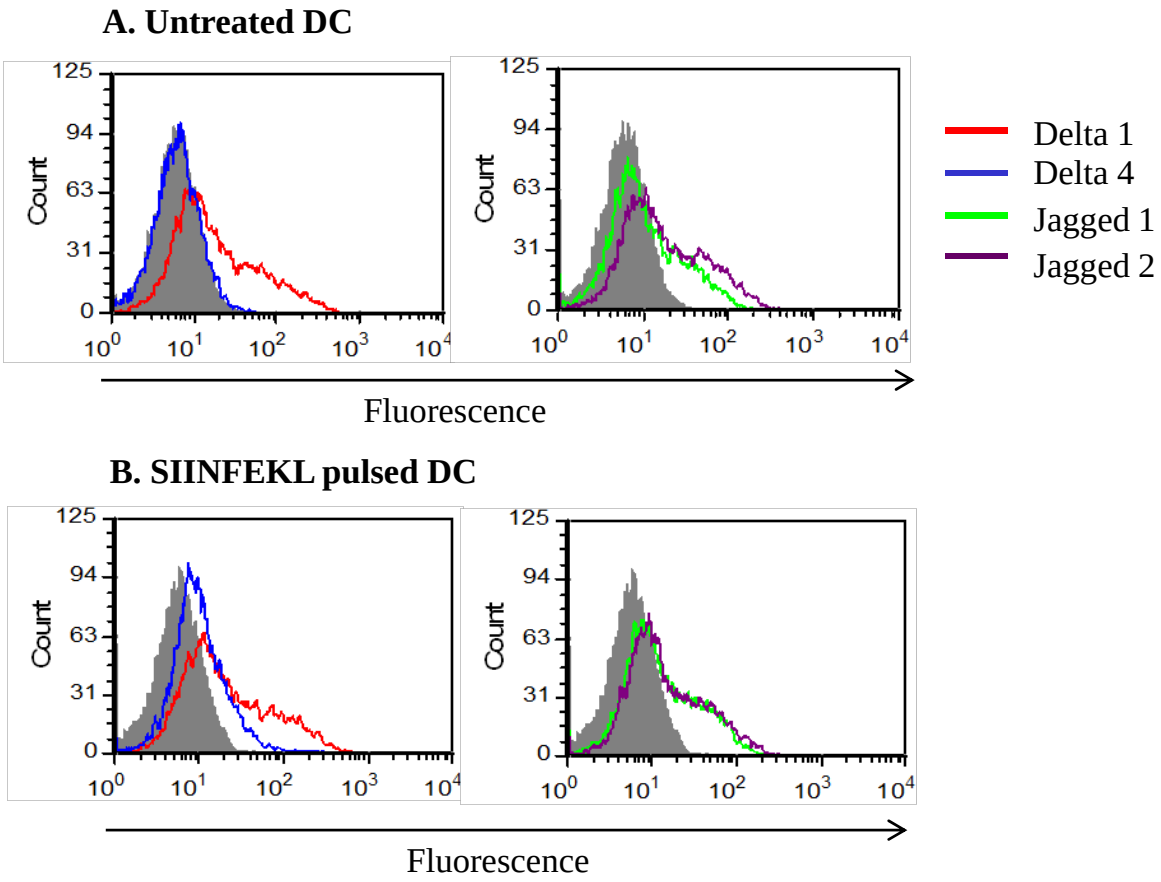


Figure S1: Bone-marrow derived dendritic cells were stained with biotin-conjugated hamster monoclonal antibodies to mouse Delta 1, Delta 4, Jagged 1 and Jagged 2 (kindly supplied by Hideo Yagita) and detected with a secondary streptavidin-PE antibody (BD Biosciences) and analysed by flow cytometry. **A.** Untreated BM-derived dendritic cells. **B**. BM-derived dendritic cells were first incubated with 1 μM SIINFEKL peptide at 37ºC for 1 hour, then stained for the above antibodies.

