

## Rheb1 loss leads to increased hematopoietic stem cell proliferation and myeloid-biased differentiation *in vivo*

Xiaomin Wang,<sup>1\*</sup> Yanan Gao,<sup>1\*</sup> Juan Gao,<sup>1\*</sup> Minghao Li,<sup>1</sup> Mi Zhou,<sup>2</sup> Jinhong Wang,<sup>1</sup> Yakun Pang,<sup>1</sup> Hui Cheng,<sup>1</sup> Chase Yuan,<sup>3</sup> Yajing Chu,<sup>1</sup> Yu Jiang,<sup>4</sup> Jianfeng Zhou,<sup>2</sup> Hongbo R. Luo,<sup>1,5</sup> Zhenyu Ju,<sup>6</sup> Tao Cheng<sup>1</sup> and Weiping Yuan<sup>1</sup>

<sup>1</sup>State Key Laboratory of Experimental Hematology, Institute of Hematology and Blood Diseases Hospital, Center for Stem Cell Medicine, Chinese Academy of Medical Sciences and Peking Union Medical College, Tianjin, China; <sup>2</sup>Department of Hematology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, China; <sup>3</sup>College of Arts and Sciences, University of North Carolina at Chapel Hill, NC, USA; <sup>4</sup>Department of Pharmacology and Chemical Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA; <sup>5</sup>Department of Pathology, Harvard Medical School, Dana-Farber/Harvard Cancer Center, Boston, MA, USA and <sup>6</sup>Institute of Aging, Hangzhou Normal University, Hangzhou, China

\*XM, YG and JG contributed equally to this work.

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Correspondence: wpyuan@ihcams.ac.cn or chengtao@ihcams.ac.cn or zhenyuju@163.com

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**This document includes two supplementary tables, supplementary methods and six supplementary figures and their legends.**

**Supplementary Table 1** GO analysis of down-regulated genes (*Rheb1<sup>Δ/Δ</sup>* HSCs VS *Rheb1<sup>fl/fl</sup>* HSCs)

| GO Id      | Name                                      | Hits | p value   |
|------------|---|------|-----------|
| GO:0007155 | cell adhesion                             | 50   | 1.958E-14 |
| GO:0016337 | cell-cell adhesion                        | 12   | 4.616E-06 |
| GO:0031589 | cell-substrate adhesion                   | 6    | 2.485E-03 |
| GO:0030155 | regulation of cell adhesion               | 6    | 1.312E-05 |
| GO:0045785 | positive regulation of cell adhesion      | 6    | 3.806E-04 |
| GO:0048870 | cell motility                             | 31   | 1.007E-13 |
| GO:0016477 | cell migration                            | 30   | 2.863E-14 |
| GO:0060326 | cell chemotaxis                           | 10   | 3.741E-15 |
| GO:0051093 | regulation of developmental process       | 56   | 4.516E-10 |
| GO:0010942 | regulation of cell death                  | 3    | 1.206E-03 |
| GO:0045597 | regulation of cell differentiation        | 5    | 1.307E-06 |
| GO:0045766 | positive regulation of angiogenesis       | 1    | 7.128E-10 |
| GO:0045778 | positive regulation of ossification       | 3    | 0.0325    |
| GO:0048869 | cellular developmental process            | 124  | 1.977E-10 |
| GO:0030154 | cell differentiation                      | 116  | 4.703E-11 |
| GO:0032989 | cellular component morphogenesis          | 27   | 0.0014    |
| GO:0008152 | metabolic process                         | 299  | 1.00E-04  |
| GO:0006807 | nitrogen compound metabolic process       | 27   | 1.00E-04  |
| GO:0009056 | catabolic process                         | 35   | 0.0022    |
| GO:0009058 | biosynthetic process                      | 131  | 0.0063    |
| GO:0019748 | secondary metabolic process               | 15   | 0.0409    |
| GO:0042440 | pigment metabolic process                 | 3    | 0.0205    |
| GO:0042445 | hormone metabolic process                 | 5    | 0.0316    |
| GO:0008283 | cell proliferation                        | 67   | 3.00E-04  |
| GO:0008285 | negative regulation of cell proliferation | 53   | 1.00E-04  |
| GO:0048144 | fibroblast proliferation                  | 4    | 0.0342    |

