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Distinct and combined interferon- α / β -receptor-1 loss in neurons and astrocytes disrupt brain energy metabolism and drive Parkinsonian dementia

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Abstract

Background Dysregulated interferon-alpha/beta-receptor 1 (IFNAR1) signaling was recently identified to contribute to the development of sporadic Parkinson's disease (PD) into PD with Dementia (PDD). The molecular, cellular, and phenotypic impacts of brain IFNAR1 loss in aging have not been explored in vivo, which may reveal novel disease mechanisms and therapeutic targets.

Methods Single nuclei RNA sequencing (snRNA-seq), liquid chromatography tandem mass spectrometry (LC-MS/MS), functional metabolic mapping, flow cytometry, quantitative PCR (qPCR), in situ hybridization, immunofluorescence and immunohistochemistry, Western blotting, and behavior analyses were used to investigate the molecular, cellular, and phenotypic impacts of IFNAR1 loss in vivo.

Results Baseline IFNAR1 expression varies among major brain cell types, including neurons and astrocytes, and is differentially affected in PD and Lewy Body Dementia patients compared to unaffected controls. Neuron- and astrocyte-specific transcriptomic and proteomic alterations in *Ifnar1*^{-/-} mice implicate mitochondrial defects, defective mitophagy, and synergistic dysfunctional neurotransmission upon IFNAR1 loss, leading to glucose hypermetabolism measured by functional metabolic analysis. Consequently, *Ifnar1*^{-/-} mice exhibited PDD-like pathogenesis, including dopaminergic cell loss in the substantia nigra, cortical neurodegeneration, Lewy-body-like inclusions, neuroinflammation, and progressive PDD-like behavior deficits. Brain cell-specific IFNAR1 loss examined in vivo revealed delayed but distinct development of PDD-like phenotypes, where neuropathology, motor, and cognitive behavior deficits were recapitulated only in mice lacking neuronal IFNAR1, and behavior resembling neuropsychiatric abnormalities recapitulated only in mice lacking astrocytic IFNAR1.

Conclusions IFNAR1 plays a crucial role in brain and mitochondrial homeostasis, loss of which results in neurodegeneration and neuropathology resembling PDD. Differential neuropathology and behavioral outcomes upon neuronal

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vs astrocytic IFNAR1 loss emphasizes a need for understanding neurodegenerative pathophysiology in cell-specific contexts.

Trial registration Not applicable as the study does not include a clinical trial.

Highlights

- *IFNAR1* and related type-I IFN genes are differentially expressed among major brain cell types in Parkinson's Disease, Lewy Body Dementia, and unaffected controls.
- Early molecular alterations in *Ifnar1*^{-/-} mice show lack of immunomodulation contributing to neuroinflammation, mitochondrial defects, and dysregulated energy metabolism.
- *Ifnar1* loss induces defective mitophagy and is reversed with IFNAR overexpression.
- *Ifnar1*^{-/-} mice develop a progressive Parkinsonian-like disease phenotype, including dopaminergic cell loss in substantia nigra, cortical neurodegeneration, phosphorylated (p)alpha-synuclein⁺ and pTau⁺ Lewy-body-like inclusions, neuroinflammation, and progressive motor, cognitive, and neuropsychiatric disturbance-like behavior deficits.
- Neuropathologies, motor, and cognitive deficits are recapitulated in mice lacking neuronal IFNAR1 (*Syn1*^{Cre};*Ifnar1*^{fl/fl}) whereas neuropsychiatric abnormalities are recapitulated in mice lacking astrocytic IFNAR1 (*GFAP*^{Cre};*Ifnar1*^{fl/fl})

Keywords Parkinson's disease dementia, IFNAR1, Mitochondria, Brain energy metabolism, SnRNA-seq, LC-MS/MS

Introduction

The molecular mechanisms underlying dementia formation in sporadic Parkinson's disease (PD), which can develop more than 10 years after motor symptom onset, are not well understood [1]. A large percentage of PD with dementia (PDD) cases have neuropathological similarities to other dementias such as Lewy Body Dementia (LBD) and Alzheimer's disease (AD), including neuroinflammation and gliosis associated with phosphorylated-tau (pTau) and amyloid-beta (Aβ) proteinopathies, the latter correlating with severity of dementia development [1–4]. Brain energy metabolism disturbances have been reported in Parkinsonian disorders [5–8] and AD [9], with region-specific alterations in glucose metabolism corresponding with severity of both motor symptoms [7, 8] and dementia [5]. As metabolic alterations have been observed prior to dementia onset in both PD [6] and AD [9], understanding the complex connection between neuroinflammation, energy metabolism, proteinopathy, neurodegeneration, and clinical outcomes is imperative for designing disease-modifying therapeutics.

Dysregulated type-I interferon (IFN) signaling, specifically the interaction between the interferon-beta (IFNβ) cytokine and the interferon alpha/beta receptor (IFNAR)1, has been implicated in the conversion of human sporadic PD to PDD [10], and we have investigated its role in brain homeostasis through a series of studies. Dysfunctional type-I IFN in PD patients and lack of IFNβ-IFNAR signaling in mice causes extrusion

of damaged mitochondrial DNA, which was found to be sufficient to initiate and propagate PDD-like neuropathology upon injection into healthy brains [11]. Lack of IFNβ in mice (*Ifnb*^{-/-}) was sufficient to induce spontaneous and progressive behavioral abnormalities and neuropathologies resembling PDD [12, 13]. Mice lacking IFNAR1 (*Ifnar1*^{-/-}) in general or specifically in Nestin⁺ neural ectodermal cells (*Nes*^{Cre};*Ifnar1*^{fl/fl}) also develop alpha-synuclein (α-syn)⁺ Lewy body (LB)-like pathology upon aging [13], implicating loss of neuronal and/or glial IFNAR1 signaling in progressive neurodegeneration. An independent study found that young *Ifnb*^{-/-} and *Ifnar1*^{-/-} mice have compromised synaptic plasticity due to impaired astrocytic glutamate transporter function [14]; however, specific neuronal versus astrocytic dysfunction in *Ifnar1*^{-/-} mice upon aging has not been explored. Identifying early brain cell-specific molecular alterations upon IFNAR1 loss in vivo may aid in understanding pathogenesis resembling PDD.

Here, publicly available single nuclei RNA sequencing (snRNA-seq) datasets were used to establish baseline expression of *IFNAR1* in major human brain cell types of healthy individuals, which were compared with samples from patients with LBD and PD. Investigating both human and mouse brains unveiled differential *IFNAR1* and related type-I IFN gene expression patterns in neurons, astrocytes and other brain cell types which were further distinguished in LBD and PD, suggesting unique requirements in brain homeostasis and potentially distinct impacts on neurodegenerative disease progression

and dementia development. Next, cell-specific molecular differences were identified in *Ifnar1*^{-/-} versus wild-type (Wt) mouse brains using an integrative approach, combining global and unbiased snRNA-seq, liquid chromatography tandem mass spectrometry (LC-MS/MS)-based proteomics, and functional metabolic ¹³C isotope tracing, which together revealed mitochondrial dysfunction and disturbed brain energy metabolism upon IFNAR1 loss. Consequently, *Ifnar1*^{-/-} mice developed PDD-like neuropathology, neuroinflammation, and progressive motor, neuropsychiatric, and cognitive behavior deficits. Furthermore, cell-specific IFNAR1 loss in neurons (*Syn1*^{Cre};*Ifnar1*^{fl/fl} mice) or astrocytes (*GFAP*^{Cre};*Ifnar1*^{fl/fl} mice) developed distinct aspects of Parkinsonian disease-like phenotypes observed in *Ifnar1*^{-/-} mice: neuronal IFNAR1 loss was sufficient to induce PDD-like neuropathologies and subsequent motor and cognitive dysfunctions, whereas astrocytic IFNAR1 loss led to specific manifestation of behavior resembling neuropsychiatric abnormalities. These results support a pivotal role of IFNAR1 in brain homeostasis, which can lead to distinct pathological outcomes depending on brain cell type dysfunction, while their concerted dysregulations are required for development of full-blown PDD-like disease features. Our findings highlight a need for understanding neurodegenerative pathophysiology in cell-specific contexts. Concerted and targeted restoration of dysfunctional Type-I-IFN signaling through IFNAR1 in neurons and astrocytes may therefore mitigate development of dementia resembling PDD.

Materials and methods

Human single nuclei (sn)RNA-seq data analysis

The RNA single cell type data released by the Allen Brain Institute (<https://portal.brain-map.org/atlas-and-data/rnaseq/human-m1-10x>) was used to obtain IFNAR1 expression values (nTPM) in specific brain cell subsets, including excitatory and inhibitory neurons, astrocytes, Oligodendrocyte Precursor Cells (OPCs), oligodendrocytes and microglia [15]. To compare IFNAR1 and related Type-1 IFN gene expression between unaffected controls and Lewy Body dementia (LBD) or Parkinson's disease (PD) patients within specific brain cell subsets, data from the publicly accessible snRNA-seq study ([16], available at: https://singlecell.broadinstitute.org/single_cell/study/SCP1768/) were used. Scaled mean expression values of IFNAR1 from each individual donor ID were extracted for human dopaminergic (DA) neurons, non-DA neurons, astrocytes, OPCs, oligodendrocytes and microglia cluster datasets.

Mice

Mice were housed in standard facilities [13]. *Ifnb*^{-/-} [13] and *Ifnar1*^{-/-} [17] mice were backcrossed more than 20 generations on to a C57BL/6J background. Wild-type (Wt) mice were *Ifnb*^{+/+} and *Ifnar1*^{+/+} mice on the C57BL/6J background. C57BL/6J mice carrying loxP-flanked *Ifnar1* (*Ifnar1*^{fl/fl}) [18] were crossed with C57BL/6J transgenic mice expressing Cre recombinase under neuron-specific synapsin-1 promoter (*Syn1*^{Cre}) [19] or astrocyte-specific glial fibrillary acidic protein promoter (*GFAP*^{Cre}) [20] to obtain *Syn1*^{Cre}; *Ifnar1*^{fl/fl} and *GFAP*^{Cre}; *Ifnar1*^{fl/fl} mice, respectively. Behavior cohort numbers and sample sizes for biochemical analyses were determined as previously described [12]. Males and females of roughly equal proportions were used for snRNA-seq, flow cytometry, qPCR, behavior, and immunohistochemistry. As higher disease prevalence and severity occurs in males in human PD [21], male behavior-tested mice were used for further evaluations, including proteomics, and metabolic studies, electron microscopy, and immunoblots. Five familial AD mutation-containing (5xFAD) transgenic mice [22], which develop significant extracellular Aβ pathology, were used for Aβ antibody verification.

Brain tissue preparation

For snap-frozen tissues, mice were sacrificed by cervical dislocation. Brains were removed swiftly and placed on a petri dish over ice and microdissected for cortex, hippocampus, and basal ganglia. Tissues were snap-frozen in liquid nitrogen and kept at - 80 °C until use for snRNA-seq, LC-MS/MS, or immunoblots. A summary of the brain regions investigated in this study, their relevance to known pathology in PD and PDD, investigation methods used, and the age of mice at the time of investigation, is shown in Table 1.

For paraffin-embedded tissues, mice were placed under isoflurane-induced anesthesia and intracardially perfused for 1 min with phosphate-buffered saline (PBS), followed by freshly prepared aqueous PBS-buffered 4% paraformaldehyde (PFA, Sigma, P6148) fixative solution. Brains were removed and kept in the same fixative at 4 °C for 18 h before alcohol dehydration and paraffin embedding. A microtome (Thermo Scientific, HM355S) was used to prepare 6 μm thick serial coronal sections onto glass slides (Thermo Scientific, 10,149,870).

Mouse snRNA-seq

Nuclear isolation

Extraction of nuclei from snap-frozen cortical tissue was performed using a modified version of previously described protocols [29, 30]. Briefly, nuclei isolation media (NIM) (250 mM sucrose, 25 mM KCl, 5 mM

Table 1 PD/PDD-like neuropathologies by brain region and their investigation in this study

Brain region	Sub region	Relevance to PD/PDD	Investigations in this study	IFNAR1 strain	Age (months)
Basal ganglia	Substantia nigra <i>pars compacta</i>	DA cell loss, dopamine defects detectable prior to symptom onset	DA cell loss (TH + cell counts); a-syn and pTau accumulation (IF)	<i>Ifnar1^{-/-}</i> <i>Syn1^{Cre};Ifnar1^{fl/fl}</i> <i>GFAP^{Cre};Ifnar1^{fl/fl}</i>	3
	Substantia nigra <i>pars reticulata</i>	Neurite retraction	DA neurite retraction (TH labeling)	<i>Ifnar1^{-/-}</i>	
	Ventral tegmental area (VTA)	Degenerated in PD/PDD	DA cell loss (TH + cell counts)		
	Striatum	Loss of DA terminals	TH density (IHC)		
Mid-brain and Thalamus	Whole region	LB inclusions [23]	LB-like inclusion detection and morphology (IHC + EM); extracellular A β deposits (IHC)	<i>Ifnar1^{-/-}</i>	12
Cortex	Sensorimotor cortex	Thinning and A β pathology in PDD [24–26]	snRNA-seq-based transcriptomics	<i>Ifnar1^{-/-}</i>	1.5
			LC MS/MS-based proteomics		3
			Acute brain slice metabolic isotope labelling		
			Mitophagy deficits and oxidative stress (IF)		6
			Neurodegeneration (NeuN + cell counts)		
			Gliosis and extracellular A β deposits (IHC/IF)		12
Hippocampus	Dentate gyrus, CA1 and CA3	Atrophied in PD/PDD [26, 27]	Acute brain slice metabolic isotope labelling	<i>Ifnar1^{-/-}</i>	3
			LC MS/MS-based proteomics		
			Neurodegeneration (NeuN + cell counts)		6
			Gliosis (IHC/IF)		9, 12
Olfactory bulb	Whole region	Early dysfunction in PD [28]	Neurodegeneration (NeuN + cell counts)	<i>Ifnar1^{-/-}</i>	6

MgCl₂, and 10 mM tris buffer, pH 8) was prepared. Each cortex was transferred to a precooled Dounce homogenizer containing 1 mL of ice-cold homogenization buffer (NIM supplemented with 1 mM DTT (Invitrogen), 0.4 U/ μ L RNase inhibitor (Takara), 0.2 U/ μ L Suprasin (Invitrogen), and 0.1% v/v Triton X-100) and dissociated on ice using five strokes of the loose pestle, followed by fifteen strokes of the tight pestle. Homogenates were filtered through 40 μ m strainers and nuclei were collected by centrifugation (1000g, 10 min at 4 °C). Pellets were gently resuspended in 250 μ L homogenization buffer each and mixed with 250 μ L 50% iodixanol (50% vol/vol iodixanol, 150 mM KCl, 30 mM MgCl₂, 60 mM tris buffer, pH 8, 0.4 U/ μ L RNase inhibitor, 0.2 U/ μ L Suprasin, and 1 mM DTT (Invitrogen)). 500 μ L 29% iodixanol (29% vol/vol iodixanol, 150 mM KCl, 30 mM MgCl₂, 60 mM tris buffer, pH 8, 0.4 U/ μ L RNase inhibitor, 0.2 U/ μ L Suprasin, and 1 mM DTT) was added to a 1 mL Beckman tube,

precoated with 0.5% BSA in PBS with RNase inhibitor, and 500 μ L of the nuclei suspension was slowly layered on top. Samples were spun at 14,000g for 22 min at 4 °C in a TLS 55 rotor (MAX-XP ultracentrifuge, Accel 3, Decel 2). Supernatants were removed, and nuclei were resuspended in 0.5% BSA in phosphate-buffered saline (PBS) with RNase inhibitor and filtered through a 35 μ m strainer. Nuclei counts were determined by trypan blue staining and hemocytometer count.

10× Chromium sequencing

16,000 nuclei per sample were loaded on the 10×Chromium chip for library preparation using the Single Cell 3' v3 chemistry according to manufacturer instructions. For cDNA amplification, 12 PCR cycles were applied. For each sample, 230 ng of purified cDNA was used for library construction. The libraries were diluted to a concentration of 10 nM and pooled for sequencing. After denaturation,

the library pool was diluted to a loading concentration of 300 pM and sequenced on a Novaseq6000 in paired-end mode (2×100) (read 1:28 cycles, read 2:91 cycles, and i7 index: 8 cycles) at an average depth of 50,000 reads per nucleus.

Processing and quantification of 10× Chromium single-nuclei transcriptomes

Raw data were demultiplexed, aligned, and quantified using Cell Ranger version (v)3.1.0. To allow for counting of unspliced pre-mRNA, a custom version of the mm10-3.0.0 mouse reference genome provided by 10×Genomics was used, where the feature ‘type of transcripts’ was changed from ‘transcript’ to ‘exon’.

