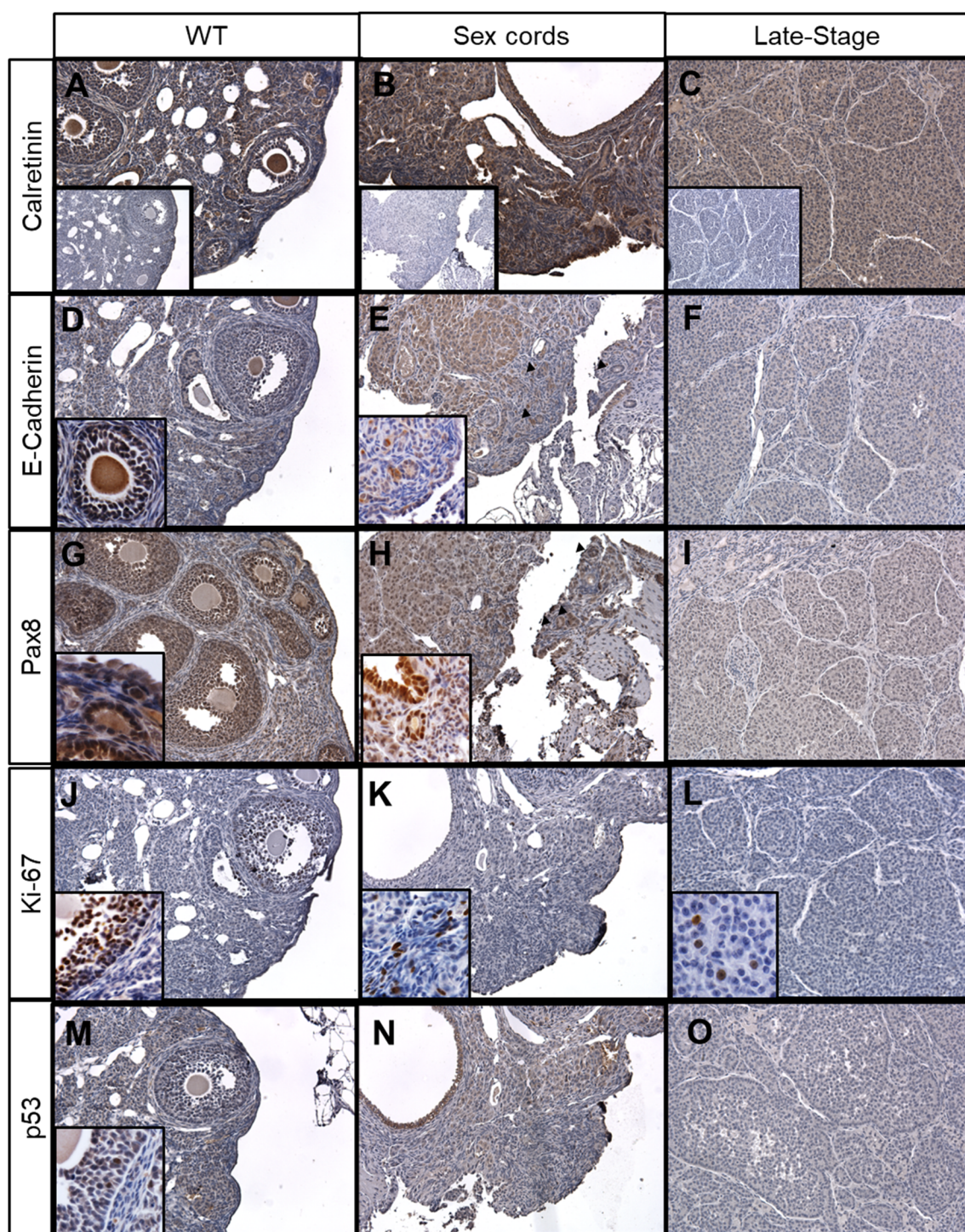*Article*

The Validation of a Precursor Lesion of Epithelial Ovarian Cancer in *Fancd2*-KO Mice

