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 $p16^{INK4A}$ staining. Decreased Lyve1 signal indicates progressive lymphatic endothelial network regression by 30 days following IRI. (Scale bar = 200μm for CollI+F4/80, 100μm for $p16^{INK4A}$ and Lyve1.)

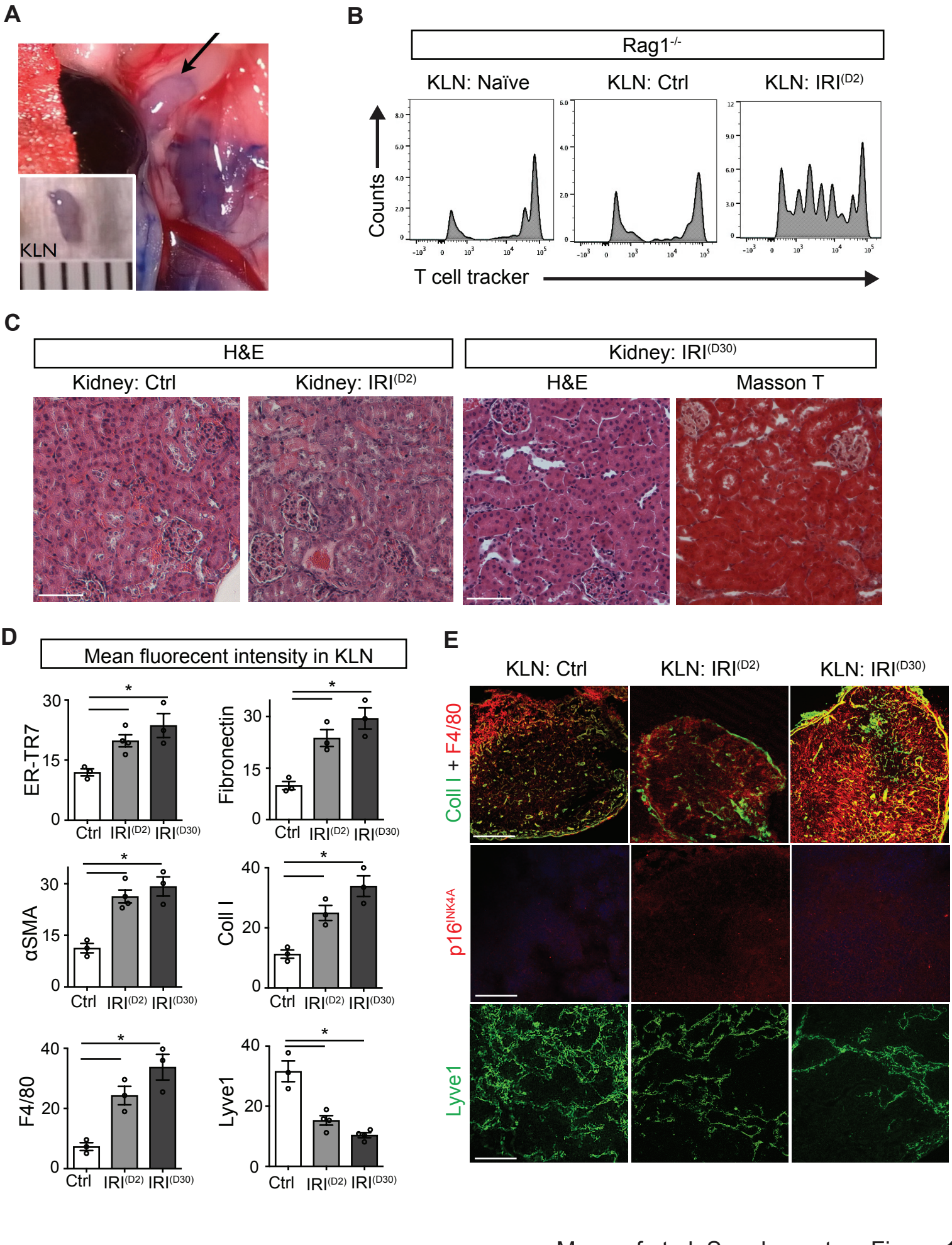
Supplementary Figure 2. Repetitive IRI results in inflammation and fibrosis of kidney and KLN

