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Supplemental information

Bexmarilimab-induced macrophage activation leads to treatment benefit in solid tumors: The phase I/II first-in-human MATINS trial

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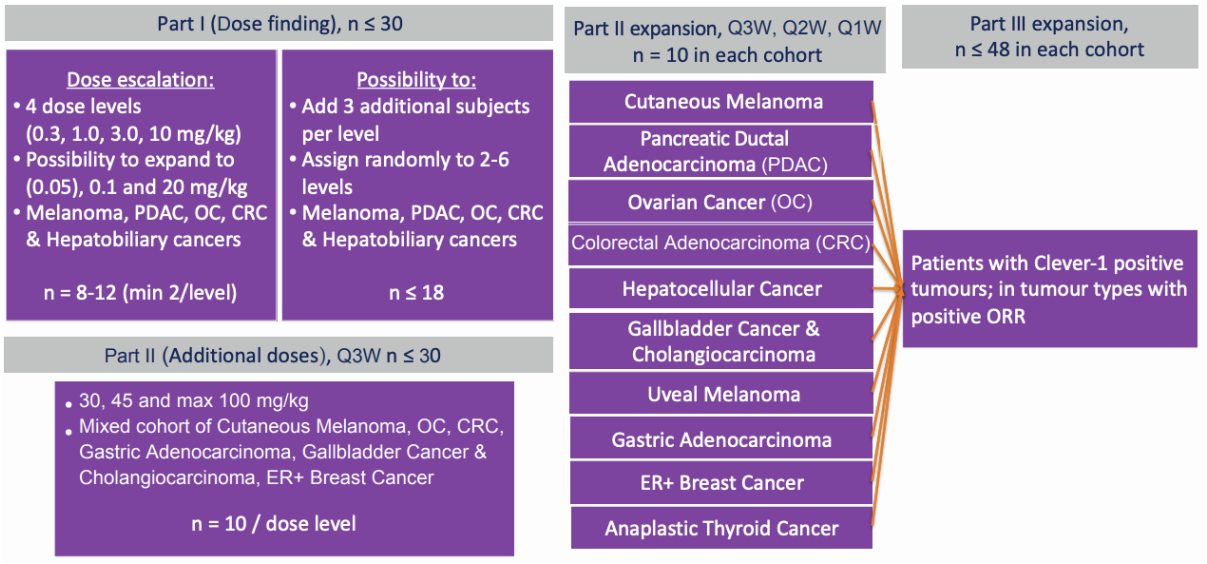

Figure S1. MATINS study design. Related to STAR methods.

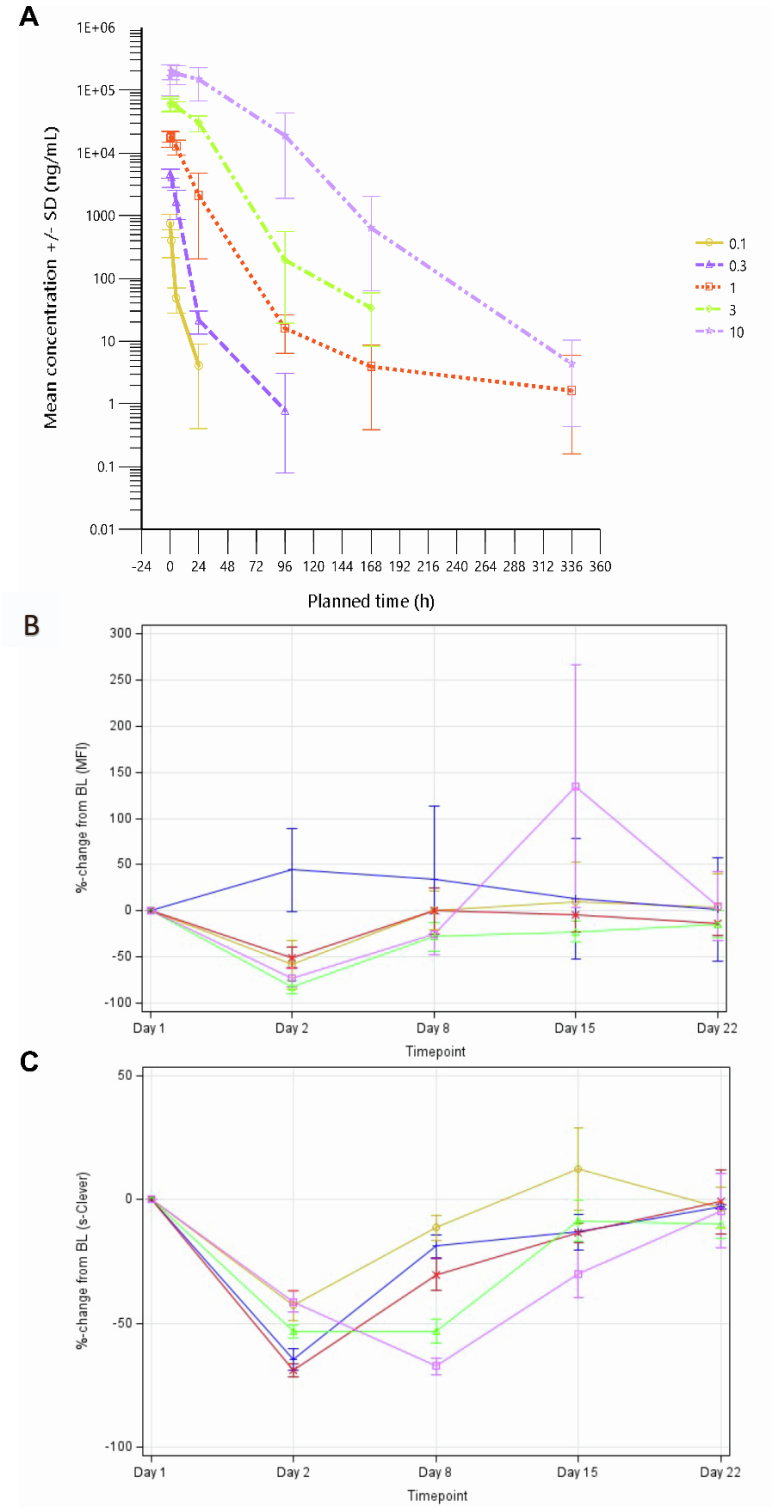

Figure S2. Pharmacokinetics, receptor occupancy and target engagement after the first dose of bexmarilimab. Related to Figure 1.

