Supporting Information

Jézégou et al. 10.1073/pnas.1211198109

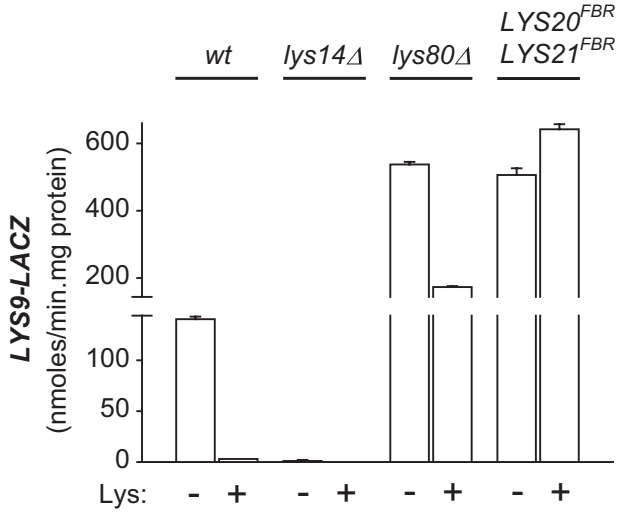

Fig. S1. Transcriptional regulation of the *LYS9* gene, a known member of the lysine-repressible *LYS* regulon. Strains of the indicated genotypes were transformed with a centromere-based plasmid expressing the *lacZ* reporter gene under the control of the *LYS9* gene promoter. Cells were grown on a minimal glucose/ammonium medium with (+) or without (–) lysine (1 mM). β-Galactosidase activities are the means of at least two independent experiments.

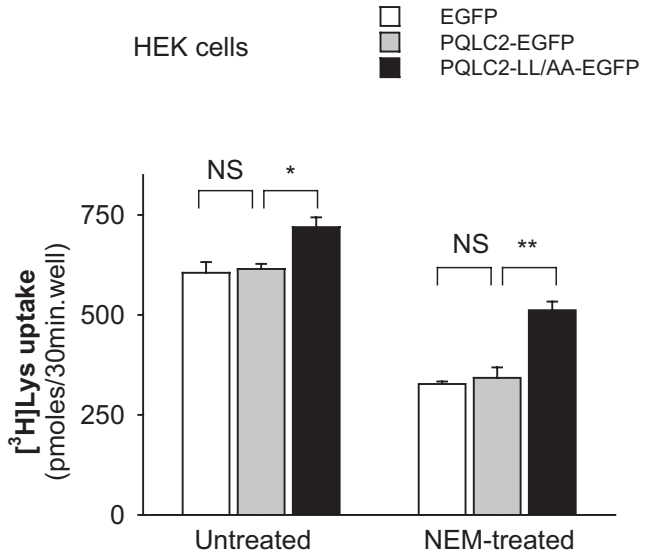

Fig. S2. Expression of PQLC2 in mammalian cells induces lysine uptake. HEK-293 cells (300,000 cells per well) were transfected with an empty pEGFP-N1 vector or with plasmids encoding WT PQLC2-EGFP or PQLC2-LL290/291AA mutation (LL/AA)-EGFP. Two days later, cells were washed and pretreated for 30 min at room temperature with 1 mM *N*-ethylmaleimide (NEM) to decrease the endogenous transport of cationic amino acid. Untreated and NEM-treated cells were then washed, incubated for 30 min with [^{3}H]lysine (0.1 mM) at pH 5.0, washed twice in ice-cold buffer, and subjected to scintillation counting. *$P < 0.02$; **$P < 0.01$; NS, not significant by the Student *t* test ($n = 3$).

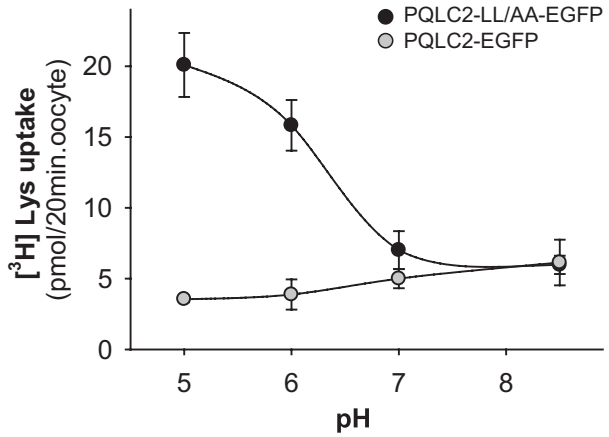

Fig. S3. Extracellular pH dependence of PQLC2-mediated lysine uptake. [^{3}H]Lysine (0.1 mM) uptake into oocytes expressing WT PQLC2 or the LL290/291AA (LL/AA) mutant was measured at distinct pH values. Uptake is activated in conditions mimicking the lysosomal environment of PQLC2. Means ± SEMs of four to five oocytes are shown.

