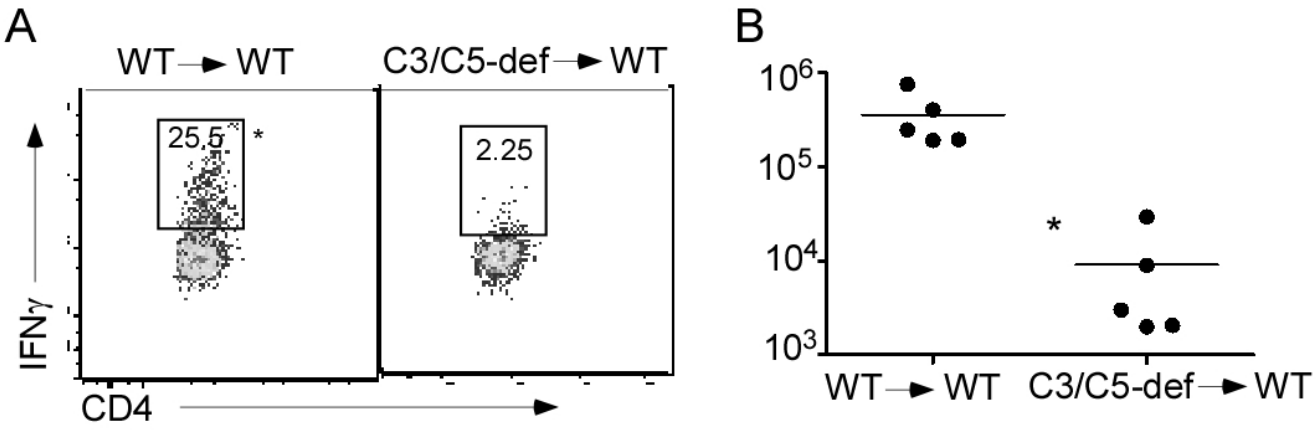
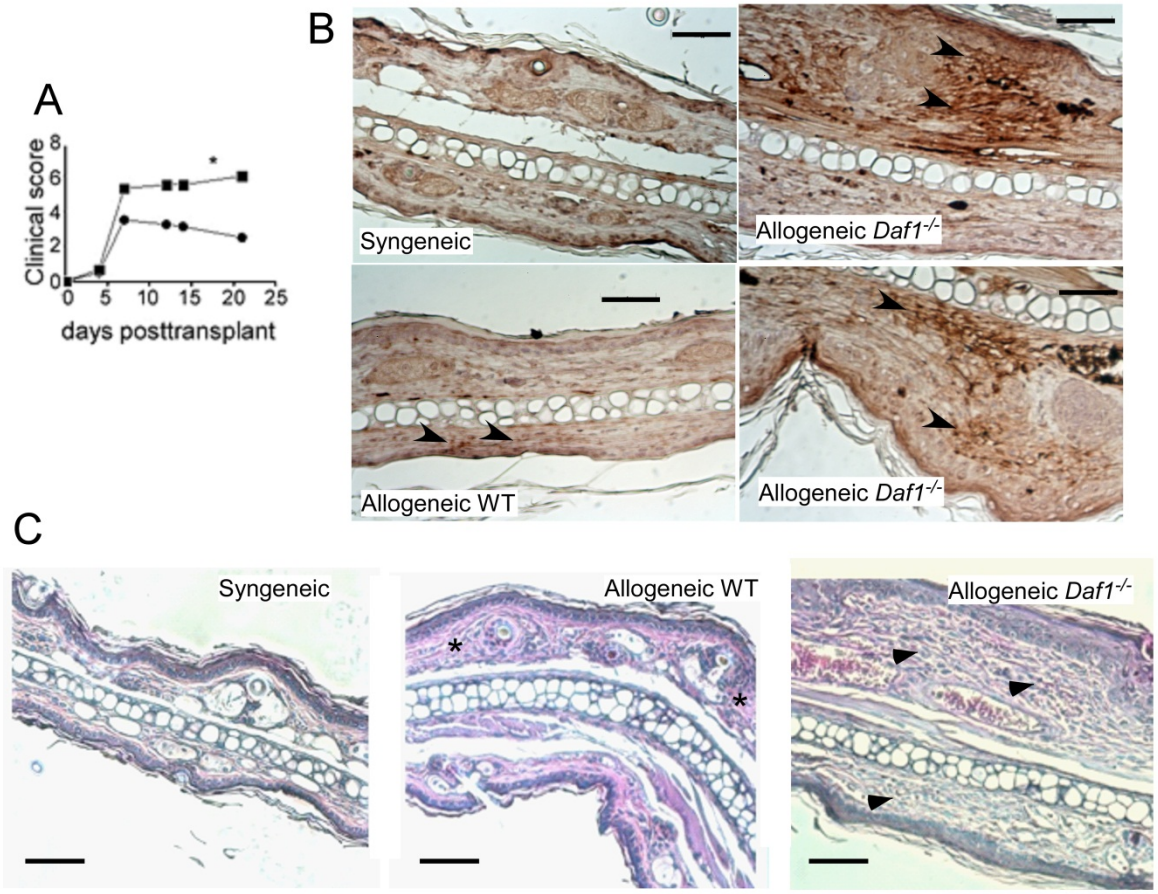
Supplemental Fig S1



