Supporting Information

Norgett et al. 10.1073/pnas.1204257109

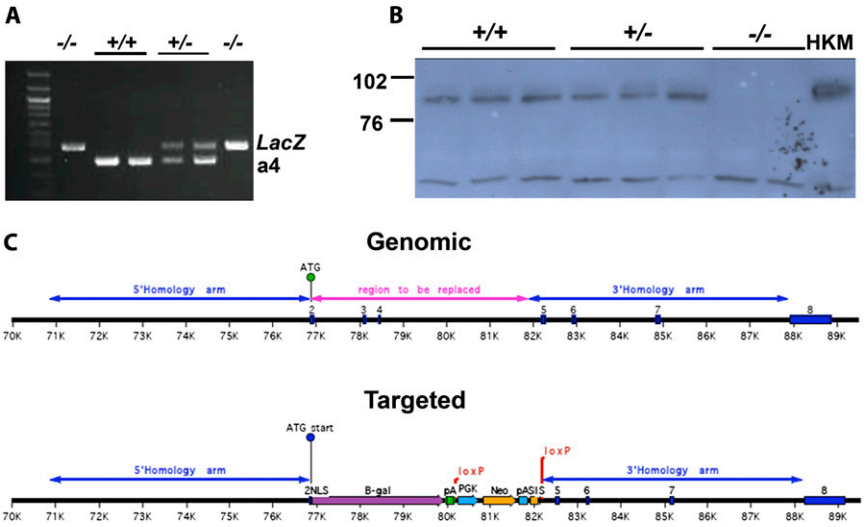

Fig. S1. Generation of the *Atp6v0a4*-null line. (*A*) Genotyping PCR showing homozygous WT a4 allele in +/+ animals, homozygous targeted *LacZ* allele in −/− animals, and both alleles in +/− animals. (*B*) Western blot of total kidney lysates showing a4 protein between the 76- and 102-kDa markers in +/+ and +/− but not −/− animals. HKM, human kidney membrane control. An internal loading control is provided by the previously described nonspecific band at around 50 kDa (1), present in all lanes. (*C*) Schematic of the *Atp6v0a4* locus and targeting construct.

1. Smith AN, et al. (2000) Mutations in *ATP6N1B*, encoding a new kidney vacuolar proton pump 116-kD subunit, cause recessive distal renal tubular acidosis with preserved hearing. *Nat Genet* 26:71–75.

