Supporting Information

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SI Text

cDNAs. cDNA of SLC17A9 was cloned by PCR from Mammalian Gene Collection clone 4563089. For the construction of cDNA, we used pENTR Directional TOPO cloning kits (Invitrogen). The primers used were based on database sequences (GenBank accession no. CAC28600): sense primer, CACCATGACCCTGACAAGCAGGCGCCAGGA; antisense primer, CTAGAGGTCCTCATGGGTAGAGCTC. The sequence was confirmed by comparison with the human genome sequence and any differences were corrected. cDNAs of mouse SLC17A9 was cloned as described above. cDNA of mouse SLC17A9 was amplified by RT-PCR using RNA derived from mouse adrenal gland. The following primers were used: sense primer, 5′-CACCATGCCATCCCAGCGCTCTA-3′, antisense primer, 5′-TTCACTGACAGGGTTGTTATCC -3′.

Northern Blot Analysis. Human and mouse multiple-tissue Northern blots (MTN) were purchased from Clontech. For Northern blot analysis, PCR fragments encoding the N-terminal 425 bps (residues 223–647) of human SLC17A9 and C-terminal 758 bps (residues 891-1648) of mouse SLC17A9 labeled with ^{32}P-dCTP were used as probes. Hybridization was performed at 68°C for 1 h in Express Hyb hybridization solution (Clontech) and washed under high-stringency conditions at 50°C.

Antibodies. Site-specific polyclonal antibodies against human and mouse SLC17A9 were prepared by repeatedly injecting GST-fusion polypeptides encoding either M1- I40 or L8-R97, respectively. The antiserum (100 μl) was preabsorbed by incubating it with the antigen (1 mg) at 4°C overnight. mAbs against synaptotagmin were kindly supplied by M. Takahashi (1). Anti-His-tag mAb was from Novagen. Alexa Fluor 488-labeled goat anti-rabbit IgG was obtained from Invitrogen (Molecular Probes) and colloidal gold-conjugated goat anti-rabbit IgG was from British Biocell International.

Nucleotide Binding. Binding of nucleotides to the purified transporter was assayed by UV light illumination according to the published procedures (2). In brief, the reaction was performed in 50 μl of reaction mixture containing 20 mM Mops·Tris (pH 7.0), 50 mM potassium acetate, 2 mM magnesium acetate, 4 mM KCl, 7 μg protein, and 5 μM concentration of [α-^{32}P] ATP (15 TBq/mmol). The reaction mixture was placed in flat-bottom plastic test tubes, on ice, and at a 5-cm distance from an unfiltered UV lamp (UVP; 15 W). After a 10-min illumination, the reaction was terminated by the addition of 10 μl of concentrated dissociation buffer to give a final concentration of 2% SDS and β-mercaptoethanol. Samples (50 μl) were elecrtrophoresed in the presence of SDS and exposed to an IP image plate.

Immunohistochemistry. Indirect immunofluorescence microscopy was performed as described (3). Adrenal glands were obtained from adult C57BL/6 mice perfused intracardially with 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. The first antibody treatment was performed with 1:500 diluted antibodies in PBS containing 0.5% BSA for 1 h at room temperature. The specimens were observed under an Olympus FV300 confocal laser microscope.

Immunoelectronmicroscopy. The LR White embedding immunogold method was used (4). Adult C57BL/6 mice were anesthetized with ether and then perfused intracardially with saline, followed by 0.1% glutaraldehyde and 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. Adrenal gland was washed with PBS, dehydrated in ethanol series, and then embedded in LR White. Ultrathin sections (80 nm) on nickel grids were incubated with PBS containing 2% goat serum and 2% BSA for 10 min, and then treated with either rabbit antiserum against mouse SLC17A9 diluted 1:20 or normal rabbit serum diluted 1:20 for 1 h at room temperature. The sections were washed and treated with secondary antibodies conjugated with 10-nm colloidal gold diluted 1:50. After washing with sodium cacodylate buffer (pH 7.4), the sections were postfixed in 2.5% glutaraldehyde in the cacodylate buffer and stained sequentially uranyl acetate for 10 min and lead citrate for 1 min. Specimens were observed under a Hitachi H-7100S electron microscope.

