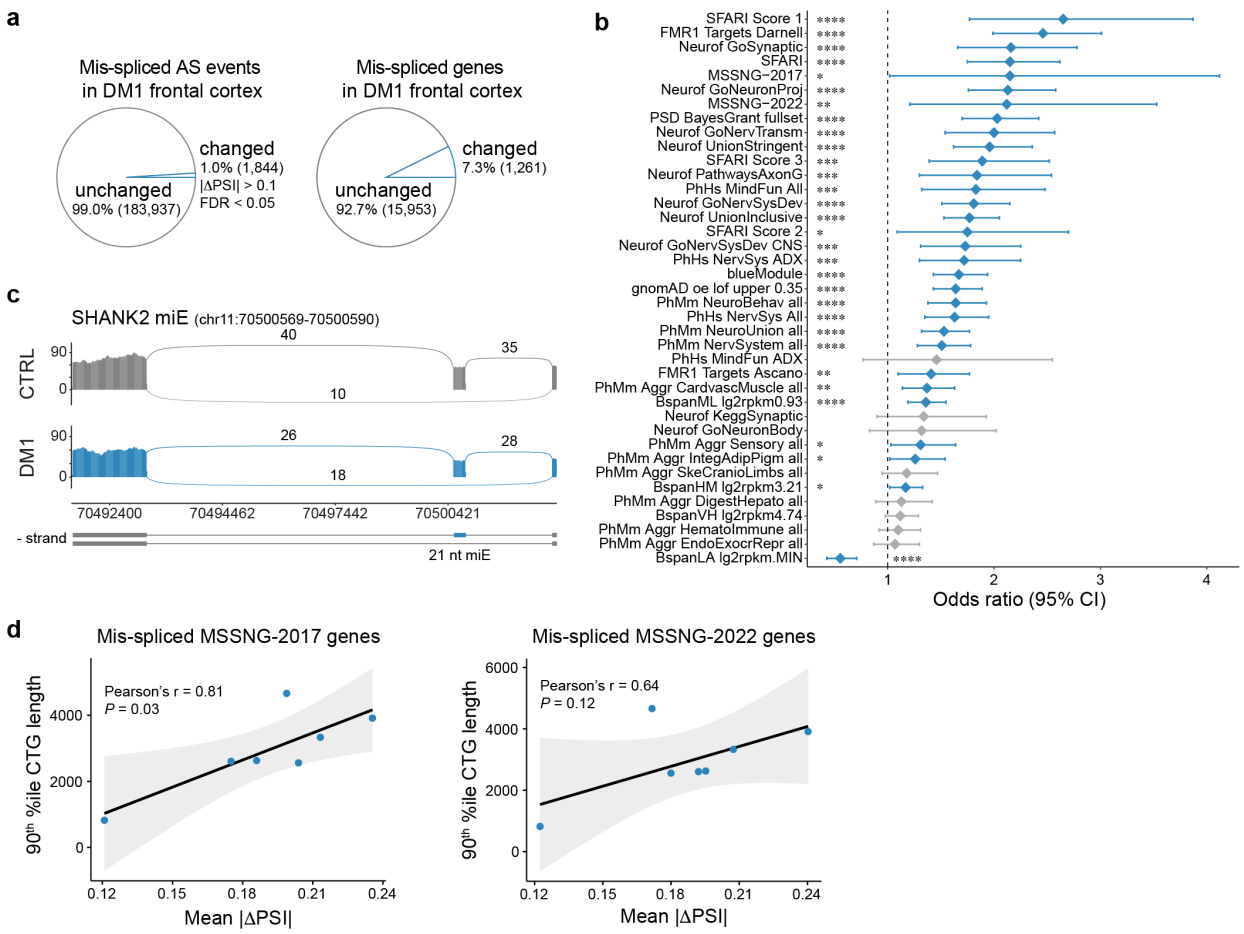
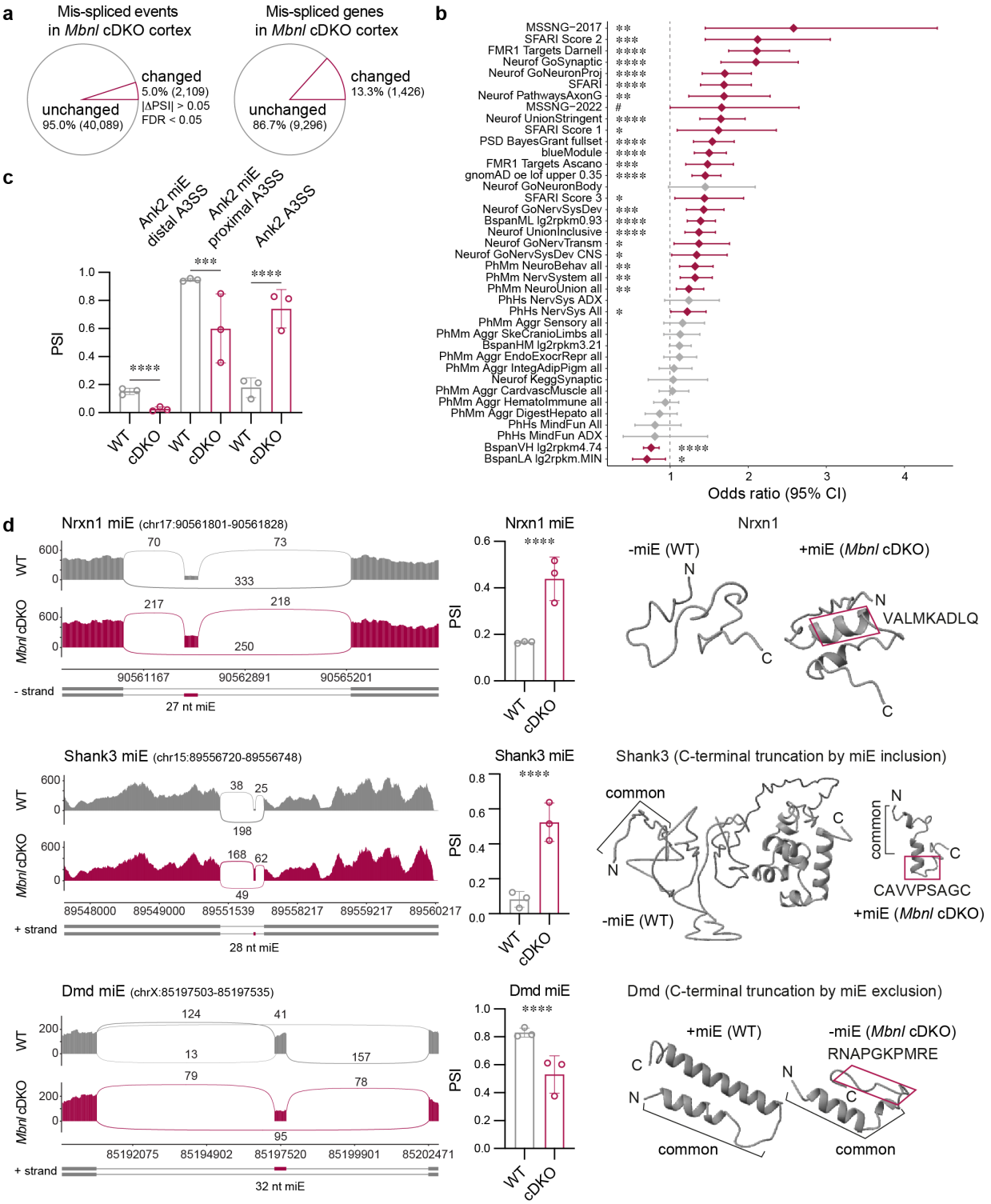
