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Article

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Neoadjuvant atezolizumab plus chemotherapy in gastric and gastroesophageal junction adenocarcinoma: the phase 2 PANDA trial

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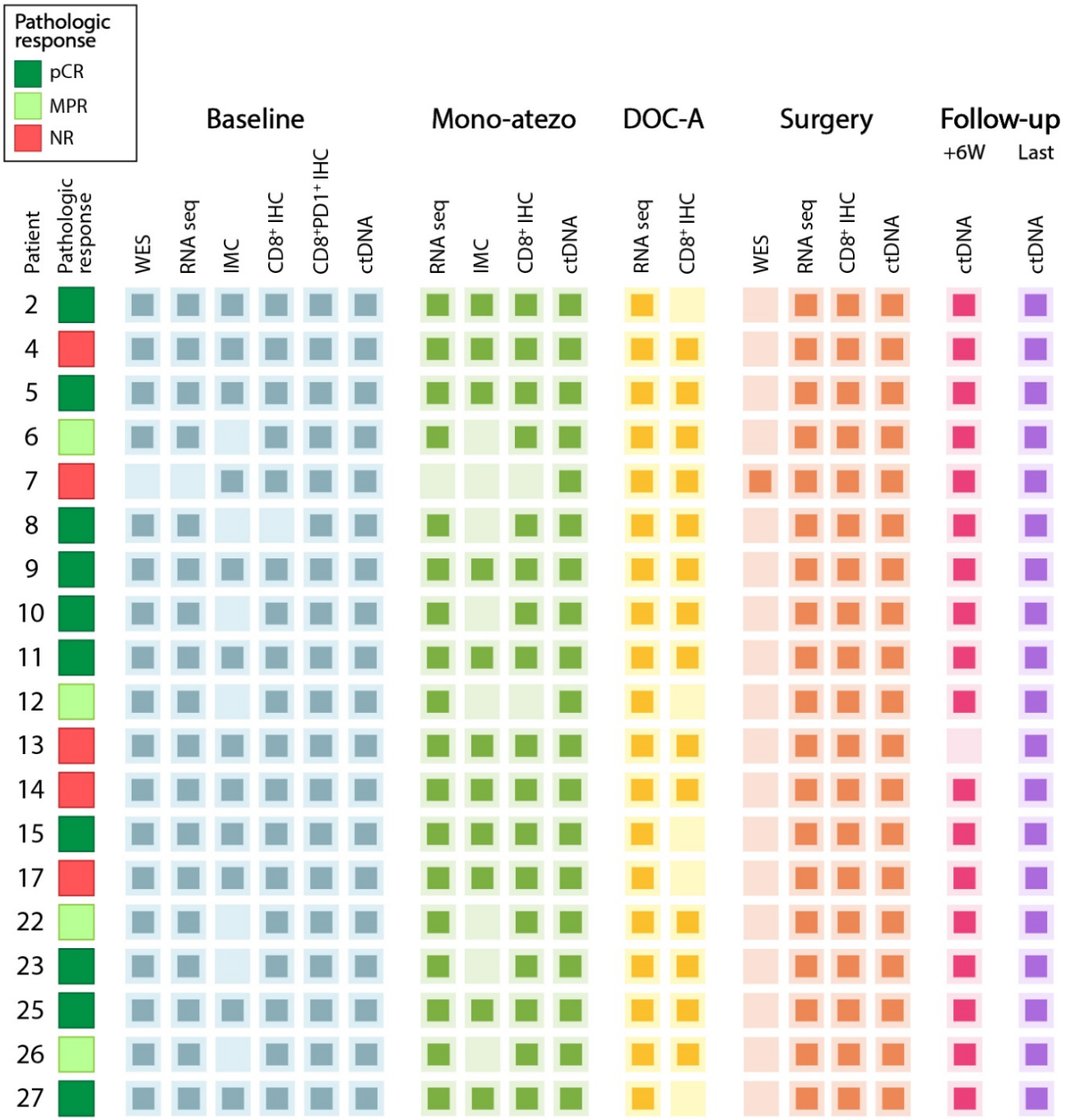
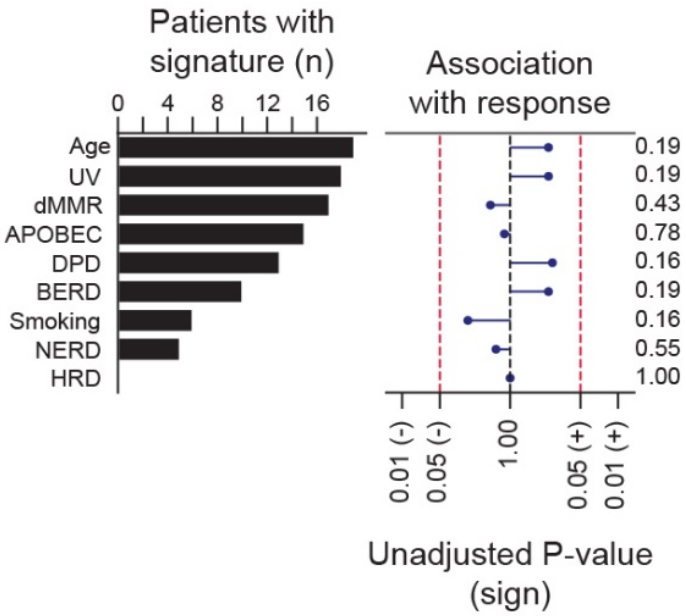
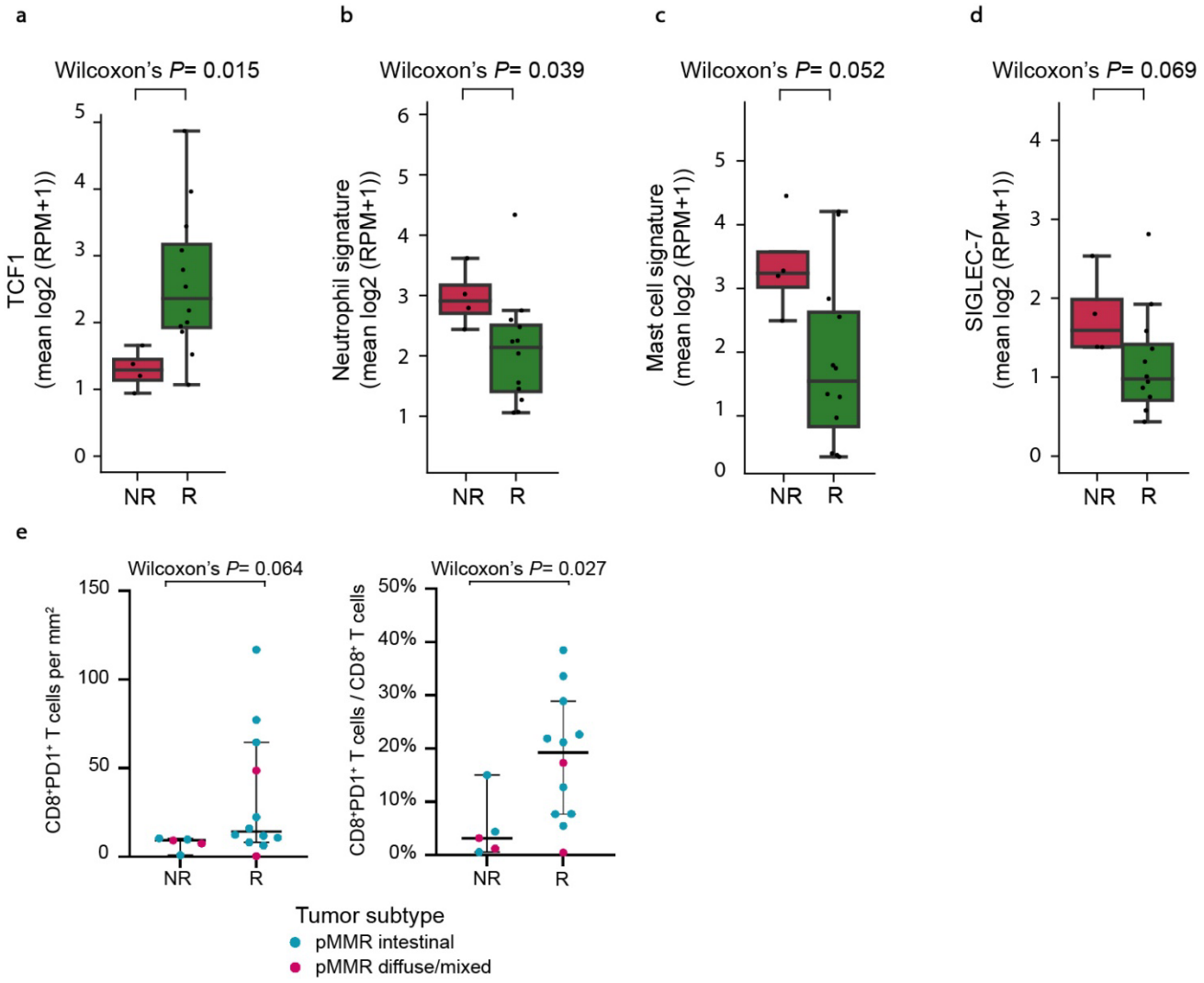
Table of content



