Supplementary Figure legends

Supplementary Figure 1

Pqbp1-cKO in NSPCs causes microcephaly with normal cortical structure.

(A) Construction of the targeting vector and restriction maps of the *Pqbp1* gene in four mouse alleles. Black arrows indicate the PCR primers used for genome typing. DT_A denotes the diphtheria toxin A gene. 5′ and 3′ probes were used for Southern blot.

(B) Pqbp1 protein expression was checked in nestin-Cre and synapsin1-Cre derived cKO mice. Pqbp1 was detected by western blot in the total brain tissue of nestin-Cre cKO mice (at 2 months) or control mice, and Pqbp1 in the cerebral cortex of synapsin1-Cre cKO was stained by immunohistochemistry. In the western blot, non-neuronal tissues in the brain such as vessels produced a faint band in nestin-Cre cKO mice.

(C) Low-power magnification of coronal sections of cerebral cortex. Lower panels show the magnification of the hippocampus (CA1) and the dentate gyrus (DG). The bars indicate the thickness of the pyramidal and granular cell layers, both of which were smaller in *Pqbp1*-cKO mice.

(D) Thickness of cerebral cortex (CTX), CA1 (CA1 field), CA1 pyramidal cell layer (CA1 pcl), dentate gyrus molecular layer (DG mol), or dentate gyrus granular cell layer (DG gcl) analyzed quantitatively (mice n = 10) and compared for each genotype. The coronal slice position was -1.82 mm from the bregma. Asterisks indicate significance ($p < 0.01$) in ANOVA with post hoc Bonferroni's test.

Supplementary Figure 2

Layer structure and thickness in the _Pqbp1_-cKO mouse embryonic brain

(A) Upper panel: sagittal sections of E15 embryonic brains (300 μm lateral from midline). Middle panel: higher magnification of squared areas in upper panels. Lower panel: quantitative analyses of the thickness of the ventricular and subventricular zones (VZ+SVZ) and the intermediate zone and cortical plate (IZ+CP). The ratios of IZ+CP to VZ+SVZ were not significantly altered, indicating that deregulated neurogenesis did not contribute to microcephaly.

(B) Sagittal sections of E10 *Pqbp1*-cKO or control mice immunostained for Pax6 and Tbr2. Inlays show larger magnification of the marked areas in phase contrast views. Quantitative data of the thickness of the Pax6-positive layer are shown in the graph.
(C) Immunostaining of *Pqbp1*-cKO or control mice at E15. The embryonic ventricular zone was co-stained for Pqbp1 and Sox2, with a 4',6-diamidino-2-phenylindole (DAPI) co-stain. Loss of Pqbp1 expression in *Pqbp1*-cKO mice, with the retention of the Sox2-positive layer of NSPCs, was apparent. Quantitative data of the thickness of Sox2-positive layer are shown in the graph.
(D) Immunostaining of *Pqbp1*-cKO or control mice at E15. The embryonic ventricular zone was co-stained for Pax6 and Tbr2 (sagittal section). The Pax6- and Tbr2-positive NSPC layers were unaffected by *Pqbp1* loss. Quantitative data of the thickness of Pax6-positive layer are shown in the graph.

Supplementary Figure 3

PQBP1/Pqbp1 does not induces centrosome-spindle pathology

(A) Apical progenitor cells (APs) of *Pqbp1*-cKO (Nes-Cre; $X^{Flox}Y$) mice showed normal spindle pole and centrosome morphology *in vivo* and *in vitro*, which were not different from those in control (nestin-Cre). Three mice of each genotype were used for these analyses (n = 100 for each mouse).
(B) Human fibroblasts stained by anti-α-tubulin or γ-tubulin antibody with DAPI. Spindle pole and centrosome structures were normal in patients with mutated *PQBP1*, and no difference was observed between control and patients with mutated *PQBP1* by quantitative analysis. Three fibroblast cell lines were used for analysis (n = 100 for each cell line).
(C) Mitotic spindle orientation of NSPCs at the ventricular surface was examined at E10.5, E14.5, and E15.5. No difference was observed between control (nestin-Cre; XY) and *Pqbp1*-cKO (Nes-Cre; $X^{Flox}Y$) mice.
(D) Asymmetric division of APs was evaluated by N-cadherin staining. Unequal distribution of the apical membrane to sister cells could be judged by the displacement of the mitotic plane from the cadherin hole. No difference was observed between control (nestin-Cre; XY) and *Pqbp1*-cKO (Nes-Cre; $X^{Flox}Y$)

mice (n = 3, 100 cells were counted in each mouse).

