

1 Supplementary Tables

2 Supplementary table 1. Clinicopathological characteristics of the patients from whom
 3 the 25 metastatic nasopharyngeal carcinoma (MNPC) and 29 non-metastatic
 4 nasopharyngeal carcinoma (NMNPC) paraffin embedded tissue microarrays were
 5 constructed.

	NNPC (n = 29)	MMPC (n = 25)
Characteristics	No. (%)	No. (%)
Age, years		
Median (range)	39 (28-56)	44 (30–69)
< 45	20 (69.0)	13 (52.0)
≥ 45	9 (31.0)	12 (48.0)
Sex		
Female	9 (31.0)	6 (24.0)
Male	20 (69.0)	19 (76.0)
Histological type		
WHO type III	29 (100)	25 (100)
T stage*		
T1–2	9 (31.0)	4 (16.0)
T3–4	20 (69.0)	21 (84.0)
N stage*		
N0–1	16 (55.2)	6 (24.0)
N2–3	13 (44.8)	19 (76.0)
Overall stage*		
II–III	23 (79.3)	14 (56.0)

IV	6 (20·7)	11 (44·0)
Radiotherapy		
IMRT	29 (100)	25 (100)
Chemotherapy		
IC+CCRT	16 (55·2)	11 (44·0)
CCRT	9 (31·0)	9 (36·0)
RT alone/IC+RT/CCRT+AC	4 (13·8)	5 (20·0)

6 WHO, world health organization; TNM, tumor-node-metastasis; IMRT,
7 intensity-modulated radiation therapy; IC, induction chemotherapy; CCRT, concurrent
8 chemoradiotherapy; RT, radiotherapy; AC, adjuvant chemotherapy.

9 * According to the 8th edition of UICC/AJCC staging system.

10

11

12

13

14

15

16

17

18

19

20

21

22

23 Supplementary table 2. List of immunomarkers used for multiOmyx fluorescent multiplex immunohistochemical staining of nasopharyngeal
 24 carcinoma tissue microarrays.

Gene	Clone	Dilution	Antigen Retrieval	Positive Signals	Product code	Source
CD39	Rabbit monoclonal	1:100	EDTA, PH = 9	Membranous	ab223842	Abcam
CD8 α	Mouse monoclonal	1:300	EDTA, PH = 9	Membranous	CST70306	Cell Signaling Technology
PD-1	Mouse monoclonal	1:200	EDTA, PH = 9	Membranous	CST43248	Cell Signaling Technology
TIM-3	Rabbit monoclonal	1:200	EDTA, PH = 9	Membranous	CST452308	Cell Signaling Technology
Galectin9	Rabbit monoclonal	1:200	EDTA, PH = 9	Membranous, Nuclear, Cytoplasmic	CST54330	Cell Signaling Technology
PD-L1	Rabbit monoclonal	1:200	EDTA, PH = 9	Membranous	CST13684	Cell Signaling Technology
CD68	Rabbit monoclonal	1:400	EDTA, PH = 9	Membranous	CST76437	Cell Signaling Technology
CD163	Rabbit monoclonal	1:50	EDTA, PH = 9	Membranous	CST93498	Cell Signaling Technology
T-bet	Rabbit monoclonal	1:200	EDTA, PH = 9	Nuclear	CST13232	Cell Signaling Technology
LAG-3	Rabbit monoclonal	1:200	EDTA, PH = 9	Membranous	CST15372	Cell Signaling Technology
CXCR5	Rabbit monoclonal	1:50	EDTA, PH = 9	Membranous	CST72172	Cell Signaling Technology
Foxp3	Rabbit monoclonal	1:100	EDTA, PH = 9	Nuclear	CST98377	Cell Signaling Technology
CD117	Rabbit monoclonal	1:100	EDTA, PH = 9	Membranous, Cytoplasmic	CST37805	Cell Signaling Technology

CD66b	Mouse monoclonal	1:500	EDTA, PH = 9	Membranous	GTX19779	Genetex
CD3	Rabbit monoclonal	1:200	EDTA, PH = 9	Membranous	ab16669	Abcam
CD4	Recombinant rabbit monoclonal	1:400	EDTA, PH = 9	Membranous	BX50023	BioLynx
Galectin1	Rabbit monoclonal	1:200	EDTA, PH = 9	Extracellular	CST13888	Cell Signaling Technology

25 Abbreviations: EDTA, ethylene diamine tetraacetic acid; PH, pondus hydrogenii.

26

27

28

29

30

31

32

33

34

35

36

37

38

39 Supplementary table 3. information about expression percentage of immune markers and univariable analysis of 17 immune markers in 54 NPC
 40 patients.

Immune Markers	Intratumor(n=54)			Stromal(n=40)		
	Positive No (%)	Hazard ratio (95% CI)	<i>P</i>	Positive No (%)	Hazard ratio (95% CI)	<i>P</i>
CD39	54(100%)	1.382(0.627–3.049)	0.423	40(100%)	2.400 (0.886–6.503)	0.085
CD8	52(96.3%)	0.966(0.441-2.118)	0.931	40(100%)	1.051(0.394-2.800)	0.921
PD1	54(100%)	1.137(0.519-2.493)	0.748	39(97.5%)	1.017(0.392-2.637)	0.973
CD39+CD8+*	52(96.3%)	1.139(0.519-2.498)	0.745	38(95.0%)	0.948(0.366-2.459)	0.913
PD1+CD8+*	52(96.3%)	0.727(0.330-1.601)	0.428	38(95.0%)	0.530(0.196-1.436)	0.212
TIM3	52(96.3%)	1.712(0.767-3.817)	0.189	40(100%)	1.880(0.682-5.181)	0.222
TIM3+CD8+*	33(61.1%)	2.493(1.096-5.669)	0.029	36(90.0%)	1.484(0.552-3.988)	0.434
Galectin9	54(100%)	0.753(0.341-1.660)	0.481	40(100%)	1.329(0.512-3.447)	0.559
CD68	54(100%)	0.647(0.293-1.425)	0.280	40(100%)	0.494(0.183-1.338)	0.165
PDL1	52(96.3%)	1.806(0.809-4.029)	0.149	40(100%)	1.491(0.555-4.008)	0.429
CD163	48(88.9%)	2.134(0.941-4.843)	0.070	39(97.5%)	2.765(0.958-7.983)	0.060

