

1 **Single-cell analysis of human ovarian cortex identifies distinct cell populations but no**
2 **oogonial stem cells.**

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30 Cell Ranger metrics and gene expression analysis of unsorted ovarian cortex cells.

31 **Supplementary Data 2**

32 Cell Ranger metrics and gene expression analysis of sorted ovarian cortex cells.

33 **Supplementary Data 3**

34 Upregulated genes in cultured human ovarian DDX4 Ab+ and DDX4 Ab- cells.

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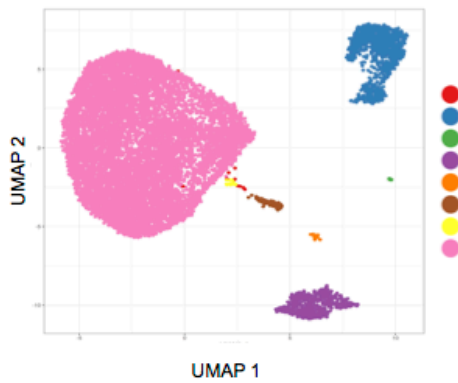
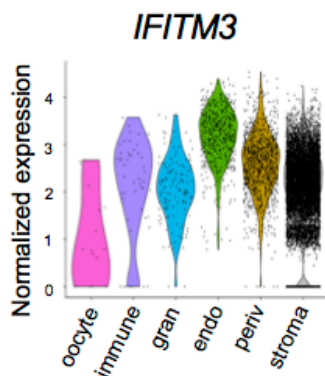
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38 **Supplementary Figure 1. ScRNA-seq analysis of unsorted ovarian cortical cells.****a**

	GRP	C-sec
oocyte	4	14
immune	19	29
granulosa	5	132
endothelial	140	494
perivascular	422	785
stroma	5,125	4,991
total	5,715	6,445

b

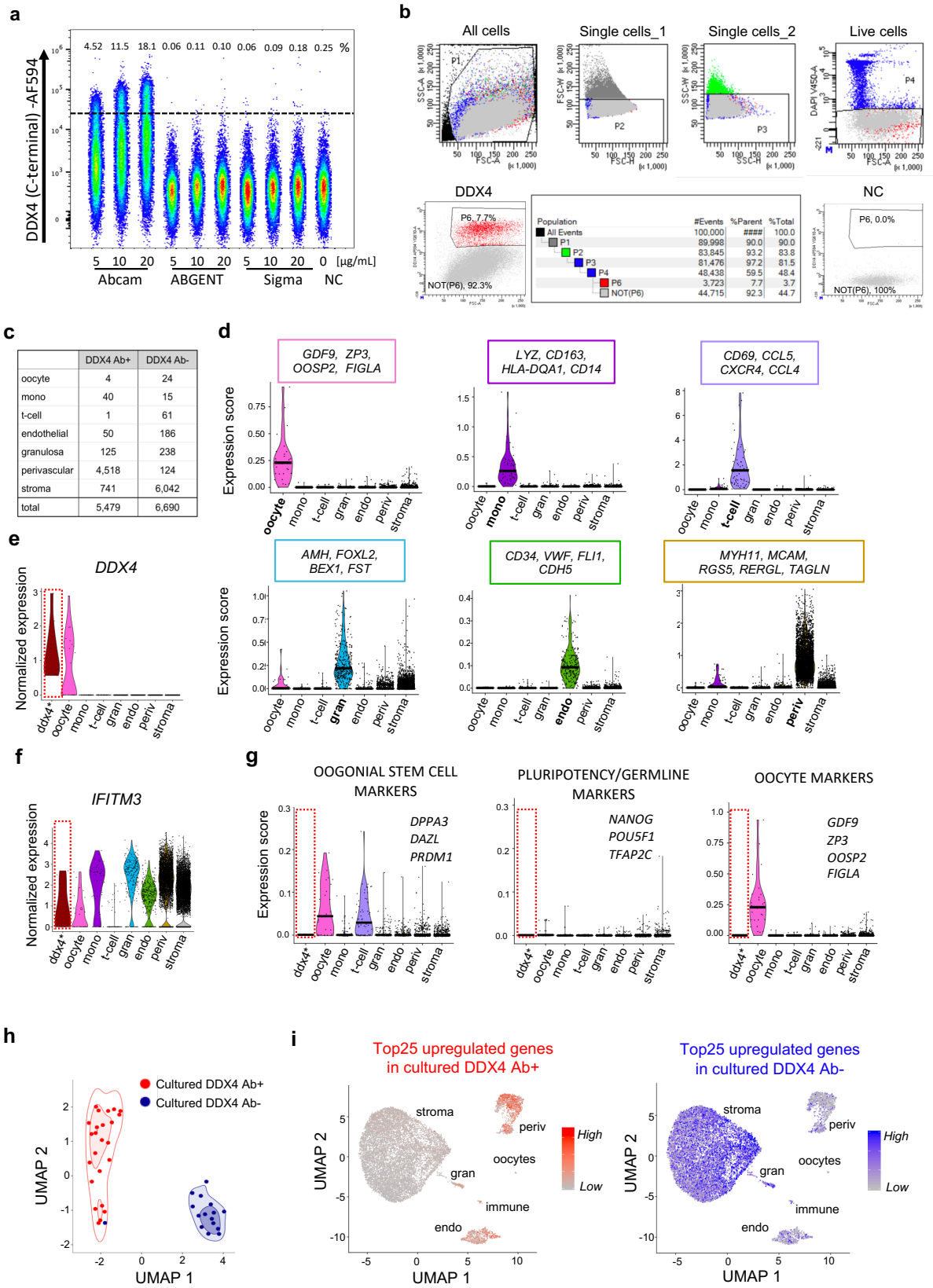
Density based hierarchical clustering (HDBSCAN)

