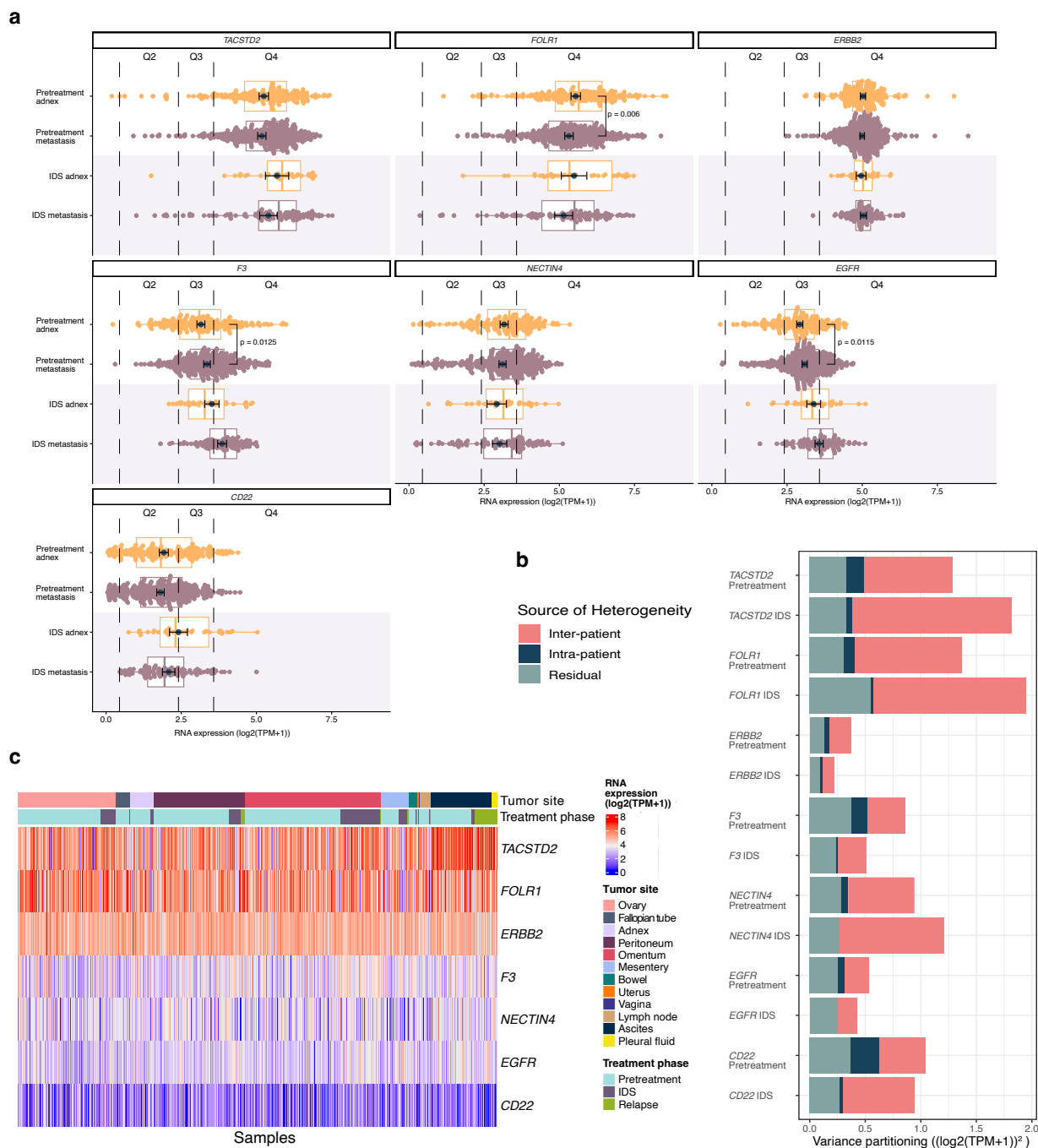


Supplementary Files

Multi-Omic Profiling Reveals Antibody-Drug Conjugate Targetability in Ovarian Cancer

