Figure S1 **Generation of *γ-GT* DTR transgenic mice**. (**A**) Schematic construct of the transgene. (**B**) PCR identified expected hHB-EGF band (left panel) and HA tag band (right) in kidneys of transgenic (TG) mice but not wild type (WT) mice. (**C**) PCR demonstrated expected hHB-EGF band in kidney but not liver of transgenic mice.

Figure S2 **Macrophages/dendritic cells played key roles in recovery from ischemia/reperfusion injury.** Macrophage/dendritic cell depletion with clodronate delayed kidney functional and histologic recovery from ischemia/reperfusion injury (arrows). H&E staining: 5 days after injury, original magnification: x 160.

Figure S3 DT induced epithelial apoptosis in *γ-GT* DTR mice. Immunohistochemistry demonstrated increased nucleus cleaved caspase-3 staining (a marker of apoptosis) in kidney epithelial cells 2 days after DT injection (arrow). Original magnification: x250.

Figure S4 **Kidney functional recovery was delayed in DT-mediated acute kidney injury**. 24-hour urinary albumin excretion (UAE) was still elevated in *γ-GT* DTR mice after 4 weeks of DT administration (100 and 1000 ng/kg) (**P*<0.01 vs. wild type, †*P*<0.01 vs. TG-100 group, n = 6 in each group).

Figure S5 **Clodronate effectively depleted macrophages in different organs.** (**A**) Three days after clodronate injection (40 mg/kg, i.p.), the animals were perfused and paraffin slides were stained with F4/80, a marker of macrophage. Clodronate effectively depleted F4/80 positive cells in kidney, liver, and spleen. Original magnification: x 160. (**B**) Quantitative data indicates that vehicle (liposome) did not deplete kidney macrophages/dendritic cells while clodronate depleted kidney macrophages/dendritic cells by ~ 80% (**P*<0.01 vs. control and liposome, n = 4 in each group).

Figure S6 **Diphtheria toxin induced more severe acute kidney injury in *γ-GT/CD11c* DTR mice than in *γ-GT* DTR mice.** Both *γ-GT* DTR mice and *γ-GT/CD11c* DTR mice were injected with 1000 ng/kg

DT, and sacrificed 10 days later. Histological injury was more severe in *γ-GT/CD11c* DTR mice than in *γ-GT* DTR mice (arrow). Original magnification: x100.

Figure S7 **Expression of Markers of M1 and M2 macrophages in macrophages isolated with CD11c beads from kidney of AKI.** (**A**) Macrophage/dendritic cell depletion with clodronate had no effect on the mRNA levels of iNOS, CCL3 and IL-23, but significantly reduced mRNA levels of arginase (Arg), mannose receptor (MR), and IL-4Rα in macrophages/dendritic cells isolated with CD11c beads from kidneys after DT administration 4 days (**P*<0.01 vs. control, ** *P*<0.01 vs. corresponding liposome group, n = 3-5). The expression levels in macrophages isolated from non-DT treated group was treated as 100%. **(B)** Renal macrophages/dendritic cells isolated with CD11b beads from 5 different control mice were pooled, and macrophages/dendritic cells from 3 DT-treated mice (5 d) were pooled (2 sets, 6 mice). Renal macrophage/dendritic cell arginase protein expression increased markedly after DT injection.

Figure S8 **Splenectomy had no effect on kidney function and kidney macrophage and neutrophil number in DT-mediated AKI**. *γ-GT* DTR mice with or without splenectomy were injected with DT (100 ng/kg, i.p.). (**A**) Splenectomy had no effect on DT-mediated BUN increases (n = 6 in each group). (**B**) Flow cytometry indicated that splenectomy had no effect on kidney macrophage and neutrophil infiltration 5 days after DT injection (n=6 in each group).