**Supplementary Table 2** GO analysis of up-regulated genes (*Rheb1*<sup>Δ/Δ</sup> HSCs VS *Rheb1*<sup>fl/fl</sup> HSCs)

| GO Id      | Name   | Hits | p value |
|------------|--|------|---------|
| GO:0007154 | cell communication                                     | 94   | 0.0012  |
| GO:0007165 | signal transduction                                    | 85   | 0.0019  |
| GO:0007267 | cell-cell signaling                                    | 15   | 0.0089  |
| GO:009605  | response to external stimulus                          | 18   | 0.0264  |
| GO:0016477 | startle response                                       | 2    | 0.0372  |
| GO:0060326 | regulation of response to external stimulus            | 8    | 0.0247  |
| GO:0003001 | generation of a signal involved in cell-cell signaling | 56   | 0.0367  |
| GO:0007269 | neurotransmitter secretion                             | 4    | 0.0458  |
| GO:0046879 | hormone secretion                                      | 6    | 0.0271  |
| GO:0007162 | negative regulation of cell adhesion                   | 3    | 0.0361  |
| GO:0032616 | interleukin-13 production                              | 2    | 0.0484  |
| GO:0010817 | regulation of hormone levels                           | 10   | 0.0306  |

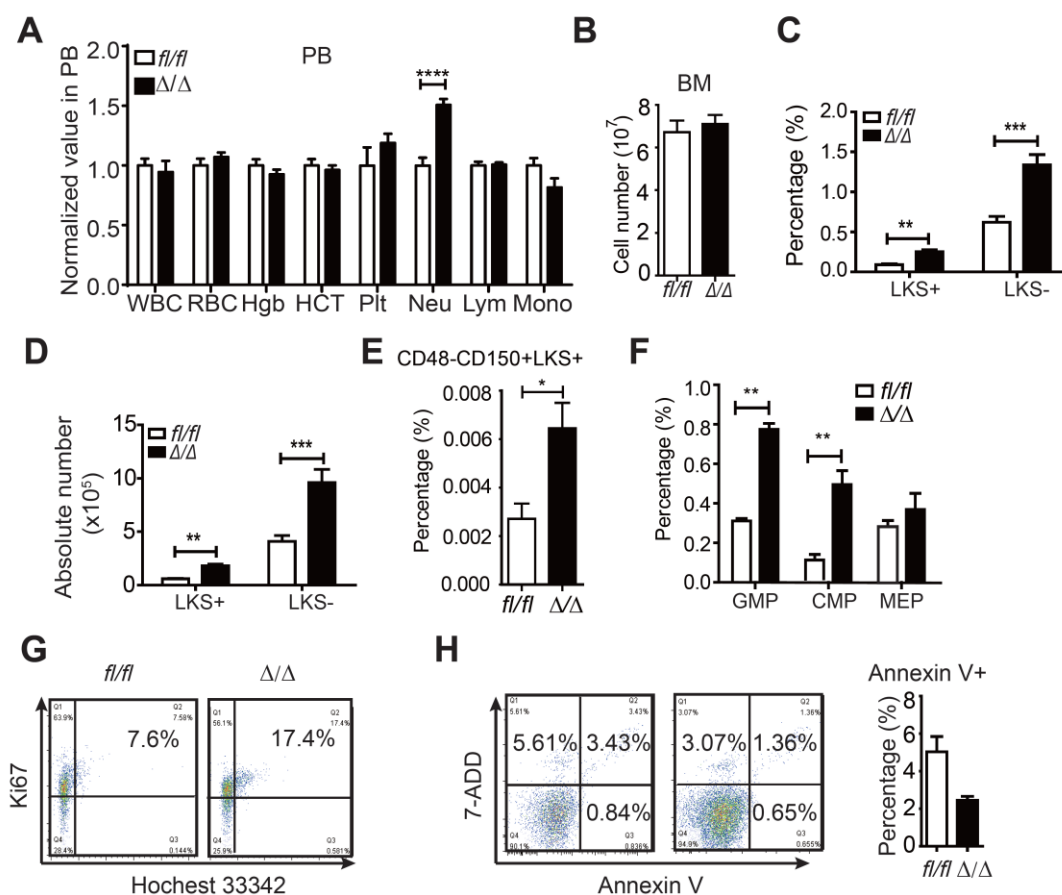
## Supplementary Methods

**Competitive bone marrow transplantation and 3BDO treatment.** Whole bone marrow cells (WBMCs) were isolated from the tibias, femurs and ilia of 8- to 10-week-old female *Rheb1<sup>fl/fl</sup>* (CD45.1) or *Rheb1<sup>Δ/Δ</sup>* mice (CD45.1). 3BDO was dissolved in DMSO at 60 mM. WBMCs were treated with 3BDO (60 nM) or DMSO for 30 min. Five million WBMCs (CD45.1) together with  $5 \times 10^5$  WBMCs from CD45.2 mice were intravenously injected into the lethally irradiated recipient mice (CD45.2). Then, the reconstitution of PB cells was analyzed by flow cytometry every four weeks after transplantation.

