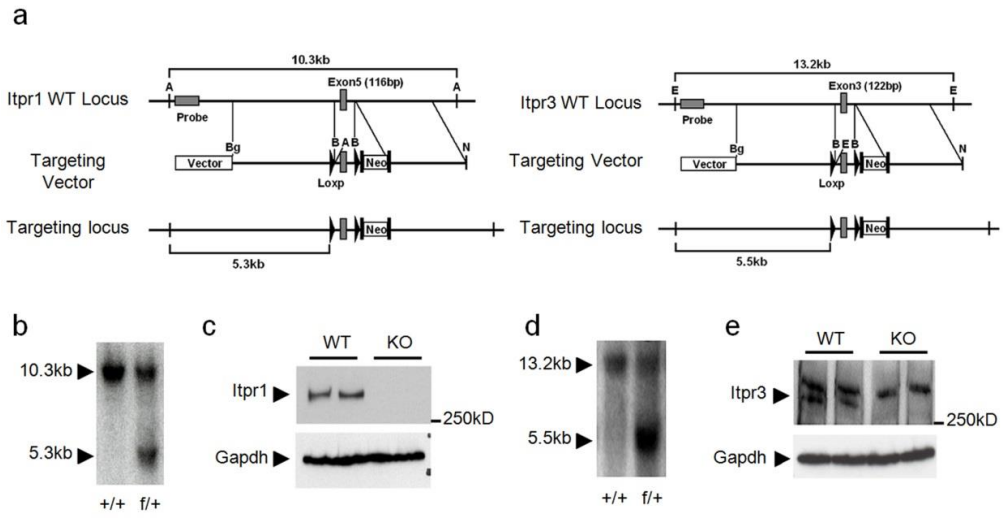
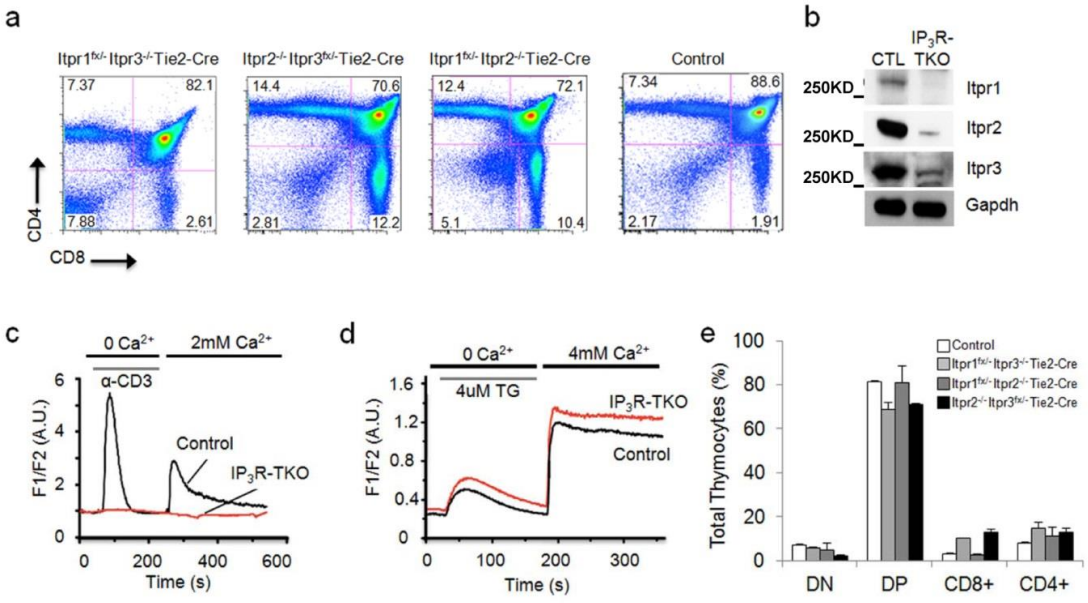


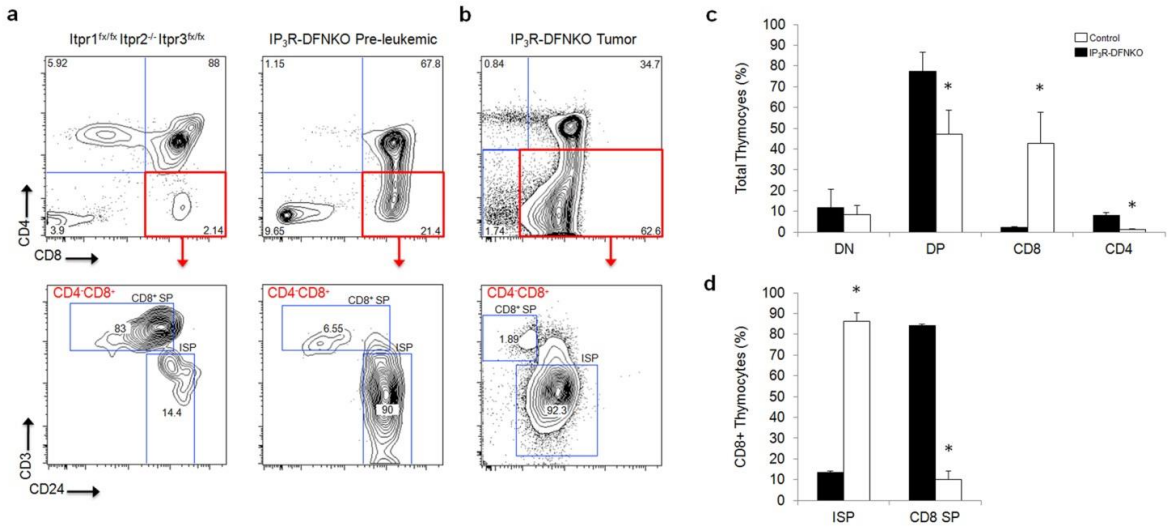
Supplementary Figure 1: Gene targeting strategies to generate *Itpr1 and Itpr3* knockout mice.

