

Expanded View Figures

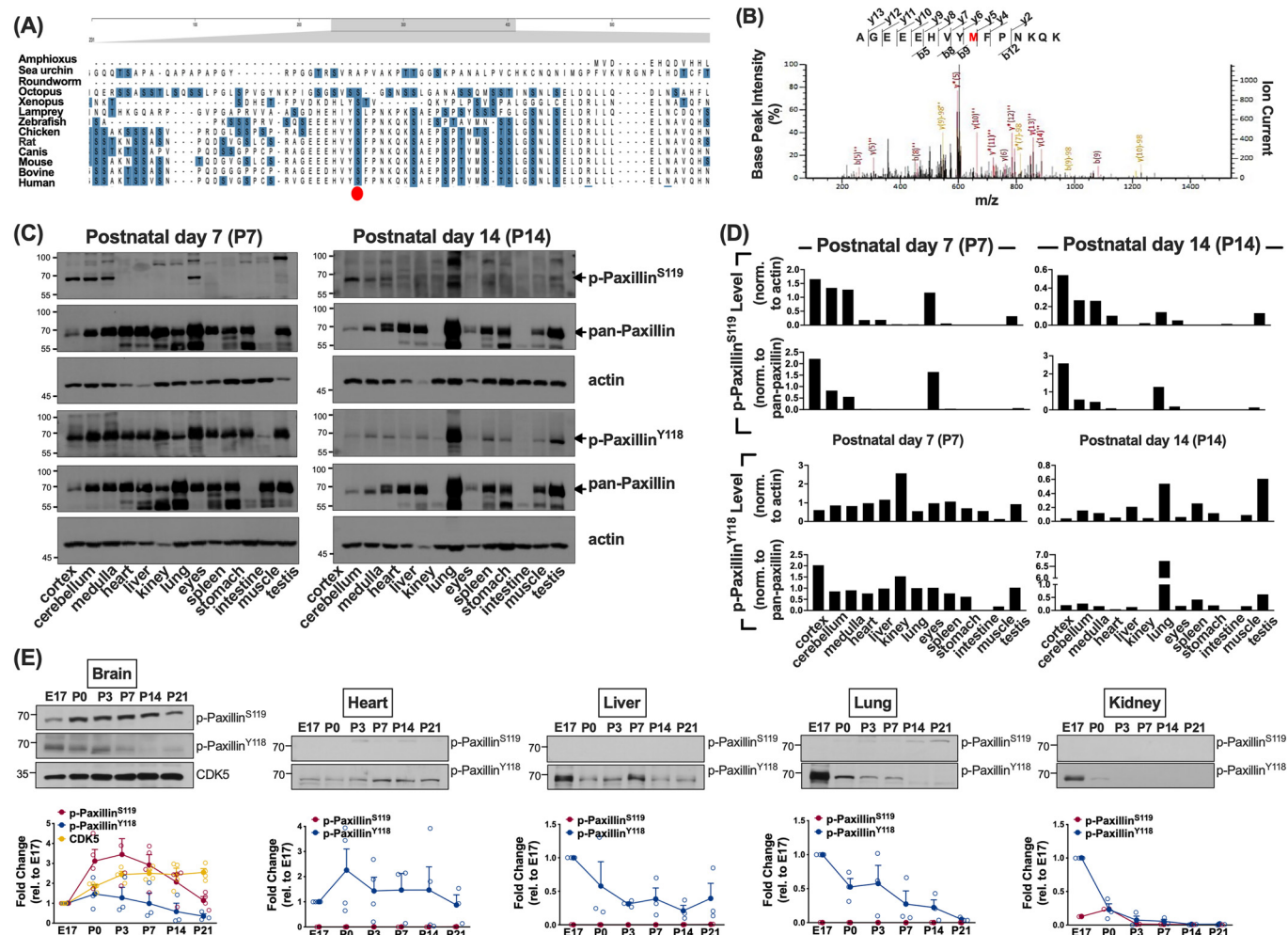


Figure EV1. Identification of a brain-enriched phosphorylation site at paxillin serine119.