Single-nuclei data integration and analysis

Data analysis was performed in R using Seurat v3.1.5 [31] and Conos v1.2.1 [32]. Briefly, the -barcode matrices were filtered to include cells meeting the following criteria: 200 < nFeature_RNA < 6000, nCount_RNA > 1000, and percent_MT < 10. Doublets were filtered out using the Scrublet package [33] and clusters containing mixed cell signatures were removed. Data were log-normalized, and individual samples were integrated using the fast mutual nearest neighbors (fastMNN) method [34] based on the top 3000 variable features. Clustering and UMAP visualization were performed using the first 40 dimensions of the MNN reduction. The integrated dataset was annotated using canonical correlation analysis (CCA) based label transfer in Seurat with the Allen Brain Map Mouse Whole Cortex and Hippocampus SMART-seq dataset [<https://portal.brain-map.org/atlas-and-data/rnaseq/mouse-whole-cortex-and-hippocampus-smart-seq>] as the reference. Of note, very few astrocytes were detected in two samples (one Wt and one *Ifnar1*^{-/-}) and were therefore excluded from astrocyte analysis to avoid technical overrepresentation of missing values. Differential gene expression analysis for each cortical cell type was performed using the getPerCellTypeDE() function in Conos. For pseudobulk data, differential gene expression analysis was performed using the getPerCellTypeDE() function in Conos, with all nuclei assigned to the same label for global pseudobulk comparison. Gene set enrichment analysis was performed using GSEA software (UC San Diego and Broad Institute) [35] and gene set collections from MsigDB [36]. Total number of genes and DE genes per class were visualized using InteractiVenn [37]. The upregulated KEGG pathway hits were visualized using ggplot2, for KEGG_PARKINSONS_DISEASE visualization the genes from GOBP_Oxidative_Phosphorylation were used to annotate mitochondria related genes.

Liquid chromatography tandem mass spectrometry (LC–MS/MS)

Sample preparation for mass spectrometry

Snap-frozen cortex and hippocampus tissues were lysed in SDC alkylation and reduction lysis buffer (1% (w/v) sodium deoxycholate, 100 mM Tris pH 8, 40 mM CAA, 10 mM TCEP) using mechanical homogenizer (IKA® ULTRA-TURRAX® disperser, Merck) for 20 s. Lysates were boiled at 95 °C for 10 min with continuous vortexing at 1200 rpm on a thermomixer (Eppendorf) to denature proteins. Samples were then sonicated using the Covaris Adaptive Focused Acoustics (AFA) sonication system (450W power, 50% duty factor, 200 cycles per burst) (Covaris, USA). Protein concentrations were measured using BCA assay (ThermoFisher Scientific). Proteins were digested overnight using trypsin (1:100 w/w, Sigma-Aldrich) and LysC (1/100 w/w, Wako) at 37 °C. The next day fresh enzyme was added for another 2 h. The peptides were acidified by 1% trifluoroacetic acid (TFA) (Merck) to quench digestion. Peptide concentration was estimated using Nanodrop and peptide mixture was purified by solid phase extraction in Stage-Tips (SDB-RPS material, two 14-gauge plugs) for desalting and concentration. Peptides were washed twice with isopropanol/1% TFA and subsequently twice with 0.2% TFA, then eluted with 80% acetonitrile/1% ammonia. Eluted samples were reduced by vacuum centrifugation at 60 °C and peptide concentration was determined by Nanodrop (ThermoFisher Scientific) measurement at A280 nm. Sample concentrations were adjusted to 200 ng per injection in buffer A* (5% ACN/0.1% TFA) for LC–MS/MS analysis.

Preparation of mouse brain spectral library

High-pH reversed-phase fractionation was used to generate a deep precursor library for data-independent (DIA) MS analysis. Purified peptides were pooled from 6 brain regions, which were fractionated at pH 10 with the spider-fractionator (24 fractions from both cortex and hippocampus and 8 fractions from striatum, cerebellum, mid brain, and the olfactory bulb, respectively (80 fractions in total) [38]. 6–18 µg of purified peptides were separated on a 30 cm C18 column in 100 min gradients and concatenated automatically into either 8 or 24 fractions with either 60- or 90-s exit valve switches for 24 or 8 fractions, respectively. Peptide fractions were vacuum-dried and reconstituted in buffer A* for LC–MS analysis.

LC–MS/MS analysis

Samples were analyzed with nanoflow Easy-nLC 1200 (Thermo Fisher Scientific, Denmark) connected to a trapped ion mobility spectrometry quadrupole time-of-flight mass spectrometer (TimsTOF Pro, Bruker Daltonik GmbH, Germany) with a nano-electrospray ion source

(Captive spray, Bruker Daltonik GmbH). Peptides were separated on a 50 cm in-house packed column (75 μm inner diameter $\times$ 50 cm length) with 1.9 μm ReproSilPur C18-AQ silica beads (Dr. Maisch, Germany). Column temperature was kept at 60 °C using an in-house made column oven. Peptide separation was achieved by 120 min gradients. Peptides were loaded and eluted with a nonlinear gradient of increasing buffer B (0.1% formic acid and 80% acetonitrile) and decreasing buffer A (0.1% formic acid) at a flow rate of 300 nL/min. Buffer B was increased slowly from 5 to 30% over 95 min and ramped to 60% over 5 min, up to 95% over 5 min and held for another 5 min before reducing to 5% for column re-equilibration for 5 min. Sample acquisition was randomized to avoid bias. Mass spectrometric analysis was performed as described in Brunner et al. [39], either in data-dependent (ddPASEF) or data-independent (diaPASEF) mode. The ddPASEF method was used for library generation. For ddPASEF, 1 MS1 survey TIMS-MS and 10 PASEF MS/MS scans were acquired per acquisition cycle. Ion accumulation and ramp time in the dual TIMS analyzer was set to 100 ms each and the ion mobility range was analyzed from $1/K0 = 1.6 \text{ Vs cm}^{-2}$ to 0.6 Vs cm^{-2} . Precursor ions for MS/MS analysis were isolated with a 2 Th window for $m/z < 700$ and 3 Th for $m/z > 700$ in a total m/z range of 100–1700 by synchronizing quadrupole switching events with the precursor elution profile from the TIMS device. Collision energy was lowered linearly as a function of increasing mobility starting from 59 eV at $1/K0 = 1.6 \text{ Vs cm}^{-2}$ to 20 eV at $1/K0 = 0.6 \text{ Vs cm}^{-2}$. Singly charged precursor ions were excluded with a polygon filter (Bruker Daltonik GmbH). Precursors for MS/MS were picked at an intensity threshold of 1.000 AU and re-sequenced until reaching a ‘target value’ of 20.000 AU considering a dynamic exclusion of 40 s elution. For diaPASEF analysis, the correlation of Ion Mobility (IM) with m/z was used and the elution of precursors from each IM scan was synchronized with the quadrupole isolation window. The collision energy was ramped linearly as a function of the IM from 59 eV at $1/K0 = 1.6 \text{ Vs cm}^{-2}$ to 20 eV at $1/K0 = 0.6 \text{ Vs cm}^{-2}$.

Spectronaut data processing

Data analysis of mass spectrometric raw files acquired in ddPASEF mode were analyzed with Spectronaut Pulsar (version 2.4, Biognosys AG, Schlieren, Switzerland) to generate the library using the default settings. The library consisted of 175,301 precursors, 115,684 peptides, 10,191 protein groups. The ddPASEF library were used in the targeted analysis of diaPASEF data for the CNS data against the *Mus musculus* UniProt fasta database (2019 release, UP00000589_10090). Peptide spectral match (PSM) and protein level FDR of 1%. A minimum of seven

amino acids was required including N-terminal acetylation and methionine oxidation as variable modifications and carbamidomethylation as a fixed modification. Enzyme specificity was set to trypsin/P with a maximum of two allowed missed cleavages. Peptide identifications by MS/MS were transferred by matching four-dimensional isotope patterns between the runs (MBR) with a 0.7 min retention time match window and a 0.05 $1/K0$ ion mobility window. diaPASEF analysis was conducted with the Spectronaut software (v14.8, Biognosys AG, Schlieren, Switzerland) under default settings. Search parameters were according to default settings. ‘Cross run normalization’ was enabled with strategy of ‘local normalization’ based on rows with ‘Qvalue complete’. FDR was set to 1% at peptide precursor level and 1% at protein level. Decoy hits and proteins, which did not pass the Q-value threshold, were filtered out prior to data analysis.

MS data analysis and visualization

Quantitative protein abundance data generated in Spectronaut was analyzed in a Python-based version of the Clinical Knowledge Graph (CKG) (v1.0b1 BETA, <https://CKG.readthedocs.io>) [40], where data handling including filtering, normalization, annotation, statistics, enrichment analysis, and visualization were conducted. A minimum of 3 quantified values out of 4 replicates in at least one group were required when analyzing the data. Missing values were imputed using a mixed model of k-Nearest Neighbor’s imputation method (KNN) which assumes that the values are Missing Completely At Random (MCAR), and Probabilistic Minimum Imputation approach (MinProb) for missing values that are considered Missing Not At Random (MNAR) (downshift of 2.4 standard deviations and a width of 0.3 standard deviations). Protein intensities were log2-transformed for further analysis. For the two-samples *t*-tests, each region was analyzed separately to eliminate noise from imputation and to achieve high stringency and confidence. For generation of the abundance distribution curves, median protein abundances across all samples within a proteome were used. Coefficients of variation (CVs) were calculated and reported as overall coefficient of variation. Correlations between LFQ intensities within biological replicates and regions were done by Pearson correlations. The subsequent data analysis includes a dimensionality reduction step to enable visualization of the high dimensional proteomic datasets using two or three-dimensional representations. Linear dimensionality reduction [Principal Component Analysis (PCA)] was implemented. Statistical tests across 4 groups (2 genotypes and 2 regions) were performed by a one-way

ANOVA using Benjamini–Hochberg False Discovery Rate (BH-FDR) to correct for multiple hypothesis testing. All *t*-tests performed were two-sided, unpaired, and corrected for multiple testing. Volcano plots were generated using FDR < 0.05.

Cell culture

Primary cells

Primary cortical neurons, cortical astrocytes or mixed glia were isolated from cortices or rest of the brain of P0/P1 *Syn1^{Cre}*; *Ifnar1^{fl/fl}*, *GFAP^{Cre}*; *Ifnar1^{fl/fl}*, and control Cre negative *Ifnar1^{fl/fl}* pups ($n = 3–7$ per genotype). For *Syn1^{Cre}*; *Ifnar1^{fl/fl}* and Cre negative controls cortices were used for cortical neuron cultures, while the rest of the brain was used for primary glial culture. For *GFAP^{Cre}*; *Ifnar1^{fl/fl}*, and control Cre negative *Ifnar1^{fl/fl}* half of the cortex was used for cortical neurons and the other half was used for cortical astrocytes. Primary cortical neurons were generated and cultured as previously described for 5–6 days in vitro (DIV) [10, 31]. Primary cortical astrocytes and glial cultures were prepared by mechanically and enzymatically digesting associated brain areas in PBS with 10ng/ml DNase (Roche, 11,284,932,001) and 0.8mg/ml Collagenase D (Roche, 11,088,866,001) and incubating for 30 min at 37°C. Enzymatic dissociation was stopped with medium, followed by straining through a 100µm strainer. Astrocytes and glia were cultured in DMEM + GlutaMAX™ (Gibco, 31,966–021), 10% HI FBS (Sigma Aldrich, F7524) and 100U/ml penicillin/streptomycin (Gibco, 15,140,122) until >90% confluency was reached, typically 7–10 DIV.

N2a cells

Mouse *δIfnar1* Neuro2a (N2a) cells were cultured in DMEM (Gibco, #31,966–021) with GlutaMAX (Gibco, #31,966–047) supplemented with 10% Fetal Bovine Serum (FBS) (Sigma, F7524).

CRISPR/Cas9 gene editing

Ifnar1 depleted (*δIfnar1*) N2a cells were generated using CRISPR/Cas9 editing. One pair of 24 nucleotide oligos were designed using the online CRISPR Design Tool (<http://crispr.mit.edu/>) for mouse *Ifnar1* (exon 2): Fwd: CACCGCACTGCCCATTTGACTCTCC and Rev: AAA CCGGAGAGTCAATGGGCAGTG. SgRNA oligos were cloned into the lentiviral LentiCRISPR.V2 vector and constructs were validated by sequencing. Plasmids including either the cloned sgRNAs or non-targeting sgRNAs, and packaging plasmids (Pmd2G and PsPAX2) were transfected within HEK293 FT cells for virus production. Viruses were then transduced in N2a cells and Cas9 expression and the efficiency of gene knockout was checked with immunoblotting.

Transfection

For overexpression of IFNAR1, transfections were performed 24 h after seeding cells in 12-well plates. 1.6 µg of eGFP vector control or full-length IFNAR1 plasmids were transfected into N2a cells using Lipofectamine 2000 as per manufacturer's recommendations. Overexpression vectors for eGFP (pLV[Exp]-Puro-CMV>EGFP) and Full IFNAR1 (pLV[Exp]-Puro-CMV>mIfnar1 [NM_010508.2](ns):3xGGGGS:EGFP) were constructed by VectorBuilder (<https://en.vectorbuilder.com/design.html>). All constructs were amplified by NucleoBond® Xtra Maxi-prep per manufacturers' instructions (Macherey–Nagel, #740,414.10) and confirmed by sequencing.

Microscope imaging and immunoblotting

Immunofluorescence (IF), Immunohistochemistry (IHC), Transmission Electron Microscopy (TEM), and immunoblotting were performed using previously described methods [12, 13, 41]. Primary antibodies used in this study are listed in Table 2, and secondary antibodies for immunoblots are listed in Table 3.

In situ hybridization assay

The *in-situ* hybridization assay RNAscope (ACDbio) was used to detect neuronal and astrocytic *Ifnar1* expression in mouse brain, which was performed according to the manufacturers protocol with the Mm-*Ifnar1* probe (cat# 512,971-C2) and RNAscope Multiplex Fluorescent Reagent Kit v2 (cat# 323,100). In brief, paraffin embedded coronal brain slices were deparaffinized with Xylene and ethanol washes (99%), followed by hydrogen peroxide treatment. Antigen retrieval was conducted with the supplied solution by microwave boiling, followed by protease treatment (supplied with the kit). The hybridization with mm-*Ifnar1* probe was done in the HyBEZ oven at 40°C for 15 min, followed by 3 amplification steps (AMP1, AMP2 and AMP3) at 40°C 30 min each, and treatment with HRP-C1 for 15 min at 40°C. Finally, slides were incubated with the fluorescent label for the probe (Opal570) for 30 min at 40°C, followed by HRP blocker for 15 min at 40°C. Conventional immunofluorescent staining with antibodies against cell specific markers GFAP and NF200 (outlined in Table 2) was then performed as previously described.

Flow cytometry (FC)

All kits were used according to manufacturer's protocols. Briefly, snap-frozen brains from 3-month-old Wt, *Ifnb^{-/-}* and *Ifnar1^{-/-}* mice ($n = 4$, 2M and 2F per genotype) were prepared in a single cell suspension using the Invent Biotechnologies inc. Minute™ Cell Suspension Isolation Kit from Fresh/Frozen Tissues (#CS-031). For myelin removal, a 33% Percoll gradient was run at

Table 2 Primary antibody uses and concentrations in this study

Antigen	Clone	Host	Company	Catalog no	Application	References
A β ₁₋₁₆	6E10	Mouse	Biolegend	803,015	IHC (1:100), EM (1:100)	IHC [12]
A β ₁₋₄₂	H31L2	Rabbit	Invitrogen	Ab5078P	IHC (1:50)	IHC (manufacturer)
A β ₁₋₄₂	Polyclonal	Rabbit	Millipore	Ab5078P	WB (1:2500), IHC (1:35), EM (1:20)	WB, IHC (manufacturer)
Actin	ACTN05 (C4)	Mouse	ThermoFisher	MA5-11,869	WB (1:5000)	WB (manufacturer)
α -syn	Polyclonal	Rabbit	Cell Signaling	2642S	WB (1:2000)	WB (manufacturer)
α -syn, phospho Ser129	D1R1R	Rabbit	Cell Signaling	23706S	WB (1:1000), IHC (1:200), EM (1:200)	WB (manufacturer); IHC, EM [12]
CD31	MEC 13.3	Rat	BD Pharmingen	550,274	IF (1:400)	IHC (manufacturer); IF [42]
COX1 (MTCO1)	1D6E1A8	Mouse	Abcam	ab14705	IF (1:100), WB (1:1000)	IF, IHC, WB (manufacturer)
GFAP	2.2B10	Rat	Invitrogen	13-0300	IF (1:100)	IF, IHC (manufacturer)
GLAST-APC	ACSA-1	Mouse	Miltenyi Biotec	130-095-184	FC (1:100)	FC (manufacturer)
Iba1	Polyclonal	Rabbit	Wako	019-19741	IF (1:500)	IF in mouse [12]
ISG15	Polyclonal	Rabbit	Thermo Fisher	PA5-17,461	IF (1:500)	IF, IHC (manufacturer)
	F-9	Mouse	SantaCruz	sc-166755	IF (1:500)	IF, IHC (manufacturer)
ISG15-PE			R&D Systems	IC8044P	FC (1:100)	
Mac-3	M3/84	Rat	BD Pharmingen	550,292	IHC (1:200)	IHC (manufacturer)
NeuN	A60	Mouse	Millipore	MAB377	IHC (1:400)	IF, IHC (manufacturer)
NF200/NFH	Polyclonal	Rabbit	Sigma-Aldrich	N4142	IF (1:80)	IF, IHC (manufacturer)
	Polyclonal	Chicken	Abcam	ab4680	IF (1:500)	IF, IHC (manufacturer)
	SMI 32	Mouse	BioLegend	801,701	IF (1:500)	IF, IHC (manufacturer)
NF200/NFH-AF488	NE14	Mouse	NovusBio	NBP2-47834AF488	FC (1:100, 1:200)	FC (manufacturer)
Optineurin	C-2	Mouse	SantaCruz	sc-166576	IF (1:100)	IF, IHC [43]
Optineurin, phospho Ser177	E8L9I	Rabbit	Cell signaling	31304S	WB (1:1000)	WB (manufacturer)
OxDJ1	M149	Mouse	Millipore	mabn1773	IF (1:100)	IF, IHC [43]
Tau, pan	Tau-5	Mouse	Abcam	Ab80579	WB (1:1000)	WB (manufacturer)
Tau, phospho Thr205	Polyclonal	Rabbit	Abcam	Ab4841	WB (1:500); IF (1:200); IHC (1:200); EM (1:200)	WB (manufacturer); IF, IHC [12]
TNF- α	MP6-XT3	Rat	eBioscience	16-7322-85	IF (1:500)	IF, IHC (manufacturer)
TOMM20	EPR15581-39	Rabbit	Abcam	ab186734	IF (1:500), WB (1:1000)	IF, IHC, WB [43]
Vinculin	hVIN-1	Mouse	Sigma	V9131	WB (1:20,000)	WB (manufacturer)
8OHdG	Polyclonal	Rabbit	Bioss	BS-1278R	IF (1:100)	IF, IHC (manufacturer)

