

The ARRIVE Essential 10

These items are the basic minimum to include in a manuscript. Without this information, readers and reviewers cannot assess the reliability of the findings.

Item	Recommendation	Section/line number, or reason for not reporting
Study design	1 For each experiment, provide brief details of study design including: - The groups being compared, including control groups. If no control group has been used, the rationale should be stated. - The experimental unit (e.g. a single animal, litter, or cage of animals).	used/used (CXCL12 loss-of function model). Control mice consisted of both wild-type (WT) and Cre-negative littermates from the same crosses, ensuring an equivalent genetic background. Section: Materials and Methods, 'Mice'; Figures 1-3. Levels per mouse were averaged to generate a single data point. Section: Materials and Methods, 'Quantification and statistical analysis'.
Sample size	2 - Specify the exact number of experimental units allocated to each group, and the total number in each experiment. Also indicate the total number of animals used. - Explain how the sample size was decided. Provide details of any <i>a priori</i> sample size calculation, if done.	pooled to generate a single-cell library per group (Control and Smad4-ECKO). Section: Materials and Methods, 'scRNA-seq data analysis' and 'Quantification and statistical analysis'. b. No formal <i>a priori</i> sample size (power) calculation was performed. The sample size (n = 4 mice per genotype) was determined based on our established laboratory protocols for developmental studies and no sample sizes used in remarkable n.b.
Inclusion and exclusion criteria	3 - Describe any criteria used for including and excluding animals (or experimental units) during the experiment, and data points during the analysis. Specify if these criteria were established <i>a priori</i>. If no criteria were set, state this explicitly. - For each experimental group, report any animals, experimental units or data points not included in the analysis and explain why. If there were no exclusions, state so. - For each analysis, report the exact value of <i>n</i> in each experimental group.	a. low-quality cells (abnormally low UMIs counts or a high percentage of mitochondrial-gene-derived UMIs) were filtered out, and doublets were removed using DoubletFinder (v2.0). Section: Materials and Methods, 'scRNA-seq data analysis'. b. No animals or experimental units were excluded from the histological analyses. For scRNA-seq, low-quality cells and doublets were removed during QC (see Item 3a). c. Histological analyses: n = 4 mice per group (Control and mutant genotypes). scRNA-seq: n = 2 pooled E14.5 embryos (one library) per group.
Randomisation	4 - State whether randomisation was used to allocate experimental units to control and treatment groups. If done, provide the method used to generate the randomisation sequence. - Describe the strategy used to minimise potential confounders such as the order of treatments and measurements, or animal/cage location. If confounders were not controlled, state this explicitly.	re defined by genotype: 'Smad4-ECKO mice' (Em1-Cre; Smad4 ^{supfl/+} /+; sup; Gad67-GFP) and control littermates (wild-type and Cre-negative mice) were generated from the same genetic crosses, and assignment to experimental groups was therefore determined by genotype. No random allocation sequence was generated. b. Caudal levels were selected and averaged per animal to account for intra-individual anatomical variance. Embryos and postnatal mice of different genotypes were processed in parallel under identical conditions. Order of measurements and cage location were not specifically randomised.
Blinding	5 Describe who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis).	blinding, tissue processing, immunostaining, image acquisition and quantitative analyses were blinded to the genotype of the animals throughout the conduct of the experiment and the outcome assessment. Group allocation was revealed only after all data had been collected and the quantitative analyses were completed.
Outcome measures	6 - Clearly define all outcome measures assessed (e.g. cell death, molecular markers, or behavioural changes). - For hypothesis-testing studies, specify the primary outcome measure, i.e. the outcome measure that was used to determine the sample size.	a. (i) Number and proportional distribution of Gad67-GFP ⁺ interneurons across cortical zones (marginal zone, MZ; cortical plate, CP; intermediate zone, IZ; subventricular zone, SVZ) at E14.5 and P0. (ii) Percentages of GFP ⁺ cells in each zone. b. The primary outcome measure was the number and spatial distribution of Gad67-GFP ⁺ cortical interneurons. No <i>a priori</i> sample size calculation was performed on the basis of this outcome (see Item 2b).
Statistical methods	7 - Provide details of the statistical methods used for each analysis, including software used. - Describe any methods used to assess whether the data met the assumptions of the statistical approach, and what was done if the assumptions were not met.	a. Histological quantifications: GraphPad Prism 10; statistical significance between Control and mutant groups was determined using an unpaired Student's t-test. All b. The assumptions of the unpaired Student's t-test (approximate normality and homogeneity of variance) were not formally tested. Based on inspection of the data distribution (approximately normal).
Experimental animals	8 - Provide species-appropriate details of the animals used, including species, strain and substrain, sex, age or developmental stage, and, if relevant, weight. - Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures.	a. Species: mouse (<i>Mus musculus</i>). Strains: Smad4 ^{supfl/+} (JAX no. 017462), Emx1-Cre (JAX no. 005628), Gad67-GFP (JAX no. 003718) and Cxcl12-DsRed (JAX no. 022458). Developmental stages: b. Provenance: all mouse lines were obtained from The Jackson Laboratory (JAX numbers as above). Genetic modification status: conditional knockout (Emx1-Cre;Emx1-Cre;Smad4 ^{supfl/+}).
Experimental procedures	9 For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including: - What was done, how it was done and what was used. - When and how often. - Where (including detail of any acclimatisation periods). - Why (provide rationale for procedures).	objectives'. Section: Materials and Methods, 'Tissue preparation', 'Immunofluorescence', 'scRNA-seq data analysis', 'Image acquisition and analysis'. b. Time points: E14.5, E15.5 and P0; four matched coronal sections per mouse per analysis. Section: Materials and Methods; Figure legends. c. All experiments were performed in the animal facility of the authors' institution (Affiliated Hospital of Hebei University of Engineering) under SPF conditions (details: Smad4 controls CXCL12-dependent interneuron migration and coordination; scientific rationale provided in the Introduction and Results).
Results	10 For each experiment conducted, including independent replications, report: - Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g. mean and SD, or median and range). - If applicable, the effect size with a confidence interval.	a. Mean and SD (n = 4 mice per group); exact p-values are reported in the results text and Figure legends. Section: Results; Figure legends; Materials and Methods, 'Quantification and statistical analysis'. b. Effect sizes with confidence intervals were not reported. Data are presented as mean ± SD with exact p-values obtained from unpaired Student's t-tests; effect sizes (e.g., Cohen's d) and 95% confidence intervals were not calculated for the outcome assessed. If randomised by

