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Supplemental Information

FcRγ Gene Editing Reprograms Conventional NK Cells to Display Key Features of Adaptive Human NK Cells

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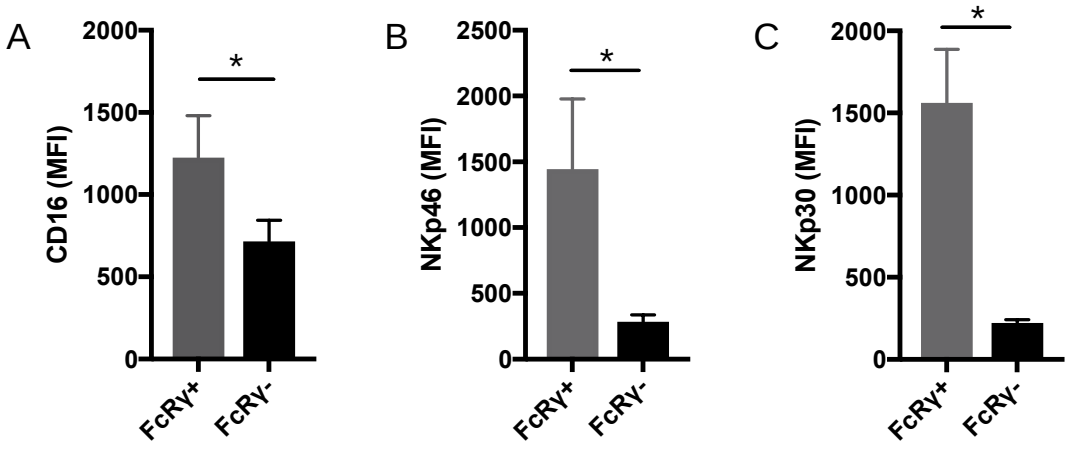

Figure S1. Loss of FcRγ leads to reduced cell-surface expression of CD16, NKp46 and NKp30, Related to Figure 3. Bar graphs show CD16 (A), NKp46 (B) and NKp30 (C) median fluorescence intensity (MFI) of FcRγ+ and FcRγ- NK subsets from 7 donors. *p<0.01.

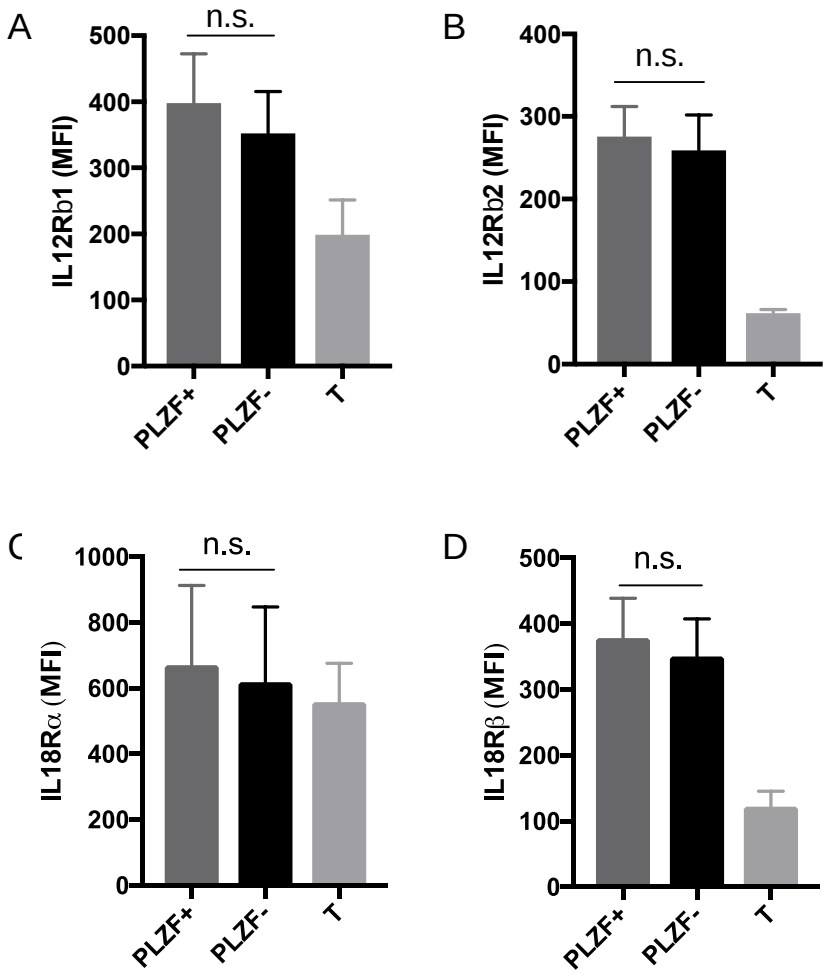

Figure S2. Loss of PLZF does not change the expression of receptors for IL-18 or IL-12, Related to Figure 4.
Bar graphs show median fluorescence intensity (MFI) of indicated markers on FcRγ+, FcRγ- NK subsets and T cells from 6 donors. n.s.: not significant.