a) Targeting strategies for *Itpr1* and *Itpr3* genes. A restriction map of the relevant genomic regions (top), the targeting vectors (middle) and the targeted locus after recombination (bottom) is shown. The targeting construct was generated by flanking exon 5 of *Itpr1* (**a**) and exon 3 of *Itpr3* (**d**) with loxP sites, while frt sites flank the Neo-cassette. A, *Acc65I*; B, *BamHI*; Bg, *BglII*; E, *EcoRV*; N, *NotI*. Neo represents the neomycin resistance gene; while the arrowheads represent LoxP sites and the long boxes represent frt sites. **b)** Detection of wild type (WT) and targeted alleles for the *Itpr1* gene by DNA Southern blot analysis. DNAs isolated from neo positive electroporated ES cell clones were digested with *Acc65I* and analyzed by DNA blot analysis with the probe as shown in (**a**). The 10.3- and 5.3-kb bands represent the WT and targeted alleles, respectively. **c)** Detection of Itpr1 by protein analysis. Proteins were prepared from the brains of neonatal WT and Itpr1-knockout (KO) mice, and analyzed with Itpr1 antibodies. **d)** Detection of WT and targeted alleles for the *Itpr3* gene by DNA Southern blot analysis, DNAs were digested with *NotI*. The 13.2- and 5.5-kb bands represent WT and targeted alleles, respectively. **e)** Detection of Itpr3 by protein analysis. Proteins were prepared from the placenta of WT and Itpr3-null (KO) mice, and analyzed with Itpr3 antibodies.



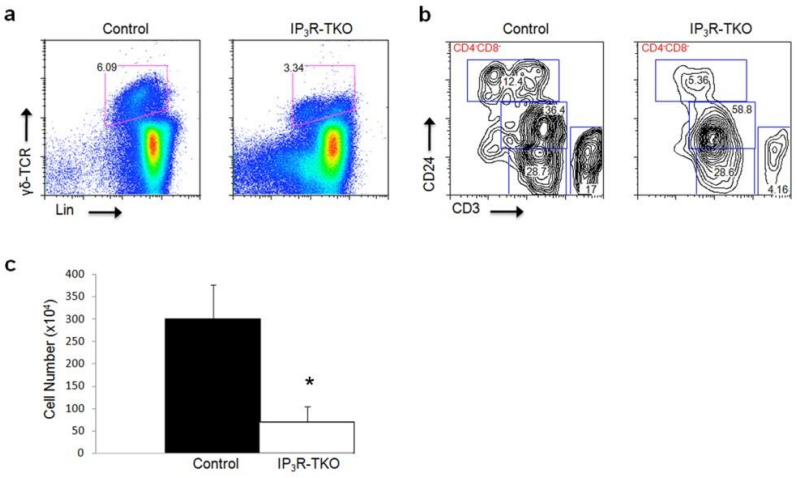
Supplementary Figure 2: Conditional deletion of *Itpr* in mouse thymocytes.

a) Surface CD4 and CD8 expression of adult *Itpr* conditional double knockout thymi. **b)** Western blot of Itpr expression from ED 17.5 control (CTL) and IP_3R-TKO thymi. **c)** Ca^{2+} influx in adult control and IP_3R-TKO thymocytes in response to crosslinking of TCR with anti-CD3 antibody and **d)** after passive depletion of intracellular Ca^{2+} stores by thapsigargin (TG), followed by reintroduction of extracellular Ca^{2+}. Relative Ca^{2+} levels were determined by ratiometric measurement with Fluo-5 (F1) and Fura-red (F2) Ca^{2+} indicator dyes. A.U., arbitrary units. Data are representative of a minimum of three independent experiments. All animals displaying signs of malignant disease were excluded from these analyses. **e)** Quantification of surface CD4 and CD8 expression of adult *Itpr* conditional double knockout thymi in (**a**). Data are expressed as percentage of total thymocytes (mean ± SEM, n=3 for all genotypes).



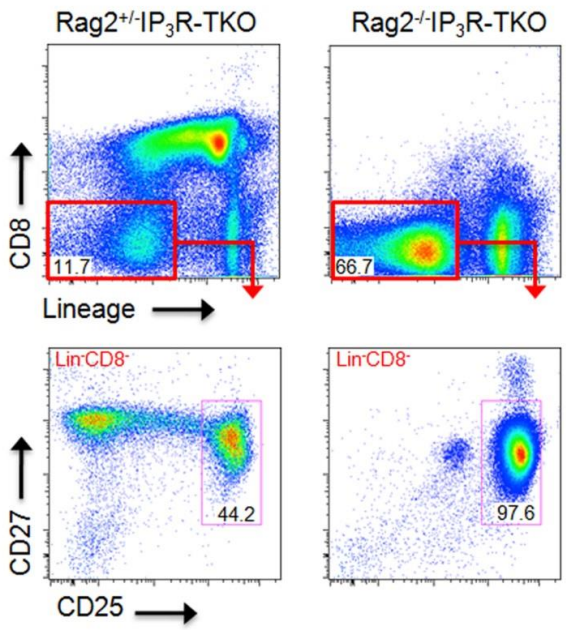
Supplementary Figure 3: **Accumulation of ISP thymocytes in pre-leukemic and tumor-burdened IP$_3$R-DFNKO thymi.**

CD4 and CD8 surface expression (top) in thymi of pre-leukemic **(a)** and tumor-burdened (**b**) IP$_3$R-DFNKO adult mice. CD8$^+$CD4$^-$ thymocytes were subgated and surface expression of CD24 and CD3 (bottom) was examined (as in **Figure 1**). Numbers in plots indicate percentage of cells. Data are representative of a minimum of ten independent experiments. Age was not used to define these stages, as we observed significant variability in the age of onset and age of progression from normal (left column), pre-leukemic mice (middle column) and T-LBL burdened (right column) IP$_3$R-TKO mice. **c**) Quantification of surface CD4 and CD8 expression of pre-leukemic IP$_3$R-DFNKO adult thymi. Data are expressed as percentage of total thymocytes (mean ± SEM, Control, n=5; IP$_3$R-DFNKO, n=4.). **d)** ISP and CD8$^+$ SP T cells as a percentage of total CD8$^+$ cells from data in (**c**). *, $p < 0.05$, measured with two-tailed Student's t test.

