ATP13A2 deficiency disrupts lysosomal polyamine export

<https://doi.org/10.1038/s41586-020-1968-7>

Received: 21 December 2018

Accepted: 2 December 2019

Published online: 29 January 2020

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ATP13A2 (PARK9) is a late endolysosomal transporter that is genetically implicated in a spectrum of neurodegenerative disorders, including Kufor-Rakeb syndrome—a parkinsonism with dementia¹—and early-onset Parkinson's disease². ATP13A2 offers protection against genetic and environmental risk factors of Parkinson's disease, whereas loss of ATP13A2 compromises lysosomes³. However, the transport function of ATP13A2 in lysosomes remains unclear. Here we establish ATP13A2 as a lysosomal polyamine exporter that shows the highest affinity for spermine among the polyamines examined. Polyamines stimulate the activity of purified ATP13A2, whereas ATP13A2 mutants that are implicated in disease are functionally impaired to a degree that correlates with the disease phenotype. ATP13A2 promotes the cellular uptake of polyamines by endocytosis and transports them into the cytosol, highlighting a role for endolysosomes in the uptake of polyamines into cells. At high concentrations polyamines induce cell toxicity, which is exacerbated by ATP13A2 loss due to lysosomal dysfunction, lysosomal rupture and cathepsin B activation. This phenotype is recapitulated in neurons and nematodes with impaired expression of ATP13A2 or its orthologues. We present defective lysosomal polyamine export as a mechanism for lysosome-dependent cell death that may be implicated in neurodegeneration, and shed light on the molecular identity of the mammalian polyamine transport system.

ATP13A2 is a P5B-ATPase that belongs to the family of P-type ATPases, which couple ATP hydrolysis to substrate transport while transiently forming a catalytic phospho-intermediate⁴. ATP13A2 is generally described as a heavy-metal transporter⁵, but Ca²⁺ (ref. ⁶) and the polyamine spermidine (SPD)^{7,8} have also been proposed as potential substrates. To screen for the transported substrate(s) of ATP13A2, we measured ATPase activity in the presence of various candidate substrates in solubilized microsomal membrane fractions of SH-SY5Y cells that overexpress wild-type human ATP13A2 (hereafter denoted WT-OE) or comparable levels of the catalytically dead mutant ATP13A2(D508N) (containing an aspartic-acid-to-asparagine mutation at position 508; denoted D508N-OE)^{9,10}.

The ATPase activity of wild-type ATP13A2 was significantly stimulated by the polyamines SPD and spermine (SPM) (Fig. 1a), whereas SPM had no effect on the activity of the D508N mutant (Extended Data Fig. 1a). MnCl₂, ZnCl₂, FeCl₃, CaCl₂, diamines, monoamines and amino

acids exerted no effect on activity (Extended Data Fig. 1a–d), but the polyamines SPM, N¹-acetylspermine and SPD stimulated the ATPase activity of ATP13A2 in a concentration-dependent manner (Fig. 1b, Extended Data Fig. 1e), with the highest apparent affinity observed for SPM (Extended Data Table 1).

The catalytic autophosphorylation and/or dephosphorylation reactions of P-type ATPases occur in response to binding of the transported substrate⁴. ATP13A2 forms a phospho-intermediate on the D508 residue in the absence of SPM supplementation^{9,10}, whereas the addition of SPM leads to a dose-dependent reduction in the levels of the ATP13A2 phosphoenzyme (Fig. 1c), which is not seen with ornithine (Extended Data Fig. 1f). The dephosphorylation rate after a chase with non-radioactive ATP increased in the presence of SPM (Fig. 1d), which further indicates that SPM could be the transported substrate.

SPM also stimulated the ATPase activity of purified human ATP13A2 (for purification, see Extended Data Fig. 2a–e). However, purified

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Fig. 1 | ATP13A2 is a polyamine transporter. a, Chemical structures of ornithine (ORN), putrescine (PUT), spermidine (SPD) and spermine (SPM). **b–d**, Measurements on solubilized SH-SY5Y microsomes overexpressing ATP13A2 (WT-OE). **b**, Dose-response curves showing the effect of SPM, SPD, PUT and ORN on the ATPase activity of ATP13A2. **c**, ATP13A2 phosphoenzyme (EP) levels in WT-OE cells in the presence of increasing SPM concentrations. Top, representative autoradiogram; bottom, quantification of EP. **d**, Pulse ([³²P]ATP) chase (cold ATP) of dephosphorylation in the presence and absence of 1 mM SPM in WT-OE microsomes. **e**, ATPase activity of purified ATP13A2 under increasing concentrations of SPM (in the presence and absence of phosphatidic acid (PA) and/or PtdIns(3,5)P₂). **f**, Dose-response curves showing the effect of SPM, SPD and PUT on the ATPase activity of purified ATP13A2 supplemented with phosphatidic acid and PtdIns(3,5)P₂ (as a reference, SPM + phosphatidic acid + PtdIns(3,5)P₂ from **e** is also shown). **g**, Pulse ([³²P]ATP) chase (cold ATP) of dephosphorylation measured for

purified ATP13A2 (wild-type or E343A mutant) in the presence or absence of 1 mM SPM. **h**, Illustration of vesicle reconstitution and the ³H-spermine (³H-SPM) transport assay. **i**, Immunoblot of reconstituted vesicles. **j**, Uptake of ³H-SPM in reconstituted vesicles derived from yeast overexpressing BAD-tagged ATP13A2 (wild-type or E343A mutant) supplemented with phosphatidylcholine and phosphatidic acid, in the presence or absence of intraluminal ATP and an ATP-regenerating system. Data are presented as mean ± s.e.m. in **b–g**, or as box and whisker plots with overlaid individual data points representing replicates (**j**; horizontal line, median; box boundaries, 25th and 75th percentiles). The number of independent biological experiments were as follows: **n** = 3 **b** (ORN, SPD), **d–g** (E343A), **i, j** (wild-type ATP outside; E343A); **n** = 4 **c**, **e** (no lipid); **n** = 5 **b** (PUT), **g** (wild-type); **n** = 6 **j** (wild-type no ATP), **b** (SPM); **n** = 8 **j** (wild-type ATP inside). Analysis by two-way (**g**) or one-way (**j**) ANOVA with Tukey's corrections. Fitted lines indicate nonlinear allosteric sigmoidal (**b**, **e**, **f**) or two-phase (**c**, **d**, **g**) decay. For gel source data, see Supplementary Fig. 1.

ATP13A2 presented similar properties to microsomal ATP13A2 only in the presence of the regulatory lipids phosphatidylinositol(3,5)bisphosphate (PtdIns(3,5)P₂) and phosphatidic acid, which bind to the N terminus of ATP13A2^{9–11} (Fig. 1e, f, Extended Data Fig. 2f, Extended Data Table 1). SPM-induced ATPase activity was blocked by orthovanadate, a general P-type ATPase inhibitor (Extended Data Fig. 1g). Finally, we also purified the mutant ATP13A2(E343A), which carries a mutation in the conserved catalytic motif for dephosphorylation (³⁴¹TGES) (Extended Data Fig. 2g). The E343A mutant underwent autophosphorylation (Fig. 1g) but displayed limited SPM-induced ATPase activity (Extended Data Fig. 2h). Notably, when the phosphoenzyme was chased with cold ATP, SPM clearly stimulated dephosphorylation of purified wild-type ATP13A2, but not of the E343A mutant (Fig. 1g, Extended Data Fig. 1h, 6a).

ATP13A2 is a lysosomal polyamine exporter

Next, we performed transport assays with ³H-labelled SPM (³H-SPM) in reconstituted vesicles from solubilized yeast membranes expressing biotin acceptor domain (BAD)-labelled wild-type ATP13A2 or the

mutant ATP13A2(E343A), supplemented with the activating lipid phosphatidic acid. This reconstitution rendered two populations of ATP13A2 proteins that were inserted either right-side-out (ATP-binding domain in the extraluminal space) or inside-out (ATP-binding domain in the lumen) (Fig. 1h, i). Uptake of ³H-SPM was detected for wild-type ATP13A2—but not for the E343A mutant—only when ATP was present inside the vesicles, together with an ATP-regenerating system (Fig. 1j); that is, when ATP13A2 was positioned inside-out ((2) in Fig. 1h). Extending these insights to the cellular context, in which ATP13A2 is present in the late endolysosomal compartment^{1,12}, ATP13A2 most probably operates as a lysosomal SPM exporter.

Notably, the functionality of ATP13A2 affects the cellular polyamine content. We generated two independent ATP13A2-knockout SH-SY5Y cell lines using CRISPR–Cas9 genome editing (denoted KO; Extended Data Fig. 3a) and, upon analysis by mass spectrometry, demonstrated that the total cellular polyamine content was lower in KO than in control cells (Fig. 2a). Expression of either the wild-type ATP13A2 in the KO background (denoted KO/WT) for rescue, or the D508N mutant (KO/D508N) as a negative control (Extended Data Fig. 3b), resulted in

Fig. 2 | ATP13A2 transport affects cellular polyamine uptake, which is impaired by catalytic and disease mutations. a, b, Metabolomics of cellular polyamines in ATP13A2 knockout cells (KO) compared with SH-SY5Y controls (a), or compared with rescue cell lines expressing wild-type ATP13A2 (KO/WT) or ATP13A2(D508N) (KO/D508N) (b). **c,** Uptake of BODIPY-SPM in the presence or absence of the endocytosis inhibitors Dynasore, genistein and/or Pitstop 2, alone or in combination (combo). **d,** Confocal microscopy of BODIPY-SPM distribution (see Methods). Scale bar, 5 μ m. The arrow head shows the region that is expanded in the insets; the dashed line shows the region analysed in Extended Data Fig. 1j. **e, f,** Immunoblotting (e) and SPM-induced ATPase activity (f) of microsomes expressing wild-type ATP13A2, the D508N mutant, or catalytic mutants in M4 (A467V), M6 (D962N) and M8 (K1062A)³⁰. **g,** Representative autoradiogram depicts phosphoenzymes of wild-type ATP13A2 compared with the indicated mutants in the presence or absence of 1 mM SPM. **h,** Uptake of BODIPY-SPM in cells expressing the indicated mutant proteins. **i, j,** Immunoblotting (i) or SPM-induced ATPase activity (j) of SH-SY5Y microsomes overexpressing mutants associated with Kufor-Rakeb syndrome (T512I and G872R) or early-onset Parkinson's disease (T12M, G528R, A741T).

a significantly higher SPD and SPM content in KO/WT cells than in KO or KO/D508N cells (Fig. 2b).

