

Supplemental Information

**Intra-lineage Plasticity and Functional
Reprogramming Maintain Natural Killer
Cell Repertoire Diversity**

Aline Pfefferle, Benedikt Jacobs, Herman Netskar, Eivind Heggernes Ask, Susanne Lorenz, Trevor Clancy, Jodie P. Goodridge, Ebba Sohlberg, and Karl-Johan Malmberg

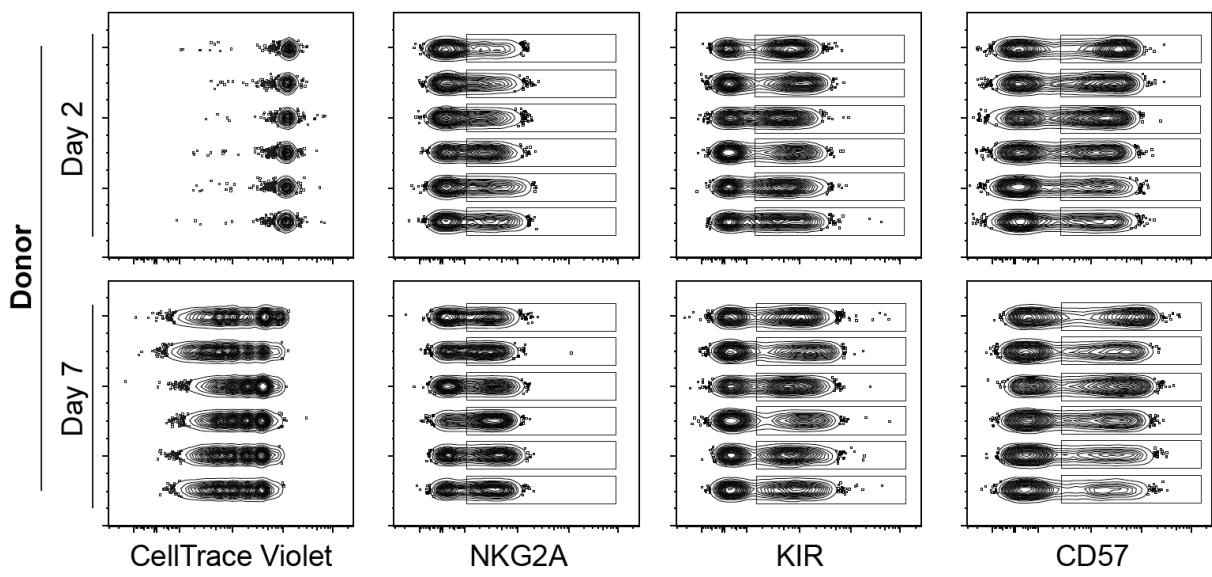


Figure S1. Inter-donor variation in response to IL-15 stimulation (Related to Figure 1)

Concatenated FACS plots showing CTV dilution and surface expression of NKG2A, KIR and CD57 of the total NK cell population on day 2 and day 7 in IL-15 stimulated cells. n = 6 from one representative experiment.

