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The Wave2 scaffold Hem-1 is required for transition of fetal liver hematopoiesis to bone marrow

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The transition of hematopoiesis from the fetal liver (FL) to the bone marrow (BM) is incompletely characterized. We demonstrate that the Wiskott–Aldrich syndrome verprolin-homologous protein (WAVE) complex 2 is required for this transition, as complex degradation via deletion of its scaffold Hem-1 causes the premature exhaustion of neonatal BM hematopoietic stem cells (HSCs). This exhaustion of BM HSC is due to the failure of BM engraftment of Hem-1⁻/⁻ FL HSCs, causing early death. The Hem-1⁻/⁻ FL HSC engraftment defect is not due to the lack of the canonical function of the WAVE2 complex, the regulation of actin polymerization, because FL HSCs from Hem-1⁻/⁻ mice exhibit no defects in chemotaxis, BM homing, or adhesion. Rather, the failure of Hem-1⁻/⁻ FL HSC engraftment in the marrow is due to the loss of c-Abl survival signaling from degradation of the WAVE2 complex. However, c-Abl activity is dispensable for the engraftment of adult BM HSCs into the BM. These findings reveal a novel function of the WAVE2 complex and define a mechanism for FL HSC fitness in the embryonic BM niche.
hematopoietic stem cells (HSCs) migrate from their sites of origin to the fetal liver (FL) on embryonic day (E) 9.5–10.5 in murine development1–3. This transient residence in the FL is essential for the maturation of adult HSCs and functional adult hematopoiesis1,2,4,5. After expansion in the FL, HSCs complete their epic journey by migrating to the bone marrow (BM) on E16.5 to 17.51–11. Several key BM environmental signals mediate the migration of FL HSCs, including CXCL12, VEGF, Slit, SCF, collagen, N-cadherin, VCAM, selectins, and fibronectin12–14,17. However, the FL HSC intracellular signaling that drives the transition to BM hematopoiesis is poorly defined1–3. The two Wiskott–Aldrich syndrome verprolin-homologous protein complexes (WAVE1 and 2) are crucial regulators of cell movement, but their role in FL HSC migration has not been explored12–14.

The two heteropentameric WAVE complexes both can activate the actin-nucleating Arp2/3 complex in an intracellular location-specific manner15–17. The WAVE2 complex is composed of the ABI-1, SRA-1, BRK-1, HEM-2 (also termed NCKAP1), and WAVE2 proteins14–16. WAVE2 is the only WAVE complex expressed in HSCs17. The WAVE complexes are further distinguished by tissue-specific components (HEM-1 versus HEM-2)18–20 that are the inner membrane scaffolds upon which the WAVE complexes directionally assemble. In all hematopoietic cells, HEM-1 (also termed NCKAP1) replaces HEM-221–24. P-element insertion in Drosophila Hem-2 resulted in failure of maternal RNA segregation, a cytoskeletal function, and subsequent embryo malformation and death18. Repression of HEM-1 in human neutrophils impairs, but does not completely abrogate their attractant-induced actin polymerization, polarity, and chemotaxis20,21. Moreover, a chemically induced point mutation in Hem-1 in mice caused defective actin polymerization and defects in leukocyte development and function31. In addition to regulating the localization of the WAVE complexes, HEM-1 and HEM-2 regulate WAVE stability. When HEM-1 or HEM-2 is depleted in multiple model organisms, the other WAVE complex components are also degraded21–24. This co-dependent stability may be an important mechanism to prevent aberrant actin polymerization31,22,24. As well as actin polymerization and cell migration, the WAVE2 complex component ABI-1 propagates c-Abl signaling25–30. The SH3 domain of ABI-1 interacts with the proline-rich region of c-ABL and mediates the dimerization of c-ABL, which can activate c-ABL kinase activity26,27. c-ABL also feeds back to enhance WAVE complex activation12,13,20,29.

We examined the role of the WAVE2 complex scaffold Hem-1 in the migration of FL HSC to the BM. Deletion of Hem-1 resulted in degradation of the WAVE2 complex21–24, but surprisingly the migration of FL HSC to the fetal BM was not altered. Rather, after arriving in the fetal marrow niche, Hem-1−/− FL HSC underwent apoptosis. Within 6–8 weeks Hem-1−/− mice underwent marrow fibrosis and hematopoietic failure, and subsequently died. Neither FL nor young marrow Hem-1−/− HSC could engraft irradiated wild-type (wt) adult mice. Without the WAVE2 complex present, Abi-1 was not present to activate downstream c-Abl signaling. Reconstituting c-Abl expression rescued Hem-1−/− HSC survival in the adult marrow niche. This study implies that FL HSCs are not fit for BM occupancy until after a niche survival signal mediated by the WAVE complex through c-Abl. This defines a novel WAVE complex function, survival signaling, and sheds light on the regulation of the transition of hematopoiesis from the FL to the marrow during development.

Results

Premature death and hematopoietic defects in Hem-1−/− mice. We hypothesized that the hematopoietic-specific WAVE complex scaffold Hem-1 is important for FL HSC transition to the BM. In the present study, Hem-1 was constitutively deleted in a murine model to assess fetal HSC development and migration (Supplementary Fig. 1a–d). Constitutive deletion permitted study of whether Hem-1 was essential for the development of any other organ system outside the hematopoietic system. In addition, it ensured that all HSCs had the gene deleted, and therefore a small number of HSC escaping conditional deletion could not skew the study. Intercessions of Hem-1−/− mice produced Hem-1−/−, Hem-1+/−, and Hem-1−/− E14.5 fetuses at the expected Mendelian ratio (Fig. 1a). However, Hem-1−/− mice exhibited growth retardation and died prematurely after birth, with an average life expectancy of 6 weeks (Fig. 1b, c). These abnormalities were associated with a dramatic defect in BM hematopoiesis, including a significant reduction in the number of total BM nucleated cells (BMCs), BM phenotypic HSCs and hematopoietic progenitor cells (HPCs), and BM cobblestone area-forming cells (CAFCs) in 5-week-old Hem-1−/− compared with littermate Hem-1+/− mice of the same age (Fig. 1d, h). In addition, Hem-1−/− mice developed a myelofibrosis-like disease with BM fibrosis, as demonstrated by an increase in reticulin staining; extra-medullary hematopoiesis, neutrophilia, and lymphopenia (Supplementary Fig. 2a–d). Heterozygote Hem-1−/+ mice developed normally, similar to Hem-1+/+ mice, and showed none of the abnormalities observed in Hem-1−/− mice.

