Extended Methods

Plasmids and antibodies

The following oligonucleotides were cloned into pBluescript/U6 to generate RNAi plasmids as described (Gaudilliere et al., 2002): PQBP1ia, 5'-GAG CCA GAG GAA GAG ATT ATA caagttaac AAT AAT CTC TTC CTC TGG CTC ctttttg-3'; PQBP1ib, 5'-TGA CAA GGC AGA CCG GGA AGT caagttaac TCT TCC CGG TCT GCC TTG TCA ctttttg-3'; Dynamin 2ia, 5'-GAA TGT GTT TGC CAA CAA TGC caagttaac TCA TTG TTG GCA AAC ACA TTC ctttttg-3'; Dynamin 2ib, 5'-CCA TTA TCC GCC CAG CCG AGA caagttaac GCT CGG CTG GGC GGA TAA TGG ctttttg-3'; Scrambled shRNA, 5'-AAG TGC CAA TTT CGA TGA TAT caagttaac CTA TCA TCG AAA TTG GCA CTT ctttttg-3' (target sequence is capitalized). The oligonucleotides for Dynamin 2ia and Scrambled were cloned into pLL3.7/Syn-mCitrine to generate pLL3.7 Dynamin2i/Syn-mCitrine and pLL3.7 Scrambled/Syn-mCitrine, respectively. The Y65C mutation of PQBP1 was introduced by PCR site-directed mutagenesis (Stratagene). IFT20 and IFT88 were amplified from rat cDNA with PCR and cloned into pEGFP-C1 (Clontech).

The following antibodies were used: rabbit PQBP1 (Sigma and Santa Cruz), mouse γTubulin (Sigma), mouse and rabbit GFP (NeuroMab and Invitrogen), rabbit AC3 (Santa Cruz), mouse Tuj1 (Sigma), rabbit Pericentrin (Sigma), rabbit Erk1/2 (Cell Signaling Technology), mouse GM130 (BD), goat Dynamin 2 (Santa Cruz), mouse 14-3-3 (Santa Cruz), rabbit SnoN (Santa Cruz), goat SSTR3 (Santa Cruz), goat MCHR1 (Santa Cruz), mouse Actin (Santa Cruz), mouse BiP (BD), mouse BCL2 (BD), and mouse GM130 (BD).

Analysis of ciliary morphology and imaging

To analyze the ciliary morphology of primary neurons and brain slices, images of individual neurons were captured randomly in a blinded manner on a Nikon Eclipse TE2000 epifluorescence microscope using a digital CCD camera (Diagnostic Instruments) with SPOT software. Images of brain sections were collected with a Nikon Ti-E microscope with a

60x/1.45NA objective lens and a spinning disk confocal unit CSU22 (Yokogawa), using Volocity software (Perkin Elmer).

Cell lines

To investigate ciliary morphology in non-neuronal cell lines, we used NIH3T3 cells and MDCK cells. NIH3T3 cells were cultured in DMEM supplemented with 10% calf serum. MDCK cells were cultured in DMEM supplemented with 10% fetal bovine serum. The cells were transfected using Lipofectamine 2000 (Life technologies) according to the manufacturer's protocol. Cells were replated on glass coverslips 24 hrs after transfection. After an additional 2 days, the primary cilium in transfected cells was analyzed as described above for neurons. To induce ciliary formation by starvation in MDCK cells, growth medium was replaced with DMEM 24 hours before fixation. NIH3T3 cells were analyzed without serum starvation.

GST pull-down

PQBP1 was cloned into pGEX-4T1 vector (Promega) to generate the GST-PQBP1 expression plasmid. GST and GST-PQBP1 were produced in BL21 *E. Coli* and purified using glutathione beads (GE healthcare). The beads were washed with high salt solution [500 mM NaCl, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA] and then low salt solution [150 mM NaCl, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA]. The washed beads were incubated with nuclear and cytoplasmic fractions from E18 rat cortical neurons. Bound proteins were eluted with the high salt solution and analyzed on a SDS-PAGE followed by Coomassie brilliant blue staining. Peptides were recovered by in-gel digestion of excised bands with trypsin, and analyzed by mass spectrometry in the Proteomics Center at Boston Children's Hospital to identify proteins.

