Supplemental files for
Research article:

Autoantibody spark response predicts prognosis in patients receiving chemoradiation followed by durvalumab therapy

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Table S1. Antigen list for autoantigen test panel (130 antigens)

No.	Antigen information				Vector
	Protein name	Uniprot ID	CT No.	Expression	
1	A1AT_Native	P01009-1 (25 - 418 aa)	–	S	CMV
2	A1AT_SCT	P01009-1 (25 - 418 aa)	–	I	pET
3	A2B1_Native	P22626-1	–	S	pET
4	A2B1_SCT	P22626-1	–	I	pET
5	AARS_SCT	P49588-1	–	I	pET
6	EEF1A1_SCT	P68104	–	I	pET
7	EEF1G_SCT	P26641-1	–	I	pET
8	EEF2K_SCT	O00418	–	I	pET
9	FKBP4_Native	Q02790	–	S	pET
10	HARS_SCT	P12081-1	–	I	pET
11	HNRNPR_Native	O43390	–	S	pET
12	HNRNPR_SCT	O43390	–	S	pET
13	KRT18_Intact	P05783	–	I	pET
14	KRT8_Intact	P05787-1	–	I	pET
15	LMNA_SCT	P02545-1	–	I	pET
16	PCNA_SCT	P12004	–	I	pET
17	PRDX2_Native	P32119-1	–	S	pET
18	PRDX2_SCT	P32119-1	–	I	pET
19	RO52_SCT	P19474-1	–	I	pET
20	RO60_SCT	P10155-1	–	I	pET
21	RPL7A_SCT	P62424	–	I	pET
22	TKT_SCT	P29401-1	–	I	pET
23	TPI1_Native	P60174-1	–	S	pET
24	TPI1_SCT	P60174-1	–	I	pET
25	Vim_SCT	P08670	–	I	pET
26	Wdr36_SCT	Q8NI36	–	I	CMV

27	TPO_Native	P07202	–	S	CMV
28	TG_Native	P01266	–	S	CMV
29	AFP_Native	P02771 (19 - 609 aa)	–	S	CMV
30	AFP_SCT	P02771 (19 - 609 aa)	–	S	CMV
31	CA125_Native	Q8WXI7 (13360 - 14347 aa)	–	S	CMV
32	CA125_SCT	Q8WXI7 (13360 - 14347 aa)	–	S	CMV
33	CA19-9_Native	Q969X2-1 (65 - 333 aa)	–	S	CMV
34	CA19-9_SCT	Q969X2-1 (65 - 333 aa)	–	I	pET
35	CEA_Native	P06731-1 (35 - 685 aa)	–	S	CMV
36	CEA_SCT	P06731-1 (35 - 685 aa)	–	S	CMV
37	CGB3_SCT	P0DN86	–	I	pET
38	CYFRA_Intact	P08727	–	I	pET
39	Elastase-1_SCT	Q9UNI1	–	I	pET
40	HE4_Native	Q14508	–	S	pET
41	PSA_SCT	P55786	–	I	pET
42	SCC_Native	P29508-1	–	S	CMV
43	ALK.cd_SCT	Q9UM73 (1060 - 1620 aa)	–	I	pET
44	ANXA1_Native	P04083	–	S	pET
45	ANXA1_SCT	P04083	–	I	pET
46	CCNB1_SCT	P14635-1	–	I	pET
47	CEACAM19_Native	Q7Z692-1 (33 - 300 aa)	–	S	CMV

48	CEACAM19_SCT	Q7Z692-1 (33 - 300 aa)	–	I	pET
49	CEACAM4_SCT	O75871 (36 - 244 aa)	–	I	pET
50	EMD_SCT	P50402	–	I	pET
51	ENO1_SCT	P06733-1	–	I	pET
52	EZR_Native	P15311	–	S	pET
53	EZR_SCT	P15311	–	I	pET
54	gp100_SCT	P40967-1 (25 - 588 aa)	–	I	pET
55	Her2-ECD_Native	P04626-1 (1 - 652 aa)	–	S	CMV
56	HSP105_SCT	Q92598-1	–	I	pET
57	HSP20_SCT	O14558	–	I	pET
58	HSP27_SCT	P04792	–	I	pET
59	HSPD1_SCT	P10809	–	S	pET
60	MSLN_Native	Q13421-4 (298 - 580 aa)	–	S	CMV
61	NPM1_Native	P06748-1	–	S	pET
62	NPM1_SCT	P06748-1	–	I	pET
63	p53_SCT	P04637-1	–	I	pET
64	PDZD11_Native	Q5EBL8	–	S	pET
65	PPIB_Native	P23284 (34 - 216 aa)	–	S	pET
66	PPP1CA_SCT	P62136-1	–	I	pET
67	PSG5_SCT	Q15238 (35 - 335 aa)	–	I	pET
68	PSG8_Native	Q9UQ74-1 (35 - 426 aa)	–	S	CMV
69	PSG8_SCT	Q9UQ74-1 (35 - 426 aa)	–	I	pET

70	RalA_SCT	P11233 (1 - 203 aa)	–	I	pET
71	RhoGDI_Native	P52565-1	–	S	pET
72	RhoGDI_SCT	P52565-1	–	I	pET
73	SOX2_SCT	P48431	–	I	pET
74	Survivin2b_SCT	O15392-2	–	I	pET
75	TCP1_SCT	P17987	–	I	pET
76	WT-1_SCT	P19544-7 (7 - 522 aa)	–	I	pET
77	ACRBP_SCT	Q8NEB7	23	I	pET
78	ACTL8_SCT	Q9H568	57	I	pET
79	BAGE_SCT	Q86Y29 (18 - 109 aa)	2.3	I	pET
80	BORIS_SCT	Q8NI51-1	27	I	CMV
81	CABYR_Native	O75952-1	88	S	pET
82	CCDC36_SCT	Q8IYA8-1 (11 - 594 aa)	74	I	pET
83	CCDC62_SCT	Q6P9F0-1	109	I	pET
84	CEP55_SCT	Q53EZ4-1	111	I	pET
85	CSAG2_SCT	Q9Y5P2-1	24.2	I	pET
86	DNAJB8_SCT	Q8NHS0	156	I	pET
87	CT45A1_SCT	Q5HYN5	45-1	I	pET
88	Cxorf61_SCT	Q5H943	83	I	pET
89	DCAF12_SCT	Q5T6F0	102	I	pET
90	DDX53_SCT	Q86TM3	26	I	pET
91	DPPA2_SCT	Q7Z7J5	100	I	pET
92	FTHL17_SCT	Q9BXU8	38	I	pET
93	HORMAD1_Native	Q86X24-1	46	S	pET
94	HORMAD1_SCT	Q86X24-1	46	I	pET
95	HSPB9_SCT	Q9BQS6	51	I	pET
96	LUZP4_SCT	Q9P127-1	28	I	pET

