

Supplemental Methods

Gating Strategies

Cell type	Organ	Gating
NK	Bone Marrow, PBMC, spleen	Live, TCR β -, B220-, NK1.1+, NKp46+
NK	Liver	Live, TCR β -, B220-, NK1.1+, NKp46+, TRAIL-
ILC1	Liver	Live, TCR β -, B220-, NK1.1+, NKp46+, CD49b-, TRAIL+
ILC1	Spleen	Live, TCR β -, B220-, NK1.1+, NKp46+, CD127+
CD8 T-cell	Spleen, liver, PBMC,	Live, TCR β +, CD4-, CD8 α +
CD4 T-cell	Bone Marrow	Live, TCR β +, CD4+, CD8 α -
Monocytes	Spleen	Live, CD11b ^{high} , SSC-A ^{low} , TCR β -, B220-, NK1.1-

Genotyping & qPCR primers

Gene	Reaction	Primer	Sequence
<i>Il18bp</i>	WT	Forward	GGGAAGCTGGGCAGTAGAATGG
<i>Il18bp</i>	WT	Reverse	CAGACAACTGCCACTGTCTTAACTGG
<i>Il18bp</i>	KO	Forward	CGGTCCTAAGGTAGCGACTCGAG
<i>Il18bp</i>	KO	Reverse	TTGCCTTGGGGACAGTACATTAGGG
<i>Il18tg</i>	TG	Forward	AACCGGGCCCCCTCTGCTAACCATG
<i>Il18tg</i>	TG	Reverse	CACCTAACTTTGATGTAAGTTAGTGAGAGTGAACAT
<i>Il18tg</i>	Internal Control	Forward	GCTTAGCACGTTACCCCTGGATGG
<i>Il18tg</i>	Internal Control	Reverse	GTTATCAGCCCAACTAATTACAC
<i>Il18</i>	KO	Forward	CTCAGTGGTCATTTCTCTCTCTGA
<i>Il18</i>	KO	Reverse	CAGACCAACCCTTGATCTAGAGC
<i>Evm003</i>		Forward	TCT GTC CTT TAA CAG CAT AGA TGT
<i>Evm003</i>		Reverse	TGT TAA CTC GGA AGT TGA TAT GGT A
<i>18s</i>		Taqman	Applied Biosystems, Hs99999901_s1

Complete Medium: RPMI 1640 with 2.05mM L-Glutamine (Cytiva HyClone) supplemented with 10mM HEPES (Gibco), 10% FBS (GenClone), 100U/mL Penn Strep (Gibco), 10mM Non-essential Amino Acids (Gibco), 0.05mM of B2-mercaptoethanol (Gibco), and 1mM Sodium Pyruvate (Gibco).

RNAseq data analysis: Pooled cDNA libraries were sequenced on the Illumina NextSeq500, mapped to Mus_musculus_ensembl_v80 reference sequence and gene track Mus_musculus_ensembl_v86, and quantified using CLC Genomics Workbench software (Version 22, Qiagen). Differentially Gene Expression (DGE) was defined as having a maximum group mean transcripts per million (TPM) ≥ 5 , absolute fold change ≥ 2 , and false discovery rate ≤ 0.01 . Fold changes were not calculated directly from TPM values but from the generalized linear model, which corrects for differences in library size between the samples and the effects of confounding factors. DGE analysis of NK cells was adjusted for tissue of origin.

Custom gene sets: NK IL-18 response: Alongside the NK cell ex vivo cultures performed in *Sciume et al*⁴⁷, sorted primary murine splenic NK cells were separately cultured with [IL-18 alone] (50ng/mL, R&D) and [IL-18 + IL-12] (IL-12 10ng/mL, R&D) for 6 hours before preparation of bulk RNAseq libraries. We combined samples from IL-18-exposed conditions ([IL-18 alone] and [IL-18 + IL-12]) and control conditions ([media alone], [IL-12 alone], and [IL-2+IL12]), and generated “Up with 18” and “Down with 18” gene sets by comparing these two groups and extracting genes with mean TPM>5 (in either group) and False Discovery Rate p-value < 0.001 (CLC genomics software as above, **Supplemental Data 2**). GSEA was performed as above using these custom gene sets. **Immature NK/ILC:** We also generated custom gene sets of genes Up and Downregulated in immature vs mature NK-like cells by setting stringent p-value and fold-change cutoffs from relevant bulk RNAseq datasets from GSE77695⁴⁸ (Thresholds and resulting gene sets as in **Supplemental Data 3**).