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Supplementary Figure 1. ScRNA-seq analysis of unsorted ovarian cortical cells. **a**, Number of cells contributing to the different cell clusters from GRP and C-sec tissue (in Fig. 2b). **b**, Confirmation of cluster identification using HDBSCAN. **c**, Violin plot showing the expression levels of *IFITM3* in unsorted ovarian cortex cells.

46 **Supplementary Figure 2. Sorted ovarian DDX4 Ab+ and Ab- cortex cells.**

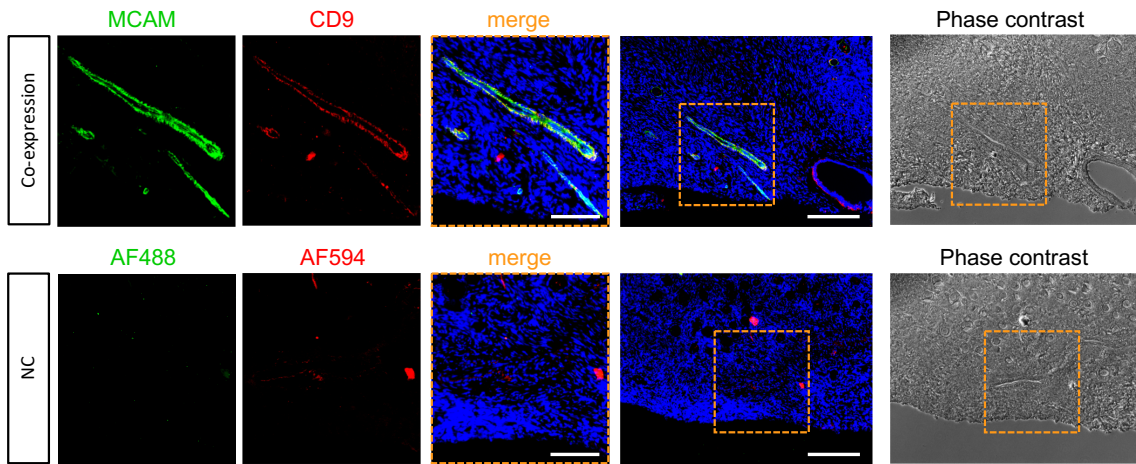


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48 **Supplementary Figure 2. Sorted ovarian DDX4 Ab+ and Ab- cortex cells.** a, FACS plots
49 of three different DDX4 C-terminus antibodies (Abcam, ABGENT, and Sigma) in three different
50 staining concentrations (5, 10 and 20 µg/mL per sample containing max 1×10^6 cells in 100

