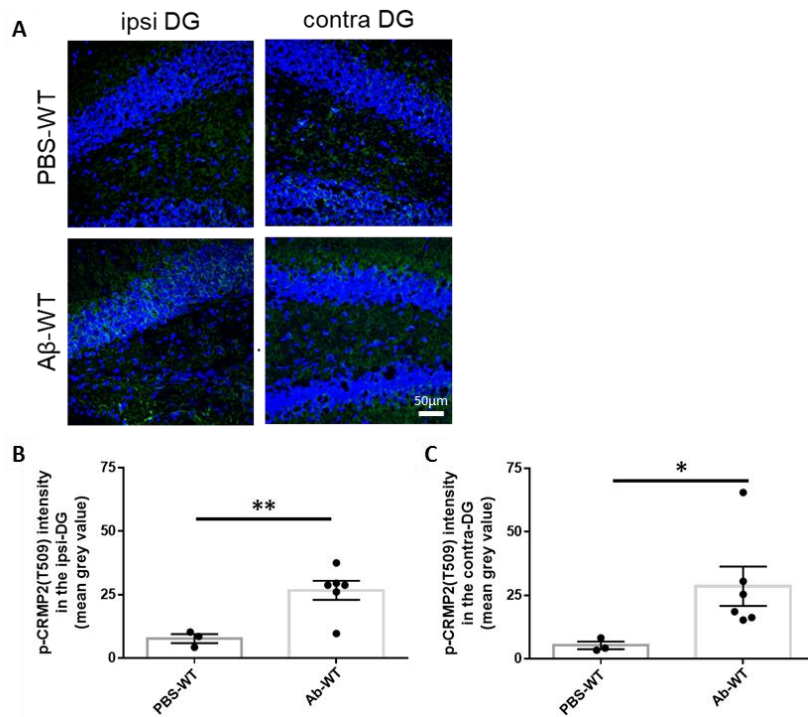


Supplemental Information

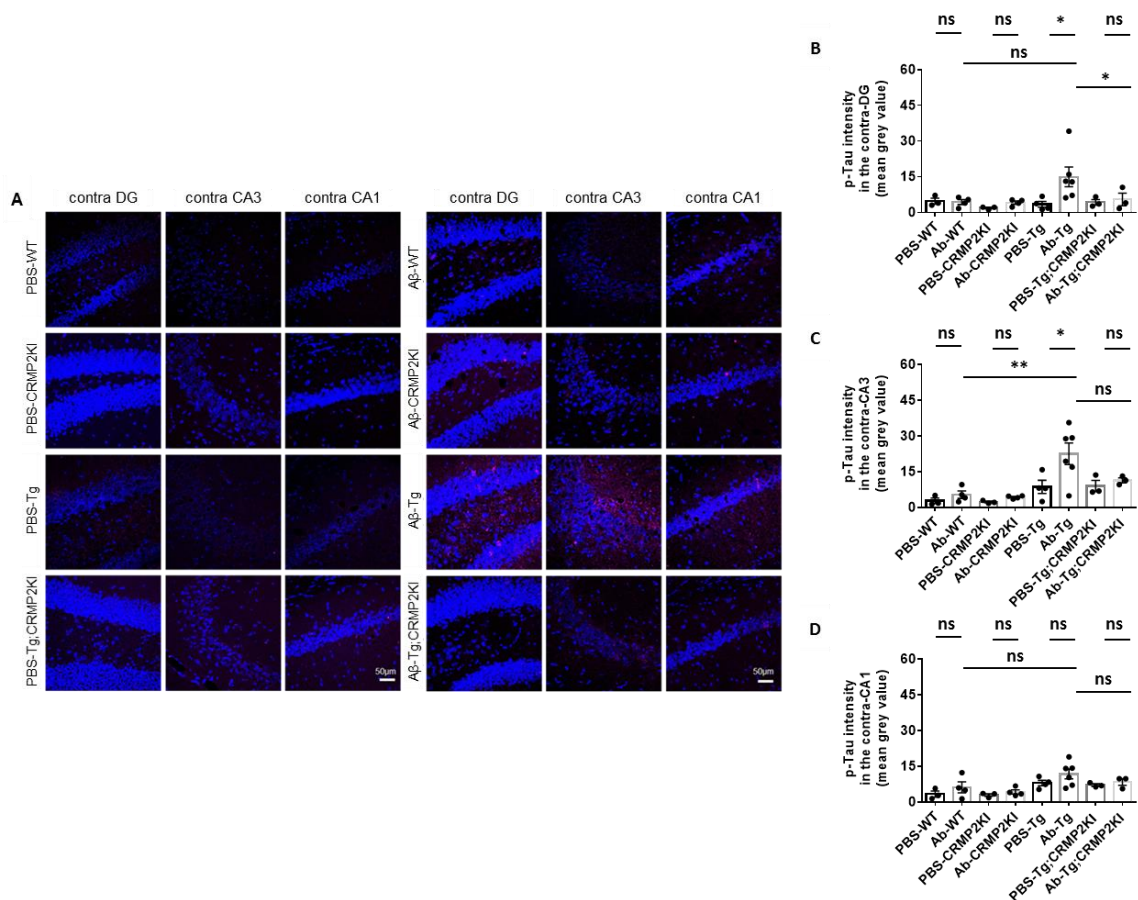
Involvement of CRMP2 phosphorylation in amyloid beta-induced tau phosphorylation of hippocampal neurons in Alzheimer's disease mouse models