Flow cytometry experiments revealed that ATP13A2 promotes the cellular uptake of BODIPY-labelled polyamines¹³. WT-OE cells took up more BODIPY-SPD and BODIPY-SPM than D508N-OE cells or control cells that expressed firefly luciferase (Fluc; Extended Data Fig. 4a, b). Similarly, KO/WT cells displayed a twofold higher uptake of BODIPY-SPM than ATP13A2 KO and KO/D508N cells, both of which took up less than control cells (Fig. 2c). The comparable uptake of the fluorescein isothiocyanate (FITC)-dextran conjugate in the KO/WT and the KO/D508N cells (Extended Data Fig. 4c) demonstrated that the higher BODIPY-SPM uptake in KO/WT cells compared with KO/D508N cells is not explained by an increased endocytic rate, but depends on the transport activity of ATP13A2. The stimulatory effect of ATP13A2 on endocytosis appears as a transport-independent phenotype¹⁴.

The observation that ATP13A2 transports SPM towards the cytosol (Fig. 1j) and increases the cellular SPM content (Fig. 2a, b) suggests that it could transport endocytosed polyamines into the cytosol. Indeed, endocytosis inhibitors prevented the uptake of FITC-dextran

The SPM dose-response curve from Fig. 1b is shown as a reference in f and j. **k,** Representative autoradiogram depicting phosphoenzymes of wild-type ATP13A2 compared with the disease-related mutants in the presence and absence of 1 mM SPM. **l,** BODIPY-SPM uptake in cells containing the indicated mutant proteins. MFI, mean fluorescence intensities; (-), vehicle-treated sample. Data are presented as individual data points (representing replicates) overlaid on box and whisker plots (a, b, horizontal line, median; box boundaries, 25th and 75th percentiles) or mean (c, h, i) or mean $\pm$ s.e.m. (f, j). The number of independent biological experiments were as follows: $n = 3$ d-f, g (D508N, A467V, D962N, K1062A (SPM)), h, j (T12M, T512I, G872R), k (T12M (-), T12M, T512I, G528R, A741T, G872R (SPM)), l; $n = 4$ a-c (KO, KO/WT, KO/DN), g (D508N, A467V, D962N, K1062A (-)), i, j (A741T, G528R), k (wild-type, T512I, G528R, A741T, G872R (-)); $n = 5$ c (control), g (wild-type (-)); $n = 6$ g (wild-type (SPM)), k (wild-type (SPM)). Analysis was performed using one-way ANOVA with Tukey's (a-c) or Dunnett's (h-l) corrections. Fitted lines indicate nonlinear allosteric sigmoidal (f (D962N, D508N), j) or one-phase (f (A467V, K1062A)) association. For gel source data see Supplementary Fig. 1.

(Extended Data Fig. 4c) and blocked that of BODIPY-SPM (Fig. 2c). Using confocal microscopy, we confirmed that KO/WT cells had a higher BODIPY-SPM content than KO/D508N cells (Extended Data Fig. 1i). In the KO/D508N cells, BODIPY-SPM mainly colocalized with LAMP1-positive vesicles (Fig. 2d, Extended Data Fig. 1j, k); this is indicative of accumulation in the late endolysosomes, which is not a lysosomotropic effect¹⁵. By contrast, cells with functional ATP13A2 displayed a broader distribution of BODIPY-SPM, and it was more abundant in the cytosol and nucleus; this is consistent with the transport direction from lysosomal lumen to cytosol (Fig. 2d, Extended Data Fig. 1l). By stimulating cellular SPM uptake and transporting SPM into the cytosol, ATP13A2 complements endogenous SPM synthesis, which depends on the enzymes ornithine decarboxylase (ODC) and the SPD and SPM synthases. Consistent with this, KO/WT cells were protected against pharmacological inhibition of ODC and the SPD and SPM synthases, and a lack of ATP13A2 activity sensitized KO and KO/D508N cells to inhibition (Extended Data Fig. 4d-g); this is consistent with the negative genetic interactions between ATP13A2 orthologues and ODC in yeast¹⁶ and *Caenorhabditis elegans*⁸.

Fig. 3 | ATP13A2 protects against lysosome-dependent SPM toxicity.

a, SPM-induced cell death (PI, propidium iodide) in SH-SY5Y control, ATP13A2 knockout (KO) and rescue cell lines with wild-type ATP13A2 (KO/WT) or ATP13A2(D508N) (KO/D508N). **b–f**, Effect of SPM (10 μ M, 4 h) and acidic nanoparticles (NP, 100-nm diameter; Extended Data Fig. 8j) on lysosomal functionality. **b**, Lysosomal pH measured by ratiometric FITC–dextran (standard curve, Extended Data Fig. 8i). **c**, **d**, Lysosomal degradation capacity analysed by DQ-BSA (**c**) or cathepsin B (CTSB) (**d**) activity. **e**, **f**, Assessment of lysosomal membrane integrity (LMI) via acridine orange (AO) staining (**e**), or galectin-3 (Gal-3) punctae formation (lysosomal rupture) (**f**). In **f**, the confocal images depict representative images (DAPI staining for nuclei reference; scale

bars, 2.5 μ m). The box and whisker plots depict the size (left) and number (right) of punctae. **g**, **h**, The effect of nanoparticles (**g**) or the cathepsin B inhibitor CA-074 (25 μ M) (**h**) on SPM-induced cytotoxicity. Data are presented as individual data points (representing replicates) overlaid on box and whisker plots (**b–f**, horizontal line, median; box boundaries, 25th and 75th percentiles) or means (**g**, **h**) or mean $\pm$ s.e.m. (**a**). The number of individual biological experiments were as follows: **n** = 3 **a**, **b–d** (NP, NP + SPM), **f–h**; **n** = 4 **e**, (NP, NP + SPM); **n** = 6 **b–d** (–), SPM; **n** = 7 **e** (–), SPM). Analysis was performed using two-way ANOVA with Dunnett's test (**a**) or one-way ANOVA with Tukey's (**b–e**, **g**, **h**) or Sidak's (**f**) test. Fitted lines indicate nonlinear log(inhibitor) versus response (variable slope) (**a**).

Mutations disrupt SPM-induced ATP13A2 activity

We next used mutagenesis techniques to confirm that the SPM-dependent activation of ATP13A2 depends on residues in the predicted substrate-binding site near transmembrane segment M4. We introduced mutations in transmembrane segments M4 (A467V), M6 (D962N) and M8 (K1062A) (Fig. 2e, Extended Data Fig. 5a, b; see Methods for the rationale in choosing these residues). In comparison with the wild type, the three mutants displayed a lower SPM-induced ATPase activity and apparent affinity for SPM (Fig. 2f), as well as a reduced cellular uptake of BODIPY–SPD and BODIPY–SPM (Extended Data Fig. 5c, Fig. 2h), which suggests that these residues contribute to SPM coordination in the membrane region. SPM-induced dephosphorylation was completely abolished in the D962N mutant (Fig. 2g, Extended Data Figs. 5d, 6), indicating that D962 may couple SPM binding to the dephosphorylation reaction.

More than thirty disease-associated mutations have been identified in ATP13A2 (Extended Data Fig. 7). We determined the activity of mainly lysosomal-localized mutants of ATP13A2 arising from point mutations in ATP13A2 that are linked to early-onset Parkinson's disease or Kufor-Rakeb syndrome. The T512I and G872R mutants—associated with Kufor-Rakeb syndrome—did not exhibit ATPase or autophosphorylation

activity and were SPM-insensitive, which is in line with their strongly reduced BODIPY–SPD and BODIPY–SPM uptake (Fig. 2i–l, Extended Data Fig. 5e–g). The T12M, G528R and A741T mutants—associated with early-onset Parkinson's disease—had a less severe effect. Compared with wild type, a reduction in the apparent SPM affinity was observed and phosphoenzyme levels were more (T12M) or less (G528R and A741T) sensitive to SPM. In cells, the uptake of BODIPY–SPD and BODIPY–SPM was less severely impaired with the mutations associated with early-onset Parkinson's disease than those associated with Kufor-Rakeb syndrome (Fig. 2i–l, Extended Data Fig. 5e–g). In summary, ATP13A2-dependent polyamine transport is disturbed in all mutants tested, and the degree of functional impact correlates with the phenotypic differences between early-onset Parkinson's disease and Kufor-Rakeb syndrome; however, the mutation type is not the sole determinant of the clinical phenotype¹⁷.

ATP13A2 protects against polyamine toxicity

We further investigated whether defective lysosomal polyamine export explains the lysosomal phenotype in ATP13A2 KO cells³. SPM and SPD are abundant organic polycations that support cell function, but at

Fig. 4 | Loss of ATP13A2 orthologues exacerbates the toxicity of polyamines in primary neurons and in *C. elegans*. **a**, Lentiviral knockdown of *Atp13a2* in isolated mouse cortical neurons (miR-3 or miR-5) was confirmed by quantitative PCR relative to *Gapdh*. miRNA against firefly luciferase (miR-Fluc) was used as a negative control. **b**, miR-Fluc and *Atp13a2* knockdown neurons were transduced with Fluc, wild-type human ATP13A2 or ATP13A2(D508N). SPM-induced cytotoxicity was assayed via TUNEL staining. Left, representative confocal images depicting TUNEL-positive cells; right, box and whisker plots showing quantification of the TUNEL staining. **c**, The indicated worm strains were assessed for SPD toxicity. Worm lengths were determined as a read-out

high concentrations they become toxic¹⁸. Consistent with this, high levels of SPM or SPD reduced the viability of control cells after 24 hours (Extended Data Fig. 8a, b), which was paralleled by an increase in cell death (Fig. 3a); ornithine and putrescine were not cytotoxic (Extended Data Fig. 8c, d). Notably, loss of ATP13A2 activity exacerbated the toxicity of SPM and SPD (Fig. 3a, Extended Data Fig. 8a, b), which may be a direct consequence of lysosomal polyamine accumulation (Fig. 2d) leading to lysosomal dysfunction. At a time point preceding cell death (4 hours; Extended Data Fig. 8e), lysosomal acidification was compromised in KO and KO/D508N cells, which was aggravated upon SPM exposure (Fig. 3b). This pH-neutralizing effect of SPM was absent in control and KO/WT cells (Fig. 3b).

Lysosomal alkalization may explain the decreased lysosomal degradation potential (Fig. 3c) and cathepsin D activity (Extended Data Fig. 8f) that is observed in KO and KO/D508N cells. However, the activity of cathepsin B increased at toxic SPM levels (Fig. 3d), in line with its higher pH optimum and most likely due to impaired lysosomal membrane integrity¹⁹, which is a driver of lysosome-dependent cell death²⁰. We then confirmed, using an acridine orange-based assay, that lysosomal membrane integrity in ATP13A2 KO and KO/D508N cells was impaired; this was more prominent after SPM challenge,

for toxicity. Scale bar, 100 μm. Top, quantification; bottom: representative images. **d**, Illustration of the proposed mechanism of endolysosomal polyamine uptake and transfer into the cytosol via ATP13A2. (–), vehicle-treated sample; EE, early endosome; LE, late endosome; LYS, lysosome. Data are presented as mean (**a**) or as box and whisker plots (**b**, **c**, horizontal line, median; box boundaries, 25th and 75th percentiles) with individual data points representing replicates. The number of independent biological replicates were as follows: *n* = 2 (**a**); *n* = 3 (**b**, **c**). Analysis was performed using one-way ANOVA with Tukey's test (**b**, **c**).

a phenotype that was absent in control and KO/WT cells (Fig. 3e). SPM treatment also increased the number and size of endogenous galectin 3 punctae, representative of lysosomal rupture, in KO and KO/D508N cells only (Fig. 3f). Lysosomal rupture was further confirmed by the loss of endolysosomal FITC–dextran punctae and a more diffuse, cytosolic cathepsin B staining in the KO and KO/D508N cells (Extended Data Fig. 8g, h). In line with reported findings²¹, exposure to acidic nanoparticles restored lysosomal pH and functionality (Fig. 3b, c), prevented cathepsin B activation (Fig. 3d) and recovered the intactness of lysosomal membranes (Fig. 3e), ultimately reducing SPM-induced cell death (Fig. 3g). Moreover, pharmacological inhibition of cathepsin B significantly reduced SPM toxicity in the KO and KO/D508N cells (Fig. 3h).