Supplementary Reference

Gaudilliere, B., Shi, Y., and Bonni, A. (2002). RNA interference reveals a requirement for myocyte enhancer factor 2A in activity-dependent neuronal survival. J Biol Chem *277*, 46442-46446.

Supplementary Figure Legends

Figure S1, related to Figure 1.

(A-D) The primary cilium was visualized in hippocampal neurons by immunocytochemistry using an AC3 antibody together with Centrin-GFP (A), acetylated tubulin (AcTub) (B), SSTR3 antibody (C) or MCHR1 antibody (D). Scale bar = 5 μm. Arrows indicate the primary cilium, and arrowheads indicate the centrosome.

(E) Time-course analysis of ciliary morphogenesis in hippocampal neurons. Percentage of neurons bearing a primary cilium was quantified in cohorts of neurons at the indicated time points. Total of 2476 neurons were quantified.

(F) Lysates of 293T cells transfected with the epitope-tagged XLID protein expression plasmids along with the cognate RNAi or control U6 RNAi plasmid were immunoblotted with the antibodies against the epitope-tag as indicated in the figure together with the 14-3-3 or Erk1/2 antibody, the latter serving as loading controls.

Figure S2, related to Figure 2.

(A) Additional representative images for Figure 2B.

(B) Additional representative images for Figure 2D.

(C) Hippocampal neurons transfected with the PQBP1 RNAi or control U6 RNAi plasmid together with the GFP expression plasmid at DIV1 were subjected to immunocytochemistry using the GFP and AC3 antibodies at DIV14. The percentage of neurons harboring a primary cilium was significantly reduced upon PQBP1 knockdown ($p < 0.01$; ANOVA). Total of 122 neurons were counted.

(D-G) NIH3T3 (D and E) and MDCK (F and G) cells transfected with the PQBP1 RNAi or control U6 RNAi plasmid together with the GFP expression plasmid were subjected to immunocytochemistry using the GFP and acetylated tubulin antibodies 3 days after transfection. PQBP1 knockdown had no effect on cilium formation in NIH3T3 or MDCK cells in the presence of serum (D and F) or after serum starvation for 24 hours (E and G).

(H-K) NIH3T3 (H and I) and MDCK (J and K) cells were subjected to immunocytochemistry using the acetylated tubulin (AcTubulin) and PQBP1 antibodies. Cells were analyzed in the presence of serum (H and J) or after serum deprivation for 24 hours (I and K). PQBP1 localizes in nucleus in NIH3T3 and MDCK cells.

(L) Additional representative images for Figure 2F.

Figure S3, related to Figure 3.

(A) Hippocampal neurons were subjected to immunocytochemistry with the PQBP1 antibody together with the Tuj1 (neuron specific class III beta-tubulin) antibody. Arrows indicate PQBP1 immunoreactivity at the cilium. Scale bar = 5 μm.

(B) Hippocampal neurons were subjected to immunocytochemistry using the PQBP1 antibody in the presence of GST-PQBP1 or control GST protein. Arrow indicates PQBP1 immunoreactivity. Scale bar = 5 μm.

(C) Hippocampal neurons transfected with the PQBP1 RNAi or control U6 RNAi plasmid together with the Centrin-GFP plasmid were subjected to immunocytochemistry with the PQBP1 and GFP antibodies. Arrows indicate PQBP1 immunoreactivity, and arrowheads indicate the centrosome. Scale bar = 5 μm.

(D) Hippocampal neurons were subjected to immunocytochemistry with a second rabbit PQBP1 (Santa Cruz) antibody together with the Tuj1 (neuron specific class III beta-tubulin) antibody. Arrows indicate PQBP1 immunoreactivity at the cilium. Scale bar = 5 μm.

Figure S4, related to Figure 4.

(A) GST, GST-PQBP1, and GST-PQBP1 W75A P78G used for Figure 4A were analyzed by SDS-PAGE and Coomassie Brilliant Blue staining.

