Flutter et al. Supplementary Data

Supplementary Figure S1

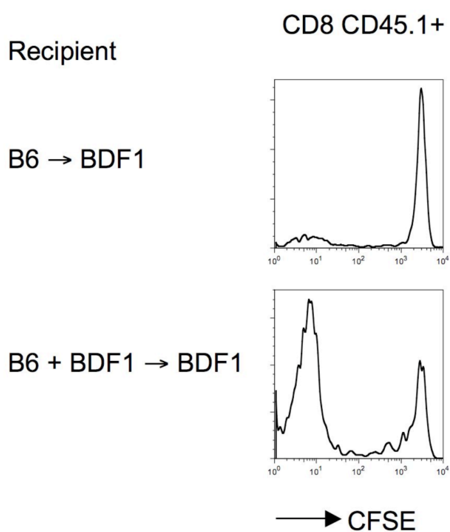

Figure S1 Proliferation of transferred CD8 cells requires direct priming by host-strain BM-derived APCs. 10 weeks after BMT, 3 x 10^7 CFSE-labelled CD45.1 B6 SC were transferred to established [B6 + BDF1 → BDF1] or [B6 chimeras → BDF1] chimeras. 6d after transfer, *in vivo* proliferation was assessed by the dilution of CFSE within gated CD8+ CD45.1+ T cell populations.

Supplementary Figure S2

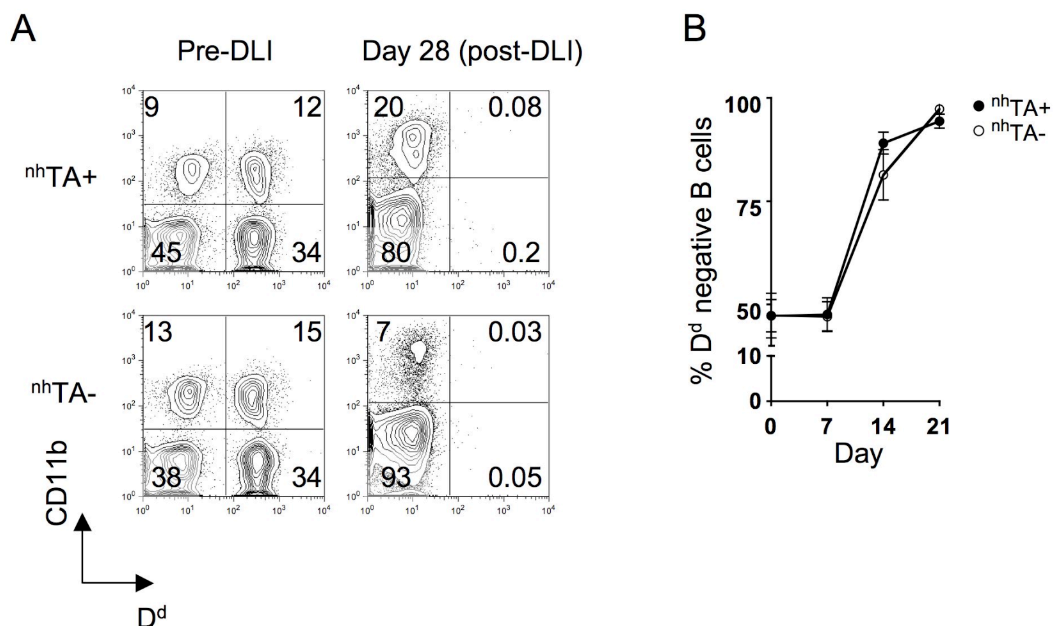

Figure S2 Eradication of BDF1 hematopoietic cells following allogeneic T cell transfer to ^{nh}TA+ and ^{nh}TA- chimeras. ^{nh}TA+ and ^{nh}TA- chimeras were established as shown in Figure 2. ***A)*** Six weeks after transplant (pre-SC transfer) or 28d after SC transfer, blood chimerism was assessed by flow cytometry using D^d as a marker of BDF1-derived cells. Representative contour plots show the chimerism of the CD11b+ myeloid compartment. Similar patterns of chimerism were also seen in B and T cell compartments. ***B)*** Graph showing kinetics of eradication of BDF1-derived B cells in ^{nh}TA+ and ^{nh}TA- chimeras.

