**Statistics reporting, by figure**

- Please specify the following information for each panel reporting quantitative data, and where each item is reported (section, e.g. Results, & paragraph number).

- Each figure legend should ideally contain an exact sample size (n) for each experimental group/condition, where n is an exact number and not a range, a clear definition of how n is defined (for example x cells from x slices from x animals from x litters, collected over x days), a description of the statistical test used, the results of the tests, any descriptive statistics and clearly defined error bars if applicable.

- For any experiments using custom statistics, please indicate the test used and stats obtained for each experiment.

- Each figure legend should include a statement of how many times the experiment shown was replicated in the lab; the details of sample collection should be sufficiently clear so that the replicability of the experiment is obvious to the reader.

- For experiments reported in the text but not in the figures, please use the paragraph number instead of the figure number.

Note: Mean and standard deviation are not appropriate on small samples, and plotting independent data points is usually more informative. When technical replicates are reported, error and significance measures reflect the experimental variability and not the variability of the biological process; it is misleading not to state this clearly.

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**Test Descriptions**

1. **WT:** n=32; Celsr2-/-: n = 16; Celsr3f/-; Wnt1::Cre: n = 16; Celsr3f/-; Isl1::Cre: n = 30; Celsr3f/-; Olig2::Cre: n = 20; Celsr3f/-; n = 52; Celsr2-/-; Celsr3f/-; n = 14.

2. **E12.5 whole embryos**

3. **E13.5 embryos**

4. **E11.5 embryos**

5. **LMC explants from E12.5 embryos**

6. **Results, para 4**

**Significance Levels**

- Mann-Whitney test: U = 5.0
- Mann-Whitney test: U = 8.0
- t-test: p<0.0001
- t-test: t(20)=10.73
- t-test: t(20)=1.926
- t-test: t(20)=1.987
- t-test: t(20)=0.2422
- t-test: t(20)=0.0608
- t-test: t(20)=0.0685
- t-test: t(20)=0.01921
- t-test: t(20)=0.8111
- t-test: t(20)=0.0011
- t-test: t(20)=0.0022
- t-test: t(20)=6.983
- t-test: t(20)=8.371
- t-test: t(20)=10.73
- t-test: t(20)=1.926
- t-test: t(20)=1.987
- t-test: t(20)=0.2422
- t-test: t(20)=0.0608
- t-test: t(20)=0.0685
- t-test: t(20)=0.01921
- t-test: t(20)=0.8111
- t-test: t(20)=0.0022
- t-test: t(20)=6.983
- t-test: t(20)=8.371

**Fig. legend**
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Nature Neuroscience: doi:10.1038/nn.3784
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Supplementary Fig. 4d

Mann-Whitney test

WT: n=22; Celsr3 mutants: n=72

single LMC neurons from E12.5 embryos

Fig. legend
error bars are mean +/- SEM

p=0.8971

Supplementary Fig. 6e

unpaired t-test

six columns: n = 63, 46, 44, 67, 68

LMC motor neurons from E12.5 embryos

Fig. legend
error bars are mean +/- SEM

p=0.6851

Supplementary Fig. 6f

unpaired t-test

wild-type: n = 12; Celsr2 +/-; Celsr3 +/-; EphA4 +/-: n = 14; EphA4 +/-; n = 22; Celsr3-/-; n = 20; Celsr3-/-; EphA4 +/-; n = 18; Celsr3-/-; EphA4 +/-; n = 12

E12.5 embryos

Fig. legend
error bars are mean +/- SEM

ns: p = 0.8637; *: p = 0.026; **: p = 0.008; ***: p < 0.0001

Supplementary Fig. 6g

unpaired t-test

wild-type: n = 10; Celsr3 +/-; Fzd3 +/-; n = 16; Celsr2 +/-; Celsr3 +/-; Fzd3 +/-; n=10

E12.5 embryos

Fig. legend
error bars are mean +/- SEM

p=0.6337

p=0.2250

Supplementary Fig. 7e

unpaired t-test

wild-type: n = 12; Celsr2 +/-; Celsr3 +/-; EphA4 +/-: n = 14; EphA4 +/-; n = 22; Celsr3-/-; n = 20; Celsr3-/-; EphA4 +/-; n = 18; Celsr3-/-; EphA4 +/-; n = 12

E12.5 embryos

Fig. legend
error bars are mean +/- SEM

ns: p = 0.8637; *: p = 0.026; **: p = 0.008; ***: p < 0.0001

Supplementary Fig. 7f

unpaired t-test

wild-type: n = 10; Celsr3 +/-; Fzd3 +/-; n = 16; Celsr2 +/-; Celsr3 +/-; Fzd3 +/-; n=10