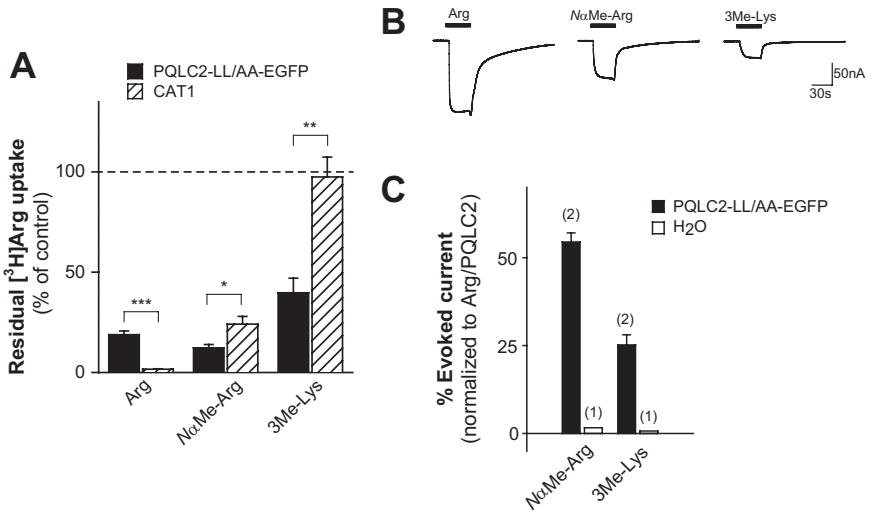

Fig. S4. Selectivity of PQLC2 toward methylated cationic amino acids resembles that of lysosomal system c. (*A*) Arginine uptake into paired sets of oocytes expressing either PQLC2-LL290/291AA mutation (LL/AA)-EGFP or the plasma membrane transporter CAT-1 was analyzed at pH 5.0, in the absence or presence of 10 mM unlabeled L-arginine, *N*-α-methyl-L-arginine (NαMe-Arg), or ε-*N*-trimethyl-L-lysine (3Me-Lys) added simultaneously to [^{3}H]L-arginine (40 nM). NαMe-Arg and 3Me-Lys preferentially inhibit PQLC2 rather than CAT-1, in agreement with earlier biochemical studies on lysosome fractions. Means ± SEMs of 8–14 oocytes from two batches are shown. ***$P < 10^{-9}$; **$P < 0.01$; *$P < 0.02$ by the Student *t* test. (*B* and *C*) Two-electrode voltage clamp recording of PQLC2-LL/AA oocytes at −40 mV and pH 5.0 shows that the two methylated compounds (10 mM) are translocated by PQLC2. Raw traces from a PQLC2-LL/AA-EGFP oocyte and mean current responses normalized to the L-arginine current from two oocytes are shown in *B* and *C*, respectively.

