Supplementary data

Supplementary Note

Clinical information of the seven patients with copy number gain in the MDS region

Case 1 (BAB 2678)

Case 1 is a six-year-old boy born at term via Caesarean section for occiput posterior with birth weight of 3.9 kg (75^{th} centile). His immediate neonatal period was complicated by respiratory distress requiring an extended nursery stay for 5 days. Subsequent feeding and physiologic growth appeared to be unremarkable. He was referred to genetics clinic at age 5½ years with concerns for developmental delay. On physical examination his growth parameters were 22.7 kg (90^{th} centile) for weight, 118 cm (90^{th} centile) for height, and 52.8 cm (75^{th} centile) for head circumference. He was noted to have thick eyebrows, synophrys, a full periorbital region, long straight eyelashes, large ears with thick fleshy earlobes, squared nose with overhanging columella, thin upper lip, and narrow and high palate. He had large hands with small distal phalanges (14½ cm, 97^{th} centile). Neurological examination showed mild muscle hypotonia and mild-to-moderate global developmental delay. He could not sit until 12 months of age and walked at 18 months. He started speaking in single words between the age of 3 and 3½ years and had only started speaking in sentences at 5 years of age. He attained toilet-training at age of 4 years. He also exhibited some behavior problems such as aggressive tendencies and disobedience. Both parents appeared to be healthy. Clinical laboratory tests that were normal included: chromosome analysis, fragile X testing, fluorescence *in situ* hybridization (FISH) for 22q deletion, and methylation testing for Angelman syndrome.

Nature Genetics: doi:10.1038/ng.302

Case 2 (BAB 2720)

Case 2 is an eight-year-old girl who was seen for evaluation of cerebral palsy, global developmental delay, and diffuse muscular hypotonia. She was born at term following an uncomplicated pregnancy to a 37-year-old mother with birth parameters (10 lbs 2 oz and 22½ inches). Her immediate neonatal period was uncomplicated. Patient's developmental milestones were delayed: she first sat by herself at 14 months, stood at 18 months, and walked at 22 months of age. In the second year of life, she relied more on sign language, until she could use words for communication at about 3 years of age. Behavioral difficulties included extreme pre-occupation with food to the point of stealing it. There was no family history of mental retardation, learning disabilities, birth defects, recurrent miscarriages, stillbirth, or bleeding disorders. Her past medical history was significant for von Willebrand disease, sleep apnea, adenoidectomy, placement of tympanostomy tubes and a non-diagnostic workup for seizures. On physical examination at 9 years of age, her weight was 68.5 kg (+3 SD), height 157.1 cm (+3 SD), and head circumference 56 cm (+2.5 SD). Craniofacial anatomic abnormalities included broad forehead, upslanting palpebral fissures, wide nasal bridge, synophrys, squared nasal tip, large ears, thin upper lip, and prominent chin. Skin exam was significant for multiple nevi. Extremities were remarkable for large hands, with mid-palm length greater than 97th centile, and the middle finger length between 75-95th centiles. Her muscle tone was diffusely decreased, gait and sensorium were intact. Her previous diagnostic workup included chromosome analysis revealing normal female complement, and a normal Fragile X DNA test. A brain MRI at age one year showed minimal prominence of third and lateral ventricle, thin

Nature Genetics: doi:10.1038/ng.302

corpus collosum, periventricular T2-hyperintensity in the posterior temporal and parietal lobes consistent with periventricular leukomalacia. However a follow up brain MRI scan at age 6 years revealed normal anatomy. There were no anatomic features consistent with lissencephaly.

Case 3 (BAB 2705)

Case 3 is a five-year-old boy of European descent with mild developmental delay with expressive language more severely affected, preserved receptive language, avoidant social behavior, and reduced eye contact. At age 18 months, he was not cooing, babbling, or talking. He was able to say two single words at age 28 months: “yes” and “no”. He sat up at age of 9 months, crawled at 12 months, and walked at 16 months. Stereotypic sensory behaviors included frequent jumping and putting his hands over the ears. These developmental issues showed improvement after speech therapy and occupational therapy. His past medical history is significant for multiple ear infections requiring bilateral tympanostomies. Physical examination revealed mild facial anatomic abnormalities including prominent and wide nasal bridge, mildly deep set eyes, prominent eyebrows, and mild prognathia. Laboratory studies included an elevated venous lactic acid of 3.5 mEq/L likely caused by the patient’s agitation, mild elevation of alanine on plasma amino analysis, unremarkable acylcarnitine profile, creatine phosphokinase, and thyroid studies; non-diagnostic fragile X DNA test, normal *SNRPN* methylation pattern. Additional diagnostic studies included unremarkable audiology evaluation at age 3 years, normal voiding cystourethrogram, and renal ultrasound revealing bilateral increase in kidney size and mild thickening of the uroepithelium.

