Supporting Information

Yuan et al. 10.1073/pnas.0812538106

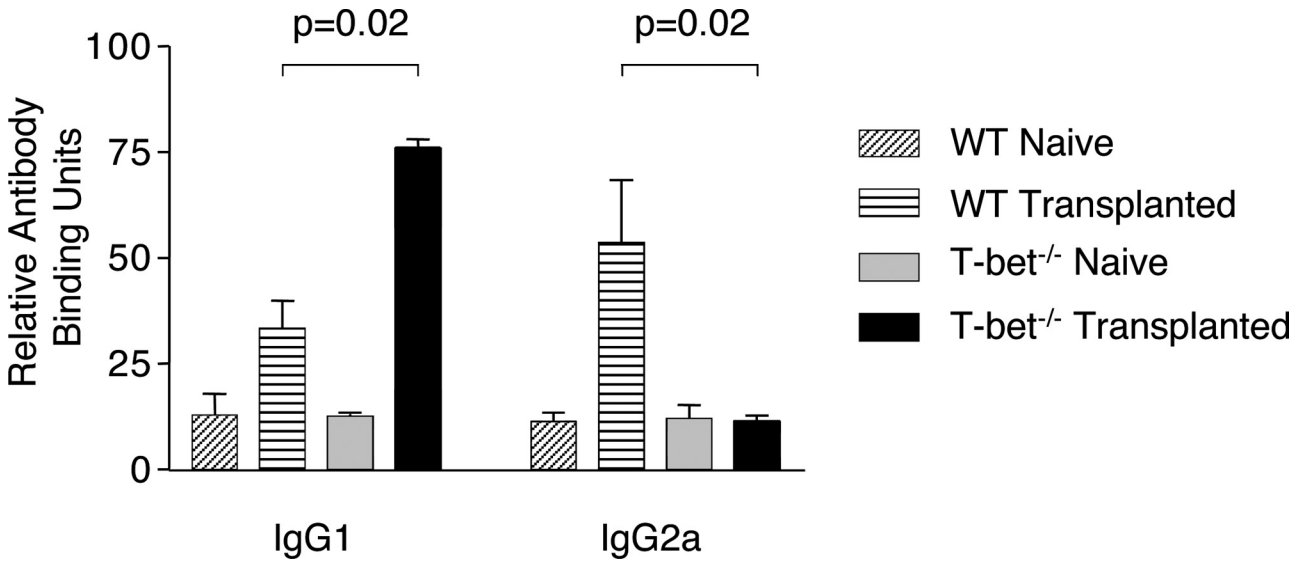

Fig. S1. Increased Th2-associated isotype (IgG1) and deficiency of the Th1 associated isotype (IgG2a) in Tbet KO recipients. To measure serum alloantibody, naive splenocytes (10^6) of donor strain BALB/c were incubated for 30 min at 4 °C with 50 μL of serially diluted sera obtained from naive BALB/c or C57BL/6 mice (controls) or C57BL/6 or T-bet$^{-/-}$ heart recipients after rejection of the allograft. Cells were washed twice; incubated with 1:25 dilution of FITC-conjugated anti-mouse IgM, IgG1, or IgG2a (all from Pharmingen) at 4 °C for 30 min; and analyzed by flow cytometry using a FACSCalibur (Becton Dickinson) and CellQuest software (Becton Dickinson). The percentage of donor cells stained at each serum dilution and the relative median fluorescence intensity (MFI) was determined and compared with that of control samples. Baseline IgM, IgG1, and IgG2a alloantibody levels were similar in WT and T-bet$^{-/-}$ allograft recipients. However, after allograft rejection, significantly higher levels of Th2-associated IgG1 isotype were noted in sera harvested from T-bet$^{-/-}$ recipients of BALB/c cardiac allografts compared with WT controls (MFI relative units 79.3 $\pm$ 2.7 vs. 34.1 $\pm$ 6.7, $P < 0.05$). In contrast, there was a striking reduction in the level of the Th1 associated IgG2a isotype in T-bet$^{-/-}$ recipients compared with WT (MFI 11 $\pm$ 2 vs. 61 $\pm$ 17, $P < 0.05$).