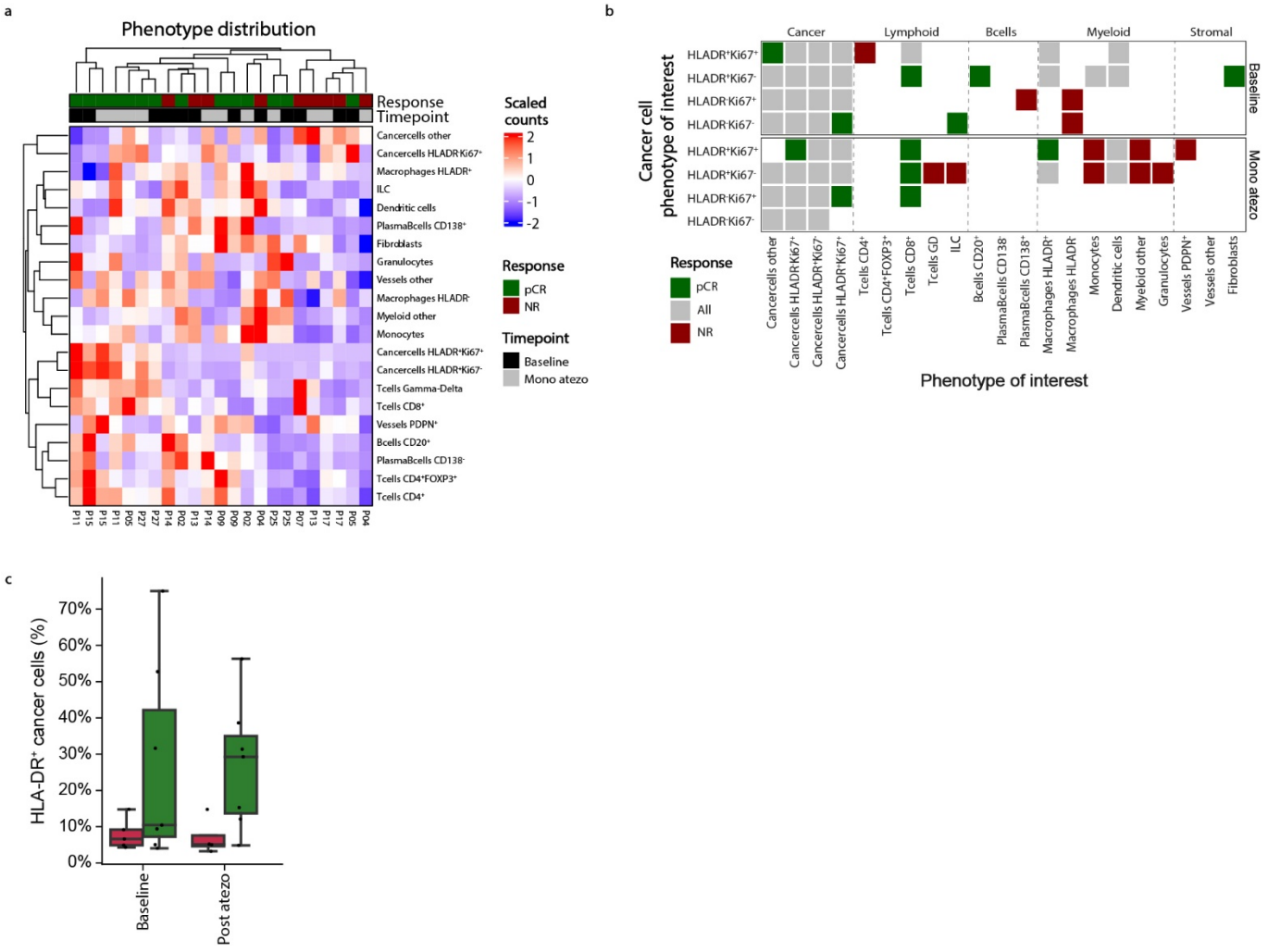
Supplementary Figure 1 | Overview of samples used for translational analyses. Dark squares indicate samples that were included in the analysis annotated per column. pCR: pathologic complete response; MPR: major pathologic response; NR: nonresponder; WES: whole exome sequencing; RNA seq: RNA sequencing; IMC: imaging mass cytometry; IHC: immunohistochemistry.



