SUPPLEMENTAL MATERIAL

Nuvolone et al., http://www.jem.org/cgi/content/full/jem.20151610/DC1

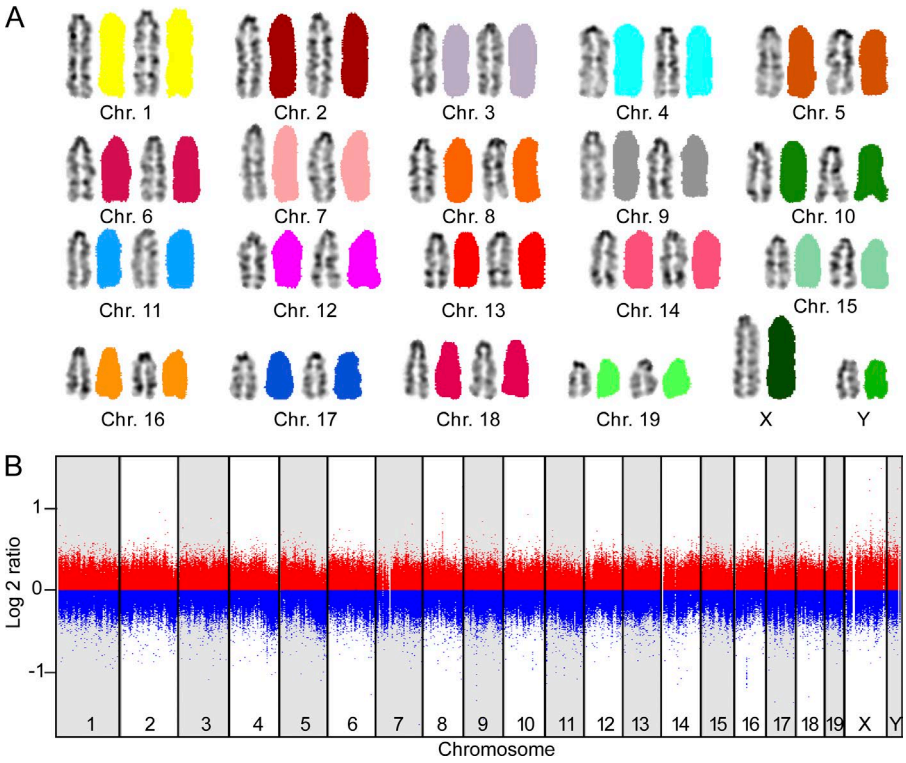

Figure S1. **C57BL/6J-*Prnp*$^{ZH3/ZH3}$ mice do not have chromosomal aberrations.** (A) G-banding and spectral karyotyping from a fibroblast cell line obtained from a *Prnp*$^{ZH3/ZH3}$ mouse show a normal 40,XY karyotype. For each chromosome, G-banded image (left) and false-colored spectral image (right) are shown. (B) Whole-genome copy number variation profile obtained with aCGH. gDNA from an individual *Prnp*$^{ZH3/ZH3}$ mouse and an individual C57BL/6J control mouse were analyzed. The ordinate represents $\log_2$-transformed ratios between the intensities of *Prnp*$^{ZH3/ZH3}$ and C57BL/6J DNA. White intervals represent genomic regions with low probe coverage in the array.

