Fibroblast expression of an IκB-dominant negative transgene attenuates renal fibrosis

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Supplementary Figure Legends

Figure S1. *FSP1.Cre;EGFP* and *γGT.Cre;EGFP* cells in the kidneys. **A.** Compared with untreated kidneys, a larger number of *FSP1* promoter-driven $EGFP^+$ fibroblasts (in Green) were found in the widened interstitium of obstructed kidneys of *FSP1.Cre;EGFP* mice. $EGFP^+$ tubular epithelia (in Green) were observed in untreated kidneys of *γGT.Cre;EGFP* mice. In the obstructed kidney, in contrast, *γGT* promoter-driven $EGFP^+$ cells were found in tubular epithelia and among solitary cells in the widened interstitium; nuclei were stained blue with TO-PRO-3. When the basement membrane was stained by periodic acid-Schiff reaction (in Red), many of all the $EGFP^+$ cells were identified as interstitial cells surrounding tubules, which were likely to be derived from tubular epithelia by EMT. **B.** Most of *FSP1* promoter-driven $EGFP^+$ fibroblasts (in Green) were simultaneously positive for HSP47 (in Red) (arrows), suggesting production of collagen proteins. Some of *γGT* promoter-driven $EGFP^+$ interstitial cells were simultaneously positive for HSP47 (arrows), suggesting production of collagen proteins. *FSP1* promoter-driven $EGFP^+$ fibroblasts were not macrophages since they were not positive for F4/80. (Objective lens 40x; bar=100 μm)

Figure S2. Inhibition of NF-κB activation suppressed fibrosis-related gene transcription in cultured mouse renal fibroblasts, TFB. The TFB cells[14] were seeded in 6-well plates ($1x10^5$ cells/well) and incubated overnight in growth medium, after which the medium was replaced with D-MEM containing 0.5% FCS and the cells were incubated for 48 hr prior to subsequent experiments. To examine the effects of NF-κB activation in these cells, an IκK inhibitor, BMS-345542 (Calbiochem, San Diego, CA) (2 μM) was added to the cultures 30 min prior to stimulation with rhTGF-β1 (3.0 ng/mL) or rhTNF-α (3.0 ng/mL). After an incubation of another 1 and 6 hr, the cells were harvested for nuclear protein and RNA extraction, respectively.

Electrophoretic mobility shift assay was performed according to the method reported previously.[41] One hr treatment with TGF-β1 induced nuclear translocation of NF-κB in TFB cells as TNF-α did, and pretreatment with BMS-345542 suppressed it (A). B. Six hr treatment with TNF-α significantly increased the levels of MCP-1 mRNA in TFB cells, while treatment with TGF-β1 significantly increased the mRNA levels of PAI-1 and FN-EIIIA, but not MCP-1 in TFB cells, the former of which was suppressed by BMS-345542 pretreatment. Data shown in B were obtained from 3 independent experiments.

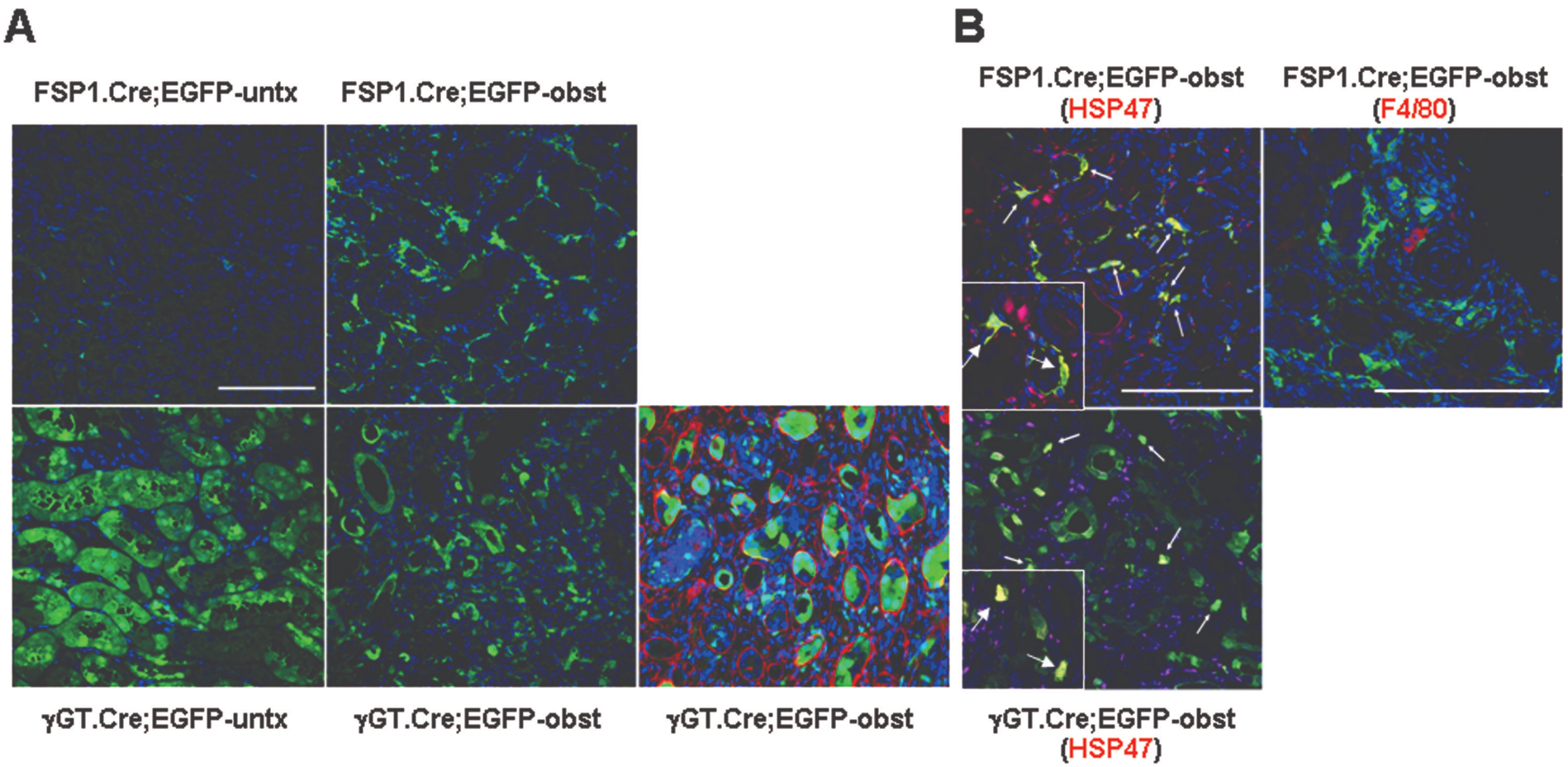
A
FSP1.Cre;EGFP-untx
FSP1.Cre;EGFP-obst
γGT.Cre;EGFP-untx
γGT.Cre;EGFP-obst
γGT.Cre;EGFP-obst
B
FSP1.Cre;EGFP-obst
(HSP47)
FSP1.Cre;EGFP-obst
(F4/80)
γGT.Cre;EGFP-obst
(HSP47)

