SUPPLEMENTAL MATERIALS

CONTENTS

Supplemental Table1. Primer sets used for real-time reverse transcription-polymerase chain reaction

Gene	Primer sequence (5'-3')	Annealing temperature (°C)
IL-1β	F: ACTCATTGTGGCTGTGGAGA R: TTGTTCATCTCGGAGCCTGT	60
IL-6	F: CTGGGGATGTCTGTAGCTCA R: CTGTGAAGTCTCCTCTCCGG	60
MCP-1	F: AGGTGTCCCAAAGAAGCTGT R: ACAGAAGTGCTTGAGGTGGT	60
IFN-γ	F: GATTGCGGGGTTGTATCTGG R: GCTTTCTTTCAGGGACAGCC	60
IL-17A	F: ACTCTCCACCGCAATGAAGA R: CTCTCAGGCTCCCTCTTCAG	60
IL-10	F: GGTGAGAAGCTGAAGACCCT R: TGTCTAGGTCCTGGAGTCCA	60
GAPDH	F: CAACTCCCACTCTTCCACCT R: GAGTTGGGATAGGGCCTCTC	60

F, forward; IFN-γ, interferon-γ; IL-1β, interleukin-1β; IL-6, interleukin-6; IL-10, interleukin-10; IL-17, interleukin-17; MCP-1, monocyte chemoattractant protein-1; R, reverse

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. Flow cytometric gating strategies for various leukocyte populations in the spleen and kidneys. (A) Gating strategies for live, singlet leukocytes in the spleen and kidneys. (B) Flow cytometry 2-dimensional diagrams for $Foxp3^+CD4^+$ regulatory T cells and $Tim\text{-}1^+CD19^+$ regulatory B cells, $Gr\text{-}1^+$ neutrophils, and $F4/80^+$ macrophages in the spleen and kidneys. 7-AAD, 7-Amino Actinomycin D; FSC, forward scatter; PBS, phosphate-buffered saline; SSC, side scatter.

Supplemental Figure 2. Adoptively-transferred regulatory B cells were found in the spleen and kidneys after IRI. (A) Sorted $CD45.1^+Tim\text{-}1^+CD19^+$ Breg cells were transferred to $CD45.2^+$ mice 1 day prior to IRI. When mice were harvested 1 day after IRI, transferred $CD45.1^+$ Bregs were found in the spleen and kidneys. (B-C) Proportions (B) and numbers (C) of transferred $CD45.1^+$ Bregs in the spleen and kidneys. Results were expressed as dot plots with the mean ± standard error of the mean. N = 3. *P<0.05, **P<0.01 compared to the PBS control group. Breg, regulatory B cell; PBS, phosphate-buffered saline.

Supplemental Figure 3. Impact of Treg depletion on the renoprotective effects of Breg transfer against IRI. (A) Depleting anti-CD25 was administered to mice where Bregs or PBS were transferred prior to IRI; mice were harvested 1 day after IRI. (B) Levels of serum creatinine and BUN after IRI. (C) Proportions of splenic and renal $CD4^+Foxp3^+$ Tregs after IRI. (D) Proportions of splenic and renal $Tim\text{-}1^+CD19^+$ or $IL\text{-}10^+CD19^+$ Bregs after IRI. Results were expressed as dot plots with the mean ± standard error of the mean. N = 3–9. *P<0.05, **P<0.01 comparison between the PBS control group and the anti-CD45RB group. $^{\#}$P<0.05, $^{\#\#}$P<0.01,

comparison between the PBS/anti-CD25 group and the anti-CD45RB/anti-CD25 group. Bregs, regulatory B cells; BUN, blood urea nitrogen; IRI, ischemia-reperfusion injury; PBS, phosphate-buffered saline; Tregs, regulatory T cells; WT, wild-type.

