

Figure S1. Peripheral Blood IFN- γ ⁺CD8⁺ T cells Predominantly Express a Terminally Differentiated Effector Phenotype. Peripheral blood lymphocytes from kidney transplant recipients were analyzed using flow cytometry to determine the composition of naïve (CD62L⁺CD45RO⁻), central memory (T_{CM}; CD62L⁺CD45RO⁺), effector memory (T_{EM}; CD62L⁻CD45RO⁺), or terminally differentiated effector CD8⁺ T cells (T_{EFF}; CD62L⁻CD45RO⁻). Cells were initially gated on lymphocytes and CD8⁺ T cells. **A)** Representative data (DSA-negative recipient, 3 months posttransplant) shows that circulating CD8⁺ T cells consist of 61% naïve CD8⁺ T cells, 12% central memory CD8⁺ T cells, 3% effector memory CD8⁺ T cells, and 21% terminally differentiated effector cells. **B)** When CD8⁺ T cells are further gated on IFN- γ ⁺ cells, IFN- γ ⁺CD8⁺ T cells predominantly express a terminally differentiated effector phenotype (CD62L⁻CD45RO⁻). Terminally differentiated effector IFN- γ ⁺CD8⁺ T cells (CD62L⁻CD45RO⁻; red histogram) also express CD44 when compared to naïve cells (orange histogram) and CD44 FMO control (blue histogram). Expression of CD45RO, CD62L, CD44, and IFN- γ are shown in comparison to fluorescence minus one (FMO) controls for CD45RO, CD62L, CD44, and IFN- γ . Flow antibodies included anti-CD62L (clone DREG-56) and anti-CD45RO (clone UCHL1, both from BD).