(B) Cytoplasmic and nuclear fractions isolated from rat cortical neuron were immunoblotted using the Pericentrin, AC3, PCM1, PQBP1, Dynamin 2, 14-3-3 and SnoN antibodies. Pericentrin, AC3,

are PCM1 are proteins associated with the centrosome or primary cilium. 14-3-3 and SnoN served as the cytoplasmic and nuclear marker, respectively.

(C) Lysates of 293T cells transfected with Flag-Dynamin 1, Flag-Dynamin 2 or Flag-Dynamin 3 were subjected to a pull-down assay with GST-PQBP1 or GST and immunoblotted with the Flag antibody.

(D) Centrosomal fractions isolated from rat cortical neurons were immunoblotted using the Dynamin 2 (Dnm2), PQBP1, γTubulin, Pericentrin, PCM1, SnoN, BiP, GM130 and BCL2 antibodies. Dynamin 2, PQBP1, Pericentrin, PCM1 and γTubulin cofractionated, suggesting PQBP1 and Dynamin 2 are present at the centrosome. Nuclear-localized protein SnoN, ER-localized protein BiP, golgi-localized protein GM130, and mitochondrial protein BCL2 served as negative controls.

(E and F) Hippocampal neurons transfected with Dynamin 2-GFP were subjected to immunocytochemistry with the GFP antibody together with the Pericentrin (E) or PQBP1 (F) antibody. Arrows indicate Pericentrin or PQBP1 immunoreactivity. Scale bar = 5 μm.

(G) The percentage of hippocampal neurons bearing a primary cilium was significantly higher at DIV3 in neurons transfected with GFP-IFT20-WW compared to control vector, GFP-IFT20, or GFP-NLS-WW ($p < 0.05$; ANOVA). Total of 207 neurons were measured.

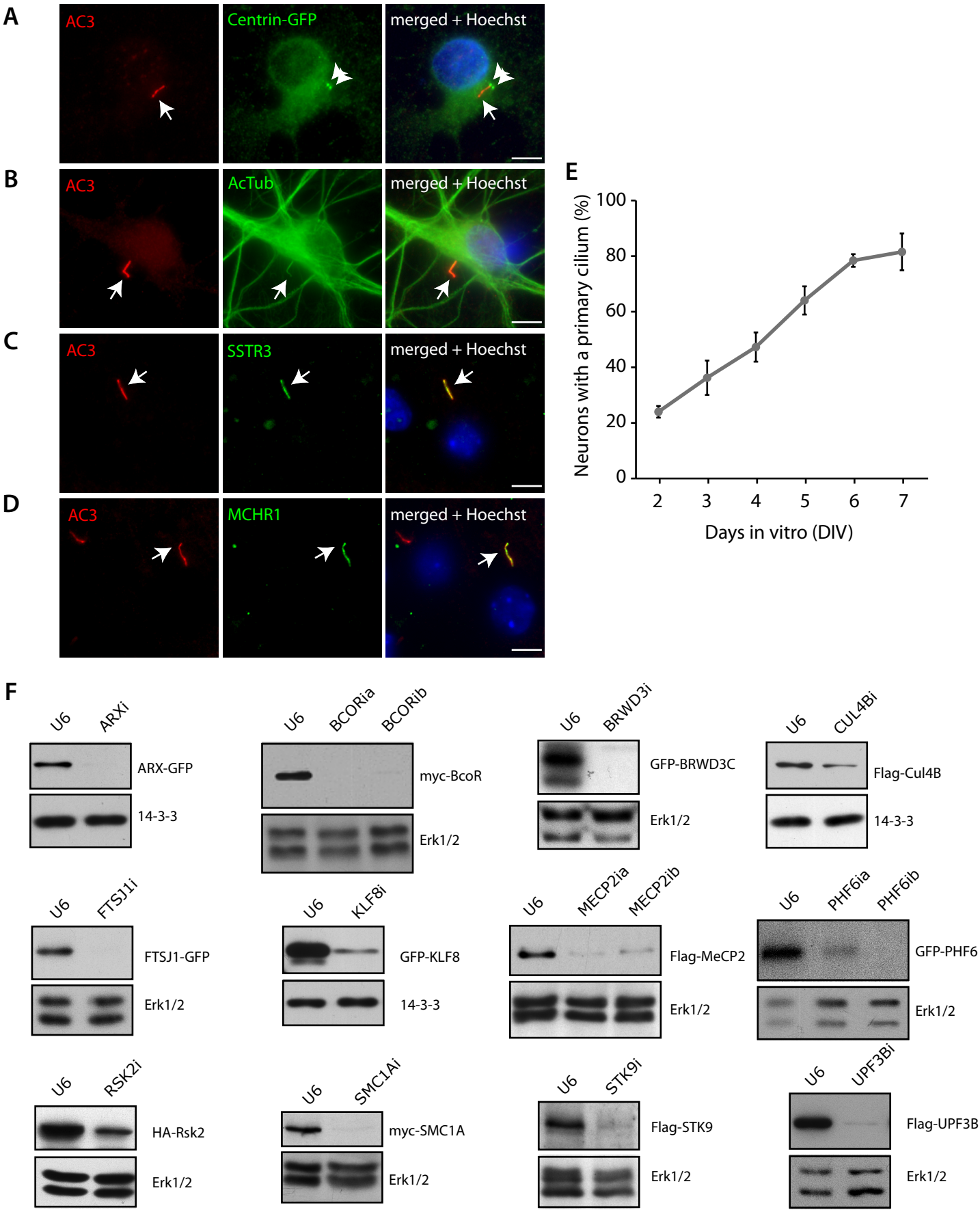
(H and I) Hippocampal neurons transfected with GFP-IFT20 (H) and GFP-IFT20-WW (I) were subjected to immunocytochemistry with the GFP antibody together with the AC3 antibody. Arrows indicate GFP immunoreactivity at the cilium. Scale bar = 5 μm. The insets show the enlarged primary cilium.

