Figure S1

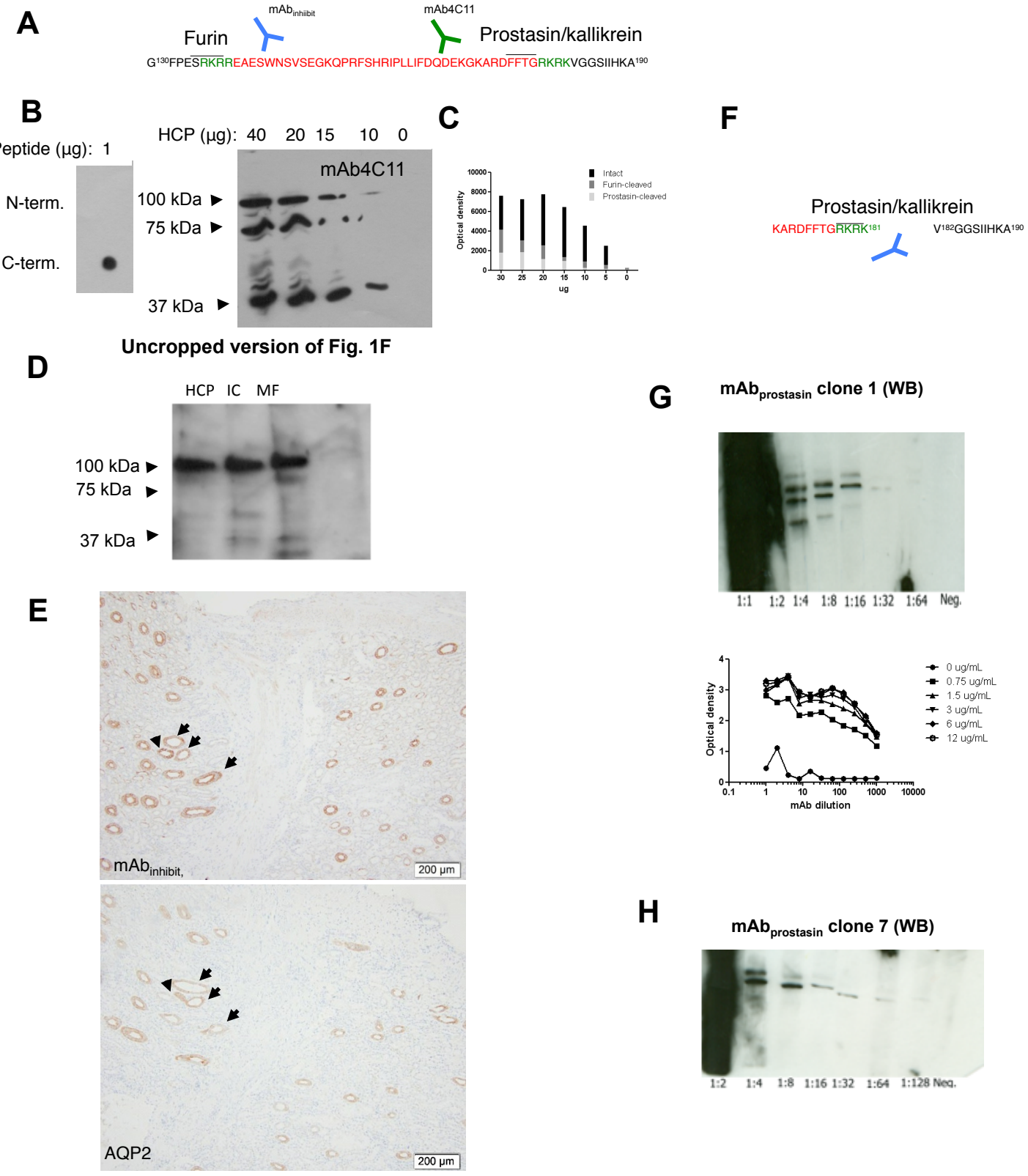

Figure S1. Characterization of mAb$_{Inhibit}$ and mAb$_{prostasin}$
(A) The primary sequence from residue 130 – 190 of the human γENaC subunit is shown. The protease cleavage sites for Furin and Prostasin/kallikrein are indicated with green. The monoclonal antibody mAb$_{inhibit}$ (blue) is directed against the N-terminal half of the inhibitory peptide (red), and mAb4c11 (green) is directed against the C-terminal half. (B) Dot blot showing that mAb4c11 only reacts with the C-terminal half of the inhibitory tract peptide (C-term), but not the N-terminal (N-term). Western blot of HCP using mAb4c11 directed against the C-terminal half of γENaC shows identical immunoreactivity as mAb$_{inhbit}$ directed against the N-terminal half. (C) Densitometric analysis of the western blots of human kidney cortex samples in Figure 1D. (D) Uncropped version of the western blot shown in