

Supplementary material

Table S1. Details of the NP, AK-RSA and URSA patients (** $P < 0.01$, *** P

< 0.001 , **** $P < 0.0001$, NS, not significant).

Parameters	NP	AK-RSA	URSA	P Value		
Maternal age (y)	31.04 ± 0.8220, n=24	32.19 ± 0.5494, n=16	30.35 ± 0.9812, n=17	NP vs URSA $P = 0.05933$	AK-RSA vs URSA $P = 0.1189$	NP vs AK-RSA $P = 0.3063$
Gestation age (weeks)	6.58 ± 0.2030, n=24	6.73 ± 0.4179, n=16	6.60 ± 0.3748, n=17	NP vs URSA $P = 0.9615$	AK-RSA vs URSA $P = 0.8104$	NP vs AK-RSA $P = 0.7152$
Number of miscarriage	0.0 ± 0.0, n=24	1.44 ± 0.1281, n=16	2.65 ± 0.2090, n=17	NP vs URSA $P < 0.0001$	AK-RSA vs URSA $P < 0.0001$	NP vs AK-RSA $P < 0.0001$
Number of live births	1.33 ± 0.0982, n=24	0.06 ± 0.0625, n=16	0.35 ± 0.2090, n=17	NP vs URSA $P < 0.0001$	AK-RSA vs URSA $P = 0.2043$	NP vs AK-RSA $P < 0.0001$
Number of pregnancies	3.04 ± 0.2039, n=24	1.62 ± 0.1797, n=16	3.056 ± 0.2775, n=17	NP vs URSA $P = 0.9596$	AK-RSA vs URSA $P < 0.0001$	NP vs AK-RSA $P < 0.0001$

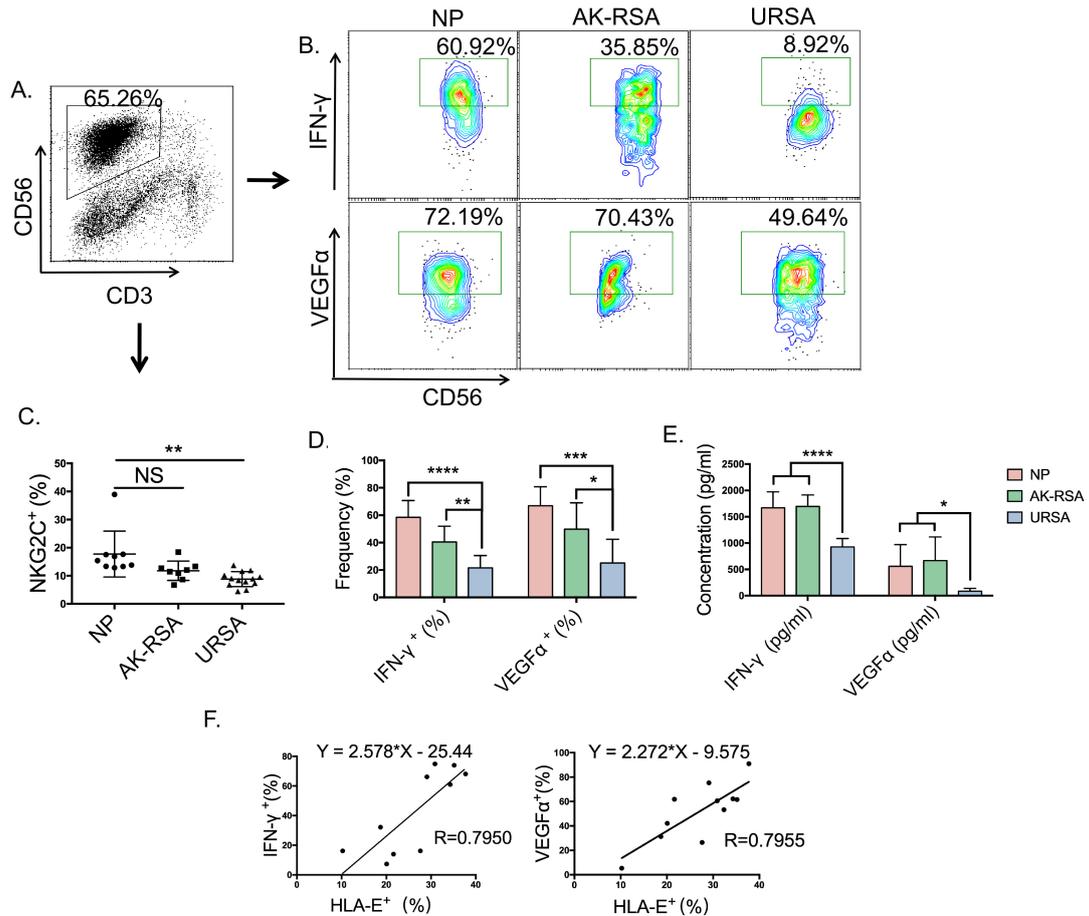


Figure S1. The secretion of IFN- γ and VEGF α by dNK cells from URSA donors decreased and was positively correlated with the levels of HLA-E in T-EVs.