ATP13A2 is protective in higher disease models

Lysosomal polyamine toxicity may be relevant in the context of neurodegeneration, because isolated mouse cortical neurons with miRNA-mediated *Atp13a2* knockdown (Fig. 4a) were more susceptible to SPM-induced cell death than control neurons (Fig. 4b). Notably, the increased sensitivity to SPM observed for neurons in which *Atp13a2* was knocked down was attenuated either by inhibition of cathepsin B

(Extended Data Fig. 9) or by rescue with wild-type human ATP13A2, but not by the D508N mutant (Fig. 4b).

Finally, SPD exposure was found to hamper growth capacity in *C. elegans* N2 (wild-type); this was marked by developmental delay and hence reduced worm length. These effects were exacerbated in worms deficient in ATP13A2 orthologues (*catp-5(O)*, *catp-6(O)* or *catp-7(O)*²²) (Fig. 4c). The phenotype of CATP-6- and CATP-7-deficient strains was rescued by wild-type CATP-6 or CATP-7, but not by a catalytically dead variant, showing that the transport activity is required (Fig. 4c).

Discussion

Polyamines are physiologically important polycations that are tightly regulated by a complex interplay of import, export, synthesis and degradation¹⁸. As a polyamine transporter that controls the cellular polyamine content, ATP13A2 emerges as a member of the mammalian polyamine transport system¹⁸. Extracellular polyamines most probably bind to plasma membrane heparan sulfate proteoglycans²³ and enter the cell through endocytosis²⁴ before transport into the cytosol by ATP13A2 (Fig. 4d). In addition, the other P5B-ATPases (ATP13A3, ATP13A4 and ATP13A5) that reside in the endosomal system share high sequence similarity in the substrate-binding region¹², and may belong to the mammalian polyamine transport system.

Genetic insights suggest a major role for lysosomal dysfunction in Parkinson's disease, contributing to α -synuclein aggregation and mitochondrial dysfunction²⁵. Here we demonstrate that impaired lysosomal polyamine export represents a lysosome-dependent cell death pathway that may be implicated in ATP13A2-associated neurodegeneration. In addition, defective ATP13A2 leads to a reduction in cellular polyamine content, which may potentiate the disease phenotype because polyamines are scavengers of heavy metals and reactive oxygen species²⁶ and regulate autophagy²⁷. The dual effect of ATP13A2 on both lysosomal and cytosolic polyamine levels may explain the broad phenotype that is associated with its loss of function⁵. Other genes that are related to Parkinson's disease may also affect ATP13A2 functionality, or may be affected by disrupted polyamine homeostasis. Polyamine levels decline with age, whereas polyamine supplementation increases lifespan in several model organisms²⁷. Conversely, defective SPM synthase causes Snyder–Robinson syndrome—a form of X-linked intellectual disability²⁸—and reduced expression of SPD/SPM *N*¹-acetyltransferase 1 has been implicated in Parkinson's disease²⁹. Modulation of polyamine homeostasis may therefore be considered for neuroprotective therapy.

In conclusion, ATP13A2 dysfunction prevents late endolysosomal polyamine export and sensitizes cells to lysosomal disruption by exogenous polyamines.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41586-020-1968-7>.

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Methods

Data reporting

No statistical methods were used to predetermine sample size. The experiments were not randomized and the investigators were not blinded to allocation during experiments and outcome assessment.

Materials

The following reagents were purchased from Sigma-Aldrich: sodium orthovanadate (S6508), CaCl_2 (C3881), ZnCl_2 (Z0152), MnCl_2 (M3634), FeCl_3 (I57740), SPM (S3256), SPD (S2626), N^1 -acetylspermine trihydrochloride (O1467), N^1 -acetylspermidine hydrochloride (9001535-1), N^6 -acetylspermidine dihydrochloride (A3658), putrescine dihydrochloride (P7505), L-arginine (A5006), L-ornithine monohydrochloride (O2375), histamine (H7250), agmatine sulfate salt (A7127), dopamine hydrochloride (H8502), cadaverine (D22606), yeast nitrogen base without amino acids (Y0626), yeast drop-out mix without uracil (Y1501), glucose (G8720), streptavidin sepharose (GE17-5113-01), thrombin (GE27-0846-01), DMSO (276855), DL- α -difluoromethylornithine (DFMO; D193), Dynasore (D7693), Pitstop 2 (SML1169), 4-methylumbelliferyl heptanoate (MUH, M2514), propidium iodide (P4170), CA-074 (C5732), fluorescein isothiocyanate-dextran (FITC-dextran, 46945), DAPI (D9542), anti-ATP13A2 antibody (A3361), anti-GAPDH antibody (G8795), Resomer RG 503H (719870) and SigmaFast protease inhibitor (S8820). In addition, 18:1 PtdIns(3,5)P₂ (1,2-dioleoyl-*sn*-glycero-3-phospho-(1'-myo-inositol-3',5'-bisphosphate) (ammonium salt); 850154), 18:1 phosphatidic acid (1,2-dioleoyl-*sn*-glycero-3-phosphate (sodium salt); 840875) and egg phosphatidylcholine (840051) were obtained from Avanti Polar Lipids. Bovine serum albumin (BSA; 3854.3) was obtained from C. Roth. Yeast extract (103753.0500) was purchased from VWR, and *n*-dodecyl- β -D-maltopyranoside (DDM; 1758-1350) was purchased from Inalco. We obtained Bio-Beads SM-2 resin (1523920) from Bio-Rad. ³H-SPM (ART 0471) was ordered from ARC. APCHA (*N*-(3-aminopropyl) cyclohexylamine; sc-202715) and 4MCHA (*cis*-4-methylcyclohexylamine; sc-272662) were purchased from Santa Cruz Biotechnology. Genistein (ab120112), anti-galectin-3 antibody (ab2785), anti-LAMP1 antibody (ab24170) and anti-cathepsin B antibody (ab58802) were purchased from Abcam. TRYPLE (12604021), AO (A1372) and DQ-Green BSA (D12050) were ordered from Life Technologies.

HEK-293T cells were purchased from ATCC and certified by ATCC via STR genotype analysis. SH-SY5Y cells were purchased from ATCC and certified by ATCC via STR genotype analysis. SH-SY5Y cells from an in-house collection were authenticated via DNA fingerprinting (Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH).

Preparation of compounds and inhibitors

All polyamines, diamines, monoamines and amino acids were prepared to a final stock concentration of 500 mM (200 mM in the case of SPM) in 0.1 M MOPS-KOH (pH 7.0). DFMO was prepared to a final stock concentration of 500 mM in Milli-Q H₂O. The inhibitors 4MCHA and APCHA were dissolved in DMSO to a final stock concentration of 200 mM. The endocytosis inhibitors Dynasore, genistein and Pitstop 2 were dissolved in DMSO to final concentrations of 50 mM, 25 mM and 25 mM, respectively. The cathepsin B inhibitor CA-074 was dissolved in DMSO to a final concentration of 25 mM.

Generation of SH-SY5Y cell models

SH-SY5Y human neuroblastoma cells were transduced with lentiviral vectors to obtain stable overexpression of firefly luciferase (Fluc) or human ATP13A2 (isoform 2, wild-type (ID: NP_001135445), indicated disease or catalytic mutants) and maintained as described previously^{9–11}. The catalytic mutants A467V on M4, D962N on M6 and K1062A on M8 were generated by mutagenesis. P5-type ATPases were discovered by genome sequence analysis 20 years ago and contain highly conserved

motifs for function and substrate binding³¹. The A467V mutation converts PPALP of the predicted substrate-binding site in transmembrane segment M4 into PPVLP that is present in ATP13A5^{4,30}. Also, neighbouring membrane helices contribute to substrate coordination in P-type ATPases, which often relies on conserved and charged residues, such as D962 in M6 and K1062 in M8 of ATP13A2⁴. Furthermore, mutants associated with Kufor-Rakeb syndrome (T512I^{3,32,33} and G872R³⁴) or early-onset Parkinson's disease (T12M², G528R² and A741T^{35–37}) were generated. All cell lines were produced at varying viral vector titres and assessed for equal expression to wild-type ATP13A2.

For CRISPR-Cas9-mediated knockout of ATP13A2, the lentiviral vector lentiCRISPRv2 (Addgene, 52961)³⁸ was used. First, the Cas9 cassette was transformed into a high-fidelity Cas9 by Gibson assembly with a gBlock gene fragment (Integrated DNA Technologies) of the Cas9 portion encoding a protein product with high-fidelity mutations (N497A/R661A/Q695A/Q926A). This Cas9 variant triggers fewer off-target events while retaining its on-target activity³⁹. A single-guide RNA (sgRNA) targeted to *Atp13a2* was designed taking into account a high on-target efficiency, using sgRNA Designer (<https://portals.broadinstitute.org/gpp/public/analysis-tools/sgRNA-design>)⁴⁰, and a low off-target efficiency, via CRISPR Design (<http://crispr.mit.edu/>). A lentiviral CRISPR-Cas9 high-fidelity expression plasmid was created by inserting fragments that contained an sgRNA sequence of ATP13A2 (forward, 5'-CACCGTCAGGGTCCCATAACCGGT; reverse, 5'-AAACACCGGTTATGGGACCCTGACC) into the lentiCRISPRv2 vector. The generated CRISPR-Cas9 high-fidelity ATP13A2 plasmid (1,000 ng), the packaging plasmid pCMV- Δ R8.91 (900 ng) and the envelope plasmid pMD2G-VSV-G (Addgene, 12259) (100 ng) were mixed together with 200 μ l of JetPrime buffer and 4 μ l of JetPrime reagent (Polyplus-transfection) for transfection of HEK-293T cells according to the manufacturer's protocol. After 4 h at 37 °C and 5% CO₂, the serum-free medium was replaced with DMEM/F12 (Dulbecco's modified Eagle's medium, Nutrient Mixture F-12) supplemented with 10% fetal calf serum (heat-inactivated). After 48 h, the lentiviral vectors were collected by passing the medium through a 0.45- μ m filter, and 0.5 ml of this medium was used to transduce SH-SY5Y cells supplemented with 8 μ g ml⁻¹ polybrene (Sigma-Aldrich). 24 h after transduction, cells were selected in 3 μ g ml⁻¹ puromycin (Sigma-Aldrich) and passaged three times before single clones were isolated via serial dilution. The resulting cells were examined by quantitative PCR (qPCR) (*ATP13A2* forward, 5'-ACCGGTTATGGGACCCTGAC; *ATP13A2* reverse, 5'-GTGATAGCCGATGACCCTCC) with *HPRT* and *TBP* as internal controls (*HPRT* forward, 5'-TGAGGATTTGAAAGGGTGT; *HPRT* reverse, 5'-ACATCTCGA GCAAGACGTTTC; *TBP* forward, 5'-CGGCTGTTAACTTCGCTTC; *TBP* reverse, 5'-CACACGCCAAGAACAGTGA) and western blotting. For rescue experiments, ATP13A2 knockout cells were stably transduced with lentiviral vectors expressing wild-type ATP13A2 or the D508N mutant in which both cDNAs were modified with synonymous mutations at the sgRNA target site. All cell lines were routinely assessed for mycoplasma and cultured for a maximum of 20 passages.

