

Supplementary information for the article;

Dnd knockout ablates germ cells and demonstrates germ cell independent sex differentiation in Atlantic salmon

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Supplementary material

Supplementary Figure S1. Representative analysis of CRISPR-Cas9-mediated knock out of *dnd* and *alb*: chromatograms showing sequencing results of amplicons of *dnd* and *alb* around the CRISPR site (light green arrows). A: fish with no mutations; B: a male and C: a female fish with mutations indicated by multiple peaks starting in the CRISPR site and proceeding downstream.

Supplementary Figure S2. Gross morphology and histology of six fish (n=3 of each sex) showing the germ cell free phenotype. Numbers on fish refer to the fish presented in Supplementary table S2, where more information on each fish is found (length, weight, age, sampling date and phenotype of sectioned gonad). (a) Picture of fish, (b) gross morphology of gonads and (c) section of respective gonad. Scale bar in 7c, 8c, 10c, 20c, 18c and 28c, 100 μ M.

Supplementary Figure S3. Gross morphology and histology of one control fish and 2 mosaic *dnd*KO fish, numbers on *dnd* KO fish refers to the fish presented in Supplementary table S2, (listing length, weight, age, sampling date and phenotype of sectioned gonad). (a) picture of fish, (b) gross morphology of gonads and (c) section of respective gonad. Scale bar in control histology, 25c and 27c, 100 μ M.

Supplementary Figure S4. Relative gene expression of *vasa* (a), *cyp19a1a* (b) and *amh* (c) in adult Atlantic salmon tissues (n=3 per tissue; ovary, testis, liver, brain, head kidney, intestine, muscle and spleen). Samples assayed are listed on the x-axis, while the y-axis indicates the relative abundance of the transcripts in relation to the normalization factor *elongation factor1 α* . For each gene all values were calibrated to the sample with the lowest Δ Ct; therefore the highest gene expression value was set to 1. All data are shown as SEM. n=3.

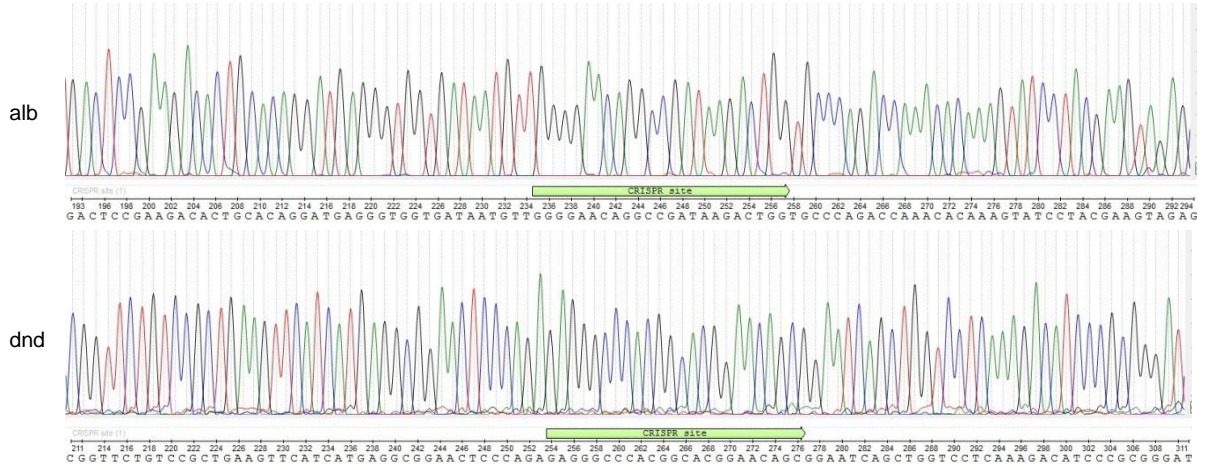
Supplementary Figure S5. Relative gene expression of *dnd* (a), *foxl2* (b) and *sox9a* (c) in adult Atlantic salmon tissues in gonads of male and female *dnd* KO (n=4 per sex) and control fish (n=3 per sex). Gonads were obtained from 12-18 months old fish. Individual fish used for qPCR are indicated in Supplementary table S2. Samples assayed are listed on the x-axis, while the y-axis indicates the relative abundance of the transcripts in relation to the normalization factor *elongation factor1 α* . Data are presented as \pm SEM. Significant gene expression differences between groups are indicated by letters (a, b and c); *n.d.* - not detected.

