Figure S1. Uaf, TD1 and TD2 iPSC lines exhibited normal karyotypes

Figure S2. Expression of pluripotency markers OCT4 and TRA-1-60 in iPSCs

iPSC lines were double stained for pluripotency markers OCT4 and TRA-1-60. The percentage of double positive cells for each line was analyzed by flow cytometry.

A. iPSCs of unrelated unaffected subject;

B. iPSCs of unaffected subject (4002);

C. iPSCs of TD1 patient (5001);

D. iPSCs of TD2 patient (5002);

E. Percentage of OCT4 and TRA-1-60 double positive cells for each iPSC line.

Figure S3. Expression of neural progenitor markers NESTIN and SOX2 in iPSC-derived NPCs

NPC lines were stained for neural progenitor markers NESTIN and SOX2. Percentage of NESTIN positive or SOX2 positive cells for each line was analyzed by flow cytometry.

A. NPCs of unrelated unaffected subject;

B. NPCs of unaffected subject (4002);

C. NPCs of TD1 patient (5001);

D. NPCs of TD2 patient (5002).

Figure S4. Characterization of iPSC, iPSC-derived NPCs and neurons by ICC

A. The iPSC colonies expressed pluripotency markers: Nanog and Oct4.

B. Neural progenitor cells (NPCs) derived from iPSCs were stained positive for NPCs markers: Nestin and Musashi1. Also, NPCs expressed neuronal marker: Class III β-tubulin.

C.Glutamatergic neuron marker VGLUT1, GABAergic neuron marker VGAT or glial cell marker GFAP was detected in subpopulations of iPSC-derived D30 neurons.

Figure S5. Functional neurons were differentiated from iPSC-derived NPCs

A-E. Calcium imaging showed that the D30 neurons exhibit spontaneous and potassium-stimulated calcium influx.

F and G. These functional Day 30 neurons expressed neuronal markers MAP2 and Class III β-tubulin as well as synaptic vesicle protein synapsin I (SYN1).

Figure S6. Inhibition of NMD eliminated the expression difference of the *PNKD* (L) transcript between unaffected and TD-affected neurons.

A. Sanger Sequencing confirmed that the unrelated unaffected (Urel_Uaf) subject doesn't carry the PNKD nonsense mutation.

B. The Urel_Uaf D30 neurons expressed PNKD (L) protein which was comparable to Uaf D30 neurons.

C. Without NMD inhibition (untreated and DMSO), TD1 and TD2 D30 neurons expressed about half of *PNKD* (L) transcript compared to the Uaf D30 neurons. Inhibition of NMD by treating D29 neurons with 100ug/ml CHX for ~15 hrs led to increase of *PNKD* (L) transcript in all lines. Importantly, inhibition of NMD eliminated the difference of *PNKD* (L) transcript level between TD and Uaf D30 neurons.

Figure S7. PNKD (L) protein is plasma membrane-associated and co-localizes with the RIMS1α protein in the iPSC-derived neurons.

A. iPSC-derived neurons were transfected with plasmid expressing the FLAG-PNKD(L) protein. anti-FLAG antibody was used to detect the PNKD (L) protein whose expression was enriched in the plasma membrane and the neurites.

B. RIMS1α-Myc was overexpressed in the iPSC-derived neurons by transfection. The anti-Myc antibody was used to detect the RIMS1α protein which is localized in the cell body and neurites of the iPSC-derived neurons.

C. Plasmids expressing the FLAG-PNKD(L) and RIMS1α-Myc were co-transfected into the iPSC-derived neurons. anti-PNKD(L)(C-terminus) and anti-Myc antibodies were used to detect the PNKD (L) and the RIMS1α proteins which were co-localized in the cell body and the neurites of the iPSC-derived neurons.

Figure S8 *PNKD* expression in developmental human brains

A. The "RNA-Seq Genecode v10 summarized to genes" dataset was downloaded from the BrainSpan website. RPKM values of *PNKD* gene were extracted and plotted using the "age" and "structures". There are 31 ages shown at the x-axis and 26 brain

structures represented by color coded lines. In general, the *PNKD* gene expression increases during human brain development.