97	LY6K_SCT	Q17RY6-1 (18 - 138 aa)	97	I	pET
98	MAEL_SCT	Q96JY0-1	128	I	pET
99	MAGE-A1_SCT	P43355	1.1	I	pET
100	MAGE-A3_Native	P43357	1.3	S	pET
101	MAGE-A4_Native	P43358	1.4	S	pET
102	MAGE-A6_Native	P43360	1.6	S	pET
103	MAGE-B3_SCT	O15480	3.5	I	pET
104	MAGE-C1_Native	O60732-2	7.1	S	pET
105	MAGE-C2_Native	Q9UBF1	10	S	pET
106	MORC1_SCT	Q86VD1-1	33	I	CMV
107	NUF2_SCT	Q9BZD4	106	I	pET
108	NXF2_SCT	Q9GZY0	39	I	pET
109	NY-ESO-1_SCT	P78358-1	6.1	I	pET
110	OIP5_SCT	O43482	86	I	pET
111	PAGE5_Native	Q96GU1-1	16.1	S	pET
112	PBK_SCT	Q96KB5-1	84	I	pET
113	PLAC1_SCT	Q9HBJ0 (23 - 212 aa)	92	I	pET
114	SEMG1_Native	P04279-1	103	S	CMV
115	SEMG1_SCT	P04279-1	103	I	pET
116	SPA17_Native	Q15506	22	S	pET
117	SPEF2(2)_SCT	Q9C093-4	122	I	CMV
118	SPNAXA_Native	Q9NS26	11.1	S	pET
119	SPNAXA_SCT	Q9NS26	11.1	I	pET
120	SPANXD_SCT	Q9BXN6	11.4	I	CMV
121	SSX2_SCT	Q16385-1	5.2	I	pET
122	SSX4_SCT	O60224-1	5.4	I	pET
123	SYCE1_SCT	Q8N0S2-2	76	I	pET
124	SYCP1_SCT	Q15431	8	I	CMV
125	TEKT5_SCT	Q96M29	149	I	pET

126	TFDP3_SCT	Q5H9I0	30	I	CMV
127	TSSK6_SCT	Q9BXA6	72	I	pET
128	XAGE-1b_SCT	Q9HD64-2	12.1	I	pET
129	XAGE2_Native	Q96GT9	12.2	S	pET
130	ZNF165_SCT	P49910	53	I	pET

Table S1. Antigen list for autoantigen test panel (130 antigens)

Antigens labeled _Native were purified by immobilized metal affinity chromatography from the soluble fraction of cell lysates or secreted proteins in medium. Antigens labeled _SCT were solubilized antigens by S-cationization techniques from the insoluble fraction followed by reverse phase HPLC purification. Antigens labeled _Intact were refolded from the insoluble fraction without chemical modification. CT No. indicates designated number in the cancer-testis antigen database (http://www.cta.lncc.br/). S and I in Expression column indicate soluble (S) and insoluble (I) in recombinant protein expression.

Fig S1

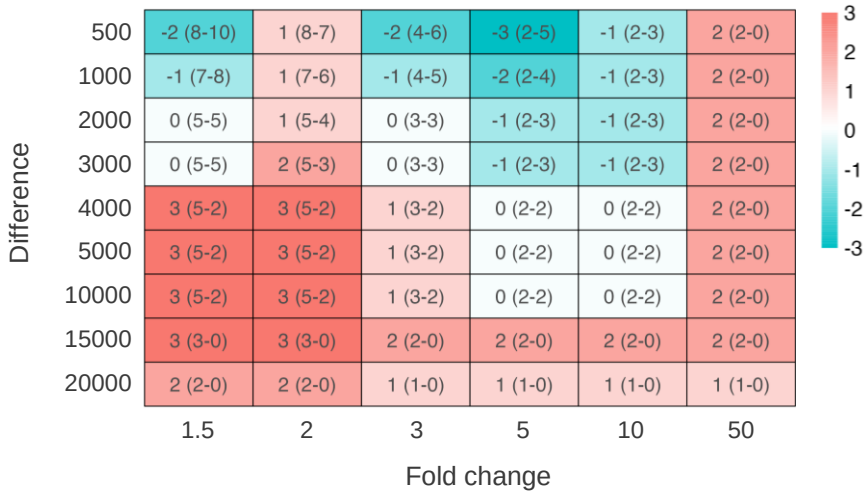
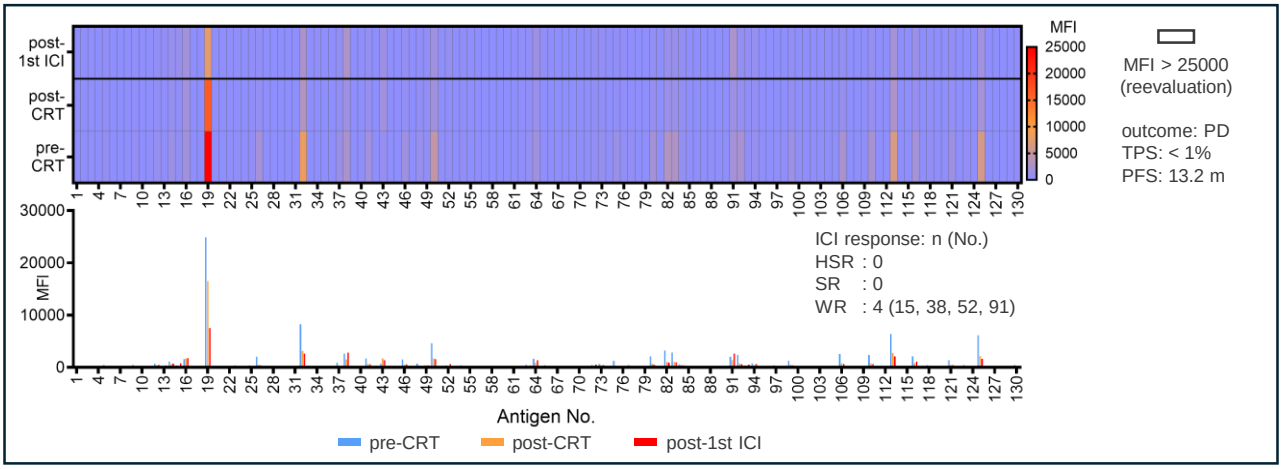


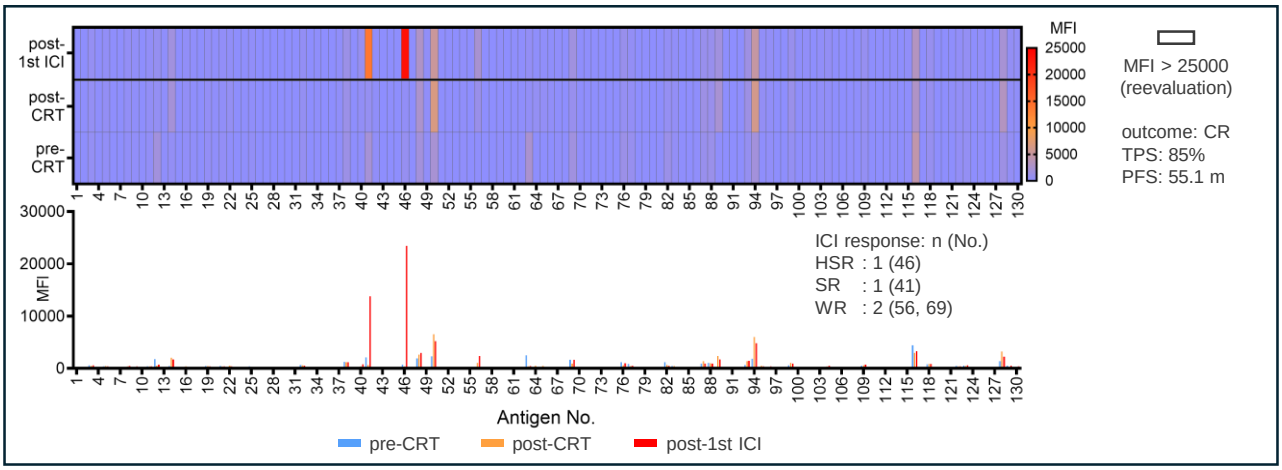
Figure S1
Categories of various cutoff combinations based on fold changes and differences in MFIs for autoantibodies. The number in the panel indicates the number of patients exhibiting values above both cutoff thresholds for the MFI difference and fold changes (the number of patients indicates CR - PD).

