

Single-cell and bulk transcriptional profiling of mouse ovaries reveals novel genes and pathways associated with DNA damage response in oocytes.

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Keywords: DNA Damage Response, CHEK2, oocyte, ovary, single-cell transcriptomics

Supplementary Materials:

Supplementary Figures (S1-S7)

Supplementary Tables (S1-S4)

Figure S1.

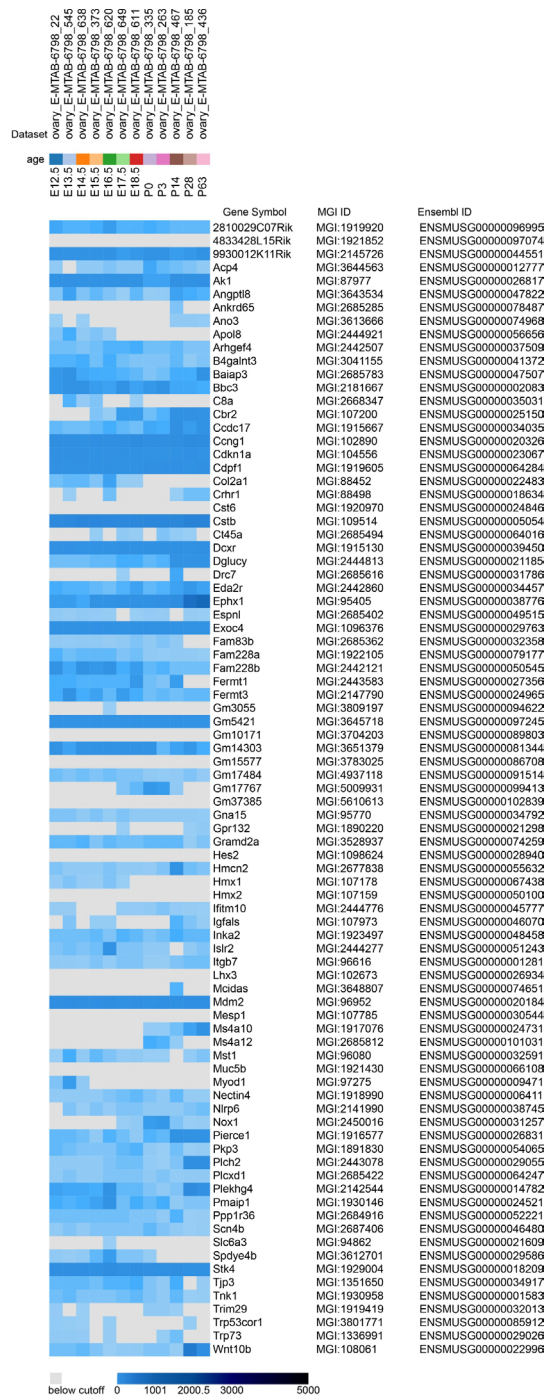


Figure S1. Expression of RRGs identified in the study during ovary development generated using published data and Gene Expression Database (GXD) at Mouse Genome Informatics Portal <https://www.informatics.jax.org/expression.shtml>

Figure S2.

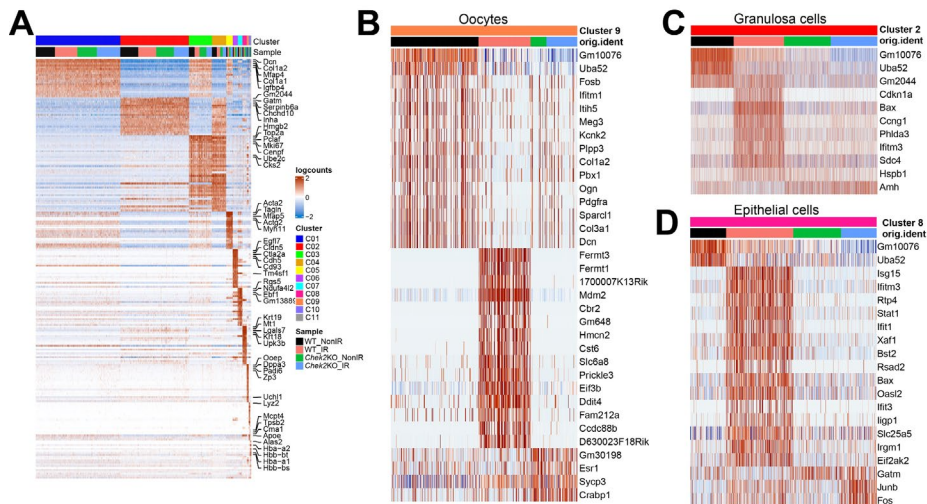


Figure S2. A) Heatmap of marker gene expression from single cells obtained from wildtype and *Chek2*^{-/-} ovaries with and without radiation organized into 11 clusters. B-D) Heatmaps of differentially expressed genes in oocyte, granulosa, and epithelial cell clusters.

Figure S3.

