Supplemental material

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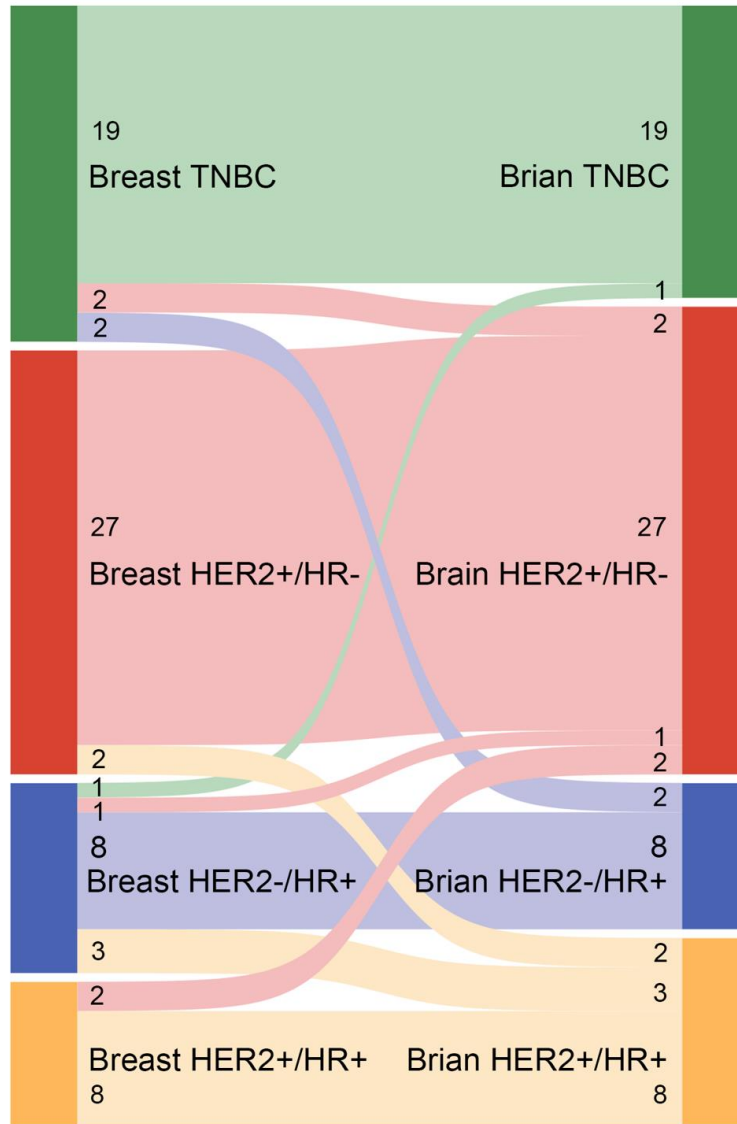
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Supplementary Table S1

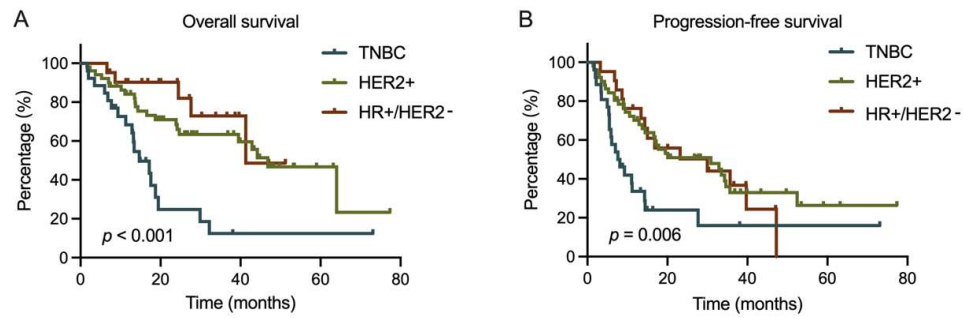Antibodies used in panel 1 of Figure 5 and panel 2 of Supplementary Figure S3

