Supplemental Material

Calbindin-D_{9k} is a Novel Risk Gene for Neurodegenerative Disease

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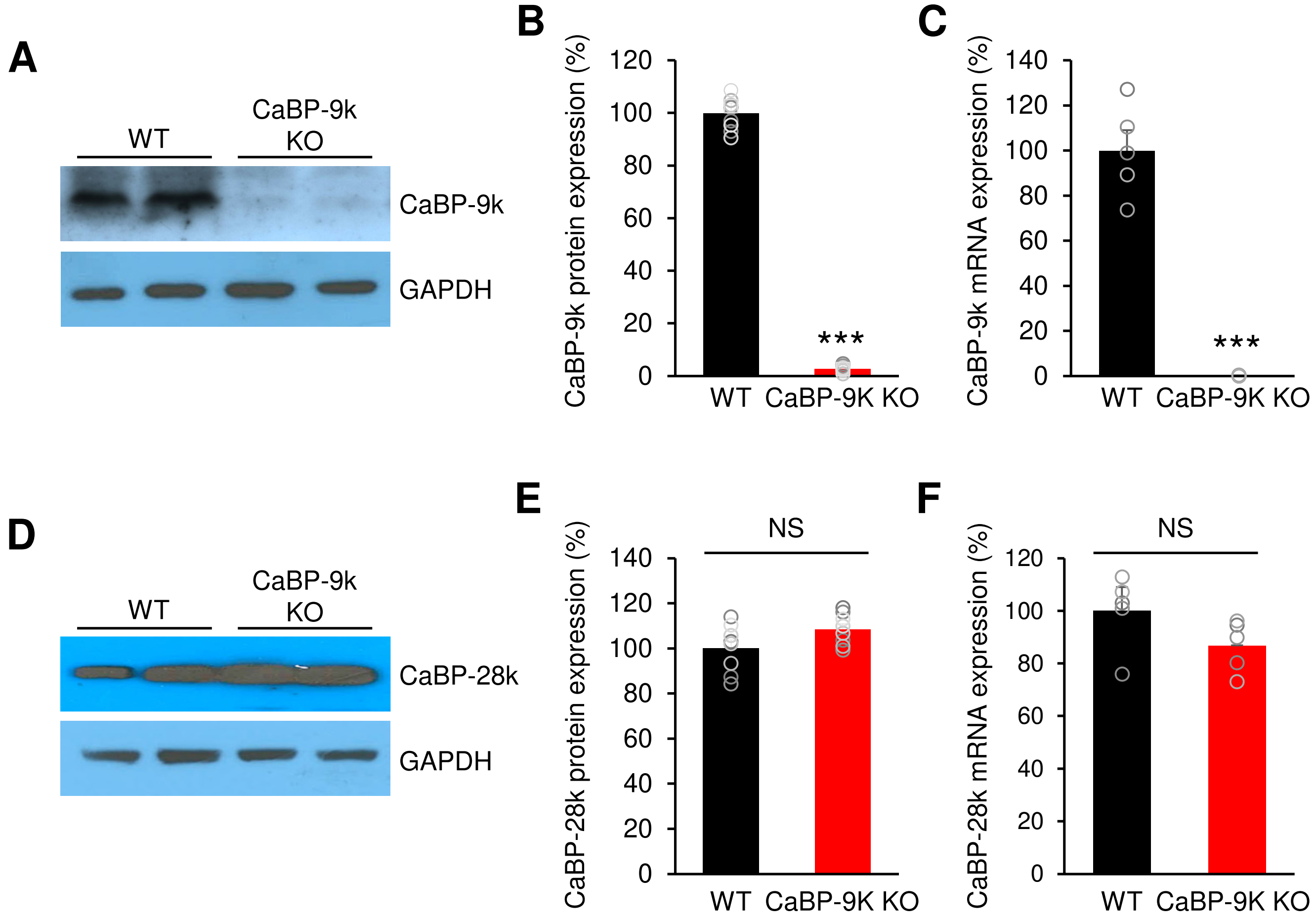
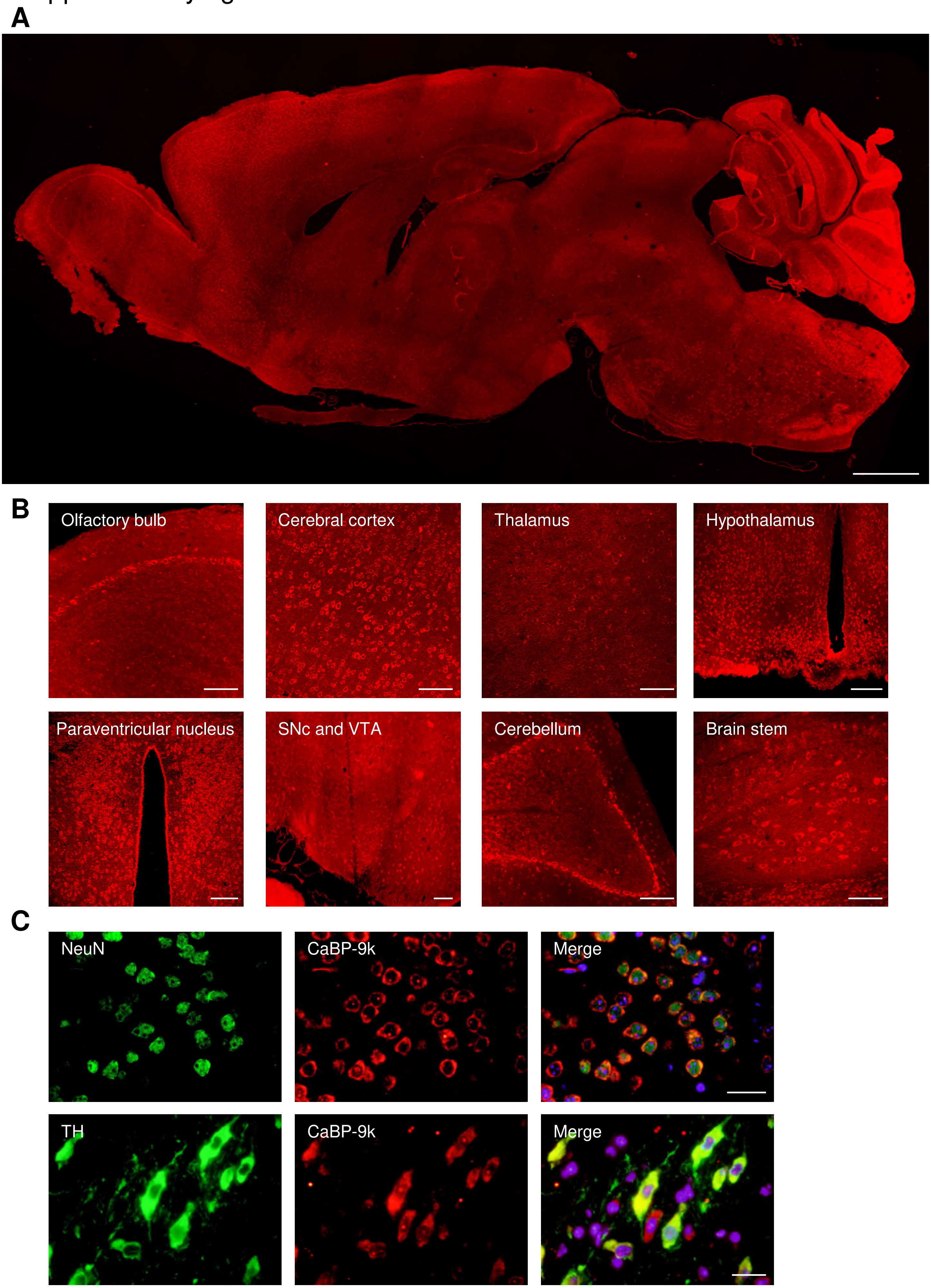
Supplementary figure-1



Supplementary Fig. 1. Study of CaBP-9k KO mouse model.

