Supplemental Figure 1

A

B

mUBE3A-Iso2	**M**ATACKRS**P**GES**QS**E DIEASRAKRAAAK-	cytosolic
mUBE3A-Iso3	**M**KRAAAK-	nuclear
hUBE3A-Iso1	**M**KRAAAK-	nuclear
hUBE3A-Iso2	**M**EKLHQCYWKSGEPQSDDIEASRAKRAAAK-	cytosolic
hUBE3A-Iso3	**M**ATACKRS**–**GE**P**QS**D**DIEASRAKRAAAK-	nuclear
HA-mUBE3A-Iso3/ HA-hUBE3A-Iso1	**M**AYPYDVPDYASGASGAPMKRAAAK-	cytosolic
HA-hUBE3A-Iso2	**M**AYPYDVPDYASGASGAPMEKLHQCYWKSGEPQSDDIEASRAKRAAAK-	cytosolic
HA-hUBE3A-Iso3	**M**AYPYDVPDYASGASGAPMATACKRS**–**GE**P**QS**D**DIEASRAKRAAAK-	nuclear

UBE3A N-terminal extension variations:

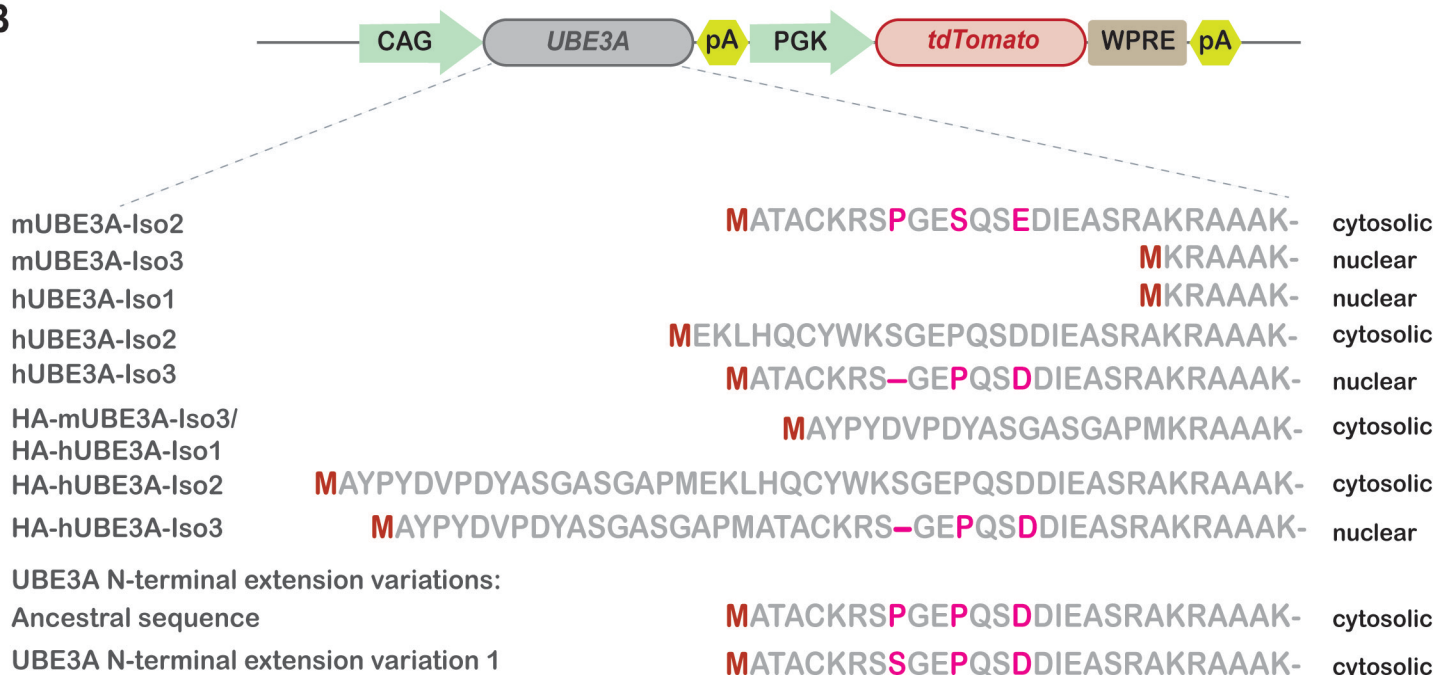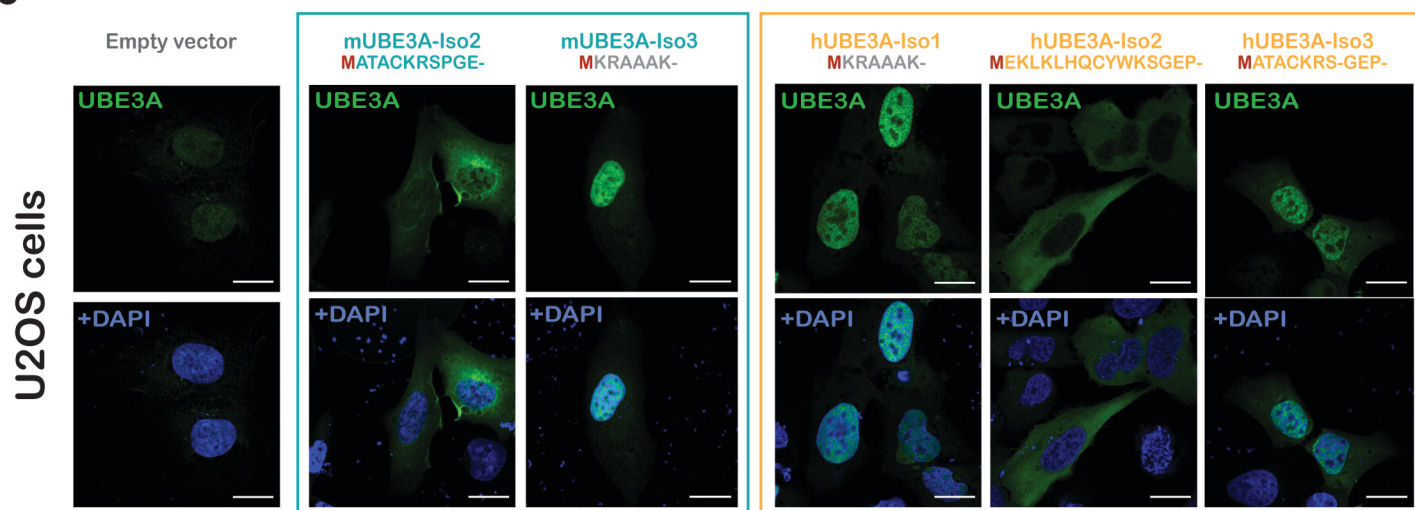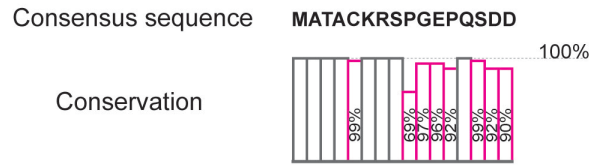|		
Ancestral sequence	**M**ATACKRS**P**GE**P**QS**D**DIEASRAKRAAAK-	cytosolic
UBE3A N-terminal extension variation 1	**M**ATACKRS**S**GE**P**QS**D**DIEASRAKRAAAK-	cytosolic

