

Supplemental material

Methods

PCR-based off-target analysis of CRISPR-mediated editing in Phf21b^{Δ4/Δ4} mice. We used a PCR-based approach to validate the precision of CRISPR-mediated gene editing in generating *Phf21b^{Δ4/Δ4}* mice. We first searched for potential off-targets by the guide sequences in the genome of the animals by using Cas-OFFinder (1) (Fig. S1A). PCR primers were designed for the three highest ranked off-target sites for each guide sequence (Table S1). The PCR was performed by using genomic DNA samples from the *Phf21b^{Δ4/Δ4}* mice. PCR products were separated via agarose gel electrophoresis (Fig. S1B) and extracted for sequence confirmation via Sanger DNA sequencing (Fig. S1C). The selected sites did not show evidence of off-target CRISPR editing in the *Phf21b^{Δ4/Δ4}* mice.

Behavioral assays: Open field. In the open field test, mice were placed in a 40 x 40 x 40 cm arena and allowed to explore freely for 10 min. For analysis, the center of the arena is prescribed as the 20 x 20 cm area in the middle.

Behavioral assays: Elevated-plus maze. In the elevated-plus maze test, mice were allowed to freely explore the elevated-plus maze for 5 min, during which the amount of time that they spent in the open arms versus the closed arms was measured.

Behavioral assays: Rotarod assay. Mice were placed on an accelerating (from 4 to 40 rpm) rotarod (IITC Inc.). Latency to fall from the rotarod was measured and recorded. The animals were given four trials a day for four consecutive days. Inter-trial interval was at least 15 min, during which the animals were returned to their home cages to rest.

Behavioral assays: Sucrose preference test. Mice were allowed free choice to drink from two identical bottles containing either water or 1% sucrose solution. The amount of intake from each bottle was measured after 48 h. The sucrose preference index is calculated as the amount of sucrose intake divided by the total intake of water and sucrose.

Behavioral assays: Tail suspension test. The test was performed as described (2). Mice were suspended from their tails for a period of 6 min. The amount of time they were mobile was scored. Immobility time is calculated by subtracting the mobility time from the total testing time of 6 min.

Behavioral assays: Forced swim test. The test was performed as described (3). Mice were placed in a clear Plexiglas cylinder filled with water for a period of 6 min. The amount of time they were mobile for the last 4 min of the test session was scored. Immobility time is calculated by subtracting the mobility time from the total scored time of 4 min.

Behavioral assays: Y-maze. Mice were allowed to freely explore all three arms of the Y-maze for a period of 10 min. Percentage of spontaneous alterations was calculated as a measure of spatial working memory, which is given by the total number of spontaneous alternations divided by the total number of arm entries minus 2, multiplied by 100.

Behavioral assays: Novel object recognition test. To test long-term recognition memory, the mice were allowed to explore two identical objects for 10 min. 24 h after the initial training session, one of the objects was replaced with a novel one, and the mouse was allowed to explore the two objects for 10 min. The novel object recognition index is given by the amount of time that the animal spends exploring the novel object divided by the total amount of time it spends exploring both objects in the test session.

Behavioral assays: Morris water maze. To test long-term spatial reference memory, the protocol as described in (4) was used. Briefly, mice were first trained for five consecutive days to reach an underwater platform in the Morris water maze. Four trials were given on each training day, with an inter-trial interval of 15 s, during which the mice were left on the platform. The start location was randomized for each trial. Mice were guided to the platform if they failed to reach it within the 1-min trial limit. On the sixth day, a probe trial was conducted, where the underwater platform was removed, and the mice were scored on their latencies to reach the platform location from a novel start location.

Behavioral assays: Social preference and social novelty tests. The social preference and social novelty tests were performed as described (5). Mice were habituated to the three-chambered setup and allowed to freely explore for 10 min. For the social preference test, the subject mouse was allowed to interact with either a juvenile stranger mouse placed in one of the side chambers, or an inanimate object in the other side chamber for a test period of 10 min. The social preference index is given the amount of time that the subject mouse spends interacting with the stranger mouse, divided by the total amount of time it spends interacting with both the stranger mouse and the object in the test session. For the social novelty test, the inanimate object was replaced with a new juvenile stranger mouse, and the subject animal was allowed to interact with either the familiar stranger or the new stranger for a test period of 10 min. The social novelty index is given by the amount of time that the subject mouse spends interacting with the new stranger, divided by the total amount of time that the subject mouse spends interacting with both the familiar and new stranger mice in the test session. All stranger mice were sex-matched to the subject animal, and the placements of the object and the stranger mice were counter-balanced between subjects.

Behavioral assays: Five-trial social memory test. The five-trial social memory test was performed as described in (6, 7). The subject animal was allowed to freely interact with a juvenile stranger animal of the same sex for four 1-min trials, with an inter-trial interval of 10 min. The same stranger animal was used for the four trials. For the fifth trial, a new stranger mouse was introduced to the subject mouse for 1 min. The interaction time of the subject animal with the stranger mouse in each trial was recorded.

Behavioral assays: Olfactory habituation/dishabituation test. Mice were tested for their ability to habituate and dishabituate to different odors, as described (8). Cotton swabs soaked with different odorants (water, vanilla, and urine from a conspecific animal) were presented to each mouse for three 2-min trials, with an inter-trial interval of 1 min. The amount of time that the animal spent investigating the odorant was recorded.