(A,D) Western blotting for CaBP-9k and CaBP-28k in CaBP-9k KO mice. (B,E) Quantification of A,C. (C,F) Real-time PCR analysis for genes encoding CaBP-9k and CaBP-28k in in CaBP-9k KO brain. $n = 10$ mice for each group. Data shown are the means ± SEMs and were analysed by two-tailed unpaired Student's t-tests.

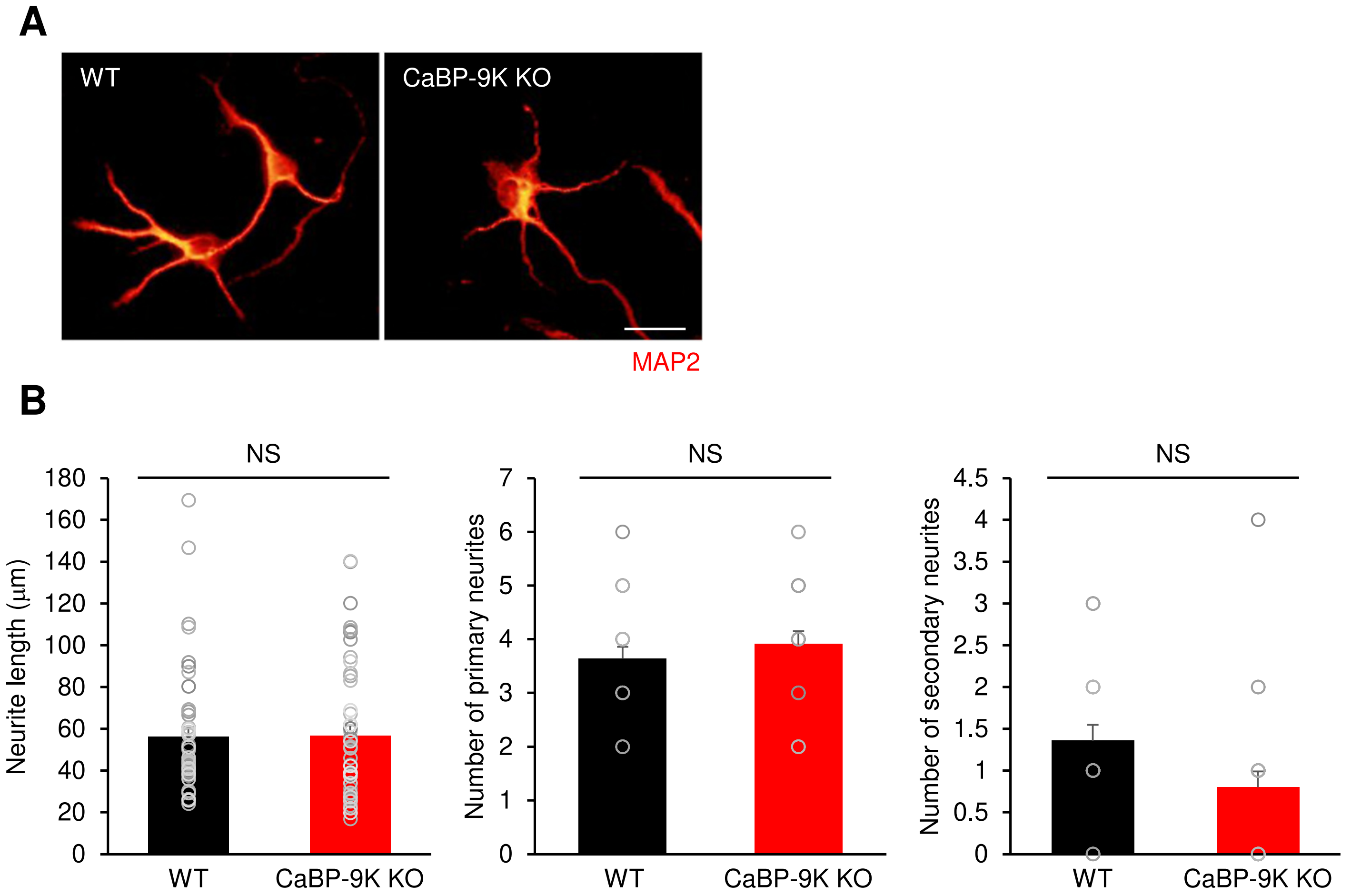
Supplementary figure-2



Supplementary Fig. 2. Distribution of CaBP-9k-positive neurons in the mouse brain.

(A,B) Fluorescence photomicrographs showing CaBP-9k expression in a sagittal section. Scale bar= 1 mm in A; Scale bar= 100 μm in B. (C) Confocal microscopy of double immunofluorescence staining for CaBP-9k colocalized with the NeuN neuronal marker in the cerebral cortex and with TH in the SNc and VTA. Scale bar= 20 μm.

