Functional brain defects in a mouse model of a chromosomal t(1;11) translocation that disrupts DISC1 and confers increased risk of psychiatric illness

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Abstract

A balanced t(1;11) translocation that directly disrupts DISC1 is linked to schizophrenia and affective disorders. We previously showed that a mutant mouse, named Der1, recapitulates the effect of the translocation upon DISC1 expression. Here, RNAseq analysis of Der1 mouse brain tissue found enrichment for dysregulation of the same genes and molecular pathways as in neuron cultures generated previously from human t(1;11) translocation carriers via the induced pluripotent stem cell route. DISC1 disruption therefore apparently accounts for a substantial proportion of the effects of the t(1;11) translocation. RNAseq and pathway analysis of the mutant mouse predict multiple Der1-induced alterations converging upon synapse function and plasticity. Synaptosome proteomics confirmed that the Der1 mutation impacts synapse composition, and electrophysiology found reduced AMPA:NMDA ratio in hippocampal neurons, indicating changed excitatory signalling. Moreover, hippocampal Parvalbumin-positive interneuron density is increased, suggesting that the Der1 mutation affects inhibitory control of neuronal circuits. These phenotypes predict that neurotransmission is impacted at many levels by DISC1 disruption in human t(1;11) translocation carriers. Notably, genes implicated in schizophrenia, depression and bipolar disorder by large-scale genetic studies are enriched among the Der1-dysregulated genes, just as we previously observed for the t(1;11) translocation carrier-derived neurons. Furthermore, RNAseq analysis predicts that the Der1 mutation primarily targets a subset of cell types, pyramidal neurons and interneurons, previously shown to be vulnerable to the effects of common schizophrenia-associated genetic variants. In conclusion, DISC1 disruption by the t(1;11) translocation may contribute to the psychiatric disorders of translocation carriers through commonly affected pathways and processes in neurotransmission.
**Introduction**

Psychiatric illnesses such as schizophrenia and recurrent affective disorders have a substantial underlying genetic component. Considerable progress has been made in recent years towards identification of the multitude of genes involved using large-scale studies of genome-wide association (GWAS) and recurrent copy number variants (CNVs). GWAS tends to identify genomic loci with common, but small, individual effects that encompass several genes, leaving the specific causal genes unidentified, unless further refinements are applied. In contrast, recurrent CNVs are rare, tending to exert a strong effect (most likely due to large changes in expression levels of the genes at fault), but also usually encompass multiple genes. Chromosomal rearrangements, such as translocations, linked to psychiatric disorders are rarer still, but can have the advantage of strong effects and accurate pinpointing of genes due to their disruption by the breakpoints of the rearranged genomic segments. It is likely that convergence of data arising from genomic events such as these will assist in revealing the genes and mechanisms that predispose to major mental illness.

One example of a chromosomal rearrangement linked to psychiatric disorders is a t(1;11) translocation that substantially increases risk of developing schizophrenia or affective disorders in a large Scottish family. The psychiatric symptoms presented by t(1;11) translocation carriers are typical, that is, they are within the range of current diagnostic criteria, and are accompanied by reduced white matter integrity, cortical thickness and prefrontal cortex gyrification, all typical of schizophrenia. Carriers of the t(1;11) translocation also have decreased glutamate levels in the dorsolateral prefrontal cortex. Moreover, transcriptome analysis of induced pluripotent stem cell (IPSC)-derived cortical neurons from t(1;11) translocation carriers found enrichment for dysregulated genes at putative schizophrenia and depression loci discovered through large scale GWAS and CNV studies, potentially identifying some of the genes of interest at those loci, and indicating that the t(1;11) translocation may trigger disease pathways shared with schizophrenic patients who are not translocation carriers.
The t(1;11) translocation directly disrupts the \textit{DISC1} gene on chromosome 1\textsuperscript{13}. \textit{DISC1} encodes a potential molecular scaffold protein involved in multiple critical functions in the developing and adult brain\textsuperscript{14, 15}, including neurogenesis\textsuperscript{16-18}, neuronal cargo trafficking\textsuperscript{19-23} and neurotransmission\textsuperscript{23-26}. \textit{DISC1} disruption is therefore likely to contribute substantially to mechanisms leading to psychiatric illness in t(1;11) translocation carriers.

Two apparently non-coding genes of unknown function, \textit{DISC2} and \textit{DISC1FP1} (otherwise known as Boymaw), are also disrupted on chromosomes 1 and 11, respectively\textsuperscript{13, 27}, and potential genetic modifier loci have been identified within the family\textsuperscript{28}, all of which may additionally impact disease mechanisms in t(1;11) translocation carriers. It is now important to discover how each of these disruptions and putative modifiers relate to the gene expression changes, brain structure alterations and psychiatric symptoms of t(1;11) translocation carriers.

To examine the impact of \textit{DISC1} disruption in isolation from the additional complexities of \textit{DISC2} disruption and loss of normal \textit{DISC1FP1} function, and of potential genetic modifiers, we have utilised a mutant mouse which accurately recapitulates the effects of the translocation upon DISC1 expression\textsuperscript{12}. IPSC-derived neural precursor cells and cortical neurons from t(1;11) translocation carriers exhibit reduced \textit{DISC1} expression\textsuperscript{12}. Chimeric transcripts encoding aberrant C-terminally truncated chimeric forms of \textit{DISC1} are also produced in the IPSC-derived neural cells as a result of fusion between the \textit{DISC1} and \textit{DISC1FP1} genes on the derived chromosome 1\textsuperscript{12, 29}. The mutant mouse was precisely engineered to mimic the fusion between \textit{DISC1} and \textit{DISC1FP1} on the derived 1 chromosome and exhibits reduced Disc1 levels plus chimeric transcript expression\textsuperscript{12}. This mutant mouse is referred to as Der1.

Heterozygous Der1 mice express reduced levels of wild-type Disc1 plus the aberrant chimeric transcripts\textsuperscript{12, 29}. Because Disc1 multimerises\textsuperscript{30}, there is thus potential in heterozygotes for dominant-negative effects due to interaction between wild-type and mutant Disc1. Homozygotes, however, lack any wild-type Disc1 and may express high levels of aberrant Disc1. Despite heterozygotes corresponding most closely to t(1:11) translocation
carriers, we opted to study both mutant genotypes in order to obtain the most complete understanding of the likely effects of DISC1 disruption. A flow chart (Supplementary Figure 1) illustrates the experimental approach taken, with the aim of allowing the results described here to be compared with previously published t(1:11) translocation studies and integrated with psychiatric genetic association studies of single nucleotide polymorphism (SNP) and CNV variants in the general population. We combine magnetic resonance imaging (MRI), histology, transcriptomics, synaptosome proteomics and electrophysiology to demonstrate that the Der1 mutation primarily affects cellular properties rather than brain structure, and that it targets a variety of cell types including neurons. Patterns of gene expression and predictions of altered biological processes substantially overlap between Der1 cortex and IPSC-derived cortical neuron cultures from t(1:11) translocation carriers. We find widespread dysregulation of genes implicated as potential common risk factors for schizophrenia, depression and bipolar disorder. We therefore propose that DISC1 disruption targets common pathways shared with psychiatric patients who do not carry the t(1;11) translocation, to contribute to the elevated risk of major mental illness displayed by t(1:11) translocation carriers.

Materials and methods

Detailed materials and methods are provided in the Supplementary information file.

Results

Adult Der1 mutant mice show no overt changes in brain structure

Using ex vivo structural MRI, we found no evidence for effects of the Der1 mutation on overall brain volume or the volumes of 51 brain regions analysed individually (Supplementary Table 1). In the absence of hypotheses arising from the MRI analysis of brain structure, and given that DISC1 is highly expressed in the hippocampus from early development through to adulthood, and that prefrontal cortex (PFC) is affected in t(1:11) translocation carriers, these regions were explored in further detail. The Der1 mutation does
not affect cell densities in the hippocampal Stratum, Radiatum, Lacunsum and Moleculare
or prefrontal cortex, nor the thickness of individual cortical layers within the barrel cortex, nor
the total cortical thickness in either the barrel cortex or the PFC (Supplementary Figure 2, 3,
4).

RNAseq analysis of adult Der1 cortex and hippocampus

We next conducted RNASeq using wild-type and heterozygous ‘cortex’ (consisting of
cortices minus hippocampus, cerebellum and olfactory bulbs) and hippocampus. The
resulting data were analysed at the whole gene and single exon levels using DESeq2 and
DEXSeq, respectively. Full-length Disc1 expression is reduced in heterozygous Der1
mouse whole brain as detected by quantitative RT-PCR and immunoblotting. RNAseq also
found reduced Disc1 expression in heterozygous Der1 cortex and hippocampus (Figure 1a,
Supplementary Table 2a, c), confirming the validity of these datasets.

Expression of 30,121 genes was detected in cortex, of which 2,124 and 3,568 are
differentially expressed in heterozygotes at the whole gene or exon level respectively (all
corrected p<0.05, Figure 1b, Supplementary Table 2a, b). Expression of 28,049 genes was
detected in hippocampus, of which 175 and 52 are differentially expressed in heterozygotes
at the whole gene or exon level respectively (all adjusted p<0.05, Supplementary Table 2c,
d).

Expression Weighted Cell-type Enrichment (EWCE) analysis of RNASeq data

suggests specific cell types are targeted by the Der1 mutation

EWCE analysis was used to look for evidence that certain cell types are especially
vulnerable to the Der1 mutation. We utilised gene expression profiles generated by
hierarchical clustering of single cell RNASeq profiles from 9,970 mouse brain cells and
around 15,000 of the most abundantly expressed genes, resulting in 24 cell classes, referred
to as the KI Superset. The authors of that study calculated ‘specificity values’ for each
gene within each cell class, to indicate enrichment for expression of that gene in a cell class
compared to the other classes in the Superset. EWCE analysis was used here to determine whether there is enrichment for Der1-induced dysregulation of genes with high specificity values for Superset cell classes in cortex (Figure 1c). Statistical significance was observed for pyramidal neurons (‘pyramidal somatosensory’, ‘pyramidal CA1’ [which also encompasses neurons from CA2 and the subiculum]), interneurons (‘cortical interneuron’, ‘striatal interneuron’), dopaminergic neurons (‘dopaminergic adult neurons’, ‘hypothalamic dopaminergic neurons’), ‘oxytocin/vasopressin-expressing neurons’ and ‘astrocytes/ependymocytes’. Der1 hippocampus dysregulated genes are also highly enriched in several cell classes (Figure 1d). Of these, pyramidal neurons (‘pyramidal somatosensory’), ‘medium spiny neuron’ and ‘interneuron’ achieved statistical significance. ‘Pyramidal CA1’ reached initial significance in Der1 hippocampus, but did not survive multiple testing correction. Pyramidal CA1, pyramidal somatosensory and medium spiny neurons should reside primarily in the hippocampus, cortex and striatum, respectively, thus some of these findings were initially unexpected. However, the previously published hierarchical clustering of cell classes indicated that ‘pyramidal CA1’ and ‘pyramidal somatosensory’ are highly similar, with medium spiny neurons the next most closely related cell type. We therefore infer that in cortex and hippocampus the Der1 mutation may target general features shared between these three neuron classes.

Based on these findings, Parvalbumin-expressing interneuron density was quantified in adult PFC and hippocampus. PFC shows no change (Supplementary Figure 5), however there is a trend towards an increase in the dentate gyrus ($p=0.07$), and a significant increase in Der1 heterozygotes when the whole hippocampus is examined (34% increase, Figure 1e, f). The EWCE analysis data pointing to hippocampal interneuron targeting could therefore be due, at least partially, to increased density of interneurons expressing Parvalbumin at high levels. This contrasts with previous reports of reduced Parvalbumin-positive cell density in mice expressing mutant DISC1, or in response to endogenous Disc1 knockdown.

