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Supplementary Materials

CDK14-dependent phosphorylation of neuronal FAM19A1 activates DIP2A signaling in oligodendrocytes to maintain myelin homeostasis in the adult brain

Soonbong Baek¹, Seungeun Yeo¹, Hyun Jin Jung¹, Jaemyung Jang¹, Jae Young Seong² and Youngshik Choe^{1*}

¹Department of Brain Health & Disease, Korea Brain Research Institute, Daegu, Republic of Korea.

²Department of Biomedical Sciences, Graduate School of Medicine, Korea University, Seoul 02841, Republic of Korea

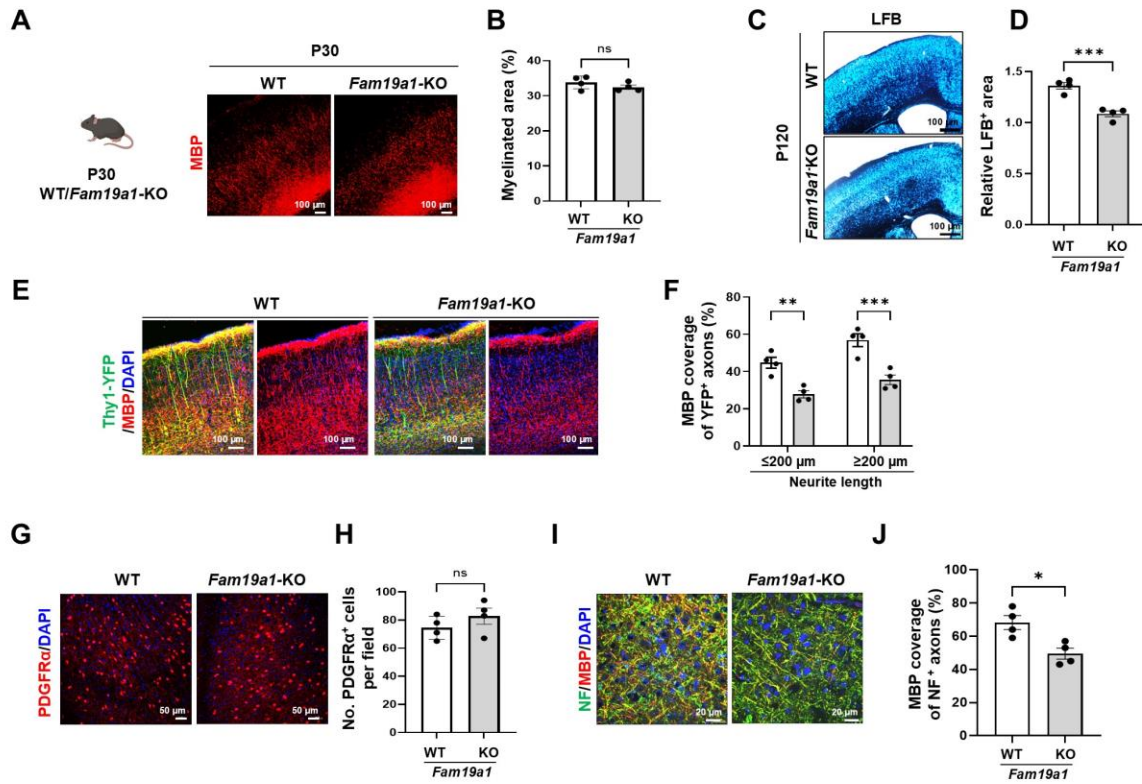
***Correspondence**

Youngshik Choe, Ph.D.

