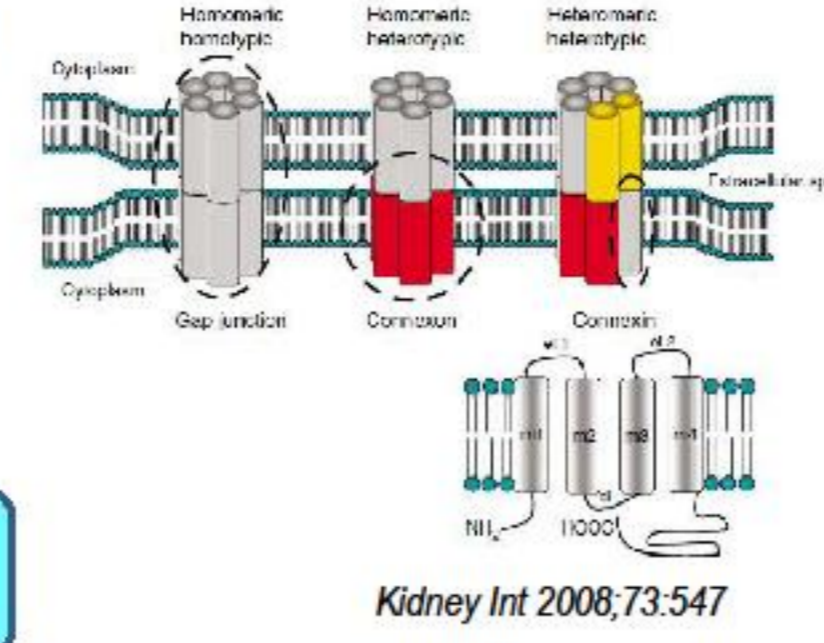


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## 【Introduction】

- Connexins (Cx) are transmembrane proteins that form either gap junctions (GJs) or hemichannels (HCs).
- Both GJs and HCs allow passage of ions and molecules < 1kDa (e.g. ATP, Ca<sup>2+</sup>, Na<sup>+</sup>, cAMP, IP<sub>3</sub>, ROS).
- GJs are essential for many physiological processes which require intercellular communication.
- HCs are involved in autocrine & paracrine signaling.
- In previous studies, we demonstrated that Cx43 was upregulated in the kidney during the progression of human CKDs and in animal models (RenTg mice, anti-serum GN, UUO). Furthermore, targeting Cx43 expression improved renal function and structure. *AJP Renal Physiol* 2011;301:F24 *Kidney Int* 2014;86:768



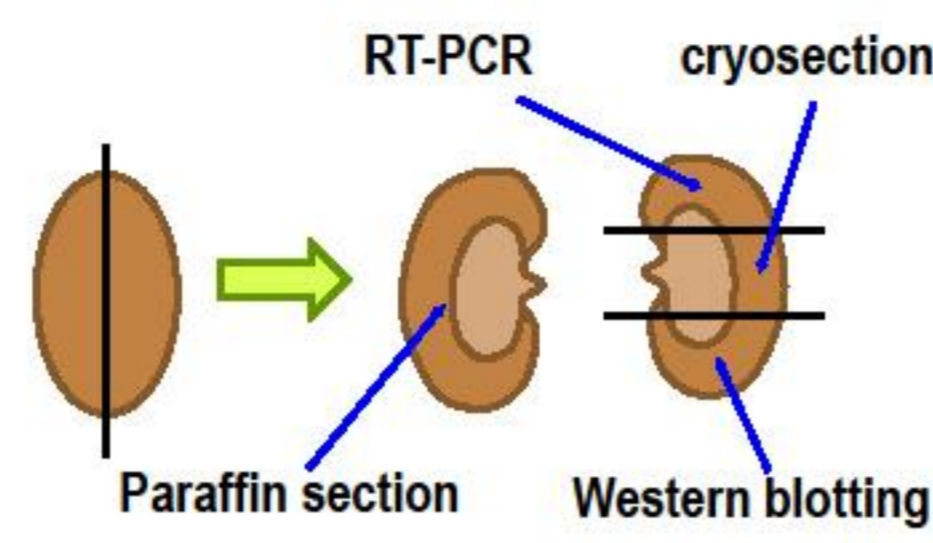
Aim of the present study:

To investigate the role of Cx43 in AKI due to renal ischemia-reperfusion injury (I/R)

## 【Methods】

Animals: C57BL/6, male, 2 mo, WT and Cx43 heterozygous knockout  
 Ischemia 25 min + Reperfusion for 3-72 hours. The contralateral kidney was nephrectomized.