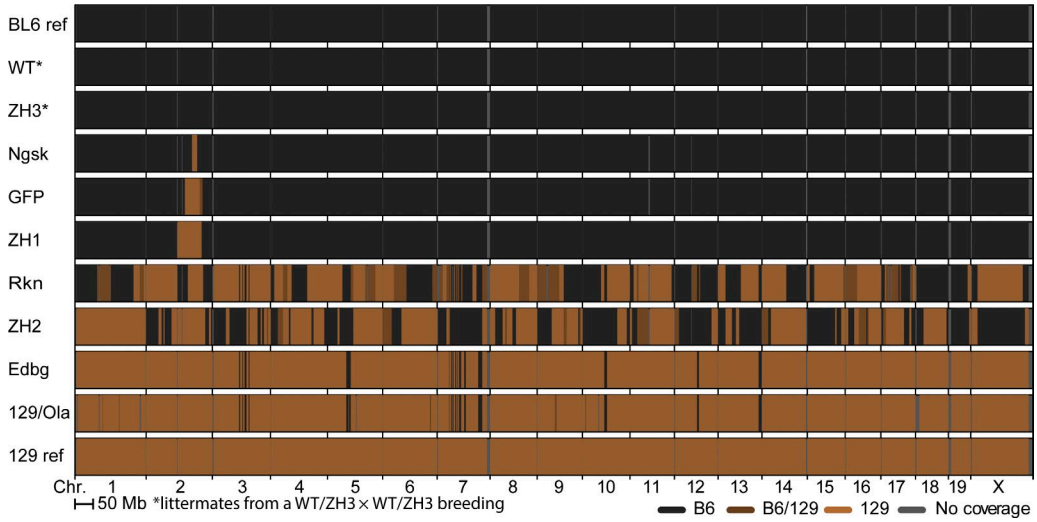

Figure S2. **Whole-genome SNP analysis.** Of 1,449 single SNPs analyzed, 865 mapped SNPs (concordant for C57BL/6 substrains and informative between C57BL/6 and 129 strains) are shown, based on their physical location. Color code for SNPs is indicated in the legend. BL6 ref. and 129 ref.: reference data for C57BL/6 and 129S6/SvEvTac strains, respectively. For each line, one mouse was analyzed. WT, $Prnp^{WT/WT}$ littermate from $Prnp^{WT/ZH3}$ x $Prnp^{WT/ZH3}$ breeding; ZH3, $Prnp^{ZH3/ZH3}$; Ngsk, $Prnp^{Ngsk/Ngsk}$; GFP, $Prnp^{GFP/GFP}$; ZH1, $Prnp^{ZH1/ZH1}$; Rkn, $Prnp^{Rkn/Rkn}$; ZH2, $Prnp^{ZH2/ZH2}$; Edbg, $Prnp^{Edgb/Edbg}$; 129/Ola, 129, 129/Ola strain.

