SUPPLEMENTARY INFORMATION FOR MANUSCRIPT:

N-CADHERIN SPECIFIES FIRST ASYMMETRY IN DEVELOPING NEURONS

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N-CADHERIN DIRECTS NEURONAL POLARITY

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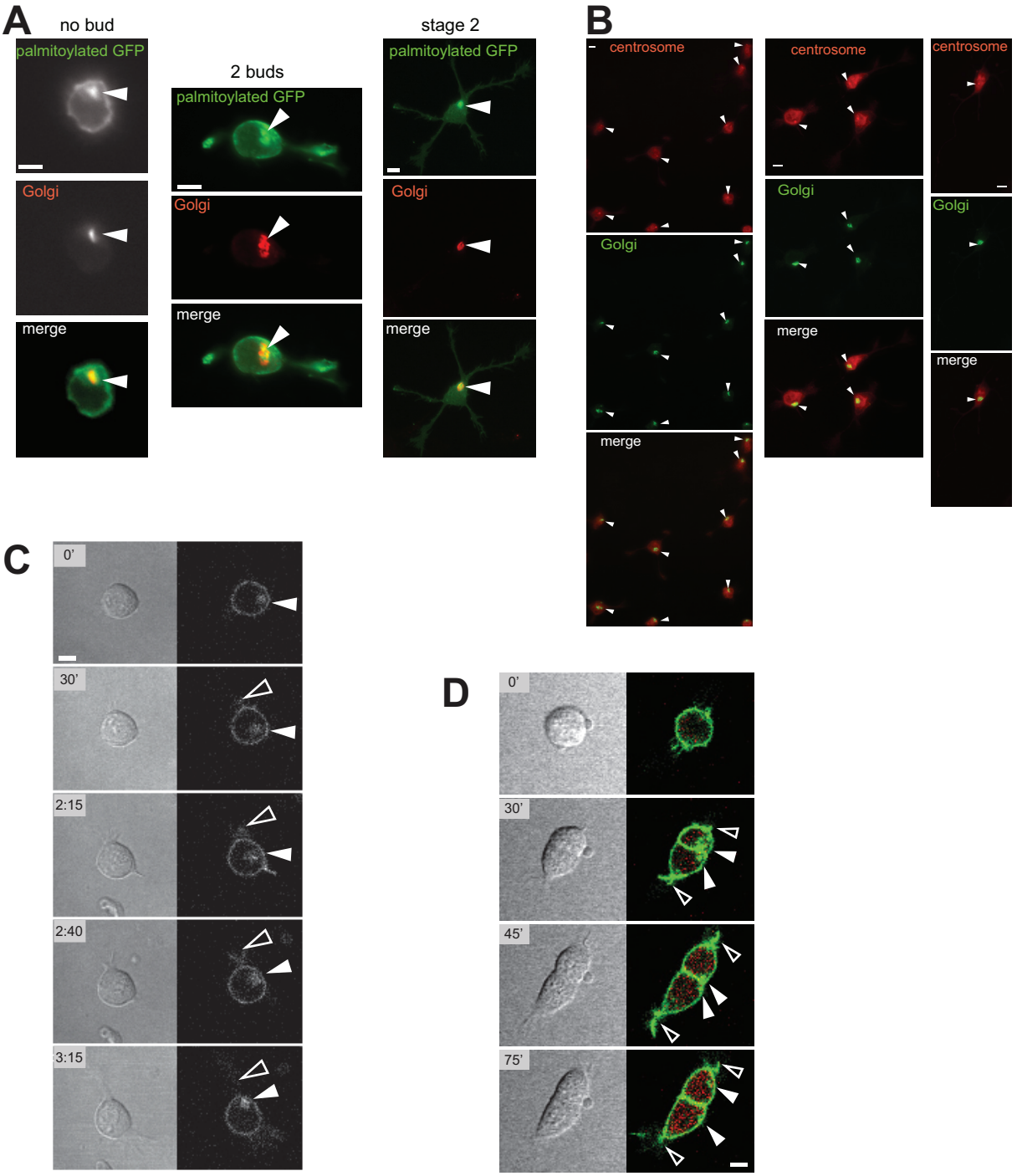
1 SUPPLEMENTARY DATA

