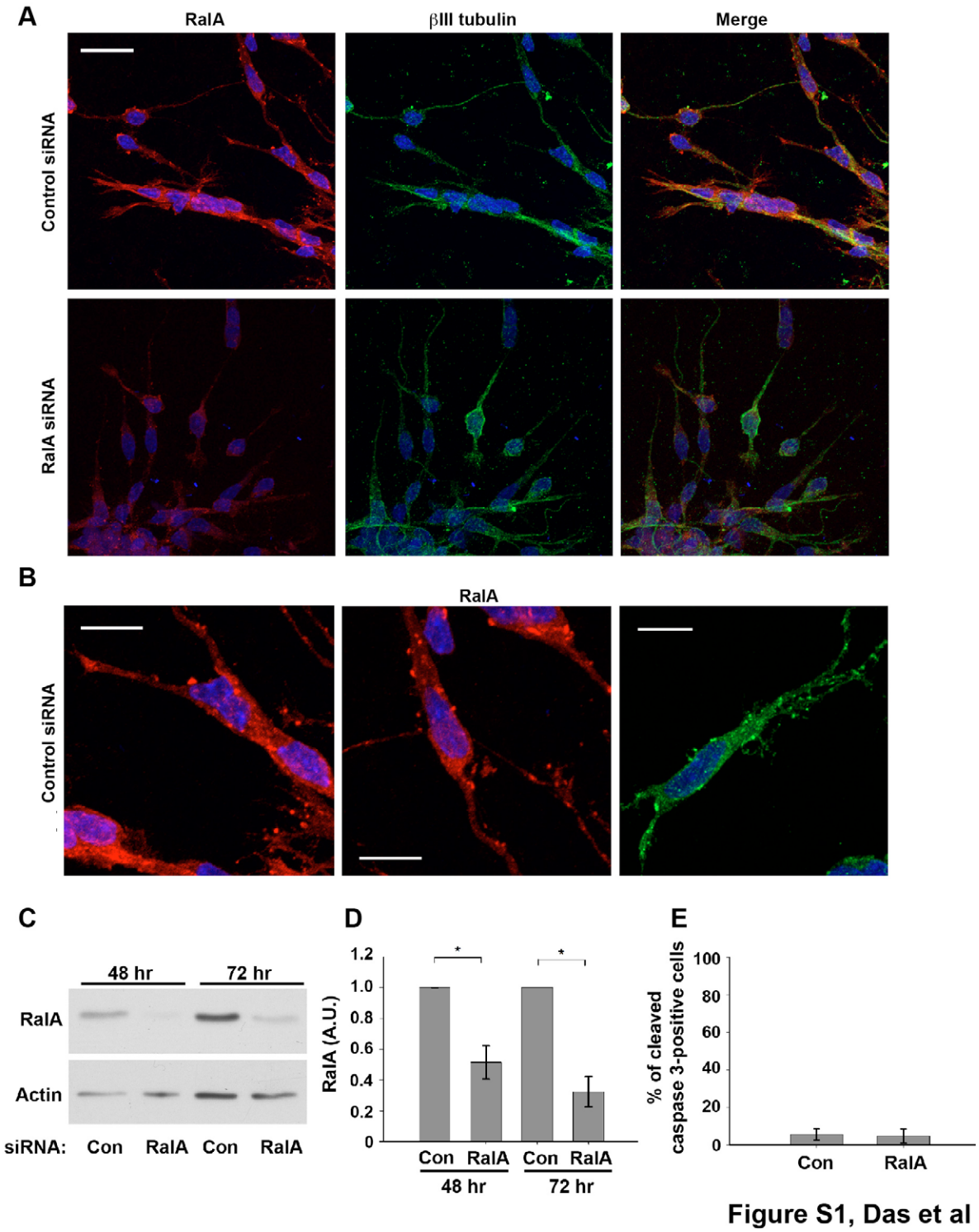

Fig. S1. RalA depletion in RMS neuroblasts. (A) Rat RMS neuroblasts were nucleofected with a control or RalA siRNA oligo and re-aggregated into clusters, embedded in Matrigel 48 h later and allowed to migrate for 24 h before immunostaining for RalA (red) and β III tubulin (green). Cell nuclei (blue) are visualized by Hoechst dye. RalA expression was significantly reduced by the RalA siRNA oligo. Bar, 20 µm. (B) High magnification pictures showing reproducible RalA punctate distribution (red or green) in neuroblasts nucleofected with control siRNA in two different experiments. Cell nuclei (blue) are visualized by Hoechst. Bars, 10 µm. (C) Representative western blot showing reduced expression of RalA at both 48 and 72 h after nucleofection with a RalA siRNA oligo compared to a control (Con) oligo. (D) Densitometric western blot analysis showing significant RalA reduction 48 and 72 h after RalA siRNA nucleofection. Mean ± SEM; *$p < 0.05$; n = 4 independent experiments. (E) Quantification of cleaved caspase 3-positive cells after nucleofection with control or RalA siRNA shows no significant difference in the percentage of cells undergoing apoptosis. Mean ± SEM; n = 3 independent experiments.