Measurement of $\Delta\psi$ and ΔpH by Fluorescence Quenching. $\Delta\psi$ (positive inside) was assayed by measuring the fluorescence quenching of oxonol V as described (5).

Preparation of Chromaffin Granule Membrane Vesicles from Bovine Adrenal Glands. Chromaffin granules were isolated from bovine adrenal glands by differential centrifugation and successive sucrose density gradient centrifugation as described (5). After isolation, the granules were immediately disrupted under low osmotic conditions, and the resulting membrane vesicles suspension was stocked at -80°C.

Other Procedures. PAGE in the presence of SDS and Western blotting were performed as described (6). Protein concentration was assayed by using BSA as a standard (7).

1. Shouji-Kasai Y, *et al.* (1992) Neurotransmitter release from synaptotagmin-deficient clonal variants of PC12 cells. *Science* 256:1820–1824.
2. Moriyama Y, Nelson N (1987) Nucleotide binding sites and chemical modification of the chromaffin granule proton ATPase. *J Biol Chem* 262:14723–14729.
3. Morimoto R, *et al.* (2006) Secretion of L-glutamate from osteoclasts through transcytosis. *EMBO J* 25:4175–4186.
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5. Nelson N, Cidon S, Moriyama Y (1988) Chromaffin granule proton pump. *Methods Enzymol* 157:619–633.
6. Juge N, Yoshida Y, Yatsushiro S, Omote H, Moriyama Y (2006) Vesicular glutamate transporter contains two independent transport machineries. *J Biol Chem* 281:39499–39506.
7. Schaffner W, Weissmann C (1973) A rapid, sensitive, and specific method for the determination of protein in dilute solution. *Anal Biochem* 56:502–514.