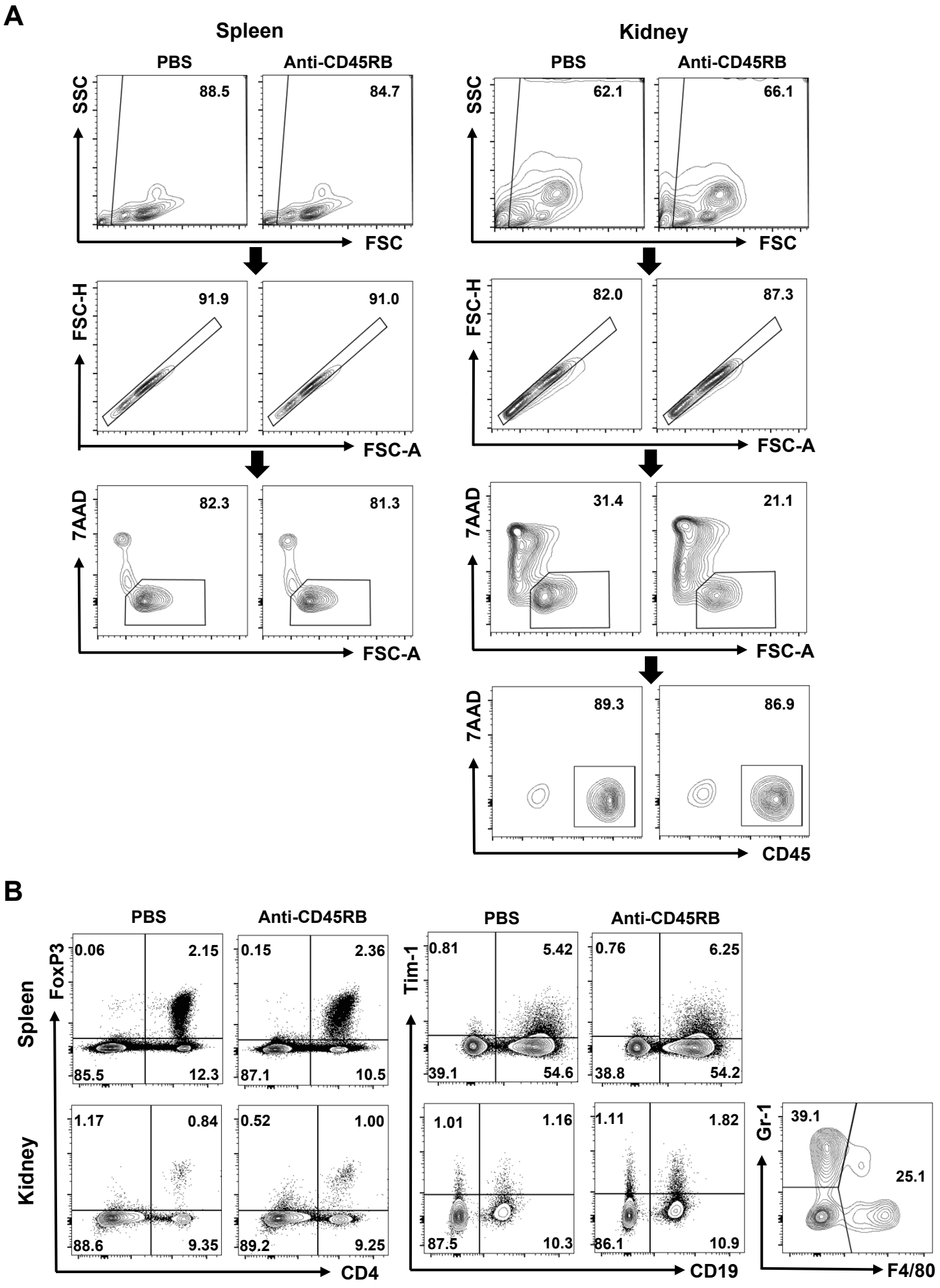
Supplemental Figure 4. Comparison of the renoprotective effects of anti-CD45RB and anti-Tim-1 treatment against renal IRI. (A) Anti-CD45RB, anti-Tim-1 or PBS was administered to WT mice 1 day prior to IRI; mice were harvested 1 day after IRI. (B) Levels of serum creatinine and BUN after IRI. (C) Flow cytometry analysis of splenic Bregs and Tregs after IRI. (D) Flow cytometry analysis of renal Bregs and Tregs after IRI. Results were expressed as dot plots with the mean ± standard error of the mean. N = 3–5. *P<0.05, **P<0.01 compared to the PBS control group. $^{\#}$P<0.05, comparison between the anti-CD45RB group and the anti-Tim-1 group. Bregs, regulatory B cells; BUN, blood urea nitrogen; IRI, ischemia-reperfusion injury; PBS, phosphate-buffered saline; Tregs, regulatory T cells; WT, wild-type.

Supplemental Figure 5. Flow cytometric diagrams for regulatory B cells and depletion of B or T cells. (A) Flow cytometric diagrams for Tim-1^{+}CD19^{+} Bregs in the spleen and the kidney 1 day after IRI. (B) Flow cytometric diagrams for IL-10^{+}CD19^{+} Bregs in the spleen and the kidney 1 day after IRI. (C) Treatment of anti-CD4 and anti-CD8 nearly completely depleted both CD4^{+} and CD8^{+} T cells in the spleen. (D) Anti-CD20 treatment depleted splenic CD19^{+} B cells effectively. Results were expressed as dot plots with the mean ± standard error of the mean. N = 3–9, *P<0.05, **P<0.01 compared to the PBS control group. $^{\#}$P<0.05, anti-CD45RB group compared to the anti-CD45RB/anti-Tim-1 group. Bregs, regulatory B cells; IRI, ischemia-reperfusion injury; PBS, phosphate-buffered saline.

Supplemental Figure 6. Renal infiltration of regulatory B cells after IRI. Anti-CD45RB or PBS was administered to WT mice 1 day prior to IRI; mice were harvested 1 day after IRI. Representative immunofluorescence images for renal infiltration of Tim-1^{+}CD19^{+} Bregs in the sham, PBS, and anti-CD45 groups. Magnification ×200; magnification in right-upper quadrant of merge views ×400. Bregs, regulatory B cells; DAPI, 4′,6-diamidino-2-phenylindole; IRI, ischemia-reperfusion injury; PBS, phosphate-buffered saline; WT, wild-type.