**Isolation of murine neutrophils.** WBMCs were added on a three-layer Percoll gradient (76%/62%/52%). After centrifugation, cells between the 78% and 69% layer were obtained, centrifuged, and resuspended. The cells were then added to the top of a Histopaque1119 layer. After centrifugation, the neutrophil-containing layer was separated and collected.

## Supplementary figures and their legends.

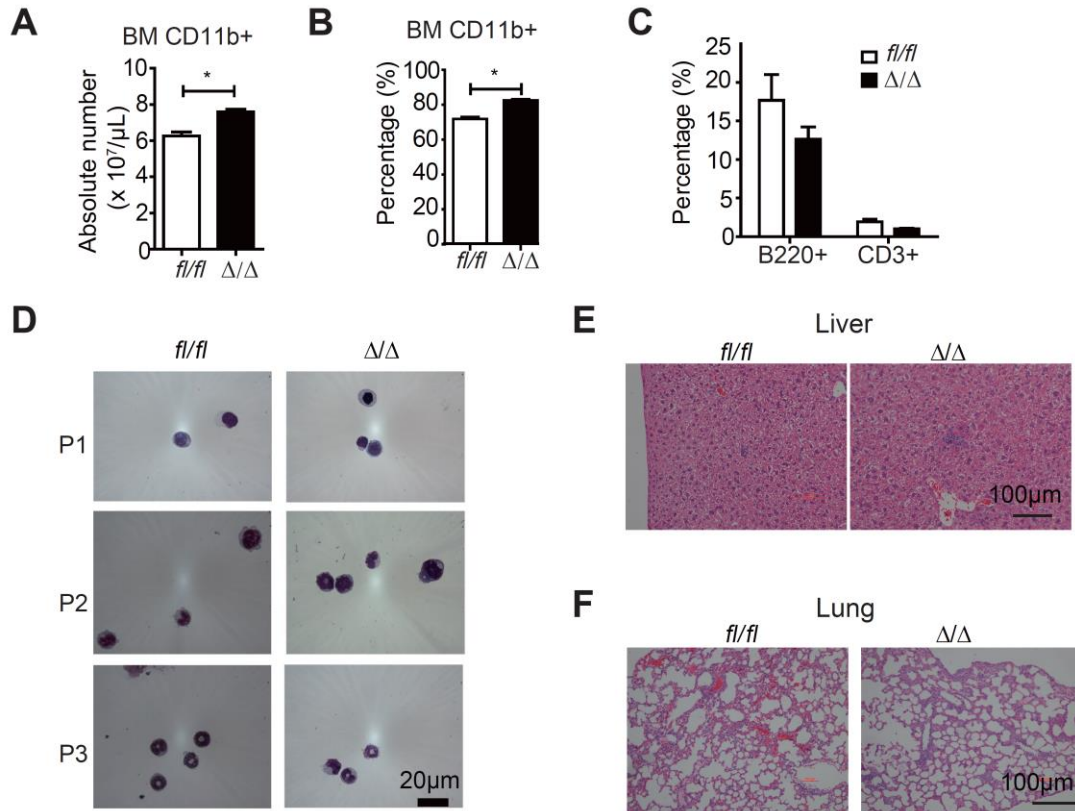
### Supplementary figure 1



### Supplementary Figure 1. The absolute number of HSC/HPCs was increased in BM of *Rheb1*<sup>Δ/Δ</sup> mice.

(A) The normalized value of various parameters in the peripheral blood (PB) of 8-week-old mice. (WBC: white blood cell, RBC: red blood cell, Hgb: hemoglobin, HCT: hematocrit, Plt: platelet, Neu: neutrophil, Lym: lymphocyte, Mono: monocyte), n=3. (B) The absolute cell number in *Rheb1*<sup>fl/fl</sup> and *Rheb1*<sup>Δ/Δ</sup> BM, n=3. (C-D) The absolute number and percentage of LKS<sup>+</sup> and LKS<sup>-</sup> populations in the BM of *Rheb1*<sup>fl/fl</sup> and *Rheb1*<sup>Δ/Δ</sup> mice, n=3. (E) Percentage of CD48<sup>-</sup>CD150<sup>+</sup>LKS<sup>+</sup> population in the BM of *Rheb1*<sup>fl/fl</sup> and *Rheb1*<sup>Δ/Δ</sup> mice, n=3. (F) Percentage of GMP, CMP and MEP populations in the BM of *Rheb1*<sup>fl/fl</sup> and *Rheb1*<sup>Δ/Δ</sup> mice, n=3. (G) Cell cycle of *Rheb1*<sup>fl/fl</sup> or *Rheb1*<sup>Δ/Δ</sup> LKS<sup>+</sup>. (H) The percentage of Annexin V-positive *Rheb1*<sup>fl/fl</sup> or *Rheb1*<sup>Δ/Δ</sup> LKS<sup>+</sup>. The data are presented as the mean ± SEM.

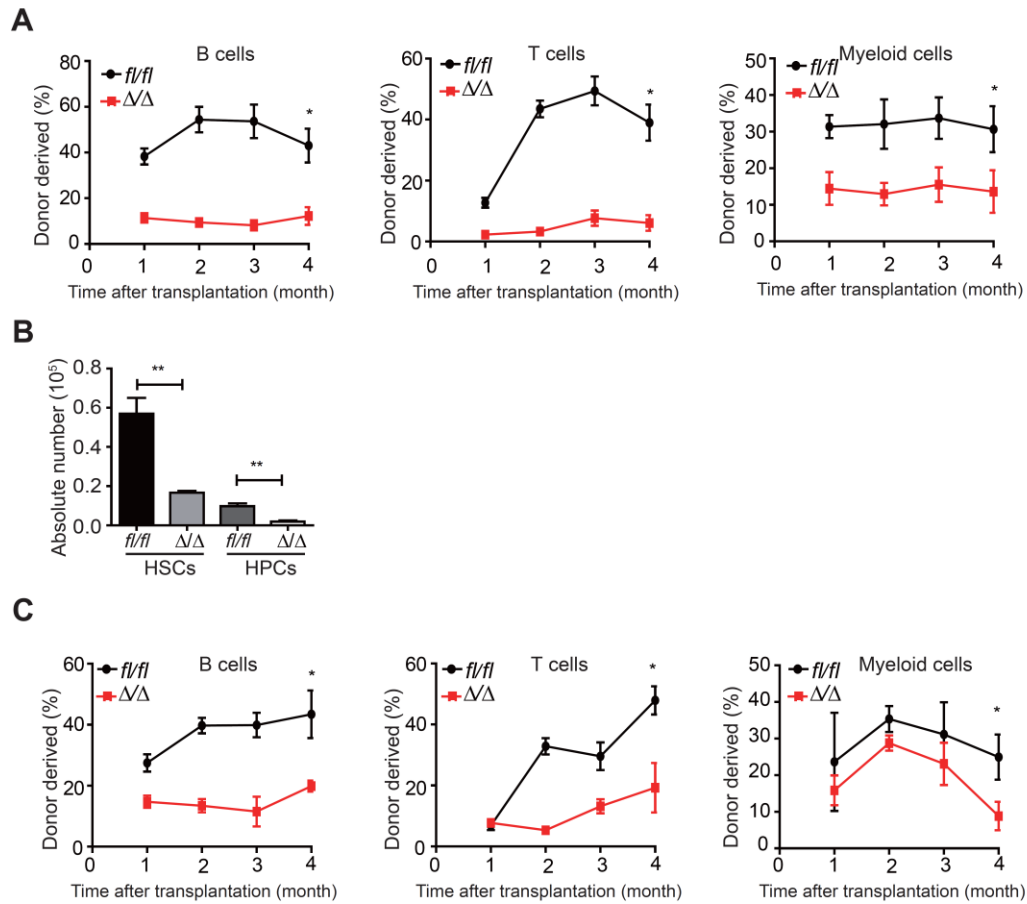
## Supplementary figure 2



### Supplementary Figure 2. The absolute number of myeloid cells was increased in BM of *Rheb1* <sup>$\Delta/\Delta$</sup> mice.

