SUPPLEMENTAL CONCISE METHODS

Induction of apoptosis

S1pr3$^{-/-}$ BMDC in serum-free medium were treated with 150 mJ cm^{-2} ultraviolet C irradiation (Stratalinker 2400). After irradiation cells were returned to a 37°C incubator for 6h prior to injection. The level of apoptosis in control and UV-irradiated BMDCs was determined by flow cytometry using 7-AAD and Annexin-V. BMDCs were resuspended in 1× binding buffer (BD Pharmingen, cat. no. 556454); 100 µl of cells were incubated with 5 µl of FITC-conjugated Annexin V and 3 µl of 7-AAD for 15 min at room temperature in the dark, the reaction was stopped by addition of 400 µl of 1× binding buffer, and cells were analyzed by FACS.

Generation of bone marrow chimeric mice and diphtheria toxin–mediated dendritic cell (DC) depletion

CD11c-DTRtg mice (B6.FVB-Tg Itgax-DTR/GFP 57Lan/J) harboring a transgene encoding a simian diphtheria toxin receptor (DTR)-GFP fusion protein under the transcriptional control of mouse CD11c promoter were purchased (Jackson Laboratory). Bone marrow chimeric mice were generated as described.[1] Briefly, lethally irradiated recipient wild type C57BL/6 mice were injected with 10^7 CD11c-DRTtg donor BM cells (i.v.), and the resulting chimeric mice were maintained for 6–8 wk before experimentation. Donor CD11c-DRTtg DCs were then depleted in these chimeric mice by a single injection of DT (Sigma Aldrich, St. Louis, MO; 4 ng/g body weight, i.p.), and ablation was confirmed by immunohistochemical staining for CD11c$^+$MHCII$^+$ DCs in kidney.[2] Chimeric mice generated in the same way but using donor BM cells from CD11c-DTRtg littermates negative for the transgene (CD11c-DTR$^-$) were used as controls and were also treated with DT. LPS-treated WT or *S1pr3*$^{-/-}$ BMDCs ($0.5x10^6$) were injected i.v. 20h

after DT injection into CD11c-DTRtg→WT B6 chimeras and control mice, and IRI was performed 20h after DC injection. In some experiments DT was administered 20h after BMDC adoptive transfer, and IRI was performed 20h later.

Supplemental References

1. Bajwa A, Huang L, Ye H, Dondeti K, Song S, Rosin DL, Lynch KR, Lobo PI, Li L, Okusa MD: Dendritic cell sphingosine 1-phosphate receptor-3 regulates Th1-Th2 polarity in kidney ischemia-reperfusion injury. *J Immunol*, 189**:** 2584-96, 2012
2. Li L, Okusa MD: Macrophages, dendritic cells, and kidney ischemia-reperfusion injury. *Semin Nephrol,* 30**:** 268-77, 2010

Supplemental Figure Legends

Supplemental Figure 1. Apoptotic *S1pr3*$^{-/-}$ BMDC do not protect kidneys from IRI. (A) UV irradiation resulted in ~80% cell death as determined by FACS. Control or apoptotic *S1pr3*$^{-/-}$ BMDC ($0.5X10^6$) were injected (i.v.) 1d prior to kidney IRI. Recipient mouse kidneys were exposed to 26min ischemia followed by 24h reperfusion and samples were collected 24h later. (A) Percent of apoptosis (late + early) at 6h after UV irradiation. (B) Plasma creatinine 24h after kidney IRI. (C) H&E staining of kidney sections from the same mice. (D) Recipient mouse kidneys were exposed to 26min ischemia followed by *S1pr3*$^{-/-}$ BMDC at 1h or 4h after IR. **, $p<0.01$. values are mean ± SEM. n=3-4 mice. **$p<0.01$, values are mean ± SEM. n=3-4.

