Supplementary Information

Prenatal vitamin D deficiency alters immune cell proportions of offspring in young adult age

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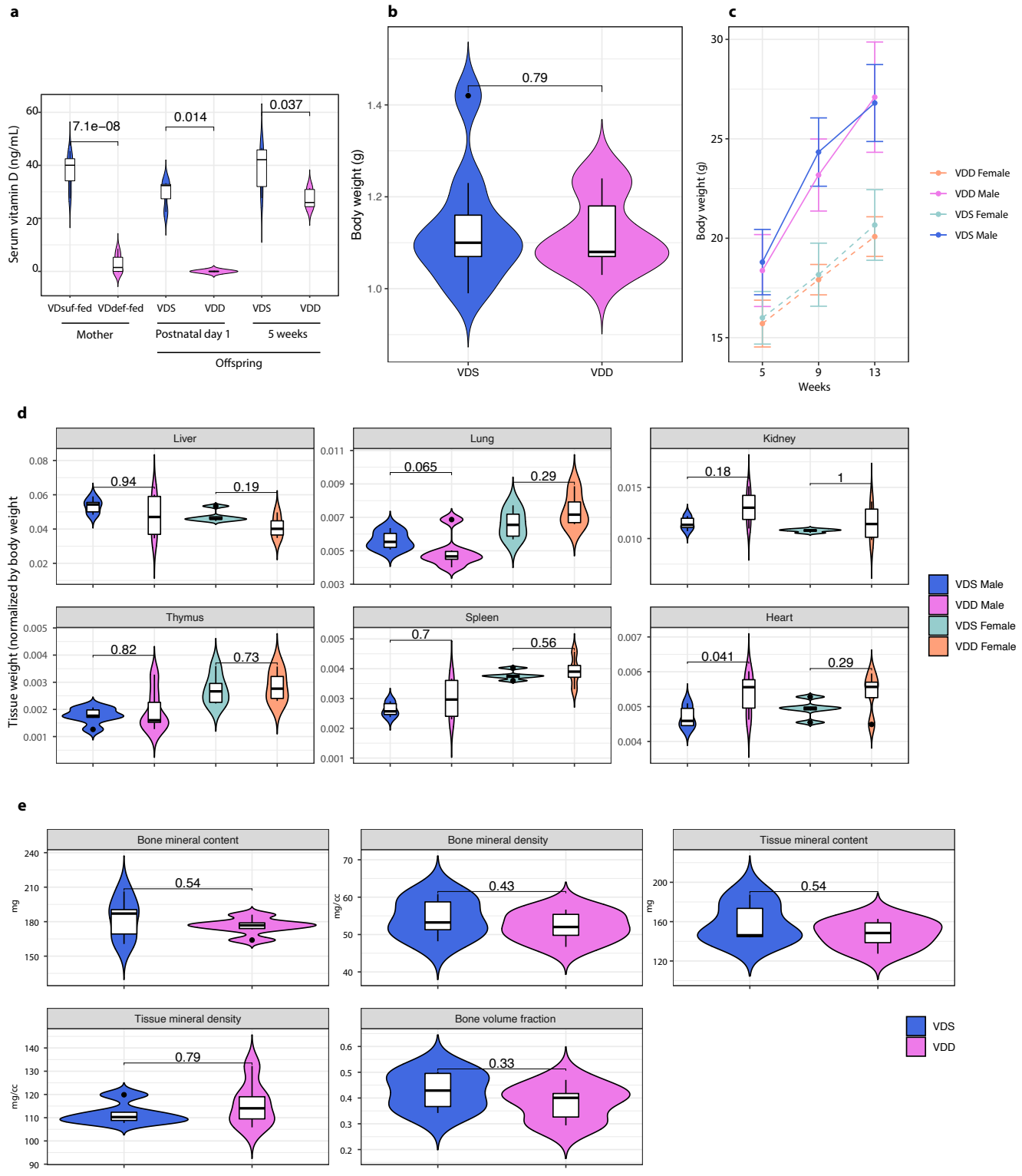
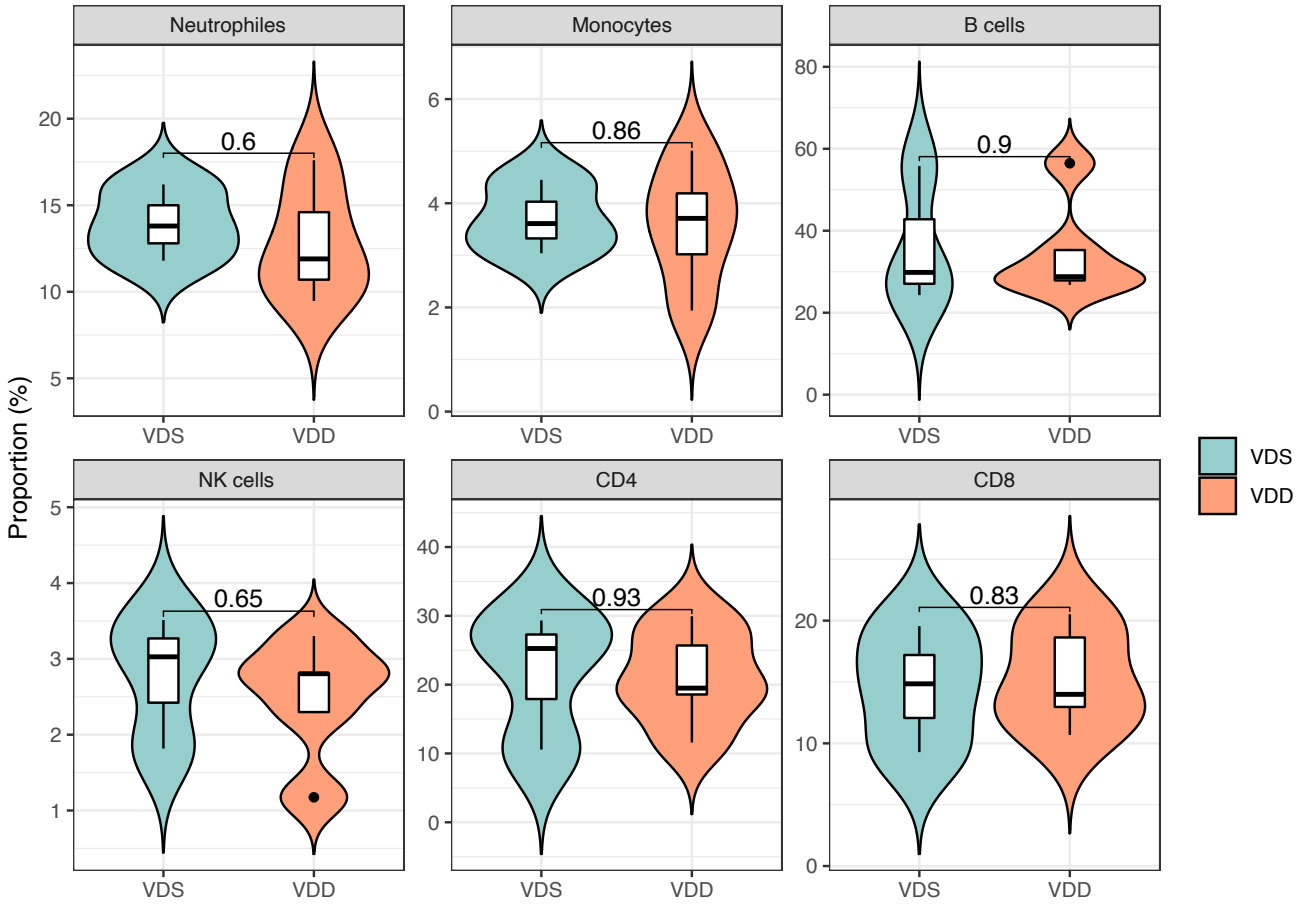
Supplementary Fig. 1