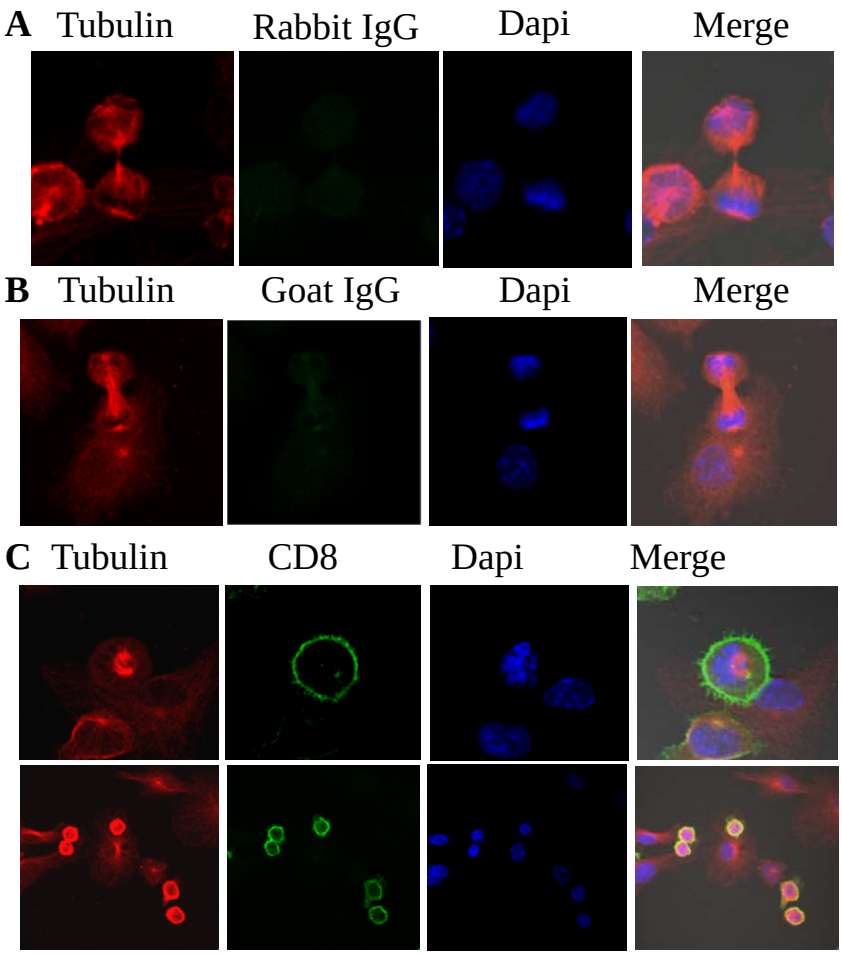


Figure S2: Control antibody staining. For immunofluorescent staining of DC-T cell conjugates, DCs were adhered overnight onto 8-well chamber slides (Nalgene Nunc, IL, USA) and incubated with 1 μM SIINFEKL for 1 hour at 37°C. Naïve OT-I T cells were overlayed for 40 hours and non adherent cells washed off. Cells were then fixed and permeabilised (see materials and methods) and labelled with **(A)** goat polyclonal IgG antibodies (Chemicon) or **(B)** rabbit polyclonal antibodies to CD46 (Crimeen-Irwin et al. (2003) JBC 278:46927-46937) or **(C)** anti-CD8 antibody (BD Biosciences) followed by detection with Alexa Fluor-conjugated secondary antibodies (Molecular Probes) and mounted in Prolong antifade with Dapi (Molecular Probes). The slides were examined at room temperature using a Fluoview FV1000 confocal microscope (Olympus) mounted with a 60x oil immersion objective (NA 1.42). Each image was taken with identical settings.