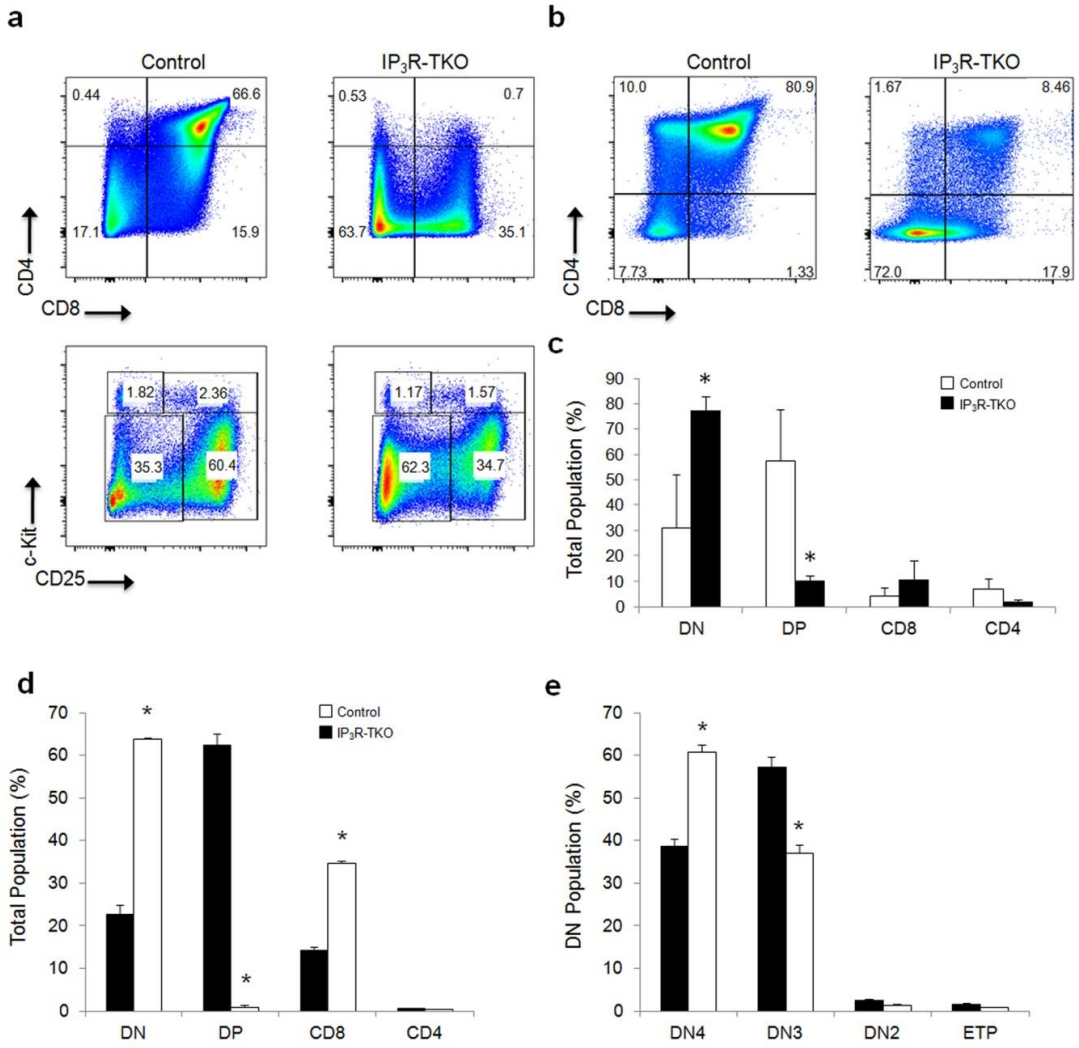


Supplementary Figure 4: **γδ T cell development is disrupted in IP_3R-TKO mice.**

a) Expression of surface γδ-TCR after staining with lineage markers (CD3, CD4, CD8, B220, CD19, Mac-1, Gr-1, Ter-119) of 1-week-old neonate control and IP_3R-TKO thymocytes. **b)** CD3 and CD24 expression in DN thymocytes of 1-week-old neonate control and IP_3R-TKO mice. **c)** Total thymic cellularity of γδ-TCR^+ T cells in 1-week-old neonate control and IP_3R-TKO mice. Data are representative of three independent experiments (mean ± SEM). *, $p < 0.05$, measured with two-tailed Student's t test.

