

LEGENDS FOR SUPPLEMENTAL FIGURES

Supplemental Figure 1. Expression of EGFR in renal epithelial cells and renal interstitial fibroblasts. Confocal photomicrographs (1X400) illustrating EGFR and α -SMA staining of cortex kidney tissue collected at day 7 after UUO injury. A, α -SMA; B, EGFR; C, DAPI; D, merge.

Supplemental Figure 2. Location of EGFR in renal tubular cells. The kidney tissue collected at day 7 after UUO injury was processed for immunofluorescence of EGFR, followed by staining with FITC-labeled PNA and DAPI. Photographs were taken by confocal microscopy (1X400) from the cortex to the medulla.

Supplemental Figure 3. Location of p-EGFR in renal tubular cells. The kidney tissue collected at day 7 after UUO injury was processed for immunofluorescence of p-EGFR (Tyr-1073), followed by staining with antibodies against megalin, Aquaporin2 (AQP2) and Tamm-Horsfall Protein (THP), respectively. Photographs (1X400) were taken by a confocal microscopy. Arrows: collecting ducts.

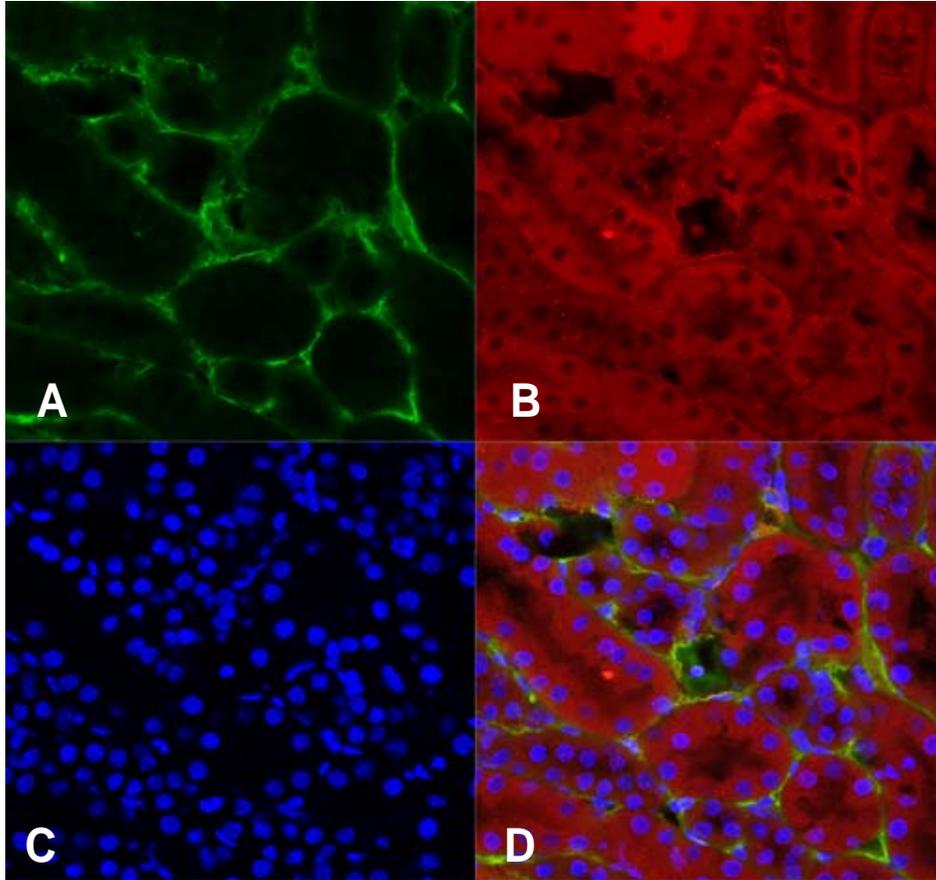
Supplemental Figure 4. The kidney tissue collected at day 7 after shamed surgery or UUO injury was processed for immunofluorescence of FSP-1. Photographs (1X400) were taken by an immunofluorescent microscopy.

