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SUPPLEMENTARY MATERIAL

corresponding to:

Advanced microinjection protocol for gene manipulation using the model newt *Pleurodeles waltl*

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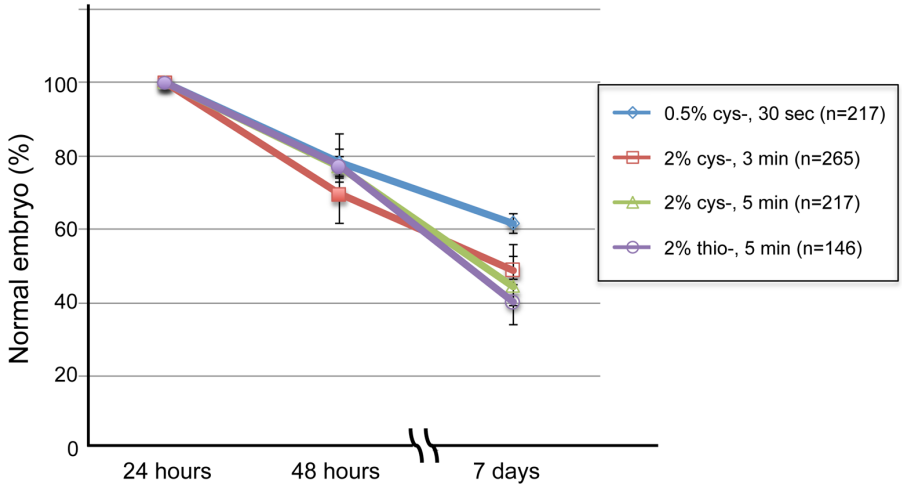
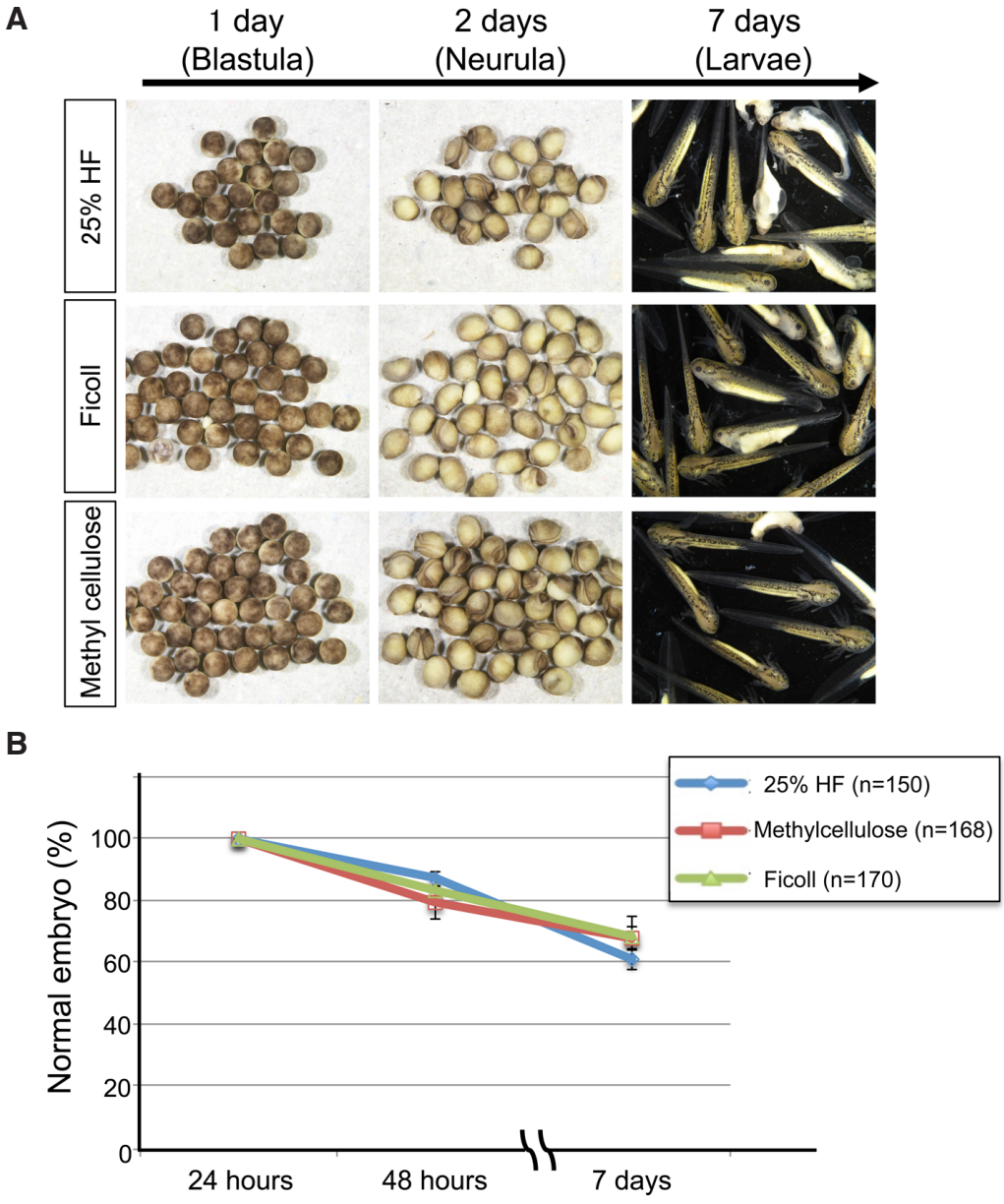
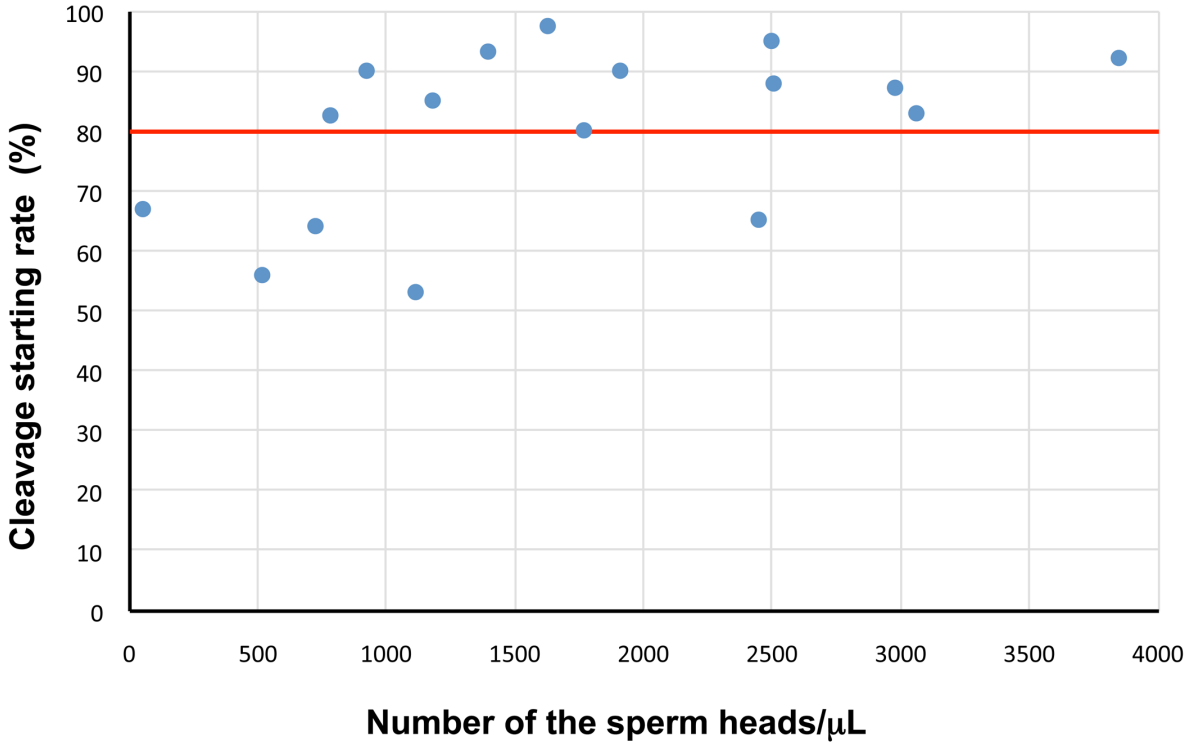


