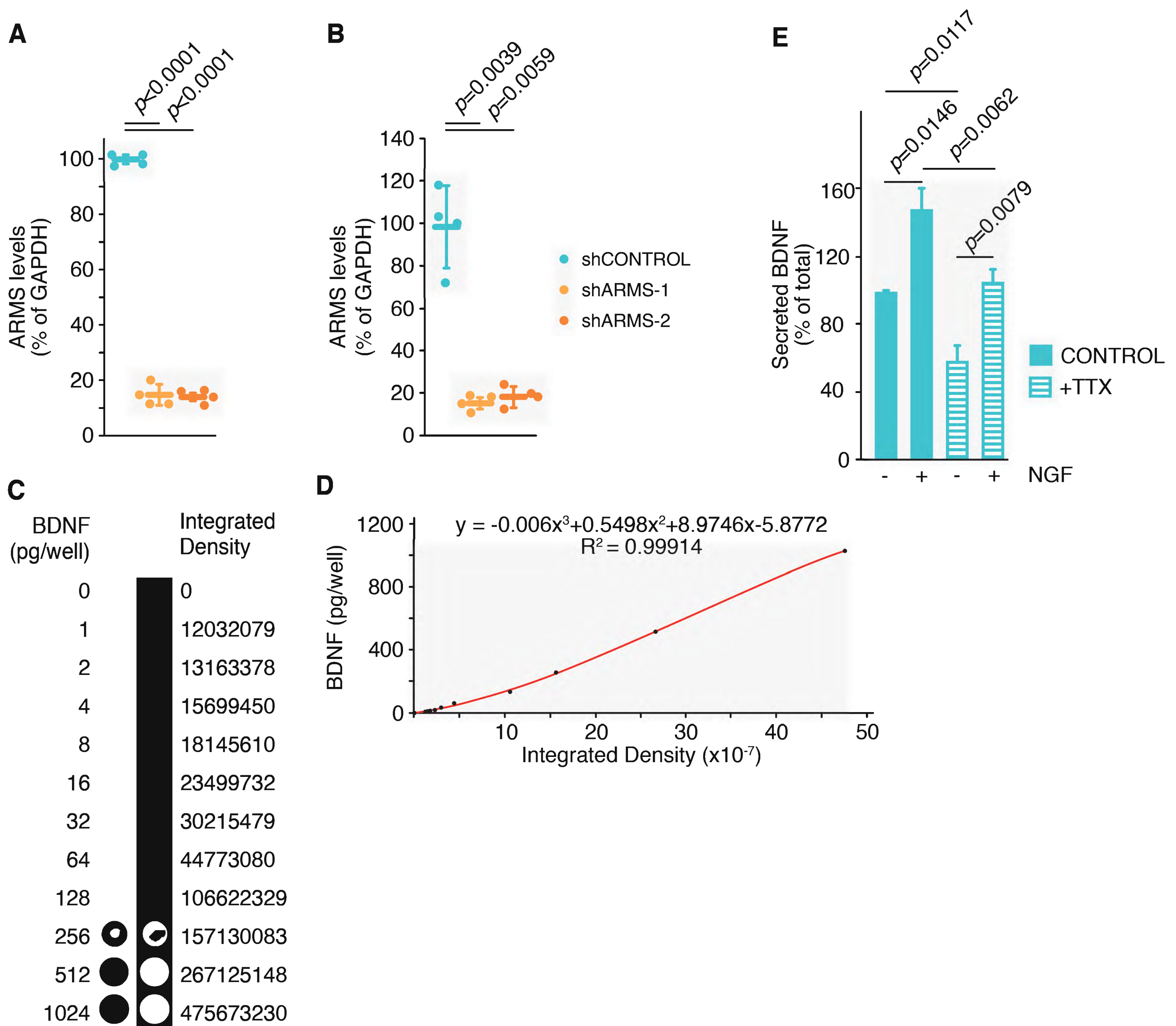


Figure 1-1. ARMS downregulation by shRNAs and sensitivity of the BDNF immunoassay

A) Quantification of ARMS depletion in cultured DRG neurons corresponding to the experiments performed in Fig. 1A,B. (n = 4). Paired Student´s t-test, mean ± s.e.m. shC vs shARMS-1 t = 8.078, df = 3; shC vs shARMS-2 t = 7.03, df = 3.