Supplemental Figure 5. Effect of gefitinib on serum-induced interstitial fibroblast activation, as well as activation of EGFR, STAT3 and ERK1/2. NRK-49F cells were cultured with 5% FBS for 24 h and then exposed to the indicated concentrations of gefitinib (0-10 nM) for 36 h (A, B, E, G, I). Cell lysates were subjected to immunoblot analysis using antibodies to fibronectin (A), α -SMA (C), collagen 1(E), α -tubulin, p-EGFR, EGFR (G), p-STAT3, STAT3, p-ERK1/2, ERK1/2 (I).

Representative immunoblots from three experiments are shown (A-J). Expression levels of all these proteins were quantified by densitometry and expressed as means \pm SEM. The ratios of p-EGFR/EGFR (H), p-STAT3/STAT3 or p-ERK/ERK (J) were shown. Fibronectin, collagen 1, or α -SMA were normalized with α -tubulin (B, D, F). Means with different superscript letters are significantly different from one another ($P < 0.05$).

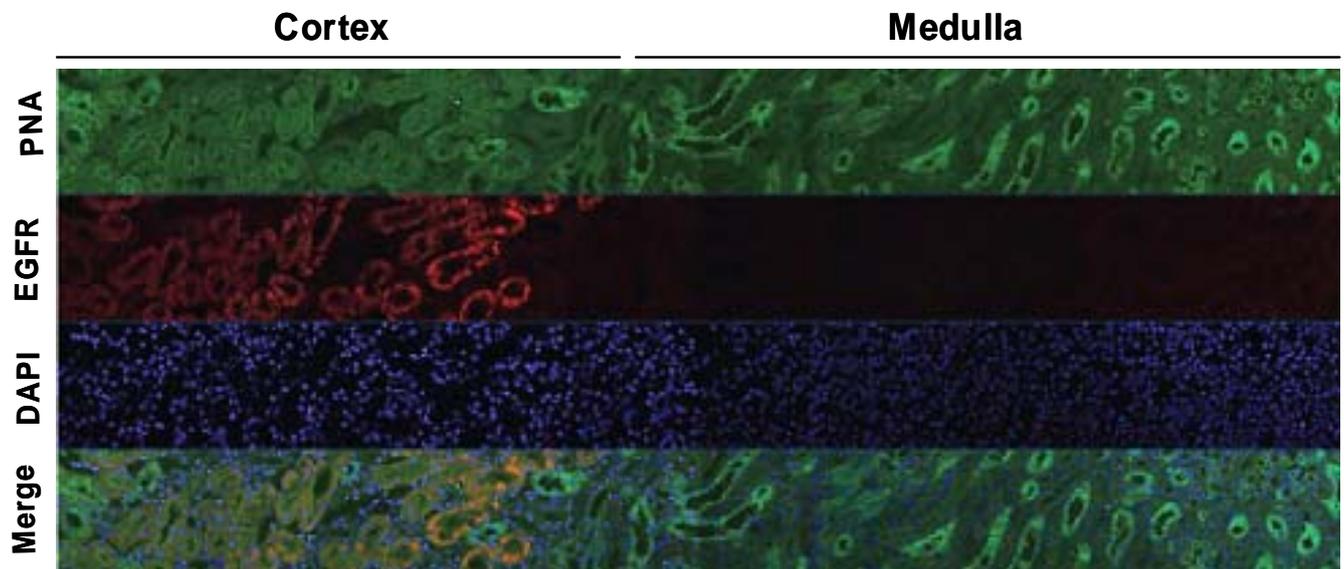
Supplemental Figure 6. Gefitinib treatment does not induce cell death in interstitial renal fibroblasts. NRK-49F cells were cultured with 5% FBS in the absence or presence of gefitinib 10 nM for 36 h or H₂O₂ 250 μ M for 3 h. Cells were harvested for immunoblot analysis of cleaved PARP and cleaved caspase-3.

