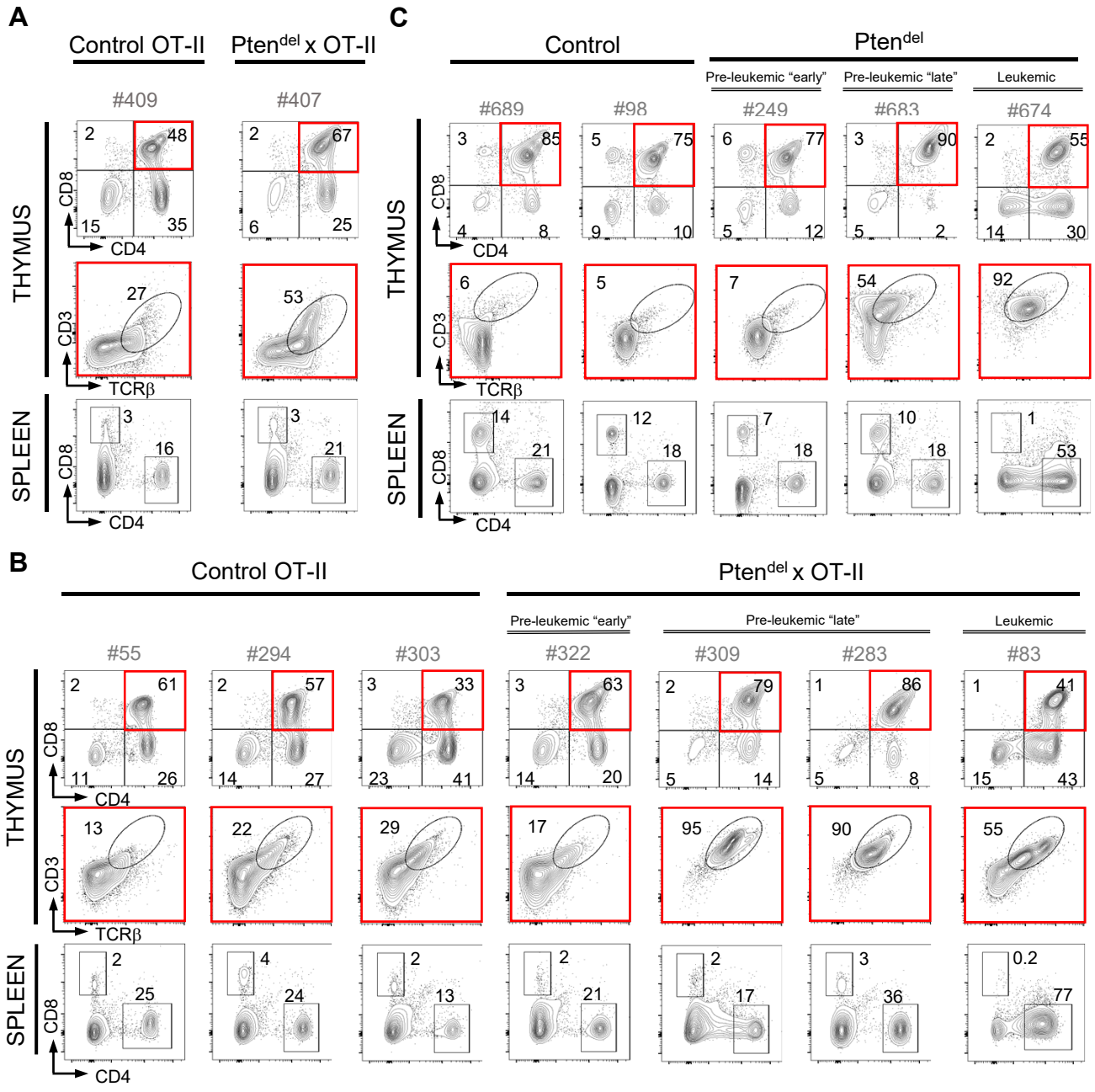
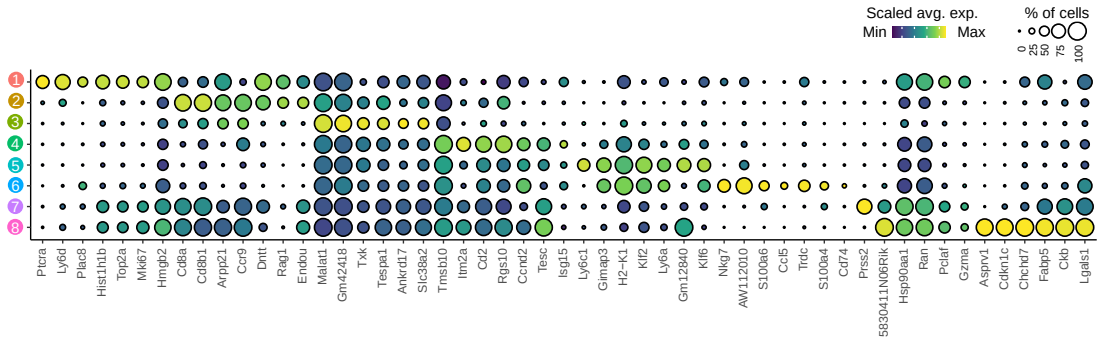
Supplementary Material