Supplementary Figure S3

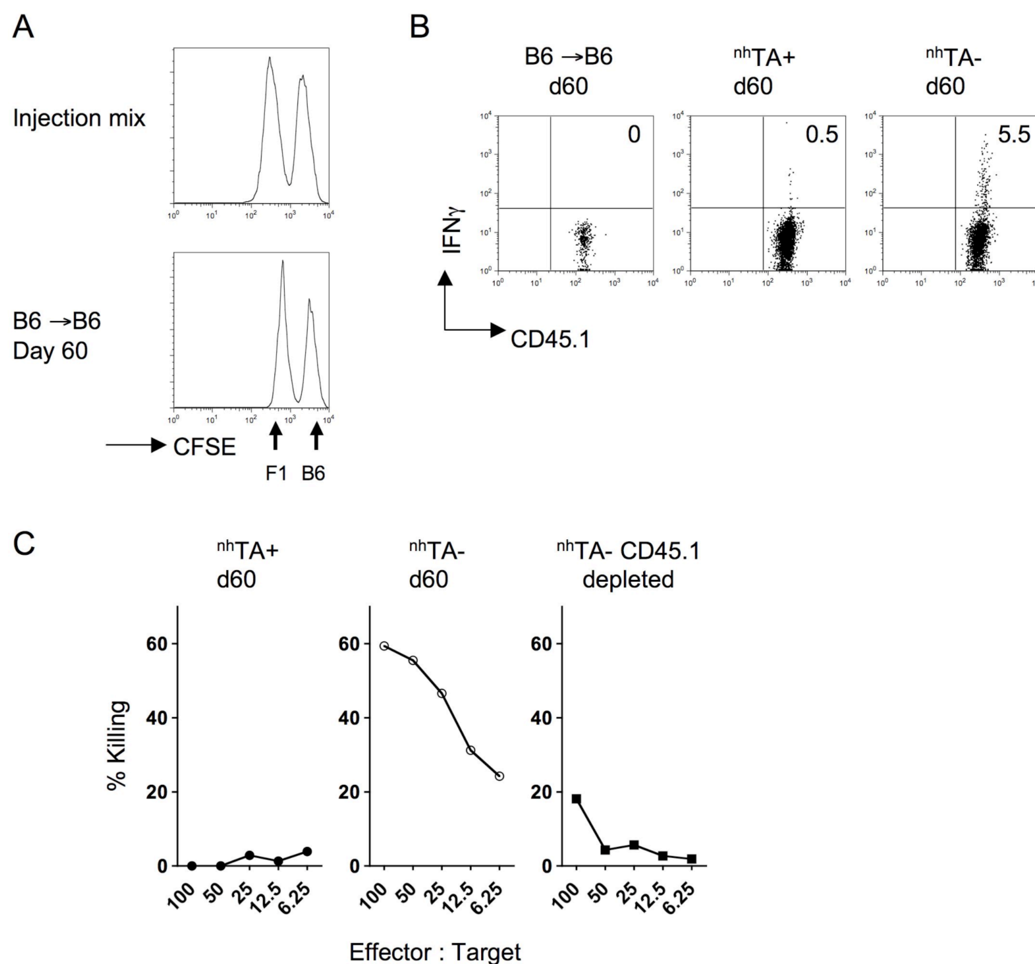

Figure S3 Contribution of naïve or non-transferred CD8 cells to cytotoxicity and IFN-γ generation in recall assays. ***A)*** *In vivo* cytotoxicity measured by loss of CFSE labelled BDF1 B cells 60d after B6 SC transfer to established B6→B6 full chimeras. Representative histograms show CFSE labelled B cell populations injected into B6 full chimeras at 59d after DLI and recovered from the spleen 15 hours later. ***B)*** Cells recovered from the spleens of ^{nh}TA+, ^{nh}TA- or B6→B6 chimeras 60 days after B6 SC transfer were stimulated overnight with BDF1 SC. Representative dot plots show IFN-γ production by CD45.1+ CD8 T cells (B6 →B6 chimeras, n=4 from 2 independent experiments). ***C)*** Cells were recovered from spleens of ^{nh}TA+ and ^{nh}TA- chimeras, 60d after CD45.1 B6 SC transfer. Either whole SC or SC depleted of CD45.1+ cells by magnetic selection were re-stimulated for 5d with irradiated BDF1 SC. Representative graphs show specific cytotoxicity assessed by the release of chromium from target BDF1 blasts in a CML assay (n=4-5 from 2 independent experiments). No killing of B6 Con A blasts was observed.

