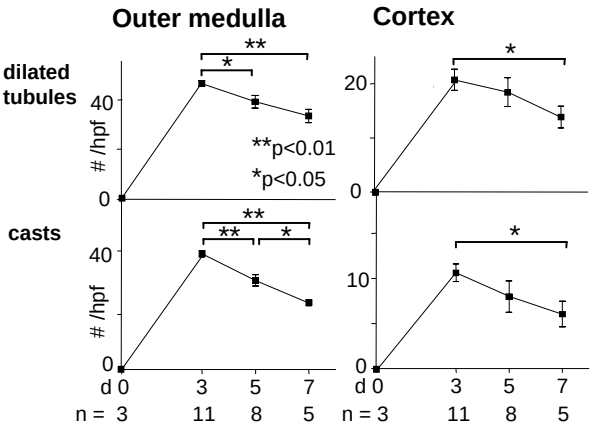
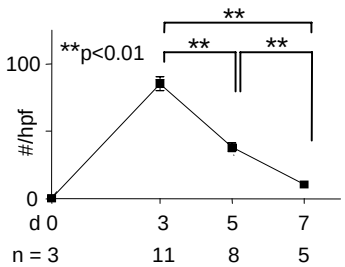
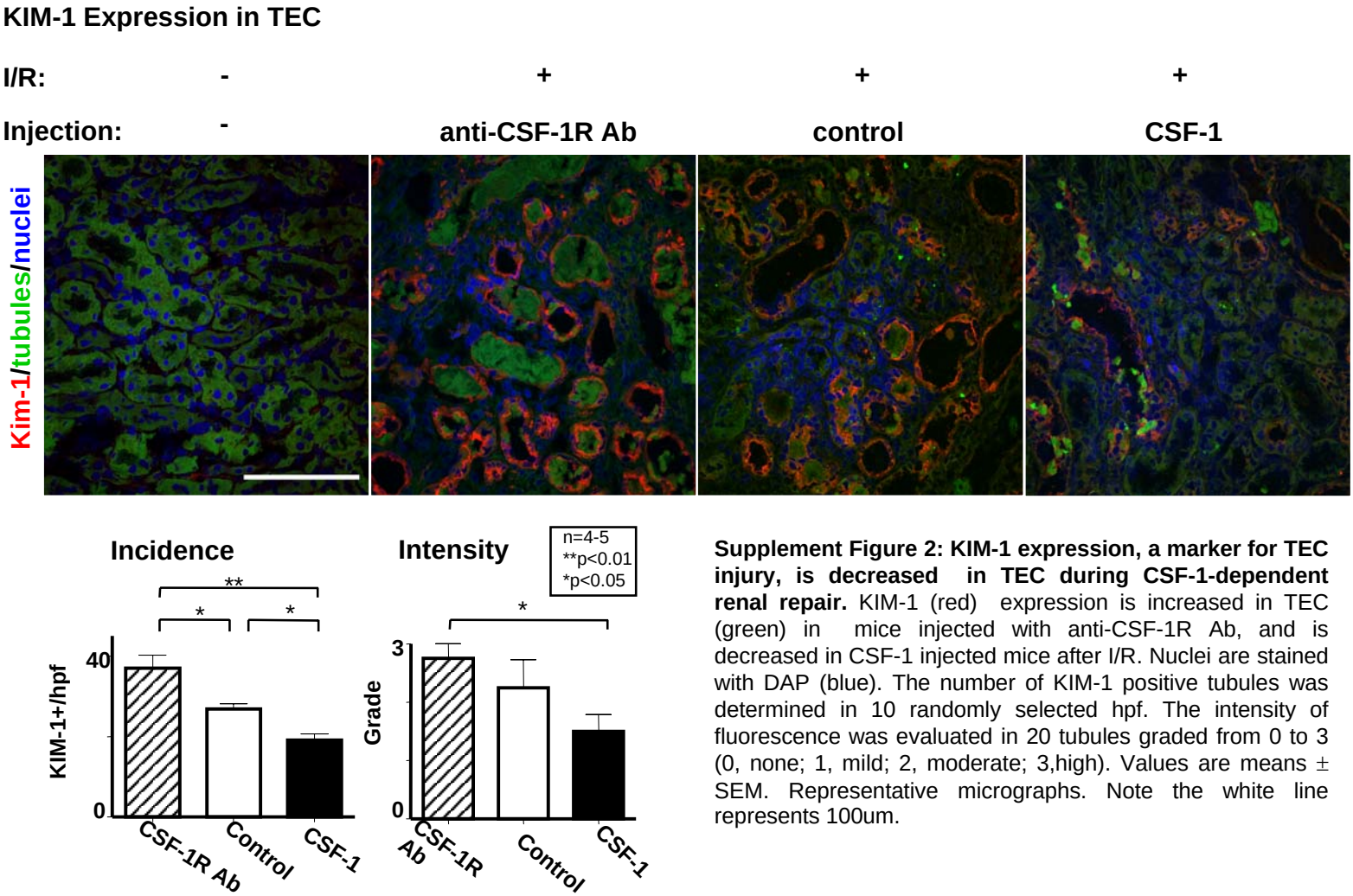
A. Tubular Histopathology