Daisuke Noguchi¹, Naoto Watamura¹, Miyu Nikkuni¹, Takaomi C Saido², Yoshio Goshima³, Toshio Ohshima^{1,4}



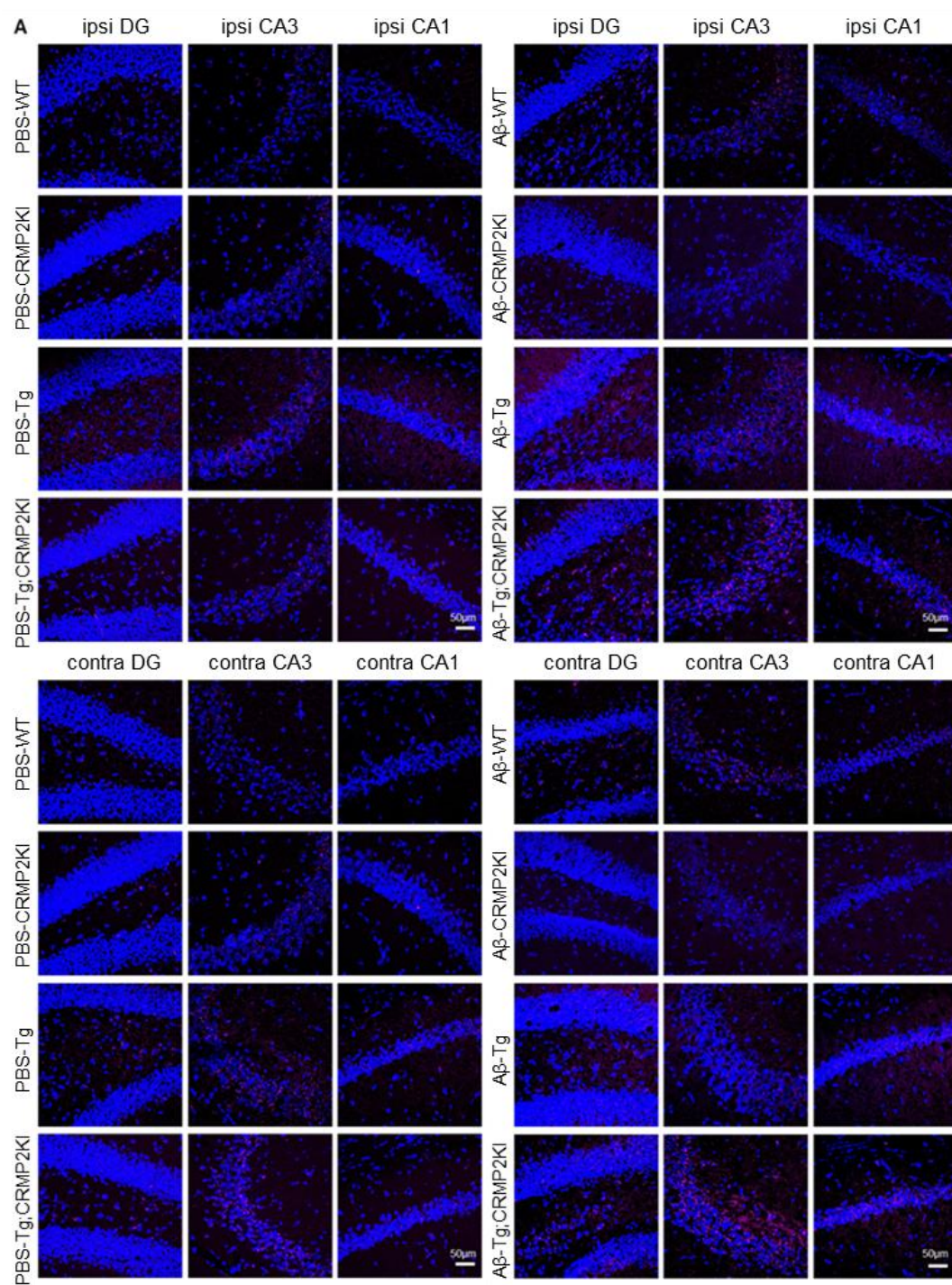
Supplementary Fig1. CRMP2 phosphorylation at T509 was significantly upregulated in the hippocampus of Aβ-injected WT mice.