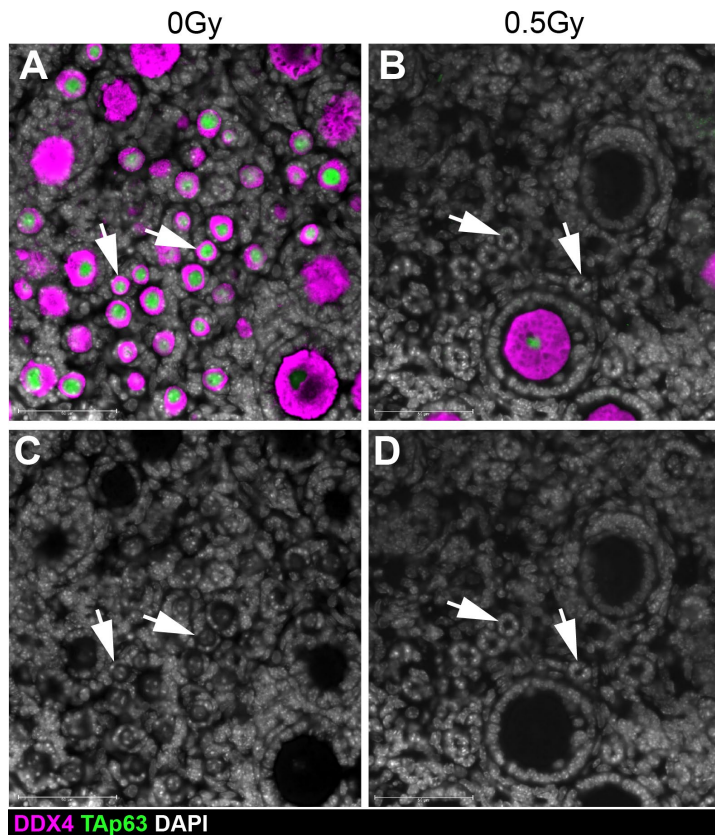


Figure S3. Example image of non-irradiated (A) and irradiated (B) ovaries immunostained with oocyte markers DDX4 and TAp63. C-D) Grayscale image for DAPI channel to visualize nuclei. Arrows indicate primordial follicles with an oocyte inside in non-irradiated (C) and empty follicles with visible granulosa cells but without the oocyte in the irradiated ovary (D).

Figure S4.

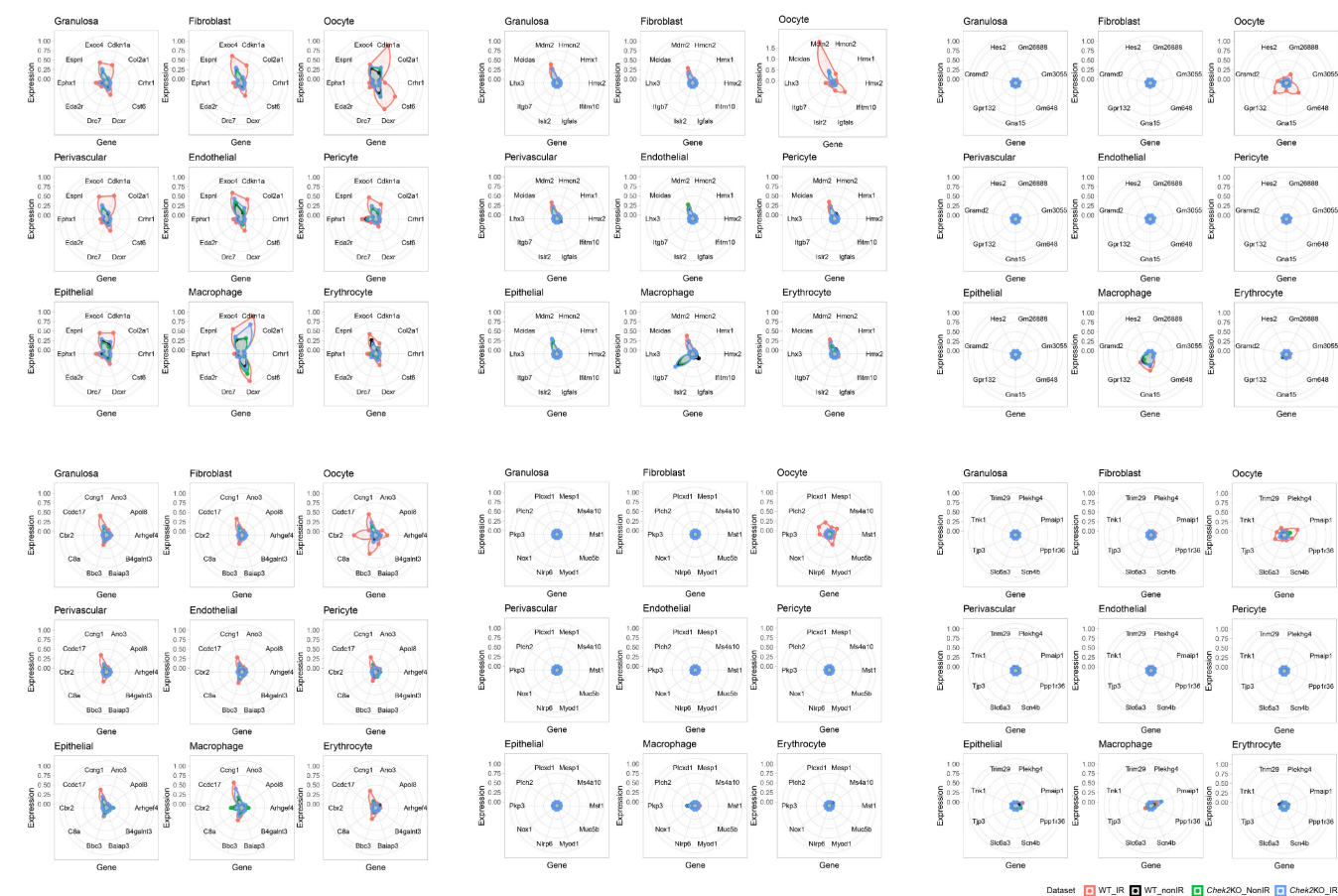


Figure S4. Radar charts showing expression of RRGs in different cell types.