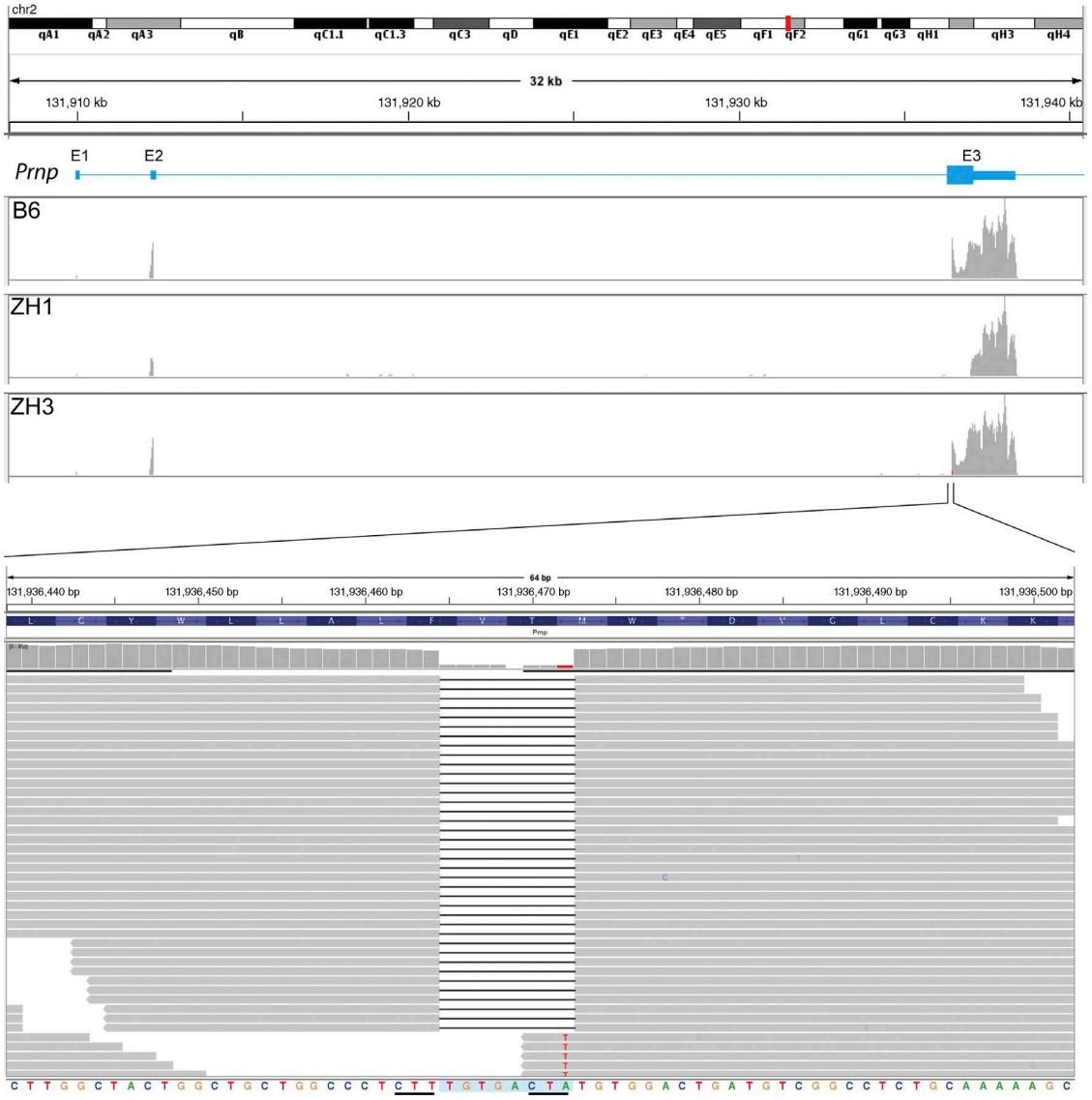

Figure S3. ***Prnp* mRNA coverage.** Visualization of RNA sequencing coverage at the *Prnp* locus, adapted from Integrative Genome Viewer. Top to bottom: ideogram of chromosome 2 region containing the *Prnp* locus; schematic RefSeq annotation of *Prnp* gene structure (in blue), with individual exons represented by bars (thin bar, 5′- or 3′-UTR; thick bar, coding region) and introns represented by the connecting lines; representative RNA sequencing read coverage plots of C57BL/6J (B6), $Prnp^{ZH1/ZH1}$ (ZH1), and $Prnp^{ZH3/ZH3}$ (ZH3); for each line, four mice were analyzed. Zoom with RNA sequencing read coverage plot and a subset of individual reads of part of *Prnp* exon 3 for the $Prnp^{ZH3/ZH3}$ mouse. In $Prnp^{ZH1/ZH1}$ mice, mRNA molecules with a sequence compatible with the 3′-portion of *Prnp* exon 3 have been reported both in the brain and in peritoneal macrophages in the original description of the line and in subsequent studies. These transcripts have been suggested to represent a fused neo/Prnp mRNA resulting from the transcription of the targeted PrnpZH1 allele where a neo cassette replaces part of Prnp exon 3. *In* $Prnp^{ZH3/ZH3}$ mice, reads aligned to the 5′ region of exon 3 indicate the presence of the 8-bp deletion characteristic of the $Prnp^{ZH3}$ allele (TGTGACTA, in light blue). A minority of reads is shown to align to the last three bases of the 8-bp deletion (CTA) and to carry a mismatch (CTT instead of CTA). This is due to a misalignment of the visualization software and, indeed, this triplet (CTT) should align to the triplet preceding the 8-bp deletion (CTT). Integrative Genome Viewer was used for visualization purposes, whereas read counts and variant identification were performed with different procedures, as detailed in Materials and methods. Within the $Prnp^{ZH3}$ allele, the deletion results in a frame shift and in the formation of a premature stop codon within the sequence encoding PrP^{C} signal peptide. Because the entire protein-coding DNA sequence of *Prnp* is located in the last exon of the *Prnp* gene (exon 3), the premature stop codon lies after the last exon–exon junction (junction between exon 2 and 3) and, as such, is not expected to be a strong driver of the non-sense–mediated mRNA decay pathway. This would explain why, in $Prnp^{ZH3/ZH3}$ mice, $Prnp^{ZH3}$ mRNA levels (consisting of Prnp mRNA bearing the 8-bp deletion) are only slightly reduced compared to $Prnp^{WT/WT}$ mice.

