Symmetric neural progenitor divisions require chromatin-mediated homologous recombination DNA repair by *Ino80*

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Chromatin regulates spatiotemporal gene expression during neurodevelopment, but it also mediates DNA damage repair essential to proliferating neural progenitor cells (NPCs). Here, we uncover molecularly dissociable roles for nucleosome remodeler *Ino80* in chromatin-mediated transcriptional regulation and genome maintenance in corticogenesis. We find that conditional *Ino80* deletion from cortical NPCs impairs DNA double-strand break (DSB) repair, triggering p53-dependent apoptosis and microcephaly. Using an in vivo DSB repair pathway assay, we find that *Ino80* is selectively required for homologous recombination (HR) DNA repair, which is mechanistically distinct from *Ino80* function in YY1-associated transcription. Unexpectedly, sensitivity to loss of *Ino80*-mediated HR is dependent on NPC division mode: *Ino80* deletion leads to unrepaired DNA breaks and apoptosis in symmetric NPC-NPC divisions, but not in asymmetric neurogenic divisions. This division mode dependence is phenocopied following conditional deletion of HR gene *BrcA2*. Thus, distinct modes of NPC division have divergent requirements for *Ino80*-dependent HR DNA repair.
Emerging human genetic findings have implicated dysregulation of chromatin as a contributor to developmental brain disorders\(^1\,2\). Altered chromatin function can perturb neural cell fates, maturation, or plasticity via transcriptional dysregulation\(^3\,4\). Chromatin, however, also plays key roles in DNA-damage repair\(^5\,6\). How disruption of chromatin-mediated genome maintenance affects brain development and whether it contributes to neurodevelopmental disorders remains largely unexplored.

Safeguarding genome integrity in proliferating neural progenitor cells (NPCs) is essential for neurodevelopment\(^6\,7\). In fetal cerebral cortex, neurons and glia are generated by NPCs via successive rounds of cell division\(^8\,9\). In this process, genome damage, notably DNA double-strand breaks (DSBs), inevitably arises during DNA synthesis and mitosis\(^7\). DSBs are mainly repaired by one of two major pathways: homologous recombination (HR) and non-homologous end joining (NHEJ)\(^10\)\(^\text{11}\). HR uses homologous sequence in S and G2 phases of the cell cycle to seamlessly repair DSBs, whereas NHEJ ligates free DNA ends throughout the cell cycle in a homology-independent manner that can introduce indels or structural rearrangements\(^11\). DSB repair occurs in the context of chromatin. Repair pathway choice and efficiency are regulated by nucleosome remodelers that control the accessibility and mobility of chromatin\(^5\,6\). Disruption of chromatin regulators may therefore contribute to neurodevelopmental disorders by impairing DNA repair in dividing NPCs, which could trigger DNA-damage response or lead to brain somatic mutations. Although emerging evidence suggests that somatic mutations can contribute to disorders of the brain\(^14\), whether post-zygotic mutations can arise as a result of chromatin dysregulation remains unknown.

Here, we uncover mechanistically distinct roles of \(\text{Ino80}\) in chromatin-mediated transcriptional regulation and genome maintenance in corticogenesis. \(\text{Ino80}\) encodes the catalytic subunit of the INO80 complex that mediates nucleosome remodeling and histone variant exchange in gene regulation and DNA repair\(^15\)\(^\text{19}\). \(\text{INO80}\) was recently identified as a candidate gene for microcephaly and intellectual disability\(^20\). The neurodevelopmental roles of \(\text{INO80}\) and how its disruption contributes to disordered brain development had not been explored.

We find that conditional deletion of \(\text{Ino80}\) from embryonic cortical NPCs leads to accumulation of unrepaired DSBs, which trigger p53 target activation, robust apoptotic responses, and microcephaly. These \(\text{Ino80}\) deletion phenotypes are extensively rescued following co-deletion of \(\text{Trp53}\), confirming their dependence on p53 response to DNA damage. Using an in vivo assay for DSB repair pathway choice, we find that \(\text{Ino80}\) is selectively required for HR DNA repair, which is mechanistically distinct from \(\text{Ino80}\) function in YY1-associated transcriptional regulation. Surprisingly, NPC sensitivity to loss of \(\text{Ino80}\)-mediated HR is not universal. At the onset of neocortex, NPCs transition from symmetric NPC–NPC divisions to asymmetric neurogenic divisions\(^9\,10\). By systematic deletion of \(\text{Ino80}\) from NPCs pre-, peri-, and post transition, we find that deletion of \(\text{Ino80}\) during exclusively symmetric divisions leads to unrepaired DNA breaks and widespread apoptosis. In contrast, deletion of \(\text{Ino80}\) after NPC transition to asymmetric divisions does not. Consistent with a requirement for HR DNA repair selectively in symmetrically dividing NPCs, conditional deletion of well-characterized HR gene \(\text{Bra2}\) phenocopies the division mode dependence of \(\text{Ino80}\) deletion. Thus, \(\text{Ino80}\) plays mechanistically dissociable roles in chromatin-mediated gene regulation and DNA repair in corticogenesis, and distinct modes of NPC division have divergent requirements for HR.

**Results**

**Neuroanatomical defects following \(\text{Ino80}\) deletion from NPCs.** In developing forebrain, \(\text{Ino80}\) is expressed on embryonic day (E) 11.5, throughout the neurogenic period, and at birth\(^22\) (Supplementary Fig. 1a). By immunoblotting, we found INO80 expression in developing cortex at E12.5, E17.5, postnatal day (P2), and P7 (Supplementary Fig. 1b). Constitutive \(\text{Ino80}\) deletion causes embryonic lethality between E8.5 and E10.5\(^13\)\(^\text{34}\). We therefore leveraged a conditional \(\text{Ino80}\) allele\(^24\) to study \(\text{Ino80}\) in corticogenesis.

To distinguish potential \(\text{Ino80}\) functions in proliferating NPCs versus postmitotic neurons, we used two complementary Cre lines for \(\text{Ino80}\) deletion. \(\text{Emx1}^{\text{Cre}+}\) mediates recombination in cortical NPCs starting at E10.5\(^25\), near the onset of excitatory neurogenesis, thus affecting subsequent NPCs, neurons, and astrocytes of \(\text{Emx1}\) lineage. Deletion of \(\text{Ino80}\) was confirmed by immunoblotting, which revealed loss of INO80 from E12.5 \(\text{Emx1}^{\text{Cre}+}\) cortex (Supplementary Fig. 1c). \(\text{Neurod6}^{\text{Cre}+}\) (\(\text{Nex}^{\text{Cre}+}\)) mediates recombination in newly postmitotic excitatory neurons and spares NPCs\(^26\). To visualize cells that have undergone recombination, we used the Cre-dependent fluorescent reporter allele \(\text{ROSA}26\text{T}^\text{mG}^{\text{Cre}+}\)\(^27\).

