Forbes et. al. Supplemental Figures.

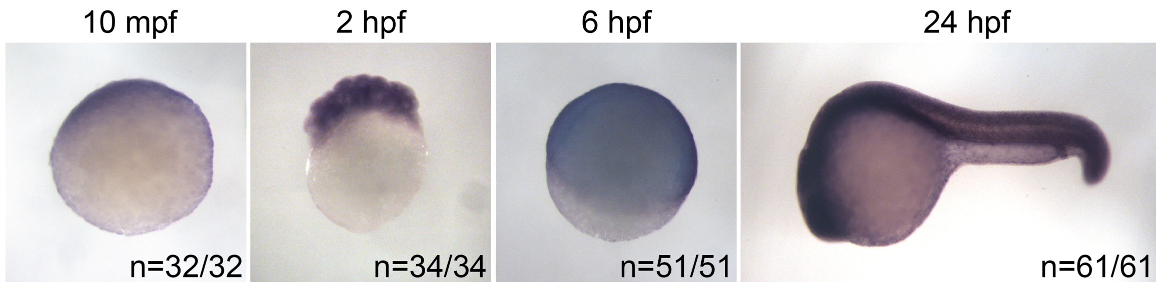

Figure S1, related to Figure 1. Whole mount *in situ* hybridization of *dazap2* transcripts at 10 mpf, 2 hpf, 6 hpf, and 24 hpf shows that *dazap2* transcripts are maternally provided and ubiquitously expressed throughout the embryo. All images are lateral views except for 6 hpf, which is an animal-lateral view.

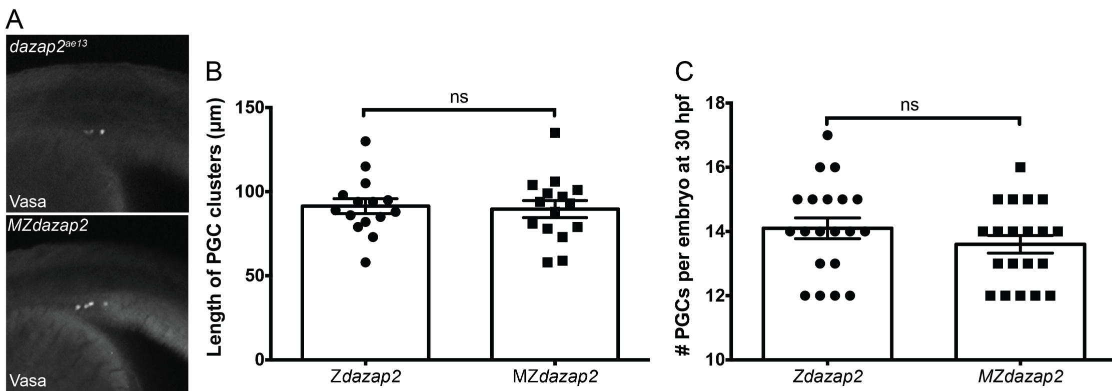

Figure S2, related to Figure 1. Maternal and zygotic *dazap2* is not essential for PGC specification and migration. A) Images are lateral views, rostral to the left, showing PGCs in the gonad anlage of zygotic and *MZdazap2* mutants. B) Graphs show length of PGC clusters and number of PGCs per embryo (C) in zygotic and *MZdazap2* mutants. For (B), p value = 0.2198 and for (C) p value = 0.7849. All error bars represent SEM.

