This supplemental section contains:

Supplemental Figures S1-S10

Supplemental Table S1

SUPPLEMENTARY FIGURE LEGENDS

Figure S1. *Kat8* expression in the developing cerebrum and its loss in cerebrum-specific knockouts. (**A)** Immunostaining with the anti-H4K16ac antibody in E12.5 embryonic sections and postnatal brain sections (P5 and adult). Only a portion of a representative para-sagittal section is shown here. DAPI staining showed that H4K16ac fluorescence signals were localized to the nuclei. Scale bars, 200 μm. **(B-C)** FPKM (fragments per kilobase of transcript per million mapped reads) values of transcripts for *Kat8* and genes encoding its associated subunits at the wild-type neonatal cerebrum (B) and E18.5 neurospheres (C). Duplicates of RNA-Seq datasets (GEO, GSE133195) were used for generation of the panels by Tophat. Note that the datasets were from bulk RNA-Seq, which does not reveal expression in individual cell types or at the early or later timepoints than the analyzed days (i.e. E16.5 and P0). **(D)** Immunostaining with the anti-H4K16ac antibody in the E12.5 wild-type and mutant embryonic sections. Merged panels represent co-localization of anti-H4K16ac fluorescence and counterstained DAPI signals. The boxed areas of wild-type and mutant cerebrocortical neuroepithelia are enlarged at the right. Three red arrowheads demarcate the boundary of the deleted and non-deleted areas. Residual positive cells in the mutant cerebrocortical neuroepithelium are perhaps related to or derived from interneuron (or microglial) precursors. Results are representative of two independent experiments. Scale bars, 500 μm. Abbreviations: CA1, Cornu Ammonis area 1 of the hippocampus; Ch, choroid plexus; DG, dentate gyrus; GE, ganglionic eminence; Hp-Pri,

hippocampus primordium; LV, lateral ventricule; PP, pre-cortical plate; SVZ, subventricular zone; VZ, ventricular zone.

Figure S2. Photos of the control and mutant mice or brains. (**A**) Photos of wild-type and cKO mouse at P6. (**B**) Photos of wild-type and cKO mouse at P14. (**C**) Representative brain images for the wild-type and cKO mice at P5. This is a different pair from that shown in Figure 1F. (**D-F**) Brain images of another pair at P1, E18.5 and E16.5. Each image is representative of at least five different experiment. Scale bars, 1 mm. Abbreviations: Cb, cerebellum; CP, cortical plate; Cx, cerebral cortex; Hp, hippocampus; Ob, olfactory bulb; Th, thalamus.

Figure S3. ***Kat8* deletion causes defective cerebral lamination.** (**A**) Immunostaining analysis of wild E16.5 brain sections with anti-CTIP2 and -CUX1 antibodies. Enlarged images of the boxed areas are shown at the right. (**B**) Same as (A) but mutant brain sections were analyzed. The results revealed cerebral lamination defects in the mutant brain. The images are representative from four different experiments. Scale bars: left panels, 500 μm; middle and right panels, 100 μm. See the Figure 1 legend for abbreviations.

Figure S4. **Immunostaining analysis of control and mutant embryonic sections with an anti-TBR2 antibody.** (**A**) Immunostaining analysis of E13.5 control and mutant embryonic sections with an anti-TBR2 antibody. Enlarged images of the squared areas are shown at the middle and right. (**B**) Same as (A) except that E12.5 embryonic sections were analyzed. Shown images are representatives of three (A) or four (B) experiments. Scale bars, 500 μm (left panels) and 100 μm (middle and right panels).

Figure S5. **Effect of *Kat8* deletion on cell proliferation and DNA damage response at E12.5.** (**A**) Immunostaining analysis of E12.5 control and mutant embryonic sections with anti-BrdU and Ki67 antibodies. Enlarged images of the squared areas are shown at the middle and right. For BrdU labeling, mating was timed and BrdU was injected intraperitoneally into E12.5 pregnant mice. After 1 h, mice were euthanized for embryo retrieval, genotyping, section preparation and subsequent immunostaining with the indicated antibodies. (**B**) Immunostaining analysis of E12.5 control and mutant embryonic sections with an anti-phospho-Ser139 H2A.X (γH2A.X) antibody. Images are representative of 4 (A) or 3 (B) independent experiments.

Figure S6. **Impact of *Kat8* deletion on apoptosis at E12.5.** (**A**) TUNEL staining of E12.5 embryonic sections uncovered massive apoptosis at the mutant cerebrocortical neuroepithelium. Each image is representative of three different experiment. (**B**) Immunostaining analysis of E12.5 embryonic sections with the anti-cleaved caspase 3 antibody confirmed massive apoptosis at the mutant neuroepithelium. Images are representative of 3 independent experiments. Scale bars, 500 μm (left panels) and 100 μm (middle and right panels).

Figure S7. **Histone H4K16 acetylation and propionylation in wild-type and mutant embryos. (A-B)** High-magnification images of representative cells from E13.5 wild-type cerebrocortical neuroepithelia immunostained with anti-H4K16ac (A) and -H4K16pr (B) antibodies. Scale bar, 10 μm. **(C-D)** Immunostaining of E12.5 wild-type (C) and mutant (D) embryonic sections with the anti-H4K16pr antibody. Two green arrowheads demarcate the boundary of the deleted and non-deleted areas. Residual positive cells in the mutant

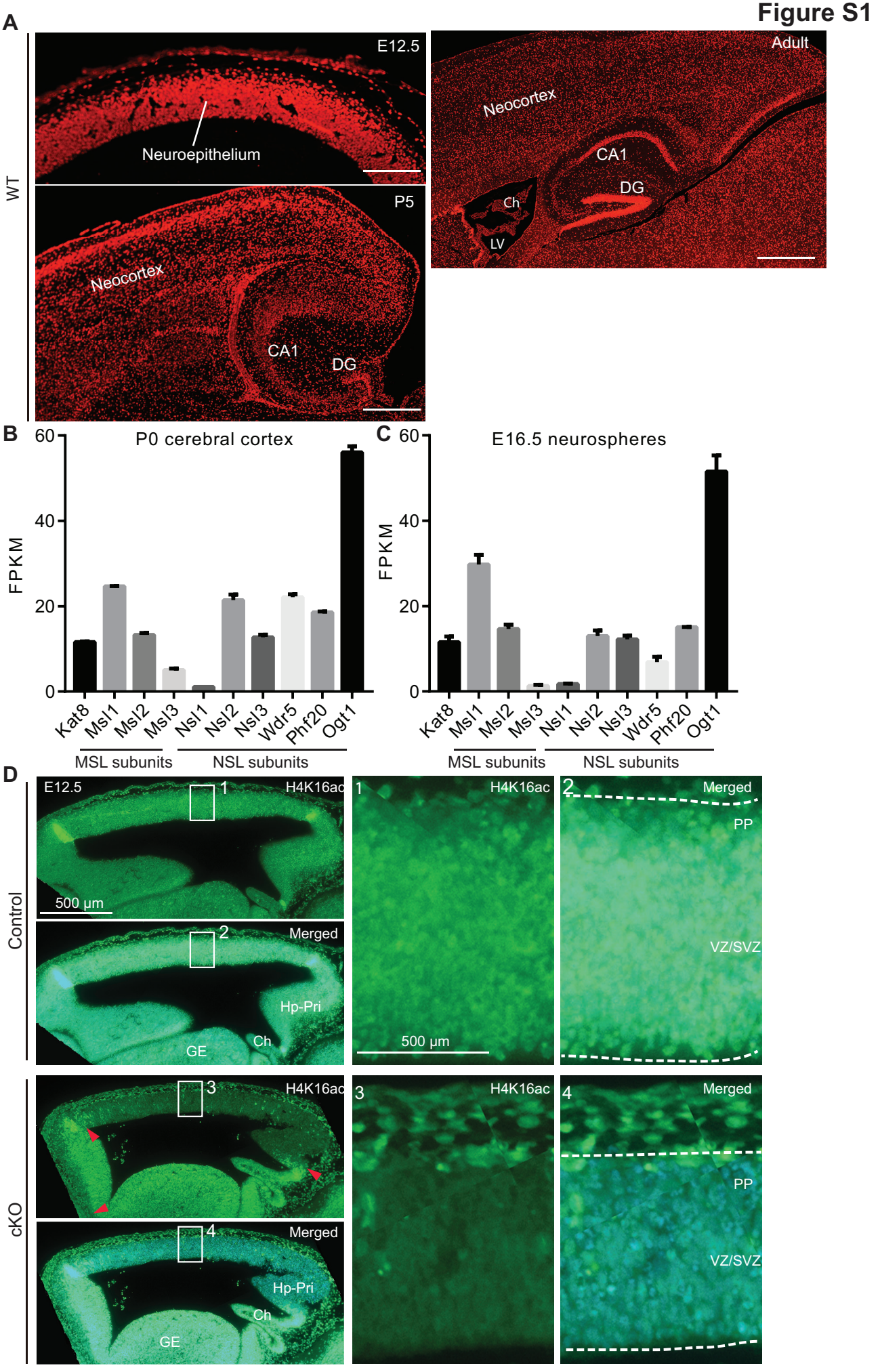
cerebrocortical neuroepithelium are perhaps related to or derived from interneuron (or microglial) precursors. Images in (A-D) are representative of at least two different experiments. GE, ganglionic eminence; pr, propionylation. Scale bars, 500 μm (left panels) and 100 μm (middle and right panels). (**E**) Model on how various metabolites may differentially regulate H4K16 acetylation and propionylation by KAT8. Only pyruvate and acetate are illustrated for events upstream from acetyl-CoA, a central player downstream from diverse metabolic pathways. Based on relative concentrations of acetyl-CoA and propionyl-CoA *in vivo*, H4K16 acetylation may thus play a major role whereas H4K16 propionylation complements acetylation. As for functional impact, the two modifications may bind to protein readers differently.

