Supporting information:

Additional Supporting Information may be found in the online version of this article:

Supplemenatry figures

Figure S1. Spleen and BM plasma cells have similar viability and apoptotic rates during 48 h culture. Spleen and BM cells were isolated from B6 recipients of BALB/c heart allografts on d. 7 post transplant and cultured for 48 hours without restimulation, as in the ELISPOT and supernatant antibody assays. Annexin V and 7-AAD staining was performed immediately after cell isolation (0 h) and at 24 and 48 hours after cell culture. The data are representative of 3 mice per group. Similar results were obtained after culture of spleen and BM cells from heart graft recipients sacrificed on d. 14, and 21 post transplant and from naïve B6 mice (not shown).

Figure S2. Frequencies of donor antigen-specific IgG producing ASCs among total PCs from different compartments. A. Heterotopic BALB/c heart transplants were placed into non-sensitized B6 mice or into sensitized B6 recipients four weeks after immunization with BALB/c alloantigens. The frequencies of cells secreting D^d-binding IgG were determined by ELISPOT assay. The percentages and numbers of B220^{lo}CD138⁺ plasma cells in each compartment were evaluated by flow cytometry. The results were calculated as numbers of D^d-specific ASCs per 1 x 10⁶ of total PCs. B. The kinetics of D^d-reactive ASC frequencies in the spleen and in the BM of sensitized versus non-sensitized recipients. N=4-5 recipients per group at each time point.

Figure S3. Secretion of donor MHC binding IgG by individual ASCs in WT versus CCR5-/recipients. A. The cumulative spot size histograms illustrate the higher production of D^d-binding antibody by individual ASCs at day 7 post transplantation in CCR5-/- recipients compared to WT recipients. B. The kinetics of average D^d-binding spot size. All ELISPOT assays were performed in duplicate, average spot sizes were determined for 4-5 recipients per group at each time point.

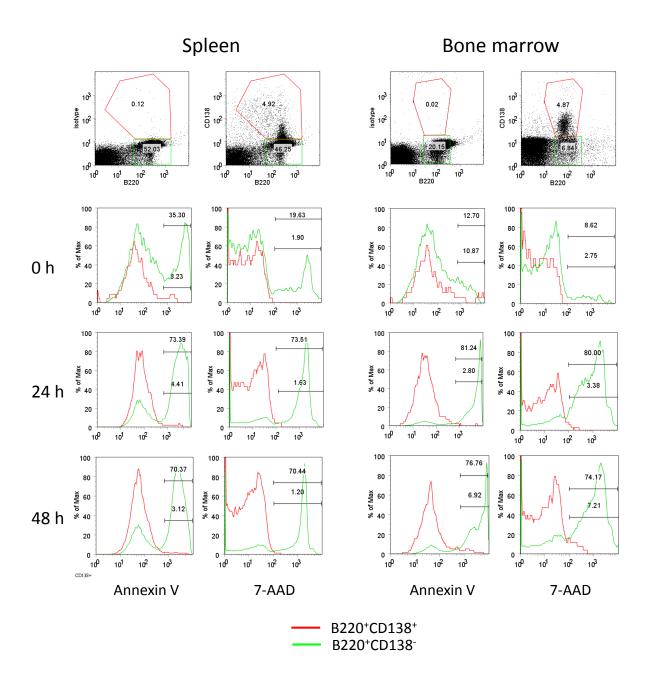


Figure S1. Spleen and BM PCs have similar viability and apoptotic rates during 48 h culture. Spleen and BM cells were isolated from B6 recipients of BALB/c heart allografts on d. 7 post transplant and cultured for 48 hours without restimulation, as in the ELISPOT and supernatant antibody assays. Annexin V and 7-AAD staining was performed immediately after cell isolation (0 h) and at 24 and 48 hours after cell culture. The data are representative of 3 mice per group. Similar results were obtained after culture of spleen and BM cells from heart graft recipients sacrificed on d. 14, and 21 post transplant and from naïve B6 mice (not shown).

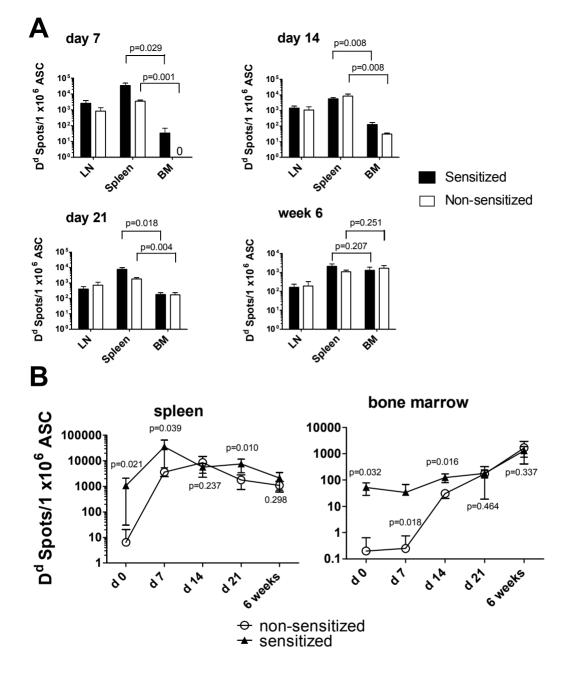


Figure S2. Frequencies of donor antigen-specific IgG producing ASCs among total PCs from different compartments. A. Heterotopic BALB/c heart transplants were placed into non-sensitized B6 mice or into sensitized B6 recipients four weeks after immunization with BALB/c alloantigens. The frequencies of cells secreting D^d -binding IgG were determined by ELISPOT assay. The percentages and numbers of B220^{lo}CD138⁺ plasma cells in each compartment were evaluated by flow cytometry. The results were calculated as numbers of D^d -specific ASCs per 1 x 10⁶ of total plasma cells. B. The kinetics of D^d -reactive ASC frequencies in the spleen and in the BM of sensitized versus non-sensitized recipients. N=4-5 recipients per group at each time point.

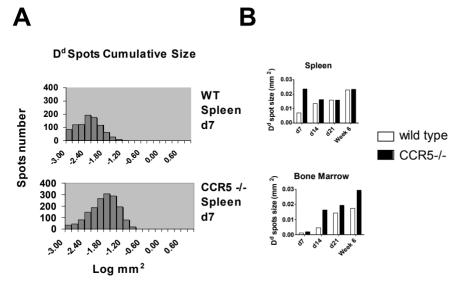


Figure S3. Secretion of donor MHC binding IgG by individual ASCs in WT versus CCR5-/- recipients.

A. The cumulative spot size histograms illustrate the higher production of D ^d-binding antibody by individual ASCs at day 7 post transplantation in CCR5-/- recipients compared to WT recipients.

B. The kinetics of average D $^{\rm d}$ -binding spot size. All ELISPOT assays were performed in duplicate, average spot sizes were determined for 4-5 recipients per group at each time point.