

Involvement of inflammasomes in hypertensive renal injury model

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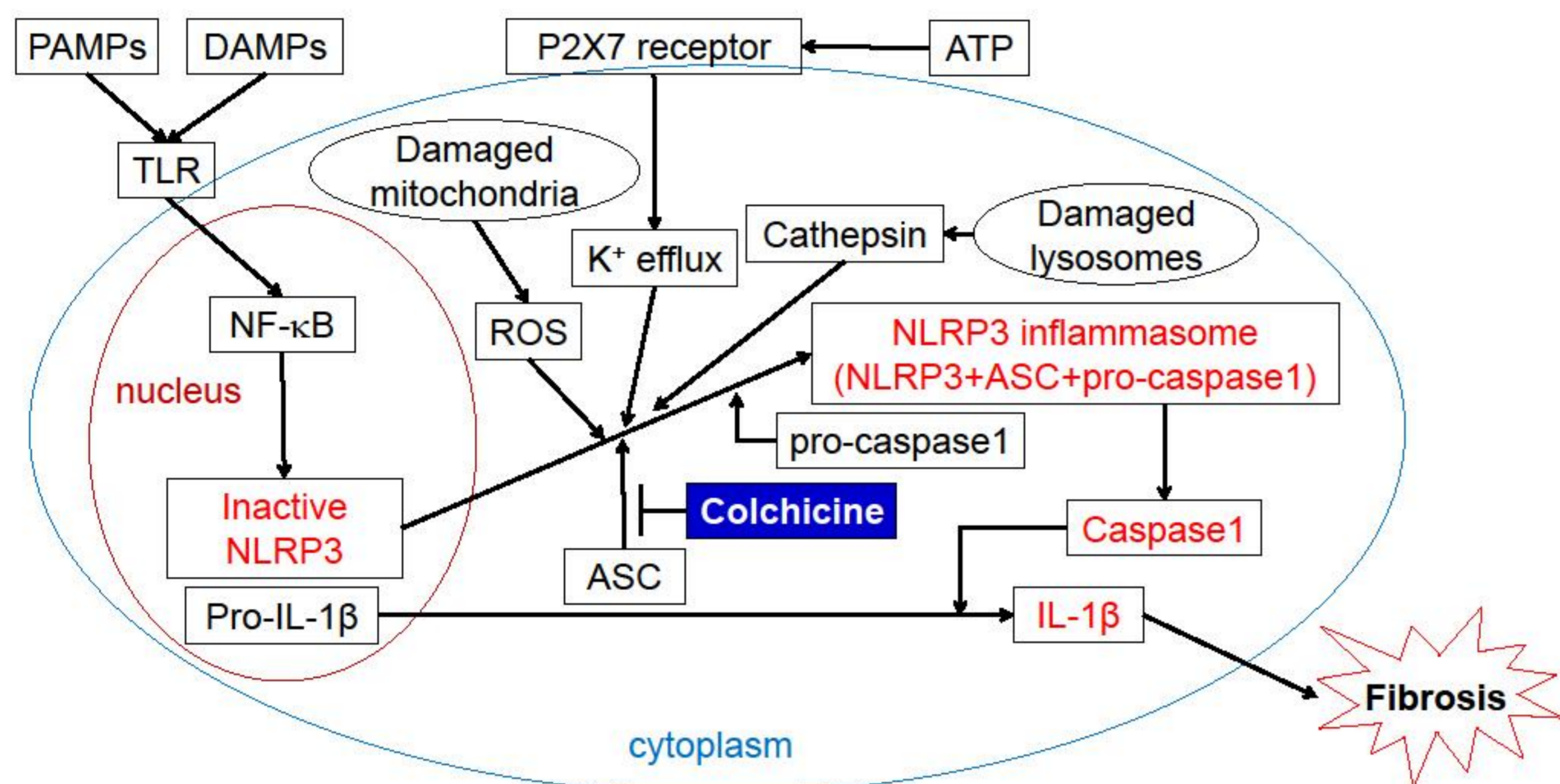
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Introduction

- In the development and progression of hypertensive renal injury, not only arteriosclerosis but also chronic inflammation are involved. Interleukin 1 β (IL-1 β), one of inflammatory cytokines, is increased in hypertension¹.
- Inflammasomes have been known to be involved in the production of IL-1 β and play an important role in the induction and progression of inflammatory reactions².
- Inflammasomes may be involved in the progression of hypertensive renal injury.

1. Krishnan SM, et al. *Br J Pharmacol.* 2014; 171: 5589–5602.
2. Guo H, et al. *Nat Med.* 2015; 21: 677–687.



Objectives

- Using the Dahl salt-sensitive rats (DS rats) and Dahl salt-resistant rats (DR rats), we examined the involvement of inflammasomes in the development of hypertensive renal injury.
- We investigated that colchicine (Col), an inhibitor of tubulin polymerization which is essential for activation of inflammasomes, attenuated hypertensive renal injury.

Methods

- Animals: DS or DR rat - 6 week old, male

Groups

- DR + normal-salt diet (NS) group : 0.21% NaCl + vehicle (n=3)
- DR + high-salt diet (HS) group : 8% NaCl + vehicle (n=3)
- DS + NS group : 0.21% NaCl + vehicle (n=3)
- DS + HS group : 8% NaCl + vehicle (n=4)
- DS + NS + Col group : 0.21% NaCl + Col (500 μ g/kg) (n=5)
- DS + HS + Col group : 8% NaCl + Col (500 μ g/kg) (n=7)

- Morphologic changes:

Masson's Trichrome staining, Periodic acid-Schiff (PAS) staining

- Immunohistochemistry:

