

Title

IFN- γ modulates Ly-49 receptors on NK cells in IFN- γ -induced pregnancy failure

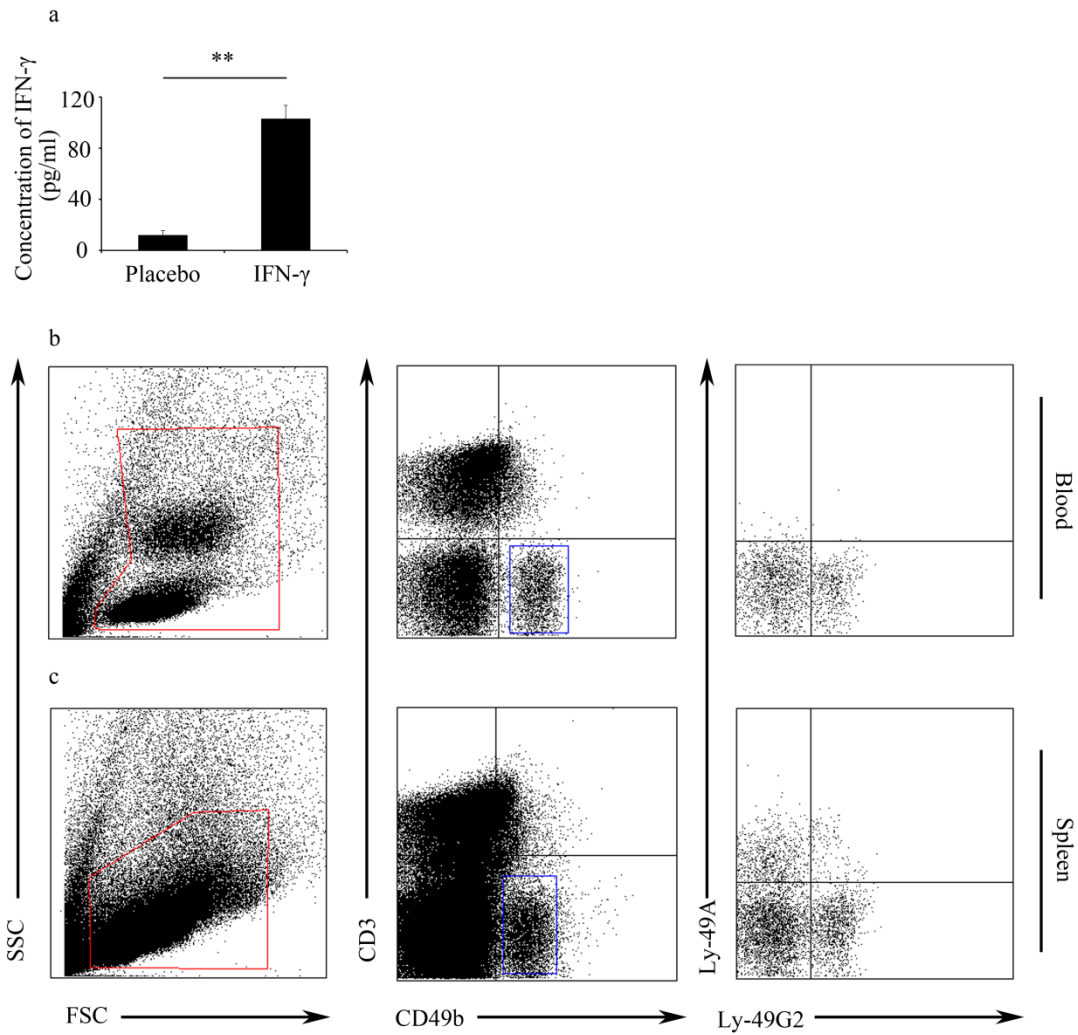
Authors

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