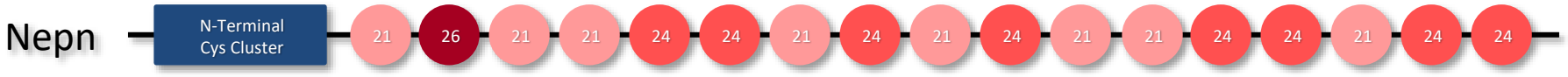
Nephrocan

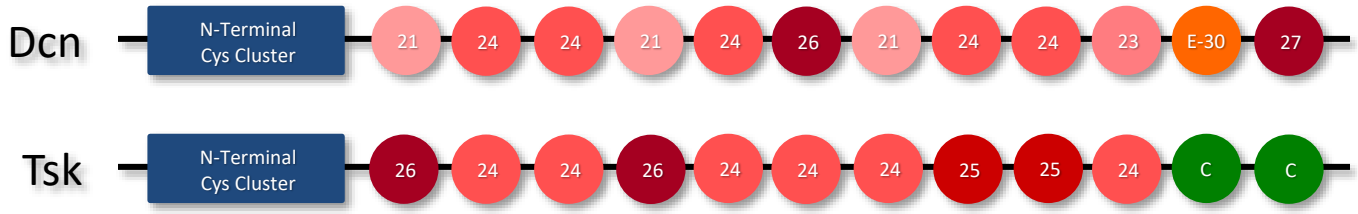
- 512-amino-acid protein
- Belongs to the Small Leucine-rich Repeat Proteglycan/Protein family (SLRP)
  - 17 Leucine-rich repeat (LRR) regions
  - N-terminal cysteine cluster
  - 3 exons
- Contains 5 potential N-glycosylation sites
- Secreted protein
- TGF-β inhibitor
- Expressed in adult kidney
- Identified as a marker for definitive endoderm and midgut endoderm



Modified from Dellet et al., 2012

Small Leucine-rich Repeat Protein Family

- Secreted, extracellular proteins
- Structural roles in the ECM
  - Bind collagens
  - Influence the structural and functional environment of cells
- Cellular proliferation
  - Decorin and biglycan can both promote and inhibit proliferation (thought to be tissue-type specific)
  - Decorin prevents apoptosis in tubular epithelial, endothelial, and bone marrow stromal cells



Modified from Dellet et al., 2012

Small Leucine-rich Repeat Protein Family

- Participation in signaling pathways
  - Interact with cytokines (like TGF-β and BMP) as well as with signaling receptors
  - Decorin, biglycan, asporin, and fibromodulin bind TGF-β
    - Decorin may be able to sequester TFG-β from its receptors
  - Biglycan-deficient osteoblasts were shown to have a differentiation defect because of reduced BMP4 sensitivity
  - Tsukushi influences ectodermal patterning and neural crest specification by controlling BMP signaling

Nephrocan, a Novel Member of the Small Leucine-rich Repeat Protein Family, Is an Inhibitor of Transforming Growth Factor-β Signaling*

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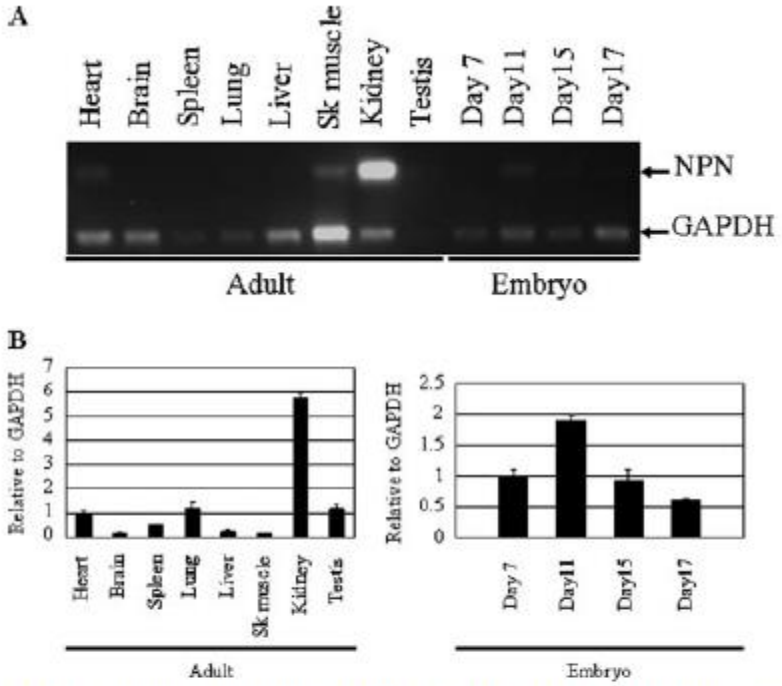


FIGURE 2. **Tissue distribution of NPN.** *A*, RT-PCR analysis with NPN-specific primers or glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*)-specific primers was performed. The PCR products were analyzed on 1.2% TAE-agarose gel and photographed under UV light. *Sk muscle*, skeletal muscle. *B*, quantitative real time PCR analysis of NPN mRNA expression. The mean fold changes in the expression of NPN relative to that of GAPDH were calculated using the value of the expression of either heart or 7 day-embryo as a calibrator. The values are shown as mean ± S.D. based on triplicate assays. Sk muscle; skeletal muscle.