Figure S9 **Inhibition of CSF-1 pathway augmented DT-induced acute kidney injury in *γ-GT* DTR mice. (A)** CSF-1 mRNA levels were increased as early as 2 days after DT injection (**P*<0.01 vs. control, n = 4). Immunostaining indicates increased CSF-1 expression in the epithelial cells (arrow). Original magnification: x 250. (**B**) DT (100 ng/kg) treated *γ-GT* DTR mice were given either vehicle (water) or GW2580 (an inhibitor of CSF-1 receptor/c-fms) and were sacrificed at 12 days. GW2580 delayed histologic recovery and caused interstitial fibrosis (arrow). Original magnification: x160.

Figure S10 **CSF-1 deficiency delayed functional recovery from ischemia/reperfusion injury as indicated by increased serum BUN** (**P*<0.05 vs. wild type, n = 4 in each group).

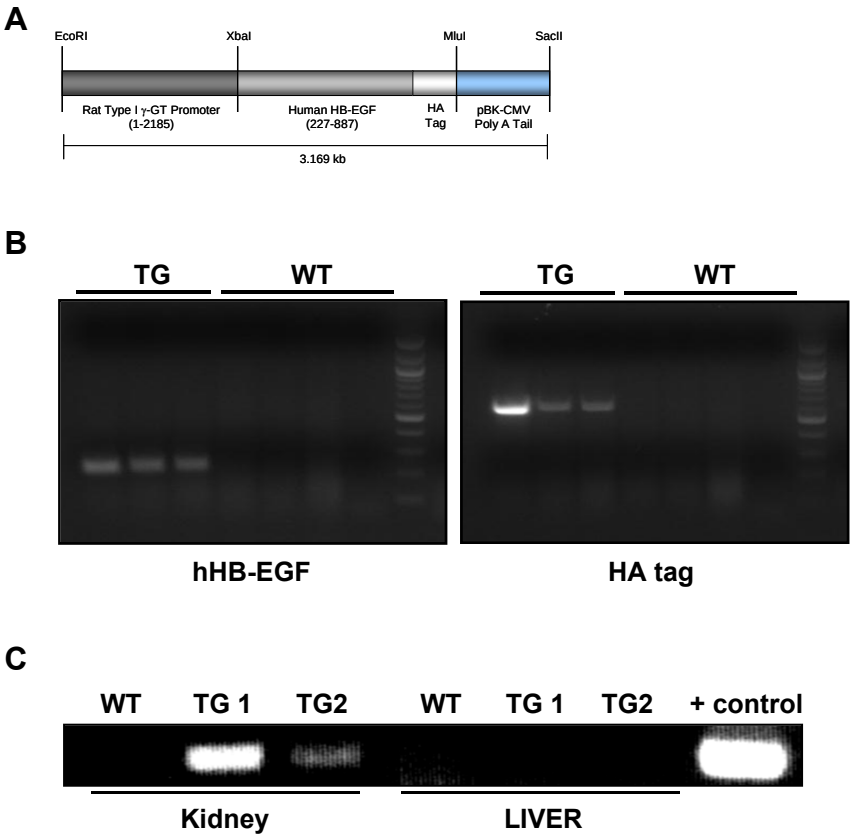
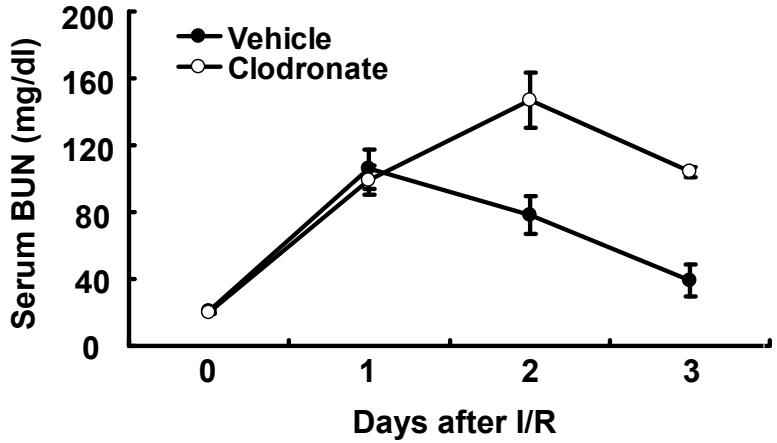