B. The "RNA-Seq Genecode v10 summarized to exons" dataset was downloaded from the BrainSpan website. RPKM values of the 13 exons of the *PNKD* gene were extracted and plotted using the "age" and "structures". There are 30 ages shown at the x-axis and 26 brain structures represented by color coded lines. The exon 3 is only included in the *PNKD* (S) transcript. The panel B shows the RPKM values of the exon 3 expression, which has higher expression in the 6-month and 4-year old brains.

C. Panel C shows the RPKM values of the exon 4 which is only included in the *PNKD* (M) transcript. The expression of the exon 4 is low in the human brain and doesn't exhibit a significant increase along with the brain maturation.

D. Panel D shows the PRKM values of the exon 6 which is shared by the *PNKD* (L) and the *PNKD* (M) transcripts. The expression of the exon 6 increases dramatically during human brain development. Since the *PNKD* (M) transcript is expressed low in the human brain, the expression increase of the exon 6 is probably due to the expression increase of the *PNKD* (L) transcript.

RPKM: Reads Per Kilobase of transcript per Million mapped reads

Figure S9 *RIMS*1 expression in developmental human brains

A. The "RNA-Seq Genecode v10 summarized to genes" dataset was downloaded from the BrainSpan website. RPKM values of the *RIMS1* gene were extracted and plotted using the "age" and "structures". There are 31 ages shown at the x-axis and 26 brain structures represented by color coded lines. In general, the *PNKD* gene expression increases during human brain development.

B. The "RNA-Seq Genecode v10 summarized to exons" dataset was downloaded from the BrainSpan website. RPKM values of exon 3 of the *RIMS1* gene were extracted and plotted using the "age" and "structures". The exon 3 is only included in the *RIMS1α.* There are 30 ages shown at the x-axis and 26 brain structures represented by color coded lines.

Figure S10 *PNKD* and *RIMS1* expression from the Human Brain Transcriptome (HBT) database.
Gene expression was measured in 6 brain regions at 15 developmental periods. For each developmental period, multiple samples were analyzed (experimental details can been found in http://hbatlas.org/files/nature10523-s1.pdf)

NCX: neocortex; STR: striatum; HIP: hippocampus; MD: mediodorsal nucleus of the thalamus; AMY: amygdala; CBC: cerebellar cortex.

A. *PNKD* expression increases from period 1 to period 12 during human brain development. STR might have higher *PNKD* expression from period 8 to 12 than other brain regions.

B. *RIMS1* expression increases dramatically from period 1 to period 6 and reaches to a plateau in all brain regions except for CBC. The CBC has higher *RIMS1* expression from period 8 to period 15 than other brain regions.

Period 1 (Embryonic development, 4< Age <8 PCW)

Period 2 (Early fetal development, 8< Age <10 PCW)

Period 3 (Early fetal development, 10< Age <13 PCW)

Period 4 (Early mid-fetal development, 13< Age <16 PCW)

Period 5 (Early mid-fetal development, 16< Age <19 PCW)

Period 6 (Late mid-fetal development, 19< Age <24 PCW)

Period 7 (Late fetal development, 24< Age <38 PCW)

Period 8 (Neonatal and early infancy, birth ≤ Age <6 postnatal months)

Period 9 (Late infancy, 6< Age <12 postnatal months)

Period 10 (Early childhood, 1< Age <6 years)

Period 11 (Middle and late childhood, 6< Age <12 years)

Period 12 (Adolescence, 12< Age <20 years)

Period 13 (Young adulthood, 20< Age <40 years)

Period 14 (Middle adulthood, 40< Age <60 years)

Period 15 (Late adulthood, 60 years +)

Figure S11 *PNKD* mutant transcript was detected in TD neurons and was translated into truncated protein in 293FT cells.

A. *PNKD* (L) transcript harboring the nonsense variant was detected in the TD1 and TD2 iPSC-derived neurons by Sanger sequencing, suggesting the nonsense-mediated decay was not complete.

B. By overexpressing the *PNKD* (L) wildtype and mutant transcripts together in 293FT cells, localization of the PNKD (L)_wt and PNKD (L)_truncated proteins can be visualized by immunocytochemistry.