Supplementary information for:

Fate mapping reveals compartment-specific clonal expansion of mononuclear phagocytes during kidney disease

Junping Yin[1], Qi Mei[2], Marco Prinz[3,4,5], Zeinab Abdullah[1], Ulf Panzer[6], Jian Li[1], Sibylle von Vietinghoff[7], Christian Kurts[1,8]

[1]Institute of Molecular Medicine and Experimental Immunology, University Hospital Bonn, Germany.

[2]Department of Oncology, Tongji Hospital, Tongji Medical College, Huazhong, University of Science and Technology, Wuhan, Hubei, China

[3]Institute of Neuropathology, University of Freiburg, Germany

[4]Center for Basics in NeuroModulation (NeuroModulBasics), University of Freiburg, Germany

[5]Signalling Research Centres BIOSS and CIBSS, University of Freiburg, Germany.

[6]Division of Translational Immunology, III Medizinische Klinik, University clinic Hamburg-Eppendorf, Hamburg, Germany.

[7]Nephrology Section, Medical Clinic 1, University Hospital Bonn, Germany.

[8]Department of Microbiology and Immunology, Doherty Institute for Infection and Immunity, University of Melbourne, Victoria, Australia

Address correspondence to: Christian Kurts, Biomedical Center II, University Clinic, Rheinische Friedrich-Wilhelms-University, 53127 Bonn, Germany. Email: ckurts@web.de.

Table of content

Supplementary methods

Mice

A female Cx3cr1$^{creER/creER}$ R26R$^{Confetti/Confetti}$ mouse was kindly provided by Prof. Marco Prinz from Freiburg University, which was bred with C57BL/6J mice to obtain the Cx3cr1$^{creER/+}$ R26R$^{Confetti/+}$ offspring (Microfetti) in the specific pathogen free (SPF) area of the central animal facility of University Clinic of Bonn. To induce NTN or generate HAD related nephritis kidney of Microfetti mice, one week after application of TAM in the Microfetti mice, nephrotoxic serum (NTS) was injected (i.p.) or mice were feed with high adenine diet as previously described[S1, S2]. This animal study was approved by the Landesamt für Natur, Umwelt und Verbraucherschutz of North Rhine-Westphalia, Germany.

Tamoxifen treatment

20mg/ml tamoxifen (T5648, Sigma) was prepared by using corn oil (C8267, Sigma) in the light-protective condition at 40°C on a shaker overnight. 10mg tamoxifen was injected to each Microfetti mice subcutaneously.

Histology and immunofluorescence microscopy

Mice were sacrificed by CO_2 and perfused with 50mM EDTA in DPBS. Kidneys were collected and cut into two halves along with the corticopapillary axis. Kidney halves were fixed in 4% paraformaldehyde in PBS for 6 hours followed by 30% sucrose in PBS for 24 hours at 4°C in the light-protective condition. Afterwards, each kidney half was embedded in the OCT and stored in -80 freezer and cut into 30um sections on the slides. Sections were rehydrated in 0.1M Tris solution (PH, 7.4) followed by blocking in blocking solution (1% BSA, 1% normal mouse serum (10410, Invitrogen), 1% GCWFS (G7041, sigma), dissolved in 0.1M Tris) for 1 h at room temperature. For labelling the BrdU, 0.3% Triton-x 100 was added in incubation to permeabilize the tissue sections at RT for 1 hour, followed by DNA denaturation with 2M HCl for 1 hour at 37°C. Sections were washed 3 times with PBS to re-equilibrate and subsequently blocked with blocking solution. All blocked sections were incubated at 4°C overnight with Alexa-Fluo 647-conjugated anti-mouse F4/80 (123122, Biolegend), Alexa-Fluo 647-conjugated ant-mouse CD86 (105019, Biolegend), Alexa-Fluo 647-conjugated anti-mouse CD206 - (141711, Biolegend), Alexa-Fluo 647-conjugated anti-mouse I-A/I-E antibodies (107617, Biolegend), APC-conjugated anti-mouse CD11b (17-0112-83, eBioscience), Alexa-Fluo 647-conjugated anti-mouse CD11c (117312, Biolegend), Alexa-Fluo-488 conjugated anti-BrdU - (364106, Biolegend), Alexa-Fluo 488-conjugated anti GFP (A-21311, Invitrogen) and, Alexa-Fluo 647-conjugated anti BrdU (364108, Biolegend) antibodies. On the following day, sections were counterstained with 4′,6-diamidino-2-phenylindole (DAPI, Sigma) and mounted with Fluoromount mounting medium (Invitrogen).

Confocal microscopy

Confocal images were captured using Leica SP8 confocal microscope system. To image the Confetti$^+$ MNPs with DAPI and AF647 anti-F4/80 or the antibodies against CD86, CD206, MHCII, CD11b, CD11c, a total four virtual channels were set up: channel 1, DAPI, excitation was 405nm and emission signals were collected at 419-469nm; channel 2, CFP and YFP, excitation were 458nm, 514nm and emission signals were collected at 465-495nm, 522-562nm, respectively; channel 3, GFP, excitation was 488nm and emission signals were collected at 496-515nm; channel 4, RFP and AF647, excitation were 561nm, 633nm and emission signals were collected at 570-620nm, 650-750nm, respectively. A 30um z stack with 2um per step image was captured for each sample. To image the BrdU labelled Confetti$^+$ MNPs with DAPI and AF647 anti-F4/80, a single channel was set up to excite DAPI, AF488 and AF647 with 458 nm, 488 nm and 635 nm laser and the emission signals were collected at 466-485 nm, 500-560 nm and 650-750 nm, respectively. To determine the proportion of CD86$^+$ M1 or CD206$^+$ M2 clones in the Confetti$^+$ clones, we evaluated 20-28 clones in each sample. A clone was defined as a cellular cluster of at least 3 same color cells and a clone with more than 50% of cells expressing CD86 or CD206 marker was counted as CD86$^+$ M1 or CD206$^+$ M2 clone. All captured confocal images were processed in Fiji software.

