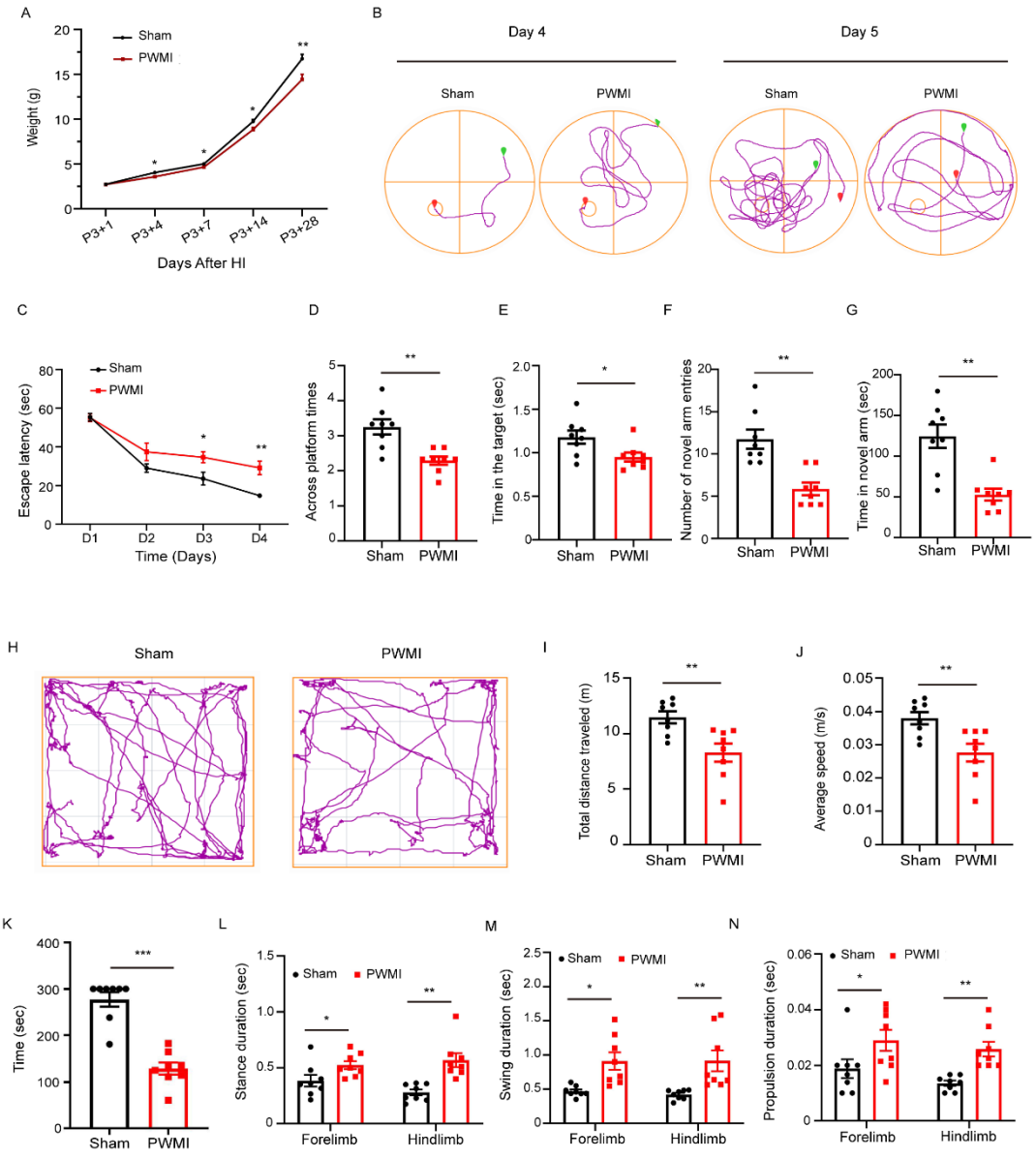