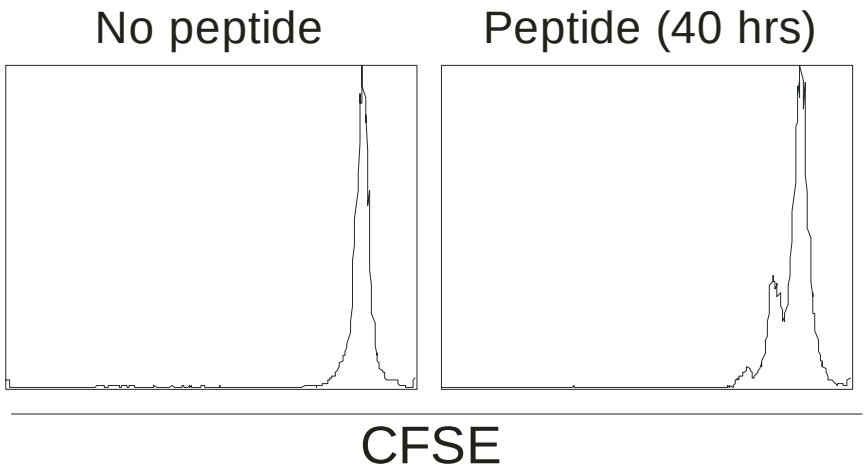
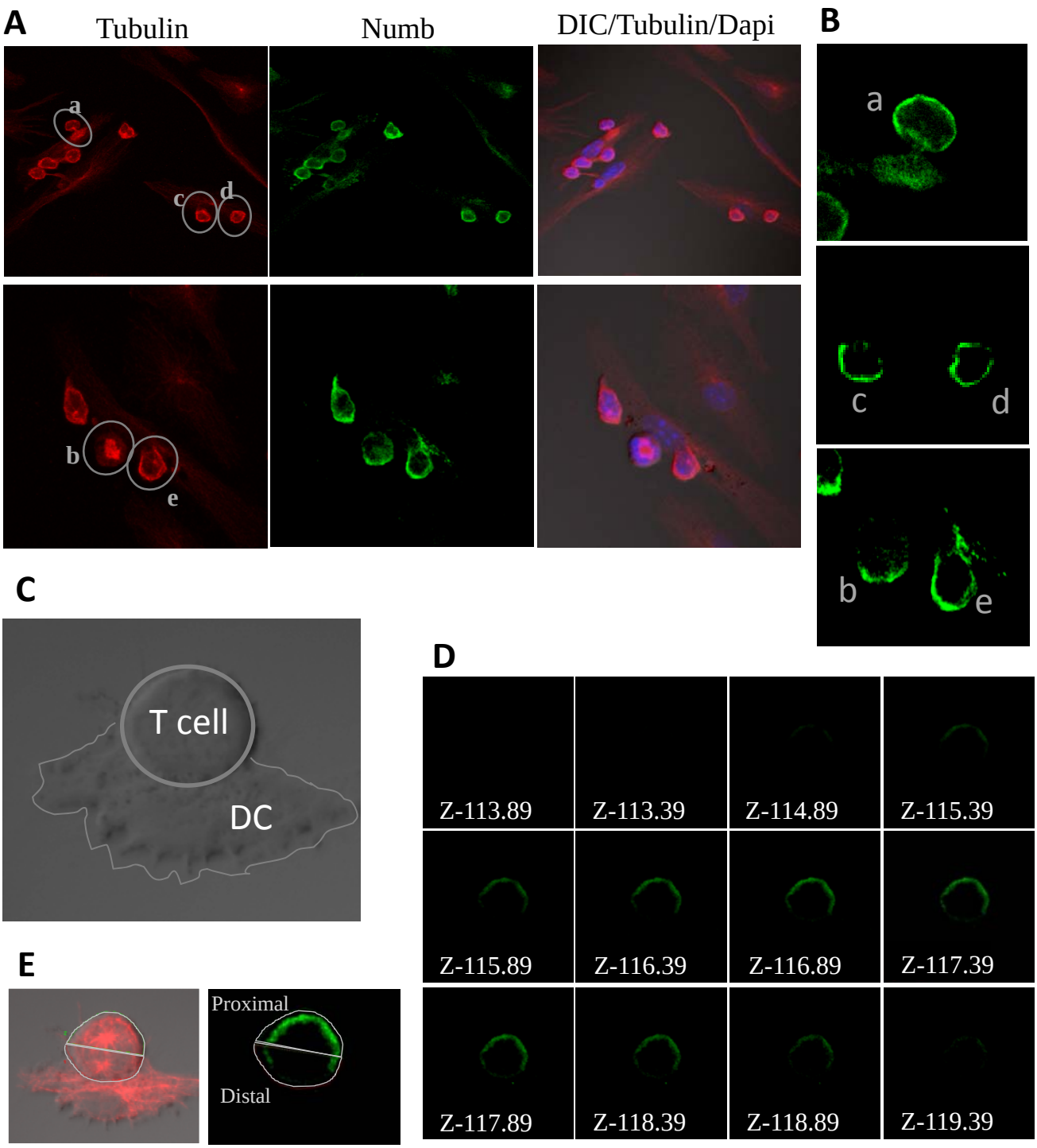