Supplementary Figures



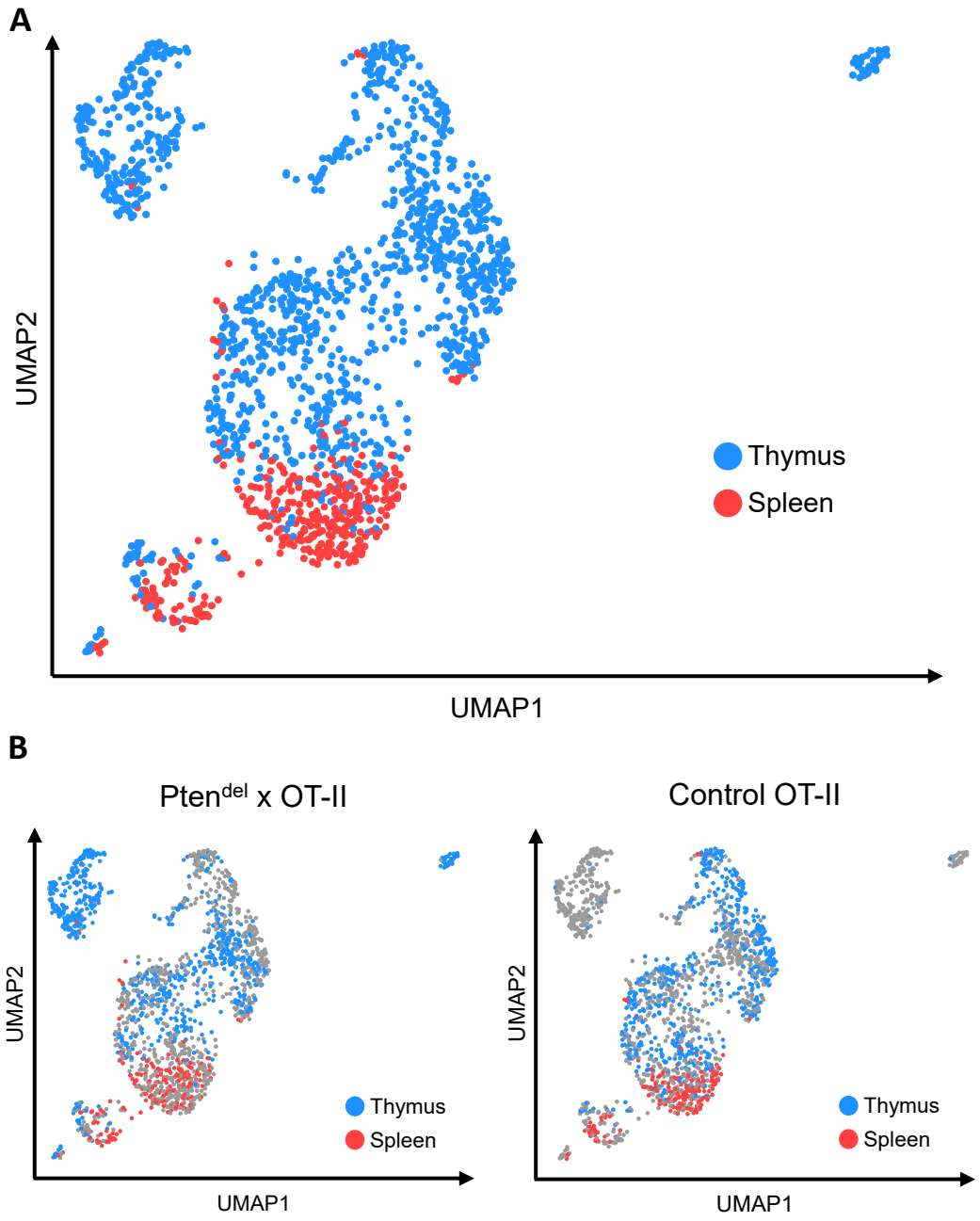
Supplementary Figure 1. Phenotypes of thymus and spleens of Pten^{del} mice from different genetic backgrounds

(**A, B, C**) FACS contour plots showing CD4 and CD8 expression on thymocytes (top panels) and splenocytes (bottom panels) from the indicated mice. Percentages of cells in depicted gates are shown. In the middle panels, the FACS contour plots show CD3 and TCRβ expression on DP thymocytes. The percentage of TCR positive cells is indicated. The identification (#number) of analyzed mice is shown. **(A)** Phenotypes of the control OT-II and Pten^{del} x OT-II mice used for scRNA-seq analysis. **(B-C)** Phenotypes of additional control and Pten^{del} mice in OT-II background **(B)** and non OT-II background mice **(C)**. Analysis were performed at the different stages from disease-free to full-blown leukemia. In pre-leukemic stages, mice do not display clinical signs of leukemia and the spleen is not invaded by leukemic blasts. Typically in the 'pre-leukemic early' stage, thymus from Pten^{del} mice are similar to Control thymus. While in 'pre-leukemic late' stage a large part of DP cells are TCRαβ^{+} and the thymus contains tumor blasts.