A. B. dNK cells were purified from decidual tissues from NP, AK-RSA or URSA patients and seeded into 96-well plates at 1×10^5 cells/well in complete medium containing 50 ng/ml IL-15 for 48 h. Intracellular expression of IFN- γ and VEGF α in CD3⁻CD56⁺ dNK cells obtained from NP, AK-RSA or RSA patients was detected by FCM. **C.** The expression of NKG2C in CD3⁻CD56⁺ dNK cells obtained from NP, AK-RSA or RSA patients was detected by FCM and statistically analyzed. **D.** Intracellular expression of IFN- γ and VEGF α in

CD3⁻CD56⁺ dNK cells obtained from NP, AK-RSA or RSA patients was statistically analyzed. **E.** Supernatants of the dNK cells in **B.** were evaluated by ELISA, and the results were statistically analyzed. **F.** The correlations between the HLA-E level in T-EVs and intracellular IFN- γ and VEGF α expression in dNK cells were analyzed by Spearman correlation analysis using GraphPad Prism 6 (n=11). P values were generated by one-way analysis of variance (ANOVA) followed by the Newman-Keuls multiple comparison test using GraphPad Prism 6 (n=11 in NP group; n=11 in AK-RSA patient group; n=8 in URSA patient group, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$, NS, not significant).

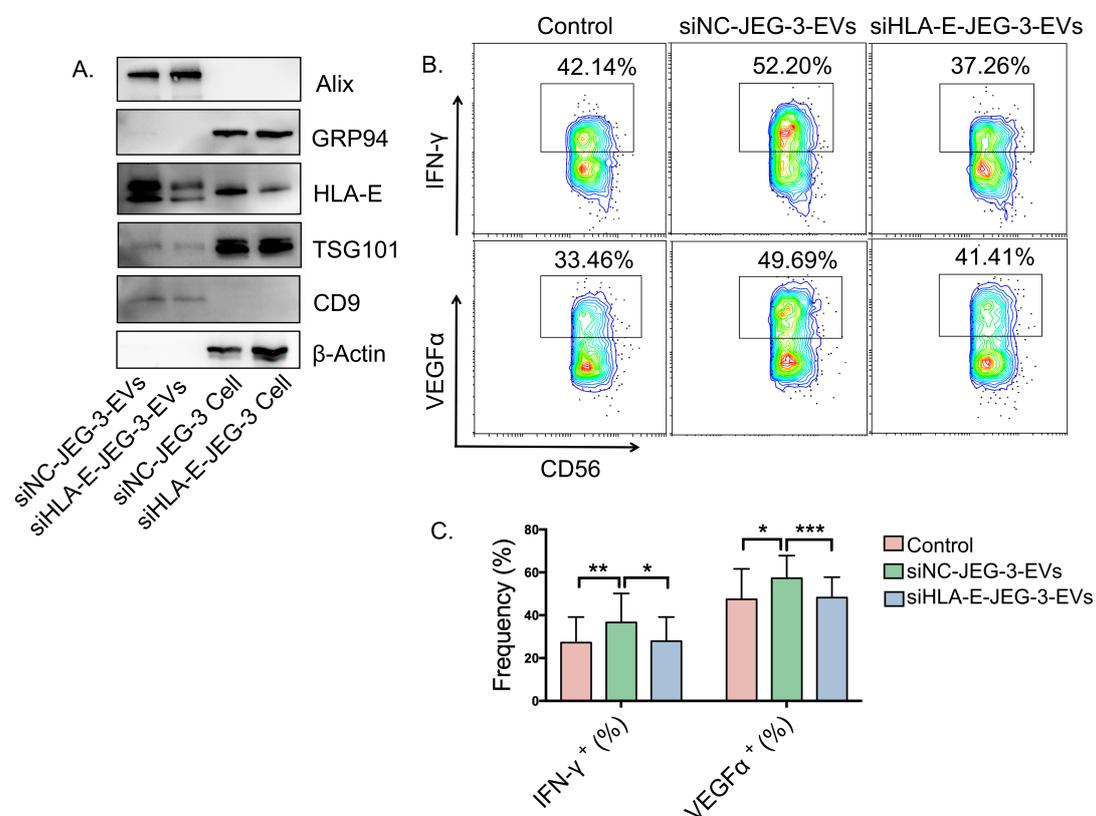


Figure S2. Exosomes from JEG-3 cells promote the secretion of IFN- γ

and VEGF α by dNK cells via HLA-E in vitro.

