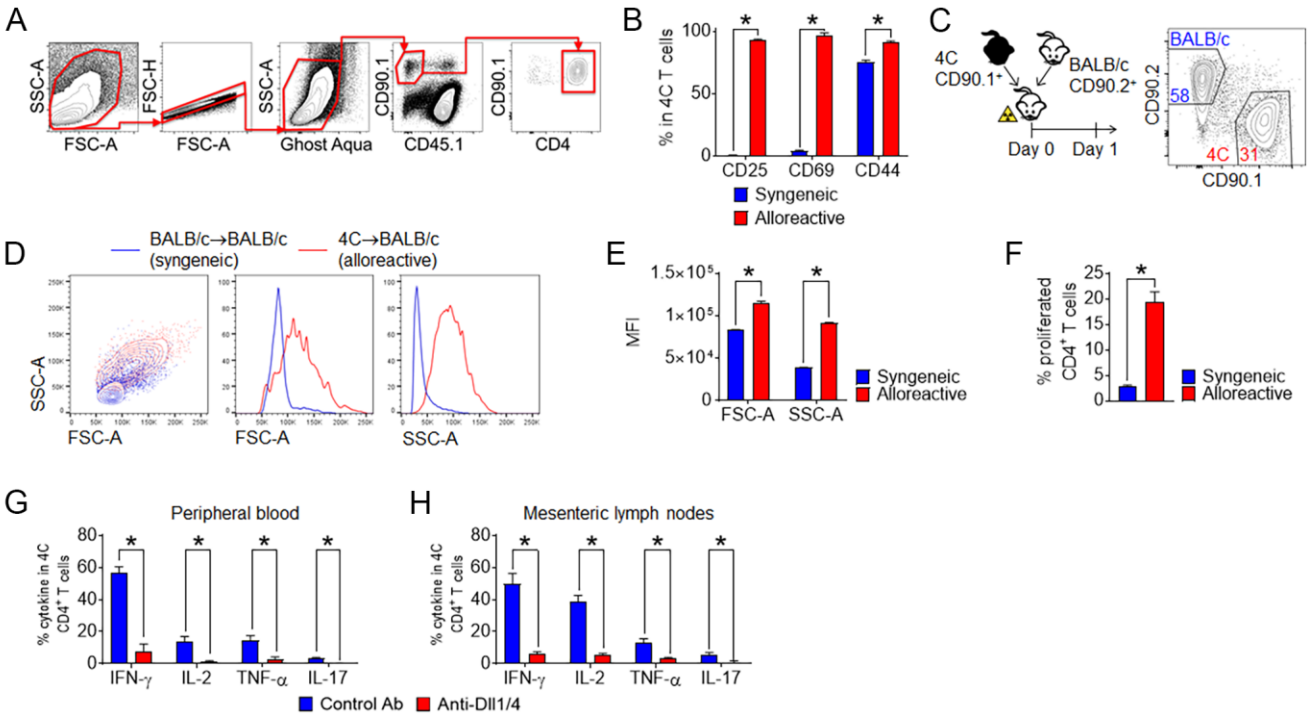

Supplemental figure 1. Early hallmarks of the alloreactive 4C T cell response and cytokine production regulation by Dll1/4 blockade. (A-B) BALB/c-CD45.1 and B6-SJL mice were lethally irradiated (8 Gy and 12 Gy, respectively) and transplanted with B6 WT TCD BM and 5×10^6 4C T cells, with lymphoid organs harvested on day 1.5 for flow cytometric analysis. **(A)** Gating strategy to identify 4C T cells. **(B)** Percentage of 4C T cells expressing the activation markers CD25, CD69, and CD44 retrieved from syngeneic (B6-SJL) and allogeneic (BALB/c) recipients (*$p < 0.001$, Student's t-test). CD25 and CD69 upregulation were specific to alloantigen-mediated T cell activation, while CD44 expression was also increased after transfer into irradiated syngeneic recipients. **(C-F)** Irradiated BALB/c mice were transplanted with B6 WT TCD BM and a mixture of 4C (alloreactive) and BALB/c (syngeneic) T cells before analyses on day 1.5 post-transplantation. **(C)** Experimental design. **(D-E)** 4C, but not BALB/c T cells showed increased cell size (FSC-A) and granularity (SSC-A), hallmarks of alloantigen-driven activation. **(F)** Robust alloantigen-driven proliferation occurred early post-transplant with faster kinetics than homeostatic proliferation. *$p < 0.001$, Student's t-test. Data are representative of at least 2 experiments with 10 animals per group. **(G-H)** Lethally irradiated BALB/c mice were transplanted with TCD BM supplemented with 2×10^3 4C T cells and treated with isotype control or anti-Dll1/4 antibodies on day 0. Animals were sacrificed 5 days after allo-HCT and peripheral blood and mesenteric lymph nodes retrieved for assessment of cytokine production. Notch blockade led to impaired cytokine production in 4C Tconv in peripheral blood **(G)** and mesenteric lymph nodes **(H)**. *$p < 0.05$, Student's t-test.