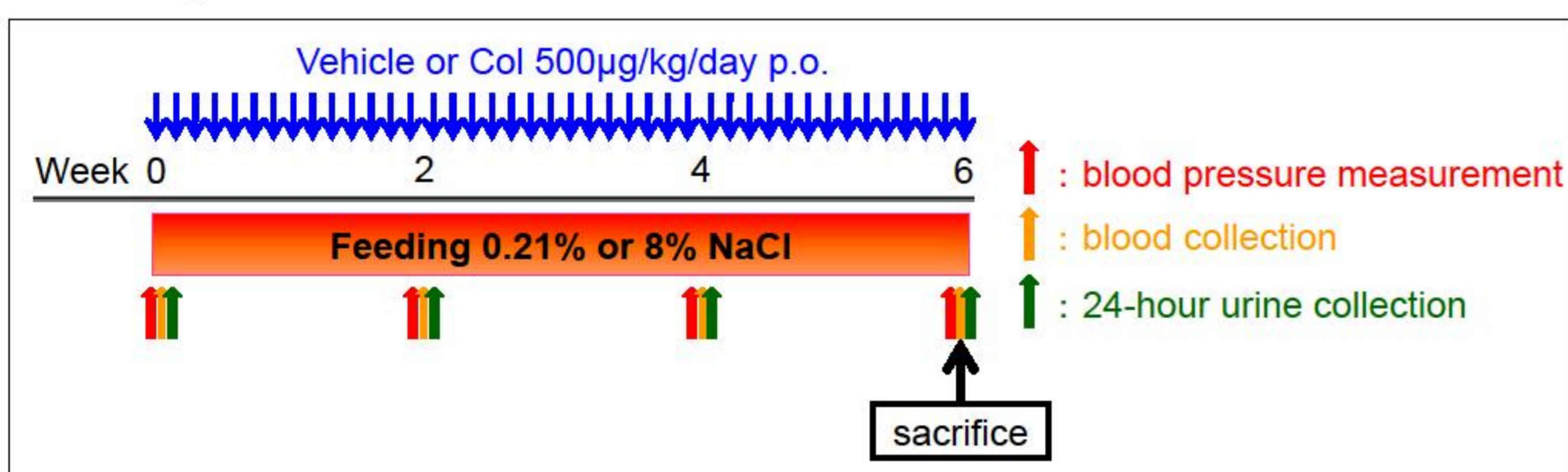
NLRP3, Caspase1

- Immunofluorescent double staining:

Caspase1, Aquaporin-1 (AQP-1), Tamm-Horsfall protein (THP), Calbindin, Aquaporin-2 (AQP-2)

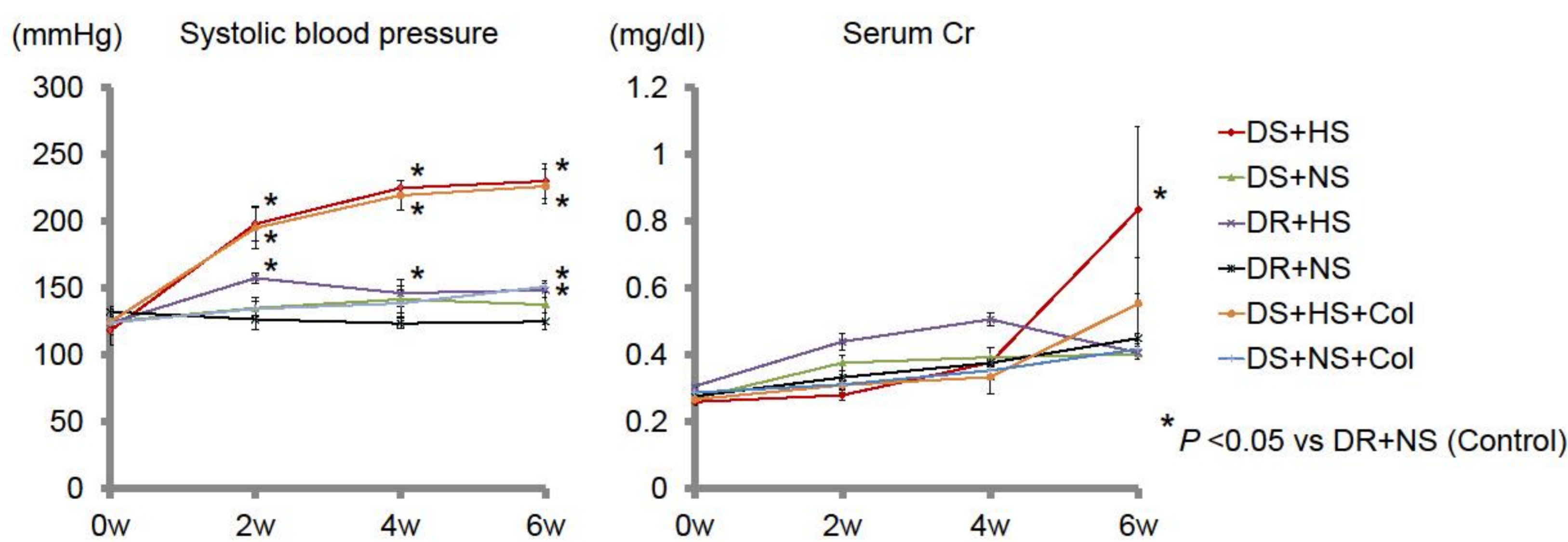
- Enzyme-linked immunosorbent assay (ELISA):