Figure S5.

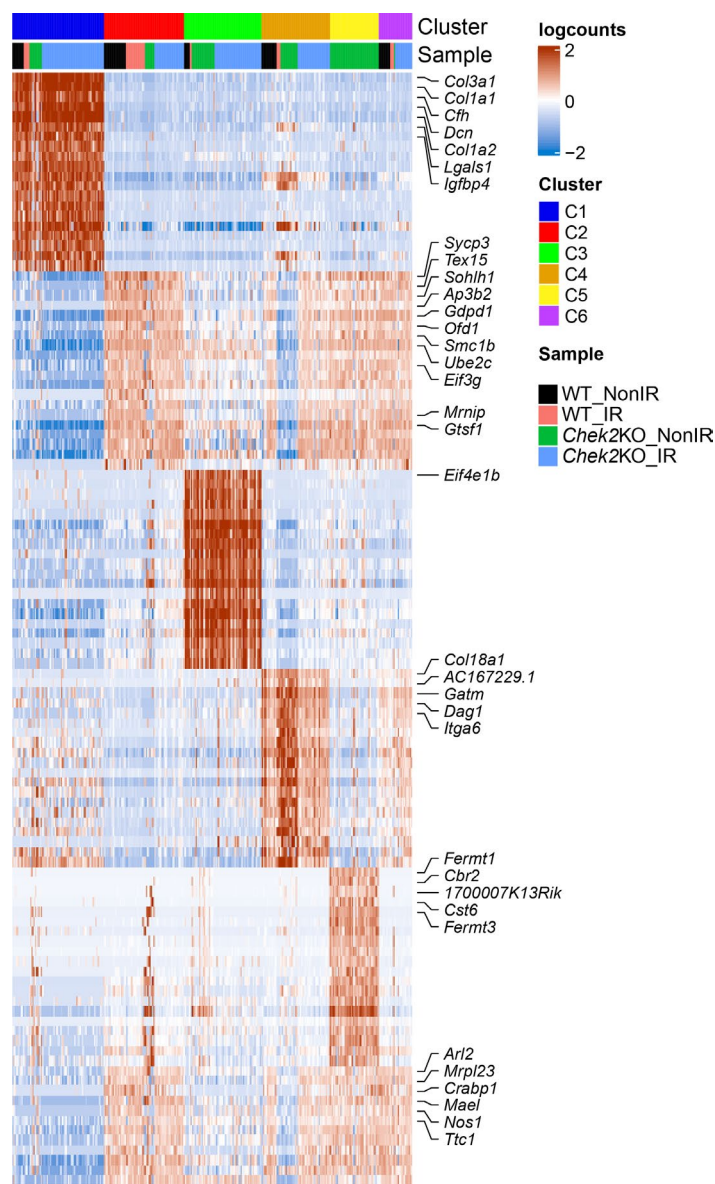


Figure S5. A) Heatmap of marker gene expression for six oocyte subclusters identified by de novo re-clustering.

A

Mean (Z-score) Percent of values > 0.0

WT_nonIR WT_IR Chek2KO_nonIR Chek2KO_IR

Gm17484 Gm17767 Ak1 Cngl1 Anos3 Drc7 Ephx1 Espnl Pmalp1 Islr2 Hmx1 Igfals Ms4a10 Arhgef4 Hmx2 Mst1 Fam83b Ilgfb7 Ifitm10 Tjp3 Gramd2 Trim29 Bialap3 Trp73 Wnt10b B4galnt3 Kpk3 Colza1 Hmcn2 Tnk1 Fermt3 Chnr1 Ankrd65 Gpr132 Chr2 Gnal5 Gnat15 4833428L15Rik Fermt1 Gm15577 Hes2 Myod1 Lhx3 Plcn2 Csl6 1700007K13Rik Gmb48 Gmz6888 Acpi4 Plcxdl Apol6 Mdm2 Ppp1r3b Muc5b Eda2r Ccdc17 Plekhg4 Cba Nlrpb Sch4b Ddxr Fam212b Cdkn1a Bcl3 Mespl Slc6a3 Slc22a8 Fam103a Ectoc4 AC102815.1 Gm3055