(A) Paxillin S119 is phylogenetically conserved. Alignment of sequences found around paxillin S119 (red dot), illustrating conservation across indicated species. (B) Mass spectrometric identification of phosphopeptides in paxillin protein IP'd from P14 mouse brain lysates. The phosphopeptide AGEEHVYMFNPKQK (phospho-paxillin^{S119}; p-Paxillin^{S119}) was identified by precursor ion scanning for neutral loss of phosphoric acid (M-H₃PO₄). M, peptide precursor ion. (C) Western blots show p-Paxillin^{S119} enrichment in mouse brain lysates at P7 and P14. Lysates were prepared from multiple tissues, as indicated. The pan-Paxillin blots shown here were derived from membranes that were first probed with either anti-p-Paxillin^{S119} or anti-p-Paxillin^{Y118}, and after antibody stripping were subsequently re-probed with anti-pan-Paxillin to serve as total Paxillin controls for the same samples. (D) Quantification of data shown in blots in (C). (E) Developmental profiles of p-Paxillin^{S119} and p-Paxillin^{Y118} across multiple tissues. Western blots showing p-Paxillin^{S119} and p-Paxillin^{Y118} levels over various developmental stages in various tissues. Graph shows average protein intensity $\pm$ SEM, compared with corresponding levels at E17 ($n = 3-4$ mice per group). Source data are available online for this figure.

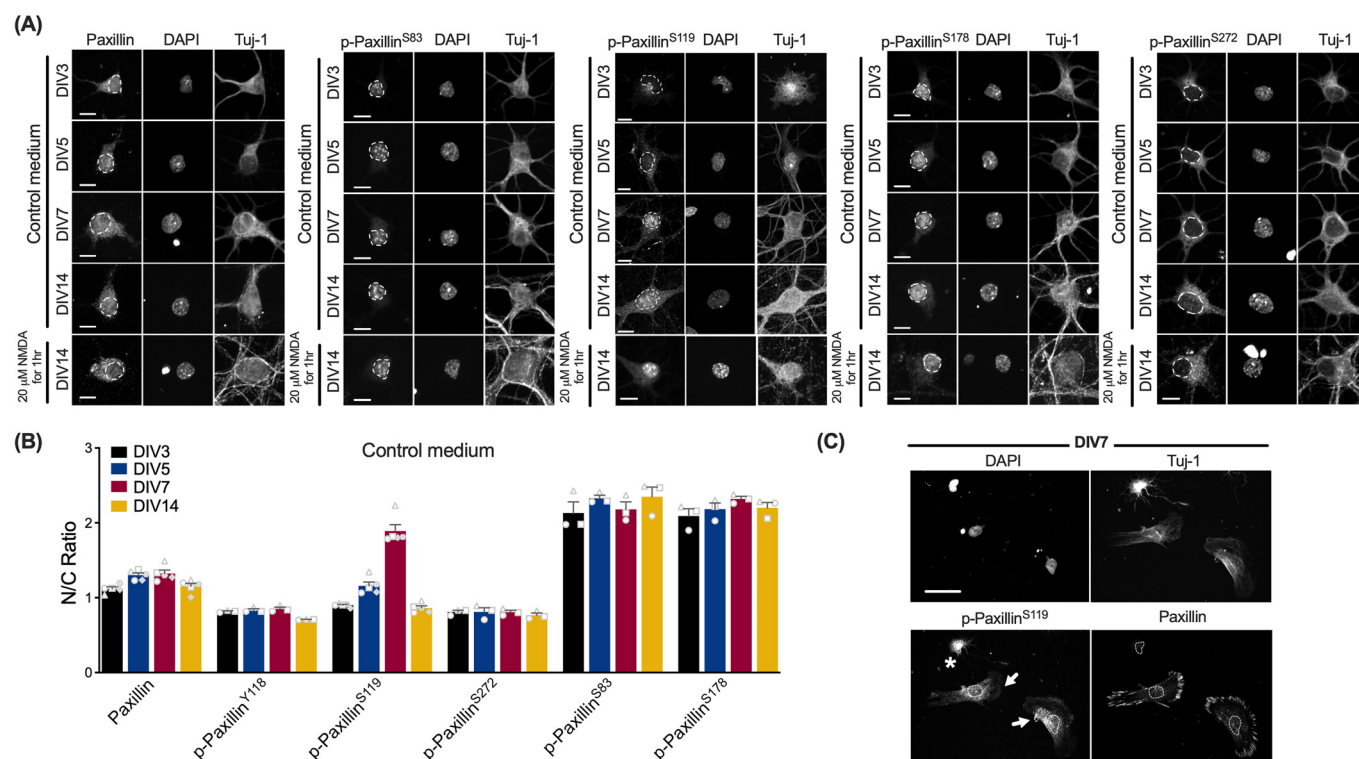


Figure EV2. Site-specific phosphorylation determines paxillin subcellular localization in neurons and glia.

