

Supplemental Figure S1

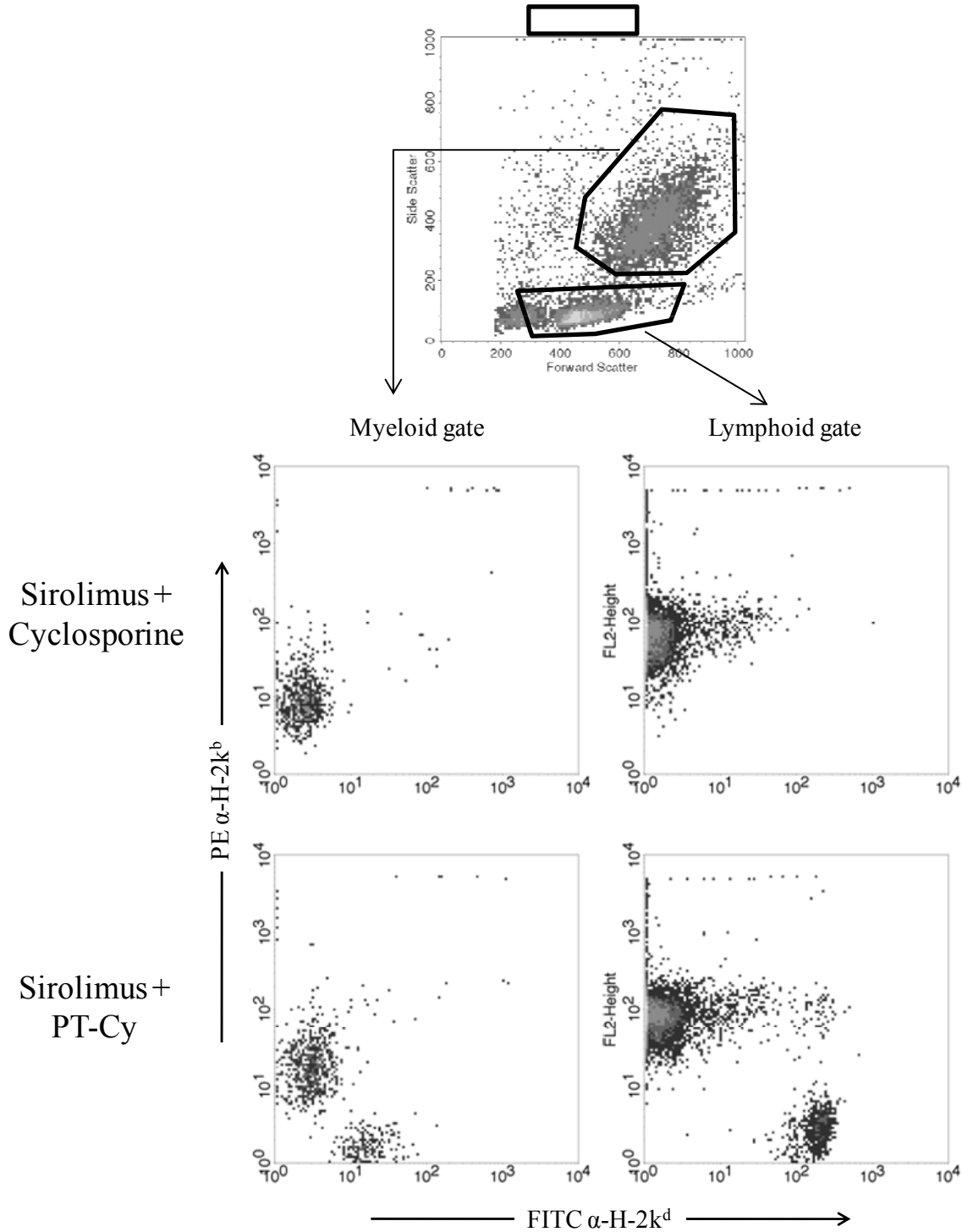


Figure S1. Flow cytometry to evaluate donor chimerism levels. Flow cytometry results from two representative mice performed at 16 weeks post-transplant. After red blood cell lysis, peripheral blood obtained from recipient mice was stained with a FITC-conjugated monoclonal antibody to the K^d antigen (donor) and a PE-conjugated monoclonal antibody to the K^b antigen (recipient). The left panels reveal myeloid gating and the right panels lymphoid gating. The upper panels are from one representative mouse that received sirolimus and cyclosporine post-transplant and subsequently rejected its graft. The bottom panels are from another mouse that was treated with sirolimus and PT-Cy post-transplant and maintained mixed chimerism. Recipient cells are located in the upper left and donor cells in the lower right.

