

FIGURE 1S: Multiple alignments of N-termini of (A) AQP3, (B) SLC6a2, and (C) SLC13a3 among different species. Regions of homology are indicated with shades (dark gray for identical residues and light gray box for conserved substitutions).

FIGURE 2S: Representative gel images and HPLC profile during the purification of expressed hSVCT2 N-terminus. (A) 14% SDS gels stained by Coomassie Blue. Lane 1: 2  $\mu$ l total *E. coli* lysate; Lane 2: 1  $\mu$ l imidazole-eluted fraction from the first Ni<sup>2+</sup>-column, concentrated maltose binding protein (MBP)-His8 hSVCT2 N-terminus (hSVCT2N); Lane 3: 1  $\mu$ l dialyzed imidazole-eluted fraction; Lane 4: 1  $\mu$ l enterokinase-digested MBP-His8 hSVCT2N showing that most protein has been cut into two fragments, MBP-His8 and hSVCT2N; Lane 5: 1.5  $\mu$ l flow-through fraction from the second Ni<sup>2+</sup>-column which trapped the MBP-His8 fragment. (B) HPLC profile showing OD220 in black line and % buffer B (75% acetonitrile, 0.1% trifluoroacetic acid) in the mobile phase in grey line. Buffer A is 0.1% trifluoroacetic acid. A 20-60-20 step gradient of buffer B was used with a flow rate of 1 ml/min.

FIGURE 3S: The alignment of C-termini of (A) SVCT, (B) AQP, (C) SLC6, and (D) SLC13 gene families. With the exception of AQP, which is based on mouse sequence, all other sequences are human. Regions of homology are indicated with shades (dark gray for identical residues and light gray box for conserved substitutions). There were no significant sequence similarities among the C-termini from different gene families.

Table 1S: Isoelectric points (pI) of N- and C-termini of apical and basolateral membrane protein pairs<sup>a</sup>

| <b>pI of basolateral-targeted membrane protein</b> |              |             |               |                |
|--|--------------|-------------|---------------|----------------|
|  | <b>SVCT2</b> | <b>AQP3</b> | <b>SLC6A2</b> | <b>SLC13A3</b> |
| <b>N-terminus</b>                                  | 3.90         | 10.03       | 9.10          | 12.02          |
| <b>C-terminus</b>                                  | 9.52         | 5.80        | 7.03          | 3.93           |
| <b>pI of apical-targeted membrane protein</b>      |              |             |               |                |
|  | <b>SVCT1</b> | <b>AQP2</b> | <b>SLC6A3</b> | <b>SLC13A2</b> |
| <b>N-terminus</b>                                  | 4.60         | 5.90        | 8.93          | 5.27           |
| <b>C-terminus</b>                                  | 5.72         | 8.90        | 9.43          | 7.12           |

<sup>a</sup>The isoelectric points were predicted using web-based ProtParam program (45).

Figure 1S, Supporting Information

### A.AQP3

human MGRQKELVSRGEMLHIRYRLLRQALAE-TM  
mouse MGRQKELMNRGEMLHIRYRLLRQALAE-TM  
rat MGRQKELMNRGEMLHIRYRLLRQALAE-TM  
cattle MGRQKELVNRGEMLHIRYRLLRQALAE-TM  
pig MGRQKELVTRGEMLHIRYRLLRQALAE-TM

### B.SLC6A2

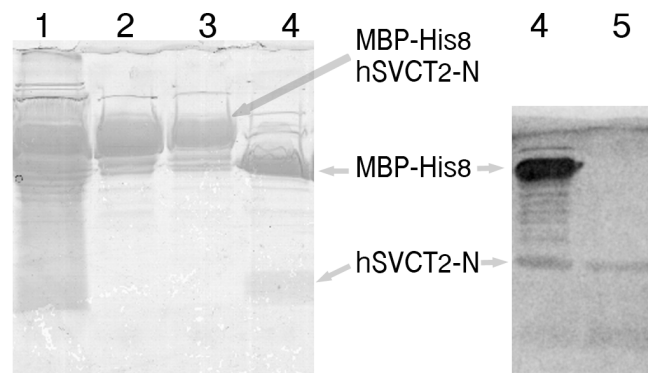
human MLLARMNPQVQPENNGADTGPEQPLRARKTAEELLVVKERNGVQCLLAPRDGDAQPRETVGKKID-TM  
mouse MLLARMNPQVQPELGGADPLPEQPLRPCKTADLLVVKERNGVQCLLASQSDAQPRETVGKKID-TM  
rat MLLARMKPQVQPELGGADQLPEQPLRPCKTADLLVVKERNGVQCLLASQDGDGDAQPRETVGKEID-TM  
cattle MLLARMNPQVQPENGGAGPGSEQPP--RKREEVLVVKERNGVQCLLASRDGDEQPRETVGKKID-TM  
dog MLLARMKPQVQPENSGAAPGPEPPGQGRKAVELLVVKERNGVQCLLASGAGDEQPRETVGKKID-TM

### C.SLC13A3

human MAALAAA~~A~~AKKVSARR-TM  
mouse MAALAA~~L~~AKKVSARR-TM  
rat MAALAA~~L~~AKKVSARR-TM  
cattle MAALAT~~V~~AKKVSARR-TM

Figure 2S, Supporting Information

**A.**



**B.**

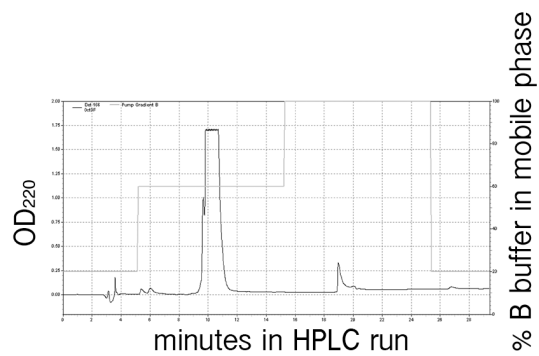


Figure 3S, Supporting Information

### A. SVCT C-terminal alignment

SVCT1 TM-DNTVPGSPEERGLIQWKAGAHANS DMSSSLKSYDFPIGMGIVK RITFLKYI  
SVCT2 TM-DNTIPGTPEERGI R KWKKGVGKGNKSLDGMESYNLPFGMNIKKYRCFSYL

SVCT1 PICPVFKGFSSSSKDQIAIPEDTIPENTETASVCTKV  
SVCT2 PISPTFVG YTWKGLRKSDNSRSSDEDSQATG

### B. AQP C-terminal alignment

AQP2 TM- PSTKSLQE---RLAVLKGLEPDTDWEEREVRRRQSVELHSPQSLPRGSKA  
AQP3 TM-IGCHLEQPPST---EEENVKLAHMKHKEQI

### C. SLC6 C-terminal alignment

SLC6A2 TM-YKFLSTQGSLWERLAYGITPEN EHHLVAQRDIRQFQLQHWLAI  
SLC6A3 TM-YKFCSLPGSFREK LAYAI APEKDR ELVDRG EVRQFTLRHWLKV

### D. SLC13 C-terminal alignment

SLC13A2 TM-PLFSLHSFPSWAQ---SNTTAQCLPSLAN TTTTPSP  
SLC13A3 TM-TIIFQLGTFPDWADMYSVNVTA-LPPTLANDTFR TL