SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. *Sphk2*$^{-/-}$ mice are more susceptible to kidney ischemia-reperfusion injury than WT or *Sphk1*$^{-/-}$ mice. Mice were subjected to 26 min of bilateral ischemia and 24 hr reperfusion. Like the *Sphk2*$^{tr/tr}$ used previously,[1] *Sphk2*$^{-/-}$ mice have significantly higher plasma creatinine compared to WT and *Sphk1*$^{-/-}$ mice at 24 hr after ischemia. Compared to *Sphk2*$^{-/-}$ lack of Ifng results in significantly lower kidney injury in *Sphk2*$^{-/-}$ *Ifng*$^{-/-}$ and *Ifng*$^{-/-}$ N=5-8,* $p<0.05$, **$p<0.01$.

Supplemental Figure 2. Increased mRNA expression of sphingosine 1-phosphate receptors (S1pr1-5) and sphingosine kinases (Sphk1, Sphk2) in kidneys in two models of chronic kidney injury in mice. Wild type C57BL/6 mice were (A) treated with vehicle (0.3 M sodium bicarbonate) or folic acid (FA; 250 mg/ml, ip) or (B) exposed to unilateral kidney ischemia (26 min) followed by reperfusion. In the unilateral IRI model, the contralateral uninjured kidney was removed on d 13 and saved as control kidney. 14 days after FA or unilateral IRI, mice were euthanized and kidneys were harvested. Kidney mRNA was extracted and expression was measured by quantitative real time RT-PCR (see Methods and Table 1 for primer sequences). Values (mean ± SE) are expressed relative to glyceraldehyde 3-phosphate (GAPDH). Values for S1pr4 and S1pr5 were at or below the level of detection in the control groups and increased sufficiently to become measureable in the injured groups. N = 3. *$p < 0.05$ compared to the respective control group for each marker.

Supplemental Figure 3. Timeline of treatments and procedures in folic acid and unilateral ischemia-reperfusion models in mice. A. Folic Acid (FA) Model: FA (250 mg/kg (ip) in a vehicle of sodium bicarbonate, pH 7.4 buffer) or vehicle was administered once on day 0. Blood was collected on d3 and 14 to measure plasma creatinine (PCr). After collecting blood on d14, mice were euthanized and kidneys were harvested. In some experiments, a sphingosine kinase 2 inhibitor (SLP120701, prepared in a vehicle of 2% hydroxypropyl-beta-cyclodextrin in PBS; 1, 3, 10, or 30 mg/kg, ip) or vehicle was administered daily beginning 2 d after FA. In other experiments an IFN-γ blocking antibody was administered as shown in panel B for unilateral IRI experiments except that treatment began 1 d before FA. B. Unilateral IRI (Uni-IRI) Model: Mice were subjected to unilateral IRI in which only one kidney was clamped for 26 min on day 0 and then allowed to reperfuse; in control mice the kidney was exposed but not clamped. To assess kidney function in the chronic model after unilateral IRI, when renal reserve ordinarily would mask changes in the injured kidney, the contralateral kidney was removed (nephrectomy; NPX) 13 d after IRI and saved as the control kidney. After collecting blood on d14 to measure PCr, mice were euthanized and kidneys were harvested. In some experiments rat anti-mouse IFN-γ or rat IgG1 isotype control antibody was administered (150 µg/mouse) 2 d after IRI and every 3 d thereafter (d2, 5, 8, and 11, as well as on d13).

Supplemental Figure 4. Folic acid induces expression of fibrotic markers in kidneys of WT and *Sphk1*$^{-/-}$ but not *Sphk2*$^{-/-}$ mice. Same treatments (same mice) as Figure 1. Mice were treated with vehicle or folic acid and kidneys were harvested on day 14. Expression of a-smooth muscle actin (αSMA) and vimentin (A) and fibronectin (B) in WT, *Sphk1*$^{-/-}$ and *Sphk2*$^{-/-}$ mice measured by western blotting. The experiment was performed by preparing sufficient sample for

5 gels and loading an equal volume of sample into the appropriate well of those 5 gels: 1) 4 separate 10% SDS-PAGE gels for αSMA, vimentin and tubulin (x2) and 2) 1 separate 7.5% gel for fibronectin, each of which were then transferred to PVDF membranes and probed with the respective primary antibodies. Western blots were then analyzed using the chemilluminescent method. Numbers under each band are arbitrary units of absorbance normalized to tubulin.

Supplemental Figure 5. Bone marrow (BM) chimeric mice deficient in Sphk2 in either hematopoietic or non-hematopoietic cells, like global *Sphk2*$^{-/-}$ mice, are protected from folic acid-induced increase in expression of fibrotic markers and pro-inflammatory markers in kidney. Recipient mice were lethally irradiated, were immediately reconstituted with 10^7 donor BM cells, and were maintained for 8 weeks prior to experiments. *Sphk2*$^{-/-}$ mice (which were bred on the WT C57BL/6 background expressing the Ptrpcb (CD45.2) pan leukocyte marker) were reconstituted with BM from WT CD45.1 (Ptrpca-expressing) mice to discriminate donor and recipient cells and demonstrate by flow cytometry that the efficiency of reconstitution after irradiation was at least 90% (data not shown). Vehicle or folic acid (250 mg/kg, ip) was administered, and kidneys were harvested 14 d later. mRNA expression of fibrotic and inflammatory markers is shown relative to that of GAPDH. N = 3-6. *p<0.05. Acta2: actin, alpha 2, smooth muscle, aorta; Col3a1: collagen, type III, alpha 1; Cxcl1: chemokine (C-X-C motif) ligand 1; Ifng: interferon gamma; Vim: vimentin.

Supplemental Figure 6. Administration of IFN-γ blocking antibody reconstitutes folic acid-induced fibrosis in *Sphk2*$^{-/-}$ mice and exacerbates fibrosis in WT (not shown) and *Sphk1*$^{-/-}$ mice but the increase in injury leads to mortality. Rat anti-mouse IFN-γ (α-IFN-γ) or rat IgG1 (IgG) isotype control antibody was administered (150 μg/mouse) 1 d before FA and every 3 d thereafter (d -1, 2, 5, 8, 11); mice that survived were euthanized on day 14. Because of the high rate of mortality (>60%), kidneys were collected and stained with Masson's trichrome (blue indicates fibrosis), as shown in this figure, but no other measures were made. Instead, the experiment was repeated in the unilateral IRI model (Figure 6). Scale bar = 1 mm.

Supplemental Figure 7. Generation of *Sphk2*$^{-/-}$ *Ifng*$^{-/-}$ mice from *Sphk2*$^{-/-}$ and *Ifng*$^{-/-}$ breeders. *Sphk2*$^{-/-}$ and *Ifng*$^{-/-}$ mice were crossed to yield progeny with both genes deleted, as confirmed by the genotyping results shown here. Agarose gel analysis of PCR products amplified from tail DNA. Lane 1, molecular weight markers; remaining lanes as shown. Size of predicted PCR products of *Sphk2*$^{-/-}$ *Ifng*$^{-/-}$ mice (number of bp) is shown at right side of gel and immediately below gel for other lanes.