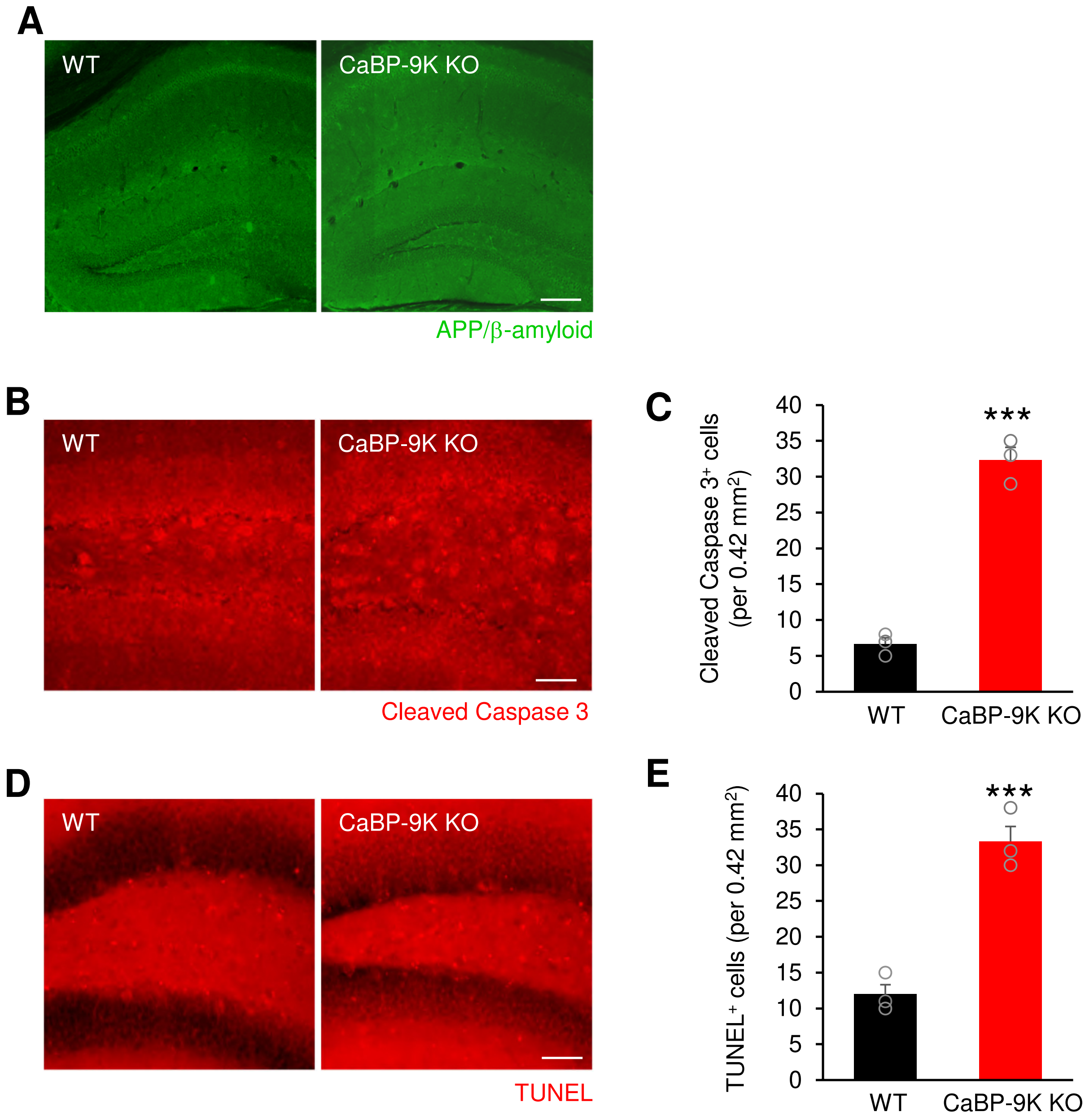
Supplementary figure-3



Supplementary Fig. 3. Neurite development in CaBP-9k KO mice.

(A) MAP2 immunofluorescence showing neurite morphology in primary neuronal cells at 5 days *in vitro* from wild-type and CaBP-9k KO mice. Scale bar= 25 μm. (B) Quantification of the length and number of neurites. (Neurite length: $n = 47$ neurites from 5 mice for each group; Neurite number: $n = 25$ neurites from 5 mice for each group). NS, nonsignificant. Data shown are the means ± SEMs and were analysed by two-tailed unpaired Student's t-tests.

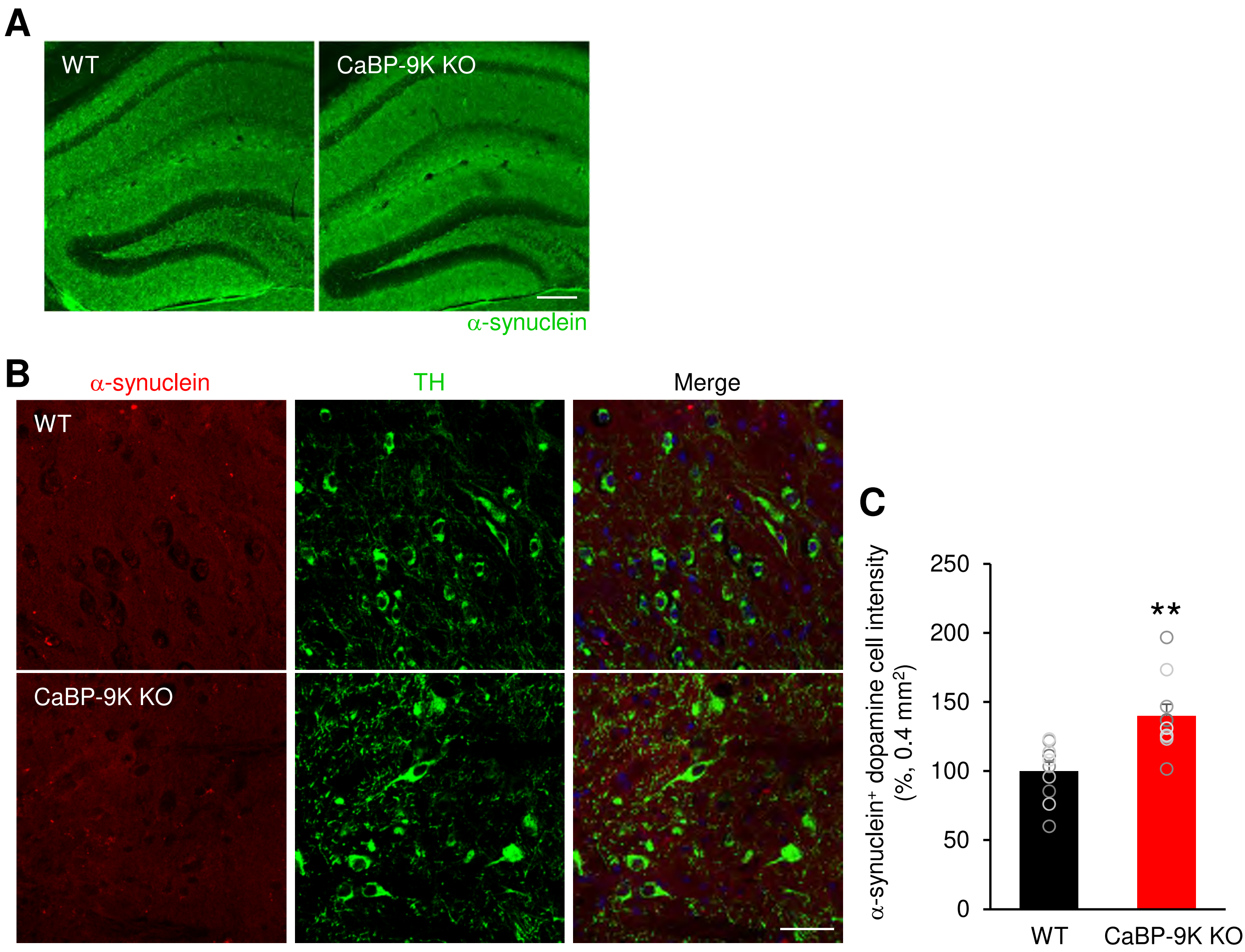
Supplementary figure-4



Supplementary Fig. 4. APP/β-amyloid expression and cell death in the hippocampal of young CaBP-9k KO mice.