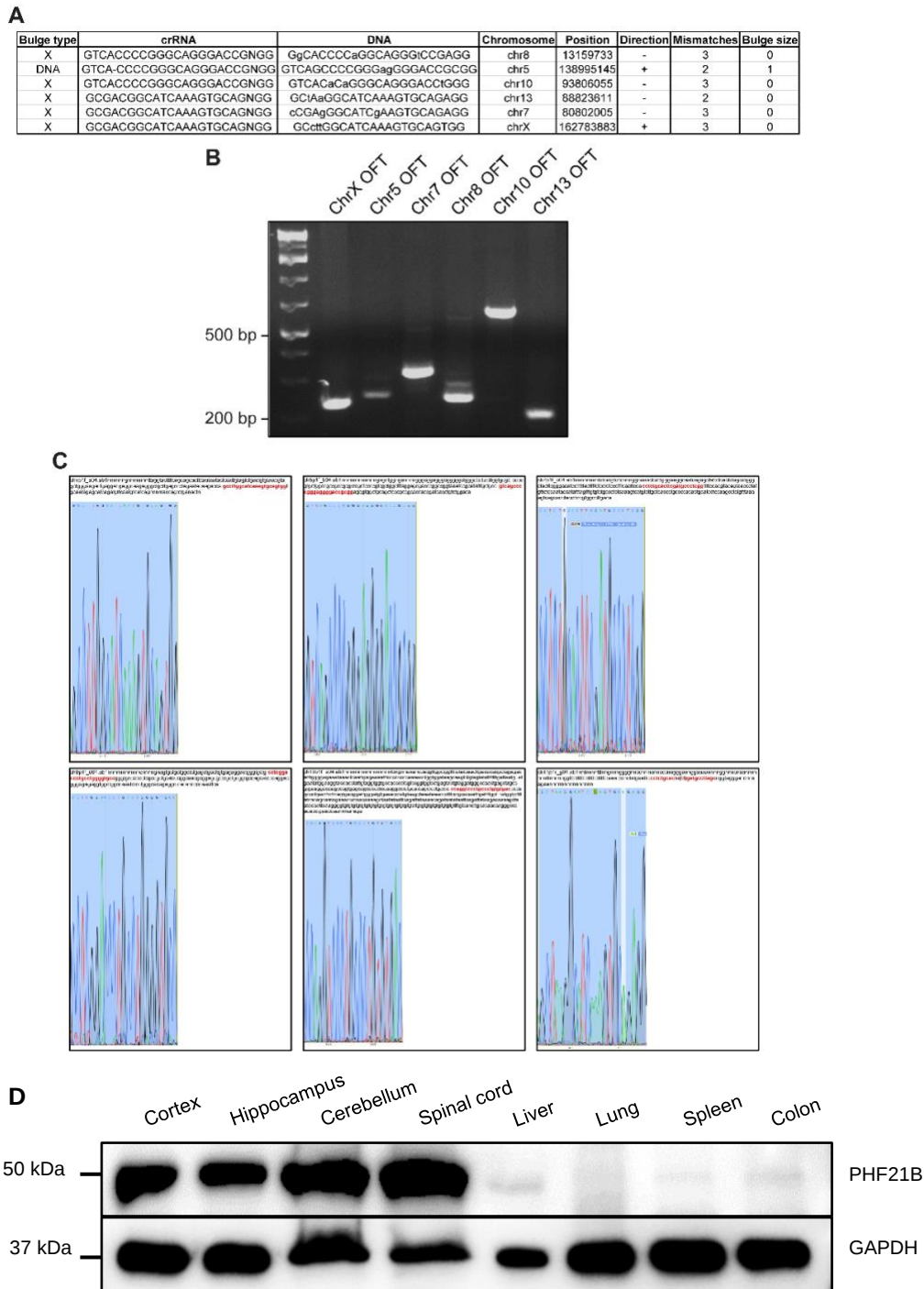


Figure S1. PCR-based off-target analysis of CRISPR-mediated editing in *Phf21b*^{Δ4/Δ4} animals and verification of PHF21B expression. (A) Potential off-target sites identified via Cas-OFFinder. **(B)** Representative agarose gel image of PCR products from primers targeting potential off-target (OFT) sites in the genomic DNA of *Phf21b*^{Δ4/Δ4} animals. **(C)** Sequence confirmation of intact off-target sites in the genomic DNA of *Phf21b*^{Δ4/Δ4} mice. **(D)** Western blot of the wild-type mouse brain and peripheral tissues expressing PHF21B.

