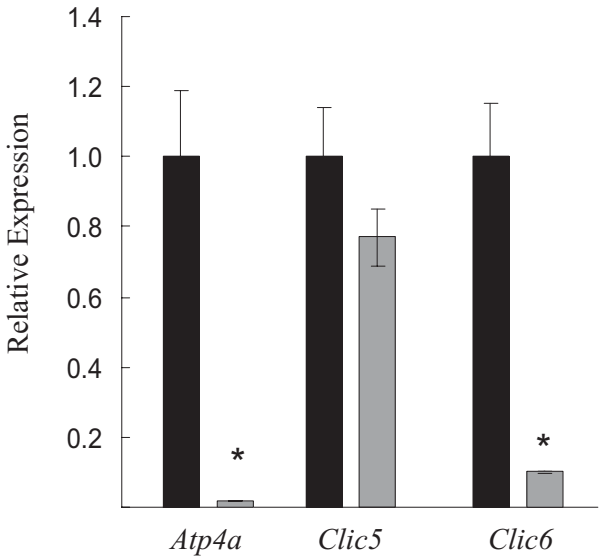
Relative Expression
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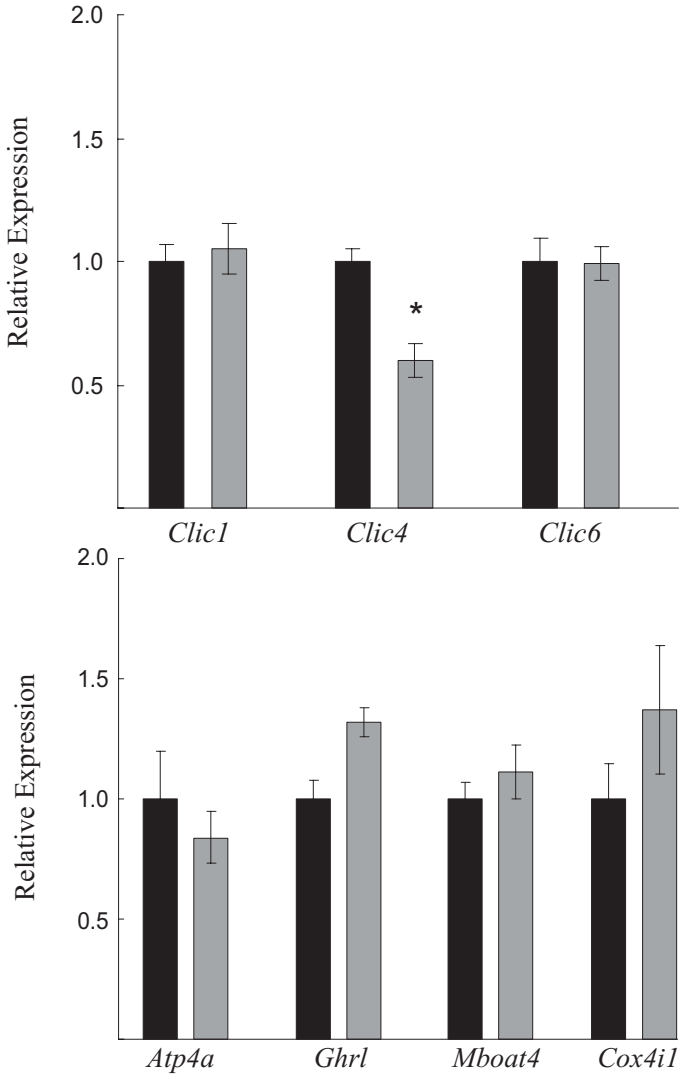
1.2
1.0
0.8
0.6
0.4
0.2
*
*
Atp4a
Clic5
Clic6


2.0
1.5
1.0
0.5
Relative Expression
*
Clic1
Clic4
Clic6
2.0
1.5
1.0
0.5
Relative Expression
Atp4a
Ghrl
Mboat4
Cox4i1

