

mRNA vaccine platform demonstrates potent immunotherapeutic targeting of amyloid- β and tau pathologies in Alzheimer's disease

Hui Wu

topwuhui@jlu.edu.cn

Jilin University

Yunyu Hou

Jilin University

Xuejian Feng

Jilin University

Xue Xia

National Engineering Laboratory for AIDS Vaccine, School of Life Sciences, Jilin University

Xianghui Yu

Jilin University

Wei Kong

Jilin University

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Abstract

Current Alzheimer's disease (AD) immunotherapeutics face efficacy and safety limitations with antibody-based approaches. While active immunization targeting A β and Tau pathologies represents a promising therapeutic avenue, existing peptide/protein vaccines fail to sustain adequate antibody titers or durable immune responses. mRNA vaccines offer a promising alternative, combining sustained immunogenicity, flexible antigen design, and favorable safety profiles. We developed an AD mRNA vaccine platform generating A β - and Tau-targeting candidates. These engineered vaccines induced robust, persistent antigen-specific antibodies in AD models without harmful T-cell responses or cerebrovascular complications. They mediated broad clearance of pathogenic aggregates across brain regions, resolved chronic neuroinflammation, and halted neurodegeneration-driven cognitive decline. Mechanistically, vaccine-induced antibodies crossed the blood-brain barrier, directly neutralized pathological proteins, enhanced microglial phagocytic activity, and modulated neuroinflammatory pathways, collectively restoring neuronal homeostasis. This mRNA platform overcomes key constraints of current clinical candidates by achieving potent pathological clearance while circumventing treatment-limiting toxicities, positioning mRNA-based active immunization as a transformative therapeutic strategy with near-term translational potential for AD.

Introduction

Alzheimer's disease (AD), the most prevalent neurodegenerative disorder, is the leading cause of dementia worldwide. With increasing global life expectancy, AD has emerged as a critical public health priority among aging populations, now ranking alongside cardiovascular disease and cancer in global disease burden. Clinically characterized by progressive, irreversible cognitive decline, AD neuropathology features extracellular amyloid β (A β) plaques, intracellular neurofibrillary tangles

composed of hyperphosphorylated tau (pTau), and widespread neuronal loss¹. Despite extensive research, the pathogenic mechanisms of AD remain incompletely understood. Dominant theories implicate cholinergic dysfunction, dysregulation of the amyloid cascade, and tau pathology². Current treatments, primarily cholinesterase inhibitors based on the cholinergic hypothesis, offer only symptomatic relief without modifying disease progression³. Immunotherapies targeting A β and tau have emerged as promising disease-modifying approaches, particularly passive immunization involving repeated administration of monoclonal antibodies. FDA-approved anti-A β therapies, including aducanumab, lecanemab, and donanemab, demonstrate clinical potential but carry significant safety concerns, notably amyloid-related imaging abnormalities (ARIA) encompassing cerebral edema and microhemorrhages, alongside high treatment costs⁴. These limitations underscore the urgent need for safer, more efficacious therapeutic interventions.

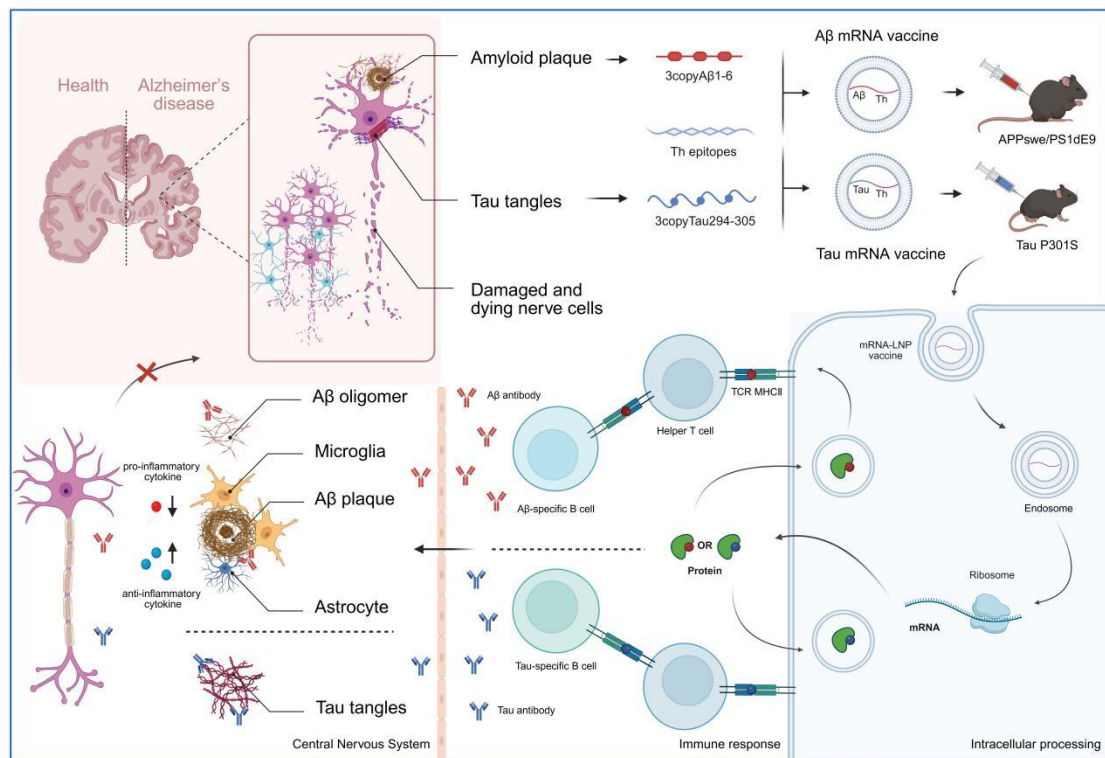
Active immunization represents a compelling therapeutic approach, capable of inducing durable antibody responses with fewer dosing requirements than passive strategies. Contemporary A β -targeting active immunotherapies primarily utilize peptide or recombinant protein platforms, although clinical translation has been hampered by inadequate efficacy or safety limitations. For example, the first-generation AN1792 A β 42 vaccine was discontinued due to T cell-driven inflammatory complications⁵. Subsequent peptide and protein vaccines, such as CAD106 and UB-311, targeted the N-terminal region of A β 42 to avoid A β -specific T cell activation^{6,7}. While these demonstrated improved safety profiles, their clinical benefits remained limited, potentially attributable to suboptimal immunogenicity. Currently, only two tau-targeting peptide vaccines are undergoing clinical evaluation. Results from the pTau vaccine ACI-35 are still pending, while the AADvac1 vaccine, designed against the pathogenic tau interaction site, elicits high-titer anti-tau antibodies in AD patients⁸. Nevertheless, despite AADvac1's ability to reduce tau pathology, it has failed to demonstrate meaningful cognitive benefits, highlighting the

57 persistent challenge of achieving robust, sustained immunity through conventional
58 vaccine platforms.

59 mRNA vaccines represent a next-generation nucleic acid platform that utilizes
60 lipid nanoparticles (LNPs) to deliver mRNA encoding antigens into host cells⁹. This
61 facilitates in situ antigen expression and subsequent immune activation¹⁰. The
62 platform elicits potent immune responses at low doses, driven by germinal center B
63 cell activation and the development of long-lived plasma cells for sustained antibody
64 production, coupled with rapid and flexible antigen design¹¹. The established efficacy
65 of mRNA vaccines against infectious diseases, including COVID-19, combined with
66 their expanding oncology applications, underscores their broad therapeutic
67 versatility¹²⁻¹⁴. Recent preclinical studies have begun exploring mRNA vaccine
68 applications for AD. Notably, Wongsodirdjo et al. developed mRNA encoding RNJ1
69 antibodies targeting intracellular tau aggregates, while Agadjanyan's group engineered
70 an A β -specific mRNA vaccine (AV-1959LR). This vaccine induced robust antibody
71 responses in wild-type mice and non-human primates, confirming the platform's
72 immunogenic potential for AD^{15,16}. Nevertheless, the therapeutic efficacy and
73 prospects for clinical translation of mRNA vaccines in AD models remain to be fully
74 characterized.

75 Leveraging mRNA's potent immunogenicity through optimized antigen design,
76 we established an AD mRNA vaccine platform to address current immunotherapy
77 shortcomings and developed two distinct candidates: an A β mRNA vaccine (AmV)
78 and a tau mRNA vaccine (TmV). To preferentially stimulate humoral over cellular
79 immunity, we engineered AmV by incorporating B cell epitopes fused with
80 heterologous T helper motifs. This mRNA was then formulated into lipid
81 nanoparticles (LNPs) via plasmid construction, in vitro transcription, and LNP
82 encapsulation. Correspondingly, TmV encodes the tau fibril core domain fused to T
83 helper epitopes to enhance antigen specificity and immunogenicity. Both vaccines
84 demonstrate potent immunogenicity and favorable safety profiles, effectively
85 reducing amyloid deposits or tau pathology in AD models while improving cognitive

86 function. Mechanistically, AmV promotes antibody-mediated clearance through
 87 central and peripheral pathways, enhancing microglial phagocytosis of A β plaques
 88 and ameliorating neuroinflammation. Similarly, TmV-induced antibodies cross the
 89 blood-brain barrier, directly neutralize pathological tau species, inhibit glial activation,
 90 and reprogram neuroinflammatory signaling to preserve neuronal integrity. These
 91 findings support the therapeutic promise of AmV and TmV as mRNA-based
 92 immunotherapies for Alzheimer's disease.



93
 94 **Fig. 1: AD mRNA vaccines prevent neurodegeneration via potent immune**
 95 **response.**

96 This study developed an mRNA vaccine platform for Alzheimer's disease (AD) to
 97 overcome the limitations of traditional immunization. In AD models, A β - and
 98 Tau-targeting vaccines generated durable, high-affinity antibodies that crossed the
 99 blood-brain barrier and selectively targeted neurotoxic aggregates. A β mRNA
 100 immunization significantly reduced plaque burden and soluble A β 42 levels, while Tau
 101 mRNA immunization markedly diminished PHF-positive areas and pathological Tau
 102 species. Concurrently, excessive glial activation and neuroinflammatory signaling

were suppressed following mRNA vaccination. Significant cognitive and motor improvements were observed in behavioral tests, with efficacy patterns varying by sex. Critically, both vaccines demonstrated exceptional safety, promoting CD4⁺ over CD8⁺ T-cell responses without inducing cerebrovascular complications, establishing mRNA nanomedicine as a transformative immunotherapy for neurodegeneration. Created with BioRender.com.

Results

Design, development, and validation of A β - and Tau-targeting mRNA vaccines

To precisely target A β and pathological tau, we engineered vaccine antigens featuring three tandem repeats of either the A β 1-6 B-cell epitope or the tau fibril core domain (Tau294-305) (Fig. 2a). To maximize population coverage, we incorporated heterologous T-helper (Th) epitopes, including a universal DR epitope, three tetanus toxoid-derived sequences, and two norovirus-derived motifs, thereby broadening immune activation and reinforcing antigen-specific protection (Table S1). A tissue plasminogen activator (tPA) signal peptide was fused to each antigen to bias immunity toward antibody production rather than cytotoxic responses by directing extracellular secretion (Fig. 2b). These optimized sequences were cloned into plasmid vectors for *in vitro* transcription, generating A β - and tau-specific mRNAs (Fig. 2, b to d; Fig. S1, a to c). Transient transfection of HEK-293T cells demonstrated robust antigen expression, with Western blotting confirming ~20 kDa bands corresponding to predicted antigen sizes and immunofluorescence visualizing intracellular localization (Fig. 2, c and d; Fig. S1e). Critically, ELISA quantification of culture supernatants validated efficient antigen secretion, confirming the functional efficacy of the antigen design (Fig. 2, c and d).

Following transcription, mRNAs were complexed with lipid components to achieve high-efficiency encapsulation into lipid nanoparticles (LNPs), leveraging their dual roles in delivery and adjuvanticity for robust, durable immunity. Dynamic light scattering (DLS) analysis revealed that A β mRNA-LNPs exhibited a hydrodynamic diameter of 182.2 ± 0.551 nm, a polydispersity index (PDI) of 0.158,

132 and a zeta potential of -2.54 mV, with tau mRNA-LNPs displaying comparable
133 physicochemical properties (Fig. 2, e and g). Given the critical importance of LNP
134 stability for cold chain logistics and global deployment, we assessed mRNA-LNP
135 integrity in phosphate-buffered saline (PBS) at 4°C over 30 days and observed no
136 appreciable change in particle size, confirming excellent colloidal stability under
137 refrigeration (Fig. S1d). Transmission electron microscopy (TEM) further validated
138 structural integrity, showing uniform spherical morphology for both formulations (Fig.
139 2f). Collectively, these results establish the successful generation of structurally stable
140 Aβ- and tau-specific mRNA-LNP vaccines with favorable physicochemical
141 characteristics and low-temperature storage stability.

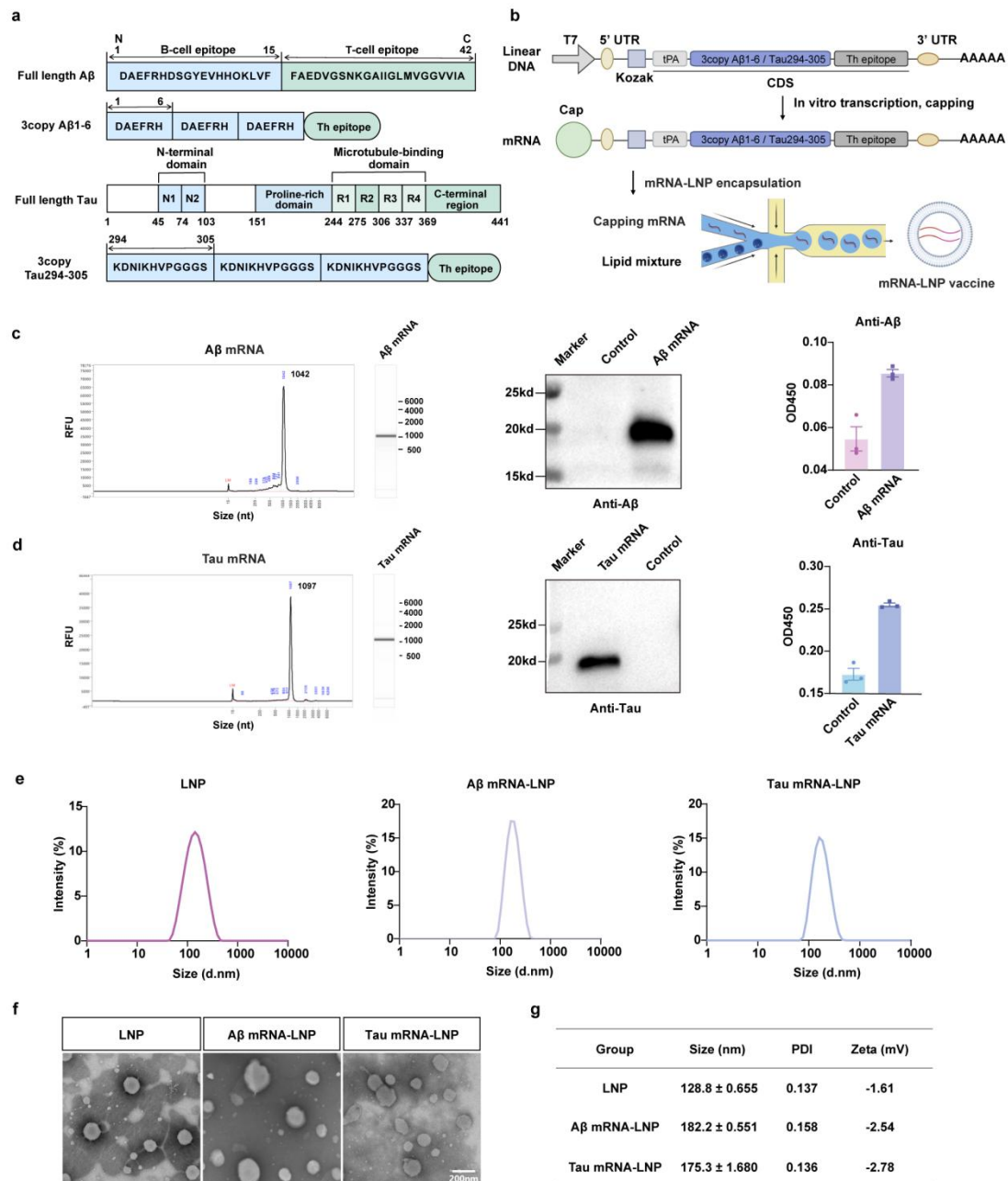


Fig. 2: Design, preparation, and characterization of Aβ- and Tau-targeting mRNA vaccine.

a Epitope selection and vaccine design schematic. The Aβ mRNA vaccine incorporates the Aβ1-6 B cell epitope, while the Tau mRNA vaccine targets the Tau294-305 epitope. Both constructs contain three copies of their respective epitopes fused to tandem T helper (Th) epitopes. **b** mRNA-LNP vaccine preparation workflow. The linearized plasmid (coding strand) comprises a T7 promoter, 5'/3' UTRs, Kozak sequence, coding sequence (CDS), and poly (A) tail. Capped, polyadenylated mRNA

is generated by in vitro transcription and capping. This mRNA is encapsulated with mixed lipid formulations via microfluidic technology to form mRNA-LNP vaccines. **c-d** Analysis of A β and Tau mRNA via capillary electrophoresis and validation of mRNA-encoded antigen expression. Untransfected cells were used as the control. ELISA data are presented as the means $\pm$ SD (n = 3). **e** Representative intensity-size graph of nanoparticles at immunization concentrations measured by dynamic light-scattering method. **f** Transmission electron microscopy (TEM) images of mRNA-LNP (scale bars, 200 nm). **g** Intensity-size, PDI, and zeta potential of mRNA-LNP. Data are presented as the means $\pm$ SD (n = 3).