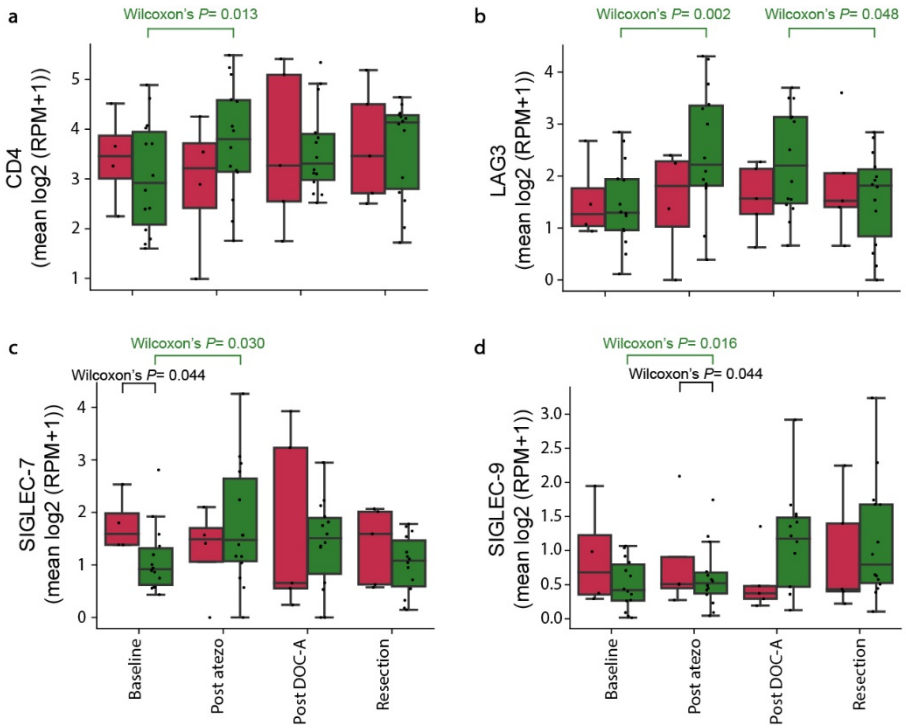
Supplementary Figure 2 | Association of mutational signatures with response
Barplot (left) of the number of patients (x-axis) with activity of specific mutational signatures (y-axis) and a lollipop plot (right) showing the signed P-value for association of mutational signature activity with response. Significance was tested using a two-sided Wilcoxon Signed-Rank sum test. X-axis on log10-scale.



