Supplementary Information

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Supplementary Methods Table 1. Participant selection and cohort descriptions for analysis of phenotype-genotype analysis at the *PTCHD1-PTCHD1-AS* risk locus.

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Supplementary Data Table 1. Summary of behavioral phenotypes in *Ptchd1-as* and other extensively studied ASD mouse models.

Gene (cytoband)	**ClinGen curation** - SFARI score - EAGLE score	**Mouse models**	**Social interaction**	**Social USV communication**	**Repetitive behavior**	**Learning and memory**	**Motor functions**	**Ref**
PTCHD1-AS (Xp22.11)	NA - SFARI: 2 - EAGLE: 17.6	*Ptchd1-as*$^{-Ex3/Y}$ (Exon3 KO)	Impaired	Impaired	Impaired	Normal	Normal	Current study
		Ptchd1-as$^{PolyA/Y}$ (PolyA KI)	Impaired	Impaired	Impaired	Normal	Normal	
NRXN1 (2q16.3)	Complex NDD/ASD - SFARI: 1 - EAGLE: 143.75	Nrxn1α (Δexon1)	Mildly impaired*	Pup call: impaired	Impaired	Impaired*	Enhanced	1,2,3,4,5
		Nrxn1α (Δexon9/intron17)	Mildly impaired (novelty ↓)	NA	NA	NA	Enhanced	5
		Nrxn1βΔC (CaMKII-insertion)	Impaired	NA	Impaired	Normal	Normal	6
SCN2A (2q24.3)	Complex NDD/Epilepsy - SFARI: 1 - EAGLE: 109.3	Scn2a (Δexon1)	Mildly impaired	Impaired	Impaired	Impaired	Hyperactivity (Anxiolytic)	7,8,9
		Scn2a (Δexon4-6)	Mildly impaired (novelty ↓)	Normal	Normal	Impaired	Hypoactivity	10
		Scn2a^{T1898NfsX27} (Δexon27)	Impaired (interaction↑)	NA	Normal	NA	Hyperactivity (Anxiolytic)	11
CHD8 (14q11.2)	Complex NDD/ASD - SFARI: 1S - EAGLE: 97.65	Chd8ΔSL (Δexon1)	Mildly impaired (novelty ↓)	NA	Normal	Enhanced	Hypoactivity (Anxiety↑)	12,13
		Chd8 KO (Δexon5)	Normal	Normal	Normal	Impaired	Normal	14(20)
		Chd8ΔL (Δexon11-13)	Mildly impaired (novelty ↓)	NA	Normal	Mildly impaired	Mildly impaired (Anxiety↑)	12
		Chd8^{N2373Kfs*2} (Δexon36)	Normal	Normal (pup call: impaired)	Impaired	Normal	Hypoactivity (Anxiety↑)	15
SHANK3 (22q13.33)	Phelan-McDermid syndrome/ ASD - SFARI: 1S - EAGLE: 74.85	Shank3 KO (Δexon4-22)	Impaired	Impaired	Impaired	Impaired	Impaired	16
		Shank3A (Δexon4-9)	Impaired	Impaired	Impaired	Impaired	Impaired	17,18,19
		Shank3A (Δexon4-7)	Mildly impaired (novelty ↓)	NA	NA	NA	NA	20
		Shank3B (Δexon13-16)	Impaired	NA	Impaired	Normal	Normal (Anxiety↑)	20,21
		Shank3ΔC/ Shank3^{G} (Δexon21)	Impaired/ Normal	NA	Impaired	Mildly impaired	Normal	22,23,24
NLGN4X (Xp22.32-p22.31)	X-linked complex NDD/ASD - SFARI: 1 - EAGLE: 12	Nlgn4X KO (Δintron1)	Impaired	Impaired	Impaired	Normal	Normal	25,26,27
NLGN3 (Xq13.1)	X-linked complex NDD/ASD - SFARI: 1 - EAGLE: 6.5	Nlgn3 KO (Δexon2)	Mildly impaired (novelty ↓)	Impaired	Normal	Mildly impaired	Hyperactivity	28,29,30
		Nlgn3^{R451C} (Δexon7)	Impaired	Pup call: impaired	Impaired	Enhanced	Hyperactivity	28,30,31, 32

* Controversial between studies

Supplementary Table 4. Summary of statistics for behavioral phenotypes.

Mouse line: *Ptchd1-as*$^{-Ex3/Y}$ (KO-1)