Supplemental Figure 2. Depletion of CD11c$^+$ DCs prevents *S1pr3*$^{-/-}$ BMDC-dependent protection in kidney IRI. Administration of diphtheria toxin depletes CD11c$^+$ cells in CD11c-DTR$^+$ mice and causes robust protection from IRI.[2,1] To direct the selectivity of diphtheria toxin to bone marrow derived CD11c expressing cells, we generated CD11c-DTR$^+$→WT bone marrow chimeric mice. In control (CD11c-DTR$^-$(WT)→WT) mice *S1pr3*$^{-/-}$ BMDC were able to protect kidney from IRI. (A) CD11c-DTR$^+$→WT and CD11c-DTR$^-$(WT)→WT chimeric mice were treated with diphtheria toxin (DT) 2d prior to sham or IRI. Mice depleted of DCs were injected (i.v.) with PBS (no cells, NC), WT BMDC, or *S1pr3*$^{-/-}$ BMDC 1d prior to IRI. Mice negative for DTR (CD11c-DTR$^-$(WT)→WT) but treated with DT were used as controls. (B) H&E staining of kidney sections from the same mice. Scale bar = 100 μm. *, $p<0.05$. ***, $p<0.001$. Values are mean ± SEM. n=5-6 for IRI mice and n=2 for sham-operated mice. (C) Plasma creatinine in *Rag-1*$^{-/-}$ mice treated with WT BMDC or *S1pr3*$^{-/-}$ BMDC 1d prior to IRI.

(D) H&E staining of kidney sections from the same mice. Depletion of DCs 1d after injection of WT-BMDC or *S1pr3*$^{-/-}$ BMDC also prevented protection by *S1pr3*$^{-/-}$ BMDC (data not shown). Scale bar = 100 μm. n=4. *, $p<0.05$. Values are mean ± SEM.

Supplemental Figure 3. Splenectomy reconstitutes injury in *S1pr3*$^{-/-}$ mice exposed to IRI. Mice were subjected to sham surgery (control) or splenectomy (SPLX) 7d before kidney sham or IRI surgery. (A) Plasma creatinine 24h after kidney IRI. n=4-5 for IRI mice and n=2-3 sham. (B) H&E staining of kidney sections from the same mice. Scale bar = 100 μm. ***, $p<0.001$. Values are mean ± SEM.

Supplemental Figure 4. Low dose liposome clodronate depletes marginal zone CD169^{+} macrophages but not CD11b^{+} cells, CD11b^{+}Ly6G^{high} neutrophils, or F4/80^{+} cells in the spleen. WT mice were treated (i.p.) with low dose (167 μg) liposome clodronate or PBS-liposomes. (A) To assess specificity of cell depletion, spleens were harvested 1d after injection, and myeloid cell populations were evaluated by flow cytometry. (B) A low dose of liposome clodronate did not reduce levels of CD11b^{+}Ly6G^{high}, CD11b^{+} and F4/80^{hi+lo} populations in the spleen. By contrast, the high dose of liposome clodronate (1000 μg) depleted CD11b^{+}Ly6G^{high}, CD11b^{+} and F4/80^{hi+lo} compared to liposomes alone. (C) Using this low dose of liposome clodronate, depletion of marginal zone CD169^{+} macrophages was demonstrated by immunofluorescence labeling. Sections were labeled with CD169 (red, MZ macrophages), CD4 (green, T cells), F4/80^{+} (magenta, red pulp macrophages) or DAPI (blue, nuclei), White pulp (WP), red pulp (RP) and marginal zone (MZ). Scale bar = 100 μm.

Supplemental Tables

Supplemental Table 1: ATN scores of WT and *S1pr3*$^{-/-}$ mice treated with WT BMDC.

WT		***S1pr3***$^{-/-}$	
NC	**WT BMDC**	**NC**	**WT BMDC**
3.0±0.2	3.3±0.1	1.0±0.03[a]	4.0±0.1

Timing for ischemia/reperfusion and BMDC injection is as in Figure 2A-B. WT BMDCs stimulated with LPS were injected into naïve WT or *S1pr3*$^{-/-}$ mice 24h before IRI. Tissues were harvested 24h after kidney IRI. Values are mean ± SEM. n=3-4 in each group. [a]$p<0.05$ compared to WT BMDC-LPS in *S1pr3*$^{-/-}$ mice.

Supplemental Table 2: ATN scores of WT mice treated with NC, WT BMDC or *S1pr3*$^{-/-}$ BMDC 7d before kidney IRI.