Urinary IL-1 β



Results

1. Changes in systolic blood pressure and serum Cr



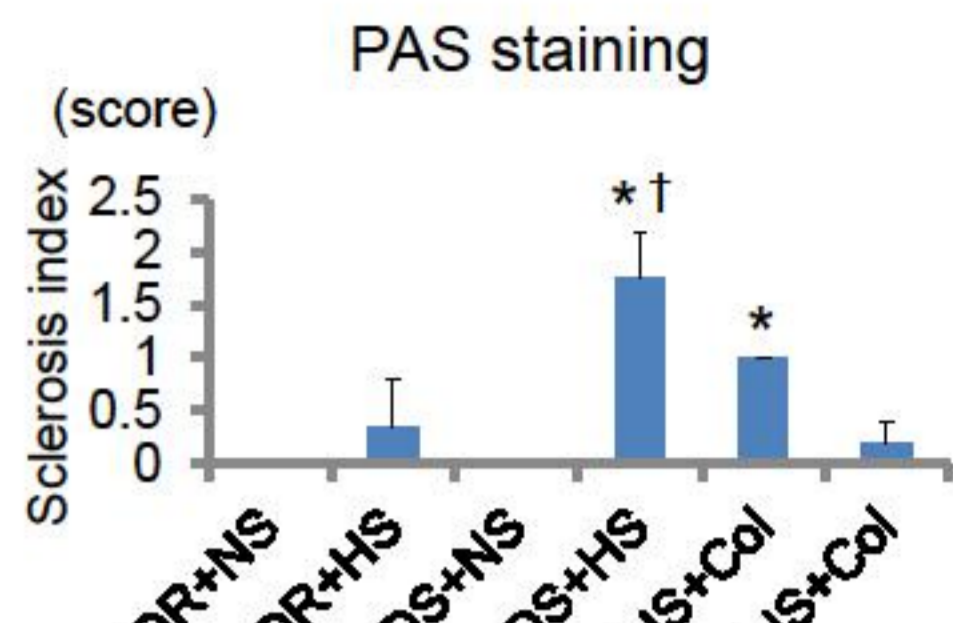
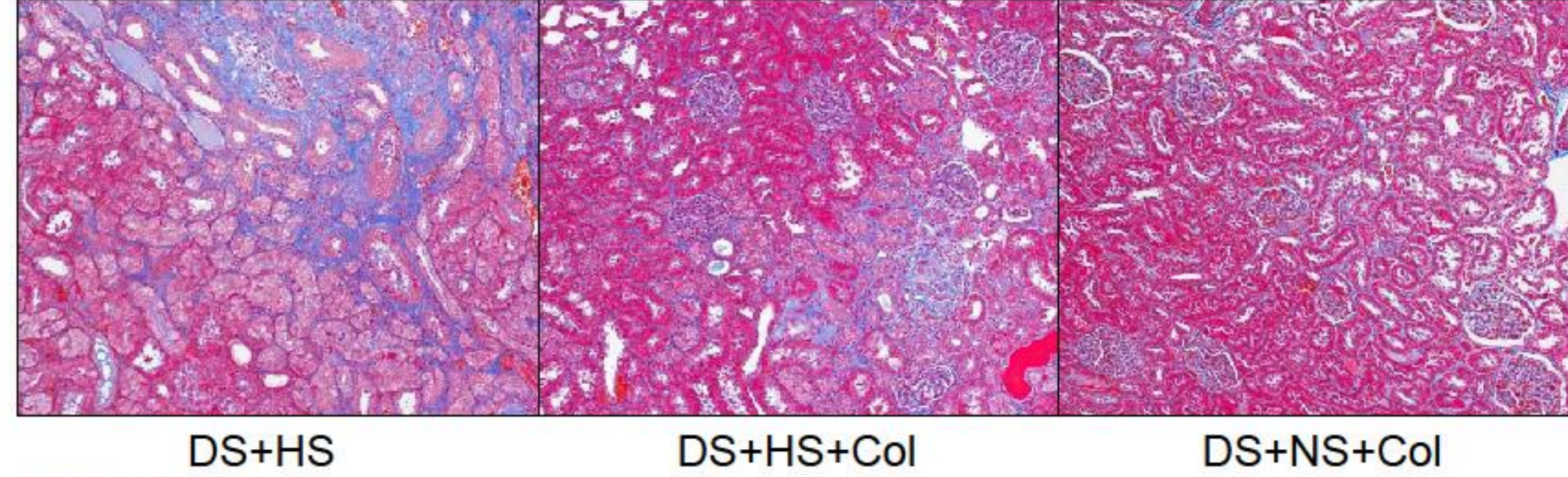
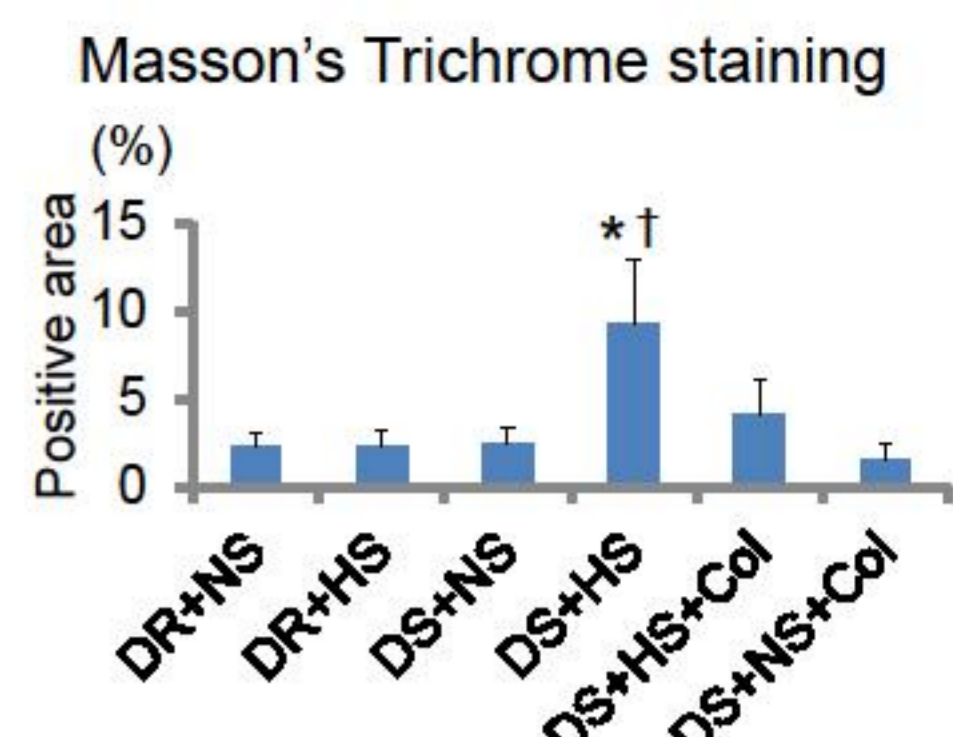
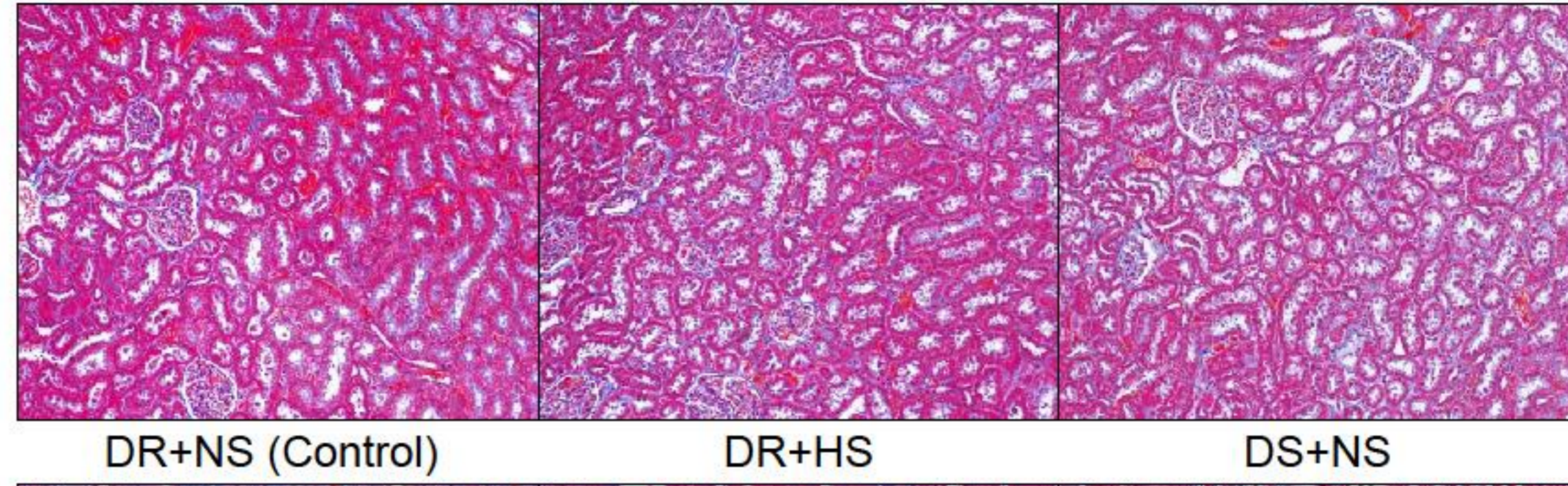
Systolic blood pressure significantly increased in the DS+HS group and the DS+HS+Col group compared with that in the DR+NS group from 2 weeks. Serum creatinine significantly increased in the DS+HS group compared with that in the DR+NS group at 6 weeks.

