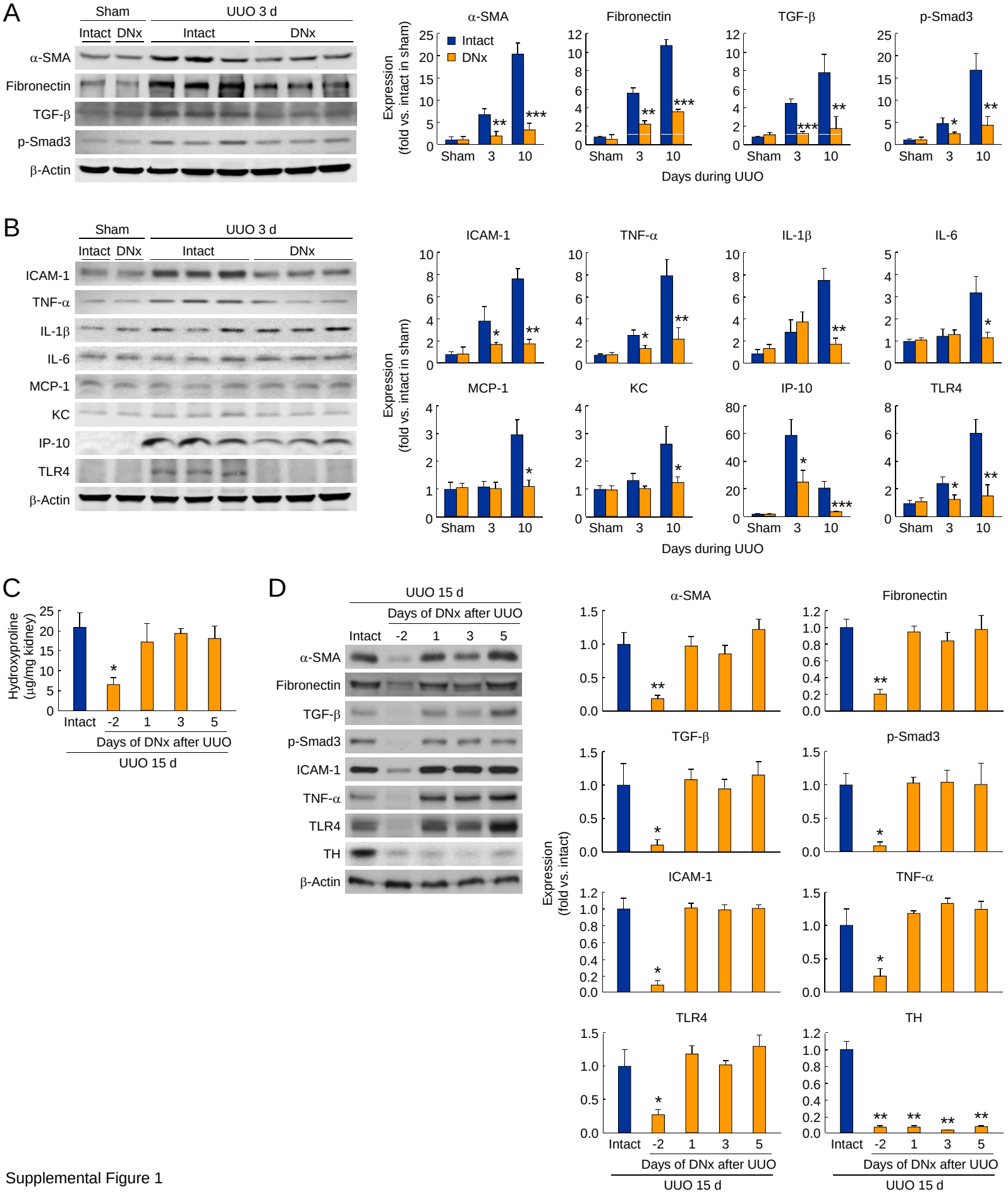
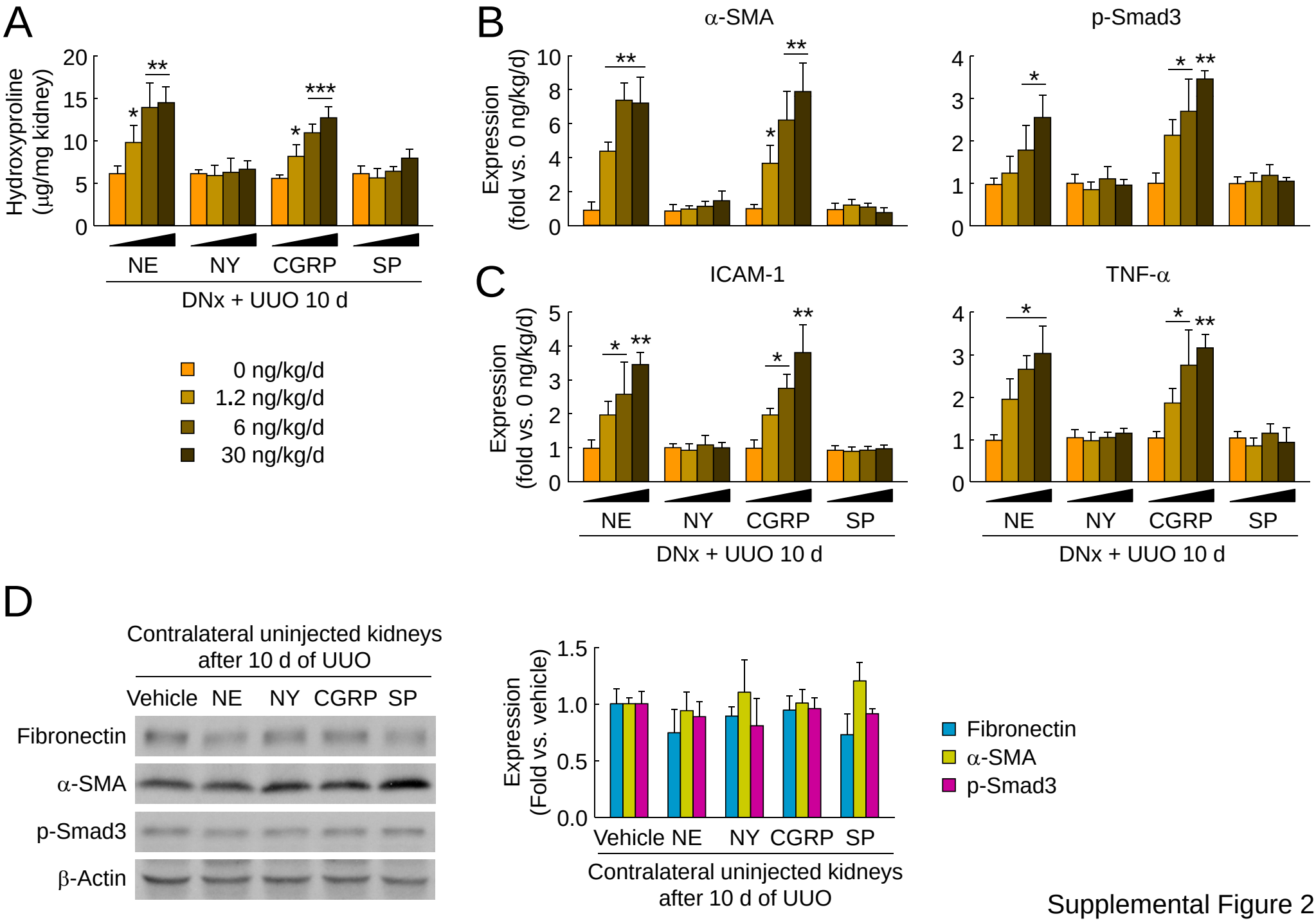


Supplemental Figure 1



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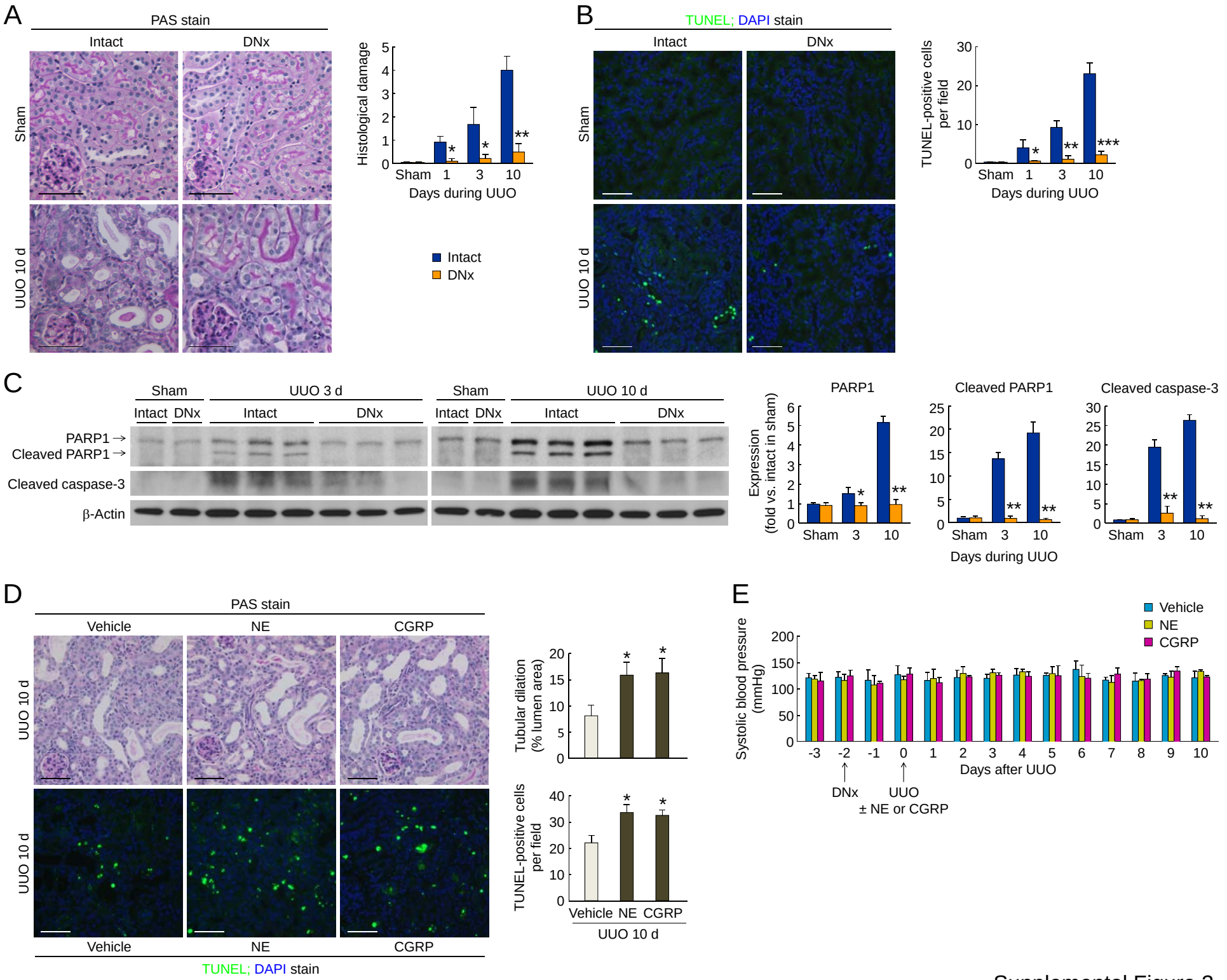

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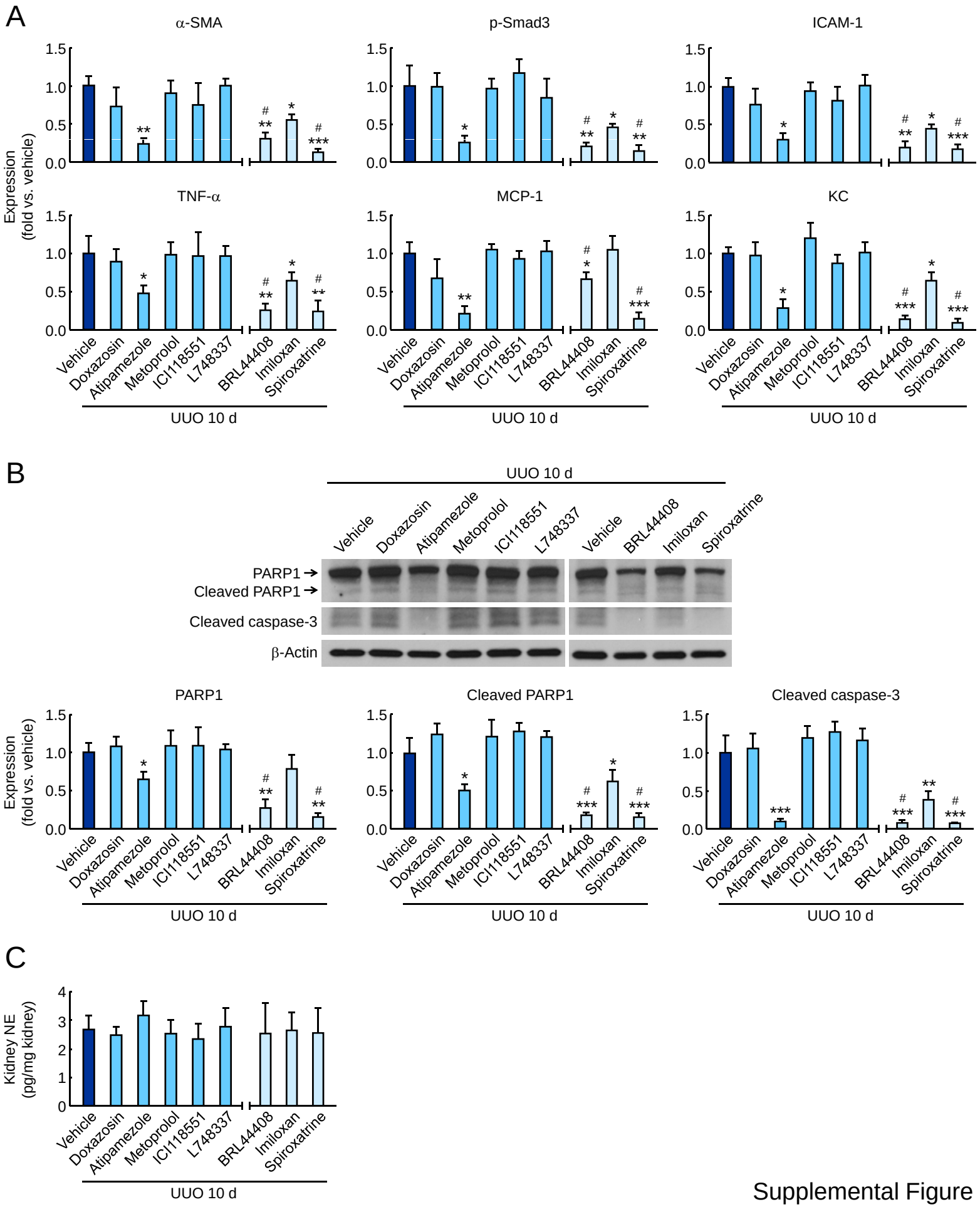