(A-B) The absolute number and percentage of myeloid cells in the BM of *Rheb1*<sup>*fl/fl*</sup> and *Rheb1* <sup>$\Delta/\Delta$</sup>  mice, n=3. (C) The percentages of B (B220<sup>+</sup>) and T (CD3<sup>+</sup>) cells in the BM of *Rheb1*<sup>*fl/fl*</sup> and *Rheb1* <sup>$\Delta/\Delta$</sup>  mice. (D) Representative images of Wright-Giemsa staining of the sorted P1-P3 neutrophils from BM. (E-F) HE-stained liver and lung sections of *Rheb1*<sup>*fl/fl*</sup> and *Rheb1* <sup>$\Delta/\Delta$</sup>  mice. The data are presented as the mean  $\pm$  SEM.

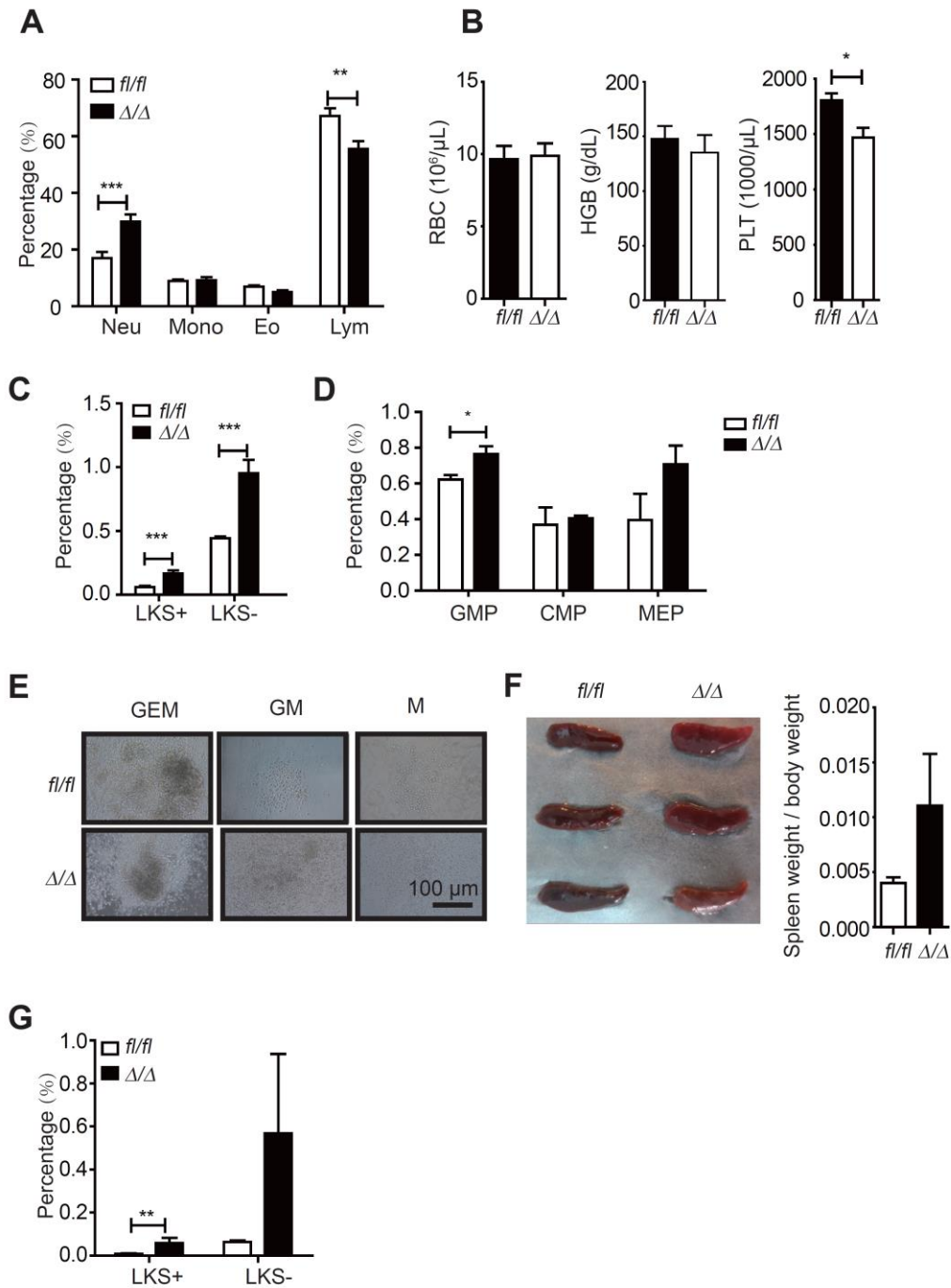
## Supplementary figure 3



### Supplementary Figure 3. Rheb1 deficiency reduced all lineages of hematopoietic cell upon transplantation.

(A) Donor-derived-cell chimerism in the PB of mice transplanted with *Rheb1<sup>fl/fl</sup>* or *Rheb1<sup>Δ/Δ</sup>* whole bone marrow cells (WBMCs), n=6. (B) The absolute number of HSCs and HPCs in BM of *Rheb1<sup>fl/fl</sup>* and *Rheb1<sup>Δ/Δ</sup>* mice under sublethal irradiation, n=3. (C) Donor-derived-cell chimerism in the PB of mice transplanted with *Rheb1<sup>fl/fl</sup>* or *Rheb1<sup>Δ/Δ</sup>* LKS<sup>+</sup> cells, n=6. The data are presented as the mean ± SEM.

## Supplementary figure 4



### Supplementary Figure 4. The phenotypes of aged *Rheb1* <sup>$\Delta/\Delta$</sup> mice.

(A) The percentage of neutrophils, monocytes, erythrocytes, and lymphocytes in PB of 2 year-old *Rheb1*<sup>*fl/fl*</sup> and *Rheb1* <sup>$\Delta/\Delta$</sup>  mice, n=6. (B) The number of RBC, HGB and PLT in PB, n=6. (C) The percentage of LKS<sup>+</sup> cells and LKS<sup>-</sup> in the BM, n=3. (D) The percentage of GMP, CMP and MEP populations in the BM of 2 year-old *Rheb1*<sup>*fl/fl*</sup> and