A β - and tau-mRNA vaccines elicit potent and sustained antibody responses in wild-type mice

We first evaluated the immunogenicity of A β and tau mRNA vaccines in wild-type C57BL/6J mice using mRNA-free lipid nanoparticles as negative controls (Fig. 3a). Both vaccines elicited high-titer, epitope-specific antibodies. Notably, A β mRNA-vaccinated mice achieved serum anti-A β 42 titers of 1:200,000 by two weeks post-second immunization, with titers remaining elevated throughout the observation period (Fig. 3b). Tau mRNA vaccination generated robust but comparatively less persistent antibody responses (Fig. 3c). Both vaccines induced Th2-skewed immunity in wild-type mice, evidenced by IgG1: IgG2a ratios >1 , indicating preferential humoral activation (Fig. 3, b and c). Critically, while humoral responses were markedly enhanced, A β /tau-specific CD8⁺ T cell responses remained undetectable. This was confirmed by ELISpot showing very little IFN- γ production in antigen-stimulated splenocytes (Fig. S2, a and b). Flow cytometry further revealed elevated plasma cell and T follicular helper (Tfh) cell populations following mRNA vaccination, indicating robust humoral immunity activation (Fig. S3). These results demonstrate that AmV and TmV induce potent, sustained antibody responses without activating cytotoxic T cells in wild-type mice.

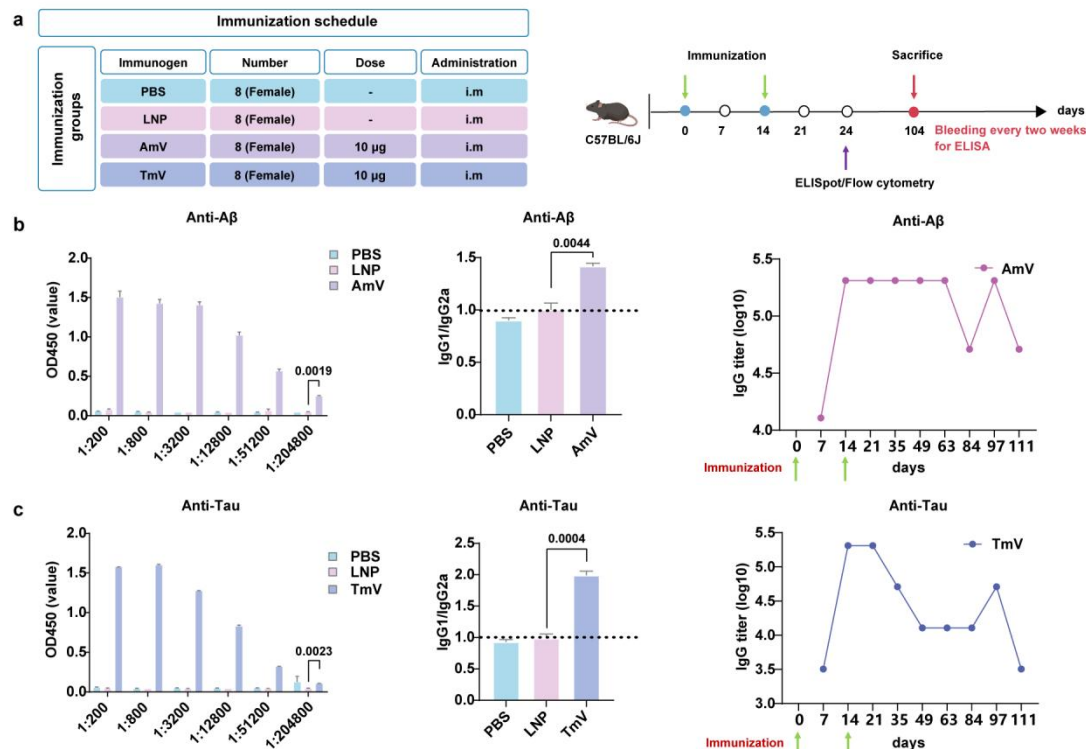


Fig. 3: Humoral immunity characterization of A β and Tau mRNA vaccines in C57BL/6J mice.

a Immunization scheme. Wild-type C57BL/6J mice received mRNA vaccines at green-arrowed timepoints, with immunological assessments including ELISpot and flow cytometry at purple arrows, and terminal endpoints at red arrows. **b** A β -specific antibody responses. Serum antibody concentrations 2 weeks post-second immunization, isotype distribution, and longitudinal titer persistence profiles. **c** Tau-specific antibody responses. Serum antibody levels 2 weeks after the 2nd immunization, isotype profiling, and sustained titer kinetics. Data represent mean $\pm$ SD (n = 3).

A β mRNA vaccine induces robust and durable antibody responses in APP/PS1 transgenic mice

To systematically evaluate AmV's therapeutic efficacy in AD, we conducted a vehicle-controlled immunization study in 3-month-old APP/PS1 transgenic mice, a well-established model exhibiting progressive A β pathology and cognitive decline. Mice received AmV or LNP vehicle according to a predefined immunization schedule (Fig. 4a). AmV induced rapid seroconversion, with serum anti-A β 42 IgG

concentrations reaching 200 µg/mL after the second dose and peaking at 600 µg/mL after booster administration (Fig. 4, b and c). Longitudinal analysis confirmed sustained antibody levels exceeding 200 µg/mL for at least two months in vaccinated cohorts, demonstrating durable therapeutic antibody concentrations essential for amyloid clearance. Furthermore, antibody isotyping revealed a Th2-biased humoral response consistent with wild-type observations (Fig. S4). Flow cytometry showed substantial germinal center (GC) B cell expansion in AmV-vaccinated mice, with concomitant increases in plasma cells and memory B cells (Fig. S5, a to c). Notably, Tfh cells were also elevated in draining lymph nodes, indicating enhanced GC-Tfh crosstalk that likely underpins the platform's superior immunogenicity (Fig. S5d). Collectively, these data demonstrate that AmV effectively activates B cell immunity and elicits durable, high-titer Aβ-specific antibodies in APP/PS1 mice.

Aβ mRNA vaccine mitigates cognitive deficits in APP/PS1 mice with enhanced efficacy in males

We next evaluated Aβ mRNA vaccine (AmV) efficacy against cognitive deficits through comprehensive behavioral testing in APP/PS1 mice. Among the tests administered, results from the Nest building test showed that vaccinated mice exhibited enhanced nesting performance, a hippocampus-dependent task indicative of Alzheimer's disease-related functional impairment, with increased material usage and higher nesting scores versus controls (Fig. 4d). Notably, vaccinated males achieved nest scores comparable to wild-type mice, while females showed only modest improvements, suggesting preferential mitigation of hippocampal injury in male APP/PS1 mice by AmV.

In Novel object recognition (NOR) test, AmV-treated mice exhibited enhanced exploration of the novel object, indicating preserved hippocampal-dependent short-term memory (Fig. 4e). This effect was more pronounced in males than females, reinforcing the gender-specific cognitive benefit. During Morris Water Maze (MWM) assessment of long-term spatial memory, vaccinated mice exhibited a 29.86% reduction in escape latency by day four versus controls, reflecting enhanced spatial

memory retention (Fig. 4f). Probe trials revealed AmV-treated mice spent more time in target quadrants than controls, without observed gender differences (Fig. 4f). Concurrent open field testing detected no intergroup differences in center zone occupancy or distance traveled, confirming cognitive improvements were independent of anxiety reduction or locomotor changes (Fig. S6). Taken together, these data confirm that AmV ameliorates multiple cognitive abilities in APP/PS1 mice with heightened efficacy in males.

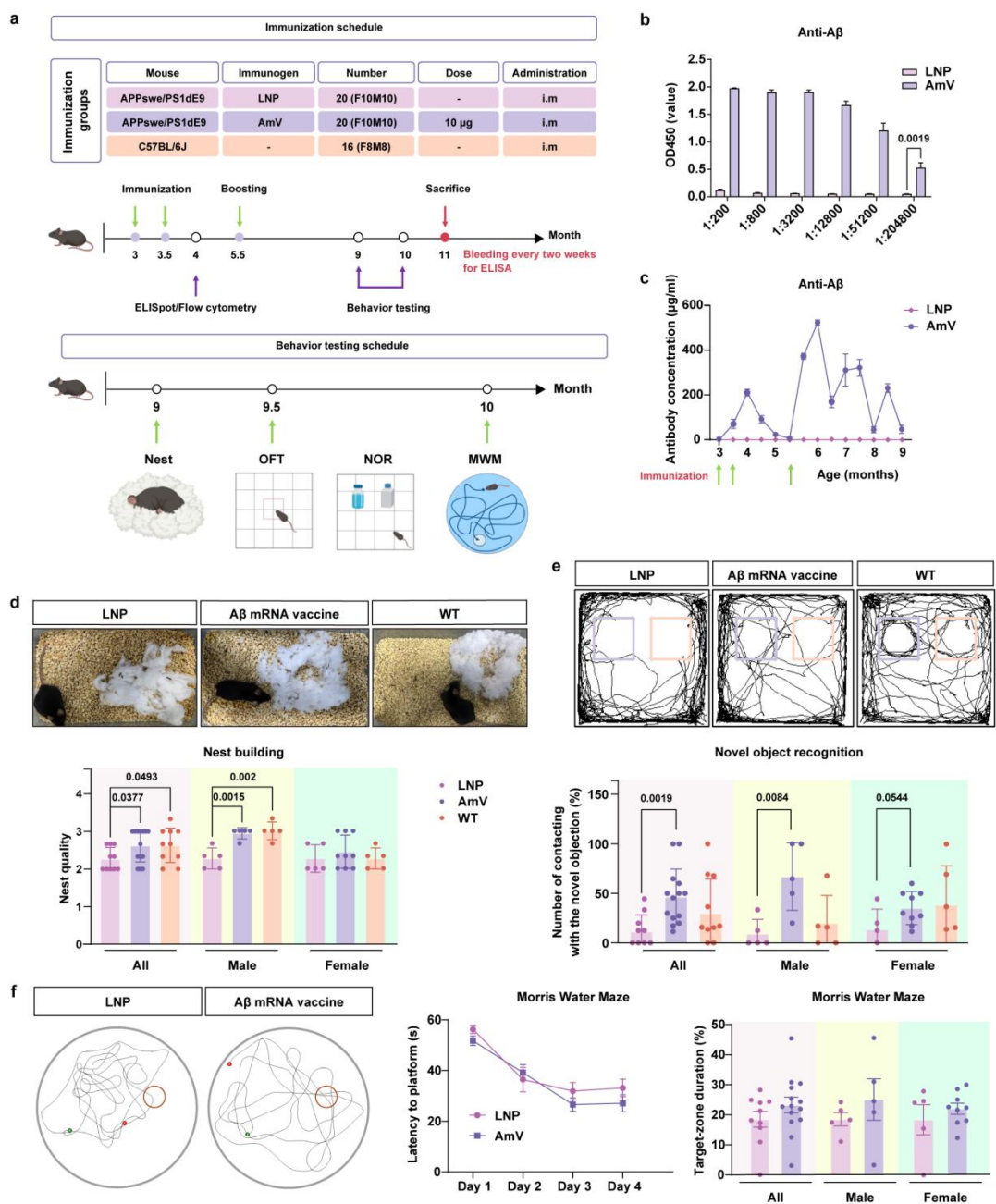


Fig. 4: Immune response and therapeutic effect of A β mRNA vaccine in APP/PS1 transgenic mice.

a Immunization and behavior testing schedule for APP/PS1 mice. Green arrows indicate mRNA vaccination time points. Purple arrows denote ELISpot and flow cytometry analyses. Double-headed purple arrows indicate the behavioral testing windows. Red arrows mark euthanasia endpoints. Serum was collected biweekly post-immunization for antibody quantification. Behavioral assessments include the Nest Building Test, Open Field Test (OFT), Novel Object Recognition (NOR), and Morris Water Maze (MWM). **b-c** A β -specific antibody kinetics. **(b)** Serum antibody concentrations 2 weeks post-boost. **(c)** Longitudinal antibody persistence profiles. Both quantified by ELISA using the 6E10 reference standard. ($n = 3$) **d** Representative nest construction images and quantitative scores. Results are shown for all mice combined, the male cohort, and the female cohort. Data are presented as means $\pm$ SD ($n=9, 10, 14$). **e** Representative novel object recognition exploration patterns and quantification of novel object contacts. Purple squares denote novel object zones. Orange squares denote familiar object zones. Data are presented as means $\pm$ SD ($n=9, 10, 14$). **f** Representative swim paths, quantification of escape latency during training, and target quadrant occupancy during the probe test. Red circles denote hidden platform locations. Data are presented as means $\pm$ SD ($n=9, 10, 14$). Statistical significance was calculated via unpaired t test (**b**) and one-way analysis of variance (ANOVA) (**d, e**) in GraphPad Prism.

A β mRNA vaccine demonstrates potent clearance of diverse A β conformers in APP/PS1 mice.

To assess the impact of AmV on A β pathology in APP/PS1 mice, we quantified amyloid plaque burden using integrated immunohistochemistry (IHC), western blotting, and ELISA analyses (Fig. 5a). IHC revealed that AmV remarkably reduced amyloid deposition, decreasing hippocampal plaque coverage by 53.39% and cortical coverage by 55.19% compared to controls, with comparable efficacy observed in both male and female mice (Fig. 5, b and e; Fig. S7a). Thioflavin-S staining confirmed a

54.8% reduction in dense-core plaques after vaccination (Fig. 5c). A β 42 analysis showed vaccinated mice exhibited a 40.92% decrease in soluble hippocampal A β 42 and a 19.43% reduction in insoluble hippocampal A β 42 (Fig. 5, d and e; Fig. S7b). Cortical soluble and insoluble A β 42 levels were similarly reduced following AmV immunization (Fig. 5, d and e; Fig. S7b). These results demonstrate potent efficacy of AmV in reducing amyloid burden across key AD-relevant brain regions.

A β mRNA vaccine alleviates neuroinflammation and exhibits a robust safety profile in APP/PS1 mice

Beyond pathological protein accumulation, neuroinflammation marked by microglial overactivation, reactive astrogliosis, and elevated proinflammatory cytokines critically contributes to AD progression. Specifically, in AmV-treated APP/PS1 mice, the hippocampal Iba1⁺ area decreased by 39.98% versus controls (Fig. 5f). Western blotting analysis confirmed decreased Iba1 protein levels and concomitant downregulation of the activated microglia marker CD68 in the brain lysates of AmV-treated mice (Fig. S8a). Three-dimensional morphometric analysis revealed increased microglial arbor complexity and enhanced terminal branching following AmV treatment, indicating a transition toward a homeostatic surveillance phenotype (Fig. 5g). These findings demonstrate that AmV remodels microglial activation to mitigate neuroinflammation. Comparatively, GFAP quantification showed no evident differences in astrocyte reactivity, indicating minimal impact on astrogliosis (Fig. S8b).

Furthermore, cytokine profiling revealed comparable brain concentrations of TNF- α , IL-1 β , and TGF- β , along with similar mRNA levels for IL-6, IFN- γ , and IL-1 β between different groups (Fig. 5h, Fig. S8c). Notably, transcriptional analysis showed downregulated level of pro-inflammatory TNF- α and upregulated anti-inflammatory TGF- β in the vaccine group (Fig. 5h). Additionally, vaccinated mice exhibited lower plasma TNF- α and elevated TGF- β compared to controls (Fig. S8d). These findings suggest that A β mRNA vaccination modulates inflammatory cytokine profiles both transcriptionally and translationally, promoting an

291 anti-inflammatory microenvironment without inducing central pro-inflammatory
292 responses. Moreover, immunohistochemical staining indicated increased neuronal
293 numbers in vaccinated mice, suggesting a neuroprotective effect (Fig. 5i). Hence,
294 these analyses establish the A β mRNA vaccine's dual efficacy in reducing A β species
295 and ameliorating neuroinflammation, thereby attenuating neurodegenerative
296 pathology in APP/PS1 mice.

297 Rigorous safety assessment is essential for preclinical AD immunotherapies. We
298 systematically evaluated epitope-specific T cell responses, cerebrovascular integrity,
299 and systemic toxicity in AmV-treated mice. No discernible long-term body weight
300 differences were observed between groups, indicating no adverse effects on growth or
301 general health (Fig. S9a). Crucially, vaccinated mice exhibited minimal A β 42-specific
302 CD8⁺ T cell activity alongside robust CD4⁺ T cell responses to vaccine-encoded Th
303 epitopes (Fig. S9b). Prussian blue staining revealed a 68% reduction in cerebral
304 microhemorrhages versus controls, likely attributable to amyloid clearance mediated
305 by the vaccine (Fig. S9c). Furthermore, histopathological examination confirmed
306 intact organ architecture without evidence of systemic toxicity, comparable to LNP
307 controls (Fig. S9d). Collectively, these results indicate that AmV elicits a favorable
308 immunogenic profile while preventing cerebrovascular or systemic adverse effects.

