



**Supplementary Figure 4** Treg specific for directly presented alloantigens prevent acute but not chronic cardiac allograft rejection. **(a)** B6 recipient mice were preconditioned i) with sublethal irradiation only ( $\blacksquare$ ,  $n=6$ ), ii) with irradiation and injection of BALB/c (H-2<sup>d</sup>) bone marrow ( $\blacktriangle$ ,  $n=5$ ), or iii) with irradiation and injection of donor bone marrow and Treg pre-activated *in vitro* ( $\blacklozenge$ ,  $n=5$ ). Three to eight weeks later, mice were transplanted with BALB/c hearts. Allograft survival was monitored. **(b)** Representative features of cardiac histopathology 100 days after transplantation (HE, hematoxylin and eosin; Masson, Masson's trichrome stains). Scale bar represents 200  $\mu$ m in left panels, 100  $\mu$ m in upper right panels, and 40  $\mu$ m in lower right panels. **(c)** Clinical score of BALB/c heart graft rejection 100 days post-transplantation. Clinical scores of B6 hearts transplanted into sublethally irradiated B6 hosts are shown as comparison ( $n=5$ ).  $**P<0.01$  (Student's  $t$  test). **(d)** PBMC from mice treated as described in the legend of Fig. 3 were analyzed by FACS at 100 days post cardiac transplantation, the day mice were euthanized and hearts taken for histological analysis. Indicated are the percentages of donor cells (H-2K<sup>d+</sup>) among PBMC.