*Rheb1<sup>Δ/Δ</sup>* mice, n=3. (E) Whole bone marrow cells were isolated from 2 year-old mice and plated in M3434 methylcellulose. Representative colonies were shown. Colonies are defined as colony forming unit monocyte (CFU-M), granulocyte-macrophage (CFU-GM), and granulocyte-erythroid-macrophage (CFU-GEM). Scale bars, 100 μm. (F) The weight of spleens in 2 year-old *Rheb1<sup>fl/fl</sup>* and *Rheb1<sup>Δ/Δ</sup>* mice, n=6. (G) The percentage of LKS<sup>+</sup> and LKS<sup>-</sup> cells in spleen of 2 year-old *Rheb1<sup>fl/fl</sup>* and *Rheb1<sup>Δ/Δ</sup>* mice, n=3. The data are presented as the mean ± SEM.

## Supplementary figure 5

**A**

<https://dcc.icgc.org/>

| Project | site  | Tumor Type                   | Donors affected | Mutations |
|---------|-------|------------------------------|-----------------|-----------|
| AML     | Blood | Acute myeloid leukemia       | 2/117 (1.71%)   | 2         |
| CLL     | Blood | Chronic Lymphocytic leukemia | 2/218 (0.92%)   | 2         |

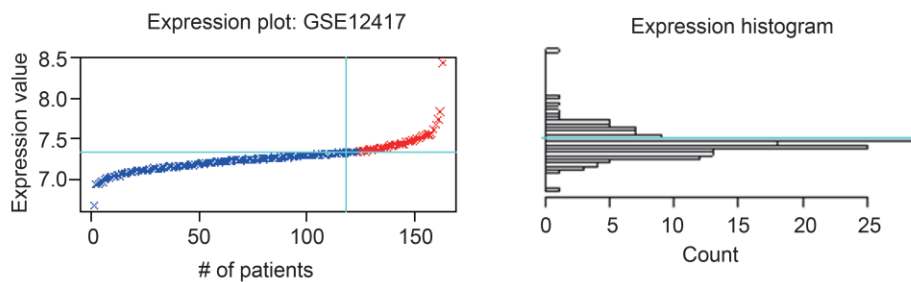
Mutations in Acute myeloid leukemia

| ID         | DNA change          | Type                     | Consequence |
|------------|---------------------|--------------------------|-------------|
| MU67505397 | chr7:g.151180972G>A | single base substitution | Intron:Rheb |
| MU67566392 | chr7:g.151202183G>A | single base substitution | Intron:Rheb |