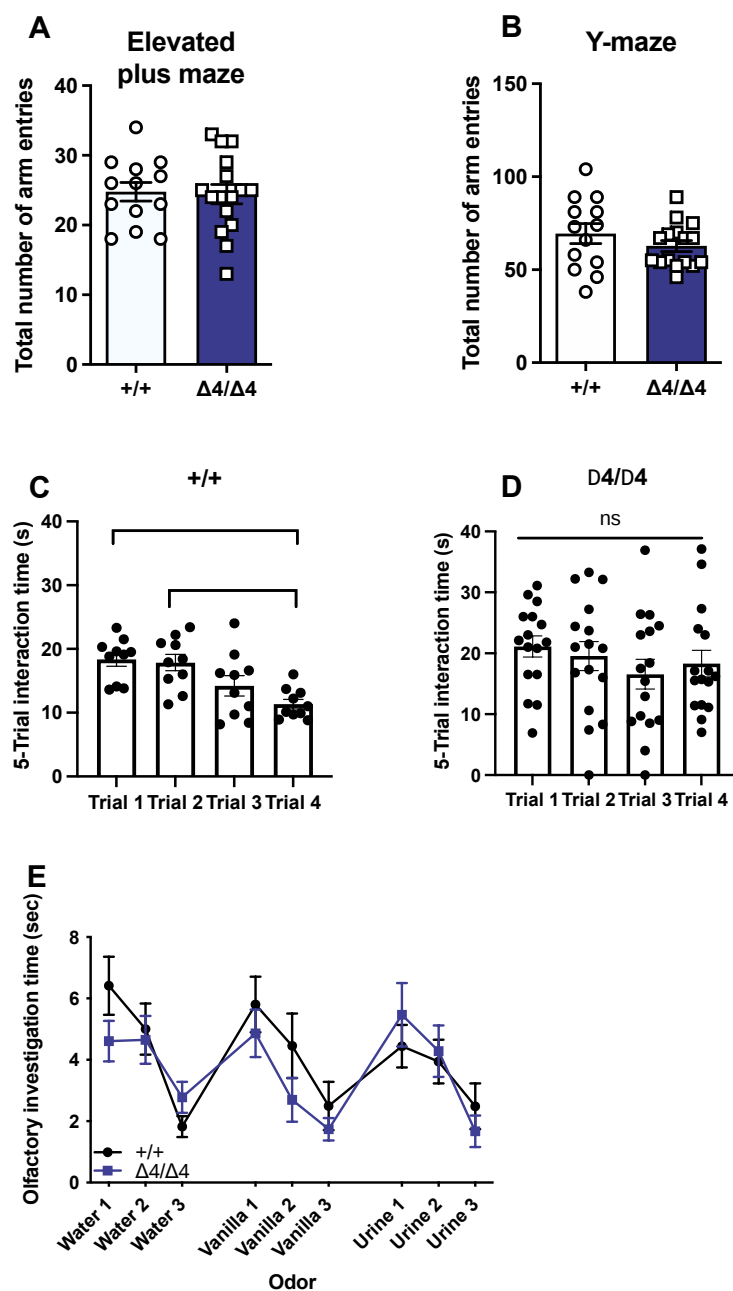


Figure S2. Behavioral characterization of *Phf21b*^{+/+} (+/+) and *Phf21b*^{Δ4/Δ4} (Δ4/Δ4) animals. (A) Total number of arm entries in the elevated plus maze test. (B) Total number of arm entries in the Y-maze test. (C) Interaction time in the 5-trial social test, one-way ANOVA with repeated measures in +/+ mice *P<0.05, **P<0.01 and (D) in Δ4/Δ4 mice. (E) Olfactory habituation/dishabituation results from +/+ and Δ4/Δ4 animals. n = 13-16. Values are presented as mean ± SEM.

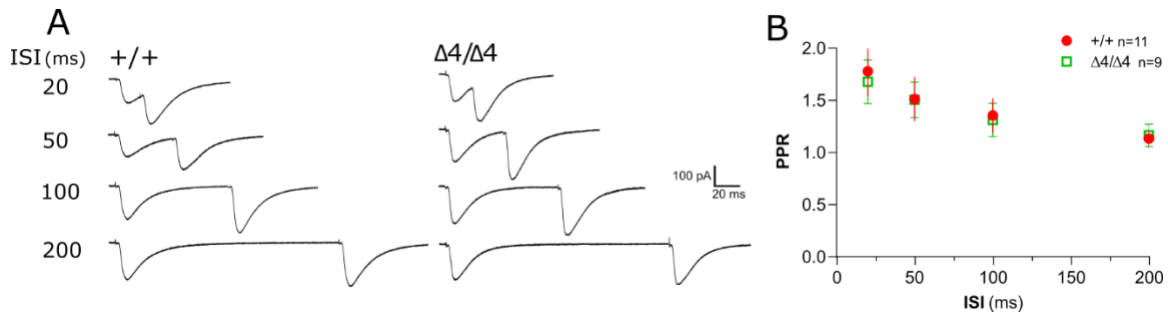


Figure S3. *Phf21b* ^{$\Delta4/\Delta4$} ($\Delta4/\Delta4$) mice showed a normal pair-pulse ratio (PPR) in hippocampal CA1 synapses. **A.** Representative traces of twin-EPSCs recorded from CA1 pyramidal neurons in *Phf21b* wild-type (+/+) and $\Delta4/\Delta4$ mice. ISI: inter-stimuli-intervals. **B.** Summary data averaged at same ISI across different neurons recorded from +/+ or $\Delta4/\Delta4$ mice. There is no difference between $\Delta4/\Delta4$ and +/+ mice, indicating that $\Delta4/\Delta4$ status does not alter the presynaptic release.