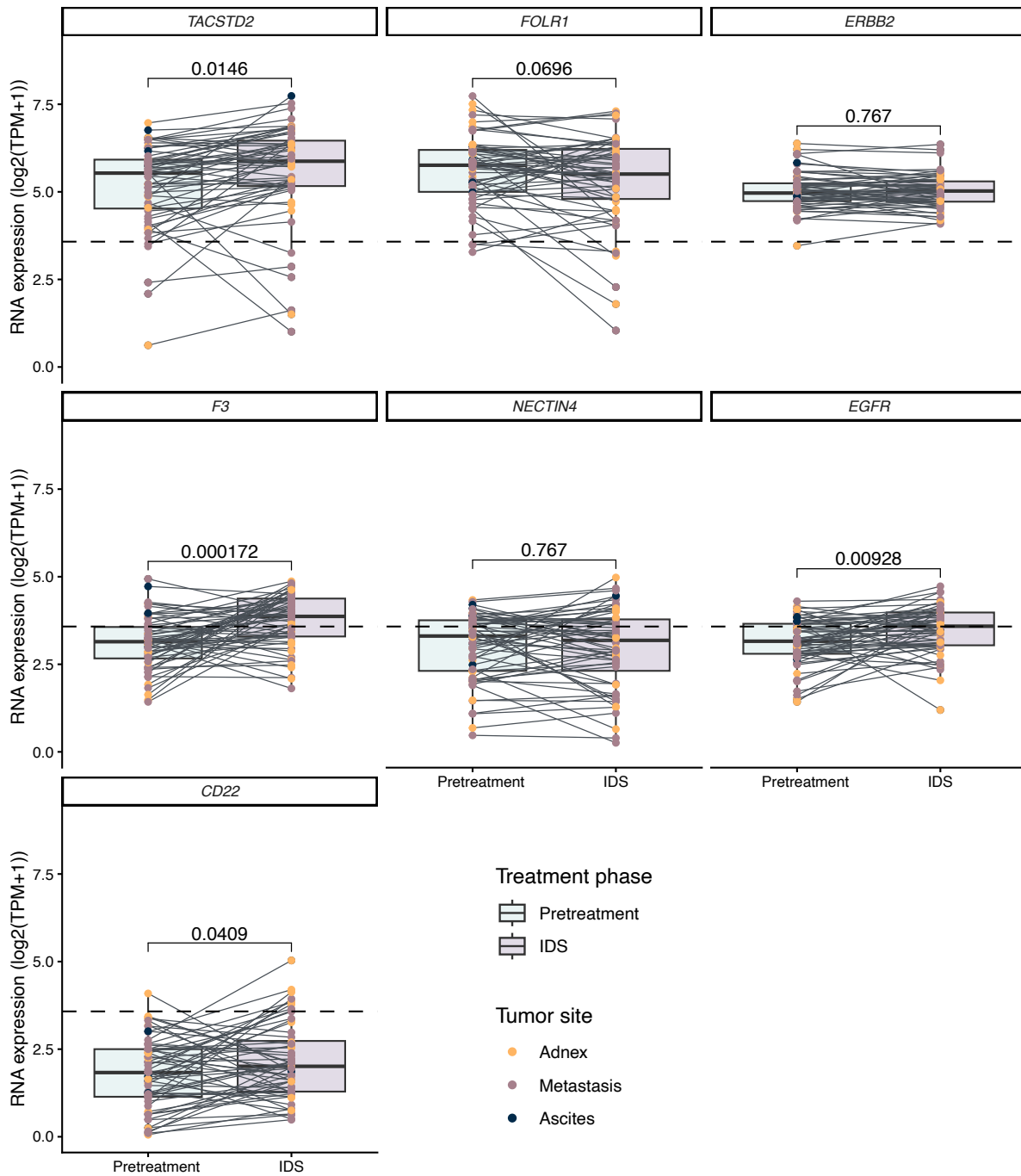
Elsi Pöllänen¹, Taru A. Muranen¹, Alexandra Lahtinen¹, Kaiyang Zhang¹, Daria Afenteva¹, Anna Pirttikoski¹, Susanna Holmström¹, Yilin Li¹, Kari Lavikka¹, Jaana Oikkonen¹, Jenni Söderlund², Johanna Hynninen², Anni Virtanen^{1,3}, Sampsa Hautaniemi^{1,2,*}

