



Figure 1 – Figure supplement 4. Phenotypic classes of *MZgdf3* rescued with *gdf3* RNA.

(A) Class I -27 hpf un-injected *MZgdf3* mutant. Embryos have greatly reduced anterior neural tube, a cyclopic eye, no notochord and poorly formed somites. (B) Class II –‘minimal’ rescue of *MZgdf3* with *gdf3* RNA. Embryos show rescue of posterior notochord and somites but no change in anterior neural tube or eye compared to un-injected mutants (C) Class III –‘partial’ rescue of *MZgdf3* with *gdf3* RNA. In addition to rescue of posterior morphology embryos show rescue of anterior neural tube including complex brain folds such as the midbrain-hindbrain junction but the eyes remain fused or incompletely separated. (D) Class IV –‘complete’ rescue of *MZgdf3*. Embryos have morphologically normal rostral structures including fully separated eyes.

The above classification scale was used to determine the optimal dose of *gdf3* used in the rescue experiments presented in this report. Injecting *MZgdf3* embryos with 25 pg of *gdf3* RNA resulted in 0% Class I, 39% Class II, 36% Class II and 25% Class IV embryos (n=56). Injecting *MZgdf3* embryos with 50 pg of *gdf3* RNA resulted in 0% Class I, 11% Class II, 39% Class II and 50% Class IV embryos (n=54). Injecting *MZgdf3* embryos with 100 pg of *gdf3* RNA resulted in 0% Class I, 0% Class II, 9% Class II and 91% Class IV embryos (n=68). Doses of 200 pg gave rescue percentages similar to 100 pg (0%, 0%, 3%, 88%, Class I-IV respectively), but 9% of injected embryos (n=65) showed tissue necrosis associated with anterior neural structures suggesting toxicity due to too much injected RNA. A dose of 100 pg was chosen for the reported rescue experiments.