B. Tubular Proliferation

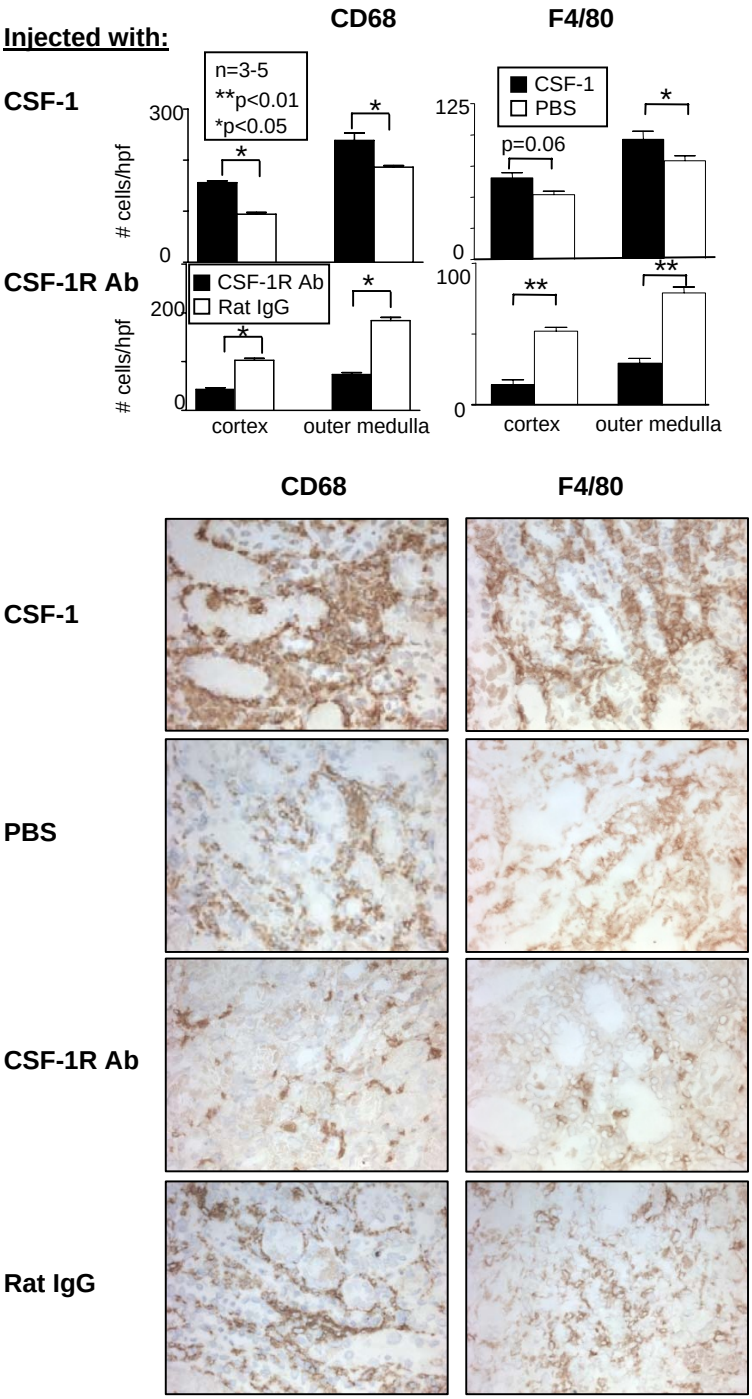


Supplement Figure 1. Tubular pathology and proliferation following I/R peaks at day 3 and returns rapidly to normal values. A. Tubular dilation and casts are readily apparent at 3 days and progressively decline at days 5 and 7. B. Tubular proliferation (Ki67) also peaks at 3 days, but returns to normal more rapidly than tubules. Values were determined in 10 hpf. Means ± SEM.



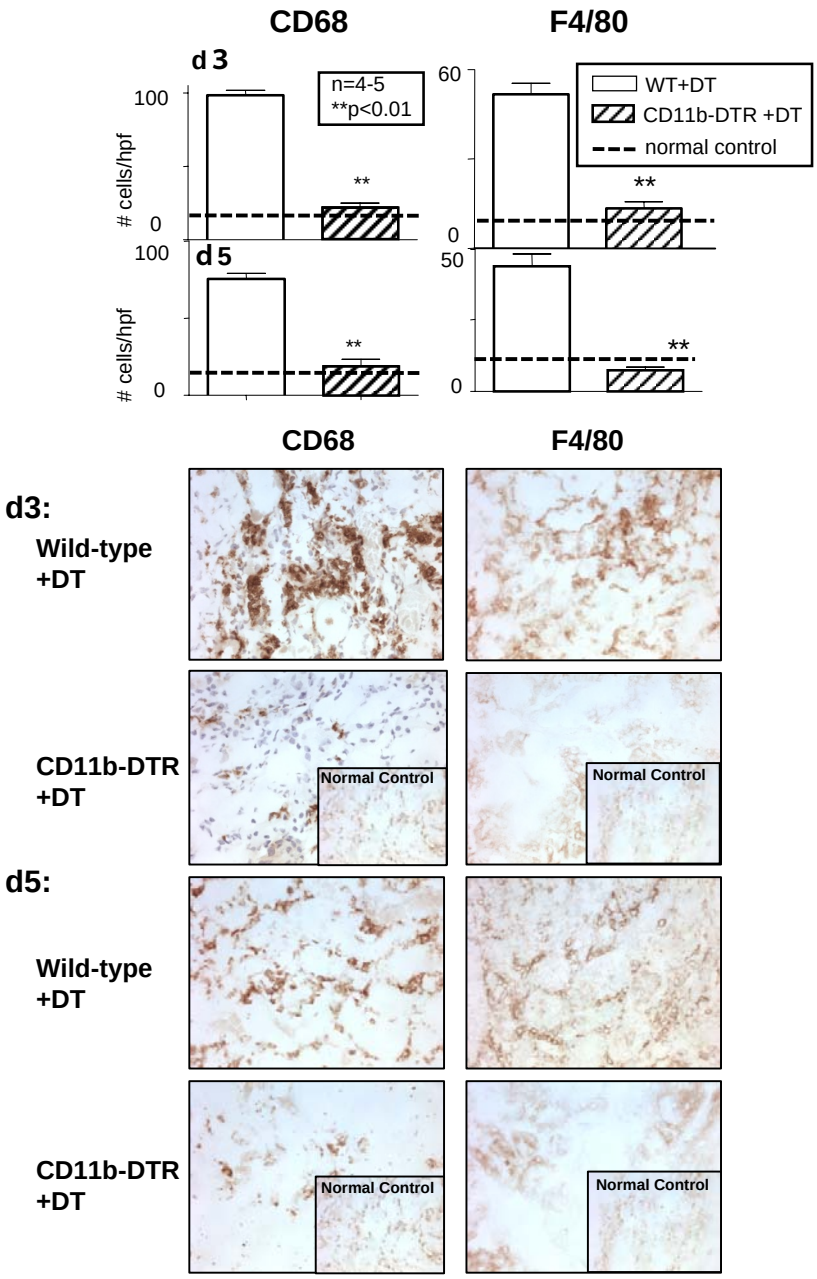
Supplement Figure 2: KIM-1 expression, a marker for TEC injury, is decreased in TEC during CSF-1-dependent renal repair. KIM-1 (red) expression is increased in TEC (green) in mice injected with anti-CSF-1R Ab, and is decreased in CSF-1 injected mice after I/R. Nuclei are stained with DAP (blue). The number of KIM-1 positive tubules was determined in 10 randomly selected hpf. The intensity of fluorescence was evaluated in 20 tubules graded from 0 to 3 (0, none; 1, mild; 2, moderate; 3,high). Values are means ± SEM. Representative micrographs. Note the white line represents 100um.