Table 3 Secondary antibodies used for immunoblots

Antibody	Concentration	Catalogue No
ECL™ donkey anti-rabbit, Horseradish peroxidase	WB 1:10,000	Amersham, NA934V
Goat anti-mouse DyLight™ 800	WB 1:10,000	Invitrogen, SA5-35,521
Goat anti-mouse DyLight™ 680	WB 1:10,000	Invitrogen, #35,518
Goat anti-rabbit Alexa Fluor™ 800	WB 1:10,000	Invitrogen, SA5-10,036

800g for 15 min (low break). The single cells suspension was counted and stained using the antibodies outlined in Table 2. Briefly, cells were first stained with LIVE/DEAD™ Fixable Violet Dead Cell Stain Kit, for 405 nm excitation (#L34955) and blocked with an BD Fc block (#553,142, 1:200). Next, cells were stained for surface markers (GLAST) for 30 min at 4 °C, after which cells were fixed and permeabilized using BD Cytofix/Cytoperm™ Fixation/Permeabilization Kit (#554,714) and stained for intracellular epitopes (NF200 and ISG15) for 30 min at 4 °C. Compensation using UltraComp eBeads™ Compensation Beads (01-2222-41) and Arc™

Amine Reactive Compensation Bead Kit (# A10346). The samples were analyzed by the BD LSRFortessa™ X-20 Cell Analyzer with HTS. The analysis was performed in FlowJo 10.8.2, using fluorescence minus one (FMO controls) for gating.

Quantitative PCR (qPCR)

All kits were used according to manufacturer's protocols. For validation of *Ifnar1* knockdown, primary neurons and astrocytes were isolated from cortices of P0/P1 *Syn1^{Cre};Ifnar1^{fl/fl}*, *GFAP^{Cre};Ifnar1^{fl/fl}*, and control Cre negative *Ifnar1^{fl/fl}* pups (n=3–7 per genotype) and cultured as previously described [13, 41]. For investigation of immune cytokines, snap-frozen cortices from 3-month-old Wt, *Ifnb^{-/-}* and *Ifnar1^{-/-}* mice (n=3, 2 M and 1 F per genotype) were used. Briefly, RNA was isolated from snap-frozen cortices of or primary neurons and astrocyte cultures using Qiagen AllPrep kit (#80,004). Biorad iScript™ cDNA Synthesis Kit (#1,708,891) was used for reverse transcription of RNA in complementary DNA (cDNA), using 350 ng of template RNA. For the qPCR reaction Roche Faststart SYBR green master (#4,673,484,001) was used on 10 ng of cDNA and 10 μM primers (Table 4) per reaction. Technical replicates averages were used for subsequent analysis, standardizing results to two housekeeping genes (*Beta Actin* and *Rpl13a*). Data were further normalized to the average of Wt replicates, shown as fold change. Statistical tests used are outlined in the figure legend of each experiment.

Functional metabolic mapping

Metabolic mapping using isotope tracing of ¹³C enriched substrates of acutely isolated brain slices were performed as previously described [47–49]. Briefly, male Wt or *Ifnar1^{-/-}* mice were euthanized by cervical dislocation after which the brain was quickly removed and micro-dissected in ice-cold artificial cerebrospinal fluid (ACSF) into cortical and hippocampal sections. The dissected

sections were sliced (350 μm) on a McIlwain tissue chopper (The Vibratome Company, O'Fallon, MO, USA). The slices were submersed in ACSF containing either [U-¹³C] glucose (5 mM), [1,2-¹³C]acetate (5 mM), [U-¹³C]glutamate (0.2 mM), or [U-¹³C]glutamine (0.2 mM) and incubated for 1 h. The ¹³C enrichment of metabolites in tissue extracts were determined by gas chromatography-mass spectrometry (GC–MS). Data is presented as the cycling ratio, describing the rate of ¹³C accumulation, i.e. reflecting the rate of the TCA cycle [47, 50]. High-performance liquid chromatography (HPLC) was used to assess amino acid amounts in slices and was performed as previously described [47, 50].

Behavior

All behavior tests were performed and analyzed with the experimenter blinded to genotype. Mice were habituated to the behavior room 24 h prior to testing. Behavior of *Ifnar1^{-/-}* mice were evaluated against Wt and *Ifnb^{-/-}* mice. Behavior of *Syn1^{Cre};Ifnar1^{fl/fl}* mice were evaluated against *Syn1^{Cre};Ifnar1^{+/fl}* and *Syn1^{Cre};Ifnar1^{+/+}* littermates. Behavior of *GFAP^{Cre};Ifnar1^{fl/fl}* mice were evaluated against *GFAP^{Cre};Ifnar1^{+/fl}* and *GFAP^{Cre};Ifnar1^{+/+}* littermates. Ethovision XT v11 software (Noldus) was used to measure performances on the open field (OF), elevated plus maze (EPM), Morris water maze (MWM), and Barnes maze. To minimize potential confounding stress of conducting multiple behavior tests during one time point, anxiety-like behavior assessments (OF and EPM) were conducted first, followed by motor (wire suspension and rotarod) tests, cognitive measures (Barnes maze and MWM), and pain sensitivity (tail-flick) tests [12]. The novelty-suppressed feeding test and sucrose preference test assessing depression-like phenotypes were conducted on separate cohorts.

Wire suspension, rotarod, MWM, and tail-flick tests were performed as previously described [13]. EPM and

Table 4 qPCR Primers used in the study

Gene	Forward Primer (5'–3')	Reverse Primer (5'–3')	References
<i>Beta Actin</i>	CTGTGTGGATTGGTGGCTCTAT	GTAACAGTCCGCCTAGAAGCAT	[44]
<i>Rpl13a</i>	GCAGATCTTGAGGTTACGGA	ATTGGGTTACACCAGGA	In house primer design
<i>Il6</i>	AGAGTTGTGCAATGGCAATTCT	CCTTCTGTGACTCCAGCTTATCTG	[45]
<i>Il1b</i>	ATGGCAACTGTTCTGAACT	GTATTGCTTGGGATCCACACT	[45]
<i>Ifnar1</i>	AGCCACGGAGAGTCAATGG	GCTCTGACACGAACTGTGTTTT	[46]
<i>Ifnar1^{fl/fl}</i>	AGC GTC TGG AAA TAC CTG TG	CTC AGC CGT CAG AAG TAC AAG	In house primer design
<i>Tgfb</i>	CACCGGAGAGCCCTGGATA	TGTACAGCTGCCGCACACA	In house primer design
<i>Isg15</i>	GCAGACTCCTTAATTCCAGGG	TTC AGT TCT GAC ACC GTC ATG	In house primer design
<i>Il12a</i>	ACG GCC AGA GAA AAA CTG AA	CTA CCA AGG CAC AGG GTC AT	Real-Time PCR.com

Barnes maze were performed as described in [12]. OF test was performed as described in [51].

Novelty-suppressed feeding test was adapted from [52] and [53]. Mice were fasted 24 h prior to the test, then assessed individually for 5 min on their behavior when given sudden access to a food pellet of known weight in a novel open arena. Reduced time spent eating and food intake were measured as reduced motivation and depression-like behaviors. Six mice (two Wt and four *Ifnar1*^{-/-}) did not eat throughout the test duration and were thus excluded from analysis.

Sucrose preference test was adapted from [54]. Mice were given free access to both water and a solution containing 1.5% weight/volume sucrose for seven consecutive days. Total liquid intake, sucrose preference ratio, and total sucrose intake were measured at the same time-point each day. Depression-like behavior was measured as a reduced preference a rewarding activity (i.e. sucrose consumption).

Rearing/climbing test was adapted from [55]. Mice were placed in a 10×15 cm-tall wire mesh cylinder, and video was recorded for 5 min. Rearing activity was defined as both forepaws stretched above the midline of the mouse and on the apparatus. Climbing activity was defined as all four paws lifted off the ground. Number of rearing and climbing events and cumulative time spent rearing or climbing were assessed as measures of spontaneous motor activity.

Statistical analysis

Prism software (v9) was used for statistical analysis of results from behavior tests, FC, qPCR, immunohistochemistry, immunoblotting, and GC-MS metabolic labelling experiments. Parametric tests were applied based on passing the Shapiro–Wilk normality test. Unpaired Student's *t*-tests were used for two-group comparisons, and one- or two-way ANOVAs were applied to multiple-group comparisons. Statistical tests used for each experiment are specified in the figure legends. *P* values < 0.05 were considered statistically significant.

Results

IFNAR1 is differentially expressed in human brain cell types and is affected in patients with PD and LBD

Though we previously reported that Type I-IFN signaling is dysfunctional in PD patients upon dementia progression [10], the primary cellular sources of general *IFNAR1* expression in the entire brain had not been identified; thus, baseline cellular expression patterns of *IFNAR1* in the human brain were examined. 10×snRNA-seq expression data from the Allen Brain Institute [15] revealed differential *IFNAR1* expression among the major human brain cell types, including excitatory and inhibitory

neurons, astrocytes, oligodendrocytes, oligodendrocyte precursor cells (OPCs), and microglia (Fig. 1A). In general, astrocytic *IFNAR1* expression was notably lower compared to neurons and the other brain cell types. Wild-type (Wt) mice also demonstrated significantly lower astrocytic *Ifnar1* expression compared to neurons using in situ hybridization combined with immunofluorescent staining using GFAP vs NF200 for visualization of astrocytic vs. neuronal *Ifnar1* expression, respectively (Fig. 1B). We next used a publicly available single cell RNA-seq dataset [16] to compare PD and LBD cortical samples to unaffected controls and determined how *IFNAR1* and associated Type-I IFN signaling gene [10] expression were affected among different brain cells with or without dementia (Fig. 1C). Notably, *IFNAR1* is specifically lower in LBD vs control and PD in dopaminergic (DA) neurons and microglia, which supports our previous observations in PD and PDD cortical samples where *IFNAR1* expression was significantly reduced in PDD versus PD [10]. IFNβ, lack of which we previously observed to result in PD and LBD-like progression in mice, had reduced expression in both LBD and PD dopaminergic neurons compared to controls [13]. *IFNAR1* and several related genes were also reduced in astrocytes, oligodendrocytes, and OPCs in both LBD and PD compared to controls, implicating *IFNAR1* signaling dysregulation in neurodegenerative mechanisms involved in both neurodegenerative conditions.

These results show that *IFNAR1* expression is subject to varying regulation across different brain cell types in both healthy human and mouse brains, warranting further investigation into the relationship between brain cell-specific changes in *IFNAR1* expression and dementia development.

Single nuclei (sn)RNA-seq in *Ifnar1*^{-/-} brain revealed PD-related changes in neuronal and astrocytic mitochondrial genes and neurotransmission

To identify potential brain cell-specific changes upon *IFNAR1* loss in vivo, snRNA-seq was utilized on cortical nuclei isolated from young adult (1.5-month-old) Wt and *Ifnar1*^{-/-} mice. Cortical cell types were grouped into five broad cell classes based on their gene expression profile (Fig. 2A; Supplementary Fig. 1A). Glutamatergic neurons (Glut) had the highest number of significant (*P* < 0.05) differentially expressed (DE) genes (396), followed by oligodendrocytes (Oligo, 294), GABAergic neurons (GABA, 242), microglia (Micro, 206), and astrocytes (Astro, 52) (Fig. 2B). Unbiased hierarchical clustering of gene expression showed tight clustering of biological replicates within genotypes for each cell class (Supplementary Fig. 1B).

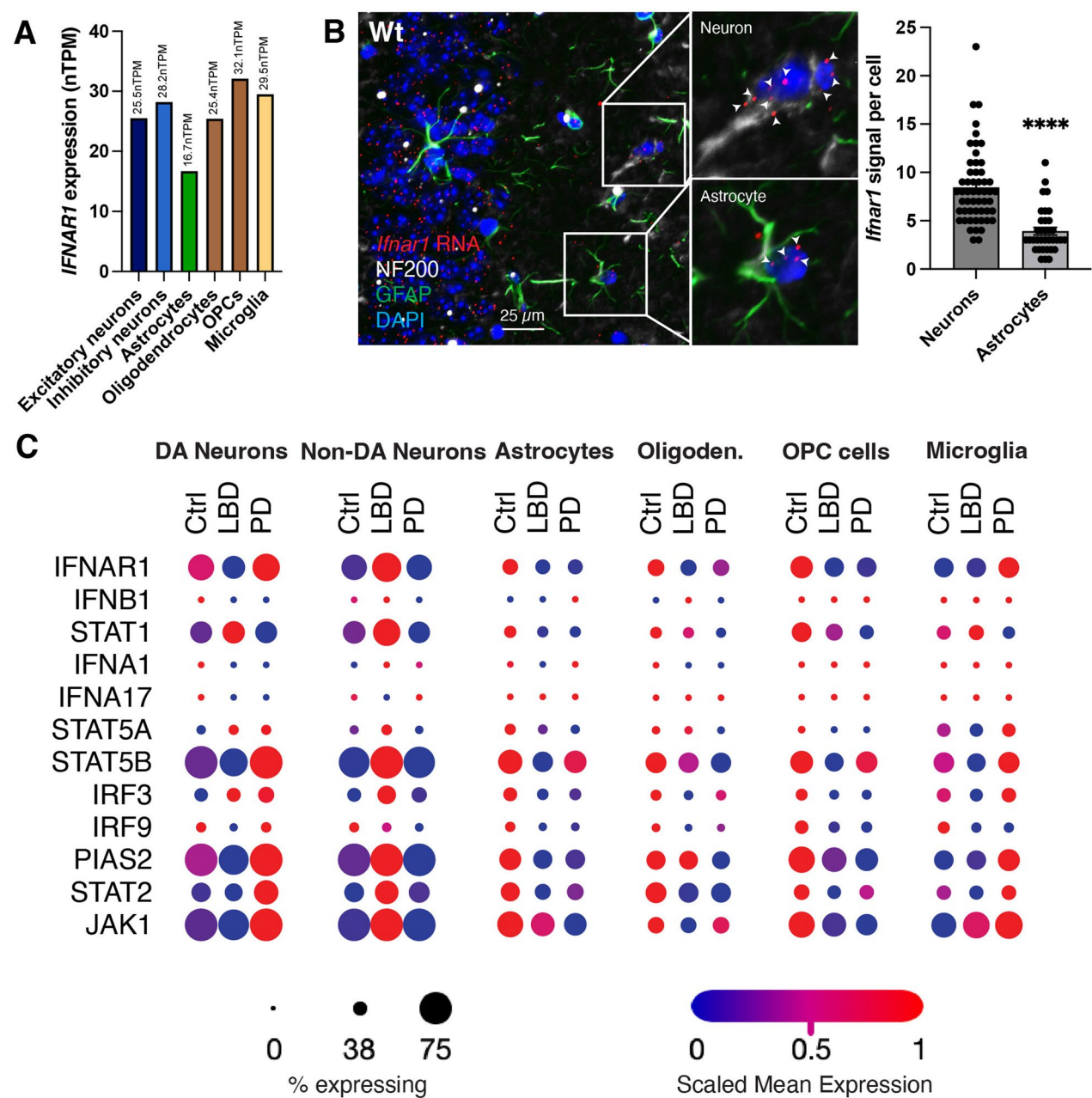


Fig. 1 *IFNAR1* expression in human brain varies by cell type and is differentially affected in PD and LBD. **A** *IFNAR1* RNA expression in human brain cell types. OPCs=Oligodendrocyte Precursor Cells. **B** Differential RNA expression of *Ifnar1* (red) in wild-type (Wt) mouse NF200⁺ neurons (white; *n*=54) and GFAP⁺ astrocytes (green; *n*=35) using in situ hybridization. DAPI=blue. *****P*<0.0001 by unpaired *t*-test. Scale bar, 25 μm. **C** Dot plot comparing *IFNAR1* and related Type-I IFN gene expression in different brain cells from an single cell RNA-seq dataset [16] derived from human PD and LBD patients compared to unaffected controls (*n*=3–8). DA=Dopaminergic. Scaled mean expression = relative gene expression across the annotated cell type within each patient category

Next, gene set enrichment analysis (GSEA) was conducted on all cell classes to examine genotypic differences. Expression of interferon-related genes in each cell class was assessed to determine the validity of the experimental approach. Glutamatergic and GABAergic

neuronal classes expressed dysregulated type-I IFN or related pathways involving inflammation and cytokine receptor signaling, inclusive of IFN- α , IFN- γ , IL-6, TNF- α , and TGF- β (Supplementary Fig. 1C). Glutamatergic neurons had the highest expression of *Ifnb* in Wt

mice, which was reduced in *Ifnar1*^{-/-} compared to Wt (Fig. 2C). *Isg15* (interferon-stimulated gene 15), an E3 ubiquitin ligase directly regulated by IFNAR1 signaling [56], was also downregulated in general in *Ifnar1*^{-/-} cortical cells (Fig. 2D), which was confirmed with qPCR and FC in *Ifnar1*^{-/-} brains (Supplementary Fig. 1D, E). Proinflammatory interleukin-1beta (*Il-1b*), normally is downregulated by IFNAR1 signaling [57], was upregulated in *Ifnar1*^{-/-} microglia (Fig. 2E), suggesting potential neuroinflammation and has been associated with dysregulated Type-I IFN signaling PDD [10]. Accordingly, *Ifnar1*^{-/-} microglia showed negative enrichment of interferon signaling-related Reactome and KEGG (Kyoto encyclopedia of genes and genomes) pathways, but significant positive enrichment in the KEGG pathways 'Parkinson's disease', 'Alzheimer's disease', and 'Huntington's disease', collectively demonstrating that *Ifnar1*^{-/-} microglia adopt a neurodegenerative phenotype (Supplementary Fig. 1F).