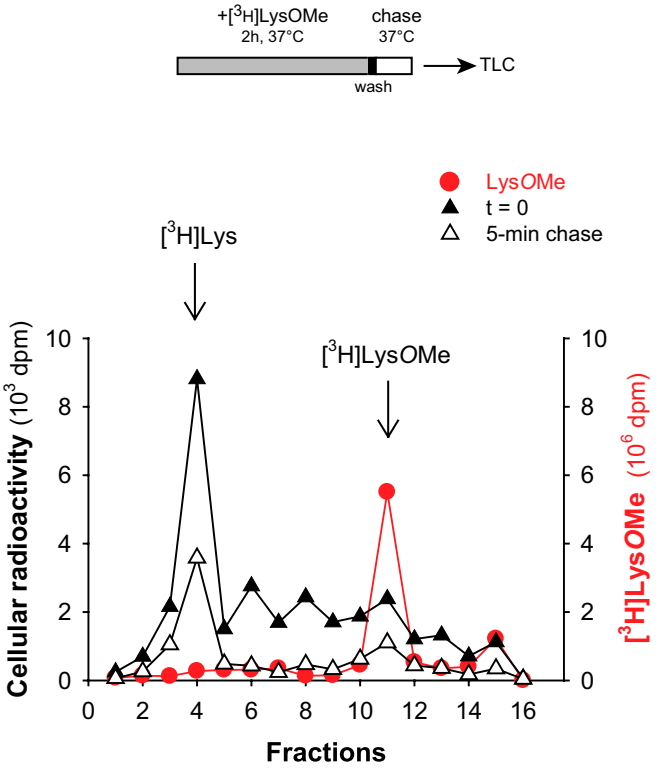

Fig. S5. L-[^{3}H]lysine methyl ester ([^{3}H]Lys*O*Me) applied to human fibroblasts is predominantly converted to lysine. Fibroblasts were incubated for 2 h at 37 °C in 5% CO_2 with 0.2 mM [^{3}H]Lys*O*Me, washed, and further incubated at 37 °C (chase period). Water-soluble cell extracts (triangles) were then analyzed by TLC and compared with an equivalent amount of [^{3}H]Lys*O*Me solution (red circles). Most of the water-soluble radioactivity was recovered as lysine. This intracellular [^{3}H]lysine pool decays with a time scale of minutes.

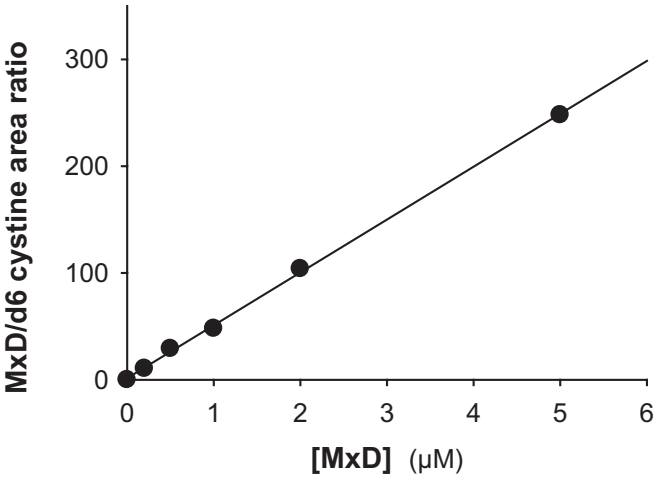

Fig. S6. Calibration curve of the mixed disulfide (MxD) liquid chromatography tandem MS assay. Increasing dilutions from a known MxD solution were tested. The assay is linear up to 5 μM (R^2 = 0.9992).

Table S1. Yeast plasmids used in this study

Plasmid	Description	Source
pFL38	*CEN-ARS (URA3)*	Bonneaud et al. (1)
pLL063	*CEN-ARS (URA3) YPQ1-GFP*	This study
pLL161	*CEN-ARS (URA3) YPQ2-GFP*	This study
pLL106	*CEN-ARS (URA3) GAL-YPQ3-GFP*	This study
pCJ502	*CEN-ARS (URA3) GAL-rPQLC2-GFP*	This study

1. Bonneaud N, et al. (1991) A family of low and high copy replicative, integrative and single-stranded S. cerevisiae/E. coli shuttle vectors. *Yeast* 7(6):609–615.

Table S2. Yeast strains used in this study

Strain	Genotype	Source
23344C	*ura3*	Laboratory collection
EL029	*ura3 ypq1Δ*	This study
EL031	*ura3 ypq2Δ*	This study
LL118	*ura3 ypq3Δ*	This study
LL178	*ura3 ypq1Δ ypq2Δ ypq3Δ*	This study
LL159	*ura3 vba1Δ vba2Δ vba3Δ*	This study
JA965	*ura3 vba1Δ vba2Δ vba3Δ ypq2Δ*	This study

All strains derive from the Sigma1278b WT strain. Mutant strains were isolated by insertion of a *kanMX2* geneticin resistance gene that was amplified by PCR assay using pUG06 plasmid as a template.