Intra-renal Mø after I/R



Supplement Figure 3. Intra-renal Mø are increased in CSF-1 injected mice during hastened renal repair. Intra-renal Mø (CD68+, F4/80+) are increased in CSF-1 injected mice during hastened renal repair and are decreased in anti-CSF-1R Ab injected mice as compared to controls during retarded repair. CD68+ and F4/80+ Mø (10hpf) were counted in the cortex and outer medulla 5 days after I/R. Means ± SEM. Representative photomicrographs (Magnification 40x).

Eliminating Intra-renal Mø after I/R

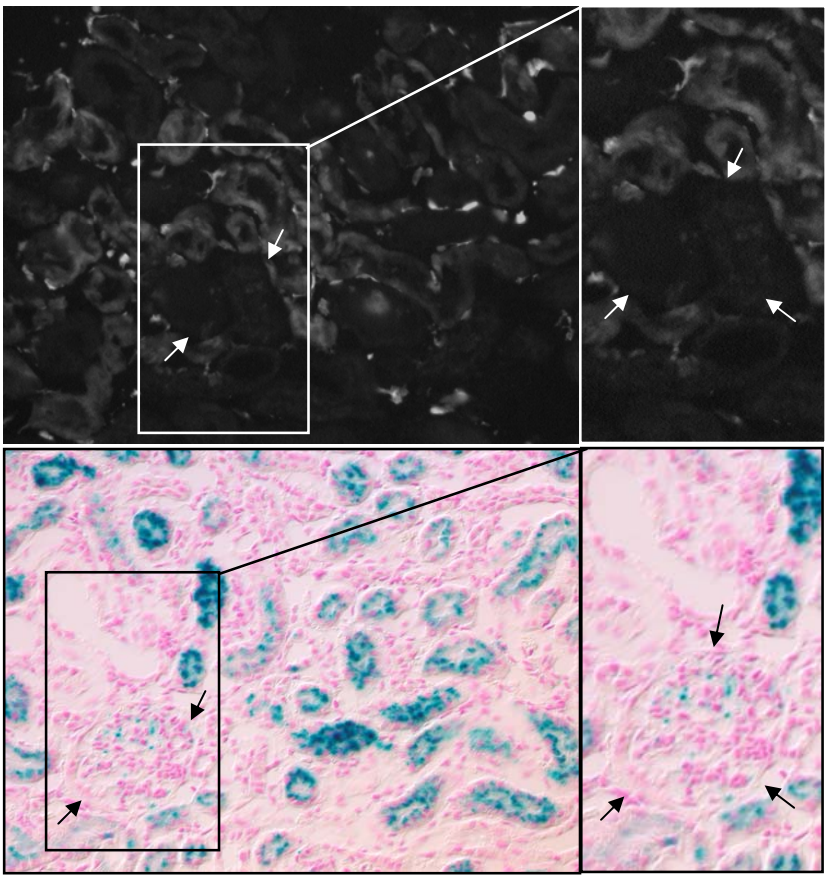


Supplement Figure 4. Intra-renal Mø that are increased after I/R, are depleted in DT sensitive CD11b-DTR mice. Injecting DT in CD11b-DTR mice effectively depletes intra-renal Mø (CD68+, F4/80+) after I/R. WT and CD11b-DTR mice were injected with DT at days 1 and 3 after I/R and Mø enumerated at days 3 and 5. Means ± SEM. Normal controls are unmanipulated mice. Means ± SEM. Representative photomicrographs (Magnification 40x).