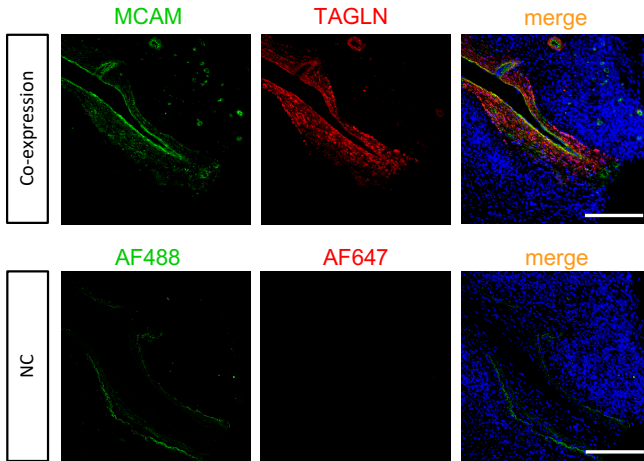
51 μL) are shown. The Abcam Ab labeled an increasing percentage of a distinct cell population
52 (4.51% - 18.1%) with increasing concentration while ABGENT and Sigma antibodies did not.
53 Gating of DDX4 Ab⁺ cells was performed on cells stained for rabbit polyclonal isotype control
54 Ab. **b**, Representative FACS plots showing the gating strategy of dissociated ovarian tissue
55 cells for sorting of DDX4 Ab⁺ cells for scRNA-seq analysis. After exclusion of debris, single
56 cells were gated based on FSC and SSC properties. Live cells were selected based on DAPI
57 signal. Gating of DDX4 Ab⁺ cells was based on FSC and distinct Ab signal. Several negative
58 control samples were included in each sort (DAPI only, primary Ab omitted, FMO as well as
59 rabbit polyclonal isotype control, labelled here as NC) to avoid false positive DDX4 Ab⁺ cells.
60 On average, a DDX4 Ab⁺ cell population of 5.5 – 11.5% was observed. **c**, Contribution of cells
61 from sorted DDX4 Ab⁺ and DDX4 Ab⁻ populations to each cluster (in Fig. 3b). **d**, Color-coded
62 violin plots showing expression scores of selected signature genes among the top highly
63 expressed genes defining the cell type of each cluster. **e**, Somatic cells expressing *DDX4*
64 ($n=12$; $n=1$ in Ab⁺ population and $n=11$ in Ab⁻ population) were manually clustered to form a
65 separate cluster (*ddx4**). **f**, Violin plot showing the expression levels of *IFITM3* in all sorted
66 ovarian cortex cells. **g**, Violin plots showing OSC (*DPPA3*, *DAZL*, *PRDM1*), pluripotency and/or
67 germline (*NANOG*, *POU5F1*, *TFAP2C*) and oocyte markers (*GDF9*, *ZP3*, *OOSP2*, *FIGLA*) as
68 expression score. None of the markers were expressed in somatic DDX4 expressing cells
69 (except from *IFITM3*). **h**, UMAP based on single-cell transcriptomes of cultured DDX4 Ab⁺ and
70 Ab⁻ cells showing that the populations cluster separately even after extended time in cell
71 culture in OSC culture conditions. Contour lines indicate the 2D kernel density for each cell
72 population divided into three bins. **i**, Feature plots showing the expression score of the top 25
73 upregulated genes of cultured DDX4 Ab⁺ (scale bar depicting low expression in grey and high
74 expression in red) and DDX4 Ab⁻ cells (scale bar depicting low expression in grey and high
75 expression in blue) in our freshly sequenced unsorted ovarian cells.
76 **Horizontal bars in violin plots indicate median gene expression per cluster.**
77 Ab, Antibody; FACS, Fluorescence Activated Cell Sorting; FMO, Fluorescence Minus One;
78 FSC, Forward Scatter; NC, Negative Control; OSC, Oogonial Stem Cell; PCA, Principal
79 Component Analysis; SSC, Side Scatter.

