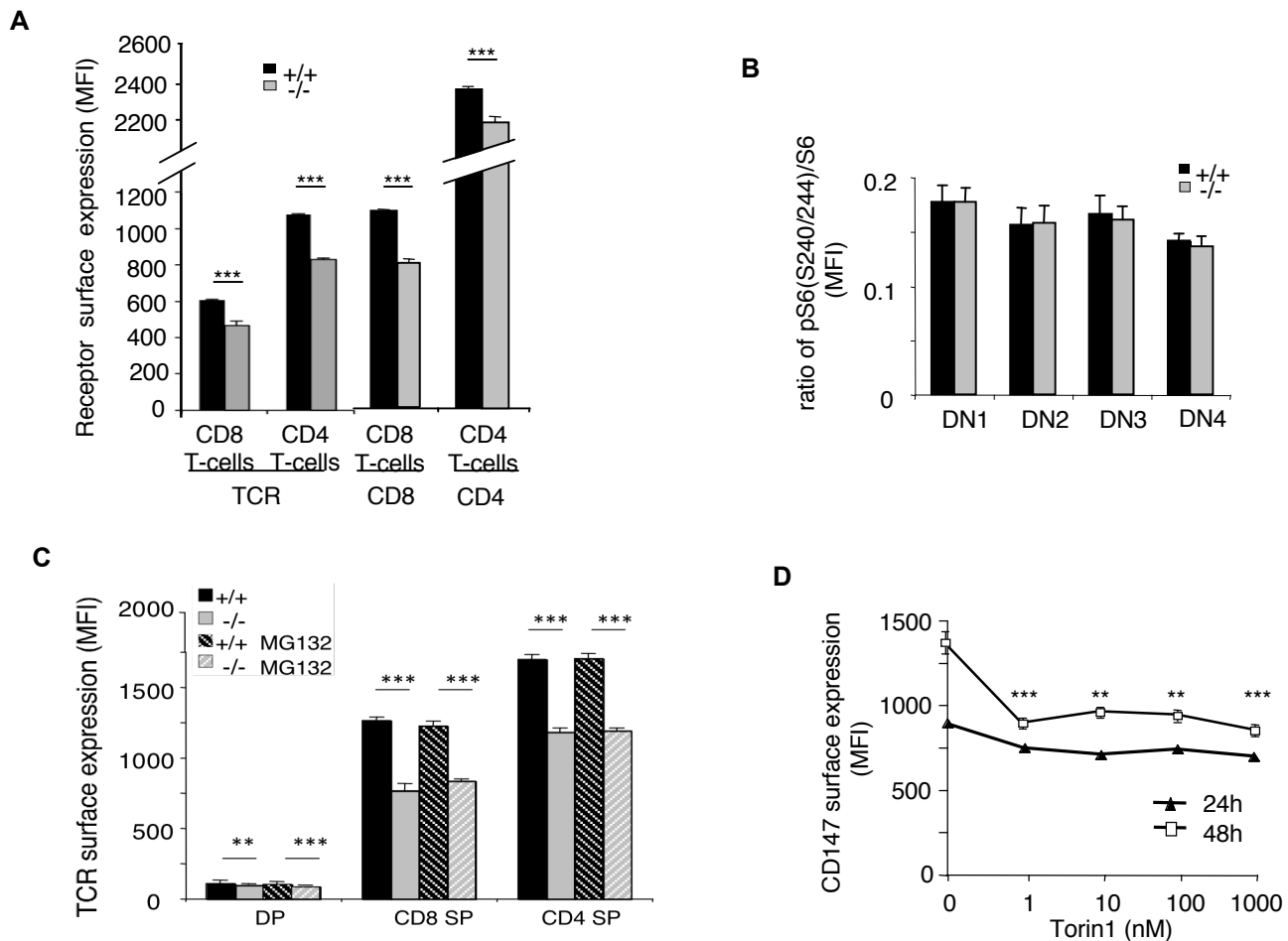


**Supplemental Fig. 1. Cartoon of thymocyte development and concurrent *rictor* deletion.**

Thymocyte development is depicted from the CD4-CD8- double negative (DN) to the CD8 or CD4 single positive (SP). CD8-ISP: immature CD8 expressing single positive cells, CD4CD8 DP: CD4 & CD8 expressing double positive thymocytes. The main surface receptors that characterize each stage are also depicted, as well as the level of pre-TCR and TCR expression. Functionality of the pre-TCR is assessed at the pre-TCR checkpoint between the DN3 and DN4 stages. Functionality of the  $\alpha\beta$ TCR is assessed at the TCR checkpoint during positive selection of DP thymocytes and their differentiation to either the CD4 or the CD8 lineage.

Due to the stage-specific expression of the tyrosine kinase, Lck, rictor deletion started at the DN2 stage prior to the  $\beta$ -selection checkpoint and lasted throughout the entire phase of thymocyte development.



**Supplemental Fig. 2. Rictor-deficiency or the mTORC2 inhibitor, Torin1 but not the proteasomal inhibitor MG132 significantly decreased TCR, CD4, CD8 and CD147 expression on the surface of peripheral or Jurkat T-cells, while no change in phosphorylation of the ribosomal protein S6 was observed in rictor<sup>T-/-</sup> DN cells as compared to wild-type subsets.** **A.** Peripheral T-cells were enriched from wild-type (+/+) and rictor-deficient (-/-) splenocytes. Cells were counted, stained and analyzed by flow cytometry. The MFI of TCR, CD4 or CD8 expressed on peripheral CD4 or CD8 T-cells were plotted from 4 independent experiments. **B.** Thymocytes were fixed with 0.5% paraformaldehyde, permeabilized with 90% MetOH and stained for the intracellular expression and phosphorylation of S6 (n=5). **C.** Thymocytes were cultured ex vivo for 4 hrs in the presence of 50  $\mu$ M of the proteasome inhibitor MG132, stained and analyzed by flow cytometry for TCR expression. The MFI of TCR surface expression was plotted (n=3). **D.** Jurkat T-cells were cultured for 24 or 48 hrs in the presence of the indicated concentrations of Torin1, or its vehicle, harvested, stained and analyzed by flow cytometry for CD147 expression. MFI of CD147 surface expression was plotted from triplicates of 1 representative experiment. Error bars denote standard error of the mean (SEM) and statistical significance was determined by Student's t test. \*\*, p<0.01; \*\*\*, p<0.001.

