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Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size

Describe how sample size was determined.

No statistical methods were used to pre-determine sample sizes for our histological, behavioural and cellular experiments but our sample sizes are similar to those reported in previous publications (Keays, Tian et al. 2007, Oliver, Keays et al. 2007, Breuss, Heng et al. 2012, Bejarano, Yuste et al. 2014).

2. Data exclusions

Describe any data exclusions.

Our initial experiments on human dermal fibroblasts were excluded as the cells tested positive for mycoplasm infection. These cells were treated with a Mycoplasm Removal Reagent containing a 4-oxoquinoline-3-carboxylic acid derivative. Following antibiotic treatment we confirmed on multiple occasions that our treated cells are mycoplasm negative (using a PCR based method). For our behavioural experiments animals and their corresponding littermates were excluded in instances where the software tracking failed.

3. Replication

Describe whether the experimental findings were reliably reproduced.

This is indicated in the figure legends for each Figure. Specifically, we replicated the data presented in:
- Figure 1 (d-i), Nissl staining and Marble rescue three times with similar results.
- Figure 2a, Quantification of Vps15, Vps34 and Beclin 1 from Mbe/Mbe, Mbe/+, +/+ animals twice with similar results.
- Figure 3d, quantification of Nischarin protein levels in Mbe/Mbe and +/+ animals twice with similar results.
- Figure 3g, quantification of activated Pak1 levels in Mbe/Mbe and +/+ animals twice with similar results.
- Figure 4a-i, NeuN and Calbindin staining on the hippocampus of Mbe/Mbe, Mbe/+, +/+ animals three times with similar results.
- Figure 5a-l, NeuN, Er81, Foxp2, and Cux1 staining on the cortex of Mbe/Mbe, Mbe/+, +/+ animals twice with similar results.
- Figure 6a-f, Nissl staining of +/fl, +/fl EMX1-Cre, fl/fl EMX1-Cre Vps15 animals twice with similar results.
- Figure 7e-i, western blot analysis of VPS15, VPS34, BECLIN1, and P62 in patients, were performed twice with similar results.
- Figure 7j-n, western blot analysis of VPS15, VPS34, BECLIN1, and P62 twice with similar results.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

Subjects were defined by the genotype - so randomization was not necessary.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

We employed strict blinding procedures. For all our behavioral experiments phenotyping was performed blind to genotype. For all histological analysis and cell counting imaged sections were blinded randomly, and then analysed before the condition/genotype was revealed. This is stated in the methods.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ A statement indicating how many times each experiment was replicated
☐	☒ The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☐	☒ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals noted
☐	☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒ Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.

We employed Graphpad PRISM (v7.0c) for our statistical analysis, and ImageJ (v1.51) for our image analysis . For the analysis of our mouse exome sequencing data we used Burrows-Wheeler Aligner (BWA) v0.5.9 and variants were called using mPileUp within the SAMTools v0.1.19 suite39. For analysis of our proteomic data we used Proteome Discoverer (version 1.4.1.14) and MS Amanda (version 1.4.14.5564). For our analysis of human data we employed SamTools (v0.1.18), Illumina Real Time Analysis software (RTA 1.13.48), snpEff (v2.0.5d), dbNSFP (v1.3), GEM Mapper and Annovar (2011).

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

There are no restrictions on the availability of materials.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

