SUPPLEMENTARY MATERIALS

Genomic profiling of primary and recurrent adult granulosa cell tumors of the ovary

Da Cruz Paula et al.

Supplementary Figures S1 – S2

Supplementary Tables S1 – S4
Supplementary Figure S1. **Cancer cell fractions of mutations identified in primary and recurrent adult-type granulosa cell tumors of the ovary.** Cancer cell fractions and clonality of non-synonymous somatic mutations identified in primary adult-type granulosa cell tumors (aGCTs), including primary non-recurrent aGCTs (n=7, left), primary aGCTs that developed recurrences (n=9, middle) and aGCT recurrences (n=19, right). Cancer cell fractions are color-coded according to the legend. Clonal mutations are depicted by a yellow box.
Supplementary Figure S2. Clonal composition of additional primary and recurrent adult granulosa cell tumors of the ovary. Representative hematoxylin and eosin micrographs (magnification, 200x) and clonal frequency heatmaps of the matched primary and recurrent adult-type granulosa cell tumors (A) aGCT18, (B) aGCT43, (C) aGCT76 and (D) aGCT81. Cancer cell fractions are color-coded according to the legend. Scale bars, 200 μM. Clonal mutations are depicted by a yellow box.
**Supplementary Table S1.** Clinico-pathologic features of primary and recurrent adult-type granulosa cell tumors of the ovary, sequencing methods employed and TERT promoter mutation status.

| Sample type | Case ID | Sample ID | Case description | Sample Description | Sequencing method | FOXL2 status | TERT promoter status | Age at diagnosis (yrs) | Stage at diagnosis | Tumor size (cm) | Menopausal status | Adjuvant chemotherapy |
|-------------|---------|-----------|------------------|--------------------|-------------------|---------------|----------------------|------------------------|-------------------|----------------|-----------------|---------------------|
| **Primary non-recurrent** | aGCT6 | aGCT6-P | Primary tumor | Primary Non-Recurrent-aGCT | IMPACT | C134W | C250T | 46 | IA | 14.0 | N/A | N |
| | aGCT7 | aGCT7-P | Primary tumor | Primary Non-Recurrent-aGCT | IMPACT | C134W | C228T | 66 | IA | 1.7 | Post | N |
| | aGCT8 | aGCT8-P | Primary tumor | Primary Non-Recurrent-aGCT | IMPACT | C134W | WT | 68 | IC | 2.6 | Post | N |
| | aGCT9 | aGCT9-P | Primary tumor | Primary Non-Recurrent-aGCT | IMPACT | C134W | WT | 49 | IA | 9.0 | Post | N |
| | aGCT10 | aGCT10-P | Primary tumor | Primary Non-Recurrent-aGCT | IMPACT | C134W | WT | 56 | IA | 5.5 | Post | N |
| | aGCT11 | aGCT11-P | Primary tumor | Primary Non-Recurrent-aGCT | IMPACT | C134W | WT | 34 | IA | 6.5 | Pre | N |
| | aGCT12 | aGCT12-P | Primary tumor | Primary Non-Recurrent-aGCT | IMPACT | C134W | WT | 43 | IA | 11.5 | Pre | N |
| | aGCT13 | aGCT13-P | Primary tumor | Primary Non-Recurrent-aGCT | Sanger | C134W | WT | 57 | IA | 3.7 | Post | N |
| | aGCT14 | aGCT14-P | Primary tumor | Primary Non-Recurrent-aGCT | Sanger | C134W | WT | 87 | IC | 12.2 | Post | N |
| | aGCT15 | aGCT15-P | Primary tumor | Primary Non-Recurrent-aGCT | IMPACT | C134W | WT | 41 | IA | 5.5 | N/A | N |
