

# Immunohistochemistry-based Taxonomical Classification of Bladder Cancer Predicts Response to Neoadjuvant Chemotherapy

Supplementary material

Supplementary Tables 1-5

Supplementary Figures 1-5

**Supplementary Table 1.** Characteristics of the subjects included and excluded from the analyses.

Characteristics	All N = 215	Excluded N = 83	Included* N = 132	P
Centre				0.00016
HUGTiP	168 (78.1%)	76 (91.6%)	92 (69.7%)	
Manresa	47 (21.9%)	7 (8.43%)	40 (30.3%)	
Sex				0.33
M	199 (92.6%)	75 (90.4%)	124 (93.9%)	
F	16 (7.44%)	8 (9.64%)	8 (6.06%)	
Age (cont)	66.0 [59.0;71.5]	65.0 [58.0;70.5]	66.0 [61.0;72.0]	0.34
Age (tertiles, years)				0.25
[35,62]	76 (35.3%)	35 (42.2%)	41 (31.1%)	
[62,69]	72 (33.5%)	25 (30.1%)	47 (35.6%)	
[69,83]	67 (31.2%)	23 (27.7%)	44 (33.3%)	
Morphology				0.37
Urothelial	183 (85.1%)	70 (84.3%)	113 (85.6%)	
Mixed	23 (10.7%)	9 (10.8%)	14 (10.6%)	
Adenocarcinoma	6 (2.79%)	4 (4.82%)	2 (1.52%)	
Other	3 (1.40%)	0 (0.00%)	3 (2.27%)	
Lymphovascular invasion				0.261
No	188 (87.4%)	74 (89.2%)	114 (86.4%)	
Yes	24 (11.2%)	7 (8.43%)	17 (12.9%)	
Unknown	1 (0.47%)	1 (1.20%)	0 (0.00%)	
'Missing'	2 (0.93%)	1 (1.20%)	1 (0.76%)	
Grade				0.55
Low	6 (2.79%)	3 (3.61%)	3 (2.27%)	
High	208 (96.7%)	79 (95.2%)	129 (97.7%)	
'Missing'	1 (0.47%)	1 (1.20%)	0 (0.00%)	
cTNM				0.16
0-3	16 (7.44%)	3 (3.61%)	13 (9.85%)	
4-6	157 (73.0%)	61 (73.5%)	96 (72.7%)	
7+	41 (19.1%)	19 (22.9%)	22 (16.7%)	
'Missing'	1 (0.47%)	0 (0.00%)	1 (0.76%)	
pTNM:				0.73
Not assessable	9 (4.19%)	8 (9.64%)	1 (0.76%)	
Complete	58 (27.0%)	18 (21.7%)	40 (30.3%)	
Partial	27 (12.6%)	10 (12.0%)	17 (12.9%)	
Not responder	117 (54.4%)	43 (51.8%)	74 (56.1%)	
'Missing'	4 (1.86%)	4 (4.82%)	0 (0.00%)	
Lymph node involvement				0.04
No	161 (74.9%)	59 (71.1%)	102 (77.3%)	
Yes	29 (13.5%)	5 (6.02%)	24 (18.2%)	
'Missing'	25 (11.6%)	19 (22.9%)	6 (4.55%)	
Lymph nodes resected				0.18
0-9	112 (52.1%)	42 (50.6%)	70 (53.0%)	
10+	78 (36.3%)	22 (26.5%)	56 (42.4%)	

Characteristics	All N = 215	Excluded N = 83	Included* N = 132	P
'Missing'	25 (11.6%)	19 (22.9%)	6 (4.55%)	
<b>Treatment</b>				0.04
CMV	66 (30.7%)	34 (41.0%)	32 (24.2%)	
CG	122 (56.7%)	38 (45.8%)	84 (63.6%)	
CaG	20 (9.30%)	8 (9.64%)	12 (9.09%)	
Other**	5 (2.33%)	1 (1.20%)	4 (3.03%)	
'Missing'	2 (0.93%)	2 (2.41%)	0 (0.00%)	

\*This group includes 6 cases from whom tumour tissue was used for TMA construction but results for all 4 IHC markers were not available. \*\*Other: Dose-dense MVAC.

