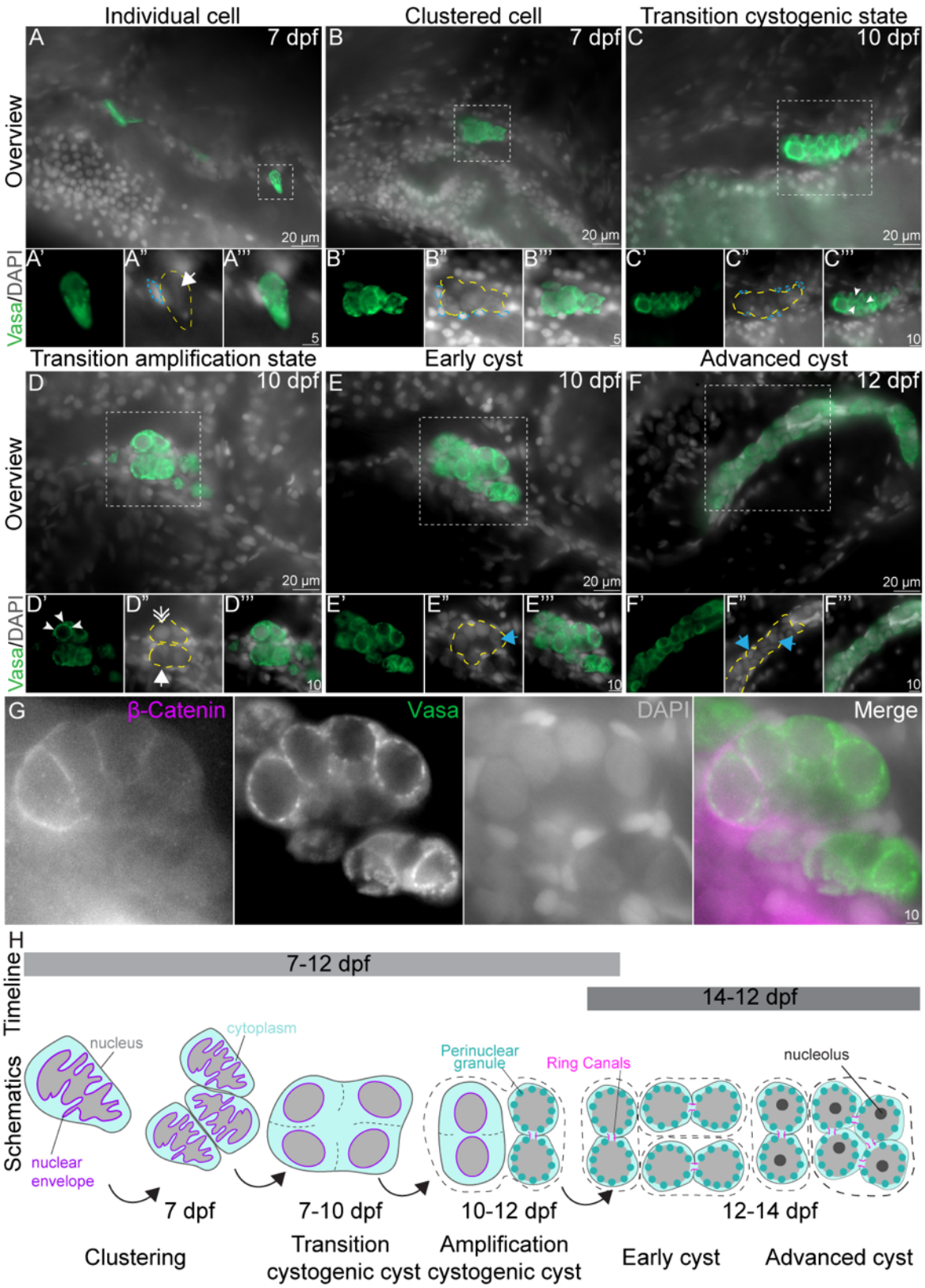


Figure S1. Overview of zebrafish cystogenesis. GCs are labeled with anti-Vasa antibody (Green), and nuclei (gray) are marked with DAPI. Merged overviews and magnified insets showing each channel (Vasa, in green; DAPI, in grey; merge in green/grey). Yellow dotted line delineates GC cytoplasm. Blue dotted line delineates the presumptive somatic cells shown in (A''-B''-C''). (A-A''') GCs are individualized in the germinal epithelium and display compact DNA structures indicated by a white arrow. (B-B''') Individual GCs form a cluster of cells with condensed nuclei. Clustering is observed between 7-10dpf (days post fertilization). (C-C''') GCs transit to a cystogenic state marked by compacted germ cells with irregular cytoplasmic and nuclear boundaries and diminished DAPI staining. Regions devoid of Vasa-immunostaining are apparent during cell division and are indicated by a white arrowhead (1C'''). (D-D''') Cell amplification by division (white filled arrowhead) and formation of germline cyst cells with round nuclei (two-chevron arrowheads), perinuclear cytoplasm adopts a spherical shape at the end of the division and contains Vasa+ granules (white arrowhead, 1D'). (E-E''') Early cyst cells emerge with a clearly-defined cytoplasm, germ granules, and nuclei with an apparent nucleolus, visible as a dark sphere within the nucleus (aqua arrow). The transient-amplification process occurs between 10-12dpf. (F-F''') As cyst formation progresses, cystoblast cells have a well-defined cytoplasm with perinuclear granules, and prominent nucleoli (aqua arrow). Scale bar, 10 μm. Inset 5 to 10 μm. (G) An early cyst labeled with ß-Catenin (Magenta) from Figure 1E-E''' (above) revealed incomplete partitioning between cells labeled with the germ cell marker Vasa (Green) and nuclear marker DAPI (greyscale) within a cyst (Figure 1G). (H) Schematics representing the cystogenesis process from individual cells that cluster, then divide, to form a premeiotic cyst. Germ cells at all stages are represented with green cytoplasm, and a grey nucleus with a purple nuclear envelope. At 7dpf, single germ cells characterized by their folded, raisin-like nuclear morphology cluster together. Next, the germ cells divide mitotically (transition-amplification) to form germline cysts between 7 to 12 days. During this period, the nucleus and cytoplasm adopt several different configurations, culminating in cells with a round nucleus and a defined cytoplasm and perinuclear

Vasa granules. Incomplete cytokinesis leads to formation of ring canals (pink). The early cyst (12-14dpf) progress through to advanced cyst stages (12-14dpf) through rounds of incomplete cell divisions**.** Finally, nucleoli emergence indicates the transition from mitotic cyst to premeiotic cyst.

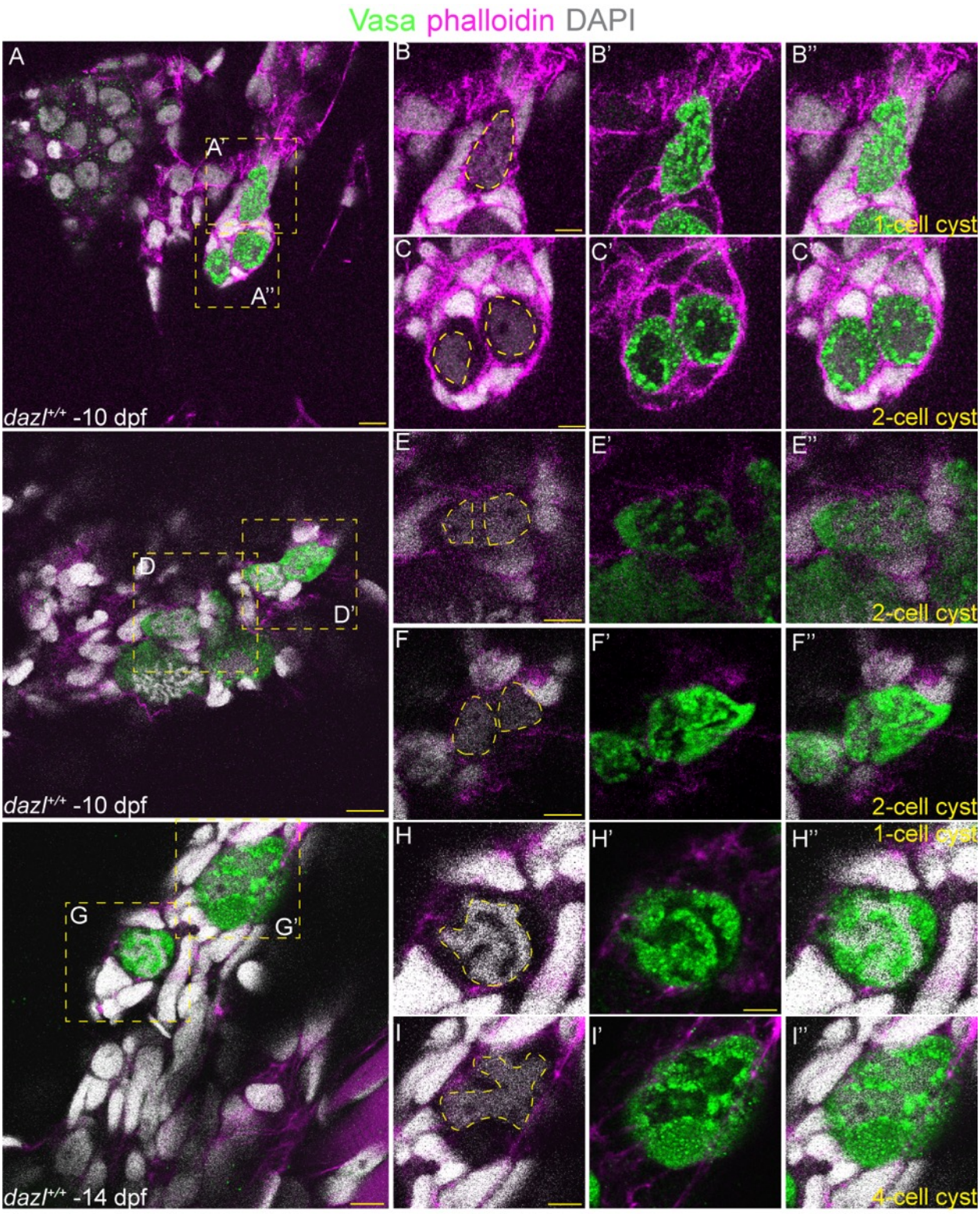


Figure S2. Cyst formation in zebrafish. (A-A'; D-D'; G-G') Single confocal plane of a larval gonad at 10dpf (A-A'; D-D') and the tip of a 14dpf (G-G') gonad with nuclei labeled with

