Supplemental Figures.

Figure S1. PACSIN2 knock down does not perturb gastrulation

(A) Morpholino blocks PACSIN2 translation. Embryos were injected with 1ng of transcript encoding a myc-tagged (MT) version of PACSIN2 alone (-) or together with 80 ng of either control morpholino (MO Ctl) or morpholino against PACSIN2 (MO 2). At stage 20, protein were extracted and analyzed by western blot with the anti-myc mAb 9E10. Only the PACSIN2 morpholino successfully prevents the translation of PACSIN2-MT. (B-C) The morpholino against PACSIN2 and 2' have a modest effect on the endogenous protein levels. (B) Embryos were injected in both blastomeres at the 2-cell stage with 80 ng of control MO (MO Ctl) or a mix of antisense morpholino against PACSIN2 and the pseudo allele PACSIN2' (40 ng each, MO 2+2'). The equivalent of half an embryo was analyzed by western blot at early gastrula (st.10.5) and tail bud (st.21) stages with the PACSIN2 mAb 3D8. All 3 species detected by PACSIN2 mAb were visible even with the injection of PACSIN2 and 2' morpholino. (C) The protein extract of embryos at stage 2, 10 and 22 and embryos injected with the antisense morpholino against PACSIN2 and 2' at stage 22 were analyzed by two dimensional gel electrophoresis followed by western blot using the PACSIN2 mAb. The orientation of the isoelectrofocalization is indicated (-; +). A quantification of the basic (B) and acidic (A) species of PACSIN recognized by the mAb 3D8 was performed using the Histograms of Photoshop software. The embryos injected with the morpholino display a reduction of the acidic species of PACSIN while the basic remains the same. Together, these results indicate that the PACSIN2 antibody recognize several species of PACSIN that can't be

knocked down by the morpholino used. (D) PACSIN2 and 2' knock down has no effect on gastrulation. Embryos injected in both blastomeres with the control morpholino or the mix of morpholino against PACSIN2 and 2'. At gastrula stage, each embryo was scored for its exact stage according to the blastopore sizes (Nieuwkoop and Faber). The picture shows a representative embryo of control and morphant at gastrula stage. The histogram represents the distribution of embryos at different gastrula stage of a representative experiment. The number of embryos analyzed was as follow: MO Ctl= 126; MO 2+2'=104. PACSIN 2 and 2' knock down does not significantly affect gastrulation. Arrowhead: blastopore lip.

