Supplementary Figures

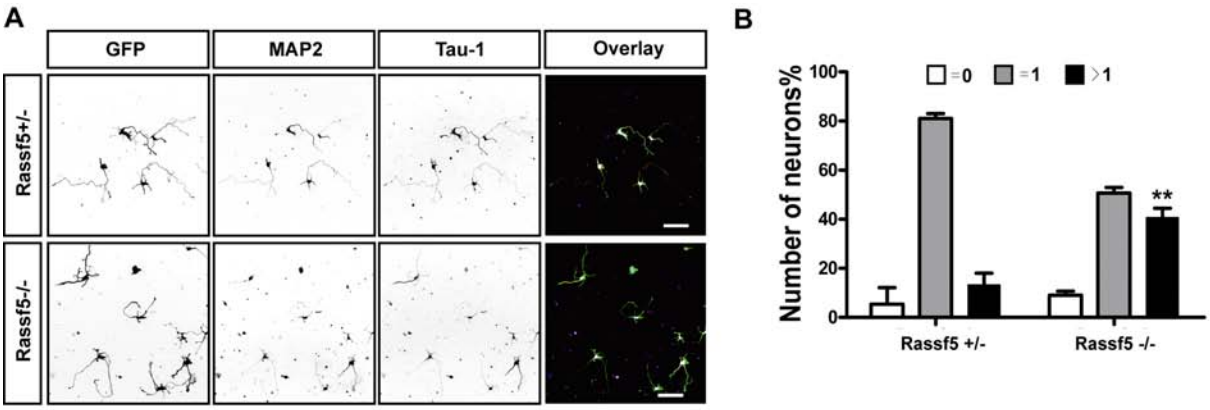


Fig. S1 Neurons from *Rassf5*$^{-/-}$ embryos form supernumerary axons. Dissociated neurons from the hippocampus of ***Rassf5***$^{+/-}$ **or *Rassf5***$^{-/-}$ **e**mbryos were transfected with a vector for GFP (green) and stained at 3 d.i.v. with the Tau-1 (blue) and an anti-MAP2 antibody (red). (C) The percentage of unpolarized neurons without any axon (0, white), polarized neurons with a single axon (1, grey) and hyperpolarized neurons with multiple axons (>1, black) is shown (3 experiments, n>40, values are means ± s.e.m.; **p ≤ 0.01 compared to control (*Rassf5*$^{+/-}$) by two-way ANOVA). Scale bars: 100 μm.