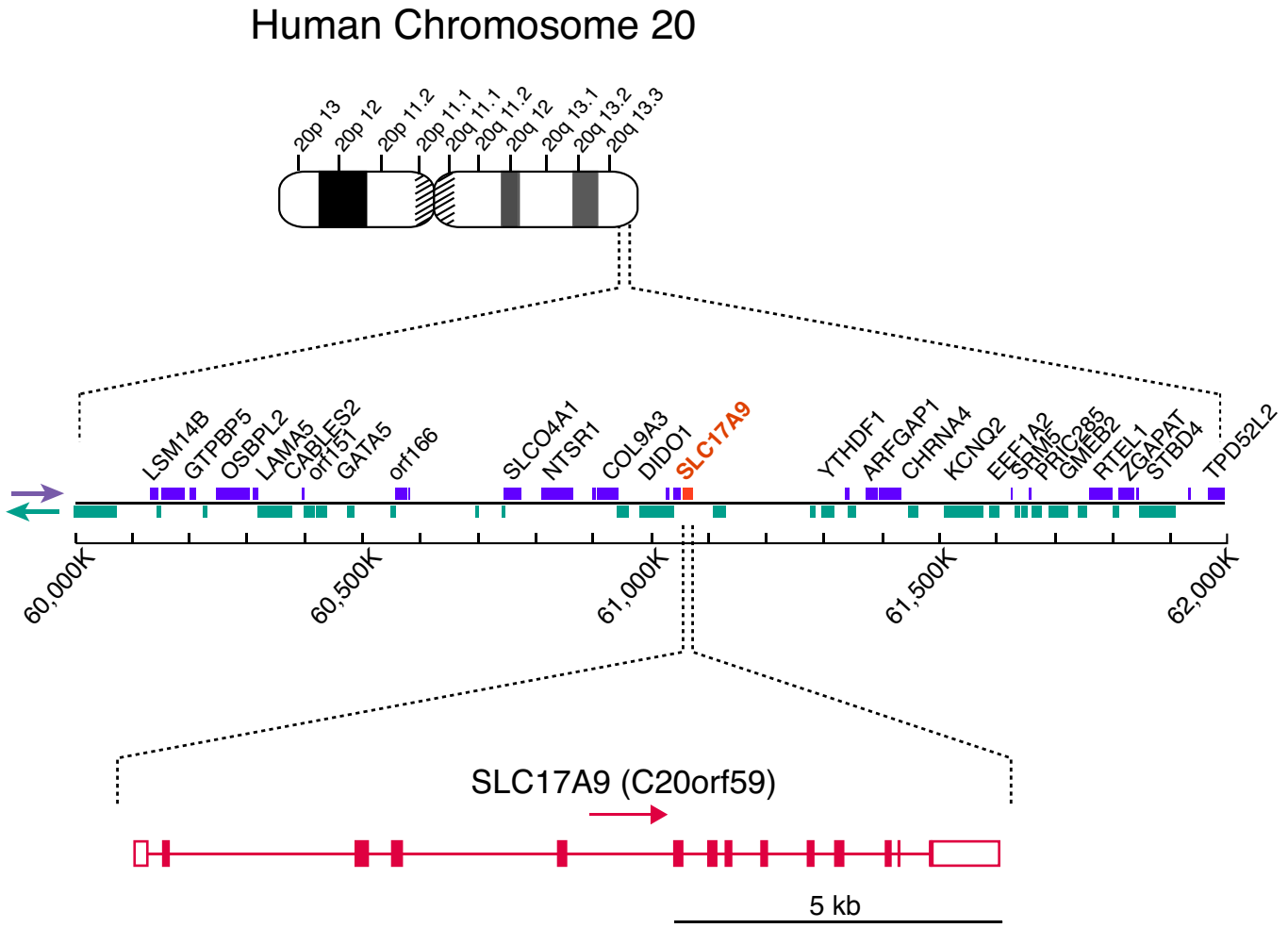

Fig. S1. Chromosomal localization and gene organization of SLC17A9. The exon and intron organization of SLC17A9 is also shown. The open and shaded boxes show noncoded and coded exons, respectively.