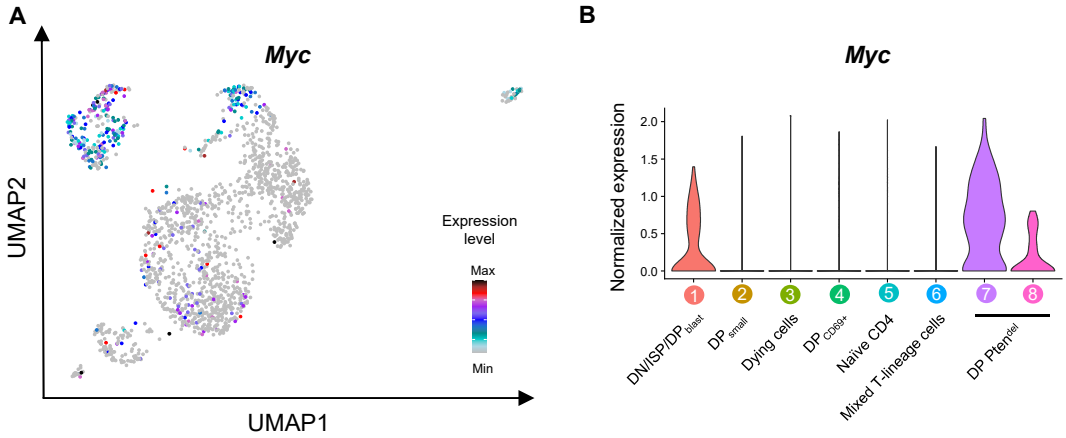
Supplementary Figure 2. Expression of marker genes across clusters

Dot plot showing the expression level of top 7 marker genes of each cluster. Dot size represents the percentage of cell expressing the gene of interest, while dot color represents the average expression of the gene of interest across the 8 clusters.



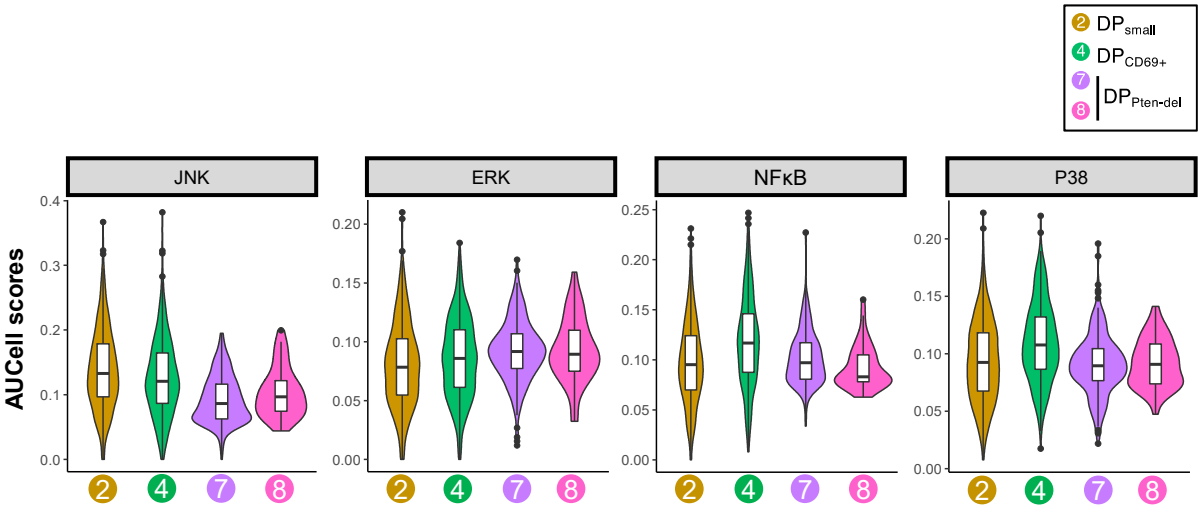
Supplementary Figure 3. Visualization of thymic and splenic cells

(A) UMAP plot colored according to tissue of origin. **(B)** Same as panel A except that each mouse genotype is highlighted.