Figure 1F. (E) Serial sections of human kidney displayed labeling of the same epithelial structures with mAb$_{inhibit}$ (top) and AQP2 (bottom). The arrows indicate examples of tubules stained in both sections. (F) The primary sequence from residue 170 – 190 of the human γENaC subunit is shown. Prostasin cleaves after residue 181 (green), generating a neo-epitope. mAb$_{prostasin}$ (blue) is raised against this epitope. Two independent clones of mAb$_{prostasin}$ (G and H) showed similar immunoreactivity in western blot of human kidney cortex in experiments with serial dilution of the primary antibody. (G) ELISA with the immunogenic peptide and mAb$_{prostasin}$ using different concentration of the immunogenic peptide using various mAb concentrations.

Figure S2

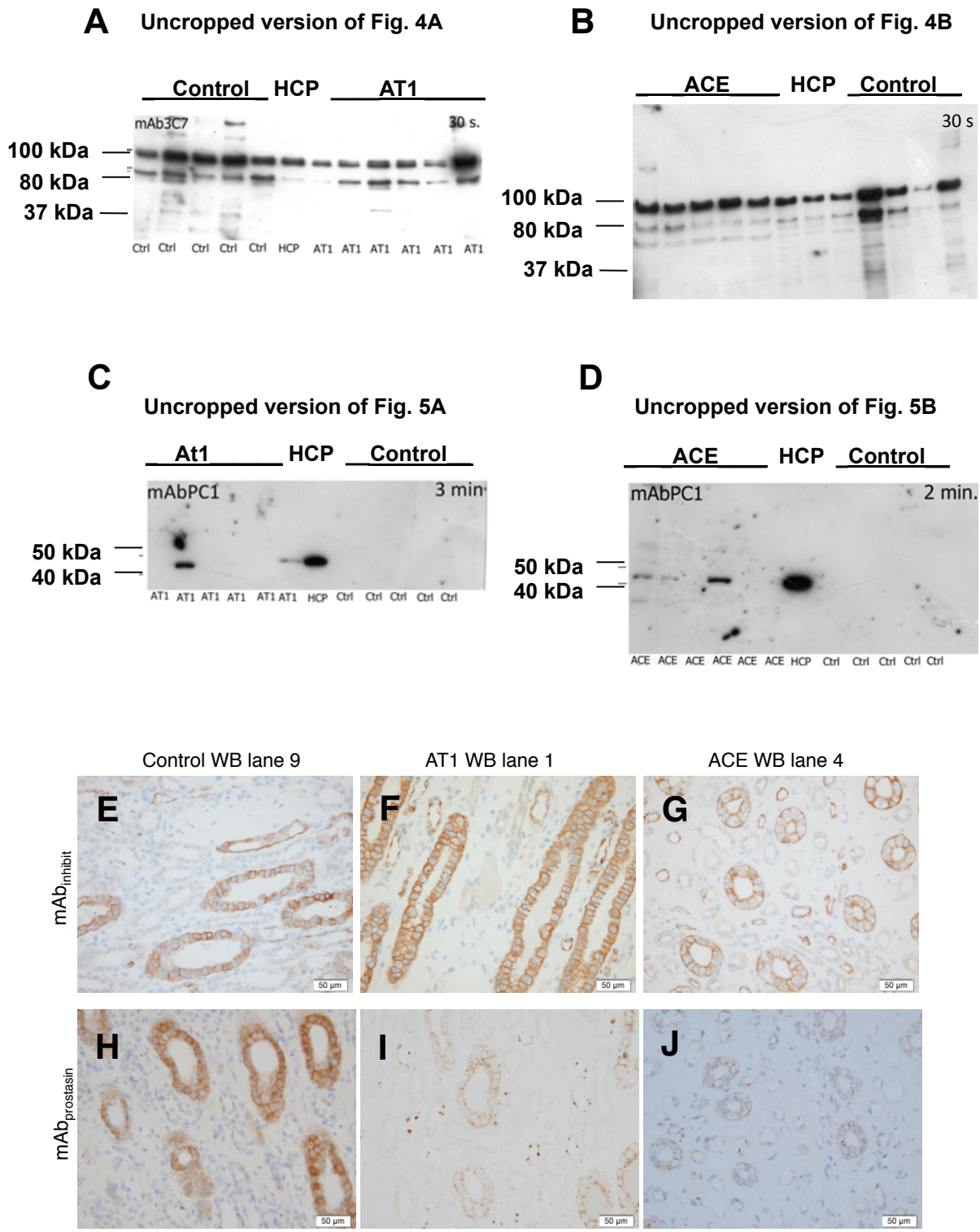

Figure S2. Western blots and IHC of kidney tissue from patients treated with AT1 receptor antagonist and ACE inhibitors.