(A) Pharmacokinetics of bexmarilimab. Y-axis is showing the concentration of bexmarilimab while X-axis presents the time in hours from the IMP infusion. (B) Receptor occupancy (RO) of bexmarilimab for Clever-1 on circulating monocytes as measured by decreased cell surface binding of fluorochrome-conjugated bexmarilimab (AF647) competitor antibody in a flow cytometry-based assay. The RO is depicted as %-decrease from baseline samples. Y-axis is percent of change from baseline while Y-axis the time in days from the IMP infusion. No significant differences between doses in the RM ANOVA model were observed, but the changes at day 2 are statistically significant ($p<0.05$) in all other doses than 0.3 mg/kg. (C) Target engagement of bexmarilimab using circulating soluble Clever-1 as a surrogate marker. Graph showing decreased binding of

biotinylated bexmarilimab in a sandwich ELISA assay. Target engagement is depicted as %-decrease from baseline samples. Y-axis is percent of change from baseline while X-axis the time in days from the IMP infusion. Investigated dose levels were 0.1 (yellow), 0.3 (blue), 1 (red), 3 (green), and 10mg/kg (purple). Significant differences between doses ($p=0.0098$ for dose effect in RM ANOVA), and also significant interaction between dose and time ($p<0.0001$ for dose*time) were observed. For all doses the changes at day 2 are statistically significant ($p<0.0001$). BL, Baseline; IMP, investigational medicinal product; MFI, median fluorescence intensity; s-Clever, soluble Clever-1.

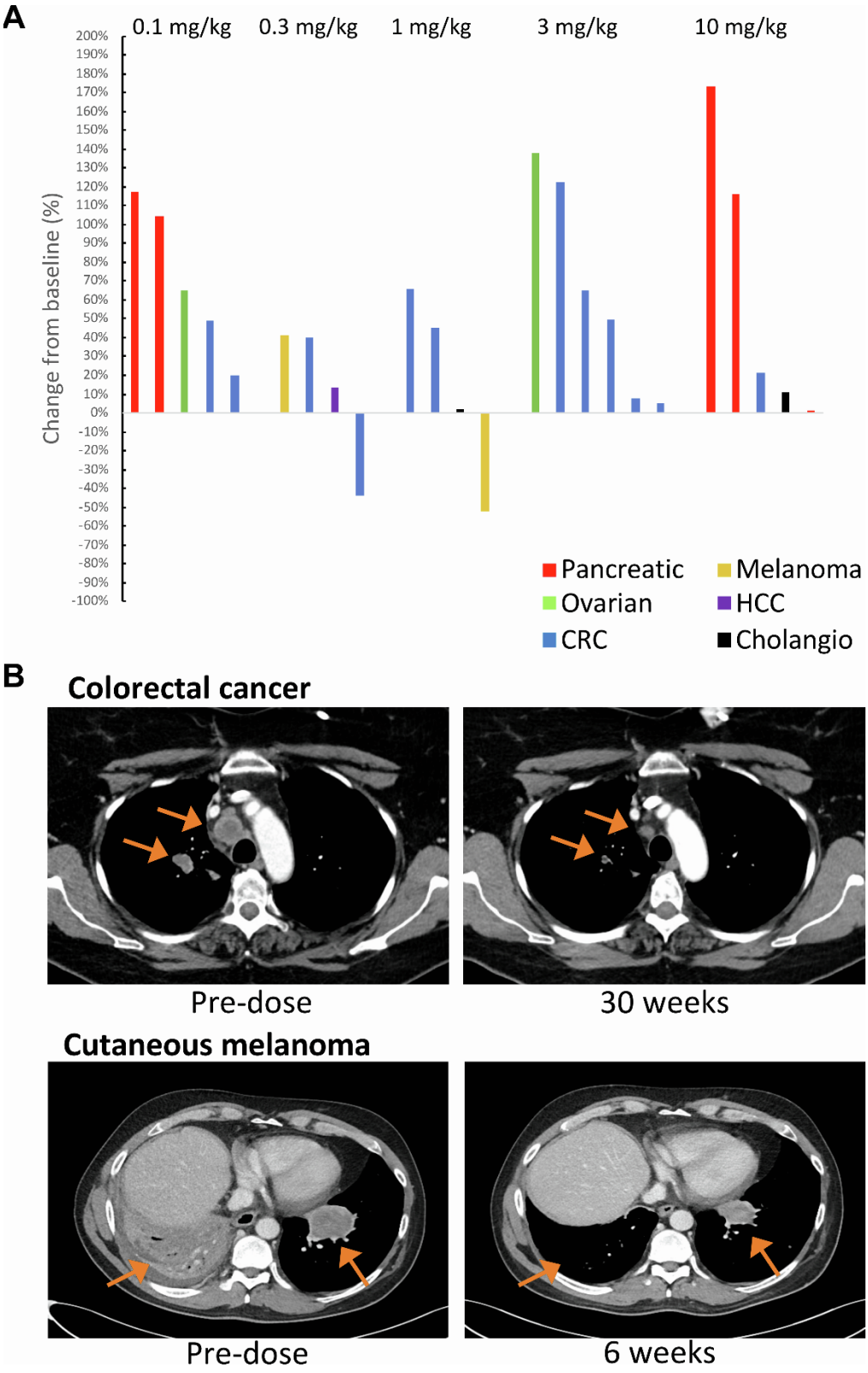

Figure S3. Preliminary anti-tumor efficacy for bexmarilimab in part I. Related to Table 3.
(A) Waterfall plot for the best target lesion responses (%) according to dose and tumor type in RECIST 1.1 evaluable patients (n=24). (B) CT-scans for the selected responding patients in baseline and at the time of the best response. Colorectal cancer patient with PR and melanoma patient with PR in target lesions are presented. CRC, colorectal cancer; HCC, hepatocellular cancer; PR, partial response.