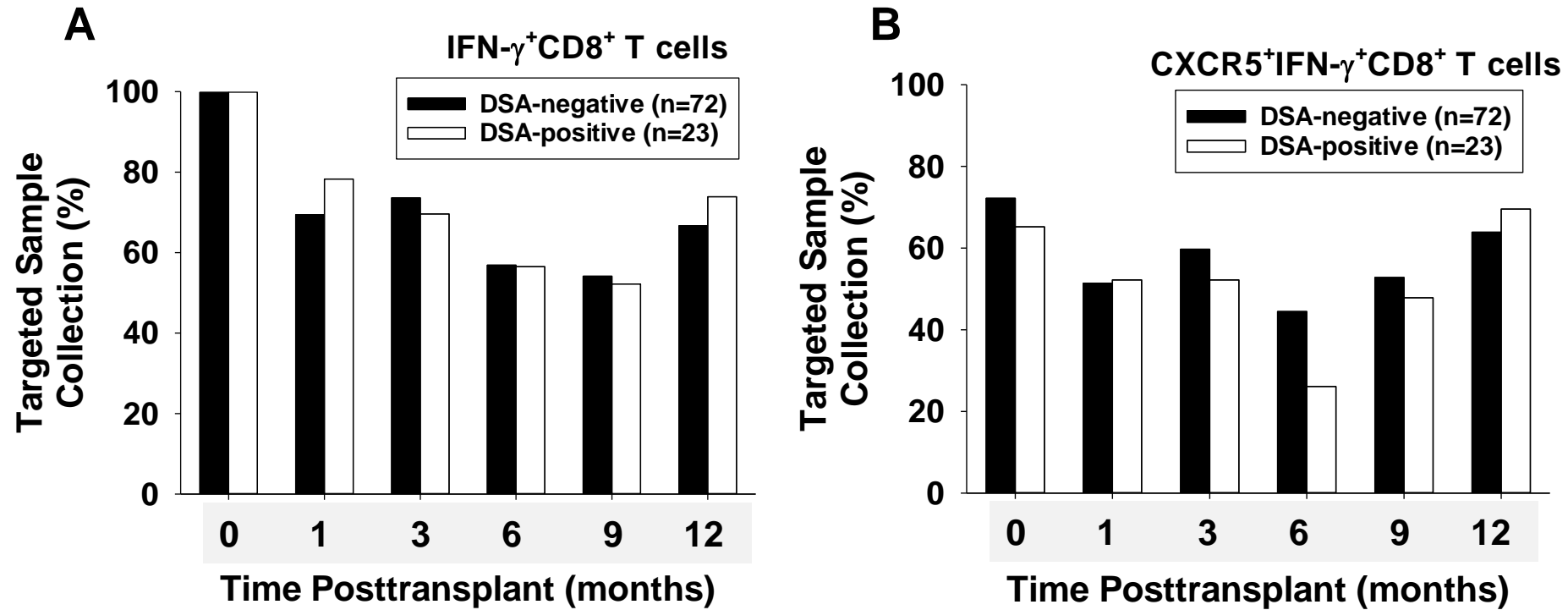


Figure S2. Capture Rate for Peripheral Blood Samples to Assess Quantity of IFN- γ ⁺CD8⁺ T cells and CXCR5⁺IFN- γ ⁺CD8⁺ T cells in Primary Kidney Transplant Recipients Over the First Year Posttransplant. Peripheral blood sample collection was targeted for 6 time-points (pretransplant as well as follow-up visits at 1, 3, 6, 9, and 12 months posttransplant) from 95 consenting kidney transplant recipients (570 potential samples). **A)** Samples to analyze peripheral IFN- γ ⁺CD8⁺ T cells were collected with overall 71% (402/570 samples) capture rate of targeted samples including 100% pretransplant, 72% 1 month, 73% 3 month, 57% 6 month, 54% 9 month, and 68% 12 month samples. Sample capture rates were similar for DSA-negative (70%) and DSA-positive (72%) recipients over the 1-year follow up ($p=0.44$). **B)** The peripheral blood sample collection for analysis of CXCR5⁺IFN- γ ⁺CD8⁺ T cells was initiated later in the study and included 56% (320/570) capture rate of the targeted study samples including 72% pretransplant, 52% 1 month, 58% 3 month, 40% 6 month, 52% 9 month, 65% 12 month samples. Sample capture rates were similar for DSA-negative (57%) and DSA-positive (52%) recipients over the 1-year follow up ($p=0.26$).

	HLA Class I antigen specificity	HLA Class II antigen specificity
HLA Class I only	A2, A3, A11, B27, B44, B51, C1, C2, C15	N/A
HLA Class II only	N/A	DR4, DR7, DR17, DR53, DQ2, DQ5, DQ6, DQ7
HLA Class I and II	A1, A2, A23, B7, B8, B44, B51, B57	DR7, DR10, DR51, DR53, DQ2, DQ7, DQ8

Figure S3. HLA Class I and HLA Class II Specificity of all DSA at the Time of Initial Detection. Initial DSA was detected, on average, 6.0 ± 3.7 months posttransplant in 23 of the 95 recipients (24.2%). Of the 23 DSA-positive recipients, recipients developed specificity to HLA I (7 recipients), HLA II (10 recipients), or to both HLA I and HLA II (6 recipients). HLA antigen specificity is listed for all recipients in each group.

