

# Supplemental Data

## **NKp46 Identifies an NKT Cell Subset Susceptible to Leukemic Transformation in Mouse and Man**

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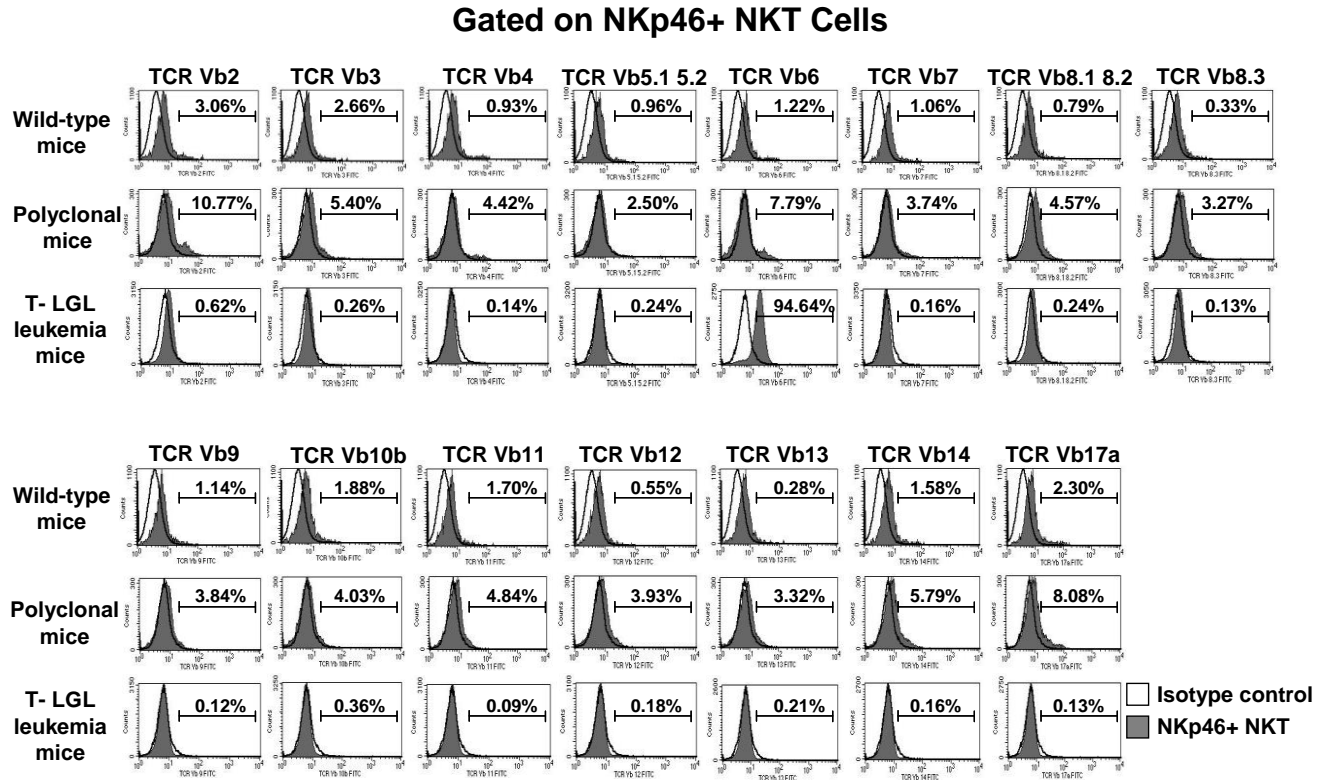
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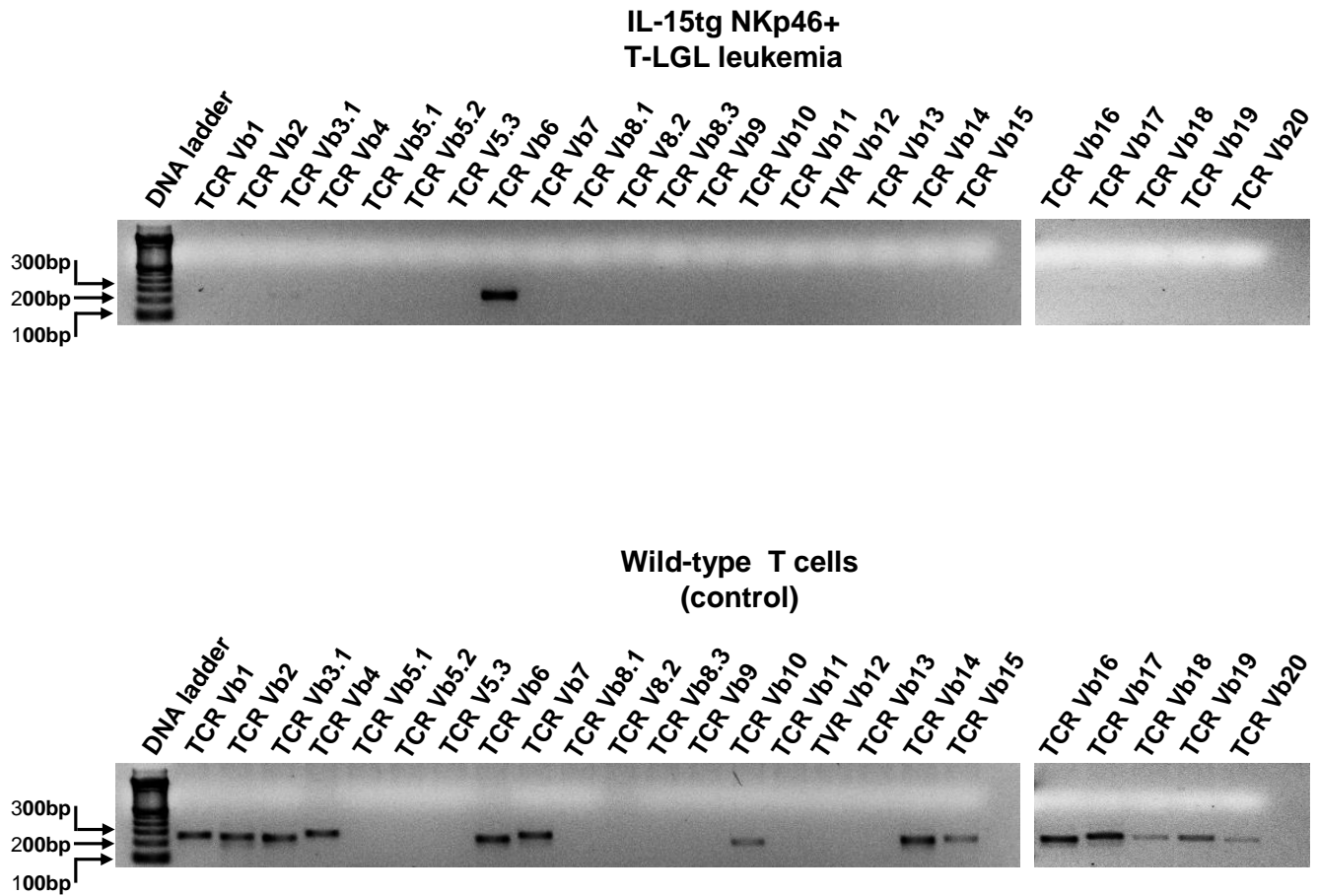
\*These authors equally contributed to this work.