A
EcoRI
XbaI
MluI
SacII
Rat Type I γ-GT Promoter
(1-2185)
Human HB-EGF
(227-887)
HA
Tag
pBK-CMV
Poly A Tail
3.169 kb
B
TG
WT
TG
WT
hHB-EGF
HA tag
C
WT
TG 1
TG2
WT
TG 1
TG2
+ control
Kidney
LIVER

Figure S1


200
160
120
80
40
0
Serum BUN (mg/dl)
Vehicle
Clodronate
0
1
2
3
Days after I/R

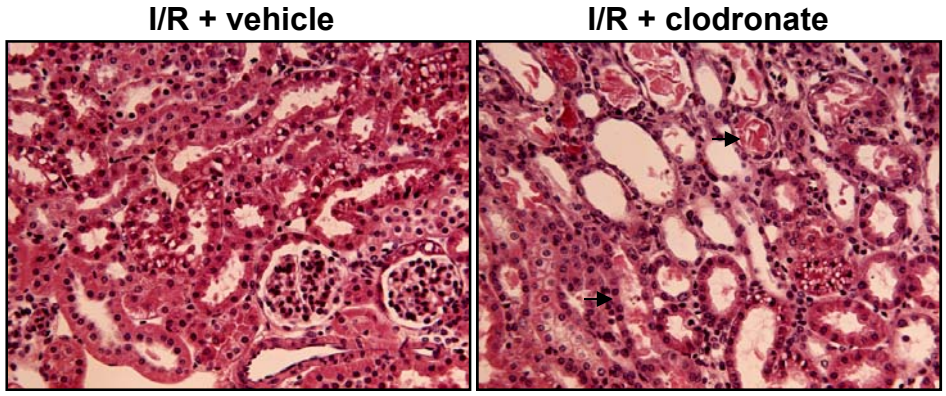

I/R + vehicle
I/R + clodronate

Figure S2

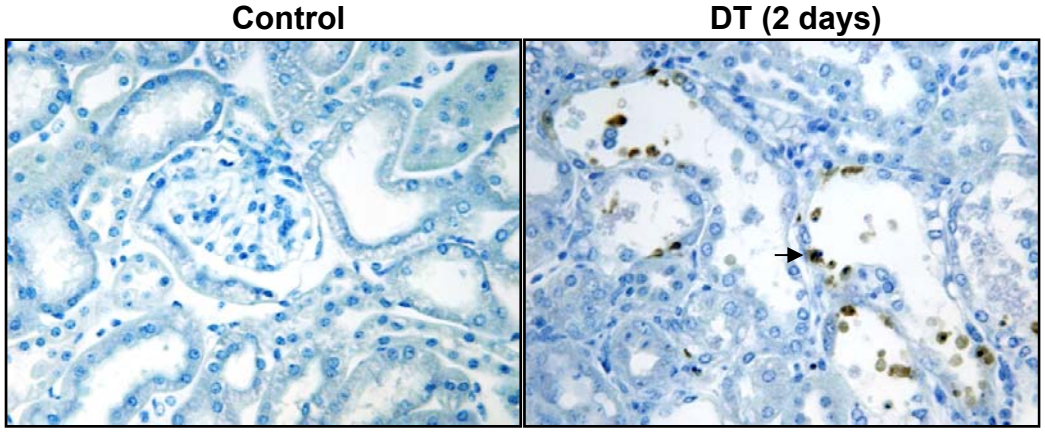


Figure S3

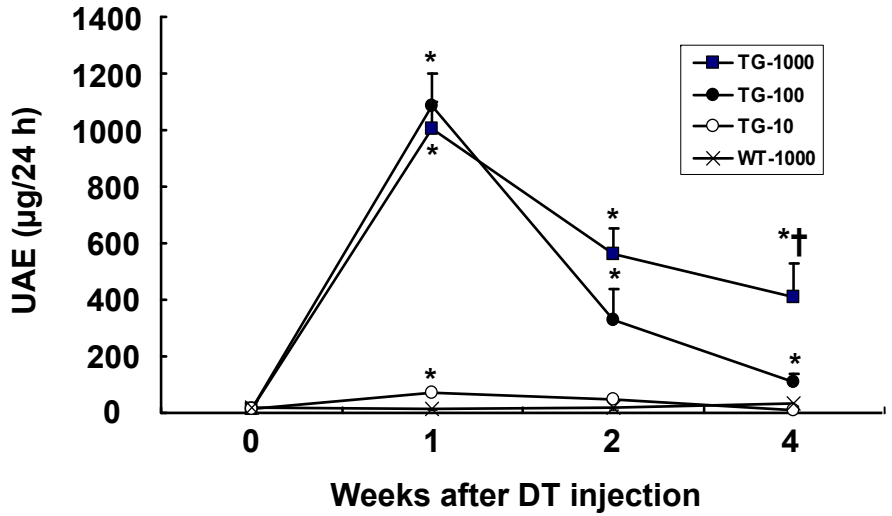

1400
1200
1000
800
600
400
200
0
UAE (µg/24 h)
0
1
2
4
Weeks after DT injection
TG-1000
TG-100
TG-10
WT-1000
*
*
*
*
*
*
*†

