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Supplemental information

ILF3 prion-like domain regulates gene expression and fear memory under chronic stress

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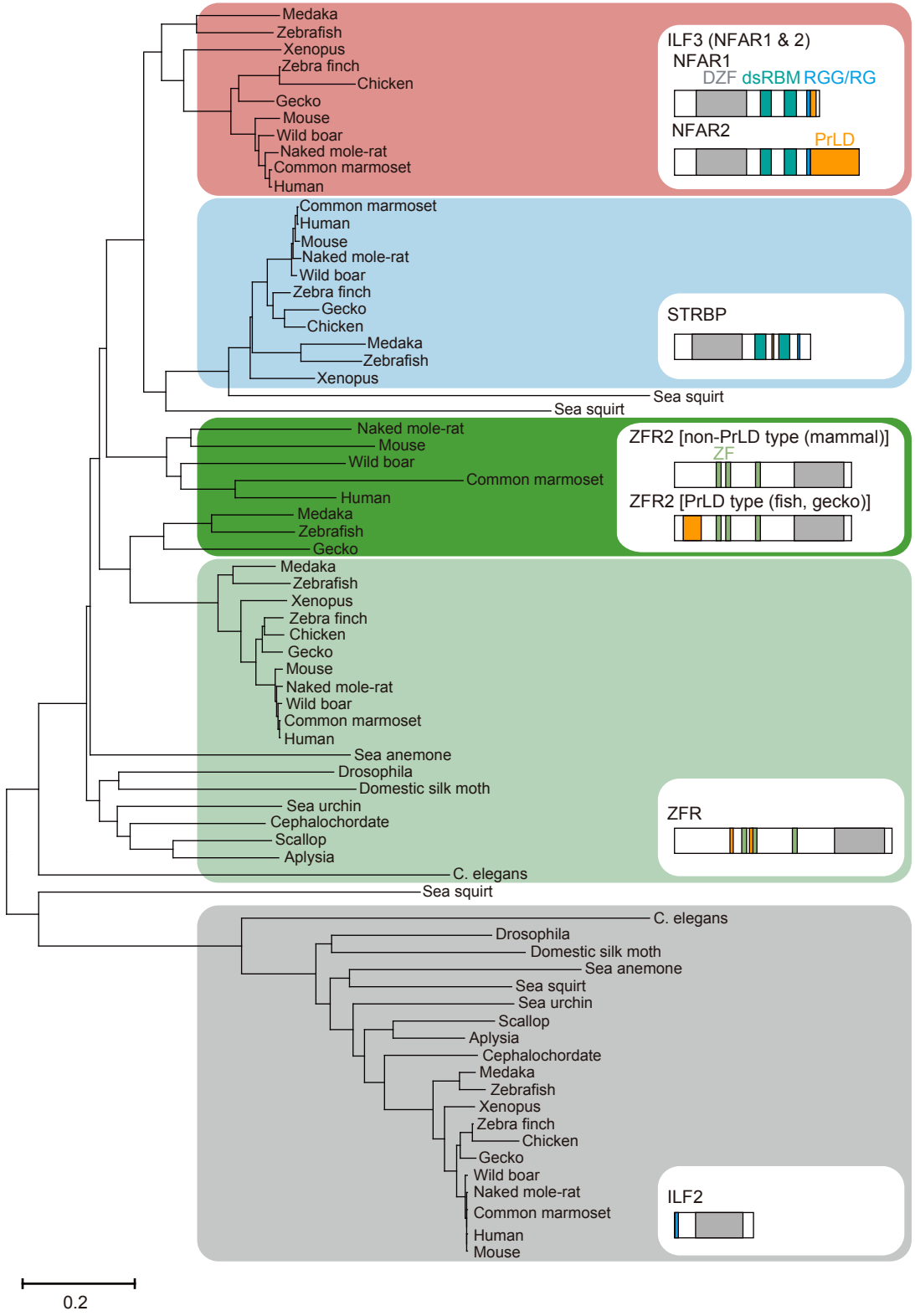


Figure S1. Phylogenetic tree of DZF-containing proteins among species, related to Figure 1
The domain structures of the DZF family proteins, ILF3, STRBP, ZFR2, ZFR, and ILF2 are shown. DZF, double zinc-finger domain; dsRBM, dsRNA-binding motif; RGG/RG, Arg-Gly-Gly/Arg-Gly-rich motif; PrLD, Prion-like domain; ZF, C2H2-type zinc-finger domain. The scale bar indicates the number of amino acid substitutions per site. Note that medaka, zebrafish, and gecko ZFR2 have medium length PrLDs, whereas mammalian ZFR2, including humans and mice, lack PrLDs. There are four family proteins in sea squirts, one of which is classified as ILF2. The other three have non-ZF-type dsRNA-binding domains similar to ILF3 and STRBP, but their DZF domains are at the C-terminus like ZFR and ZFR2, therefore they are not classified in ZFR, ZFR2, STRBP, or ILF3.

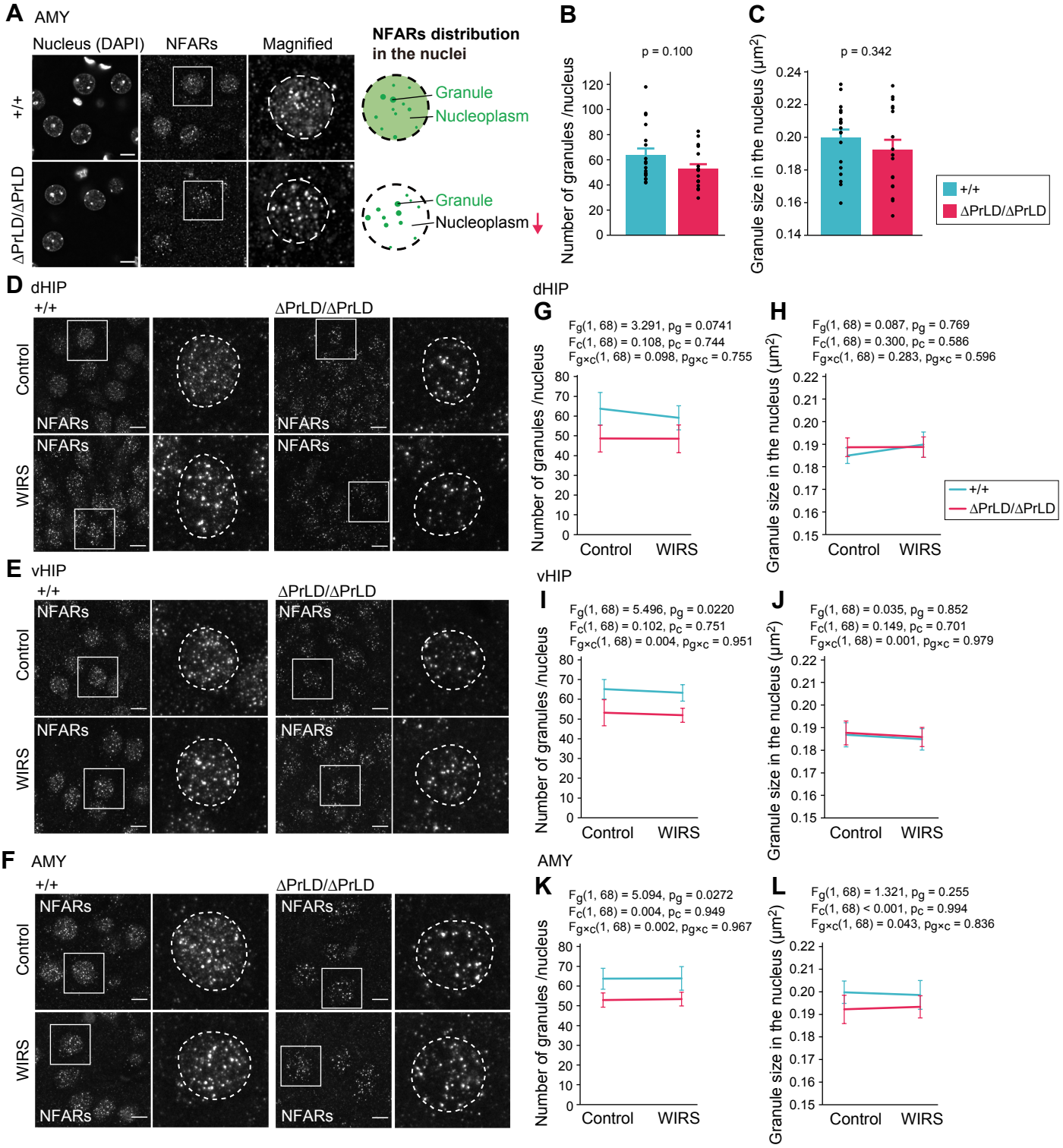


Figure S2. NFARs-containing nuclear granules tend to be reduced in number by PrLD deletion, but not by WIRS, related to Figure 2

(A) Representative images of DAPI and anti-ILF3 (NFARs) antibody staining in the amygdala (AMY) of *Ilf3*$^{+/+}$ and *Ilf3*$^{\Delta PrLD/\Delta PrLD}$ mice, and schematic diagram showing different distributions of NFARs in the nucleus between *Ilf3*$^{+/+}$ and *Ilf3*$^{\Delta PrLD/\Delta PrLD}$ mice.