Supplementary Figure 1



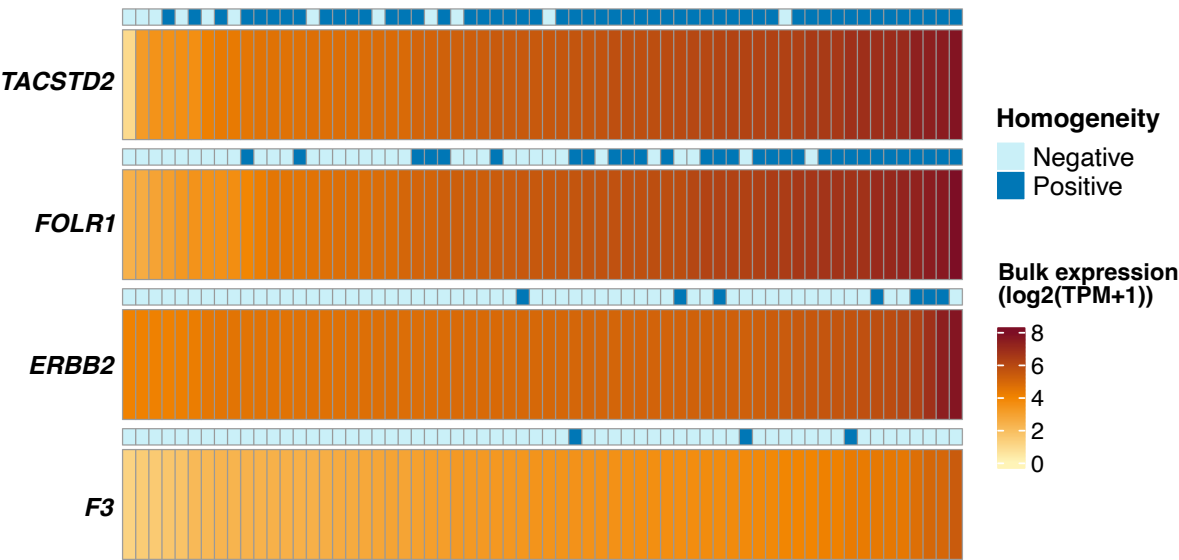
Supplementary Figure 1. Effects of the varying tumor sites. **a.** Input data as swarm- and boxplots, individually for each target. Results are divided into the studied treatment phases (pretreatment samples on white background and interval debulking surgery (IDS) samples on pale grey) and tumor sites (adnex samples with yellow and metastatic samples in taupe). Estimated marginal means, confidence intervals, and significant differences between the tumor sites are marked for each target. The quartiles from the expression landscape are illustrated with vertical dashed lines. **b.** Total variances explained by intra-patient, inter-patient and residual heterogeneity, which reflects the unexplained variance. Variances of each target are reported for pretreatment and interval debulking surgery (IDS) samples separately. **c.** Bulk RNA-sequencing data for the whole cohort described as a heatmap. Samples are ordered based on tumor sites and treatment phases.

Supplementary Figure 2



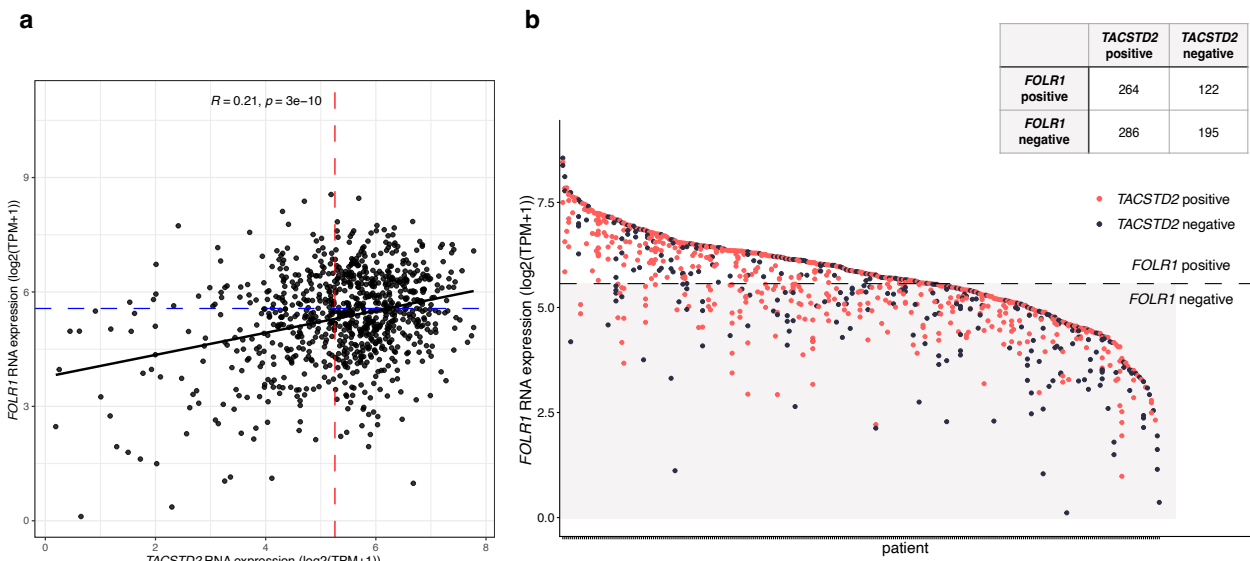
Supplementary Figure 2. Target expression across pretreatment and interval debulking surgery (IDS) samples. The figure illustrates the bulk RNA expression of patient matched pretreatment and IDS samples. Each sample is colored based on the collected tumor site, and each pretreatment-IDS pair relates to a line. High expression threshold has been marked with a horizontal dashed line. Benjamini-Hochberg adjusted p-values are shown for all targets.