Figure S3: Time of first division of OT-I T cells in response to peptide-pulsed dendritic cells. Naive $CD8^+$ T cells were purified from the spleens of OT-I mice by MACS negative selection and labelled with carboxyfluorescein succinimidyl ester (CFSE: Sigma), such that each cell division results in a detectable halving of the fluorescence. T cells were co-cultured with bone-marrow derived DC and analyzed by flow cytometry to determine the time of first division. Histograms show naive OT-I cells cultured with non-pulsed DCs (no proliferation) and SIINFEKL-pulsed DC showing approximately 10% of cells in first division and approx 1% of cells in second division.



Daughter	Area	Fluorescent Intensity	(P-D)/(P+D)
Proximal	15145	137246	-0.5
Distal	16776	405908	

Figure S4: Confocal analysis of dividing OT-1 T cells attached to dendritic cells (DC). **A.** Images represent two fields of T cells and DCs following co-culture *in vitro*. Each image shows one dividing cell (a,b) and undivided T cells (c,d,e) stained for Numb (green) and Tubulin (red). **B.** Magnified images of T cells highlighted in left panels demonstrating polarization of Numb to the distal daughter/side in a and b respectively, distal polarization of Numb in undivided T cells attached to a DC (c,d) and a T cell that would not be analyzed as it sits on top of the DC (e) and polarization relative to the DC cannot be defined. **C**. Shows the detailed analysis of the image shown in Figure 1 (top panel). The DCs are identified and distinguished from the OT-1 T cells based on morphology and membrane characteristics. The DC is orientated at bottom of all figures in the manuscript (DC is cropped for better emphasis on T cells) and dividing T cells were only selected for analysis if they had a single contact site with the DC enabling designation of proximal versus distal regions. All images in the figures were taken using a Fluoview FV1000 confocal microscope (Olympus) mounted with a 60x oil immersion objective (NA 1.42). 3D images of the cells were acquired with an optical distance of 0.5 μm between slices. **D**. Example of individual Z sections for the protein of interest, aPKC, shown in C. **E**. Tubulin staining overlaid on DIC was used to draw regions to define the two poles of the mitotic spindle in early mitotic cells and the proximal or distal daughter cells in late mitosis using MetaMorph® software. The regions were then overlaid onto the image of the protein of interest (eg aPKC). In order to remove background fluorescence from the images, a top hat filter was applied using a circular structure element with a radius equal to the average radius of the two marked cells. For quantitation, MetaMorph® software was using to calculate the integrated fluorescence in the proximal (P) or distal (D) region, the ratio (P-D/P+D) was applied.

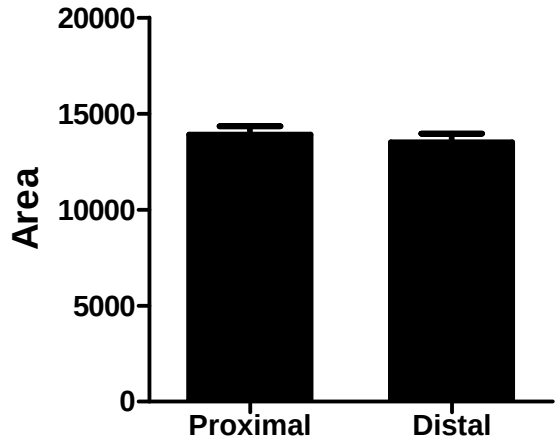


Figure S5: Area of proximal and distal daughter cells of dividing OT-I T cells at late telophase. $CD8^+$ T cells from OT-I mice were cultured with peptide-pulsed DC and at 40 hrs, the cells were fixed and stained for α-tubulin and protein markers. MetaMorph software was used to draw regions around the proximal (closest to the DC) and distal (away from the DC) daughter cells and the area calculated in arbitrary units. The column graph shows no significant difference in area measurement between proximal and distal daughter cells at late telophase. SEM of 191 cells from at least 7 experiments is shown.