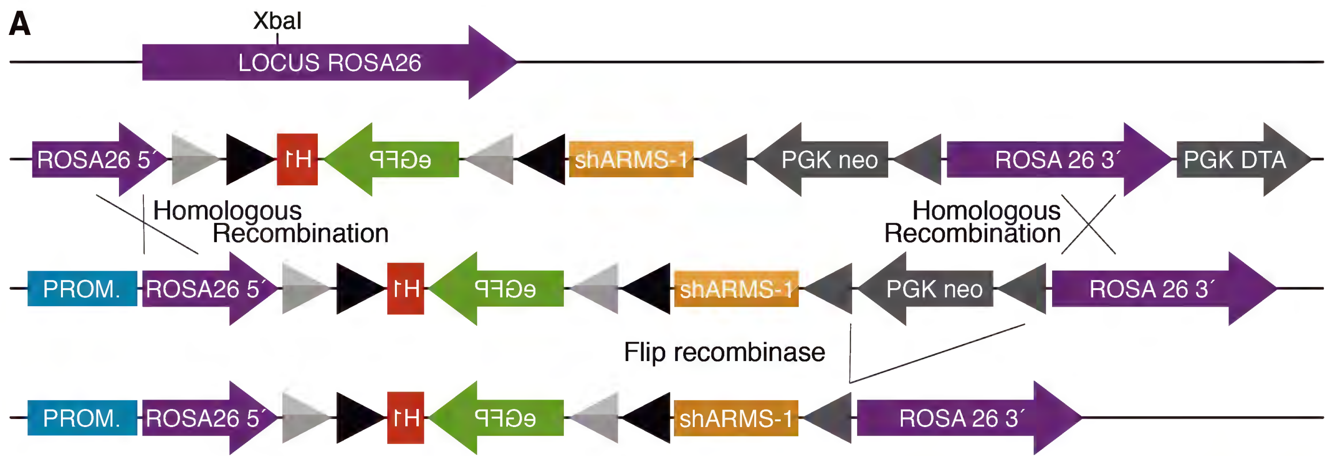
B) Quantification of ARMS depletion in cultured cortical neurons corresponding to the experiments performed in Fig. 1C,D. (n = 4). Paired Student´s t-test, mean ± s.e.m. shC vs shARMS-1 t = 44.19, df = 3; shC vs shARMS-2 t = 111.8, df = 3.