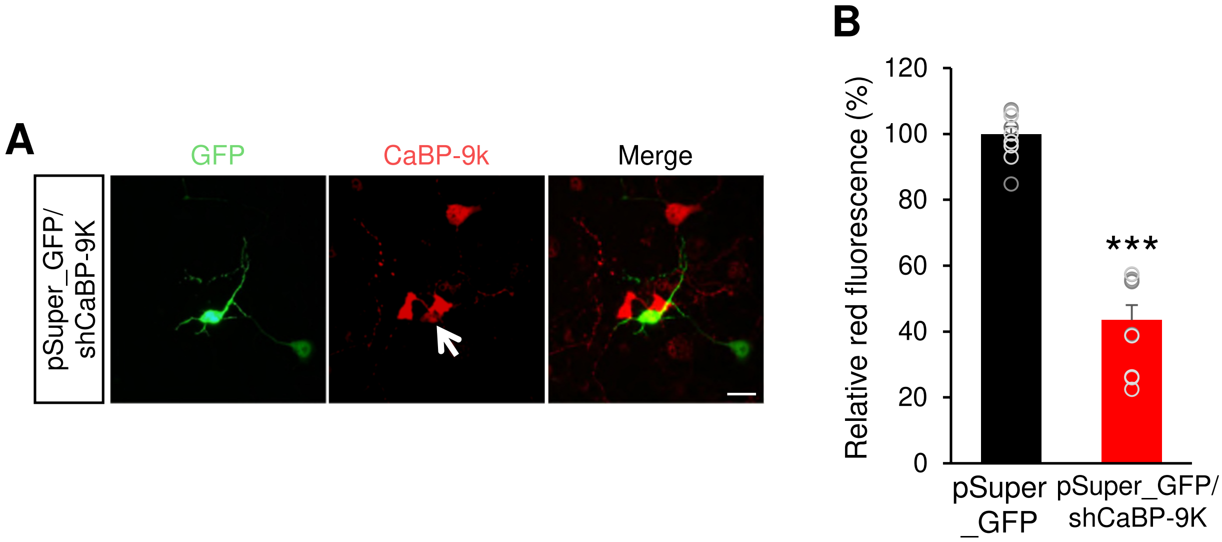
(A) Immunofluorescence staining for APP/β-amyloid plaques in the hippocampi of young wild-type and CaBP-9k KO mice. Scale bar= 200 μm. $n = 4$ for mice for each group. (B,D) Cell death was assessed in the hippocampi of young wild-type and CaBP-9k KO mice by immunostaining for cleaved caspase-3 or by TUNEL assays. Scale bar= 50 μm. (C,E) Quantification of B and D. $n = 3$ for mice for each group. Data shown are the means ± SEMs and were analysed by two-tailed unpaired Student's t-tests.

Supplementary figure-5



Supplementary Fig. 5. α-synuclein-positive neurons in the SNc and VTA of young CaBP-9k KO mice. (A) Immunofluorescence for α-synuclein aggregates in the hippocampi of young wild-type and CaBP-9k KO mice. Scale bar= 200 μm. $n = 4$ mice for each group. (B) Immunofluorescence staining for α-synuclein and TH in dopaminergic cells in the SNc and VTA of young wild-type and CaBP-9k KO mice. Scale bar= 40 μm. (C) Quantification of B. Histograms show the intensity of α-synuclein-positive and TH-positive cells in the SNc and VTA of old CaBP-9k KO mice. $n = 10$ for mice for each group. Data shown are the means ± SEMs and were analysed by two-tailed unpaired Student's t-tests.

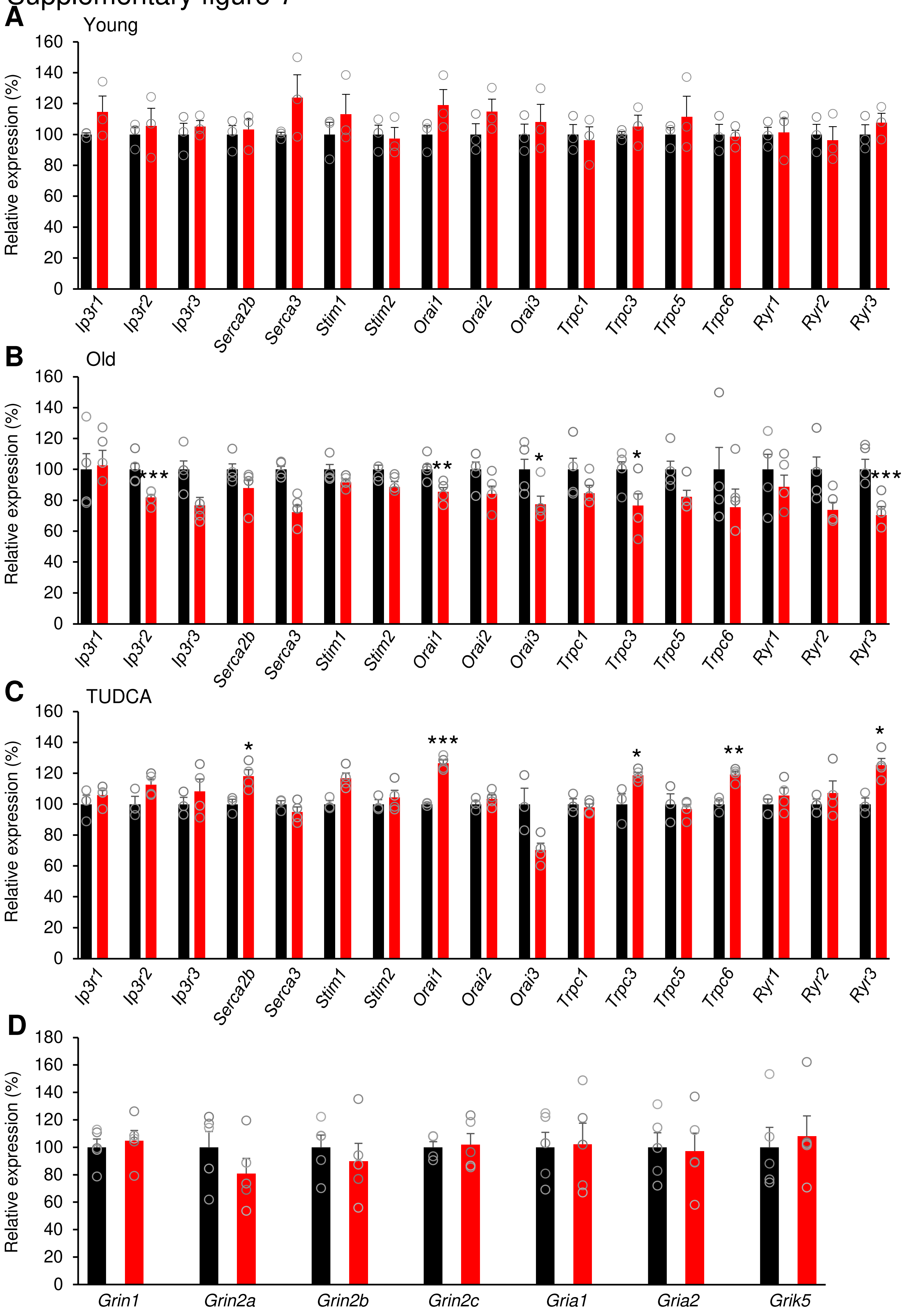
Supplementary figure-6



Supplementary Fig. 6. shCaBP-9k mediated knockdown of endogenous CaBP-9k in primary neuronal cells

(A) shCaBP-9k knockdown endogenous CaBP-9k. Expression of shCaBP-9k transfected cultured primary neuronal cells. (B) Quantification of A. The intensities were measured using ImageJ. n = 7 cell culture replicates using 7 mice for each condition. Data shown are the means ± SEMs and were analysed by two-tailed unpaired Student's t-tests.