Supplemental Figure 1

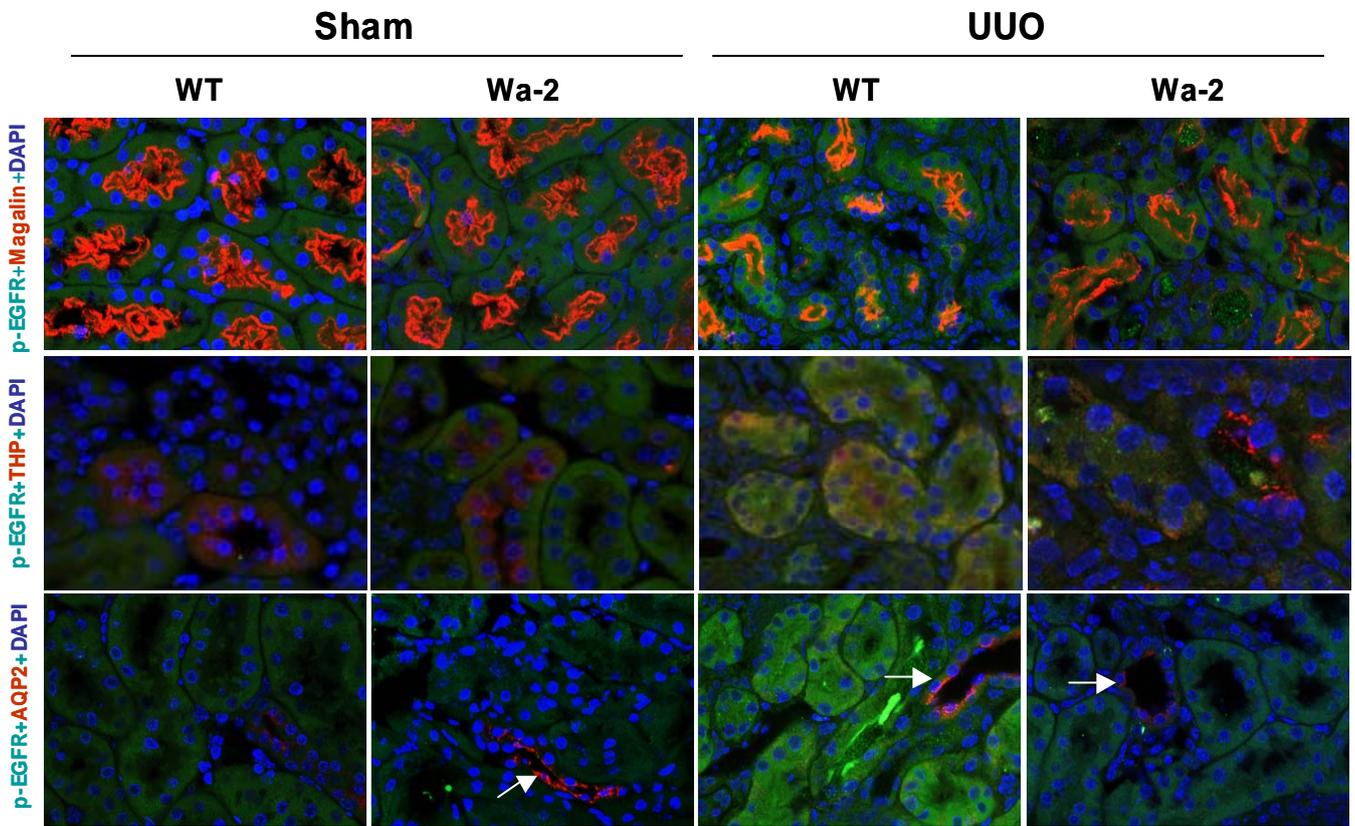


α -SMA+EGFR+DAPI

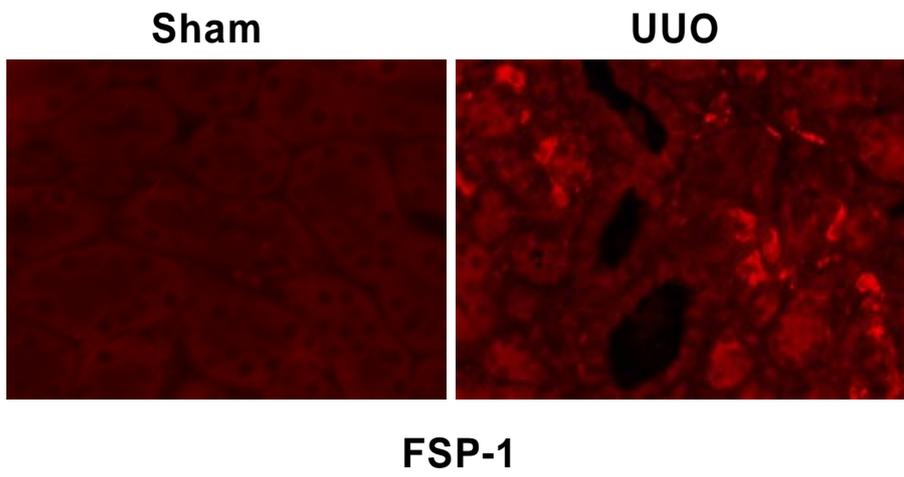