Membrane fractionation

SH-SY5Y cells were seeded in 15-cm dishes at a density of 6×10^6 cells per plate. The cells were collected 24 h later after trypsinization and brief centrifugation (300g, 5 min). Subcellular fractionation was performed by differential centrifugation, as described previously^{9–11}. The microsomal protein concentration was measured using the bicinchoninic acid assay (Thermo Fisher Scientific, Pierce) according to the manufacturer's instructions.

ATPase assay

The ATPase activity of ATP13A2 was assessed using a commercially available luminescence assay (ADP-Glo Max assay, Promega) that monitors the production of ADP via luciferase activity. Substrate screen was designed to include candidates previously postulated in

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the literature^{6–8,41–45}. The reactions were performed for 30 min (37 °C) in a final volume of 25 µl. The assay reaction mixture contained 50 mM MOPS-KOH (pH 7), 100 mM KCl, 11 mM MgCl₂, 1 mM DTT, 195 µM DDM, various concentrations of the indicated compound, and either microsomes (5 µg) collected from SH-SY5Y cells overexpressing ATP13A2 (wild-type or mutants) or purified ATP13A2 (0.3–0.5 µg). When purified ATP13A2 was used, we included 125 µM phosphatidic acid, 125 µM PtdIns(3,5)P₂ and 19.5 µM DDM in the reaction buffer. The assay was started by the addition of 5 mM ATP and was terminated by adding 25 µl of ADP-Glo Reagent. The 96-well plate was then incubated for 40 min at room temperature, followed by the addition of 50 µl of ADP-Glo Max Detection Reagent. After 40 min, luminescence was detected using a FlexStation 3.0 system (Molecular Devices). Dose–response curves and n , K_m and V_{max} values were calculated using GraphPad Prism Software (GraphPad Software).

Autophosphorylation assay

The autophosphorylation activity of ATP13A2 on the conserved D508 residue was measured as described previously^{9,12,17}. In brief, microsomes (20 µg) or purified ATP13A2 (1 µg) were incubated with radioactively labelled ATP in the presence of the indicated SPM or ornithine concentrations, and after 1 min the reaction was stopped. In the case of purified ATP13A2, 125 µM phosphatidic acid and 125 µM PtdIns(3,5)P₂ were included in the reaction mixture. To determine the sensitivity of the ATP13A2 phosphoenzyme to ATP or a combination of ATP and SPM, 30 s after adding ³²P-ATP samples were incubated with non-radioactive ATP (5 mM in experiments using microsomes, 1 mM for purified ATP13A2) and SPM (1 mM) before the reaction was stopped at the indicated time points. The incorporation of ³²P was visualized after SDS–PAGE under acidic conditions and subsequently detected by autoradiography (microsomes) or liquid scintillation counting (purified ATP13A2) (Liquid Scintillation Analyzer TRI-CARB 2900TR).

Transformation and overexpression of ATP13A2 in yeast

The *S. cerevisiae* W303-1B/Gal4-ΔPep4 strain (leu2-3, his3-11,15, trp1-1::TRP1-GAL10-GAL4, ura3-1, ade2-1, canr, cir⁺, ΔPep4 MAT; a gift from R. Lopez Marques) was transformed according to the lithium acetate/single-stranded carrier DNA/polyethylene glycol method⁴⁶, with the pYEP60 vector containing a yeast codon-optimized version of human ATP13A2 variant 2 (wild-type or the catalytically dead E343A mutant) followed by a thrombin cleavage site and a C-terminal BAD tag^{47,48}. The transformation mixture was grown for 48 h at 30 °C on minimal medium agar plates lacking uracil (0.54% yeast nitrogen base without amino acids, 0.12% yeast drop-out mix without uracil, 2% glucose and 2% agar) to select yeast colonies that acquired the plasmid. These colonies were then cultured in 20 ml of MM-Ura medium (0.67% yeast nitrogen base without amino acids, 0.19% yeast drop-out mix without uracil, and 2% glucose) and grown for 24 h at 28 °C and 200 rpm. The MM-Ura yeast pre-culture was used to inoculate 100 ml of MM-Ura medium to a final OD₆₀₀ of 0.2, followed by a 12-h incubation period (28 °C and 200 rpm). The second pre-culture was inoculated into 4.5 l of YPGE2X medium (2% yeast extract, 2% bactopectone, 1% glucose and 2.7% ethanol) to a final OD₆₀₀ of 0.05 and grown for 36 h (28 °C and 175 rpm). ATP13A2-BAD expression was induced with 2% galactose, followed by a second galactose induction 12 h later. After another 12 h, the pellet was collected (1,000g, 10 min, 4 °C).

Yeast membrane preparation

Yeast cells were broken with glass beads using a BeadBeater (BioSpec products). The lysis buffer contained 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.6 M sorbitol, 1 mM phenylmethylsulfonyl fluoride and SigmaFast protease inhibitor. To remove cell debris and nuclei, the crude extract was centrifuged at 2,000g for 20 min (4 °C). The supernatant (S1) was centrifuged at 20,000g for 20 min (4 °C) to pellet the heavy membrane fraction (P2), and the resulting supernatant (S2) was further

centrifuged at 200,000g for 1 h (4 °C). The resulting pellet (that is, the light membrane fraction, P3) was resuspended in 20 mM HEPES-Tris (pH 7.4), 0.3 M sucrose and 0.1 mM CaCl₂. The total protein concentration was determined using a Bradford assay (B6916, Sigma-Aldrich).

Purification of ATP13A2–BAD

The method used to purify ATP13A2 was based on the purification of overexpressed Drs2p from yeast membranes⁴⁷. Yeast P3 membranes were diluted to 5 mg of total protein per ml in SSR buffer (50 mM MOPS-KOH (pH 7), 100 mM KCl, 20% glycerol, 5 mM MgCl₂, 1 mM DTT, and SigmaFast protease inhibitor cocktail) and solubilized using DDM, with a detergent-to-protein ratio of 1:1. The samples were stirred on ice for 30 min, followed by centrifugation (100,000g, 1 h, 4 °C) to pellet non-solubilized membranes. The solubilized material was incubated with streptavidin beads for 4 h (4 °C) to enable binding of BAD-tagged ATP13A2 to the resin. To eliminate unbound material, the resin was washed four times with three resin volumes of SSR buffer supplemented with 0.5 mg ml^{−1} DDM. Subsequent cleavage by thrombin (0.0625 U per mg total protein) enabled the release of ATP13A2 from the beads by overnight incubation at 4 °C. Finally, a Vivaspin Turbo 4 concentrator (100 kDa molecular weight cut-off, Sartorius) was used to concentrate the sample. The protein concentration was determined using a Bradford assay. The quality of the purification was evaluated via SDS–PAGE followed by Coomassie staining or immunoblotting, as described previously^{9–11}. Furthermore, the purified ATP13A2 sample was analysed by linear mode MALDI–TOF MS (matrix-assisted laser desorption time-of-flight mass spectrometry; Applied Biosystems 4800 Proteomics Analyzer) in the presence of α-cyano-4-hydroxycinnamic acid as matrix and after C4 omix (Agilent) pipette tip purification.

Reconstitution of yeast membranes

To reconstitute yeast membranes, we followed a similar strategy as described before⁴⁹. P3 membranes from the yeast membrane preparation expressing the ATP13A2–BAD construct were solubilized in buffer T (10 mM Tris-HCl (pH 7.4) and 1 mM EDTA) supplemented with 1.4% DDM. After removing the insoluble fraction by ultracentrifugation (30 min, 200,000g), the detergent extract was supplemented with 4.5 mM egg phosphatidylcholine and 0.5 mM 18:1 phosphatidic acid (in buffer T containing 0.7% DDM). The extract was then treated with Bio-Beads to remove the DDM and reconstitute proteoliposomes (that is, ‘no ATP inside’ condition). To generate proteoliposomes that contained intraluminal ATP (that is, ‘ATP inside’ condition), we added 5 mM ATP and an ATP-regenerating system before incubation with the Bio-Beads. Finally, the vesicles were recovered by ultracentrifugation (1 h, 200,000g) and resuspended in buffer T. The protein concentration was determined using a Bradford assay.

Transport assay using reconstituted vesicles

Uptake of ³H-SPM into freshly prepared vesicles was measured within 60 min. The above-described vesicles (‘no ATP inside’ or ‘ATP inside’) were diluted to 1 µg µl^{−1} in buffer T. The reactions were performed for 10 min (30 °C) in a final volume of 1 ml. The assay reaction mixture contained 50 mM MOPS, 100 mM KCl, 11 mM MgCl₂, 1 mM DTT and reconstituted vesicles (45 µg). The reaction was started by adding 1 mM ³H-SPM. For the condition ‘no ATP inside’, 5 mM ATP and an ATP-regenerating system were added after Bio-Bead treatment, before the addition of ³H-SPM. The reaction was stopped by filtering the samples through Millipore filters (0.45 µm). After washing of the filters with assay buffer, radioactivity retained on the filters was counted using a liquid scintillation counter (Liquid Scintillation Analyzer TRI-CARB 2900TR).

Cellular transport assay and endocytosis assessment

BODIPY–SPD and BODIPY–SPM were synthesized as described previously (compounds 14 and 15, respectively)¹³. The cells were seeded in 12-well plates (1.0 × 10⁵ cells per well) and the next day the cells were

incubated with 5 μM BODIPY-SPM or BODIPY-SPD for 2 h before collection. To assess endocytosis, the cells were pre-treated (30 min) with the endocytosis inhibitors Dynasore (100 μM), genistein (50 μM) and/or Pitstop 2 (50 μM) before the addition of either 20 $\mu\text{g ml}^{-1}$ FITC-dextran or BODIPY-SPM (2 h and 37 °C). The cells were then collected (300g, 5 min), washed and resuspended in PBS containing 1% BSA. Finally, an Attune Nxt (Thermo Fisher Scientific) flow cytometer was used to record the mean fluorescence intensities (MFI) of 10,000 events per treatment.