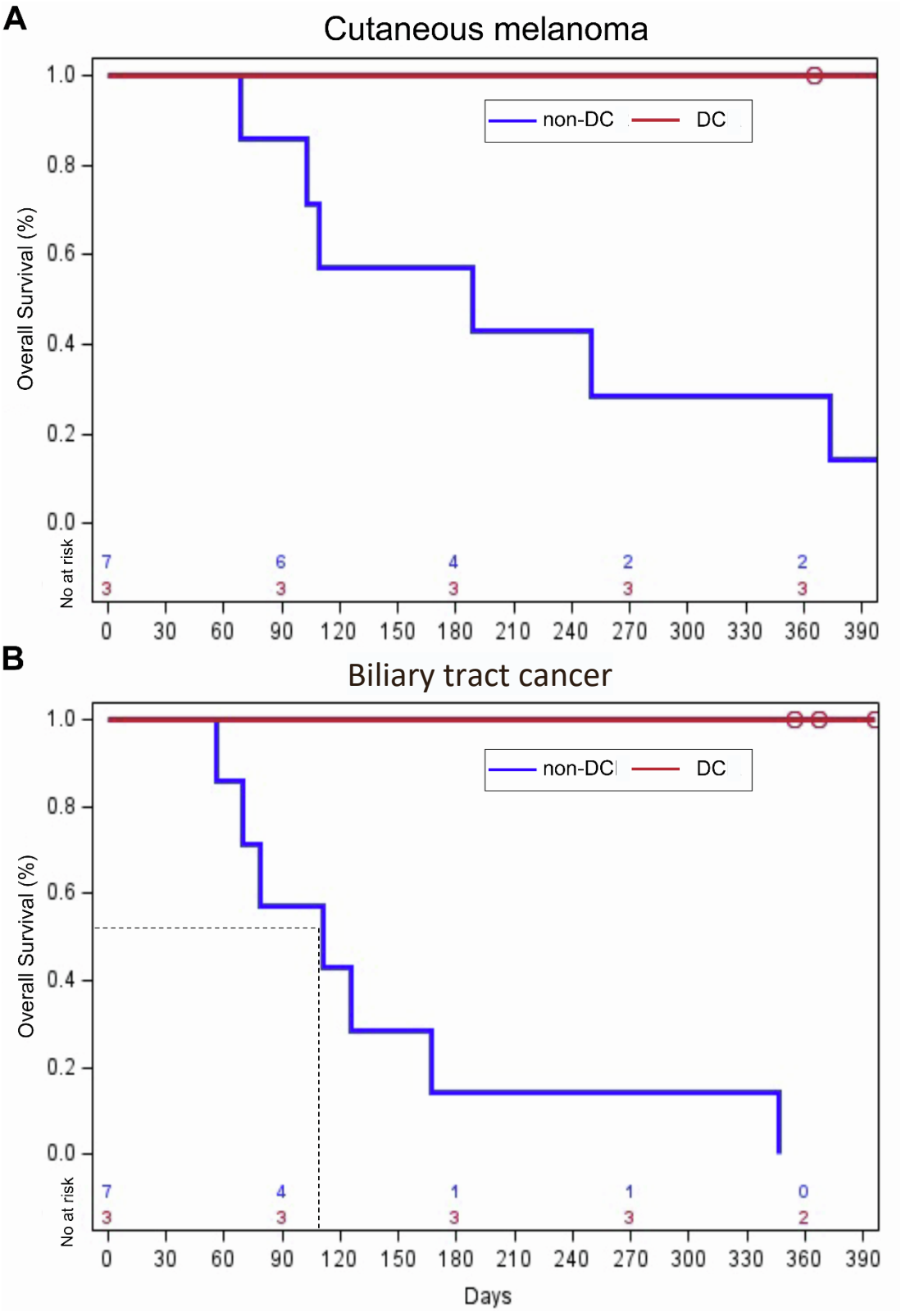

Figure S4. Overall survival analysis according to DC in selected cancer types. Related to Figure 1.
(A-B) Overall survival according to DC in cutaneous melanoma (A) and biliary tract cancer (B). Circles indicate censored events. DC, disease control.

