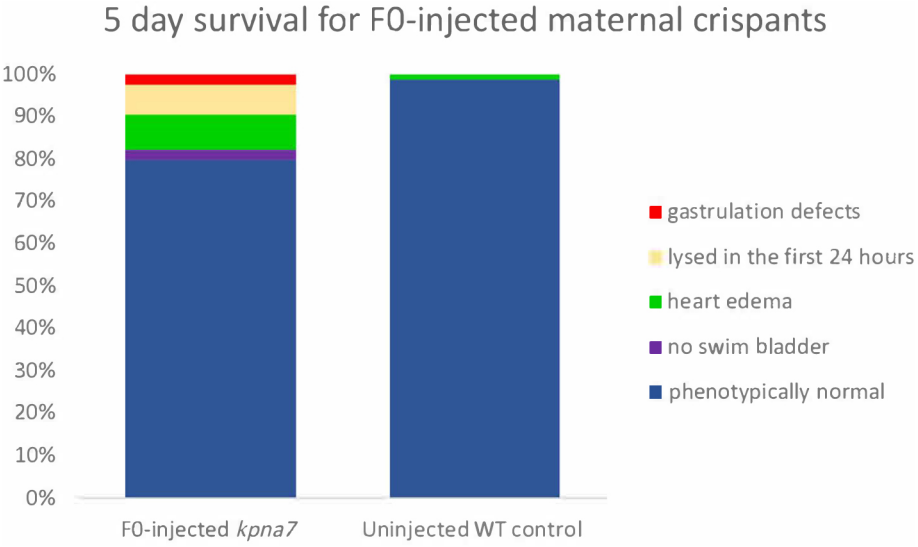


Fig. S1. Injection of multiple guide RNAs into one-cell embryos has a minimal effect on viability compared to uninjected controls, as scored by the presence of a swim bladder at five days.