Metabolomics

Cells were grown into a 6-well plate and extracted as described previously⁵⁰. In brief, the medium was removed and cells were washed with a 0.9% NaCl solution. The washing solution was removed and 150 μl of a 6% trichloroacetic acid (Sigma) was added for the extraction. Using a cell scraper, the full extract was transferred into an eppendorf and incubated for 30 min on ice. Insoluble material, such as precipitated proteins, was removed by centrifugation for 20 min at 20,000g at 4 °C. To 100 μl of the supernatant, 900 μl of a 100 mM sodium carbonate buffer (pH 9.0) was added. Next, 25 μl of isobutyl chloroformate (Sigma) was added and the mixture was incubated for 30 min at 35 °C. 800 μl of the reaction mixture was transferred to a 2 ml eppendorf tube and 1 ml of diethylether (Sigma) was added. The mixture was vortexed vigorously and placed for 15 min at 25 °C. 900 μl of the upper phase was transferred into an eppendorf and dried using a vacuum centrifuge. Finally, the dried extract was dissolved in 125 μl of a 50% acetonitrile (LC-MS grade, Merck) solution in water containing 0.2% acetic acid.

15 μl of the extract was loaded onto a Thermo Scientific Liquid Chromatography QQQ (Quantiva, Thermo Fisher Scientific) equipped with an ACQUITY UPLC BEH C18 (1.7 μm , 2.1 $\times$ 100 mm) column from WATERS. Solvent A consisted of ultrapure H₂O with 0.2% acetic acid while solvent B was acetonitrile (Merck) with 0.2% acetic acid; all solvents used were LC-MS grade. Flow rate remained constant at 250 $\mu\text{l min}^{-1}$, and the column temperature remained constant at 30 °C. A gradient for the separation of modified polyamines was applied as follows: from 0 to 2 min 20% B, from 2 to 10 min a linear increase to 85% B was carried out and 85% B was maintained until 17 min. At 18 min the gradient returned to 20% B. The method stopped at 22 min. The mass spectrometer operated in positive ion mode (3,500 V); the source settings were as follows: sheath gas at 50, aux gas at 10, the ion transfer tube was heated at 325 °C and the vaporizer temperature was set at 350 °C. The mass spectrometer operated in multiple reaction monitoring mode and used the following transitions: putrescine (parent m/z at 289.2 $\rightarrow$ fragment 215.2, collision energy at 10.25 V), SPD (parent m/z at 446.4 $\rightarrow$ fragment 198.2, collision energy at 23.6 V) and SPM (parent m/z at 603.4 $\rightarrow$ fragment 455.1, collision energy at 19.91 V). Peak area was integrated using the XCalibur Quan tool (version 4.2.28.14, Thermo Fisher Scientific).

Preparation and characterization of acidic nanoparticles

Acidic nanoparticles were prepared as previously described²¹. In brief, 31 mg of Resomer RG 503H (lactide to glycolide ratio 50:50, molecular weight 24–38 kDa) was dissolved in 3.1 ml of tetrahydrofuran and subsequently 200 μl of this solution was added to 20 ml of ultrapure water under sonication. The suspension was then concentrated using the rotary evaporator to a final volume of approximately 12 ml, resulting in a concentration of 0.167 mg ml^{-1} . The size distribution of prepared nanoparticles was measured using a Wyatt DynaPro DLS plate reader (Wyatt), using an 830-nm laser in a flat-bottom 384-well plate (Greiner) at 25 °C and 10 measurements were averaged per experiment.

MUH cytotoxicity assay

SH-SY5Y cells were seeded in 96-well plates (1×10^4 cells per well) and allowed to adhere overnight. The cells were subsequently treated with increasing doses of the indicated compounds for 24–48 h. After

exposure, the cells were washed with PBS and stained with 300 $\mu\text{g ml}^{-1}$ MUH (prepared in DMSO, dissolved in PBS) for 30 min at 37 °C. Cytotoxicity was read using a FlexStation 3.0 multi-well plate reader (Molecular Devices; excitation 360 nm, emission 460 nm, cut-off 455 nm). Data were expressed relative to control.

Propidium iodide exclusion assay

Cells were seeded in 12-well plates (1.0×10^5 cells per well) and the next day the cells were treated with increasing doses of SPM, alone or in combination with the cathepsin B inhibitor CA-074 (25 μM , 1 h pre-incubation) or acidic nanoparticles (180 ng ml^{-1} , 1 h pre-incubation), and incubated for 24 h at 37 °C. Thereafter, the cells were collected following trypsinization and a brief centrifugation (300g; 5 min), washed with PBS and stained with 1 $\mu\text{g ml}^{-1}$ propidium iodide (in PBS containing 1% BSA). An Attune Nxt (Thermo Fisher Scientific) flow cytometer was used to determine the proportion of propidium iodide-positive cells (10,000 events per treatment).

FITC-dextran-based lysosomal pH

The protocol was adapted from refs.^{51,52}. SH-SY5Y cells were seeded in 12-well plates (1.0×10^5 cells per well) and allowed to adhere overnight. Cells were exposed to 50 $\mu\text{g ml}^{-1}$ FITC-dextran for 72 h. Samples were then washed and placed in fresh medium for 2 h before treatment with SPM (10 μM) alone or in combination with acidic nanoparticles (180 ng ml^{-1} , 1 h pre-incubation) for a further 4 h. Samples were then collected by centrifugation (300g, 5 min) and washed in PBS. Cells were finally resuspended in 500 μl of PBS containing 1% BSA and FITC dual emission was assessed by flow cytometry (excitation 488 nm, emission 530 nm (BL1) and 600 nm (BL2)) of 10,000 events per condition using an Attune NXT flow cytometer (Thermo Fisher Scientific). The emission ratio (BL1/BL2) of all samples were compared to a standard curve, whereby signals were obtained from untreated cells resuspended in monensin (100 μM) containing Britton Robinson buffer with increasing pH (3.0–8.0).

Lysosomal degradative capacity

Cell lines were seeded in 12-well plates (1.0×10^5 cells per well) and the next day the cells were pre-treated with SPM (10 μM) for 1 h at 37 °C. For samples requiring exposure to acidic nanoparticles, cells were treated with 180 ng ml^{-1} 1 h before the addition of SPM. Subsequently, 5 $\mu\text{g ml}^{-1}$ DQ-Green BSA was added to the cells for a further 3 h (37 °C). Finally, the cells were collected (300g, 5 min), and the MFI of 10,000 events were assessed using an Attune Nxt (Thermo Fisher Scientific) flow cytometer.

Cathepsin activity assays

SH-SY5Y cells were seeded in 10-cm plates (2×10^6 cells per plate) and allowed to adhere overnight before treatment with 10 μM SPM for 4 h at 37 °C. For samples requiring the addition of acidic nanoparticles, 180 ng ml^{-1} nanoparticles were added 1 h before the addition of SPM. Next, the samples were collected using TRYPLE and a brief centrifugation (300g, 5 min). The activities of cathepsin B (ab65300) and cathepsin D (ab65302) were assessed using commercially available kits (Abcam) according to the manufacturer's instructions. MFI values were acquired using a FlexStation 3.0 multi-well plate reader (Molecular Devices).

Lysosomal membrane integrity

SH-SY5Y cells were seeded in 12-well plates (1.0×10^5 cells per well) and the next day the cells were incubated with 5 $\mu\text{g ml}^{-1}$ acridine orange (dissolved in medium) for 15 min at 37 °C. Thereafter, the medium was discarded, the cells were washed, and fresh medium was added. For samples requiring acidic nanoparticles, cells were treated with 180 ng ml^{-1} 1 h before the addition of SPM. The cells were then treated with 10 μM SPM for 4 h at 37 °C. Finally, the cells were collected and resuspended in PBS containing 1% BSA. The MFI of 10,000 events was

captured using an Attune Nxt (Thermo Fisher Scientific) flow cytometer.

BODIPY-SPM localization and lysosomal rupture analysis

For all immunofluorescent stainings, SH-SY5Y cell lines were seeded in 12-well plates (0.25×10^5 cells per well on coverslips) and the next day the cells were incubated with the indicated compounds for 4 h at 37 °C. For BODIPY-SPM analysis, cells were pulsed with 5 μ M BODIPY-SPM for 15 min, washed and chased in fresh medium for a further 105 min at 37 °C. After treatment, the cells were washed twice in PBS, fixed in 4% paraformaldehyde for 30 min (37 °C), washed in PBS and stored at 4 °C. For immunofluorescence staining, the cells were washed in PBS containing 0.5% Tween 20 (PBS-T), permeabilized in PBS containing 0.1% Triton X-100 (30 min) and blocked first in 0.1 M glycine (1 h) and then in PBS-T containing 1% fetal calf serum and 10% BSA (30 min). Gal-3-, LAMP1- and cathepsin B-specific antibodies were used at a dilution of 1:100–200 (in PBS-T containing 1% BSA) overnight at 4 °C. Subsequently, the samples were washed and incubated with Alexa Fluor secondary antibody (1:1,000; 30 min). To assess FITC-dextran release, cells were loaded with 50 μ g ml⁻¹ FITC-dextran for 72 h, cells were then washed and placed in fresh medium for 1 h before the addition of SPM (10 μ M). To visualize the nucleus, all samples were stained with DAPI (200 ng ml⁻¹, 15 min). After staining, the samples were fixed to slides, and images were acquired using an LSM780 or LSM880 confocal microscope (Zeiss). For the acquisition of BODIPY-SPM, images were taken with equal settings to confirm uptake potential of ATP13A2 (Extended Data Fig. 1i). To assess the intracellular distribution of BODIPY-SPM (Fig. 2d, Extended Data Fig. 1j–l), microscope settings were optimized per cell type to enable a comparable assessment of BODIPY-SPM localization in KO/WT and KO/D508N cells.

Neuron isolation

Primary cortical neurons were derived from FVB/N mice embryos at embryonic day (E)16. Pregnant mice were euthanized on gestation day 16 by cervical dislocation. The brains of E16 mouse pups were collected and placed in a dish containing calcium- and magnesium-free Hanks' Balanced Salt Solution (HBSS, Life Technologies, 14180-046) on ice. Both cerebral hemispheres were separated from the cerebellum. Meninges were removed from the cerebral hemispheres and the brain cortices dissected. Brain cortices were collected, washed twice and digested with 0.05% trypsin (Life Technologies, 25300-054, 10 min at 37 °C). The trypsin reaction was terminated by the addition of 7 ml HBSS and 1 ml of horse serum. Cells were separated by pipetting and filtration through a cell strainer (40 μ m, Falcon, 352340). Cells were centrifuged at 1,000 rpm for 5 min (4 °C), the supernatant discarded and the pellet suspended in 5 ml Dulbecco's modified Eagle's medium (DMEM; Sigma-Aldrich, D6546) containing GlutaMAX (Life Technologies, 31966-021), 5% horse serum (Life Technologies, 26050-088) and 20 mM glucose (Sigma-Aldrich, 8270). Primary cortical neurons were plated in 12-well plates, on coverslips coated with poly-D-lysine (Sigma-Aldrich, P6407). After an overnight incubation, cell medium was exchanged for Neurobasal medium (Life Technologies, 21103-049) supplemented with 2 mM L-glutamine (Life Technologies, 25030-24) and 2% B27 (Life Technologies, 17504-044).

