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# Supplementary Materials for

## Retinoic acid synthesis by ALDH1A proteins is dispensable for meiosis initiation in the mouse fetal ovary

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**This PDF file includes:**

**SUPPLEMENTARY MATERIALS**

**Fig. S1: Validation of an ATRA-reporter inducible mouse model.**

A. Schematic maps of the *Tg(RARE-Hspa1b-cre/ER$^{T2}$)* construct, in which ATRA-response elements (RARE) coupled to the *Hspa1b* minimal promoter (Yellow rectangles) drive the expression of the tamoxifen (TAM) inducible cre$^{ERT2}$ recombinase (blue rectangles), thus allowing ATRA-responsive cell lineage tracing. Blue triangles indicate *loxP* sequences. *RARE-Hspa1b-cre/ER$^{T2}$* allows *Gt(ROSA)26Sor$^{tm4(ACTB-tdTomato,-EGFP)Luo}$* (noted *mTmG*) recombination in ATRA-responsive cells upon Tamoxifen (TAM) administration. The *Gt(ROSA)26Sor$^{tm4(ACTB-tdTomato,-EGFP)Luo}$* reporter line triggers membrane GFP-staining in the cells where the CRE recombinase is active. mG: membrane GFP.

B. Immunodetection of CDH1 (epithelial cells) (red) and GFP (cells where the cre$^{ERT2}$ is active) (green) at 13.5 d*pc* in the developing eye and heart of *Tg(RARE-Hspa1b-cre/ER$^{T2}$)* embryos. DAPI (blue): nuclei.

Fig. S1

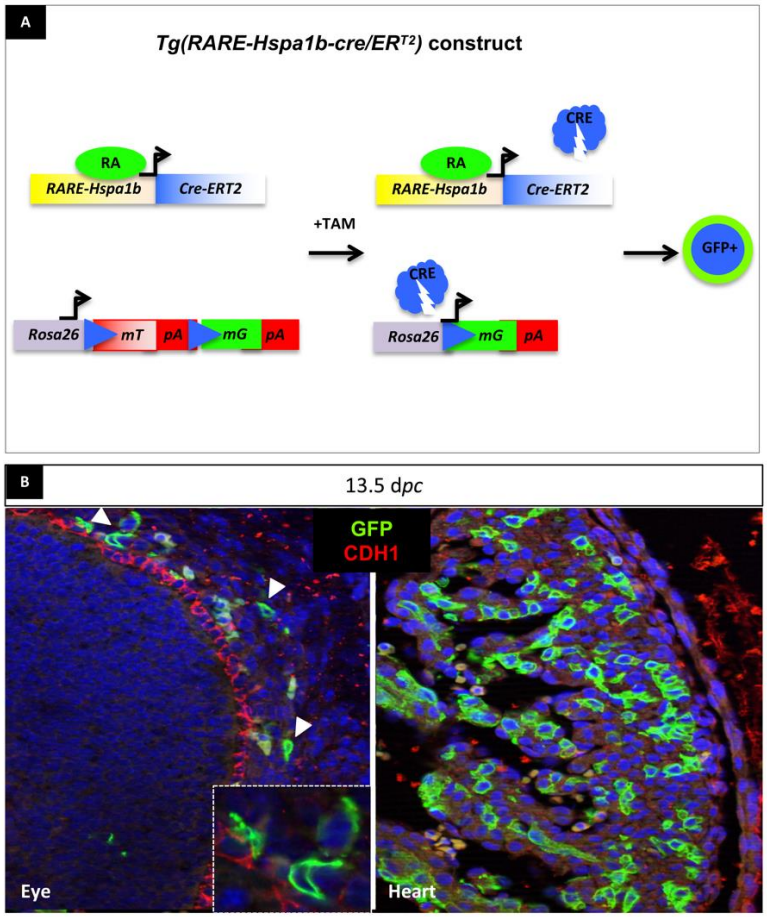

A
Tg(RARE-Hspa1b-cre/ERT2) construct
RA
RARE-Hspa1b
Cre-ERT2
CRE
+TAM
GFP+
Rosa26
mT
pA
mG
pA
B
13.5 dpc
GFP
CDH1
Eye
Heart

**Fig. S2: Construction of a stable loss-of-function of *Aldh1a1-3* in mouse embryos.**

A. Schematic maps of the targeted *Aldh1a1*, *Aldh1a2* and *Aldh1a3* alleles (left panel) and the CRE-recombined resultant alleles (right panel). Exons are shown as black (not recombined) or grey (recombined exons) boxes, whereas intronic sequences are shown as solid lines. Red triangles sandwiching exons in grey indicate *loxP* sequences.