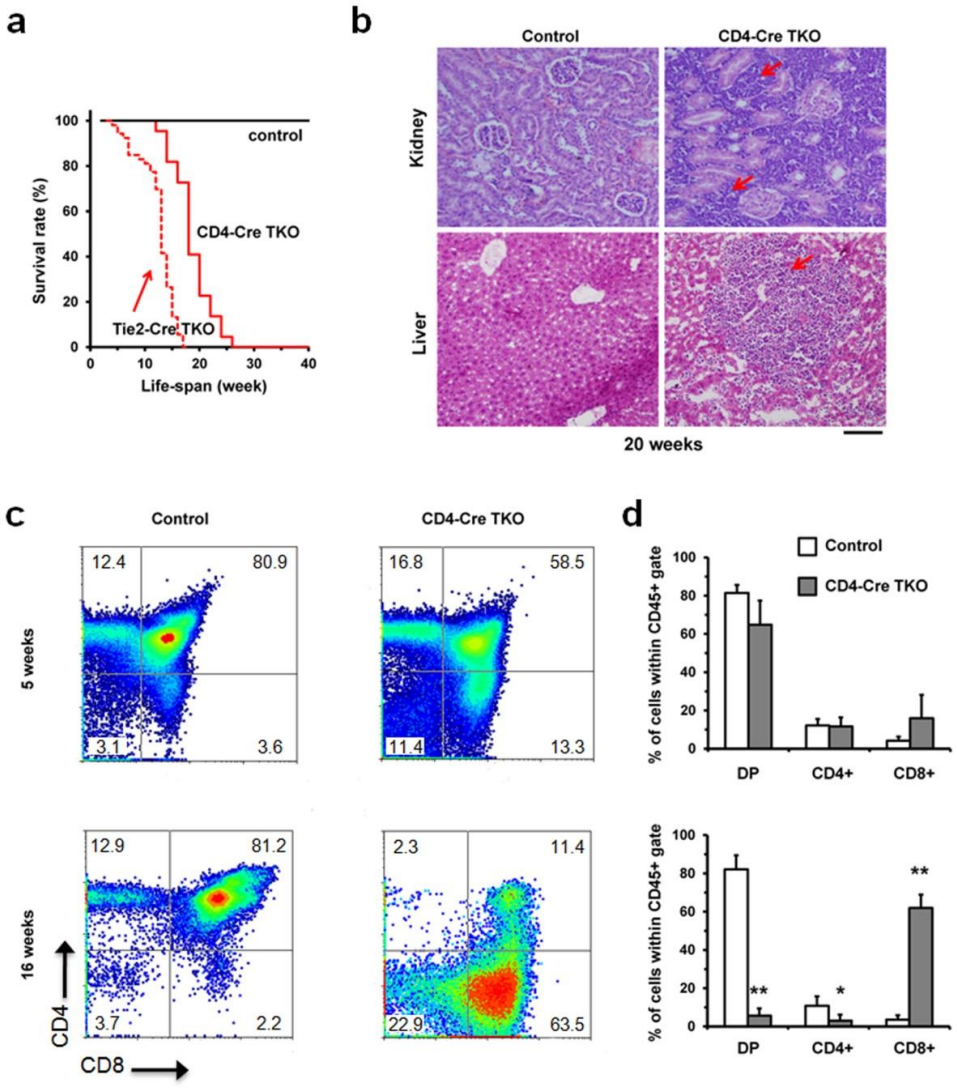


Supplementary Figure 5: IP_3R-Ca^{2+} store depletion is not required for the enforcement of β-selection. Thymocytes from 6-week-old IP_3R-TKO $Rag2^{+/-}$ and IP_3R-TKO $Rag2^{-/-}$ were stained for lineage markers (CD3, CD4, Mac1, Gr1, Ter119, B220, γδ-TCR) and surface CD8 expression (top). $CD8^-Lineage^-$ thymocytes were then sub-gated and examined for surface CD27 and CD25 expression (bottom). Data are representative of three independent experiments.



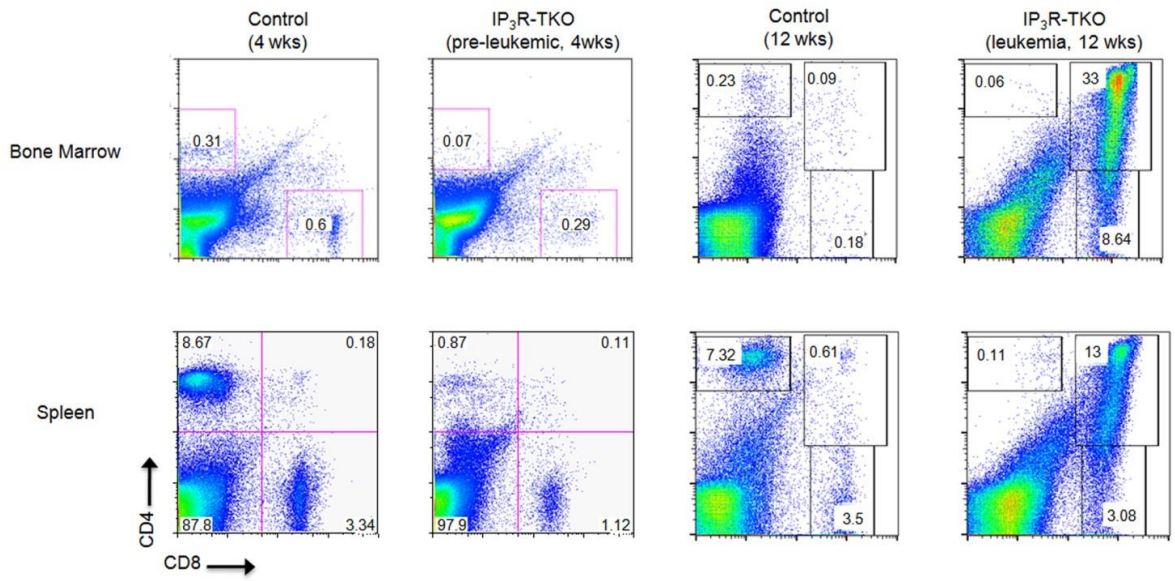
Supplementary Figure 6: **Thymocyte accumulation in IP$_3$R-TKO mice is due to inefficient transition of precursors through the post β-selection stages of development.**

a) Expression of surface CD4 and CD8 in ED 17.5 thymocytes (top) of control and IP$_3$R-TKO mice. CD3$^-$CD4$^-$CD8$^-$ (DN) thymocytes were then sub-gated and examined for surface CD25 and c-Kit expression (bottom). **b)** Sca-1$^+$ hematopoietic stem and progenitor cells purified from ED 14.5 fetal livers of control and IP3R-TKO mice were cultured on OP9-DL1 stromal cells for twelve days and examined for expression of surface CD4 and CD8. Data are representative of (**a**) two and (**b**) three independent experiments. **c)** Surface CD4 and CD8 expression in (**b**), expressed as percentage of total population (mean ± SEM, n=3). **d**) Surface CD4 and CD8 expression in (**a,** top row), expressed as a percentage of total cells (mean ± SEM, control, n=6; IP$_3$R-TKO, n=3). **e**) Surface CD25 and c-Kit expression of CD3$^-$DN cells (**a**, bottom row), expressed as a percentage of total CD3$^-$DN cells (mean ± SEM, control, n=6; IP$_3$R-TKO, n=3). *, $p < 0.01$, measured with two-tailed Student's t test.