WT		
NC	**WT BMDC-LPS**	***S1pr3*$^{-/-}$ BMDC-LPS**
3.5±0.02	3.7±0.3	1.5±0.3[a]

Timing for ischemia/reperfusion and BMDC injection is as in Figure 2E-F. WT or *S1pr3*$^{-/-}$ BMDCs stimulated with LPS were injected into naïve WT mice 7d before IRI. Tissues were harvested 24h after kidney IRI. Values are mean ± SEM. n=3-4 in each group. [a]$p<0.05$, compared to NC and WT BMDC-LPS groups; n=3.

Supplemental Table 3: ATN scores in kidneys of diphtheria toxin-pretreated CD11c-DTR^{-} (WT)→WT and CD11c-DTR^{+}→WT chimeric mice subsequently treated with NC, WT BMDC and *S1pr3*$^{-/-}$ BMDC.

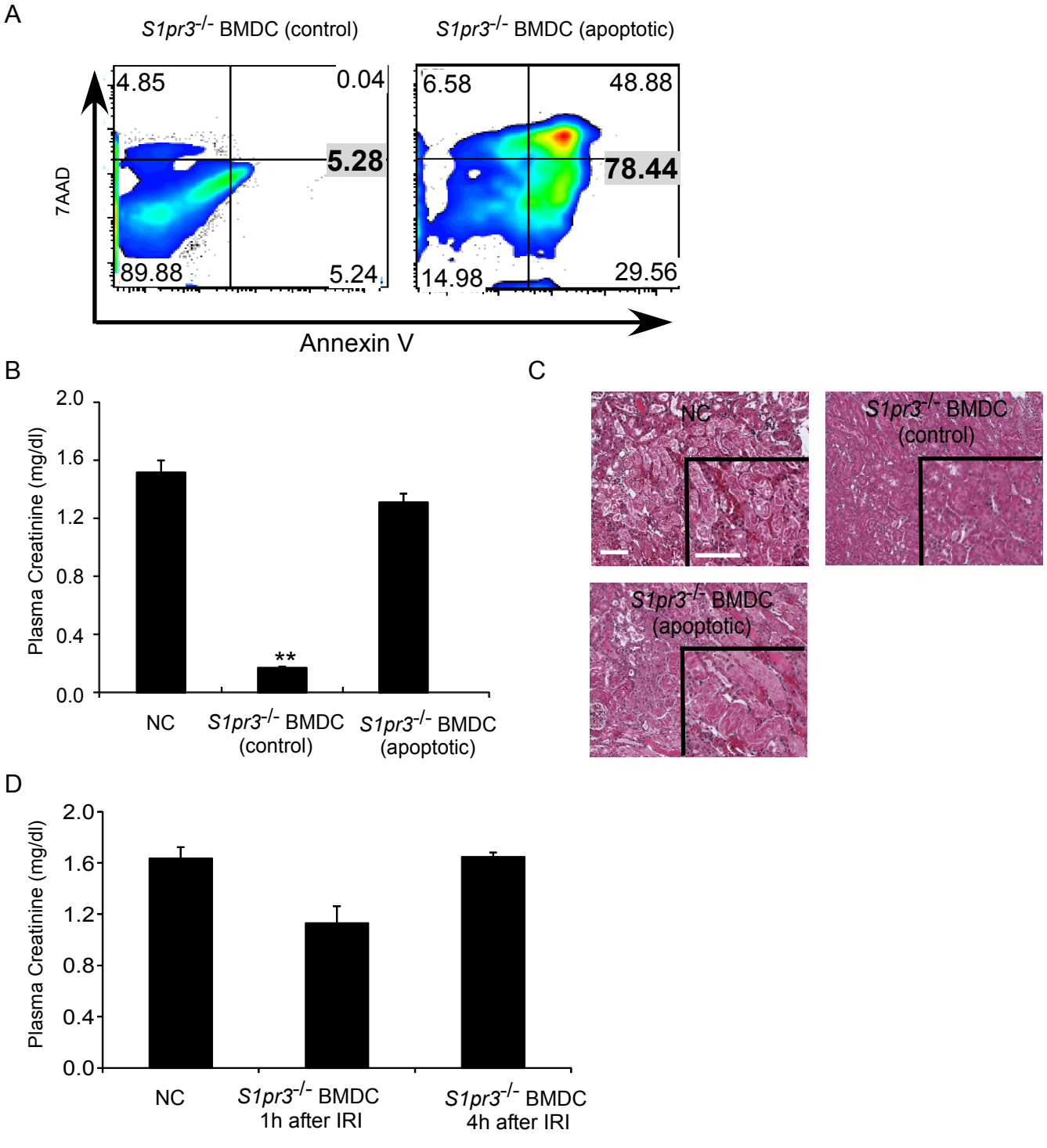
CD11c-DTR^{-}→WT				**CD11c-DTR^{+}→WT**			
Sham	**NC**	**WT BMDC**	***S1pr3*$^{-/-}$ BMDC**	**Sham**	**NC**	**WT BMDC**	***S1pr3*$^{-/-}$ BMDC**
1.0±0.01	3.3±0.02	3.3±0.17	1±0.35[a]	1±0.17	1.5±0.17	2.8±0.16	2.8±0.36