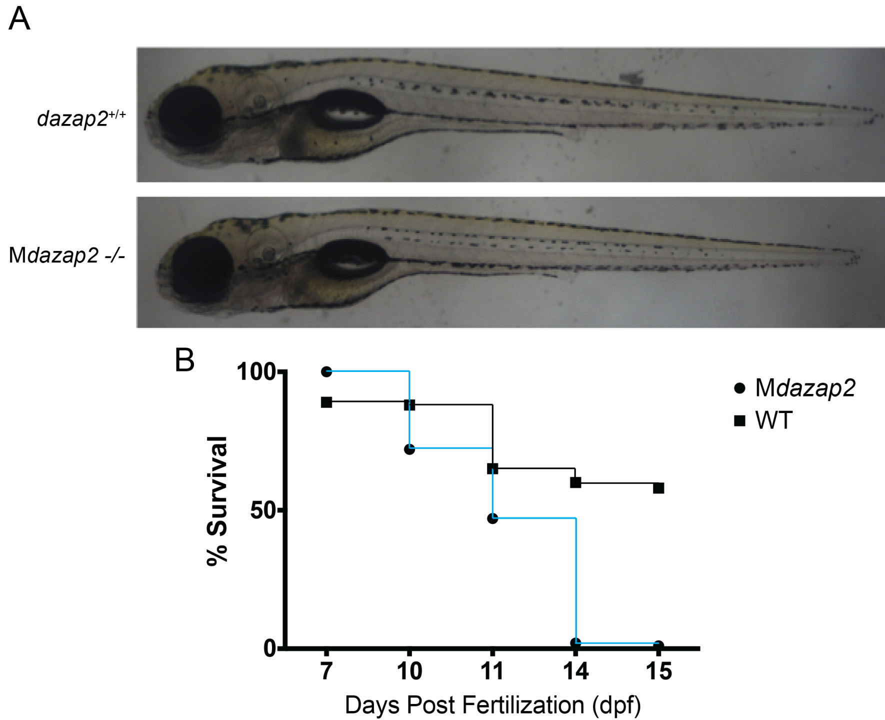

Figure S3, related to Figure 2. A) Loss of maternal *dazap2* is embryonic lethal; however, MZ*dazap2* mutants appear morphologically normal and inflate their swim bladder at 5 dpf. B) M*dazap2* mutants die between 10 and 15 dpf.

Supplemental Methods

Table 1. Primers.

Cloning primers	**Sequence (5' to 3')**
dazap2_ATG	ATGAACAACAAAGGTTCATATCC
dazap2_93bp	GTGTCTCAGTATCCAGACGCC
dazap2_180bp	CCCCAGATGACCTCTGCTTA
dazap2_195bp	ACAAATGCCCCAGATGACCTCT
dazap2_294bp	TGGCGTATTATCCGATGGGG
dazap2_364bp	GGCACTGGTAGTTCCATTCC
dazap2_ae13_STOP	GCAGGGTGCAAATGTTGTGA
dazap2_STOP	GTGGTTACACCATCTGGTAA
CRISPR-Cas9 primers	
dazap2_exon3_gRNA_F	AGACTGTGCCAATGGGGGCCTA
dazap2_exon3_gRNA_R	CCCCCATTGGCACAGTCTGTTT
Genotyping primers	
dazap2_XbaI_dCAPs_F	CCCATGCATGCCCAGACTGTGCCAATGGGGGCCATGTCTAG
QuickChange primers	
SH2.2 Y94A_F	TAGTGTCCCGATGGCGGCCTATCCGATGGGGCCCG
SH2.2 Y94A_R	CGGGCCCCATCGGATAGGCCGCCATCGGGACACTA
SH2.3 Y167A_F	GTGGCTCCAGCGGTGGTGCCACCATCTGGTAAAAGG
SH2.3 Y167A_R	CCTTTTACCAGATGGTGGCACCACCGCTGGAGCCAC

Immunohistochemistry

Zebrafish embryos and larvae were euthanized in Tricaine and then fixed overnight in 4% PFA/1XPBS. Dissected ovaries from euthanized females were fixed overnight in 4% PFA/1XPBS, Samples were then washed 4 x 5 min in 1X PBS.

For $DiOC_6(3)$ staining (D-273,Life Technologies) of oocytes, samples were stained with 0.5 µg/ml in PBST (0.1% Triton X-100) in the dark for 2 hrs at room temperature, washed 6 x 10 min in PBS, and then cleared in glycerol.

For anti-Buc staining (Y1165)(Heim et al., 2014), samples were dehydrated in Methanol and stored at -20°C overnight. Samples were rehydrated in PBS and then washed in PBST 4 x 10 min and then blocked in antibody blocking solution (PBST +0.5% TritonX-100 + 5% NGS) for 1 hr at room temperature. Samples were then incubated overnight at 4°C in anti-Buc antibody diluted 1:1000 in antibody blocking solution. Samples were then washed 6 x 10 min in PBST and then incubated overnight at 4°C in goat α-rabbit Alexa 568 diluted at 1:500 in antibody blocking solution. Finally, samples were washed 6 x 10 min in PBST and cleared in glycerol.