Supplemental Figure 8. Identification of leukocyte subsets and IFN-γ-producing cells in mouse kidneys after unilateral IRI. Mice were subjected to unilateral IRI, as described in Supplemental Figure 3, except that brefeldin A (250 μg/mouse, ip) was administered 5 hr before euthanizing to enable evaluation of intracellular cytokines. Kidneys were removed at day 14 and evaluated by histologic and immunofluorescent methods (in frozen sections) and by flow cytometry. A. WT and *Sphk1*$^{-/-}$ mice had substantial deposition of collagen (picrosirius red birefringence), proliferation of interstitial immune cell (Ki67$^+$CD45$^+$) or fibroblasts (Ki67$^+$PDGFRβ$^+$), transformation of fibroblasts to myofibroblasts (α-SMA$^+$PDGFRβ$^+$), infiltration of leukocytes (CD45$^+$) and numbers of IFN-γ$^+$ cells (some were also CD45$^+$) whereas *Sphk2*$^{-/-}$ mice were

protected from fibrosis. The protective effect of Sphk2 deletion was lost in the absence of Ifng (*Sphk2*$^{-/-}$ *Ifng*$^{-/-}$ mice). Black and white panels on the right are IFN-γ labeling pulled out separately from the multicolor CD45/CD3/ IFN-γ /DAPI photo to better reveal IFN-γ^{+} cells alone. Scale bar = 100 μm. B-E. Flow cytometry of kidneys. B. There were increased numbers of neutrophils (CD11b^{+}Ly6G^{high}) and macrophages (CD11b^{+}F4/80^{low}) in IRI kidneys compared to contralateral control kidneys of WT and Sphk1-/- mice (with macrophage numbers substantially higher in *Sphk1*$^{-/-}$ mice than in either WT or *Sphk2*$^{-/-}$ mice) but neutrophil numbers did not increase in *Sphk2*$^{-/-}$ mice while macrophage numbers in *Sphk2*$^{-/-}$ IRI kidneys were comparable to WT kidneys. The pattern of leukocyte infiltration (i.e. relative increases in cell numbers) was somewhat different in kidneys from the unilateral model compared to the folic acid model (Figure 3). There were small (but N.S.) differences in T cell numbers between control and IRI kidneys and between mouse strains in the unilateral IRI model. C. The percentage of CD45^{+}IFN-γ^{+} cells was lower in IRI kidneys of *Sphk2*$^{-/-}$ than in WT and *Sphk1*$^{-/-}$ mice, whereas CD45^{-l}FN-γ^{+} cells were about the same in all 3 groups. D. T cell subsets. There were more CD8 T cells in IRI kidneys of *Sphk2*$^{-/-}$ mice than in WT and more CD4 T cells in *Sphk1*$^{-/-}$ than in WT kidneys, but the total numbers of CD4 (though N.S.) and CD8 T cells were lower in *Sphk2*$^{-/-}$ than in WT mouse kidneys E. *, $p<0.05$; **, $p<0.01$. N = 5.N.S. not significant.

References

[1] Jo SK, Bajwa A, Ye H, Vergis AL, Awad AS, Kharel Y, Lynch KR, Okusa MD: Divergent roles of sphingosine kinases in kidney ischemia-reperfusion injury. *Kidney Int* 75**:** 167-75, 2009.