Following \(\text{Ino80}\) deletion from NPCs (cKO-E), or excitatory neurons (\(\text{Neurod6}^{\text{Cre}+}\)-\(\text{Ino80}\)\(^0\)/\(^0\), cKO-N), mice were viable at birth, and fertile as adults. On P0, cKO-E, but not cKO-N, was microcephalic, with significant decreases in cortical area, thickness, and mediolateral extent (Fig. 1a–c). In addition, cKO-E cortex showed agenesis of corpus callosum and hypoplasia of hippocampus (Fig. 1b; Supplementary Fig. 1d–f). In contrast to cKO-E, \(\text{Ino80}\) deletion from neurons (cKO-N) did not lead to microcephaly, callosal defects, or hippocampal hypoplasia (Fig. 1a–c; Supplementary Fig. 1e, f). Thus, \(\text{Ino80}\) functioned in NPCs during corticogenesis.

**\(\text{Ino80}\) deletion from NPCs disrupted medial corticogenesis.** To assess neocortical laminarization, we analyzed layer markers by immunostaining. This revealed a striking mediolateral regional difference in layer formation in \(\text{Ino80}\) cKO-E. In lateral neocortex, TLE4+ layer (L)6, BCL11B (CTIP2)+ L5, and LHX2+ L2–5 neurons were properly ordered in cKO-E (Fig. 1d). Analysis of cumulative distribution of neurons labeled by each marker through the thickness of the cortex revealed correct lamination in both ctrl and cKO-E lateral cortex (Fig. 1e). cKO-E medial cortex, however, was characterized by disrupted layer organization (Med, Fig. 1d, e). The consequences of \(\text{Ino80}\) deletion from NPCs were therefore regionalized and graded on the mediolateral axis in cKO-E; lateral neocortex was grossly normal in lamination, medial neocortex was significantly disorganized, and hippocampus, a cortical structure medial to neocortex, was severely hypoplastic. In contrast, postmitotic deletion of \(\text{Ino80}\) in cKO-N did not alter medial or lateral neocortical laminarization (Fig. 1d, e). Together, these data suggested that \(\text{Ino80}\) deletion from NPCs, but not neurons, preferentially disrupted medial corticogenesis.

**Loss of medial NPCs to apoptosis following \(\text{Ino80}\) deletion.** The cKO-E phenotypes implicated \(\text{Ino80}\) function in NPCs. In E15.5 cKO-E cortex, SOX2+ apical progenitors (APs) and EOMES (TBR2)+ intermediate progenitors (IPs) were each significantly reduced in number in medial, but not lateral, cortex (Fig. 2a, b). Analysis of 5-phase NPCs by a 1-h pulse of thymidine analog ethynyl deoxyuridine (EdU) confirmed selective loss of cycling NPCs in medial cortex in cKO-E by E13.5 (Supplementary Fig. 2a). This regional NPC loss did not affect the cortical hem, a signaling center (Fig. 2a). Next, we assessed whether NPC loss was associated with apoptosis. At E13.5, cleaved-caspase 3 (CC3), a marker of apoptosis, and pyknosis, condensation of chromatin during apoptosis, were widespread in medial, but not lateral, cortex (Fig. 2c, d). Together, these results showed that the
NPCs (cKO-E) led to microcephaly, whereas conditional mutant (cKO) brains. Nuclear (n)GFP (green) was expressed Cre-dependently from ROSA^Cre^i-mediated Ino80 deletion from postmitotic excitatory neurons (cKO-N) did not. Sample measurements of cortical area (red) quantified in c are indicated (ctrl: n = 4, cKO-E: n = 4, cKO-N: n = 3 animals). OB olfactory bulb, Nctx neocortex, Mb midbrain. B MAP2 (magenta) and nGFP (green) immunostaining of coronal P0 brain sections. cKO-E, but not cKO-N, was characterized by microcephaly and severe hippocampal hypoplasia. Sample measurements of cortical thickness (yellow) and mediolateral extent (blue) quantified in c are indicated (ctrl: n = 4, cKO-E: n = 3, cKO-N: n = 3 animals). CPu caudate putamen, Hp hippocampus, Th thalamus. C Cortical area (red), thickness (yellow), and mediolateral extent (blue) were each significantly decreased in cKO-E, but not cKO-N, compared with ctrl (data are mean, one-way ANOVA with Tukey’s post hoc test, Cortical area, ctrl: nGFP = 1.6E-6). DAPI staining (cyan) of coronal P0 sections revealed altered lamination of medial neocortex and severe hypoplasia of hippocampus in cKO-E (n = 3 animals). Analyzed by marker immunostaining (insets), the lamination of LHX2 + (L2-5, green), BCL11B + (L5, blue), and TLE4 + (L6, red) neurons was correctly ordered in lateral (Lat) neocortex of cKO-E, but severely disrupted in medial (Med) neocortex. In cKO-N, normal lamination was present in medial and lateral neocortex. E Analysis of cumulative distribution of layer marker-expressing neurons through thickness of cortex from white matter (WM) to marginal zone (MZ) revealed disrupted laminations in medial cKO-E cortex (n = 3 animals). Scale bar: 1 mm in a, 500 μm in b, d, 50 μm in d inset. The mediolateral difference in apoptosis in cKO-E embryonic cortex was remarkably consistent, showing a similar pattern in all animals we analyzed (Supplementary Fig. 2b, c). To determine whether this was a reflection of spatial difference in normal Ino80 expression in embryonic cortex, we microdissected medial and lateral cortex from E11.5, E13.5, and E15.5 wild-type embryos and used droplet digital (dd)RT-PCR to analyze Ino80 mRNA levels (Fig. 2e). This revealed no regional difference in Ino80 expression between medial and lateral cortex. To ascertain that the spatial sensitivity to Ino80 deletion was independent of potential nonuniformity in Emx1Cre activity, we used the Cre-dependent reporter gene ROSA^nT-nGFP^ to analyze Cre recombination, which showed uniform mediolateral expression of nuclear (n)GFP (Fig. 2c, f; Supplementary Fig. 2b, c). This is consistent with other studies using Emx1Cre, a widely used Cre driver line with over 700 citations25. Furthermore, we analyzed the extent of cell ablation by Emx1Cre and Cre-dependent suicide gene ROSA^DTA^, which led to cell ablation throughout the mediolateral extent of Emx1Cre:ROSA^DTA/nT-nGFP^ cortex (Fig. 2f). Together, these data indicated that cKO-E phenotypes were not the result of nonuniform Cre activity or regionalized Ino80 expression, and implicated an alternative explanation for the preferential sensitivity of medial cortex to Ino80 deletion.

