Supplementary Information for

IGF-1 promotes the development and cytotoxic activity of human NK cells regulated by miR-483-3p

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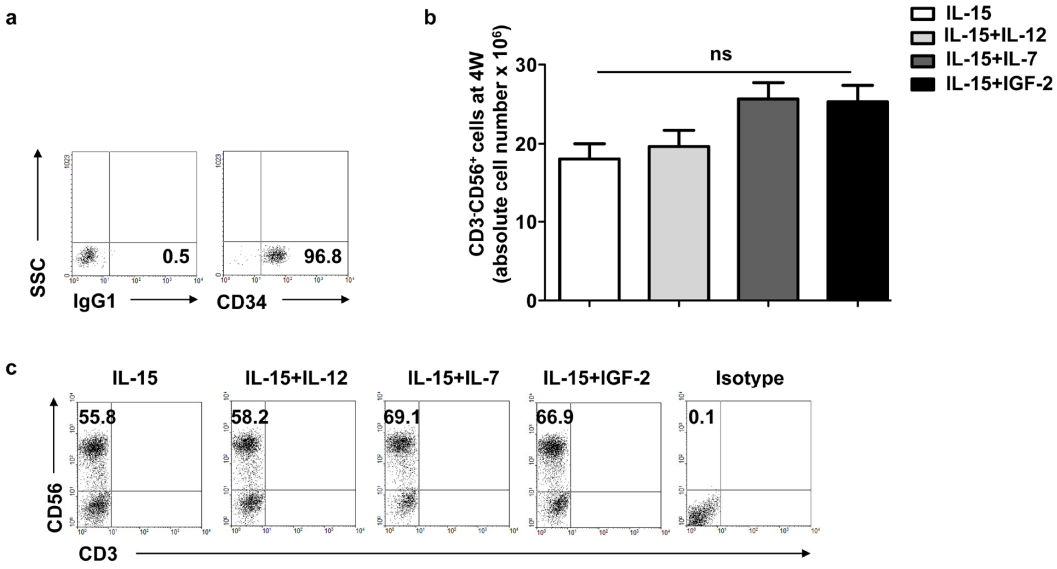
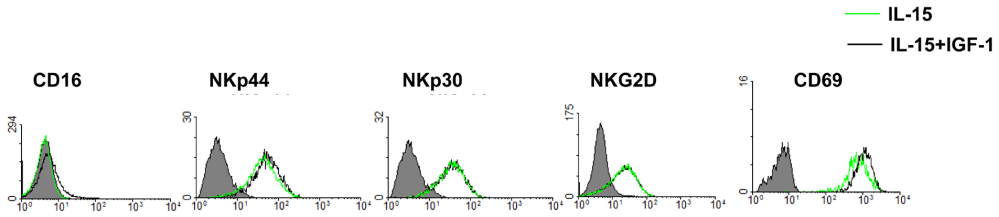
Supplementary Figures S1-S9