Computational analysis of cellular distribution

To determine the distribution of renal MNPs, an algorithm was used from a previous report[S3]. All analyses in this study were performed in R software. Distance (*Distance$_{ij}$*) from Confetti$^+$ MNP *i* to the same-color Confetti$^+$ MNP *j* was calculated based on the following equation (X_i or X_j denotes the position of MNP *i* or *j* in the X axis, while Y_i or Y_j denotes the position of MNP *i* or *j* in the Y axis):

$$Distance_{ij} = \sqrt{(X_i - X_j)^2 + (Y_i - Y_j)^2}$$

To determine the properties of distribution of Confetti+ MNPs, a Confetti+ cell-centered density estimate (*Density*) of the number of same-color Confetti$^+$ MNPs in a concentric ring of radius *r* and width *w*. In each sample *s*, an individual Confetti$^+$ MNP *i* was set as the center of the ring. Based on the center, a series of concentric ring which have radius started from 10um to 300um and width at 20um was generated. In each concentric ring, the same-color cell *j* was counted if its absolute distance (*Distance$_{ij}$*) to *i* minus *r* less than 1/2*w*. The number of same-color Confetti+ MNPs *C* in each concentric ring was calculated, in which process the counted cells were excluded in the subsequent count. Density for each sample was determined using the sum of count of same-color cells divided by volume *V* (*r2* was denoted as the radius of outer ring and *r1* was the radius of inner ring).

$$Density_r = \frac{\sum C_{(r,i)}\{|Dis_{ij} - r| < 1/2w\}}{V_{(r)} = (\pi {r_2}^2 - \pi {r_1}^2) * h_{(s)}}$$

Calculated densities of each group were then averaged for using in plotting and comparing with Monte Carlo data.

Monte Carlo simulation was employed to simulate the random distribution of Confetti$^+$ MNPs. During the simulation, positions of renal MNPs were taken as fixed positions while the position of Confetti$^+$ MNPs were randomly selected. A total 100 iterations of Monte Carlo simulation were performed. For each iteration, the distance and density of same-color Confetti$^+$ MNPs were calculated by using the algorithm abovementioned. The 2th percentile and 98th percentile values of the total 100 iterations were calculated and used for plotting and the 98th percentile values of each group were utilized for comparisons with recorded data.

Flow cytometry

To perform flow cytometry for characterization of confetti$^+$ cells in blood monocytes and renal MNPs, blood was collected, mice were perfused with cold PBS and afterwards the perfused kidneys were collected as described[S4]. Blood samples were incubated with ACK buffer twice at room temperature to remove the red blood cells. Kidneys were digested with DNase and collagenase at 37 °C incubator and filtered with 100um cell strainer. Blood cells were stained with BUV conjugated anti mouse CD45 (564279, BD), APC conjugated anti mouse Ly6c (128015, Biolegend) and Live/dead (65-0865-18, eBioscience). Kidney cells were analyzed with BUV conjugated anti mouse CD45, BV421 conjugated anti mouse CD206 (141717, Biolegend), AF700 conjugated anti mouse CD86 (105122, Biolegend), AF647 conjugated anti mouse F4/80 (123122, Biolegend) and Live/dead. To detect the BrdU$^+$ cells, kidney cell samples were digested with DNase for 1hour at 37 °C. Intracellular staining was performed using a BD intracellular staining kit and cells were then stained with AF647-conjugated anti BrdU. All cells were acquired and recorded by using SONY ID7000 spectral analyzer. The unmixing settings were calculated by using the single-color control of cells from GFP, YFP, CFP, RFP transgenic mice and wild type cells. Recorded data were analyzed in Flowjo software 10.