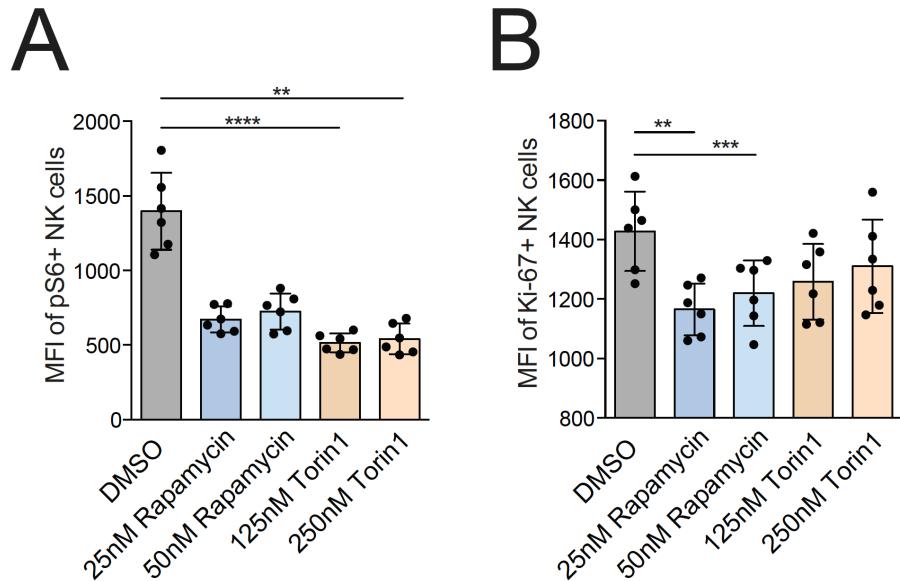


Figure S2. mTOR inhibition in proliferating NK cells (Related to Figure 2)

(A and B) Mean fluorescent intensity of pS6 (A) and Ki-67 (B) on day 6 in IL-15 stimulated NK cells treated with DMSO, 25nM Rapamycin, 50nM Rapamycin, 125nM Torin-1 or 250nM Torin-1 for 48h prior to readout. n = 6 from one representative experiment. Data are represented as mean (SD). Significance was calculated using a Friedman test followed by Dunn's multiple comparisons test (A-B). p-values: * < 0.05, ** < 0.01, *** < 0.001, **** < 0.0001.

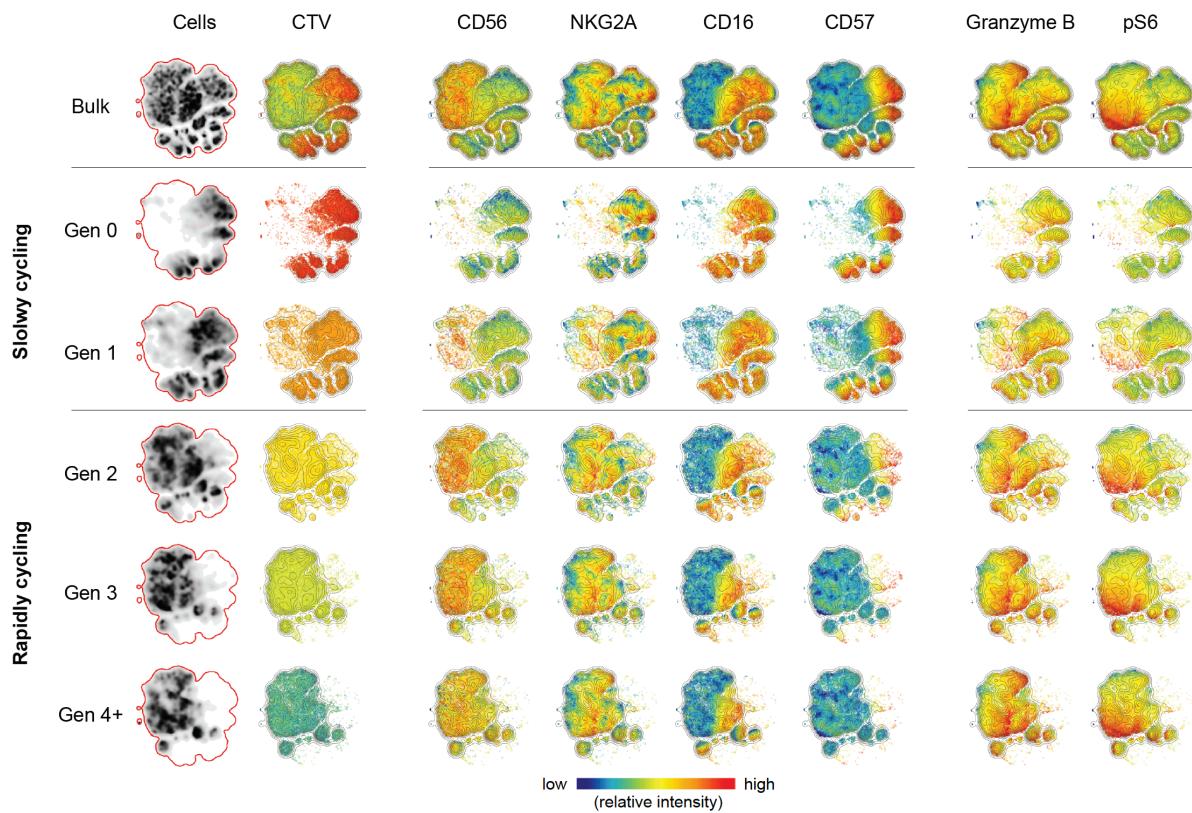


Figure S3. Phenotype of proliferating NK cells observed at the generation level (Related to Figure 3)

t-SNE plots showing the relative intensity of CD56, NKG2A, CD16, CD57, Granzyme B and pS6 expression in the total NK cell population and gated on individual generations using CTV dilution in one representative donor on day 5. n = 1 from one representative experiment.