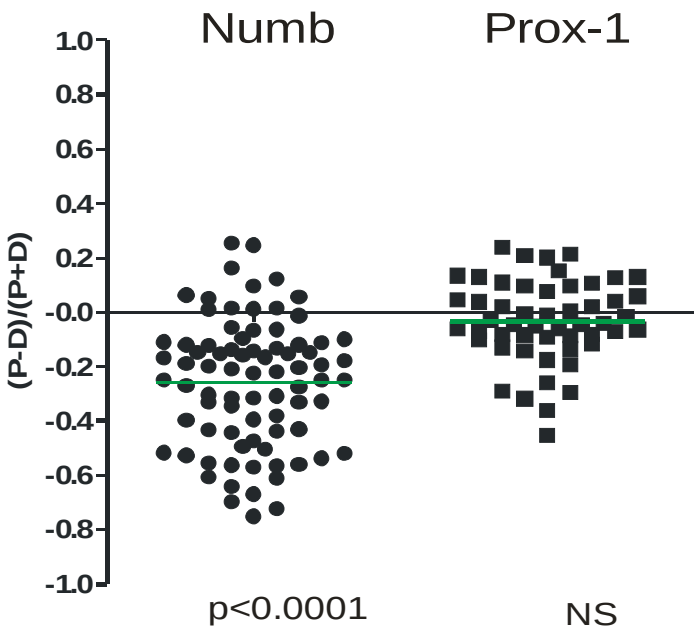


Figure S6: Polarization of Numb and Prox-1 in naive T cells dividing on a DC. Naive OT-I T cells dividing in response to antigen presentation by DC were fixed and stained to determine the ration of proximal and distal polarization of Numb (80 cells, 3 experiments) and Prox-1 (52 cells, 2 experiments). Positive and negative values indicated proximal and distal polarization respectively. Each point on the graph represents an individual cell and the green bar represents the mean. Data shows distal polarization of Numb and symmetric polarization of Prox-1.