- Histological evaluation (PAS staining)
- RT-PCR
- Immunofluorescence (cryosections): Cx43
- IHC for detection of inflammatory cell infiltration: GR1, F4/80
- Western blotting



### Histological grading scores

Histological findings in renal tubules were evaluated using a semi-quantitative score we developed. Pictures of PAS stained 3um kidney sections were evaluated by a blinded observer for the outer stripe (OSOM) and the inner stripe (ISOM) of the outer medulla (magnification x200 and x400, respectively).

The % of tubules with either:

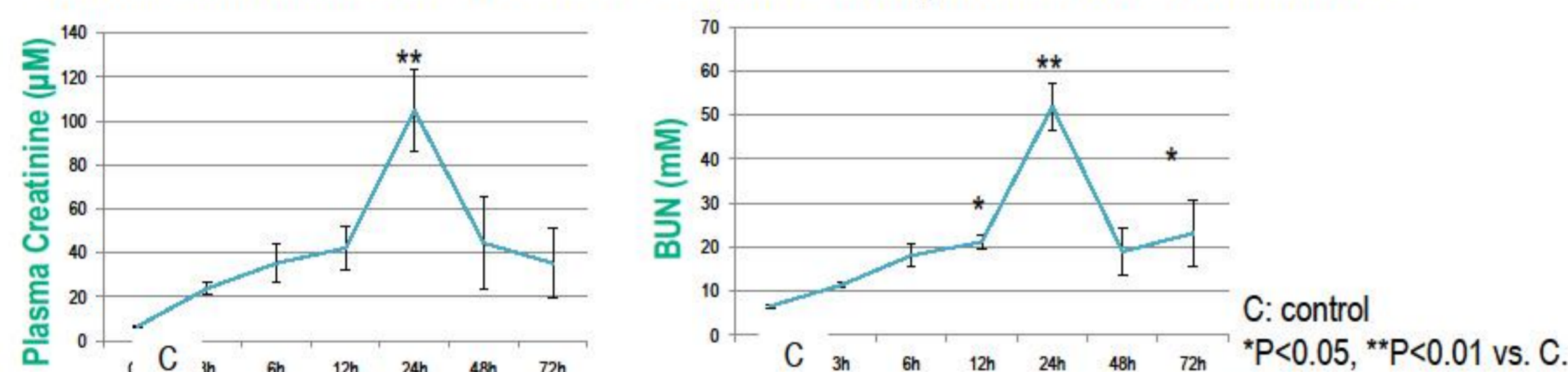
- ❖ epithelial necrosis
- ❖ sloughing (necrotic debris) in lumen
- ❖ tubular casts

was calculated, and classified according to the following criteria:

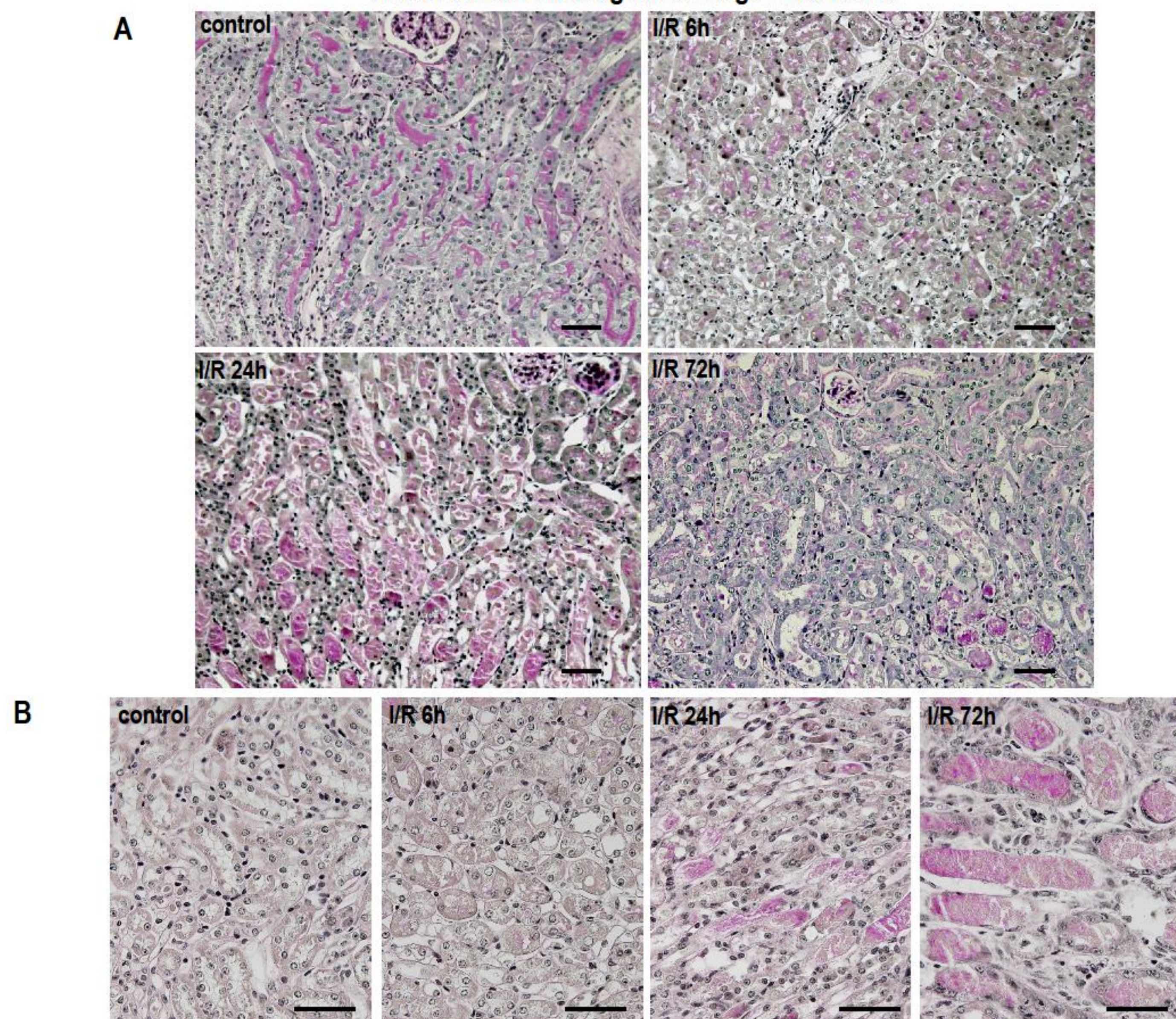
Grade 0: 0%  
 Grade 1: <10%  
 Grade 2: 10-25%  
 Grade 3: 25-50%  
 Grade 4: 50-75%  
 Grade 5: >75%

## 【Results】

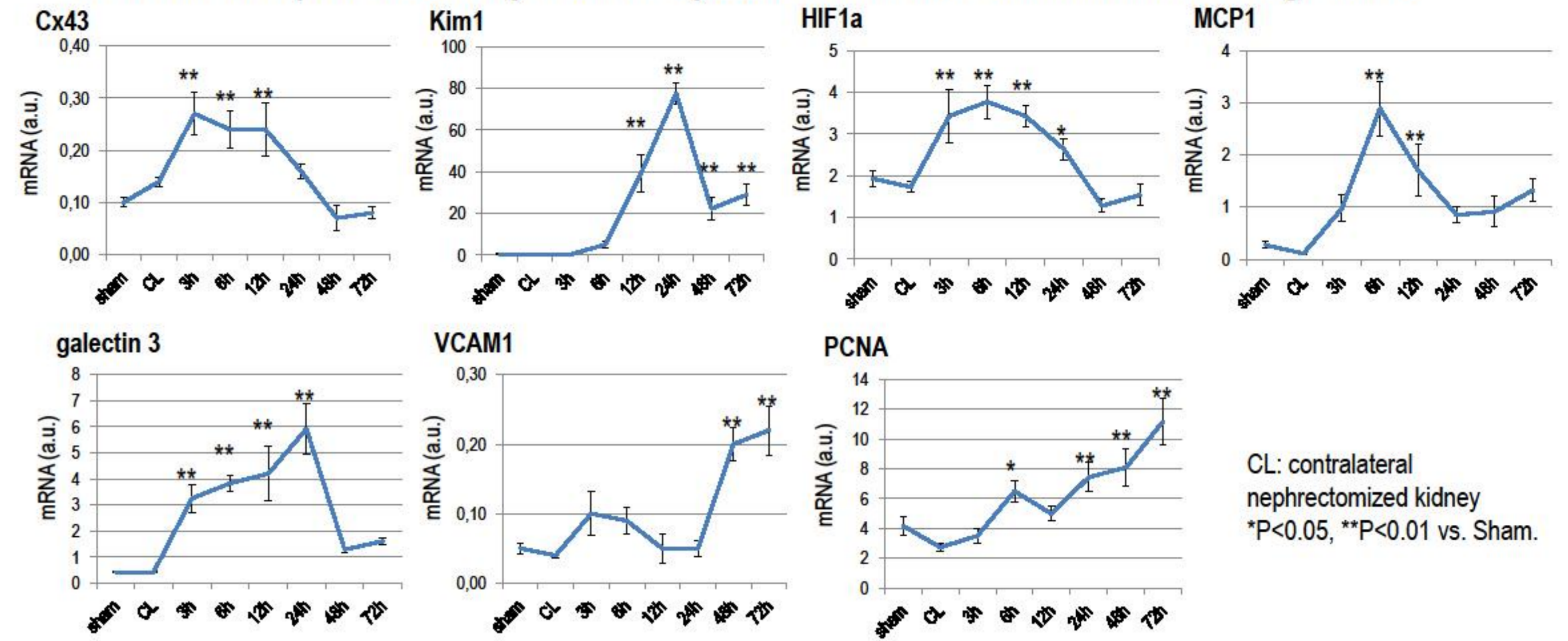
### Plasma creatinine and BUN levels at various time points after renal ischemia.