Fig S2 A (1/5)

L-01



L-02



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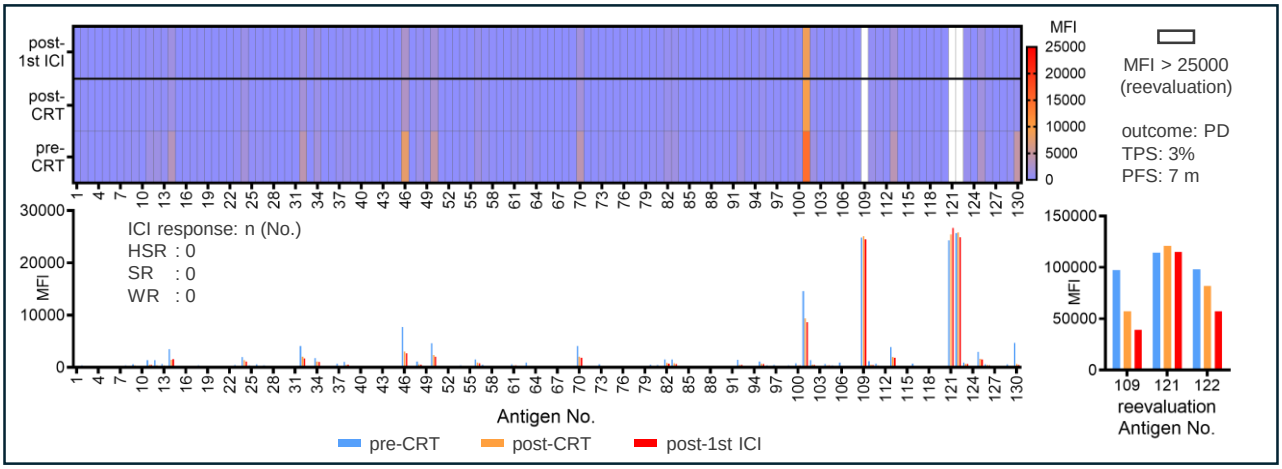

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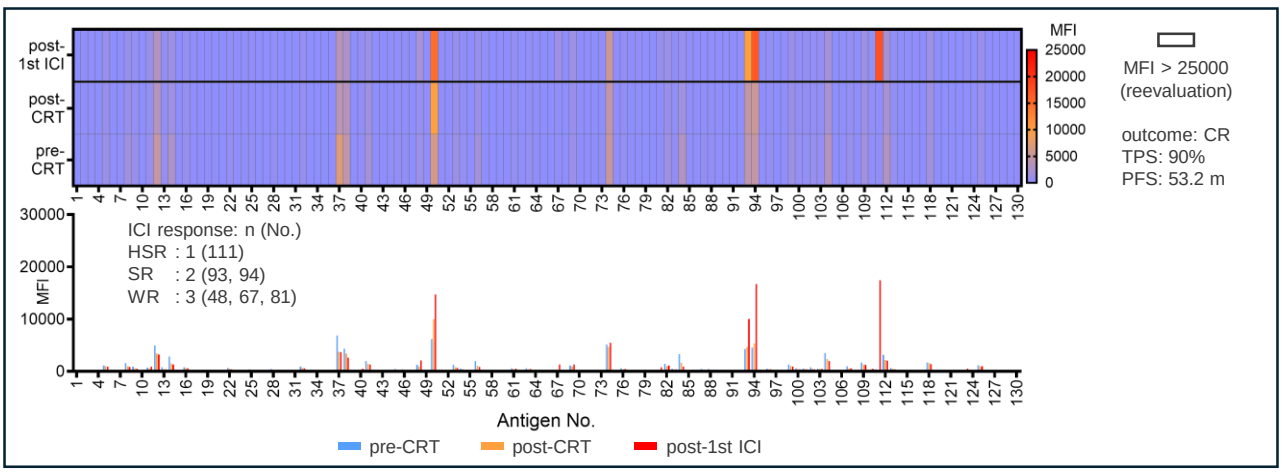
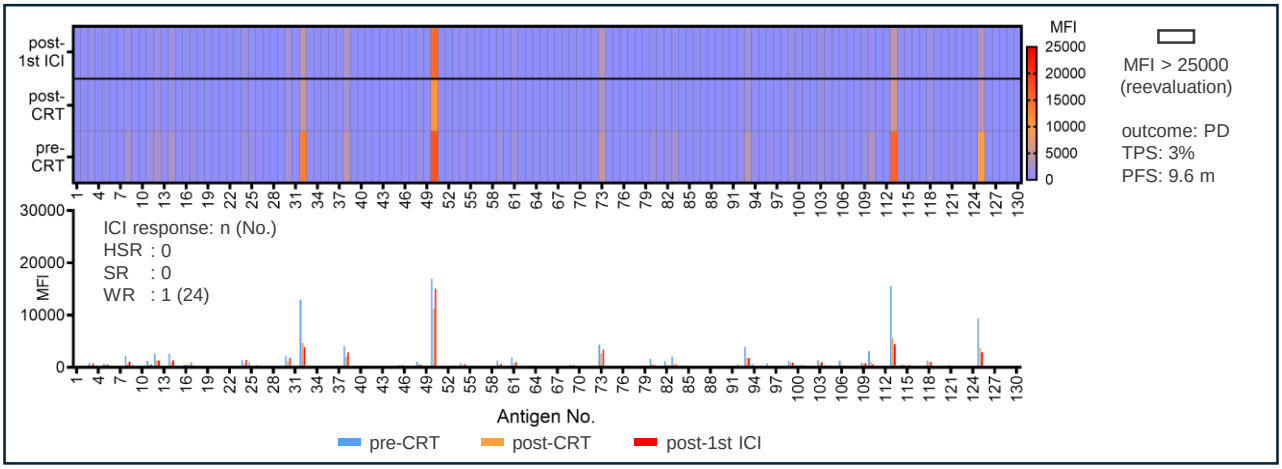


Fig S2 A (2/5)

L-06



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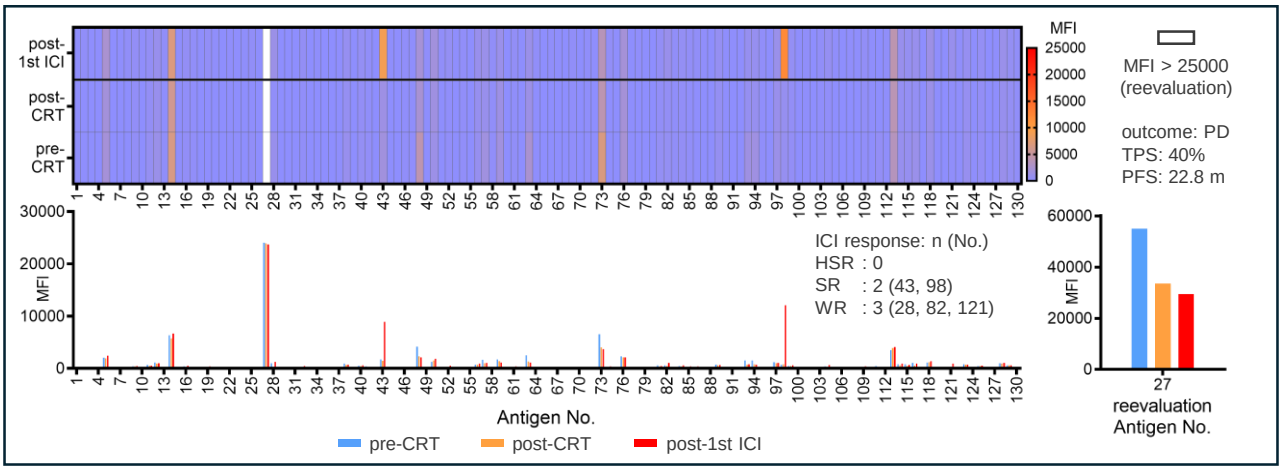