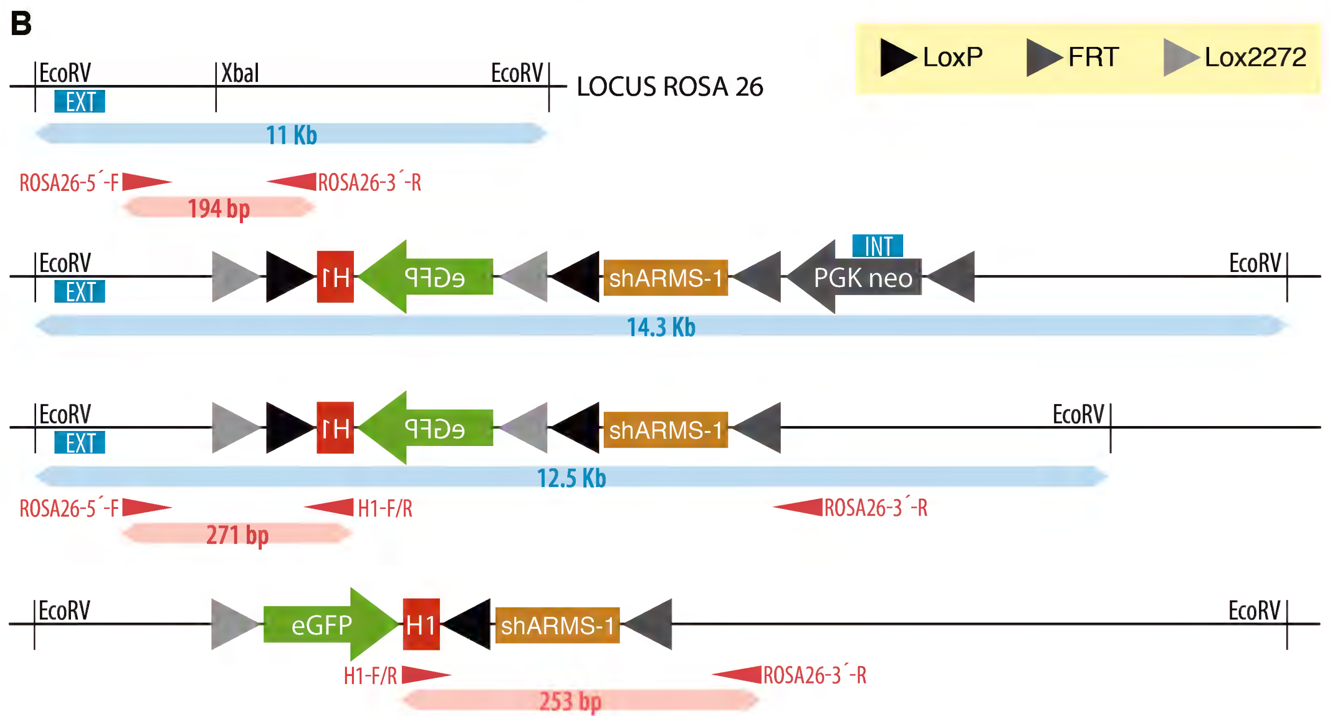
C) Different amounts of recombinant BDNF (1–1024 pg/well) were incubated on ELISA plates coated with 1 μg/well of mAb#1. 12.5 ng of mAb#9 HRP were incubated for 3 hours at 30 ºC, washed with TBST and developed with SuperSignal ELISA Femto Substrate diluted 50% in water. A representative standard curve is shown.

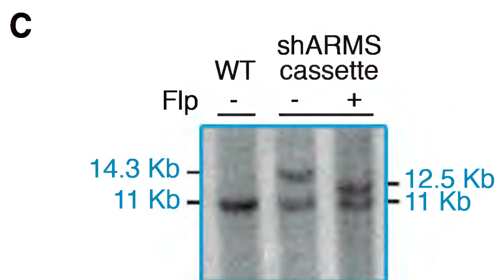
D) Adjusted standard curve of BDNF ELISA using recombinant BDNF.

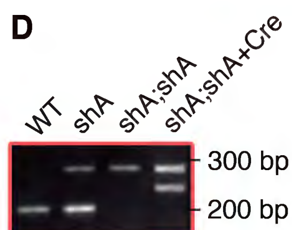
E) Neuronal activity is required for BDNF release in response to NGF. BDNF secretion experiments were performed in DRGs in a similar way as described in Fig. 1B in presence (striped bars) and absence (solid bars) of TTX with or without NGF. (n = 7). Paired Student´s t-test, mean ± s.e.m. Control vs TTX t = 9.157, df = 6; Control-NGF vs TTX-NGF t = 4.466, df = 6.