GSEA of glutamatergic and GABAergic neuronal classes featured the KEGG pathway 'Parkinson's disease' among the top 20 upregulated KEGG pathways (Fig. 2F, G), which was reflected in the majority of annotated cortical neuronal subtypes within each class (Supplementary Fig. 1B and Supplementary Fig. 2A, B), with Parvalbumin⁺ (PValb) interneurons shown as representative subtype (Fig. 2H). Notably, mitochondrial genes (belonging to the GOBP-OXPHOS pathway) were overrepresented among KEGG 'Parkinson's disease' for glutamatergic (Fig. 2I) and GABAergic (Fig. 2J) neurons, making up 86% and 78% of core enrichment genes respectively, compared to 70% overall genes shared between KEGG 'Parkinson's disease' and GOBP-OXPHOS (Fig. 2K). Accordingly, several mitochondrial and metabolic pathways were among the top 15 dysregulated Reactome pathways in both glutamatergic and GABAergic neuronal classes (Fig. 2L, M, Supplementary Fig. 2C).

Reactome pathways related to neuronal trafficking were among the top 15 negatively enriched pathways in neurons, while neurotransmission and receptor signaling-related pathways were among the top 15 positively enriched pathways (Fig. 2L, M). Dysregulation of genes in processes relating to glutamatergic signaling and cholinergic neurotransmission (Fig. 2L, Supplementary Fig. 2D, E) suggests dysfunction of excitatory neurotransmission upon IFNAR1 loss [58]. Of note, *Ifnar1*^{-/-} astrocytes and oligodendrocytes also showed positive enrichment of excitatory neurotransmission pathways (Fig. 2N; Supplementary Fig. 1G); however, *Ifnar1*^{-/-} astrocytes also demonstrated negative pathway enrichment relating to energy metabolism and mitochondrial function (Fig. 2N), along with several pathways involved in neurotransmission and inflammation (Supplementary Fig. 2F). Downregulation of pyruvate metabolism genes in *Ifnar1*^{-/-} astrocytes and other brain energy-related processes may indicate altered brain metabolism and subsequent disturbances in neurotransmission events (Supplementary Fig. 2G).

These findings suggest that IFNAR1 loss results in cell-specific transcriptomic alterations that may collectively affect neuronal function and homeostasis. Many DE genes were unique between neuronal and astrocytic classes (Fig. 2B), suggesting distinct cell-specific dysfunctions upon IFNAR1 loss.

Protein alterations in *Ifnar1*^{-/-} brains suggest disrupted neurotransmission

To investigate how cell-specific transcriptional changes affect brain protein expression, proteome comparisons were made between Wt and *Ifnar1*^{-/-} cortex and hippocampus. Evaluation of technical and biological quality showed a strong correlation between replicates (Pearson correlation ranging from 0.94 to 0.95) (Fig. 3A) and a clear separation of brain regions and genotypes by principal component analysis (PCA)

(See figure on next page.)

Fig. 2 snRNA-seq revealed early PD-related changes in neuronal mitochondrial genes, neurotransmission, and glial support upon *Ifnar1*^{-/-}. **A** Uniform manifold approximation and projection (UMAP) of broad cell classes isolated from cortex from 1.5-month-old Wt vs *Ifnar1*^{-/-} mice, *n* = 4 per genotype. A total of 26,020, 22,821, 24,268, 21,069, and 15,185 genes identified in glutamatergic neurons, GABAergic neurons, astrocytes, microglia, and oligodendrocytes, respectively. **B** Venn diagram showing overlap of significant (*P* < 0.05) differentially expressed (DE) genes identified in glutamatergic neurons (Glut), GABAergic neurons (GABA), astrocytes (Astro), oligodendrocytes (Oligo), and microglia (Micro), and volcano plots for each cell class showing significance and fold-change (FC) of DE genes. **C–E** Specific expression of interferon-related genes (**C**) *Ifnb*, (**D**) *Isg15*, and (**E**) *Il1b* in each cell class. Purple circles represent *Ifnar1*^{-/-} and black bars represent Wt average expression. **F–H** Top 20 upregulated KEGG pathways in *Ifnar1*^{-/-} vs Wt (**F**) glutamatergic neurons, (**G**) GABAergic neurons, and (**H**) parvalbumin inhibitory neurons, with KEGG 'Parkinsons Disease' pathway indicated in a black box. **I, J** Core enrichment genes within the KEGG 'Parkinsons Disease' (KEGG_PD) pathway for (**I**) glutamatergic neurons and (**J**) GABAergic neurons, with mitochondrial genes (belonging to the 'GOBP Oxidative Phosphorylation' (GOBP_OXPHOS) pathway) labelled in yellow. **K** Overlap between KEGG_PD core enrichment genes for glutamatergic neurons, GABAergic neurons, and the KEGG_PD pathway overall with GOBP_OXPHOS genes. **L, M** GSEA-Reactome enrichment tables showing top 15 positively and negatively enriched pathways for (**L**) glutamatergic neurons, (**M**) GABAergic neurons, and (**N**) astrocytes. Pathways relevant to neuronal and astrocytic function are highlighted, where blue = trafficking affecting neurotransmission; magenta = neurotransmission and receptor signaling; green = energy metabolism and mitochondrial function. Pathways enclosed in a black box have heatmaps featured by cell type in Supplementary Fig. 2D–G

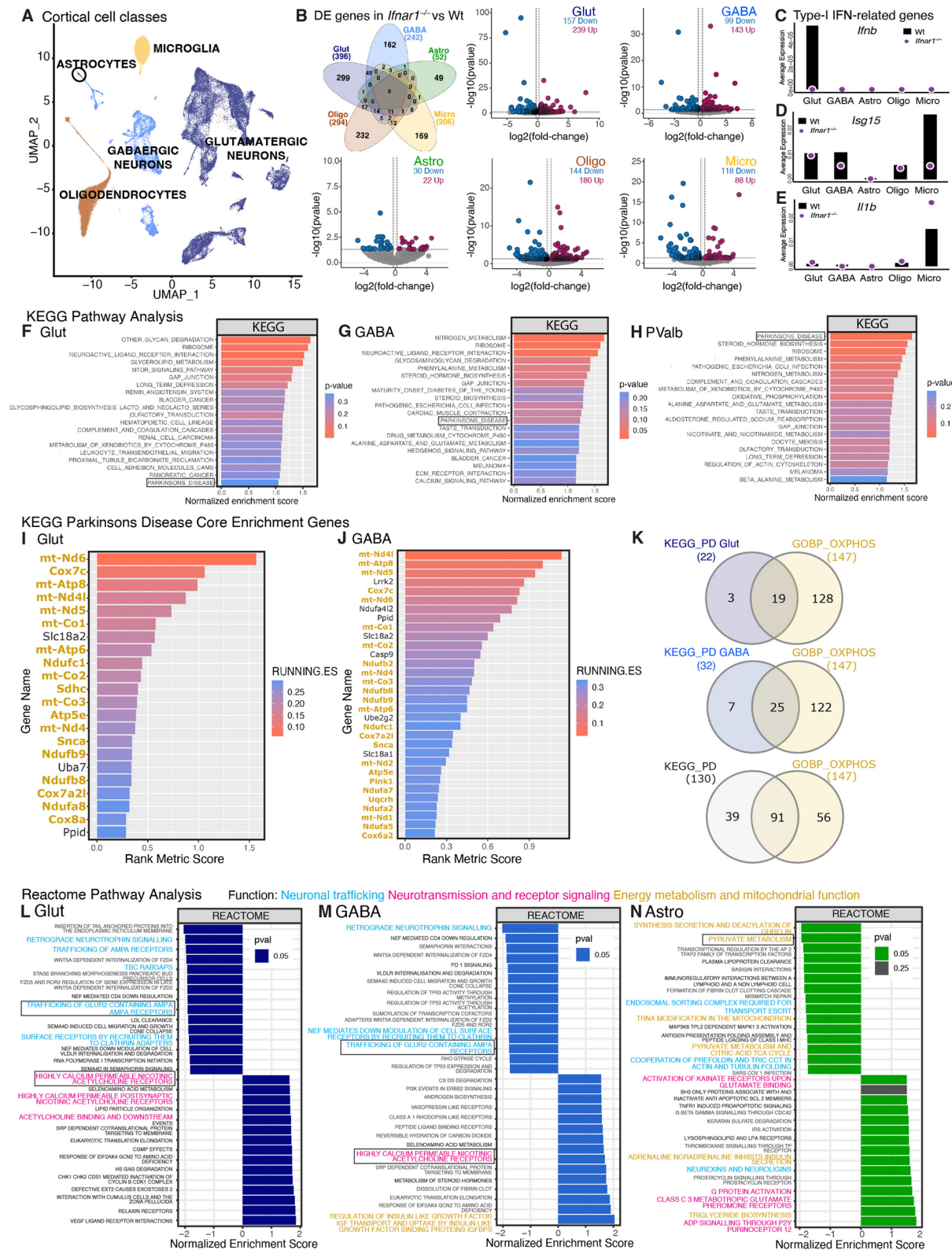


Fig. 2 (See legend on previous page.)

(Fig. 3B). An average of 7657 protein groups were identified after data preparation and filtering (Supplementary Fig. 3A). Proteome coverage and dynamic range were comparable across cortex and hippocampus in both genotypes (Supplementary Fig. 3B), as well as coefficients of variation (CVs) (Wt cortex: 9.74%; *Ifnar1*^{-/-} cortex: 10.97%; Wt hippocampus: 14.27%; and *Ifnar1*^{-/-} hippocampus: 12.01%) (Supplementary Fig. 3C). In total, 1560 significant (FDR < 0.05) protein differences were found (Fig. 3C), most of which reflected regional differences and were comparable to a previous report [48]. Genotypic differences within each brain region were analyzed and visualized as differential expression on volcano plots (Fig. 3D, E).

Consistent with snRNA-seq, qPCR, and FC data (Fig. 2D, Supplementary Fig. 1D, E), ISG15 was significantly downregulated in both *Ifnar1*^{-/-} cortex and hippocampus (Fig. 3F). Cortical changes included proteins involved in synaptic remodeling [Nrxn2, a pre-synaptic protein, reduction of which reduces N-methyl-D-aspartate (NMDA) receptor activity and spontaneous cortical neurotransmitter release [59]], endosomal sorting [Chmp1a, an essential subunit of the endosomal sorting complex required for transport (ESCRT)-III, dysfunction of which is associated with increased PDD risk [60] and pTau NFT development [61]], and microtubule dynamics [Myo5c, which facilitates actin-dependent organelle trafficking, dysregulation of which is associated with NFT manifestation [62]] (Fig. 3G). As with cortex, proteins significantly altered in *Ifnar1*^{-/-} hippocampus are involved in neuronal survival, function, and synaptic regulation, including mitochondrial regulation [Them4, regulator of mitochondrial fission, mitochondrial membrane permeability, and fatty acid metabolism [63, 64]], cholinergic neurotransmission [Slc5a7, essential for synthesis of acetylcholine and mitochondria-derived acetyl-CoA [65]], and synapse remodeling [Dock10, required for synaptic morphogenesis of hippocampal neurons [66]] (Fig. 3H). Of note, protein expression and transcription patterns in both regions appeared to differ by cell type (Fig. 3G, H).

Here it was observed that cell-specific transcriptional alterations upon IFNAR1 loss affect expression of brain

proteins involved in several processes required for effective neurotransmission and mitochondrial function, implicating IFNAR1 loss with general dysregulation of brain homeostasis.

Integrated Omics revealed mitochondrial dysfunction, defective mitophagy and elevated oxidative stress upon IFN β -IFNAR signaling loss

To identify shared and potentially central pathways disrupted upon IFNAR1 loss, analysis of cortical transcriptomic and proteomic datasets from *Ifnar1*^{-/-} mice were integrated. GSEA of the proteomics dataset identified 'Gene Ontology (GO) Cellular Component (GOCC) Mitochondrion' and metabolic pathways as major top hits (Fig. 4A, orange and green boxes, respectively), which supported the mitochondrial and metabolic related Reactome pathways identified by GSEA in the neuronal and astrocytic transcriptomic data (Fig. 2L–N, Supplementary Fig. 2G). To directly compare cell-specific transcriptomic data with bulk proteomic data, all cellular transcriptomic data were declassified into a pseudobulk dataset. For validation of the approach, gene ontologies related to IFNAR1 depletion were checked, namely 'GO Biological Process (GOBP) Response to Interferon beta' (IFNb-response) and 'GOBP regulation of viral process' (Viral response), which appeared among the top altered GSEA pathways in the pseudobulk snRNA-seq data of *Ifnar1*^{-/-} group (Fig. 4B), supporting single cell findings for related Type-I IFN genes (Fig. 2C–E) and proteomic analysis (Fig. 3F). There was also significant overlap between mitochondrial gene/protein expression and Type-1 IFN-related pathways associated with IFN β -IFNAR responses (Fig. 4C), supporting further investigation of mitochondrial dysfunction in *Ifnar1*^{-/-} mice.

To characterize potential mitochondrial defects, optineurin, a receptor for removal of damaged mitochondria [67], was investigated in *Ifnar1*^{-/-} mice compared to Wt and *Ifnb*^{-/-} mice, as accumulation of mitochondria in *Ifnb*^{-/-} brains have been associated with defects in mitophagy [43]. Optineurin colocalization with mitochondrial marker TOMM20 was elevated in brains of mice lacking IFN β -IFNAR signaling compared to Wt (Fig. 4D). Higher levels of cytochrome C

(See figure on next page.)