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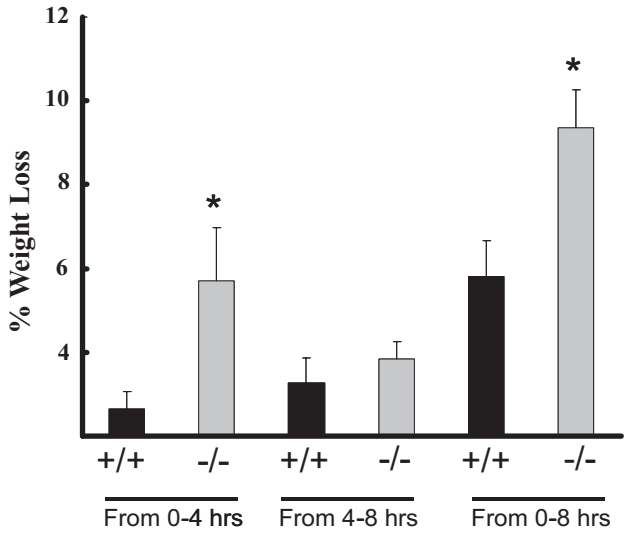
Supp Fig 3



Supplementary Fig. 1. Real-time PCR analysis of gene expression in stomachs of wild-type and *Nhe4*-null mice. Quantitative PCR analysis (see Methods) of *Clic1*, *Clic4*, *Clic5*, *Clic6*, and *Atp4a* (gastric H^+-K^+ATPase alpha subunit) mRNA levels revealed that only *Clic6* and *Atp4a* were down-regulated in stomachs of *Nhe4*-null mice (n = 3, grey bars) relative to that of wild-type mice (n = 3, black bars). Microarray data (see Supplementary Table 2) indicated that expression of *Clic5* was down-regulated 76-fold; however, verification using real-time PCR revealed that *Clic5* was down-regulated only 1.3-fold (wild-type = 1.0 ± 0.1, *Nhe4*-null = 0.77 ± 0.08), which was not statistically significant ($p = 0.2$). The expression levels of each gene were normalized to the expression of mRNA for the L32 ribosomal subunit. The level of expression in wild-type mice was set to 1.0, and expression levels for *Nhe4*-null mice were normalized to wild-type values. * $p < 0.05$ using a Student's *t*-test.

Supplementary Fig. 2. Real-time PCR analysis of gene expression in stomachs of wild-type and *Clic5* mutant mice. Quantitative PCR analysis was performed as described in Methods. The upper panel shows mRNA expression for *Clic* isoforms 1, 4, and 6, normalized to mRNA for the L32 ribosomal subunit. *Clic4* was significantly downregulated. The bottom panel shows mRNA expression for *Atp4a* (gastric H^+-K^+ATPase alpha subunit), *Ghrl* (ghrelin), *Mboat4* (membrane bound O-acyltransferase domain containing 4; also known as GOAT or ghrelin O-acyltransferase), and *Cox4i1* (cytochrome c oxidase subunit IV isoform 1). * $p < 0.05$.

Supplementary Fig. 3. *Clic5* mutant mice lose weight more rapidly than wild-type mice during fasting. When wild-type (+/+, black bars) and *Clic5* mutant mice (-/-, grey bars) were fasted for 8 hours, the *Clic5* mutant mice lost a greater percentage of their starting body weight than wild-

type mice, with the greatest differences occurring during the first 4 hours of fasting. During fasting, mice were individually housed in cages on wire racks and had free access to water. Differences were statistically significant (*$p < 0.05$) during the first 4 hours (0-4 hrs) and during the entire 8 hour period (0-8 hrs). n = 7 adult mice of each genotype.