| | aGCT16 | aGCT16-P | Primary tumor | Primary Non-Recurrent-aGCT | IMPACT | C134W | C250T | 41 | I | N/A | Pre | N |
| | aGCT17 | aGCT17-P | Primary tumor | Primary Non-Recurrent-aGCT | IMPACT | C134W | C228T | 65 | IB | 18.0 | Post | Y |
| | aGCT18 | aGCT18-P | Primary tumor | Primary Non-Recurrent-aGCT | IMPACT | C134W | WT | 62 | IB | 18.0 | Pre | N |
| | aGCT19 | aGCT19-P | Primary tumor | Primary Non-Recurrent-aGCT | IMPACT | C134W | WT | 83 | IA | 10.0 | Post | N |
| | aGCT20 | aGCT20-P | Primary tumor | Primary Non-Recurrent-aGCT | IMPACT | C134W | C250T | 42 | I | 29.0 | Pre | N |
| | aGCT21 | aGCT21-P | Primary tumor | Primary Non-Recurrent-aGCT | IMPACT | C134W | WT | 50 | N/A | 3.3 | Post | N |
| | aGCT22 | aGCT22-P | Primary tumor | Primary Non-Recurrent-aGCT | IMPACT | C134W | WT | 36 | N/A | 1.5 | Pre | Y |
| | aGCT23 | aGCT23-P | Primary tumor | Primary Non-Recurrent-aGCT | IMPACT | C134W | WT | 40 | N/A | 1.5 | Pre | N |
| | aGCT24 | aGCT24-P | Primary tumor | Primary Non-Recurrent-aGCT | IMPACT | C134W | WT | 56 | N/A | 0.7 | Post | N |
| | aGCT25 | aGCT25-P | Primary tumor | Primary Non-Recurrent-aGCT | IMPACT | C134W | WT | 45 | N/A | 8.0 | Pre | N |
| | aGCT26 | aGCT26-P | Primary tumor | Primary Non-Recurrent-aGCT | IMPACT | C134W | WT | 57 | N/A | 6.5 | Post | N |
| | aGCT27 | aGCT27-P | Primary tumor | Primary Non-Recurrent-aGCT | IMPACT | C134W | WT | 34 | N/A | 4.0 | Pre | Y |

N, no; N/A, not available; WT, wild-type; Y, yes.
Supplementary Table S2. Sequencing statistics of the adult-type granulosa cell tumors of the ovary subjected to MSK-IMPACT sequencing.

| Sample ID | Tissue type | Total Reads | Mean Target Coverage (X) | Target Bases 2X | Target Bases 50X | Target Bases 100X |
|-----------|-------------|-------------|--------------------------|-----------------|-------------------|-------------------|
| aGCT6-P   | Primary tumor | 67545102 | 760 | 99.0% | 98.1% | 97.8% |
| aGCT6-N   | Normal | 18141499 | 244 | 99.0% | 96.9% | 89.7% |
| aGCT35-P  | Primary tumor | 76593776 | 931 | 99.3% | 98.7% | 98.2% |
| aGCT38-P  | Primary tumor | 114407263 | 1223 | 99.4% | 98.9% | 98.5% |
| aGCT30-P  | Primary tumor | 48729140 | 396 | 99.0% | 97.9% | 96.7% |
| aGCT30-N  | Normal | 33891814 | 366 | 98.9% | 97.4% | 92.6% |
| aGCT32-P  | Primary tumor | 6223237 | 238 | 98.9% | 97.3% | 92.8% |
| aGCT16-P  | Primary tumor | 58939726 | 549 | 99.0% | 98.0% | 96.8% |
| aGCT16-N  | Normal | 14522831 | 199 | 98.9% | 93.6% | 74.5% |
| aGCT17-P  | Primary tumor | 18861672 | 510 | 99.1% | 98.1% | 96.8% |
| aGCT18-P  | Primary tumor | 28101497 | 440 | 99.3% | 96.8% | 90.1% |
| aGCT18-R  | Recurrence | 33508372 | 713 | 99.3% | 98.3% | 97.2% |
| aGCT43-P  | Primary tumor | 41997488 | 820 | 99.5% | 98.1% | 95.2% |
| aGCT43-R  | Recurrence | 48973084 | 923 | 99.4% | 98.8% | 98.2% |
| aGCT43-N  | Normal | 15746115 | 438 | 99.4% | 98.0% | 94.3% |
| aGCT76-P  | Primary tumor | 20780770 | 242 | 98.8% | 89.0% | 70.9% |