E12.5 embryos

Fig. legend
error bars are mean +/- SEM

p=0.6337

p=0.2250

Supplementary Fig. 8i

unpaired t-test

wild-type: n = 12; Celsr2 +/-; Celsr3 +/-; EphA4 +/-: n = 14; EphA4 +/-; n = 22; Celsr3-/-; n = 20; Celsr3-/-; EphA4 +/-; n = 18; Celsr3-/-; EphA4 +/-; n = 12

E12.5 embryos

Fig. legend
error bars are mean +/- SEM

ns: p = 0.8637; *: p = 0.026; **: p = 0.008; ***: p < 0.0001

Supplementary Fig. 8f

unpaired t-test

wild-type: n = 10; Celsr3 +/-; Fzd3 +/-; n = 16; Celsr2 +/-; Celsr3 +/-; Fzd3 +/-; n=10

E12.5 embryos

Fig. legend
error bars are mean +/- SEM

p=0.6337

p=0.2250

Supplementary Fig. 8g

unpaired t-test

wild-type: n = 12; Celsr2 +/-; Celsr3 +/-; EphA4 +/-: n = 14; EphA4 +/-; n = 22; Celsr3-/-; n = 20; Celsr3-/-; EphA4 +/-; n = 18; Celsr3-/-; EphA4 +/-; n = 12

E12.5 embryos

Fig. legend
error bars are mean +/- SEM

ns: p = 0.8637; *: p = 0.026; **: p = 0.008; ***: p < 0.0001

Supplementary Fig. 8h

unpaired t-test

wild-type: n = 10; Celsr3 +/-; Fzd3 +/-; n = 16; Celsr2 +/-; Celsr3 +/-; Fzd3 +/-; n=10

E12.5 embryos

Fig. legend
error bars are mean +/- SEM

p=0.6337

p=0.2250

Supplementary Fig. 8i

unpaired t-test

wild-type: n = 12; Celsr2 +/-; Celsr3 +/-; EphA4 +/-: n = 14; EphA4 +/-; n = 22; Celsr3-/-; n = 20; Celsr3-/-; EphA4 +/-; n = 18; Celsr3-/-; EphA4 +/-; n = 12

E12.5 embryos

Fig. legend
error bars are mean +/- SEM

ns: p = 0.8637; *: p = 0.026; **: p = 0.008; ***: p < 0.0001

Supplementary Fig. 8j

unpaired t-test

wild-type: n = 10; Celsr3 +/-; Fzd3 +/-; n = 16; Celsr2 +/-; Celsr3 +/-; Fzd3 +/-; n=10

E12.5 embryos

Fig. legend
error bars are mean +/- SEM

p=0.6337

p=0.2250

Supplementary Fig. 8k

unpaired t-test

wild-type: n = 12; Celsr2 +/-; Celsr3 +/-; EphA4 +/-: n = 14; EphA4 +/-; n = 22; Celsr3-/-; n = 20; Celsr3-/-; EphA4 +/-; n = 18; Celsr3-/-; EphA4 +/-; n = 12

E12.5 embryos

Fig. legend
error bars are mean +/- SEM

ns: p = 0.8637; *: p = 0.026; **: p = 0.008; ***: p < 0.0001

Supplementary Fig. 8l

unpaired t-test

wild-type: n = 10; Celsr3 +/-; Fzd3 +/-; n = 16; Celsr2 +/-; Celsr3 +/-; Fzd3 +/-; n=10

E12.5 embryos

Fig. legend
error bars are mean +/- SEM

p=0.6337

p=0.2250

Supplementary Fig. 8m

unpaired t-test

wild-type: n = 12; Celsr2 +/-; Celsr3 +/-; EphA4 +/-: n = 14; EphA4 +/-; n = 22; Celsr3-/-; n = 20; Celsr3-/-; EphA4 +/-; n = 18; Celsr3-/-; EphA4 +/-; n = 12

E12.5 embryos

Fig. legend
error bars are mean +/- SEM

ns: p = 0.8637; *: p = 0.026; **: p = 0.008; ***: p < 0.0001

Supplementary Fig. 8n

unpaired t-test

wild-type: n = 10; Celsr3 +/-; Fzd3 +/-; n = 16; Celsr2 +/-; Celsr3 +/-; Fzd3 +/-; n=10

E12.5 embryos

Fig. legend
error bars are mean +/- SEM

p=0.6337

p=0.2250

Supplementary Fig. 8o

unpaired t-test

wild-type: n = 12; Celsr2 +/-; Celsr3 +/-; EphA4 +/-: n = 14; EphA4 +/-; n = 22; Celsr3-/-; n = 20; Celsr3-/-; EphA4 +/-; n = 18; Celsr3-/-; EphA4 +/-; n = 12