Timing for ischemia/reperfusion, diphtheria toxin (DT, 4ng/g, i.p -2d) and BMDC injections ($0.5X10^6$, i.v. -1d) . Tissues were collected 24h after kidney IRI. Values are mean ± SEM. n=5-6 in each group. [a]$p<0.05$ compared to NC and WT BMDC-LPS in CD11c-DTR^{-} mice.

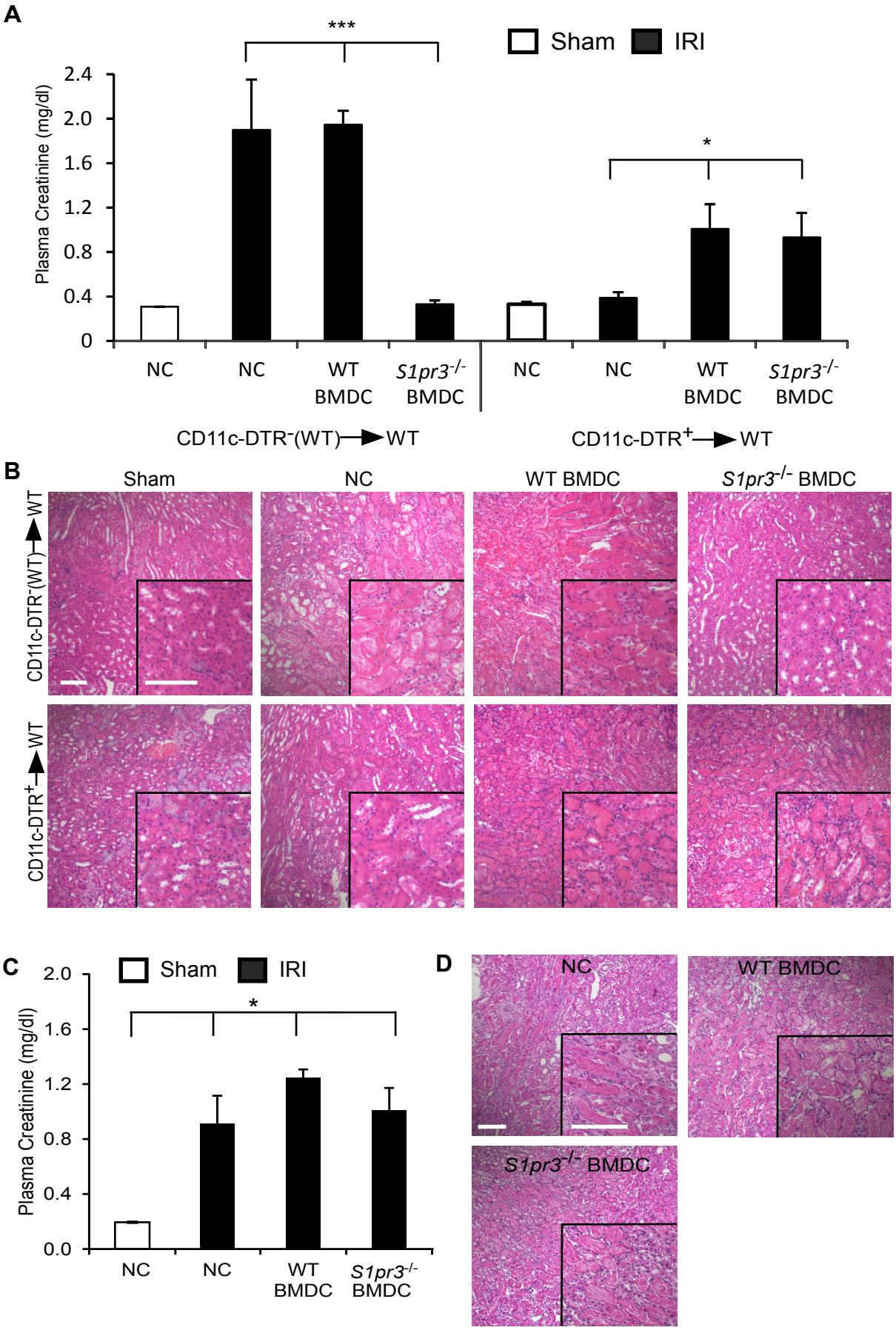
Supplemental Table 4: Acute tubular necrosis (ATN) scores in kidneys of control and splenectomized (SPLX) WT and *S1pr3*$^{-/-}$ mice after IRI.