Figure S4

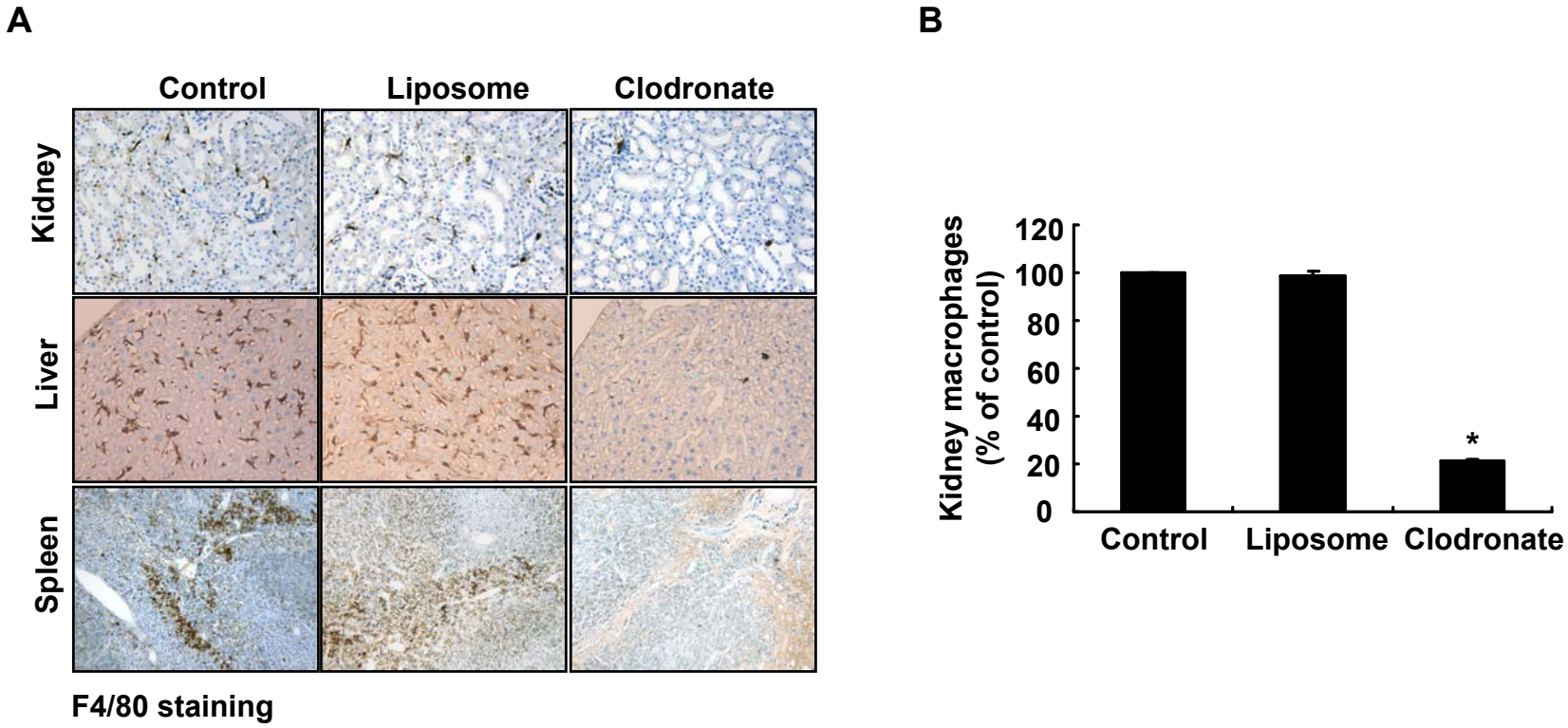

A
Control
Liposome
Clodronate
Kidney
Liver
Spleen
F4/80 staining
B
Kidney macrophages
(% of control)