Transcriptomic signature of p53 activation in Ino80 cKO-E. To gain mechanistic insights into the sensitivity of medial NPCs to Ino80 deletion, we explored molecular functions of Ino80. As a chromatin remodeler, INO80 plays a role in transcriptional regulation17,18. We performed transcriptome analysis of E13.5 cortex by unique molecular identifier (UMI) RNA-seq using Click-seq29. In UMI RNA-seq, Click addition of UMI tag to each cDNA molecule enabled post-sequencing deduplication30. Analysis of spike-in ERCC standards revealed excellent quantification, and deletion of Ino80 exons 2–4 by Emx1Cre was confirmed (Supplementary Fig. 3a–c). Analysis of differential gene expression using edgeR31 revealed 205 significantly upregulated and 418 significantly downregulated genes in cKO-E with a stringent false discovery rate (FDR) of <0.001 (Fig. 3a; Supplementary Tables 1 and 2). Strikingly, of the 205 upregulated genes, 36 are known to be transcriptionally

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**Fig. 1 Microcephaly and disrupted medial corticogenesis following Ino80 deletion from NPCs.** a Dorsal view of whole-mount P0 control (ctrl) and Ino80 conditional mutant (cKO) brains. Nuclear (n)GFP (green) was expressed Cre-dependently from ROSA^nT-nGFP^, Emx1Cre-mediated Ino80 deletion from cortical NPCs (cKO-E) led to microcephaly, whereas Neurod6Cre-mediated Ino80 deletion from postmitotic excitatory neurons (cKO-N) did not. Sample measurements of cortical area (red) quantified in c are indicated (ctrl: n = 4, cKO-E: n = 4, cKO-N: n = 3 animals). OB olfactory bulb, Nctx neocortex, Mb midbrain. b MAP2 (magenta) and nGFP (green) immunostaining of coronal P0 brain sections. cKO-E, but not cKO-N, was characterized by microcephaly and severe hippocampal hypoplasia. Sample measurements of cortical thickness (yellow) and mediolateral extent (blue) quantified in c are indicated (ctrl: n = 4, cKO-E: n = 3, cKO-N: n = 3 animals). CPu caudate putamen, Hp hippocampus, Th thalamus. c Cortical area (red), thickness (yellow), and mediolateral extent (blue) were each significantly decreased in cKO-E, but not cKO-N, compared with ctrl (data are mean, one-way ANOVA with Tukey’s post hoc test, Cortical area, ctrl: nGFP = 1.6E-6). DAPI staining (cyan) of coronal P0 sections revealed altered lamination of medial neocortex and severe hypoplasia of hippocampus in cKO-E (n = 3 animals). Analyzed by marker immunostaining (insets), the lamination of LHX2 + (L2-5, green), BCL11B + (L5, blue), and TLE4 + (L6, red) neurons was correctly ordered in lateral (Lat) neocortex of cKO-E, but severely disrupted in medial (Med) neocortex. In cKO-N, normal lamination was present in medial and lateral neocortex. e Analysis of cumulative distribution of layer marker-expressing neurons through thickness of cortex from white matter (WM) to marginal zone (MZ) revealed disrupted laminations in medial cKO-E cortex (n = 3 animals). Scale bar: 1 mm in a, 500 μm in b, d, 50 μm in d inset.
activated by tumor suppressor protein p53 (TRP53), bound at their genomic loci by p53, or both. p53 is activated in response to DNA damage and turns on target genes that mediate cell-cycle arrest, DNA repair, or apoptosis. We used ddRT-PCR and validated upregulation of three p53-target genes (Fig. 3b). To determine whether p53 targets were overrepresented, we intersected cKO-E-upregulated genes with known p53 targets. Analysis of hypergeometric distribution revealed significant enrichment of p53-target genes identified by genome-wide studies. p53 can upregulate diverse target genes depending on the trigger for activation. Intersection of cKO-E-upregulated genes with p53 targets activated by X-radiation-induced DSBs revealed a highly significant overrepresentation ($P_{\text{hyper}} = 3.60E-20$). Therefore, our transcriptomic analysis uncovered a signature of p53 activation that was consistent with DSBs as a trigger for p53. In an unactivated state, p53 is monoubiquitinated by MDM2 and degraded. DSBs trigger abrogation of MDM2–p53 interaction, thus blocking degradation and stabilizing p53. Consistent with p53 stabilization, nuclear p53 immunostaining was significantly increased 17-fold in E13.5 medial, but not lateral, cortex (Fig. 3d, e), a gradient highly similar to that of apoptosis in cKO-E. We also found a signature consistent with microglia in cKO-E; 31 of the 205 upregulated genes are selectively expressed in microglia in wild-type brain (Fig. 3a, c). Microglia are resident phagocytes of the brain and can increase in number and undergo activation in response to apoptosis. Immunostaining for microglia marker ADGRE1 (F4/80) showed a significant increase in number of activated microglia with phagocytic morphology in medial, but not lateral, cortex (Fig. 3f), consistent with uniform expression of $Emx^{Cre}$ activity throughout the cortex. Scale bar: 100 μm in a, c, 20 μm in c inset; 10 μm in d, 200 μm in f.
Unrepaired DNA double-strand breaks in \textit{Ino80} cKO-E. To assess DSBS, we immunostained for phospho-(p)KAP1 (TRIM28), a marker of unrepaired heterochromatic DSBS\textsuperscript{36}. At E13.5, cKO-E was characterized by a marked 38-fold increase in pKAP1 + cells in medial cortex (arrowheads, Fig. 4a), but not lateral cortex. Notably, pKAP1 immunostaining was largely localized near the ventricular surface. During corticogenesis, apical NPCs (radial glia) undergo interkinetic nuclear migration (IKNM), where NPC nuclei migrate to upper VZ for S phase and ventricular surface for mitosis. pKAP1 localization in cKO-E suggested that NPCs in G2 or M phase carried unrepaired DSBS. We next co-immunostained pKAP1 (white, Fig. 4b) with a 1-h pulse of Edu (red), a marker of S phase, and phospho-histone H3 (pHH3, cyan), a marker of mitosis. pKAP1 was present in 1-h Edu-positive (S-phase, solid arrowheads) and Edu-negative (post-S-phase, open arrowheads) cells near the ventricular surface, but did not colocalize with pHH3. Together, these data suggested that \textit{Ino80} cKO-E NPCs accumulated, in late S or G2 phase, DSBS that were not properly repaired, impairing or delaying progression through the cell cycle into mitosis.