WT		***S1pr3***$^{-/-}$	
Control-IRI	**SPLX-IRI**	**Control-IRI**	**SPLX-IRI**
3.5±0.3	4.0±0.2	1.5±0.1[a]	3.5±0.3

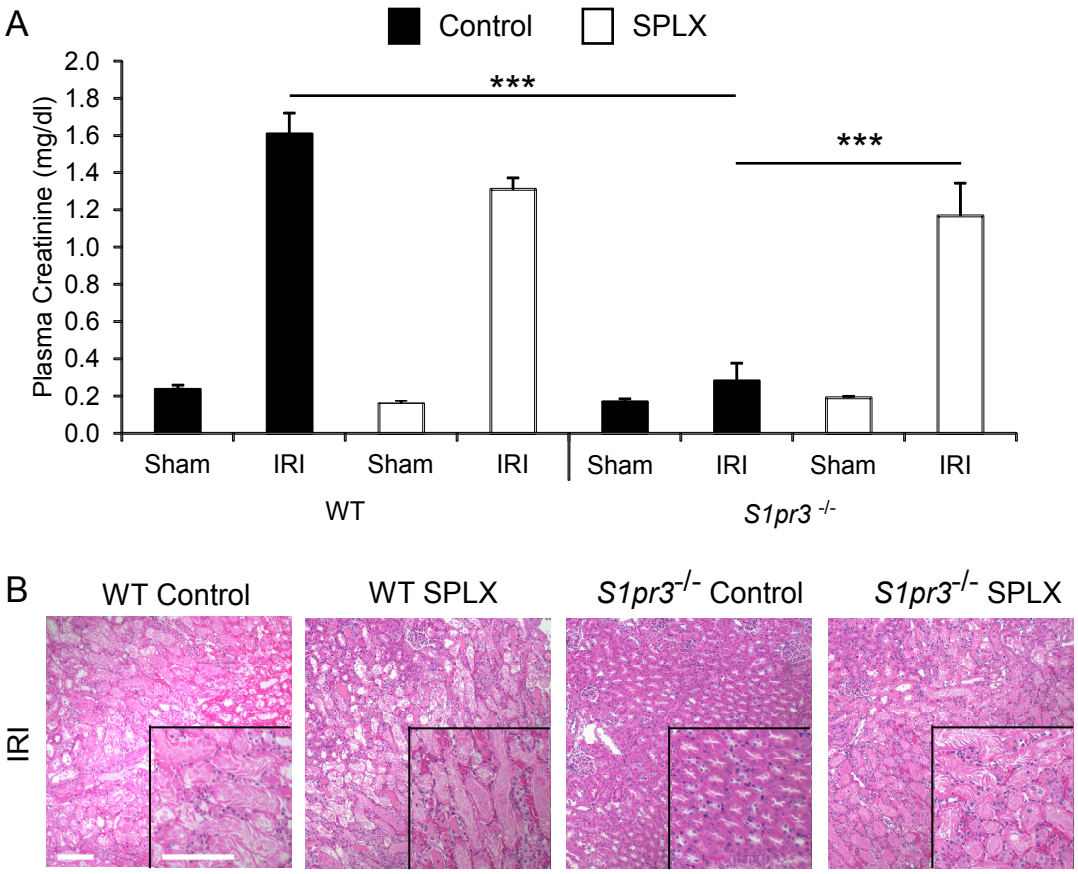
Timing for ischemia/reperfusion and SPLX as in Supplemental Figure 3A. Tissues were collected 24h after kidney IRI. Values are mean ± SEM. n=4-5 in each group. [a]$p<0.05$ compared to control or SPLX-IRI in WT mice and to SPLX-IRI in *S1pr3*$^{-/-}$ mice.



