Classical Dendritic Cells Mediate Hypertension by Promoting Renal Oxidative Stress and Fluid Retention

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Short title: Classical DCs mediate Ang II-induced hypertension

Supplemental Materials

Supplemental Methods

Cell preparations and flow cytometry

The spleens or kidneys were harvested and digested into single cell suspensions. For the T cell panel, cells were stained with fluorescently-labeled anti-CD45, anti-CD3, anti-CD11b, anti-CD11c, and anti-CD19 and subjected to flow cytometric analysis.

ELISA

The blood of mice was collected and centrifuged 6000 rpm for 10 minutes in microtainer tubes (Cat#: VT365967, VWR international). The supernatant was collected to determine the serum levels of FLT3L according to kit instructions (Cat#: MFK00, R&D systems).

In vitro study

Splenocytes were harvested from 12-week old naïve FVB mice and 129/SvEv mice. T cells and dendritic cells were isolated with a Pan T Cell Isolation Kit II (Cat#: 130-095-130, MACS miltenyi Biotec) and the mouse CD11c positive selection kit (Cat#: 18780A, STEMCELL), respectively. T cells from the FVB mice were labeled with CSFE (Cat#: 423801, BioLegend), the cell division tracker dye, and seeded into the 6-well plates. Cell culture media (Vehicle) or dendritic cells (DCs) from the 129/SvEv mice were added into the wells containing same amount of T cells for 72 hours. The culture media for the cells was RPMI-1640 (Cat#: R-7638, Sigma) containing 10% heat-inactivated fetal calf serum (FCS), 100 U/ml penicillin/streptomycin, 2 mM L-glutamine. Cells were collected and stained with fluorescently-labeled anti-CD3, anti-CD44, and anti-CD62L, and subjected to flow cytometric analysis.

Histopathological analysis

Kidneys were fixed in 10% neutral buffered formalin and processed for staining. Briefly, the tissue samples were

dehydrated and embedded in paraffin. After deparaffinization, thin sections (5 μm) were processed for NOX2 staining. After antigen retrieval, the sections were blocked in 1% BSA for 1 hour and then incubated with NOX2 antibody (Cat#: ab80508, Abcam) at 4°C overnight. After washing off the primary antibody, sections were incubated with HRP-conjugated secondary antibody (Cat#: 7074S, Cell Signaling Technology) at room temperature for 1 hour. Following three washes with PBS, the sections were incubated with 3,3'-diaminobenzidine chromogen substrate resulting in positive brown staining of the target protein. The nuclei were counterstained with Mayer's hematoxylin before dehydration and mounting. Images were randomly captured from each section, and positive staining signals were analyzed using ImageJ 1.38 (NIH, USA). Scores were averaged for each animal and then across each group.

Statistical analysis.

The values of each parameter within a group are expressed as the mean ± the standard error of the mean (SEM). For the blood pressure measurement experiment, comparisons between the groups were performed using the two-way ANOVA with repeated measures. For comparisons between two groups with normally distributed data, statistical significance was assessed using an unpaired student' *t*-test. For comparisons between more than two groups with normally distributed variables, the one-way ANOVA was employed. $p<0.05$ was considered statistically significant. *, $p<0.05$. #, $p<0.05$. **, $p<0.01$. ***, $p<0.005$.