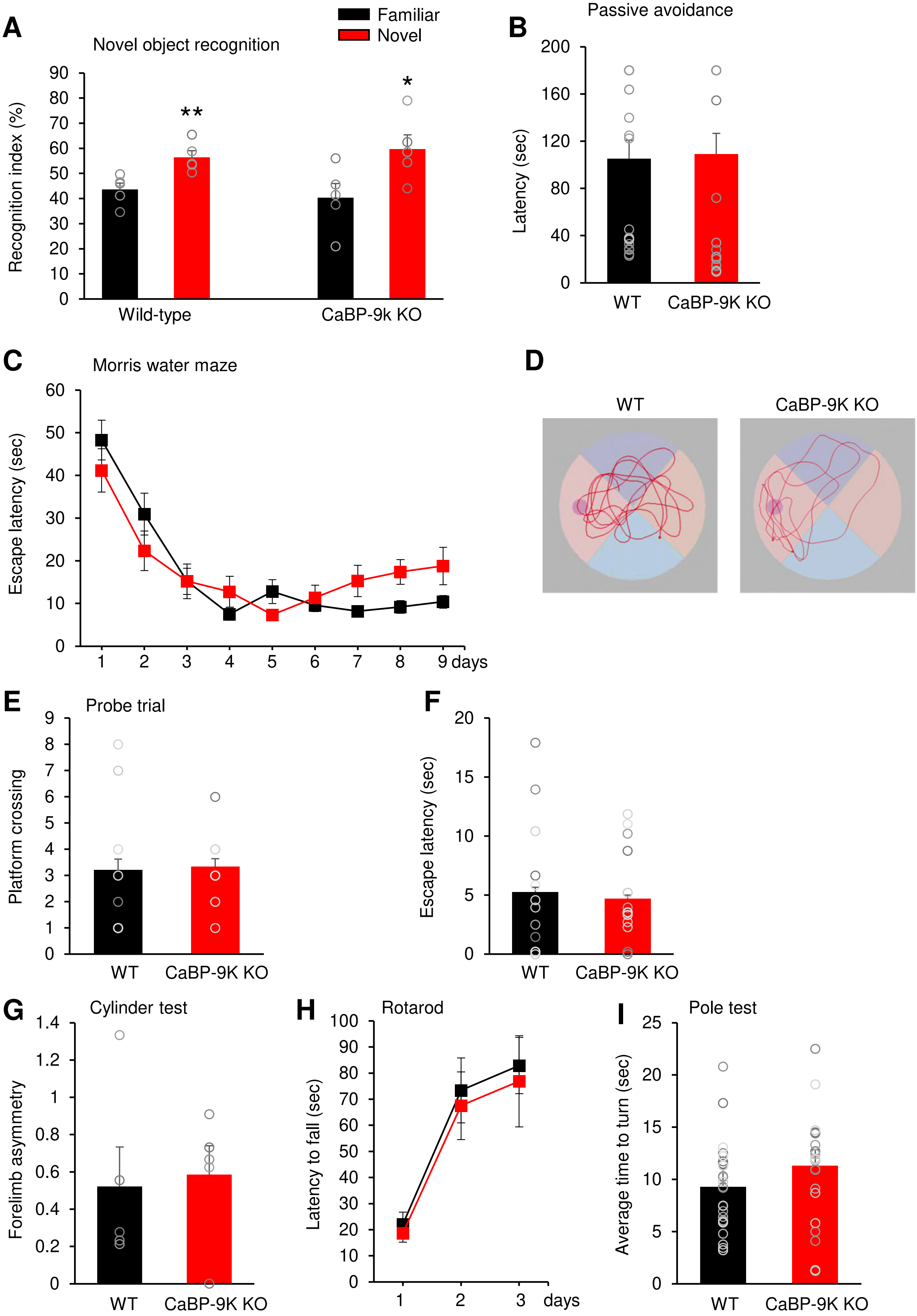
Supplementary figure-7



Supplementary Fig. 7. Altered calcium-channel genes in CaBP-9k KO mice.

The mRNA levels of genes encoding calcium channels were assessed by real-time PCR using brain lysates from (A) young, (B) old, and (S) TUDCA-treated mice. Young group: n = 3 for mice for each group; Old group: n = 5 for mice for each group; TUDCA-treated groups: n = 3 for TUDCA-treated wild-type mice; n = 4 for TUDCA-treated CaBP-9k KO mice. (D) The mRNA levels of glutamate receptors were assessed by real-time PCR using brain lysates from old wild-type and CaBP-9k KO mice. The level of *Gapdh* mRNA was used for normalization. Data shown are the means ± SEMs and were analysed by two-tailed unpaired Student's t-tests.