Supplemental Figure 7. Role of immunosuppressive molecules of regulatory B cells in the renoprotective effects of anti-CD45RB treatment against IRI. (A) Role of TGF-β of B cells in the renoprotective effects of anti-CD45RB treatment against IRI. First, LAP expression in the splenic and renal B cells after IRI was assessed. Next, anti-TGF-β antibody was administered to WT mice with anti-CD45RB or PBS 1 day prior to IRI; mice were harvested on 1 day after IRI. Then, levels of serum creatinine and BUN were assessed along with proportions of splenic and renal Bregs. (B) Role of IDO of B cells in the renoprotective effects of anti-CD45RB treatment against IRI. First, IDO expression in the splenic and renal B cells after IRI was assessed. Next, B cells sorted from IDO KO mice were transferred to RAG1 KO mice 2 weeks before IRI. Anti-CD45RB or PBS was administered to RAG1 KO mice with transferred IDO-deficient B cells 1 day prior to IRI; mice were harvested on 1 day after IRI. Then, levels of serum creatinine and BUN were assessed along with proportions of splenic and renal Bregs. (C-E) Changes in expression of PD-L1 (C), FasL (D), and IL-35 (IL-12α, Ebi3) (E) in the splenic and renal B cells in response to anti-CD45RB treatment in IRI. Results were expressed as dot plots with the mean ± standard error of the mean. N = 4–6. *P<0.05, **P<0.01 compared to the PBS control group. $^{\#}$P<0.05, comparison between the PBS/anti-TGF-β group and anti-

CD45RB/anti-TGF-β group. Bregs, regulatory B cells; BUN, blood urea nitrogen; FasL, Fas ligand; IDO, indoleamine-pyrrole 2,3-dioxygenase; IRI, ischemia-reperfusion injury; KO, knock out; LAP, latency associated peptide; PBS, phosphate-buffered saline; PD-L1, programmed death-ligand 1; RAG1, recombination activating gene 1; TGF, transforming growth factor; WT, wild-type.



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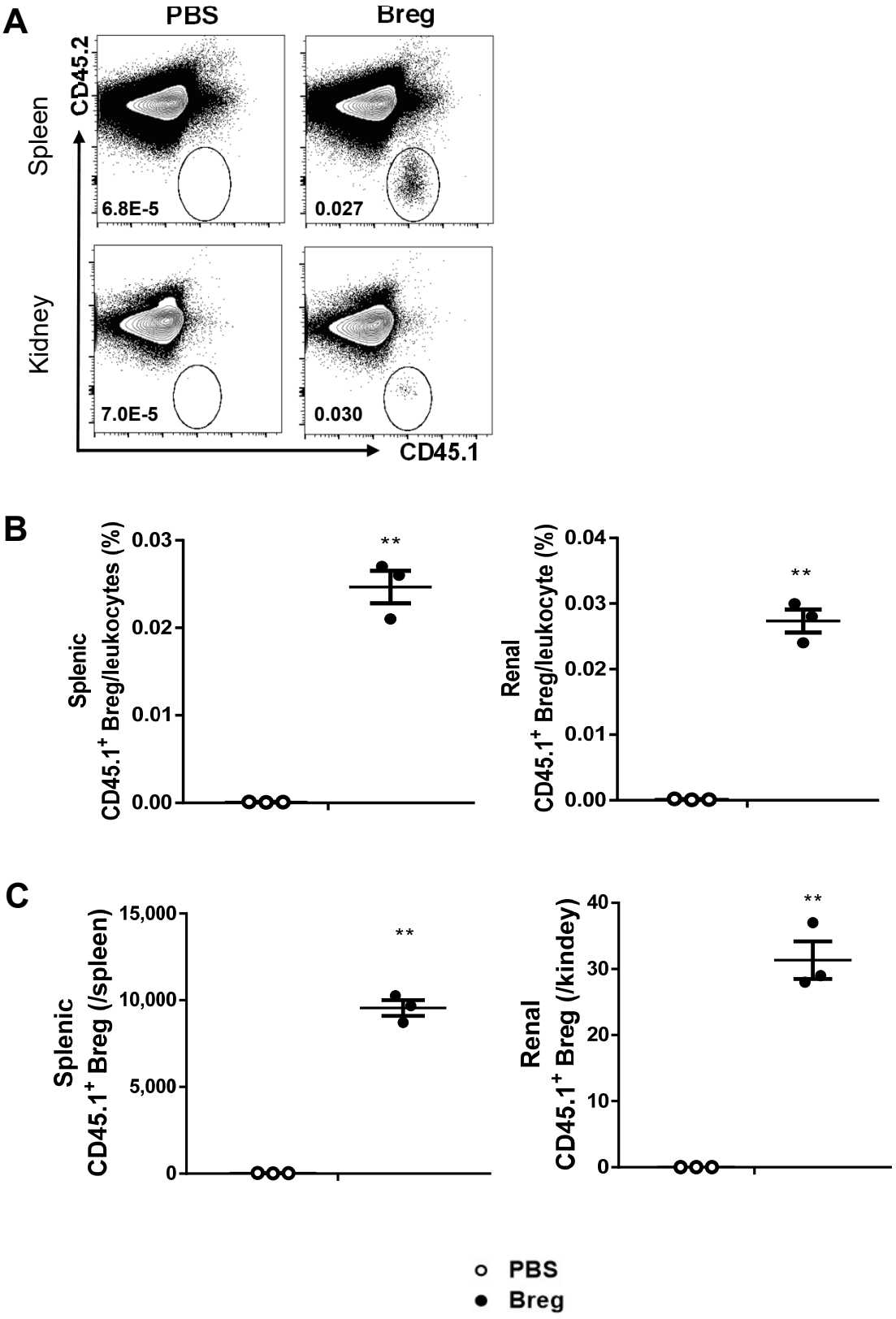