Supplementary Figure 3



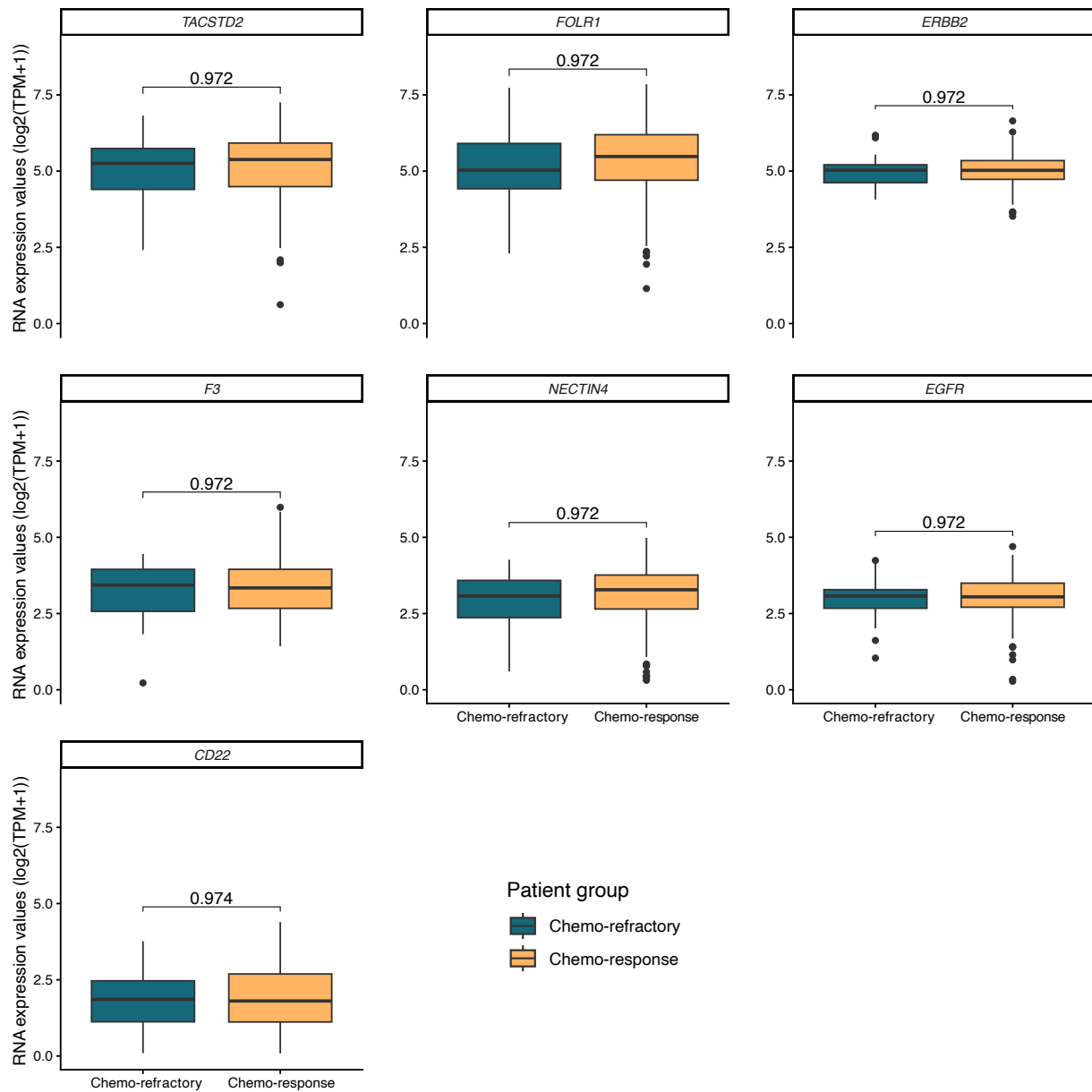
Supplementary Figure 3. The concordance of intra-tumor heterogeneity and bulk RNA expression. Bulk RNA expression compared to the intra-tumor heterogeneity from the matching single cell RNA-sequencing samples. Each of the genes is ordered based on descending bulk RNA expression, and the corresponding intra-tumor heterogeneity is marked in the annotation column.

