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Supplemental Data

Biallelic Mutations in *PDE10A* Lead to Loss of Striatal PDE10A and a Hyperkinetic Movement Disorder with Onset in Infancy

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Supplemental Note: Case Reports

Family 1 is a UK family of Pakistani origin.

IV:2 was the proband. He presented to the department of clinical genetics at age 5 with generalised developmental delay, motor delay had been evident since age 3 months. At age 5 he was noted to be ataxic with a wide based stamping gait and frequent falls. He was unable to manage tandem gait and had a marked intention tremor. On closer examination he had a generalised movement disorder with dyskinesia of all four limbs in all positions. There was also an orofacial dyskinesa. There was no obvious muscle weakness, there was no peripheral neuropathy or sensory deficit. He was markedly dysarthric, without dysphagia, and in fact had good general comprehension. There was no facial weakness and cranial nerve examination was otherwise normal. Tendon reflexes were brisk but plantars were downgoing.

A diagnosis of a generalised dyskinetic movement disorder was made, with little evidence of a cognitive impairment. He was investigated but there was no evidence of acanthocytes in blood. Urinary organic acid analysis was normal, in particular glutarica ciduria was excluded. Serum copper and ceruloplasmin levels were normal. Thyroid function tests and serum lipids were normal. Routine karyotype was also normal.

He was further reviewed throughout childhood and adult life. Last review was at age 38, on examination he had a subtle dyskinesia of the arms, hands and fingers which is made worse when he was asked to raise his hands above his head. On walking his gait was reasonable, but he had clear difficulty maintaining progressive gait on heel-toe testing. Accompanying this difficulty in walking he had subtle but obvious dyskinesia of the fingers. He had a clear orofacial dyskinesia and his speech is slightly dysarthric. Tendon reflexes were brisk but plantars were downgoing and there was no sensory component or neuropathy.

IV:4 was the sister of the proband and presented at age 3 to the Clinical genetics department. She was again felt to have generalised delay from age 3 months but like her brother her manifestations were principally of a motor disturbance. She had the generalised dyskinetic movements of face trunk and limbs seen in her brother. She was dysarthric, but there were no other cranial nerve abnormalities apparent on examination, there was no real evidence of muscle weakness, there was no evidence of a peripheral neuropathy or sensory loss. She also had brisk tendon reflexes and downgoing plantars. Cognition was normal. Investigations revealed no obvious cause for her deficit. Routine karyotype was normal. She was reassessed throughout childhood and adult life. At her most recent assessment at age 31 findings were stable with no evidence of progression or improvement.

Individuals III:4 and III:5, the parents of IV:2 and IV:4 were examined and neither had any evidence of motor abnormalities. In view of the family structure and the absence of

disease in the parents a diagnosis of an autosomal recessive benign chorea was made. At a later date analysis of *NKX2-1* was performed in IV:2 but no mutation was identified.

V:1 was the first daughter of IV:2 and his first cousin. She presented in a very similar fashion to her father with delay in motor development first noted at the age of 4 months. She then presented with repeated falls and unsteady gait at age 3. She was followed up throughout childhood and was most recently seen at age 16 years. She attended a normal school and at her most recent assessment aged 16 was managing well, planning to complete school examinations. The major issue for her in class had always been her dysarthria which was very debilitating. Despite this her symptoms have remained stable through childhood. On examination aged 16 she displayed a generalized hyperkinetic movement disorder characterized by dyskinesia of the limbs and trunk, with facial involvement, orolingual dyskinesia and drooling. The hyperkinesis has a jerky quality, with occasional short almost myoclonic jerks of the upper limbs. She kept her mouth open during part of the examination, possibly suggestive of jaw dystonia. Her gait was abnormal, with some gait instability, reduced arm swing and impaired foot clearance and a possible shuffling nature to her gait. On walking backwards, she showed some distal upper limb posturing. Heel-toe walking was relatively stable, with heel-toe strike but was associated with upper limb posturing She found jumping difficult and postures her hands (R>L) when performing this task. There were no overt cerebellar features on examination but tasks such as hand-tapping and finger-nose testing seem to exacerbate the hyperkinesia. She had brisk tendon reflexes with downgoing plantars

She was dysarthric but cranial nerve examination was normal. Results of investigations including cranial MRI, anti thyroid antibodies, and array CGH were normal. DNA studies for SCA (types 1, 2, 3, and 6), HD, and DRPLA were normal. Urinary organic acid analysis was normal, in particular glutaric aciduria was excluded. Serum copper and ceruloplasmin levels were normal. Thyroid function tests and serum lipids were normal. Routine karyotype was also normal.