Mouse kidney
CSF-1R / CSF-1 expression-TEC and glomeruli

CSF-1R

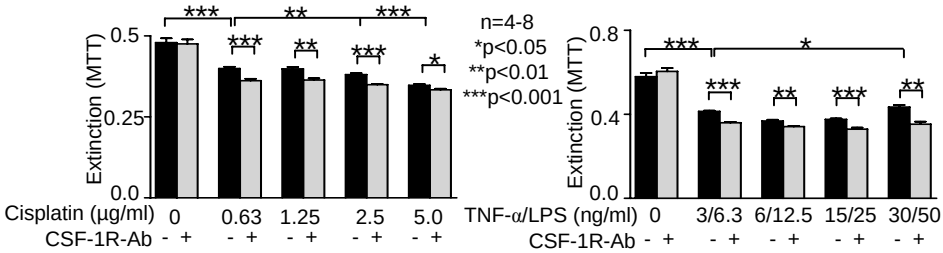
CSF-1



Supplement Figure 5. Mouse renal parenchymal cell expression of CSF-1 is mainly on TEC, with sparse amounts in glomeruli, while CSF-1R is exclusively on TEC after renal injury. Kidney sections from mice following I/R from MacGreen mice depicting CSF-1R expression (EGFP) in the top panels and TgZ mice depicting CSF-1 expression (b-gal expression, blue). Note: Mø expression of CSF-1R is far brighter than TEC expression (faint, broad). Magnification 20x, enlargements (40x) Glomeruli indicated with arrows.