(B and C) Number (B) and size (C) of nuclear NFARs-containing granules in the AMY.

(D-F) Representative images of NFARs immunostaining in the dorsal hippocampus (dHIP) (D), ventral HIP (vHIP) (E), and AMY (F) of *Ilf3*$^{+/+}$ and *Ilf3*$^{\Delta PrLD/\Delta PrLD}$ mice with and without WIRS. In the magnified images, the white dotted lines outline the nuclei. Scale bars, 10 μm.

(G-L) Number and size of nuclear NFARs-containing granules in the dHIP (G and H), vHIP (I and J), and AMY (K and L) with and without WIRS.

The data are presented as mean ± SEM. n = 18 cells from 3 mice in each group. In B and C, the p-values in the Student's t-test are indicated. In G-L, the data were analyzed by two-way ANOVA. The F-values and p-values for the main effects of genotype (F_g and p_g, respectively) and condition (with or without WIRS) (F_c and p_c, respectively), and the interaction effect between genotype and condition ($F_{g\times c}$ and $p_{g\times c}$, respectively) are indicated.

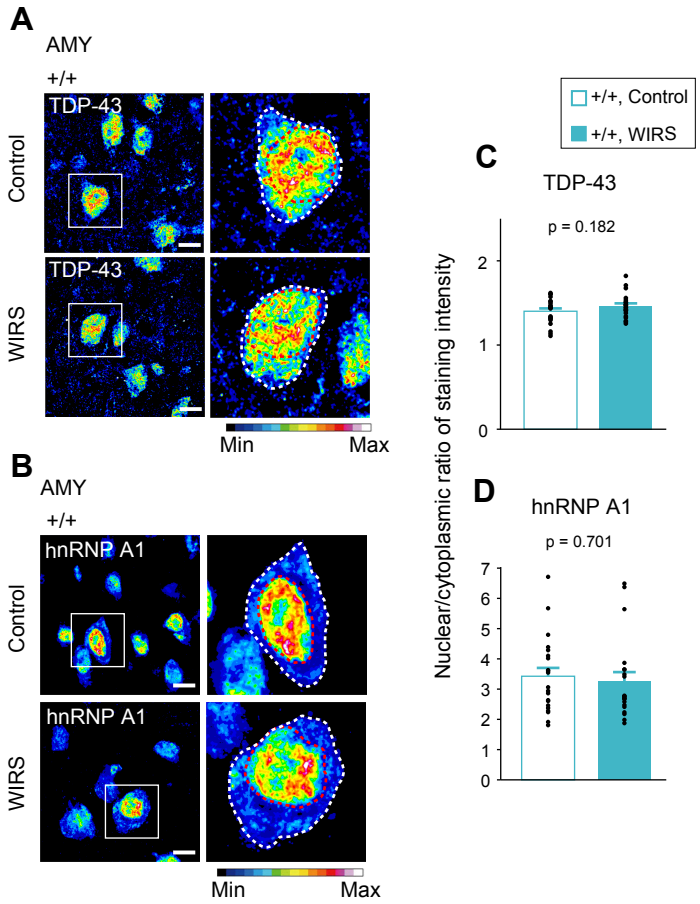


Figure S3. PrLD-bearing proteins TDP-43 and hnRNP A1 do not show WIRS-induced translocation in the AMY, related to Figure 2

(A and B) Representative heatmap images of TDP-43 (A) and hnRNP A1 (B) immunostained in the AMY of *Ilf3*$^{+/+}$ mice with and without WIRS. In the right magnified images, the white and red dotted lines outline the cells and nuclei, respectively. Scale bars, 10 μm.

(C and D) Nuclear-cytoplasmic ratio of TDP-43 (C) and hnRNP A1 (D) staining intensity in the AMY. The data are presented as mean ± SEM. n = 21 pictures from three mice in each group. Student's t-test p-values are indicated.

