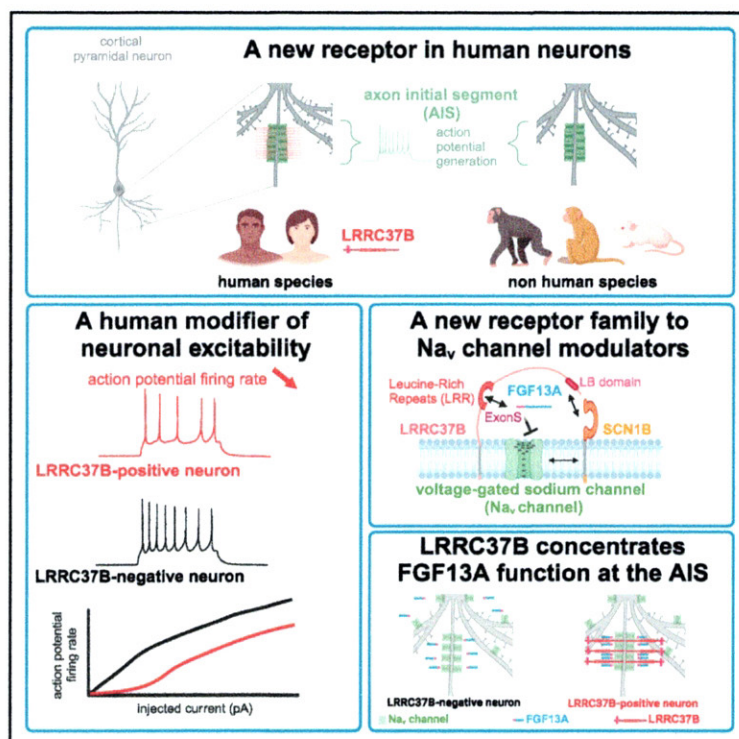


LRRC37B is a human modifier of voltage-gated sodium channels and axon excitability in cortical neurons

Graphical abstract



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In brief

The hominid-specific duplicated gene LRRC37B encodes a membrane receptor expressed selectively at the level of the axon initial segment of pyramidal neurons of the human cerebral cortex. LRRC37B acts as a receptor for FGF13A and thereby concentrates its inhibitory effects on axonal voltage-gated sodium channels, resulting in decreased intrinsic excitability of human cortical pyramidal neurons.

Highlights

- The hominid-specific gene LRRC37B is a human modifier of neuronal excitability
- LRRC37B is selectively localized to the axon initial segment in human cortical neurons
- LRRC37B is a receptor for FGF13A, which inhibits voltage-gated sodium channels
- LRRC37B reduces intrinsic excitability specifically at the axon initial segment



Article

LRRC37B is a human modifier of voltage-gated sodium channels and axon excitability in cortical neurons

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SUMMARY

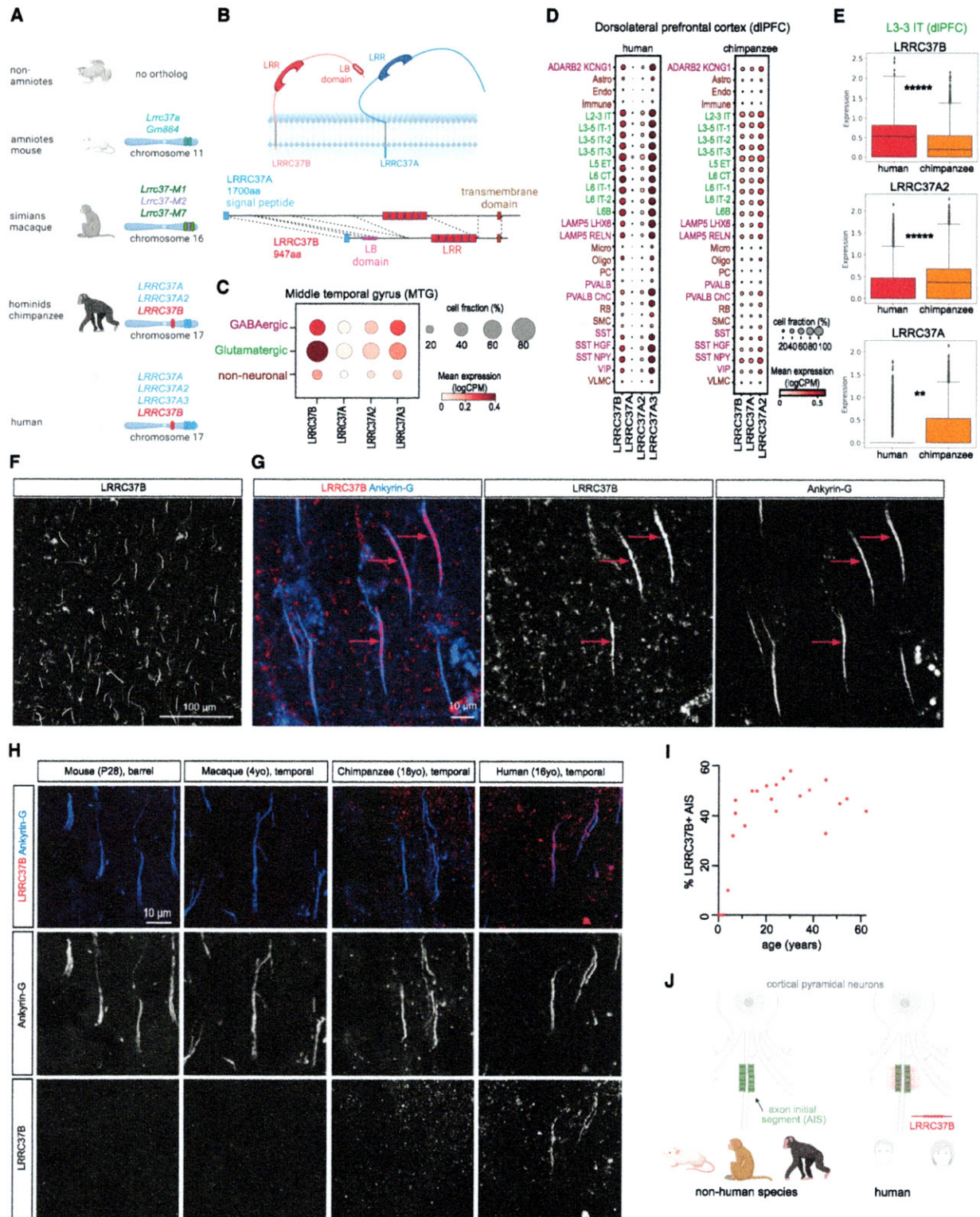
The enhanced cognitive abilities characterizing the human species result from specialized features of neurons and circuits. Here, we report that the hominid-specific gene *LRRC37B* encodes a receptor expressed in human cortical pyramidal neurons (CPNs) and selectively localized to the axon initial segment (AIS), the subcellular compartment triggering action potentials. Ectopic expression of *LRRC37B* in mouse CPNs *in vivo* leads to reduced intrinsic excitability, a distinctive feature of some classes of human CPNs. Molecularly, *LRRC37B* binds to the secreted ligand FGF13A and to the voltage-gated sodium channel (Nav) β -subunit SCN1B. *LRRC37B* concentrates inhibitory effects of FGF13A on Nav channel function, thereby reducing excitability, specifically at the AIS level. Electrophysiological recordings in adult human cortical slices reveal lower neuronal excitability in human CPNs expressing *LRRC37B*. *LRRC37B* thus acts as a species-specific modifier of human neuron excitability, linking human genome and cell evolution, with important implications for human brain function and diseases.

INTRODUCTION

Humans display distinctive cognitive abilities compared with other animals, including our closest hominid relatives, chimpanzees and bonobos.¹ These specialized functions rely on features that distinguish the human brain from those of other species, particularly at the level of the cerebral cortex. Distinctive properties of the human cortex include an increased number of neurons and an increased number of synapses between cortical neurons.^{2–5} These changes are linked to the evolution of mechanisms underlying cortical development, which have started to be unraveled by convergent results of genomics, genetics, and developmental neurobiology.^{4–7}

Next to these global changes, species-specific features of human brain circuits may be linked to evolutionary changes at the level of individual neurons, as described in other species.^{4,8} Human cortical pyramidal neurons (CPNs) display distinctive morphological properties, including larger and more branched dendritic arbors⁹ and increased numbers of synapses per neuron compared with all other primates.¹⁰ Electrophysiological recordings of *ex vivo* human and non-human brain slices have uncovered functional properties of human CPNs that distinguish them from those in other species. Human CPNs display specialized synaptic features leading to stronger and more plastic synapses.^{11–14} Some classes of human CPNs display distinct biophysical and intrinsic properties, which have been linked to the





(legend on next page)

unusual large size of their dendritic arbors, but also to differences in membrane properties, such as the distribution and biophysical properties of ion channels.^{15–24} These changes could impact neuronal intrinsic excitability, which is indeed decreased in some classes of human CPNs compared with other species.^{15,19} This could have significant consequences for circuit function,^{15–23} as excitability determines whether and how neurons integrate external synaptic influences (or input) to generate specific action potential (AP) trains at the axonal level (or output).^{25–27} The input/output relationship can critically affect information processing and neural circuit plasticity, for instance, through neuronal gain modulation, by which neurons adapt to changing inputs, through Hebbian or homeostatic mechanisms.^{25,26}

Changes in neuronal intrinsic properties could thus contribute importantly to the enhanced information processing that characterize our species, but the underlying mechanisms remain essentially unknown.