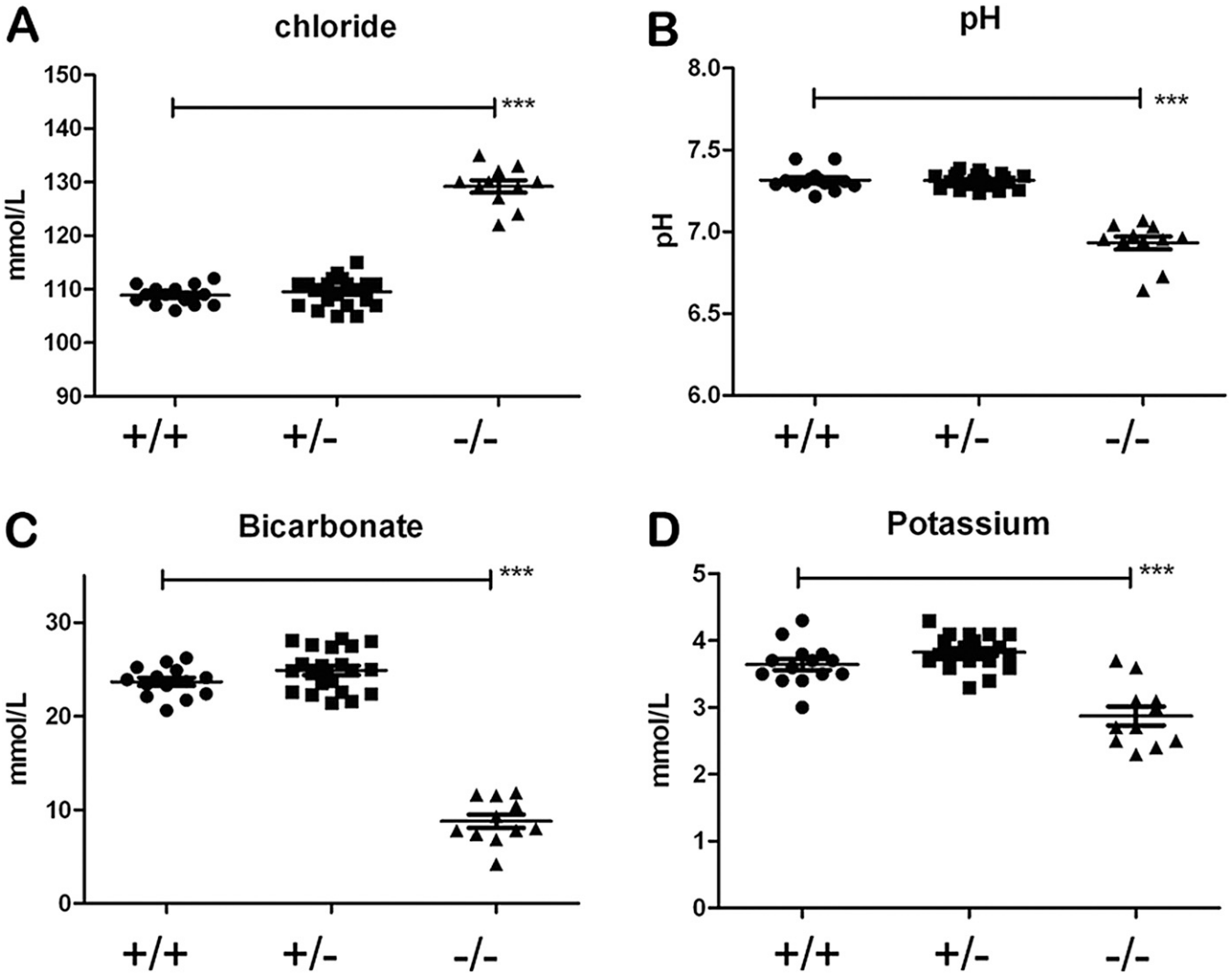

Fig. S2. Whole blood analysis from P21 untreated mice of all three genotypes. The –/– animals exhibited a hyperchloremic acidosis (*A*–*C*) with hypokalemia (*D*), in comparison with +/+ and +/– animals. All values are means ± SEM.

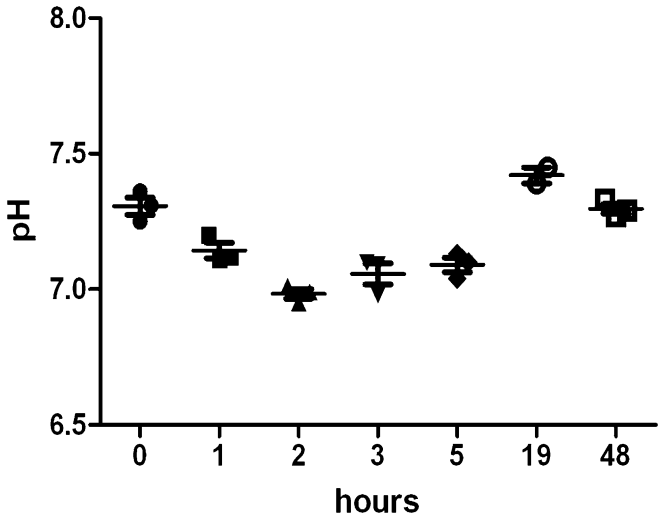

Fig. S3. To verify metabolic acidosis, whole-blood pH of *Atp6v0a4*$^{+/+}$ animals was measured in a time course after acid gavage. Maximal acidification was reached after 2 h.