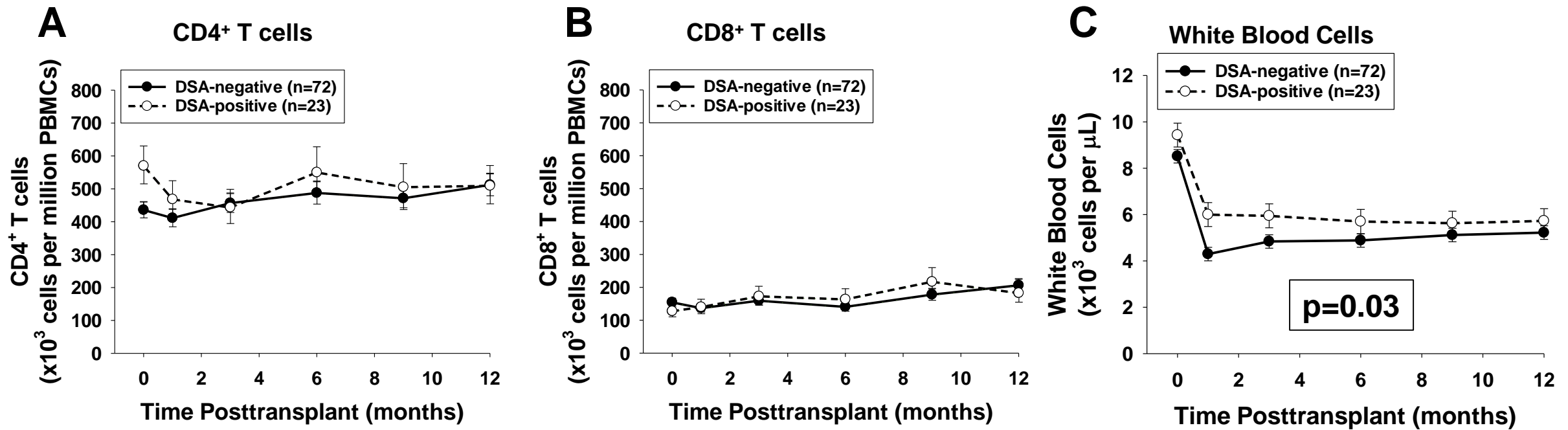


Figure S4. CD4⁺ T cells, CD8⁺ T cells, and White Blood cells in Peripheral Blood of DSA-positive and DSA-negative Kidney Transplant Recipients Over the First Year Posttransplant. Peripheral blood from DSA-positive (n=23) and DSA-negative recipients (n=72) was analyzed for quantity of total CD4⁺ T cells and CD8⁺ T cells and white blood cell count (WBC) (pretransplant and 1, 3, 6, 9, and 12 months posttransplant). **A)** Total peripheral CD4⁺ T cells and **B)** Total peripheral CD8⁺ T cells by flow cytometric analysis in DSA-positive and DSA negative recipients were similar (p=0.27 and p=0.86, respectively). **C)** WBC counts were slightly higher in DSA-positive recipients (p=0.03) but were in the normal range ($4\text{--}10 \times 10^3$) over the first year posttransplant. Graphed data represents geometric mean \pm standard error.