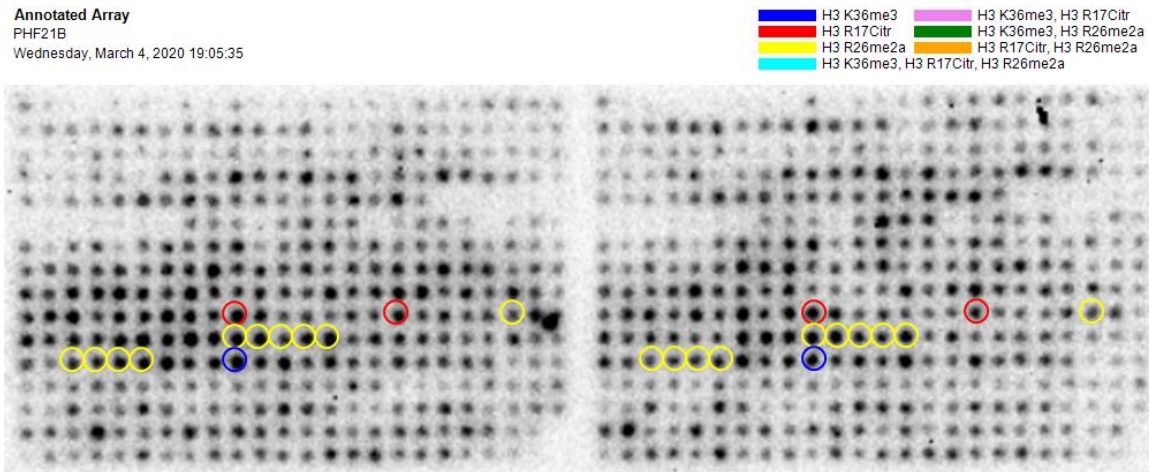


Figure S4. Enhanced chemiluminescence image of the MODiFied™ Histone Peptide Array. This array was used to screen purified human recombinant PHF21B for its interactions with histone and their post-translational modifications. Single histone modifications were color coded.

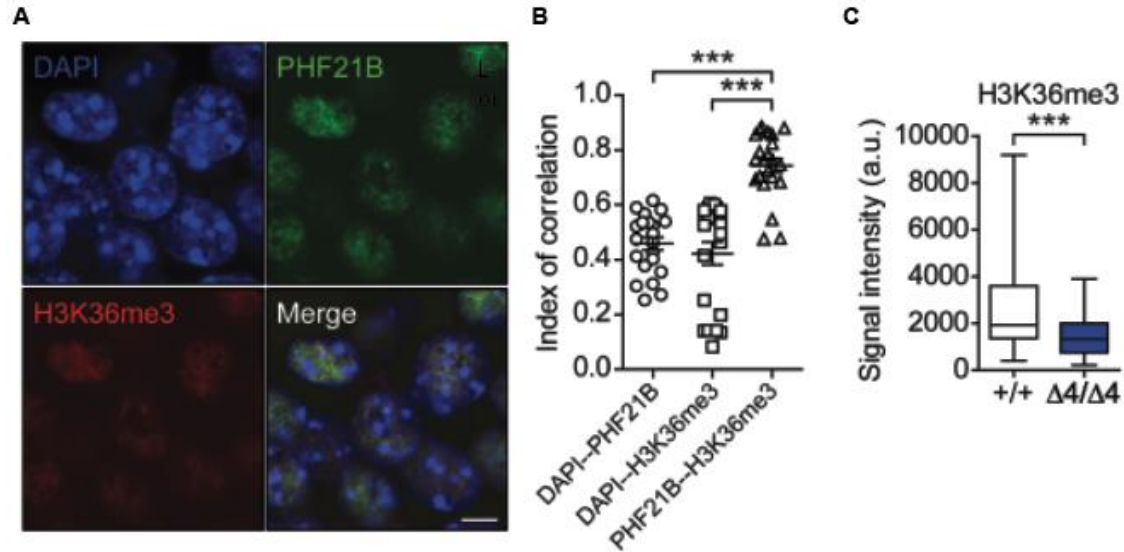


Figure S5. Colocalization of PHF21B with H3K36me3. (A) Representative images of PHF21B and H3K36me3 expression in the nuclei of wild-type CA1 neurons. (B) Index of correlation as a measure of co-localization of DAPI-, PHF21B-, and H3K36me3-positive immunofluorescence signals in the nuclei of wild-type CA1 neurons; $n = 5$; One-way ANOVA; Tukey's test *post hoc*. (C) Expression of H3K36me3 in +/+ and $\Delta 4/\Delta 4$ hippocampal tissues measured by its immunofluorescence signal intensity; $n = 5$; Student's *t*-test.

Table S1. Sequences of primers used for PCR-based off-target analysis of CRISPR-mediated *Phf21b* deletion.