| aGCT76-R  | Matched Recurrence | 25981138 | 291 | 98.9% | 95.0% | 84.8% |
| aGCT77-P  | Primary tumor | 12488514 | 193 | 98.6% | 91.7% | 76.5% |
| aGCT77-R  | Matched Recurrence | 32279005 | 696 | 99.3% | 98.3% | 97.2% |
| aGCT78-P  | Primary tumor | 21932811 | 309 | 99.1% | 96.9% | 90.4% |
| aGCT79-R  | Matched Recurrence | 14641112 | 246 | 98.7% | 95.9% | 87.4% |
| aGCT79-N  | Normal | 10911225 | 152 | 99.2% | 91.8% | 69.1% |
| aGCT79-P  | Primary tumor | 45111281 | 423 | 99.3% | 97.8% | 93.7% |
| aGCT79-R  | Matched Recurrence | 39151357 | 361 | 99.3% | 97.6% | 93.6% |
| aGCT79-N  | Normal | 18615212 | 404 | 99.3% | 97.5% | 93.0% |
| aGCT80-P  | Primary tumor | 11933495 | 121 | 96.6% | 87.1% | 56.9% |
| aGCT80-R  | Matched Recurrence | 42168428 | 1033 | 99.4% | 98.9% | 98.5% |
| aGCT80-N  | Normal | 3523541 | 48 | 98.1% | 37.9% | 8.3% |
| aGCT81-P  | Primary tumor | 22799785 | 280 | 99.1% | 96.8% | 89.1% |
| aGCT81-R  | Matched Recurrence | 29886363 | 550 | 99.2% | 98.1% | 96.9% |
| aGCT81-N  | Normal | 10761336 | 117 | 99.0% | 70.6% | 35.8% |
| aGCT82-P  | Primary tumor | 21778878 | 518 | 99.2% | 98.0% | 95.4% |
| aGCT82-R  | Matched Recurrence | 45114749 | 1012 | 99.5% | 98.9% | 98.5% |
| aGCT82-N  | Normal | 13377769 | 367 | 99.4% | 97.9% | 93.5% |
| aGCT83-P  | Recurrence | 24949134 | 379 | 99.3% | 97.1% | 88.3% |
| aGCT83-N  | Normal | 13663124 | 250 | 99.2% | 96.7% | 86.3% |
| aGCT58-R  | Recurrence | 30097844 | 532 | 99.4% | 98.3% | 95.7% |
| aGCT59-N  | Normal | 19279986 | 397 | 99.4% | 97.9% | 93.5% |
| aGCT100-R | Recurrence | 34194042 | 611 | 99.4% | 98.4% | 96.8% |
| aGCT100-N | Normal | 32130500 | 510 | 99.3% | 98.0% | 95.6% |
| aGCT111-R | Recurrence | 20899010 | 467 | 99.4% | 98.3% | 95.1% |
| aGCT111-N | Normal | 12330450 | 310 | 99.3% | 97.5% | 90.7% |
| aGCT122-R | Recurrence | 28086816 | 481 | 99.4% | 97.8% | 93.3% |
| aGCT148-R | Recurrence | 39630138 | 465 | 99.4% | 98.3% | 96.1% |
| aGCT148-N | Normal | 16247196 | 384 | 99.3% | 97.4% | 89.2% |
| aGCT199-R | Recurrence | 22005986 | 519 | 99.4% | 98.2% | 95.0% |
| aGCT199-N | Normal | 17133311 | 421 | 99.4% | 98.0% | 93.0% |
| aGCT200-R | Recurrence | 78768625 | 953 | 99.5% | 98.9% | 96.3% |
| aGCT211-R | Recurrence | 50487662 | 855 | 99.4% | 96.8% | 97.9% |
| aGCT75-R2 | Recurrence | 23813248 | 649 | 99.2% | 98.6% | 98.1% |
| Sample ID | Gene symbol | Amino acid change | Chromosome | Genomic position | Reference allele | Alternate allele | Type of mutation | Depth at mutational hotspots | Hotspot mutation | Loss of heterozygosity (LOH) | Cancer Cell Fraction (ABSOLUTE) | Closal Status | Pathogenicity | Cancer driver genes (Baby et al) |
|-----------|-------------|------------------|------------|-----------------|----------------|----------------|----------------|-----------------------------|----------------|-----------------------------|-----------------------------|--------------|--------------|-------------------------------|