We also hypothesised that the cell types most affected by the Der1 mutation might be susceptible to apoptosis, as quantified using Activated-Caspase-3. Of the adult PFC and
hippocampal regions examined, there is a trend towards increased apoptosis in CA1 (p=0.06, Supplementary Figure 6), which may indicate that CA1 cells are particularly vulnerable. This could lead to reduced cell density in CA1, a parameter that unfortunately could not be adequately examined due to the prohibitively tight packing of cells in this region.

RNASeq deconvolution suggests that cell class proportions are unaltered by the Der1 mutation

RNASeq deconvolution was carried out utilising gene expression data (rather than the specificity values used above for EWCE analysis) for the most highly enriched genes from the 24 cell classes of the Superset\(^3\). First, the ability of the deconvolution programme, CIBERSORT\(^3\), to deconvolve the 24 cell classes was examined by generating artificial in silico samples with varying proportions of each cell class (Supplementary Figure 7). Using two specificity value thresholds, CIBERSORT was able to deconvolve most cell types. The exceptions include embryonic cell types, which should be absent from our Der1 samples, and rarer cell types in adult brain such as neural progenitors and neuroblasts. When used to deconvolve Der1 cortex and hippocampus whole gene DESeq2 RNASeq data, CIBERSORT found no evidence for an effect of the mutation upon the relative proportion of any of the cell classes examined (Supplementary Figure 8). The increased density of hippocampal Parvalbumin-positive interneurons (Figure 1e, f) therefore may not represent a general effect upon all hippocampal interneuron types. Likewise, the trend towards increased hippocampal CA1 apoptosis (Supplementary Figure 6) does not translate to a detectably decreased density of pyramidal CA1 neurons, possibly because this Superset class also contains pyramidal neurons from CA2 and the subiculum\(^3\).

Molecular pathway analysis predicts wide-ranging effects of the Der1 mutation

Since the patterns of gene dysregulation are not explained by overtly altered cell proportions, the RNASeq data were next used to predict effects upon canonical pathways. Ingenuity Pathway Analysis (IPA), an unbiased method for examining transcriptomic data
using statistical significance and magnitude plus direction of fold-change, was carried out
using the whole gene level DESeq2 data, or combined DESeq2 plus exon level DEXSeq
data. This analysis predicts effects upon diverse pathways including metabolic, stress-
response and important neurosignalling processes (Figure 2a). A selection of these
pathways, based on statistical significance or relevance to later parts of this study, are
discussed below.

Mitochondrial dysfunction, including increased oxidative phosphorylation is strongly
predicted, based largely upon upregulated whole gene expression of multiple complex I, III
and IV components (Supplementary Figure 9), consistent with DISC1’s known role in
regulating oxidative phosphorylation\textsuperscript{37, 38}. Moreover, the chimeric transcripts expressed by
Der1 mice encode aberrant mitochondrial species that induce mitochondrial dysfunction\textsuperscript{29}.

Also upregulated at the whole gene level is the mitochondrial pathway ‘Fatty acid β-
oxidation I’ (Supplementary Figure 10) which degrades fatty acids to release energy.
Dysregulated enzymes feeding into this pathway are involved in fatty acid synthesis and
break down. Together these changes imply altered levels of lipids, which are critical for
many brain processes.

The ‘CREB signalling in neurons’ pathway (Figure 2b) is activated by cell surface
glutamate receptors, including AMPA and NMDA receptors, and calcium channels. It
regulates gene expression changes that are critical for synaptic plasticity and long-term
potentiation (LTP), both known to be DISC1-modulated\textsuperscript{30}. DISC1 is also already known to
regulate CREB signalling\textsuperscript{30}, and in our study IPA predicts that downregulation of Creb1
activity is responsible for many of the gene expression changes (p=9e-9, z=-3). Indeed,
there is enrichment (hypergeometric p=0.02) for dysregulation of genes containing
conserved cAMP-Response Elements (CREs, http://natural.salk.edu/creb/, Supplementary
Table 3) in heterozygous Der1 cortex, with 203 (9.6%) of the genes dysregulated at the
whole gene level having CREs. The Der1 mutation potentially affects activation of the
pathway via AMPA receptor subunit degradation\textsuperscript{39}, and NMDA receptor membrane
dynamics and surface expression\textsuperscript{12}. Moreover, genes encoding glutamate receptors,
including AMPA and NMDA receptor subunits, and several synaptic scaffolds are
dysregulated (Figure 2b).

Using the combined dysregulated DESeq2 plus DEXSeq data, IPA also determined
that many cellular functions are enriched for differentially expressed genes (Table 1,
Supplementary Table 4a). Predictions relating to neurotransmission, synaptic plasticity and
LTP are related to the ‘CREB signalling in neurons’ pathway above, plus genes encoding
inhibitory signalling factors, such as subunits of GABA_A and GABA_B receptors. Predictions
relating to vesicle transport and exo/endocytosis are based on dysregulated genes encoding
vesicle trafficking factors; voltage-gated calcium channel subunits as well as synaptotagmins
and syntaxins that together mediate calcium-dependent neurotransmitter release;
components of the exocyst complex; and components of the endocytic Clathrin-associated-
Adaptor-Protein-Complex. The wide-ranging neuronal morphology and cytoskeleton-related
predictions are based on multiple genes involved in diverse relevant processes. Similarly,
the cell-cell contact/adhesion-related functions are widespread, but notably encompass
genes required for early synapse formation, such as latrophilins, as well as maintenance of
trans-synaptic connections, for example neuroligins and neurexins. Other predictions relate
to cell proliferation, neuronal migration and circadian rhythms. All of these processes are
already known to involve DISC1^12, 16, 18, 21, 25, 35, 40-43.

Der1 hippocampus RNASeq data were similarly analysed. IPA did not strongly
predict any canonical pathway changes due to the relatively small number of changes, but
did predict altered functions that largely reflect those for cortex (Supplementary Table 4b, 5),
and there is enrichment for dysregulation of 86 shared genes (39% of the total dysregulated
hippocampal genes, Supplementary Table 2c, d) in both regions (p=7e-11). Myelination is
also predicted to be affected, consistent with previous studies demonstrating DISC1
involvement in oligodendrocyte differentiation and function^44-46.

Numerous processes are thus predicted to be affected by the Der1 mutation in cortex
and hippocampus, with striking convergence upon neurotransmission.
Molecular pathway analysis of targeted cell types identified by EWCE analysis

EWCE analysis identified cell classes that may be targeted by the Der1 mutation (Figure 1c, d). We reasoned that the cell class-enriched gene expression changes may inform on the impact of the Der1 mutation in each cell type. Pathway analysis was therefore carried out using the cell class-enriched dysregulated genes (Supplementary Table 6, 4c). Der1 cortex pyramidal neuron (CA1 and somatosensory) and interneuron terms relate to synaptic transmission. Der1 cortex astrocyte/ependymocyte terms relate to lipid metabolism and uptake of glutamine/glutamate. The lipid metabolism predictions are based on upregulation of genes encoding enzymes involved in fatty acid β-oxidation, and other aspects of brain lipid metabolism. This is related to the Der1 cortex RNASeq canonical pathway prediction ‘Fatty Acid β-oxidation I’ (Figure 2a), and indicates a potential imbalance between lipid synthesis and oxidation. Since astrocytes are a major source of brain lipid which is widely utilised, including for synapse function and myelination by oligodendrocytes, these processes may be compromised via astrocyte dysfunction. The glutamine/glutamate uptake predictions are based on dysregulated expression of genes such as Slc1a2, which encodes the synaptic glutamate transporter Eaat2. Astrocytes are critical regulators of glutamine and glutamate homeostasis in the brain, which includes glutamate clearance from synapses, and consequent regulation of glutamatergic neurotransmission and synaptic plasticity. There were no convincing findings for the other cell classes examined.

Shared gene dysregulation in heterozygous Der1 cortex and t(1:11) translocation carrier-derived cortical neuron cultures confirms the relevance of the Der1 RNASeq findings to psychiatric illness

To determine how the above RNASeq data analyses of the Der1 mouse relate to the t(1:11) translocation, we compared the Der1 mouse data to previously published RNASeq data generated from t(1:11) translocation carrier-derived neuron cultures. Human iPSC-derived neurons grown in culture are not directly comparable to adult mouse brain tissue. Even so, a
trend towards enrichment for shared gene expression changes was evident from the 20
dysregulated genes in common between IPSC-derived cortical neuron cultures from t(1:11)
translocation carriers\(^{12}\) and heterozygous Der1 hippocampus (p=0.06, Supplementary Table
2c, d), while 511 genes are differentially expressed in both heterozygous Der1 mouse cortex
and the IPSC-derived cortical neuron cultures (Supplementary Table 2a, b), demonstrating
significant enrichment (p=1e-14), and further validating the Der1 mouse as an accurate
model for the effect of the t(1:11) translocation upon DISC1 expression. An overlapping set
of cellular functions were also identified in the human cortical neuron cultures and
heterozygous Der1 mouse cortex (Table 1, Supplementary Table 4a, d). Moreover, for most
of the shared functions there is either significant enrichment or a trend towards enrichment
for a common set of differentially expressed genes (Table 1). This convergence indicates
that disruption of DISC1 likely contributes substantially to the altered molecular pathways in
the human neuron cultures.

Nonetheless, several functions are enriched in the Der1 cortex data, but not in the
human cortical neuron data. Many relate specifically to synaptic plasticity and LTP,
processes that are constitutive in brain, but which require stimulation to be detected in
neuronal cultures. A number of other changes relate specifically to development of
dendrites, which may not reach maturity in IPSC-derived neuronal cultures\(^{50}\).

**Mass spectrometry and SynGO analysis of adult Der1 synaptosomes confirm synaptic
changes**

To complement the RNASeq analysis, synaptosome fractions were prepared from hetero-
or homozygous Der1 mice and mass spectrometry was used to determine whether
synaptosomal protein expression profiles differ between mutant and wild-type mice. Of the
2,783 detected proteins in cortex, no changes survived multiple correction testing in
synaptosomes prepared from Der1 mice (Supplementary Table 7a, Supplementary Figure
11). Of the 2,183 proteins detected in hippocampus, 62 were found to be dysregulated in
homozygotes (FDR adjusted p-value < 0.05, Supplementary Table 7b, Supplementary
These proteins were annotated to well-established synaptic genes using the SynGO database\textsuperscript{51} (Figure 2c, Supplementary Table 7c). This is an expert-curated database of gene ontology terms relating to synapses. From the 62 regulated proteins, 26 were found annotated in SynGO, 24 with cellular component annotation and 19 with biological processes annotation. Dysregulated proteins were found annotated across a wide spectrum of pre- and post-synapse functions. For instance, several proteins were annotated to the postsynaptic density, such as Camk2a (downregulated); AMPA receptor subunits (downregulated); the DISC1 interactor Trio\textsuperscript{52}, which modulates AMPA receptor currents in hippocampal CA1 pyramidal neurons\textsuperscript{53}; vesicle proteins Exoc4, an exocyst component, and the SNARE STX7; and Gad2, a presynaptic protein that synthesises GABA in interneurons. These changes point to effects upon similar synaptic processes to those highlighted by RNASeq analysis. However, fewer changes were detected in the synaptosomes, probably due to the lower number of proteins identified in comparison with the RNASeq analysis, in which many relevant RNASeq changes were detected at the isoform level.

