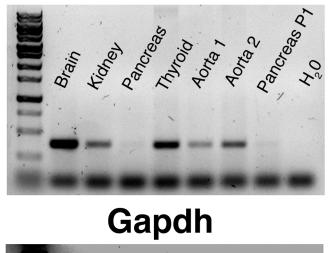


suppl. Figure S1

supplementary Figure S1 Legend:

Expression of the SIc4a8 gene encoding NDCBE. In a multiple tissue northern blot from organs of adult wild-type mice probed with a Slc4a8-cDNA probe (nucleotides 777-1522 according to NM 021530), transcripts were abundant in brain and testis, and detectable in several other tissues including the kidney. b) Targeting of the SIc4a8 locus. The partial genomic structure of the *Slc4a8* gene is shown in the upper panel. The middle panel displays the targeted Slc4a8 locus. A neomycin selection cassette flanked by loxP sites (grey arrows) was inserted into intron 11. A third loxP site was introduced into intron 12. Two correctly targeted ES cell clones were transiently transfected with a Cre-recombinase expression plasmid. ES cell clones with a Cremediated excision of the DNA fragment between the outer loxP sites (lower panel) were subsequently used for the generation of chimeric mice. c) Verification of SIc4a8 disruption. Southern blot analysis of genomic DNA of wild-type (+/+), heterozygous (+/-) and knockout (-/-) mice. Northern blot analysis of wild-type (+/+), heterozygous (+/-) and knockout (-/-) brain tissue with the Slc4a8-cDNA probe as described in a) revealed no detectable residual aberrant transcripts in knockout tissue. A membrane protein immunoblot with a NDCBE-antibody confirmed the absence of NDCBE in brain homogenates of KO mice. Actin was used as a loading control.

Ndcbe exons 5-8



3----

Supplementary Figure S2

Analyzes of NDCBE transcripts by RT-PCR on various mouse tissue.

Semiquantitative PCR on cDNAs of various tissues reveals strong expression of NDCBE in brain, kidney and thyroid and to a lesser extent in the aorta. NDCBE transcripts could not be detected in the pancreas of newborn (P1) or adult mice. GAPDH served as a loading control. Total RNA was isolated from different mouse using trizol (Invitrogen). Reverse transcription of total RNA (1 µg) was performed using standard protocols with random hexanucleotide primers and Superscript II reverse transcriptase (Invitrogen). Semiquantitative PCR was performed using intron spanning primers with the following sequences: NDCBE Exon 5-8 forw 5′-cgtgcaagtagcatagaggag-3′, rev 5′-gtatccacctctcccaccag-3′, GAPDH forw 5′- caacagcaactcccactcttc-3′ and rev 5′- aaggagtaagaaaccctggacc-3′.