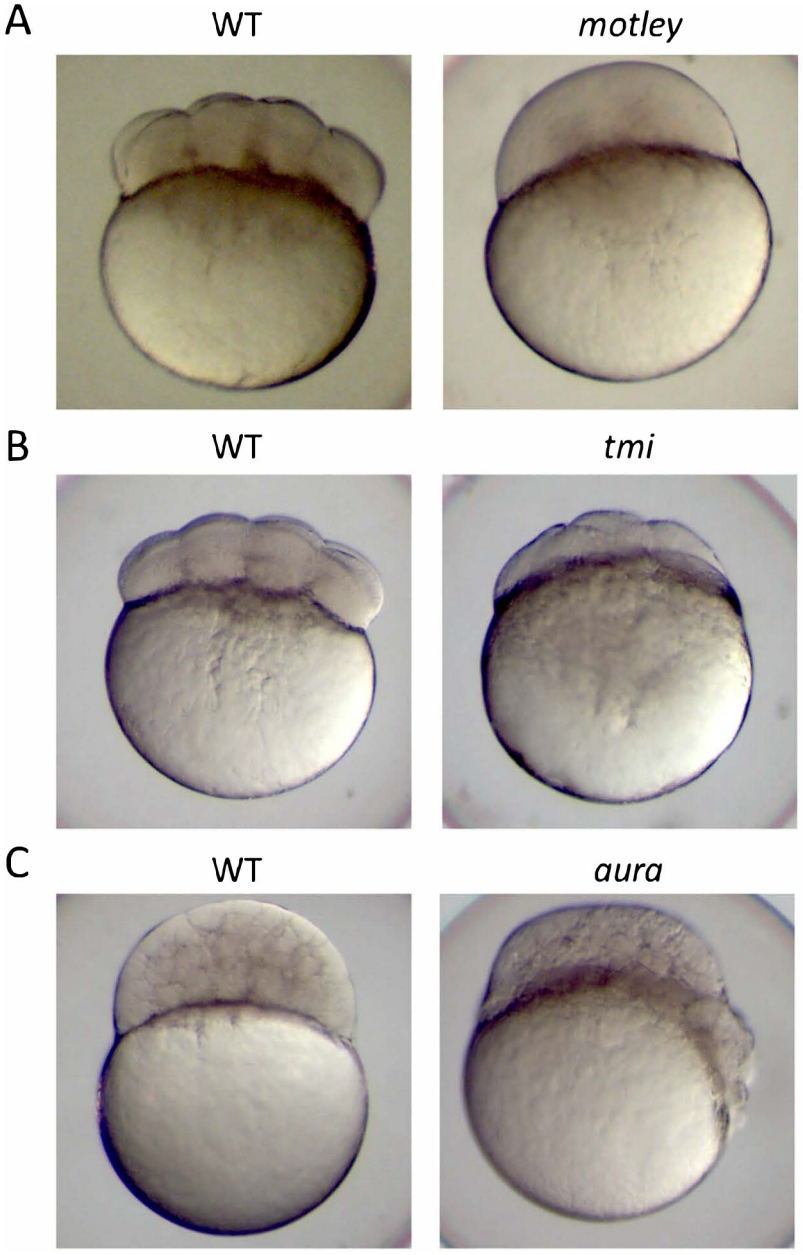


Fig. S2. Representative images of known maternal-effect mutants: *motley* (A), *too much information* (*tmi*) (B), and *aura* (C).