DAPI (gray), GCs marked with Vasa (green), and F-actin with phalloidin (pink). Each cystogenesis stage is boxed with yellow dotted lines. (B-B”; H-H”) 1 cell stage with a highly folded nucleus (B;H), high cytoplasm/nucleus ratio (B’;H’). 2-cell stage cyst with 2 nuclei surrounded by perinuclear Vasa (C-C”; E-E”; F-F”, I-I”). Scale bar, 5μm.

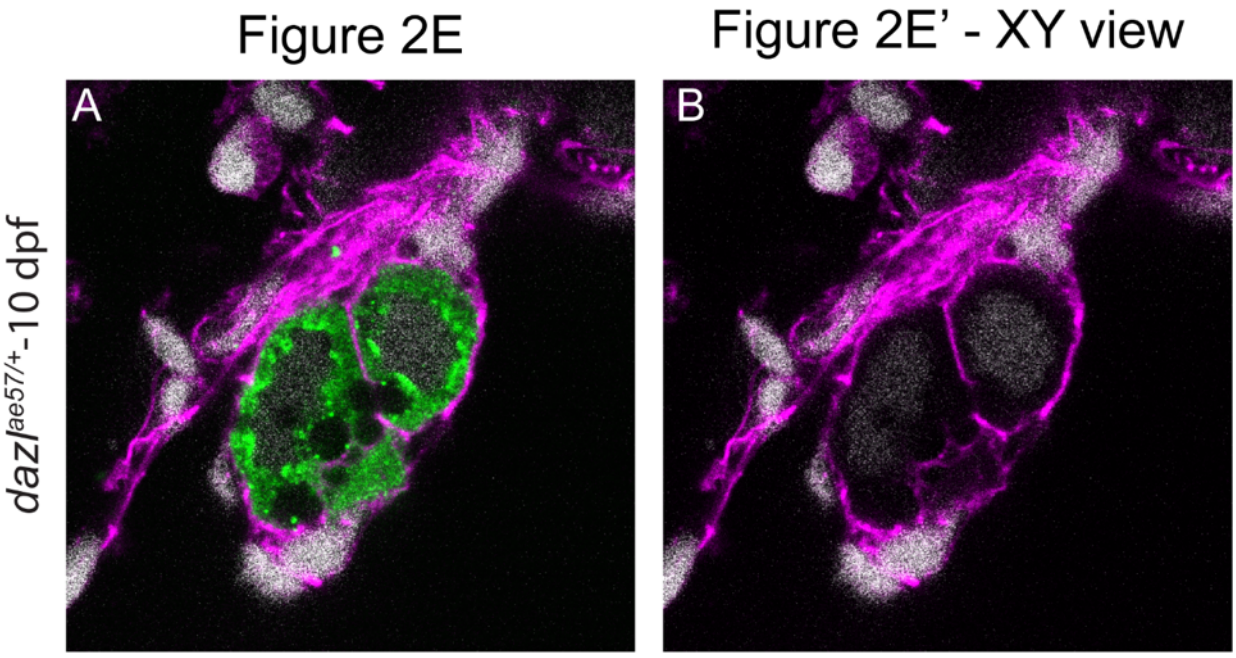


Figure S3. Unlabeled ring canal – Fig.1E (A) Intercellular bridges in 10dpf larval gonad. (B) XY view without Vasa (green) and the previous white arrowhead pointing toward the intercellular bridge.

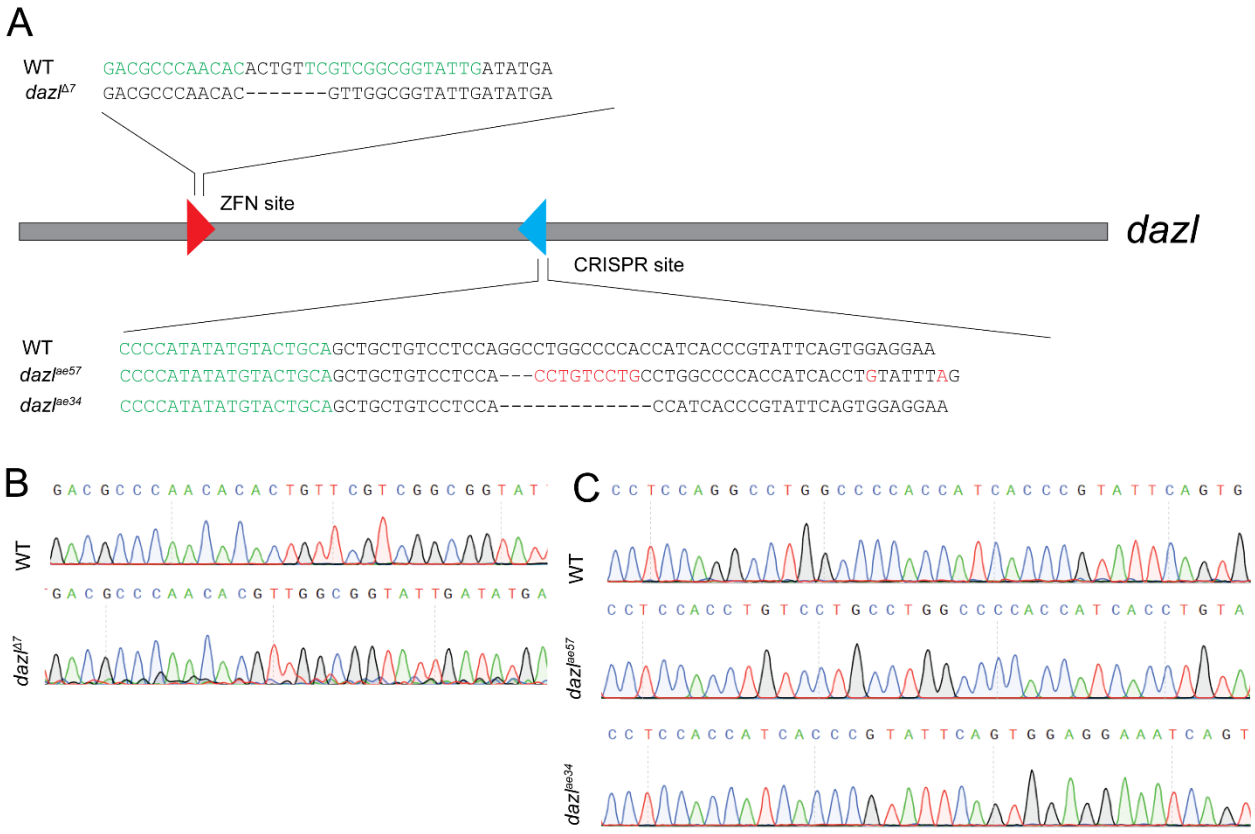


Figure S4. Generation of *dazl* mutants using zinc fingers nucleases and CRISPR-Cas9 mutagenesis. A schematic representation of the endogenous *dazl* locus targeted by zinc finger nucleases (ZFN, red triangle) and Crispr-Cas9 (blue triangle) with the target sequences indicated. Zinc finger nucleases and Cas9 binding sites are highlighted in green. *dazl* alleles generated by ZFN (*dazl*$^{\Delta 7}$) and CRISPR (*dazl*^{ae57}and *dazl*^{ae34}). Deletions are represented by a dashed line and the substitution is highlighted in blue. (B) Sequence and the corresponding chromatogram of wild-type (wt) and *dazl*$^{\Delta 7}$ alleles. (C) Sequence and chromatogram alignment of wild-type, *dazl*^{ae57}, *dazl*^{ae34} alleles.