## Supplemental Fig. 1A



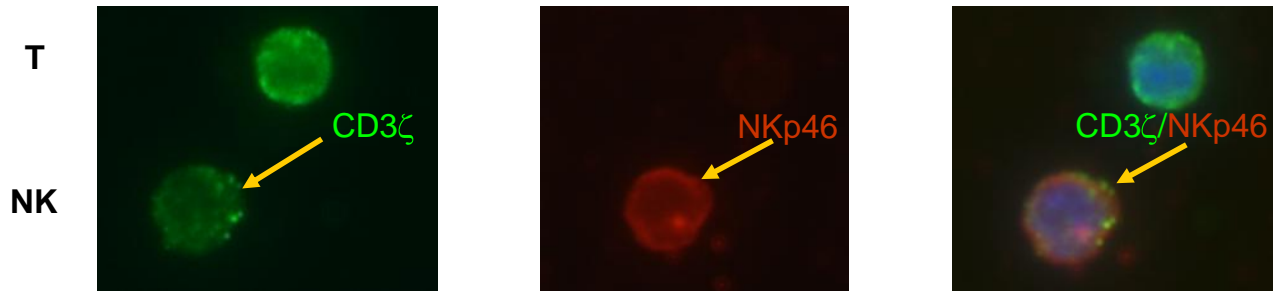
**Supplemental Figure 1A. Flow cytometric analysis of TCR Vβs expression on NKp46+ NKT cells in WT, polyclonal and IL-15tg T-LGL leukemia mice.** Skewing of TCR Vβ expression was observed within the gated NKp46+NK1.1+CD3+ population in IL-15tg polyclonal mice (note TCR Vβ2 and TCR Vβ6 compared to WT mice). Monoclonal expansion of the TCR Vβ6 population was detected in a particular IL-15tg T-LGL leukemia. Representative plots from one of three independent experiments are shown. Percentages of cells positive for each TCR Vβ are indicated.

## Supplemental Fig. 1B

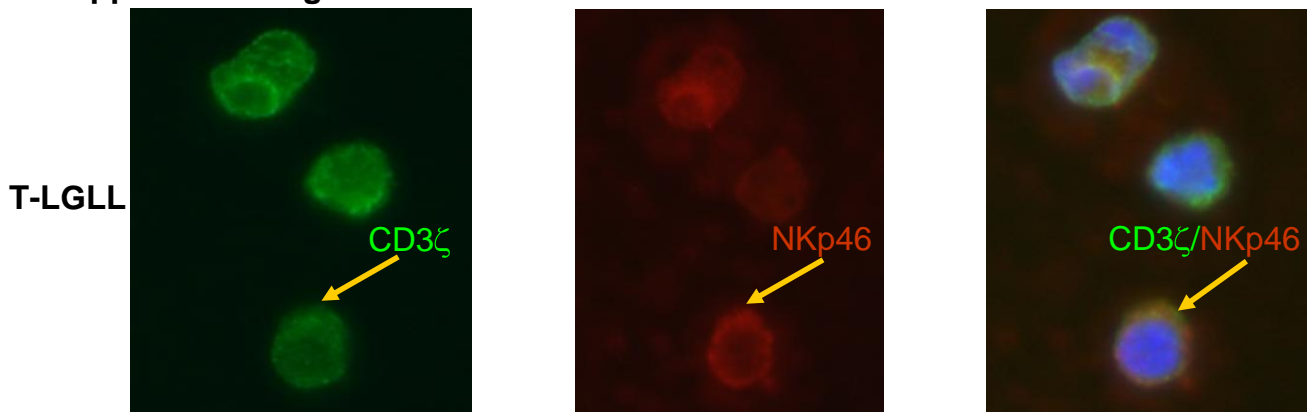


**Supplemental Figure 1B. PCR analysis of TCR V $\beta$ s expression on NKp46+ T-LGL leukemia cells.** TCR V $\beta$ 6 monoclonal expansion of NKp46+CD3+NK1.1+ NKT cells in the leukemic mouse shown in Supplemental Figure 1A was confirmed by PCR analysis of 24 TCR V $\beta$ s. FACS-purified T cells from wild-type (WT) mice served as a control.