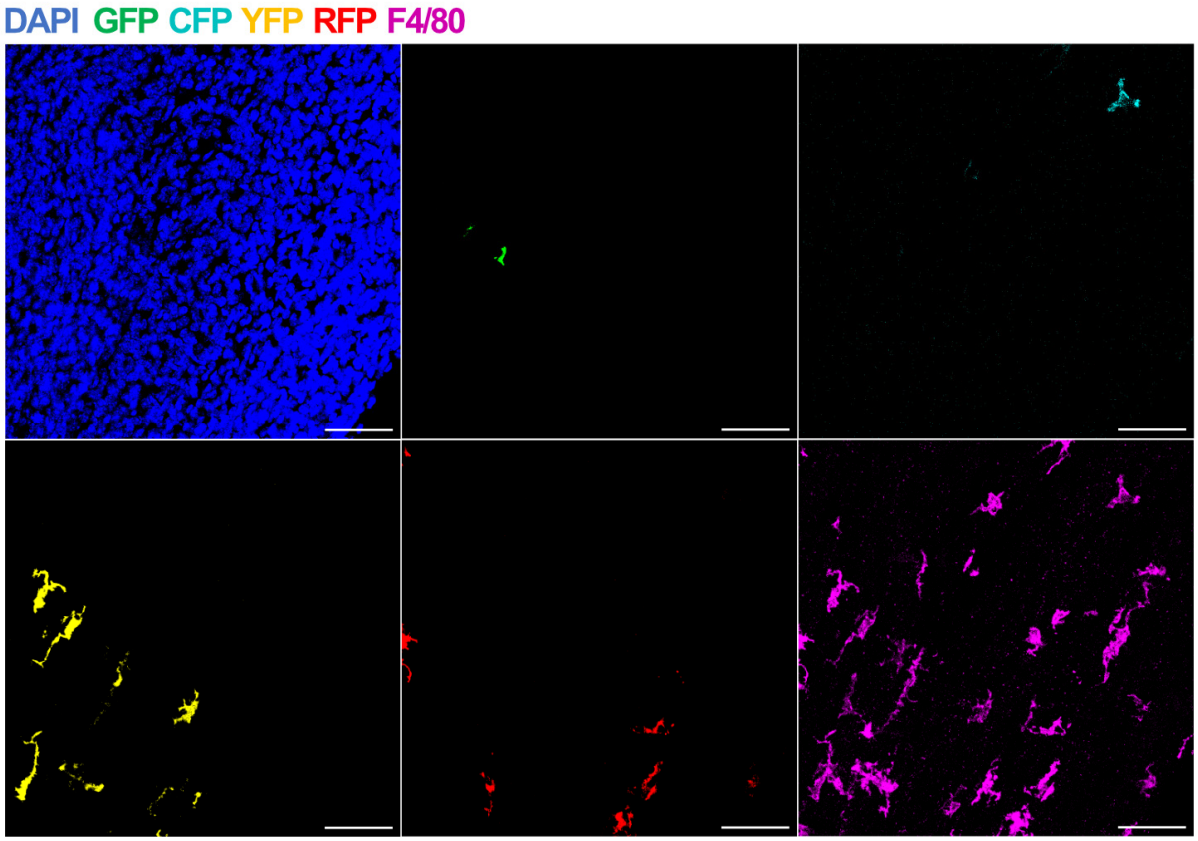
***In vivo* labelling with BrdU**

100mg BrdU (5-Bromo-2´-Desoxyuridin, B23151, Invitrogen) was dissolved in 5ml PBS on the shaker until all particles are dissolved. Mice were injected (i.p.) with 5mg of BrdU at 5 and 6 days after application of nephrotoxic serum or 8 and 9 days after feeding with high adenine diet to induce inflammatory disease in kidney.