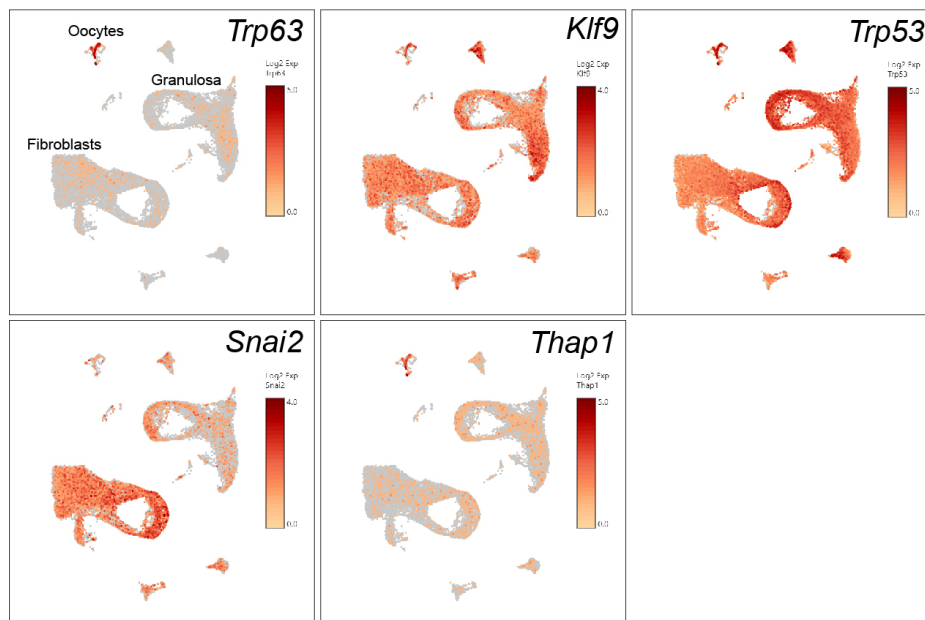
B

Mean (Z-score) Percent of values > 0.0

WT_nonIR WT_IR Chek2KO_nonIR Chek2KO_IR

Anvcf Pou3f1 Macrod1 Cspg5 Ajuba Lmf1 Nudt18 Perp Necab2 Ick Rapgef1 Tchp Traf3 Tmem8 Glse1 Hps4 Efna1 Casz1 Mia2 Cdc88b Mcam Procr Micall1 Pycard Mrohi1 Slc6a8 D630023F18Rik Cpne2 Tuft1 Frs3 Ndura412 Cdc42bpq Galnt6 Trim7 Prickle3 Ly6a Phlda3 Fam212a Ddit4 Lama5 Sfrn Espn Mob3a Nle1 Baiap2 Sp2 AC149090.1 Ptgd5 Gm15389

Figure S7.



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Supplementary Tables:

Supplementary Table S1. RRGs identified by bulk RNA-seq.

