SUPPLEMENTARY FIGURES

Supplementary Figure 1: immunoprecipitation with anti-Casr antibody

The Casr protein was expressed in transiently transfected HEK cells. Cell lysates from HEK cells were subjected to immunoprecipitation (IP) using either anti-Casr antibodies ab 10-12 or MA1-934. Samples were submitted to SDS-Page and blotted with anti Casr antibodies. The Casr protein was detected as 2 bands: one at 150 kDa and the other one at 250 kDa. Note that the Casr protein was not detected in mock preparations.

Supplementary Figure 2: Casr staining extinction by the immunizing peptide

(A) Immunoperoxidase staining in rat kidney sections using 1:500 anti Casr Ab 10-12 showing a cytoplasmic and basolateral staining of tubular cells in the cortex. The stained tubules were present in the medullary rays and in the juxta-glomerular apparatus. Proximal tubules and glomeruli showed no detectable Casr staining.
Immunohistochemistry showing a cytoplasmic peroxidase staining of rat kidney cortex using 1:500 anti Casr Ab 10-12.

(B) Control experiments using a 1:500 dilution of the affinity purified anti-Casr antibody preadsorbed with the immunizing peptide (100 µg/ml). The resulting medium was used as a negative control. Note that a non-specific background is seen in all tubules lumen in the presence of immunizing peptide

Supplementary Figure 3: Casr localization in mice kidney

(A) Casr localization in renal mice cortex at low magnification (X100)

(B) Casr localization in renal mice cortex at high magnification (X1000)

(C) Casr localization in mice TAL by immunofluorescence and confocal microscopy showing a colocalization between Casr and Thp.

(D) Casr localization in mice CCD (Double staining with anti-Casr and anti-Aqp2 antibodies showing no colocalization between the 2 proteins).

Supplementary Figure 4: CaSR localization in human kidney showing a basolateral tubular CaSR staining without staining of proximal tubules individualized by brush borders (Panel A and B) and a colocalisation with Tamm Horsfall protein, demonstrating the CaSR localization in human TAL (Panel C) without costaining with AQP2 (Panel C).

Supplementary Figure 5: Immunoblot of Casr peptide in mouse renal cortex preparation. Each lane was loaded with 15 µg of protein. Anti-mouse Casr antibody detected Casr in kidney cortex but not in purified CCD preparation. Anti-mouse pendrin antibody used as a control, detected pendrin in both the kidney cortex and the purified CCD preparations. This experiment confirms the western blot experiment performed in rat CCD (Figure 5 Panel F).