Conclusions

- We demonstrated that NLRP3 inflammasomes were activated in hypertensive renal injury model and that Col suppressed caspase1 expression and attenuated hypertensive renal injury.

2. Masson's Trichrome staining and PAS staining

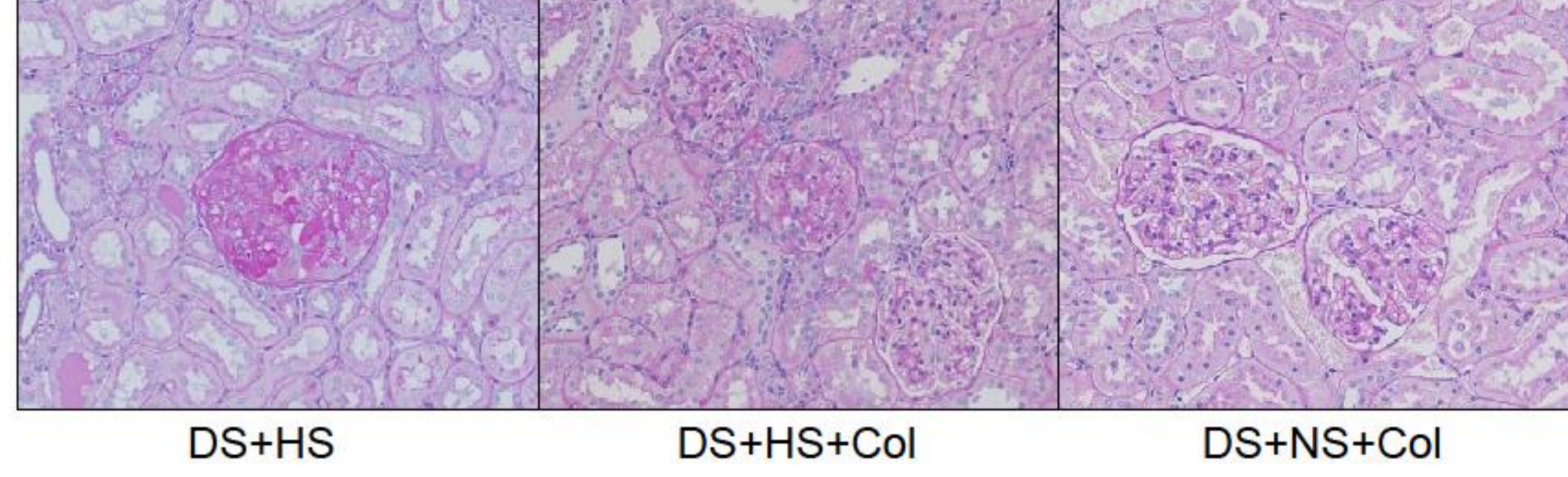
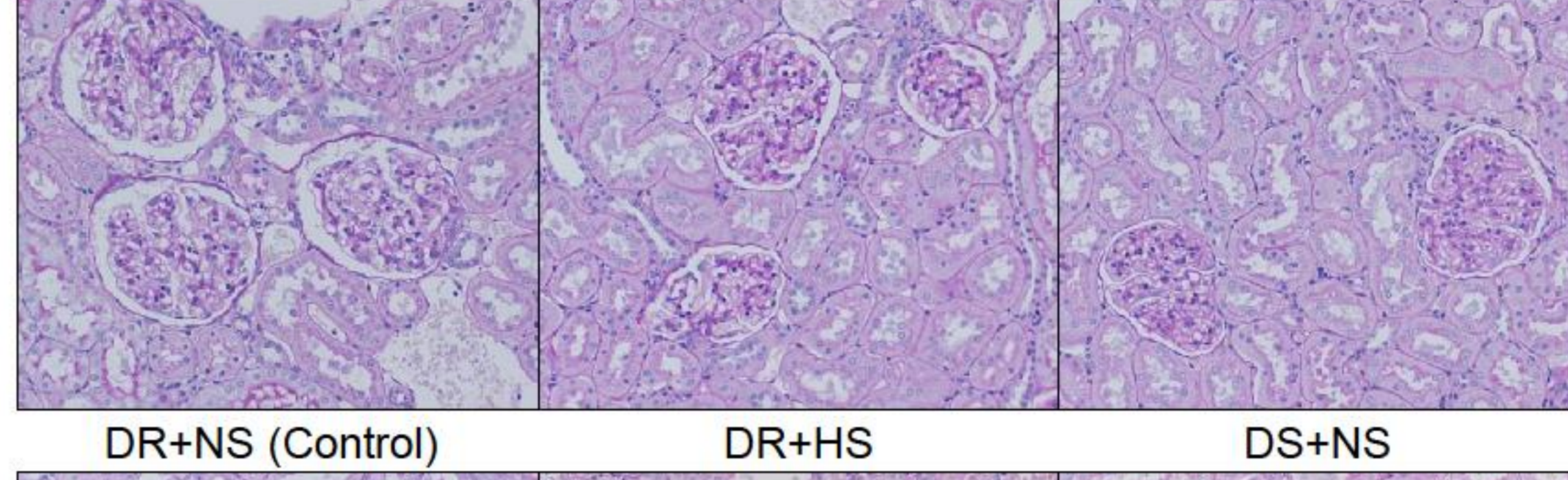
Masson's Trichrome staining