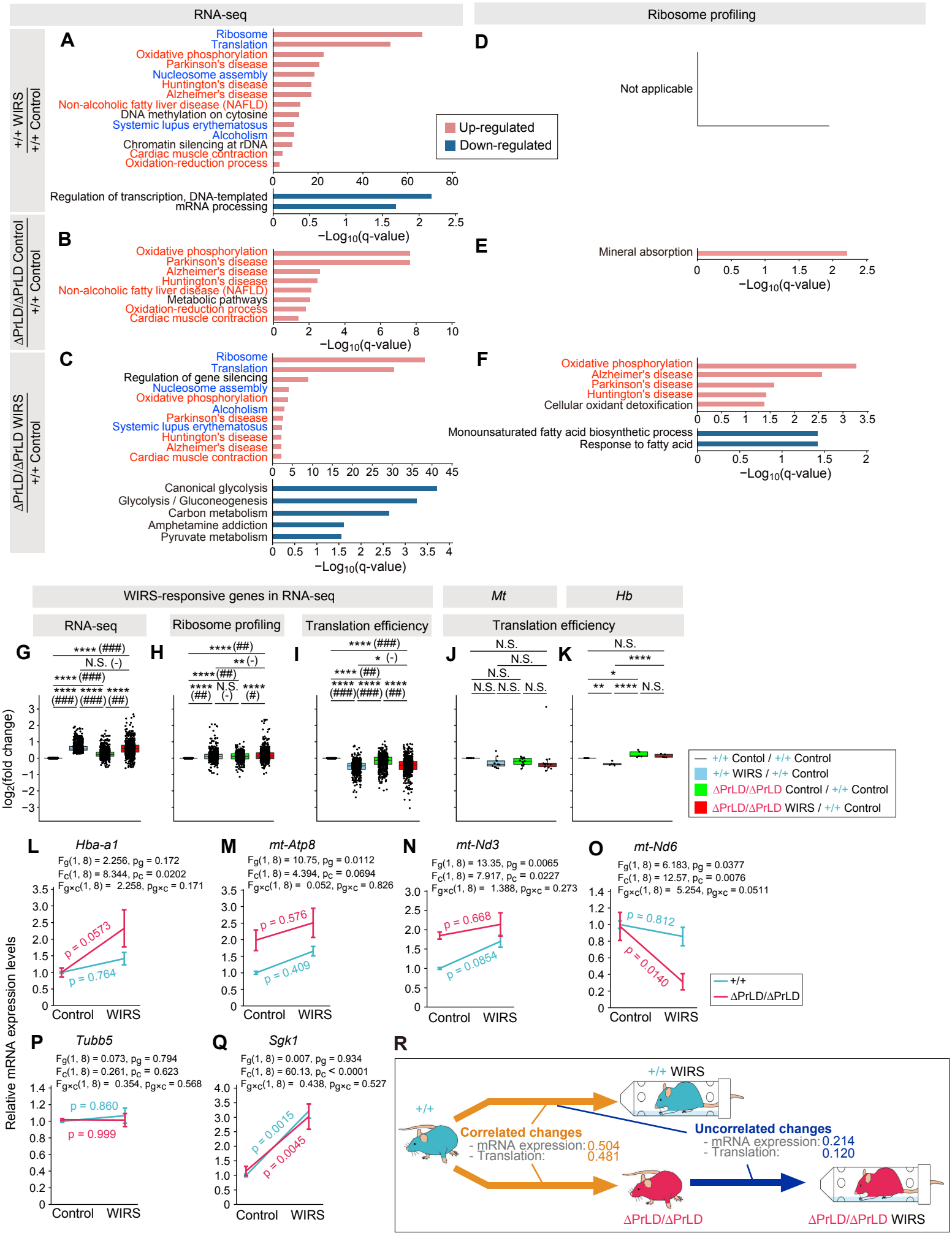

RNA-seq
Ribosome profiling
+/+ WIRS / +/+ Control
ΔPrLD/ΔPrLD Control / +/+ Control
ΔPrLD/ΔPrLD WIRS / +/+ Control
A
Ribosome
Translation
Oxidative phosphorylation
Parkinson's disease
Nucleosome assembly
Huntington's disease
Alzheimer's disease
Non-alcoholic fatty liver disease (NAFLD)
DNA methylation on cytosine
Systemic lupus erythematosus
Alcoholism
Chromatin silencing at rDNA
Cardiac muscle contraction
Oxidation-reduction process
Regulation of transcription, DNA-templated
mRNA processing
Up-regulated
Down-regulated
−Log10(q-value)
B
Oxidative phosphorylation
Parkinson's disease
Alzheimer's disease
Huntington's disease
Non-alcoholic fatty liver disease (NAFLD)
Metabolic pathways
Oxidation-reduction process
Cardiac muscle contraction
−Log10(q-value)
C
Ribosome
Translation
Regulation of gene silencing
Nucleosome assembly
Oxidative phosphorylation
Alcoholism
Parkinson's disease
Systemic lupus erythematosus
Huntington's disease
Alzheimer's disease
Cardiac muscle contraction
Canonical glycolysis
Glycolysis / Gluconeogenesis
Carbon metabolism
Amphetamine addiction
Pyruvate metabolism
−Log10(q-value)
D
Not applicable
E
Mineral absorption
−Log10(q-value)
F
Oxidative phosphorylation
Alzheimer's disease
Parkinson's disease
Huntington's disease
Cellular oxidant detoxification
Monounsaturated fatty acid biosynthetic process
Response to fatty acid
−Log10(q-value)
WIRS-responsive genes in RNA-seq
Mt
Hb
RNA-seq
Ribosome profiling
Translation efficiency
Translation efficiency
G
H
I
J
K
log2(fold change)
+/+ Contol / +/+ Control
+/+ WIRS / +/+ Control
ΔPrLD/ΔPrLD Control / +/+ Control
ΔPrLD/ΔPrLD WIRS / +/+ Control
L
Hba-a1
Fg(1, 8) = 2.256, pg = 0.172
Fc(1, 8) = 8.344, pc = 0.0202
Fg×c(1, 8) = 2.258, pg×c = 0.171
p = 0.0573
p = 0.764
M
mt-Atp8
Fg(1, 8) = 10.75, pg = 0.0112
Fc(1, 8) = 4.394, pc = 0.0694
Fg×c(1, 8) = 0.052, pg×c = 0.826
p = 0.576
p = 0.409
N
mt-Nd3
Fg(1, 8) = 13.35, pg = 0.0065
Fc(1, 8) = 7.917, pc = 0.0227
Fg×c(1, 8) = 1.388, pg×c = 0.273
p = 0.668
p = 0.0854
O
mt-Nd6
Fg(1, 8) = 6.183, pg = 0.0377
Fc(1, 8) = 12.57, pc = 0.0076
Fg×c(1, 8) = 5.254, pg×c = 0.0511
p = 0.812
p = 0.0140
+/+
ΔPrLD/ΔPrLD
Control
WIRS
Relative mRNA expression levels
P
Tubb5
Fg(1, 8) = 0.073, pg = 0.794
Fc(1, 8) = 0.261, pc = 0.623
Fg×c(1, 8) = 0.354, pg×c = 0.568
p = 0.860
p = 0.999
Q
Sgk1
Fg(1, 8) = 0.007, pg = 0.934
Fc(1, 8) = 60.13, pc < 0.0001
Fg×c(1, 8) = 0.438, pg×c = 0.527
p = 0.0015
p = 0.0045
R
+/+
+/+ WIRS
Correlated changes
- mRNA expression: 0.504
- Translation: 0.481
Uncorrelated changes
- mRNA expression: 0.214
- Translation: 0.120
ΔPrLD/ΔPrLD
ΔPrLD/ΔPrLD WIRS

Figure S4. mRNAs and GO terms that were differentially regulated by WIRS and PrLD deletion in the AMY, related to Figure 3

(A-F) GO enrichment analysis of mRNAs of which expression (A-C) and translation (D-F) up- or down-regulated ($q < 0.05$). Shown are the overrepresented GO terms of mRNAs altered by WIRS in *Ilf3*$^{+/+}$ mice (A and D), PrLD deletion under control conditions (B and E), and the combinational effects of PrLD deletion and WIRS (C and F). Blue letters indicate GO terms that commonly appear in A and C, and red letters, in A-C and F.

(G-I) Box plots showing the changes in mRNA expression (G), translation (H), and translation efficiency (I) of WIRS-responsive genes in RNA-seq (defined as genes up-regulated in mRNA abundance by WIRS in *Ilf3*$^{+/+}$ mice, Figure 3B, red dots).

(J and K) Box plots showing the changes in translation efficiency of the *Mt* (J) and *Hb* (K) genes.

In G-K, ****$p < 0.0001$, **$p < 0.01$, *$p < 0.05$, Welch's t-test with Bonferroni correction. N.S., no significant difference ($p \geq 0.05$). Due to the high number of transcripts (620) in G-I, the effect size (Hedges' g) is also shown. ###, $|g| \geq 0.8$; ##, $0.5 \leq |g| < 0.8$; #, $0.2 \leq |g| < 0.5$; -, $|g| < 0.2$.

(L-Q) Quantitative RT-PCR of representative genes. Equal amounts of mRNA extracted from the AMY in each group of mice were subjected to the analysis. The levels of mRNA expression are shown in folds compared with those in *Ilf3*$^{+/+}$ mice under control conditions. *Tubb5* and *Sgk1* mRNAs are WIRS-unaffected and WIRS-induced controls, respectively (P and Q). *Hba-a1* (L), *mt-Atp8* (M), and *mt-Nd3* (N) mRNA levels were increased by PrLD deletion and/or WIRS, whereas *mt-Nd6* (O) mRNA levels were decreased, which confirmed the results in RNA-seq. Data are presented as mean ± SEM. n = 3. The data were analyzed by two-way ANOVA. The F-values and p-values for the main effects of genotype (F_g and p_g, respectively) and condition (with or without stress) (F_c and p_c, respectively), and the interaction effect between genotype and condition ($F_{g\times c}$ and $p_{g\times c}$, respectively) are indicated. The p-values for the Tukey-Kramer test after the two-way ANOVA are shown in blue (*Ilf3*$^{+/+}$) and red (*Ilf3*$^{\Delta PrLD/\Delta PrLD}$).

(R) A model diagram summarizing the correlation of changes in mRNA expression and translation in the AMY caused by WIRS and PrLD deletion. Numbers indicate Pearson correlation coefficients between the genotypes shown in Figures 3K, 3M, 3Q, and 3S.

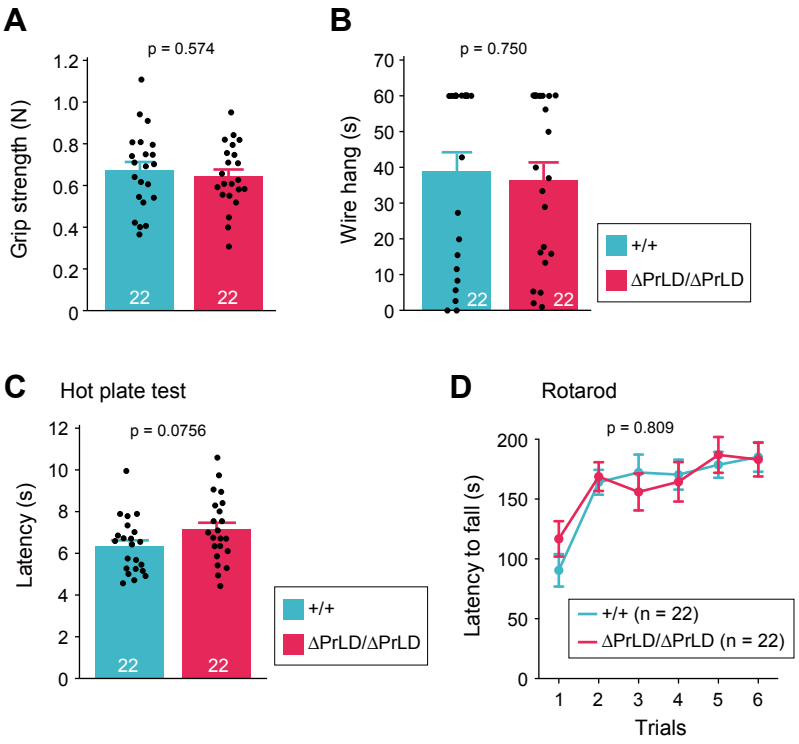


Figure S5. *Ilf3*$^{\Delta PrLD/\Delta PrLD}$ mice exhibit normal physical and sensory abilities, related to Figure 4

(A and B) Grip strength (A) and latency to fall in the wire hang test (B) for assessment of neuromuscular strength.
(C) Latency to withdraw the paw from the hot plate (paw shaking or licking) to assess the reaction to pain.
(D) Latency to fall from the rod in the rotarod test to evaluate motor coordination.
Data are presented as mean ± SEM. The numbers in the bars indicate the number of mice tested. The p-values for one-way ANOVA (A-C) and the main effect of genotype in two-way repeated measures ANOVA (D) are indicated.