Table S1. Radiation Responsive Genes in the ovary					
Part 1. Response in wildtype ovaries.					
MGI Gene/ Marker ID	Symbol	LogFC WT	Adj p- value	Feature Type	Gene Ontology terms
MGI:5610613	<i>Gm37385</i>	22.7	8.77E-05	unclassified gene	
MGI:3809197	<i>Gm3055</i>	20.6	1.02E-03	protein coding gene	
MGI:102673	<i>Lhx3</i>	7.8	1.14E-10	protein coding gene	cell differentiation; DNA-binding transcription factor activity, RNA polymerase II-specific
MGI:1921852	<i>4833428L15Rik</i>	6.9	1.06E-07	lncRNA gene	
MGI:2685285	<i>Ankrd65</i>	6.7	1.22E-18	protein coding gene	
MGI:107159	<i>Hmx2</i>	6.5	3.27E-05	protein coding gene	cell differentiation; DNA-binding transcription factor activity, RNA polymerase II-specific
MGI:2444921	<i>Apol8</i>	5.8	6.63E-08	protein coding gene	chloride channel activity; extracellular space; high-density lipoprotein particle; intracellular membrane-bounded organelle
No associated gene	<i>Gm43301</i>	5.7	1.22E-04	lncRNA gene	
MGI:3613666	<i>Ano3</i>	5.6	1.27E-30	protein coding gene	calcium activated phospholipid scrambling; chloride channel activity; chloride transmembrane transport
MGI:3783025	<i>Gm15577</i>	5.5	5.92E-03	lncRNA gene	
MGI:107785	<i>Mesp1</i>	5.5	2.99E-03	protein coding gene	DNA-binding transcription activator activity, RNA polymerase II-specific; Notch signaling pathway
MGI:1098624	<i>Hes2</i>	5.5	6.45E-08	protein coding gene	DNA-binding transcription factor activity, RNA polymerase II-specific; DNA-binding transcription repressor activity, RNA polymerase II-specific
MGI:2443078	<i>Plch2</i>	5.3	8.57E-51	protein coding gene	calcium ion binding; intracellular signal transduction; phosphatidylinositol-mediated signaling; phospholipase C activity; plasma membrane; signal transduction
MGI:94862	<i>Slc6a3</i>	5.2	2.50E-11	protein coding gene	cell surface; dopamine transport; integral component of membrane; signaling receptor binding
MGI:107200	<i>Cbr2</i>	5.1	2.91E-05	protein coding gene	carbonyl reductase (NADPH) activity; glucose metabolic process; mitochondrion; oxidoreductase activity
MGI:97275	<i>Myod1</i>	4.8	6.15E-07	protein coding gene	cell differentiation; cellular response to estradiol stimulus; cellular response to oxygen levels; cellular response to tumor necrosis factor; DNA-binding transcription activator activity, RNA polymerase II-specific; ubiquitin protein ligase binding
MGI:88498	<i>Crrh1</i>	4.7	2.07E-09	protein coding gene	adenylate cyclase-activating G protein-coupled receptor; signaling pathway; cell surface receptor signaling pathway; endosome; transmembrane signaling receptor activity; vesicle
MGI:2685402	<i>Espnl</i>	4.7	2.42E-18	protein coding gene	actin binding; cell projection; stereocilium tip; stereocilium tip
MGI:2666347	<i>C8a</i>	4.7	1.39E-03	protein coding gene	complement activation; cytolysis; immune response; membrane attack complex
MGI:2443583	<i>Fermt1</i>	4.5	9.82E-06	protein coding gene	cell adhesion; cell junction; focal adhesion; integrin-mediated signaling pathway; negative regulation of; canonical Wnt signaling pathway; positive regulation of cell adhesion mediated by integrin
MGI:1890220	<i>Gpr132</i>	4.5	1.42E-15	protein coding gene	G1/S transition of mitotic cell cycle; G protein-coupled receptor signaling pathway; negative regulation of G2/M; transition of mitotic cell cycle; plasma membrane; signal transduction
MGI:2685616	<i>Drc7</i>	4.4	5.93E-07	protein coding gene	cell differentiation; cell projection; cilium
MGI:88452	<i>Col2a1</i>	4.3	1.35E-06	protein coding gene	cellular response to BMP stimulus; extracellular region
MGI:3648807	<i>Mcidas</i>	4.2	2.60E-02	protein coding gene	cell cycle; negative regulation of cell cycle; negative regulation of DNA replication
MGI:2444776	<i>Ifitm10</i>	4.2	5.11E-37	protein coding gene	integral component of membrane; membrane
MGI:1916577	<i>Pierce1 (1700007K13Rik)</i>	4.1	1.10E-18	protein coding gene	cellular response to DNA damage stimulus; regulation of gene expression
MGI:2685494	<i>Gm648</i>	4.1	2.26E-10	protein coding gene	
MGI:95770	<i>Gna15</i>	4.0	3.05E-05	protein coding gene	adenylate cyclase-modulating G protein-coupled receptor signaling pathway; GTPase activity membrane; phospholipase C-activating G protein-coupled receptor signaling pathway
MGI:1918990	<i>Nectin4 (Pvrl4)</i>	3.9	3.12E-10	protein coding gene	cell junction
MGI:1919920	<i>2810029C07Rik</i>	3.9	9.10E-37	lncRNA gene	
MGI:2444277	<i>Islr2</i>	3.8	2.02E-11	protein coding gene	cell surface; integral component of membrane; protein binding
MGI:2687406	<i>Scn4b</i>	3.7	2.07E-11	protein coding gene	ion transport; plasma membrane; sodium channel activity
MGI:2684916	<i>Ppp1r36</i>	3.7	1.11E-09	protein coding gene	negative regulation of phosphatase activity; phosphatase binding
MGI:3612701	<i>Spdye4b (4933411G11Rik)</i>	3.7	3.15E-03	protein coding gene	protein kinase binding
MGI:2147790	<i>Fermt3</i>	3.6	4.15E-10	protein coding gene	cell adhesion; cell junction; integrin-mediated signaling pathway
MGI:3645718	<i>Gm5421</i>	3.6	1.67E-02	pseudogene	
MGI:2685362	<i>Fam83b</i>	3.3	3.85E-23	protein coding gene	phosphatidylinositol 3-kinase catalytic subunit binding; signal transduction
MGI:1921430	<i>Muc5b</i>	3.3	4.79E-08	protein coding gene	extracellular matrix; regulation of macrophage activation
MGI:2444813	<i>Dglucy (9030617O03Rik)</i>	3.3	5.40E-16	protein coding gene	D-glutamate cyclase activity; lyase activity; mitochondrion
MGI:107178	<i>Hmx1</i>	3.2	2.56E-05	protein coding gene	DNA-binding transcription factor activity, RNA polymerase II-specific
MGI:3801771	<i>Trp53cor1</i>	3.2	2.03E-04	lncRNA gene	negative regulation of gene expression; ribonucleoprotein complex
MGI:1920970	<i>Cst6</i>	3.2	4.08E-04	protein coding gene	epidermis development
MGI:1930958	<i>Tnk1</i>	3.0	7.58E-06	protein coding gene	ATP binding; kinase activity; negative regulation of Ras protein signal transduction; transmembrane receptor protein tyrosine kinase signaling pathway
MGI:108061	<i>Wnt10b</i>	3.0	1.04E-02	protein coding gene	canonical Wnt signaling pathway; G2/M transition of mitotic cell cycle; negative regulation of transcription by RNA polymerase II; regulation of cell cycle
MGI:96080	<i>Mst1</i>	3.0	4.59E-04	protein coding gene	extracellular region; negative regulation of epithelial cell apoptotic process; receptor tyrosine kinase binding; regulation of receptor signaling pathway via JAK-STAT
MGI:1915667	<i>Ccdc17</i>	3.0	7.06E-18	protein coding gene	serine-type endopeptidase activity
MGI:5477382	<i>Gm26888</i>	2.8	4.60E-09	lncRNA gene	
MGI:1336991	<i>Trp73</i>	2.8	8.25E-11	protein coding gene	apoptotic process; cell cycle; cell junction; cellular response to DNA damage stimulus; DNA-binding transcription activator activity, RNA polymerase II-specific; intrinsic apoptotic signaling pathway in response to DNA damage by p53 class mediator; MDM2/MDM4 family protein binding; negative regulation of JUN kinase activity; p53 binding; positive regulation of apoptotic process; positive regulation of MAPK cascade
MGI:1930146	<i>Pmaip1</i>	2.7	1.16E-16	protein coding gene	activation of cysteine-type endopeptidase activity; involved in apoptotic process; positive regulation of apoptotic process; positive regulation of DNA damage response, signal transduction by p53 class mediator; reactive oxygen species metabolic process; regulation of apoptotic process
MGI:3643534	<i>Angptl8 (Gm6484)</i>	2.6	3.32E-04	protein coding gene	extracellular region; negative regulation of lipoprotein lipase activity
MGI:1891830	<i>Pkp3</i>	2.6	6.08E-07	protein coding gene	adherens junction; alpha-catenin binding; cell-cell adhesion; cell-cell junction assembly
MGI:2142544	<i>Plekha4</i>	2.6	6.96E-03	protein coding gene	activation of GTPase activity
MGI:1351650	<i>Tjp3</i>	2.5	4.90E-06	protein coding gene	cell-cell adhesion; cell-cell junction; cell surface; regulation of G1/S transition of mitotic cell cycle
MGI:3644563	<i>Acp4 (Acpt)</i>	2.5	1.09E-07	protein coding gene	acid phosphatase activity; negative regulation of ERBB4 signaling pathway; peptidyl-tyrosine dephosphorylation involved in inactivation of protein kinase activity; protein tyrosine phosphatase activity