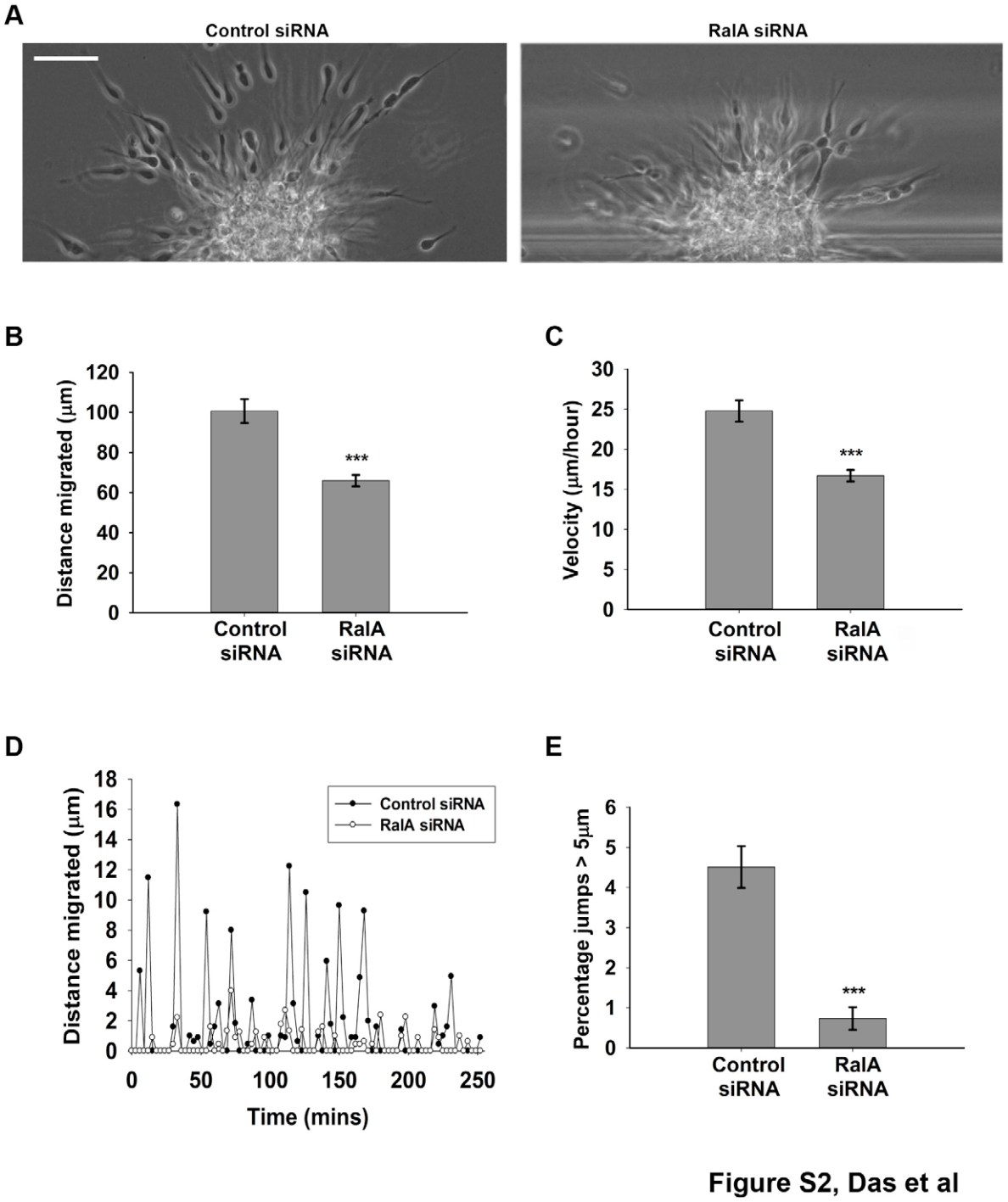

Fig. S2. ***In vitro*** **RalA depletion impairs neuroblast migration.** (A) Snapshots of control and RalA-depleted rat neuroblasts migrating in Matrigel taken 55 h after siRNA nucleofection. Depleting RalA decreased neuroblast migration distance (B) and velocity (C). (D) Representative nuclear displacement traces for control and RalA-depleted neuroblasts. (E) RalA knockdown significantly decreased the percentage of large nuclear jumps. Mean ± SEM; ***$p < 0.001$; for each condition 80 cells were tracked from 3 independent experiments.

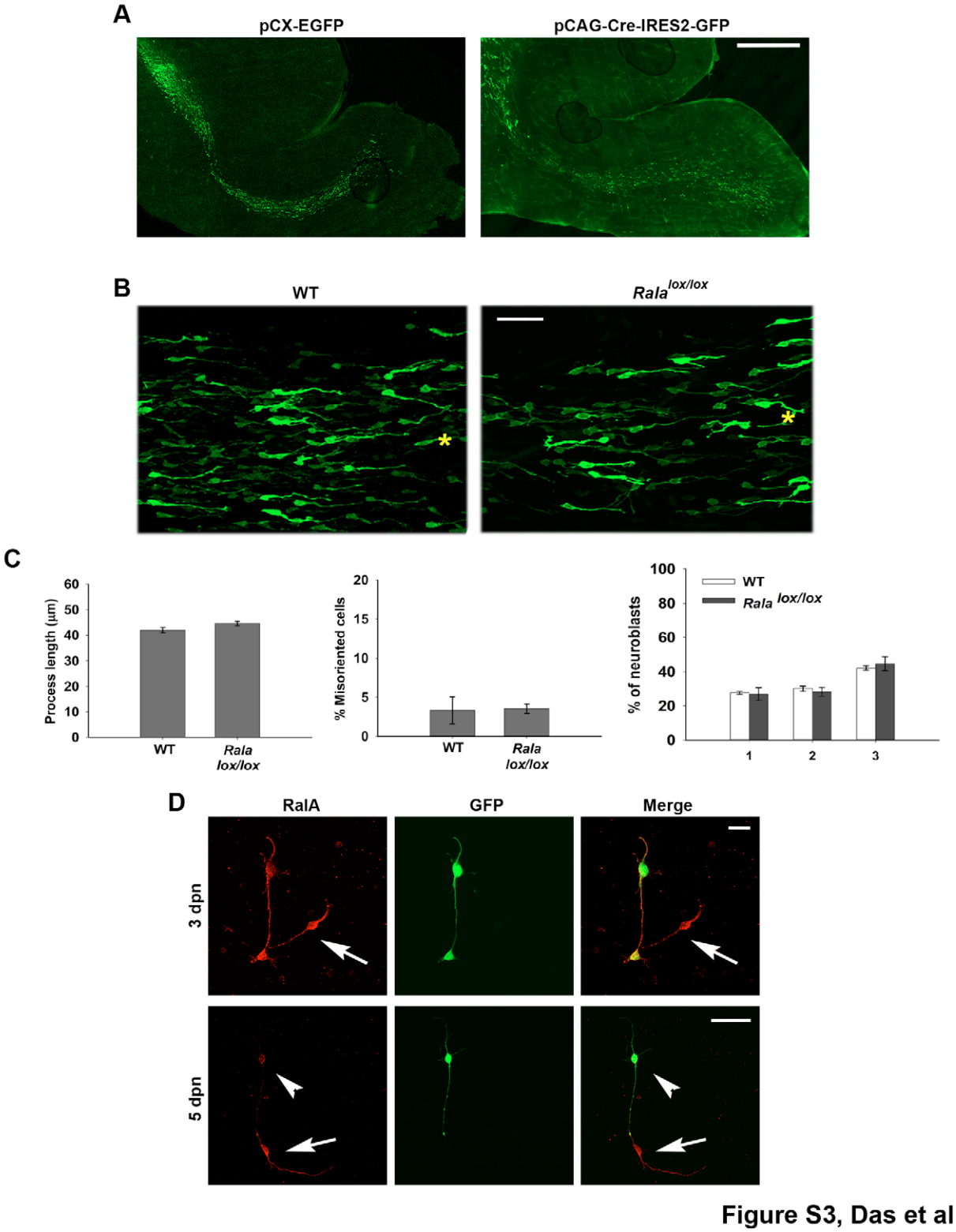

Fig. S3. Cre-induced genetic deletion of *Rala*. (A) Confocal projections of sagittal slices from wt mouse brains immunostained for GFP 5 d after *in vivo* electroporation of