Biallelic mutations in the 3′ exonuclease TOE1 cause pontocerebellar hypoplasia and uncover a role in snRNA processing

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Deadenylases are best known for degrading the poly(A) tail during mRNA decay. The deadenylase family has expanded throughout evolution and, in mammals, consists of 12 Mg2+-dependent 3′-end RNases with substrate specificity that is mostly unknown1. Pontocerebellar hypoplasia type 7 (PCH7) is a unique recessive syndrome characterized by neurodegeneration and ambiguous genitalia2. We studied 12 human families with PCH7, uncovering biallelic, loss-of-function mutations in TOE1, which encodes an unconventional deadenylase3,4. toe1-morphant zebrafish displayed midbrain and hindbrain degeneration, modeling PCH-like structural defects in vivo. Surprisingly, we found that TOE1 associated with small nuclear RNAs (snRNAs) incompletely processed splicosomal. These pre-snRNAs contained 3′ genome-encoded tails often followed by post-transcriptionally added adenoses. Human cells with reduced levels of TOE1 accumulated 3′-end-extended pre-snRNAs, and the immunosilosided TOE1 complex was sufficient for 3′-end maturation of snRNAs. Our findings identify the cause of a neurodegenerative syndrome linked to snRNA maturation and uncover a key factor involved in the processing of snRNA 3′ ends.

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The onset of pontocerebellar neurodegeneration occurs so early that it overlaps with neurodevelopment, and it is thus alternatively referred to as pontocerebellar hypoplasia (PCH)5. PCH7 (MIM 614969) is characterized by neurological deterioration, atrophy or hypoplasia of the pons and cerebellum, muscular hypotonia and breathing abnormalities, in combination with hypogonadism2. This combination of rare conditions suggests a unique syndromic association due to mutation of a single gene, but no locus or causative gene has been identified to date. We recruited 12 families meeting criteria for PCH7, including the index family on the basis of which the condition was defined (Fig. 1a), and we confirmed that the clinical features of these families matched those published for PCH7 (Supplementary Table 1). These features included reduced pons and cerebellum parenchyma (Fig. 1b), ventriculomegaly, thin corpus callosum and variable hypogonadism—ranging from absent gonads to ovarian and uterine remnants or atrophic and undescended testes (Supplementary Fig. 1a, b). All patients and families were enrolled in institutional review board (IRB)-approved protocols at referral institutions and provided consent for study. We performed whole-exome sequencing in the proband and parents from Egyptian families 1275 and 1603. Aligning the genomic variants uncovered a homozygous mutation in TOE1 encoding p.Glu220Lys (NC_000001.11) within a shared haplotype of 500 kb (Fig. 2a and Supplementary Fig. 2a, b), which is indicative of shared ancestry for these families. All ten additional families enrolled subsequently proved positive for biallelic mutations in TOE1 (Fig. 2a and Supplementary Table 2). The variants were all predicted to impair expression of full-length TOE1 or affect protein function6 by altering well-conserved amino acids (Fig. 2b) and were not observed
in our in-house database of 4,000 ancestry-matched exomes. We confirmed that each variant segregated according to a recessive mode of inheritance in all genetically informative members of each family, suggesting that TOE1 biallelic mutations underlie PCH7.

To test whether TOE1 missense mutations were likely to interfere with protein function, we modeled them on the structure of the protein encoded by paralogous CNOT7 (UniProt, Q9UIV1) (Supplementary Fig. 3a). Most variant residues were on the surface rather than within the RNA-binding cleft, suggesting that they are not likely to directly affect deadenylase activity (Fig. 2c). Next, we established primary fibroblast cultures from individuals carrying homozygous mutations encoding p.Glu220Lys, p.Phe148Tyr and

![Figure 1](https://example.com/figure1.png)

**Figure 1** TOE1 mutations lead to pontocerebellar hypoplasia with abnormal genitalia (PCH7). (a) Pedigrees of affected families showing recessive inheritance. Double bar, consanguineous marriage; open circle, unaffected female; open square, unaffected male; filled circle, affected female; filled square, affected male; triangle, spontaneous abortion; open diamond, unaffected individual of unknown sex; diagonal line, deceased. Arrows indicate probands. (b) Magnetic resonance midline sagittal (top) and axial (bottom) images showing reduced cerebellar volume in affected individuals (yellow arrows).
p.Ala103Thr, as well as their unaffected relatives (families 1603, 4127 and 4128, respectively). Patient-derived fibroblasts had reduced levels of TOE1 protein as determined by protein blot (Fig. 2d and Supplementary Fig. 3b). Additionally, neural progenitor cell (NPC) lines that were derived from an affected individual of family 1603 showed less TOE1 protein than NPC lines from an unaffected relative and control fibroblast cell lines (Fig. 2e and Supplementary Fig. 3b,c). Together, these results indicate that TOE1 amino acid substitutions negatively affect protein accumulation.