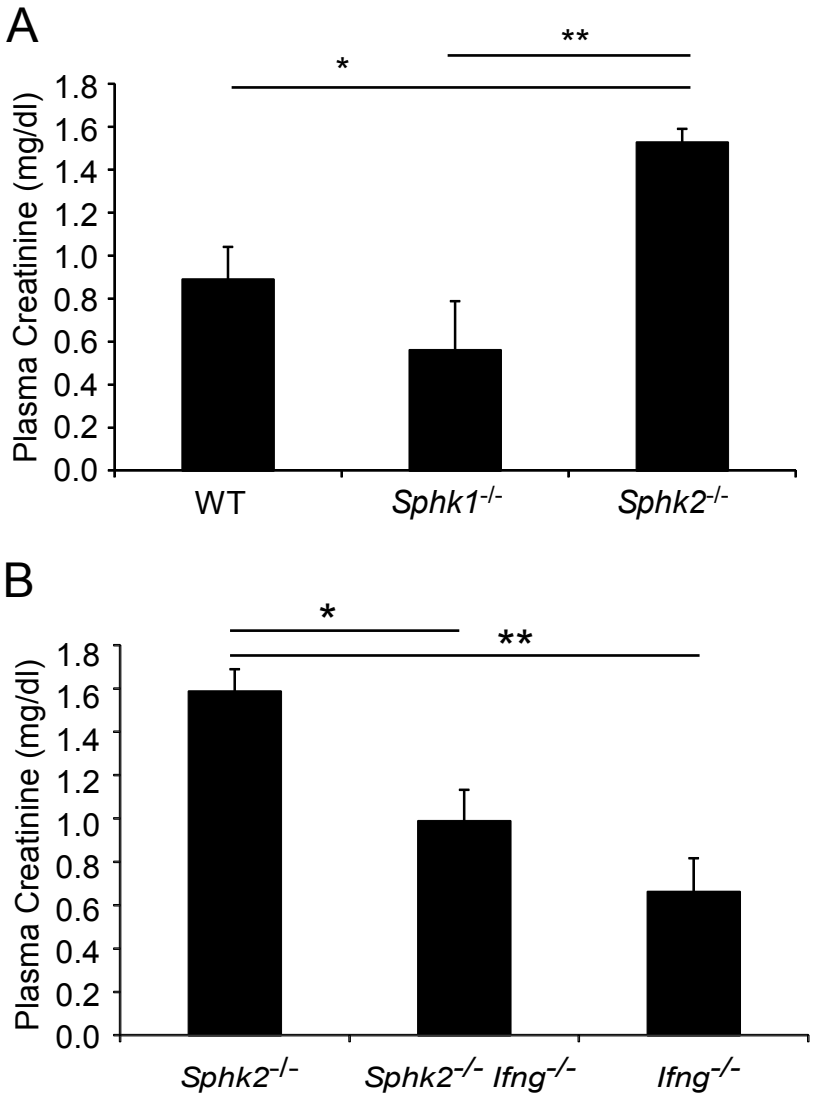
Supplemental Tables

Supplemental Table 1. Primer sequences for quantitative real time RT-PCR

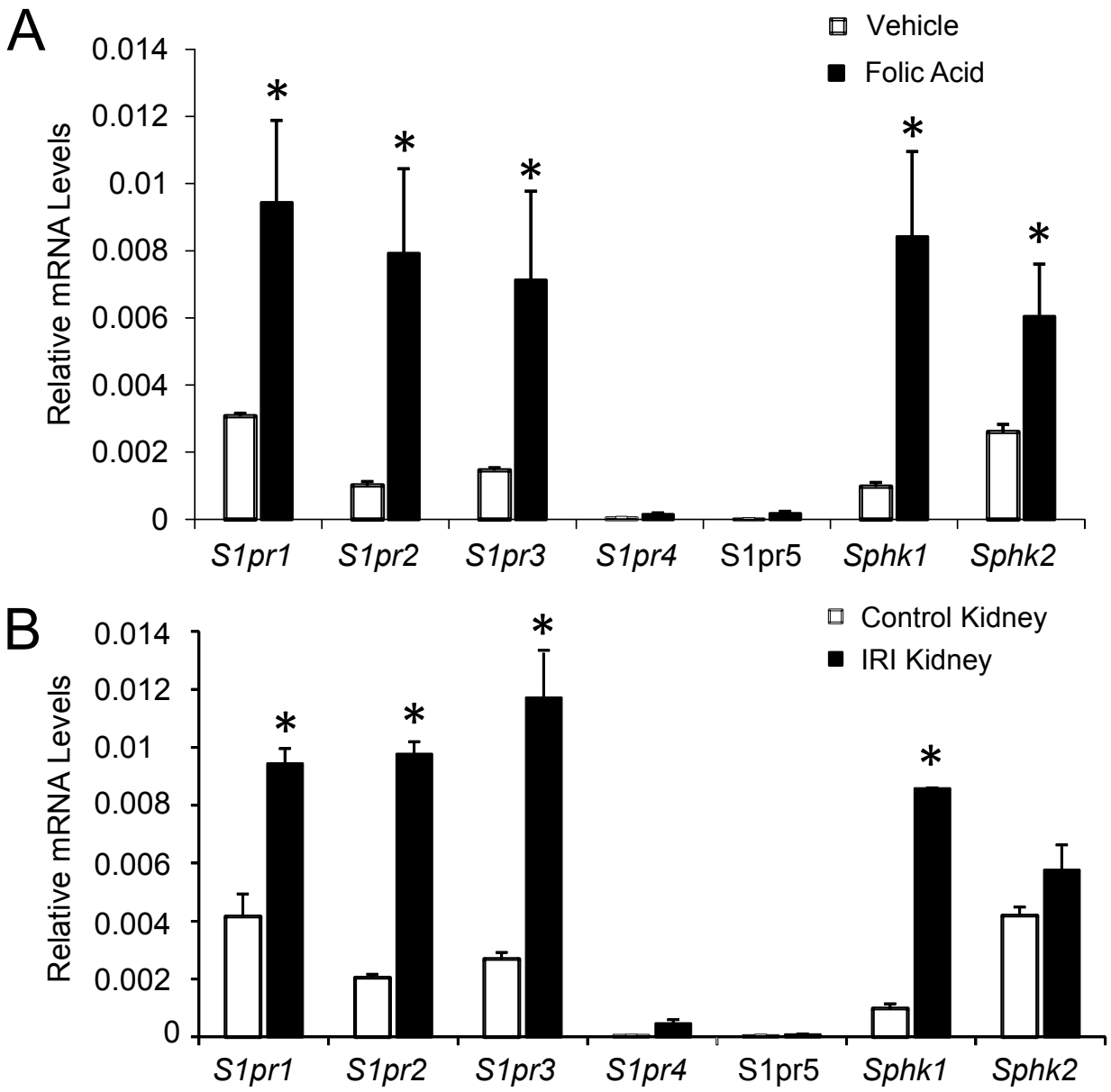
Gene name	Forward 5' to 3'	Reverse 5' to 3'
Gapdh	ACG GCA AAT TCA ACG GCA CAG TCA	TGG GGG CAT CGG CAG AAG G
Fn1	ACC AAC CTT AAT CCG GGC AC	TCA GAA ACT GTG GCT TGC TGG
Col1a2	GAG CGG AGA GTA CTG GAT CG	TAC TCG ACC GGG AAT CCA TC
Col3a1	TCC TAA CCA AGG CTG CAA GAT GGA	ACC AGA ATC TGT CCA CCA GTG CTT
Acta2	ATT GTG CTG GAC TCT GGA GAT GGT	TGA TGT CAC GGA CAA TCT CAC GCT
Vim	AGA TGG CTC GTC ACC TTC GTG AAT	TTG AGT GGG TGT CAA CCA GAG GAA
Ctgf	GTT GTT CAT TAG CGC ACA GTG CCA	TAG AGC AGG TCT GTC TGC AAG CAT
Tgfb	TAA AGA GGT CAC CCG CGT GCT AAT	ACT GCT TCC CGA ATG TCT GAC GTA
Cxcl1	TGG CTG GGA TTC ACC TCA AGA ACA	TGT GGC TAT GAC TTC GGT TTG GGT
Cxcl2	ACA TCC CAC CCA CAC AGT GAA AGA	TCC TTC CAT GAA AGC CAT CCG ACT
Cxcl9	TGT GGA GTT CGA GGA ACC CT	TGC CTT GGC TGG TGC TG
Cxcl10	GGA TGG CTG TCC TAG CTC TG	TGA GCT AGG GAG GAC AAG GA
Il10	GCTCTTACTGACTGGCATGAG	CGCAGCTCTAGGAGCATGTG
Ifng	GCATAGATGTGGAAGAAAAGAGTC	GGTGTGATTCAATGACGCTTATG
Mcp-1	TCA CCT GCT GCT ACT CAT TCA CCA	TAC AGC TTC TTT GGG ACA CCT GCT
Tlr4	AAC CAG CTG TAT TCC CTC AGC ACT	ACT GCT TCT GTT CCT TGA CCC ACT
Pcna	ATA AAG AAG AGG AGG CGG T	GTG GAG TGG CTT TTG TGA
S1pr1	TTC TCA TCT GCT GCT TCA TCA TCC	GGT CCG AGA GGG CTA GGT TG
S1pr2	TTA CTG GCT ATC GTG GCT CTG	ATG GTG ACC GTC TTG AGC AG
S1pr3	GCG TGT TCC TTC TGA TTG G	GCA AGA TGG TAG AGC AGT C
S1pr4	CTG TCA GGG ACT CGT ACC	CGT GAA GAG CAG ACT GAA G
S1pr5	TTG CTA TTA CTG GAT GTC GCG TGC	AGA TGA TGG GAT TCA GCA GCG AGT
Sphk1	GGA ACT TGA CTG TCC ATA CC	TAC CAT CAG CTC TCC ATC C
Sphk2	GCA CGG CGA GTT TGG TTC	GAG ACC TCA TCC AGA GAG ACT AG

Acta2, α-smooth muscle actin; Col1a2, collagen 1 alpha 2; Col3a1,collagen 3 alpha1; Ctgf, connective tissue growth factor; Fn1, fibronectin; Gapdh, glyceraldehyde 3-phosphate dehydrogenase; Mcp-1, monocyte chemoattractant protein-1; Pcna, proliferating cell nuclear antigen; S1pr1-5, sphingosine 1-phosphate receptors 1-5; SphK1 & 2, sphingosine kinases 1 and 2; Tgfb, TGF-β; Vim, vimentin