Sarah Sczelecki and Janet L. Pitman

Supplemental Figures

Figure S1. – Immunohistochemistry of common epithelial ovarian cancer markers to characterise a late-stage tumour phenotype in *Fancd2$^{-/-}$* mice. (A,D,G,J,M) depicts ovarian tissue from a 3-month *Fancd2$^{+/+}$* (wild-type; WT) mouse including primordial, primary and mature follicles, as well as stroma samples. (B,E,H,K,N) depicts ovarian tissue from a 3-month *Fancd2$^{-/-}$* ovary that includes sex cords (E,H arrowheads) and stroma. (C,F,I,L,O) depicts ovarian tissue from a 1-year *Fancd2$^{-/-}$* ovary with a later stage ovarian cancer neoplasm. (A,B,C) Calretinin is strongly expressed within all tissues and insets display secondary antibody only controls. (D) E-Cadherin and (G) Pax8 are both present in the ovarian surface epithelium and stroma, with Pax8 more strongly labelled in all granulosa cells, including primary follicles of WT mice. (E) E-Cadherin and (H) Pax8 are present in sex cords and stroma of 3-month *Fancd2$^{-/-}$* animals, but (F,I) are only present as weak and diffuse staining in the 1-year *Fancd2$^{-/-}$* phenotype. (J,K,L) Ki-67 staining is limited to a few cells within all samples and (M,N,O) p53 is present, and its weak and diffuse staining suggests normal function. Photomicrographs taken at 20x magnification or 40x for higher magnification in inset images in (D,E,G,H,J,K,L,M).
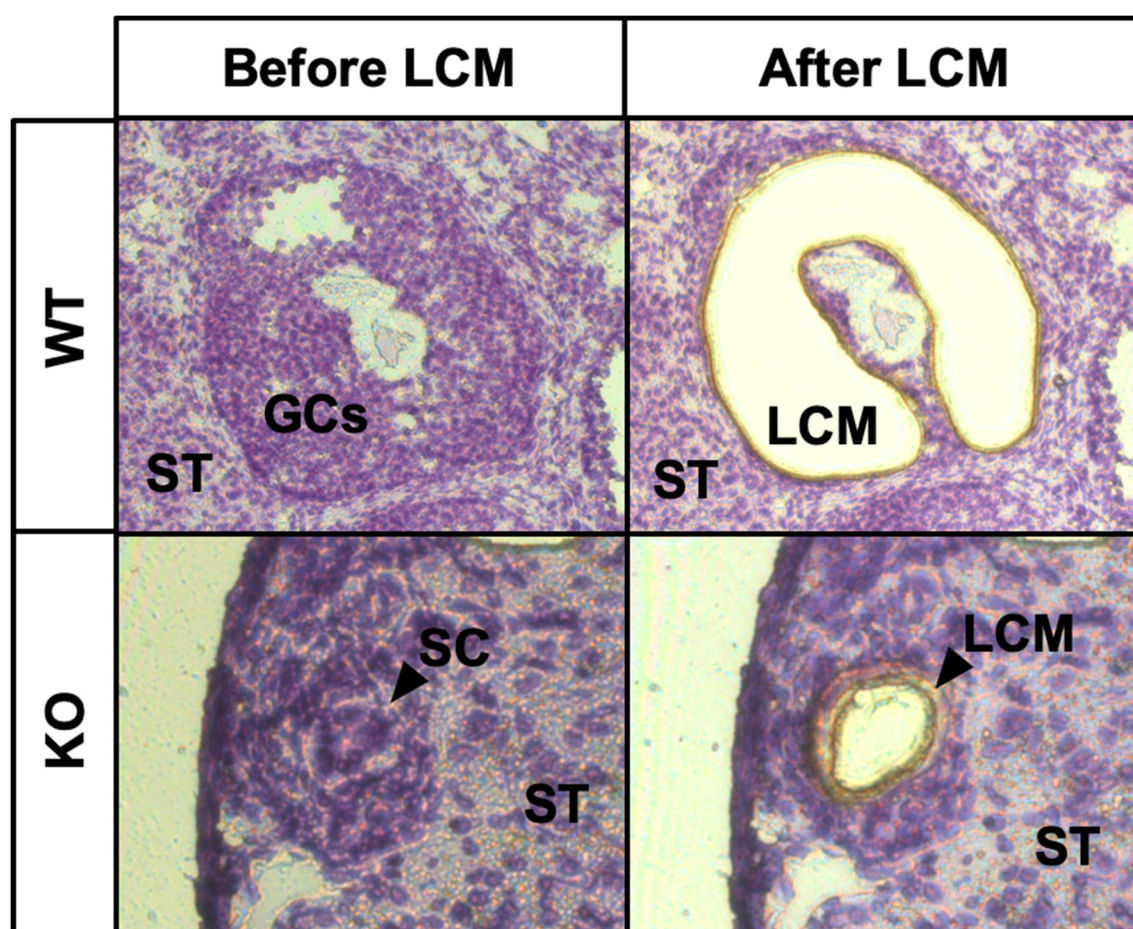
Figure S2. - LCM of mature GCs and sex cords. Representative images of tissue before and after laser capture micro-dissection (LCM) performed on *Fancd2$^{+/+}$* (WT) and *Fancd2$^{-/-}$* (KO) ovaries to collect mature GCs and sex cords (SC), respectively. ST = ovarian stroma.
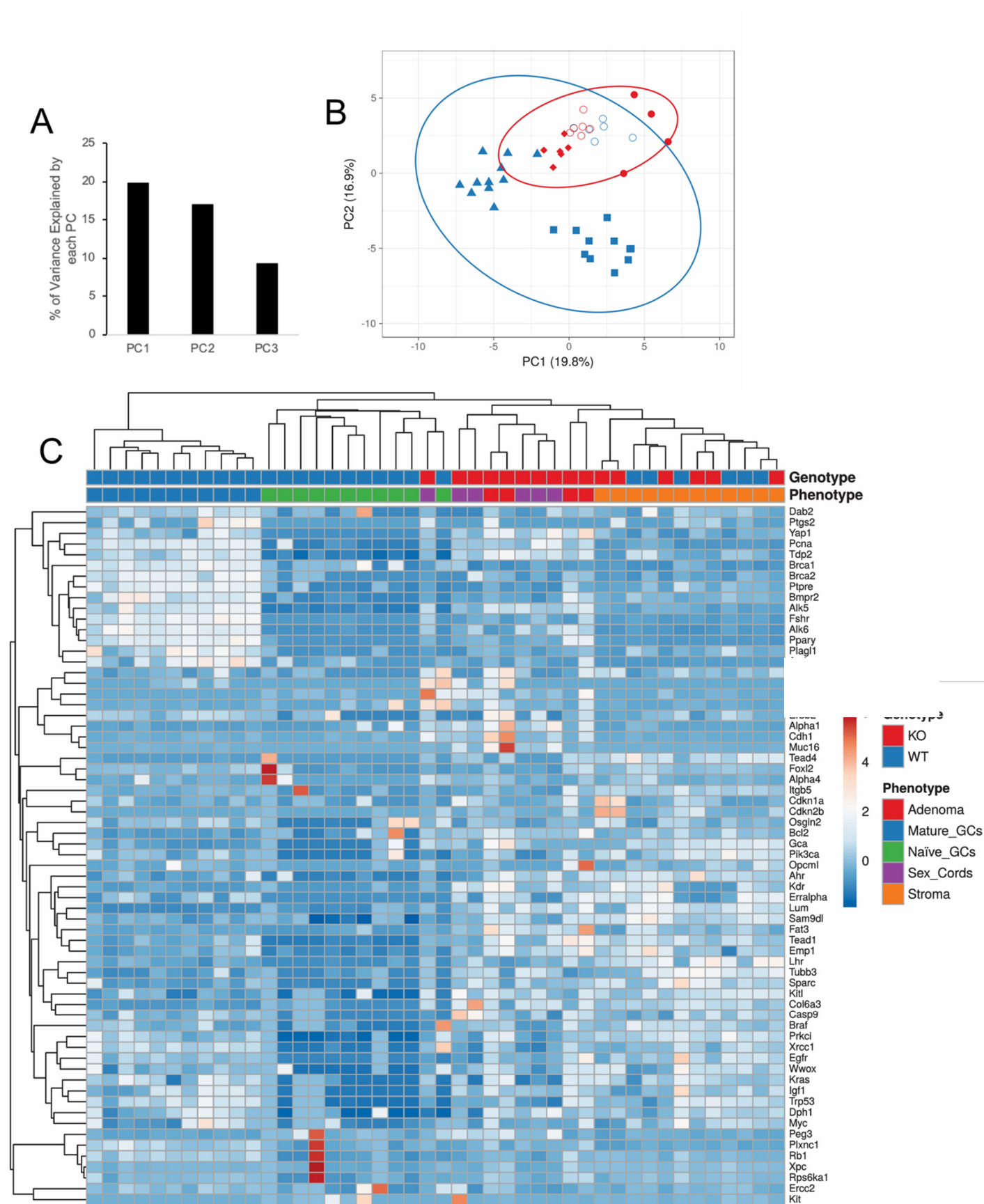
Figure S3. Unbiased analyses of relative gene expression data of sex cords and ovarian stroma from 3-month-old *Fancd2*⁻ᐟ⁻ (KO) mice, tubulostromal adenomas and ovarian stroma from 1-year old KO mice, naïve granulosa cells (GC), mature GC and stroma from 3-month-old *Fancd2*⁺ᐟ⁺ (WT) mice and