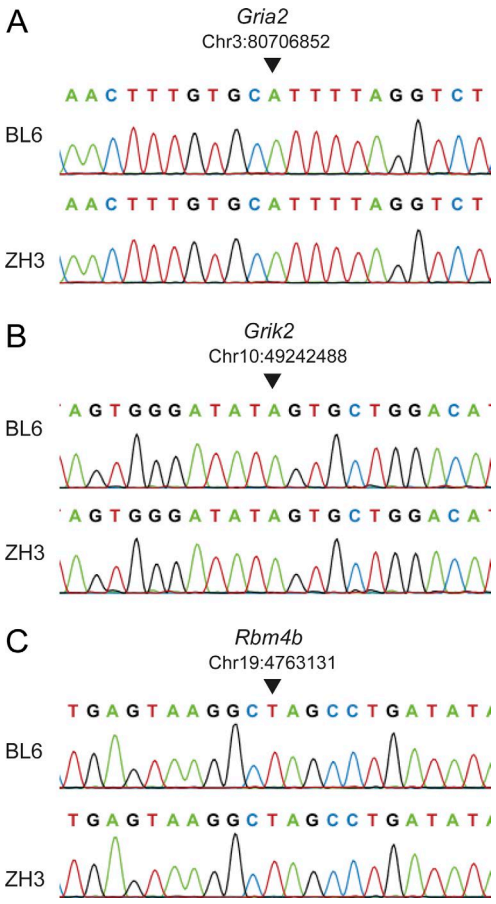

Figure S4. **Sanger sequencing of selected genomic sites undergoing RNA editing.** Sanger sequencing reads of selected genomic loci identified by RNA sequencing of hippocampus as undergoing RNA editing. For each line, one C57BL/6 (BL6) mouse and one *Prnp*$^{ZH3/ZH3}$ mouse (ZH3) belonging to the group included in the RNA sequencing experiment was analyzed. (A) Sequencing of *Gria2*. Arrowhead indicates adenosine at position chr3:80706852, shown by RNA sequencing as edited to a guanosine. (B) Sequencing of *Grik2*. Arrowhead indicates adenosine at position chr10:49242488, shown by RNA sequencing as edited to a guanosine. (C) Sequencing of *Rbm4b*. Arrowhead indicates thymidine at position chr19:4763131, shown by RNA sequencing as edited to a cytosine.

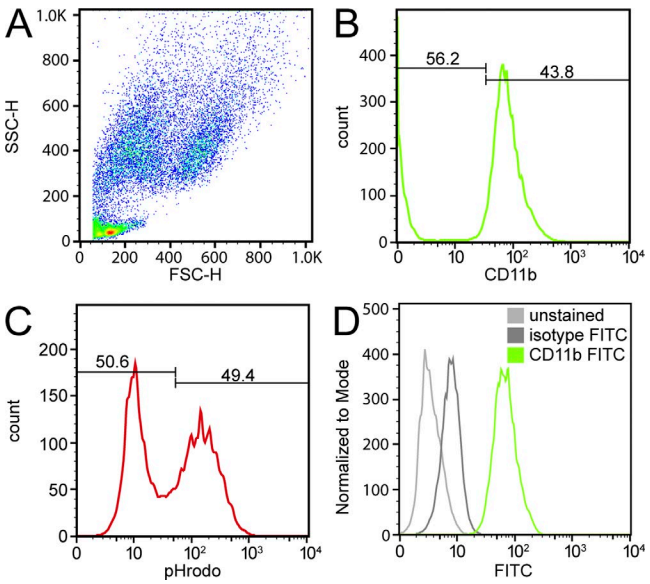

Figure S5. **Flow cytometry analysis of phagocytic activity.** (A) Forward scatter (FSC) and side scatter (SSC) dot plot. (B) Gating for CD11b$^+$ cells. (C) Gating for pHrodo$^+$ cells among CD11b$^+$ cells. (D) Overlay of unstained, isotype control–stained, and CD11b-stained cells.

Table S1. **Predicted TALEN OTs analyzed**

Site	Chr.	TALEN left binding site	TALEN right binding site	Spacer length
Prnp	2	**T**GGCTGCTGGCCCTCT	**T**GCAGAGGCCGACATCA	16
OT1	19	**T**GcCTGCcGaCCCTCT	**T**aaAaAGcCCcACAgCA	18
OT2	X	**T**GcCTaCccaCagTCT	**T**aCAaAaGCCaACATCA	14
OT3[a]	X	**T**GaCTGCTGaCaCTCT	**T**GgAGAtGCCtACATCA	22
OT4	5	**T**GGCTtCTGGCCCTCT	**T**GCAGAaaCCGAtATgc	19
OT6	5	**T**caCTGCTGaCCtTCT	**T**GaAGAaGtCcACATCA	16
OT7	8	**T**ccCTcCTGcCCCTCT	**T**cCAGAtGCCaACcTCc	23
OT11	11	**T**atCTaCTtcCCCTCT	**T**GCAcAGGtCtACATCA	18
OT12	6	**T**aaCTGCTGGCCCTCT	**T**GCAaAcaaacAaAaCA	18

Potential TALEN *Prnp* OTs were predicted using the TALE-NT algorithm. All sites are 5′→3′. The initial T bound by the TALEN backbone is shown in bold; mismatches between OTs and the *Prnp*-binding sites are lowercase. Chr., chromosome.