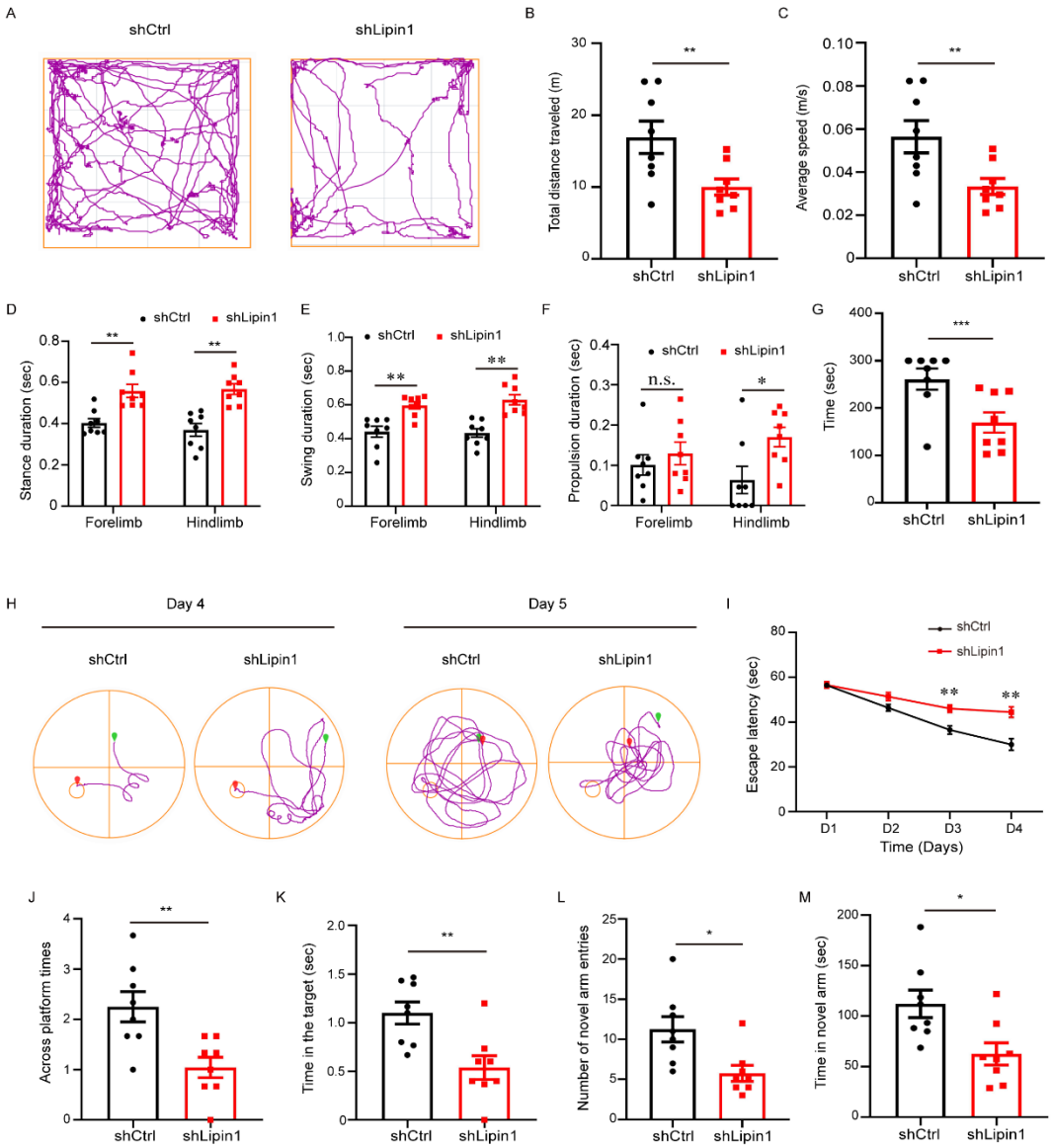
Supplementary Figure 1