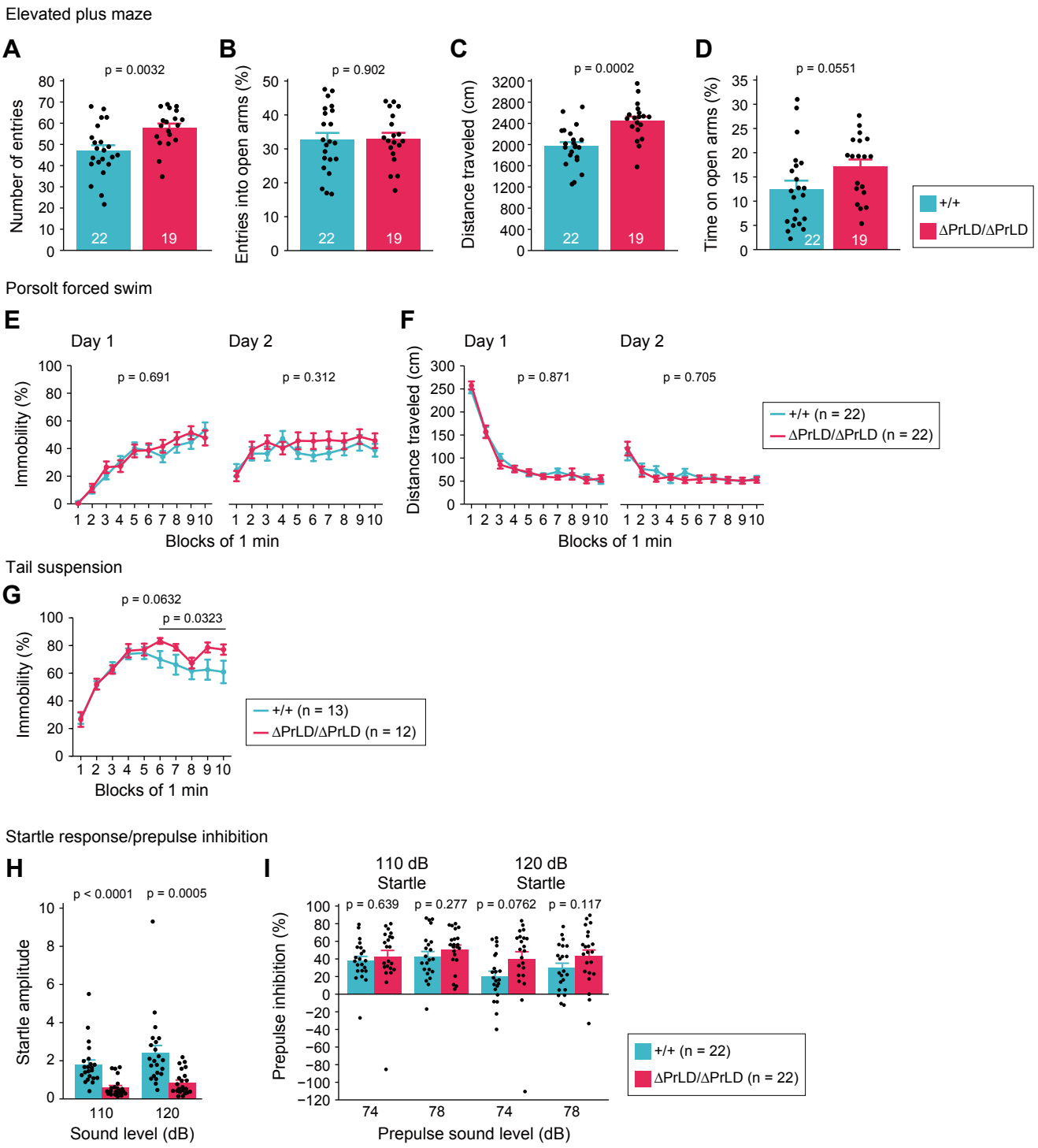


Figure S6. Results of emotion-related behavioral tests, related to Figure 4

(A-D) Elevated plus maze test for the evaluation of anxiety-related behaviors. The number of arm entries (A), percentage of entries to open arms (B), total distance traveled (C), and percentage of time spent on open arms (D). *Ilf3*$^{\Delta PrLD/\Delta PrLD}$ mice showed hyperactivity (A and C) and tended to reduce anxiety-like behavior (D).

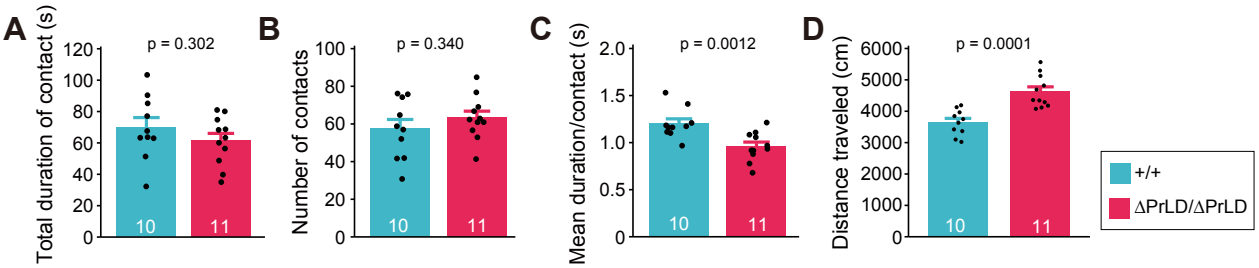
(E and F) Porsolt forced swim test to assess depression-related behaviors. Percentage of immobility time on days 1 and 2 (E), and distance traveled on days 1 and 2 (F).

(G) Percentage of immobility time in the tail suspension test. *Ilf3*$^{\Delta PrLD/\Delta PrLD}$ mice showed more depression-related immobility than *Ilf3*$^{+/+}$ mice during the last 5 min.

(H and I) Startle response/prepulse inhibition test. Startle response to 110 dB and 120 dB startle stimuli (H) and inhibition of the startle response by 74 dB and 78 dB prepulse sounds (I). *Ilf3*$^{\Delta PrLD/\Delta PrLD}$ mice showed a marked reduction in startle response (H).

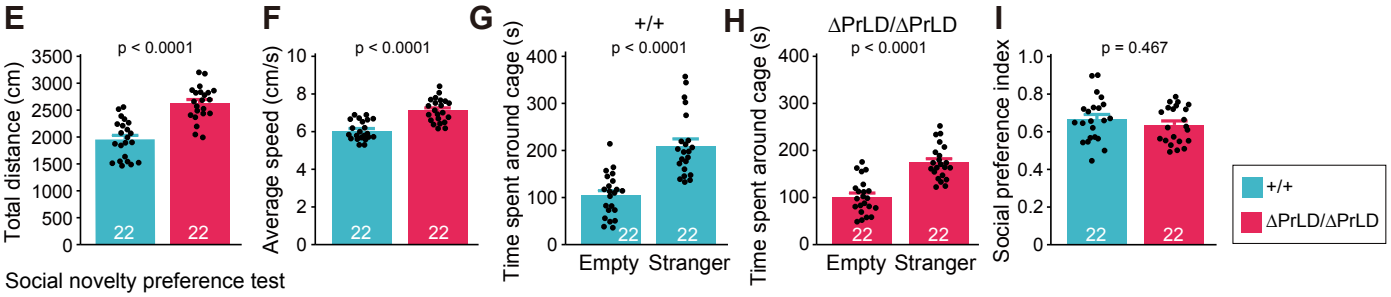
Data are presented as mean ± SEM. The numbers in the bars indicate the number of mice tested. The p-values for one-way ANOVA (A-D, H, and I) and the main effect of genotype in two-way repeated measures ANOVA (E-G) are indicated.