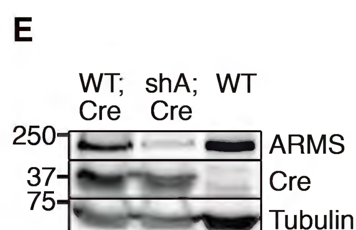
FIGURE 1-1

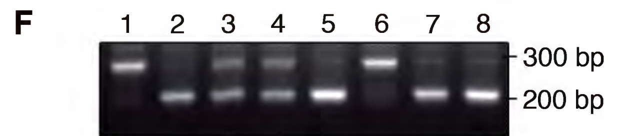


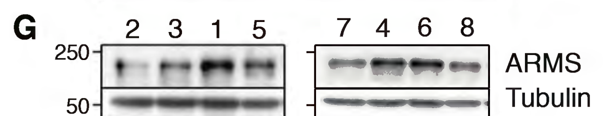


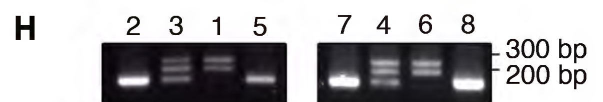












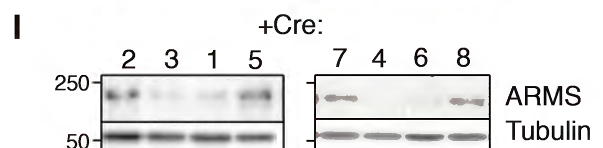


FIGURE 2-1

Figure 2-1. Generation, genotyping, and functionality of shARMS mice

A) Schematics of the wild-type ROSA26 allele, the targeting vector and the targeted allele before and after Flp recombination. DTA: diphtheria toxin; eGFP: enhanced GFP; H1: promoter H1; PGK neo: neomycin resistance gene; PROM: promoter ROSA26.

B) Schematics of the wild-type ROSA26 allele and the targeted allele before and after Flp recombination with the restriction sites EcoRV and XbaI used to digest genomic DNA and to clone ARMS shRNA cassette, respectively. The size of the fragments generated after EcoRV digestion are shown (blue). For genotyping, primers are denoted with red arrowheads and the fragment sizes amplified after PCR depending on the genotype are: 194 base pairs for WT mice, 271 base pairs and 253 base pairs for shARMS mice before and after Cre recombinase, respectively (red).

C) Southern blot analysis of genomic DNA from WT and targeted mice before and after Flip-mediated recombination, upon digestion with EcoRV. As indicated in the schematics in panel B, the 5´ external probe detects an 11 kb DNA fragment from WT alleles and a 14.3 kb and 12.5 kb DNA fragment from mutant alleles with or without the Neo cassette, respectively. A representative blot is shown.

D) PCR analysis of genomic tail DNA from WT, shARMS, shARMS;shARMS cultured cortical neurons infected with Cre-expressing lentiviruses. As indicated in the schematics in B, the fragments amplified were of 194 bp and 271 bp for WT and shARMS mice, respectively, and 253 bp for shARMS mice in presence of Cre recombinase. A representative PCR is shown.