Supplementary Figure 1. Impaired Spatial Memory and Motor Function in PWMI Model Mice. A: Comparison of weight gain between PWMI group and Sham group. B: Representative trajectory plots of both groups of mice on days 4 and 5 of the Morris water maze task. C: The average time spent to locate the hidden platform during the first four days of the spatial navigation training in both groups of mice. D-E: The number of crossings and the time spent in the original platform area during the spatial probe test on day 5 in both groups of mice. F-G: The number of entries into the novel arm and the time spent in the novel arm in the Y-maze test for both groups of mice. H: Representative trajectory plots of both groups of mice in the open field test. I-J: Total distance traveled and average speed of both groups of mice in the open field. K: The time spent on the rotating rod by both groups of mice. L-N: Gait analysis of both groups of mice, including the duration of stance, swing, and propulsion phases for the forelimbs

and hindlimbs. n = 8. Statistics, two-tailed Student's t-test (A, D, E, G, I, J, L, M, N), two-way ANOVA with Tukey's multiple comparisons test (C) and Mann-Whitney test (F, K). The results were expressed as Mean ± SEM, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

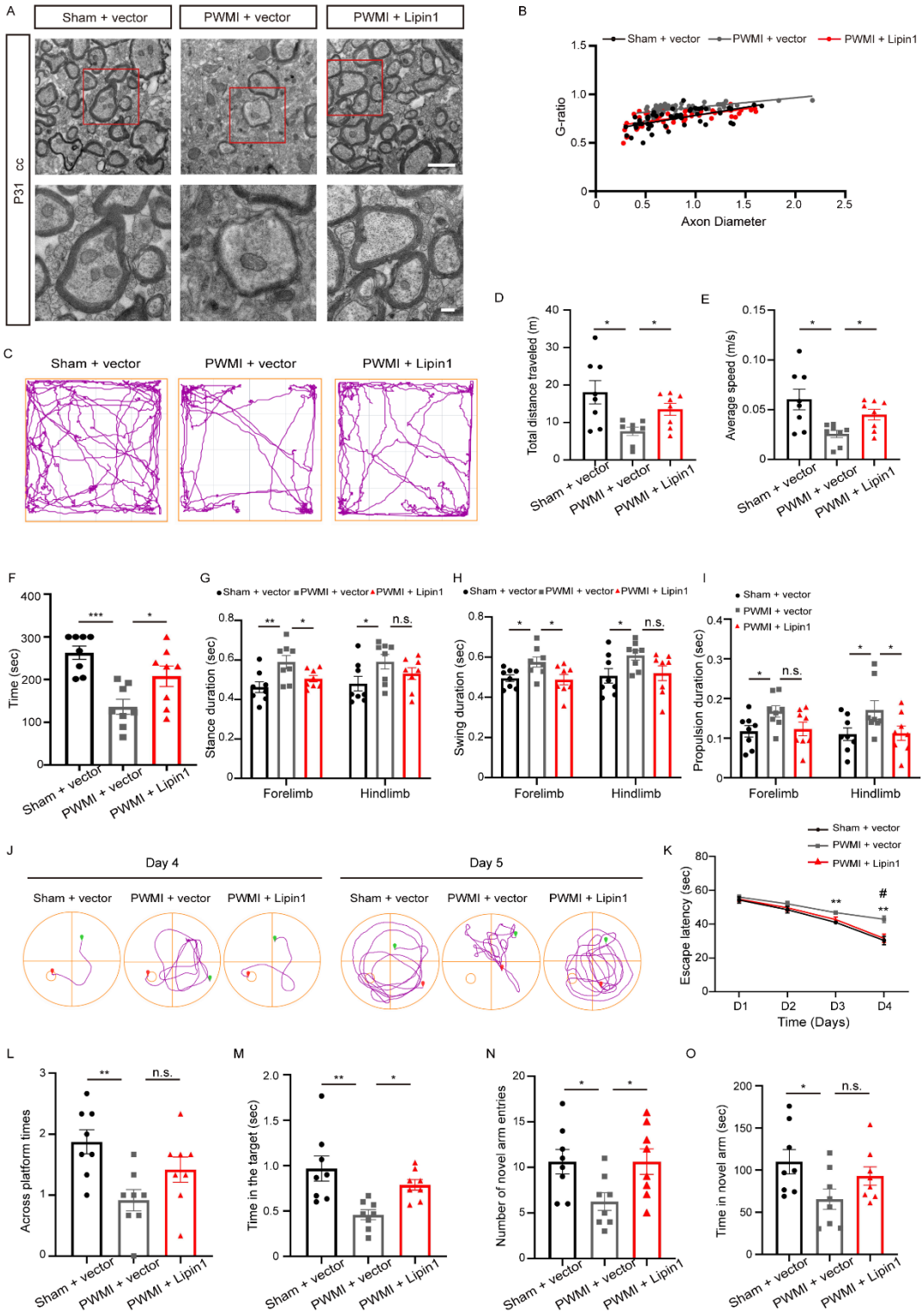
Supplementary Figure 2



Supplementary Figure 2. Downregulation of Lipin1 in Vivo in the Corpus Callosum Resulted in Impaired Spatial Memory and Motor Function in Mice. A: Representative track diagram showing the movement of mice from both groups in the open field. B-C: Total distance traveled and average speed of mice from both groups in the open field. D-F: Gait analysis showing the standing duration, swing duration, and propulsive duration of the forelimb and hindlimb in both groups of mice. G: Duration of time spent by mice from both groups on the rotating rod. H: Morris water maze experiment on days 4 and 5, showing the representative trajectory of mice from both groups. I: The average time taken by both groups of mice to locate the hidden platforms during the first 4 days of the orientation navigation training. J-K: The number of times both groups of mice crossed the original platform and the time spent on it during the 5th day of the spatial exploration experiment. L-M: In the Y-maze experiment, the number of entries into the new arm and the time spent in the new arm by both groups of mice. Statistics, two-tailed Student's t-test (B, C, F, J, K, M), Mann-Whitney test (D,

E, G, L) and two-way ANOVA with Tukey's multiple comparisons test (I). The results were expressed as Mean ± SEM, n = 8, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Supplementary Figure 3