80 **Supplementary Figure 3. Validation of markers identified in transcriptomic and**
 81 **proteomic analysis.**

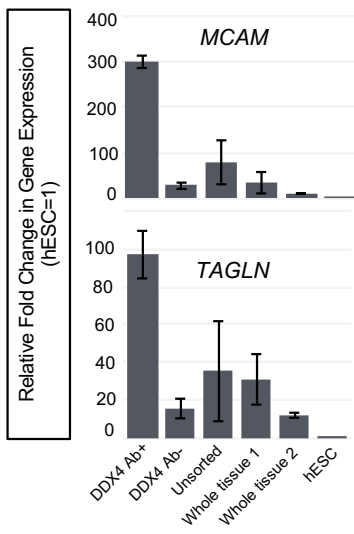
a



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84 **Supplementary Figure 3. Validation of markers identified in transcriptomic and**
85 **proteomic analysis. a**, Immunostaining of human ovarian cortical tissue sections from GRP
86 donor showing the expression of MCAM (green) and CD9 (red) on a protein level. DAPI (blue)
87 was used as nuclear counterstain. Co-expression was detected in cells of blood vessels
88 (zoomed-out, scale bars: 200 μm). Orange rectangles demarcate the zoomed-in area (scale
89 bars: 100 μm). Immunostaining was performed in three histological sections from three
90 patients and a representative image is shown. **b**, Immunostaining of human ovarian cortical
91 tissue sections from a GRP donor showing the protein expression of MCAM (green) and
92 TAGLN (red) in cells of a blood vessel. Nuclei are counterstained using DAPI (blue). Scale
93 bars: 200 μm . Immunostaining was performed in three histological sections from two patients.
94 All vessels stained positive for MCAM/TAGLN and a representative image is shown. **c**, Gene
95 expression analysis of *MCAM* and *TAGLN* in sorted ovarian tissue cells from pooled GRP
96 donors (DDX4 Ab+/-), unsorted ovarian tissue cells (DDX4 ab+ and Ab-) from three GRP
97 donors dissociated during independent experiments and three pieces of whole tissue from
98 two GRP donors, respectively. Values were normalized to *GAPDH* expression and displayed
99 relative to hESC sample. Mean \pm SD is shown from three undissociated pieces of whole ovary
100 tissue from two donors, respectively, three independently dissociated ovarian tissue samples,
101 and three independently sorted DDX4 Ab+ and Ab- cell fractions.
102 As negative control (NC) in immunostaining, primary antibodies were omitted.
103 hESCs, human Embryonic Stem Cells.

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105**Supplementary Figure 4. Germline-like cells in the adult human ovary.**

Integration with unsorted GRP data

Integrated cluster ID	mitotic FGCs	RA resp. FGCs	meiotic FGCs	Oo-genesis	NA	endo-thelial	granulosa (w7-10)	granulosa (w10-20)	granulosa (w20-26)	GRP (5,715 cells)
	mitotic FGCs	426	14	0	0	17	0	0	0	0
RA responsive FGCs	0	78	0	0	8	0	0	0	0	0
meiotic FGCs	2	2	90	6	27	0	0	0	0	0
oocytes/oogonia	0	0	0	25	1	0	0	0	0	3
immune	0	0	0	0	33	0	0	1	0	17
granulosa	0	0	0	0	9	0	2	2	42	1
endothelial	0	0	0	0	4	7	0	0	0	131
perivascular	12	0	0	0	14	0	2	21	2	408
stroma	5	0	3	0	67	1	33	47	120	5,155