Figure S5, related to Figure 5.

(A) Additional representative images for Figure 5B.

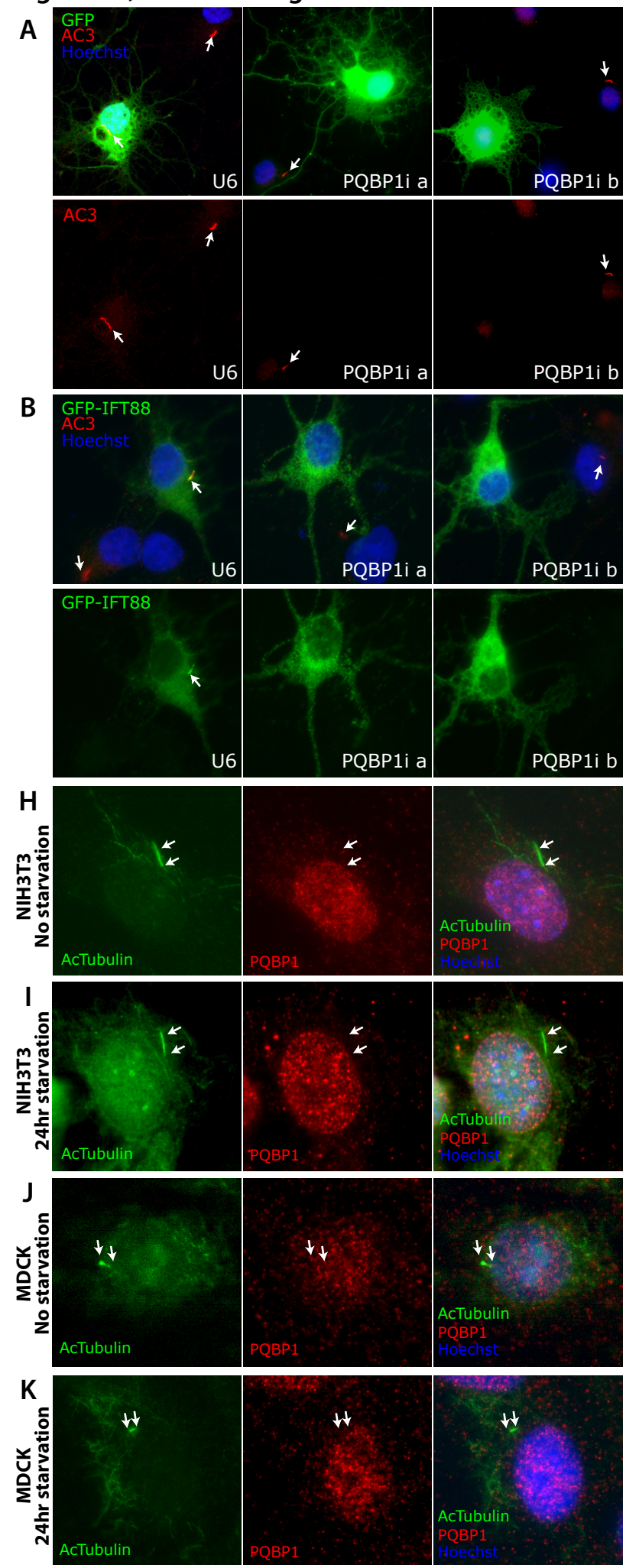
(B) The percentage of hippocampal neurons with PQBP1 immunoreactivity at the base of the cilium was analyzed at DIV7 in neurons transfected with Dynamin 2 RNAis or the control vector. Total of 112 neurons were measured.