Behavior test	Measurement	Number of animals	Statistical test & p-value	Post-hoc test & p-value
Social three chambered interaction	Sociability (Time spent in M1 vs O chamber)	WT-1 = 9, KO-1 = 11	Two-way repeated measures ANOVA Interaction: F (2,36) = 2.161, p = 0.1299 Genotype: F (1,18) = 0.1500, p = 0.7031 Chamber: F (1.479, 26.63) = 29.92, p < 0.0001	Tukey WT-1 [M1 vs O]: p < 0.05 KO-1 [M1 vs O]: p = 0.4855 Sidak M1 [WT- vs KO-1]: p = 0.3415 O [WT- vs KO-1]: p = 0.9652
	Close interaction (Time spent for direct interaction with M1 vs O)		Two-way repeated measures ANOVA Interaction: F (1,18) = 7.392, p = 0.0141 Genotype: F (1,18) = 2.311, p = 0.1458 Subject: F (1,18) = 27.29, p < 0.0001	Sidak WT-1 [M1 vs O]: p < 0.0001 KO-1 [M1 vs O]: p = 0.1504 M1 [WT- vs KO-1]: p < 0.05 O [WT- vs KO-1]: p = 0.8846
	Preference for novelty (Time spent in M2 vs M1 chamber)		Two-way repeated measures ANOVA Interaction: F (2,36) = 6.829, p = 0.0031 Genotype: F (1,18) = 0.8127, p = 0.3792 Chamber: F (1.814, 32.64) = 21.52, p < 0.0001	Tukey WT-1 [M2 vs M1]: p < 0.05 KO-1 [M2 vs M1]: p = 0.9752 Sidak M2 [WT- vs KO-1]: p < 0.01 M1 [WT- vs KO-1]: p = 0.6713
	Close interaction (Time spent for direct interaction with M2 vs M1)		Two-way repeated measures ANOVA Interaction: F (1,18) = 9.766, p = 0.0058 Genotype: F (1,18) = 1.049, p = 0.3192 Subject: F (1,18) = 24.09, p = 0.0001	Sidak WT- [M2 vs M1]: p < 0.001 KO-1 [M1 vs M2]: p = 0.3606 M2 [WT- vs KO-1]: p < 0.05 M1 [WT- vs KO-1]: p = 0.8007
Reactivity to social odor cues	Time spent sniffing odors (%)	WT-1 = 8, KO-1 = 7	Two-way repeated measures ANOVA Interaction: F (6,78) = 3.288, p = 0.0062 Genotype: F (1,13) = 12.12, p = 0.0041 Session: F (6,78) = 14.35, p < 0.0001	Sidak 1st F odor: p < 0.01 1st M odor: p < 0.0001
Repetitive grooming	Time spent grooming (%)	WT-1 = 12, KO-1 = 8	Unpaired t-test, p = 0.0006	-
USV	Number of syllables	WT-1 = 9, KO-1 = 11	Unpaired t-test, p = 0.0055	-
	Mean frequency (kHz)		Unpaired t-test, p = 0.0436	-
	Total syllable energy (dB)		Unpaired t-test, p = 0.0031	-
	Repertoire comparison		Kruskal-Wallis, p = 0.2071	Dunn [WT-1-WT-2] vs [WT--KO-1]: p = 0.5260
Pre-pulse inhibition	Prepulse inhibition (%)	WT-1 = 8, KO-1 = 6	Two-way repeated measures ANOVA Interaction: F (4,48) = 1.310, p = 0.2797 Genotype: F (1,12) = 11.28, p = 0.0057 Session: F (4,48) = 77.61, p < 0.0001	Sidak [WT- vs KO-1] 77 dB: p < 0.01 81 dB: p < 0.01 85 dB: p < 0.05
Open field test	Distance traveled (timed)	WT-1 = 9, KO-1 = 9	Two-way repeated measures ANOVA Interaction: F (2,32) = 0.6939, p = 0.5070 Genotype: F (1,16) = 0.0007, p = 0.9792 Time: F (2,32) = 45.31, p < 0.0001	Sidak 10 mins: p = 0.9563 20 mins: p = 0.9959 30 mins: p = 0.9970
	Total distance traveled		Unpaired t-test, p=0.9792	-
	Time spent in zones (%)		Two-way repeated measures ANOVA Interaction: F (2,32) = 0.3013, p = 0.7419 Genotype: F (1,16) = 1.231, p = 0.2837 Zone: F (2,32) = 1014, p < 0.0001	Sidak [WT- vs KO-1] Outer: p = 0.8946 Middle: p = 0.8636 Inner: p = 0.9998
Gait analysis	Stride length	WT-1 = 9, KO-1 = 7	Unpaired t-test, p = 0.8702	-
	Stride width		Unpaired t-test, p = 0.5926	-
Ladder walking test	Number of foot faults	WT-1 = 8, KO-1 = 7	Unpaired t-test, p = 0.8053	-
Rotarod	Time on rotarod	WT-1 = 6, KO-1 = 9	Two-way repeated measures ANOVA Interaction: F (4,52) = 0.0859, p = 0.9864 Genotype: F (1,13) = 0.8261, p = 0.3799 Day: F (4,52) = 7.880, p < 0.0001	Sidak [WT- vs KO-1] Day 1: p = 0.9373 Day 2: p = 0.9459 Day 3: p = 0.9749 Day 4: p = 0.8295 Day 5: p = 0.9766

Mouse line: *Ptchd1-as*$^{-Ex3is/Y}$ (KO-2)

