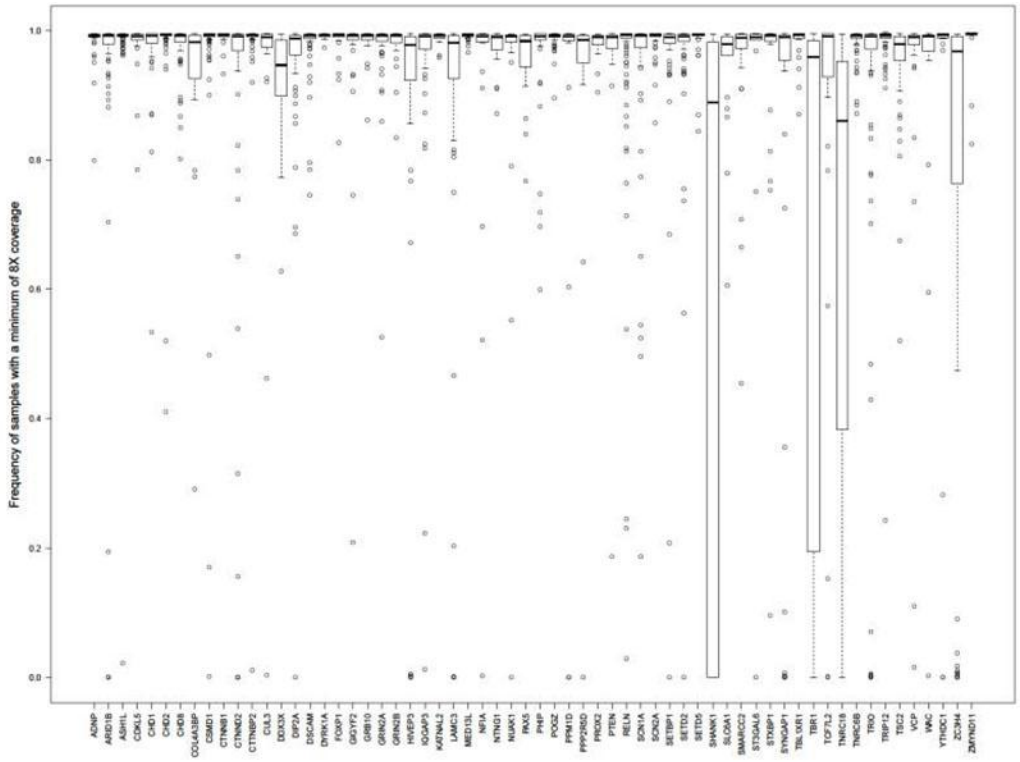
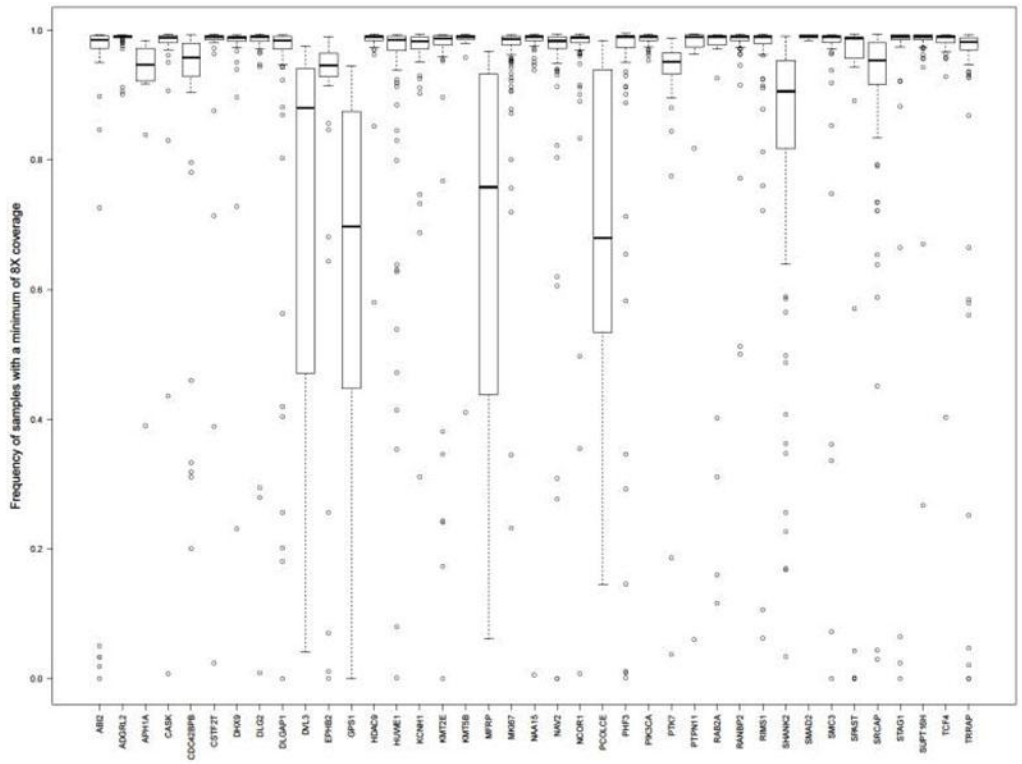


Supplementary Figure 1

smMIP quality control for the Gold pool.

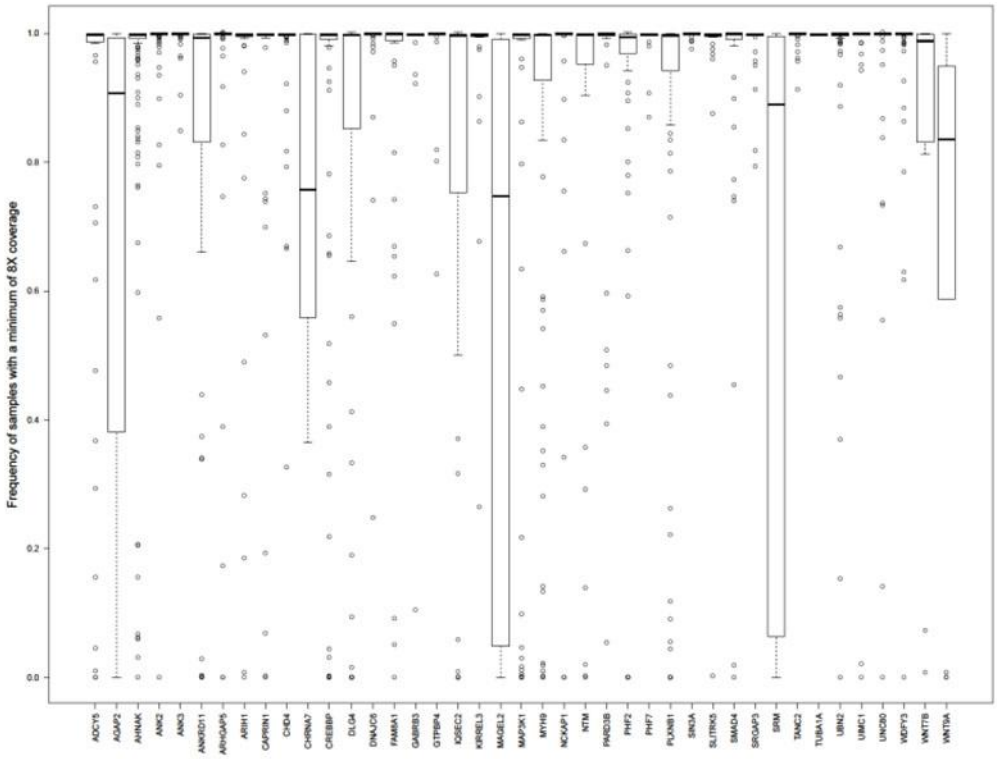
For 960 sibling control samples, the frequency of samples (*y*-axis) that reach at least 8X coverage for each individual smMIP (*x*-axis) is plotted as a boxplot by gene.



Supplementary Figure 2

smMIP quality control for the ASD4 pool.