Supplementary Figure 4



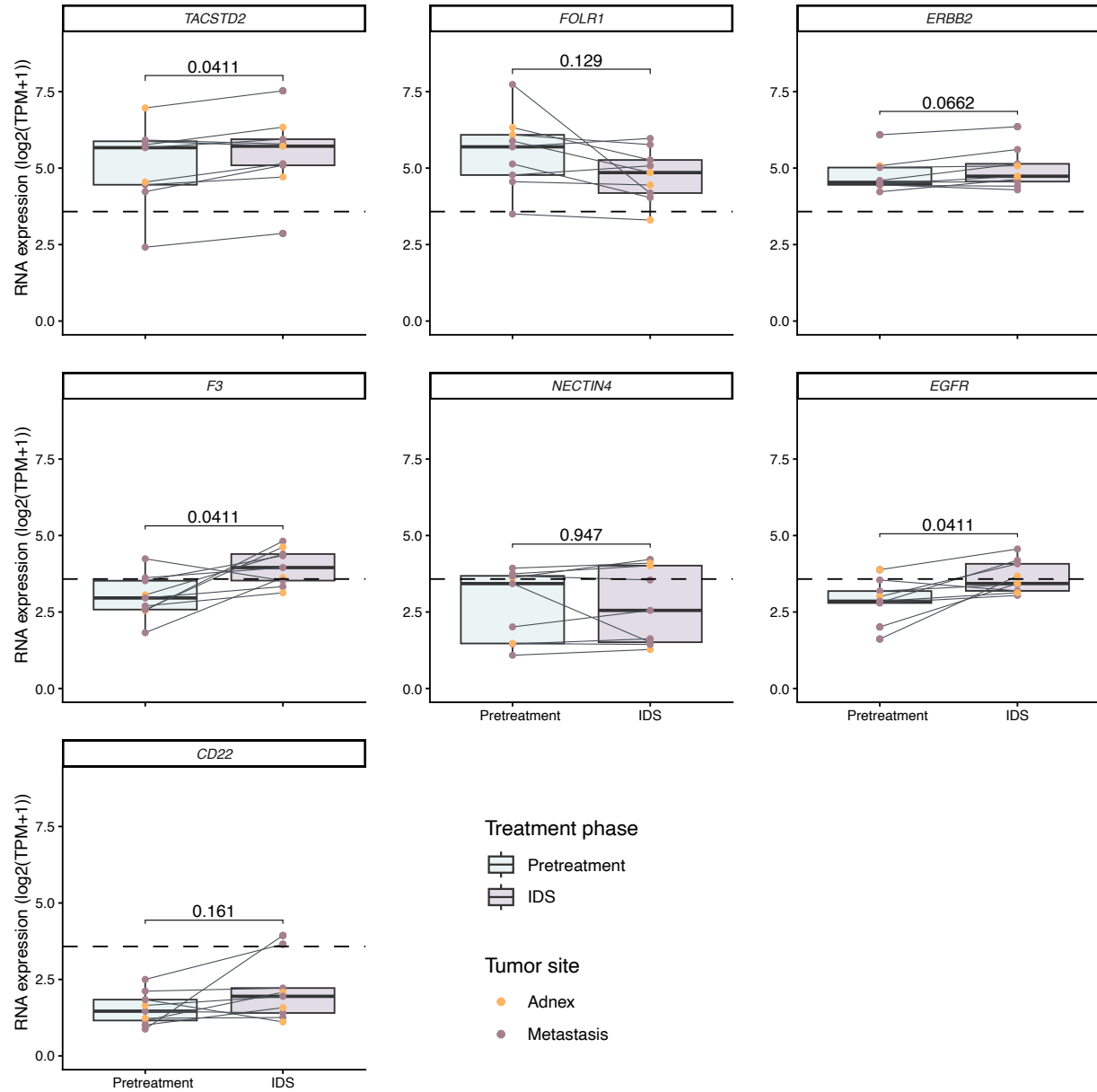
Supplementary Figure 4. Expression relationship between *FOLR1* and *TACSTD2*. **a.** Spearman correlation of *TACSTD2* and *FOLR1* expressions. ROC-derived thresholds are marked with blue dashed line for *FOLR1* and red dashed line for *TACSTD2*. **b.** *FOLR1* and *TACSTD2* expression across all samples. Points are ordered by *FOLR1* expression and classified as intratumor positive (homogeneous) or negative (heterogeneous) using ROC-derived thresholds from matched bulk and intratumor heterogeneity analysis samples (Supplementary Figure 3); *TACSTD2* positivity is indicated by color. Summary statistics are shown in the contingency table.