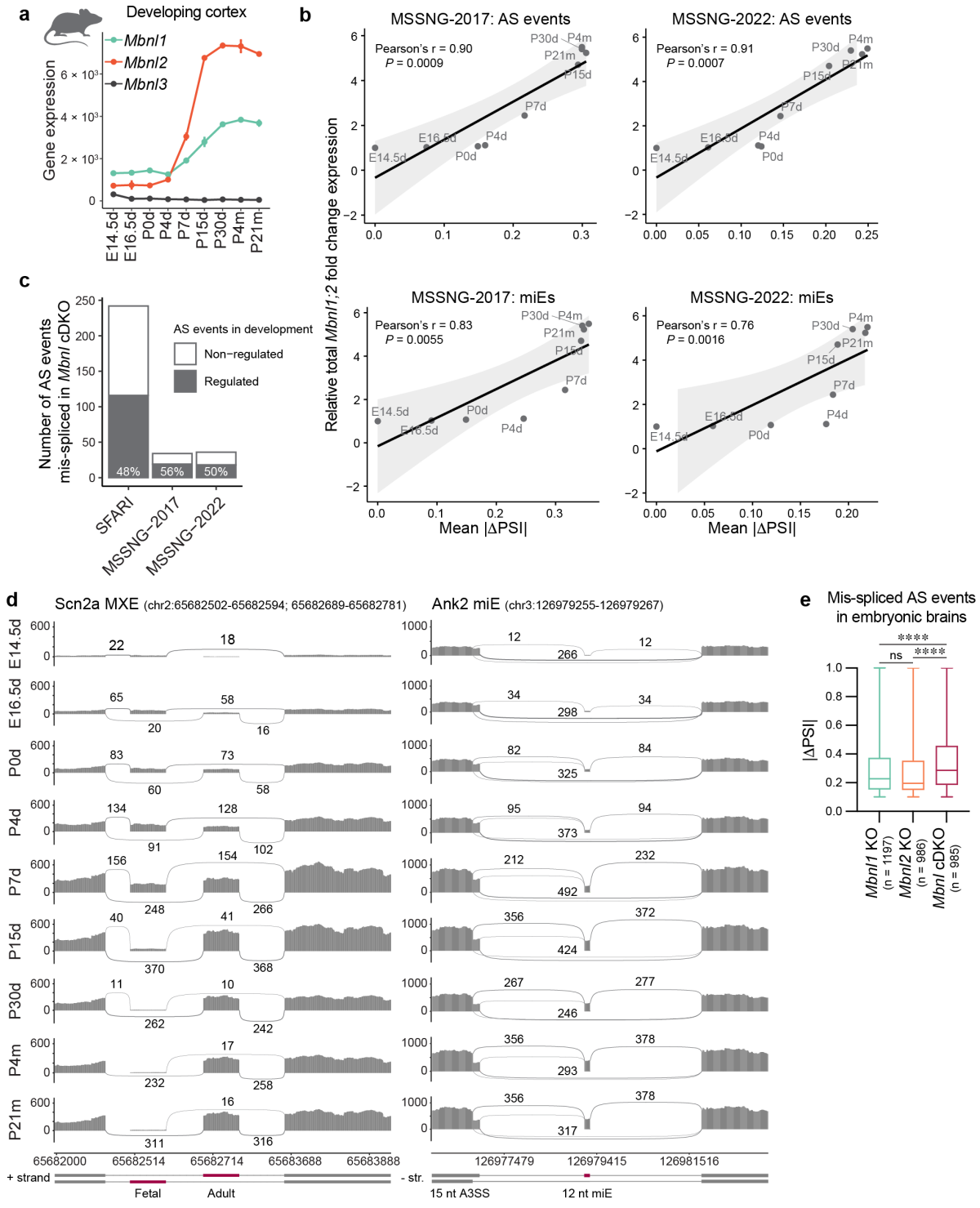


