Supplementary Figures

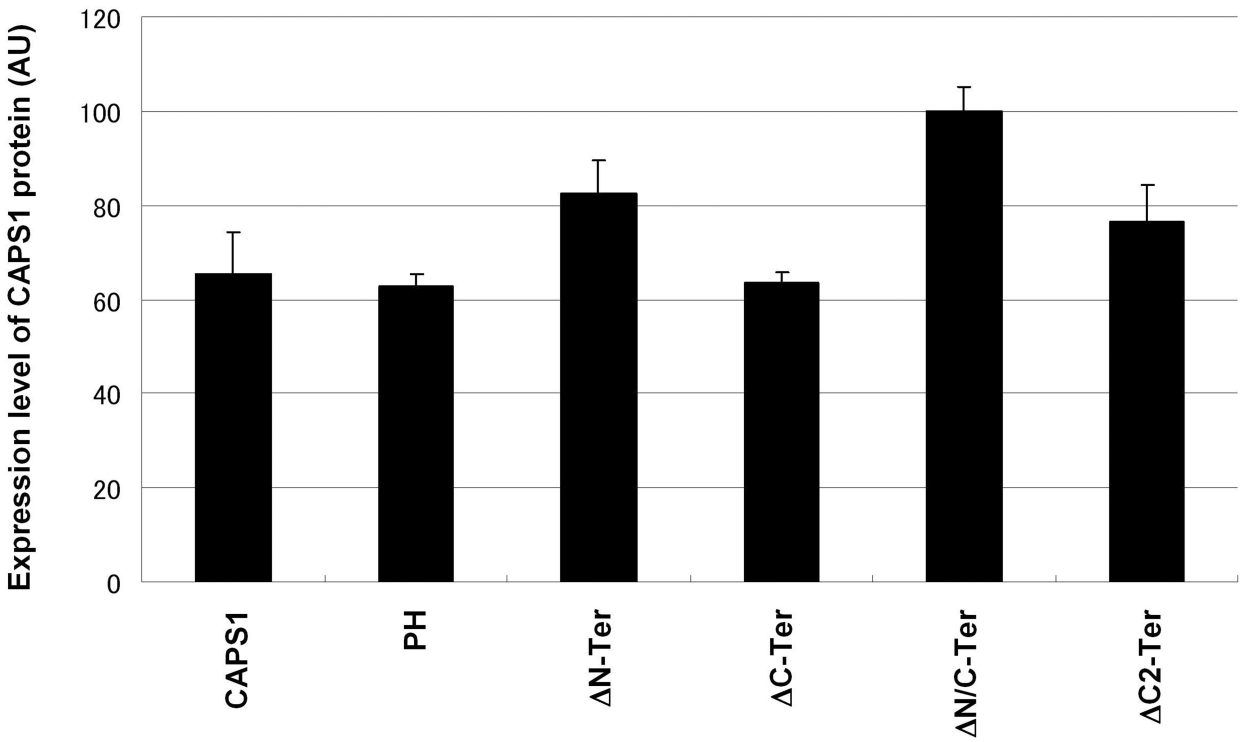
Figure S1. Protein expression level of six deletion derivatives of CAPS1-YPet-HA expressed in PC12 cells. Wild-type CAPS1-YPet-HA, PH domain alone (PH)-YPet-HA, PH domain and Munc13-1-homologous domain (ΔN-Ter)-YPet-HA, the region from the 1st amino acid to the PH domain (ΔC-Ter)-YPet-HA, C2 and PH domain (ΔN/C-Ter)-YPet-HA, C2-domain-skipped CAPS1 (ΔC2)-YPet-HA were expressed in PC12 cells and the cell lysates were electrophoresed and analyzed by western blotting using anti-HA antibody followed by densitometric analysis. The error bars indicate the SD.

Figure S2. Distribution of the ARF family mRNAs in whole mouse brain. *A*-*J*, Sagittal sections of P7 and P21 whole brain were analyzed by in situ hybridization. (*A*-*E*) ARF1 (*A*), ARF3 (*B*), ARF4 (*C*), ARF5 (*D*), and ARF6 (*E*) mRNA distribution in the whole P7 brain. (*F*-*J*) ARF1 (*F*), ARF3 (*G*), ARF4 (*H*), ARF5 (*I*), and ARF6 (*J*) mRNA distribution in the whole P21 brain.

Figure S3. Specificity of anti-ARF antibodies and siRNA of class II ARFs. *A*-*D*, The lysates of COS-7 cells expressing mouse ARFs tagged with C-terminal FLAG epitopes were blotted with anti-ARF antibody (clone. no. 1D9) (*A*), anti-ARF5 antibody (*B*), or anti-FLAG antibody (*C*). The asterisk indicates immunoreactivity for endogenous ARF5. *D*, The lysates of COS-7 cells co-transfected with pEF-BOS-ARF4-FLAG and the siRNA of ARF4 (cat. no. 128374 D05) were probed with anti-FLAG antibody. *E*, The lysates of COS-7 cells co-transfected with pEF-BOS-ARF5-FLAG and the siRNA of ARF5 (cat. no. 128374 C09) were probed with anti-FLAG antibody.