E12.5 embryos

Fig. legend
error bars are mean +/- SEM

ns: p = 0.8637; *: p = 0.026; **: p = 0.008; ***: p < 0.0001

Supplementary Fig. 8p

unpaired t-test

wild-type: n = 10; Celsr3 +/-; Fzd3 +/-; n = 16; Celsr2 +/-; Celsr3 +/-; Fzd3 +/-; n=10

E12.5 embryos

Fig. legend
error bars are mean +/- SEM

p=0.6337

p=0.2250

Nature Neuroscience: doi:10.1038/nn.3784
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<tr>
<td>Example</td>
<td>Number</td>
<td>Provided</td>
<td>No</td>
<td>Yes, Fig. 1-7; Supplementary Fig. 1-5,7-9, 11</td>
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<td>Fig. 1-7</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
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<td>2. For each representative image, is there a clear statement of how many times this experiment was successfully repeated and a discussion of any limitations in repeatability?</td>
<td>Yes</td>
<td>Yes</td>
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<td>Yes, Fig. 1-7; Supplementary Fig. 1-5,7-9, 11</td>
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<td>IHC experiments have been performed at least three times, with identical results. Co-IP assays (Fig. 6, 7, Supplementary Fig. 9) were repeated at least twice.</td>
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### Statistics and general methods

|   |   |   |   |   |
|---|---|---|---|
| 1. Is there a justification of the sample size? | No | No | No |
| No | No | No |
| Even if no sample size calculation was performed, authors should report why the sample size is adequate to measure their effect size. |
| Yes, unpaired t-tests were used when the data meet the normality criterion otherwise non-parametric statistical tests (Mann-Whitney test) were used. Details are provided in the figure legends and in the checklist. |

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<td>2. Are statistical tests justified as appropriate for every figure?</td>
<td>Yes</td>
<td>Yes</td>
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a. If there is a section summarizing the statistical methods in the methods, is the statistical test for each experiment clearly defined? | No | No | No |
| No | No | No |
| We checked the normality of the data, and for Fig. 3g, 3h, 5c and Supplementary Fig 6e,7e, 8i, they are normally distributed, and we used unpaired t-test for them. For the other data (non normal or n is not enough to test normality), we used an non-parametric test: the Mann-Whitney test. |
c. Is there any estimate of variance within each group of data?  
Is the variance similar between groups that are being statistically compared?  
Where is this described (section, paragraph #)?  
N/A

d. Are tests specified as one- or two-sided?  
two side T-tests were used (indicated in legends)

3. Are criteria for excluding data points reported?  
Was this criterion established prior to data collection?  
Where is this described (section, paragraph #)?  
N/A

4. Define the method of randomization used to assign subjects (or samples) to the experimental groups and to collect and process data.  
If no randomization was used, state so.  
Where does this appear (section, paragraph #)?  
N/A

5. Is a statement of the extent to which investigator knew the group allocation during the experiment and in assessing outcome included?  
If no blinding was done, state so.  
Where (section, paragraph #)?  
N/A

6. For experiments in live vertebrates, is a statement of compliance with ethical guidelines/regulations included?  
Yes, Methods section, Mutant mice

7. Is the species of the animals used reported?  
Where (section, paragraph #)?  
Yes, Abstract and throughout the paper: Mice (Mus musculus).

8. Is the strain of the animals (including background strains of KO/transgenic animals used) reported?  
No. Mice of mixed background were used to increase yield of embryos.

9. Is the sex of the animals/subjects used reported?  
No

10. Is the age of the animals/subjects reported?  
Yes, embryonic and adult ages are defined in the text and legends when appropriate.

11. For animals housed in a vivarium, is the light/dark cycle reported?  
N/A
12. For animals housed in a vivarium, is the housing group (i.e. number of animals per cage) reported?
   Where (section, paragraph #)?
   N/A

13. For behavioral experiments, is the time of day reported (e.g. light or dark cycle)?
   Where (section, paragraph #)?
   N/A

14. Is the previous history of the animals/subjects (e.g. prior drug administration, surgery, behavioral testing) reported?
   Where (section, paragraph #)?
   N/A
   a. If multiple behavioral tests were conducted in the same group of animals, is this reported?
      Where (section, paragraph #)?
      N/A

15. If any animals/subjects were excluded from analysis, is this reported?
   Where (section, paragraph #)?
   No animal was excluded from analysis
   a. How were the criteria for exclusion defined?
      Where is this described (section, paragraph #)?
   b. Specify reasons for any discrepancy between the number of animals at the beginning and end of the study.
      Where is this described (section, paragraph #)?

Reagents

1. Have antibodies been validated for use in the system under study (assay and species)?
   All antibodies used are commercially available and have been validated in previous studies in our lab and elsewhere.
   a. Is antibody catalog number given?
      Yes, in Methods.
   b. Where were the validation data reported (citation, supplementary information, Antibodypedia)?
      No new validation is reported because those antibodies have all been extensively used in several publications.

