

Supplementary Table 8. List of top25 up (red) and down (blue) regulated proteins differentially expressed between PARENTAL and CRISPR clones.

GENE	Function and relationship with cancer	Relation with granulosa cells and/or FOXL2	Common in Transcriptomic & Proteomic DEG
RARRES2 Retinoic Acid Receptor Responder 2	Secreted chemotactic protein that initiates chemotaxis via the ChemR23 G protein-coupled seven-transmembrane domain ligand. This protein is involved in regulation of adipogenesis, energy metabolism, and inflammation. Related to ovarian cancer among others.	Regulates granulosa cells apoptosis during folliculogenesis by upregulating p53 and p21 (doi: 10.1002/mrd.23241). (doi: 10.1095/biolreprod.113.117044)	Yes
FABP3 Fatty Acid Binding Protein 3	Participates in uptake, transport, and metabolism of long-chain fatty-acids in the cells. Tumor suppressor in breast cancer.	Participates in KGN cell proliferation and also increases the expression of aromatase CYP19 (doi: 10.1093/jmcb/mjaa044)	Yes
LMOD1 Leiomodinin-1	Required for proper contractility of visceral smooth muscle cells, it mediates nucleation of actin filaments. Deregulated in endometrial cancer (doi: 10.1038/s41598-020-66872-3).	Involved in ovulation in mouse ovary (doi: 10.1101/2023.08.21.554210).	Yes (top-25)
COL1A2 Pro-alpha2 chain of type I collagen	Found in connective tissues, bone, cornea, dermis and tendon. Mutations and abnormal expression can cause instability of the extracellular matrix, increasing proliferation and invasion of tumoral cells.	Indicates oocyte quality, being upregulated in granulosa cells (doi: 10.1071/RD14452; 10.1016/j.rbmo.2021.05.018). FoxL2 (wild-type form) affects its transcription and expression, being increased when its knock-down and affecting follicular depletion during reproductive aging. Mutation FoxL2 C134W does not affect expression levels (doi: 10.1016/j.ydbio.2016.05.022)	Yes
CSPG4 Chondroitin Sulfate Proteoglycan 4	It can mediate intracellular signalling downstream of growth factor receptor and integrin interactions, potentiating communication between the extracellular and intracellular compartments of the cell. Related to several tumor types.	Upregulated in TGFβ-induced GCTs, when compared with wild type granulosa cells (doi: 10.3390/cancers14092184)	Yes (top-25)
CAV1 Caveolin 1	Main component of the caveolae plasma membranes. Negative regulator of RAS signaling. Links integrin to Ras-ERK signaling. It has been related to various cancer types, including head and neck squamous cell carcinoma, melanoma, and Ewing's sarcoma family tumors.	It plays a role in folliculogenesis and female reproduction (doi: 10.1093/HUMREP/DEY299). In bovine ovaries, CAV1 is expressed in granulosa and theca cells of the follicle and large and small cells of the corpus luteum (doi: 10.1002/MRD.20513).	Yes (top-25)
ALDH1L2 Aldehyde Dehydrogenase 1 Family Member L2	This mitochondrial enzyme takes part in the folate metabolism (doi: 10.1074/jbc.M110.128843).	Related to cell cycle and apoptosis in primordial follicles (doi: 10.1093/toxsci/kfs137)	Yes