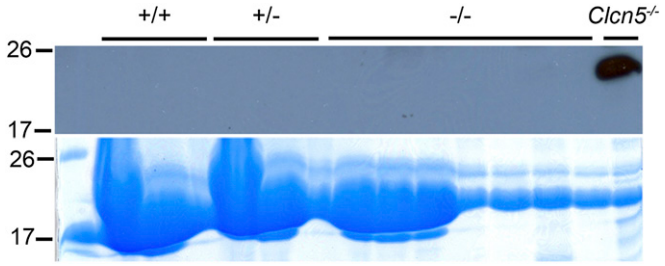

Fig. S4. Western blot analysis of RBP in 3-mo animals showed the typical band of 21 kDa in urine from *Clcn5*$^{-/-}$ mice (which have proximal tubulopathy) and no bands present in a 10-fold load of urine in any *Atp6v0a4* samples, regardless of genotype.

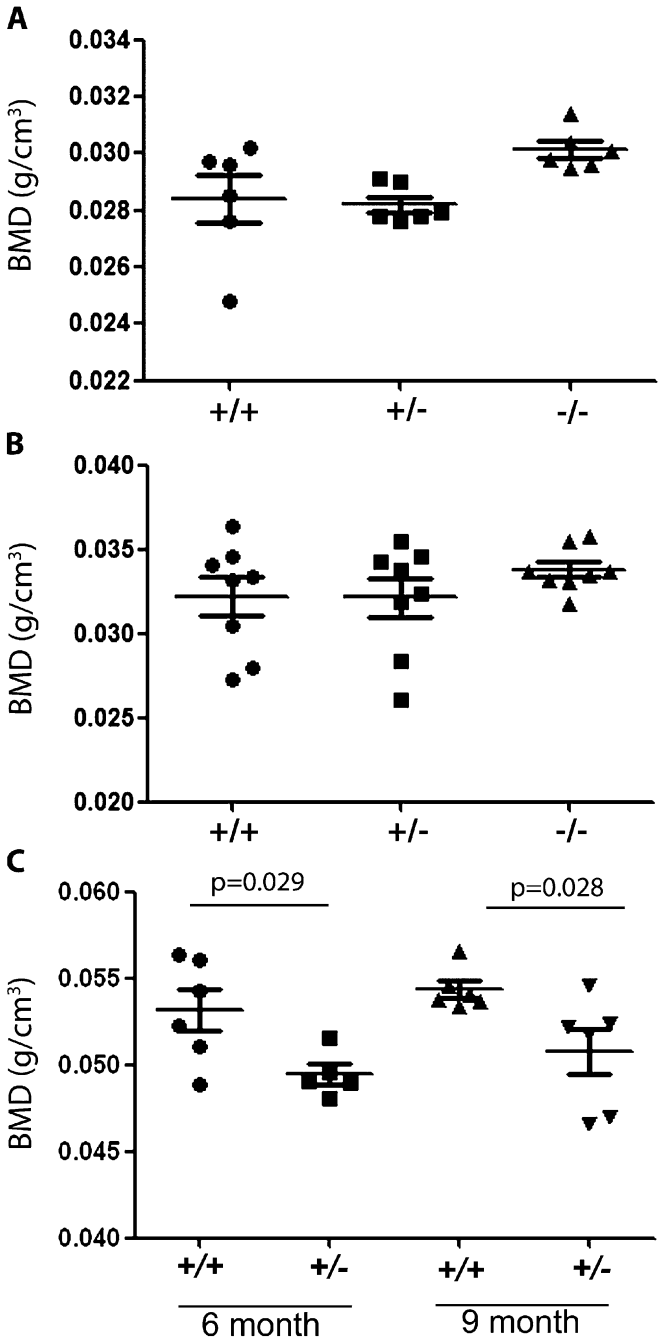

Fig. S5. BMD was the same in +/+, +/–, and –/– animals at 14 d (*A*) and 21 d (*B*). At 6 and 9 mo, BMD was reduced in chronically acidified +/– animals compared with +/+ (*C*).