C

U2OS cells

Figure S1. **A.** Staining of endogenous UBE3A in 12-week-old iPSC-derived control neurons. Neurons were stained for UBE3A (green, left panel) together with MAP2 (pink) and DAPI (blue)(right panel). Scale bars, 20μm. **B.** Schematic representation of the dual promoter construct structure and the UBE3A N-terminal sequences that were inserted in the vector. **C.** Localization of the mouse and human isoforms in (non-neuronal) U2OS cells. Scale bars, 20μm.

Supplemental Figure 2

A

Consensus sequence **MATACKRSPGEPQSDD**

Conservation — 100%

Conservation values: 99%, 69%, 97%, 66%, 92%, 99%, 99%, 60%

Species	Sequence	Subgroup	Order
Human (Homo sapiens)	MATACKRS-GEPQSDD	Hominidae	Primates
Chimpanzee (Pan troglodytes)	MATACKRS-GEPQSDD		
Bonobo (Pan paniscus)	MATACKRS-GEPQSDD		
Orangutan (Pongo abelii)	MATACKRS-GEPQSDD		
Macaque (Macaca mulatta)	MATACKRS-GEPQSDD	Old World monkeys	
Sooty mangabey (Cercocebus atys)	MATACKRS-GEPQSDD		
Ugandan red colobus (Piliocolobus tephrosceles)	MATACKRS-GEPQSDD		
Black snub-nosed monkey (Rhinopithecus bieti)	MATACKRS-GEPQSDD		
Nancy Mas night monkey (Aotus nancymaae)	MATACKRSPGEPQSDN	New World monkeys	
Black-capped squirrel monkey (Saimiri boliviensis boliviensis)	MATACKRSPGEPQSDD		
Common marmoset (Callithrix jacchus)	MATACKRSPGEPQSDD		
Philippine tarsier (Carlito syrichta)	MATACKRSPEEPQSDD	Tarsiiformes	
Northern greater galago (Otolemur garnettii)	MATACKRSPGEPQSDD	Lemuriformes	
Gray mouse lemur (Microcebus murinus)	MATACKRSPGEPQSDD		
Coquerels sifaka (Propithecus coquereli)	MATACKRSPREPQSDD		
Sunda flying lemur (Galeopterus variegatus)	MATACKRSPGEPQSDD		Demroptera
Chinese tree shrew (Tupaia chinensis)	MATACKRSPGEPQSDD		Scandetia
American beaver (Castor canadensis)	MATACKRSPGEPQSDD	Castoridae	Rodentia
Alpine marmot (Marmota marmota marmota)	MATACKRSPGEPQSDN		
Rat (Rattus norvegicus)	MATACKRSSGESQSED	Muridae	
Mouse (Mus musculus)	MATACKRSPGESQSED		
Algerian mouse (Mus spretus)	MATACKRSPGESQSED		
Mongolian gerbil (Meriones unguiculatus)	MATACKRSPGEPQSED		
Long-tailed chinchilla (Chinchilla lanigera)	MATASKRSPGEPQSDD	Hystricognathi	
Common degu (Octodon degus)	MATASKRSPGEPQSDD		
Damara Mole-rat (Fukomys damarensis)	MATASKRSPGEPQSDD		
Thirteen-lined ground squirrel (Ictidomys tridecemlineatus)	MATACKRSPGEPQSDN	Sciuridae	
European rabbit (Oryctolagus cuniculus)	MATACKRSPGEPQSDD		Lagomorpha
Cow (Bos taurus)	MATACKRSSGEPQSDD	Bovidae	Cercatiodactyla
Zebu (Bos indicus)	MATACKRSSGEPQSDD		
Goat (Capra hircus)	MATACKRSSGEPQSDD		
Sheep (Ovis aries)	MATACKRSSGEPQSDD		
Minke whale (Balaenoptera acutorostrata scammoni)	MATACKRSSGEPQSDD	Cetacea	
Sperm whale (Physeter catodon)	MATACKRSSGEPQSDD		
Killer whale (Orcinus orca)	MATACKRSSGEPQSDD		
Narwhal (Monodon monoceros)	MATACKRSSGEPQSDD		
Baiji (Lipotes vexillifer)	MATACKRSSGEPQSDD		
Bottlenose dolphin (Tursiops truncatus)	MATACKRSSGEPQSDD		
Wild boar (Sus scrofa)	MATACKRSSGEPQSDD	Suidae	
Alpaca (Vicugna pacos)	MATACKRSSGESQSDD	Camelidae	
Arabian camel (Camelus dromedarius)	MATACKRSSGESQSDD		
Pacific walrus (Odobenus rosmarus divergens)	MATACKRSPGEPQSDD		Carnivora
Polar bear (Ursus maritimus)	MATACKRSPGEPQSDD		
Dog (Canis lupus familiaris)	MATACKRSPGEPQSDD		
Red fox (Vulpes vulpes)	MATACKRSPGEPQSDD		
Cheetah (Acinonyx jubatus)	MATACKRSPGEPQSDN		
Canada lynx (Lynx canadensis)	MATACKRSPGEPQSDN		
Iberian lynx (Lynx pardinus)	MATACKRSPGEPQSDN		
Cat (Felis catus)	MATACKRSPGEPQSDN		
Ferret (Mustela putorius furo)	MATACKRSPGEPQSDD		
Meerkat (Suricata suricata)	MATACKRSPGEPQSDD		
Sunda pangolin (Manis javanica)	MATACKRSPGEPQSDD		Pholidota
Donkey (Equus asinus asinus)	MATACKRSPGKPQSDD		Perissodactyla
Przewalski horse (Equus przewalskii)	MATACKRSPGKPQSDD		
Horse (Equus caballus)	MATACKRSPGKPQSDD		
Greater horseshoe bat (Rhinolophus ferrumequinum)	MATACKRSPGEPQSDD		Chiroptera
Natal long-fingered bat (Miniopterus natalensis)	MATACKRSPGEPQSDD		
Pale spear-nosed bat (Phyllostomus discolor)	MATACKRSPGEPQSDD		
David myotis (Myotis davidii)	MATACKRSPGEPQSDD		
Little brown bat (Myotis lucifugus)	MATACKRSPGEPQSDD		
Brandts bat (Myotis brandtii)	MATACKRSPGEPQSDD		
Great roundleaf bat (Hipposideros armiger)	MATACKRSPGEPQSDD		
European hedgehog (Erinaceus europaeus)	MATACKRSPGEPQPDD		Eulipotyphla
Common shrew (Sorex araneus)	MATACKRSPGEPQSDD		
Star nosed mole (Condylura cristata)	MATACKRSPGEPQSDD		
Nine-banded armadillo (Dasypus novemcinctus)	MATACKRSPGEPQSDD		Xenarthra
Aardvark (Orycteropus afer afer)	MATACKRSPGEPQSDD		
Lesser hedgehog tenrec (Echinops telfairi)	MATACKRSPGERQSDD		
Cape golden mole (Chrysochloris asiatica)	MATACKRSPGEPQSDD		
West Indian manatee (Trichechus manatus latirostris)	MATACKRSPGEPQSDD		Afrotheria
African bush elephant (Loxodonta africana)	MATACKRSPGEPQSED		