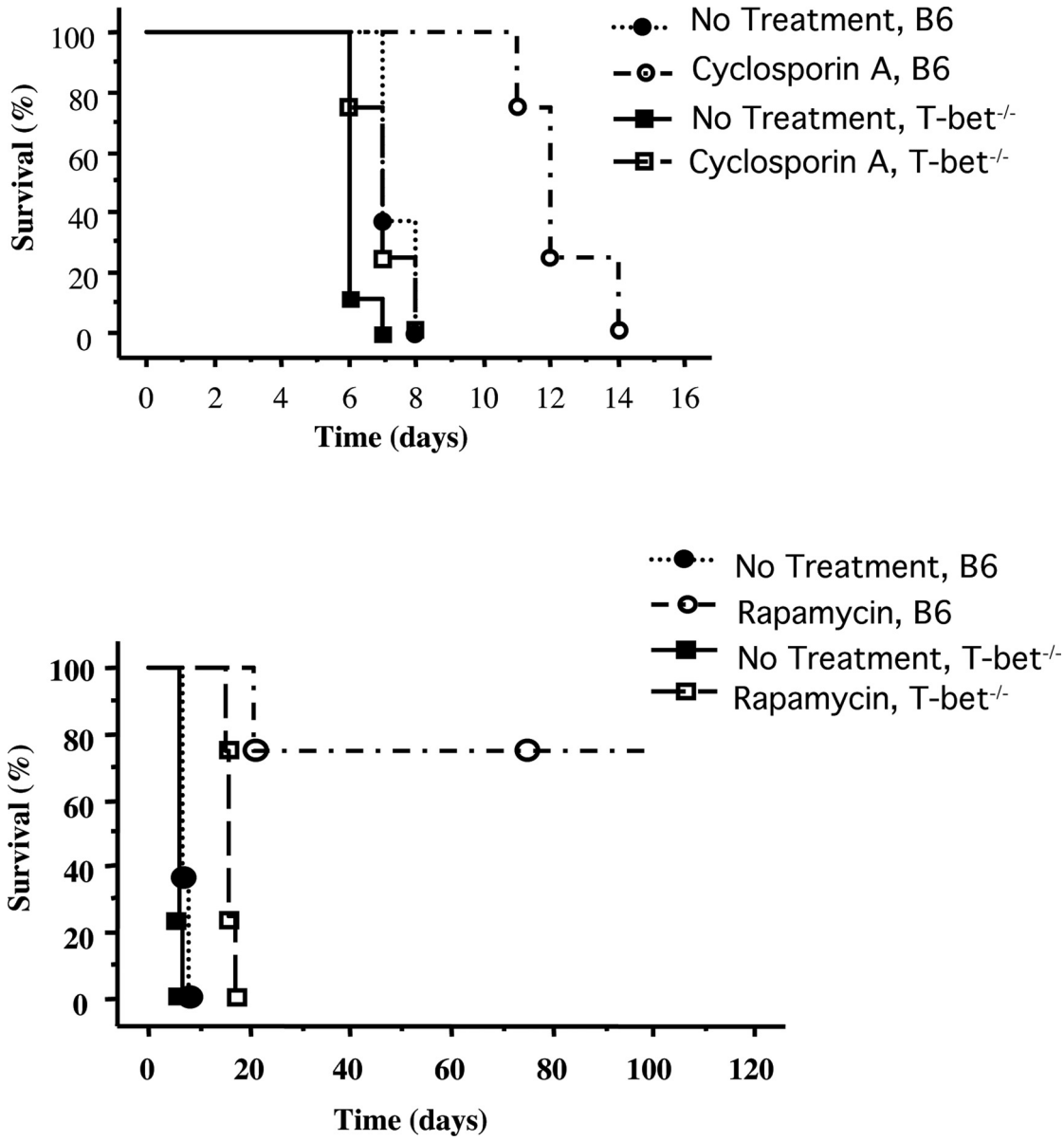

Fig. S2. Kaplan–Meier survival curves of fully mismatched cardiac allografts in WT and Tbet KO recipients treated with CsA (*Upper*) MST Tbet KO 7 days vs. WT 12 days; $P < 0.01$) or SRL (*Lower*) MST Tbet KO 16 days vs. WT >63; $P < 0.01$). Each group had 4 to 7 animals.