Target	Forward sequence (5' → 3')	Reverse sequence (5' → 3')	Expected product size (bp)
ChrX	GCAGTGTGCTGATCCAGAGC	CAGACTGGTACAAGCTGATGAC	231
Chr5	ACAGAGTCGCTCCGGG	GTCCAGACAGGTGATCGGTG	244
Chr7	CAGGGCCTGTGGACTTTACC	CAAGGCCATGGGAAGGTAGG	320
Chr8	GTCTGAGCCGCTCTGGAAG	GAATTTGGGGTGTGAGCCT	238
Chr10	CCAAGGACCATCTGACATCT	CCTTTACTGGTTCAGGTGTGG	653
Chr13	ATGTTTTCGGAGTGGGTCGC	GAACAGAGGCGGGGATAGGA	201

Table S2. Posthoc Sidak's multiple comparison test of mixed ANOVA test of +/- and $\Delta 4/\Delta 4$ mice

Fixed effects (type III)	P value	P value summary	F (DFn, DFd)
Intersections	<0.0001	****	F (78, 4368) = 143.0
Genotype	0.0662	ns	F (1, 56) = 3.510
Intersections x Genotype	<0.0001	****	F (78, 4368) = 1.744

Distance from soma (μm)	Predicted (LS) mean diff.	95.00% CI of diff.	Summary	Adjusted P Value
10	0.6952	-0.8174 to 2.208	ns	>0.9999
15	0.2548	-1.258 to 1.767	ns	>0.9999
20	0.04286	-1.470 to 1.555	ns	>0.9999
25	0.481	-1.032 to 1.994	ns	>0.9999
30	-0.03333	-1.546 to 1.479	ns	>0.9999
35	-0.3405	-1.853 to 1.172	ns	>0.9999
40	-1.017	-2.529 to 0.4959	ns	0.8254
45	-0.7571	-2.270 to 0.7555	ns	0.9993
50	-1.605	-3.117 to -0.09215	*	0.0232
55	-0.231	-1.744 to 1.282	ns	>0.9999
60	-1.448	-2.960 to 0.06499	ns	0.0832
65	-0.6595	-2.172 to 0.8531	ns	>0.9999
70	-0.681	-2.194 to 0.8317	ns	>0.9999
75	-1.545	-3.058 to -0.03263	*	0.0383
80	-0.4	-1.913 to 1.113	ns	>0.9999
85	-1.248	-2.760 to 0.2650	ns	0.3217
90	-1.107	-2.620 to 0.4055	ns	0.6307
95	-1.652	-3.165 to -0.1398	*	0.0153
100	-1.252	-2.765 to 0.2602	ns	0.313
105	-1.612	-3.125 to -0.09929	*	0.0218
110	-1.357	-2.870 to 0.1555	ns	0.1605
115	-1.231	-2.744 to 0.2817	ns	0.3535
120	-0.95	-2.463 to 0.5626	ns	0.9243
125	-1.207	-2.720 to 0.3055	ns	0.4019
130	-1.126	-2.639 to 0.3864	ns	0.5857

135	-0.7333	-2.246 to 0.7793	ns	0.9997
140	-0.8286	-2.341 to 0.6840	ns	0.9934
145	-0.5333	-2.046 to 0.9793	ns	>0.9999
150	-0.5762	-2.089 to 0.9364	ns	>0.9999
155	-0.2381	-1.751 to 1.275	ns	>0.9999
160	-0.03571	-1.548 to 1.477	ns	>0.9999
165	0.02143	-1.491 to 1.534	ns	>0.9999
170	-0.01429	-1.527 to 1.498	ns	>0.9999
175	0.2905	-1.222 to 1.803	ns	>0.9999
180	0.1905	-1.322 to 1.703	ns	>0.9999
185	0.08333	-1.429 to 1.596	ns	>0.9999
190	0.1286	-1.384 to 1.641	ns	>0.9999
195	0.05476	-1.458 to 1.567	ns	>0.9999
200	0.2048	-1.308 to 1.717	ns	>0.9999
205	0.1071	-1.405 to 1.620	ns	>0.9999
210	0.1238	-1.389 to 1.636	ns	>0.9999
215	0.1048	-1.408 to 1.617	ns	>0.9999
220	0.1214	-1.391 to 1.634	ns	>0.9999
225	0.1333	-1.379 to 1.646	ns	>0.9999
230	-0.02381	-1.536 to 1.489	ns	>0.9999
235	0.04762	-1.465 to 1.560	ns	>0.9999
240	-0.01429	-1.527 to 1.498	ns	>0.9999
245	-0.01429	-1.527 to 1.498	ns	>0.9999
250	-0.181	-1.694 to 1.332	ns	>0.9999
255	-0.007143	-1.520 to 1.505	ns	>0.9999
260	-0.1786	-1.691 to 1.334	ns	>0.9999
265	-0.2857	-1.798 to 1.227	ns	>0.9999
270	-0.2952	-1.808 to 1.217	ns	>0.9999
275	-0.3048	-1.817 to 1.208	ns	>0.9999
280	-0.281	-1.794 to 1.232	ns	>0.9999
285	-0.1881	-1.701 to 1.325	ns	>0.9999
290	-0.1024	-1.615 to 1.410	ns	>0.9999
295	-0.119	-1.632 to 1.394	ns	>0.9999
300	-0.03571	-1.548 to 1.477	ns	>0.9999
305	-0.01667	-1.529 to 1.496	ns	>0.9999
310	0.1095	-1.403 to 1.622	ns	>0.9999
315	0.119	-1.394 to 1.632	ns	>0.9999
320	0.2119	-1.301 to 1.725	ns	>0.9999
325	0.169	-1.344 to 1.682	ns	>0.9999
330	0.2643	-1.248 to 1.777	ns	>0.9999

