1 **Supplementary data:**

2 Supplementary Tables: 4

3 Supplementary Figures: 9

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5 **Supplementary Table S1. Establishment of CTC Cutoff Value in Training Cohort**

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CTC cutoff	Group*	No. in each group	36-Month PFS (%)	Survival difference (%)	HR	95%CI	*P*
1	0	3	100.0	52.2	1.7	0.4-7.1	0.4700
	≥ 1	67	47.8				
2	0-1	21	85.7	51.0	2.2	1.1-4.4	0.0210
	≥ 2	49	34.7				
3	0-2	30	80.0	52.5	4.7	2.1-11.0	0.0002
	≥ 3	40	27.5				
4	0-3	45	73.3	65.3	5.1	2.4-11.0	.0000
	≥ 4	25	8.0				
5	0-4	56	60.7	53.6	5.1	2.1-12.0	0.0003
	≥ 5	14	7.1				
6	0-5	62	56.5	-	6.1	1.8-20.0	0.0032
	≥ 6	8	-				
7	0-6	64	54.7	-	11.0	3.2-39.0	0.0002
	≥ 7	6	-				
8	0-7	67	52.2	-	13.0	2.5-65.0	0.0023
	≥ 8	3	-				
9	0-8	67	52.2	-	13.0	2.5-65.0	0.0023
	≥ 9	3	-				
10	0-9	69	50.7	-	42.0	3.7-480.0	0.0025
	≥ 10	1	-				

7 *Split into below and above different CTC cutoffs in rows.

8 *P* indicates significance according to Cox proportional hazards regression.

9 CTC indicates circulating tumor cell; HR, hazard ratio; PFS, progression-free survival; CI,

10 confidence interval.

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Supplementary Table S2. Establishment of CTC Cutoff Value in Training Cohort

CTC cutoff	Group	No. in each group	36-Month OS (%)	Survival difference (%)	HR	95%CI	P
1	0	3	100.0	28.4	4.9	0.7-36.0	0.1200
	≥ 1	67	71.6				
2	0-1	21	100	38.8	5.0	2.3-11.0	.0000
	≥ 2	49	61.2				
3	0-2	30	96.7	41.7	8.7	4.0-19.0	.0000
	≥ 3	40	55.0				
4	0-3	45	97.8	69.8	10.0	4.6-23.0	.0000
	≥ 4	25	28.0				
5	0-4	56	83.9	60.7	8.2	3.4-20.0	.0000
	≥ 5	14	28.6				
6	0-5	62	80.6	68.1	10.0	3.9-28.0	.0000
	≥ 6	8	12.5				
7	0-6	64	78.1	61.4	9.6	3.5-27.0	.0000
	≥ 7	6	16.7				
8	0-7	67	76.1	-	47.0	7.5-290.0	.0000
	≥ 8	3	-				
9	0-8	67	76.1	-	47.0	7.5-290.0	.0000
	≥ 9	3	-				
10	0-9	69	73.9	-	22.0	2.3-220.0	0.0070
	≥ 10	1	-				

*Split into below and above different CTC cutoffs in rows.

P indicates significance according to Cox proportional hazards regression.

CTC indicates circulating tumor cell; HR, hazard ratio; OS, overall survival; CI, confidence interval.

Supplementary Table S3. The Associations Between Different CTC Levels and Clinicopathological Characteristics in 212 Stage IB LUAD Patients