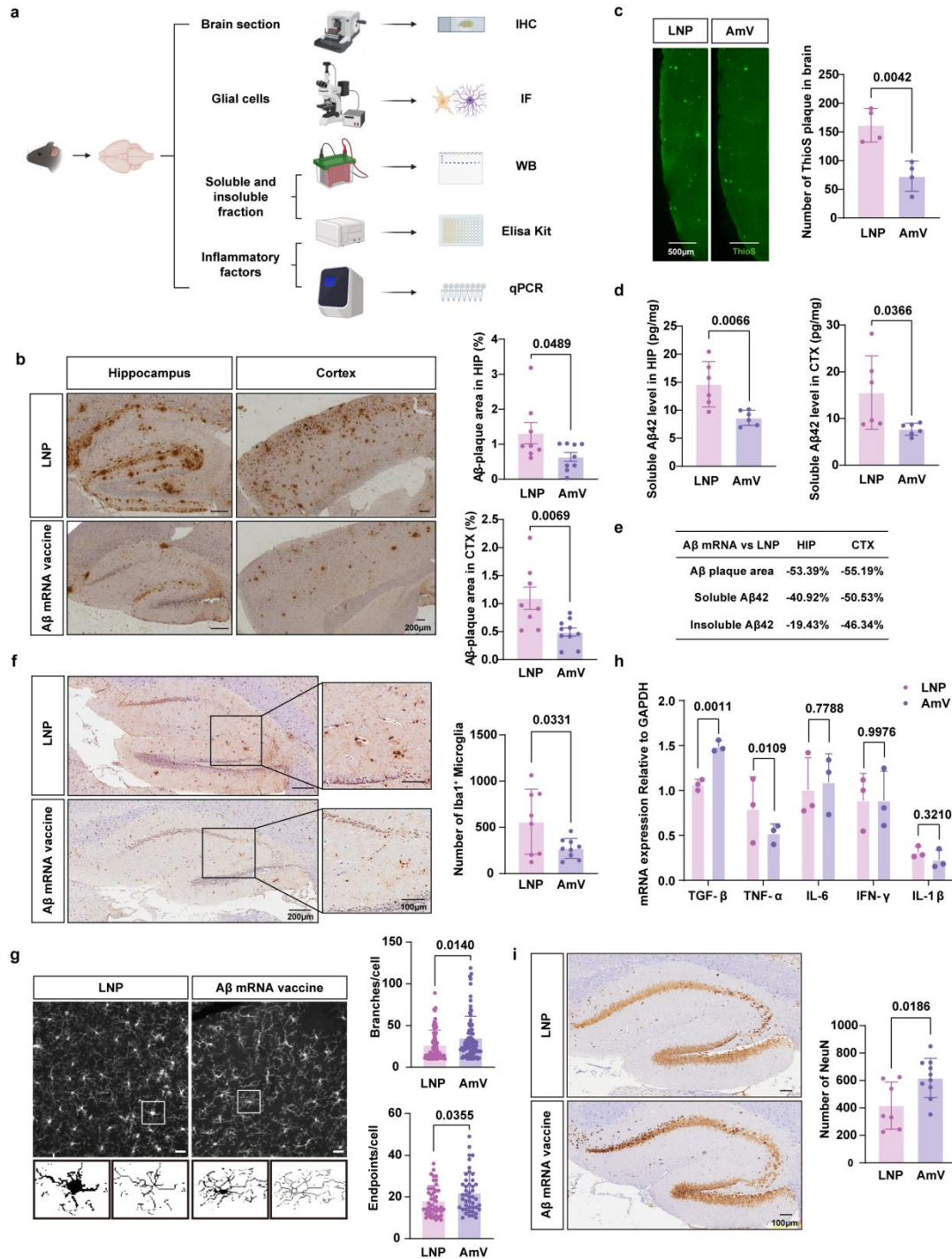


Fig. 5: A β pathology and neuroinflammation in immunized APP/PS1 transgenic mice

a Brain pathological evaluation workflow. Immunohistochemistry (IHC) assessed A β pathology. Immunofluorescence (IF) evaluated glial activation. Western blot and ELISA quantified soluble and insoluble pathological proteins in brain homogenates. Cytokine profiles were analyzed by ELISA and quantitative real-time PCR

(qRT-PCR). Created with BioRender.com. **b** Representative IHC images and quantification of A β plaque-positive area. Data are presented as means $\pm$ SD (Scale bars, 200 μ m, **n = 8, 9**). **c** Thioflavin S (ThioS)-stained plaques in APP/PS1 brains. Data are presented as means $\pm$ SD (Scale bars, 500 μ m, **n = 4**). **d** A β 42 levels in soluble brain homogenates in the hippocampus and cortex regions by ELISA. Data are presented as means $\pm$ SD (**n = 6**). **e** Quantitative A β pathology reduction following AmV intervention. **f** Iba1⁺ microglia distribution and density in the hippocampus. Data are presented as means $\pm$ SD (Scale bars, 100/200 μ m, **n = 8, 9**). **g** Microglial morphometric analysis. Quantitative comparison of soma size and branching complexity in AmV-treated versus LNP-control cohorts. Data are presented as means $\pm$ SD (**n = 40-80**). **h** qRT-PCR analysis of inflammatory factors normalized to GAPDH. Data are presented as means $\pm$ SD (**n = 3**). **i** Assessment of hippocampal NeuN⁺ neurons across different groups. Data are presented as means $\pm$ SD (Scale bars, 100 μ m, **n = 7, 10**). Statistical significance was calculated via unpaired t test (**b-d, f-i**) in GraphPad Prism.

A β mRNA vaccine mediates multifaceted anti-A β mechanisms in APP/PS1 mice

To elucidate the potential mechanism underlying AmV-mediated amelioration of A β pathology, we first quantified brain-tissue A β -specific antibodies, detecting concentrations from 0.314 to 5.343 μ g/mL (average: 1.22 μ g/mL) with approximately 0.23% blood-brain barrier penetration (**Fig. 6, a and b**). Immunohistochemistry confirmed specific antibody binding to A β plaques in the brains of APP/PS1 mice (**Fig. 6, a and c**). Further characterization by dot blot analysis revealed that the induced A β antibodies exhibited low antibody affinity for monomers but high specificity for neurotoxic oligomeric and fibrillar A β species (**Fig. 6d**). Concordantly, serum A β 42 levels substantially decreased post-vaccination versus sustained high levels in LNP controls, indicating peripheral A β clearance facilitated by vaccine-induced antibodies (**Fig. 6, a and e**). Additionally, reduced APP and BACE1 levels suggested suppression of upstream amyloidogenic processing upon the administration of the A β mRNA vaccine (**Fig. 6f**).

Microglia plays the key role in A β clearance via Fc γ receptor-dependent phagocytosis (Fig. 6a). Notably, vaccinated mice exhibited enlarged microglial coverage surrounding plaques with encapsulating morphology, suggesting enhanced phagocytic activity (Fig. 6g). We also observed astrocyte accumulation near plaque-microglia complexes, indicating glial crosstalk during clearance. In vitro, antibodies derived from AmV-immunized mice enhanced BV2 microglial uptake of A β oligomers, demonstrating antibody-mediated phagocytic enhancement (Fig. S10).

We next characterized the transcriptome profiles of hippocampal tissues from AmV-immunized APP/PS1 mice. Differential gene expression analysis revealed a distinct transcriptional signature in AmV-treated mice compared to LNP controls, identifying 365 upregulated and 329 downregulated genes. Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis indicated that the enriched gene profiles corresponded to neuroactive ligand-receptor interactions and ECM-receptor interactions, pathways critical for regulating neuronal signal transduction and synaptic function (Fig. S11a). Gene Ontology (GO) analysis further demonstrated enrichment in transmembrane transporter/ion channel activity, suggesting a potential restoration of neuronal homeostasis following vaccination (Fig. S11b). In all, these results demonstrate that the A β mRNA vaccine mediates anti-amyloid effects through coordinated mechanisms, including enhanced clearance of toxic A β species, augmented microglial phagocytosis, and suppression of amyloid production.

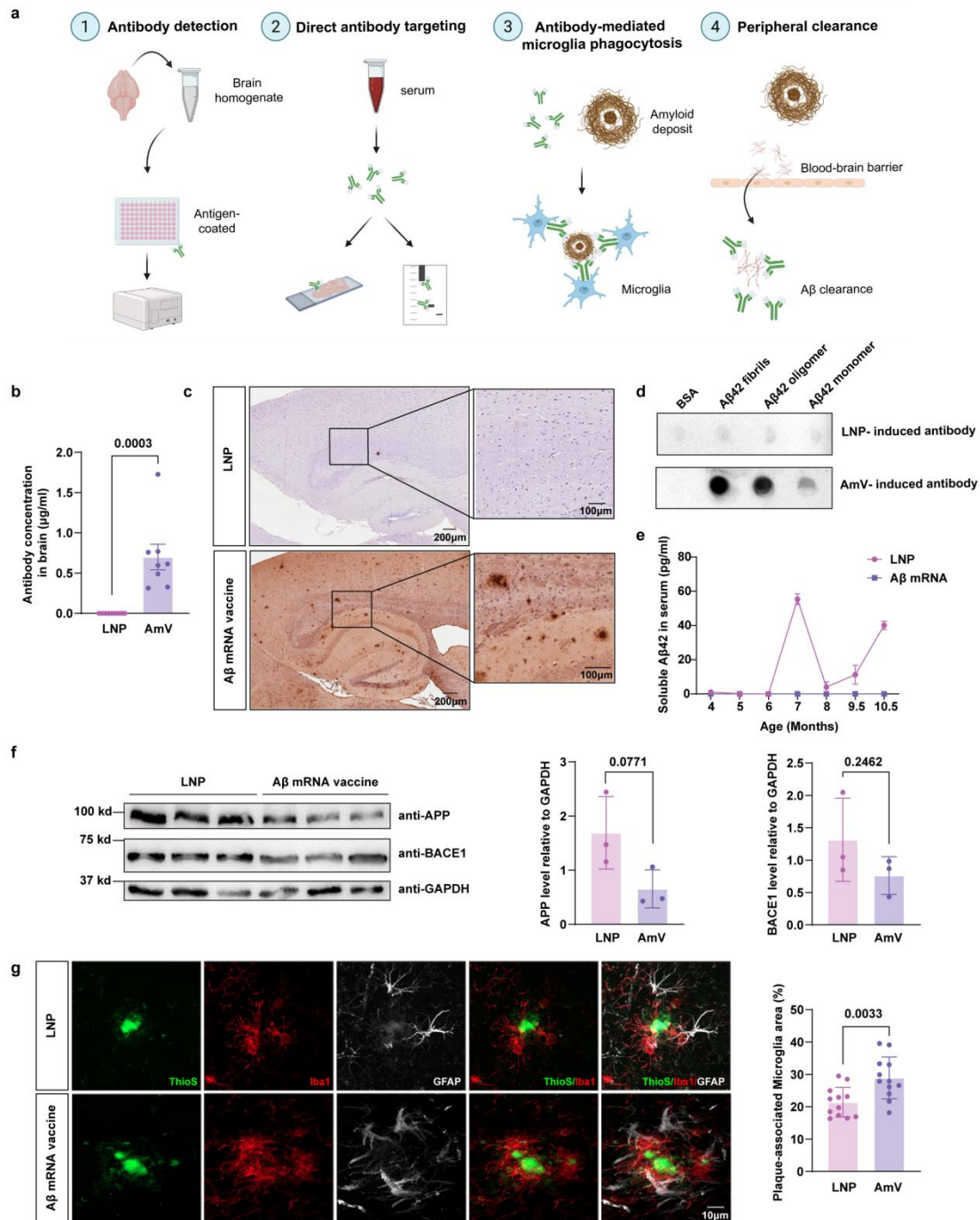


Fig. 6: Multifaceted anti-Aβ actions of Aβ mRNA vaccine in APP/PS1 mice

a Schematic diagram of Aβ clearance mechanisms. These mechanisms include direct antibody-targeted clearance, microglia-mediated phagocytosis, and peripheral clearance. The diagram includes a flowchart showing how ELISA was used to detect antibodies within the brain homogenate of the Aβ mRNA vaccine group. Created with BioRender.com. **b** Antibody concentration against Aβ in brain homogenates of AmV-treated mice. Data are presented as means ± SD (n = 8). **c** IHC staining of

APP/PS1 brain sections using serum-derived antibodies from LNP- or AmV-immunized mice. (Scale bars, 100/200 μ m). **d** Dot blot analysis of serum antibody reactivity against A β 42 monomer, oligomer, and fibrils. **e** Longitudinal plasma A β levels monitored by ELISA. Data are presented as means $\pm$ SD (n = 3). **f** Western blot analysis of APP and BACE1 expression in brain lysates with quantification normalized to GAPDH. Data are presented as means $\pm$ SD (n = 3). **g** Confocal microscopy showing A β plaques, microglia, and astrocytes, along with quantification of microglial plaque engagement. A β deposits are shown by ThioS green staining. Microglia are shown by Iba1 red staining. Astrocytes are shown by GFAP white staining. Data are presented as means $\pm$ SD (Scale bars, 10 μ m, n = 12). Statistical significance was calculated via unpaired t test (**b**, **f**, **g**) in GraphPad Prism.

Tau mRNA vaccine elicits robust anti-Tau immunity in TauP301S transgenic mice

Building on the potent immunogenicity of Tau mRNA vaccine observed in wild-type mice, we evaluated whether the Tau mRNA vaccine would maintain strong immunogenicity in tauopathy models to mediate pathological protein clearance (Fig. 7a). In TauP301S transgenic mice, a single TmV immunization elicited detectable Tau-specific antibodies within 7 days (Fig. 7c). Following booster immunization, endpoint titers reached 1:204,800, demonstrating durable immunogenicity (Fig. 7, b and c). Flow cytometry revealed specific expansion of memory B cells and plasma cells in vaccinated mice compared to LNP controls (Fig. S12, a and b). Critically, plasma cell frequency correlated directly with antibody titers, while memory B cell expansion predicted enhanced antibody responses post-boost. Furthermore, vaccinated mice exhibited elevated frequencies of CD4⁺ T helper cells, indicating coordinated T cell support for humoral immunity (Fig. S12c). Collectively, these data demonstrate that Tau mRNA vaccination effectively engages both B cell and T helper cell compartments, generating sustained, high-titer anti-Tau immunity in a tauopathy model.

Tau mRNA vaccine ameliorates cognitive and motor deficits with sex-dependent therapeutic efficacy in TauP301S mice

To further evaluate the therapeutic effects of the Tau mRNA vaccine on cognition and motor function, we performed behavioral tests in 8-9-month-old TauP301S and wild-type mice (Fig. 7a). Initial assessment using the open field test revealed no evident treatment effects on overall locomotor activity or anxiety-like behavior between vaccinated and control groups. Particularly, sex-stratified analysis indicated marked behavioral differences in female mice (Fig. 7d). Vaccinated females exhibited increased path length and center zone time, suggesting enhanced exploratory drive, whereas no marked differences were observed in males (Fig. 7d, Fig. S13a). Comparatively, the nest building experiment detected no intergroup differences (Fig. S13b).

Consistent with this sex-dependent cognitive pattern, novel object recognition testing demonstrated that vaccinated females displayed increased novel object interaction time and exploratory path length compared to LNP controls (Fig. 7e, Fig. S13c). In contrast, male cohorts showed no treatment-related differences in exploratory behavior (Fig. 7e, Fig. S13c). In addition, we assessed spatial learning and long-term memory using the Morris water maze (MWM) test. During training, vaccinated mice exhibited consistently shorter escape latencies than controls, indicating enhanced spatial learning. Vaccinated mice also demonstrated superior performance in the probe trial, reflecting improved long-term spatial memory retention, with no evident sex differences observed (Fig. 7f, Fig. S13d).

Given the progressive motor deficits characteristic of the TauP301S model, we further assessed neuromuscular function. Stride length analysis at 9 months showed TmV-treated transgenic mice achieved longer strides than LNP controls, approaching wild-type levels and suggesting reversal of muscle atrophy. This improvement was specifically more pronounced in males (Fig. 7g). Hindlimb clamping tests revealed greater extension capability in vaccinated mice versus controls, particularly in males (Fig. 7h). Moreover, grip strength quantification confirmed considerably enhanced

strength in TmV-treated mice, again with male-preferential enhancement (Fig. 7i).
These findings provide compelling evidence for TmV's efficacy in preserving neuromuscular function.

Taken together, our results demonstrate that TmV effectively ameliorates both cognitive and motor deficits in a tauopathy model through a sexually dimorphic therapeutic profile, in which cognitive improvements are predominantly observed in females, whereas motor enhancements are more pronounced in males.

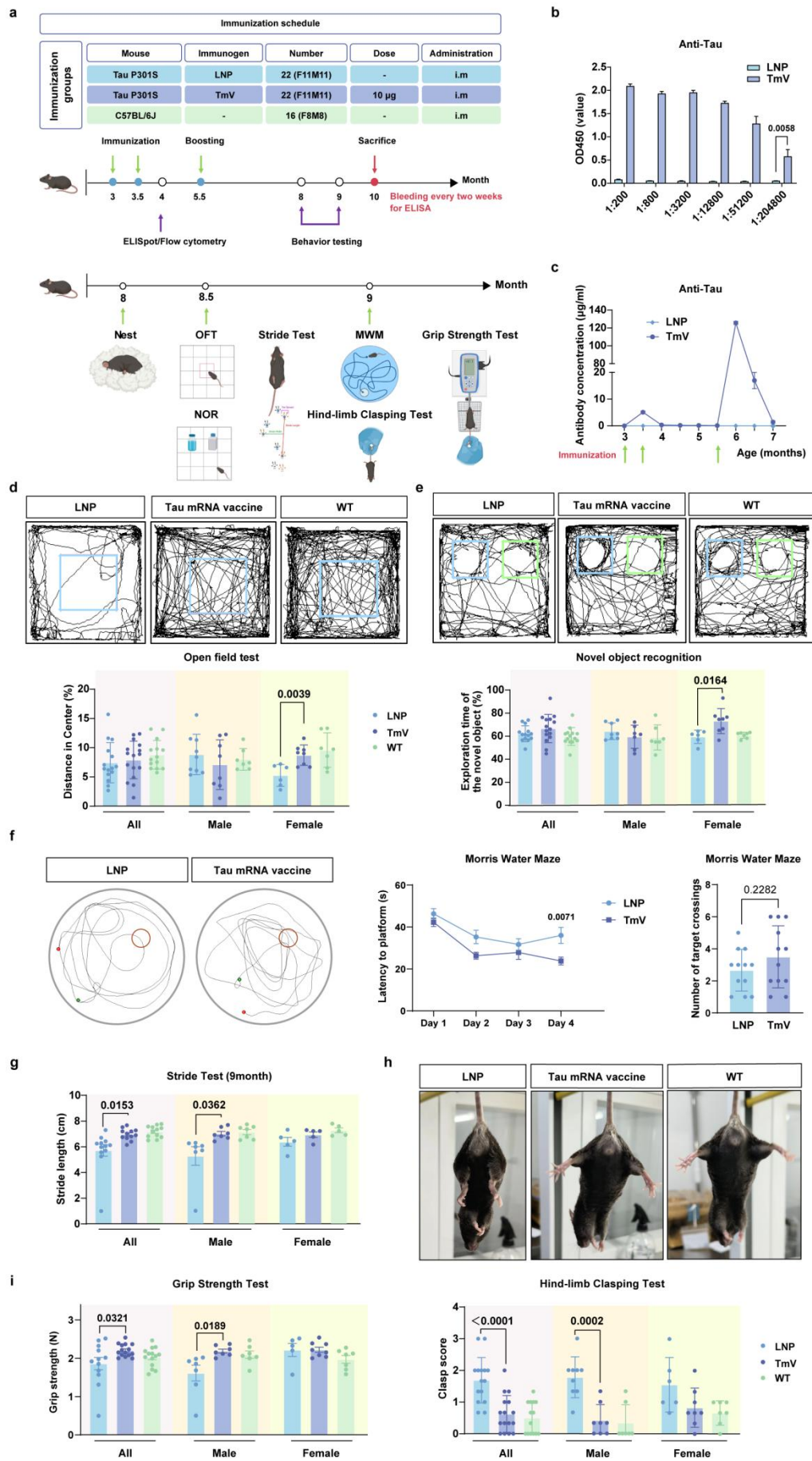


Fig. 7: Immune activation and behavioral improvement induced by Tau mRNA vaccine in TauP301S transgenic mice.

a Immunization and behavior testing schedule for Tau transgenic mice. **b** Serum Tau-specific antibody concentrations 2 weeks after booster immunization. **c** Longitudinal Tau-specific antibody profiles (n=3). **d** Representative locomotor trajectories and center zone occupancy in the open field test. Blue zones indicate center regions. Data are presented as means $\pm$ SD (n = 14, 15, 16). **e** Representative novel object recognition exploration patterns and exploration time of the novel object. Blue squares denote novel object zones. Green squares denote familiar object zones. Data are presented as means $\pm$ SD (n = 14, 15, 16). **f** Representative swim paths, quantification of latency during training, and platform-crossing number during the probe test. Red circles denote hidden platform locations. Data are presented as means $\pm$ SD (n = 12). **g** Stride length quantification. Data are presented as means $\pm$ SD (n = 12). **h** Representative hind-limb clasping status and quantification of clasp scores. Data are presented as means $\pm$ SD (n = 14, 15, 16). **i** Forelimb grip strength measurements. Data are presented as means $\pm$ SD (n = 12, 14, 15). Statistical significance was calculated via unpaired t test (**b**, **f**), one-way ANOVA (**d**, **e**, **g-i**), and two-way ANOVA (**f**) in GraphPad Prism.