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V:2 was the second child of IV:2. She presented at age 6 months with motor delay, unable to sit unsupported with marked truncal ataxia. She went on to develop a dyskinetic movement disorder accompanied by dysarthria. She was most recently reviewed at age 15. On examination she had a hyperkinetic disorder characterised by dyskinetic movements of all four limbs and trunk. She had orofacial dyskinesia with some lingual dyskinesia and drooling. She was dysarthric. She had an abnormal gait with a shuffling nature and poor arm swing and and floor clearance of her feet. She was unable to maintain a good heel-toe gait, and on this test she had upper limb posturing like her sister. She was unable to jump, hand posturing accompanied her attempts. She had brisk tendon reflexes with downgoing plantars. She had no overt cerebellar signs. Although she displayed a worse dysarthria than her sister, cranial nerve examination was normal. She attended the same mainstream school as her elder sister V:1

V:3 was the third child to the couple, the younger sister of V:1 and V:2. She presented with motor delay aged 3 months, with poor head control and orofacial movements noted

by her parents. She developed a dyskinetic movement disorder during childhood similar to that seen in her sisters and father. She was most recently reviewed at age 12 years. . She had a similar degree of dysarthria to her sisters and attended the same school.
On examination she had the jerky hyperkinetic movement disorder seen in her older sisters with dyskinesia of all four limbs and trunk. She had orofacial dyskinesia and some drooling. Like her sisters she had an unstable gait and upper limb posturing on heel-toe walking. Again she had brisk tendon reflexes with downgoing plantars. Results of all inveistigations were normal, in particular brain MRI was normal. Careful review of the basal ganglia and cerebellum in particular revealed no abnormality.

She had a similar degree of dysarthria to her sisters and attended the same school.

V:4 was the first male child to the couple. At age 2 months his mother noted abnormal facial movements and very messy feeding when he was weaned. This pattern was very similar to that noted by her in his sister, V:3. He went on to develop a very similar hyperkinetic movement disorder with dyskinesia of all four limbs and trunk. His speech was clearly dysarthric and and at age 2 he was very difficult to understand. His comprehension of speech was age appropriate. He was unable to stand unsupported at that age and had jerky dyskinetic movements of his limbs, which were worse when he was trying to pull himself to stand. He still had truncal ataxia and dyskinetic movements of his face. He had marked orolingual dyskinesia and severe drooling. His clinical signs at that age were very similar to his siblings at similar ages.

In summary all five individuals had a very stable movement disorder, characterised by hyperkinetic dyskinesia and dysarthria, however there was little evidence of a cognitive component. All investigations, including testing for mutations in *NKX2-1*, the cause of benign hereditary chorea (BCH OMIM 118700) were normal.

Family 2 is of Caucasian origin and has two affected sons who were born at 35-38 weeks (with birth weights of 3550 g and 3890 g) to healthy parents who are the first cousins. Both male individuals (Individuals 1 and 2) presented with profound axial hypotonia and athetotic movements at four months of age. Brain MRI, EEG, ENMG and muscle biopsy did not show any specific abnormal findings. Mitochondrial respiratory chain enzyme activities of skeletal muscle sample were normal, and there were no deletions in muscle mitochondrial DNA (mtDNA). Sequencing of the mtDNA and nuclear genes encoding mitochondrial twinkle helicase (*PEO1*) and polymerase gamma (*POLG1*) were negative. Furthermore, *XNP* and *HPRT* genes were analyzed to exclude ATR-X and Lesch-Nyhan syndromes, but no pathogenic mutations were found. Extensive metabolic investigations including dopamine metabolites and other neurotransmitters in cerebrospinal fluid were unyielding. Motor and speech development of both individuals has been markedly delayed, but there has been no loss of skills. Both individuals suffer from severe dysarthria and they started to express a few words at the age of seven years, but they mainly communicate with picture symbols and sign language. Their understanding of speech has been estimated to be close to normal. They learned to wheel their wheel chairs independently at 7 years of age as well. Individual 1 was also diagnosed with insulin-dependent autoimmune diabetes at 7 years of age. At the age of 9 years he is able take a

couple of steps with support (Supplementary Video 1 on Individual 1). The younger brother (Individual 2) presents with a more severe neurological phenotype including more severe movement disorder, he has not learnt to stand up, his cognitive development is more delayed compared to his brother (Supplementary Video 2 on Individual 2). Furthermore, due to difficulties in eating a gastrostomy tube was inserted at 2.5 years of age, and he was diagnosed with focal epilepsy at 3.5 years presenting with focal dyscognitive seizures (impairment of consciousness, interruption of ongoing activities, staring,and swallowing). During carbamazepine (CBZ) treatment he has been seizure free except for occassional seizures with similar seizure type related to too low dose of CBZ. EEG revealed arrhytmic delta activity without focal epileptiformic discharges. Both individuals present with axial hypotonia and ongoing movement disorder with generalized hyperkinesis and dyskinesia affecting all four limbs, trunk and face that is described in more details in the videos (Supplementary Videos 1 and 2).