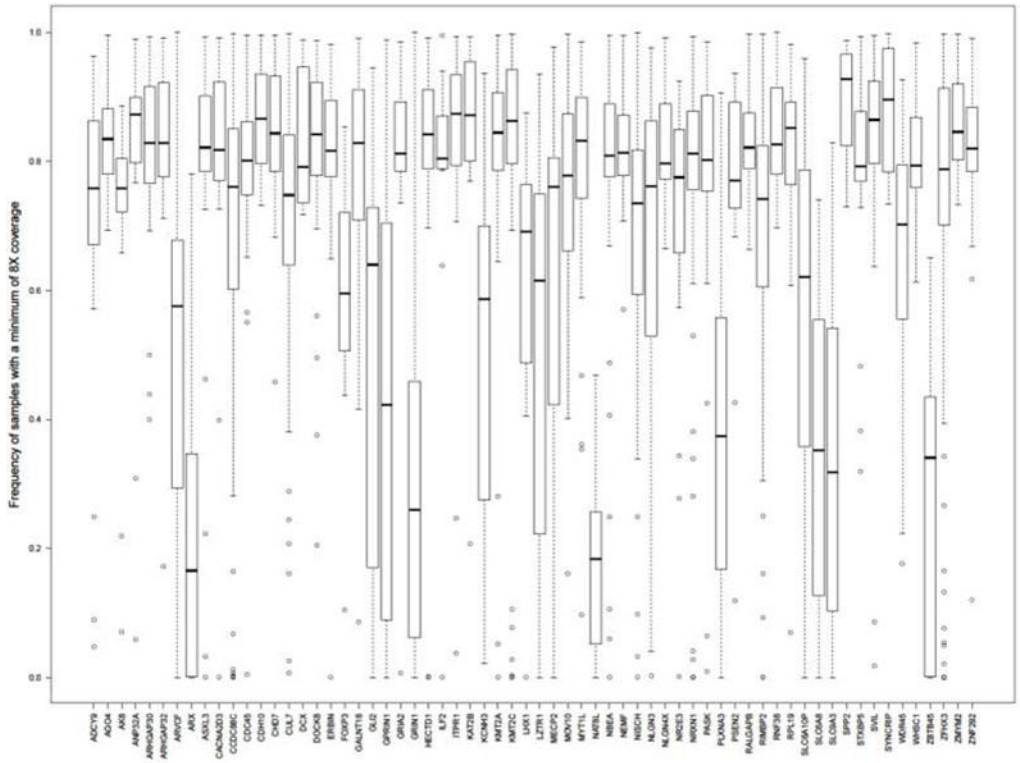
For 960 sibling control samples, the frequency of samples (*y*-axis) that reach at least 8X coverage for each individual smMIP (*x*-axis) is plotted as a boxplot by gene.



Supplementary Figure 3

smMIP quality control for the ASD5 pool.

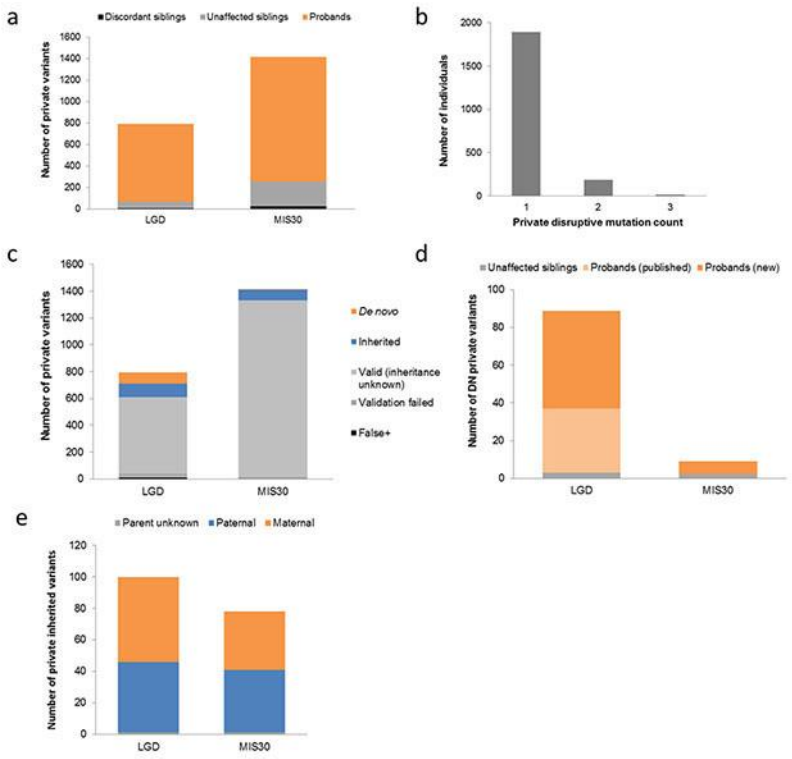
For 960 sibling control samples, the frequency of samples (*y*-axis) that reach at least 8X coverage for each individual smMIP (*x*-axis) is plotted as a boxplot by gene.



Supplementary Figure 4

smMIP quality control for the ASD6 pool.

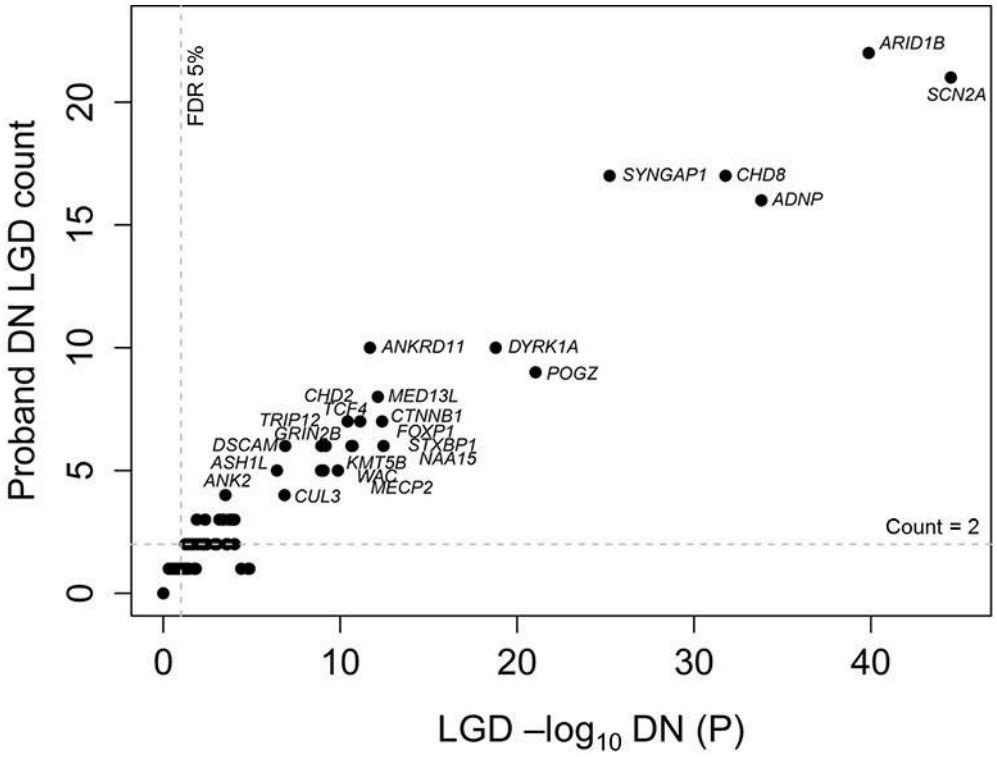
For 960 sibling control samples, the frequency of samples (*y*-axis) that reach at least 8X coverage for each individual smMIP (*x*-axis) is plotted as a boxplot by gene.



Supplementary Figure 5

Summary of private events identified in the study.