Antibodies used for flow cytometry

Marker	Fluorophore	Clone	Manufacturer	Catalog Number
B220/ CD45R	BV605	RA3-6B2	Biolegend	103243
B8R H2-Kb (sequence: TSYKFESV)	APC		NIH Tetramer Core	
CD11b	BV711	M1/70	Biolegend	101241
CD11b/ MAC-1	APC-Cy7	M1/70	Biolegend	101225
CD127/ IL-7r	BV605	A7R34	Biolegend	135025
CD127/ IL-7r	PE-Cy7	SB/199	Biolegend	121120
CD178 (Fas Ligand)	APC-eFluor™ 780	MFL3	eBio	47-5911-82
CD19	APC	1D3/CD19	Biolegend	152409
CD19	PE/DAZZLE594	6D5	Biolegend	115553
CD253 (TRAIL)	Biotin	N2B2	Biolegend	109303
CD27	Pacific Blue	LG.3A10	Biolegend	124217
CD279 (PD-1)	PerCP-e710	RMP1-30	eBio	46-9981-80
CD279/PD-1	BV421	RMP1-30	Biolegend	109121
CD279/PD-1	PerCP-Cy5.5	RMP1-30	Biolegend	109119
CD279/PD-1	PE-Dazzle	RMP1-30	Biolegend	109115
CD314 (NKG2D)	PE	CX5	BioLegend	130207
CD335/NKp46	APC	29A1.4	Biolegend	137627
CD335/NKp46	AF647	29A1.4	BD	560755
CD335/NKp46	BV605	29A1.4	Biolegend	137619
CD39	BV605	Y23-1185	BD	567266
CD39	PE-Cy7	Duha59	Biolegend	143805
CD4	BUV737	RM4-5	BD	612844
CD4	BV785	GK1.5	BioLegend	100453
CD4	FITC	RM4-5	Biolegend	100509
CD4	PerCP-Cy5.5	RM4-5	BioLegend	116012
CD4	APC-Cy7	RM4-5	Tonbo	25-0042
CD44	BV650	IM7	Biolegend	103049
CD44	PerCP-Cy5.5	IM7	BD	560570
CD44	AF700	IM7	bd	560567
CD49b	APC-Cy7	DX5	Biolegend	108920
CD62L	BV711	MEL-14	Biolegend	104445
CD8a	Pacific Blue	53-6.7	Biolegend	100728

CD8a	PerCP-Cy5.5	53-6.7	eBio	45-0081-82
CD8a	AF700	53-6.7	BD	557959
CD8a	APC-Cy7	53-6.7	BD	557654
CD8a	APC-eFluor™ 780	53-6.7	eBio	47-0081-80
CD8b	APC-Cy7	YTS156.7. 7	Biolegend	126619
CD8b/Ly3	PE-Dazzle	YTS 156.7.7	BioLegend	126621
FC Block		93	Biolegend	101320
IL-18Ra (CD218a)	APC	P3TUNYA	eBio	17-5183-82
IL-18Ra (CD218a)	PE-Cy7	P3TUNYA	Thermo Fisher	25-5183-82
IL-18Ra (CD218a)	PE	P3TUNYA	eBio	2460173
KLRG1	BV785	2F1/KLRG1	Biolegend	138429
KLRG1	PE-Cy7	2F1/KLRG1	Biolegend	138416
KLRG1	PE	2F1	eBio	12-5893-80
LAG-3	BV650	C9B7W	Biolegend	125257
Fixable LIVE/DEAD	Aqua		Invitrogen	L34957
Ly6C	AF700	HK1.4	Biolegend	128024
NK1.1	BV421	pk136	Biolegend	108731
NK1.1	FITC	PK136	Biolegend	108705
NK1.1	PerCP-Cy5.5	PK136	Biolegend	108727
NK1.1	APC-eFluor™ 780	PK136	eBio	47-5941-82
NK1.1	APC	PK136	Tonbo	20-5941
NK1.1	PE-Cy7	PK136	eBio	25-5941-82
NK1.1	PE-Dazzle	PK136	Biolegend	108747
Streptavidin	BV421		Biolegend	405225
Streptavidin	BV605		Biolegend	405229
TCRb	Pacific Blue	H57-597	Biolegend	109225
TCRb	BUV737	H57-597	BD	612821
TCRb	FITC	H57-597	Tonbo	35-5961
TCRb	AF700	H57-597	Biolegend	109224
TCRb	APC	H57-597	Biolegend	109212
TCRb	PE-Cy7	H57-597	Tonbo	60-5961
TIGIT	BV421	1G9	Biolegend	142111