

Supp Figure S1. Deletion of slc26a6 upregulates intestinal Na⁺-dependent dicarboxylate uptake. The figure shows succinate uptake by intestinal epithelium obtained from wild-type and slc26a6⁻ /- mice incubated either in the absence or presence of 140 mM Na⁺. Total uptake (a) was used to calculate the Na⁺-dependent uptake (b).



Supp. Fig. S2. NaDC-1 does not transport oxalate and slc26a6 does not transport succinate. Panel (a) shows the current recorded in oocytes expressing NaDC-1 and panel (b) shows the current recorded in oocytes expressing slc26a6 exposed to 1mM succinate and 1mM oxalate, as indicated by the bars.



Supp. Fig. S3. SIc26a2 does not inhibit NaDC-1. Panel (a) shows example traces and panel (b) is the mean±S.E.M of the NaDC-1 current measured in the presence of absence of SIc26a2 and sIc26a6, as indicated. In comparable conditions sIc26a6 inhibits while SIc26a2 on NaDC-1 activity.



Supp. Fig. S4. Reciprocal activation of human NaDC-1 and slc26a6 activity and expression and Co-IP of slc26a6 with human NaDC-1. Panel (a) shows activation of slc26a6 by hNaDC-1 and panel (b) shows inhibition of hNaDC-1 activity by slc26a6. Results are shown mean±S.E.M of the indicated number of experiments. Panel (c) shows a western blot analysis of expression of slc26a6(mKate) and hNaDC-1(His-myc) in Xenopus oocytes. Panel (d) shows Co-IP of slc26a6(mKate) and hNaDC-1(His-myc) expressed in HEK cells.

hNaDC	MATCWQALWAY <mark>RSYLIVFFVPILLLPLPILVP</mark> SKEAYCAYAIIIMALFWCTEALPLAVTA	60		
RabNaD	MATCWQGLWAYRMYLLVFLLPISLLPLPILVPRKEAYCAYAIIIMALFWCTDALPLAVTA	60		
opNaDC	MVNLWRLLWAYRKYLMSILFPILLLPLPLLVPTKEAKCAYSIILMALLWCTETLPLAVTA	60		
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hNaDC	LFPLILFPMMGIVDASEVAVEYLKDSNLLFFGGLLVAIAVEHWNLHKRIALRVLL <mark>IVG</mark> VR	120		
RabNaD	LLPLCLFPMMGIMEASEVGLEYLKDTNVLFIGGLLLAIAVEHWNLHKRIALRVLLLTGYR	120		
opNaDC	FLPIIFFPMMGIMDASEVSIEYLKDTNILFIGGLLVAIAVEHWNLHKRIALRVLLII <mark>G</mark>			
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hNaDC	PAPLILGFMLVTAFLSMWISNTATSAMMVPIAHAVLDQLHSSQASSNVEEGSNNPTFELQ			
RabNaD	PALLILGFMVVTAFLSMWISNTASTAMMVPIAHAVLQELNNTQSNVEEGSDNPTFELQ			
opNaDC	PAFLILGFMVVTAFLSMWISNTATTAMMVPIAHAVLEQLHKGPEEKDTEMGHVNISFELQ			
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hNaDC	EPSPQKEVTKLDNGQALPVTSASSEGRAHLSQK <mark>HLHLTQCMSLCVC</mark>	226		
RabNaD	EPSPQKETSKVDEKDNGQAQPLPAVPLESGEHMTQEQLRFSQGMSLCVC	227		
opNaDC	EPHGNPKKEPPSLREKENSPVPTSMPPEYKEKEEEEKEENKEEREKEHFKLSQGMSLCVC	240		
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hNaDC	YSASIGGIATLTGTAPNLVLQGQINSLFPQNGNVVNFASWFSFAF <mark>PTMVILLLLAWLWLQ</mark>	286		
RabNaD	YSASIGGIATLTGTTPNLVLQGQMTSLFPQNPNVVNFASWFGFAFPIMVILLLLSWLWLQ	287		
opNaDC	YAASIGGIATLTGTTPNLVLQGQMNSLFPKNPSVVNFASWFGFAFPTMVLLLLLSWIWLQ	300		
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hNaDC	ILFLGFNFRKNNFGIGEKMQEQQQAAYCVIQTEHRLLGPMTFA <mark>E</mark> KAISILFVILVLLWF	344		
RabNaD	ILFLGINFRKNFGIREQEHEQQRKQAAYRVIQTQYRLLGPMSFAEKAVFILFVILVLLWF	347		
opNaDC	ILFLGFNFRKNFNCGRKSQEKERAAYQVIQTEHKKLGPMSFAEIAVTFLFFLLVVLWF	358		
	*****:********			
		404		
	TREPGFFLGWGNLAFPNARGESMVSDGTVAFFLGLIMFILPSKFPGLTODPENPGRLKAP	404		
RADNAD opNoDC				
ормарс		41/		
hNaDC	LCLLDWKTWNOKMDWNTVLLLCCCVALAKCSEPSCLSEWLCNKLTDLOSVDA DATATIS	464		
RahNaD	DALL NUKLYNKKMPUNTYLLIGGGYALAKGSERSGLSOWI, GNKLMPI, OHVDPDATVETTG	467		
nNaDC	DALLOWNTVNKKMDWNTLFLLCCCFALAKCSFVSCLSWUCNKLTPLOSTDSDATAFTLC	477		
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hNaDC	LLVATFTECTSNVATTTIFLPILASMAOAICLHPLYVMLPCTLATSLAFMLPVATPPNAI	524		
RabNaD	LLVATFTECTSNAATTTLLLPILASMAOAICLHPLYVMLPCTLASSLAFMLPVATPPNAI	527		
opNaDC	LLVATFTECASNVATTTLFLPILASMAOAICLNPLYVMLPCTLSASLAFMLPVATPPNAI	537		
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hNaDC	VFSFGDLKVLDMARAGFLLNIIGVLIIALAINSWGIPLFSLHSFPSWAQSNTTAOCLPSL	584		
RabNaD	VFSFGGLRVSDMARAGIMLNIIGVLVIMLAINSWGVPMFQLHTFPSWAHSNSTTHCLASP	587		
opNaDC	VFSYGOLKVIDMAKTGFLLNIIGVLTITLAINTWSYPIFOLDOFPTWAOINSTSCOVNGO 5			
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hNaDC	ANTTTPSP 592			
RahNaD	PTAPSP 593			

Supp. Fig. S5. Multiple sequence alignment and secondary structure of NaDC-1. The secondary structure of rabbit NaDC-1 was used to map the topology of human and opossum NaDC-1. The alignment was generated by T-coffee program. Relevant domains are indicated, including the site where stop codons were inserted (light green).

ſ	TMD
	in->out TMD out->in TMD
	Stop codons
	Deletion

GNTNITSP 605

opNaDC



b) Intestine slice

a) Kidney slice

Supp. Fig. S6. Images of kidney and intestinal jejunum epithelium pieces used for measurement of Succinate uptake. The kidneys were placed in an agarose gel (insert) and 1mm slices were cut. The cortex was isolated by cutting out the medulla and the cortical slices were used to measure succinate uptake. The size of kidney and intestinal slices was determined from the images as shown.