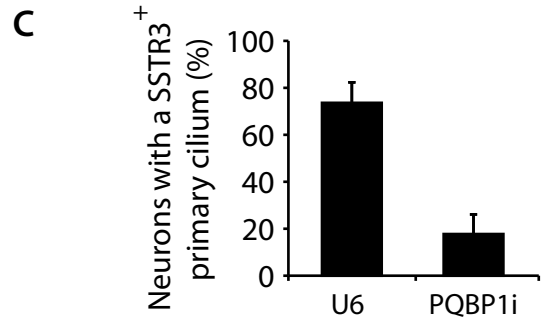
Figure S1, related to Figure 1

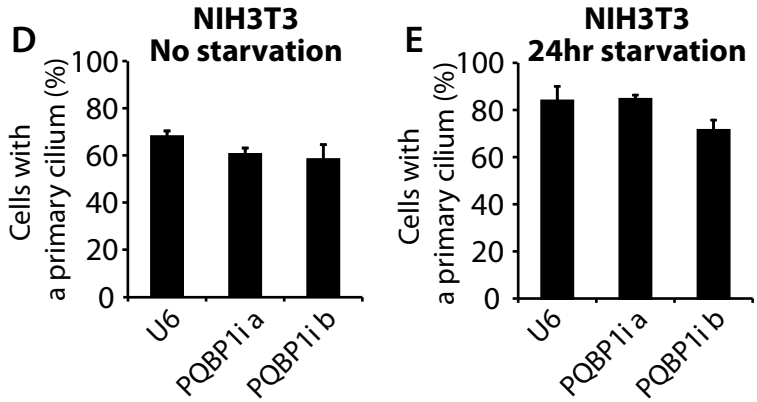


