended Experimental Procedures for details.

Hematological Analyses

Hematological assays were performed in peripheral blood, platelets, bone marrow, spleen, thymus, and lymph nodes. Where needed, cells were sorted by flow cytometry and analyzed with FlowJo software. For in vivo HSC analysis, total bone marrow cells from adult WT and *Clasp2* KO male mice were intravenously injected into irradiated recipients. After 1 and 4 months, donor chimerism was analyzed by semiquantitative PCR on peripheral blood. For

the in vivo homing assay, bone marrow cells from WT and *Clasp2* KO mice were labeled with CFSE, analyzed by flow cytometry to estimate the percentage of $CFSE^+$ LSK cells (time point 0 hr), and intravenously injected into irradiated recipients. After injection (15 hr), bone marrow cells were collected, and the number of $CFSE^+$ LSK cells was again determined. The fraction of LSK cells homing into the bone marrow was calculated by dividing the number of donor $CFSE^+$ LSK cells at 15 hr by the number of $CFSE^+$ LSK cells injected at 0 hr. See Extended Experimental Procedures for details.

Genome-wide Transcriptome Analyses

RNA was isolated from LSK and $CD150^+$ LSK fractions from WT and *Clasp2* KO mice and sequenced using the Illumina platform. See Extended Experimental Procedures for further details.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Extended Experimental Procedures, four figures, and one table and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2012.08.040.

LICENSING INFORMATION

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