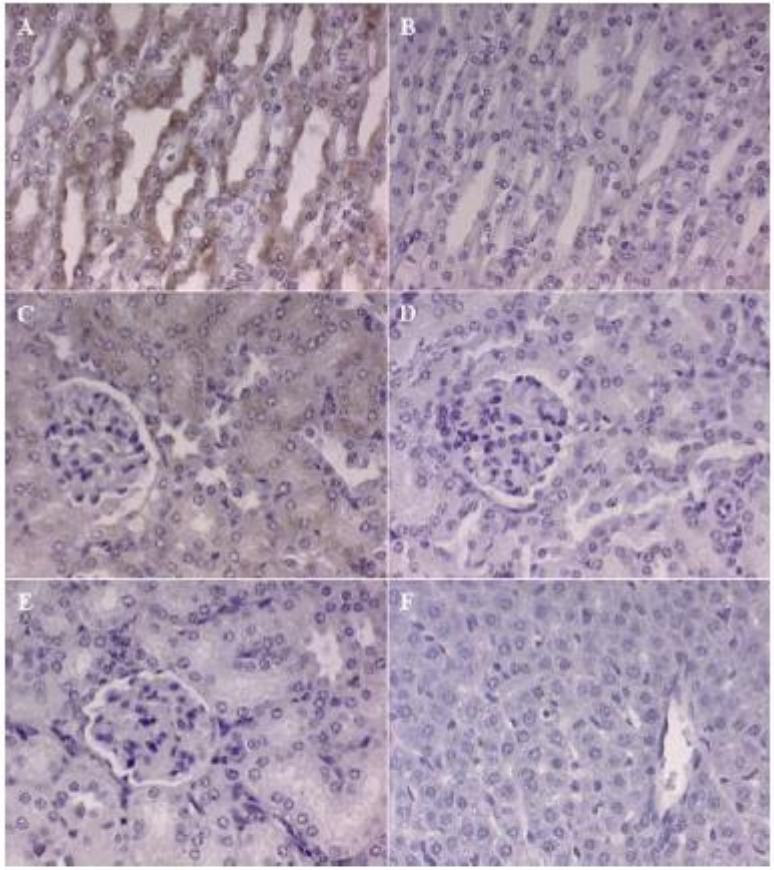
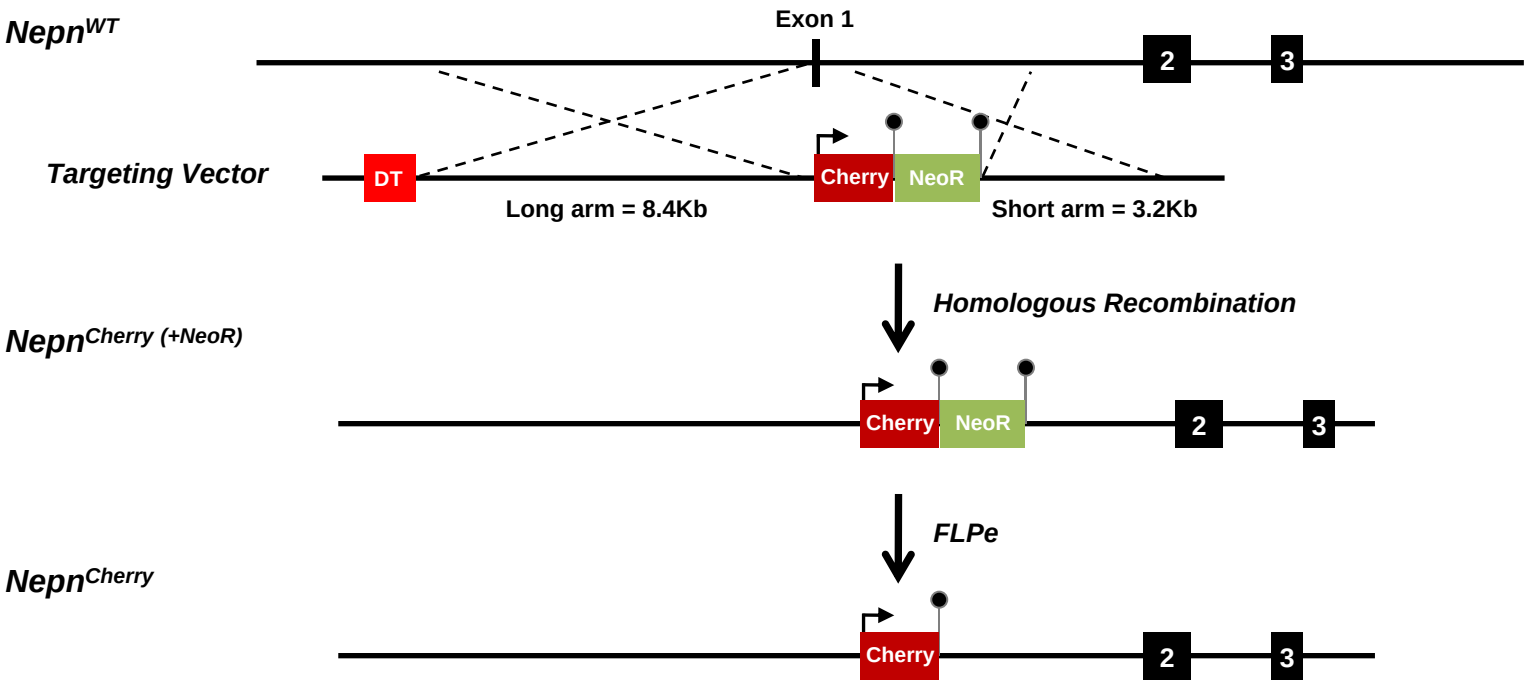