Nature Genetics: doi:10.1038/ng.302

Case 4 (BAB 2668)

Case 4 is a 15-year-old boy of Northern and Western European/ Native American ancestry initially referred to Genetics for evaluation of suspected Marfan syndrome with physical findings of tall stature, mild aortic root dilatation, mitral valve prolapse, and scoliosis. He was born at 36 weeks gestation via spontaneous vaginal delivery with birth weight 3.75 kg (95% centile) and length 55.9 cm (+3 SD). Pregnancy was complicated by nicotine exposure. Family history was notable for two healthy paternal half siblings and hyperthyroidism in the mother. On physical examination at age 15 years his weight was 75.8 kg (95^{th} centile), height 187.5 cm (>97^{th} centile), and head circumference 57 cm (90^{th} centile). He had a long face, mild synophrys, mild hypotelorism, upslanting palpebral fissures, prominent nasal bridge, overhanging columella, short philtrum, thin upper lip, dental malocclusion, sloping shoulders, prominent proximal interphalangeal joints, mild scoliosis, widened atrophic knee scar, and ruptured and fibrosing folliculitis over the chest. His developmental milestones of gross motor and speech development appeared to be within normal range, but he showed mild fine motor delay. His past medical history was notable for recurrent otitis media requiring placement of myringotomy tubes, recurrent nosebleeds necessitating cauterization, mild aortic root dilatation treated with losartan, mitral valve prolapse, attention deficit disorder with hyperactivity (ADHD), and testicular torsion at age 12 years. His bone age was advanced by 2 years and 3 months compared to his chronological age. Laboratory findings include a normal male karyotype, negative fragile X testing, and normal total plasma

Nature Genetics: doi:10.1038/ng.302

homocysteine. Workup revealed that the patient did not meet the revised diagnostic criteria required for making the diagnosis of Marfan or Loeys-Dietz syndrome.

Case 5 (BAB 2665)

Case 5 is a three-year-old adopted boy, born to non-consanguineous parents at 42 weeks gestation by caesarean section for failure to progress. Birth weight was 2.97 kg (10^{th} centile). His prenatal history was complicated by decreased amniotic fluid. At age 3 months his head circumference was 40 cm (10^{th} centile). On examination at 2 years 8 months, his growth appeared retarded: weight 23.8 kg (-3 SD), height 32 cm (-3.5 SD), and head circumference 40 cm (-4 SD). His physical exam was remarkable for microcephaly, prominent forehead, triangular face, mild jaw retraction, and thin upper lip. His past medical history was significant for corrective surgeries of gut malrotation and sagittal craniosynostosis, correction of dolichocephaly using a helmet, failure to thrive, feeding difficulty requiring placement of a gastric feeding tube, and sleep apnea. Family history was notable for diagnosis of seizures and ADHD in the mother. One of the proband's paternal cousins was reportedly "blind", another had suffered from a heart defect.

Case 6 (BAB 2721)

Case 6 is a 17-year-old young man with a long history of chronic neurologic and developmental problems. His birth weight was 2753 grams (5%) and he had dislocated hips and hypotonia. His growth parameters reveal severe growth restriction: weight 26.7 kg (-5 SD), height 128.2 cm (-6 SD). At 12 years of age, his head circumstance was 50