Supplementary Fig. 1 | **Mis-splicing in DM1 prefrontal cortex. a**, Differential AS analysis in DM1 (N = 21: N_{XX} = 12, N_{XY} = 9; sampling age: median = 56 years (y), min = 39y, max = 77y; unknown ASD status) compared to age-matched control (CTRL; N = 8: N_{XX} = 4, N_{XY} = 4; sampling age: median = 63y, min = 48y, max = 71y) prefrontal cortex (Brodmann area 10; BA10) RNA-seq samples. Pie charts represent the percentage of mis-spliced AS events (left) and genes (right) in the DM1 BA10. **b**, ASD-risk gene-set enrichment analysis for mis-spliced genes in DM1 BA10. Points represent the OR and error bars represent the 95% CI. The vertical dashed line represents OR = 1. * FDR < 0.05, ** FDR < 0.01, *** FDR < 0.001, and **** FDR < 0.0001. **c**, Sashimi plots of DM1 (N = 8) and CTRL (N = 8) BA10 RNA-seq samples for SHANK2 miE. **d**, Correlation between estimated 90th percentile of CTG repeat lengths (N = 7)[38] and mean |ΔPSI| values for mis-spliced MSSNG-2017 and MSSNG-2022 genes in DM1 BA10.



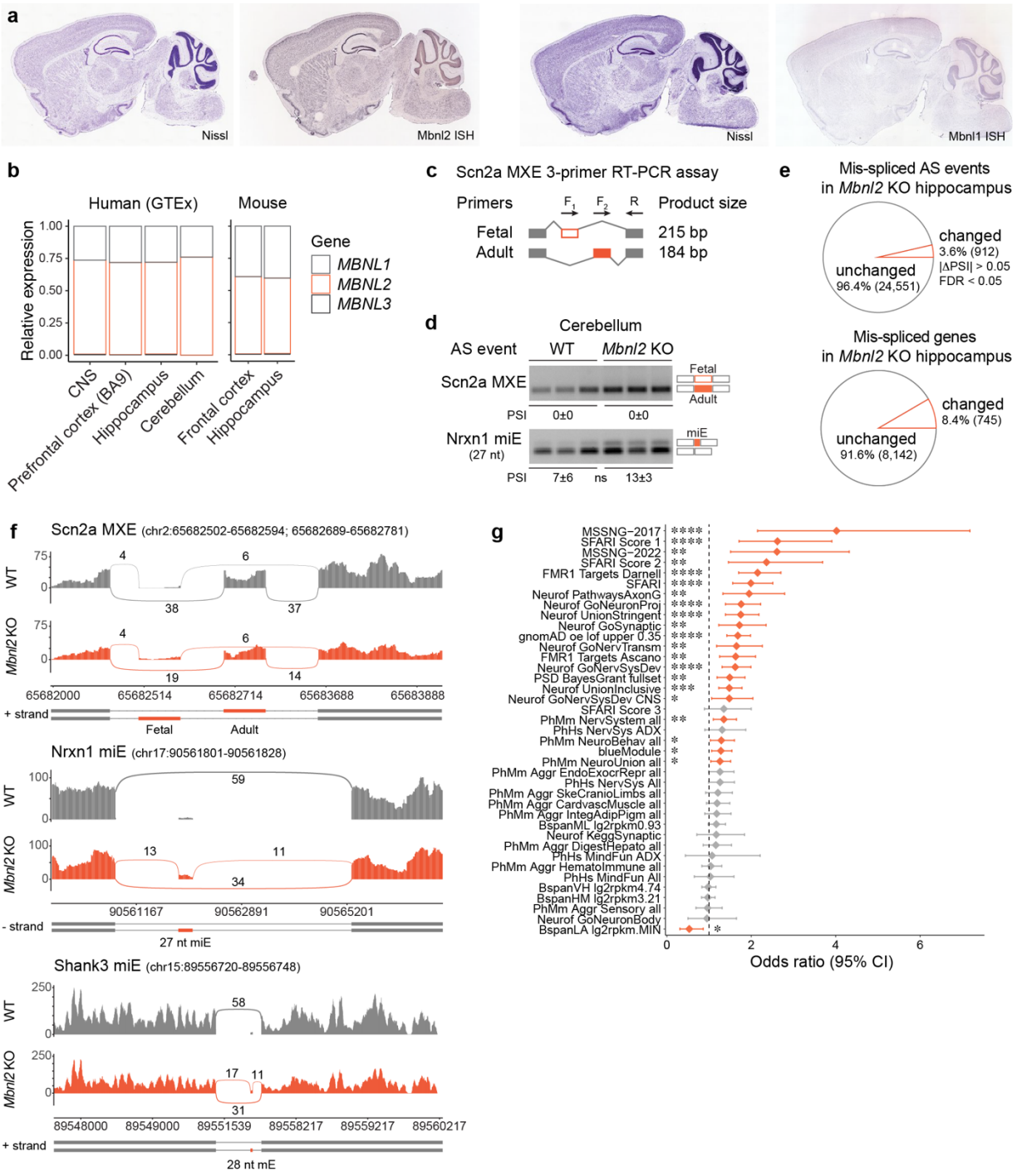
Supplementary Fig. 2 | MiE mis-splicing in *Mbnl* cDKO frontal cortex. a, Differential AS analysis in *Mbnl* cDKO (N_{XY} = 3) compared to littermate WT control (N_{XY} = 3) frontal cortex RNA-seq samples. Pie charts represent the percentage of mis-spliced and AS events (left) and genes

(right) in the *Mbnl* cDKO frontal cortex. **b**, ASD-risk gene-set enrichment analysis for mis-spliced genes in *Mbnl* cDKO frontal cortex. Points represent the OR and error bars represent the 95% CI. The vertical dashed line represents OR = 1. **c**, The quantification of coordinate Ank2 miE and A3SS mis-splicing. The bar graphs show the mean PSI ± SD. **d**, Examples of miE mis-splicing in ASD-risk genes. Sashimi plots of *Mbnl* cDKO and WT RNA-seq samples for Nrxn1 miE, Shank3 miE, and Dmd miE. The bar graphs show the mean PSI ± SD. Modeled structures of mouse protein isoform regions containing a sequence encoded by miE (magenta box). **b,c,d**, # FDR = 0.056, * FDR < 0.05, ** FDR < 0.01, *** FDR < 0.001, **** FDR < 0.0001.