SUPPLEMENTAL FIGURE 1

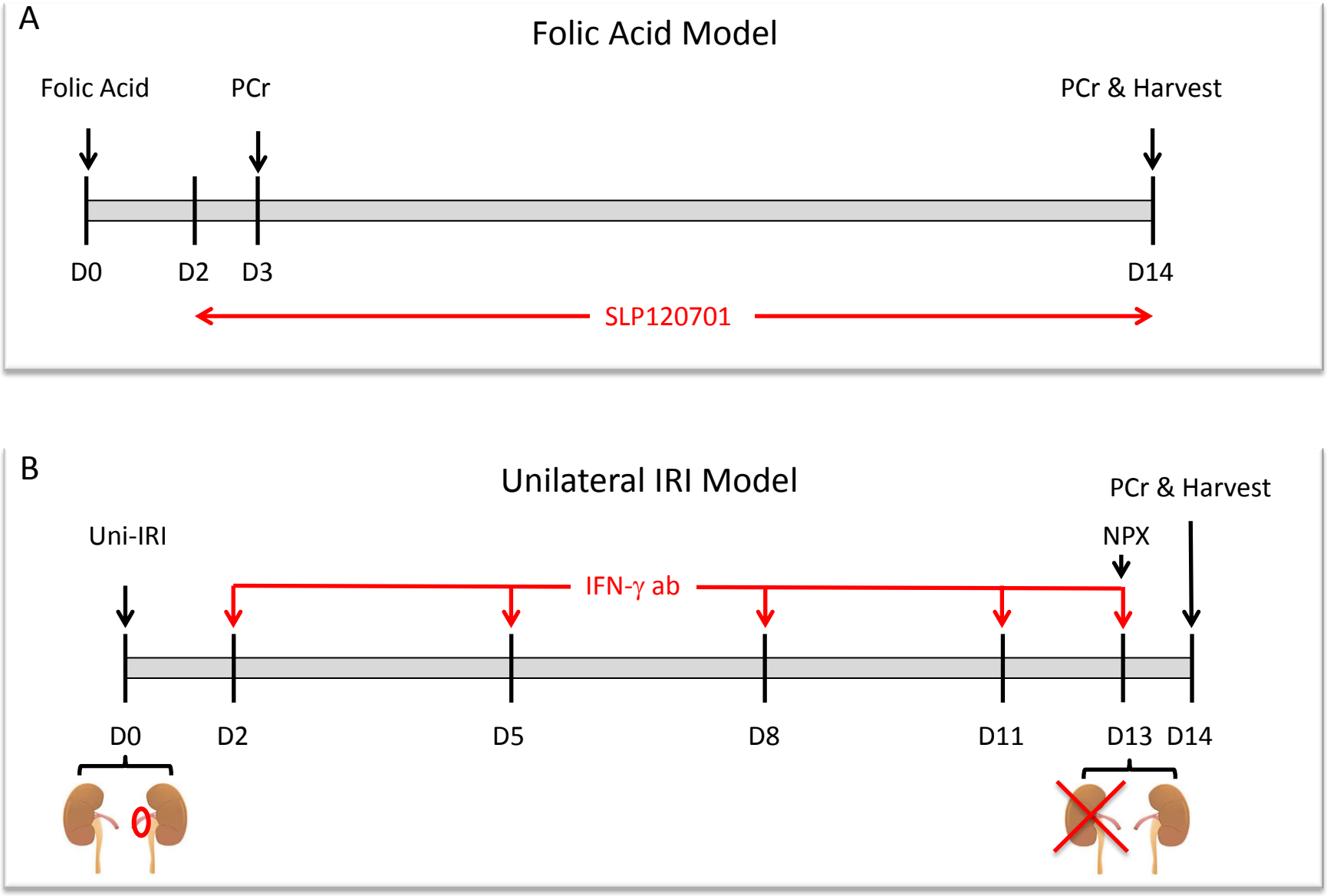


Supplemental Figure 1. *Sphk2*$^{-/-}$ mice are more susceptible to kidney ischemia-reperfusion injury than WT or *Sphk1*$^{-/-}$ mice. Mice were subjected to 26 min of bilateral ischemia and 24 hr reperfusion. Like the *Sphk2*$^{tr/tr}$ used previously,[1] *Sphk2*$^{-/-}$ mice have significantly higher plasma creatinine compared to WT and *Sphk1*$^{-/-}$ mice at 24 hr after ischemia. Compared to *Sphk2*$^{-/-}$ lack of Ifnγ results in significantly lower kidney injury in *Sphk2*$^{-/-}$*Ifng*$^{-/-}$ and *Ifng*$^{-/-}$ N=5-8,* $p<0.05$, **$p<0.01$.



Supplemental Figure 2. Increased mRNA expression of sphingosine 1-phosphate receptors (*S1pr1-5*) and sphingosine kinases (*Sphk1, Sphk2*) in kidneys in two models of chronic kidney injury in mice. Wild type C57BL/6 mice were (A) treated with vehicle (0.3 M sodium bicarbonate) or folic acid (FA; 250 mg/ml, ip) or (B) exposed to unilateral kidney ischemia (26 min) followed by reperfusion. In the unilateral IRI model, the contralateral uninjured kidney was removed on d 13 and saved as control kidney. 14 days after FA or unilateral IRI, mice were euthanized and kidneys were harvested. Kidney mRNA was extracted and expression was measured by quantitative real time RT-PCR (see Methods and Table 1 for primer sequences). Values (mean ± SE) are expressed relative to glyceraldehyde 3-phosphate (GAPDH). Values for *S1pr4* and *S1pr5* were at or below the level of detection in the control groups and increased sufficiently to become measureable in the injured groups. N = 3. *$p < 0.05$ compared to the respective control group for each marker.

