Supplementary material

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Supplementary Figure 6: Flow cytometric analysis of kidney infiltrating immune cells.

Supplementary Figure 7: Gene array analysis on IL-6 transcript numbers.

Supplementary Table 1: Whole blood counts of wild type, *Notch3* knockout and chimeric animals.

Supplementary Figure 1. Complete blood counts with immune cell composition as well as kidney function in Notch3 knockout and wild type mice. No differences between wild type and *Notch3* knockout animals were seen in **(A)** complete blood counts of circulating leukocytes, **(B)** cellular composition of the bone marrow, **(C)** kidney morphology (PAS staining, scale bars equal 50 µm) and **(D)** kidney function (blood urea nitrogen (BUN), serum creatinine).

Supplementary Figure 2: Flow cytometry on bone marrow chimerism from transplanted mice. (A) CD45.1/CD45.2 chimeras were generated by lethally irradiating the recipient mice and transplanting bone marrow cells from the donor mice as indicated. **(B)** Kaplan-Meier survival curves revealed no difference in lethality of the mice due to the transplantation procedure. **(C-E)** Flow cytometric analysis of CD45.1 and CD45.2 expression of isolated cells from spleen **(C)**, bone marrow **(D)**, and blood **(E)** reveal a level of chimerism that exceeds 80%.

Supplementary Figure 3: Confirmation of successful homing, reconstitution, as well as chimerism in bone marrow transplanted mice. (**A**) BM transplantation was carried out with 8 weeks old mice by *i.v.* injection of $1x10^6$ bone marrow-derived cells 4 hours after irradiation. To investigate the contribution of Notch3 expression on bone marrow-derived cells and tissue resident cells UUO was performed 4 weeks following transplantation. Kidneys were analyzed on days 5 and 14 following disease induction. **(B)** The percent survival of the chimeric animals is shown for each of the four strains. A 10% mortality rate is seen in all strains, likely due to complications within the transplantation procedure. **(C)** Weight analysis of the mice reveals no difference, irrespective of the transplantation procedure. **(D)** To determine complete depletion of bone marrow cells by irradiation the number of circulating leukocytes 72 hours post intervention was analyzed and Pappenheim staining of the irradiated bone marrow was performed without bone marrow transfer. 72 hours post irradiation the number of circulating leukocytes was less than $0.1x10^9$/L without bone marrow transfer. Following injection of $1x10^6$ bone marrow cells the pool of circulating leukocytes reached values within “normal” range 6 weeks later. The receptor *Notch3* knockout in bone marrow cells did not affect erythropoiesis. Pappenheim staining of bone marrow isolated cells was used to exclude phenotypic differences between the groups. (**E)** Successful homing of the transferred wild type and *Notch3* knockout cells

to the bone marrow and lymph nodes was seen within 16 hours post transplantation. We analyzed the repopulation of the bone marrow and the lymph nodes by injected bone marrow-derived DID labeled cells into irradiated wild type mice and receptor *Notch3*$^{-/-}$ mice. The cells were detected 16 hours after transplantation in the organ systems bone marrow and lymph nodes by flow cytometry. As a significant difference, $p < 0.05$ was considered. (n=4). **(F)** Chimerism was confirmed by PCR of isolated bone marrow cells and isolated cells of the tail tip (representing the genotype of the non-transplanted cells of the recipients) using the primers described for genotyping. Sensitivity of the PCR was determined by sequential dilution of stock solutions. **(G)** In the chimera an analysis of transcripts for Notch1, Notch2 and Notch4 was performed by means of gene array (Agilent 4x44k mouse v2 design ID:026655). **(H)** Following UUO no differences in the transcript numbers for *Notch1* and *Notch2* were observed. However, the *Notch4* receptor seemed to be differently regulated in knockout recipients compare to wild type. **(I)** Kidney function of bone-marrow chimeric mice was assessed by quantifying blood urea nitrogen (BUN) and serum creatinine levels. Animals that received folic acid had strongly impaired renal function and served as positive control.