At the cellular level, the input/output function is known to be modulated in all neuronal compartments, and particularly at the level of the axon initial segment (AIS), the specialized axonal compartment site of AP generation.²⁸ The size and location of the AIS, together with the density and modulation of ion channels concentrated at this level, in particular voltage-gated sodium channels (Nav channels), are critical components of neuronal excitability.^{29–31} However, whether and how the AIS contributes to human neuron specialization remains unknown.

At the molecular level, the specialization of human CPNs could be driven by evolutionary changes in the gene regulatory networks underlying their identity and properties.^{32,33} Human and non-human orthologous cortical neuronal types have been shown to display evolutionary divergence at the transcriptional levels, which could underlie functional species-specific functionality.^{34–37} Evolutionary novelties in human neurons could also emerge from new genes, many of which arose through recent segmental genomic duplications in the human lineage.³⁸ Dozens of hominid-specific gene duplicates are transcriptionally active in the human fetal cortex,³⁹ some of which can act as human-specific modifiers of human brain development.^{39–45} However, none of them have been linked to human cortical neuron function so far.

Here, we focus on the *Leucine-Rich Repeat Containing 37* (*LRRC37*) gene family,⁴⁶ which was amplified in hominid and hu-

man species.⁴⁶ *LRRC37* genes belong to the larger structural group of leucine-rich repeat (LRR) receptors,⁴⁷ but so far, *LRRC37* receptors have no known function or ligands. We found that the hominid-specific gene *LRRC37B* encodes a receptor localized to the AIS in human CPNs. *LRRC37B* decreases intrinsic excitability following overexpression in the mouse, and endogenous expression of *LRRC37B* in human CPNs is associated with decreased neuronal excitability. *LRRC37B* acts as a co-receptor for fibroblast growth factor 13, isoform 1 (hereafter named FGF13A) and thereby inhibits the function of Nav channels, specifically at the AIS level. Our data identify a molecular pathway acquired in the human lineage that regulates neuronal excitability.

RESULTS

LRRC37 gene family displays selective genomic amplification and gene expression in simian and human species

We previously identified >30 hominid-specific genes expressed during human corticogenesis,³⁹ including the *LRRC37* gene family encoding putative membrane receptors.⁴⁶ The number of *LRRC37* genes increased through independent segmental duplications in simian and hominid species, leading to many paralogs.⁴⁶ The human genome carries at least 15 partial duplications that encode several pseudogenes, and at least 4 protein-encoding genes, compared with 2 in the mouse genome (Figure 1A).⁴⁶ *LRRC37* genes encode two types of proteins, A- and B-types. Both A- and B-types are transmembrane proteins that contain an extracellular LRR domain (Figure 1B). The B-type proteins carry an additional specific domain, hereafter referred to as LB domain, located near the extracellular N-terminal part of the protein (Figure 1B). *LRRC37B* types emerged in the simian genomes and are thus not found in the mouse (Figures 1A, S1A, and S1B). Alignment of the amino acid sequences of *LRRC37B*-types found in hominids revealed strong conservation between orthologous human and other hominid (chimpanzee, bonobo, gorilla, and orangutan) proteins, especially at the level of the LB and LRR domains (89%–99% of identity; Figure S1A). The macaque genome contains a single *LRRC37B*-type paralog (*LRRC37-M2*), which displays lower levels of homology with human *LRRC37B* at the amino acid level (65%; Figures S1A and S1B) and does not seem to possess a human orthologous counterpart.

Figure 1. *LRRC37* gene family evolution and expression in the cerebral cortex

- (A) *LRRC37* genes in mouse, macaque, and hominids.⁴⁶
 (B) Protein structure of human *LRRC37* proteins (LRRs, leucine-rich repeats; dashed lines indicate positions of aligned protein sequences; note the presence of a *LRRC37B* specific “LB domain”).
 (C) Expression of *LRRC37* transcripts from scRNA-seq from human MTG.³⁵
 (D) *LRRC37* expression (snRNA-seq) from human and chimpanzee dLPFC⁴⁸; CPNs, green; GABAergic neurons, purple; non-neuronal cells, brown.
 (E) Expression of *LRRC37A*, *LRRC37A2*, and *LRRC37B* in upper-layer CPNs (expression in logCPM, log counts per million; median ± quartiles; Deseq2 test with pseudo-bulk approach; adjusted p value: **p < 0.01, ****p < 10^{−7}).
 (F and G) Immunofluorescence of *LRRC37B* (G, arrows for *LRRC37B*+ neurons) and ankyrin-G (a marker for the axon initial segment [AIS]) in the human cerebral cortex (upper layer, temporal cortex vibratome section, 22 years old).
 (H) Immunofluorescence on cryosections of *LRRC37B* and ankyrin-G in upper layers of mouse barrel cortex (postnatal day 28, P28), and of temporal cortex from 4-year-old macaque, 18-year-old chimpanzee, and 16-year-old human.
 (I) Proportion of CPNs positive for *LRRC37B* at the AIS from neonates to 62 years old (n = 25 cases).
 (J) Schematic summary: *LRRC37B* is localized to the AIS specifically in human.
 See also Figures S1 and S2.

The *LRRC37B* gene studied here thus appears to be present only in hominid genomes. Many hominid-specific genes may be of little functional relevance, which is typically associated with variable copy number (CN), indicative of relaxed evolutionary constraints. We examined CN variation of *LRRC37* human paralogs using whole-genome sequencing⁴⁹ across thousands of diverse humans from the 1000 Genomes Project.⁵⁰ This revealed that *LRRC37B*, shared between hominids, and *LRRC37A3*, for which no ortholog could be found in the chimpanzee, were nearly fixed in the tested population (diploid CN = 2) (Figure S1C), strongly suggesting conservation and functionality, while *LRRC37A* and *LRRC37A2* exhibit CN polymorphism (diploid CN between 0 and 5).

We next examined the pattern of expression of *LRRC37* genes, first in the human cerebral cortex, then in comparison with other species. Inspection of postnatal developmental expression in the human prefrontal cortex (PFC)⁵¹ revealed that all four *LRRC37* genes are expressed throughout development until adulthood (Figure S1D for upper-layer neurons). Inspection of single-cell RNA sequencing (scRNA-seq) data in the human adult middle temporal gyrus (MTG)³⁵ and in the dorsolateral PFC (dlPFC)⁴⁸ revealed that *LRRC37* genes were expressed in most subclasses of CPNs and gamma-aminobutyric acid (GABA)ergic interneurons, but in a fraction of neurons in each subclass (Figures 1C, 1D, and S1E). *LRRC37B* transcript levels were higher in CPNs compared with GABAergic interneurons (1.47-fold higher, adjusted *p* value < 10^{-81} ; Wilcoxon ranked sum test) (Figures 1C and S1E; Table S1). All *LRRC37* genes were expressed at lower levels in non-neuronal cells (at least 1.4-fold lower for the four paralogs, adjusted *p* values < 10^{-31} ; Wilcoxon rank-sum test) (Figures 1C and S1E; Table S1).

Bulk RNA-seq data⁵² revealed expression of *LRRC37* paralogs in the adult human and macaque cerebral cortex, while no *Lrrc37* transcripts were detected in the mouse cortex in any cell type at any stage (Figure S2A), as previously described.⁴⁶ snRNA-seq of human and chimpanzee dlPFC⁴⁸ revealed species differences in the levels of expression of the *LRRC37* paralogs. *LRRC37B* RNA levels were consistently higher in the human than in the chimpanzee cortical neurons (Figures 1D, 1E, and S2B). For instance, in upper-layer CPNs (L2/3 IT), *LRRC37B* was expressed at 1.9-fold higher levels in human than in chimpanzee counterparts (Deseq2 test with pseudo-bulk approach, adjusted *p* value < 6.9×10^{-8}) (Figure 1E). Conversely, *LRRC37A* and *LRRC37A2* were expressed at higher levels in the chimpanzee than in the human neurons (Figures 1D and 1E; Deseq2 tests with pseudo-bulk approach, adjusted *p* values < 0.01 for L2/3 IT neurons).

Overall, these data indicate that within simians the *LRRC37* gene family members display divergent genomic amplification and gene expression in the cerebral cortex. Among them, *LRRC37B* is only present in the hominid genomes, with a fixed CN in the human modern population, and enriched at the transcript level in human cortical neurons (including CPNs) compared with chimpanzee cortical neurons.

LRRC37B is localized to the AIS of human CPNs

We next examined *LRRC37B* at the protein level, using a specific antibody raised against the LB domain (see STAR Methods). This

antibody recognizes *LRRC37B*-type proteins encoded in human, chimpanzee, and macaque, but none of the *LRRC37A* types (Figures S2C and S2D). Using this antibody, we confirmed that *LRRC37B* can be targeted to the cellular membrane with the expected topology in heterologously transfected HEK293T cells (Figure S2C).