Nature Genetics: doi:10.1038/ng.302

cm (2%). His past medical history includes mild-to-moderate mental retardation, episodic ear infections, feeding difficulty requiring supplemental G-tube feedings, poor to no bladder control and constipation often requiring ememas to achieve evacuation, asymptomatic stones in the left renal pelvis, and prominent scoliosis. On physical examination, his head examination was notable for high, but rounded palate and tongue without fasciculation. He also showed asymmetric chest, muscle atrophy in the upper and lower extremities, and medial leg deviation. He developed spasticity with minimal movements in the arms and no movement in the legs and tight ankle ligaments and was followed by orthopedics receiving Botox injections. The remainder of internal organ evaluation was unremarkable. His additional diagnoses are ADHD and Obsessive-Compulsive Disorder (OCD). A brain MRI documented the presence of a right subarachnoid cyst and gross dysgenesis of corpus collosum especially affecting the splenium. In addition there is quite marked cerebellar atrophy which appeared non-progressive and mild cerebral volume loss. The review of previous brain MRIs demonstrated that cerebellar atrophy is non-progressive, suggesting that it is likely to be a defect in development, rather than a postnatal regression. Laboratory evaluation included normal chromosome analysis, negative studies for chromosome 15 deletion and fragile X syndrome, normal electromyogram and nerve conduction velocity studies, creatine kinase within the normal limits, non-diagnostic workup for congenital disorders of glycosylation and creatinine-guanidinoacetate synthesis. He requires occupational therapy and physiotherapy, attends special education with Life Skills classes. He is not able to work. He has a younger biological brother who had a stroke in early childhood with non-diagnostic workup and subsequent seizure.

Nature Genetics: doi:10.1038/ng.302

Case 7 (BAB 2719)

Case 7 is a 7-year-old girl born at 38 weeks of gestation via spontaneous vaginal delivery with birth weight 3.06 kg (25^{th} centile), length 53 cm (75^{th} centile), head circumference 31 cm (10^{th} centile). Her perinatal history was complicated by advanced maternal age, *in vitro* fertilization, fraternal twin gestation, gestational diabetes managed with diet, use of aspirin and subcutaneous heparin in the first trimester for concerns about occult thrombotic problems. Delivery was complicated by repeat C-section. Prenatal ultrasound revealed choroid plexus cysts. Her immediate neonatal period was notable for stable vital organ function. Her twin-brother has no physical or behavioral abnormalities. Physical evaluation at age 7 months revealed developmental delay without regression, diffuse muscle hypotonia, significant drooling, and borderline microcephaly. She walked at about 18 months of age with a walker and braces and was not able to negotiate stairs safely until age of 3 years. There have been prominent sleep problems with the patient often not sleeping more than two hours at any one time at age range of 2 - 3 years. On physical examination at 5 years of age, her weight was 20.3 kg (75^{th} centile), height 115.8 cm (50^{th} centile), and head circumference 49.3 cm (50^{th} centile). At 10 years 5 months of age, her weight was 43 kg (95^{th} centile), height 145 cm ($>75^{th}$ centile), and head circumference 51.4 cm (10^{th} centile). Her facial appearance was not particularly dysmorphic, but notable for strabismus. There were no cardiac, pulmonary, or abdominal abnormalities. Her deep tendon reflexes were decreased. She had considerable language impairment using about 150 words at 5 years of age. The patient has apparent behavioral difficulties: unprovoked screaming, self-abusive behaviors such as hitting herself in the

Nature Genetics: doi:10.1038/ng.302

head. She liked music and water contact. A brain MRI at one year of age demonstrated thinning of the splenium of the corpus collosum and a mild cerebellar volume loss.

Supplementary Methods

Barrel labeling

Essentially the published procedure was followed [1]. Briefly, the intact cortical sheet was removed, sandwiched between two pieces of glass slides and submerged overnight in 0.25% glutaraldehyde, 0.5% PFA and 5% sucrose over night. Samples were cryprotected overnight in 15% sucrose and sliced parallel to the barrel by sliding microtome (50 μm). Slices washed in PBS and reacted for cytochrome oxidase activity by immersion in a solution of 10 ml PO_4 containing 0.75 mg of cytochrome C, 40 μl catalase and 5 mg Diaminobenzidine (DAB). Slices were incubated in the dark at room temperature under constant agitation for approximately 2h. Histochemical reaction continued until the patches became visible. Slices were mounted and images were taken using light microscopy.

DiO labeling

To examine radial glia cell shapes, DiO DiO: 3,3'-dioladecyloxacarbocyanine perchlorate (fluoresces orange/yellow-FITC filter set, Molecular probes, Inc., Eugene, OR) crystals were deposited on the pial surface of a dissected telencephalon (E14.5). To ensure better diffusion shallow scratches were done at the site of the deposition. The dye was left to

Nature Genetics: doi:10.1038/ng.302

diffuse overnight prior to vibrotome sectioning (60 μm) that were later imaged using standard fluorescent microscopy.