Increased accumulation of DSBS in cKO-E could result from increased DNA damage or compromised DNA repair. We immunostained for histone variant H2AX, which is recruited to DSBS, with 1-h EdU (magenta, Supplementary Fig. 4a). This revealed that S-phase NPCs were normally characterized by an abundance of \textit{H2AX} foci (green), consistent with a previous study that \textit{~50 DSBS are sustained by each normal cell division}\textsuperscript{37}. To quantify S-phase DSBS, we co-immunostained for \textit{H2AX} and the DNA repair protein 53BP1, which co-localize at DSBS (Supplementary Fig. 4b). This revealed no increase in \textit{H2AX} +/53BP1 + foci in EdU + NPCs in E12.5 cKO-E, suggesting that \textit{Ino80} deletion did not induce additional DNA breaks in S phase. In contrast, many post-S-phase (EdU-negative) ventricular NPCs were characterized by pan-nuclear \textit{H2AX} staining, a marker of apoptosis following substantial DNA damage during replication\textsuperscript{38} (Supplementary Fig. 4a, c). Together, these data suggested that in the absence of \textit{Ino80}, no additional DNA damage occurred during replication, but repair of synthesis-associated DNA damage was impaired in dividing NPCs, leading to accumulation of unrepaired DSBS and p53-dependent apoptosis in cKO-E medial cortex.
Impaired homologous recombination DNA repair in Ino80 cKO-E. Ino80 has been shown to mediate removal of histone subunit H2A.Z and exchange of RPA for RAD51, important steps in DSB repair by HR39,40. To assess HR in cKO-E, we designed an assay to interrogate DSB repair pathway choice in vivo. We used CRISPR–Cas9 to generate a DSB at the C terminus of Actb coding region41 and provided two reporter repair templates. The homology-dependent repair (HDR) template contained sgRNA target sites resulting in ACTB-3xMYC expression (Fig. 4c). The NHEJ template contained ssDNA target sites flanking a 3xHA epitope tag. CRISPR–Cas9 cleavage would generate a short repair template without homology, and NHEJ repair in forward orientation would lead to ACTB-3xHA expression. By providing both repair templates, relative pathway choice can be assessed using an HDR/NHEJ ratio.

To perform the assay in vivo, we used in utero electroporation (IUE) to transfect CRISPR–Cas9 and repair template constructs into E12.5 cortical NPCs (Fig. 4c). Analyzed at E17.5, control brains showed an HDR/NHEJ ratio of 0.87 (Fig. 4d, e). In cKO-E, the HDR/NHEJ ratio was 0.31, a significant 64% decrease. These results support a selective impairment of HR in Ino80 cKO-E and are consistent with previous studies demonstrating INO80 function in H2A.Z removal and RPA/RAD51 exchange during HR39,40,43. Our analysis of E13.5 cKO-E transcriptome revealed no significant change in expression of HR genes (Supplementary Fig. 4d), suggesting that Ino80 function in HR was not mediated via transcriptional regulation. Together, our data indicated that following Ino80 deletion, disruption of HR DNA repair led to an accumulation of DSBs in NPCs.