mature GC and stroma from 1-year old WT mice. (A) Bar graph displays the percent variance represented by the first three principal components. (B) X and Y axis show principal components 1 and 2, which explain 19.8% and 16.9% of the total variance, respectively. Data segregation based on genotype, WT vs KO, however stromal controls are intermediately mixed. Prediction ellipses are such that with a probability of 0.95, a new observation from the same group will fall inside the ellipse. N = 44 data points (C) Heat map of unsupervised hierarchical clustering with minimal misclassification. Data mirrors results of PCA plot. Rows are centred; unit variance scaling is applied to rows. Imputation is used for missing value estimation. Both rows and columns are clustered using correlation distance and average linkage. 65 rows, 44 columns.
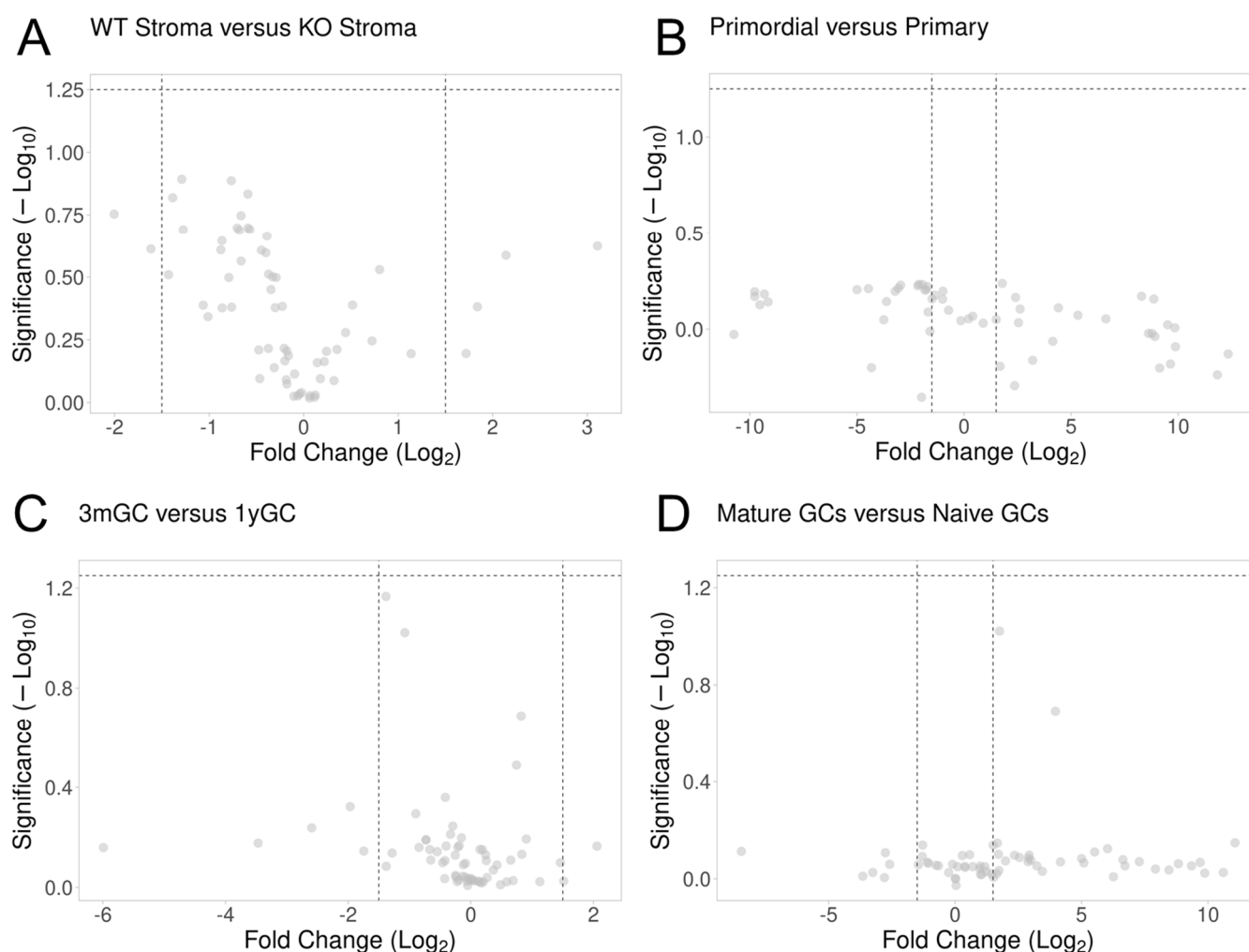
Figure S4. – Volcano plots representing non-significant gene expression changes following pairwise comparisons. (A) Ovarian stroma from 3-month-old *Fancd2*^{+/+} (wild type; WT) and *Fancd2*^{-/-} (knock out; KO) mice exhibited similar levels of expression of all genes tested and served as a genotype control. (B) Granulosa cells (GC) from primordial and primary follicles from WT mice exhibited similar levels of expression of all genes tested and confirmed that these sample types could be combined as naïve GCs. (C) GC from growing follicles of 3-month-old and 1-year old WT mice exhibited similar levels of expression of all genes tested and confirmed these sample types could be combined as mature GCs. (D) Naïve and mature GCs from WT mice exhibited similar levels of expression of all genes tested and supports the grouping of these phenotypes for further analyses. Thresholds represented as dashed lines, were set at a fold change of > 1.5 and p-value of < 0.05 for relative gene expression data from all GeXP gene sets. No genes for any of the control comparisons were significant based on designated threshold. .