Integration with sorted DDX4 Ab+ data

Integrated cluster ID	mitotic FGCs	RA resp. FGCs	meiotic FGCs	Oo-genesis	NA	endo-thelial	granulosa (w7-10)	granulosa (w10-20)	granulosa (w20-26)	DDX4 Ab+ (5,479 cells)
	mitotic FGCs	409	1	2	0	31	0	0	25	10
RA responsive FGC	2	92	1	1	13	0	0	0	0	0
meiotic FGCs	0	1	89	7	26	0	0	0	0	0
oocytes/oogonia	0	0	0	23	1	0	0	0	0	2
immune	0	0	0	0	32	0	0	1	0	40
granulosa	1	0	1	0	30	1	14	3	111	124
endothelial	0	0	0	0	4	7	0	0	0	50
perivascular	32	0	0	0	29	0	0	38	24	4,495
stroma	1	0	0	0	14	0	23	4	19	767

Integration with sorted DDX4 Ab- data

Integrated cluster ID	mitotic FGCs	RA resp. FGCs	meiotic FGCs	Oo-genesis	NA	endo-thelial	granulosa (w7-10)	granulosa (w10-20)	granulosa (w20-26)	DDX4 Ab- (6,690 cells)
	mitotic FGCs	437	2	2	0	24	0	1	0	0
RA responsive FGC	0	90	0	0	10	0	0	0	0	0
meiotic FGCs	1	2	88	8	18	0	0	0	0	0
oocytes/oogonia	0	0	0	23	1	0	0	0	0	21
immune	0	0	0	0	34	0	0	1	0	74
granulosa	0	0	2	0	30	0	3	17	79	244
endothelial	0	0	0	0	4	7	0	0	0	188
perivascular	0	0	0	0	0	0	0	0	0	108
stroma	7	0	1	0	59	1	33	53	85	6,053

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Supplementary Figure 4. Germline-like cells in the adult human ovary. Our adult ovarian cortex datasets were integrated with a publicly available fetal ovary scRNA-seq dataset [1] in order to find any adult cells that would cluster with pre-meiotic germ cells. Integration was carried out sample by sample in order to avoid bias caused by disproportionate cell numbers between the datasets. Numbers of cells contributing to the integrated clusters from fetal and adult samples are shown for each comparison. Integration with unsorted C-sec sample is displayed in Fig. 5. Adult cells clustering with pre-meiotic germ cells are highlighted in red.

117 **Supplementary Table 1. First surface marker screening of human ovarian cortex cells.**
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Screen 1

Surface Marker	% CD-POS	MFI
CD47	99,4	57332
CD59	99,4	99797
CD147	99,3	18778
CD55	96,3	13128
CD58	93,6	8389
CD95	83,2	8813
CD46	82,2	10896
CD29	80,4	18113
CD166	80,2	13714
CD49c	80,1	14038
CD44	79,7	8886
CD151	79,2	12902
CD63	78,5	6336
CD164	77,7	7817
CD140b	77,2	19179
CD81	77,1	7447
CD165	71,5	9929
CD73	68,1	30713
CD268	67,4	3064
CD49a	66,1	7902
B2M	65,6	10503
CD90	65,5	24181
CD321	64,6	5080
CD54	63,9	5922
CD340	63,6	3193
CD9	59,9	9134
CD26	59,8	31591
HLA-A,B,C	59,4	39782
EFR-R	59,3	3458
CD49e	57,7	9602
CD105	57,2	11868
CD62E	51	5876
CD98	50,6	2857
CD221	50,6	2538
CD200	49,5	10743
CD146	48,9	10298
HLA-DQ	46,1	4438
CD141	43,8	2941
CD61	40,3	2857
CD91	40	3508
CD142	38,1	2749
CD326	37,5	5511
CD51/61	35,1	2913
HPC	34,4	3424
HLA-A2	34,1	12284
CD338	32,5	2569
CD34	31,1	4051
CD71	30,8	2519
CD140a	30,1	2453
CD56	27,9	3500