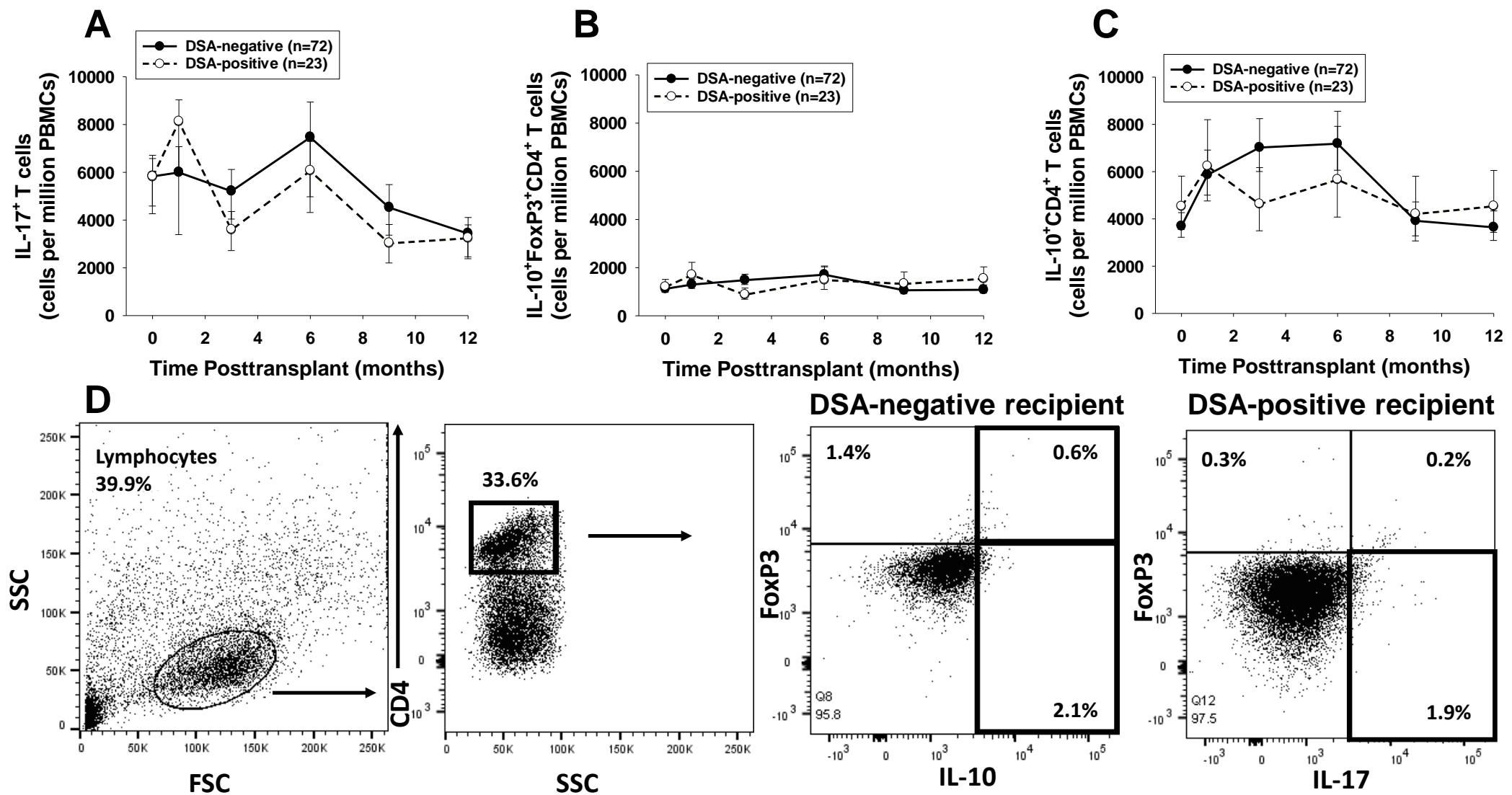


Figure S5. Quantity of IL-17⁺CD4⁺ T cells, FoxP3⁺IL-10⁺CD4⁺ T cells, and IL-10⁺CD4⁺ T cells in DSA-positive and DSA-negative recipients Over the First Year Posttransplant. Peripheral blood from DSA-positive recipients (n=23) and DSA-negative recipients (n=72) was analyzed using flow cytometry to determine the quantity of IL-17⁺CD4⁺T cells, FoxP3⁺IL-10⁺CD4⁺T cells, and IL-10⁺CD4⁺ T cells. **A-C)** The quantities of IL-17⁺CD4⁺T cells, FoxP3⁺IL-10⁺CD4⁺T cells, and IL-10⁺CD4⁺ T cells were not significantly different between DSA-positive and DSA-negative recipients over the first year posttransplant (p=0.47, p=0.83, and p=0.94, respectively). Graphed data represents geometric mean \pm standard error. **D)** Flow plots for IL-17, FoxP3, and IL-10 expression on CD4⁺ T cells are shown from representative DSA-negative and DSA-positive recipients (3 months posttransplant). Cells were gated on lymphocytes and CD4⁺ T cells. Flow antibodies included anti-IL-17 (clone N49-653), anti-FoxP3 (clone 259D/C7), and anti-IL-10 (clone JES3-9D7, all from BD).