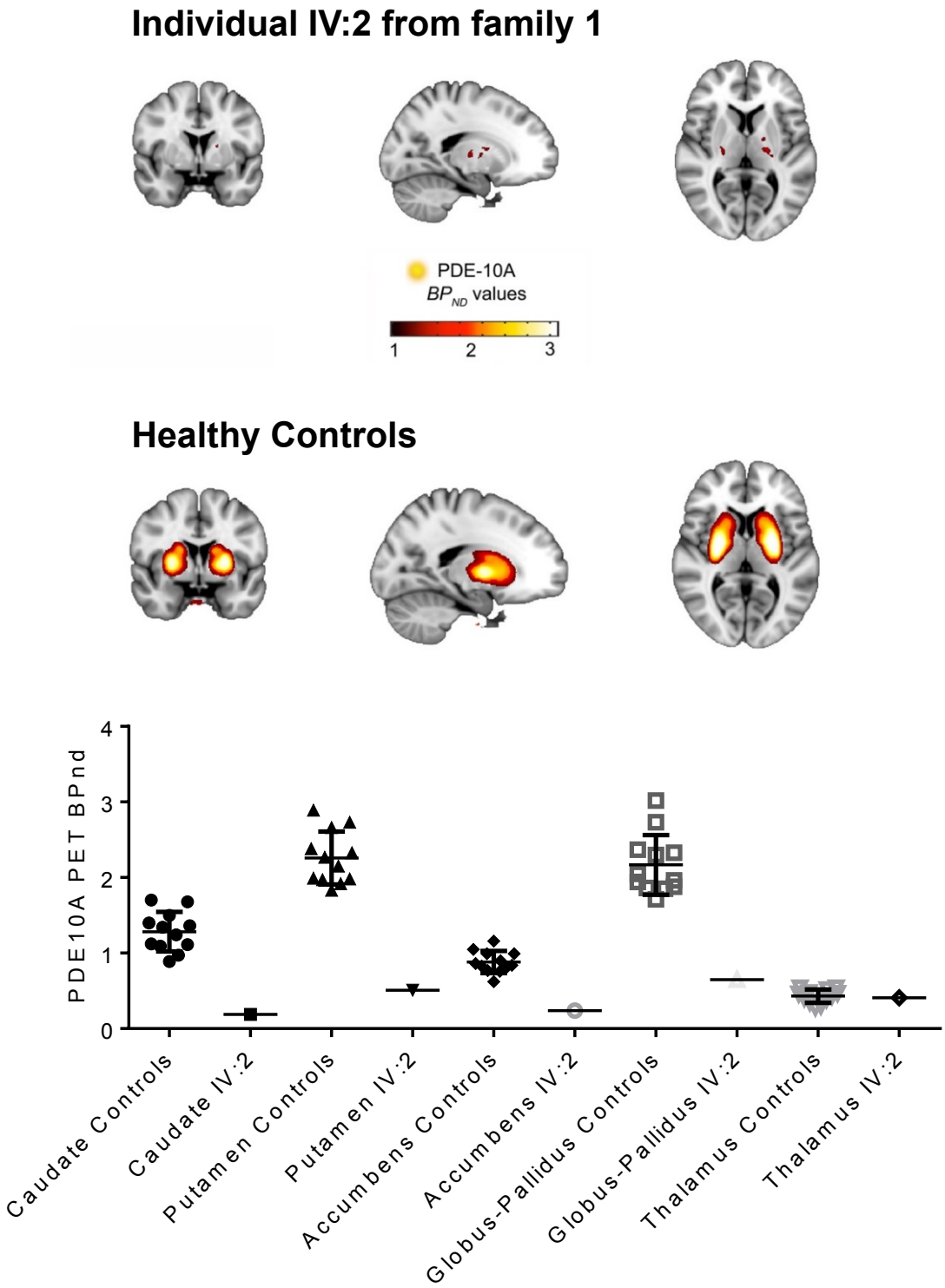