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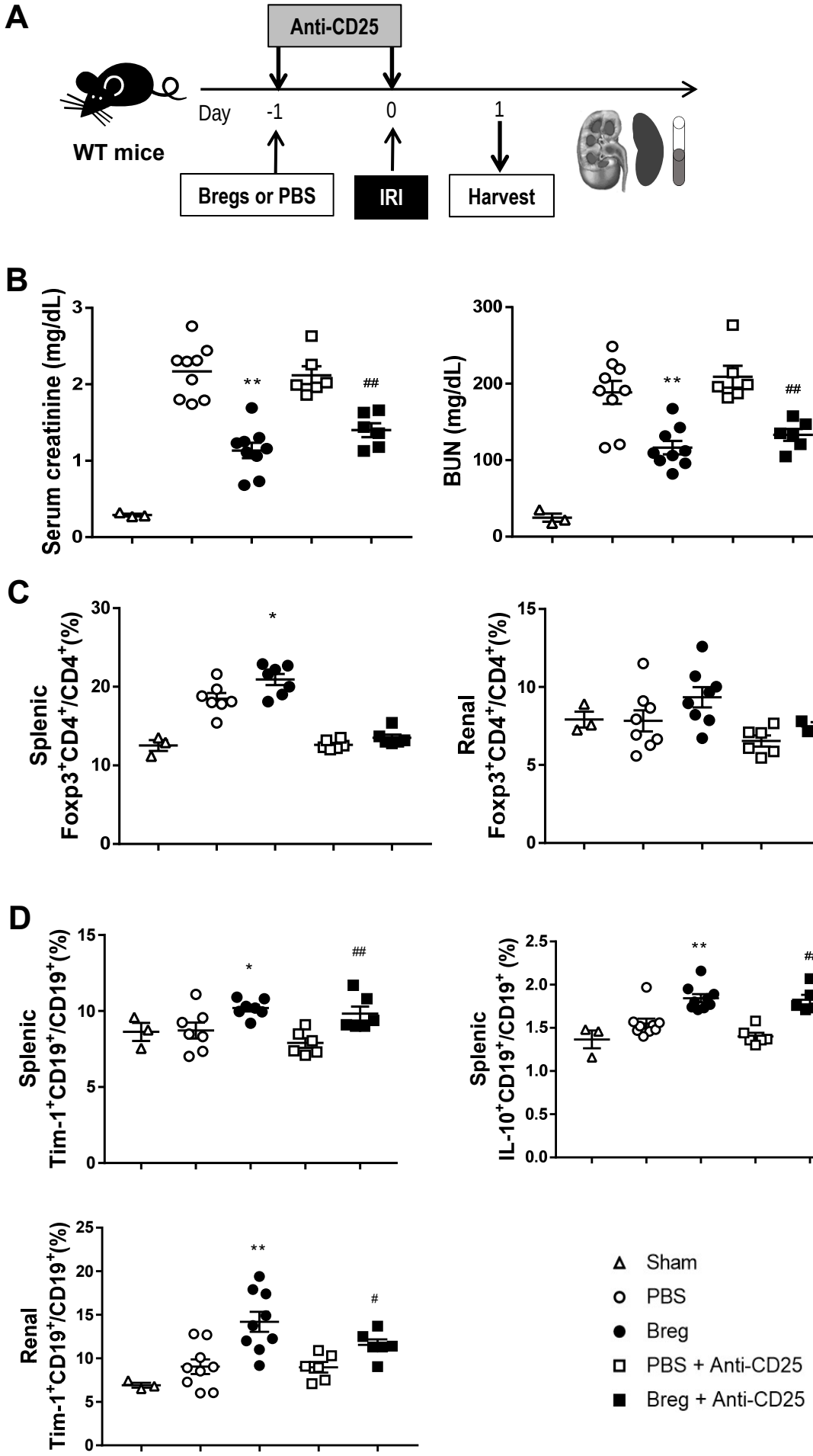

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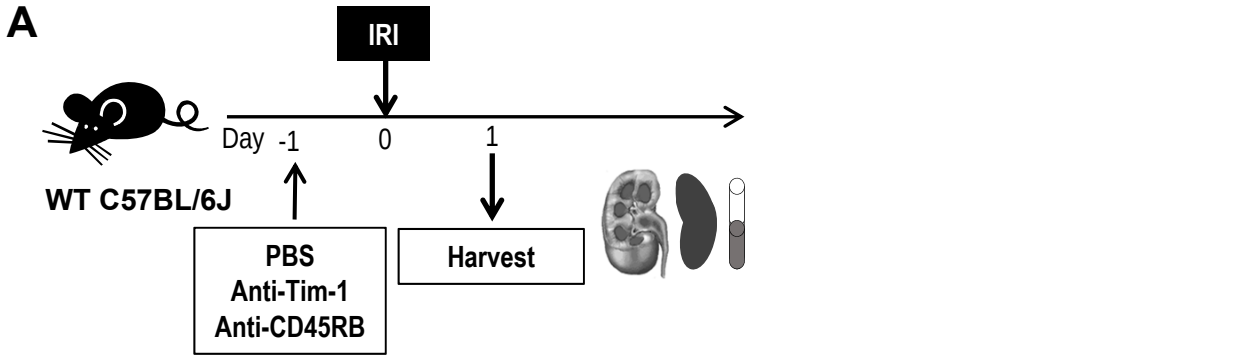

