

# Supplementary Table 1

## qPCR primer list

Target	PrimerBank ID	Amplicon Size	Sequence (5' -> 3')	Length	Tm (°C)	Location
Mouse $\beta$ Actin	145966868b1	245	F: GTGACGTTGACATCCGTAAAGA	22	60.3	854-875
			R: GCCGGACTCATCGTACTCC	19	61.6	1098-1080
Mouse Cyclophilin	13385854a1	187	F: AACCCGCGAGTCTTCTTTGAC	21	62.6	43-63
			R: TAATTCGGTGGAAAGGGCATC	21	60.1	229-209
Mouse GAPDH	126012538b1	95	F: AGGTCGGTGTGAACGGATTTG	21	62.6	8-28
			R: GGGGTCGTTGATGGCAACA	19	62.6	102-84
Mouse CLCN2	164698431b2	130	F: CCAGATGTCGCATTTGTTCTGT	22	61.1	233-254
			R: GCGATGGCATAGTCCATAGCC	21	62.7	362-342
Mouse $\alpha$ ENaC	33859618a1	206	F: GCTCAAOCCTTGACCTAGACCT	21	60.9	27-47
			R: GGTGGAACCTCGATCAGTGCC	20	62.6	232-213
Mouse $\beta$ ENaC	6755412a1	125	F: GGCCCAGGCTACACCTACA	19	63	49-67
			R: AGCAGCGTAAGCAGGAACC	19	62.3	173-155
Mouse $\gamma$ ENaC	6755414a1	118	F: GCACCGACCATTAAGGACCTG	21	62.7	64-84
			R: GCGTGAACGCAATCCACAAC	20	62.8	181-162

Primer sequences for SYBR Green qPCR analysis of the seven target genes were obtained from Primer Bank (<http://pga.mgh.harvard.edu/primerbank>)

## Supplementary Table 2

Fold target gene expression relative to housekeeping gene

	Wildtype	<i>Clcn2</i> <sup>-/-</sup>
$\alpha$ ENaC/ $\beta$ Actin	$7.7 \times 10^{-4} \pm 1.7 \times 10^{-6}$	$9.1 \times 10^{-4} \pm 1.8 \times 10^{-6}$
$\alpha$ ENaC/GAPDH	$6.1 \times 10^{-4} \pm 1.0 \times 10^{-6}$	$1.19 \times 10^{-3} \pm 2.8 \times 10^{-6}$
$\alpha$ ENaC/Cyclophilin	$1.0 \times 10^{-2} \pm 5.6 \times 10^{-4}$	$6.1 \times 10^{-3} \pm 5.0 \times 10^{-5}$
$\beta$ ENaC/ $\beta$ Actin	$6.3 \times 10^{-2} \pm 8.5 \times 10^{-3}$	$4.6 \times 10^{-2} \pm 4.7 \times 10^{-3}$
$\beta$ ENaC/GAPDH	$5.0 \times 10^{-2} \pm 5.0 \times 10^{-3}$	$6.1 \times 10^{-2} \pm 7.5 \times 10^{-3}$
$\beta$ ENaC/Cyclophilin	$8.5 \times 10^{-1} \pm 3.1 \times 10^1$	$3.1 \times 10^{-1} \pm 1.4 \times 10^{-1}$
$\gamma$ ENaC/ $\beta$ Actin	$6.1 \times 10^{-2} \pm 7.9 \times 10^{-3}$	$4.8 \times 10^{-2} \pm 5.0 \times 10^{-3}$
$\gamma$ ENaC/GAPDH	$4.8 \times 10^{-2} \pm 4.7 \times 10^{-3}$	$6.4 \times 10^{-2} \pm 8.0 \times 10^{-3}$
$\gamma$ ENaC/Cyclophilin	$8.2 \times 10^{-1} \pm 2.9 \times 10^1$	$3.2 \times 10^{-1} \pm 1.4 \times 10^{-1}$

Relative expression levels of  $\alpha$ -ENaC,  $\beta$ -ENaC, and  $\gamma$ -ENaC genes were successfully determined by SYBR Green qPCR in colonic cDNA samples from wildtype and *Clcn2*<sup>-/-</sup> mice. Uniform expression profiles between all samples were seen for  $\beta$ -actin and GAPDH, and somewhat less so for cyclophilin. Data are given as average  $\pm$  S.D. from three independent experiments.