B. Schematic representation of the experimental mating strategy for obtention of triple conditional mutant mice. Both parents are carrying the *Aldh1a1-3* triple floxed alleles, and the father only is carrying the *Cre*-encoding gene (upper panel), which allows collecting 50% of *Cre*-positive; *Aldh1a1-3* triple floxed embryos (25% being females).

C. Haematoxylin and Eosin staining at 13.5 d*pc* in control (left panel) and *CAGG-CreER*$^{TM}$*;Aldh1a1-3*$^{flox/flox}$ (right panel) hearts.

Fig. S2

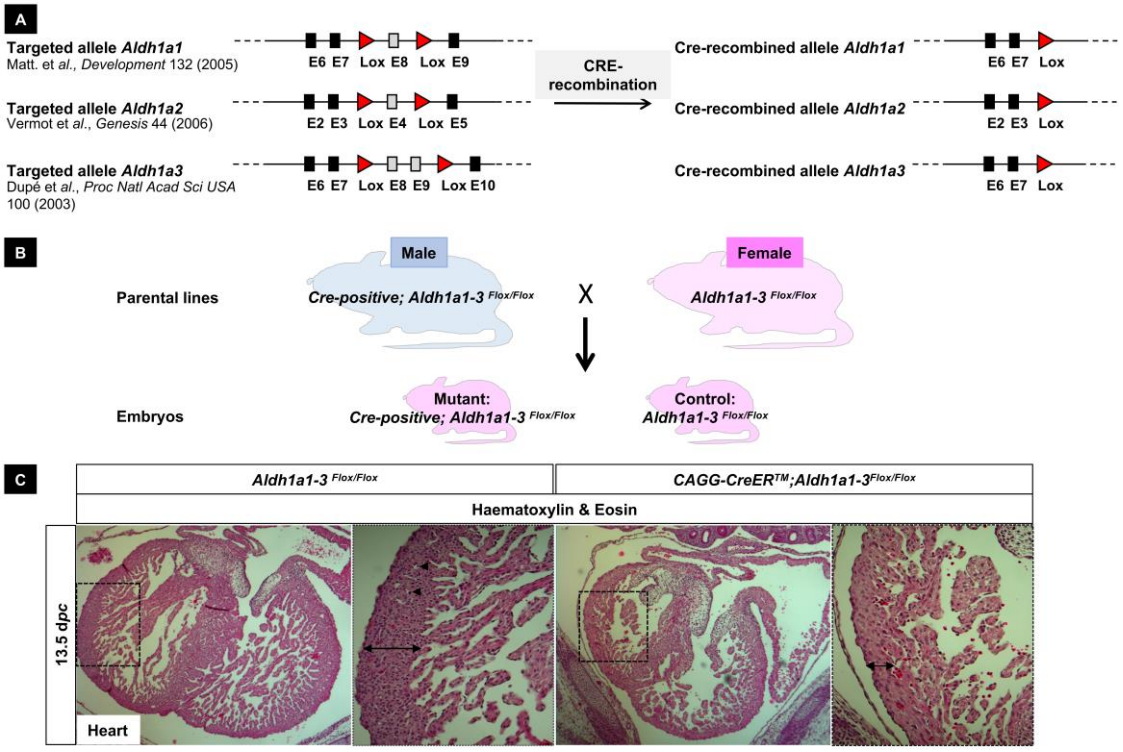

A
Targeted allele Aldh1a1
Matt. et al., Development 132 (2005)
E6 E7 Lox E8 Lox E9
CRE-recombination
Cre-recombined allele Aldh1a1
E6 E7 Lox
Targeted allele Aldh1a2
Vermot et al., Genesis 44 (2006)
E2 E3 Lox E4 Lox E5
Cre-recombined allele Aldh1a2
E2 E3 Lox
Targeted allele Aldh1a3
Dupé et al., Proc Natl Acad Sci USA 100 (2003)
E6 E7 Lox E8 E9 Lox E10
Cre-recombined allele Aldh1a3
E6 E7 Lox
B
Parental lines
Male
Cre-positive; Aldh1a1-3 Flox/Flox
X
Female
Aldh1a1-3 Flox/Flox
Embryos
Mutant:
Cre-positive; Aldh1a1-3 Flox/Flox
Control:
Aldh1a1-3 Flox/Flox
C
Aldh1a1-3 Flox/Flox
CAGG-CreER™;Aldh1a1-3Flox/Flox
Haematoxylin & Eosin
13.5 dpc
Heart