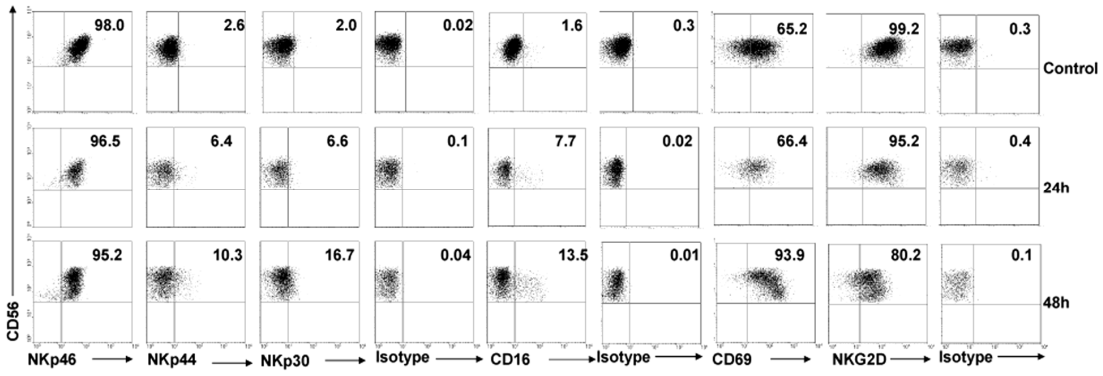
Supplementary Table S1



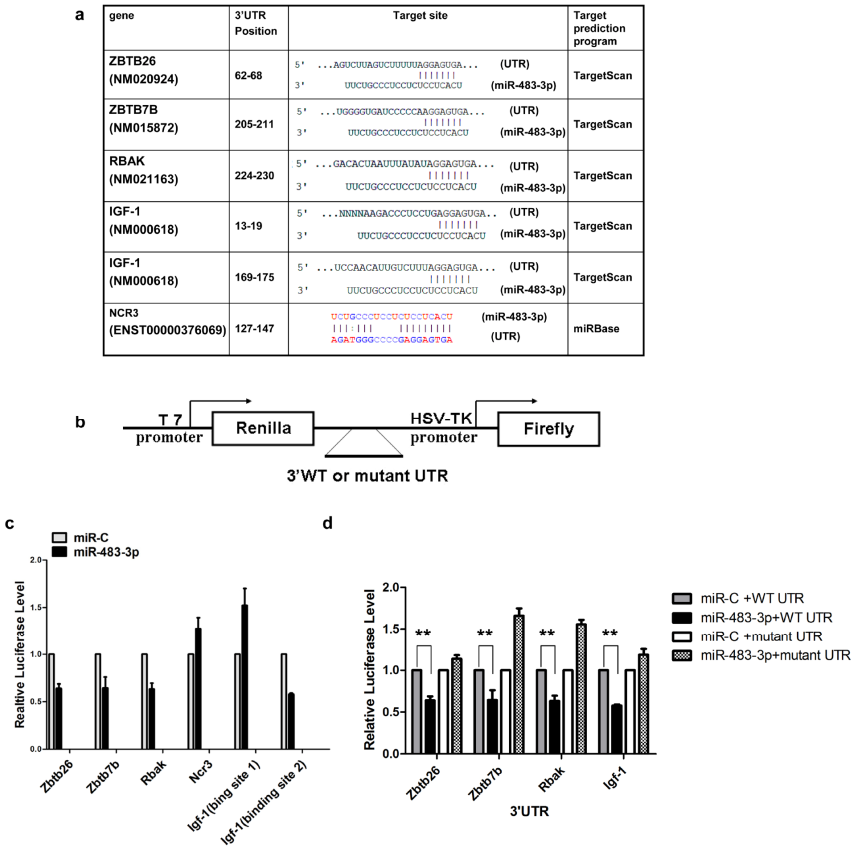
Supplementary Figure S1 IL-7, IL-12 and IGF-2 only slightly enhanced NK cell expansion from human CD34$^+$ HSCs. (a) CD34$^+$ cells were isolated from CBMCs with the MACS isolation system according to the manufacturer's instructions. The purified cells were stained with PE conjugated anti-CD34 or isotype-matched negative control antibodies labeled with PE and analyzed by flow cytometry. **(b)** Fold expansion of UCB CD34$^+$ cells after 4 weeks of culture with different cytokine combinations. **(c)** Flow cytometry analysis of the relative ratio of NK cells cultured with different cytokine combinations at day 28. Data are representative of three independent experiments.