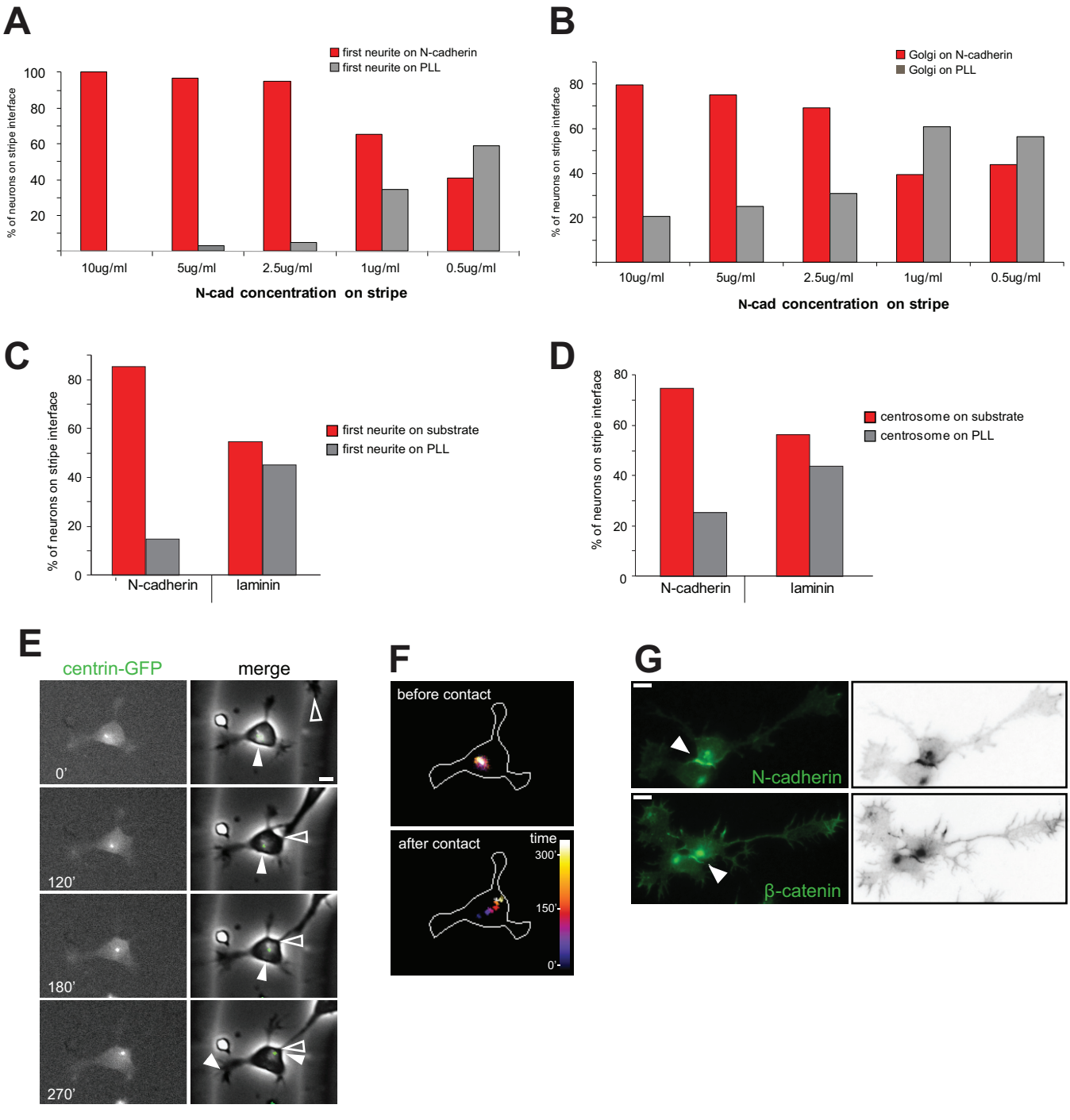
Supplementary Figure 1



Supplementary Figure 1: ***Golgi and centrosome follow the formation of the first sprout in polarizing neurons.***

A: Palmitoylated GFP labels efficiently the Golgi in all neurons we analysed during the early developmental stages (round, 1 bud, 2 bud and stage 2 and 3 neurons). The three panels depict example neurons expressing palmitoylated GFP at different developmental stages which clearly co-localises with the Golgi specific marker GM-130. Therefore we utilized this construct in order to visualize Golgi localization and - since palmitoylated GFP also binds to the cell membrane- also cell morphology in nascent polarizing neurons (Fig. 1B, Fig. S1C/D). **B:** At all developmental stages observed (round, 1 bud, 2 bud and stage 2/3 neurons) we could see in all neurons a clear overlap of the Golgi position (GPP130 antibody) and the centrosome (gamma Tubulin antibody). Several examples are shown in panel B. **C:** A second example of a polarizing hippocampal neuron (see Fig. 1A) in which the Golgi followed the first bud is shown. Neurons were transfected with a palmitoylated version of GFP (see Fig. S1A) visualized and were imaged at 5 min frames immediately after transgene expression and plating. **D:** A second example of Golgi movements (see Fig. 1B) in polarizing neurons immediately after cell division is shown. Mouse embryonic 12.5 cortical neurons were nucleofected with palmitoylated GFP and Cherry fluorescent protein expressed under the control of the neuron-specific tubulin alpha promoter. Cherry progressively accumulates after neurons have been generated. Confocal pictures were obtained at 15 min frames. C/D: First neurite (open arrowhead); Golgi position (filled arrowheads). Scale bar in all panels 5 μm.