Social interaction test in a novel environment

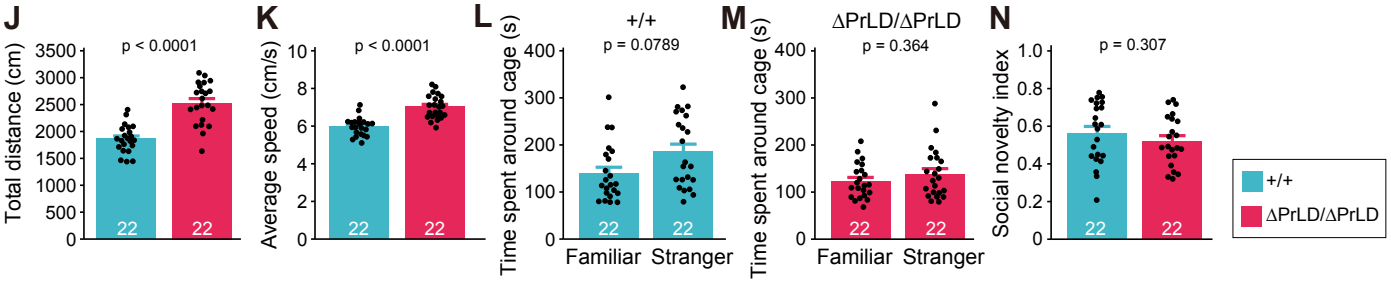


Three-chambered social approach test (100 lux)

Sociability test

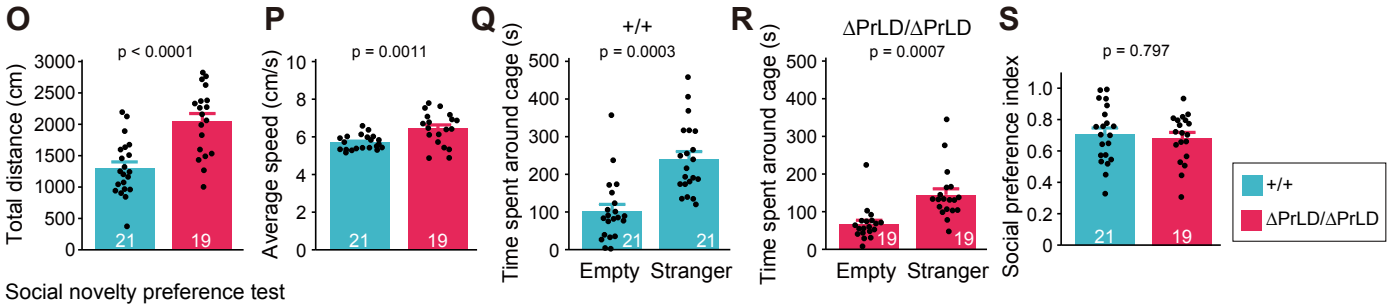


Social novelty preference test

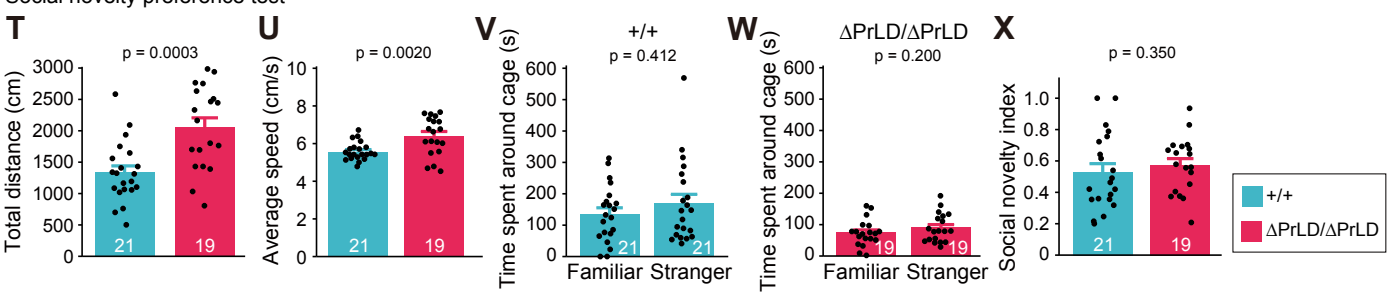


Three-chambered social approach test (5 lux)

Sociability test



Social novelty preference test



Social interaction test in a home cage

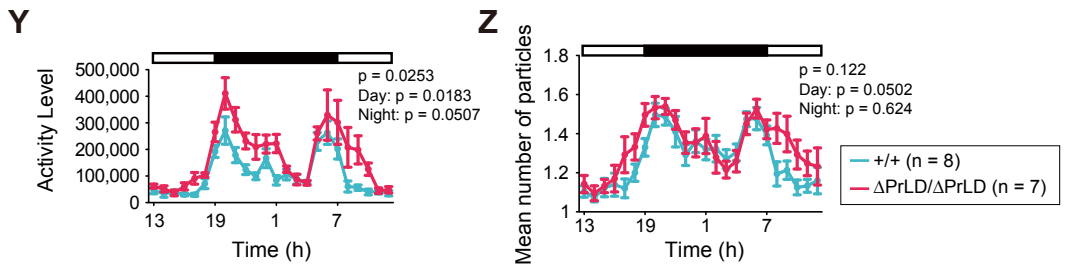


Figure S7. *Ilf3*$^{\Delta PrLD/\Delta PrLD}$ mice show normal sociality but hyperactivity in social behavior tests, related to Figure 4

(A-D) Social interaction test in a novel environment. Pairs of mice of the same genotype were tested. The total duration of contact (A), number of contacts (B), mean duration per contact (C), and total distance traveled (D).
(E-X) Three-chambered social approach test at 100 lux (E-N) and 5 lux (O-X). The results of the sociability test (E-I and O-S) and the social novelty preference test (J-N and T-X) are shown. Total distance traveled (E, J, O, and T); average locomotor speed (F, K, P, and U); time spent around the indicated cages by *Ilf3*$^{+/+}$ mice (G, L, Q, and V) and *Ilf3*$^{\Delta PrLD/\Delta PrLD}$ mice (H, M, R, and W); social preference index (I and S) and social novelty index (N and X). The social preference index and the social novelty index were calculated as the ratio of the time spent around the stranger's cage to the time spent around both cages.
(Y and Z) Social interaction test in a home cage. White and black bars indicate lights on and off, respectively. The data are represented as the average of three days (day 3 to day 5) from the 7-day experiment. Locomotor activity level (Y), and mean number of particles for determining social interaction between two mice (Z). When the mice are apart from each other, the particle number is two, and the particle number is one when they are close to each other.
Data are presented as mean ± SEM. The numbers in the bars indicate the number of pairs (A-D) and mice (E-X) tested. P-values for one-way ANOVA (A-X) and the main effect of genotype in two-way repeated measures ANOVA (Y and Z) are indicated.