Tau mRNA Vaccine effectively clears multiple pathological Tau species in Tau transgenic mice

Subsequently, we assessed tau pathology in TmV-treated versus control Tau transgenic mice after 7 months of continuous administration. Immunohistochemistry using the PHF antibody revealed that TmV prominently reduced tau aggregates in both the cortex and hippocampus, with a near fourfold reduction in pathological burden specifically within the hippocampal CA1 region (Fig. 8a). We next analyzed human Tau and pTau levels in Sarkosyl-soluble fractions from hippocampal and cortical homogenates of vaccinated mice. Remarkably, human Tau was readily detectable by western blot in LNP-treated controls but nearly undetectable in TmV-treated mice, indicating near-complete elimination of pathological human Tau

after vaccination. This clearance was confirmed using the Tau5 antibody, excluding epitope masking and demonstrating a substantial reduction in transgenic tau protein (Fig. 8b, Fig. S14a). Concurrently, levels of pTau181 and PHF were also markedly diminished in hippocampal and cortical extracts. This reduction was paralleled by decreases in both soluble and insoluble pTau181 fractions, as quantified by ELISA (Fig. 8 c, Fig. S14b). Given the pronounced clearance efficacy observed, we investigated potential effects on physiological tau function. Notably, endogenous mouse tau levels, essential for biological functions, were preserved in vaccinated mice (Fig. 8b, Fig. S14a). In all, these analyses confirm that TmV effectively reduces multiple pathological tau forms, clearing not only the immunogen-targeted aggregates but also reducing phosphorylation at specific epitopes. This broad-spectrum efficacy represents an advance over current intervention strategies, highlighting the potential of mRNA vaccines as novel therapeutics for tauopathies.

Tau mRNA vaccine confers neuroprotection through attenuated neuroinflammation and demonstrates safety in Tauopathy mice

Following confirmation of extensive pathological tau clearance, we investigated TmV's effects on neuroglial activation and inflammatory responses in Tau transgenic mice. Compared to controls, vaccinated mice exhibited pronounced reduced levels of Iba1⁺ microglia and GFAP⁺ astrocytes in the hippocampus and cortex (Fig. 8d, Fig. S15). Immunofluorescence further revealed markedly decreased glial coverage, confirming suppressed microglial and astrocytic activation (Fig. 8e). Furthermore, ELISA analysis showed comparable levels of TNF- α , IL-1 β , and TGF- β in brain homogenates across groups (Fig. 8, f-h). At the transcript level, however, qPCR demonstrated markedly downregulation of pro-inflammatory transcripts, including IL-6, IFN- γ , TNF- α , and IL-1 β , in vaccinated mouse brains (Fig. 8i). These findings indicate that TmV concurrently suppresses neuroglial activation and pro-inflammatory transcription, promoting a homeostatic neuroimmune environment.

We next assessed TmV's direct neuroprotective effects. Immunohistochemistry revealed increased neuronal density in the hippocampus of vaccinated mice (Fig. 8j).

Western blotting confirmed considerable NeuN upregulation, indicating preserved neuronal integrity likely mediated by combined tau clearance and anti-inflammatory actions (Fig. 8k).

To evaluate the in vivo safety of TmV, we performed comprehensive multiparameter analysis. Vaccinated mice maintained stable body weights throughout the study, with weights comparable to or slightly exceeding controls (Fig. S16a). ELISpot assays detected only background Tau-specific CD8⁺ T cell activity, confirming the absence of cytotoxic T cell responses (Fig. S16b). Crucially, TmV administration caused no increase in cerebral microhemorrhages versus controls, and histopathological examination revealed normal organ morphology without evidence of vaccine-related toxicity (Fig. S16, c and d). Taken together, these data establish TmV as a well-tolerated therapeutic strategy that drives comprehensive neuroprotection through synergistic mechanisms of pathological clearance and neuroglial modulation.

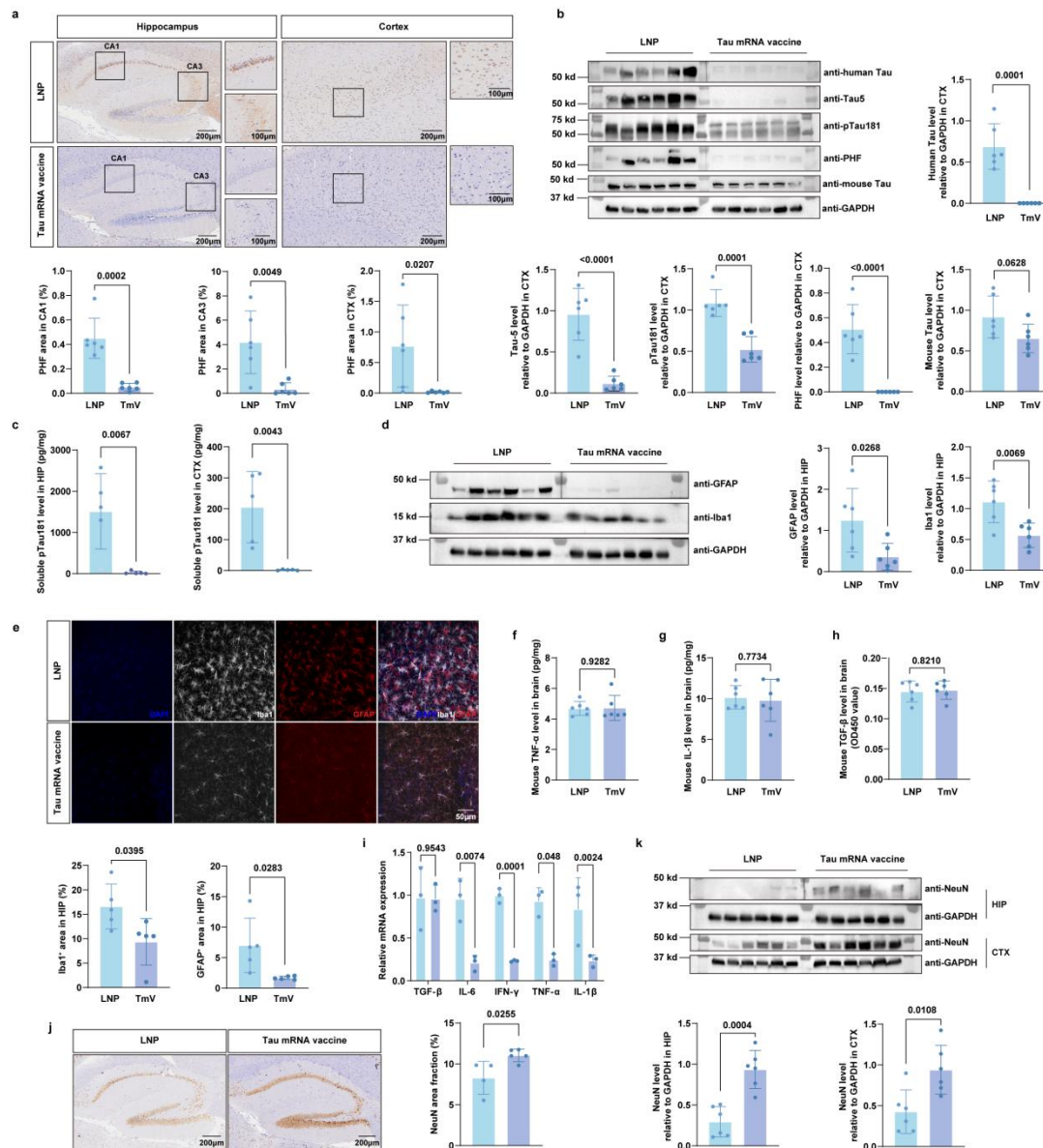


Fig. 8: Tau pathology and neuroinflammation in immunized Tau transgenic mice

a Immunohistochemical analysis and quantification of PHFs in the hippocampus and cortex of TmV-immunized TauP301S mice. Data are presented as means $\pm$ SD (Scale bars, 100/200 μ m, n = 6). **b** Western blot analysis and quantification of human Tau, Tau5, pTau181, PHF, and mouse Tau protein levels in cortical homogenates. Data are presented as means $\pm$ SD (n = 6). **c** Soluble pTau181 levels in the hippocampus and cortex of TmV-treated mice. Data are presented as means $\pm$ SD (n = 5). **d** Western blot and quantification of GFAP and Iba1 in the hippocampus. Data are presented as means $\pm$ SD (n = 6). **e** Immunofluorescence images and quantification of astrocytes

shown by GFAP staining and microglia shown by Iba1 staining in the hippocampus. Data are presented as means $\pm$ SD (Scale bars, 50 μ m, n = 5). **f-h** Levels of TNF- α (**f**), IL-1 β (**g**), and TGF- β (**h**) in brain lysates. Data for each cytokine are presented as means $\pm$ SD (n = 6). **i** qRT-PCR analysis of inflammatory factors normalized to GAPDH. Data are presented as means $\pm$ SD (n = 3). **j** NeuN IHC in the hippocampus across groups. Data are presented as means $\pm$ SD (Scale bars, 200 μ m, n = 4, 5). **k** Western blot and quantification of NeuN in the hippocampus and cortex. Data are presented as means $\pm$ SD (n = 6). Statistical significance was calculated via unpaired t test (**a-k**) in GraphPad Prism.

Tau mRNA vaccine specifically targets pathological tau aggregates and restores neuroimmune homeostasis

To elucidate TmV's mechanism of tau pathology clearance, we quantified tau-specific antibodies in brain tissue. Vaccinated mice exhibited an average cerebral antibody concentration of 0.152 μ g/mL, corresponding to a blood-brain barrier penetration rate of 0.21% (Fig. 9a). This pharmacodynamic evidence demonstrates that systemic vaccination achieves therapeutic antibody titers with appreciable CNS bioavailability. We further confirmed that vaccine-generated antibodies specifically bound tau neurofibrillary tangles in brain sections from Tau mice (Fig. 9b). Western blot analyses revealed these antibodies selectively recognized pathological tau aggregates while sparing physiological tau isoforms, enabling precise immunological targeting (Fig. 9c). In contrast, a commercially available anti-human Tau antibody bound both NFT and full-length tau simultaneously (Fig. 9c).

Next, we performed transcriptomic sequencing of hippocampal tissue to investigate TmV's impact on brain signaling networks. This analysis identified 183 differentially expressed genes post-vaccination. Notably, 166 downregulated genes showed robust enrichment in neuroinflammatory pathways, including Toll-like receptor signaling, complement activation, cytokine-receptor interactions, and chemokine signaling pathways (Fig. 9, d and e). Expression of key genes involved in excessive microglial activation and pro-inflammatory factor secretion, specifically

Ccl9, *C3ar1*, *Itgax*, *Ifit3*, and *Cd22*, was markedly suppressed following vaccination. Subsequent RT-PCR validation robustly confirmed downregulation of critical neuroinflammatory mediators, including *Oasl2*, *Clec7a*, *Ccl9*, *C3ar1*, *Itgax*, *Ifit3*, and *Cd22*, which contributes to the alleviation of AD-related neuroinflammation (Fig. 9f). Collectively, these findings demonstrate that TmV exerts therapeutic efficacy through synergistic mechanisms, such as generating brain-penetrant antibodies for selective pathological tau clearance while concurrently suppressing detrimental neuroinflammatory pathways. This coordinated action restores neuroimmune homeostasis and ameliorates disease pathology in Tan transgenic mice.

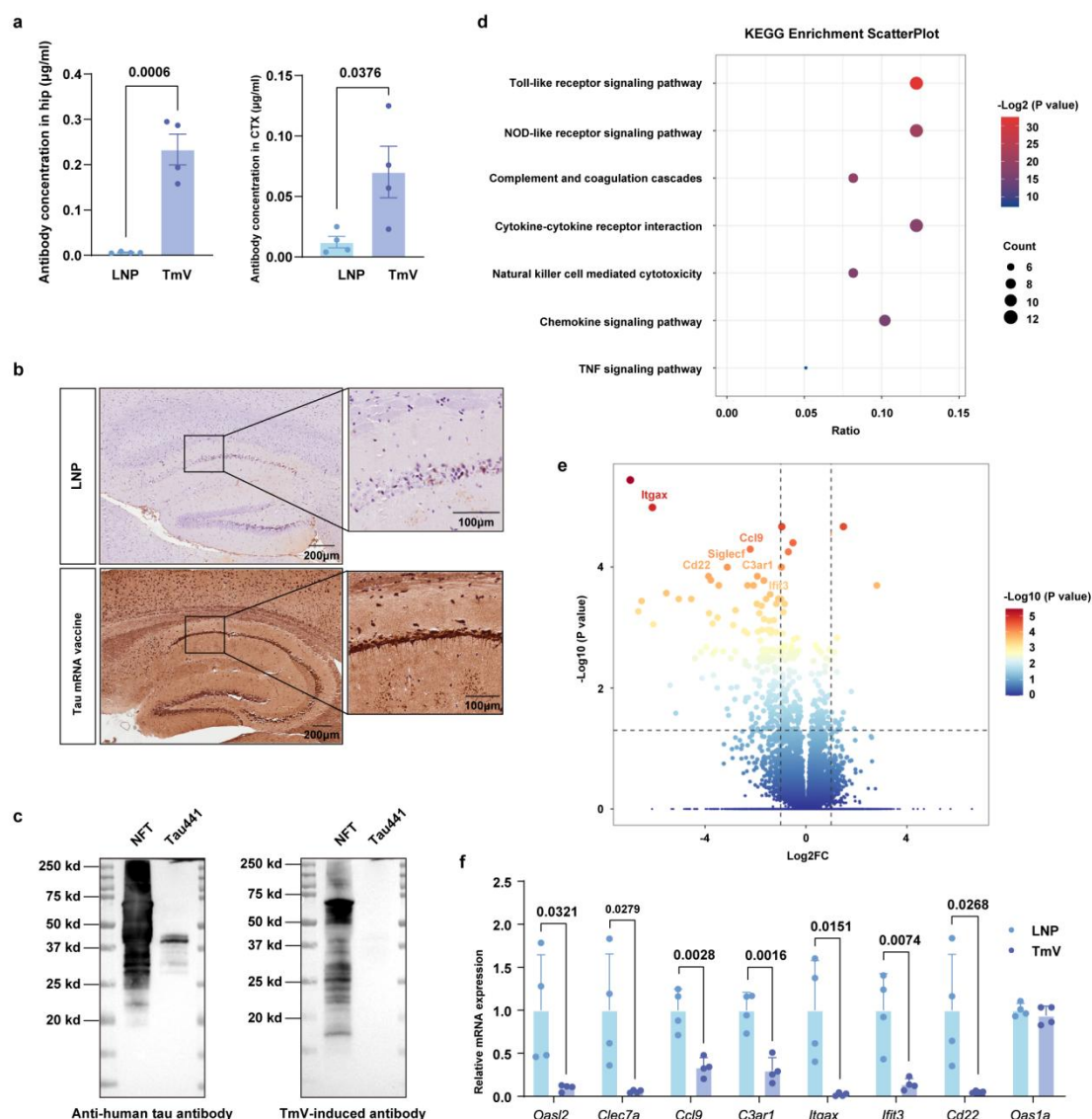


Fig. 9: Dual therapeutic mechanisms of Tau mRNA vaccine in Tau P301S transgenic mice

a Anti-Tau antibody concentration in hippocampal and cortical tissues of TmV-treated mice. Data are presented as means $\pm$ SD (n = 4). **b** Immunostaining of brain sections from 12-month-old Tau P301S transgenic mice using TmV-induced antibodies. LNP-induced antibody served as a negative control (Scale bars, 100/200 μ m). **c** Specificity of purified TmV-induced polyclonal antibodies against Tau isoforms. Human tau monoclonal antibody served as a positive control. **d** KEGG pathway analysis of differentially expressed genes (DEGs) between TmV and LNP groups. **e** Volcano plot of DEGs between TmV and LNP groups. **f** qRT-PCR validation of hippocampal gene expression normalized to GAPDH. Data are presented as means $\pm$ SD (n = 4). Statistical significance was calculated via unpaired t test (**a**, **f**) in GraphPad Prism.