120
100
80
60
40
20
0
*
Control
Liposome
Clodronate

Figure S5

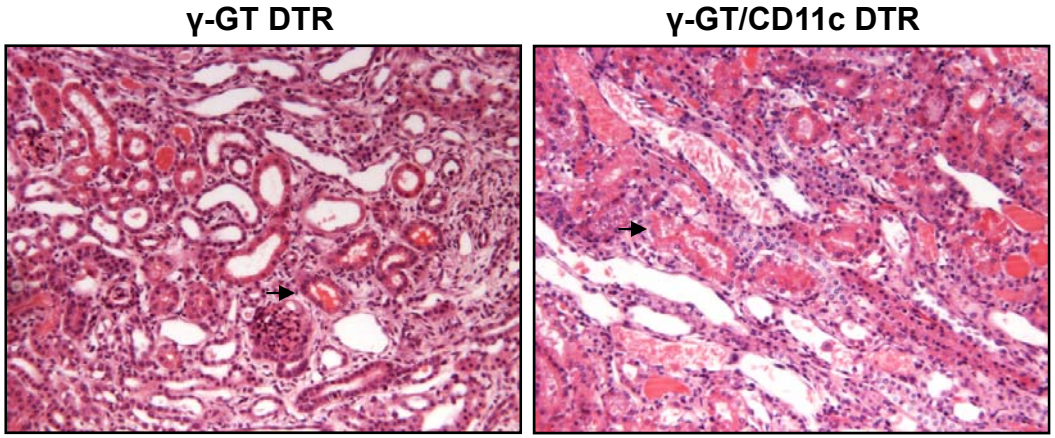
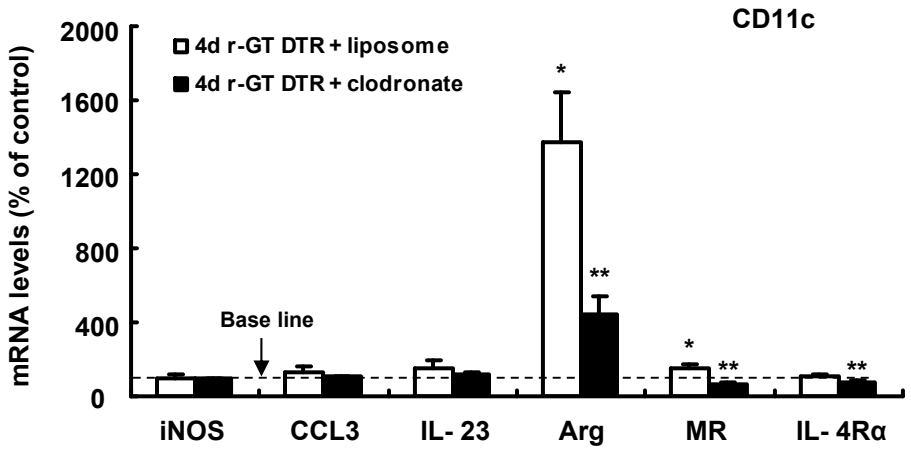