Behavior test	Measurement	Number of animals	Statistical test & p-value	Post-hoc test & p-value
Social three chambered interaction	Sociability (Time spent in M1 vs O chamber)	WT-2 = 9, KO-2 = 10	Two-way repeated measures ANOVA Interaction: F (2,34) = 4.426, p = 0.0196 Genotype: F (1,17) = 0.3441, p = 0.5652 Chamber: F (1.904, 32.36) = 34.15, p < 0.0001	Tukey [M1 vs O] WT-: p < 0.01 KO-2: p = 0.9355 Sidak [WT- vs KO-2] M1: p < 0.05 O: p = 0.2517
	Close interaction (Time spent for direct interaction with M1 vs O)		Two-way repeated measures ANOVA Interaction: F (1,17) = 5.034, p = 0.0385 Genotype: F (1,17) = 2.492, p = 0.1328 Subject: F (1,17) = 24.54, p = 0.0001	Sidak WT- [M1 vs O]: p <0.001 KO-2 [M1 vs O]: p = 0.1266 M1 [WT- vs KO-2]: p = 0.0238 O [WT- vs KO-2]: p = 0.9619
	Preference for novelty (Time spent in M2 vs M1 chamber)		Two-way repeated measures ANOVA Interaction: F (2,34) = 7.981, p = 0.0014 Genotype: F (1,17) = 0.5752, p = 0.4586 Chamber: F (1.767, 30.04) = 24.30, p < 0.0001	Tukey WT- [M2 vs M1]: p < 0.01 KO-2 [M2 vs M1]: p = 0.9505 Sidak M2 [WT- vs KO-2]: p < 0.01 M1 [WT- vs KO-2]: p = 0.1328
	Close interaction (Time spent for direct interaction with M2 vs M1)		Two-way repeated measures ANOVA Interaction: F (1,17) = 21.33, p = 0.0002 Genotype: F (1,17) = 12.65, p = 0.0024 Subject: F (1,17) = 51.46, p < 0.0001	Sidak WT- [M2 vs M1]: p < 0.0001 KO-2 [M1 vs M2]: p = 0.1550 M2 [WT- vs KO-2]: p < 0.0001 M1 [WT- vs KO-2]: p = 0.9902
Reactivity to social odor cues	Time spent sniffing odors (%)	WT-2 = 8, KO-2 = 7	Two-way repeated measures ANOVA Interaction: F (6,78) = 8.476, p < 0.0001 Genotype: F (1,13) = 15.56, p = 0.0017 Session: F (6,78) = 21.06, p < 0.0001	Sidak [WT- vs KO-2] 1st F odor: p < 0.0001 2nd F odor: p < 0.05 1st M odor: p < 0.0001
Repetitive grooming	Time spent grooming (%)	WT-2 = 10, KO-2 = 10	Unpaired t-test, p = 0.0446	-
USV	Number of syllables	WT-2 = 9, KO-2 = 10	Unpaired t-test, p = 0.0153	-
	Mean frequency (kHz)		Unpaired t-test, p = 0.0004	-
	Total syllable energy (dB)		Unpaired t-test, p = 0.0363	-
	Repertoire comparison		Kruskal-Wallis, p = 0.2071	Dunn [WT-1 vs WT-2] vs [WT- vs KO-2]: p = 0.2848 [WT- vs KO-1] vs [WT- vs KO-2]: p > 0.9999
Contextual fear conditioning	Time spent freezing (%)	WT-2 = 7, KO-2 = 13	Unpaired t-test, p = 0.2357	-
Pre-pulse inhibition	Prepulse inhibition (%)	WT-2 = 8, KO-2 = 8	Two-way repeated measures ANOVA Interaction: F (4,56) = 2.541, p = 0.0497 Genotype: F (1,14) = 14.62, p = 0.0019 Session: F (4,56) = 48.12, p < 0.0001	Sidak [WT- vs KO-2] 77 dB: p < 0.001 81 dB: p < 0.05 85 dB: p <0.01
Open field test	Distance traveled (timed)	WT-2 = 8, KO-2 = 9	Two-way repeated measures ANOVA Interaction: F (2,30) = 0.7296, p = 0.4905 Genotype: F (1,15) = 0.0126, p = 0.9122 Time: F (2,30) = 45.36, p < 0.0001	Sidak 10 mins: p = 0.9734 20 mins: p = 0.9622 30 mins: p = 0.9335
	Total distance traveled		Unpaired t-test, p=0.9122	-
	Time spent in zones (%)		Two-way repeated measures ANOVA Interaction: F (2,30) = 0.0154, p = 0.9848 Genotype: F (1,15) = 3.971, p = 0.0648 Zone: F (2,30) = 792.8, p < 0.0001	Sidak [WT- vs KO-2] Outer: p = 0.9995 Middle: p = 0.9998 Inner: p = 0.9974
Gait analysis	Stride length	WT-2 = 7, KO-2 = 8	Unpaired t-test, p = 0.4032	-
	Stride width		Unpaired t-test, p = 0.9354	-
Ladder walking test	Number of foot faults	WT-2 = 9, KO-2 = 11	Unpaired t-test, p = 0.9047	-
Rotarod	Time on rotarod	WT-2 = 8, KO-2 = 7	Two-way repeated measures ANOVA Interaction: F (4,52) = 1.936, p = 0.1184 Genotype: F (1,13) = 0.1227, p = 0.7317 Day: F (4,52) = 8.450, p < 0.0001	Sidak [WT- vs KO-2] Day 1: p = 0.7580 Day 2: p = 0.9846 Day 3: p > 0.9999 Day 4: p = 0.9699 Day 5: p = 0.9824
Touchscreen assay	PD Acquisition	WT-2 = 14, KO-2 = 10	Two-way repeated measures ANOVA	Tukey [WT- vs KO-2] Session 1: p = 0.5246