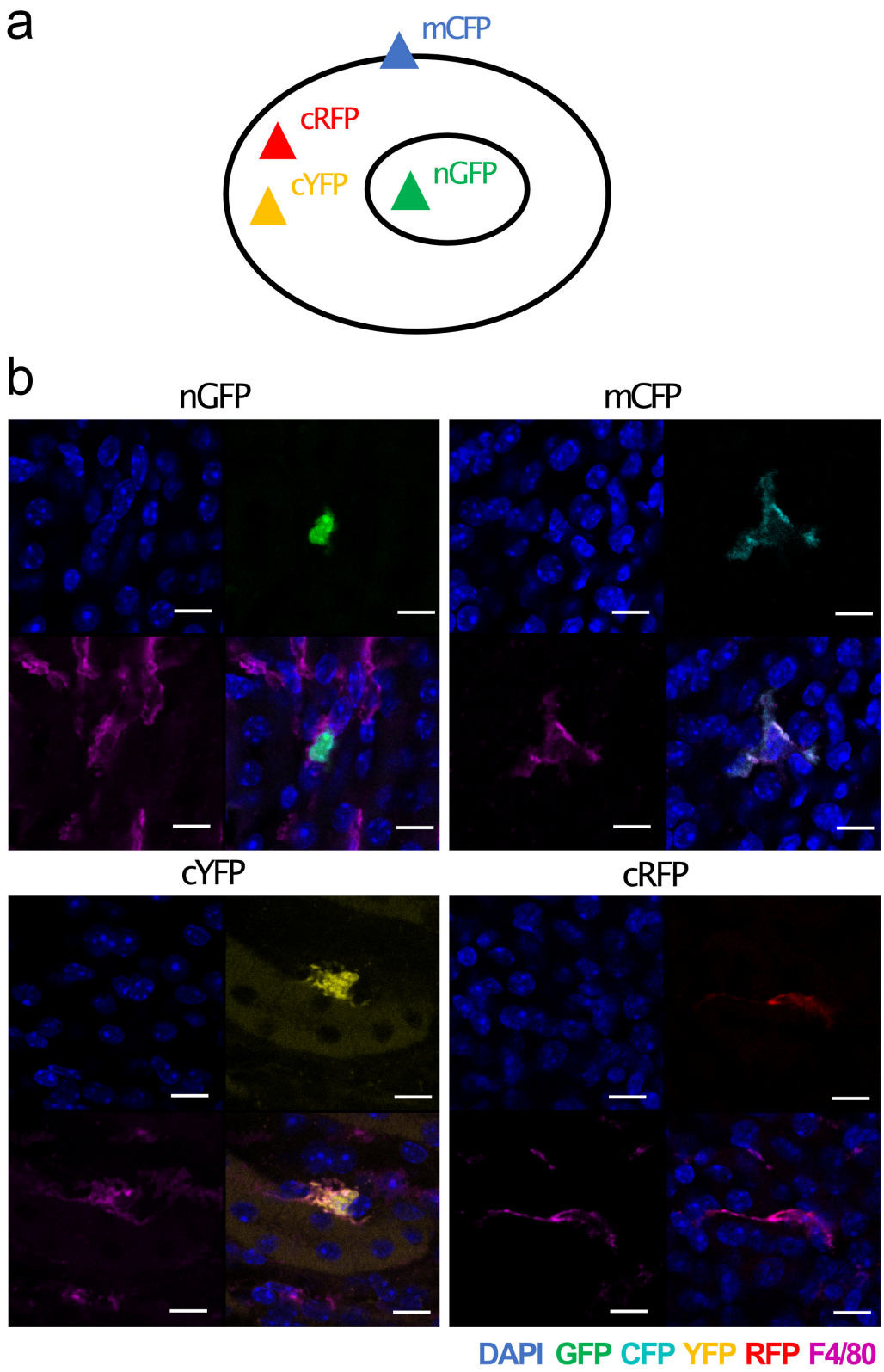
Software and statistical analysis

All schematic plots were generated by BioRender. Bar plots and corresponding statistical analysis were performed in Graphpad Prism 9. The statistical significance of two individual groups was calculated by using the Student's t test or Mann Whitney u test depending on the normality of distribution, and a p value less than 0.05 was considered statistically significant.