| AGCT2-F  | FBXW7       | p.R484X         | 9          | 123622947       | G              | A              | Missense_Mutation | 56% Clonal, likely pathogenic | FALSE          | TRUE                        | 100% Clonal, likely pathogenic | FALSE        | TRUE         | Clonal, likely pathogenic     |
| AGCT2-F  | FBXW7       | p.R484X         | 9          | 123622947       | G              | A              | Missense_Mutation | 56% Clonal, likely pathogenic | FALSE          | TRUE                        | 100% Clonal, likely pathogenic | FALSE        | TRUE         | Clonal, likely pathogenic     |
| AGCT2-F  | FBXW7       | p.R484X         | 9          | 123622947       | G              | A              | Missense_Mutation | 56% Clonal, likely pathogenic | FALSE          | TRUE                        | 100% Clonal, likely pathogenic | FALSE        | TRUE         | Clonal, likely pathogenic     |
| AGCT2-F  | FBXW7       | p.R484X         | 9          | 123622947       | G              | A              | Missense_Mutation | 56% Clonal, likely pathogenic | FALSE          | TRUE                        | 100% Clonal, likely pathogenic | FALSE        | TRUE         | Clonal, likely pathogenic     |
| AGCT2-F  | FBXW7       | p.R484X         | 9          | 123622947       | G              | A              | Missense_Mutation | 56% Clonal, likely pathogenic | FALSE          | TRUE                        | 100% Clonal, likely pathogenic | FALSE        | TRUE         | Clonal, likely pathogenic     |
| AGCT2-F  | FBXW7       | p.R484X         | 9          | 123622947       | G              | A              | Missense_Mutation | 56% Clonal, likely pathogenic | FALSE          | TRUE                        | 100% Clonal, likely pathogenic | FALSE        | TRUE         | Clonal, likely pathogenic     |
| AGCT2-F  | FBXW7       | p.R484X         | 9          | 123622947       | G              | A              | Missense_Mutation | 56% Clonal, likely pathogenic | FALSE          | TRUE                        | 100% Clonal, likely pathogenic | FALSE        | TRUE         | Clonal, likely pathogenic     |
| AGCT2-F  | FBXW7       | p.R484X         | 9          | 123622947       | G              | A              | Missense_Mutation | 56% Clonal, likely pathogenic | FALSE          | TRUE                        | 100% Clonal, likely pathogenic | FALSE        | TRUE         | Clonal, likely pathogenic     |
| AGCT2-F  | FBXW7       | p.R484X         | 9          | 123622947       | G              | A              | Missense_Mutation | 56% Clonal, likely pathogenic | FALSE          | TRUE                        | 100% Clonal, likely pathogenic | FALSE        | TRUE         | Clonal, likely pathogenic     |
| AGCT2-F  | FBXW7       | p.R484X         | 9          | 123622947       | G              | A              | Missense_Mutation | 56% Clonal, likely pathogenic | FALSE          | TRUE                        | 100% Clonal, likely pathogenic | FALSE        | TRUE         | Clonal, likely pathogenic     |
### Supplementary Table S4. Pathway analysis using MsigDB and DAVID in recurrent adult granulosa cell tumors of the ovary.