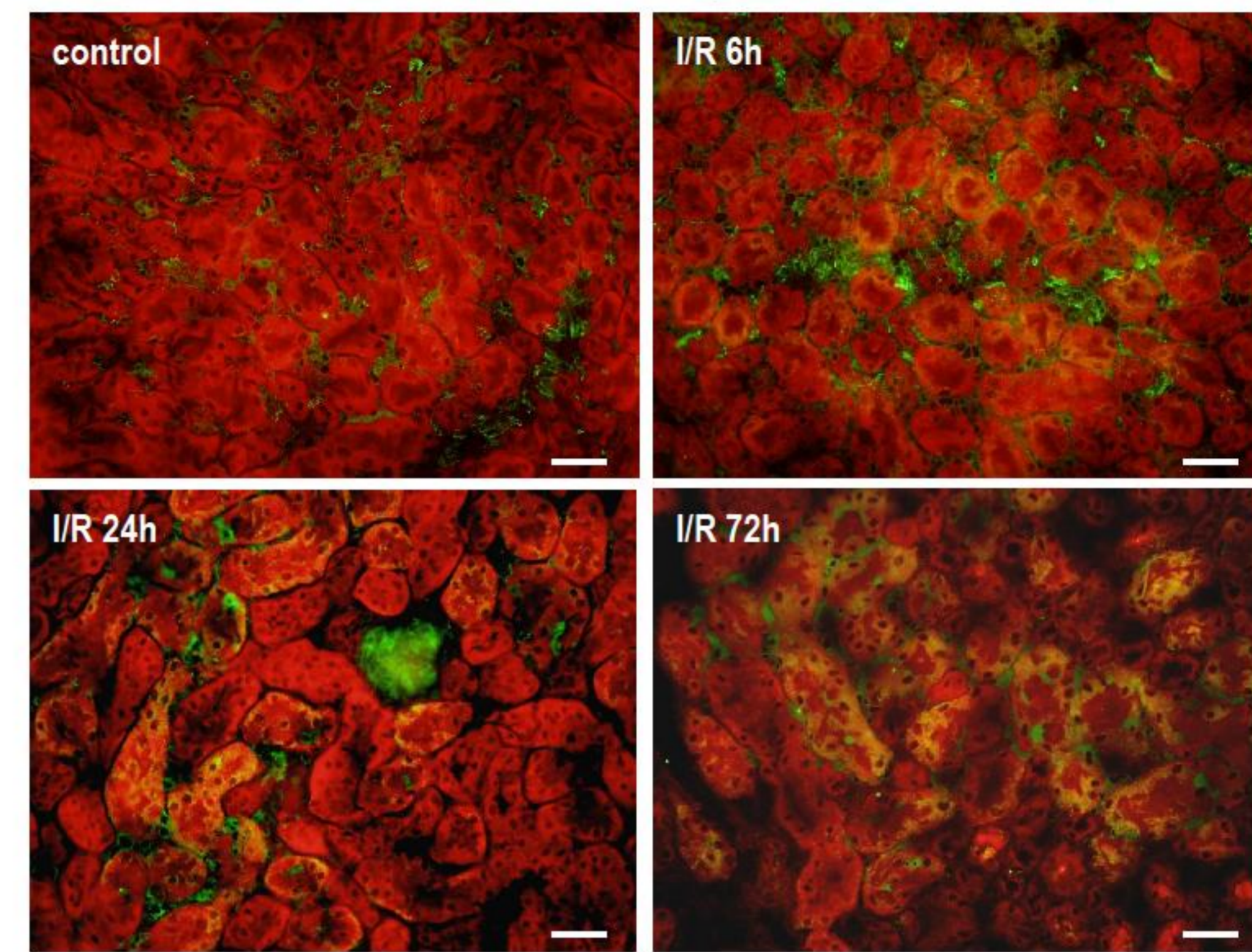
### Evaluation of histological damage in renal I/R.