335	0.181	-1.332 to 1.694	ns	>0.9999
340	0.2429	-1.270 to 1.755	ns	>0.9999
345	0.0619	-1.451 to 1.575	ns	>0.9999
350	-0.0119	-1.525 to 1.501	ns	>0.9999
355	-0.01667	-1.529 to 1.496	ns	>0.9999
360	-0.01905	-1.532 to 1.494	ns	>0.9999
365	-0.02381	-1.536 to 1.489	ns	>0.9999
370	0.009524	-1.503 to 1.522	ns	>0.9999
375	-0.02619	-1.539 to 1.486	ns	>0.9999
380	-0.02619	-1.539 to 1.486	ns	>0.9999
385	-0.09762	-1.610 to 1.415	ns	>0.9999
390	-0.09762	-1.610 to 1.415	ns	>0.9999
395	-0.03095	-1.544 to 1.482	ns	>0.9999
400	0.002381	-1.510 to 1.515	ns	>0.9999

Table S3. List of all genes (n=139) found to be differentially expressed in *Phf21b*^{+/-} and *Phf21b*^{Δ4/Δ4} hippocampal tissues, with their respective fold changes, p-values, and false discovery rates. RNAseq data were deposited under the accession number GSE201477, NCBI GEO repository.

Gene ID	Fold change	P-value	False discovery rate (FDR)
<i>Gm5741</i>	-3247.63	0.00015	0.058641
<i>Irx2</i>	-493.272	8.69E-07	0.004523
<i>Pou4f1</i>	-340.463	1.25E-06	0.004523
<i>Chat</i>	-255.711	1.20E-05	0.015308
<i>D130079A08Rik</i>	-230.363	0.000223	0.064576
<i>Slc18a3</i>	-136.325	8.48E-05	0.043867
<i>Shox2</i>	-133.375	0.000527	0.078629
<i>D130009I18Rik</i>	-130.756	1.88E-05	0.01675
<i>Gm38534</i>	-89.0792	9.75E-06	0.015308
<i>Nppa</i>	-80.2295	1.97E-05	0.01675
<i>Chrbnb4</i>	-52.218	1.27E-05	0.015308
<i>Chrna3</i>	-42.4243	9.45E-07	0.004523
<i>Gpr151</i>	-34.6766	2.38E-07	0.003446
<i>Wfikkn2</i>	-28.6555	0.000178	0.061679
<i>Irx1</i>	-22.8385	0.000199	0.061679
<i>Irx5</i>	-20.6858	0.000188	0.061679
<i>Cytip</i>	-16.9811	4.74E-05	0.028581
<i>Slc5a7</i>	-15.5758	1.74E-05	0.01675
<i>Gng8</i>	-15.5672	1.13E-05	0.015308
<i>Tyrp1</i>	-14.57	0.000129	0.053711

<i>Tmem182</i>	-13.7779	2.29E-05	0.018439
<i>Tafa4</i>	-13.1165	1.08E-05	0.015308
<i>Gm13373</i>	-11.9992	3.49E-05	0.023739
<i>Tac2</i>	-11.3515	3.79E-06	0.009145
<i>Cubn</i>	-10.6913	3.67E-05	0.023739
<i>Chrn3</i>	-9.74694	0.000319	0.068212
<i>Irx3</i>	-9.4892	0.000329	0.068212
<i>Syt9</i>	-9.17583	0.000496	0.075841
<i>Nhlh2</i>	-9.03027	8.93E-06	0.015308
<i>Tcf7l2</i>	-8.68022	3.39E-05	0.023739
<i>Avil</i>	-8.63494	0.000259	0.068176
<i>Crabp2</i>	-8.29422	0.000771	0.092211
<i>Syt15</i>	-8.05554	0.000124	0.053711
<i>Sncg</i>	-6.55309	1.78E-05	0.01675
<i>Cldn19</i>	-6.27332	0.000316	0.068212
<i>Ano1</i>	-6.17137	1.96E-06	0.005679
<i>Ascl4</i>	-6.07173	0.00054	0.078629
<i>Pla2r1</i>	-5.50737	0.000268	0.068207
<i>Scx</i>	-5.30418	0.000319	0.068212
<i>Pld5</i>	-5.23611	0.000731	0.089708
<i>Syt6</i>	-5.16257	0.000104	0.050138
<i>Lrrc55</i>	-5.06769	0.000379	0.071256
<i>Fzd10</i>	-5.02458	0.000171	0.061679
<i>Wif1</i>	-4.5528	0.000198	0.061679
<i>Cftr</i>	-4.53162	0.000715	0.088883

