Repetitive ischemic injuries to the kidneys result in lymph node fibrosis and impaired healing

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**Supplementary Figure legend**

**Supplementary Figure 1. Acute effects of IRI on the kidney and KLN**

A) Live image of KLN following injection of India ink (black arrow). B) CFSE-labeled CD4+ T cells injected into Rag1−/− mice preferentially enter and proliferate within the KLN draining ischemic kidney (KLN: IRI(D2)), in comparison to contralateral KLN draining non-ischemic kidney (KLN: Ctrl) and naïve KLN (KLN: Naïve). C) H&E staining of kidney section shows mild tubular injury 2 days following IRI (kidney: IRI(D2)). 30 days following IRI, histologic signs of renal injury have resolved (kidney: IRI(D30)) by H&E and Masson’s Trichrome stains. (Scale bar = 75µm.) D) A single episode of IRI leads to prolonged changes in KLN. Sustained increases in ER-TR7, fibronectin, αSMA and Collagen I signals are present 30 days following IRI. A persistent increase in macrophage density is seen 30 days following IRI. Regression of lymphatic endothelium is sustained 30 days following IRI. Representative data of mean fluorescent signal (n=3-4/group, mean ± SEM, student t-test; *p<0.05). E) Increased staining for Collagen I and F4/80+ macrophages are seen in KLN both 2 days (KLN: IRI(D2)) and 30 days (KLN: IRI(D30)) following IRI, in comparison to KLN draining kidney without IRI (KLN: Ctrl). Senescence of cells in KLN does not increase following IRI, as assessed by p16INK4A staining. Decreased Lyve1 signal indicates progressive lymphatic endothelial network regression by 30 days following IRI. (Scale bar = 200µm for CollI+F4/80, 100µm for p16INK4A and Lyve1.)

**Supplementary Figure 2. Repetitive IRI results in inflammation and fibrosis of kidney and KLN**

A) H&E and Masson’s trichrome stains of kidney tissue following repetitive IRI show interstitial fibrosis. (Scale bar = 75µm.) B) Gene expression of kidney tissue shows significant increase in
pro-inflammatory cytokines and markers of fibrosis following repetitive IRI (n=3/group, mean ± SEM, student’s t-test, *p<0.05, **p<0.01). C) Gene expression of KLN tissue shows significant increase in markers of fibrosis and macrophages following repetitive IRI (n=3/group, mean ± SEM, student’s t-test, *p<0.05, **p<0.01).

**Supplementary Figure 3. DT administration shows no morphologic changes in kidney**

A) No structural change in the glomeruli or tubules was noted in H&E staining of CCL19^{Cre}x iDTR kidney tissue following DT administration. (Scale bar = 75µm.) B) PDPN and megalin staining of CCL19^{Cre}x iDTR kidney tissue shows intact glomeruli and tubules following DT administration. (Scale bar = 50µm.) C) As compared to KLN, the expression of CCL19 was almost undetectable in podocytes (n=4/group, mean ± SEM, student’s t-test, ***p<0.01).
A

Kidney: IRI\(^{\text{rep}}\)

H&E  
Masson T

B

Kidney: Ctrl  
Kidney: IRI\(^{\text{rep}}\)

IFNγ  
TNFα  
IL-1β  
IL-6  
FN  
Coll I  
αSMA  

C

KLN: Ctrl  
KLN: IRI\(^{\text{rep}}\)

Coll I  
αSMA  
FN  
F4/80  

** Maarouf et al. Supplementary Figure 2**
A

CCL19\textsuperscript{Cre} \texttimes iDTR

<table>
<thead>
<tr>
<th>Without DT</th>
<th>With DT</th>
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B

CCL19\textsuperscript{Cre} \texttimes iDTR

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>PDPN Megalin</td>
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C

Fold-change in CCL19 gene expression

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<tr>
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<th>Podocytes</th>
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