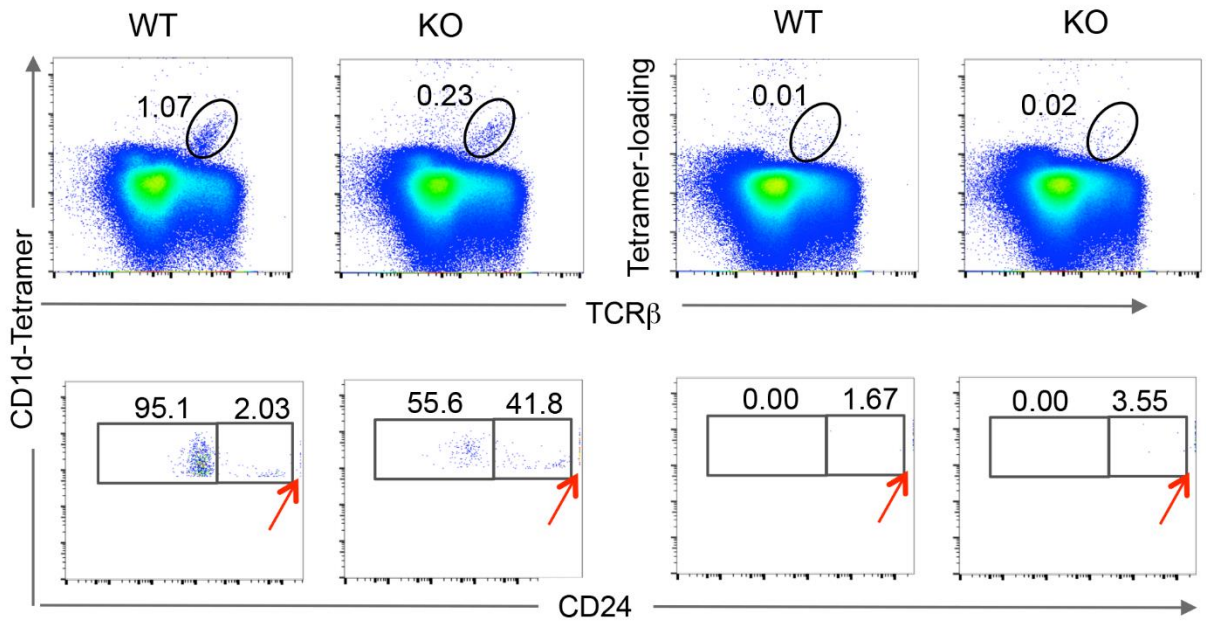
Supplemental information

The lysine acetyltransferase GCN5 is required for iNKT cell development through catalyzing EGR2 acetylation

Yajun Wang[1,3,10], Chawon Yun [1,10], Beixue Gao [1,10], Yuanming[1] Xu, Yana Zhang[1,2,6], Yiming Wang [4], Qingfei Kong [1], Fang Zhao [1], Chyung-Ru Wang [7] , Sharon Y.R. Dent [8], Jian Wang [9], Xiangping Xu[3, *], Hua-Bin Li [2, *] and Deyu Fang [1,4, 5, *]

Figure S1. Analysis of iNKT cell development using the tetramer loading control—related to Figure 2.



Single-cell suspensions from the thymus of WT and GCN5 KO mice were isolated. iNKT cells were identified with CD1d-tetramer and TCRb staining using the tetramer loading control (top panels). The iNKT development in the thymus of WT and GCN5 KO mice was further analyzed by their expression of CD24 (bottom panels).

Table s1. Antibodies used for the flow cytometry analysis—related to Figures 1-3 & 7.

Fluorescence	**Antigen**	**Company**	**Cat. #**
Alexa 647	Annxin V	Biolegend	640912
FITC	Annxin V	Biolegend	640906
PerCP/Cy5.5	CD4 (GK1.5)	Biolegend	100433
APC	CD4 (RM4-5)	eBbioscience	17-0042-82
PerCPCy5.5	CD8 (53-6.7)	Biolegend	100733
PE	CD8 (53-6.7)	eBbioscience	12-0081-83
PerCP/Cy5.5	CD24 (M1/69)	Biolegend	101823
APC	CD24 (M1/69)	Biolegend	101814
PE Cy7	CD44 (IM7)	Biolegend	103030
FITC	CD44 (IM7)	eBbioscience	11-0441-81
FITC	CD122 (TM-b1)	Biolegend	123208
PE	CD122 (5H4)	Biolegend	105905
PE	NK1.1 (PK136)	Biolegend	123505
FITC	CD1d	Biolegend	**108709**
Pacific Blue	T-Bet (4B10)	Biolegend	644807
PE	PLZF (9E12)	Biolegend	145803
PerCP/Cy5.5	PLZF (9E12)	Biolegend	145803
PE	Runx1	eBioscience	12-9816-80
PE	Egr2	eBioscience	12-6691-80
unconjugated	Ac-H3	Millipore	06-599
Alexa Fluor 488	Rabbit IgG	BD	544020
Pacific Blue	CD45.1 (A20)	Biolegend	110722
PE/CY7	Cd45.2 (104)	Biolegend	109829

* All antibodies were used at concentrations as recommended by the manufactures.

Table S2: Primers used for the study—related to Figures 3 & 5-7.