To assess whether TOE1 mutations affect protein levels, we generated single-site-integration T-REx-293 cell lines for tetracycline-regulated expression of small interfering RNA (siRNA)-resistant transcripts for wild-type human TOE1 and the Glu220Lys, Phe148Tyr, Val173Gly and Ala103Thr variants. When cells were depleted of endogenous TOE1 and induced with a concentration of tetracycline that promoted accumulation of wild-type TOE1 to near-endogenous levels, we observed reduced levels of mutant TOE1 as compared with wild-type TOE1, despite similar mRNA levels (Supplementary Fig. 3d,e). In contrast, a previously characterized, catalytically inactive mutant of TOE1 (DE) accumulated to similar levels as wild-type protein (Supplementary Fig. 5a). These results are consistent with our findings from patient-derived fibroblast cell lines and indicate that the mutations in affected individuals are deleterious by reducing TOE1 levels.

Because human TOE1 and mouse Toe1 were expressed in all tested tissues (Supplementary Fig. 4a,b), we generated Toe1-mutant mice to test for defects in vivo. Embryos with homozygous Toe1 frameshift mutations showed uniform lethality before embryonic day (E) 11.5 (Supplementary Fig. 4c), demonstrating that Toe1 is required for mouse development. Because the mutations in human patients allow for partial expression of TOE1 protein, we next turned to morpholino (MO)-based knockdown in zebrafish, where protein dosage could be regulated, to create a PCH7 disease model (Supplementary Fig. 5a). Knockdown of the single toe1 ortholog (NM_001256682.1) led to a reproducible phenotype comprising microcephaly, small eyes and
curly tail in 90% of embryos by 48 hours post-fertilization (h.p.f.), which was rescued by co-injection with human TOE1 mRNA but not mRNA encoding the catalytically inactive DE mutant or the mutants identified in patients (Fig. 3a and Supplementary Fig. 6a). To visualize neurons, we performed whole-mount immunofluorescence for the neuronal marker HuC. Like the human patients, zebrafish injected with toe1 MO showed structural defects of the developing midbrain, cerebellum and hindbrain (Fig. 3b). This phenotype was largely rescued by co-injection with wild-type zebrafish toe1 or human TOE1, but not the mutant mRNAs (Fig. 3b and Supplementary Figs. 5b and 6b). To determine whether neuronal loss in the zebrafish injected with toe1 MO was due to cell death, we performed staining for cleaved caspase-3 (Casp3) at 24 h.p.f. While zebrafish injected with control MO showed few Casp3-positive cells, those injected with toe1 MO showed a dramatic apoptotic response that was consistent with neurodegeneration (Supplementary Fig. 5c). We conclude that reduced expression of TOE1 leads to neurodegeneration and PCH-like structural brain defects in vivo.

TOE1 was originally identified as a growth suppressor and a direct target gene of ERG1, an immediate-early transcription factor. We reported TOE1, which is alternatively called CAF1z because of homology with CAF1 deadenylases, as a 3′-to-5′ exonuclease with a preference for adenosines in vitro. Unlike characterized mRNA deadenylases, TOE1 is concentrated in nuclear Cajal bodies. 