(A and B) Uncropped version of the western blots shown in Figure 4A and B, respectively (C and D) Uncropped version of the western blots shown in Figure 5A and B, respectively. $mAb_{inhibit}$ staining of the medullary regions of kidney sections from (E) control, (F) AT1 blocker and (G) ACE inhibitor treated patients. $mAb_{prostasin}$ staining of medullary regions of kidney sections from (H) control, (I) AT1 blocker and (J) ACE inhibitor treated patients. Scale bar 50 μm.

Figure S3

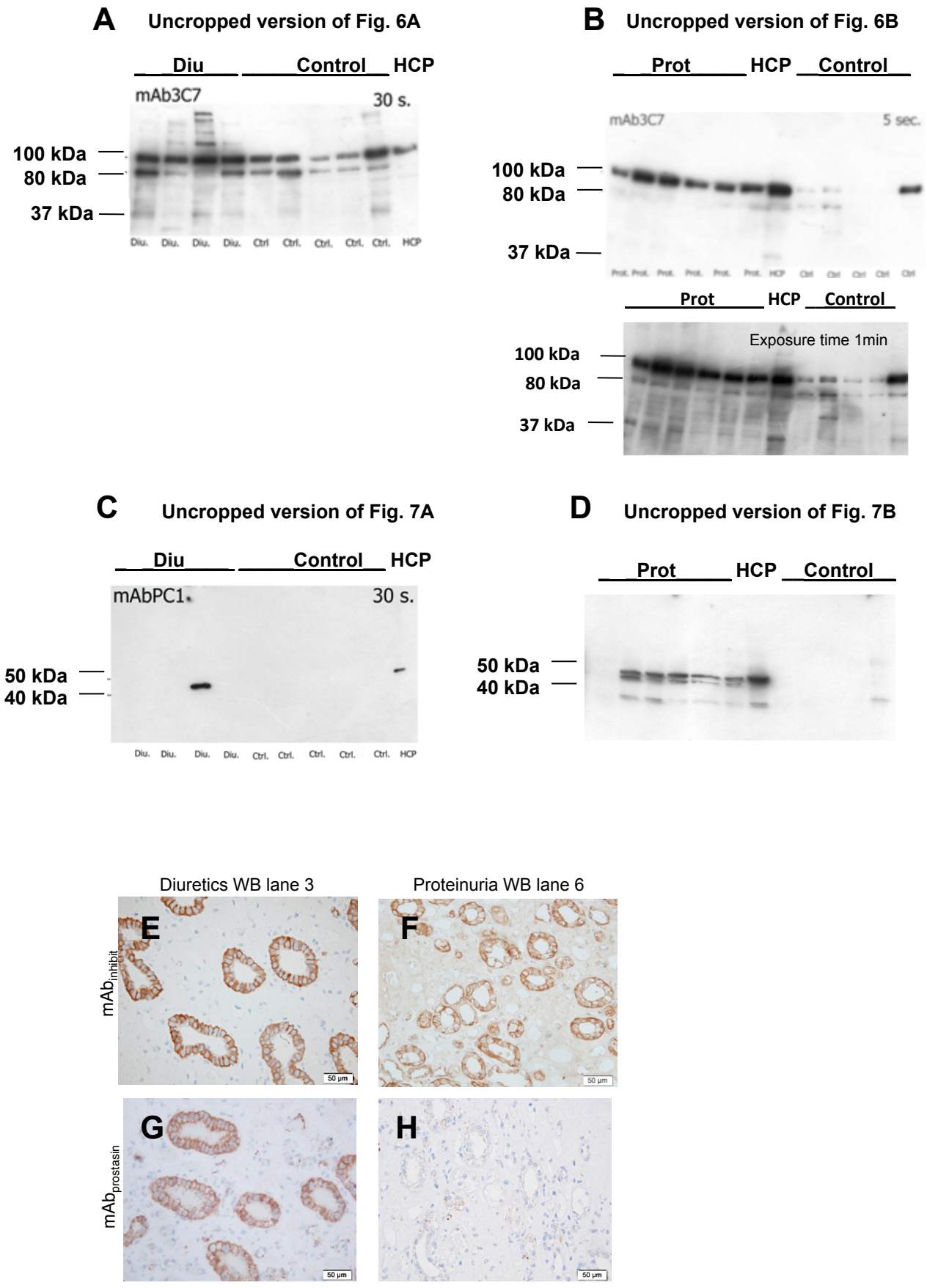

Figure S3. Western blot and IHC of kidney tissue from patients treated with diuretics and patients with proteinuria
(A and B) Uncropped version of the western blots shown in Figure 6A and B, respectively. Additionally, a longer exposure time (1 min) is shown for the western blot in Figure 6B. (C and D) Uncropped version of the western blots shown in Figure 7A and B, respectively. $mAb_{inhibit}$ staining of the medullary regions of kidney sections from (E) diuretics treated and (F) proteinuric patients. $mAb_{prostasin}$ staining of medullary regions of kidney sections from (G) diuretics treated and (H) proteinuric patients. Scale bar 50 μm.