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

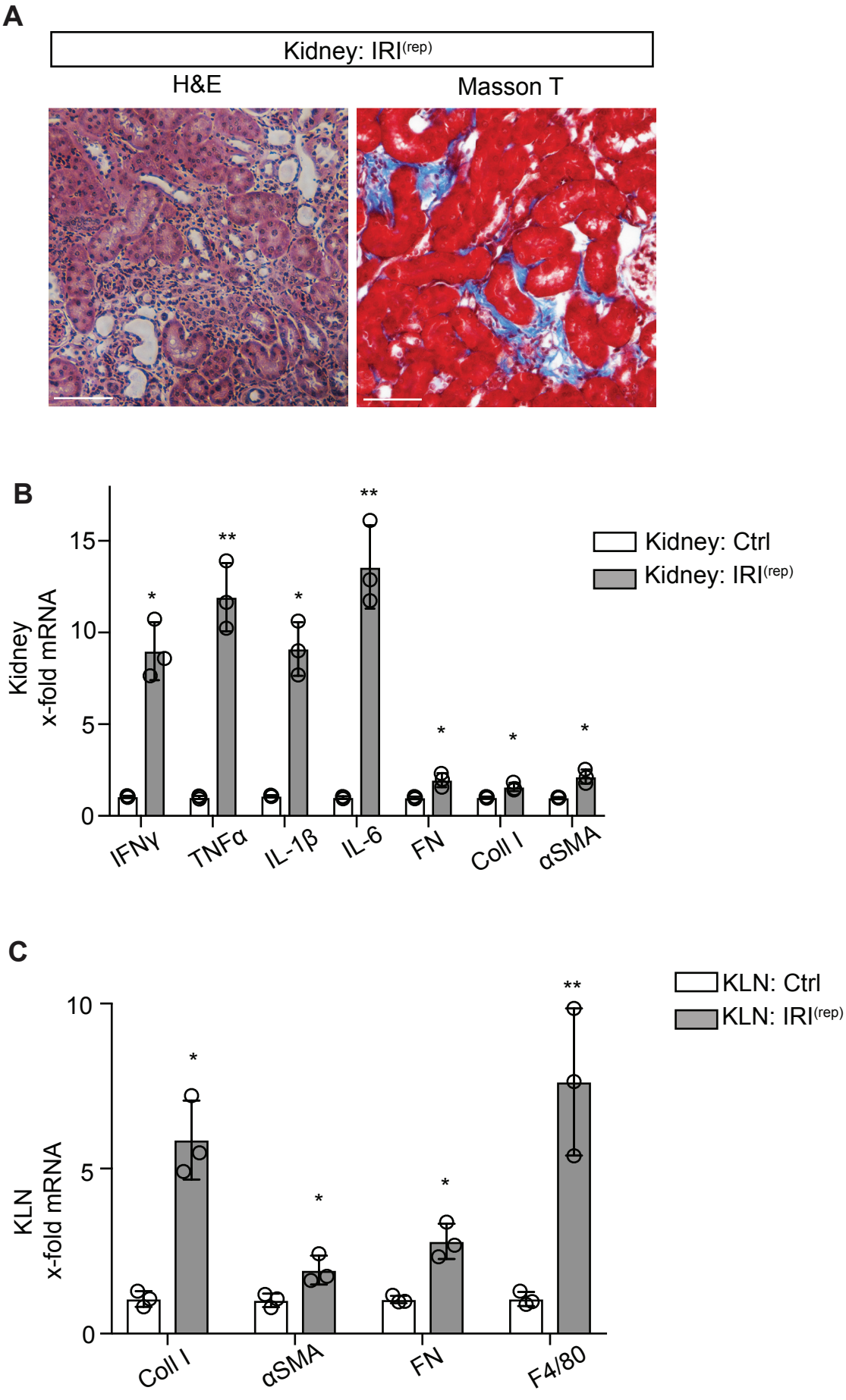
pro-inflammatory cytokines and markers of fibrosis following repetitive IRI (n=3/group, mean ± SEM, student's *t*-test, **p*<0.05, ***p*<0.01). C) Gene expression of KLN tissue shows significant increase in markers of fibrosis and macrophages following repetitive IRI (n=3/group, mean ± SEM, student's *t*-test, **p*<0.05, ***p*<0.01).