pCX-EGFP or pCAG-Cre-IRES2-EGFP. Cre expression *per se* does not affect neuroblast morphology and migration. Bar, 500 µm. (B) wt and *Rala*$^{lox/lox}$ mice were electroporated with pCX-EGFP. Representative confocal projections of fixed RMS sections showing GFP-labeled neuroblasts 5 d after electroporation. Yellow asterisks indicate relative position of the OB. (C) There was no significant difference in process length, orientation, and migration of neuroblasts between wt and *Rala*$^{lox/lox}$ mice. Mean ± SEM; n = 6 brains for wt and *Rala*$^{lox/lox}$. (D) Neuroblasts from P7 *Rala*$^{lox/lox}$ mouse pups were nucleofected with pCAG-Cre-IRES2-GFP, fixed 3 or 5 d later (3dpn/5dpn) and immunostained for RalA (red) and GFP (green). Downregulation of RalA expression is visible in GFP-positive cells only at 5 dpn (arrowhead), while GFP-negative cells retain strong RalA immunoreactivity (arrows). Bars, (3 dpn) 20 µm; (5 dpn) 50 µm.

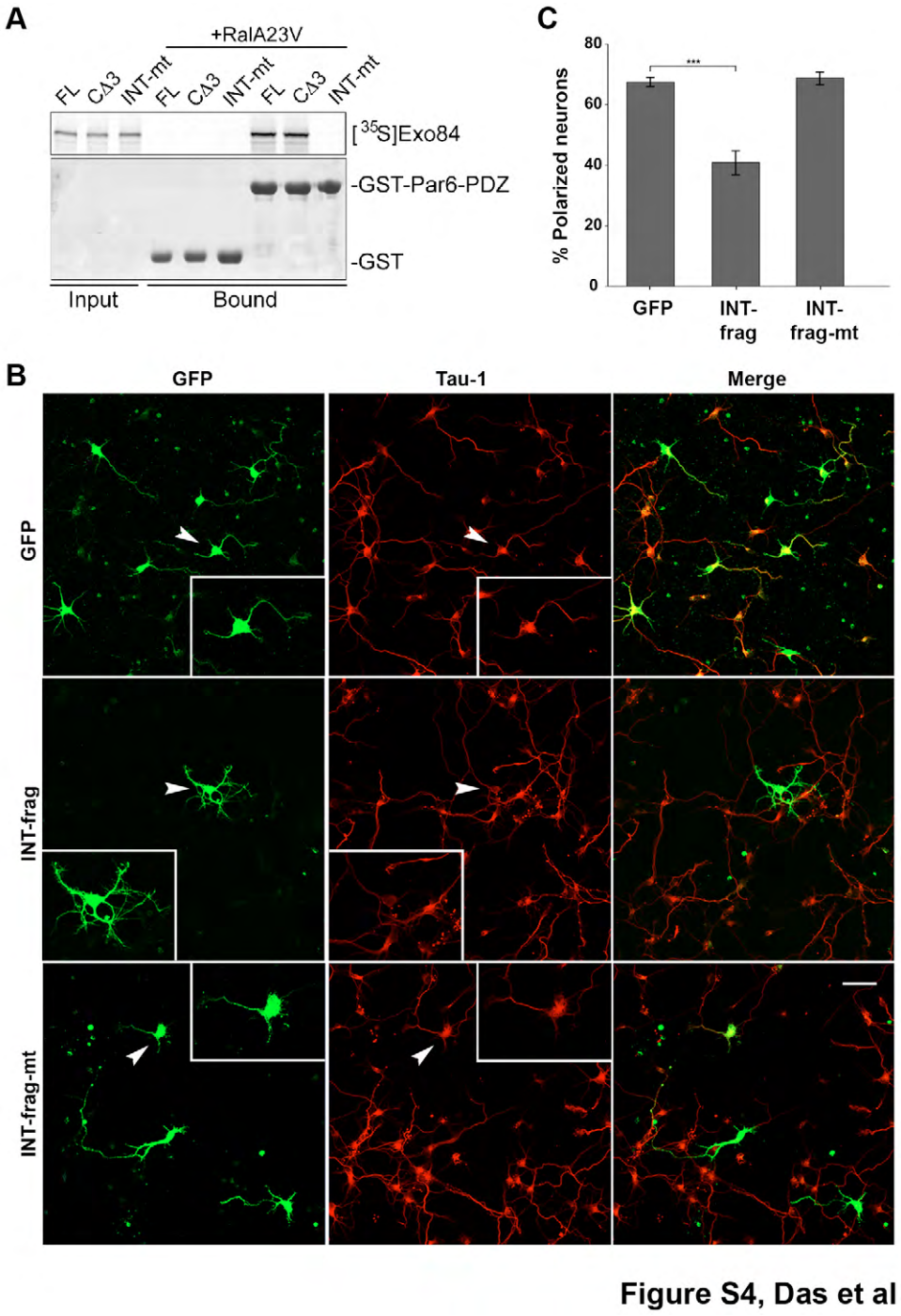

Fig. S4. Disrupting the Exo84-Par6 interaction affects neuronal polarization. (A) The internal PDZ-binding motif of Exo84 is required for RalA-promoted interaction between Exo84 and Par6. *In vitro* translated full length Exo84 (FL), Exo84 lacking the C-terminal PDZ binding domain (CΔ3) and Exo84 mutated in the internal PDZ-binding motif (INT-mt) were incubated with GST-Par6-PDZ in presence of RalA23V. Both Exo84FL and Exo84CΔ3 bound to GST-Par6-PDZ, whereas Exo84INT-mt was unable to interact with Par6-PDZ. The lower panel is a Coomassie blue-stained gel showing the amounts of GST-Par6-PDZ and GST (as control). (B) Embryonic rat cortical neurons were nucleofected with plasmids encoding GFP or GFP-tagged Exo84 fragments and cultured for 48 h before immunostaining for GFP and the axonal marker tau-1. Representative confocal images of neurons nucleofected with the indicated constructs. Most of GFP-labelled control neurons extend a tau-1-positive axon (top row). Expression of GFP-tagged Exo84 INT-frag interacting with Par6-PDZ impairs axonal specification, causing cells to extend minor neurites negative for tau-1 (middle row). Expression of Exo84 INT-frag-mt unable to bind Par6 does not affect neuronal polarization (bottom row). Arrowheads indicate neurons shown at higher magnification in the insets. Bar, 50 µm. (C) Quantitative analysis of neuronal polarization. Mean ± SEM; ***$p < 0.001$; n = 3 independent experiments.