CD275	26,1	2218
CD49d	24,3	3367
CD107a	23,6	2262
CD144	23,5	6303
HLA-DR	23,4	4831
HLA-DR,DP,DQ	21,1	2736
CD39	17,8	34067
CD40	16	2405
CD97	14,4	2477
CD220	13,6	2073
CD49b	13,4	2120
CD243	12,5	4438
CD226	11,9	2042
CD227	11,6	2588
CD235a	10,2	2142
CD152	9,59	2104
CD183	9,14	1996
CD112	8,56	1996
CD161	8,31	2042
CD130	7,97	2062
CD107b	7,82	2115
CD126	7,82	1996
SSEA-4	7,54	6643
CD102	7,25	2441
CD77	7,17	2690
CD181	6,64	2062
CD109	5,74	1945
CD184	5,66	2174
CDw329	5,53	1991
CD124	5,19	1935
CD314	4,92	1911
CD271	4,84	3334
CD205	4,41	2057
CD309	4,35	7388
CD336	4,32	2223
Invariant NK	4,25	2062
Gamma delta TCR	4,12	5801
CD162	4,03	1975
CD11a	4,01	2089
CD72	3,97	1980
CD22	3,91	1955
BLTR-1	3,72	1950
CD66(a,c,d,e)	3,63	3391
CD121b	3,6	2158
CD13	3,52	2837
CD274	3,26	2026
CD10	3,14	2370
GD2	2,91	3208
CD4v4	2,85	2268
CD31	2,77	4770
CD27	2,53	2115
CD206	2,36	2447
CD45RA	2,29	2037
CD14	2,21	2052
CD195	2,19	2163
CD83	2,18	2089
CD197	2,17	2201

CD244	2,16	6125
CLIP	2,15	2052
CD231	2,13	2324
CD150	2,11	2136
CD38	1,9	2394
CD74	1,89	2364
CD36	1,88	3508
CD80	1,87	2201
CD117	1,82	2330
CD45RO	1,79	2251
CD41b	1,74	2465
CD134	1,72	2207
CD84	1,7	2115
CD123	1,69	2330
CD75	1,65	2550
CD53	1,64	2613
CD32	1,55	2256
CD273	1,53	2723
CD85	1,49	2544
CD177	1,46	2273
MIC A/B	1,45	2163
CD88	1,45	2336
CD196	1,45	2229
CD229	1,39	2526
CD62P	1,34	2857
CD118	1,33	2152
CD172b	1,32	2229
CD18	1,31	2394
CD64	1,3	2723
CD171	1,3	3247
CD57	1,29	2507
CD193	1,29	2749
CD45	1,27	2313
CD79b	1,26	2347
CD163	1,26	2532
CD337	1,23	2083
CDMR-56	1,23	2336
CD50	1,23	2899
CD137 Ligand	1,21	2459
CDw328	1,2	2158
CD15	1,2	2417
CD62L	1,19	2399
CD153	1,17	2388
CD278	1,17	2913
CD69	1,15	2716
NKB1	1,11	2532
CD178	1,11	2697
CD209	1,11	2465
CMRF-44	1,1	2626
CD11b	1,09	2471
CD45RB	1,09	2268
CD99	1,09	2423
CD158b	1,09	2581
CDw327	1,08	2273
CD279	1,06	2411
CD108	1,06	2569
SSEA-1	1,05	2296

CD48	1,05	2613
CD282	1,03	2262
CD5	1,03	2899
CD70	1,03	2683
CD20	1,01	2290
CD30	1,01	2405
CD35	1,01	2405
CD99R	1,01	2110
Vbeta8	1	2229
CD16	1	2313
Alpha beta TCR	0,99	2581
CD128	0,99	2507
CD305	0,98	2223
CDw93	0,98	2613
CD135	0,97	3278
CD66b	0,95	2600
CD11c	0,94	2174
CD43	0,94	2756
CD106	0,94	2483
fMLP-R	0,91	2477
CD28	0,91	2330
CD42a	0,91	2483
CD3	0,9	2544
CD21	0,88	2399
CD42b	0,87	2489
CD138	0,87	3050
CD158a	0,87	2336
CD33	0,85	2330
CD122	0,85	2664
CD137	0,85	2749
TRA-1-81	0,84	2142
CD1d	0,84	2158
CD1a	0,82	2336
CD8b	0,82	2495
CD23	0,81	2588
CD103	0,81	2083
Vbeta23	0,8	2313
CD66f	0,79	2168
CD89	0,79	2364
CD41a	0,78	2207
CD86	0,78	2658
C119	0,78	2218
CD25	0,77	2716
CD7	0,76	2723
CD154	0,76	2501
CD37	0,75	2429
CD87	0,74	2201
CD180	0,74	2453
CD255	0,74	6875
CD6	0,73	2262
CD114	0,73	3154
CD2	0,72	2126
CD1b	0,71	2273
CD121a	0,71	2423
TRA-1-60	0,69	2519
CD19	0,69	2556
CD127	0,68	2364