Figure S6

A


CD11c
4d r-GT DTR + liposome
4d r-GT DTR + clodronate
mRNA levels (% of control)
2000
1600
1200
800
400
0
Base line
*
**
*
**
**
iNOS
CCL3
IL- 23
Arg
MR
IL- 4Rα

B

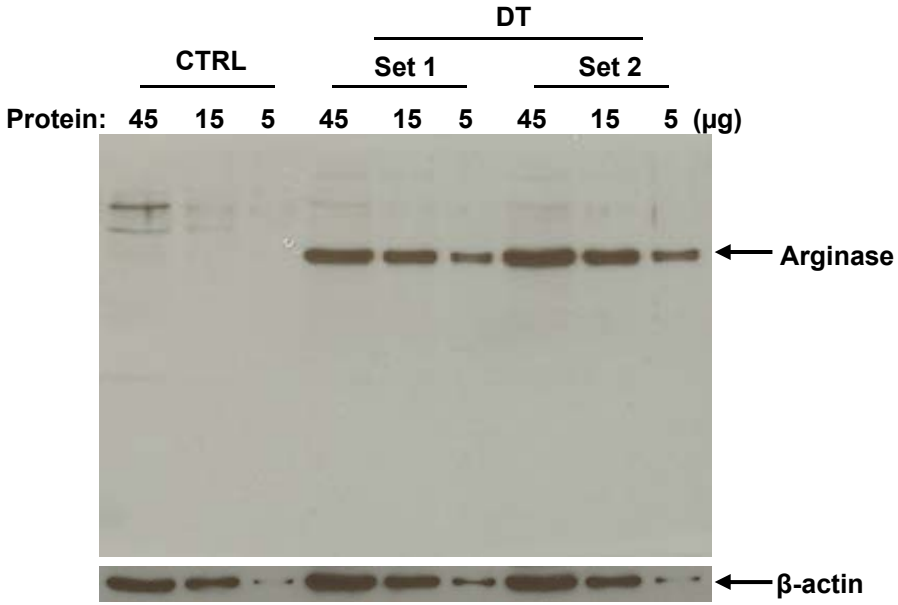
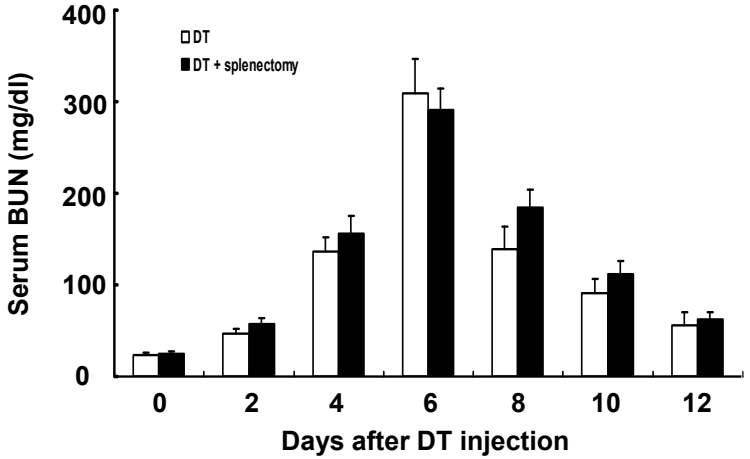
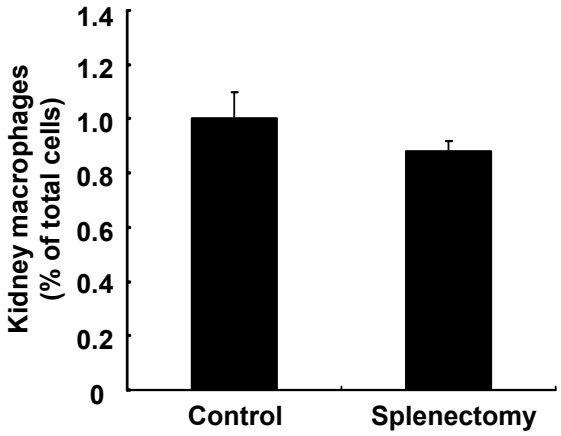