* $P < 0.05$ vs DR+NS (Control)
† $P < 0.05$ vs DS+HS+Col

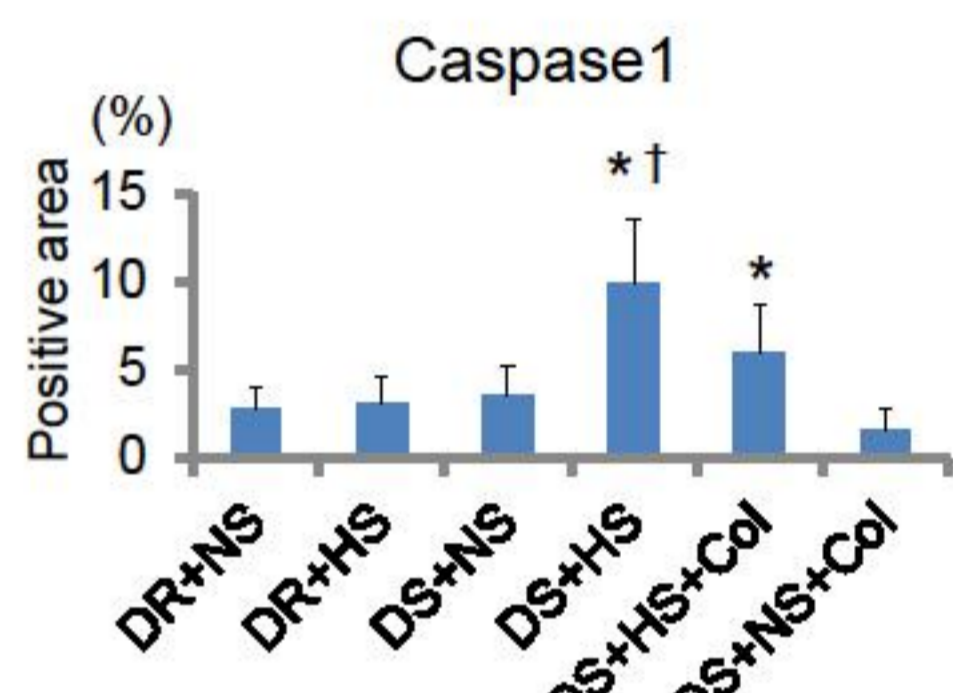
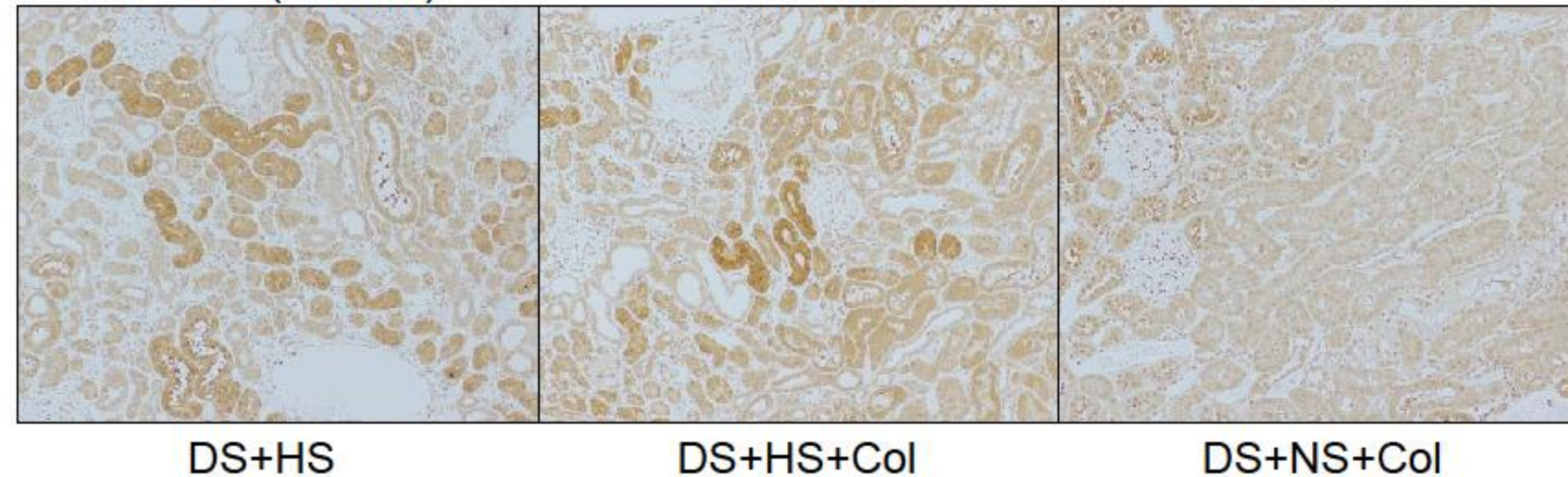
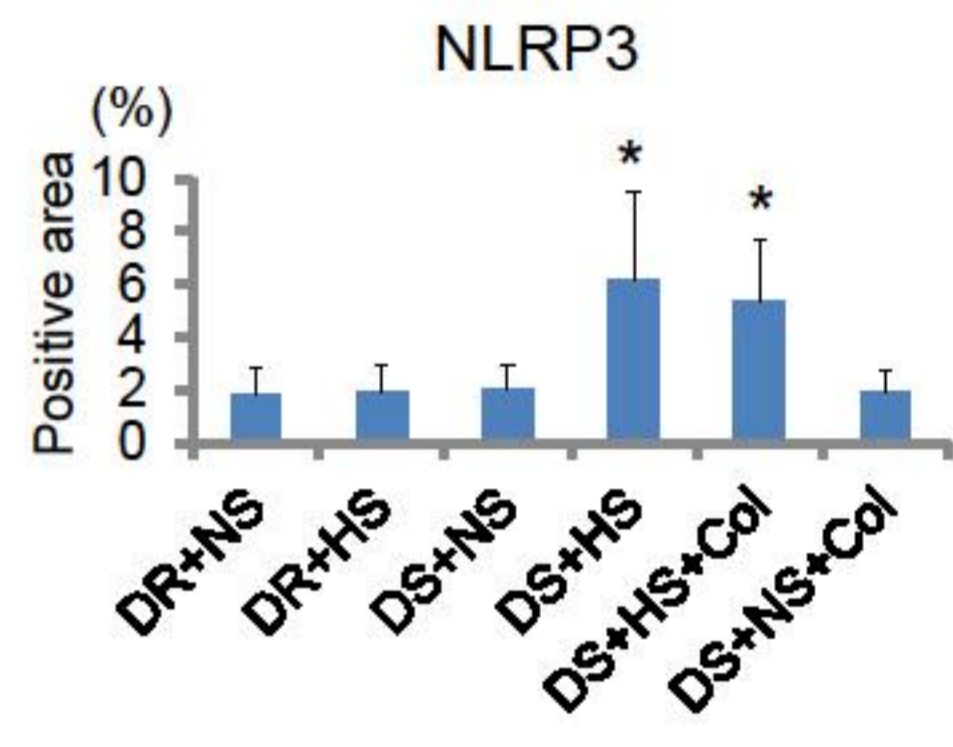
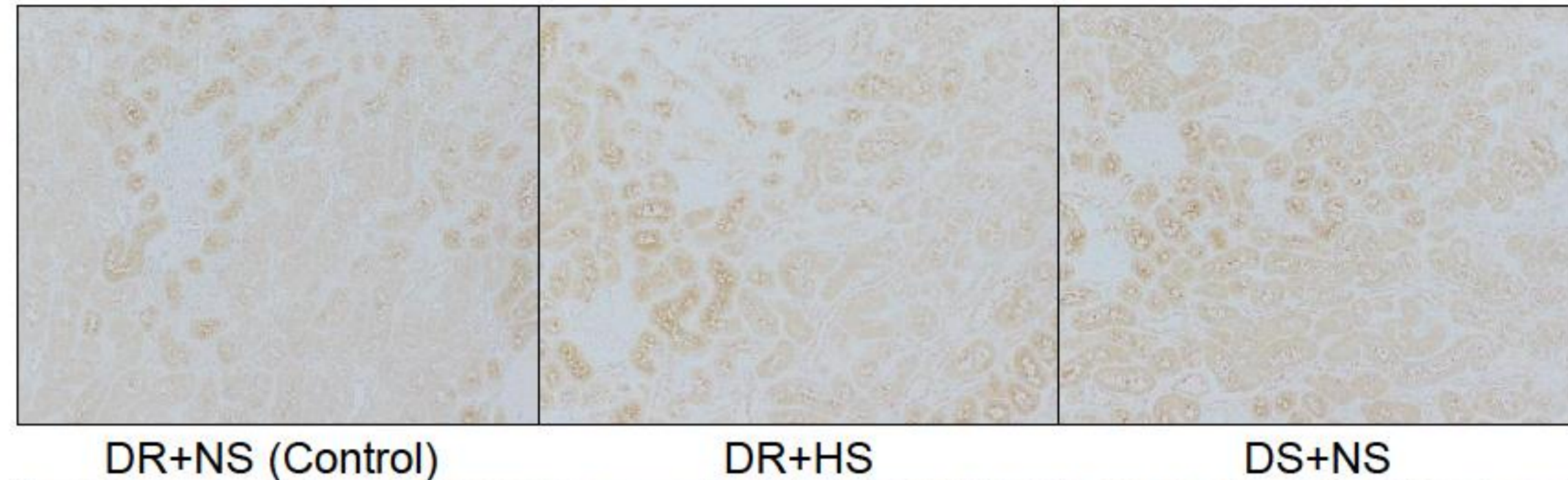
Interstitial fibrosis and glomerulosclerosis significantly developed in the DS+HS group compared with that in the DR+NS group. These changes were significantly suppressed in the DS+HS+Col group compared with that in the DS+HS group.