Hem-1−/− FL HSCs are unable to engraft BM. To investigate whether Hem-1−/− mice die from premature exhaustion of BM HSCs as a result of an intrinsic defect in HSCs or a defect in their BM microenvironment, we performed rescue stem cell transplantation (SCT) by infusing E14.5 FLCs from Hem-1+/+ or Hem-1−/− fetuses into lethally irradiated normal C57BL/6 congenic (CD45.1) mice. Transplantation of littermate Hem-1−/−/+ FL cells (FLCs) fully rescued the irradiated recipients, whereas all the recipients that received Hem-1−/− FLCs died within 11 days after BMT (Fig. 2a). Competitive repopulation assays (CRAs) found that E14.5 FLCs from Hem-1−/−/+ mice failed to compete with normal CD45.1 BMCSs for engraftment after SCT (Fig. 2b; Supplementary Fig. 3a, b). More importantly, we found that transplantation of Hem-1−/−/+ CD45.1 BMCSs into non-ablated CD45.2 Hem-1−/− mice at 3 weeks of age out-competed endogenous HSCs to repopulate their hematopoietic system, and rescued the Hem-1−/− development defects and survival (Fig. 2c, d; Supplementary Fig. 3c, d). These results suggest that the lack of Hem-1 does not affect fetal development, but causes growth retardation and premature death after birth due to an intrinsic defect in HSCs. The Hem-1−/− BM HSCs exhibit premature exhaustion, which we postulated may be due to an inability of Hem-1−/− FL HSCs to engraft in the BM.

Hem-1−/− FL HSCs can migrate to the BM. FL HSCs transition to the BM starting around E16.5–17.5, and continues briefly after birth1–3. This transition requires significant cell migration and adherence. Therefore, we next examined whether Hem-1 deletion leads to defects in FL HSC actin polymerization, migration, adherence, and homing to the BM. Unexpectedly, HSC-enriched Lin−/Sca-1−/Kit+ (LSK) E14.5 Hem-1−/− FLCs showed no defects in F-actin polymerization, actin capping, and migration in response to the HSC chemokine stromal derived factor-1 alpha (SDF-1α), compared to littermate Hem-1−/−/+ equivalent cells (Fig. 3a, b). Hem-1−/− FL Lin− cells could adhere to fibronectin equally as well as the cells from Hem-1−/−/+ littermates (Fig. 3b). In addition, E14.5 Hem-1−/− FL LSK cells expressed levels of HSC adhesion and BM homing components (CXCR4, VLA-4, VLA-5, Tie2) equal to or greater than E14.5 Hem-1−/−/+ FL LSK cells.
There was no difference in the numbers of E14.5 FL LSK cells failed to survive and/or move closer to the endosteum between the 16 and 48 h time points. We then measured the ability of Hem-1−/− E14.5 CD45.2 FL LSK cells to cells were half the numbers of their Hem−1+/+ counterparts (Fig. 3c). Next, we assessed Hem−1−/− E14.5 FL LSK migration to the BM osteoblast niche. There were equivalent numbers of 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiD)-labeled Hem−1+/+ E14.5 CD45.2 FL LSK within the osteoblastic niche 16 h after injection into non-ablated CD45.1 col2-3-EGFP reporter mice as littermate Hem−1+/+ equivalent cells (Fig. 3d). However, 48 h after injection, there were more than twice the numbers of Hem−1−/− E14.5 FL LSK within the niche compared to equivalent Hem−1−/− FL LSK cells (Fig. 3d), implying that the Hem−1−/− FL LSK cells failed to survive and/or failed to proliferate once arriving in the BM niche. Using three-dimensional analysis, the distance to the nearest col2-3-EGFP osteoblast and endosteum was also not significantly different between Hem−1+/+ and Hem−1−/− E14.5 FL LSK 16 and 48 h after the injection (Supplementary Fig. 6), indicating that Hem−1−/− E14.5 FL LSK can adhere to the osteoblastic niche equally well as Hem−1+/+ E14.5 FL LSK. Interestingly, both cell types moved closer to the endosteum between the 16 and 48 h time points.

Hem−1−/− FL HSCs cannot survive in the BM. We then measured the ability of Hem−1−/− E14.5 CD45.2 FL LSK cells to

(Supplementary Fig. 4). In contrast, neutrophils from Hem−1−/− mice are defective in fMLP-stimulated F-actin polymerization, actin capping and migration, and adhesion to fibronectin as the cells from Hem−1 mutant mice reported previously (Supplementary Fig. 5)21. Furthermore, we found that inhibition of CDC42 with a specific inhibitor, CASIN, suppressed both E14.5 Hem−1+/+ and Hem−1−/− FL LSK cell adhesion and migration in vitro (Fig. 3b). These findings suggest that unlike neutrophils and other hematopoietic cells in adult mice, FL HSCs can migrate and home to the BM independent of the WAVE complex, possibly via the CDC42 Wiskott-Aldrich syndrome protein (WASP) pathway. This is consistent with the observation that HSCs from WASP-deficient mice had decreased BM homing capability in association with a defect in adhesion to collagen31.

We next assessed whether FL hematopoietic stem/progenitor cells (HSPCs) were able to migrate to the BM in vivo after transplantation. 5- and 6- Carboxyfluorescein succinimidyl ester (CFSE)-labeled E14.5 Hem−1+/− CD45.2 FL LSK cells homed to the adult BM as well as their normal littermate counterparts after they were transplanted into lethally irradiated CD45.1 mice (Fig. 3c)32. There was no difference in the numbers of Hem−1−/− E14.5 FL LSK cells in the BM 16 h after injection as equivalent Hem−1+/+ cells. However, 48 h after arrival in the BM, Hem−1−/− E14.5 FL LSK

Fig. 1 Hem−1−/− mice exhibit decreased HSCs and HSCs, and die prematurely. a Genetic characterization of Hem−1−/− offspring, demonstrating that Hem−1−/− mice are born with appropriate Mendelian frequency. b Hem−1−/− mice have growth retardation compared to littermate Hem−1+/+ mice ( Hem−1−/− mice, n = 6; Hem−1−/− mice, n = 8, * p < 0.01, Student’s t test). c Hem−1−/− mice die an average of 6 weeks after birth (n = 13, p < 0.001, log-rank test). d Flow cytometric analytic schematic of hematopoietic stem and progenitor cells in Hem−1−/− and littermate Hem−1+/+ mice. (FSC: forward scattered light, Lin−/CD3ε−/CD11b−/CD45R−/B220−/Ter-199−/Gr-1+, LSK: Lin−/Sca-1−/c-Kit+, HPC: Lin−/Sca-1−/c-Kit−, HSC: LSK/CD150+/CD48−). e E14.5 fetal liver hematopoietic stem and progenitor cell adhesion and migration, and adhesion to collagen31.