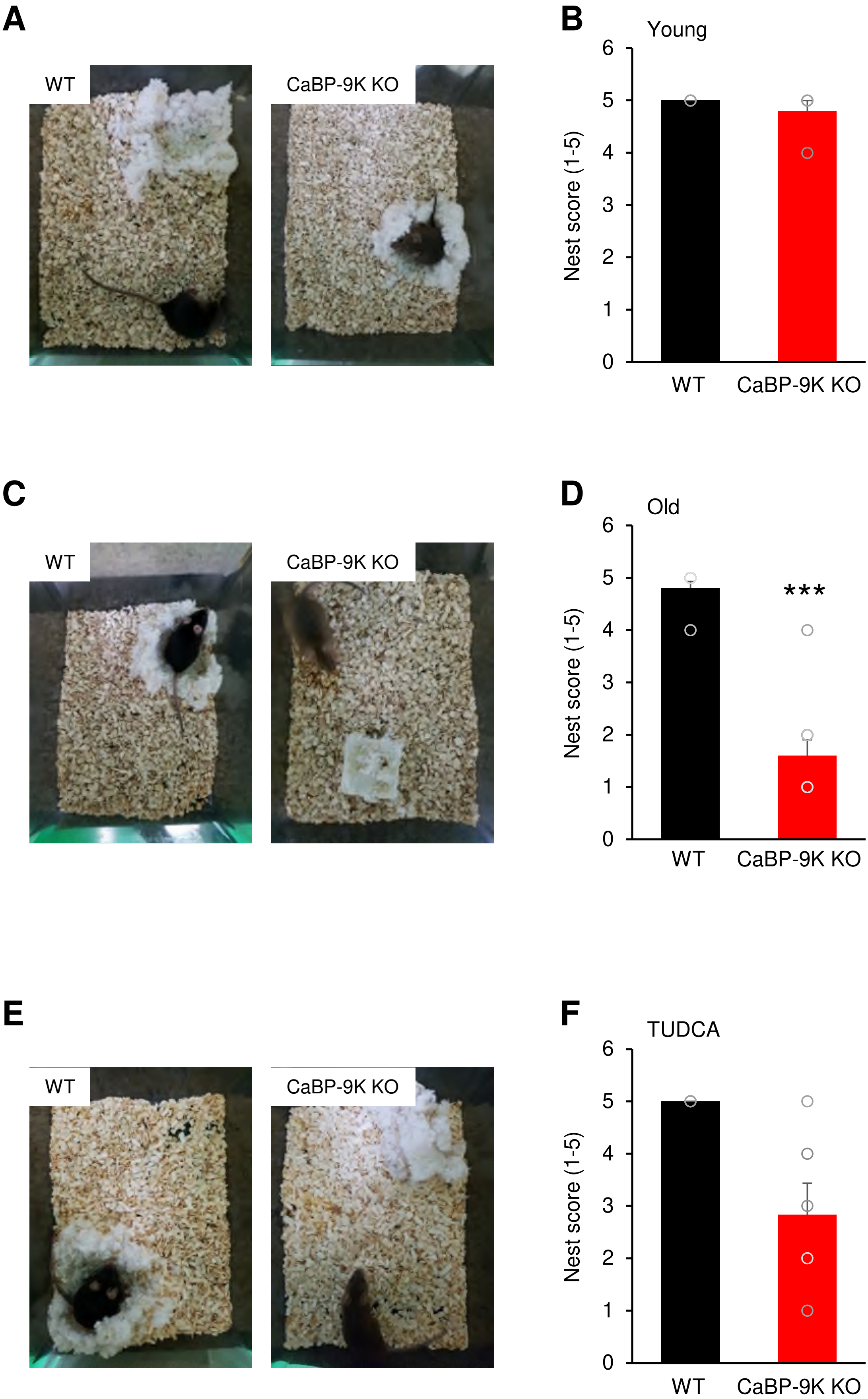
Supplementary figure-8



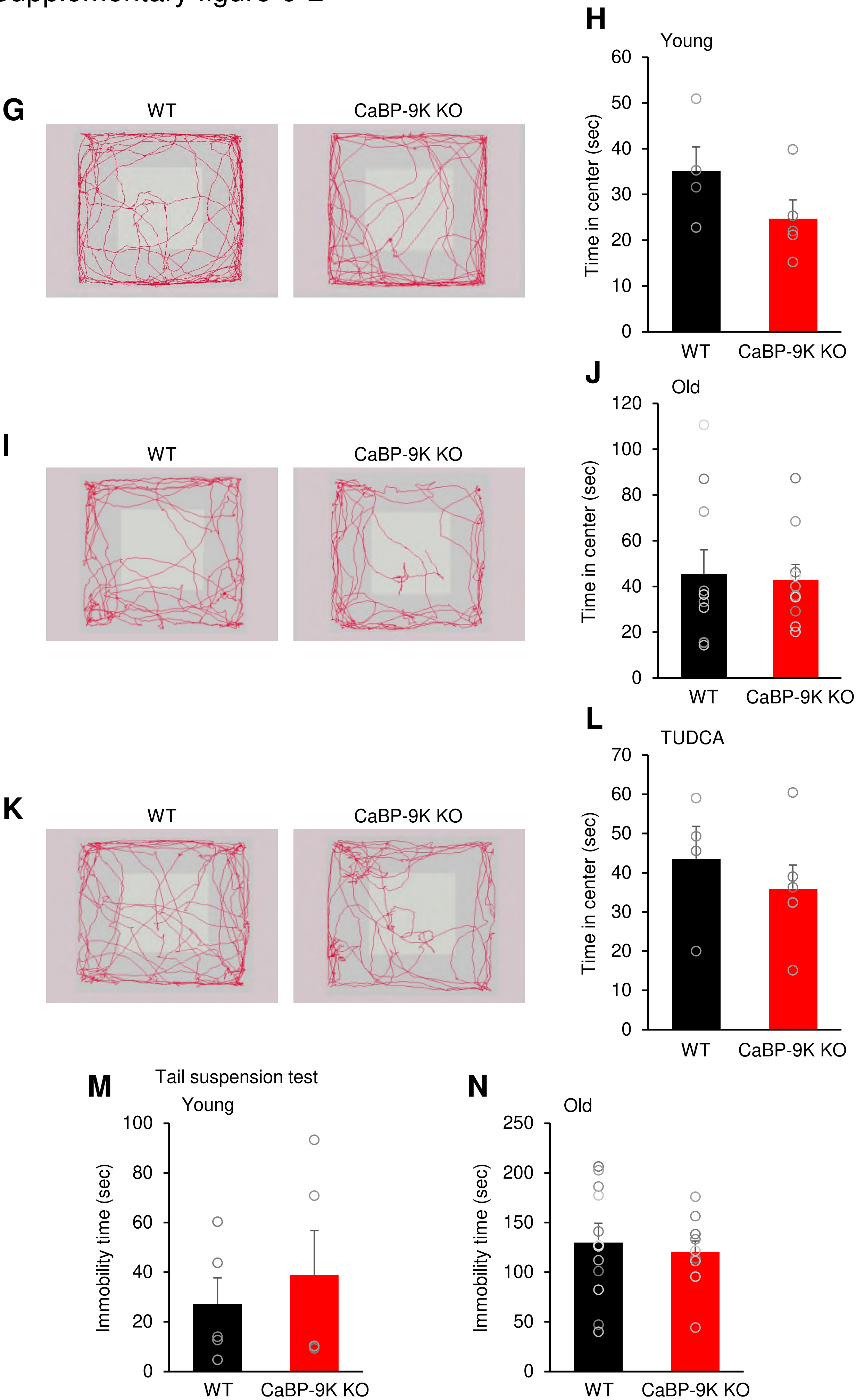
Supplementary Fig. 8. Memory and motor behaviors in young CaBP-9k KO mice.

(A) Young wild-type and CaBP-9k KO mice were assessed in the novel object recognition test. $n = 5$ for mice for each group. (B) Young wild-type and CaBP-9k KO mice were assessed in the passive avoidance test; the latency to enter the dark compartment was recorded. $n = 5$ for mice for each group. (C) The Morris water maze reveals the performance during training trials in young wild-type and CaBP-9k KO mice. $n = 5$ for mice for each group. (D) Representative swim paths of young mice during a probe trial after training. (E,F) Quantification of D. Young CaBP-9k KO mice showed no differences the platform crossing times or escape latency in probe trial. (G) Contralateral forelimb use was assessed in young wild-type and CaBP-9k KO mice in the cylinder test. (H) The latency to fall was assessed in young mice in the rotarod test. (I) In the pole test, the turning time was assessed in young mice in the pole test. $n = 5$ for mice for each group. Data shown are the means ± SEMs and were analysed by two-tailed unpaired Student's t-tests.

Supplementary figure-9-1



Supplementary figure-9-2



Supplementary Fig. 9. Nesting, anxiety and depression behaviors in CaBP-9k KO mice.

(A,C,E) Representative photographs of the nests at 24 h. (B,D,F) The quality of the nest construction was assessed on a rating scale of 1–5. Young group: $n = 5$ for mice for each group; Old group: $n = 10$ for mice for each group; TUDCA group: $n = 4$ for TUDCA-treated wild-type mice; $n = 6$ for TUDCA-treated CaBP-9k KO mice. (G,I,K) Representative tracing of mouse movement in the open field test. (H,J,L) Quantification of G,I and K. There were no differences in time spent in the center area among the groups. (M,N) CaBP-9k KO mice showed similar immobility times in the tail suspension test. Young group: $n = 5$ for mice for each group; Old group: $n = 10$ for mice for each group; TUDCA group: $n = 4$ for TUDCA-treated wild-type mice; $n = 6$ for TUDCA-treated CaBP-9k KO mice. Data shown are the means ± SEMs and were analysed by two-tailed unpaired Student's t-tests.