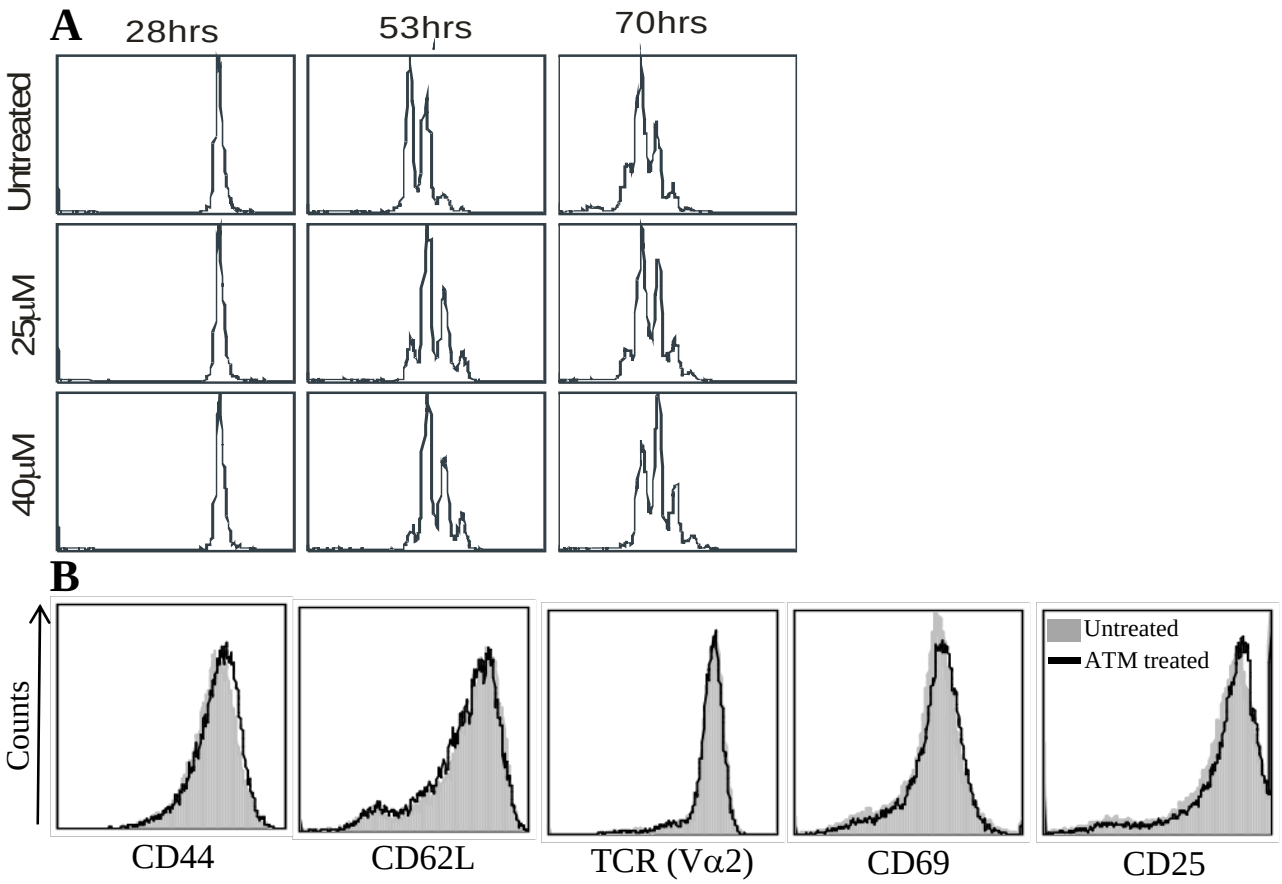


Figure S7: Effect of ATM treatment on OT-1 T cells. **A**. Proliferation of OT-1 T cells in the presence or absence of aurothiomalate (ATM). $CD8^+$ T cells from OT-1 mice were CFSE-labelled and cultured with peptide-pulsed DC for 3 days. In some instances, 25 μM or 40 μM ATM was added to the co-cultures 20 hrs following addition of the T cells. At each time point, the cells were harvested and analyzed by flow cytometry. OT-1 T cells proliferated under each condition, with a slight delay in proliferation of T cells treated with ATM. **B.** Phenotype of T cells treated with 40 μM ATM 48 hrs post activation with peptide-pulsed DC. T cells were harvested and stained with fluorophore-conjugated antibodies to the surface proteins as labelled.

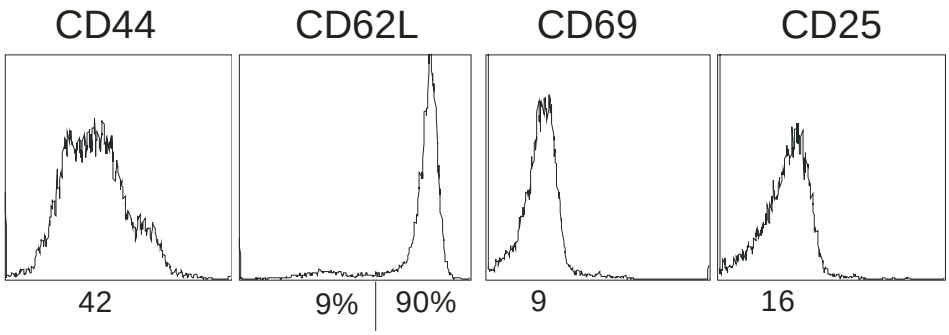


Figure S8: Expression of the surface markers, CD44, CD62L, CD69 and CD25 on naive OT-I T cells. $CD8^+$ T cells were purified from the spleens of OT-I mice by MACS negative selection and stained for CD44, CD62L, CD69 and CD25 and analyzed by flow cytometry. Ninety percent of cells were positive for CD62L and negative for CD69 and CD25. Geometric means, or % cells gated, are shown below the peaks.