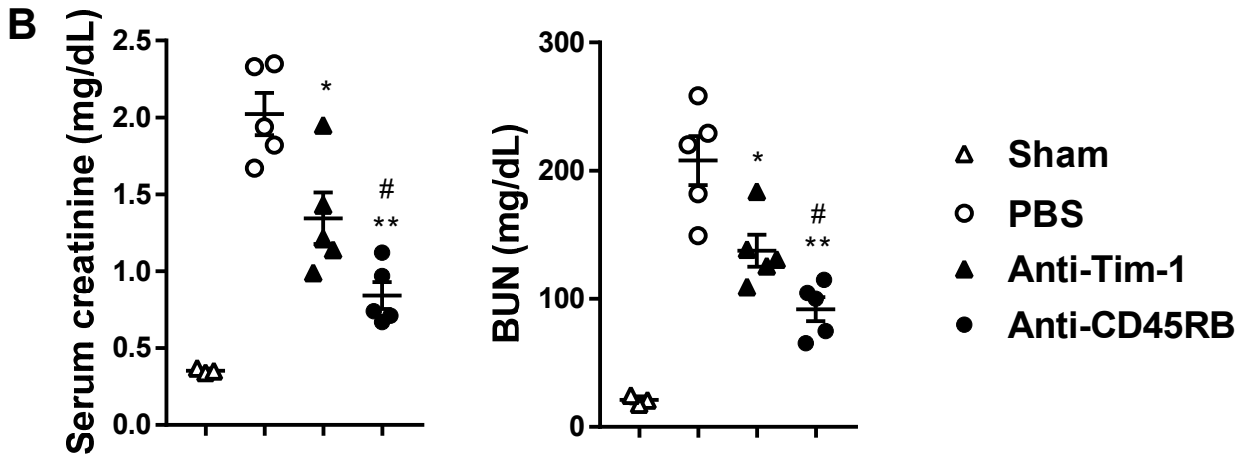

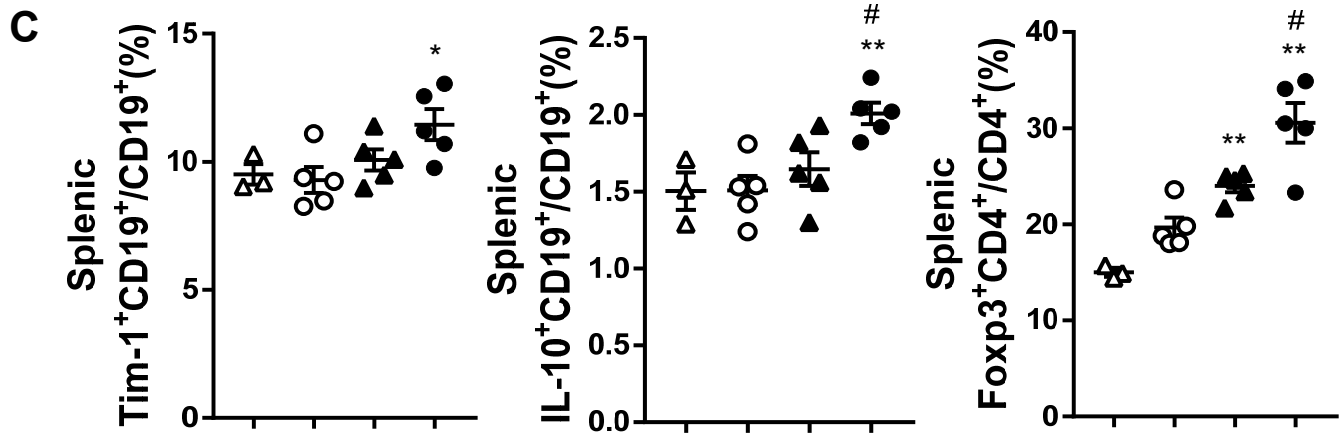

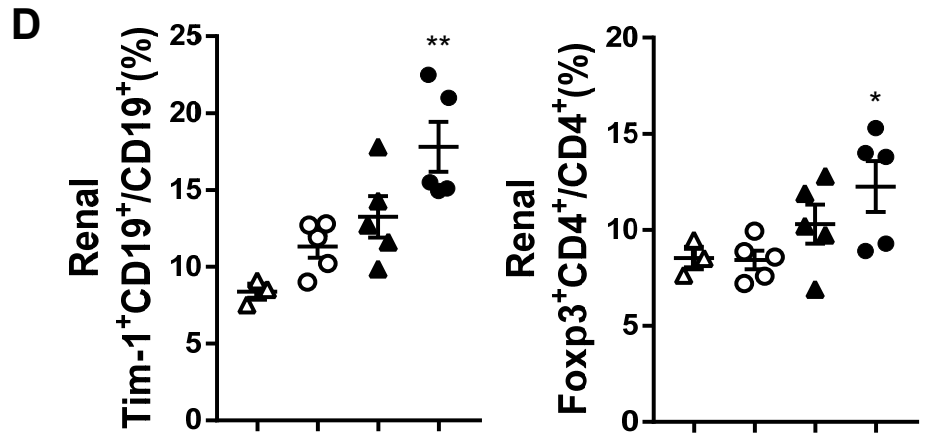

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