For our histological studies the following antibodies were used 1:1000 GFP (ab13970) (Kita, Kawakami et al. 2013), 1:400 caspase3 (Cell Signaling, #9661) (Chu, Ko et al. 2016), 1:500 LC3 (Nanotools, 0260-100/LC3-2G6), 1:500 p62 (Novus Biologicals, H00008878-M01), 1:300 Sox2 (Santa Cruz, sc-17320) (Navarro-Quiroga, Hernandez-Valdes et al. 2006), 1:300 Tbr2 (Millipore, AB15894) (Vasistha, Garcia-Moreno et al. 2014), 1:100 DCX (Santa Cruz, sc-8066) (Boekhoorn, Sarabdjitsingh et al. 2008), 1:500 pH3 (Millipore, 06-570) (Hendzel, Wei et al. 1997), 1:300 nestin (BD Biosciences, #611658) (Shah, Lutter et al. 2016), 1:300 NeuN (Millipore, MAB377) (Mullen, Buck et al. 1992), 1:250 calbindin (Millipore, AB1778) (Nobrega, Nascimento-Ferreira et al. 2013), 1:100 Cux1 (Santa Cruz, sc-6327) (Keays, Tian et al. 2007), 1:50 Nischarin (Santa Cruz, sc-365364), 1:1000 Er81 (Jessell lab) (Keays, Tian et al. 2007), 1:300 FoxP2 (abcam, ab16046)(Keays, Tian et al. 2007), 1:400 Prox1 (Millipore, MAB5654) (Keays, Cleak et al. 2010), 1:500 GFAP (Dako, Z0334) (Castellano, Gonzalez et al. 1991), 1:2000 Calretinin (Swant, 6B3) (Gonchar, Wang et al. 2007), 1:500 Parvalbumin (Millipore, MAB1572) (Gandal, Sisti et al. 2012). For our western blot studies the following antibodies were used: 1:2000 Vps15 for mouse studies (Novus Biologicals NBP1-30463) (Bejarano, Yuste et al. 2014), 1:1000 Vps15 for human studies (abcam ab124817, abcam ab128903) (Sun, Li et al. 2015), 1:500 Vps34 (Cell Signaling, #3811) (Ma, Zhang et al. 2012, Feng, Chang et al. 2014), 1:1000 Beclin1 (Cell Signaling #3738) (Lee, Lee et al. 2014), 1:500 p62 (Novus Biologicals, H00008878-M01) (Willy, Young et al. 2017), 1:2000 GAPDH (Millipore, MAB374) (Jana, Tanaka et al. 2000, Akizu, Cantagrel et al. 2015), 1:1000 Ubiquitin (Santa Cruz, sc-8017) (Santoh, Sanoh et al. 2016), 1:10000 alpha-tubulin (Sigma Aldrich, T6199) (Rinaldi, Bott et al. 2012), 1:100 LC3 (Nanotools, 0260-100/LC3-2G6) (Walls, Ghosh et al. 2010), 1:600 EGFR (Cell Signaling #4267S) (Bertrand, Lesept et al. 2015), 1:200 Nischarin (Santa Cruz, sc-365364) (Alahari, Reddig et al. 2004), 1:500 PAK1 T423 (Cell Signaling, #2601S) (Li, Leshchyns'ka et al. 2013), 1:500 N-terminal Vps15 antibody (Proteintech, 17894-1-AP).

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

Neuro2A cells were employed to assess the efficiency of shmiRNA constructs and for antibody validation (Vps15 N-term). These cells were obtained from an in house supply at the IMP.

b. Describe the method of cell line authentication used.

qPCR analysis of neuronal markers is consistent with a mouse cell line of neuronal origin.

c. Report whether the cell lines were tested for mycoplasma contamination.

Cells were tested for mycoplasm contamination by our in house molecular biology service that employs a PCR based assay. All data presented in this manuscript is from mycoplasm negative cells.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

None of the cells used are in the ICLAC database.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

For our ENU screen male C57BL/6 male mice (Mus musculus) were given ENU injections and subsequently crossed with C3H/HeH females to generate G1 founder males. These founders were then out-crossed to C3H/HeH and the G2 daughters backcrossed to the G1 Founder male. Adult G3 mice aged approximately 16 weeks were sacrificed and subject to phenotypic analysis. Following the identification of the Marble line it was maintained on a C3H/HeH background. Vps15 KO animals were maintained on a C57/BL6J background. For our behavioral experiments we employed n=20 animals with sex matched littermate pairs (n=9 females, n=11 males). Behavioral testing was performed on animals aged 12 weeks. Histological analysis of the Marble and Vps15 KO lines was performed on animals aged E14.5, P0, P11, 8 weeks and 4 months (n=3-6). Our histological analysis employing the Golgi method was performed on animals aged 4 months (n=3 animals per genotype). For our cellular experiments mouse embryonic fibroblasts (MEFs) were prepared from E13.5 embryos of all three genotypes (+/+, +/Mbe, Mbe/Mbe) (n=3). For our qPCR studies we employed tissue isolated from C57/BL6J animals aged E10.5, E12.5, E14.5, E16.5, E18.5 and postnatal mice P0, P6 and 8 weeks (n=3). For our in utero electroporation studies we used pregnant C57/BL6J females. Embryos were electroporated at E14.5, and then analyzed a E17.5 (n=3-6). The Vps15 rescue line was created by injection of a BAC (RP24-281C16) into the pronuclei of B6CBAF1 zygotes.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

We report all the relevant clinical details with respect to the patient with the L1224R mutation in Vps15 in the results of this manuscript. Specifically, the affected individual presented with severe cortical and optic nerve atrophy, localized cortical dysplasia, intellectual impairment, spasticity, ataxia, psychomotor delay, muscle wasting, pseudobulbar palsy, a mild hearing deficit and late onset epilepsy. He was homozygous for the L1224R mutation, whereas he parents were heterozygous.