Discussion

The rising prevalence of age-related neurodegenerative disorders, particularly Alzheimer's disease (AD), underscores an urgent need for disease-modifying therapies that transcend the symptomatic relief offered by current acetylcholinesterase inhibitors and NMDA antagonists. While recent FDA-approved disease-modifying antibodies represent a conceptual advance, debates surrounding their marginal efficacy and safety risks persist. This landscape highlights the necessity for innovative therapeutic paradigms. Active immunization strategies targeting A β and Tau pathologies have emerged as promising alternatives. However, traditional peptide- or protein-based vaccines fail to sustain therapeutic antibody levels or safely engage the central nervous system (CNS).

Here, we present an mRNA vaccine platform engineered to overcome these limitations through rational antigen design, potent immune activation, and sustained immune memory. In AD transgenic models, both A β - and Tau-targeting mRNA vaccines elicited sustained high-affinity antibodies that coordinately cleared pathogenic aggregates via multimodal mechanisms while preserving neuronal

architecture and rescuing cognitive deficits. Crucially, the absence of CD8⁺ T cell activation or cerebrovascular complications demonstrates unprecedented CNS safety for an active immunization strategy. By integrating potent immunogenicity with stringent neuroimmune tolerance, our mRNA platform establishes a blueprint for pathology-targeting neurodegenerative immunotherapies. This approach offers a clinically translatable strategy to address AD's multifactorial pathology without compromising brain homeostasis.

Current active A β immunotherapies predominantly rely on polypeptide or recombinant protein vaccines. However, most have stumbled in clinical translation owing to inadequate efficacy or safety concerns. For instance, CAD106, a recombinant protein vaccine displaying A β 1-6 peptides on phage capsids, induced only modest antibody levels (approximately 100 μ g/mL) in APP/PS1 mice¹⁷. Although it generated A β antibodies in AD patients with reasonable tolerance, it failed to significantly ameliorate cognitive decline¹⁸. Similarly, the A β peptide vaccine UB-311 elicited an antibody response yet did not translate to clinical cognitive improvement⁶. These outcomes underscore a critical limitation of traditional platforms, such as inherently weak immunogenicity and poor persistence, yielding subtherapeutic antibody concentrations incapable of modifying disease trajectory. Parallel challenges exist for Tau vaccines. AADvac1, which is a clinical-stage peptide vaccine targeting the Tau294-305 epitope conjugated to KLH, induced antibodies that bound tau aggregates in AD patients. Nevertheless, it failed to demonstrate cognitive benefit in its phase II trial, further highlighting the immunogenicity hurdle common to peptide-based approaches⁸.

Consequently, novel immunization strategies capable of eliciting sustained high antibody titers without compromising safety are therefore urgently needed for AD immunotherapeutics. Our study demonstrates that mRNA vaccines elicit potent immune responses against both A β and Tau pathologies. Critically, the A β mRNA vaccine exhibited superior immunogenicity compared to conventional platforms like CAD106, inducing higher antibody titers (approximately 600 μ g/mL) in APP/PS1

mice. Flow cytometry revealed robust plasma B cell and T follicular helper (Tfh) cell activation, indicative of germinal center reactions essential for high-affinity antibody production. Similarly, the Tau mRNA vaccine elicited exceptional antibody titers ($\log_{10} > 6$) in TauP301S mice, with flow cytometry confirming antigen-specific plasma B and T helper cell activation.

A key advantage of mRNA vaccines is their capacity to induce durable immune responses. For the A β vaccine, antibody levels persisted near peak concentrations three months post-booster, surpassing the rapid decline seen in conventional vaccines like CAD106 and PP-based nanoparticle vaccines, which exhibit a 60% antibody decline by 4 weeks¹⁹. Elevated memory B cells provided cellular evidence for this longevity. Likewise, the Tau vaccine induced persistent humoral responses after boosters, implying the establishment of immunological memory. Such durability underscores mRNA vaccines' superiority in generating long-lived memory cells over traditional platforms, a critical feature for combating chronic neurodegeneration.

This potent and durable immunity translated to substantial therapeutic efficacy across pathological, inflammatory, and behavioral domains. Pathologically, A β mRNA vaccination reduced cerebral A β plaque coverage by more than 50%, decreased soluble and deposited A β 42, and lowered serum A β 42, suggesting peripheral sink involvement. Tau mRNA vaccination robustly reduced PHF-positive areas as well as soluble and insoluble human Tau, including pTau181, indicating broad-spectrum clearance of pathogenic Tau species throughout brain regions in AD models.

Regarding neuroinflammation, A β mRNA vaccination attenuated microgliosis by approximately 50% and promoted the transition of microglia to a homeostatic phenotype. Simultaneously, it markedly reduced plasma TNF- α levels while elevating TGF- β levels. This modulation of peripheral immunity occurred without inducing central pro-inflammatory responses, thereby mirroring the neuroprotective anti-neuroinflammatory effects observed with anti-A β antibodies²⁰. Similarly, the Tau mRNA vaccine potently reduced the activation of microglia and astrocytes and

downregulated the transcriptional levels of pro-inflammatory cytokines. Hippocampal transcriptomic analysis further revealed suppressed cytokine/chemokine signaling pathways, indicative of a shift toward an anti-inflammatory CNS microenvironment. Hence, our mRNA vaccination strategy effectively modulates glial activation states and CNS inflammation, as well as potential peripheral-central immune interactions.

Behaviorally, A β mRNA vaccination improved nest building and novel object recognition performance in APP/PS1 mice, suggesting hippocampal rescue and enhanced short-term memory. These cognitive benefits were more pronounced in males than females, indicating that the vaccine triggers gender-specific cognitive benefits. In parallel, Tau mRNA vaccination ameliorated motor deficits, muscle atrophy, and cognitive impairment in Tau transgenic mice while demonstrating sex-dependent efficacy, with females achieving superior cognitive improvements and males exhibiting enhanced motor recovery. This differential response likely reflects intrinsic sex-based variations in Tau pathology progression and manifestation within this model, as males develop earlier and more severe motor deficits and neuronal loss, which may be influenced by sex hormones that impact brain circuits and function^{21,22}. Although cognitive improvements confirm functional relevance, particularly regarding short-term memory after A β vaccination and combined motor-memory functions following Tau vaccination, the limited long-term memory rescue underscores a key translational gap requiring improvement.

Both vaccines demonstrate multimodal therapeutic mechanisms. Crucially, they generate brain-penetrant antibodies that achieve substantial blood-brain barrier penetration, with the A β mRNA vaccine reaching 0.23% and the Tau mRNA vaccine 0.21%, which exceeds the 0.1% penetration typical of systemic antibodies. These antibodies exhibit precise pathological selectivity. A β antibodies preferentially bind oligomers and fibrils rather than monomers, while Tau antibodies recognize pathological aggregates without cross-reacting with physiological isoforms. This selective targeting enables pathogen-specific clearance while minimizing off-target

effects, which represents a pivotal advancement over earlier vaccines that were prone to non-specific binding²³.

Antibody-dependent microglial phagocytosis constitutes a core mechanism underlying AD active immunization. A β mRNA immunization promoted microglial transformation, accompanied by increased plaque coverage and enhanced phagocytic activity. This phenomenon is consistent with Fc γ receptor (Fc γ R)-dependent “phagocytic synapse” formation²⁴. Moreover, the recruitment of astrocytes to plaque-microglia complexes implies coordinated glial crosstalk during A β clearance²⁴. Similarly, Tau mRNA vaccine-induced pTau antibodies crossed the blood-brain barrier (BBB), underwent neuronal endocytosis, bound intracellular tau, and facilitated its lysosomal degradation, although definitive degradation data require further validation²⁵⁻²⁷.

Moreover, transcriptomic analysis revealed that AD mRNA vaccine administration restores neuroimmune homeostasis through anti-inflammatory reprogramming. The A β mRNA vaccine increased expression of *Cxcl5*, which is involved in microglial activation and recruitment, aligning with the observed enhancement of antibody-mediated phagocytosis. Meanwhile, the Tau vaccine efficiently suppressed key neuroinflammatory mediators including *CCL9* and *C3AR1* along with their associated pathways. This immunomodulatory effect aligns with the established concept that pathological protein clearance interrupts innate immune activation cascades^{28,29}. Furthermore, A β mRNA vaccination suppressed amyloidogenesis by reducing APP/BACE1 expression, indicating that it also modulates upstream pathological processes.

Safety remains paramount for any Alzheimer's disease therapeutic. Notably, both A β and Tau mRNA vaccines demonstrated favorable safety profiles in our preclinical studies. This profile is attributed primarily to their biased CD4⁺ T cell responses, avoiding potentially damaging CD8⁺ T cell activation, thereby minimizing risks associated with excessive cytotoxic inflammation. Critically, treated AD model mice exhibited no evidence of severe adverse events, such as brain hemorrhages.

Furthermore, comprehensive histopathological analysis of multiple organs versus wild-type controls revealed no apparent lesions or damage. This observed safety profile aligns with established clinical data from approved mRNA-LNP vaccines against infectious diseases (e.g., COVID-19 and RSV vaccines), which have undergone billions of administrations, demonstrating the platform's overall tolerability^{30, 31}. While our preclinical data are promising, vigilance regarding potential clinical safety concerns remains imperative. These include transient liver or spleen toxicity following intravenous or intramuscular LNP administration, complexities associated with LNP components, and the inherent immunostimulatory nature of mRNA itself.

In conclusion, our A β and Tau mRNA-LNP vaccines demonstrate compelling therapeutic efficacy in AD model mice. These vaccines exhibit exceptional immunogenicity, driving robust Tfh-dependent B cell activation and high-affinity antibody production, alongside highly durable immune responses indicative of potent immune memory. Critically, they confer substantial efficacy in mitigating core pathological hallmarks, including A β plaques, PHFs, and pTau, while ameliorating neuroinflammation and eliciting tangible, albeit complex, improvements in behavioral deficits. Their safety profile is further supported by the absence of major adverse events and alignment with broader mRNA vaccine safety experience. This work not only establishes a foundational framework for precision immunotherapies across neurodegenerative disorders but also redefines mRNA nanomedicine's potential against proteinopathies. Looking forward, enhancing therapeutic efficacy remains our priority, which will include optimizing mRNA antigen design and T helper epitopes, engineering LNPs to target lymphoid organs for enhanced immune cell priming, and critically developing dual-targeting A β /Tau mRNA vaccines.

Materials and Methods

Animals

For immunogenicity evaluation, eight-week-old female C57BL/6J wild-type mice were obtained from Changsheng Biotechnology Co., Ltd. (Liaoning, China). To

assess the therapeutic efficacy of the vaccines, transgenic APP/PS1 and TauP301S mouse models (Changzhou Cavens Laboratory Animal Co., Ltd.) were employed. All animal experiments were conducted according to the Guide for the Care and Use of Laboratory Animals (Jilin University, China). All the experimental procedures were reviewed and approved by the Animal.

Welfare and Research Ethics Committee of Jilin University (ethics number: LACCAES2023024).

Experimental protocols/Animal immunization

C57BL/6J mice immunization

C57BL/6J mice were intramuscularly immunized with A β or Tau mRNA vaccines, with PBS and LNP groups serving as negative controls. Each group included 8 female mice, receiving two 10 μ g doses administered intramuscularly at 2-week intervals. Serum samples were collected bi-weekly post-immunization for ELISA-based quantification of antibody titers. Ten days after the second immunization, 4 mice per group were randomly selected for ELISpot and flow cytometry analyses to characterize T-cell responses and immune cell activation, while the remaining mice underwent continuous blood sampling to monitor antibody persistence. Mice were euthanized on day 104.

A β mRNA Vaccine in APP^{swe}/PS1^{dE9} transgenic mice

Three-month-old APP^{swe}/PS1^{dE9} transgenic mice were immunized with the A β mRNA vaccine, with LNP and littermate wild-type (WT) mice as negative controls. Each group (vaccine and LNP) consisted of 20 mice (10 females/10 males), receiving two 10 μ g intramuscular doses at 2-week intervals. ELISpot and flow cytometry assays were performed at 4 months, followed by bi-weekly blood sampling and body weight monitoring. A booster immunization was administered at 5.5 months, with blood collection continuing until 9 months. Behavioral assessments of memory and cognitive function were conducted from 9 to 10 months, and mice were euthanized at 11 months for histological and pathological analysis of brain and peripheral organs.

Tau mRNA Vaccine in TauP301S transgenic mice

TauP301S transgenic mice at 3 months of age were immunized with the Tau mRNA vaccine, using LNP and littermate WT mice as negative controls. Each group (vaccine and LNP) included 22 mice (11 females/11 males), receiving two 10 µg intramuscular doses at 2-week intervals. At 4 months, five mice per group were randomly selected for ELISpot and flow cytometry analyses. The remaining mice received a booster immunization at 5.5 months, with bi-weekly blood sampling to monitor antibody kinetics. Behavioral evaluations were performed between 8 and 9 months, and mice were euthanized at 10 months for histopathological analysis of brain and organ tissues.

Cell Culture

HEK293T cells and BV2 cells were maintained in DMEM/F12 medium supplemented with 10% FBS (Zhejiang Tianhang Biotechnology Co., Ltd.) and 1% penicillin-streptomycin at 37 °C in a humidified incubator with 5% CO₂. When cell confluency reached 80-90%, cells were harvested using trypsin-EDTA and passaged for further culture.

mRNA Vaccine Design and Product

Alzheimer's disease (AD) mRNA vaccines were designed based on the human sequences of Aβ and Tau proteins. To generate the recombinant constructs, three tandem copies of the coding sequences for human Aβ1-6 (DAEFRH) or Tau294-305 (KDNIKHVPGGGS) were synthesized and fused in-frame to a nucleotide sequence encoding a T helper (Th) epitope. Each repeat was separated by a flexible glycine-serine linker (GGGGS), with an additional GGGGS linker positioned between the antigenic region and the Th epitope. All sequences were codon-optimized and synthesized by GenScript (Nanjing, China).

Linearized DNA templates for in vitro transcription included a 5' untranslated region (UTR), a 3' UTR, and a poly(A) tail, designed based on the mRNA architecture used by Moderna³². mRNA synthesis was performed using T7 RNA polymerase (E131, Novoprotein) in the presence of N1-methyl-pseudouridine triphosphate (DD4503, Vazyme) to enhance transcript stability and reduce innate

immune activation. Following transcription, a Cap1 structure was enzymatically added using the Cap1 capping system (M082, Novoprotein). The mRNA products were purified, quantified, and stored at -80°C until use.

Antigen Expression and Validation (mRNA Transfection and IF, WB, ELISA)

mRNA Transfection

HEK293T cells were seeded in 6-well plates and transfected at 70-80% confluency with synthesized mRNA encoding A β or Tau antigens using Lipofectamine 2000 (11668-019, Thermo Fisher Scientific) according to the manufacturer's protocol. Cells were further cultured for 48 h prior to downstream analysis.

Western Blot

Transfected cells were lysed in RIPA buffer supplemented with a protease inhibitor cocktail (4693116001, Roche). Protein concentrations were determined using a BCA Protein Assay Kit (P0009, Beyotime). Equal amounts of protein were separated by SDS-PAGE and transferred to nitrocellulose membranes. Membranes were blocked with 5% (w/v) non-fat milk and incubated with primary antibodies against A β or Tau (GenScript, Laboratory preparation) overnight at 4°C , followed by incubation with HRP-conjugated secondary antibodies (C31460100, C31430100, Thermo Fisher Scientific). Signals were visualized using enhanced chemiluminescence (ECL) reagents (34577, Thermo Fisher Scientific).

Immunofluorescence

Transfected cells were fixed in 4% paraformaldehyde and incubated with anti-A β antibody or anti-Tau antibody (GenScript, Laboratory preparation) at 4°C for at least 8 hours or overnight, followed by FITC-conjugated goat anti-mouse IgG (H + L) (A0568, Beyotime) for 1 h. Nuclei were stained with DAPI, and images were obtained using a fluorescence microscope.

ELISA

For quantification of secreted antigen levels, cell culture supernatants were collected at 48 h post-transfection and analyzed using ELISA. Briefly, cell culture

media were collected after transfection and centrifuged at 5000 rpm for 10 min to remove cellular debris. The supernatants were concentrated and exchanged into coating buffer to a final volume of 200 μ L. Samples (100 μ L/well) were added to ELISA plates and incubated overnight at 4 °C. After washing with PBST, wells were blocked with 3% BSA at 37 °C for 1 h. Primary antibodies against A β ₁₋₆ or Tau were applied and incubated at 37 °C, followed by HRP-conjugated secondary antibody. After TMB substrate development and termination with 2 M H₂SO₄, absorbance was measured at 450 nm.

mRNA-LNP Formulation and Characterization

mRNA-LNPs were prepared using a microfluidic mixing system by encapsulating in vitro-transcribed mRNA with lipid components. LNP formulations were prepared as described previously³³. Briefly, lipids were formulated in anhydrous ethanol (SM-102: DSPC: cholesterol: DMG-PEG2000 = 50:10:38.5:1.5, mol/mol) (O02010, S01005, O01001, O02005, Shanghai Advanced Vehicle Technology L.T.D. Co). mRNA was diluted in 100 mM sodium citrate buffer (pH 4.0) prior to mixing. The resulting mRNA-LNPs were buffer-exchanged into PBS and concentrated to appropriate immunization doses. Particle size, polydispersity index (PDI), and zeta potential were measured using a nanoparticle analyzer. Morphology was examined by transmission electron microscopy (TEM) following negative staining with 1% phosphotungstic acid. Encapsulation efficiency was assessed using the Quant-iTTM RiboGreen® RNA assay (R11490, Thermo Fisher Scientific). Based on the RNA standard curves with and without Triton, the encapsulation efficiency was calculated using the following formula: EE% = ((Total mRNA – unencapsulated mRNA)/total mRNA) $\times$ 100%. For all groups, the mRNA encapsulation efficiencies were above 85%.