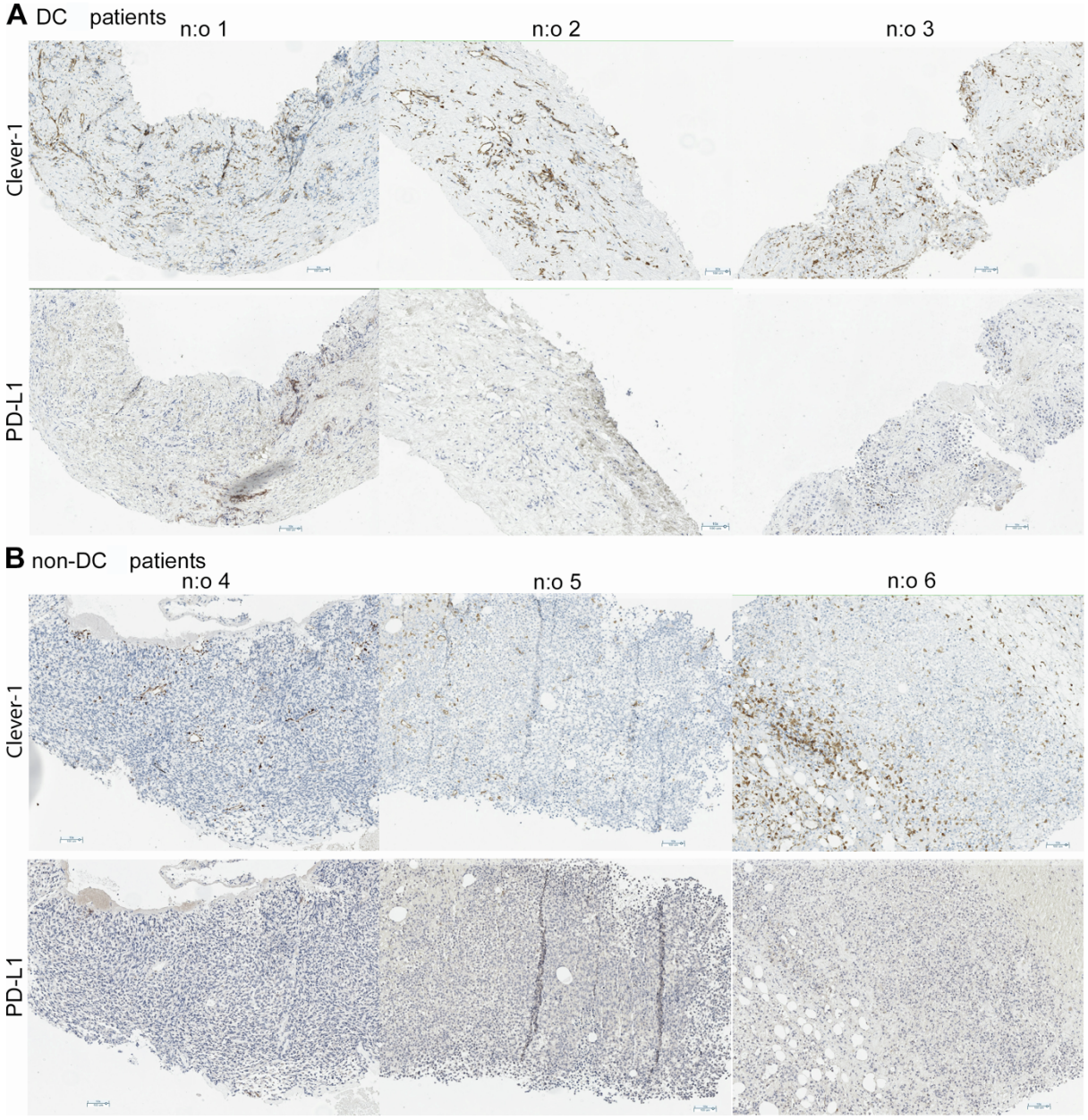

Figure S5. Tumor Clever-1 and PD-L1 expression in selected DC and non-DC patients. Related to Table 4.

(A-B) Immunohistochemical staining of pre-treatment tumor samples for Clever-1 and PD-L1 in selected DC patients (A, 1-3) and non-DC patients (B, 4-6). Line segments 100 µm. DC, disease control.

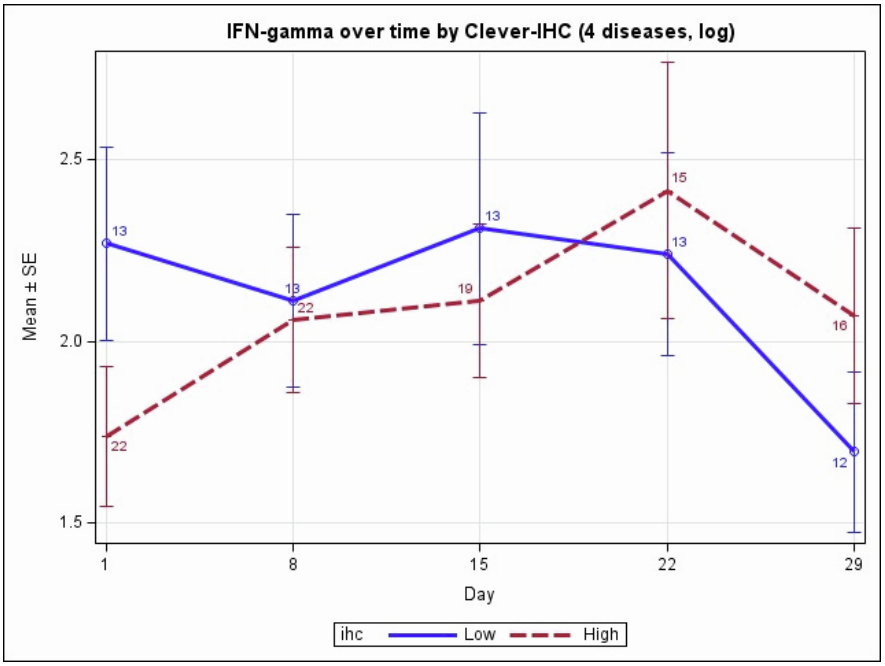

Figure S6. Changes in IFNγ levels in cancer cohorts with high DC according to the intratumoral Clever-1 scores. Related to Figure 1 and Table 4.
Significant increases of IFNγ (p=0.018) were observed in Clever-1 high (cut-off 3%) patients compared to Clever-1 low patients during the first cycle of treatment using RM ANOVA model.

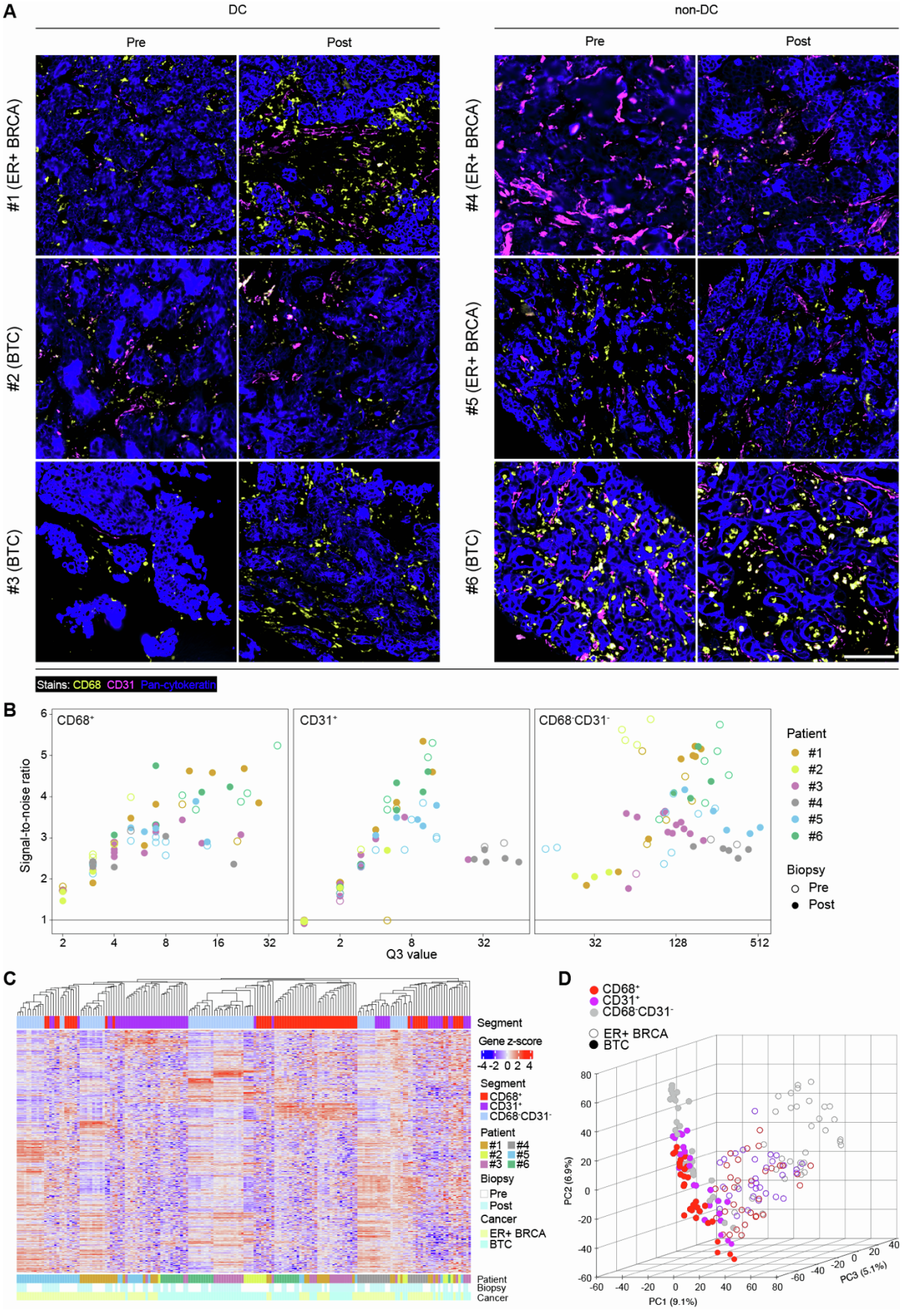
A
DC
non-DC
Pre
Post
#1 (ER+ BRCA)
#2 (BTC)
#3 (BTC)
#4 (ER+ BRCA)
#5 (ER+ BRCA)
#6 (BTC)
Stains: CD68 CD31 Pan-cytokeratin
B
CD68+
CD31+
CD68-CD31-
Signal-to-noise ratio
Q3 value
Patient
#1
#2
#3
#4
#5
#6
Biopsy
Pre
Post
C
Segment
Gene z-score
Cancer
ER+ BRCA
BTC
D
CD68+
CD31+
CD68-CD31-
ER+ BRCA
BTC
PC1 (9.1%)
PC2 (6.9%)
PC3 (5.1%)