Figure S2. PACSIN2 knock down delay neurulation

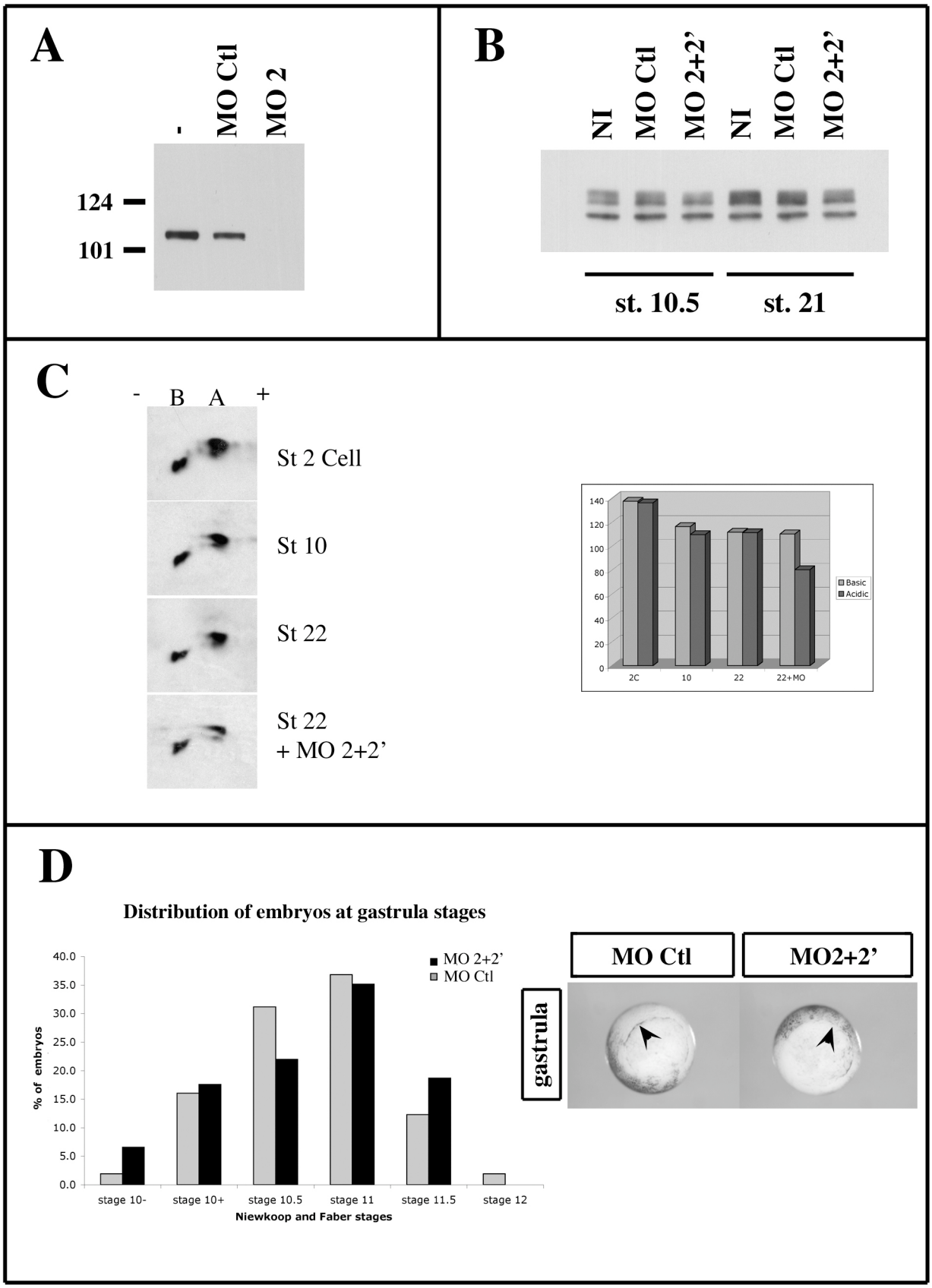
(A) The PACSIN2 double morphant delay neurulation. Embryos were injected at the 2-cell stage in both blastomeres with 80 ng of either the control morpholino (MO Ctl) or the mix of AS morpholino against PACSIN2 and 2' (MO2+2'). The embryonic development was carefully monitored until tail bud stage and a picture of representative embryo was taken at neurula (dorsal view, anterior up) and tailbud stage (lateral view, anterior left and dorsal up). No differences were noted during gastrulation between embryos injected with MO Ctl and MO2+2'. When control embryos reached mid-neurula stage, each embryo was scored for its exact stage according to the Nieuwkoop and Faber Table. The histogram represents the distribution of embryos at different neurula stage of a representative experiment. The number of embryos analyzed was as follow: MO Ctl= 39; MO 2+2'=36. During neurulation, MO2+2' injected embryos displayed a delay in neurulation. However, embryos eventually closed their neural tube and appeared to

continue developing normally. (B) The PACSIN2 morpholino does not prevent neural induction. Dorsal view of embryos treated by whole mount hybridization at stage 18 and 22 with the neural marker N-tubulin. Anterior is up. When control embryo reached stage 18, embryos injected with MO2+2' appeared to be at a younger stage. The N-tubulin pattern indicates that neural tissue is present. At stage 22, embryos injected with MO2+2' have close the neural tube and express N-tubulin in the two dorsal rows of neurons in the neural tubes like the controls (white arrows). Unlike control embryos, groups of N-tubulin positive cells are seen outside of the neural tube in the morphant embryos (arrowheads), indicating that those neurons failed to move dorsally. This suggests that PACSIN2 could be involved in cell movement during neurulation.