Figure 3 toe1 depletion in zebrafish results in structural brain defects, mimicking human PCH7 pathology. (a) Comparison of zebrafish at 48 h.p.f. injected with 6 ng of control MO, toe1 ATG MO, toe1 splice-blocking MO, or toe1 splice-blocking MO together with 0.1 pg of mRNA encoding zebrafish (z) Toe1 or human (h) TOE1. Zebrafish injected with toe1 MO have abnormal head shape and thin, curved tails; this phenotype is rescued by addition of wild-type mRNA for zebrafish or human TOE1 but not mRNA for the human DE mutant. Scale bars, 500 µm. Quantification is shown at the top right. Normal, no observable phenotype; moderate, small eyes, slight reduction in head size; severe, small eyes, small head, thin curved tail. n = 100 embryos per. (b) Maximum confocal projection of whole-mount pan-neuronal HuC immunofluorescence at 48 h.p.f. A diagram of zebrafish anatomy is shown to the upper left. The midbrain, cerebellum and hindbrain regions of zebrafish injected with toe1 MO have reduced HuC protein levels, which were restored with mRNA encoding wild-type zebrafish or human TOE1 but not the human DE mutant. Scale bars, 100 µm. n = 6 embryos per condition.
are rich in enzymes that process RNAs not known to have poly(A) tails, suggesting that TOE1 might target non-poly(A) RNA substrates. More recently, TOE1 was shown to associate with spliceosomal proteins\(^5,6\), which are known to localize to Cajal bodies, and TOE1 depletion caused defective splicing of a pre-mRNA reporter\(^7\). To validate TOE1 association with the spliceosome, we performed TOE1 knockdown together with expression at near-endogenous levels of FLAG-tagged wild-type or DE mutant TOE1 in T-REX-293 cells followed by immunoprecipitation (IP). As assessed by RNA blotting (Fig. 4a) and tandem mass spectrometry (MS) (Supplementary Fig. 7 and Supplementary Table 3), TOE1 assembled with snRNAs and, in near-complete overlap with previously reported immunoprecipitation and mass spectrometry (IP–MS) results\(^8\), with spliceosomal proteins. The CCR4-like protein ANGEL2 (also known as CCR4D), which we previously reported in complex with TOE1 (ref. 4), was not detected in our IP–MS analysis, possibly owing to low cellular abundance of this protein\(^9\).

Inspection of RNA blots for snRNAs associated with the DE TOE1 catalytic mutant revealed slower-migrating U1, U2 and U5 snRNA species (Fig. 4a). Processing of RNA polymerase II (Pol II)-transcribed snRNAs (U1, U2, U4 and U5) initiates with co-transcriptional cleavage by the Integrator complex downstream of the mature 3’ end\(^10\), but the mechanism mediating the removal of the 3’ tail to produce a mature-length snRNA is unknown\(^11,12\). To characterize the snRNAs that migrated slower in the RNA blots, we performed 3’-end sequencing of TOE1-associated snRNAs and found that TOE1 bound to Pol II–transcribed snRNAs that were incompletely processed at the 3’ end (Fig. 4b,c and Supplementary Fig. 8), suggesting that TOE1 may mediate snRNA 3’-tail processing.

The 3’-tail sequences of TOE1-associated pre-snRNAs consisted of both genome-encoded and post-transcriptionally added nucleotides, henceforth referred to as templated and untemplated tails, respectively (Fig. 4b,c and Supplementary Fig. 9a). The untemplated snRNA tails, which were previously observed by global 3’-end sequencing of non-coding RNA\(^13\), were found almost exclusively on snRNAs that were either longer or shorter than their annotated mature length and consisted primarily of uridines and adenosines, with snRNAs associated with DE TOE1 predominantly enriched for untemplated 3’ adenosines (Supplementary Fig. 9a,b). Interestingly, 3’-maturation of U2 snRNA finishes with the addition of an untemplated 3’ adenosine\(^14\), but this modification was entirely absent from TOE1–associated U2 snRNAs (Supplementary Fig. 9b). Taken together, these observations suggest that TOE1 associates specifically with pre-snRNAs that are not fully processed at the 3’ end and have often acquired untemplated tails.

snRNAs associated with DE TOE1 contained longer tails than those associated with wild-type TOE1, suggesting that TOE1 may catalytically process these tails. In accordance with this hypothesis, 3’-end sequencing of the total snRNA pool showed an increased fraction of 3’-end-extended snRNAs upon depletion of TOE1 (Fig. 4b and Supplementary Fig. 8). Notably, complementation with exogenous wild-type TOE1 rescued the snRNA 3’-end defect, while addition of DE TOE1 failed to rescue the defect (Fig. 4b and Supplementary Fig. 8). There was little to no accumulation of 3’ tails for C/D-box U3 and H/ACA-box SNORA63 small nucleolar RNAs (snoRNAs), 5.8S rRNA, tRNAs and U6 snRNA (Supplementary Figs. 8 and 10), which, like Pol II–transcribed snRNAs, are processed from 3’-extended precursor RNAs\(^15–18\). These results support a catalytic role for TOE1 as a 3’-to-5’ exonuclease with specificity for snRNA processing.