Characteristic	Patients with CTCs (%)							
	≥1	≥2	≥3	≥4	≥5	≥6	≥7	≥10
All (n=212)	94.3	89.6	56.6	41.5	20.8	10.4	7.5	1.4
Sex								
Male	94.5	76.1	56.0	39.4	22.9	14.7	11.0	1.8
Female	94.2	74.8	57.3	41.7	18.4	5.8	3.9	1.0
P	1	0.874	0.890	1	0.499	0.043	0.068	1
Age (years)								
<60	90.9	63.6	42.7	30.0	10.9	3.6	2.7	0.0
≥60	98.0	88.2	71.6	53.9	31.4	17.6	12.7	2.9
P	0.035	<0.001	<0.001	<0.001	<0.001	0.001	0.008	0.110
Smoke								
Yes	95.0	82.5	55.0	42.5	27.5	12.5	10.0	5.0
No	93.9	72.7	56.4	41.8	18.8	9.7	6.7	0.6
P	1	0.230	1	1	0.274	0.569	0.499	0.098
Differentiation								
Poorly	100.0	83.3	50.0	33.3	8.3	0.0	0.0	0.0
Moderately	94.0	75.4	56.9	41.3	22.2	12.0	8.4	1.8
Well	93.9	72.7	57.6	45.5	18.2	6.1	6.1	0.0
P	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.333
Tumor size (cm)								
<3.5	93.9	73.5	56.4	40.9	21.0	11.0	7.7	1.7
≥3.5	96.8	87.1	58.1	45.2	19.4	6.5	6.5	0.0
P	1	0.539	0.859	0.655	0.835	0.749	1	0.097

P indicates significance according to Chi-square test or Fisher's exact test.

CTC indicates circulating tumor cell; LUAD, lung adenocarcinoma.

59 **Supplementary Table S4: The C-Index of the Five Enrolled Factors in the Nomogram in**
60 **the SYSUFH Training Cohort**
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Factors	C-index	95% CI	*P*
Sex	0.507	0.423-0.590	0.883
Age	0.682	0.606-0.759	< .001
Smoke	0.536	0.472-0.600	0.274
Differentiation	0.530	0.464-0.560	0.374
CTC	0.708	0.685-0.785	< .001

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63 *P* indicates significance according to Cox proportional hazards regression
64 C-index indicates concordance index; CI, confidence interval; CTC, circulating tumor cell;
65 SYSUFH, The First Affiliated Hospital of Sun Yat-sen University.
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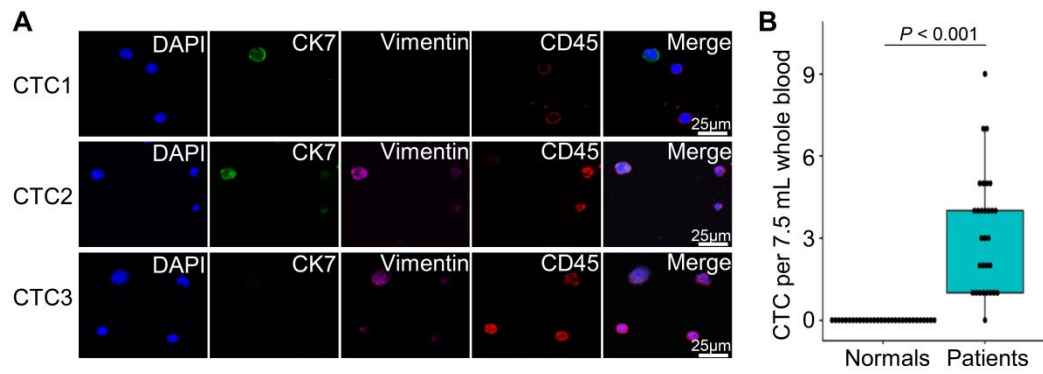
Supplementary Figure S1: Gallery of CTC images from the aptamer-modified

NanoVelcro system obtained from 7.5 mL of blood from 30 healthy donors and 30 stage

IB LUAD patients. A, CTC1 shows that epithelial CTCs express CK7. CTC2 shows that hybrid

CTCs express CK7 and Vimentin. CTC3 shows that mesenchymal CTCs express Vimentin. B,

CTC number in 7.5 mL of peripheral blood from stage IB LUAD patients was significantly

higher than that in heathy controls ($P<0.001$). CTC indicates circulating tumor cell; LUAD, lung

adenocarcinoma.