We looked at the pattern of endogenous *LRRC37B* protein expression in the adult human cerebral cortex. This revealed that *LRRC37B* is mostly localized at the level of the AIS (marked with ankyrin-G) in a subset (>40%) of CPNs (Figures 1F–1I; Table S2). Similar patterns were observed throughout all cortical areas tested (frontal, temporal, occipital, parietal, and in both upper layer; Figures 1F–1I and S2E; Table S2) and deep-layer CPNs (data not shown). The AIS subcellular compartment is the main site of generation of AP through the opening of voltage-gated sodium channels (Nav channels) concentrated at this location.²⁹ Time course analysis from early postnatal ages to >60 years old human specimens, revealed an absence of AIS immunoreactivity in neonates while the proportion of CPNs displaying *LRRC37B* at the AIS sharply increased from childhood to stabilize at puberty (Figure 1I; Table S2). Finally, we examined the cortical white matter, in which ankyrin-G and Nav channels are co-localized at the nodes of Ranvier.⁵³ Ankyrin-G was moderately co-localized with Nav1.6 ($r = 0.33 \pm 0.07$), as expected, but it was not co-localized with *LRRC37B* ($r = 0.09 \pm 0.04$) (Figure S1F).

We next examined *LRRC37B* expression in other species, using the same antibody (directed against the LB domain; Figure S1A) on similarly prepared cryosections of adult cerebral cortex of mouse (barrel cortex), macaque, and chimpanzee (temporal cortex) (Figures 1H and S2E; Table S3). This failed to reveal any immunoreactivity in the mouse, as expected given the absence of the gene in this species. More surprisingly, there was no detectable immunoreactivity at the AIS levels in macaque or chimpanzee samples, although a weak, diffuse punctate pattern of expression could be detected in the chimpanzee cerebral cortex (Figures 1H and S2E). The species-specific targeting of *LRRC37B* at the AIS level could be related to the higher levels of RNA expression in human CPNs (Figures 1D, 1E, and S2B), or to differential post-transcriptional control of *LRRC37B* in human CPNs.

Thus, *LRRC37B* is an AIS protein selectively expressed in human CPNs (Figure 1J).

LRRC37B negatively regulates CPN excitability

We next performed gain of function of *LRRC37B* in mouse CPNs through *in utero* electroporation (IUE) in the barrel cortex (at embryonic day 15.5, thereby targeting mostly cortical layer II/III CPNs). We electroporated vectors leading to a cre-dependent bicistronic expression of *LRRC37B* and EGFP (Enhanced Green Fluorescent Protein) (*LRRC37B*-expressing neurons), or EGFP alone (control neurons), followed by analysis at postnatal day (P)28. We found that the human *LRRC37B* protein (h*LRRC37B*) was enriched at the AIS, as in human CPNs (Figures 2A and S3A), while control neurons displayed no *LRRC37B* immunoreactivity, as expected (Figure S3A). We detected no differences in the AIS length and localization in *LRRC37B*-expressing neurons compared with control neurons (Figures S3B and S3C).

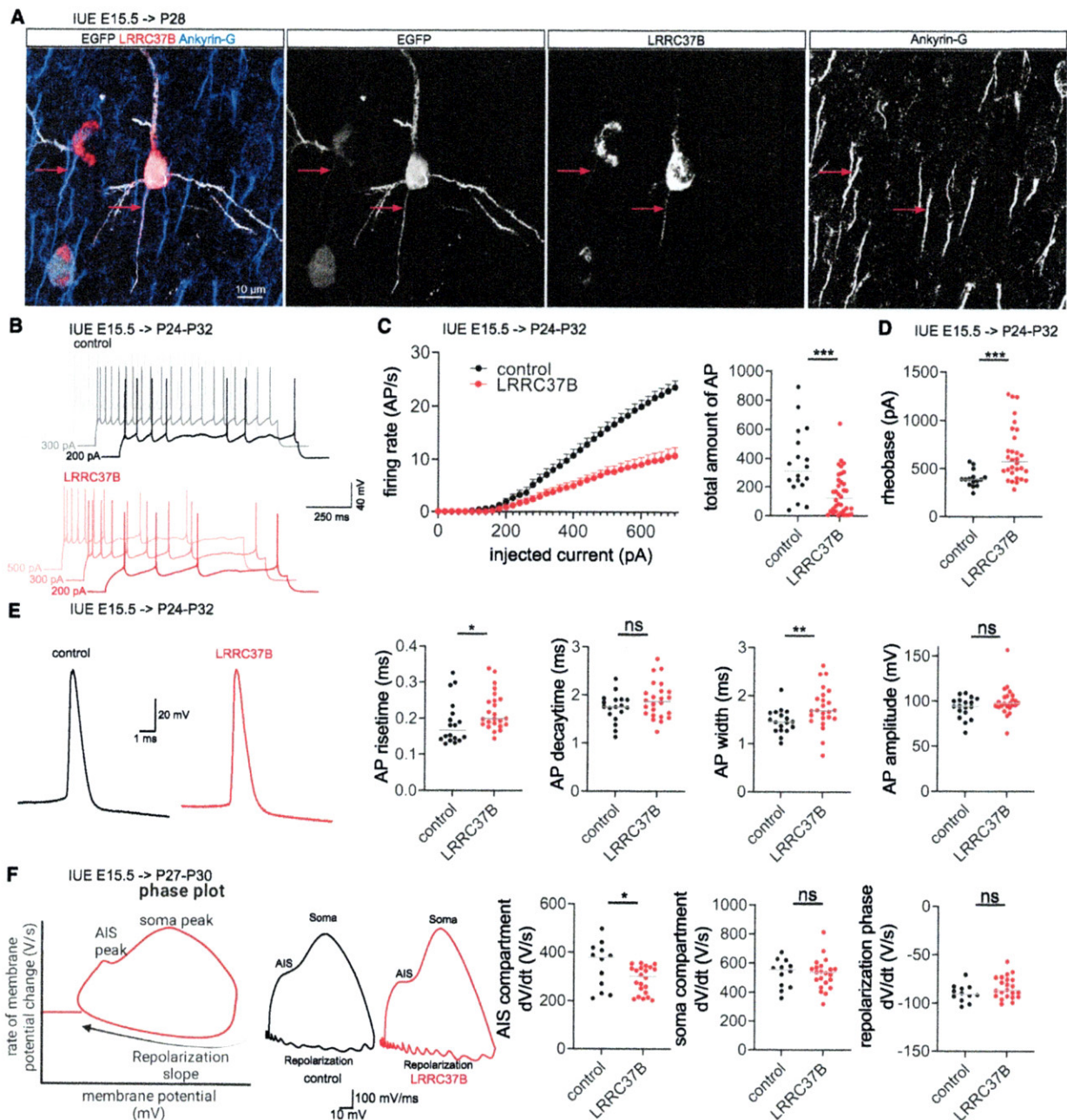


Figure 2. LRR37B is sufficient to decrease neuronal excitability at the AIS

(A) Immunodetection of LRR37B (arrows) and ankyrin-G in the mouse cerebral cortex (barrel cortex) after IUE with plasmids driving bicistronic expression of LRR37B and EGFP (LRR37B neurons), compared with EGFP only (control neurons).

(B) Examples traces of evoked action potential (AP) of LRR37B and control neurons.

(C) Firing rates (mean ± SEM) as a function of injected current of LRR37B neurons (36 neurons from 12 animals from 4 litters) compared with control (18 neurons from 7 animals from 4 litters) with corresponding quantification (line at median, Mann-Whitney test).

(D) Rheobase of LRR37B neurons compared with control neurons (line at median, Mann-Whitney test).

(E) Single evoked AP examples and properties of LRR37B neurons (n = 24 neurons) compared with control neurons (n = 18 neurons) (lines at median, Mann-Whitney tests).

(F) Phase plot analysis of single evoked AP of LRR37B neurons (n = 22 neurons from 11 animals from 4 litters) and control neurons (n = 12 neurons from 5 animals from 4 litters) (lines at median, Mann-Whitney tests) (Method 2). ns, non-significant; *p < 0.05; **p < 0.001; ***p < 0.001.

See also Figure S3.

IUE of *LRRC37A2*, belonging to the more ancestral *LRRC37* A-type, did not reveal a selective localization at the AIS (Figure S3D). On the other hand, IUE of chimpanzee *LRRC37B* (cLRRC37B) led to similar results of AIS subcellular localization (Figures S3A and S3B), further suggesting that the absence of protein detection of *LRRC37B* in the chimpanzee cerebral cortex (Figures 1H and S2E) is not solely due to sequence differences between human and chimpanzee orthologous proteins.

Given the importance of the AIS in neuronal excitability, we next compared the electrophysiological properties of control and *LRRC37B*-expressing mouse cortical CPNs, using patch-clamp recordings in *ex vivo* cortical slices (barrel cortex, cortical layer II/III CPNs) of P24–P32 electroporated mice (Figures 2B–2F and S3E–S3G). *LRRC37B* gain of function led to a sharp decrease in neuronal excitability, characterized by a lower AP firing rate and a higher rheobase (Figures 2B–2D). It also led to a decreased input resistance and an increased capacitance (Figure S3E), which could in principle contribute to changes in the AP firing rates. Furthermore, *LRRC37B*-expressing neurons also displayed specific changes in two critical features of APs, rise-time and width, pointing to changes in intrinsic excitability of the neurons (Figure 2E). Phase plot analyses^{27,54}—used to study the temporal profile of single evoked APs (using 3 independent approaches; see “Methods 1–3” in STAR Methods for details)—revealed that *LRRC37B*-expressing neurons display a selective decrease in the AIS component of APs (between –16% and –20%), while the somatic and repolarization components remained unchanged (Figures 2F and S3F–S3H). Current-voltage IV-curve-based sodium and potassium ionic currents that reflect mostly currents at the level of the cell body⁵⁵ (Figure S3I) also remained unchanged.