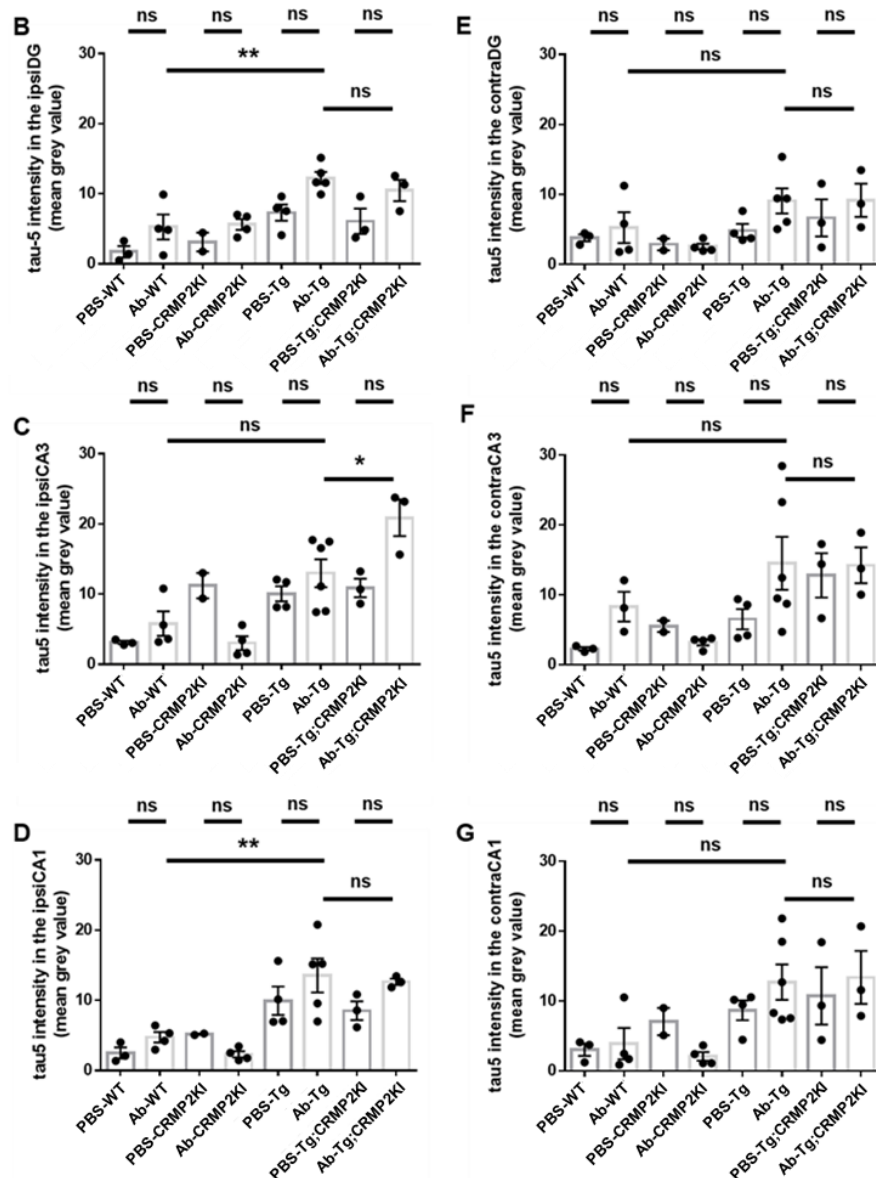
(A) Representative images of immunostaining with anti-CRMP2 (phospho T509) antibody (green) in the ipsilateral and contralateral sides of the hippocampus dentate gyrus (DG) from PBS-injected and Aβ25-35-injected WT mice. Nuclear counterstaining is shown in blue. Scale bar 50 μm. (B)-(C) Quantification of the mean gray value of p-CRMP2(T509)-positive intensity in the ipsilateral DG (B) and contralateral DG (C). Groups compared using unpaired two-tailed Student's t-tests with Welch's correction and confidence intervals of 95% used. Data expressed as mean ± standard error of the mean (SEM). PBS-WT, n=3; Aβ-WT, n=6. *P<0.05, and **P<0.01.