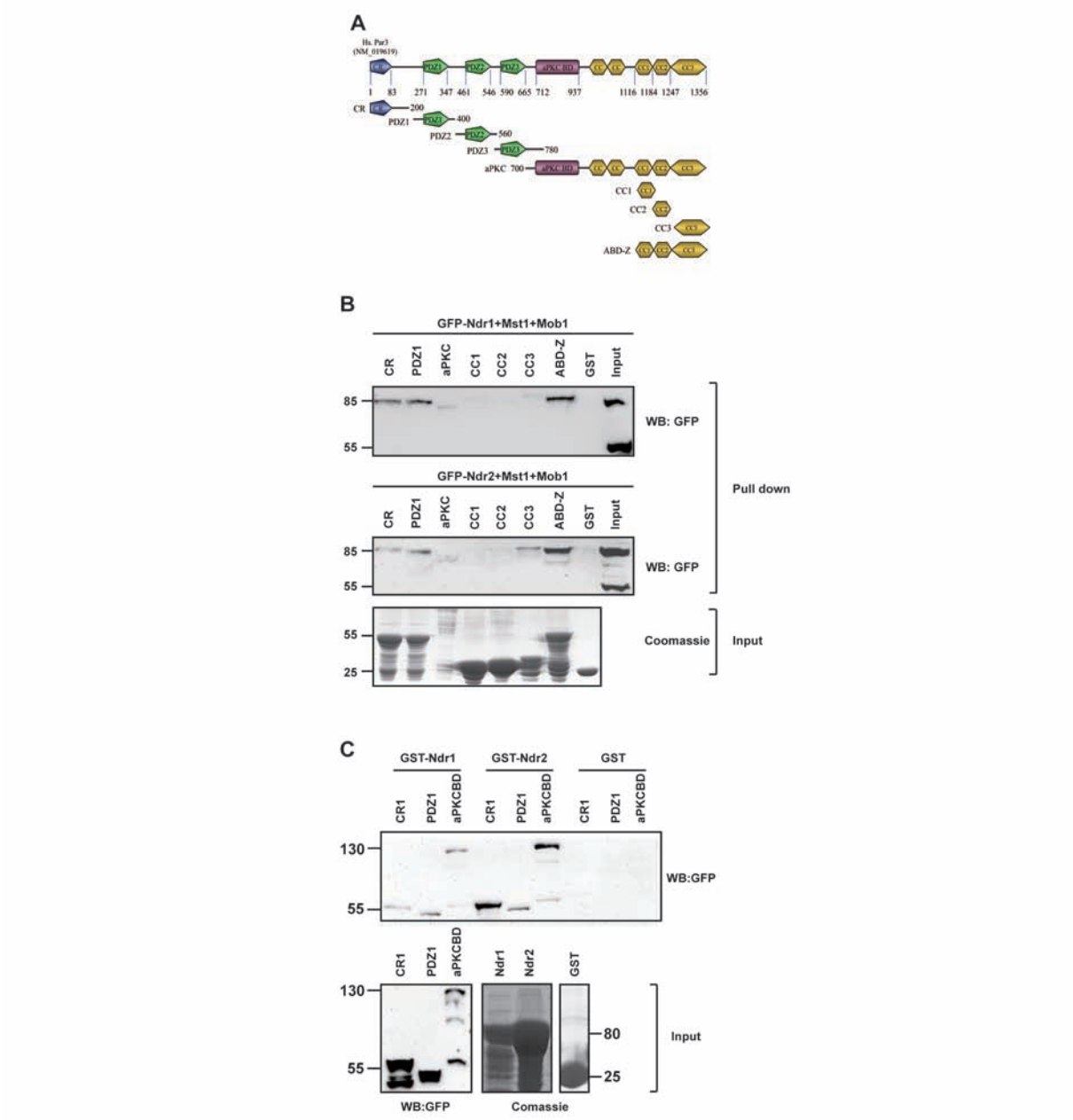


Figure S2. Ndr1 and 2 interact with Par3 domains.

(A) A schematic representation of the Par3 domains is shown. Numbers indicate the position of the domains in the amino acid sequence.

(B) The indicated bacterially expressed GST-fusion proteins for different Par3 domains were coupled to glutathione-sepharose beads and incubated with lysates of HEK 293T cells transfected with the expression vectors for GFP-Ndr1 and -Ndr2. Bound proteins and the expression of comparable amounts of GFP- and GST-tagged proteins were analyzed by Western blot (WB) using anti-GFP antibodies and Coomassie blue staining. Numbers indicate the molecular weight in kDa.

(C) The indicated bacterially expressed GST-fusion proteins for Ndr1 and Ndr2 were coupled to glutathione-sepharose beads and incubated with lysates of HEK 293T cells transfected with the expression vectors for GFP-Par3-CR1, -PDZ1 and -aPDCBD. Bound proteins and the expression of comparable amounts of GFP- and GST-tagged proteins were analyzed by Western blot (WB) using anti-GFP antibodies and Coomassie blue staining. Numbers indicate the molecular weight in kDa.