Finally, we examined the synaptic properties of *LRRC37B*-expressing neurons. As the AIS is the synaptic target of chandelier GABAergic interneurons that regulate CPN output,⁵⁶ we quantified GABAergic synapses at the level of the AIS. This revealed no difference between *LRRC37B*-expressing and control mouse CPNs in the density of VGAT (Vesicular GABA Transporter) or Gephyrin puncta (Figures S3J and S3K). Whole-cell voltage clamp recordings revealed no difference in frequency or amplitude of spontaneous excitatory and inhibitory postsynaptic currents compared with control neurons (Figure S3L).

These data indicate that *LRRC37B* can act as a negative regulator of intrinsic excitability when overexpressed in mouse CPNs, acting specifically on AP generation at the AIS level.

LRRC37B is a receptor for extracellular FGF13A

To identify the molecular mechanisms underlying the function of *LRRC37B*, we searched for its endogenous ligands. We first performed an ELISA-based interactome screen.^{57,58} We used as a bait the predicted extracellular sequence of *LRRC37B* (*LRRC37B*_ECTO) fused with alkaline phosphatase (ALP).⁵⁹ Using *LRRC37B*_ECTO-ALP immobilized in each well of 384-well plates, 920 transmembrane or secreted proteins (including 24 proteins enriched at the AIS or in chandelier interneurons; see STAR Methods), fused to an Fc domain, were used as preys.

The screen led to a single reproducible hit from three independent experiments: FGF13A (Figure S4A). This result first came as a surprise, as FGF13 is as a member of the non-ca-

nonical FGF (Fibroblast Growth Factor) FHF (FGF Homologous Factor) family, previously reported to encode non-secreted proteins.^{60–62} FGF13 encodes several splice isoforms (FGF13A, FGF13B, FGF13V, FGF13Y, and FGF13VY) that share a C-terminal domain (encoded by coding exons 2–5, hereafter named “core domain”) but differ by their first coding exon (Figure 3A).⁶³ We hypothesized that alternatively spliced mRNAs could encode secreted or non-secreted isoforms of FGF13. We tested this using heterologous expression in HEK293T cells of all major FGF13 isoforms, followed by analysis of cell lysates vs. medium. Only FGF13A could be detected as a secreted protein in the culture medium, but none of the other tested isoforms, nor the core domain (Figure 3A). We next tested whether FGF13A could bind to *LRRC37B* using co-immunoprecipitation (coIP). *LRRC37B* could be coIP only with FGF13A, and not with any of the other tested isoforms, nor with the FGF13 core domain (Figure 3B). Adding recombinant FGF13A protein to the culture medium of cells expressing *LRRC37B* also resulted in coIP of FGF13A with *LRRC37B* (Figure 3C). Similar binding results were obtained with a synthetic peptide corresponding to the first exon of FGF13A (ExonS; Figure 3C), indicating that the ExonS-encoded domain alone is sufficient to bind to *LRRC37B*.

These data indicate that FGF13A is an extracellular ligand of *LRRC37B*, to which it binds to through its isoform-specific N-terminal domain, henceforth referred to as F13ExonS.

In order to identify the domains of *LRRC37B* receptor responsible for the interaction with FGF13A, we generated a series of *LRRC37B* constructs containing deletions in the extracellular domain of *LRRC37B*, fused at the N-terminal with the prolactin leader peptide and an hemagglutinin (HA) tag, and at the C-terminal with the transmembrane domain of PDGF-R (Platelet-Derived Growth Factor Receptor) (Figure 3D). We performed coIPs of the different *LRRC37B* deletion mutants transfected in HEK293T cells, following application of FGF13A to the culture medium. This revealed that the LRR domain (amino acids 468–841) was necessary and sufficient to bind to FGF13A (Figure 3E). Similar coIPs were observed following co-transfection of *LRRC37B* and FGF13A cDNAs and FGF13A IP (Figure S4B). We also compared the molecular properties of human *LRRC37B* with macaque and chimpanzee *LRRC37B* proteins. This revealed that the chimpanzee protein could bind to FGF13A, while the macaque *LRRC37B* did not (Figure S4C), in line with its shorter LRR domain structure (Figure S1B). Similarly, human *LRRC37A2*, which displays an LRR domain very similar to *LRRC37B* (Figure S1A), could bind to FGF13A (Figure S4C). To assess the specificity of this interaction, we performed the same approach with the extracellular domains of other membrane receptors (GPR158, SLITRK2, LRRTM1, and CD4), including several LRR receptors. None of them coIPed FGF13A (Figure S4D).

We next performed interaction measurements between the purified recombinant LRR domain (amino acids 468–841) fused at its N terminus with a FLAG tag (Figure S4E) and a synthetic peptide corresponding to F13ExonS. As negative controls, we used synthetically produced random peptides, and the ExonU of FGF13B (F13ExonU; Figure 3A). Fluorescent polarization (FP) assays showed that LRR binds strongly to F13ExonS (dissociation

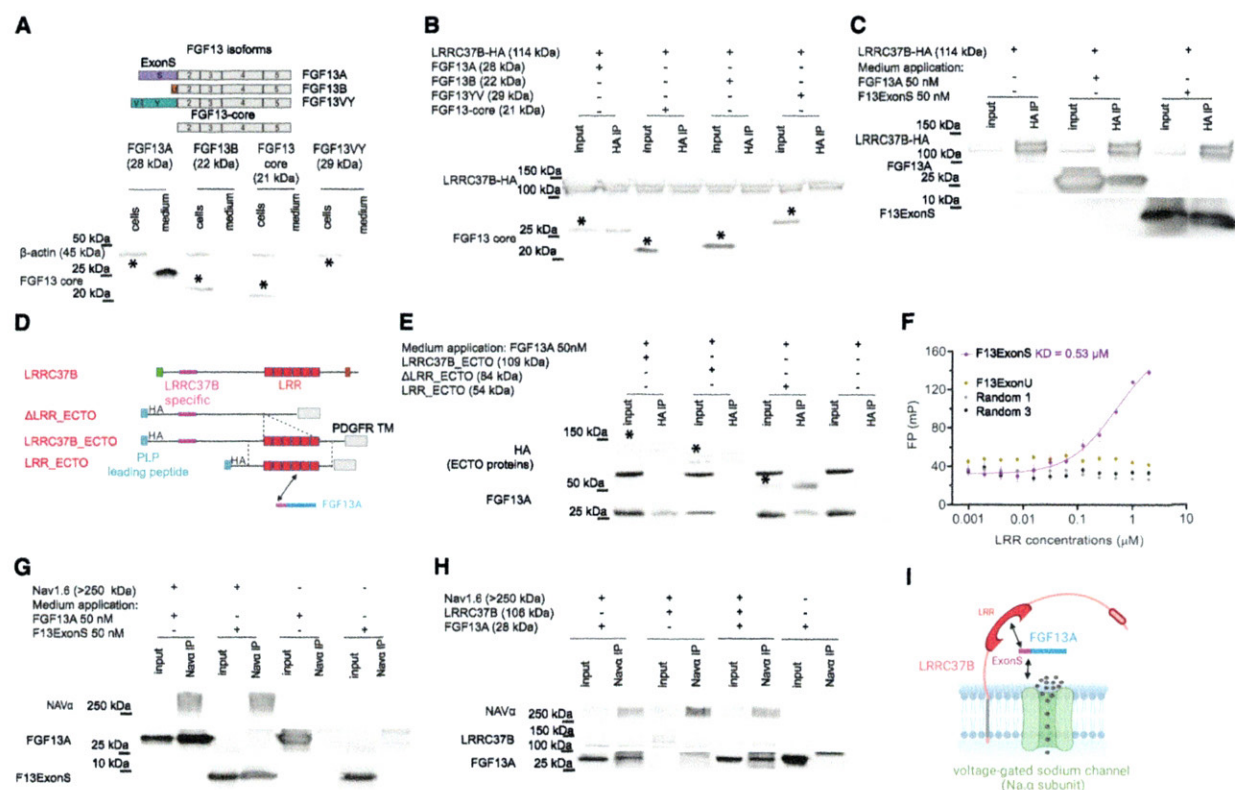


Figure 3. LRR37B is a receptor for FGF13A

(A) FGF13 codes for several spliced isoforms; cell lysate and medium samples of HEK293T cells transfected for FGF13A, FGF13B, FGF13VY, and FGF13-core cDNAs (stars indicate each isoform in the cell extracts) detected with FGF13 antibody.

(B) LRR37B-HA IP from HEK293T cells co-transfected for LRR37B-HA cDNA and cDNAs coding for the different FGF13 isoforms (stars indicate each isoform in the inputs) detected with FGF13 antibody.

(C) LRR37B-HA IP from HEK293T cells transfected for LRR37B-HA cDNA and with recombinant FGF13A or its synthetic F13ExonS applied in the culture medium detected with a FGF13A-specific antibody (used in next panels).

(D and E) IP from HEK293T cells transfected with LRR37B and mutants lacking or carrying LRR of the extracellular domain and treated with recombinant FGF13A in the culture medium (stars indicate each LRR37B protein in the inputs).

(F) Fluorescence polarization (FP) assay between LRR recombinant protein and F13ExonS peptide, F13ExonU peptide (part of FGF13B) and two random peptides (n = 9 measures; mean ± SD).

(G) IP of Nav1.6 (SCN8A) from HEK293T cells transfected for SCN8A cDNA with recombinant FGF13A or its synthetic F13ExonS applied in the culture medium.

