

Supplementary Table 1: Characteristics of ovarian patients that provided serum samples.

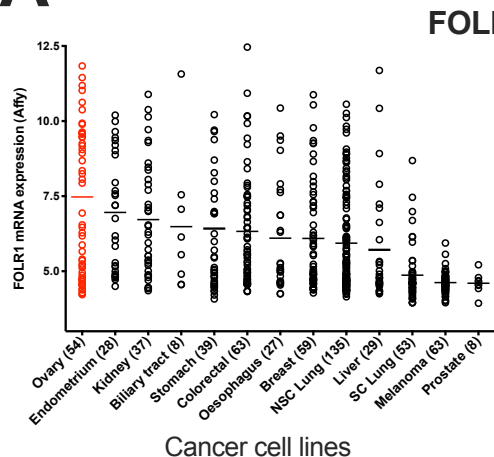
	Neo-adjuvant	Adjuvant	Palliative
Number of patients	57	33	53
Age – median (range)	65 (25-86)	59 (28-79)	64 (43-87)
Tumour histology – total number (%)			
Epithelial ovarian			
High grade serous	54 (94.7)	18 (54.5)	44 (83.0)
Low grade serous	1 (1.8)	2 (6.1)	1 (1.9)
Endometrioid	1 (1.8)	1 (3.0)	1 (1.9)
Clear cell	0 (0)	9 (27.3)	3 (5.7)
Mixed	0 (0)	1 (3.0)	1 (1.9)
Adult granulosa cell	0 (0)	0 (0)	1 (1.9)
High grade serous endometrial	1 (1.8)	0 (0)	0 (0)
Carcinosarcoma	0 (0)	2 (6.1)	2 (3.8)
FIGO-stage – total number (%)			
Stage I	0 (0)	10 (30.3)	8 (15.1)
Stage II	0 (0)	5 (15.2)	1 (1.9)
Stage III	31 (54.4)	17 (51.5)	37 (69.8)
Stage IV	26 (45.6)	1 (3.0)	7 (13.2)
Debulking surgery – total number (%)			
Optimal	29 (50.9)	30 (90.9)	N/A
Suboptimal	21 (36.8)	2 (6.1)	N/A
No debulking surgery	7 (12.3)	1 (3.0)	N/A
Primary tumour – total number (%)			
Ovary	45 (78.9)	24 (72.7)	42 (79.2)
Fallopian tube	8 (14.0)	9 (27.3)	7 (13.2)
Primary peritoneal	3 (5.3)	0 (0)	4 (7.5)
Endometrium	1 (1.8)	0 (0)	0 (0)
Menopausal status – total number (%)			
Post-menopausal	45 (78.9)	22 (66.7)	45 (84.9)
Pre-menopausal	2 (3.5)	2 (6.1)	0 (0)
Unknown	10 (17.5)	9 (27.3)	8 (15.1)