Fig. 3 Protein alterations in *Ifnar1*^{-/-} brains support snRNA-seq results suggesting dysregulated neurotransmission. **A** Pearson correlation plot indicating high reproducibility between samples. **B** Principal component analysis (PCA) showing high correlation of samples within genotype and brain region ($n = 4$ per genotype and per region). **C** Unbiased hierarchical clustering of differentially expressed proteins indicates distinct signatures for each brain region and some changes between genotypes within each region. **D, E** Volcano plots showing significant hits by false discovery rate (FDR < 0.05)-adjusted p values in *Ifnar1*^{-/-} vs Wt (**D**) cortex and (**E**) hippocampus. **F** Relative expression (Log₂ FC intensity) of Isg15 in both cortex and hippocampus. **G** Relative protein expression of Nrxn2, Chmp1a, and Myo5c in 3-month-old *Ifnar1*^{-/-} cortex compared to 1.5-month-old *Ifnar1*^{-/-} cortical cell snRNA-seq expression. **H** Relative protein expression of Them4, Slc5a7, and Dock10 in 3-month-old *Ifnar1*^{-/-} hippocampus compared to 1.5-month-old *Ifnar1*^{-/-} cortical cell snRNA-seq expression. * $P < 0.05$ and ** $P < 0.01$ by FDR-adjusted ANOVA

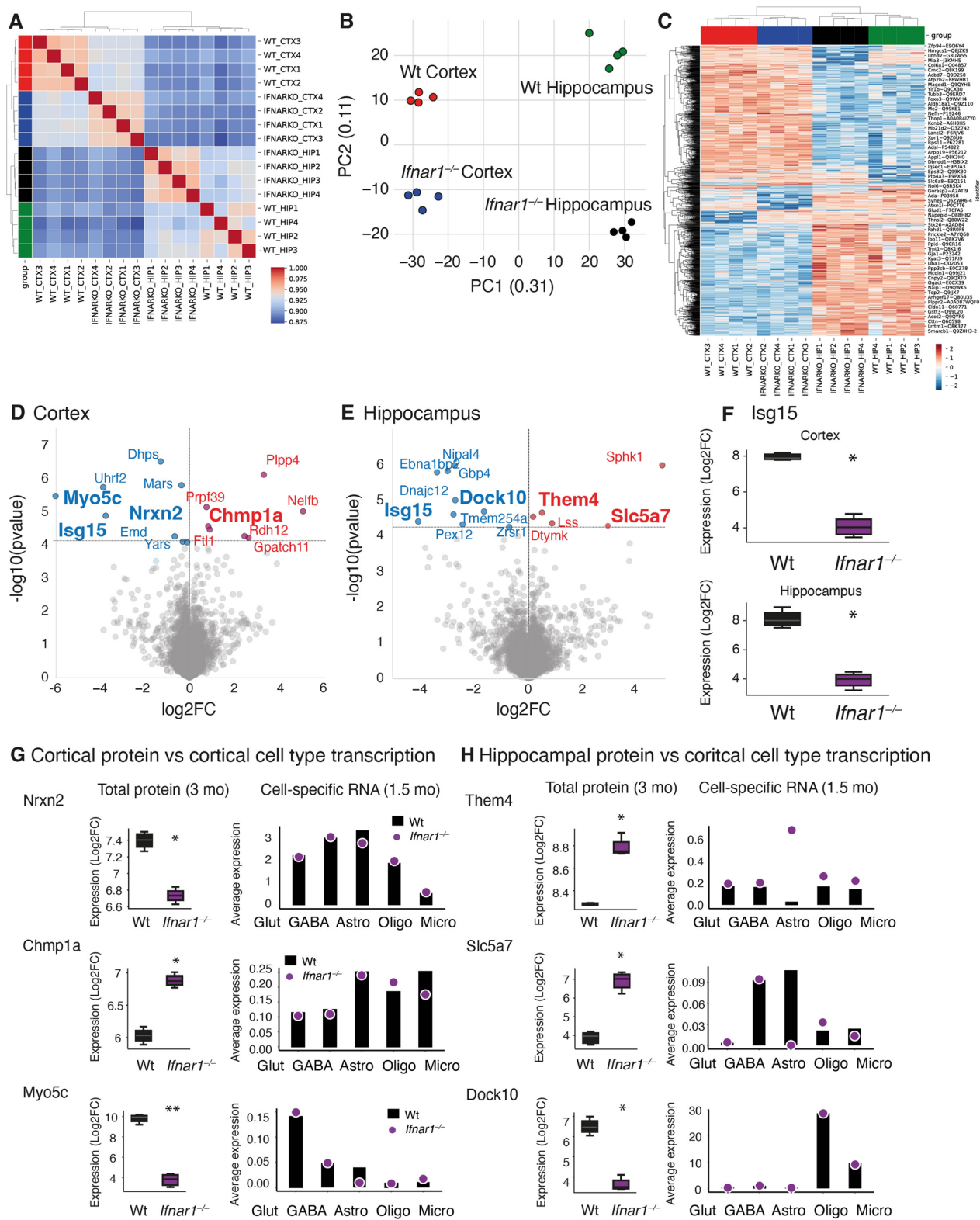


Fig. 3 (See legend on previous page.)

oxidase I (COXI) were also observed in both *Ifnb*^{-/-} and *Ifnar1*^{-/-} mice (Fig. 4E), collectively indicating defects in mitophagy [43]. Oxidized DJ-1 (oxDJ-1) and 8-hydroxy-2'-deoxyguanosine (8OHdG) were measured by immunohistochemistry as indicators of oxidative stress resulting from mitochondrial dysfunction [68]. Increased oxDJ1 and high cytoplasmic expression of 8OHdG colocalized to COXI were found in both *Ifnar1*^{-/-} and *Ifnb*^{-/-} cortex (Fig. 4F, G), showing that disrupted IFN β -IFNAR signaling results in mitochondrial dysregulation and increased oxidative stress.

We next investigated whether lack of *Ifnar1* in primary culture of astrocytes and cortical neurons show similar trends regarding mitochondrial dysfunction by measuring levels of phosphorylated S177 optineurin (p-optineurin), which is positively associated with clearance of mitochondria via mitophagy [69, 70]. Defects in *Ifnar1* resulted in disturbed p-optineurin levels, resulting in either high turnover in astrocytes (Fig. 4H) or lack of mitophagy in neurons similar to neurons lacking *Ifnb* [43], as depicted by accumulation of both COXI and TOMM20 (Fig. 4I).

To functionally address the role of *Ifnar1* in mitophagy, we generated Crisp/Cas9-mediated deletion of *Ifnar1* in Neuron2a (N2a) cells. Similar to primary neurons, N2a cells lacking *Ifnar1* (δ *Ifnar1*) exhibited reduced p-optineurin (Fig. 4J, top) and increased TOMM20 (Fig. 4J, bottom) compared to WT N2a cells, demonstrating defective mitophagy with *Ifnar1* loss. Importantly, both p-Optineurin and TOMM20 were restored to WT levels with IFNAR1 overexpression (IFNAR1^{OE}) in δ *Ifnar1* cells (Fig. 4J), showing direct involvement of IFNAR1 on the promotion of mitophagy.

Together, these data support that both independent omics datasets identified shared signaling defects and corresponding malfunctional pathways related to a lack

of IFN β -IFNAR signaling causing mitochondrial dysfunction. These data were further verified to establish that lack of type I signaling in both *Ifnar1*^{-/-} and *Ifnb*^{-/-} brains results in dysregulated mitochondrial homeostasis, oxidative stress and mitophagy.

Mitochondrial defects upon *Ifnar1*^{-/-} alters neurotransmitter synthesis and glucose hypermetabolism

As neurotransmission, synaptic activity, and energy metabolism are closely linked and dependent on tricarboxylic acid (TCA) cycle activity and mitochondrial function in both neurons and astrocytes, it was next determined whether the identified mitochondrial defects affected brain energy metabolism [71]. Baseline measurement of amino acids related to TCA cycle in Wt and *Ifnar1*^{-/-} brains were similar in cortex or hippocampus (Fig. 5A, B), indicating that potential metabolic changes in *Ifnar1*^{-/-} mice reflect functional alterations due to IFNAR1 loss, rather than genotypic differences in baseline amino acid pools.

To functionally probe TCA cycle capacity upon IFNAR1 loss, cortical and hippocampal slices from Wt and *Ifnar1*^{-/-} mice were incubated with ¹³C-labelled substrates (Fig. 5C, D; Supplementary Fig. 4A, B). Elevated metabolic activity, or hypermetabolism, in *Ifnar1*^{-/-} cortex and hippocampus was revealed upon incubation with [U-¹³C]glucose, leading to significantly increased ¹³C accumulation in the TCA cycle intermediates citrate, α -ketoglutarate, malate, and the amino acid aspartate (Fig. 5C, green boxes). Increased TCA cycle activity resulted in elevated glutamate synthesis in both cortex and hippocampus (Fig. 5C, orange box), whereas GABA synthesis was significantly increased in cortex while less pronounced in hippocampus (Fig. 5C, purple box).

Though no changes were observed in [U-¹³C]glutamate or [U-¹³C]glutamine metabolism (Supplementary

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Fig. 4 Integrated Omics revealed lack of IFN β -IFNAR signaling results in mitochondrial dysfunction. **A** Top 10 GSEA pathways in the proteomics dataset with mitochondrial (yellow box) and metabolic (green boxes) pathways indicated. **B** Top 10 GSEA pathways in the proteomics dataset with Type-I IFN-related (yellow boxes) pathways indicated. **C** Heatmap indicating yes/no (Y/N) overlap and differential expression (DE) of proteins from the proteomic dataset (DE.Prot) and genes from the pseudobulk snRNA transcriptomic dataset (DE.pseudo) within 'GOCC Mitochondrion', 'GOBP Response to Interferon beta', and 'GOBP regulation of viral process' pathways, alongside individual protein (Log2FC.Prot) and gene (Log2FC.pseudo) expression patterns. **D** Representative images and quantification of average Optineurin (green) and TOMM20 (Red) colocalization in NF200⁺ (white) cortical neurons of 6-month-old Wt, *Ifnb*^{-/-}, and *Ifnar1*^{-/-} mice ($n=3-4$ mice per genotype). * $P<0.05$ by one-way ANOVA and Dunnett's post hoc correction test. Scale bars, 5 μ m. Representative images and quantification of **E** COXI (green) mean intensity, **F** 8OHdG (red) mean intensity, and COXI/8OHdG %colocalization in NF200⁺ (white) cortical neurons of 6-month-old Wt, *Ifnb*^{-/-}, and *Ifnar1*^{-/-} mice ($n=3-4$ mice per genotype). * $P<0.05$ and ** $P<0.01$ by one-way ANOVA and Dunnett's post hoc correction test. Scale bars, 5 μ m. **G** Representative images and quantification of %OxDJ1⁺(green)/DAPI⁺(blue)/total DAPI+ cells in cortex of 6-month-old Wt, *Ifnb*^{-/-}, and *Ifnar1*^{-/-} mice ($n=3-4$ mice per genotype). * $P<0.05$ and ** $P<0.01$ by one-way ANOVA and Dunnett's post hoc correction test. Scale bars, 10 μ m. Representative immunoblots and quantifications of **(H)** p-Optineurin and TOMM20 in Wt vs *Ifnar1*^{-/-} primary astrocytes ($n=4-6$), **(I)** p-Optineurin, COXI, and TOMM20 in Syn1^{Cre};*Ifnar1*^{+/+} vs Syn1^{Cre};*Ifnar1*^{-/-} primary cortical neurons ($n=3-5$), **(J)** p-Optineurin (top) and TOMM20 (bottom) in WT and δ *Ifnar1* N2a cells ($n=3-6$) with (+) or without (-) IFNAR1 overexpression (IFNAR1^{OE}). * $P<0.05$, and *** $P<0.001$ by Welch's t test. Data in all graphs are mean $\pm$ SEM

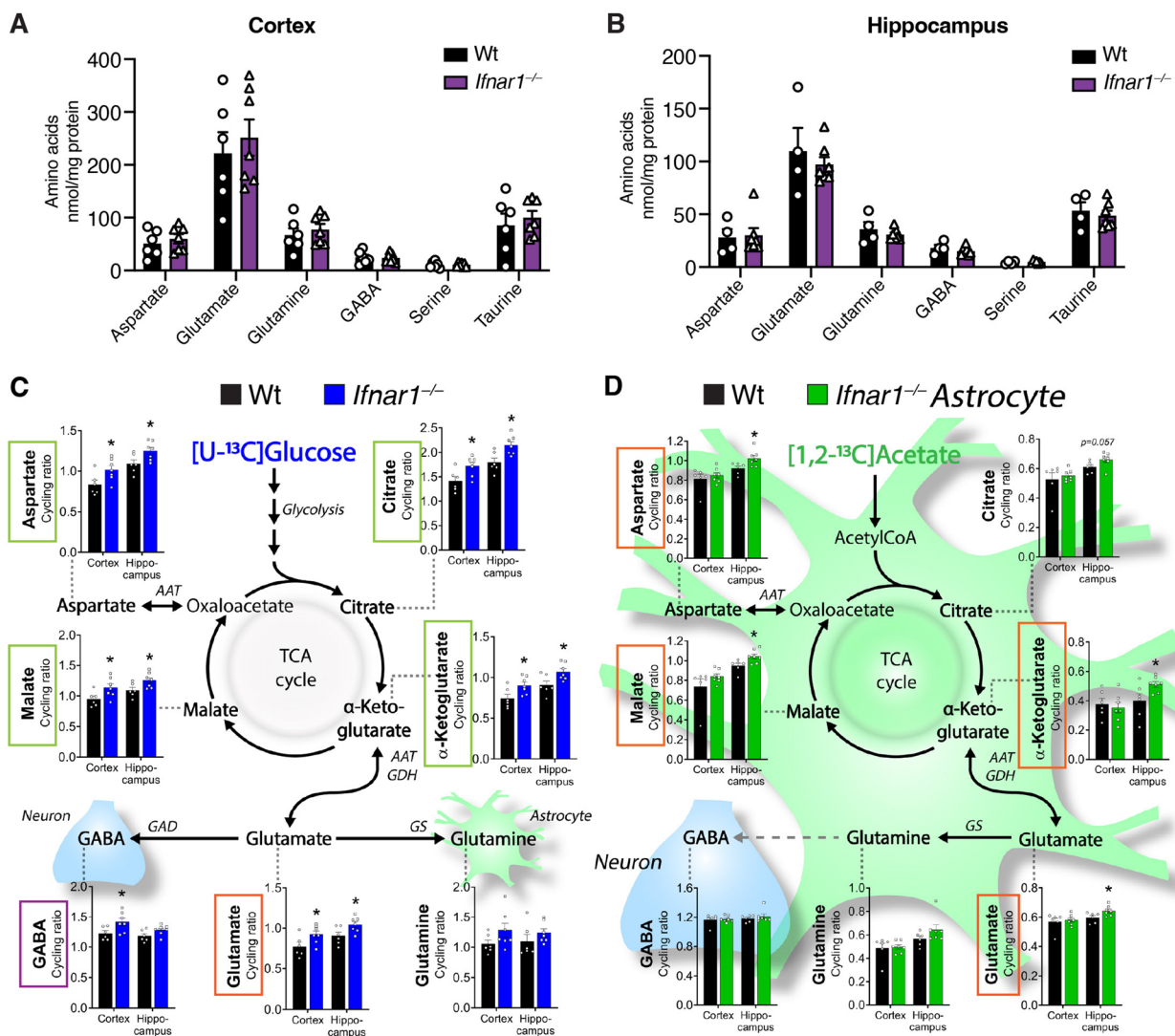


Fig. 5 Mitochondrial defects upon *Ifnar1*^{-/-} alters neurotransmitter synthesis and glucose hypermetabolism. Baseline amino acid levels in (A) cortex and (B) hippocampus of Wt and *Ifnar1*^{-/-} mice ($n=6-7$ per genotype), measured by HPLC. Cortical and hippocampal slices from Wt and *Ifnar1*^{-/-} mice ($n=6-7$ per genotype) were incubated with ¹³C-labelled energy substrates (C) [U-¹³C]glucose or (D) [1,2-¹³C]acetate. Cycling ratios of TCA cycle intermediates citrate, α-ketoglutarate, and malate, and amino acids aspartate, glutamine, glutamate, and GABA, in cortical and hippocampal slices, reflecting TCA cycling rate. Data are mean ± SEM. * $P < 0.05$ by t -test

Fig. 4A, B), there were slight increases in ¹³C accumulation in glutamine derived from [U-¹³C]glucose metabolism (Fig. 5C). Increased ¹³C accumulation in glutamine, produced from glutamate selectively in astrocytes [47, 48], could reflect alterations in astrocyte-specific TCA cycle activity and metabolism. When probed directly with [1,2-¹³C]acetate, a substrate predominantly metabolized by astrocytes [47, 48, 72], several metabolites including aspartate, malate, α-ketoglutarate, and glutamate (Fig. 5D, orange boxes) exhibited elevated ¹³C accumulation selectively in hippocampal slices of *Ifnar1*^{-/-} mice,

which could reflect changes in hippocampal energy requirements from astrocytes lacking IFNAR1.

These results indicate that loss of IFNAR1 signaling results in brain glucose hypermetabolism affecting glutamate and GABA synthesis. Dysregulated excitatory neurotransmission may require greater energy demands in *Ifnar1*^{-/-} neurons and astrocytes, prompting compensatory glucose metabolism and neurotransmitter production, and could indicate neuronal dysfunction and potential neurodegeneration.

***Ifnar1*^{-/-} mice develop PDD-like neuropathology, behavior deficits, and neuroinflammation**

Neuronal counts in cortex, hippocampus, and olfactory bulb were quantified to determine whether the loss of IFNAR1 signaling, and subsequent mitochondrial and metabolic dysfunction, contributes to a broad neurodegenerative phenotype similar to previous observations in *Ifnb*^{-/-} brains [12, 13]. Though IFN β binds to IFNAR to conduct signaling, the lack of the *Ifnb* gene could potentially be counteracted by *Ifna* genes to compensate loss of cellular functions. Nevertheless, the number of NeuN⁺ neurons were reduced in all regions investigated (cortex, hippocampus and olfactory bulb combined) both in *Ifnar1*^{-/-} and *Ifnb*^{-/-} brains (Fig. 6A, Supplementary Fig. 5A–C). *Ifnar1*^{-/-} mice also exhibited neuropathological hallmarks of PDD as observed in *Ifnb*^{-/-} mice [12, 13], featuring DA neuronal loss and reduction in TH⁺ neurites in the substantia nigra (Fig. 6B) with concurrent loss of TH⁺ immunoreactivity in the striatum compared to age-matched Wt mice (Fig. 6C). Notably, TH⁺ cell loss in the substantia nigra pars compacta was approximately 10% greater than loss in the ventral tegmental area (Supplementary Fig. 5D), resembling PD neuropathology [73]. Significant accumulation of α -syn, phosphorylated α -syn at serine 129 (p α -syn), Tau (pan), and phosphorylated Tau

at threonine 205 (pTau) was observed in aged (12-month-old) *Ifnar1*^{-/-} but not Wt or young (1.5-month-old) mice (Supplementary Fig. 5E). Importantly, LB-like structures staining positive for both p α -syn and pTau were confirmed in neurons of *Ifnar1*^{-/-} thalamus using both light and electron microscopy (Fig. 6D). Fibrillar structures containing A β were also observed by electron microscopy (Fig. 6E), and A β ⁺ plaques like those in 5xFAD mice and *Ifnb*^{-/-} mice [12] were detected in *Ifnar1*^{-/-} but not Wt mice (Fig. 6F).