Supplemental Tables

Table S1. Genome GeXP Multiplex Assay Set 1.

Gene Symbol	Accession Number	Primer Sequences (5′ – 3′)	[Primer μM] F R	Amplicon Length (bp)
Pax8	NM_011040	F- AGGTGACACTATAGAATAAGATCCTTGGCAGGTACT R- GTACGACTCACTATAGGGATGTAGTCTCCTATCTTCTCCA	2 0.5	145
Rpl19	NM_009078	F- AGGTGACACTATAGAATACCCACAAGCTCTTTCC R- GTACGACTCACTATAGGGAATCCAACCAGACCTTCTT	2 0.5	150
Hprt	NM_013556	F- AGGTGACACTATAGAATACAGGTGTTCTAGTCCTGTG R- GTACGACTCACTATAGGGAGTGCAAATCAAAAGTCTGG	2 0.0039	157
Col6α3	NM_001243008	F- AGGTGACACTATAGAATAGCCATTTCTAACTCGTTTCT R- GTACGACTCACTATAGGGATTAGCACCATATTGACAGG	2 0.0039	163
Notch3	NM_008716.3	F- AGGTGACACTATAGAATACTCGTATGTACCAAGTAGCC R- GTACGACTCACTATAGGGACTTTCCCAGTGAGTCTACA	2 0.5	174
Braf	NM_139294	F- AGGTGACACTATAGAATAGTCTTCCTGCCCAACA R- GTACGACTCACTATAGGGAGTTTCTTCTCTCCATCCTG	2 0.0078	182
Dph1	NM_144491	F- AGGTGACACTATAGAATACAACCCTAATATACCTGCTT R- GTACGACTCACTATAGGGAAAGGTTCCCAGAATAAGG	2 0.5	187
Erbb2	NM_001003817	F- AGGTGACACTATAGAATAAACTGGTGTGTTCAGATTG R- GTACGACTCACTATAGGGAATTCAGTCTCATCAATGTCC	2 0.0078	197
Ccne1	NM_007633	F- AGGTGACACTATAGAATAGAAATCTACCCTCCAAAG R- GTACGACTCACTATAGGGATGGACATAGACATTCAGC	2 0.5	202
Brca1	NM_009764	F- AGGTGACACTATAGAATAGACCCCCAAAGAAGTAAT R- GTACGACTCACTATAGGGAACTATCCACTTTCCTCCTG	2 0.5	208
Cdkn1a	NM_007669	F- AGGTGACACTATAGAATAAGACATTCAGAGCCACAG R- GTACGACTCACTATAGGGAGACGAAGTCAAAGTTCCAC	2 1	219
Casp3	NM_001284409	F- AGGTGACACTATAGAATAGTGCGAGATGAGGTGTT R- GTACGACTCACTATAGGGATCTTAGCGTACCGTTCC	2 0.5	224
Myc	NM_001177352	F- AGGTGACACTATAGAATACTCAGTGGTCTTTCCCTAC R- GTACGACTCACTATAGGGATTCTTGCTCTTCTTCAGAGT	2 0.5	233
Igf1	NM_010512	F- AGGTGACACTATAGAATATGTGACATTGCTCTAACATC R- GTACGACTCACTATAGGGACAGGTAGAAGAGGTGTGAAG	2 0.0039	240
Ercc2	NM_010512	F- AGGTGACACTATAGAATAGGAGGTGACCAAACTTATCTA R- GTACGACTCACTATAGGGAGACACTTCCCATCAACATC	2 0.5	246
Kit	NM_010512	F- AGGTGACACTATAGAATAACAAAGAGCAAATCCAG R- GTACGACTCACTATAGGGAGGAAACTCCCATTTGTG	2 1	257
Dlec1	NM_177117	F- AGGTGACACTATAGAATACTCTCCGTCTGTATAACTCC R- GTACGACTCACTATAGGGAGGGACAGTGTGGCTAGA	2 1	263
Aurka	NM_011497	F- AGGTGACACTATAGAATAGAGGGTTTGACTCCTATTTT R- GTACGACTCACTATAGGGAAGGGAACAGTGGTCTTAAC	2 0.5	273
Ppia	NM_008907	F- AGGTGACACTATAGAATAGATGACGAGCCCTTG R- GTACGACTCACTATAGGGAGGACCTGTATGCTTTAGGA	2 0.5	285
Kitl	NM_013598	F- AGGTGACACTATAGAATAGGTCCCTTAGTAAAAGTGAA R- GTACGACTCACTATAGGGATCCTTAGTAGCACCCTACAT	2 0.125	291
Arl11	NM_177337	F- AGGTGACACTATAGAATATGCCCTAACCACTCG R- GTACGACTCACTATAGGGACCCTAGTAAATCCTCCTG	2 0.5	299
Bax2	NM_007527	F- AGGTGACACTATAGAATACTACAGGGTTTCATCCAG R- GTACGACTCACTATAGGGAAAAGTAGAAGAGGGCAACC	2 0.5	307
Dab2	NM_023118	F- AGGTGACACTATAGAATAAGTCCGTGTACTTTGTGG R- GTACGACTCACTATAGGGACATCATCAATACCGATTAGC	2 0.0078	318
Trp53	NM_011640	F- AGGTGACACTATAGAATACCGAAGACTGGATGACT R- GTACGACTCACTATAGGGAAGAAGGGACAAAAGATGAC	2 0.5	336
Kras	NM_021284	F- AGGTGACACTATAGAATACCTGTCTCTTGGATATTCTC	2	345

Gene Symbol	Accession Number	Primer Sequences (5′ – 3′)	[Primer µM] F R	Amplicon Length (bp)
		R- GTACGACTCACTATAGGGAAACACCCTGTCTTGTCTTT	0.125	
Diras2	NM_001024474	F- AGGTGACACTATAGAATACCTACCCCAAGTTTGTAAC R- GTACGACTCACTATAGGGAGCTGTAACTGAGCTTTATTG	2 0.5	363
Casp9	NM_015733	F- AGGTGACACTATAGAATATCCACTTGAGTTTAGATGG R- GTACGACTCACTATAGGGACCTCTCTCCTGTGTGTC	2 0.5	377
Brca2	NM_001081001	F- AGGTGACACTATAGAATAGGGTGCTGTGATGTATCTAGT R- GTACGACTCACTATAGGGAGATTCCTCTGTGGTGTTTT	2 0.5	383

Table S2. Genome GeXP Multiplex Assay Set 2.