TAGLN Transgelin	It is involved in calcium-independent smooth muscle contraction. It acts as a tumor suppressor, and the loss of its expression is an early event in cell transformation and the development of some tumors, coinciding with cellular plasticity.	Upregulated in TGF β -induced GCTs when compared with wild type granulosa cells (doi: 10.3390/cancers14092184).	Yes (top-25)
ALDH2 Aldehyde Dehydrogenase 2 or Mitochondrial	Mitochondrial isoform that participates in alcohol metabolism.	Deregulated in granulosa cells from PCOS patients (at protein level) (doi: 10.1177/11795514231206732)	No
PTN Pleiotrophin	Secreted heparin-binding growth factor that participates in cell growth, migration, angiogenesis and tumorigenesis.	In the ovary is associated with stromal cell genes with COL1A1 (doi: 10.1038/s41421-022-00492-1). Under FOXL2 knock-down, this gene is upregulated in granulosa cells (doi: 10.1186/1471-213X-9-36)	Yes
TNC Tenascin C	Component of the extracellular matrix. Contributes to elongation and migration of endothelial cells during angiogenesis in tumors. In ovarian tumors it appears to be overexpressed in the stroma and is also involved in invasion (doi: 10.1371/journal.pone.0136473).	None	Yes
MCAM Melanoma Cell Adhesion Molecule	Involved in glomerular filtration and vascular wound healing. Acts upstream of or within angiogenesis. Biomarker of uveal melanoma.	Protein involved in neovascularization during the formation of corpus luteum in the human ovary, and defined as a FOXL2 target gene (doi: 10.1093/molehr/gag042; 10.7554/elife.04207)	No
CD99 T-Cell Surface Glycoprotein E2	Cell surface glycoprotein involved in leukocyte migration, transmembrane protein transport, rearrange cytoskeleton, T-cell adhesion and cell death. Also plays an important role in the identification and diagnosis of different ovary tumors.	Granulosa cell tumors are positive for this cell marker, that is characteristic of sex cord-stromal tumors (SCSTs) (PMID: 9720506.) Granulosa cell tumor marker along with activin and inhibin- α (doi: 10.5858/2000-124-0563-IOISIA)	No
PODXL Podocalyxin	Involved in cell adhesion and morphology in cancer progression by its interactions with actin-binding protein EZR, that increases activation of MAPK/PI3K pathway.	Related to ovarian cancer via activation by a miRNA (doi: 10.1007/s43032-020-00366-5)	Yes
SDPR Serum Deprivation Response Protein (Cavin-2)	Protein increased in serum-starved cells and required for caveola formation.	None	No

CD36 Platelet glycoprotein 4	Glycoprotein of the platelet surface, it serves as a receptor of thrombospondin in various cell lines	Overexpressed in granulosa cells of obese patients, causing malfunction of these cells and problems in fertility and ovarian function (doi: 10.1071/RD18292). Takes part in steroidogenesis and cholesterol pathways in granulosa cells by increasing its expression as a fatty acid transporter (doi: 10.1186/s40104-021-00660-5). Increased expression of CD36 reduces FOXL2 (doi: 10.1186/s12958-017-0276-z)	No
CYSTM1 Cysteine-rich and transmembrane domain-containing protein 1	Implicated in immune system pathways. Biomarker of hepatocellular carcinoma	None	Yes
FLNC Filamin C	Related to myopathies and other muscular phenotypes.	Differentially expressed at transcriptomic level, when compared with <i>wild-type</i> FOXL2. It is associated with tumorigenesis due to its interaction with the extracellular matrix (doi: 10.1186/s12967-021-02754-0)	Yes
COL1A1 Collagen Type I Alpha 1 Chain	Most abundant protein in the extracellular matrix.	Implicated in follicular phase, accumulating in cells due to its regulation via TGF β signaling (doi: 10.1096/fj.202001377R).	No
NCALD Neurocalcin Delta	It is thought to be a regulator of G protein-coupled receptor signal transduction. Associated with ovarian cancer. Its lower expression in this case is related to chemotherapy resistance (doi: 10.1002/jcb.29670; 10.1186/s13048-020-00635-6)	None	Yes
CAV2 Caveolin 2	Major component of the inner surface of caveolae, small invaginations of the plasma membrane, and is involved in essential cellular functions, including signal transduction, lipid metabolism, cellular growth control and apoptosis. It plays a significant role in cancer progression and metastasis in various types of cancer, including head and neck squamous cell carcinoma (HNSCC), breast cancer, and pancreatic cancer.	None	Yes (top-25)
TPST2 Tyrosylprotein Sulfotransferase 2	This type II integral membrane protein is found in the Golgi body catalyzes the O-sulfation of tyrosine residues within acidic regions of proteins.	None	Yes
ALDH1B1 Aldehyde	Aldehyde dehydrogenase involved in involved in the metabolism of	Downregulated in cumulus cells (doi: 10.1093/biolre/ioab163)	Yes