Supplementary Fig. 1: The effects of vitamin D deficient diet feeding on the mothers and the impacts of prenatal vitamin D deficiency on the growth and bone density of offspring at the adult stage.

a The deficient status of VDD mothers was confirmed by the serum vitamin D level of female mice (n=8 VDD mothers and n=10 VDS mothers) after five weeks of vitamin D-deficient diet feeding before the mating. The deficient status was maintained in the offspring at postnatal day 1 (n=3 per group). After weaning to the vitamin D sufficient diet, the vitamin D status of VDD offspring becomes sufficient at 5 weeks old (n=5 per group). **b** The body weight at postnatal day 1 was comparable between VDD and VDS offspring. **c** The growth was also comparable between VDD and VDS offspring in both males (n=28 VDD and n=15 VDS) and females (n=12 VDD and n=12 VDS). **d** The weight of each tissue was comparable in VDD (6 males and 4 females) and VDS (6 males and 5 females), except for the heart in males. **e** The micro CT analyses show that prenatal vitamin D deficiency does not affect the offspring's bone and tissue mineral status in adults (n=5 VDD and n=6 VDS).

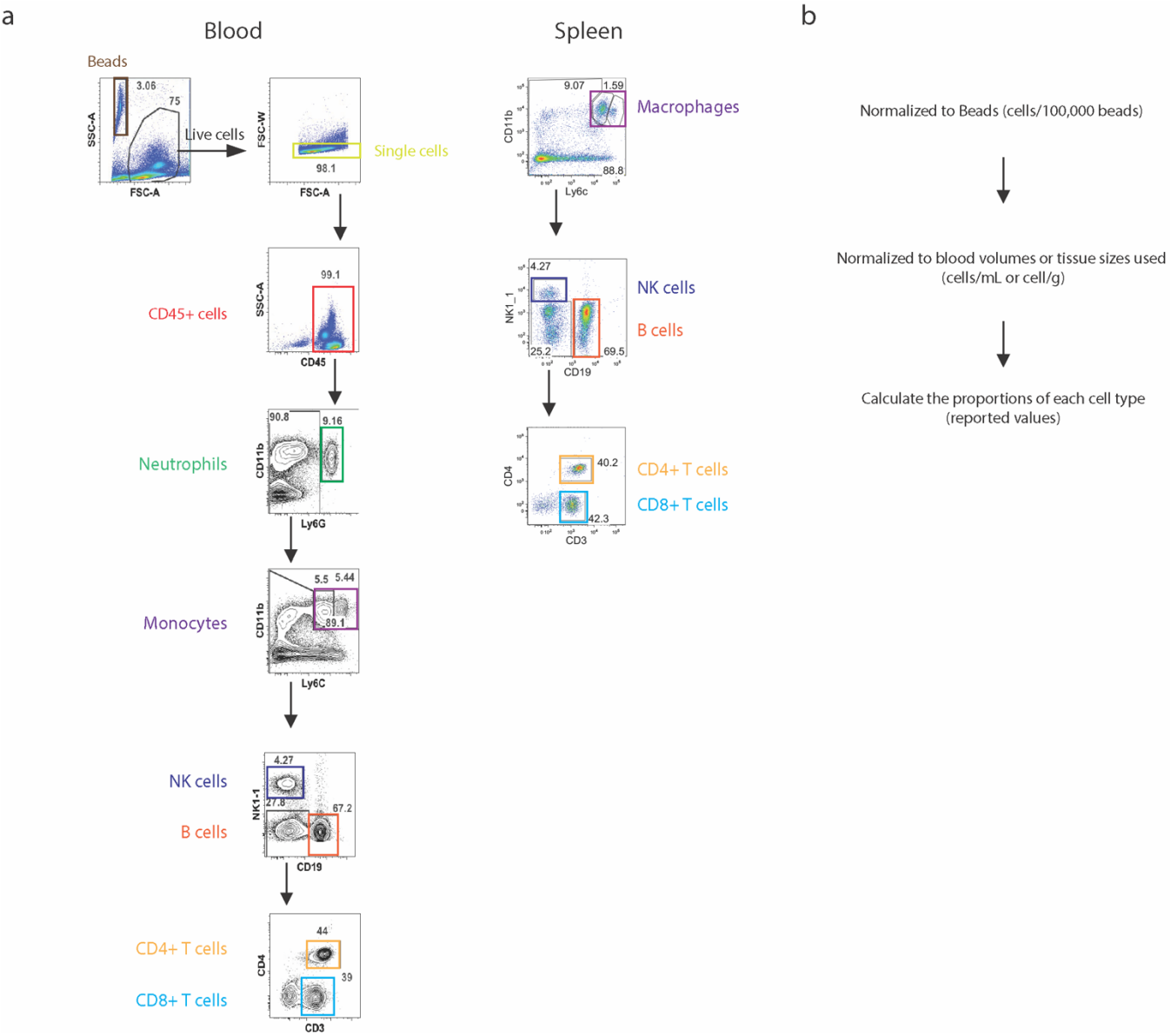
Supplementary Fig. 2



Supplementary Fig. 2: Prenatal vitamin D deficiency doesn't alter the immune cell proportions of female offspring at the adult stage.

The violin plots illustrate the proportions of immune cells in the peripheral blood of female mice at the adult stage, showing no significant alterations in the female samples (n=5 VDD and n=3 VDS). The white box shows the range between the first and third quartiles. The upper and lower whiskers represent the 1.5x inter-quantile range, while the black bars show the median. The values in the plot are p-values (Student's t-test).