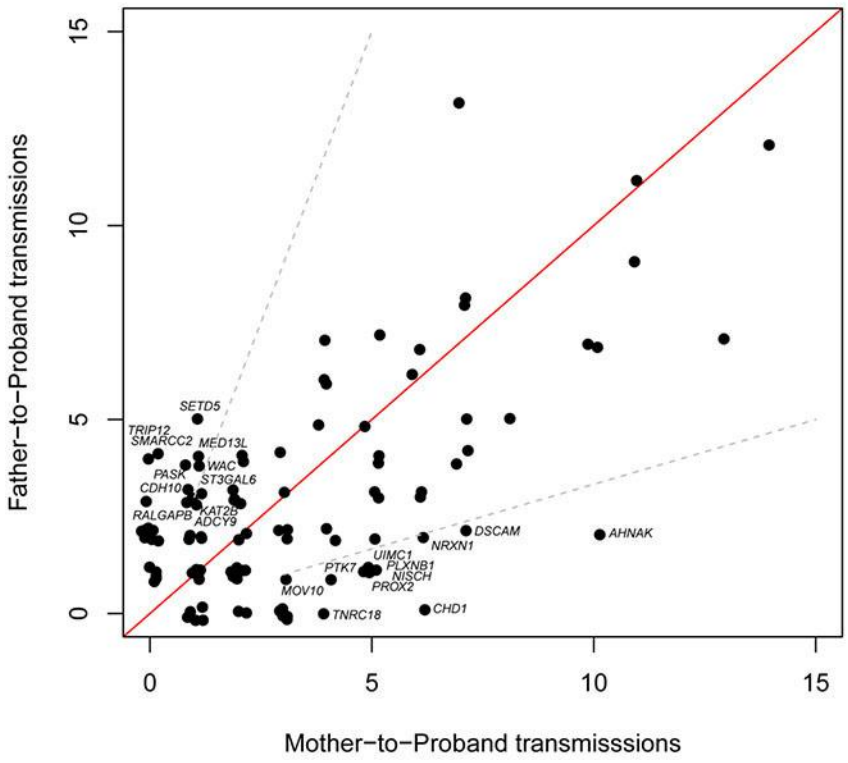
(**a**) Private events identified split by LGD and MIS30 variants in probands (orange), unaffected siblings (gray), and discordant siblings (i.e., a proband and sibling in the same family both share the event; black). (**b**) Number of private events identified per individual. (**c**) Private events split by LGD and MIS30 variants found to be *de novo* (orange), inherited (blue), validated by Sanger with unknown inheritance (light gray), Sanger validation failed (dark gray), and false+ (black). (**d**) *De novo* private events split by LGD and MIS30 variants into probands (orange) and unaffected siblings (gray). Dark orange represents new events in the study and light orange published events (all found in probands). (**e**) Inherited private events split by LGD and MIS30 variants into paternal (blue), maternal (orange) and unknown parent (gray).



Supplementary Figure 6

De novo (DN) significance is correlated with the number of ultra-rare/private DN variants identified.

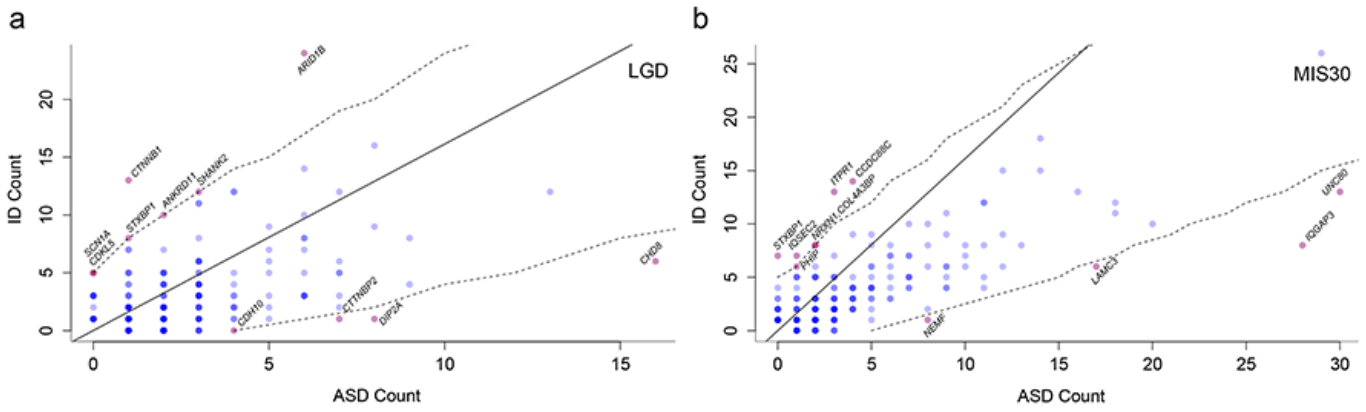
The total number of DN proband LGD mutations is plotted on the *y*-axis against the FDR-corrected DN LGD *P* value on the *x*-axis for each gene. New DN events identified in this study were considered in addition to published studies of ASD, ID, and DD (**Supplementary Table 15**). Dashed gray lines indicate an FDR cutoff of 5% ($q = 0.1$) and a DN LGD proband count = 2.



Supplementary Figure 7

Inheritance patterns by gene count.

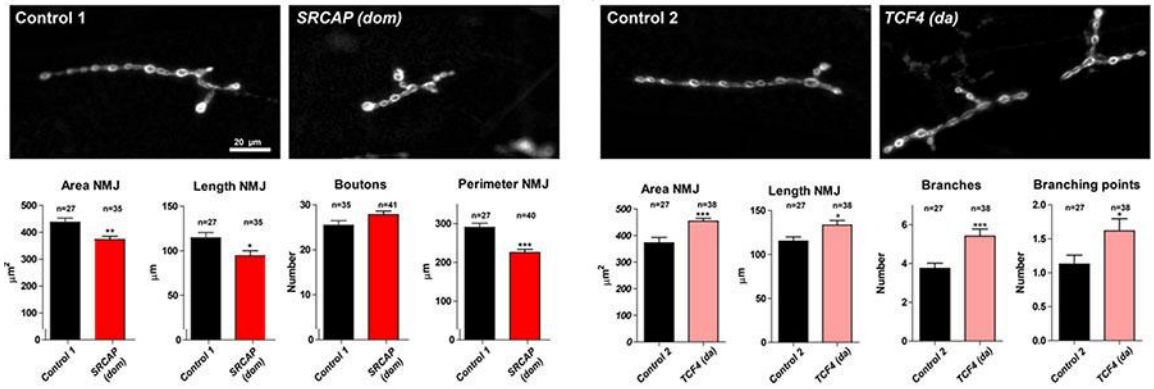
Plot of paternal (*y*-axis) or maternal (*x*-axis) inheritance counts by gene where at least one inherited event was identified in the smMIP dataset combined with published private inherited events in the SSC. Gene labels identify genes with a frequency >0.75 for either paternal or maternal inheritance where at least four inherited events have been identified.



Supplementary Figure 8

Genes exhibiting ASD and ID specificity by mutation type.

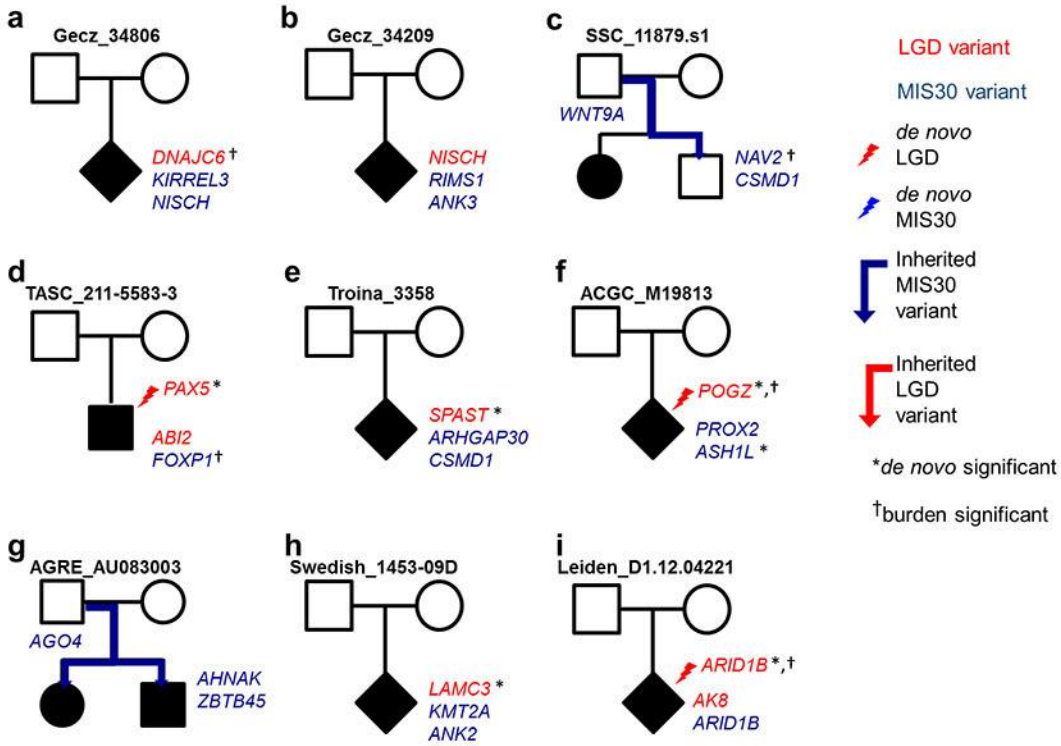
(**a**,**b**) Shown are the combined counts of private LGD (**a**) and MIS30 (**b**) events for each gene in our panel from probands in our study, published *de novo* events from ASD, ID, and DD proband studies, and published private inherited events from the SSC. Probands were scored as having ASD or ID (including DD) based on the primary ascertainment diagnosis of the cohort from which the case was sampled (**Fig. 1** and published reports). Genes were tested for a bias of LGD and MIS30 events to one phenotype (ASD or ID) by two one-tailed binomial tests ($P < 0.025$ for either bias). The solid line indicates equal proportions of mutations corrected for the screened population size. Significant genes are indicated in red and labeled with gene names while the significance threshold is indicated as a dashed line.