To determine whether the identified neuropathologies resulted in discernible phenotypes, *Ifnar1*^{-/-} mouse behaviors were assessed over time. 3-month-old *Ifnar1*^{-/-} mice exhibited significantly reduced motor performances on the wire suspension test compared to Wt mice (Fig. 6G). Though 3-month-old *Ifnar1*^{-/-} mice performed marginally better than *Ifnb*^{-/-} mice on the Morris water maze (MWM) (Supplementary Fig. 5F–H), both 6-month-old *Ifnb*^{-/-} mice and *Ifnar1*^{-/-} mice in the Morris water maze spent less time in the platform quadrant after training (Supplementary Fig. 5I–K). These deficits were sustained in 12-month-old *Ifnb*^{-/-} and *Ifnar1*^{-/-} mice on the Barnes maze, where both strains of mice spent more time finding the target escape hole (Fig. 6H, I). Importantly, there were no significant differences

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Fig. 6 *Ifnar1*^{-/-} leads to PDD-like neuropathology and behavior. **A** Immunohistochemical (IHC) images and collective quantification of NeuN⁺ cells per mm² in 6-month-old Wt, *Ifnb*^{-/-}, and *Ifnar1*^{-/-} cortex, hippocampus, and olfactory bulb as %Wt ($n=3-4$ per genotype). **** $P<0.0001$ by one-way ANOVA and Bonferroni's post hoc correction test. Scale bars, 50 μ m. IHC images and quantifications of tyrosine hydroxylase (TH)⁺ DA neurons and neurites in 3-month-old Wt versus *Ifnar1*^{-/-} **(B)** substantia nigra and **(C)** striatum. ($n=4$ per genotype). * $P<0.05$ and ** $P<0.01$ by unpaired t -test. Scale bars, 200 μ m (substantia nigra), 20 μ m (insert), and 1 mm (striatum). **D** IHC (left) and immunogold-labelled (white arrows) TEM images (right) for p α -syn or pTau of Lewy body (LB)-like inclusions in 12-month-old *Ifnar1*^{-/-} thalamus. Nu = nucleus. Scale bars, 5 μ m (IHC) and 500 nm (TEM). **E** TEM micrograph of immunogold-labelled A β ₁₋₁₆ (white arrows) in fibrillar structures (black arrows) in 12-mo *Ifnar1*^{-/-} thalamus. **F** Representative images of 12-month-old 5xFAD, Wt, and *Ifnar1*^{-/-} cortex stained for A β ₁₋₁₆, with extracellular A β ⁺ plaques per mm² cortex quantified in Wt versus *Ifnar1*^{-/-} mice ($n=4-7$ per genotype). Scale bars, 50 μ m. **G** Wire hang performances of 3-month-old mice ($n=10-16$ per genotype). * $P<0.05$ and ** $P<0.01$ by one-way ANOVA and Tukey's post hoc correction test. **H, I** Barnes maze results of 12-month-old mice ($n=6-10$ per genotype). **H** Barnes maze spatial learning performances. # $P<0.01$ for training effect and $^5P<0.05$ for genotype effect by two-way ANOVA. Genotype effect vs Wt * $P<0.05$ and ** $P<0.01$ by Tukey's post hoc test. **I** Memory probe on the Barnes maze as percent total test time spent in the escape zone. * $P<0.05$ and ** $P<0.01$ by one-way ANOVA and Tukey's post hoc correction test. **J** Novelty-suppressed feeding test results of 3-month-old mice ($n=8-9$ per genotype), showing latency to first bite (seconds, s), amount of food consumed (grams, g), and eating duration (s). **K** Sucrose preference test results of 6-month-old Wt and *Ifnar1*^{-/-} mice ($n=10-15$ per genotype), comparing total liquid intake per mouse weight (g), sucrose intake per mouse weight (g), and sucrose preference ratio over water. * $P<0.05$ and ** $P<0.01$ by unpaired t -test. **L** Rearing activity of 3-month-old Wt, *Ifnb*^{-/-}, and *Ifnar1*^{-/-} mice ($n=17-20$ per genotype). **M** Climbing activity of 3-month-old mice ($n=17-20$ per genotype), quantified by latency to first climbing event (s) and number of climbing events. **N** Open field (OF) performance of 3-month-old mice as cumulative time (s) spent in maze center ($n=17-20$ per genotype). **O** Elevated plus maze (EPM) performances of 3-month-old mice ($n=17-20$ per genotype). Time spent in the maze open arms (top diagram, left graph) and number of side edge investigations (bottom diagram, right graph) were quantified. **P** Heat-induced tail flick latencies (nociception) of 3-month-old mice ($n=17-20$ per genotype) as %Wt. **Q** Gene expression of cytokines IL-1 β , IL-6, IL-12, and TGF- β by qPCR in 3-month-old mouse brain, shown as Log2FC of Wt ($n=3$ per genotype). ** $P<0.01$, *** $P<0.001$, and **** $P<0.0001$ by two-way ANOVA and Tukey's post hoc correction test. **R** IF images and quantification of TNF- α ⁺Iba1⁺ as %total Iba1⁺ microglia in the hippocampus of 9-month-old Wt, *Ifnb*^{-/-}, and *Ifnar1*^{-/-} mice ($n=3$ per genotype). Previously published [12] Wt and *Ifnb*^{-/-} data (blue text) were generated concurrently with the *Ifnar1*^{-/-} data shown and included for comparison. *** $P<0.001$ and **** $P<0.0001$ by one-way ANOVA and Bonferroni's post hoc correction test. Scale bars, 25 μ m. **S** Representative IHC images and quantifications Mac-3⁺ microglia (black arrows) per mm² in Wt vs *Ifnar1*^{-/-} cortex ($n=4-7$ per genotype). Scale bars, 100 μ m; insert, 10 μ m. * $P<0.05$ by unpaired t -test. **T** IF images of GFAP⁺ astrocytes, Iba1⁺ microglia, or CD31⁺ endothelial cells (green) associated with A β ⁺ (red) aggregates (white arrows) in 12-month-old *Ifnar1*^{-/-} cortex. DAPI = blue. Scale bars, 25 μ m. Data in all graphs are mean $\pm$ SEM

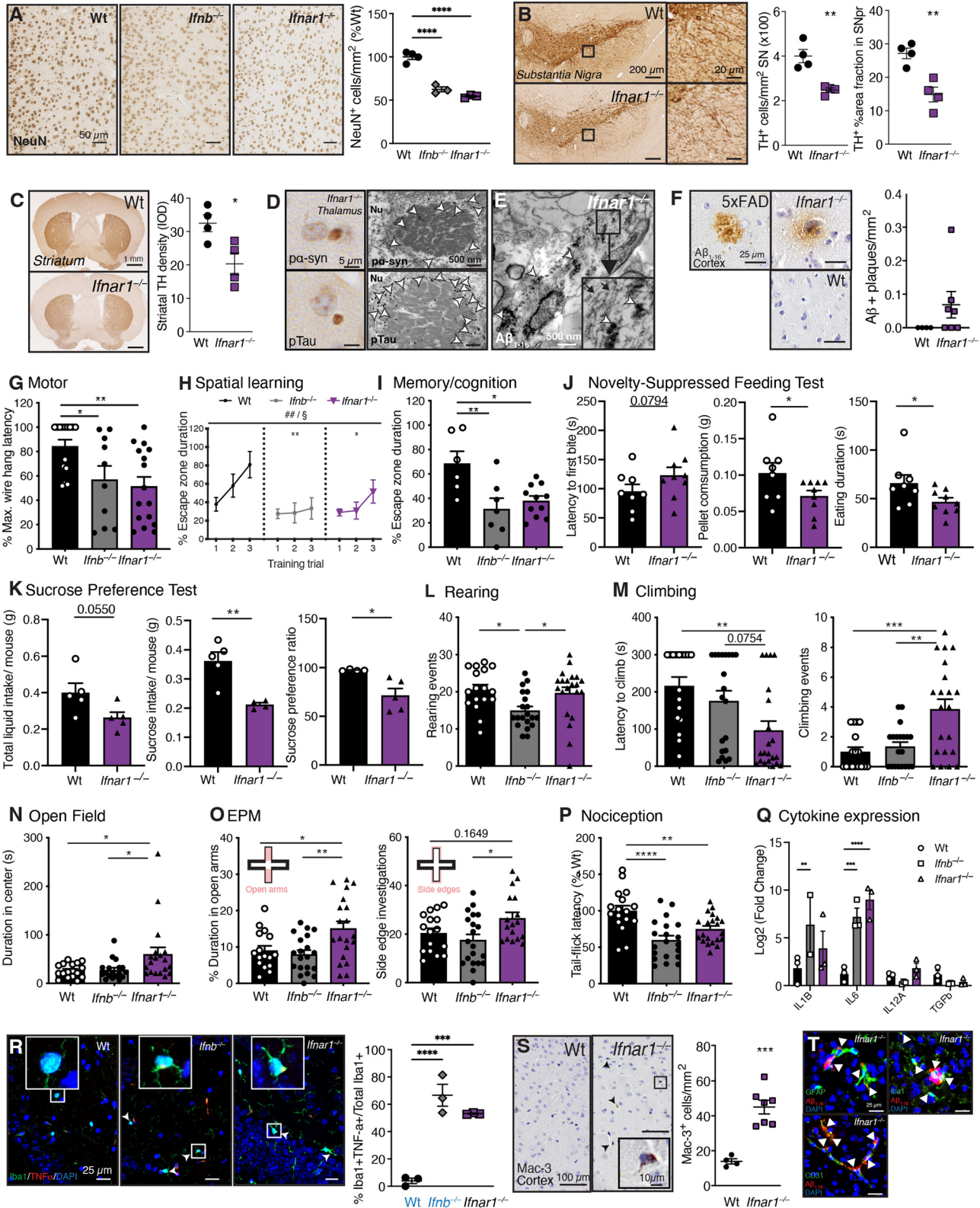


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found in distance travelled or velocity between Wt and either *Ifnb*^{-/-} and *Ifnar1*^{-/-} during any of the cognitive tests (Supplementary Fig. 5L–Q), suggesting that both *Ifnb*^{-/-} and *Ifnar1*^{-/-} mice have impaired spatial learning and memory.

Non-motor neuropsychiatric behaviors such as depression, anxiety, and pain, which can develop prior to PD diagnosis in humans [74, 75], were assessed in *Ifnar1*^{-/-} mice. Depression-like behavior was observed on the novelty-suppressed feeding test, where 3-month-old *Ifnar1*^{-/-} mice spent significantly less time eating and consumed less when exposed to a food pellet in a novel open arena after a 24 h fasting period (Fig. 6J). Similar behavior was sustained in 6-month-old *Ifnar1*^{-/-} mice with the sucrose preference test, which manifested as significantly reduced sucrose preference ratio and reduced total sucrose intake compared to Wt mice (Fig. 6K). *Ifnb*^{-/-} mice spontaneously reared less and for shorter durations than Wt and *Ifnar1*^{-/-} mice (Fig. 6L; Supplementary Fig. 5R), corresponding with reduced motor capacity on the wire suspension test (Fig. 6G) and on the rotarod [12, 13]; however, *Ifnar1*^{-/-} mice initiated climbing behavior significantly faster and more frequently than Wt and *Ifnb*^{-/-} mice (Fig. 6M). To determine whether *Ifnar1*^{-/-} climbing behavior was due to increased anxiety, mouse behavior was assessed in open field (OF) and elevated plus maze (EPM). Genotypic differences were observed for distance travelled or velocity on either test (Supplementary Fig. 5S, T). In contrast with *Ifnb*^{-/-} mice, which previously exhibited classic anxiety-like behaviors at a later age (Supplementary Fig. 5U), 3-month-old *Ifnar1*^{-/-} mice spent more time in the OF center (Fig. 6N), on the EPM open arms, and had significantly more EPM side edge investigations than Wt and *Ifnb*^{-/-} mice (Fig. 6O). Though conventionally interpreted as anxiolytic behavior, these data reflect similar escape-induced anxiety-like behavior on the EPM observed in the early stages of neurodegeneration in the 6-OHDA and A53T mouse models of PD [76, 77]. Finally, both 3-month-old *Ifnb*^{-/-} mice and *Ifnar1*^{-/-} mice demonstrated faster heat-induced nociception, suggesting that IFN β -IFNAR defects may be involved in early-onset pain sensitivity (Fig. 6P).

Lack of IFN β -IFNAR signaling could impact expression of other brain cytokines like increased IL-1 β , IL-6 and TNF- α [10, 12, 78, 79]. To assess whether anxiety-related phenotypes could be associated with differential regulation of brain cytokines, a panel of pro- and anti-inflammatory cytokines were investigated by qPCR, among which IL-1 β , IL-6, IL-12A and TGF β were detectable (Fig. 6Q). Increased expression of proinflammatory IL-1 β and IL-6 gene were found in cortex of mice lacking IFN β -IFNAR signaling, however no significant differences were

found between *Ifnb*^{-/-} and *Ifnar1*^{-/-} strains to correlate with differential anxiety-like behavior. Furthermore, as IFN β loss was associated with elevated neuronal TNF- α [12], *Ifnar1*^{-/-} TNF- α ⁺/Iba1⁺ counts were concurrently-quantified alongside Wt and *Ifnb*^{-/-} counts (previously published [12], indicated in blue text). Like *Ifnb*^{-/-} mice, higher numbers of TNF- α ⁺ microglia in *Ifnar1*^{-/-} mice were found compared to Wt controls (Fig. 6R), confirming neuroinflammation upon IFN β -IFNAR loss. Significantly more phagocytic microglia, marked by lysosomal Mac-3 expression [80], were seen in *Ifnar1*^{-/-} cortex compared to Wt (Fig. 6S). Interestingly, GFAP⁺ astrocytes and Iba1⁺ microglia were detected surrounding A β plaques (Fig. 6T, top panels), supporting astrocytic involvement in A β processing and microgliosis. A β was also found localized to CD31⁺ endothelial cells lining blood vessels in 12-month-old *Ifnar1*^{-/-} cortex (Fig. 6T, bottom panel), mimicking what is observed in dementia [81, 82].

Collectively, these results show that molecular and functional differences observed due to IFNAR1 loss results in Parkinsonian dementia-like neuropathology, behavior deficits, and neuroinflammation.

Neuronal and astrocytic IFNAR1 loss recapitulate distinct aspects of PDD-like neuropathology and behavior

As the transcriptomic and functional metabolic results suggested differential effects of IFNAR1 loss in neurons and astrocytes, PDD-like neuropathology and behavioral phenotypes were investigated in mice lacking either neuronal or astrocytic IFNAR1. Neuron-specific Cre recombinase (Cre)-floxed *Ifnar1* gene *Syn1*^{Cre};*Ifnar1*^{fl/fl} mice and astrocyte-specific GFAP^{Cre};*Ifnar1*^{fl/fl} mice were generated and validated for target-cell specific knock-down of *Ifnar1* using qPCR (Fig. 7A). Though the potential for GFAP^{Cre}-based knockout to affect neuronal populations has been reported and discussed [83, 84], cell-specific deletion of *Ifnar1* in both astrocytes and neurons was confirmed, and no off-target reduction of *Ifnar1* were observed in either conditional mouse strains. Moreover, ISG15 downregulation was found in target cells but not in other brain cell types (Fig. 7B, C), supporting cell-specific loss of IFNAR signaling.

As Cre expression can in itself alter anxiety-related behaviors [85], *Syn1*^{Cre};*Ifnar1*^{+/+} and GFAP^{Cre};*Ifnar1*^{+/+} littermates were used as controls for both molecular and behavioral comparisons. Significant DA neuronal loss in the substantia nigra was observed specifically in 3-month-old neuronal *Syn1*^{Cre};*Ifnar1*^{fl/fl} mice but not in astrocytic GFAP^{Cre};*Ifnar1*^{fl/fl} mice or *Syn1*^{Cre};*Ifnar1*^{+/+} littermate controls (Fig. 7D). Accumulation of p α -syn and pTau was found in TH⁺ neurons of both *Syn1*^{Cre};*Ifnar1*^{fl/fl}

fl and GFAP^{Cre};*Ifnar1*^{fl/fl} mice (Fig. 7E); however, only neuronal Syn1^{Cre};*Ifnar1*^{fl/fl} mice had indications of motor and cognitive deficits at 3 months of age (Supplementary Fig. 6A, B), which became fully penetrant at 12 months of age (Fig. 7F–I) as shown by significant motor impairment on the rotarod (Fig. 7F, G) and reduced cognitive performance on the Barnes maze (Fig. 7H, I). Though cognitive deficits were only significant in 12-month-old neuronal heterozygous Syn1^{Cre};*Ifnar1*^{+/fl} mice (Fig. 7H), both heterozygous and homozygous groups showed significantly inhibited Barnes maze performances compared to their respective 3-month-old groups (Fig. 7I). Aging differences were not significant between 3- and 12-month-old control Syn1^{Cre};*Ifnar1*^{+/+} mice (Fig. 7I). Of note, neither astrocytic GFAP^{Cre};*Ifnar1*^{+/fl} nor GFAP^{Cre};*Ifnar1*^{fl/fl} demonstrated motor deficits or cognitive decline at either time point (Fig. 7F, H; Supplementary Fig. 6A, B).