Figure S8. Photographs and brain MRI images of individual T3. (**A-B**) Facial and hand photos taken at the age of 12 years and 3 months. (**C**) Brain MRI scans were performed at the age of 11 years and 7 months. See Table S1 for MRI image assessment.

Figure S9. MRI images from three individuals. (**A**) Brain images of individuals T6, T7 and T9. (**B**) Head and additional brain images of individual T7. (**C**) Skeletal images of individual T7. See Table S1 for MRI image assessment.

Figure S10. Sequence, domain organization and function of KAT8. (**A**) Sequence comparison of human KAT8 with fly Mof. Residues altered in the variants from the nine individuals (Figure 7A) are marked, along with an autoacetylation site (Lys-274) and a key catalytic residue (Glu-338). Five acetylated lysine residues in the KAT8/of-specific domain are shown in light green. (**B**) Schematic illustration of KAT8 domains and its two stoichiometric multisubunit complexes.

See Figure S1B-C for expression of mouse genes encoding KAT8 and its associated subunits. KAT8 possesses a chromobarrel domain and an acetyltransferase domain. The acetyltransferase domain is sufficient for formation of both complexes and contains a small KAT8/Mof-specific region adjacent to the MYST domain (A). The position of an acetyl-CoA binding motif is marked with a short bar. The domain organization is also illustrated for the p.Lys175* truncation variant from individual T9 (Figure 7A) and the artificial N-terminal truncation mutant 88-458. (**C**) Nucleosomal histone H4 acetylation