Sildenafil Citrate Downregulates PDE5A mRNA Expression in Women with Recurrent Pregnancy Loss without Altering Angiogenic Factors—A Preliminary Study

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Characetristics	Value (range)
Age (years), mean (range)	36 (33-42)
Number of pregnancies – mean (range)	4.5 (2-7)
• number of miscarriages – mean (range)	3.09 (2-5)
Vitamin D (ng/ml)	32 (16.6-52.8)
AMH (ng/ml)	5.5 (1.9-12.4)
Homocysteine (mcmol/L)	9.38 (6.2-14.94)
Hashimoto's disease (number of patients)	n=1
Insulin resistance (number of patients)	n=3
Antiphospholipid syndrome (number of patients)	n=0
Thrombophilia (number of patients with confirmed thrombophilia)	
• Factor V Leiden pathogenic variant	n=0
• Prothrombin G20210A pathogenic variant	n=0
• Antithrombin deficiency (activity <60%)	n=0
• Protein S deficiency (functional assay <55%)	n=0
• Protein C deficiency (activity <65%)	n=0
• Antiphospholipid antibodies	n=0
MTHFR gene polymorphisms (number of patients with confirmed polymorphisms)	
• A1298C	n=4 (heterozygous)
• C677T	n=4 (heterozygous)
PAI-1 Gene Polymorphism (number of patients with confirmed polymorphisms)	
• 4G/5G	n=4 (heterozygous)

Table S1. Characteristics of RPL patients included to the study group.

a)
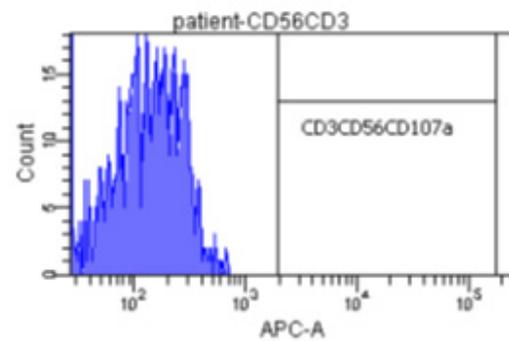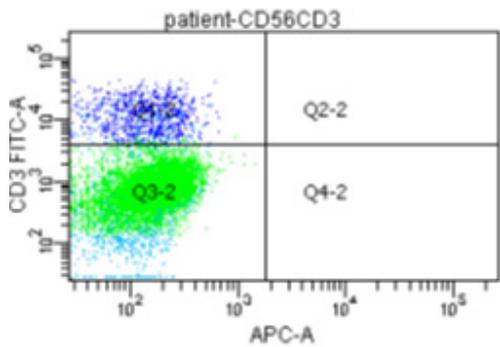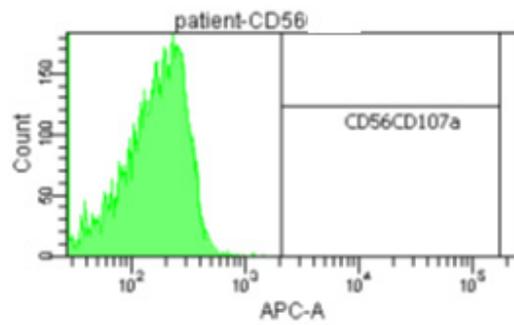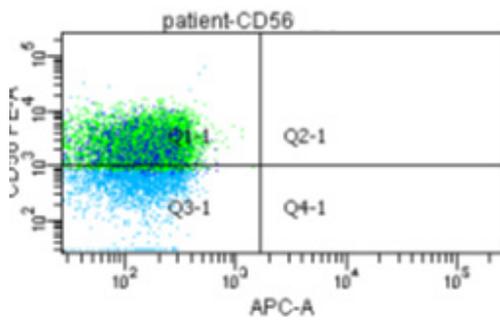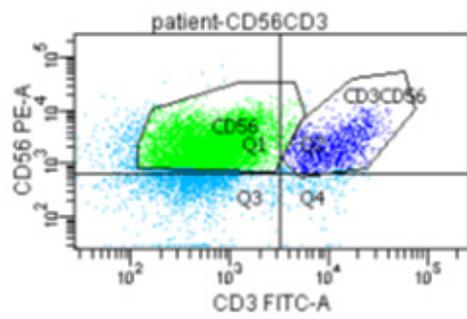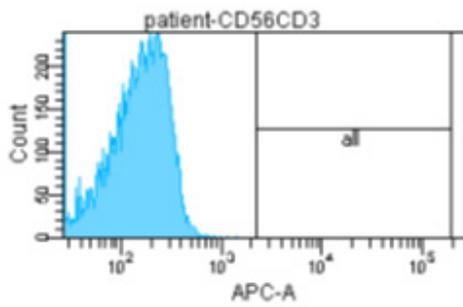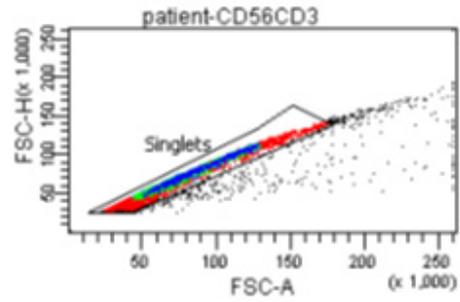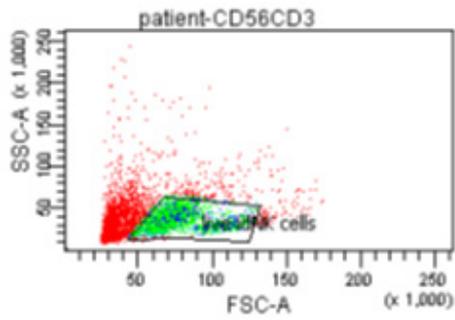
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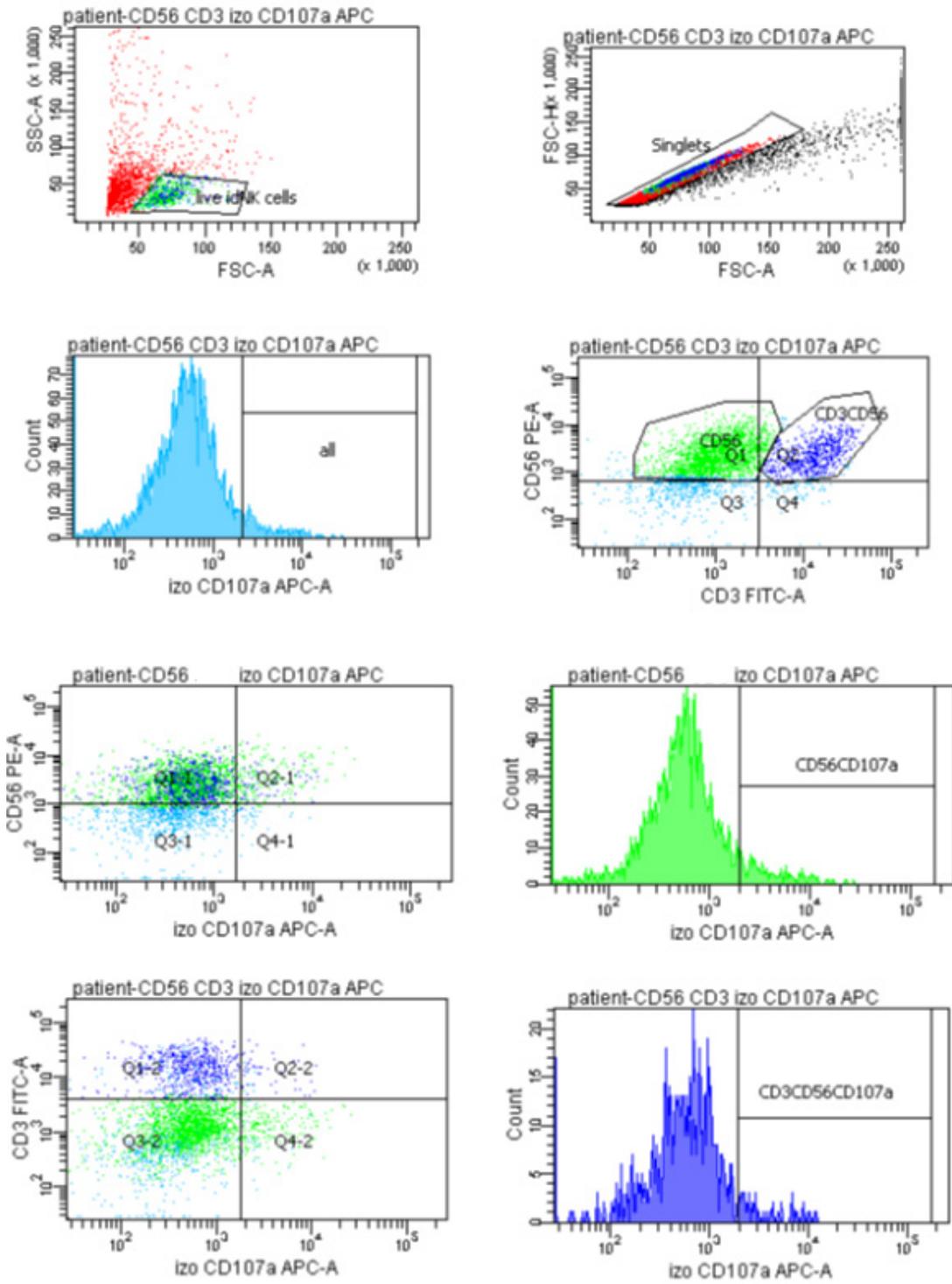
