Supplemental Figure 1: Design and function of the Splice Blocking (SB) FAK MO.

A. Two SB FAK MOs (materials and methods) were designed to inhibit splicing at the exon 8 - intron junction and the intron - exon 9 junction of the primary FAK mRNA transcript.

B. Primers for sqRT-PCR analysis were designed to detect a splice product (300bp), spanning the exon 8 and exon 9 regions.

C. The SB *FAK* MO inhibited *FAK* mRNA splicing. Injection of 18 ng of SB *FAK* MO significantly decreased levels of the 300 bp product (lane 4) compared to control embryos (lane 2) or embryos injected with the translational blocking *FAK* MO (60 ng, lane 3). *EF1* α , was used as a positive control for RNA loading level in each sample. Total RNA from was isolated from pools of five embryos for sqRT-PCR analysis.

Supplemental Figure 1



Normal product



Supplemental Figure 2: Comparisons of the *FAK* mm-MO and *Wnt3a* MO phenotypes to FAK morphant embryos.

A. Control neurula stage had a normal phenotype in 96% (n=102) of the embryos.

B. Embryos were injected with FAK MO (35-50 ng) at the one-cell stage. FAK MO injected embryos are anteriorized, having a shortened A-P axis and open neural folds in 90% (n=209) of the embryos.

C. The *FAK* mm-MO (35-50 ng) injected embryos had the normal phenotype as in (A) in 90% (n=284) of the embryos.

D. Control neurula stage embryo.

E. *Wnt3a* MO (30 ng) embryos were injected animally at the one-cell stage. The Wnt3a morphant phenotype is similar to (B), having a shortened A-P axis and open neural folds in 98% (n=90) of the embryos.

F. Posterior neural marker expression is unaltered in *FAK* mm-MO injected embryos. Embryos were injected at the one-cell stage with *FAK* MO (35ng) and the *FAK* mm-MO (35ng). As determined by sqRT-PCR, posterior neural marker expression (*N-tubulin, Krox20, HoxB9*) was unaltered by the *FAK* mm-MO in comparison to the *FAK* MO. Expression levels of the *FoxD3* neural crest marker is unaltered by either MO injection.

G. Embryos were injected at the one-cell stage with *FAK* MO (35ng) and the *FAK* mm-MO (35ng). As determined by Western analysis, at early neurula stages, the *FAK* MO strongly reduces endogenous FAK protein levels, but the *FAK* mm-MO levels are similar to the uninjected control embryos (compare lanes 1-3). Loading per sample is determined by α -tubulin protein

Supplemental Figure 2A-E





Supplemental Figure 2F-G