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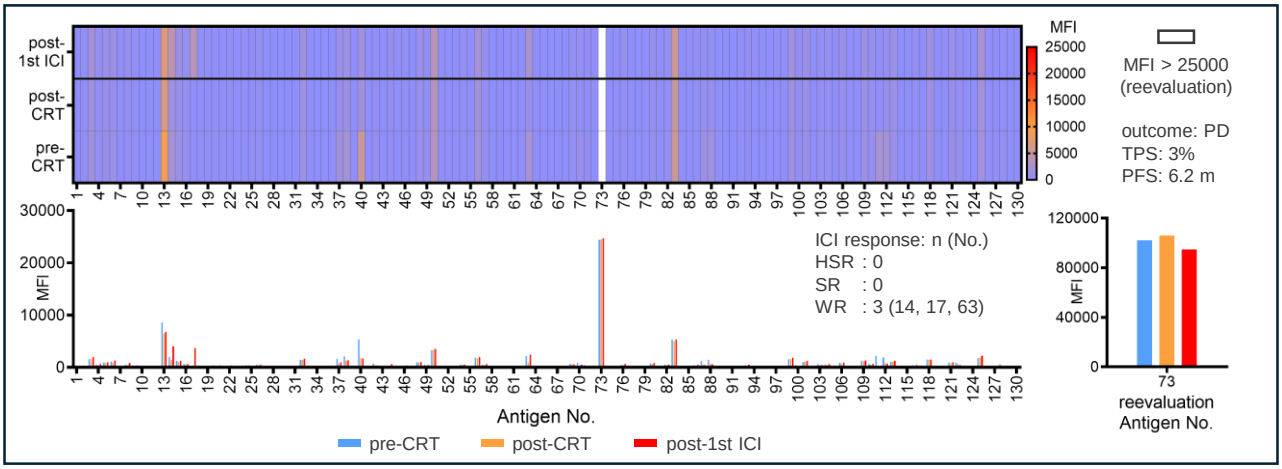

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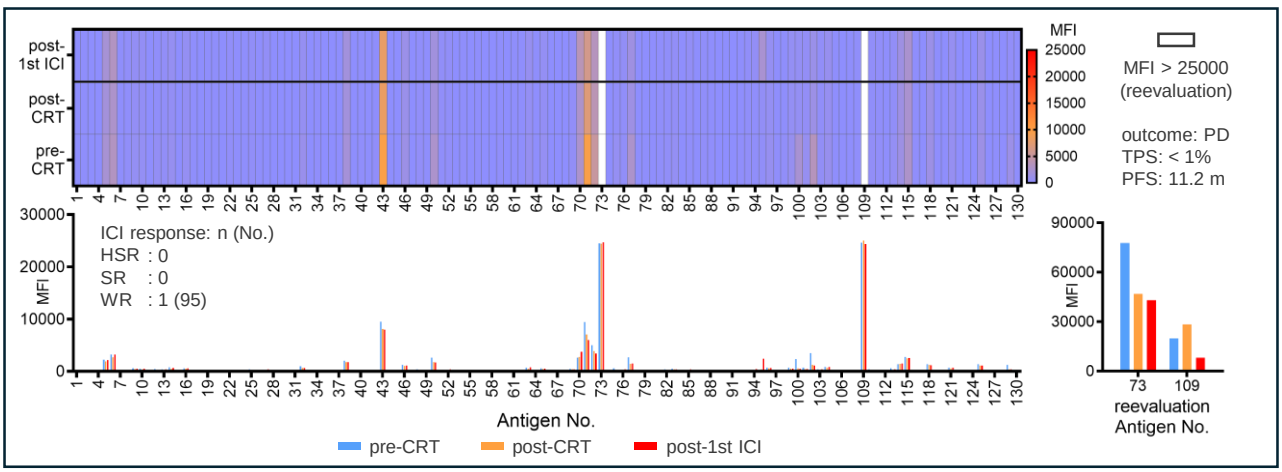
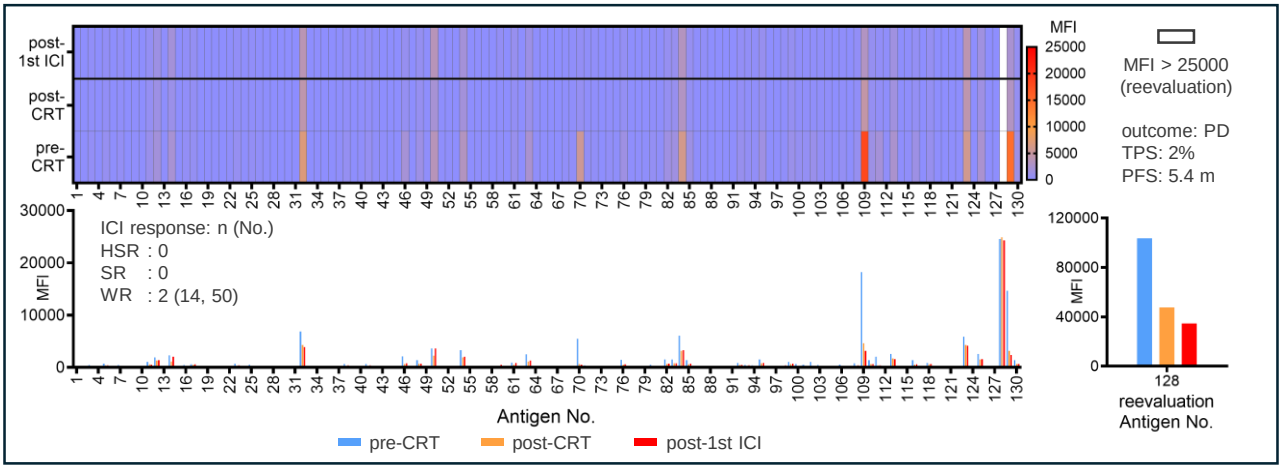


Fig S2 A (3/5)

L-13



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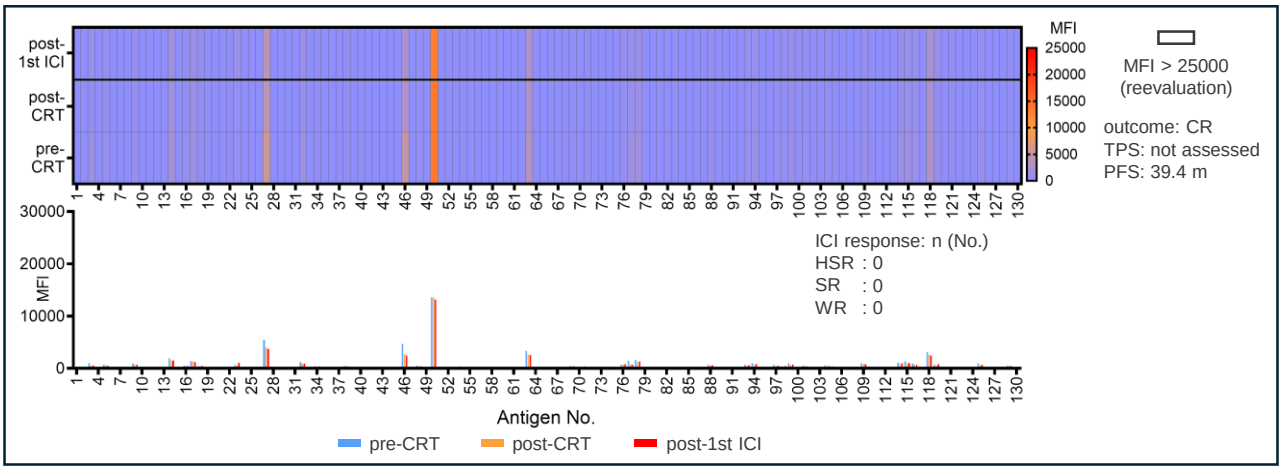