Supplementary Figure S4

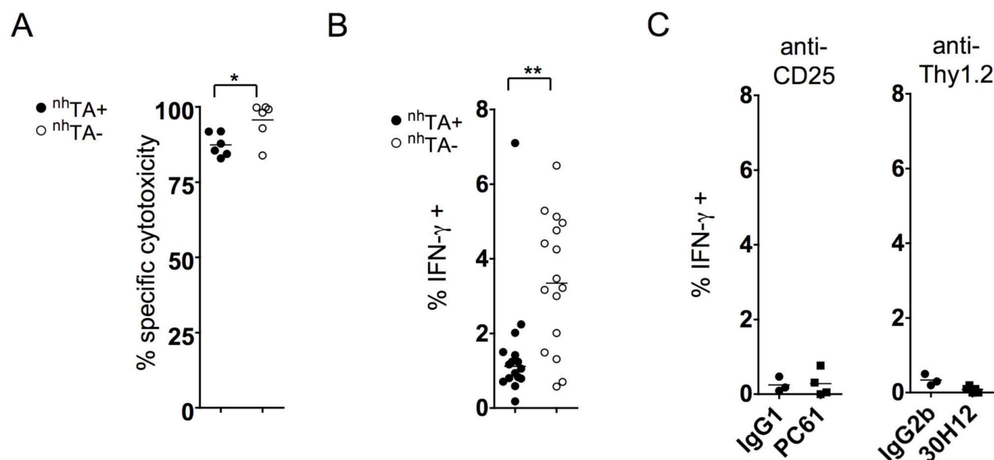

Figure S4 *Non-haematopoietic antigen impairs early, intrinsic CTL functions. A) In vivo* cytotoxicity of CFSE labelled BDF1 B cells in nhTA+ and nhTA- hosts at 14d after SC transfer. *B)* 14d after SC transfer, production of IFN-γ was measured following overnight stimulation with irradiated BDF1 SC. A-B show pooled data from 3-5 independent experiments. *C)* nhTA+ chimeras received a transfer of 3 x 10^7 CD45.1 (left panel) or Thy1.1 SC (right panel). 14d after SC transfer, production of IFN-γ was measured following overnight stimulation with irradiated BDF1 SC. *Left- In vivo* depletion of CD25+ cells was performed at the time of transfer by i.p. injection of 0.5mg PC61 antibody at d-2, and 0.25mg on days d1, d4 and d7. Depletion of donor (CD45.1) and recipient (CD45.1-) CD4+Foxp3+ cells by 13d was 4-fold and 3-fold respectively compared to isotype control. This lack of effect was not due to depletion of effector cells, since activated CD8 cells do not express CD25 following delayed DLI in this model and depletion had no effect upon donor CD8 expansion or *in vivo* cytotoxicity (data not shown). *Right-* Some mice, which received Thy1.1 SC transfer were depleted of host resident T cells by i.p. injection of 0.5mg of 30H12 antibody at d0 and d7. Depletion of non-transferred T cell populations on day 14 was >2-fold for Thy1.2 CD4 and >4-fold for Thy1.2 CD8 compared to isotype control. Data in *C* derived from 3 independent experiments. Statistical comparisons using the Mann-Whitney test: * $p<0.05$; ** $p<0.01$.