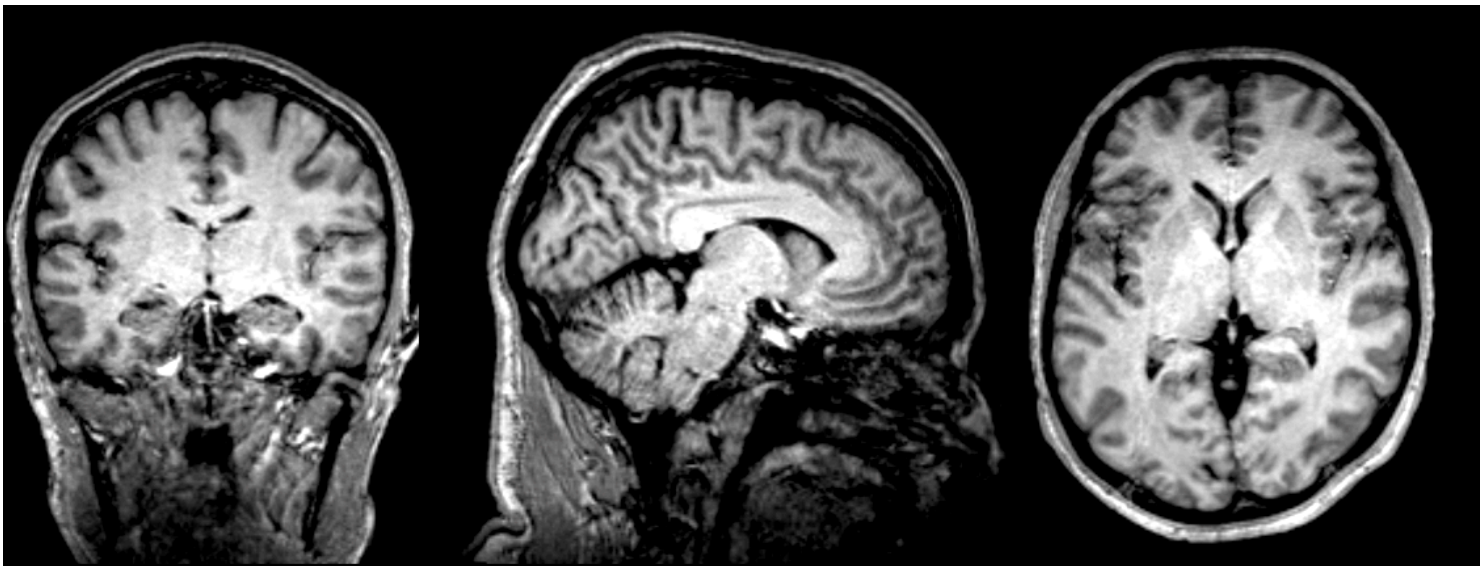