Discovery phase

Selecting method of CTC enumeration: NanoVelcro system

Collecting blood samples for CTC detection and gathering clinical materials from multi-center (n=223)

Patients out of follow-up (n=11)

Patients from multi-center meet the inclusion criterion and join the follow-up process (n=212)

Training phase

Using Kaplan-Meier method, log-rank test and X-tile software evaluating a series of CTC thresholds

Establishing the most appropriate prognostic threshold for CTCs in 70 LUADs from SYSUFH with univariate and multivariate analysis

Validation phase

Validating the optimum prognostic CTC cut-off

Internal validation set: 71 stage IB LUAD patient samples from SYSUFH

External validation set: 71 stage IB LUAD patient samples from WHCH

Nomogram was conducted to evaluate prognosis for guiding adjuvant chemotherapy in clinic

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123 **Supplementary Figure S2: Study flowchart of the development and evaluation of the**

124 **CTC-based classifier in stratifying the risk of stage IB LUAD.** CTC indicates circulating

125 tumor cell; LUAD, lung adenocarcinoma; SYSUFH, First Affiliated Hospital of Sun Yat-sen

126 University; WHCH, Wuhan Central Hospital.

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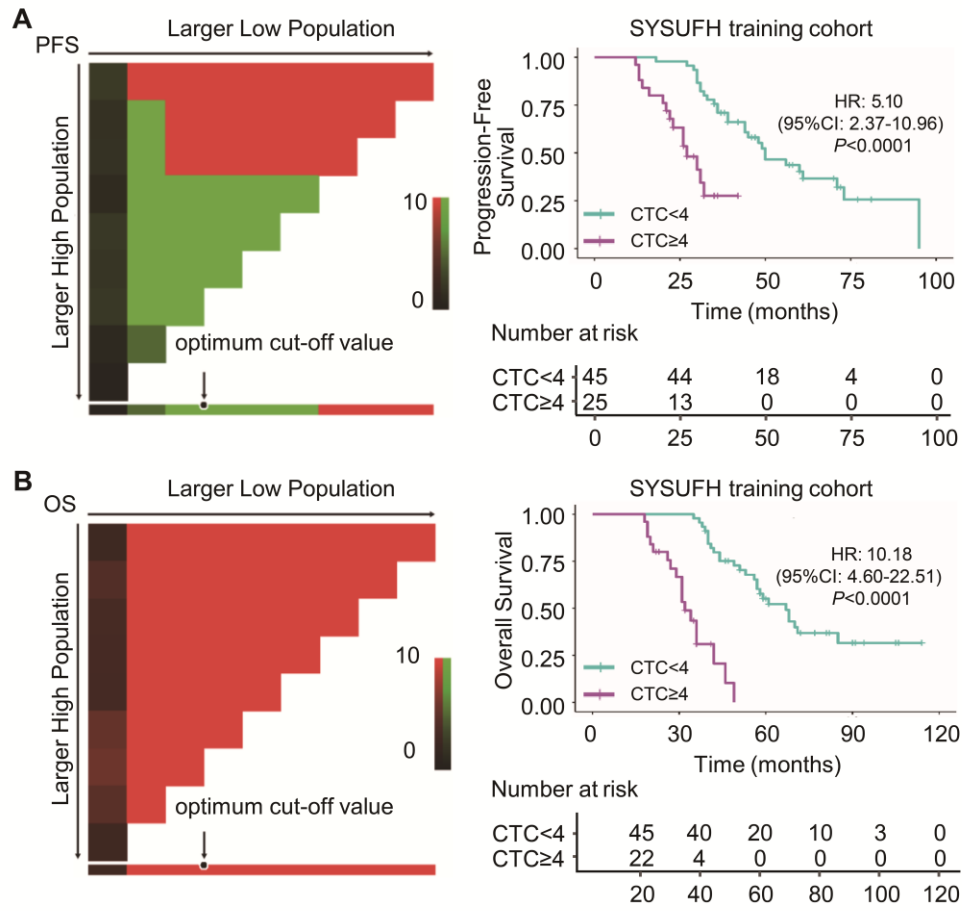
Supplementary Figure S3: The optimum cutoff value of CTC count and Kaplan-Meier

curves for predicting PFS and OS in the training cohort with 70 stage IB LUAD patients.

A, Progression-free survival; B, Overall survival. The optimum cutoff value was defined using

the X-tile program**,** and the coloration of the plot represents the strength of the association at

each division. Red represents a positive association between the risk score and PFS or OS,

whereas green represents an inverse association. CTC indicates circulating tumor cell; PFS,

progression-free survival; OS, overall survival; HR, hazard ratio.