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survive and proliferate after migration to the BM compared to littermate equivalent \textit{Hem-1}^{+/+} cells (Fig. 3c). We found that \textit{Hem-1}^{−/−} E14.5 CD45.2 FL LSK had both a higher rate of apoptosis and a decreased rate of cycling cells compared to equivalent cells from \textit{Hem-1}^{+/+} littermate controls. This suggests that HSC-enriched LSK cells from the E14.5 \textit{Hem-1}^{−/−} FL are just as capable of migrating to the BM and homing to the osteoblast niche as their normal counterparts, but cannot survive and proliferate once there\textsuperscript{31}. Thus, \textit{Hem-1} deletion does not impair FL to BM hematopoietic cell homing or adherence to the niche, suggesting that the WAVE2 complex has a distinct function in FL HSPCs besides regulating cell migration and adhesion by mediating survival and expansion after migration from the FL to the BM. This is consistent with the observation that knockdown of WAVE2 had no significant effect on HSC migration to the BM but prevented HSCs from expanding in the BM\textsuperscript{17}.

However, the mechanism by which the WAVE complex regulates HSC expansion in the BM was unknown, and thus was studied further. First, we performed a detailed analysis of HSCs in the BM from E18.5 through postnatal day (PD) 1, PD3 and PD7 in both \textit{Hem-1}^{−/−} and \textit{Hem-1}^{+/+} fetuses and neonates. As expected, we found that the number of HSCs in the BM was low in E18.5 \textit{Hem-1}^{−/−} fetuses and then increased rapidly from PD1 to PD7 in \textit{Hem-1}^{+/+} neonates (Fig. 4a). By contrast, \textit{Hem-1}^{−/−} neonates exhibited a moderate increase in the number of BM HSCs on PD3 and then an abrupt decrease on PD7 to a level similar to that in E18.5 fetuses (Fig. 4a). We then measured the cell cycle distribution and apoptotic fraction of \textit{Hem-1}^{−/−} BM HSPCs compared with those of \textit{Hem-1}^{+/+} littermate equivalent cells on E18.5, PD1, PD3 and PD7 using Ki-67/7-aminoactinomycin-D (7-AAD) and Annexin V staining, respectively. The cell cycle distribution was not significantly different between the cells from \textit{Hem-1}^{−/−} and \textit{Hem-1}^{+/+} littermates until PD7 (Fig. 4b). Interestingly, a high fraction of BM HSCs from both E18.5 \textit{Hem-1}^{−/−} and \textit{Hem-1}^{+/+} fetuses were apoptotic (Fig. 4c), indicating that FL HSCs arriving at the BM were not fit for their new environment. However, in \textit{Hem-1}^{−/−} BM, the apoptotic fraction fell rapidly over the ensuing days, whereas in \textit{Hem-1}^{−/−} BM the apoptotic fraction remained at a high level. This implies that there is an intrinsic survival signal the incoming FL HSCs require, but lack when \textit{Hem-1} is deleted.

\textbf{\textit{Hem-1}^{−/−} FL HSCs lack c-Abl survival signaling.} We therefore sought to identify the FL HSC intrinsic survival signal required for engraftment after HSC transition to the BM. In addition to regulating actin polymerization, which mediates cell migration and adherence, the WAVE complex can also promote c-Abl signaling via c-Abl’s interaction with Abi-1\textsuperscript{25–30}. Deletion of c-Abl results in defects in embryonic hematopoiesis resembling those seen here\textsuperscript{33,34}, and constitutive activation of c-Abl via the t(9;22) increases HSC survival and proliferation and generates chronic myeloid leukemia (CML)\textsuperscript{35–37}. Therefore, we investigated whether the defect in the \textit{Hem-1}^{−/−} FL HSC transition to BM hematopoiesis was due to a lack of c-Abl survival signaling.

Without \textit{Hem-1} as the assembly scaffold, the other components of the WAVE2 complexes are reported to degrade\textsuperscript{21,23,24}. As expected, deletion of the WAVE scaffold \textit{Hem-1} here also resulted in degradation of the WAVE2 components Abi-1, Abi-2, WAVE2 and Sra-1 in FL Lin\textsuperscript{−} cells (Fig. 5a). As a result of the loss of its interacting partner Abi-1, the expression of c-Abl protein was significantly reduced in Lin\textsuperscript{−} cells from \textit{Hem-1}^{−/−} FL Lin\textsuperscript{−} cells (Fig. 5a and Supplementary Fig. 7). Similar findings were also observed in PD3 BM Lin\textsuperscript{−} cells. In E14.5 \textit{Hem-1}^{−/−} FL LSK cells, the loss of c-Abl did not affect the phosphorylation of Crkl, Jak2, Stat3, Stat5, Erk, Akt, and S6, downstream effectors of HSC survival and hematopoiesis (Fig. 5b, c and Supplementary Fig. 8). However, by PD3, \textit{Hem-1}^{−/−} BM LSK cells exhibited a significant reduction in the phosphorylation of all of these signaling components (Fig. 5d and Supplementary Fig. 8). These results suggest that: (1) the c-Abl protein relies on the WAVE2 complex for stability in FL HSPCs\textsuperscript{25–30} and (2) there are other signals in FL HSPCs for survival besides c-Abl\textsuperscript{58–40}. However, c-Abl signaling becomes essential at the transition of hematopoiesis from the FL to the neonatal BM. The differential phosphorylation status of downstream signalers in E14.5 \textit{Hem-1}^{−/−} FL LSK cells.
and PD3 Hem−1−/− BM LSK cells correlated with their expression of anti-apoptotic and pro-apoptotic proteins and the level of apoptosis (Fig. 5b–e and Supplementary Fig. 8). The PD3 Hem−1−/− BM LSK cells had decreased Bcl-2 and increases in Puma, Bak, and Bax messages, indicating that there was significant apoptotic signaling in the Hem−1−/− PD3 BM LSK cells compared to littermate Hem−1+/− equivalent cells.