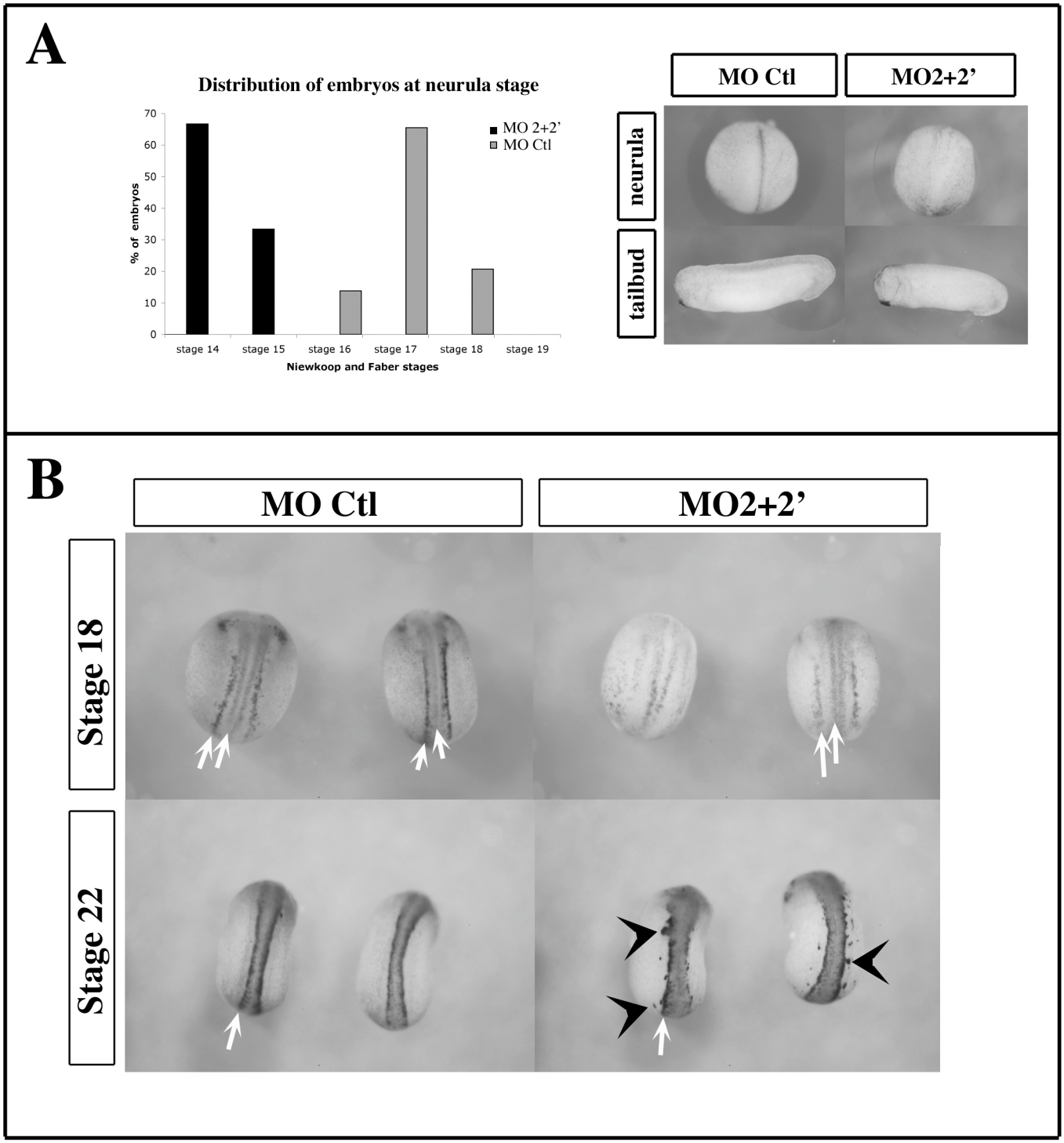
Figure S3. PACSIN2 does not bind α5β1.

(A) Immunoprecipitations of protein extract of 10 gastrulas was performed using mAb against PACSIN2 (3D8), and α5β1 (P8D4). The precipitates were analyzed by western blot using biotinylated mAb against PACSIN2 (3D8biot, left panel). The blots were stripped in 200 mM glycin pH 2.8 and re-blotted with a rabbit polyclonal antibody against α5 integrin (881, right panel) (Joos et al. 1995). The P8D4 antibody successfully precipitated the α5β1 integrin but failed to co-immunoprecipate PACSIN2. Note that the PACSIN2 signal was incompletely stripped and therefore appear in the integrin β1 blot. (B) Pull Down experiment was performed using protein extract of 10 gastrula and 1μg of GST alone or GST fusion proteins of integrin α5 or β1 tail. The proteins bound were analyzed by western blot using the PACSIN2 mAb (3D8). The protein extract of 1 embryo equivalent was loaded as a positive control (extract). A ponceau staining of the

