

Supplementary Note 1

Rational for the use of in vitro cultured Treg

In this report we used Treg prestimulated *in vitro* with donor-type APC. Freshly isolated Treg did not induce tolerance to bone marrow allografts in our previous study, probably for quantitative reasons¹. Moreover, very large numbers of non-specifically expanded Treg were required to inhibit bone-marrow allograft rejection in another report². Also in reports on Treg-mediated prevention of GvHD it was shown that Treg *in vitro* cultured with specific APC are more potent than cells expanded *in vitro* with third-party APC^{3,4}. *In vitro* culture therefore has two advantages: it increases the available numbers of Treg and improves their *in vivo* reactivity. Moreover, the use of *in vitro* cultured cells allows for evaluation of their *in vivo* antigen-specificity during the suppressor-effector phase (**Fig. 1e**).

1. Joffre, O., Gorsse, N., Romagnoli, P., Hudrisier, D. & van Meerwijk, J.P.M. Induction of antigen-specific tolerance to bone marrow allografts with CD4+CD25+ T lymphocytes. *Blood* **103**, 4216-4221 (2004).
2. Taylor, P.A. *et al.* L-Selectin(hi) but not the L-selectin(lo) CD4+25+ T-regulatory cells are potent inhibitors of GVHD and BM graft rejection. *Blood* **104**, 3804-3812 (2004).
3. Cohen, J.L., Trenado, A., Vasey, D., Klatzmann, D. & Salomon, B.L. CD4(+)CD25(+) Immunoregulatory T Cells: New Therapeutics for Graft-Versus-Host Disease. *J. Exp. Med.* **196**, 401-406. (2002).
4. Trenado, A. *et al.* Recipient-type specific CD4+CD25+ regulatory T cells favor immune reconstitution and control graft-versus-host disease while maintaining graft-versus-leukemia. *J. Clin. Invest.* **112**, 1688-1696 (2003).