assays showing truncation mutant 88-458 (B) is inactive in acetylation of nucleosomal histone H4K16. KAT8 and the truncation mutant were expressed as FLAG-tagged fusion proteins in HEK293 cells along with HA-tagged MSL1/2/3 subunits for immunoprecipitation (IP) on the anti-FLAG M2 agarose and elution with the FLAG peptide. Eluted proteins were detected by immunoblotting (IB) with anti-FLAG and -HA antibodies (top two panels). Acetylation of H4K5 and H4K16 was detected by immunoblotting with antibodies recognizing histone H4 or its acetylated forms (bottom three panels). Impact on H4K5ac was less dramatic than that on H4K16ac. Results are representative of two different experiments.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Neocortex
CA1
DG
Adult
Neocortex
CA1
DG
Ch
LV
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FPKM
60
40
20
0
Kat8
Msl1
Msl2
Msl3
Nsl1
Nsl2
Nsl3
Wdr5
Phf20
Ogt1
MSL subunits
NSL subunits
C
E16.5 neurospheres
FPKM
60
40
20
0
Kat8
Msl1
Msl2
Msl3
Nsl1
Nsl2
Nsl3
Wdr5
Phf20
Ogt1
MSL subunits
NSL subunits
D
Control
E12.5
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H4K16ac
500 μm
2
Merged
Hp-Pri
GE
Ch
1
H4K16ac
500 μm
2
Merged
PP
VZ/SVZ
cKO
3
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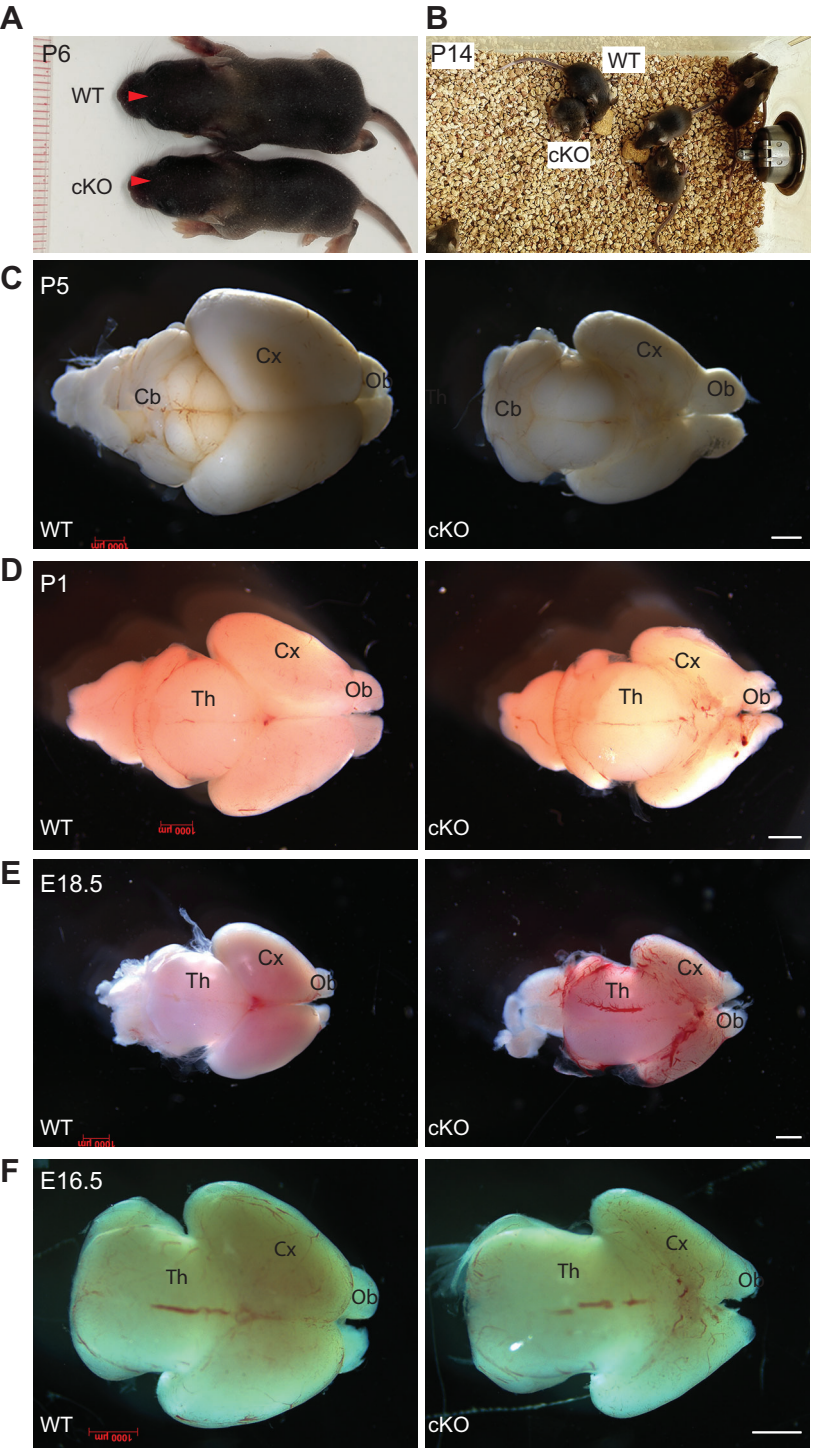
VZ/SVZ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D
P1
Cx
Th
Ob
WT
Cx
Th
Ob
cKO
E
E18.5
Th
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WT
Th
Cx
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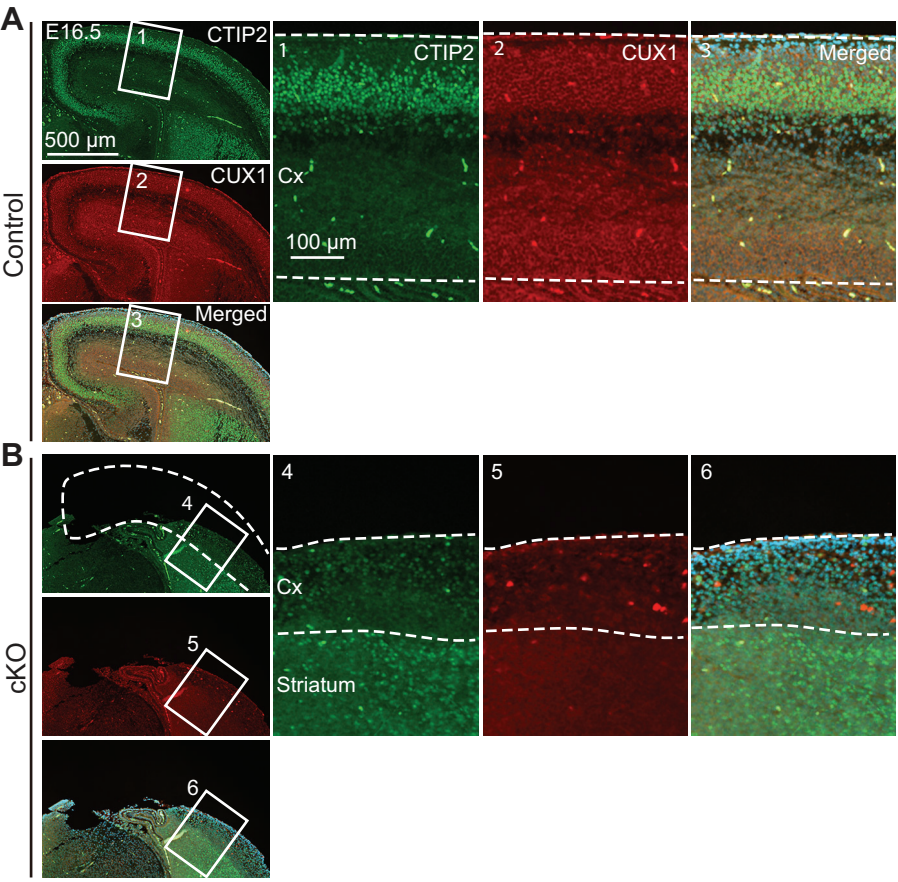