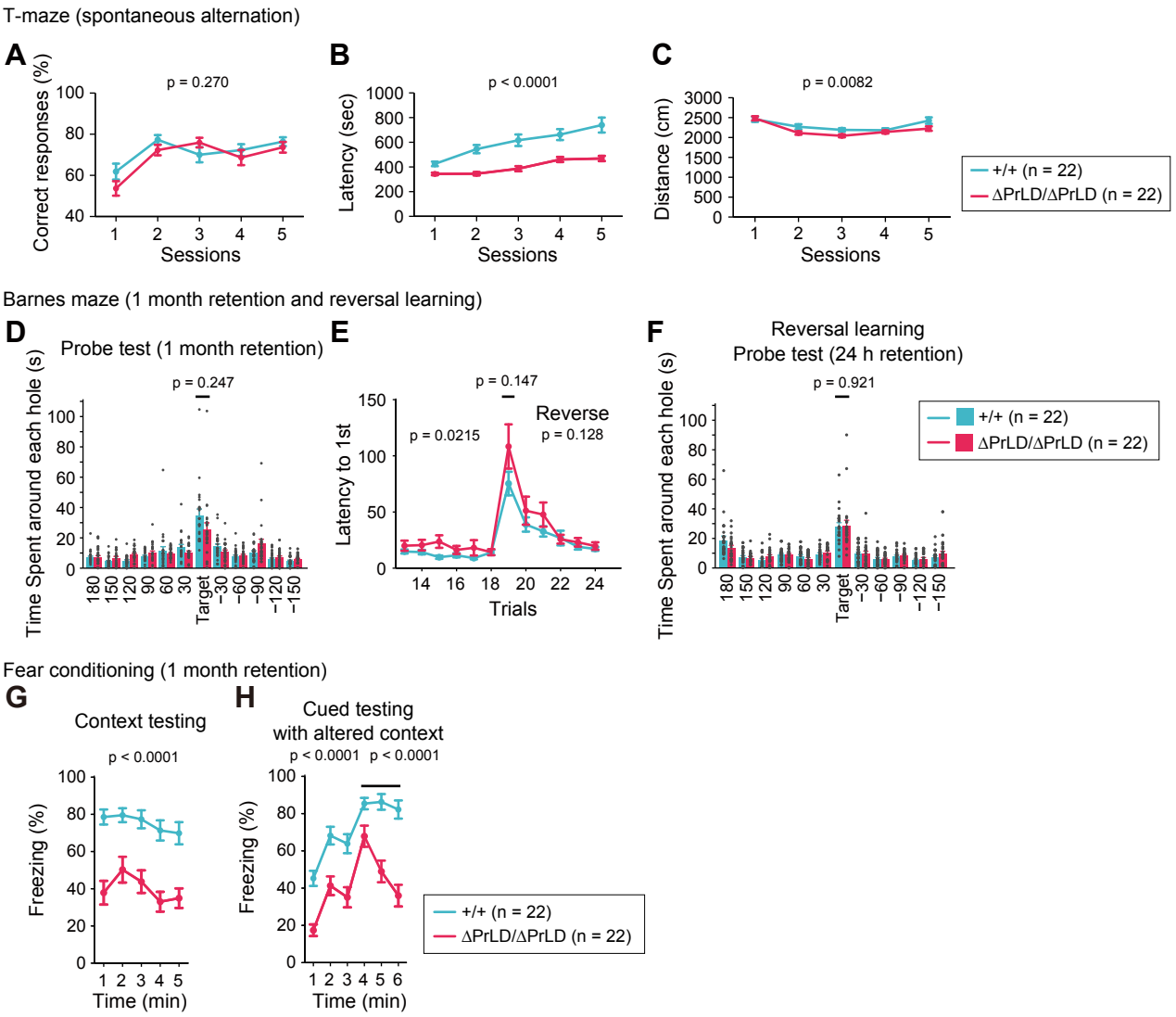


Figure S8. Results of learning and memory tests, related to Figure 4

(A-C) T-maze spontaneous alternation task. Percentage of correct responses (A), latency to finish each trial (B), and total distance traveled (C). *Ilf3*$^{\Delta PrLD/\Delta PrLD}$ mice showed equivalent working memory (A) but performed faster than *Ilf3*$^{+/+}$ mice (B and C).

(D-F) The 1-month memory retention and reversal learning in the Barnes maze test. (D) Time spent around each hole in the 2nd probe test conducted one month after the 1st probe test (see Figures 4L-4N). (E) The latency to the first visit to the correct hole in 6 additional trials after the 2nd probe test (trials 13-18) and subsequent reversal training (trials 19-24). (F) Time spent around each hole 24 h after the last reversal training. *Ilf3*$^{\Delta PrLD/\Delta PrLD}$ mice showed normal memory retention for one month (D) although subsequent task performance was low (trials 13-18 in E). Their spatial reversal learning and memory were also normal (trials 19-24 in E, and F).

(G and H) The 1-month retention of contextual and cued fear conditioning memory. One month after the fear conditioning (see Figures 4O-4R), mice were tested for contextual memory (G) and cued memory (H). The results were similar to those one day after the conditioning (see Figures 4P-4R).

Data are presented as mean ± SEM. The p-values for the main effect of genotype in two-way repeated measures ANOVA (A-C, E, G, and H) and one-way ANOVA (D, F, and trial 19 in E) are indicated.

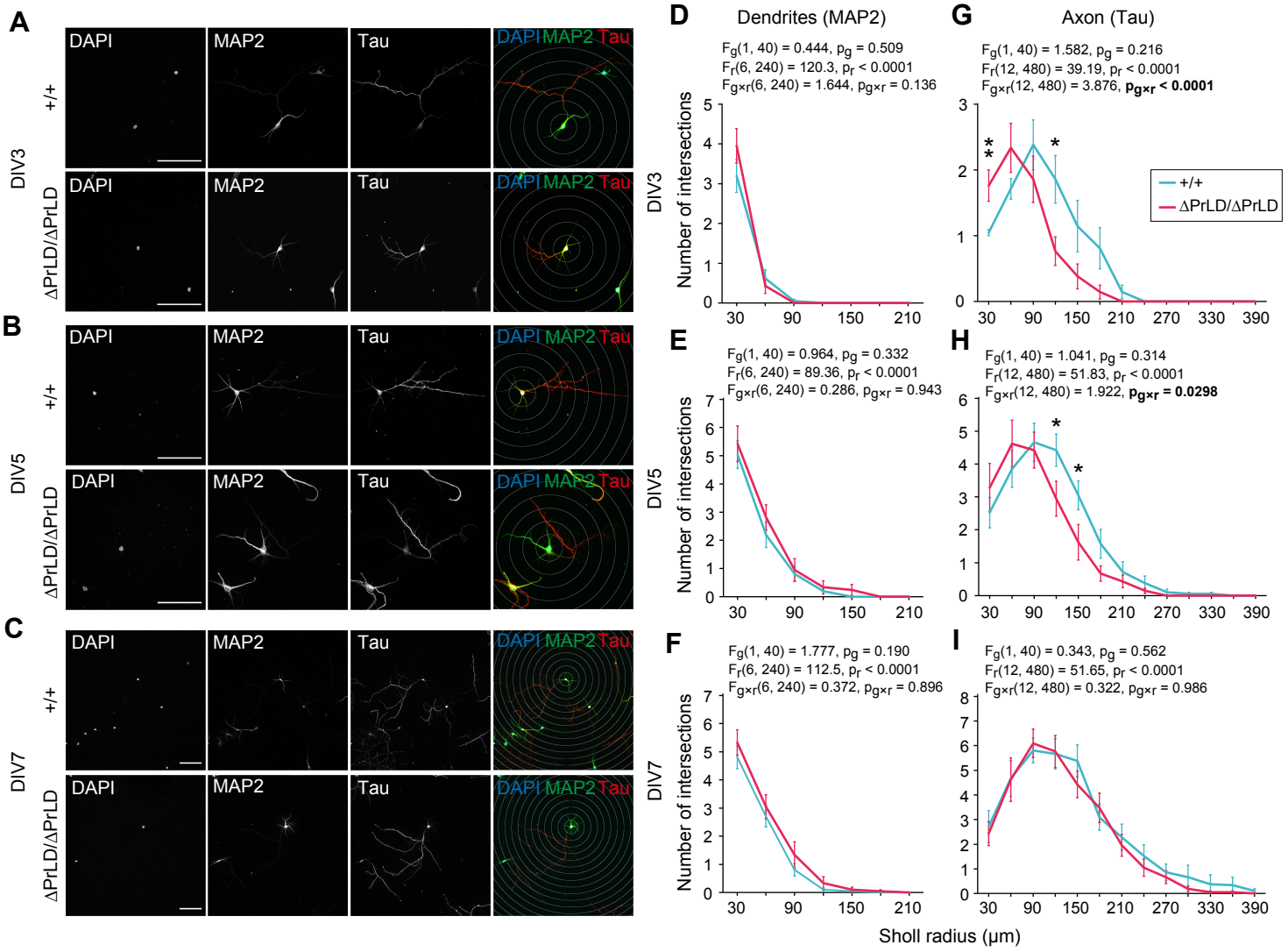


Figure S9. Delayed early development of axons in *Ilf3*$^{\Delta PrLD/\Delta PrLD}$ cultured neurons, related to Figure 5

(A-C) Representative images of staining with DAPI, anti-MAP2, and anti-Tau antibodies in cultured neurons from the AMY of *Ilf3*$^{+/+}$ and *Ilf3*$^{\Delta PrLD/\Delta PrLD}$ littermates at 3, 5, and 7 days *in vitro* (DIV). The right panels show merged images with concentric circles with 30 μm spacing used for Sholl analysis. Scale bars, 100 μm.

(D-I) Sholl analysis of dendrites (MAP2) (D-F) and axons (Tau) (G-I) in cultured neurons at DIV3 (D and G), DIV5 (E and H), and DIV7 (F and I). n = 21 cells from three littermates in each group. The data were analyzed using two-way repeated measures ANOVA. The F- and p-values for the main effects of the genotype (F_g and p_g, respectively), radius (F_r and p_r, respectively), and interactions between the genotype and radius ($F_{g\times r}$ and $p_{g\times r}$, respectively) are indicated. In G and H, **p < 0.01, *p < 0.05 in simple effect analysis after significant interaction in the two-way repeated measures ANOVA.