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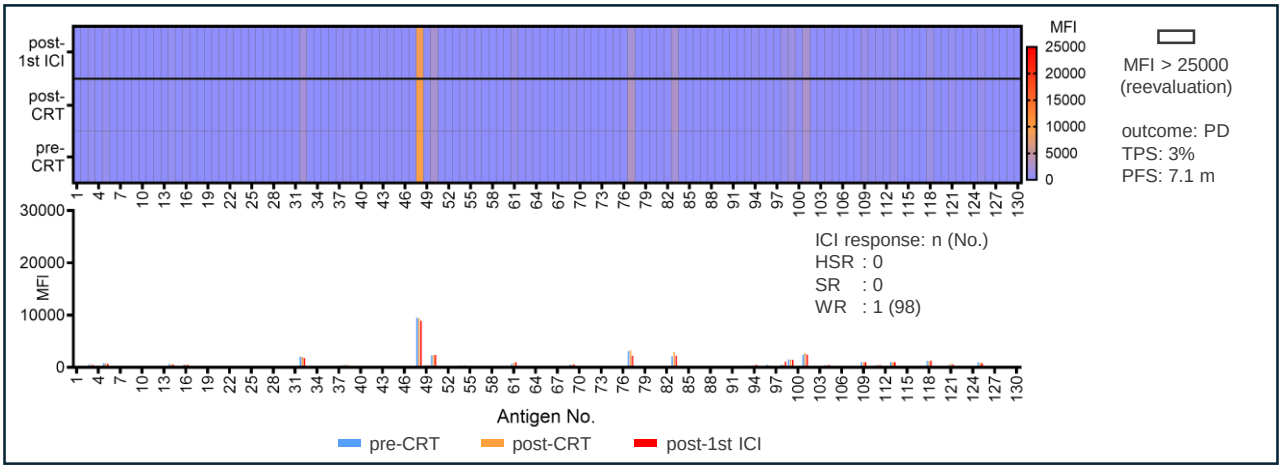


L-18

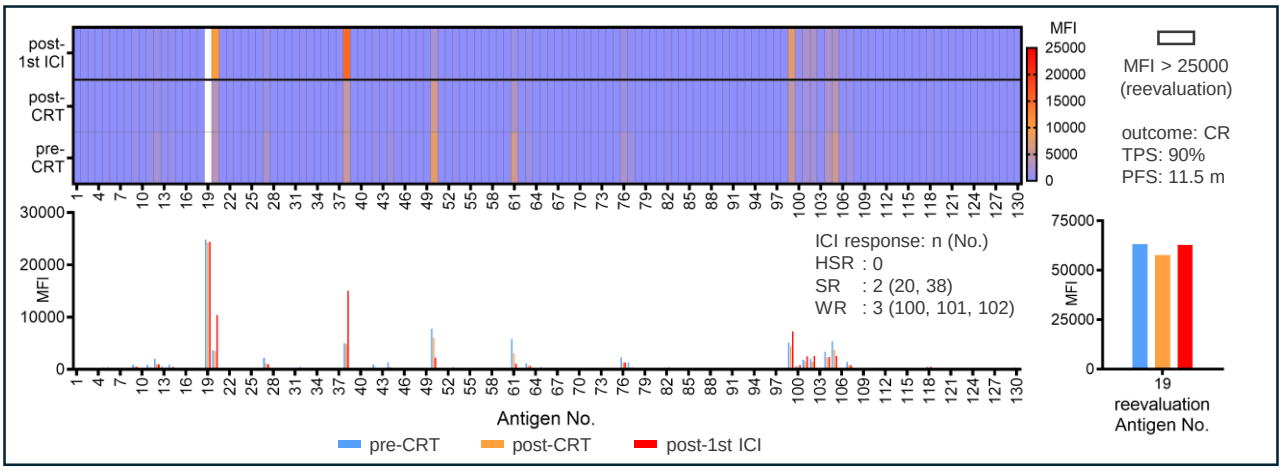


Fig S2 A (4/5)

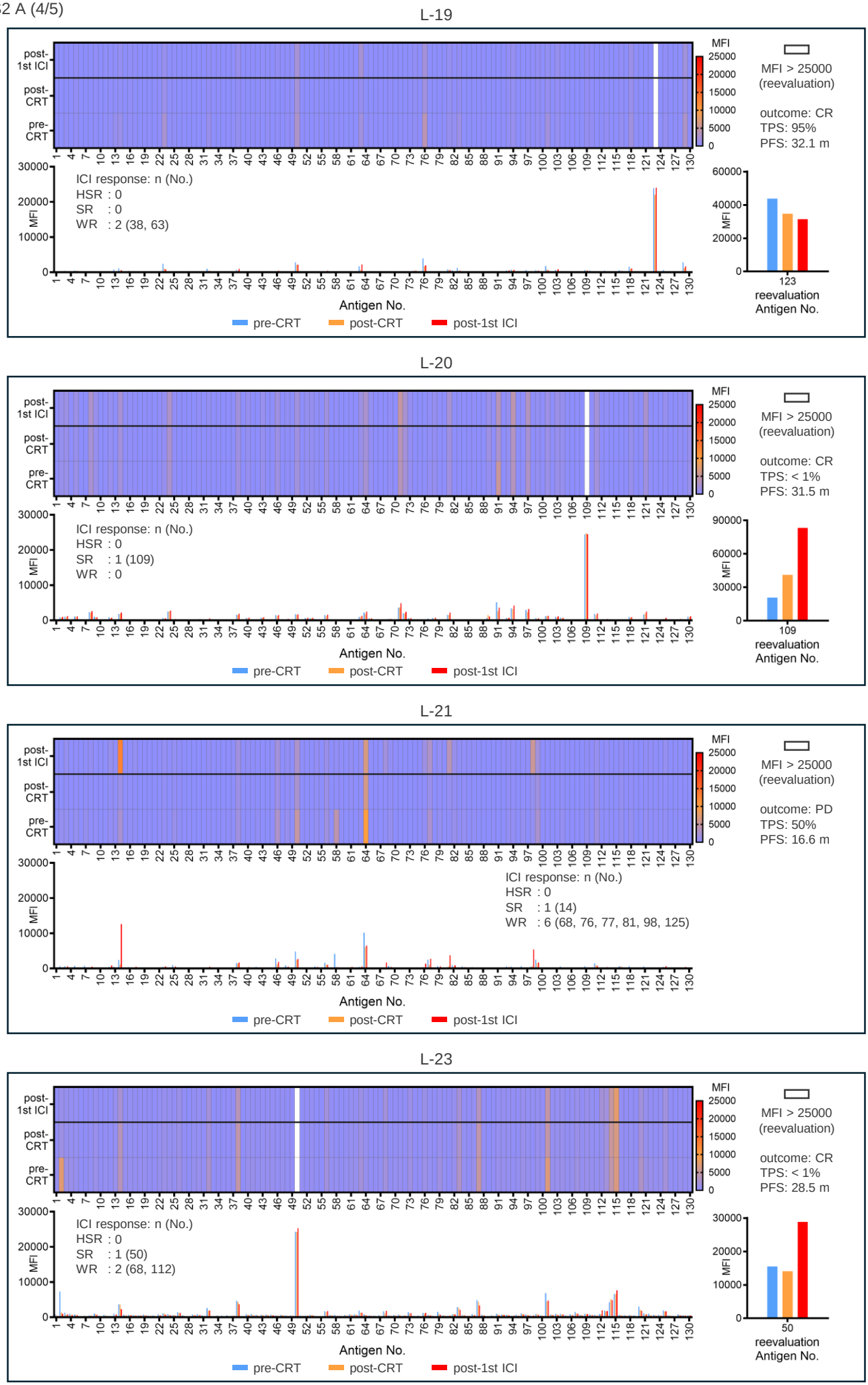
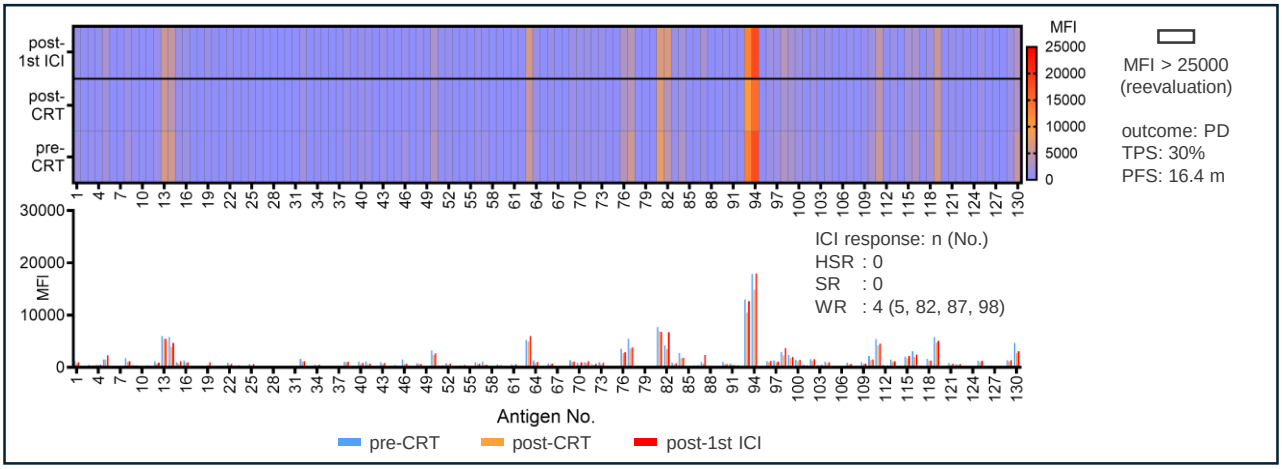
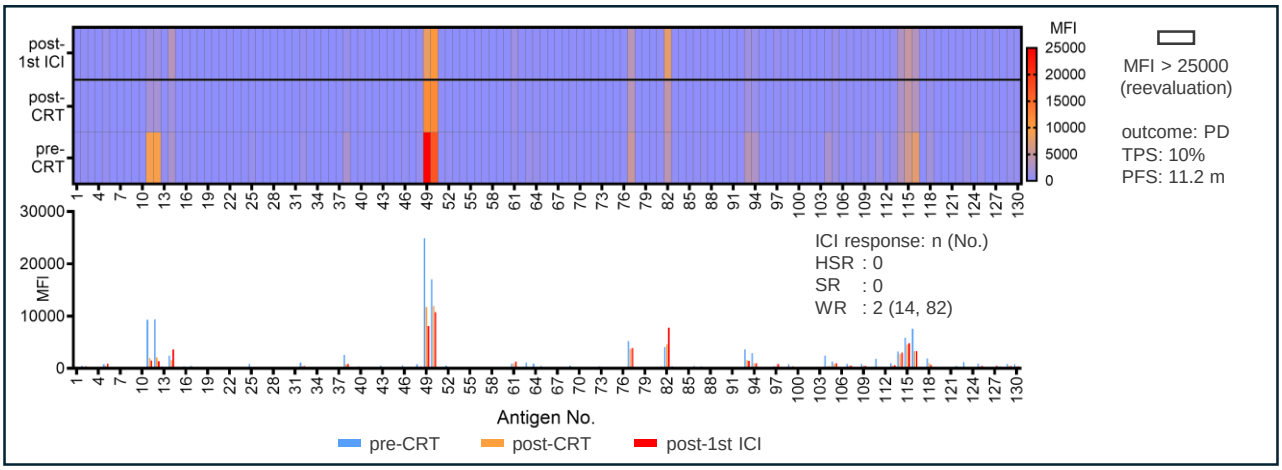