Figure S3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CUX1
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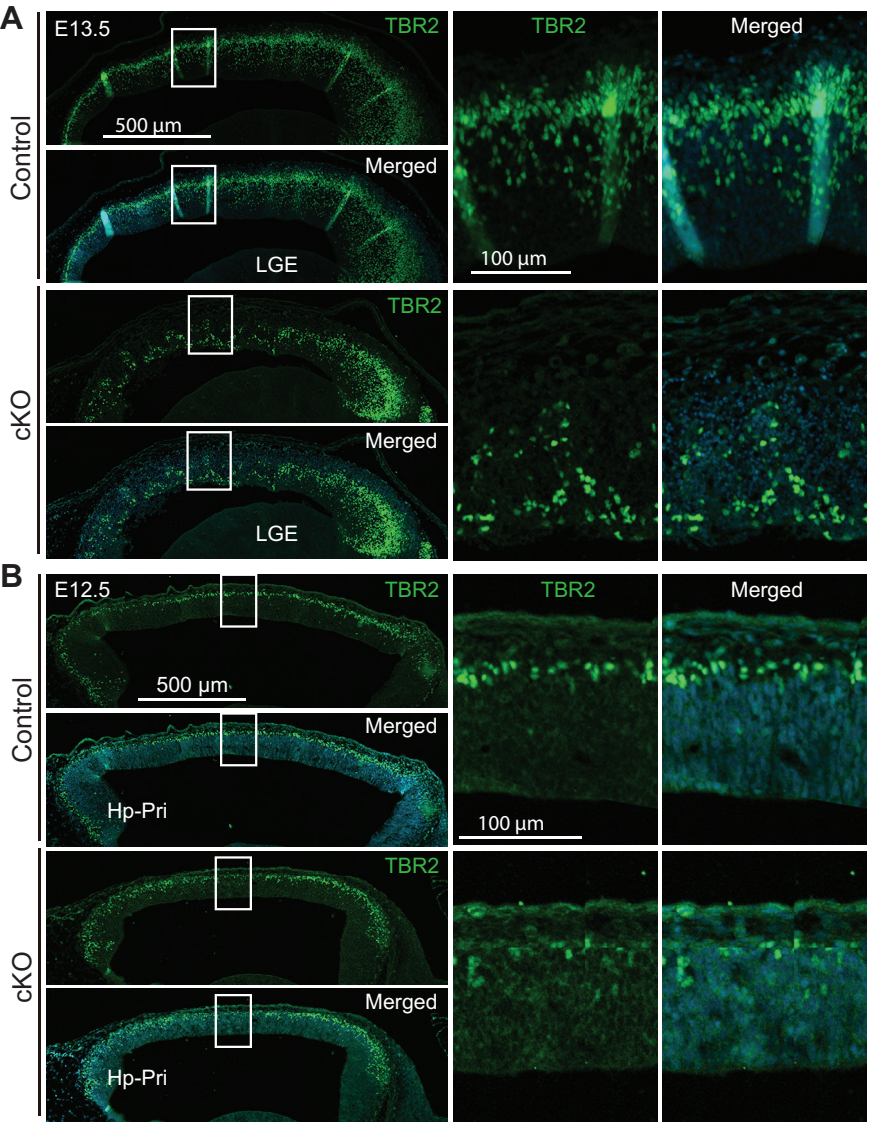
B
cKO
4
5
6
4
Cx
Striatum
5
6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500 µm
Merged
LGE
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100 µm
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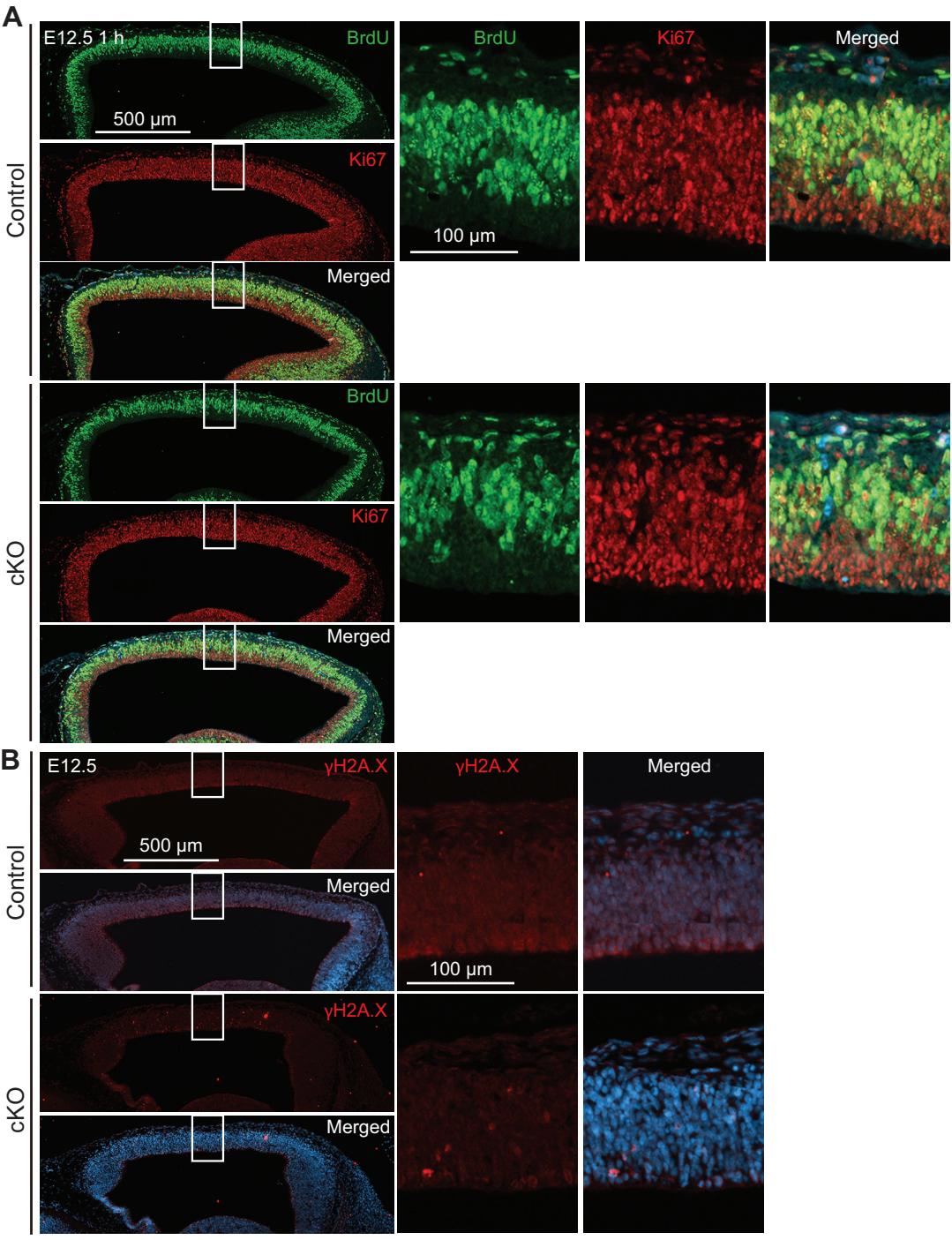
Merged
LGE
cKO
B
E12.5
TBR2
500 µm
Merged
Hp-Pri
TBR2
Merged
100 µm
Control
TBR2
Merged
Hp-Pri
cKO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Ki67
Merged
BrdU
Ki67
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100 μm
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cKO
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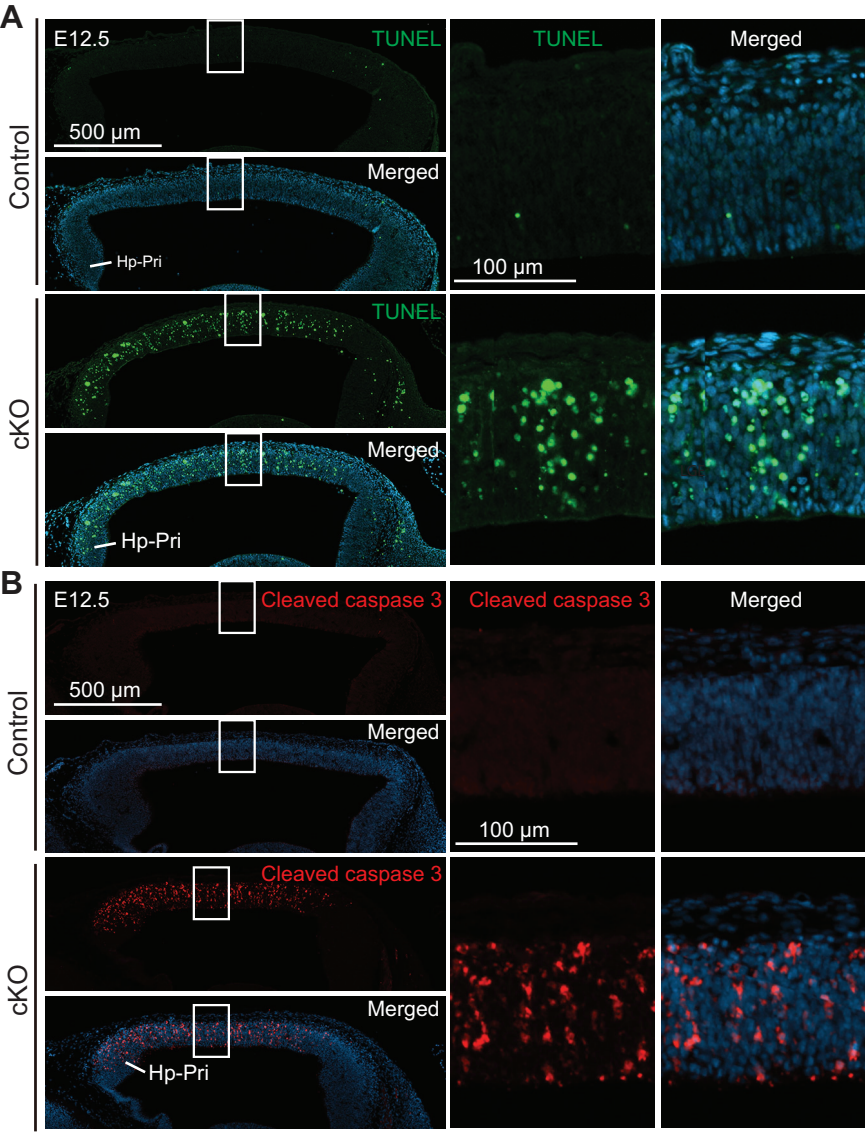
γH2A.X
500 μm
Merged
γH2A.X
Merged
100 μm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100 μm
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cKO
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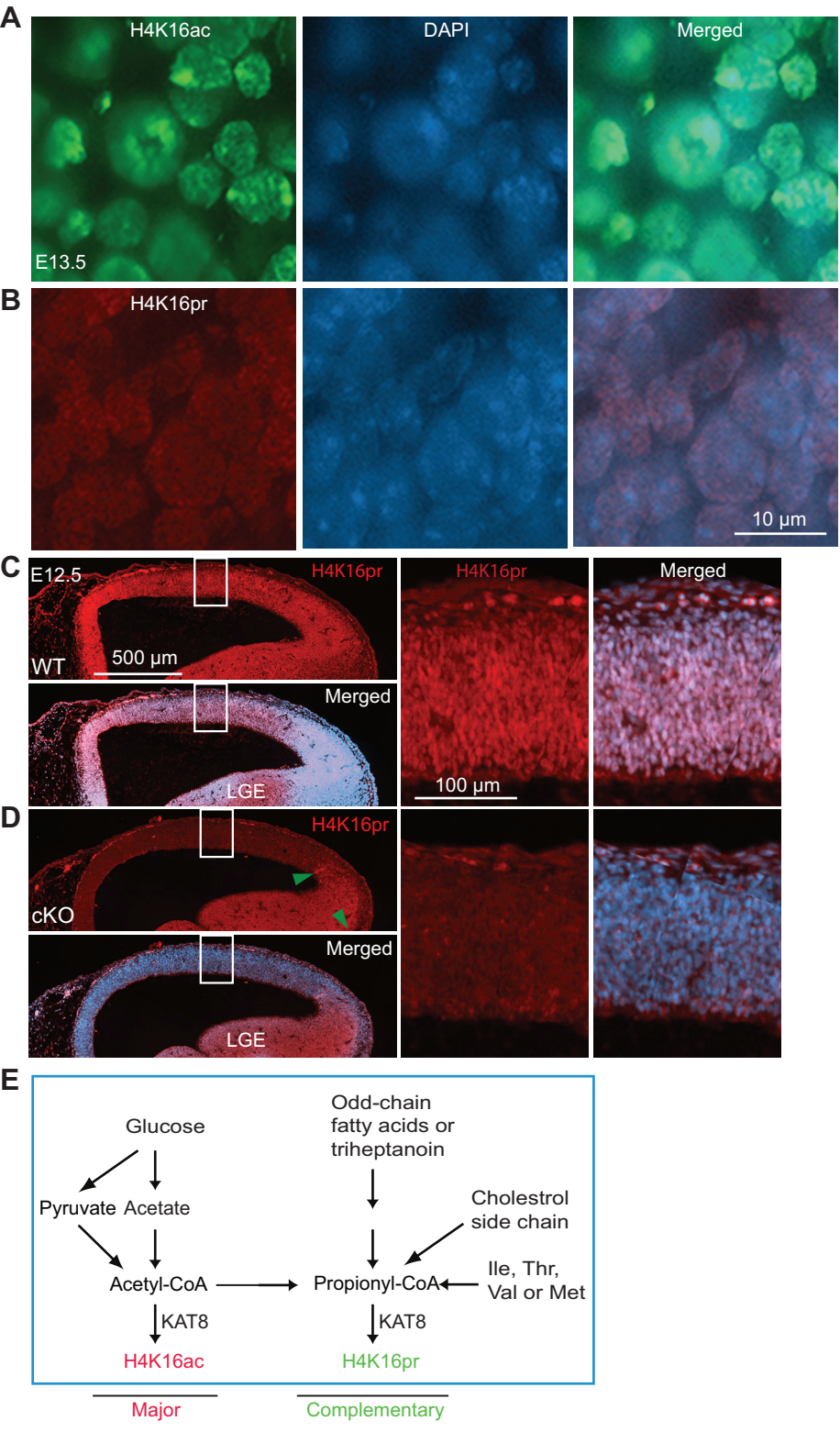
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Merged
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B
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10 μm
C
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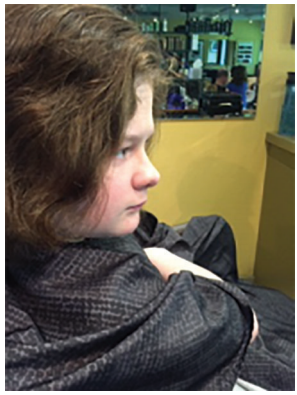
H4K16pr
H4K16pr
Merged
WT
500 μm
Merged
LGE
100 μm
D
H4K16pr
cKO
Merged
LGE
E
Glucose
Odd-chain
fatty acids or
triheptanoin
Pyruvate
Acetate
Cholestrol
side chain
Acetyl-CoA
Propionyl-CoA
Ile, Thr,
Val or Met
KAT8
KAT8
H4K16ac
H4K16pr
Major
Complementary

