RAP-536 induces reticulocyte maturation in wild-type mice, increases survival of β-thalassemic reticulocytes, and increases red blood cells in a mouse model of α-thalassemia

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Presenting author disclosures

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Introduction, objectives, and methods

Introduction

- Luspatercept, and its murine analog RAP-536, have been shown to act as erythroid maturation agents via their effects on late-stage erythropoiesis by inducing erythroblast maturation, leading to increases in RBCs and Hb levels[1–5]

Objectives

- To investigate the effects of RAP-536 in mice by mathematical modeling, to test whether the observed increase in reticulocyte maturation is relevant in a murine model of β-thalassemia, and to assess whether RAP-536 is effective in a murine model of α-thalassemia

Methods

- RAP-536 was administered (s.c. or i.p.) at doses of 10 mg/kg and 30 mg/kg to 8–14-week-old, both male and female mice (WT control, α-thalassemia model [129S-*Hba-a1*^{tm1Led}/J], and β-thalassemia model [th3/+; B6.129P2-*Hbb-b1*^{tm1Unc} *Hbb-b2*^{tm1Unc}/J])
- Complete blood counts and reticulocyte analyses were conducted via hematology analyzer
- Flow cytometry analysis of BM, blood, and spleen cell suspensions were conducted
- A mathematical model was used to describe the successive terminal stages of erythropoiesis in BM, blood, and spleen

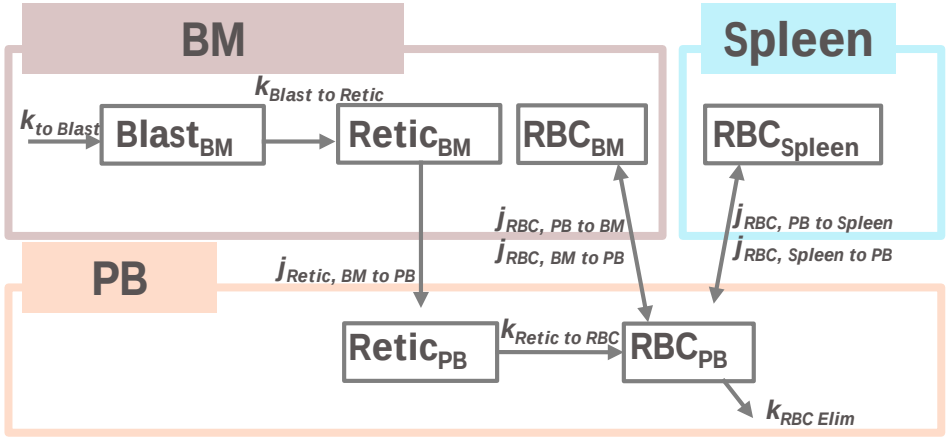
BM, bone marrow; Hb, hemoglobin; i.p., intraperitoneal; RBC, red blood cell; s.c., subcutaneous; WT, wild type.

1. Suragani RNVS, et al. *Nat Med* 2014;20:408–414. 2. Attie KM, et al. *Am J Hematol* 2014;89:766–770. 3. Cappellini MD, et al. *N Engl J Med* 2020;382:1219–1231. 4. Fenaux P, et al. *N Engl J Med* 2020;382:140-151. 5. Suragani RNVS, et al. *Blood* 2014;123:3864–3872.

Results

Figure 1. Mathematical model of late-stage erythropoiesis in BM, blood, and spleen

Model compartmental structure



- Maturation and translocation parameters are reported as *k* and *j*, respectively
- Mathematically, the model is a system of ordinary differential equations, with first-order transfer processes (only erythroblast formation [$k_{to\ Blast}$] is zero-order)
- Under homeostatic conditions, all compartments have a stable cell level, whereas upon RAP-536 dosing, parameter values are perturbed according to model fit to data