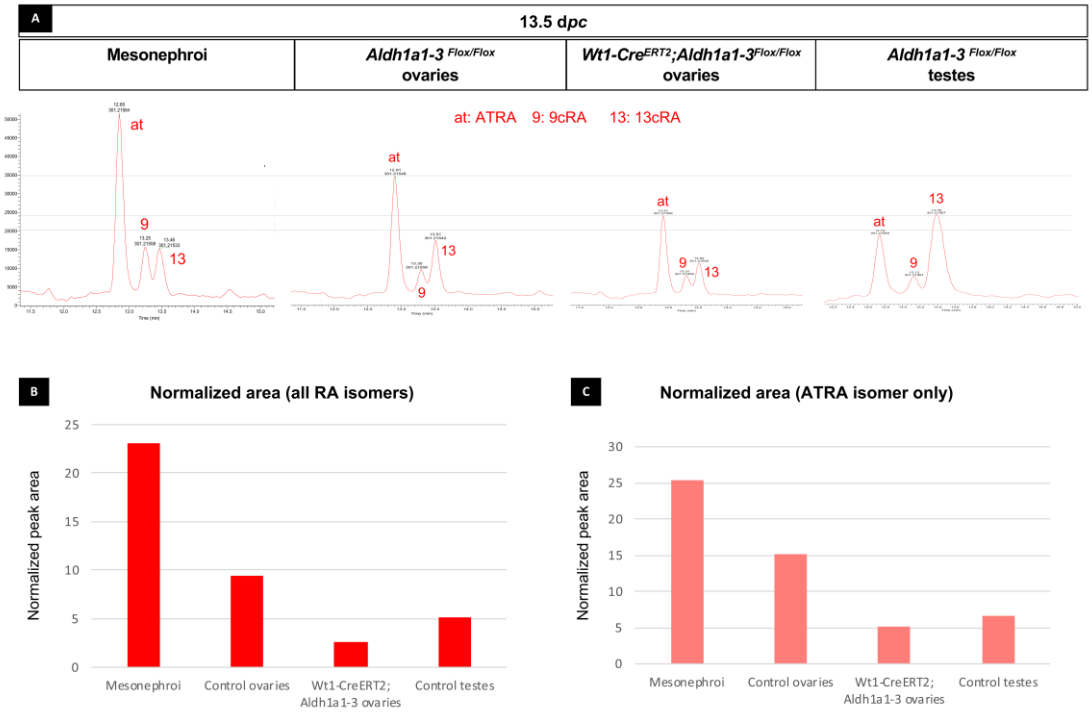
**Fig. S3: Retinoic Acid isomer separation by mass spectrometry in control and *Wt1-Cre$^{ERT2}$;Aldh1a1-3$^{flox/flox}$* embryos.**

A. Mass spectrometry chromatograms of All-*Trans* Retinoic Acid (ATRA, at), 9-*Cis* Retinoic Acid (9cRA, 9) and 13-*Cis* Retinoic Acid (13cRA, 13) isomers in *Aldh1a1-3$^{flox/flox}$* mesonephroi, *Aldh1a1-3$^{flox/flox}$* ovaries, *Wt1-Cre$^{ERT2}$;Aldh1a1-3$^{flox/flox}$* ovaries and *Aldh1a1-3$^{flox/flox}$* testes from 13.5 d*pc* littermate embryos. n = 12 individual gonads or mesonephroi.

B. Histograms: quantification of all three RA isomers using total peak area normalized by the protein concentration of each sample (*Aldh1a1-3$^{flox/flox}$* mesonephroi, *Aldh1a1-3$^{flox/flox}$* ovaries, *Wt1-Cre$^{ERT2}$;Aldh1a1-3$^{flox/flox}$* ovaries and *Aldh1a1-3$^{flox/flox}$* testes).

C. Histograms: quantification of ATRA isomer only using ATRA (at) peak area normalized by the protein concentration of each sample (*Aldh1a1-3$^{flox/flox}$* mesonephroi, *Aldh1a1-3$^{flox/flox}$* ovaries, *Wt1-Cre$^{ERT2}$;Aldh1a1-3$^{flox/flox}$* ovaries and *Aldh1a1-3$^{flox/flox}$* testes).