Supplementary Figure 4: Analysis of polarization efficiency, cell proliferation, IL-6 receptor and integrin expression of wild type and Notch3 knockout bone marrow-derived macrophages. Bone marrow from wild type or *Notch3* knockout mice was isolated and cultured in media supplemented with 10ng/ml M-CSF alone or together with IL-6 or IL-4 and IL-13. **(A)** Bone marrow-derived macrophages were stimulated with IL-6 for 2 or 30 min in the presence or absence of the IL-6 blocking antibody. Cellular lysates were prepared and analyzed for the tyrosine-phosphorylated (p)STAT3. *Notch3* knockout macrophages were less responsive to IL-6 compared to wild type macrophages. **(B)** IL-6-receptor expression on wild type and *Notch3* knockout bone marrow-derived macrophages was analyzed by flow cytometry. IL-6-receptor expression differed between non-polarized (M0), IL-6-polarized (M1), and IL4/IL13-polarized (M2) cells, but no difference in IL6-receptor expression was found between wild type and knockout cells for any given population. **(C)** Expression of β1-integrin (CD29) and **(D)** β2-integrin (CD18) was analyzed. The histogram shows the mean fluorescence intensity (MFI) of the CD29 and CD18 expression in wild type and *Notch3* knockout bone marrow-derived macrophages. **(E)**

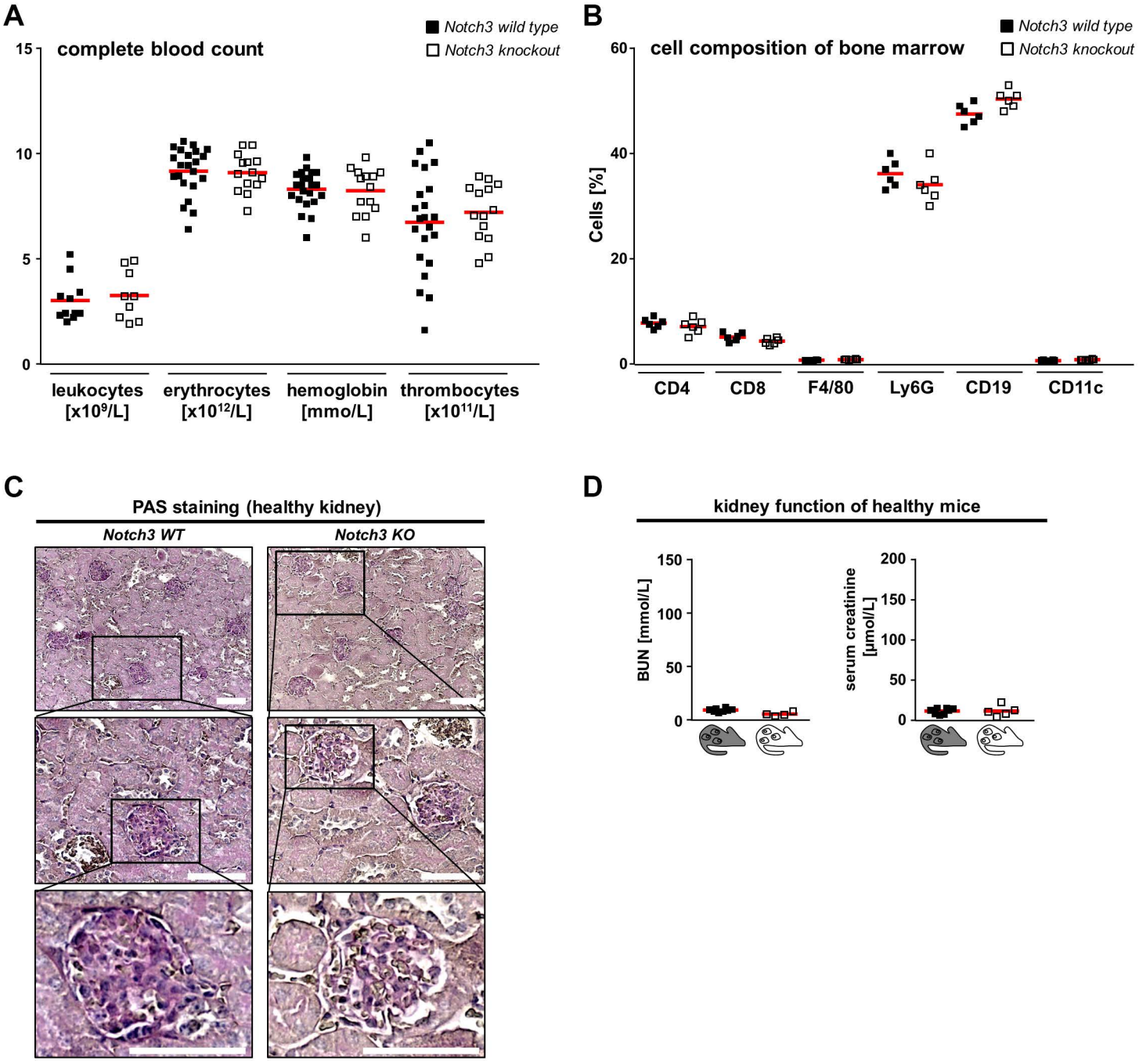
Phenotypical analysis of macrophages from wild-type and knockout cultures before and after polarization using F4/80 and CD11b revealed that almost all cells are macrophages. **(F)** Bone marrow cells were cultured for 14 days and cell numbers quantified on days 2, 5, 11, and 14. (n=3). **(G)** Cell cycle analysis was performed using propidium iodide staining of fixed cells at day 9. The proportion of cells in G1, S, and G2/M is indicated in the inset Table (n=3). **(H)** Analysis of arginase 1 (Arg1) expression by Western blot confirms successful IL-4/IL-13-polarization for both wild type and *Notch3* knockout macrophages (n=3).