Atp13a2 knockdown and rescue in isolated cortical neurons

For knockdown, microRNA (miR)-based short-hairpin lentiviral vectors were generated as described⁵³. The two most potent miRs against mouse *Atp13a2* (mouse miR-3, CCACGCCGAAACACTCGTTATA and mouse miR-5, CGCCGAAACACTCGTTATAGAA) were used to induce knockdown in mouse primary neurons. A miR targeting Fluc was used as a control (miR-Fluc, ACGCTGAGTACTTCGAAATGTC). Primary neurons were transduced 4 days after isolation, 72 h before experimentation. For rescue of ATP13A2 expression in knockdown conditions, neurons were subjected to a second round of transductions with either human

wild-type or the D508N variant of ATP13A2, 24 h after the addition of the miR. Fluc was used as an overexpression control. At day 7 post-isolation, cells were treated with 10 μ M SPM for 24 h. To test the contribution of cathepsin B to SPM-induced cell death, neurons were pre-treated (30 min) with 10 μ M CA-074. Knockdown efficiency was validated with qPCR on mRNA levels 72 h after transduction using the following primers (*Atp13a2* forward, CATGGCCCTCTACAGCCTGA; *Atp13a2* reverse, CTCATGAGCACCGCAACCGT) with *Gapdh* as internal control (forward, TGTGTCCGTCGTGGATCTGA; reverse, CCTGCTTACCACCTTCTTGA). All mouse primary neuron experiments were carried out in accordance with the European Communities Council Directive of November 24 1986 (86/609/EEC) and approved by the Bioethical Committee of the KU Leuven (Belgium) (ECD project P185-2014).

TUNEL staining

TUNEL staining was assessed according to the manufacturer's protocol for the Click-iT Plus TUNEL assay (Thermo Fisher Scientific, C10617). DAPI was used as a nuclear counterstain and images were acquired using a LSM780 confocal microscope.

Caenorhabditis elegans

During routine culture, nematodes were grown on NGM (nematode growth medium) using *Escherichia coli* strain AMA1004 as food source^{54,55}. In order to reduce the likelihood that bacterial catabolism and divalent cations would interfere with SPD assays, these were done using DCDA (divalent-cation-depleted agar) plates, which do not permit bacterial growth. This medium contains 2% agar (Carl Roth 5210) that has been washed with 50 mM EDTA, followed by multiple washes with reverse osmosis-purified water; 50 mM HEPES pH 7.4; and 20 μ g ml⁻¹ kanamycin. SPD trihydrochloride (1 M stock solution; Sigma S2501) was added to a final concentration of 5 mM after micro-waving to melt the agar. Assays were performed in 35-mm plates that contained 1 ml of DCDA. Approximately 2 μ l of *E. coli* was transferred to each assay plate from the lawn of a seeded NGM plate. In each case, multiple adult hermaphrodites were added to the assay plate and allowed to lay eggs for 8 h (datasets 1 and 3) or 15 h (dataset 2) at 23.5 °C, then removed. Incubation of plates at 23.5 °C was continued until the 0 mM SPD N2 plates contained many mid-late stage L4s, at which point worms were rinsed off the plates and stored in microfuge tubes at -20 °C. Worm lengths were determined by mounting the thawed (dead) animals on an agarose pad covered by a coverslip, capturing images using a Leica M205 FA microscope equipped with digital camera plus software, and measuring the length of each worm from snout to tail tip using ImageJ.

C. elegans expresses three ATP13A2 orthologues: CATP-5, CATP-6 and CATP-7. Therefore, in this study the following mutant alleles were used, each of which was backcrossed to N2 Bristol at least three times: *catp-6(ok3473)* IV, *catp-7(tm4438)* IV, *catp-5(tm4481)* X⁵⁶. Each of these is a null allele, so they are referred to in the text as *catp-#(0)*.

Transgenic strains carrying extrachromosomal arrays with the pRF4 *rol-6(su1006)* plasmid were generated by microinjection and maintained as described previously⁵⁷. Injection mixes typically contained 100 μ g ml⁻¹ pRF4, plus 30 μ g ml⁻¹ of the test construct. In some cases, *Pmyo-2::gfp* was included at approximately 5 μ g ml⁻¹ as an additional method for detecting transgenic animals. To generate transport-defective versions of *catp-6* and *catp-7*, plasmids carrying *catp-6::mKate2* and *catp-7::GFP*²² were modified to change the coding sequence for the conserved DKTGT autophosphorylation motif to NKTGT. Proper expression and subcellular localization of CATP-6::mKate2 and CATP-7::GFP was verified in the case of all transgenic strains that were used. (*catp-6(0);[catp-6(+)]*) and *catp-7(0);[catp-7(+)]* express CATP-6 and wild-type CATP-7 in the null background, respectively; *catp-6(0);[catp-6(D465N)]* and *catp-7(0);[catp-7(D503N)]* express CATP-6(D465N) and CATP-7(D503N) in the null background).

Statistics and reproducibility

Data are expressed as the mean $\pm$ s.e.m. or with individual data points (replicates of multiple independent experiments) shown on group means or box and whisker plots (indication of median, 25th percentile, 75th percentile and minimum to maximum value range). Flow cytometry was set up and gated as described in Supplementary Fig. 2. GraphPad Prism 7.04 was used to plot all graphs and to perform all of the required statistical and quantitative assessments. Statistical tests for each graph are described in the legend. The number of independent biological experiments for each panel is highlighted in the figure legends. For the cell biological experiments using the SH-SY5Y control, ATP13A2 KO, KO/WT and KO/D508N cell lines, each cell model is the sum of two independent clones, each performed a minimum of three independent times. For the quantification of immunoblots and radiograms, ImageJ and ImageQuant programmes were used. Experiments on various model systems were executed by different researchers, which provided consistent results that independently confirmed the major conclusions.

Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability

Gel source data for immunoblots and radiograms (Figs. 1, 2, Extended Data Figs. 1–3, 6) are available with the online version of the paper (Supplementary Fig. 1). All other datasets generated within this study are presented and analysed within this manuscript and are available from the corresponding author upon reasonable request.

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Acknowledgements This work was funded by the Fonds Wetenschappelijk Onderzoek (FWO, Research Foundation Flanders) (G094219N to P.V., G092714 and G080517N to V. Baekelandt, SBO Neuro-TRAFFIC S006617N to V. Baekelandt, P.V., W.A. and J.V.S. and 1503117N to S.M.), the KU Leuven (OT/13/091; LysoCaN C16/15/073 to P.V., J.V.S., W.A. and P.A.; OT/14/120 to V. Baekelandt) and the Queen Elisabeth Medical Foundation for Neurosciences (P.V. and V. Baekelandt). S.v.V. is an aspirant FWO research fellow (11Y7518N) and J.P.K. is the recipient of a strategic basic research doctoral grant of the FWO (1S18518N). J.L. received Lundbeck postdoctoral fellowships R209-2015-2704 and R171-2014-663. Funding for E.L. and J.Z. was provided by DFG grant LA3380/2-1. Some nematode strains were provided by the Caenorhabditis Genetics Center, which is funded by NIH Office of Research Infrastructure Programs (P40 OD010440). We thank P. P. Van Veldhoven (KU Leuven) for discussions; P. Chaltin (Center for Drug Design and Discovery) for financial support for V. Benoy; and M. Schuermans, T. Arslan, A. Florizoone, J. Van Asselberghs, J. Chen and N. Hamouda for technical assistance. We also acknowledge our frequent use of the facilities and equipment of the Leuven Viral Vector Core facility (KU Leuven), Mass Spectrometry Core (R. Derua, KU Leuven), Cell and Tissue Imaging Cluster (P. Vanden Bergh, KU Leuven) and the FACS Core (KU Leuven/VIB).

Author contributions The study was designed by P.V., S.M., S.v.V. and J.E.; C.V.d.H. generated all stable cell lines and analysed ATP13A2 knockdown; J.-P.D. performed and analysed the CRISPR experiments; R.V. and J.P.K. synthesized BODIPY-labelled polyamine analogues; J.P.K. performed and analysed acidic nanoparticle preparations; S.v.V. performed and analysed the biochemical experiments (except Extended Data Fig. 2f by V. Benoy); J.L. contributed to the ATP13A2 purification protocol; S.M. performed and analysed the cell biology experiments; B.G. performed metabolomics; G.G. isolated mouse neurons; E.L. and J.Z. performed *C. elegans* experiments; and P.V., S.v.V. and S.M. wrote the manuscript, which was reviewed by all authors.

Competing interests Patent WO-2018002350-A1 of KU Leuven describes methods for detecting compounds with therapeutic use that target ATP13A2 or related isoforms using biological material and assays described in the current manuscript. A second patent of KU Leuven describing ATP13A2 cell models described in this manuscript has also been filed.

Additional information

Supplementary information is available for this paper at <https://doi.org/10.1038/s41586-020-1968-7>.

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Peer review information Nature thanks Guillaume Lenoir, Hanne Poulsen, Taiji Tsunemi and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

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Extended Data Fig. 1 | ATP13A2 is a polyamine transporter. **a–d**, The ATPase activity of ATP13A2 was measured in solubilized microsomes (5 μ g) collected from SH-SY5Y cells stably overexpressing wild-type ATP13A2 (WT-OE) in the presence of 100 nM CaCl₂, MnCl₂, ZnCl₂ or FeCl₃ and 100 μ M SPD or SPM (D508N-OE as a negative control, wild type was referenced from Fig. 1b) (**a**) or in the presence of the indicated doses of inorganic ions and heavy metals CaCl₂, MnCl₂, ZnCl₂ or FeCl₃ (**b**), diamines (cadaverine, agmatine and the amino acid L-arginine) (**c**), monoamines (dopamine and histamine) (**d**) and acetylated polyamines (N¹-acetylspermine, N⁸-acetylspermidine or N¹-acetylspermidine) (**e**). As a reference for **c–e**, we plotted the dose–response curve of SPM from Fig. 1b. **f**, Microsomes (20 μ g) collected from SH-SY5Y cells that overexpress ATP13A2 were incubated for 60 s with [γ -³²P]ATP in the presence of 10 mM ornithine (ORN) or SPM (referenced from Fig. 1c). Left, a representative autoradiogram of the phosphoenzymes (EP); right, quantification. CON, control. **g**, The ATPase activity of purified ATP13A2 was assessed after 1 mM SPM was administered in the presence or absence of 0.25 mM orthovanadate (ORTH), a general P-type ATPase inhibitor (supplemented with 125 μ M phosphatidic acid/PtdIns(3,5)P₂; conditions (–) and (–)/SPM refer to Fig. 1f).

h, Purified ATP13A2 was incubated with [γ -³²P]ATP in the presence of 1 mM SPM, and radioactivity of the phospho-intermediate was assessed by scintillation counting. **i**, Comparison of the pulse (5 μ M, 15 min) chase (105 min, medium) BODIPY–SPM uptake in KO/WT and KO/D508N cell lines by confocal microscopy. Cells were subsequently stained with LAMP1 and imaged with the same laser settings by confocal microscopy. DAPI was used to visualize the nuclei. Scale bar, 5 μ m. **j**, Line intensity plots of the indicated dashed lines in Fig. 2d. **k**, Analysis of the Pearson's coefficient of LAMP1 and BODIPY–SPM for the images in Fig. 2d (KO/WT, 78 images; KO/D508N, 85 images). **l**, Mean fluorescence intensities (MFI) of BODIPY in DAPI-positive regions of samples shown in Fig. 2d (KO/WT, 233 nuclei; KO/D508N, 243 nuclei). Data are presented as the mean $\pm$ s.e.m. or mean with individual data points shown (points represent replicates), with $n = 3$ independent biological experiments. Analysis was carried out using one-way ANOVA with Dunnett's (**f**) or Tukey's (**a, g**) corrections, or by two-tailed t -tests (unpaired, **h** or Welch's, **k, l**). Fitted lines are semi-log lines (**b**) or nonlinear allosteric sigmoidal association (**c–e**). For gel source data, see Supplementary Fig. 1.