(A, B) DIV7 neurons show transient increases in nuclear p-Paxillin^{S119} levels. (A) Representative images of rat primary neuronal cultures stained with antibodies against different phosphorylated forms of paxillin at the indicated time points. Scale bar, 5 μ m. (B) Histograms summarizing the nucleus-to-cytoplasm (N/C) ratios of various phosphorylated paxillin forms from experiments similar to those in (A). Data represent mean $\pm$ SEM ($n = 3$ –5 independent cultures; >6 cells per group). (C) Differential distribution of p-Paxillin^{S119} in neurons vs. glia. Representative image of a DIV7 primary neuronal culture showing p-Paxillin^{S119} signals in a neuron (asterisk) versus in neuroglia (arrows). Scale bar, 10 μ m.

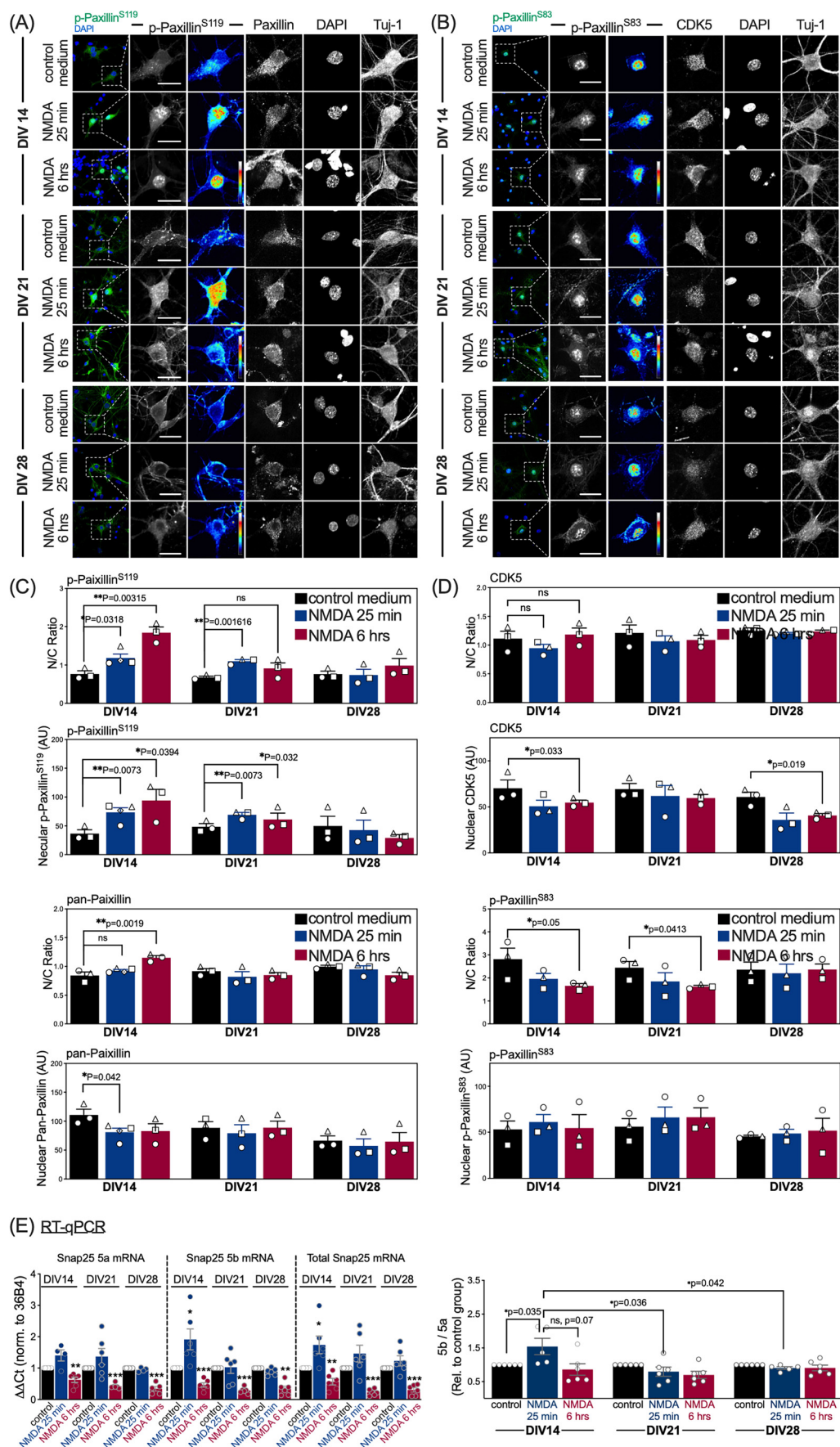


Figure EV3. Both NMDA-induced nucleocytoplasmic translocation of p-Paxillin^{S119} and Snap25 exon 5b-to-5a inclusion in neurons decrease after DIV14.

(A–D) Subcellular distribution of endogenous p-Paxillin^{S119}, pan-Paxillin, p-Paxillin^{S83}, and CDK5 in primary cortical neurons at the indicated days in vitro (DIV), following acute (“25 min”) or prolonged (“6 h”) stimulation with 20 μ M NMDA. (A, B) Representative images of neurons cultured in standard Neurobasal medium under the indicated conditions. Cells were immunostained for p-Paxillin^{S119} (A, green), pan-Paxillin, p-Paxillin^{S83} (B, green), CDK5 and the neuronal marker Tuj-1, with DAPI counterstain (blue) at the indicated conditions and time points. Scale bar, 15 μ m. The intensity of p-Paxillin^{S119} is also displayed using a “royal” look-up table, highlighting the range from low (blue) through medium (green) to high (red) intensity. (C, D) Histogram summarizing nuclear to cytoplasmic (N/C) ratio and nuclear abundance of p-Paxillin^{S119}, pan-Paxillin, p-Paxillin^{S83}, and CDK5 in primary neuronal cultures at indicated conditions and time points. Data represent mean $\pm$ SEM ($n = 3$ independent cultures; >30 cells per group; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns not significant compared to control, by one-way ANOVA with Dunnett’s multiple comparisons test). (E) Real-time qPCR analysis of Snap25 exon 5a, 5b, and total mRNA expression in neuronal cultures at the indicated time points under control or NMDA treatment conditions. Data represent mean $\pm$ SEM ($n = 5$ –6 cultures per group; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns not significant compared to DIV14 NMDA 25 min group, multiple t -tests). Source data are available online for this figure.