Figure S7. GeoMx spatial transcriptomics profiling of pre- and post-treatment tumor biopsies. Related to Figure 2.

(A) Morphology marker staining for GeoMx analysis (n = 6 patients) showing $CD68^+$ macrophages (yellow), $CD31^+$ vessels (magenta) and pan-cytokeratin$^+$ cancer cells (blue). Images of representative ROIs were selected based on CD68 staining in each biopsy and 400μm × 400μm square regions are displayed from the center of the ROIs. Scale bar 100 μm. (B) Signal-to-noise ratio (Q3 value / geoMean[NegativeProbes]) is shown separately for $CD68^+$, $CD31^+$ and $CD68^-CD31^-$ segments with points representing analyzed ROIs. Segments with signal-to-noise ratio ≤1 were excluded from further analyses. (C-D) Clustering of all QC-passing segments (n = 180) based on Q3-normalized and log_2-transformed counts (n = 10,612 genes). Heatmap of unsupervised hierarchical clustering with columns representing segments (C) and a scatter plot of the first three principal components with each point representing a single segment (D). BTC, biliary tract cancer; ER+ BRCA, estrogen receptor positive breast cancer; DC, disease control; Pre, pre-treatment biopsy; Post, post-treatment biopsy; Q3, 75^{th} percentile of counts; ROI, region of interest.

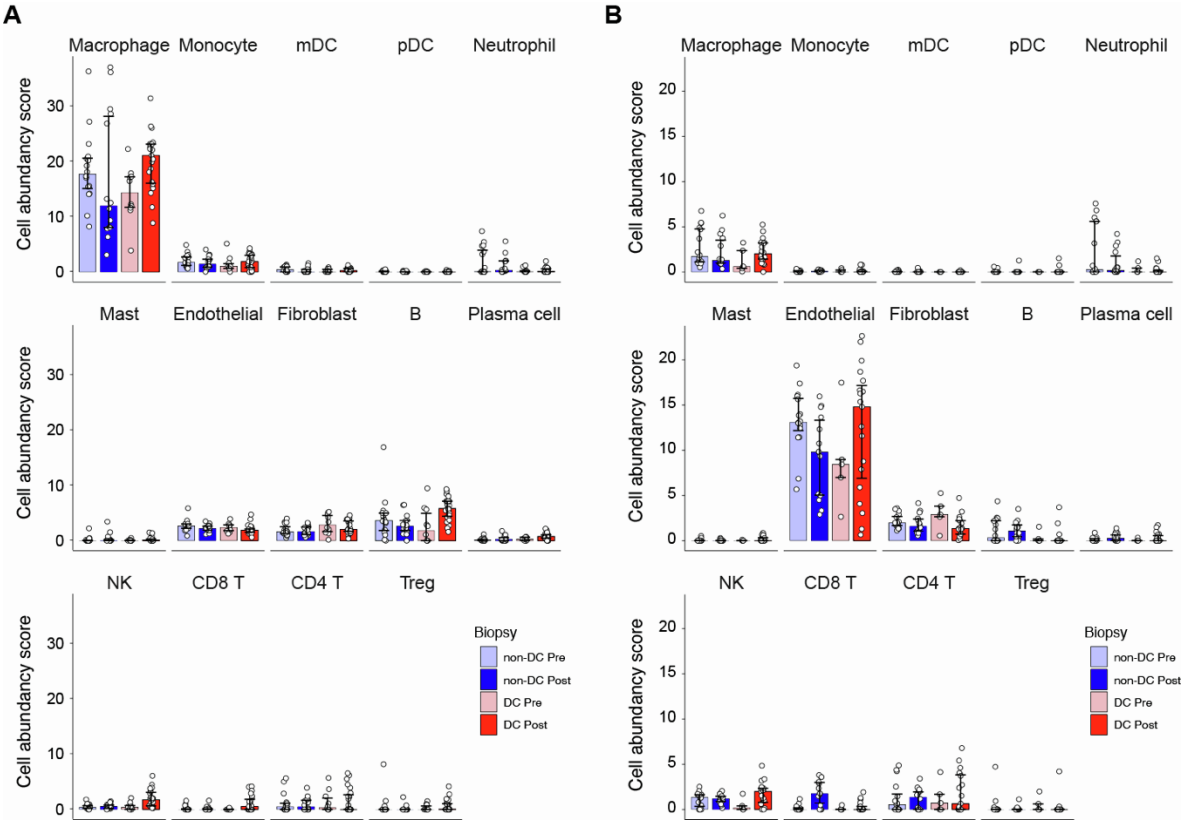

Figure S8. Cell type deconvolution of GeoMx-profiled tumor areas. Related to Figure 2.
(A-B) Cell abundancy scores for the indicated cell types were calculated based on Q3-normalized gene expression from $CD68^{+}$ (A) and $CD31^{+}$ (B) tumor areas using SpatialDecon. Each point represents a single ROI, n = 3 patients per group. Median ± interquartile range. DC, disease control; NK, natural killer cell; mDC, myeloid dendritic cell; pDC, plasmacytoid dendritic cell; Treg, regulatory T-cell; Pre, pre-treatment biopsy; Post, post-treatment biopsy; ROI, region of interest.

