Supplemental Methods

Animals and human tissues. NOD.CB17-Prkdc^{scid}/J (NOD/SCID), NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ (NOD/SCID/$\gamma c^{-/-}$ or NSG), and B6.129-Itgp^{tm1Fpl} (CD47 KO B6) mice were purchased from the Jackson Laboratory (Bar Harbor, ME), and were housed in a specific pathogen-free microisolator environment and used in experiments at 6 to 10 weeks of age. GFP-transgenic CD47 KO mice were generated by crossing CD47 KO mice with C57BL/6-transgenic (UBC-GFP)30Scha/J mice (The Jackson Laboratory). Human fetal thymus and liver tissues of gestational age of 17 to 20 weeks were obtained from Advanced Bioscience Resource (Alameda, CA). Protocols involving the use of human tissues and animals were approved by the Human Research Committee and Subcommittee on Research Animal Care of Columbia University Medical Center (New York, NY).

Humanized mice preparation. Humanized mice were made as described in our previous studies.[1; 2] In brief, NOD/SCID or NSG mice were conditioned with sublethal (2.25 Gy) total body irradiation, and received intravenous infusion of 1-5×10^5 human CD34$^+$ fetal liver cells (FLCs) alone, or with a 1 mm^3 human fetal thymic tissue fragment transplanted under the recipient kidney capsule from the same fetal donor, as previously described.[1;3;4] Levels of human hematopoietic cells in humanized mice were determined by flow cytometric (FCM).

Macrophage depletion in mice. Macrophage depletion *in vivo* was performed by intravenous injection of liposome-encapsulated clodronate (dichloromethylene diphosphonate; CL2MDP).[5] Clodronate-liposomes were given at 100 μl per mouse for the 1st injection, and either 50 μl or 100 μl per mouse thereafter, with an interval of 2-7 days as indicated. Control mice were injected with liposome-encapsulated PBS (PBS-liposomes) at the same time points. Depletion of macrophages was determined by measuring the ability of mice to clear CD47$^{-/-}$ mouse RBCs and/or by assaying F4/80$^+$ macrophages via immunohistochemical staining in randomly selected mice, and efficient depletion was shown by the lack of rejection of CD47$^{-/-}$ RBCs and of F4/80$^+$cells in the treated mice, as previously described [2].

Flow cytomeric analysis. Levels of human hematopoietic cells in humanized mice were determined by flow cytometric (FCM) analysis using various combinations of the following mAbs: anti-human CD45, CD19, CD3, CD4, CD8, CD61, CD42a; anti-mouse CD45 and CD41; and isotype controls (all purchased from BD PharMingen, San Diego, CA). Peripheral blood was collected from tail vein into heparinized tubes. Mononuclear cells were purified by density gradient centrifugation with Histopaque 1077 (Sigma-Aldrich, St. Louis, MO). Platelets were prepared as previously described.[6] Briefly, blood was diluted with PBS and spun at 100g for 15 min. Supernatant containing platelets was collected, washed and pelleted (1,000g for 10 min) before use. All samples were collected on a FACSCalibur or LSR II (Becton Dickinson,

Mountain View, CA) and analysis performed with FlowJo (TreeStar, Ashland, OR). Dead cells were excluded from the analysis by gating propidium iodide negative cells. Small numbers of human and mouse platelet aggregates (i.e., platelets stained positive for both huCD42a$^+$ and muCD41$^+$) were excluded from the calculation of human platelet chimerism.

Immunohistochemistry

Femur and tibia collected from control and humanized mice were fixed in 10% buffered formalin and embedded in paraffin. Immunohistochemistry was performed with primary antibody mouse anti-human CD61 (clone Y2/51, DAKO) and biotinylated secondary antibody horse anti mouse IgG, and human CD61$^+$ cells (brown color) were visualized by the avidin-biotin peroxidase complex (ABC) technique. Sections were counterstained with Hematoxylin. The slides were examined under a Zeiss microscope equipped with a 20×/0.5 or 40×/0.75 objective lens and photographed using a Nikon Coolpix 5000 digital color camera.

Reference

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2. Hu Z, Van Rooijen N, Yang YG. Macrophages prevent human red blood cell reconstitution in immunodeficient mice. *Blood*. 2011;118:in press.
3. Lan P, Wang L, Diouf B, et al. Induction of human T-cell tolerance to porcine xenoantigens through mixed hematopoietic chimerism. *Blood*. 2004;103(10):3964-3969.
4. Tonomura N, Habiro K, Shimizu A, Sykes M, Yang YG. Antigen-specific human T-cell responses and T cell-dependent production of human antibodies in a humanized mouse model. *Blood*. 2008;111(8):4293-4296.
5. Van Rooijen N, Sanders A. Liposome mediated depletion of macrophages: mechanism of action, preparation of liposomes and applications. *J Immunol Methods*. 1994;174(1-2):83-93.
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Supplemental Figure

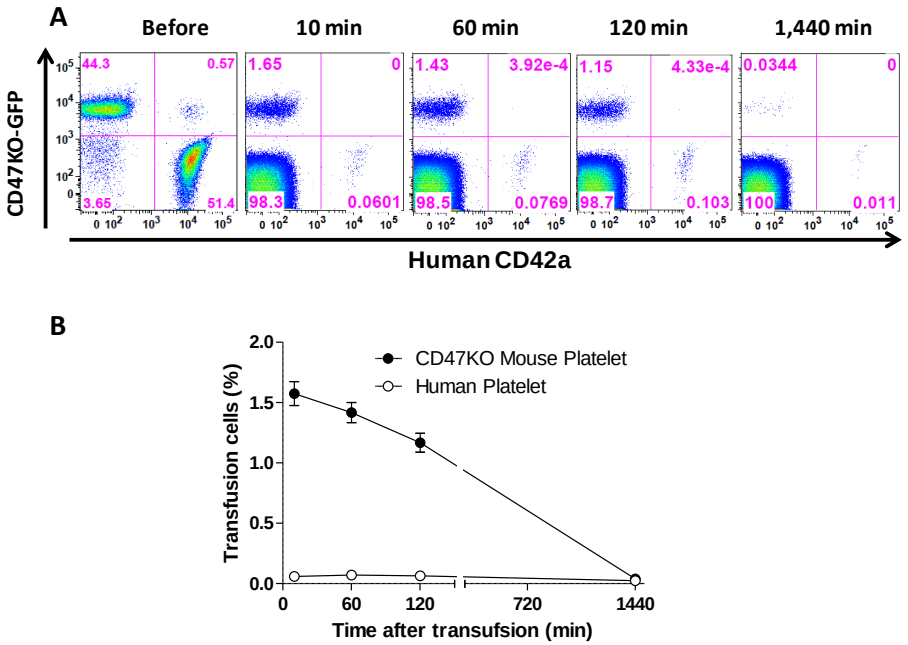

Figure S1. Human platelets are more rapidly cleared than CD47 KO mouse platelets in NSG mice. Human platelets were mixed with CD47 KO-GFP C57BL/6 mouse platelets at 1:1 ratio, and $4x10^7$ platelets (2×10^7 each) were injected i.v. into NSG mice (n=4). Blood was collected as the indicated times and the levels of surviving human and CD47 KO mouse platelets were measured by flow cytometry. (A) Representative flow cytometric profiles. (B) Percentages (mean ± SEMs) of human $CD42a^+$ and mouse $CD41^+$ platelets in blood.