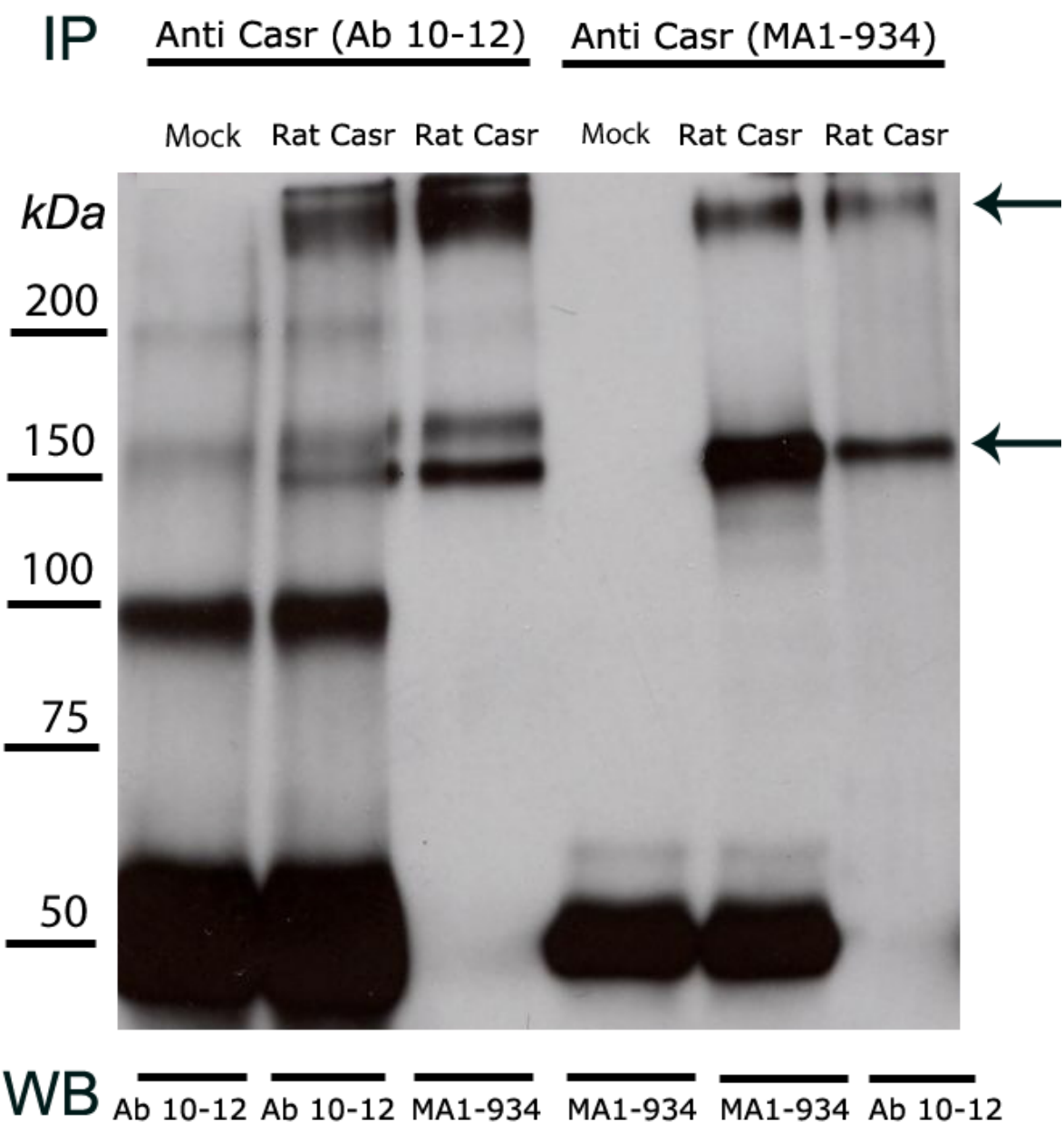
Supplementary Figure 6: Functional expression of Casr in the rat kidney.
A: cortical thick ascending limbs were dissected from rat kidney and perfused *in vitro* as explained (see « Methods »). Peritubular addition of 1 µM NPS-R568 or 300 µM neomycin elicited a sudden increase in cytosolic free calcium concentration.
B: cortical collecting ducts were dissected from rat kidney and perfused *in vitro*. Peritubular addition of 1 µM NPS-R568 or 300 µM neomycin were unable to induce a significant increase in cytosolic free calcium concentration. Conversely, peritubular addition of 10 µM 2-furoyl-LIGRL-NH_2 (F-AP), a Protease Activated Receptor (PAR) 2 agonist (positive control), elicited an increase in cytosolic free calcium concentration.
C: cortical collecting ducts were dissected from rat kidney and perfused *in vitro*. Luminal addition of 1 µM NPS-R568, 300 µM neomycin, or 3 mM calcium chloride were unable to induce a significant increase in cytosolic free calcium concentration. Conversely, peritubular addition of 10 µM F-AP, a PAR2 agonist, elicited an increase in cytosolic free calcium concentration.

Supplementary Figure 7: Bone markers measurement in TPTX, PTH-supplemented rats treated or not by Casr antagonist NPS2143 (A, B) as compared to TPTX, PTH-supplemented rats treated by the anti-bone resorptive agent sodium pamidronate (C and D)