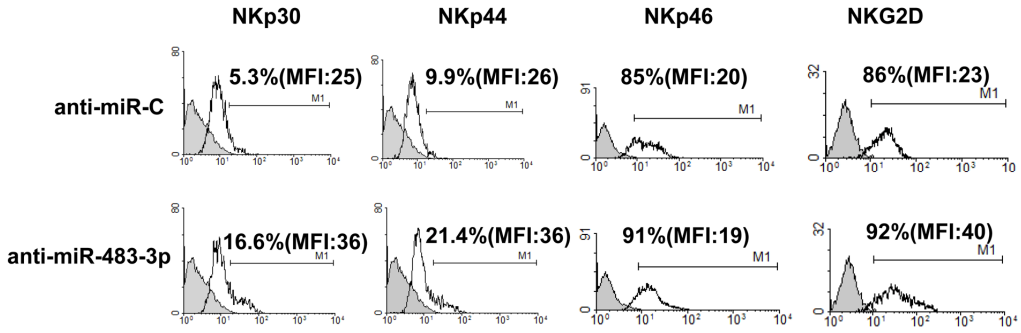
Supplementary Figure S2 Phenotypic features of cytokine-differentiated CD34$^+$ cells. Flow cytometry for expression of CD16, NKp30, NKp44, NKG2D, and CD69 in human UCB-CD34$^+$ derived CD3$^-$CD56$^+$ NK cells cultured for 28 days in the presence (black line) or absence (green line) of IGF-1. The gray histogram represents the isotype-matched negative controls. Data are representative of three independent experiments.



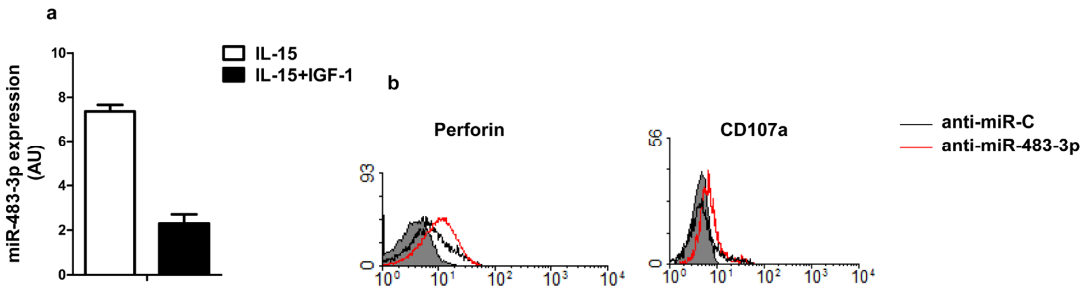
Supplementary Figure S3 Activation markers of human dNK cells treated with IGF-1. The purified decidual NK cells were treated with IGF-1 (100 ng/ml) for 24 h or 48 h, then cells were stained for NKp30, NKp44, NKp46, NKG2D, CD16, and CD69, and analyzed by flow cytometry. Fluorochrome-conjugated, isotype-matched mAb from the same manufacturers were used to measure background fluorescence. Freshly isolated dNK cells were used as controls for antigen expression. The percentage of cells staining positively for each given antigen is indicated in the dot plots. Data are representative of three independent experiments.