Supplementary table S1. Oligonucleotides and primers used in the study

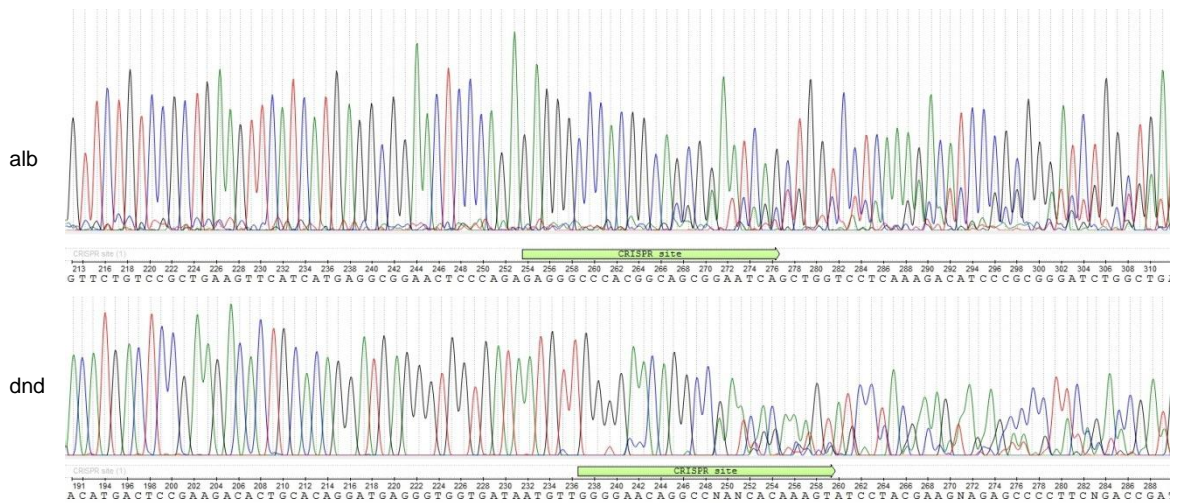
Supplementary table S2. Overview of all fish sampled and used in the analysis of mutated and control fish. The table shows fish number (No), sample type (Sample), sex (F/M), phenotype of gonad, if sample was included in qPCR analysis, sampling date and length, weight and age (months) of fish.