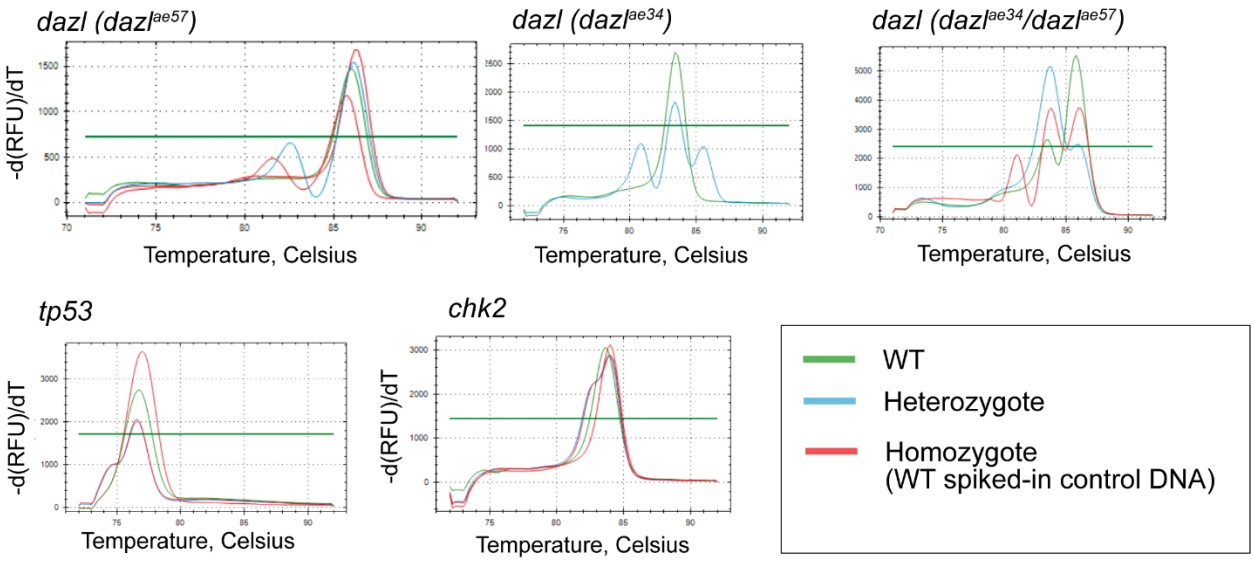


Figure S5. HRMA genotyping assays. High resolution melt analysis of progeny of heterozygotes intercrosses for *dazl*^{ae57} (A); *dazl*^{ae34} (B)*; dazl*$^{ae34/57}$ (C)*; p53* (D) *and chk2* (E). Green, blue lines represent a wild-type or heterozygous genotype respectively. Homozygous mutants are identified by a double red line, which appear heterozygote when wild-type genomic DNA is spiked in a secondary reaction. (F) Legend.

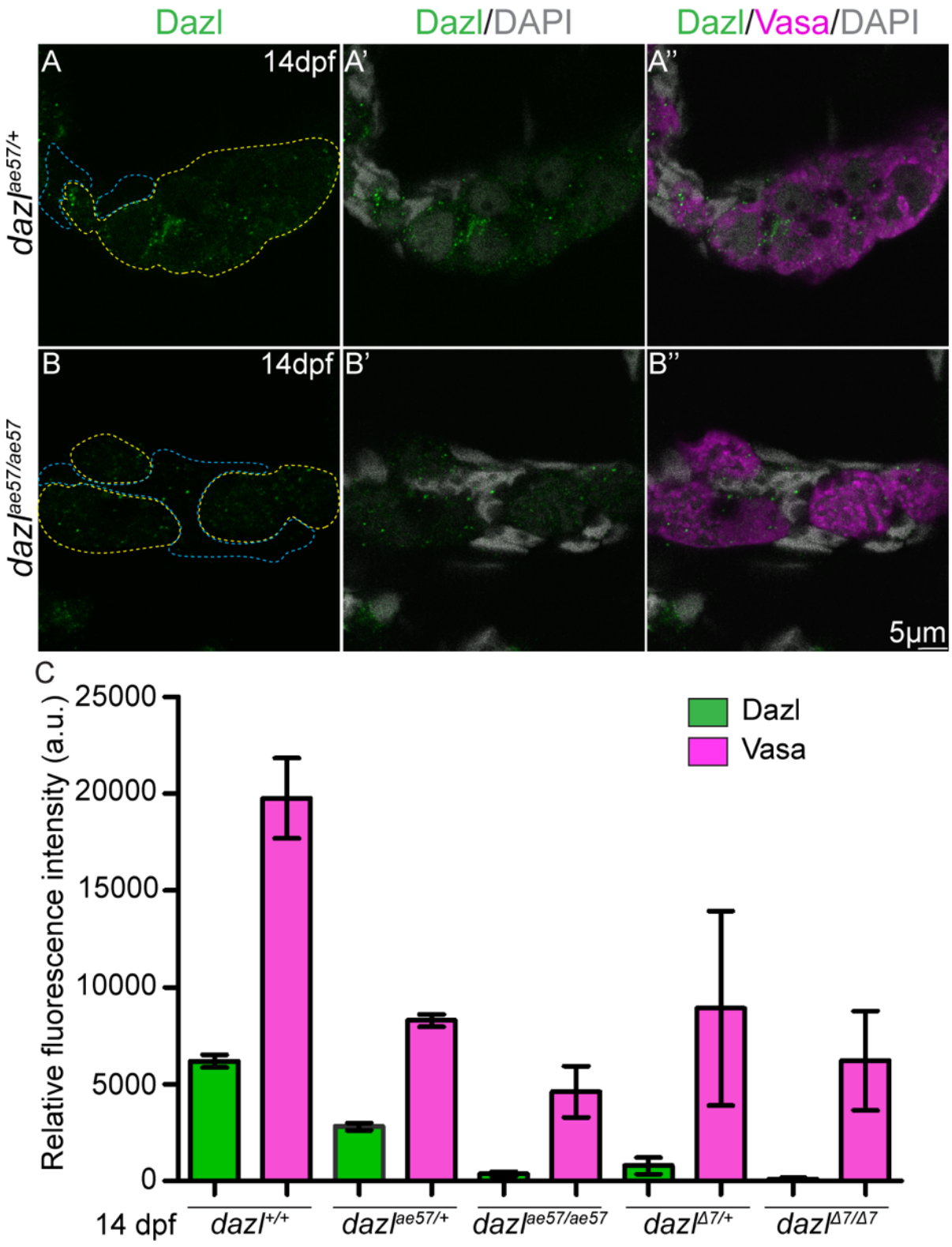


Figure S6. Dazl protein in 14dpf gonads. Single confocal plane of representative *dazl*$^{ae57/+}$, *dazl*$^{ae57/ae57}$ gonads at 14dpf immunostained with Dazl (green, germ cells), Vasa

(magenta) as a germ cell specific cytoplasmic marker and DAPI (grey) as nuclear marker. (A-A'') Localization of Dazl in Vasa+ GC of *dazl*$^{ae57/+}$ (n=2). Note the abundance of Dazl is less than in wild-type (Figure 3). (B-B'') Localization of Dazl in Vasa+ GC of *dazl*$^{ae57/ae57}$ (n=2). Note that Dazl is barely detectable. Yellow dotted line delineates the gonad in this focal plane and blue dotted line indicate somatic gonad. Scale bar, 5 μm. (C) Bar plot displaying the relative fluorescent intensities of Dazl (green) or Vasa (magenta) in 14dpf gonads (wildtype (WT), *dazl*$^{ae57/+}$, *dazl*$^{ae57/ae57}$, *dazl*$^{\Delta 7/+}$, *dazl*$^{\Delta 7/\Delta 7}$). Intensities were measured from the samples shown in Figure 4 and above. Intensities were calculated by measuring the integrated density in 3 germ cell areas of the sample followed by subtraction of 3 mean value of background intensities. Mean ± s.d.