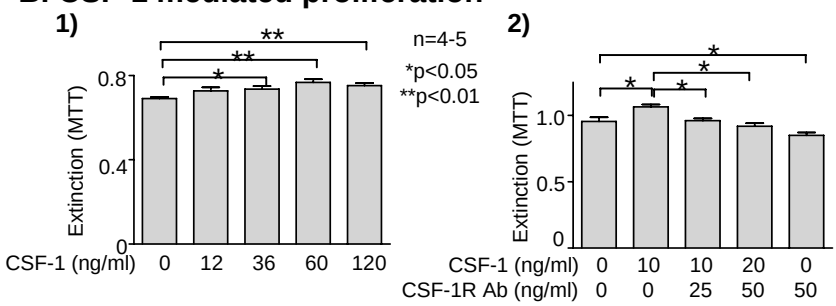
Human CSF-1R+ TEC *(in vitro)*

A. CSF-1 mediated survival/proliferation (HK2 cell line)

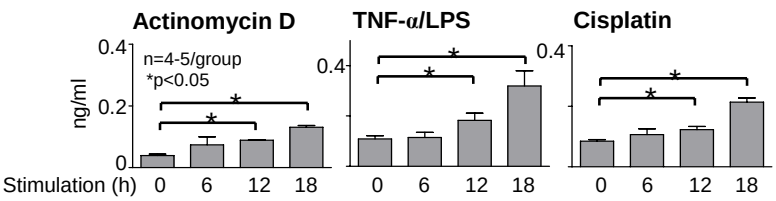


Mouse CSF-1R+ TEC *(in vitro)*

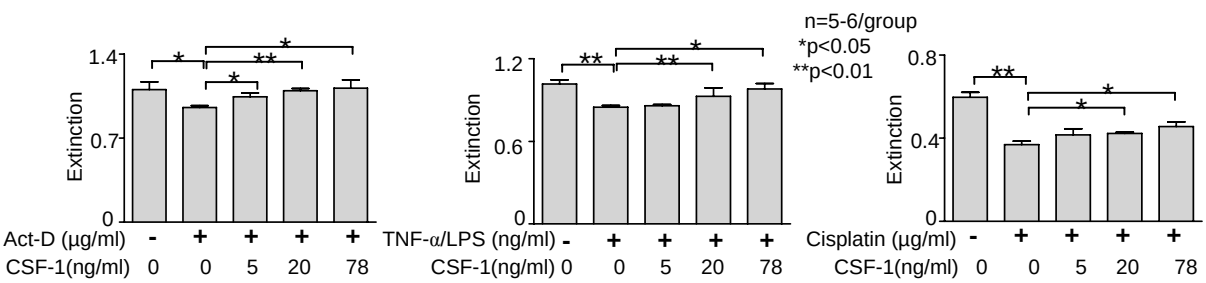
B. CSF-1 mediated proliferation



C. TEC injury - CSF-1 expression



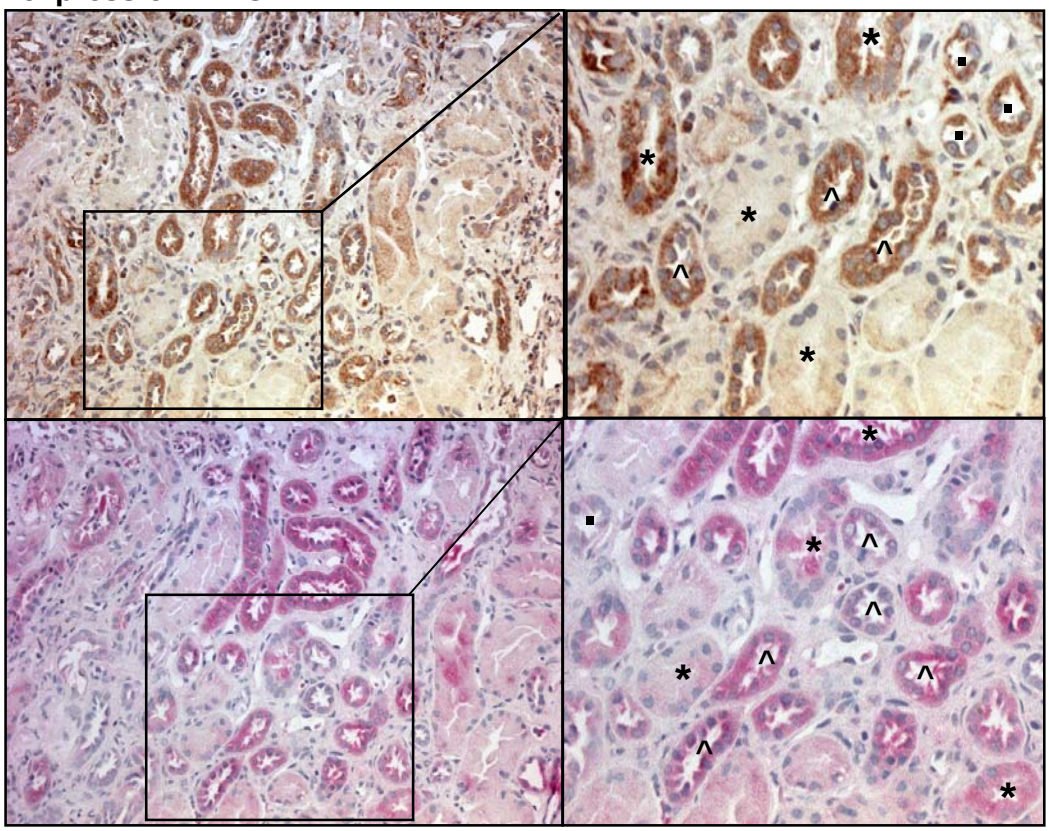
D. TEC injury; CSF-1 mediated proliferation