PAS staining



3. Immunohistochemistry for NLRP3 (inactive and active) and Caspase1

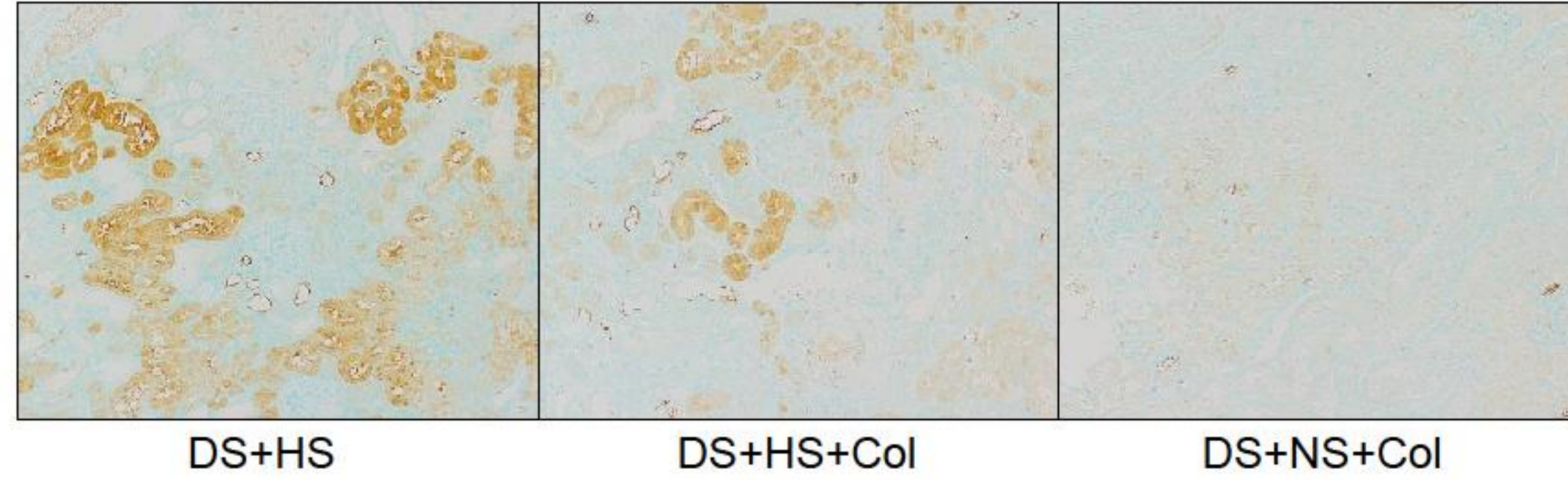
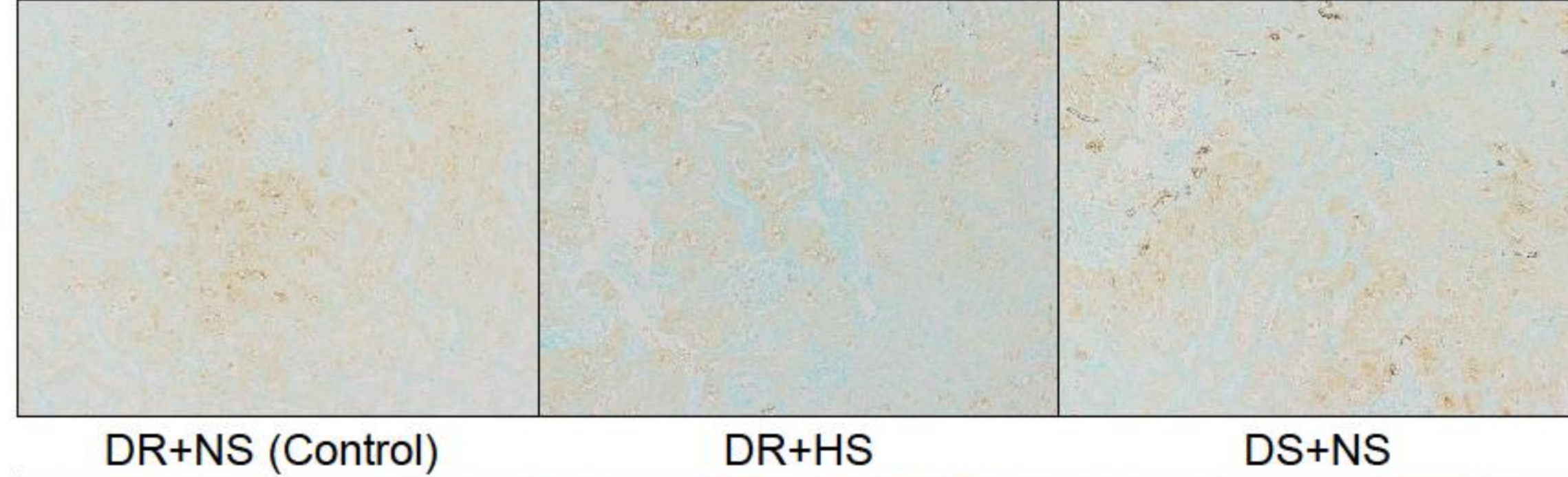
NLRP3



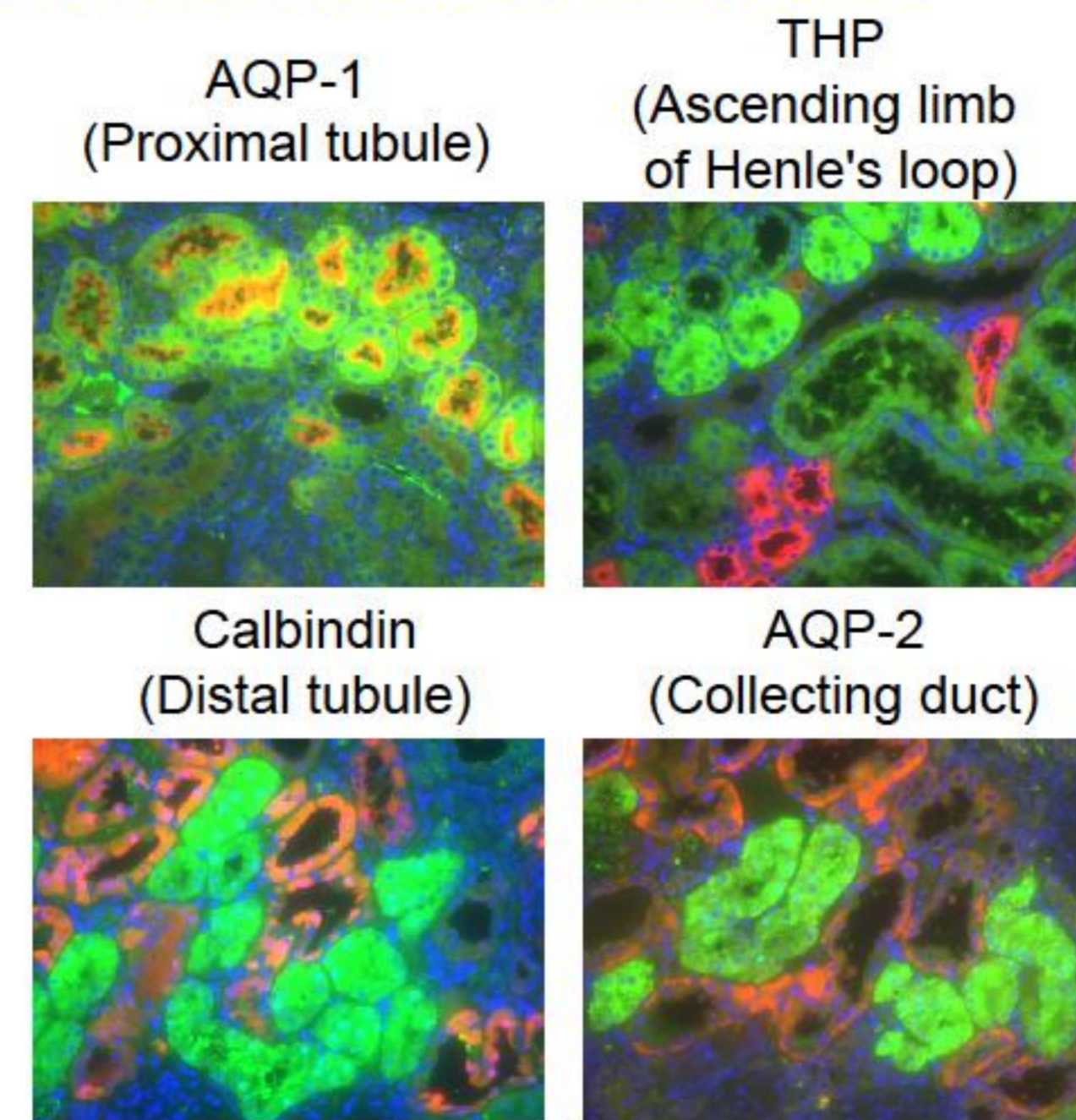
* $P < 0.05$ vs DR+NS (Control)
† $P < 0.05$ vs DS+HS+Col