***In situ* hybridization** was conducted as described [2].

Gel filtration

Brain extracts from mice expressing LIS1-DsRed were subjected to gel filtration using Superdex 200 column (Amersham Biosciences, Uppsala, Sweden) essentially as previously described [3]. Samples were dialyzed against TMDS (50 mM Tris-HCl, pH 7.5 150mM NaCl, 6mM $MgCl_2$, 75mM sucrose and 1mM dithiothreitrol) buffer that was also used for column equilibration. As reference, a mixture of size markers (MW-GF-200; Sigma, Rehovot, Israel) was separated under the same conditions. Blue dextran marked the void volume.

References

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2. Yaylaoglu, M.B. et al. Comprehensive expression atlas of fibroblast growth factors and their receptors generated by a novel robotic in situ hybridization platform. *Dev Dyn* **234**, 371-386 (2005).

3. Gerlitz, G., Darhin, E., Giorgio, G., Franco, B. & Reiner, O. Novel functional features of the Lis-H domain: role in protein dimerization, half-life and cellular localization. *Cell Cycle* **4**, 1632-1640 (2005).

Nature Genetics: doi:10.1038/ng.302

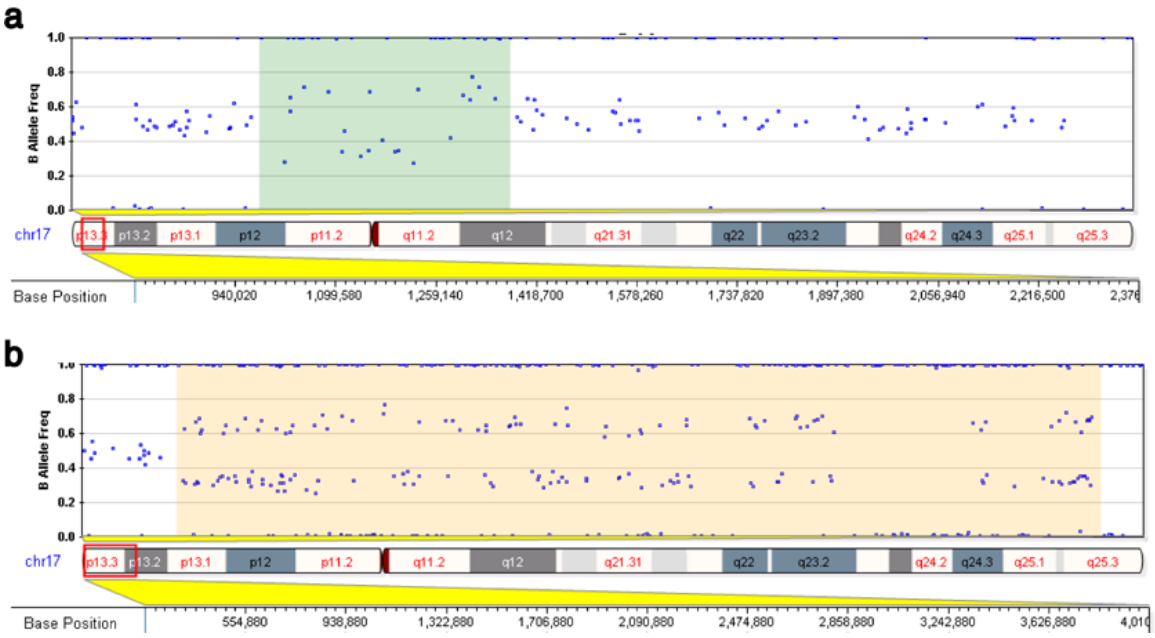
Supplementary Table 1

Expression analyses of genes within the MDS critical region

Gene		Case 4		Case 5	
		Genomic variation	Comparative expression ratio	Genomic variation	Comparative expression ratio
14-3-3ε	Primer 1	duplicated	1.55 (±0.06)	normal	1.16 (±0.02)
	Primer 2	duplicated	1.57 (±0.07)	normal	1.10 (±0.04)
CRK		duplicated	1.56 (±0.07)	normal	1.07 (±0.04)
LIS1	Primer 1	normal	1.06 (±0.12)	duplicated	1.60 (±0.09)
	Primer 2	normal	1.15 (±0.13)	duplicated	1.64 (±0.06)