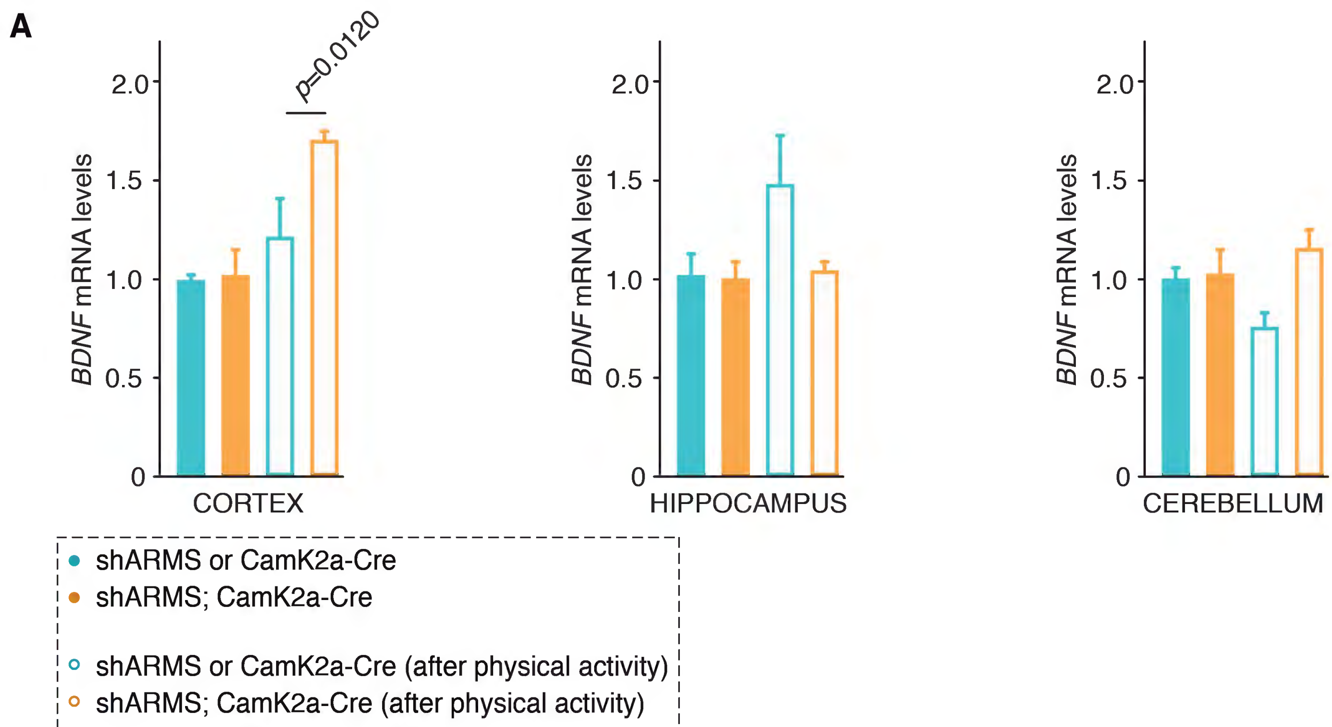
E) Efficient ARMS downregulation in cortical neurons from shARMS mouse in the presence of Cre recombinase. Cortical neurons were obtained from E16.5 mouse embryos with the corresponding genotype, and infected or not with lentiviruses expressing Cre recombinase. Western blot analyses were performed to detect ARMS, Cre recombinase and tubulin as a loading control.

F) PCR genotyping of E13.5 embryos from shARMS matings. Genomic DNA was obtained from tails and PCR reaction was performed. Samples 2, 5, 7, and 8 are WT; 3 and 4 have one copy, and 1 and 6 two copies of the cassette. A representative PCR is shown.

G) ARMS expression is unchanged in shARMS mice. Western blot analyses from lysates of cultured DRG neurons corresponding to E13.5 embryos of panel F. Tubulin was used as loading control.

H) PCR analysis of cultured DRGs from E13.5 embryos corresponding to panel F infected with lentivirus expressing Cre recombinase. DRG neurons were cultured and infected with lentiviruses expressing Cre recombinase. Afterwards, genomic DNA was obtained and PCR reaction was carried out. Note the presence of the 253 bp fragment in those samples with the shARMS cassette (samples # 3, 1, 4, and 6). A representative PCR is shown.

I) Knockdown of ARMS expression using cultured DRG neurons from shARMS embryos expressing Cre recombinase corresponding to panel H (samples # 3, 1, 4 and 6). Samples were collected and Western blot analyses were performed to assess ARMS expression. Tubulin was used as a loading control.