TOE1 could promote either maturation or degradation of pre-snRNAs. To distinguish between these possibilities, we tested the activity of immunoisolated TOE1 on co-purifying pre-snRNA
Figure 5 TOE1 enzymatic activity processes snRNA 3′ ends. (a) RNA from TOE1 activity assays. Left, input RNA samples from cells treated with siCtrl or siTOE1 and then induced to express either wild-type or DE FLAG-TOE1 at near-endogenous levels. Right, samples from RNA immunoprecipitation with antibody to FLAG were divided and treated with either 2 mM Mg2+ or 2 mM EDTA on beads after IP; asterisks mark TOE1-depletion did not affect the levels of unstable snRNAs mutated in the snRNA tails in NPCs. Bottom, percentage of reads with 3′-end tails.

Figure 6 Patient-derived fibroblasts and neuronal progenitor cells show defects in snRNA 3′ ends. (a) Top, cumulative U1 and U2 snRNA 3′-end sequence reads for fibroblasts from affected (A) and unaffected (U) individuals, showing that TOE1 mutations result in snRNA tails. Bottom, bar graph showing the percentage of reads with 3′ tails. (b) Top, cumulative U1 and U2 snRNA 3′-end sequence reads for NPCs from affected and unaffected individuals showing that TOE1 mutations result in snRNA tails in NPCs. Bottom, percentage of reads with 3′-end tails.

sugging that TOE1 has a function in maturation rather than degradation. 3′-end sequencing confirmed that the in vitro processing of U1, U2 and U5 by TOE1 halted at or before the mature 3′ end (Fig. 5a and Supplementary Fig. 11a), including at an alternative U5 3′-end also present in total cellular snRNA (Supplementary Fig. 8b). In accordance with TOE1 having a function in maturation rather than degradation, TOE1 depletion did not affect the levels of unstable endogenous variants of U1 snRNA (Supplementary Fig. 11b)8, nor did it affect the levels or 3′ processing of unstable U1 snRNAs mutated in the Sm-binding site (Supplementary Fig. 11c)20, suggesting that the latter are targeted for degradation before 3′ processing by TOE1. We conclude that TOE1 acts in snRNA 3′-end maturation (Fig. 5c).

To determine whether TOE1 mutations affect snRNA processing in the individuals in whom they are present, we performed snRNA 3′-end sequencing in the patient-derived cell lines. This identified an increased fraction of U1 and U2 snRNAs, as well as U5 snRNA to a lesser extent, that contained tails in fibroblasts derived from patients with TOE1 mutations when compared to those derived from their unaffected relatives, which were indistinguishable from the control (Fig. 6a and Supplementary Fig. 12a). Similarly, patient-derived NPC lines showed enrichment for snRNA extensions (Fig. 6b and Supplementary Fig. 12b). We conclude that cells derived from patients with PCH7 accumulate incompletely processed snRNAs.

In summary, we have shown that individuals with PCH7 harbor biallelic, damaging mutations in TOE1 that result in the accumulation of incompletely processed snRNAs. The involvement of TOE1 in snRNA maturation was surprising, given the homology of TOE1 with CAF1 mRNA deadenylases and its preference for poly(A) RNA in vitro4. However, this result could provide a mechanistic explanation for
the observed defect in pre-mRNA splicing upon TOE1 depletion. The enrichment of snRNAs with untemplated 3′ adenosines associated with catalytically inactive TOE1 suggests that 3′ adenylation by an unknown 3′-terminal nucleotidyltransferase (3′ Tnt) serves as a mechanism to recruit TOE1 to pre-snRNAs (Fig. 5c). The abundance of Sm subunits in the TOE1 IP–MS analysis (Supplementary Fig. 7 and Supplementary Table 3) and the absence of processing of U1 snRNA mutated in the Sm-binding site (Supplementary Fig. 11d) suggest that the Sm complex is another component important for TOE1 recruitment. PARN, which has been linked to familial pulmonary atrophy, and PARN-like DEDD deadenylases were recently found to promote 3′ processing of snRNAs and PIWI-interacting RNAs (piRNAs), respectively, and, like snRNAs, RNA targets had templated and untemplated tails upon depletion of PARN15. Thus, the adaptation of deadenylases to function in 3′ processing of noncoding RNAs in conjunction with poly(A) polymerases might be a general principle in RNA metabolism.