Head Researcher

Korea Brain Research Institute

Supplementary Fig. 1



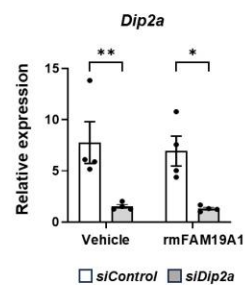
Supplementary Fig. 1. *Fam19a1* deficiency is associated with a reduced cortical myelin area. **A and B** Visual representation (A) with associated quantification (B) of MBP⁺ myelinated area in the cortex of P30 WT and *Fam19a1*-KO mice. n = 4 mice per group. Each dot represents the mean MBP-positive area quantified from three coronal sections per mouse. **C and D** Representative images (C) and relative value (D) of LFB-stained myelin area in the cortex of 4-month-old WT and *Fam19a1*-KO mice. n = 4 mice per group. **E and F** Representative immunofluorescence images (E) and quantification (F) of cortical myelin in Thy1-YFP;WT and Thy1-YFP;*Fam19a1*-KO mice. n = 4 mice per group. Each dot represents the mean MBP-positive coverage of YFP-positive axons, calculated from 90–100 axons per mouse. **G and H** Representative immunofluorescence images (G) and quantification (H) of PDGFR α -positive OPCs in the cerebral cortex of WT and *Fam19a1*-KO mice. n = 4 mice per group. Each dot represents the mean number of PDGFR α -positive cells quantified from 10 randomly selected 0.5-mm² fields per mouse. **I** Visual representation of MBP and NF immunostaining in the somatosensory cortex of WT and *Fam19a1*-KO mice. **J** Quantification of MBP coverage along NF⁺ axons. n = 4 mice per group. Each dot represents the mean MBP-positive coverage of NF⁺ axons, calculated from 90–110 axons per mouse. Differences between the two groups were evaluated using an unpaired two-tailed Student's *t*-test. Data are presented as mean $\pm$ SEM. **P* < 0.05, ***P* < 0.01, and ****P* < 0.001.

Supplementary Fig. 2

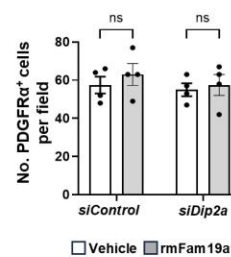
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Rank	Accession	Gene Symbol	Log2 abundance ratio (52 °C / 62 °C)
1	Q80TA9	Epg5	2.57
2	Q61696	Hspa1a	2.15
3	Q99LT0	Dpy30	1.7
4	P99027	Rplp2	1.49
5	Q3TWC7	Gpnmb	1.34
6	Q6IRU2	Tpm4	1.32
7	G3UYF9	Pfdn6	1.28
8	Q3TNG2	Nsmf	1.26
9	Q9D1L9	Lamtor5	1.24
10	Q9JHL1	Slc9a3r2	1.17
11	Q88WT5	Dip2a	1.15
12	H3BIX7	Atp2c1	1.12
13	A2A4P3	Gm27029	1.1
14	Q8BJW6	Elf2a	1.04
15	Q8CG71	P3h2	1.01

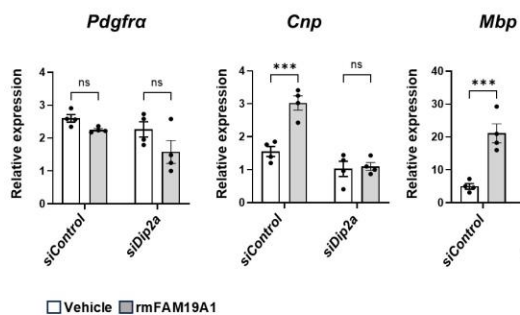
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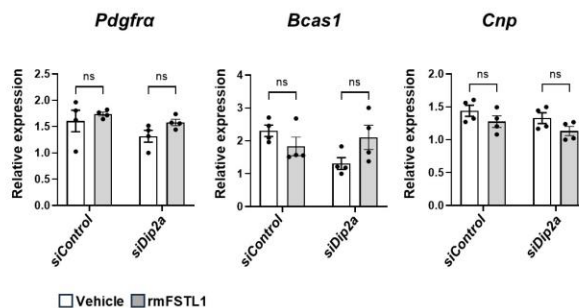
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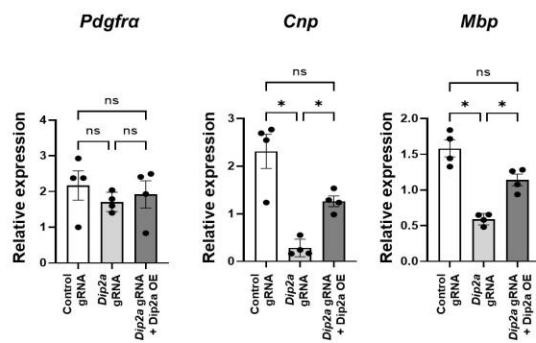
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E