## Supplemental Fig. 2A

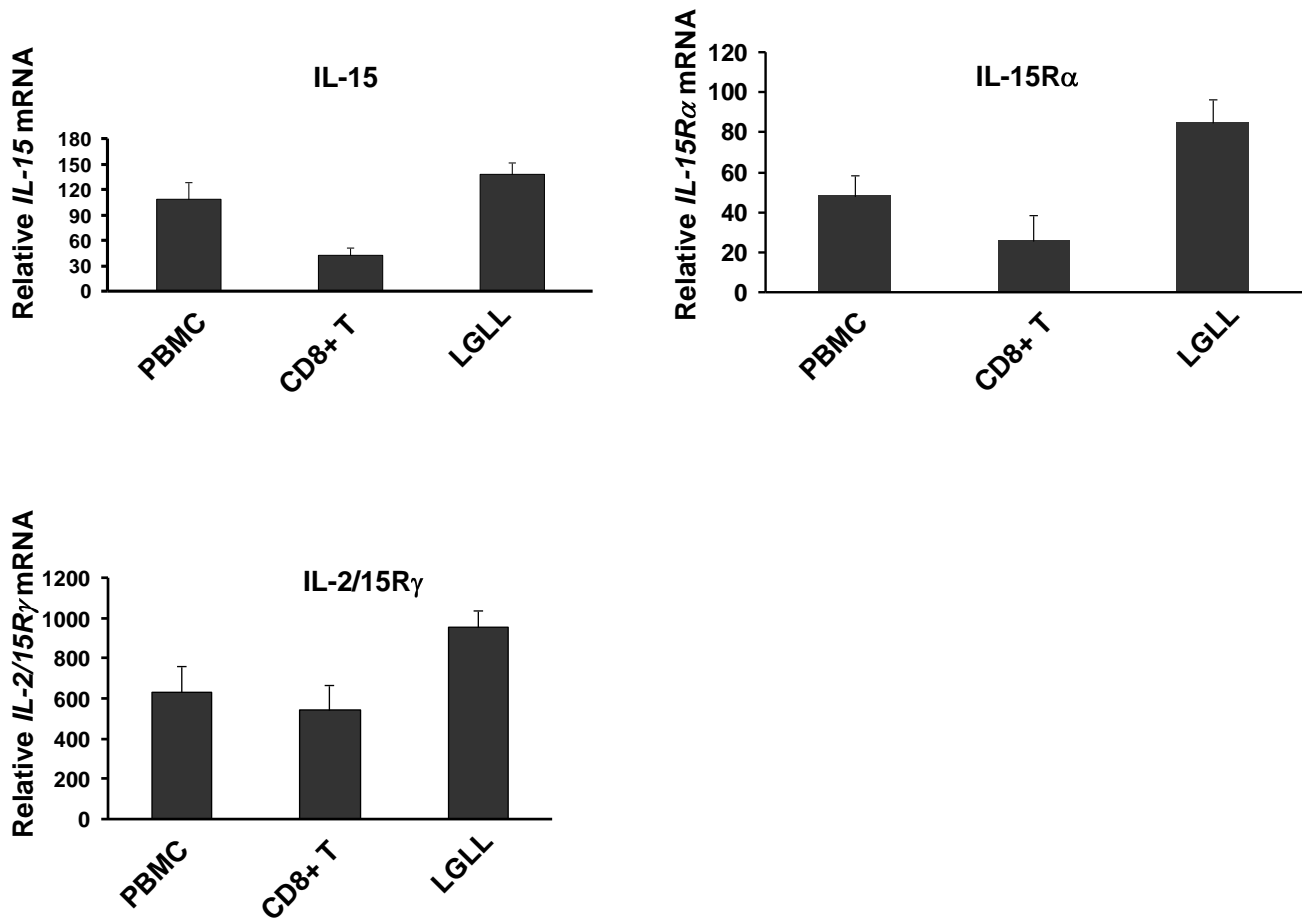


## Supplemental Fig. 2B



**Supplemental Figure 2. Expression of NKp46 and CD3 $\zeta$  co-exists in the same region in an NK cell and a T-LGL leukemia cell.** (A) Enriched NK cells by RosetteSep cocktail (Stem Cell Technologies) were stained with rabbit anti-human CD3 $\zeta$  and goat anti-human NKp46 (R & D) primary mAbs, followed by staining with donkey anti-rabbit AF488 and donkey anti-goat AF594 secondary mAbs (Invitrogen), respectively. DAPI (blue) was included to stain nuclei. Representative pictures from one of at least three independent experiments indicated that expression of some NKp46 (red) and CD3 $\zeta$  (green) co-existed in the same region in an NKp46+ human NK cell. The T cell in the same field did not stain for NKp46. Magnification, 630x. (B) CD3+CD56+ LGL leukemia (T-LGLL) cells were FACS-enriched and stained using the exact method that was described in (A).

Supplemental Fig. 3



**Supplemental Figure 3. Expression of IL-15 and its receptors in T-LGL leukemia cells.**

Microarray analysis comparing IL-15, IL-15 receptor  $\alpha$  and IL-15 receptor  $\gamma$  expression in isolated PBMC ( $n = 4$ ) and CD8+ T cells ( $n = 4$ ) from healthy donors to those in T-LGL leukemia patient samples (majority of the 34 samples are CD8+). IL-15 expression