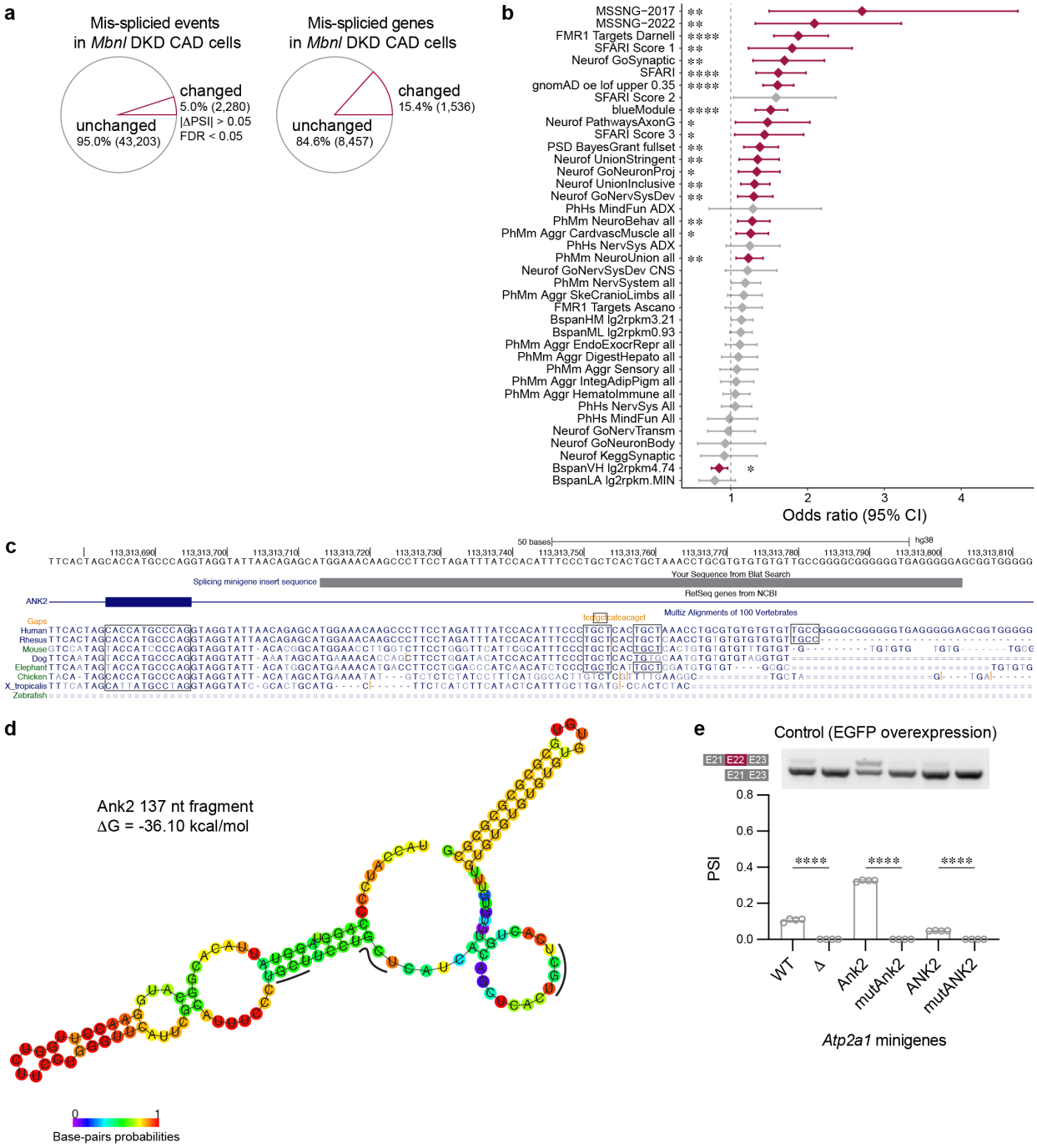
Supplementary Fig. 3 | MBNL-mediated splicing transitions in ASD-risk genes during brain development. **a**, *Mbnl1*, *Mbnl2* and *Mbnl3* gene expression levels during mouse cortex development. Points connected by lines show mean expression ± SD. **b**, Correlations between

Mbnl gene expression and mean |ΔPSI | values for MBNL-sensitive splicing changes in MSSNG-2017 and MSSNG-2022 genes in developing WT mouse cortex. MBNL-sensitive AS events (top) and miEs only (bottom) were selected based on differential AS analysis in the *Mbnl* cDKO cortex. The Pearson correlation coefficient (r) and *P* values are shown. **c**, Bar graph shows the proportion of MBNL-sensitive AS events differentially spliced (gray) in the developing cortex (E14.5d, E16.5d vs P4m, P21m). **d**, Sashimi plots show Scn2a MXE and Ank2 miE splicing transitions during WT cortical development. RNA-seq was performed (n = 2 for each time point) at embryonic days: E14.5d, E16.5d, postnatal days: P0d, P4d, P7d, P15d, P30d, and postnatal moths: P4m, P21m. **e**, RNA mis-splicing in *Mbnl1* KO (N = 2), *Mbnl2* KO (N = 2) and *Mbnl* cDKO (N = 2) mouse E18.5 cortical neuron RNA-seq samples. The box plot shows the lower (25^{th} %ile), middle (median, 50^{th} %ile) and upper (75^{th} %ile) quartiles. Whiskers show minimum and maximum. Number of mis-spliced AS events are provided as n value. Statistical differences were determined by Kruskal-Wallis test followed by Dunn's multiple comparison test: ns $P = 0.11$, and **** $P < 0.0001$.