Supplementary figure-10-1

Figure 1I

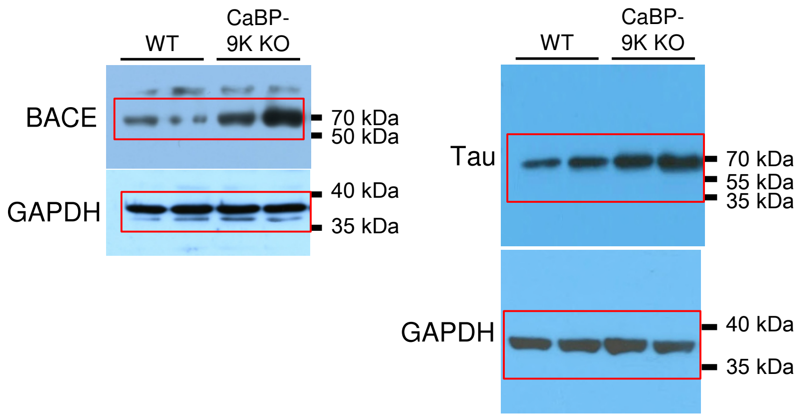


Figure 3A

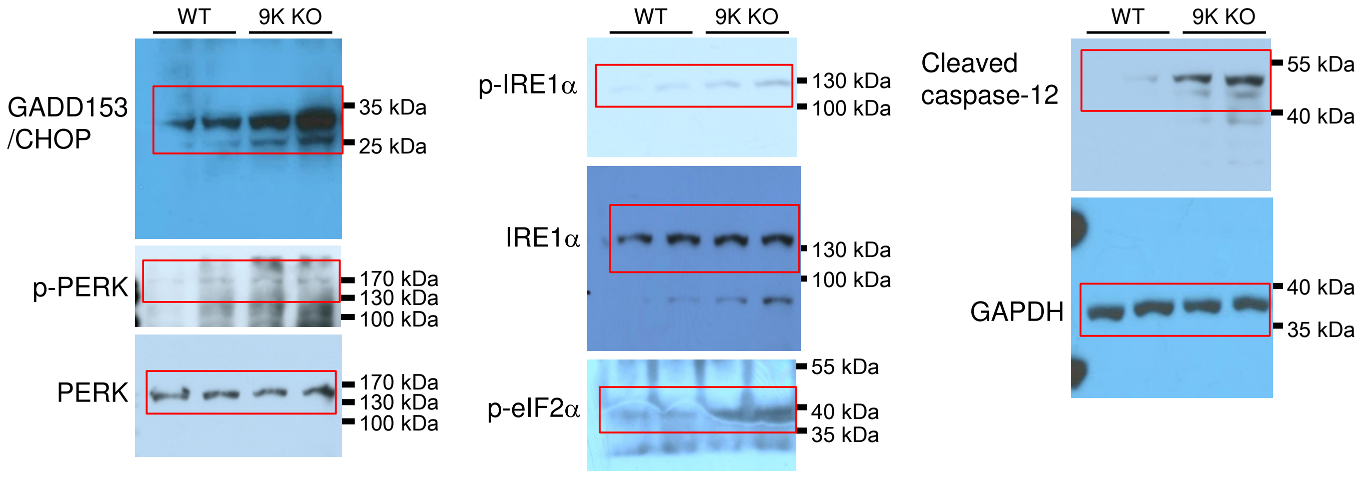


Figure 3C

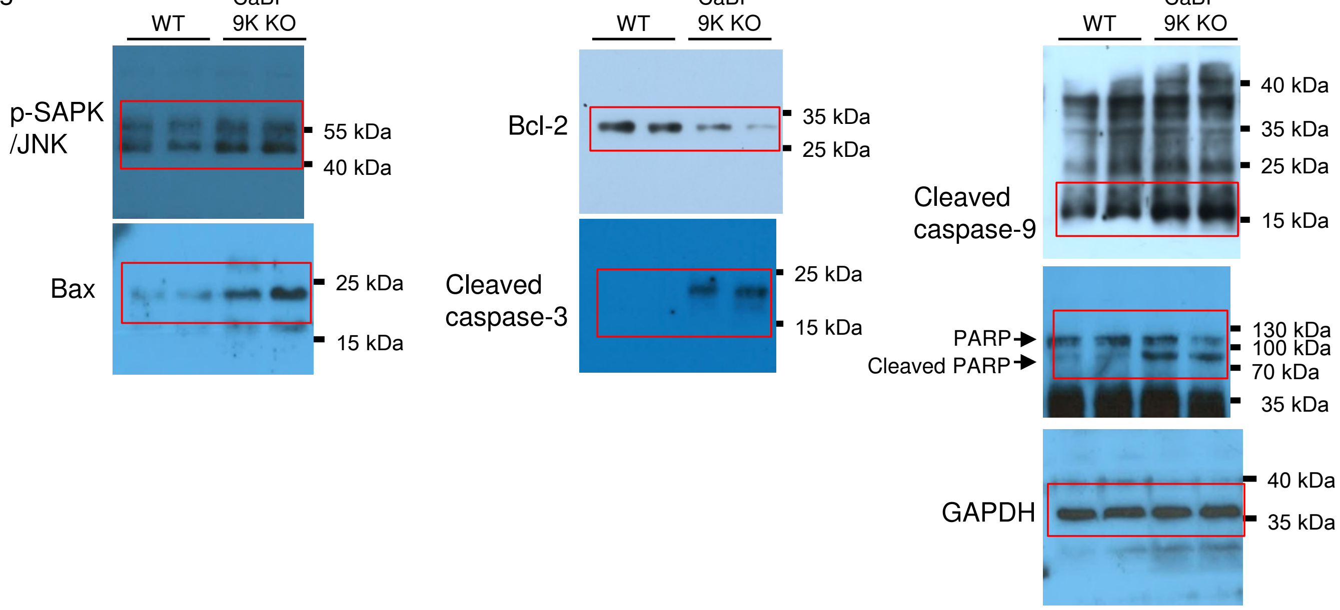
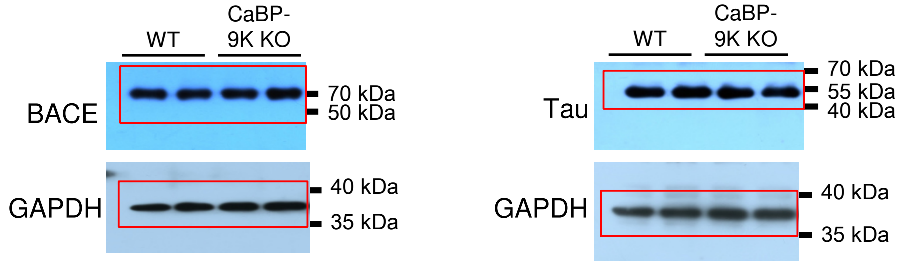