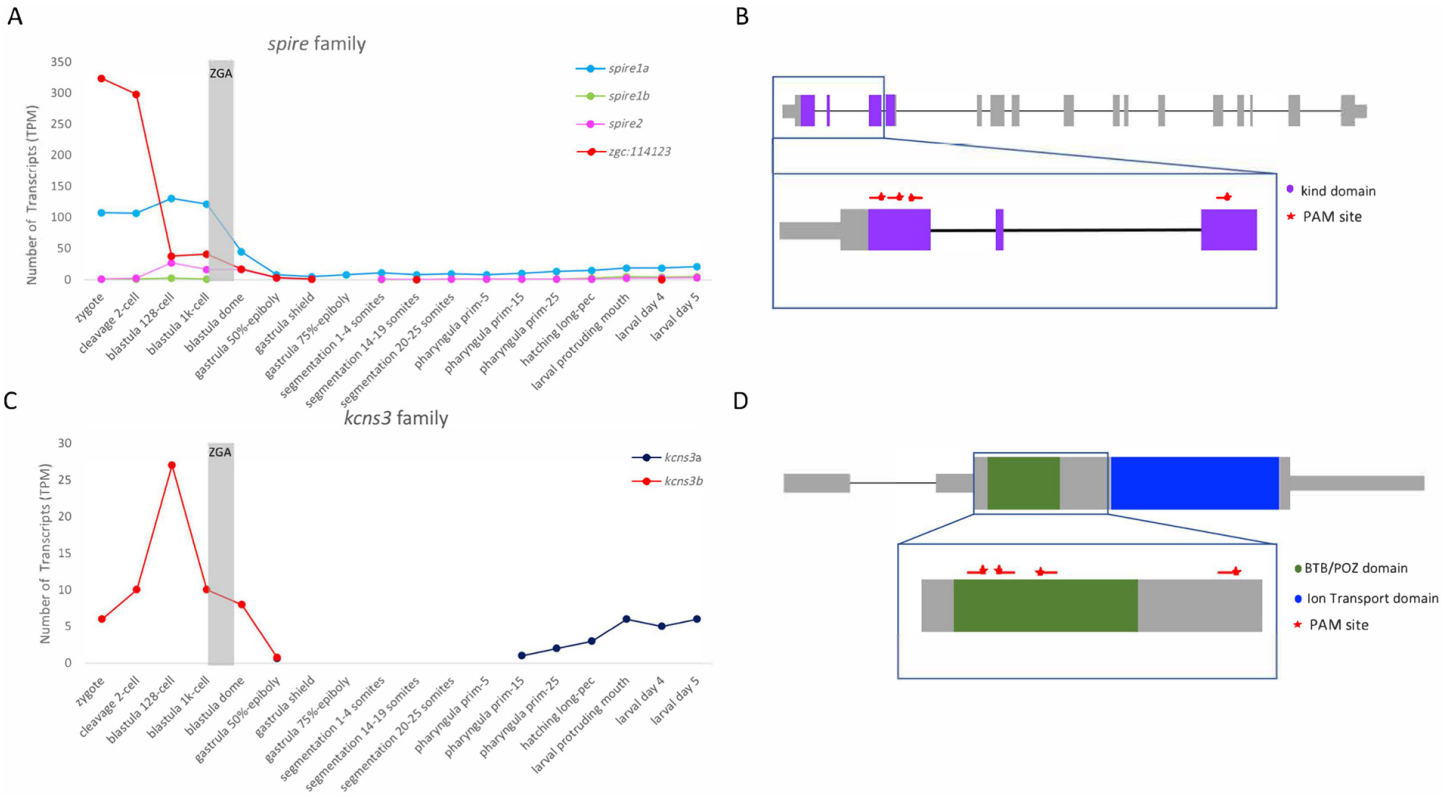


Fig. S3. mRNA expression levels and gene structures for candidate maternal-effect genes, *zgc:114123* and *kcns3b*, did not display a phenotype in the maternal crispants. A, C) Expression pattern for the *spire* (A) and *kcns3*(C) families during early development, from zygote to larval day 5. Expression of maternal-specific transcripts for each family is represented in red, and the gray bar marks zygotic genome activation. (B, D) Gene structure diagrams of *zgc:114123* (B) and *kcns3b* (D), with guide RNA target sites (red lines) and PAM sites (red stars).

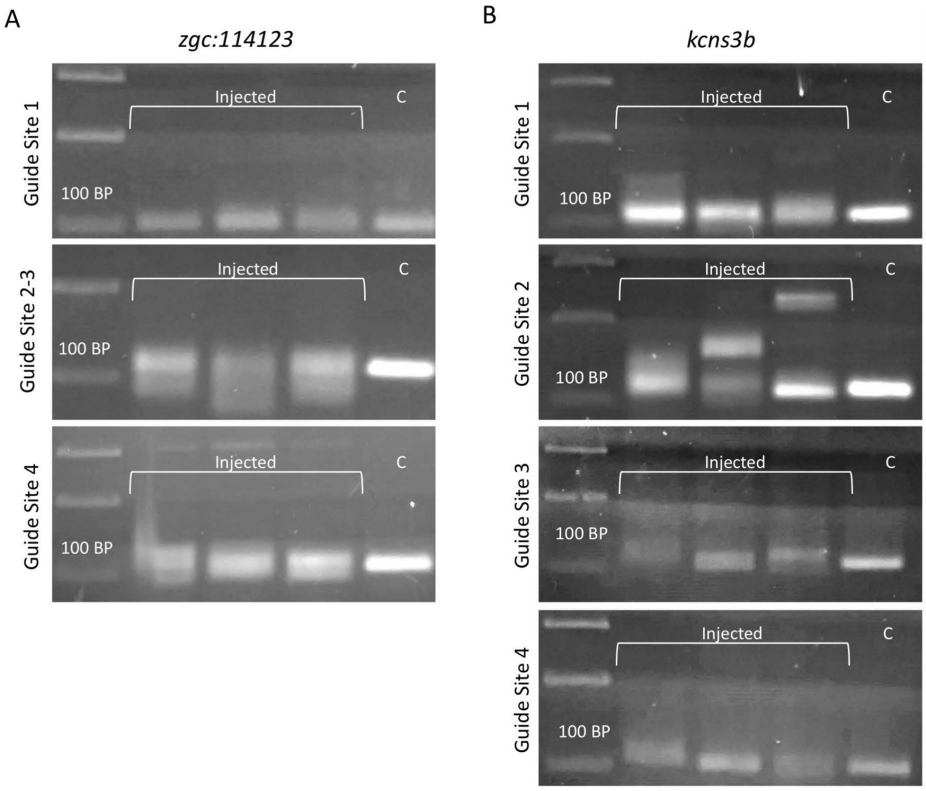


Fig. S4. INDELS found in the somatic tissue of F0-injected embryos at 24 hpf for *zgc:114123*(A) and *kcns3b* (B).