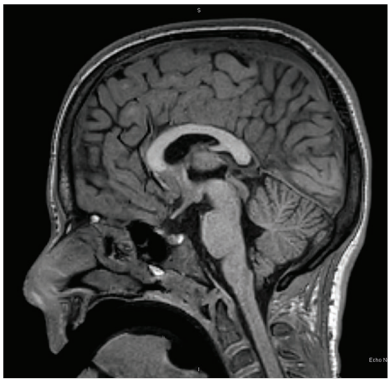
A

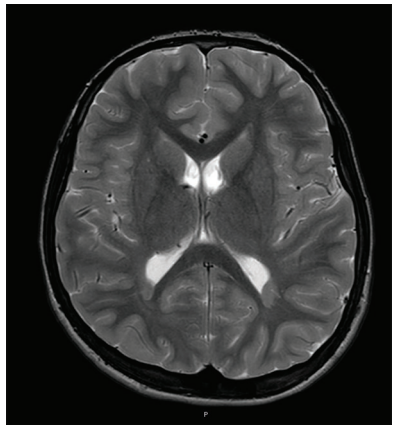
Subject T3 (p.Tyr90Cys)



B

C





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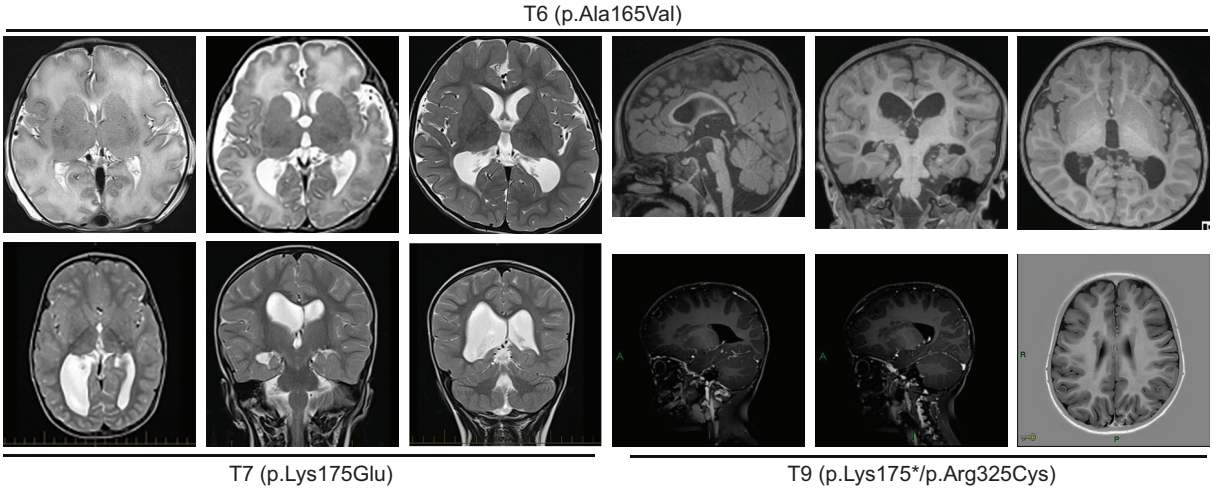
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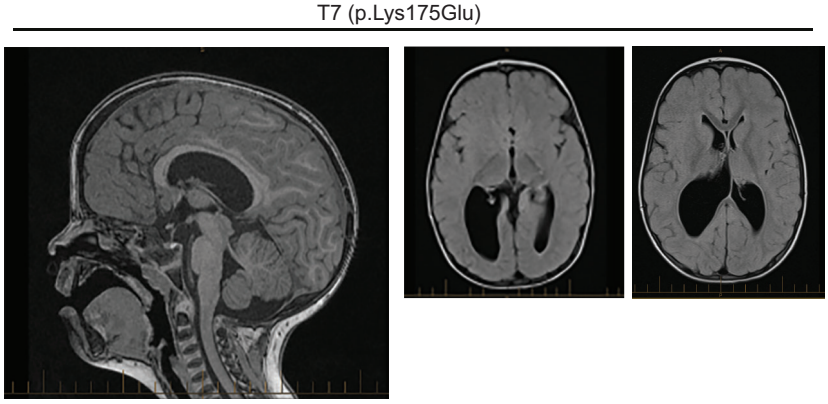
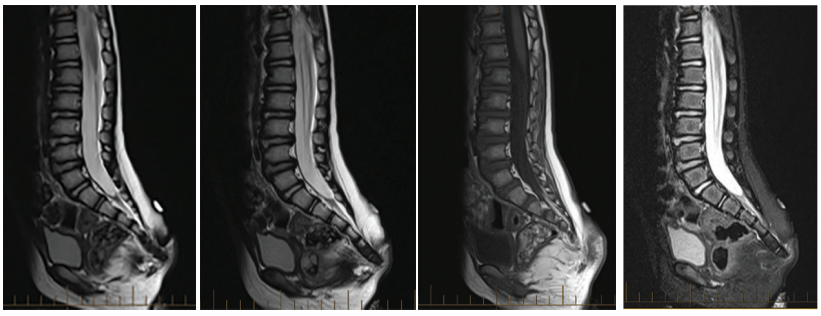
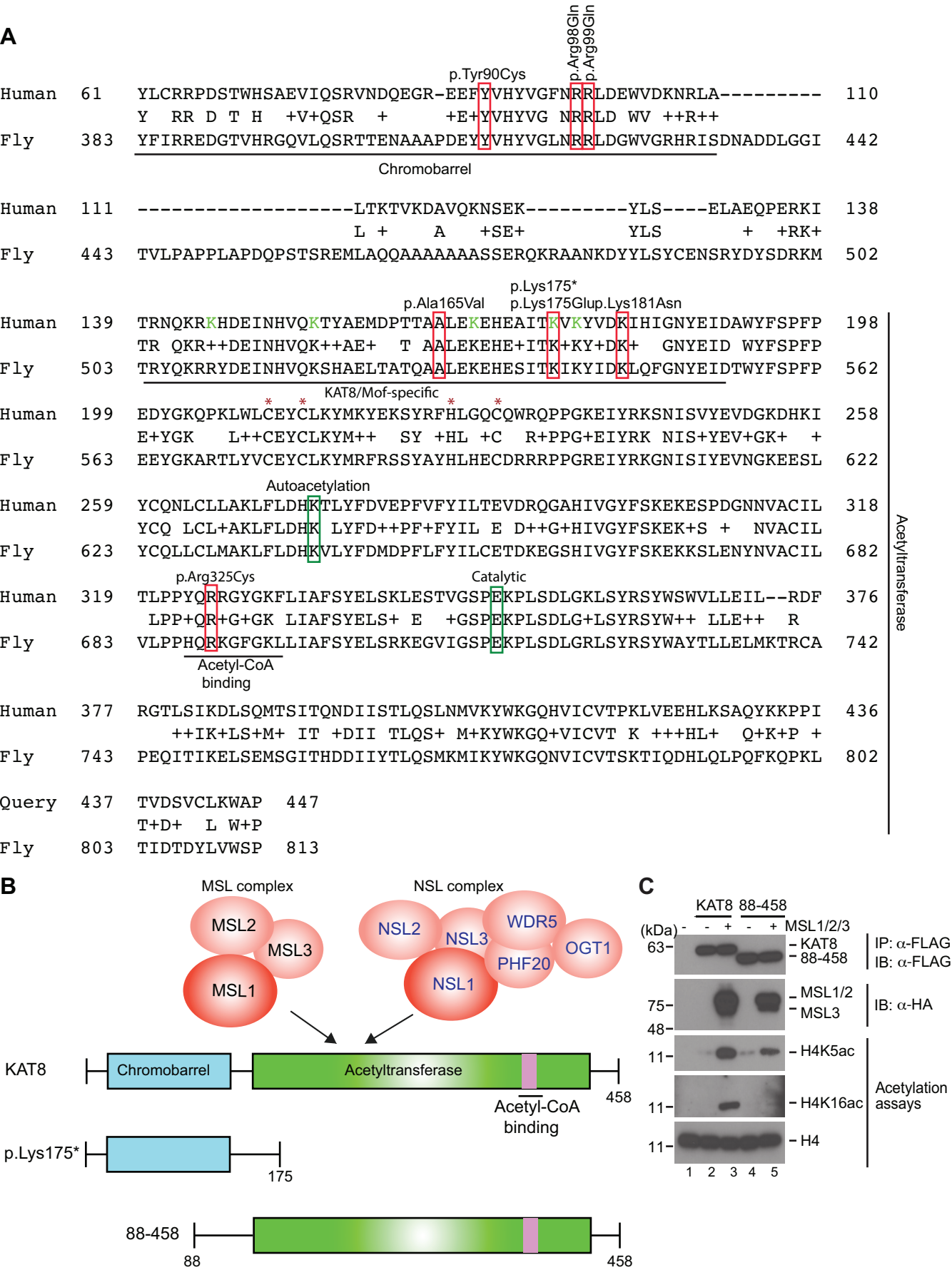
T7 (p.Lys175Glu)
T9 (p.Lys175*/p.Arg325Cys)