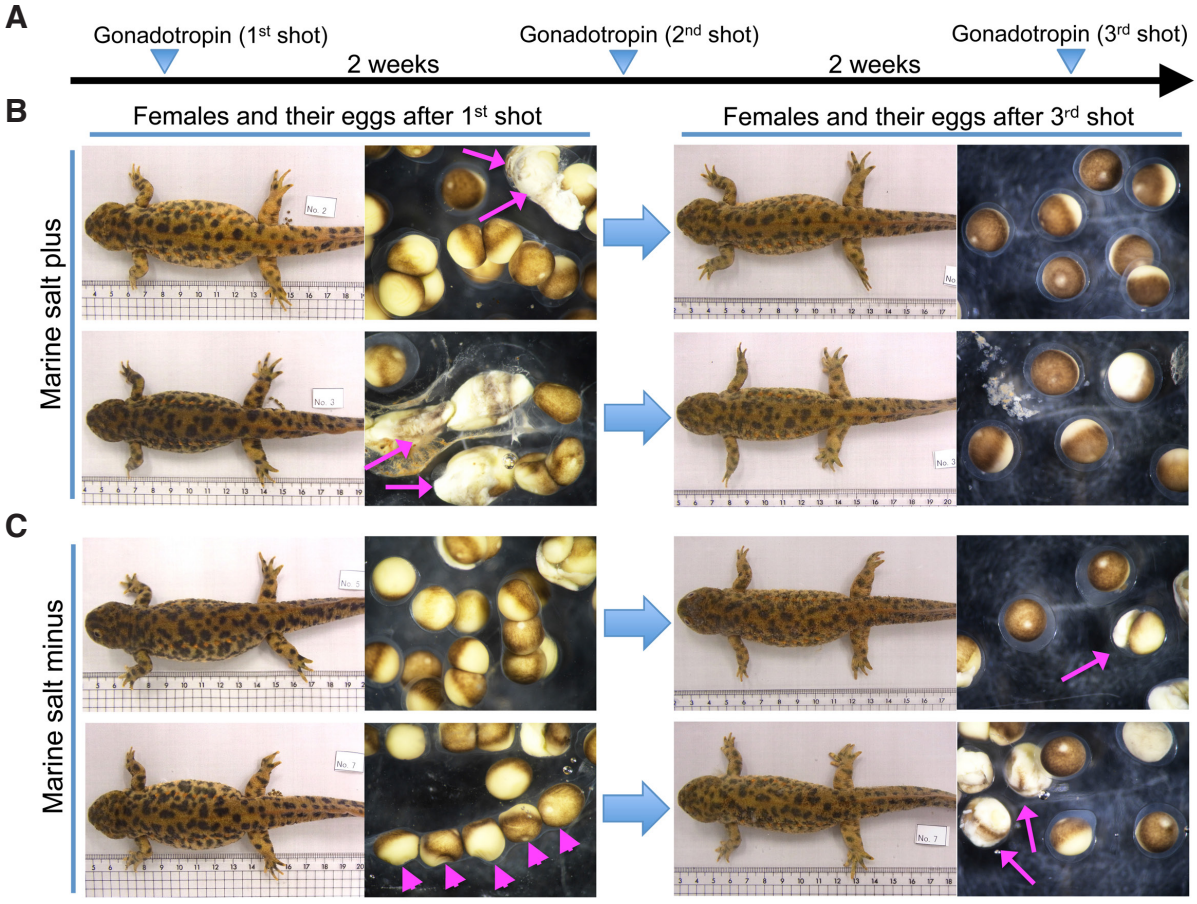
Suppl. Fig 1. Comparison of cysteine and sodium thioglycolate for de-jelly treatment. *The fertilized eggs were treated with 0.5% or 2% cysteine or 2% Sodium Thioglycolate for 30 sec, 3 min, or 10 min. The eggs were then sorted and incubated in the injection medium up to 24 hours (blastula stage), and then the embryos were kept in 25% HF for 7 days (hatching stage). The graph shows the rates of normal developing embryos up to 7 days post fertilization. Three independent experiments were conducted. Error bars indicate standard deviations. "n" means total numbers of fertilized eggs examined.*