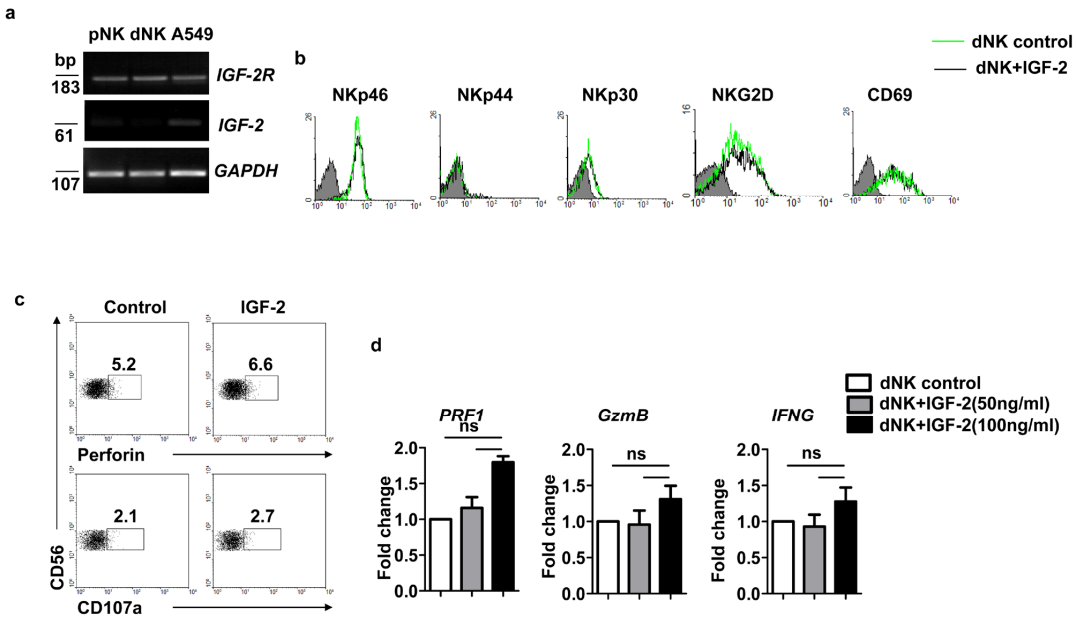
Supplementary Figure S4 Targets prediction of miR-483-3p and verification by reporter gene system. **(a)** The 3'UTRs of ZBTB26, ZBTB7B, RBAK, NCR3 and IGF-1 mRNA contain binding sites of miR-483-3p. **(b)** The 3'UTR reporters in target validation. WT, wild-type. The mutant UTR has the deletion of the miR-483-3p target site. **(c)** Dual-luciferase activity in lysates of HEK293T cells transfected with the 3'UTR constructs for the genes in **a**., along with either the negative control (miR-C), or the miR-483-3p mimic (miR-483-3p). **(d)** Dual-luciferase assay (as in **c.**) of HEK293T cells transfected with luciferase constructs containing wild-type 3′UTR (3'WT UTR) or mutated 3′UTR (3'mutant UTR) (with deletion of the miR-483-3p-responsive element) from ZBTB7B, ZBTB26, RBAK or IGF-1, plus miRNAs (as in **c.**). Data are shown as the mean ± SEM (**c-d**). $^{**}P < 0.01$.



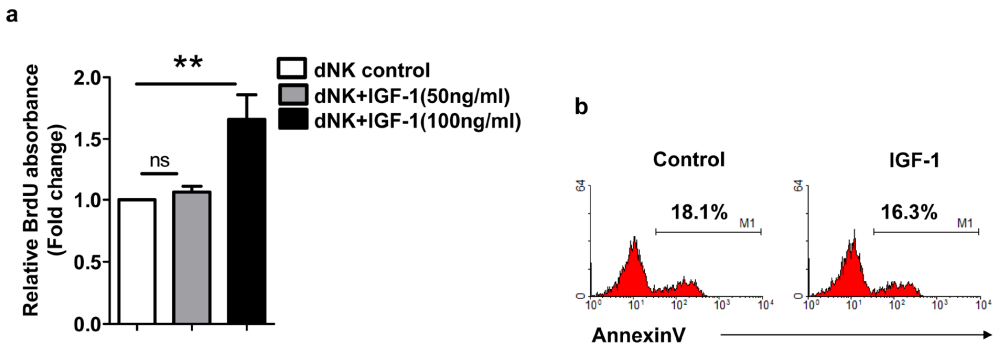
Supplementary Figure S5 Phenotypic features of human dNK cells transfected with miR-483-3p inhibitor. Flow cytometry analysis of NKp30, NKp44, NKp46 and NKG2D expression in human purified dNK cells transfected with control inhibitor (anti-miR-C) or mir-483-3p inhibitor (anti-miR-483-3p).The data are representative of three independent experiments.