To determine whether the hematopoietic transition from the FL to the BM is dependent on c-Abl signaling for HSC survival, we incubated E14.5 FL and adult BM Lin− cells from Hem−1+/+ mice with Imatinib (c-Abl kinase inhibitor)35–37, and then analyzed FL and BM LSK cell proliferation and apoptosis (Fig. 6a–d). Imatinib resulted in decreased cultured Hem−1+/+ FL LSK cell proliferation and increased apoptosis, but not in BM LSK cells. Significantly, the inhibition of c-Abl activity with Imatinib reduced the long-term engraftment of competitively transplanted Hem−1+/+ FL HSCs (Fig. 6e–i). The inhibition of c-Abl activity by imatinib had little effect on the engraftment of competitively transplanted adult Hem−1+/+ CD45.2 BM Lin− cells, but markedly decreased the competitive engraftment of E14.5 Hem−1+/+ CD45.2 BM Lin− cells (Fig. 6c–g; Supplementary Fig. 9a–d). To control the target specificity of imatinib, we next depleted endogenous c-Abl by transducing E14.5 Hem−1+/+ FL Lin− cells with a lentiviral short hairpin RNA (shRNA) against c-Abl (Fig. 6h, i; Supplementary Fig. 9e, f). We demonstrated that depletion of c-Abl using a lentiviral short hairpin RNA (shRNA) against c-Abl had little effect on the engraftment of competitively transplanted adult Hem−1+/+ CD45.2 BM Lin− cells, but markedly decreased the competitive engraftment of E14.5 Hem−1+/+ CD45.2 BM Lin− cells (Fig. 6c–g; Supplementary Fig. 9a–d). To control the target specificity of imatinib, we next depleted endogenous c-Abl by transducing E14.5 Hem−1+/+ FL Lin− cells with a lentiviral short hairpin RNA (shRNA) against c-Abl (Fig. 6h, i; Supplementary Fig. 9e, f). We demonstrated that depletion of c-Abl using a lentiviral shRNA against c-Abl had little effect on the engraftment of competitively transplanted adult Hem−1+/+ CD45.2 BM Lin− cells, but markedly decreased the competitive engraftment of E14.5 Hem−1+/+ CD45.2 BM Lin− cells (Fig. 6c–g; Supplementary Fig. 9a–d). To control the target specificity of imatinib, we next depleted endogenous c-Abl by transducing E14.5 Hem−1+/+ FL Lin− cells with a lentiviral short hairpin RNA (shRNA) against c-Abl (Fig. 6h, i; Supplementary Fig. 9e, f). We demonstrated that depletion of c-Abl using a lentiviral shRNA against c-Abl had little effect on the engraftment of competitively transplanted adult Hem−1+/+ CD45.2 BM Lin− cells, but markedly decreased the competitive engraftment of E14.5 Hem−1+/+ CD45.2 BM Lin− cells (Fig. 6c–g; Supplementary Fig. 9a–d). To control the target specificity of imatinib, we next depleted endogenous c-Abl by transducing E14.5 Hem−1+/+ FL Lin− cells with a lentiviral short hairpin RNA (shRNA) against c-Abl (Fig. 6h, i; Supplementary Fig. 9e, f). We demonstrated that depletion of c-Abl using a lentiviral shRNA against c-Abl had little effect on the engraftment of competitively transplanted adult Hem−1+/+ CD45.2 BM Lin− cells, but markedly decreased the competitive engraftment of E14.5 Hem−1+/+ CD45.2 BM Lin− cells (Fig. 6c–g; Supplementary Fig. 9a–d). To control the target specificity of imatinib, we next depleted endogenous c-Abl by transducing E14.5 Hem−1+/+ FL Lin− cells with a lentiviral short hairpin RNA (shRNA) against c-Abl (Fig. 6h, i; Supplementary Fig. 9e, f). We demonstrated that depletion of c-Abl using a lentiviral shRNA against c-Abl had little effect on the engraftment of competitively transplanted adult Hem−1+/+ CD45.2 BM Lin− cells, but markedly decreased the competitive engraftment of E14.5 Hem−1+/+ CD45.2 BM Lin− cells (Fig. 6c–g; Supplementary Fig. 9a–d). To control the target specificity of imatinib, we next depleted endogenous c-Abl by transducing E14.5 Hem−1+/+ FL Lin− cells with a lentiviral short hairpin RNA (shRNA) against c-Abl (Fig. 6h, i; Supplementary Fig. 9e, f).
c-Abl restored the ability of Hem-1<sup>−/−</sup> E14.5 CD45.2 Fl Lin<sup>−</sup> cells to competitively engraft lethally irradiated CD45.1 wt recipients, whereas lentiviral c-Abl had minimal effect on the engraftment of transplanted equivalent cells from littermate controls (Fig. 6j, k). These findings demonstrate that FL HSCs depend on c-Abl for survival and engraftment in the BM, and that Hem-1<sup>−/−</sup> FL HSCs lack this signal.

**Discussion**

In this study, we constitutively deleted Hem-1 in mice to originally assess two questions: First, was Hem-1 function specific for hematopoietic cells, or were other organs affected by its deletion? Second, were Hem-1 and Hem-2 interchangeable, or did they have specific functions? In the initial characterization of the Hem-1<sup>−/−</sup> mice we found that all other organ systems developed normally, and functioned into adulthood. The only defects were in the hematopoietic system. Thus, Hem-1 function was indeed specific for blood cells, and was not required for the normal development and function of any other organ systems. Second, consistent with the previous report by Park et al. that a Hem-1 point mutation affected blood cell development, we found multiple hematopoietic defects in the Hem-1<sup>−/−</sup> mice. Thus, Hem-2 cannot replace Hem-1 function in hematopoiesis.