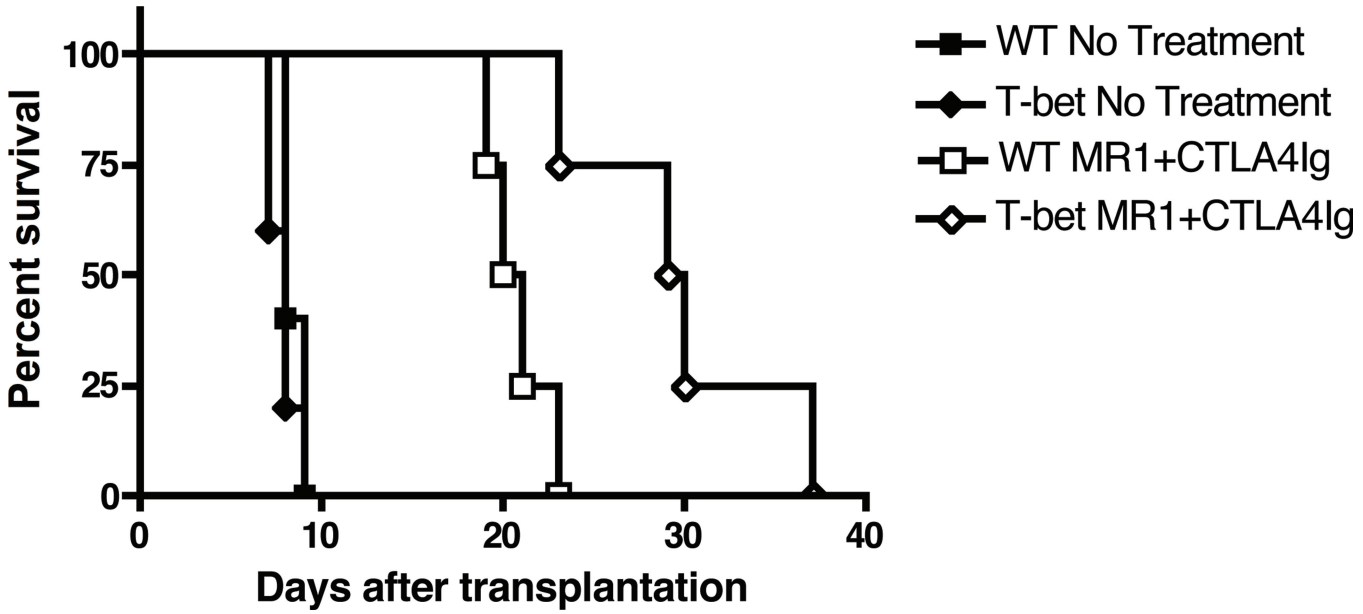

Fig. S3. Kaplan–Meier survival curves of fully mismatched skin allografts in WT and Tbet KO recipients treated with CTLA4Ig+MR1 (MST Tbet KO 29 vs. WT 21; $P = 0.237$). Each group had 4 to 5 animals.

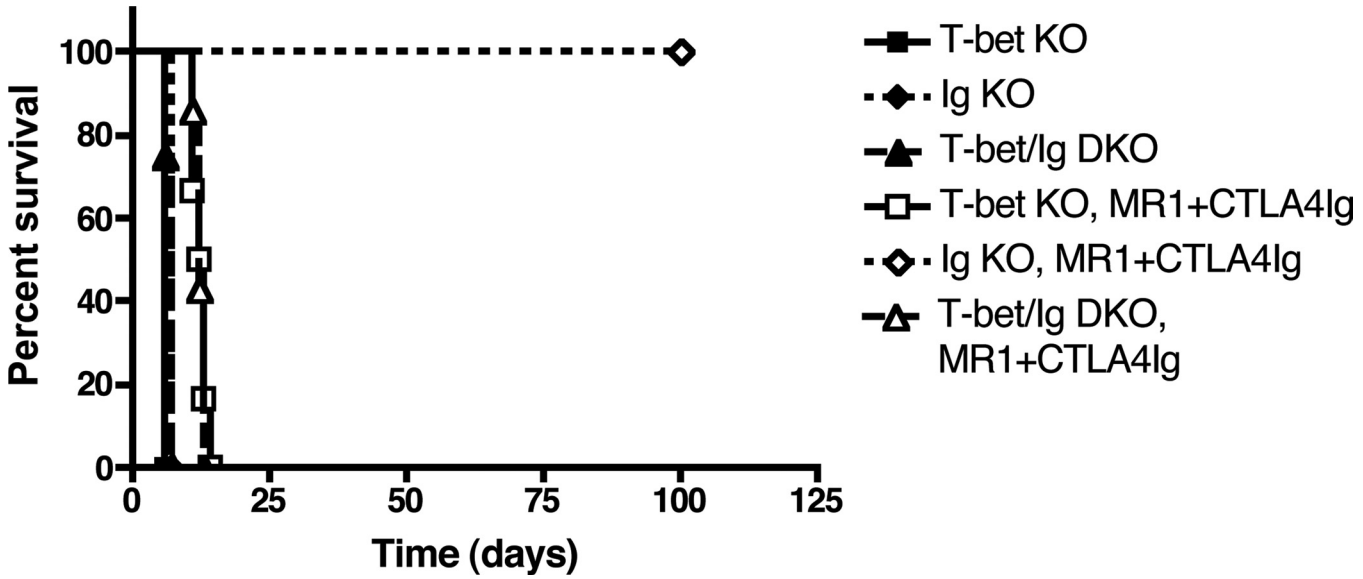

Fig. S4. Kaplan–Meier survival curves of fully mismatched cardiac allografts in Ig KO, Tbet KO, and Tbet/Ig DKO recipients treated with CTLA4Ig+MR1 [MST with CTLA4Ig+MR1: Ig KO >100 days; Tbet KO 13 and Tbet/Ig DKO 12 days; $P < 0.01$ (Ig KO vs. Tbet KO or Tbet/Ig DKO); $P > 0.05$ (Tbet KO vs. Tbet/Ig DKO)]. Each group had 4 to 7 animals.