B


T7 (p.Lys175Glu)

C

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p.Arg98Gln
p.Arg99Gln
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Fly 383 YFIRREDGTVHRGQVLQSRTTENAAAPDEYYVHYVGLNRRLDGWVGRHRISDNADDLGGI 442
Chromobarrel
Human 111 ----------LTKTVKDAVQKNSEK---------YLS----ELAEQPERKI 138
Fly 443 TVLPAPPLAPDQPSTSREMLAQQAAAAAAASSERQKRAANKDYYLSYCENSRYDYSDRKM 502
p.Ala165Val
p.Lys175*
p.Lys175Glup.Lys181Asn
Human 139 TRNQKRKHDEINHVQKTYAEMDPTTAALEKEHEAITKVKYVDKIHIGNYEIDAWYFSPFP 198
Fly 503 TRYQKRRYDEINHVQKSHAELTATQAALEKEHESITKIKYIDKLQFGNYEIDTWYFSPFP 562
KAT8/Mof-specific
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Fly 563 EEYGKARTLYVCEYCLKYMRFRSSYAYHLHECDRRRPPGREIYRKGNISIYEVNGKEESL 622
Autoacetylation
Human 259 YCQNLCLLAKLFLDHKTLYFDVEPFVFYILTEVDRQGAHIVGYFSKEKESPDGNNVACIL 318
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Catalytic
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Fly 683 VLPPHQRKGFGKLLIAFSYELSRKEGVIGSPEKPLSDLGRLSYRSYWAYTLLELMKTRCA 742
Acetyl-CoA binding
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Fly 743 PEQITIKELSEMSGITHDDIIYTLQSMKMIKYWKGQNVICVTSKTIQDHLQLPQFKQPKL 802
Query 437 TVDSVCLKWAP 447
Fly 803 TIDTDYLVWSP 813
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MSL2
MSL3
MSL1
NSL complex
NSL2
NSL3
WDR5
OGT1
PHF20
NSL1
KAT8
Chromobarrel
Acetyltransferase
458
Acetyl-CoA binding
p.Lys175*
175
88-458
88
458
C
KAT8
88-458
(kDa)
MSL1/2/3
63
KAT8
88-458
IP: α-FLAG
IB: α-FLAG
75
48
MSL1/2
MSL3
IB: α-HA
11
H4K5ac
11
H4K16ac
11
H4
Acetylation assays
1 2 3 4 5

Table S1 Genetic information and clinical features of nine individuals with *KAT8* variants

Subject ID	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**
Mutation on NM_032188.2	c.269A>G	c.269A>G	c.269A>G	c.293G>A	c.296G>A	c.494C>T	c.523A>G	c.543G>C	c.523A>T & c.973C>T
Protein alteration	p.Tyr90Cys	p.Tyr90Cys	p.Tyr90Cys	p.Arg98Gln	p.Arg99Gln	p.Ala165Val	p.Lys175Glu	p.Lys181Asn	p.Lys175* & p.Arg325Cys
Transmission	*De novo*	*De novo*	*De novo*	*De novo*	*De novo*	*De novo*	*De novo*	*De novo*	Inherited
Family history	Non-contributory, but patient has multiple regions of homozygosity detected by chromosome microarray.	One brother: autism, ODD and ADHD; another with autism and ADHD. A sister: ADD and seizures. Father: dyslexia but no intellectual disability. Mother may have learning disability.	Non-contributory.	Negative for developmental delay or seizures. Mother's orbital frontal cortex + 3 SD. Parents are of Moroccan descent.	First child of nonconsanguineous parents.	Mother's sibling with fragile X syndrome, for which the mother is the carrier.	At birth of this subject, both parents were 35 years old, with no known exposure to teratogens.	Non-consanguineous. Parents have mild learning difficulties and 2 children, w/ the proband as the 2nd. Brother has mild learning and behavioral difficulties.	Parents, asymptotic monoallelic carriers of the mutations. A sister has the c.973C>T mutation and is asymptotic. One missed abortion at 9 weeks of gestation, with no material available for genetic testing.
Pregnancy issues	Maternal stress, poor nutrition.	None	31-year old mother with subchorionic hemorrhage during the first trimester.	None	History of rheumatic disease in the mother.	IUGR, reduced fetal movements prior to delivery.	Normal vaginal delivery, full-term. Prenatal ultrasound examination normal.	Reduced fetal movements. Poor prenatal growth.	None
Delivery issues (specify gestational age)	40-week gestation.	None, full term.	Induced vaginal delivery, due to fluid leakage at 38-week gestation.	Born at term.	42 weeks, delivery with a vacuum extractor. APGAR scores 9 and 9 after 1 and 5 minutes.	37 weeks 4 days, poor biophysical profile, C-section. Mother: perinatal depression, concern for prenatal etiology e.g. metabolic and infectious cause. Hydropic at birth: ascites, pleural effusions.	None. Delivered at 40 weeks and 5 days.	None recorded. At term, via normal vaginal delivery.	None. Delivered at 40 weeks.
Birth Weight	2.86 kg	3.26 kg	2.84 kg	N/A	3.1 kg	2.22 kg (<3rd centile)	2.89 kg	2.8 kg	2.59 kg
Birth Length	Unknown	50.8 cm	45.7 cm	N/A	Unknown	47 cm (6th centile)	50.8 cm	Not recorded	48 cm
Birth Head circumference	Unknown	45.5 cm at 9.5 months (75^{th} centile)	Unknown	N/A	Unknown	32 cm (3rd centile)	Unknown	In 1^{st} 12 months, increased from	35 cm

								50th to 98th centile.	
Neonatal issues	Weak, poor feeding, poor weight gain, jaundice, in NICU (newborn intensive care unit) at 2 weeks of age.	Atrial and ventricular septal defects.	Congenital unilateral hip dysplasia Jaundice.	None	None	Very sick for several months, hydrops, transient hepatosplenomegaly, hypoglycemia, transaminitis, coagulation defects, congenital vascular anomalies, differential diagnosis included lysosomal storage disorder, mitochondrial disorder, congenital disorders of glycosylation, sulfite oxidase/molybdenum cofactor deficiency, Niemann-Pick and other metabolic disorders, connective tissue disorders, channelopathy, nitric oxide defect or congenital infection.	None	Poor weight gain, needed high calorie milk.	N/A
Gross motor delay	Yes	Yes First walked after 2 years.	Yes, sat independently at 14 months, crawled at 15 months, and walked independently at 20 months Was in Spica cast from 7-9 months and Rhino brace until 12 months due to congenital hip dysplasia.	Yes, mild	Walking at 18 months of age.	Yes	Yes, did not roll until 5-6 months of age. Sitting unassisted and pulling to stand at 9 months. Walked at 15 months but required AFOs due to pronation. At age 3, ran with awkward gait, but was very clumsy, w/ both feet not off the floor at the	Yes, hypotonia. Tired easily and required wheelchair for longer journeys. Unsteady gait.	Yes