Suppl. Fig. 2. Comparison of rate of normal development with Ficoll based- or methylcellulose based-injection media. (A) *The de-shelled fertilized eggs were incubated in the injection dish filled with 25% HF, 6% Ficoll-based or 0.75% methylcellulose-based injection media until 24 hours post fertilization (Blastula stage). Next, the blastula embryos were kept in 25% HF up to 7 days (hatching stage). The embryos were photographed after 2 days and 7 days. Dead embryos were removed.* **(B)** *The rates of normal developing embryos were graphed. Three independent experiments were conducted. Error bars indicate standard deviations. "n" means total numbers of fertilized eggs examined. There was no significant difference in the rate of normal developing embryos between 25% HF, 6% Ficoll or methylcellulose. Since 0.75% methylcellulose has higher viscosity than 6% Ficoll, it is easy to handle for eggshell removal as well as the microinjection. Furthermore, the cost of producing the injection medium is less than 1/10 that of Ficoll-based injection medium. Therefore, we concluded that methylcellulose-based injection medium is better than Ficoll-based medium.*



Suppl. Fig. 3. Relationship between sperm density and fertilization rate. *The fertilization rate was judged according to whether or not cleavage started 16-20 hours after insemination. In this period, embryos that are normally developing were in the blastocyst stage. Each dot indicates a value for each independent experiment. Sperm density was estimated by the method described below. Five microliters were taken from the sperm suspension and a smear sample was prepared using 18 mm square cover glass, and then photographed. Density of sperm per mL of the sperm suspension was calculated based on the sum of sperm heads taken by the photographs.*



Suppl. Fig. 4. Pretreatment for obtaining good quality eggs. (A) *Summary of gonadotropin injection schedule. Fat females or no spawning experience females (left column in* **(B,C)***) lay low quality eggs. Typical signatures of low quality are ribbon-shaped eggs (arrowheads) and crushed eggs (arrow). In ribbon-shaped eggs, since many eggs are packed in a single elongated eggshell, the fertilization rate is extremely low. After repeating the injection of the gonadotropin several times (right column), the female would lay high quality eggs. Each egg was packed in an independent eggshell. At that moment, the female might become slim in shape. The fragility of the eggs depends on water quality. When the rearing water is supplemented with marine salt, the newt eggs become stronger and do not break as easily* **(B)** *compared to the non-marine salt group* **(C)**.