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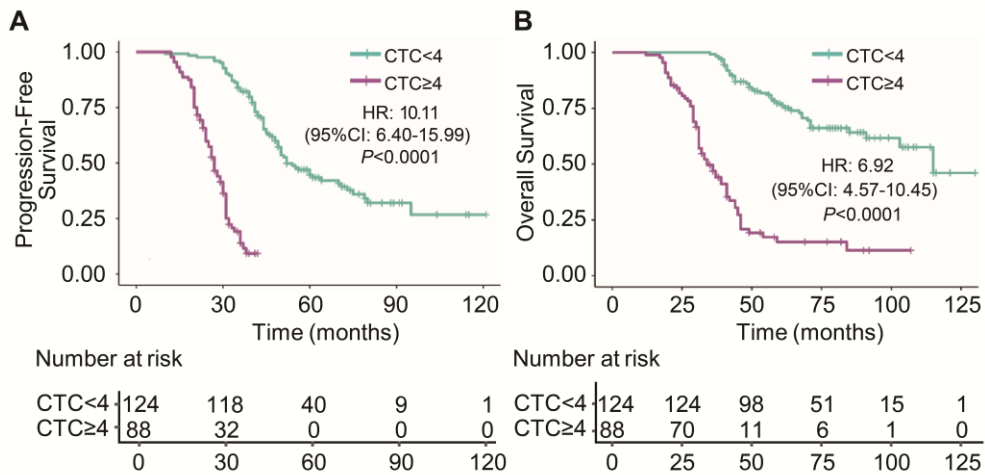
Supplementary Figure S4. Kaplan-Meier curves for predicting PFS and OS according to the cutoff value of 4 CTCs per 7.5 mL of blood in all 212 patients with stage IB LUAD. A, Progression-free survival according to the number of CTCs detected in the total patient cohort; B, Overall survival according to the number of CTCs detected in the total patient cohort. *P* indicates significance according to log Rank test. CTC indicates circulating tumor cell; PFS, progression-free survival; OS, overall survival; HR, hazard ratio.