Supplementary Figure 9

NMJ morphology changes in *Drosophila* knockdown models.

NMJ morphology is affected in *dom* (fly ortholog of *SRCAP*, VDRC #7787) and *da* (ortholog of *TCF4*, VDRC #105258) pan-neuronal knockdown flies. Two further *da* RNAi lines (VDRC #51297, #51300) confirmed a significant increase of branches and branching points (not shown). Top: representative Dlg staining of L3 wandering larva NMJs, body wall muscle 4, segment 3 of *dom* (*SRCAP*) and *da* (*TCF4*) knockdown larvae and their genetic background controls, respectively. Bottom: quantifications of NMJ area, perimeter, length, branching, bouton numbers for over 30 NMJs per genotype. *Dom* knockdown data is shown in dark red on the left and *da* knockdown data in light red on the right. Error bars are standard error of the mean. **P* < 0.05, ***P* < 0.01, ****P* < 0.001 (two-tailed Student's *t*-test). Exact statistical values: *SRCAP* (*dom*), NMJ area *P* = 0.0012 df = 60, length *P* = 0.0184 df = 65, boutons *P* = 0.0771 df = 73, perimeter *P* = 0.0001 df = 60; *TCF4* (*da*), NMJ area *P* = 0.0003 df = 63, length *P* = 0,0128 df = 68, branches *P* = 0.0009 df = 68, branching points *P* = 0.0390 df = 68.



Supplementary Figure 10

Probands carrying three private events in the study.

(**a**-**i**) Pedigrees show individuals carrying three private LGD (red) or MIS30 (blue) events identified in this study. Where available, inheritance is indicated (*de novo* or inherited). *Genes that reach DN significance in the study. †Genes that show private disruptive burden in the study.

Case Reports

NAA15

Patient ID: D1.11.09640 (Leiden) – Patient 1

Event: inheritance unknown *NAA15* nonsense mutation

Child (gender unknown) was born in 1994 presented with ID, cutis marmorata, and small head circumference with proportionate short stature (both -2 SDS). Mother also has ID.

Patient ID: 04147-8645 (Troina) – Patient 2

Event: inheritance unknown *NAA15* frameshift mutation

Girl born in 2002 seen for the first time at 8 years and diagnosed as Moderate ID (ICD-9-CM Diagnosis Code 318.0) and Other Specified Pervasive Developmental Disorder, Current or Active State (ICD-9-CM Diagnosis Code 299.80). ID and psychiatric illness are reported in her pedigree, but further detail is unavailable. She was born by dystocic term delivery, and neonatal cyanosis was reported. Phenotype at 8 years showed growth retardation (weight and height on the 3^{rd}-10^{th} percentile ranges), microcephaly (OFC <2^{nd} percentile), facial asymmetry, hypertelorism, prominent anthelix on the ears, a single café-au-lait spot on the left side of the trunk, joint hyperlaxity, genu recurvatum, right congenital hip dysplasia, hyperopia. EEG and brain MRI were normal. Agilent 60K array CGH was normal, and MLPA test for 11p11.2 chromosomal band didn't show any deletion/duplication.

Patient ID: AU031003 (AGRE, NIMH) – Patient 3

Event: *de novo NAA15* frameshift mutation

Female patient has been diagnosed with ASD.

Patient ID: 115149 (Antwerp) – Patient 4

Event: inheritance unknown *NAA15* nonsense mutation

Patient is a 7 year old boy with global developmental delay. Speech and language are also delayed (level of 2-2.5 years old at age 4) with inadequate communication and contact and autistic traits. He also has cognitive delay: SON-IQ 72, SON-RS 79, SON-PS 72: harmonic IQ profile.

Patient was delivered at 41 gestational weeks with a birth weight of 3280 g and length of 52 cm. He began crawling at 6.5 months and walking independently at 13 months. Potty training was delayed. He experiences behavioral problems at school, and had a transient period of hair-pulling at night. He has normal hearing (BERA). Mother followed special education.

Patient ID: 100046 (Antwerp) – Patient 5

Event: paternally inherited *NAA15* missense mutation

Patient is a 10 year old female. She was a vaginal delivery at 40 weeks with placental detachment. Birth weight and length were 3700 g and 53 cm, respectively. She began walking at 15 months and her first words were at 12 months. At age 6: W 21.2 kg (P50), L 121 cm (P75), OFC 48.9cm (P25-50), ICD 3cm, OCD 9,8cm. Patient has hypertelorism and a broad nasal bridge as well as behavioral problems including: selective mutism, pervasive developmental disorder.

Patient ID: 211-5353-3 (TASC) – Patient 6

Event: maternally inherited *NAA15* missense mutation

Patient is an 11-year old female. Patient has a height of 148 cm, weight of 34 kg, and head circumference of 52 cm. Patient was diagnosed with autism spectrum disorder (confirmed with ADOS, ADI, and clinical judgment using DSM-IV criteria). Patient's verbal cognitive ability falls

in the extremely low range (Verbal IQ = 36). Other cognitive ability scores could not be computed due to patient being out of age range of the normed sample. Patient's overall cognitive ability appears to fall in the extremely low range. Per parent report, patient's adaptive abilities were impaired (Adaptive composite = 41). Abnormalities were first noted in her development at 12 months of age. She first used single words at 66 months and has not developed phrase speech (since last assessment).

Patient ID: 105005 (Antwerp) – Patient 7

Event: *de novo NAA15* nonsense mutation

Patient is a 6 year old boy delivered at 38w1d weighing 2905 g(L 47 cm and OFC 35 cm). The child was sitting at 8.5 months, spoke his first words at 14 months, and was walking at 19 months. MRI shows no brain abnormalities. Patient has unilateral cryptorchidia, ptosis of the left eye, mild hypertelorism, flat philtrum, mild pectus carinatum upper side and excavatum below, and low-set ears. He had relative macrocephaly at age 2y4m: L84cm (P3), Weight 12,4kg (P10-25), OFC 51cm (P75-90).

Patient has global developmental delay, speech and language delay, and possible autism spectrum disorder including aggressive behavior and traits of Noonan syndrome.

Patient ID: D2.11.05195 (Leiden) – Patient 8

Event: inheritance unknown *NAA15* frameshift mutation

Patient (gender unknown) was born in 2005 and presented with ASD, IQ 50, and hyperactivity, yet no obvious dysmorphisms. Mother has followed special education.

Patient ID: 106663 (Antwerp) – Patient 9

Event: *de novo NAA15* nonsense mutation

Patient is a 31 year old male with intellectual disability, ASD, and no facial dysmorphism except 3 pre-auricular tags on the right side. His current biometrics are: L 165 cm, W 75-80 kg, and OFC 54.3cm.

Patient ID: 4114-08D (Swedish) – Patient 10

Event: inheritance unknown *NAA15* nonsense mutation

Patient has severe ID.

Patient ID: AU025403 (AGRE) – Patient 11

Event: *De novo NAA15* frameshift mutation

Patient is male with a diagnosis of ASD.

Patient ID: 146.03 (SAGE) – Patient 12

Event: inheritance unknown *NAA15* missense mutation

Patient is a 15 year old male with Asperger's syndrome.

Patient ID: 3211 (Troina) – Patient 13

Event: inheritance unknown *NAA15* stop-gained mutation

Boy born in 2001 and seen for a single time at 6 years, diagnosed as NOS ID (ICD-9-CM Diagnosis Code 319) and NOS Pervasive Developmental Disorder (ICD-9-CM Diagnosis Code 299.9). Epilepsy, Delayed Speech, and ID are shown by some relatives (one maternal uncle, two 1st degree cousins on the paternal line, one 2nd degree cousin on the maternal line). On the paternal line both grandparents showed endocrine disorders (Addison disease in the

grandmother and Thyroid disease in the grandfather). He was delivered by CS for early placental detachment after 36 weeks of uneventful pregnancy. Birth asphyxia and cyanosis required Oxygen supplementation. The Apgar score was 6 at 1' and 8 at 5'. Weight at birth was 2600 grams. Weight and length thrived normally in the first months of life. Psychomotor development was delayed (independent sitting at 8 months, walking at 18 months, first words at 30-36, bladder control at 3 years, bowel control at 5 years). Weight and height at 6 years were on the 97th and 75th percentile, respectively (28 kg and 122 cm). Brain MRI and EEG were normal. Agilent 180K Array-CGH was normal.