**Fig. S3**


A
13.5 dpc
Mesonephroi
Aldh1a1-3 Flox/Flox ovaries
Wt1-CreERT2;Aldh1a1-3Flox/Flox ovaries
Aldh1a1-3 Flox/Flox testes
at: ATRA 9: 9cRA 13: 13cRA
at
9
13


B
Normalized area (all RA isomers)
Normalized peak area
25
20
15
10
5
0
Mesonephroi
Control ovaries
Wt1-CreERT2; Aldh1a1-3 ovaries
Control testes


C
Normalized area (ATRA isomer only)
Normalized peak area
30
25
20
15
10
5
0
Mesonephroi
Control ovaries
Wt1-CreERT2; Aldh1a1-3 ovaries
Control testes

**Fig. S4: Genetic ablation of all three *Aldh1a1, Aldh1a2* and *Aldh1a3* does not impair germ cell differentiation *in vivo*.**

A- Protocol of induction of *Aldh1a1-3* deletion (9.5 d*pc* onwards). TAM: Tamoxifen.

B- RT-qPCR analysis of *Pou5f1*, *Sox2* and *Dazl* expression in 11.5 and, C- 12.5 d*pc* control (orange) and *Wt1-Cre$^{ERT2}$;Aldh1a1-3$^{flox/flox}$* (blue) gonads. Student's *t*-test, unpaired. Bars represent mean +SEM, n = 10 individual gonads. ns: not significant.

D. Immunodetection of GATA4 (cyan, gonadal somatic cells) and POU5F1 (pluripotent germ cells, red); Middle panels: GATA4 (cyan), DAZL (germ cells, red) and FUT4 (or SSEA1, germ cells, green); SOX2 (pluripotent germ cells, green) and FUT4 (germ cells, red); Lower panel: DAZL (germ cells, red) and POU5F1 (pluripotent germ cells, green) in 11.5 and 12.5 d*pc* control and *Wt1-Cre$^{ERT2}$;Aldh1a1-3$^{flox/flox}$* ovaries. DAPI (blue): nuclei. Scale bars (white): 50 micrometers.