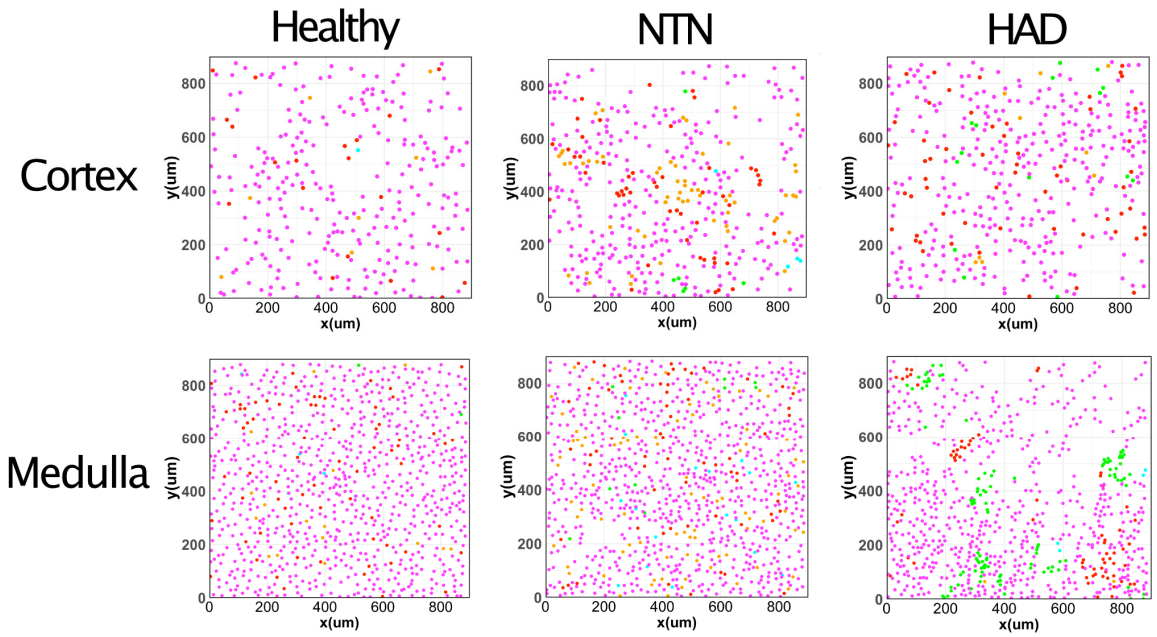
Supplementary Figures



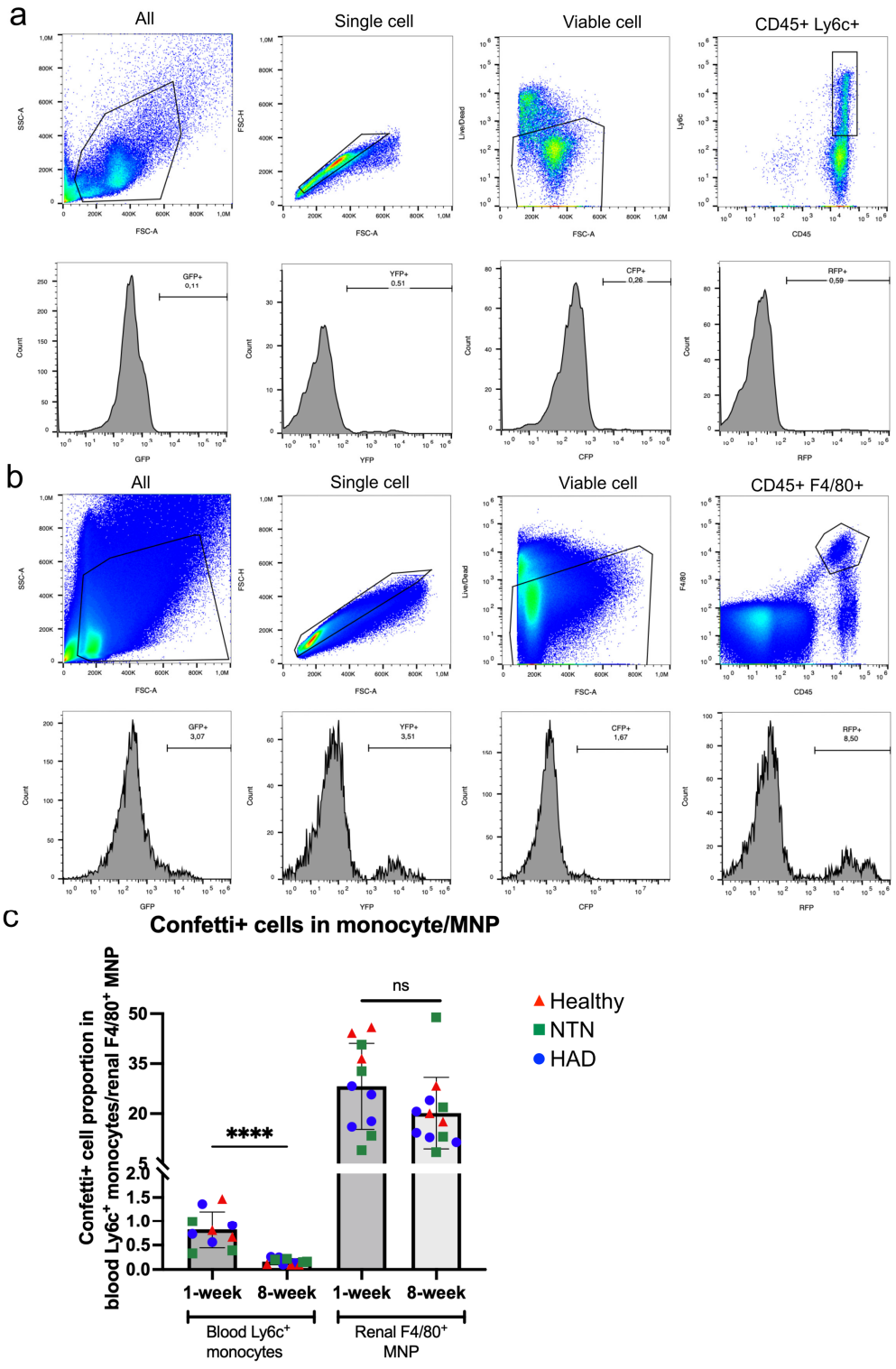
Supplementary Figure S1. Multichannel micrograph of Confetti^{+} MNPs in the kidney of Microfetti mice. Scale bar, 50um



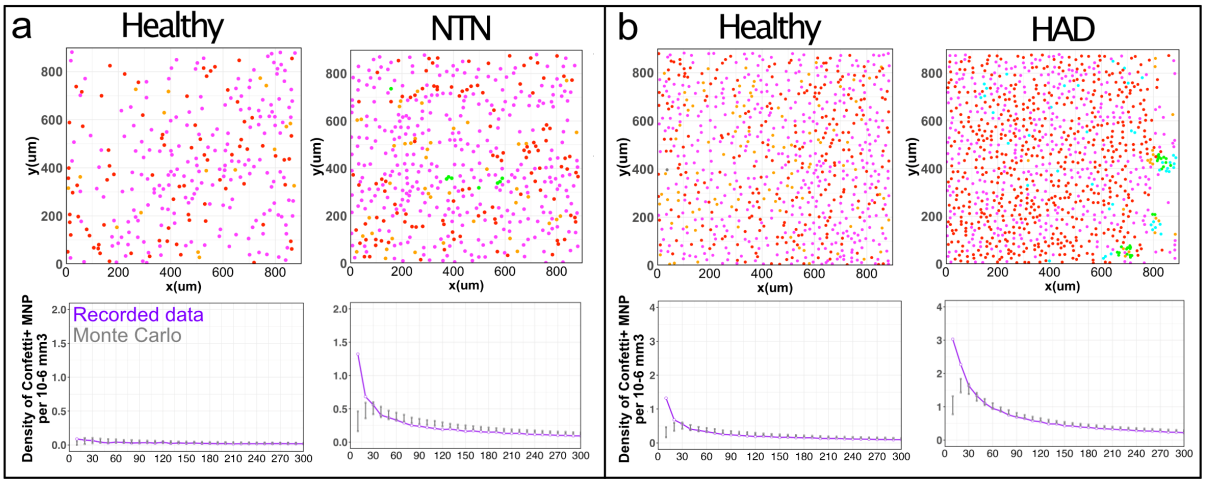
Supplementary Figure S2. Location of cellular compartments of Confetti labelled MNPs. (a) Scheme of the location of Confetti^{+} MNPs. (b) Representative micrograph of Confetti channels. Scale bar, 10um.