Figure S1 PDE10A signal is reduced across different regions of the striatum in individual 1 (IV:2 Family 1) Mean 11C-IMA107 BP_{ND} parametric images derived from individual IV:2 Family 1 and 12 healthy controls in stereotaxic space overlaid onto the T1-weighted MNI template showing significant loss of striatal and pallidal PDE10A signal in IV:2.Scatterplots showing individual 11C- IMA107 BP_{ND} values in the striatum and motor thalamic nuclei for healthy controls (Caudate controls et seq) , and individual IV:2 Family 1 (Caudate IV:2 et seq). Solid lines represent 11C-IMA107 BP_{ND} mean and SD values for healthy controls and individual IV:2 Family 1.

MRI of Individual IV:2 from family 1



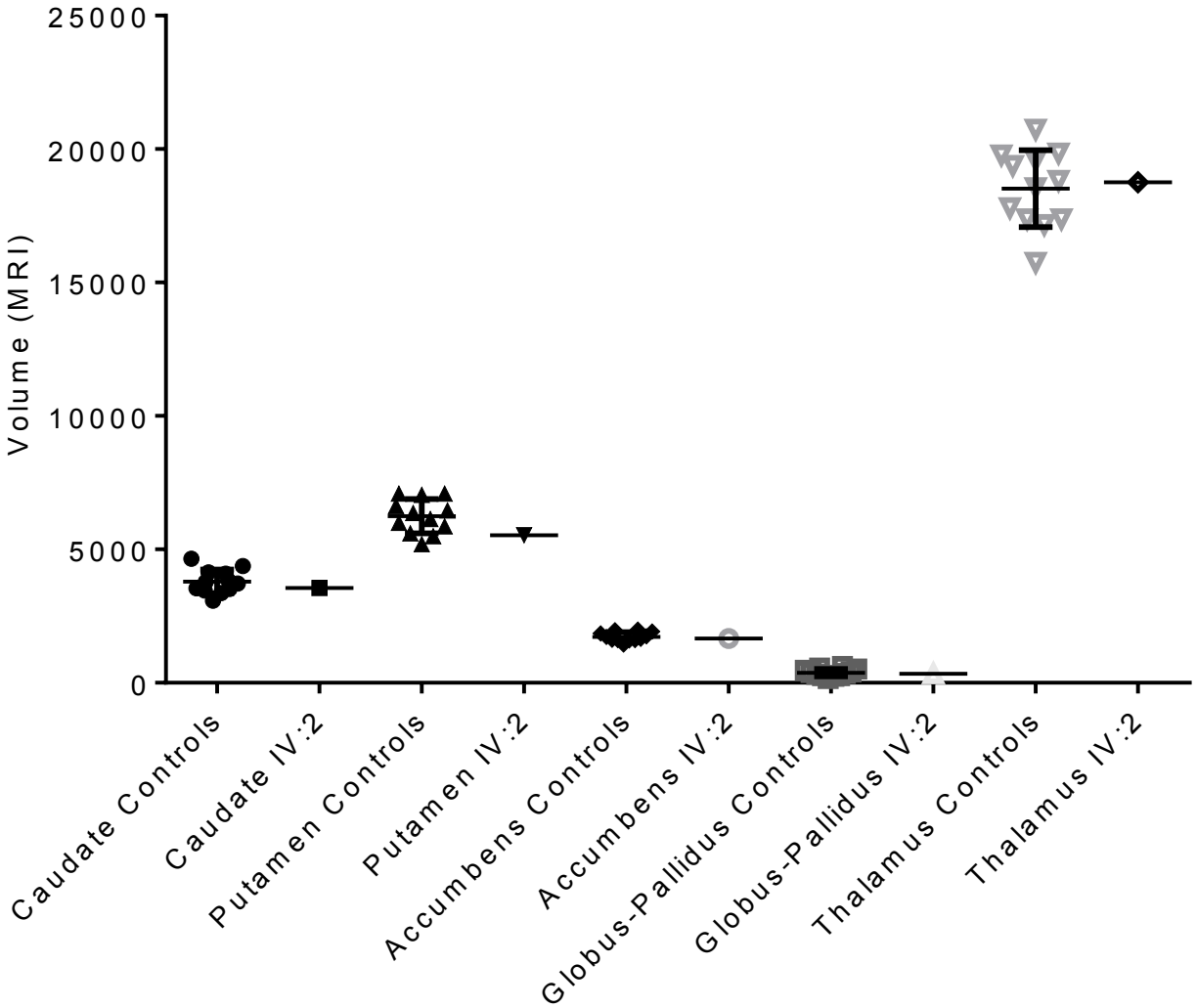


Figure S2 Normal MRI from individual IV:2 from family 1. Scatterplots showing individual volume of caudate, putamen, nucleus accumbens, globus pallidus and thalamus for healthy controls (Caudate controls et seq) and individual IV: 2 Family 1 (Caudate IV:2 et seq). Solid lines represent mean and SD for healthy controls and individual IV:2 Family 1.