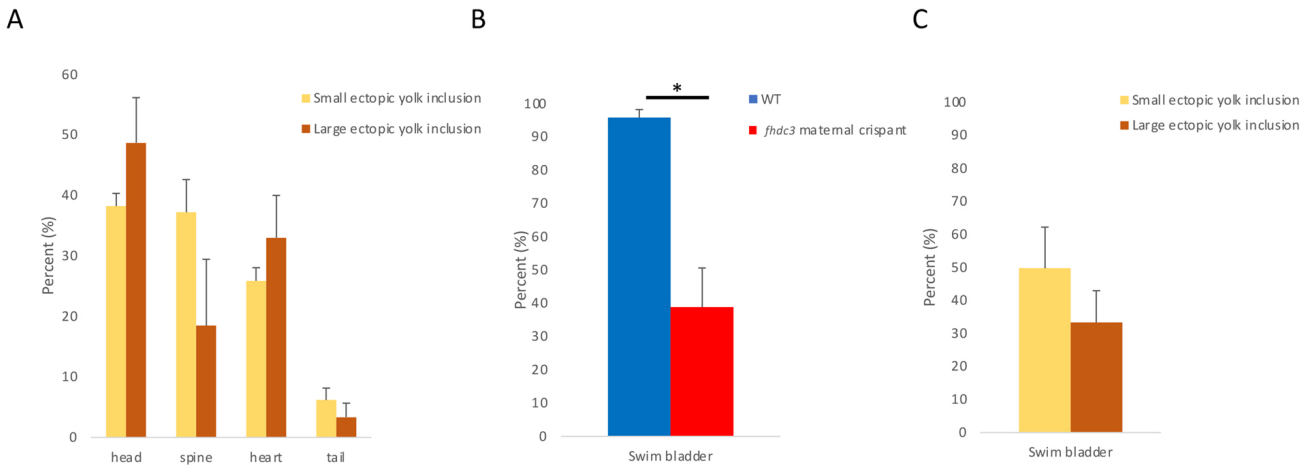
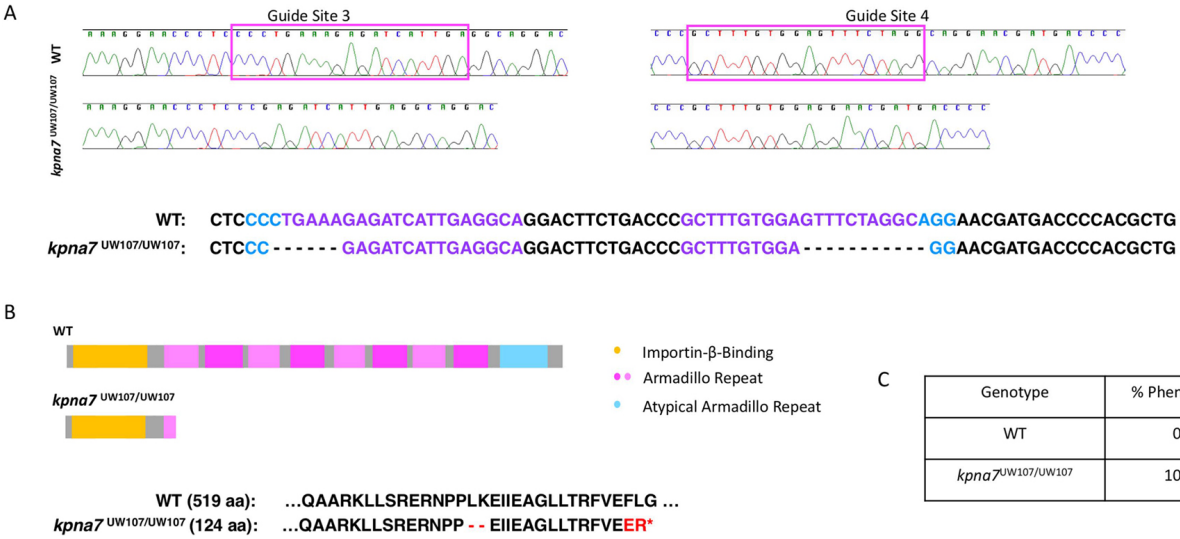


Fig. S5. Viability of the *fhdc3* maternal crispant embryos is not influenced by the amount of ectopic yolk in the embryo. A) In *fhdc3* maternal crispant embryos, there is no difference in the location of the yolk at one day between large and small ectopic yolk inclusions. B) *fhdc3* maternal crispant embryos have decreased viability when compared to wild-type controls as scored by the presence of the swim bladder at 5 days (P-value =.0091). C) No difference was observed when comparing the size of yolk and viability in the *fhdc3* maternal crispant embryos, as scored by the presences of the swim bladder at 5 days (swim bladder inflation (> 1/8 of blastodisc volume: 34%, n=54; < 1/8 of blastodisc volume: 45%, n=57; P-value = 0.4235).