Figure S4. Amino acid sequence comparisons of mouse ARF proteins. *A*, Amino acids that are identical in two or more family members are shown against a gray background. Amino acids that are identical in the class II ARFs are shown against a black background. *B*, Three-dimensional structure of mouse ARF1. Figure was generated using Cn3D software (http://www.ncbi.nlm.nih.gov/Structure/CN3D/cn3d.shtml) from the structure 1R8Q in the Protein Data Bank (http://www.rcsb.org/pdb/). The N-terminal region between the 3rd and 6th amino acids (*yellow*), which contains the critical amino acids for CAPS1 binding to ARF4/5, is shown in the ARF1 3D structure.

Fig. S1


Expression level of CAPS1 protein (AU)
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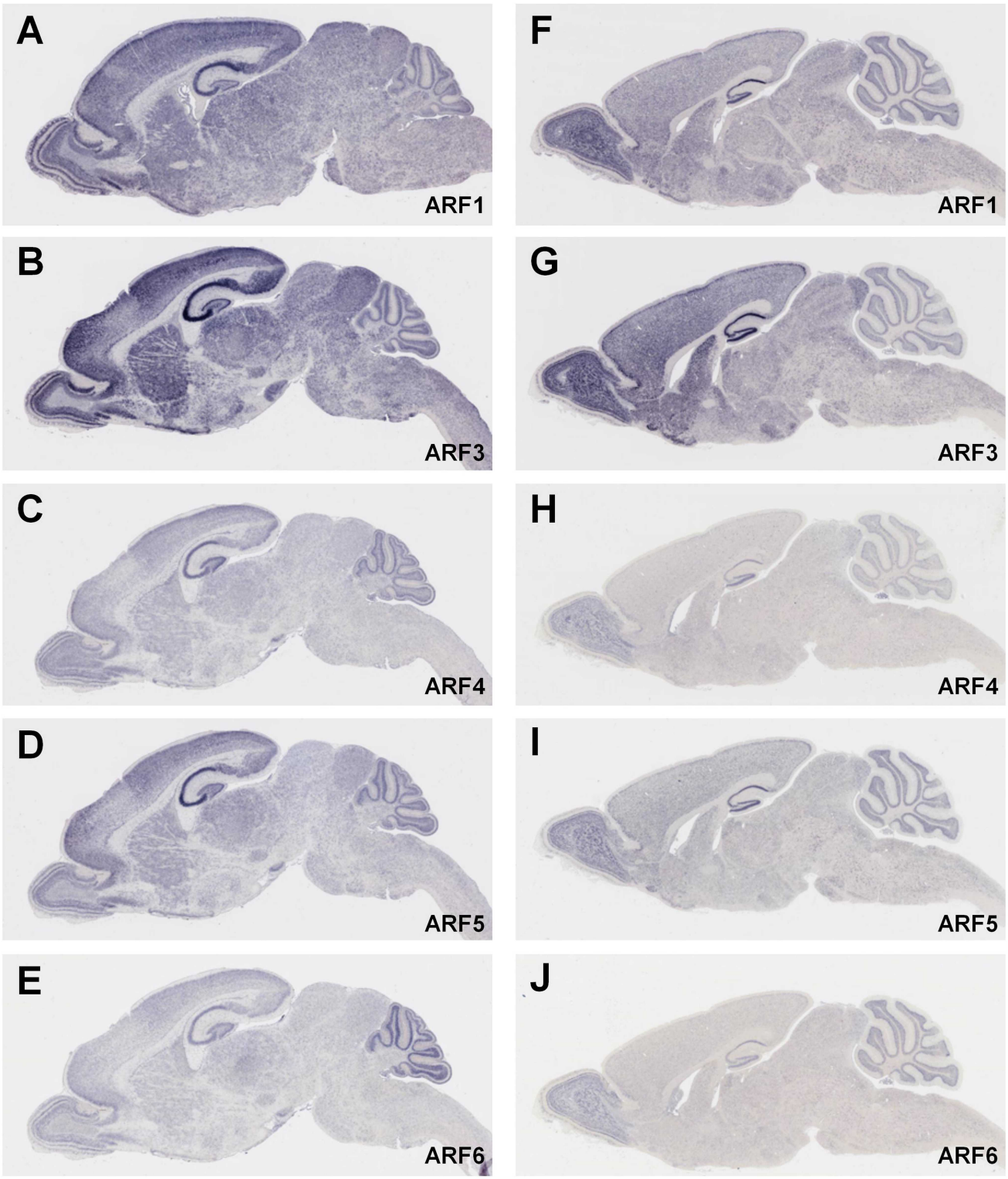
40
20
0
CAPS1
PH
ΔN-Ter
ΔC-Ter
ΔN/C-Ter
ΔC2-Ter