Supplementary Figure S1

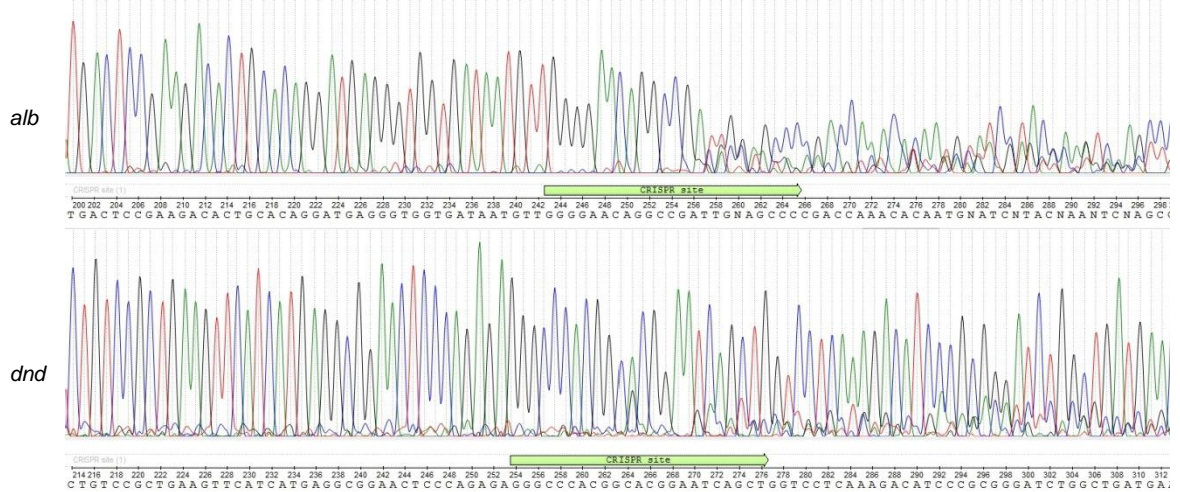
A control fish



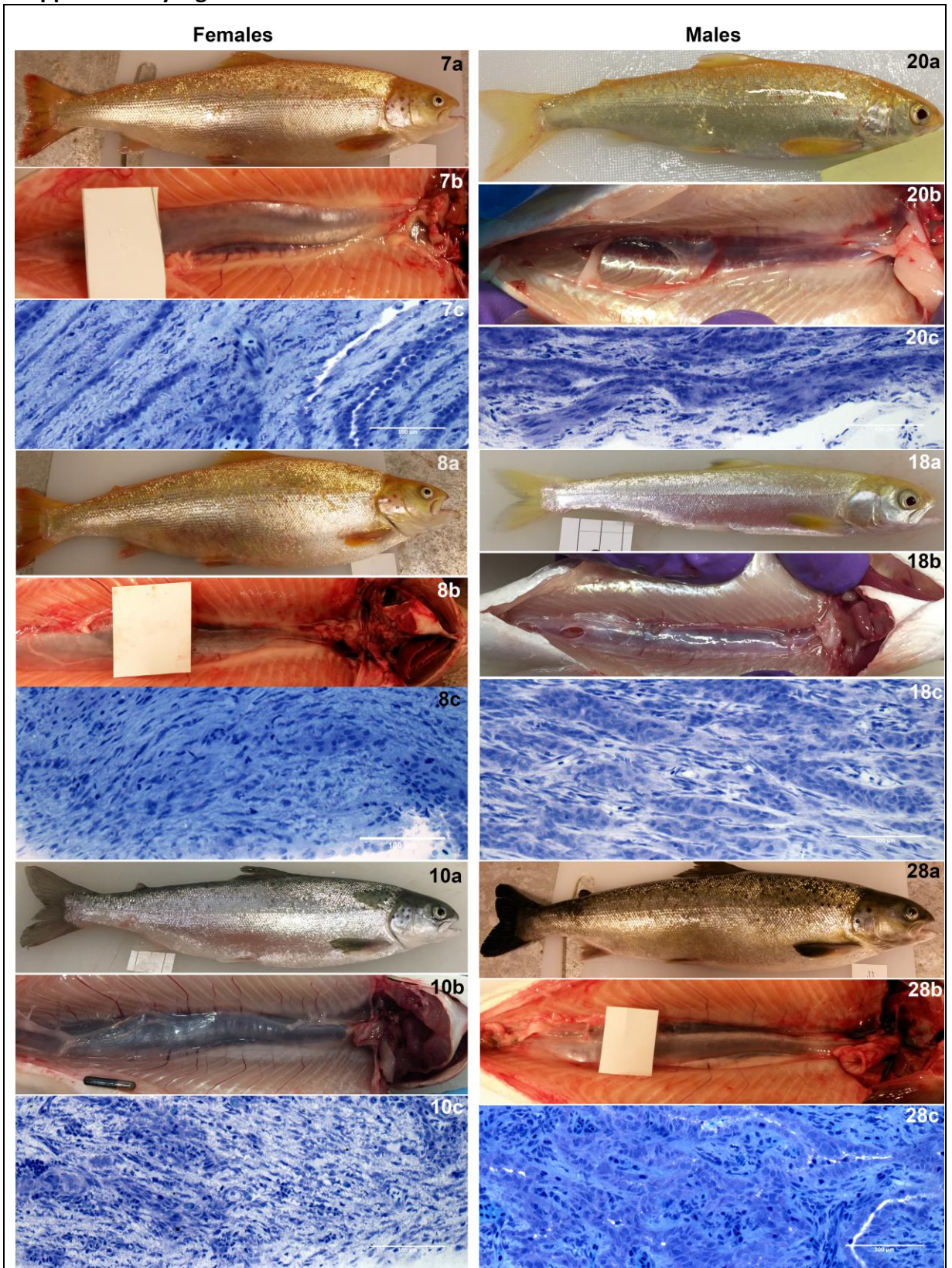