**Supplementary Table 2.** Patient characteristics according to the response to treatment (N = 131)\*.

Characteristics	Complete N = 40	Partial/ Non-responders N = 91	P value
<b>Centre</b>			
Can Ruti	28 (70.0%)	64 (70.3%)	1.000
Manresa	12 (30.0%)	27 (29.7%)	
<b>Sex</b>			
M	39 (97.5%)	84 (92.3%)	0.442
F	1 (2.50%)	7 (7.69%)	
<b>Age (cont.), median [1<sup>st</sup>-3<sup>rd</sup> quartile]</b>			
Age (tertiles, years)	66.0 [62.8;69.8]	67.0 [60.5; 72.0]	0.777
[35,62]	10 (25.0%)	31 (34.1%)	0.067
[62,69]	20 (50.0%)	26 (28.6%)	
[69,83]	10 (25.0%)	34 (37.4%)	
<b>Morphology</b>			
Urothelial	37 (92.5%)	75 (82.4%)	0.465
Mixed	3 (7.50%)	11 (12.1%)	
Adenocarcinoma	0 (0.00%)	2 (2.20%)	
Other	0 (0.00%)	3 (3.30%)	
<b>Lymphovascular invasion</b>			
No	36 (90.0%)	77 (84.6%)	0.568
Yes	4 (10.0%)	13 (14.3%)	
Unknown	0 (0.00%)	1 (1.10%)	
<b>Grade</b>			
Low	1 (2.50%)	2 (2.20%)	1.000
High	39 (97.5%)	89 (97.8%)	
<b>cTNM</b>			
0-3	6 (15.0%)	6 (6.59%)	0.303
4-6	27 (67.5%)	69 (75.8%)	
7+	6 (15.0%)	16 (17.6%)	
'Missing'	1 (2.50%)	0 (0.00%)	
<b>Lymph node involvement</b>			
No	38 (95.0%)	63 (69.2%)	0.002
Yes	0 (0.00%)	24 (26.4%)	
'Missing'	2 (5.00%)	4 (4.40%)	
<b>Lymph nodes resected</b>			
0-9	19 (47.5%)	51 (56.0%)	0.449
10+	19 (47.5%)	36 (39.6%)	
'Missing'	2 (5.00%)	4 (4.40%)	
<b>Hydronephrosis</b>			
No	27 (67.5%)	52 (57.1%)	0.339
Yes	13 (32.5%)	39 (42.9%)	
<b>Treatment</b>			
CG	25 (62.5%)	58 (63.7%)	1.000
CaG	4 (10.0%)	8 (8.79%)	
CMV	10 (25.0%)	22 (24.2%)	
'Missing'	1 (2.50%)	3 (3.30%)	

\*For one patient, response could not be assessed.

**Supplementary Table 3.** Distribution of bladder cancer gene mutations according to the 3 clusters identified (N = 126).

MARKER	MIXED N = 35	LUMINAL-LIKE N = 44	BASAL-LIKE N = 47	P VALUE
<i>FGFR3</i>				0.015
wt	23 (69.7%)	35 (79.5%)	44 (93.6%)	
mut	8 (24.2%)	4 (9.09%)	1 (2.13%)	
'Missing'	2 (6.06%)	5 (11.4%)	2 (4.26%)	
<i>PIK3CA</i>				0.132
wt	21 (63.6%)	35 (79.5%)	35 (77.8%)	
mut	7 (21.2%)	3 (6.82%)	2 (4.44%)	
'Missing'	5 (15.2%)	6 (13.6%)	8 (17.8%)	
<i>HRAS</i>				0.314
wt	13 (39.4%)	23 (54.8%)	19 (42.2%)	
mut	1 (3.03%)	2 (4.76%)	0 (0.00%)	
'Missing'	19 (57.6%)	17 (40.5%)	26 (57.8%)	
<i>KRAS</i>				0.131
wt	14 (42.4%)	25 (58.1%)	16 (35.6%)	
mut	0 (0.00%)	1 (2.33%)	3 (6.67%)	
'Missing'	19 (57.6%)	17 (39.5%)	26 (57.8%)	
<i>NRAS</i>				0.241
wt	13 (39.4%)	26 (60.5%)	18 (40.0%)	
mut	1 (3.03%)	0 (0.00%)	1 (2.22%)	
'Missing'	19 (57.6%)	17 (39.5%)	26 (57.8%)	
<i>RAS-any</i>				0.415
wt	12 (36.4%)	22 (52.4%)	15 (33.3%)	
mut	2 (6.06%)	3 (7.14%)	4 (8.89%)	
'Missing'	19 (57.6%)	17 (40.5%)	26 (57.8%)	

