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Supplemental Data

Homozygous Truncating Variants in *TBC1D23* Cause Pontocerebellar Hypoplasia and Alter Cortical Development

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Supplemental Note: Case Reports

Family 1 (MCD-S001)

Affected sister and brother were referred to the Pediatric and Genetics Departments for neurodevelopmental delay, microcephaly and agenesis of the corpus callosum (2/2) associated with imperforate anus and bilateral talipes equinovarus (only present for the boy). They were the second and third children born to healthy consanguineous parents (first cousins) originating from Turkey. Of particular interest was the description of a first-degree maternal cousin presenting with the same symptomatology. Regarding the male subject, pregnancy was marked by discovery of corpus callosum agenesis at 32WA (weeks of amenorrhea). Delivery occurred at 38WA and birth measurements were in the normal range with weight: 2760g (P15), length: 46cm (P5), Occipital Frontal Circumference (OFC): 32.5cm (P25). Imperforate anus and bilateral talipes equinovarus were noticed and surgically removed. Development was marked by severe ID associated with postnatal microcephaly, poor ocular contact, neurologic abnormalities and autistic behavior. Brain MRI revealed PCH with agenesis of the corpus callosum. Ophthalmologic examination found severe hyperopia, astigmatism and strabismus. Audition was normal.

For the affected sister, prenatal factors and birth parameters were not available. She was examined by a clinical geneticist at 10 years old as she presented the same symptomatology as her brother. The microcephaly and the developmental delay were diagnosed at an age of 9 months. The language is limited to few syllables. The neurological evolution was similar to her brother with severe developmental delay, microcephaly, poor ocular contact, autistic behavior and stereotypic behavior. Imaging showed PCH with thin corpus callosum.

Family 2 (M268)

The two subjects were born from second cousin healthy parents originating from Khuzestan province in the south-western part of Iran. Both individuals were born with an unremarkable pregnancy, delivery, and neonatal period. They had normal birth OFC but height had not been documented. The male individual started to speak few simple words at 4 years and to walk at 5 years. He had strabismus, prominent incisors, and protruding ears. He had a slender body and was able to walk slowly. Serum CK (creatine kinase) level was normal and EMG (electromyogram) showed no muscular deficit pattern. Developmental testing at age 13 years using an adapted version of WISC-IV showed an IQ of 55, in the range of moderate ID. He died at age 18 years as a result of severe pneumonia.

The female individual started to sit at age 9 months, to stand at 18 months, to speak a few simple words at age 4 years, and to walk at age 3 years. She had also a slender body, strabismus, prominent incisors, happy expression, and protruding ears. Brain MRI showed a mild abnormal white matter, and hypoplastic corpus callosum with severe cerebellar hypoplasia and vermis agenesis. Dandy-Walker variant anomaly was noted and the junction of pons and cerebral peduncles showed signs of a molar tooth. Developmental testing at age 4 years using WISC-IV showed an IQ of 30, in the range of severe ID.

Family 3 (PKMR52)

Three affected siblings (one male and two females) born to healthy consanguineous parents of Pakistani origin were evaluated for ID. Two other sibs (one male and one female) were unaffected. Prenatal factors and birth parameters were not available due to birth of these individuals at home. All affected individuals had developmental milestones (motor, speech and social) delay. The oldest affected girl could sit at 10 years of age but was unable to walk. Her younger sister was able to sit at the age of 5 years and walk at the age of 7 years. The boy just started to stand at 14 years of age. All three could hardly repeat sounds. There was no history of a seizure disorder. At the time of last evaluation, physical examination was remarkable

for growth retardation and microcephaly in all affected individuals. There was also marked motor weakness and spasticity. Attention deficit hyperactivity disorder (ADHD) was observed in one patient. Both affected females had poor social communication and interaction and persistent repetitive motor behavior suggestive of Autism Spectrum Disorder (ASD). Dysmorphic features included coloboma (3/3), convergent squint (1/3), wide prominent nasal bridge (1/3) and widely spaced dysplastic teeth (1/3). Brain MRI was only available for the male patient and revealed PCH and mega cisterna magna.