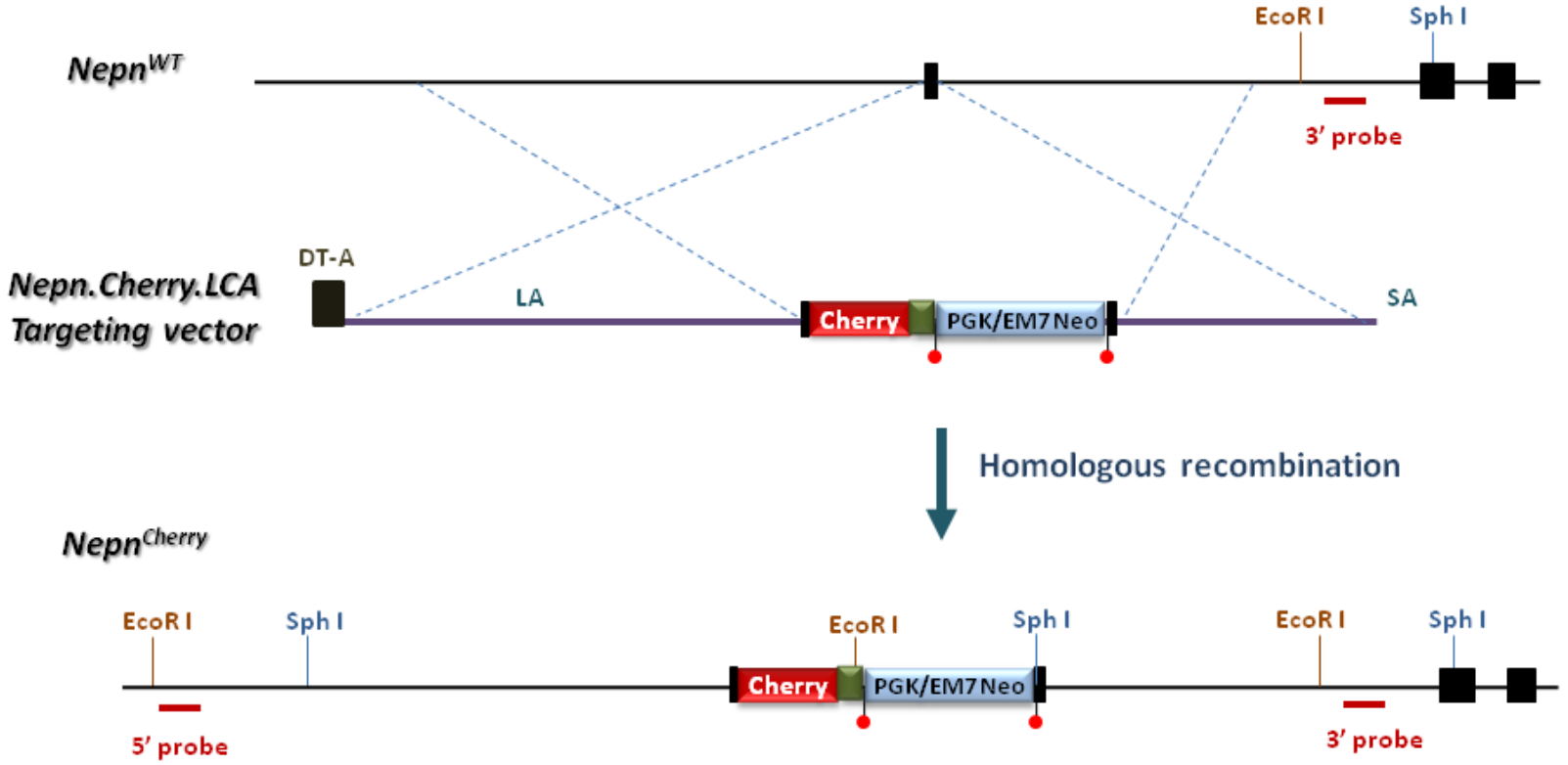