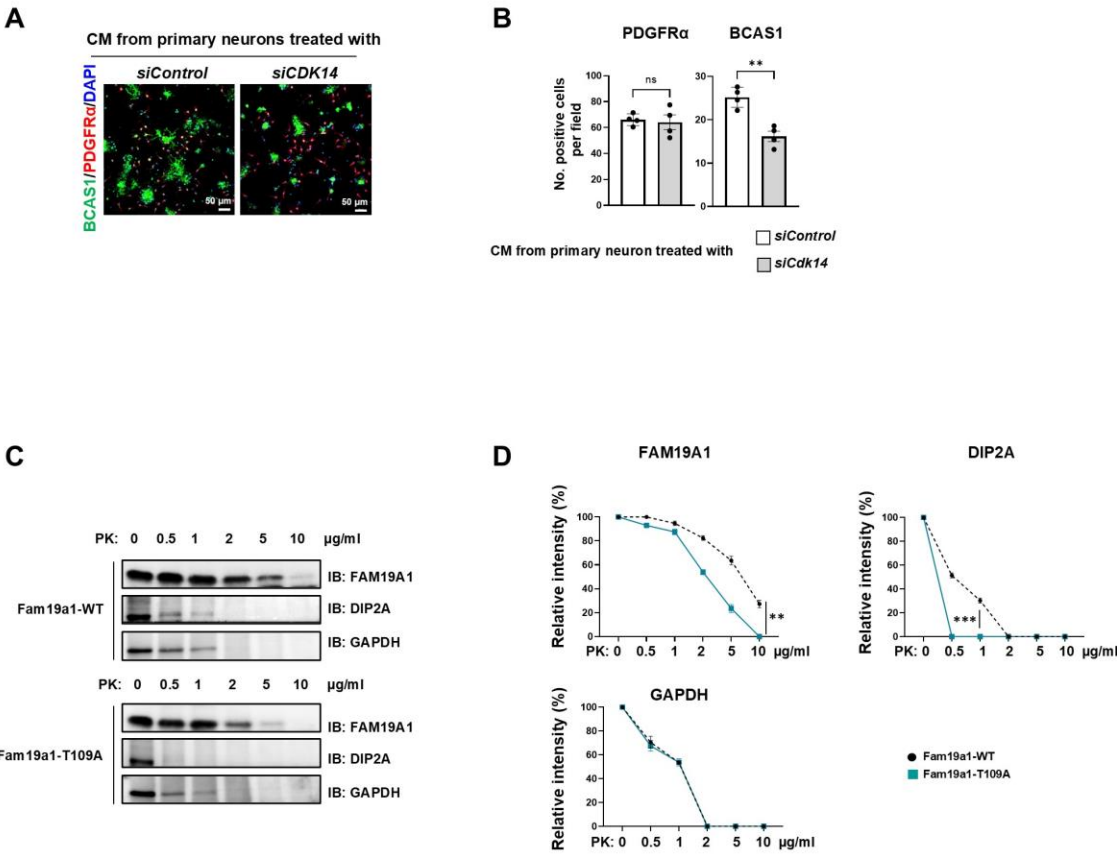
F



Supplementary Fig. 2. DIP2A mediates FAM19A1-induced OL maturation. **A** List of proteins showing differential enrichment in the Fam19a1 interactome at 52 °C compared with 62 °C. **B** qRT-PCR quantification of *Dip2a* mRNA expression in OLs transfected with *siControl* or *siDip2a* and subsequently treated with vehicle (0.1% DMSO) or rmFAM19A1 (10 ng/ml). *Dip2a* mRNA expression was normalized to *Gapdh*. n = 4 independent experiments. Statistical significance was assessed by two-way ANOVA followed by Bonferroni's multiple-comparisons test. *siControl*: $P > 0.999$. *siDip2a*: $P > 0.999$. Interaction: $F(1, 12) = 0.05696$, $P = 0.8154$. **C** Quantification of PDGFR α^+ OPCs in *siControl*- and *siDip2a*-treated OL cultures following treatment with vehicle or rmFAM19A1. n = 6 independent experiments. Each dot represents the mean number of PDGFR α^+ cells quantified from six randomly selected 0.5-mm² fields per independent experiment. Statistical significance was assessed by two-way ANOVA followed by Bonferroni's multiple-comparisons test. Vehicle: $P > 0.999$. rmFAM19A1: $P > 0.999$. Interaction: $F(1, 6) = 0.1179$, $P = 0.7430$. **D** qRT-PCR analysis showing that *Dip2a* KD attenuated rmFAM19A1-induced upregulation of OL marker genes, *Cnp* and *Mbp*, while expression of *Pdgfra*, an OPC marker, remained unaffected. Statistical significance was assessed by two-way ANOVA followed by Bonferroni's multiple-comparisons test. *Pdgfra*: Bonferroni-adjusted P values were 0.5966 (Vehicle) and 0.1016 (rmFAM19A1). Interaction: $F(1, 12) = 0.5861$, $P = 0.4587$. *Cnp*: Bonferroni-adjusted P values were 0.1331 (Vehicle) and < 0.001 (rmFAM19A1). Interaction: $F(1, 12) = 14.33$, $P = 0.0026$. *Mbp*: Bonferroni-adjusted P values were 0.8070 (Vehicle) and < 0.001 (rmFAM19A1). Interaction: $F(1, 12) = 31.55$, $P = 0.0001$. **E** Treatment with rmFSTL1 (10 ng/ml), a known ligand of Dip2a, did not affect the expression of OPC (*Pdgfra*), pre-OL (*Bcas1*), and OL (*Cnp*) markers. n = 4 independent experiments. *Pdgfra*: Bonferroni-adjusted P values were 0.2427 (Vehicle) and 0.7584 (rmFSTL1). Interaction: $F(1, 12) = 0.5861$, $P = 0.4587$. *Bcas1*: Bonferroni-adjusted P

