Supplemental Information

The Fz-Dsh Planar Cell Polarity Pathway

Induces Oriented Cell Division via Mud/NuMA

in Drosophila and Zebrafish

Marion Ségalen, Christopher A. Johnston, Charlotte A. Martin, Julien G. Dumortier, Kenneth E. Prehoda, Nicolas B. David, Chris Q. Doe, and Yohanns Bellaïche



Figure S1. The orientation of the polarity is not affected in *mud* mutant cells

A: Cumulative plot of the anterior-posterior orientation of the Pins crescent in wild-type (n=26) and *mud* mutant pI cells (n=30).

B: Cumulative plot of the anterior-posterior orientation of the Numb crescent in wild-type (n=32) and *mud* mutant pI cells (n=46).

C: Cumulative plot of the anterior-posterior orientation of the Pon::GFP crescent in wild-type (n=17) and *mud* mutant pI cells (n=15).

D-**I**: Orientation of the mitotic spindle relative to the Pins crescent. The angle α is defined by the vector perpendicular to the middle of Pins crescent (green) and the spindle axis defined by the position of the two centrosomes (red) (**D**). Mean and standard deviation of the α angle in wild-type pI cells (n=25), *mud* (n=25), *dsh*¹ (n=25) and double *mud*, *dsh*¹ (n=25) mutant pI cells; (**E**). Pins crescent (green in **F**-**I**), Senseless (blue in **F**-**I**), centrosomes (labeled by γ -tubulin, red in **F**-**I**) and DNA (labeled by DAPI counterstaining, white in **F**-**I**) in wild type (**F**), *mud* (H), *dsh*¹ (**G**, asterisk indicate the centrosome of a neighboring dividing cell) and *mud*, *dsh*¹ (**I**). Note that in *dsh*¹ mutant pI cells, the mitotic spindle is not as well as oriented as in the wild-type pI cells and that Pon::GFP segregates in both daughter cells in 10% of the *dsh*¹ mutant pI cells (not shown, see also Bellaïche et al., 2001).



Figure S2. NuMA is expressed during zebrafish gastrulation.

A-F: Expression of NuMA. *In situ* hybridization for the NuMA sense probe (**A-C**) or anti-sense probe (**D-F**) at the sphere (**A**, **D**), shield (**B**, **E**) and 75% epiboly (**C**, **F**) stages. Animal pole to the top, (**C**, **F**) dorsal to the right.

G-H'': The efficiency of the NuMA MO was controlled by showing that the expression of a GFP gene harboring the NuMA MO sequence was abrogated by NuMA MO injection (**G-G**'') but not

by the NuMA control MO (**H**-**H**'') in zebrafish embryo injected at the 1 cell stage. GFP channel (**G** and **H**), bright field image (**G**' and **H**') and merge (**G**'' and **H**'').

I-J'': Dividing epiblast cells in control (**I-I''**) and NuMA MO injected embryos (**J-J''**) stained for α -tubulin (**I**, **I''**, **J** and **J''**, green), γ -tubulin (**I'**, **I''**, **J'** and **J''**, red) and DNA (Hoechst, **I''** and **J''**, blue).

SUPPLEMENTAL REFERENCE

Bellaïche, Y., Gho, M., Kaltschmidt, J.A., Brand, A.H., and Schweisguth, F. (2001). Frizzled regulates localization of cell-fate determinants and mitotic spindle rotation during asymmetric cell division. Nat Cell Biol *3*, 50-57.