Supplementary Figure 4. Cells from Pten^{del}-specific DP clusters express *Myc*

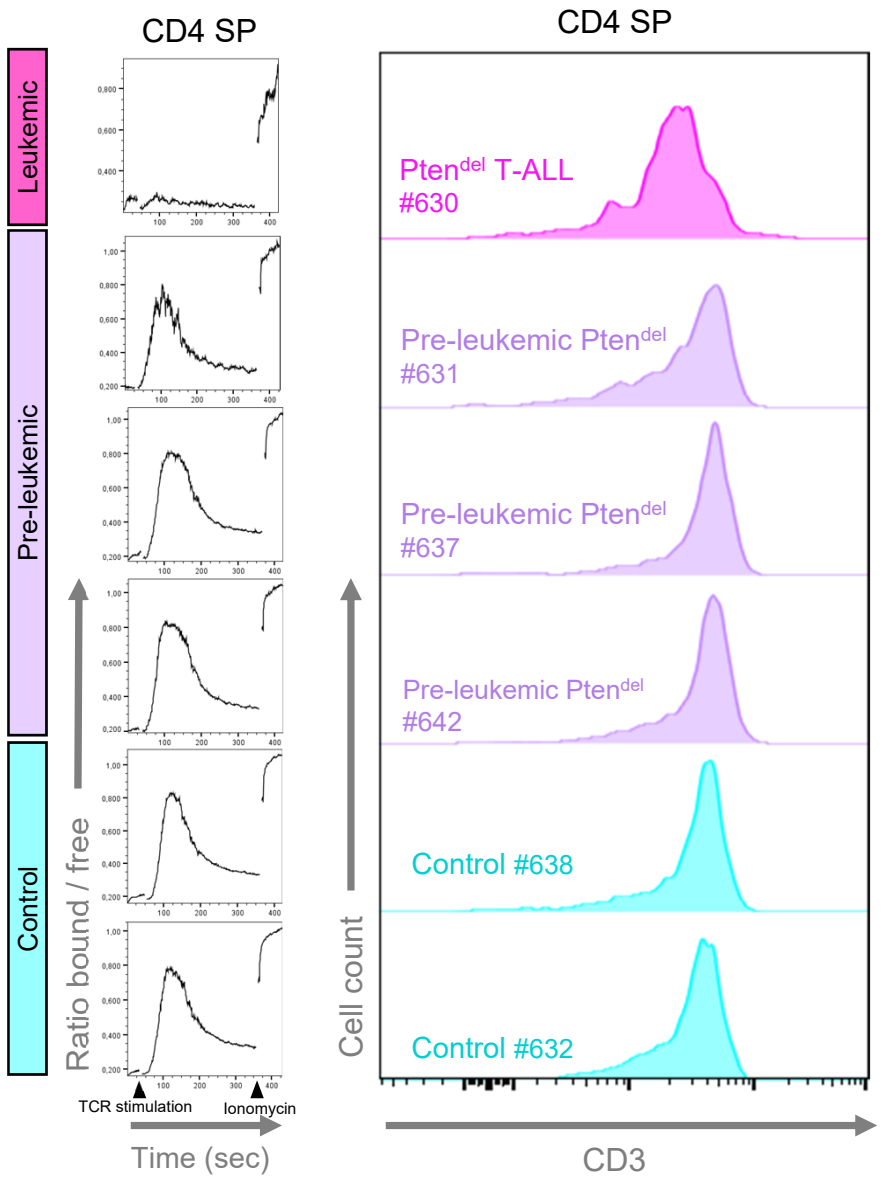
(A) UMAP plot colored according to the scaled *Myc* mRNA expression. **(B)** Violin plots of normalized *Myc* expression for the 8 annotated clusters.



Supplementary Figure 5. Inferred JNK, ERK, NFκB and P38 pathways' activities using scRNAseq data

