Fig. 2. *NIS mRNA and protein expression along the villus-crypt axis.*

A) Total RNA was extracted from enterocytes sequentially isolated, in nine fractions, from the small intestine along the villus-crypt axis as described in Materials and Methods. Differential expression of NIS mRNA was analyzed by RT-PCR and standardized with respect to β-actin mRNA expression. Purity of the villus-crypt fraction separation was confirmed by analysis of the expression of ALP (a villus marker) and PCNA (a crypt marker) mRNAs. Densitometric ratios of NIS, PCNA, and ALP over β-actin expression are shown; **B**) ALP activity from villus-tip epithelial cells in fractions A (homogenate); B (nonpurified apical membranes); and C (enriched apical membranes). **C)** NIS immunoblot: Lane 1: FRTL-5 cell membranes (10 μg); lanes 2-4, fractions A, B, or C (50 μg). *Lower panel*: Ezrin immunoblot as loading control after stripping anti-NIS antibodies. Boxes indicate different gels.

Fig. 5. *A high-I^- diet reduces intestinal I- transport and NIS protein* in vivo.

Rats were provided water (control) or 0.05% KI-supplemented water. After the indicated times, BBMV were purified as described in Materials and Methods and **A**) a steady-state I^- uptake assay was performed with 50 μg protein and 20 μM $^{125}I^-$ alone (gray bars) or in the presence of 80 μM ClO_4^- (dark bars). **B**) BBMV (100 μg) were also used for immunoblot and probed with anti-NIS and, after stripping the nitrocellulose, anti-ezrin antibodies. Ezrin was probed as a loading control, as described in Materials and Methods. Boxes indicate different gels. **C)** Quantitation of the NIS/ezrin densitometric signal was done with ImageJ (NIH).