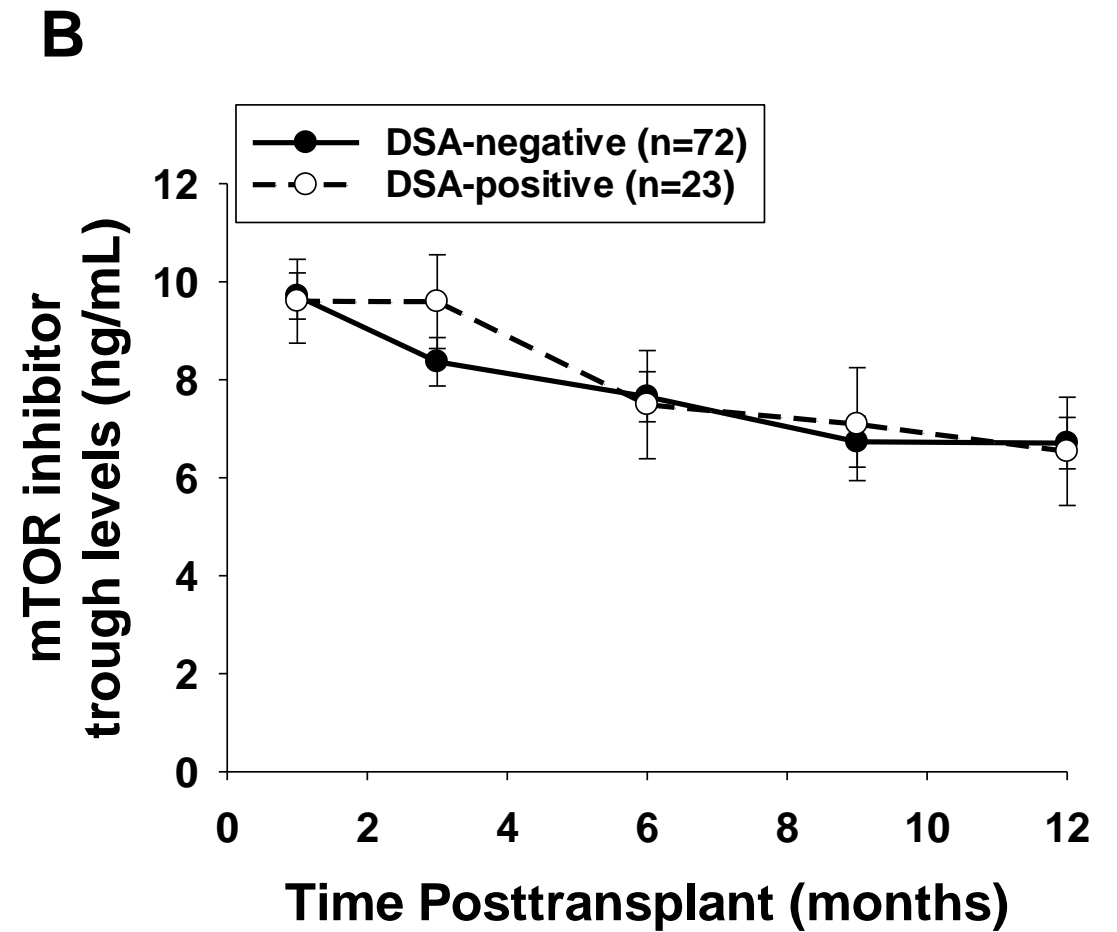
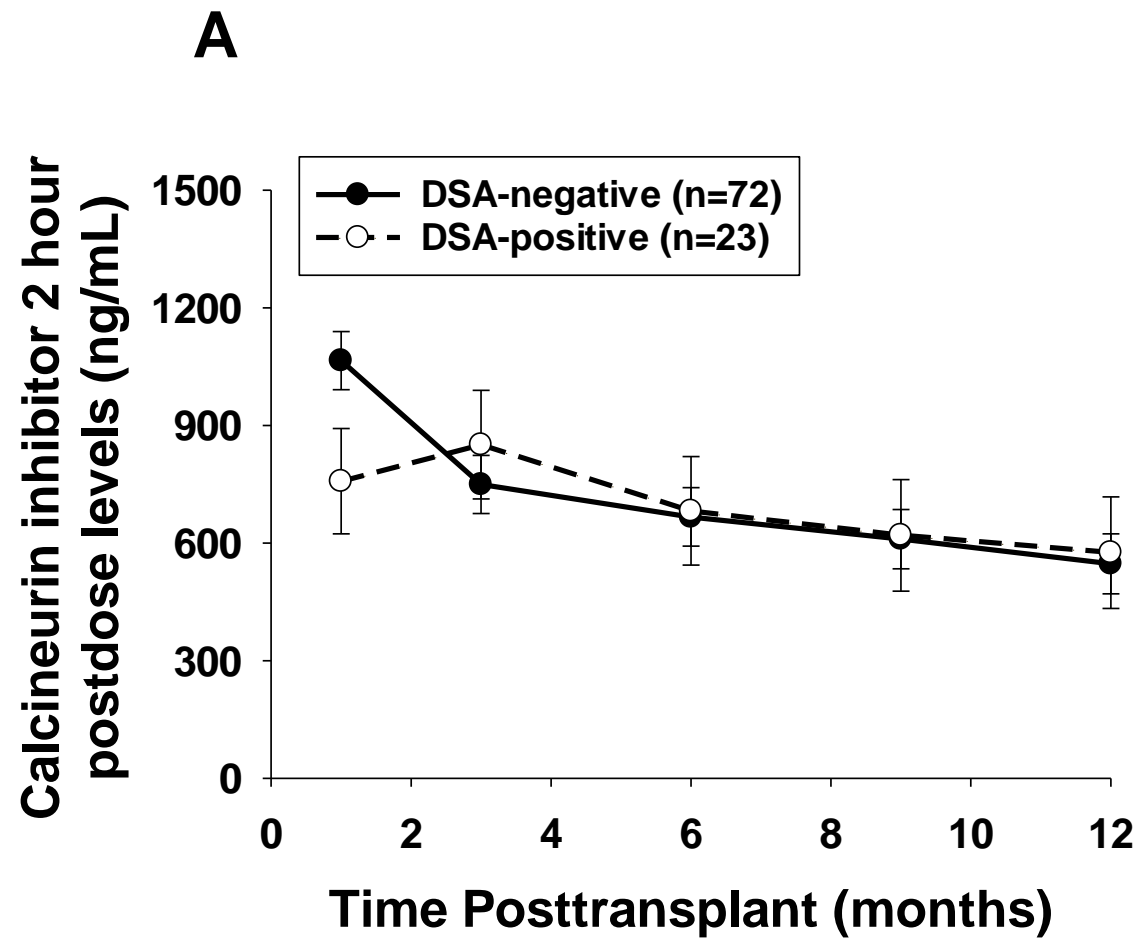