### Reverse transcriptase-PCR assay of various cytokines and markers of inflammation during renal I/R.

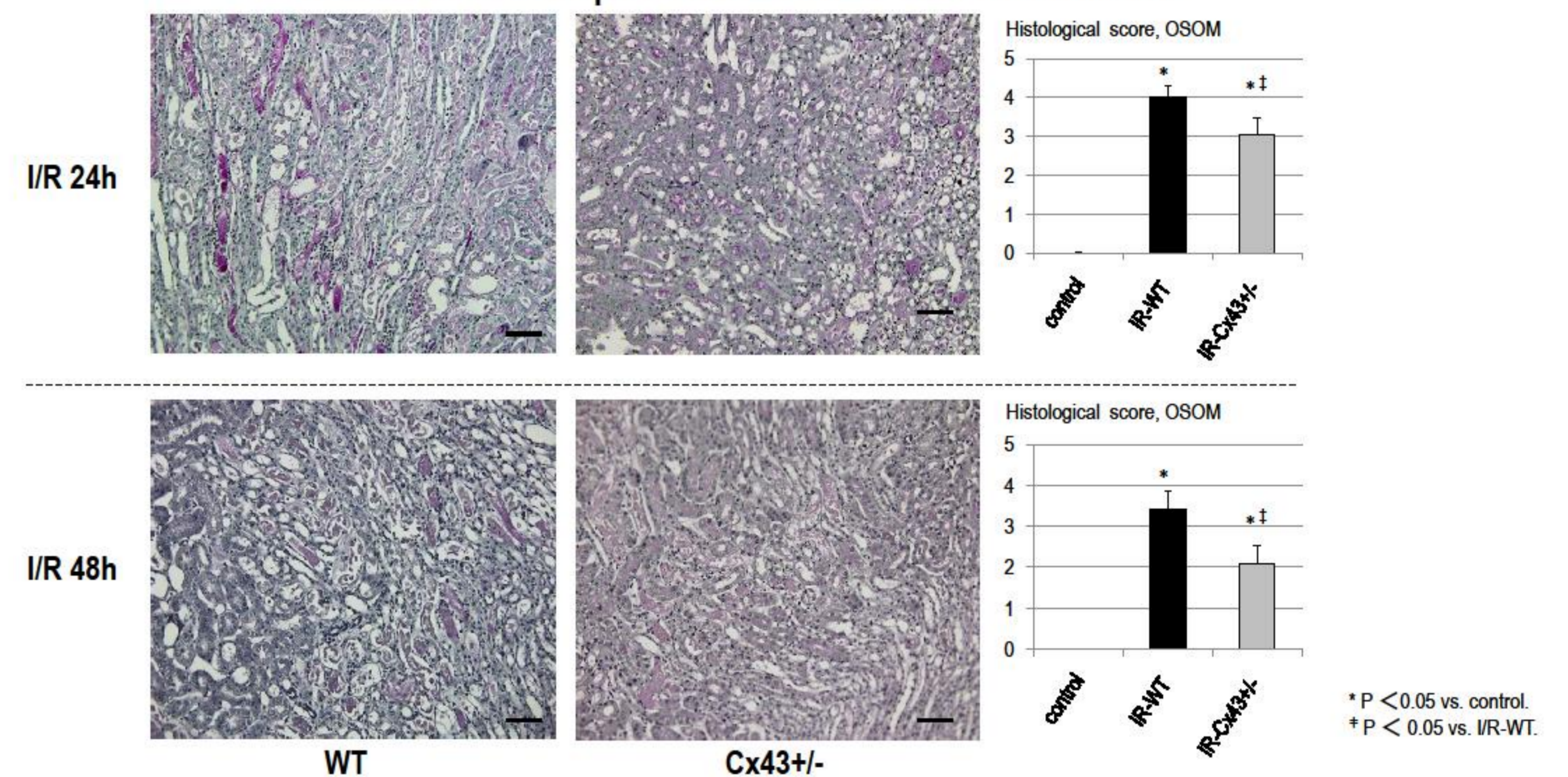


### Immunofluorescent staining for Connexin 43

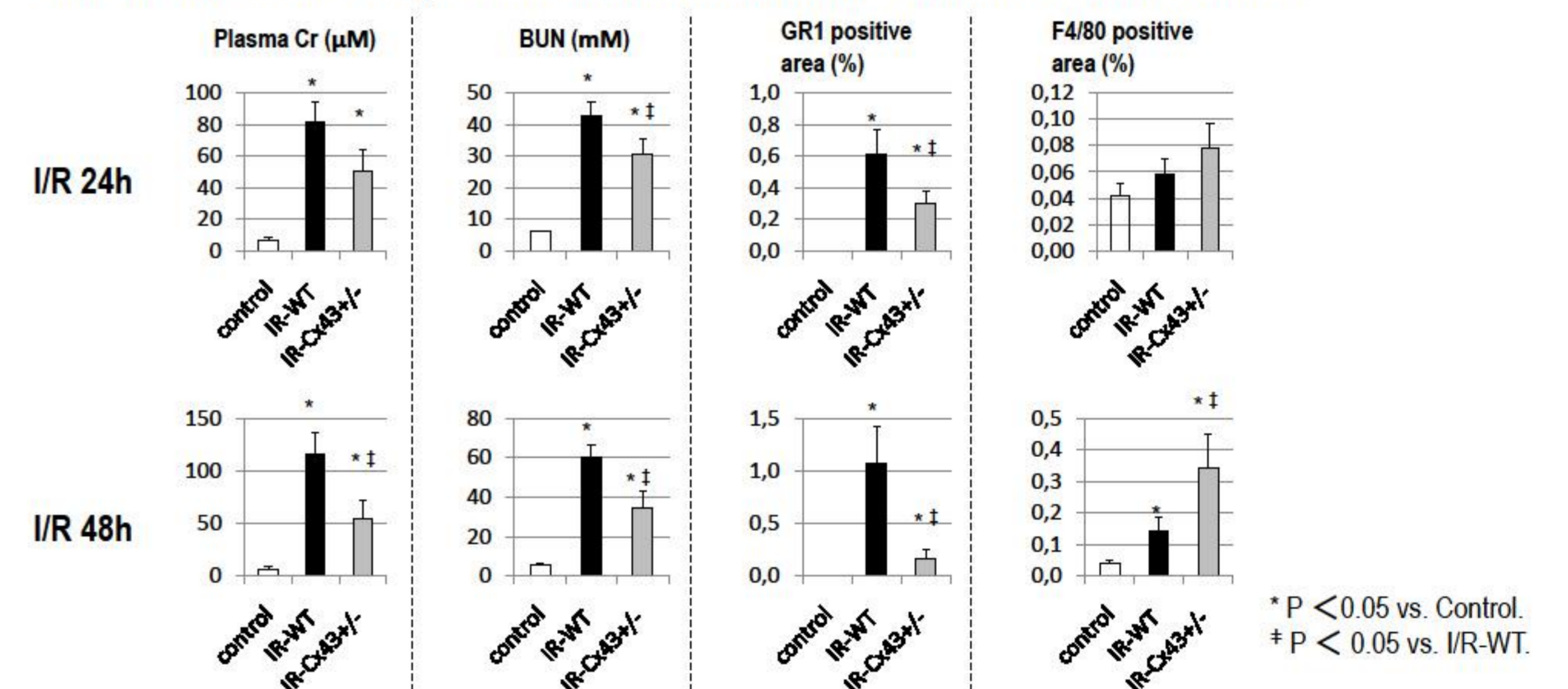


Cx43 (green) was de novo expressed in the proximal tubules of the OSOM compared to controls in I/R mice, and the amount of expression increased with time. Cryosections were counterstained with Evans blue (red). Bar: 50 µm.