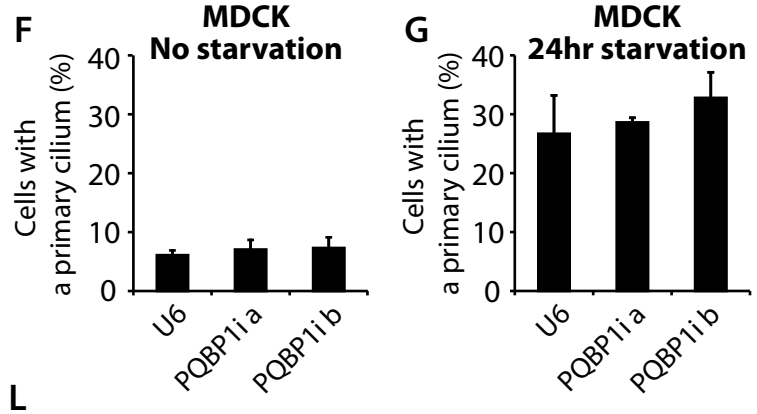
Supplemental Figure S2

Figure S2, related to Figure 2









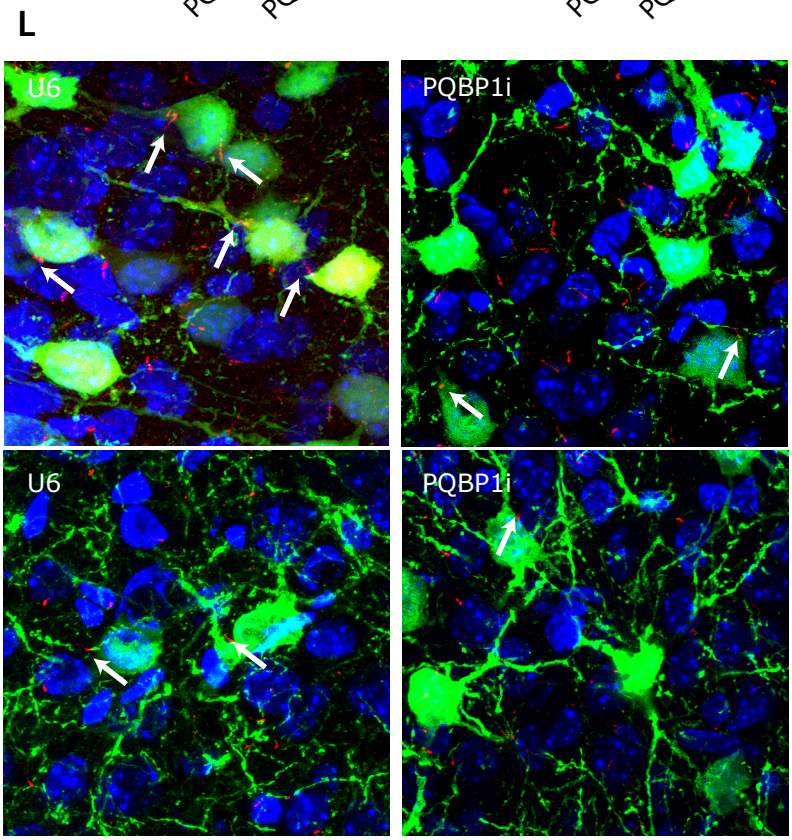