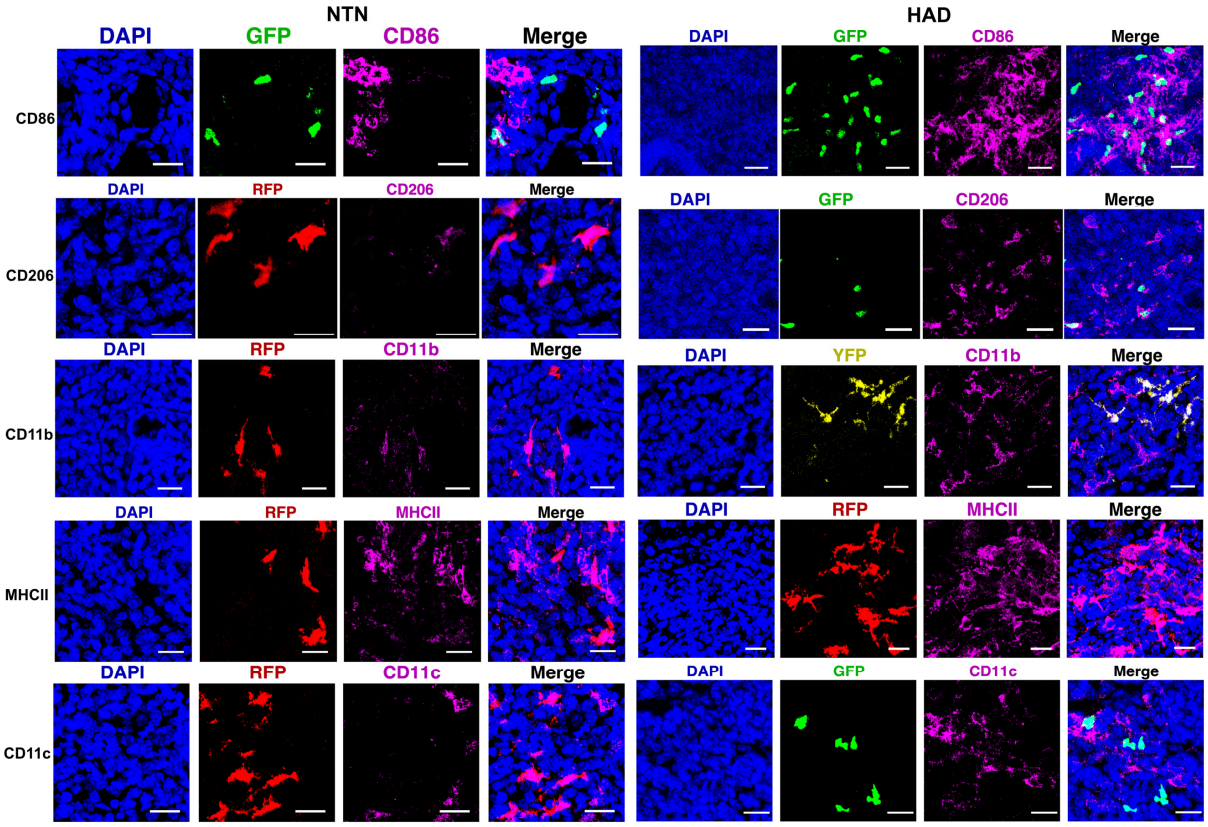
Supplementary Figure S3. Rendering model of confocal stack image of kidney from Microfetti mice, showing colored points representing each individual MNP in the cortex (top) and medulla (bottom) from healthy (left), NTS (nephrotoxic serum)-treated (middle) and HAD (high adenine diet)-treated (right) mice.



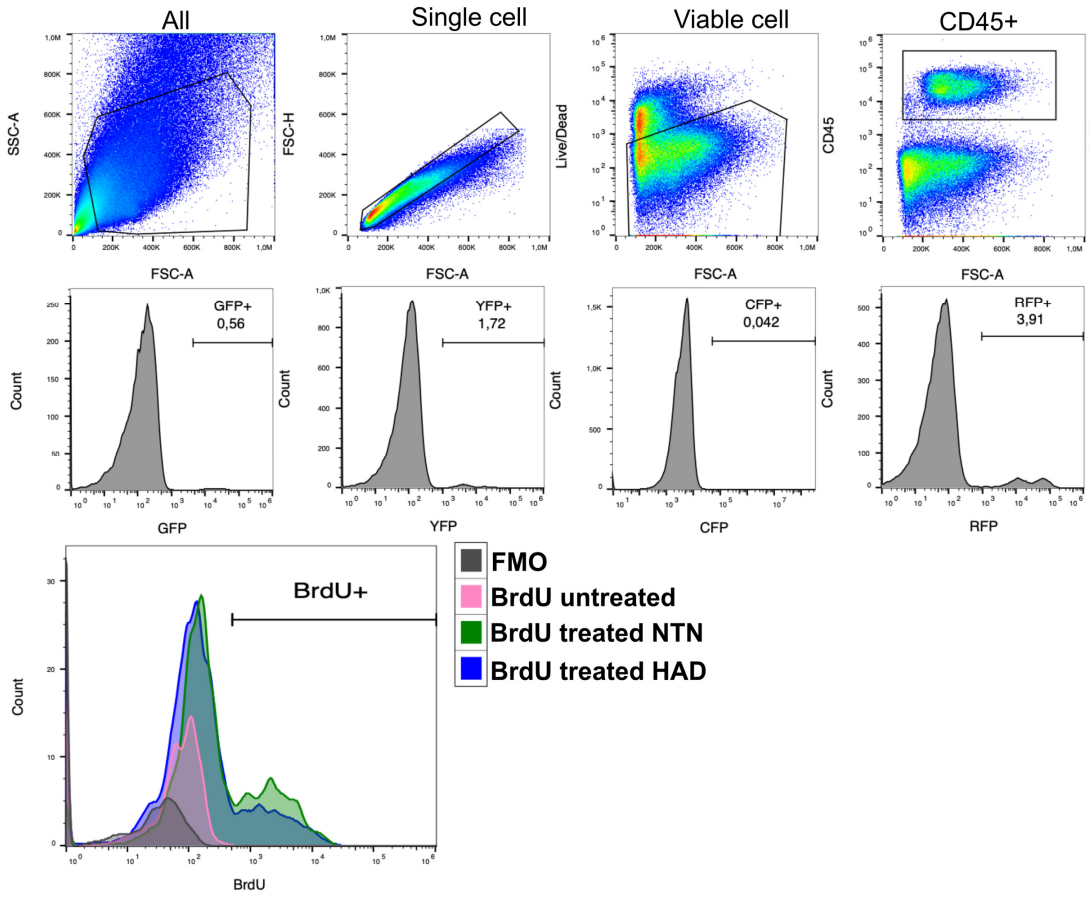
Supplementary Figure S4. Flow cytometry analysis of Confetti labeling in Ly6c$^+$ blood monocytes and F4/80$^+$ renal MNPs. (a) Gating strategy of Confetti$^+$ cells from Ly6c$^+$ blood monocytes. (b) Gating strategy of Confetti$^+$ MNPs from F4/80$^+$ renal MNPs. (c) Quantitative measurement of the Confetti$^+$ cell proportion in blood monocytes and renal MNPs. Statistical analyses were done by Student's t test or Mann Whitney U test. ****, P<0.0001, ns, not significant.