PDL1 ⁺ D163 ⁺ *	44(81.5%)	2.991(1.284-6.969)	0.011	36(90.0%)	2.784(0.964-8.037)	0.058
T-bet	54(100%)	1.677(0.752-3.740)	0.206	40(100%)	2.019(0.733-5.562)	0.174
LAG3	52(96.3%)	3.494(1.452-8.404)	0.005	38(95.0%)	2.835(0.983-8.174)	0.054
CXCR5	52(96.3%)	1.353(0.539-3.393)	0.520	37(92.5%)	3.930(1.416 -10.909)	0.009
Foxp3	52(96.3%)	1.377(0.624-3.039)	0.428	40(100%)	3.845(1.322-11.187)	0.013
CD117	52(96.3%)	2.620(1.126-6.093)	0.025	39(97.5%)	1.876(0.681-5.166)	0.224
CD66b	52(96.3%)	1.233(0.559-2.717)	0.604	24(60%)	0.525(0.199-1.381)	0.192
CD3	54(100%)	0.616(0.266-1.429)	0.259	54(100%)	0.853(0.329-2.212)	0.744
CD4	48(88.9%)	0.606(0.272-1.349)	0.220	39(97.5%)	0.747(0.284-1.962)	0.554
Galectin1	53(98.1%)	1.775(0.783-4.023)	0.169	38(95.0%)	0.602(0.229-1.583)	0.304

41 *The data form used was percentage multiplied by 1000.

42

43

44

45

46 Supplementary table 4. Coefficients of the algorithm for the final intratumor prediction model.

	coef	Se (coef)	z	p
CD163 ⁺ PD-L1 ⁺ *	0.013479	0.007955	1.694	0.090
CXCR5	0.057512	0.039632	1.451	0.147
CD117	0.011048	0.004090	2.701	0.007

47 *The form of the data used was the percentage multiplied by 1000.

48

49

50

51

52

53

54

55

56

Supplementary table 5. Five-year DMFS and PFS estimates for different risk groups in training and validation cohorts.

	Training cohort (n = 194)		Validation cohort (n = 304)	
	Low-risk (n = 152)	High-risk (n = 42)	Low-risk (n = 234)	High-risk (n = 70)
DMFS	89.1	61.4	87.8	63.1
(95% CI)	(82.2–96.0)	(45.9–76.9)	(81.1–94.5)	(48.2–78.0)
PFS	74.1	54.0	66.8	44.5
(95% CI)	(62.9–85.3)	(35.2–72.8)	(54.8–78.8)	(21.8–67.2)

57 DMFS, distant metastasis-free survival; PFS, progression free survival; CI, confidence interval.

58

59

60

61

62

63

64

Supplementary table 6. Five-year DMFS, and PFS estimates concerning treatment method in different risk groups .

	Low-risk (n = 303)		High-risk (n = 85)	
	CCRT (n = 146)	IC+CCRT (n = 157)	CCRT (n = 41)	IC+CCRT (n = 44)
DMFS	78.1	93.5	68.7	64.1
(95% CI)	(65.0–91.2)	(88.2–98.8)	(49.5–87.9)	(47.0–81.2)
PFS	70.4	76.2	60.4	59.4
(95% CI)	(61.0–79.8)	(66.6–85.8)	(41.4–79.4)	(42.0–76.8)

DMFS, distant metastasis-free survival; PFS, progression free survival; CI, confidence interval; IC, induction chemotherapy; CCRT, concurrent radiochemotherapy.

Supplement table 7: The C-index of three significant risk factors in the training and validation cohorts.

Factors	Training cohort (n=194)		Validation cohort (n=304)	
	C-index	95% CI	C-index	95% CI
HGB	0.516	(0.436–0.596)	0.565	(0.479-0.651)
N category	0.605	(0.519–0.691)	0.607	(0.529-0.685)
Immune signature A	0.703	(0.613–0.793)	0.636	(0.551-0.721)

HGB, hemoglobin; CI, confidence interval.

Supplement table 8: The C-index of Nomogram A and Nomogram B to predict the DMFS in the training cohort and validation cohort.

	Training cohort (n = 194)		Validation cohort (n = 304)	
	C-index (95% CI)	<i>P</i>	C-index (95% CI)	<i>P</i>
Nomogram A	0.791 (0.720–0.862)		0.729 (0.630–0.828)	
Nomogram B	0.805 (0.718–0.892)		-	
EBV DNA	0.663 (0.587–0.739)		-	
N category	0.605 (0.519–0.691)		0.607 (0.529–0.685)	
Nomogram A vs. Nomogram B		0.386		-
Nomogram A vs. EBV DNA		0.002		-
Nomogram A vs. N category		<0.00001		0.003

DMFS, distant metastasis-free survival; CI, confidence interval.