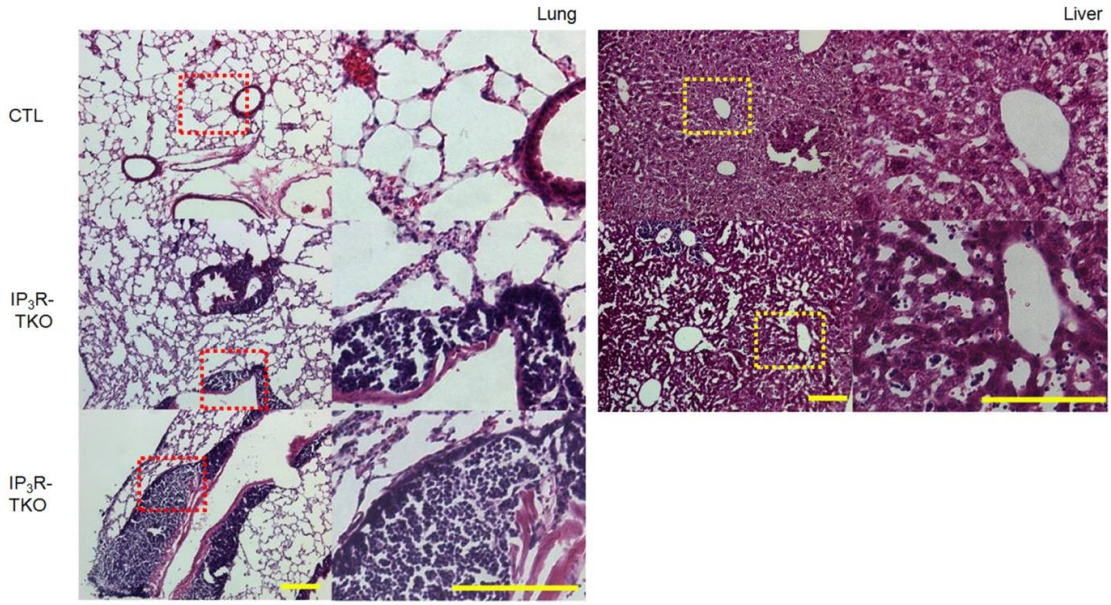
Supplementary Figure 7: CD4-Cre IP$_3$R TKO mice develop premature lethality, tumors, and abnormal T cell development.

a) Kaplan-Meier survival curve of control, CD4-Cre TKO and Tie2-Cre TKO mice (control, n>100; CD4-Cre TKO, n=22; Tie-Cre TKO, n>100). **b)** Histological analysis showed leukocyte infiltration (arrow) in kidney and liver of CD4-Cre TKO mice at the age of 20 weeks. Scale bars: 100 μm. **c)** Expression of surface CD4 and CD8 in control and CD4-Cre TKO thymocytes at the age of 5 weeks (left, upper; control, n = 5; CD4-Cre TKO, n = 3) and 16 weeks (left, bottom; control, n = 4; CD4-Cre TKO, n = 4), and the percentage of each cell subset (d). Data are presented as mean ± SEM. *, $p<0.05$; **, $p<0.01$, measured with two-tailed Student's t test.

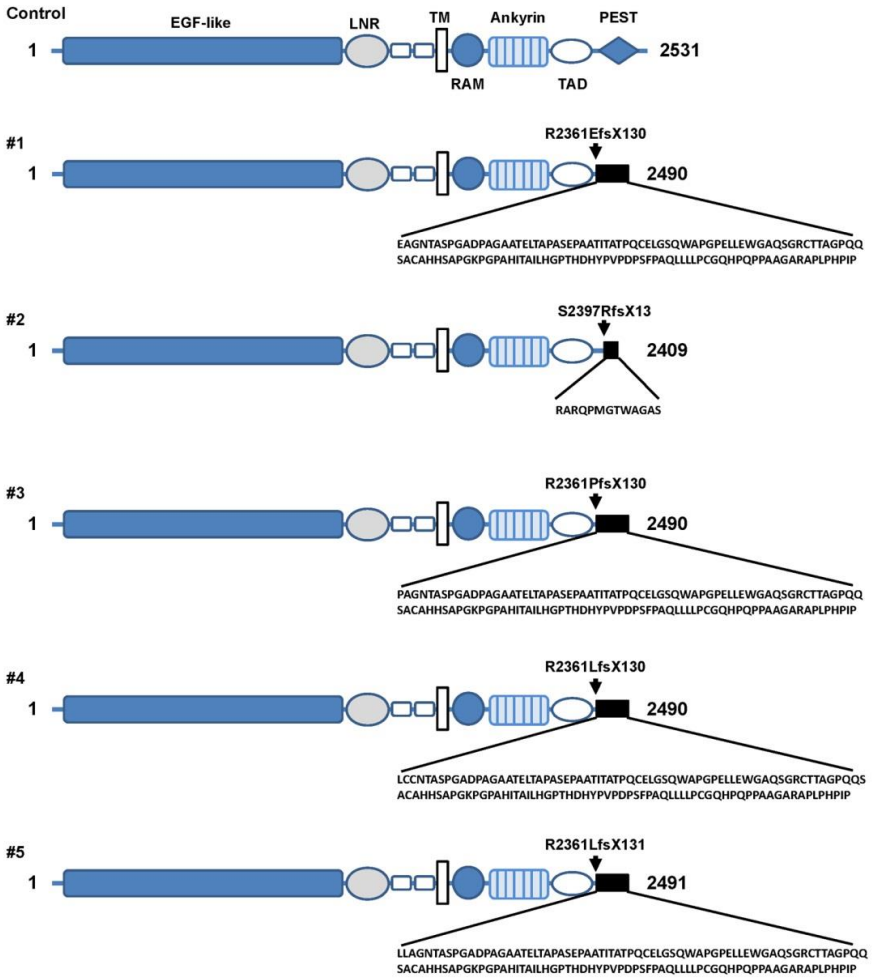


Supplementary Figure 8: **Leukemic blasts are present in bone marrow and spleen of moribund IP_3R-TKO mice.**

Expression of surface CD4 and CD8 reveal T-ALL blasts present in IP_3R-TKO bone marrow (**top**, leukemia, 12wks) and spleen (**bottom**, leukemia, 12wks). Adult (4wks) Cre-negative control and pre-leukemic IP_3R-TKO bone marrow and spleen are included for comparison, as well an age-matched Cre-negative littermate control (12wks). Data are representative of a minimum of five experiments.

