Supplementary Material:

Supplementary Methods:

Creation of an MHC-defined rhesus macaque model of transplantation: We have performed an immunogenetic analysis on >350 animals from the Yerkes National Primate Research Center and >800 animals from the NIAID-sponsored colony at Yemassee, SC, including family tree and MHC haplotype determination via DNA microsatellite testing. To accomplish this, we employed a two-part strategy based on microsatellite genotypes. DNA was first purified from 1cc of citrate-anticoagulated blood, and eight multiplexed PCR reactions were then performed to obtain genotype data for 41 markers, 15 of which spanned the MHC region. Details of markers, primers and multiplex groups are summarized in Supplementary Table 1. PCR reactions were combined as described in Supplementary Table 1 for separation by capillary electrophoresis on ABI 3730 instruments (Applied Biosystems, Foster City, CA). Pedigrees were then established based on analysis of the inheritance patterns of 26 microsatellite markers. (17, 23) Once animals were placed in a pedigree, MHC sharing was determined between full and half-siblings by comparing the inheritance of parental MHC haplotypes based on high-density microsatellite analysis of the MHC region from the rhesus chromosome 4. This process allowed us to unambiguously assign macaque offspring to a family tree and to assign them two unique MHC haplotype codes. A typical family pedigree display is shown in Supplementary Figure 1a. As demonstrated in this Figure, rhesus pedigrees contain a large number of full and half-sibling pairs that share either one or two MHC haplotypes. It is from this large pool of potential MHC-matched and -mismatched transplant pairs that our experimental cohorts were chosen. Figures 1b and 1c show a detailed view of the microsatellite haplotypes in each of the transplant pairs that were studied.

454-Sequencing of MHC Haplotypes: To define the MHC class I alleles transcribed on each of the MHC haplotypes, 454 pyrosequencing was performed as previously described. (24, 25) Briefly, a diagnostic amplicon of 367 b.p spanning the highly polymorphic peptide binding domain encoded by exons 2 and 3 was amplified from total RNA by cDNA-PCR. Pooled amplicons were pyrosequenced on a Roche/454 GS FLX instrument (Branford, CT) using FLX chemistry. After image processing the resultant transcript sequences were identified by comparison to a custom library of previously described rhesus macaque MHC class I alleles using NCBI blastn.

Supplementary Results:

MHC disparity predicts the degree of pre-transplant alloreactivity in a pedigreed, MHCdefined rhesus macaque colony. Having established a system to determine the degree of MHC disparity between transplant pairs, we were able to rigorously determine the degree to which MHC sharing impacted alloproliferation. We performed an analysis of alloproliferation as measured by CFSE-MLR, and compared four cohorts: (1) two MHChaplotype matched pairs ($n= 8$), (2) one MHC-haplotype-matched pairs ($n = 48$), (3) autologous controls (with responder and stimulator cells derived from the same animal, n = 61) and (4) a cohort of animals for whom haplotype information was not available at the time of the CFSE MLR (n=99). Examples of representative CFSE MLR assays for each of the groups are shown in Supplementary Figure 2a. Supplementary Figure 2b shows that pairs matched at both MHC haplotypes displayed minimal pre-transplant alloproliferation (1.1% \pm 1%), not statistically different than autologous controls (1.3% \pm 1.5%). As expected, pairs matched at just one MHC haplotype displayed significantly more alloproliferation (8.4% + 8.5%) than either the two MHC haplotype-matched pairs or the autologous controls (p<0.01). The percentage of cells that had proliferated in the MLR cohort with unknown MHC disparity (17% \pm 14.9%) was higher than for either the one MHC-haplotype- or two MHC-haplotype-matched pairs (p<0.01). However, the

proliferation measured in this untyped cohort was not uniform, with these MLR pairs displaying a high standard deviation around the mean (Supplementary Figure 2b), with many animals demonstrating surprisingly low levels of alloproliferation. These examples of low levels of alloproliferation in the cohort that had not been pedigreed or MHC typed led us to hypothesize that some pairs included in this group serendipitously possessed significant degrees of relatedness or MHC similarity.

To test this hypothesis, we determined the profiles of MHC class I allele transcripts in a subset of donor/recipient animal pairs by pyrosequencing (24, 25) This analysis allowed us to (1) confirm the results of the microsatellite-based MHC analysis shown in Supplementary Figure 1 and (2) determine if serendipitous MHC similarity could accompany low alloproliferation by CFSE MLR. As shown in Supplementary Figure 2c, the degree of MHC identity predicted by microsatellite analysis for RDe9/RNs9 or DK5P/DL74 (both two MHC haplotype-matched pairs) as well as RWk10/RMn10 (one MHC haplotype-matched) was confirmed by pyrosequencing. This comprehensive analysis of expressed alleles also confirmed that high degrees of MHC similarity could be found in the untyped cohort. Thus, CX6X and CX2A, who showed significant allo-proliferation as measured by MLR (22.4%), were found to have totally disparate MHC haplotypes (Figure 2c). In contrast, pyrosequencing analysis of CX3M and RMn10, led to a significantly different outcome. Although these two animals had no known familial relationship, and were derived from two different primate colonies (RMn10 from the Yerkes National Primate Research Center and CX3M from NIAIDsponsored colony at Yemassee, SC), they demonstrated low levels of alloproliferation by CFSE MLR (1.2%). Pyrosequencing analysis detected a shared MHC haplotype between these animals, and a resultant high degree of MHC similarity (Supplementary Figure 2c). These results confirm the utility of rapid microsatellite-based MHC typing to determine the degree of MHC disparity *within a pedigreed colony* and, perhaps most

importantly, point to a significant potential confounder in transplant analysis of untyped and unpedigreed animals: serendipitously high levels of MHC similarity, even between animals from different colonies, might significant impact alloreactivity and potentially, transplant outcome.