FIGURE 4. **Immunohistological detection of NPN in mouse adult kidney.** The mouse adult kidney sections were immunostained using anti-NPN antibody with (*B* and *D*) or without (*A* and *C*) the synthetic peptide used for antibody generation. The epithelial cells of distal tubules and collecting ducts were extensively stained (*A*), and proximal epithelial cells were slightly stained (*C*). There was no significant staining in glomeruli (*C*). The immunoreactivities were not observed using anti-NPN antibody in the presence of the synthetic peptide (*B* and *D*). Preimmune serum was also used as a negative control, and no immunoreactivities were observed (*E*). In liver, no immunoreactivities were observed (*F*). The specimens were observed and photographed under a light microscope at a magnification of ×20.

Gene targeting design for reporter allele and global deletion of Nephrocan



Nepn.Cherry gene targeting strategy



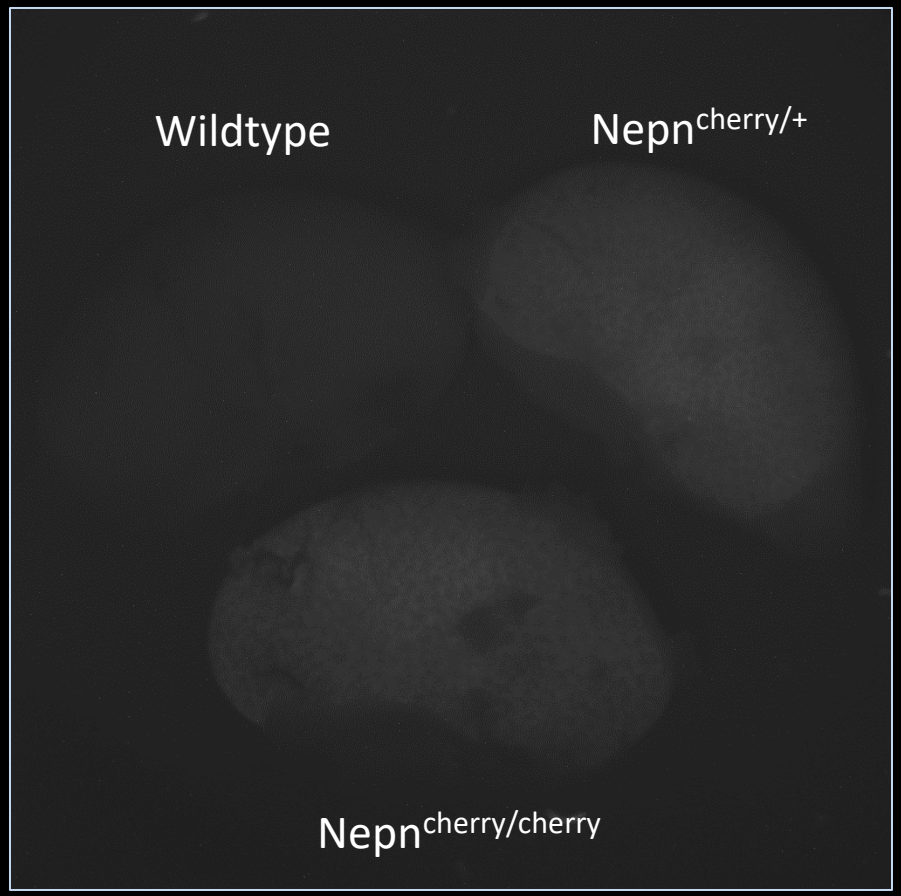
rabbit β–globin polyA; FRT site

5' screening : EcoRI digest
WT: 14,36 kb
TM: 12,11 kb

3' screening : SphI digest
WT: 16,77 kb
TM: 9,1 kb

Whole Mount Images of Kidney

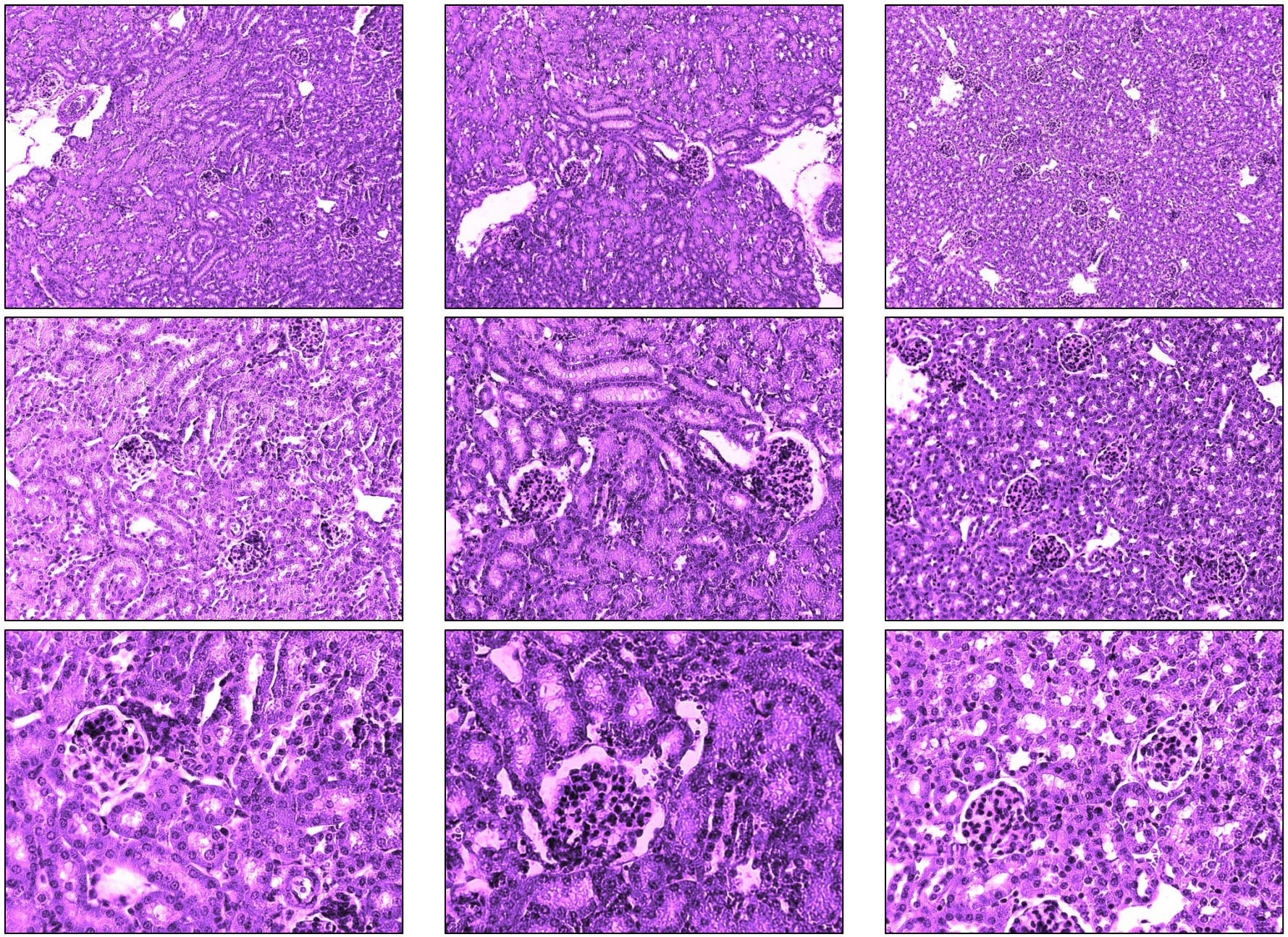
Brightfield