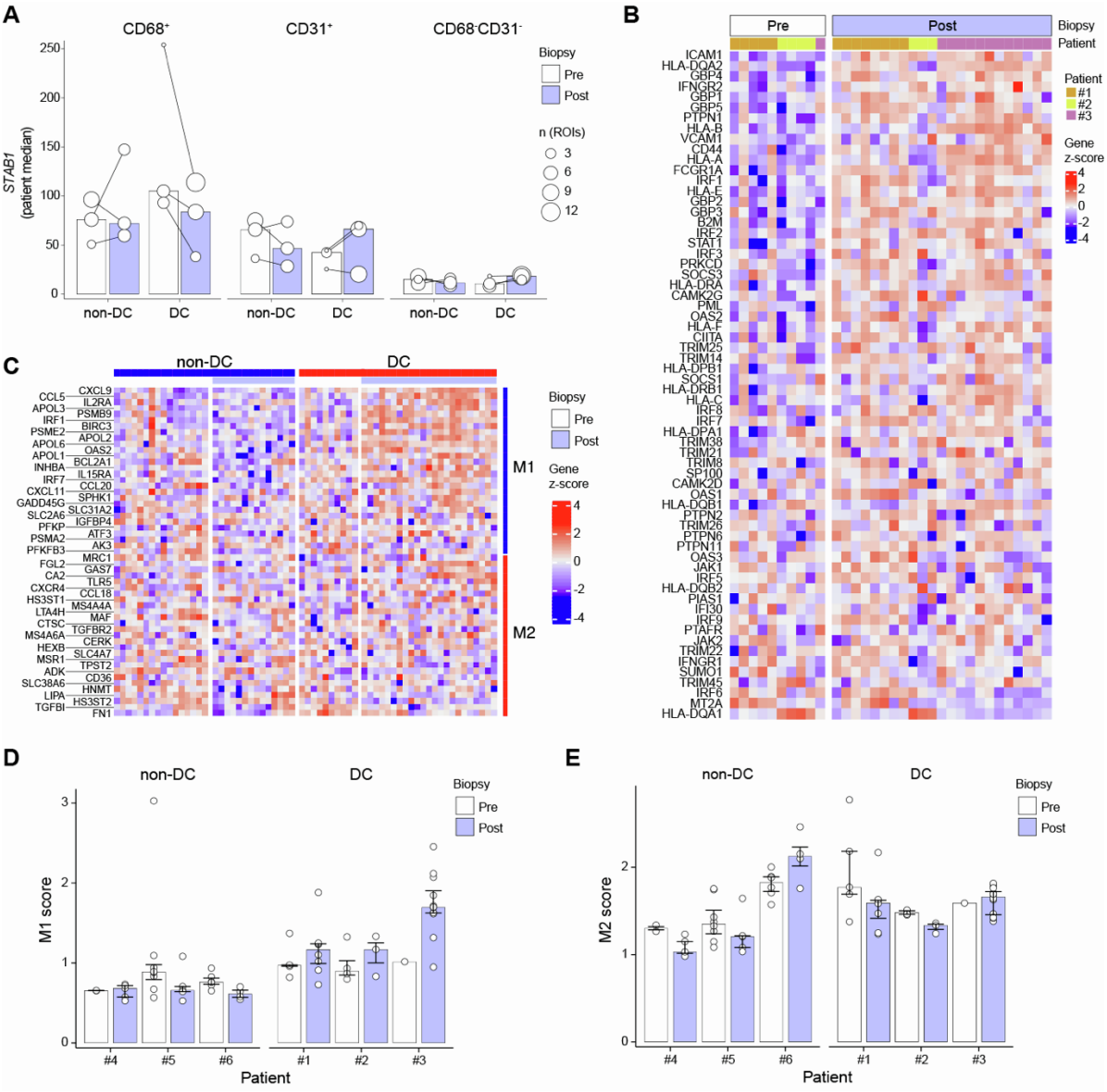

Figure S9. GeoMx profiling of biopsy CD68+ area transcriptome after bexmarilimab therapy. Related to Figure 2.

(A) Clever-1 mRNA (*STAB1*) levels in $CD68^+$, $CD31^+$ and $CD68^-CD31^-$ areas of DC and non-DC patient biopsies. Points indicate median expression across patient's ROIs and bars represent patient group median. (B) Expression levels of interferon gamma signaling pathway genes measured from $CD68^+$ areas of DC patient biopsies. (C) Heatmap of M1 and M2 macrophage marker gene expression levels calculated from $CD68^+$ biopsy areas. In (B-C), color gradient represents gene z-scores calculated from Q3-normalized and log_2-transformed counts with red corresponding to higher expression, and columns represent individual ROIs. (D-E) Bar graphs of M1 (D) and M2 (E) scores calculated based on mean expression level of macrophage marker genes shown in (C). Score of 1 indicates marker gene expression level equal to overall gene expression level on $CD68^+$ biopsy area. Median ± interquartile range, points represent individual ROIs. DC, disease control; Pre, pre-treatment biopsy; Post, post-treatment biopsy, ROI: region of interest.