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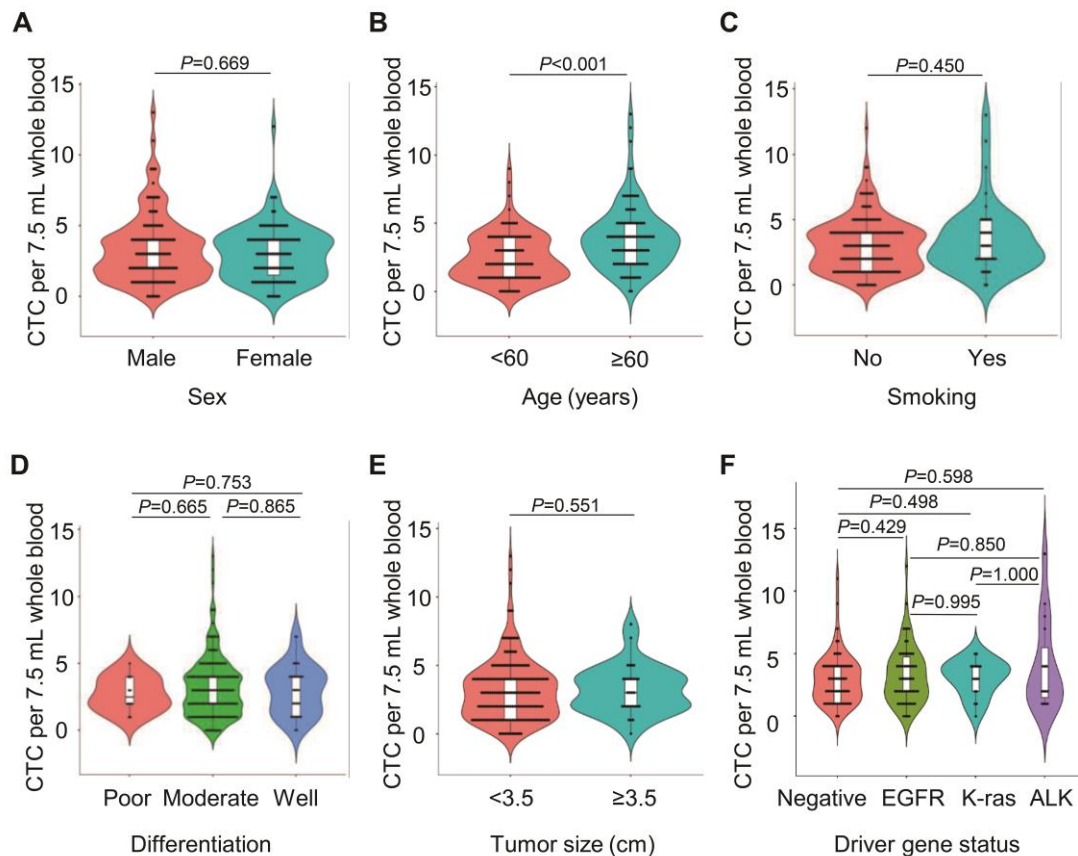
Supplementary Figure S5. Relationship between total CTC number and different clinicopathological characteristics. A, Prevalence of CTCs in 7.5 mL of blood in sex: male (n = 109) and female (n = 103). B, Prevalence of CTCs in 7.5 mL of blood in age (years): <60 (n = 110) and ≥ 60 (n= 102). C, Prevalence of CTCs in 7.5 mL of blood in status of smoking: no (n=165) and yes (n = 40). D, Prevalence of CTCs in 7.5 mL of blood in different differentiation: poor (n = 12), moderate (n = 167) and well (n = 33). E, Prevalence of CTCs in 7.5 mL of blood in different tumor size: < 3.5 cm (n = 181) and ≥ 3.5 cm (n = 31). F, Prevalence of CTCs in 7.5 mL of blood from different driver gene groups: negative (n= 65), EGRF mutation (n = 62), K-ras mutation (n = 17), ALK rearrangement (n = 15). Other 53 patients have no gene testing reports. CTC indicates circulating tumor cell.