Table S1. Urine biochemistry of 3-mo-old animals following chronic alkalinization

	Untreated*				Chronically alkalinized†			
	+/+ (n = 9)	+/− (n = 10)	−/− (n = 8)	P	+/+ (n = 6)	+/− (n = 5)	−/− (n = 7)	P
Sodium	0.4 ± 0.05	0.4 ± 0.04	0.5 ± 0.07	NS	1.4 ± 0.19	1.1 ± 0.08	1.7 ± 0.27	NS
Potassium	0.7 ± 0.08	0.9 ± 0.07	0.7 ± 0.09	NS	0.8 ± 0.05	0.7 ± 0.09	0.8 ± 0.13	NS
Chloride	0.7 ± 0.10	0.9 ± 0.05	0.8 ± 0.12	NS	1.0 ± 0.13	0.8 ± 0.14	1.1 ± 0.15	NS

Urine was collected from pairs of animals; the number of pairs (n) is indicated in parentheses. All values are means ± SEM. Units are mmol/24 h.
*−/− animals withdrawn from alkali treatment 7 d before acute treatment.
†−/− animals had received lifelong alkali treatment.

Table S2. Whole-blood biochemistry of 3-mo animals following acute alkalinization or acidification

	Acute-alkalinized				Acute-acidified			
	+/+ (n = 9)	+/− (n = 8)	−/− (n = 8)*	P	+/+ (n = 8)	+/− (n = 8)	−/− (n = 8)*	P
Sodium	145.1 ± 0.48	144.0 ± 0.46	145.9 ± 0.67	NS	144.5 ± 1.15	143.3 ± 0.37	147.5 ± 0.57	0.0346
Potassium	4.2 ± 0.19	4.1 ± 0.20	3.6 ± 0.09	0.0096	5.3 ± 0.17‡	4.9 ± 0.13	4.2 ± 0.16	0.0004
Chloride	109.4 ± 1.00†	108.8 ± 0.59	110.0 ± 0.65	NS	127.1 ± 1.38	125.4 ± 1.12	130.3 ± 0.84	NS
Urea	4.6 ± 0.24†	6.1 ± 0.41	7.7 ± 1.18	0.0229	10.3 ± 1.85	8.9 ± 0.72	12.2 ± 0.67	NS
Creatinine	28.9 ± 2.74	33.3 ± 2.25	35.1 ± 1.48	NS	24.1 ± 1.53‡	22.0 ± 0.71	27.3 ± 1.07	NS
pH	7.44 ± 0.02	7.43 ± 0.04	7.38 ± 0.02	NS	7.05 ± 0.04	7.07 ± 0.02	7.03 ± 0.04	NS
pCO_2	5.3 ± 0.39	5.6 ± 0.55	6.9 ± 0.22	0.0026	4.8 ± 0.47	5.2 ± 0.33	4.8 ± 0.40	NS
Bicarbonate	26.2 ± 1.20	27.3 ± 0.83	30.5 ± 0.74	0.0096	9.9 ± 1.08	11.4 ± 0.75	9.6 ± 0.71	NS
Osmolality	296.9 ± 0.90†	299.5 ± 0.93	301.9 ± 2.10	0.0459	303.8 ± 1.42	299.1 ± 0.97	311.3 ± 0.84	0.0005
BE	2.0 ± 1.20	3.1 ± 1.03	5.5 ± 1.02	0.0446	−20.9 ± 1.52	−18.8 ± 0.98	−21.1 ± 1.14	NS

All values are means ± SEM P values relate to the difference between +/+ groups and −/−. All units are mM, except creatinine (μM); pCO_2 (kPa); pH; and BE.
*−/− animals withdrawn from alkali treatment 7 d before acute treatment.
†n = 8.
‡n = 7.

Table S3. Urine biochemistry of 3-mo-old animals following chronic acidification

	+/+	+/−	P
Sodium	0.3 ± 0.05	0.4 ± 0.03	NS
Potassium	0.6 ± 0.07	0.8 ± 0.06	NS
Chloride	1.4 ± 0.22	1.7 ± 0.11	NS

Urine was collected from nine pairs of animals of each genotype. All values are means ± SEM. Units are mmol/24 h.

Table S4. Urine calcium:creatinine ratio before and after in vitro sample acidification

	Unacidified	Acidified
+/+ (n = 10)	0.7 ± 0.03	0.9 ± 0.05
+/− (n = 12)	0.8 ± 0.16	0.9 ± 0.17
−/− (n = 12)	0.3 ± 0.03	0.7 ± 0.08

All values are means ± SEM. Urine was collected from pairs of animals.