DT
CTRL
Set 1
Set 2
Protein: 45 15 5 45 15 5 45 15 5 (μg)
Arginase
β-actin

Figure S7

A


DT
DT + splenectomy
Serum BUN (mg/dl)
400
300
200
100
0
0
2
4
6
8
10
12
Days after DT injection

B


Kidney macrophages
(% of total cells)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
Control
Splenectomy

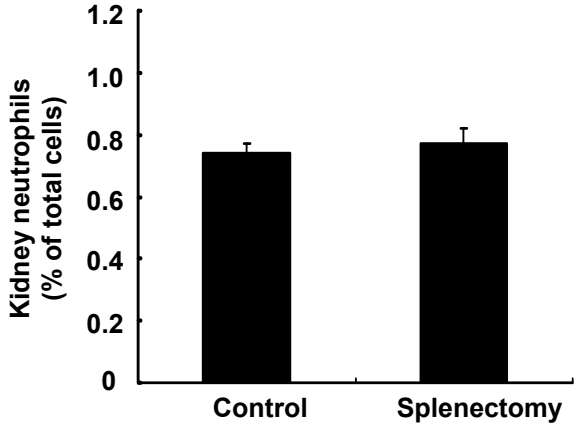

Kidney neutrophils
(% of total cells)
1.2
1.0
0.8
0.6
0.4
0.2
0
Control
Splenectomy