The Hem-1<sup>−/−</sup> FL HSC were essentially normal in numbers and proliferative function, but after migration to the marrow osteoblast niche, marrow HSC became rapidly depleted, resulting in a myelofibrosis-like phenotype, with anemia, myeloid metaplasia, and marrow reticulin. The Hem-1<sup>−/−</sup> mice lacked other WAVE2 components, consistent with earlier findings in flies, amoeba, and mice, that WAVE complexes are obligate heteropentamers, probably to prevent unregulated actin polymerization, and cell paralysis. Crystal structures of HEM-2 in the WAVE complex structure indicate that it likely attaches to the membrane and serves as a scaffold for the other WAVE components to assemble. Thus, without the scaffold and the protection of the assembled heteropentamer, it is not surprising that the individual WAVE protein components are prone to destruction.

c-Abl-null mice also exhibit neonatal lethality, and if they survive they become running, splenic, and marrow atrophy, with lymphopenia and increased susceptibility to infections, similar to the Hem-1<sup>−/−</sup> mice here. Consistent with previous reports, when Abi-1 is degraded in the Hem-1<sup>−/−</sup> cells c-Abl fails to signal, resulting in decreased phosphorylation of downstream c-Abl targets, such as Crkl, Stat3, and S6, both in FL and marrow stem/progenitor cells. Depleting or inhibiting c-Abl inhibit wt FL HSC engraftment capability, but they do not harm engraftment of adult marrow HSC. Restoring c-Abl
phosphorylation of c-Abl downstream signaling pathway effectors in that pro-apoptotic effectors were increased and anti-apoptotic effectors were decreased in analysis of regulators of apoptosis between cells as measured by western blot.

However, Rac1/2 and cdc42 deletion prevents FL HSC from reaching the marrow for transition of hematopoiesis, consistent with our data demonstrating that the cdc42 inhibi-
tion blocked FL HSC migration and adhesion.

Since the WAVE2 complex is not needed for FL HSC to migrate to the marrow osteoblast niche, there must be alternative pathways that can mediate appropriate actin polymerization for FL HSC migration and adherence. WASP complexes are a potential candidate to mediate an alternative pathway for actin polymerization besides WAVE utilized by the FL HSC to reach the marrow36. After activation by rac or cdc42, WASP also activates Arp2/3, and stimulates actin polymerization39,40. However, Rac1/2 and cdc42 deletion prevents FL HSC from reaching the marrow for transition of hematopoiesis, consistent with their importance in upstream regulation of actin polymer-
ization, epistatically above both WAVE and WASP39,40. This is consistent with our data demonstrating that the cdc42 inhibition blocked FL HSC migration and adhesion.

The data here also have implications for the molecular pathogenesis of CML. The 9;22 chromosomal translocation in CML fuses BCR to c-Abl and results in HSC immortality, but these HSCs are addicted to c-Abl for survival35,36. This implies that the CML HSCs more closely resembles the FL HSCs than the marrow HSCs. It is therefore possible that WAVE2 might be important for BCR-Abl signaling, and WAVE2 could be an additional target for therapy in CML. In addition, this finding suggests that pregnant CML patients should not be treated with a c-Abl inhibitor at late gestation. It might inhibit the survival of fetal HSC after migration from the FL to the marrow, and result in a decreased HSC population into adulthood. Exposure to imatinib during pregnancy results in an increased incidence of fetal malformations41, but there is little data on blood counts as children of such pregnancies mature42. Such children could have a higher risk of marrow aplasia or myelofibrosis in adulthood.

In summary, the scaffold upon which the WAVE2 complex assembles in hematopoietic cells, Hem-1, is required for the engrafment of FL HSC in the marrow during embryonic develop-
ment. Surprisingly, the engrafment failure of Hem-1−/− FL HSC is not due to decreased actin polymerization resulting in poor migration or adherence to the marrow niche. Rather, this engrafment requires c-Abl signaling, which is lost when its partner Abi-1 is degraded with the rest of the WAVE complex when Hem-1 is deleted. After the FL HSC has engrafed the marrow, the c-Abl survival signal is no longer needed, indicating that FL HSCs are specifically for and modified by the marrow microenvironment.

Methods
Model generation. For the generation of Hem-1-deleted mice, a mouse Hem-1 genomic clone was obtained from the mouse 129/sv BAC library42. A lacZ-neo cassette, in which the neomycin phosphotransferase gene is linked to the lacZ gene placed between the independent ribosomal entry sequences and an SV40 poly-
adenylation signal, replaced a sequence covering the fi rst exon. The gene-targeting construct was electroporated into embryonic stem (ES) cells, and the cells were selected with neomycin. Recombinant ES cell clones were then injected into C57BL/6 mouse blastocysts, and Hem-1+/− mice were
was performed monthly after transplantation. At 4 months post transplantation, CD45.2 BMCs were assessed to analyze long-term engraftment.

Hem-1 obtained using standard protocols. All of the mice were backcrossed to C57BL/6 mice for more than eight generations before being used in our study. Constitutive deletion was chosen to: (1) ensure complete Hem-1 deletion in embryonic HSC, and (2) analyze whether Hem-1 had any non-hematopoietic effects.

Male C57BL/6 (CD45.2) mice, B6.SJL-PtprcaPep3bBoyJ (CD45.1) mice, and B6.129P2-BoyJ (CD45.1) knockouts were purchased from Jackson Laboratories (Bar Harbor, MA, USA). The mice were housed at the University of Arkansas for Medical Sciences (UAMS). Tibiae, spleen, and other organs were obtained using standard protocols. All of the mice were backcrossed to C57BL/6 mice for more than eight generations before being used in our study. Constitutive deletion was chosen to: (1) ensure complete Hem-1 deletion in embryonic HSC, and (2) analyze whether Hem-1 had any non-hematopoietic effects.

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Genotyping. Genomic DNA was isolated from mouse tails or embryo brains and digested in DNA lysis buffer (VIAGEN Biotech, Los Angeles, CA, USA)42,43. The genotype of each mouse or embryo was determined by PCR using LacZ and Hem-1 primers. The LacZ forward (LACZFWD: 5′-TTCACTGGCCGTGTTTTA-CAACGTGTTGTA-3′) and LacZ reverse (LACZREV: 5′-ATGTGAGCGAGTAAC-3′) primers recognize the LacZ reporter gene from the Knockout vector. The Hem-1 forward (HEMFWD: 5′-GGTGGGTTGGAAAA GAGATATTGTGGTGG-3′) and Hem-1 reverse (HEMRV: 5′-TACCACGCCCC AACACTACCTTAGAAAACACC-3′) primers recognize the Hem-1 gene. The LacZ product is 364 bp and the Hem-1 product is 228 bp, as shown in Supplementary Fig. 1b.