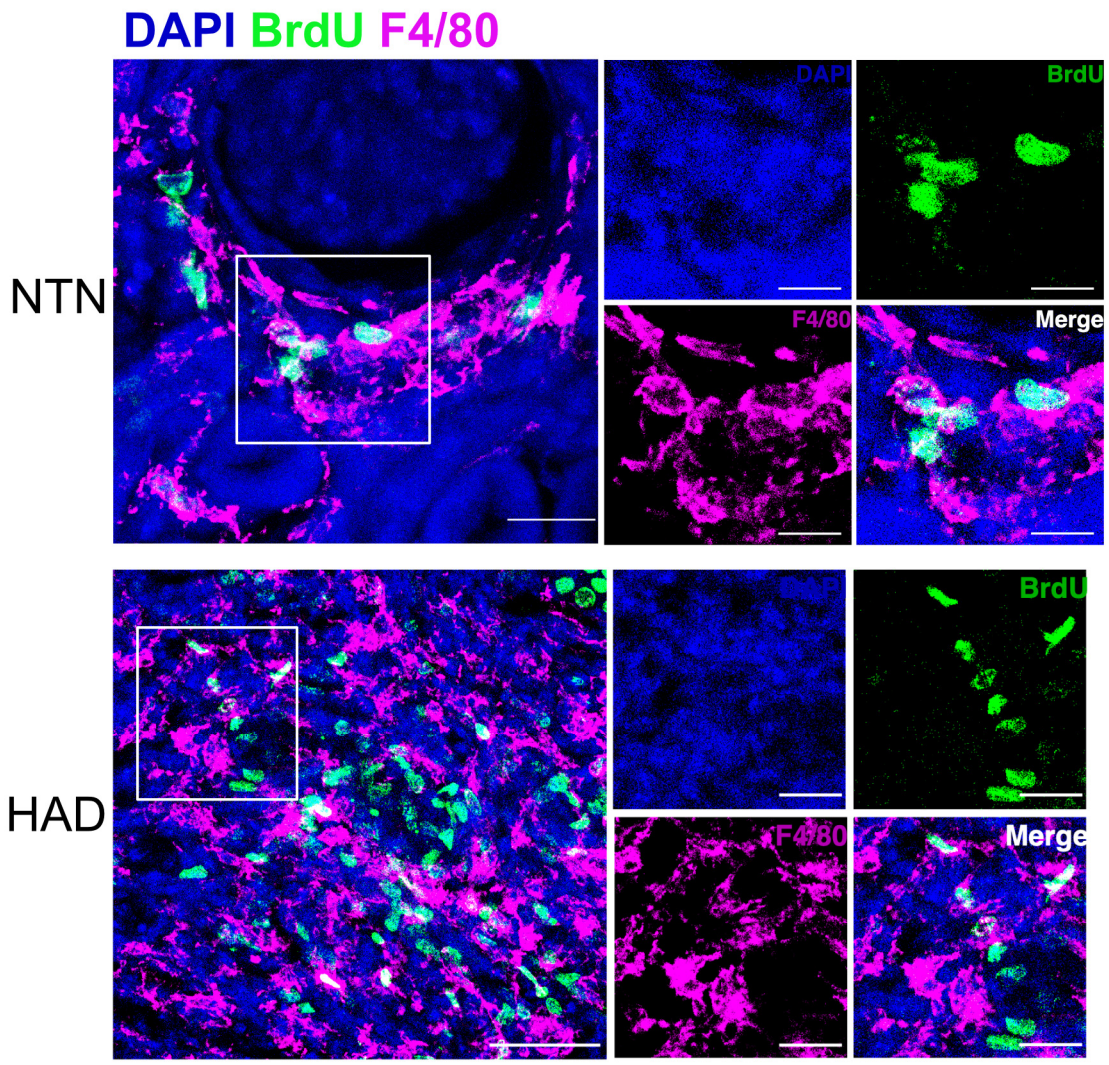
Supplementary Figure S5. (a) Rendering model (top row) of confocal stack image of kidney from healthy and NTN Microfetti mouse at 8 weeks after TAM injection and corresponding confetti^{+} cell density from recorded data (purple) and Monte Carlo simulation (grey) (bottom row). Densities of recorded data and Monte Carlo simulation were used for calculation of division events in Figure 2h. (b) Rendering model (top row) of confocal stack image of kidney from healthy and HAD Microfetti mouse at 8 weeks after TAM injection and corresponding confetti^{+} cell density from recorded data (purple) and Monte Carlo simulation (grey) (bottom row). Densities of recorded data and Monte Carlo simulation were used for calculation of division events in Figure 2i.