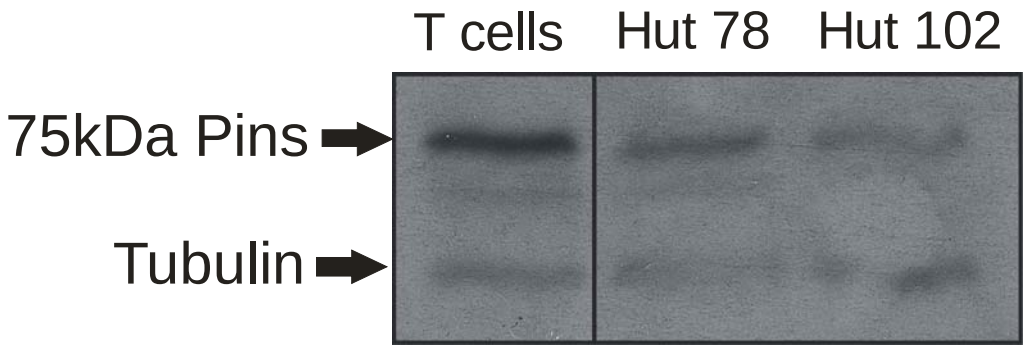


Figure S9: Detection of Pins protein in naive T cells and T cell lines. Cell lysates were prepared from purified splenic naive $CD8^+$ T cells, Hut78 and Hut102 T cell lines. Pins expression was detected by Western blot using a rabbit anti-Pins antibody. Western blot analysis using this antibody shows detection of a major 75 kDa band as previously reported for mouse liver and brain (Yu et al., (2002) Journal of Cell Science 116: 887-896).

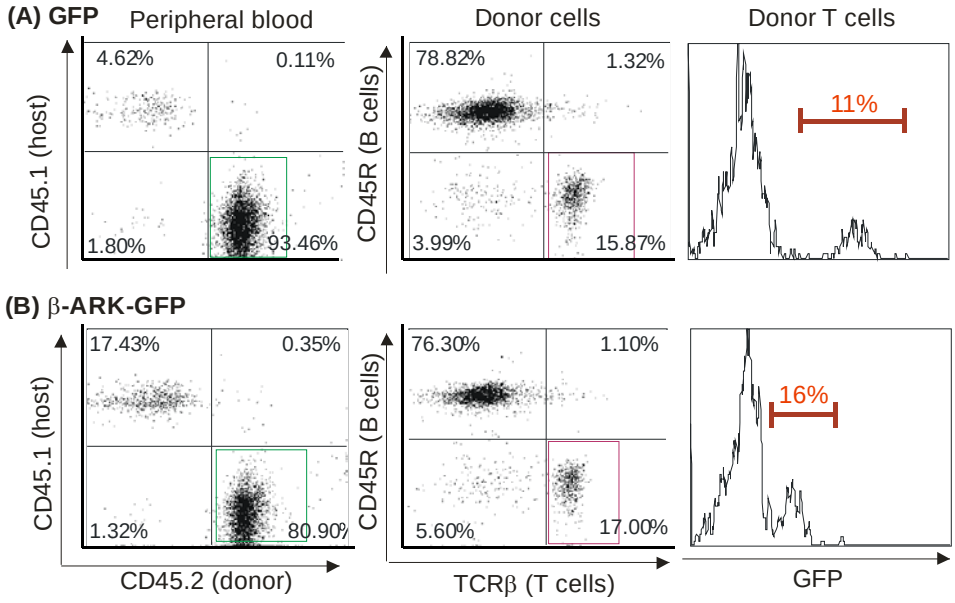


Figure S10: Analysis of the T cells in the peripheral blood of mice reconstituted with GFP constructs. OT-I hematopoietic stem cells were retrovirally transduced with either GFP alone or β-ARK-C-terminal GFP constructs and used to reconstitute lethally irradiated syngeneic mice. At 6 weeks post-injection of transduced cells, the peripheral blood of the mice was analyzed for reconstitution efficiency (donor vs host cells) and the donor cells analyzed for ratios of B and T cells based on CD45R and TCRβ expression respectively. TCRβ positive cells were then assessed for GFP expression.