Supplementary Figure 4

Pqbp1 KD delays cell cycle of NSPCs

(A) Schematic of *Pqbp1*-shRNA plasmids.

(B) Western blot analyses confirmed the suppressive effect of transient shRNA expression on Pqbp1. Each shRNA plasmid efficiently suppressed Pqbp1 expression in P19 cells, 48 hours after transfection. Left: RNAi-Ready pSIREN-DNR DsRed-express, right: RNAi-Ready pSIREN-RetroQ–ZsGreen, Lane 1: PQBP1-shRNA 1, Lane 2: PQBP1-shRNA 2, Lane 3: PQBP1-shRNA 3, Lane 4: PQBP1-shRNA 4, Lane 5: non-silencing shRNA, Lane 6: mock-transfected P19, Lane 7: non-treated P19, Lane 8: hPQBP1 expressed in Drosophila Schneider cells.

(C) Confirmation of Pqbp1 suppression in cultured NSPCs by immunocytochemistry.

(D) Pqbp1 stain signal intensities in NSPCs transfected with *Pqbp1*-shRNA or non-silencing shRNA; estimated by AQUACOSMOS software (HAMAMATSU). Mean +SE (n = 100). *: $p < 0.01$ (Student's *t*-test).

(E) Twenty-four hours after *in utero* electroporation with *Pqbp1*-shRNA-ZsGreen or non-silencing shRNA-ZsGreen, E16 cortices were immunostained with anti-PQBP1 antibody (red). VZ, ventricular zone; SVZ, subventricular zone; IZ, intermediate zone. Arrowheads and arrows denote PQBP1-negative and positive transfectants in the VZ/SVZ, respectively. PC, phase contrast image. Bar, 10 μm.

Supplementary Figure 5

Pqbp1-KD delays neurogenesis and increases cell death at the M phase

(A) *Pqbp1*-shRNA-ZsGreen or non-silencing shRNA-ZsGreen was electroporated into NSCs *in utero* at E14 and the brain was fixed at E16. The ratio of ZsGreen-positive cells remaining in the NSC layer were quantified by Sox2 immunostaing. The ratio of transfected cells remaining in the Sox2 layer after 48 hours was remarkably increased by Pqbp1-KD. A portion of the remaining cells transfected with *Pqbp1*-shRNA-ZsGreen (magenta arrow) still

expressed Sox2 (16%) while the remaining cells transfected with non-silencing shRNA-ZsGreen did not express Sox2 (0%). The results were consistent with the delay in the cell cycle of NSCs and in the exit to differentiated BPs or neurons. VZ: ventricular zone, SVZ: subventricular zone, IZ: intermediate zone, CP: cortical plate.

(B) The brains were also examined by immunostaining for a radial glia marker, RC2. The right graph shows a remarkable increase by Pqbp1-KD in the ratio of ZsGreen-positive cells remaining in the RC2 layer (arrow). Most of the remaining cells transfected with *Pqbp1*-shRNA-ZsGreen (magenta arrow) still expressed RC2 (82%) while the remaining cells transfected with non-silencing shRNA-ZsGreen did not express RC2 (0%).

(C) *Pqbp1*-shRNA-ZsGreen-transfected cells shifted to the Tuj1-negative layer were clearly decreased, consistently with the delay of NSPCs in cell cycle.

Supplementary Figure 6

Nestin-Cre Pqbp1 cKO does not affect the cell volume of neurons or cilia of NSPCs *in vivo*

(A) Representative images of neurons at layer V of the retrosplenial dysgranular area in three genotypes are shown. Images acquired with two-photon microscopy were analyzed using IMARIS for quantification of dendrite length and total cell volume.

(B) Quantitative analysis of the results obtained in (A). Nsetin-Cre Pqbp1-cKO did not affect neuronal cell volume, while synapsin-1-Cre Pqbp1-cKO decreased the cell volume, unexpectedly. Dendrite length was not affected by synapsin-1-Cre cKO.