**Functional effects of the *Der1* mutation upon synapses**

The RNAseq data point towards effects of the *Der1* mutation upon synapses, which was confirmed by subsequent synaptic proteomics analysis. The observed changes include subtly altered expression of NMDA receptor isoforms and reduced AMPA receptor subunit levels. Moreover, we previously demonstrated that cultured hippocampal neuron dynamics and cell surface/synaptic expression of NMDA receptors are dysregulated by the *Der1* mutation\textsuperscript{12}.

To examine these receptors functionally, whole cell patch-clamping was used to record currents from both receptor types in mature cultured hippocampal neurons (Figure 2d). The AMPA:NMDA ratio is decreased in homozygous *Der1* neurons indicating functional imbalance between these two receptor subtypes. This may be due in part to altered AMPA receptor currents, which although not statistically significant, are decreased in hetero- and homozygous neurons. To discover whether this whole cell patch-clamp finding extends to
receptors located at synapses in heterozygous Der1 hippocampus, and in cortex, will require future in-depth electrophysiological measurements. If it does indeed extend to synapses, the decreased AMPA:NMDA ratio could have many consequences including impaired triggering of NMDA receptor-dependent LTP, which is initiated by AMPA receptor-induced release of the magnesium block on NMDA receptors.

**Enrichment for dysregulation of putative schizophrenia, bipolar disorder and depression risk genes in heterozygous Der1 cortex and hippocampus**

A large number of putative schizophrenia risk genes have been identified from two large-scale GWAS and one large-scale CNV study\(^1\)-\(^3\). IPA maps many of these genes to shared molecular pathways. The top canonical pathway (Figure 3a) is ‘CREB signalling in neurons’. Others include ‘Synaptic long-term potentiation’ and ‘Synaptic long-term depression’, both mechanisms underlying synaptic plasticity. These findings largely agree with previous observations\(^5\)

The heterozygous Der1 cortex combined RNASeq DESeq2 plus DEXSeq data were compared to the list of putative schizophrenia risk genes used above\(^1\), but including only genes encoding synaptic proteins from the CNV study\(^3\) as defined by its authors. This identified significant enrichment for dysregulation of schizophrenia candidate gene orthologues (Table 2, Supplementary Table 2a, b). The top canonical pathways identified using this set of genes for IPA are ‘Synaptic long-term depression’, ‘CREB signalling in neurons’, ‘Synaptic long-term potentiation’ and ‘Calcium signalling’ (Figure 3a-c). These predictions are among the top five of those obtained using the full set of putative schizophrenia risk genes (Figure 3a), indicating that the Der1 mutation and genetic risk factors for schizophrenia converge upon the same pathways.

Enrichment for dysregulation of schizophrenia candidate gene orthologues was also apparent using the heterozygous Der1 hippocampus combined RNAseq DESeq2 plus DEXSeq data (Table 2, Supplementary Table 2c, d), although there were too few genes to carry out meaningful pathway analysis.
Large-scale genetic data are also available for bipolar disorder and depression\textsuperscript{4-6}. IPA did not find that the genes identified from these studies converge strongly upon any canonical pathways, although a subset of depression-associated genes are involved in synaptic structure and activity\textsuperscript{6}. Nonetheless, there is enrichment for dysregulation of the orthologues of candidate genes for both disorders in Der1 cortex, and for depression in Der1 hippocampus (Table 2, Supplementary Table 2). Moreover, the dysregulated putative depression risk gene orthologues in Der1 cortex predict effects upon the 'CREB signalling in neurons' pathway (Figure 3a).

We also examined overlaps between genes dysregulated in the Der1 mouse and two non-psychiatric illness related large-scale GWAS. For Alzheimer's Disease\textsuperscript{55} there is enrichment for dysregulation of candidate gene orthologues in Der1 cortex (Table 2, Supplementary Table 2), with six of the nine gene matches (ABCA7, APOE, CLU, FERMT2, PTK2B/PYK2, SORL1) involved in Amyloid-Beta (A\textbeta) -related processes\textsuperscript{56-61}. This effect may be explained by observations that DISC1 interacts with Amyloid Precursor Protein\textsuperscript{62}, and regulates A\textbeta generation\textsuperscript{63, 64}. The second comparison was to a study of cerebral cortex architecture\textsuperscript{65}. Again, there is enrichment for dysregulation of candidate gene orthologues in Der1 cortex (Table 2, Supplementary Table 2), although no molecular pathways are highlighted.

The enrichment for dysregulation of orthologues of candidate genes for brain disorders (which is particularly striking for schizophrenia) when combined with convergence upon specific molecular pathways already implicated in those disorders, indicates that the Der1 mutation may exert effects that are directly relevant to these human brain illnesses.

**Discussion**

Heterozygous Der1 mutant mice accurately recapitulate the effects of the t(1:11) translocation upon DISC1 expression in IPSC-derived neural precursors and cortical neurons\textsuperscript{12}. We now demonstrate that patterns of gene expression dysregulation and
pathway predictions are similar between heterozygous Der1 cortex, and IPSC-derived
cortical neuronal cultures from t(1:11) translocation carriers. Together these observations
suggest that DISC1 disruption is an important factor in the increased risk of major mental
illness displayed by t(1:11) translocation carriers, and argue that the Der1 mouse model can
be used to study the neuronal effects of DISC1 disruption upon brain function to understand
disease mechanisms in these individuals.

Many of the findings reported here are consistent with known DISC1 biology and
brain function, but observations such as the lack of overt brain structural changes, and of
increased density of hippocampal Parvalbumin-expressing interneurons were unexpected on
the basis of previously described DISC1 mutant mice which model aspects of the effects of
the t(1;11) translocation upon DISC1 expression35 (Supplementary Table 8). Such
differences, and the many phenotypic differences between previously published mutants
(Supplementary Table 8), accentuate the critical importance of studying a mutant that
accurately mimics all effects of the t(1;11) translocation in order to understand disease
mechanisms in t(1;11) translocation carriers. Other findings, such as the predicted
dysregulation of astrocyte lipid metabolism, have not been reported previously. This is the
first, and only, mutant mouse to accurately model effects of the t(1:11) translocation, and it
therefore provides important and new insights into molecular mechanisms underlying the
increased disease risk and psychiatric symptoms of t(1:11) translocation carriers.

Structural and functional brain abnormalities have been reported in human t(1:11)
translocation carriers11, whereas none were detected in the adult Der1 mice studied here.
This difference may reflect fundamental species differences in brain structure and
development, and/or secondary genetic or environmental factors consequent upon, or
interacting with, the t(1:11) translocation event. Genetic effects may include loss of normal
function of the additional disrupted genes DISC2 and DISC1FP113, 29, or an influence of
genetic modifiers28. Environmental effects may include greater relative age, and duration of
chronic mental illness with associated long-term exposure to medication such as
antipsychotic drugs. The latter progressively decreases grey matter volume in schizophrenia patients\textsuperscript{66}, and decreases cortical volume in rats\textsuperscript{67}.

The absence of brain structural changes, together with the lack of evidence for altered cell class proportions from RNASeq data deconvolution, indicates that the subtle transcriptomic and proteomic alterations identified in the \textit{Der1} mouse are principally due to altered cellular properties that are largely conserved between it and \textit{t(1:11)} translocation carriers. EWCE analysis of RNASeq data suggests that the \textit{Der1} mutation may target distinct cell types including pyramidal neurons (CA1 and somatosensory) and interneurons. These findings correlate well with a previous EWCE analysis using large-scale schizophrenia GWAS data\textsuperscript{2,34} which found that schizophrenia-associated SNPs map to genomic loci containing genes that are highly expressed in a limited number of brain cell types including CA1 and somatosensory pyramidal neurons, and interneurons\textsuperscript{34}, thus implicating these cell types in the aetiology of schizophrenia. The additional cell types that appear to be targeted by the \textit{Der1} mutation: dopaminergic neurons, oxytocin/vasopressin-expressing neurons and astrocytes/ependymocytes, were not implicated in schizophrenia by the genomic EWCE analysis. However, dopamine signalling is heavily implicated in schizophrenia, in part because all antipsychotic drugs in clinical use target the dopamine D2 receptor\textsuperscript{68}, while \textit{DRD2} is located at a genetic locus repeatedly found to associate with schizophrenia\textsuperscript{1-2} and also with depression\textsuperscript{6}. The neuropeptides oxytocin and vasopressin regulate many processes, including social behaviour and anxiety\textsuperscript{69}, and are widely implicated in psychiatric disorders\textsuperscript{70}. Astrocyte abnormalities have also been reported in relation to psychiatric disorders\textsuperscript{71}. Thus, even if not directly targeted by genomic risk variants, these additional cell types do apparently contribute to psychiatric illness.

Pyramidal neurons are the major excitatory neurons in the brain. Interneurons are inhibitory and regulate neuronal network excitability, primarily of pyramidal neurons. Our analyses suggest widespread targeting of pyramidal neurons and interneurons by the \textit{Der1} mutation, thus excitation and inhibitory control of neuronal networks may be impaired. Neuronal activity could be further impaired if the EWCE predictions are correct and
glutamate uptake by astrocytes is dysregulated. Our findings and predictions relating to
pyramidal neurons, which are glutamatergic cells, and to astrocytic glutamate uptake, may
be related to the decreased glutamate levels detected by brain imaging of t(1:11)
translocation carriers. Altered lipid production by astrocytes may be an additional factor
affecting neuronal activity. Lipids are required for many processes, including synaptic
activity and myelination. We have previously demonstrated impaired myelination in Der1
cortex which is presumably due, at least partially, to direct effects of the mutation upon
oligodendrocytes because the corresponding IPSC-derived oligodendrocytes from t(1:11)
translocation carriers are abnormal. EWCE analysis did not, however, find evidence that
oligodendrocytes are strongly targeted by the Der1 mutation, although some genes highly
specific for this cell type are dysregulated, such as Myelin-Oligodendrocyte-Glycoprotein in
cortex (Supplementary Table 2a), while genes that impact myelination are dysregulated in
hippocampus (Supplementary Table 4b, 5). Altered lipid production by astrocytes could
therefore be a contributory factor in the myelination phenotype.

Consistent with the targeting of cell types implicated in schizophrenia, the Der1
mutation dysregulates orthologues of many genes implicated as risk factors for
schizophrenia and depressive disorders through large-scale genome-wide association and
CNV studies, as we have previously shown for the t(1:11) translocation in IPSC-derived
neurons. The pathways by which the t(1:11) translocation causes major mental illness may
therefore overlap those targeted by common genetic risk factors for schizophrenia and
depression. We speculate that disruption of the gene encoding the molecular scaffold
DISC1, with knock-on effects for its numerous binding partners and functions can, at least
partially, recapitulate the consequences of the more common scenario in psychiatric patients
whereby multiple interacting common genetic risk factors are inherited, with both scenarios
converging upon the same biological pathways. In agreement with this, the symptoms of
t(1:11) translocation carriers are indistinguishable from the typical spectrum of clinical
presentation of the psychiatric disorders with which they are diagnosed.
The convergence of the Der1 mutation with a subset of putative common genetic risk factors for schizophrenia and depressive disorders, and the convergence of this subset of genes upon synapses and synaptic plasticity\textsuperscript{6, 54} implies that, of all the Der1 cortex pathway predictions, dysregulated neurotransmission and synaptic plasticity are among the most critical factors in the psychiatric symptoms of t(1:11) translocation carriers. Notably, synaptic plasticity underpins cognition, which is characteristically impaired in schizophrenia.