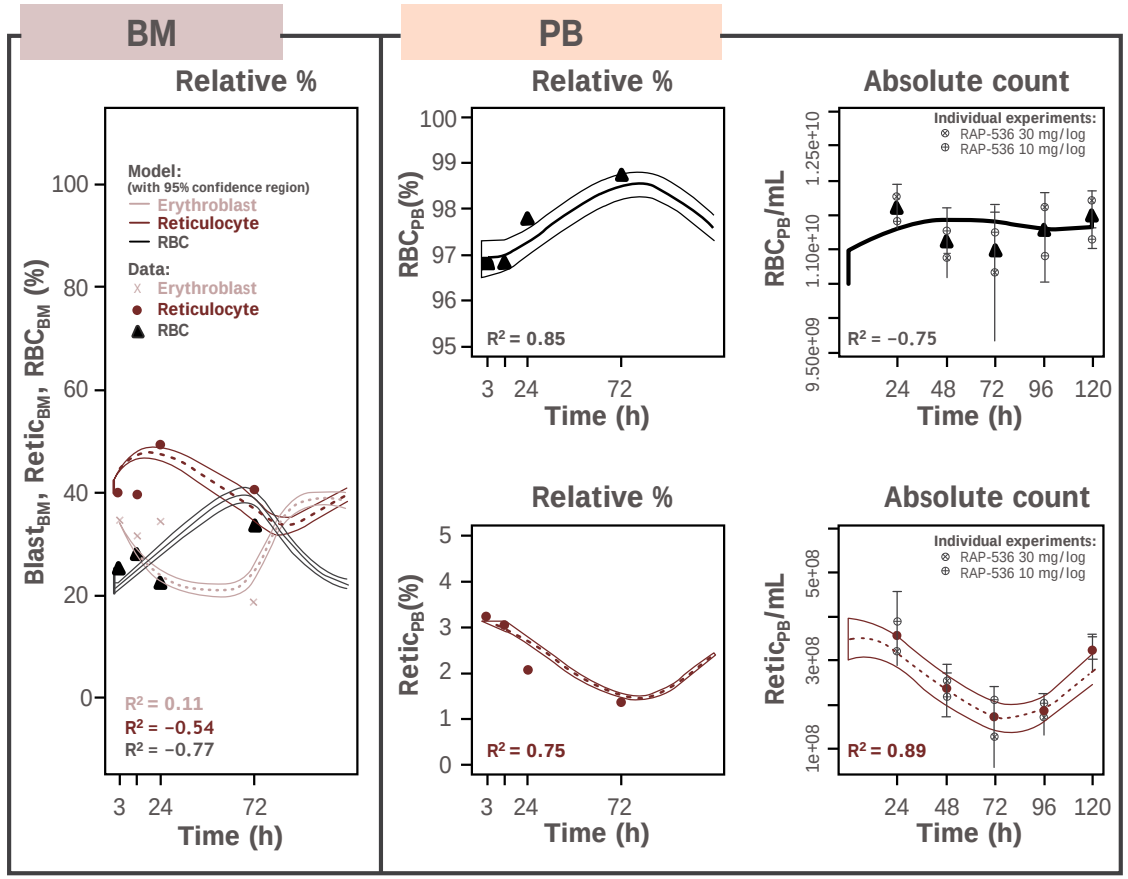
Parameter	Definition
$k_{to\ Blast}$	Rate of erythroblast formation in BM
$k_{Blast\ to\ Retic}$	Rate of erythroblast conversion to reticulocyte in BM
$j_{Retic,\ BM\ to\ PB}$	Rate of reticulocyte transfer from BM to PB
$k_{Retic\ to\ RBC}$	Rate of reticulocyte conversion to erythrocyte in PB
$k_{RBC\ Elim}$	Rate of erythrocyte elimination in PB
$j_{RBC,\ PB\ to\ BM}$	Rate of erythrocyte transfer from PB to BM
$j_{RBC,\ BM\ to\ PB}$	Rate of erythrocyte transfer from BM to PB
$j_{RBC,\ PB\ to\ Spleen}$	Rate of erythrocyte transfer from PB to spleen
$j_{RBC,\ Spleen\ to\ PB}$	Rate of erythrocyte transfer from spleen to PB

Blast, erythroblast; Elim, elimination; PB, peripheral blood; Retic, reticulocyte.