Figure S1

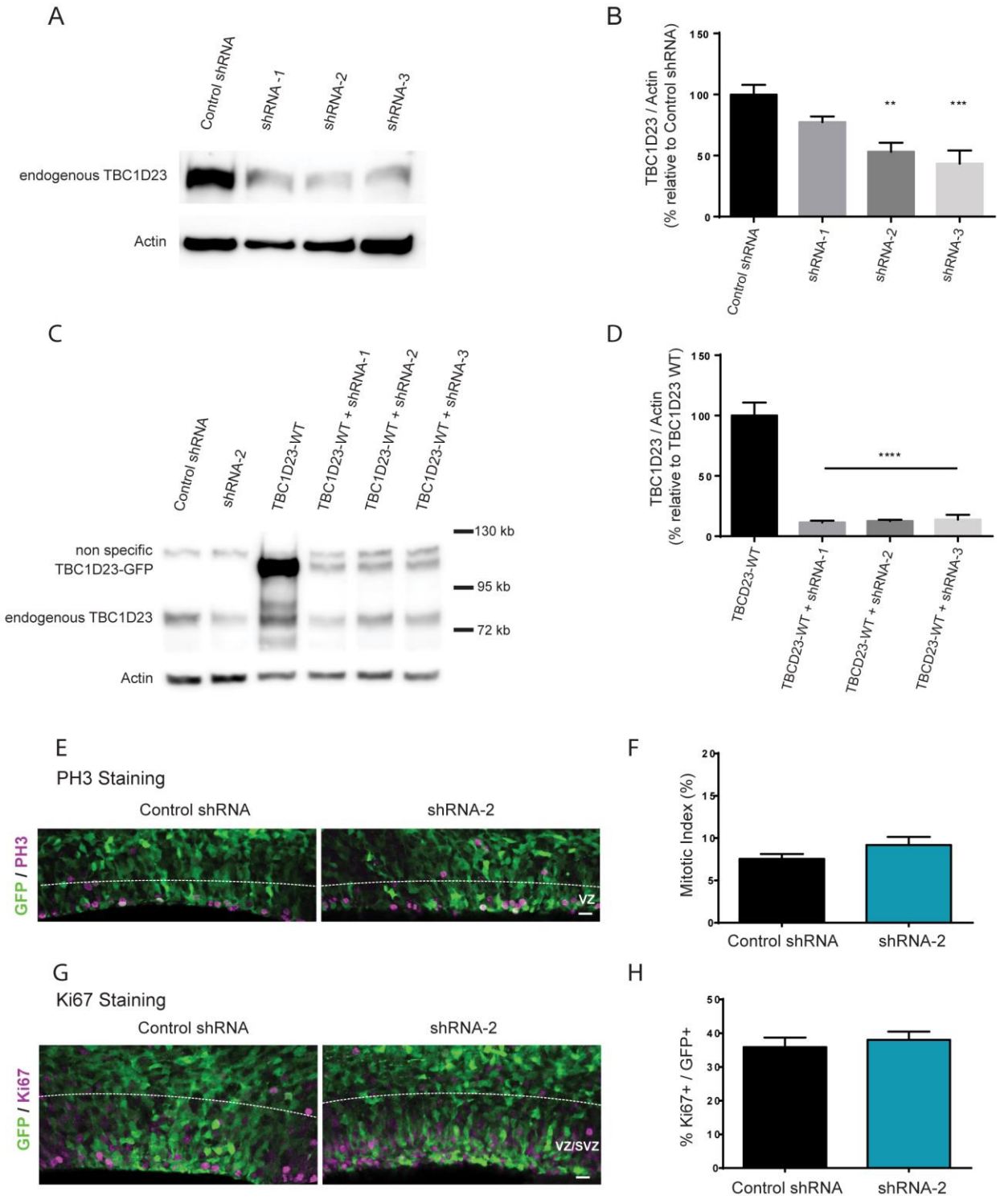
A
Control shRNA
shRNA-1
shRNA-2
shRNA-3
endogenous TBC1D23
Actin
B
TBC1D23 / Actin
(% relative to Control shRNA)
150
100
50
0
**
***
Control shRNA
shRNA-1
shRNA-2
shRNA-3
C
Control shRNA
shRNA-2
TBC1D23-WT
TBC1D23-WT + shRNA-1
TBC1D23-WT + shRNA-2
TBC1D23-WT + shRNA-3
non specific
TBC1D23-GFP
endogenous TBC1D23
Actin
130 kb
95 kb
72 kb
D
TBC1D23 / Actin
(% relative to TBC1D23 WT)
150
100
50
0
****
TBCD23-WT
TBCD23-WT + shRNA-1
TBCD23-WT + shRNA-2
TBCD23-WT + shRNA-3
E
PH3 Staining
Control shRNA
shRNA-2
GFP / PH3
VZ
F
Mitotic Index (%)
20
15
10
5
0
Control shRNA
shRNA-2
G
Ki67 Staining
Control shRNA
shRNA-2
GFP / Ki67
VZ/SVZ
H
% Ki67+ / GFP+
50
40
30
20
10
0
Control shRNA
shRNA-2

Figure S1. *TBC1D23* knockdown immunoblotting analysis and effects on progenitor's proliferation.

(A) Representative immunoblots showing the effect of *TBC1D23* shRNAs on endogenous TBC1D23 levels in Nero2a cells transfected respectively with: a control shRNA, shRNA-1, shRNA-2, shRNA-3. (B) Relative quantification of band intensity as compared to average control band intensity and normalized to respective actin band. Data are represented as mean ± SEM. shRNA-2 vs Control P = 0.0014; shRNA-3 vs Control P = 0.0002.