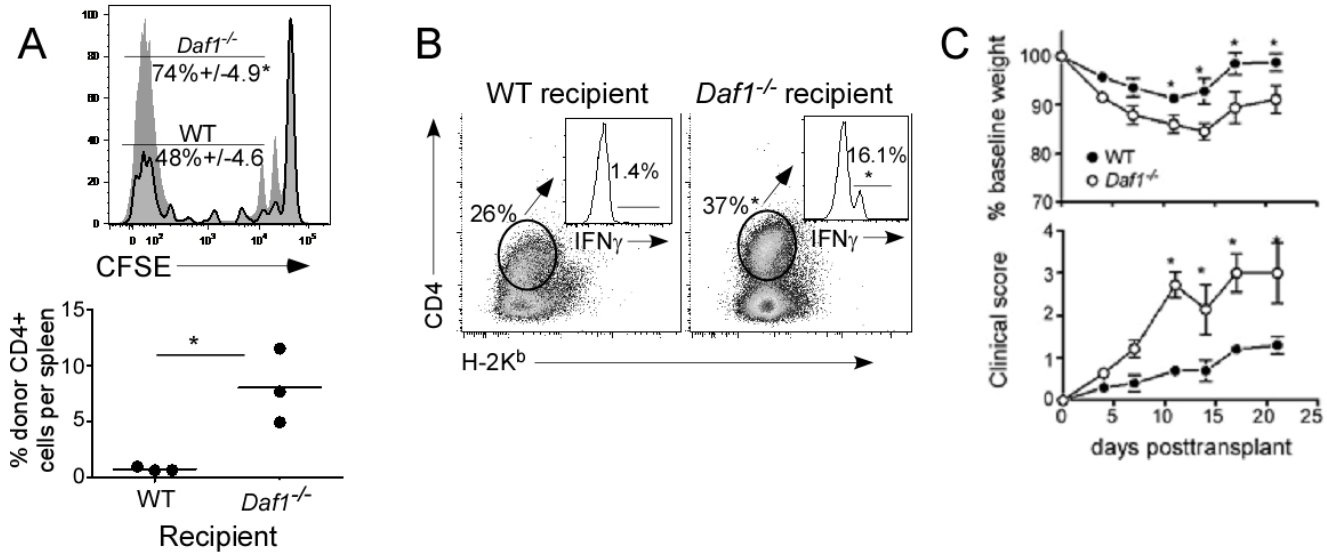
Allogeneic T cells differentiate into IFNγ producers at a lower rate and expand less in irradiated mice with C3/C5-deficient BM. Groups of BALB/c mice were irradiated and transplanted with either control WT (*H-2*^{d}) BM or C3/C5-deficient (*H-2*^{d}) BM. >8 weeks later, the mice were given TBI followed by adoptive transfer of 400,000 WT B6 (*H-2*^{b}) CD4+ T cells. Spleen cells were isolated on d 5 posttransfer and analyzed by flow cytometry. A. Representative flow plots gated on *H-2*^{d}-neg cells stained for CD4+ and IFNγ. Numbers represent mean values of IFNγ-producing cells per group (n=5), $p<0.05$. B. Total numbers of *H-2*$^{b+}$ CD4 cells in each animal. *$p<0.05$.