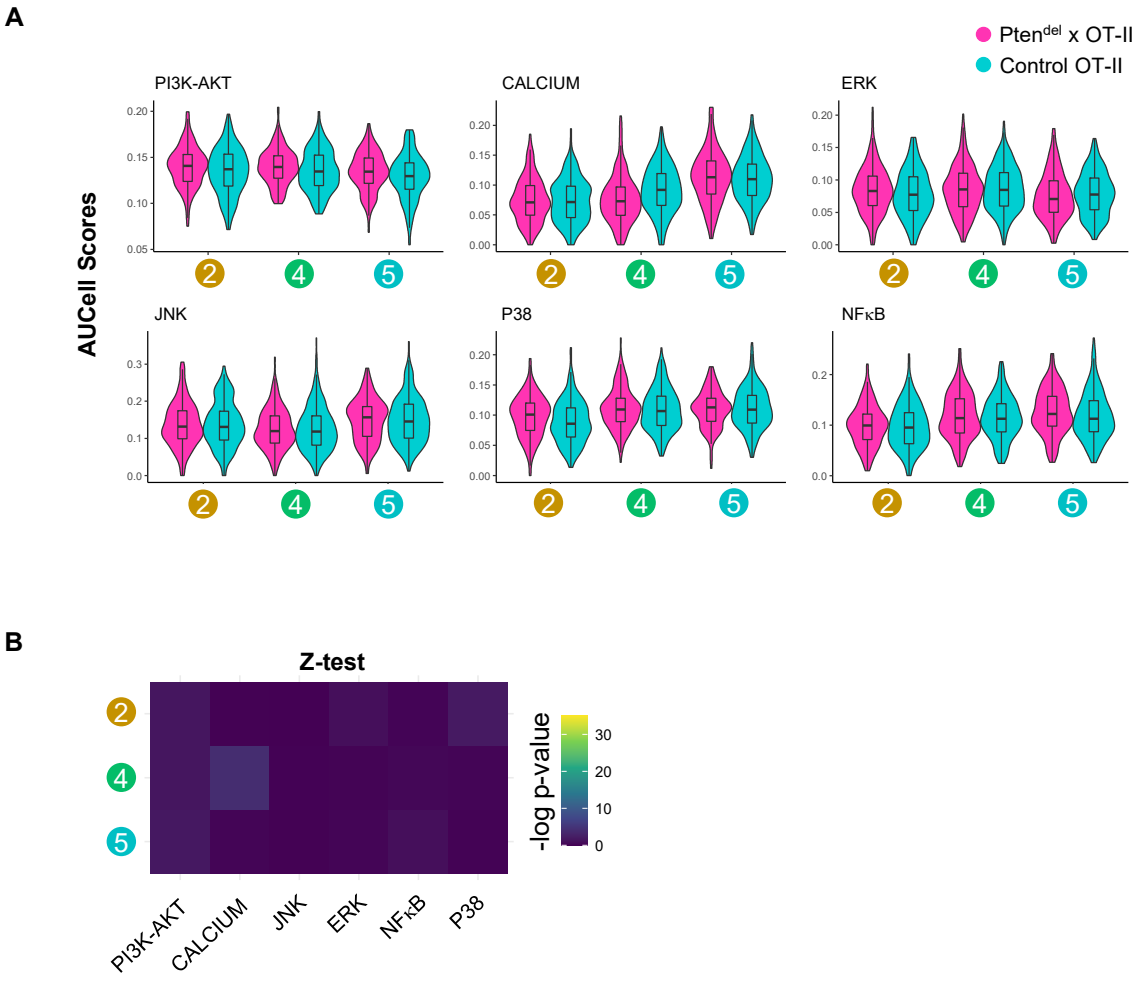
Violin plots displaying AUCell scores for the inferred activity of selected pathways (ERK, P38, JNK, and NFκB) in DP clusters (clusters 2, 4, 7 and 8). The genes lists featuring each pathway were retrieved from MSigDB and are shown in Supplementary Table 2. This analysis suggests that JNK pathway is down-regulated in Pten^{del} DP cluster. It would be interesting to confirm this initial observation by additional investigations. Indeed, as JNK pathway is known to be involved in the process of negative selection of thymocytes [1], its down-regulation may impair thymocytes apoptosis.

1. Bommhardt U, Scheuring Y, Bickel C, Zamoyska R, Hünig T. MEK Activity Regulates Negative Selection of Immature CD4+CD8+ Thymocytes. *The Journal of Immunology* (2000) 164:2326–2337. doi:10.4049/jimmunol.164.5.2326



Supplementary Figure 6. Calcium flux is not impaired in non leukemic Pten^{del} thymocytes

Flow cytometry analysis of calcium flux was performed as described in Figure 2. The left panels display the assays performed with thymocytes from control mice (aged 7 and 8 weeks), pre-leukemic (aged 7 and 8 weeks) and leukemic (aged 8 weeks) Pten^{del} mice. The right panel displays flow cytometry histograms showing CD3 expression in CD4 SP thymocytes. The identification of analyzed mice is indicated (#number).



Supplementary Figure 7. Analysis of pathways activity in non-tumor cell clusters

Pathways analysis were performed as described in Figure 1. (**A**) Violin plots reporting AUCell scores for PI3K-AKT, calcium, ERK, JNK, P38 and NF-kB pathways activities in Control OT-II (blue violins) and Pten^{del}×OT-II (pink violins) cells of clusters 2, 4, and 5 (depicted in Figure 1C). (**B**) Heatmap of –log p-value calculated by the z-test of pathway activity, for Control OT-II *versus* Pten^{del}×OT-II cells within the 3 clusters indicated on the left. The analyzed pathways are indicated at the bottom of each column. Color scale is identical to Figure 1F.

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P70227|ITPR3_MOUSE    436  VSEIRDLDFANDASSMLASAVEKLNEGFISQNDRRFVIQLLEDLVFFVSDVPNNGQNVLD  495
Q9Z329|ITPR2_MOUSE    436  LSEVRDLDFANDANKVLATTVKKLENGSITQNERRFVTKLLEDLIFFVADVTNNGQDVLD  495
P11881|ITPR1_MOUSE    437  PAEVRDLDFANDASKVLGSIAGKLEKGTITQNERRSVTKLLEDLVYFVTGGTNSGQDVLE  495
                            :*:*********..:*.: . **::* *:**:** * :*****::**:.  *.**:**:

P70227|ITPR3_MOUSE    496  IMVTKPNRERQKLMREQNILKQIFGILKAPFRDKGGEGPLVRLEELSDQKNAPYQYMFRL  555
Q9Z329|ITPR2_MOUSE    496  VVITKPNRERQKLMREQNILAQVFGILKAPFKEKAGEGSMLRLEDLGDQRYAPYKYVLRL  555
P11881|ITPR1_MOUSE    497  VVFSKPNRERQKLMREQNILKQIFKLLQAPFTD-CGDGPMLRLEELGDQRHAPFRHICRL  555
                            ::.:*************** *:* :*:*** :  *:* ::***:*.**: **:::: **