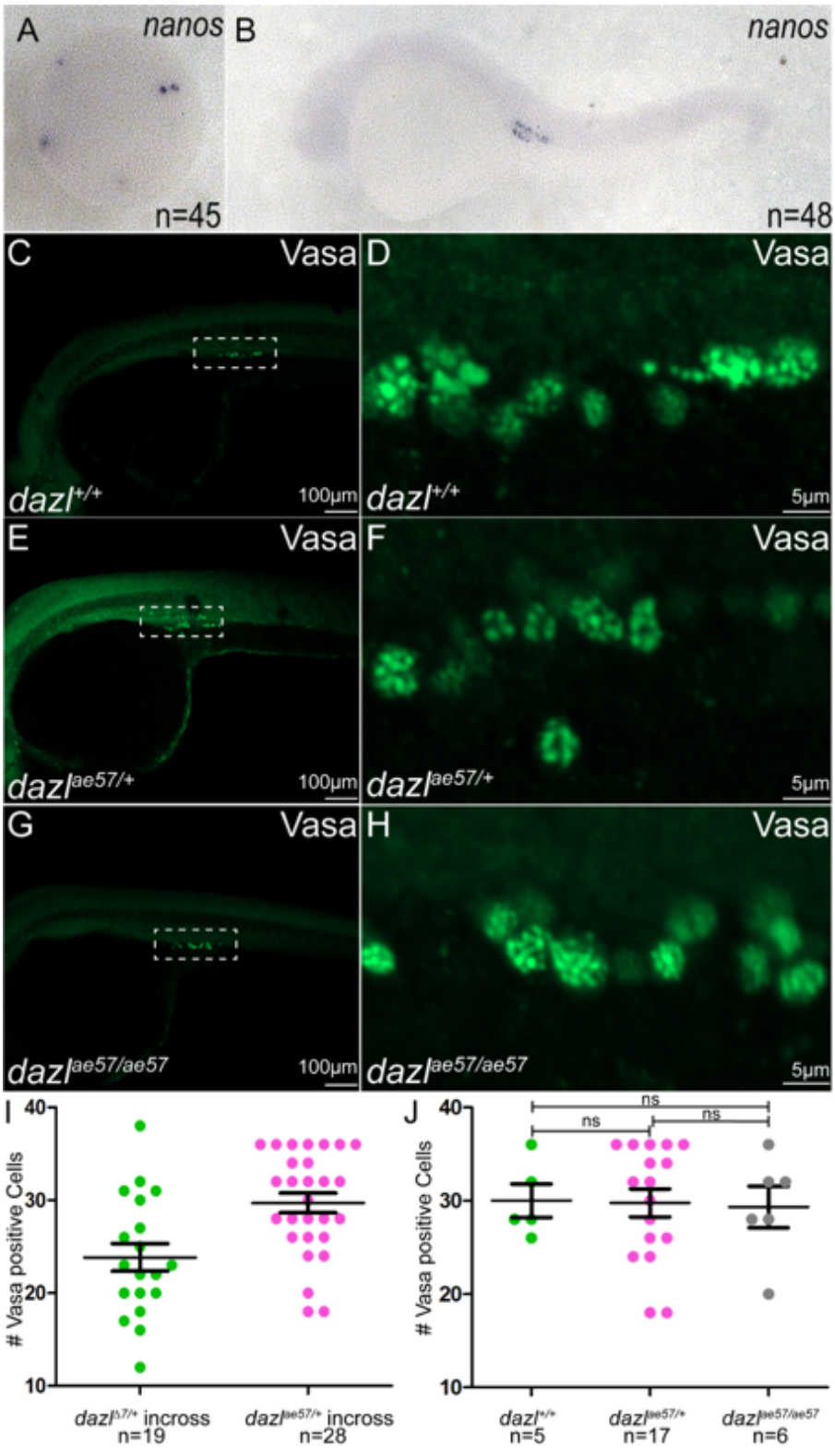


Figure S7. Zygotic *dazl* is dispensable for PGC specification and migration. (A, B) *In situ* of *nanos3* RNA in representative progeny of *dazl*$^{ae57/+}$ intercrosses *nanos3* at sphere

(A) stage (animal pole view) and 30hpf (B; lateral view). (C, H) Vasa immunostaining of *dazl*$^{+/+}$ (C, D), *dazl*$^{ae57/+}$ (E, F) and *dazl*$^{ae57/ae57}$ (G, H) embryos at 30hpf. A magnified view of the GC region of each genotype is shown in each inset reveals no difference in germ granules (D, F, H). Dorsal is oriented toward the top in panels (B-H). (I) Quantification of Vasa+ GCs in progeny of a *dazl*$^{\Delta 7/+}$ (left) or *dazl*$^{ae57/+}$ heterozygote intercross. (J) Quantification of Vasa+ cells in a *dazl*$^{ae57/+}$ heterozygote intercross grouped according to genotype (*dazl*$^{+/+}$; *dazl*$^{ae57/+}$; *dazl*$^{ae57/ae57}$). Statistical analyses were performed between *dazl*$^{+/+}$ and *dazl*$^{ae57/+}$ (ns:0.9375), *dazl*$^{+/+}$ and *dazl*$^{ae57/ae57}$(ns:0.8261) *dazl*$^{ae57/+}$ and *dazl*$^{ae57/ae57}$ (ns:0.8818).

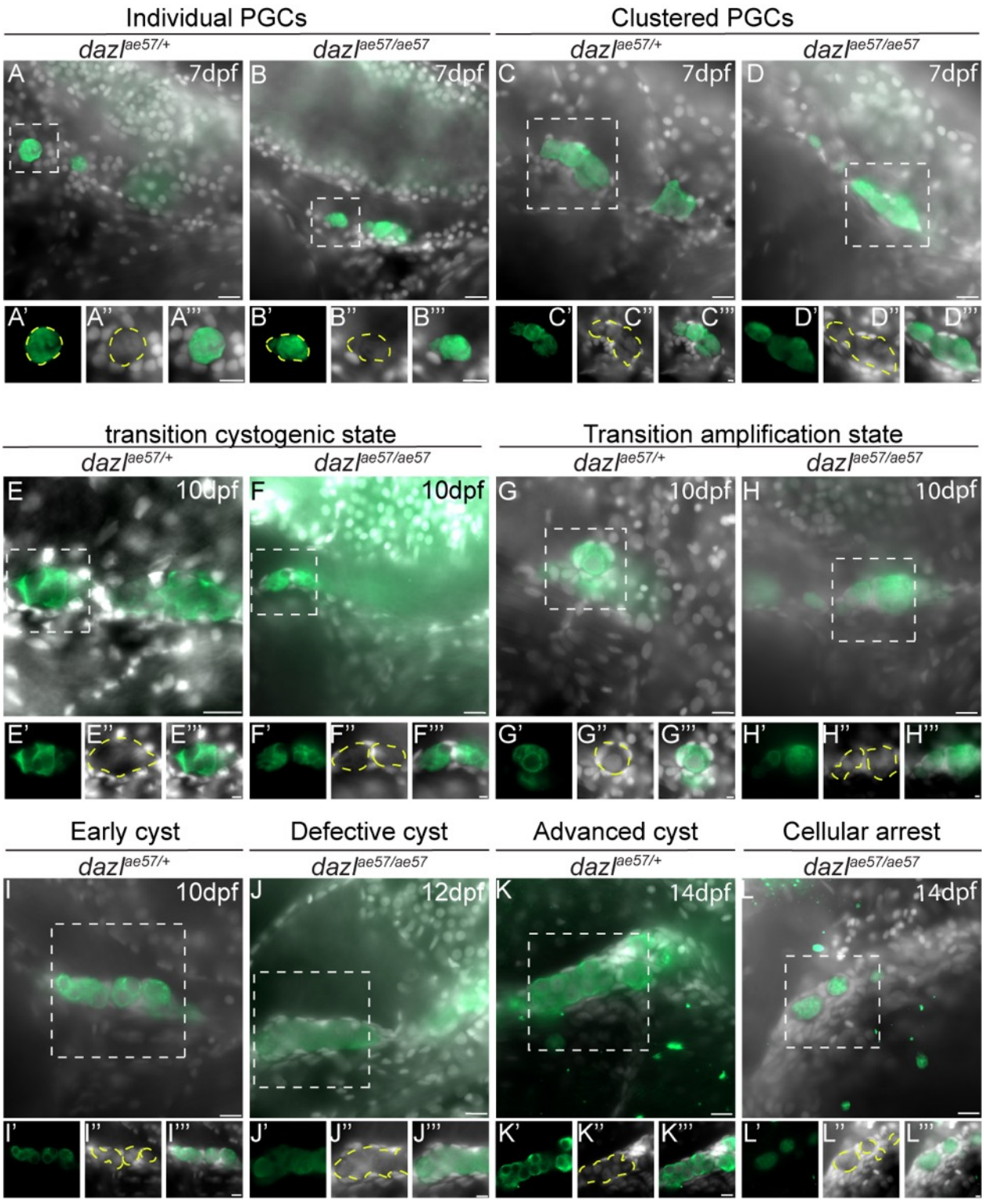


Figure S8. Zygotic *dazl* is required for germline cyst formation and germ cell maintenance. (A-C’’’) At 7 and 10dpf, Vasa labels individual germ cells which are dispersed along

the germinal epithelium of *dazl*$^{ae57/+}$ or *dazl*$^{ae57/ae57}$ zebrafish larvae (AA''' and B-B''', respectively). The boxed cell in A and B are magnified in the bottom inset to show the Vasa signals (green) (A'), DAPI (grayscale), the cell outlines are depicted with yellow dashed lines in the magnified views. Individual PGCs at this stage are characterized by condensed chromatin (white arrowhead), a high nucleus/cytoplasm ratio, and a highly folded nucleus surrounded by cytoplasm. Somatic gonadal cells surround the germ cells. Next, individual germ cells group together, termed clustered PGCs hereafter, in wild-type (C-C''') and mutant genotypes (D-D'''). In Clustered GCs Vasa is cytoplasmic but does not appear to be uniform throughout the cytoplasm (C' and D'), and the nucleus becomes more compact (C'' and D''). This step precedes the transition to amplifying divisions and cyst formation (E-E'''-H-H'''). In E and F, synchronous compartmentalized cells, characterized by an enlarged and irregular cell morphology are apparent in wild-type genotypes (E-E''') and in *dazl* mutants (F-F'''). Compartments are apparent as voids in the cytoplasm. During this phase of cyst formation Vasa is diffuse throughout the cytoplasm but granules are not apparent during the transition from individual cells to cystogenesis. Once the 2-cell cyst forms subsequent synchronous cystocyte divisions generate larger cysts in wild-type genotypes (G-G''', II''', K-K'''), but not in *dazl* mutants (H-H''', J-J''', L-L'''). The germline cyst cells in wild-type genotypes are characterized by the presence of perinuclear Vasa granules (G'), more uniform chromatin staining (G''). Between 10 to 12dpf, numerous 2-cell cysts (early cyst) (I) defined by perinuclear Vasa positive aggregates (I'), nuclei symmetrically opposed (I'') fill the gonad. Increasing numbers of somatic gonad cells encapsulate the cysts (I'''). By 12-14dpf closely spaced premeiotic cells become apparent (K) among the advanced cysts. Boxed cells are represented in the inset (K'-K'''). Advanced cysts are defined by the premeiotic germ cell with cytoplasmic and perinuclear Vasa aggregates (K'), and round nuclei with a large nucleolus (K''), encapsulated by elongated somatic gonad cells (K'''). In *dazl*$^{ae57/ae57}$ mutants cysts fail to form, instead GCs return to the PGC like morphology and arrest ((H-H''', J-J''', L-L'''). bar, 20 μm for the

overview and 5 µm for the insets. *dazl*$^{ae57/+}$ (7dpf, n=5; 10dpf, n=7; 12dpf, n=11; 12dpf, n=8; 14dpf, n=6), *dazl*$^{ae57/ae57}$ (7dpf, n=4; 10dpf, n=8; 12dpf, n=4; 14dpf, n=4).