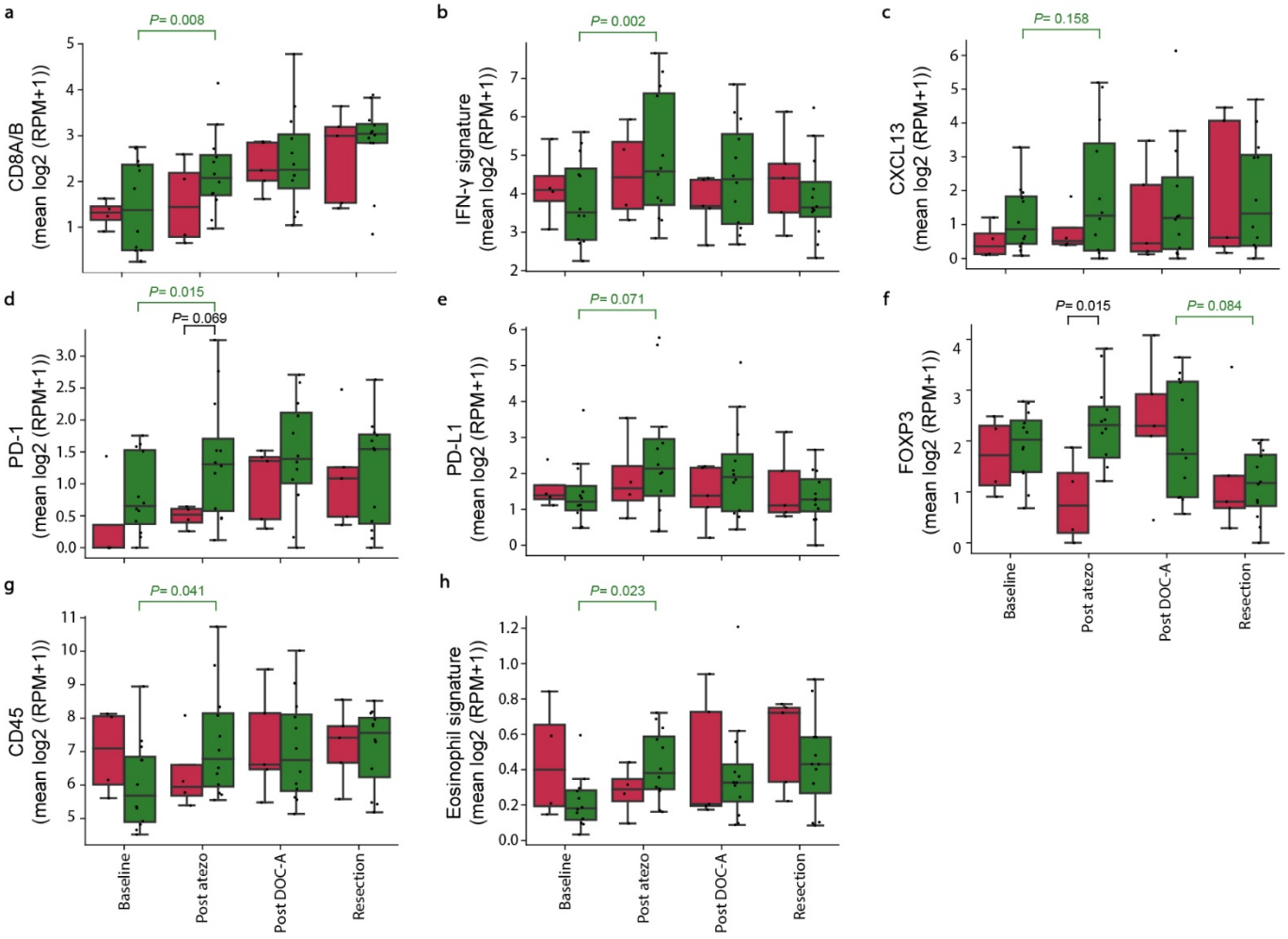
Supplementary Figure 3 | Comparisons between pMMR responders and pMMR nonresponders of pretreatment *TCF1* and *SIGLEC-7* gene expression, neutrophil and mast cell signature plus immunohistochemistry for CD8$^+$PD1$^+$. a-d, Pretreatment gene expression in nonresponders (NR, *n*=4) and responders (R, *n*=12) of **a**, *TCF1*. **b**, Neutrophil signature. **c**, Mast cell signature. **d**, *SIGLEC-7*. **a-d**, Boxplots represent the median, 25th and 75th percentiles; whiskers extend from the hinge to the largest value below 1.5 * IQR from the hinge. For comparison between NR and R, significance was tested using a two-sided Wilcoxon Rank-sum test. **e**, Pretreatment CD8$^+$PD1$^+$ T-cells using immunohistochemical double staining for CD8 and PD-1, shown per mm2 (left) and as percentage of all CD8$^+$ cells (right) in nonresponders (NR, *n*=5) versus responders (R, *n*=12). Dots represent individual patients and the colors represent the Lauren subtype (blue= intestinal type; purple= diffuse/mixed type). The horizontal line represents the median; whiskers show the 95% confidence interval. The difference between R and NR tested using a two-sided Wilcoxon Rank-sum test.