Supplemental Figure S2

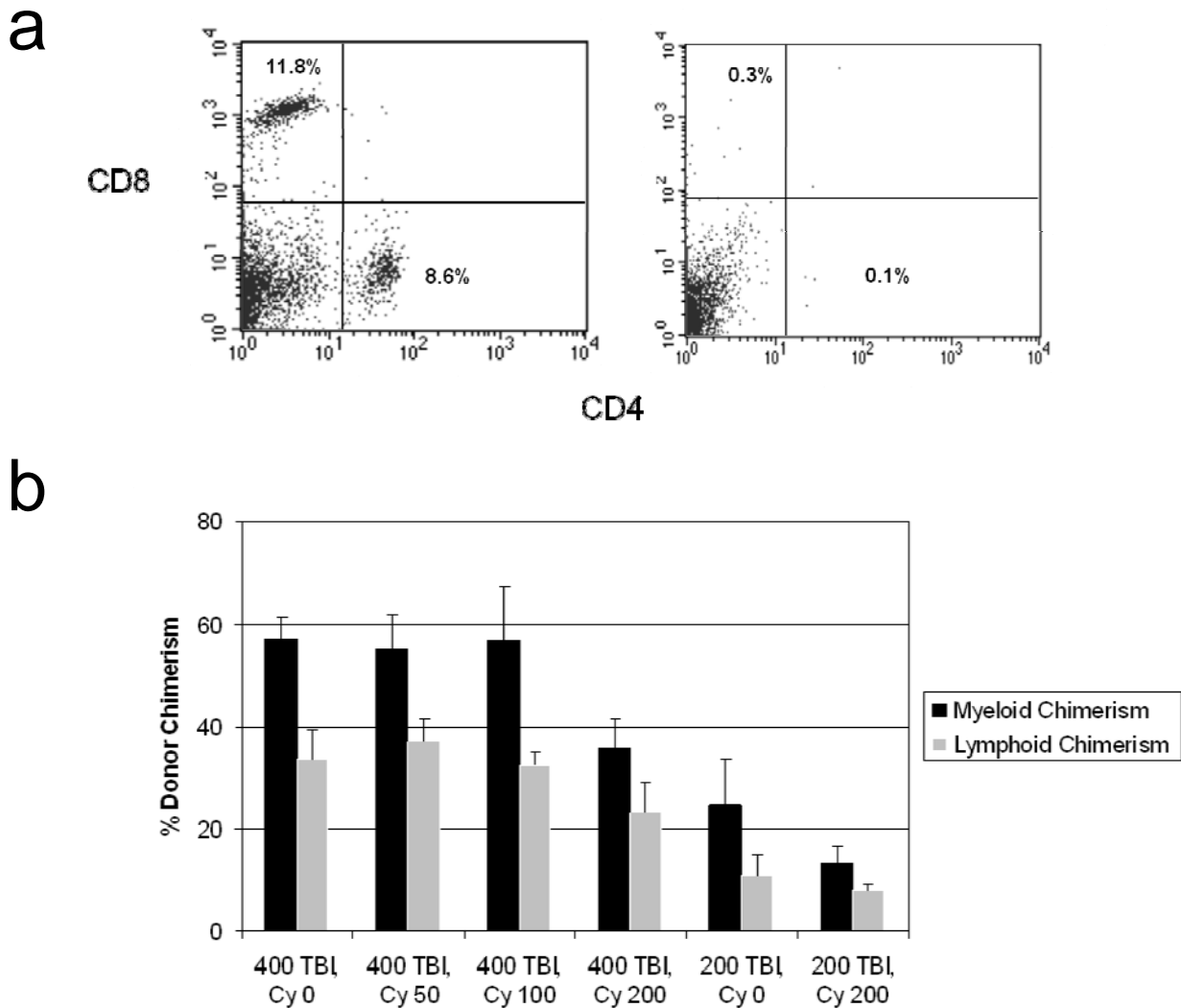


Figure S2. Evaluation of whether cyclophosphamide given in addition to sirolimus improves engraftment in the setting of profound lymphocyte depletion. **(a)** C57BL/6 mice (n=3) were given the lymphocyte depleting agent Thy 1.2 monoclonal antibody (mAb, 1 mg i.p.). Flow analysis was performed to evaluate the percentage of CD4⁺ and CD8⁺ cells in 2 littermate controls (left panel) and the treated mice (right panel). **(b)** C57BL/6 mice (n=42) received Thy 1.2 mab (1mg i.p.), sirolimus (3mg/kg/day i.p.), 200 or 400cGy TBI, 100 x 10⁶ donor splenocytes, and cyclophosphamide (Cy) doses ranging from 0 to 200mg/kg i.p. given 2 days post transplant. These data show donor myeloid and lymphoid chimerism levels at 9 months post-transplant and represent a compilation of 2 experiments.