Supplementary Figure S5

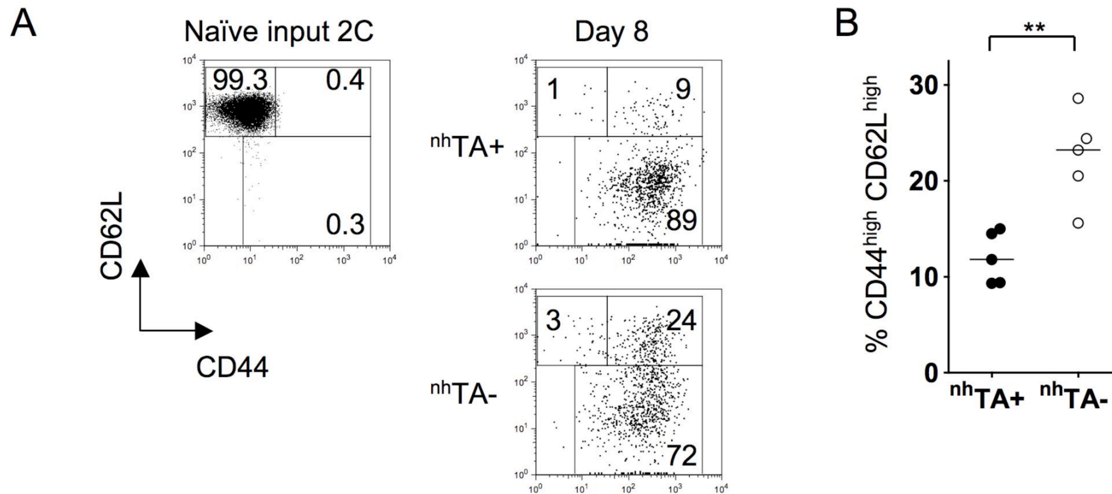

Figure S5 Initial, direct recognition of alloantigen upon non-hematopoietic cells blocks the generation of CD44^{high}CD62L^{high} CD8 cells. *A)* CD8 cells bearing the 2C TCR and displaying a naïve (CD44^{low} CD62L^{high}) phenotype were flow-sorted to high purity. 10^6 naïve 2C CD8 + 8 x10^6 B6 CD3 cells were then injected into ^{nh}TA+ and ^{nh}TA- chimeras and the phenotype of the tg cells was examined on d8. *B)* Summary data showing the proportion of cells with a CD44^{high}CD62L^{high} phenotype. Data derived from 3 independent experiments. Statistical comparisons using the Mann-Whitney test: ** $p<0.01$.

Supplementary Figure S6

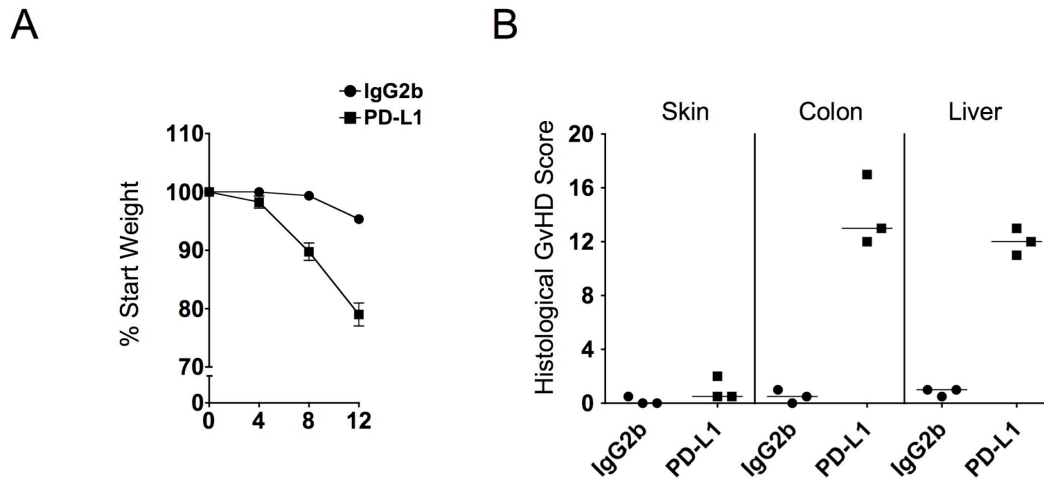

Figure S6 Early PD-L1 blockade following DLI induces GVHD. 3 x 10^7 CFSE-labelled CD45.1 B6 SC were transferred to ^{nh}TA+ chimeras given 0.2mg anti-PD-L1 antibody or isotype control by i.p. injection on d0, d3, d6 and d9. *A)* Change in weights and *B)* day 12 histology scores on skin, colon and liver scored single blind.