<i>Cnmd</i>	-4.34976	0.000781	0.092451
<i>Vav3</i>	-4.26307	0.000156	0.059417
<i>Npr1</i>	-4.25244	0.000188	0.061679
<i>Kcng4</i>	-4.03663	0.000203	0.061679
<i>Spx</i>	-3.99011	0.000377	0.071256
<i>Kcnh8</i>	-3.9395	0.000858	0.094412
<i>Gpr179</i>	-3.93778	0.000457	0.075246
<i>Adcyap1</i>	-3.68078	0.000557	0.079838
<i>Scn9a</i>	-3.56366	0.000241	0.067738
<i>Tex15</i>	-3.17861	0.000258	0.068176
<i>Cdca7l</i>	-3.17687	0.000407	0.072325
<i>Amotl1</i>	-2.93533	0.000371	0.071256
<i>Aebp1</i>	-2.75947	0.000247	0.067738
<i>Ptgds</i>	-2.63231	6.39E-05	0.035566
<i>Cep112</i>	-2.56008	0.00052	0.07843
<i>Gpr153</i>	-2.47073	0.000662	0.086294
<i>Prkch</i>	-2.32653	0.000543	0.078629
<i>Wls</i>	-2.1938	0.000442	0.073622
<i>Rflnb</i>	-2.02805	0.000861	0.094412
<i>Gm50431</i>	-2.01919	0.000292	0.068207
<i>Hhip</i>	-1.97454	0.00033	0.068212
<i>Cdyl</i>	-1.94559	0.000248	0.067738
<i>Id3</i>	-1.93144	0.00032	0.068212
<i>Fzd1</i>	-1.88878	0.000598	0.083266
<i>Lca5</i>	-1.82995	0.000819	0.092575

<i>Cnnm2</i>	-1.76155	8.98E-05	0.044807
<i>Isoc1</i>	-1.74687	0.000677	0.086798
<i>Slc29a1</i>	-1.6707	0.000309	0.068212
<i>Pdzd2</i>	-1.61176	0.000417	0.072325
<i>Aqp4</i>	-1.59437	0.000405	0.072325
<i>Shisa1</i>	-1.55267	0.000653	0.086001
<i>Axin2</i>	-1.51971	0.000176	0.061679
<i>Arhgap24</i>	-1.50305	0.000787	0.092451
<i>Nek7</i>	-1.46064	0.000564	0.08003
<i>Rpl39</i>	-1.317	0.000884	0.094781
<i>Adgrb3</i>	1.360114	0.000917	0.097078
<i>Cdh11</i>	1.369075	0.000577	0.081114
<i>Sel1l</i>	1.419391	0.000403	0.072325
<i>Csrnp2</i>	1.424667	0.000617	0.083948
<i>Cdh10</i>	1.428154	0.000205	0.061679
<i>Usp46</i>	1.455037	0.000753	0.091154
<i>Pld3</i>	1.455462	0.000478	0.075841
<i>Large1</i>	1.460375	0.000835	0.09349
<i>Tmem74</i>	1.471975	0.000919	0.097078
<i>Homer1</i>	1.488299	0.000282	0.068207
<i>Rap2b</i>	1.493232	0.000286	0.068207
<i>Lrrn2</i>	1.499845	0.000498	0.075841
<i>Adam23</i>	1.508529	0.000711	0.088883
<i>St8sia3</i>	1.527343	0.000277	0.068207
<i>Gad2</i>	1.541043	0.000756	0.091154

<i>Brinp2</i>	1.599715	7.21E-05	0.038658
<i>Ppm1e</i>	1.608113	0.000494	0.075841
<i>Pls3</i>	1.610765	0.000374	0.071256
<i>Ext1</i>	1.615763	0.000482	0.075841
<i>Ap2b1</i>	1.620352	0.00071	0.088883
<i>Mal2</i>	1.637022	0.000386	0.071598
<i>Pcdhga2</i>	1.649238	0.000951	0.09907
<i>Klhl3</i>	1.658838	0.000814	0.092575
<i>Sertm1</i>	1.675461	0.000128	0.053711
<i>Prkcb</i>	1.678694	0.000882	0.094781
<i>Nr4a1</i>	1.688614	6.29E-05	0.035566
<i>Rasl11b</i>	1.696932	0.000127	0.053711
<i>Sept9</i>	1.702277	0.00013	0.053711
<i>Zdhhc23</i>	1.704707	0.000485	0.075841
<i>Synj2</i>	1.727667	0.000434	0.073622
<i>Plxna1</i>	1.748143	0.00042	0.072325
<i>Htr2a</i>	1.757264	0.000638	0.084698
<i>Tmem121b</i>	1.765785	0.000718	0.088883
<i>Sh3rf1</i>	1.772809	0.000196	0.061679
<i>Cacna2d3</i>	1.777189	0.000473	0.075841
<i>Tmem200a</i>	1.802365	0.00067	0.086551
<i>Crhbp</i>	1.821505	3.77E-05	0.023739
<i>Akap5</i>	1.834138	0.000325	0.068212
<i>Sstr4</i>	1.834562	0.000815	0.092575
<i>Ar</i>	1.836513	0.000441	0.073622

<i>Vgf</i>	1.882551	0.000947	0.09907
<i>Mchr1</i>	1.887289	0.000807	0.092575
<i>Sorcs3</i>	1.919584	1.44E-05	0.016027
<i>Dlx1as</i>	1.927339	0.000881	0.094781
<i>Camk4</i>	1.929184	0.000792	0.092451
<i>Galntl6</i>	1.962446	0.000272	0.068207
<i>Fam189a1</i>	1.993603	0.000347	0.06966
<i>Htr1a</i>	2.020346	0.000149	0.058641
<i>Foxp1</i>	2.090582	0.000336	0.068554
<i>Lancl3</i>	2.104651	0.000629	0.084286
<i>Vxn</i>	2.186427	0.00084	0.09349
<i>Galnt9</i>	2.205406	0.000351	0.06966
<i>Fyb2</i>	2.359708	0.000215	0.063635
<i>Gpr161</i>	2.53297	3.58E-05	0.023739
<i>Hcn1</i>	2.593056	0.00062	0.083948
<i>Gm10754</i>	2.604448	0.000539	0.078629
<i>Mpped1</i>	2.70499	0.000608	0.08377
<i>Fibcd1</i>	2.921538	0.000289	0.068207
<i>Ptpru</i>	2.977622	0.000413	0.072325

Table S4. Sequences of primers used in qRT-PCR experiments.