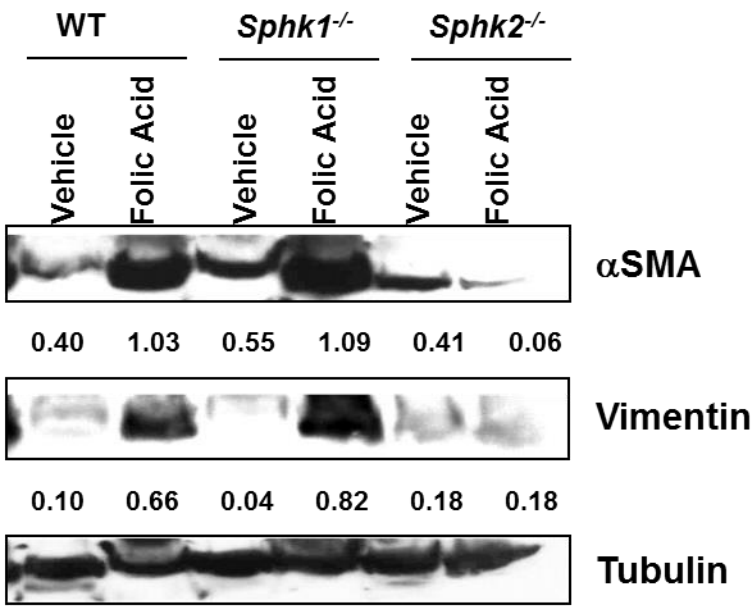
SUPPLEMENTAL FIGURE 3



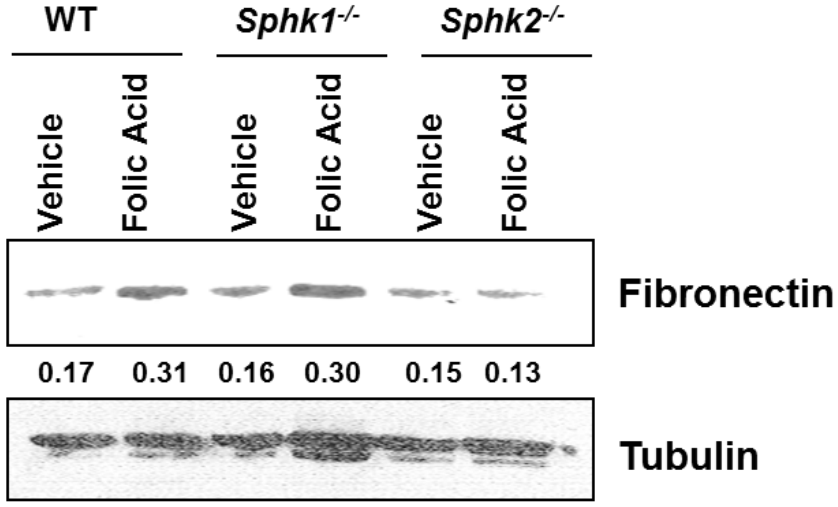
Supplemental Figure 3. Timeline of treatments and procedures in folic acid and unilateral ischemia-reperfusion models in mice. A. Folic Acid (FA) Model: FA (250 mg/kg (ip) in a vehicle of sodium bicarbonate, pH 7.4 buffer) or vehicle was administered once on day 0. Blood was collected on d3 and 14 to measure plasma creatinine (PCr). After collecting blood on d14, mice were euthanized and kidneys were harvested. In some experiments, a sphingosine kinase 2 inhibitor (SLP120701, prepared in a vehicle of 2% hydroxypropyl-beta-cyclodextrin in PBS; 1, 3, 10, or 30 mg/kg, ip) or vehicle was administered daily beginning 2 d after FA. In other experiments an IFN-γ blocking antibody was administered as shown in panel B for unilateral IRI experiments except that treatment began 1 d before FA.

B. Unilateral IRI (Uni-IRI) Model: Mice were subjected to unilateral IRI in which only one kidney was clamped for 26 min on day 0 and then allowed to reperfuse; in control mice the kidney was exposed but not clamped. To assess kidney function in the chronic model after unilateral IRI, when renal reserve ordinarily would mask changes in the injured kidney, the contralateral kidney was removed (nephrectomy; NPX) 13 d after IRI and saved as the control kidney. After collecting blood on d14 to measure PCr, mice were euthanized and kidneys were harvested. In some experiments rat anti-mouse IFN-γ or rat IgG1 isotype control antibody was administered (150 μg/mouse) 2 d after IRI and every 3 d thereafter (d2, 5, 8, and 11, as well as on d13).

A



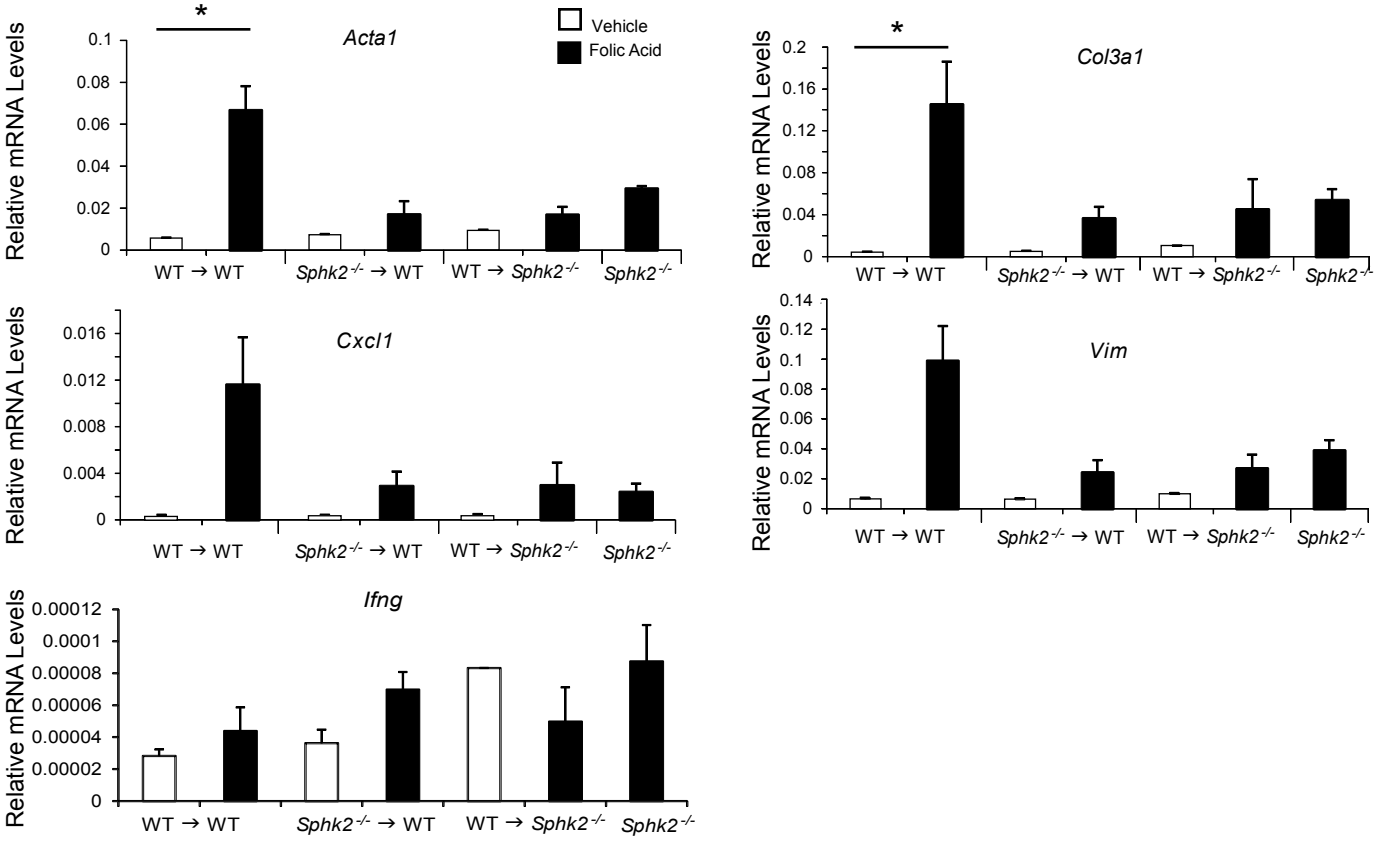
B



Supplemental Figure 4. Folic acid induces expression of fibrotic markers in kidneys of WT and *Sphk1*$^{-/-}$ but not *Sphk2*$^{-/-}$ mice. Same treatments (same mice) as Figure 1. Mice were treated with vehicle or folic acid and kidneys were harvested on day 14. Expression of α-smooth muscle actin (αSMA) and vimentin (A) and fibronectin (B) in WT, *Sphk1*$^{-/-}$ and *Sphk2*$^{-/-}$ mice measured by western blotting.