Supplementary Figure 3. Overexpression of Lipin1 in the Corpus Callosum Improves Spatial Memory and Motor Function in PWMI Mice. A: TEM images of myelin in the corpus callosum, with high magnification images shown as insets of low magnification images. Low magnification Bar = 1 μm, high magnification Bar = 200 nm. B: Distribution and statistical analysis of G-ratio of myelinated axons in the three

groups of mice. n = 3. C-E: Representative track diagram, total distance, and average speed of the three mouse groups in the open field. F: The time spent by the three mouse groups on the rotating rod. G-I: Gait analysis measured the standing, swing, and propulsive durations of the forelimb and hindlimb in the three mouse groups. J: Morris water maze experiment on days 4 and 5, showing the representative trajectory of the three mouse groups. K: The average time taken by the three mouse groups to locate the hidden platform during the first 4 days of orientation navigation training, * Sham + vector vs. PWMI + vector; # PWMI + vector vs. PWMI + Lipin1. L-M: The number of times the three mouse groups crossed the original platform and the time spent on it during the space exploration experiment on day 5. N-O: In the Y-maze experiment, the number of entries into the new arm and the time spent in the new arm by the three mouse groups. n = 8. Statistics, one-way ANOVA with LSD post-hoc analysis (D, E, F, G, H, I, L, M, N, O) and two-way ANOVA with Tukey's multiple comparisons test (K). The results were expressed as Mean ± SEM, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Supplementary Figure 4

A

Primer pair 1

	Sequence (5'->3')	Length	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	AAGAGACTGACAACGATCAGGA	22	58.84	45.45	4.00	2.00
Reverse primer	TTCCCCAGAGAACCAGTGGAT	21	60.49	52.38	5.00	5.00

Products on target templates

>NM_001130412.1 Mus musculus lipin 1 (Lpin1), transcript variant 3, mRNA

```
product length = 190
Forward primer  1    AAGAGACTGACAACGATCAGGA  22
Template        475  ......................  496

Reverse primer  1    TTCCCCAGAGAACCAGTGGAT  21
Template        664  ......................  644
```

B

Primer pair 1

	Sequence (5'->3')	Length	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	GGAGAGTGTTTCCTCGTCCC	20	59.75	60.00	4.00	1.00
Reverse primer	ATGAAGGGGTCGTTGATGGC	20	60.39	55.00	3.00	2.00

Products on target templates

>NM_001289726.2 Mus musculus glyceraldehyde-3-phosphate dehydrogenase (Gapdh), transcript variant 1, mRNA

```
product length = 136
Forward primer  1    GGAGAGTGTTTCCTCGTCCC  20
Template        84   ....................  103

Reverse primer  1    ATGAAGGGGTCGTTGATGGC  20
Template        219  ....................  200
```

Supplementary Figure 4. Verification of primer specificity by NCBI Primer-BLAST. A-B: Representative screenshots of Primer-BLAST results for the *Lpin1* and *Gapdh* primers used in RT-qPCR. The analyses confirm that each primer pair generates a single, specific amplicon corresponding to the target gene in *Mus musculus*, without significant off-target alignments. Target transcript IDs include *Lipin1* (GenBank: NM_001130412.1) and *Gapdh* (GenBank: NM_001289726.2).

Supplementary Table S1 List of Lipin1 mRNA-associated Proteins Identified by Mass Spectrometry

RNA pull down	Peptide (No.)
RNA helicase (Ddx3x)	13
Serpine1 mRNA binding protein 1 (Serbp1)	8
Small ribosomal subunit protein (RACK1)	7
RRM domain-containing protein (Hnrnpc)	6
RNA cytidine acetyltransferase (Nat10)	4
DNA-(apurinic or apyrimidinic site) endonuclease (Apex1)	4
Probable ATP-dependent RNA helicase (DDX6)	4
CCN family member 1 (Ccn1)	4
Keratin, type I cytoskeletal 13 (Krt13)	4

Supplementary Table S2 Primer Sequences and qPCR Parameters

Gene	Forward Primer (5’ to 3’)	Reverse Primer (5’ to 3’)	Amplicon Size (bp)	GenBank Accession
Lipin1	AAGAGACTGACA ACGATCAGGA	TTCCCCAGAGA ACCAGTGGAT	190	NM_001130 412.1
Gapdh	GGAGAGTGTTTC CTCGTCCC	ATGAAGGGGTC GTTGATGGC	136	NM_001289 726.2

Supplementary Table S3 List of Lipin1 shRNA and its blank vector sequences

Name	Sequence 5’ to 3’
Lipin1 shRNA	GGAGACAACGGAGAAGCAT
Control shRNA	GTAGCGCGGTGTATTATAC