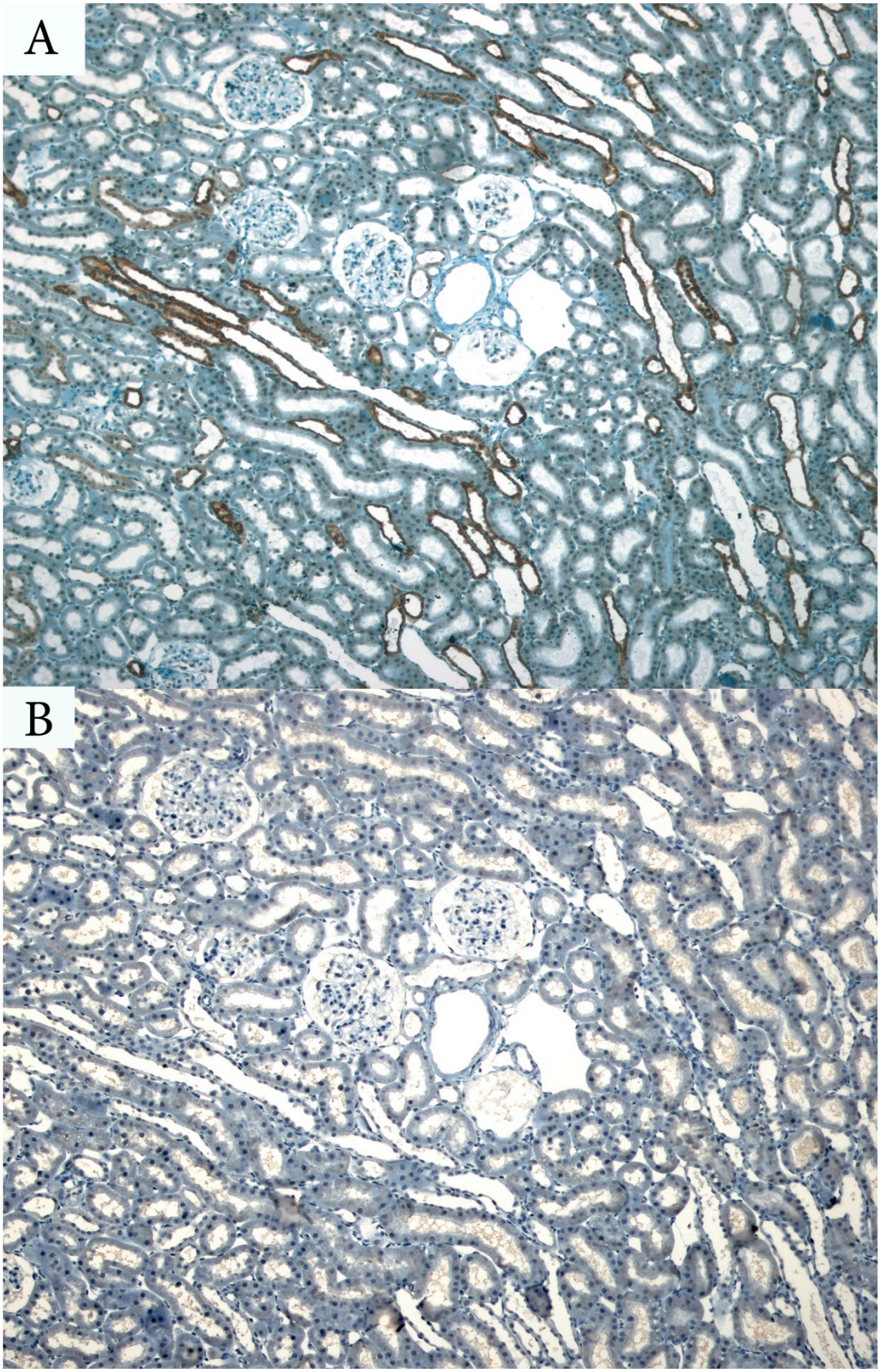
Supplementary table 1: Urinary parameter values in both groups of rats during acute studies with NPS2143

Supplementary table 2: Blood, urine and clinical parameter values in both groups of rats during chronic studies with NPS 2143