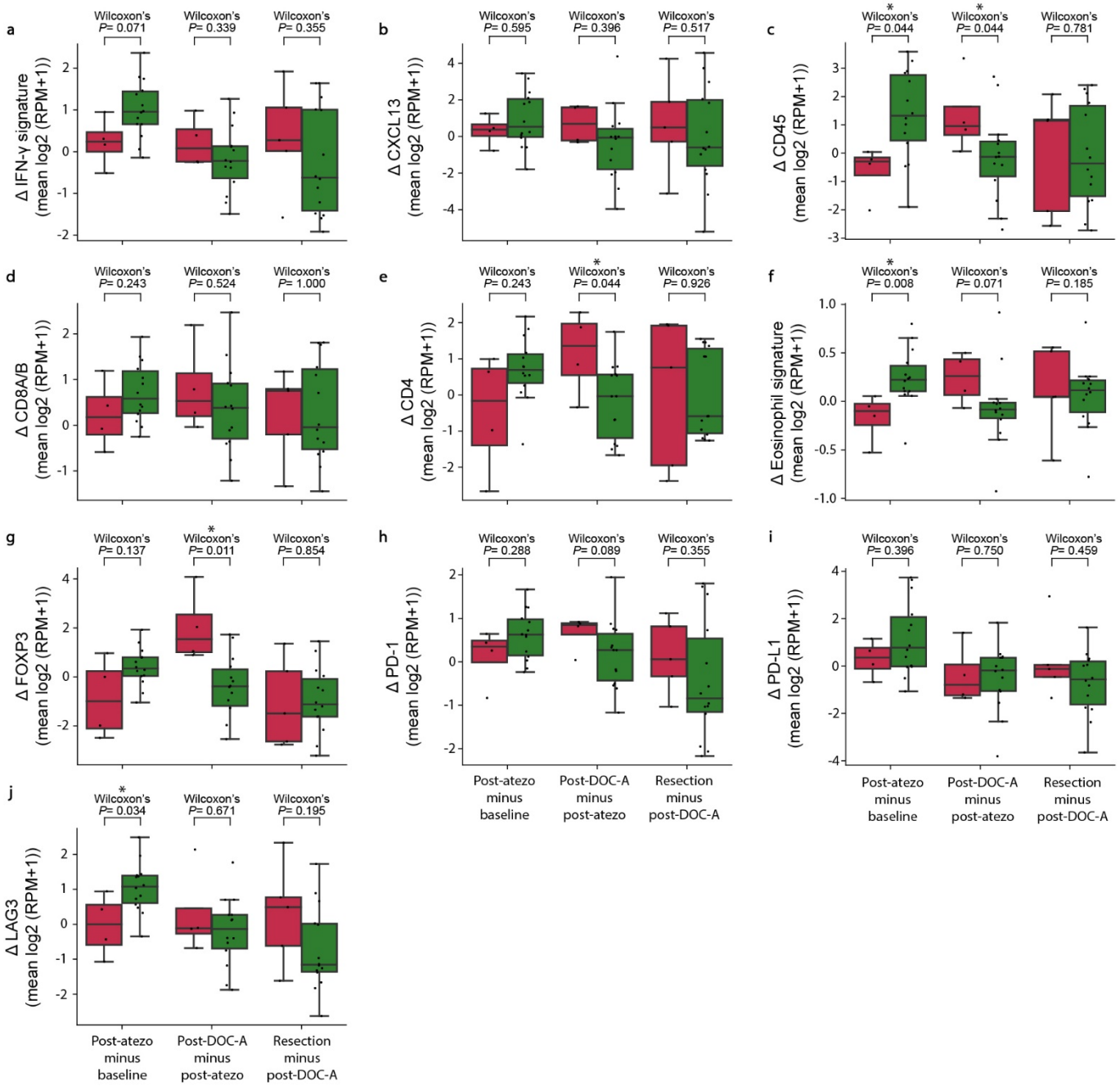
Supplementary Figure 4 | Imaging mass cytometry of pMMR nonresponders and pMMR responders with a pCR. a, Relative distribution of cellular phenotypes identified by IMC at baseline (pCR, *n*=7; NR, *n*=5) and after monotherapy atezolizumab (post atezo; pCR, *n*=7; NR, *n*=4). **b,** Spatial interactions between all populations types and cancer cell subsets. Interactions were considered specific if the colocalization of cell types was at least 2 z-scores above random cell distribution. Interactions specifically occurring in complete responders (pCR) are depicted in green, interactions specific for nonresponders (NR) in red and significant interactions that occurred in both groups are depicted in grey. **c,** Percentage of cancer cells expressing HLA-DR at baseline (pCR, green, *n*=7; NR, red, *n*=5) and after monotherapy atezolizumab (post atezo; pCR, green, *n*=7; NR, red, *n*=4). Boxplots represent the median, 25th and 75th percentiles; whiskers extend from the hinge to the largest value below 1.5 * IQR from the hinge. Dots represent individual patients.