Figure S8

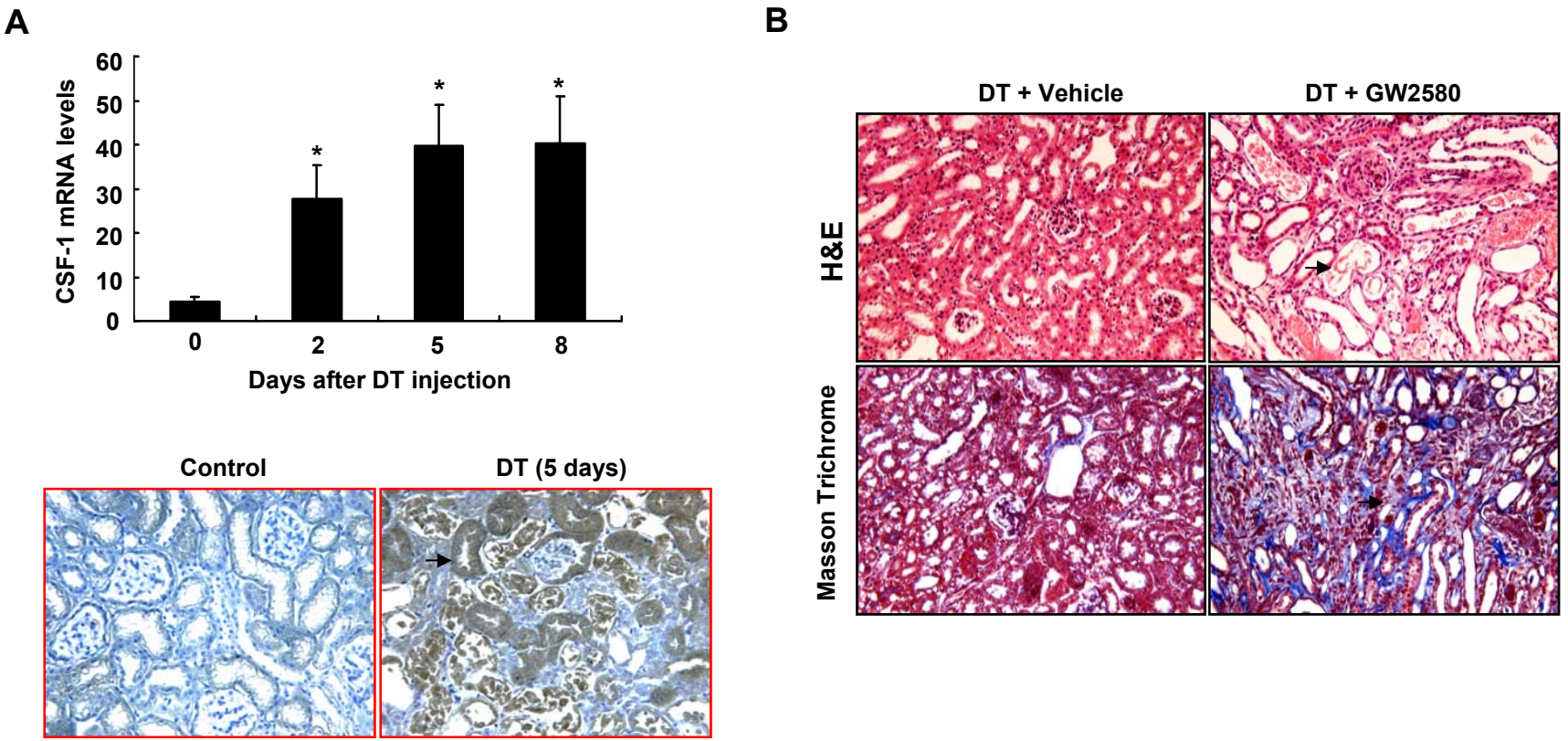

A
CSF-1 mRNA levels
60
50
40
30
20
10
0
*
*
*
0
2
5
8
Days after DT injection
Control
DT (5 days)
B
DT + Vehicle
DT + GW2580
H&E
Masson Trichrome

Figure S9

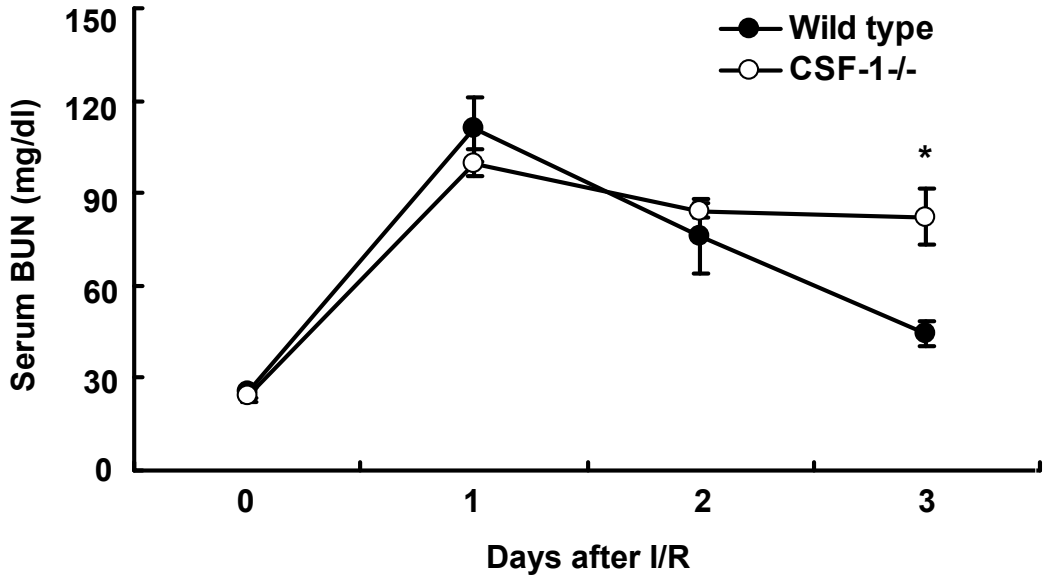

Wild type
CSF-1-/-
Serum BUN (mg/dl)
150
120
90
60
30
0
0
1
2
3
Days after I/R
*

Figure S10