Total RNA was extracted from the transformed lymphoblast cells by Trizol followed by further purification using a QIA RNeasy kit (Qiagen, Valencia, CA). 1 μg of RNA was reverse transcribed in a 20 μl reaction with random hexamers using SuperScript^{TM} First-Strand Synthesis System (Invitrogen, Carlsbad CA). Real-time PCR reaction was performed with 12.5 μl reaction containing 1 μl of diluted (1:5) reverse transcribed products using an ABI Prism 7900HT sequence detection system. Primers were designed using Primer express software from Applied Biosystems. Two pairs of primers were examined for *14-3-3ε* and *LIS1* genes. Expression level was estimated by relative quantification using a standard curve generated by serially dilution (1:3, 1:10, 1:30, 1:100, 1:300, 1:1000) of one control sample. Comparative expression ratios were calculated by dividing the averaged values of the triplicates of each patient sample by the average values for the control group consisting four samples of parents without duplications in 17p13.3. The comparative expression ratio is mean ±S.D.

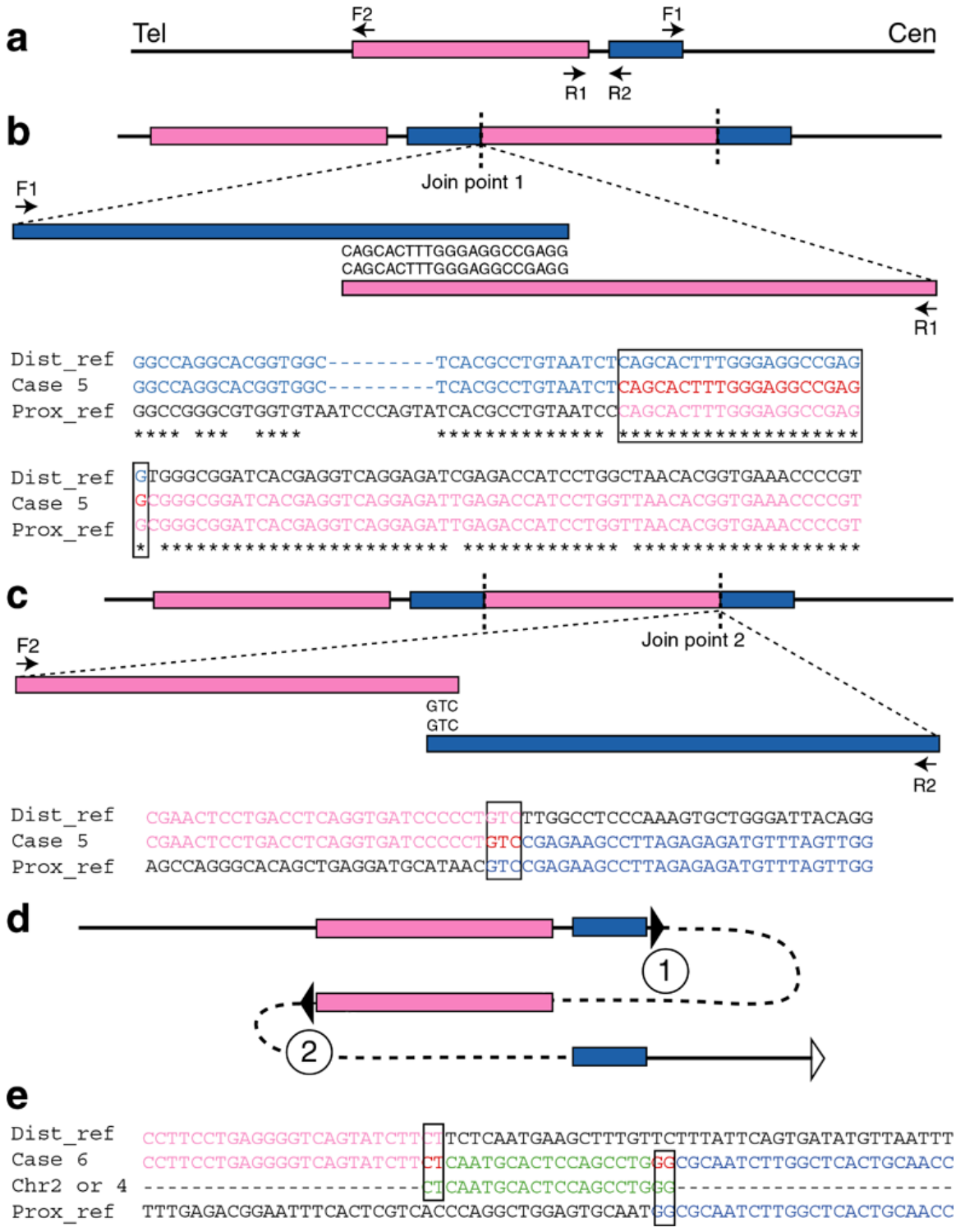
Nature Genetics: doi:10.1038/ng.302



Supplementary Figure 1

Fine mapping the duplicated region by SNP genotyping using the HumanHap300 BeadChip. The SNP array (Illumina, Inc, San Diego, CA) contains 317,000 oligonucleotide single nucleotide polymorphism (SNP) markers and the resolution is 50 kb using a 10-SNP moving average along the genome. (a) The duplication in case 2 spans an ~ 378 kb region from nucleotide position 1064096 to 1442939 in 17p13.3 within the MDS critical region. In addition, a heterozygous deletion was detected in chromosome 21 from nucleotide 23372476 to 23573227 which contains no genes. The copy number loss of this region is present in the Toronto CNV database. (b) The duplication in case 7 was mapped to a 3.6 Mb region from nucleotide position 515,591 to 3,860,158.