Figure S1

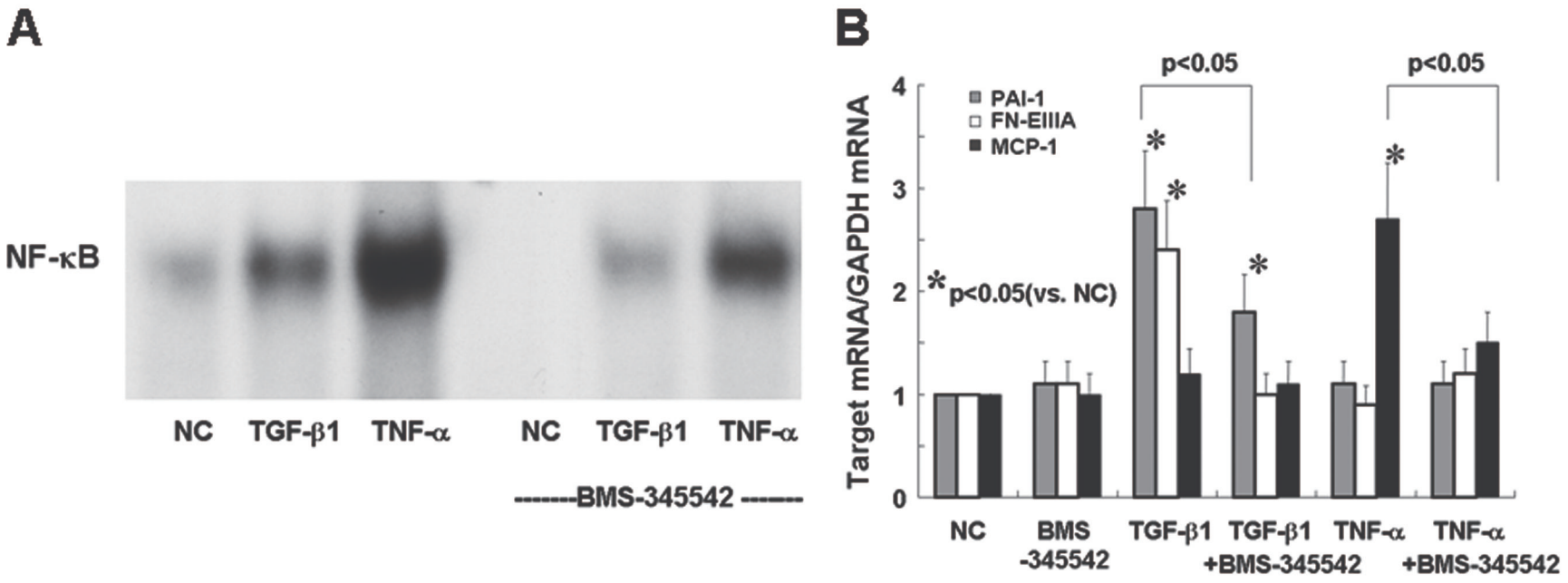
A
NF-κB
NC
TGF-β1
TNF-α
NC
TGF-β1
TNF-α
-------BMS-345542 -------
B
Target mRNA/GAPDH mRNA
PAI-1
FN-EIIIA
MCP-1
p<0.05
p<0.05
*p<0.05(vs. NC)
NC
BMS -345542
TGF-β1
TGF-β1 +BMS-345542
TNF-α
TNF-α +BMS-345542

Figure S2