Supplementary Figure 5: Sirius Red staining performed with obstructed kidneys on days 14. Sirius red staining for collagen deposits showed an increase of collagen deposition in *Notch3*$^{wt>wt}$ or *Notch3*$^{ko>wt}$ mice. Representative Sirius red stainings of obstructed kidneys on day 14 from chimeric mice are shown. A polarization filter allowed to distinguish collagen 1 (red) and 3 (green). Scale bar equals 50 µm.

Supplementary Figure 6: Flow cytometric analysis of kidney infiltrating immune cells. (A) Gating strategy for flow cytometric analysis of infiltrating immune cells. Renal lymphocytes were defined by doublet exclusion of live cells (using the forward and side scatter properties) followed by gating for $CD45^+$ leukocytes. The different subsets were gated out of the $CD45^+$ population using the markers $CD45^+/CD11b^+$ for myeloid cells; $CD45^+/CD11b^+/GR1^-/F4/80^+$ for macrophages; $CD45^+/CD11b^{int}/GR1^+$ for monocytes; $CD45^+/CD11b^+/GR1^+$ for neutrophils; and $CD45^+/CD3^+$ for T cells. **(B)** CD11c cells were described as $CD11b^+$ and $Ly6G^-$. The number of $CD11c^+$ cells within kidney appeared to be independent of Notch3 expression.

Supplementary Figure 7: Gene array analysis on IL-6 transcript numbers. Gene transcript analysis of healthy contralateral wild type and Notch3 knockout kidneys was compared to obstructed kidneys following UUO. IL-6 transcripts were more abundant following UUO, but there was no significant difference between wild type and Notch3 knockout genetic background.

Supplementary Figure 1

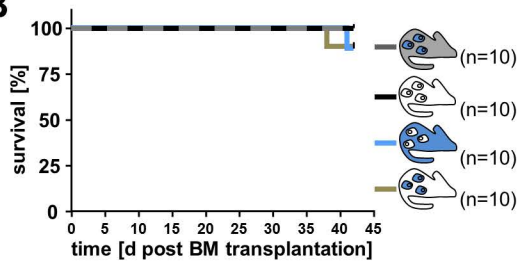


Supplementary Figure 2

A

description	Notch3$^{wt>wt}$	Notch3$^{ko>wt}$	Notch3$^{wt>ko}$	Notch3$^{ko>ko}$	CD45.1^{wt}
donor	CD45.1	CD45.2	CD45.1	CD45.2	CD45.1
recipient	CD45.2	CD45.1	CD45.2	CD45.2	CD45.1

