

Fig. S1. NK cell depletion induces strong alloreactive CD4 T-cell priming in allogeneic DC-immunized but not naïve mice. B6 CD8<sup>-/-</sup> mice treated or not with anti-NK1.1 PK136 mAb, were injected subcutaneously into the hind foot pads with either PBS (A) or with allogeneic BALB/c BM-DCs ( $0.5 \times 10^{6}$ /mouse) (B). Six days after immunization, draining lymph nodes were harvested and purified CD4 T cells were cultured ( $2 \times 10^{5}$  cells/well) in the presence or absence of irradiated allogeneic BALB/c splenocytes ( $1 \times 10^{5}$  cells/well) for 72 hours. CD4 T-cell proliferation was evaluated by <sup>3</sup>H-TdR incorporation and results are expressed as mean  $\pm$  SEM of quadruplicate cultures. Data are from one representative experiment of two performed.



Fig. S2. Selectivity of Ly49D<sup>+</sup> NK cell depletion.

B6 CD8<sup>-/-</sup> mice were treated with anti-Ly49A/D 12A8, anti-Ly49D 4E5, isotype control rat IgGa or anti-NK1.1 PK136 mAb as described in Materials and Methods. Mice were then injected subcutaneously into the hind footpads with allogeneic BALB/c DCs (10<sup>6</sup>/mouse). At 24 h after immunization, draining popliteal lymph nodes (A) and spleen (B) were collected and stained for TCR $\beta$ , DX5, CD127 and Ly49D expression. The frequency of TCR $\beta$ -DX5+ NK cells and the expression of CD127 and Ly49D on NK cells (TCR $\beta$ <sup>neg</sup> NK1.1<sup>pos</sup>) are shown for one representative animal per group.



**Fig. S3. Allogeneic BALB/c DCs persistence in draining lymph nodes is impaired in the presence of host Ly49D<sup>+</sup> NK cells.** Representative CFSE profiles of injected DCs obtained at 48 hours from untreated or PK136-treated mice are shown. B6 CD8<sup>-/-</sup> mice were treated, or not, with anti-NK1.1 PK136 mAb or anti-Ly49D 4E5 mAb, and injected with a mixture of CFSE<sup>low</sup>-B6 DCs and CFSE<sup>high</sup>-BALB/c DCs. 48 hours after immunization, the presence of CFSE positive DCs (gated on CD11c<sup>pos</sup> MHC II<sup>high</sup>) was analyzed in the draining lymph nodes and the percentage of CFSE<sup>high</sup> BALB/c DCs normalized to control CFSE<sup>low</sup> B6 DCs was determined [(%BALB/c DCs : %B6 DCs) x 100].



Fig. S4. Perforin-mediated killing, but not IL-12RB2-signaling, is critical for allogeneic DC elimination by host NK cells *in vivo*. (A) B6 WT, B6 pfp<sup>-/-</sup> or (B) IL-12R $\beta$ 2<sup>-/-</sup> mice, were all injected with anti-CD8 mAb, and then treated or not with anti-NK1.1 PK136 mAb before immunization with equal numbers of CFSE<sup>low</sup>-B6 DCs and CFSE<sup>high</sup>-BALB/c DCs. 48 hours after immunization, the presence of CFSE positive DCs (gated on CD11c<sup>pos</sup> MHC II<sup>high</sup>) was analyzed in the draining lymph nodes and the percentage of BALB/c DCs among control B6 DCs was determined. Representative CFSE profiles of injected DCs obtained at 48 hours from untreated or PK136-treated mice are shown. Results are expressed as mean ± SEM of 3 to 4 mice per group.



Fig. S5: Alloreactive CD4 T cell priming in response to allogeneic H-2<sup>d</sup> DCs is not affected by host NK cells in pfp-deficient mice. B6 CD8<sup>-/-</sup> (A) or B6 CD8<sup>-/-</sup> pfp<sup>-/-</sup> (B) mice, treated or not with anti-NK1.1 PK136 mAb, were injected s.c. into the hind foot pads with allogeneic BALB/c DCs (10<sup>6</sup>/mouse). Six days after immunization, CD4 T cells were purified from draining lymph nodes, and restimulated (at 2 x 10<sup>5</sup> cells/well) with the indicated amounts of irradiated allogeneic BALB/c splenocytes for 72 hours. CD4 T-cell proliferation was evaluated by <sup>3</sup>H-TdR incorporation during the last 8 hours of culture. Results are expressed as mean  $\pm$  SEM of 3-4 mice per group. Data are from one representative experiment of three performed.



**Fig. S6.** Absence of allogeneic DC killing in perforin-deficient B6 CD8-/- mice despite normal recruitment of NK cells in draining lymph nodes. (A) B6CD8-/- or B6CD8-/-pfp-/- mice were injected with a mixture of CFSE-labeled BALB/c DCs (green) and CMTMR-labeled B6 DCs (red). Draining lymph nodes were removed at 48 h. Acetone fixed frozen sections were stained with goat NKp46-specific antibodies followed by donkey anti-goat Alexa 633- conjugated antibodies. (B) Histograms indicate the percentage of CFSE BALB/c cells among control CMTMR B6 cells (left) and the number of NKp46<sup>+</sup> cells per lymph node sections (right). Results are expressed as mean ± SEM of individual lymph nodes (3 mice per group).



**Fig. S7. Adoptively transferred NK cells reduces allogeneic DC numbers in perforin-deficient B6 CD8**<sup>-/-</sup> **mice.** (A) B6CD8<sup>-/-</sup> pfp<sup>-/-</sup> mice were reconstituted or not with purified wild-type NK cells i.v. (5 x 10<sup>6</sup>/mouse, see supporting Materials and Methods below) before immunization with 1.5 x 10<sup>6</sup> CMTMR-labeled BALB/c DCs (red). Draining lymph nodes were removed at 72 hours. Acetone fixed frozen sections were stained with APC conjugated anti-B220 (blue) mAb and goat NKp46-specific antibodies followed by donkey anti-goat Alexa 488-conjugated antibodies (green). (B) Histograms indicate the numbers of CMTMR BALB/c DCs cells per lymph node sections and results are expressed as mean ± SEM of individual lymph nodes (2-3 mice per group).