**Supplementary Table 4.** Predictive factors of complete response to neoadjuvant treatment. Multivariate logistic regression models (N = 126). Model 1 and Model 2 differ regarding adjustment for by lymphovascular and lymph node invasion.

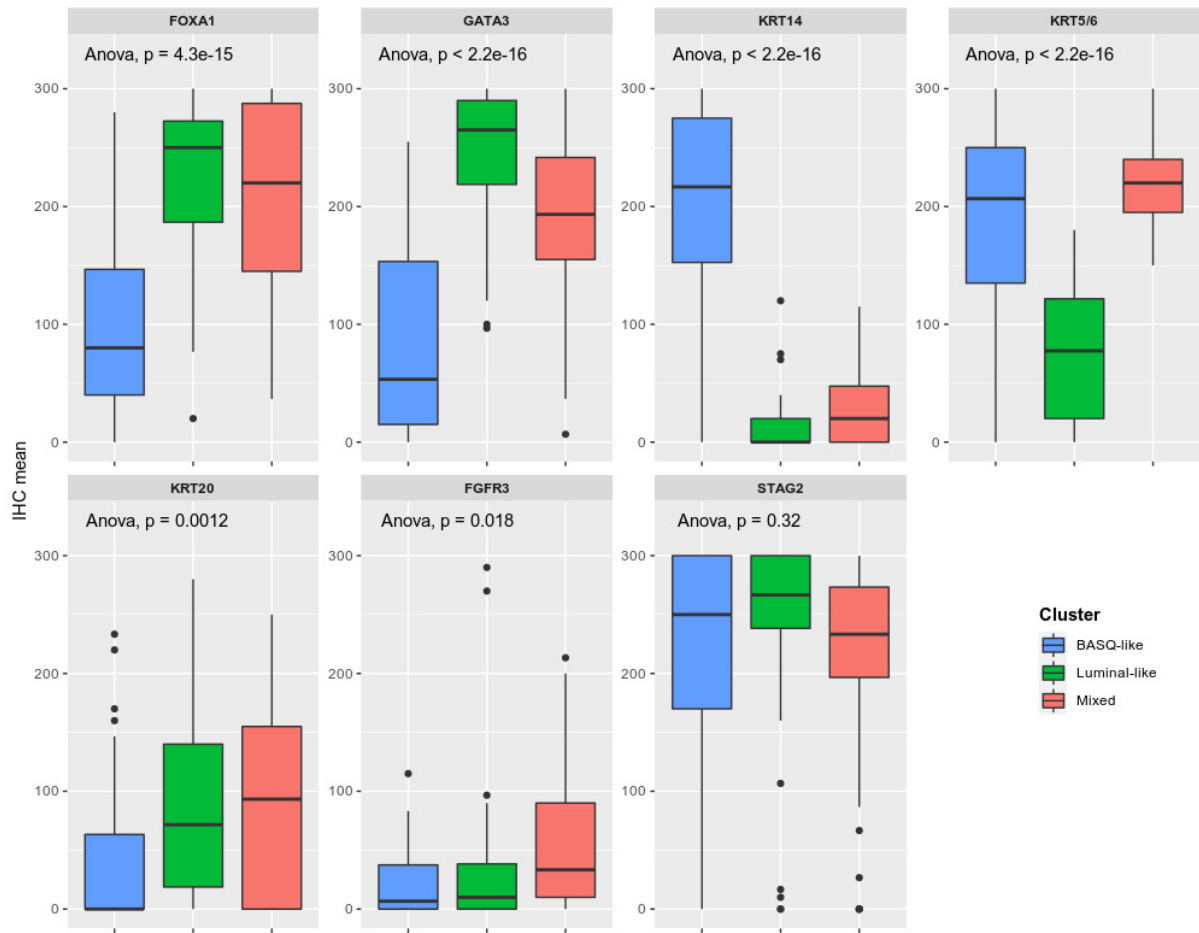
Factors	Model 1			Model 2		
	OR	P value	95% CI	OR	P value	95% CI
Morphology						
Urothelial	Ref			Ref		
Mixed	0.27	0.083	(0.06, 1.19)	0.32	0.159	(0.06, 1.56)
Lymphovascular invasion						
No	-			Ref.		
Yes	-			0.61	0.531	(0.13, 2.90)
Lymph node invasion						
No	-			Ref		
Yes	-			0.07	0.014	(0.00, Inf)
cTNM						
T2 N0	Ref			Ref		
T3/4 N0	0.31	0.121	(0.07, 1.37)	0.22	0.093	(0.04, 1.28)
TxN1Mx	0.36	0.245	(0.06, 2.01)	0.33	0.293	(0.04, 2.59)
Clusters						
Mixed	Ref			Ref		
Luminal-like cluster	1.28	0.699	(0.37, 4.49)	1.34	0.672	(0.34, 5.26)
BASQ-like cluster	3.96	0.017	(1.28, 12.20)	4.06	0.026	(1.18, 13.99)