Altogether, the EWCE and pathway analyses pointing to potential pyramidal neuron and interneuron dysfunction in hippocampus; the evidence that the number of apoptotic cells in CA1 may be increased; the higher density of hippocampal Parvalbumin-positive interneurons; the extensive changes to synaptic protein expression in hippocampus synaptosomes; and the electrophysiology data indicating reduced AMPA:NMDA ratio in cultured hippocampal neurons, suggest that hippocampal circuits are especially sensitive to the mutation, although effects upon other brain regions are also likely.

The hippocampus has multiple input/output pathways from/to other brain regions which are regulated by various neurotransmitters. Hippocampal dysfunction in Der1 mice could thus have numerous extrinsic/intrinsic causes, and knock-on effects. CA1 pyramidal neurons provide the major hippocampal output, including the hippocampal-to-PFC pathway that regulates NMDA receptor-dependent LTP and cognition\textsuperscript{72}. This pathway is widely implicated in psychiatric disorders\textsuperscript{72}. It is thus an exemplar of the mechanisms by which DISC1 disruption could confer susceptibility to major mental illness by bringing together the diverse effects described here, and elsewhere\textsuperscript{12, 46}, in our studies of neural cells derived from t(1:11) translocation carriers, and of the corresponding Der1 mouse. Our findings thus provide important insights into potential disease mechanisms involving specific molecular pathways/functions and cell types in t(1:11) translocation carriers that are likely relevant to schizophrenia and affective disorders in general.

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Conflict of interest statement

The authors declare no competing financial interests. AMM has received research support from Eli Lilly and Company, Janssen and the Sackler Trust, and speaker fees from Illumina and Janssen. ACV has received research support from F. Hoffman La Roche and UCB Biopharma SPRL. ML is a full time employee of Sylics (Synaptologics B.V.), a private company that offers mouse phenotyping services. ABS is shareholder of Alea Biotech B.V., a holding of Sylics (Synaptologics B.V.). MD is based at Sanofi.
Table 1 Top relevant altered cellular functions in heterozygous Der1 mouse cortex and human IPSC-derived neurons from members of the t(1;11) translocation family predicted using DESeq2+DEXSeq data. All Der1 mouse and human t(1;11) neuron functions are listed in Supplementary Table 4.

| Function (no. of molecules) | Der1 cortex score (no. of genes) | Human t(1:11) translocation neuron culture score (no. of genes) | Hypergeometric p value for enrichment (no. of shared genes) |
|-----------------------------|----------------------------------|---------------------------------------------------------------|-------------------------------------------------------------|
| General cell morphology     |                                  |                                                               |                                                             |
| Development of neurons (1,423) | p=2e-53 (457)                  | p=2e-13 (148)                                                   | p=1e-3 (63)                                                   |
| Morphogenesis of neurons (1,080) | p=1e-47 (360)                  | p=4e-13 (119)                                                   | p=3e-4 (56)                                                   |
| Morphology of neurons (1,123) | p=7e-37 (303)                   | p=1e-4 (80)                                                     | p=6e-5 (37)                                                   |
| Morphology of cells (4,370)  | p=1e-29 (902)                   |                                                               |                                                             |
| Abnormal morphology of neurons (923) | p=6e-25 (212)                  |                                                               |                                                             |
| Cell contact                |                                  |                                                               |                                                             |
| Cell-cell contact (1,118)    | p=5e-26 (299)                   | p=4e-6 (92)                                                     | p=6e-4 (38)                                                   |
| Development of gap junctions (327) | p=1e-18 (123)                  | p=2e-4 (37)                                                     | p=0.08 (17)                                                   |
| Formation of cell-cell contacts (414) | p=6e-16 (138)                  | p=8e-6 (48)                                                     | p=0.08 (19)                                                   |
| Formation of intercellular junctions (409) | p=1e-15 (136)                  | p=1e-5 (47)                                                     | p=0.07 (19)                                                   |
| Formation of plasma membrane (406) | p=1e-15 (134)                  | p=3e-6 (48)                                                     | p=0.05 (20)                                                   |
| Cytoskeleton                |                                  |                                                               |                                                             |
| Organization of cytoplasm (2,832) | p=2e-64 (791)                  | p=3e-16 (257)                                                   | p=2e-6 (104)                                                   |
| Organization of cytoskeleton (2,624) | p=4e-57 (720)                  | p=3e-16 (240)                                                   | p=1e-8 (101)                                                   |
| Microtubule dynamics (2,247) | p=6e-54 (627)                   | p=1e-14 (206)                                                   | p=1e-6 (87)                                                   |
| Development of cytoplasm (873) | p=9e-19 (233)                  | p=2e-4 (71)                                                     | p=1e-5 (35)                                                   |
| Formation of cytoskeleton (733) | p=5e-14 (179)                   |                                                               |                                                             |
| Cellular protrusions/neurites|                                  |                                                               |                                                             |
| Neurogenesis (1,067)         | p=2e-46 (354)                   | p=4e-12 (115)                                                   | p=2e-4 (55)                                                   |
| Formation of cellular protrusions (1,645) | p=1e-46 (488)                  | p=3e-15 (170)                                                   | p=2e-4 (70)                                                   |
| Growth of neurites (910)     | p=5e-30 (261)                   | p=1e-7 (81)                                                     | p=6e-4 (36)                                                   |
| Morphology of cellular protrusions (522) | p=3e-25 (166)                  |                                                               |                                                             |
| Morphology of neurites (414)  | p=6e-25 (139)                   |                                                               |                                                             |
| Axons                       |                                  |                                                               |                                                             |
| Axonogenesis (338)           | p=1e-18 (122)                   | p=2e-7 (45)                                                     | p=2e-3 (25)                                                   |
| Morphology of axons (169)    | p=2e-16 (65)                    |                                                               |                                                             |
| Growth of axons (281)        | p=2e-12 (87)                    |                                                               |                                                             |
| Abnormal morphology of axons (133) | p=4e-11 (46)                   |                                                               |                                                             |
| Guidance of axons (202)      | p=5e-10 (71)                    | p=2e-5 (29)                                                     | ns (12)                                                      |
| Dendrites                   |                                  |                                                               |                                                             |
| Formation of dendrites (209) | p=9e-19 (90)                    |                                                               |                                                             |
| Dendritic growth/branching (446) | p=8e-18 (131)                  |                                                               |                                                             |
| Density of dendritic spines (143) | p=1e-11 (49)                   |                                                               |                                                             |
| Morphology of dendrites (138) | p=3e-9 (49)                     |                                                               |                                                             |
| Abnormal morphology of dendrites (75) | p=3e-8 (32)                    |                                                               |                                                             |
| Cell proliferation          |                                  |                                                               |                                                             |
| Proliferation of neuronal cells (1066) | p=5e-28 (290)                  | p=2e-9 (98)                                                     | p=2e-3 (39)                                                   |
| Neuronal migration           |                                  |                                                               |                                                             |
| Migration of neurons (362)   | p=1e-16 (125)                   | p=8e-6 (43)                                                     | p=0.03 (20)                                                   |
| Circadian rhythm            |                                  |                                                               |                                                             |
| Circadian rhythm (132)       | p=3e-8 (55)                     |                                                               |                                                             |
| Transport                   |                                  |                                                               |                                                             |
| Organisation of organelle (948) | p=1e-23 (270)                  |                                                               |                                                             |
| Transport of vesicles (192)  | p=1e-14 (69)                    |                                                               |                                                             |
| Endocytosis (924)            | p=8e-10 (202)                   | p=3e-6 (76)                                                     | p=2e-3 (27)                                                   |
| Secretory pathway (367)      | p=8e-10 (93)                    |                                                               |                                                             |
| Formation of vesicles (307)  | p=4e-9 (70)                     |                                                               |                                                             |
| Neurotransmission           |                                  |                                                               |                                                             |
| Neurotransmission (716)      | p=5e-31 (233)                   | p=5e-5 (62)                                                     | p=0.03 (26)                                                   |
| Potentiation of synapse (546) | p=1e-28 (165)                   |                                                               |                                                             |
| Long-term potentiation (539) | p=4e-28 (163)                   |                                                               |                                                             |
| Synaptic transmission (558) | p=4e-27 (191)                   | p=7e-0 (55)                                                     | p=0.04 (24)                                                   |
| Developmental process of synapse (303) | p=2e-18 (117)                  | p=1e-4 (36)                                                     | p=0.08 (17)                                                   |
| Excitatory postsynaptic potential (166) | p=2e-15 (72)                   |                                                               |                                                             |
| Long-term potentiation of brain (281) | p=2e-13 (74)                   |                                                               |                                                             |
| Plasticity of synapse (170)  | p=2e-12 (66)                    |                                                               |                                                             |
| Long-term potentiation of cerebral cortex (254) | p=6e-12 (66) |
|-----------------------------------------------|---------------|
| Miniature excitatory postsynaptic currents (71) | p=1e-11 (38)  |

A full list of functions is provided in Supplementary Table 4a, d. Related functions are grouped, with top functions shown for each group. a, total number of molecules relating to each IPA function; b, number of dysregulated genes relating to each function; c, number of genes relating to function that are dysregulated in both Der1 cortex and human t(1;11) translocation neurons; italics, trend; ns, not significant.
Table 2 Enrichment for dysregulated expression of putative psychiatric illness risk gene orthologues in Der1 cortex and hippocampus.

| Study                                      | Loci | Genes  | Dysregulated in cortex | Hypergeometric p value for enrichment in cortex | Dysregulated in hippocampus | Hypergeometric p value for enrichment in hippocampus |
|--------------------------------------------|------|--------|------------------------|-----------------------------------------------|----------------------------|-----------------------------------------------------|
| GWAS, schizophrenia¹                      | 108  | 348    | 121 genes at 61 loci   | p=1e-13 (p=8e-19)                              | 6 genes at 6 loci           | p=0.04 (p=2e-4)                                      |
| GWAS, schizophrenia²                      | 143  | 481    | 127 genes at 73 loci   | p=3e-6 (p=8e-19)                               | 6 genes at 6 loci           | p=0.09 (p=8e-4)                                      |
| MAGMA, schizophrenia¹¹                   | 535  | 210    |                        | p=3e-30                                       | 15                          |                                                     |
| CNV (synapse genes), schizophrenia¹¹      | 52   | 25     |                        | p=8e-7                                        | 4                           |                                                     |
| GWAS, depression¹⁴                        | 44   | 70     | 19 at 19 loci          | p=0.02 (p=9e-5)                               | 1                           | ns                                                  |
| MAGMA, depression¹⁴                       | 153  | 33     |                        | p=0.047                                       | 1                           | ns                                                  |
| MAGMA meta-analysis, depression¹⁴         | 269  | 94     |                        | p=4e-11                                       | 6                           | p=0.01                                              |
| GWAS, bipolar disorder¹⁵                  | 30   | 218    | 73 at 21 loci          | p=4e-8 (p=8e-10)                              | 3 genes at 3 loci           | ns (p=2e-3)                                         |
| MAGMA, bipolar disorder¹⁵                 | 152  | 49     |                        | p=2e-6                                        | 3                           |                                                     |
| GWAS, Alzheimer’s Disease¹⁵               | 21   | 102    | 9 at 9 loci            | p=3e-3 (p=6e-3)                               | 1                           | ns                                                  |
| GWAS cerebral cortex architecture¹⁵       | 193  | 57     |                        | p=5e-5                                        | 1                           | ns                                                  |

Loci indicates the number of associated genomic loci identified by GWAS. Genes indicates the total number of genes at the associated loci, or the total number identified by MAGMA. Bracketed p values indicate enrichment for loci containing at least one dysregulated gene orthologue. italics, trend; ns, not significant.
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Legends to figures