P70227|ITPR3_MOUSE    556  CYRVLRHSQEDYRKNQEHIAKQFGMMQSQIGY  587
Q9Z329|ITPR2_MOUSE    556  CYRVLRHSQQDYRKNQEYIAKNFCVMQSQIGY  587
P11881|ITPR1_MOUSE    556  CYRVLRHSQQDYRKNQEYIAKQFGFMQKQIGY  587
                           *********:*******:***:* .**.****
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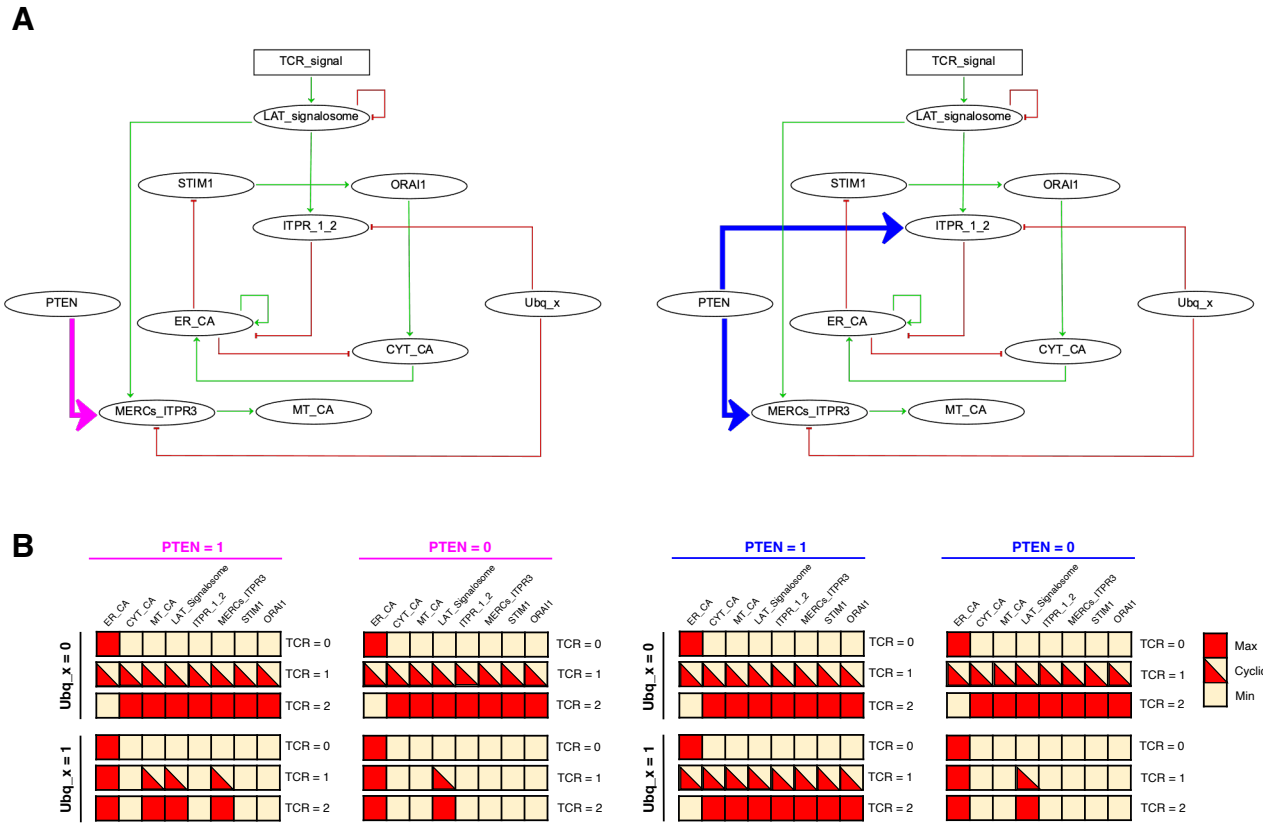
	Identity with ITPR3	Similarity with ITPR3
ITPR2	72.4% (110/152)	90.0% (137/152)
ITPR1	67.1% (102/152)	85.5% (130/152)

Supplementary Figure 8. Protein sequence comparison of PTEN-binding region across IP3 receptors