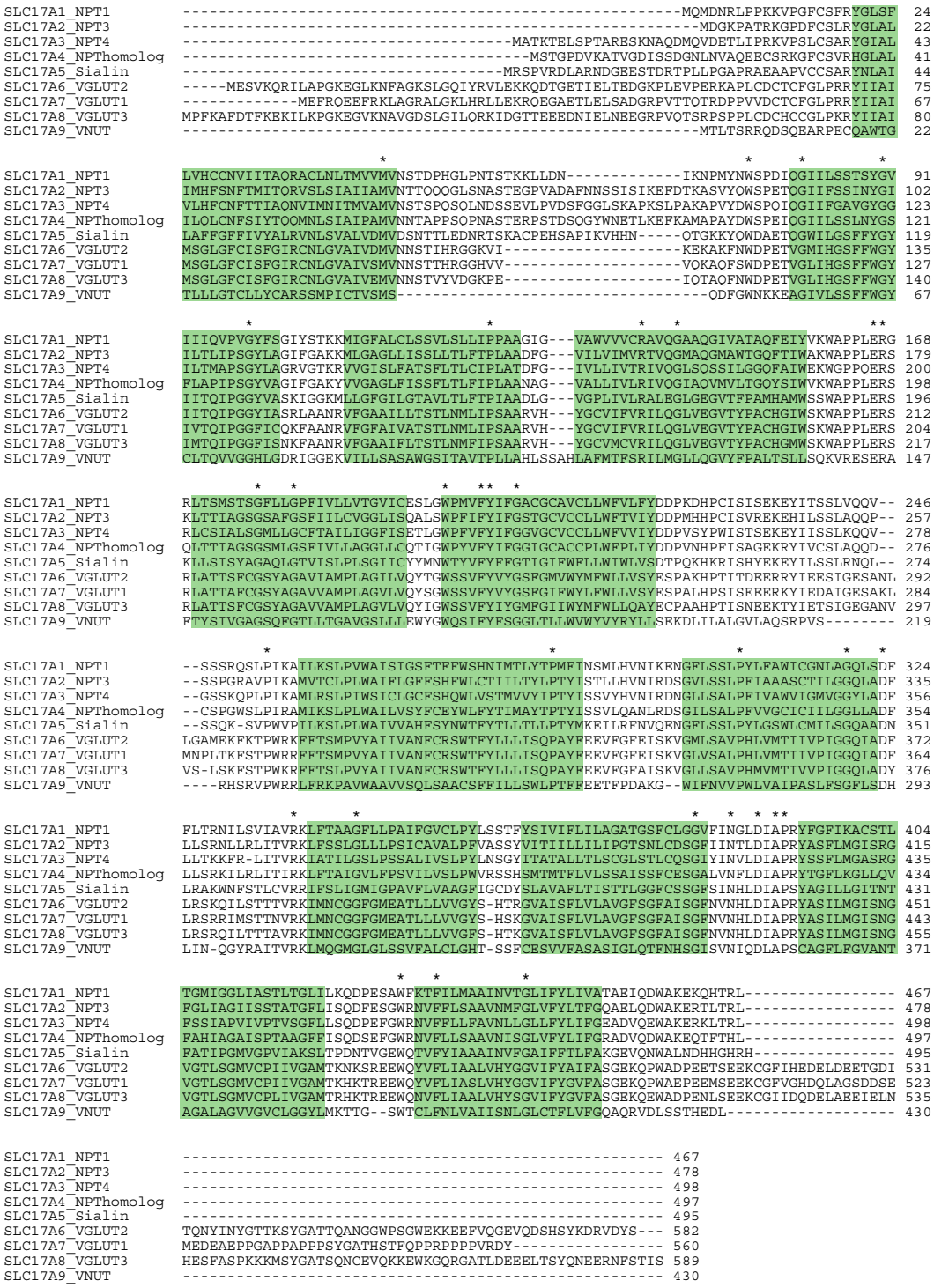

Fig. S2. Amino acid sequences comparison of members of the SLC17 family. Identical residues are indicated by asterisks. Predicted transmembrane regions are shaded.