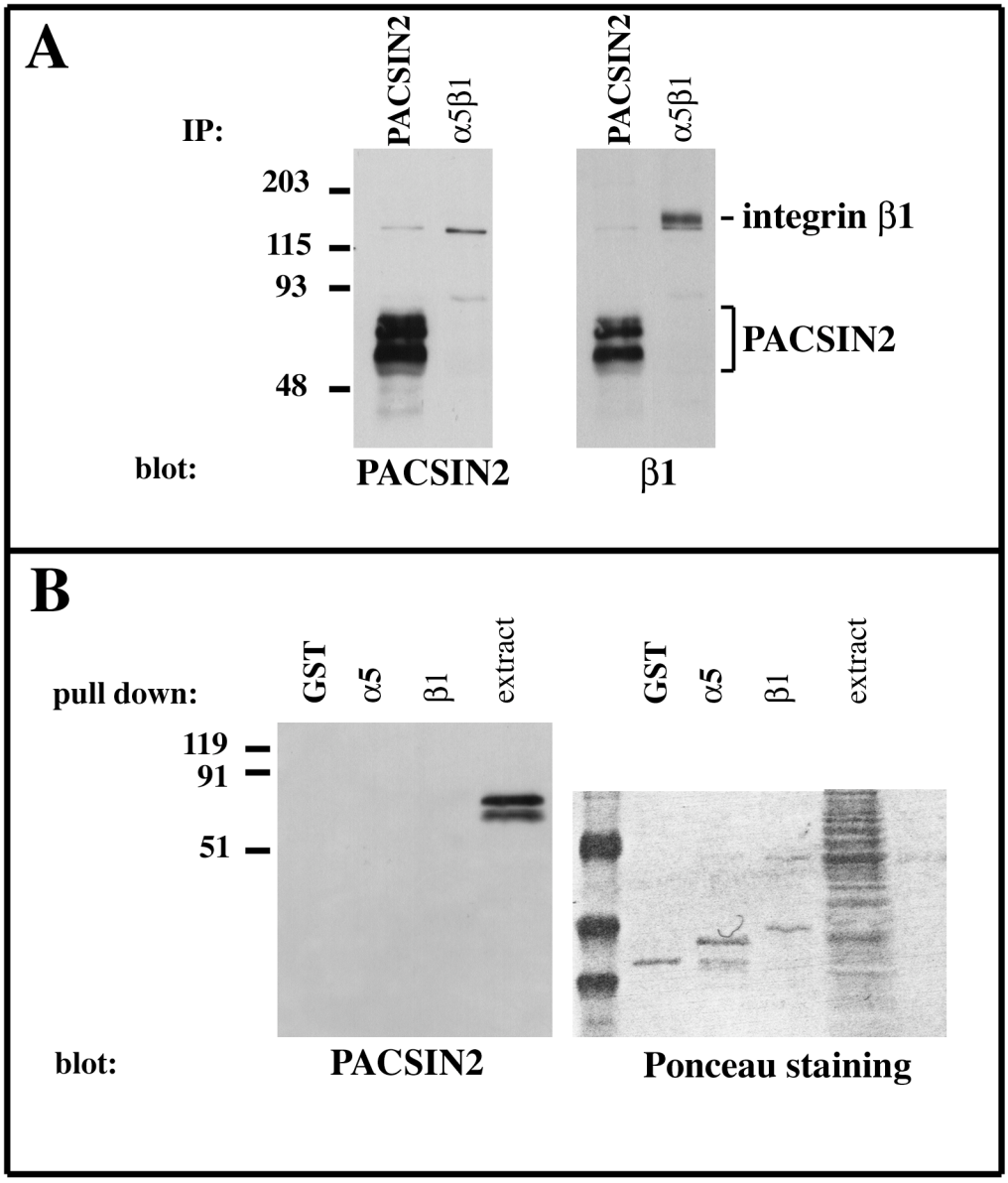
membrane is shown on the right. The fusion protein corresponding to the α5 or β1 cytoplasmic tail of integrin does not precipitate PACSIN2.



Cousin et al Figure S1



Cousin et al Figure S2



Cousin et al. Figure S3