Our results suggest that perturbations in snRNA pools may contribute to the features manifested by patients with PCH7, although other currently unknown functions of TOE1 cannot be ruled out as being causal in the disease. A defining feature of pontocerebellar degeneration is loss of motor neurons, akin to spinal muscular atrophy (SMA). The gene associated with SMA, SMN1 (survival of motor neuron 1), encodes a well-established snRNP assembly factor23,24, and this association, together with our results, suggests that maintaining proper snRNP biogenesis is important for neuronal survival. EXOSC3, which encodes a core component of the 3′-RNA-exonuclease exosome complex, is mutated in PCH type 1 (ref. 25), suggesting that there may be shared RNA targets for TOE1 and the exosome, perhaps snRNAs. In accordance with the idea that snRNAs are important for neuronal survival, a recent study identified recessive cerebellar degeneration resulting from a mutation in the Rnu2-8 U2 snRNA gene in mice26. We and others recently described mutations in genes involved in protein synthesis, including CLP1 and other TRNA splicing endonuclease (TSEN) complex components, leading to misprocessing of pre-tRNAs in cells derived from patients with PCH27–30. While we found no defect in tRNA 3′-end processing in TOE1-mutant cells (Supplementary Fig. 10), CLP1 has also been implicated in snRNA 3′-end processing31, suggesting a possible link between TOE1 and CLP1 mutations in PCH. Our data, along with data from other recent studies26,32, suggest defects in the processing of small noncoding RNAs as a common cause of severe, early-onset neurodegenerative conditions.

**METHODS**

Methods, including statements of data availability and any associated accession codes and references, are available in the online version of the paper.

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**AUTHOR CONTRIBUTIONS**

A.E.S., S.M., N.A., A.G.-G., L.D.H. and A.G. performed fibroblast, iPSC and knockout mouse experiments. R.M.L. generated stable cell lines and performed all snRNA/P experiments. V.R.C.E., N.A., E.S., D. Musaev, R.M., A.W., A.E.S., J.L.S., E.D., R.O.R. and H.T.C. analyzed clinical and exome results. S.G., A.E.S., Z.S., B.R., A.G.-G., I.M.-V. and L.D.H. generated zebrafish data, and N.C.C. and D.T. provided resources for zebrafish experimentation. M.S.Z., N.F., W.B.D., E.S., S.M.B., E.M.V., J.H.D., L.M.K., H.U.A., M.L.F., L.W., D.C., S.B., C.F., M.K., K.A.A., D. Manchester, N.M., A.O.Q., K.B., H.P., M.-C.N., H.S., M.S., K.O. and K.M. conceived of the genetic investigation. S.B.G. and M.G. supported exome sequencing. E.L.V.N., S.S., T.L.S. and G.W.Y. supported RNA sequencing and computational analysis. E.J.B. performed mass spectrometry. R.L.M., A.E.S., J.L.-A. and J.G.G. wrote the manuscript. R.M.L., A.E.S., V.R.C.E., Z.S., N.C.C., D.T., G.W.Y., F.B., J.L.-A. and J.G.G. edited the manuscript. J.L.-A., F.B. and J.G.G. directed the project.

**COMPETING FINANCIAL INTERESTS**

The authors declare no competing financial interests.

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ONLINE METHODS

Patient recruitment. Patients were enrolled and sampled according to standard local practice in approved human subjects protocols at the University of California, The Rockefeller University and The Academic Medical Center (AMC) in Amsterdam for blood, saliva and skin biopsy sampling.

Sequencing. Blood was acquired from informed, consenting individuals according to institutional guidelines, and DNA was extracted using established protocols. Exome sequencing was performed on both the parents and affected member(s) from each family as previously described34. All variants were prioritized by allele frequency, conservation and predicted effect on protein function, and were tested by Sanger sequencing for segregation with disease. Sequence data were analyzed with Sequencer 4.9 (Gene Codes).