Supplementary Figure 5



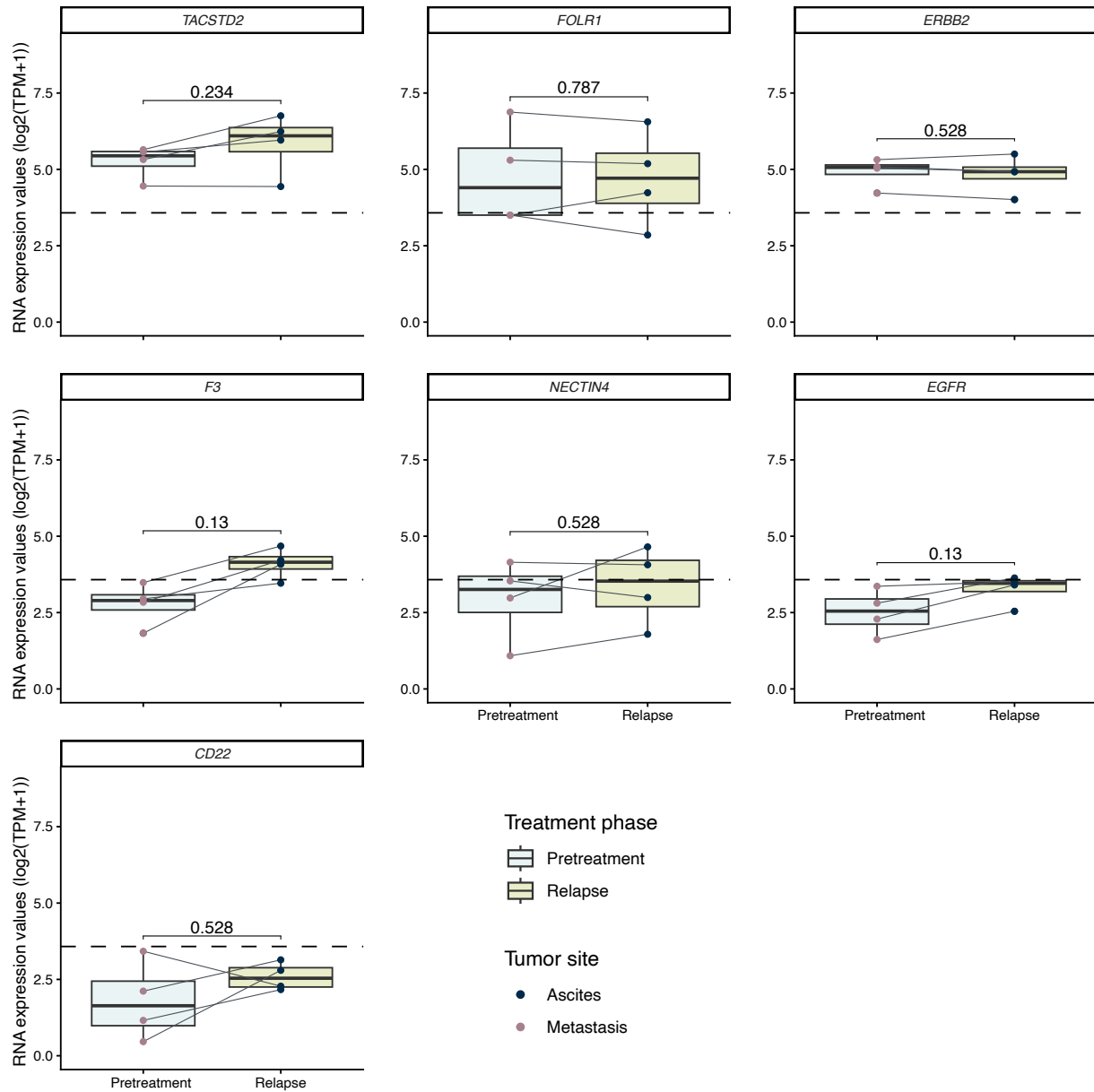
Supplementary Figure 5. Antibody-drug conjugate target expression in chemo-refractory patients. We divided cases into chemo-refractory and chemotherapy-responsive groups to evaluate target expression in chemo-resistant tumors. The refractory group was defined as progressive- or stable disease after primary therapy (RECIST 1.1 criteria) patients with platinum-free interval (PFI) < 45 days, whereas the responsive group was defined as complete- or partial response (RECIST 1.1 criteria) to primary therapy and PFI > 6 months. Statistical significance was tested with two-sided Student's t-test and the Benjamini-Hochberg adjusted p-values are shown for all targets.