Gene Symbol	Accession Number	Primer Sequences (5′ – 3′)	[Primer µM] F R	Amplicon Length (bp)
Lum	NM_008524	F- AGGTGACACTATAGAATATTGGCATTAGTCGGTAGT R- GTACGACTCACTATAGGGAGGGGCAGTTACATTCTG	2 0.0078	142
Rpl19	NM_009078	F- AGGTGACACTATAGAATACCCACAAGCTCTTTCC R- GTACGACTCACTATAGGGAATCCAACCAGACCTTCTT	2 0.5	150
Hprt	NM_013556	F- AGGTGACACTATAGAATACAGGTGTTCTAGTCCTGTG R- GTACGACTCACTATAGGGAGTGCAAATCAAAAGTCTGG	2 0.0156	157
Prkci	NM_008857	F- AGGTGACACTATAGAATAGTGAGTTGTGTCAAGTTTGT R- GTACGACTCACTATAGGGACAGGCATCATCAGGTC	2 0.0156	169
Peg3	NM_008817	F- AGGTGACACTATAGAATAGTGCGTAGAGTGCTGTG R- GTACGACTCACTATAGGGACTGATCTCCCCTTGCT	2 0.5	176
Rb1	NM_009029	F- AGGTGACACTATAGAATAGGCATCTGCATCTTTATC R- GTACGACTCACTATAGGGACATAGCATTATCAACCTTGG	2 0.5	184
Samd9l	NM_010156	F- AGGTGACACTATAGAATACGAAACTGGAAACTGAAG R- GTACGACTCACTATAGGGAGTCCGTGACAAACAACTT	2 0.5	196
Rab25	NM_016899	F- AGGTGACACTATAGAATATATCTCCACCTCCCTTACT R- GTACGACTCACTATAGGGACCAGGCACAATACAAAAC	2 0.5	206
Tubb3	NM_023279	F- AGGTGACACTATAGAATACAAGACAAGCAGCATCT R-GTACGACTCACTATAGGGAATACAGAGGTGGCTAAAATG	2 0.5	212
Bcl2	NM_009741	F- AGGTGACACTATAGAATAGATGCCTTTGTGGAACT R- GTACGACTCACTATAGGGACTGCTTTAGTGAACCTTTTG	2 0.5	221
Kdr	NM_010612	F- AGGTGACACTATAGAATATATGAGCCAAGTGTTAAGTG R- GTACGACTCACTATAGGGAGTAACCCGACTGTGAAGA	2 0.5	230
Osgin2	NM_145950	F- AGGTGACACTATAGAATAGTTTGGGTATTGGCAGA R- GTACGACTCACTATAGGGAGCAGTAACACATTTCACAG	2 0.5	236
Gca	NM_145523	F- AGGTGACACTATAGAATAGATACCTGGGTTATTCTGAC R- GTACGACTCACTATAGGGATTTCCTGTGTAGTCTCTATCC	2 0.0156	245
Xpc	NM_009531	F- AGGTGACACTATAGAATAGGCTGTGTCCAGATGAC R- GTACGACTCACTATAGGGACCTTTTTCTCCTTCTCCT	2 0.5	251
Ptpre	NM_001316678	F- AGGTGACACTATAGAATAATCCTTCATAGATGGCTAC R- GTACGACTCACTATAGGGAGGTGTCGCTCTTTATCTCT	2 0.5	263
Itgb5	NM_001145884	F- AGGTGACACTATAGAATATACAAAACTGCTAAGGACTG R- GTACGACTCACTATAGGGACGCTCACTTTGGAACT	2 0.0156	270
Sparc	NM_009242	F- AGGTGACACTATAGAATACGAGACTTTGAGAAGAACTAC R- GTACGACTCACTATAGGGAGATGTCCTGCTCCTTGAT	2 0.0156	277
Ppia	NM_008907	F- AGGTGACACTATAGAATAGATGACGAGCCCTTG R- GTACGACTCACTATAGGGAGGACCTGTATGCTTTAGGA	2 0.5	285
Plxnc1	NM_018797	F- AGGTGACACTATAGAATAGTTTGCATCCAGTTTGAC R- GTACGACTCACTATAGGGAAATTAGGAGCTAGAAACCTG	2 0.5	290
Xrcc1	NM_009532	F- AGGTGACACTATAGAATATTTGTGGAGGTGCTAGTG R- GTACGACTCACTATAGGGAAGGAGGGCTGTGAAACT	2 0.5	304