Extended Data Fig. 2 | Streptavidin-based purification of wild-type ATP13A2

and the catalytically dead E343A mutant. **a**, Coomassie staining showing the purification process for wild-type ATP13A2, starting from solubilized yeast membrane fractions, followed by streptavidin affinity chromatography and on-column thrombin cleavage to elute the protein. **b**, Western blot analysis of stages in the purification of ATP13A2. **c**, Bar graph depicting protein purity as determined by densitometry from Coomassie-stained SDS-PAGE. **d**, Mass spectrometry analysis of the purified ATP13A2 sample. Singly, doubly and triply charged species are indicated. **e**, To evaluate phosphoenzyme formation, yeast P3 membranes (20 µg) and purified ATP13A2 (1 µg) were incubated for 60 s with [γ - 32 P]ATP. As a positive control, microsomes collected from SH-SY5Y cells that overexpress wild-type ATP13A2 (20 µg) were used. The image is a

representative radiogram depicting the ATP13A2 phosphoenzyme. **f**, The ATPase activity of purified ATP13A2 (0.3 µg) was measured in the presence of 2 mM SPM and the indicated concentrations of the ATP13A2 regulatory lipids phosphatidic acid (PA) and PtdIns(3,5)P₂. **g**, Coomassie staining showing the purification process for ATP13A2(E343A). **h**, The ATPase activity of purified wild-type ATP13A2 or ATP13A2(E343A) (0.5 µg) was measured in the presence of the indicated concentrations of SPM with 125 µM phosphatidic acid and 125 µM PtdIns(3,5)P₂. Data are expressed as mean with individual data points (points represent replicates) (**c**, **h**). The number of independent biological experiments were as follows: $n = 3$ (**b**, **e**–**h**); $n = 6$ (**d**); $n = 22$ (**a**, **c**). Analysis was performed using one-way ANOVA with Tukey's post-hoc correction (**h**). For gel source data, see Supplementary Fig. 1.

Extended Data Fig. 3 | Confirmation of CRISPR–Cas9-mediated ATP13A2 knockout and subsequent rescue with wild-type ATP13A2 or the D508N mutant. a, The ATP13A2 knockout cell lines (KO) were generated by CRISPR–Cas9 in SH-SY5Y cells and confirmed by qPCR (top) and immunoblotting (bottom). *Atp13a2* mRNA expression was normalized to hypoxanthine phosphoribosyltransferase (HPRT) and TATA-sequence-binding protein (TBP), and GAPDH was used as a loading control for the ATP13A2 protein levels. Two

fragments of the same blot are depicted and separated by a dotted line. **b**, Rescue of ATP13A2 knockout was performed by lentiviral transduction to express wild-type ATP13A2 (KO/WT) and the catalytically dead mutant D508N, which was used as a negative control (KO/D508N). The expression of the ATP13A2 constructs was confirmed via immunoblotting. The number of biologically independent experiments were as follows: $n = 1$ (**a**, top panel); $n = 3$ (**a**, bottom panel, **b**). For gel source data, see Supplementary Fig. 1.

Extended Data Fig. 4 | Polyamine uptake by ATP13A2 complements cytosolic polyamine synthesis. **a–c**, Assessment of the cellular uptake of BODIPY-labelled polyamine analogues (**a**, **b**) or FITC-dextran (**c**) by flow cytometry. Uptake of BODIPY-SPD (**a**) or BODIPY-SPM (**b**) in SH-SY5Y cells overexpressing Fluc (negative control), wild-type ATP13A2 (WT-OE) or the catalytically dead mutant D508N (D508N-OE). The cells were incubated with 5 μ M BODIPY-SPM or BODIPY-SPD for 2 h before analysis by flow cytometry. **c**, Analysis of FITC-dextran uptake (as a measure of endocytic capacity) was performed in SH-SY5Y control (CON) cells with endogenous ATP13A2 expression, ATP13A2 knockout cells (KO) and rescue cell lines with expression of wild-type ATP13A2 (KO/WT) or the D508N mutant (KO/D508N). The cells were pre-treated for 30 min with a combination (combo) of endocytosis inhibitors Dynasore (100 μ M), genistein (50 μ M) and Pitstop 2 (50 μ M). The cells were incubated for an additional 2 h

with FITC-dextran at 37 °C, followed by flow cytometry. **d**, Schematic representation of polyamine synthesis. SRM, spermidine synthase; SMS, spermine synthase. Specific inhibitors are indicated in red. Control, KO, KO/WT or KO/D508N cells were subjected to inhibition of polyamine synthesis by DFMO (**e**), 4MCHA (**f**) and APCHA (**g**) before measuring cell viability via the MUH assay. All data represent the average of two independent CRISPR-Cas9 knockout and control clones. All data are presented as mean with data points overlaid (points represent replicates) or mean $\pm$ s.e.m. The number of biologically independent experiments were as follows: $n = 3$ (**a**, **b**); $n = 4$ (**c**, **e–g**). Analysis was performed using one-way ANOVA with Dunnett's (**a**, **b**) or Tukey's (**c**) post-hoc correction or two-way ANOVA with Dunnett's post-hoc correction (**e–g**). Fitted lines indicate nonlinear log(inhibitor) versus response (variable slope) (**e–g**).

Extended Data Fig. 5 | Catalytic and clinical mutations of ATP13A2 perturb polyamine function. **a**, Sequence alignment of predicted transmembrane helices M4 (left), M6 (middle) and M8 (right). The alignment was generated using Clustal Omega. We generated mutants in M4 (A467V), M6 (D962N) and M8 (K1062A). The A467V mutation converts the protein sequence PPALP of the predicted substrate-binding site in transmembrane segment M4 into the protein sequence PPVLP that is present in ATP13A5^{4,30}. Neighbouring membrane helices also contribute to substrate coordination in P-type ATPases, which often relies on conserved and charged residues, such as D962 in M6 and K1062 in M8 of ATP13A2⁴. **b**, Densitometry of the expression of catalytic mutants presented in Fig. 2e. **c**, Flow cytometric analysis of cellular BODIPY-SPD uptake in SH-SY5Y cells overexpressing wild-type ATP13A2, the D508N mutant or catalytic mutants. **d**, Quantification of ATP13A2 phosphorylation levels (EP) presented in Fig. 2g. **e**, Densitometry analysis of the expression of

disease-related mutants presented in Fig. 2i. **f**, Flow cytometric analysis of cellular BODIPY-SPD uptake in SH-SY5Y cells overexpressing wild-type ATP13A2, D508N or disease mutants. **g**, Quantification of ATP13A2 phosphorylation levels presented in Fig. 2k. All data are depicted as mean with individual data points (points represent replicates). The number of independent biological experiments were as follows: **n** = 3 **b, c, d** (D508N (SPM), A467V (SPM), D962N (SPM), and K1062A (SPM)), **e** (T12M, G872R), **f, g** (T12M (-), T12M (SPM), T512I (SPM), G528R (SPM), A741T (SPM), and G872R (SPM)); **n** = 4 **d** (D508N (-), A467V (-), D962N (-), and K1062A (-)), **e** (wild-type, T512I, G528R and A741T), **g** (wild-type (-), T512I (-), G528R (-), A741T (-), and G872R (-)); **n** = 5 **d** (wild-type (SPM)) and **g** (wild-type (SPM)). Analysis by one-way ANOVA with Dunnett's (**b, c, e, f**) or two-way ANOVA with Sidak's (**d, g**) post-hoc corrections.

a

	Source	K ± SEM (s ⁻¹) ATP sensitivity	K ± SEM (s ⁻¹) ATP/SPM sensitivity
ATP13A2 WT	Microsomes	0.066 ± 0.010	0.098 ± 0.008
ATP13A2 D962N	Microsomes	0.022 ± 0.019	0.020 ± 0.012
ATP13A2 WT	Purified protein	0.050 ± 0.012	0.136 ± 0.030
ATP13A2 E343A	Purified protein	0.035 ± 0.031	0.039 ± 0.015

Extended Data Fig. 6 | The ATP sensitivity of ATP13A2(D962N) and ATP13A2(E343A) is independent of SPM. **a**, Overview of rate constants of ATP13A2 phosphoenzyme decay following a chase with non-radioactive ATP with or without 1 mM SPM. **b**, After 30 s of incubating D962N microsomes (20 µg) with [γ -³²P]ATP, the time course of dephosphorylation after an ATP chase was measured in the presence or absence of SPM. The top panel shows a

b

representative autoradiogram of the phosphoenzymes (EP), whereas the bottom panel depicts the quantification of ATP13A2 phosphorylation levels. As a reference we plotted the wild-type curve, shown in Fig. 1d. Data are presented as the mean $\pm$ s.e.m. of $n = 4$ biologically independent experiments. Analysis by two-way ANOVA with Tukey's test (**b**). The fitted line indicates two-phase decay (**b**). For gel source data, see Supplementary Fig. 1.

Extended Data Fig. 7 | Predicted topology of ATP13A2. a, Homology model of ATP13A2 based on the structure of Na⁺/K⁺-ATPase (ATP1A1, PDB ID: 3A3Y) as a template, generated by iTASSER (<https://zhanglab.ccmb.med.umich.edu/I-TASSER/>)^{58,59}. **b,** Predicted membrane topology of ATP13A2 visualized by Protter⁶⁰ (<http://wlab.ethz.ch/protter>). ATP13A2 consists of 10 transmembrane helices (M1–10) and an N-terminal membrane-associated region (Ma)⁹. Kufor-Rakeb syndrome (KRS)-associated mutations are highlighted in red; early-

onset Parkinson's disease (PD)-associated mutations in green; hereditary spastic paraplegia (HSP)-associated mutations in light blue; neuronal ceroid lipofuscinosis (NCL)-associated mutations in orange. Catalytic mutations and mutations in the predicted substrate-binding region are highlighted in dark blue. Residues that were subjected to mutagenesis in this study are labelled (only in **a**). P-type ATPase signature motifs in the cytosolic domains are indicated in pink (only in **b**).