MGI:96616	<i>Itgb7</i>	2.4	2.61E-04	protein coding gene	cell adhesion; cell adhesion mediated by integrin; focal adhesion; integrin-mediated signaling pathway
MGI:1919419	<i>Trim29</i>	2.4	5.51E-03	protein coding gene	innate immune response; lysosome; negative regulation of transcription by RNA polymerase II; p53 binding
MGI:1922105	<i>Fam228a</i>	2.4	1.30E-02	protein coding gene	
MGI:3528937	<i>Gramd2</i>	2.3	5.77E-08	protein coding gene	extrinsic component of cytoplasmic side of plasma membrane; integral component of membrane; phosphatidylinositol binding
MGI:1917076	<i>Ms4a10</i>	2.1	2.42E-03	protein coding gene	cell surface receptor signaling pathway; integral component of membrane; membrane
MGI:4937118	<i>Gm17484</i>	2.1	5.54E-05	lncRNA gene	
MGI:2145726	<i>9930012K11Rik</i>	2.1	1.26E-12	protein coding gene	
MGI:102890	<i>Ccng1</i>	2.0	5.64E-105	protein coding gene	cell cycle; cell division; cyclin-dependent protein; serine/threonine kinase regulator activity; cytoplasm; mitotic G2 DNA damage checkpoint signaling; negative regulation of apoptotic process
MGI:87977	<i>Ak1</i>	2.0	1.64E-05	protein coding gene	adenylate kinase activity; regulation of G1/S transition of mitotic cell cycle
MGI:2685783	<i>Baiap3</i>	2.0	1.95E-05	protein coding gene	calcium ion binding; G protein-coupled receptor signaling pathway; late endosome membrane; regulation of dense core granule exocytosis
MGI:2685422	<i>Plcx1</i>	1.9	1.10E-07	protein coding gene	lipid metabolic process
MGI:2181667	<i>Bbc3</i>	1.9	3.41E-11	protein coding gene	activation of cysteine-type endopeptidase activity; involved in apoptotic process; cellular response to DNA damage stimulus; cellular response to ionizing radiation; intrinsic apoptotic signaling pathway; intrinsic apoptotic signaling pathway by p53 class mediator; PUMA-BCL-xL complex; response to endoplasmic reticulum stress
MGI:107973	<i>Igfals</i>	1.9	2.92E-02	protein coding gene	cell adhesion; extracellular region
MGI:2677838	<i>Hmncn2</i>	1.8	1.92E-03	protein coding gene	basement membrane; cell junction; collagen-containing extracellular matrix; response to stimulus
MGI:1923497	<i>Inka2 (Fam212b)</i>	1.8	2.95E-13	protein coding gene	negative regulation of catalytic activity; protein serine/threonine kinase inhibitor activity
MGI:2442860	<i>Eda2r</i>	1.8	2.19E-21	protein coding gene	cell differentiation; integral component of membrane; intrinsic apoptotic signaling pathway by p53 class mediator; positive regulation of I-kappaB kinase/NF-kappaB signaling; positive regulation of JNK cascade
MGI:3041155	<i>B4galnt3</i>	1.7	3.26E-03	protein coding gene	programmed cell death; tumor necrosis factor-mediated signaling pathway
MGI:95405	<i>Ephx1</i>	1.7	3.26E-03	protein coding gene	acetylgalactosaminyltransferase activity; glycosyltransferase activity; Golgi apparatus
MGI:104556	<i>Cdkn1a</i>	1.7	5.54E-05	protein coding gene	integral component of membrane; plasma membrane; response to toxic substance
MGI:1915130	<i>Dcrr</i>	1.6	2.91E-03	protein coding gene	cell cycle; cellular response to DNA damage stimulus; cellular response to ionizing radiation; cellular response to UV-B; cyclin-dependent protein serine/threonine kinase inhibitor activity; DNA damage response, signal; transduction by p53 class mediator resulting in cell cycle arrest; mitotic G2 DNA damage checkpoint signaling; PCNA-p21 complex; regulation of cell cycle G1/S phase transition
MGI:2442507	<i>Arhgef4</i>	1.5	6.22E-04	protein coding gene	carbonyl reductase (NADPH) activity; L-xylulose reductase (NADP+) activity; membrane; oxidoreductase activity; positive regulation of reactive oxygen species; metabolic process
MGI:1096376	<i>Exoc4</i>	1.4	9.61E-06	protein coding gene	intracellular signal transduction; lamellipodium assembly; protein domain specific binding
MGI:96952	<i>Mdm2</i>	1.3	3.40E-07	protein coding gene	cell projection; exocytosis; regulation of protein transport; small GTPase binding; vesicle tethering involved in exocytosis
MGI:5009931	<i>Gm17767</i>	-2.2	4.93E-03	lncRNA gene	apoptotic process; cellular response to gamma radiation; DNA damage response, signal transduction by p53 class mediator resulting in cell cycle arrest; NEDD8 ligase activity; p53 binding
MGI:2450016	<i>Nox1</i>	-2.6	7.06E-05	protein coding gene	cell junction; cellular response to hyperoxia; endosome; extracellular matrix organization; NADPH oxidase complex; oxidoreductase activity; positive regulation of JNK cascade; positive regulation of MAPK cascade; positive regulation of oxidative stress-induced intrinsic; apoptotic signaling pathway; small GTPase binding
MGI:2141990	<i>Nlrp6</i>	-3.1	8.69E-03	protein coding gene	activation of cysteine-type endopeptidase activity; acute inflammatory response; ATP binding; inflammasome complex; necroptotic process; negative regulation of ERK1 and ERK2 cascade; negative regulation of I-kappaB kinase/NF-kappaB signaling; NLRP6 inflammasome complex
No associated gene	<i>AC102815.1</i>	-15.2	1.96E-02	unclassified gene	
MGI:3704203	<i>Gm10171</i>	-20.2	1.64E-07	pseudogene	
MGI:3651379	<i>Gm14303</i>	-22.2	1.71E-04	pseudogene	