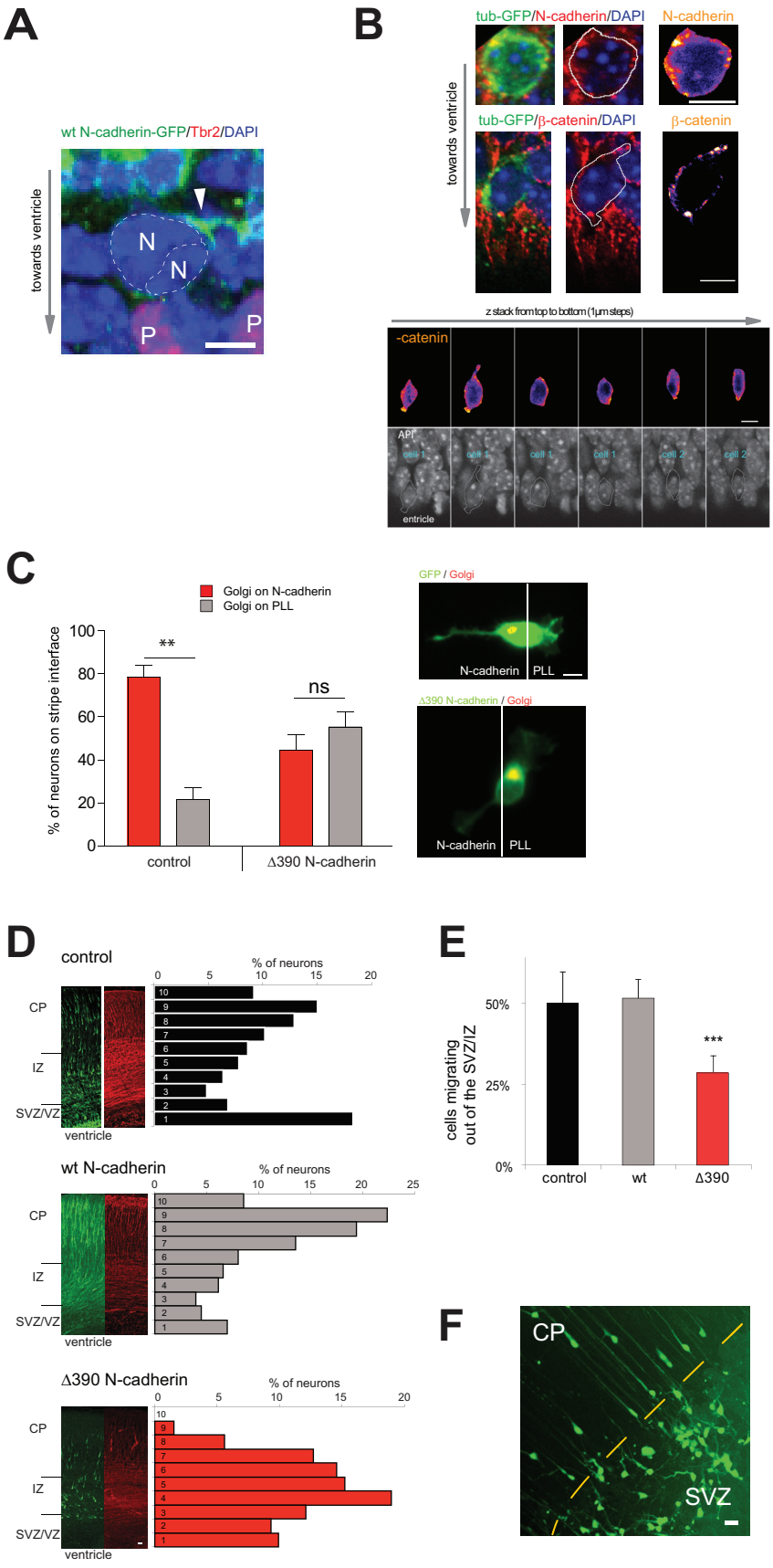
Supplementary Figure 2



Supplementary Figure 2: ***N-Cadherin recruits first sprout and centrosome/Golgi towards N-cadherin substrate and organelles to cell-cell contacts***