Pairwise discrimination task & reversal			Interaction: F (11, 242) = 0.9331, p = 0.5091 Genotype: F (1, 22) = 1.296, p = 0.2671 Session: F (4.897, 107.7) = 25.54, p < 0.0001	Session 2: p = 0.1173 Session 3: p < 0.01 Session 4: p = 0.5024 Session 5: p = 0.6574 Session 6: p = 0.7932 Session 7: p = 0.6303 Session 8: p = 0.8990 Session 9: p = 0.5218 Session 10: p = 0.4755 Session 11: p = 0.1362 Session 12: p = 0.2847
	Sessions to criteria	WT-2 = 14, KO-2 = 10	Two-way repeated measures ANOVA Interaction: F (1, 22) = 3.234, p = 0.0859 Genotype: F (1,22) = 3.109, p = 0.0918 Trial: F (1, 22) = 10.35, p < 0.01	Uncorrected Fisher's LSD [WT- vs KO-2] PD: p = 0.7190 PD-Rev: p < 0.05
Puzzle box	Latency time to goal	WT-2 = 12, KO-2 = 13	Two-way repeated measures ANOVA Interaction: F (8, 184) = 0.692, p = 0.6983 Genotype: F (1,23) =0.6232, p = 0.4379 Trial: F (5.420, 124.7) = 30.7, p < 0.0001	Sidak [WT- vs KO-2] T1 Baseline: p = 0.9806 T2 Underpass: p = 0.9992 T3 (STM) Underpass: p = 0.9998 T4 (LTM) Underpass: p > 0.9999 T5 Burrow: p = 0.9781 T6 (STM) Burrow: p = 0.8991 T7(LTM) Burrow: p > 0.9999 T8 Plug: p = 0.8660 T9 Plug (STM): p = 0.9990

Supplementary Table 6. Nuclei counts per cell type in *Ptchd1-as* KO, KO-1 and KO-2 snRNA-seq experiments.

Experiment Group	Cell Type	WT littermate group (nuclei initial)	Ptchd1-as mutation group (nuclei initial)	Down-sampled nuclei	Sampling Repetitions
KO-1	D1 MSN	1625	680	680	3
	D2 MSN	1591	604	604	3
	Ecc MSN	457	190	190	3
	Oligo	2001	341	341	6
	Astrocyte	198	112	112	2
	Poly	329	97	97	4
	Microglia	215	42	42	6
	IN	1079	331	331	4
	Glia_Union	2743	592	592	5
	Pseudo-bulk	7495	2397	2397	4
KO-2	D1 MSN	374	596	374	2
	D2 MSN	381	530	381	2
	Ecc MSN	105	88	88	2
	Oligo	656	198	198	4
	Astrocyte	61	94	61	2
	Poly	148	93	93	2
	Microglia	85	18	18	5
	IN	263	239	239	2
	Glia_Union	950	403	403	3
	Pseudo-bulk	2073	1856	1856	2
KO	D1 MSN	1999	748	748	5
	D2 MSN	1972	762	762	5
	Ecc MSN	562	176	176	6
	Oligo	2657	396	396	11
	Astrocyte	259	122	122	4
	Poly	477	186	186	4
	Microglia	300	36	36	12
	IN	1342	478	478	5
	Glia_Union	3693	806	806	7
	Pseudo-bulk	9568	3712	3712	5

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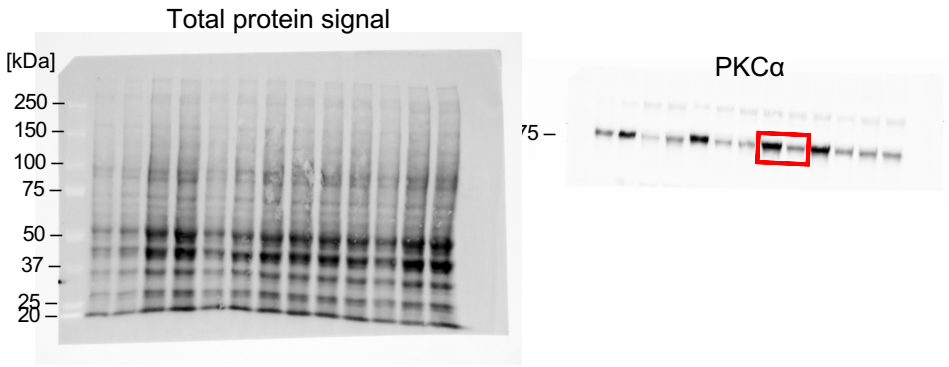
Supplementary Figure 1.

a

Striatum PKCα

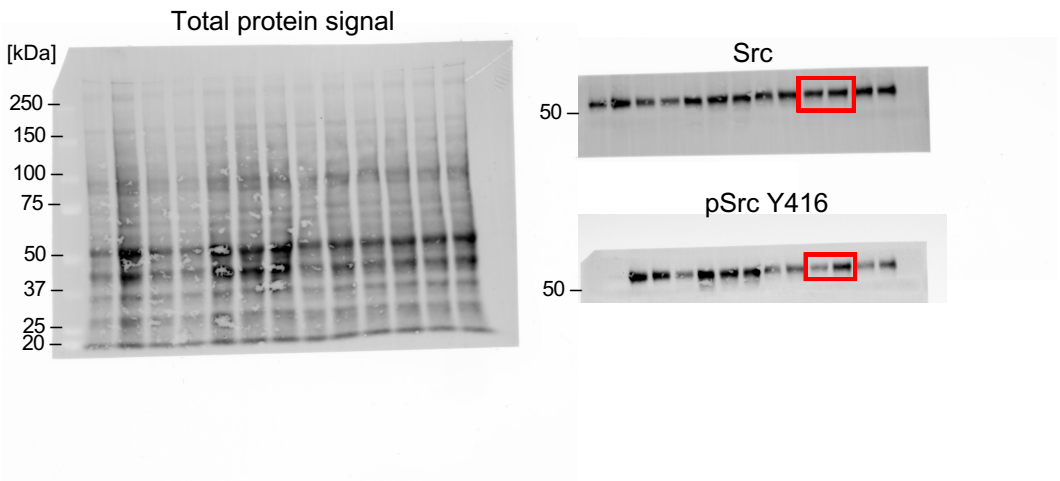
Well order	
1	SM
2	IC
3	WT-2
4	KO-2
5	WT-2
6	KO-2
7	WT-2
8	KO-2
9	WT-2
10	KO-2
11	WT-2
12	WT-2
13	KO-2
14	WT-2