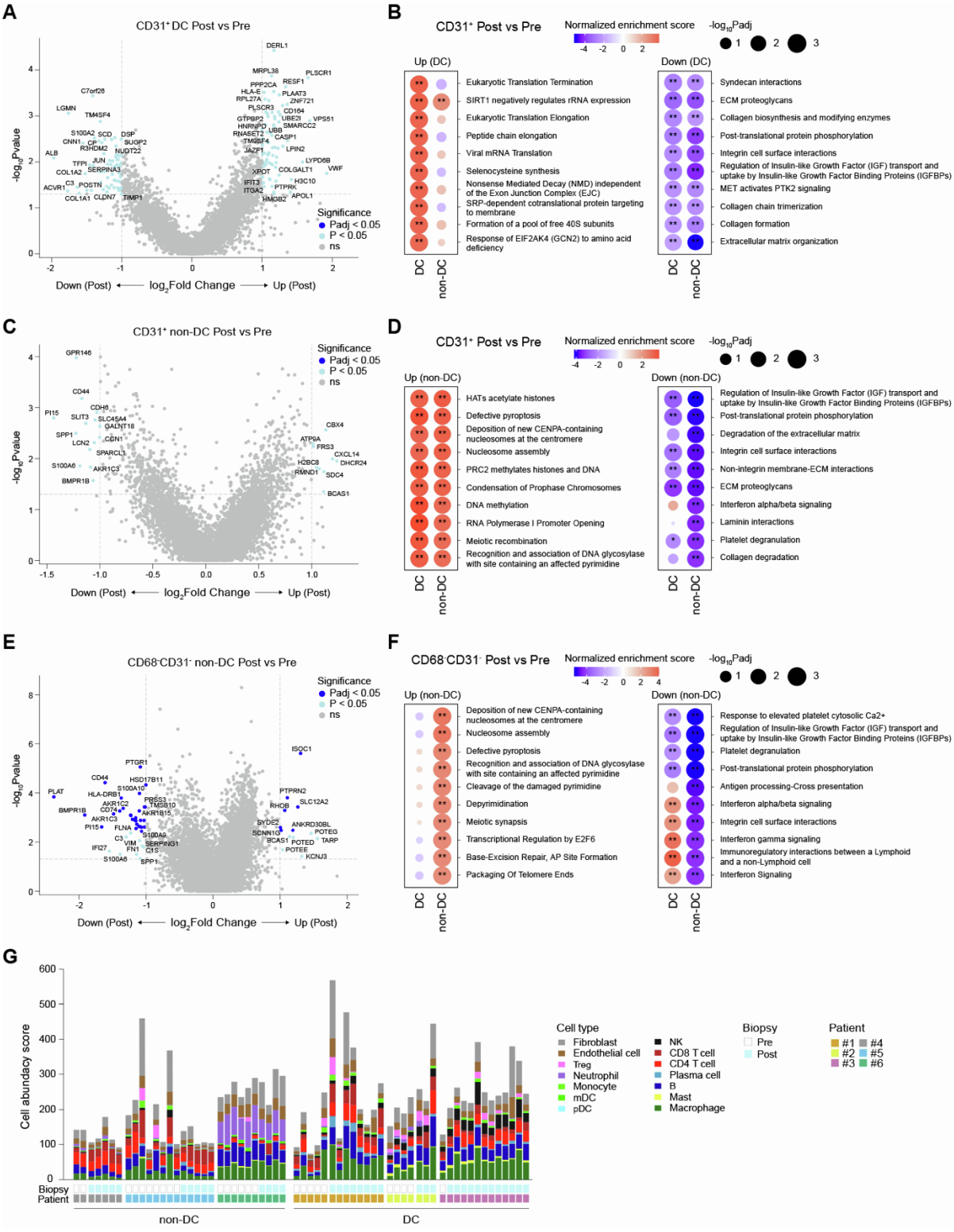

Figure S10. GeoMx profiling of CD31+ and CD68-CD31- tumor area transcriptomes after bexmarilimab therapy. Related to Figure 3.

(A-F) Analysis of gene expression changes after bexmarilimab therapy in CD31$^+$ tumor areas of DC patients (A-B), CD31$^+$ tumor areas of non-DC patients (C-D) and CD68$^-$CD31$^-$ tumor areas of non-DC patients (E-F), n = 3 patients per group with paired biopsies. Volcano plots show differentially expressed genes (A, C and E) and bubble plots top up- and downregulated pathways (B, D and F; gene set enrichment analysis). In B, D, and F, red color denotes pathway activation and blue downregulation. (G), Cell abundancy scores for the indicated cell types were calculated based on Q3-normalized gene expression on CD68$^-$CD31$^-$ area using SpatialDecon. Each stacked

bar represents a single ROI. DC, disease control; Pre, pre-treatment biopsy; Post, post-treatment biopsy; ROI, region of interest. Padj, Benjamini-Hochberg-adjusted p-value; *, Padj < 0.05; **, Padj < 0.01; ns, not significant.

Table S1. Treatment-related adverse events in part I and part II separated by dose. Related to Table 2.

Dose (mg/kg)	0.1	0.3	1.0	3.0	10	Total
	(n = 5)	(n = 13)	(n = 97)	(n = 17)	(n = 6)	(n = 138)
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Any	3 (60.0)	8 (61.5)	49 (50.5)	6 (35.3)	3 (50.0)	69 (50.0)
Blood and lymphatic system disorders						
Anemia	1 (20.0)	0 (0.0)	6 (6.2)	1 (5.9)	0 (0.0)	8 (5.8)
Gastrointestinal disorders						
Nausea	1 (20.0)	1 (7.7)	5 (5.2)	0 (0.0)	0 (0.0)	7 (5.1)
Vomiting	0 (0.0)	0 (0.0)	4 (4.1)	0 (0.0)	0 (0.0)	4 (2.9)
General disorders						
Fatigue	1 (20.0)	5 (38.5)	14 (14.4)	2 (11.8)	1 (16.7)	23 (16.7)
Pyrexia	1 (20.0)	2 (15.4)	6 (6.2)	2 (11.8)	1 (16.7)	12 (8.7)
Investigations						
Blood alkaline phosphatase increased	0 (0.0)	0 (0.0)	5 (5.2)	2 (11.8)	0 (0.0)	7 (5.1)
Alanine aminotransferase increased	0 (0.0)	0 (0.0)	3 (3.1)	1 (5.9)	0 (0.0)	4 (2.9)
Aspartate aminotransferase increased	0 (0.0)	0 (0.0)	2 (2.1)	2 (11.8)	0 (0.0)	4 (2.9)
Metabolism and nutrition disorders						
Decreased appetite	0 (0.0)	0 (0.0)	3 (3.1)	1 (5.9)	0 (0.0)	4 (2.9)

*Included are treatment-related adverse events with National Cancer Institute-Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 5.0 that occurred in at least four patients.

Table S2. Treatment-emergent adverse events in part I and part II. Related to Table 2.