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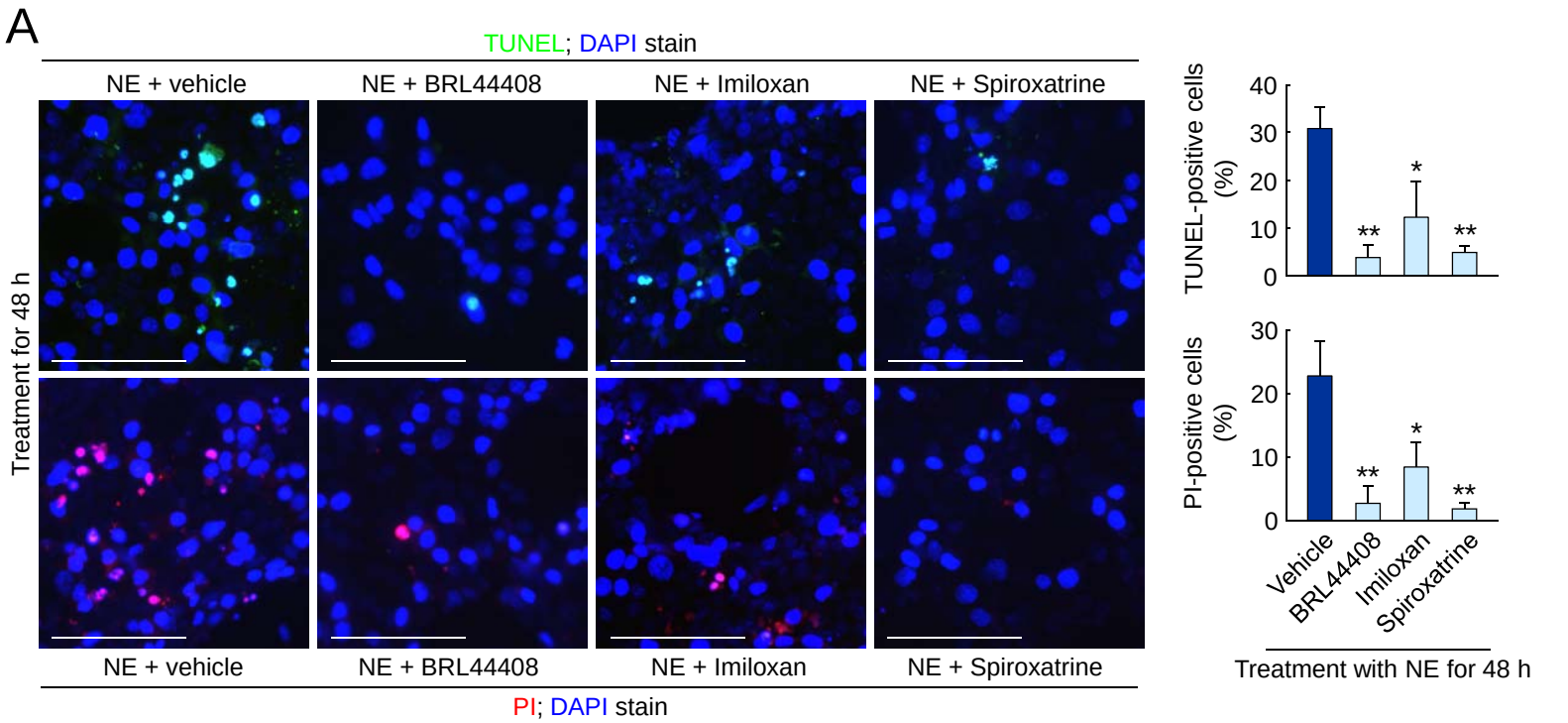
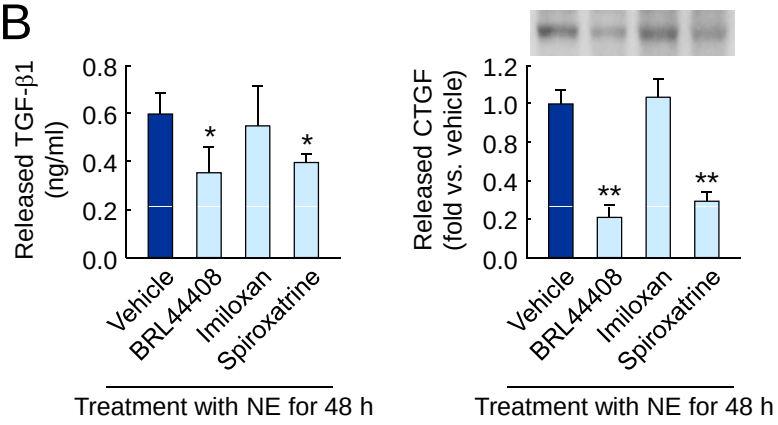
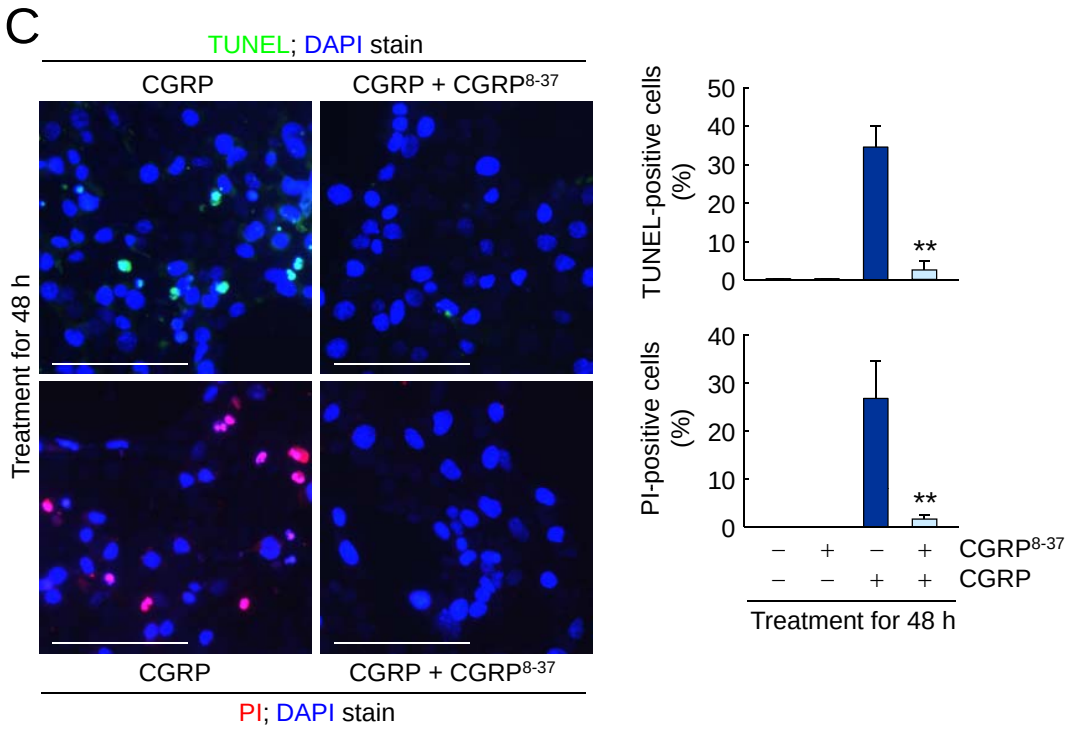
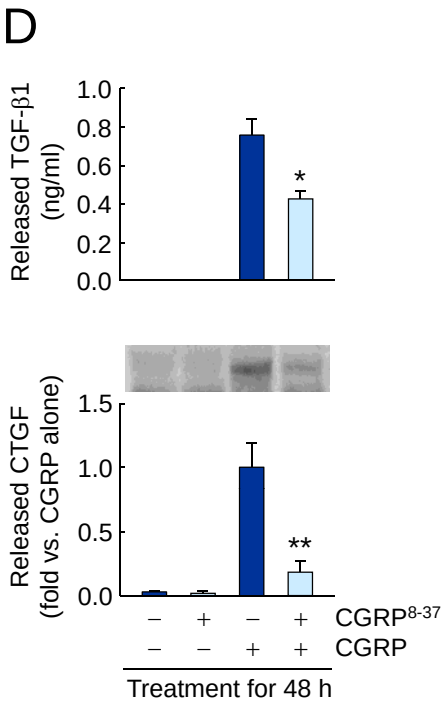




Supplemental Figure 5





SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1: DNx preconditioning prevents expressions in pro-fibrotic proteins