							same time. Can now go up and down stairs using a railing but cannot jump.		
Fine motor delay	Yes	Yes	Yes	Yes	Unknown	Yes	Yes	Yes	Yes
Language delay	Yes	Yes	Yes- had a few words at 20 months, with receptive and expressive language moderately delayed, as well as mild articulation problems.	Yes	Time of first words: 18 months. Difficulty with pronunciation, Ability to form sentences: 4 years.	Yes Does not speak & can follow few simple commands,	Yes, 1th word ("dada") at 12-13 months. At age 2, did some sign language but still had the single word "dada." Very good receptive language skills but had difficulty with expressive language.	Yes First word at 4; Short, simple sentences at 5.	Yes At 2, had no words or syllables but understood simple instructions. At 4, still spoke no words, with autistic features.
Developmental delay or intellectual disability (see below for additional issues)	GDD and moderate intellectual disability (estimated). Speaks in short phrases, simple directions. Writes first but not last name. Does not know phone #, but knows address, state, country. Does not understand money. Cannot count to 100. Parents are requesting legal power of attorney.	Yes – GDD & ADHD	GDD and mild intellectual disability. Full scale IQ using the Wechsler Intelligence Scale (WISC-V) at the age of 11 years and 7 months was 55.	IQ 50	Yes, no formal IQ-test available. Memory: very good ("perfect"). Interests: music, cars, trains, planes Knows numbers and can count; Problems with arithmetic Behavior: social, kind, shy. Does not know the value of money.	Receiving speech therapy, PT, OT every week.	Yes, diagnosed with autism spectrum disorder at the age of 2 years and 3 months. At 6 months, not responding to her name. At 12 months, not clapping, waving, or following objects, and only interested in reading. At 18 months, still not pointing, clapping, or waving; still only interested in books and not playing with other toys. At 2, now clapping and occasionally pointing.	Intellectual disability – moderate. Struggle a lot with numbers and with money.	Severe and autism
Seizures	No	One febrile seizure.	Yes- mixed, first grand mal seizure requiring	Yes	No	Yes, absence and clonic. Treated with leviracetam,	Yes Seizures EEG reports:	Yes Treated with sodium	Yes Due to recurrent

			hospitalization at the age of 8 years and 5 months, currently taking keppra with clonazepam as rescue medication. At the age of 8 years and 11 months: EEG normal during the awake and sleep state At the age of 6 years and 11 months: EEG abnormal, because of the bursts of delta activity, right greater than left, and greater activity on the right side independently. Suggestive of focal to diffuse cerebral irritation. This may be seen in any toxic, metabolic, or diffuse structural lesion. Because of the focality seen, a focal lesion should be ruled out.			Oxycarbazine. H/o status epilepticus with breakthrough seizures needing diastat and ativan and sometimes admission.	abnormal, it reveals relatively slower occipital dominant rhythm for age, finding suggesting the presence of mild diffuse disturbance in cerebral function. Intermittent superimposed left temporal, and less frequently left occipital spike, sharp and slow wave discharges were seen, finding suggesting the presence of epileptiform activity in these regions. The increased fast (beta) activity is a finding that could be related to medication the patient has received.	valproate. Presented with nocturnal generalized seizures around 3. No generalized seizures for some years, but still has absence seizures	epileptic seizures hydantoin was first added to topiramate with slow down tapering. At 25 months, Levetiracetam was started with slow increase but frequent absence episodes were reported and she had episodes of status epilepticus. At 4, she was treated with valproic acid (depalept, 38 mg/kg) and Levetiracetam with better seizure control, yet still episodes of brief absence and eye blinking. At 32 months, papilledema was diagnosed during a hospitalization and pseudotumor cerebri diagnosed with increased LP pressure and acetazolamide started. Was treated for ~6 months with

									acetazolamide but stopped due to adverse effect.
Other neurological abnormalities	No ataxia, but it's hard for her to balance. Cannot run. All responses/movements are slow. Otherwise non-focal exam.	Some aggressive behaviors, temper tantrums, rocking, self-stimulation. Significant head injury secondary to a fall; no imaging done.	Neuronal migration disorder Generalized hypotonia.	Nasal speech	No	Low appendicular tone with increased passive range of motion, deep tendon reflexes present 2+, wide based gait	Barely able to draw lines due to difficulty in pencil grip. Unable to jump with two feet, but able to run or climb stairs. Motor imitation is very impaired and cannot imitate others' actions.	Autistic features Delayed visual maturation.	N/A
Brain MRI	Unremarkable brain MRI.	Focal subcortical white matter lesion in right posterior frontal lobe. Most recent MRI indicated mild enlargement of the sella – suggested in her age group likely secondary to incompetent diaphragmatic sella; gland was otherwise unremarkable.	At the age of 11 years and 7 months: 1) There is a new area of high T2 and T2 FLAIR signal within the right mesial temporal lobe. This could be post seizure affect or quite possibly seizure focus amongst multiple abnormal foci of subependymal gray matter heterotopia. 2) Otherwise stable multiple subependymal gray matter heterotopias involving bilateral medial temporal lobes/hippocampi, and temporal horns of the lateral ventricles.	Normal	N/A	At 2: possible polymicrogyria of left inferior frontal lobe, bilateral small hippocampi, moderate ventriculomegaly, diffuse white matter volume loss with periventricular leukomalacia	At the age of 2 years and 2 months: 1) Multiple foci of subependymal heterotopic gray matter. Seizures are common clinical sequelae of heterotopic gray matter, but do not occur in all patients. 2) Ventriculo-megaly, right greater than left with associated decreased periventricular white matter volume and thinning of the corpus callosum. 3) Dysmorphic shape to coccyx with superficial induration/inflammation of the subcutaneous fat. This could be due to pressure	At 11 months: prominent ventricular system and extra cerebral CSF spaces. Non-specific small foci of high signal intensity in left caudate nucleus, thought to represent enlarged perivascular CSF spaces. Slight underdevelopment of the corpus callosum.	Delayed myelinization. A recent MRI suggested heterotopia.

			3) Subtle asymmetry to the right pituitary gland. This is not dedicated pituitary protocol. Dedic ated pituitary imaging could be performed if clinical concern exists. At the age of 9 years and 3 months: Multiple posterior subependymal gray matter heterotopias adjacent to the posterior bodies of the lateral ventricles and in the medial temporal lobes. Normal MR venogram. Mild bulging of the optic papilla bilaterally consistent with the given clinical diagnosis of papilledema. At the age of 8 years and 5 months: No acute interval changes. Patient with multiple neuronal migration anomalies in the form of				from the underlying bone versus infected fibers no open sinus tract or presacral meningocele is seen. 4) No tethered cord.		

			subependymal heterotopic gray matter bilaterally along the temporal horns and periatrial region. Multiple foci of gray matter heterotopia are seen along bilateral hippocampus.						
Age at last follow-up	18 years	13 years	11 years 10 months	11 years	6 years and 6 months	2 years	5 years	11 years	2.6 years
Gender	Female	Female	Female	F	Male	Male	Female	Male	Female
Weight at last follow-up	48.7 kg	47.4kg	43.1kg	NA	24.9 kg (+0.47 SD)	12.2 kg (24th centile)	14.7 kg (36th centile)	21 kg at age of 6 years 6 months	9 kg
Height at last follow-up	156.9 cm	151.2 cm	141.1cm	140.2 cm (-1.4 SD)	125 cm (+0.3 SD)	87.2 cm (23rd centile)	96.3 cm (37th percentile).	118 cm at 6 years 6 months	81 cm
Head circumference	55.5 cm	57.4cm		57.5 cm (+2.5 SD)	55 cm (+1.82SD)	46 cm (2.5 centile)	53.5 cm (>97th centile)	56 cm (at the age of 6 years and 6 months)	48.2 cm
Cranial shape	Mild brachycephaly	Normal	Relative macrocephaly	Normal	Normal	Microcephaly, flat occiput	Normal skull shape	Asymmetric skull shape with wide anterior fontanelle	Occipital flattening
Forehead	Narrow	N/A	High and broad	N/A	Normal	N/A	N/A	Broad	Frontal bossing upper part
Face	Asymmetric, decreased facial expression, severe micrognathia	Not dysmorphic	N/A	N/A	Normal	Elongated face (coarse during infancy).	Malar hypoplasia and bitemporal narrowing	Mild facial dysmorphism	Flat midface
Hair	Low anterior hairline		N/A	Thick curly hair	Normal	No issues	Thick, wiry, curly hair. Slightly sparse at temples. Sparse eyebrows	Normal	fine
Eyes (dysmorphisms)	Hypotelorism, shallow and asymmetric orbits, endpoint nystagmus, intermittent left esotropia,	WNL	N/A	Short upslanted palpebral fissures	No	Inner epicanthal folds, ptosis, cross eyed	Telecanthus. Brown irides.	Bilateral squint	almond shaped upslant eyes with epicanthal folds