2. If cell lines were used to reflect the properties of a particular tissue or disease state, is their source identified?
   N/A
   Where (section, paragraph #)?
a. Were they recently authenticated?
   Where is this information reported (section, paragraph #)?

Data deposition

Data deposition in a public repository is mandatory for:
   a. Protein, DNA and RNA sequences
   b. Macromolecular structures
   c. Crystallographic data for small molecules
   d. Microarray data

Deposition is strongly recommended for many other datasets for which structured public repositories exist; more details on our data policy are available here. We encourage the provision of other source data in supplementary information or in unstructured repositories such as Figshare and Dryad.

1. Are accession codes for deposit dates provided?
   Where (section, paragraph #)?
   N/A

Computer code/software

Any custom algorithm/software that is central to the methods must be supplied by the authors in a usable and readable form for readers at the time of publication. However, referees may ask for this information at any time during the review process.

1. Identify all custom software or scripts that were required to conduct the study and where in the procedures each was used.
   N/A

2. Is computer source code/software provided with the paper or deposited in a public repository? Indicate in what form this is provided or how it can be obtained.
   N/A

Human subjects

1. Which IRB approved the protocol?
   Where is this stated (section, paragraph #)?
   N/A

2. Is demographic information on all subjects provided?
   Where (section, paragraph #)?
   N/A

3. Is the number of human subjects, their age and sex clearly defined?
   Where (section, paragraph #)?
   N/A

4. Are the inclusion and exclusion criteria (if any) clearly specified?
   Where (section, paragraph #)?
   N/A
5. How well were the groups matched?
   Where is this information described (section, paragraph #)?
   N/A

6. Is a statement included confirming that informed consent was obtained from all subjects?
   Where (section, paragraph #)?
   N/A

7. For publication of patient photos, is a statement included confirming that consent to publish was obtained?
   Where (section, paragraph #)?
   N/A

fMRI studies

For papers reporting functional imaging (fMRI) results please ensure that these minimal reporting guidelines are met and that all this information is clearly provided in the methods:

1. Were any subjects scanned but then rejected for the analysis after the data was collected?
   N/A

   a. If yes, is the number rejected and reasons for rejection described?
      Where (section, paragraph #)?
      N/A

2. Is the number of blocks, trials or experimental units per session and/or subjects specified?
   Where (section, paragraph #)?
   N/A

3. Is the length of each trial and interval between trials specified?
   N/A

4. Is a blocked, event-related, or mixed design being used? If applicable, please specify the block length or how the event-related or mixed design was optimized.
   N/A

5. Is the task design clearly described?
   Where (section, paragraph #)?
   N/A

6. How was behavioral performance measured?
   N/A

7. Is an ANOVA or factorial design being used?
   N/A

8. For data acquisition, is a whole brain scan used?
   If not, state area of acquisition.
   N/A

   a. How was this region determined?
      N/A
9. Is the field strength (in Tesla) of the MRI system stated?
   a. Is the pulse sequence type (gradient/spin echo, EPI/spiral) stated? N/A
   b. Are the field-of-view, matrix size, slice thickness, and TE/TR/flip angle clearly stated? N/A

10. Are the software and specific parameters (model/functions, smoothing kernel size if applicable, etc.) used for data processing and pre-processing clearly stated? N/A

11. Is the coordinate space for the anatomical/functional imaging data clearly defined as subject/native space or standardized stereotaxic space, e.g., original Talairach, MNI305, ICBM152, etc? Where (section, paragraph #)? N/A

12. If there was data normalization/standardization to a specific space template, are the type of transformation (linear vs. nonlinear) used and image types being transformed clearly described? Where (section, paragraph #)? N/A

13. How were anatomical locations determined, e.g., via an automated labeling algorithm (AAL), standardized coordinate database (Talairach daemon), probabilistic atlases, etc.? N/A

14. Were any additional regressors (behavioral covariates, motion etc) used? N/A

15. Is the contrast construction clearly defined? N/A

16. Is a mixed/random effects or fixed inference used?
   a. If fixed effects inference used, is this justified? N/A

17. Were repeated measures used (multiple measurements per subject)?
   a. If so, are the method to account for within subject correlation and the assumptions made about variance clearly stated? N/A

18. If the threshold used for inference and visualization in figures varies, is this clearly stated? N/A

19. Are statistical inferences corrected for multiple comparisons?
   a. If not, is this labeled as uncorrected? N/A
20. Are the results based on an ROI (region of interest) analysis? N/A
   a. If so, is the rationale clearly described? N/A
   b. How were the ROI’s defined (functional vs anatomical localization)? N/A

21. Is there correction for multiple comparisons within each voxel? N/A

22. For cluster-wise significance, is the cluster-defining threshold and the corrected significance level defined? N/A

▶ Additional comments

Additional Comments

Nature Neuroscience: doi:10.1038/nn.3784