and pro-inflammatory proteins. (A and B) DNx in left kidneys of male 129S1/SvImJ mice aged 8 to 10 weeks was carried out; then 2 d after the onset, the left ureters were obstructed for 3 or 10 d (n=4 in each sham-group, n=6 mice in each UUO-group). (A) Kidney levels of α-SMA, fibronectin, TGF-β and p-Smad3 protein were measured by Western blot analysis after UUO or sham-operation in DNx or intact kidneys. Anti-β-actin antibody served as a loading control. The bands were quantified using Lab Works analysis software. (B) Kidney levels of ICAM-1, TNF-α, IL-1β, IL-6, MCP-1, KC, IP-10 and TLR4 protein expression were measured by Western blot analysis after UUO or sham-operation in DNx or intact kidneys. Anti-β-actin antibody served as a loading control. The bands were quantified using Lab Works analysis software. (C and D) UUO in left ureters of male 129S1/SvImJ mice aged 8 to 10 weeks were obstructed for 15 d, and DNx was carried out 1, 3 or 5 d after UUO (n=4 in each group). (C) Collagen deposition using hydroxyproline measurement after UUO in DNx or intact kidneys (n=4 in each sham-group, n=6 in each UUO-group). (D) The Sirius red-positive area was measured in 5 randomly chosen high-power (x 200) fields per kidney using NIH ImageJ software. *P < 0.05, **P < 0.01, ***P < 0.001 versus intact. Error bars represent SD.