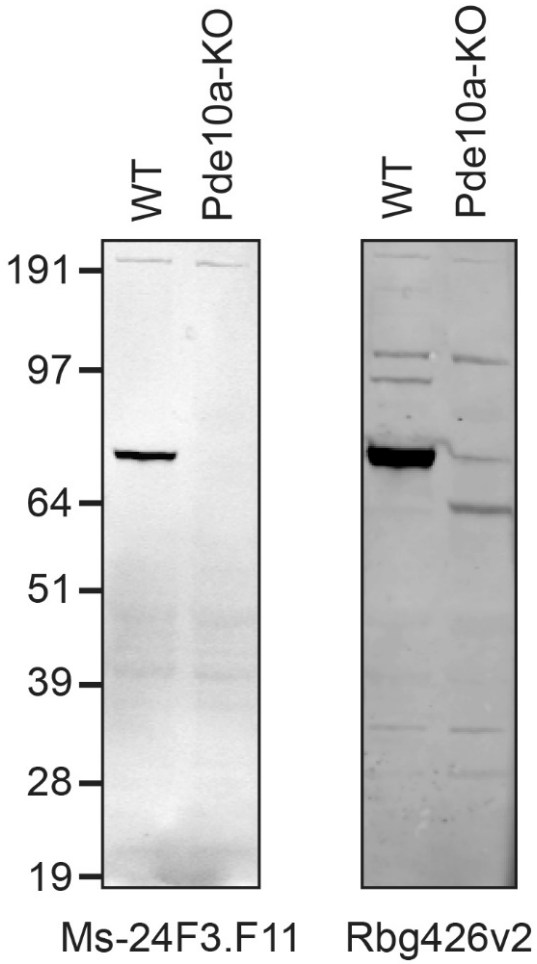


Figure S3 Characterization of PDE10A antibodies.
Striatal lysates from either WT or Pde10a knock-out mice were analyzed for PDE10A expression with a specific PDE10A monoclonal antibody (mouse-anti- PDE10Ag24F3.F11) or a specific PDE10A polyclonal antibody (Rbg426v2).

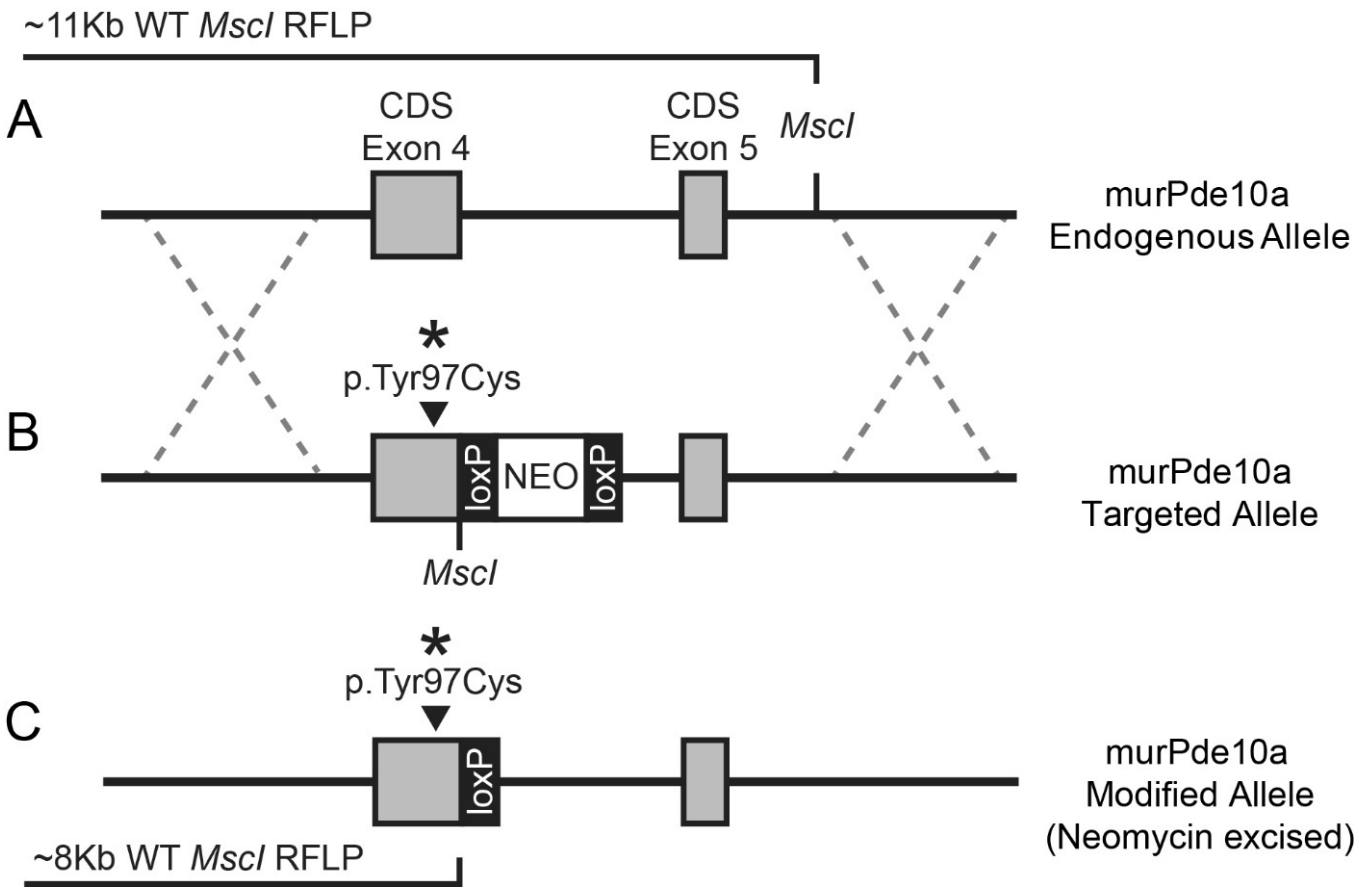


Figure S4 Schematic of construct and genomic regions for Pde10A p.Tyr97Cys knock-in mouse. Please see Methods above for details on the targeting construct