B *dnd/alb* KO fish, male



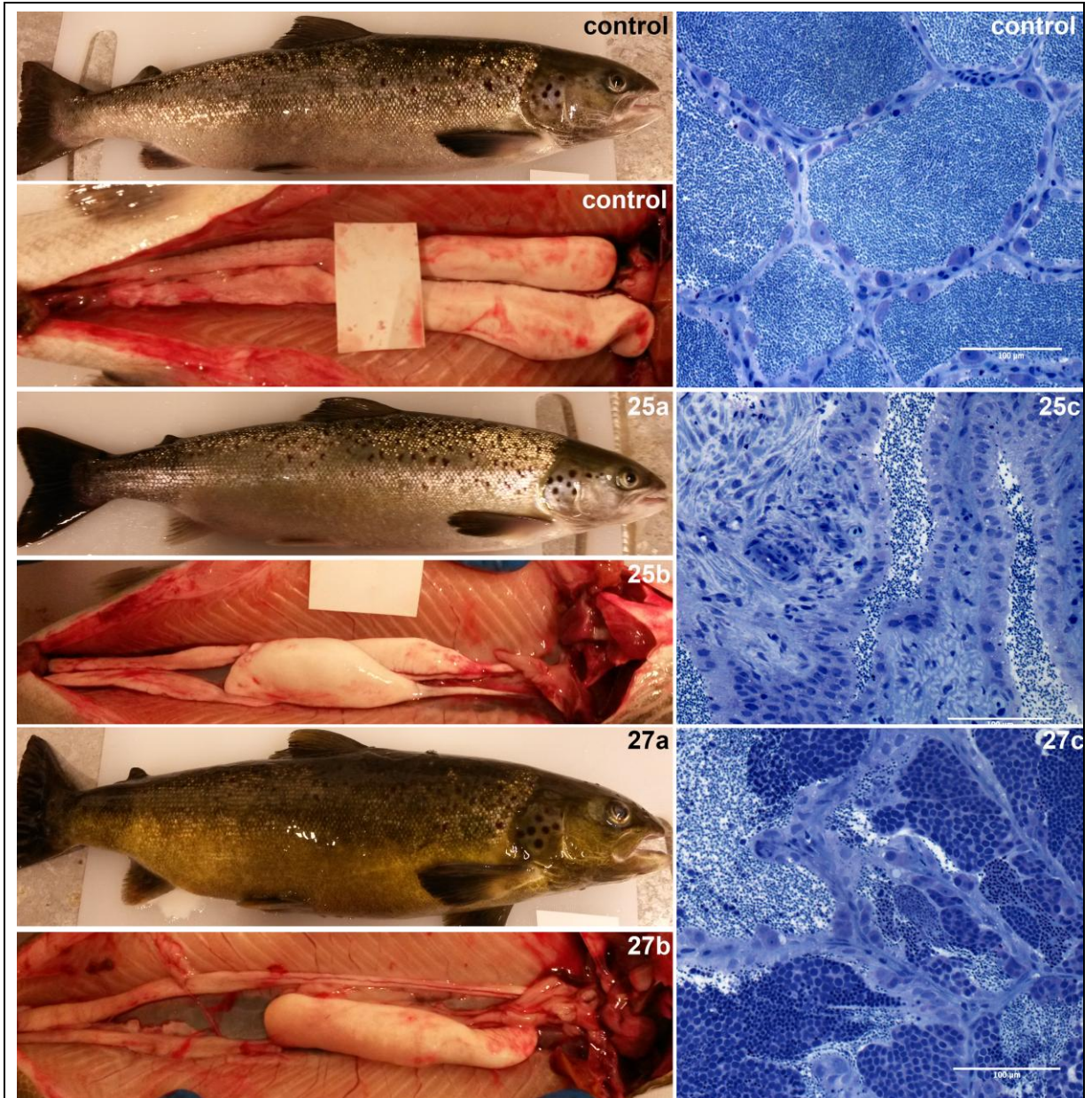
C *dnd/alb* KO fish, female



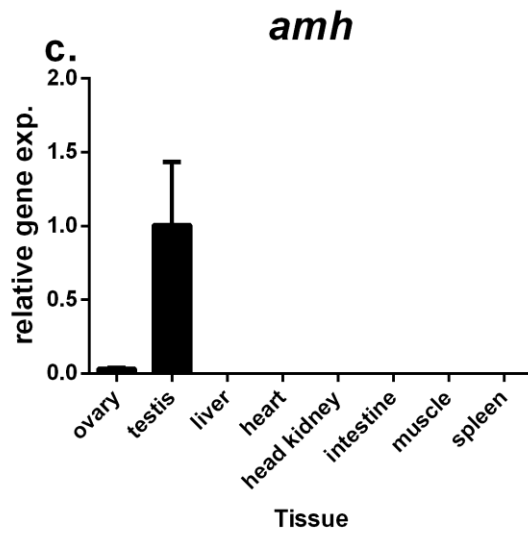