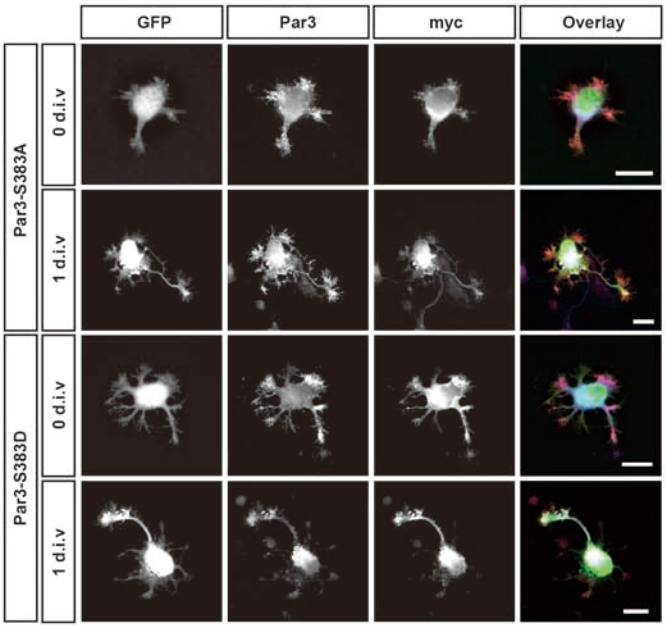
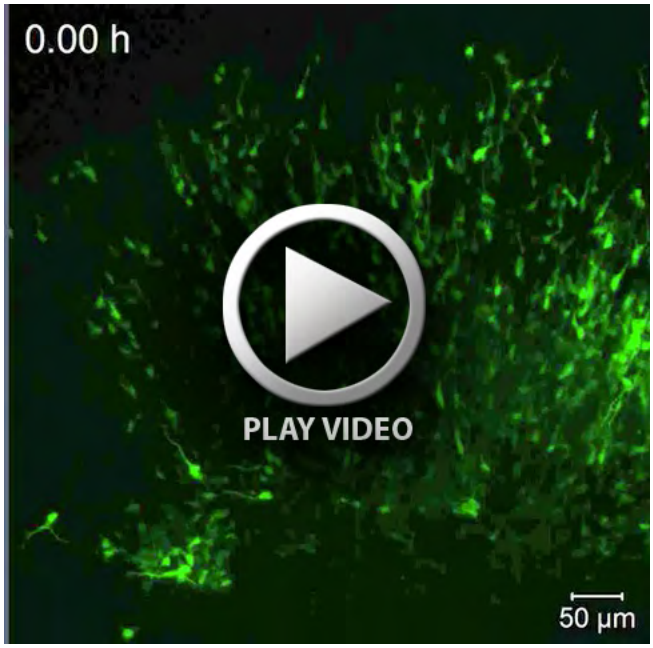
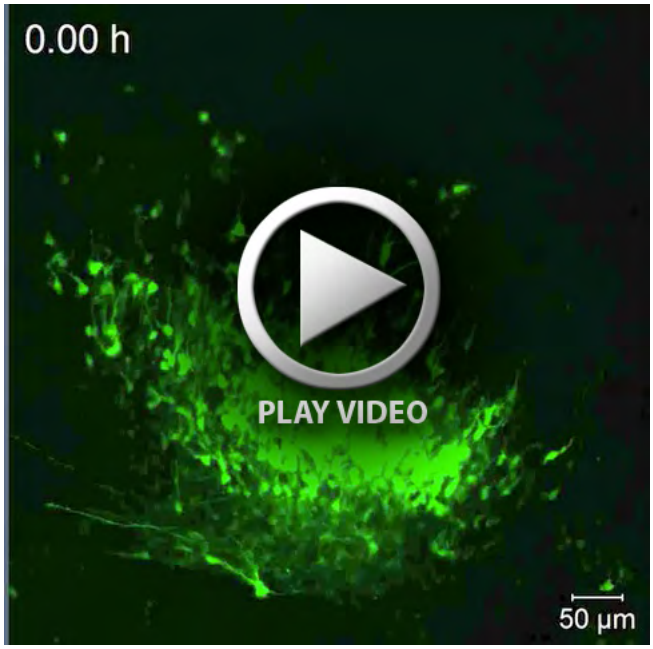


Figure S3. Uniform localization of Par3-S383A and Par3-S383D in unpolarized neurons. Hippocampal neurons were transfected at 0 d.i.v. with vectors for GFP (green) and myc-Par3-S383A or myc-Par3-S383D. Neurons were fixed at 0 or 1 d.i.v. and stained with anti-Par3 (red) and anti-myc (blue) antibodies. Scale bars: 50 μm.



Movie 1. The knockdown of Rassf5 interferes with the polarization of neurons. E14.5 brains were transfected by *ex vivo* electroporation with empty *pCAGGS-U6* (control) and live cell imaging of slice cultures was performed 36 hrs after electroporation. A maximum intensity projection is shown.



Movie 2. The knockdown of Rassf5 interferes with the polarization of neurons. E14.5 brains were transfected by *ex vivo* electroporation with *pCAGGS-U6-Rassf5* with an shRNA against Rassf5 (Rassf5 RNAi) and live cell imaging of slice cultures was performed 36 hrs after electroporation. A maximum intensity projection is shown.