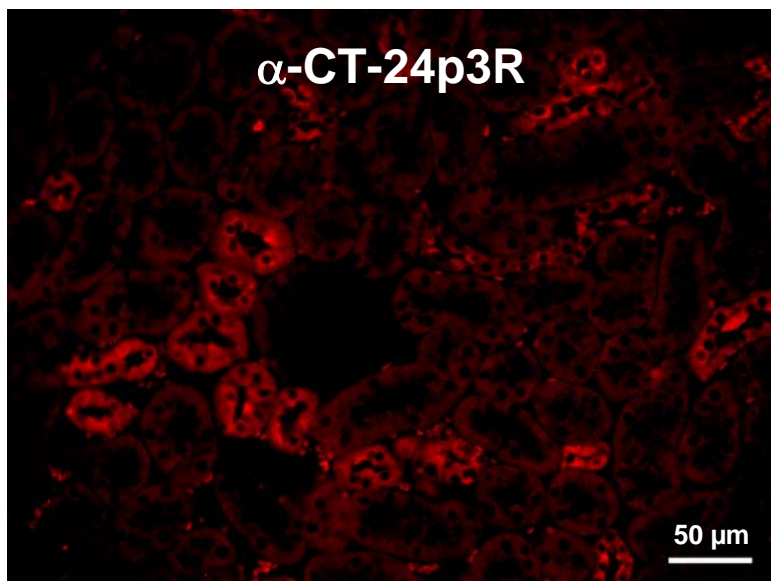
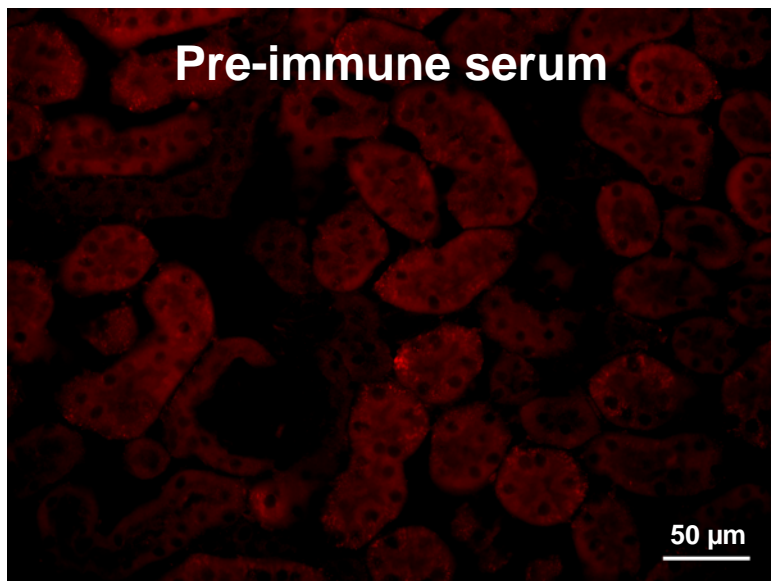
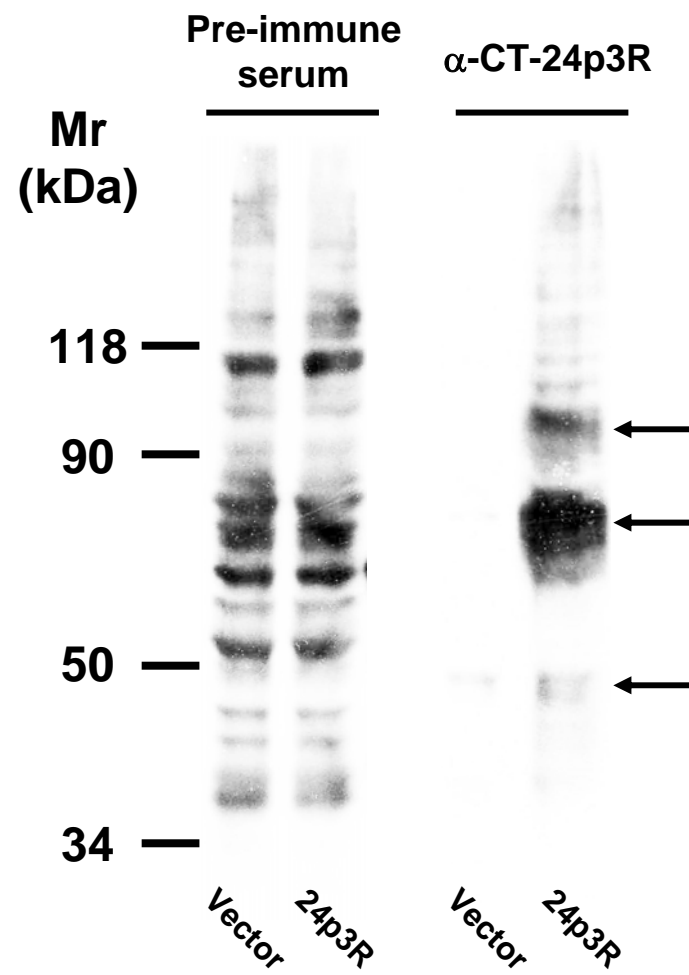
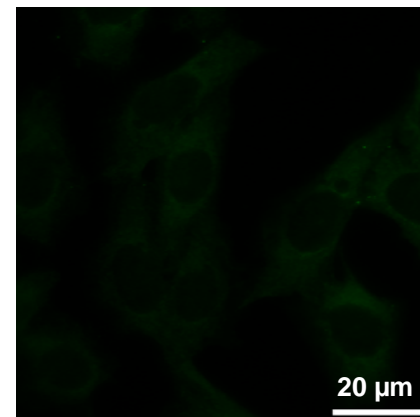
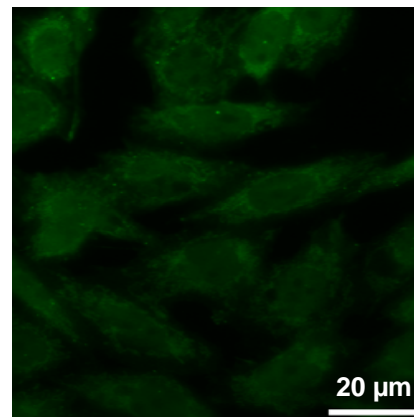
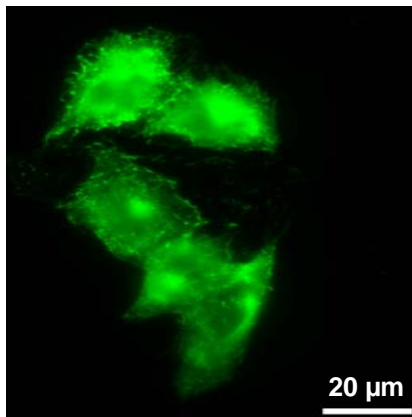