Extended Data Fig. 8 | Lysosomal functionality and recovery. a–d. The impact of exogenous polyamines on cell toxicity (24 h) and lysosomal functionality (4 h) was assessed in SH-SY5Y control cells (CON) with endogenous ATP13A2, ATP13A2 knockout cells (KO) and rescue cell lines with wild-type expression (KO/WT) or expression of the catalytically dead mutant D508N (KO/D508N) on the KO background. Cytotoxicity of SPD (**a**), SPM (**b**), ORN (**c**) and PUT (**d**) were assessed via a MUH cell-viability assay. **e**, Death of the aforementioned cells was assessed after 4 h of SPM exposure (10 μ M) by propidium iodide (PI)-based flow cytometry. **f**, Measurement of cathepsin D activity. **g, h**, Lysosomal rupture under basal (–) and SPM (10 μ M) conditions was assessed via loss of FITC–dextran (FITC–DEX) punctae (**g**) or loss of cathepsin B (CTSB)/LAMP1 colocalization (**h**). Confocal images depict representative images with or without SPM exposure (4 h, DAPI staining for nuclei was included as a reference). Scale bars, 10 μ m. The box and whisker plots in **g** depict the frequency (left) and size (right) of FITC–DEX punctae; in **h**

the Pearson coefficient of colocalization of cathepsin B and LAMP1. **i**, Lysosomal pH (Fig. 3b) was evaluated using the fluorescent probe FITC–dextran and a dual-emission ratiometric technique. FITC is excited at 488 nm and emission is analysed at 530 nm (BL1) and 610 nm (BL2). A pH calibration curve was generated using FITC–dextran in cells permeabilized with 100 μ M monensin and equilibrated with calibration buffers (pH 3–8). **j**, Representative size distribution of the acidic nanoparticles used in this study. Data are presented as the mean $\pm$ s.e.m. (**a–d, i**) or individual data points (representing replicates) overlaid on group means (**e**) or box and whisker plots (**f–h, line, median; box boundaries, 25th and 75th percentiles**). The number of independent biological experiments were as follows: $n = 3$ (**e–j**); $n = 4$ (**a–d**). Analysis was performed using two-way ANOVA with Dunnett’s (**a, b**) or Bonferroni’s (**c, d**) post-hoc corrections, or one-way ANOVA with Dunnett’s (**e**), Sidak’s (**f, g** (right)) or Tukey’s (**g** (left), **h**) post-hoc corrections. Fitted lines indicate nonlinear log(inhibitor) versus response (variable slope) (**a, b**).

Extended Data Fig. 9 | Inhibition of cathepsin B activity attenuates SPM-induced neuronal death. The effect of a cathepsin B inhibitor (CA-074, 10 μ M) on SPM-induced (10 μ M, 24 h) cell death in control (miR-Fluc) and *Atp13a2* knockdown (miR-3 and miR-5) neurons was assayed via TUNEL-based staining. Left, representative confocal images depicting TUNEL-positive neurons; right,

box and whisker plots with the quantification of the TUNEL staining. Data are presented as box and whisker plots (line, median; box boundaries, 25th and 75th percentiles) for which individual data points (representing replicates) are shown. $n = 3$ biologically independent experiments. Analysis by one-way ANOVA with Tukey's post-hoc correction.

Extended Data Table 1 | Apparent K_m and V_{max} values for ATP13A2 in the presence of various polyamines

	$K_m \pm \text{SEM} (\mu\text{M})$		$V_{max} \pm \text{SEM} (\text{nmol ADP/mg/min})$	
	Microsomal ATP13A2	Purified ATP13A2	Microsomal ATP13A2	Purified ATP13A2
Spermine	149 ± 34	76 ± 26	140 ± 6	159 ± 10
N ¹ -acetylspermine	286 ± 48	not tested	98 ± 5	not tested
Spermidine	~ 1700*	890 ± 501	~ 106*	194 ± 26

Data are derived from Fig. 1b, f and Extended Data Fig. 1e. Data represent $n = 3$ (spermidine, N¹-acetylspermine), or $n = 6$ (spermine) biologically independent experiments. *Estimated values (could not be accurately determined following fitting of the curve).

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a Confirmed

- ☐ ☒ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- ☒ ☐ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- ☐ ☒ The statistical test(s) used AND whether they are one- or two-sided
Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- ☐ ☒ A description of all covariates tested
- ☐ ☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- ☐ ☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- ☐ ☒ For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
Give P values as exact values whenever suitable.
- ☒ ☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- ☒ ☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- ☒ ☐ Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection

Typhoon TM FLA 9500 (phosphorimaging)
Flexstation 3 microplate reader (fluorescent and luminescent assays)
Bio-Rad ChemiDoc (western blot imager)
QuantaSmart TM (v2.03) (scintillation counting)
Attune Cytometric Software (v2.1) (flow cytometry)
ZEN SP5 2012 (confocal imaging)

Data analysis

GraphPad Prism Version 7.04 was used for data representation and statistical analysis. Image J and Zen 2.3 Lite were used for image analysis. Image Quant TL (version 8.1) was used for analysis of gel-based auto-phosphorylation assays. Image Lab (5.2.1) was used to analyze Western blots. XCalibur Quan tool (version 4.2.28.14) was used for polyamine quantification (LC MS). Flowing Software (v2.5.1) was used for flow cytometry analysis. i-TASSER (Protein structure and function predictions, <https://zhanglab.ccmb.med.umich.edu/I-TASSER/>). PyMOL (structure visualization, version 2.3)

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Source data for immunoblots and radiograms (Fig. 1-2, Extended data Fig. 1-3, 6) are available with the online version of the paper. All other datasets generated

within this study are presented and analysed within this manuscript and are available from the corresponding author upon reasonable request.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No statistical methods were used to pre-determine sample size. All fundamental experiments were performed a minimum of three times (independent biological experiments), including all relevant controls, to allow for the generation of S.E.M. Confirmation of ATP13A2 knockout (SH-SY5Y) was performed only once by qRT-PCR and N = 3 by western blot analysis. Knockdown of ATP13A2 in neurons was only confirmed two times by qRT-PCR.
Data exclusions	No data were excluded.
Replication	All experimental findings were reproduced in several independent biological experiments (N) with multiple technical replicates. For each figure panel, the number of independent experiments N is indicated in the figure legends. Conclusions were independently confirmed in different model systems (in vitro, in cell lines, isolated neurons and in vivo) handled by multiple researchers and across several laboratories.
Randomization	Samples were not randomized, but appropriate controls were included in each figure. For in vivo studies (nematodes), animals were evenly distributed such that each group had a similar mean/density at the start of the study.
Blinding	Analyses were not blinded because experiments were performed and analyzed by the same researchers. Biochemistry, cell biology and in vivo research were performed independently by different researchers and their findings support one another providing independent confirmation.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	<p>Primary antibodies used in this study with supplier, catalogue number and lot number, as well as the used dilution.</p> <p>SIGMA anti-ATP13A2 antibody; catalogue number A3361, lot # 076M4839, (Western Blotting, Rabbit, 1:1000) anti-GAPDH antibody; catalogue number G8795, lot # 076M4785V, (Western Blotting, Mouse, 1:5000)</p> <p>ABCAM anti-galectin-3 antibody; catalogue number ab2785, lot # GR3200865-11, (Immunofluorescence, Mouse, 1:200) anti-cathepsin B antibody; catalogue number ab58802, lot # GR3181508-13, (Immunofluorescence, Mouse, 1:100) anti-lamp1 antibody; catalogue number ab24170, lot # GR3235359-1, (Immunofluorescence, Rabbit, 1:200)</p>
Validation	<p>Concerning antibody specificity, we kindly refer to the supplier's websites and datasheets to find statements on specificity and citations for the use of the antibodies:</p> <p>anti-ATP13A2 antibody; https://www.sigmaaldrich.com/catalog/product/sigma/a3361?lang=en&region=BE anti-GAPDH antibody; https://www.sigmaaldrich.com/catalog/product/sigma/g8795?lang=en&region=BE anti-galectin-3 antibody; https://www.abcam.com/galectin-3-antibody-a3a12-ab2785.html</p>

anti-cathepsin B antibody; <https://www.abcam.com/cathepsin-b-antibody-ca10-ab58802.html>
 anti-lamp1 antibody; <https://www.abcam.com/lamp1-antibody-lysosome-marker-ab24170.html>

Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)	SH-SY5Y from ATCC (ATCC number CRL-2266™) (lot number 62431864) SH-SY5Y in house collection HEK-293T from ATCC (https://www.lgcstandards-atcc.org/Products/All/CRL-11268.aspx?geo_country=be)
Authentication	SH-SY5Y (ATCC); were certified by ATCC by STR genotype analysis (https://www.lgcstandards-atcc.org/en/Products/All/CRL-2266.aspx#documentation). SH-SY5Y cells; (from the in house collection) were authenticated via DNA fingerprinting (Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH). HEK-293T (ATCC); were certified by ATCC by STR genotype analysis (https://www.lgcstandards-atcc.org/Products/All/CRL-11268.aspx?geo_country=be#documentation)
Mycoplasma contamination	All cell lines were routinely tested for mycoplasma contamination using the MycoAlert Mycoplasma Detection kit (LT07-418) and no contaminations have been detected.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals	Mice - E16 FVB/N mice (females for embryos, neuronal isolation). C. elegans - N2 Bristols (Males and females, larvae to adult growth analysis)
Wild animals	No wild animals were used within this research
Field-collected samples	No field collected samples were used within this research
Ethics oversight	All mouse experiments were carried out in accordance with the European Communities Council Directive of November 24, 1986 (86/609/EEC) and approved by the Bioethical Committee of the KU Leuven (Belgium) (ECD project P185-2014).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Flow Cytometry

Plots

Confirm that:

- ☐ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- ☐ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- ☐ All plots are contour plots with outliers or pseudocolor plots.
- ☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Samples were harvested by TRYPLE, washed and stained. Prior to FACS, cells were resuspended in PBS containing BSA, filtered and stored on ice. Analysis was performed on living (non-fixed) cells.
Instrument	Attune NxT - Thermo Fisher Scientific
Software	Attune Cytometric Software (v2.1)
Cell population abundance	Flow cytometry was performed on cell lines and at no point tracked a subpopulation of a heterogeneous cell mixture. We assessed 10,000 events of the total cell population, including both living and dead cells.
Gating strategy	Total cells were gated (R1) to remove any signal contamination from debris. Upon selection live/dead cells were characterized in (RX/R1) or fluorescence acquired (R2). In all cases samples were gated on the position of the negative population (unstained) of each cell line. Detection was obtained within the relevant detection window.

- ☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.