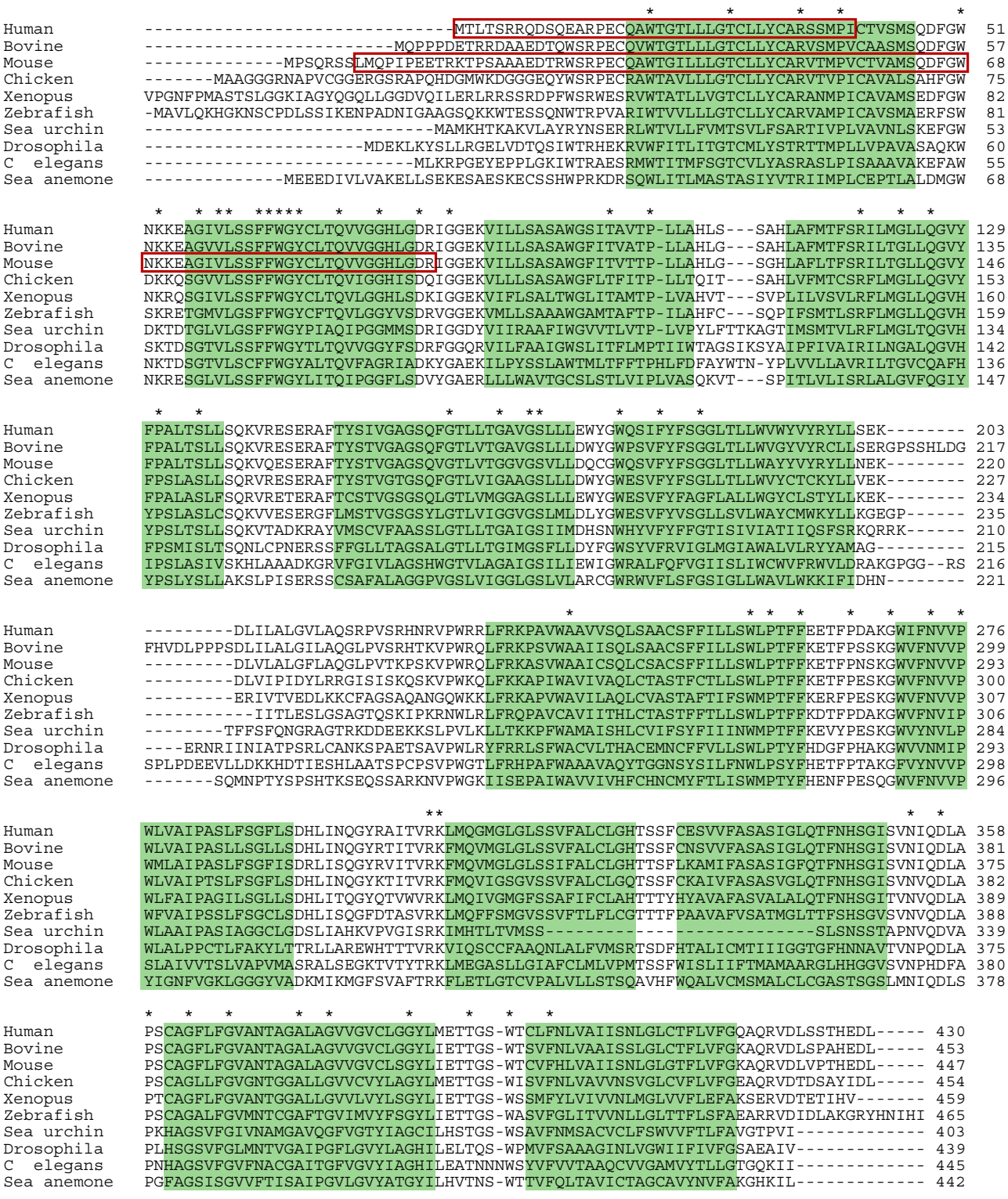

Fig. S3. The amino acid sequences of SLC17A9 orthologues are aligned. Identical residues are indicated by asterisks. Predicted transmembrane regions are shaded. The positions of polypeptide used for preparation of antibody are boxed in red.