STAR★Methods

Key Resources Table

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Anti-Fc epsilon R1gamma - AF488	MBL International	Cat# M191-A48; RRID:AB_11160966
anti-CD247 (TCRζ, CD3ζ) - PE	BioLegend	Cat# 644106; RRID:AB_2565721
Anti-CD3 - AF700	BD Biosciences	Cat# 557943; RRID:AB_396952
Anti-CD14 - APC/Cyanine7	BioLegend	Cat# 301820; RRID:AB_493695
Anti-CD19 - APC/Cyanine7	BioLegend	Cat# 302218; RRID:AB_314248
Anti-CD56 - PE/Cyanine7	Beckman Coulter	Cat#A51078; RRID:N/A
Anti-CD107a - BV786	BD Biosciences	Cat# 563869; RRID:AB_2738458
Anti-IFN-gamma - APC	BioLegend	Cat# 502512; RRID:AB_315237
Anti-TNF-alpha - Pacific Blue	BioLegend	Cat# 502920; RRID:AB_528965
Ultra-LEAF Purified anti-human CD16 Antibody	BioLegend	Cat# 302050; RRID:AB_2561481
Anti-CD16 - Pacific Blue	BioLegend	Cat# 302032; RRID:AB_2104003
Anti-NKp30 - APC	BioLegend	Cat# 325210; RRID:AB_2149449
Anti-NKp46 - APC	BioLegend	Cat# 137608; RRID:AB_10612758
Mouse IgG1, κ Isotype ctrl Antibody - APC	BioLegend	Cat# 400121; RRID:AB_326443
Anti-NKG2C - PE	R and D Systems	Cat# FAB138P; RRID:AB_2132983
Anti-NKG2A - APC	Miltenyi Biotec	Cat# 130-114-089; RRID:AB_2726447
Anti-CD2 - APC	BioLegend	Cat# 300214; RRID:AB_10895925
Anti-CD11a - PE	BD Biosciences	Cat# 555380; RRID:AB_395781
Anti-CCR5 - APC	BD Biosciences	Cat# 560748; RRID:AB_1937308
Anti-PLZF - AF647	BD Biosciences	Cat# 563490; RRID:AB_2738238
Bacterial and Virus Strains		
Biological Samples		
Healthy donor whole blood	Gulf Coast Regional Blood Centern	N/A

Chemicals, Peptides, and Recombinant Proteins		
RPMI 1640 medium, with L-glutamine and sodium bicarbonate	Sigma Aldrich	Cat# R8758-24X500ML
L-Glutamine (200 mM)	Thermo Scientific	Cat# 25030081
Penicillin-Streptomycin (10,000U/ml)	Gibco	Cat# 15-140-122
Phytohemagglutinin-L (PHA-L)	Sigma Aldrich	Cat# 11249738001
Human AB serum	Corning	Cat# 35060CI
Recombinant Human IL-2	Peprotech	Cat# 200-02
Sodium pyruvate (100mM)	Gibco	Cat# 11-360-070
MEM Non-Essential Amino Acid Solution (100X)	Gibco	Cat# 11-140-050
Fetal Bovine Serum (FBS), qualified, heat inactivated	Fisher Scientific	Cat# 16140089
Recombinant Human IL-12 p70 (CHO derived)	Peprotech	Cat# 200-12
Recombinant Human IL-18	MBL International	Cat# B001-5
DPBS, without calcium chloride and magnesium chloride	Sigma Aldrich	Cat# D8537
Lymphoprep	STEMCELL technologies	Cat# 07851
Dimethyl sulfoxide (DMSO)	Sigma Aldrich	Cat# D4540
Brefeldin A Solution (1000X)	BioLegend	Cat# 420601
Formaldehyde solution	Sigma Aldrich	Cat# F8775
Bovine Calf Serum (BCS)	Sigma Aldrich	Cat# 12133C
Sodium azide (NaN3)	Sigma Aldrich	Cat# 71289
Saponin	Sigma Aldrich	Cat# 47036-250G-F
Critical Commercial Assays		
EasySep Human NK Cell Isolation Kit	STEMCELL technologies	Cat# 17955
LIVE/DEAD™ Fixable Far Red Dead Cell Stain Kit, for 633 or 635 nm excitation	Invitrogen	Cat# L34973
human natural killer cell Nucleofector Kit	Lonza	Cat# VPA-1005
Deposited Data		
Experimental Models: Cell Lines		
RPMI 8866 Cell Line human	Sigma aldrich	Cat# 95041316-CDNA-20UL
K562 ATCC® CCL-243	ATCC	N/A
Raji Cell Line human	Sigma aldrich	Cat# 5011429-CDNA-20UL
Experimental Models: Organisms/Strains		
Oligonucleotides		