Fig. S4

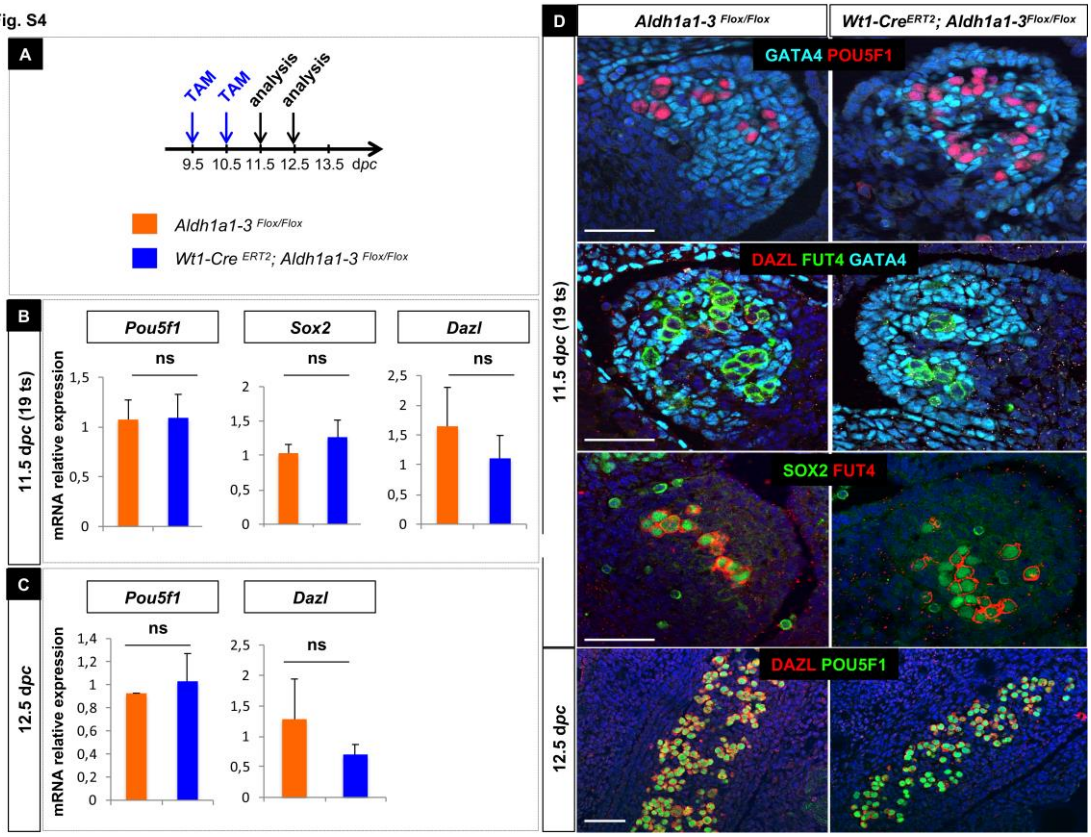

A
TAM
TAM
analysis
analysis
9.5 10.5 11.5 12.5 13.5 dpc
Aldh1a1-3 Flox/Flox
Wt1-Cre ERT2; Aldh1a1-3 Flox/Flox
B
11.5 dpc (19 ts)
Pou5f1
Sox2
Dazl
ns
mRNA relative expression
C
12.5 dpc
Pou5f1
Dazl
ns
mRNA relative expression
D
Aldh1a1-3 Flox/Flox
Wt1-CreERT2; Aldh1a1-3Flox/Flox
11.5 dpc (19 ts)
12.5 dpc
GATA4 POU5F1
DAZL FUT4 GATA4
SOX2 FUT4
DAZL POU5F1

**Fig. S5: Genetic ablation of all three *Aldh1a1, Aldh1a2* and *Aldh1a3* genes using either *Sf1-cre* or *Wt1-cre$^{ERT2}$* induced at 9.5 d*pc* does not impair germ cell entry into meiosis.**

A. Left panel: RT-qPCR analysis of *Stra8* expression in 13.5 d*pc* control (orange) and *Sf1-Cre*;*Aldh1a1-3$^{flox/flox}$* (pale blue) ovaries. Student's *t*-test, unpaired. Bars represent mean +SEM, n = 10 individual gonads. * p<0.05. Right panel: *In situ* hybridization using *Stra8* riboprobe at 13.5 d*pc* in control (left panel) and *Sf1-Cre;Aldh1a1-3$^{flox/flox}$* (right panel) ovaries. Scale bars (black): 50 micrometers.

B. Left panel: Protocol of induction of *Aldh1a1-3* deletion (9.5 d*pc* onwards). TAM: tamoxifen. RT-qPCR analysis of *Stra8* expression in 13.5 d*pc* control (orange) and *Wt1-Cre$^{ERT2}$;Aldh1a1-3$^{flox/flox}$* (blue) gonads. Student's *t*-test, unpaired. Bars represent mean +SEM, n = 10 individual gonads. **p<0.01. Right panel: *In situ* hybridization using *Stra8* riboprobe at 13.5 d*pc* in control (left panel) and *Wt1-Cre$^{ERT2}$;Aldh1a1-3$^{flox/flox}$* (right panel) gonads. Scale bars (black): 50 micrometers.