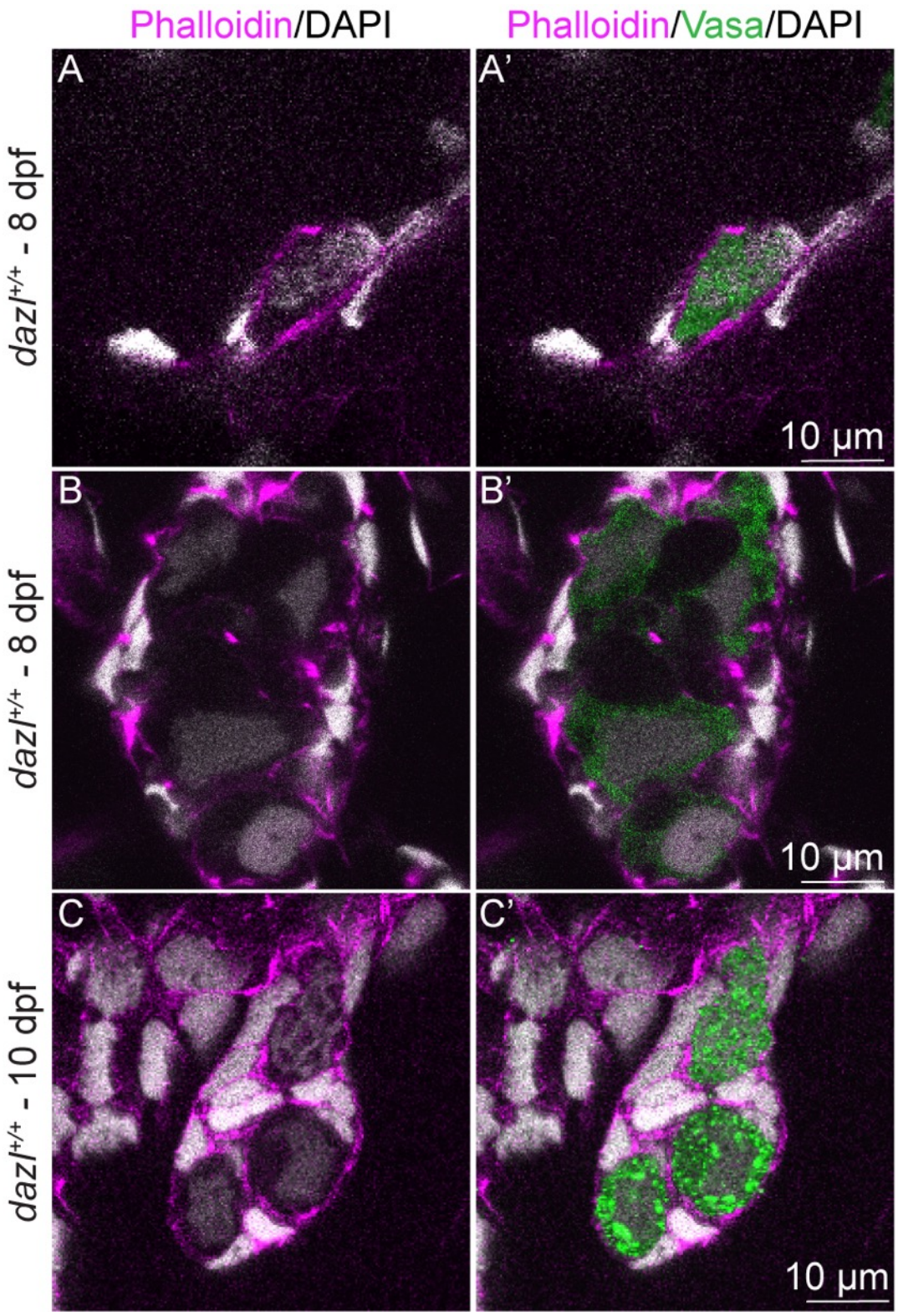


Figure S9. Additional wild-type gonads at 8dpf and 10dpf. Single confocal plane of a larval gonad at 8dpf and 10dpf immunostained with Vasa (green) , F-actin labelled with

phalloidin (magenta) and DAPI marks the nuclei (grey) (A-A') 8dpf gonad displaying individual cells with highly folded nucleus, high nucleus/cytoplasm ratio. (B-B') 8dpf gonad displaying the amplification step. (C-C') 10dpf gonad displaying an individual cell on the top, and on the bottom a cyst. 8dpf gonads (n=4), 10dpf gonads (n=6). Note the gonads were taken under a 63x objective, zoom 1, resolution 512x512. Scale bar, 10 μm.

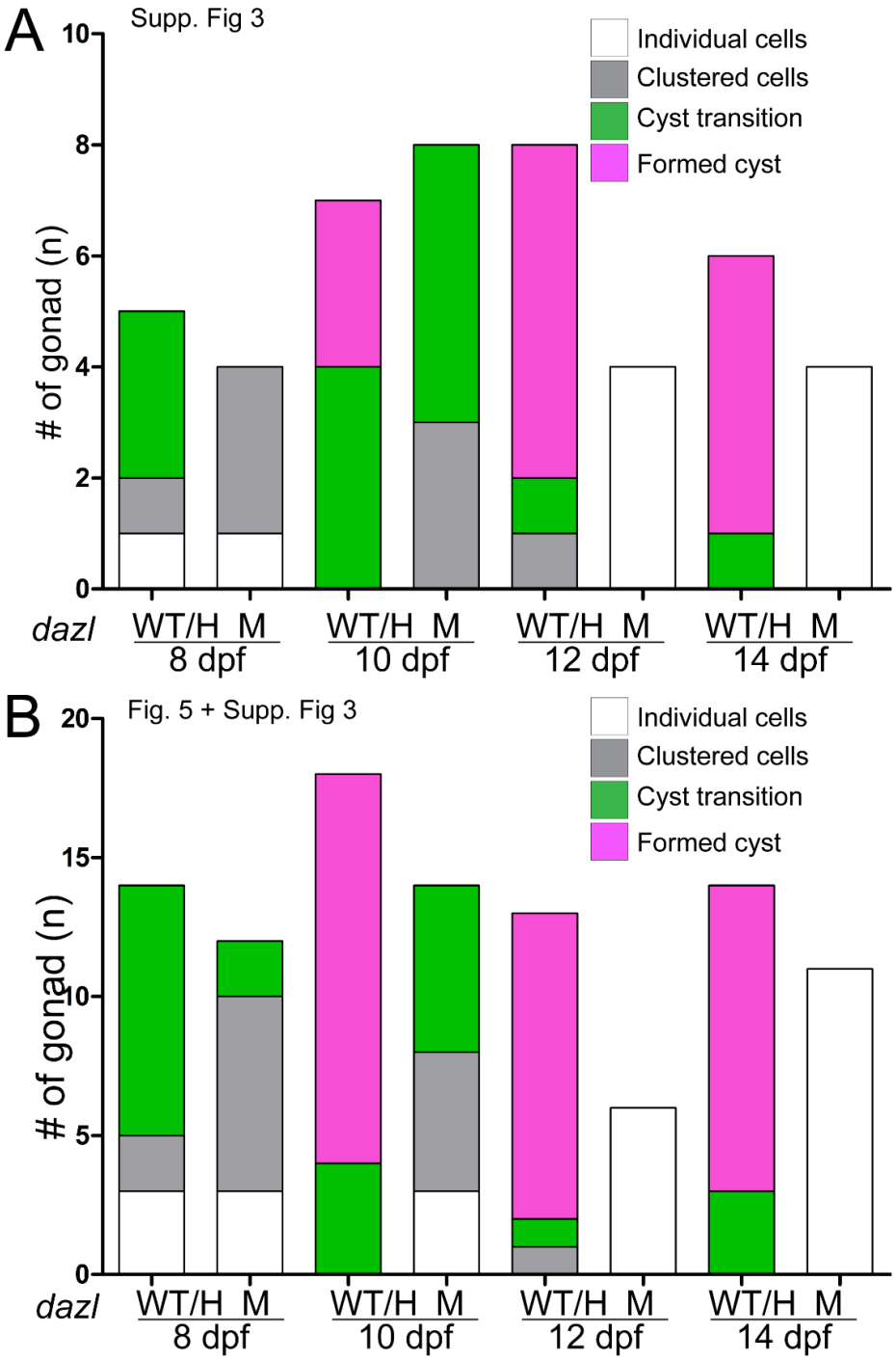


Figure S10 Defective cyst formation in *dazl* mutants. Bar plots quantifying the gonads analyzed at 8dpf, 10dpf, 12dpf and 14dpf in wild-type (WT), *dazl*$^{ae57/+}$ and *dazl*$^{ae57/ae57}$ mutants represented in (A) Supplemental Figure 3 and (B) in Figure 5 and Supplemental Figure 3.