Treatment-emergent adverse event*	All grades (n=138) number (percent)	Grade ≥3 (n=138) number (percent)
Any	138 (100.0)	111 (80.4)
Blood and lymphatic system disorders		
Anemia	32 (23.2)	8 (5.8)
Gastrointestinal disorders		
Abdominal pain	33 (23.9)	4 (2.9)
Constipation	24 (17.4)	1 (0.7)
Nausea	21 (15.2)	0
Diarrhea	16 (11.6)	0
Vomiting	15 (10.9)	1 (0.7)
Ascites	13 (9.4)	8 (5.8)
Intestinal obstruction	3 (3.1)	3 (2.2)
Small intestinal obstruction	2 (1.4)	2 (1.4)
Large intestinal obstruction	2 (1.4)	2 (1.4)
Ileus	2 (1.4)	2 (1.4)
General disorders and administration site conditions		
Fatigue	51 (37.0)	4 (2.9)
Pyrexia	20 (14.5)	0
Edema peripheral	7 (5.1)	0
Death	7 (5.1)	7 (5.1)
General physical health deterioration	4 (2.9)	3 (2.2)
Hepatobiliary disorders		
Cholestasis	6 (4.3)	0
Hepatic failure	2 (1.4)	2 (1.4)
Infections and infestations		
Pneumonia	3 (2.2)	2 (1.4)
Investigations		
Blood alkaline phosphatase increased	21 (15.2)	3 (2.2)
Aspartate aminotransferase increased	17 (12.3)	3 (2.2)
Alanine aminotransferase increased	16 (11.6)	1 (0.7)
Blood bilirubin increased	8 (5.8)	3 (2.2)
Transaminases increased	3 (2.2)	3 (2.2)
Metabolism and nutrition disorders		
Decreased appetite	24 (17.4)	0
Hyperglycemia	6 (4.3)	3 (2.2)
Hyponatremia	5 (3.6)	2 (1.4)
Musculoskeletal and connective tissue disorders		
Back pain	13 (9.4)	2 (1.4)
Flank pain	9 (6.5)	0
Myalgia	6 (4.3)	0
Pain in extremity	5 (3.6)	0
Arthralgia	5 (3.6)	0
Neoplasms benign, malignant, and unspecified		
Tumor pain	4 (2.9)	2 (1.4)
Nervous system disorders		
Headache	8 (5.8)	0
Dizziness	5 (3.6)	0
Psychiatric disorders		
Insomnia	7 (5.1)	0
Respiratory, thoracic and mediastinal disorders		
Dyspnea	13 (9.4)	1 (0.7)
Cough	6 (4.3)	0
Pleural effusion	4 (2.9)	3 (2.2)
Vascular disorders		
Hypertension	4 (2.9)	2 (1.4)

*Included are treatment-emergent adverse events that occurred in at least five patients or treatment-emergent grade ≥3 adverse events that occurred in at least two patients.

Table S3. Potential immune-related adverse events in part I and part II. Related to Table 2.

Subject	Dose (mg/kg)	Adverse event preferred term	irAE class	Onset date (cycle)	Severity	Relationship to study drug
#1	0.3	Rash erythematous	Dermatitis	Day 110(C5D1)	Grade 1	Possibly related
#1	0.3	Hyperthyroidism	Thyroiditis	Day 133 (C7D1)	Grade 1	Possibly related
#1	0.3	Pneumonitis	Pneumonitis	Day 133 (C7D1)	Grade 1	Probably related
#1	0.3	Hand dermatitis	Dermatitis	Day 153 (C8D1)	Grade 1	Possibly related
#1	0.3	Hypothyroidism	Dermatitis	Day 176 (C8D1)	Grade 2	Possibly related
#1	0.3	Hand dermatitis	Dermatitis	Day 181 (C8D1)	Grade 2	Possibly related
#1	0.3	Myositis	Myositis	Day 181 (C8D1)	Grade 2	Probably related
#1	0.3	Autoimmune thyroiditis	Thyroiditis	Day 181 (C8D1	Grade 2	Probably related
#1	0.3	Rash erythematous	Dermatitis	Day 217 (C8D1)	Grade 1	Possibly related
#2	3	Aspartate aminotransferase increased	Hepatitis	Day 71 (C3D1)	Grade 1	Possibly related
#2	3	Alanine aminotransferase increased	Hepatitis	Day 71 (C3D1)	Grade 2	Possibly related
#2	3	Drug-induced liver injury	Hepatitis	Day 240(C11D1)	Grade 4	Probably related
#3	0.3	Alanine aminotransferase increased	Hepatitis	Day 65 (C3D1)	Grade 2	Possibly related
#3	0.3	Aspartate aminotransferase increased	Hepatitis	Day 65 (C3D1)	Grade 2	Possibly related
#4	1	Diarrhea	Colitis	Day 78 (C4D1)	Grade 1	Possibly related
#4	1	Pancreatic failure	Pancreatitis	Day 122 (C5D1)	Grade 2	Possibly related
#5	1	Hepatic failure	Hepatitis	Day 33 (C2D1)	Grade 4	Probably related
#6	1	Hyperparathyroidism	Parathyroiditis	Day 22 (C1D1)	Grade 1	Possibly related
#7	1	Aspartate aminotransferase increased	Hepatitis	Day 43 (C1D1)	Grade 1	Probably related
#7	1	Alanine aminotransferase increased	Hepatitis	Day 64 (C3D1)	Grade 1	Probably related
#8	3	Aspartate aminotransferase increased	Hepatitis	Day 22 (C1D1)	Grade 1	Possibly related
#8	3	Diarrhea	Colitis	Day 27(C2D1)	Grade 1	Possibly related
#8	3	Rash macular	Dermatitis	Day 54 (C3D1)	Grade 2	Possibly related
#9	1	Hypothyroidism	Thyroiditis	Day 64 (C3D1)	Grade 2	Possibly related
#10	1	Transaminases increased	Hepatitis	Day 22 (C1D1)	Grade 4	Probably related

irAE, immune-related adverse event.

Table S4. PFS on bexmarilimab/duration of previous treatment line ratio of > 1.3. Related to Figure 1.

		n (%)	p-value*
All		130 (100)	
	Yes	21 (16)	
	No	109 (84)	
Non-DC		111 (100)	
	Yes	13 (12)	
	No	99 (88)	
DC		19 (100)	
	Yes	8 (42)	0.0031
	No	11 (58)	

DC, disease control; PFS, progression-free survival; *Two-sided Fisher's exact test.