values were 0.0405 (Vehicle) and 0.9492 (rmFSTL1). Interaction: $F(1, 12) = 5.824$, $P = 0.0327$. *Cnp*: Bonferroni-adjusted P values were 0.7066 (Vehicle) and 0.4765 (rmFSTL1). Interaction: $F(1, 12) = 0.03799$, $P = 0.8487$. **F** qRT-PCR analysis of *Pdgfra*, *Cnp* and *Mbp* mRNA expression following CRISPRi-mediated *Dip2a* repression in primary OLs with or without Dip2a OE. mRNA expression was normalized to *Gapdh*. $n = 4$ independent experiments. One-way ANOVA, *Pdgfra*: $F(1.629, 4.887) = 0.4993$ ($P = 0.5998$). *Cnp*: $F(1.260, 3.779) = 27.13$ ($P = 0.0069$). *Mbp*: $F(1.703, 5.109) = 25.26$ ($P = 0.0025$). Data are presented as mean $\pm$ SEM. Differences between two groups were evaluated using an unpaired two-tailed Student's t -test. Multiple-group comparisons were performed using one-way or two-way ANOVA followed by Tukey's or Bonferroni's multiple-comparisons test, as appropriate. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

Supplementary Fig. 3



Supplementary Fig. 3. T109 phosphorylation enhances the proteolytic stability of FAM19A1 and Dip2a. **A** Representative immunofluorescence images of BCAS1 and PDGFR α in pre-OLs cultured with CM collected from primary neurons treated with either *siControl* or *siCdk14*. **B** Quantification of BCAS1⁺ and PDGFR α ⁺ cells. CM derived from *siCdk14*-treated neurons reduced the number of BCAS1⁺ OLs, indicating impaired OL maturation. Each dot represents the mean number of PDGFR α ⁺ or BCAS1⁺ cells, quantified from six randomly selected 0.5-mm² fields per independent experiment. n = 4 independent experiments. **C** Representative western blot analysis of limited proteolysis of Fam19a1 and Dip2a following treatment with increasing concentrations of PK in lysates from HEK293T cells expressing Fam19a1-WT or the phospho-deficient Fam19a1-T109A mutant. **D** Quantification of the remaining FAM19A1 and DIP2A protein levels following PK treatment. Protein levels were normalized to the corresponding untreated control. n = 3 independent experiments. Statistical significance was assessed by two-way ANOVA followed by Tukey's multiple-comparisons test. FAM19A1: $P < 0.001$ (PK 10 μ g/ml). Interaction: $F(5, 20) = 91.74, P < 0.001$. DIP2A: $P < 0.001$ (PK 1 μ g/ml). Interaction: $F(5, 20) = 1249, P < 0.001$. GAPDH: $P > 0.99$ (PK 1 μ g/ml). Interaction: $F(5, 20) = 0.5257, P = 0.075$. Data are presented as mean $\pm$ SEM. Differences between two groups were evaluated using an unpaired two-tailed Student's *t*-test. Multiple-group comparisons were performed using one-way or two-way ANOVA followed by Tukey's multiple-comparisons test, as appropriate. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.