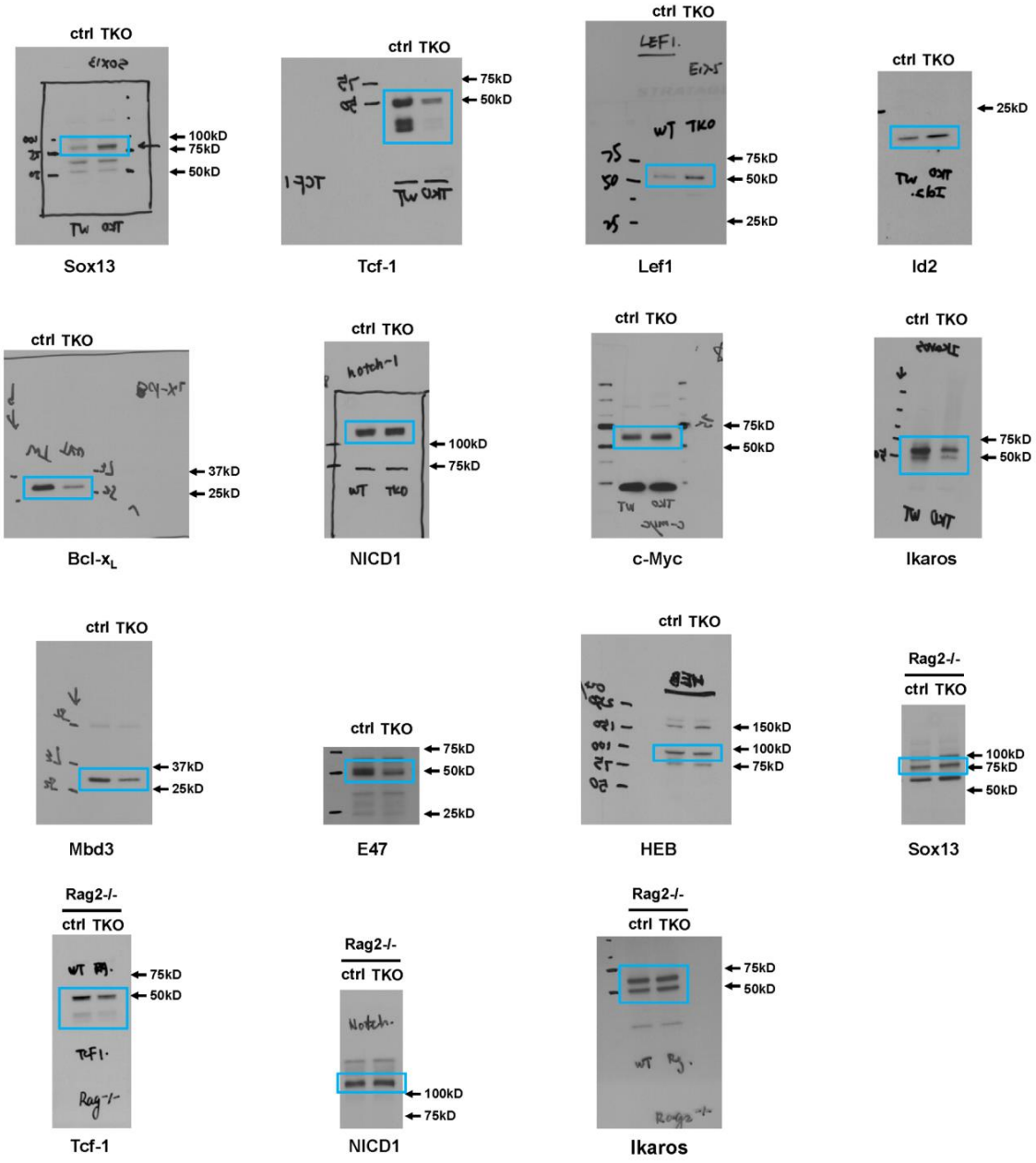


Supplementary Figure 9: **Leukocyte infiltration into lung and liver of moribund IP_3R-TKO mice.**
H&E staining of lung (left) samples (red dashed boxes represent image area magnified on right) and liver (yellow dashed boxes represent image area magnified on right). Scale bars: 200μm.



Supplementary Figure 10: Mutations in the Notch1 gene identified in IP_3R-TKO thymi.

Schematic representation of control and predicted truncated Notch1 proteins translated from mRNA carrying frame-shift mutations. Black boxes indicate amino acid sequences (elaborated below) predicted from the new reading frame following the identified mutations.



Supplementary Figure 11: The original Western blots in the paper. Uncropped Western blots of Figure 3d, e.

Supplementary Table 1. KEGG pathway analysis: GO terms enriched in ISP thymocyte gene set.

ID	Term	Count	%	P-Value	Fold Enrichment	Bonferroni	Benjamini	FDR
mmu04510	Focal adhesion	47	0.47	1.72E-17	4.20	2.67E-15	2.67E-15	2.07 E-14
mmu04512	ECM-receptor interaction	29	0.29	2.01E-15	6.19	3.10E-13	1.55E-13	2.40 E-12
mmu05410	Hypertrophic cardiomyopathy (HCM)	25	0.25	1.30E-11	5.27	2.01E-09	6.69E-10	1.56 E-08
mmu05412	Arrhythmogenic right ventricular cardiomyopathy (ARVC)	22	0.22	3.93E-10	5.19	6.10E-08	1.52E-08	4.72 E-07
mmu05414	Dilated cardiomyopathy	24	0.24	6.76E-10	4.62	1.05E-07	2.09E-08	8.11 E-07
mmu04020	Calcium signaling pathway	34	0.34	4.22E-09	3.15	6.54E-07	1.09E-07	5.07 E-06
mmu02010	ABC transporters	13	0.13	4.41E-06	5.12	6.83E-04	9.76E-05	5.29 E-03
mmu05222	Small cell lung cancer	17	0.17	1.58E-05	3.54	2.44E-03	3.06E-04	0.019
mmu04530	Tight junction	22	0.22	1.72E-05	2.89	2.67E-03	2.97E-04	0.021
mmu04810	Regulation of actin cytoskeleton	29	0.29	2.68E-05	2.37	4.14E-03	4.15E-04	0.032
mmu04730	Long-term depression	14	0.14	1.55E-04	3.44	0.024	2.18E-03	0.19
mmu04260	Cardiac muscle contraction	14	0.14	3.55E-04	3.18	0.054	4.57E-03	0.43
mmu04010	MAPK signaling pathway	30	0.30	3.79E-04	2.00	0.057	4.50E-03	0.45
mmu05200	Pathways in cancer	33	0.33	1.10E-03	1.81	0.16	0.012	1.31
mmu05416	Viral myocarditis	14	0.14	2.15E-03	2.64	0.28	0.022	2.55
mmu04360	Axon guidance	16	0.16	6.46E-03	2.16	0.63	0.061	7.49
mmu04270	Vascular smooth muscle contraction	15	0.15	7.13E-03	2.21	0.67	0.063	8.23
mmu04912	GnRH signaling pathway	13	0.13	7.82E-03	2.37	0.70	0.065	9.00
mmu04520	Adherens	11	0.11	9.63E-03	2.56	0.78	0.076	10.97