Supplemental Figure Legends

Figure S1

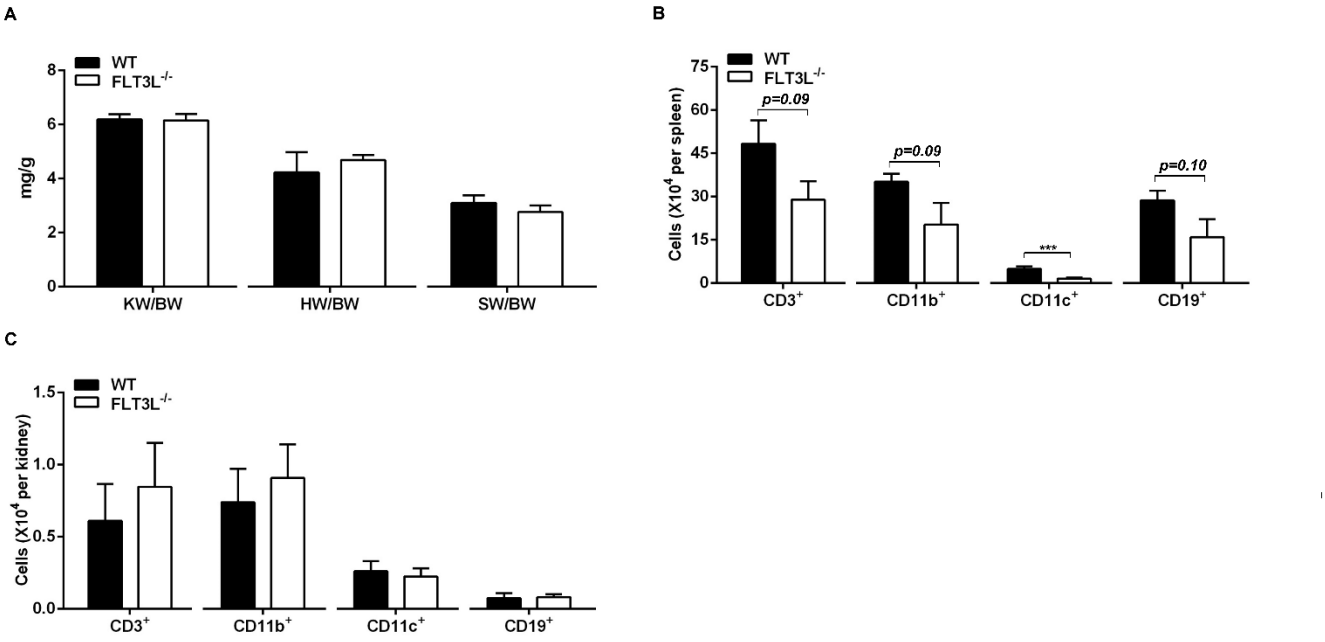

Figure S1. Physiological parameters of the FLT3L KO mice and the numbers of cell populations at baseline. (A) The ratios of kidney weight/body weight (KW/BW), heart weight/body weight (HW/BW), and spleen weight/body weight (SW/BW) in the WT and FLT3$^{-/-}$ cohorts at baseline. (B) The numbers of specific immune cell populations in the spleen at baseline. (C) The numbers of specific immune cell populations in the kidney at baseline. N=6 mice/group. Data are mean ± SE.

Figure S2

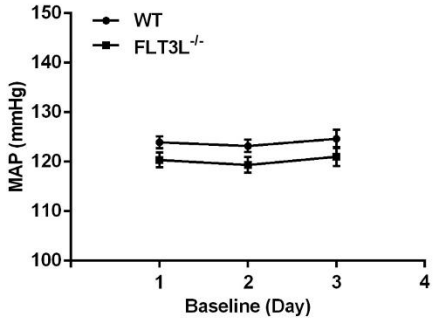

Figure S2. Genetic deletion of FLT3L does not impact baseline blood pressures. Mean arterial blood pressures measured by radio-telemetry at baseline. Wild-type ("WT"), circles. FLT3L KO ("FLT3$^{-/-}$"), squares. N=15 mice/group. Data are mean ± SE.

Figure S3

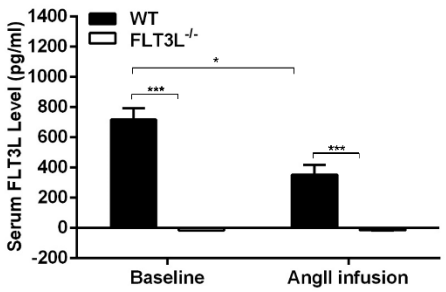

Figure S3. Circulating FLT3L levels are reduced in WT mice during Ang II-induced hypertension. Serum was collected from mice at baseline and following chronic Ang II infusion. FLT3L levels were quantitated by ELISA. N=5-6 mice/group. Data are mean ± SE.