Peripheral blood counts and organ histology. Blood was obtained through retro-orbital bleeding and transferred to ethylenediaminetetraacetic acid (EDTA)-coated tubes43–45. Peripheral blood cell numbers were determined using a Vet Abc Hematological analyzer (Sci Animal Care, Gurnee, IL, USA). Tibiae, spleen, and
lung from 5-week-old Hem−/− or Hem+−/− mice were fixed in 4% paraformaldehyde (Fisher Scientific, Pittsburgh, PA, USA) for 24 h. Tibiae were decalcified in 14% EDTA for 7 days. The bones were then embedded in paraffin and 5-μm longitudinal sections were obtained. After de-paraffinization and rehydration, the sections were processed for staining with hematoxylin and eosin for the histologic assessment of organ morphology, silver for reticulin, or myeloperoxidase for myeloid cells.

Isolation of BM and FL hematopoietic cell subsets. The femora and tibiae were harvested from mice immediately after they were euthanized with CO2. BM cells were flushed from the bones into Hank’s balanced salt solution (HBSS) containing 2% fetal calf serum (FCS) using a 21-gauge needle and syringe. FLCs were obtained from E14.5 embryos. The cells were centrifuged through Histopaque 1083 (Sigma, St. Louis, MO, USA) to isolate mononuclear cells (MNCs). For the isolation of Lin− cells, MNCs from the BM or FL were incubated with biotin-conjugated rat-specific antibodies specific for murine CD3e, CD11b, CD45R/B220, anti-Gr-1, anti-CD11b, and anti-Ter-119 antibodies, with anti-MNCs from BM or FL were pre-incubated with biotin-conjugated anti-CD3e, anti-CD11b, and anti-CD45R/B220–receptors and then stained with anti-Sca-1-PE and c-Kit-APC antibodies. Dead cells were excluded by gating out the cells stained with propidium iodide (PI).

Analysis of the frequencies of hematopoietic cell subsets by flow cytometry. MNCs from BM or FL were pre-incubated with biotin-conjugated anti-CD3e, anti-CD45R/B220, anti-Gr-1, anti-CD11b, and anti-Ter-119 antibodies, with anti-CD16/32 antibody to block the Fcγ receptors and then stained with antibodies against various cell-surface markers. The BrdU incorporation was measured by flow cytometry using the FITC BrdU Flow Kit provided in Supplementary Table 1.

Homing assay. Thirty thousand FL LSK cells from E14.5 Hem−/− or Hem+−/− embryos were stained with 5 μM CFSE-mixed isomers (Thermo Fisher Scientific, Waltham, MA, USA) for 10 min according to the manufacturer’s instructions. The stained cells were re-suspended in 100 μl IMDM medium containing 30,000 cells and retro-orbitally transplanted into lethally irradiated male CD45.1 recipient mice. The mice were euthanized at 16 and 48 h after transplantation. The BMCs were harvested and analyzed by flow cytometry using an LSR II flow cytometer (BD Biosciences) for the presence of CFSE− cells in FL negative cells. The absolute numbers of CFSE+ cells in the BM from femora and tibiae were calculated by multiplying the total numbers of BMCs by the percentages of CFSE+ cells.
1×10^4 embryos were pre-incubated with vehicle or 10 μM CASIN (Sigma) for 2 h to inhibit the activity of CDC42. They were plated in the upper well of a 24-well Transwell chamber separated with a filter containing 3.0 μm pore size (Corning, NY, USA) in IMDM medium with 2% bovine serum albumin (BSA). After a 4-h incubation against an SDF-1 gradient (100 ng/ml) in the lower chamber, all cells that migrated through the filter were collected. These cells were then stained with anti-β2 integrin c-Kit-APC and Sca-1-PE and analyzed using an LSRII flow cytometer (BD Bioscience) for the percentages of LSK cells within F1 negative cells. The numbers of migrating LSK cells were calculated by multiplying the total number of migrating Lin− cells by the percentage of migrating LSK cells.

Adhesion assays. To determine the ability of hematopoietic cells to adhere to a substrate in vitro, 10,000 FL LSK cells from E14.5 Hem−/− and Hem−/− embryos in Stemspan medium with 10 ng/ml thrombopoietin (TPO) and 10 ng/ml stem cell factor (SCF) were plated in triplicate in well non-tissue-cultured plates previously coated with fibronectin (CH-296, 20 μg/ml; Clontech Laboratories, Mountain View, CA, USA)42. The cells were incubated for 1 h at 37 °C, after which the supernatant was removed, and the wells were washed once with phosphate-buffered saline (PBS) to remove non-adherent cells. The number of adherent cells was counted under a light microscope.

F-actin fluorescence staining. To characterize F-actin polymerization and capping, FL LSK cells from E14.5 Hem−/− and Hem−/− embryos were serum-starved in HBSS and stimulated with the chemokine SDF-1α (10 ng/ml) for 10 min. The cells were fixed with 4% paraformaldehyde for 15 min and permeabilized with 0.1% Triton X-100 (Sigma) for 15 min. After blocking in 2% BSA, the cells were stained with tetramethylrhodamine-phalloidin (Thermo Fisher Scientific, Waltham, MA, USA) and 4.6-diamidino-2-phenylindole (DAPI) (Sigma) and mounted for fluorescence imaging analysis on a Zeiss fluorescence microscope equipped with a ×40 oil-immersion objective lens, an AxioCam MRm camera, and the AxioVision Rel. 4.8 software (Jena, Germany). The images shown are representative of more than 100 cells examined for each genotype.

Neutrophil F-actin adhesion, migration, and polarization assays. Neutrophil isolation was performed as follows: BM cells (2×10^5) from 5-week-old Hem−/− and Hem−/− mice were suspended in 3 ml HBSS-Eydta buffer and transferred on the top of pre-prepared Percoll gradient separation solution containing 78% Percoll (1.11 g/ml), 69% Percoll (1.09 g/ml), and 52% Percoll (1.083 g/ml). Cells were separated by centrifugation at 1300 g for 30 min. The neutrophil layer was collected from the 78%/69% Percoll interface and red blood cells were lysed with 0.83% NH4Cl solution. Neutrophils were counted and used for testing the capacities of adhesion, migration, and polarization.

