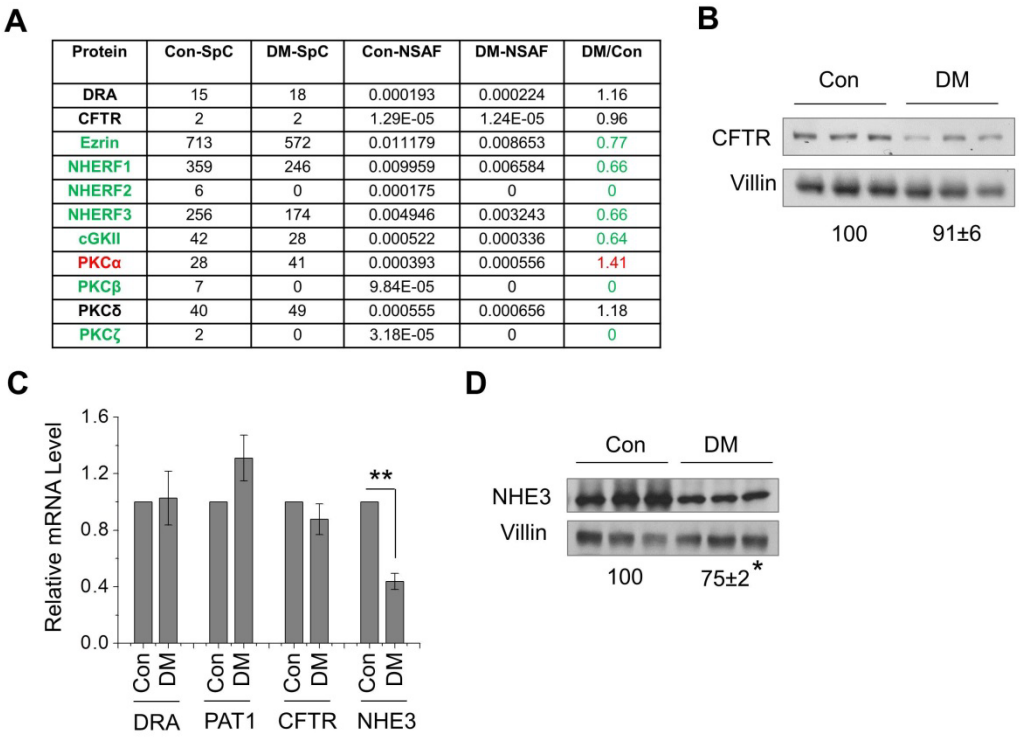
Supplemental Information

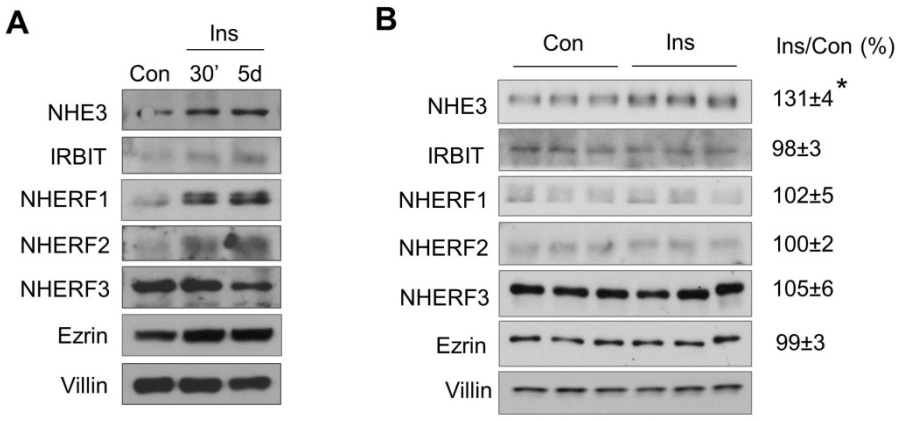
A

Protein	Con-SpC	DM-SpC	Con-NSAF	DM-NSAF	DM/Con
DRA	15	18	0.000193	0.000224	1.16
CFTR	2	2	1.29E-05	1.24E-05	0.96
Ezrin	713	572	0.011179	0.008653	0.77
NHERF1	359	246	0.009959	0.006584	0.66
NHERF2	6	0	0.000175	0	0
NHERF3	256	174	0.004946	0.003243	0.66
cGKII	42	28	0.000522	0.000336	0.64
PKCα	28	41	0.000393	0.000556	1.41
PKCβ	7	0	9.84E-05	0	0
PKCδ	40	49	0.000555	0.000656	1.18
PKCζ	2	0	3.18E-05	0	0