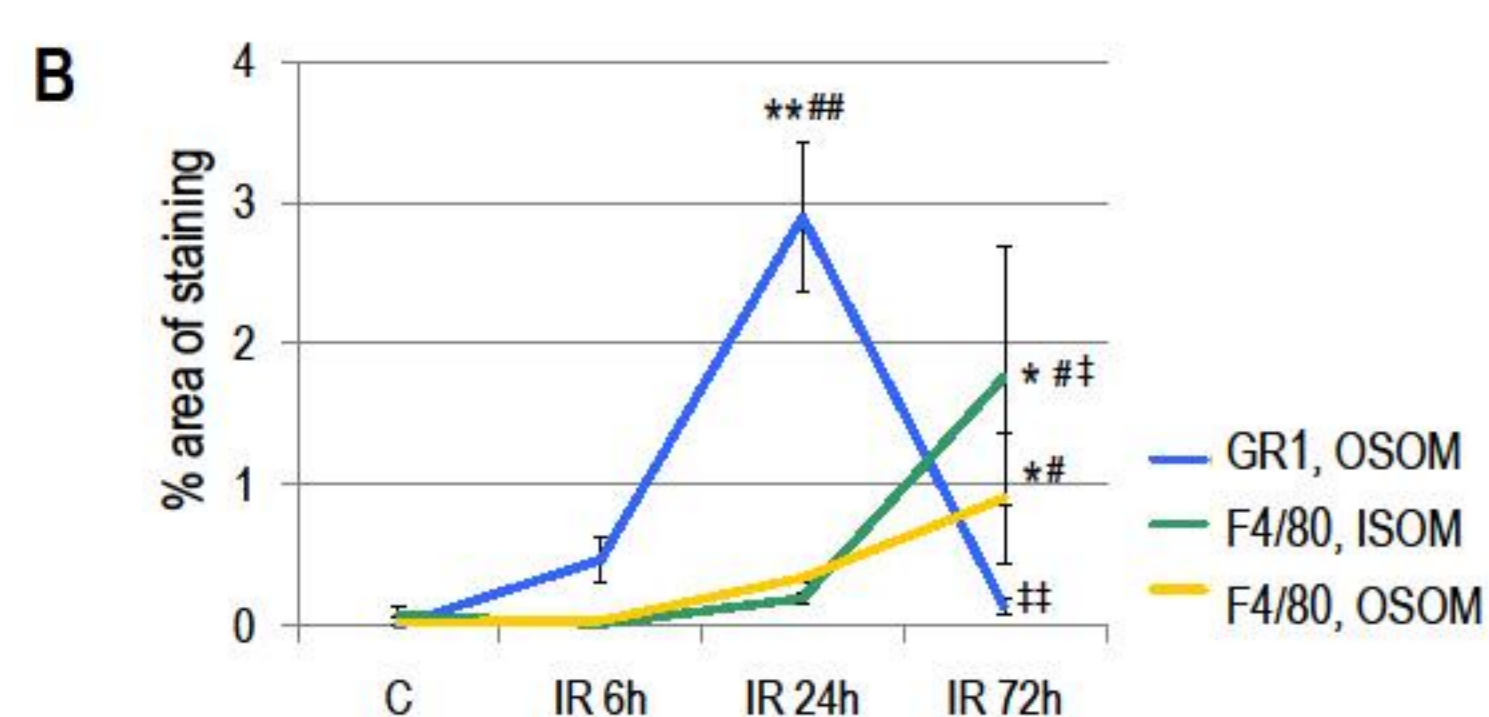
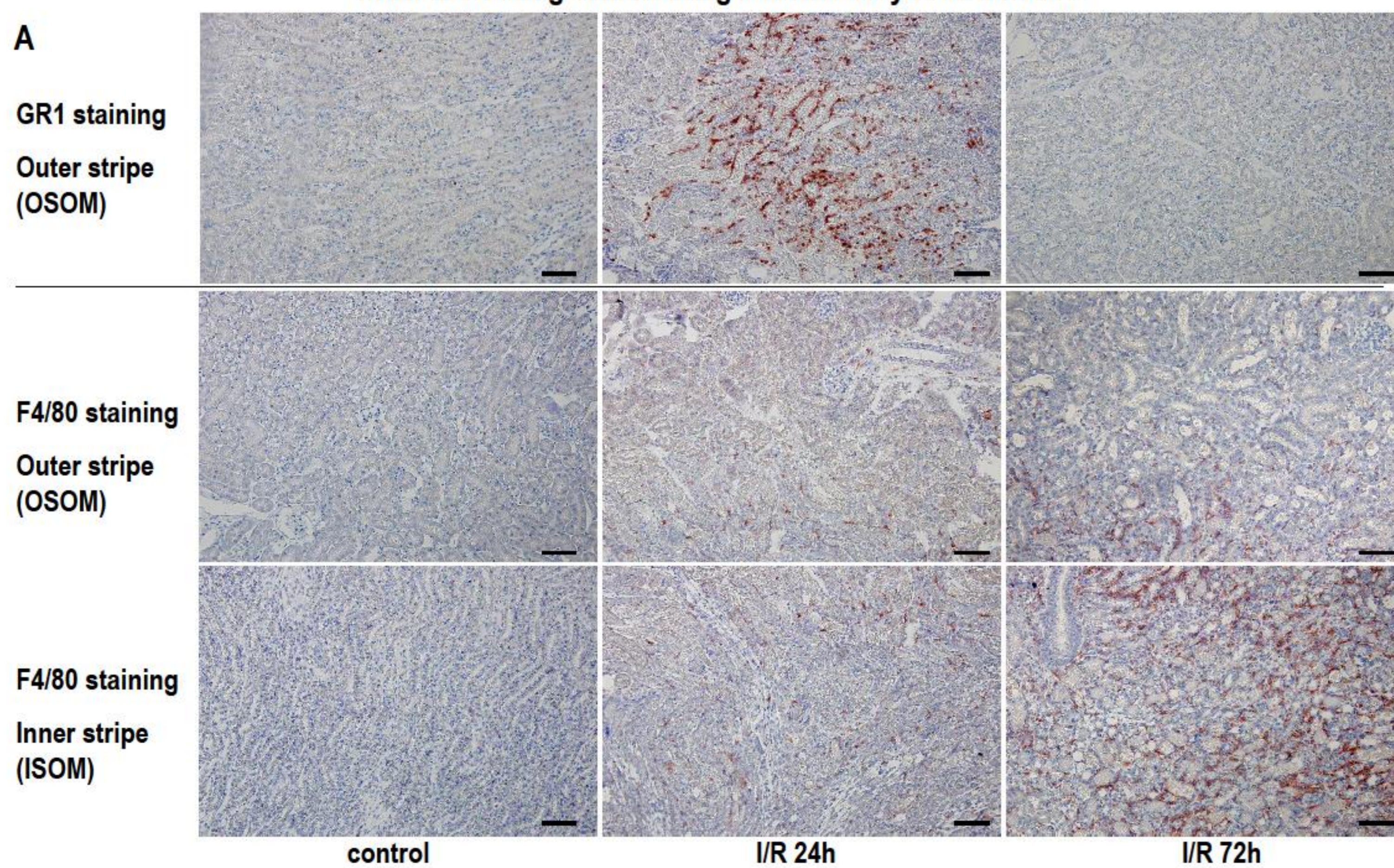
### Renal structure was preserved in Cx43 +/- mice after renal I/R.