B



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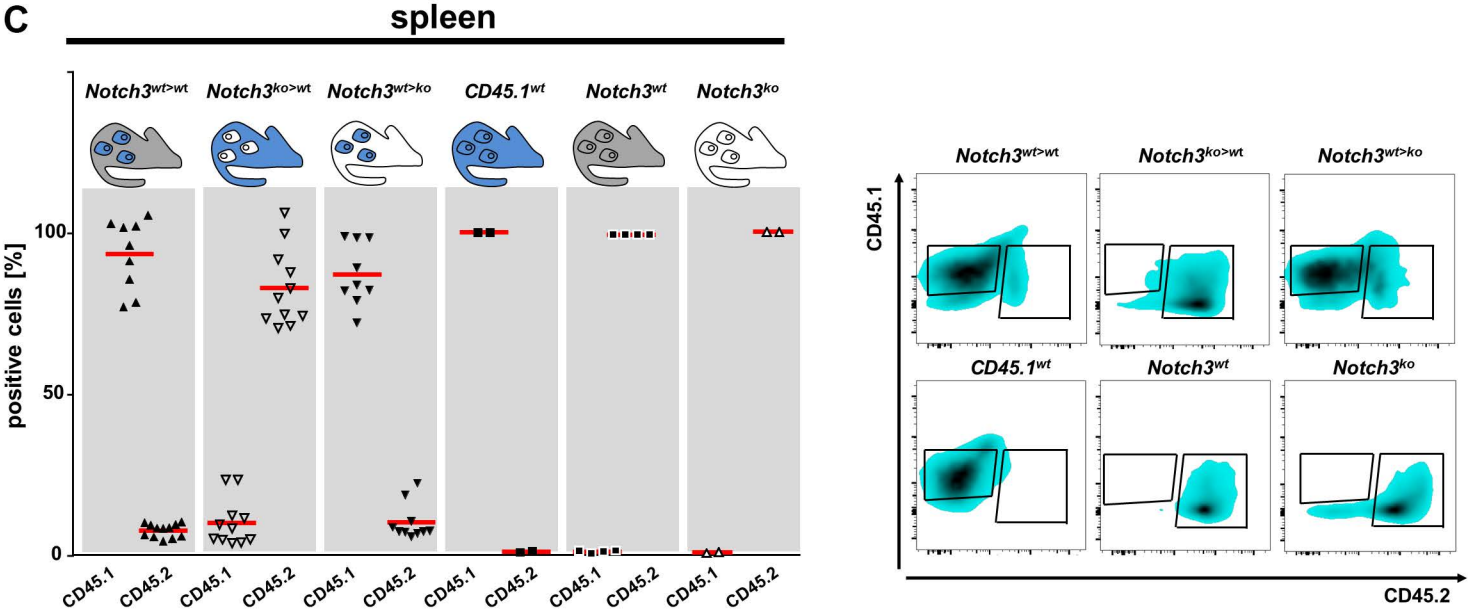

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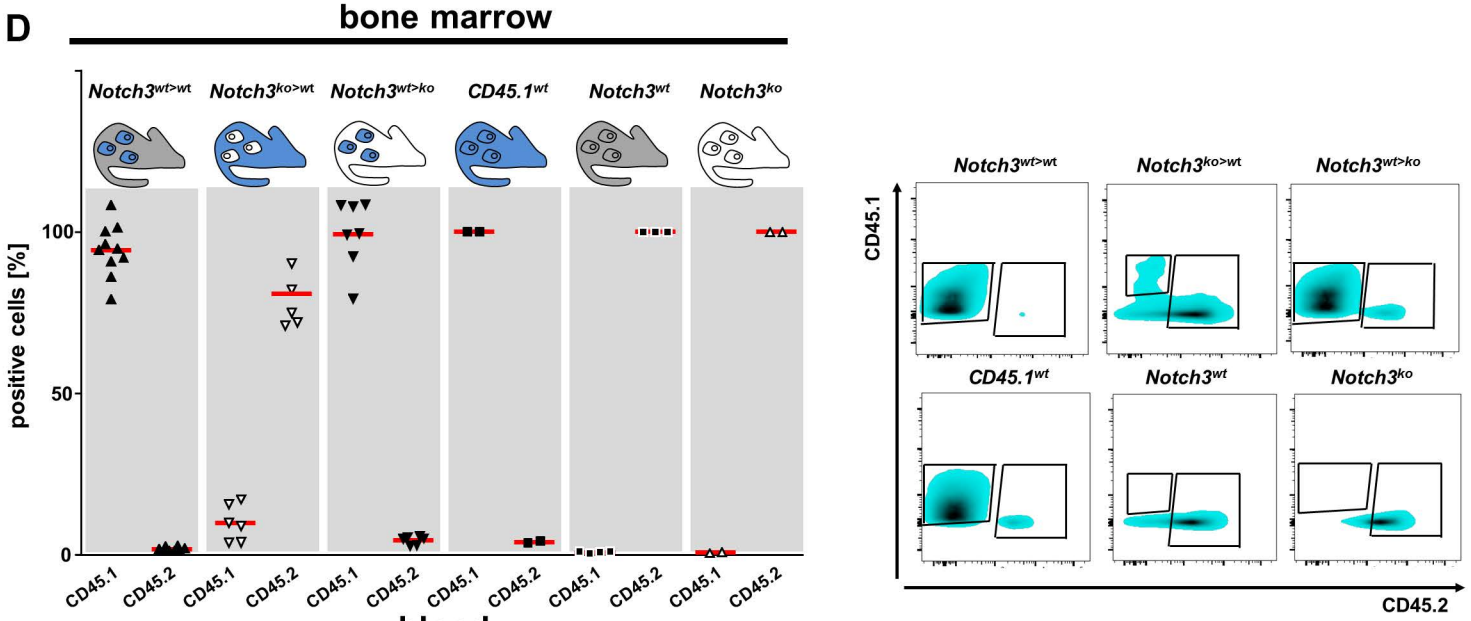