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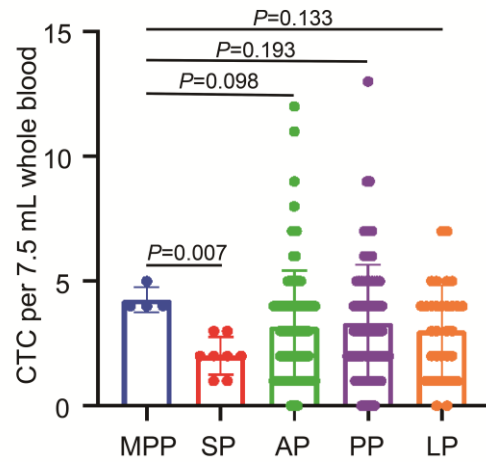
Supplementary Figure S6. Prevalence of CTCs in 7.5 mL of blood from different histologic subtypes of LUAD. MPP (n = 4), SP (n = 8), AP (n = 88), PP (n =79), LP (n = 33). n indicates the number of patients in each group. CTC indicates circulating tumor cell; MPP, Micropapillary predominant; SP, solid predominant; AP, acinar predominant; PP, papillary predominant; LP, Lepidic predominant.
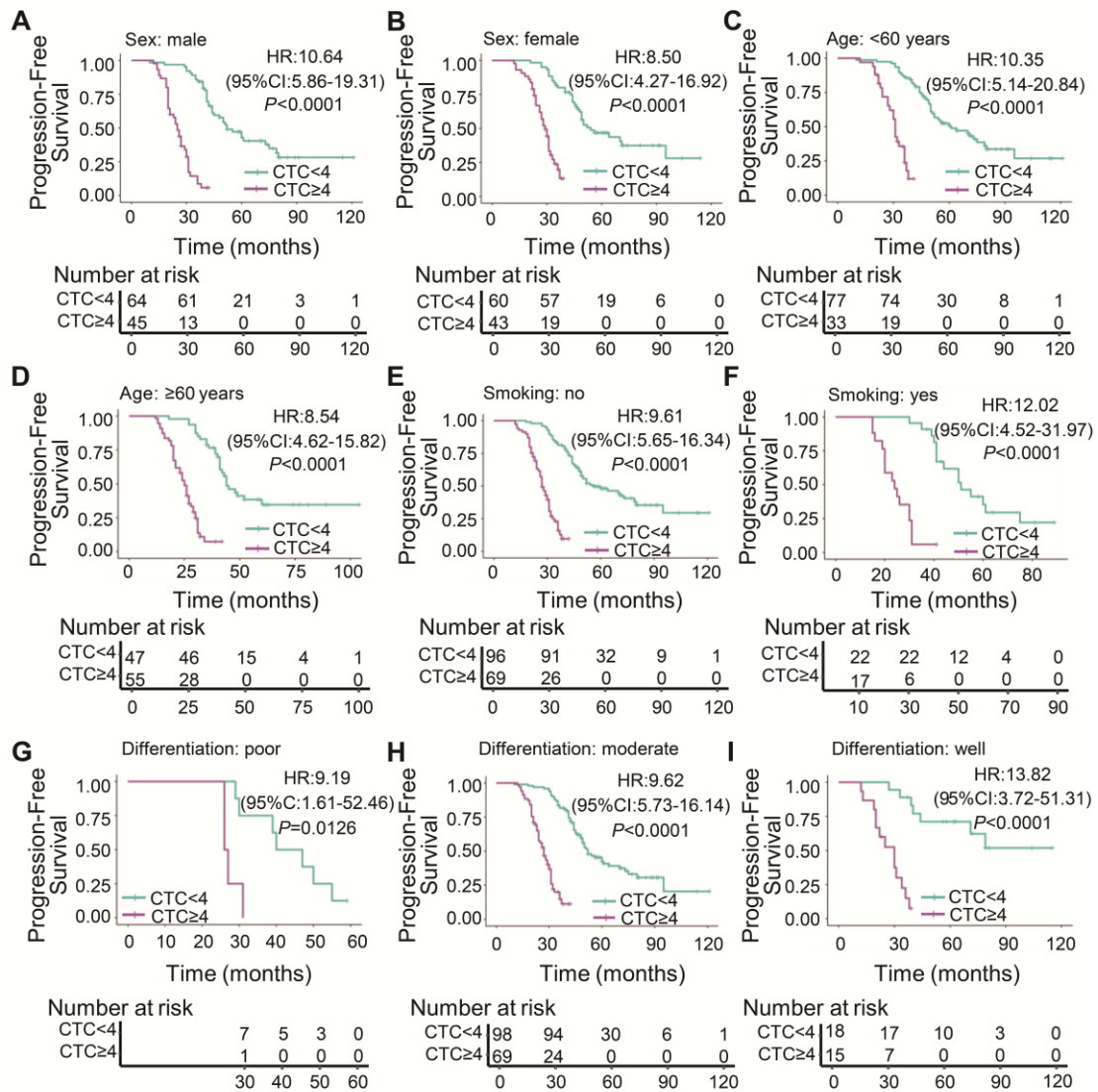