b

Striatum Src

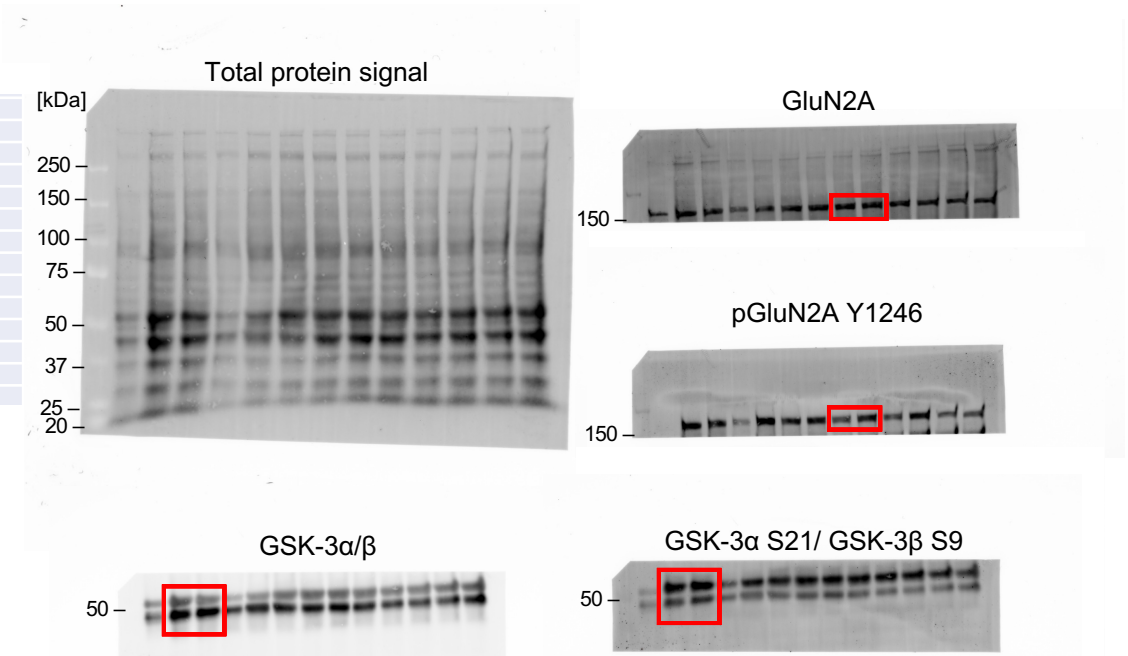
Well order	
1	SM
2	IC
3	WT-2
4	KO-2
5	WT-2
6	KO-2
7	WT-2
8	KO-2
9	WT-2
10	KO-2
11	WT-2
12	KO-2
13	WT-2
14	WT-2



c

Striatum GluN2A

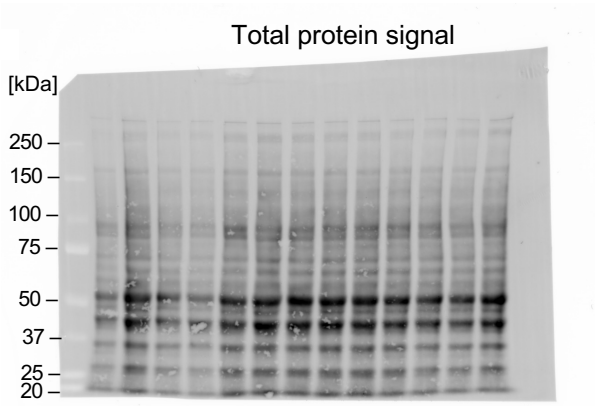
Well order	
1	SM
2	IC
3	WT-2
4	KO-2
5	WT-2
6	KO-2
7	WT-2
8	KO-2
9	WT-2
10	KO-2
11	WT-2
12	KO-2
13	WT-2
14	WT-2

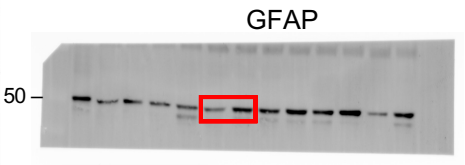


d

Striatum GFAP

Well order	
1	SM
2	IC
3	WT-2
4	KO-2
5	WT-2
6	KO-2
7	WT-2
8	KO-2
9	WT-2
10	KO-2
11	WT-2
12	KO-2
13	WT-2
14	WT-2

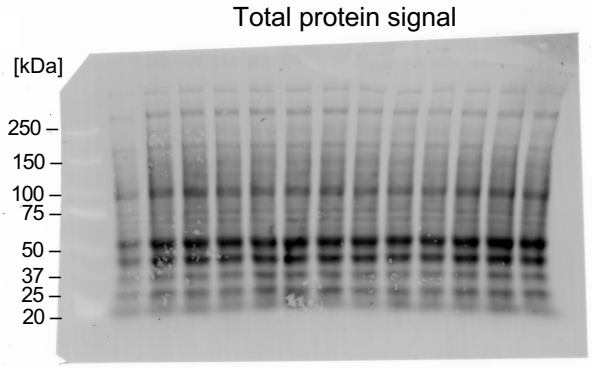


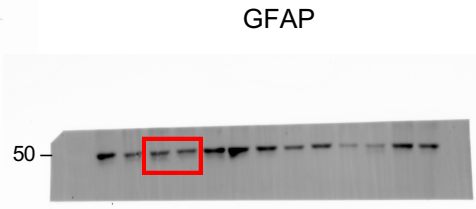


e

Cortex GFAP

Well order	
1	SM
2	IC
3	WT-2
4	KO-2
5	WT-2
6	KO-2
7	WT-2
8	KO-2
9	WT-2
10	KO-2
11	WT-2
12	KO-2
13	WT-2
14	WT-2