Fig. 1 The Der1 mutation targets specific cell types in heterozygous cortex and hippocampus. a Disc1 RNASeq reads normalised to total reads per sample in wild-type versus heterozygous Der1 cortex and hippocampus. b Heat maps of the top 500 dysregulated genes identified by RNASeq of wild-type versus heterozygous Der1 cortex and hippocampus. c d EWCE analysis of heterozygous Der1 cortex and hippocampus, respectively, in mouse brain cell classes. asterisk, significance after Bonferroni correction; Emb, embryonic; Hyp, hypothalamic; SD, standard deviation e Parvalbumin expression in hippocampal sections from nine week old mouse brain. Enlarged regions showing Parvalbumin-expressing interneurons are indicated by boxes. scale bars, 100µm f Average density of Parvalbumin-expressing interneurons. Hippocampus refers to the whole hippocampal formation. Data were analysed by Kruskal-Wallis one-way ANOVA, p=0.07 for the dentate gyrus; p=0.049 for the hippocampal formation. Horizontal line on graphs for each sample, average of values; WT, wild-type; HET, heterozygous Der1; HOM, homozygous Der1; DG, dentate gyrus; *, p<0.05

Fig. 2 Consequences of the Der1 mutation. a Top relevant canonical pathway predictions for heterozygous Der1 cortex using whole gene, DESeq2, or whole gene and exon level, DESeq2+DEXSeq data. Asterisks indicate pathways highlighted in both cases. Where IPA predicts a direction of change this is indicated by a z score, with positive z scores indicating upregulation. b Altered gene expression in the ‘CREB signalling in neurons’ canonical pathway in heterozygous Der1 cortex, determined using whole gene and exon level DESeq2+DEXSeq data. To provide additional information, genes encoding calcium channels (CaCh), metabotropic glutamate receptors (mGLUR), ionotropic glutamate receptor subunits (iGLUR) and structural synaptic components have been added to the pathway using the IPA ‘Build’ tool. Transcripts encoding components from the whole pathway are dysregulated at the whole gene and/or isoform level, including ionotropic AMPA and NMDA glutamate receptor subunits, metabotropic glutamate receptors and voltage-gated calcium channels, all
of which can control the calcium ion influx or G-protein activation that initiates the pathway.

Genes encoding several synaptic scaffolds that are required to generate and maintain synapse structure/size and/or anchor glutamate receptors and calcium channels are also dysregulated, including Shank1, Homer1 and Dlg1/3/4, neurexins and neuroligins. Also dysregulated are genes encoding various factors downstream of glutamate receptors and calcium channels that activate the cAMP-dependent transcription factor CREB, such as various forms of Camk2, and adenyl cyclases. The transcriptional machinery is additionally affected, including the cAMP-dependent transcription factor complex. Double outlines indicate protein complexes and classes, the components of which can be found in Supplementary Table 2a, b. Colour intensity represents strength of gene expression change, with graded colour within double outlined symbols representing overall direction of change within protein complexes. green, downregulated; red, upregulated; *genes identified by DEXSeq; ** genes identified by DEXSeq and DESeq2 c Sunburst plots showing SynGO annotated synaptic functions of the dysregulated proteins found in homozygous Der1 hippocampus synaptosomes (FDR adjusted p-value < 0.05). Note that synaptosomes are enriched for the complete presynaptic terminal, the postsynaptic membrane and the postsynaptic density, as well as membranes originating from organelles such as the Golgi and endoplasmic reticulum73. d Quantification of AMPA and NMDA receptor currents by whole-cell patch clamping of neurons cultured from Der1 hippocampus. Data were analysed by one-way ANOVA, p=0.03. Horizontal line on graphs for each sample, average of values; WT, wild-type; HET, heterozygous Der1; HOM, homozygous Der1; *, p<0.05

Fig. 3 The Der1 mutation dysregulates canonical pathways and genes related to schizophrenia and depression in heterozygous Der1 cortex. a Canonical pathway predictions for putative schizophrenia risk genes, and for orthologues of putative schizophrenia and depression risk genes that are dysregulated at the whole gene and exon level, as identified using DESeq2+DEXSeq data. b, c Altered schizophrenia risk gene orthologue expression in the ‘Synaptic long-term depression’ and ‘CREB signalling in neurons’ canonical pathways,
respectively. Double outlines indicate protein complexes and classes, the components of which can be found in Supplementary Table 2a, b. To provide additional information, genes encoding ionotropic glutamate receptor δ subunits (Grid), AMPA receptor subunits (AMPAR), voltage-gated calcium channel subunits (VGCC), calcium channels (CaCh), ionotropic glutamate receptor subunits (iGLUR) and structural synaptic components have been added to the pathways using the IPA ‘Build’ tool. *genes identified by DEXSeq; red, dysregulated putative schizophrenia risk gene orthologue
Functional brain defects in a mouse model of a chromosomal t(1;11) translocation that disrupts DISC1 and confers increased risk of psychiatric illness

Supplementary information

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Materials and methods

Mouse colony maintenance

Mice were housed in the Biomedical Research Facility at the University of Edinburgh. All mice were maintained in accordance with Home Office regulations, and all protocols were approved by the local ethics committee of the University of Edinburgh. Mouse genotyping was carried out as previously described.

Perfusion fixation and brain isolation

Mice were anaesthetized with intraperitoneal injection of 0.1ml/10g Fentanyl/Fluanisone (Hypnorm®) and Midazolam (Hyponovel®). Deep anaesthesia was ensured by measuring withdrawal reflexes. The mice were then transcardially perfused with 4% paraformaldehyde at a rate of 0.2-0.5 ml/second. Brains were dissected out and the olfactory bulbs and cerebellum removed. Brains were transferred to 4% neutral buffered formalin for 24 hr, then stored in 70% ethanol.

Magnetic resonance imaging

Brains were taken from twelve same-sex littermate genotype trios (one wild-type, one heterozygote, one homozygote from the same litter, six male and six female trios). Brains were removed from 70% ethanol and incubated for three weeks in 8mM gadolinium contrast agent. Brains were then transferred to a 2ml Eppendorf tube filled with Fomblin and scanned in pairs using a three-dimensional gradient echo pulse sequence and an Agilent 7T DirectDrive MRI scanner, with acquisition parameters as follows; matrix 512x192x192 (reconstructed to 512x256x256); field of view 40x10x10 mm; repetition time/echo time (TR/TE) 30/10 ms; 20 signal averages; total scan time 8.2 hours. A 26mm radiofrequency coil was used for signal transmission and reception. Magnetic resonance images were processed blind to genotype using a combination of FSL, ANTs and in-house C++ software utilizing the ITK library, available from https://github.com/spinicist/QUIT. In brief, multi-
head scans were bias-field corrected before being split into individual sample images.

Registration was then performed between each subject and the Dorr atlas image to ensure all samples were aligned. An average study template image was then constructed using MR images from all animals. The resulting template was then non-linearly registered to the atlas image. All subject images were then non-linearly registered to the study template. The inverse transforms from the atlas to the study template and from the study template to each subject were applied to calculate the total brain volume and individual brain region of interest (ROI) volumes of each subject. ROIs match those found in the Dorr atlas.

**Histology**

Five 9 week old male littermate genotype trios were used for histological analysis except where indicated below. Perfused brains were removed from 70% ethanol and paraffin wax-embedded, then sections were cut from three different zones of the brain; Bregma ≈ 2.46 (prefrontal cortex); Bregma ≈ 0.75 (lateral ventricles and corpus callosum); Bregma ≈ -1.94 (hippocampus).Brains were processed by the University of Edinburgh Shared University Research Facilities (SURF), using a Leica RM2235 base sledge microtome. Twenty coronal sections of 10μm were cut for each block. Sections were mounted on to Superfrost Plus slides (ThermoFisher Scientific) and oven-dried. Two successive sections were used per location for each procedure.

To visualize cytoarchitecture by Nissl staining, sections were dewaxed in xylene, then rehydrated through graded alcohols. Rehydrated slides were incubated for 2 minutes in 0.2 % Cresyl fast violet solution containing 10 drops of acetic acid per 100 ml. Sections were dehydrated through graded alcohols, then cleared in xylene and cover-slipped with the xylene-based mounting solution DPX (Fisher Scientific).

To examine Parvalbumin-expressing neurons, sections were dewaxed and endogenous peroxidase activity quenched by incubating in methanol containing 1% hydrogen peroxide for 30 minutes. Sections were incubated in 10 mM sodium citrate buffer
at room temperature, then microwaved for 20 minutes at high power. Sections were cooled on ice for 20 minutes, then blocked using 20% normal goat serum (Vector) for 1 hour. Sections were next incubated overnight at 4°C with mouse anti-Parvalbumin primary antibody (Sigma-Aldrich P3088, 1:400 dilution), followed by incubation at room temperature for one hour with biotinylated goat anti-mouse secondary antibody (Sigma-Aldrich, 1:200 dilution). Next, sections were incubated in Vectastain Elite Avidin-Biotin Complex (Vector Laboratories) for 30 minutes before visualization by incubation with 0.05% 3,3’-diaminobenzidine tetrahydrochloride (DAB, Sigma-Aldrich) containing 0.001% hydrogen peroxide. Finally, sections were dehydrated through graded alcohol, cleared in xylene, and cover slipped with DPX. To analyse the distribution of interneurons, the prefrontal cortex sections were initially separated into different regions of interest, distinguished using anatomical features and the Mouse Brain Atlas in stereotaxic coordinates, then combined.

In the hippocampal sections interneurons were counted in CA1 and the dentate gyrus, as well as the whole hippocampal area. ROIs were set at the same position on each section and the cells were counted manually, blind to genotype, using the Fiji ‘Cell Counter’ plugin.

To examine apoptotic cells, the same procedure was used except the primary antibody was specific for active (cleaved) caspase-3 (Sigma-Aldrich, AB3623, 1:70 dilution), the secondary antibody was biotinylated goat anti-rabbit (Sigma-Aldrich, 1:200 dilution), with nickel was added to the DAB solution. Sections were counterstained with Nuclear Fast Red (Vector Laboratories).

For cortical layer measurements in barrel cortex, brains were taken from three to five month old mice and immediately placed in 4% paraformaldehyde overnight. Fixed brains were sectioned using a cryostat (Leica) coronally at 50µm from rostral to caudal. Sections were mounted onto Superfrost slides (VWR) in gelatin solution. The slices were washed in acetone and water, then stained in 1% thionin-Nissl and dehydrated in increasing concentrations of alcohol, cleared in xylene and then coverslipped using DPX (Fluka).

**Image analysis**
For most purposes, images of brain sections were captured using a dotSlide scanner (Olympus). Equivalent areas of both hemispheres were quantified on each slide, blind to genotype, then averaged per animal. Cell density was determined within the regions of interest shown (Supplementary Figure 2), which were manually drawn and set using the Fiji region of interest manager, with area determined and cells counted using the Fiji ‘Cell Counter’ plugin.

For cortical layer measurements in barrel cortex, brain sections were imaged using a light microscope (Olympus). Barrel cortex could be identified by the presence of barrels in layer IV cortex (Bregma anterior-posterior 0.38 to -1.94mm) whilst sections of brain containing prefrontal cortex (Bregma 2.80 to 2.10mm) were identified using a mouse brain atlas. Analysis of cortical thickness was performed within distinct brain areas. For cortical layer thickness, layers I, II/III, IV and V/IV were measured as distances perpendicular to the pial surface in addition to the total cortical thickness in barrel cortex. Total cortical thickness between the central sulcus and white matter for limbic cortex was measured for prefrontal cortex using Camera Lucida (Olympus).