Nature Genetics: doi:10.1038/ng.302



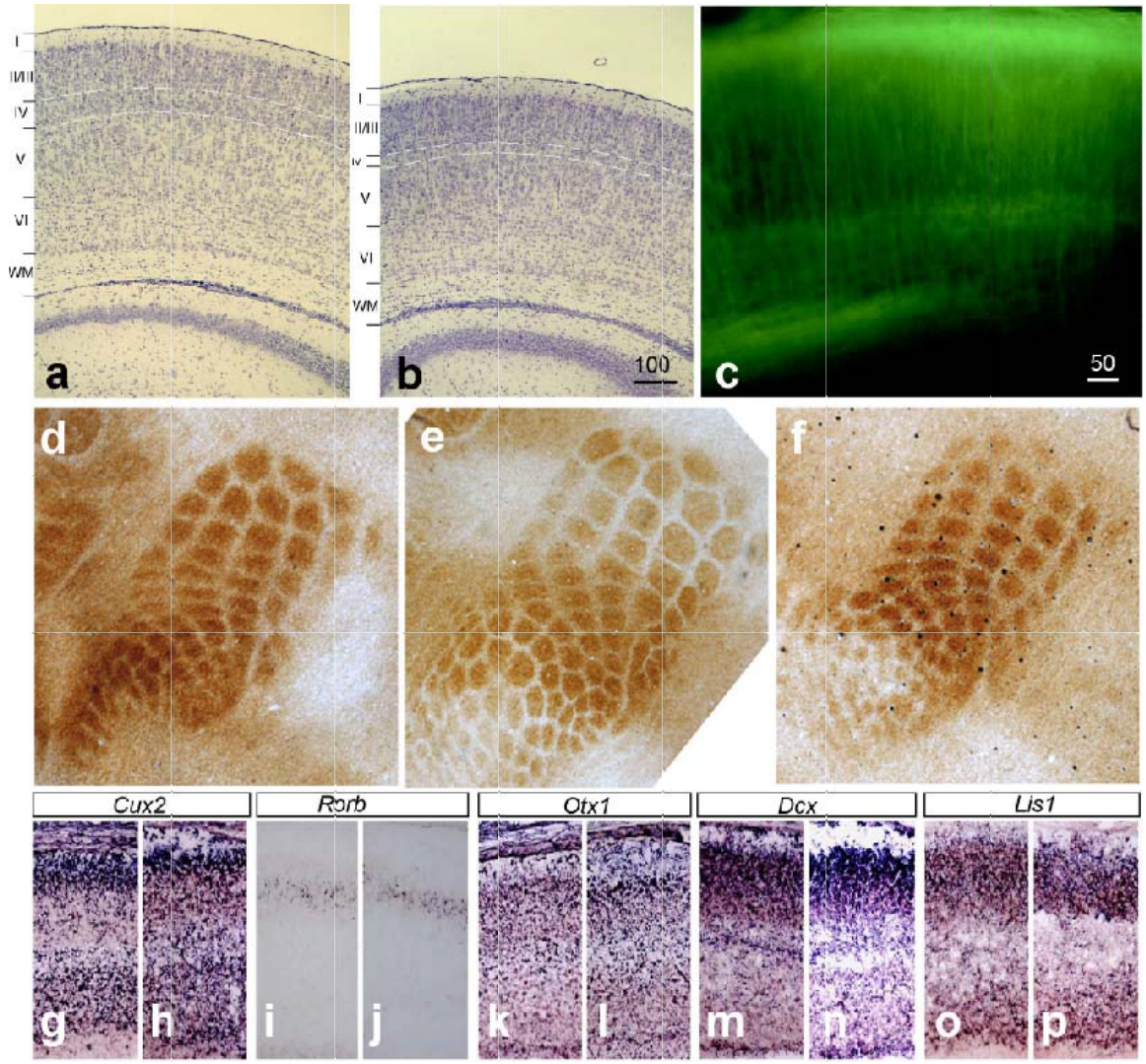
Supplementary Figure 2

Sequence analysis of the join points of the complex rearrangements in case 5 (a-d) and case 6 (e). (a) Genomic rearrangement in case 5 with duplicated region indicated by pink

Nature Genetics: doi:10.1038/ng.302

and blue horizontal boxes. The location and orientation of the primers for junction analysis are indicated. (b) DNA sequencing of the breakpoint junction showed 21 base pairs of microhomology in join point 1. (c) A 3 base pairs of microhomology is present in join point 2. (d) An illustration of two FoSTeS events leading to the duplication/ normal/ duplication rearrangement. Arrowheads show direction of DNA relative to the positive strand; closed arrowheads with circled numbers below depict where fork stalling and template switching presumably occurred; open arrowhead depicts resumption of replication