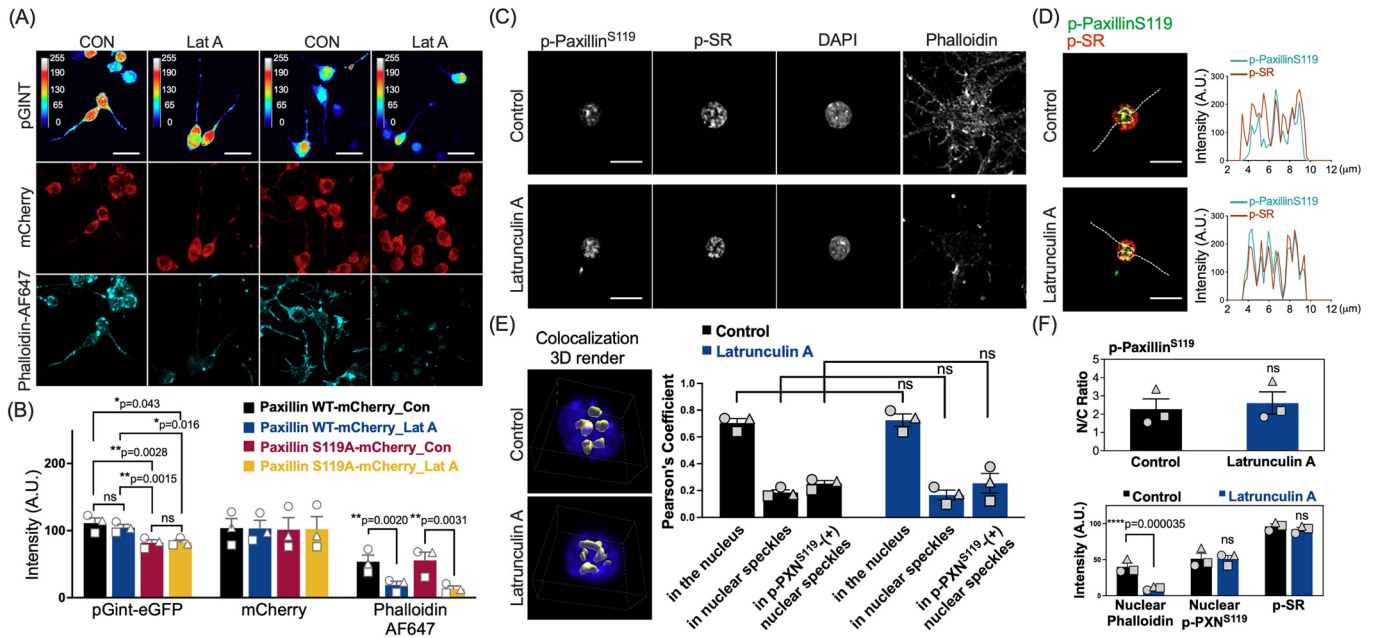


Figure EV4. Disruption of cytoplasmic actin polymerization does not alter mRNA splicing efficiency.

(A) Representative images showing differentiated Neuro2A cells transduced with either mCherry-tagged paxillin wild-type (WT) or its S119A variant via lentiviral transduction, followed by transfection with the pGint splicing reporter. Cells were treated with or without the actin polymerization inhibitor Latrunculin A ("Lat A"; 330 nM for 1 h) and then stained with Phalloidin-AF647 to visualize F-actin. eGFP fluorescence intensity is shown using a "royal" look-up table, highlighting the range from low (blue) through medium (green) to high (red) intensity. Scale bar, 20 μ m. (B) Plot summarizing average eGFP intensity, mCherry signal, and Phalloidin-AF647 staining ($\pm$ SEM; $n = 21$ -30 cells from three independent experiments; **** $p < 0.0001$ compared to WT by Two-way ANOVA with Tukey's multiple comparisons test). (C-F) p-Paxillin^{S119} colocalization with phospho-SR proteins (p-SR) is maintained after disruption of actin polymerization. (C) Immunofluorescence images of DIV7 neurons treated with or without latrunculin A (330 nM, 1 h), stained for p-Paxillin^{S119}, p-SR, DAPI, and Phalloidin. (D) Fluorescence intensity profiles (right panel) across p-Paxillin^{S119} puncta show colocalization with p-SR. Scale bar, 10 μ m. (E) Left: 3D renderings showing colocalization of p-Paxillin^{S119} with p-SR in nuclear speckles. Right: Bar graphs quantify Pearson's correlation coefficients ($\pm$ SEM; $n = 3$ cultures; 15 cells per condition; ns not significant, by multiple unpaired t -tests) for p-Paxillin^{S119} and p-SR colocalization within the nucleoplasm, nuclear speckles, and p-Paxillin^{S119}-positive nuclear speckles. (F) Quantification of the N/C ratio of p-Paxillin^{S119} (top), and nuclear intensity of p-Paxillin^{S119}, p-SR, and Phalloidin staining (bottom) in DIV7 neurons with or without Lat A treatment. Data represent mean ($\pm$ SEM; $n = 3$ independent cultures; >30 cells per group; **** $p < 0.0001$; ns not significant, by multiple unpaired t -tests). Source data are available online for this figure.