Trp53 co-deletion extensively rescued Ino80 cKO-E phenotypes. As a nucleosome remodeler, Ino80 is positioned to regulate gene expression and DNA repair. To distinguish these distinct aspects of Ino80 function in contributing to cKO-E phenotypes, we simultaneously deleted Trp53 (the p53 gene) with Ino80 (Emx1Cre; Ino80fl/fl;Trp53fl/fl, dKO-E) to block p53-dependent response to DNA damage. Co-deletion of Trp53 led to a strikingly complete rescue of cKO-E phenotypes. At P0, microcephaly (Fig. 5a, b), hippocampal hypoplasia, callosal agenesis, and medial cortical laminar defects (Supplementary Fig. 5a–c) were each markedly rescued by Trp53 co-deletion in dKO-E. At E13.5, dKO-E cortex showed no increase in apoptosis or activated microglia compared with control (Fig. 5c; Supplementary Fig. 5e, f), and at E15.5, no loss of NPCs (Fig. 5d). This extensive rescue following Trp53 co-deletion indicated that these Ino80 cKO-E phenotypes were underpinned by Ino80 function in DNA repair and p53-dependent response to the resulting DNA damage.
Fig. 5 Trp53 co-deletion rescued Ino80 phenotypes and revealed mechanistically distinct Ino80 roles. a Dorsal view of P0 whole-mount brains and BCL11B immunostaining (black) of P0 coronal sections. Co-deletion of Trp53 with Ino80 (dKO-E) rescued major Ino80 deletion (cKO-E) phenotypes, including microcephaly, severe hippocampal hypoplasia, and disrupted neocortical lamination. Sample measurements quantified in b are indicated (ctrl cortical area: n = 6, all other measurements: n = 4 animals). b Cortical area (red), thickness (yellow), and mediolateral extent (blue) were restored in dKO-E and not significantly different compared with ctrl (data are mean, one-way ANOVA with Tukey’s post hoc test, ctrl cortical area: n = 6, all other measurements: n = 4 animals). c CC3 immunostaining (magenta) revealed no increase in apoptosis in dKO-E E13.5 neocortex (n = 3 animals). d The number of SOX2+ (red) and EOMES+ (cyan) NPCs in E15.5 dKO-E cortex were restored to levels not significantly different compared with ctrl (data are mean, one-way ANOVA with Tukey’s post hoc test, ctrl: n = 6, all other: n = 4 animals). e UMI RNA-seq volcano plots comparing cKO-E with ctrl, and dKO-E (n = 9 animals) with ctrl E13.5 cortex. In the cKO-E versus ctrl comparison (left panel), differentially regulated genes are indicated (upregulated, red dots; downregulated, blue dots). The same genes are labeled in the dKO-E versus ctrl comparison (right panel). Genes that remained differentially regulated (FDR < 0.001) following Trp53 co-deletion are indicated by dots of the same color. Genes that lost differential expression (FDR ≥ 0.001) are indicated by black dots. The vast majority of cKO-E-upregulated genes (202/205) were rescued by Trp53 co-deletion, indicating that their upregulation was p53-dependent. About one-third of cKO-E-downregulated genes (134/418), however, remained significantly downregulated in dKO-E, consistent with p53-independent gene regulation by Ino80. f Intersectional analysis of the 134 p53-independent downregulated genes with published ChIP-seq data from mouse neural stem cells (NSCs) revealed an enrichment of genes bound at their transcriptional start site (TSS) by transcription factor YY1, a known binding partner of INO80. This enrichment was absent from the 42 cKO-E downregulated genes that were reversed by Trp53 co-deletion. Scale bar: 500 μm in a; 100 μm in c, d.
**Dissociable roles of Ino80 in transcription and DNA repair.** Our transcriptome analysis of Ino80 cKO-E revealed p53-target activation and microglial gene expression. Using Ino80 cKO-E, potential direct transcriptional effects of Ino80 could not be isolated from those related to p53 activation. Ino80/Trp53 dKO-E, however, could be leveraged to identify primary effects of Ino80 on expression. We performed UMI RNA-seq of E13.5 Ino80/Trp53 dKO-E cortex (n = 7 animals) for direct comparison with cKO-E (Fig. 5e; Supplementary Tables 3 and 4). In single Ino80 cKO-E, a majority of upregulated genes were attributed to p53 activation or microglia. In double Ino80/Trp53 dKO-E, these transcriptomic signatures were nearly completely reversed; only 3/205 genes remained significantly upregulated (Fig. 5e). The increased expression of these genes in cKO-E was therefore p53-dependent. In contrast to upregulated genes, a substantial subset of downregulated genes from cKO-E (134/418) remained significantly downregulated in dKO-E (Fig. 5e). The expression of these 134 genes was therefore p53-independent and potentially under direct Ino80 regulation. Previous studies have shown that INO80 binds YY1 and mediates YY1-associated gene expression.\(^{37,44}\) Intersectional analysis with available YY1 ChIP-seq data from mouse NPCs revealed that, of the 134 downregulated genes unaffected by Trp53 co-deletion (down in both cKO-E and dKO-E, p53-independent, Supplementary Table 5) 67% were characterized by a YY1 peak within 500 bp of the transcriptional start site (TSS, Fig. 5f; Supplementary Fig. 5f, g), typical of YY1-regulated genes. In contrast, for five sets of randomly selected genes, only 10–18% of genes were characterized by a YY1 peak (Supplementary Fig. 5f, g). Thus, Ino80 regulated YY1-associated transcription independently of p53, thereby playing mechanistically dissociable roles in gene expression and DNA repair during corticogenesis (Supplementary Fig. 6). Importantly, despite persistence of YY1-associated transcriptomic dysregulation in Ino80/Trp53 dKO-E, we found no overt cellular or anatomical phenotypes (Fig. 5; Supplementary Fig. 5). The robust apoptosis and microcephaly in Ino80 cKO-E were therefore consequences of p53-dependent responses to impaired DNA repair.

INO80 is a chromatin remodeler. To assess whether genome-wide chromatin accessibility was altered, we carried out ATAC-seq in E13.5 cortex. Analysis of genome-wide ATAC-seq peaks\(^{46}\) (arrows in IGV\(^{47}\); Supplementary Fig. 5h) revealed a 91.3% overlap between Ino80 cKO-E and control. Chromatin accessibility at TSSs was similar and peak-to-peak correlation of reads per peak was high (\(R = 0.88\), Supplementary Fig. 5i, j), with a modest reduction in accessibility in cKO-E. To assess correlation between accessibility and transcriptomic changes in cKO-E, we identified differentially accessible regions (DARS) using GFO\(^{48}\). Consistent with modest changes, 0.75% of ATAC-seq peaks met GFO\(^{-}\)cutoff of 1.0 for increased accessibility, and 1.02% of peaks for decreased accessibility (Supplementary Fig. 5k). We identified ATAC-seq peaks within predicted E14.5 brain enhancers (EnhancerAtlas 2.0\(^{49}\)) or at TSSs of genes that showed differential gene expression (DEX) in cKO-E compared with ctrl. Intersectional analysis revealed that 0.78% (15/1658) of brain enhancers and 0.24% (2/832) of TSSs of DEX genes contained DARS. Thus, DARS did not correlate with DEX in Ino80 cKO-E. Together, these data are consistent with previous work that Ino80 can regulate HR independently of chromatin accessibility.\(^{39,40,43}\)

**Phenotypic severity correlates with symmetric NPC divisions.** Our analyses revealed a markedly higher sensitivity of medial cortex to Ino80 deletion than was not a result of nonuniform Cre activity or Ino80 expression (Fig. 2e, f), implicating an alternative explanation. During normal corticogenesis, NPCs transition from symmetric NPC–NPC divisions to asymmetric neurogenic divisions\(^{48}\) from approximately E11.5 to E13.5. This transition does not occur simultaneously throughout the cortex\(^{49}\). Lateral NPCs transition to asymmetric divisions earlier than medial cortex following a transverse neurogenetic gradient (TNG, lateral–rostral → medial–caudal)\(^{50,51}\). Consistent with the TNG, our analysis of wild-type E12.5 revealed in lateral cortex a more developmentally advanced cortical plate (CP) comprising rows of RBFOX3 + neurons (red, Fig. 6a), suggesting that lateral SOX2 + NPCs (cyan) have initiated asymmetric division and neurogenesis. In contrast, the medial CP was not developed and did not contain RBFOX3 + neurons, suggesting that medial NPCs were largely dividing symmetrically (schematic, Fig. 6b). We also visualized the TNG by immunostaining for NEUROG2 (NGN2), a pro-neural gene expressed by NPCs undergoing neurogenic divisions.\(^{52}\). In E11.5 wild-type cortex, a large majority of NPCs were NEUROG2-negative (Fig. 6c), consistent with largely symmetric divisions at this age. From E12.5 to E15.5, wild-type cortex was characterized by an increase in the number of NEUROG2 + NPCs following a lateral to medial gradient. At E12.5, a transition zone delineated NEUROG2-positive and NEUROG2-negative NPCs along the mediolateral axis (arrowhead, Fig. 6c). By E15.5, the entire mediolateral extent showed high NEUROG2 expression, consistent with asymmetric divisions at this age.