(H) Nav1.6 IPs from HEK293T cells transfected for SCN8A ± FGF13A ± LRR37B cDNAs.

(I) Schematics of the LRR37B-FGF13A-Nav1.6 interaction.

See also Figure S4.

constant, $K_d = 530$ nM), whereas no binding was observed for the negative controls, including F13ExonU (Figure 3F). The affinity of the interaction between F13ExonS and LRR37B was also estimated by performing cell-surface-binding assays using a biotin-labeled F13ExonS synthetic peptide and HEK293T cells expressing the extracellular domain of LRR37B and deletion mutants (Figure S4F). This revealed a specific binding of F13ExonS to the extracellular domain of LRR37B ($K_d = 1.105 \pm 0.261$ nM) and to the LRR domain alone ($K_d = 1.179 \pm 0.280$ nM), while no binding was detected with LRR37B mutant devoid of LRR (Figure S4F).

Overall, these experiments indicate that the LRR domain of LRR37B interacts directly and with high affinity with F13ExonS.

Extracellular FGF13A and F13ExonS bind to Nav channel SCN8A/Nav1.6

FGF13 was previously reported to modulate the function of Nav channels through binding of its core domain to an intracellular domain of the channel.^{64–67} Given our data above, we hypothesized that extracellular FGF13A could potentially bind to Nav channels. We first tested whether FGF13A and F13ExonS could bind to the Nav channel Nav1.6 (encoded by *sodium-voltage-gated channel alpha subunit 8*, SCN8A), a major Nav channel that initiates AP at the AIS of CPNs.^{29,68,69} FGF13A and F13ExonS, when applied in the medium of HEK293T cells transfected for Nav1.6, could be coIPed with Nav1.6 (Figure 3G). Moreover, binding assays using a biotin-labeled F13ExonS peptide and HEK293T cells expressing Nav1.6 revealed specific

binding of F13ExonS to Nav1.6 ($K_d = 49.826 \pm 0.261$ nM) (Figure S4G).

Finally, co-transfecting the three proteins in HEK293T cells revealed that LRRC37B could not be colP with Nav1.6 alone, while it could be found in the same complex in the presence of FGF13A (Figure 3H).

These data indicate that FGF13A when applied extracellularly can bind directly to LRRC37B and to the Nav channel Nav1.6, allowing the three proteins to form a complex when co-expressed together (Figure 3I).

Extracellular FGF13A and F13ExonS decrease the intrinsic excitability of CPNs

We next tested whether extracellular FGF13A may affect the physiological properties of mouse CPNs *in vivo*. We performed patch-clamp recordings of mouse non-electroporated cortical slices (barrel cortex at P24–P32) combined with extracellular bath application of recombinant protein FGF13A (Figures 4 and S5A–S5D; Table S4). This revealed a dose-dependent effect of FGF13A on neuronal excitability at 10–50 nM, leading to decreased AP firing rate, increased rheobase, increased AP risetime and width, as observed following LRRC37B gain of function (Figures 4A–4E, S5A, and S5B; Table S4). FGF13A addition had no effect on membrane resistance and capacitance (Figure S5B; Table S4). Phase plot analyses on multiple trains of AP revealed that FGF13A extracellular application led to a decrease in the AIS compartment (–17.8%), as observed following LRRC37B gain of function (Figures 4F and S5D). However, unlike for LRRC37B, we also detected effects on the soma (–9.4%) and during repolarization phases (Figure 4F). Moreover, we observed a global decrease in ionic currents, reflecting mostly cell body currents (Figure S5C),⁵⁵ two effects not seen following LRRC37B gain of function alone (Figure S3I). F13ExonS elicited the same effects as FGF13A except for the AP width (Figures 4A and S5A–S5C; Table S4).

In contrast, the same experiments performed with intracellular application of FGF13A at the same concentration (50 nM) did not lead to detectable effects on neuronal excitability, except a small decrease in AP amplitude and sodium currents (Figures S5E–S5I), which could be due to leakage of internal medium from the recording pipette during the cellular approach phase. These results further indicate that at this concentration, FGF13A exerts its effects essentially at the extracellular level.

Thus, extracellular FGF13A can decrease mouse CPN excitability, but at global levels not restricted to the AIS, unlike LRRC37B.

LRRC37B concentrates FGF13A function at the AIS of CPNs

We next examined the functional interaction between LRRC37B, Nav1.6, and FGF13A *in vivo* in LRRC37B-expressing mouse CPNs versus control CPNs. We performed colP of LRRC37B from electroporated cortex, which confirmed *in vivo* the interaction between LRRC37B and FGF13A (Figure S6A). We then examined the distribution of the proteins by immunofluorescence. This revealed that FGF13A was present also at the level of the AIS (Figures 5A and 5B), but more abundantly in LRRC37B-expressing CPNs than in control CPNs, in distinct patches along the AIS (Figures 5B and S6B). STED (stimulated

emission depletion) microscopy confirmed that LRRC37B was expressed at the level of the AIS in the vicinity of Nav1.6 and FGF13A, with a high co-occurrence between LRRC37B and Nav α ($r = 0.57 \pm 0.17$), and moderate co-occurrence between LRRC37B and FGF13A ($r = 0.32 \pm 0.17$) (Figure 5A). We measured autocorrelation and periodicity of LRRC37B, FGF13A, and Nav α signals. We obtained similar periodicity values for all proteins in the range of 190 nm (Figure S6C), as typically found for AIS protein clusters.^{29,70}

To test the impact of FGF13A–LRRC37B on neuronal function, we combined FGF13A extracellular application (50 nM) with LRRC37B gain of function in mouse CPNs *in vivo*. The effects of FGF13A application and LRRC37B overexpression were similar but non-additive, leading to similarly decreased AP firing rate, increased rheobase, increased AP risetime and width, and decreased AP generation at the AIS level (Figures 5C–5G and S6D–S6F). The effects of FGF13A on ionic currents were unaffected by LRRC37B overexpression (Figure S6G), consistent with global effects of FGF13A on all neuronal compartments (Figures 4F, 5G, S5C, and S6G), and those of LRRC37B restricted to the AIS (Figures 2F and 5G).

Altogether, these data indicate that LRRC37B interacts with FGF13A at the AIS and thereby concentrates its effects on neuronal excitability at this level.

LRRC37B binds to the Nav β subunit 1 (SCN1B)

To identify additional binding partners of LRRC37B, we performed affinity purification of rat brain extracts using as a bait the ectodomain of LRRC37B fused to the Fc portion of human IgG (LRRC37B_ECTO-Fc),⁷¹ followed by liquid chromatography–tandem mass spectrometry (LC-MS/MS) analysis, compared with Fc alone. Several membrane proteins of the AIS were detected uniquely in the LRRC37B_ECTO-Fc condition, at low levels, including the Nav channel Nav1.1 (encoded by SCN1A [sodium voltage-gated channel α subunit 1]), but also the regulatory Nav β subunit SCN1B (encoded by sodium voltage-gated channel β subunit 1) (Figure S7A). AIS proteins are resistant to detergents, which makes their extraction challenging,⁷² hence the generally low number of spectral counts.

Nav β -subunits are not channels but cell adhesion molecules that can directly bind to and positively regulate the trafficking and function of Nav channels, and are thereby critically involved in neuronal excitability.^{73,74} We therefore tested their potential interactions with LRRC37B. Using heterologous co-expression in HEK193T cells, we found that the extracellular part of LRRC37B could colP SCN1B (Figures 6A–6C). We next tested for the interaction of SCN1B with several LRRC37B deletion mutants. We found that the LB domain (amino acids 133–186 of LRRC37B) was necessary to mediate the binding between LRRC37B and SCN1B (Figures 6A and 6B). Consistent with the importance of the LB domain for SCN1B interaction, we detected no interaction between SCN1B and LRRC37A2 that lacks this domain, while macaque and chimpanzee LRRC37B receptors both displayed binding to SCN1B (Figure S7B).

Overall, these data show that the LB domain of LRRC37B is necessary and sufficient to mediate the binding to SCN1B.

Finally, we tested the interactions between LRRC37B and its binding partners FGF13A and SCN1B, as well as Nav1.6. We