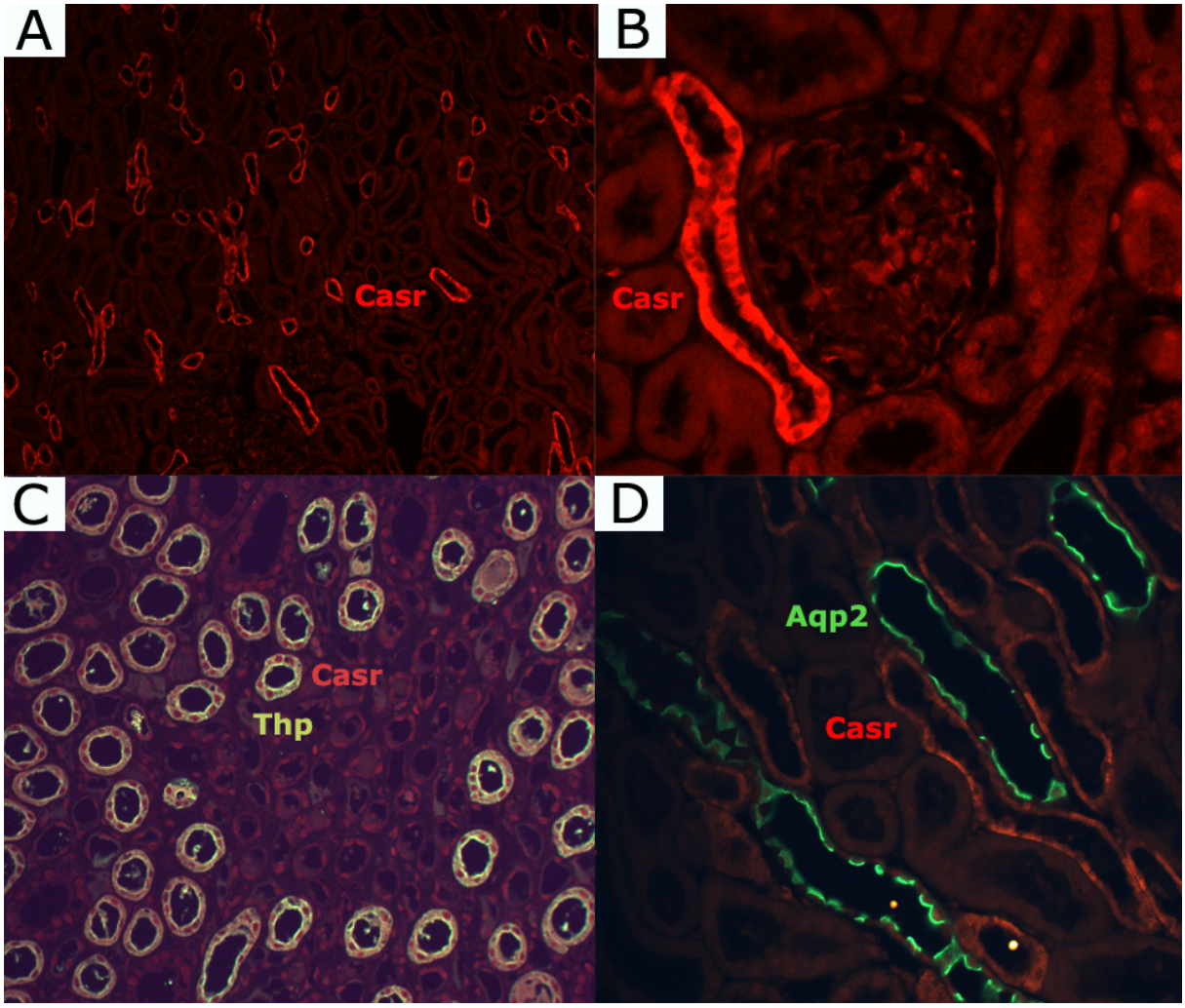
Supplementary Figure 1



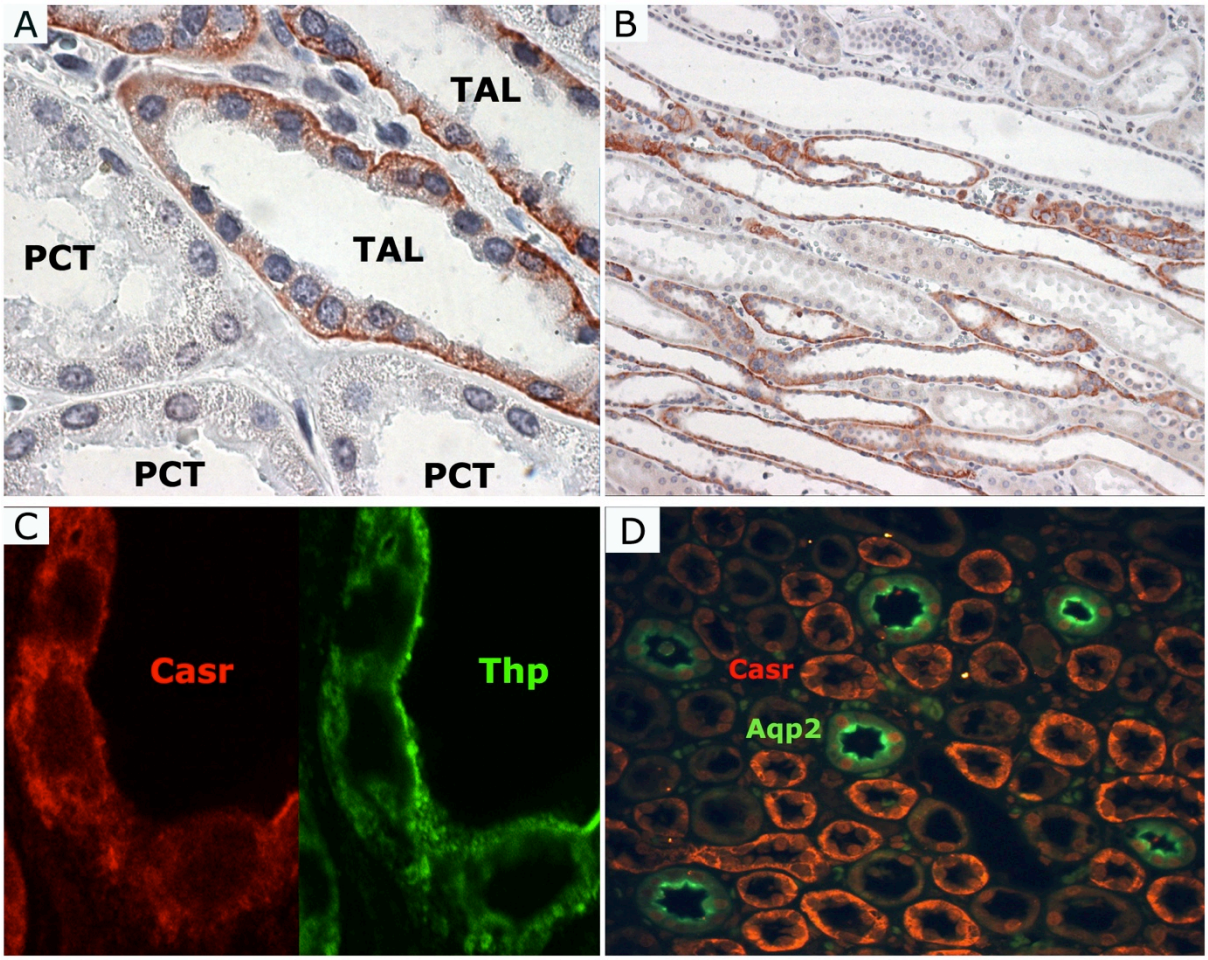
Supplementary Figure 2



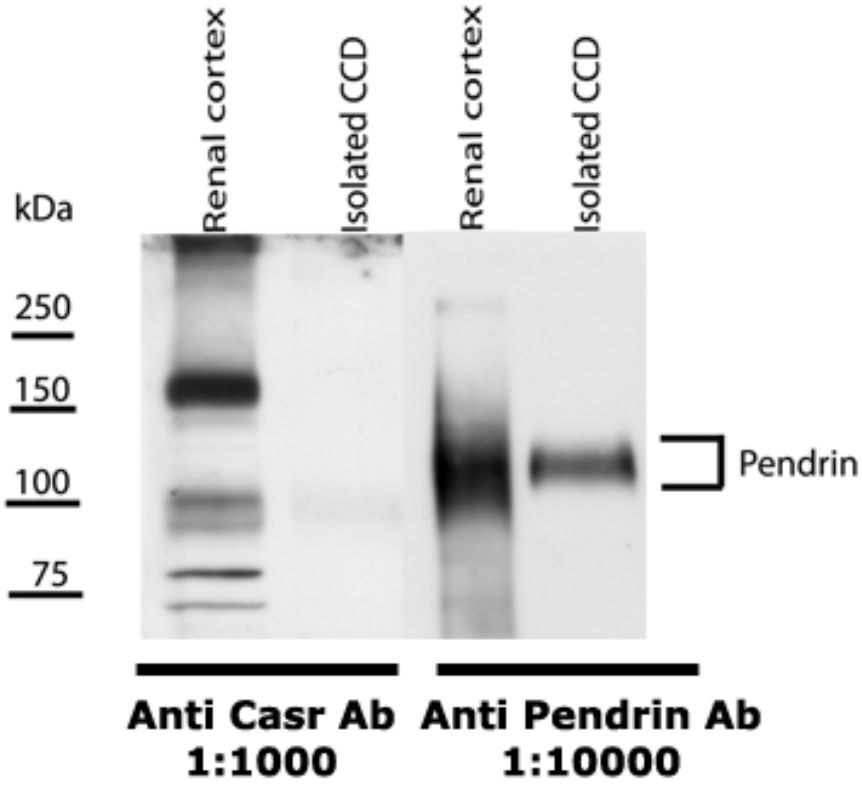