KMT5B

Patient ID: D1.12.00933 (Leiden) – Patient 1

Event: maternally inherited *KMT5B* missense mutation

Proband was born in 2007 and has developmental delay, motor delay, and intellectual disability (IQ ~57, dysharmonic profile). Patient also has bilateral epicanthal folds.

Patient ID: 2135-09D (Swedish) – Patient 2

Event: *de novo KMT5B* frameshift mutation

10 year old girl born to healthy consanguineous parents. She has six siblings. None of them has the same diagnosis as the proband.
There is a diagnosis of mild-moderate intellectual disability. She has symptoms of attention deficit disorder, but she does not have a formal diagnosis.
In addition, the girl has seizures linked to fever episodes. The seizures have not required prophylactic antiepileptic drug treatment. EEG has shown mild epileptic abnormalities. Imaging of the brain using CT and MRI have shown wide ventricles.
She is taller than the median (+1SD).

Patient ID: 1895-11D (Swedish) – Patient 3

Event: *de novo KMT5B* frameshift mutation
Patient is a seven year old boy born to healthy consanguineous parents. He has healthy half-siblings on the paternal side. He has mild-moderate intellectual disability and autism. MRI of the brain showed enlarged perivascular areas. He has had unilateral cryptorchidism, and he is taller than the mean (+2SD).

Patient ID: 1720-08D (Swedish) – Patient 4

Event: inheritance unknown *KMT5B* frameshift mutation

Patient is a 19 year old boy who is the only child born to healthy non-consanguineous parents. Pregnancy and delivery was without remarks. He had unilateral pes equinovarus requiring surgery, and bilateral pes plano valgus. Psychomotor development was delayed, he walked independently at the age of 28 months. He has a diagnosis of intellectual disability and autism and has no language. In infancy there were seizures linked to fever episodes. These did not require any prophylactic drug treatment. EEG showed slow activity without epileptic activity. CT of the brain was without remarks. He is taller than the mean (+2SD).

Patient ID: T169.03 (11519.p1, SSC) – Patient 5

Event: *de novo KMT5B* missense mutation

Patient is a 14-year-old Caucasian male. Facial features include a high forehead, horizontal palpebral fissures, and sparse lateral eyebrows. Patient's ears are small and slightly low; he has a wide nasal bridge, wide nasal base, and low columella. Physical examination reveals long toes, long feet, and notable fetal finger pads. Patient has a below average height and a significantly below average weight (*14 years:* height = 150 cm, z = -1.78; weight = 35.83 kg, z = -2.17). Patient currently has an above average head circumference (*14 years*: head circumference= 57.7 cm, z = 1.69)

Patient was diagnosed with autism spectrum disorder (confirmed with ADOS, ADI, and clinical judgment using DSM-5 criteria), moderate intellectual disability (confirmed with cognitive and

adaptive testing), and developmental coordination disorder. He primarily speaks in 3-4 word phrases, which are sometimes difficult to understand, and has a concurrent diagnosis of Speech Sound Disorder. Patient showed autism-related impairments in social communication, including limited variation in vocalizations, poor quality in social overtures, limited conversation skills, and atypical eye contact. He also displayed autism-related impairments in restricted and repetitive behaviors, including complex motor mannerisms (i.e., flapping and rocking while flipping hand on floor; flapping and jumping at the same time), and possible sensory interests. Patient showed shared enjoyment during activities, spontaneously showed objects to others, and responded to joint attention. Patient has a strong history of use of another person's body as a tool, little reciprocal play, unusual preoccupations, unusual sensory interests (i.e., water and soap), complex motor mannerisms, and aggression towards other peers (i.e., pinching, pushing). Patient's cognitive and adaptive abilities fall in the extremely impaired range (Verbal Ratio IQ = 35, Nonverbal Ratio IQ = 45, Full Scale Ratio IQ = 42, and Adaptive Composite = 57). Patient first used single words at 14 months, but lost that language after going into allergic-anaphylactic shock with peanuts. Abnormalities were first noted in his development at 12 months of age when patient would lie on his back and flap his hands. Patient started walking at 15 months of age. Patient has extremely low receptive vocabulary (PPVT Standard Score = 44) and extremely low expressive vocabulary (EVT Standard Score = 43). He also has significantly impaired fine motor coordination (Purdue Pegboard T scores all <10 and Movement ABC Manual Dexterity Scaled Score = 1) as well as difficulty with gross motor coordination (Movement ABC Aiming & Catching subtest Scaled Score = 1 and Balance subtest Scaled Score = 1). Parent report about his social responsiveness on the SRS-2 suggests moderately impaired restricted interests and repetitive behavior, social cognition, and social awareness, as well as mild impairment in social awareness and social motivation.

The patient was born full-term via C-section due to large head and failure to progress. Patient was somewhat floppy after birth, had meconium aspiration and issues with temperature regulation. Mother reports that patient had poor suck and feeding difficulties. Patient has significant food allergies (i.e., gluten, casein, wheat, milk, nuts, egg), environmental allergies (i.e. grass and trees), and allergies to medication (i.e. omnicef, zithromax, amoxicillin, sulfrotrim). Patient has a history of gastrointestinal illnesses, including chronic diarrhea, unusual stools, and excessive gas, which have all resolved. Patient currently experiences chronic constipation and bloating. Patient was suspected of having Celiac disease as an infant and hypothyroidism at 12 years of age. Given multiple allergies and infections in early life, patient

was eventually diagnosed with combined variable immune deficiency (CVID) at 8 years of age. Per parent report, at 15 months of age, patient suffered anaphylactic shock while hospitalized for pneumonia and seemed to lose babbling after that. Patient suffered from chronic otitis media (50-60 occurrences in the first 8 years), resulting in two rounds of Myringotomy tube placement and removal of adenoids. Patient was diagnosed with Developmental Coordination Disorder at 3 years of age and with movement abnormalities ("feet turned in") at 6 years of age. Additional medical diagnoses include: asthma at 9 months of age and failure to thrive at 13 years of age. Parent reports some sleep problems including incontinence at night and frequent/prolonged awakenings at night but these have gradually improved.

Patient ID: 11729.p1 (SSC) – Patient 6

Event: *de novo KMT5B* missense mutation

Patient is a 9-year old Caucasian male. Patient has an average height (*110 months:* height = 132 cm, z = -0.51) and an underweight BMI (*110 months*: BMI = 14.6, z = -1.13; weight = 25.4 kg, z = -0.91). Patient currently has a below average head circumference measurement (*110 months*: Head circumference = 51 cm, z = -1.53). Patient was diagnosed with autism spectrum disorder (confirmed with ADOS, ADI, and clinical judgment using DSM-IV criteria). Patient's cognitive and adaptive abilities fall in the extremely impaired range (Verbal Ratio IQ = 30, Nonverbal Ratio IQ = 45, Full Scale Ratio IQ = 37, and Adaptive Composite = 57). Abnormalities were first noted in his development at 9 months of age. He first used single words at 78 months and first used phrases at 84 months of age. Parents report loss of language skills in association with an unspecified physical illness). Parents report attention and affective problems. Parent report about his social responsiveness on the SRS suggests severely impacted social communication, social cognition, social motivation, social awareness, and autistic mannerisms.

Patient was born vaginally at 39 weeks gestation following a labor induced by Pitocin for unspecified reasons. Mother was anemic throughout all three trimesters and had the flu and upper respiratory virus during the 2nd trimester. Patient was diagnosed with chronic constipation at 2 years of age, but no other gastrointestinal disturbances were reported. Patient was diagnosed with chicken pox at a young age. In terms of neurological conditions, patient had a positive lead screening at 4 years of age and was also diagnosed with Tourette's/Tics at 6 years

of age. Patient had had febrile seizures and, per parent report, demonstrates improvements in repetitive behaviors when he has a fever. He has also been diagnosed with kidney/urinary problems. Patient has a history of significant sleep problems including difficulties falling asleep, incontinence, and frequent awakenings at night and currently still takes sedatives.