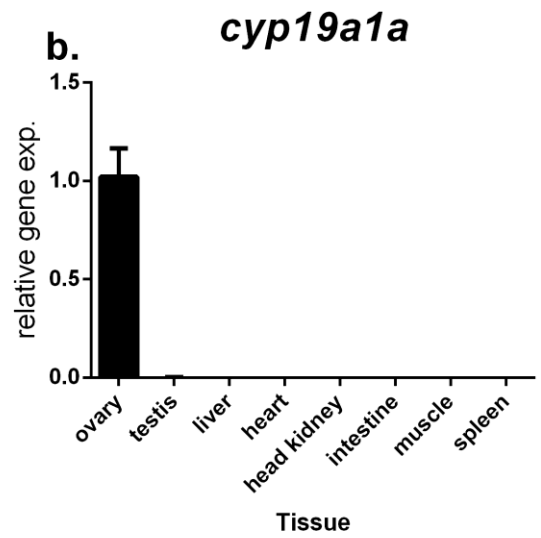
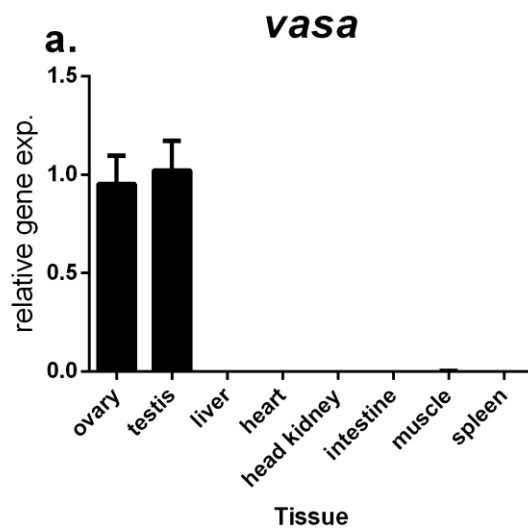
Supplementary Figure S2