Supplemental Figure S2



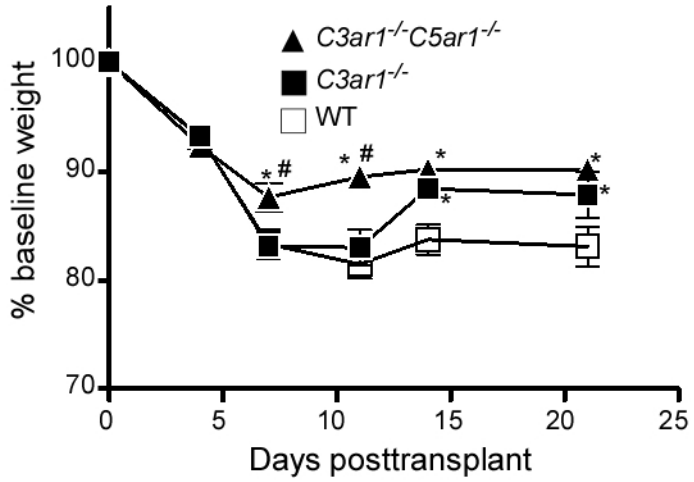
Recipient DAF deficiency worsens GvHD in a fully MHC disparate combination. A. Clinical scores in WT (circles) or *Daf1*$^{-/-}$ (squares) B6 recipients transplanted with WT BALB/c BM supplemented with 1 x 10^6 WT BALB/c spleen cells. n=5 per group. *p<0.05. Repeated with the same results (n=5/group).**B.** C3d staining in skin with GvHD is found at sites of cellular infiltration**.** Representative C3d stained ear skin tissue obtained 6 weeks after transplantation. Observed essentially no staining in normal dermis of syngeneic B6 to B6 transplants but positive intra-dermal C3d staining at sites of cellular infiltration (arrowheads, red staining) in allogeneic transplants, particularly in the context of DAF deficiency. Fully representative of 3 animals per group. scale bar 50 microns. Staining for C3d was performed with technical assistance from William M. Baldwin III, MD, PhD, and Nina Volokh, Cleveland Clinic, Cleveland OH using anti-mouse complement component C3d Antibody, R&D Systems, catalog #: AF2655 in 1/100 dilution. Biotin-SP-conjugated AP (or HRP) Donkey Anti-Goat IgG (H+L) antibody (Jackson Lab, catalog #: 705-065-147) in 1:1000 was used as the secondary. **C.** representative H&E stained sections of same ear skin tissue illustrating normal histology of recipients of syngeneic transplants (left), moderate mononuclear cell infiltration in WT recipients of allogeneic transplants (*) and severe mononuclear cell infiltration in *Daf1*$^{-/-}$ recipients of allogeneic transplants (arrow head).