Characterization of Serum Antibody Responses

Serum A β - or Tau-specific antibody levels were quantified by ELISA. 96-well plates were coated with A β ₄₂ or Tau₂₉₄₋₃₀₅ peptides (0.1 μ g/well) and incubated overnight at 4 °C. Plates were blocked and then incubated with serially diluted mouse

serum samples or standard antibodies at 37 °C for 2 hours. After washing with PBST, HRP-conjugated anti-mouse IgG was added and incubated at 37 °C for 1 hour. Following additional washes, TMB substrate (PA107-01, Tiangen) was applied and developed in the dark. Reactions were terminated with 2 M H₂SO₄, and absorbance was measured at 450 nm using a microplate reader.

Antibody isotypes in mouse sera were determined using an antibody isotyping ELISA kit (ISO2, Sigma), which enables detection of IgG1, IgG2a, IgG2b, IgG3, IgA, and IgM subclasses. The procedure followed the manufacturer's instructions and was based on a standard ELISA workflow with the addition of isotype-specific secondary antibodies.

ELISpot Assay of Mouse Splenocytes

Th Protein Expression and Purification

The coding sequence of the T helper (Th) protein was cloned into the pET-30a(+) vector and expressed in *E. coli* with an N-terminal His tag. Recombinant protein was purified using nickel-nitrilotriacetic acid (Ni-NTA) affinity chromatography on an ÄKTA protein purification system. After column equilibration and sample loading, bound proteins were eluted using a stepwise imidazole gradient (50, 100, and 300 mM). Eluted fractions were assessed by SDS-PAGE and confirmed by Western blot analysis.

ELISpot Assay

Multiscreen 96-well ELISpot plates were coated overnight at 4 °C with 5 µg/mL capture antibodies specific for mouse IFN-γ or IL-4 (551083, 551017, BD Biosciences). On the following day, plates were washed and blocked with R10 medium (RPMI-1640 supplemented with 10% FBS) for 2 h at room temperature. Mouse splenocytes were seeded at 1 × 10⁷ cells/mL per well and stimulated with 5 µg/mL Th protein or 10 µg/mL Aβ42 peptide. Control wells received R10 medium (negative control) or 2 µL concanavalin A (positive control). After 24 h of incubation at 37 °C with 5% CO₂, plates were developed according to the manufacturer's protocol. The number of spots was read and analyzed with an ImmunoSpot S6

analyzer (Cellular Technology Limited, USA).

Sample Processing and Flow Cytometry

The mice were euthanized by CO₂ inhalation, and both inguinal lymph nodes were harvested on day 10. Tissue fragments were digested with collagenase (0.04 mg/mL) and DNase I (10 U/mL) at 37 °C for 1 hour. The cell suspension was gently pipetted, centrifuged at 400 g for 5 min, and the supernatant discarded. Pellets were resuspended in R10 medium and filtered through a 200-mesh cell strainer, followed by centrifugation at 350 g for 5 min. Cells were then stained for flow cytometry to assess GC B cells, plasma B cells, memory B cells, and T follicular helper (Tfh) cells. Antibody markers used are listed in Table S2.

Behavioral Assessments

Nesting Test

Four equal-sized cotton nesting pads were placed in the same location in each cage. Mice were individually housed in clean cages in the dark. Nesting behavior was scored at 16 and 24 hours by three independent investigators using a 1-4 scale, with higher scores indicating more complete nests and better hippocampal function, as described by Sun et al³⁴.

Open Field Test

Mice were acclimated to the test room for 30 minutes before testing. The open field apparatus was calibrated, and the central zone was defined using tracking software. Each mouse was gently placed along the edge of the arena, and locomotion was recorded for 10 minutes. Time spent in the center zone was analyzed as a measure of anxiety-like behavior. The arena was cleaned with ethanol between tests.

Novel Object Recognition

The test included three phases: habituation, training, and testing. In the habituation phase, mice freely explored the empty arena for 10 minutes. In the training phase, two identical objects were presented for exploration. One hour later, in the testing phase, one familiar object was replaced with a novel one. Exploration time and contact frequency were recorded to assess short-term memory and novelty

906 preference.

907 **Morris Water Maze**

908 A circular pool was divided into four quadrants with distinct visual cues on the
909 walls. A hidden platform was submerged 2 cm below the surface in one quadrant.
910 During the four-day training phase, each mouse was given four trials per day from
911 different quadrants to locate the platform within 60 s; latency was recorded. On day 5,
912 the platform was removed for a probe trial, and the number of platform crossings was
913 recorded to assess spatial memory retention.

914 **Footprint Analysis**

915 A 30 cm-long, 5 cm-wide, and 10 cm-high runway ending in a dark box was
916 used. Fore- and hindpaws were coated with red and blue paint, respectively. Mice
917 walked along the runway, and footprints were recorded on removable white paper.
918 Stride length was calculated by measuring the distance between successive same-side
919 footprints, excluding the maximum and minimum values.

920 **Hindlimb Clasping Test**

921 Each mouse was suspended by the tail for 10 s, and hindlimb clasping was
922 scored independently by three observers on a 0-3 scale, with higher scores indicating
923 more severe neuromuscular deficits³⁴.

924 **Grip Strength Test**

925 Forelimb and hindlimb grip strength was measured using a digital force gauge.
926 Mice were acclimated for at least 30 minutes before testing. Each mouse was allowed
927 to grasp the bar with all paws and was gently pulled backward until it released. Peak
928 force was recorded. Each mouse underwent at least three trials with appropriate rest
929 between tests to prevent fatigue.

930 **Pathological Protein Detection in Brain Homogenates**

931 **Tissue Collection**

932 Mice were deeply anesthetized with sodium pentobarbital (150 mg/kg) and
933 transcardially perfused with 40 mL of cold 0.9% saline. Brains were carefully
934 removed, bisected, and processed as follows: one hemisphere was fixed in 4%

paraformaldehyde for histological analysis, while the cortex and hippocampus from the other hemisphere were dissected, snap-frozen in liquid nitrogen, and stored at -80°C for biochemical assays. Major organs (heart, liver, spleen, lung, and kidney) were also collected and fixed in 4% paraformaldehyde.

Brain Homogenization of APP/PS1 Mice

Cortical and hippocampal tissues were thawed on ice and homogenized in ice-cold Buffer I (50 mM Tris, 150 mM NaCl, pH 7.6) containing protease inhibitors and magnetic beads (5.65 m/s, 45 s on/30 s off, 2 cycles). Homogenates were incubated on ice for 10-20 min and centrifuged at $50,000 \times g$ for 20 min at 4°C . The supernatant was collected as the soluble fraction. The pellet was washed with Buffer I, then resuspended in Buffer II (5 mM guanidine HCl, 50 mM Tris, pH 8.0), and shaken at room temperature for 3 h. After centrifugation ($50,000 \times g$, 4°C , 20 min), the supernatant was collected as the insoluble fraction. Protein concentrations were determined by BCA assay, and samples were aliquoted and stored at -80°C .

Brain Homogenization of TauP301S Mice

Brain tissue was homogenized in ice-cold Tris-HCl buffer (20 mM Tris, 800 mM NaCl, 1 mM EGTA, 1 mM EDTA, 10% sucrose, pH 7.4) containing protease and phosphatase inhibitors, using magnetic beads (5.65 m/s, 45 s on/30 s off, 2 cycles). Homogenates were centrifuged at $20,000 \times g$ for 20 min at 4°C , and the supernatant (S1) was collected. Sarkosyl was added to S1 to a final concentration of 1%, followed by shaking at room temperature for 60-90 min³⁵. The mixture was centrifuged at $100,000 \times g$ for 90 min. The supernatant (S2) was collected as the soluble fraction, while the pellet was resuspended in 50 mM Tris-HCl (pH 7.4) as the insoluble fraction. All samples were quantified using the BCA assay, aliquoted, and stored at -80°C .

Western Blot

Brain homogenates were analyzed by Western blot. Proteins were separated on 4.5-12.5% SDS-PAGE gels, transferred to nitrocellulose membranes, and blocked with 5% non-fat milk in TBS. Membranes were incubated overnight at 4°C with the

following primary antibodies: Iba-1 Antibody (1:1000, ab178846, Abcam), CD68 Antibody (1:1000, ER1901-32, HUABIO), Purified anti-GFAP Antibody (1:1000, 801103, Biolegend), APP/ β -Amyloid Recombinant Rabbit Monoclonal Antibody (1:1000, HA722139, HUABIO), BACE1 Recombinant Rabbit Monoclonal Antibody (1:1000, HA722244, HUABIO), Human Tau Antibody (1:1000, MN1000, Thermo Fisher Scientific), PHF Antibody (1:1000, 836103, Biolegend), and Human Tau (pT181) Antibody (1:1000, ab223192, Abcam). After washing, membranes were incubated for 1 h at room temperature with HRP-conjugated goat anti-mouse or anti-rabbit secondary antibodies (1:10,000, Thermo Fisher Scientific). Signal detection was performed using ECL substrate (34577, Thermo Fisher Scientific), and images were acquired with the Tanon 5200 automatic chemiluminescence imaging system. GAPDH (1:5000, 10494-1-AP, Proteintech) was used as a loading control. Band intensities were quantified using ImageJ software. For multiplex detection, membranes were stripped (PS107, Epizyme) and reprobed with additional primary antibodies as needed.

ELISA kit

A β ₄₂ levels in brain homogenates from APP/PS1 mice were quantified using an A β ₄₂ ELISA kit (E-EL-H0543, Elabscience). Human total Tau and phosphorylated Tau (pT181) levels in Tau P301S mouse brain homogenates were measured using Human Tau (E-EL-H0948, Elabscience) and Human Tau [pT181] ELISA kits (KHO0631, Invitrogen), respectively, according to the manufacturers' protocols.

Inflammatory cytokines in mouse brain homogenates and serum samples were tested using the following ELISA kits according to the manufacturer's instructions: TNF- α (E-EL-M3063, Elabscience), IL-1 β (E-EL-M0037, Elabscience), and Mouse TGF- β 1 ELISA Kit (E-EL-0162, Elabscience).

Immunohistochemistry (IHC)

Brain sections were deparaffinized through a graded xylene and ethanol series, followed by antigen retrieval using citrate buffer. After cooling, sections were washed four times with TBST and sequentially incubated with 3% hydrogen peroxide to

quench endogenous peroxidase activity and 10% goat serum to block nonspecific binding. The sections were then incubated overnight at 4 °C with primary antibodies, including Iba-1 Antibody (1:500, ab178846, Abcam), Purified anti-GFAP Antibody (1:500, 801103, Biolegend), 3D6 Antibody (1:500, Genscript), Anti-NeuN Antibody (1:500, 834501, Biolegend), and Anti-PHF Antibody (1:500, 836103, Biolegend). After washing with TBST and TBS, sections were incubated at 37 °C for 30 minutes with a biotin-labeled rabbit anti-human IgG antibody included in the human immunohistochemistry kit (ZN1829, Biolab). Following three washes with TBS (3 × 5 min), sections were incubated with SABC reagent at 37 °C for 30 minutes. After an additional three TBS washes, DAB substrate was applied for color development, and hematoxylin was used for nuclear counterstaining. The sections were then dehydrated, cleared, and mounted with neutral balsam. After drying, the slides were scanned using a panoramic digital slide scanner for whole-slide imaging.

Immunofluorescence Staining

One series of the 40 µm sections was rewarmed for 20 minutes. After three washes with TBS, sections were permeabilized and blocked with TBS containing 0.3% Triton X-100 for 15 minutes at room temperature. Subsequently, they were incubated with the following primary antibodies: Iba-1 Antibody (1:300, ab178846, Abcam) and purified anti-GFAP Antibody (1:500, 801103, Biolegend) at 4°C for 24 h, followed by incubation with the corresponding fluorescent secondary antibodies at 37°C for 2 h, including Alexa Fluor® 647 Donkey pAb to Rabbit IgG (1:300, ab150075, Abcam) and Alexa Fluor® 555 Goat Anti-Mouse IgG (1:400, 115-565-146, Jackson ImmunoResearch). For Aβ staining, an additional step involved incubating the samples with 1% thioflavin S working solution at room temperature for 5 minutes, followed by treatment with 70% anhydrous ethanol for 5 minutes. This was then followed by three additional rinses with TBS. Sections were stained with DAPI and sealed with an anti-fluorescence quencher. Images were acquired using a VS200 automated slide scanner (Olympus) with a 20 × objective lens and on a confocal laser scanning microscope (Zeiss) with spectral unmixing to resolve fluorescence signals.

Prussian Blue Staining

Paraffin-embedded brain sections were deparaffinized through a graded series of xylene and ethanol, followed by incubation in Prussian blue staining solution for 1 h. After rinsing with distilled water, nuclear counterstaining was performed for 5 min. Sections were then washed again under running water and mounted with neutral balsam.

Hematoxylin and Eosin (H&E) Staining

For H&E staining, deparaffinized sections were stained with hematoxylin for 5 min, rinsed in tap water, and subjected to differentiation and bluing steps. Slides were then stained with eosin for 3 min, followed by dehydration and mounting.

Immunohistochemistry Image Analysis

The percentage of antibody-positive area was quantified using ImageJ software. Images were first threshold-adjusted for optimal contrast, and the “Analyze Particles” function was used to calculate the proportion of stained area.

For microglial morphological analysis, the skeleton analysis method was applied. Individual microglia were segmented from IHC images, and the ImageJ Skeleton plugin was used to extract cellular skeletons. The “Analyze Skeleton” function was then employed to quantify branch numbers and endpoints, enabling assessment of microglial structural changes under pathological conditions.

Purification of Serum Antibody from Immunized Mice

IgG antibodies were purified from the serum of immunized mice using a saturated ammonium sulfate (SAS) precipitation method³⁶. Briefly, 0.5 mL of high-titer serum was mixed with an equal volume of 0.9% NaCl, followed by the dropwise addition of 1 mL of saturated ammonium sulfate solution. The mixture was incubated at 4 °C for 1 hour and centrifuged at 1000 rpm for 20 minutes. The supernatant was discarded, and the pellet was resuspended in 2 mL of 0.9% NaCl. A second round of SAS precipitation was performed by adding 1 mL of saturated ammonium sulfate dropwise, followed by incubation and centrifugation under the same conditions. This step was repeated once more to enhance purity. The final pellet

was resuspended in 200 μ L of 0.9% NaCl to obtain the purified IgG antibody fraction.

Purification of Serum Antibody from Immunized Rabbit

Rabbits were immunized with the Tau294-305 peptide 3 times at a dose of 600 μ g per rabbit per immunization. Complete Freund's adjuvant was used for the first immunization, while incomplete Freund's adjuvant was applied for subsequent immunizations. Antibody titers were detected after each immunization. Once the titers met the requirements, large-volume blood collection was performed. Polyclonal antibodies were purified from the serum separated from whole blood using a HiTrap Protein G HP column: the column was washed and equilibrated until the UV280 value stabilized, followed by serum loading. Elution was conducted with 0.1 M glycine, and eluted samples were collected. The purified polyclonal antibodies were precipitated by saturated ammonium sulfate: the harvested polyclonal antibodies were slowly added dropwise to the saturated ammonium sulfate solution in a volume ratio of 1:1 with continuous stirring. After incubation at 2-8°C for 2 hours, the mixture was centrifuged at $16,000 \times g$ for 30 minutes at 4°C. The precipitate was completely dissolved in an appropriate amount of antibody storage solution and stored at -20°C.

In Vitro Preparation of Pathological Protein Aggregates

Preparation of A β Monomers, Oligomers, and Fibrils

A β aggregates were prepared following a modified protocol as previously described³⁷.

Briefly, 1 mg of synthetic A β ₄₂ peptide was dissolved in 220 μ L of pre-chilled hexafluoroisopropanol (HFIP) and incubated at room temperature in the dark for 1 hour. The solution was aliquoted into Eppendorf tubes (55 μ L/tube) and air-dried under a fume hood for 2 hours to completely evaporate the solvent. Each tube was then resuspended in 11 μ L of DMSO, vortexed to dissolve the peptide, followed by the addition of 539 μ L of DMEM.

For oligomer preparation, the solution was incubated at 4 °C for 24 hours, then centrifuged at 14,000 rpm for 10 minutes. The supernatant containing soluble A β oligomers was collected.

For fibril formation, following DMSO/DMEM dilution, samples were incubated at 37 °C for 7 days. After centrifugation at 14,000 rpm for 10 minutes, the supernatant was discarded, and the pellet was resuspended in 550 µL of DMEM to obtain Aβ fibrils.

For monomer preparation, synthetic Aβ₄₂ peptide was used, and the solution obtained after DMSO/DMEM dilution was directly used as Aβ monomers.

Preparation of Tau Fibrils (Paired Helical Filament-like Aggregates)

Full-length Tau₄₄₁ protein was diluted in 20 mM sodium phosphate buffer (pH 7.4) containing 1 mM EDTA, 1 mM PMSF, 1 mM DTT, and 50 mM NaCl. Heparin was added at a molar ratio of 1:3 (Tau: heparin). The mixture was gently mixed and incubated at 37 °C for 24 hours to allow the formation of Tau fibrils³⁸.

Dot Blot

A nitrocellulose membrane was pre-soaked in electrotransfer buffer for 5 minutes and placed into a dot blot apparatus. Protein samples (10 µL each) were loaded into the wells of the dot blot manifold. After sample application, the membrane was blocked with 5% (w/v) skim milk in TBS for 30 minutes at room temperature with gentle agitation. Primary and secondary antibody incubations, as well as ECL signal detection, were carried out following the procedure described in the Western blotting section.

Aβ uptake assay

As previously described, FITC-conjugated Aβ₄₂ peptide was dissolved in 10 µL of 1% NH₄OH and diluted with PBS to a final concentration of 1 mg/mL, as previously described³⁹. The solution was incubated at 37 °C for 12 h to facilitate oligomer formation and subsequently diluted in DMEM/F12 medium to 5 µg/mL for application to BV2 microglial cells.

BV2 cells were pretreated with IL-4 (40 ng/mL) and dexamethasone (100 ng/mL) for 24 h, followed by incubation with 5 µM FITC-labeled Aβ₄₂ oligomers and 1 µM serum antibodies in DMEM for an additional 24 h. Cells were then washed with PBS and fixed with 4% paraformaldehyde for 15 minutes at room temperature. After

fixation, a final PBS wash was performed, and cells were counterstained with DAPI-containing antifade mounting medium for 5 minutes at room temperature.

