Supporting Information

The two splice variant forms of Cdc42 exert distinct and essential functions in neurogenesis

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Figure S1

Figure S2

Figure S3

Figure S4

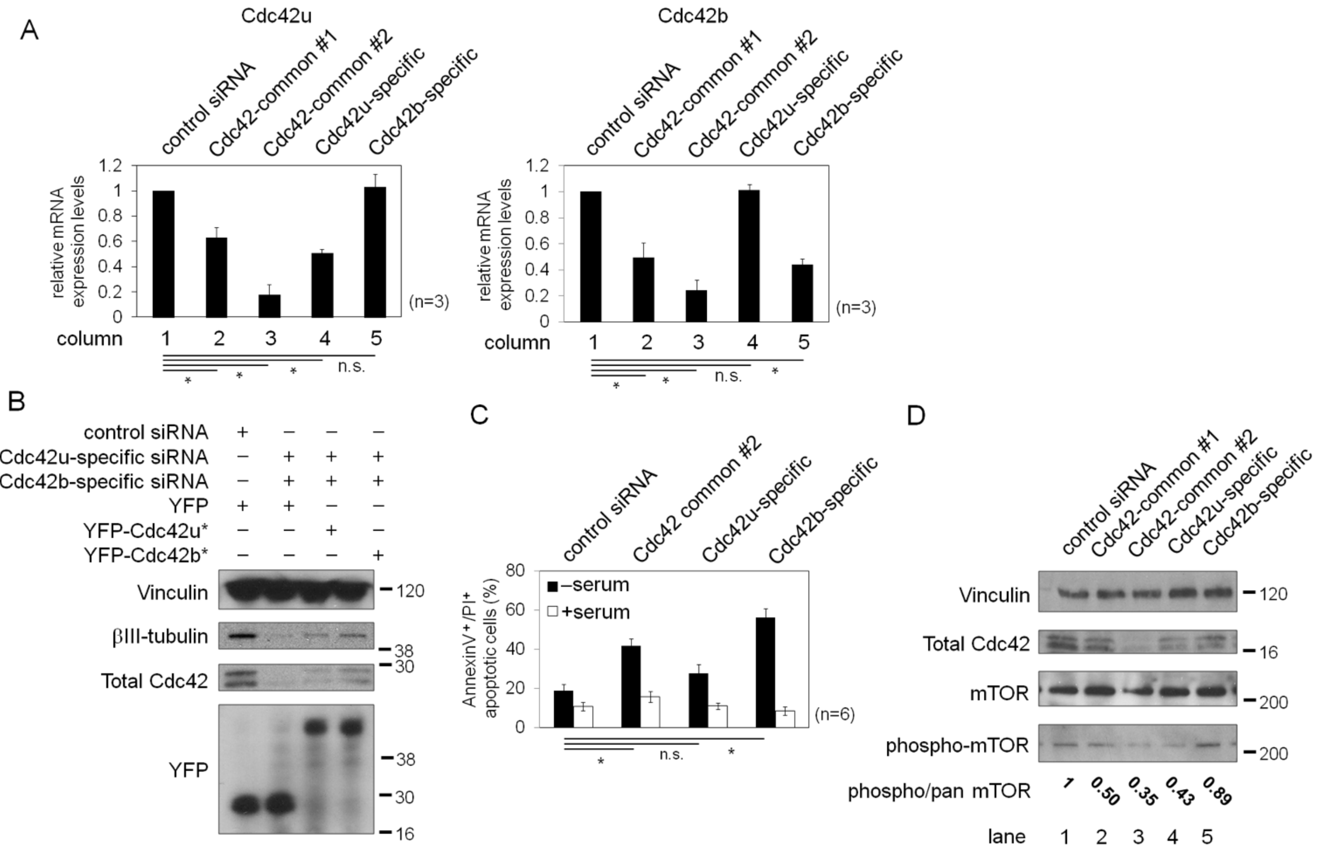

Figure S1. (A) Histograms showing the relative mRNA expression levels of Cdc42u (left) and Cdc42b (right) in the RNAi background. P19 cells were treated with control siRNA or the siRNAs that targeted both Cdc42 splice variants (Cdc42 common #1 and #2) or specific splice variants (Cdc42u- or Cdc42b-specific), and then were subjected to the RA-dependent neural differentiation protocols. Total mRNAs were collected at day 4 and subjected to semi-quantified real time-PCR. The mRNA levels are represented relative to that of control siRNA-treated cells. Error bars indicate s.e.m. (n=3). In Figures S1B and C, P19 cells were subjected to the RA-dependent neural differentiation protocol until day 4 and then, treated with siRNAs and/or lentivirus. After transfection and/or transduction, cells were subjected to the neural differentiation protocol until day 9. (B) Immunoblotting images showing the expression levels of βIII-tubulin, endogenous total Cdc42 proteins, and RNAi-resistant YFP-tagged Cdc42 isoforms. The bars and numbers next to each panel indicate the positions and sizes (k Da) of molecular weight markers, respectively. (C) Histograms showing the percentage of apoptotic cells, upon treatment with siRNAs. Error bars indicate s.e.m. (n=6). Significance of differences is indicated by n.s. (not significant, $p > 0.05$) and * ($p < 0.05$), using a *t*-test. (D) Immunoblotting images showing the expression levels of total Cdc42 proteins, and the expression and activation levels (pS2448) of mTOR. P19 cells were treated with control siRNA or the siRNAs that targeted both Cdc42 splice variants (Cdc42 common #1 and #2) or specific splicing variants (Cdc42u- or Cdc42b-specific), and then were subjected to the RA-dependent neural differentiation protocols. Cell lysates were collected at day 4 and subjected to Western blotting. Vinculin served as a loading control. The numbers under the immunoblotting images of mTOR indicate the fold changes, based on densitometry.

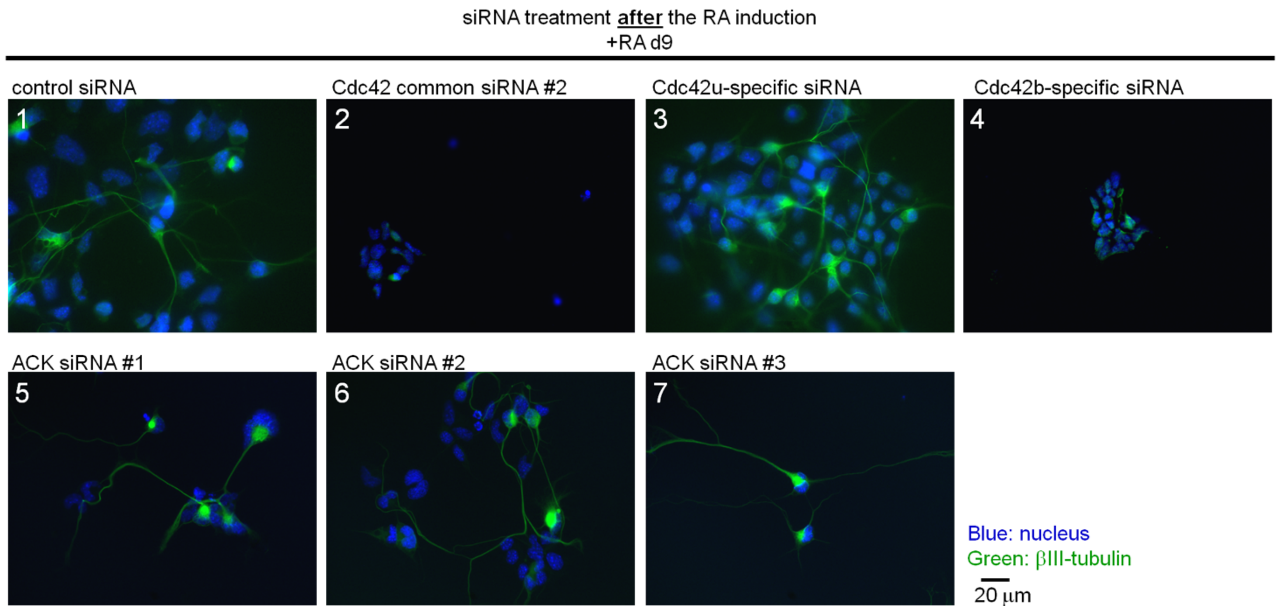

Figure S2. Epifluorescence images showing the neuronal cell differentiation and morphology of siRNA-treated P19 cells. Cells were subjected to the RA-induced neural differentiation protocol until day 4, and then treated with siRNAs. After transfection, cells were subjected to the normal neural differentiation protocol. At day 9, cells were fixed and stained with Hoechst (blue) and anti-βIII-tubulin antibody (green). The black bar to the right of the images indicates a scale (20 μm).

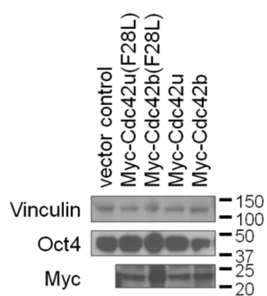

Figure S3. Immunoblotting images showing the expression levels of the indicated proteins in undifferentiated P19 stable cell lines. Vinculin served as a loading control, and Oct4 as a marker showing the undifferentiated status of stable cell lines. The bars and numbers next to each immunoblotting panel indicate the positions and sizes (k Da) of molecular markers, respectively.