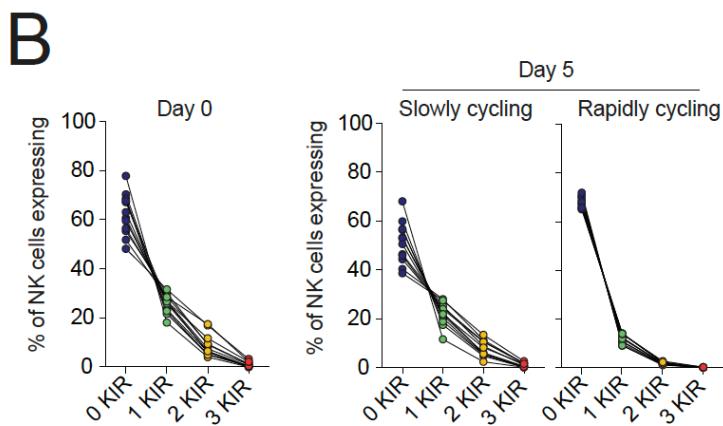
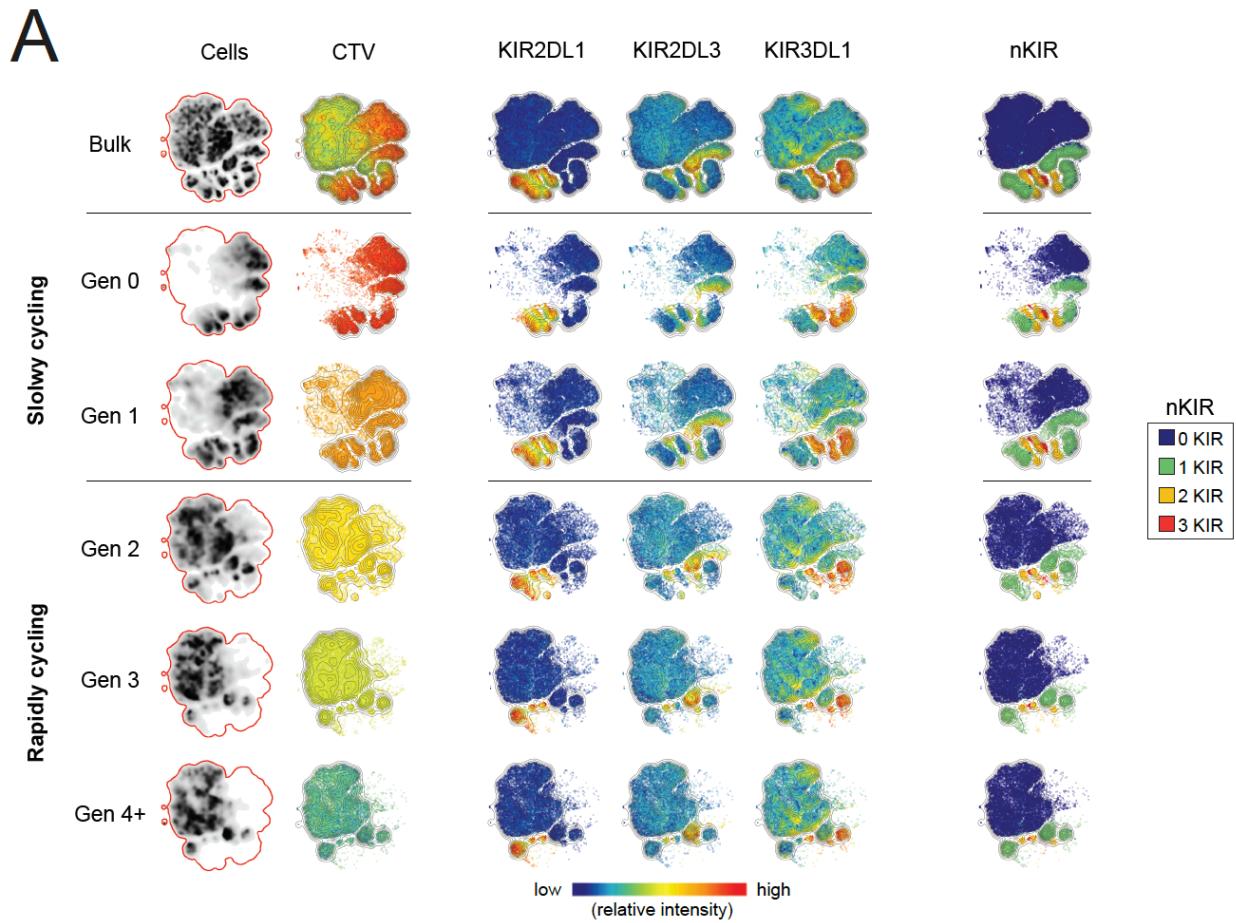