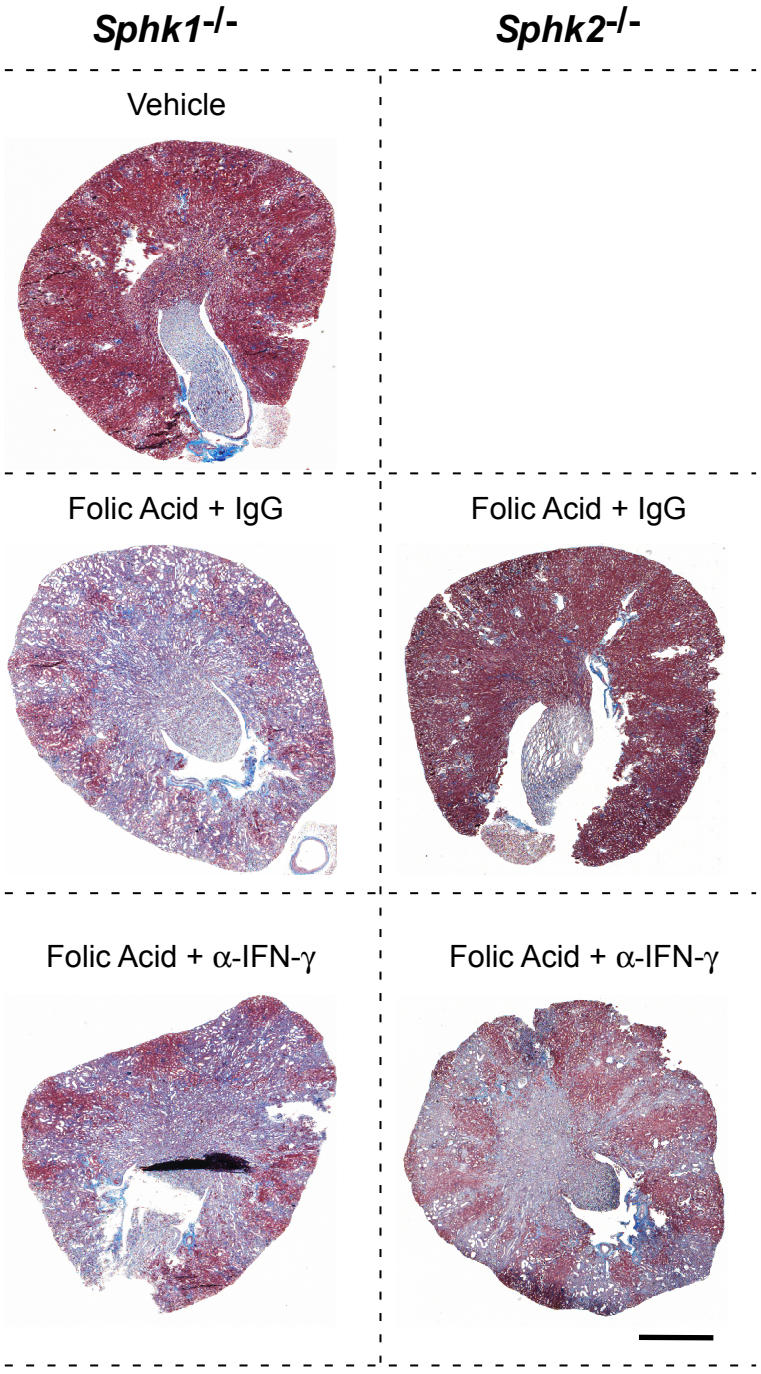
The experiment was performed by preparing sufficient sample for 5 gels and loading an equal volume of sample into the appropriate well of those 5 gels: 1) 4 separate 10% SDS-PAGE gels for αSMA, vimentin and tubulin (x2) and 2) 1 separate 7.5% gel for fibronectin, each of which were then transferred to PVDF membranes and probed with the respective primary antibodies. Western blots were then analyzed using the chemilluminescent method. Numbers under each band are arbitrary units of absorbance normalized to tubulin.

SUPPLEMENTAL FIGURE 5



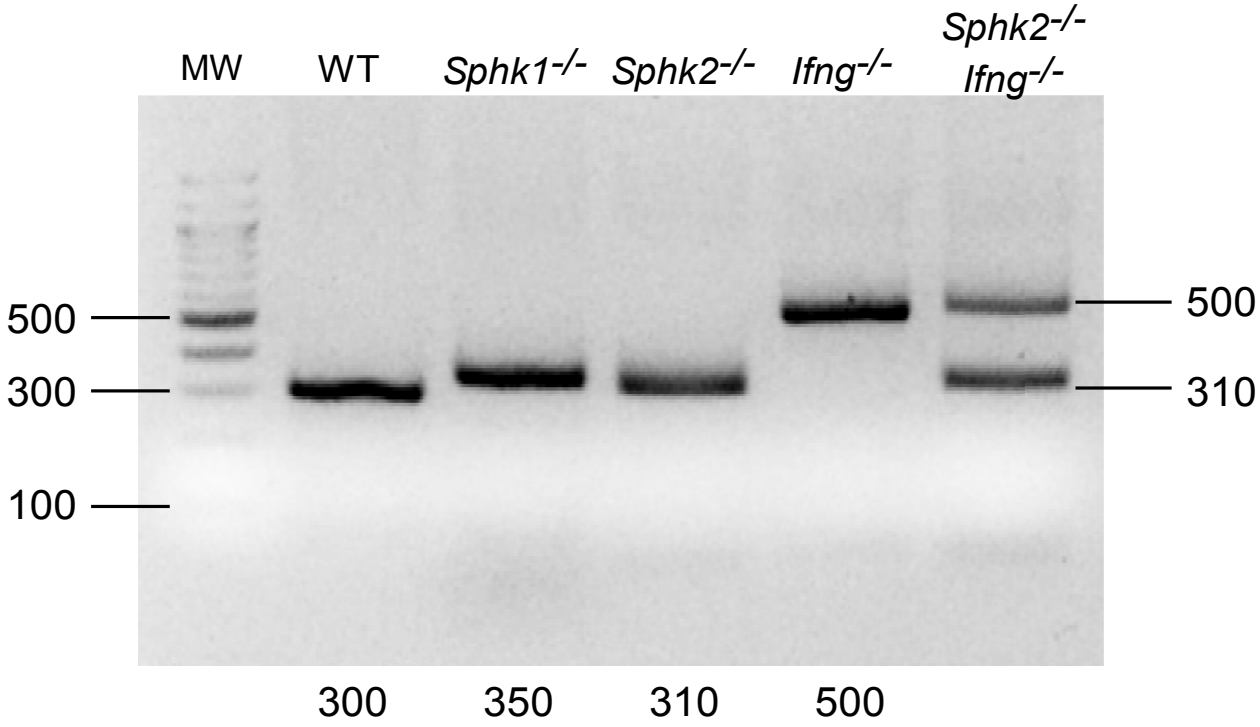
Supplemental Figure 5. Bone marrow (BM) chimeric mice deficient in *Sphk2* in either hematopoietic or non-hematopoietic cells, like global *Sphk2*$^{-/-}$ mice, are protected from folic acid-induced increase in expression of fibrotic markers and pro-inflammatory markers in kidney. Recipient mice were lethally irradiated, were immediately reconstituted with 10^7 donor BM cells, and were maintained for 8 weeks prior to experiments. *Sphk2*$^{-/-}$ mice (which were bred on the WT C57BL/6 background expressing the *Ptrpcb* (CD45.2) pan leukocyte marker) were reconstituted with BM from WT CD45.1 (*Ptrpca*-expressing) mice to discriminate donor and recipient cells and demonstrate by flow cytometry that the efficiency of reconstitution after irradiation was at least 90% (data not shown). Vehicle or folic acid (250 mg/kg, ip) was administered, and kidneys were harvested 14 d later. mRNA expression of fibrotic and inflammatory markers is shown relative to that of GAPDH. N = 3-6. *p<0.05. *Acta2*: actin, alpha 2, smooth muscle, aorta; *Col3a1*: collagen, type III, alpha 1; *Cxcl1:* chemokine (C-X-C motif) ligand 1; *Ifng*: interferon gamma; *Vim*: vimentin.