was significantly upregulated when compared to the normal CD8+ T cell control ( $p < 0.01$ ), while predictably not upregulated when compared to the normal PBMC control (which includes antigen presenting cells that normally express IL-15) ( $p = 0.25$ ). Both IL-15 receptor  $\alpha$  and IL-15 receptor  $\gamma$  expression in T-LGL leukemia patient samples were upregulated when compared to both the CD8+ T cell control and the PBMC control ( $p < 0.05$  for each comparison).

<b>Supplemental Table 1. Percent CD3+NKp46+ cells among lymphocyte populations</b>	
<b>Organ</b>	<b>Percent CD3+NKp46+</b>
<b>Spleen</b>	<b>0.20 ± 0.01</b>
<b>Bone marrow</b>	<b>0.35 ± 0.02*</b>
<b>Peripheral blood</b>	<b>0.16 ± 0.01</b>
<b>Thymus</b>	<b>0.06 ± 0.005</b>

These results are averaged from three mice. \* The percentage of NKp46+CD3+ T cells in bone marrow is relatively higher than that in other organs ( $p < 0.001$ ,  $n = 3$ )

**Supplemental Table 2 : Differential gene expression between NKp46+ and NKp46- NKT cells****A. Up-Regulated Gene in NKp46+ Cells**

Gene Name	Gene Symbol	Fold Change	P value
natural cytotoxicity triggering receptor 1	Ncr1	112.2621279	0.0000276
acyloxyacyl hydrolase	Aoah	18.3563842	0.0000533
TYRO protein tyrosine kinase binding protein	Tyrobp	18.0097344	0.0001219
elastin microfibril interfacier 2	Emilin2	12.1054894	0.0000241