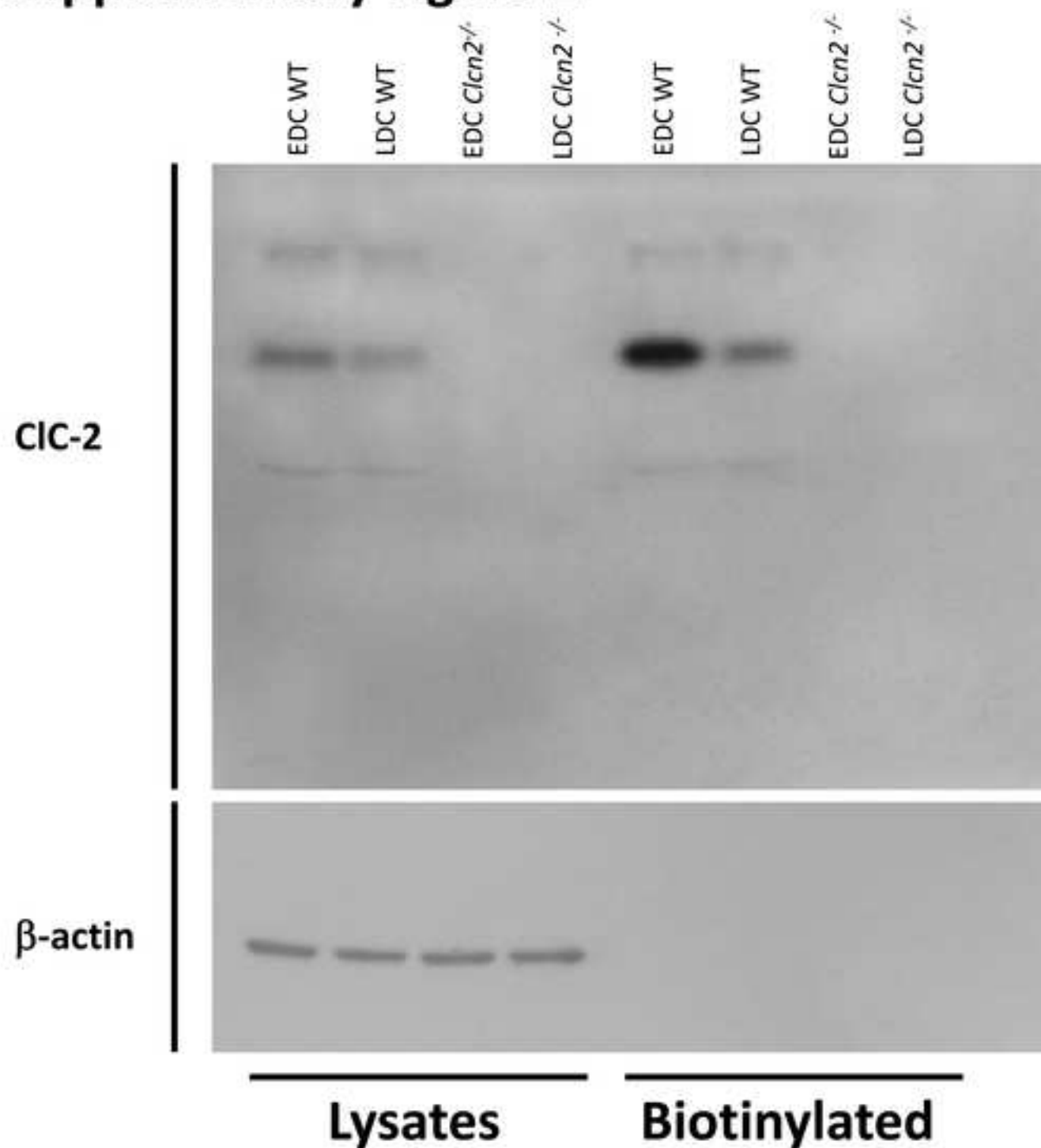
# Supplementary Table 3

Comparison of relative *Clcn2* expression to housekeeping gene between EDC and LDC

	EDC	LDC
<i>Clcn2</i> /β Actin	$3.2 \cdot 10^{-2} \pm 1.6 \cdot 10^{-3}$	$3.1 \cdot 10^{-2} \pm 1.5 \cdot 10^{-3}$
<i>Clcn2</i> /GAPDH	$2.6 \cdot 10^{-2} \pm 6.8 \cdot 10^{-4}$	$2.3 \cdot 10^{-2} \pm 6.8 \cdot 10^{-4}$
<i>Clcn2</i> /Cyclophilin	$6.1 \cdot 10^{-1} \pm 6.5 \cdot 10^{-1}$	$2.2 \cdot 10^{-1} \pm 6.3 \cdot 10^{-2}$

Relative expression levels of *Clcn2* gene was successfully determined by SYBR Green qPCR in colonic cDNA samples from wildtype EDC and LDC, respectively. Uniform expression profiles between all samples were seen for β-actin and GAPDH, and somewhat less so for cyclophilin. Data are given as average ± S.D. from three independent experiments.

## Supplementary Figure 1



The membrane blotted for CIC-2 expression that is showed in Figure 5, was incubated in stripping buffer (100 mM 2-Mercaptoethanol, 2% SDS, 62.5 mM Tris-HCl pH 6.7) for 30 min at 50°C. After washing with TBS containing 0.1% Tween-20 (TBST), the membrane was incubated overnight at 4°C with  $\beta$ -actin (residues 2-15; Abcam Inc.) at a dilution of 1:2,500 in TBS/2.5% non-fat dry milk solution.