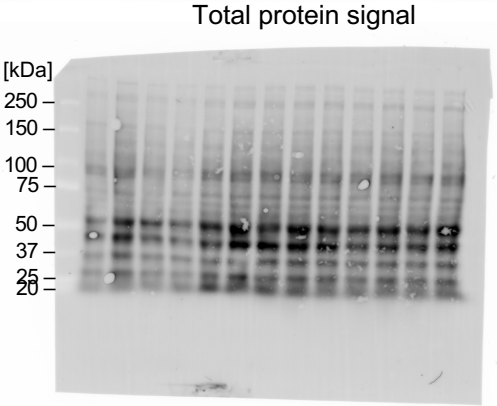


NOTE: Orientation flipped horizontal to change WT-2 and KO-2 arrangement for cortical GFAP blot.

f

Striatum FMRP

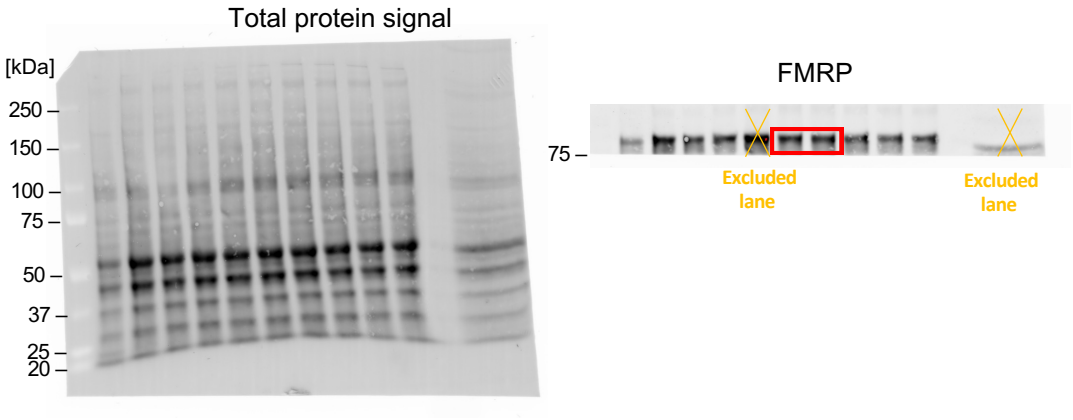
Well order	
1	SM
2	IC
3	WT-2
4	KO-2
5	WT-2
6	KO-2
7	WT-2
8	KO-2
9	WT-2
10	KO-2
11	WT-2
12	KO-2
13	WT-2
14	WT-2



g

Cortex FMRP

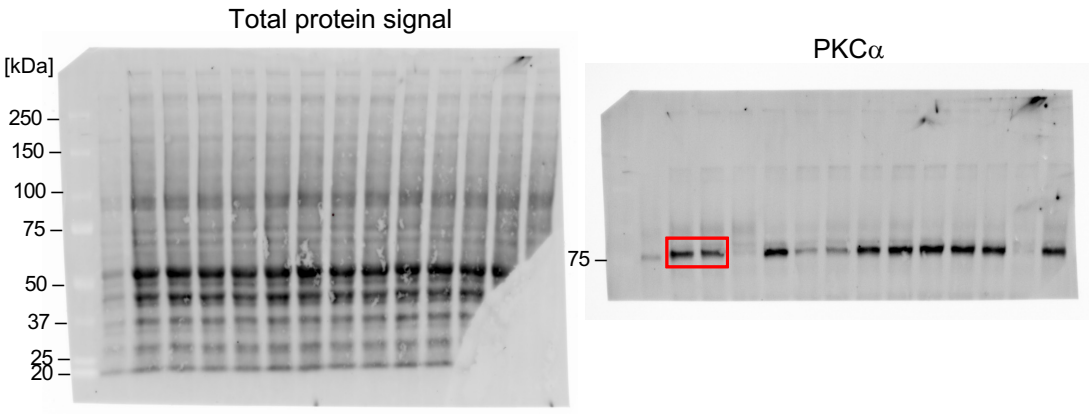
Well order	
1	SM
2	IC
3	WT-2
4	KO-2
5	WT-2
6	KO-2
7	WT-2
8	KO-2
9	WT-2
10	KO-2
11	WT-2