Part 2. Response in <i>Chek2</i> ^{-/-} ovaries.					
MGI Gene/Marker ID	Symbol	LogFC <i>Chek2</i> ^{-/-}	Adj p-value	Feature Type	Gene Ontology terms
MGI:5610613	<i>Gm37385</i>	26.4	1.77E-05	unclassified gene	
MGI:1338893	<i>Padi1</i>	-6.7	0.032901	protein coding gene	calcium ion binding; hydrolase activity; protein-arginine deiminase activity
MGI:102673	<i>Gm43520</i>	-22.9	1.77E-05	unclassified gene	

Supplementary Table S2. Gene enrichment analysis of RRGs with g:Profiler.

Source	Term Name	Term ID	Adj.pvalue	-log10 adj.pvalue	Genes
GO:BP	intrinsic apoptotic signaling pathway by p53 class mediator	GO:0072332	0.001	3.15	<i>Eda2r, Pmaip1, Bbc3, Trp73, Mdm2, Cdkn1a</i>
GO:BP	signal transduction by p53 class mediator	GO:0072331	0.019	1.71	<i>Eda2r, Pmaip1, Bbc3, Trp73, Mdm2, Cdkn1a</i>
KEGG	p53 signaling pathway	KEGG:04115	0.000	6.62	<i>Ccng1, Pmaip1, Bbc3, Trp73, Mdm2, Cdkn1a</i>
KEGG	Platinum drug resistance	KEGG:01524	0.001	3.05	<i>Pmaip1, Bbc3, Mdm2, Cdkn1a</i>
KEGG	Apoptosis - multiple species	KEGG:04215	0.013	1.88	<i>Pmaip1, Bbc3</i>
KEGG	Human papillomavirus infection	KEGG:05165	0.024	1.62	<i>Hes2, Mdm2, Col2a1, Cdkn1a, Itgb7, Wnt10b</i>
KEGG	Colorectal cancer	KEGG:05210	0.036	1.44	<i>Pmaip1, Bbc3, Cdkn1a</i>
WP	p53 signaling	WP:WP2902	0.000	7.07	<i>Ccng1, Pmaip1, Bbc3, Trp73, Mdm2, Cdkn1a</i>
WP	Apoptosis	WP:WP1254	0.004	2.41	<i>Pmaip1, Trp73, Mdm2</i>
WP	Hypoxia-dependent self-renewal of myoblasts	WP:WP5023	0.009	2.04	<i>Myod1, Cdkn1a</i>
MIRNA	mmu-miR-23a-3p	MIRNA:mmu-miR-23a-3p	0.030	1.52	<i>Pmaip1, Bbc3</i>

Supplementary Table S3. Gene Set Enrichment Analysis of RRGs (GSEA).