B

ATGGAGAAGCTGCACC**C**AGTaTTATTGGAA
↳ **out of frame exon**

ATGGAGAAGCTGCACC**C**AGTaTTATTGGAA
↳ **out of frame exon**

Lemuriidae

Tarsiidae

Callitricidae

Cebidae

Cercopithecidae

Hylobatidae

Hominidae

Primates

MEKLHQCYWK-

ATGGAGAAGCTGCACC**C**AGTaTTATTGGAA
↳ **out of frame exon**

Lagomorpha

Scadentia

ATGGAGAAGCTGCACC**C**AGTaTTATTGGAA
↳ **out of frame exon**

Euarchontoglires

ATGGAGAAGCTGCACCAGTGTTATTGGAA
↳ **in frame exon**

Boreoeutheria

Rodentia

c**T**GGAcAAGCTGCACC**C**AtTaTTATTGGAA
↳ **mutated initiation codon**

Cecartiodactyla

c**T**GGAcAAGCTGCACC**C**AtTaTTATTGGAA
↳ **mutated initiation codon**

Carnivora

c**T**GGAcAAGCTGCACC**C**AtTaTTATTGGAA
↳ **mutated initiation codon**

Laurasitheria

Atlantogenata

ATGGAGAAGCTGCACC**C**AGTaTTATTGGAA
↳ **out of frame exon**

Placentalia

Marsupialia

ATGGAGAAGCTGCACC**C**AGTaTTATTGGAA
↳ **out of frame exon**

Theria

Chiroptera

c**T**GGAGAAG-
↳ **mutated initiation codon**

Perissodactyla

c**T**GGAGAAG-
↳ **mutated initiation codon**

Monotremata

ATGGAGAAGCTGCACC**C**AGTaTTATTGGAA
↳ **out of frame exon**

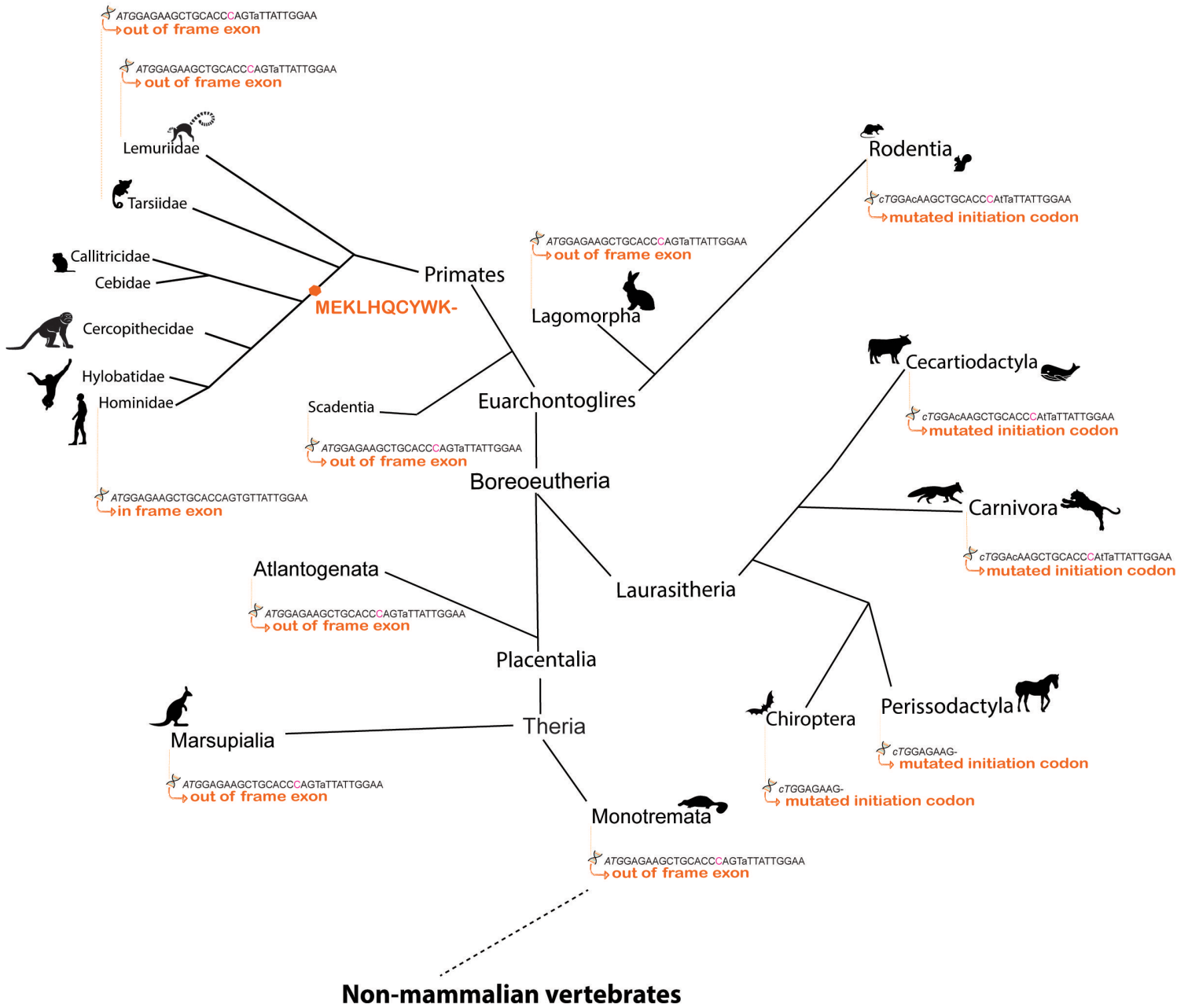**Non-mammalian vertebrates**