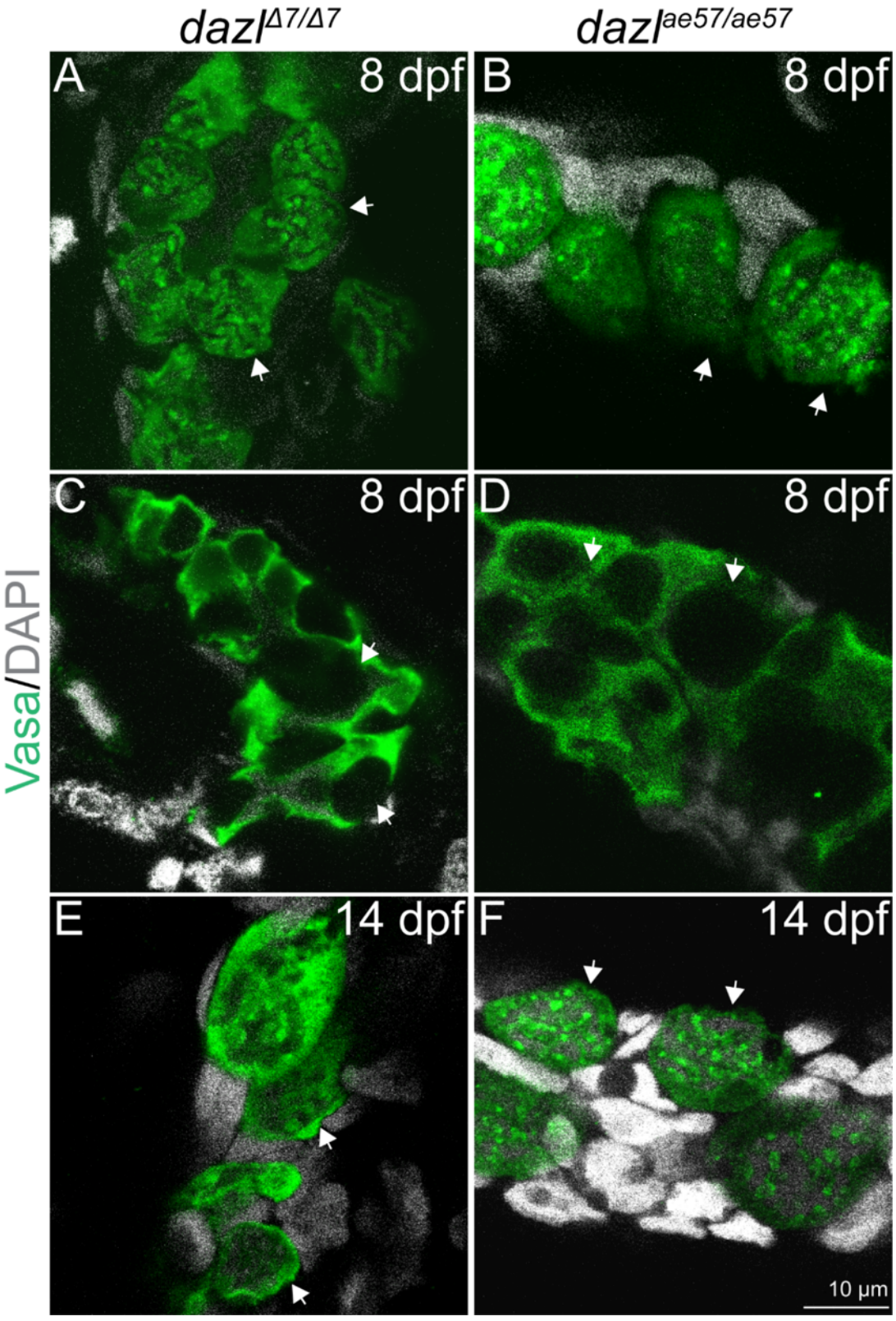


Figure S11. Comparison of cystogenesis in *dazl* mutant alleles. Gonads from

dazl$^{\Delta 7/\Delta 7}$ *or dazl*$^{ae57/ae57}$ between 8dpf and 14dpf immunostained with Vasa and DAPI. Individual germ cells of (A) *dazl*$^{\Delta 7/\Delta 7}$ (n=2) and (B) *dazl*$^{ae57/ae57}$ (n=6) mutant gonads. (C,D) Germ cells mutant for either allele initiate cystogenesis as evident from cellular and nuclear morphology, (C) *dazl*$^{\Delta 7/\Delta 7}$ (n=1) and (D) *dazl*$^{ae57/ae57}$ (n=2). At 14dpf dazl$^{\Delta 7/\Delta 7}$ (n=2) (E) or *dazl*$^{ae57/ae57}$ (n=7) (F) mutant germ cells remain as individual cells, indicative of failed cystogenesis.

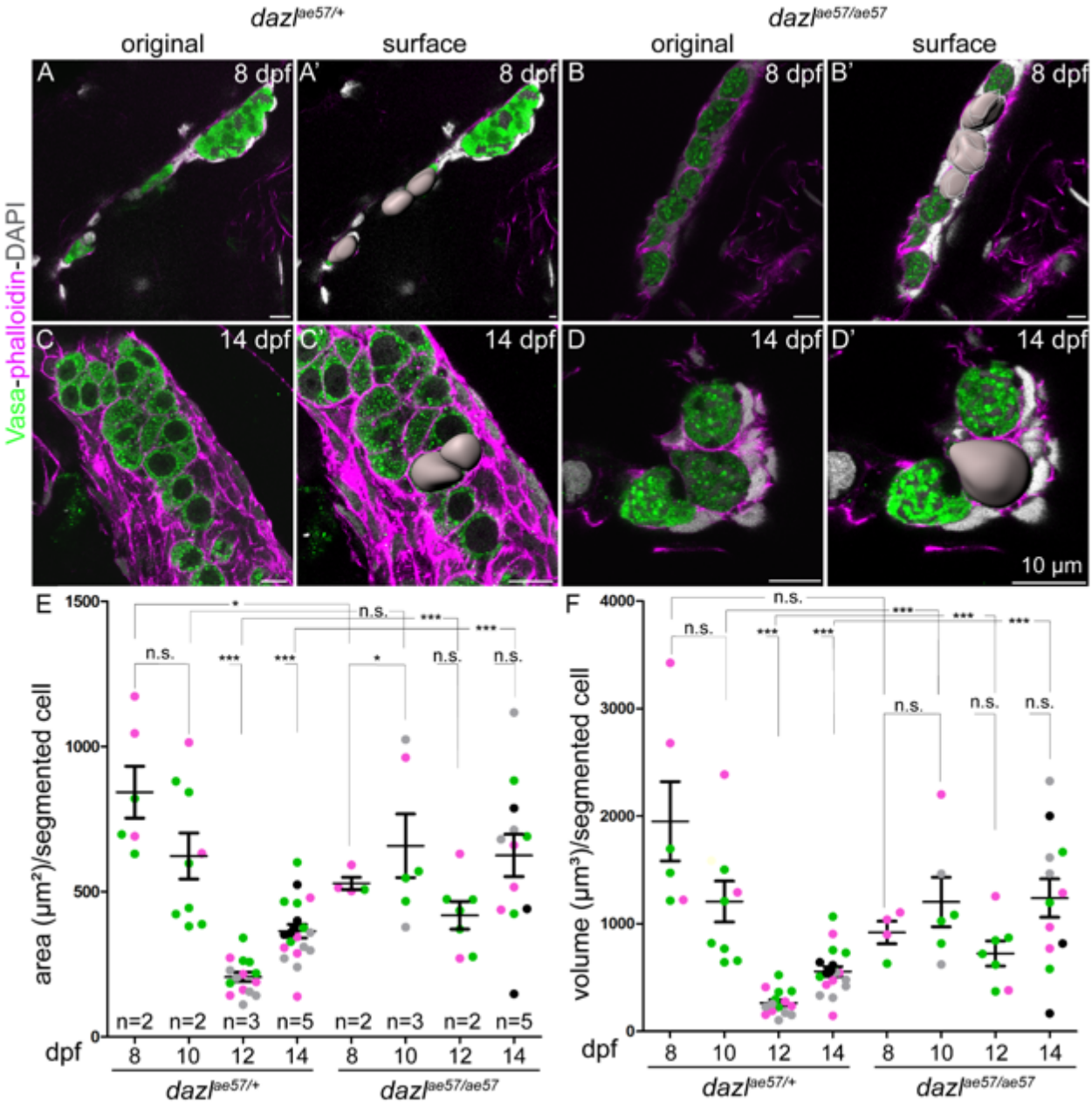


Figure S12. *dazl* mutant cell size between 8 and 14dpf. Gonads of *dazl*$^{ae57/+}$ or *dazl*$^{ae57/ae57}$ at 8, 10, 12 and 14dpf were immunostained with Vasa for GCs; F-actin was stained with phalloidin and nuclei with DAPI. Cells within the Z-stack were manually segmented using Imaris software to measure cell area and cell volume. (A-A') Gonad from *dazl*$^{ae57/+}$ at 8dpf (A) (Note: The resolution is 512x512) and the segmented cells are in grey. (A-D') Gonads from *dazl*$^{ae57/+}$ at 8dpf (A) and 14dpf (C) or *dazl*$^{ae57/ae57}$ (B) and (D). The respective segmented cells are

in grey (A', B', C', D'). (E) Quantification of cell area at 8, 10, 12 and 14dpf. The number of gonads is indicated at the bottom of each data point. Individual sample values are indicated by the colored dots. (F) Quantification of cell volume at 8, 10, 12 and 14dpf. The number of gonads is as in (E). Each sample value is differently colored. Two-tailed Student's *t*-test; $*P \leq 0.05$, $***P \leq 0.001$, n.s. not significant. Mean ± s.d.