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Supplementary Figure S7. Kaplan-Meier curves for predicting PFS with different clinicopathological characteristics according to the cutoff value of 4 CTCs per 7.5 mL of blood in all 212 patients.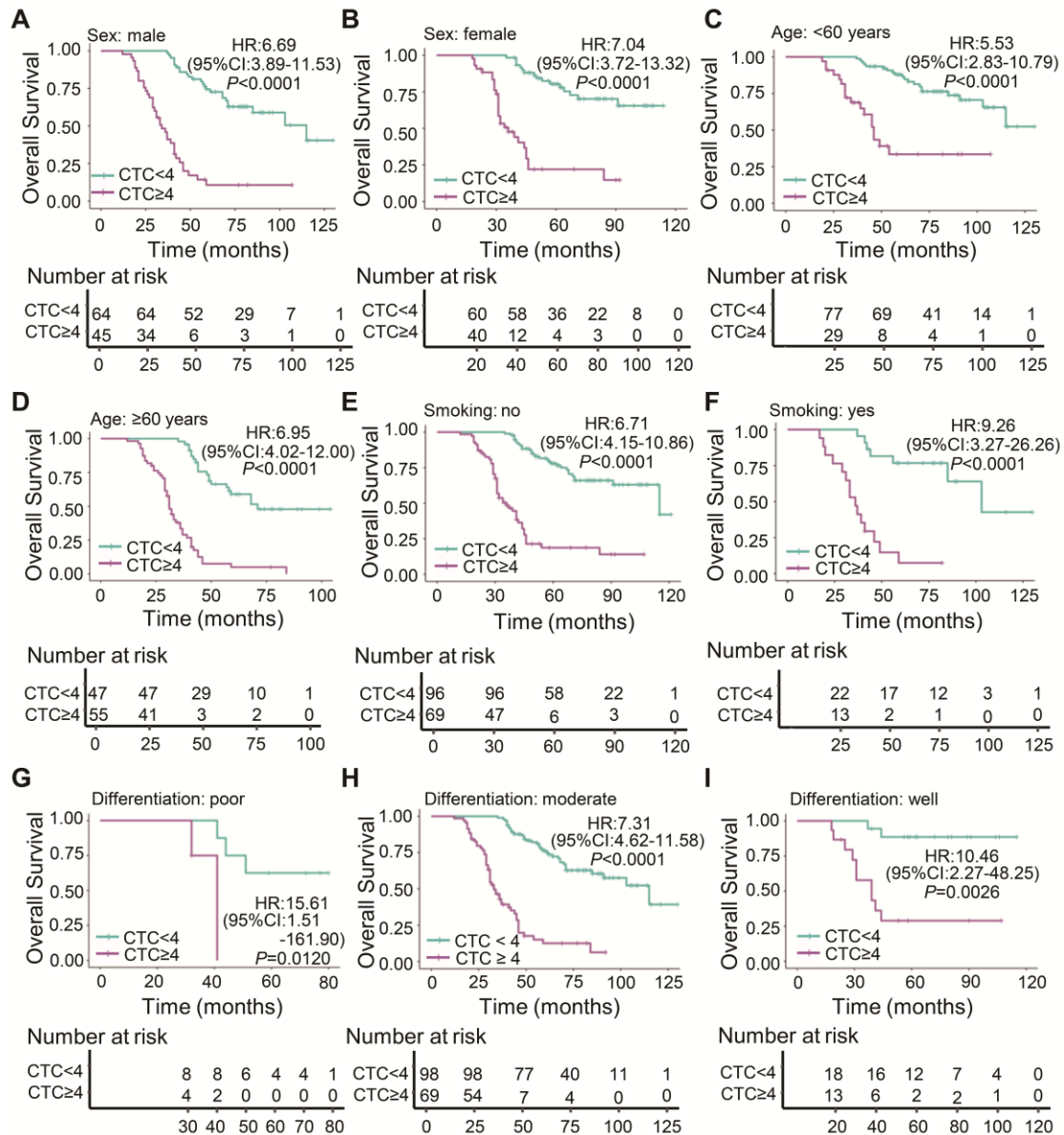 A, male with CTC<4 or CTC≥4 groups; B, female with CTC<4 or CTC≥4 groups; C, age<60 with CTC<4 or CTC≥4 groups; D, age≥60 with CTC<4 or CTC≥4 groups; E, smoking with CTC<4 or CTC≥4 groups; F, no smoking with CTC<4 or CTC≥4 groups; G, poor differentiation with CTC<4 or CTC≥4 groups; H, moderate differentiation with CTC<4 or CTC≥4 groups; I, well differentiation with CTC<4 or CTC≥4 groups. *P* indicates

