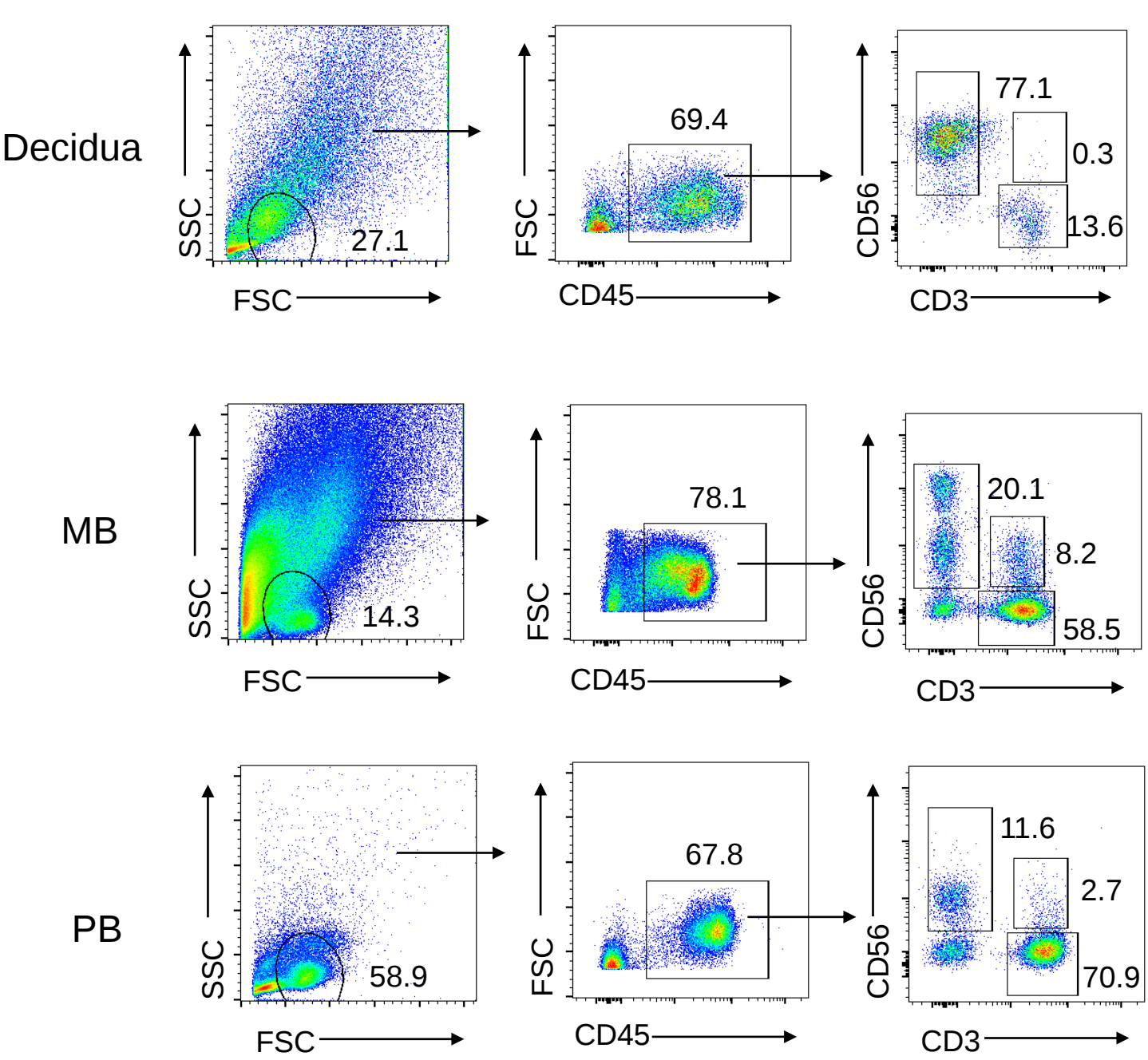


