



Supplemental Figure S1: *Pard3* deletion does not disrupt junction integrity initially. (A)

Representative confocal images of E12.5 control and *Pard3* cKO cortices stained for PARD3 (red) and counter-stained for DAPI (blue). High-magnification images of the VZ surface (broken line rectangles and arrows) are shown at the bottom. Asterisks indicate the residual PARD3 signal in the cortical plate likely in migrating interneurons originated from the ventral telencephalon or post-mitotic neurons produced prior to the *Cre* recombination. Scale bars: 150 μm (top) and 20 μm (bottom). **(B)** Western blot assay of PARD3 expression in control and *Pard3* cKO cortices using an antibody against the N-terminus of PARD3. Note that the expression of both the long (~180 KDa) and short (~100 KDa) isoforms of PARD3 is largely abolished in the *Pard3* cKO cortex. **(C)** Representative confocal images of E12.5 control and *Pard3* cKO cortices stained for centrosomal marker PCNT (red) and junction marker ZO-1 (green) and counter-stained for DAPI (blue). High-magnification images of the VZ surface (broken line rectangles) are shown at the bottom. Note no obvious junction disruption in the *Pard3* cKO cortex at this stage. Scale bars: 20 μm (top) and 10 μm (bottom). **(D, E)** Representative confocal images of E12.5 control and *Pard3* cKO cortices stained for N-CADHERIN (green, D) and β -CATENIN (red, E), and counter-stained for DAPI (blue). Scale bars: 10 μm . **(F)** Representative Nissl-stained images of P21 control and *Nex-Cre;Pard3* cKO brain coronal sections. Note no obvious defect in cortical development in the *Nex-Cre;Pard3* cKO brain. Scale bar: 1.25 mm. **(G)** Representative confocal images of P21 control and *Nex-Cre;Pard3* cKO cortices stained for layer V/VI neuronal marker CTIP2 (green) and layer II-IV neuronal marker CUX1 (red), and counter-stained with DAPI (blue). Scale bars: 70 μm . **(H, I)** Quantification of the number of CTIP2⁺ cells (H) and CUX1⁺ cells (I) per 300 μm radial column in control and *Nex-Cre;Pard3* cKO mice at P21 (n=6 per genotype; unpaired two-tailed t-test

with Welch's correction). Box-whisker plot: center line, median; box, interquartile range; whiskers, minimum and maximum. **(J)** Representative confocal images of P21 control and *Pard3* cKO coronal sections stained for pan-axonal neurofilament marker SMI-312 (green), and blood vessel marker ISOLECTIN B4 (red), and counter-stained with DAPI (blue). High-magnification images of the cortices (broken line rectangles) are shown at the bottom. Asterisks indicate the giant SBH. Note the two axonal tracts surrounding the SBH and the abundance of blood vessels within the SBH. Scale bars: 150 μm (top) and 70 μm (bottom). **(K)** Representative confocal images of control and *Pard3* cKO cortices stained for centrosomal marker PCNT (green) and junction marker ZO-1 (red), and counter-stained for DAPI (blue) at E13.5 (left), E15.5 (middle) and E17.5 (right). Arrows indicate the junction disruption at E15.5 and E17.5. Scale bar: 30 μm .