A/B: Hippocampal neurons growing on coverslips coated with alternating stripes of N-cadherin and PLL were immuno-labelled with a neuron-specific antibody (anti-βIII tubulin) and with an antibody recognizing the Golgi (anti-GM130). The effects of decreasing concentrations of N-cadherin were tested. **A:** The first sprout position and **B:** the Golgi position in neurons with the cell body in contact with both substrates was quantified (experimental n=3, >100 cells/experiment). Golgi and first sprout are reproducibly attracted by N-cadherin from a concentration of 2.5 mg/ml on. **C/D:** Cortical neurons growing on coverslips coated with alternating stripes of N-cadherin and PLL or Laminin and PLL were immuno-labelled with a neuron-specific antibody (anti-βIII tubulin) and with an antibody recognizing the centrosome (anti-gamma tubulin). The effects of the different substrates on the early polarity of cortical neurons (see same experiment for hippocampal neurons: Fig. 2 and supplementary Fig. 3) was tested. **C:** The first sprout position (N-cadherin: n= 20; Laminin: n=31) and **D:** the centrosome position in neurons with the cell body in contact with either N-cadherin or Laminin and PLL was quantified (N-cadherin: n= 115; Laminin=35). **E:** Hippocampal neurons transfected with an EGFP-tagged centrin1 translocate the centrosome (white arrowhead) towards the site of cell-cell contacts (open arrowhead). **F:** Pseudocolor time sequence representation of the centrosome recruitment shown in E. **G:** Hippocampal neurons were fixed after 20 h in culture and the localization of endogenous N-cadherin and β-catenin analyzed by immuno-fluorescence. In black and white the negative fluorescence image is shown. Both proteins are accumulated at cell-cell contact sites. Scale bar in all panels 5 μm.

Supplementary Figure 3



Supplementary Figure 3: ***Interference with endogenous asymmetrically localised N-cadherin prevents Golgi/centrosome recruitment and leads to migration defects vivo.***

A: A pair of neurons (N) just generated in the SVZ (note the adjacent Tbr2 positive basal precursors (P) show a crescent of N-cadherin GFP. **B:** Paraffin sections from Tubb3-mGFP mice, expressing mGFP in freshly generated neurons. Cells were labelled with N-cadherin or beta-catenin. The left picture shows the co-labelling of mGFP (new neuron), N-cadherin and DAPI (nucleus). The middle picture shows N-cadherin and DAPI with the outline of the GFP positive cell drawn and the right picture the intensity of N-cadherin only in this GFP positive selected neuron. The lower panel shows a pair of neurons in a z-scan. **C:** In order to interfere and reduce endogenous N-cadherin function, as demonstrated in Fig. 6, we used a mutant carrying a deletion in a large part of the extracellular domain of N-cadherin (Δ390). This mutant blocks N-cadherin function *in vitro*, since neurons failed to re-orient the centrosome towards N-cadherin stripes. Hippocampal neurons transfected with a control GFP construct or a dominant negative Δ390 N-cadherin-GFP were plated on coverslips coated with alternating stripes of N-cadherin and PLL, fixed and immuno-labelled with an antibody recognizing the Golgi (anti-GM130) and with a neuron specific antibody (βIII tubulin). The recruitment of the Golgi N-cadherinΔ390 expressing cells is strongly reduced in comparison to neurons transfected with the wt control (experimental n=3, >20 cells/experiment). **D:** *In utero* electroporation of the lateral ventricles of E14.5 mice with GFP control vectors, wt N-cadherin-GFP or dominant negative Δ390N-cadherin-GFP. The fate of transfected cells was followed after fixation at different time points. Δ390N-cadherin-GFP expressing neurons migrate less far in the cortical plate (in utero electroporation E14.5-E17.5; experimental n=5) with respect to neurons transfected with a GFP (experimental n=7) or wild type N-Cadherin-GFP (n=6) expressing vector (Scale bar 100 μm). **E:** Neurons transfected and developing *in utero* as in D. The percentage of neurons exiting the SVZ is displayed and is significantly reduced when neurons express Δ390N-cadherin-GFP (mean ± SEM; n>450 neurons per treatment from >3 mice, ANOVA followed by Tukey's post-test, *$p<0.05$, **$p<0.05$ Δ390N-cadherin-GFP vs. control and vs. wt N-cadherin-GFP). **F:** Cortical E14 neurons were grown on top of E14 vibratome sections. Neurons align according to the slice architecture: in a radial direction in the cortical plate region (CP) and randomly in the subventricular zone region (SVZ). Scale bars 20 μm (D/F); others 5μm.