

Online Research @ Cardiff

This is an Open Access document downloaded from ORCA, Cardiff University's institutional repository: <http://orca.cf.ac.uk/123704/>

This is the author's version of a work that was submitted to / accepted for publication.

Citation for final published version:

Mohamed, Noha-Ehssan, Hay, Trevor, Reed, Karen R., Smalley, Matthew J. and Clarke, Alan R. 2019. APC2 is critical for ovarian WNT signalling control, fertility and tumour suppression. BMC Cancer 19 , 677. 10.1186/s12885-019-5867-y filefilefilefilefilefilefilefilefile

Publishers page: <http://dx.doi.org/10.1186/s12885-019-5867-y> <<http://dx.doi.org/10.1186/s12885-019-5867-y>>

Please note:

Changes made as a result of publishing processes such as copy-editing, formatting and page numbers may not be reflected in this version. For the definitive version of this publication, please refer to the published source. You are advised to consult the publisher's version if you wish to cite this paper.

This version is being made available in accordance with publisher policies. See <http://orca.cf.ac.uk/policies.html> for usage policies. Copyright and moral rights for publications made available in ORCA are retained by the copyright holders.



Supplementary Information

Supplementary Figure Legends

Supplementary Figure 1: APC2 is dispensable for oviduct and uterine gross morphology. Microphotographs of **(a,b)** oviducts and **(c,d)** uteri from **(a,c)** *Apc2*^{+/+} and **(b,d)** *Apc2*^{-/-} 10-week-old female mice collected at diestrus stage. Bars = 100 μ m for oviducts and 200 μ m for uteri.

Supplementary Figure 2: APC2 is dispensable for corpora lutea. **(a,b)** Representative photomicrographs of corpus luteum in **(a)** *Apc2*^{+/+}, and **(b)** *Apc2*^{-/-} ovarian sections. **(c,d)** corpora lutea size **(c)**, and Ki67 cell counts **(d)** in the two genotypes (mean \pm SE; n = 4; no significant differences, t-test). **(e,f)** Representative photomicrographs of Ki67-positive cells (black arrows) in **(e)** *Apc2*^{+/+} and **(f)** *Apc2*^{-/-} corpora lutea. **(g,h)** Absence of cleaved caspase 3 staining in corpora lutea of **(g)** *Apc2*^{+/+}, and **(h)** *Apc2*^{-/-} ovarian sections. Positive cleaved caspase 3 staining, as a control, is indicated by red arrows in atretic follicles. **(i, j)** Representative photomicrographs of CD34 staining in corpora lutea of **(i)** *Apc2*^{+/+} and **(j)** *Apc2*^{-/-} ovaries. Bars = 200 μ m.

Supplementary Figure 3: Constitutive loss of APC2 has no effect on fertility hormones produced by pituitary gland or on the ovulation process. **(a,b)** Serum levels of **(a)** FSH and **(b)** LH measured by ELISA (mean \pm SE; n = 4 for LH, 6 for FSH; no significant differences, t-test). **(c)** Quantitation of atretic follicles in ovaries of *Apc2*^{+/+} and *Apc2*^{-/-} mice (mean \pm SE; n = 4 no significant differences, t-test). **(d)** Size distribution of atretic antral and pre-ovulatory follicles (mean \pm SE; n = 4; no significant differences, t-test). **(e)** Comparable proliferation of *Apc2*^{+/+} and *Apc2*^{-/-} follicular cells (mean \pm SE; n = 4; no significant differences, t-test). **(f)** Representative photomicrograph of follicles approaching the pre-ovulatory stage in *Apc2*^{+/+} and *Apc2*^{-/-} ovarian sections. Bar = 100 μ m. **(g)** qRT-PCR gene

expression levels of a subset of *Egf* ligands and the *Egfr* receptor, known to regulate COC expansion, in *Apc2*^{+/+} and *Apc2*^{-/-} ovarian extracts (mean \pm 95% confidence intervals; n=4; **P<0.01, significance determined from confidence intervals) (Cumming *et al.* 2007).

Supplementary Figure 4: Immunohistochemical localization of β -catenin in ovaries. (a-

g) 10-week-old female *Apc2*^{+/+} mice. **(h-n)** 10-week-old female *Apc2*^{-/-} mice. OSE: ovarian surface epithelium. 1ry: primary follicle. 2ry: secondary follicle. SA: small (early) antral follicle. LA: large antral follicle. PO: pre-ovulatory follicle. Cl: corpus luteum. AF: atretic follicles. Bars = 100 μ m, except **(f, l)** bars = 200 μ m. Insets shows granulosa cells of growing follicles at 4X original magnification.

Supplementary Figure 5: Gene expression analysis of GCTs formed in a subset of

APC2-deficient ovaries. qRT-PCR analysis of gene expression levels in one *Apc2* null tumour from a 12-month old animal and one *Apc2* heterozygote tumours from 18-month old animals compared to age-matched *Apc2* wild type ovaries (n=3 at both ages). Expression levels of GCT molecular marker *Foxl2* **(a)** was elevated in tumours while *Foxo1* expression **(b)** was reduced. WNT signalling target genes *Wif1* **(c)** and *Axin2* **(d)** were strongly increased in tumours, as was *Esr1* **(e)**, which is also typical for GCTs. n=3 for 12-months and 18-months *Apc2* wild type ovaries. Two independent samples from the each tumour were analysed and results from both these samples are shown. The small numbers of tumour samples available means that statistical analysis is not possible. T: Tumour.