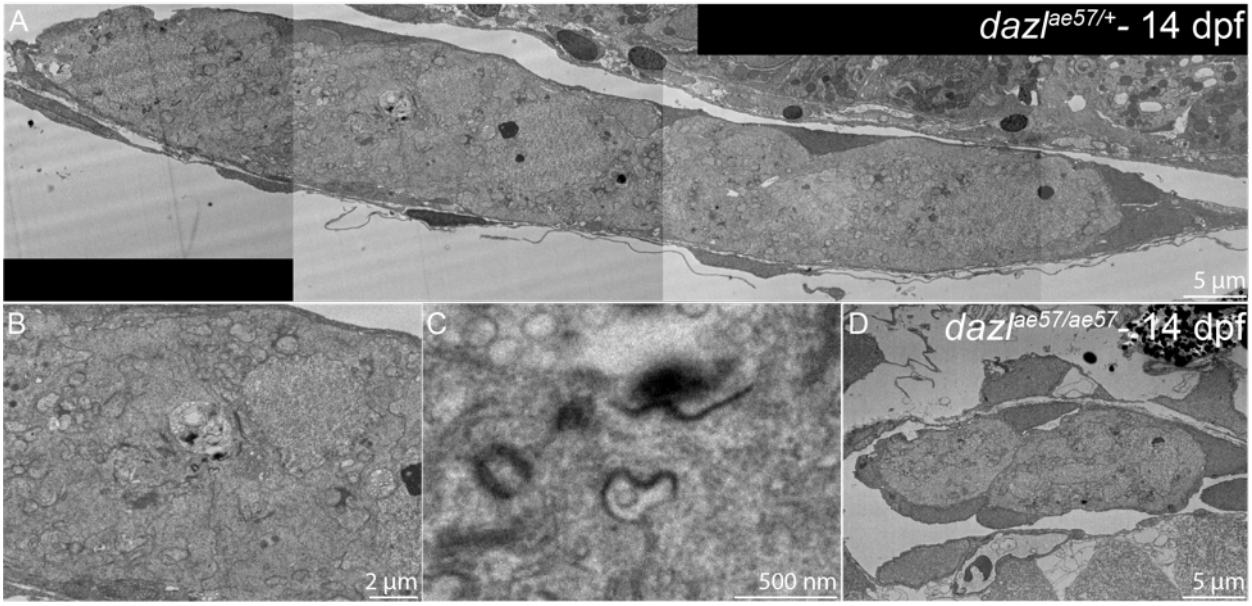


Figure S13. Actin rings are not maintained in *dazl* mutants (Main Figure 8 without pseudocolor). (A) TEM image of 14dpf wild-type gonad stitched from tiled images (Magnification:700x). (B) Magnified image of (A) (pink dashed line box) showing ring canal connecting sister GCs within a cyst. (C) Higher magnification of (B) (pink dashed line box). (D) TEM image of 14dpf *dazl* mutant gonad stitched from tiled images (Magnification: 700x); No cysts or ring canals were detected.

Figure S14. *chk2sa30250* is a nonsense allele. (A) *chk2sa20350* sequence at the mutant locus compared to the wild-type (*wt*) and the deduced protein sequence of Chk2^{sa20350}. C to A substitution triggers a stop codon. (B) Chromatogram from the sequenced *chk2*^{sa20350} locus.

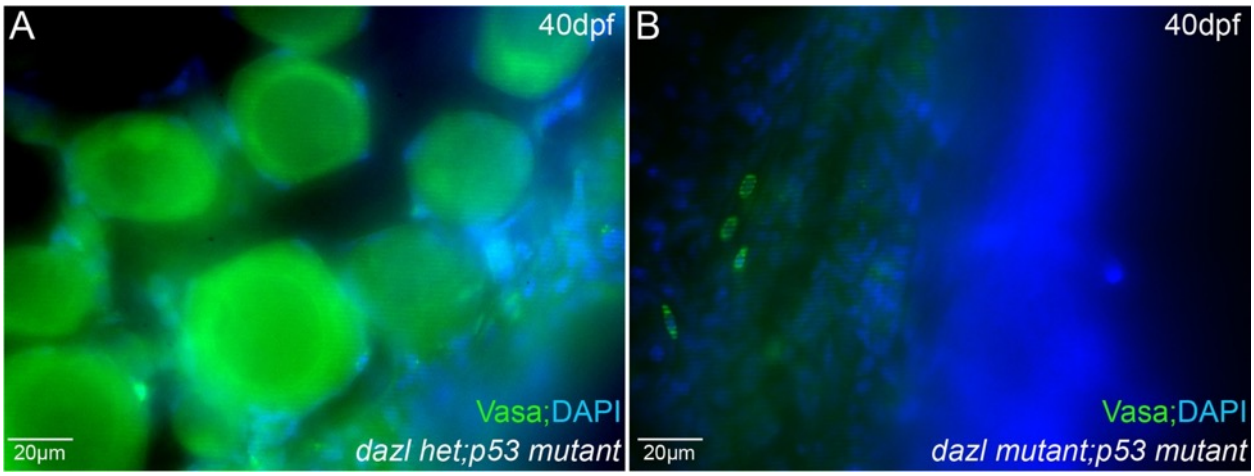


Figure S15. Mutation of *p53* cannot suppress germline loss in *dazl* mutants. Vasa staining of *dazl*$^{ae57/+}$ and *dazl*$^{ae57/ae57}$ in a *p53* mutant background at 40dpf. (A) Presence of Vasa positive (green) germ cells in a *dazl*$^{ae57/+}$*;Tp53* mutant gonad (n=6). (B) Absence of Vasa positive germ cell in a representative *dazl*$^{ae57/ae57}$*;Tp53* double mutant gonad (n=4).

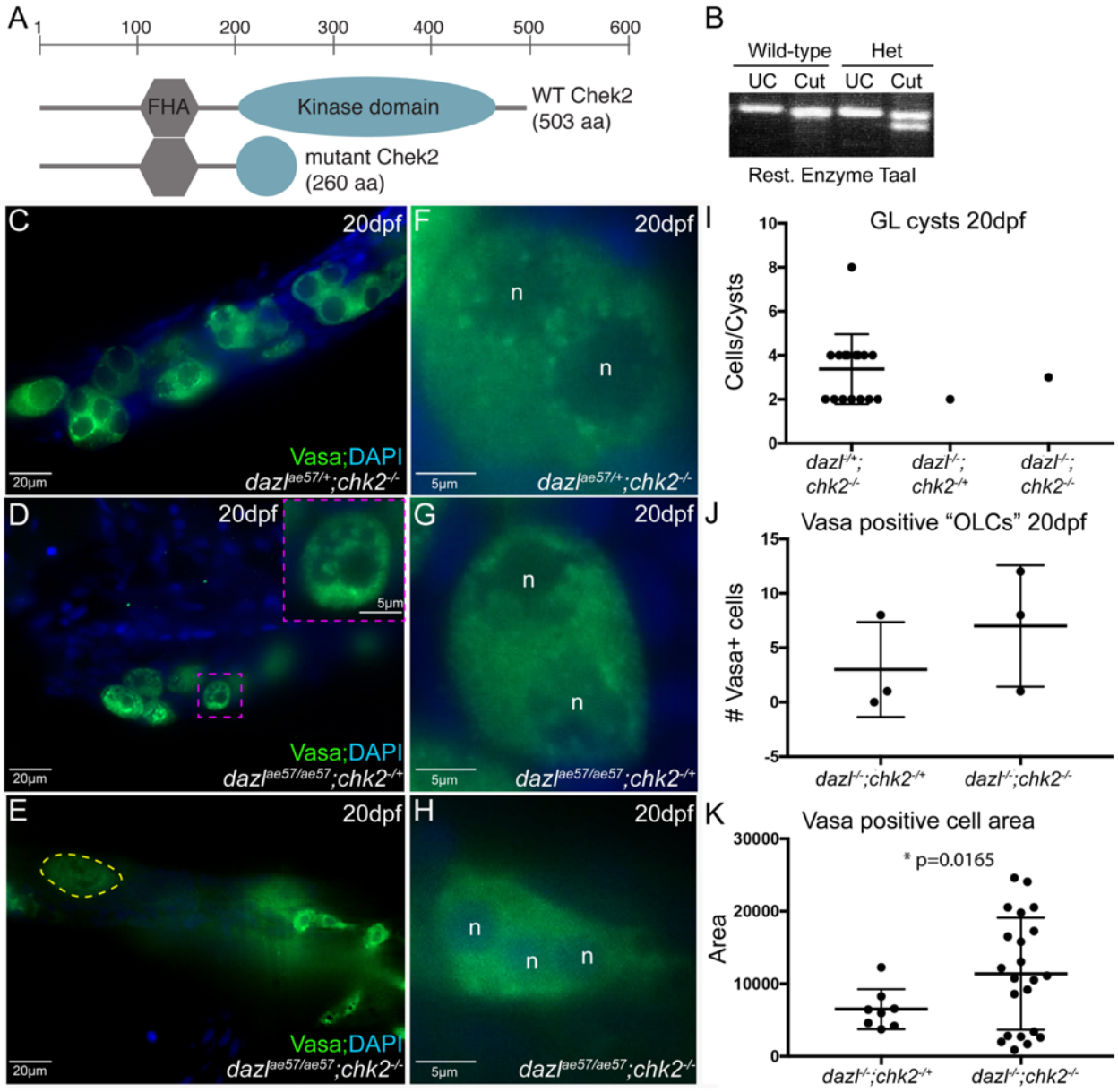


Figure S16. *chk2* inactivation does not prevent GC loss. (A) Schematic of wild-type and mutant Chk2 proteins. (B) Representative genotyping assay. TaaI cuts the mutant allele. (C-D) GC clusters of *dazl*$^{ae57/+}$;*chk2*$^{sa20350/sa20350}$. Presence of Vasa granules surrounding the GC nucleus. (D-G) Vasa+ GCs of *dazl*$^{ae57/ae57}$; *chk2*$^{sa20350/+}$. Note the vacuolization of the cell enlarged in the inset (E-H) Vasa+ GCs of *dazl* $^{ae57/ae57}$;

chk2$^{sa20350/sa20350}$. (I) Quantification of germline cysts for *dazl* $^{ae57/+}$; *chk2*$^{sa20350/+}$, dazl$^{ae57/ae57}$; chk2$^{sa20350/+}$ and *dazl* $^{ae57/ae57}$; *chk2*$^{sa20350/+}$ (n=3 gonads/genotype). (J) Quantification of large Vasa+ GCs of *dazl*$^{ae57/ae57}$; *chk2*$^{sa20350/+}$ and *dazl*$^{ae57/ae57}$; *chk2*$^{sa20350/sa20350}$. (K) Quantification of the cell area in Vasa+ GCs in *dazl*$^{ae57/ae57}$; *chk2*$^{sa20350/+}$ and *dazl*$^{ae57/ae57}$*; chk2*$^{sa20350/sa20350}$. Scale bars, 20 μm (C-E) and 5μm (F-H).