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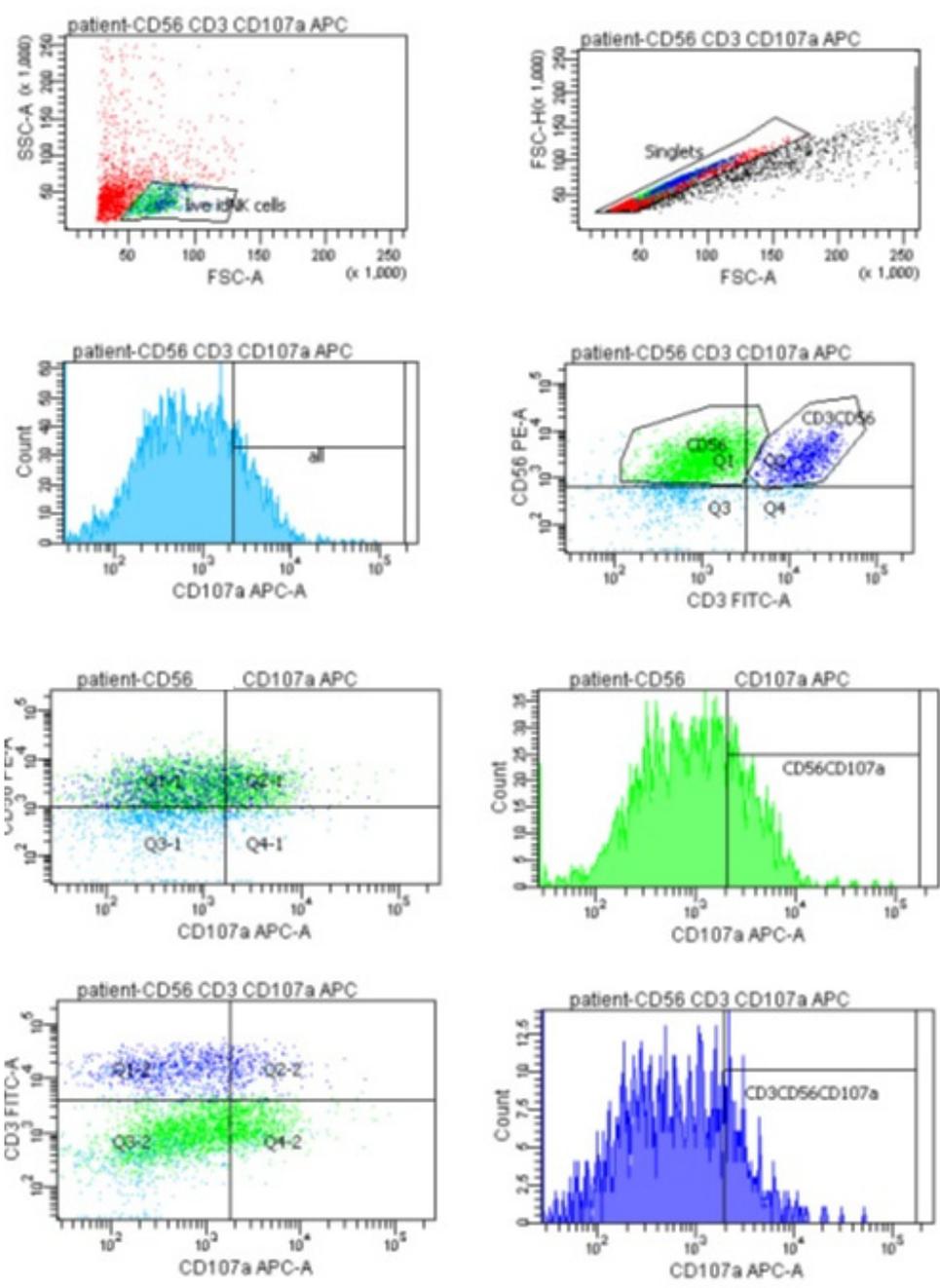
Figure S1. Gating strategy for CD107a expressing idNK cells (CD56$^+$CD3$^-$) and NKT cells (CD56$^+$CD3$^+$), a) CD3CD56 and CD56-positive cell gating, singlet distributions were determined on the FSC-H/FSC-A axis, lymphocytes were identified as 50-150 x 10^3 cells on FSC, CD56-positive cells were identified among lymphocytes with red PE – fluorescence above 10^3 on Y axis on a logarithmic scale, CD3CD56-positive cells were identified with 8x10^2 red PE-fluorescence on Y axis and 2x10^3 green FITC-fluorescence on X axis c) FMO control for CD107a-APC expressed on CD3-FITC and CD56-PE-positive cells after K562 stimulation, APC-negative cells were identified with APC fluorescence below 1x10^3 on a dot plot as well as on histograms for CD56 and for CD3CD56-positive cells, d) CD107a