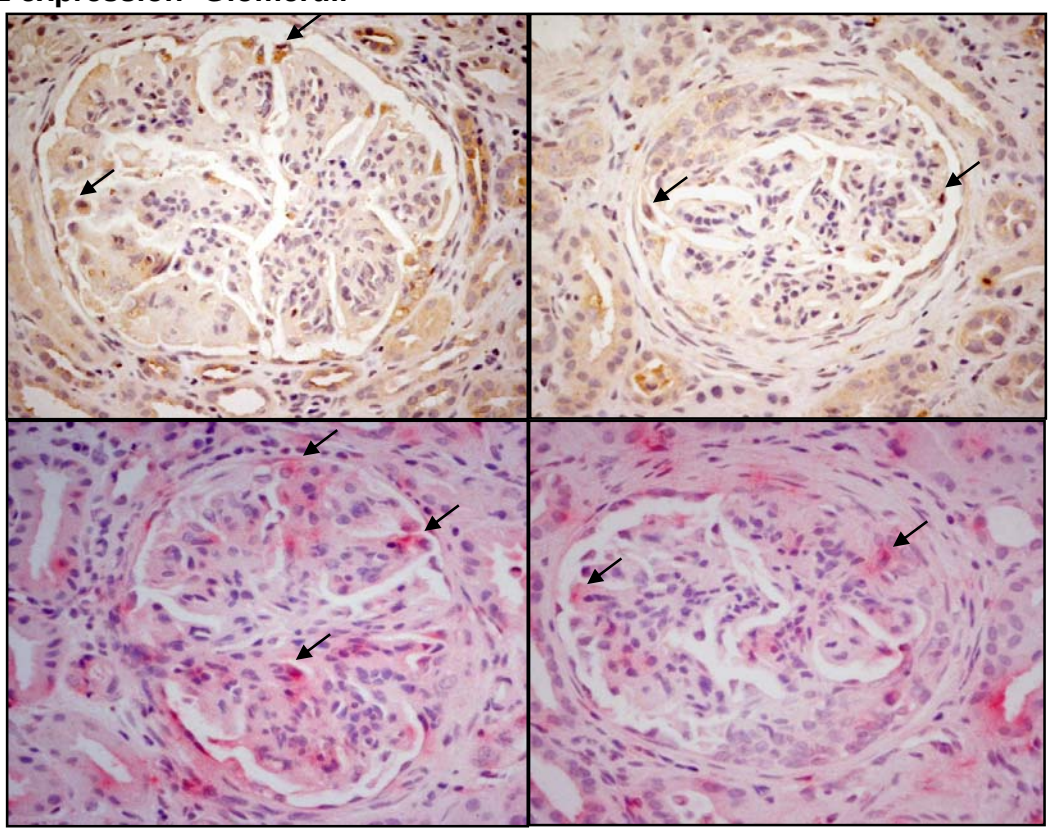
Supplemental Figure 6. CSF-1R+ human/mouse TEC are protected from induced apoptosis and proliferate in response to CSF-1. A. Cisplatin and TNF-a/LPS induced TEC survival/proliferation is CSF-1-mediated. Blocking with CSF-1R Ab (50ng/ml) decreased TEC survival/proliferation following cisplatin or TNF-a/LPS-induced HK2 TEC injury. Means ± SEM. B. 1) CSF-1 mediates TEC survival/proliferation in primary mouse TEC (MTT assay). B. 2) Blocking the CSF-1R reduces primary TEC proliferation (MTT assay). Means ± SEM. C. Actinomycin D (0.05µg/ml), cisplatin (1µg/ml) or TNF-a (6ng/ml)/LPS (12.5ng/ml) treated mouse primary TEC secrete CSF-1. Determination of supernatant CSF-1 by ELISA. Mean ± SEM. C. CSF-1 enhances the survival/proliferation of primary mouse TEC treated with to actinomycin D, cisplatin or TNF-a/LPS (MTT). Means ± SEM.