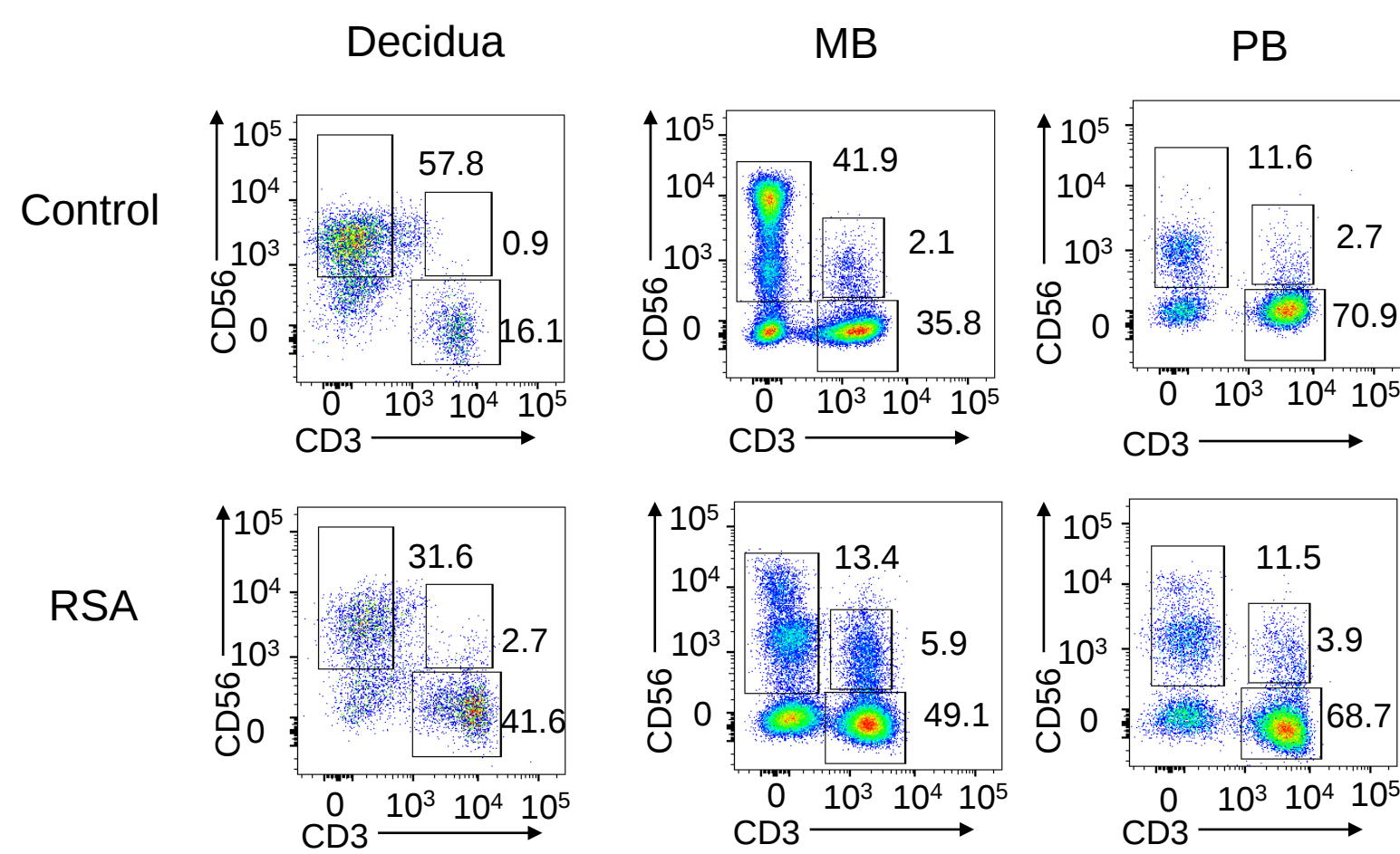
SFigure 1.