Patient ID: 12864.p1 (SSC) – Patient 7

Event: de novo KMT5B splice-donor mutation

Patient is a 5-year old Hispanic female. Patient has a significantly above average height (*69 months*: height = 124 cm; *z* = 2.06) and an average BMI (*69 months*: BMI = 15.9, *z* = 0.4; weight = 24.49 kg, *z* = 1.27). Patient currently has macrocephaly (*69 months:* HC = 56 cm; *z* = 4.0). Patient was diagnosed with autism spectrum disorder (confirmed with ADOS, ADI, and clinical judgment using DSM-IV criteria). Patient's cognitive and adaptive abilities fall in the low range (Verbal IQ = 73, Nonverbal IQ = 74, Full Scale IQ = 74, by DAS-2; Adaptive Composite = 68). Per parent report, she first used single words at 34 months and first used phrases at 48 months of age. She currently speaks in phrases. Abnormalities were first noted in her development at 30 months of age and parents report a regression in language skills. Patient also has a history of elevated attention and withdrawal problems. Patient had low average receptive vocabulary abilities (PPVT Standard Score = 88). Parent report about her social responsiveness on the SRS suggests severe impairment in social awareness, social motivation, social cognition, social communication, and autistic mannerisms.

Patient was born via planned C-section at 40 weeks gestation. No pregnancy or labor complications were observed. Patient has a history of sleep problems including difficulties falling asleep and laying down with parent to fall asleep. Patient has an unremarkable medical history; no gastrointestinal or neurological disturbances were reported.

ASH1L

Patient ID: 1206-11D (Swedish) – Patient 1

Event: de novo ASH1L frameshift mutation

Female patient was originally evaluated at six years of age. She has ID, hearing deficits, hypothyroidism, hyperopia, astigmatism, normal growth, smooth filtrum, hypertelorism, and a low hairline.

Patient ID: 3145 (Troina) – Patient 2

Event: inheritance unknown *ASH1L* missense mutation

Male born in 1996, seen by us for the first time at age 5 years, and the last time at age 16. The last diagnosis is NOS Pervasive Developmental Disorder, mild ID and epilepsy. He was born by eutocic delivery at term of an uneventful pregnancy. After a regular psychomotor development, he showed at 3 years a regression in the verbal speech, and some behavioral changes, such as hyperactivity and tendency to isolation. At age 6 first seizures and EEG anomalies prompted towards a diagnosis of epilepsy and anticonvulsant treatment. Some months afterwards a diagnosis of intellectual disability was made. Brain MRI showed only a mild asymmetry in the temporal horns of lateral ventricles, with the right one wider than the left one. His phenotype showed dolicocephaly, normal weight and height, lumbar hyperlordosis, winged scapulae.

Patient ID: 2235-13D (Swedish) – Patient 3

Event: de novo ASH1L frameshift mutation

17 year old male born to healthy non-consanguineous parents. He has a healthy brother. Presented with mild intellectual disability and no other features.

Patient ID: 11282.p1 (SSC) – Patient 4

Event: *de novo ASH1L* frameshift mutation

Patient is an 8-year old Caucasian male. Patient has an average height (*105 months*: height = 128 cm; *z* = -0.45) and an average BMI (*105 months*: BMI = 15.5 *z* = -0.31; weight = 25.7 kg). Patient currently has an average head circumference of 53.9 cm (*z* = 0.75). Patient was diagnosed with autism spectrum disorder (confirmed with ADOS, ADI, and clinical judgment

using DSM-IV criteria). Patient's cognitive abilities fall in the below average to low range (Verbal IQ = 83, Nonverbal IQ = 74, Full Scale IQ = 75, by DAS-2) and adaptive abilities fall in the moderately low range (Adaptive Composite = 78). Per parent report, he first used single words at 27 months and first used phrases at 33 months of age. Abnormalities were first noted in his development at 15 months of age. Patient also has a history of elevated externalizing, internalizing, withdrawal, affective, social, and thought problems. Patient has average receptive vocabulary abilities (PPVT Standard Score = 89). Parent report about his social responsiveness on the SRS suggests severe impairment in social awareness, social motivation, social cognition, social communication, and autistic mannerisms. No neurological disturbances or other medical problems were reported. Patient has a history of sleep problems including excessive daytime sleepiness and unusually frequent naps during the day. Patient was born via vaginal delivery with cephalic presentation at 38 weeks gestation. No pregnancy or labor complications were observed. Patient was irritable and inconsolable as an infant.

Patient ID: 13678.p1 (SSC) – Patient 5

Event: *de novo ASH1L* nonsense mutation

Patient is a 13-year old Caucasian male. Patient has an average height (*162 months*: height = 156 cm; z = -0.64) and an overweight BMI (*162 months*: BMI = 26.3, z = 2.14; weight = 64 kg). Patient currently has an average head circumference of 55 cm (z = 0.36). Patient was diagnosed with autism spectrum disorder (confirmed with ADOS, ADI, and clinical judgment using DSM-IV criteria) and mild intellectual disability (confirmed with cognitive and adaptive testing). Patient's verbal cognitive ability falls in the below average range, while his nonverbal cognitive and adaptive abilities fall in the low range (Verbal IQ = 89, Nonverbal IQ = 67, Full Scale IQ = 60, by DAS-2; Adaptive Composite = 61). Per parent report, he first used single words at 18 months and first used phrases at 22 months of age. Abnormalities were first noted in his development at 6 months of age. Patient also has a history of elevated attention, externalizing, internalizing, affective, social, and thought problems. Patient has average vocabulary abilities (PPVT Standard Score = 93). Parent report about his social responsiveness on the SRS suggests severe impairment in social awareness, social motivation, social cognition, social communication, and autistic mannerisms. Patient has a history of sleep problems including sleepwalking, difficulty going to bed, long or frequent daytime naps, and incontinence at night. Patient has a history of seizures. Medical history is significant for chicken pox, herpes

1, otitis media. No gastrointestinal disturbances were reported. Patient was born at 39 weeks via vaginal delivery with cephalic presentation; labor was induced via an amniotomy and prostaglandins due to failure to progress. Mother reports that patient was irritable and inconsolable as an infant.

Supplementary Table 1. Published sequencing cohorts used for candidate gene selection.

Study	Official collection name	Primary ascertainment	Number of individuals	Type of sequencing	PMID
de Ligt_2012	NA	ID	100	Exome	23033978
De Rubeis_2014	Autism Sequencing Consortium (ASC)	ASD	1,445	Exome	25363760
Deciphering Developmental Disorders_2015	Deciphering Developmental Disorders	DD	1,133	Exome	25533962
Iossifov_2014	Simons Simplex Collection	ASD	2,508 probands; 1,909 unaffected siblings	Exome	25363768
Krumm_2015		ASD		Exome	25961944
O'Roak_2012		ASD		Exome	23160955
O'Roak_2014[α]	The Autism Simplex Collection (TASC)	ASD	921	Targeted sequencing	25418537
Rauch_2012	NA	ID	51	Exome	23020937

ASD: autism spectrum disorder; ID: intellectual disability; DD: developmental delay

[α]Study also included individuals from the Simons Simplex Collection shown in the studies above and were not included in the number of individuals for this study to avoid double counting.

Supplementary Table 2. Published DN SNVs and indels from ASD, ID, and DD exome sequencing studies.

Table provided as a separate .xlsx spreadsheet.

Supplementary Table 3. Selection criteria and smMIP pool composition.

Table provided as a separate .xlsx spreadsheet.

Supplementary Table 4. Gold pool probes.

Table provided as a separate .xlsx spreadsheet.

Supplementary Table 5. ASD4 pool probes.

Table provided as a separate .xlsx spreadsheet.

Supplementary Table 6. ASD5 pool probes.

Table provided as a separate .xlsx spreadsheet.

Supplementary Table 7. ASD6 pool probes.

Table provided as a separate .xlsx spreadsheet.

Supplementary Table 8: Patient cohorts.