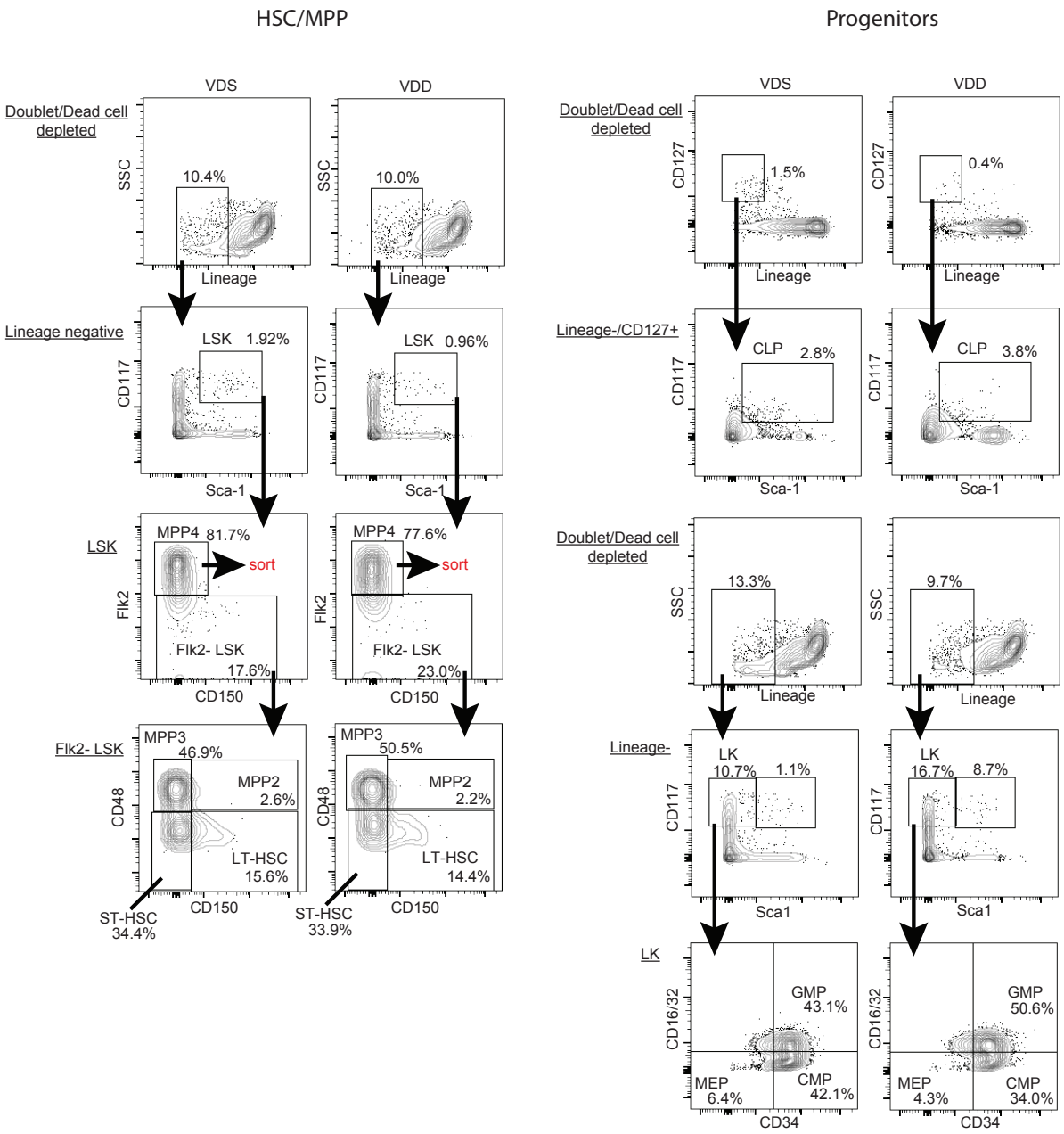
Supplementary Fig. 3



Supplementary Fig. 3: Gating and analytical strategies to assess the immune cell profiles in peripheral blood and spleen.

Representative FACS traces show the gating strategies to assess peripheral blood (**a**) and spleen (**b**). **c**. A flowchart outlines the process for calculating cell proportions. The panels on the left display the results from the VDS male mouse, while the panels on the right show the results from the VDD male mouse. The cell numbers obtained from the FACS results were first normalized by the number of beads we spiked into the samples before the wash steps and then further normalized by the blood volumes or tissue weight (spleen). The obtained cell numbers were used to calculate immune cell proportions.