Supplemental Figure 2



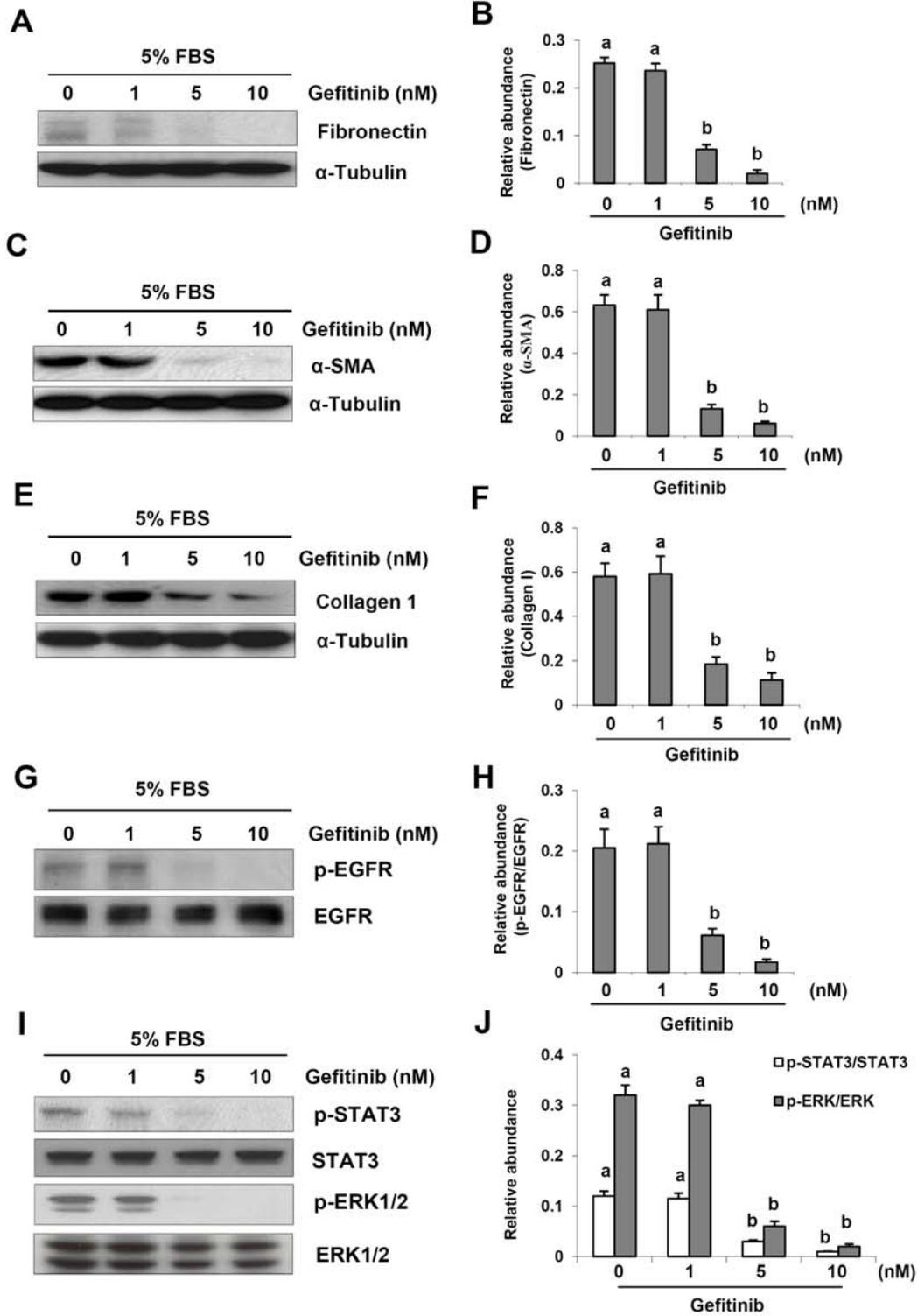
Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 5



Supplemental Figure 6