Supplemental Fig2. Tau phosphorylation in the contralateral hippocampus was significantly upregulated Aβ-injected tau Tg mice

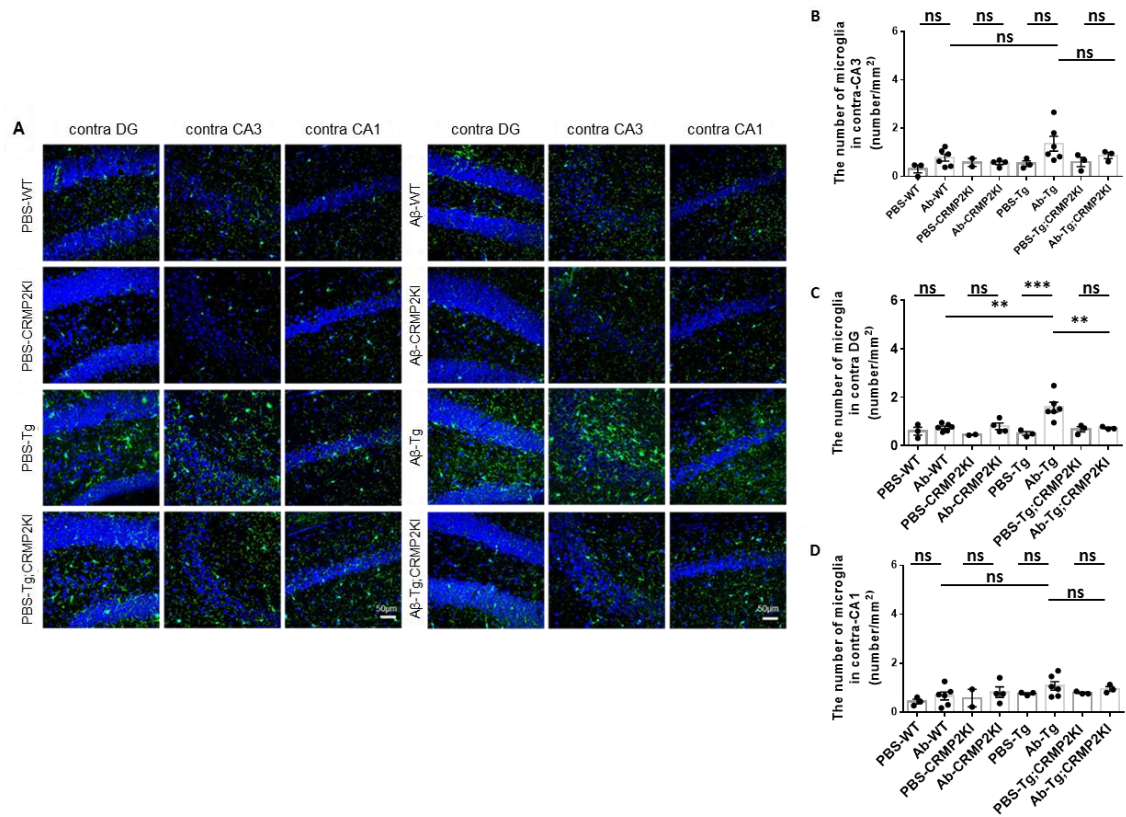
(A) Representative images of immunostaining with anti-AT8 antibody (magenta) in the contralateral hippocampus from WT, CRMP2KI, tau Tg (Tg), and tau Tg; CRMP2KI mice injected with PBS or Aβ₂₅₋₃₅. Nuclear counterstaining is shown in blue. Scale bar = 50 μm. (B)-(D) Quantification of the mean gray value of AT8 positive intensity in the contralateral DG (B), CA3 region (C), CA1 region (D). Tukey's multiple comparison test with Geisser-Greenhouse correction used to compare multiple groups. Data expressed as mean ± standard error of the mean (SEM). PBS-WT, n=3; Aβ-WT, n=4; PBS-2KI, n=3; Aβ-2KI, n=4; PBS-Tg, n=4; Aβ-Tg, n=6; PBS-Tg;2KI, n=3 and Aβ-Tg;2KI, n=3. ns, not significant. *P<0.05, **P<0.01.





Supplementary Fig3. Immunostaining with anti-tau5 antibody in the hippocampus from PBS and A β 25-35 injection samples.

(A) Images of immunostaining with anti-tau-5 antibody (magenta) in the contralateral hippocampus from WT, CRMP2KI, tau Tg (Tg), and tau Tg; CRMP2KI mice injected with PBS or A β 25-35. Scale