HALLMARK PATHWAY	ES	NES	NOM p-val	FDR q-val	TOP GENES (HUMAN SYMBOLS)
P53 PATHWAY	0.67	2.20	0.00	0.00	CCNG1, AK1, CLCA2, CDKN1A, DCXR, EPHX1, MDM2, PIDD1, PHLDA3, BTG2, ZMAT3, ZNF365, GLS2, MAPKAPK3, EPHA2, SFN, BAX, SLC19A2, DEF6, DDIT4
INTERFERON ALPHA RESPONSE	0.67	2.05	0.00	0.00	DHX58, RTP4, TRIM14, IRF9, LGALS3BP, TMEM140, TAP1, EIF2AK2, SAMD9L, PARP9, IRF7, HELZ2, PROCR, DDX60, TRIM21, ISG15, PSMB8, IFITM3, GBP4, HERC6
INTERFERON GAMMA RESPONSE	0.57	1.86	0.00	0.00	CDKN1A, ITGB7, DHX58, OAS2, RTP4, TRIM14, IRF9, LGALS3BP, TAP1, EIF2AK2, SAMD9L, DDX58, XAF1, STAT1, IRF7, HELZ2, DDX60, CSF2RB, TRIM21, ISG15
APOPTOSIS	0.53	1.70	0.00	0.01	PMAIP1, GNA15, CDKN1A, BTG2, BAX, TAP1, CD14, GUCY2D, IFITM3, CASP7, MMP2, GADD45A, IER3, CASP1, PLCB2, GSR, FAS, HGF, DPYD, TNFSF10
TNFA SIGNALING VIA NFKB	0.51	1.69	0.00	0.01	CDKN1A, BTG2, TAP1, EGR2, KYNU, DDX58, EGR1, DRAM1, LIF, F3, DUSP2, PLEK, PTGER4, EFNA1, IFIH1, LAMB3, SOCS3, IL7R, FOS, PLK2
INFLAMMATORY RESPONSE	0.49	1.61	0.00	0.02	GPR132, GNA15, CDKN1A, BTG2, RTP4, EIF2AK2, IRF7, LIF, RNF144B, BEST1, F3, CD14, ADORA2B, PTGER4, P2RX7, SEMA4D, HRH1, IL7R, ITGB8, CD48
IL6 JAK STAT3 SIGNALING	0.52	1.56	0.01	0.03	IRF9, STAT1, IL1R2, CSF2RB, CD14, CCR1, SOCS3, CXCL9, DNTT, IL18R1, SOCS1, STAT2, CSF3R, FAS, PIK3R5, EBI3, MAP3K8, ACVR1B, PF4, CD44
APICAL JUNCTION	0.47	1.54	0.00	0.03	EXOC4, NECTIN4, COL17A1, NFASC, MAP3K20, CLDN4, ACTA1, PPP2R2C, CDH4, PARD6G, CLDN14, LIMA1, GRB7, VCL, BAIAP2, ICAM5, THY1, MAP4K2, CDH3, LAMB3
ESTROGEN RESPONSE LATE	0.47	1.53	0.00	0.03	PKP3, DCXR, TRIM29, TJP3, RAPGEFL1, OVOL2, SFN, ASS1, CLIC3, ST14, CA2, CACNA2D2, TOB1, SCNN1A, MAPT, PERP, DUSP2, TFAP2C, CELSR2, PGR
COMPLEMENT	0.46	1.52	0.00	0.03	TMPRSS6, ADRA2B, CDK5R1, CA2, KLK1, MMP15, KYNU, IRF7, F3, PLEK, CASP7, PSMB9, GCA, LCP2, F7, ITIH1, CFB, ACTN2, ITGAM, TFPI2
COAGULATION	0.46	1.47	0.02	0.04	C8A, MST1, TMPRSS6, MMP15, F3, PLEK, CTSE, MMP2, ITIH1, F13B, HPN, CFB, P2RY1, TFPI2, C1QA, F10, KLK8, LEFTY2, WDR1, GP9
ESTROGEN RESPONSE EARLY	0.44	1.47	0.01	0.05	PMAIP1, ESRP2, TJP3, RAPGEFL1, OVOL2, SFN, CLIC3, SLC19A2, SYT12, TOB1, SCNN1A, MAPT, ELF3, TFAP2C, CELSR2, ADCY1, PGR, TTC39A, MUC1, FOS

ES- Enrichment Score; NES- Normalized Enrichment Score; NOM p-val- Nominal pvalue; FDR q-val- False discovery rate

Supplementary Table S4. Primers used in this study.

RT-qPCR Primers		
Gene	Sequence	Direction
<i>Ankrd65</i>	AGTGGCTAAGGGCATTGAAATA	Forward
<i>Ankrd65</i>	GGAGATGGCTGACAACCTTCTAC	Reverse
<i>Cbr2</i>	GGTAGCCAGGGACATGATTAAC	Forward
<i>Cbr2</i>	GAGCTGTAGGTGATCAAGTTAGG	Reverse
<i>Cdkn1a</i>	TTAGGCAGCTCCAGTGGCAACC	Forward
<i>Cdkn1a</i>	ACCCCCACCACCACACACCATA	Reverse
<i>Fermt1</i>	CATGCAAATGGAGAGCAGCAG	Forward
<i>Fermt1</i>	TTCCCACCACAGAGCATAGTC	Reverse
<i>Fermt3</i>	ATGGAGGCTCAGGGAACAAA	Forward
<i>Fermt3</i>	CTTGGCCTTGAACCTTCGCT	Reverse
<i>Gapdh</i>	TCCATGACAACCTTTGGCATTG	Forward
<i>Gapdh</i>	CAGTCTTCTGGGTGGCAGTGA	Reverse
<i>Ddx4</i>	GTGGAAATACTGGCAGAGCG	Forward
<i>Ddx4</i>	ATCCTGTTGAGCGTCTGACA	Reverse

Genotyping primers			
Gene	Probe	Sequence	Direction
<i>Chek2</i>	Wildtype	CTTGTCCTGCTGGACTCACA	Forward
<i>Chek2</i>	Wildtype	GCAACCGTTACCTACCCTGA	Reverse

<i>Chek2</i>	<i>Mutant</i>	CGGTCGCTACCATTACCAGT	Forward
<i>Chek2</i>	<i>Mutant</i>	CAGCGCTTATCCCAACACT	Reverse
<i>Trp53</i>	<i>Wildtype</i>	CAGCCTCTGTTCCACATACACT	Forward
<i>Trp53</i>	<i>Mutant</i>	AGGCTTAGAGGTGCAAGCTG	Forward
<i>Trp53</i>	<i>Common</i>	TGGATGGTGGTATACTCAGAGC	Reverse
<i>Trp63</i>	<i>Common</i>	ACCTGGCTTCCTTCTCATTG	Forward
<i>Trp63</i>	<i>Common</i>	CTTTGATACGCTGCTGCTTG	Reverse

Supplementary Data Files.

Supplementary Data 1: Bulk RNAseq_Ovary_Gene_Counts_DEGs_groupwise

Supplementary Data 2: scRNAseq_Ovary_Clusters_Marker_Genes_DEGs_groupwise

Supplementary Data 3: scRNAseq_Oocyte_Subclusters_Marker_Genes_DEGs