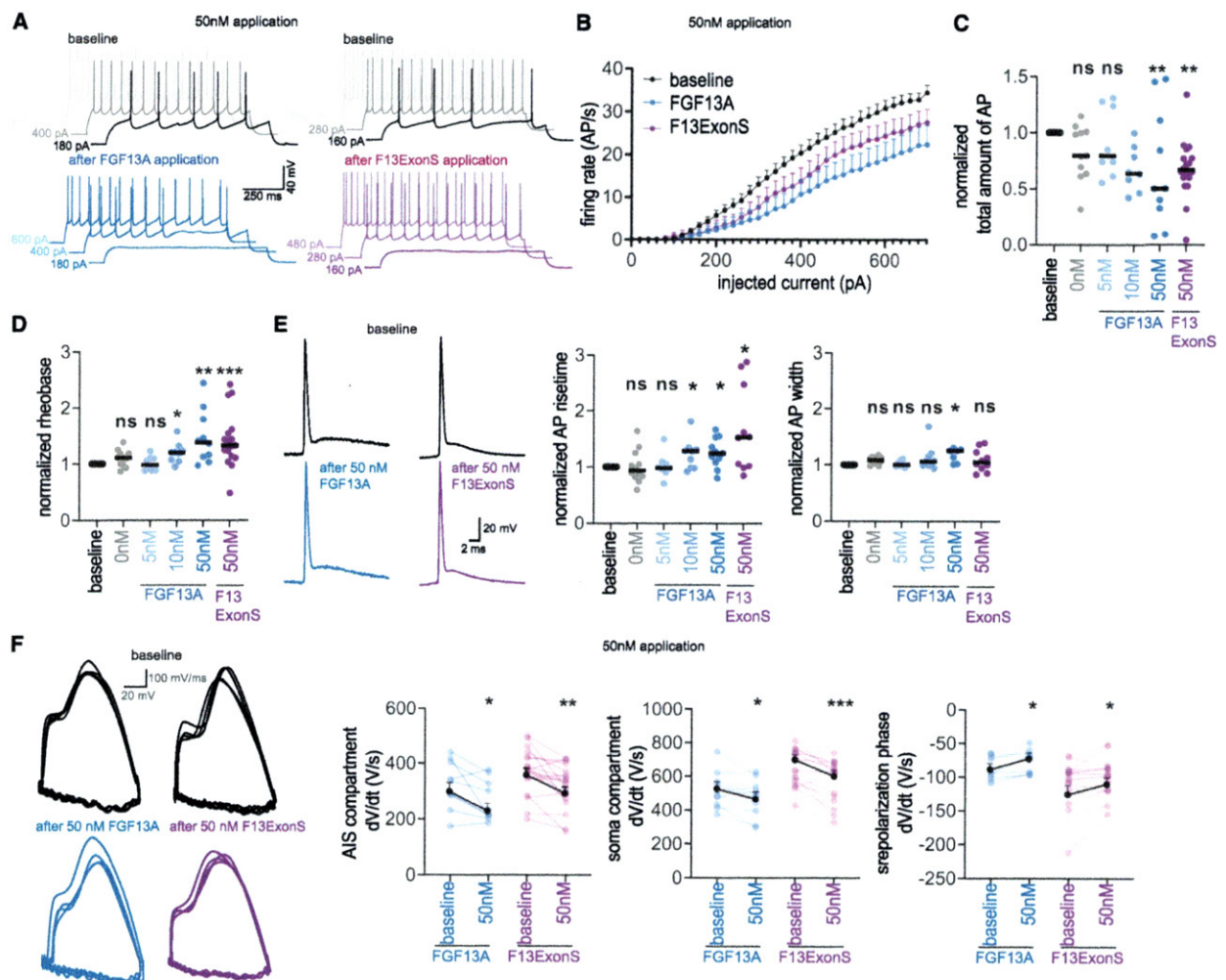


Figure 4. FGF13A regulates neuronal excitability through its F13ExonS peptide

(A) Examples traces of evoked AP of mouse CPNs (barrel cortex, P24–P32) with 50 nM recombinant FGF13A or synthetic F13ExonS extracellular application. (B) Corresponding firing rates (mean + SEM). (C) Dose-response effect of recombinant FGF13A extracellular application on mouse CPNs at 0 nM (11 neurons from 3 animals), 5 nM (8 neurons from 3 animals), 10 nM (9 neurons from 2 animals), and 50 nM (10 neurons from 4 animals) or synthetic F13ExonS at 50 nM (18 neurons from 8 animals) (values for each neuron are normalized to their initial value before application; lines at median; for each dose, paired Wilcoxon test). (D) Similar dose-response effects on rheobase. (E) Single evoked AP examples and dose-response effects on AP properties (shown as in C). (F) Phase plot analysis of AP generation (multiple trains, Method 4) with 50 nM FGF13A or F13ExonS extracellular application on mouse CPNs (each replicate in blue or purple; mean + SEM in black; paired Wilcoxon tests). ns, non-significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. See also Figure S5.

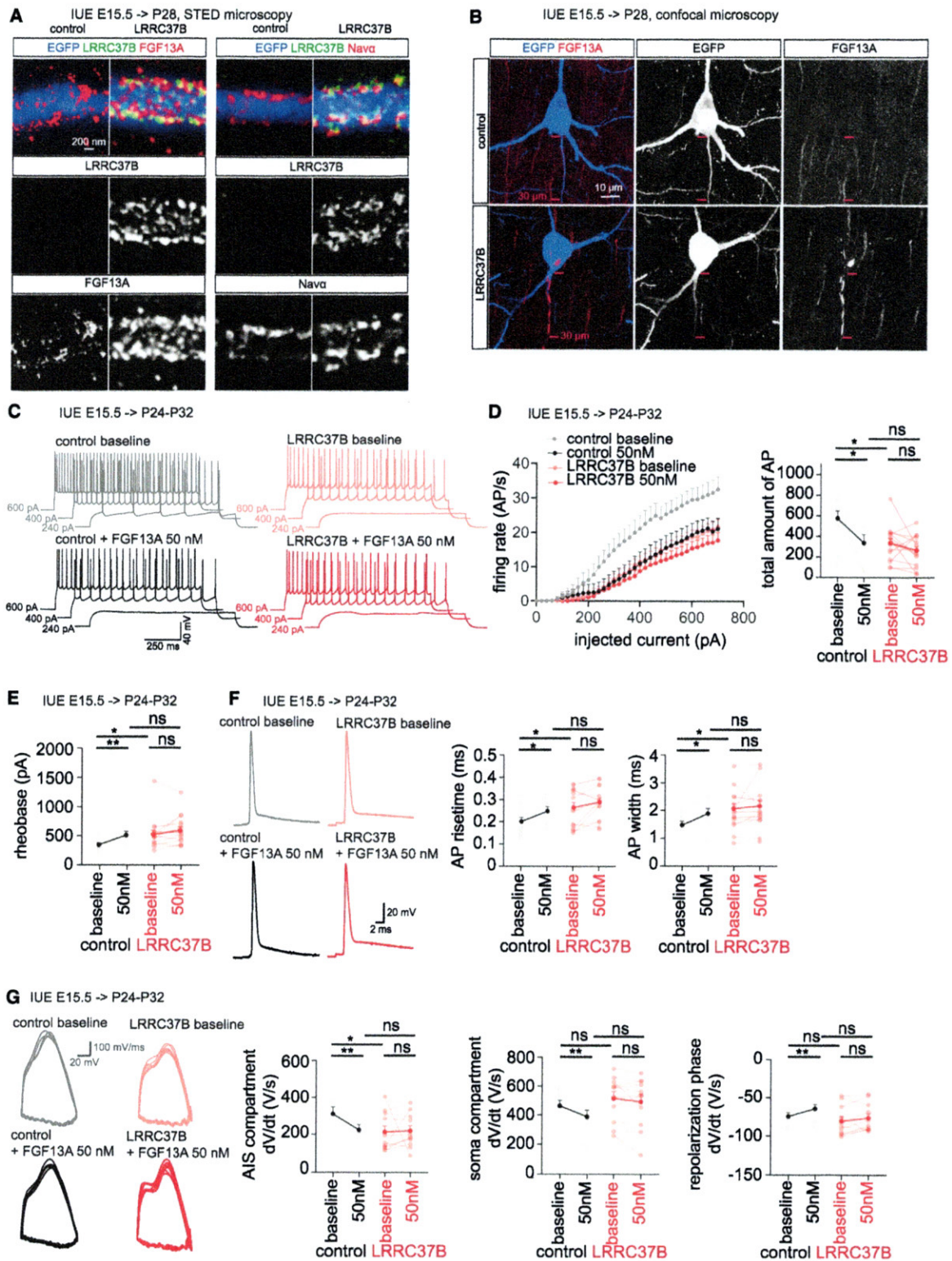
found that LRRC37B, SCN1B, and FGF13A could be coIPed in the same complex, while SCN1B alone could not bind to FGF13A (Figure 6C). SCN1B was found to bind to Nav1.6, as expected,⁷³ but this interaction was abolished when co-transfecting LRRC37B (Figure 6D).

These results indicate that LRRC37B binds to SCN1B through its specific LB domain and that LRRC37B and Nav1.6 can compete for their binding to SCN1B, the functional consequence of which remains to be explored.

LRRC37B regulates human CPN excitability

Could LRRC37B interactions with Nav1.6 regulators FGF13A and SCN1B be physiologically relevant in the human cerebral cortex *in vivo*?

By examining scRNA-seq databases³⁶ we found that FGF13, SCN1B, SCN8A (Nav1.6), and LRRC37B were co-expressed in most types of human cortical neurons (Figure S7C). FGF13 was expressed at higher levels in GABAergic interneurons compared with CPN (>2-fold, adjusted p value < 10^{-7} ,



(legend on next page)

Wilcoxon rank-sum test) (Figure S7C). SCN8A and SCN1B were expressed at higher levels in cortical neurons compared with non-neuronal cells (>3-fold for each, adjusted p values < 2×10^{-56} , Wilcoxon rank-sum tests). We next performed co-IP experiments from human cortical tissue, which revealed that Nav α subunits (Nav channels), LRRC37B, SCN1B, and FGF13A were found in the same IP complex (Figure 7A).

Finally, we performed acute patch-clamp recordings on human *ex vivo* temporal cortex biopsies (focusing on cortical layer II/III CPNs) (Figure 7B; Table S2). We confirmed that human L2/3 CPNs display a lower excitability compared with mouse counterparts, as previously reported (Figure S7D).¹⁵ Next, taking advantage of the fact that only a subset (>40%) of human L2/3 CPNs express LRRC37B (Figure 1I), we compared the properties of LRRC37B-positive versus LRRC37B-negative neurons. Cells were filled with biocytin during electrophysiological recordings, followed by LRRC37B immunostainings (Figure 7C).