Genetic mapping. Chromosomal ideogram plots were generated using the Bioconductor package quantsomeshape. Red represents homozygous regions that segregate between affected and unaffected family members.

Fibroblast culture, and iPSC and NPC generation. Fibroblasts were generated from unaffected and affected dermal biopsy explants. Induced pluripotent stem cells (iPSCs) and NPCs were obtained as previously described35. Mycoplasma testing was routinely performed, and all cell lines were negative.

cDNA synthesis and RT–PCR. cDNA was synthesized with the Superscript III First-Strand cDNA synthesis system for RT–PCR (Life Technologies) and used for real-time PCR or cloning. qRT–PCR for intron-containing tRNAs was performed in triplicate on 10 ng of human cDNA27. RT–PCR to assess toe1 intron inclusion in zebrafish embryos morphant for toe1 splice-blocking MO was performed with 10 ng of zebrafish cDNA and primers flanking the first intron of zebrafish toe1 (Supplementary Table 4).

Plasmid constructs and stable cell line generation. The ORF of human TOE1 was subcloned into pcDNA5/FRT/TO-FLAG with BamHI and NotI restriction sites from pcDNA3-FLAG3. The ORF of zebrafish toe1 or human TOE1 was subcloned into pCS2+ (ref. 36) with BamHI and XhoI. Missense mutations were generated using the QuikChange Mutagenesis kit (Agilent). For expression of N-terminally FLAG-tagged proteins, stable HEK FLP-In T-REX-293 cell lines were generated (Invitrogen). Mycopolysome testing was routinely performed, and all cell lines were negative.

RNA blotting. RNA was extracted using TRIzol and separated by electrophoresis on 9% 10:1 polyacrylamide, 0.6× Tris/borate/EDTA (TBE). 8 M urea gels at 20 mA per gel for 2 h. RNA was transferred in 0.5× TBE to nylon membrane at 25 V for 16 h. Membranes were UV cross-linked, blocked with UltraHyb Oligo (Life Technologies) and hybridized with 5′-end-radiolabeled DNA oligonucleotides (Supplementary Table 4).

3′ RNA tail sequencing and analysis. RNA adaptors containing barcodes and 10–11 nt randomers (Supplementary Table 4) were ligated to the 3′ ends of total and eluted RNA with T4 RNA ligase (New England BioLabs) at 16°C for 16 h, treated with RQ1 RNase-free DNase (Promega) for 30 min at 37°C and extracted with PCA (Affymetrix). cDNA was generated using AR-17 primer with Superscript III (Life Technologies). 3′ ends were amplified by Q5 DNA polymerase (New England BioLabs) with siRNA-gene-specific primers and AR-17, and then with primers D501 and D702 (Illumina; Supplementary Table 5). For tRNA 3′-end sequencing, tRNAs were gel purified from a 9% agarose gel. tRNA 5′ ends were amplified by qRT–PCR (Life Technologies) and used for real-time PCR or cloning. qRT–PCR for intron-containing tRNAs was performed in triplicate on 10 ng of human cDNA27. RT–PCR to assess toe1 intron inclusion in zebrafish embryos morphant for toe1 splice-blocking MO was performed with 10 ng of zebrafish cDNA and primers flanking the first intron of zebrafish toe1 (Supplementary Table 4).

Protein blotting. Protein blotting was performed with rabbit polyclonal antibody to Cafliz/Toe1 (ref. 4), rabbit polyclonal antibody to GADPH (Cell Signaling, 2118s) and mouse antibody to vinculin (Sigma, V9264) at 1:1,000 dilutions in 5% nonfat milk in PBST. The secondary antibodies were HRP-conjugated anti-mouse and anti-rabbit secondary antibodies used at 1:20,000 dilutions in 5% nonfat milk in PBST.

Animals. All animal experiments complied with the Institutional Animal Care and Use Committee at the University of California San Diego and were carried out in a non-blinded fashion. The Toe1-mutant mice were generated using CRISPR/SaCas9 technology. Briefly, pronuclear co-injection with 5 ng of sgRNA targeting sequence, 5′-CTGTGTGAGATGTTCCCAGC-3′ and 7 ng of gRNA (targeting sequence, 5′-CTGTGTGAGATGTTCCCAGC-3′) was performed on 143 embryos. A total of 126 embryos were transferred into host dams for implantation, resulting in 23 live pups. Sanger sequence genotyping identified only one mouse with a heterozygous single-base-pair frameshift mutation (chr. 4: 116806688–69; c.668_669insA), resulting in a Toe1 null allele. Mutagenesis was performed on C57BL/6J single-cell blastocysts. Positive founders were bred to establish lines transmitting the Toe1 null allele. Male and female carriers were intercrossed at 6–8 weeks of age to assess the embryonic phenotype associated with Toe1 mutation.