We examined the possibility that this spatiotemporal gradient of neurogenesis contributed to the mediolateral difference in NPC sensitivity to Ino80 deletion. Strikingly, at E12.5 and E13.5, the border of medial apoptotic and lateral nonapoptotic cells in cKO-E aligned with the NEUROG2+/NEUROG2– transition zone (red arrowheads, Fig. 6d). Laterally, where NPCs have initiated asymmetric division, CC3 immunostaining was largely absent (inset, Fig. 6d). In contrast, medially, where NPCs were largely dividing symmetrically, CC3 immunostaining was abundant, suggesting that the symmetric mode of division was associated with NPC sensitivity to Ino80 deletion. Analysis of immunofluorescent pixel intensity from lateral to medial neocortex revealed strikingly complementary, opposing gradients of NEUROG2 (green) versus CC3 (magenta) in cKO-E (Fig. 6c). To determine whether apoptosis persisted through transition into asymmetric divisions, we analyzed cKO-E cortex at E15.5, when both medial and lateral NPCs have largely transitioned to asymmetric divisions. Remarkably, cKO-E showed no persistent apoptosis at E15.5 (Fig. 6d, f), suggesting that the remaining NPCs, which continued to cycle (Supplementary Fig. 2a), were no longer sensitive to Ino80 deletion. Together, these data supported a spatiotemporal correlation between NPC sensitivity to Ino80 deletion and mode of NPC division. Near the onset of Ino80 deletion in cKO-E, the severely affected medial NPCs were largely undergoing asymmetric NPC–NPC divisions, whereas the unaffected lateral NPCs have initiated transition to asymmetric neurogenic divisions. By mid-neurogenesis, NPCs have largely transitioned to asymmetric divisions and no longer underwent apoptosis in cKO-E.

**Ino80 function in symmetric versus asymmetric NPC divisions.** To directly test the possibility that division symmetry contributed to medial NPC sensitivity following Ino80 loss, we systematically compared Ino80 deletion from NPCs pre-, peri-, and post transition from symmetric to asymmetric division. To delete Ino80 pre-transition, we used Foxg1\(^{Cre}\) (Foxg1\(^{Cre+}\)/;Ino80\(^{fl/fl}\), cKO-F), which mediates deletion from forebrain NPCs starting at E8.5\(^{53}\), an early stage when NPC divisions were exclusively symmetric NPC–NPC. Remarkably, Foxg1\(^{Cre}\) deletion of Ino80 led to widespread apoptosis throughout the entire mediolateral extent of...
cKO-F cortex at E11.5 (Fig. 7a). Therefore, at a stage when all NPCs were undergoing symmetric divisions, medial and lateral NPCs were equally sensitive to Ino80 deletion. This manifested postnatally as forebrain agenesis (Fig. 7b, c). Immunostaining revealed p53 activation throughout the mediolateral axis of E11.5 cKO-F cortex (Supplementary Fig. 7a), indicating DNA damage in both medial and lateral NPCs. We further generated Foxg1Cre; Ino80fl/−; Emx1Cre/−; Trp53−/− (dKO-F), which was characterized by a near complete rescue of the forebrain agenesis phenotype of cKO-F (Supplementary Fig. 7c). Thus, the phenotypes of cKO-F, similar to those of cKO-E, were also underpinned by DNA damage and p53 activation. Furthermore, lateral NPCs, during exclusively symmetric divisions, were equally sensitive to loss of Ino80 as medial NPCs. Together, these data suggested that the spatiotemporal difference in phenotypic severity in the Emx1Cre cKO-E (peri-transition) was underpinned by a mediolateral difference in mode of NPC division.

To assess the consequence of Ino80 deletion post transition to asymmetric division, we generated Ino80 cKO using Tg(hGFAP-Cre) and Tg(hGFAP-Cre); Ino80fl/−; Foxg1cre; Emx1Cre; Trp53−/− (cKO-hG). Tg(hGFAP-Cre) mediates deletion in cortical NPCs beginning at E12.5 and extending throughout the cortex at E13.5, a stage when NPCs transition to asymmetric neurogenic divisions. Remarkably, the E15.5 cKO-hG cortex showed no increase in apoptosis compared with ctrl (Fig. 7a, d) and did not activate p53 (Supplementary Fig. 7b). At P0, cKO-hG cortex was similar to ctrl in size and morphology (Fig. 7b, c). Thus, after transition to asymmetric divisions, NPCs were no longer sensitive to Ino80 deletion. Together, our systematic analysis of Ino80 deletion from NPCs pre-, peri-, and post transition from symmetric to asymmetric division convergently supported that distinct modes of NPC division have divergent requirements for Ino80-mediated HR.

Despite impaired HR DNA repair, Ino80 deletion did not significantly disrupt asymmetrically dividing NPCs, suggesting...
usage of alternative DSB repair pathways. To test this, we used the in vivo DSB repair pathway choice assay to compare, in control animals, symmetric (E12.5) versus asymmetric (E15.5) NPC divisions. We found that under wild-type conditions, HR could occur after NPC transition to asymmetric divisions, but at a significantly reduced frequency (Supplementary Fig. 7d). Thus, the balance of DSB repair pathways used by NPCs was not constant throughout development; HR became less frequent relative to NHEJ as NPCs transitioned to asymmetric divisions.

Deletion of HR gene Brca2 phenocopies Ino80 deletion. To determine the extent to which Ino80 cKO phenotypes were based on impaired HR, we orthogonally disrupted HR by conditional deletion of Brca2. Brca2 is required for HR DNA repair, where it mediates the switch on ssDNA from RPA to recombination RAD51. It is not required for NHEJ. We reasoned that if Ino80 deletion phenotypes were consequences of disrupted HR, they should be extensively phenocopied following deletion of Brca2, a bona fide HR gene.

At E12.5, the Brca2 cKO-E cortex was characterized by apoptosis in medial cortex, where NPCs were largely NEUROG2-negative, but not lateral cortex, where NPCs were largely NEUROG2-positive (Fig. 8c). These mediolateral gradients are strikingly reminiscent of Ino80 cKO-E (Fig. 6). Furthermore, at E13.5, we found preferential pyknosis and accumulation of pKAP1-labeled DSBs in medial Brca2 cKO-E cortex (Supplementary Fig. 8d, e). Compared with Ino80 cKO-E, Brca2 lateral cortex showed a modest increase in apoptosis (Supplementary Fig. 8d, e). Brca2 has been shown to also play a role in chromosome segregation, defects in which it may contribute to the mild cell death in lateral NPCs. Overall, the mediolateral patterns in phenotypic severity of Ino80 cKO-E (Figs. 1 and 2) were extensively phenocopied in Brca2 cKO-E.