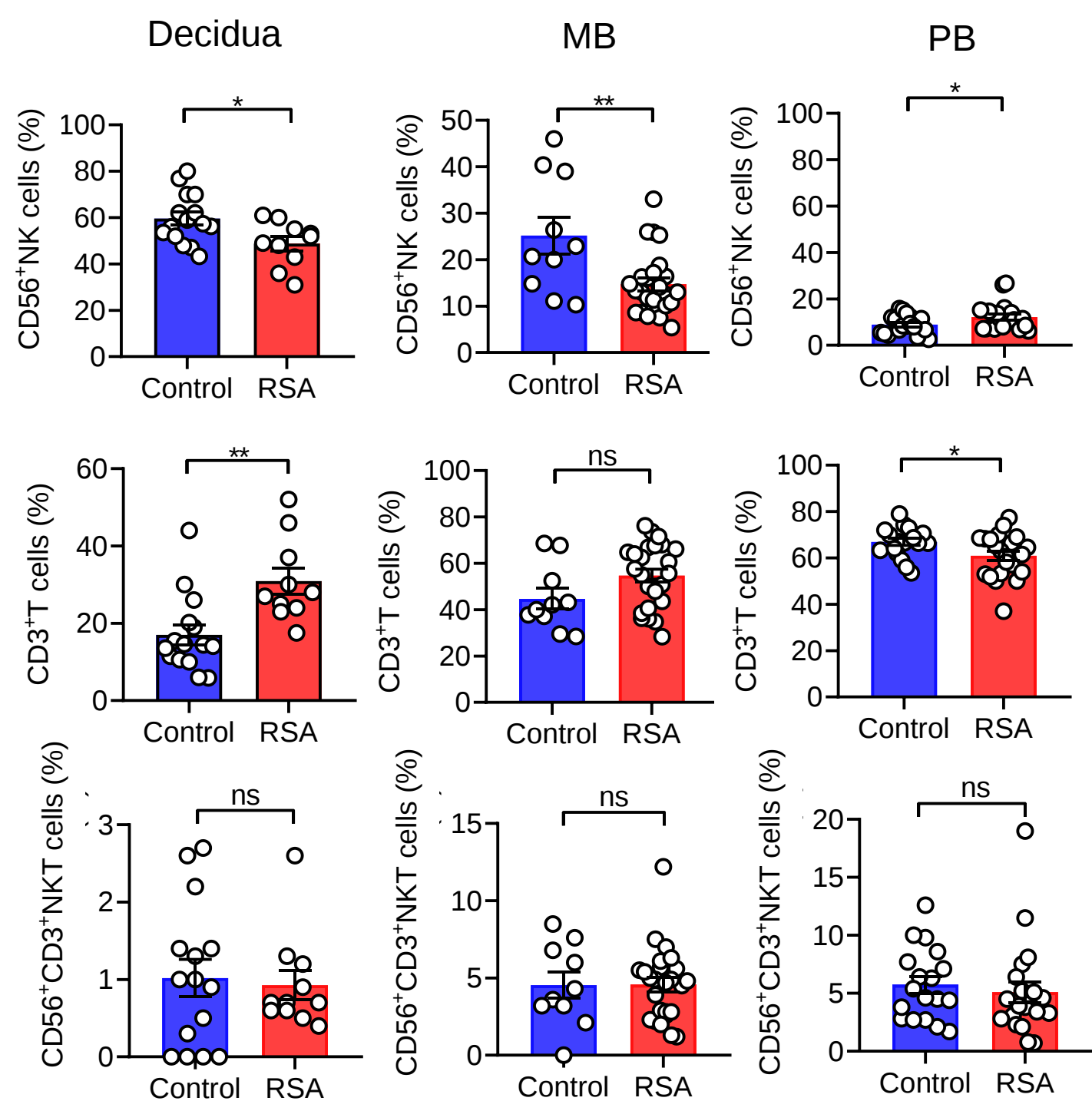
SFig1. Representative flow cytometry dot plots of decidua, PB and MB cells. Initial gating was gated on FSC/SSC to determine CD45⁺lymphocyte. CD56⁺NK cells, CD3⁺T cells and C56⁺CD3⁺NKT cells were gated on CD45⁺ lymphocyte.

SFigure 2.

A.



B.