For the F-actin polymerization and capping assay, neutrophils (2×10^5) from 5-week-old Hem−/− and Hem−/− mice were suspended in 3 ml HBSS-EYdtA buffer and transferred on the top of pre-prepared Percoll gradient separation solution containing 78% Percoll (1.11 g/ml), 69% Percoll (1.09 g/ml), and 52% Percoll (1.083 g/ml). Cells were separated by centrifugation at 1300 g for 30 min. The neutrophil layer was collected from the 78%/69% Percoll interface and red blood cells were lysed with 0.83% NH4Cl solution. Neutrophils were counted and used for testing the capacities of adhesion, migration, and polarization.

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Transduction of constitutive c-Abl in hematopoietic cells. The pEGFP-c-Abl plasmid (generous gift from Dr. Zhi-min Yuan, Harvard T.H. Chan School of Public Health) was digested with BglII and Hpal sites of the pLent-GFP-vector to generate the pLent-GFP-c-Abl vector. To up-regulate c-Abl expression in hematopoietic cells, FL Lin− cells from E14.5 Hem−1/1+ embryos were maintained in Stemspan medium with TPO (10 ng/ml) and SCF (10 ng/ml) and infected twice with viral particles containing pLKO.1-c-Abl shRNA or pLKO.1-CTL shRNA under centrifugation (900 x g) at 32 °C for 30 min. Stably transduced cells were selected with puromycin (2 mg/ml) for 2 days. The infected cells were transplanted into 9.5 G lymphoid irradiated CD45.1 recipient mice along with 5 x 10^6 BMCs from the recipients. The analysis of peripheral blood was performed monthly after transplantation. At 4 months post transplantation, the BMCs were used to analyze the engraftment ability. c-Abl knockdown using different shRNA clones in Lin− cells was determined by western blotting as shown in Supplementary Fig. 9e.

Statistical analysis. The data exhibited normal variation. No data sets were excluded from the analysis. Past experimentation was used to predict sample size. The experiments were not randomized, except for the in vivo animal studies with mice as described. The investigators were not blinded to allocation during experiments and outcome assessment. The data were analyzed by analysis of variance (ANOVA). Differences among the group means were analyzed by Student–Newman–Keuls multiple comparisons test after one-way or two-way ANOVA. For experiments in which only single experimental and control groups were used, the group differences were examined by an unpaired Student’s t test. The frequencies of CAFC were analyzed by using Poisson statistics. The differences in the distribution of the cell cycle phases were determined by chi2 test. Survival curves were constructed using the Kaplan–Meier method and compared using the log-rank test. Differences were considered significant at p < 0.05. All analyses were performed with GraphPad Prism from the GraphPad software.

Data availability. All data are available from the authors upon written request.