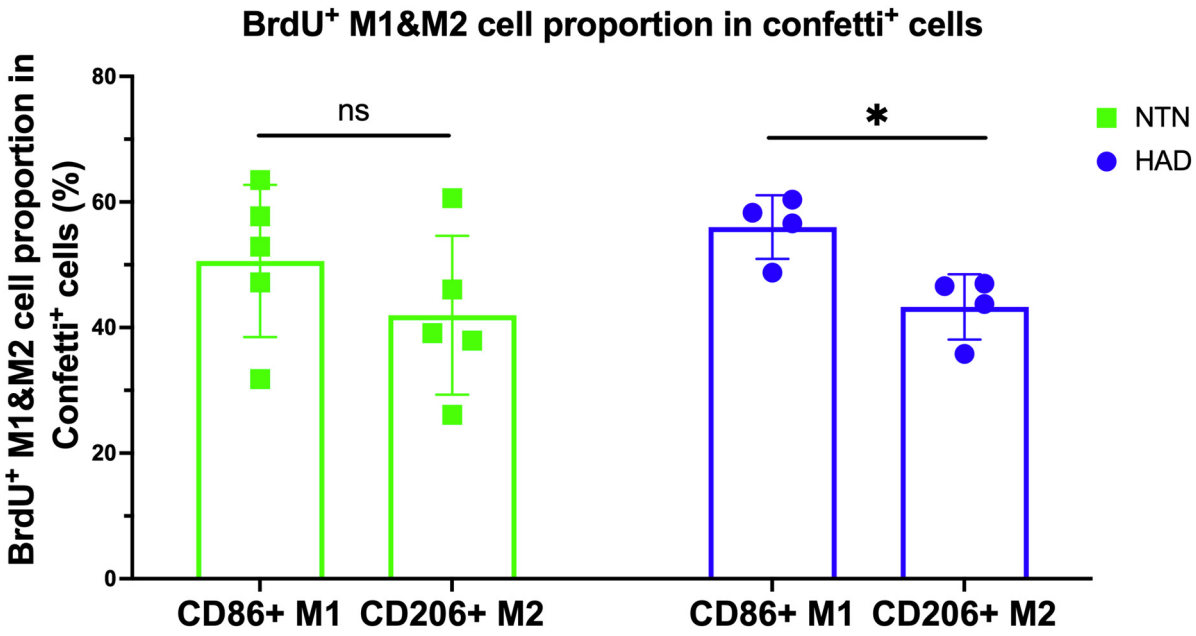
Supplementary Figure S6. Confocal images showing the expression of surface markers CD86, CD206, CD11b, MHCII and CD11c in confetti clones from NTN (left) and HAD (right) treated Microfetti mice. Scale bar, 20um.



Supplementary Figure S7. Gating strategy of BrdU^{+} cells (Figure 2k) among Confetti^{+} cells in kidneys of Microfetti mice.



Supplementary Figure S8. Confocal images of BrdU^{+} F4/80^{+} MNPs in kidneys from NTN and HAD treated Microfetti mice. Scale bar, main panel, 50um, inset panel, 20um



Supplementary Figure S9. Cell proportion of BrdU$^+$ CD86$^+$ M1 and CD206$^+$ M2 cells within Confetti$^+$ BrdU$^+$ cells in kidneys of NTN (green) and HAD (blue) treated Microfetti mice. Data presented as mean ± SD and statistical analysis was performed by using Student's t test. *, P<0.05, ns, not significant.

Supplementary references

S1. Ludwig-Portugall I, Bartok E, Dhana E*, et al.* An NLRP3-specific inflammasome inhibitor attenuates crystal-induced kidney fibrosis in mice. *Kidney Int* 2016; **90:** 525-539.

S2. Hochheiser K, Engel DR, Hammerich L*, et al.* Kidney Dendritic Cells Become Pathogenic during Crescentic Glomerulonephritis with Proteinuria. *J Am Soc Nephrol* 2011; **22:** 306-316.

S3. Tay TL, Mai D, Dautzenberg J*, et al.* A new fate mapping system reveals context-dependent random or clonal expansion of microglia. *Nat Neurosci* 2017; **20:** 793-803.

S4. Hochheiser K, Heuser C, Krause TA*, et al.* Exclusive CX3CR1 dependence of kidney DCs impacts glomerulonephritis progression. *J Clin Invest* 2013; **123:** 4242-4254.