SUPPLEMENTAL FIGURE 6



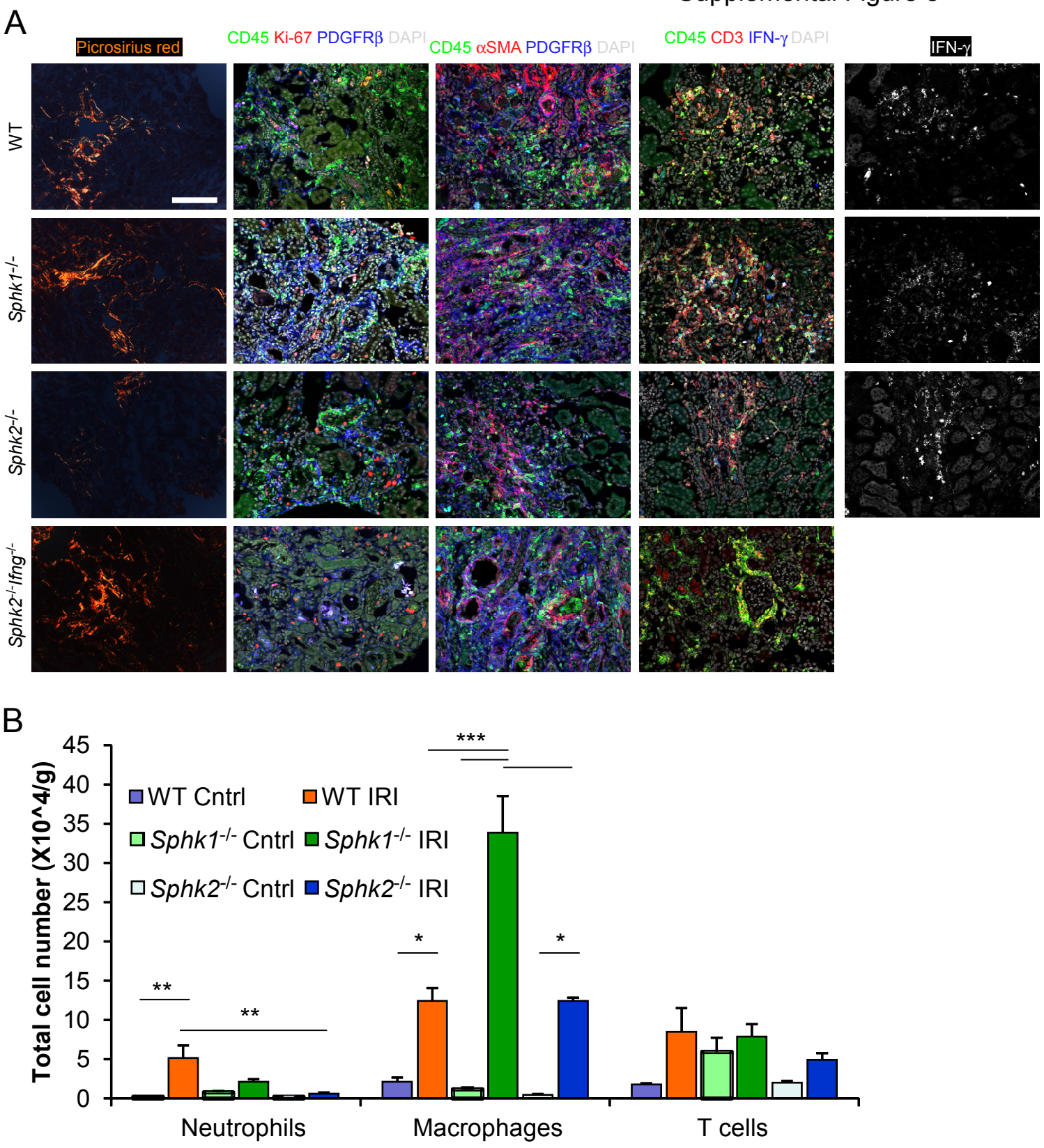
Supplemental Figure 6. Administration of IFN-γ blocking antibody reconstitutes folic acid-induced fibrosis in *Sphk2*$^{-/-}$ mice and exacerbates fibrosis in WT (not shown) and *Sphk1*$^{-/-}$ mice but the increase in injury leads to mortality. Rat anti-mouse IFN-γ (α-IFN-γ) or rat IgG1 (IgG) isotype control antibody was administered (150 μg/mouse) 1 d before FA and every 3 d thereafter (d -1, 2, 5, 8, 11); mice that survived were euthanized on day 14. Because of the high rate of mortality (>60%), kidneys were collected and stained with Masson's trichrome (blue indicates fibrosis), as shown in this figure, but no other measures were made. Instead, the experiment was repeated in the unilateral IRI model (Figure 6). Scale bar = 1 mm.