| Method | Database/ gene set ID | Description Term | Number of genes in gene set | Number of in overlap | P value |
|--------|-----------------------|-------------------|----------------------------|----------------------|---------|
| **MsigDB** | KEGG_PATHWAYS_IN_CANCER | Pathways in cancer | 328 | 11 | 9.16 e-13 |
| | KEGG_PROSTATE_CANCER | Prostate cancer | 89 | 7 | 4.07 e-11 |
| | KEGG_CELL_CYCLE | Cell cycle | 128 | 7 | 5.35 e-10 |
| | BIOCARTA_G1_PATHWAY | Cell Cycle: G1/S Check Point | 28 | 6 | 1.91 e-12 |
| | BIOCARTA_CTCF_PATHWAY | CTCF: First Multivalent Nuclear Factor | 23 | 5 | 1.36 e-10 |
| | BIOCARTA_ARF_PATHWAY | Tumor Suppressor Arf Inhibits Ribosomal Biogenesis | 17 | 4 | 7.53 e-9 |
| | BIOCARTA_ATM_PATHWAY | ATM Signaling Pathway | 20 | 4 | 1.53 e-8 |
| | KEGG_PATHWAY_hsa05203 | Viral carcinogenesis | 205 | 36 | 1.11E-16 |
| | KEGG_PATHWAY_hsa04622 | RIG-I-like receptor signaling pathway | 70 | 18 | 3.91E-11 |
| | KEGG_PATHWAY_hsa04140 | Regulation of autophagy | 39 | 14 | 1.06E-10 |
| | KEGG_PATHWAY_hsa04623 | Cytosolic DNA-sensing pathway | 64 | 15 | 9.55E-09 |
| | KEGG_PATHWAY_hsa05202 | Transcriptional misregulation in cancer | 168 | 23 | 1.53E-08 |
| | KEGG_PATHWAY_hsa05320 | Autoimmune thyroid disease | 82 | 13 | 5.98E-08 |
| | KEGG_PATHWAY_hsa04620 | Toll-like receptor signaling pathway | 106 | 17 | 2.13E-07 |
| | KEGG_PATHWAY_hsa04060 | Cytokine-cytokine receptor interaction | 230 | 25 | 2.78E-07 |
| | KEGG_PATHWAY_hsa04650 | Natural killer cell mediated cytotoxicity | 122 | 17 | 1.50E-06 |
| | KEGG_PATHWAY_hsa04630 | Jak-STAT signaling pathway | 145 | 18 | 3.42E-06 |
| | KEGG_PATHWAY_hsa04191 | PI3K-Akt signaling pathway | 345 | 27 | 3.87E-05 |
| | KEGG_PATHWAY_hsa04110 | Cell cycle | 124 | 10 | 0.018011772 |
| | KEGG_PATHWAY_hsa04919 | Thyroid hormone signaling pathway | 114 | 9 | 0.029906665 |
| | BIOCARTA_h_arfPathway | Tumor Suppressor Arf Inhibits Ribosomal Biogenesis | 18 | 6 | 1.29E-04 |
| | BIOCARTA_h_g1Pathway | Cell Cycle: G1/S Check Point | 30 | 7 | 1.89E-04 |
| | BIOCARTA_h_ctcfPathway | CTCF: First Multivalent Nuclear Factor | 25 | 6 | 6.83E-04 |
| | BIOCARTA_h_i11rPathway | Signal transduction through IL1R | 33 | 6 | 0.00254416 |
| | BIOCARTA_h_cellcyclePathway | Cyclins and Cell Cycle Regulation | 25 | 5 | 0.005790756 |
| | BIOCARTA_h_sggf1mrPathway | Skeletal muscle hypertrophy is regulated via AKT/mTOR pathway | 20 | 4 | 0.020476084 |
| | BIOCARTA_h_ifnaPathway | IFN alpha signaling pathway | 9 | 3 | 0.028015574 |
| | BIOCARTA_h_mtorPathway | mTOR Signaling Pathway | 26 | 4 | 0.041158217 |