expression after activation with K562 cells on CD3 and CD56-positive cells, CD56-positive cells were identified with red PE-fluorescence above 10^3 on Y axis on a logarithmic scale, CD3CD56-positive cells were identified with 8x10^2 red PE-fluorescence on Y axis and 2x10^3 green FITC-fluorescence on X axis, CD56CD107a and CD3CD56CD107a APC-positive cells were identified with APC fluorescence above 1x10^3 on X axis on dot plots for CD56 and CD3CD56 cells as well as on histograms.
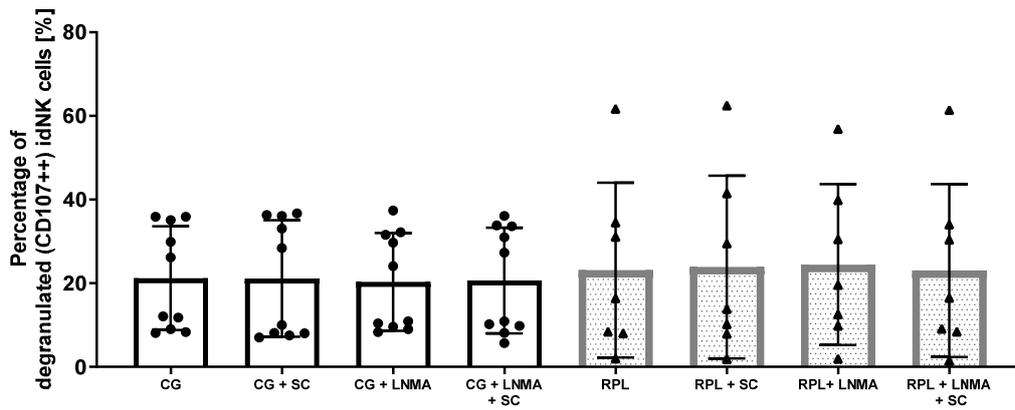
Figure S2. The effect of sildenafil on the degranulation (CD107a expression) of idNK cells determined after 5 days of culturing in transformation media and hypoxia, results are showed as the median and IQR, CG – control group, RPL – study group, 500µM L-arginine iNOS inhibitor (L-NMMA), SC – sildenafil citrate 400 ng/ml).