Mutations in Chronic lymphocytic leukemia

| ID         | DNA change          | Type                     | Consequence |
|------------|---------------------|--------------------------|-------------|
| MU54194519 | chr7:g.151207184C>G | single base substitution | Intron:Rheb |
| MU52863043 | chr7:g.151167362C>- | deletion of <=200bp      | Intron:Rheb |

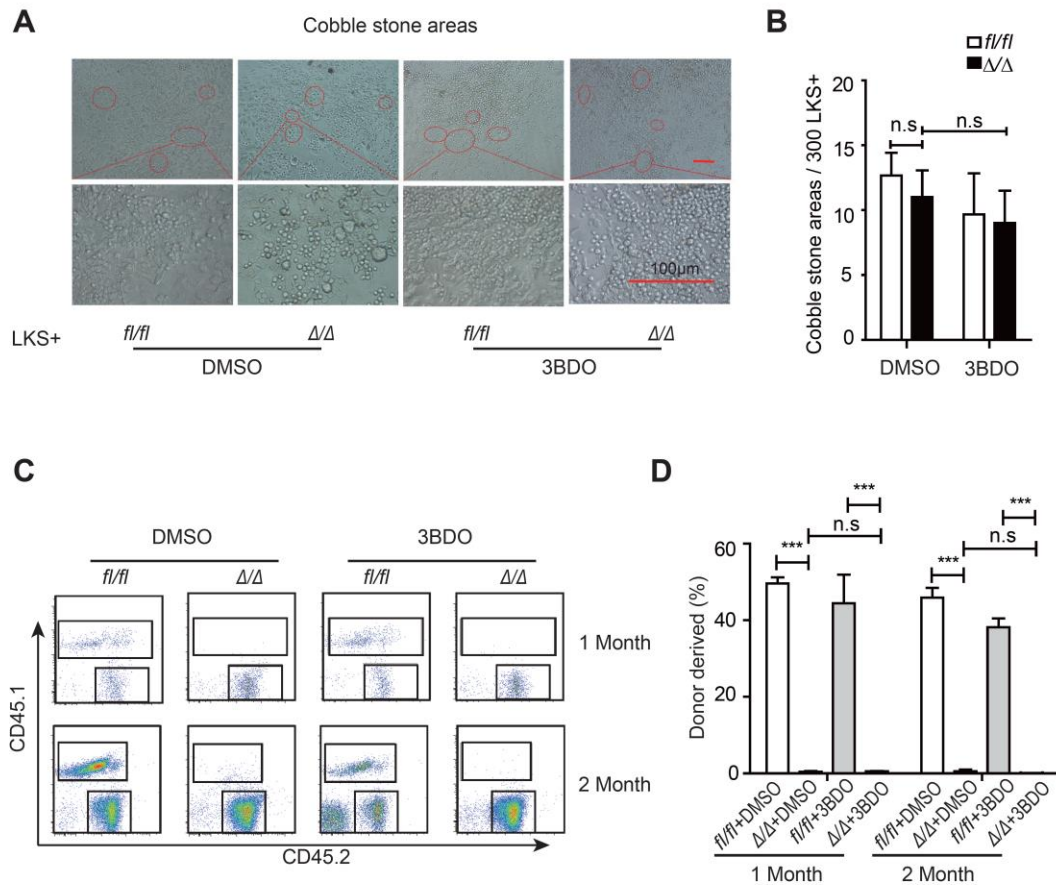
**B**



### Supplementary Figure 5. The mutations of *RHEB* gene in leukemia patients.

(A) The mutation of *RHEB* gene in AML patients and ALL patients (<http://www.cbioportal.org/>). (B) The expression value of *RHEB* in AML patients with normal karyotype (<http://www.abren.net/PrognoScan-cgi/>, GSE12417).

## Supplementary figure 6



### Supplementary Figure 6. 3BDO did not rescue the defect of *Rheb1* <sup>$\Delta/\Delta$</sup> HSCs upon transplantation.

(A-B) LKS<sup>+</sup> cells were treated with 3BDO or DMSO and were inoculated on the MSCs in M5300 for 5 weeks. Colonies were counted 5 weeks after plating, n=3. Representative cobblestone-forming areas were shown. (C-D) Donor-derived-cell chimerism in the PB of mice transplanted with *Rheb1* <sup>$fl/fl$</sup>  or *Rheb1* <sup>$\Delta/\Delta$</sup>  WBMCs treated with 3BDO or DMSO (see method in supplemental method section), n=3. The data are presented as the mean  $\pm$  SEM.