h

Cortex PKCα

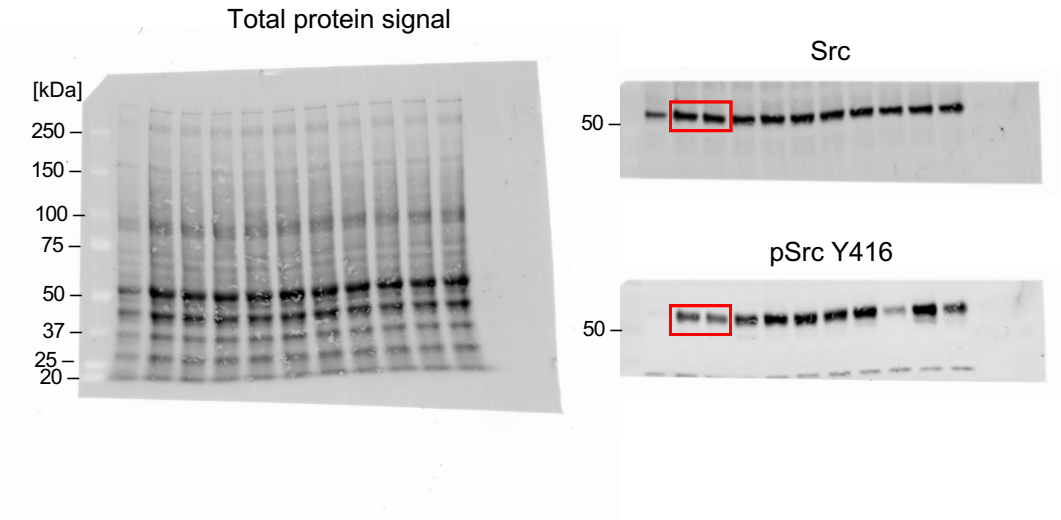
Well order	
1	SM
2	IC
3	WT-2
4	KO-2
5	WT-2
6	KO-2
7	WT-2
8	KO-2
9	WT-2
10	KO-2
11	WT-2
12	KO-2
13	WT-2
14	KO-2
15	WT-2



i

Cortex Src

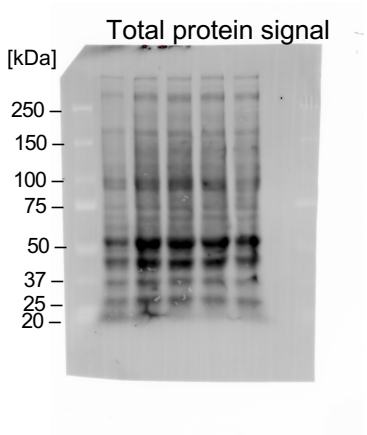
Well order	
1	SM
2	IC
3	WT-2
4	KO-2
5	WT-2
6	KO-2
7	WT-2
8	KO-2
9	WT-2
10	KO-2
11	WT-2
12	KO-2

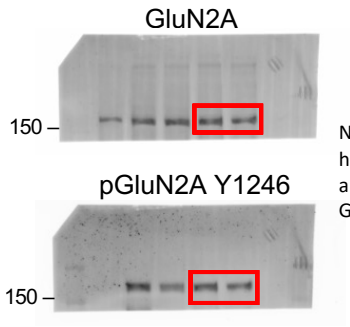


j

Cortex GluN2A:

Well order	
1	SM
2	IC
3	KO-2
4	WT-2
5	KO-2
6	WT-2



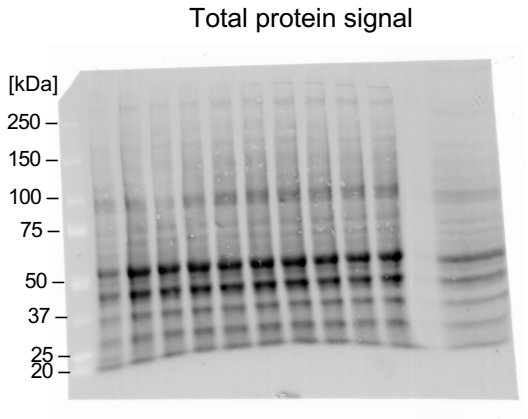


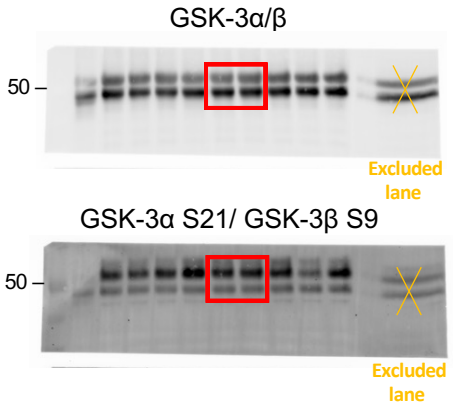
NOTE: Orientation flipped horizontal to change WT-2 and KO-2 arrangement for GluN2A & pGluN2A Y1246

k

Cortex GSK3α/β

Well order	
1	SM
2	IC
3	WT-2
4	KO-2
5	WT-2
6	KO-2
7	WT-2
8	KO-2
9	WT-2
10	KO-2
11	WT-2





Supplementary Figure 1. Complete western blot images showing well lane order (left table), total protein stain normalization signals across the whole blot (middle membrane), and primary antibody targets (right-side cut membranes) presented in Figure 4 (c-e) and Extended Data Figure 12 (d-k). Red square, region containing representative pairs of bands from each genotype selected for insertion into the respective figures described above. SM, size marker lane and IC, internal control.

Supplementary information methods

Supplementary Methods Table 1. Particpant selection and cohort descriptions for analysis of phenotype-genotype analysis at the *PTCHD1-PTCHD1-AS* risk locus.

Cohort	**Subjects (M:F)**	**Primary Inclusion Criteria**
MSSNG [1]	5,289 (4.5:1)	ASD diagnosis
Simons Simplex Collection (SSC[2]; Sanders *et al.*, 2011[3])	1,124 (6.2:1)	ASD diagnosis
Chaudhry *et al.*, 2015[4]	18 (17:1)	Presence of developmental disorder
DECIPHER[5]	35,541 (N/A)	Presence of developmental disorder
Gambin *et al.*, 2018[6]	63,127 (N/A)	Presence of developmental disorder
Lineagen, Inc.	19,591 (2.4:1)	Presence of developmental disorder
Control individuals (MSSNG[1] and SSC[2])	11,909 (0.9:1)	Non-psychiatric (adult)

Supplementary Methods Table 2. Taqman probes used in droplet digital PCR.

