

Supplementary Material

1 **Supplementary Table 1.** Utilized antibodies for flow cytometry

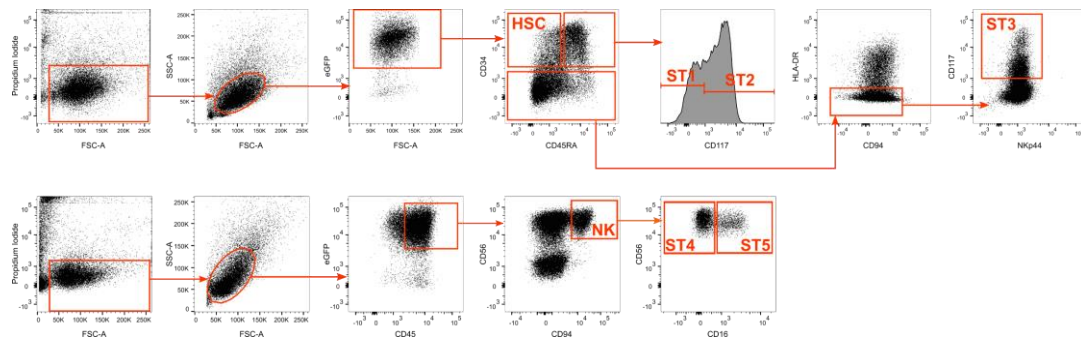
Marker	Alternative name	Fluorochrome	Clone	Supplier
CD3	CD3	APC	SK7	BD Biosciences
CD11a	LFA-1	PE, Alexa Fluor 700	HI111	Biologend
CD14	CD14	APC	REA599	Miltenyi Biotec
CD16	Fc γ RIIIA	PE, APC	B73.1	Biologend
CD19	CD19	APC	SJ25C1	Thermo Fisher Scientific
CD34	CD34	PE, PB	AC136	Miltenyi Biotec
CD45	CD45	APC/Fire TM 750 (APC-Fire)	2D1	Biologend
CD56	NCAM1	VioBlue (VB), APC	5.1H11	Biologend
CD94	KLRD1	PerCP-Cy5.5	DX22	Biologend
CD117	KIT	PECy7	104D2	Thermo Fisher Scientific
CD158a,h	KIR2DL1/KIR2DS1	PE	REA1010	Miltenyi Biotec
CD158b1/b2	KIR2DL2/KIR2DL3	PE	REA1006	Miltenyi Biotec
CD158e1/e2	KIR3DL1/KIR3DS1	PE	REA168	Miltenyi Biotec
CD158i	KIR4DS1	PE	REA860	Miltenyi Biotec
NKG2A	CD159a	APC	REA110	Miltenyi Biotec
NKG2C	CD159c	PE	FAB138P	R&D systems
CD226	DNAM-1	Alexa Fluor 647	DX11	BD Biosciences
NKG2D	CD314	APC	1D111	Biologend
NKp46	CD335;NCR1	PECy7	9E2	Biologend
NKp44	CD336;NCR2	eFluor450, APC	44.189	Thermo Fisher Scientific
NKp30	CD337	PE	P30-15	Biologend
SLAMF6	NTB-A/CD352	PE	NT-7	Biologend
Granzyme B	Granzyme B	PE	GB11	Thermo Fisher Scientific
HLA-DR	HLA-DR	eFluor780	LN3	Thermo Fisher Scientific
Perforin	Perforin	PE	dG9	Thermo Fisher Scientific
CD45RA	CD45RA	APC, Brilliant Violet 421	HI100	Biologend
AHR	AHR	PE	FF3399	Thermo Fisher Scientific
AIOS	IKZF3	PE	16D9C97	Biologend
CBFB	CBFB	unconjugated	JG39-35	Thermo Fisher Scientific
Donkey Anti-Rabbit	Donkey Anti-Rabbit	PE	Poly4064	Biologend
EOMES	EOMES	PE, eFluor660	WD1928	Thermo Fisher Scientific
GATA2	GATA2	PE	W17242A	Biologend
HELIOS	IKZF2	APC	22F6	Biologend
HOBIT	ZNF683	Alexa Fluor 647	Sanquin-Hobit/1	BD Biosciences
ID2	ID2	APC	ILCID2	Thermo Fisher Scientific
IFN-γ	IFN- γ	eFluor 660	4S.B3	Thermo Fisher Scientific
IKZF2	HELIOS	APC	22F6	Biologend

IRF8	IRF8	APC	V3GYWCH	Thermo Fisher Scientific
NFIL3	E4BP4	PE	MABA223	Thermo Fisher Scientific
PRDM1	Blimp-1	PE	6D3	BD Biosciences
RUNX2	AML3, CBFA1	PE	D1L7F	Cell Signalling Technologies
RUNX3	AML42 CBFA3	PE	R3-5G4	BD Biosciences
TBET	TBET	PE	4B10	Thermo Fisher Scientific
TNF-α	TNF- α	PECy7	Mab11	Thermo Fisher Scientific
TOX	TOX	PE	TXRX10	Thermo Fisher Scientific