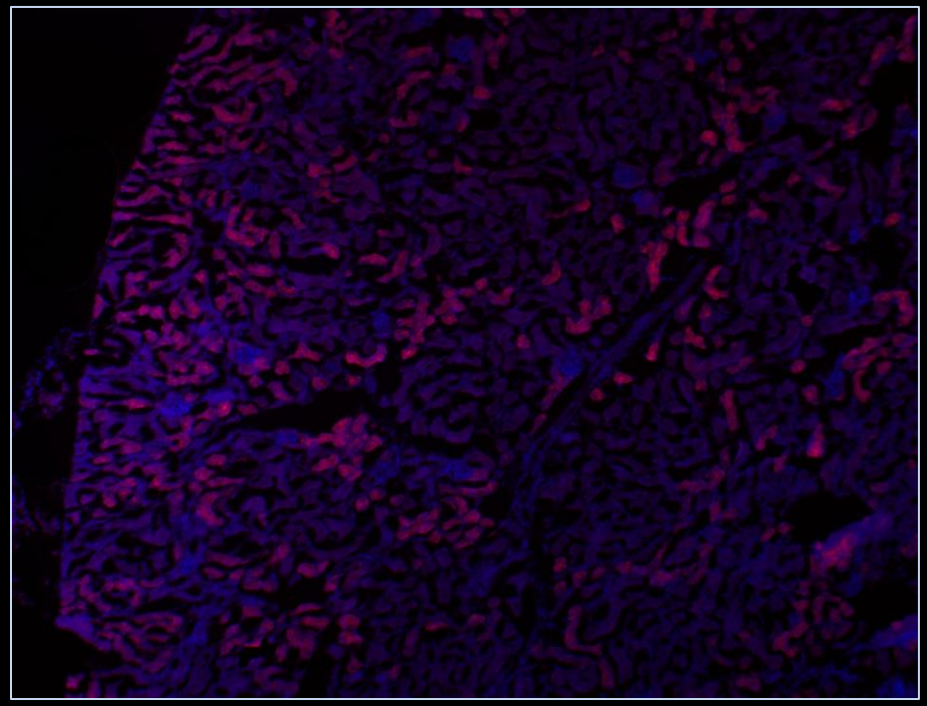
DSRed

6 weeks of age

H&E Staining of Kidney Paraffin Sections at 4 weeks of age

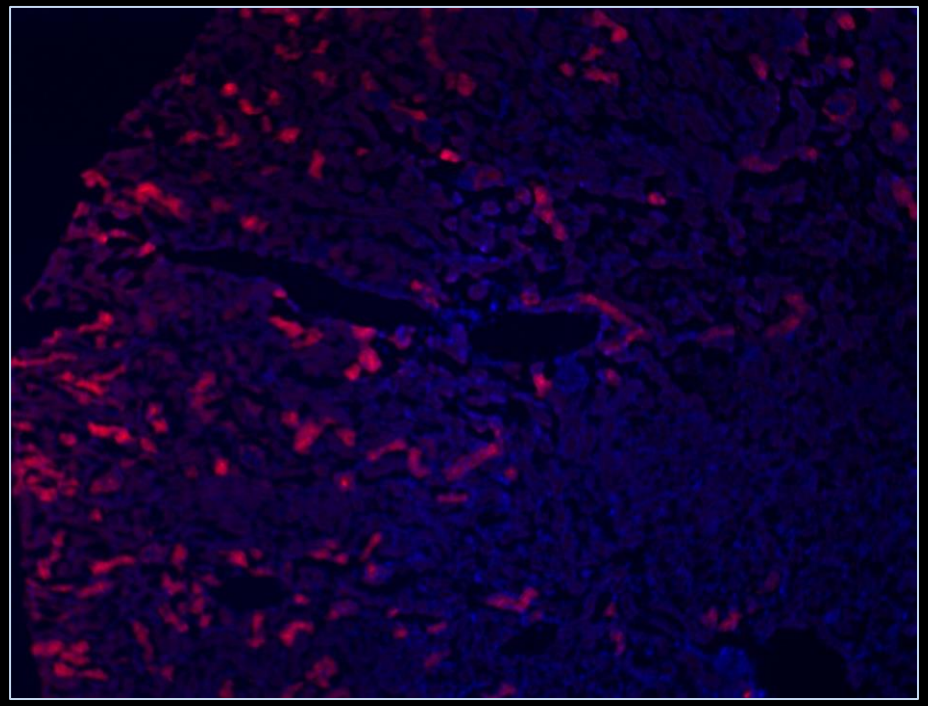


Nepn$^{cherry/+}$ Kidney, 6 weeks



DAPI RFP

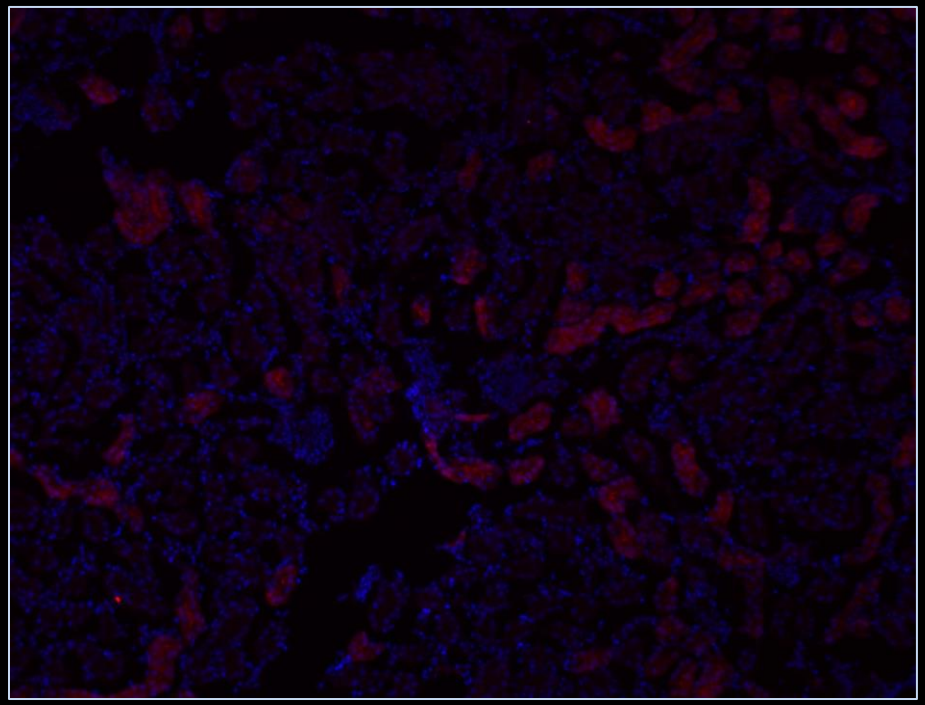
Nepn$^{cherry/cherry}$ Kidney, 10 weeks



DAPI RFP

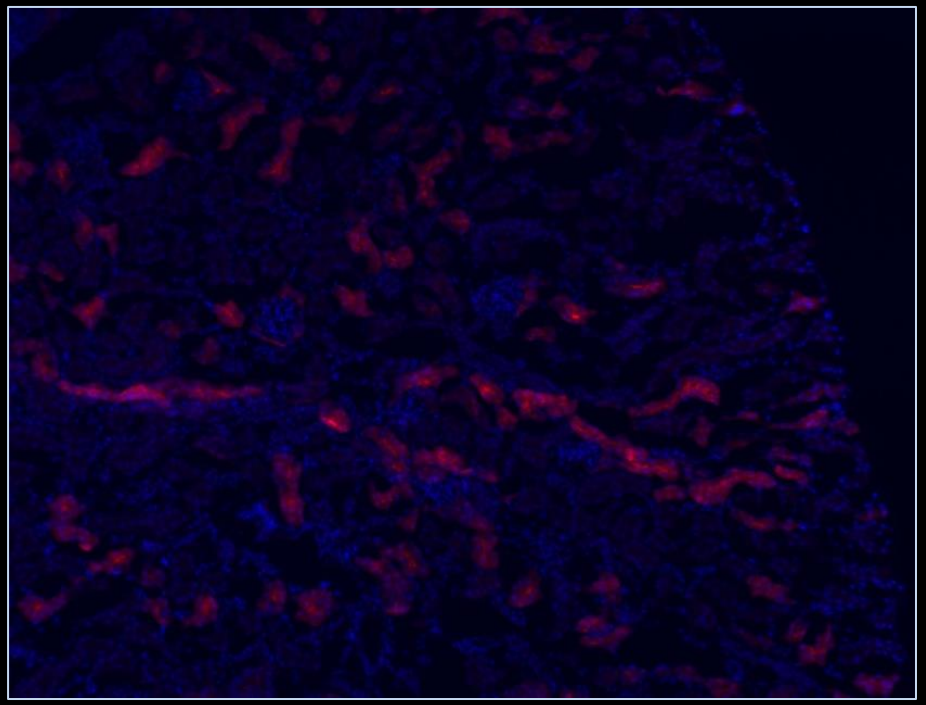
5X

Nepn$^{cherry/+}$ Kidney, 6 weeks



DAPI RFP

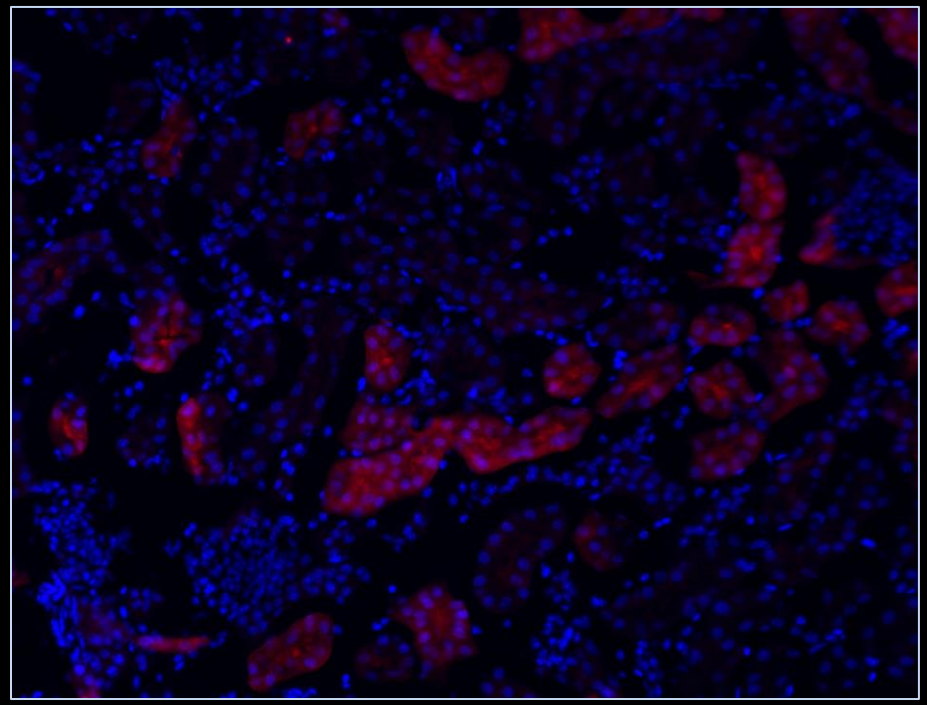