Supplementary Figure 6



Supplementary Figure 6. Target expression between pretreatment and interval debulking surgery (IDS) samples in chemo-refractory patients. The figure illustrates the bulk RNA expression of patient matched pretreatment and IDS samples for chemo-refractory patients. Each sample is colored based on the collected tumor site, and each pretreatment-IDS pair relates to a line. High expression threshold has been marked with a horizontal dashed line. Statistical significance was tested with paired Student's t-test and the Benjamini-Hochberg adjusted p-values are shown for all targets.

Supplementary Figure 7



Supplementary Figure 7. Target expression between pretreatment and relapse samples in chemo-refractory patients. The figure illustrates the bulk RNA expression of patient matched pretreatment and relapse samples for chemo-refractory patients. Each sample is colored based on the collected tumor site, and each pretreatment-relapse pair relates to a line. High expression threshold has been marked with a horizontal dashed line. Statistical significance was tested with paired Student's t-test and the Benjamini-Hochberg adjusted p-values are shown for all targets.

Supplementary Table 1. Model fit and heterogeneity proportions.

Gene	R2m	R2c	Inter-patient heterogeneity	Intra-patient heterogeneity	Residual heterogeneity	Sample subset
<i>TACSTD2</i>	0.001	0.742	0.624	0.119	0.258	Pretreatment
<i>FOLR1</i>	0.009	0.775	0.707	0.068	0.225	Pretreatment
<i>ERBB2</i>	0	0.664	0.54	0.124	0.336	Pretreatment
<i>F3</i>	0.011	0.564	0.394	0.171	0.436	Pretreatment
<i>NECTIN4</i>	0.001	0.704	0.633	0.071	0.296	Pretreatment
<i>EGFR</i>	0.012	0.524	0.422	0.102	0.476	Pretreatment
<i>CD22</i>	0.003	0.649	0.398	0.251	0.351	Pretreatment
<i>TACSTD2</i>	0.008	0.818	0.789	0.028	0.182	IDS
<i>FOLR1</i>	0.011	0.717	0.706	0.011	0.283	IDS
<i>ERBB2</i>	0.005	0.587	0.491	0.096	0.413	IDS
<i>F3</i>	0.041	0.544	0.503	0.041	0.456	IDS
<i>NECTIN4</i>	0.001	0.779	0.778	0.001	0.221	IDS
<i>EGFR</i>	0.014	0.419	0.405	0.014	0.581	IDS
<i>CD22</i>	0.02	0.718	0.69	0.028	0.282	IDS

Supplementary Table 2. Mean differences (D_{mean}) for low and high expression regions. Regions are marked in Figure 1b.

Gene	D_{mean} in low expression region	D_{mean} in high expression region
<i>TACSTD2</i>	0.95924463	0.589347664
<i>FOLR1</i>	0.764187347	0.643901691
<i>ERBB2</i>	0.470231512	0.326785524
<i>F3</i>	0.808957584	0.81231554
<i>NECTIN4</i>	0.784161222	0.43539891
<i>EGFR</i>	0.755137613	0.510763307
<i>CD22</i>	0.921095357	0.732146007

Supplementary Table 3. The numbers of cases changing classification between pretreatment and interval debulking surgery (IDS) samples. Classification changes for each target were assessed using high expression threshold to assign samples into high and low expression groups. For each target, four classifications were defined; High, when both samples of the pair were above high-expression threshold; Low, when both samples of the pair were below high-expression threshold; Increase, when expression was below the threshold in pretreatment sample and above it in IDS sample; Decrease, when expression was above the threshold in the pretreatment sample and below it in IDS sample. Numbers indicate the cases belonging to each category.

Gene	High	Low	Increase	Decrease
<i>TACSTD2</i>	51	2	3	4
<i>FOLR1</i>	52	1	2	5
<i>ERBB2</i>	59	0	1	0
<i>F3</i>	12	19	26	3
<i>NECTIN4</i>	13	29	8	10
<i>EGFR</i>	13	26	17	4
<i>CD22</i>	0	50	9	1

Supplementary Table 4. The numbers of cases changing classification between pretreatment and relapse samples. Classification changes for each target were assessed using high expression threshold to assign samples into high and low expression groups. For each target, four classifications were defined; High, when both samples of the pair were above high-expression threshold; Low, when both samples of the pair were below high-expression threshold; Increase, when expression was below the threshold in pretreatment sample and above it in relapse sample; Decrease, when expression was above the threshold in the pretreatment sample and below it in relapse sample. Numbers indicate the cases belonging to each category.