Fluorescent images were captured using a CKX41-32PH automated microscope (Olympus). The fluorescence intensity of internalized A β 42 oligomers was quantified on a per-cell basis and expressed as fold change relative to untreated control cells. A minimum of 30 cells per group was analyzed for statistical evaluation.

Transcriptomic Sequencing of Mouse Brain Tissue

Frozen hippocampal tissues from mice were sent to Novogene Co., Ltd. (Beijing, China) for transcriptome sequencing. Differential gene expression analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were subsequently performed based on the sequencing data.

To validate the differentially expressed genes, quantitative polymerase chain reaction (qPCR) was conducted. Total RNA extracted from hippocampal samples was reverse transcribed into cDNA using the PrimeScript™ RT Reagent Kit with gDNA Eraser (Takara) according to the manufacturer's instructions. qPCR was then performed using TB Green® Premix Ex Taq™ II (Takara) with gene-specific primers and cDNA templates to quantify mRNA expression levels. All gene expression data were normalized to the internal control gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

Statistical Analysis

All statistical analyses and data visualizations were performed using GraphPad Prism version 9.5.0. Data are presented as mean $\pm$ standard deviation of the mean (SD). Differences between two groups were assessed using unpaired two-tailed Student's t-tests. For comparisons among multiple groups, one-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used. A p-value < 0.05 was considered statistically significant.

Data and materials availability

All data are available in the main text or the supplementary materials.

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1261 **Author contributions**

1262 Project design: H.W., Y.H., X.F. Investigation: H.W., Y.H., X.F., X.X., X.Y., W.K. Data
1263 analysis: Y.H. Funding acquisition: H.W. Project administration: H.W.
1264 Writing—original draft: Y.H., X.F. Writing—review & editing: H.W.

1265 **Competing interests**

1266 Authors declare that they have no competing interests.

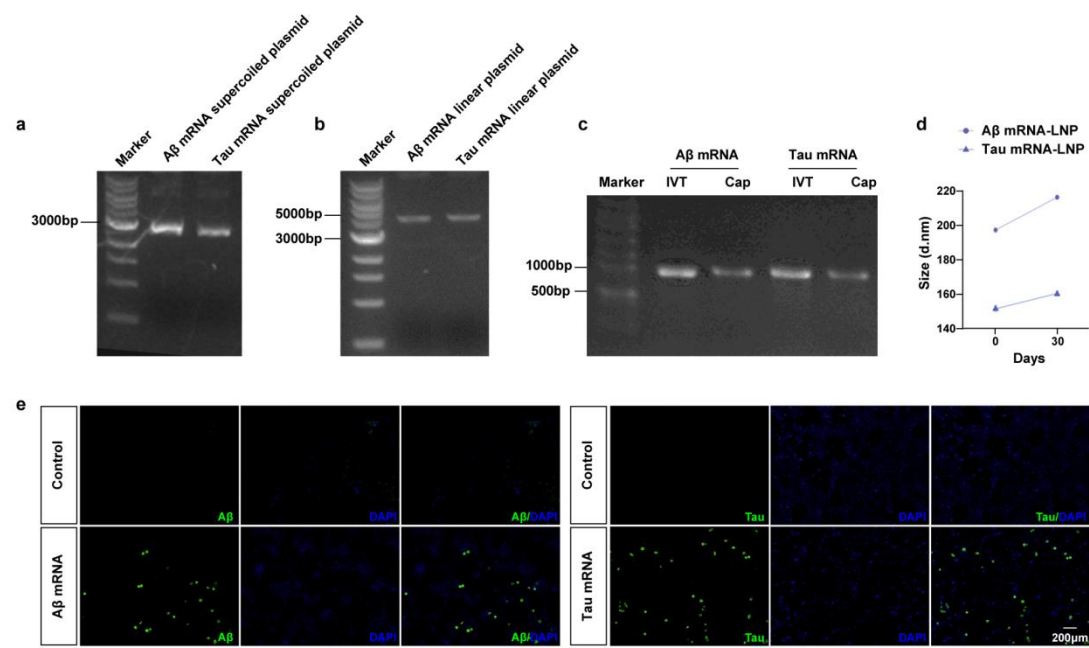


Fig. S1. Preparation and expression analysis of Aβ and Tau mRNA vaccines.

a Agarose gel electrophoresis analysis of supercoiled Aβ and Tau mRNA plasmids. **b** Agarose gel electrophoresis analysis of linear Aβ and Tau mRNA plasmids. **c** Agarose gel electrophoresis analysis of in vitro transcribed and capped Aβ and Tau mRNA products. **d** Size distribution stability of mRNA-LNP vaccines following 30-day storage at 4°C. **e** Immunofluorescence analysis of antigen expression in HEK293T cells transfected with Aβ mRNA (left) and Tau mRNA (right) (Scale bars, 200 μm).

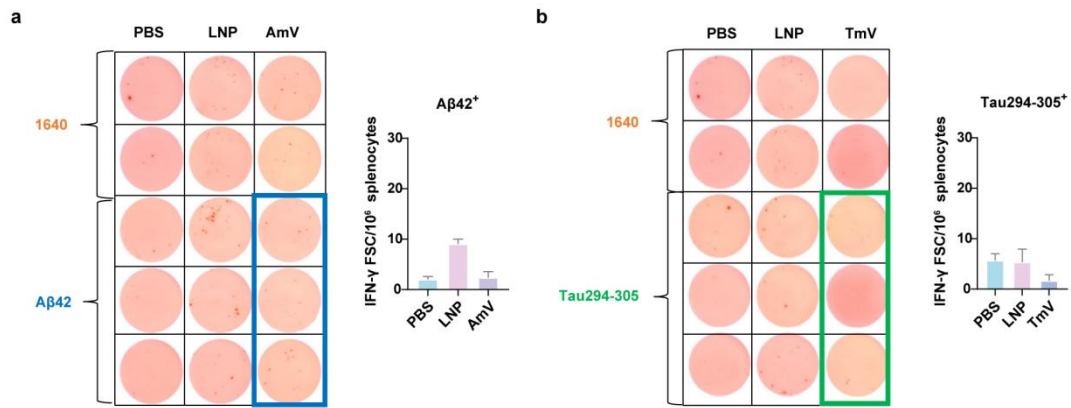


Fig. S2. T cell response analysis to Aβ and Tau antigens in mRNA-vaccinated C57BL/6J mice.

a ELISpot analysis of Aβ42-specific IFN-γ-secreting T cells. **b** ELISpot analysis of Tau-specific IFN-γ-secreting T cells. Data are presented as means ± SD from the number of 3 technical replicates.

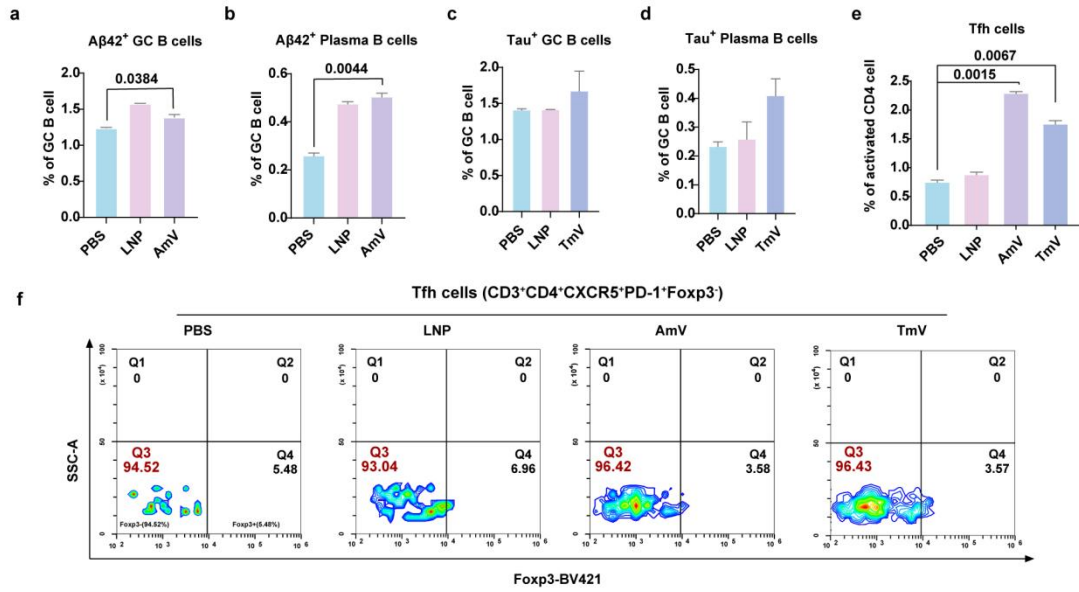


Fig. S3. Cellular immune activation in C57BL/6J mice immunized with AD mRNA vaccine.

a-b Quantification analysis of lymph node-resident germinal center (GC) B cells (**a**) and plasma cells (**b**) from immunized mice. **c-d** Flow cytometric analysis of Tau-specific germinal center (GC) B cells (**c**) and plasma cells (**d**) induced by vaccination. **e** Quantification of lymph node-resident Tfh cells from immunized mice. **f** Representative flow cytometric plots of Tfh cells. Data are presented as mean $\pm$ SD ($n = 3$). Statistical significance was calculated via one-way ANOVA (**a**, **b**, **e**) in GraphPad Prism.

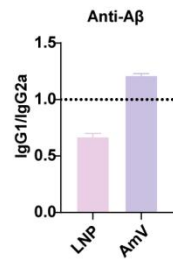


Fig. S4. Th2-biased antibody isotyping of A β mRNA-vaccinated APP/PS1 transgenic mice.

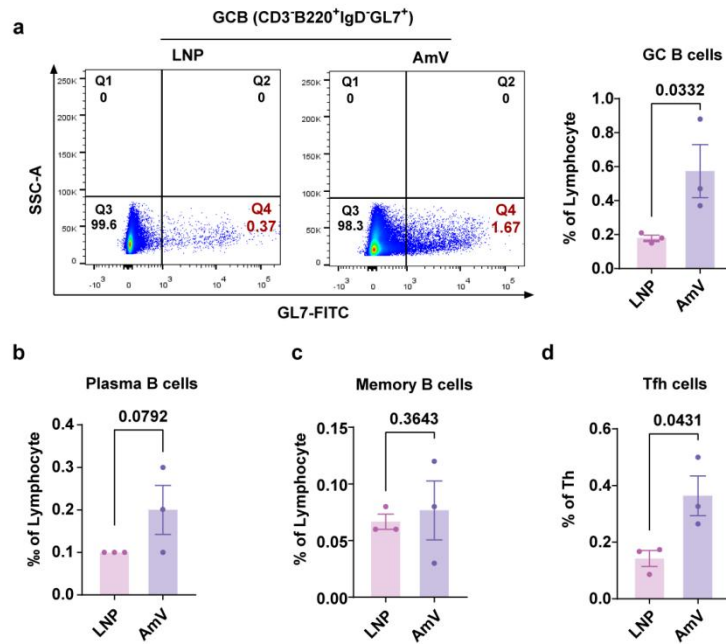


Fig. S5. Immune activation in A β mRNA-vaccinated APP/PS1 transgenic mice.

a Representative flow cytometric plots and quantification of lymph node-resident germinal center (GC) B cells from immunized mice. Data are presented as means $\pm$ SD ($n = 3$). **b-d** Quantification of lymph node-resident plasma B cells (**b**), memory B cells (**c**), and Tfh cells (**d**). Data are presented as mean $\pm$ SD ($n = 3$). Statistical significance was calculated via unpaired t test (**a-d**) in GraphPad Prism.

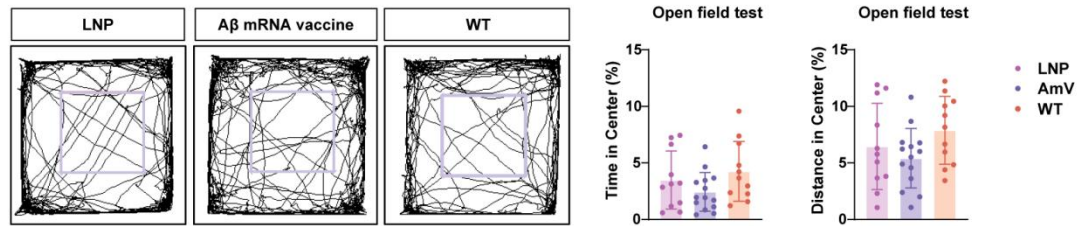


Fig. S6. Representative open field testing movement traces, quantification of time spent, and distance traveled in the center zone across experimental groups in Tau P301S mice (n = 11, 14, 11).

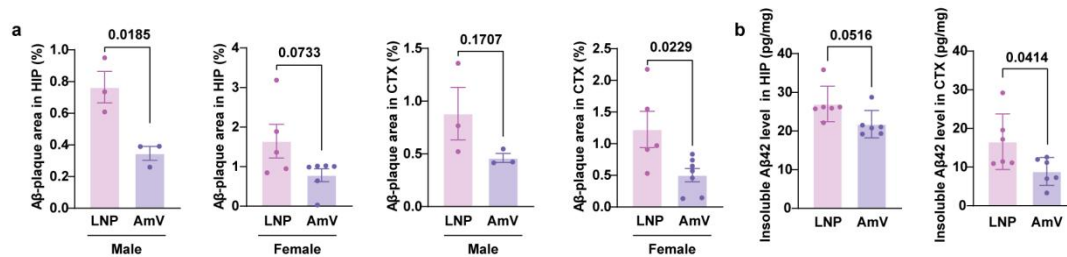


Fig. S7. Quantitative Assessment of Aβ Pathology in APP/PS1 Transgenic Mice.

a Quantification of Aβ plaque-positive area in hippocampal and cortical regions of APP/PS1 transgenic mice by IHC analysis. Data are organized by gender and brain region: male hippocampus, female hippocampus, male cortex, and female cortex. Data are presented as mean ± SD ($n = 3, 5, 6, 7$). **b** Quantification of Aβ42 levels in hippocampal (left) and cortical (right) homogenates from transgenic mice by ELISA. Data are presented as mean ± SD ($n = 6$). Statistical significance was calculated via unpaired t test (**a, b**) in GraphPad Prism.

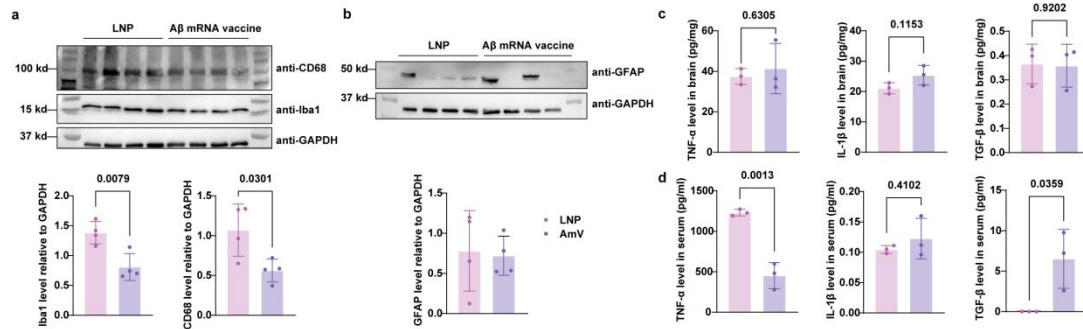


Fig. S8. Glial markers and cytokine levels in Aβ mRNA-vaccinated APP/PS1 mice.

a Western blot analysis and quantification of CD68 and Iba1 levels in the brain. Data are presented as means $\pm$ SD (n=4). **b** Western blot analysis and quantification of GFAP levels in the brain. Data are presented as means $\pm$ SD (n=4). **c** TNF- α , IL-1 β , and TGF- β levels in brain lysates of APP/PS1 mice. Data are presented as means $\pm$ SD (n=3). **d** TNF- α , IL-1 β , and TGF- β levels in serum of APP/PS1 mice. Data are presented as means $\pm$ SD (n=3). Statistical significance was calculated via unpaired *t* test (**a**, **c**, **d**) in GraphPad Prism.

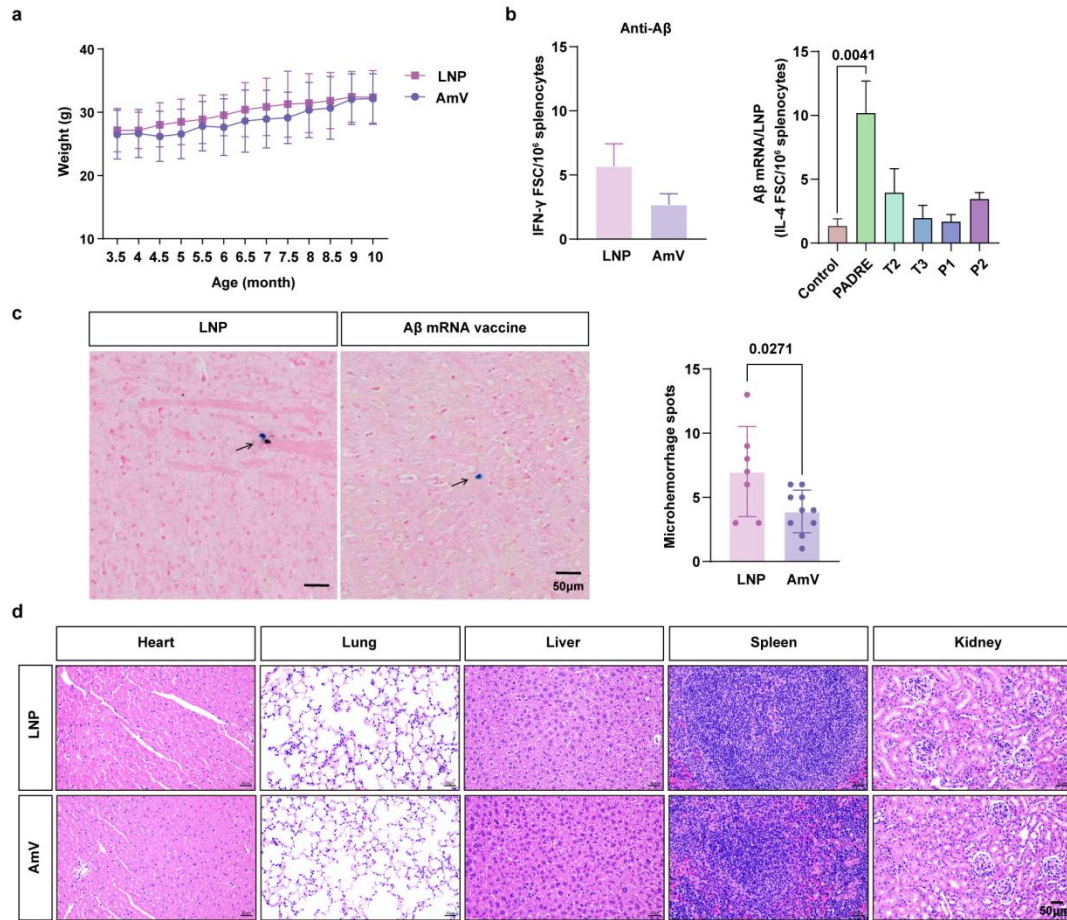


Fig. S9. Comprehensive safety profile of AmV immunization in APP/PS1 mice.

a Body weight monitoring of vaccine group versus LNP control group mice. Data are presented as means $\pm$ SD ($n = 12, 16$). **b** ELISpot analysis of A β 42-specific IFN- γ -secreting and Th epitope-specific IL-4-secreting T cells. Data are presented as means ($\pm$ SD) from the number of 3 technical replicates. **c** Prussian blue staining of cerebral sections. Data are presented as mean $\pm$ SD (scale bar, 50 μ m, $n = 7, 10$). **d** Representative hematoxylin and eosin (H&E) staining images of major organs across experimental groups (scale bar, 50 μ m). Statistical significance was calculated via two-way ANOVA (**a**), one-way ANOVA (**b**), and unpaired t test (**c**) in GraphPad Prism.