Gene name	**Supplier**	**Catalogue#**	**Forward primer (5' to 3')**	**Taqman probe**	**Reverse primer (5' to 3')**
ptchd1-as (Gm15155 exon2-3)	Thermo Fisher (Custom)	Gm15155 Ex2-3 (15155-23 CD9HJCX, Fam)	GGAGCTGACAG GAAAGGAAGTG	TTGGTTTT GATTCCAG AAAT	GGGAGGACGCTGGTT GGT
Ptchd1-as (Gm15155 exon1-2)	Thermo Fisher (Custom)	Gm15155 Ex1-2.2 CDXGRER	CACTTCCCCTCG GGAGATTT	ACGCACA GTGATCCT	CGAACTCAGCAGACA GTCCTATTG
Ptchd1-as (Gm15155 exon5-6)	Thermo Fisher (Custom)	15155-65_CDAAAPN	TCCTCCCCTGAG GTTTCTTTG	CCTTAGCT GTCTTGG AAC	TCAACACCAGTTTCGT CTACAGAGT
Ptchd1-as (XR.1-exon1-2)	Thermo Fisher (Custom)	XR.1-1-2_CDAAAR6	TGACATTAGTTG AAGAAGAAGCA TCTAAC	TCACAGA CACTTGAT GTGC	CATCTGTTCCACCTGT CACTCTCT

Ptchd1-as (XR.3 - exon2-3)	Thermo Fisher (Custom)	XR.3-2-3_CD7DPUH	CAATAGGACTGTCTGCTGAGTTCGT	CTGACAGGAAAGGAAG	ACAAGTAGATCTGGAATCAAAACCAA
Ptchd1-as (XR.3 -exon 3-4)	Thermo Fisher (Custom)	XR.3-3-4_CD9HJEF	AAGGAATACTCTGGTATATGAATTGATGAA	TAAGTGGAAAATGGAAGAAA	GGAGGACGCTGGTTGGTTT
Ptchd1-as (XR.1 - exon2-3)	Thermo Fisher (Custom)	XR.1-2-3_CDCE4C3	GGGATGAATCGAAAATATGTCACA	TCCTAAGATCGCGTGGAGA	GGTCACCTTGGGCACGAA
Ptchd1 (exon1-3)	Thermo Fisher (Custom)	Ptchd1 1_3 CD47WPK(Fam)	ACGGCCGGGTCATTGTC	CTCCTACCAGAAAGC	GTCCCGTATAATCCATGACCTTT
Ptchd1-as (Gm15155 exon3-4)	Thermo Fisher	Mm1164232_m1			
Ptchd1-as (Gm15155 exon4-5)	Thermo Fisher	Mm01164233_m1			
Ptchd1 (exon1-2)	IDT	Mm.PT.58.42663566			
Ptchd1 (exon2-3)	Thermo Fisher	Mm01138948_m1			
Tfrc	IDT	Mm.PT.58.9333140			

Supplementary Methods Table 3. Antibodies used for western blotting.

Antibody Target	**Ab Dilution**	**Catalogue Number**	**Company**	**Species**
GFAP	1:1000	75-240	Neuromab	Ms
FMRP	1:1000	4317	Cell Signaling Technology	Rb
GluN2A	1:500	MA5-27692	Invitrogen	Ms
pGluN2A Y1426	1:500	4206	Cell Signaling Technology	Rb

GluN2B	1:1000	66565-1	Cell Signaling Technology	Ms
pGluN2B Y1472	1:1000	4208	Cell Signaling Technology	Rb
PKC alpha	1:1000	5578-MSM2-P0	Thermo Fisher Scientific	Ms
Src	1:500	2110	Cell Signaling Technology	Ms
pSrc Y416	1:500	6943	Cell Signaling Technology	Rb
pGSK-3α/β S21/9	1:1000	9331	Cell Signaling Technology	Rb
GSK-3α/β	1:2000	44-610	Invitrogen	Ms
StarBright Blue 700 Anti-Rb	1:3000	12004161	BioRad	Goat
StarBright Blue 520 Anti-Ms	1:3000	12005866	BioRad	Goat
StarBright Blue 700 Anti-Ms	1:3000	12004158	BioRad	Goat
StarBright Blue 520 Anti-Rb	1:3000	12005869	BioRad	Goat

References

1. Trost, B. *et al.* Genomic architecture of autism from comprehensive whole-genome sequence annotation. *Cell* **185**, 4409-4427.e18 (2022).
2. Fischbach, G. D. & Lord, C. The Simons Simplex Collection: A Resource for Identification of Autism Genetic Risk Factors. *Neuron* **68**, 192–195 (2010).
3. Sanders, S. *et al.* Multiple recurrent de novo CNVs, including duplications of the 7q11.23 Williams syndrome region, are strongly associated with autism. *Neuron* **70**, 863–885 (2011).
4. Chaudhry, A. *et al.* Phenotypic spectrum associated with*PTCHD1*deletions and truncating mutations includes intellectual disability and autism spectrum disorder. *Clinical Genetics* **88**, 224–233 (2014).
5. DECIPHER v11.27: Mapping the clinical genome. *www.deciphergenomics.org* https://www.deciphergenomics.org/.

6. Gambin, T. *et al.* Identification of novel candidate disease genes from de novo exonic copy number variants. *Genome Medicine* **9**, (2017).