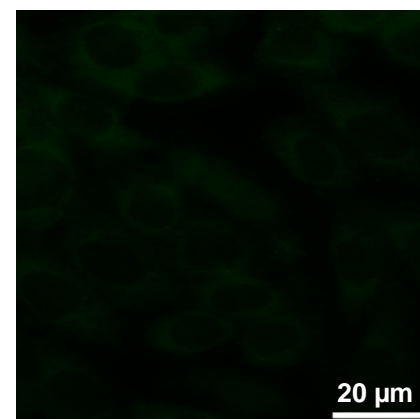
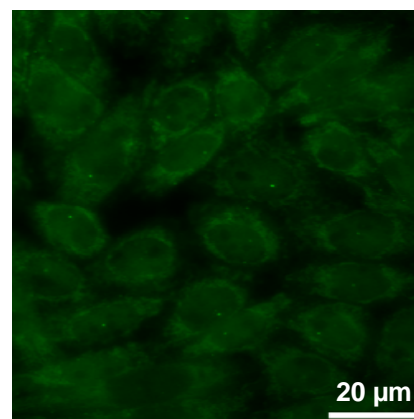
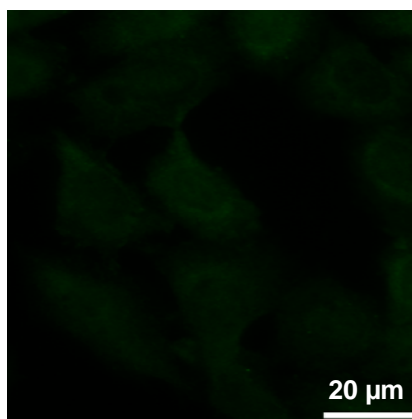
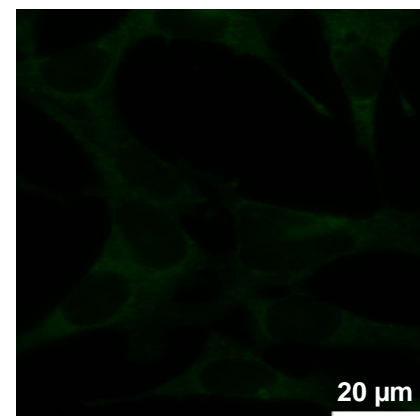
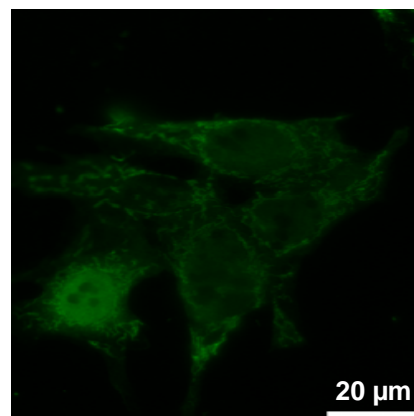
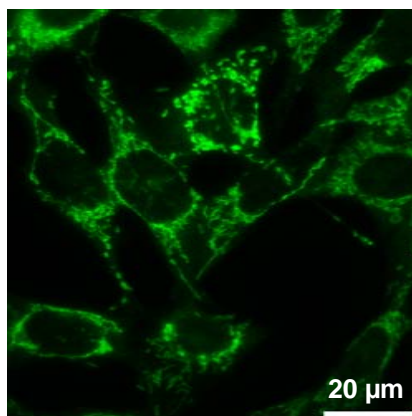