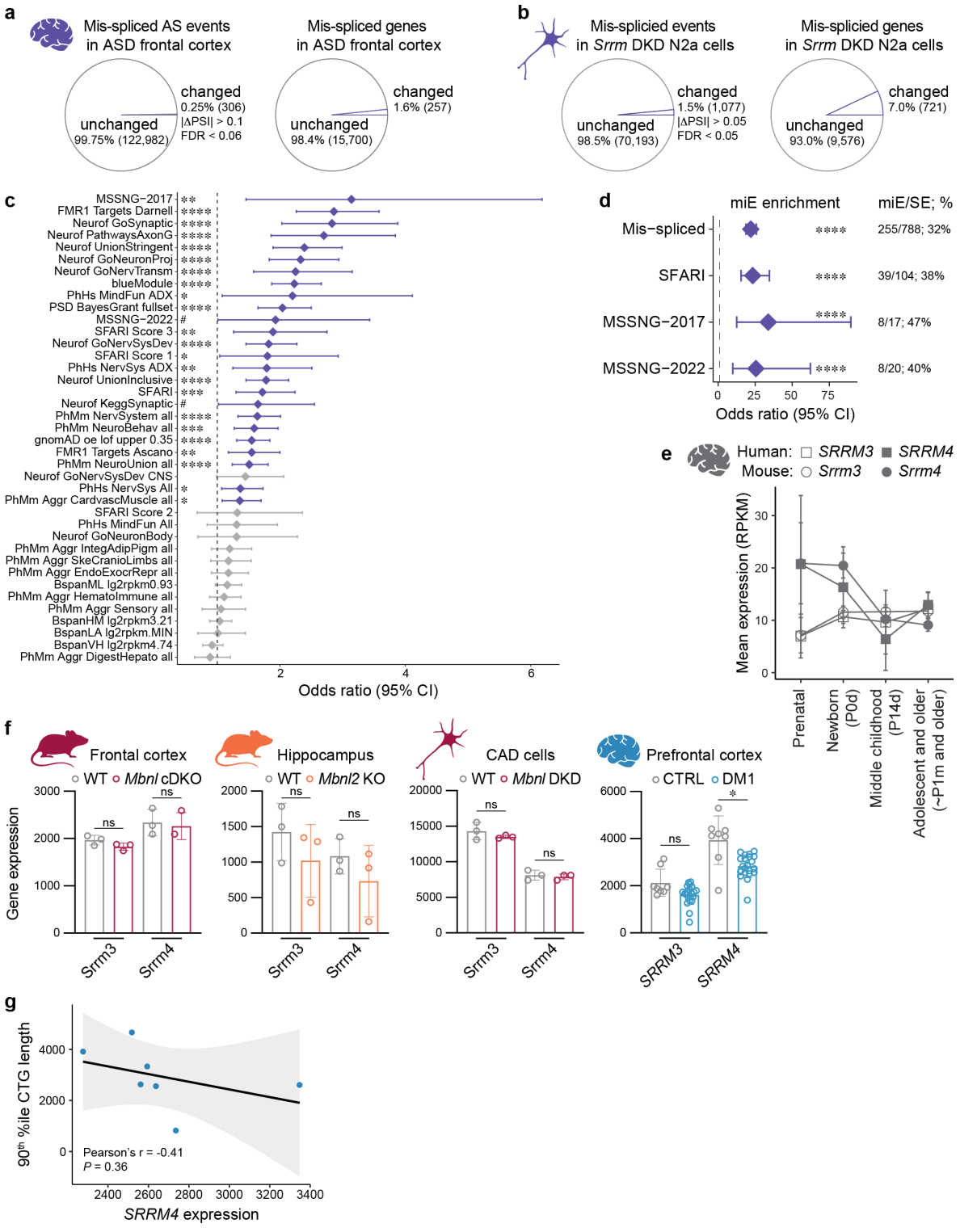
Supplementary Fig. 4 | Mis-splicing in the mouse *Mbnl2* knockout brain. a, Nissl from the Allen Mouse Brain Atlas at the same slice position as Mbnl1 and Mbnl2 RNA *in situ* hybridization (ISH) results. Nissl staining labels Nissl bodies and serves as a reference for the ISH data. For ISH-specific digoxigenin (DIG) tagged RNA probes were used to label cells expressing Mbnl1

(mouse.brain-map.org/gene/show/36037) and Mbnl2 (mouse.brain-map.org/gene/show/69724) transcripts[75]. **b**, Relative *MBNL1*, *MBNL2* and *MBNL3* expression in multiple human and mouse brain regions. CNS - central nervous system. **c**, Schematic of the 3-primer Scn2a RT-PCR assay. **d**, Representative RT-PCR assay gels of Scn2a MXE and Nrxn1 miE in *Mbnl2* KO (N = 5) and littermate WT (N = 3) cerebellum samples. Mean PSI ± SD are shown below the gels. The lack of significant differences was determined by an unpaired two-tailed t-test: ns $P \geq 0.05$. **e**, Differential AS analysis in *Mbnl2* KO (N_{XX} = 3) compared to littermate WT control (N_{XX} = 3) hippocampus RNA-seq samples. The bar graph shows the number and percentage of significantly mis-spliced AS event types ($|\Delta PSI| > 0.1$, FDR < 0.05). Pie charts represent the percentage of mis-spliced and AS events (left) and genes (right) in the *Mbnl2* KO. **f**, Sashimi plots of *Mbnl2* KO and WT hippocampus RNA-seq samples for Scn2a MXE, Nrxn1 miE, Shank3 miE. **g**, ASD-risk gene-set enrichment analysis for mis-spliced genes in *Mbnl2* KO hippocampus. Points represent the OR and error bars represent the 95% CI. The vertical dashed line represents OR = 1. * FDR < 0.05, ** FDR < 0.01, *** FDR < 0.001, **** FDR < 0.0001.



Supplementary Fig. 5 | Mis-splicing in the *Mbnl1;2* double knockdown CAD cells. **a**, Differential AS analysis in *Mbnl* DKD (N = 3) compared to control (N = 3) CAD RNA-seq samples. Pie charts represent the percentage of mis-spliced and AS events (left) and genes (right) in the *Mbnl* DKD CAD cells. **b**, ASD-risk gene-set enrichment analysis for mis-spliced genes in