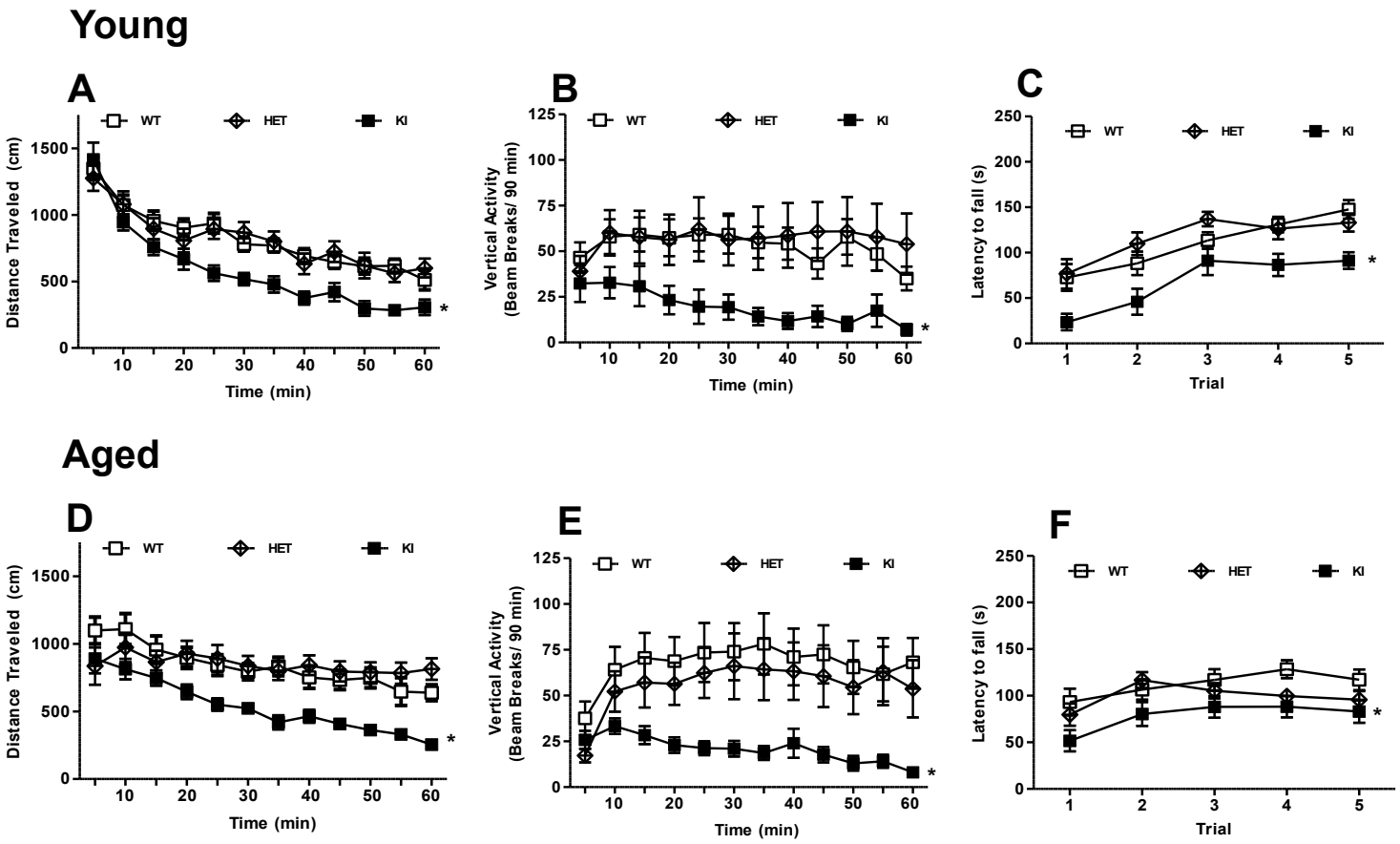


Figure S5 Pde10A p.Tyr97Cys Knock-in mice demonstrate motor abnormalities. WT, HET and KI mice were evaluated at different ages (young; A-C and aged; D-F). Total distance traveled (A, D) and rearing behavior as measured by vertical activity (B, E) were analyzed by two-way repeated measures ANOVA (genotype x time), using Prism Statistical SoPware (version 5.0; GraphPad Software Inc, California USA), with Bonferroni post hoc tests as appropriate. Latency to fall (C, F) was recorded and analyzed by a two-way repeated measures ANOVA (genotype x trial) using Prism Statistical Software (version 5.0; GraphPad Software Inc, California USA) with Bonferroni post hoc tests as appropriate. All data are presented as group mean +/- s.e.m. WT (young, n=13; adult, n=7; aged, n=13), HET (young, n=12; aged, n=12), KI (young, n=11; aged, n=11) *$p < 0.05$