L-19
post-1st ICI
post-CRT
pre-CRT
MFI
25000
20000
15000
10000
5000
0
MFI > 25000 (reevaluation)
outcome: CR
TPS: 95%
PFS: 32.1 m
ICI response: n (No.)
HSR : 0
SR : 0
WR : 2 (38, 63)
60000
40000
20000
0
123
reevaluation Antigen No.
Antigen No.
pre-CRT
post-CRT
post-1st ICI
L-20
MFI > 25000 (reevaluation)
outcome: CR
TPS: < 1%
PFS: 31.5 m
ICI response: n (No.)
HSR : 0
SR : 1 (109)
WR : 0
90000
60000
30000
0
109
reevaluation Antigen No.
L-21
MFI > 25000 (reevaluation)
outcome: PD
TPS: 50%
PFS: 16.6 m
ICI response: n (No.)
HSR : 0
SR : 1 (14)
WR : 6 (68, 76, 77, 81, 98, 125)
L-23
MFI > 25000 (reevaluation)
outcome: CR
TPS: < 1%
PFS: 28.5 m
ICI response: n (No.)
HSR : 0
SR : 1 (50)
WR : 2 (68, 112)
50
reevaluation Antigen No.

Fig S2 A (5/5)

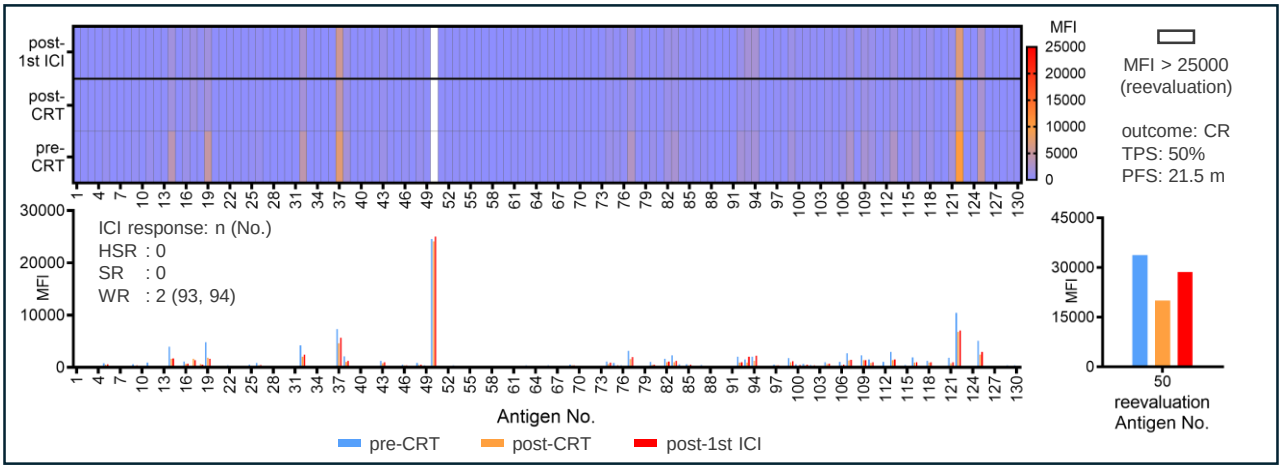
L-24



L-26



L-27



L-30

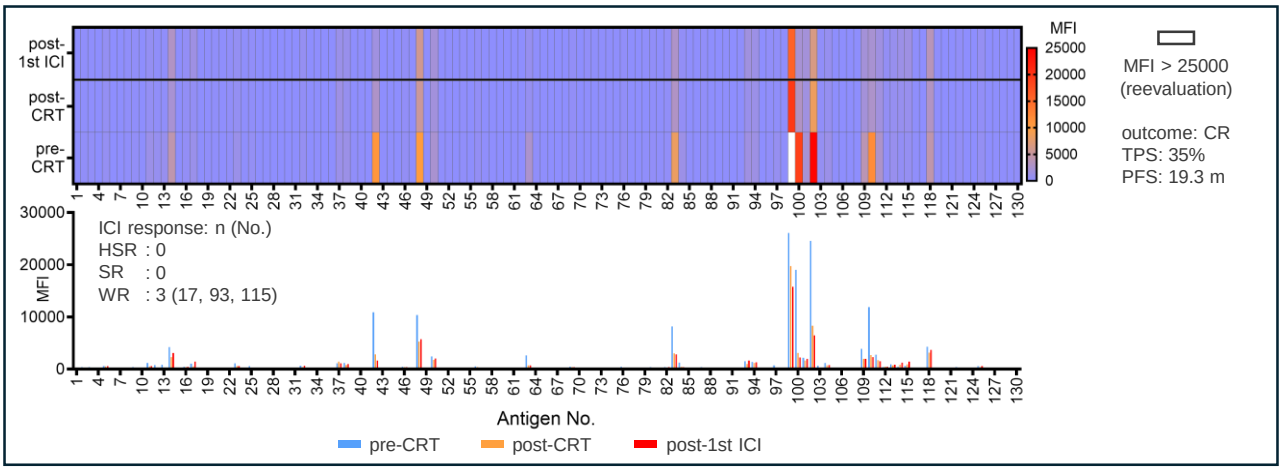


Fig S2 B (1/2)