Genotype	% Phenotype	N
WT	0	238
kpna7$^{UW107/UW107}$	100	365

Fig. S6. Validation of the *kpna7* maternal crispant phenotype using a stable Crispr-Cas9 line. A) Chromatograms and sequencing alignments for wild-type and *kpna7*$^{UW107/UW107}$ samples lesions in edited guide sites. In the sequencing alignment, the purple text represents the guide sites, and the blue text is the PAM site. B) Diagrams highlighting known conserved domains in the Kpna7 protein and the predicted truncated product encoded by the *kpna7*^{UW107} allele and amino acid sequences showing the predicted changes and appearance of a premature stop codon. C) Embryos from *kpna7*$^{UW107/UW107}$ females exhibit a fully penetrant phenotype identical to that observed in *kpna7* maternal crispants.

Table S1. candidate genes *zgc:114123* and *kcns3b* did not result in any apparent maternal crispant embryos.

Gene	Fish	Number of embryos in F1 generation	% Phenotype (gross morphological changes)
zgc:114123	WT	63	0
	1	78	0
	2	63	0
kcns3b	WT	92	0
	1	136	0
	2	62	0
	3	51	0

Table S2. List of DNA oligos for guide RNAs and primers used in this study.

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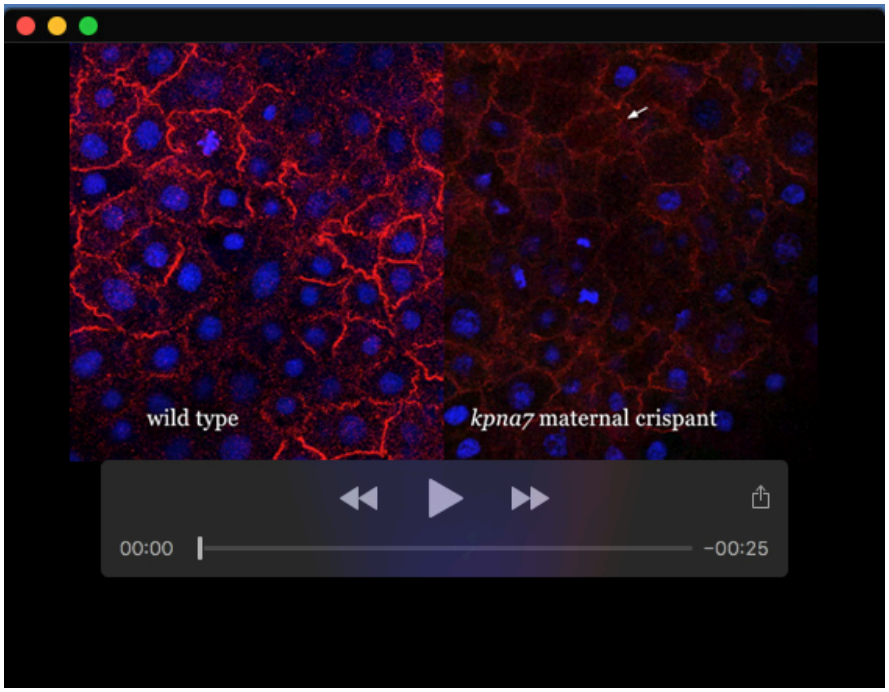

Movie 1. Cellular cortex of a *kpna7* maternal crispant and wild-type embryo at 6 hpf. Individual focal planes of confocal *Z*-stack of a 6 hpf embryo (shown as a 2D *Z*-projection in Figure 4B). The cell membranes are labeled with anti-β-catenin antibodies (red) and DNA is stained with DAPI (blue). Visualization of sequential focal planes demonstrates that the *kpna7* maternal crispants contain cells that lack nuclei.