**RNA sequencing**

Hippocampi, and cortices minus hippocampus, cerebellum and olfactory bulbs, were dissected from the right brain hemisphere mice at nine weeks of age. Samples were snap frozen in liquid nitrogen and stored at -80°C, then processed in batches of mixed genotypes to extract the RNA. Total RNA samples were assessed with a Fragment Analyser (Agilent) for quality and integrity of total RNA. Libraries were prepared using 100ng of each total RNA sample using the TruSeq Stranded mRNA Library Prep Kit (Illumina). Single end RNA Sequencing was carried out to a depth of approximately 60 to more than 100 million reads. Demultiplexing of sequencing reads was carried out using CASAVA (version 1.8.2, Illumina), with adapters trimmed using Skewer (version 0.1.116). Raw sequence reads were mapped to mouse reference genome mm10 using STAR (version 2.4.0h).
Raw counts at gene level were obtained using htseq-count\textsuperscript{12} (version 0.7.2, in the default union mode) on the alignment bam files and the Ensembl release 85 mouse gtf file. Differential gene expression was analysed using DESeq2 from the R statistical package\textsuperscript{13}. Differential exon expression was analysed using DEXSeq\textsuperscript{14} (version 1.19.4) using exon counts obtained by running the script “dexseq_count.py” provided by the Deseq package. Adjusted-p-values were calculated via a Benjamini-Hochberg Procedure to get False Discovery Rate (FDR), the default in Deseq2 package. Raw count data for all samples were together subjected to a regularised logarithm transformation\textsuperscript{10} using the DESeq2 package version 1.16.1. For each heat map, the transformed counts for each gene were normalised to Z-scores across all samples.

Heat maps of gene expression were generated using R (version 3.4.2) and RStudio (version 1.0.143).

**Expression-weighted cell-type enrichment (EWCE) analysis**

This analysis used the Karolinska Institute ‘Superset’ of RNASeq profiles generated from six independent single cell RNASeq studies of several brain regions and cell types. The Superset consists of 24 cellular classes generated by hierarchical clustering of nearly 9,970 mouse brain single cell RNASeq profiles (all generated by exactly the same method) followed by cell type identity assignment\textsuperscript{15}. Profiles consist of a set of specificity values which provide a measure of gene expression enrichment (calculated from mean expression of each gene in a cellular class divided by its mean expression in all cellular classes) for each gene detected in that class\textsuperscript{15}. Some cells were isolated from mouse cortex, hippocampus, striatum, hypothalamus and midbrain, while others were the result of specifically isolating cortical Parvalbumin-positive interneurons or oligodendrocytes from multiple brain regions including somatosensory cortex and hippocampus. The ages of the mice used to generate the profiles include embryonic and a range from P14 to P90. Each class and profile is therefore an amalgamation of single cell profiles from closely related cell
types, not all necessarily from the same brain region or age. Superset profiles were downloaded from http://hjerling-leffler-lab.org/data/scz_singlecell using specificity table: ctd[[1]]$specificity and expression table: ctd[[1]]$mean_exp. Analysis was carried out in the R package using script downloaded from https://github.com/NathanSkene/EWCE/ (version 0.99.2), and default options with Bonferroni multiple testing correction. The full list of expressed Der1 cortex or hippocampus genes was used as background, as appropriate. The script was run with 10,000 repetitions. The Superset samples were sequenced at a lower depth than the Der1 samples, and using unique molecular identifiers. Consequently only the most abundantly expressed genes (up to 14,581) were detected. For cortex, 1,794 of 2,125 dysregulated genes are present in the Superset profiles. For hippocampus, 151 of 175 dysregulated genes are present in the Superset profiles. Der1 whole gene DESeq2 RNASeq data were used for this analysis.

RNASeq deconvolution

RNASeq deconvolution was carried out using the cell Karolinska Institute ‘Superset’ of RNASeq profiles described above under EWCE analysis as reference. Specificity value thresholds of 0.75 and 0.6 were set to ensure that the most highly enriched genes were used in this analysis, thus profile signatures consisted of genes with at least one specificity value in one cell type above these thresholds. To provide context, specificity value=1 represents 100% specificity for one cell class, the astrocyte marker Gfap exhibits a specificity value of 0.87 in the astrocyte/ependymocyte class, the oligodendrocyte marker Mbp exhibits a specificity value of 0.6 in the oligodendrocyte class, the interneuron marker Parvalbumin exhibits specificity values of 0.41 and 0.27 in the interneuron and striatal interneuron classes, respectively, and the synapse marker Dlg4 (Psd95) exhibits specificity values of ~0.1 in pyramidal neurons and <0.1 in other neuron classes. This resulted in the use of 346 genes for threshold=0.75, and 752 genes for threshold=0.6, of which 285 and 653 are present in our wild-type RNASeq data.
Deconvolution was carried out using CIBERSORT Jar Version 1.06 (May 5th 2017)\textsuperscript{16}, available at the web interface \url{https://cibersort.stanford.edu}. The ability of CIBERSORT to accurately deconvolute the 24 cell types in the Superset was examined by creating artificial cell mixes by combining Superset gene expression values in various proportions from 0 to 0.5. CIBERSORT input was then compared to output to determine the efficiency of artificial sample deconvolution for the 24 cell types.

**Pathway analysis**

DESeq2 data were examined separately or combined with DEXSeq data by Ingenuity Pathway Analysis (IPA, Qiagen), using corrected $p$ values and log2 fold changes, and the corresponding full list of expressed genes for each brain region as the background gene set. Human t(1;11) translocation neuron RNASeq data\textsuperscript{1} were similarly analysed using IPA. Pathway analysis of putative schizophrenia or depression risk genes was carried out using IPA and the full cortical gene expression list as the background gene set. Pathway analysis of dysregulated orthologues of putative schizophrenia or depression risk genes used the corresponding full list of expressed genes for each brain region as the background set. Adjusted $p<0.05$, and $z>2$ or $z<-2$ were used as thresholds throughout. Pathway analysis of dysregulated genes from cell class profiles used the full Superset profile as background. A specificity value threshold of 0.2 was set to ensure that a sufficient number of the most specific genes were used in the analysis. For context regarding specificity values see deconvolution, above. Where DEXSeq identified dysregulated sequences that did not unambiguously map to a single gene, or mass spectrometry identified peptides that could not be unambiguously mapped to a single protein (due to close homology with other proteins), all possible genes and proteins were included in the pathway analysis.

**Synaptosome preparation and mass spectrometry**

Synaptosomes were prepared from 8-10 week old Der1 cortex and hippocampus (six wild-type, five heterozygous, five homozygous) as previously described\textsuperscript{17}. Tissue was
homogenized in HEPES buffer (5 mM HEPES, pH 7.4, 0.32 M sucrose supplemented with protease inhibitor cocktail, Roche) and centrifuged at 1000 x g for 10 min at 4°C. The supernatant was subsequently centrifuged in a 0.85/1.2 M sucrose gradient at 100,000 x g for 2 hours. Synaptosomes were recovered from the 0.85/1.2 M sucrose interface and concentrated by centrifugation at 18,000 x g for 30 min.

Samples were digested using filter aided sample preparation (FASP) with some modifications\textsuperscript{18}. Briefly, 20 μg of each protein sample were incubated with 75 μL 2% SDS, 1 mM Tris(2-carboxyethyl)phosphine at 55°C for 1 hour, after which samples were incubated with 0.5 μL 200 mM methyl methanethiosulfonate for 15 min. Next, 200 μL 8 M Urea in Tris pH 8.8 were added and the samples were transferred to Microcon-30 filter tubes (Millipore). Samples were washed 4 times with 8M Urea in Tris buffer and 4 times with 50 mM ammonium bicarbonate by centrifugation at 14,000 x g for 10 min each. Proteins were digested with 0.7 μg Trypsin/Lys-C Mix (MS grade, Promega) overnight at 37°C. Peptides were eluted with 200 μL 50 mM ammonium bicarbonate, dried in SpeedVac and stored at -20°C.

Peptides were analysed by micro LC MS/MS using an Ultimate 3000 LC system (Dionex, Thermo Scientific) and the TripleTOF 5600 mass spectrometer (Sciex). Peptides were trapped on a 5 mm Pepmap 100 C18 column (300μm i.d., 5μm particle size, Dionex) and fractionated on a 200 mm AlltimaC18 column (300μm i.d., 3μm particle size). The concentration of acetonitrile in the mobile phase was increased at a flow rate of 5μL/min from 5 to 18% in 88 min, to 25% at 98 min, 40% at 108 min and to 90% in 2 min. Peptides were electro-sprayed into the mass spectrometer with a micro-spray needle (at 5500 V). The mass spectrometer was operated in a data-independent mode, as described in\textsuperscript{19}. Each cycle consisted of a parent ion scan of 150 msec and 8 Da MS/MS windows (80 msec scan time each), throughout a 450-770 m/z mass range. The collision energy for each window was calculated for a 2+ ion centered upon the window (spread of 15 eV).

The data were analysed with Spectronaut Pulsar v 12.0.20491.21.28109\textsuperscript{20} and using a spectral library created by data-dependent acquisition from hippocampal synapse-enriched
samples containing spike-in iRT peptides (Biognosys). Cross-run normalization was enabled using local normalization strategy. Only peptides quantified with a Q-value ≤ $10^{-2}$ and $10^{-3}$ (for hippocampus and cortex datasets, respectively) across all samples in at least two groups were considered. Limma R package was used to Loess normalize protein abundance (‘normalizeCyclicLoess’ function, ‘fast’ method and 10 iterations). Volcano plots were generated using R (version 3.6.2). Protein were annotated to synaptic genes and sunburst plots were generated using SynGO 1.0 database and online tool\(^{21}\).

**Hippocampal Cell Culture and Electrophysiological recordings**

Primary hippocampal cultures were prepared from individual E17.5 DER littermate pups as described\(^{22}\). Briefly, hippocampi were dissected from pups, incubated in Papain, dissociated and grown in Neurobasal A growth medium containing 1% Rat Serum and supplemented with B-27, and maintained until Days In Vitro (DIV) 21.

Whole cell patch clamp recordings were performed as described\(^{23}\). Briefly, coverslips containing DIV 21 hippocampal neurons were transferred to a recording chamber with a constant (3-5ml/min) perfusion of external recording solution containing: 150 mM NaCl, 2.8 mM KCl, 10 mM HEPES, 2 mM CaCl\(_2\), 10 mM D-glucose and 100 μM glycine, pH 7.35, 320 mOsm. Tetrodotoxin citrate (300 nM) was included to block action-potential driven excitatory events. Patch-pipettes were pulled from borosilicate glass (Harvard Apparatus, Kent, UK) with a resistance of 3-5 MΩ, and filled with a K-gluconate-based internal solution containing: 141 mM Potassium Gluconate, 2.5 mM NaCl, 10 mM HEPES, 11 mM EGTA, pH 7.35. Currents were evoked by S-AMPA (50 μM) and NMDA (150 μM). All currents were recorded at room temperature, using an axopatch 200B amplifier (Molecular Devices, Union City, CA). Neurons were voltage-clamped at -60 mV. Whole-cell currents were analysed using WinEDR v3.2 software (John Dempster, University of Strathclyde, UK), with currents normalised to cell capacitance. For statistical analysis, n was taken as the number of pups, with n=3 WT, 4 HET and 3 HOM. A total of 12 WT, 14 HET and 11 HOM genotype coverslips were recorded from.
Statistical analysis

For analysis of MRI data, a multivariate general linear model 2-way MANCOVA statistical test was performed using SPSS statistics 22 (IBM) to determine group-level differences in brain ROI volumes with genotype as fixed effect, total brain volume and brain region as dependent variable, and littermate trio groupings as covariate.