Figure S4. KIR repertoires of proliferating NK cells at the generation level (Related to Figure 4)

(A) t-SNE plots showing the relative intensity of KIR2DL1, KIR2DL3 and KIR3DL1 expression, as well as the number of KIR (nKIR) expressed per cell, in the total NK cell population and gated on individual generations using CTV dilutions in one representative donor on day 5. **(B)** The frequency of NK cells expressing 0-3 KIR at baseline and within slowly (Generation 0-1) and rapidly cycling (Generation 2+) cells on day 5. $n = 1-12$ from 2 independent experiments. In **(B)** 3 longitudinal samples from 4 donors each were analyzed with >1 year in between sampling.

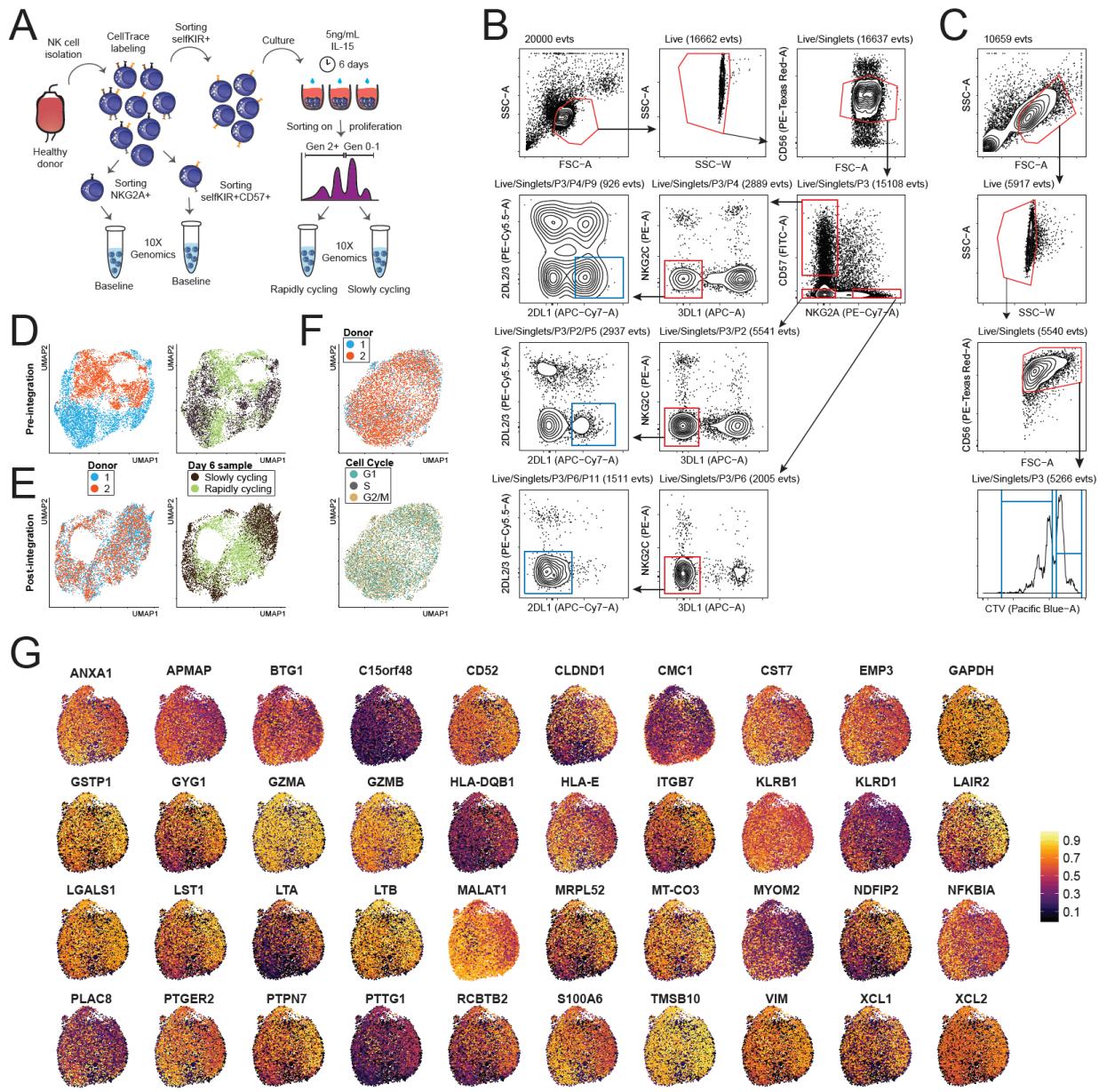


Figure S5. Design and analysis of single-cell RNA sequencing experiments (Related to Figure 5)

(A) Graphical methodology outline for upstream sample preparation before single-cell RNA sequencing using the 10x Genomics platform. (B) The gating strategy used to sort distinct $CD56^{\text{dim}}$ NK cell populations at baseline from healthy blood donors. $NKG2A^+$ KIR $^-$ CD57 $^+$ and $NKG2A^{-/-}$ selfKIR $^+$ CD57 $^+$ NKG2C $^{-/-}$ subsets were sorted for single-cell RNA sequencing. $NKG2A^-$ selfKIR $^+$ CD57 $^+$ cells were sorted and placed in culture with IL-15 for 6 days to induce proliferation. Blue gates denote the sorted populations. (C) The gating strategy used to re-sort the