Supplementary Figure 5 | Dynamics of CD4 T-cells, *LAG3*, *SIGLEC-7* and *SIGLEC-9* and comparison between responders and nonresponders. Dynamics of gene expression of **a**, *CD4.* **b**, *LAG3*. **c**, *SIGLEC-7*. **d**, *SIGLEC-9*. **a-d**, Dynamics of gene expression in responders (R, green) and nonresponders (NR, red) at baseline (R, *n*=14; NR, *n*=4), post atezo (R, *n*=14; NR, *n*=4), post DOC-A (R, *n*=14; NR, *n*=5), and at resection (R, *n*=14; NR, *n*=5). Boxplots represent the median, 25th and 75th percentiles; the whiskers extend from the hinge to the largest value below 1.5 * IQR from the hinge. Differences between R and NR were tested using a two-sided Wilcoxon Rank-sum test. Differences between time points in R and NR separately were tested using a two-sided Wilcoxon Signed-Rank test. Significant p-values are depicted with accolades, colors indicate a significant increase/decrease in responders (green) or a significant difference between responders and nonresponders (black).



Supplementary Figure 6 | **Dynamics of immune-related gene expression in predicting response to ICB plus chemotherapy comparing pMMR responders and pMMR nonresponders. a-h**, Dynamics of gene expression of **a**, *CD8* (=*CD8A*+*CD8B*). **b**, IFN-γ signature. **c**, *CXCL13*. **d**, *PD-1*. **e**, *PD-L1*. **f**, *FOXP3*. **g**, *CD45*. **h**, Eosinophil signature. **a-h**, Dynamics of gene expression in pMMR responders (green) and pMMR nonresponders (red) at baseline (R, *n*=12; NR, *n*=4), after monotherapy atezolizumab (post atezo; R, *n*=12; NR, *n*=4), after DOC plus atezolizumab (post DOC-A; R, *n*=12; NR, *n*=5) and at resection (R, *n*=12; NR, *n*=5). Boxplots represent the median, 25th and 75th percentiles; whiskers extend from the hinge to the largest value below 1.5 * IQR from the hinge. Differences between R and NR were tested using a two-sided Wilcoxon Rank-sum test. Differences between time points in R and NR separately were tested using a two-sided Wilcoxon Signed-Rank test. Significant p-values are depicted with accolades, colors indicate a significant increase/decrease in responders (green) or a significant difference between responders and nonresponders (black).