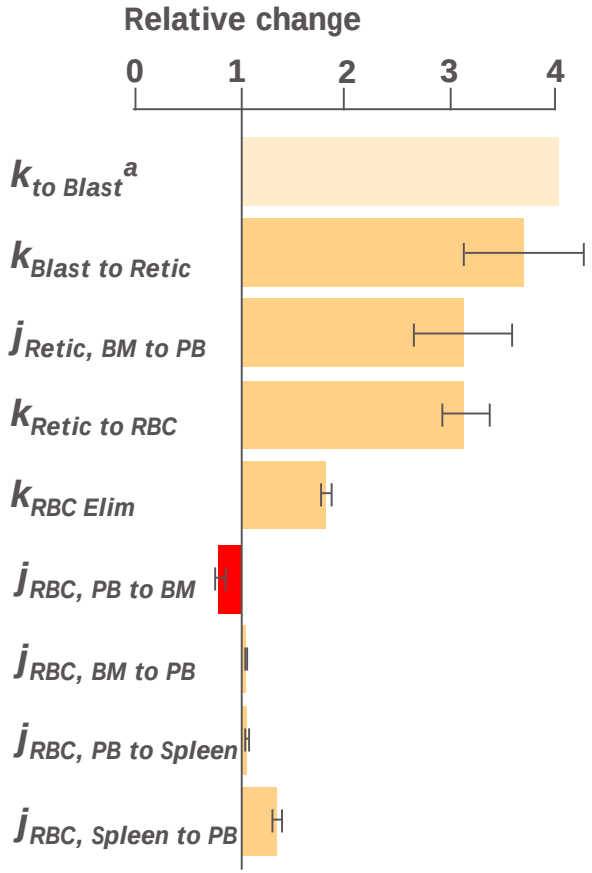
Results (cont.)

Figure 2. Mathematical modeling of RAP-536–induced modulations of late-stage erythropoiesis in BM, blood, and spleen

A Model best fit to experimental data of RAP-536 administration in WT mice



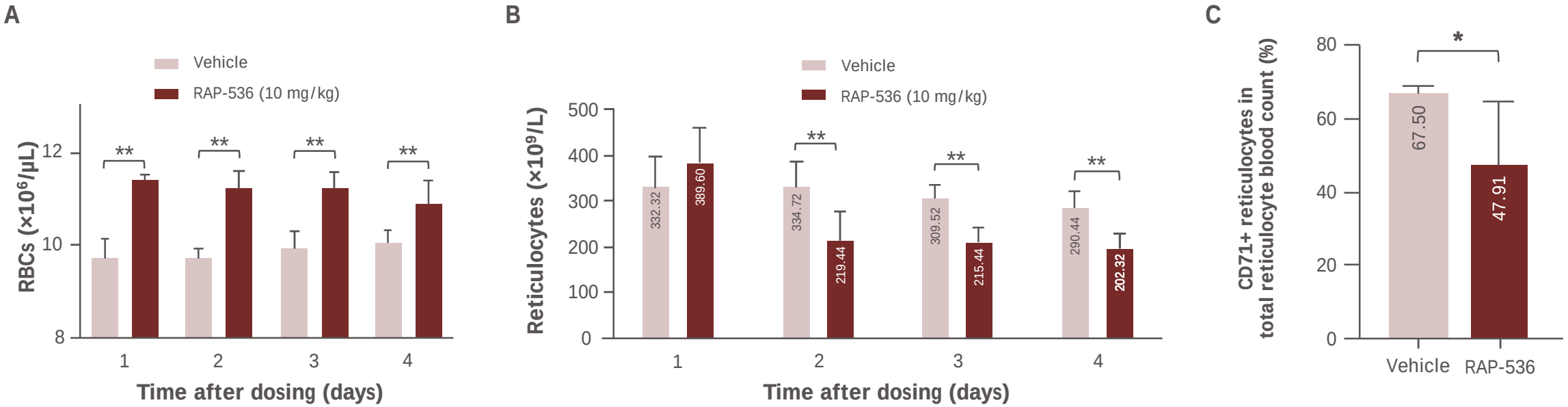
B Comparison of homeostatic versus RAP-536-perturbed parameter values



[a] $k_{to\ Blast}$ change occurs with a time delay as opposed to all other parameters.