	junction							
mmu00230	Purine metabolism	17	0.17	0.015	1.92	0.90	0.11	16.37
mmu04144	Endocytosis	20	0.20	0.018	1.75	0.94	0.13	20.05
mmu04012	ErbB signaling pathway	11	0.11	0.023	2.24	0.97	0.15	24.80
mmu04070	Phosphatidylinositol signaling system	10	0.10	0.024	2.36	0.98	0.15	25.29
mmu05214	Glioma	9	0.09	0.026	2.49	0.98	0.16	27.18
mmu00562	Inositol phosphate metabolism	8	0.08	0.030	2.62	0.99	0.17	31.02
mmu04720	Long-term potentiation	9	0.09	0.042	2.28	1.00	0.22	40.02
mmu04640	Hematopoietic cell lineage	10	0.10	0.045	2.11	1.00	0.23	42.77
mmu04666	Fc gamma R-mediated phagocytosis	11	0.11	0.048	1.99	1.00	0.24	44.54
mmu04930	Type II diabetes mellitus	7	0.07	0.055	2.53	1.00	0.26	49.48
mmu05010	Alzheimer's disease	16	0.16	0.087	1.56	1.00	0.38	66.57
mmu04514	Cell adhesion molecules (CAMs)	14	0.14	0.092	1.61	1.00	0.38	68.75
mmu05014	Amyotrophic lateral sclerosis (ALS)	7	0.07	0.099	2.17	1.00	0.40	71.54

Supplementary Table 2. KEGG pathway analysis: Genes enriched within ISP thymocyte gene set.

ID	Term	Genes
mmu04510	Focal adhesion	TLN2, ERBB2, ITGB4, ITGA10, COL2A1, VCL, IGF1R, DOCK1, ITGB8, COL6A2, COL6A1, COL11A1, THBS3, FN1, SHC4, EGFR, COL4A4, PIK3CG, COL4A2, VAV3, FLT1, TNXB, ITGA2, ACTN2, ITGA3, FLNC, KDR, LAMA2, VWF, ITGA9, LAMA1, CCND1, LAMA4, LAMA3, ITGA5, LAMA5, LAMC3, RASGRF1, CCND2, ITGA7, COL1A2, PDGFRA, PDGFRB, RELN, LAMC2, LAMC1, MYLK
mmu04512	ECM-receptor interaction	ITGB4, ITGA10, COL2A1, ITGB8, COL6A2, COL6A1, AGRN, COL11A1, THBS3, FN1, COL4A4, COL4A2, TNXB, ITGA2, ITGA3, LAMA2, VWF, ITGA9, LAMA1, LAMA4, LAMA3, ITGA5, LAMC3, LAMA5, ITGA7, COL1A2, RELN, LAMC2, LAMC1
mmu05410	Hypertrophic cardiomyopathy (HCM)	ITGB4, ITGA10, CACNB2, TTN, TGFB2, ACE, ITGB8, PRKAA2, CACNA2D1, ITGA2, CACNG4, ITGA3, MYH7, MYH6, CACNA2D2, CACNA1S, CACNA2D4, LAMA2, ITGA9, ITGA5, ITGA7, RYR2, CACNA1F, CACNA1C, CACNA1D
mmu05412	Arrhythmogenic right ventricular cardiomyopathy (ARVC)	CACNA2D1, ITGB4, CACNG4, ITGA2, ITGA10, CACNB2, ACTN2, ITGA3, CTNNA1, CACNA1S, CACNA2D2, CACNA2D4, JUP, LAMA2, ITGA9, ITGB8, ITGA5, ITGA7, RYR2, CACNA1F, CACNA1C, CACNA1D
mmu05414	Dilated cardiomyopathy	CACNA2D1, ITGB4, CACNG4, ITGA2, ITGA10, CACNB2, MYH7, ITGA3, MYH6, TTN, CACNA1S, CACNA2D2, TGFB2, CACNA2D4, LAMA2, ITGA9, ITGA5, ITGB8, ITGA7, RYR2, CACNA1F, CACNA1C, CACNA1D, IGH-VJ558
mmu04020	Calcium signaling pathway	PHKA2, ERBB4, ERBB3, ERBB2, PHKA1, PLCB3, PLCB4, PTK2B, PDE1C, PDE1A, NOS2, PPP3CA, IGH-VJ558, EGFR, NOS1, CACNA1I, ITPR3, CACNA1S, ITPR2, P2RX7, RYR3, PLCG2, RYR1, CACNA1G, PDGFRA, RYR2, PDGFRB, CACNA1E, CACNA1F, CACNA1C, CACNA1D, MYLK, CACNA1A, CACNA1B
mmu02010	ABC transporters	ABCB11, ABCA8A, CFTR, ABCA1, ABCA4, ABCA3, ABCA6, ABCA5, ABCC9, ABCC3, ABCC4, ABCA13, ABCA12
mmu05222	Small cell lung cancer	PIK3CG, COL4A4, COL4A2, ITGA2, ITGA3,