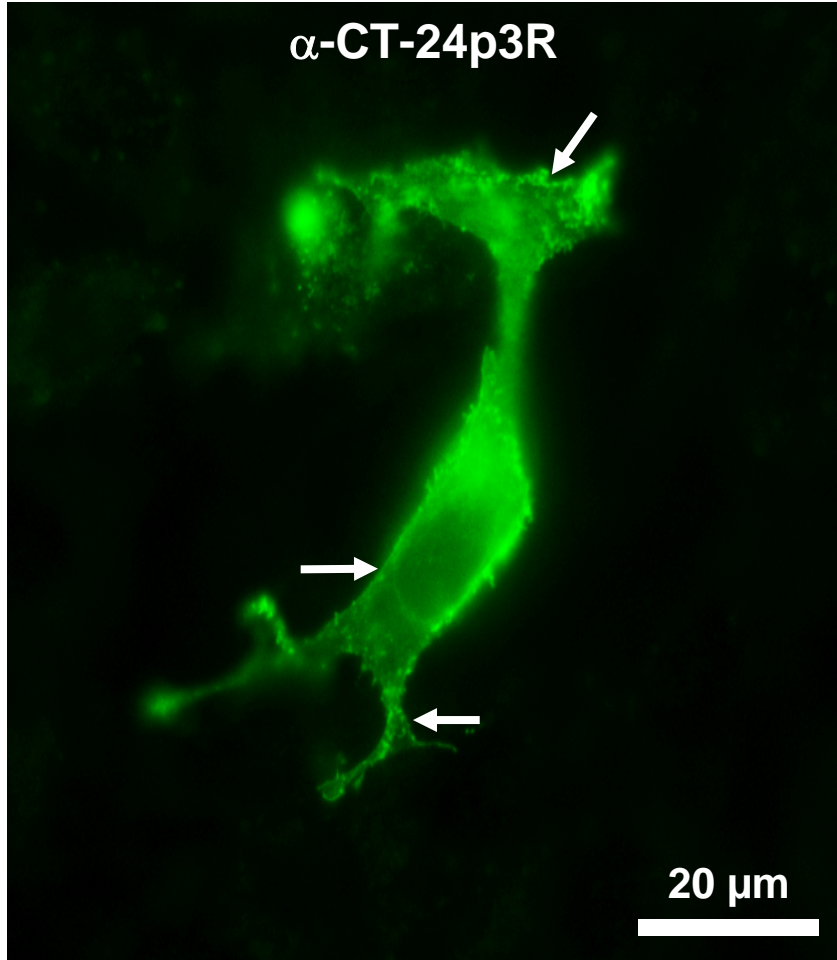
**A****Mouse kidney****B****HEK293 cells**