Both models were adjusted for age and centre.

**Supplementary Table 5.** Predictive factors of pCR to NAC. Multivariate logistic regression models (N = 100) \*.

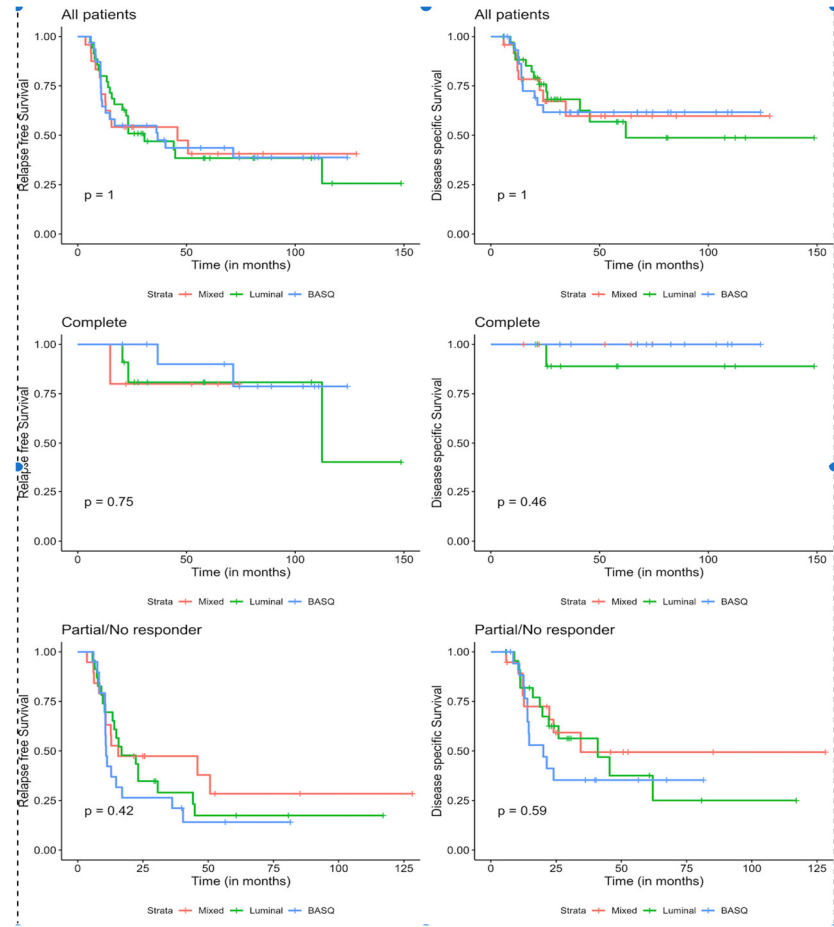
Factor	Model 1		Model 2	
	OR	P value	OR	P value
Age (years)				
<62	Ref		Ref	
62-69	2.10	0.186	2.16	0.206
70-83	0.69	0.560	0.76	0.693
Morphology				
Urothelial	Ref		Ref	
Mixed	0.43	0.310	0.54	0.471
Lymphovascular invasion				
No	–	–	Ref	
Yes	–	–	1.38	0.723
Lymph node involvement				
No	–	–	Ref	
Yes	–	–	0.08	0.03
cTNM				
T2 N0	Ref		Ref	
T3/4 N0	0.33	0.190	0.19	0.093
Clusters				
Mixed	Ref		Ref	
Luminal-like cluster	0.93	0.916	0.81	0.790
BASQ-like cluster	3.22	0.062	3.28	0.076