NLRP3 and Caspase1 expressions were limited to renal tubules, not glomerulus, and these expressions in the DS+HS group were significantly elevated compared with those in the DR+NS group. Caspase1 expression was significantly suppressed in the DS+HS+Col group compared with that in the DS+HS group.

Caspase1



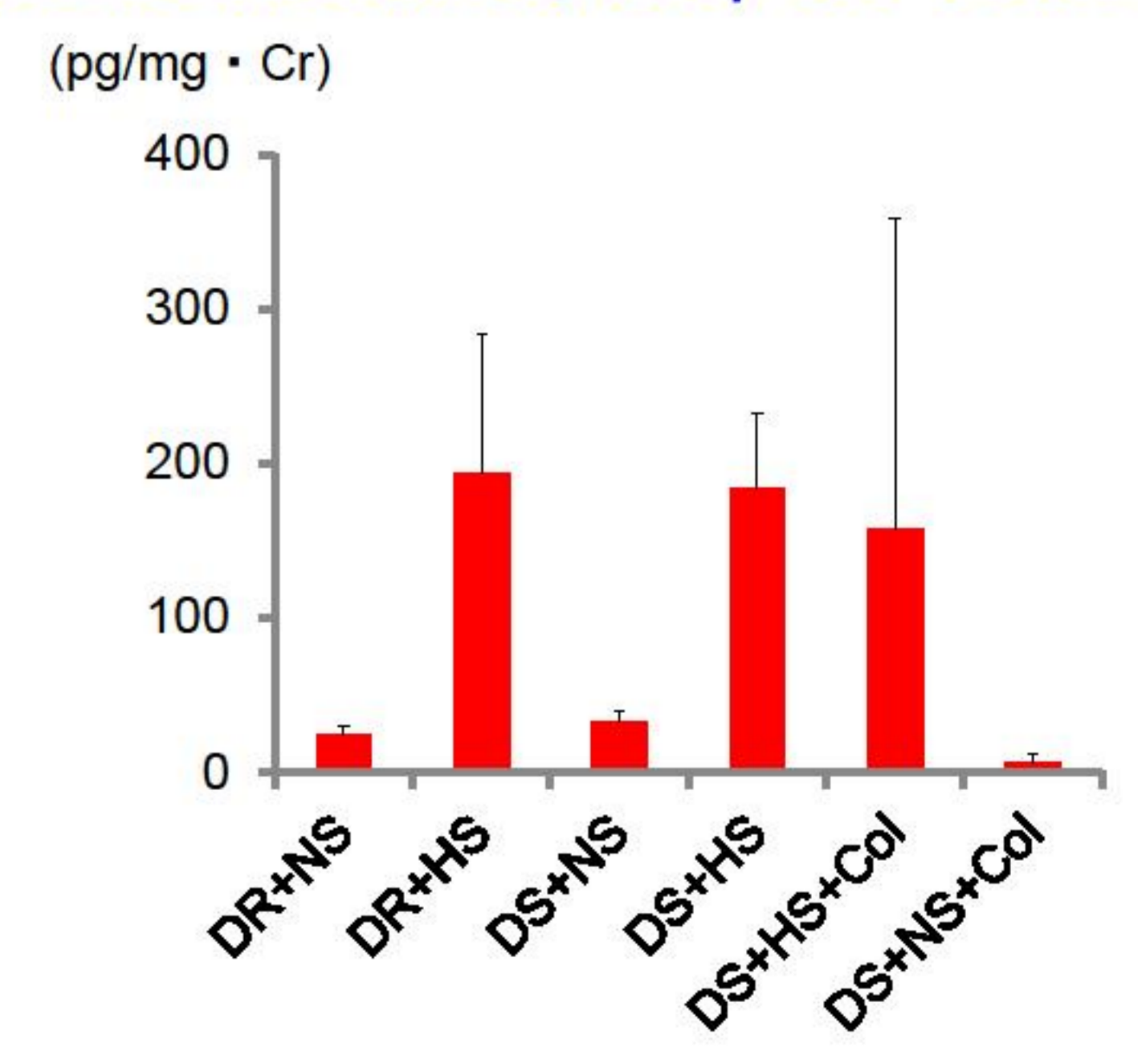
4. Localization of Caspase1



red: AQP-1/THP/Calbindin/AQP-2, green: Caspase1, blue: DAPI

Caspase1 expression was localized in the proximal tubules.

5. ELISA for urinary IL-1 β at 6 weeks



Urinary IL-1 β was tended to increase in the DS+HS group compared with that in the DR+NS group. This change was slightly suppressed in the DS+HS+Col group compared with that in the DS+HS group.

Conflict of interest: We have no conflicts of interest.