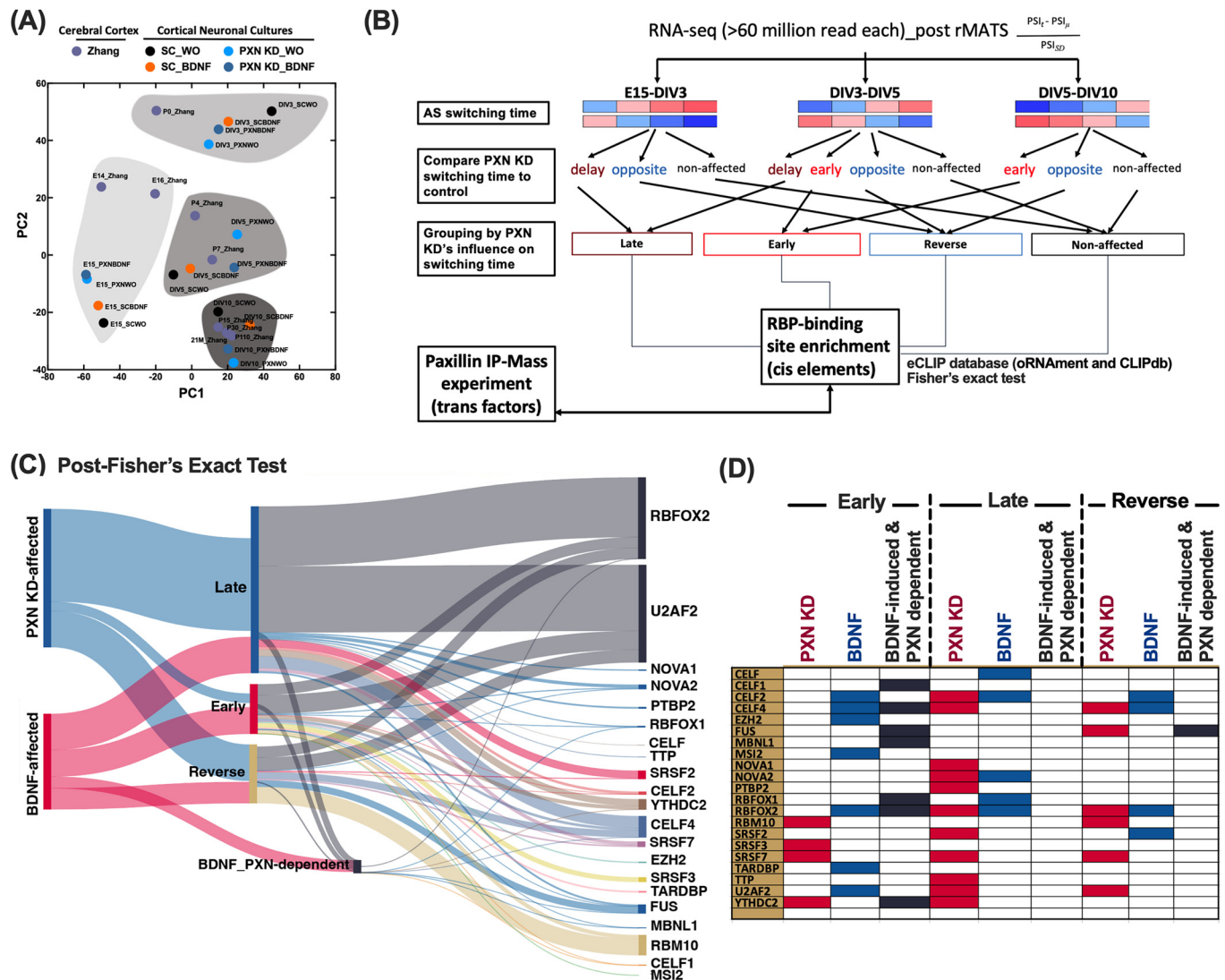


Figure EV5. Identification of alternative splicing events associated with paxillin knockdown and/or BDNF treatment in primary neuronal cultures.

(A) PCA analysis of cassette exon profiles. Cassette exon usage patterns from E15 mouse primary neuronal cultures at various DIV were compared with those seen in brain lysates (“Zhang”; dataset from (Weyn-Vanhenhenryck et al, 2018) at corresponding postnatal days. Note that DIV3 clustered with P0, DIV5 with P4 and P7, and DIV19 with >P15. (B) Analysis pipeline for splicing event identification. Schematic illustrating workflow used to detect cassette exon switching events altered by paclitaxel knockdown (“PXN KD”) and/or BDNF treatment, followed by prediction of trans-acting RNA-binding proteins (RBPs). (C) Sankey diagram of splicing time switches and RBPs. Diagram indicating how timing of cassette exon switching is influenced by PXN KD and/or BDNF treatment, and highlighting associated RBPs. Notably, most late-switched cis-elements affected by PXN KD contain U2AF2 and RBFOX2 binding sites. (D) Table listing RBPs significantly linked to the PXN KD- and/or BDNF-affected splicing events compared with those in the non-affected group ($p < 0.05$ by Fisher’s exact test).