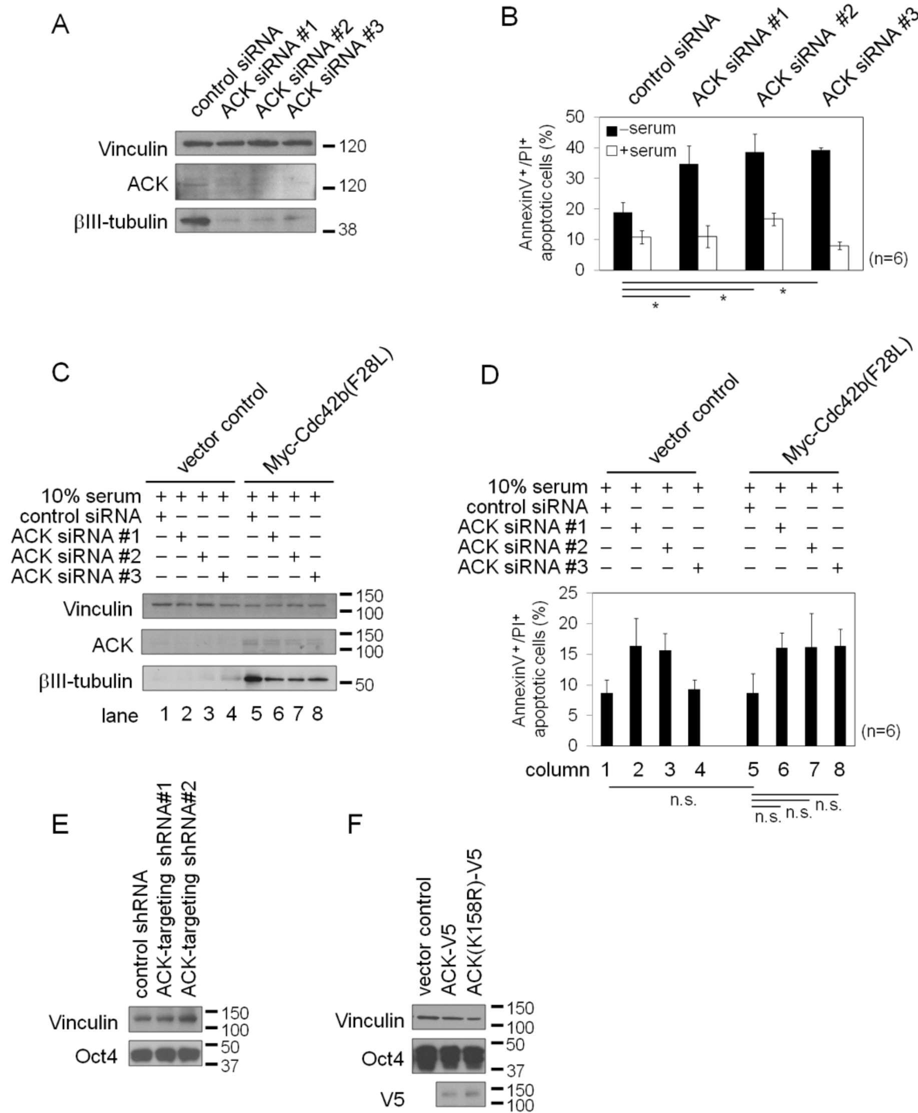

Figure S4. (A-D) P19 cells or the indicated P19 cell lines were subjected to the RA-dependent neural differentiation protocol until day 4 and then treated with control or ACK-targeting siRNAs. After transfection, cells were subjected to the neural or neural progenitor cell differentiation protocol until day 9. (A) Immunoblotting images showing the expression levels of ACK and βIII-tubulin. Vinculin served as a loading control. The bars and numbers next to each panel indicate the positions and sizes (k Da) of molecular markers, respectively. (B) Histograms showing the percentages of apoptotic cells. Error bars indicate s.e.m. (n=6). Significance of differences is indicated by * ($p < 0.05$) using a *t*-test. (C) Immunoblotting images showing the expression levels of ACK and βIII-tubulin in P19 stable cell lines. (D) Histogram showing the percentages of apoptotic cells. Error bars indicate s.e.m. (n=6). Significance of differences is indicated by n.s. (not significant, $p > 0.05$), using a tukey's test. Immunoblotting images showing the expression levels of the indicated proteins in undifferentiated P19 stable cell lines. (E) ACK-targeting shRNA-expressing cells. (F) V5-tagged ACK protein-expressing cells. Oct4 served as a marker showing the undifferentiated status of stable cell lines. In the undifferentiated status, P19 cells showed very low expression of endogenous ACK proteins, such that they were not detected by Western blotting. Therefore, the effectiveness of ACK-targeting shRNAs on ACK protein expression was examined after the RA-induction (E).