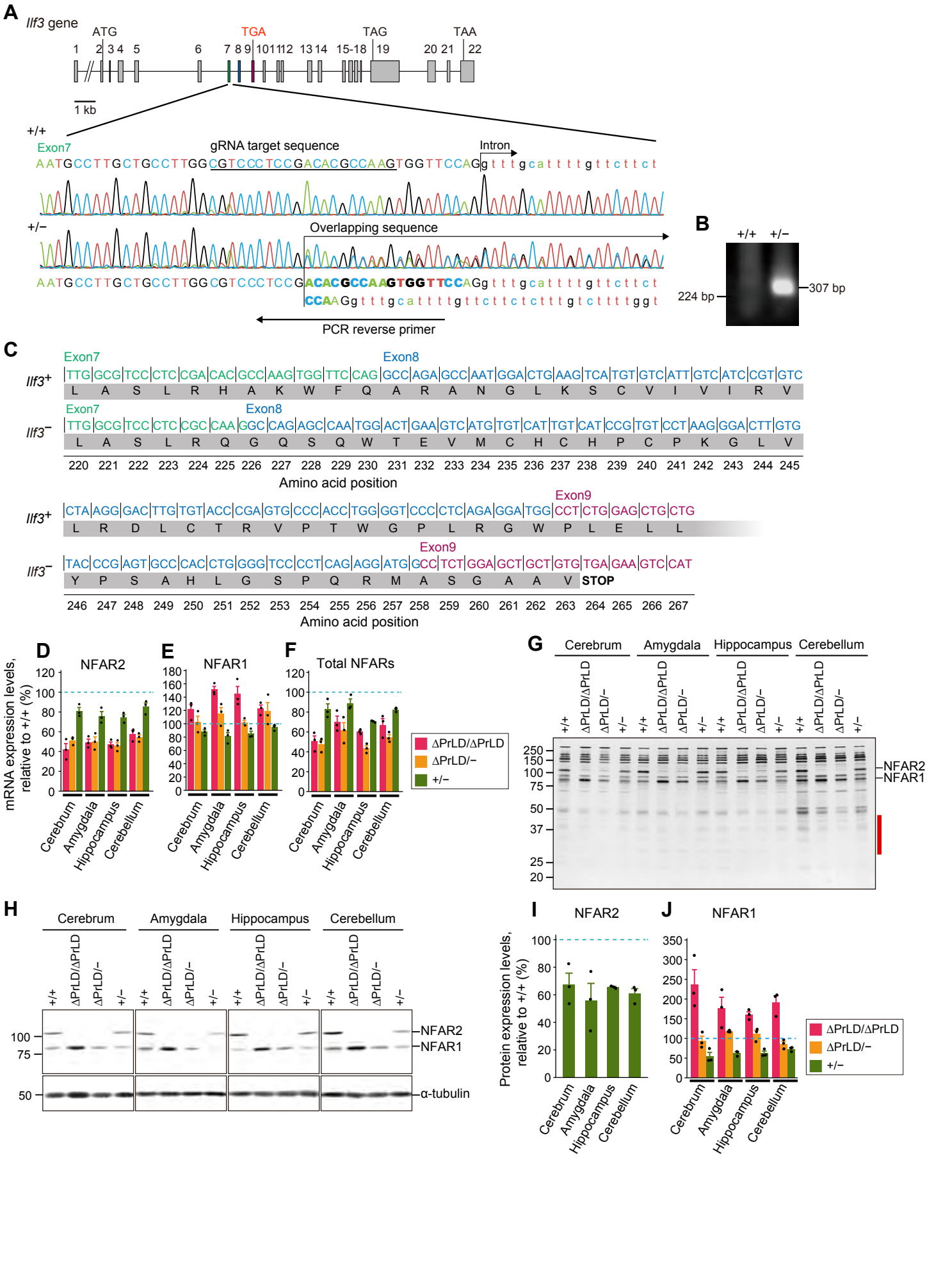

A
Ilf3 gene
ATG
TGA
TAG
TAA
1 kb
+/+
Exon7
gRNA target sequence
Intron
+/−
Overlapping sequence
PCR reverse primer
B
+/+
+/−
224 bp
307 bp
C
Exon7
Exon8
Ilf3+
Ilf3−
Amino acid position
Exon9
STOP
D
NFAR2
E
NFAR1
F
Total NFARs
mRNA expression levels, relative to +/+ (%)
Cerebrum
Amygdala
Hippocampus
Cerebellum
ΔPrLD/ΔPrLD
ΔPrLD/−
+/−
G
NFAR2
NFAR1
H
α-tubulin
I
NFAR2
Protein expression levels, relative to +/+ (%)
J
NFAR1

Figure S10. Generation of *Ilf3*$^{+/-}$ and *Ilf3*$^{\Delta PrLD/-}$ mice, related to Figure 6

(A) *Ilf3* gene structure and representative DNA sequencing in exon 7 of the *Ilf3* genome from *Ilf3*$^{+/+}$ and *Ilf3*$^{+/-}$ mice. Bold letters in the sequence indicate different sequences between the *Ilf3*$^{+}$ allele and the *Ilf3*$^{-}$ allele in the *Ilf3*$^{+/-}$ mice. A 14 bp deletion in the exon 7 sequence in the *Ilf3*$^{-}$ allele causes a frameshift, generating a downstream premature stop codon in exon 9 (red letter TGA in the gene structure). A PCR reverse primer for detecting the *Ilf3*$^{-}$ allele used in genotyping in (B) is indicated.
(B) PCR genotyping of the indicated genotypes using the reverse primer.
(C) Nucleotide and amino acid sequences encoded by exons 7-9 of the *Ilf3*$^{+}$ and *Ilf3*$^{-}$ alleles. The premature stop codon was generated in exon 9 of the *Ilf3*$^{-}$ allele.
(D-F) The expression levels of NFAR2, NAFA1, and total NFARs mRNAs in the indicated regions of the brain in *Ilf3*$^{\Delta PrLD/\Delta PrLD}$, *Ilf3*$^{\Delta PrLD/-}$, and *Ilf3*$^{+/-}$ mice were normalized to GAPDH mRNA expression levels, and then compared with the expression levels of those mRNAs in *Ilf3*$^{+/+}$ mice. Relative expression levels of NFAR2 (D), NFAR1 (E), and total NFARs (F) mRNAs are shown. n = 3.
(G) Western blotting of the indicated regions of the brain in *Ilf3*$^{+/+}$, *Ilf3*$^{\Delta PrLD/\Delta PrLD}$, *Ilf3*$^{\Delta PrLD/-}$, and *Ilf3*$^{+/-}$ mice for the *Ilf3* gene products with a polyclonal antibody against the N-terminal region of ILF3 (amino acid 8-343 of human ILF3). The red line indicates the region where proteins produced from the *Ilf3*$^{-}$ allele were expected to appear, but no different bands were detected among genotypes.
(H) Western blotting of the indicated regions of the brain in *Ilf3*$^{+/+}$, *Ilf3*$^{\Delta PrLD/\Delta PrLD}$, *Ilf3*$^{\Delta PrLD/-}$, and *Ilf3*$^{+/-}$ mice for the *Ilf3* gene products with a monoclonal antibody against ILF3 and an α-tubulin antibody as a control.
(I and J) The expression levels of NFAR2 (I) and NFAR1 (J) proteins in the brain regions in *Ilf3*$^{\Delta PrLD/\Delta PrLD}$, *Ilf3*$^{\Delta PrLD/-}$, and *Ilf3*$^{+/-}$ mice were normalized by the expression levels of α-tubulin and then compared with those in *Ilf3*$^{+/+}$ mice. n = 3.