Supplementary Table 1. Microsatellite marker panels used to establish pedigrees and MHC haplotypes. a: Names with D prefix correspond to human nomenclature for markers; b: Rhesus macaque chromosome number in Jan 2006 MSGC Merged 1.0/rheMac2 draft assembly (http://genome.ucsc.edu/cgi-bin/hgGateway); c: Multiplexes with same group letter (e.g. A1 and A2) were combined for electrophoresis; ^d: bases in lower case are tails added to primers; ^e: allele size range does not include tails.

Supplementary Figure Legends:

Supplementary Figure 1: Creation of a pedigreed and MHC-defined rhesus macaque transplant model.

Supplementary Figure 1a: Pedigree and MHC Inheritance Map for N5K, his mating pairs and his offspring. The sire, N5K, is depicted at the center of the circular pedigree. Each dam with whom he mated is depicted on the edge of the circle, with a linear connection to N5K. Offspring from each of the mating pairs are depicted emanating from these connections. Circles indicate female offspring and dams. Squares indicate male offspring and sires. MHC haplotypes are indicated by color coded bars associated with each animal. N5K's MHC haplotypes are indicated as either a red or a black bar. MHC haplotypes for each of the dams are indicated by unique color-coded bars. The inheritance of an MHC haplotype from the sire or dam is indicated by the color of the bars for each of the offspring.

Supplementary Figure 1b: Detailed view of the DNA microsatellite-based MHC haplotypes for the two MHC haplotype-matched transplant pairs. Animals were analyzed using 8-15 microsatellites that spanned the MHC and haplotypes were derived from their inheritance between parents and offspring. Individual MHC haplotypes are color-coded.

Supplementary Figure 1c: Detailed view of the DNA microsatellite-based MHC haplotypes for the one MHC-haploptye-matched transplant pairs. Animals were analyzed using 8-15 microsatellites that spanned the MHC and haplotypes were derived from their inheritance between parents and offspring. Individual MHC haplotypes are color-coded.

Supplementary Figure 2: MHC disparity predicts alloproliferation as measured by CFSE MLR.

Supplementary Figure 2a. CFSE MLR analysis reveals increasing alloproliferation with increasing MHC disparity. This figure shows CFSE fluorescence for both CD4+ and CD8+ cells after a 5 day MLR culture. Shown (left to right, top row) are a representative autologous control, a two MHC-haplotype matched pair, and a one MHC-haplotype matched pair. The bottom row shows a representative pair with unknown MHC disparity and high proliferation (left) and a representative pair with unknown MHC disparity and low proliferation (right).

Supplementary Figure 2b. The amount of CD8+ T cell alloproliferation correlated with the degree of MHC disparity. CFSE MLR analysis was performed on two-MHC haplotype matched pairs (n= 8), one MHC-haplotype-matched pairs (n=48), autologous controls (n=61), and animals for whom MHC disparity Information was not available (n=99). The percent of CD8+ T cells remaining at the end of the five day MLR incubation period that had undergone at least one round of cell division (% proliferation) was then determined using the FloJo flow cytometry analysis program. Shown are the average % proliferation and the standard deviation for all four groups. Statistical significance was determined by ANOVA analysis of the log-transformed data followed by a post-hoc Tukey HST test to determine significant differences for pair-wise comparisons.

Supplementary Figure 2c: Class I transcript profiles for animals with varying degrees of MHC disparity. MHC class I genotypes were determined by pyrosequencing of cDNA-PCR amplicons for representative pairs of animals used in the CFSE-MLR assays. The number of sequence reads identified for specific MHC class I alleles or closely related allele lineages are indicated for each animal. MHC haplotypes shared between animals are highlighted with open boxes.

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Supplementary Figure 1b: Detailed view of the DNA microsatellite-based MHC haplotypes for the two MHC haplotype-matched

transplant pairs. Animals were analyzed using 8-15 microsatellites that spanned the MHC and haplotypes were derived from their inheritance between parents and offspring. Individual MHC haplotypes are color-coded.

Supplementary Figure 1c: Detailed view of the DNA microsatellite-based MHC haplotypes for the one MHC-haploptye-matched

transplant pairs. Animals were analyzed using 8-15 microsatellites that spanned the MHC and haplotypes were derived from their inheritance between parents and offspring. Individual MHC haplotypes are color-coded.

Supplementary Figure 2a. CFSE MLR analysis reveals increasing alloproliferation with increasing MHC disparity. This figure shows CFSE fluorescencefor both CD4+ and CD8+ cells after a 5 day MLR culture. Shown (left to right, top row) are a representative autologous control, a two MHC-haplotype matched pair, and a one MHC-haplotype matched pair. The bottom row shows a representative pair with unknown MHC disparity and high proliferation (left) and a representative pair with unknown MHC disparity and low proliferation (right).

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