previously sorted $NKG2A^-$ selfKIR $^+$ CD57 $^+$ into slowly (generation 0-1) and rapidly (generation 2+) cycling cells based on CellTrace Violet dilution. Blue gates denote the sorted populations. (D and E) UMAP embedding of the four day 6 scRNA-seq samples depicting donor and sample information for each cell before (D) and after (E) data integration. (F) UMAP embedding of the four baseline scRNA-seq samples depicting donor information and cell cycle phase for each cell after data integration. (G) Imputed gene expression of a selection of the 63 DEGs plotted onto the UMAP embedding of baseline and day 6 samples. $n = 4-8$ from 4 independent experiments.

63 differentially expressed genes at day 6

↑ slowly cycling	↑ rapidly cycling	
FGFBP2	PTTG1	ITGB7
CCL5	PTPN7	NDFIP2
MALAT1	BCL7C	GZMB
S100A4	TMSB10	TNFSF10
KLRF1	GZMA	GZMK
FCGR3A	XCL2	HLA-DQB1
LITAF	CEBPD	MRPL52
KLRD1	ENTPD1	KLRC1
CST7	MT-CO3	LGALS1
CMC1	HSPD1	RCBTB2
BTG1	FBXO6	XCL1
PTPRC	LAIR2	LTA
TIMP1	CCND2	C15orf48
KLRB1	GSTP1	CAPG
ZFP36L2	ASB2	COTL1
MYOM2	CLDND1	
PLAC8	NME1	
HLA-E	GYG1	
ANXA1	GAPDH	
PTGER2	TESC	
S100A6	LST1	
EMP3	VIM	
NFKBIA	LTB	
APMAP	CD52	

Table S1. Differentially expressed genes between slowly and rapidly cycling cells (Related to Figure 5)

Genes that were differentially expressed between slowly and rapidly cycling cells day 6 within the G1 phase of the cell cycle. The 63 genes are ordered from highest to lowest fold.