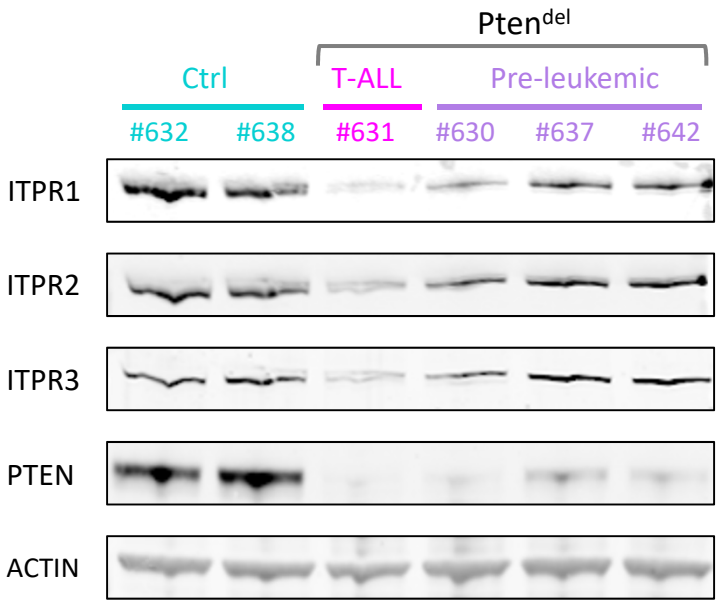
The PTEN-binding region in ITPR3 was defined by Kuchay et al. (2017); it comprises 152 amino acids (AA) ranging from AA436 to AA587. This amino acid sequence from ITPR3 was compared with the corresponding sequence from ITPR1 and ITPR2 using CLUSTAL Omega V. 1.2.4 [1]. The asterisk (*) indicates positions which have a single, fully conserved residue. The colon (:) indicates conservation between groups of strongly similar properties. The period (.) indicates conservation between groups of weakly similar properties. Percentages of identities and similarities with ITPR3 are indicated.

1. Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Li W, Lopez R, McWilliam H, Remmert M, Söding J, et al. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol Syst Biol* (2011) 7:539. doi:10.1038/msb.2011.75



Supplementary Figure 9. Mathematical models assessing the putative role of PTEN in TCR-induced calcium flux in thymocytes

(A) Two regulatory networks representing TCR-induced calcium flux with (left) and without (right) the activation of ITPR_1_2 by PTEN. Nodes of the network represent calcium signalling component (ellipse for Boolean node, rectangular for multilevel node). The role of PTEN in each model was labelled in pink (without activation of PTEN on ITPR_1_2) and blue (with activation of PTEN on ITPR_1_2). **(B)** Attractors of the logical model for each combination of inputs: PTEN = 1 (left) and PTEN = 0 (right), Ubq_x = 0 (top) and Ubq_x = 1 (bottom), according to the 3 values of the TCR input (0/1/2). The color of each square represents the activity levels, red for active (1), yellow inactive (0). Bicolored case represents an oscillating node (cyclical attractor). The left panel shows the attractors from the model without the activation of PTEN on ITPR_1_2 and the right panel shows the attractor from model with the activation of PTEN on ITPR_1_2. A comparison of these simulations with the corresponding experimental data validates the presence of the activation of PTEN on ITPR_1_2.



Supplementary Figure 10. Analysis of ITPRs protein expression levels in pre-leukemic Pten^{del} thymocytes

Immunoblotting assays were performed with antibodies specific for ITPR1, ITPR2, ITPR3, PTEN and ACTIN as a loading control. Thymic cells from Control (Ctrl), pre-leukemic and leukemic Pten^{del} mice were analyzed. The identification of analyzed mice (same mice as used in Supplementary Figure 6) is indicated (#number). According to our definition in Supplementary Figure 1, mice #637 and #642 were considered as 'pre-leukemic early' (normal thymus), while #630 as 'pre-leukemic late' (abnormal thymus).

Supplementary Tables

Supplementary Table 1. Antibodies used for Flow Cytometry analysis

Antibody	Company	Catalog number
CD3e biotin	BD Pharmingen	553059
CD3 APC Cy7	BD Pharmingen	560590
CD4 V450	BD Horizon	560468
CD4 APC	BD Pharmingen	553051
CD8a PE	BD Pharmingen	553033
CD8a PE Cy7	BD Pharmingen	561097
TCR αβ PE	BD Pharmingen	553172

Supplementary Table 2. Gene lists of pathways of interest obtained from MSigDB database