Supplementary Figure 7 | **Dynamics of immune-related gene expression presented as delta (Δ) values in responders and nonresponders. a**, Dynamics of gene expression or gene signature expression in responders (R, green) and nonresponders (NR, red) presented as delta (Δ) expression values of **a**, IFN-γ signature. **b**, *CXCL13*. **c**, *CD45*. **d**, *CD8* (= *CD8A/CD8B*) **e**, *CD4*. **f**, Eosinophil signature. **g**, *FOXP3*. **h**, *PD-1*. **i**, *PD-L1*. **j**, *LAG3*. **a-j**, Dynamics of gene expression represented as delta (Δ) expression values in responders (green) and nonresponders (red) at baseline, showing post atezo (R, *n*=14; NR, *n*=4) minus baseline (R, *n*=14; NR, *n*=4), post-DOC-A (R, *n*=14; NR, *n*=5) minus post atezo (R, *n*=14; NR, *n*=4), and resection (R, *n*=14; NR, *n*=5) minus post-DOC-A (R, *n*=14; NR, *n*=5). Boxplots represent the median, 25th and 75th percentiles; the whiskers extend from the hinge to the largest value below 1.5 * IQR from the hinge. The difference between R and NR was tested using a two-sided Wilcoxon Rank-sum test. All p-values are depicted with an accolade and significant value are identified with an asterisk.

Supplementary table 1 | FDG-PET response evaluation, SUV_{max} values and ΔTLG per patient.

Patient	**Response evaluation on FDG-PET**	**SUV_{max} baseline**	**SUV_{max} posttreatment**	ΔTLG
1	PR	17.7	5.8	-100%
2	CR	9.0	3.4	-100%
4	PR	11.4	5.8	-100%
6	Near-CR	4.8	2.3	-100%
8	Near-CR	9.8	4.5	-100%
9	PR	20.8	6.5	-100%
10	Near-CR	6.9	4.7	NE
11	Near-CR	34.0	3.9	-100%
13	SD	4.7	5.7	NE
14	PR	10.9	4.5	-100%
15	CR	15.7	4.7	-100%
17	Near-CR	5.8	4.7	NE
22	CR	9.4	2.8	-100%
23	Near-CR	10.6	4.6	-100%
25	CR	5.9	2.5	-100%
26	SD	4.0	5.1	NE
27	CR	8.7	3.7	-100%
This table shows FDG-PET results per patient, including response evaluation, maximum standardized uptake value (SUV_{max}) at baseline and posttreatment as well as the difference in total lesion glycolysis (ΔTLG) between baseline and posttreatment FDG-PET scan. PR: partial response; CR: complete response; NE: FDG-PET scan not evaluable for ΔTLG				

Supplementary table 2 | Response among patients with a CPS using a cutoff of 1, 5, and 10.

	PD-L1 CPS <1 (*n* = 2)	**PD-L1 CPS ≥1 (*n* = 18)**	***P*-value ***
			1.000
Responders	2	12	
Nonresponders	0	6	
	PD-L1 CPS <5 (*n* = 6)	**PD-L1 CPS ≥5 (*n* = 14)**	***P*-value ***
			1.000
Responders	4	10	
Nonresponders	2	4	
	PD-L1 CPS <10 (*n* = 16)	**PD-L1 CPS ≥10 (*n* = 4)**	***P*-value ***
			0.549
Responders	12	2	
Nonresponders	4	2	

CPS = combined positive score; * P value was calculated using a two-sided Fisher's exact test.

Supplementary table 3 | Antibody panel used for imaging mass cytometry.

Target	Clone	Lot	Company	Metal	Incubation time	Temp	Dilution
CD4	EPR6855	1014578-6	Abcam, Cambridge, United Kingdom	145 Nd	Indirect ON	4°C	100
TCRgd	H41	D3021	Santa Cruz biotechnology, Dallas, United states	148 Nd	Indirect ON	4°C	25
Anti-rabbit IgG	Polyclonal	GR3215731-15	Abcam	145 Nd	1h	RT	100
Anti-mouse IgG	Polyclonal	GR3300461-1	Abcam	148 Nd	1h	RT	100
CD8a	D8A8Y	2	Cell signaling technology, Danvers, United states	146 Nd	5h	RT	50
PD-1	D4W2J	1	Cell signaling technology	160 Gd	5h	RT	50
ICOS	D1K2T(tm)	4	Cell signaling technology	161 Dy	5h	RT	50
CD204	J5HTR3	2518439	Thermo Fisher Scientific, Waltham, United States	164 Dy	5h	RT	50
CD103	EPR4166(2)	GR3399209-2	Abcam	168 Er	5h	RT	50
Tbet	4B10	B298378	Thermo Fisher Scientific	170 Er	5h	RT	50
Caspase	D4V4B	25	Cell signaling technology	172 Yb	5h	RT	50
CD163	D6U1J	1	Cell signaling technology	173 Yb	5h	RT	50
HLA-DR	TAL 1B5	GR3424852-2	Abcam	141 Pr	5h	RT	100
CD11b	D6X1N	1	Cell signaling technology	144 Nd	5h	RT	100
Granzyme B	D6E9W	7	Cell signaling technology	150 Nd	5h	RT	100
CD138	5A1E	1	Cell signaling technology	155 Gd	5h	RT	100
CD39	EPR20627	GR3274485-6	Abcam	157 Gd	5h	RT	100
VISTA	D1L2G(TM)	7	Cell signaling technology	158 Gd	5h	RT	100
CD14	D7A2T	2	Cell signaling technology	163 Dy	5h	RT	100