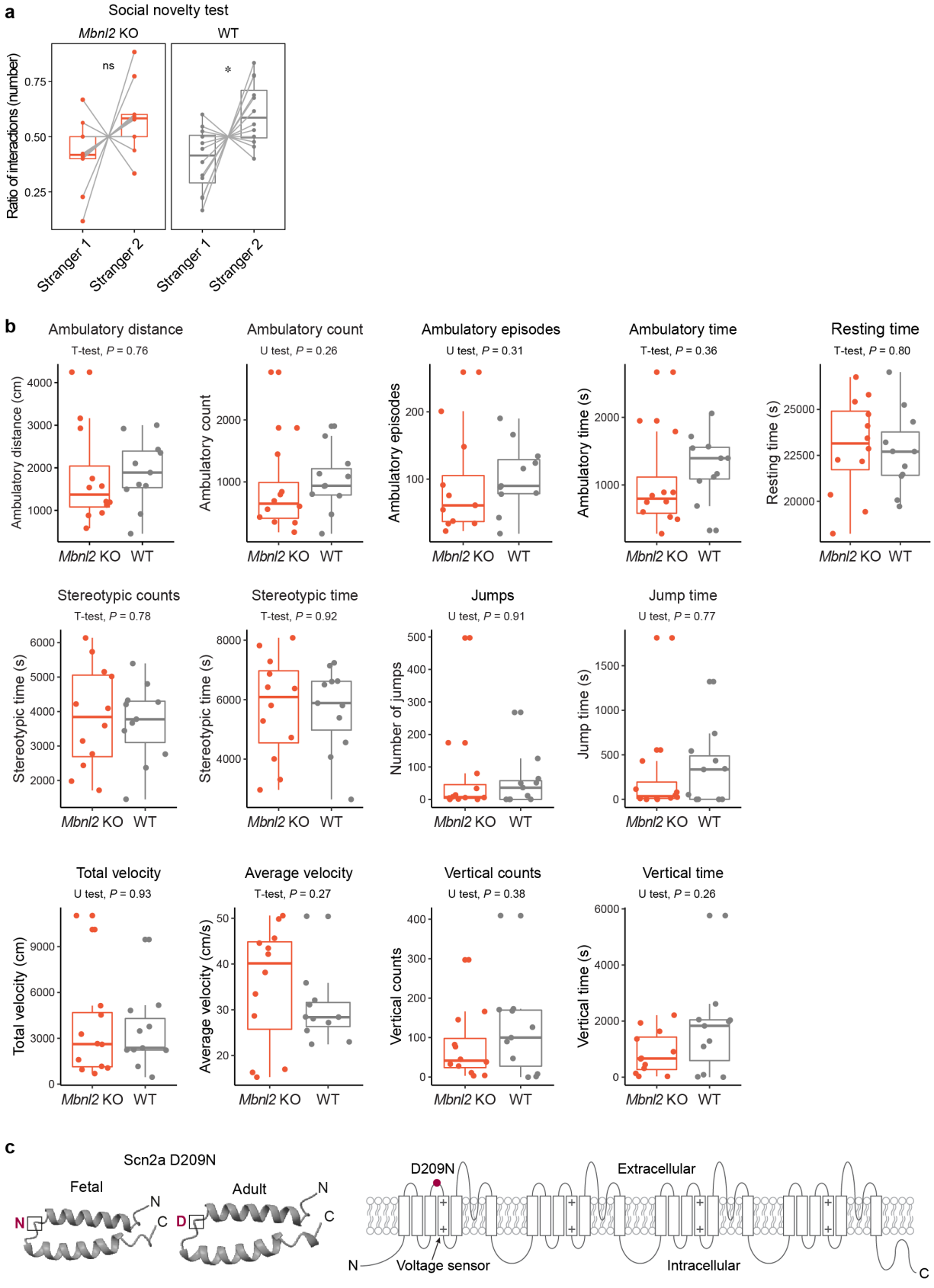
Mbnl DKD cells. Points represent the OR and error bars represent the 95% CI. The vertical dashed line represents OR = 1. * FDR < 0.05, ** FDR < 0.01, **** FDR < 0.0001. **c**, UCSC Genome browser view of human *ANK2* illustrates the conservation of miE 12 bp and predicted intronic MBNL-binding motifs across multiple species. **d**, The proposed centroid secondary structure for the 137 nt fragment of Ank2. It contains miE and downstream intron with indicated (black line) three potential MBNL-binding motifs and a $(TG)_n(CG)_n$ tandem repeat sequences. The optimal thermodynamic stability of the structure is expressed in Gibbs free energy (ΔG) in kcal/mol for the reaction at 37°C using RNAfold. The color annotation represents the pair probabilities of the nucleotides while for unpaired regions, the color denotes the probability of being unpaired. **e**, *Atp2a1*-derived minigenes splicing profiles in HeLa cells transfected with EGFP expressing vector (N = 4). The bar graph shows the mean Atp2a1 E22 PSI ± SD. Significant differences were determined by unpaired t-test: **** $P < 0.0001$.



Supplementary Fig. 6 | Mis-splicing in the *Srrm3;4* double knockdown Neuro2a cells.

a, Differential AS analysis in ASD (N = 10: N_{XX} = 1, N_{XY} = 9; sampling age: median = 51y, min =

38y, max = 67y; ASD confirmed by the Autism Diagnostic Interview-Revised (ADI-R; N = 8) or supported by records) compared to age-matched CTRL (N = 10: N_{XX} = 2, N_{XY} = 8; sampling age: median = 50y, min = 41y, max = 60y) frontal cortex (BA9) RNA-seq samples. Pie charts represent the percentage of mis-spliced and unchanged AS events (left) and genes (right) in the ASD frontal cortex. **b**, Differential AS analysis in *Srrm* DKD (N = 2) compared to control (N = 2) N2a RNA-seq samples. Pie charts represent the percentage of mis-spliced and AS events (left) and genes (right) in the *Srrm* DKD N2a cells. **c**, ASD-risk gene-set enrichment analysis for mis-spliced genes in *Srrm* DKD N2a cells. **d**, MiE enrichment analysis for SFARI, MSSNG-2017 and MSSNG-2022 mis-spliced SE events. **c,d**, Points represent the OR and error bars represent the 95% CI. The vertical dashed line represents OR = 1. * FDR < 0.05, ** FDR < 0.01, *** FDR < 0.001, **** FDR < 0.0001. **e**, *SRRM3* and *SRRM4* gene expression levels in developing human and mouse brains. Points show mean expression ± SD. **f**, SRRM3 and SRRM4 gene expression levels in *Mbnl* DKO frontal cortex, *Mbnl2* KO hippocampus, *Mbnl* DKD CAD cells, and DM1 prefrontal cortex; ns P_{adj} > 0.05, * P_{adj} = 0.01. **g**, The lack of correlation between estimated 90th percentile of CTG repeat lengths (N = 7)[38] and *SRRM4* gene expression in DM1 BA10 RNA-seq.