bar = 50 μ m. (B)–(F) Quantification of the mean gray value of tau-5 positive intensity in the ipsilateral and contralateral DG, CA3, and CA1 region. Tukey's multiple comparison test with Geisser-Greenhouse correction used to compare multiple groups. Data expressed as mean \pm standard error of the mean (SEM). PBS-WT, n=3; A β -WT, n=4; PBS-2KI, n=3; A β -2KI, n=4; PBS-Tg, n=4; A β -Tg, n=6; PBS-Tg;2KI, n=3 and A β -Tg;2KI, n=3. ns, not significant. *P<0.05, and **P<0.01.



Supplemental Fig4. Microglial activation was alleviated in the contralateral hippocampus DG in Aβ-injected tau Tg; CRMP2KI than in Aβ-injected tau Tg mice.

(A) Representative images of immunostaining with anti-Iba1 antibody (green) in the contralateral hippocampus from WT, CRMP2KI, tau Tg (Tg), and tau Tg; CRMP2KI mice injected with PBS or Aβ₂₅₋₃₅. Scale bar = 50 μm. (B-D) Quantification of the mean gray value of Iba1-positive intensity in the contralateral DG (B), CA3 (C) and CA1 region (D). Tukey's multiple comparison test with Geisser-Greenhouse correction was used to compare multiple groups. Data are shown as mean ± standard error of the mean (SEM). ns, not significant. **P<0.01, and ***P<0.005.