Supplementary Data

Methods for Figure 3

A list of known xCT genes was established from entries in both the NCBI (www.ncbi.nlm.nih.gov) and Ensembl (www.ensembl.org/index.html) databases. These genes were then used as queries for a BLAST search in the NCBI database (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Searches with the TBLASTN algorithm were performed in the nucleotide collection (nr/nt) database restricted to selected species to complete the overview and/or verify the absence of a known xCT ortholog. Multiple sequence alignments were created using MAFFT version 6 (http://mafft.cbrc.jp/alignment/server) using the E-INS-i setting (3). The phylogenetic analysis was done using the PhyML algorithm, which is based on maximum likelihood after trimming the multiple sequence alignment with JalView 2.5 (www.jalview.org) at a cut-off value of 85% gaps. This algorithm is available online (www.hiv.lanl.gov/content/sequence/PHYML/interface.html), and the Blosum62 substitution matrix and 100 bootstrap trials were chosen as settings (2). The resulting phylogenetic tree was visualized with NJplot (http://pbil.univ-lyon1.fr/software/njplot.html).

Methods for Figure 14

Cryosections of adult C57/Bl6 mouse eyes were fixed with ice-cold acetone; nonspecific binding was blocked using Powerblock. Sections were incubated for 3 h at 37°C with a polyclonal antibody prepared against xCT (1). Negative control experiments were performed by blocking the anti-xCT antibody with 20 μg/ml of the antigenic peptide for 30 min before incubation with slides. After rinsing, all sections were incubated for 1 h at 37°C with Alexa Fluor 555-conjugated anti-rabbit IgG (Life Technologies, Grand Island, NY; 1:1000). Slides were coverslipped using Fluoroshield (Sigma Chem. Corp., St. Louis, MO). xCT was detected by epifluorescence using a Zeiss Axioplan-2 microscope equipped with the Axiovision program and an HRM camera. The anterior portion of the eye was examined to detect xCT in the cornea, ciliary body, and iris.

Supplementary References

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2. Guindon S and Gascuel O. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst Biol* 52: 696–704, 2003.
3. Katoh K, Kuma K, Toh H, and Miyata T. MAFFT version 5: improvement in accuracy of multiple sequence alignment. *Nucleic Acids Res* 33: 511–518, 2005.