Panel	Cycle	Marker	Conjugate	Vendor	Catalog	Dilution
1	1	Autofluorescence	AF488	None	None	None
1	1	Autofluorescence	AF555	None	None	None
1	1	TIM-3	None	Abcam	ab241332	1/100
1	1	Goat anti-rabbit IgG	AF647	Abcam	ab150083	1/200
1	2	CD163	AF488	Abcam	ab218293	1/100
1	2	CD68	AF555	CST	79594	1/100
1	2	CD20	AF647	eBioscience	50-0202-80	1/100
1	3	CD3D	AF555	Abcam	ab208514	1/100
1	3	CD8a	AF647	eBioscience	50-0008-82	1/100
1	4	PD-L1	AF555	Abcam	ab213358	1/50
1	4	PD-1	AF647	Abcam	ab201825	1/100
1	5	pan-CK	AF555	CST	3478	1/200
1	5	Foxp3	AF647	BioLegend	320113	1/50
2	1	Cleaved Caspase-3	None	CST	9661	1/100
2	1	BCL-6	None	GeneAb	IHC029-100	1/100
2	1	Goat anti-mosue IgG	AF555	Abcam	ab150118	1/200
2	1	Goat anti-rabbit IgG	AF647	Abcam	ab150083	1/200
2	2	CD4	AF488	Abcam	ab196372	1/100
2	2	TCF1/TCF7	AF555	CST	17404	1/100
2	2	CXCR5	AF647	Abcam	ab316179	1/100
2	3	HLA-DR	AF488	BioLegend	307619	1/100
2	3	CD8a	AF647	eBioscience	50-0008-82	1/100
2	4	Ki-67	AF488	CST	11882	1/100
2	4	pan-CK	AF555	CST	3478	1/200
2	4	HLA-A	AF647	Abcam	ab199837	1/200
2	5	CD20	AF647	eBioscience	50-0202-80	1/100
2	5	CD57	AF555	BioLegend	393307	1/100

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The code used for the analyses of the nomogram in Figure 4 is shared in the
link below.

https://gitee.com/tao-taos-warehouse/nomo.-r-score-sur.-r.git

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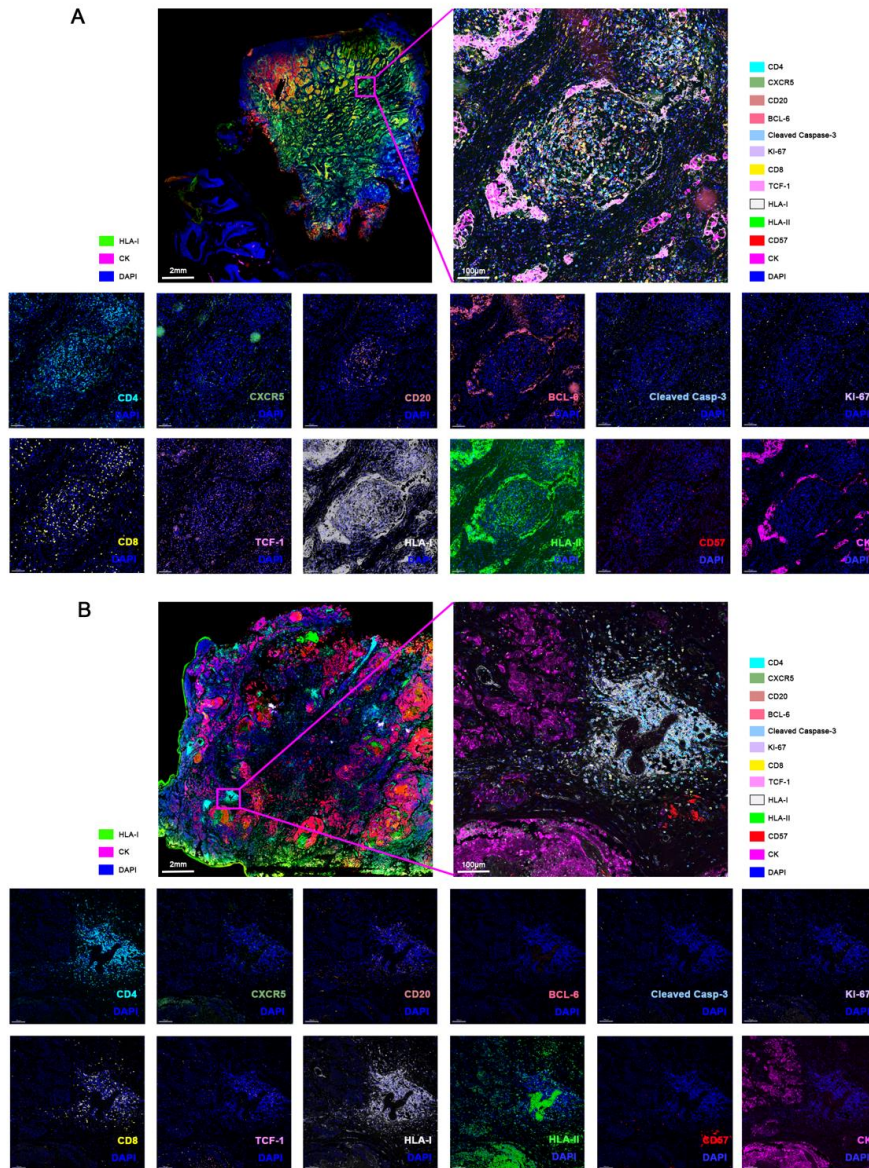
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Supplementary Figure S1: Subtype switching. Width of the bands indicates number of patients crossing over. + = positive; − = negative; HR = hormonal receptor; HER2 = human epidermal growth factor receptor 2.

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Supplementary Figure S2: Kaplan-Meier curves for overall survival (A) and progression-free survival (B) according to breast subtype. + = positive; − = negative; HR = hormonal receptor; HER2 = human epidermal growth factor receptor 2.