Supplemental Figure S3



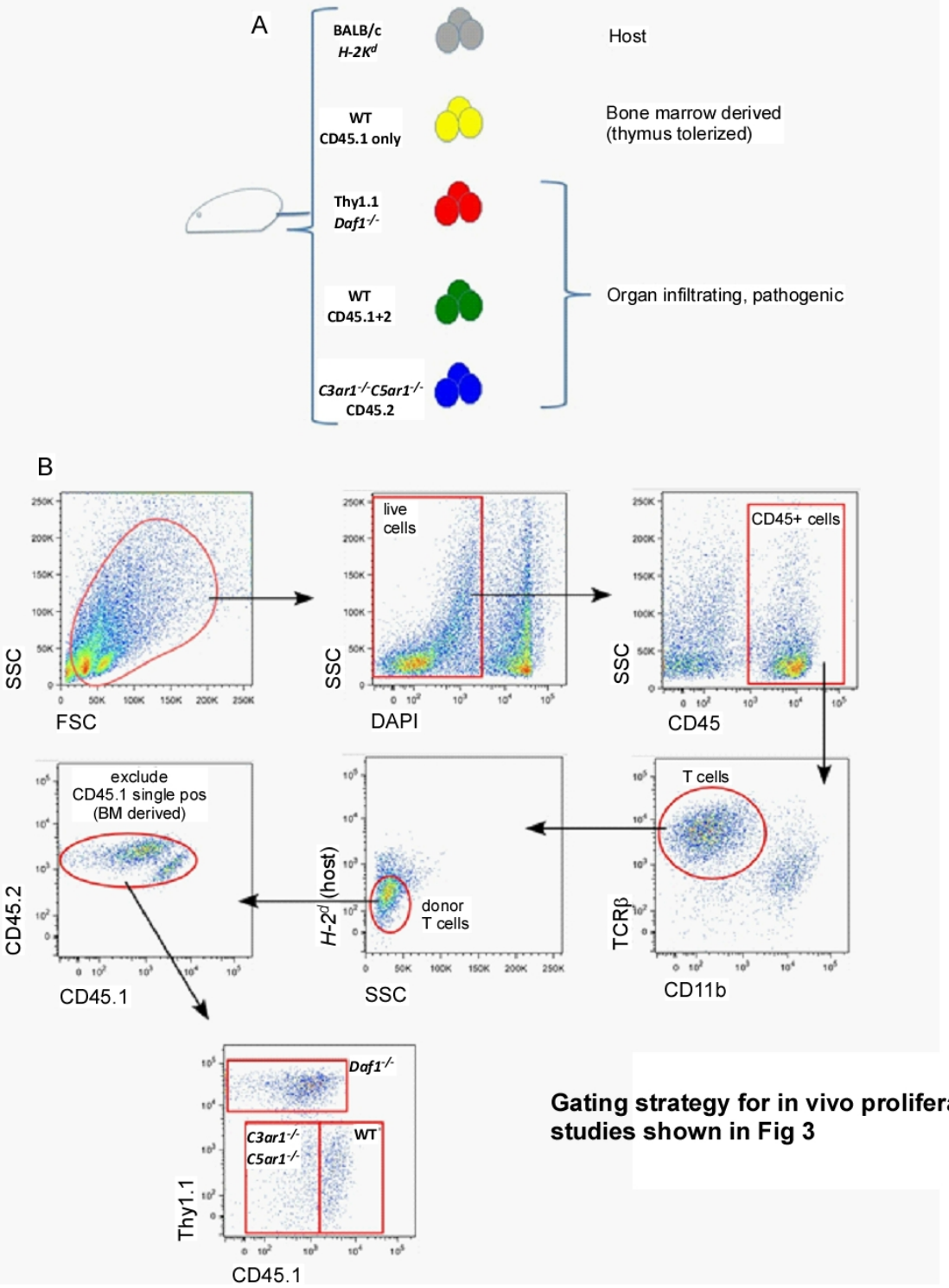
A. Recipient DAF deficiency augments primary proliferation and expansion of naive allogeneic T cells. 4x10^6 CFSE-labeled BALB/c (*H-2^d*) CD4+ T cells were injected into TBI treated mice. 5 d later spleen cells were analyzed by flow cytometry gating on *H-2^{d+}* cells. TOP: representative flow plots for responses d5 in WT (black line) or *Daf1$^{-/-}$* (grey filled) mice, numbers represent means+s.e. of % that proliferated >1 division (n=3/group, *p<0.05 vs WT). Bottom: Total numbers of donor (*H-2^d*) CD4 cells in the spleens of each mouse n=3/group, p<0.05. Repeated with similar results. Additional experiments performed on d3 after transfer also showed more donor CD4+ T cells in *Daf1$^{-/-}$* (5.4%) vs WT (0.5%, p<0.05) recipients, not shown. **B. Recipient DAF deficiency augments proliferation and expansion allo-primed T cells.** 250,000 CD4+ BALB/c T cells were mixed with 250,000 B6 T cell depleted spleen cells in 96 well plates for 5 days. Re-isolated CD4+ cells (Miltenyi beads) were pooled and 3x10^6 were injected into TBI treated WT or *Daf1$^{-/-}$* mice. 3 d later spleen cells were analyzed by flow cytometry. Representative flow plots are shown. Intracellular IFNγ is shown (inset) within the oval gated region of *H-2^b*-negative CD4+ cells. Both the % *H-2^b*-negative CD4+ cells and the % IFNγ-producers within the gate were greater (p<0.05)in *Daf1$^{-/-}$* recipients, n=5/group. **C. Recipient DAF deficiency worsens GvHD in a mH disparate combination**. % weight change from baseline(top) and clinical scores (bottom) in WT or *Daf1$^{-/-}$* B6 recipients of mH disparate C3H.SW BM + 10 millionspleen cells. n=6 per group, *p<0.05, repeated with same results.

Supplemental Fig S4



GvHD is reduced in recipients of *C3ar1*$^{-/-}$ T cells. Weights of BALB/c recipients of WT B6 BM plus purified WT, *C3ar1*$^{-/-}$, or *C3ar1*$^{-/-}$*C5ar1*$^{-/-}$ T cells (n=10-17/group pooled from 2 experiments). The data from the WT and *C3ar1*$^{-/-}$*C5ar1*$^{-/-}$ T cell groups are the same as those shown in Fig 3 for comparison.*p<0.05

Supplemental Fig S5



Gating strategy for in vivo proliferation studies shown in Fig 3