CD56	E7X9M	2	Cell signaling technology	167 Er	5h	RT	100
CD7	EPR4242	GR3424737-2	Abcam	174 Yb	5h	RT	100
CD11c	EP1347Y	GR3357092-17	Abcam	176 Yb	5h	RT	100
CD45	D9M8I	12	Cell signaling technology	149 Sm	Overnight	4°C	50
CD3	EP449E	GR3418069-6	Abcam	153 Eu	Overnight	4°C	50
PD-L1	E1L3N(R)	2	Cell signaling technology	156 Gd	Overnight	4°C	50
FOXP3	D608R	2	Cell signaling technology	159 Tb	Overnight	4°C	50
CD27	EPR8569	GR3446729-2	Abcam	175 Lu	Overnight	4°C	50
Vimentin	D21H3	1	Cell signaling technology	194 Pt	Overnight	4°C	50
Keratin	C11 and AE1/AE3	2	Cell signaling technology	198 Pt	Overnight	4°C	50
TGFb	D10A8	157850	Cell signaling technology	89Y	Overnight	4°C	100
CD20	H1	1209781	BD Biosciences, Franklin Lakes, United states	142 Nd	Overnight	4°C	100
CD68	D4B9C	2	Cell signaling technology	143 Nd	Overnight	4°C	100
CD31	89C2	1	Cell signaling technology	147 Sm	Overnight	4°C	100
CD57	HNK-1 / Leu-7	GR3373313	Thermo Fisher Scientific,	151 Eu	Overnight	4°C	100
Ki-67	8D5	11	Cell signaling technology	152 Sm	Overnight	4°C	100
IgG1	D3W8G	1	Cell signaling technology	154 Sm	Overnight	4°C	100
IDO	D5J4E(TM)	7	Cell signaling technology	162 Dy	Overnight	4°C	100
CD45RO	UCHL1	1	Cell signaling technology	165 Ho	Overnight	4°C	100
D2-40	D2-40	B316467	Thermo Fisher Scientific,	166 Er	Overnight	4°C	100
CD38	EPR4106	GR3378690-1	Abcam	169 Tm	Overnight	4°C	100
CD15	MC480	5	Abcam	171 Yb	Overnight	4°C	100
Bcatenin	D10A8	1	Cell signaling technology	196 Pt	Overnight	4°C	100
Histone H3	D1H2	1	Cell signaling technology	209 Bi	Overnight	4°C	50
The location of each company its headquarter is provided in the first mention of the company in the table. Abbreviations: Temp: temperature; h: hour; RT: room temperature							

Supplementary table 4 | Gene sets used in transcriptional analysis.

Gene set	Number of genes	Gene symbols
B cells[81]	9	BLK, CD19, FAM30A, FCRL2, MS4A1, PNOC, SPIB, TCL1A, TNFRSF17
CD8A/B[81]	2	CD8A, CD8B
Cytotoxic T-cells[82]	6	CTSW, GNLY, GZMA, GZMB, GZMH, PRF1
Eosinophils[44]	5	SIGLEC8, RNASE2, RNASE3, IL5RA, CCR3
Exhausted CD8 T-cells[81]	4	CD244, EOMES, LAG3, PTGER4
IFN-γ signature (expanded)[36]	18	IDO1, CXCL10, HLA-DRA, STAT1, CD3D, CIITA, CD3E, CCL5, GZMK, CD2, CXCL13, IL2RG, NKG7, HLA-E, CXCR6, LAG3, TAGAP, GZMB
Macrophages[81]	4	CD163, CD68, CD84, MS4A4A
Mast cells[81]	5	CPA3, HDC, MS4A2, TPSAB1, TPSB2
Neutrophils[81]	7	CEACAM3, CSF3R, FCAR, FCGR3B, FPR1, S100A12, SIGLEC5
NK cells[82]	2	KLRF1, NCR1
Regulatory T-cells[81]	1	FOXP3
T-cells[81]	6	CD3D, CD3E, CD3G, CD6, SH2D1A, TRAT1