Figure 6E



Supplementary figure-10-2

Figure 6K

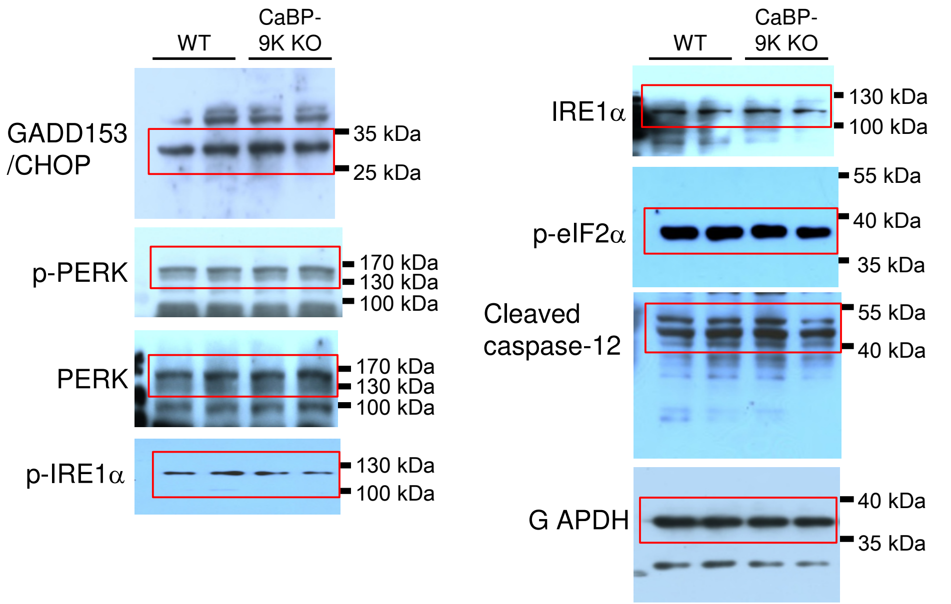
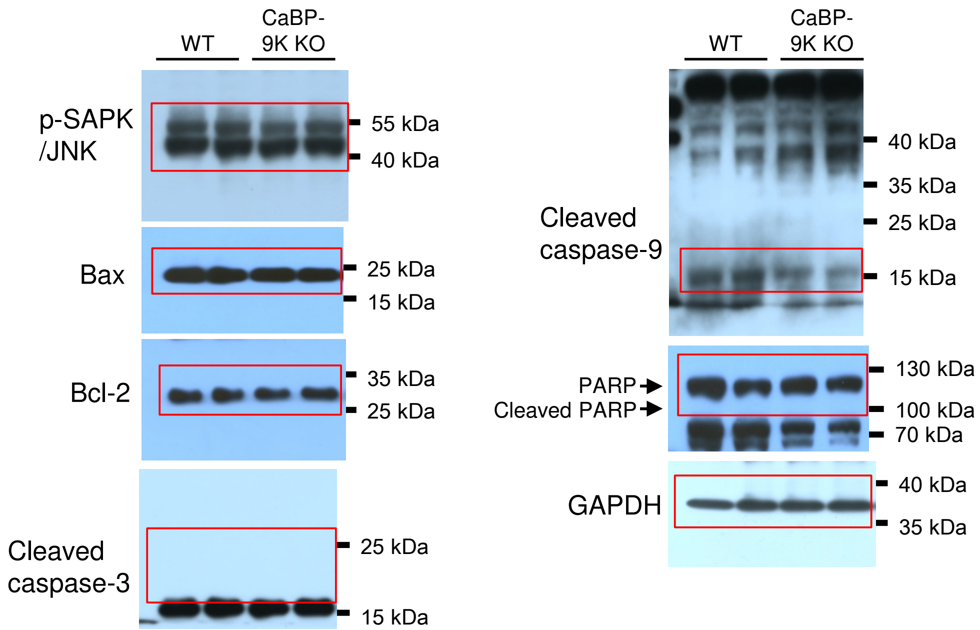
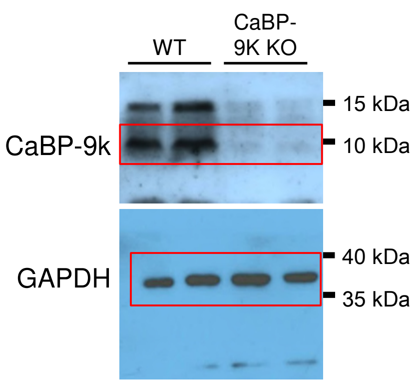


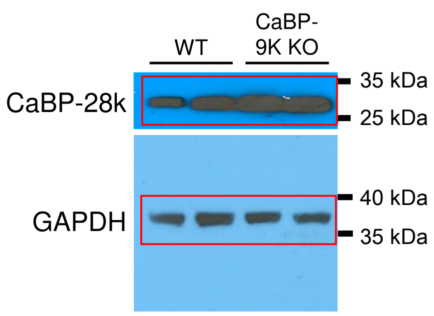
Figure 6M



Supplementary Figure 1A



Supplementary Figure 1D



Supplementary Fig. 10. Western blot images.

Western blot images for figure 1I, 3A, 3C, 6C, 6D, 6I, supplementary figure 1A and supplementary figure 1D.

Supplementary Table 1. Primer sequences for real-time PCR

Gene	Primer sequence (5`→ 3`)	Accession No.
CaBP-9k	F: tgtgtgctgagaagtctcctg	NM_009789
	R: gctggggaactctgactgaa	
Th	F: cgtcatgcctcctcacctat	NM_009377
	R: tacacagcccaaactccaca	
Dat	F: cccctgcttccttctgtatg	NM_010020
	R: gcataggccagtttctctcg	
Ddc	F: tgactacaggcactggcaga	NM_001190448
	R: agcagaccaacccaagaatg	
Gdnf	F: cctcgaagagagaggaatcg	NM_001301332
	R: acaggaaccgctgcaatatc	
Bdnf	F: gcggcagataaaaagactgc	NM_001048139
	R: cccgaacatacgattgggta	
Drd1a	F: cctccctgaaccccattatt	NM_001291801
	R: gtggctggaaaacatcacag	
Drd2	F: ctcaggagctggaaatggag	NM_010077
	R: ttttctggtttggcaggact	
Ip3r1	F: acactggtcaggccttctgt	NM_010585
	R: gaaagctcccagcagaaaca	
Ip3r2	F: gcacaacatgtggcattacc	NM_010586
	R: ccttcgttgctgacaagtga	
Ip3r3	F: gaacctcatctttggggtga	NM_080553
	R: tgctccagtttgatgtgctc	
Serca2b	F: gatcacaccgctgaatctga	NM_001110140
	R: agggagcaggaagatttggt	
Serca3	F: ccatggccttatctgtgctt	NM_001163336
	R: gtggcaccaggaggataaga	
Stim1	F: ccaggatctctggtggagaa	NM_009287
	R: ggggctaagagaatgggaag	
Stim2	F: caatggcatcctggagaaat	NM_001081103
	R: gtcatgtgggatgctgctta	
Orai1	F: ctcatgatcagcacctgcat	NM_175423
	R: agcacgacctctgctaggaa	
Orai2	F: tgagcaacatccacaacctc	NM_178751

	R: catccactgggaggaacttg	
Orai3	F: catcacaacagccttccaaa	NM_198424
	R: gcaaccaaggatcggtagaa	
Trpc1	F: atggatttgctcgcatacct	NM_001311123
	R: gtgctctgcatcttctgtcg	
Trpc3	F: ttttccaaatgcaggaggag	NM_019510
	R: gctgatatcgtgttggctga	
Trpc5	F: aggaaagccaaaatccgagt	NM_009428
	R: ctgccacatacaatgctgct	
Trpc6	F: ggccaaattgtggttttcct	NM_001282086
	R: gcatcttcttggaagccttg	
Ryr1	F: gacgtgctacctgttccaca	NM_009109
	R: tgatagccagcagaatgacg	
Ryr2	F: agtgccacatggctttgaa	NM_023868
	R: gggaaaaattcccaacacct	
Ryr3	F: catggagaccaagtgcttca	NM_001319156
	R: ctggcccgtatgttctgttt	
Grin1	F: gcatcgtagctgggatcttc	NM_001177656
	R: accgagggatctgagaggtt	
Grin2a	F: atacgggagcctgttcagtg	NM_008170
	R: cgagggacatctcccaataa	
Grin2b	F: agcaagtcgctctaccctga	NM_001363750
	R: ggctgacaccactggcttat	
Grin2c	F: ccatttctcccgctattcc	NM_010350
	R: agagggttggatcgagtgaa	
Gria1	F: cgataaaggggaatgtggaa	NM_001113325
	R: agaaacccttcatccgcttc	
Gria2	F: tttccttgggtgcctttatg	NM_001039195
	R: atcctcagcactttcgatgg	
Grik5	F: gtttgggcatggagaacatt	NM_001360067
	R: tagctcctgcagcatctcct	
Gapdh	F: aaggtcatcccagagctgaa	NM_008084
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