Figure S3, related to Figure 3

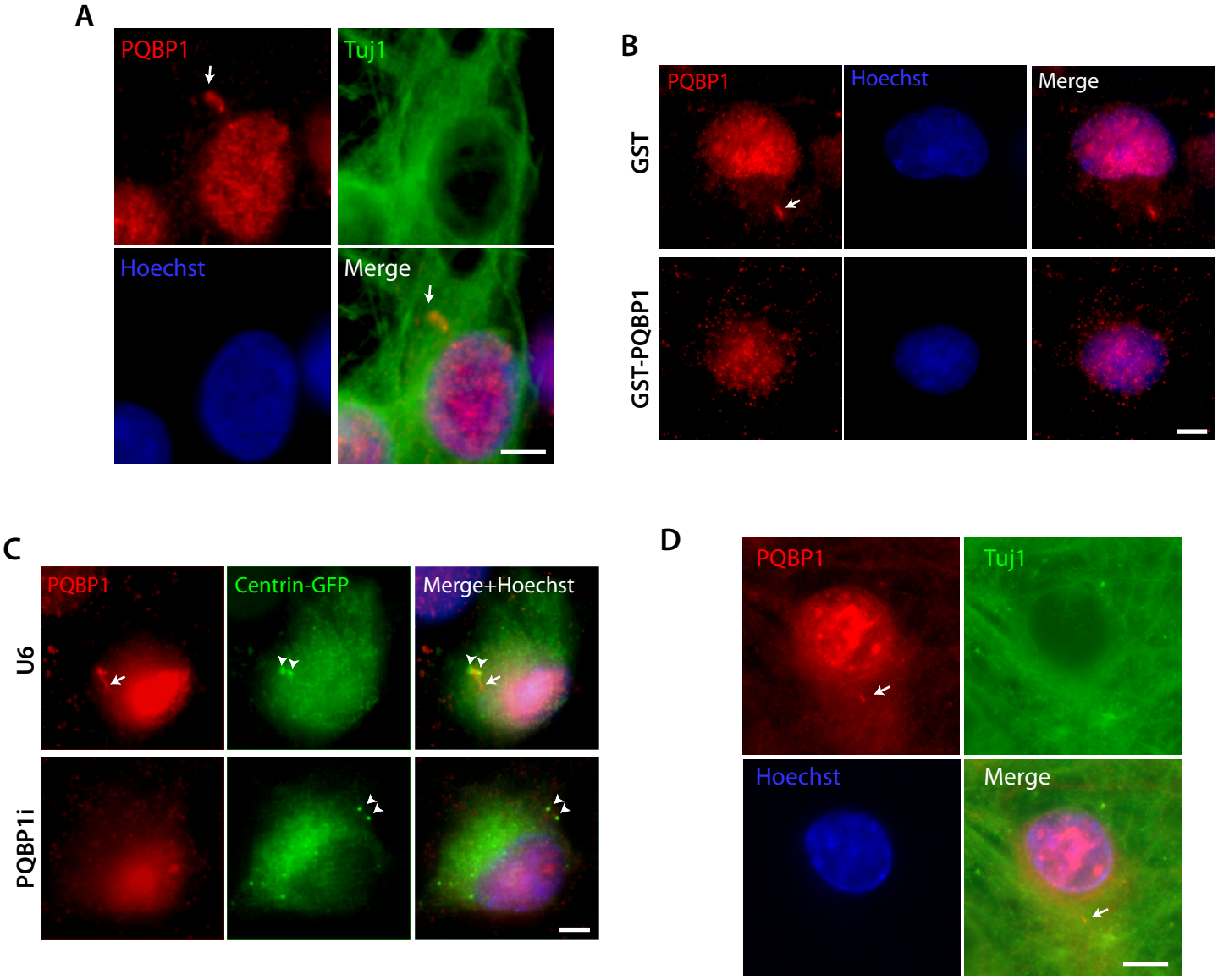


Figure S4, related to Figure 4

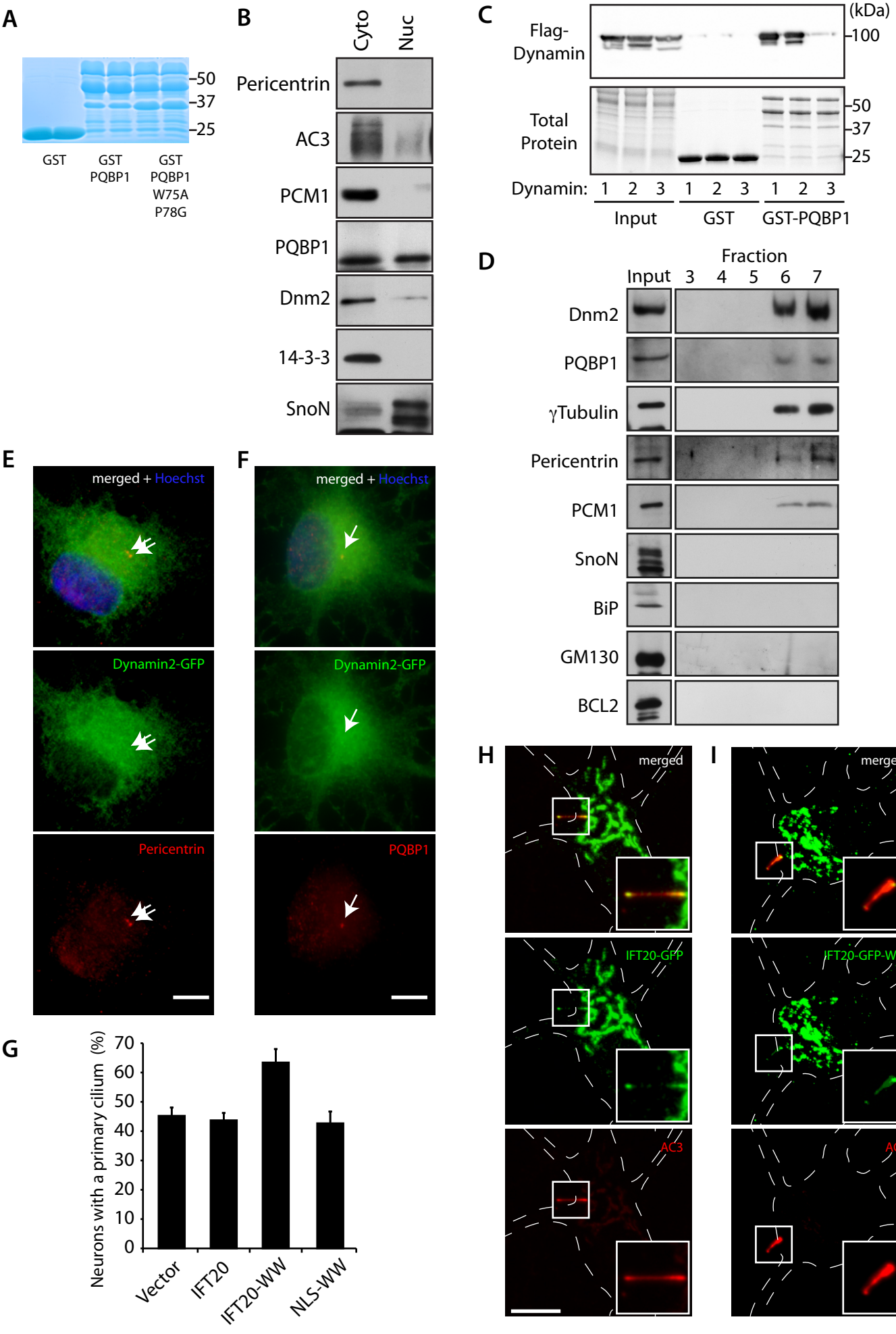