Results (cont.)

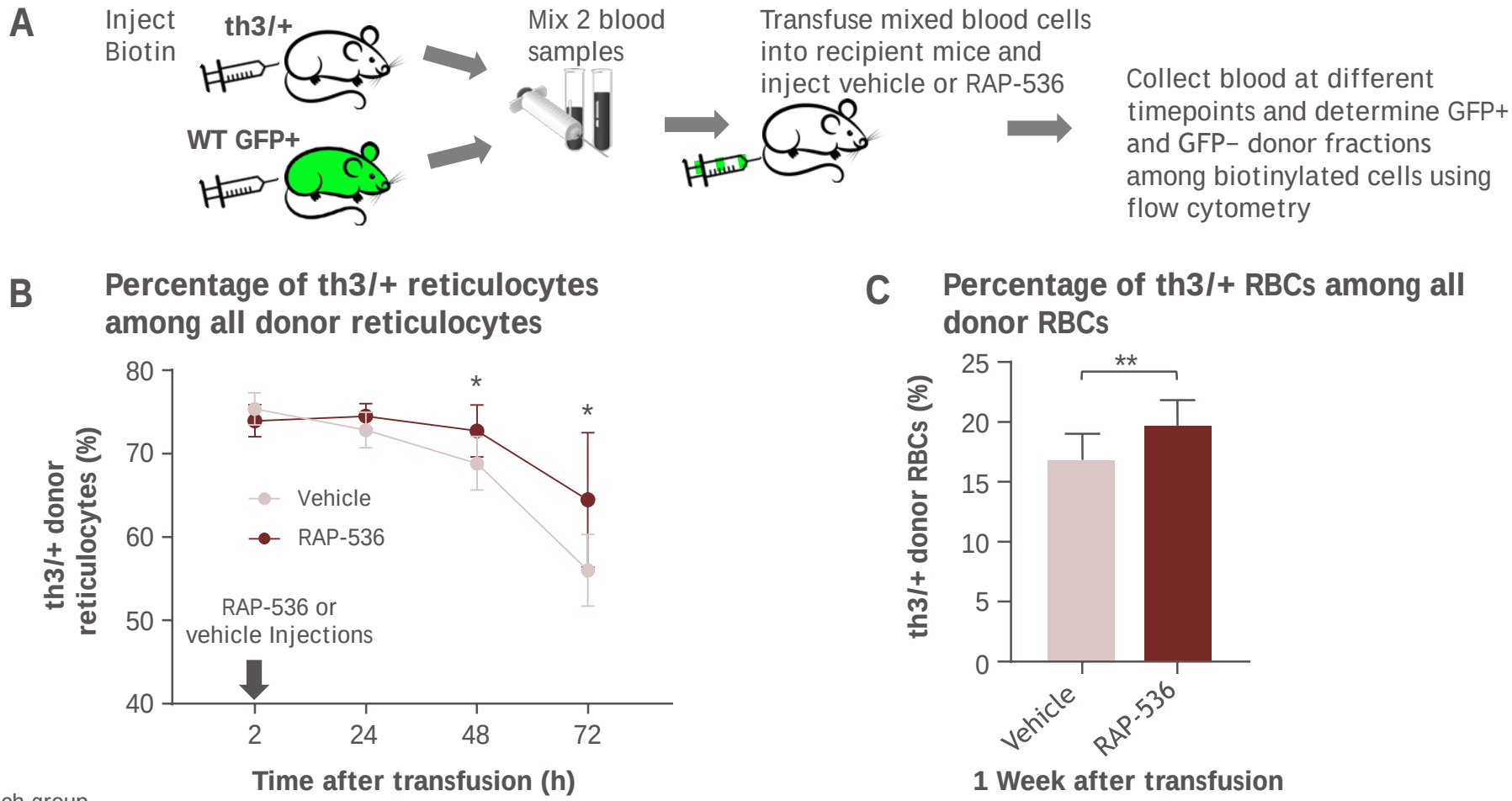
Figure 3. Effect of RAP-536 on (A) RBC[a], (B) reticulocyte levels[b], and (C) reticulocyte maturation in WT mice



[a]N = 5 for each group.
*$P < 0.05$; **$P < 0.01$.