ON-TARGETplus Human FCER1G siRNA (SMARTPool)	Horizon	Cat# L-011856-00-0005
FCERIG KO sgRNA 1: 5'-UCUAUCCCCUCAGCGGCCCU-3'	Synthego	Cat# N/A
FCERIG KO sgRNA 2: 5'-GCAGAGCUGAGGCUCUCCCA-3'	Synthego	Cat# N/A
chemically modified non-targeting (negative) control sgRNA	Synthego	Cat# N/A
ZBTB16 KO sgRNA 1: 5'-CAGAACCCUAGCCACCCCAC-3'	Synthego	Cat# N/A
ZBTB16 KO sgRNA 2: 5'-AGAACCCUAGCCACCCCACG-3'	Synthego	Cat# N/A
Recombinant DNA		
Software and Algorithms		
Prism 7	GraphPad Software	https://www.graphpad.com/scientific-software/prism/
Other		

Experimental Model and Subject Details

Human Blood Samples

All studies were approved by the Institutional Review Board of the University of California, Davis. Leukopacks from healthy donors were obtained from Gulf Coast Regional Blood Center (Houston, Texas). PBMCs were isolated using SepMate tubes (STEMCELL technologies) and Lymphoprep density gradient media by centrifugation per manufacturer's instructions. Isolated PBMCs were cryopreserved in FBS with 10% DMSO.

Cell Lines

Cell lines were maintained at 37°C in 5% CO_2 humidified incubators. RPMI 8866, K562 and Raji cells were grown in RPMI 1640 medium containing 10% FBS, 2 mM L-glutamine and 100 U/ml penicillin-streptomycin.

Method Details

NK Cell Enrichment and Expansion

NK cells were enriched from cryopreserved PBMCs by immunomagnetic negative selection using EasySep Human NK Cell Isolation Kit (STEMCELL technologies). The resulting samples were co-cultured with feeder cells (a mixture of allogeneic PBMCs and RPMI 8866 cells) that had been irradiated. These cells were cultured in NK cell culture media (RPMI1640 supplemented with 10% FBS, 2 mM L-glutamine, 100 U/ml penicillin-streptomycin, 5% human AB serum, 500 U/ml IL-2, sodium pyruvate, 1X MEM non-essential amino acids) in the presence of 10 ug/ml Phytohemagglutinin-L for 10-14 days before nucleofection.

Nucleofection of Cas9/gRNA Ribonucleoprotein

To prevent FcRγ or PLZF expression, two target-specific modified sgRNAs were used together to maximize chances for successful knockouts. The online “Knockout Guide Design” tool featured by Synthego (https://design.synthego.com/#/) was used to select sgRNAs with highest activity and least off-target rate. sgRNAs and Cas9 2NLS nuclease were pre-assembled at a 9:1 ratio in nucleofector solution provided within the human natural killer cell Nucleofector Kit. 0.5 nmol ribonuclease complex was nucleofected into two million expanded cells by Nucleofector 2b Device (Lonza) using program “X1”. Pulsed cells were cultured in NK cell culture media. Sequences of sgRNAs are listed in the Key Resources Table.

In Vitro Stimulation of Cells

Cells were resuspended in 10% FBS supplemented RPMI 1640 before any functional assay. Functional assays using immobilized anti-CD16 mAb were performed by stimulating cells in 96-well ELISA plate coated with Ultra-LEAF Purified anti-human CD16 mAb. A maximum of 0.2 million cells were seeded into each well and were stimulated for 6 hours with the addition of Brefeldin A and anti-CD107a mAb at the beginning of this assay. Functional assays using K562 and Raji cells were performed at an effector: target ratio of 5:1 for a 6-hour co-culture period. Brefeldin A and anti-CD107a mAb were added at the beginning of the co-culture. Functional assays using IL-12 and IL-18 were conducted by adding a mixture of 20 ng/ml IL-12 and 100 ng/ml IL-18 into cell culture 16 hours prior to the addition of Brefeldin A. Cells were harvested after another 6 hours.

Flow Cytometry

Cells were stained for flow cytometry using fluorochrome-conjugated mAbs, and $CD56^{+}CD3^{-}CD14^{-}CD19^{-}$ cells were gated as previously described (Hwang et al., 2012). For detection of intracellular FcRγ, CD3ζ, PLZF, IFN-γ and TNF-α, cells were fixed and

permeabilized, then stained with fluorochrome-conjugated mAbs. Unstained or isotype-matched control antibody stained cells were used to determine background staining in corresponding channels. Data were collected on an LSR Fortessa flow cytometer (BD Biosciences) and were analyzed using FlowJo Software, version 10.6.1. Antibodies are listed in the Key Resources Table.

Quantification and Statistical Analysis

All statistical analyses were performed with Graphpad Prism 7. The Wilcoxon matched pairs signed rank test was used for all assays. Differences were considered significant when $p<0.05$.