	full arched brows.								
Vision	High hyperopia, left esotropia, glasses for reading only now, vision has improved??? No ectopia lentis.	Partially accommodative esotropia	Glasses for hyperopia Papilledema	N/A	Normal	Hyperopia	Deposits of pigment on the retina. Otherwise, normal ocular health.	Good	normal
Ears (dysmorphisms)	Low set, mildly prominent	WNL	N/A	Slightly cupped and posteriorly rotated	No	Very prominent and large ears (? related to fragile X)	Thick over folded ear helices. Ears are cupped and low set Left> right. No pits or tags. Ear length: Right: 5.0 cm (25th-50th percentile). Left: 4.5 cm (<3rd percentile).	Normal	Low set
Hearing	No concerns	Normal	Normal	N/A	Normal	N/A	Normal	Normal	normal
Nose	Prominent, high nasal bridge, long deviated septum		N/A	Columnella under alae nasi	Normal	Slightly depressed nasal root and bulbous tip of the nose	Depressed nasal bridge. More prominent bulbous nasal tip. Thick alae and columella. Long deep philtral pillars and groove.	Small nose	Small, depressed nasal bridge
Mouth	Full lips, poor dental hygiene, carious teeth	Lots of cavities	N/A	N/A	Normal	Smooth philtrum, thick lips	Slight micrognathia with small mouth. Normal tongue. Thin lips.	Thin lips	Thin upper lip
Palate	high arched palate	WNL	N/A	N/A	Normal	N/A	Mildly high palate. Single uvula.	Normal	Normal
Hands	Sloped shoulders, contracted elbows with decreased carrying angle,	WNL	Bilateral contractures of the 5th digits – surgical release in 5 years of age.	Hockey-stick creases	Normal	No concerns	Wrinkling of skin on palms. Fifth finger clinodactyly. Flexible fingers. Total hand	Normal	Normal

	hyperextended wrists, slim long fingers, 2-3-4 syndactyly (mild, bilateral)						length: 10.0 cm (<3rd percentile); Middle finger length: 4.5 cm (3rd-25th percentile).		
Feet	Long, slender legs, long toes	WNL	Overlapping 2nd, 3rd and 4th toes bilaterally	N/A	Some spatulate toes (broad rounded end)	No concerns	Normal appearance of feet and toes. Mildly overlapping toes.	Normal	Normal
Heart	At one point had dilated aortic root, more recently echo was normal (2016).	ASD and VSDs; all closed spontaneously except one very small mid to low muscular VSD present in 2016 and is hemodynamic ally insignificant	At 11 years 4 moths of age: Normal sinus rhythm. Borderline prolonged QT. No previous ECGs available. No echocardiogram.	N/A	No heart murmur	Needed PDA ligation, pulmonary hypertension during infancy	Normal echocardiogram	Cardiac examination normal, no echo	Normal
Kidneys	N/A	N/A	N/A	N/A	Unknown	Normal	Normal renal ultrasound.	No concerns, no scans	Mild dilatation of left renal pelvis
Feeding difficulties	Only as infant	No	None	No	No	None	None	Yes –poor weight gain in infancy,	Failure to thrive feeding diff. after birth
Additional issues with intellectual development	N/A	N/A	N/A	N/A	N/A	N/A	At 7, can speak, but articulation is poor. Scored at 60% intelligible to unfamiliar listener. Speaking in full sentences, but mostly simple statements and questions. Repe ats many phrases. Started talking in single words at 3 and putting two phrases together at 4.	N/A	N/A

							Excellent, unusually good memory for quotes, books, movies and storytelling. Very interested in music. Can complete 60-piece jigsaw puzzle without help. Her number sense is very severely impaired. Cannot count to three.		
Other clinical features	Long, lean body build with central weight distribution. Narrow thorax. Pectus excavatum. Scoliosis (triphasic) documented in 2011. Connective tissue "flavor".	Intact hypothalamic pituitary axis Skin picking Trichotillomania PICA	Congenital unilateral hip dysplasia (Spica cast from 7-9 months and Rhino brace until 12 months) Constipation Poor sleep-difficulty initiating and maintaining, takes trazodone at night. Anxiety disorder Attention deficit hyperactivity disorder, combined type, mild growth hormone deficiency (on GHT since the age of 8 years and 10 months), short stature and delayed bone age. Skeletal surveys below: At the age of 11 years and 5 months: The patient's skeletal	N/A	N/A	Chest, abnormal protuberance of the right thoracic wall and widely spaced nipples. Several services feel that while he has fragile X, he has several other features not consistent with fragile X.	Localized superficial hemangioma of right upper back - involuting. Sacral dimple. The technical quality of the awake portion of the EEG is limited due to the presence of excessive movement, and myogenic artifacts caused by the patient's irritability. There is an occipital dominant rhythm of 7.5-8 Hz. Moderate voltage 18-22 Hz activity is seen in all head regions. Intermittent superimposed moderate voltage 4-6 Hz activity seen in the central regions. Recurrent	Eczema Bilateral pes planus Poor sleep (melatonin) Autistic features	N/A

			maturation is now normal. At the age of 8 years and 5 months: An AP radiograph of the left hand and wrist was performed and bone age was calculated using the Greulich and Pyle standard. Radiograph is normal. Chronologic age: 9 years and 5 months. Calculated bone age: 6 years and 10 months , with standard deviation of 10.7 months. Gender: Female Conclusion: Delayed bone age. At the age 7 years and 10 months: The patient's chronological age is 7 years and 11 months. The patient's approximate radiologic bone age utilizing the Brush table is 5 years and 9 months. This is slightly over the two standard deviations, consistent with				superimposed left temporal spike, sharp and slow wave discharges were seen. During sleep, the generalized moderate voltage 18-22 Hz (beta) activity were seen in addition to the intermittent superimposed left temporal spike, sharp and slow wave discharges. Also rare left occipital spike, sharp and slow wave discharges were seen during sleep. EGG abnormal, revealing relatively slower occipital dominant rhythm for age, finding suggesting the presence of mild diffuse disturbance in cerebral function. Intermittent superimposed left temporal, and less frequently left occipital spike, sharp and slow wave discharges were seen, finding suggesting the		

			delayed bone age.				presence of epileptiform activity in these regions. The increased fast (beta) activity is a finding that could be related to medication the patient has received.		
Other genetic findings	Multiple regions of homozygosity (76.4 Mb total), no dosage changes negative studies: fragile X, metabolic screening, homocysteine, FBN1, GFFBR1, TGFBR2, myotonic dystrophy.	*KMT2C,* variant of uncertain significance; *MYBPC3,* pathogenic variant.	Variants of uncertain significance: *WNK1* (NM_213655.4) c.2362C>T, p. Arg788Cys (from mother); *KIF7* (NM_198525.2) c.3944C>T, p. Pro1315Leu (from mother); *MYO5A* (NM_000259.3) c.52C>T, p.Pro18Ser (*de novo*); *PLG* (NM_000301.3) c.1259G>A, p.Gly420Asp (from mother).	N/A	SNP array: deletion of 70,5 kb in 2p16.3 and deletion of 7,4 kb in 15q13.3: both deletions were also found in DNA of mother No fragile X syndrome No mutation SLC6A8 gene Metabolic investigations in blood and urine: normal.	*FMR1* mutation-c-129CGG[>200]; Vascular tortuosity gene panel, Nieman-Pick gene sequencing, CDG transferrin and N-glycan analysis, urine sialic acid, sulfatides gene panel for lysosomal disorders. Wolman's disease enzyme assay, urine succinyl acetone, MPS screen, and serum VLCFA, 7-dehydrocholesterol: all negative.	Microarray analysis: Variant of Unknown Clinical Significance, likely benign, paternally inherited. 15q25.3(83,999,296-84,854,797)x3: 856-kb duplication on 15q25.3. Diagnostic testing via chorionic villus sampling was performed showing normal chromosomes.	46,XY.arr2p21p16.3 (46,523,660-47,974,691)x3 mat. Chromosome 2p21 duplication of uncertain significance found in proband, mother and brother.	N/A

Abbreviation: N/A, not available; IUGR, intrauterine growth restriction; GDD, general developmental delay; ADHD, attention deficit hyperactivity disorder; ADD, attention deficit disorder; ODD, oppositional defiant disorder; WNL, within normal limits; SD, standard deviation.