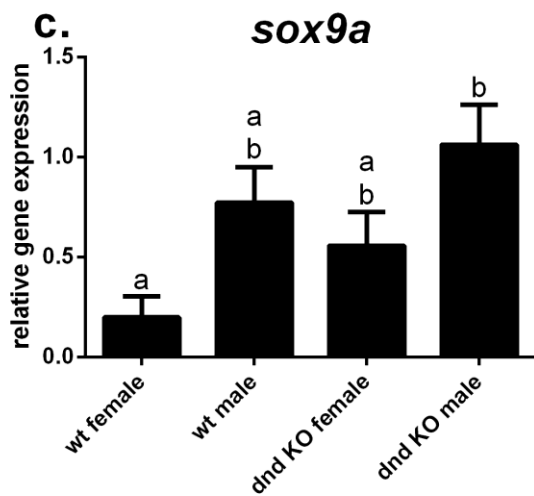
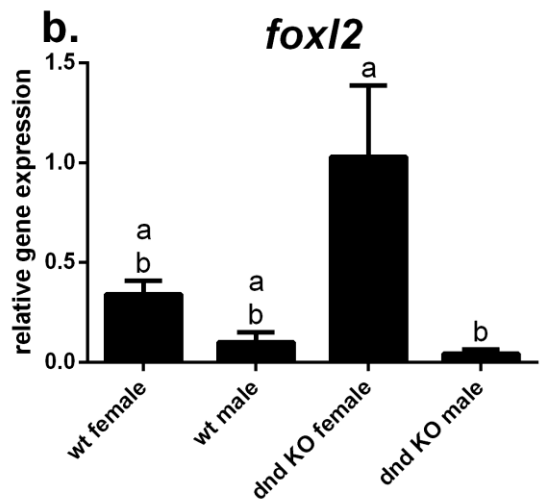
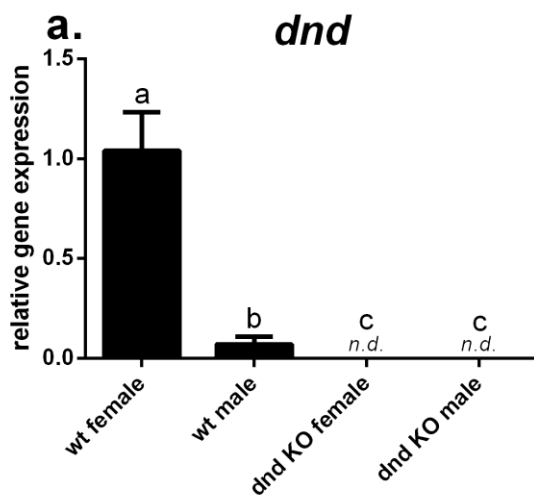
Supplementary Figure S3



Supplementary Figure S4



Supplementary Figure S5



Supplementary table S1. Oligonucleotides and primers**CRISPR target site oligonucleotides**

Oligo name	Oligo
<i>dnd target</i>	5'-GGGCCCACGGCACGGAACAGCGG-3'
<i>dnd 1F</i>	5'-TAGGGCCCACGGCACGGAACAG-3'
<i>dnd 1R</i>	5'-AAACCTGTTCCGTGCCGTGGGC-3'

PCR primers used in mutation screens for *dnd*

Primer name	Primer sequence
<i>dnd</i> PCRF	5'-TACATGCATCATTCCCACCCC-3'
<i>dnd</i> PCRR	5'-AAGTTCCACCATTACACTGCTT-3'

Primers and probes used for qPCR

Primer/ probe name	Primer sequence
<i>vasa</i> F	5'-GAGGTCCTCAAGACTACAACCTTGTGT-3'
<i>vasa</i> R	5'-GCACATTGCAGCCCTTCAGT-3'
<i>vasa</i> probe	Fam-AGCACCGGTCACACCATTTCGTGAG-TAMRA
<i>cyp19a1a</i> F	5'-TCAAACAGAACCCTGACGTAG-3'
<i>cyp19a1a</i> R	5'-GCTCCCTTTCACCTATAGCAGTGT-3'
<i>cyp19a1a</i> probe	Fam-ACAGCTCCTAGAAGAGAT-MGB
<i>amh</i> F	5'-CAGTCACTCTCTGCAGCCTTACAA-3'
<i>amh</i> R	5'-CAACATTGAATCTCCATTTCAGTTTAC-3'
<i>amh</i> probe	Fam-TTTGCCCTCGGGTTGCTTTCCTGTT-TAMRA
<i>ef1a</i> F	5'-CCCCTCCAGGACGTTTACAAA-3'
<i>ef1a</i> R	5'-CAGACGGCCCACAGGTACA-3'
<i>ef1a</i> probe	Fam-ATCGGTGGTATTGGAAC-MGB
<i>dnd</i> F	5'-TCTGTACAGGGCCTGATGGT-3'
<i>dnd</i> R	5'-TAAAACAAAGTAGGGGATCTGTG-3'
<i>foxl2</i> F	5'-TACTGGGATCACGAGAGCAAAC-3'
<i>foxl2</i> R	5'-ACAAGTGGCCCTCTGGTAAAC-3'
<i>sox9a</i> F	5'-TGCAGAACTCAGCAAAACCC-3'
<i>sox9a</i> R	5'-TTTCTCCTTCTGGGCTGGTAC-3'

Supplementary table S2. Overview of all fish sampled and used in the analysis of data.