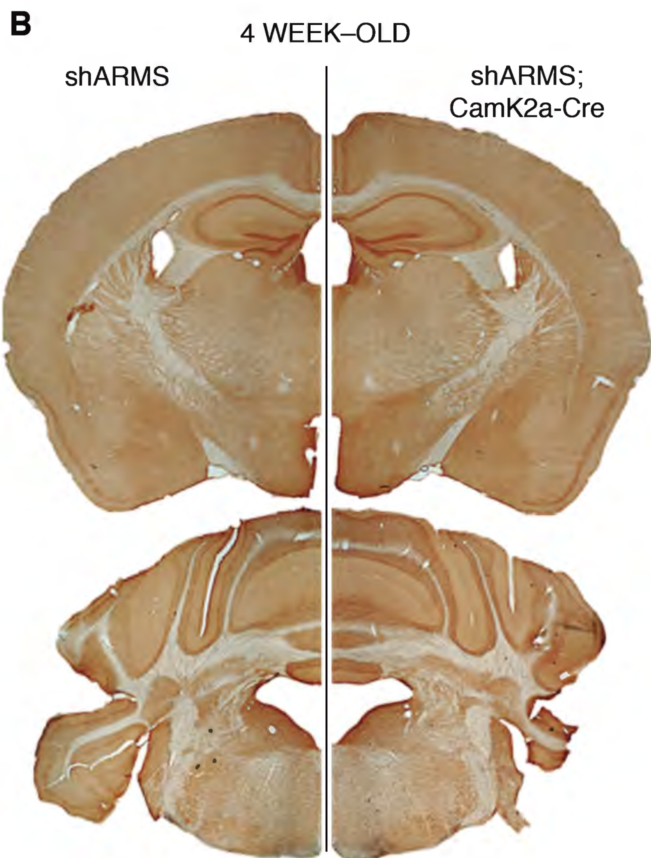


Figure 3-1.
A) Quantification of bdnf mRNA levels in mice subjected to physical exercise. Total RNA was obtained, cDNA was synthesized and quantitative PCR was performed as described in Material and Methods. The relative transcription level of bdnf was normalized to that of sdha using the 2-ΔΔCt method. Data are presented as means ± SEM. Triplicate determinations from 3-4 control and mutant mice subjected or not to exercise were quantified. P value was calculated using a two-tailed Student´s t-test.
B) Similar ARMS expression in forebrain of shARMS and shARMS;CamK2a-Cre mice at 4 weeks of age
Immunohistochemistry with ARMS antibodies of control (shARMS) (left hemisphere) and mutant mice (shARMS;CamK2a-Cre) (right hemisphere). A representative staining is shown (n = 2).