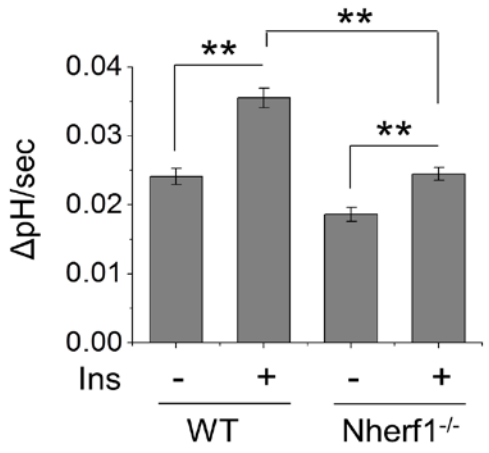
Supplemental Figure 1

NHE3 expression is down-regulated in ileum of STZ-induced diabetic mice. (A) Purified BBM from control (Con) and diabetic (DM) mice were subjected to mass spectrometry analysis. Number indicates relative abundance of listed proteins. Green highlights proteins with a decrease while red denotes an increase in DM. SpC, spectral counts; NSAF, normalized spectral abundance factor. (**B**) The BBM expression of CFTR was determined by Western blot in WT and DM mice, with villin as an internal control. n =6. (**C**) The relative expression of DRA, PAT1, CFTR and NHE3 mRNA in the ileum of control and DM mice was examined by qPCR, with β-actin as an internal control. n = 5. ** $P < 0.01$. Error bars indicate mean ± SEM. (**D**) Total NHE3 protein expression was examined by Western blot using villin as an internal control. n =6. * $P < 0.05$. Statistical analysis was performed using 2-tailed Student's *t* test.



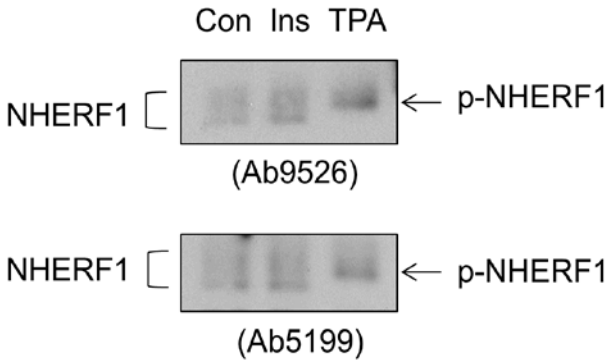
Supplemental Figure 2

Total and membrane expression of NHE3 is enhanced by insulin in diabetic mouse intestine. (**A**) Diabetic mice were treated with or without insulin (Ins) for 30 min (30') or 5 consecutive days (5d). n = 4 per group. The expression of NHE3 and its binding proteins in BBM fraction was examined by Western blot using villin as an internal control. (**B**) The expression levels of NHE3 and NHE3-scaffold proteins (mean ± SEM) were determined in diabetic mice with insulin for 5 days. n = 6. * $P < 0.05$ by 2-tailed Student's *t* test.



Supplemental Figure 3

Loss of NHERF1 attenuates but does not abolish NHE3 activation by chronic insulin treatment. WT and *Nherf1*$^{-/-}$ mice were treated with (+) or without (-) insulin for 5 consecutive days. NHE3 activity was determined in isolated villi and is presented as the rate of Na^+-dependent pH change at pH_i 6.6. n = 4. ** $P < 0.01$ by 2-tailed Student's *t* test. Error bars indicate mean ± SEM.



Supplemental Figure 4

Insulin does not increase NHERF1 phosphorylation. Serum starved Caco-2bbe/NHE3V/HA-IRBIT cells were treated with insulin (100 nM) or phorbol-12-myristate-13-acetate (TPA;100 nM) for 15 min. Changes in the electrophoretic mobility of NHERF1 protein was determined by immunoblotting using two different antibodies against NHERF1, Ab9526 (upper) and Ab5199 (lower). Both antibodies depicted the mobility shift induced by TPA, but no difference was observed in insulin treated cells. Arrow denotes phosphorylated forms of NHERF1, which migrate slower. Representative blots from three independent experiments are shown.