Gene	High	Low	Increase	Decrease
<i>TACSTD2</i>	28	2	1	2
<i>FOLR1</i>	29	2	1	1
<i>ERBB2</i>	32	0	1	0
<i>F3</i>	6	10	11	6
<i>NECTIN4</i>	12	14	5	2
<i>EGFR</i>	2	23	5	3
<i>CD22</i>	0	31	1	1

Supplementary Table 5. Average expression of targets in individual tumor sites. This table contains the average expression of each target in all tumor sites and treatment phases. One sample per patient per tumor site/treatment phase was prioritized based on highest purity. The total amount of available samples for each row is shown in the last column. Only data with at least five available samples was included.

Treatment phase	Tumor site	<i>EGFR</i>	<i>ERBB2</i>	<i>F3</i>	<i>FOLR1</i>	<i>TACSTD2</i>	<i>NECTIN4</i>	<i>CD22</i>	Number of samples
Pretreatment	Adnex	2.883954495	5.11550032	3.41379153	5.198213358	5.202115434	3.305974981	2.226922902	33
IDS	Adnex	3.669161511	5.095894189	3.486838164	4.48006722	5.330988886	3.236650958	3.050056115	6
Relapse	Ascites	3.251085347	4.911079402	3.769939645	5.146735366	6.072730886	3.445706367	1.593623462	24
Pretreatment	Ascites	3.33036952	5.061250398	3.575248557	5.09914929	6.198918287	3.432252042	1.891437861	72
IDS	Ascites	3.151077734	5.010846358	3.569447953	4.930740205	6.38820402	3.277776472	1.841085407	7
Pretreatment	Bowel	3.048987282	5.106906475	3.541594622	5.128157884	5.393097241	3.19718012	1.99867627	13
Pretreatment	Fallopian tube	2.859648146	5.128311237	2.997272486	5.643269584	5.16326351	3.325200743	1.703492401	24
Pretreatment	Lymph node	2.959080767	4.446497907	3.45169731	5.189486007	4.846170306	2.778468747	2.421573755	12
Pretreatment	Mesentery	2.823296844	4.823568525	3.177740001	4.99449478	4.947353418	2.682424751	1.762729497	32
IDS	Mesentery	3.376605724	4.990126125	3.538985405	4.382445134	4.540599659	2.335070684	2.092545486	13
Pretreatment	Omentum	3.092142565	5.019134681	3.419462112	5.346007507	5.228642671	3.113984901	1.719385741	160
IDS	Omentum	3.577960798	5.055059676	3.93422428	5.397599026	5.628986871	3.134557907	2.020661829	62
Pretreatment	Ovary	2.887798018	4.97984713	3.051748894	5.497164903	5.209720476	3.078573372	1.922261246	122
IDS	Ovary	3.113102833	4.879342743	3.487253289	5.72523633	5.73872007	3.1123433	2.332738111	22
Relapse	Peritoneum	3.157551142	4.923484689	3.147010947	5.5318329	4.945245946	3.235667938	1.079355738	7
Pretreatment	Peritoneum	3.136075258	5.075516054	3.3307806	5.3893033	5.300870845	3.194057109	1.878563381	131
IDS	Peritoneum	3.570258575	5.092828946	3.756055865	5.335129059	5.427435832	3.139401587	2.010504305	21
Relapse	Pleural fluid	3.16461429	5.080500451	3.657692832	5.521934302	6.302765036	4.090641757	1.787308358	8

Supplementary Table 6. Copy-number status of *ERBB2* and *F3* for cancer samples with high mRNA expression of either *ERBB2* or *F3*. Copy-number status is given separately for the major and minor alleles. Tumor ploidy is the average genomic copy number. Positive target specifies for which target the patient was positive.

Patient	Sample	<i>ERBB2</i> copy number (major minor)	<i>F3</i> copy number (major minor)	Ploidy	Positive target
EOC649	pretreatment omentum	<i>ERBB2</i> :15 0	<i>F3</i> :2 1	3.0	<i>ERBB2</i>
EOC204	pretreatment mesentery	<i>ERBB2</i> :3 0	<i>F3</i> :9 2	4.0	<i>F3</i>
EOC321	pretreatment peritoneum	<i>ERBB2</i> :7 7	<i>F3</i> :1 1	1.8	<i>ERBB2</i>
EOC783	relapse ascites	<i>ERBB2</i> :1 0	<i>F3</i> :1 1	1.7	<i>ERBB2</i>
EOC926	pretreatment peritoneum	<i>ERBB2</i> :3 0	<i>F3</i> :2 1	2.7	<i>ERBB2</i>