Figure S6. CNi and mTORi Immunosuppression Levels in DSA-positive and DSA-negative Recipients Over the First Year Posttransplant. Following kidney transplantation, recipients were maintained on an immunosuppressive regimen consisting of a combination of calcineurin inhibition (CNi, with Neoral) and mTOR inhibition (mTORi, with Rapamune or Zortress). Immunosuppression CNi 2 hour peak (C2) and mTORi trough levels were analyzed in 95 recipients at 1, 3, 6, 9, and 12 months posttransplant. **A)** DSA-negative (n=72) and DSA-positive recipients (n=23) had similar CNi C2 hour levels over the first year posttransplant ($p=0.68$). **B)** DSA-positive and DSA-negative recipients had similar mTORi trough levels over the first year posttransplant ($p=0.69$). Graphed data represents geometric mean \pm standard error.

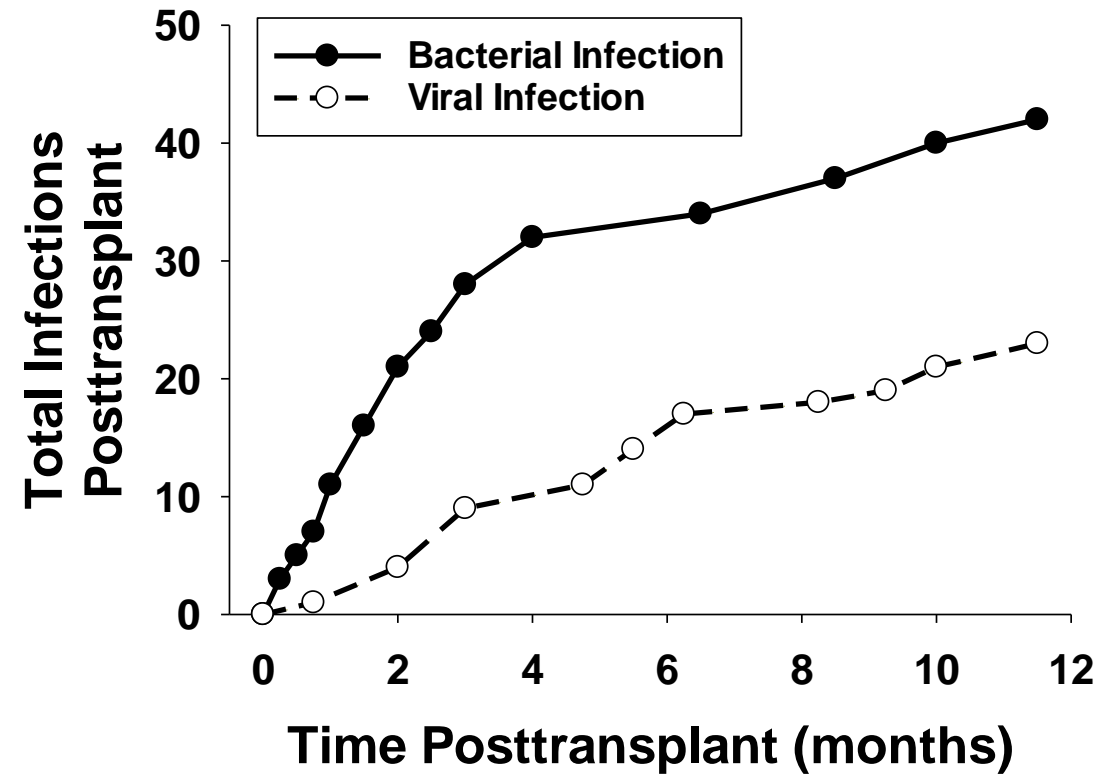


Figure S7. Cumulative Bacterial and Viral infections in 95 First-time Kidney Transplant Recipients Over the First Year Posttransplant. Following kidney transplantation (n=95), 43 recipients (45.3%) developed a bacterial (25 recipients) and/or viral (22 recipients) infection over the first year posttransplant. Most recipients with infection only developed 1 bacterial infection (17/25) and/or 1 viral infection (21/22) during the study period. Six recipients developed multiple bacterial infections, 4 recipients developed bacterial and viral infections (2 of whom also had multiple bacterial infections), and 1 recipient developed multiple viral infections. The majority of bacterial infections occurred in the first 4 months posttransplant (32 of 42 total infections; 8 patients with multiple bacterial infections). Viral infections (23 total) were less common than bacterial infections and their occurrence was more evenly distributed over the 1 year follow-up (1 recipient with 2 viral infections).