Results (cont.)

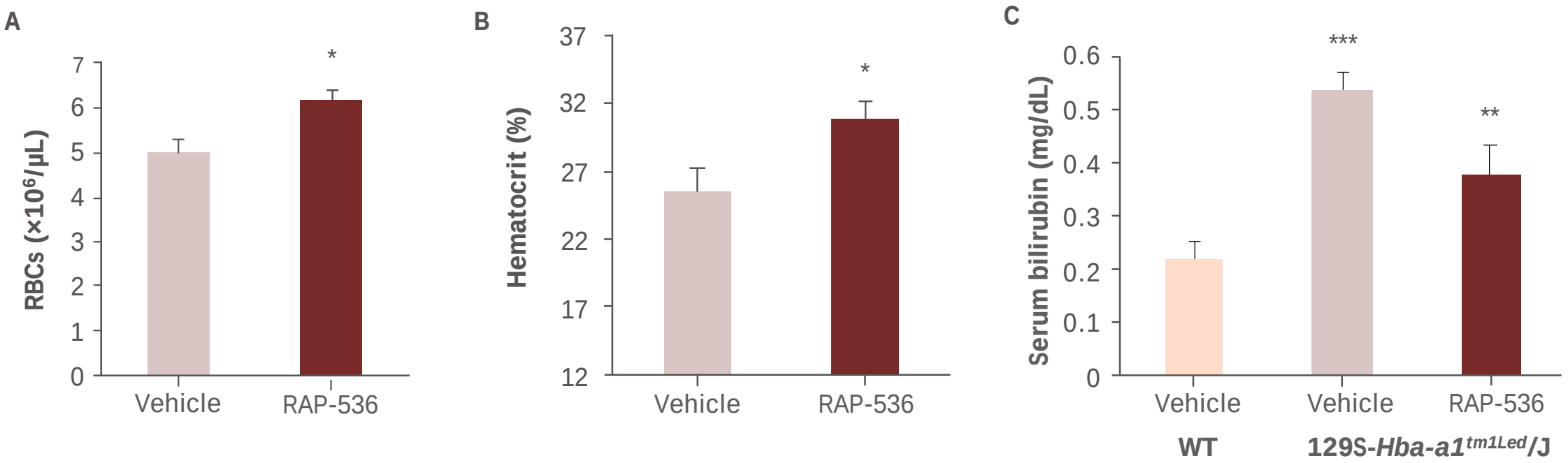
Figure 4. RAP-536 increases th3/+ reticulocyte persistence and RBC production from th3/+ reticulocytes



N = 8 recipient mice for each group.
*$P < 0.05$; **$P < 0.01$.
GFP, green fluorescent protein.

Results (cont.)

Figure 5. Effect of RAP-536 on (A) RBCs, (B) hematocrit, and (C) serum bilirubin levels in an α-thalassemia mouse model



*P < 0.05; **P < 0.01; ***P < 0.001.

Summary

- These results confirm that RAP-536 is an erythroid maturation agent, which in addition to modulating erythroblast maturation, also modulates reticulocyte maturation
- Mathematical modeling results indicated that RAP-536 stimulates erythroblast production, conversion of erythroblasts to reticulocytes and reticulocytes to RBCs, and the transfer of BM reticulocytes to blood
- RAP-536 also prolonged the peripheral persistence of th3/+ reticulocytes and produced more th3/+ RBCs from these th3/+ reticulocytes
- In a murine model of α-thalassemia, RAP-536 increased RBCs and hematocrit and reduced hemolysis, as assessed by serum bilirubin
- Overall, these preclinical data suggest that luspatercept has the potential to improve anemias associated with hemolysis

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