**C****24p3R-CHO****vector-CHO****mDCT209** **$\alpha$ -NT-24p3R****Pre-immune serum****Pre-absorption**

**D**

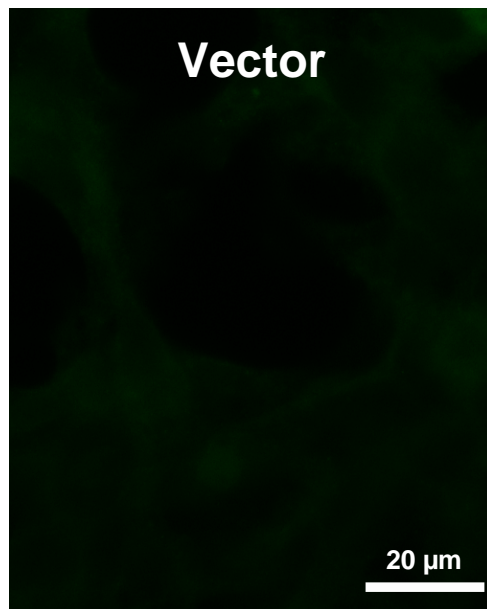
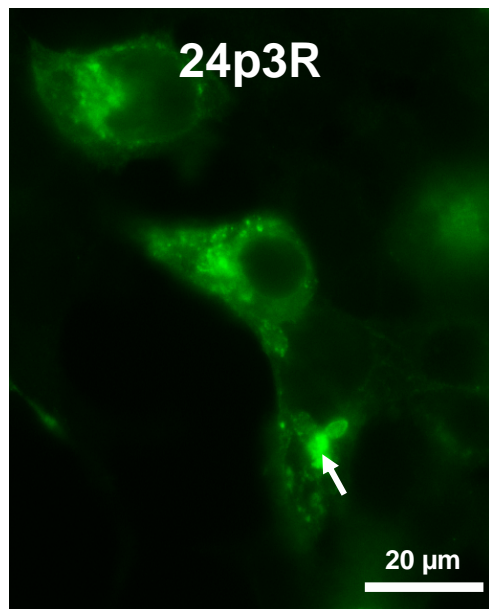
**CHO cells**

**$\alpha$ -CT-24p3R**

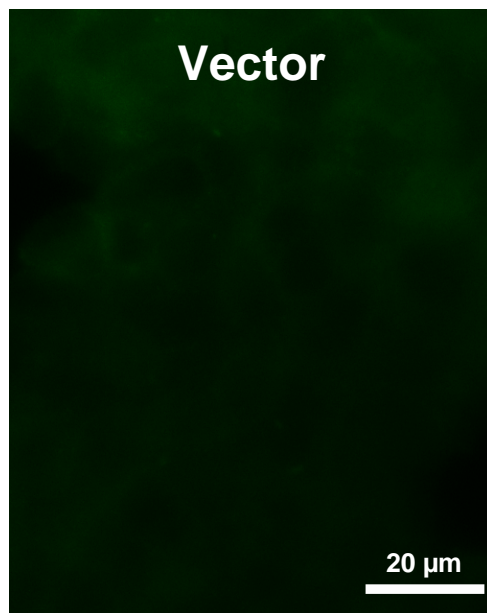
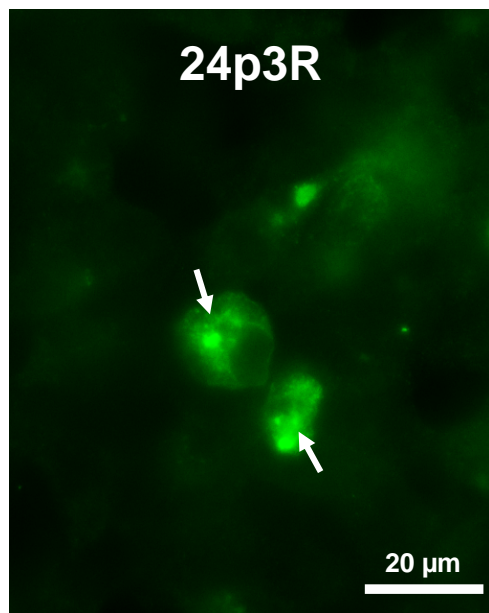