No	Sample	sex ¹	Phenotype	qPCR	Sampling date	Length	Weight	Age (months)
1 ²	Control	F	Normal immature female	Yes	20/1-2015	25,8	186	15
2	Control	F	Normal immature female	Yes	20/1-2015	29,9	309	15
3	Control	F	Normal immature female	Yes	20/1-2015	28,1	246	15
4 ²	<i>dnd/alb</i> KO	F	Female lacking ovarian bulbs.	No	20/1-2015	16	49	12
5	<i>dnd/alb</i> KO	F	Female lacking ovarian bulbs.	Yes	20/1-2015	18,9	62	12
6	<i>dnd/alb</i> KO	F	Female lacking ovarian bulbs.	No	12/8-2015	43	1063	18
7 ³	<i>dnd/alb</i> KO	F	Female lacking ovarian bulbs.	No	12/8-2015	42,5	1022	18
8 ³	<i>dnd/alb</i> KO	F	Female lacking ovarian bulbs.	No	12/8-2015	42	1051	18
9	<i>dnd/alb</i> KO	F	Female lacking ovarian bulbs.	No	12/8-2015	42,5	1072	18
10 ³	<i>dnd</i> KO	F	Female lacking ovarian bulbs.	Yes	20/1-2015	28,3	255	15
11	<i>dnd</i> KO	F	Female lacking ovarian bulbs.	Yes	20/1-2015	27	248	15
12	<i>dnd</i> KO	F	Female lacking ovarian bulbs.	Yes	20/1-2015	24	153	15
13	<i>dnd</i> KO	F	Normal immature female	No	20/1-2015	27	229	15
14	Control	M	Immature male	Yes	12/3-2015	26,5	208	17
15 ²	Control	M	Immature male	Yes	12/3-2015	20,5	95	17
16	Control	M	Immature male	Yes	12/3-2015	21,5	122	17
17	<i>dnd/alb</i> KO	M	Male lacking germ cells with tubular structure.	Yes	20/1-2015	20	77	12
18 ³	<i>dnd/alb</i> KO	M	Male lacking germ cells with tubular structure.	Yes	20/1-2015	17,3	40	12
19 ²	<i>dnd/alb</i> KO	M	Male lacking germ cells with tubular structure.	Yes	12/3-2015	13.5	31	14
20 ³	<i>dnd/alb</i> KO	M	Male lacking germ cells with tubular structure.	Yes	12/3-2015	22	110	14
21	<i>dnd</i> KO	M	Male lacking germ cells with tubular structure.	No	20/1-2015	26	191	15
22	<i>dnd</i> KO	M	Male lacking germ cells with tubular structure.	No	20/1-2015	27,5	231	15
23	<i>dnd</i> KO	M	Immature male,	No	20/1-2015	25,2	191	15
24	<i>dnd</i> KO	M	Maturing male with half gonad- mosaic	No	20/1-2015	27	254	12
25 ⁴	<i>dnd</i> KO	M	Maturing male with mosaic gonad	No	12/8-2015	34	548	20
26	<i>dnd</i> KO	M	Maturing male with mosaic gonad	No	12/8-2015	30	363	20
27 ⁴	<i>dnd</i> KO	M	Maturing male with mosaic gonad	No	12/8-2015	37	724	20
28 ³	<i>dnd</i> KO	M	Male lacking germ cells with tubular structure.	No	12/8-2015	41,5	1012	20
29	<i>dnd</i> KO	M	Male lacking germ cells with tubular structure.	No	12/8-2015	44	1322	20
30	<i>dnd</i> KO	M	Male lacking germ cells with tubular structure.	No	12/3-2015	38	781	20

¹ sdy PCR determined genetic sex

² Fish shown in Figure 1

³ Fish shown in Supplementary figure S2

⁴ Fish shown in Supplementary figure S3