Behaviors resembling neuropsychiatric abnormalities, which manifested prior to cognitive decline in *Ifnar1*^{−/−} mice, were assessed in Syn1^{Cre};*Ifnar1*^{fl/fl} mice and GFAP^{Cre};*Ifnar1*^{fl/fl} mice. Though pain sensitivity was not detected in 3-month-old conditional knock-out animals (Supplementary Fig. 6C), significantly increased heat-induced nociception manifested only in 12-month-old GFAP^{Cre};*Ifnar1*^{fl/fl} mice (Fig. 7J). Like *Ifnar1*^{−/−} mice, hyper-anxious behavior was significant in 3-month-old GFAP^{Cre};*Ifnar1*^{fl/fl} mice (Supplementary Fig. 6D) and more pronounced in 12-month-old GFAP^{Cre};*Ifnar1*^{+/fl} mice and GFAP^{Cre};*Ifnar1*^{fl/fl} compared to GFAP^{Cre};*Ifnar1*^{+/+} controls or Syn1^{Cre};*Ifnar1*^{fl/fl} mice (Fig. 7K; Supplementary Fig. 6E). The absence of neuropsychiatric phenotypes in neuronal Syn1^{Cre};*Ifnar1*^{fl/fl} mice, while being exhibited by GFAP^{Cre};*Ifnar1*^{fl/fl} mice,

suggests a unique role for astrocytic IFNAR1 dysfunction in the development of neuropsychiatric symptoms associated with PDD.

In summary, neuronal IFNAR1 loss appeared sufficient to recapitulate substantia nigral neuropathology alongside motor and cognitive behavior deficits, whereas astrocytic IFNAR1 loss recapitulated behavior resembling neuropsychiatric abnormalities. The later-onset of behavior deficits in both cell-specific IFNAR1 knock-out strains relative to genomic *Ifnar1*^{−/−} mice suggest that synergistic dysfunction occurs upon neuronal and astrocytic IFNAR1 loss, contributing to a progressive PDD-like phenotype in *Ifnar1*^{−/−} mice.

This study shows that cell-specific transcriptional alterations in mitochondrial and energy metabolism genes in *Ifnar1*^{−/−} brain are reflected in regional proteome changes and altered glucose metabolism, supporting a regulatory role of IFNAR1 in excitatory neurotransmission. These molecular changes upon IFNAR1 loss results in broad neurodegenerative processes as well as neuropathology resembling PDD in aged mice, including LB-like inclusions, pTau accumulation, Aβ pathology, gliosis with neuroinflammatory profile associated with mitochondrial dysfunctions. Importantly, neuronal IFNAR1 loss was sufficient to induce PD-like neurodegeneration and motor and cognitive behavior manifestations, whereas astrocytic IFNAR1 loss manifested as behaviors resembling pain sensitivity and hyper-anxiety, together revealing distinct brain cell-specific requirements of IFNAR1 signaling which in concert contribute to maintaining brain homeostasis (Fig. 8).

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Fig. 7 Neuron- or astrocyte-specific *Ifnar1*^{−/−} recapitulate differential aspects of PDD-like phenotypes. **A** *Ifnar1* expression in primary cortical neurons vs enriched astrocyte cultures from Syn1^{Cre};*Ifnar1*^{fl/fl} and GFAP^{Cre};*Ifnar1*^{fl/fl} pups compared to Cre-negative *Ifnar1*^{fl/fl} controls (*n* = 3–7 per genotype). ***P* < 0.01 by unpaired *t*-test. Quantification (**B**) and Representative images (**C**) and of ISG15 (green) as %target population in 3-month-old Syn1^{Cre};*Ifnar1*^{+/+} (Syn1cre Wt), Syn1^{Cre};*Ifnar1*^{fl/fl} (Syn1cre Mut), GFAP^{Cre};*Ifnar1*^{+/+} (GFAPcre Wt), and GFAP^{Cre};*Ifnar1*^{fl/fl} (GFAPcre Mut) brains (*n* = 3 per genotype). N = NF200⁺ neuron (red); A = GFAP⁺ astrocyte (white). DAPI = blue. Scale bars, 5 μm. **P* < 0.05, ***P* < 0.01, and ****P* < 0.001 by two-way ANOVA and Tukey's post hoc test. **D** Representative images and quantifications of TH⁺ DA neurons and neurites in substantia nigra of 3-month-old Syn1^{Cre};*Ifnar1*^{fl/fl} mice and astrocytic GFAP^{Cre};*Ifnar1*^{fl/fl} mice compared to littermate controls (*n* = 3–4 per genotype). ***P* < 0.01 by one-way ANOVA and Bonferroni's post hoc correction test. Scale bars, 100 μm. **E** Representative images and quantifications of pα-syn (red) and pTau (green) fluorescence intensity per TH⁺ (white) neurons of 3-month-old Syn1^{Cre};*Ifnar1*^{fl/fl} mice and astrocytic GFAP^{Cre};*Ifnar1*^{fl/fl} mice compared to littermate controls (*n* = 3–4 per genotype). Blue = DAPI. **P* < 0.05 and ***P* < 0.01 by one-way ANOVA and Bonferroni's post hoc correction test. Scale bars, 100 μm. **F** Rotarod performance of 12-month-old neuronal Syn1^{Cre};*Ifnar1*^{fl/fl} mice and astrocytic GFAP^{Cre};*Ifnar1*^{fl/fl} mice (*n* = 7–16 per genotype). **P* < 0.05 by one-way ANOVA and Tukey's post hoc correction test. **G** Rotarod comparison of 3-month-old (*n* = 8–11 per genotype) and 12-month-old (*n* = 7–16 per genotype) neuronal Syn1^{Cre};*Ifnar1*^{fl/fl} mice. ****P* < 0.001 by unpaired *t*-test. **H** Barnes maze probe performance reflecting cognitive capacity of 12-month-old neuronal Syn1^{Cre};*Ifnar1*^{fl/fl} mice and astrocytic GFAP^{Cre};*Ifnar1*^{fl/fl} mice (*n* = 7–16 per genotype). **P* < 0.05 by one-way ANOVA and Tukey's post hoc correction test. **I** Barnes maze probe performance comparison between 3-month-old (*n* = 8–11 per genotype) and 12-month-old (*n* = 7–16 per genotype) neuronal Syn1^{Cre};*Ifnar1*^{fl/fl} mice. **P* < 0.05 by unpaired *t*-test. Data in all graphs are mean ± SEM. **J** Tail flick latencies representing heat-induced nociception as %Wt (either Syn1^{Cre};*Ifnar1*^{+/+} or GFAP^{Cre};*Ifnar1*^{+/+}, respectively) of 3-month-old (*n* = 8–11 per genotype) and 12-month-old mice (*n* = 7–16 per genotype). **K** EPM end-edge investigations of 12-month-old Syn1^{Cre};*Ifnar1*^{fl/fl} and GFAP^{Cre};*Ifnar1*^{fl/fl} mice (*n* = 7–16 per genotype). **P* < 0.05 by one-way ANOVA and Tukey's post hoc correction test. Data in all graphs are mean ± SEM

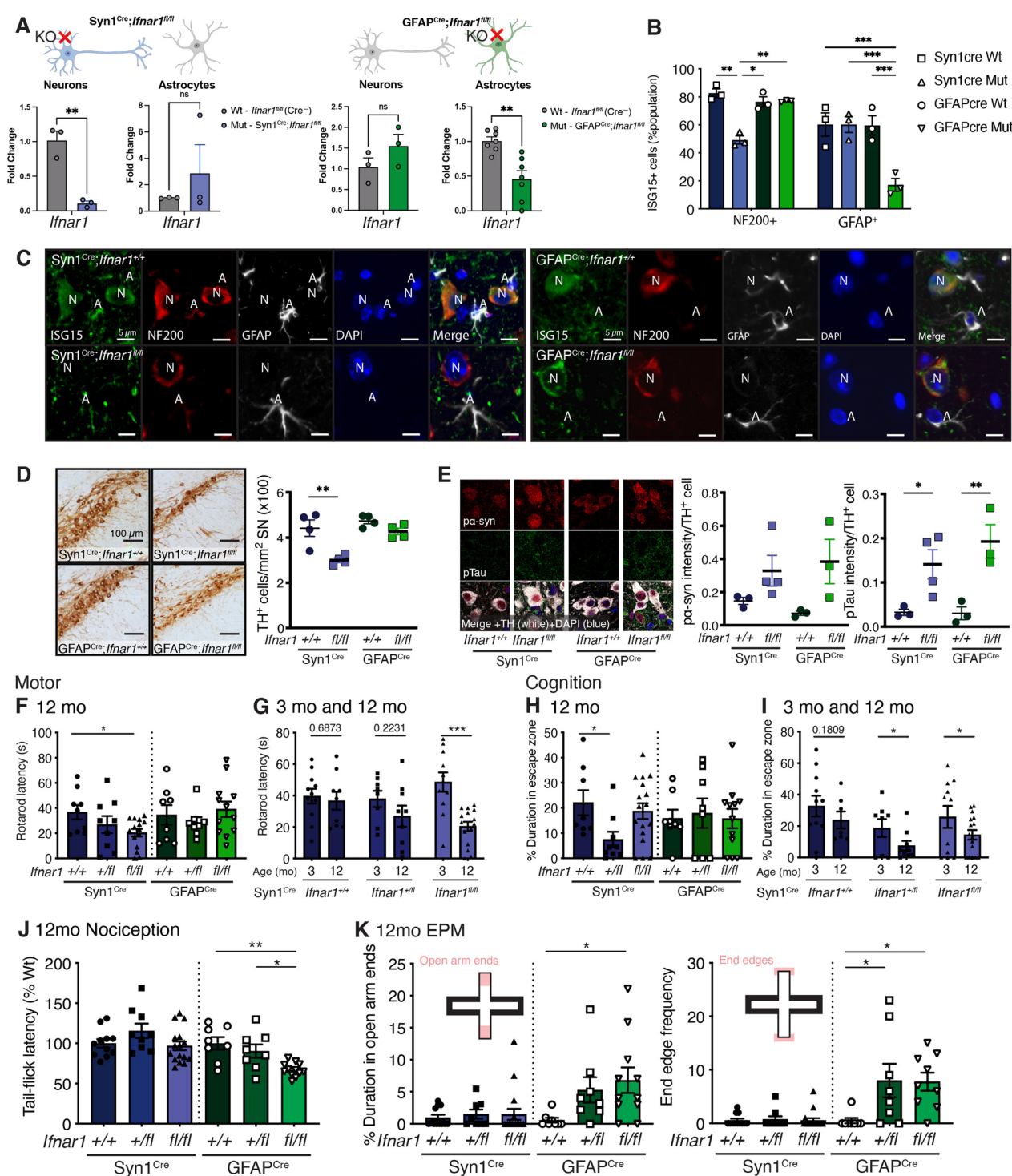


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Discussion
IFN β is a potent anti-inflammatory and antiviral cytokine that signals through IFNAR1 and IFNAR2 receptors, and defective signaling of IFN β through IFNAR is associated

with patients suffering from PD and PDD [10] and mimics PDD-like pathology in experimental models [11–13]. However, the neuropathological and neuropsychiatric outcomes resulting from *Ifnb* loss may differ in some

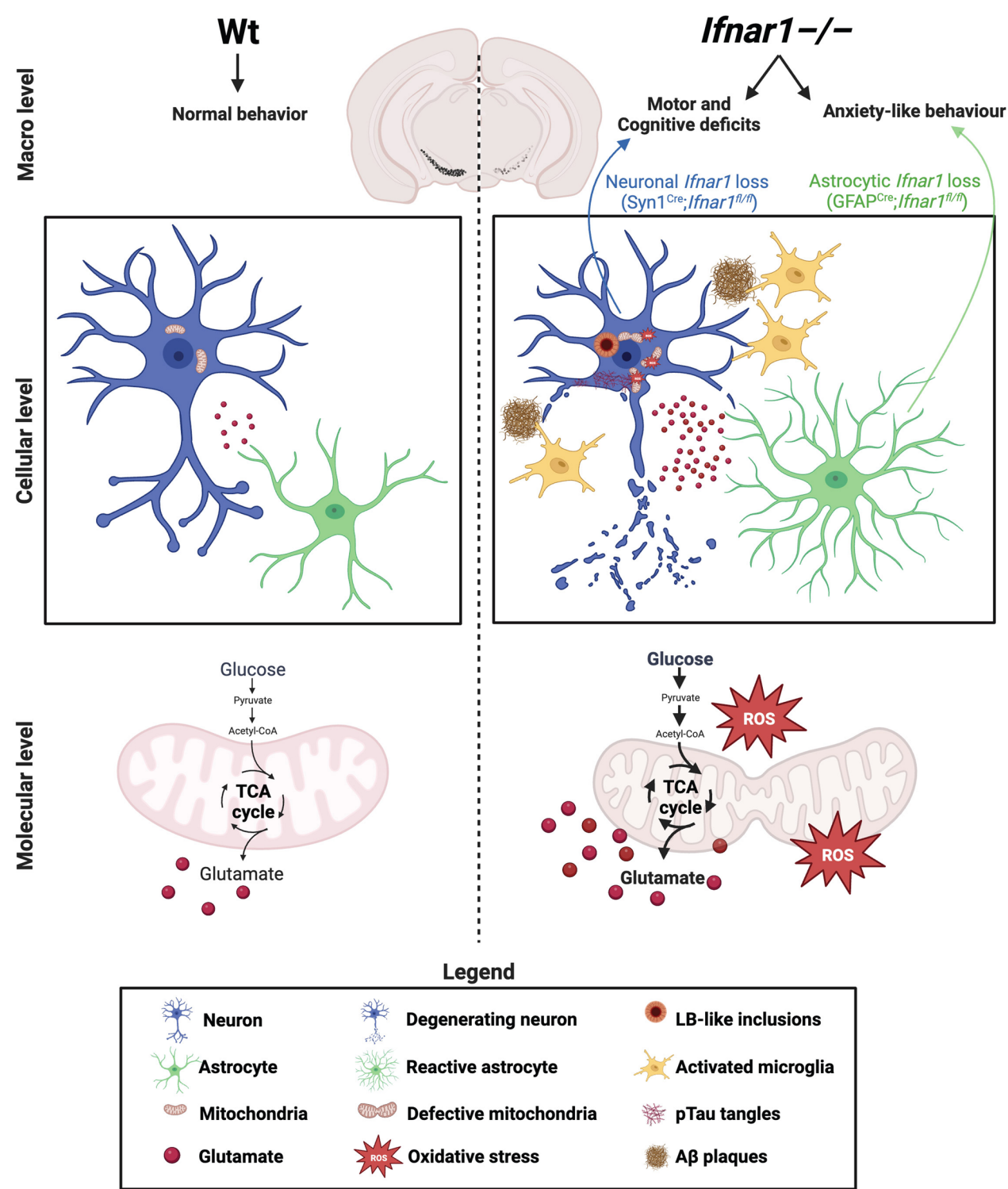


Fig. 8 Graphical summary of the study. Lack of immune modulation due to IFNAR1 loss results in mitochondrial dysfunction shown as mitophagy deficits, oxidative stress, and glucose hypermetabolism (molecular level), leading to development of neuroinflammation, neurodegeneration, LB-like inclusions, pTau accumulation, Aβ plaques, and disturbed brain energy metabolism (cellular level). These PDD-like pathologies manifest as motor, cognitive, and neuropsychiatric behavior deficits upon aging (macro level). Cardinal motor and cognitive manifestations were recapitulated by mice lacking neuronal IFNAR1, and neuropsychiatric disturbance-like behaviors were driven by astrocytic IFNAR1 loss. Therapeutics targeting the synergistic dysfunction that results from neuronal and astrocytic IFNAR1 loss may therefore prevent PDD progression and manifestation

respects from those caused by the absence of the *Ifnar1* gene, which could be due to potential compensatory mechanisms involving other type I interferon cytokines or variations related to the cellular origins of the defective genes. This report explores the role of brain cell-specific IFNAR1 and provides evidence of the importance of IFNAR1, conventionally investigated in immune cells [86] or upon viral infection [87–89], in neuronal homeostasis, including how its dysfunction could contribute to neuropathological and neuropsychiatric conditions that resemble PDD.