Supplementary Table 2: Tumour Burden Score

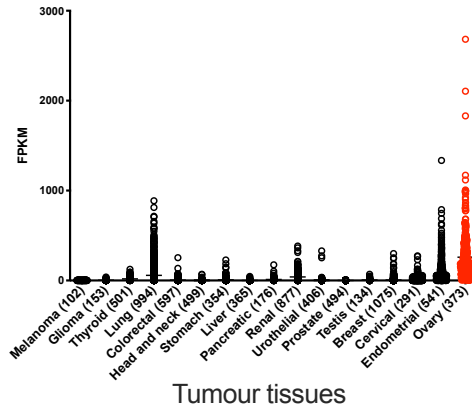
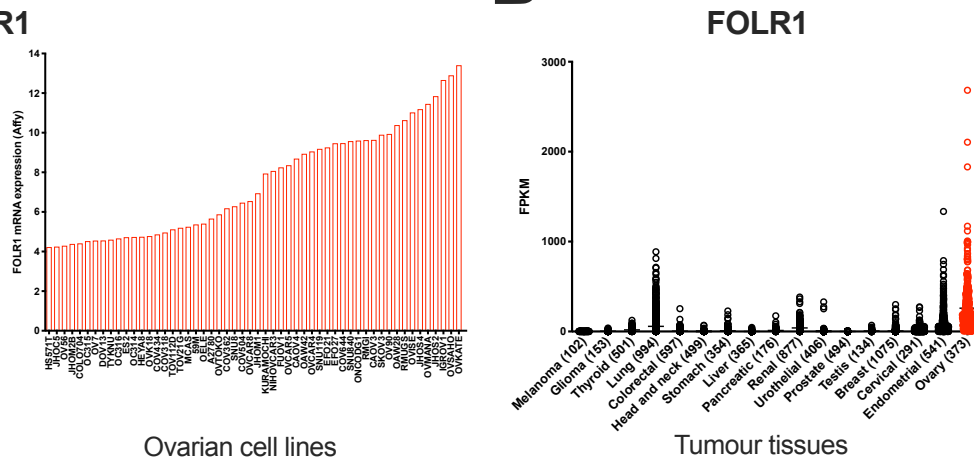
	Example 1	Example 2	Example 3
Site of disease (1 point per site)			
Lung	0	0	0
Pleural	0	0	0
Pleural Effusion	0	1	0
Liver	0	1	1
Spleen	0	0	1
Soft Tissue	0	0	0
Peritoneal Nodularity	0	1	1
Pelvic Mass	0	1	1
Ascites	1	1	1
Lymph nodes	1	1	4
Bone	0	0	0
Sub-Total	2	6	9
Number of metastases per site (1-2 = 1 point), (2-4 = 2 points), ($\geq 5 = 3$ points)			
Lung	0	0	0
Pleural	0	0	0
Pleural Effusion	0	1	0
Liver	0	1	2
Spleen	0	0	2
Soft Tissue	0	0	0
Peritoneal Nodularity	0	1	1
Pelvic Mass	0	1	1
Ascites	1	1	1
Lymph nodes	1	1	1
Bone	0	0	0
Sub-Total	2	6	8
Maximum metastasis diameter per site (<2cm = 1 point), (2-5cm = 2 points), (>5cm = 3 points)			
Lung	0	0	0
Pleural	0	0	0
Pleural Effusion	0	1	0
Liver	0	3	3
Spleen	0	0	3
Soft Tissue	0	0	0
Peritoneal Nodularity	0	1	2
Pelvic Mass	0	3	3
Ascites	1	1	1
Lymph nodes	1	1	2
Bone	0	0	0
Sub-Total	2	10	14
Total Tumour Burden Score	(2+2+2) = 6	(6+6+10) = 22	(9+8+14) = 31

Supp. Figure 1

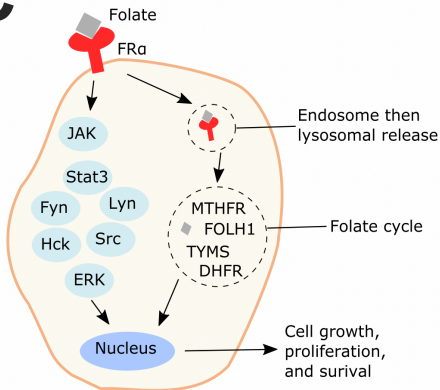
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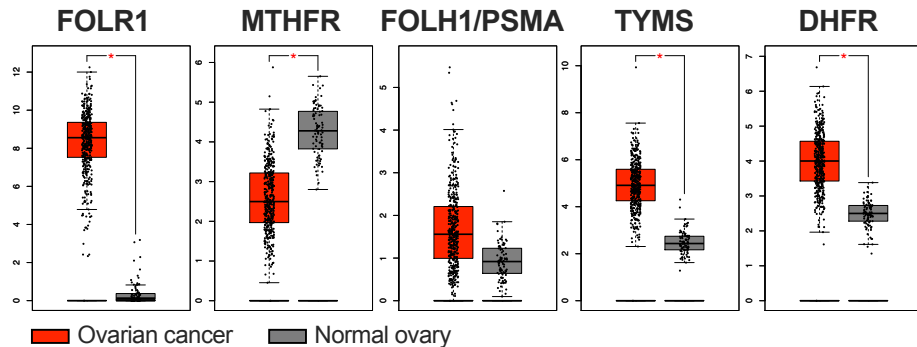
B