Supplementary Figure 3



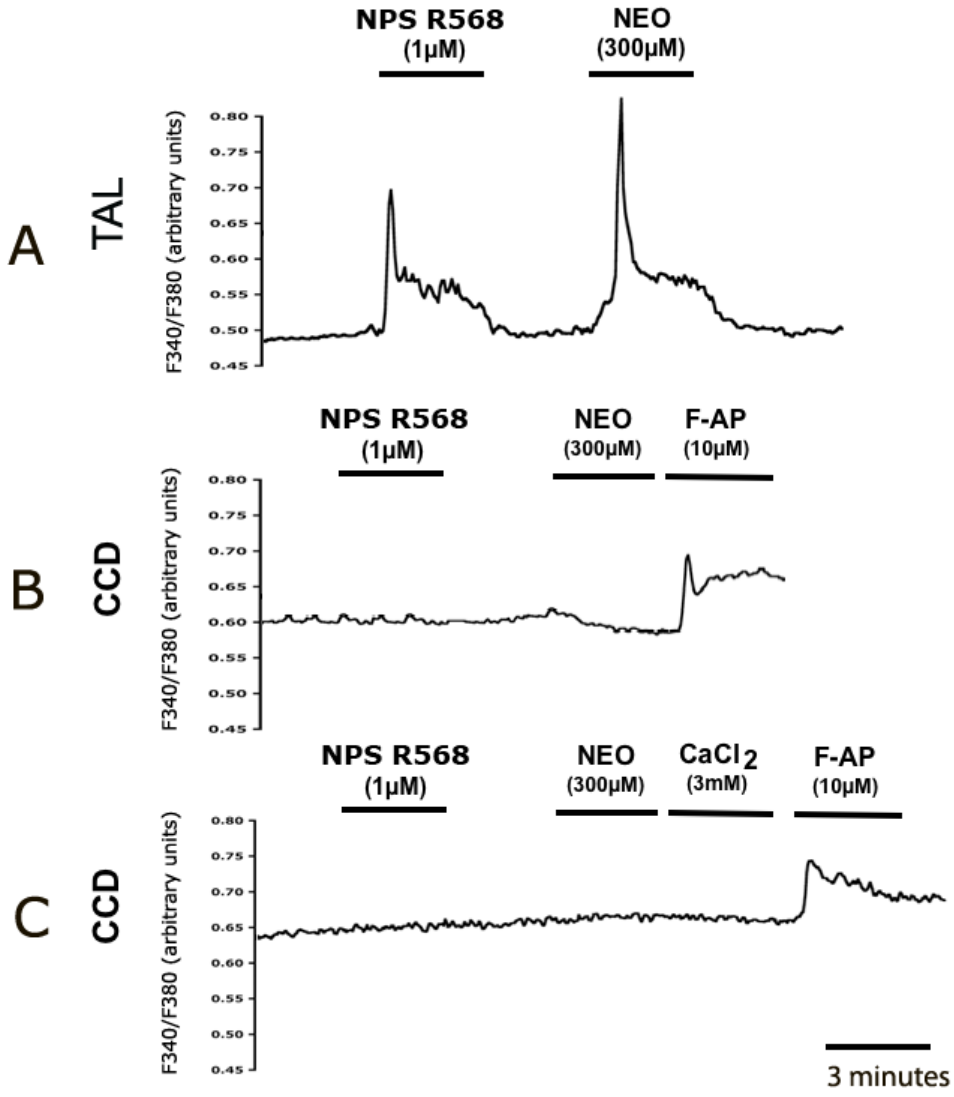
Supplementary Figure 4



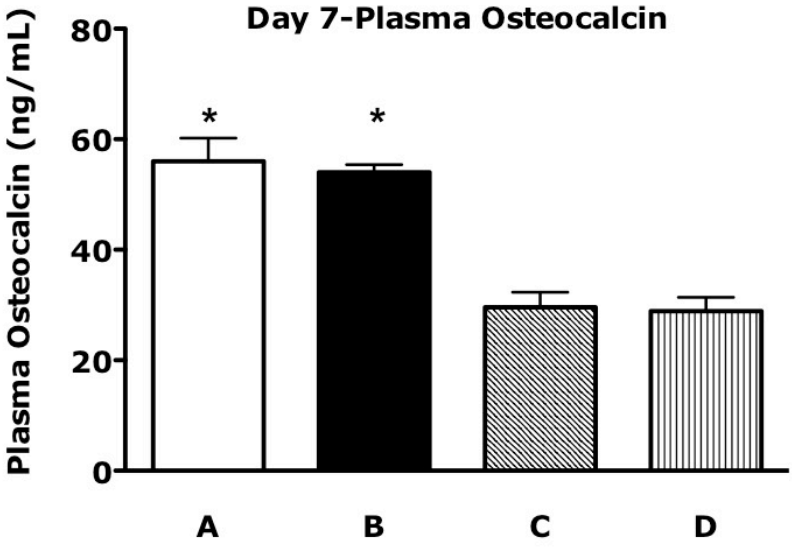
Supplementary Figure 5



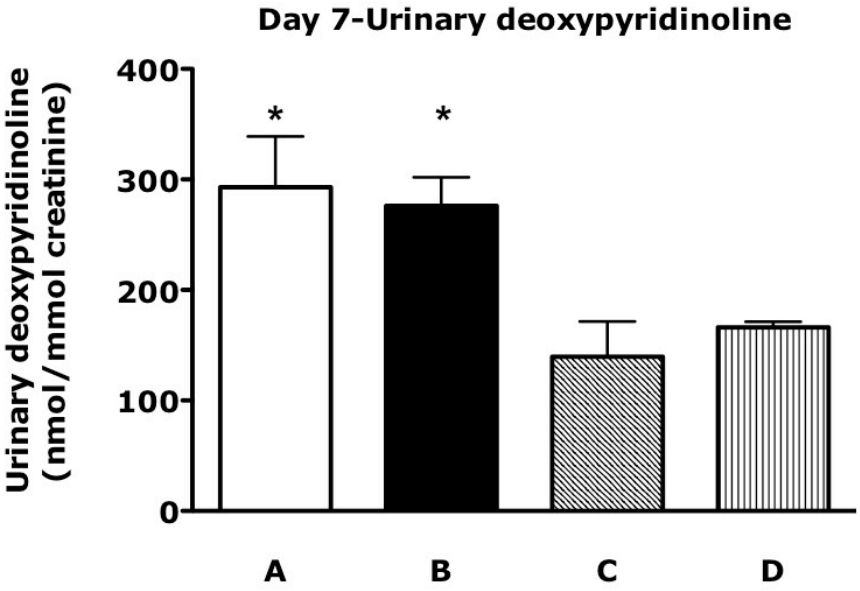
Supplementary Figure 6



Supplementary Figure 7



*: p<0.05 as compared to C and D
A vs B: p=NS



*: p<0.05 as compared to C and D
A vs B: p=NS

A- Control TPTX-PTH supplemented rats
B- TPTX-PTH supplemented rats treated by NPS2143
C- TPTX-PTH supplemented rats treated by Pamidronate
D- TPTX-PTH supplemented rats treated by Pamidronate and NPS2143