Pathways	Curator	MSigDB R pathway name	Gene list
Calcium	The Pathway Interaction Database (PID)	PID_TCR_CALCIUM_PATHWAY	Akap5, Batf3, Cabin1, Cd40lg, Chp1, Csf2, Fasl, Fkbp1a, Fos, Fosl1, Ifng, Il2, Il2ra, Il4, Jun, Junb, Nfatc1, Nfatc2, Nfatc3, Pou2f1, Ppp3ca, Ppp3cb, Ppp3r1, Prkaca, Ptgs2, Rcan1, Rcan2
PI3K/AKT	MSigDB Hallmark Gene Sets	HALLMARK_PI3K_AKT_MTOR_ SIGNALING	Acaca, Actr2, Actr3, Akt1, Akt1s1, Ap2m1, Arf1, Arhgdia, Arpc3, Atf1, Cab39, Cab39l, Calr, Camk4, Cdk1, Cdk2, Cdk4, Cdkn1a, Cdkn1b, Cfl1, Cltc, Csnk2b, Cxcr4, Dapp1, Ddit3, Dusp3, E2f1, Ecsit, Egfr, Eif4e, Fasl, Fgf17, Fgf22, Gna14, Gngt1, Grb2, Grk2, Gsk3b, Hras, Hsp90b1, Il2rg, Il4, Irak4, Itpr2, Lck, Map2k3, Map2k6, Map3k7, Mapk1, Mapk10, Mapk8, Mapk9, Mapkap1, Mknk1, Mknk2, Myd88, Nck1, Nfkbib, Nod1, Pak4, Pdk1, Pfn1, Pik3r3, Pikfyve, Pin1, Pitx2, Pla2g12a, Plcb1, Plcg1, Ppp1ca, Ppp2r1b, Prkaa2, Prkag1, Prkar2a, Prkcb, Pten, Ptpn11, Rac1, Raf1, Ralb, Ripk1, Rit1, Rps6ka1, Rps6ka3, Rptor, Sfn, Sla, Slc2a1, Smad2, Sqstm1, Stat2, Tbk1, Them4, Tiam1, Tnfrsf1a, Traf2, Trib3, Tsc2, Ube2d3, Ube2n, Vav3, Ywhab
JNK	The Pathway Interaction Database (PID)	PID_TCR_JNK_PATHWAY	Crk, Crkl, Dbnl, Grap2, Jun, Lat, Lcp2, Map2k4, Map3k1, Map3k7, Map3k8, Map4k1, Mapk8, Prkcb
ERK	REACTOME	REACTOME_SIGNALLING_TO_ ERKS	Braf, Crk, Crkl, Frs2, Grb2, Hras, Kidins220, Kras, Map2k1, Map2k2, Mapk1, Mapk11, Mapk12, Mapk13, Mapk14, Mapk3, Mapkapk2, Mapkapk3", "Nras, Rala, Ralb, Ralgds, Rap1a, Rapgef1, Rit1, Shc1, Sos1, Src, Ywhab
P38	The Pathway Interaction Database (PID)	PID_P38_ALPHA_BETA_ PATHWAY	Blk, Ccm2, Cdc42, Dusp1, Dusp10, Dusp16, Dusp8, Fgr, Fyn, Hck, Lck, Lyn, Map2k3, Map2k4, Map2k6, Map3k12, Map3k3, Mapk11, Mapk14, Pak1, Pak2, Rac1, Rala, Ralb, Ripk1, Src, Tab1, Traf6, Yes1
NFkB	The Pathway Interaction Database (PID)	PID_NFKAPPAB_CANONICAL_ PATHWAY	Atm, Bcl10, Birc2, Chuk, Cyld, Erc1, Ikbkb, Ikbkg, Malt1, Nfkb1, Nfkbia, Nod2, Prkca, Ran, Rela, Ripk2, Tnf, Tnfaip3, Tnfrsf1a, Traf6, Ube2d3, Xpo1

Supplementary Table 3. Antibodies used for immunoblotting assays

Antibody	Company	Catalog number
ACTIN Antibody (I-19)	Santa Cruz	sc-1616
PTEN Antibody	Ozyme	9559S
ITPR1 Antibody	Biolegend	817701
ITPR2 Antibody	BIO-TECHNE (Novus Biologicals)	NB100-2466
ITPR3 Antibody	BD transduction	610312
Phospho-NFAT1 (Ser54) Antibody	Invitrogen	44-944G
Phospho-PLCγ1 (Tyr783) Antibody	Cell signaling	14008S
Phospho-Akt (Ser473) Antibody	Cell signaling	9271S
Goat Anti-Rabbit IgG (H+L), Highly Cross-Adsorbed	Biotium	20078
Donkey Anti-Goat IgG (H+L), Highly Cross-Adsorbed	Biotium	20277
Goat Anti-Mouse IgG (H+L), Highly Cross-Adsorbed	Biotium	20065

Supplementary Table 4. Oligonucleotide sequences

RT-qPCR Primers	Target	Sequence
Q-Abl-S	Mouse	tgtggccagtggagataacactc
Q-Abl-AS	Mouse	ttcacaccattccccattgtg
Q-Itpr1-S	Mouse	gggccaacagcactacagat
Q-Itpr1-AS	Mouse	ggatgacggtccccaacaat
Q-Itpr2-S	Mouse	agctgagaagcgagggtgat
Q-Itpr2-AS	Mouse	tcgtccgaaggaaaatgtgc
Q-Itpr3-S	Mouse	gctcttcatccagcccttc
Q-Itpr3-AS	Mouse	agctggtgttgcagttgaca

Supplementary Table 5. Logical equation of each component in calcium model

Component	Logical Equation
TCR_signal	input {0,1,2}
PTEN	input {0,1}
Ubq_x	Input {0,1}
LAT_signalosome	TCR_signal:2 TCR_signal:1 & !LAT_signalosome
ER_CA	(ER_CA | CYT_CA) & !ITPR_1_2
CYT_CA	!ER_CA & ORAI1
MT_CA	MERCs_ITPR3
ITPR_1_2	LAT_signalosome & (PTEN | !Ubq_x)
MERCs_ITPR3	LAT_signalosome & (PTEN | !Ubq_x)
STIM1	!ER_CA
ORAI1	STIM1