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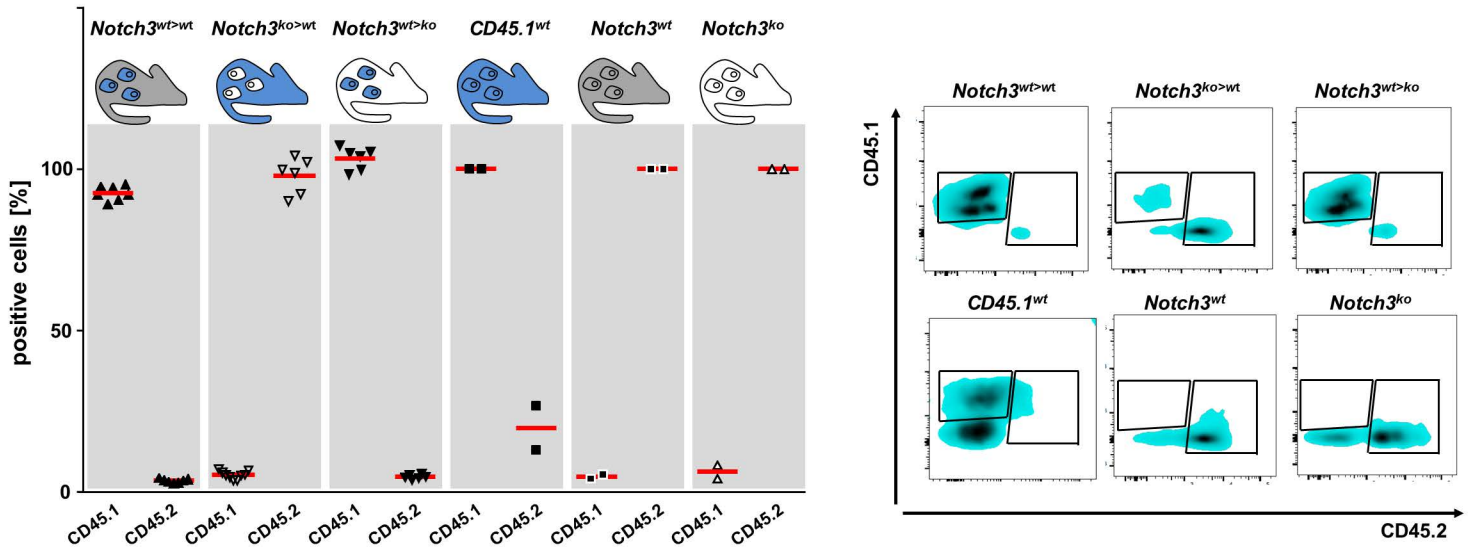