**B. Down-Regulated Gene in NKp46+ Cells**

Gene Name	Gene Symbol	Fold Change	P value
CD4 antigen	Cd4	-69.8947893	0.0000234
cytotoxic T-lymphocyte-associated protein 4	Ctla4	-50.3632682	0.0001690
forkhead box P3	Foxp3	-48.5716301	0.0000045
ankyrin repeat domain 6	Ankrd6	-48.0240610	0.0000070
chemokine (C-C motif) receptor 6	Ccr6	-27.4500905	0.0000172
signaling lymphocytic activation molecule family member 1	Slamf1	-25.4527829	0.0001805
neuritin 1	Nrn1	-24.2396506	0.0001355
coiled-coil domain containing 22	Ccdc22	-23.0392365	0.0000220
interleukin 1 receptor-like 1	Il1rl1	-22.8466067	0.0000645
megakaryocyte-associated tyrosine kinase	Matk	-22.1297385	0.0001529
actin, gamma 2, smooth muscle, enteric	Actg2	-20.9072803	0.0000117
aryl hydrocarbon receptor nuclear translocator 2	Arnt2	-20.5493307	0.0001280
chemokine (C-C motif) receptor 4	Ccr4	-19.9408362	0.0000109
cytochrome P450, family 2, subfamily s, polypeptide 1	Cyp2s1	-19.3379304	0.0000160
integrin alpha E, epithelial-associated	Itgae	-18.2175936	0.0001581
killer cell lectin-like receptor subfamily G, member 1	Klrg1	-17.9706726	0.0000787

Gene Name	Gene Symbol	Fold Change	P Value
argininosuccinate synthetase 1	Ass1	-17.8762782	0.0000455
guanylate binding protein 1	Gbp1	-17.7521087	0.0000928
Rho guanine nucleotide exchange factor (GEF) 12	Arhgef12	-17.1404193	0.0000285
src homology 2 domain-containing transforming protein E	She	-17.1392075	0.0001451
a disintegrin and metallopeptidase domain 12 (meltrin alpha)	Adam12	-17.1085371	0.0000695
chondroitin sulfate N-acetylgalactosaminyltransferase 1	Csgalnact1	-15.7891763	0.0000330
syndecan binding protein (syntenin) 2	Sdcbp2	-15.2042737	0.0001198
a disintegrin and metallopeptidase domain 12 (meltrin alpha)	Adam12	-15.048497	0.0000240
ankyrin repeat domain 6	Ankrd6	-13.6979728	0.0000294
CD81 antigen	Cd81	-12.9922465	0.0000378
Epstein-Barr virus induced gene 3	Ebi3	-12.8821947	0.0001261
nucleobindin 2	Nucb2	-12.2627658	0.0001679
RIKEN cDNA D430019H16 gene	D430019H16Rik	-12.2613549	0.0002142
RIKEN cDNA 1110059G02 gene	1110059G02Rik	-12.2430406	0.0000934
leucine-rich repeats and immunoglobulin-like domains 1	Lrig1	-11.9554829	0.0001478
syndecan 4	Sdc4	-11.9270306	0.0000268
neuropilin 1	Nrp1	-11.8832743	0.0000869
Rho guanine nucleotide exchange factor (GEF) 12	Arhgef12	-11.7251421	0.0000943
zinc finger and BTB domain containing 32	Zbtb32	-11.7195722	0.0001165
purinergic receptor P2X, ligand-gated ion channel, 7	P2rx7	-11.6992083	0.0001180
RIKEN cDNA 1110059G02 gene	1110059G02Rik	-11.6683689	0.0000383
chemokine (C-C motif) receptor 3	Ccr3	-11.2637515	0.0001989
dystonin	Dst	-11.2179333	0.0000817
trans-acting transcription factor 6	Sp6	-11.1784829	0.0002320



<b>Gene Name</b>	<b>Gene Symbol</b>	<b>Fold Change</b>	<b>P Value</b>
microtubule associated monooxygenase, calponin and LIM domain containing 3	Mical3	-10.9677182	0.0000552
serine (or cysteine) peptidase inhibitor, clade B, member 1a	Serpinb1a	-10.9240544	0.0000496
RIKEN cDNA 1110059G02 gene	1110059G02Rik	-10.8877052	0.0000537
legumain	Lgmn	-10.7073162	0.0001003
colony stimulating factor 1 (macrophage)	Csf1	-10.5349678	0.0000618
laminin, gamma 1	Lamc1	-10.2816543	0.0001809
IKAROS family zinc finger 4	Ikzf4	-10.2576511	0.0000826
tumor necrosis factor receptor superfamily, member 4	Tnfrsf4	-10.2419641	0.0000488