(C) Cilia at the ventricular surface of the cortex were stained with the AC3 antibody. No difference was observed between nestin-Cre Pqbp1-cKO mice and the control (nestin-Cre mice) in the number and morphology of cilia.

Supplementary Figure 7

Pqbp1 is not located at the centrosome in dividing NSPC and HEK293 cells

(A) PQBP1-EGFP was transfected into HEK-293T cells. PQBP1 showed diffuse

distribution during mitosis.
(B) PQBP1 was distributed homogenously during mitosis of NSPCs (E15) in primary culture. PQBP1 was co-stained with DAPI and γ-tubulin.

Supplementary Figure 8

Pqbp1 deficiency reduces the expression of Apc4 and other M phase-related genes though an RNA splicing defect

(A) From significantly changed genes on the gene chip ($p < 0.05$), functional groups, whose expression pattern was unequally shifted in GSEA (false discovery rate [FDR] q-value < 0.1) in NSPCs of nestin-Cre Pqbp1-cKO mice, were extracted and listed. All combinations of individual dataset, mean value data from three sets, and significant p-value sets were compared between wild-type and cKO mice, as shown in the lower scheme, for detection of the GSEA shift. The numbers of M phase-related gene groups (red), other cell cycle phase-related gene groups (yellow), and cell cycle-unrelated gene groups (gray) are shown below each list. M phase-related or cell cycle-related gene groups were unequally shifted at high probabilities.
P-values were calculated by one-sided *t*-test (p-value). An FDR q-value less than 0.1 is judged significant based on previous studies (47). Detailed functional group names and their q-values are shown in Supplemental Table 1.
(B) Representative M phase-related groups significantly changed in GSEA.
(C) PPI network of genes selected by GSEA as in A (m-m). The expression levels of most genes were decreased (gray) whereas those of a few genes were increased (red). The gray scale corresponds to the amount of change in microarray analyses.
(D) PPI network of GSEA-selected genes, excluding those with a log2 fold change of less than -0.2. Note that the majority of unconnected genes encode centrosomal proteins (spots aligned below).
(E) Genes in the PPI network (D) were divided into the M phase-specific group (M, red), ubiquitin/proteasome-related group (U, blue), and S phase-related group (S, yellow). Links between PQBP1 and U5-15kD, U5-15KD and APC4, and APC2 and APC4 are highlighted.

Supplementary Figure 9

Pqbp1 KD impairs splicing of *Apc4* RNA

(A) RT-PCR and Southern blot analyses detected unspliced *Apc4* RNA in *Pqbp1*-shRNA4 transfected NSPCs.

(B) Antisera against a Pqbp1-N-terminus peptide, Pqbp1-C-terminus peptide or a GST-Pqbp1 fusion protein inhibited the splicing of *Apc4* but not *crystallin* in an *in vitro* splicing assay. Antisera against Yes-associated protein (YAP) or hepatoma-derived growth factor (HDGF) (negative controls) did not affect *Apc4* splicing. Y12 antibody was used as the positive control.

(C) First-round RT-PCR showed that shRNA mediated KD of Upf1 (using Upf1#1 or Upf1#2), which controls nonsense-mediated RNA decay, inhibited the degradation of unspliced *Apc4* RNA in NSPCs following suppression of Pqbp1 (middle and lower panels, lane 5, 6). In this case, we detected that Pqbp1 KD led to intron retention between exons 22–23 and 6–7 (lower right schemes). Western blot analyses (upper panels) show the expression levels of Upf1 and Pqbp1 in NSPC transfectants used for RT-PCR analyses.

(D) Association of *APC4*-RNA with the human PQBP1 splicing complex. After transfection of HEK-293T cells with Flag-tagged *PQBP1*, PQBP1 protein-RNA complexes were immunoprecipitated (IP) using anti-FLAG agarose beads; anti-mIgG agarose beads were used as a negative control. Input lysates and 1/10 of the immunoprecipitates were resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and western blots probed with anti FLAG-HRP antibody. Experiments were performed four times.