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References

1. Ciriza, J. et al. The migration of hematopoietic progenitors from the fetal liver to the fetal bone marrow: lessons learned and possible clinical applications. Exp. Hematol. 41, 411–423 (2013).
2. Zhou, Y., Lewallen, M. & Xie, T. Stem cells: exodus: a journey to immortality. Dev. Cell 24, 113–114 (2013).
3. Coskun, S. et al. Development of the fetal bone marrow niche and regulation of HSC quiescence and homing ability by emerging osteoide cells. Cell Rep. 9, 581–590 (2014).
4. Zanjani, E., Ascensao, J. & Tavassoli, M. Liver-derived fetal hematopoietic stem cells selectively and preferentially home to the fetal bone marrow. Blood 81, 399–404 (1993).
5. Arai, T. et al. Long-term hematopoietic stem cells require stromal cell-derived factor-1 for colonizing bone marrow during ontogeny. Immunity 19, 257–267 (2003).
6. Katayama, Y. et al. PSGL-1 participates in E-selectin-mediated progenitor homing to bone marrow: evidence for cooperation between E-selectin ligands and alpha4 integrin. Blood 102, 2060–2067 (2003).
7. Kawatsuru, K. et al. A cell-autonomous requirement for CXCR4 in long-term lymphoid and myeloid reconstitution. Proc. Natl. Acad. Sci. USA 96, 5663–5667 (1999).
8. Fraser, S. et al. Definitive hematopoietic commitment within the embryonic vascular endothelial-cadherin+ population. Exp. Hematol. 30, 1070–1078 (2002).
9. Frenette, P. et al. endothelial selectins and vascular cell adhesion molecule-1 promote hematopoietic progenitor homing to bone marrow. Proc. Natl. Acad. Sci. USA 95, 14423–14427 (1998).
10. Smith-Berdan, S. et al. Robo4 cooperates with CXCR4 to regulate hematopoietic stem cell localization to bone marrow niches. Cell Stem Cell 8, 72–83 (2010).
11. Papayannopoulou, T. et al. Molecular pathways in bone marrow homing: doman tor/alpha(4)/beta(1) over beta(2)/integrins and selectins. Blood 98, 2403–2411 (2001).
12. Lebovson, A. & Kirschner, M. Activation of the WAVE complex by coincident signals controls actin assembly. Mol. Cell 36, 512–524 (2009).
13. Weiner, O. et al. An actin-based wave generator organizes cell motility. PLoS Biol. 5, e221 (2007).
14. Drevet, O. & Wozniak, H. Signaling networks that regulate cell migration. Cold Spring Harb. Perspect. Biol. 7, a009599 (2015).
15. Kurisu, S. & Takenawa, T. The WASP and WAVE family proteins. Genome Biol. 10, 226 (2009).
16. Chen, Z. et al. Structure and control of the actin regulatory WAVE complex. Nature 468, 533–538 (2010).
17. Ogami, T. et al. The actin polymerization regulator WAVE2 is required for early bone marrow repopulation by hematopoietic stem cells. Stem Cells 27, 1120–1129 (2009).
18. Baumgartner, S. et al. The HEM proteins: a novel family of tissue-specific transmembrane proteins expressed from invertebrates through mammals with an essential function in embryonic development. J. Mol. Biol. 291, 41–49 (1995).
19. Thomas, R. et al. Hem-1, a potential membrane protein, with expression restricted to blood cells. Biochim. Biophys. Acta 1090, 241–244 (1991).
20. Weiner, O. et al. Hem-1 complexes are essential for Rac activation, actin polymerization, and myosin regulation during neutrophil chemotaxis. PLoS Biol. 4, e38 (2006).
21. Park, H. et al. A point mutation in the murine Hem1 gene reveals an essential role for hematopoietic protein 1 in lymphopoiesis and innate immunity. J. Exp. Med. 205, 2899–2913 (2008).
22. Dinh, H., Scholz, G. & Hamilton, J. Regulation of WAVE1 expression in macrophages at multiple levels. J. Leukoc. Biol. 84, 1483–1491 (2008).
23. Kunda, P., Craig, G., Dominguez, V. & Baum, B. Ab, Sral, and Kette control the stability and localization of SCAR/WAVE to regulate the formation of actin-based protrusions. Curr. Biol. 13, 1867–1875 (2003).
24. Zhu, Z. & Bhat, K. The Drosophila Hem/Kettp/Nap1 protein regulates asymmetric division of neural precursor cells by regulating localization of Finside and Numb. Mech. Dev. 128, 483–495 (2011).
25. Fan, P., Cong, F. & Goff, S. Homo- and hetero-oligomerization of the c-Abl kinase and Abelson-interactor-1. Cancer Res. 63, 873–877 (2003).
26. Sato, M. et al. Identification and functional analysis of a new phosphorylation site (Y398) in the SH3 domain of Abi-1. FEBS Lett. 585, 834–840 (2011).
27. Tani, K. et al. Abi interactor 1 promotes tyrosine 296 phosphorylation of mammalian Efnan (Mena) by c-Abl kinase. J. Biol. Chem. 278, 21685–21692 (2003).
28. Jiang, J. & Hoffmann, F. Drosophila abl/onsen interacting protein (dAbi) is a positive regulator of abl/onsen tyrosine kinase activity. Oncogene 18, 5138–5147 (1999).
29. Leng, Y. et al. Abelson-interactor-1 promotes WAVE2 membrane translocation and Abelson-mediated tyrosine phosphorylation required for WAVE2 activation. Proc. Natl. Acad. Sci. USA 102, 1098–1103 (2005)
30. Maruoka, M. et al. Identification of B cell adaptor for PI3-kinase (BCAP) as an Abi interactor 1-regulated substrate of Abi kinases. FEBS Lett. 579, 2896–2890 (2005).
31. Lacout, C. et al. A defect in hematopoietic stem cell migration explains the nonrandom X-chromosome inactivation in carriers of Wiskott–Aldrich syndrome. Blood 102, 1282–1289 (2003).
32. Lo Celso, C. et al. Live-animal tracking of individual haematopoietic stem/ progenitor cells in their niche. Nature 457, 92–96 (2009).
33. Schwartzberg, P. L. et al. Mice homozygous for the abl/m1 mutation show poor viability and depletion of selected B and T cell populations. Cell 65, 1165–1175 (1991).
34. Tybulewicz, V. et al. Neonatal lethality and lymphopenia in mice homozygous disruption of the c-abl proto-oncogene. Cell 65, 1153–1164 (1991).
35. Tsifouglou, A. S., Bonovolais, I. D. & Tsifouglou, S. A. Multiple targeting of hematopoietic stem cell self-renewal, differentiation and apoptosis for leukemia therapy. Pharmacol. Ther. 122, 264–280 (2008).
36. Trautau, A., Pacheco, J. M. & Dingli, D. Reproductive fitness advantage of BCR-ABL expressing leukemia cells. Cancer Lett. 294, 43–48 (2010).
37. Sinclair, A., Latif, A. L. & Holyoke, T. L. Targeting survival pathways in chronic myeloid leukaemia stem cells. Br. J. Pharmacol. 169, 1693–1707 (2013).
38. Mani, M. et al. Wiskott–Aldrich syndrome protein is an effector of Kit signaling. Blood 114, 2900–2908 (2009).
39. Yang, F. et al. Rac and Cdc42 GTPases control hematopoietic stem cell shape, adhesion, migration, and mobilization. Proc. Natl. Acad. Sci. USA 98, 5614–5618 (2001).
40. Yang, L. et al. Rho GTPase Cdc42 coordinates hematopoietic stem cell quiescence and niche interaction in the bone marrow. Proc. Natl. Acad. Sci. USA 104, 5091–5096 (2007).
41. Pye, S. J. et al. The effects of imatinib on pregnancy outcome. Blood 111, 5505–5508 (2008).
42. Okuda, T., van Deursen, J., Hiebert, S. W., Grosveld, G. & Downing, J. R. AML1, the target of multiple chromosomal translocations in human leukemia, is essential for normal fetal liver hematopoiesis. Cell 84, 321–330 (1996).
43. Wang, Y., Schulte, B. A., LaRue, A. C., Ogawa, M. & Zhou, D. Total body irradiation selectively induces murine hematopoietic stem cell senescence. Blood 107, 358–366 (2006).
44. Shao, L. et al. Total body irradiation causes long-term mouse BM injury via induction of HSC premature senescence in an Ink4a- and Arf-independent manner. Blood 123, 3105–3115 (2014).
45. Chang, J. et al. Clearance of senescent cells by ABT263 rejuvenates aged hematopoietic stem cells in mice. Nat. Med. 22, 78–83 (2016).
46. Oostendorp, R. A., Audet, J. & Eaves, C. J. High-resolution tracking of cell division suggests similar cell cycle kinetics of hematopoietic stem cells stimulated in vitro and in vivo. Blood 95, 855–862 (2000).
47. Christopherson, K. W. II, Campbell, J. J., Travers, J. B. & Hromas, R. A. Low-molecular-weight heparins inhibit CCL21-induced T cell adhesion and migration. J. Pharmacol. Exp. Ther. 302, 290–295 (2002).
48. Yuan, Z. M. et al. Role for c-Abl tyrosine kinase in growth arrest response to DNA damage. Nature 382, 272–274 (1996).

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Author contributions
L.S. designed, performed, and analyzed most of the experiments and wrote the manuscript; J.C., W.F., X.W., E.A.W., Y.L., A.S., I.J.M. and A.P. performed and analyzed some experiments; J.D. provided Hem-1-knockout mice, interpreted the data, and revised the manuscript; I.J.M., D.S., C.P.L. and L.L. interpreted data and revised the manuscript; and D.Z. and R.H. conceived, designed, and supervised the study, analyzed and interpreted the data, and wrote the manuscript. All authors discussed the results and commented on the manuscript.

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