a
Social novelty test
Mbnl2 KO
WT
ns
*
Ratio of interactions (number)
0.75
0.50
0.25
Stranger 1
Stranger 2
Stranger 1
Stranger 2
b
Ambulatory distance
T-test, P = 0.76
Ambulatory distance (cm)
Ambulatory count
U test, P = 0.26
Ambulatory count
Ambulatory episodes
U test, P = 0.31
Ambulatory episodes
Ambulatory time
T-test, P = 0.36
Ambulatory time (s)
Resting time
T-test, P = 0.80
Resting time (s)
Mbnl2 KO WT
Stereotypic counts
T-test, P = 0.78
Stereotypic time (s)
Stereotypic time
T-test, P = 0.92
Stereotypic time (s)
Jumps
U test, P = 0.91
Number of jumps
Jump time
U test, P = 0.77
Jump time (s)
Total velocity
U test, P = 0.93
Total velocity (cm)
Average velocity
T-test, P = 0.27
Average velocity (cm/s)
Vertical counts
U test, P = 0.38
Vertical counts
Vertical time
U test, P = 0.26
Vertical time (s)
c
Scn2a D209N
Fetal
Adult
N
C
D
D209N
Extracellular
Voltage sensor
Intracellular

Supplementary Fig. 7 | **Lack of a locomotor deficit in *Mbnl2* knockout mice. a**, The ratio of interactions with novel animal (Stranger 2) and familiar animal (Stranger 1) during the social novelty test. The box plot shows the lower (25^{th} %ile), middle (median, 50^{th} %ile) and upper (75^{th} %ile) quartiles. Whiskers show minimum and maximum. Paired dots represent all sample data points, including outliers. WT mice (N = 12: N_{XX} = 9, N_{XY} = 3), *Mbnl2* KO (N = 12: N_{XX} = 9, N_{XY} = 3). Statistical differences were determined by the paired t-test: ns P = 0.078 (t = 1.941, df = 11), * P = 0.025 (t = 2.594, df = 11). **b**, Several parameters were measured during the 25 min (5-30 min) interval of the open field test. Box plots show lower (25^{th} %ile), middle (median, 50^{th} %ile) and upper (75^{th} %ile) quartiles. Whiskers show minimum and maximum, and dots represent outliers. WT mice (N = 12: N_{XX} = 8, N_{XY} = 3), *Mbnl2* KO (N = 12: N_{XX} = 9, N_{XY} = 3). Statistical differences were determined by the parametric t-test or nonparametric Mann-Whitney U test, and P values are depicted in the figure. **c**, Modeled structures of SCN2A protein fragments containing a D209N change within the loop sequence, and the $Na_V1.2$ transmembrane topology. The location of D209N is marked by the magenta dot.

Status	Age	Sex	CTG length (90 %ile)	Source
Myotonic dystrophy type 1	48	Female	2557.92	
Myotonic dystrophy type 1	41	Male	N/A	
Myotonic dystrophy type 1	56	Male	3330.64	
Myotonic dystrophy type 1	66	Female	N/A	
Myotonic dystrophy type 1	77	Female	822.267	
Myotonic dystrophy type 1	70	Male	N/A	
Myotonic dystrophy type 1	39	Male	4661.35	
Myotonic dystrophy type 1	59	Female	N/A	
Myotonic dystrophy type 1	51	Female	N/A	
Myotonic dystrophy type 1	59	Female	N/A	
Myotonic dystrophy type 1	65	Female	3910.92	
Myotonic dystrophy type 1	50	Male	N/A	
Myotonic dystrophy type 1	41	Female	N/A	
Myotonic dystrophy type 1	65	Male	N/A	
Myotonic dystrophy type 1	60	Male	2605.47	Otero *et al*., 2021 Cell Reports
Myotonic dystrophy type 1	44	Female	2627.01	
Myotonic dystrophy type 1	50	Female	N/A	
Myotonic dystrophy type 1	73	Female	N/A	
Myotonic dystrophy type 1	58	Female	N/A	
Myotonic dystrophy type 1	41	Male	N/A	
Myotonic dystrophy type 1	47	Male	N/A	
Control	48	Male		
Control	60	Male		
Control	52	Female		
Control	60	Female		
Control	65	Male		
Control	67	Male		
Control	65	Female		
Control	71	Female		
Autism - confirmed by ADI-R	38	Male		
Autism - confirmed by ADI-R	39	Male		
Autism - confirmed by ADI-R	52	Female		
Autism - confirmed by ADI-R	60	Male		
Autism - confirmed by ADI-R	51	Male		PsychENCODE
Autism - confirmed by ADI-R	54	Male		
Autism - suspected; diagnosis made in childhood	46	Male		
Autism - confirmed by ADI-R	46	Male		
Autism - supported by records	67	Male		

Autism - confirmed by ADI-R	53	Male
Control	44	Male
Control	41	Male
Control	60	Male
Control	57	Male
Control	56	Male
Control	52	Female
Control	53	Male
Control	44	Male
Control	41	Female
Control	50	Male

Supplementary Table 1 | Human subjects.