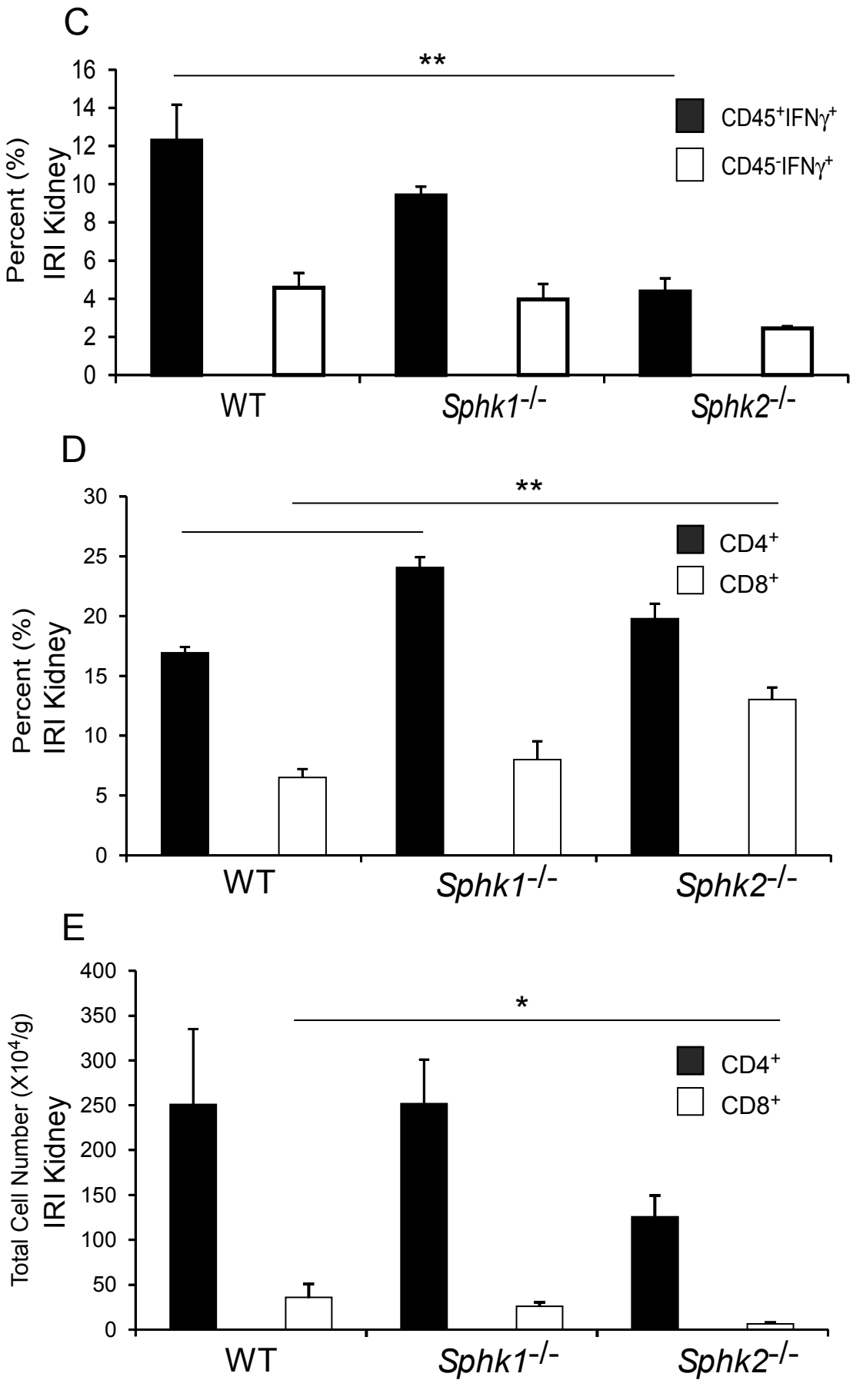
SUPPLEMENTAL FIGURE 7



Supplemental Figure 7. Generation of *Sphk2*$^{-/-}$*Ifng*$^{-/-}$ mice from *Sphk2*$^{-/-}$ and *Ifng*$^{-/-}$ breeders. *Sphk2*$^{-/-}$ and *Ifng*$^{-/-}$ mice were crossed to yield progeny with both genes deleted, as confirmed by the genotyping results shown here. Agarose gel analysis of PCR products amplified from tail DNA. Lane 1, molecular weight markers; remaining lanes as shown. Size of predicted PCR products of *Sphk2*$^{-/-}$*Ifng*$^{-/-}$ mice (number of bp) is shown at right side of gel and immediately below gel for other lanes.

Supplemental Figure 8




C
**
16
14
12
10
8
6
4
2
0
Percent (%)
IRI Kidney
CD45+IFNγ+
CD45-IFNγ+
WT
Sphk1-/-
Sphk2-/-
D
**
30
25
20
15
10
5
0
Percent (%)
IRI Kidney
CD4+
CD8+
WT
Sphk1-/-
Sphk2-/-
E
*
400
350
300
250
200
150
100
50
0
Total Cell Number (X10^4/g)
IRI Kidney
CD4+
CD8+
WT
Sphk1-/-
Sphk2-/-

Supplemental Figure 8. Identification of leukocyte subsets and IFN-γ-producing cells in mouse kidneys after unilateral IRI. Mice were subjected to unilateral IRI, as described in Supplemental Figure 3, except that brefeldin A (250 μg/mouse, ip) was administered 5 hr before euthanizing to enable evaluation of intracellular cytokines. Kidneys were removed at day 14 and evaluated by histologic and immunofluorescent methods (in frozen sections) and by flow cytometry. A. WT and *Sphk1*$^{-/-}$ mice had substantial deposition of collagen (picrosirius red birefringence), proliferation of interstitial immune cell (Ki67^{+}CD45^{+}) or fibroblasts (Ki67^{+}PDGFRβ^{+}), transformation of fibroblasts to myofibroblasts (SMA^{+} PDGFRβ+), infiltration of leukocytes (CD45^{+}) and numbers of IFN-γ^{+} cells (some were also CD45^{+}) whereas *Sphk2*$^{-/-}$ mice were protected from fibrosis. The protective effect of Sphk2 deletion was lost in the absence of Ifng (*Sphk2*$^{-/-}$ *Ifng*$^{-/-}$ mice). Black and white panels on the right are IFN-γ labeling pulled out separately from the multicolor CD45/CD3/ IFN-γ /DAPI photo to better reveal IFN-γ $^{+}$ cells alone. Scale bar = 100 μm. B-E. Flow cytometry of kidneys. B. There were increased numbers of neutrophils (CD11b^{+}Ly6G^{high}) and macrophages (CD11b^{+}F4/80^{low}) in IRI kidneys compared to contralateral control kidneys of WT and *Sphk1*$^{-/-}$ mice (with macrophage numbers substantially higher in *Sphk1*$^{-/-}$ mice than in either WT or *Sphk2*$^{-/-}$ mice) but neutrophil numbers did not increase in *Sphk2*$^{-/-}$ mice while macrophage numbers in *Sphk2*$^{-/-}$ IRI kidneys were comparable to WT kidneys. The pattern of leukocyte infiltration (i.e. relative increases in cell numbers) was somewhat different in kidneys from the unilateral model compared to the folic acid model (Figure 3). There were small (but N.S.) differences in T cell numbers between control and IRI kidneys and between mouse strains in the unilateral IRI model. C. The percentage of CD45^{+}IFN-γ^{+} cells was lower in IRI kidneys of *Sphk2*$^{-/-}$ than in WT and *Sphk1*$^{-/-}$ mice, whereas CD45^{-}IFN-γ^{+} cells were about the same in all 3 groups. D. T cell subsets. There were more CD8 T cells in IRI kidneys of *Sphk2*$^{-/-}$ mice than in WT and more CD4 T cells in *Sphk1*$^{-/-}$ than in WT kidneys, but the total numbers of CD4 (though N.S.) and CD8 T cells were lower in *Sphk2*$^{-/-}$ than in WT mouse kidneys E. *, $p<0.05$; **, $p<0.01$. N = 5.N.S. not significant.