Analysis of DSB repair pathway choice showed in Ino80 cKO-E a preferential loss of HR (Fig. 4). To assess whether the phenocopy between Ino80 deletion and Brca2 deletion was mechanistically centered on disrupted HR, we used the DSB repair pathway assay. This showed a relative decrease in HDR compared with NHEJ in Brca2 cKO-E highly reminiscent of Ino80 cKO-E (Fig. 8d). These data thus validated that the DSB repair pathway assay could measure impaired HR DNA repair, and implicated disrupted HR as a shared molecular mechanism underlying the mediolateral phenocopy between Ino80 cKO-E and Brca2 cKO-E. Together, these data strongly support that the mediolateral differences in phenotypic severity in Ino80 cKO-E were mechanistically based on disrupted HR DSB repair.

Ino80 deletion pre-, peri-, and post NPC transition from symmetric to asymmetric division revealed divergent requirements for Ino80-mediated HR during distinct modes of NPC
Fig. 8 A selective requirement for Ino80-mediated HR in symmetric NPC-NPC divisions. a P0 whole-mount brains and immunostaining for MAP2 (magenta) and nGFP (green). Emx1Cre-mediated deletion of HR gene Brca2 (Brca2 cKO-E) led to microcephaly and hippocampal hypoplasia reminiscent of Ino80 cKO-E (n = 3 animals). b The significant reductions in Ino80 cKO-E cortical area (red), thickness (yellow), and mediolateral extent (blue) were each phenocopied in Brca2 cKO-E (data are mean, one-way ANOVA with Tukey’s post hoc test, ctrl: n = 4, Ino80 cKO-E cortical area: n = 4, thickness, mediolateral extent: n = 3, Brca2 cKO-E: n = 3 animals). c Complementary gradients of asymmetric neurogenic divisions (NEUROG2, green) and apoptosis (CC3, magenta) in Brca2 cKO-E E12.5 cortex similar to those found in Ino80 cKO-E (data are LOESS curve ± 99% confidence interval, n = 3 animals). d In vivo DSB repair pathway assay revealed significant decrease in HDR (ACTB-3xMYC, magenta) relative to NHEJ (ACTB-3xHA, green) in Brca2 cKO-E compared with ctrl (data are mean, one-way ANOVA with Tukey’s post hoc test, ctrl: n = 4, Ino80 cKO-E: n = 5, Brca2 cKO-E: n = 4 animals). e Foxg1Cre deletion of Brca2 (cKO-F) during exclusively symmetric NPC-NPC divisions led to widespread CC3 (magenta in overlay, blue in monochrome) throughout the mediolateral axis of E11.5 cortex. The lateral extent of apoptosis (magenta arrowhead) reached the lateral extent of neocortex (green arrowhead). Emx1Cre deletion of Brca2 (cKO-E) near the onset of transition between symmetric and asymmetric divisions led to robust apoptosis in medial, but not lateral, neocortex. Tg(hGFAP-Cre) deletion of Brca2 (cKO-hG) after most NPCs had transitioned to asymmetric neurogenic division did not lead to widespread increase in apoptosis. These Cre-by-Cre phenotypes were reminiscent of those found in Ino80 cKOs (n = 3 animals). f During corticogenesis, synthesis-associated DSBs in dividing NPCs are repaired in S, G2, or M phase to safeguard genome integrity. Following Ino80 or Brca2 deletion from NPCs, HR was selectively disrupted. In symmetrically dividing NPCs, loss of HR led to unrepaired DSBs and apoptosis. In asymmetrically dividing NPCs, Ino80 or Brca2 deletion did not give rise to unrepaired DSBs or apoptosis, suggesting that homology-independent DNA repair pathways were sufficient. Thus, distinct modes of NPC division have divergent requirements for HR DNA repair.

division (Fig. 7). To systematically dissect the consequences of Brca2 deletion, we similarly used Foxg1Cre, Emx1Cre, and Tg (hGFAP-Cre). We found a remarkable Cre-by-Cre phenocopy between Ino80 and Brca2 cKOs. Brca2 deletion by Foxg1Cre during exclusively symmetric divisions led to widespread apoptosis throughout the mediolateral extent of E11.5 cortex (Fig. 8e), whereas Brca2 deletion by Tg(hGFAP-Cre), after NPC transition to asymmetric divisions, did not. This striking Cre-by-Cre phenocopy between Ino80 and Brca2, a bona fide HR gene, provided strong support that Ino80 mediates DNA repair by HR.
During corticogenesis, and that NPC requirement for HR DNA repair is dependent on division mode—symmetric NPC–NPC divisions, but not asymmetric NPC–neuron divisions, are highly sensitive to loss of HR.

**Discussion**

Biological processes on nuclear DNA occur in the context of chromatin. Replication, repair, and transcription are thus mediated by remodelers that control chromatin mobility and dynamics. Here, we find mechanistically distinct roles for chromatin remodeler **Ino80** in HR DNA repair and YY1-associated transcription in corticogenesis. Following **Ino80** deletion from NPCs, DNA repair by HR is selectively impaired, leading to an accumulation of DSBs, p53 activation, apoptosis, and microcephaly. Co-deletion of **Trp53** with **Ino80** led to extensive phenotypic rescue, similar to other mutants with DNA damage. Our data thus strongly support **Ino80** cKO apoptosis and microcephaly as consequences of impaired DNA repair.

In addition to DNA repair, we find a gene regulatory role for **Ino80** in YY1-associated transcription. Previous studies have shown that INO80 and YY1 interact, bind open chromatin, and mediate transcriptional activation. This role is compromised in **Ino80** cKO-E, which is characterized by downregulation of YY1-associated genes. These transcriptomic changes persisted in **Ino80**/**Trp53** dKO-E, in which we found no overt neuroanatomical abnormalities (Fig. 5). Thus, YY1-associated expression changes did not contribute to structural phenotypes in **Ino80** cKO-E. Together, these findings support the conclusion that **Ino80** plays mechanistically distinct roles in YY1-associated gene regulation and HR DNA repair in corticogenesis (Supplementary Fig. 6).