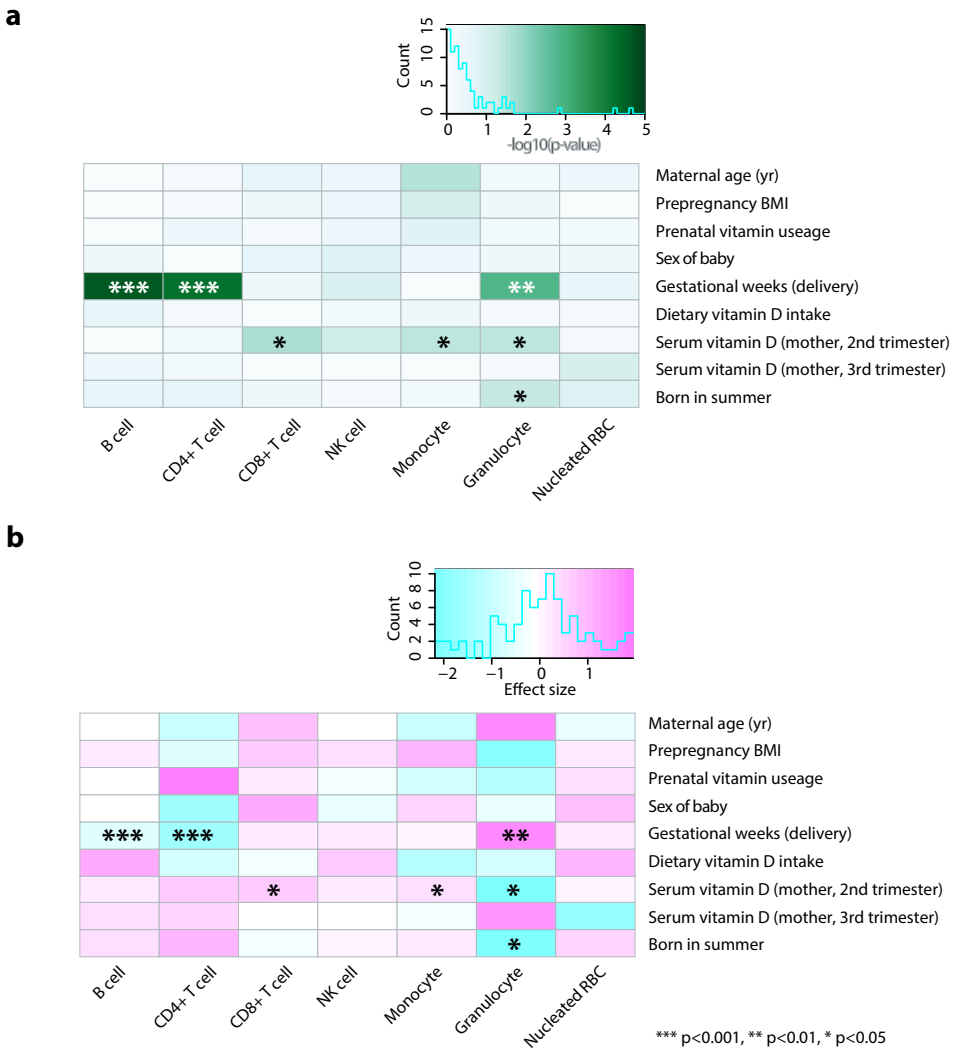
Supplementary Fig. 4



Supplementary Fig. 4: Gating and analytical strategies to assess hematopoietic stem cells, multipotent progenitor cells, and progenitor cells from bone marrow.

The gating strategies used to assess hematopoietic stem cells (HSC) and multipotent progenitor cells (MPPs) (a) and progenitor cells (b) are shown in the representative FACS traces. The results from the VDS male mouse are displayed in the panels on the left of the flow chart, while the panels on the right show the results from the VDD male mouse. The sorted cell populations used for RNA-seq analysis are indicated as "sort" in the bottom second panels of the HSC/MPP flow chart.

Supplementary Fig. 5



Supplementary Fig. 5: The association between known covariates and immune cell proportions of cord blood

The heatmaps show that the gestational week at the delivery, maternal serum vitamin D (2nd trimester), and being born in the summer season are significantly associated with the proportions of immune cells. The heatmaps represent -log10(p-value) (**a**) and the direction of the associations (**b**). Asterisks indicate the significance (*** p<0.001, ** p<0.01 and * p<0.05, Student's t-test).