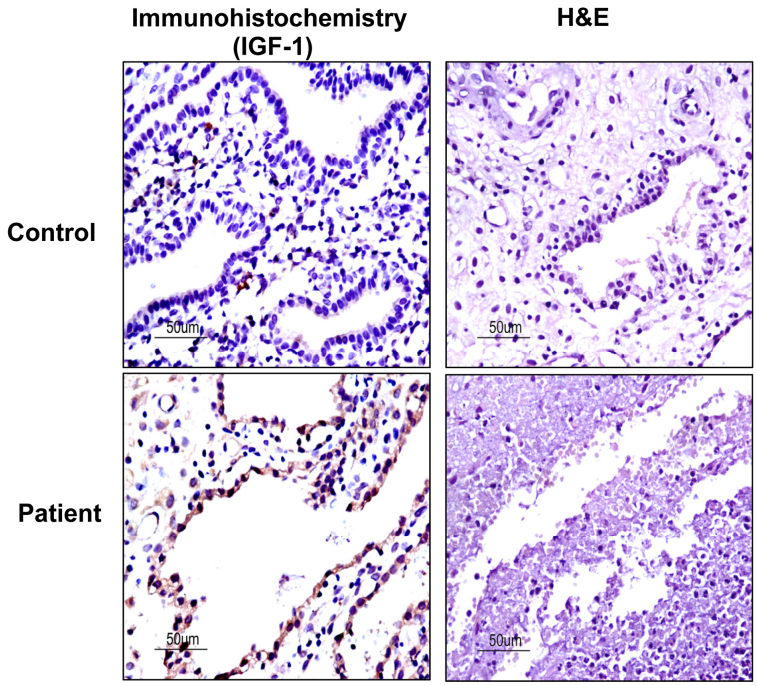
Supplementary Figure S6 miR-483-3p knockdown promotes the cytotoxic potential of human CD34$^+$ -derived NK cells. (a) Quantitative RT-PCR analysis of miR-483-3p expression in IL-15-differentiated human CD34$^+$ cells cultured for 28 days in the presence or absence of IGF-1. **(b)** Flow cytometry for expression of perforin and CD107a in miR-483-3p inhibitor (anti-miR-483-3p) / control miRNA (anti-miR-C) -transfected NK cells derived from UCB/CD34$^+$ HSCs. The gray histogram represents the isotype-matched negative controls. The data are representative of three independent experiments.



Supplementary Figure S7 IGF-2 has no significant effect on dNK cell phenotype and cytotoxic potential. **(a)** The products of qRT-PCR analysis of *IGF-2* and *IGF-2R* in purified pNK cells and dNK cells detected by agarose electrophoresis. A549 cells served as a positive control. **(b)** The purified dNK cells were treated with IGF-2 (100 ng/ml) for 24 h or left untreated, and then the cells were stained for NKp30, NKp44, NKp46, NKG2D, CD69 and analyzed by flow cytometry. **(c)** Flow cytometry for perforin and CD107a of purified dNK cells treated with IGF-2 (100 ng/ml) for 24 h or left untreated. **(d)** Relative *PRF1, GzmB* and *IFNG* expression levels in purified dNK cells cultured for 24 h with IGF-2 under indicated concentrations, as quantified by qRT-PCR. The data are representative of three independent experiments.



Supplementary Figure S8 IGF-1 promotes the proliferation of NK cells. (a) The proliferation of isolated decidual NK cells treated with IGF-1 at the indicated concentrations or left untreated for 24 hours was measured by BrdU proliferation ELISA. **(b)** Flow cytometry analysis of cell apoptosis in dNK cells treated with IGF-1 (100 ng/ml) for 24 hours or left untreated. The data are representative of three independent experiments. $^{**}P < 0.01$ (Student's *t*-test).



Supplementary Figure S9 Histology of paraffin sections of human decidual tissues.

Decidual tissues were isolated from patients who underwent spontaneous abortions and age-matched healthy controls, then fixed and paraffin embedded. Sections (5μm) were stained with H&E to assess the tissue pathology. For immunohistochemical analysis, sections were deparaffinized and subsequently exposed to a 0.3% hydrogen peroxide–methanol solution to quench the endogenous peroxidase activity before staining with IGF-1-specific mAbs (Santa Cruz Biotechnology). H&E, hematoxylin and eosin. Scale bars, 50 μm.

Supplementary Table S1 Characteristics of patients and controls

	Control	Spontaneous abortion
Sample size	18	12
Age	24.3± 2.2	26.8 ± 1.9
Pregnant weeks	8 ± 3.4	9± 2.8
Number of abortion	2±0. 27	3.62±0.56
Drug treatment	-	-

Relevant information about human subjects recruited for this study. Sample size is total number of subjects; age is presented in years ± s.e.m.; '–' indicates 'not applicable