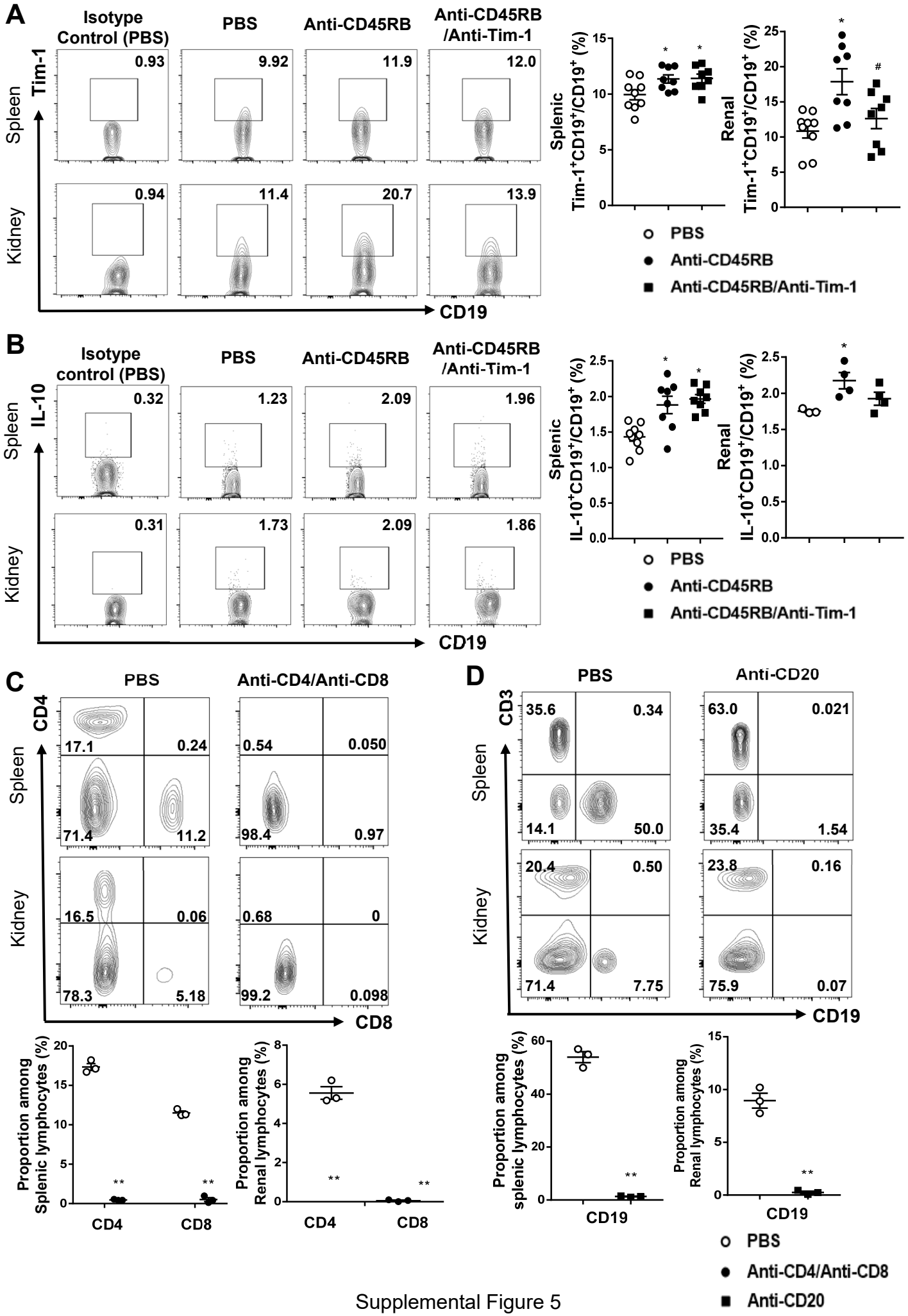

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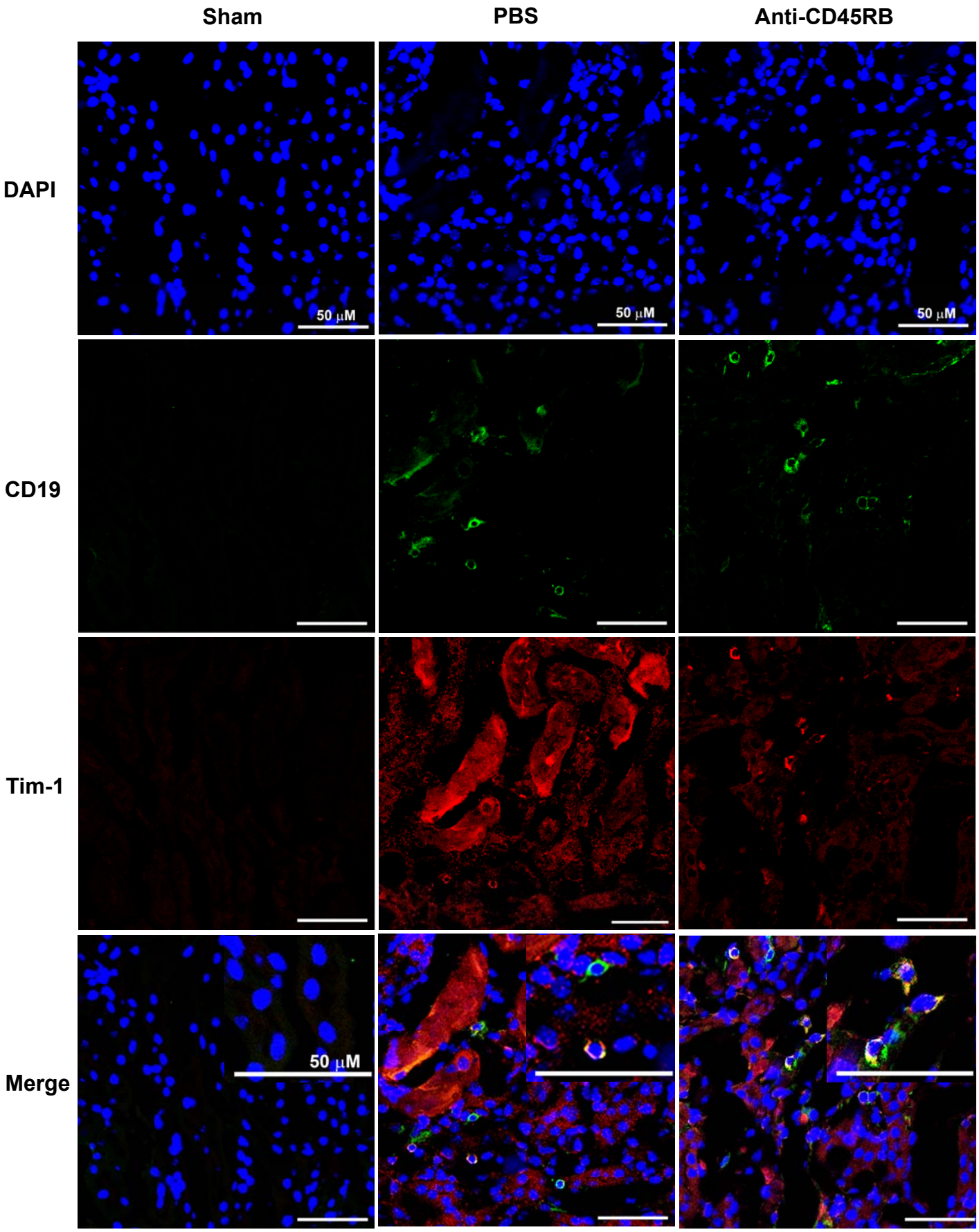