Cohort	Location	Site Lead	Individuals	Ascertainment[α]	Pools[β]	Figure number[γ]
Autism Clinical and Genetic Resources in China (ACGC)	Changsha, China	Kun Xia	1654	ASD	4, 5, 6, G	1
Autism Genetic Resource Exchange (AGRE)	NIMH, USA	Autism Speaks	1732	ASD	4, 5, 6, G	2
Autism Phenome Project (APP)	Davis, CA, USA	David Amaral	152	ASD	4, 5, G	3
Leuven	Leuven, Belgium	Hilde Peeters	906	ASD	G	4
Melbourne	Melbourne, Australia	Ingrid Scheffer	309	ASD	G	5
Murdoch	Murdoch, Australia	Ingrid Scheffer	56	ASD	4, 5, G	6
San Diego	San Diego, CA, USA	Eric Courchesne	488	ASD	4, 5, 6, G	7
The Autism Simplex Collection (TASC)	NIMH, USA	Raphael Bernier	1045	ASD	4, 5, 6, G	8
Adelaide	Adelaide, Australia	Jozef Gecz	2383	ID	4, 5, 6, G	9
Greenwood	Greenwood, SC, USA	Charles Schwartz	253	ID	4, 5, G	10
Antwerp	Antwerp, Belgium	Frank Kooy	1089	DD	4, 5, 6, G	11
Leiden	Leiden, The Netherlands	Gijs Santen	210	DD	4, 5, 6, G	12
SAGE	Seattle, WA, USA	Raphael Bernier	429	DD	4, 5, 6, G	13
Stockholm	Stockholm, Sweden	Magnus Nordenskjold	1500	DD	4, 5, 6, G	14
Troina	Troina, Italy	Corrado Romano	1201	DD	4, 5, 6, G	15
TOTAL			**13,407**			

[α]Primary diagnosis under which each cohort was broadly ascertained. Secondary diagnoses may be present. ASD: autism spectrum disorder; ID: intellectual disability; DD: developmental delay.
[β]smMIP pools for which cohort was tested. 4: ASD4; 5: ASD5; 6: ASD6; G: Gold.
[γ]Numbers correspond to the map in Figure 1.

Supplementary Table 9: Unaffected sibling cohorts.

Cohort	Location	Individuals
AGRE Siblings	NIMH, USA	644
SAGE Siblings	Seattle, WA, USA	129
SSC Siblings	New York, NY, USA	1920
TASC Siblings	NIMH, USA	174
Autism Phenome Project (APP)	Davis, CA, USA	68
TOTAL		**2,955**

Supplementary Table 10: Quality control metrics by smMIP pool

Pool	#MIPs	#Genes	Cases Screened	Controls Screened	Total variants passing QC[α]	Total private variants passing QC[β]
Gold	3341	63	13407	2955	17326	793
ASD4	2614	39	12192	2955	12937	409
ASD5	2686	41	12192	2955	13857	414
ASD6	3340	65	11731	2867	17195	569

[α]Variants passing quality and depth metrics excluding common dbSNP variants (Methods).
[β]Full annotated list of private variants can be found in Supplementary Table 11.

Supplementary Table 11. All private LGD and MIS30 events in this study.

Table provided as a separate .xlsx spreadsheet.

Supplementary Table 12. All DN smMIP events identified in the 208 genes screened in this study.

Table provided as a separate .xlsx spreadsheet.

Supplementary Table 13. Proband counts of private validated LGD and MIS30 mutations by gene and inheritance pattern.

Table provided as a separate .xlsx spreadsheet.

Supplementary Table 14. Rank-ordered observed DN p-values with corresponding odds ratios.

Table provided as a separate .xlsx spreadsheet.

Supplementary Table 15. Summary of all gene statistics for this study.

Table provided as a separate .xlsx spreadsheet.

Supplementary Table 16. Ultra-rare (AC ≤ 3) inherited smMIP events identified in the 208 genes screened in this study.

Table provided as a separate .xlsx spreadsheet.

Supplementary Table 17. Published private inherited events from the SSC.

Table provided as a separate .xlsx spreadsheet.

Supplementary Table 18. LGD and MIS30 mutations in our 208 candidate genes in ExAC.

Table provided as a separate .xlsx spreadsheet.

Supplementary Table 19. Clinical features of *NAA15* patients from all cohorts.

Individual	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8
Study ID	Leiden_D1.11.09640	Troina_04147-8645	AGRE_AU031003	Antwerp_115149	Antwerp_100046	TASC_211-5353-3	Antwerp_105005	Leiden_D2.11.05195
Child	Proband	Proband	Proband	Proband	Proband	Proband	Proband	Proband
Mutation	c.154A>T (p.Lys52*)	c.239_240del (p.His80Argfs*17)	c.532_533del (p.Gln178Thrfs*5)	c.868G>T (p.Gly290*)	c.1348A>G (p.Lys450Glu)	c.1424C>T (p.Ala475Val)	c.1695T>A (p.Tyr565*)	c.1988del (p.Pro663Argfs*2)
Frequency	Private	Ultra-rare	Private	Private	Ultra-rare	Ultra-rare	Private	Private
Inheritance	*Unknown*	*Unknown*	*De novo*	*Unknown*	*Paternal*	*Unknown*	*De novo*	*Unknown*
Age (years)	22	8		7	10	11	6	11
Gender		Female	Female	Male	Female	Female	Male	
ID/DD	ID	Moderate ID		DD with mild ID	pervasive developmental disorder	Extreme ID	Global DD	ID (IQ 50)
Speech/ language development		Delayed		Delayed	Normal	First single words at 66 mos. No phase speech	Delayed	
Motor development		Delayed		Normal	Normal			
Behavior/		pervasive developmental disorder		Behavioral problems; transient period of hair-pulling	Selective mutism and pervasive developmental disorder		Aggressive behavior, traits of Noonan syndrome	Hyperactivity
ASD			ASD	ASD traits		ASD	ASD?	ASD
HCZ	-2 SD	<2nd percentile			P25-50	52cm	P75-90	
Height/weight (z)	-2 SD height	3rd-10th percentile			P75/P50	148cm/34kg	P3/P10-25	
Seizures		No						
GI dysfunction		No						
Other	Cutis marmorata; mother also has ID			Mother followed special education	Hypertelorism, broad nasal bridge		Unilateral cryptorchidia, ptosis of the left eye, flat philtrum, mild pectus carinatum upper side and excavatum below, low set ears	Mother followed special education

Individual	Patient 9	Patient 10	Patient 11	Patient 12	Patient 13	Total (N = 13)
Study ID	Antwerp_106663	Swedish_4114-08D	AGRE_03C14733 (AU025403)	SAGE_146.03	Troina_3211	
Child	Proband	Proband	Proband	Proband	Proband	
Mutation	c.2086A>T (p.Lys696*)	c.2344C>T (p.Arg782*)	c.225_230delTGACTTinsT (p.Asp76Glufs*20)	c.334G>A (p.Asp112Asn)	c.2389A>T (p.Arg797*)	10 LGD, 3 MIS30
Frequency	Private	Private	Private	Private	Private	
Inheritance	*De novo*	*Unknown*	*De novo*	*Unknown*	*Unknown*	4 *de novo*, 1 inherited, 8 unknown
Age (years)	31			15	6	
Gender	Male		Male	Male	Male	6 Males, 4 Females
ID/DD	ID	Severe ID		-	NOS ID	10/11 (91%)
Speech/ language development					Delayed Speech age 2 years according to MacArthur questionnaire	5/6 (83%)
Motor development					Delayed	2/4 (50%)
Behavior/ ***ASD***	 ASD		 ASD	Asperger's diagnosis	NOS Pervasive Developmental Disorder. Motor instability. Opposition	 Formal ASD diagnosis 5/8 (63%)
HCZ	OFC 54.3cm				unavailable	
Height/weight (z)	165cm/75-80kg				75^{th}/97^{th} percentile	
Seizures					No	No
GI dysfunction					No	No
Other	3 pre-auricular tags on the right side				No	

All HGVS annotations were annotated on RefSeq transcript NM_057175.3.

Supplementary Table 20. Clinical features of *KMT5B* patients from all cohorts.