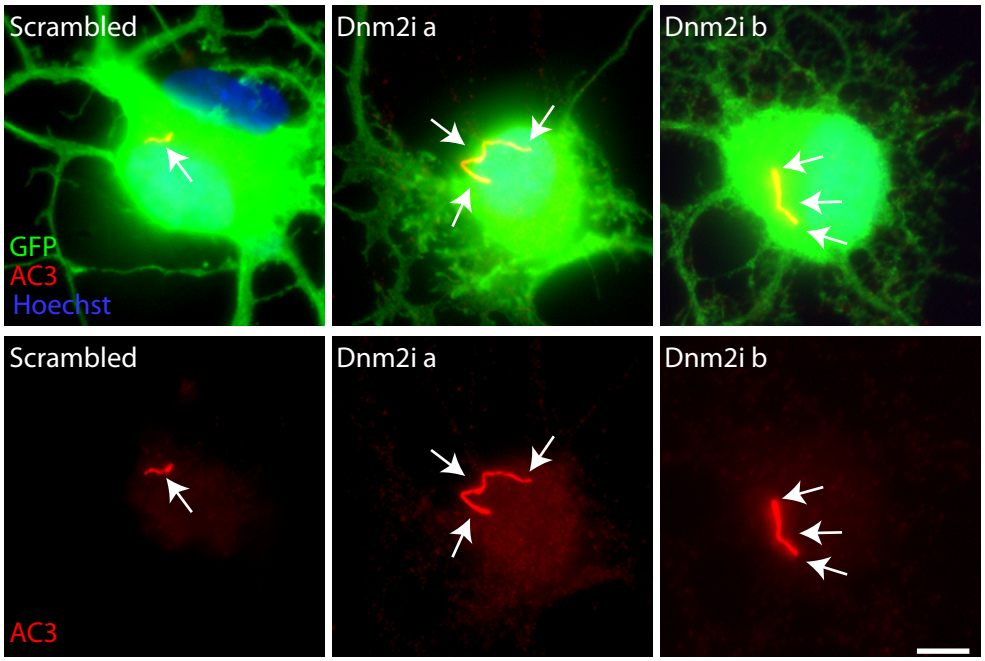


Figure S5, related to Figure 5

A



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