195   significance according to log Rank test. CTC indicates circulating tumor cell; PFS,

196   progression-free survival; HR, hazard ratio.

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198 **Supplementary Figure S8. Kaplan-Meier curves for predicting OS with different**

199 **clinicopathological characteristics according to the cutoff value of 4 CTCs per 7.5 mL of**

200 **blood in all 212 patients.** A, male with CTC<4 or CTC≥4 groups; B, female with CTC<4 or

201 CTC≥4 groups; C, age<60 with CTC<4 or CTC≥4 groups; D, age≥60 with CTC<4 or CTC≥4

202 groups; E, smoking with CTC<4 or CTC≥4 groups; F, no smoking with CTC<4 or CTC≥4

203   groups; G, poor differentiation with CTC<4 or CTC≥4 groups; H, moderate differentiation with

204   CTC<4 or CTC≥4 groups; I, well differentiation with CTC<4 or CTC≥4 groups. CTC indicates

205   circulating tumor cell; OS, overall survival; HR, hazard ratio.

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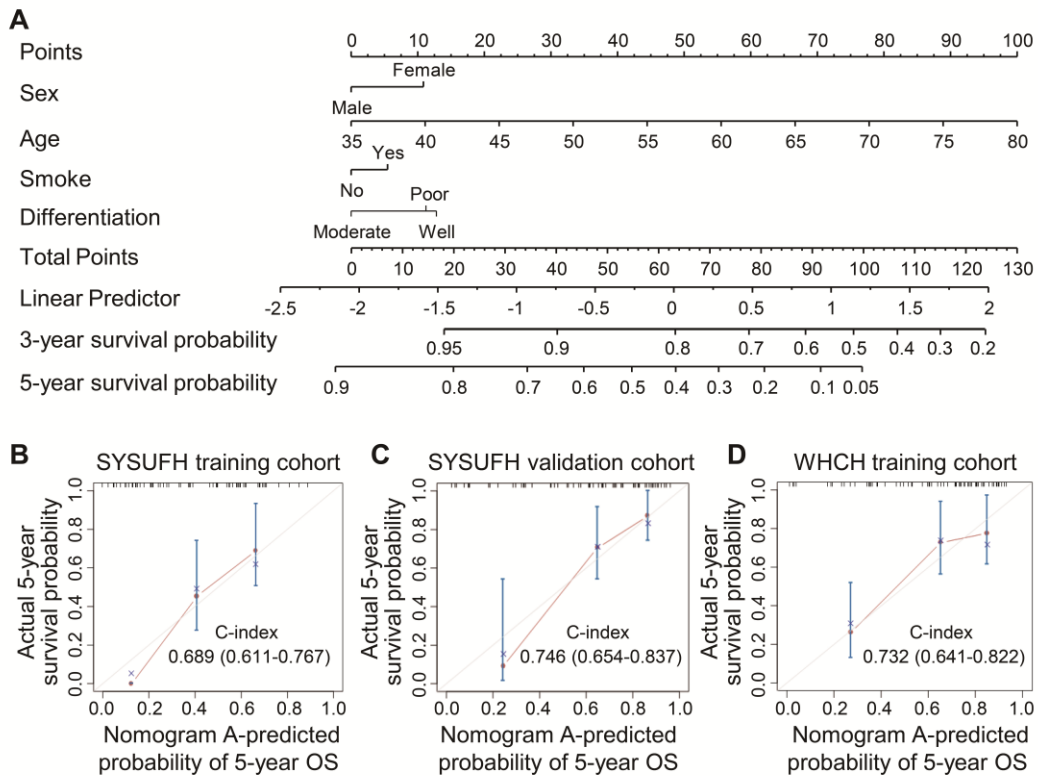
Supplementary Figure S9: Nomogram A (excluding CTC level) for predicting the 3- and

5-year survival probabilities of patients with stage IB LUAD and calibration curves for

testing the stability of nomogram. A, Nomogram A was based on the multivariate analysis

results of the SYSUFH training cohort. B, Calibration curves of the SYSUFH training cohort. C,

Calibration curves of the SYSUFH validation cohort. D, Calibration curves of the WHCH

validation cohort. CTC indicates circulating tumor cell; C-index, concordance index; SYSUFH,

First Affiliated Hospital of Sun Yat-sen University; WHCH, Wuhan Central Hospital; LUAD,

lung adenocarcinoma.