Gene Symbol	Accession Number	Primer Sequences (5′ – 3′)	[Primer μM] F R	Amplicon Length (bp)
Egfr	NM_207655	F- AGGTGACACTATAGAATATGTGCCCACTACATTGA R- GTACGACTCACTATAGGGAACGTCTTCGCATGAATAG	2 1	313
Cdkn2b	NM_007670	F- AGGTGACACTATAGAATAAACCTGGAGAGTAGATGAGA R- GTACGACTCACTATAGGGAAGAGCAACTCAAATGTAGGA	2 0.0156	331
Pik3ca	NM_008839	F- AGGTGACACTATAGAATAGAGGAGAACCCTTATGTGA R- GTACGACTCACTATAGGGATGACCCAGTAACACCAATA	2 0.0156	342
Opcml	NM_177906	F- AGGTGACACTATAGAATACCATCTCATAGTGCAAGTTC R- GTACGACTCACTATAGGGACCAGTGTTCTTGGCTTT	2 0.5	349
Plagl1	NM_009538	F- AGGTGACACTATAGAATACTGCTCTCACAGAAGATACC R- GTACGACTCACTATAGGGAGCTTATACTTGGAGACGAAG	2 0.5	358
Rps5ka1	NM_009097	F- AGGTGACACTATAGAATACGACACCTTCTACTTTGATA R- GTACGACTCACTATAGGGAGGATCTCTTTTGCTCTTGT	2 0.5	367
Wwox	NM_019573	F- AGGTGACACTATAGAATAGCTGCTACCACTGTCTACTG R- GTACGACTCACTATAGGGATCCCTGCTTCCCACTA	2 0.5	372
Rassf1	NM_001243748	F- AGGTGACACTATAGAATAAGCCAGAACTCATTGAACTAC R- GTACGACTCACTATAGGGATCGTATCCCGCTCTAGT	2 0.5	379
Akt2	NM_001110208	F- AGGTGACACTATAGAATATCCCTTGGTCTACTACGTC R- GTACGACTCACTATAGGGAGCTGTCACCTAGCTTGAG	2 0.5	387

Table S3. Genome GeXP Multiplex Assay Set 3.

Gene Symbol.	Accession Number	Primer Sequences (5′ – 3′)	[Primer μM] F R	Amplicon Length (bp)
Lhr	NM_013582.2	F- AGGTGACACTATAGAATAGTGAAAGCACAGTTAGAGAA R- GTACGACTCACTATAGGGATGGGTGAACAGAAGTCATA	2 0.002	142
Rpl19	NM_009078	F- AGGTGACACTATAGAATACCCACAAGCTCTTTCC R- GTACGACTCACTATAGGGAATCCAACCAGACCTTCTT	2 0.5	150
Hprt	NM_013556	F- AGGTGACACTATAGAATACAGGTGTTCTAGTCCTGTG R- GTACGACTCACTATAGGGAGTGCAAATCAAAAGTCTGG	2 0.0039	157
Ahr	NM_013464.4	F-AGGTGACACTATAGAATATCTGGTTTTCTGGCAAT R- GTACGACTCACTATAGGGAGGCTGAAGTGGAGTAGC	2 0.0015625	165
Pcna	NM_011045.2	F- AGGTGACACTATAGAATAGAAGCACCAAATCAAGAG R- GTACGACTCACTATAGGGAGCTAAGGTCTCGGCATA	2 0.0039	184
Pgc1α1	NM_008904.2	F- AGGTGACACTATAGAATAGGACATGTGCAGCCAAGACTCT R- GTACGACTCACTATAGGGACACTTCAATCCACCCAGAAAG CT	2 0.5	193
Foxl2	NM_012020.2	F- AGGTGACACTATAGAATAGCCCAGATGACACTCCTA R- GTACGACTCACTATAGGGACGAAAGACATTACTCAACCA	2 0.0039	201
Pgc1α4	NR_132764.1	F- AGGTGACACTATAGAATATCACACCAAACCCACAGAAA R- GTACGACTCACTATAGGGACTGGAAGATATGGCACAT	2 0.5	209
Alk6	NM_007560.4	F- AGGTGACACTATAGAATATGTCGTGTTGTCTAAATCTC R- GTACGACTCACTATAGGGATGTAAGAGGTCCATACCAG	2 0.5	216
Tead1	NM_001166584.1	F- AGGTGACACTATAGAATAGCTGAAGGTAACAAGCA R- GTACGACTCACTATAGGGATGGCTGTCCTGTCTGTAT	2 0.5	224
Cdh1	NM_009864.3	F- AGGTGACACTATAGAATATCTCTTGTCCCTTCCAC R- GTACGACTCACTATAGGGAAGTGTCCCTCCAAATCC	2 0.5	232
Tdp2	NM_019551.2	F- AGGTGACACTATAGAATAGCACCTTATTCCTCAAAGT R- GTACGACTCACTATAGGGATGTATTAGACCCAGATGTCC	2 0.125	237
Fat3	NM_001080814.1	F- AGGTGACACTATAGAATATGTCCTCAACATAACCATC R- GTACGACTCACTATAGGGAATTACCTCACCATTAGAACC	2 0.5	247