[a]OT3 in exon 3 of *Srpk3* gene, otherwise noncoding regions.

Table S2. **List of primers and probes used**

Primers used for NHEJ repair assays	
*Prnp*_NHEJ Fwd: AGATGTCAAGGACCTTCAGCC	*Prnp*_NHEJ Rev: TATGGGTACCCCCTCCTTGG
OT1 Fwd: GGAGACTAAGTGGGTGATGTTGT	OT1 Rev: AGGTGATGATTCACTGTGAGCTT
OT2 Fwd: CTAAGACCCTTGGGTCCATAAGC	OT2 Rev: GACATAGAAGGCCAATCTGACCA
OT3 Fwd: TCAAATCCAACTACCAGGGTCTG	OT3 Rev: GTGGGGTATCACAGTATCCTTCC
OT4 Fwd: CTGTCCTGGTTTGTTTAAGTGGC	OT4 Rev: AAGATCTTCTCAGCAGGCTTTCA
OT6 Fwd: AACTGTGGTCAAAGATGGAAGGA	OT6 Rev: CCCGTGCATAGGGATCAGAATTA
OT7 Fwd: CAATGCTTTTAGAGGAATGGCCC	OT7 Rev: CTGACACCTGTATCTTTGACCCA
OT11 Fwd: GAAGATGTGCACAAGGAAAGGAC	OT11 Rev: GAGACCACAGCCAATTAGACACT
OT12 Fwd: TGACACTGTTTTGCTAGGTCTGA	OT12 Rev: ATGTGAATGTGGGTATTCTGCCT
Primers and probes used for allelic discrimination assay	
*Prnp*_AD Fwd: CCTGAGGTGGGTAACGGTTG	*Prnp*_AD Rev: GTCATCATGGCGAACCTTGG
*Prnp*_AD WT probe: FAM-AGAGGCCGACATCAGTCCACATAGT-BHQ1	
*Prnp*_AD ZH3 probe: Yakima Yellow-ACATCAGTCCACAAAGAGGGCCAGC-BHQ1	
Primers used for RFLP analysis[a]	
*Prnp*_RFLP Fwd: AGGGTTGACGCCATGACTTT	*Prnp*_RFLP Rev: TATGGGTACCCCCTCCTTGG
Primers for RT-PCR analysis	
Prnd Fwd: ATCTAGCCCCCGAGTGTTCT	*Prnd* Rev: GGGAGTACTTGGGAGGGACT
Gapdh Fwd: TCCATGACAACTTTGGCATTG	*Gapdh* Rev: CAGTCTTCTGGGTGGCAGTGA
Eif2a Fwd: CAACGTGGCAGCCTTACA	*Eif2a* Rev: TTTCATGTCATAAAGTTGTAGGTTAGG
Utpc6 Fwd: TTTCGGTTGAGTTTTTCAGGA	*Utpc6* Rev: CCCTCAGGTTTACCATCTTGC
Primers for Sanger sequencing	
Gria2 Fwd: CGAGTGGCACACTGAGGAAT	*Gria2* Rev: GCTTACGCCGACGGTAAAAA
Grik2 Fwd: TCCCCATTGGCAAGGTGAAC	*Grik2* Rev: ACACCCAAGACGATCAGCAG
Rmb4b Fwd: AGAGCATCAGTACTTTGCCTATGA	*Rmb4b Rev*: GCTGTCCATTACTAGCTCCCA

All primers and probes (5′→3′) were obtained from Microsynth.
[a]Amplicons were digested with Tsp45I (NEB).

Table S3, available as an Excel file, lists DEGs between *Prnp*$^{ZH1/ZH1}$ and C57BL/6J hippocampi.

Table S4, available as an Excel file, lists DEGs between *Prnp*$^{ZH3/ZH3}$ and C57BL/6J hippocampi.

Table S5, available as an Excel file, lists differentially expressed exons between *Prnp*$^{ZH1/ZH1}$ and C57BL/6J hippocampi.

Table S6, available as an Excel file, lists loci with differential RNA editing level between *Prnp*$^{ZH1/ZH1}$ and C57BL/6J hippocampi.

Table S7, available as an Excel file, lists loci with differential RNA editing level between *Prnp*$^{ZH3/ZH3}$ and C57BL/6J hippocampi.