C

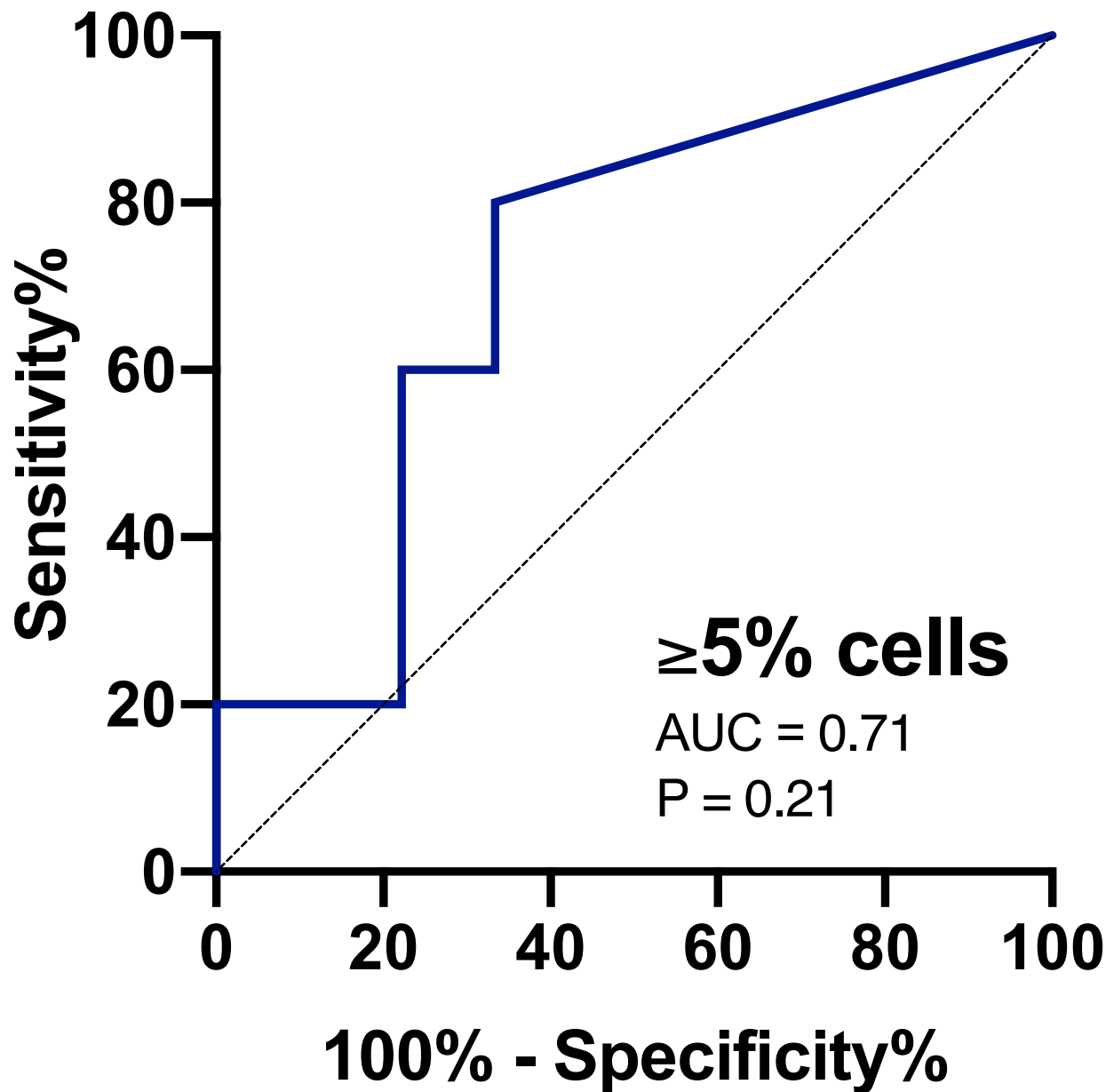


D

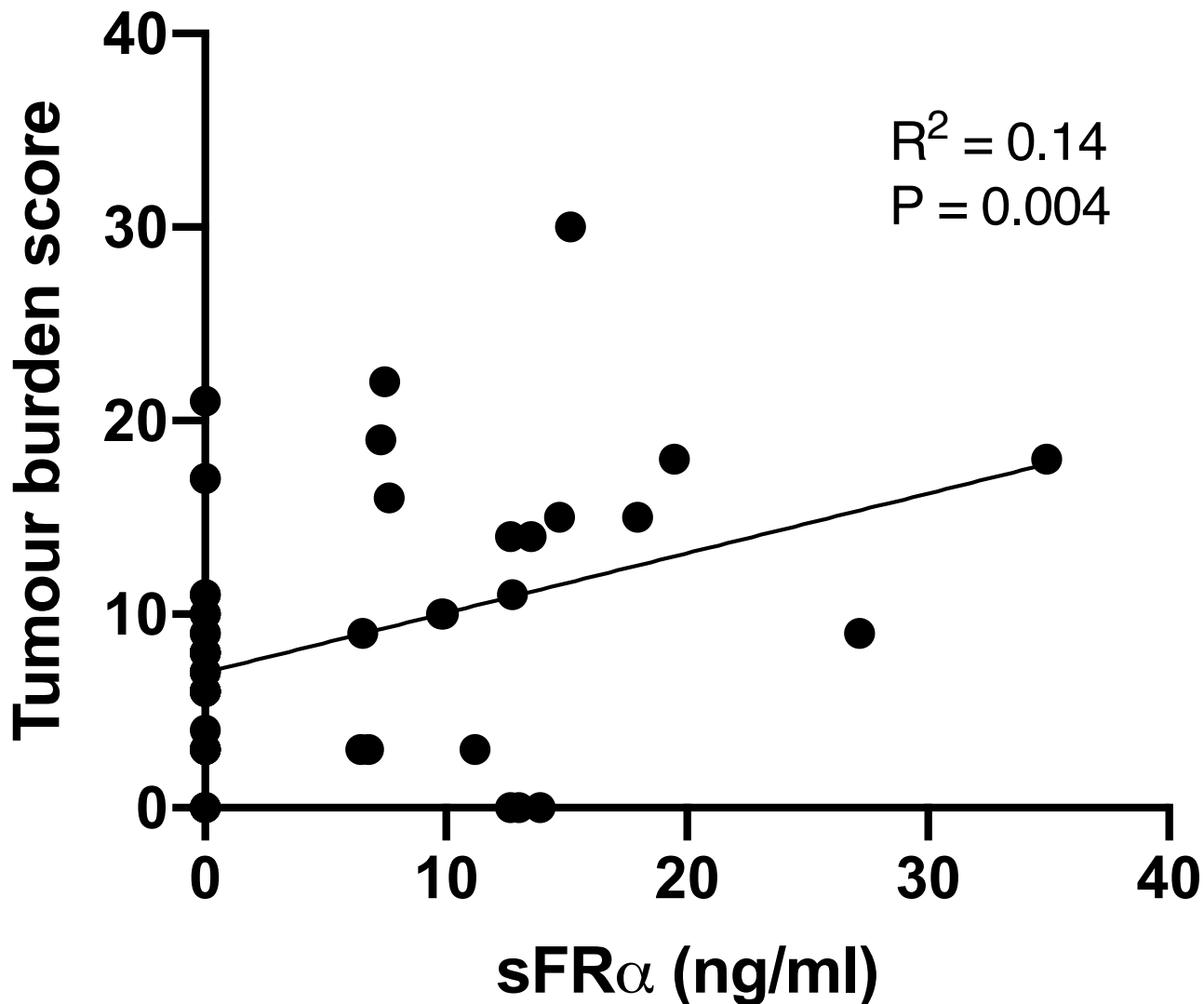


Supp. Figure 2

Tumour cytoplasmic FR α expression



Supp. Figure 3



Supplementary Figure Legends

Supplementary Figure 1: High *FOLR1* gene expression in ovarian cancers. A: Gene expression levels of *FOLR1* (encoding FR α) measured in human cancer cell lines (left, 10 top- and 3-lowest expressing cancer types shown, N numbers indicated in parentheses) and across ovarian cancer cell lines (right) (Cancer Cell Line Encyclopedia (CCLE) online tool) indicate high relative expression in ovarian cancer cells. B: Gene expression levels (fragments per kilobase of exon per million mapped fragments, FPKM) were also elevated in human ovarian cancer tissues (N numbers indicated in parentheses; The Human Protein Atlas online tool ^{1,2}). C: The folate/FR α signaling pathway involves intracellular molecules, e.g., *MTHFR*, *FOLH1*, *TYMS*, and *DHFR*, and is associated with cell growth, proliferation and survival. D: *FOLR1*, and folate pathway signaling molecules are differentially expressed in ovarian cancer in relation to normal ovary tissues (Gene Expression Profiling Interactive Analysis (GEPIA) online tool ³; ovarian tumours, N=426; normal ovary tissues, N=88).

Supplementary Figure 2: Receiver operating characteristic (ROC) curve analysis evaluating the capability of sFR α to predict cytoplasmic FR α expression in tumours. In the neo-adjuvant treatment-naïve patient group, sFR α concentration was not predictive of cytoplasmic FR α expression in the patients' tumour. Statistical test: receiver operating characteristic (ROC) curve analyses.

Supplementary Figure 3: Correlation between tumour burden score and sFR α . In the neo-adjuvant treatment-naïve patient group, a modest correlation was observed between tumour burden score and the level of circulating of sFR α .

- 1 Gyorffy, B., Lanczky, A. & Szallasi, Z. Implementing an online tool for genome-wide validation of survival-associated biomarkers in ovarian-cancer using microarray data from 1287 patients. *Endocr Relat Cancer* **19**, 197-208 (2012).
- 2 Uhlen, M., Zhang, C., Lee, S., Sjöstedt, E., Fagerberg, L., Bidkhori, G. *et al.* A pathology atlas of the human cancer transcriptome. *Science* **357** (2017).
- 3 Tang, Z., Li, C., Kang, B., Gao, G., Li, C. & Zhang, Z. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. *Nucleic Acids Res* **45**, W98-W102 (2017).