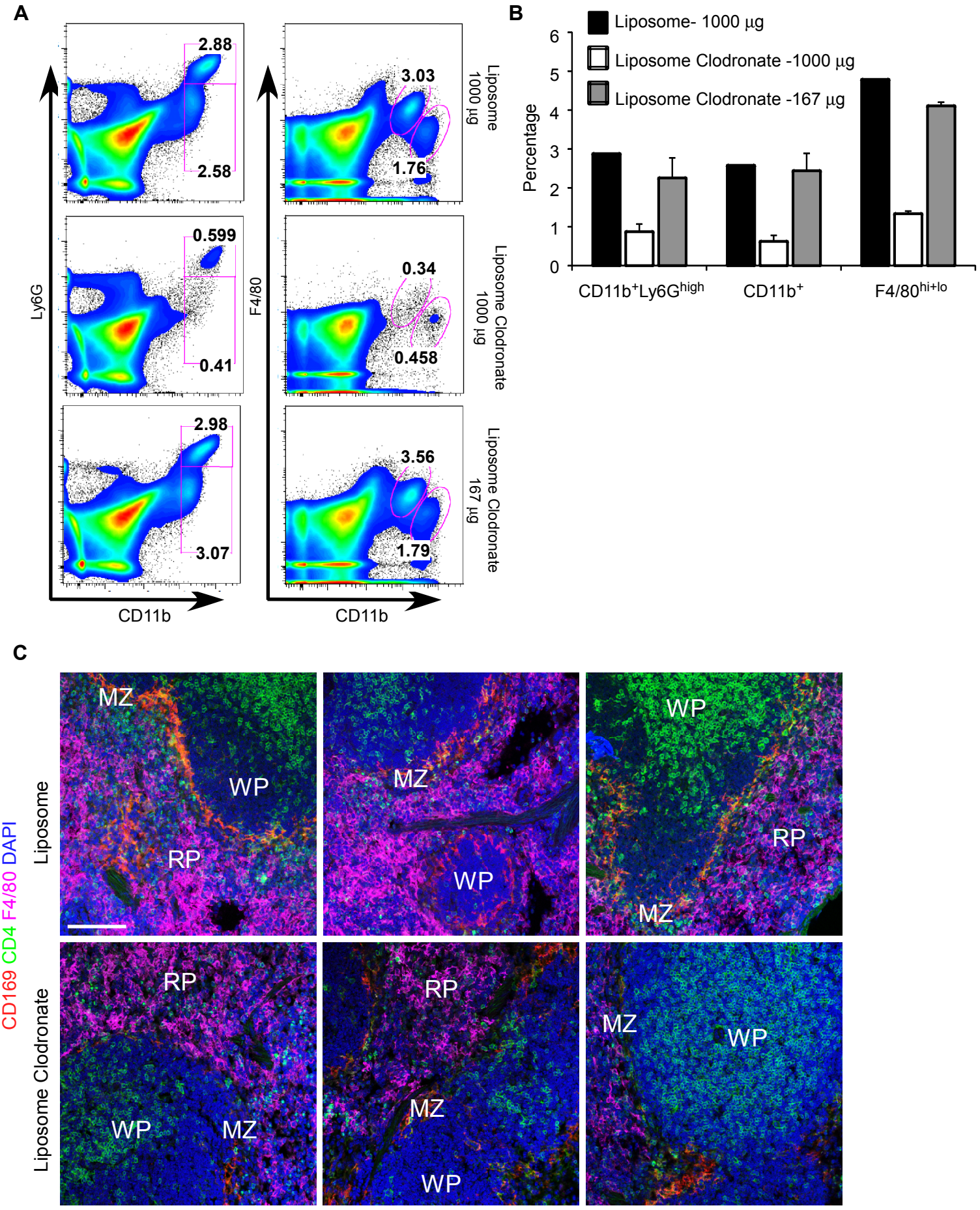
Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4