For enrichment analysis, hypergeometric probabilities were calculated using keisan.casio.com/exec/system/1180573201. As with the pathway analysis, where DEXSeq identified dysregulated sequences that did not unambiguously map to a single gene, or mass spectrometry identified peptides that could not be unambiguously mapped to a single protein (due to close homology with other proteins), all possible genes and proteins were included in the enrichment analysis.

For the proteomic analysis, empirical Bayes moderated t-statistics with multiple testing correction by false discovery rate were performed on log-transformed protein abundances (‘eBayes’ and ‘topTable’ functions from Limma R package), as previously described\textsuperscript{18, 19, 24, 25}. Proteins with a FDR adjusted p-value < 0.05 were considered significantly regulated for subsequent downstream analysis.

Other statistical analyses were carried out using GraphPad Prism, with statistical tests used stated in figure legends.

Supplementary Table 1 (Excel file) Magnetic resonance imaging data. Both hemispheres, regional volumes (mm\(^3\)) corrected to individual whole brain volumes, left and right hemispheres combined, separate hemispheres, regional volumes (mm\(^3\)) corrected to individual whole brain volumes, left and right hemispheres considered separately

Supplementary Table 2 (Excel file) RNA sequencing data. a DeSeq2 (whole gene differential expression) Der1 cortex data, b DEXSeq (exon level differential expression) Der1
cortex data, **c** DeSeq2 (whole gene differential expression) **Der1** hippocampus data, **d** DEXSeq (exon level differential expression) **Der1** hippocampus data. In each case data are provided with comparisons to human IPSC-derived cortical neuron cultures from members of the t(1;11) family, two large-scale genome-wide association studies of schizophrenia, synapse genes from a large-scale schizophrenia CNV study, two large-scale genome-wide association studies of depression, a large-scale genome-wide association study of bipolar disorder, a large-scale genome-wide association study of Alzheimer's Disease where matches were found, and a large-scale genome-wide association study of cerebral cortex architecture, where matches were found (references numbered according to main text). Overlaps are represented by a gene name in the relevant genetic study column. Non-overlaps are represented by empty cells, BaseMean, mean of normalised counts of all samples; p value, p value for wild-type versus heterozygous; adjusted p value, p value adjusted for multiple testing.

**Supplementary Table 3** (Excel file) Dysregulated genes with conserved cAMP response elements according to [http://natural.salk.edu/creb/](http://natural.salk.edu/creb/).

**Supplementary Table 4** (Excel file) Ingenuity Pathway Analysis functions. **a** functions enriched for dysregulated genes in **Der1** cortex. All functions in the categories 'Molecular and cellular function' and 'Physiological system development and function' are included. Selected top relevant functions are provided in Table 1. Data are provided with comparisons to functions predicted from human IPSC-derived cortical neuron cultures from members of the t(1;11) family (reference numbered according to main text). Overlaps are represented by an x in the human neuron column. Non-overlaps are represented by empty cells, **b**, functions enriched for dysregulated genes in **Der1** hippocampus. All functions in the categories 'Molecular and cellular function' and 'Physiological system development and function' are included. Selected top relevant functions are provided in Supplementary Table
5. **c**, functions enriched for dysregulated genes in Superset cell classes. All functions in the categories 'Molecular and cellular function' and 'Physiological system development and function' are included. Selected top relevant functions are provided in Supplementary Table 6.

6. **d**, functions enriched for dysregulated genes in human iPSC-derived cortical neuron cultures from members of the t(1;11) family. All functions in the categories 'Molecular and cellular function' and 'Physiological system development and function' are included. Selected top relevant functions are provided in Table 1. The genes listed for each function are dysregulated in the corresponding dataset.
Supplementary Table 5  Top predicted relevant altered functions in heterozygous *Der1* mouse hippocampus.

| Function (no. of molecules\(^a\)) | *Der1* hippocampus score (no. of genes\(^b\)) |
|-----------------------------------|-----------------------------------------------|
| **General cell morphology**        |                                               |
| Development of neurons (1,423)    | \(p=2e^{-9}\) (33)                           |
| Morphology of neurons (1,125)     | \(p=8e^{-7}\) (22)                           |
| Maturation of neurons (114)       | \(p=8e^{-6}\) (7)                            |
| Abnormal morphology of neurons (923) | \(p=3e^{-5}\) (16)                        |
| Differentiation of neurons (648)  | \(p=5e^{-4}\) (14)                           |
| **Cell contact**                  |                                               |
| Adhesion of neuronal cells (89)   | \(p=8e^{-9}\) (9)                            |
| Formation of plasma membrane (406) | \(p=1e^{-8}\) (17)                        |
| Cell-cell contact (1,118)         | \(p=1e^{-5}\) (22)                           |
| Cell-cell contact of neurons (24) | \(p=1e^{-5}\) (4)                            |
| Cell-cell adhesion of neurons (22) | \(p=3e^{-4}\) (3)                         |
| **Cytoskeleton**                  |                                               |
| Neurogenesis (1,067)              |                                               |
| Formation of cellular protrusions (1,645) | \(p=1e^{-4}\) (26)                     |
| Growth of neurites (910)          | \(p=2e^{-4}\) (16)                           |
| Branching of cells (746)          | \(p=2e^{-4}\) (14)                           |
| Extension of neurites (267)       | \(p=5e^{-4}\) (8)                            |
| **Axons**                         |                                               |
| Extension of axons (134)          | \(p=5e^{-3}\) (5)                            |
| Myelination of optic nerve (8)    | \(p=2e^{-3}\) (2)                            |
| Myelination (8)                   | \(p=5e^{-3}\) (7)                            |
| **Dendrites**                     |                                               |
| Formation of dendrites (209)      | \(p=2e^{-4}\) (8)                            |
| Dendritic growth/branching (446)  | \(p=4e^{-4}\) (10)                           |
| Density of dendritic spines (143) | \(p=1e^{-3}\) (5)                            |
| Morphology of dendrites (138)     | \(p=3e^{-3}\) (5)                            |
| Length of dendrites (47)          | \(p=3e^{-3}\) (3)                            |
| **Cell proliferation**            |                                               |
| Proliferation of epithelial cells (996) | \(p=3e^{-3}\) (14)                        |
| Neurogenesis of cerebral cortex (69) | \(p=5e^{-3}\) (3)                         |
| Proliferation of stem cells (372) | \(p=8e^{-3}\) (7)                            |
| **Transport**                     |                                               |
| Exocytosis (336)                  | \(p=9e^{-4}\) (8)                            |
| Transport of dopamine (76)        | \(p=8e^{-4}\) (3)                            |
| Secretion of neurotransmitter (248) | \(p=1e^{-3}\) (7)                        |
| Release of neurotransmitter (510) | \(p=1e^{-3}\) (7)                            |
| Transport of 5-hydroxytryptamine (40) | \(p=2e^{-3}\) (2)                        |
| **Neurotransmission**             |                                               |
| Developmental process of synapse (303) | \(p=3e^{-9}\) (16)                        |
| Neurotransmission (716)           | \(p=8e^{-8}\) (20)                           |
| Synaptic transmission (558)       | \(p=3e^{-7}\) (17)                           |
| Maturation of synapse (36)        | \(p=3e^{-5}\) (4)                            |
| Miniature excitatory postsynaptic currents (71) | \(p=2e^{-4}\) (5)                        |
| Plasticity of synapse (170)       | \(p=3e^{-4}\) (7)                            |
| Excitatory postsynaptic potential (166) | \(p=3e^{-4}\) (7)                        |
| Paired-pulse facilitation of synapse (55) | \(p=9e^{-4}\) (4)                        |
| Action potential of cells (238)   | \(p=1e^{-3}\) (7)                            |
| Formation of excitatory synapses (14) | \(p=3e^{-3}\) (2)                         |

A full list of functions is provided in Supplementary Table 4b. Related functions are grouped, with top functions shown for each group. \(a\), total number of molecules relating to each IPA function; \(b\), number of dysregulated genes relating to each function.
A full list of functions is provided in Supplementary Table 5c, e. The most highly enriched genes that are dysregulated in Der1 cortex for each cell class were used for IPA analysis, with specificity value cut-off=0.2 (SV=1 indicates 100% specificity, see methods for more context). In many cell classes the relatively low number of genes above this threshold was
insufficient for meaningful pathway analysis. a, total number of molecules relating to each IPA function; b, number of dysregulated genes relating to each function

Supplementary Table 7 (Excel file) Synaptosome mass spectrometry data. a, Mass spectrometry analysis of cortex synaptosomes isolated from wild-type (WT), heterozygous (HET) or homozygous (HOM) Der1 mice, b, Mass spectrometry analysis of hippocampus synaptosomes isolated from wild-type (WT), heterozygous (HET) or homozygous (HOM) Der1 mice, c, SynGo annotations. SD, standard deviation
**Supplementary table 8** Comparison between characteristics of the Der1 mouse and pertinent characteristics of mutant mice that are known or proposed to be relevant to the t(1;11) translocation.