Supplementary Figure S7

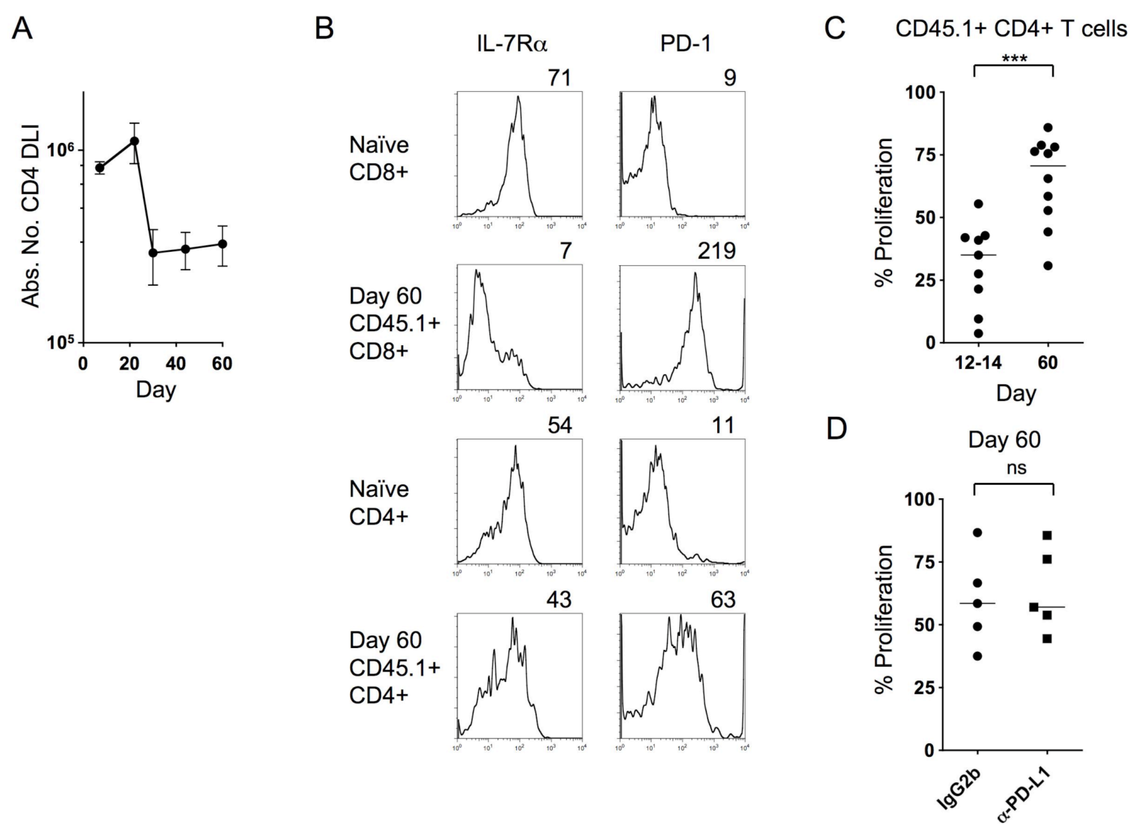

Figure S7 Donor CD4 cells do not become exhausted following delayed DLI to allogeneic chimeras *A)* Absolute numbers of CD45.1+ CD4 cells in spleen of recipient ^{nh}TA+ chimeras at timed intervals following transfer of 3 x 10^7 CD45.1 B6 SC. *B)* Representative histograms showing cell surface phenotype of CD45.1+ CD4 cell populations recovered from the spleen of ^{nh}TA+ chimeras, 60d after SC transfer. Numbers show the MCF. Staining for CD45.1+ CD8 populations from the same experiment is shown in comparison. *C-D)* SC recovered from ^{nh}TA+ chimeras 12d or 60d after SC transfer, were labelled with CFSE and stimulated for 5d with irradiated BDF1 SC. *Ex vivo* proliferation of CD45.1+ CD4 T cells was assessed by dilution of CFSE as measured by flow cytometry. Scatter plots shows the percentage of cells which had divided 2 or more times. Comparison between 12d and 60d is shown in *C*. Effect of anti-PD-L1 blocking antibody or isotype control upon proliferation at 60d is shown in *D*.