Affiliations

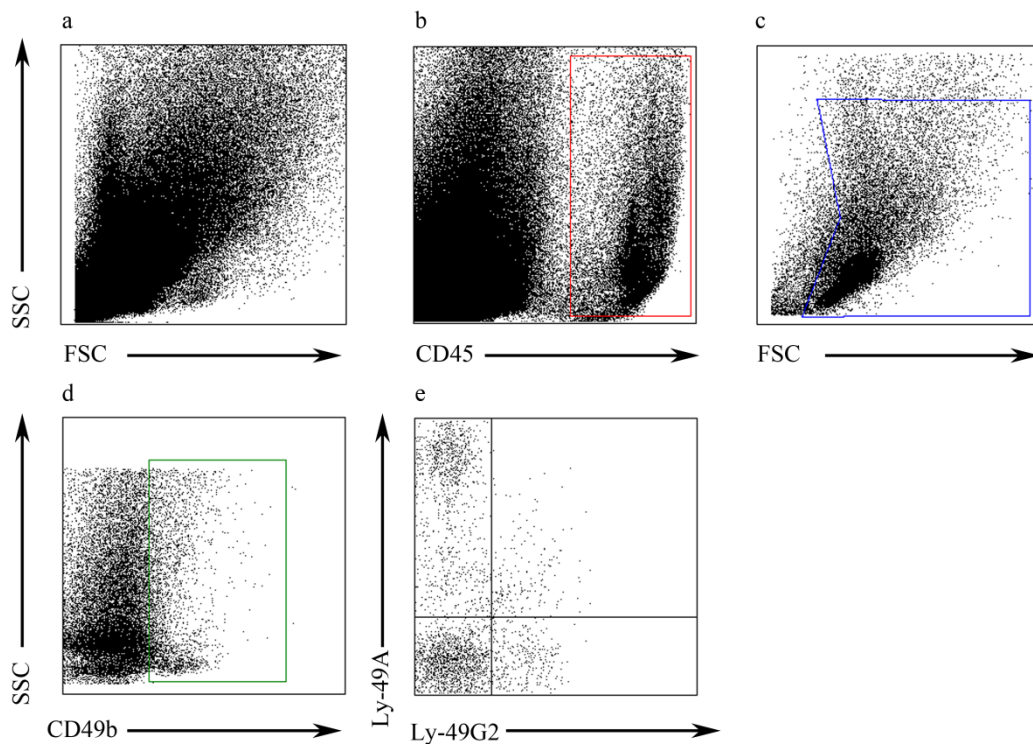
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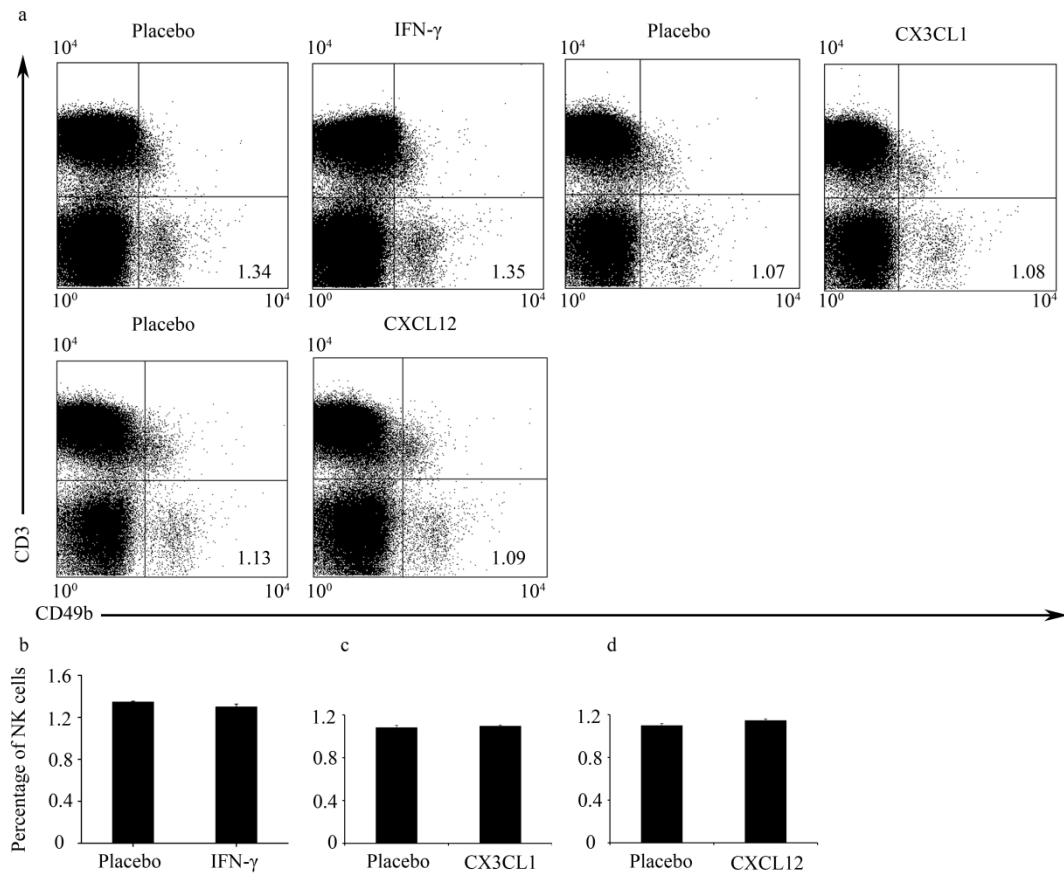