### Cx43 +/- mice showed improved renal function and less inflammation in renal I/R.



### Immunostaining of infiltrating inflammatory cells after I/R.



## 【Discussion】

### Possible Roles of Cx43 in renal ischemia reperfusion

- Propagation of inflammation/death signals through gap junctions (GJs) *Br J Pharmacol* 2010 Aug;160(8):2055-68
- Hemichannels: purinergic [Ca<sup>2+</sup>]<sub>i</sub> signaling *JCI* 2014;124:2050
- Recruitment of neutrophils & monocytes → inflammation *Biochim Biophys Acta* 2005; 1711: 197, *J Vasc Res* 2011; 48: 91
- ATP depletion → opening of GJs/HCs *Acta Physiol Scand* 2003;179:33
- HC opening → influx of ECF/death signals → cell damage/apoptosis *Acta Physiol Scand* 2003;179:33
- Cx43 expression↑ in leucocytes → adhesion ↑, migration ↑ *PNAS* 1995;92:7011, *AJP Heart Circ Physiol* 2008;295:1056
- Activation of endothelial cells → adhesion ↑ *JCI* 2006;116:2193
- Vascular permeability ↑ *JCI* 2006;116:2193
- Suppression of the PI3K/Akt Pathway (pro-survival) *JASN* 2008;9:2086
- Downregulation of Bcl2 (an anti-apoptotic protein) *J Biol Chem* 2003;278:44852

## 【Conclusion】

- The degree and distribution of renal inflammatory lesions, and the type of inflammatory cells showed a temporal change after renal I/R injury.
- The upregulation of Cx43 is associated with alterations in the expression of various cytokines and markers of inflammation.
- Cx43 is increased in renal I/R, indicating a key role in the pathophysiology of the model, and its deletion improved renal function and tubular damage.

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