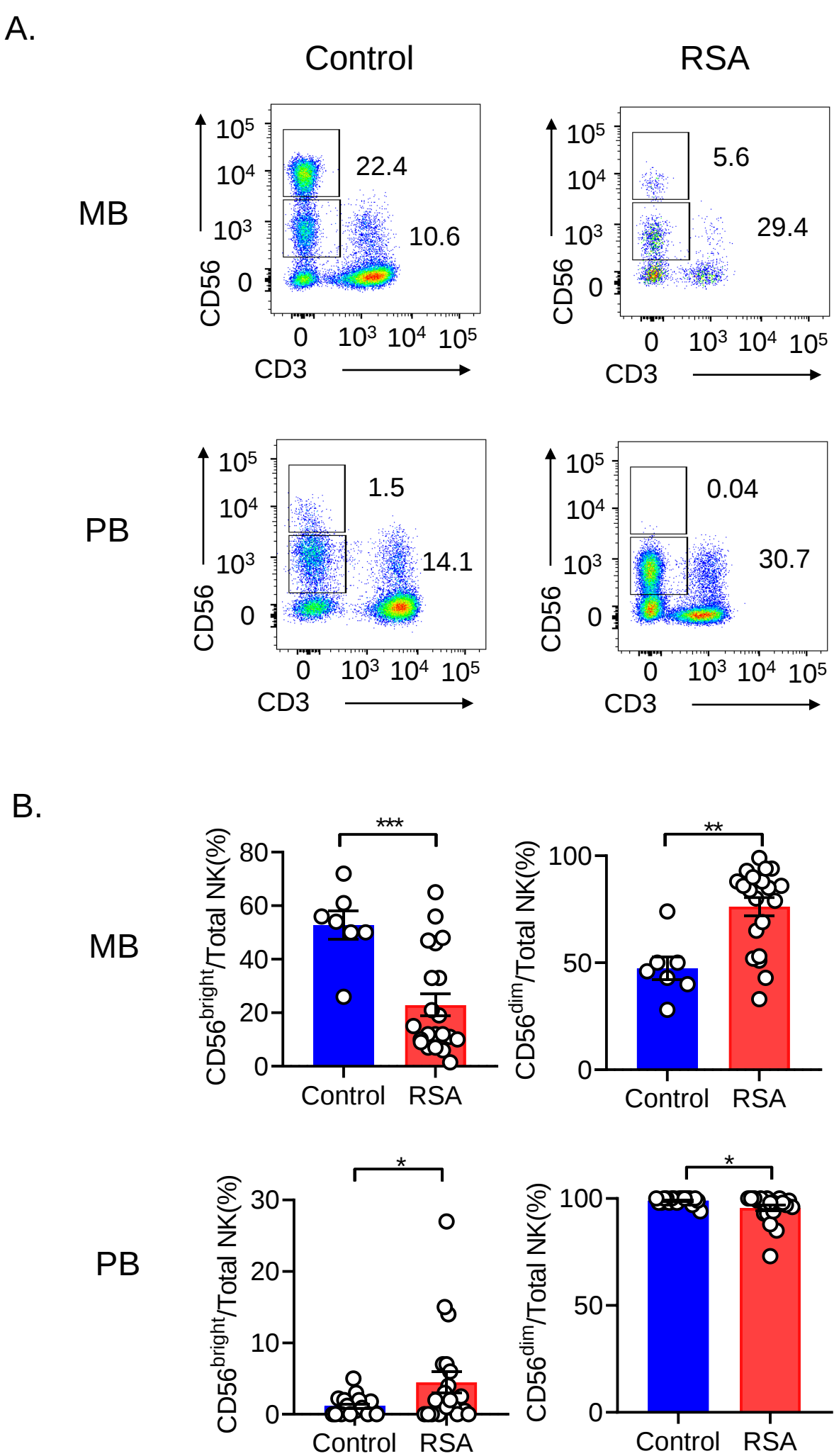


SFig.2. The percentage of NK cells from both MB and decidua but not PB decreased in RSA patients compared with normal controls.

(A) Representative dot plots showing an analysis of NK cells (CD3⁻CD56⁺), T cells (CD3⁺CD56⁻), NKT cells (CD56⁺CD3⁺) expressions in gated CD45⁺ leukocyte cells isolated from decidua, MB and PB isolated from normal controls and RSA patients.

(B) Total percentages of CD3⁻CD56⁺ NK cells , CD3⁺CD56⁻ T cells, CD56⁺CD3⁺NKT cells based on CD45⁺leukocyte of decidua , MB and PB. n = 15, 10 and 18 for decidua, MB and PB respectively in control group. n =10, 25 and 22 for decidua, MB and PB respectively in RSA group. Use the unpaired T test, *P< 0.05 , **P<0.01 , ***P<0.001, ****P<0.0001.

SFigure.3.



Sfig. 3 . Expression of CD56^{bright} ,CD56^{dim}NK in MB and PB.