Supplementary Table 1: Patient characteristics: Patients were divided into 5 groups based in their antihypertensive treatment (none, AT1 receptor antagonist, ACE inhibitors, and diuretics) and proteinuria. The antihypertensive medicine received by the individual patient is listed.

Patient number	Medication – Drug	Blood pressure (systolic/diastolic)	Age	Gender M /F	Pathological Diagnosis
		Control			
1. 172	None	139/69	41	F	Hydronephrosis
2. 165	None	136/91	45	M	Hydronephrosis
3. 152	None	155/83	72	M	Renal clear cell carcinoma
4. 229	None	153/98	71	F	Renal clear cell adenocarcinoma
5. 242	None	150/78	69	F	Renal clear cell carcinoma
		Angiotensin II AT1 receptor antagonists			
1.132	Losartan	125/81	60	M	Renal cyst
2. 136	Losartan	175/95	69	F	Urothelial carcinoma
3. 182	Losartan, Amlodipin	136/83	65	M	Acute and chronic inflammation, hydronephrosis
4.184	Eprosartan, Bendroflumethiazid, Lercanidipin	131/79	64	F	Chronic inflammation, fibrosis
5. 185	Losartan, Amlodipin	124/86	64	F	Renal clear cell carcinoma

6. 218	Losartan, Bendroflume Furosemid	146/81	67	F	Renal clear cell carcinoma
		ACE-inhibitors			
1. 126	Enalapril	128/77	68	M	Renal clear cell carcinoma
2. 173	Quinapril, Carvedilol, Furosemid, Tamsulosin	174/77	81	M	Oncocytoma
3. 186	Ramipril, amlodipine	139/83	70	M	Renal clear cell carcinoma
4. 194	Lisinopril	149/79	69	M	Renal clear cell carcinoma
5. 223	Enalapril	109/75	67	M	Renal clear cell carcinoma
6. 233	Enalapril , amlodipin	145/91	69	F	Urothelial carcinoma
		Diuretics only			
1. 95	Thiazide	190/90	66	F	Renal clear cell carcinoma
2. 130	Thiazide	140/90	56	F	Renal clear cell carcinoma
3. 227	Loop diuretic	116/60	63	F	Renal clear cell adenocarcinoma
4. 181	Thiazide, Doxazosin, Metoprolol, Amlodipin	115/56	80	M	Papillary renal cell carcinoma

Proteinuria

1. 270	Losartan	172/103, 2+	58	M	Papillary, urothelial carcinoma
2.286	Amlodipine	146/73, 3+	70	F	Renal clear cell carcinoma
3. 289	None	152/82, 2+	72	M	Fibrosis, Inflammation
4.294	Amlodipine, losartan, Furosemide	132/76, 2+	66	M	Renal clear cell carcinoma
5.301	Amlodipine	162/90, 3+	49	M	Papillary urothelial carcinoma
6. 299	Thiazide, Metoprolol	178/82, 2+	81	M	Renal clear cell carcinoma

Supplementary methods

Production and cloning of monoclonal antibodies

Thirty μg of each peptide coupled to diphtheria toxin and mixed with Freunds incomplete adjuvant was injected into BALB/c mice subcutaneously 3 times with a 14 days interval. Three days prior to the fusion the mice received an intravenous injection with 30 μg antigen administered with adrenalin. The fusion and selection were done as described by Kohler and Milstein.[1] Cells from the SP2/0-AG14 myeloma cell line (HGPRT negative) were used as a fusion partner and polyethylenglycol (PEG) as fusion media. Clones reacting with the immunogenic peptide corresponding to the cleaved γENaC, but not with the putative uncleaved form, *i.e.* peptide [CDF FTGRKRKVGGSIIHKA] for prostasin, were identified by ELISA. Cloning was performed by limited dilution. Single clones were grown in culture flasks in RPMI+10% FCS. The mAbs were subsequently purified from culture supernatant by protein A affinity chromatography using the Äkta FPLC system according to the manufacturer's instructions (Amersham Pharmacia, Uppsala, Sweden).

References

1. Kohler, G, Milstein, C: Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature,* 256**:** 495-497, 1975.