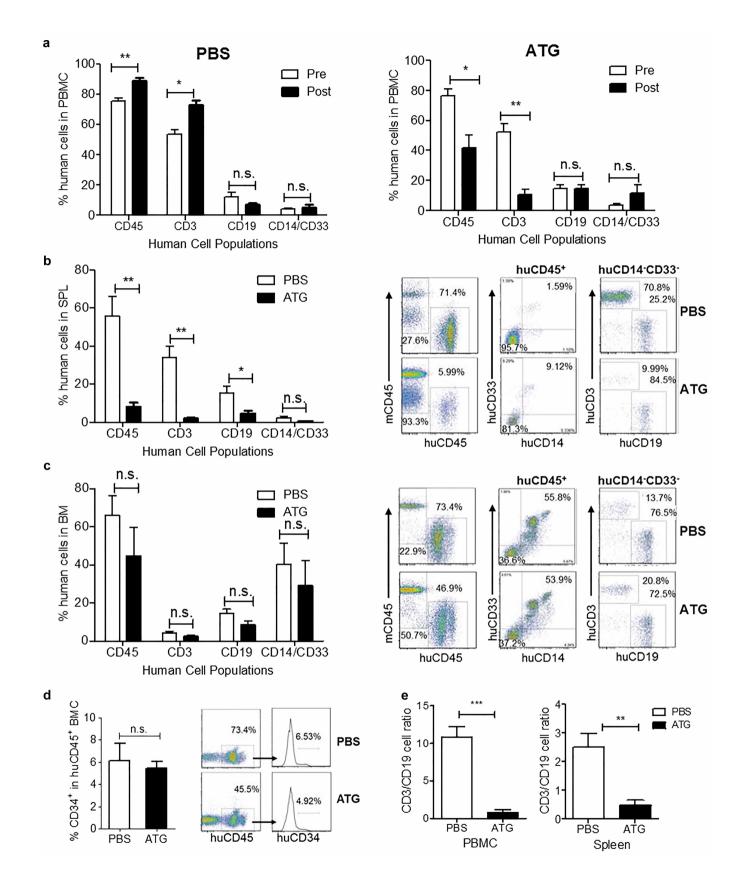
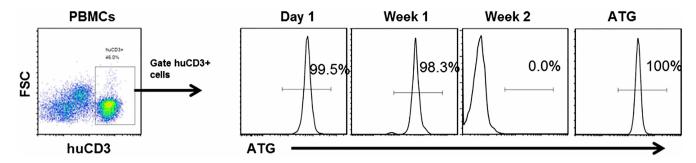


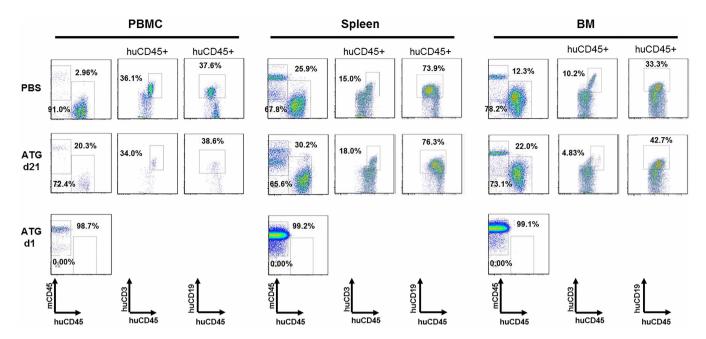
Supplementary Figure S1 Depletion of human thymocytes by ATG in humanized mice. Humanized mice were prepared by transplantation of human fetal thymus and CD34+ cells, and 13 weeks later injected i.v. with ATG-G (3 mg kg per injection at day 0 and day 2, and 30 mg kg⁻¹ at day 4) or PBS (these are the same mice as shown in Figure 1). Shown are results of a representative human thymic graft analyzed 7 days after the last injection of ATG. (a) Macroscopic appearance. (b) Histology (hematoxylin and eosin).



Supplementary Figure S2 Comparison of human cell depletion in various tissues by ATG in humanized mice. Humanized mice were constructed by transplantation of human fetal thymus and CD34+ cells, and 17 weeks later received three injections i.v. of ATG-F (3 mg kg per injection at day 0 and day 2, and 30 mg kg⁻¹ at day 4) or PBS. (a) Levels (%) of human CD45+, CD3+, and CD19+ cells in peripheral blood measured 10 days prior to the fist injection and 7 days after last injection of ATG or PBS. (b-d) Levels (%) and FACS profiles of human CD45+, CD3+, CD19+, and CD14+CD33+ cells in the spleen (b) and bone marrow (c), and of human CD34+ cells in bone marrow (d) measured 7 days after last injection of ATG or PBS. Data presented are mean ± SEMs (n = 4 per group). (e) Ratios of human CD3+ to CD19+ cells in PBMCs and spleen from PBSinjected and ATG-treated humanized mice. *P < 0.05; **P < 0.01; ***P < 0.001. Similar results were obtained from another independent experiment (not shown).



Supplementary Figure S3 Biological activity of ATG in immunodeficient mice. NSG mice were treated with ATG-F (10 mg kg^{-1} , n = 4), and sera were collected at day 1, week 1, and week 2 post-treatment for measuring ATG activity. Briefly, we incubated human PBMCs with serum samples collected at the indicated times or ATG (positive control), followed by staining with fluorochrome-conjugated goat anti-rabbit antibody, and ATG binding to human CD3+cells was assessed by flow cytometry.



Supplementary Figure S4 ATG depletes donor HSCs prior to their settling in the bone marrow. NSG mice were transplanted with human fetal thymus and CD34+ cells, followed 1 day later by injection i.v. of ATG-F (10 mg kg⁻¹; ATG d1), or 21 days later by injection i.v. of ATG-F (30 mg kg⁻¹; ATG d21) or PBS. Mice were killed 17 weeks after transplantation for measuring human chimerism. Shown are representative staining profiles of human CD45+ hematopoietic, CD3+ T, and CD19+ B cell chimerism in PBMCs, spleen, and bone marrow (BM) cells.