Gene Symbol.	Accession Number	Primer Sequences (5′ – 3′)	[Primer μM] F R	Amplicon Length (bp)
Emp1	NM_010128.4	F- AGGTGACACTATAGAATAATCTCCCTTGTGGTCTTC R- GTACGACTCACTATAGGGAAAGCTGAAACAGAAGCAG	2 0.5	252
Muc16	XM_011242634.1	F- AGGTGACACTATAGAATACCAGAGTCCAACACAGTTAT R- GTACGACTCACTATAGGGAGTGAACATCCCATTCTTCT	2 0.125	258
Pparγ	NM_001127330.2	F- AGGTGACACTATAGAATATCACCATTTGTCATCTACG R- GTACGACTCACTATAGGGAACACCATACTTGAGCAGAG	2 0.5	270
Bmpr2	NM_007561.4	F- AGGTGACACTATAGAATAAATCAGACGAAGAGCAG R- GTACGACTCACTATAGGGATCTCACCTATCCCAAGAT	2 0.0039	279
Ppia	NM_008907	F- AGGTGACACTATAGAATAGATGACGAGCCCTTG R- GTACGACTCACTATAGGGAGGACCTGTATGCTTTAGGA	2 0.125	285
Ptgs2	NM_011198.4	F- AGGTGACACTATAGAATAGGAACTAACCAAGAACAAAG R- GTACGACTCACTATAGGGAATGGGCTGGAAGACATA	2 0.5	295
Tead4	NM_011567.2	F- AGGTGACACTATAGAATAAAGATGTATGGTCGGAATG R- GTACGACTCACTATAGGGAAAACCCTGAGATTGCTG	2 1	307
Alk5	NM_009370.3	F- AGGTGACACTATAGAATAGCTTAGTGTTCTGGGAAAT R- GTACGACTCACTATAGGGACTGTTGGCTGAGTTGTG	2 0.125	318
Fshr	NM_013523.3	F- AGGTGACACTATAGAATACGTCAAGAGACACCAAGAT R- GTACGACTCACTATAGGGATTGTGGGTAATAGATGAGGT	2 0.125	338
Errα	NM_007953.2	F- AGGTGACACTATAGAATAAAAGTGAATGCCCAGGT R- GTACGACTCACTATAGGGACTGCTTGGAGTTATTGCTT	2 1	343
Mmp9	NM_013599.4	F- AGGTGACACTATAGAATAACAGCACCTCCCACTAT R- GTACGACTCACTATAGGGAGGGCTTCCTCTATGATT	2 1	348
Fcgbp	NM_001122603.1	F- AGGTGACACTATAGAATACCTGACAACAGCCACTAT R- GTACGACTCACTATAGGGACAAGGCACACTCCTCAC	2 0.5	355
Yap1	NM_001171147.1	F- AGGTGACACTATAGAATACTTGGTTATCTCCTCTCTACA R- GTACGACTCACTATAGGGAAATCAGGCTAAGGGAAGTA	2 0.5	360
Taz	NM_001173547.2	F- AGGTGACACTATAGAATACTGAAGTTGATGCGTTG R- GTACGACTCACTATAGGGAAGTAGGGTGGGCTGTTAG	2 0.5	365
Wfdc2	NM_026323.2	F- AGGTGACACTATAGAATAGCTCTCAGGGACAGATACT R- GTACGACTCACTATAGGGAGGCAGAAACAACCTCTCT	2 0.5	370
Amh	NM_007445.2	F- AGGTGACACTATAGAATAAGGGGAGACTGGAGAAC R- GTACGACTCACTATAGGGATCAGAGCCAAATAGAAAGG	2 0.5	385

Table S4. Detailed information about antibodies used for immunohistochemistry.

Supplier	Catalogue Number	Target	Dilution	Antigen Retrieval Solution
Abcam	ab92341	Calretinin	1:100	Sodium Citrate
Invitrogen	PA585088	E-Cadherin	1:500	Sodium Citrate
Abcam	ab97477	Pax8	1:500	Tris-EDTA
Abcam	ab16667	Ki-67	1:200	Sodium Citrate
Abcam	ab131442	p53	1:200	Sodium Citrate
Abcam	ab6721	Goat α Rabbit IgG HRP	1:300	N/A

Table S5. – Summary of staining intensity of immunohistochemical markers in representative samples. Three-month wild-type (WT) samples represents staining of granulosa cells within primordial, primary and growing follicles as well as stroma. Sex cords represents both sex cord structures and stroma within 3-month old *Fancd2*^{-/-} mice and late-stage represents adenomas from 1-year old *Fancd2*^{-/-} mice.

Marker	3-month WT	Sex Cords	Late-Stage
Calretinin	+++	+++	+++

E-Cadherin	+	++	+
Pax8	++	++	++
Ki-67	++	+	+
p53	++	++	++

Table S6. – Summary of all pairwise comparisons made for Gene Set Enrichment Analysis and the resulting number of enriched gene ontology (GO) terms for each comparison.

		Compared to:			
		Naïve GCs	**Mature GCs**	**Sex Cords**	**Adenoma**
GO Terms Enriched in:	Naïve GCs		0	0	0
	Mature GCs	20		10	8
	Sex Cords	23	0		1 p=0.053
	Adenoma	0	0	0	