Table S1. Genotyping and genetic ablation primers.

Genotyping primers

Restriction enzyme assay	Forward (5'-3')	Reverse (5'-3')	Enzyme
dazl^{ae57}*or dazl*^{ae34}	GGTCAGTGTCATCTCCAATGAT	TCCTGGAGGACTGGAGTAAGAA	Eco147I
dazl$^{\Delta 7}$	CGTCAGGGTTTTCCGTCCTC	AAAGGCCTCAAAAACCGAATA	AflIII
chk2^{sa30520}	AGCCACACGAAATGCTGAG	CAATGTAGTAGGATTCTTCAGACTG	TaaI

HRM assay	Forward (5'-3')	Reverse (5'-3')
dazl^{ae57}	TGGTCCATCACAGTGGGTAA	CCACTAAATACAGGTGATGGTG
dazl^{ae34}	GGTCCATCACAGTGGGTAAA	TGCATGTACTGATTTCCTCCA
p53^{M214K}	CTGAATGGACAACTGTGCTACT	ATGATTGTGAGGATGGGCCTG
chk2^{sa30520}	CCGTGCCTGATCAAAACTGA	TCCAAAACAATGTAGTAGGAGTCT

RT-PCR

	Forward	Reverse
dazl	ATGGTTCAGGGGGTTCAGTTACC	CATAAGGGTTAGCAAAGTCGC

sgRNA

	Target site	PAM
dazl	GATGGTGGGGCCAGGCCTGG	AGG

Zinc finger nuclease

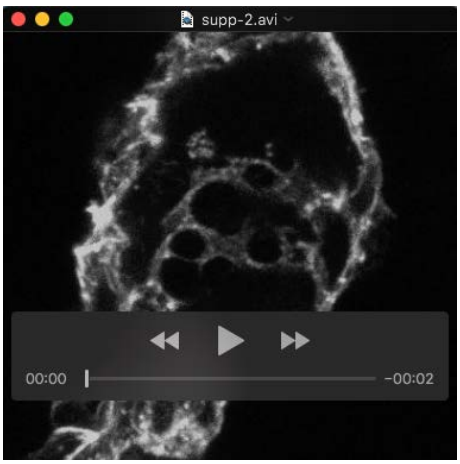
	Target site	Left primer	Right primer
dazl	GACGCCCAACACACTGTTCGTCGGCGGTATTG	GACGCCCAACAC	TCGTCGGCGGTATTG

Table S2. PGC counting and statistics

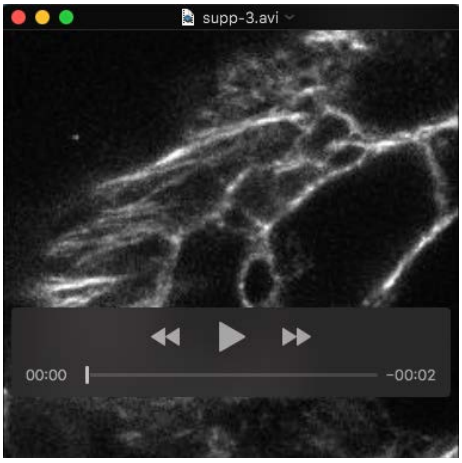
Fig.4J-30 hpf-*dazl*$^{ae57/+}$ incross		
dazl$^{+/+}$ (n=5)	*dazl*$^{ae57/+}$ (n=17)	*dazl*$^{ae57/ae57}$ (n=6)
36	34	20
28	28	36
32	30	28
28	24	32
26	18	28
	36	32
	36	
	18	
	36	
	36	
	26	
	24	
	34	
	32	
	32	
	36	
	26	

Statistics	
Unpaired t test (comparison column *dazl*$^{+/+}$ and *dazl*$^{ae57/+}$)	
P value	0.9375
P value summary	ns
Are means signif. different? (P < 0.05)	No
One- or two-tailed P value?	Two-tailed
t, df	t=0.07937 df=20
Unpaired t test (comparison column *dazl*$^{ae57/+}$ and *dazl*$^{ae57/ae57}$)	
P value	0.8818
P value summary	ns
Are means signif. different? (P < 0.05)	No
One- or two-tailed P value?	Two-tailed
t, df	t=0.1506 df=21

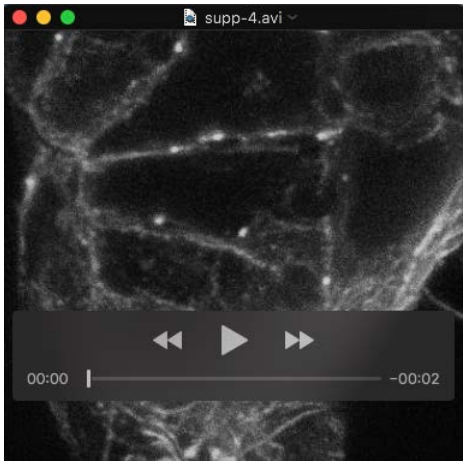
Unpaired t test (comparison column *dazl*$^{+/+}$ and *dazl*$^{ae57/ae57}$)	
P value	0.8261
P value summary	ns
Are means signif. different? (P < 0.05)	No
One- or two-tailed P value?	Two-tailed
t, df	t=0.2261 df=9



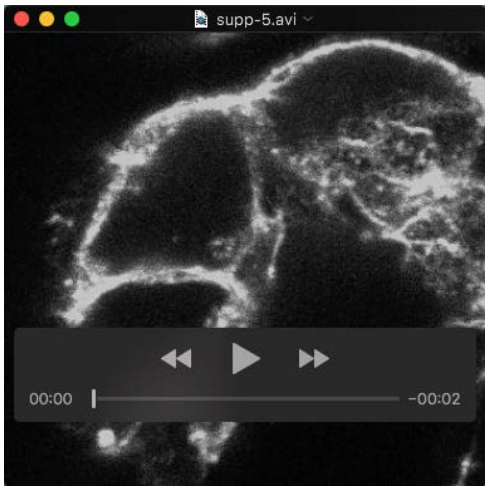
Movie 1. 3D projections of actin rings of *dazl*^{ae57} heterozygote gonad at 10dpf.



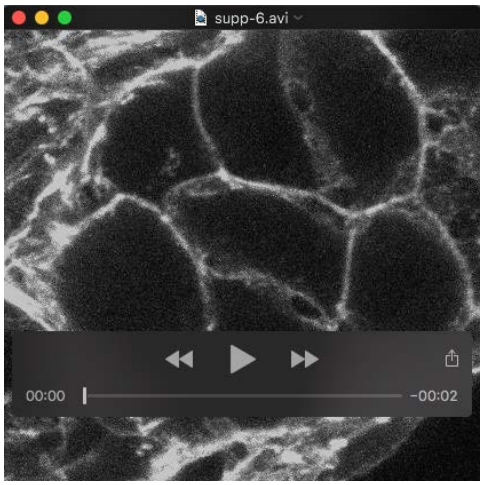
Movie 2. 3D projections of actin rings of *dazl*^{ae57ae57} mutant gonad at 10dpf.



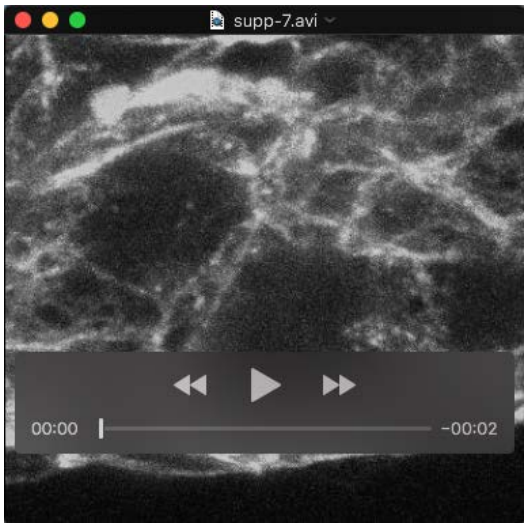
Movie 3. 3D projections of actin rings of *dazl*^{ae57} heterozygote gonad at 12dpf.



Movie 4. 3D projections of actin rings of *dazl*^{ae57ae57} mutant gonad at 12dpf.



Movie 5. 3D projections of actin rings of *dazl*^{ae57ae57} heterozygote gonad at 14dpf.



Movie 6. 3D projections of actin rings of *dazl*^{ae57} mutant gonad at 14dpf.