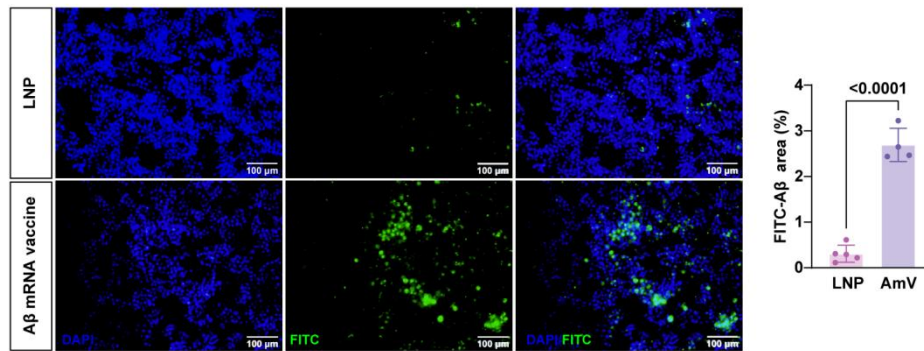


Fig. S10. AmV-induced antibodies promote microglial phagocytosis of A β oligomers.

Immunofluorescence analysis of A β oligomer phagocytosis in BV2 microglia treated with serum antibodies from AmV-immunized mice. FITC-labeled A β 42 oligomers appear green. DAPI nuclear stain appears blue. Data are presented as mean $\pm$ SD (scale bar, 50 μ m, $n = 4, 5$). Statistical significance was calculated via unpaired t test in GraphPad Prism.

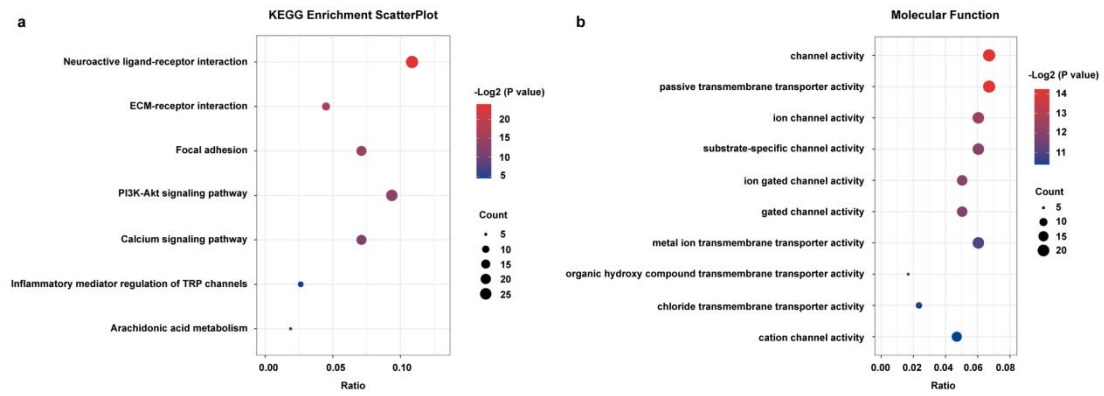


Fig. S11. Transcriptomic profiling of A β mRNA vaccine effects in APP/PS1 mice.
a KEGG pathway analysis of DEGs between AmV and LNP groups. **b** Bubble plot of GO enrichment analysis.

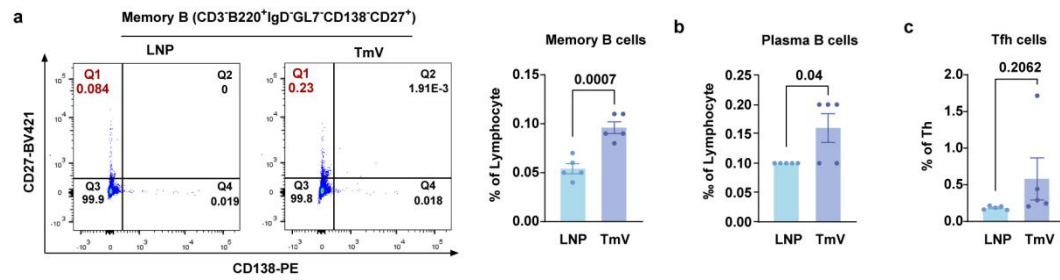


Fig. S12. Immune activation in Tau mRNA-vaccinated TauP301S mice.

a Representative flow cytometry plots and quantification of lymph node-resident memory B cells from immunized mice. Data are presented as means $\pm$ SD ($n = 5$). **b-c** Quantification of lymph node-resident plasma B cells (**b**), and Tfh cells (**c**). Data are presented as mean $\pm$ SD ($n = 5$). Statistical significance was calculated via unpaired t test (**a-c**) in GraphPad Prism.

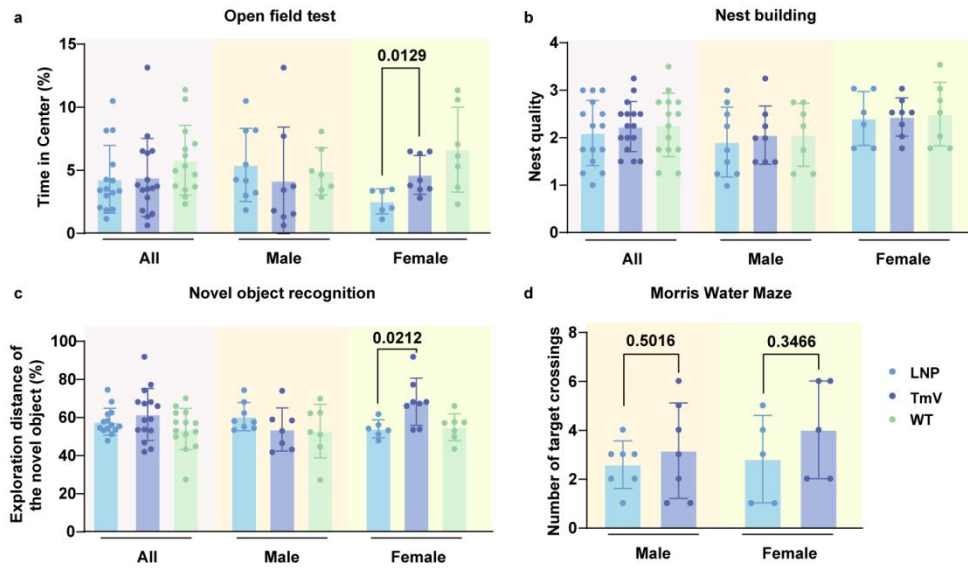


Fig. S13. Multi-dimensional behavioral assessment in TauP301S transgenic mice.

a Center zone occupancy time quantification across experimental groups during open field testing. Data are presented as means $\pm$ SD (n = 14-16). **b** Nest construction score quantification for all mice combined, male cohort, and female cohort. Data are presented as means $\pm$ SD (n = 14-16). **c** Novel object exploration distance quantification. Data are presented as means $\pm$ SD (n = 10-14). **d** Platform crossing frequency quantification for male and female cohorts. Data are presented as means $\pm$ SD (n = 5-7). Statistical significance was calculated via one-way ANOVA (**a-c**) and unpaired *t* test (**d**) in GraphPad Prism.

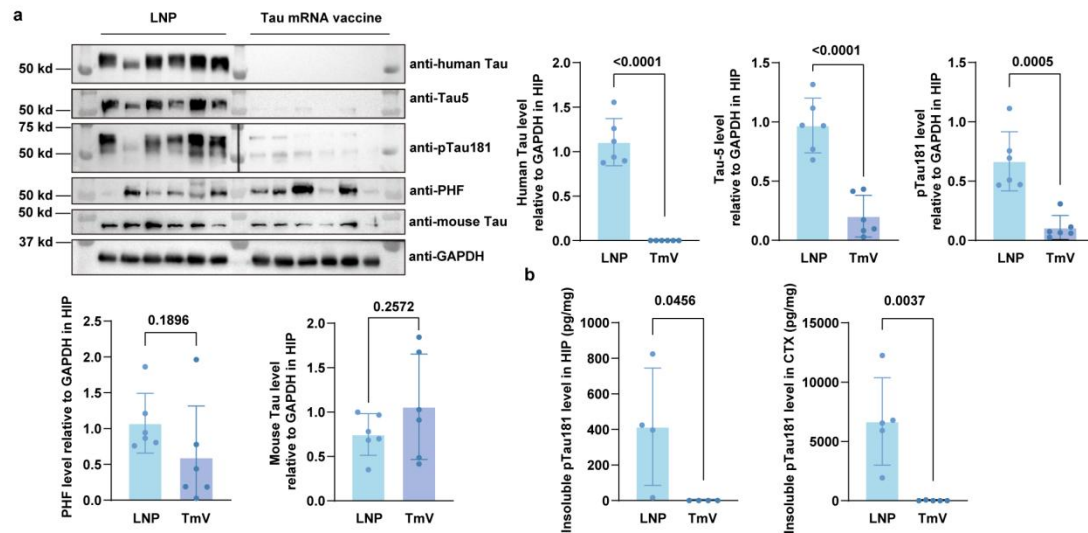


Fig. S14. Comprehensive Tau pathology quantification in Tau mRNA-vaccinated Tau transgenic mice.

a Western blot analysis and quantification of human Tau, Tau5, pTau181, PHF, and mouse Tau protein levels in hippocampal homogenates. Data are presented as means $\pm$ SD ($n = 6$). **b** Concentration of insoluble pTau181 in the hippocampus and cortex of TmV-treated Tau P301S mice. Data are presented as means $\pm$ SD ($n = 4-5$). Statistical significance was calculated via unpaired t test (**a**, **b**) in GraphPad Prism.

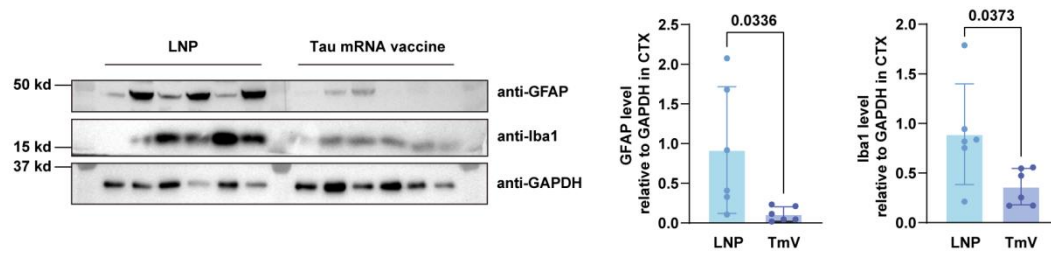


Fig. S15. Western blot analysis and quantification of GFAP and Iba1 levels in the cortex of Tau P301S mice. Data are presented as means $\pm$ SD (n = 6). Statistical significance was calculated via unpaired *t* test in GraphPad Prism.

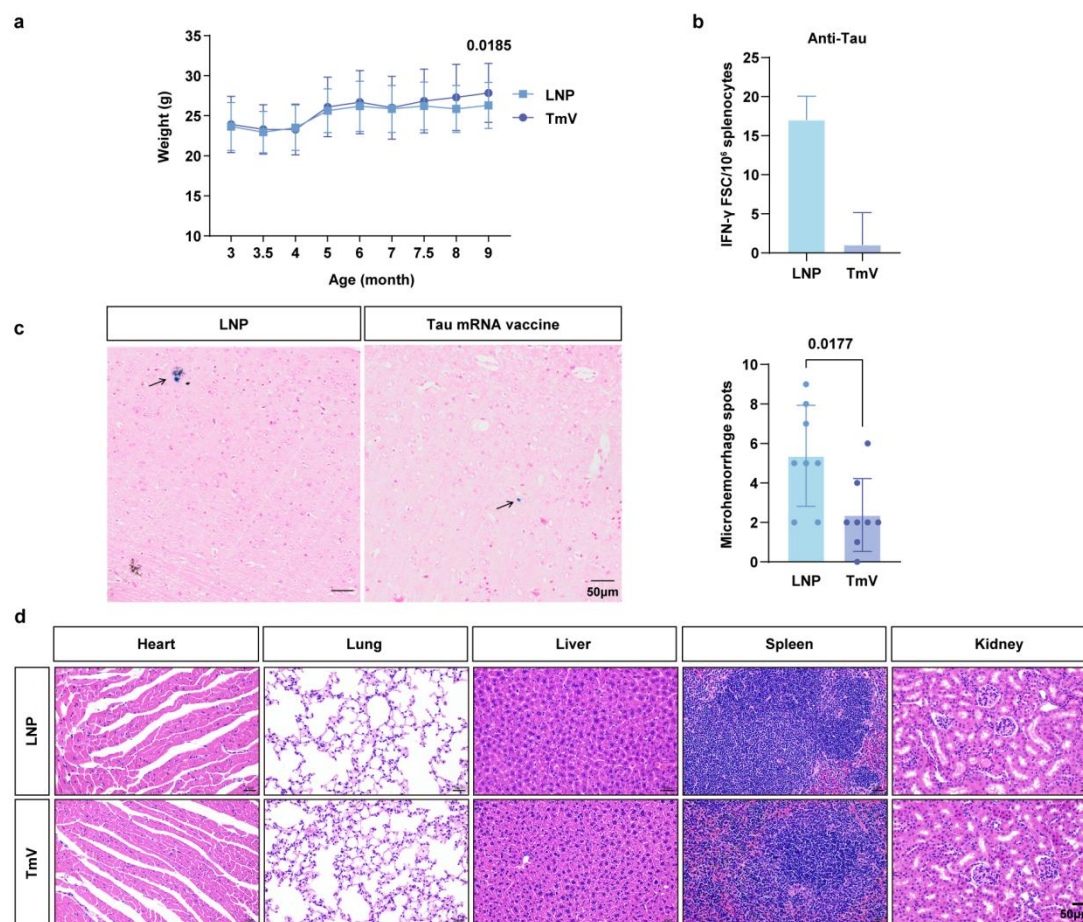


Fig. S16. Systemic safety evaluation of Tau mRNA vaccine in TauP301S transgenic mice.

a Body weight monitoring of vaccine group versus LNP control group mice. Data are presented as means $\pm$ SD ($n = 16$). **b** ELISpot analysis of Tau-specific IFN- γ -secreting and Th-specific IL-4-secreting T cells. Data are presented as means $\pm$ SD from the number of 3 technical replicates. **c** Prussian blue staining of cerebral sections. Data are presented as mean $\pm$ SD ($n = 8$). **d** Histopathological examination of major organs by H&E staining. Statistical significance was calculated via two-way ANOVA (**a**), one-way ANOVA (**b**), and unpaired t test (**c**) in GraphPad Prism.

Th epitope	Amino acid sequence
Norovirus epitope P1	ICTFRGDVTHIAGTQNY
Norovirus epitope P2	GEQLLFFRSTMPGCSG
Tetanus epitope T1	LCIKIKNEDLTFIAEKNSFS
Tetanus epitope T2	LINSTKIYSYFPSVISKVNQ
Tetanus epitope T3	IAESSTQKEKIIKTIDNFLE
PADRE	AKFVAAWTLKAAA

1389 **Table S1. Th cell epitope sequences.**

1390

Cell subtypes	Marker
GC B	CD3 ⁻ B220 ⁺ IgD ⁻ GL7 ⁺
Plasma B	CD3 ⁻ B220 ⁺ CD138 ⁺
Memory B	CD3 ⁻ B220 ⁺ IgD ⁻ GL7 ⁻ CD138 ⁻ CD27 ⁺
Tfh	CD3 ⁺ CD4 ⁺ CXCR5 ⁺ PD-1 ⁺ Foxp3 ⁻

1391 **Table S2. Flow Cytometry Detection Markers.**

1392 anti-CD3 PE/Cyanine5 (100274, Biolegend), anti-B220 APC/Cyanine7 (103224,
 1393 Biolegend), anti-IgD APC (405713, Biolegend), anti-GL7 FITC (144603, Biolegend),
 1394 anti-CD138 PE (142503, Biolegend), and anti-CD27 BV421(124223, Biolegend),
 1395 anti-CD185 (CXCR5) PE (145504, Biolegend), anti-CD4 APC/Cyanine7 (100413,
 1396 Biolegend), anti-PD-1 FITC (135213, Biolegend), anti-FOXP3 BV421 (126419,
 1397 Biolegend).