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

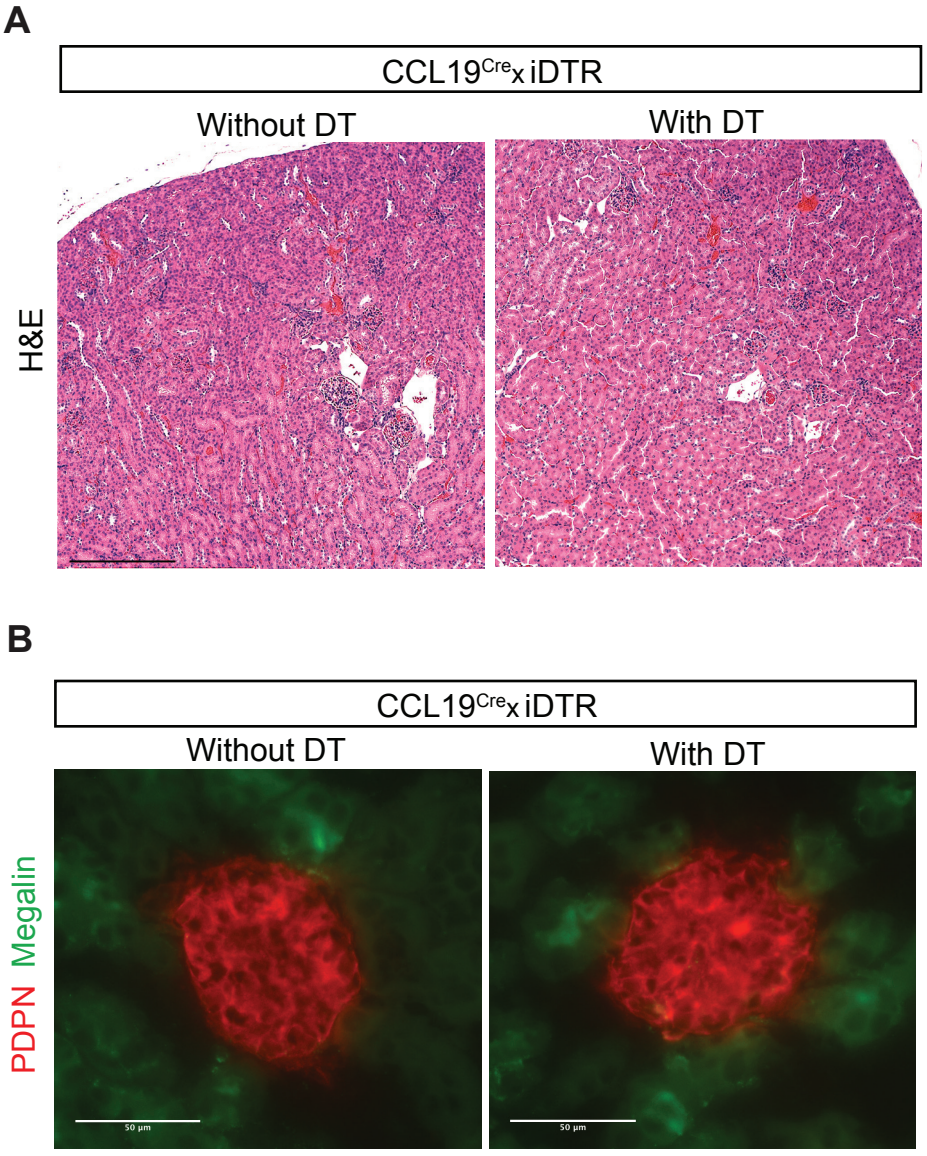
A) No structural change in the glomeruli or tubules was noted in H&E staining of $CCL19^{Cre}$x iDTR kidney tissue following DT administration. (Scale bar = 75μm.) B) PDPN and megalin staining of $CCL19^{Cre}$x iDTR kidney tissue shows intact glomeruli and tubules following DT administration. (Scale 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).



Maarouf et al. Supplementary Figure 1



Maarouf et al. Supplementary Figure 2



Maarouf et al. Supplementary Figure 3