This revealed that LRRC37B-positive neurons display lower excitability compared with LRRC37B-negative neurons, with a decreased AP firing rate, an increased rheobase and increased AP risetime and width (Figures 7D–7G), while we observed no differences in input resistance, capacitance, ionic currents (Figures S7E and S7F). Phase plot analysis of single APs (measured through 2 different independent approaches—see “Methods 2 and 3”) revealed that LRRC37B-positive neurons display a decrease in the AIS compartment (between –22% and –29%) compared with LRRC37B-negative neurons, while other compartments remained unchanged (Figures 7H, S7G, and S7H). Finally, as the cortical depth, size of the soma, and length of the AIS have been associated with changes in neuronal excitability,^{19,30,36} we examined these parameters but detected no differences between LRRC37B-positive and LRRC37B-negative neurons (Figures S7I–S7K).

Overall, these data indicate that endogenous expression of LRRC37B in human CPNs is associated with decreased intrinsic excitability at the level of the AIS.

DISCUSSION

Human brain evolution is associated with global changes in structure and function of the cerebral cortex.^{5,6} Distinctive novelties of the human cortex also likely emerged from evolutionary divergence at the level of individual neurons.⁴ Specialization in morphology and physiology of human cortical neurons is likely linked to gene expression divergence in with orthologous cortical

neuron subtypes from other species,^{34–36} but the links between human genomic and neuronal evolution have remained largely unknown.

Evolutionary changes targeting various classes of ion channels were previously reported in several species, leading to species-specific neuronal features.⁸ In humans, a human-specific gene duplicate of the $\alpha 7$ -nAChR (nicotinic Acetylcholine Receptor) was reported to inhibit the ancestral gene in heterologous systems,⁷⁵ H-channels are expressed at higher levels in human compared with mouse CPNs,¹⁶ while Nav and Kv channels display distinct biophysical properties in human CPNs, leading to a higher stability of AP kinetics.²⁴ These findings suggest that the modulation of membrane conductance properties is an important driver of neuronal evolution, for which LRRC37B constitutes a striking example in our species, by linking gene duplication with neuronal excitability.

Some classes of human neurons display distinctive functional properties when compared with other species, including decreased excitability.^{15–23} Although the reported human specializations may be partly due to differences in the identity of the neurons analyzed across species,^{23,76} some of them likely bear important evolutionary significance. The underlying mechanisms have been linked to properties of the dendrites, membrane capacitance, or ionic conductance. The involvement of the AIS had not been considered so far, even though distinct AP properties were previously described at the AIS of human CPNs.¹⁴ Here, we found that LRRC37B, by concentrating FGF13A at the AIS, contributes to decreased excitability of CPNs. As FGF13A could decrease neuronal excitability more globally than LRRC37B itself, it may act on other Nav channels located in other neuronal compartments.

We also found that LRRC37B interacts with SCN1B, but the functional meaning of this interaction remains to be clarified. Given the known positive impact of SCN1B on Nav channel function,⁷³ the ability of LRRC37B to compete with Nav1.6 for binding to SCN1B could potentially lead to an inhibitory influence. Alternatively, as SCN1B promotes the targeting of Nav channels to the AIS,⁷⁴ the binding of SCN1B to LRRC37B could similarly target LRRC37B to the AIS. Related to this, the mechanisms that underlie the species-specific localization of LRRC37B at the AIS of human CPNs remain to be elucidated. Despite strong structure conservation and ability of the chimpanzee LRRC37B protein to be targeted to the mouse CPN AIS, it could not be detected at the AIS in the chimpanzee cortex. This reflects the lower levels of LRRC37B RNA expression that we observed in the chimpanzee cortex but could also involve species-specific

Figure 5. LRRC37B concentrates FGF13A at the level of the AIS

(A) AIS of LRRC37B and control CPNs immunostained for EGFP, LRRC37B, FGF13A, and Nav α subunits (Nav channels).

(B) LRRC37B and control CPNs immunostained for EGFP and FGF13A.

(C) Examples of traces of evoked AP of control and LRRC37B neurons with 50-nM recombinant FGF13A extracellular application.

(D and E) Corresponding firing rates (mean + SEM) of LRRC37B neurons (15 neurons from 8 animals from 5 litters) and control neurons (13 neurons from 7 animals from 5 litters) with corresponding quantification (D) and rheobase (E) (in gray and pink, each replicate; in dark and red, mean + SEM; paired Wilcoxon tests to test the application per group, Mann-Whitney tests to compare groups before and after applications).

(F) Corresponding single evoked AP examples and properties (shown as in D and E).

(G) Phase plot analysis of AP (multiple train) in LRRC37B neurons (11 neurons from 6 animals from 3 litters) and control neurons (10 neurons from 4 animals from 3 litters) with 50 nM FGF13A extracellular application (shown as in D and E) (Method 4). ns, non-significant; * p < 0.05; ** p < 0.01.

See also Figure S6.

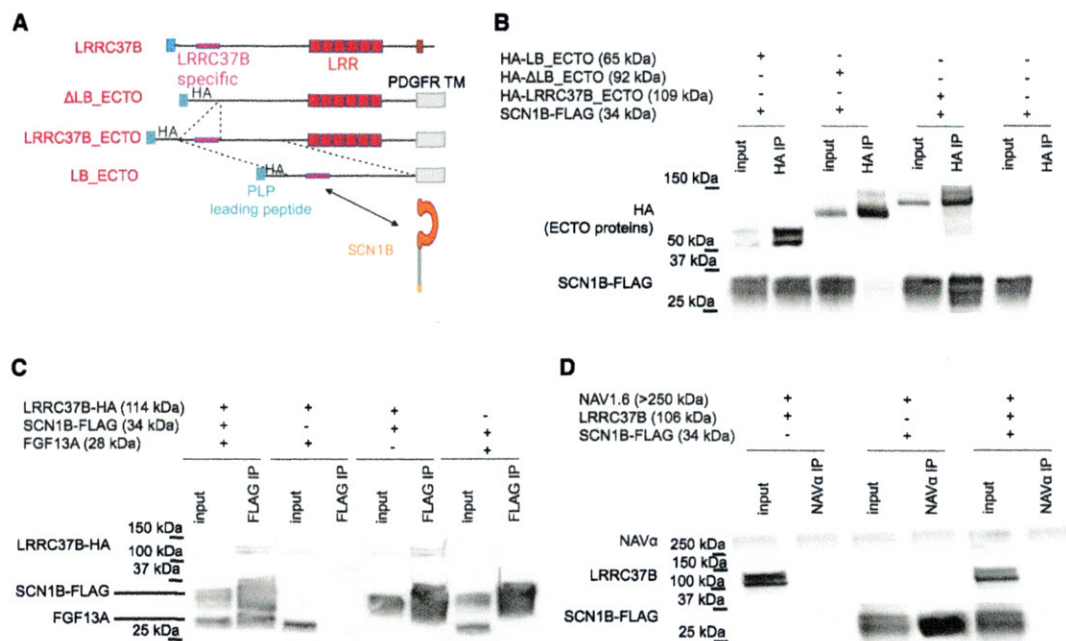


Figure 6. LRRC37B binds to SCN1B through its specific B domain

(A and B) IP of different protein lacking or carrying the LB specific domain of the extracellular domain of LRRC37B from HEK293T cells co-transfected for the SCN1B cDNA.

(C) SCN1B IPs from HEK293T cells transfected for SCN1B ± LRRC37B ± FGF13A cDNAs.

(D) Nav1.6 IPs from HEK293T cells transfected for SCN1B ± LRRC37B cDNAs.

See also Figure S7.

post-transcriptional regulation or protein trafficking. The latter could help explain our finding that early developing human cortex displays detectable LRRC37B RNA, but no protein at the level of the AIS.

We identify FGF13A as a ligand for LRRC37B and for Nav1.6/SCN8A when applied extracellularly. This was unexpected since FHF non-canonical FGFs (FGF11–13) were reported to encode non-secreted proteins, and do not display a predicted signal peptide.⁶¹ Notably however, proteins lacking N-terminal signal peptides, among them FGF2, can be secreted through non-canonical mechanisms.⁷⁷ Inhibitory effects of intracellular FGF13A on Nav channels were reported previously.^{60,67,78–81} However, these studies used either FGF13A transfection or very high (1 mM) intracellular concentrations of FGF13,^{60,67} while at the more physiological (nM) concentrations used in our study, no effect was detectable following intracellular addition of FGF13A, except a mild reduction of sodium current intensities and AP amplitude. FGF13A thus appears to act mostly extracellularly to decrease neuronal excitability, while other isoforms of FGF13 or its core domain could act intracellularly.^{67,79} The sequence of ExonS of FGF13A, or F13ExonS, which is necessary and sufficient to bind to LRRC37B and decrease CPN excitability, is highly conserved with other members of the FHF family (FGF11–14).⁸² This suggests that could they too could encode secreted ligands that bind to LRRC37B and modulate the activity of voltage-gated channels.

Given the extracellular localization of FGF13A, a remaining question is the cellular origin of the ligand. FGF13 has been found at the level of the AIS of pyramidal neurons in the hippocampus⁸³ and we find it similarly located in cortical CPNs, but it is also expressed in chandelier interneurons (ChC), which regulate CPN excitability through inhibitory synapses targeting the AIS of CPNs.⁸⁴ FGF13 was also previously implicated in ChC innervation of the AIS of mouse CPNs.⁸⁴ While we did not detect any effect of LRRC37B on inhibitory innervation of the AIS, LRRC37B and FGF13A could modulate other aspects of CPN-ChC interactions.