Gene set ID	Gene set name	No. of genes	Source
Neurof_PathwaysAxonG	Axon guidance pathways	388	union of these pathway-based gene-sets: {REACT: NCAM signaling for neurite out-growth, REACT: Axon guidance, NCI:NETRIN_PATHWAY, NCI:REELINPATHWAY, KEGG:04360 Axon guidance}
Neurof_KeggSynaptic	KEGG synaptic pathways	407	union of thse KEGG pathway-based gene-sets: {KEGG:04725 Cholinergic synapse, KEGG:04724 Glutamatergic synapse, KEGG:04728 Dopaminergic synapse, KEGG:04727 GABAergic synapse, KEGG:04726 Serotonergic synapse, KEGG:04721 Synaptic vesicle cycle, KEGG:04723 Retrograde endocannabinoid signaling, KEGG:04720 Long-term potentiation, KEGG:04730 Long-term depression}
Neurof_GoNeuronBody	GO neuron body	309	GO:0043025 neuronal cell body
Neurof_GoSynaptic	GO synapsis	622	union of these GO-based gene-sets: {GO:0045202 synapse, GO:0050808 synapse organization}
Neurof_GoNeuronProj	GO neuron projection	1230	union of these GO-based gene-sets: {GO:0043005 neuron projection, GO:0031175 neuron projection developmen}
Neurof_GoNervTransm	GO neurotransmission	716	union of these GO-based gene-sets: {GO:0019226 transmission of nerve impulse, GO:0007268 synaptic transmission}
Neurof_GoNervSysDev_CNS	GO central nervous system development	774	GO:0007417 central nervous system development
Neurof_GoNervSysDev	GO nervous system development	1874	GO:0007399 nervous system development
Neurof_UnionInclusive	Neurofunction union inclusive	2874	union of these previously defined gene-sets: {Neurof_KeggSynaptic, Neurof_GoNervTransm, Neurof_GoNeuronProj, Neurof_GoNeuronBody, Neurof_GoSynaptic, Neurof_GoNervSysDev, Neurof_PathwaysAxonG}
Neurof_UnionStringent	Neurofunction union stringent	1424	set of genes found in at least two of these previously defined gene-sets {Neurof_KeggSynaptic, Neurof_GoNervTransm, union of {Neurof_GoNeuronProj, Neurof_GoNeuronBody, Neurof_GoSynaptic}, Neurof_GoNervSysDev, Neurof_PathwaysAxonG}
FMR1_Targets_Darnell	FMR1 targets Darnell et al	840	Human homologs of mouse Fmr1 (fragile X mental retardation 1)gene targets as defined in: FMRP stalls ribosomal translocation on mRNAs linked to synaptic function and autism - Darnell et al - Cell. Jul 22, 2011; 146(2): 247–261.
FMR1_Targets_Ascano	FMR1 targets Ascano et al	927	Predicted human FMR1 (fragile X mental retardation 1) gene targets as defined in: FMR1 targets distinct mRNA sequence elements to regulate protein expression - Manuel Ascano Jr et al - Nature 2012 December 20; 492(7429): 382–386
PSD_BayesGrant_fullset	Post-synaptic density components (Bayes et al, full list)	1407	Members of the human post-synaptic density as defined in: Characterization of the proteome, diseases and evolution of the human postsynaptic density. Bayés et al. Nat Neurosci. 2011
PhHs_NervSys_All	HPO: Nervous system, any inheritance	1590	HP:0000707 Abnormality of the nervous system
PhHs_NervSys_ADX	HPO: Nervous system, AD or X-linked	651	HP:0000707 Abnormality of the nervous system, X-linked or autosomal dominant subset
PhHs_MindFun_All	HPO: Higher mental function abnormality, any inheritance	439	HP:0011446 Abnormality of higher mental function
PhHs_MindFun_ADX	HPO: Higher mental function abnormality, AD or X-linked	153	HP:0011446 Abnormality of higher mental function, X-linked or autosomal dominant subset
PhMm_NeuroBehav_all	MPO: Neurological abnormality or abnormal behavior	2123	MP:0005386 behavior/neurological phenotype
PhMm_NervSystem_all	MPO: Nervous system abnormality	2375	MP:0003631 nervous system phenotype

PhMm_NeuroUnion_all	MPO: Neurological abnormality or abnormal behavior or nervous system abnormality	3202	union of these MPO-based gene-sets: {MP:0005386 behavior/neurological phenotype, MP:0003631 nervous system phenotype}
PhMm_Aggr_IntegAdipPigm_all	MPO: Adipose or integument or pigmentation abnormality	1624	union of these MPO-based gene-sets: {MP:0005375 adipose tissue phenotype, MP:0010771 integument phenotype, MP:0001186 pigmentation phenotype)
PhMm_Aggr_EndoExocrRepr_all	MPO: Endo- or exocrine or reproductive system abnormality	2026	union of these MPO-based gene-sets: {MP:0005379 endocrine/exocrine gland phenotype, MP:0005389 reproductive system phenotype}
PhMm_Aggr_HematoImmune_all	MPO: Hematological or immune abnormality	2605	union of these MPO-based gene-sets: {MP:0005397 hematopoietic system phenotype, MP:0005387 immune system phenotype}
PhMm_Aggr_DigestHepato_all	MPO: Digestive or hepatobiliary abnormality	1493	union of these MPO-based gene-sets: {MP:0005381 digestive/alimentary phenotype, MP:0005370 liver/biliary system phenotype}
PhMm_Aggr_CardvascMuscle_all	MPO: Cardiovascular or muscle abnormality	2059	union of these MPO-based gene-sets: {MP:0005385 cardiovascular system phenotype, MP:0005369 muscle phenotype}
PhMm_Aggr_Sensory_all	MPO: Sensory system abnormality	1293	union of these MPO-based gene-sets: {MP:0005377 hearing/vestibular/ear phenotype, MP:0005394 taste/olfaction phenotype, MP:0005391 vision/eye phenotype}
PhMm_Aggr_SkeCranioLimbs_all	MPO: Skeletal or limb or cranium abnormality	1588	union of these MPO-based gene-sets: {MP:0005382 craniofacial phenotype, MP:0005371 limbs/digits/tail phenotype, MP:0005390 skeleton phenotype}
BSpan_VH_thr4.74	Brain very high expr	4600	> 3rd quartile of BrainSpan expression
BSpan_HM_thr3.21	Brain high/medium expr	4605	2nd-3rd quartile of BrainSpan expression
BSpan_ML_thr0.93	Brain medium/low expr	4596	1st-2nd quartile of BrainSpan expression
BSpan_Ab_thr.MIN	Brain low/absent expr	4601	< 1st quartile of BrainSpan expressoin
gnomAD_oe_lof_upper_0.35	gnomAD LoF intorelance	2925	gene with upper bound of CI of oe < 0.35 defined by gnomAD
MSSNG-2017	MSSNG 2017	81	List of genes in MSSNG (2017) database
MSSNG-2022	MSSNG 2022	134	List of genes in MSSNG (2022) database
SFARI	SFARI	1023	List of genes in SFARI database (as of 01-27-2022)
SFARI_Score_1	SFARI Score 1	207	List of genes in SFARI database (as of 01-27-2022) with a score of 1
SFARI_Score_2	SFARI Score 2	220	List of genes in SFARI database (as of 01-27-2022) with a score of 2
SFARI_Score_3	SFARI Score 3	515	List of genes in SFARI database (as of 01-27-2022) with a score of 3

Supplementary Table 2 | ASD-relevant gene sets.