CD24	0,67	2417
CD100	0,67	2613
CD8a	0,66	2376
CD116	0,66	2212
CD335	0,64	2313
CD94	0,64	2218
CD120a	0,62	2382
CD4	0,58	2353
CD15s	0,56	2477

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Supplementary Table 1. First surface marker screening of human ovarian cortex cells.

Percentage of ovarian cortex cells positive for 242 CD markers and corresponding MFI values.

CD, Cluster of differentiation; MFI, Mean fluorescence intensity.

125 **Supplementary Table 2. Second surface marker screening of human ovarian cortex**
 126 **cells.**
 127

Screen 2

Surface Marker	% CD-POS	MFI
CD49a	99,7	76235
CD61	99,3	467000
CD151	99,2	18996
CD56	96	16101
CD59	93	9308
CD47	87,4	9798
CD98	86,8	8263
CD141	86,5	21738
CD39	85,2	19692
CD200	83,8	17174
CD49e	83,5	15692
HLA A2	81,8	26911
CD165	81,3	13136
CD166	79	12261
CD29	74,2	27183
CD90	71,1	9798
HLA-DQ	70,5	22470
CD104	69,9	7883
CD73	69,4	5951
CD95	69,4	16953
CD26	69,1	8486
CD51/61	68,1	11002
CD63	66,5	8900
CD107a	66,5	8953
CD221	66	11559
CD81	64,5	46349
CD268	57	3003
CD49c	56,1	5310
CD336	55,6	5138
HLA-DR	53,3	5581
B2M	52,4	2979
CD55	46,7	5266
CD2	37,9	2933
HLA A,B,C	37,4	3732
CD105	34,2	2895
CD140b	31,9	2655
CD210	27,2	2979
CD147	25,4	7746
CD144	25,1	2987
CD54	23,8	3164
CD62E	23,7	3131
CD142	21,6	3139
CD340	18,4	2409
CD146	15,8	5124
HLA-DR,DP,DQ	15,7	4449
CD58	13,7	2820
CD44	13,6	41634
SSEA-4	7,94	3458
CD1b	4,81	2153

CD120b	2,45	5013
CLA	1,83	2733
SSEA-3	0,98	2987
CD309	0,93	2321
CD267	0,93	2941
CD3	0,82	2415
CD338	0,79	2820
HPC	0,76	2712
Integrin b7	0,7	2655
CD321	0,68	3018
CD1a	0,64	2676
CD1d	0,64	2513
CD132	0,64	2641
CD9	0,63	2600
CD201	0,62	2539
CD294	0,62	2655
CD212	0,61	2956
CD49f	0,33	2339
CD46	0	9253
CD71	0	7266
gdTCR	0	7860
CD326	0	5597

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Supplementary Table 2. Second surface marker screening of human ovarian cortex cells. Percentage of ovarian cortex cells positive for CD markers pre-selected in the first surface marker screening (Supplementary Table 1) and corresponding MFI values. Selected CD markers determined negative in Supplementary Table 1 are included as negative controls. CD, Cluster of differentiation; MFI, Mean fluorescence intensity.