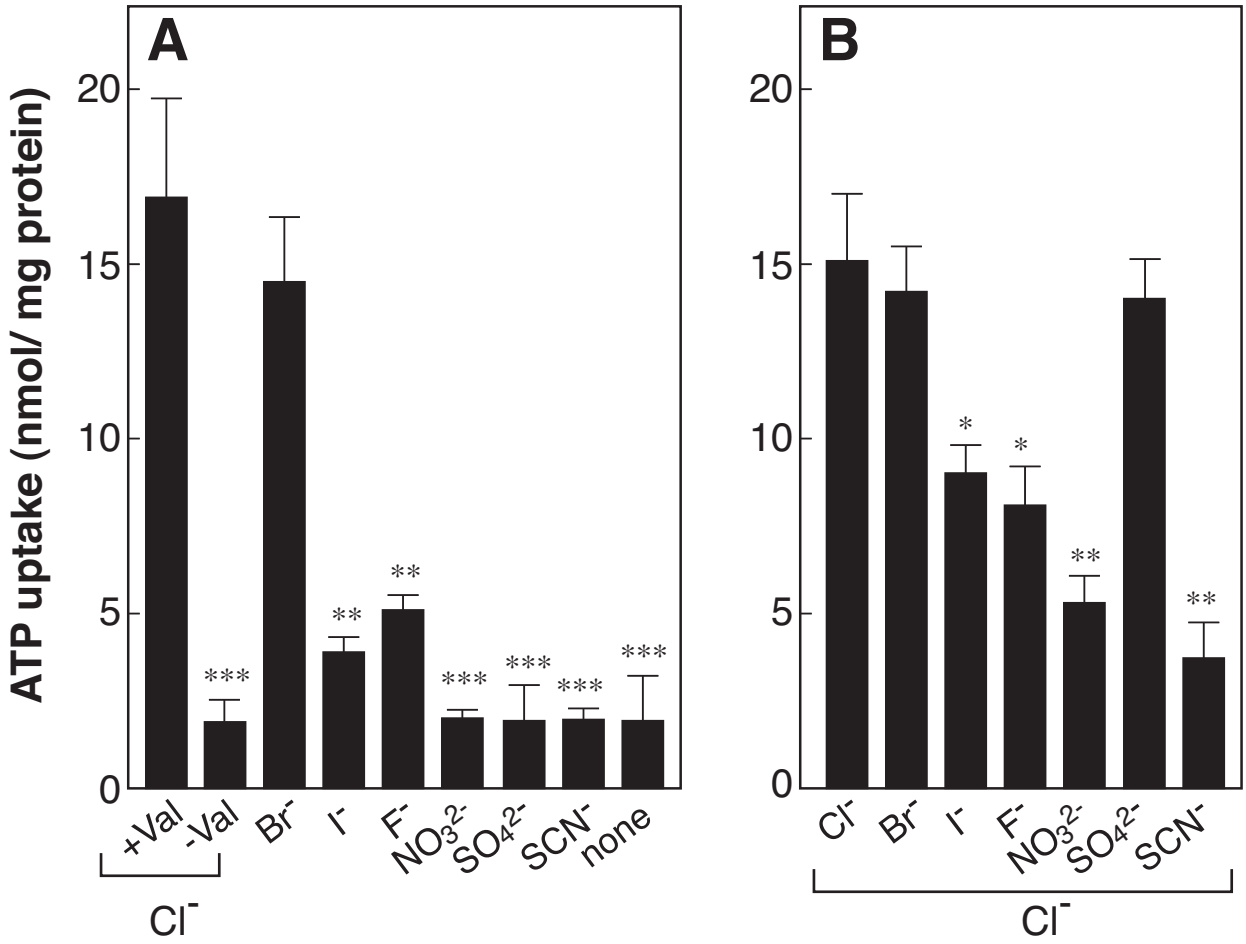

Fig. S4. The effect of various anions on $\Delta\psi$-mediated uptake of [α-^{32}P] ATP by SLC17A9 protein. (*A*) The uptake was performed under the standard assay conditions except that KCl was replaced with various potassium salts as indicated. (*B*) The effects of anion species on Cl^--dependent [α-^{32}P]ATP uptake at 2 min in the presence of 4 mM KCl plus the indicated potassium salts (4 mM) were measured.