**Supplemental Table 1:** Primer sequences used for mice genotyping and gene expression by RT-PCR :

gene	Forward primer (5'- 3')	Reverse primer (5'- 3')	Band (bp)
Rictor <sup>+/+</sup>	TTATTAAGTGTGTGGGTTG	CGTCTTAGTGTTGCTGTCTAG	197
Rictor <sup>ff</sup>	TTATTAAGTGTGTGGGTTG	CGTCTTAGTGTTGCTGTCTAG	295
Lck-Cre	GGTTCCCGCAGAACCTGAAG	GCTAAGTGCCTTCTCTACACC	480
Rag-2 <sup>+/+</sup>	GGGAGGACTCACTTGCCAGTA	AGTCAGGAGTCTCCATCTCACTGA	263
Rag-2 <sup>-/-</sup>	CGGCCGGAGAACCTGCGTGCAA	AGTCAGGAGTCTCCATCTCACTGA	350
$\beta_2m$ <sup>+/+</sup>	TATCAGTCTCAGTGGGGGTG	CTGAGCTCTGTTTTCGTCTG	230
$\beta_2m$ <sup>-/-</sup>	TATCAGTCTCAGTGGGGGTG	GCTATCAGGACATAGCGTTGG	210
V $\alpha$ 2	GACTCTCAGCCTGGAGACTCAGC	GTTAAATCCCGCTACTTTTCAG	477
V $\beta$ 5	ACGTGTATTCCCATCTCTGG	CTGTTCATAATTGGCCCGA	238
	Primers below were used for RT-PCR		
TCR $\alpha$	AAACTGTGCTGGACATGAAAG	GGTAAATCCCGCTACTTTTCAG	243
TCR $\beta$	CAGCACGGACCCTCAGGCCTA	TTTGGGTGAGCCCTCTGGCCA	166
CD3 $\epsilon$	CATTGCTGACTCAACAGCCT	GGGCTTAGCTATGAGGGCCAA	169
CD127	AGCCGAGGCTCCCTCTGACCT	TTCACCCCTTGCTGGGCGGTA	143
actin	TCCTGAGCGCAAGTACTCTGTGT	CGGACTCATCGTACTCCTGCTT	101

**Supplemental Table 2:** Antibodies used for flow cytometry and for IP/immunostaining

Antigen	clone	vendor
TCR $\beta$	H57-597	Biologend (San Diego, CA)
V $\alpha$ 2	B20.1	Biologend (San Diego, CA)
V $\beta$ 5	MR9-4	Biologend (San Diego, CA)
CD3 $\epsilon$	145-2C11	Biologend (San Diego, CA)
CD4	RM4-5	Biologend (San Diego, CA)
CD8 $\alpha$	53.6.7	Biologend (San Diego, CA)
CD8 $\beta$	YTS156.7.7	Biologend (San Diego, CA)
CD25	PC61	Biologend (San Diego, CA)
CD44	IM7	Biologend (San Diego, CA)
CD69	H1.2F3	Biologend (San Diego, CA)
CD127	A7R34	Biologend (San Diego, CA)
CD147	OX-114	Biologend (San Diego, CA)
S6 ribosomal protein	54D2	Cell Signaling Technology, MA
Phospho-S6 (240/244)	D68F8	Cell Signaling Technology, MA
Antibodies below were used for IP/western		
Rictor	53A2	Cell Signaling Technology, MA
Akt		Cell Signaling Technology, MA
phospho-Akt (S473)	D9E	Cell Signaling Technology, MA
Phospho-Akt (T450)		Cell Signaling Technology, MA
ERK2		Santa Cruz Biotechnology, CA
phospho-p44/42 (Thr202/Tyr204)		Cell Signaling Technology, MA
phospho-mTOR (S2481)		Cell Signaling Technology, MA
phospho-FoxO1/3a (Thr24)/(Thr32)		Cell Signaling Technology, MA
ubiquitin	6C1	Sigma-Aldrich, MO
CD147		Santa Cruz Biotechnology, CA
TCR $\beta$	H-197	Santa Cruz Biotechnology, CA
TCR $\alpha$	H28-710	Santa Cruz Biotechnology, CA
mTOR	N5D11	IBL, Japan
$\beta$ -actin	AC-74	Sigma-Aldrich, MO
Sin1		Bethyl, TX
S6 Ribosomal protein	54D2	Cell Signaling Technology, MA
phospho-S6 (S240/244)		Cell Signaling Technology, MA
phospho-S6 (S235/236)	2F9	Cell Signaling Technology, MA
PKC $\alpha$		Cell Signaling Technology, MA
phospho-PKC $\alpha$ / $\beta$ II (T638/641)		Cell Signaling Technology, MA
ULK1	D8H5	Cell Signaling Technology, MA
phospho-ULK1 (S757)		Cell Signaling Technology, MA
LC3		Cell Signaling Technology, MA
tubulin	YL1/2	Santa Cruz Biotechnology, CA