from the original template. (e) DNA sequencing of one join point in case 6 showed a 17 base pairs of fragment present between the distal and proximal reference sequences which has sequence homology in chromosomes 2 and 4.

Nature Genetics: doi:10.1038/ng.302

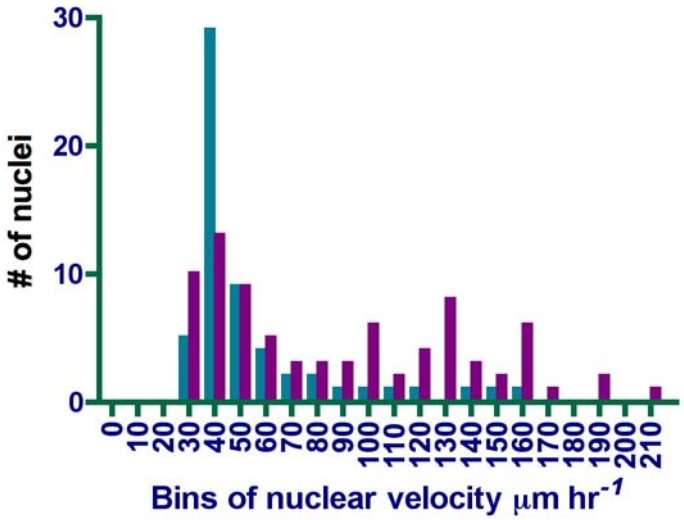


Supplementary Figure 3

LIS1 overexpressing brains display some abnormal and normal features. (a-b) Cresyl violet staining of brain sections at P6. Note the reduction of layer 4 in the LIS1 overexpressing section (b). (c) LIS1 overexpressing brain section displays normal alignment of radial glia at E14.5 using DiO backfilling. (d-f) Barrel organization at P6; (d) control with insertion of the transgene without Cre expression, *LIS1* (e) control expressing Cre without the transgene, *Foxg1(cre)* (f) LIS1 overexpression, *LIS1::Foxg1(cre)* note that the barrel boundaries are less orthagonal. (g-p) *In situ*