Allophycocyanin (APC), Peridinin Chlorophyll Protein-Cyanin5.5 (PerCP-Cy5.5), Phycoerythrin (PE), Phycoerythrin-Cyanin7 (PECy7)

2 Supplementary Table 2. Utilized qPCR primers

Gene	Sense	Sequence
IRF2	forward	5'-AAGCACACTGAGAGGGGCAC-3'
	reverse	5'-CATCCACCCATGTCTAGCC-3'
IRF2 (flanking shRNA binding sites)	forward	5'-CGATGAAGAGAGTGCCGAG-3'
	reverse	5'-TTGAAAAGGGGGCCAGGAG-3'
BTG3	forward	5'-ATGAAATTGCTGCCGTTGTCT-3'
	reverse	5'-GCCTGTCCTTTCGATGGTTTT-3'
ZWILCH	forward	5'-TCCCTCCACTCTCTTCAACTGC-3'
	reverse	5'-CCAGTCCTCAAACCATCAGCCA-3'
CLSPN	forward	5'-TGAAAACCTCAGGCAAGCAGCCTA-3'
	reverse	5'-GGCTGATAGGATGGAATCGTGG-3'
GAPDH	forward	5'-TCCTCTGACTTCAACAGCGACA-3'
	reverse	5'-GTGGTCGTTGAGGGCAATG-3'
TBP	forward	5'-CACGAACCACGGCACTGATT-3'
	reverse	5'-TTTTCTTGCTGCCAGTCTGGAC-3'
YWHAZ	forward	5'-ACTTTTGGTACATTGTGGCTTCAA-3'
	reverse	5'-CCGCCAGGACAAACCAGTAT-3'



Supplementary Figure 1. Gating strategy of NK cell developmental stages in differentiation cultures. Flow cytometric gating strategy of NK cell developmental stages, including HSC (CD34⁺CD45RA⁻), stage 1 (CD34⁺CD45RA⁺CD117⁻), stage 2 (CD34⁺CD45RA⁺CD117⁺), stage 3 (CD34⁻CD94⁻CD117⁺HLA-DR⁻NKp44⁻), stage 4 (CD45⁺CD56⁺CD94⁺CD16⁻) and stage 5 (CD45⁺CD56⁺CD94⁺CD16⁺).

Supplementary Table 3. Differentially expressed genes in d3 HSC and d7 ST3 cells in the gene ontology (GO) positive and negative regulation of mitotic cell cycle pathways. Genes containing an IRF2-binding motif are marked as bold.

HSC				ST3			
GO positive regulation of mitotic cell cycle		GO negative regulation of mitotic cell cycle		GO positive regulation of mitotic cell cycle		GO negative regulation of mitotic cell cycle	
DOWN (4)	UP (4)	DOWN (12)	UP (4)	DOWN (6)	UP (7)	DOWN (15)	UP (14)
<i>CYP11A1</i>	<i>RAB11A</i>	<i>TAOK3</i>	<i>INHBA</i>	<i>DYNLT3</i>	<i>DTL</i>	<i>CTDSP2</i>	<i>BRCA1</i>
<i>CCND2</i>	<i>NSMCE2</i>	<i>PTEN</i>	<i>BTG2</i>	<i>MEIS2</i>	<i>CDC6</i>	<i>TAOK3</i>	<i>CLSPN</i>
<i>CDK4</i>	<i>HES1</i>	<i>CTDSP1</i>	<i>BTG3</i>	<i>ANXA1</i>	<i>ANKRD17</i>	<i>CCL2</i>	<i>ZWILCH</i>
<i>CCNB1</i>	<i>EIF4E</i>	<i>TOPBP1</i>	<i>SDE2</i>	<i>PBX1</i>	<i>ANAPC5</i>	<i>ABRAXAS1</i>	<i>ZWINT</i>
		<i>HMGA2</i>		<i>PRKCA</i>	<i>AIF1</i>	<i>PTEN</i>	<i>DTL</i>
		<i>BLM</i>		<i>PFAH1B1</i>	<i>BRCA2</i>	<i>CDKN1C</i>	<i>CDC6</i>
		<i>CDK5RAP2</i>			<i>EIF4E</i>	<i>FHL1</i>	<i>TPR</i>
		<i>ZFP36L2</i>				<i>CHMP4C</i>	<i>PRKDC</i>
		<i>ORC1</i>				<i>CDKN1A</i>	<i>BABAM1</i>
		<i>CCNB1</i>				<i>ZFP36L1</i>	<i>DONSON</i>
		<i>NACC2</i>				<i>NABP1</i>	<i>NDC80</i>
		<i>FHL1</i>				<i>CCNG1</i>	<i>MSH2</i>
						<i>BTG2</i>	<i>CDK5RAP2</i>
						<i>BTN2A2</i>	<i>MAD2L1</i>
						<i>CRLF3</i>	