Supplementary Figure S1. The gating strategies to analyze the expression of Ly-49 receptors in the blood and spleen. (a) Syngeneically mated BALB/c females were injected with placebo or IFN- γ intraperitoneally on GD6 and sacrificed on GD8. IFN- γ concentration in the serum was determined by ELISA. Data show the mean \pm SEM of three independent experiments and are obtained from three mice of each group. **P<0.01 by independent samples T-test. (b) Dot plots shown are gating strategy to analyze the expression of Ly-49 receptors in the blood. Pan leucocytes are gated using FSC versus SSC (*left panel*) and NK cells are defined as CD3⁻CD49b⁺ (*middle panel*). Then, the expression of Ly-49 receptors is analyzed on CD3⁻CD49b⁺

NK cells (*right panel*). (c) Dot plots shown are gating strategy to analyze the expression of Ly-49 receptors in the spleen. Pan leucocytes are gated using FSC versus SSC (*left panel*) and NK cells are defined as $CD3^-CD49b^+$ (*middle panel*). Then, the expression of Ly-49 receptors is analyzed on $CD3^-CD49b^+$ NK cells (*right panel*). FSC, forward scatter; SSC, side scatter.



Supplementary Figure S2. The gating strategy to analyze the expression of Ly-49 receptors in the uterus. (a) Total cells are visualized using FSC versus SSC. Pan leucocytes are gated using anti-CD45 antibody versus SSC (b) and then back gate analysis of NK cells (c, d) is shown. NK cells are defined as $CD45^+CD49b^+$. Then, the expression of Ly-49 receptors is analyzed on $CD45^+CD49b^+$ NK cells (e). FSC, forward scatter; SSC, side scatter.



Supplementary Figure S3. IFN- γ , CX3CL1 and CXCL12 did not alter the percentage of CD3⁺CD49b⁺ NK cells in vitro. (a-d) Splenic leukocytes were prepared as described in Materials and Methods and then cultured with IFN- γ (250 U/ml) or CX3CL1 (250 ng/ml) or CXCL12 (500 ng/ml) for 24 h. (a) Representative flow cytometric analysis of the percentages of CD3⁺CD49b⁺ NK cells (lower-right quadrant) and numbers in dot plots indicate the percentages of CD3⁺CD49b⁺ NK cells. Data summary of the percentages of CD3⁺CD49b⁺ NK cells with IFN- γ (b), CX3CL1 (c) and CXCL12 (d) treatment. Data show the mean \pm SEM of four (IFN- γ), three (CX3CL1) or three (CXCL12) independent experiments, respectively.