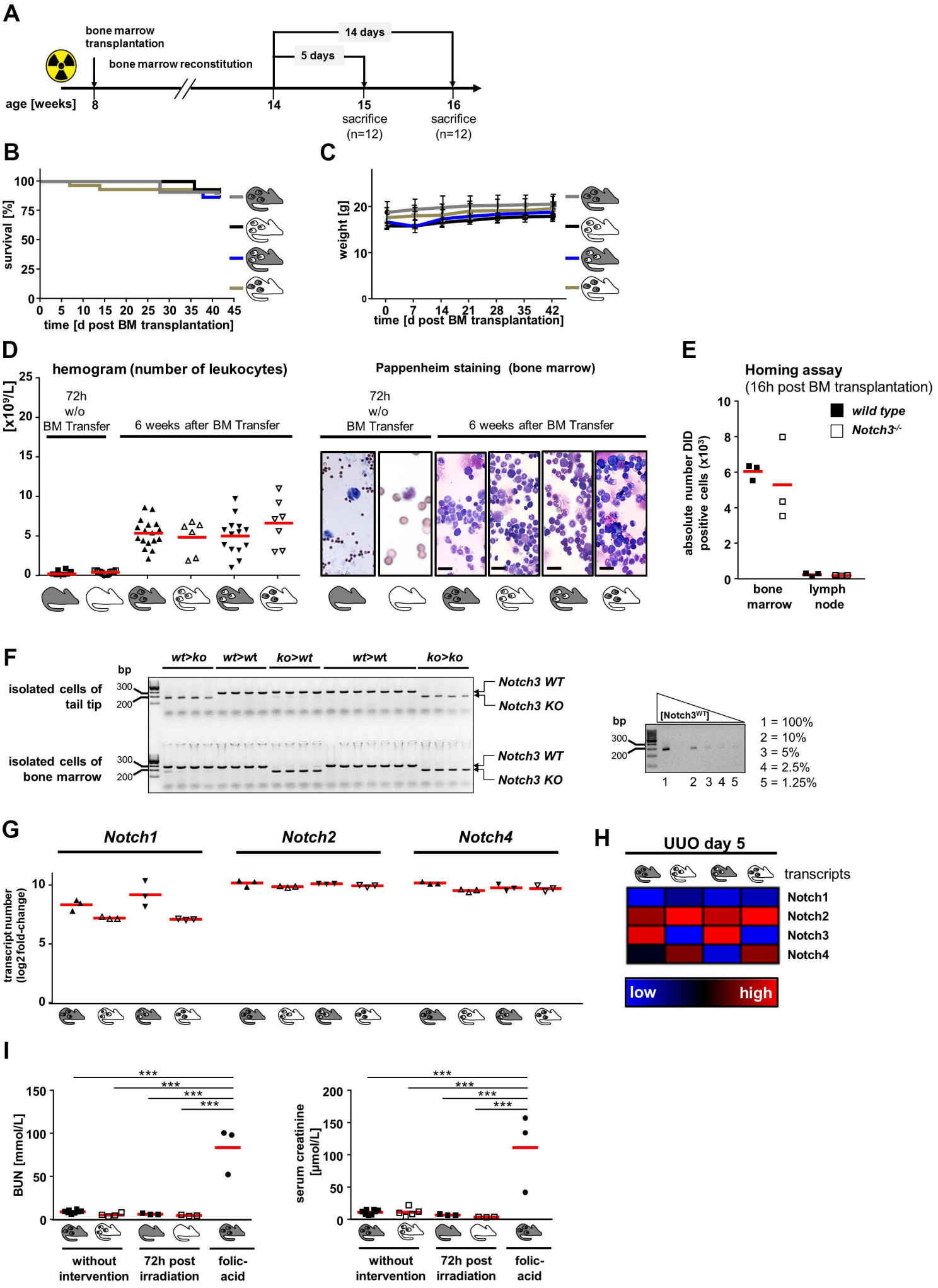
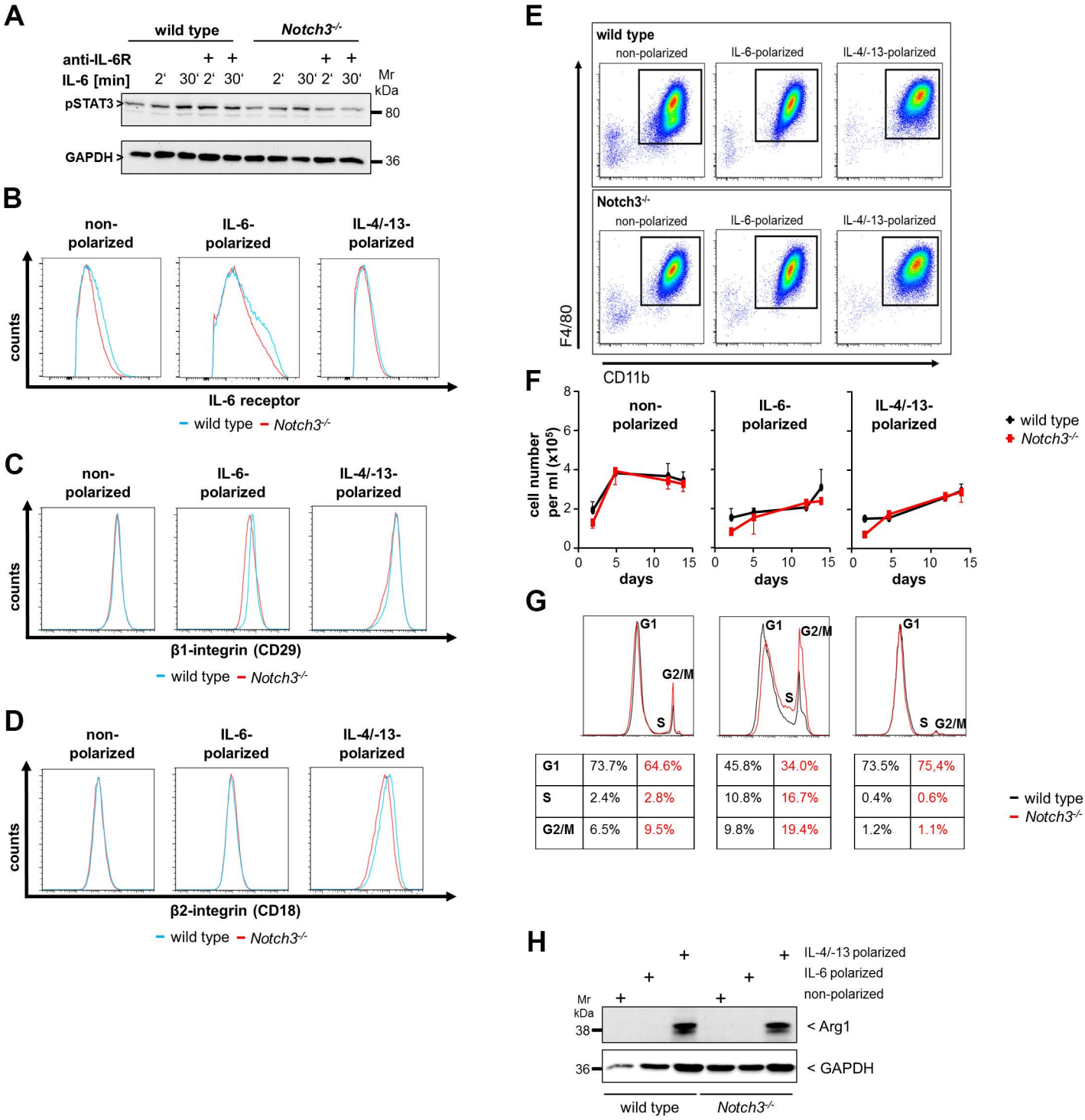