Fig. S2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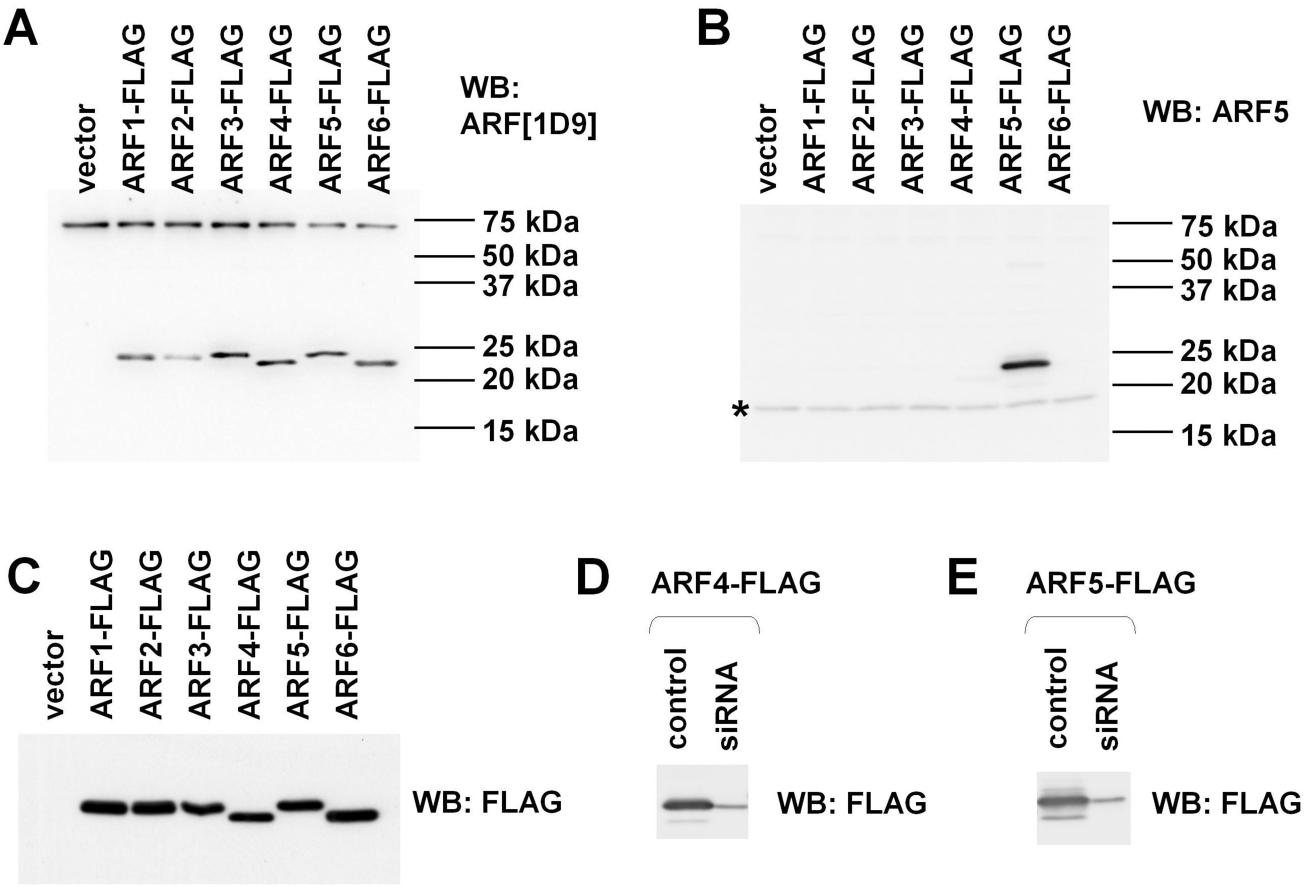
F
ARF1
B
ARF3
G
ARF3
C
ARF4
H
ARF4
D
ARF5
I
ARF5
E
ARF6
J
ARF6

Fig. S3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ARF3-FLAG
ARF4-FLAG
ARF5-FLAG
ARF6-FLAG
WB: ARF[1D9]
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50 kDa
37 kDa
25 kDa
20 kDa
15 kDa
B
vector
ARF1-FLAG
ARF2-FLAG
ARF3-FLAG
ARF4-FLAG
ARF5-FLAG
ARF6-FLAG
WB: ARF5
*
75 kDa
50 kDa
37 kDa
25 kDa
20 kDa
15 kDa
C
vector
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ARF2-FLAG
ARF3-FLAG
ARF4-FLAG
ARF5-FLAG
ARF6-FLAG
WB: FLAG
D
ARF4-FLAG
control
siRNA
WB: FLAG
E
ARF5-FLAG
control
siRNA
WB: FLAG

Fig. S4