Primer name	Sequence (5' to 3')	Use
Gcn5-F	TTG ATT GAG CGC AAA CAG GC	Real-time RT-PCR
Gcn5-R	CAG CCT GTC TCT CGA ATG CC	Real-time RT-PCR
PCAF-F	GTT CTT GGT GTT GGT CGT GA	Real-time RT-PCR
PCAF-R	ATG GCT ACA GCT TCG ACA GG	Real-time RT-PCR
Egr2-F	CTA CCC GGT GGA AGA CCT C	Real-time RT-PCR
Egr2-R	AAT GTT GAT CAT GCC ATC TCC	Real-time RT-PCR
Runx1-F	CTC CGT GCT ACC CAC TAC CT	Real-time RT-PCR
Runx1-R	ATG ACG GTG ACC AGA GTG C	Real-time RT-PCR
BclXl-F	CCT TGG ATC CAG GAG AAC G	Real-time RT-PCR
BclXl-R	CAG GAA CCA GCG GTT GAA	Real-time RT-PCR
VDR-F	TGG ACA TTG GCA TGA TGA A	Real-time RT-PCR
VDR-R	GGC CTC AGA CTG TCC TTC AA	Real-time RT-PCR
c-Myc-F	CCT AGT GCT GCA TGA GGA GA	Real-time RT-PCR
c-Myc-R	TCC ACA GAC ACC ACA TCA ATT T	Real-time RT-PCR
PLZF-F	GAC GCA CTA CAG GGT TCA CA	Real-time RT-PCR
PLZF-R	GCT TGA TCA TGG CCG AGT AG	Real-time RT-PCR
RelA-F	CAT GCG ATT CCG CTA TAA AT	Real-time RT-PCR
RelA-R	GGT CCT GTG TAG CCA TTG ATC T	Real-time RT-PCR
GATA3-F	GAC TCT TCC CAC CCA GCA	Real-time RT-PCR
GATA3-R	CCC CGC AGT TCA CAC ACT	Real-time RT-PCR
Tbet-F	GAA AGG CAG AAG GCA GCA T	Real-time RT-PCR
Tbet-R	GAG CTT TAG CTT CCC AAA TGA A	Real-time RT-PCR
Bcl2-F	GTA CCT GAA CCG GCA TCT G	Real-time RT-PCR
Bcl2-R	GGG GCC ATA TAG TTC CAC AA	Real-time RT-PCR
ITK-F	GGA AAA AGC TTG TGT CAT CCA	Real-time RT-PCR
ITK-R	CCA AAG TCG GAC ACC TTG AT	Real-time RT-PCR
RelB-F	GTG ACC TCT CTT CCC TGT CAC T	Real-time RT-PCR
RelB-R	TGT ATT CGT CGA TGA TTT CCA A	Real-time RT-PCR
cMyb-F	GCC TTC CCT GTT GGT TTG	Real-time RT-PCR

cMyb-R	CAT TCA TCC TTC TCC CGA GT	Real-time RT-PCR
P300-F	AAC AAA GCC AGC CAT CTG GA	Real-time RT-PCR
P300-R	GAG GCC ACA CCA GCA TTT TC	Real-time RT-PCR
Tip60-F	GGA GGT GGG GGA GAT AAT CG	Real-time RT-PCR
Tip60-R	CTT TCG GCC ACT GAT GTC CT	Real-time RT-PCR
β-Actin-F	CTA AGG CCA ACC GTG AAA AG	Real-time RT-PCR
β-Actin-R	ACC AGA GGC ATA CAG GGA CA	Real-time RT-PCR
WL20-EJ	CAC AGA GCT TCT TGG AGA CC	Gcn5 floxed mice genotyping
WL21-3-EJ	GGC TTG ATT CCT GTA CCT CC	Gcn5 floxed mice genotyping
oIMR1084	GCG GTC TGG CAG TAA AAA CTA TC	LCK-Cre TG mice genotyping
oIMR1085	GTG AAA CAG CAT TGC TGT CAC TT	LCK-Cre TG mice genotyping
oIMR7338	CTA GGC CAC AGA ATT GAA AGA TCT	LCK-Cre TG mice genotyping
oIMR7339	GTA GGT GGA AAT TCT AGC ATC ATC C	LCK-Cre TG mice genotyping
Tbet-p-1	CAT ACT AGC TCC CGT ATT A	ChIP
Tbet-p-2	GTA AAG CGG ACT GCG AAG T	ChIP
Runx1-p-1	CGT CTC ATG AGG GGC CAG G	ChIP
Runx1-p-2	ATG CCA AGG CTC TGG GAT AG	ChIP
PLZF-p-1	CAG CAT ATT GCA AAT GGT	ChIP
PLZF-p-2	AGA TAT CAT CCA CAC TG	ChIP
IL2RB-p-1	CAA GGC GAG CCC ACT TCC T	ChIP
IL2RB-p-2	CTA GGT CCC TGT CCA ACA	ChIP
Bclxl-p-1	CAT AGG TTT TAA GCC AAG TA	ChIP
Bclxl-p-2	TTC TGT TAT ATG GCA TGG C	ChIP

Table S3. Antibodies used for western blotting, ChIP and immunoprecipitation—related to Figures 5-7.

Antigen	Species	Company	Cat #
HA	R	Santa Cruz	SC-805
Egr-2	R	Santa Cruz	SC-20690
Tubulin	M	Calbiochem	CP06
GCN5L2	R	Millipore	07-1545
GCN5L2	R H-75	Santa Cruz	SC-20698
Myc	M	Santa Cruz	SC-40
MYC	M HRP	Santa Cruz	SC-40
Myc	R	Santa Cruz	SC-789
Flag	M	Sigma	F1804
Flag	Rb	Sigma	F7425
c-Myb	Rb	Millipore	05-175
NFkB P50	Rb	Santa Cruz	SC-7178
NF-kB P50	M	Millipore	06-886
NF-kB RelA	M	Abcom	ab16502
NF-kB RelA	Rb	Millipore	12-10060
Ac-H3	Rb	Millipore	06-599
H3	Rb	Millipore	04-928

*** All antibodies were used at concentrations as recommended by the manufactures.**