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primary rabbit polyclonal antibody to caspase-3 (Abcam, ab13847) or primary mouse monoclonal antibody to HuC/HuD (Thermo Fisher, A-21271) and Alexa Fluor 488–conjugated anti-rabbit or anti-mouse secondary antibody. Zebrafish were immobilized in agarose, and fluorescent images were acquired with a Zeiss LSM 780 and Olympus FV1000 confocal microscope. Cells were counted using ImageJ. No statistical method was used to predetermine sample size. The experiments were not randomized.

Immunocytochemistry. Cells were seeded on coverslips and fixed in 4% paraformaldehyde or 100% cold methanol, permeabilized with 0.1% Triton X-100, and blocked with 0.1% BSA and 0.5% gelatin from cold water fish skin in PBS. Cells were incubated with primary antibody in blocking solution overnight at 4 °C, washed and incubated with secondary antibody in blocking solution and 0.4 µg of Hoechst for 1 h at room temperature. The primary antibodies used were mouse antibody to nestin (EMD Millipore, MAB5326) and rabbit antibody to Pax6 (Covance, PRB-278P) at 1:1,000 dilutions. The secondary antibodies were Alexa Fluor 555–conjugated anti-rabbit and Alexa Fluor 488–conjugated anti-mouse antibody at 1:500 dilutions. Images were taken with an Olympus IX51 inverted fluorescent microscope.

Mass spectrometry. Liquid chromatography and tandem mass spectrometry (LC–MS/MS) assays were performed as described previously39 using anti-FLAG IP samples from extracts of T-Rex-293 cell lines stably expressing FLAG-tagged wild-type or DE TOE1 at near-endogenous TOE1 levels, with a parental cell line serving as a control.

Protein modeling. Needleman–Wunsch alignment of human TOE1 and CNOT7 was performed with protein–protein BLAST (pBLAST). Homologous TOE1 missense mutations, and previously published inactivating mutations6, were modeled onto the predicted protein structures of human CNOT7 (PDB 2FC6). Patient mutations predicted to result in truncated protein (for example, nonsense, splice and frameshift mutations) were excluded from analysis. Residues not in alignment were excluded (Phe148). PyMOL was used to create 3D renderings.

Statistics. Student’s two-tailed paired t test was employed to test the significance of accumulation of extended snRNAs as indicated (Fig. 4b and Supplementary Fig. 8a) (U1 snRNA: siCtrl vs. siTOE1, P = 0.0054; WT vs. DE, P = 0.0036; WT vs. RIP WT, P = 0.0069; DE vs. RIP DE, P = 0.0050; U2 snRNA: siCtrl vs. siTOE1, P = 0.014; WT vs. DE, P = 0.014; WT vs. RIP WT, P = 0.0058; DE vs. RIP DE, P = 0.012; U5 snRNA: siCtrl vs. siTOE1, P = 0.032; WT vs. DE, P = 0.014; WT vs. RIP WT, P = 0.011; DE vs. RIP DE, P = 0.0027; U4 snRNA: siCtrl vs. siTOE1, P = 0.033; WT vs. DE, P = 0.016; WT vs. RIP WT, P = 0.12; DE vs. RIP DE, P = 0.042; U6 snRNA: siCtrl vs. siTOE1, P = 0.57; WT vs. DE, P = 0.015; WT vs. RIP WT, P = 0.021; DE vs. RIP DE, P = 0.19). Student’s two-tailed unpaired t test was employed to test the significance of cleaved caspase-3-positive cells in Supplementary Figure 5c (control MO vs. toe1 ATG MO, P = 0.015).

Data availability. The exome sequencing data for all individuals consented for public release of data in this study have been deposited to the database of Genotypes and Phenotypes (dbGaP) under accession phs000288.v1.p1. RNA sequencing data have been deposited into Gene Expression Omnibus (GEO) under accession GSE71536.