Table S1. Blood glucose and body weight changes by STZ treatment

Group	Blood glucose (mg/dl)	Body Weight (g)
Control	115±2	40.1±0.3
Diabetic	380±4 **	29.8±0.4 **

Male CF-1 mice were treated with (diabetic) or without (control) streptozotocin (STZ) for 5 consecutive days. Blood glucose and body weight shown are the levels measured 3 months after the completion of STZ treatment. n = 20 per group. Values are mean ± SE. ** $P < 0.01$ compared with untreated control. Note that diabetic mice with a fasting blood glucose level between 300-400 mg/dl were chosen for studies. Statistical analysis was performed using 2-tailed Student's *t* test.

Table S2. Human ileal biopsies from type 1 diabetic patients and controls.

Biopsy #	Sex	Age	Years Since Diagnosis
Control 1	M	47	N/A
Control 2	F	23	N/A
Diabetic 1	M	32	19
Diabetic 2	M	36	26
Diabetic 3	F	30	20

Table S3. Blood glucose levels in control and diabetic mice before and after insulin treatment.

	Control				Diabetic			
Insulin treatment	30 min		5 day		30 min		5 day	
	Before	After	Before	After	Before	After	Before	After
Blood glucose (mg/dl)	111±4	110±3	109±4	107±2	386±3	280±8 **	389±4	248±5 **

Blood glucose levels of control and diabetic mice before and after insulin (5 U/kg body weight) treatment are shown. n = 5 per group. Values are mean ± SE. ** $P < 0.01$, compared with the same group of mice before insulin treatment. Statistical analysis was performed using 2-tailed Student's *t* test.

Supplemental Methods

***BBM proteomic analysis*.** BBMVs were likewise purified as the above described from three control and diabetic mice. At the last step of purification, three samples of each control and diabetes were pooled together. Protein expression profile of the control and diabetic samples was analyzed through Mass Spectrometry by MS Bioworks (Ann Arbor, MI). Briefly, samples were solubilized in 1% SDS, 50 mM Tris-HCl, pH 8.0. Protein quantitation was performed using Qubit fluorimetry (Life Technologies) and 15 µg of each sample was separated ~1.5 cm on a 10% Bis-Tris Novex mini-gel (Life Technologies) using the MES buffer system. The gel was stained with coomassie and each lane was excised into ten equally sized segments. Each gel segment was reduced using dithiothreitol, alkylated with iodoacetamide and then subjected to in-gel digestion with trypsin for 4h (Promega, Madison, WI) using ProGest digestion station (Digilab, Inc., Marlborough, MA). Peptides were acidified to 0.1% formic acid and analyzed directly by nano LC/MS/MS with an EasynLC 1000 HPLC system interfaced to a Q Exactive tandem mass spectrometer (ThermoFisher, San Jose, CA) equipped with the Easy Spray source. Data were searched against the combined forward and reverse Swissprot Mouse protein database using a locally stored copy of the Mascot search engine v2.4.1 (Matrix Science, London, U.K.) via Mascot Daemon v2.4. Peak lists were generated using the Proteome Discoverer (ThermoFisher). The database was appended with common background proteins. The criteria for accepting a protein identification were determined by calculating the false discovery rates (FDR) from the concatenated forward/reverse database. A minimum of two unique peptides per protein were required. The number of spectral counts (SpC) was output for each protein, and then converted to spectral abundance factors (SAF) by dividing by the protein MW in kDa and subsequently normalized to the sum of all SAF per sample to give the normalized spectral abundance factor (NSAF). NSAF values were used to approximate relative abundance of proteins within a given sample, and relative abundance between the control and diabetic samples.

***Quantitative RT-PCR*.** Total RNA was isolated from ileal mucosa scrapes using RNA Extraction Kit (Qiagen), and cDNA was synthesized using SuperScript III First-Strand Synthesis Kit (Life Technologies). Quantitative RT-PCR was performed as previously described using iQ SYGR Green Supermix (Bio-Rad) on the Eppendorf Mastercycler realplex. The primers are as follows: DRA: 5'- aatgctgatgcagtttgctg -3' (forward) and 5'- tgctccttccaacattagcc -3' (reverse); PAT1: 5'- tccatagcctcatcctggac -3' (forward) and 5'- cagaggcaaagacatgctgc -3' (reverse); CFTR: 5'- agagcagtttcctggacagc -3' (forward) and 5'- ccagcgaaggcttgttttag -3' (reverse); NHE3: 5'- cacagaagcggaggaatagc -3' (forward) and 5'- tatcaattcctgccccagag -3' (reverse); and β-actin: 5'- tacagcttcaccaccacagc -3' (forward) and 5'- aaggaaggctggaaaagagc -3' (reverse)