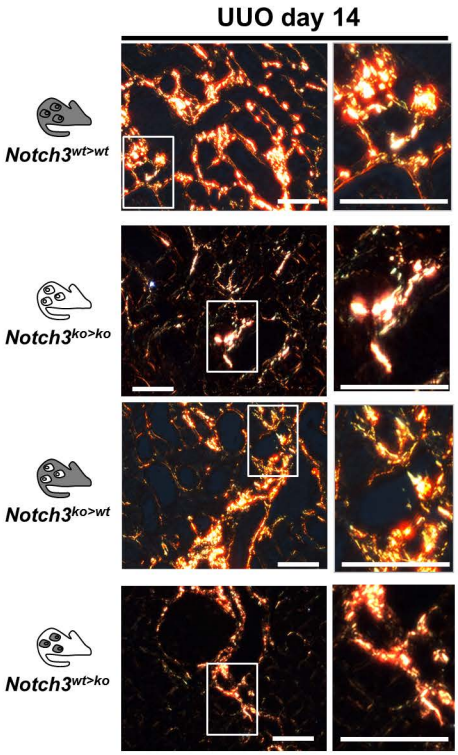
Supplementary Figure 3



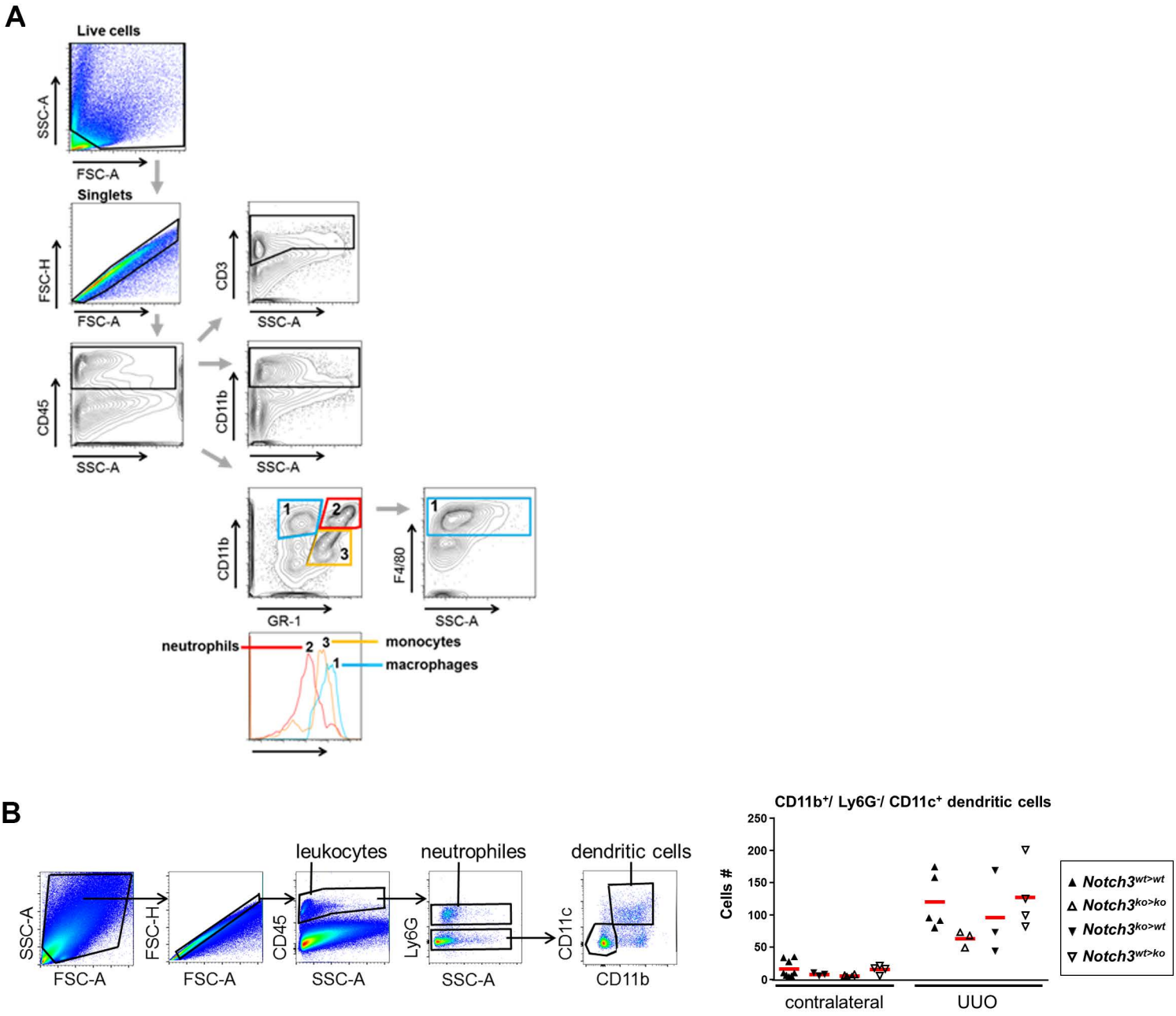
Supplementary Figure 4



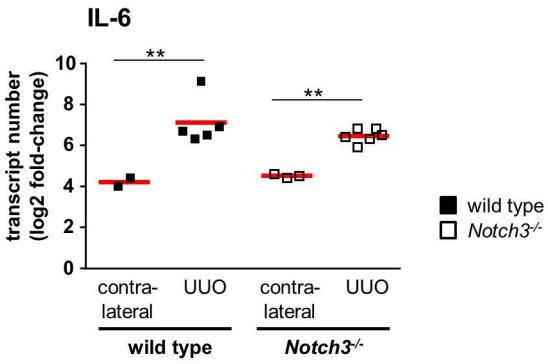
Supplementary Figure 5



Supplementary Figure 6



Supplementary Figure 7



Supplementary Table 1: Whole blood analysis of wild type, *Notch3* knockout, and chimeric mice.
All analyzed blood samples were within normal limits for mice.

		without intervention [± SD]		6 weeks following bone transfer, chimeric mice [± SD]			
parameter	normal range [unit]	***wild type***	***Notch3* knockout**	***Notch3*$^{wt>wt}$**	***Notch3*$^{ko>ko}$**	***Notch3*$^{ko>wt}$**	***Notch3*$^{wt>ko}$**
leukocyte	1.8 – 10.7 [10e9/L]	3.00 ± 1.02	3.24 ± 1.17	5.32 ± 1.63	5.36 ± 1.99	5.68 ± 1.94	6.39 ± 2.56
erythrocyte	6.36 – 9.42 [10e12/L]	9.14 ± 1.14	9.07 ± 0.90	8.16 ± 1.31	8.30 ± 0.95	8.33 ± 1.05	9.12 ± 0.95
hemoglobin	5 – 8 [mmol/L)	8.27 ± 0.89	8.22 ± 1.01	6.84 ± 1.27	7.00 ± 1.05	6.94 ± 0.91	6.83 ± 1.19
thrombocytes	592 – 2972 [10e9/L]	673 ± 237	719 ± 136	1036 ± 451	954 ± 197	953 ± 296	985 ± 367