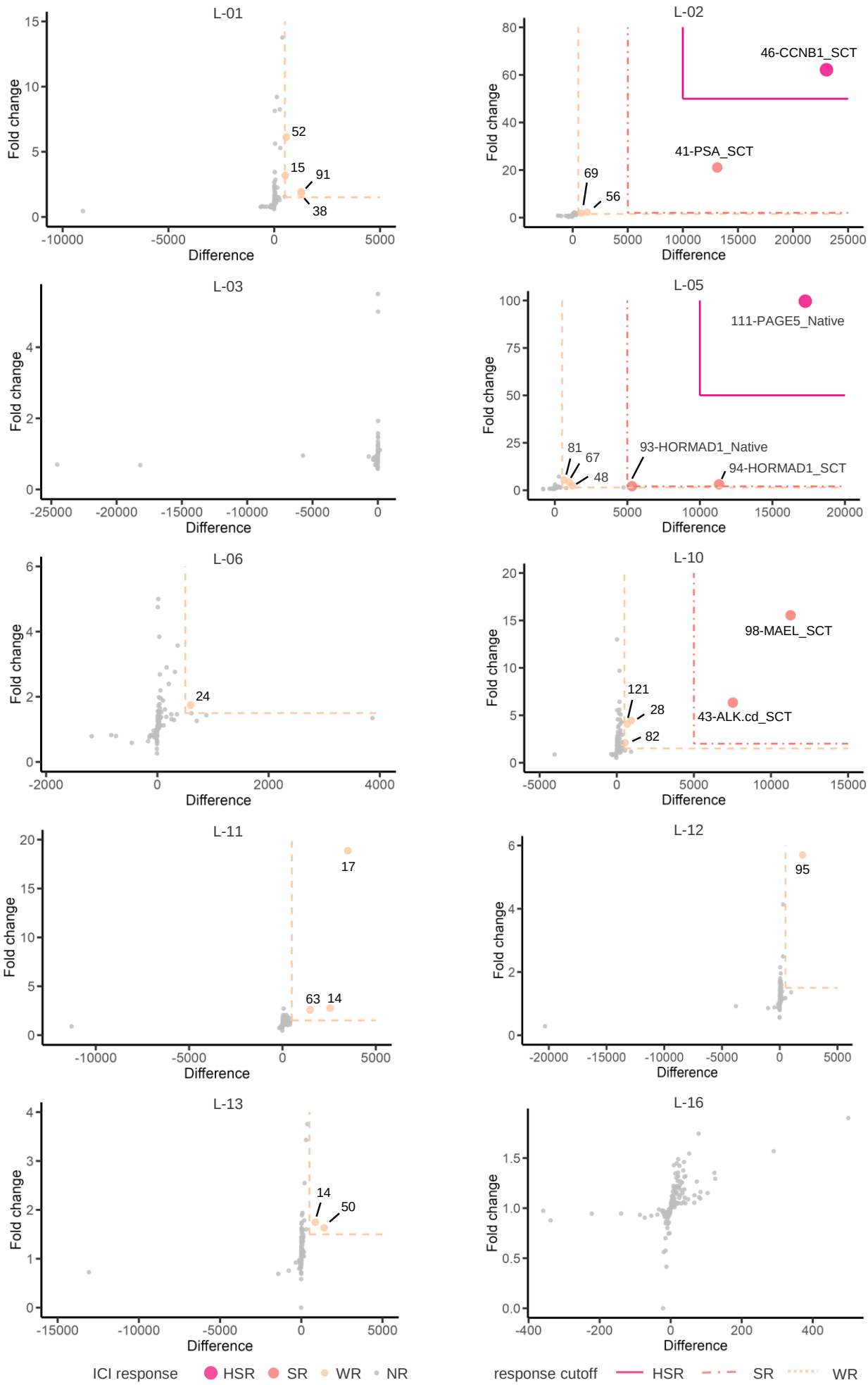

L-01
Fold change
Difference
52
15
91
38
L-02
46-CCNB1_SCT
41-PSA_SCT
69
56
L-03
L-05
111-PAGE5_Native
81
67
48
93-HORMAD1_Native
94-HORMAD1_SCT
L-06
24
L-10
98-MAEL_SCT
121
28
82
43-ALK.cd_SCT
L-11
17
63
14
L-12
95
L-13
14
50
L-16
ICI response
HSR
SR
WR
NR
response cutoff
HSR
SR
WR

Fig S2 B (2/2)