Fig. S5

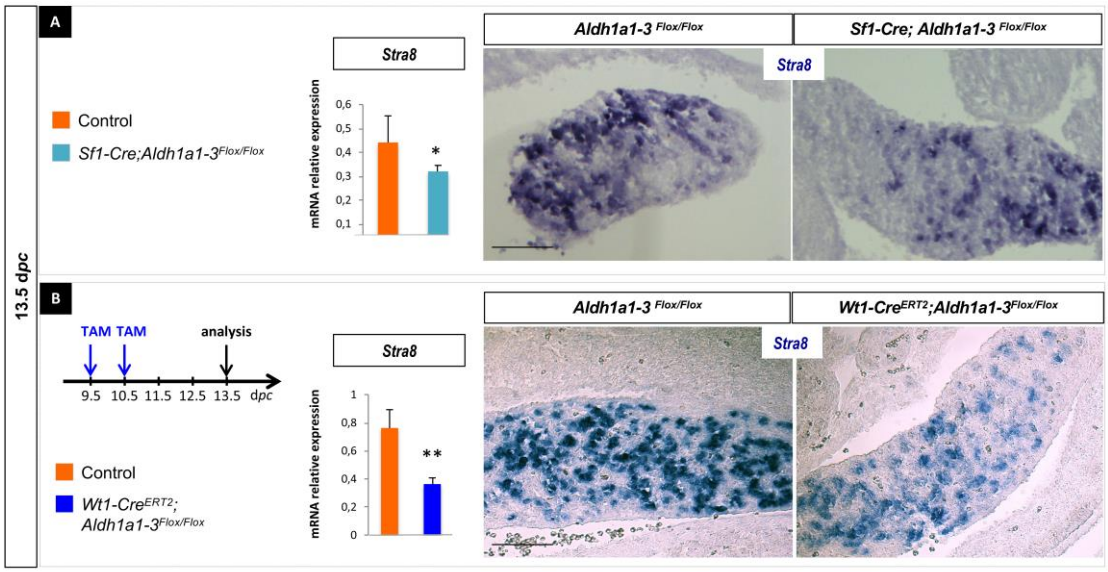

13.5 dpc
A
Control
Sf1-Cre;Aldh1a1-3Flox/Flox
Stra8
mRNA relative expression
0,6
0,5
0,4
0,3
0,2
0,1
*
Aldh1a1-3 Flox/Flox
Sf1-Cre; Aldh1a1-3 Flox/Flox
Stra8
B
TAM TAM
analysis
9.5 10.5 11.5 12.5 13.5 dpc
Control
Wt1-CreERT2; Aldh1a1-3Flox/Flox
Stra8
mRNA relative expression
1
0,8
0,6
0,4
0,2
0
**
Aldh1a1-3 Flox/Flox
Wt1-CreERT2;Aldh1a1-3Flox/Flox
Stra8

**Fig. S6: Comparison of two experimental conditions for *Aldh1a1 and Aldh1a2* deletion**

A- Protocol of induction of *Aldh1a1-3* deletion 10.5 (condition A) or 9.5 (condition B) d*pc* onwards. TAM: tamoxifen.

B- RT-qPCR analysis of *Aldh1a1 and Aldh1a2* expression in 13.5 d*pc* control (orange) and *Wt1-Cre*$^{ERT2}$*;Aldh1a1-3*$^{flox/flox}$ (blue) gonads in experimental conditions A and B. Student's *t*-test, unpaired. Bars represent mean +SEM, n = 10 individual gonads. ***p<0.001.

Fig. S6

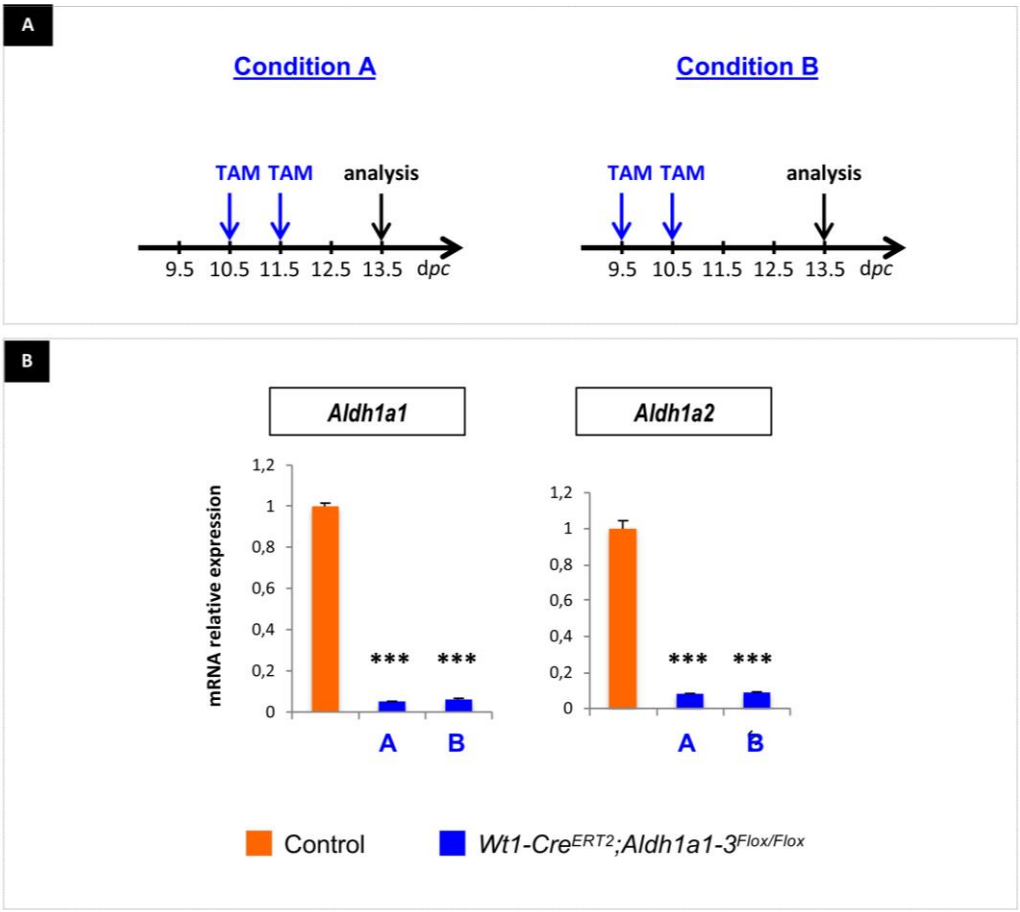

A
Condition A
TAM TAM
analysis
9.5 10.5 11.5 12.5 13.5 dpc
Condition B
TAM TAM
analysis
9.5 10.5 11.5 12.5 13.5 dpc
B
Aldh1a1
Aldh1a2
mRNA relative expression
1,2
1
0,8
0,6
0,4
0,2
0
***
***
A
B
Control
Wt1-CreERT2;Aldh1a1-3Flox/Flox