Supplementary Figure S8

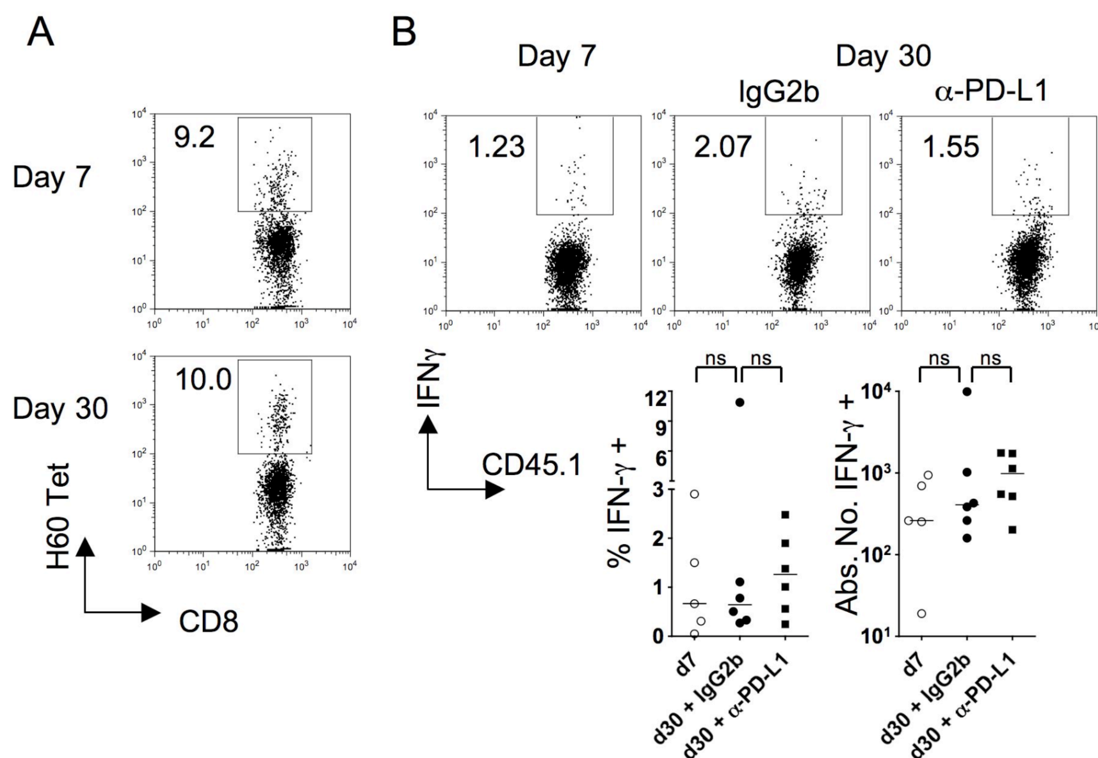

Figure S8 Donor CD8 cells specific for the immunodominant H60 minor H antigen do not become exhausted. 129 mice were lethally irradiated and reconstituted with B6 BM. At d7, $2x10^7$ CD45.1 B6 SC were given as DLI. *A)* The presence of H60-specific CD8 T cells was examined by flow cytometry at d7d and d30 after DLI using tetramer staining. *B)* Following DLI, mice were treated with 0.2mg anti-PD-L1 or isotype control antibody by i.p. injection at 24d and 27d. Representative dot plots and summary data show IFN-γ generation by d30 CD8+ CD45.1+ SC following overnight stimulation with H60 peptide. Gates set according to staining following irrelevant peptide stimulation. Data derived from 3 independent experiments.