Individual	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5*	Patient 6*	Patient 7*	Total (N = 7)
Study ID	Leiden_D1.12.00933	Swedish_2135-09D	Swedish_1895-11D	Swedish_1720-08D	SSC_11519.p1	SSC_11729.p1	SSC_12864.p1	
Child	Proband	Proband	Proband	Proband	Proband	Proband	Proband	
Mutation	c.1619G>A (p.Arg540Gln)	c.725del (p.Leu242Hisfs*30)	c.1557_1558del (p.Asn520Serfs*33)	c.1166dup (p.Asn389Lysfs*6)	c.791G>C (p.Trp264Ser)	c.1538C>T (p.Ala513Val)	c.977+1G>A (p?)	4 LGD, 3 MIS30
Frequency	Private	Private	Private	Ultra-rare	Ultra-rare	Ultra-rare	Ultra-rare	
Inheritance	*Maternal*	*De novo*	*De novo*	*Unknown*	*De novo**	*De novo**	*De novo**	5 *de novo*, 1 inherited, 1 unknown
Age (years)	9	10	7	19	14	9	5	
Gender		F	M	M	M	M	F	4 Males, 2 Females
ID/DD	ID/DD	Mild-moderate ID	Mild-moderate ID	ID	Moderate ID	ID	ID	7/7 (100%)
Speech/ language development				No language	Low receptive and expressive vocabularies; Speech Sound Disorder	cognitive and adaptive abilities extremely impaired	low average receptive vocabulary	3/4 (75%)
Motor development	Motor delay			Delayed psychomotor development	Developmental coordination disorder	-	-	3/5 (60%)
Behavior/		ADD symptoms but no formal diagnosis			Aggression towards other peers	Attention and affective problems	History of elevated attention and withdrawal problems	Attention problems in 3 patients
ASD		-	ASD	ASD	ASD	ASD	ASD	5/6 (83%)
HCZ					1.69	-1.53	4.0	
Height/weight (z)		+1 SD height	+2 SD height	+2 SD height	-1.78/-2.17	-0.51/-0.91	2.06/1.27	Trend toward taller height
Seizures		Febrile		Febrile in infancy	-	Febrile	-	Febrile seizures in 3/5 (60%)
GI dysfunction					+ (history but now resolved)	+	-	
Other	Bilateral epicanthal folds	EEG has shown mild epileptic abnormalities; CT and MRI show wide ventricles	MRI of the brain showed enlarged perivascular areas; unilateral cryptorchidism	unilateral pes equinovarus and bilateral pes plano valgus; EEG showed slow activity	Combined variable immune deficiency; sleep problems	Tourette's/Tics diagnosis improved with fever; history of significant sleep problems	history of sleep problems	

All HGVS annotations were annotated on RefSeq transcript NM_017635.4. *Events have been reported previously, but clinical information reported here is novel.

Supplementary Table 21. Clinical features of *ASH1L* patients from all cohorts.

Individual	Patient 1	Patient 2	Patient 3	Patient 4*	Patient 5*	Total (N = 5)
Study ID	Swedish_1206-11D	Troina_3145	Swedish_2235-13D	SSC_11282.p1	SSC_13678.p1	
Child	Proband	Proband	Proband	Proband	Proband	
Mutation	c.8868_8869delAAinsAAA (p.Arg2957LysfsTer19)	c.7172G>A (p.Arg2391His)	c.3704_3705delCTinsC (p.Glu1236LysfsTer6)	c.7764_7768dup (p.Asp2590Alafs*7)	c.6427G>T (p.Glu2143*)	4 LGD, 1 MIS30
Frequency	Private	Ultra-rare	Private	Ultra-rare	Ultra-rare	
Inheritance	*De novo*	*Unknown*	*De novo*	*De novo**	*De novo**	4 *de novo*, 1 unknown
Age (years)	6	16	17	8	13	
Gender	Female	Male	Male	Male	Male	4 Males, 1 Female
ID/DD	ID	Mild ID	Mild ID	Mild-moderate (VIQ=83; NVIQ=74; FSIQ=75)/DD	Mild ID	5/5 (100%)
Speech/ language development	Hearing deficits	Delayed		Delayed		2/3 (67%)
Motor development		Normal				
Behavior/		NOS Pervasive Developmental Disorder				
ASD		-		ASD	ASD	2/3 (67%)
HCZ		50th percentile, dolichocephaly		0.75	0.36	
Height/weight (z)		50th/50th percentile		-0.45/-0.31	-0.64/2.14	
Seizures		+		-	+	2/3 (67%)
GI dysfunction		-		Bloating and excessive gas	-	
Other	Hypothyroidism, hyperopia, astigmatism, smooth filtrum, hypertelorism, low hairline	Epilepsy		History of sleep problems; irritable and inconsolable as an infant	History of sleep problems; history of chicken pox, herpes 1, otitis media; irritable and inconsolable as an infant	

All HGVS annotations were annotated on RefSeq transcript NM_018489.2. *Events have been reported previously, but clinical information reported here is novel.

Supplementary Table 22. Adjustments to the de Vries scale for assessing general phenotypic severity.

Adjusted de Vries Scale (Feenstra et al., 2011)		Modified de Vries Scale for this study	
Phenotypic Trait	**Score**	**Phenotypic Trait**	**Score**
Intellectual Disability/ Developmental Delay		Intellectual Disability/ Developmental Delay	
Mild-moderate	1	Borderline	1
Severe	2	Mild-moderate	2
Prenatal onset growth retardation	2	Severe	3
Postnatal growth abnormalities		Postnatal growth abnormalities	
Microcephaly	1	Microcephaly	1
Short stature	1	Macrocephaly	1
Macrocephaly	1	DSM-5 Diagnosis	1-2
Tall stature	1	Medical Diagnosis	1-2
Facial dysmorphic features	2	Facial dysmorphic features	2
Non-facial dysmorphism and congenital abnormalities	1-2	Non-facial dysmorphism and congenital abnormalities	1-2
Total Maximum	10	Total Maximum	12

Supplementary Table 23. All fly strains tested for changes in the light-off habituation paradigm.

Human Gene	Drosophila gene[α]	VDRC #	ratio of initial jumpers	TTC fold change[β]	p-value	Neuronal defect[γ]
ASH1L	*ash1*	108832	0.13	N/A	N/A	reduced fitness
ASH1L	*ash1*	28982	0.50	1.18	3.64E-01	None
DLG2	*Dlg1*	109274	0.72	3.60	2.53E-04	habituation defect
DLG2	*Dlg1*	41136	0.76	2.03	1.42E-04	habituation defect
DSCAM	*Dscam1*	3115	0.19	1.04	7.76E-01	reduced fitness
EPHB2	*Eph*	110448	0.50	0.91	5.89E-01	None
EPHB2	*Eph*	4771	0.47	1.75	6.63E-02	None
GABRB3	*Lcch3*	109606	0.80	5.26	1.61E-11	habituation defect
GABRB3	*Lcch3*	42546	0.55	3.30	1.78E-02	habituation defect
GIGYF2	*CG11148*	105985	0.89	5.07	1.56E-12	habituation defect
GIGYF2	*CG11148*	18159	0.19	N/A	N/A	reduced fitness
GRIN2B	*Nmdar2*	12189	0.63	0.90	5.68E-01	None
GRIN2B	*Nmdar2*	3196	0.71	2.41	3.71E-03	habituation defect
LAMC3	*LanB2*	104013	0.97	3.54	3.46E-03	habituation defect
LAMC3	*LanB2*	42559	0.45	0.89	8.81E-01	None
NAA15	*Nat1*	110689	locomotor defect	N/A	N/A	adult locomotor defect
NAA15	*Nat1*	17571	0.19	N/A	N/A	reduced fitness
NCKAP1	*Hem*	103380	0.75	2.48	5.35E-06	habituation defect
NCKAP1	*Hem*	22207	0.56	3.62	4.56E-03	habituation defect
NcoR1	*Smr*	106701	0.81	3.46	1.01E-06	habituation defect
PARD3B	*baz*	2915	0.42	0.63	1.55E-01	None
PARD3B	*baz*	2914	0.23	0.71	4.17E-01	reduced fitness
PLXNB1	*PlexB*	46687	0.11	N/A	N/A	reduced fitness
PLXNB1	*PlexB*	27219	0.42	0.97	9.08E-01	reduced fitness
SCN2A	*para*	104775	embryonic lethal	N/A	N/A	embryonic lethal
SCN2A	*para*	6131	0.52	0.84	5.40E-01	None
Sin3A	*Sin3A*	105852	0.74	1.16	4.63E-01	None
Sin3A	*Sin3A*	10808	0.23	N/A	N/A	reduced fitness
SRCAP	*dom*	7787	0.66	2.99	1.17E-03	habituation defect
KMT5B	*Hmt4-20*	106849	0.74	1.43	4.50E-02	habituation defect
SYNGAP1	*CG42684*	109589	0.67	2.06	4.96E-02	habituation defect
SYNGAP1	*CG42684*	23432	0.68	1.27	4.86E-01	None
TRIP12	*ctrip*	50190	0.67	1.64	8.73E-02	None
TRIP12	*ctrip*	49703	0.45	0.98	7.05E-01	None
TCF4	*da*	51297	0.78	2.43	3.17E-03	habituation defect
WDFY3	*bchs*	110785	0.81	4.77	3.11E-11	habituation defect
WDFY3	*bchs*	45028	0.58	1.89	1.19E-02	habituation defect

VDRC=Vienna Drosophila Resource Center; TTC=N/A indicates that the strain was not scored due to low initial jump rate suggesting reduced fitness.

[α]Drosophila-human orthologs were chosen using Ensembl, Unigene, and flybase.

[β]Time to criterion (TTC) fold was calculated for each fly strain compared to their wild-type background strain.

[γ]Reduced fitness = low initial jump response (<40%)