FIGURE 3-1

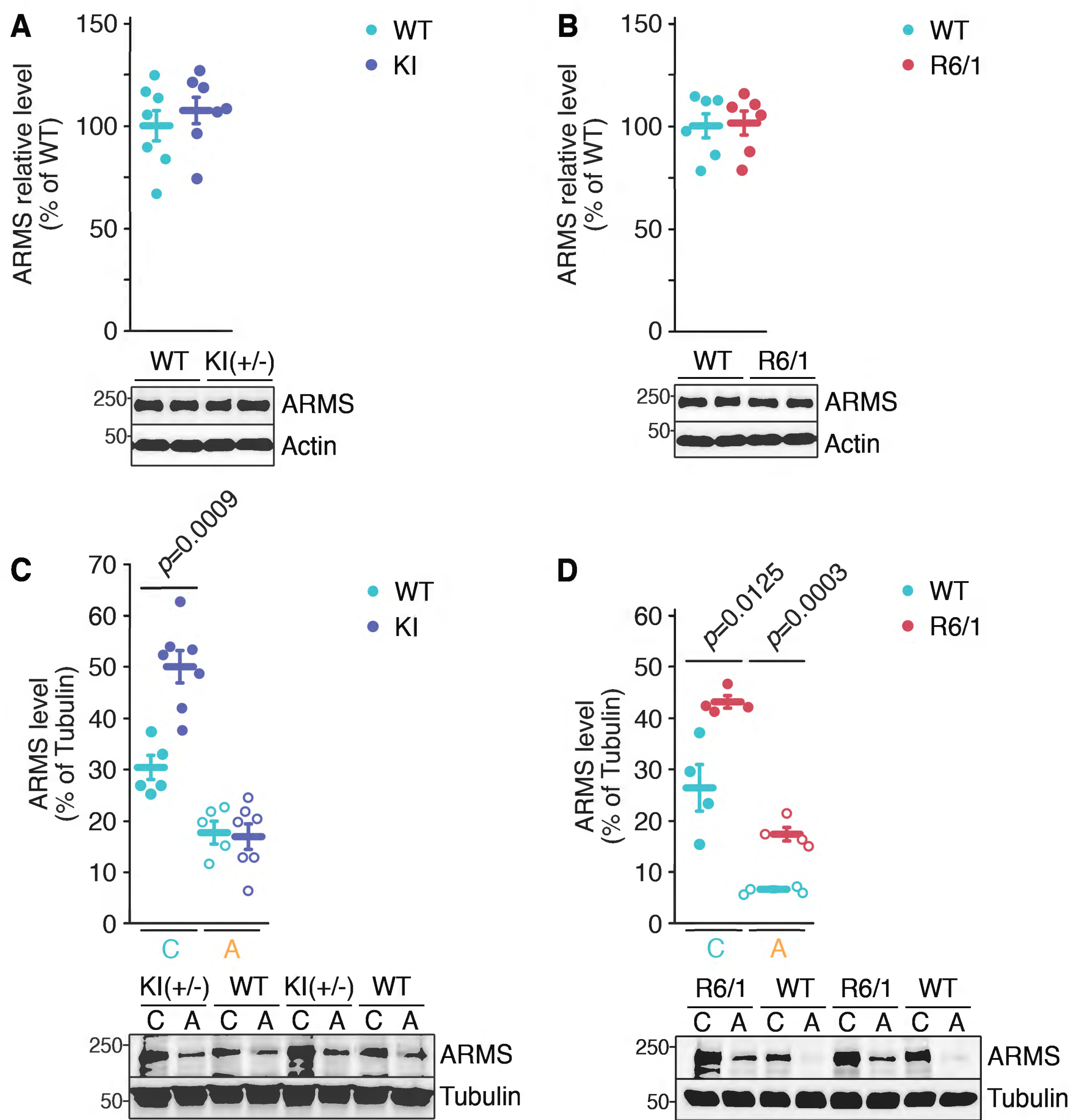


Figure 4-1. ARMS levels do not change in the cortex of HD mouse models

A) ARMS expression in the cortex of 8 month-old KI mice is not altered. Western blot for ARMS in the cortex from WT and KI mice. Actin was used as a loading control (mean ± SEM; n = 7). A representative Western blot is shown.

B) ARMS expression in the cortex of 20–30 week-old R6/1 mice is not altered. Western blot for ARMS in the cortex from WT and R6/1 mice. Actin was used as a loading control (mean ± SEM; n = 6 and 6; for WT and R6/1, respectively). A representative Western blot is shown.

C) Depletion of ARMS in organotypic hippocampal slices from WT and KI mice. Quantification of ARMS protein levels upon infection with lentivirus expressing shControl (C) or shARMS-1 (A) (n = 5 and 7 for WT and KI mice, respectively). Unpaired Student's t-test, mean ± s.e.m. Representative Western blot showing ARMS levels in hippocampal slices of WT and KI mice.

D) Depletion of ARMS in organotypic hippocampal slices from WT and R6/1 mice. Quantification of ARMS protein levels upon infection with lentivirus expressing shControl (C) or shARMS-1 (A) (n = 4). Unpaired Student's t-test, mean ± s.e.m. Representative Western blot showing ARMS levels in hippocampal slices of WT and R6/1 mice.

FIGURE 4-1