Figure S4

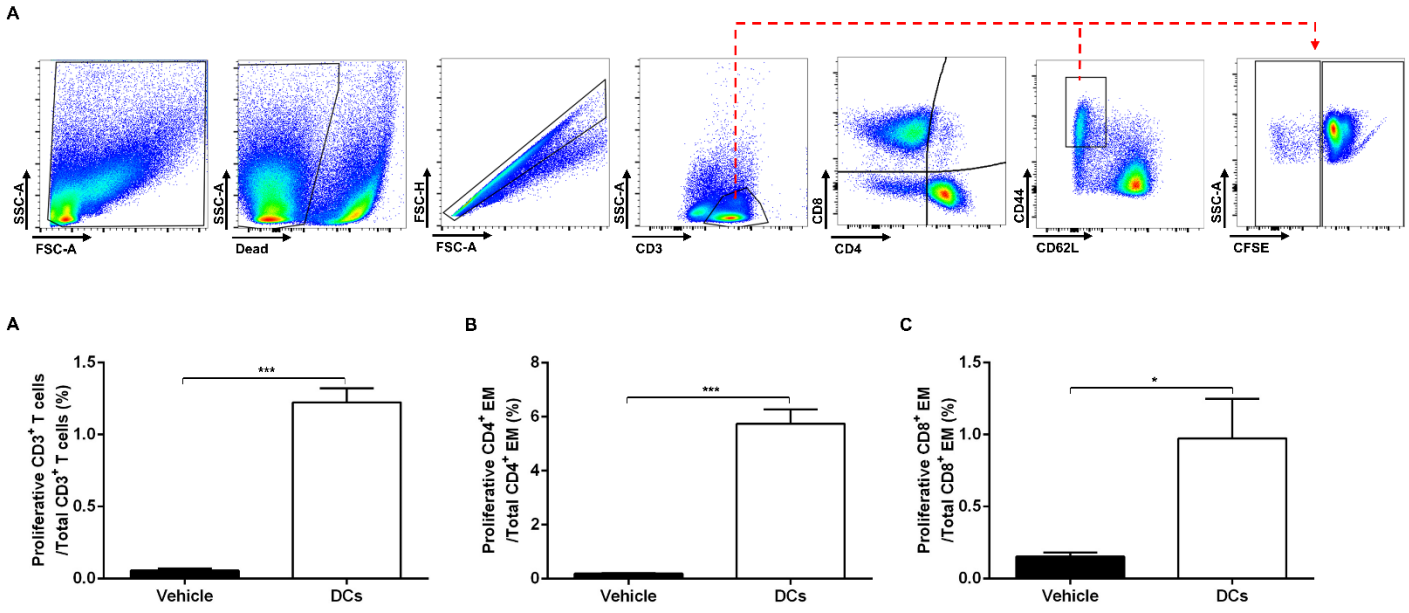

Figure S4. Dendritic cells enhance proliferation of effector memory T cells. FVB T cells were co-cultured with 129Sv/Ev dendritic cells for 72 hours, and the cells were collected for flow cytometric analysis. (A) Gating strategy for parsing T cell and effector memory T cells (EM). (B) The ratio of the proliferating $CD3^+$ T cells to total $CD3^+$ T cells. (C) The ratio of the proliferating $CD4^+$ EM to total $CD4^+$ EM T cells. (D) The ratio of the proliferating $CD8^+$ EM to total $CD8^+$ EM T cells. N=4-5 wells/group. Data are mean ± SE.