The Recommended Set

These items complement the Essential 10 and add important context to the study. Reporting the items in both sets represents best practice.

Item		Recommendation	Section/line number, or reason for not reporting
Abstract	11	Provide an accurate summary of the research objectives, animal species, strain and sex, key methods, principal findings, and study conclusions.	and study conclusions. Section: Abstract.
Background	12	a. Include sufficient scientific background to understand the rationale and context for the study, and explain the experimental approach. b. Explain how the animal species and model used address the scientific objectives and, where appropriate, the relevance to human biology.	a. Section: Introduction (rationale for studying <i>Snad4</i> in cortical development and the coordinated development of projection neurons and interneurons). b. Section: Introduction (explains how the <i>Snad4</i> conditional knockout mouse model addresses the objectives and the relevance of Pih-derived signals to interneuron migration).
Objectives	13	Clearly describe the research question, research objectives and, where appropriate, specific hypotheses being tested.	disrupts the CXCL12-CXCR4 guidance axis.'
Ethical statement	14	Provide the name of the ethical review committee or equivalent that has approved the use of animals in this study, and any relevant licence or protocol numbers (if applicable). If ethical approval was not sought or granted, provide a justification.	Laboratory Animal Ethical and Welfare Committee of Affiliated Hospital of Hebei Engineering University (Approval No. IACUC-Hebei-2025-0002)
Housing and husbandry	15	Provide details of housing and husbandry conditions, including any environmental enrichment.	ional acclimatisation period was required.
Animal care and monitoring	16	a. Describe any interventions or steps taken in the experimental protocols to reduce pain, suffering and distress. b. Report any expected or unexpected adverse events. c. Describe the humane endpoints established for the study, the signs that were monitored and the frequency of monitoring. If the study did not have humane endpoints, state this.	a. To reduce pain and distress, mice were deeply anaesthetised (pentobarbital sodium, 100 mg/kg i.p.) before tissue collection, and postnatal mice were transcardially perfused under deep anaesthesia. Section: Materials and Methods, 'Tissue preparation'. b. No expected or unexpected adverse events were observed during the course of the study. All animals survived until the scheduled tissue collection time points (E14.5, E15.5 and P90). c. The study did not establish specific humane endpoints, as all animals were euthanised at predetermined developmental time points (E14.5, E15.5 and P90) for tissue collection; this is a scheduled-endpoint study in which animals were not expected to die.
Interpretation/scientific implications	17	a. Interpret the results, taking into account the study objectives and hypotheses, current theory and other relevant studies in the literature. b. Comment on the study limitations including potential sources of bias, limitations of the animal model, and imprecision associated with the results.	a. Section: Discussion (interpretation of the findings in light of the study objectives, current theory and relevant literature on interneuron migration and chemokine signalling). b. This study has several limitations. First, the scRNA-seq analysis was performed on cortical tissues pooled from two independent embryos per group, which provides limited biological replication for the transcriptomic analysis. Second, the causal role of <i>Snad4</i> in interneuron migration requires further investigation.
Generalisability/translation	18	Comment on whether, and how, the findings of this study are likely to generalise to other species or experimental conditions, including any relevance to human biology (where appropriate).	development in other species, including humans. Given the established links between impaired interneuron migration and neurodevelopmental disorders, these findings may have implications for understanding the pathogenesis of such conditions. However, direct translational relevance to human biology requires further investigation.
Protocol registration	19	Provide a statement indicating whether a protocol (including the research question, key design features, and analysis plan) was prepared before the study, and if and where this protocol was registered.	No study protocol was registered, as protocol registration was not a requirement for this basic research study. The experimental design, methods and analysis plan are fully described in the Materials and Methods section.
Data access	20	Provide a statement describing if and where study data are available.	responding author upon reasonable request.
Declaration of interests	21	a. Declare any potential conflicts of interest, including financial and non-financial. If none exist, this should be stated. b. List all funding sources (including grant identifier) and the role of the funder(s) in the design, analysis and reporting of the study.	The authors declare no competing interests. b. This work was supported by grants from the National Natural Science Foundation of China (No. 82501435), the Natural Science Foundation of Hebei Province (No. H2025402018), and the Medical Science Research Project of Hebei (No. 20250165). The f