Supplementary Figure S3: Representative multiplex immunofluorescence images of case 1 (A) and case 2 (B) showing the positivity of CD4, CXCR5, CD20, BCL-6, Cleaved Caspase-3, Ki-67, CD8, TCF-1, HLA-I, HLA-II, CD57, and CK in intra-tumoral TLSs. Abbreviations: CD, cluster of differentiation; CXCR5, C-X-C motif chemokine receptor 5; BCL-6, BCL6 transcription repressor; HLA, histocompatibility antigen; TCF-1, T cell factor 1; CK, cytokeratin; DAPI, 4′,6-diamidino-2-phenylindole.

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Supplementary Table S2 The univariate analysis and multivariate analysis between clinicopathological characteristics and OS

Variables		Univariate analysis		Multivariate analysis	
		HR[a] （95% CI）	*p* value	HR （95% CI）	*p* value
age at time of BCBM （years）	<65	0.443 （0.183-1.072）	0.071	0.335 （0.121-0.930）	0.036
	≥65	Reference	-	Reference	-
Breast cancer subtype of BrM	TNBC	4.848 （1.781-13.193）	0.002	4.440 （1.505-13.100）	0.007
	HER2+	1.648 （0.621-4.374）	0.315	2.488 （0.872-7.101）	0.088
	HR[b]+/HER2−	Reference	-	Reference	-
TLS score	0	4.414 （1.510-12.902）	0.007	3.249 （1.050-10.055）	0.041
	1	2.195 （0.636-7.572）	0.213	1.072 （0.288-3.997）	0.917
	2	1.270 （0.357-4.522）	0.712	0.529 （0.136-2.062）	0.359
	3	Reference	-	Reference	-
KPS	90	0.342 （0.127-0.926）	0.035	0.226 （0.071-0.722）	0.012
	70-80	1.133 （0.495-2.595）	0.768	0.980 （0.380-2.529）	0.967
	60 or less	Reference	-	Reference	-
De novo Stage IV breast cancer	yes	0.301 （0.156-0.578）	<0.001	0.283 （0.129-0.620）	0.002
	no	Reference	-	Reference	-
Systemic therapy after BrM diagnosis	yes	1.994 （0.911-4.364）	0.084	2.366 （0.960-5.831）	0.061
	no	Reference	-	Reference	-
Radiotherapy after BrM diagnosis	yes	1.224 （0.597-2.512）	0.581	1.185 （0.490-2.867）	0.707
	no	Reference	-	Reference	-

OS = overall survival; HR[a] = hazard ratio; CI = confidence interval; HR[b] = hormone receptor; HER2 = epidermal growth factor receptor 2; TNBC: triple-negative breast cancer; KPS =

Karnofsky performance status; TLS = tertiary lymphoid structure; BCBM = breast cancer brain metastasis; BrM = brain metastasis.

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Supplementary Table S3 The univariate analysis and multivariate analysis between clinicopathological characteristics and PFS

Variables		Univariate analysis		Multivariate analysis	
		HR[a]（95% CI）	*p* value	HR（95% CI）	*p* value
age at time of BCBM（years）	<65	0.576（0.271-1.225）	0.152	0.580（0.250-1.345）	0.204
	≥65	Reference	-	Reference	-
Breast cancer subtype of BrM	TNBC	2.245（1.121-4.495）	0.022	2.341（1.079-5.080）	0.031
	HER2+	0.938（0.498-1.766）	0.842	1.561（0.766-3.182）	0.220
	HR[b]+/HER2−	Reference	-	Reference	-
TLS score	0	5.190（2.010-13.397）	0.001	4.268（1.595-11.417）	0.004
	1	3.594（1.276-10.121）	0.015	2.517（0.859-7.379）	0.093
	2	1.893（0.631-5.678）	0.255	1.003（0.317-3.177）	0.996
	3	Reference	-	Reference	-
KPS	90	0.324（0.157-0.669）	0.002	0.233（0.101-0.535）	0.001
	70-80	0.678（0.363-1.265）	0.222	0.622（0.321-1.204）	0.159
	60 or less	Reference	-	Reference	-
De novo Stage IV breast cancer	yes	0.312（0.172-0.564）	<0.001	0.311（0.156-0.619）	0.001
	no	Reference	-	Reference	-
Systemic therapy after BrM diagnosis	yes	1.625（0.816-3.236）	0.167	1.981（0.911-4.310）	0.085
	no	Reference	-	Reference	-
Radiotherapy after BrM diagnosis	yes	0.787（0.416-1.486）	0.460	0.594（0.277-1.274）	0.181
	no	Reference	-	Reference	-

PFS = progression-free survival; HR[a] = hazard ratio; CI = confidence interval; HR[b] = hormone receptor; HER2 = epidermal growth factor receptor 2; TNBC: triple-negative breast cancer; KPS = Karnofsky performance status; TLS = tertiary lymphoid structure; BCBM = breast cancer brain metastasis; BrM = brain metastasis.