dehydrogenases family of proteins	corticosteroids, biogenic amines, neurotransmitters, and lipid peroxidation. Contributes to proliferation of pancreatic cancer.		
LCP1 Lymphocyte Cytosolic Protein 1	Actin-binding protein. It plays a role in the activation of T-cells in response to costimulation through TCR/CD3 and CD2 or CD28 and modulates the cell surface expression of IL2RA/CD25 and CD69. Associated with cancer.	None	Yes
ENAM Enamelin	Plays a role in the development of the tooth matrix. Downregulated in renal cancer tissues and also involved in inhibition of proliferation in clear cell renal carcinoma.	None	Yes
ALDH1A3 Aldehyde dehydrogenase family member A3	Catalyzes the NAD-dependent oxidation of aldehyde substrates. Promotes cancer cell growth and invasion inducing gene expression via retinoic acid.	Participates during steroidogenesis in the ovary (10.1016/j.jare.2023.06.002). ALDH1A3 enhances expression of ovarian genes in Sertoli cells, differentiating them into granulosa cells (doi: 10.1016/j.ydbio.2017.02.015)	Yes
CALB2 Calbindin (Calretinin)	Intracellular calcium-binding protein belonging to the troponin C superfamily. This protein plays a role in diverse cellular functions, including message targeting and intracellular calcium buffering. It plays a role in cancer progression and may serve as a potential biomarker or therapeutic target in certain cancer types.	Useful marker for GCTs (doi: 10.1309/GRH4-JWX6-J9J7-QQTA)	Yes (top-25)
TXNIP Thioredoxin- interacting protein	A thiol-oxidoreductase that is a major regulator of cellular redox signaling which protects cells from oxidative stress. Overexpression of this protein induces cell cycle arrest.	Downregulated in normal granulosa cells and up-regulated in PCOS granulosa patients, generating granulosa cell dysfunction by activation of NLRP3 inflammasome (doi: 10.1016/j.mce.2022.111824)	No
LPXN Leupaxin	Focal-adhesion-associated adaptor-protein family member.	Upregulated with CD36 in PCOS patients with obesity (doi: 10.1071/RD18292)	Yes
EEF1A2 Elongation factor 1 alpha 2	Promotes binding of aminoacyl-tRNA to the A-site of ribosomes in protein synthesis. Overexpressed in breast cancer, where is involved in migration and filopodia stimulation in a PI3K-Akt dependent manner.	Critical in the development of ovarian cancer and in Juvenile Granulosa cell tumors, causing cell migration (doi: 10.1016/j.ebiom.2015.03.002)	No
SAT2 Serine acetyltransferase 2	Catalyzes the N-acetylation of the amino acid thialysine (S-(2-aminoethyl)-L-cysteine) and it is located in exosomes.	None	No