The identification of LRRC37B opens new avenues to study the impact of human-specific intrinsic neuronal properties on circuit function. LRRC37B could affect information processing through neuronal gain modulation, by which neurons adapt to changing inputs²⁵ or the amount of information transfer.¹⁴ While neuronal gain relies largely on the regulation of synaptic inputs, it can also be modified by output modulation, including at the level of the AIS,²⁸ in link with neuronal plasticity.^{25,85} LRRC37B could thus influence neuronal information processing and plasticity, and thereby contribute to human-specific properties of cortical circuits. In this context, it is intriguing that LRRC37B is expressed in only a fraction of all cortical neuron subclasses, suggesting that its levels of expression could depend on cell state or levels of neuronal activity.

The discovery of LRRC37B as a co-receptor for FGF13A and SCN1B regulating Nav channels at the AIS has

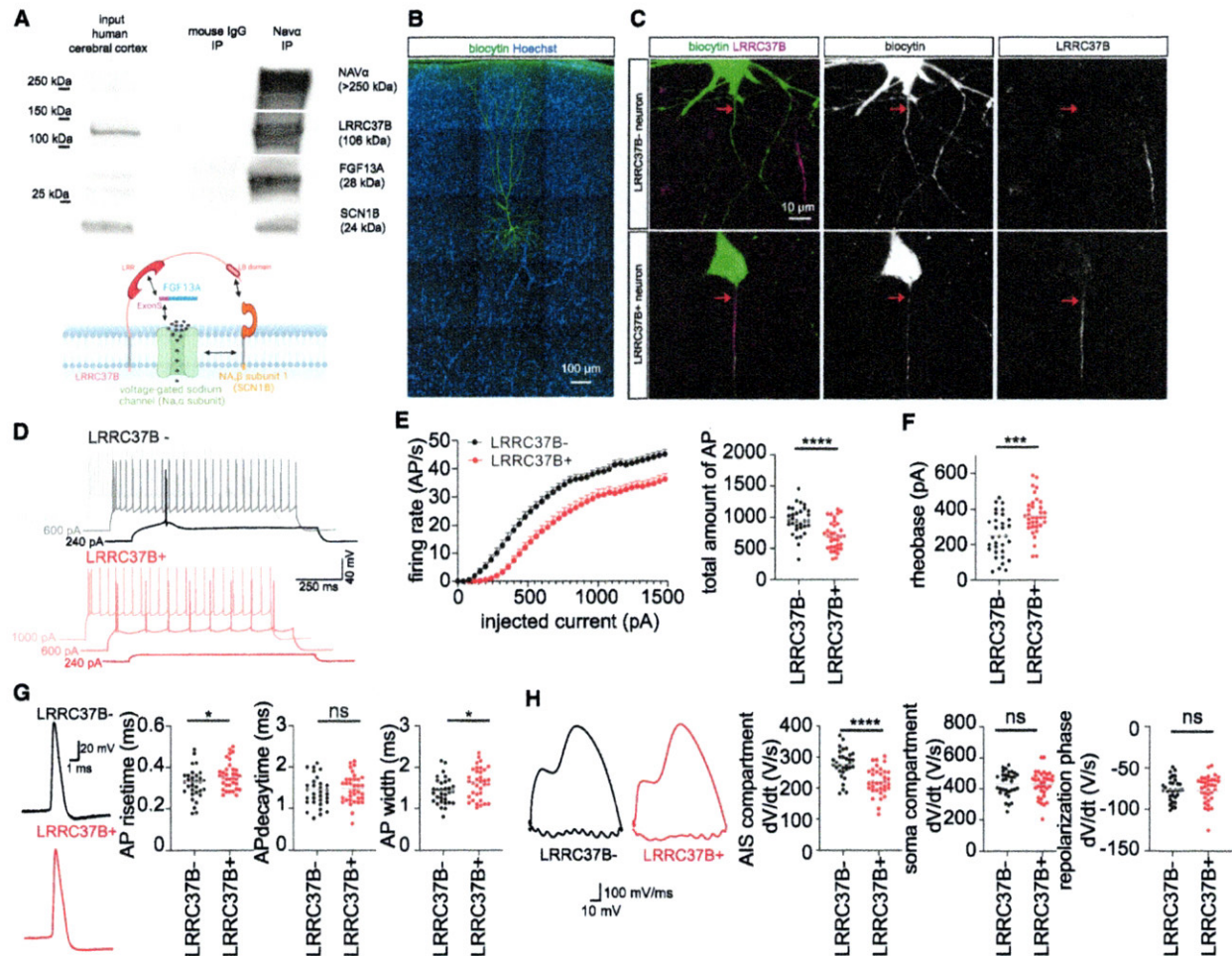


Figure 7. LRRC37B-positive human neurons display decreased neuronal excitability at the AIS

(A) Navα IP from human cerebral cortex (temporal cortex, 25 years old); the input and IP lanes are from the same gel. (B and C) Layer 2/3 human CPNs filled by biocytin which enables to correlate their properties to the LRRC37B post hoc detection (arrows) (B, 4 years old; C, 62 years old). (D) Examples of traces of evoked AP of neurons LRRC37B-positive versus LRRC37B-negative (62 years old). (E and F) Firing rates (E; left: mean + SEM) and rheobase (F) of LRRC37B-positive neurons (35 neurons from 12 individuals, 4–62 years old) versus LRRC37B-negative neurons (32 neurons from 12 individuals, 4–62 years old) (E, right, and F; lines at median; Mann-Whitney tests). (G) Individual single evoked AP examples (62 years old) and properties of LRRC37B-positive and LRRC37B-negative neurons (lines at median; Mann-Whitney test). (H) Phase plot on single evoked AP of LRRC37B-positive (n = 35 neurons from 12 individuals, 4–62 years old) and LRRC37B-negative neurons (n = 32 neurons from 12 individuals, 4–62 years old) (lines at median; Mann-Whitney tests) (Method 2). ns, non-significant; *p < 0.05; ***p < 0.001; ****p < 0.0001. See also Figure S7.

implications for brain diseases, including epilepsy and neurodevelopmental disorders.⁸⁶ Patients with specific mutations at the level of the ExonS of FGF13A, the site of interaction to LRRC37B, are affected by epilepsy and neurodevelopmental defects.^{82,87} Similarly, SCN1B is mutated in severe forms of epilepsy,^{88,89} and mutations in genes encoding Nav channels including Nav1.6 lead to neurodevelopmental disorders and epilepsy.⁸⁶ Our finding that the CN of *LRRC37B* is nearly fixed in the human population strongly suggests its importance for normal human physiology. It remains to be determined

whether sequence variants or CNV of *LRRC37* genes, including somatic mutations,⁹⁰ are associated with brain excitability diseases, alone or through interactions with FGF13 and Nav channel genes.

In conclusion, we identified a molecular pathway, acquired in the human lineage, that regulates cellular excitability at the level of the AIS in CPNs, by modulating the highly conserved Nav channels. Our data provide direct molecular links between human genome and neuronal evolution, with important implications for human brain function and diseases.

Limitations of the study

Because of limited chimpanzee sample availability, we could test only chimpanzee temporal cortex at the protein level, and the PFC at the RNA level: it remains to be determined whether and how LRRC37B is expressed and functional in the chimpanzee in other brain/cortical areas. The impact of LRRC37B on membrane resistance and capacitance in mouse but not human neurons should be clarified further. The exact contribution of SCN1B to LRRC37B/Nav functions remains to be elucidated.

STAR★METHODS

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.cell.2023.11.028>.

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AUTHOR CONTRIBUTIONS

Conceptualization and methodology, B.L.-P. and P.V.; formal analysis, B.L.-P., A.L., K.W., N.L., E.E., I.V., M.D.V., D.C.S., and M.Y.D.; investigation, B.L.-P., A.L., K.W., N.L., E.K., I.V., K.K., K.M.V., N.V., T.W.B., D.C.S., M.Y.D., D.C., J.S., J.d.W., and P.V.; transcriptome analysis, E.E. and B.L.-P.; copy-number analysis, D.C.S. and M.Y.D.; electrophysiological recording and analysis, K.W., I.V., and A.L.; *in utero* electroporations and mouse cortex processing for immunostaining, B.L.-P., A.L., and S.B.; molecular cloning assistance, A.L., V.G., and K.M.V.; cryosections, A.B. and B.L.-P.; recruitment and informed consent of human subjects and surgical resection of human cortical tissue, T.T.; human biopsy processing assistance, A.L., I.V., and H.N.; ELISA assay, T.W.B. and D.C.; recombinant protein production and SEC-MALS, B.L.-P., T.J., M.D., and M.D.V.; fluorescent polarization measures, N.L., K.K., F.R., and J.S.; protein pull-down experiment, K.M.V. and J.d.W.; affinity estimation assays, B.L.-P. and N.V.; all other experimental work and analyses, B.L.-P. and A.L.; writing, B.L.-P. and P.V.; funding acquisition, P.V.; resources, P.V.; supervision, J.d.W. and P.V.

DECLARATION OF INTERESTS

B.L.-P., P.V., J.d.W., A.L., K.W., J.S., F.R., N.L., and K.K. are inventors on a PCT application related to this work.

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