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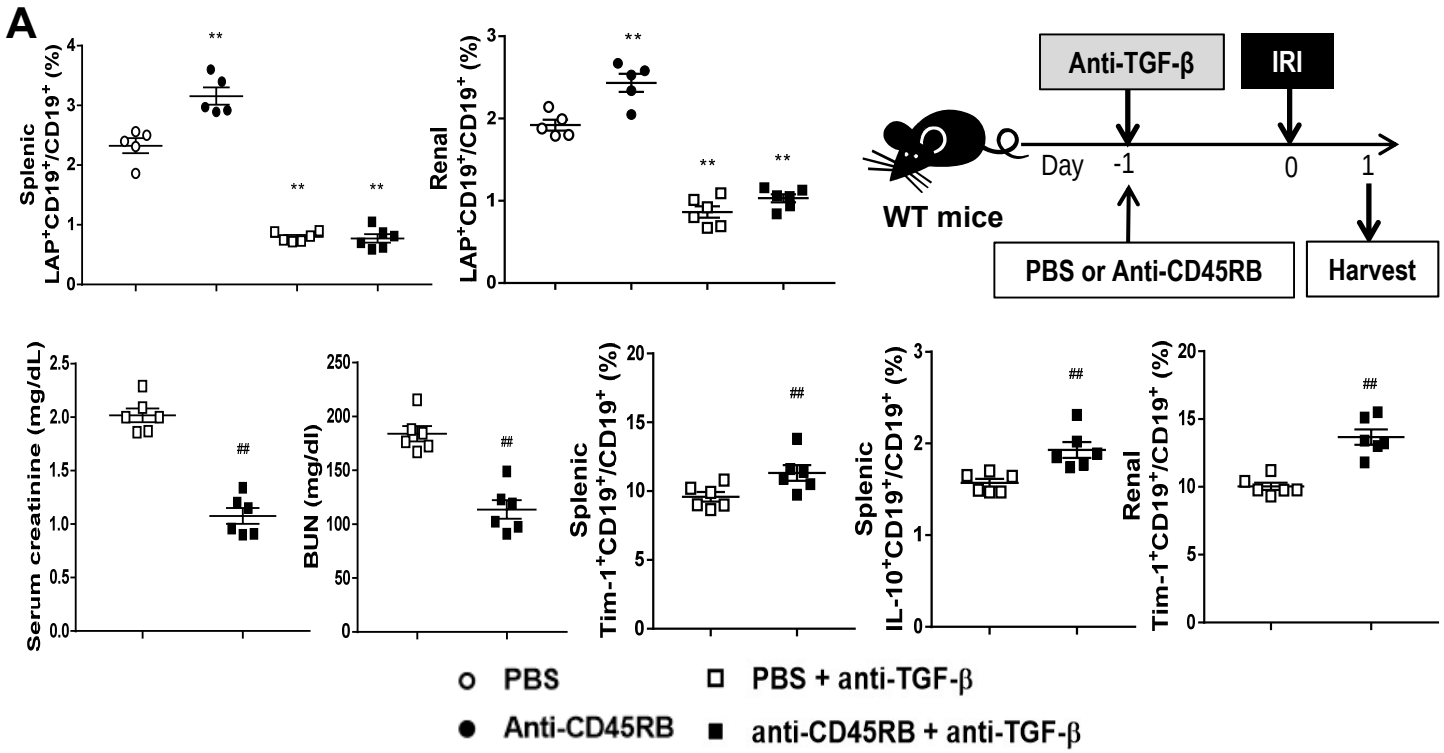