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137**Supplementary Table 3. Third surface marker screening of human ovarian cortex cells.**

Screen 3

Surface Marker	% CD-POS	MFI
CD47	99,4	55154
CD147	90,9	22055
CD55	86,6	17317
CD58	83,1	14497
CD63	72,4	10533
CD164	71,5	12249
CD46	70,1	12391
CD29	68,7	31394
CD151	68,7	23449
CD26	63,5	30331
CD95	63,4	11666
CD44	60,7	14974
CD9	59,8	18387
CD49E	58,7	19121
CD62E	57,7	18277
CD166	56	17111
HLA A,B,C	55,8	35270
CD90	54,8	33430
CD105	53,6	13997
CD49C	51,4	28230
CD140B	50,6	23812
CD54	44	9792
CD49F	41,3	11902
CD165	39,8	13875
CD200	36,7	13515
CD321	32,8	9236
CD49A	30,2	13052
CD340	30,2	6353
HLA-DQ	27,5	7212
CD221	25	6222
CD146	23,4	23739
CD201	22,7	7548
HLA-A2	19,9	29767
CD81	18,8	7467
HPC	18,3	8010
B2M	15,6	15791
CD98	14,3	6948
CD144	14	11210
CD146/MCAM	13,7	60966
CD51/61	12,1	7368
CD338	11,9	6015
HLA-DR	11,8	9365
CD39	11,6	47265
CD141	10,8	6749
CD91	10,7	6173
CD142	7,26	6488
HLA-DR,DQ,DP	7,23	8669
CD42b	5,73	6488
CD56	5,64	6141
EGF-R	5,31	5771

CD42a	5,31	6437
CD107A	5,09	5625
CD43	4,76	6336
SSEA-4	3,43	9443
CD13	2,42	7022
CD77	2,33	6505
CD11C	1,74	5984
CD268	1,61	5756
CD4v4	1,57	6173
CD4	1,17	5712
CD326	0,98	7135
Gamma delta TCR	0,66	6287

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Supplementary Table 3. Third surface marker screening of human ovarian cortex cells. Percentage of ovarian cortex cells positive for CD markers pre-selected in the first surface marker screening (Supplementary Table 1) and corresponding MFI values. Selected CD markers determined negative in Supplementary Table 1 are included as negative controls. CD, Cluster of differentiation; MFI, Mean fluorescence intensity.

146 **SUPPLEMENTARY DATA**

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148 **Supplementary Data 1. Cell Ranger metrics and gene expression analysis of unsorted**
149 **ovarian cortex cells.**

150 Sheet 'Stats': Cell Ranger metrics is listed for both samples of unsorted single cells dissociated
151 from GRP and C-sec patients' ovarian cortex, respectively. After quality control and filtering,
152 5,715 and 6,445 cells were kept for downstream analysis, respectively.

153 Sheet 'Variable genes clusters': List of markers per cluster identified using FindAllMarkers with
154 default settings in Seurat.

155 Sheet 'ddx4': List of markers expressed in *DDX4* expressing somatic cells of the ovarian
156 cortex. All markers of manually pooled somatic *DDX4* expressing cells were identified
157 compared to all other clusters using FindMarkers with default settings in Seurat.

158

159 **Supplementary Data 2. Cell Ranger metrics and gene expression analysis of sorted**
160 **ovarian cortex cells.**

161 Sheet 'Stats': Cell Ranger metrics is listed for both samples of sorted single cells dissociated
162 from GRP ovarian cortex, *DDX4* Ab+ and Ab- cells, respectively. After quality control and
163 filtering, 5,479 and 6,690 cells were kept for downstream analysis, respectively.

164 Sheet 'Variable genes clusters': List of markers per cluster identified using FindAllMarkers with
165 default settings in Seurat.

166 Sheet 'Variable genes Orig.ident': List of markers per sample (*DDX4* Ab+ versus *DDX4* Ab-)
167 identified using FindAllMarkers with default settings in Seurat.

168 Sheet 'ddx4': List of markers expressed in *DDX4* expressing somatic cells of the ovarian
169 cortex. All markers of manually pooled somatic *DDX4* expressing cells were identified
170 compared to all other clusters using FindMarkers with default settings in Seurat.

171

172 **Supplementary Data 3. Upregulated genes in cultured human ovarian *DDX4* Ab+ and**
173 ***DDX4* Ab- cells.**

174 Sheet 'Up Down': List of genes identified as upregulated in cultured *DDX4* Ab+ and Ab- ovarian
175 cortex cells, respectively, using featureCounts in EdgeR.

176 Sheet 'description': Description of all terms and abbreviations used in sheet 'Up Down'.

177 **REFERENCES**

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