Target	Forward sequence (5' → 3')	Reverse sequence (5' → 3')
<i>Chat</i> (mRNA)	CCAATGACCAGCTAAGGTTTG	CAGTCAGTGGGAATGGATTGG
<i>Fibcd1</i>	ACAAACCGCAGCGAGCAAG	TTCAGGAAGAGAACCACACCG
<i>Gapdh</i>	ATGCCATCACTGCCACCCAGAAG	TGCCAGTGAGCTTCCCGTTCAG
<i>Gm5741</i>	GGCATCAACCGCATAAAGGTATC	ACAAGGAAGGGGTGCTCTT
<i>Kdm1a</i>	GGTTGTAACAGGTCTTGGAGGG	GGAACAGCTTGTCCATTGGC
<i>Nppa</i>	CGTCTTGGCCTTTTGGCTTC	GTGGTCTAGCAGGTTCTTGAAG
<i>Phf21a</i>	TCTAACTGCATCACAGAAGACTG	AGCAATGGTAGGTCTCTGGC
<i>Phf21b</i> Primer 1 (Exon 1-2)	GCCAAAGTGAGCGCAAAGTG	GAGGTCGCCGTTCTGGTG
<i>Phf21b</i> Primer 2 (Exon 11- 12)	CAGCGGAAGGCCTTAAAGAA	CACTGTCTTGTGGGTGACATAG
<i>Slc18a3</i>	AGACTATGCCACGCTCTTCG	AGGCTCCTCGGGATACTTGT
<i>Scn1a</i>	TTCAGGGGCTATCGAGGCTG	TTCATGTGAGATTCCCCGAAA
<i>Shank3</i>	GGACCTGCAACAAACGAAGT	GGCTGGAATAGCCCGTAGTT
<i>Vav3</i>	CGGCGCAGGTGTTTCGAC	GTTCTTCAAACAGAGAACTGGGAC
<i>Chat</i> (TSS)	TGGCGTCGCTCATGCTAATA	GGAACATCTCGGAAGCGGA
<i>Chat</i> (-1000 bp)	TAACAGTGCCAAGGGAGACG	TGCGTGACCACACCCTAAG
<i>Chat</i> (-2000 bp)	TCGCGCCGTCTGAGTAAAT	AGAGAAAAGTCTGCCTCCC
<i>Chat</i> (- 3000bp)	ATGGTCTCAGGGCTCAATGC	AAAACCCACCACCACTCTG
<i>Chat</i> (+1000 bp)	CCAAGTAGGCGCTGGCATT	GTGCCCATTTGTTCCCGACTA

<i>Chat</i> (+2000 bp)	AGGAGTAGGAGTGCGTCGAA	GGGGCATCCTCTACGAGTTC
<i>Chat</i> (+3000 bp)	GAGTGTGTTTGGAGAGGGGG	GGAATCCCTTCTCCGTGACC
<i>Grin2b</i>	GCCATGAACGAGACTGACCC	GCTTCCTGGTCCGTGTCATC
<i>Gria1</i>	ACCCTCCATGTGATCGAAATG	GGTTCATTCTGGACGCTTGAG

References

1. Bae S, Park J, and Kim JS. Cas-OFFinder: a fast and versatile algorithm that searches for potential off-target sites of Cas9 RNA-guided endonucleases. *Bioinformatics*. 2014;30(10):1473-5.
2. Can A, Dao DT, Terrillion CE, Piantadosi SC, Bhat S, and Gould TD. The tail suspension test. *J Vis Exp*. 2012(59):e3769.
3. Can A, Dao DT, Arad M, Terrillion CE, Piantadosi SC, and Gould TD. The mouse forced swim test. *J Vis Exp*. 2012(59):e3638.
4. Vorhees CV, and Williams MT. Morris water maze: procedures for assessing spatial and related forms of learning and memory. *Nat Protoc*. 2006;1(2):848-58.
5. Chin EWM, and Goh ELK. Behavioral Characterization of MeCP2 Dysfunction-Associated Rett Syndrome and Neuropsychiatric Disorders. *Methods Mol Biol*. 2019;2011:593-605.
6. Thor DH, and Holloway WR. Social memory of the male laboratory rat. *J Comp Physiol Psychol*. 1982;96(6):1000-6.
7. Hitti FL, and Siegelbaum SA. The hippocampal CA2 region is essential for social memory. *Nature*. 2014;508(7494):88-92.
8. Yang M, and Crawley JN. Simple behavioral assessment of mouse olfaction. *Curr Protoc Neurosci*. 2009;Chapter 8:Unit 8 24.