Figure S2. Multiple alignment of UBE3A N-terminal extensions homologous to hUBE3A-Iso3.
A. The table shows the alignment of UBE3A N-terminal extensions in placental mammals, homologous to the N-terminal extension of hUBE3A-Iso3. Sequences were identified by BLASTP analyses with residues 1-16 of hUBE3A-Iso3. On the left side of the sequence, the common and Latin (in brackets) name for each species is given. On the right of the sequences the family/superfamily or order of the species is indicated, corresponding to Figure 3A. The graph on the top of the table shows the conservation of each amino acid residue during placental mammal evolution. Amino acid residues that are not 100% conserved compared to the 'ancestral' consensus sequence (MATACKRSPGEPQSDD, shown on top) are colored magenta and are highlighted in all sequences shown. The gradient of highlighting corresponds to the level of conservation of each residue, with light magenta indicating most conserved residues and darker magenta indicating least conserved residues. **B.** Molecular evolution of the sequence of the UBE3A 'MEKL' exon in placental mammals. Nucleotide changes compared to the human sequence are in small letters and nucleotide insertions are indicated in magenta.
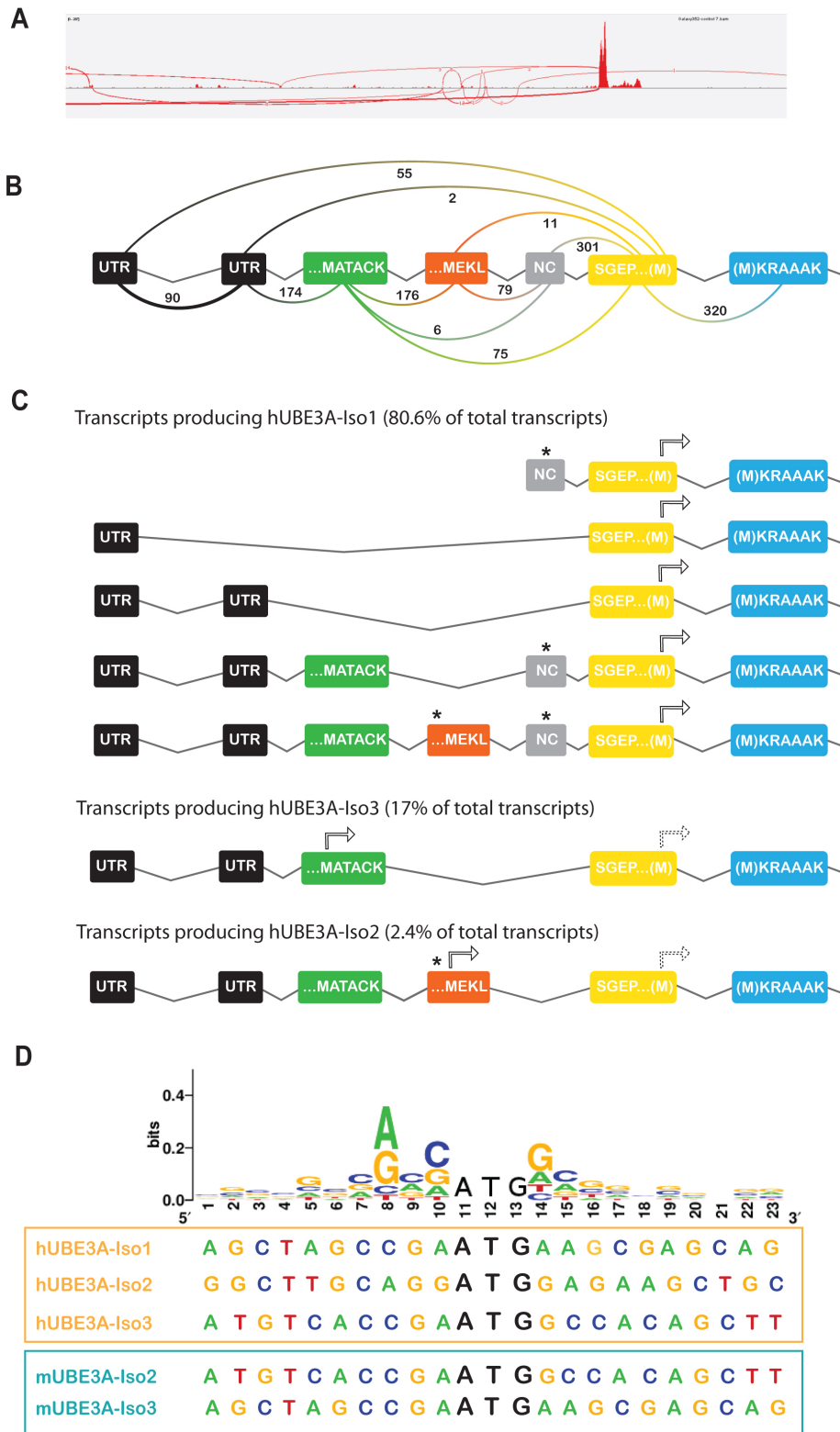
Supplemental Figure 3

Figure S3. 5' coding region of the *UBE3A* gene and predicted splicing pattern of transcripts in iPSC-derived neurons. A. Representative image of a Sashimi plot for the human cortex RNA-seq samples. **B.** Schematic representation of the number of *UBE3A* exon junctions found in the hES-derived NPC RNA-seq dataset. **C.** Splicing pattern of predicted *UBE3A* transcripts for the hES-derived NPC RNA-seq dataset. Splicing pattern and relative abundance of each isoform producing transcript were calculated as described in the legend to Figure 4. Putative initiation ATGs (arrows) and stop codons (asterisks) upstream of the initiation ATG are indicated for each predicted transcript. Solid arrows indicate the first possible translation site to produce a full length UBE3A isoform. Dashed arrows indicate downstream translation sites possible to produce full length UBE3A isoforms. **D.** Top: Consensus Kozak sequence (modified from Wegrzyn et al., 2018). Bottom: alignment of the sequences surrounding the ATGs of human and mouse UBE3A isoforms with the consensus Kozak sequence.