Supplemental Table 1

URINARY PARAMETERS	Baseline		Post-treatment	
	Control *	NPS2143 *	Control	NPS2143
Creatinine excretion (nmol/minute)	28.6 ± 3.4	27.9 ± 4.7	33.3 ± 7.5	31.6 ± 8.9
Na/creatinine (mmol/mmol)	11.3 ± 15.3	13.2 ± 4.2	13 ± 1.4	11.9 ± 0.9
K/Creatinine (mmol/mmol)	25.9 ± 9.6	23.3 ± 3	17.6 ± 1.7	20 ± 8.1
Urinary pH	7.33 ± 0.1	7.25 ± 0.1	7.52 ± 0.1	7.45 ± 0.1
Osmolarity (mOsm/Kg)	1413 ± 60	1365 ± 75	1299 ± 158	1375 ± 258
HCO_3^- (mEq/L)	22.0 ± 3	17.9 ± 2	29.0 ± 0.4	27.5 ± 0.6
*** N = 6 in each group**				

Supplemental Table 2

	Day-0		Day 1		Day 2		Day 4		Day 7	
	Control *	**NPS1243 ***	**Control**	**NPS1243**	**Control**	**NPS1243**	**Control**	**NPS1243**	**Control**	**NPS1243**
Weight (g)	328±15	355±17	330±12	354±19	329±12	354±13	327±13	355±11	325±4	317±9
Food intake (g/24H)	19.4±5	19.6±2.5	18.1±3.8	17.3±3	23.3±3.3	21.6±5.5	18.9±4	18.5±2.4	19.1±7.4	15.6±8.2
Water intake (mL/24h)	26.5±7.1	27.3±10.4	24.5±6.4	23.7±3.9	27.8±10	24.6±7	22.3±9.3	27.5±5.2	21.7±6	22.4±12
Urine output (mL/24h)	12.1±8.6	12.6±4.8	10.4±6.4	10.6±2.7	11.6±6.5	10.3±3.8	12.6±6.8	11.7±5.2	12.0±8.9	10.4±3.3
Urinary osmolarity (mOsm/kg)	1345±157	1459±232	1455±128	1328±98	-	-	-	-	1512±126	1452±214
Urinary Ca excretion (nmol/min)	32.4±11.3	36.2±9.4	34.4±8.3	22.0±5.3*	30.2±9.6	36.5±11.6	34.2±10.4	38.9±11.2	31.7±8.9	35.5±13.8
Urinary Mg (mmol/mmol creatinine)	2.2±0.3	1.9±0.4	2.4±0.4	2.1±0.6	-	-	-	-	1.7±0.5	1.9±0.7
Urinary deoxypyridinoline (nmol/mmol creatinine)	245±11	284±8							293±46	276±26
1,25$(OH)_2$D (pmol/L)									483±49	348±47
Plasma creatinine (µmol/L)	26.9±4.5	26.2±5.5	-	-	-	-	-	-	32.9±2.6	33.2±3.4
Plasma osteocalcin (ng/mL)									56±4.2	54±1.4
Dietary Ca intake (mmol/24 h)	5.2±1.0	5.3±0.6	4.8±1.0	4.7±0.8	6.3±0.8	5.8±1.5	5.1±1.0	5.0±0.6	5.1±2.0	4.2±2.0
Fecal Ca output (mmol/24h)	3.4±0.4	3.8±0.3	3.2±0.6	2.6±0.8	2.6±0.5	2.9±0.6	N/A	N/A	3.0±0.8	3.3±1
Intestinal Ca absorption (mmol/24h)	1.8±0.8	1.5±0.5	1.6±1.0	2.1±1.3	3.7±0.6	2.9±1.1	N/A	N/A	2.1±1.4	0.9±1.6

***N= 9 in each group**

*p=0.04 as compared to controls