Nature Genetics: doi:10.1038/ng.302

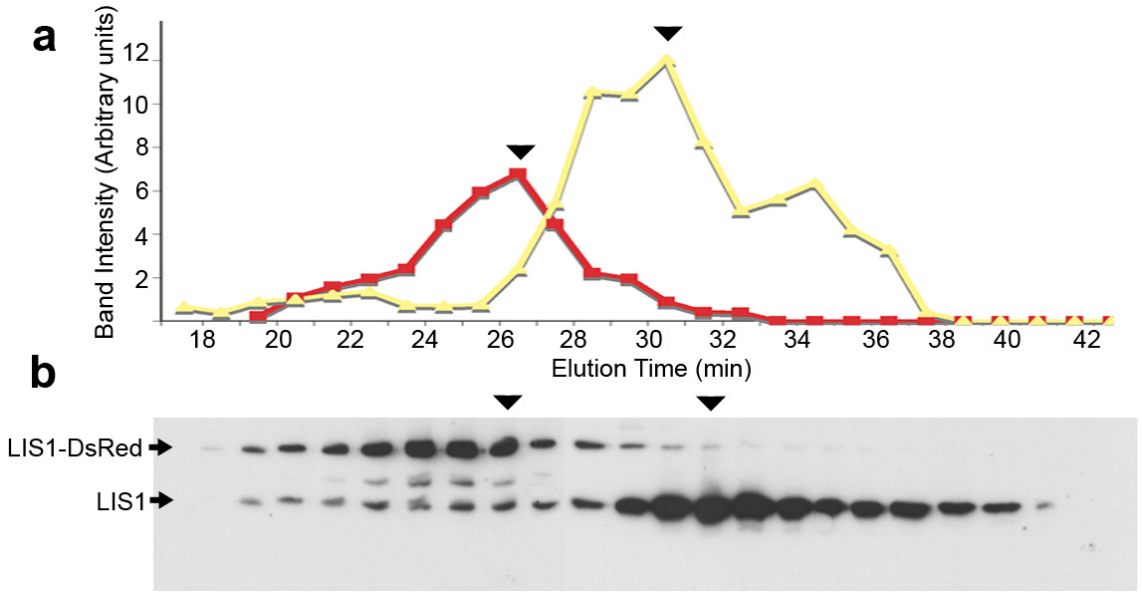
hybridization of E16.5 brain sections with markers specific for different layers. The distribution looks similar in the control and the LIS1 overexpressing mice. (g,h) *Cux2*, control (g), LIS1 (h); (i,j) *Rorβ,* control (i), LIS1 (j); (k,l) *Otx1*, control (k), LIS1 (l); (m,n) *Dcx*, control (m), LIS1 (n); (o,p) *Lis1*, control (o), LIS1 (p).



Supplementary Figure 4

Frequency distribution of cell speed. Live brain sections from LIS1 overexpressing brains or control sections were imaged and nuclear velocity was measured using Imaris. The relative proportion of nuclei moving in a particular speed bin was plotted. Note the faster velocity and the wider distribution of LIS1 overexpressing cells (purple) in comparison to control (turquoise).

Nature Genetics: doi:10.1038/ng.302



Supplementary Figure 5

Gel filtration of brain extracts from transgenic LIS1 mice. The collected fractions were analyzed by SDS/PAGE (b) and the intensity of each band was plotted against elution time in elution profiles (a). The elution profile of the wild type LIS1 (yellow) includes two peaks corresponding to a dimer (major peak) and a monomer (minor peak). The positions of the peaks as well as the position of the five size markers used were consistent with our previous studies [3]. The LIS1-DsRed eluted in one major peak (red) whose size was ~ 60 kDa larger than the LIS1 dimer due to the addition of the FLAG-linker-DsRed tag. Therefore, we concluded that LIS1-DsRed was found mainly as a dimer. The peaks are indicated by black triangles.

Nature Genetics: doi:10.1038/ng.302

Supplementary movies

Organotypic slice cultures were prepared from E13.5 LIS1 overexpressing embryos brains (*LIS1::Foxg1(cre)*, Supplementary movie 2) and control litter mates carrying a silent transgene (Cre negative, Supplementary movie 1). Slices were kept for 2 hours prior to imaging. Time-lapse microscopy was done in the presence of Hoechst dye to visualize nuclei. Images were taken every 2 minutes during the course of three hours. Control ventricular zone shows organized structure with elongated nuclei moving slowly perpendicular to the ventricular surface. LIS1 overexpressing slices are less organized with a small percentage of nuclei moving fast within the ventricular milieu. The ventricular surface is positioned up in both movies.

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