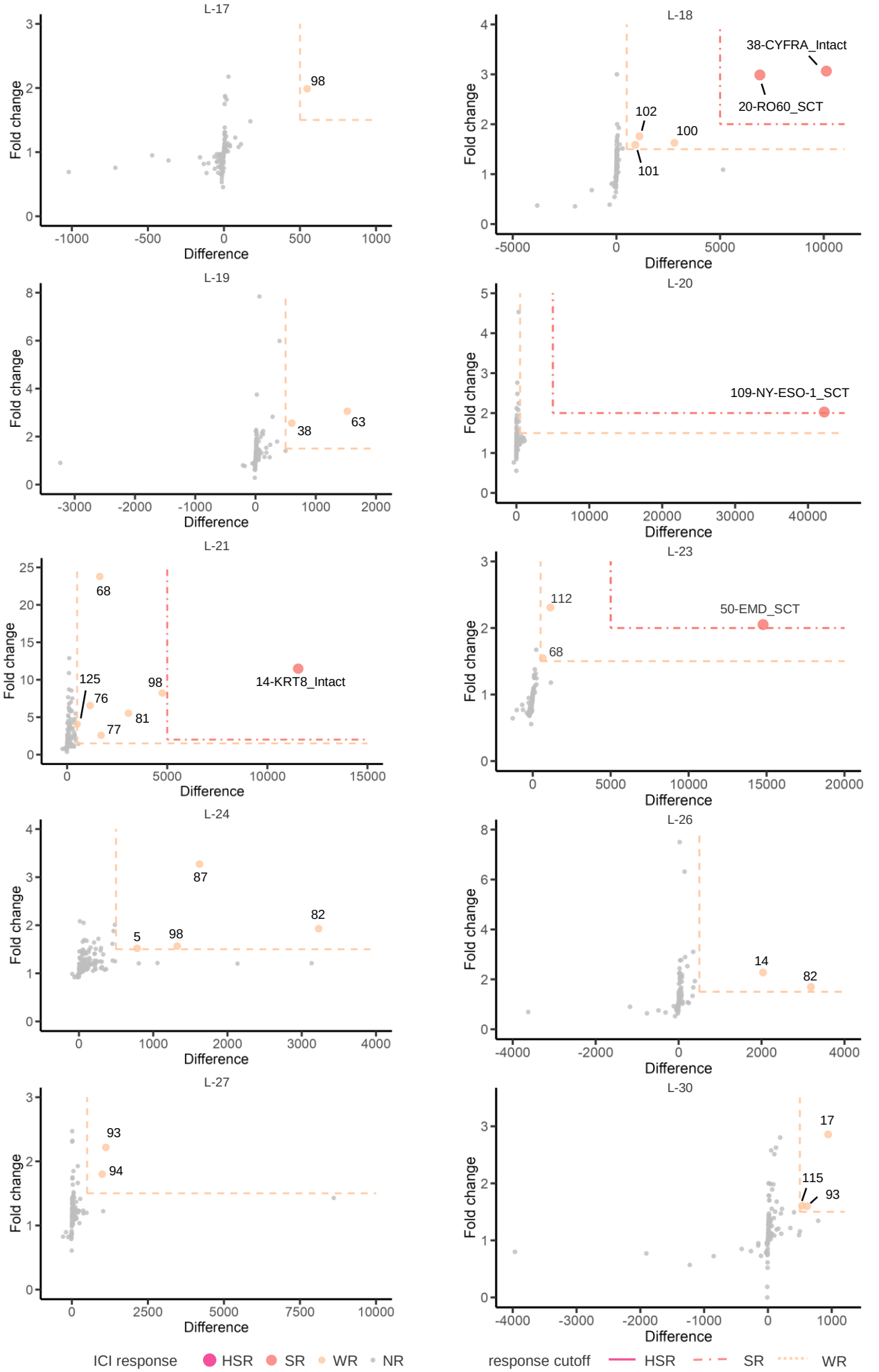

L-17
Fold change
Difference
98
L-18
38-CYFRA_Intact
20-RO60_SCT
102
100
101
L-19
38
63
L-20
109-NY-ESO-1_SCT
L-21
68
125
76
98
81
77
14-KRT8_Intact
L-23
112
68
50-EMD_SCT
L-24
87
82
5
98
L-26
14
82
L-27
93
94
L-30
17
115
93
ICI response
HSR
SR
WR
NR
response cutoff
HSR
SR
WR

Fig S3

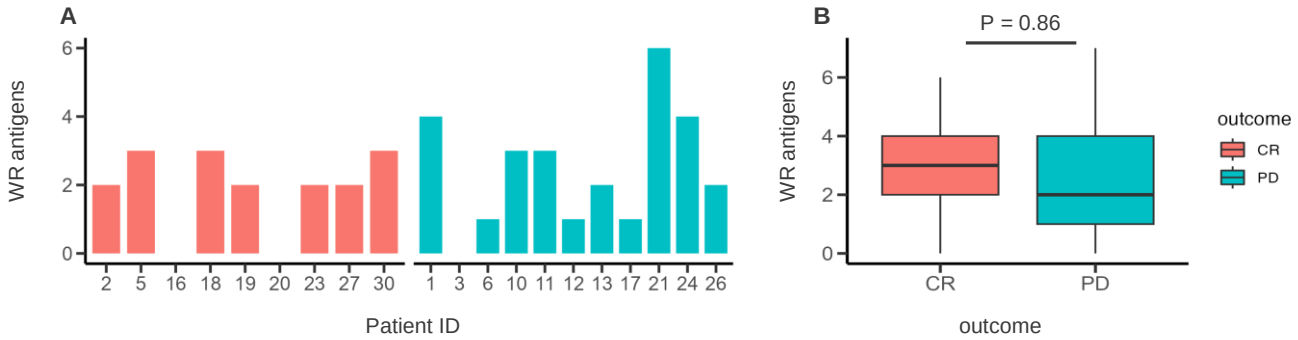


Figure S3

(A)The number of autoantibody immune responses in the category of WR in each patient. (B) The total number of distributed antigens showed WR in CR or PD.

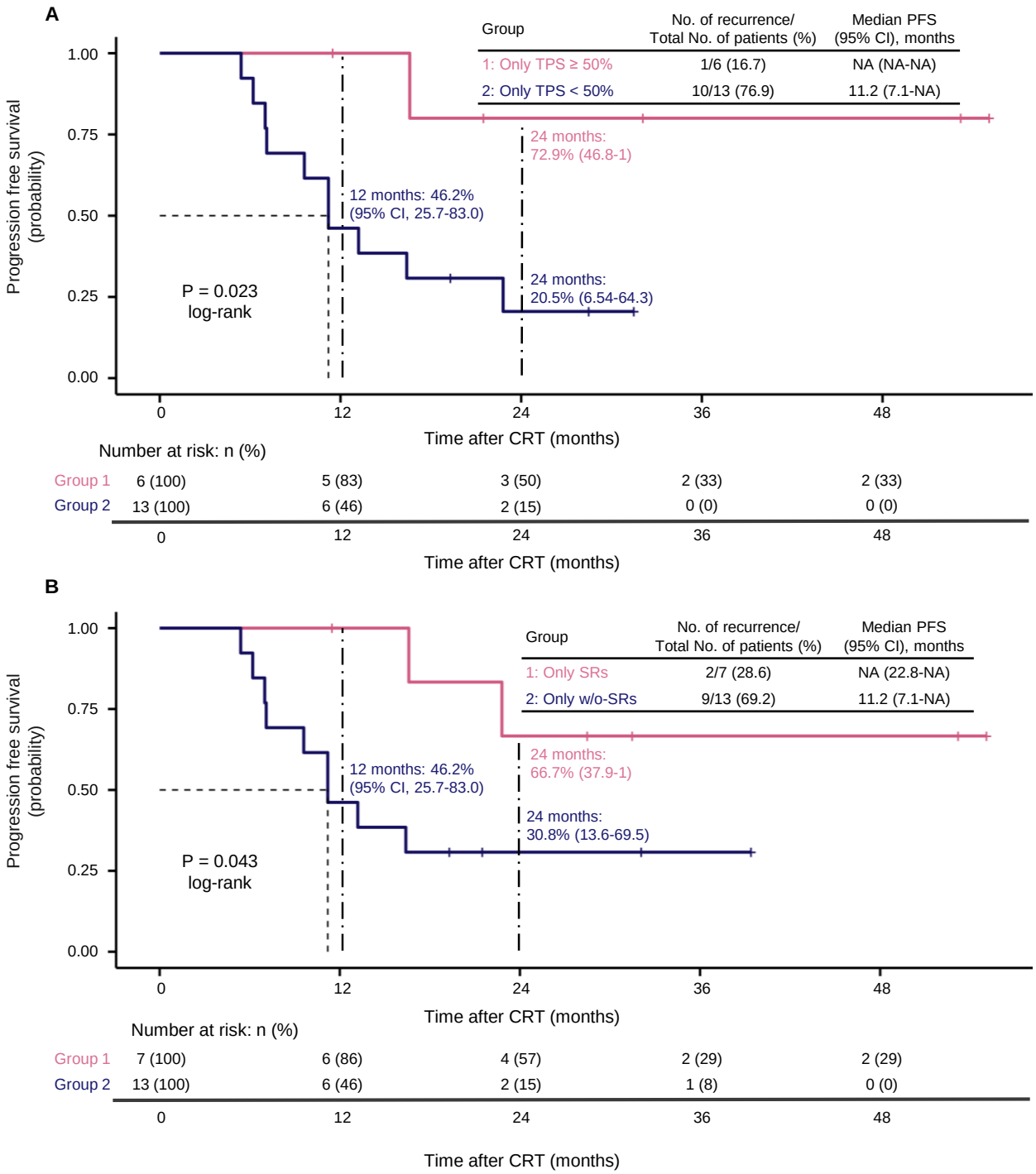


Figure S4
(Kaplan–Meier plots illustrating PFS, TPS, and autoantibody response were analyzed. (A) The relationship between PFS and TPS, determined by tumor biopsy from pre-CRT tumors, was evaluated. (B) The correlation between PFS and autoantibody response, assessed by liquid biopsy two weeks after the first injection of durvalumab, was explored.