(A) Flow cytometry of CD56^{bright},CD56^{dim}NK on gated CD56⁺CD3⁻NK cells isolated from MB and PB.

(B) Total percentages of CD56^{bright} NK cells, CD56^{dim}NK cells,CD56^{bright}/ Total NK cells, CD56^{dim}/Total NK cells of MB and PB. n = 7 ,18 for MB and PB respectively in control group, MB and PB were obtained from the same woman (n=4). n =21, 21 for MB and PB respectively in RSA group , MB and PB were obtained from the same woman (n=21). Use the unpaired T test, *P < 0.05 ,**P < 0.01 ,***P < 0.001, ****P < 0.0001.

STable 1. Comparison of clinical data between RSA patients and normal controls (Menstrual blood samples)

Group	Age (years)	Number of pregnancies	Number of children	Number of spontaneous abortion
Control (N=10)	31.0±2.49	1.51±0.53	1.40±0.52	-
RSA (N=25)	30.2±5.92	3.52±1.67	0.36±0.57	2.64±0.95

STable 2. Comparison of clinical data between RSA patients and normal controls (decidual samples)

Group	Age (years)	Number of pregnancies	Number of children	Number of spontaneous abortion	Pregnancy (days)
Control (N=15)	30.1±5.71	3.0±1.60	0.87±0.74	-	46.5±4.5
RSA (N=10)	30.6±2.70	4.0±1.80	0.60±0.52	2.2±0.42	73.1±15.1

STable 3. AUC , sensitivity , specificity , Accuracy , PPV and NPV for NK cell subsets, total NK cell percentages in menstrual samples.

NK cell	AUC (95%CI)	Accuracy (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Cut-off value(%)
CD49a ⁺ EOMES ⁺	0.92(0.83-1.00)	87.9%	91.3%	80%	91.3%	80%	32.1%
CD56 ⁺ CD49a ⁺	0.87(0.74-1.00)	85.3%	83.3%	90%	95.1%	69.2%	56.8%
CD49a ⁺ CD16 ⁻	0.85(0.72-0.98)	81.8%	78.3%	90%	94.7%	64.3%	48.2%
CD56 ⁺ CD16 ⁺	0.83(0.68-0.98)	77.1%	72%	90%	94.7%	56.2%	39.3%
CD3 ⁻ CD56 ⁺	0.77(0.59-0.95)	80%	84%	70%	87.5%	63.6%	19.4%

STable. 4. Resource table:

Antibodies	SOURCE	IDENTIFIER
Anti-human CD45 BV510	Biolegend	Cat No:368526
Anti-human CD45 APC-Cy7	BD	Cat No:557833
Anti-human CD56 BV421	Biolegend	Cat No:318328
Anti-human CD56 BV605	Biolegend	Cat No:362538
Anti-human CD3 BV605	Biolegend	Cat No:344836
Anti-human CD3 PerCP-Cy5.5	Biolegend	Cat No:300328
Anti-human CD16 PerCP-Cy5.5	Biolegend	Cat No:302028
Anti-human CD49a Alexa Fluor 647	Biolegend	Cat No:328310
Anti-human EOMES PE	eBioscience	Cat No:12-4877-42
Isotype		
Mouse IgG1, κ BV510	Biolegend	Cat No:400171
Mouse IgG1, κ BV421	Biolegend	Cat No:400157
Mouse IgG1, κ BV605	Biolegend	Cat No:400161
Mouse IgG2a, κ PerCP-Cy5.5	Biolegend	Cat No:400251
Mouse IgG1, κ PerCP-Cy5.5	Biolegend	Cat No:400149
Mouse IgG1, κ Alexa Fluor 647	Biolegend	Cat No:400130
Mouse IgG1, κ APC-Cy7	BD	Cat No:557873
Mouse IgG1, κ Alexa Fluor 647	BD	Cat No:555751
Mouse BALB/c IgM FITC	BD	Cat No:555583
Mouse BALB/c IgG2b, κ PE	BD	Cat No:555743
Mouse BALB/c IgG1, κ PE	BD	Cat No:559320
Mouse IgG ₁ FITC	R&D	Cat No:110416
Mouse IgG1 K PE	eBioscience	Cat No:12-4714-82
Mouse IgG2a K PE	eBioscience	Cat No:12-4724-82