Figure S5

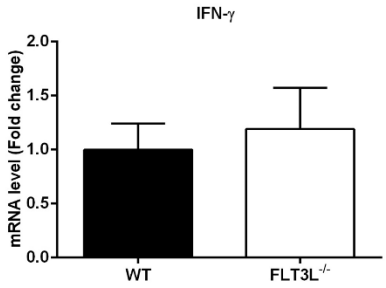

Figure S5. The expression of IFN-γ in the hypertensive kidney from WT and FLT3L KO mice. At the end of the experiment (4 weeks), kidneys from WT and FLT3L$^{-/-}$ mice were harvested for real-time QPCR analysis. Relative mRNA levels for IFN-γ are shown. N=5 mice/group. Data are mean ± SE.

Figure S6

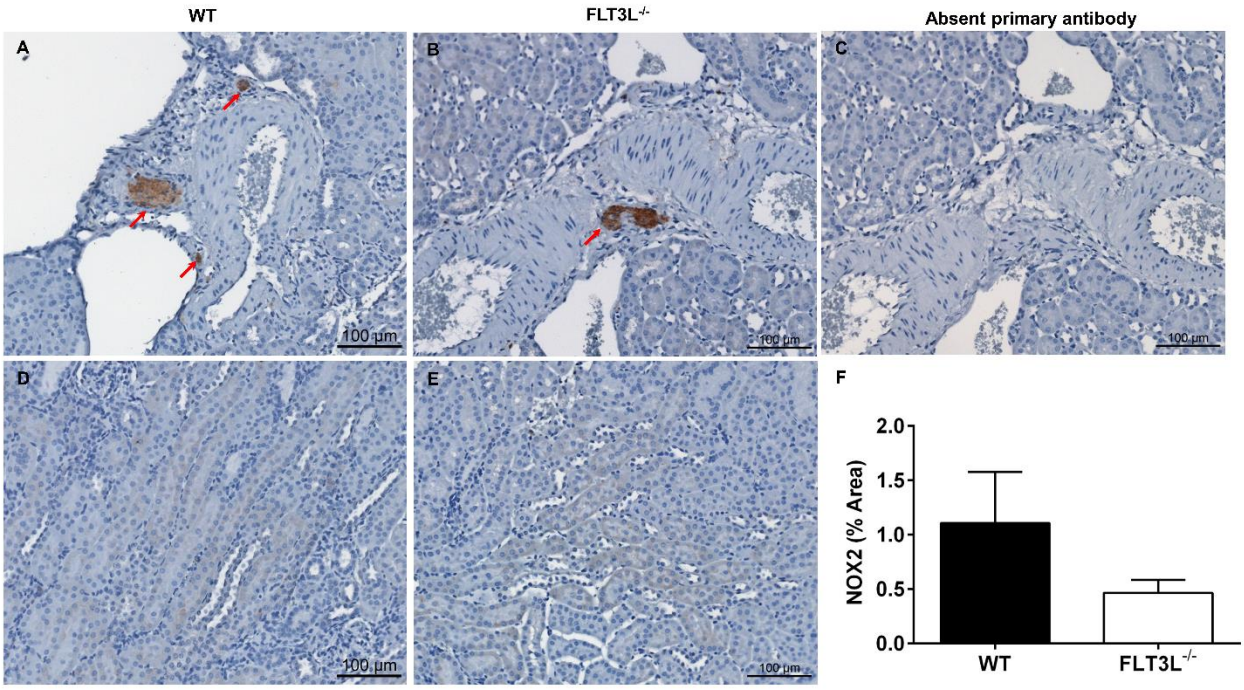

Figure S6. Immunostaining for NOX2 in the kidney during hypertension. (A-B) Representative image of staining for NOX2 around the renal arterioles in kidney from (A) WT and (B) FLT3L$^{-/-}$ mice. (C) The non-staining control image, consecutive section without incubated NOX2 primary antibody. (D-E) Representative image of staining for NOX2 in the renal tubules of (D) WT and (E) FLT3L$^{-/-}$ mice. (F) Blinded quantification of the positive NOX2 staining area. N=5 mice/group. Data are mean ± SE.