**Fig. S7: Genetic ablation of all three *Aldh1a1, Aldh1a2* and *Aldh1a3* genes does not impair germ cell entry into meiosis.**

Upper panel: Immunodetection of DAZL (red) and SYCP3 (green) in 16.5 d*pc* control and *Wt1-Cre$^{ERT2}$;Aldh1a1-3$^{flox/flox}$* ovaries. Middle panel: Immunodetection of Phospho Histone γ-H2AX (red) and DDX4 (green) in 16.5 d*pc* control and mutant ovaries. DAPI (blue): nuclei. Scale bars (white): 50 micrometers. Lower panel, Histograms: quantification of the percentage of both Phospho Histone γH2AX- and DDX4-positive cells 16.5 d*pc* in control (orange) and *Wt1-Cre$^{ERT2}$;Aldh1a1-3$^{flox/flox}$* (blue) gonads. Student's *t*-test, unpaired. Bars represent mean +SEM, ns: not significant.

Fig. S7

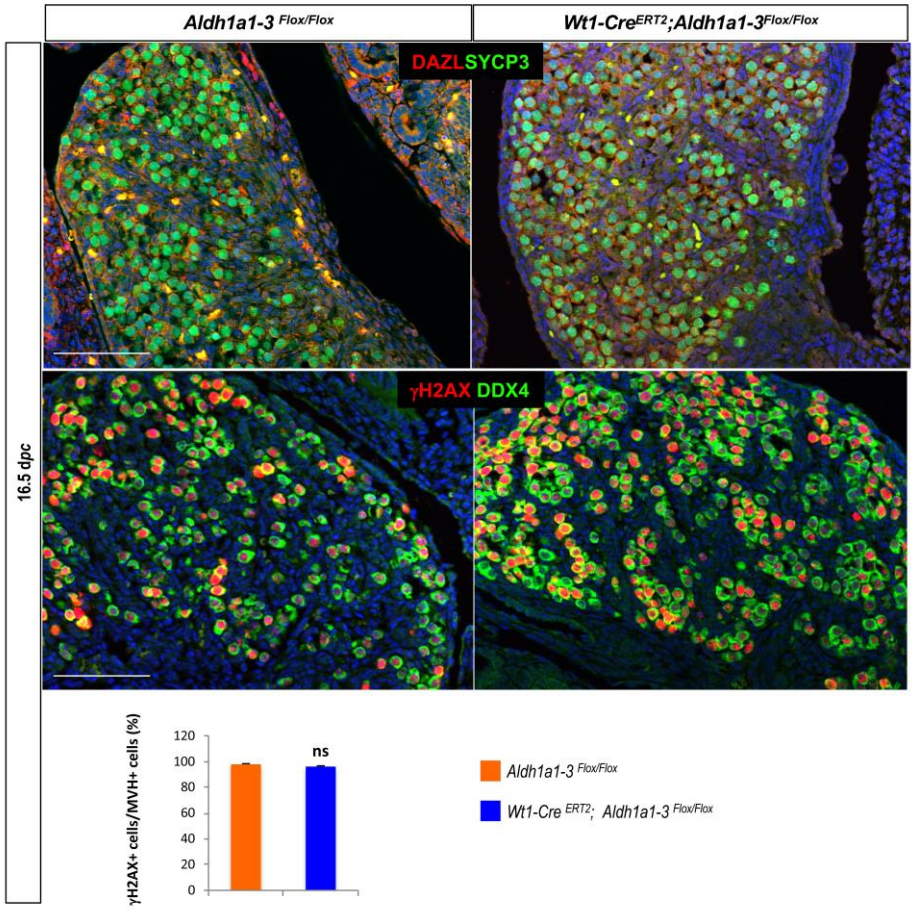

Aldh1a1-3 Flox/Flox
Wt1-CreERT2;Aldh1a1-3Flox/Flox
16.5 dpc
DAZLSYCP3
γH2AX DDX4
γH2AX+ cells/MVH+ cells (%)
120
100
80
60
40
20
0
ns
Aldh1a1-3 Flox/Flox
Wt1-Cre ERT2; Aldh1a1-3 Flox/Flox

