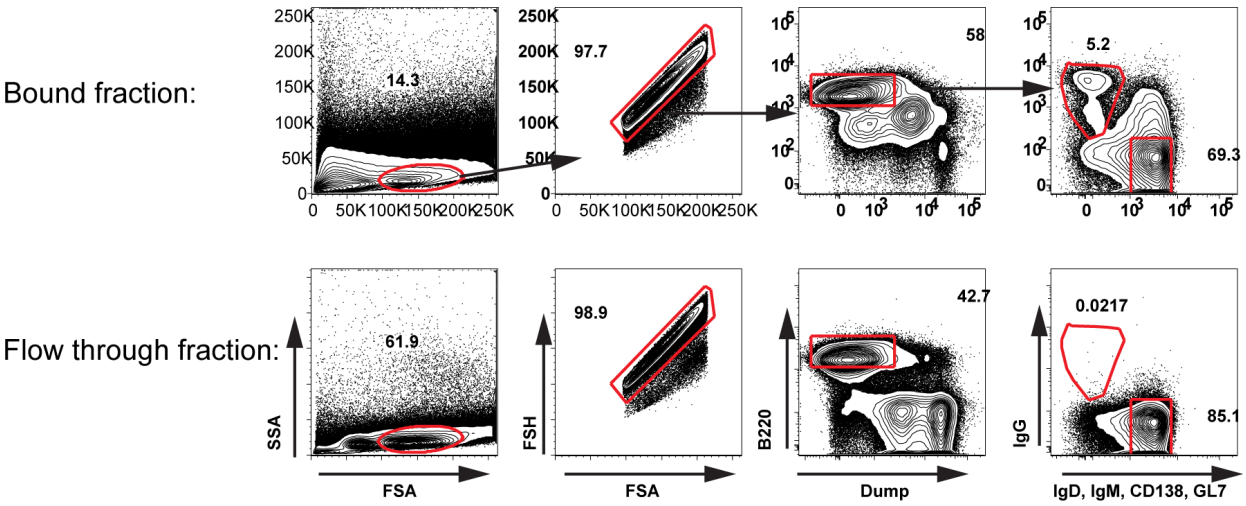
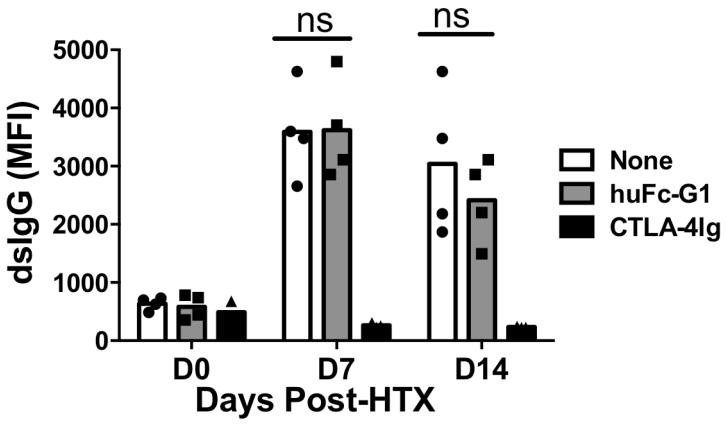
Supplemental Figures



Supplemental Figure 1: No IgG-FITC+ cells are detected in the unbound fraction after positive selection of anti-IgG coated cells and magnetic bead selection. Pooled spleen and lymph node cells were stained with FITC conjugated anti-IgG and enriched with anti-FITC magnetic beads (130-048-701; Miltenyi Biotech, Auburn, CA). The gating strategies for IgG-enriched bound fraction (top) and unbound flow through fraction (bottom) are indicated. For phenotyping of alloreactive B cells, all antibodies were from Ebioscience (San Diego, CA), unless specified. Anti-mouse IgG1 (BD, A85-1), IgG2b (R12-3), IgG3 (R40-82), anti-B220-Brilliant violet 510 (RA3-6B2; Becton Dickinson, Franklin Lakes, NJ), anti-CD80 (16-10A1; Biolegend (San Diego, CA) was conjugated with APC-Cy7 using Lighting-link APC-Cy7 kit (765-0010; Innova Biosciences, Ramona, CA), anti-CD73-Brilliant violet 605 (Biolegend, TY/11.8), anti-CD273-PerCP-eFluor 710 (122), anti-CD38-Alex Fluor 700 (90), anti-FAS-PE-Cy7 (Becton Dickinson, J02), and lineage antibody cocktail including anti-CD3-eFluor 450 (17A2), anti-CD4-eFluor 450 (GK1.5), anti-CD8-eFluor 450 (53-6.7), anti-CD138-Brilliant violet 421 (Biolegend, 281-2), anti-GL7-eFluor 450 (GL-7), anti-F4/80-eFluor 450 (BM8), anti-DX5-eFluor 450 (DX5). Cells then were stained with $H2K^d$-PE and $H2K^d$-APC tetramers. Flow cytometry was performed with an LSRII- blue (four lasers, BD) and analyzed with Flowjo VX (Tree Star, Ashland, OR).



Supplemental Figure 2: No inhibition of recall DSA response with human Fc-G1 control. C57BL/6 mice sensitized with BALB/c splenocytes were transplanted with a BALB/c heart at 2-3 months post-sensitization and treated with CTLA-4Ig or human Fc-G1 (Bioxell, cat: E0096, lot: 4961/1013) from day of transplantation (2X week). Sera were collected on days 0, 7 and 14, and donor-specific (ds) IgG quantified (N = 3-4 mice/group from two independent experiments). In brief, 0.5 μL serum were incubated with 10^6 BALB/c splenocytes in 50 μL of flow cytometry buffer for 1 h at 4°C, then with anti-IgM or anti-IgG mAbs (1021-09 & 1030-02; Southern Biotech, Birmingham, AL) for 30 minutes. For memory B cell transfer experiments, DSA were quantified with biotin-conjugated anti-mouse IgG1a, anti-mouse IgG1b (10.9 & B68-2, Becton Dickinson), anti-mouse IgMa (or anti-mouse IgMb (MA-69 & AF6-78, Biolegend), and anti-B220-FITC. After 1 h incubation, streptavidin-PE was added and the mean fluorescence intensity (MFI) on B220-negative cells was quantified by flow cytometry.