\* Patients treated with Carboplatin or with lymph node involvement were excluded from the analyses. Both models were adjusted for centre.

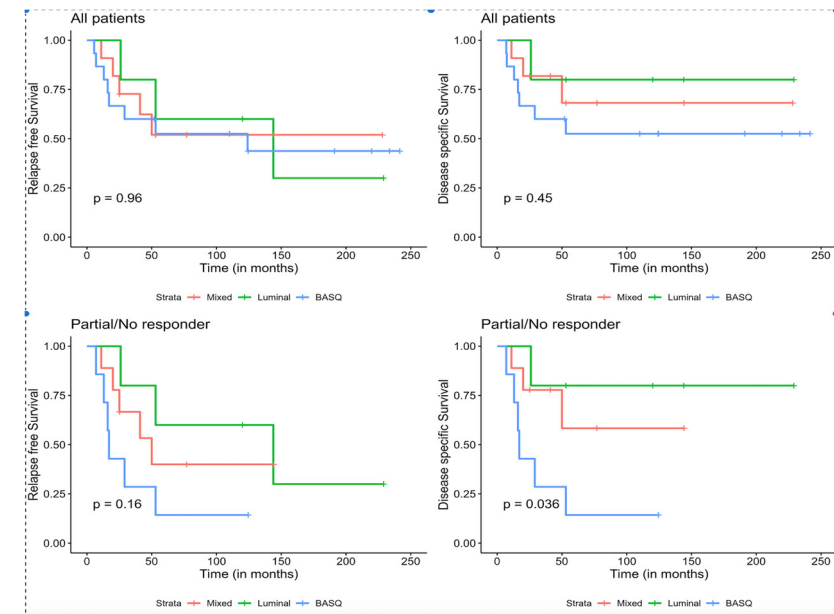


**Supplementary Figure 1.** Median and interquartile range [IQR] of HS corresponding to each tumour marker according to the 3 clusters identified (N = 126).