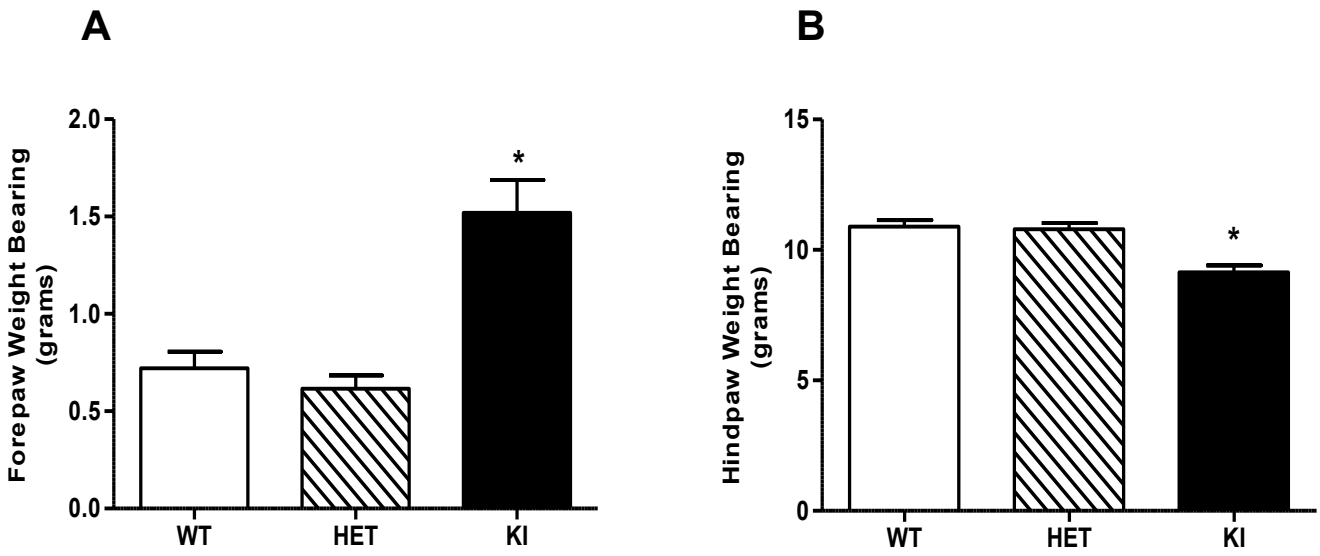


Figure S6 Pde10A p.Tyr97Cys Knock-in mice show compensatory weight shifting from hindpaws to forepaws. Adult WT (n=12), HET (n=13) and KI (n=9) mice were evaluated in a dynamic weight bearing assay for differences in forepaw (A) and hindpaw (B) weight bearing. Data are presented as group mean +/- s.e.m. Individual measures were analyzed by one-way ANOVA with Dunnets's post hoc test as appropriate. *$p < 0.05$

Table 1. **Clinical Findings of 8 Affected Cases.**

Family	Case	Sex	Age at Onset	Current Age	Hyperkinetic movements (dyskinesia, myoclonus, chorea	Dystonia	Dysarthria	Axial Hypotonia	Developmental Delay	Epilepsy	Brain MRI results
1	IV:2	M	3 mo	38yrs	+	+	+	-	-	-	Normal
	IV:4	F	3 mo	30 yrs	+	+	+	-	-	-	ND
	V:1	F	4 mo	17 yrs	++	++	++	+	-	-	Normal
	V:2	F	6 mo	16 yrs	+++	+	++	+	-	-	ND
	V:3	F	3mo	13 yrs	++	++	+++	+	-	-	Normal
	V:4	M	2 mo	2 yrs	+++	+++	+++	++	-	-	Normal
2	IV:1	M	4 mo	10 yrs	+++	+++	+++	++	++	+	Normal
	IV:2	M	4 mo	9 yrs	+++	+++	+++	++	++	-	Normal

ND = Not done