Recent genetic findings have converged on altered chromatin regulation in disorders of brain development, including autism spectrum disorder (ASD) and intellectual disability. Unraveling the functional consequences of these mutations is an active area of research. Chromatin dysregulation perturbs transcriptional control, but can also impair DNA repair. Our findings support an **Ino80** role in HR in NPCs. We note as a potential caveat that a theoretical reduction in NHEJ may not be detectable by our assay. Loss of HR in **Ino80** cKO-E, however, is consistent with INO80 function in mobilizing H2A.Z and exchanging RPA for RAD51 on resected DNA, key steps in HR.

**Ino80** cKO-E, phenotypic severity shows a remarkable complementarity to the TNG (Fig. 6a, b). At the onset of **Ino80** deletion by **Emx1**/Cre, lateral NPCs have initiated transition to asymmetric divisions; they are spared from DNA damage and cell death. Medial NPCs largely remain in symmetric divisions at this age; they are affected by unrepair DNA repair. These data support a sensitivity to **Ino80** deletion in symmetrically dividing NPCs that underpins the mediolateral phenotypes in **Ino80** cKO-E, in which NPC division mode is coupled to NPC spatial positioning at **Emx1**/Cre onset. To test the selective requirement for **Ino80** by symmetrically dividing NPCs, we leverage additional Cre lines in which NPC division mode is uncoupled from mediolateral position at Cre onset. To study NPCs dividing exclusively in symmetric mode regardless of location, we use **Foxg1**/Cre. Remarkably, **Foxg1**/Cre deletion of **Ino80** leads to widespread apoptosis throughout the entire mediolateral extent of the cortex (Fig. 7). Thus, during exclusively symmetric divisions, lateral and medial NPCs are equally sensitive to loss of **Ino80**. To study NPCs after both medial and lateral NPCs have initiated transition to asymmetric divisions, we use **Tg(hGFAP-Cre)**. Strikingly, **Tg(hGFAP-Cre)** deletion of **Ino80** leads to neither apoptosis, p53 activation, nor microcephaly (Fig. 7). Thus, following NPC transition to asymmetric divisions, neither lateral nor medial NPCs are sensitive to loss of **Ino80**. Together, our systematic analysis of **Ino80** deletion pre-, peri-, and post transition from symmetric divisions to asymmetric divisions revealed a previously unappreciated relationship between division symmetry and DNA repair pathway; symmetric, but not asymmetric, divisions are selectively sensitive to loss of **Ino80**-mediated HR (Fig. 8). This remarkable division mode-dependent requirement for HR is orthogonally validated in conditional mutants of **Brcα2**, a well-characterized HR gene. Notably, early NPCs have been previously shown to be especially sensitive to DNA damage following conditional deletion of **Brcα** or **Topbp1**. Our results suggest that the symmetry of NPC division may contribute to this temporal difference. More broadly, to support organogenesis, stem cells undergo asymmetric and asymmetric divisions. Our work thus suggests that stem cell division mode can bias DNA repair pathway choice in other developmental systems.

Symmetric and asymmetric NPC divisions are characterized by remarkable differences in cell cycle dynamics. In symmetric divisions, S-phase length is ~8 h. In contrast, S-phase length is merely 2 h in asymmetric divisions. This difference has key implications for repair of replication-associated DSBs. NHEJ can be completed within 30 min. HR requires extensive DNA processing, recruitment of proteins to resected DNA, and search for a homology template, and thus can take 7 h or longer. Given the accelerated S phase, homology-independent DNA repair may be
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Methods

Mice. All experiments were carried out in compliance with ethical regulations for animal research. Mouse strains are listed in Supplementary Table 6. Our study protocol was reviewed and approved by the University of Michigan Institutional Animal Care & Use Committee. Mice were maintained on a standard 12-h day:

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Technologies) and quantified using the NEBNext Library Quant Kit for Illumina. Libraries were sequenced on an Illumina NovaSeq S4 flow cell to obtain paired-end 150-bp reads. After trimming adapter using cutadapt57, reads were aligned to the mouse reference genome (GRCm38/mm10) using BWA with default settings58. Low-quality, mitochondrial, and duplicate reads were removed using a combination of SAMTools and Picard's MarkDuplicates program. ATAC-seq peaks were called using Macs2 with the parameters: -model --extsize 200 --shift -100, and blacklisted regions were excluded46. A consensus set of peaks (137,816) was generated using bedtools merge, and reads from each sample that fell within this consensus peak set were counted using bedtools, and normalized by library size, and to RPM (reads per million). Differentially accessible regions (DARs) were determined using GFOLD48 with a significance cutoff score of 1.0. This GFOLD score reflects a confidence interval of -99% that the observed fold change is at least log2(10). ATAC-seq peaks were annotated by overlap with TSSs from the UCSC browser (GRCm38). Peaks associated with enhancer regions were determined based on predicted enhancer–gene interaction data from the E14.5 brain dataset from EnhancerAtlas 2.047, which integrates ChIP-seq, DNaSe hypersensitivity, ChIA-Pet, Hi-C, and gene expression data. These intersections were established using PyRanges77 to find ATAC-seq peaks as called by Macs2 that overlapped with predicted enhancer regions.

**Statistical analysis.** Statistical calculations were performed in GraphPad Prism. Values were compared using a two-tailed, unpaired Student’s t test or ANOVA with Tukey's post hoc test. A P value of ≤0.05 was considered statistically significant.

**Image processing and analysis.** Images were exported as TIFF files and processed in Adobe Photoshop. Counts and measurements were performed in Photoshop or using ImageJ software.

**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**

The RNA-seq and ATAC-seq data that support the findings of this study have been deposited to NCBI GEO with the accession number GSE153062. Data from publicly available sources: GRCm38/mm10 reference genome [https://www.ncbi.nlm.nih.gov/assembly/GCF_000001635.26], ENCODE [ENCSR160IIN, ENCSR647QBV, ENCSR076WAM, ENCSR581XQW, ENCSR352IRG, ENCSR080EYZ, ENCSR362AI2], and EnhancerAtlas 2.0. Source Data are provided with this paper.
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Author contributions
K.Y.K. conceived the study. J.M.K. and K.Y.K. designed experiments, J.M.K., D.Z.D., I.D. (fellowship to J.M.K.), March of Dimes Foundation (#5-FY15-33 to K.Y.K.), and Simons Foundation Autism Research Initiative (402133 and 334586 to K.Y.K.).

Competing interests
The authors declare no competing interests.

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