A. Western blot analysis of HLA-E, Alix, GRP94, TSG101 and CD9 in exosomes and cell lysates derived from negative control siRNA-treated JEG-3 cells (siNC-JEG-3) and HLA-E-specific siRNA-treated JEG-3 cells (siHLA-E-JEG-3). **B.** The intracellular expression of IFN- γ and VEGF α in dNK cells from URSA patients was detected by FCM and **C.** statistically analyzed. P values were generated by one-way analysis of variance (ANOVA) followed by the Newman-Keuls multiple comparison test using GraphPad Prism 6 (n=7, * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$; NS, not significant).

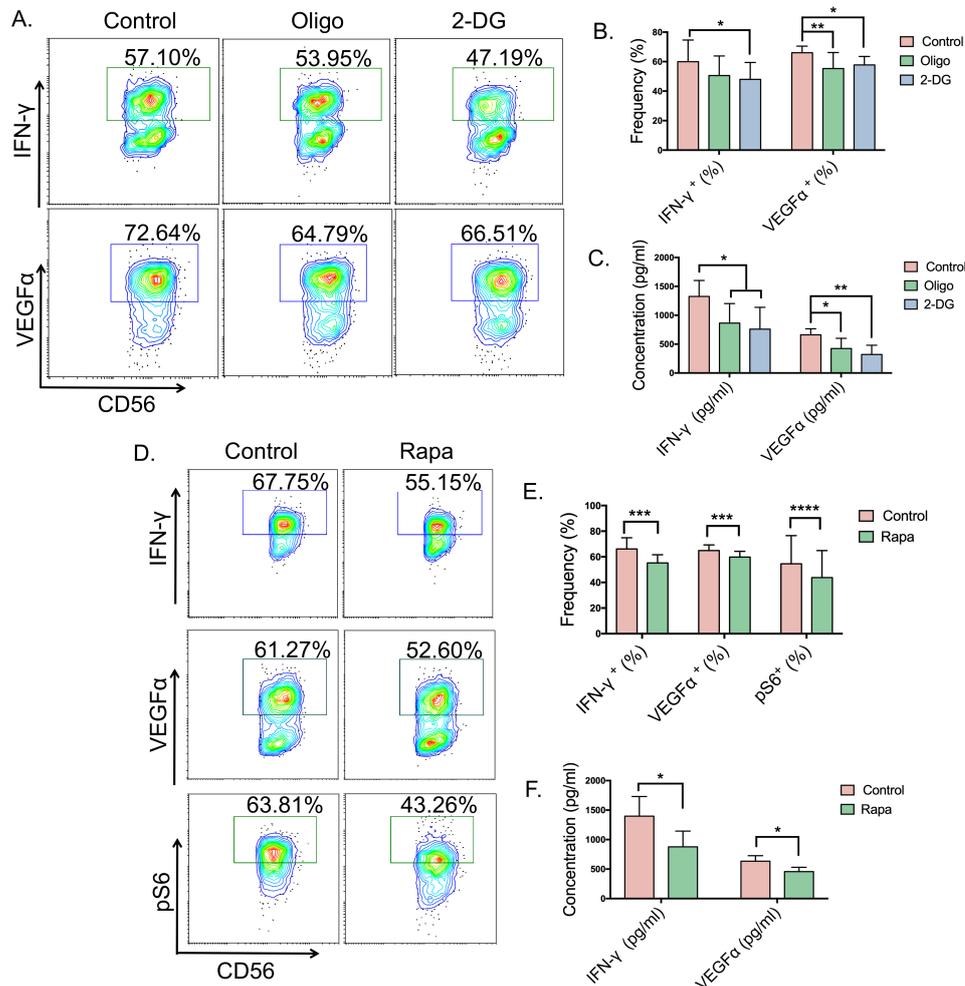


Figure S3. The secretion of IFN- γ and VEGF α by dNK cells from NP donors was dependent on cellular metabolism.

A. Intracellular expression of IFN- γ and VEGF α in dNK cells from NP donors treated with or without the glycolytic inhibitor 2-DG (1 mM) or ATP synthase inhibitor oligomycin (20 μ M) was detected by FCM and statistically analyzed in **B. C.** Supernatants of the dNK cells in **a.** were detected by ELISA, and the results were statistically analyzed. **D.** Intracellular staining for pS6, IFN- γ and VEGF α in dNK cells treated with 10 nM mTORC1 inhibitor (rapamycin) was evaluated by FCM and statistically analyzed in **E. F.** Supernatants of the dNK cells in **D** were detected by ELISA, and the results were statistically analyzed. The data are representative of three independent experiments or are shown as the mean \pm s.e.m. pooled from three independent experiments. P values were generated by one-way analysis of variance (ANOVA) followed by the Newman-Keuls multiple comparison test using GraphPad Prism 6 (n=14, * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$; NS, not significant).