		LAMA2, LAMA1, CCND1, LAMA4, LAMA3, LAMC3, LAMA5, LAMC2, LAMC1, NOS2, TRAF5, FN1
mmu04530	Tight junction	SYMPK, INADL, MYH15, MAGI2, MAGI1, MPDZ, MYH2, CASK, ACTN2, MYH7, MYH6, CTNNA1, CSDA, MYH8, TJP1, CTTN, CGN, MYH11, MYH13, TJP2, MYH7B, MYH10
mmu04810	Regulation of actin cytoskeleton	FGFR2, FGD1, ENAH, SSH1, ITGAE, ITGB4, ITGA10, ITGAM, VCL, DOCK1, ITGAX, ITGB8, GSN, FN1, PIK3CG, EGFR, VAV3, ITGA2, ACTN2, ITGA3, NCKAP1, ITGA9, ITGA5, ITGA7, PDGFRA, CYFIP1, PDGFRB, MYLK, MYH10
mmu04730	Long-term depression	NOS1, LYN, GRIA3, ITPR3, ITPR2, IGF1R, PLCB3, PLA2G4A, PLCB4, GRIA2, JMJD7, RYR1, CRH, CACNA1A
mmu04260	Cardiac muscle contraction	CACNA2D1, COX7A1, CACNG4, CACNB2, MYH7, ATP1A2, MYH6, CACNA2D2, CACNA1S, CACNA2D4, RYR2, CACNA1F, CACNA1C, CACNA1D
mmu04010	MAPK signaling pathway	FGFR2, CACNB2, TGFB2, MAP3K5, PPP3CA, EGFR, CACNA2D1, CACNA1I, CACNG4, NR4A1, FLNC, CACNA2D2, CACNA1S, CACNA2D4, RPS6KA6, PLA2G4A, RASGRF2, RASGRF1, JMJD7, CACNA1G, PDGFRA, PDGFRB, CACNA1E, CACNA1F, MAPK8IP1, CACNA1C, CACNA1D, MAP3K12, CACNA1A, CACNA1B
mmu05200	Pathways in cancer	FGFR2, DCC, ERBB2, STK36, TGFB2, IGF1R, NOS2, TRAF5, FN1, COL4A4, EGFR, PIK3CG, COL4A2, FLT3, ITGA2, ITGA3, CTNNA1, DAPK1, LAMA2, JUP, LAMA1, CCND1, LAMA4, CDKN1A, LAMA3, NCOA4, LAMC3, LAMA5, PLCG2, PDGFRA, PDGFRB, LAMC2, LAMC1
mmu05416	Viral myocarditis	MYH15, MYH2, MYH7, MYH6, MYH8, LAMA2, CCND1, H2-BL, MYH11, H2-T22, MYH13, MYH7B, IGH-VJ558, MYH10
mmu04360	Axon guidance	DCC, ABLIM2, PLXNA4, PLXNA1, PLXNA2, PLXNB1, PLXNB2, L1CAM, EPHB4, SEMA4G, SEMA4C, SRGAP3, ROBO2, PPP3CA, ROBO3, SRGAP1
mmu04270	Vascular smooth muscle contraction	NPR1, NPR2, ITPR3, CACNA1S, ITPR2, PLCB3, PLA2G4A, PLCB4, CYP4A32, JMJD7, MYH11, CACNA1F, CACNA1C, CACNA1D, MYLK

mmu04912	GnRH signaling pathway	EGFR, PLD1, ITPR3, CACNA1S, ITPR2, PLCB3, PLA2G4A, PLCB4, PTK2B, JMJD7, CACNA1F, CACNA1C, CACNA1D
mmu04520	Adherens junction	EGFR, IGF1R, TJP1, SORBS1, ERBB2, PVRL3, LMO7, ACTN2, CTNNA1, VCL, FARP2
mmu00230	Purine metabolism	XDH, ENPP1, NPR1, NPR2, PDE10A, AK7, GUCY2C, AMPD1, PDE6A, PDE7B, PDE2A, CYP4A32, PDE1C, POLD1, PDE1A, ENTPD8, PDE8B
mmu04144	Endocytosis	EGFR, FGFR2, PLD1, FLT1, ERBB4, ERBB3, PSD3, EEA1, KDR, IGF1R, RAB31, TFRC, RABEP1, WWP1, H2-BL, PDGFRA, H2-T22, ITCH, AGAP1, ARAP2
mmu04012	ErbB signaling pathway	EGFR, PIK3CG, CDKN1A, EIF4EBP1, ERBB4, ERBB3, ERBB2, PLCG2, GAB1, NRG2, SHC4
mmu04070	Phosphatidylinositol signaling system	PIK3CG, PLCB3, PLCB4, PIK3C2G, PIK3C2A, PLCG2, SYNJ2, INPP4B, ITPR3, ITPR2
mmu05214	Glioma	EGFR, PIK3CG, IGF1R, CDKN1A, CCND1, PLCG2, PDGFRA, PDGFRB, SHC4
mmu00562	Inositol phosphate metabolism	PIK3CG, PLCB3, PLCB4, PIK3C2G, PIK3C2A, PLCG2, SYNJ2, INPP4B
mmu04720	Long-term potentiation	RPS6KA6, PLCB3, PLCB4, GRIA2, GRIN2B, PPP3CA, ITPR3, CACNA1C, ITPR2
mmu04640	Hematopoietic cell lineage	CR2, TFRC, FLT3, ITGA5, ITGA2, ITGA3, IL5RA, IL7R, IGH-VJ558, ITGAM
mmu04666	Fc gamma R-mediated phagocytosis	PIK3CG, PLA2G4A, DOCK2, PLD1, VAV3, LYN, GSN, PLCG2, PPAP2B, IGH-VJ558, AMPH
mmu04930	Type II diabetes mellitus	PIK3CG, CACNA1G, CACNA1E, CACNA1C, CACNA1D, CACNA1A, CACNA1B
mmu05010	Alzheimer's disease	NOS1, COX7A1, SNCA, ITPR3, CACNA1S, ITPR2, NDUFV3, PLCB3, LRP1, PLCB4, GRIN2B, RYR3, PPP3CA, CACNA1F, CACNA1C, CACNA1D
mmu04514	Cell adhesion molecules (CAMs)	CD276, NFASC, L1CAM, ITGAM, ALCAM, ITGA9, ITGB8, PVRL3, ICOS, H2-BL, CNTNAP2, H2-T22, CNTNAP1, CD226
mmu05014	Amyotrophic lateral sclerosis (ALS)	ALS2, MAP3K5, NOS1, GRIA2, GRIN2B, PPP3CA, CAT