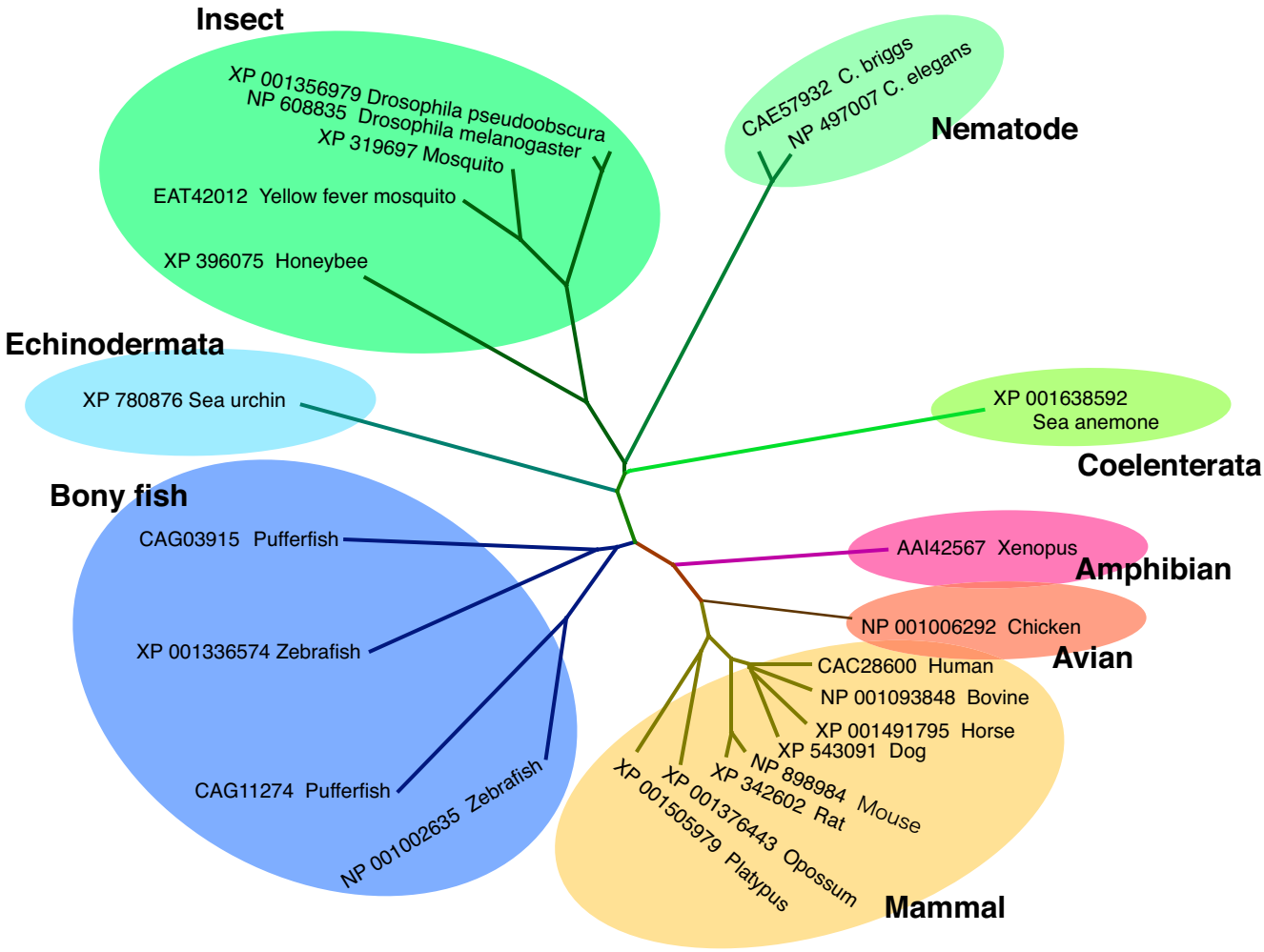

Fig. S5. VNUT orthologues are widely distributed in the animal kingdom. A phylogenetic tree of selected genes encoding VNUT-type transporters from the animal kingdom is shown. VNUT from mammals are shown in beige, birds in coral, amphibians in dark pink, fish in blue, and insects in green.

Table S1. Energetics of ATP transport

Inside	Outside	Ionophores	Inside pH	Outside pH	ATP uptake, %
		None			3.1 ± 1.7
Na^+	K^+	Valinomycin	7.0	7.0	100.0
		Nigericin			6.4 ± 2.1
		None			7.6 ± 6.8
Na^+	Na^+	Valinomycin	7.0	7.0	6.5 ± 5.0
		Nigericin			4.5 ± 4.1
		None	7.0	7.0	5.9 ± 2.3
			5.5	7.0	2.6 ± 0.1
K^+	K^+		5.5	7.5	2.4 ± 0.4
		Valinomycin	7.0	7.0	5.9 ± 5.1
		Nigericin			2.4 ± 3.0
		None			2.5 ± 0.6
K^+	Na^+	Valinomycin	7.0	7.0	3.0 ± 2.1
		Nigericin			3.3 ± 2.8

Na^+-trapped proteoliposomes or K^+-trapped proteoliposomes were prepared by dilution of purified transporter (10 μg protein, 150 μl) into the 3 ml of buffer consisting of 20 mM MOPS·Tris (pH 7.0), 0.15 M Na-acetate (Na^+-trapped proteoliposomes) or 0.15 M K-acetate (K^+-trapped proteoliposomes), and centrifuged as described. The pellets were suspended in 200 μl of the same buffer. The proteoliposomes (0.5 μg protein) was added to the buffer containing 0.15 M K-acetate and 4 mM KCl or 0.15 M Na-acetate and 4 mM NaCl in the presence or absence of listed ionophores (1 μM each). After incubation for 5 min, ATP uptake was started as described. One-hundred percent control corresponded to 16.0 ± 1.3 nmol/mg of protein for 2 min. In some experiments, proteoliposomes were prepared in 20 mM MES (pH 5.5), 0.15 M K-acetate, and 4 mM KCl. Then, proteoliposomes were added to the assay solution, pH 7.0 or 7.5, 0.15 M K-acetate, and 4 mM KCl. ATP uptake at 2 min was measured.