







- Untreated (n=5)
- □ αCD20 + CsA (n=4)



#### **Supplemental Figure Legends**

Supplemental Figure 1. Absolute B-cell and T-cell counts in peripheral blood of individual animals after anti-CD20 therapy. Peripheral blood B-cell numbers (A) are significantly reduced with  $\alpha$ CD20+CsA (brown curves; each line represents an individual animal) relative to CsA (green curve reflects CsA group mean ± SEM), whereas T-cells (B) exhibit similar trends over time in both treatment groups. DJ3M5 (dotted line, panel B) exhibited high leukocyte counts associated with acute parvovirus infection that proved lethal on day 35, and was excluded from Figure 1.

#### Supplemental Figure 2. Immunochemistry analysis of graft infiltrating B-

**cells.** Cardiac graft biopsy or explanted heart tissue specimens were stained for CD20 as described in Methods. Representative pictures from untreated and treated transplant recipients. Original magnification x100. D: day post-transplant.

Supplemental Figure 3. Rejection scores determined according to the ISHLT guidelines. (mean  $\pm$  SD) Perioperative depletion of B-cells with  $\alpha$ CD20 in CsA-treated heart allograft recipients is associated with lower ISHLT acute cellular rejection scores relative to animals treated with CsA alone (p=0.045 at day 90, including monkey ID#DJ4J7). \*, p<0.05 vs. untreated, #, p<0.05 vs. CsA

Supplemental Figure 4. Intra-graft gene expression of PAX5 in explanted cardiac allografts. Relative expression levels by real-time RT-PCR expressed as fold increase over normal heart after normalization to HRPT. The black bar displays the group median while each dot represents an individual animal. PAX5 expression correlates with CD20 gene expression and is generally decreased in surviving grafts compared to acutely rejected controls. The outlier with high PAX5 expression in the  $\alpha$ CD20+CsA group corresponds to the animal exhibiting partial depletion by flow cytometry and immunochemistry (DJ4J7).

**Supplemental Figure 5. Immunochemistry analysis of intra-graft classical pathway complement activation.** Frozen tissue sections were stained by double immuno-fluorescence for Von Willebrand factor (VWF, green) and complement deposits (C4d, red). **A**, Representative picture showing vascular deposits of C4d in an animal treated with CsA, original magnification x200. **B**, Quantitative C4d deposits scores are shown for native hearts (control) and cardiac allografts harvested from recipients treated with the indicated regimens. Each dot represents an individual animal, the turquoise bar displays the group median. Experiments for which the quality of frozen tissue was inadequate to accurately estimate C4d deposition are excluded from the figure

Supplemental Table 1.	<b>Real-time PCR reagents.</b>
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Gene symbol	Company	Catalog #	
TNFSF13B	Qiagen	QT00094759	
(BAFF)			
PAX5	Applied Biosystems	Rh_02872283_m1	
CD3E	Applied Biosystems	Rh_010662242_m1	
<b>CD28</b>	Applied Biosystems	Hs_00174796_m1	
CD80	Applied Biosystems	Hs_00175478_m1	
ΙΓΝγ	Applied Biosystems	Hs_99999041_m1	
IL-4	Applied Biosystems	Rh_02621716_m1	
IL-17	Applied Biosystems	Rh_02621750_m1	
Gene symbol	Forward primer *	Reverse primer *	Probe *
HPRT1	Rh-HPRT-740F-	Rh-HPRT-848R-	Hs-HPRT-785T-
	TGCCCTTGACTATA	CCAAACTCAACTTGA	TGTCATTAGTGAAACTGGA
	ATGAATACTTCAG	ACTCTCATCTT	AAAGCAAATACAAAGCC
FOXP3	p/h-FOXP3-467F-	p/h-FOXP3-555R-	p/h-FOXP3-526T-
	CCCTGCCCTTCTCAT	GTGGCCCGGATGTGA	AGCCAGAGGACTTCCTCA
	CCA	AAA	AGCACTGCC
<b>CD20</b>	Rh-CD20-686F-	Rh-CD20-763R-	
	CCATCTACTAATAC	AAGAAGGCAAAGAT	
	TGTTACAGCATACA	CAGCATCACTGA	

\* These reagents were designed based on macaque sequences (Accession #GI:109080344;74136394;109105983) using Primer Express.