Nepn$^{cherry/cherry}$ Kidney, 10 weeks



DAPI RFP

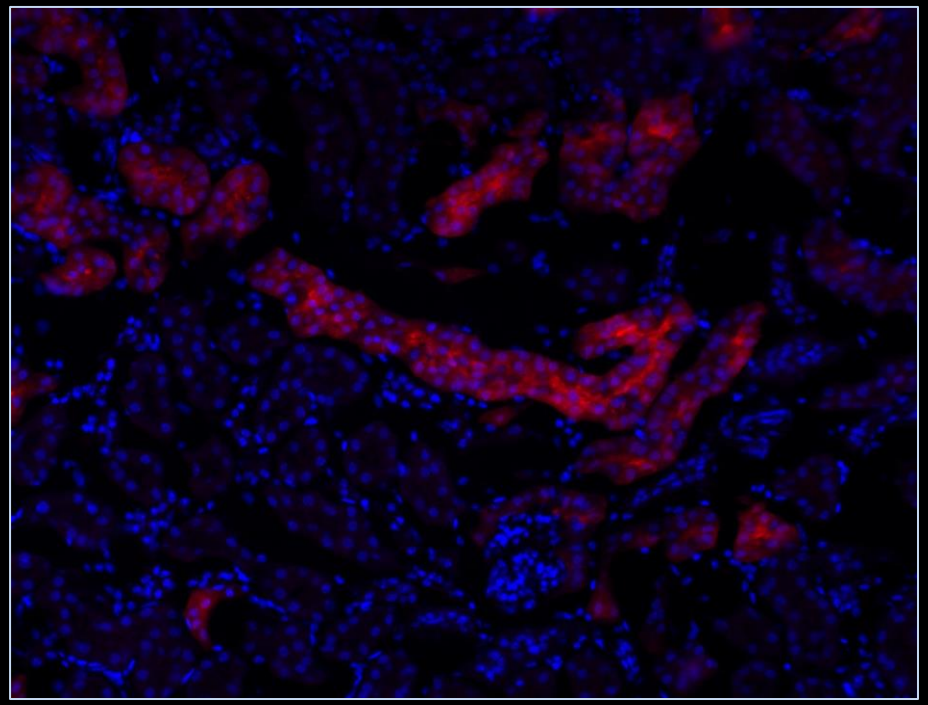
10X

Nepn$^{cherry/+}$ Kidney, 6 weeks



DAPI RFP

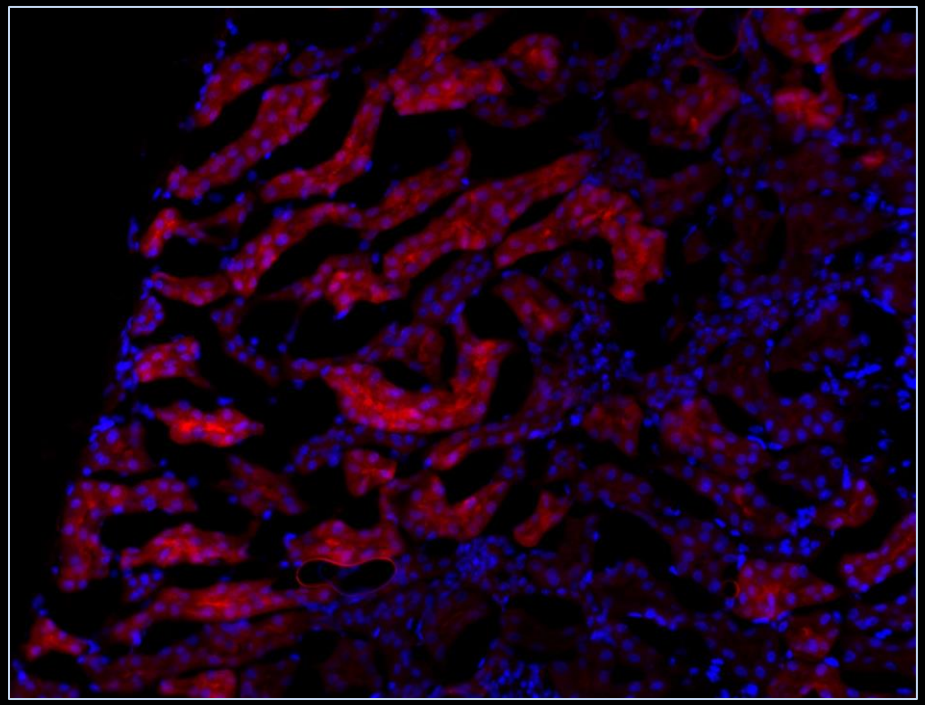
Nepn$^{cherry/cherry}$ Kidney, 10 weeks



DAPI RFP

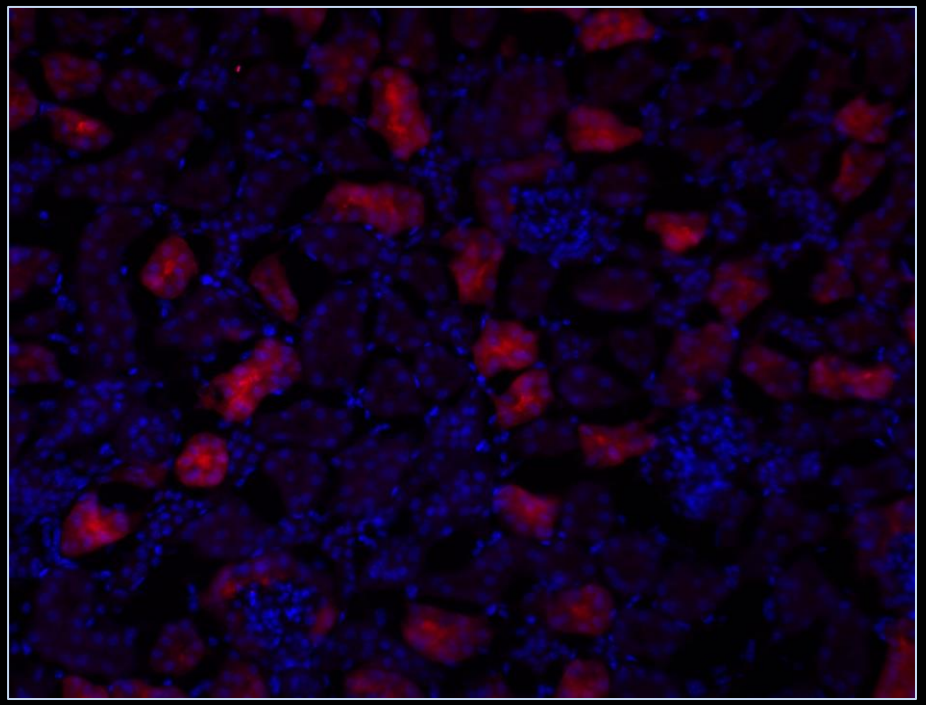
20X

Nepn$^{cherry/+}$ Kidney, 6 weeks



DAPI RFP

Nepn$^{cherry/cherry}$ Kidney, 10 weeks



DAPI RFP

20X