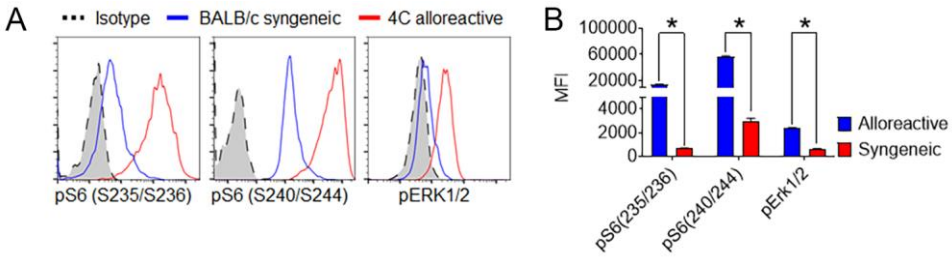

Figure S2. Alloantigen-driven T cell response promotes mTORC1 and Ras/MPAK activation. Irradiated BALB/c mice were transplanted with B6 WT TCD BM and a mixture of 5×10^6 4C (alloreactive) and 5×10^6 BALB/c (syngeneic) T cells before analysis 24 hours post-transplantation. Representative histogram plots **(A)** and cumulative data **(B)** showing the abundance of phosphorylated S6 (S235/S236 and S240/S244 residues) and pERK1/2 in alloreactive 4C and syngeneic BALB/c T cells retrieved from spleens and lymph nodes of transplanted BALB/c mice. *$p < 0.05$, Student's t-test.

Antigen	Clone	Vendor	Antigen	Clone	Vendor
H-2Kb	AF66-88.5	Biolegend	Phospho-Akt (Ser473)	D9E	Cell Signaling Technologies
H-2Kd	SF1-1.1	Biolegend			
CD25	PC61	Biolegend			
CD4	GK1.5	Biolegend			
CD44	IM7	Biolegend	Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204)	D13.14.4E	Cell Signaling Technologies
CD45.1	A20	Biolegend			
CD69	H1.2F3	Biolegend			
CD8a	53.6-7	Biolegend			
CD90.1	OX-1	Biolegend	Phospho-S6 Ribosomal Protein (Ser235/236)	D57.2.2E	Cell Signaling Technologies
CD90.2	30-H12	Biolegend			
IFN-g	XMG1.2	Biolegend			
IL-17	TC11-18H10.1	Biolegend			
IL-2	JES6-1A12	Biolegend	Phospho-S6 Ribosomal Protein (Ser240/244)	D68F8	Cell Signaling Technologies
TNF-α	MP6-XT22	Biolegend			
Nur77	12.14	eBioscience			
Vß13	MR12-4	Biolegend			

Supplemental table I. Monoclonal antibodies used for flow cytometry analysis.