CYP11A1 Cytochrome P450 Family 11 Subfamily A Member 1	Member of cytochrome P450 superfamily of enzymes, which catalyze many reactions involved in drug metabolism and synthesis of cholesterol, steroids and other lipids. This protein localizes to the mitochondrial inner membrane and catalyzes the conversion of cholesterol to pregnenolone, the first and rate-limiting step in the synthesis of the steroid hormones. It has been implicated in different cancers (breast, kidney, renal, squamous cell and skin).	It plays a crucial role in steroid hormone synthesis in granulosa cells. Increase expression in GCs undergoing luteinization in ovulation (doi: 10.1210/en.2016-1264. Epub 2016 Jul 18). No expression in granulosa cell tumors (doi: 10.1158/0008-5472.CAN-05-1024). FOXL2 represses the activity of the mouse Cyp11a1 promoter (doi: 10.1530/REP-11-0259).	Yes (top-25)
HLA-DRB5; HLA-DRB3 Major Histocompatibility Complex Class II DR Beta 3 and 5	HLA class II beta chain paralogues. They play a central role in the immune system by presenting peptides derived from extracellular proteins. Associated with gliomas with aggressive phenotypes.	None	No
SLC14A1 Solute Carrier Family 14 Member 1	Membrane transporter that mediates urea transport in erythrocytes. Biomarker in some cancers. Associated with the progression of different tumors.	None	Yes (top-25)
SERPINB2 Serpine Family B Member 2	Protein located on the external side of the plasma membrane, with endopeptidase activity, that promotes cell survival and it is associated with cytokine signaling. Overexpression inhibits apoptosis and promotes cell survival (doi: 10.1096/fj.202001377R).	Expression of this proteins depends on the stage of the follicular cycle. Highly upregulated in pregnant women (doi: 10.1016/j.theriogenology.2020.02.044). FOXL2 is defined as a positive regulator of this gene (doi: 10.3390/cancers11040499)	No
LACC1 Laccase Domain Containing 1	Oxidoreductase that promotes fatty-acid oxidation, with concomitant inflammasome activation, mitochondrial and NADPH-oxidase-dependent reactive oxygen species production.	None	No
VAT1L Vesicle amine transport 1	Oxidoreductase and acetyltransferase activity. Involved in patient distant metastasis-free survival in breast cancer.	None	Yes
RASGRF2 Ras Protein Specific Guanine Nucleotide Releasing Factor 2	Calcium-regulated nucleotide exchange factor that activates both RAS and RAS-related protein, RAC1.	Downregulated in the ovary of old mice (doi: 10.18632/aging.203150)	No
GSTP1 Glutathione S-transferases	It plays an important role in detoxification by catalyzing the conjugation of many hydrophobic and electrophilic compounds with reduced glutathione. Related to susceptibility to cancer.	Hypermethylated in granulosa cell tumors, causing inactivation of this suppressor gene and increasing tumor progression (doi: 10.1158/1078-0432.CCR-04-0228)	No
STK17B Serine/Threonine Kinase 17B	This nuclear protein enables ATP binding activity and protein serine/threonine kinase activity. Involved in intracellular signal transduction; positive regulation of fibroblast apoptotic process; and protein phosphorylation.	None	No

	Highly expressed in various malignant tumors, including epithelial ovarian cancer (doi: 10.21037/atm-21-601)		
TBCEL Tubulin Folding Cofactor E like	Predicted to enable alpha-tubulin binding activity, to be involved in microtubule cytoskeleton organization, post-chaperonin tubulin folding pathway and tubulin complex assembly.	Upregulate in large follicles, include in cell cycle category (doi: 10.1186/1471-2164-15-24; 10.1101/2022.10.24.513438)	No
TUBA1A; TUBA3E Tubulins (Alpha a1 and e3)	Major components of the microtubules that take part in the formation of the cytoskeleton. Associated with tumor processes.	None	No
HNMT Histamine N- Methyltransferase	In charge of histamine degradation. The dysregulation of its methylation involve this protein in certain cancers.	DEG in ovaries according to different environmental perturbations (doi: 10.1002/jez.b.22848)	No
CADM1 Cell Adhesion Molecule 1	Involved in cell recognition, positive regulation of cytokine production, susceptibility to NK cell mediated cytotoxicity. Implicated in cervix, prostate and ovarian tumors, where it upregulates PI3K/Akt/mTOR signaling pathway, increasing cell proliferation and migration (doi: 10.1016/j.biopha.2019.109717)	None	Yes
ACP1 Acid Phosphatase 1	Hydrolyzes protein tyrosine phosphate to protein tyrosine and orthophosphate. Not related to cancer	None	No
CAPG Capping protein actin	Contributes to the control of actin-based motility in non-muscle cells.	None	No
GLO1 Glyoxilase I	Linked to HLA. Participates in pyruvate metabolism and respiratory electron transport.	Involved in PCOS and decreased in old ovaries and ovulated oocytes, affecting their integrity (doi: 10.1016/j.fertnstert.2012.11.029)	No
DPYD Dihydropyrimidine Dehydrogenase	Catalyzes the reduction of uracil and thymine. Also involved in the degradation of the chemotherapeutic drug 5-fluorouracil	High expression in mural granulosa cells (doi: 10.1186/1471-2164-15-24)	No
GALM Galactose mutarotase	Cytoplasmic protein responsible for epimerization of hexose sugars such as glucose and galactose.	None	No
ASMTL Acetylserotonin O- Methyltransferase Like	Nucleoside triphosphate pyrophosphatase that hydrolyzes dTTP and UTP. May have a dual role in cell division arrest and in preventing the incorporation of modified nucleotides into cellular nucleic acids.	None	Yes