For anti-Vasa and anti-Ziwi antibody staining, fixed embryos and larvae were permeabilized in acetone at -20°C for 3 minutes and 7 minutes, respectively. Samples were washed in PBST 4 x 10 min and then blocked in antibody blocking solution (PBST +0.5% TritonX-100 + 5% NGS) for 1 hr at room temperature. Samples were then incubated overnight at 4°C in anti-Vasa antibody (Knaut et al., 2000) diluted 1:5,000 or anti-Ziwi antibody (Houwing et al., 2007) diluted 1:250 in antibody blocking solution. Samples were then washed 6 x 10 min in PBST and then incubated overnight at 4°C in goat α-rabbit Alexa 568 diluted at 1:500 in antibody blocking solution. Finally, samples were washed 6 x 10 min in PBST and mounted in 1% LMA or VECTASHIELD® Mounting Medium with DAPI (H-1200, Vector Labs) and then mounted in Matek-dishes.

anti-Vasa and anti-GFP double staining, was performed as described above except samples were incubated overnight at 4°C in anti-Vasa antibody (Knaut et al., 2000) diluted 1:5,000 and anti-GFP antibody (A11120, Invitrogen) diluted 1:1000 in antibody blocking solution. Samples were then incubated overnight at 4°C in goat α-rabbit Alexa 568 diluted 1:500 and goat α-mouse Alexa 488 diluted 1:500 in antibody blocking solution. To label nuclei, samples were mounted in VECTASHIELD® Mounting Medium with DAPI (H-1200, Vector Labs)

Fixed samples were imaged with Zeiss Axioobserver Apotome fluorescence microscope equipped with a 63× oil 1.4NA lens (Zeiss) and Axiovision Rel 4.7 software (Zeiss). Live samples were imaged with a Zeiss LSM5 LIVE Duoscan confocal microscope. Images were acquired with a 63x oil/1.4NA lens (Zeiss), 488-nm excitation laser, and ZEN 2011 software (Zeiss) at room temperature (RT).

Targeted mutagenesis using CRISPR-Cas System

The *dazap2* exon 3 sgRNA sequence (AGACTGTGCCAATGGGGGCC) was cloned into *pDR274* (Plasmid 42250, Addgene) using primers *dazap2_exon3_gRNA_F* and *dazap2_exon3_gRNA_R* in Table 1. The gRNA expression vector and *cas9* mRNA (MLM3613:Plasmid 42251, Addgene) were *in vitro* transcribed as described in (Hwang et al., 2013). 1nL of solution (25pg sgRNA; 600pg *cas9* mRNA) was microinjected into one-cell WT

zebrafish embryos. Genomic DNA was extracted from single injected embryos as in (Westerfield, 1995). Genomic DNA surrounding sgRNA target site in exon 3 of *dazap2* was amplified (*dazap2_180bp* and *dazap2_294_bp* primers in Table 1) followed by T7 Endonuclease I assay as in (Hwang et al., 2013). Potential founder fish were outcrossed to the AB WT strain and embryos were screened for mutations in *dazap2* using the T7E1 assay. One germline mutation was recovered herein referred to as $dazap2^{ae13}$ and confirmed by sequencing. Fish carrying this mutation were outcrossed to the Tubingen wild-type strain or intercrossed to propagate the line.

Protein binding studies –Co-immunoprecipitation assays
HEK293 cells were transfected with 3ug of *pCS3eGFP-buc*, *pCS3MT-dazap2-FL* or the specified *pCS3MT-dazap2* truncations and IP was performed with 1µg of anti-GFP antibody (A11120, Invitrogen) as in (Heim et al., 2014). Proteins were resolved (10% Protein Gel:BP7710-500, Fisher) and then transferred to PVDF membrane (IPFL00010, Millipore). Membranes were blocked in 5% Milk/TBST (0.1% Tween20) and then incubated in anti-GFP antibody (11814460001, Roche) at 1:2000 or anti-Myc antibody (13-2500, Invitrogen) at 1:1000 in blocking solution. Membranes were washed in TBST, then blocked in 5% Milk/TBST (0.1% Tween20), then incubated in Goat anti-mouse HRP (1:20,000) and processed for ECL detection (RPN2232, GE Life Sciences). Blots were imaged using a FujiImager 3000 or Amersham Hyperfilm ECL (28906836, GE Life Sciences).