Supplemental Figure 2: NE and CGRP contribute to fibrosis and inflammation in UUO kidneys. (A-C) DNx in left kidneys of male 129S1/SvImJ mice aged 8 to 10 weeks was carried out; 2 d after the onset, NE, NY, CGRP or SP (0, 1.2, 6 or 30 ng/kg/d) was continuously infused into kidneys via a mini-osmotic pump; and the left ureters were obstructed for 10 d (n=6 in each group). (A) NE- and CGRP-induced collagen deposition using hydroxyproline measurement in denervated UUO kidneys. (B and C) α-SMA, p-Smad3, ICAM-1 and TNF-α expression in either NE-, NY-, CGRP- or SP-treated UUO kidneys using Western blot analysis. The bands were quantified using Lab Works analysis software. (D) Fibronectin, α-SMA and p-Smad3 expression in either NE-, NY-, CGRP- or SP-uninjected contralateral kidneys after 10 d of UUO using Western blot analysis (n=6 in each group). The bands were quantified using Lab Works analysis software. *P<0.05, **P <0.01 versus 0 ng/kg/d. Error bars represent SD.

Supplemental Figure 3: NE and CGRP contribute to tubular cell death in UUO kidneys. (A-C) DNx in left kidneys of male 129S1/SvImJ mice aged 8 to 10 weeks was carried out; then 2 d after the onset, the left ureters were obstructed for 1, 3 or 10 d (n=4 in each sham-group, n=6 in each UUO-group). (A) The kidneys were fixed for PAS staining to measure the histological damage in tubules. The histological damage in tubules on PAS-stained kidney sections was scored by counting the percentage of tubules. Five randomly chosen high-power (x 200) fields per kidney were used for the counting. (B) The kidneys were fixed for TUNEL assay to detect apoptotic cells using In Situ Cell Death Detection kit. The visible blue color

indicates nuclei stained by DAPI. The number of TUNEL-positive apoptotic cells in tubules was counted in 5 randomly chosen high-power (x 200) fields per kidney. (C) Kidney expression levels of PARP1, cleaved PARP1 and cleaved caspase-3 protein were measured by Western blot analysis. Anti-β-actin antibody served as a loading control. *P<0.01, **P<0.05, ***P<0.001 versus intact. (D) NE or CGRP (30 ng/kg/d) was continuously infused into kidneys via a mini-osmotic pump, and the left ureters were obstructed for 10 d (n=4 in each group). The kidneys were fixed for PAS and TUNEL staining to measure the tubular dilation and detect apoptotic cells in tubules, respectively. The visible blue color indicates nuclei stained by DAPI. The lumen area of tubular dilation on PAS-stained kidney sections was measured in 5 randomly chosen high-power (x 200) fields per kidney using NIH ImageJ software. The number of TUNEL-positive apoptotic cells in tubules was counted in 5 randomly chosen high-power (x 200) fields per kidney. *P<0.05 versus vehicle. (E) Systolic blood pressure of mice was measured by a noninvasive taill cuff method. Mice were placed on a heated platform (30°C) in an isolated chamber, and systolic blood pressure levels were obtained. Mice were trained for 5 d before DNx followed by daily recording for experimental days (n=5 in each group). Scale bar, 50 μm. Error bars represent SD.