| Mutant                          | Brain structure                                      | Synapses & plasticity                          | Electrophysiology                                      | Neuronal intracellular transport |
|---------------------------------|------------------------------------------------------|------------------------------------------------|--------------------------------------------------------|----------------------------------|
| **Der1**                        | ↑ hippocampal Parvalbumin-positive interneuron density altered oligodendrocyte-myelin function\(^{26}\) no gross structural changes | ↑ surface/synaptic NMDA receptor expression in cultured hippocampal neurons\(^{1}\) altered Psd95 distribution indicative of an increased density of weaker synapses\(^{1}\) altered expression of genes involved in synapse formation, structure & function altered expression of genes critical for synaptic plasticity and long-term potentiation, including the CREB signalling pathway | ↓ AMPA/NMDA ratio in cultured hippocampal neurons | ↑ NMDA receptor motility\(^{1}\) altered expression of genes required for vesicle transport and exo/endocytosis |
| **humanised DISC1-Boyman & Boyman-DISC1\(^{27}\)** | endogenous mouse Disc1 gene replaced with human DISC1-Boyman or Boyman-DISC1 cDNA fusion transgenes (Boyman is otherwise known as DISC1FP1) resulting in Disc1 promoter-driven forced expression of putative chimeric proteins\(^{26}\) (whose expression in t(1;11) carriers remains to be established) | ↓ cortical expression of NMDA receptor subunit GluN1 and Psd95\(^{27}\) | | |
| **Disc1\(\Delta\)2-3\(^{29}\)** | deletion of exons 2 & 3 from endogenous mouse Disc1 gene, abolishes full-length Disc1 expression | ↓ density of Parvalbumin-positive interneurons in many cortical areas\(^{30}\), and in hippocampus\(^{31}\) no gross structural changes\(^{39}\) | catecholaminergic network dysfunction\(^{32}\) ↓ methamphetamine-induced dopamine release & ↑ dopamine receptor expression in nucleus accumbens\(^{31}\) | ↑ threshold for induction of long-term potentiation in hippocampus\(^{29}\) ↓ dendritic ITTPR1 mRNA transport in cultured hippocampal neurons\(^{33}\) ↓ synaptic vesicle exocytosis\(^{34}\) |
| **Disc1-L1\(^{26}\)** | deletion of exons 1-3 from endogenous mouse Disc1 gene, abolishes full-length Disc1 expression | | altered parvalbumin-positive interneuron function\(^{26}\) | | |
| **Disc1\(^{c}\)**               | C-terminally truncated Disc1 (encoded by exons) | ↓ density of Parvalbumin-positive interneurons in hippocampus and medial prefrontal | ↓ NMDA receptor GluN2A & GluN2B, ↑ GluN1 (trend) protein expression in hippocampus\(^{36}\) | ↑ long-term potentiation in Schaffer collateral commissural pathway temporoammonic long-term potentiation |
| 1-8) fused to green fluorescent protein, expressed from transgenic mouse bacterial artificial chromosome under control of Disc1 promoter | cortex, and displacement in dorsolateral prefrontal cortex\(^37\)  
† lateral ventricle volume  
↓ cerebral cortex thickness  
partial agenesis of corpus callosum\(^37\) | abolished\(^39\)  
altered hippocampus-prefrontal cortex connectivity & reduced neurotransmitter release probability in the glutamatergic hippocampal CA1–prefrontal cortex projection\(^38\) |
| hDISC1\(^\text{40}\)  
C-terminally truncated DISC1 (exons 1-8) transgene under inducible control of CaMKII promoter | ↓ density of Parvalbumin-positive cortical interneurons\(^41\)  
† lateral ventricle volume\(^40\)  
altered oligodendrocyte specification\(^42, 43\) | ↓ spontaneous excitatory postsynaptic currents in cultured cortical neurons\(^46\)  
altered expression of proteins required for vesicular transport\(^49\) |
| DN-DISC1\(^\text{50}\)  
C-terminally truncated DISC1 (exons 1-8) transgene under control of CamKII promoter | ↓ density of Parvalbumin-positive cortical interneurons\(^50\)  
† lateral ventricle volume\(^50\) | oscillations in hippocampal CA1\(^51\)  
abnormal action potentials, and dopaminergic regulation, in fast spiking parvalbumin-positive interneurons of prefrontal cortex\(^52\) |
| DN-DISC1-PrP\(^\text{53}\)  
C-terminally truncated DISC1 (exons 1-8) transgene under control of PrP promoter | no gross structural changes\(^53\) |  |
| nes-DN-DISC1\(^\text{54}\)  
C-terminally truncated DISC1 (exon 1-8) transgene inducibly expressed in neural precursor cells | ↓ density of Parvalbumin-positive interneurons in cingulate cortex, retrosplenial granular cortex, and motor cortex\(^54\) |  |
| Disc1\(^\text{TM}^{\text{55}}\)  
natural deletion within mouse Disc1 | Parvalbumin-positive interneuron density unchanged\(^55\) | ↓ dendritic spine density & altered spine morphology in cultured hippocampal and  
short-term potentiation at hippocampal CA1-CA3 synapse  
↓ synaptic vesicle volume at hippocampal CA3 synapses\(^56\) |
| exon 6 that introduces a premature termination codon, combined with targeted premature transcription termination signal in intron 8, abolishes full-length Disc1 expression and may express C-terminally truncated protein due to the termination codon within exon 7 | ↓ prefrontal cortex volume\textsuperscript{56} | altered hippocampal CREB signalling\textsuperscript{56} | altered short-term plasticity at mossy fibre-CA3 circuit\textsuperscript{56} |
| --- | --- | --- | --- |
| | | | ↑ neuronal excitability in medial prefrontal cortex\textsuperscript{59} |
| | | | ↑ short-term depression & probable ↑ neurotransmitter release probability in medial prefrontal cortex\textsuperscript{59} |
| | | | ↑ spontaneous excitatory postsynaptic currents in cultured cortical neurons\textsuperscript{49} |
| | | | altered spontaneous inhibitory postsynaptic currents in cultured cortical neurons\textsuperscript{49} |
| | | | proteomic changes suggest effects upon synaptic vesicle transport\textsuperscript{59} |

1322 The mutants fall into three main categories 1) recapitulation of the gene fusion between 1323 DISC1 and DISCFP1 (Der1, transgenic Boymaw fusions), 2) elimination of full-length Disc1 1324 expression (Der1, transgenic Boymaw fusions, Disc1Δ2-3, DISC1-LI, Disc1\textsuperscript{Tm1Kara}), 3) 1325 transgenic overexpression of a truncated form of Disc1 or DISC1 encoded by exons 1-8 that 1326 was inferred to arise from the t(1;11) prior to discovery of the DISC1/DISCFP1 gene fusion 1327 (Disc1\textsubscript{tr}, hDisc1, DN-DISC1, DN-DISC1-PrP, nes-DN-DISC1).
**THIS STUDY**

*Der1* mouse
endogenous Disc1 allele modified to recapitulate effect of t(1;11) upon DISC1 expression

**STRUCTURAL**

- Whole brain imaging
- Magnetic resonance imaging to examine brain structure
- Nissl staining of sections to examine brain structure at the cellular level

**OMICS**

- RNASeq analysis of tissue to identify deregulated genes
  - EWCE analysis of RNASeq data to identify cellular classes targeted by the Der1 mutation
  - RNASeq deconvolution of RNASeq data to examine relative cell class proportions
  - Pathway analysis to identify molecular pathways impacted by the Der1 mutation
    - Comparison to t(1;11) human neuron cultures to identify shared characteristics and confirm relevance of the Der1 mouse to studies of the t(1;11)
    - Mass spectrometry analysis of synaptosomes to confirm synaptic changes

**FUNCTIONAL**

- Electrophysiology to confirm altered synapse function

**RELEVANCE TO MAJOR MENTAL ILLNESS**

- Comparison between RNASeq and genetic data for major mental illness to confirm relevance of the t(1;11) and Der1 mouse
Supplementary Fig. 1 Flowchart indicating the experimental approach taken. Superscript numbers indicate references according to the main (not supplementary) text.
Supplementary Fig. 2 Regions of interest for image analysis of cell density, Parvalbumin and cleaved Caspase 3. a Hippocampus (HP). The box indicates the region of the Stratum, Radiatum, Lacunsum and Moleculare (SRLM) in which cell density was quantified. Dotted lines outline the hippocampal formation, CA1 and the dentate gyrus (DG) used for quantification of Parvalbumin-positive cells and cells expressing cleaved Caspase 3. b Prefrontal cortex (PFC). The box indicates the region in which cell density was quantified. The dotted line outlines the region in which Parvalbumin-positive cells and cells expressing cleaved Caspase 3 were quantified. Scale bars, 100μm
Supplementary Fig. 3 Brain structure visualised by Nissl staining. Sections through hippocampus (HP) a, prefrontal cortex (PFC) b, and corpus callosum (CC) c, were stained with Nissl to visualise cell bodies and tissue structure. scale bars, 100μm in a and b, 500μm in c d Quantification of average cell density from both sides of the brain in hippocampal
Stratum, Radiatum, Lacunosum and Moleculare, and PFC. Data were analysed by Kruskal-Wallis one-way ANOVA. Horizontal line on graphs, average of values for each sample; WT, wild-type; HET, heterozygous Der1; HOM, homozygous Der1
Supplementary Fig. 4 Cortical layers visualised by Nissl staining. Barrel cortex was used to examine layering in detail because the individual cortical layers could not be distinguished in prefrontal cortex. Sections through barrel cortex (BC) a, and prefrontal cortex (PFC) b, were stained with Nissl to visualise cell bodies and tissue structure. Cortical layers and measurements taken are indicated. c Quantification of layer thickness in barrel cortex and PFC. Two-way ANOVA found no effect of genotype on layer thickness \( (F_{1,40}=0.1959, \ p>0.05) \), nor any interaction between layer thickness and genotype \( (F_{3,40}= 0.6631, \ p>0.05) \) in
barrel cortex. Unpaired two-tailed t-test found no effect of genotype on cortical thickness in PFC (p=0.2). Horizontal line on graphs, average of values for each sample; scale bars, 200µm; WT, wild-type; HET, heterozygous Der1
Supplementary Fig. 5 No change in Parvalbumin-expressing interneuron density in Der1 prefrontal cortex. a Prefrontal cortex (PFC) sections from nine week old mouse brain were stained with an antibody specific for Parvalbumin. Enlarged regions showing Parvalbumin-expressing interneurons are indicated by boxes. Scale bars, 100μm b Average density of Parvalbumin-expressing interneurons from both sides of the brain. Data were analysed by Kruskal-Wallis one-way ANOVA. Horizontal line on graphs, average of values for each sample; WT, wild-type; HET, heterozygous Der1; HOM, homozygous Der1
a

Hippocampus

b

Hippocampus

CA1

Dentate gyrus

c

PFC

WT

HET

HOM

d

Prefrontal cortex

WT

HET

HOM
Supplementary Fig. 6 Quantification of apoptotic cells. a Hippocampal (HP) sections from nine week old mouse brain were stained with an antibody specific for Activated Caspase 3 and counterstained with Nuclear Fast Red. Enlarged regions showing apoptotic cells are indicated by white boxes. b Average density of hippocampal apoptotic cells from both sides of the brain. Hippocampus refers to the whole hippocampal formation. c Prefrontal cortex (PFC) sections from nine week old mouse brain were stained with an antibody specific for Activated Caspase 3 and counterstained with Nuclear Fast Red. Enlarged regions showing apoptotic cells are indicated by boxes. d Average density of PFC apoptotic cells from both sides of the brain. Data were analysed by Kruskal-Wallis one-way ANOVA, p=0.06 for CA1. Horizontal line on graphs, average of values for each sample; WT, wild-type; HET, heterozygous Der1; HOM, homozygous Der1; DG, dentate gyrus; scale bars, 100μm
Supplementary Fig. 7 Test deconvolution of the 24 Superset cell classes\textsuperscript{15}. Reference profiles were generated using stringent specificity value (SV) thresholds of 0.75 or 0.6 to ensure that each cell class was represented by its most specific genes. \textit{In silico} samples were created by mixing the thresholded gene expression profiles in proportions between 0 and 0.5. CIBERSORT input was compared to output and Pearson correlation coefficient and $R^2$ calculated to assess the quality of deconvolution of each artificial sample.
Supplementary Fig. 8 Deconvolution of heterozygous *Der1* cortex and hippocampus RNASeq data using the Superset cell class profiles\(^5\) as reference. Reference profiles were generated using stringent specificity value (SV) thresholds of 0.75 or 0.6 to ensure that each cell class was represented by its most specific genes. Note that although the proportions change with the threshold set, and therefore the number of specific genes used for deconvolution, the relative proportions of each cell class do not differ between genotypes.
Samples from embryonic cell types, neural progenitors and neuroblasts were not accurately deconvolved by CIBERSORT, thus their apparently high levels in the wild-type and *Der1* tissue are not an indication of true prevalence. Blue, wild-type; red, *Der1* heterozygote; Emb, embryonic
Supplementary Fig. 9 Der1 cortex gene dysregulation within the ‘Oxidative phosphorylation’ canonical pathway. Pathway impairment was predicted by IPA based on gene dysregulation at the whole gene level using DESeq2 data. Double outlines indicate protein complexes, the components of which can be found in Supplementary Table 2a, b. Colour intensity represents strength of gene expression change. green, downregulated; red, upregulated
Supplementary Fig. 10 Der1 cortex gene dysregulation within the ‘Fatty acid β-oxidation I’ canonical pathway. Pathway impairment was predicted by IPA based on gene dysregulation at the whole gene level using DESeq2 data. Double outlines indicate enzyme complexes. To provide additional information, genes encoding relevant dysregulated enzymes and a transporter have been added to the pathway using the IPA ‘Build’ tool. Colour intensity represents strength of gene expression change, with graded colour within double outlined symbols representing overall direction of change within protein complexes. green, downregulated; red, upregulated.
Supplementary Fig. 11 Volcano plots showing differentially expressed synaptosomes proteins in comparisons between Der1 mice and wild-type controls. No significant differences were found after multiple testing correction in cortex from heterozygous and homozygous Der1 mice, nor in hippocampus from heterozygous Der1 mice. In hippocampus from homozygous Der1 mice, 62 proteins were found to be significantly dysregulated (FDR adjusted p-value ≤ 0.05) as indicated by the coloured dots above the dashed line.
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