Human kidney

CSF-1R/CSF-1 expression -TEC

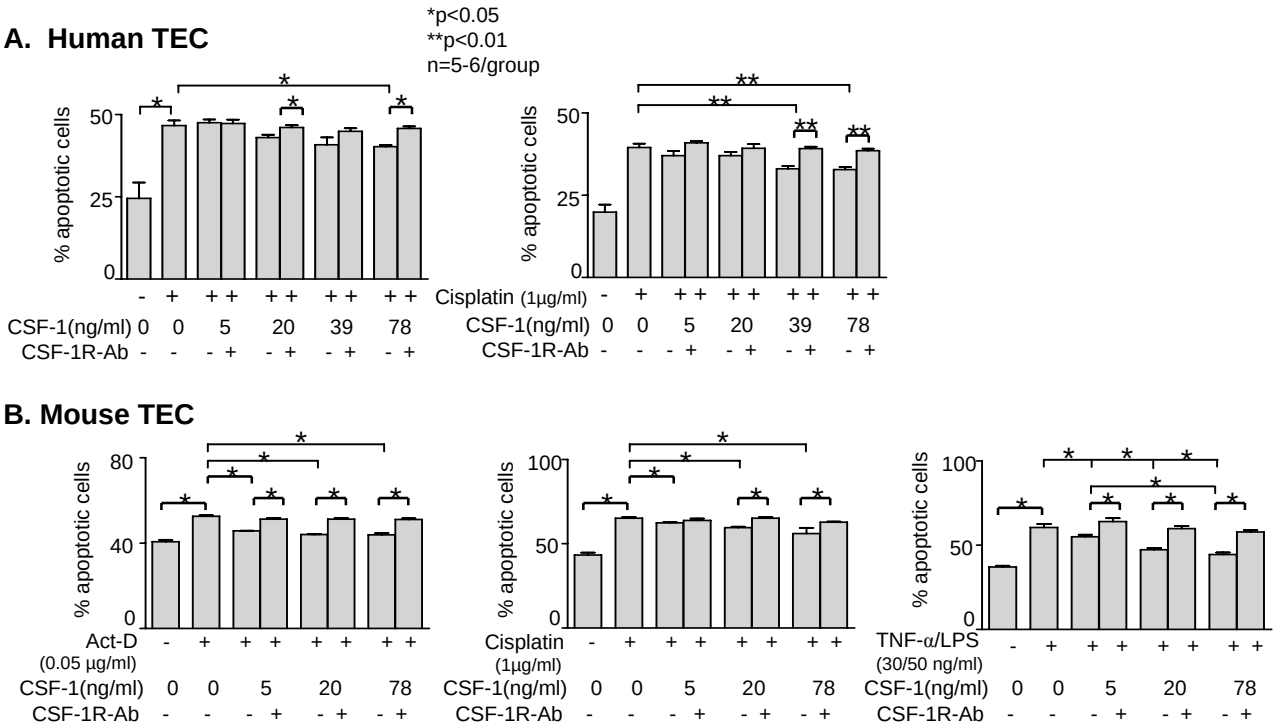


CSF-1R/CSF-1 expression -Glomeruli



Supplement Figure 7. CSF-1 and CSF-1R are expressed in human TEC (distal, proximal and loops of Henle). In contrast, CSF-1 expression is sparse in glomeruli, and CSF-1R expression is barely detectable in glomerular parenchymalcells. The distribution of CSF-1R (brown) and CSF-1 (red) expression was evaluated in sections from patients with impaired renal function and tubular pathology post transplant. We detected CSF-1R and CSF-1 in proximal tubules (*), distal tubules (^), and loops of Henle (•). In contrast, CSF-1 expression was sparsely detected in glomeruli (mesangial, podocyte distribution) and the CSF-1R was only occasionally detected on cells in the glomeruli. Magnification 20x, enlargement 40x.

TEC Injury;CSF-1 suppresses apoptosis



Supplement Figure 8. CSF-1 counters TEC (human/mouse) apoptosis following injury. CSF-1 dose-dependent attenuation of actinomycin-D, cisplatin or TNF-a/LPS induced TEC apoptosis (flow cytometry, Annexin-PI). A. Human HK2 TEC; B. Mouse C1 TEC. The CSF-1R was blocked using a specific CSF-1R Ab (50ng/ml) (41). Means ± SEM.