Supplemental Figure 4: α_2-AR antagonists reduce tubulointerstitial fibrosis and inflammation after UUO. Male 129S1/SvImJ mice aged 8 to 10 weeks was continuously treated with doxazosin (α_1-AR antagonist, 12 mg/kg/d), atipamezole (α_2-AR antagonist, 2.4 mg/kg/d), metoprolol (β_1-AR antagonist, 12 mg/kg/d), ICI118551 (β_2-AR antagonist, 2.4 mg/kg/d), L748337 (β_3-AR antagonist, 2.4 mg/kg/d), BRL44408 (α_{2A}-AR antagonist, 12 mg/kg/d), imiloxan (α_{2B}-AR antagonist, 12 mg/kg/d), spiroxatrine (α_{2C}-AR antagonist, 12 mg/kg/d) or vehicle (10% DMSO in PBS) via an intraperitoneal implantation of a mini-osmotic pump 24 h before UUO (n=5). (A) α-SMA, p-Smad3, ICAM-1, TNF-α, MCP-1 and KC expression in UUO kidneys treated with α_2-AR antagonists using Western blot analysis. The bands were quantified using Lab Works analysis software. (B) PARP1, cleaved PARP1 and cleaved caspase-3 expression in UUO kidneys treated with α_2-AR antagonists using Western blot analysis. Anti-β-actin antibody served as a loading control. The bands were quantified using Lab Works analysis software. (C) NE level in UUO kidneys using ELISA assay. *P<0.05, **P<0.01, ***P<0.001 versus vehicle; $^{\#}P$ < 0.05 versus imiloxan. Error bars represent SD.

Supplemental Figure 5: NE and CGRP induce renal tubular apoptosis via α_2-ARs and CGRP receptor, respectively. (A and B) Mouse renal proximal tubular cell line (MCT) was maintained in DMEM-high-glucose medium containing 10% FBS at 37°C with 5% CO_2. The cells were grown until 70% confluence on culture plates and then changed to serum-free medium. After serum starvation for 6 h, 1 nM of NE and either 10 μM of α_{2A}-AR antagonist BRL44408, 1 μM of α_{2B}-AR antagonist imiloxan, 100 nM of α_{2C}-AR antagonist spiroxatrine, or vehicle (10% DMSO in PBS) was co-treated to the culture (n=4). (A) The MCT cells co-treated with NE and either α_{2A}-AR antagonist, α_{2B}-AR antagonist, α_{2C}-AR antagonist, or vehicle

for 48 h were fixed for TUNEL and PI staining to detect apoptotic and necrotic cells, respectively. The visible blue color indicates nuclei stained by DAPI. The number of TUNEL-positive apoptotic or PI-positive necrotic MCT cells was counted in 5 randomly chosen high-power (x 200) fields per plate. (B) The released level of TGF-β1 and CTGF in the supernatant of MCT cells co-treated with NE- and respective antagonists of α_2-AR subtypes for 48 h was examined by ELISA assay and Western blot analysis. The protein bands were quantified using Lab Works analysis software. *P < 0.05, **P < 0.001 versus vehicle. (C and D) MCT cells were grown until 70% confluence on culture plates and then changed to serum-free medium. After serum starvation for 6 h, 1 nM of CGRP plus/minus either 10 nM of CGRP antagonist ($CGRP^{8-37}$) was incubated for 48 h (n=4). (C) The MCT cells co-treated with NE and $CGRP^{8-37}$ for 48 h were fixed for TUNEL and PI staining to detect apoptotic and necrotic cells, respectively. The visible blue color indicates nuclei stained by DAPI. The number of TUNEL-positive apoptotic or PI-positive necrotic MCT cells was counted in 5 randomly chosen high-power (x 200) fields per plate. (B) The released level of TGF-β1 and CTGF in the supernatant of MCT cells co-treated with CGRP and $CGRP^{8-37}$ for 48 h was examined by ELISA assay and Western blot analysis. The protein bands were quantified using Lab Works analysis software. *P < 0.05, **P < 0.001 versus no treatment. Error bars represent SD.