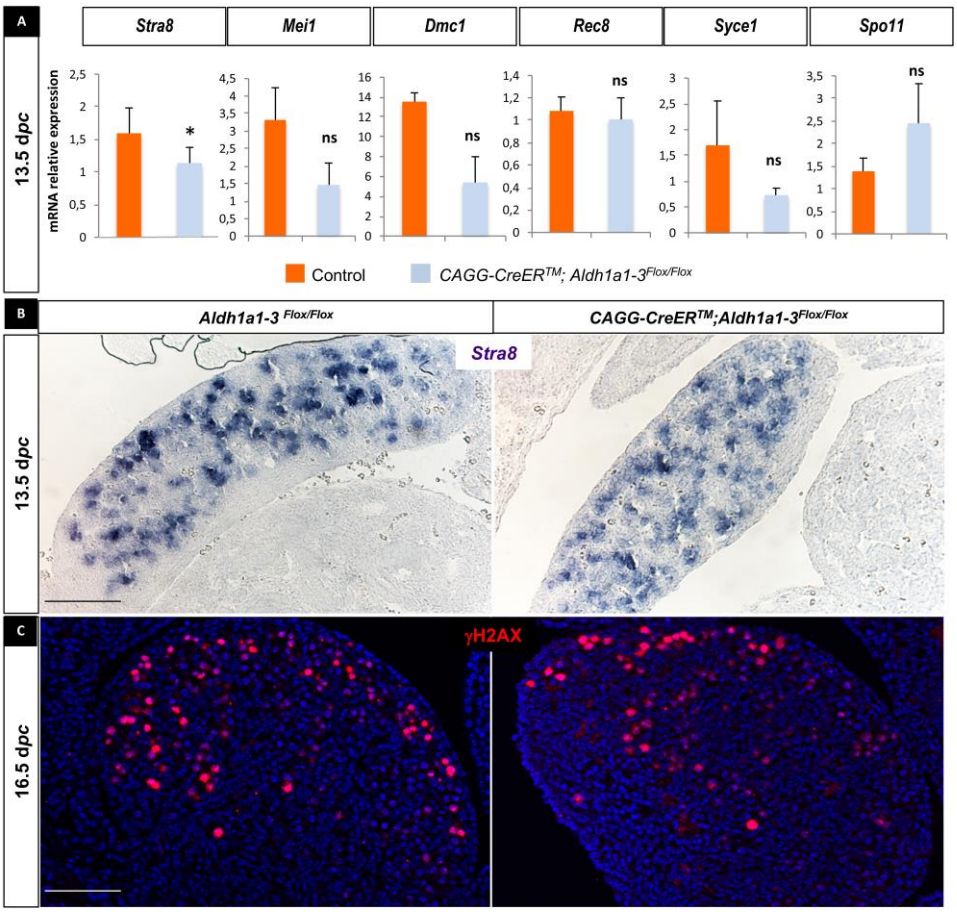
**Fig. S8: Genetic ablation of all three *Aldh1a1, Aldh1a2* and *Aldh1a3* genes in the whole gonad failed to impair meiosis *in vivo*.**

A- RT-qPCR analysis of *Stra8*, *Mei1*, *Dmc1*, *Rec8, Syce 1* and *Spo11* expression in 13.5 d*pc* control (orange) and *CAGG-CreER*$^{TM}$*; Aldh1a1-3*$^{flox/flox}$ (blue) gonads. Student's *t*-test, unpaired. Bars represent mean +SEM, n = 5 individual gonads. * $p<0.05$; ns: not significant.

B- *In situ* hybridization using *Stra8* riboprobe at 13.5 d*pc* in control (left panel) and *CAGG-CreER*$^{TM}$*;Aldh1a1-3*$^{flox/flox}$ (right panel) gonads. Scale bars (black): 50 micrometers.

C. Immunodetection of Phospho Histone γH2AX (red) in 16.5 d*pc* control and *CAGG-CreER*$^{TM}$*;Aldh1a1-3* $^{flox/flox}$ ovaries. DAPI (blue): nuclei. Scale bars (white): 50 micrometers.

Fig. S8


A
Stra8
Mei1
Dmc1
Rec8
Syce1
Spo11
13.5 dpc
mRNA relative expression
*
ns
Control
CAGG-CreER^TM; Aldh1a1-3^Flox/Flox
B
Aldh1a1-3 Flox/Flox
CAGG-CreER^TM;Aldh1a1-3^Flox/Flox
13.5 dpc
Stra8
C
16.5 dpc
γH2AX