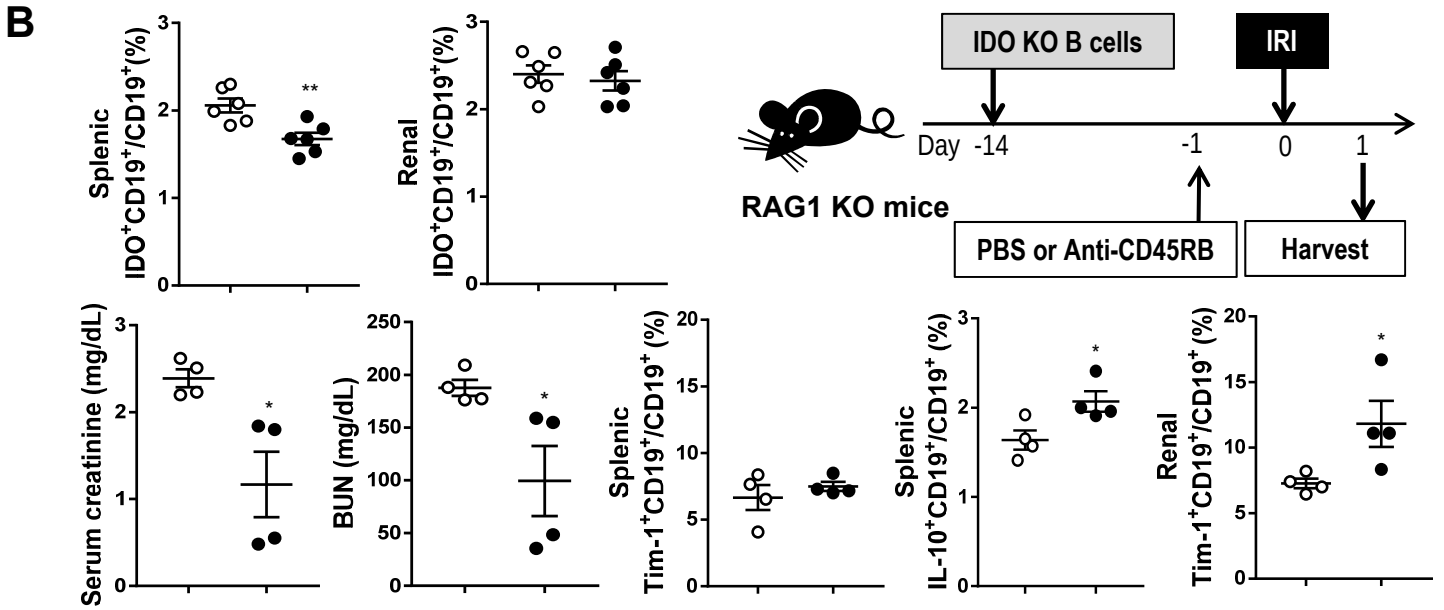

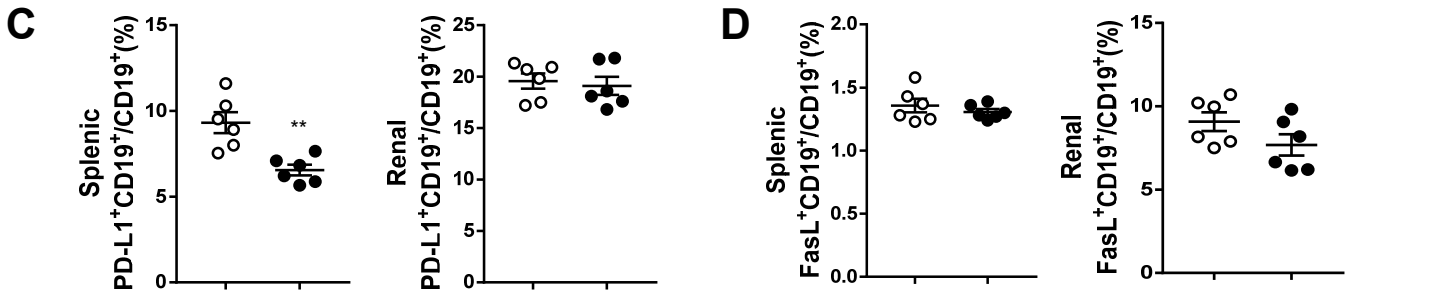

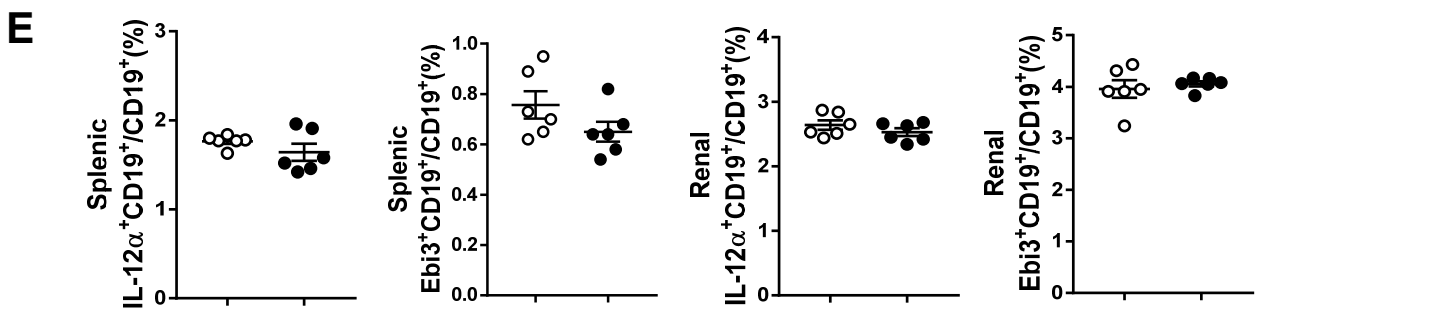

Supplemental Figure 7

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