**Suppl. Figure 1**

HEK293 cells



$\alpha$ CT-24p3R



$\alpha$ NT-24p3R

Gene	GeneBank Accession Number	Primer	Cycling Protocol		Product Size (bp)
<b>Rat 24p3R</b>	NM_177421.3	<i>for</i> 5'-AGGACTGGCGGTTCTGCAG-3' <i>rev</i> 5'-CGTTCAGATAATCCCACAGG-3'	Activation Denaturing Annealing Extension Final extension Cycles	95 °C for 15 min 94 °C for 30 sec 64.2 °C for 30 sec 72 °C for 1 min 72°C for 5 min 28	540
<b>Murine 24p3R</b>	NM_021551.4	<i>for</i> 5'-TACCTGATGCGCCTGGAGCT-3' <i>rev</i> 5'-TTCTCCAGTTCCTGCAAAGCTT-3'	Activation Denaturing Annealing Extension Final extension Cycles	95 °C for 10 min 95 °C for 30 sec 58.5 °C for 45 sec 72 °C for 10 sec 72°C for 10 min 30	332
<b>Rat <math>\beta</math>-actin</b>	NM_031144.2	<i>for</i> 5'-AGATCAAGATCATTGCTCCTC-3' <i>rev</i> 5'-ACTCATCGTACTCCTGCTTG-3'	Activation Denaturing Annealing Extension Final extension Cycles	95 °C for 15 min 94 °C for 15 sec 65 °C for 30 sec 72 °C for 30 min 72°C for 5 min 26	349
<b>Murine <math>\beta</math>-actin</b>	NM_007393.3	<i>for</i> 5'-GTATGGAATCCTGTGGCATC-3' <i>rev</i> 5'-AAGCACTTGCGGTGCACGAT-3'	Activation Denaturing Annealing Extension Final extension Cycles	94 °C for 5 min 94 °C for 25 sec 55 °C for 25 sec 72 °C for 30 sec 72°C for 7 min 28	322

**Supplementary Table 1**

## **SUPPLEMENTARY FIGURE AND TABLE LEGENDS**

**SUPPLEMENTARY FIGURE 1.** Characterization of rabbit polyclonal antibodies directed against rat 24p3R  $\alpha$ -CT- and  $\alpha$ -NT-24p3R. **(A)** Immunofluorescence microscopy demonstrates that  $\alpha$ -CT-24p3R stains mouse tubules in the cortex resembling distal convoluted tubules at the apex and intracellularly (bottom panel), which is absent with pre-immune serum (top image). **(B)**  $\alpha$ -CT-24p3R detects several bands of ~50-100 kDa in HEK293 transiently overexpressing 24p3R, which are not found in vector-transfected cells. With pre-immune serum, the pattern of cross-reacting proteins does not differ between vector- and 24p3R-transfected HEK293 cells. **(C)** Immunofluorescence microscopy of 24p3R-CHO cells and the murine distal convoluted cell line mDCT209 demonstrates labeling at the plasma membranes and intracellularly with  $\alpha$ -NT-24p3R, but labeling is absent in vector-CHO cells, using pre-immune serum or after pre-absorption of the  $\alpha$ -NT-24p3R antibody with a 10-fold excess of antigenic peptide. **(D)** Immunofluorescence microscopy of 24p3R-CHO cells shows intracellular labeling with  $\alpha$ -CT-24p3R, but labeling also extends to plasma membranes (*arrows*).

**SUPPLEMENTARY FIGURE 2.** HEK293 cells overexpress 24p3R intracellularly only. Transiently 24p3R-transfected HEK293 cells, but not vector-transfected cells, express rat 24p3R intracellularly (*arrows*), as demonstrated by immunofluorescence microscopy with  $\alpha$ -CT-24p3R or  $\alpha$ -NT-24p3R.

**SUPPLEMENTARY TABLE 1.** GeneBank accession numbers, nucleotide sequences, cycling PCR protocol and product size of the primers used for RT-PCR.