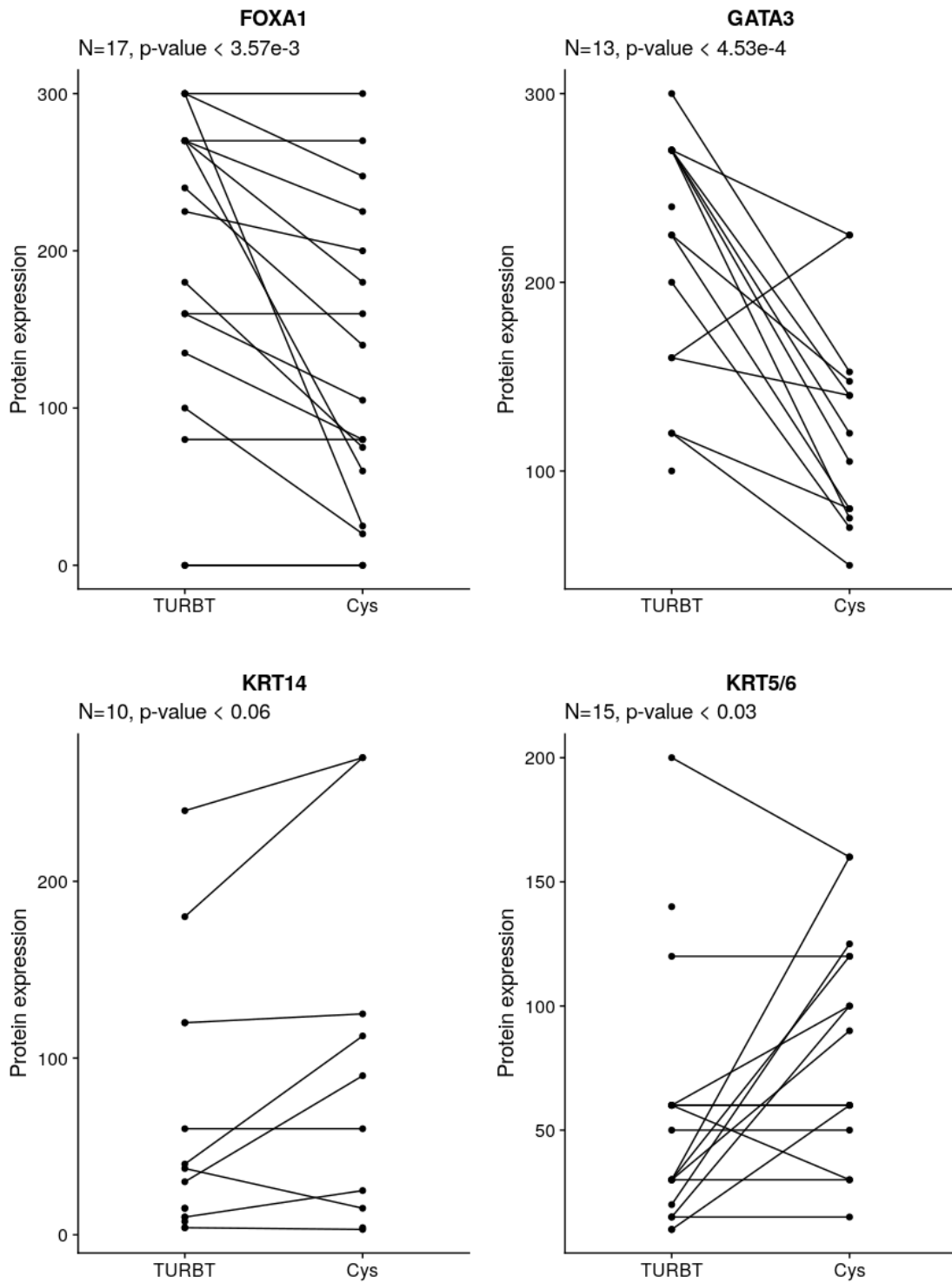
A



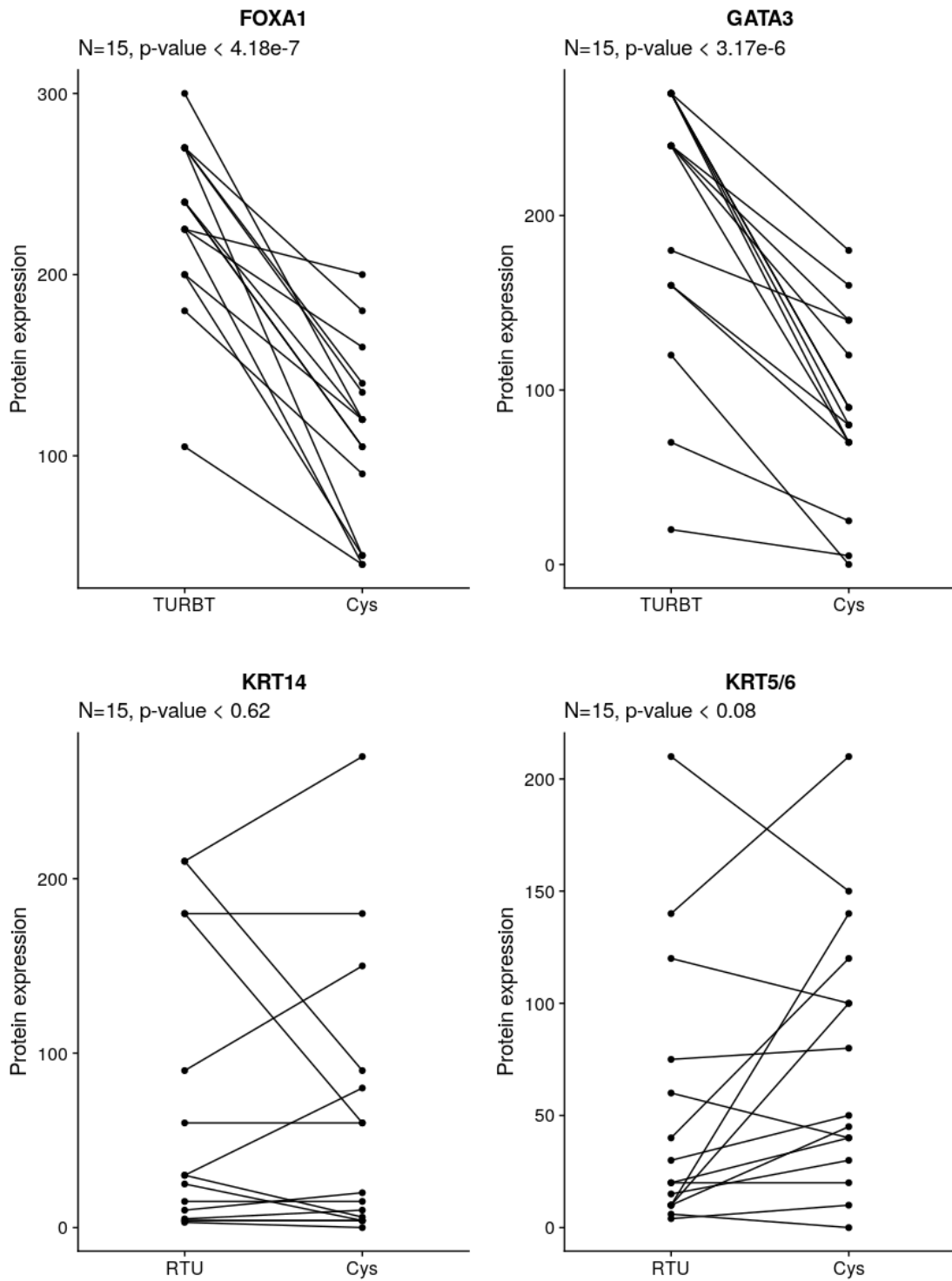
B



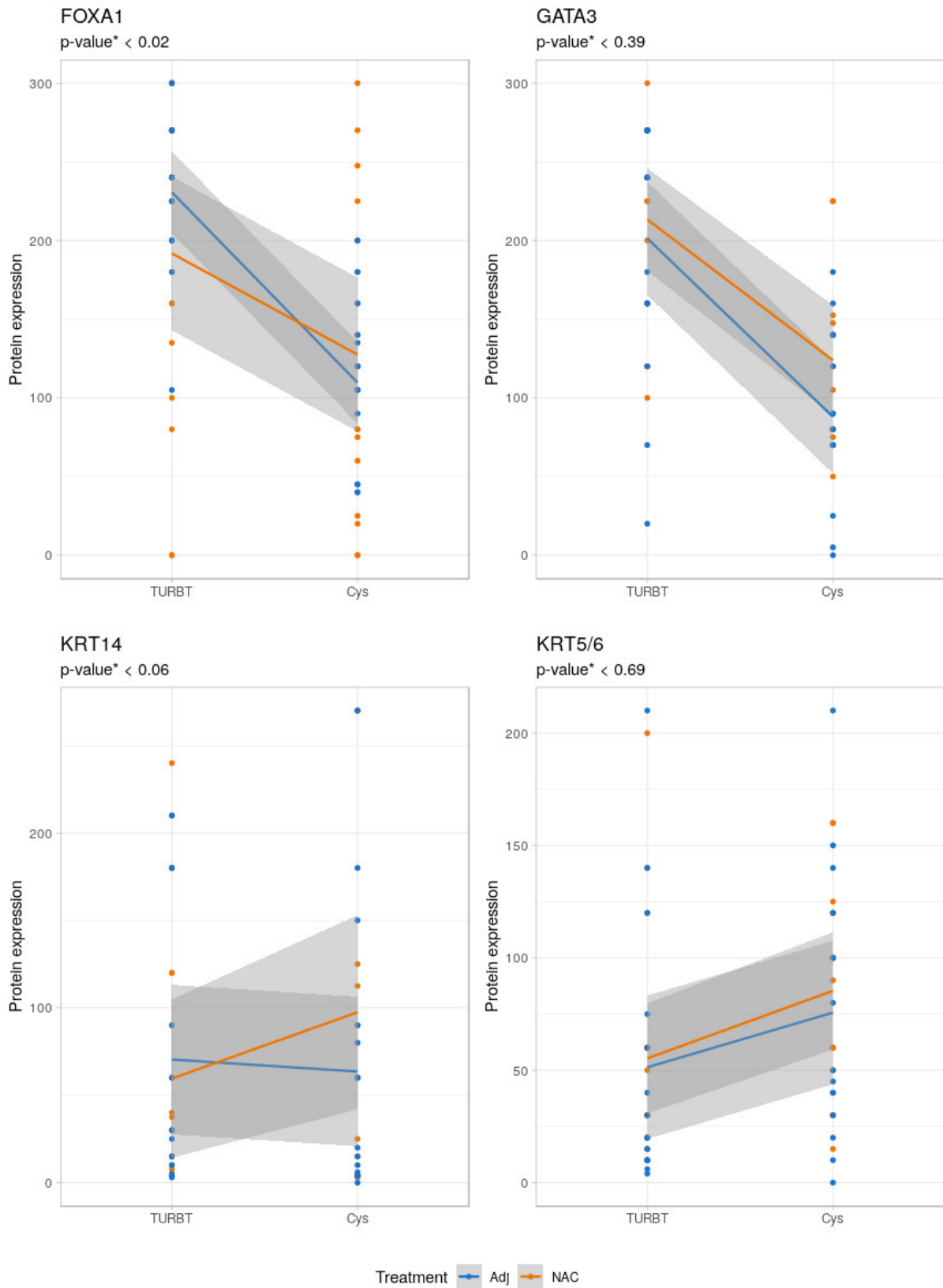
**Supplementary Figure 2.** Kaplan-Meier relapse-free survival (left panels) and disease-specific survival (right panels) curves for patients treated with CG/CaG (A) vs. CMV (B) combination therapy. While sample size did not allow stratified analyses, we found a similar trend in the association of BASQ subtype with outcome in both patient subgroups..



**Supplementary Figure 3.** Comparison of marker expression in full sections of samples obtained at TURBT and cystectomy (Cys) from patients treated with NAC. Statistical analysis: repeated measurements ANOVA.



**Supplementary Figure 4.** Comparison of marker expression in full sections of samples obtained at TURBT and cystectomy (Cys) from patients who did not receive NAC. Statistical analysis: repeated measurements ANOVA.



**Supplementary Figure 5.** Interaction analysis of marker expression in full sections of samples obtained at TURBT and cystectomy (Cys) from patients treated (orange) or not (blue) with NAC. Statistical analysis: linear mixed effects model; shown is the interaction P-value.