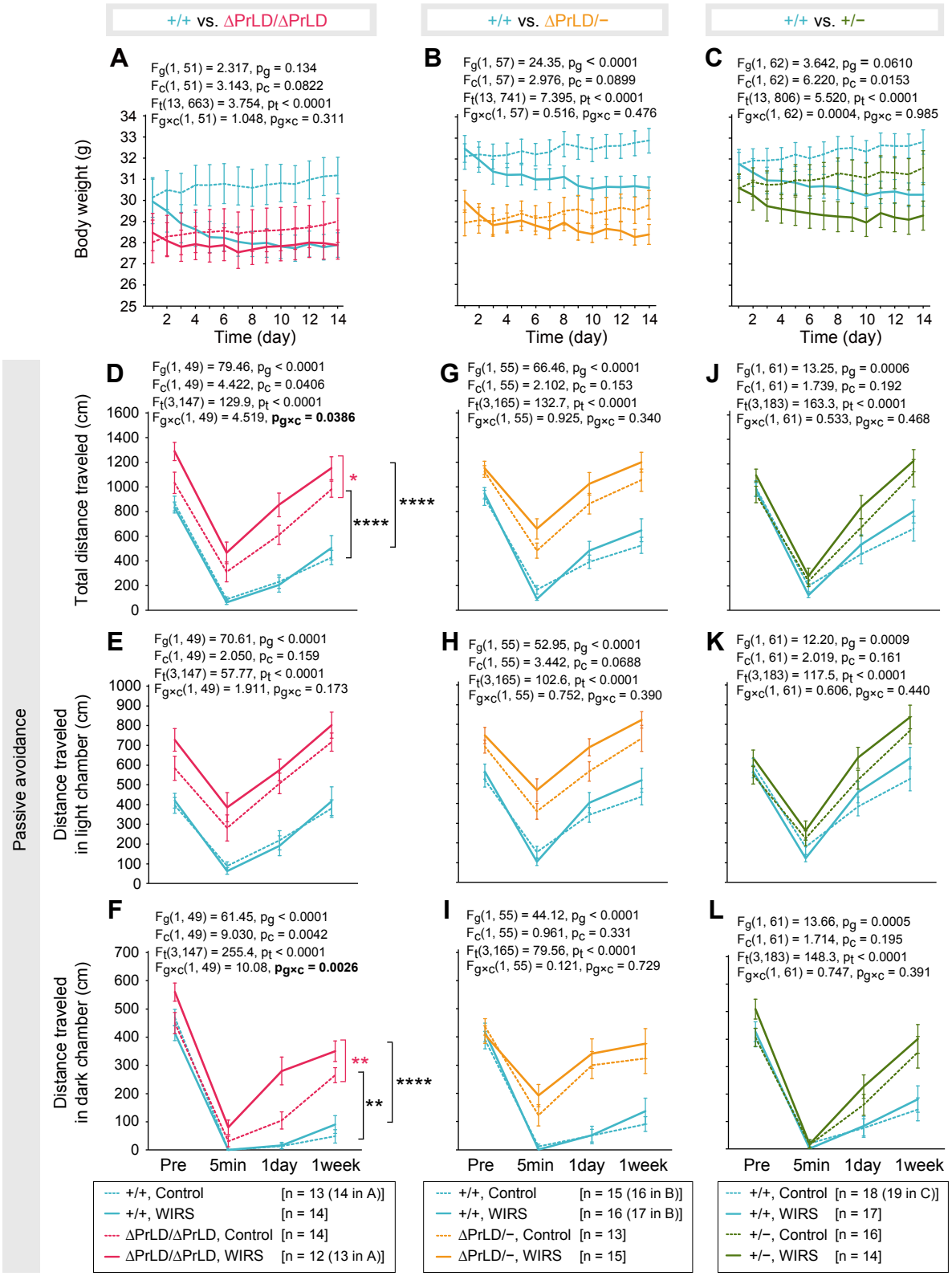

+/+ vs. ΔPrLD/ΔPrLD
+/+ vs. ΔPrLD/−
+/+ vs. +/−
Passive avoidance
A
B
C
D
E
F
G
H
I
J
K
L
Body weight (g)
Time (day)
Total distance traveled (cm)
Distance traveled in light chamber (cm)
Distance traveled in dark chamber (cm)
Pre
5min
1day
1week
Fg(1, 51) = 2.317, pg = 0.134
Fc(1, 51) = 3.143, pc = 0.0822
Ft(13, 663) = 3.754, pt < 0.0001
Fg×c(1, 51) = 1.048, pg×c = 0.311
Fg(1, 57) = 24.35, pg < 0.0001
Fc(1, 57) = 2.976, pc = 0.0899
Ft(13, 741) = 7.395, pt < 0.0001
Fg×c(1, 57) = 0.516, pg×c = 0.476
Fg(1, 62) = 3.642, pg = 0.0610
Fc(1, 62) = 6.220, pc = 0.0153
Ft(13, 806) = 5.520, pt < 0.0001
Fg×c(1, 62) = 0.0004, pg×c = 0.985
Fg(1, 49) = 79.46, pg < 0.0001
Fc(1, 49) = 4.422, pc = 0.0406
Ft(3,147) = 129.9, pt < 0.0001
Fg×c(1, 49) = 4.519, pg×c = 0.0386
Fg(1, 55) = 66.46, pg < 0.0001
Fc(1, 55) = 2.102, pc = 0.153
Ft(3,165) = 132.7, pt < 0.0001
Fg×c(1, 55) = 0.925, pg×c = 0.340
Fg(1, 61) = 13.25, pg = 0.0006
Fc(1, 61) = 1.739, pc = 0.192
Ft(3,183) = 163.3, pt < 0.0001
Fg×c(1, 61) = 0.533, pg×c = 0.468
Fg(1, 49) = 70.61, pg < 0.0001
Fc(1, 49) = 2.050, pc = 0.159
Ft(3,147) = 57.77, pt < 0.0001
Fg×c(1, 49) = 1.911, pg×c = 0.173
Fg(1, 55) = 52.95, pg < 0.0001
Fc(1, 55) = 3.442, pc = 0.0688
Ft(3,165) = 102.6, pt < 0.0001
Fg×c(1, 55) = 0.752, pg×c = 0.390
Fg(1, 61) = 12.20, pg = 0.0009
Fc(1, 61) = 2.019, pc = 0.161
Ft(3,183) = 117.5, pt < 0.0001
Fg×c(1, 61) = 0.606, pg×c = 0.440
Fg(1, 49) = 61.45, pg < 0.0001
Fc(1, 49) = 9.030, pc = 0.0042
Ft(3,147) = 255.4, pt < 0.0001
Fg×c(1, 49) = 10.08, pg×c = 0.0026
Fg(1, 55) = 44.12, pg < 0.0001
Fc(1, 55) = 0.961, pc = 0.331
Ft(3,165) = 79.56, pt < 0.0001
Fg×c(1, 55) = 0.121, pg×c = 0.729
Fg(1, 61) = 13.66, pg = 0.0005
Fc(1, 61) = 1.714, pc = 0.195
Ft(3,183) = 148.3, pt < 0.0001
Fg×c(1, 61) = 0.747, pg×c = 0.391
+/+, Control [n = 13 (14 in A)]
+/+, WIRS [n = 14]
ΔPrLD/ΔPrLD, Control [n = 14]
ΔPrLD/ΔPrLD, WIRS [n = 12 (13 in A)]
+/+, Control [n = 15 (16 in B)]
+/+, WIRS [n = 16 (17 in B)]
ΔPrLD/−, Control [n = 13]
ΔPrLD/−, WIRS [n = 15]
+/+, Control [n = 18 (19 in C)]
+/+, WIRS [n = 17]
+/−, Control [n = 16]
+/−, WIRS [n = 14]

Figure S11. Effects of WIRS on body weight and physical activity in passive avoidance tests, related to Figure 6

(A-C) Effect of WIRS on body weight of *Ilf3*$^{+/+}$ and *Ilf3* mutant mice. Mutant genotypes are *Ilf3*$^{\Delta PrLD/\Delta PrLD}$ (A), *Ilf3*$^{\Delta PrLD/-}$ (B), and *Ilf3*$^{+/-}$ (C).

(D-L) Distance traveled in the passive avoidance test. Results of comparing *Ilf3*$^{+/+}$ and *Ilf3*$^{\Delta PrLD/\Delta PrLD}$ mice (D-F), *Ilf3*$^{+/+}$ and *Ilf3*$^{\Delta PrLD/-}$ mice (G-I), and *Ilf3*$^{+/+}$ and *Ilf3*$^{+/-}$ mice (J-L). Total distance traveled (D, G, and J), distance traveled in the light chamber (E, H, and K), and distance traveled in the dark chamber (F, I, and L) before foot shock (Pre) and 5 min, 1 day, and 1 week after conditioning.

The data are presented as mean ± SEM. The data were analyzed by three-way repeated measures ANOVA. The F-values and p-values for the main effects of genotype (F_g and p_g, respectively), condition (with or without WIRS) (F_c and p_c, respectively), and time (F_t and p_t, respectively), and the interaction effect between genotype and condition ($F_{g\times c}$ and $p_{g\times c}$, respectively) are indicated. In D and F, ****$p < 0.0001$, **$p < 0.01$, *$p < 0.05$ in simple effect analysis after significant interaction in the three-way repeated measures ANOVA.