(E) Schematic of human *APC4* exons 22 to 25, which corresponds to mouse *Apc4* exons 21 to 24, with primer sequences used for RT-PCR amplification following human PQBP1 immunoprecipitation. Total RNA recovered from PQBP1 immunoprecipitation experiments was amplified by RT-PCR using a human *APC4*-specific primer pair located in exons 22 and 25. Products were resolved on 3% agarose gels. Specific human *APC4* mRNAs were present in the FLAG immunoprecipitations and absent from control IPs or mock RT-PCR experiments. RT-PCR from HEK-293T total RNA was amplified as a positive control using the same primer pair. M, 100-bp ladder.

(F, G) Synthetic small interfering RNAs (siRNA) were used to knock down

human *PQBP1* in HEK-293T cells. Non-specific siRNAs (ns siRNA) were used in parallel as controls. Experiments were performed either in the presence of cycloheximide (CHX) to block NMD or DMSO as a control. A cell aliquot from each KD experiment was lysed and analyzed by western blot for siRNA efficiency. Following the KD of PQBP1 in HEK-293T cells (F), *APC4* intron 23 containing transcripts was RT-PCR-amplified from untreated (dimethyl sulfoxide, DMSO) and cycloheximide (CHX+)-treated cells using primers located in *APC4* exon 22 and intron 23 (GenBank Acc. No. NM_013367) (G). *HPRT* RT-PCR products amplified from the same cDNA preparations were used for normalization (G).

(H) The histogram shows the fold change in *APC4*-intron 23 transcripts after cycloheximide treatment of the control or KD HEK-293T cells (G). *p ≤ 0.05 in *t*-test (two-tailed, homoscedastic).

(I) The remaining cells were used for RNA isolation and real-time RT-PCR using *APC4*-specific primers located in exons 22 and 25. The histogram shows the relative RNA amounts of *APC4* in HEK-293T cells transfected with either non-silencing control siRNA (white column) or *PQBP1*-specific siRNA (black columns), with and without CHX treatment. Columns represent the mean values of 12 samples measured in parallel ± s.d. PQBP1 was used for normalization. *t*-test (two-tailed, homoscedastic): *p ≤ 0.005.

Supplementary Figure 10

Summary of the other behavioral analyses of nestin-Cre cKO mice

Results from behavioral analyses of nestin-Cre cKO mice at 3 months are summarized.

*p ≤ 0.05 and **p ≤ 0.01 in Tukey's test.

Supplementary Figure 11

Summary of the other behavioral analyses of synapsin-1-Cre cKO mice

Results from behavioral analyses of synapsin-1-Cre cKO mice at 3 months are summarized.

*p ≤ 0.05 and **p ≤ 0.01 in Tukey's test.

Supplementary Table 1

Functional gene groups whose expression pattern was unequally shifted in GSEA, extracted from a comparison of NSPCs from nestin-Cre Pqbp1-cKO mice and from nestin-Cre mice, are shown. Refer to legend in Supplementary Figure 8 for details.

Supplementary Table 2

Number of links (degree) for each gene that is proximal to PQBP1 in the protein-protein interaction network (PPI), listed along with their functions.

Supplementary Table 3

Exon signals in NSPC or cortex tissues were normalized, and compared between cKO and the background B6 mice (exon-exon comparison). Significantly changed genes (gene symbol) and their exons (exon ID) are listed. The exon-exon comparison is affected both by transcription and splicing.

Supplementary Table 4

Variances of multiple exons were compared between cKO and the background B6 mice. The F-test was used to test whether the variance was changed between the two mouse groups in each gene. The ratio in the table is the ratio of variance between cKO and background mice, and is shown as an indicator of the change in splicing patterns. The number of probe positions indicates nucleotide number in the NCBI genome database. Variance is affected by the change in splicing.

Supplementary Table 5

The results of the exon-exon comparison analysis were compared among 3 arrays. Genes categorized to each group in Figure 3B (left panel) are listed.

Supplementary Table 6

The results of “variance” analysis were mutually compared among 3 arrays.

Genes categorized to each group in Figure 3B (right panel) are listed.

Supplementary Table 7

Summary of exon array analyses in Apc1, Apc2, Apc4, Pqbp1, and NCAM1. Hybridization was performed with cDNA probes generated from mRNA of NSPCs or cortical tissues of cKO mice, and the signals of each exon were compared between Pqbp1-cKO mice and nestin-Cre mice (first method), or variance among the exons of a single gene was compared between Pqbp1-cKO mice and nestin-Cre mice (second method).