Supplementary Table 1. Primers used for real-time PCR expression analysis

Gene	Primer Sequence
Atp4a 5'	TGTACACATGAGAGTCCCCTTG
Atp4a 3'	GAGTCTTCTCGTTTTCCACACC
Clic1 5'	AAGAACAACCTCAGGTC
Clic1 3'	TCTGAGAGAATGCCCTCATCTTC
Clic4 5'	CTGAAGGAGGAGGACAAAGAG
Clic4 3'	GCGCTTCATTAGCCTCTGGTC
Clic5 5'	GCAAAACACCGGGAATCTAA
Clic5 3'	AGGCAATGGGGAAGAAGAGT
Clic6 5'	GGATGCAAATGAGATTTATGAAAAG
Clic6 3'	CGACAATCTTAATGATATGGAGC
Ghrl 5'	CCATCTGCAGTTTGCTGCTA
Ghrl 3'	GCAGTTTAGCTGGTGGCTTC
Mboat4 5'	GTGAGTGCTGGAGCTGGACTG
Mboat4 3'	TGAGCCACAGAGCTGTGCTTC
Cox4i1 5'	AGTGTTGTGAAGAGTGAAGAC
Cox4i1 3'	GCGGTACAACTGAACTTTCTC
L32 5'	GATCTAGCGGCCGCCATCTGTTTTACGGCATCATG
L32 3'	TAGATCGCGGCCGCCGCTCCCATAACCGATGTTGG

Forward (5') and reverse (3') primer sequences for each of the mRNAs analyzed in Supplementary Figs. 1 and 2 are shown.

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Supplementary Table 2

Supplementary Table 2. Ion transport protein mRNAs down-regulated in stomachs of mice lacking the NHE4 Na^+/H^+ exchanger

Symbol	Name	-WT/KO
Clic5	CLIC5 chloride intracellular channel	-75.7
Atp4a	gastric H^+,K^+-ATPase, alpha subunit	-13.5
Kcnq2	KCNQ2 voltage-gated potassium channel	-9.97
Trpv6	transient receptor potential cation channel 6	-8.65
Atp4b	gastric H^+,K^+-ATPase, beta subunit	-7.43
Kcnj16	KCNJ16 inward rectifying potassium channel	-6.61
Asna1	arsenite transporter, ATP-binding, homolog 1	-6.26
Aqp4	aquaporin 4	-4.91
Clic6	CLIC6 chloride intracellular channel	-4.67
Kcne2	KCNE2 potassium channel	-4.24
Slc4a2	AE2 chloride bicarbonate exchanger	-2.71
Kcne2	KCNE2 voltage-gated potassium channel	-2.41
Aqp11	aquaporin 11	-2.15
Kcnj15	KCNJ15 inwardly-rectifying potassium channel	-2.01
Clca4	CLCA4 calcium activated chloride channel	-1.54

Microarray analyses were performed to compare the gene expression profiles in whole stomach from wild-type (WT) and NHE4-null (KO) mice (see Methods), which have few parietal cells. The ion transport proteins shown were identified as down-regulated in NHE4-null stomachs, consistent with their expression in parietal cells. Expression data are presented as the fold-changes (ratio of fluorescence intensity values), with negative values indicating down-regulation in the NHE4-null samples. All of the expression changes shown attained a *p*-value of less than 5×10^{-5}.

Supplementary Table 3. Analysis of blood from wild-type and *Clic5* mutant mice

	WT	KO
platelets (10^3/μl)	911	1149
red blood cells (10^6/μl)	8.6	9.2
hemoglobin (g/dl)	13.9	14.5
hematocrit (%)	49	50
mean corpuscular volume (fl)	57	54
mean corpuscular hemoglobin (pg)	16.2	15.8
mean corpuscular hemoglobin concentration (g/dl)	29	29
prothrombin time (sec)	11.1	10.6
activated partial thromboplastin time (sec)	27.4	28.3
Fibrinogen (mg/dl)	193	298
D-dimer (ng/ml)	250	250

Blood was pooled from 3 wild-type (WT) and from 3 *Clic5* mutant (KO) mice and analyzed. Note that the small increase in platelet numbers and fibrinogen in the *Clic5* mutant mouse are inconsistent with a bleeding disorder. Normal numbers of red blood cells, hemoglobin, hematocrit, and mean corpuscular volume suggest that hydration, iron levels and erythropoiesis are normal. Normal values for the prothrombin time and activated partial thromboplastin time suggest that both the intrinsic and extrinsic clotting pathways are intact. Levels of D-dimer, a fibrin degradation product used to evaluate the presence of disseminated intravascular coagulation, were normal, thus indicating an absence of chronic, low level bleeding in peripheral tissues. Since the values for pooled samples were within the normal range and showed no evidence of a coagulation defect, no additional samples were analyzed.