(C) Representative immunoblots showing the effect of *TBC1D23* shRNAs on exogenous GFP-fused *Tbc1d23* mouse cDNA in Neuro2a cells transfected respectively with: control shRNA, shRNA-2, TBC1D23-WT fused to GFP; TBC1D23-WT-GFP + shRNA-1; TBC1D23-WT-GFP + shRNA-2; TBC1D23-WT-GFP + shRNA-3. (D) Relative quantification of band intensity for lanes 3 to 6 as compared to average TBC1D23-WT band intensity and normalized to respective actin band. Data are represented as mean ± SEM. ****$P \leq 0.0001$.

(E) Coronal sections of E16.5 brains electroporated at E14.5 with either a control shRNA or shRNA-2 together with a GFP-reporter construct. Sections were immunolabeled for the mitotic marker PH3 (magenta). VZ: ventricular zone. Scalebar 10µm. (F) Double-positive GFP and PH3 cells were counted in the ventricular zone and reported to the total number of electroporated GFP-positive cells in order to calculate the mitotic index (percentage of cells currently in mitosis). Data are represented as mean ± SEM.

(G) Coronal sections of E16.5 brains electroporated at E14.5 with either a control shRNA or shRNA-2 together with a GFP-reporter construct. Sections were immunolabeled for the proliferating progenitor marker Ki67 (magenta). VZ: vebtricular zone; SVZ: subventricular zone. Scalebar 10µm. (H) Double-positive GFP and Ki67 cells were counted in the ventricular and subventricular zones and reported to the total number of electroporated GFP-positive cells in order to calculate the percentage of proliferative cells. Data are represented as mean ± SEM.

Figure S2

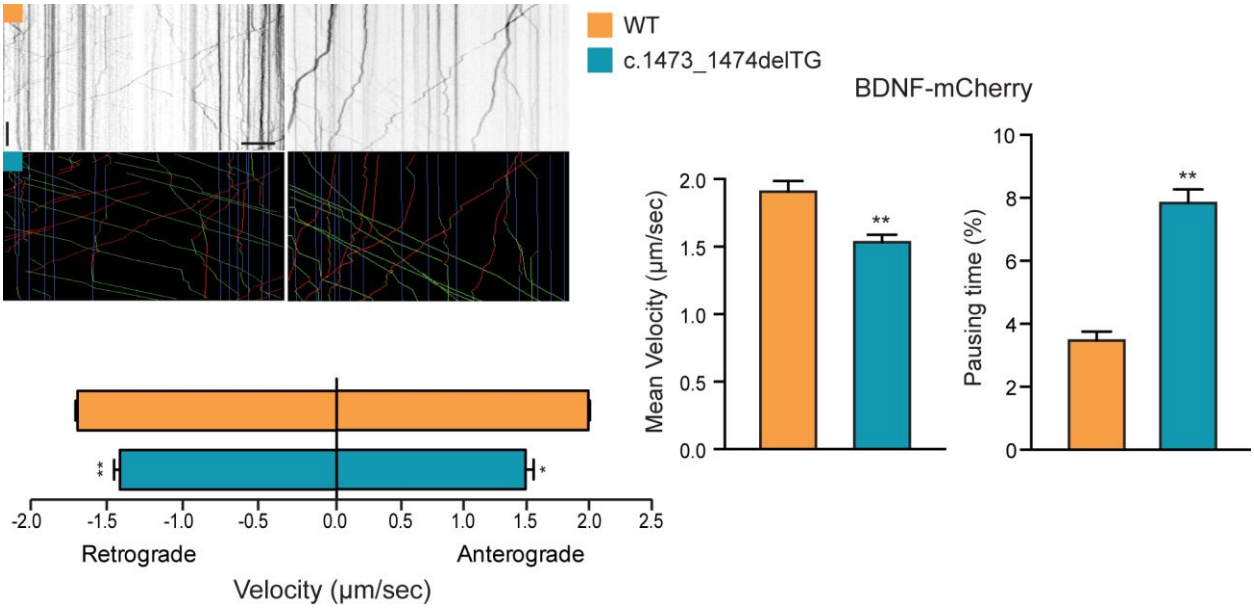

Figure S2. *TBC1D23* knockdown in primary neurons alters BDNF trafficking.

Kymographs and colored kymographs (green=anterograde, blue=static, red=retrograde) showing the trajectories of single BDNF-mCherry containing vesicles. Histograms represent mean ± SEM of average velocity (t=13.11; p=0.0058; n=3), %pausing time (t=23.53; p=0.0018; n=3) and instantaneous velocity (Anterograde: t=8.668; p=0.0131; n=3. Retrograde: t=10.14; p=0.0096; n=3) of BDNF-mCherry vesicles. Scale bars: 10µm and 10sec.