In humans, baseline IFNAR1 expression differs in neurons and astrocytes and appears to be distinctly impacted in LBD and PD along with several related Type I-IFN genes, implicating dysregulated IFNAR1 signaling in dementia development as previously observed in sporadic PDD [10]. Of note, a recent study on the use of Anifrolumab, a monoclonal antibody that inhibits IFNAR1, reported male-specific adverse events included memory impairment and altered mood with continued use of Anifrolumab for treatment of systemic lupus erythematosus [90]. In mice, unbiased transcriptomic and proteomic analysis of *Ifnar1*^{−/−} brains revealed molecular differences from Wt, affecting pathways related to mitochondrial function and PD. Mitophagy defects due to lack of *Ifnar1*, reversible with IFNAR1 overexpression, likely contribute to brain metabolic dysregulation and clinical and pathological phenotypes resembling PDD in *Ifnar1*^{−/−} mice. These findings support that lack of IFNβ-IFNAR signaling in *Ifnar1*^{−/−} mice, like in *Ifnb*^{−/−} mice, leads to dysregulated neuronal autophagy and mitophagy [11–13, 91] and immunomodulation [12, 17, 41], resulting in neuroinflammation alongside mitochondrial and metabolic malfunctions [10, 43]. Importantly, it is shown that lack of neuronal IFNAR1 (*Syn1*^{Cre};*Ifnar1*^{fl/fl}) alone mimics all *Ifnar1*^{−/−} behavioral manifestations except for behavior resembling neuropsychiatric disturbances and pain sensitivity, which manifested instead in mice lacking astrocytic IFNAR1 (*GFAP*^{Cre};*Ifnar1*^{fl/fl}), revealing distinct cell-specific requirements of IFNAR1 in brain homeostasis.

Collectively, the transcriptomic, proteomic, and functional investigations in this report suggest that excitatory neurotransmission is compromised in *Ifnar1*^{−/−} brains, and is supported by another study implicating IFNAR1 loss in the dysregulation of the glutamate-aspartate transporter GLAST and subsequent detrimental effects on synaptic strength [14]. Consistent with previous work showing that IFNβ signaling is essential for mitochondrial homeostasis [43], the transcriptomic and proteomic results here suggested dysregulated processes essential for neurotransmission upon IFNAR1 loss, including mitochondrial function [63, 64]. Additionally, we show here that lack of *Ifnar1*, like in *Ifnb*^{−/−} mice,

causes accumulation of defective/oxidatively damaged mitochondria. Importantly, these mitophagy deficits seen in both primary neurons and modified N2a cells lacking *Ifnar1* were restored with IFNAR1 overexpression. Together, these findings support the requirement of IFNβ-IFNAR signaling in mitochondrial homeostasis [10, 11, 43] and shed further light on related reports in PDD patients implicating mitochondrial dysfunction in dementia development [10, 92, 93].

Type-I IFNs have been shown to increase fatty acid oxidation and oxidative phosphorylation in peripheral immune cells [94] and it was found that IFNβ regulates cellular glucose metabolism upon viral infection [95]; however, the investigation of IFNAR1 involvement in brain energy metabolism is unprecedented. Brain glucose metabolism is closely coupled to glutamatergic neurotransmission [71, 96], which was found here to be hypermetabolic in *Ifnar1*^{−/−} cortex and hippocampus. Increased glucose metabolism has been reported prior to proteinopathy in AD mouse models [47, 97] and correlated to cognitive dysfunction severity in PD patients [7]. Brain glucose hypermetabolism upon IFNAR1 loss may therefore reflect compensatory metabolic excitatory mechanisms for declining neurotransmission that manifests upon aging as PDD-like behaviors in *Ifnar1*^{−/−} mice.

The neurodegeneration observed in *Ifnar1*^{−/−} mice, as with *Ifnb*^{−/−} mice [12, 13], is comprised of significant DA neuron loss, the hallmark of PD pathology, alongside cortical and hippocampal neurodegeneration mimicking cortical thinning and hippocampal atrophy among PDD patients [24, 98]. Though DA neurodegeneration in the substantia nigra is significant in PD patients and considered a defining neuropathology of parkinsonism [99], PD neuropathology extends throughout the brain beyond the basal ganglia [24, 98], and the impact of L-dopa treatment, which targets DA neuronal dysfunction, on prevention of non-motor symptoms is poor [100–102]. Thus, the broad behavioral phenotypes exhibited by mice lacking IFNAR1 signaling, which appear to phenocopy patients with PD/PDD, likely reflect a general failure in neuronal health and signaling abilities and highlight a need for investigating neurodegenerative mechanisms beyond DA neuropathology in PDD. Though the mechanistic observations in this report were made in mice, patient-based studies showing a reduced risk of PD development in hepatitis C patients receiving IFN-I treatment [103] as well as significant reduction of IFNAR1 in PDD compared to PD patients without dementia [10] support the importance of the homeostatic functions of IFNAR1 in maintenance of human brain health.

Lack of astrocytic IFNAR1 signaling impacted hippocampal metabolic regulation reported here to be disturbed in *Ifnar1*^{−/−} mice, which manifested as

depression-like behaviour, early-onset anxiety and pain sensitivity observed in both *Ifnar1*^{-/-} mice and GFAP^{Cre};*Ifnar1*^{fl/fl}. These observations could implicate astrocytic IFNAR1 dysfunction in neuropsychiatric abnormalities that develop in PDD. Moreover, the earlier and more pronounced manifestation of anxiety-like behavior of *Ifnar1*^{-/-} compared to *Ifnb*^{-/-} mice indicate that although IFN β and IFNAR1 work together to conduct cellular signaling, their function or lack of it do not necessarily exert the exact same effect. Depending on the cellular source of IFN β production, versus cells actively expressing IFNAR1, there might be differences in outcome upon autocrine and/or paracrine functions. In support, neuron-derived IFN β , but not astrocytic or microglial-derived IFN β , can exclusively convert encephalitogenic T cells into regulatory T cells in a neuroinflammatory context by autocrine regulation of neuronal PD-L1 expression and negative PD-1 dependent T cell suppression [41] and glioblastoma control [104]. Additionally, residual IFN α -IFNAR signaling in *Ifnb*^{-/-} mice could potentially exert functions distinct from IFN β -IFNAR [105], particularly in astrocytes versus neurons compared to complete lack of signaling or differential signaling in *Ifnar1*^{-/-} brain, thus leading to different outcome.

Though cognitive deficiencies were previously reported in young mice lacking astrocytic IFNAR1 (1–1.5-month-old) GFAP^{Cre};*Ifnar1*^{+/fl} mice compared to Cre-negative (*Ifnar1*^{fl/fl}) mice on the Morris Water Maze [14], here we did not observe cognitive deficits at any age between 3 and 12-month-old GFAP^{Cre};*Ifnar1*^{+/fl} and GFAP^{Cre} controls on the Barnes maze, which could be due to differences in age, test conditions, and the use of Cre+ in our study versus Cre- mice as controls in the previous report [14]. Furthermore, though cell-specific IFNAR1 loss was confirmed in the conditional strains both here and previously [87], more recent literature have reported some limitations in usage of the GFAP^{Cre} lines which are associated either with increased toxicity of some Cre lines or lack of efficient gene editing because of lower expression of GFAP in a subtype of astrocytes during adulthood in striatum [106, 107], as well as their potential to affect distinct neuronal populations [83, 84]. A less pronounced cognitive deficit observed in homozygous versus heterozygous Syn1^{Cre} animals may indicate activation of compensatory signaling in astrocytes or other supportive brain cell types. Nevertheless, the delayed onset of behavior deficits in either cell-specific knockout strain indicates a synergistically detrimental effect of IFNAR1 loss in both neurons and astrocytes that results in PDD-like neuropathology and behavioral phenotypes. Further study of brain cell-specific roles of IFN β -IFNAR signaling will prove essential in understanding neurodegenerative

pathophysiology and identifying more precise therapeutic targets.

Conclusions

Together, these findings provide molecular insight into cell-specific roles of IFNAR1 in brain homeostasis, loss of which results in alterations of genes and proteins indicative of lack of neuronal immunomodulation, neuroinflammation, defective mitochondria and mitophagy, and dysregulated brain energy metabolism prior to the development of PDD-like neurodegeneration, proteinopathy, and behavior abnormalities. Importantly, neuron-specific IFNAR1 loss alone induced the development of PDD-like neurodegeneration and behavioral abnormalities, highlighting the importance of functional neuronal IFNAR1 in brain homeostasis. These results also suggest a significant role for astrocytic IFNAR1 in neuronal support and modulation of neuropsychiatric-like outcomes. As dysfunctional IFN β -IFNAR signaling has been reported to be associated with human sporadic PD and its progression to PDD [10], results from this study suggest that concerted and targeted repair of dysfunctional neuronal and astrocytic IFNAR1 may be a promising therapeutic strategy in mitigating different aspects of disease associated with dementia development, particularly in PDD.

Supplementary Information

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Additional file1 **Supplementary Fig. 1.** Additional snRNA-seq analysis supplementing Fig. 2. Uniform manifold projection showing complete annotations of all cell types and sub-types defining the broad cell classes used for DE analysis. Unbiased hierarchical clustering of differentially expressed genes among all 5 cortical cell classes isolated from 1.5-month-old Wt and *Ifnar1*^{-/-} mice showing similarities between samples. GSEA pathway enrichment scores for pathways relating to interferon signaling, inflammation, and cytokine signaling in Glutamatergic and GABAergic cell classes. NES = Normalized Enrichment Score. Relative mRNA expression of IFNAR1 and ISG15 expression in the cortex of 3-month-old Wt, *Ifnb*^{-/-}, and *Ifnar1*^{-/-} mice as log2FC expression standardized to two housekeeping genes and normalized to Wt. **P* < 0.05 by two-way ANOVA and Tukey's post hoc test. Quantification of parent frequency of ISG15-PE single cells in NF200-FITC neuronal, GLAST-APC astrocyte, and 'All' single cell populations analyzed by flow cytometry in 3-month-old Wt and *Ifnar1*^{-/-} cortex, normalized to Wt. **P* < 0.05 by two-way ANOVA and Bonferroni's post hoc test. GSEA pathway tables showing top 15 positively and negatively enriched terms for microglia and oligodendrocytes. Significant neurodegenerative disease pathways are highlighted in bold text.

Additional file2 **Supplementary Fig. 2.** Dysregulated GSEA pathways highlighting Parkinson's Disease and mitochondrial metabolism pathways in *Ifnar1*^{-/-} neuronal sub-types and astrocytes. Top 20 upregulated KEGG pathways cortical glutamatergic and GABAergic neuronal subtypes annotated in the snRNA-seq dataset shown in Supplementary Fig. 1B, highlighting 'Parkinsons disease', 'oxidative phosphorylation', and other neurodegenerative disease pathways such as 'Alzheimers disease' and 'Huntingtons disease'. GSEA pathway enrichment scores for pathways relating to energy metabolism and mitochondrial function in cortical *Ifnar1*^{-/-} vs Wt glutamatergic and GABAergic neurons. NES = normalized enrichment score. Heatmaps showing differential gene expression

within the major cortical cell classes of genes within the Reactome pathways 'Trafficking of GluR2 containing AMPA receptors' and 'Highly calcium permeable nicotinic acetylcholine receptors' identified as commonly affected in *Ifnar1*^{-/-} glutamatergic and GABAergic neuronal classes shown in Fig. 4L and M, respectively. GSEA pathway enrichment scores for pathways relating to neurotransmission and inflammation in cortical *Ifnar1*^{-/-} vs Wt astrocytes. NES = normalized enrichment score showing differential gene expression among the major cortical cell classes of genes within the Reactome pathway 'Pyruvate metabolism' identified in *Ifnar1*^{-/-} astrocytes shown in Fig. 2N.

Additional file3 **Supplementary Fig. 3.** Additional LC-MS/MS proteomics analysis supplementing Fig. 3. Quality control for proteomics analysis. Total number of proteins per sample. Coefficients of variation between samples. Protein intensities, showing similarity between samples.

Additional file4 **Supplementary Fig. 4.** Additional metabolic isotope labelling data supplementing Fig. 5. Cortex and hippocampal slices from 3-month-old Wt and *Ifnar1*^{-/-} mice were incubated with [U-¹³C]glutamine or [U-¹³C]glutamate, which primarily reflect neuronal and astrocytic metabolism, respectively. **P* < 0.05 by *t*-test.

Additional file5 **Supplementary Fig. 5.** Additional neuropathological and behavior data in *Ifnar1*^{-/-} supplementing Fig. 6. Representative images and quantification of NeuN+ cells in cortex, hippocampus, and olfactory bulb of 6-month-old Wt, *Ifnb*^{-/-}, and *Ifnar1*^{-/-} mice as %Wt. **P* < 0.05 by one-way ANOVA and Dunnett's post hoc correction test. Representative images and quantification of % loss of TH+ cells in the substantia nigra pars compacta versus ventral tegmental area of 3-month-old *Ifnar1*^{-/-} mice compared to Wt mice. ****P* < 0.01 by *t*-test. Representative immunoblots and quantifications of relative protein levels of total tau, phosphorylated tau at threonine 205, alpha-synuclein, and phosphorylated α-syn at serine 129 in brain lysates from 1.5- and 12-month-old Wt vs *Ifnar1*^{-/-} mice, normalized to vinculin. **P* < 0.05 by *t*-test. MWM results for 3-month-old and 6-month-old Wt, *Ifnb*^{-/-}, and *Ifnar1*^{-/-} mice. Data are mean ± SEM, *n* = 13–20 per genotype. MWM training performances. ^{§§}*P* < 0.01 for genotype effect by two-way ANOVA. Genotype effect vs Wt **P* < 0.05 and ****P* < 0.01 by Tukey's post hoc test. Time spent in platform zone as % duration of test session. **P* < 0.05 and ****P* < 0.01 by one-way ANOVA and Tukey's post hoc correction test. Number of platform zone crossings. **P* < 0.05 by one-way ANOVA and Tukey's post hoc correction test. Distance and Velocity measurements for 3 month MWM, 6 month MWM, and 12 month Barnes maze cohorts. ****P* < 0.001 by one-way ANOVA and Tukey's post hoc correction test. Additional rearing activity measurements of 3-month-old Wt and *Ifnar1*^{-/-} mice, including latency to begin rearing and cumulative rearing duration. Distance and velocity of 3-month-old Wt, *Ifnb*^{-/-}, and *Ifnar1*^{-/-} mice on the OF, corresponding to Fig. 6, Q. Distance and velocity of 3-month-old mice on the EPM, corresponding to Fig. 6R. EPM Open arm duration and side edge investigations of 6-month-old mice.

Additional file 6 **Supplementary Fig. 6.** Additional data on conditional *Ifnar1*^{-/-} strains supplementing Fig. 7. Rotarod and Barnes Maze performances of 3-month-old neuronal Syn1^{Cre}; *Ifnar1*^{fl/fl} mice and astrocytic GFAP^{Cre}; *Ifnar1*^{fl/fl} mice. Nociception to heat-induced pain as %Wt performance of 3-month-old Syn1^{Cre}; *Ifnar1*^{fl/fl} and GFAP^{Cre}; *Ifnar1*^{fl/fl} mice. EPM performances of 3-month-old Syn1^{Cre}; *Ifnar1*^{fl/fl} and GFAP^{Cre}; *Ifnar1*^{fl/fl} mice and 12-month-old Syn1^{Cre}; *Ifnar1*^{fl/fl} and GFAP^{Cre}; *Ifnar1*^{fl/fl} mice. **P* < 0.05 by one-way ANOVA and Tukey's post hoc correction test. Data in all graphs are mean ± SEM.

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Author contributions

E.B.V., Z.Z., M.C.-S., J.L., J.V.A., F.L.Q., E.W.W., A.M., G.J.-D., L.R.-P., E.T., O.K., D.L., T.G., T.B., M.P., B.I.A., M.M., and N.H.S. generated data for the manuscript. E.B.V. collected brain tissues for immunoblots, snRNA-seq, and LC-MS/MS. Z.Z. performed neuronal and astrocytic primary cultures, qPCR, FC, GSEA of transcriptomic data, and combined visualization of transcriptomic and proteomic datasets. M.C.-S. performed neuronal and astrocytic cultures, N2a transfection experiments, immunoblots, and analysis. J.L. conducted snRNA-seq experiments, transcriptomic data processing, analysis, and visualization. F.L.Q., M.M., and N.H.S. conducted LC-MS/MS experiments, proteomic data processing, analysis, and visualization. J.V.A., E.W.W., and B.I.A. performed acute brain slice incubations, GC-MS, HPLC, and analysis. E.B.V. and A.M. conducted behavior experiments and analysis. L.R.-P. designed and generated *ΔIfnar1* N2a cells and conducted immunoblots and analysis. E.T., O.K., and D.L. conducted electron microscopy experiments. E.B.V., A.M., G.J.-D., T.G., T.B., and M.P. conducted immunohistochemical experiments and analysis. E.B.V. and S.I.-N. conceived the project and wrote the manuscript. All authors reviewed and edited the manuscript.

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Availability of data and materials

The snRNA-seq data has been deposited in the Gene Expression Omnibus (GEO) with the identifier GSE213671 [(https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE213671) (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE213671)]. MS-based proteomics data is deposited to the ProteomeXchange Consortium (http://proteomecentral.proteomexchange.org) via the PRIDE partner repository [108] with the identifier PXD037113 [(http://www.ebi.ac.uk/pride/archive/projects/PXD037113) (http://www.ebi.ac.uk/pride/archive/projects/PXD037113)]. MS-based proteomics data is deposited to the ProteomeXchange Consortium (http://proteomecentral.proteomexchange.org) via the PRIDE partner repository [108] with the identifier PXD037113 (http://www.ebi.ac.uk/pride/archive/projects/PXD037113).

Declarations

Ethics approval and consent to participate

All animal experiments were performed in accordance with Danish ethical standards in compliance with Directive 2010/63/EU, under ethical permission licenses 2013-15-2934-00807 and 2018-15-0201-01572, and are in adherence with ARRIVE guidelines (v2.0). As the study does not include a clinical trial, consent to participate is not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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